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Article

Isolation, Structure Determination, and Total Synthesis of Hoshinoamide C, an Antiparasitic Lipopeptide from the Marine Cyanobacterium *Caldora penicillata*

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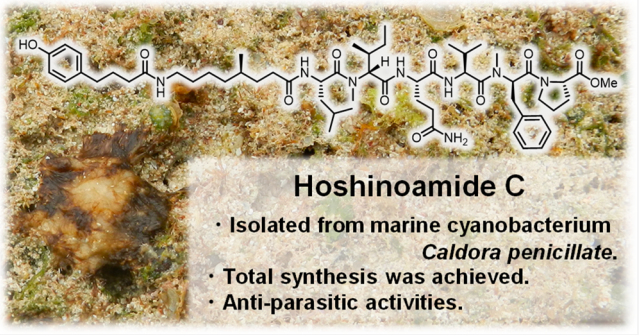
ABSTRACT: Hoshinoamide C (**1**), an antiparasitic lipopeptide, was isolated from the marine cyanobacterium *Caldora penicillata*. Its planar structure was elucidated by spectral analyses, mainly 2D NMR, and the absolute configurations of the α-amino acid moieties were determined by degradation reactions followed by chiral-phase HPLC analyses. To clarify the absolute configuration of an unusual amino acid moiety, we synthesized two possible diastereomers of hoshinoamide C and determined its absolute configuration based on a comparison of their spectroscopic data with those of the natural compound. Hoshinoamide C (**1**) did not exhibit any cytotoxicity against HeLa or HL60 cells at 10 μM, but inhibited the growth of the parasites responsible for malaria (IC_{50} 0.96 μM) and African sleeping sickness (IC_{50} 2.9 μM).



Some of the secondary metabolites from marine cyanobacteria are good sources for antiparasitic compounds. Several compounds possessing such activities, including gallinamide A[1] and the almiramides,[2] have been reported to date. Our group has also investigated such natural products from marine cyanobacteria inhabiting Japanese coastal areas and very recently reported iheyamides[3] and ikoamide.[4] In the course of our study on antiparasitic marine natural products, we discovered two lipopeptides, hoshinoamides A and B, from the marine cyanobacterium *Caldora penicillata* in 2018.[5] Although they do not show toxicity against human cells at 10 μM, they inhibited the *in vitro* growth of the malarial parasite *Plasmodium falciparum* with IC_{50} values of 0.52 and 1.0 μM, respectively. Further investigation of *C. penicillata* led to the discovery of another analogue of the hoshinoamides. Here, we report the isolation, structure determination, total synthesis, and antiparasite activities of this new analogue, hoshinoamide C (**1**).

RESULTS AND DISCUSSION

The marine cyanobacterium *C. penicillata* (2000 g, wet weight) was collected at Ikei Island, Okinawa, Japan, in March 2017, and extracted with MeOH. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with reversed-phase column chromatography (ODS silica gel, MeOH−H_2O) and repeated reversed-phase HPLC to give hoshinoamide C (**1**, 4.1 mg). From the same cyanobacterial sample, we had already isolated a new polyketide, caldorin, and had reported its structure and biological activity.[6]

Hoshinoamide C (**1**) was obtained as a colorless oil. The NMR data for **1** are summarized in Table 1. The molecular formula of **1** was found to be $C_{58}H_{90}N_8O_{11}$ by HRESIMS, indicating that **1** has 18 degrees of unsaturation. In the ^{1}H NMR spectrum, we observed three singlet methyl signals (δ_H 3.70, 3.11, and 3.00) corresponding to one *O*-Me ester and two *N*-Me amides, respectively. Also, six deshielded methine signals (δ_H 5.63, 4.90, 4.71, 4.67, 4.39, and 4.24) indicated the presence of six amino acid moieties. Meanwhile, nine deshielded signals in the ^{13}C NMR spectrum (175.1, 174.4, 173.9, 173.3, 172.7, 172.0, 171.3, 170.2, and 168.6) suggested the presence of nine carbonyl groups. Based on this information, we assumed that **1** is a peptidic compound. The detailed analyses of the 2D NMR data clarified four partial structures, as shown in Figure 1: C1−C6 (partial structure A), C7−C40 (partial structure B), C41−C48 (partial structure C),

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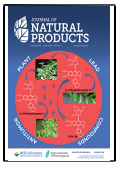

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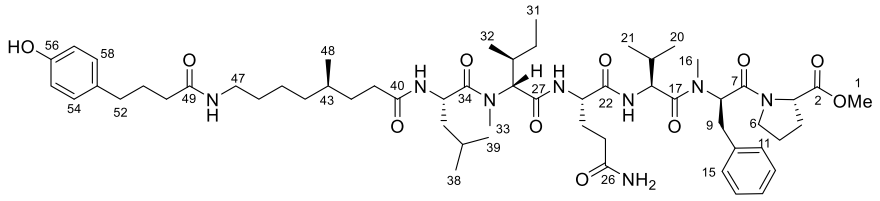

hoshinoamide C (**1**)

and C49−C58 (partial structure D). Partial structure A (C1−C6) was an *O*-Me-Pro moiety and considered to be the C-terminus of hoshinoamide C (**1**). However, its adjacent residue was not determined due to the lack of HMBC correlations. On the other hand, partial structure D (C49−C58) was a 4-(4-hydroxyphenyl)butanoic acid moiety and seemed to be the N-terminus of **1**. Partial structure B (C7−C40) was a pentapeptide moiety with a carbonyl group, *N*-Me-Phe-Val-Gln-*N*-Me-Ile-Leu-CO. Based on the chemical shift at C-40 (δ_C 173.9), it seemed that C-40 was a carbonyl carbon of an amide group. Partial structure C (C41−C48) was an alkyl chain possessing a branched methyl group at C-43. The chemical shifts of C-41 (δ_H 2.19, δ_C 34.1) indicated the presence of a carbonyl group next to it. The sum of the molecular formulas of these partial structures matched that of **1**, and, therefore, we had revealed all of the partial structures of this linear lipopeptide **1**. Next, we determined the sequence of these four partial structures. We had already discovered both termini of **1**, and, therefore, there were two possible sequences of **1**: A−B−C−D or A−C−B−D. Considering the carbon chemical shift at C-49 (δ_C 173.3), which is typical of an amide carbonyl carbon, we determined the sequence of **1** to be A−B−C−D, as shown in Figure 1.

The absolute configuration of each α-amino acid moiety in hoshinoamide C (**1**) was determined by acid hydrolysis followed by a combination of chiral-phase HPLC analyses and Marfey's method.[7] As a result, the *N*-Me-Phe and *N*-Me-Ile residues were determined to be the D-form and D-*allo* form, respectively, and the other residues were determined to be the L-forms. However, we were unable to clarify the absolute configuration at C-43 in the unusual amino acid moiety using any degradation experiments. To reveal the configuration at C-43, we synthesized two possible isomers of hoshinoamide C, (43*R*)-hoshinoamide C (**1**) and (43*S*)-hoshinoamide C (**2**).

First, we synthesized the two isomers of *N*-Boc-8-amino-4-methyloctanoic acid, (*R*)-**7** and (*S*)-**7** (Scheme 1). Protection of an amino group in 6-aminohexanoic acid with Boc followed by condensation with (*R*/*S*)-4-benzyl-2-oxazolidinone gave chiral oxazolidinones (*R*)-**3** and (*S*)-**3**, respectively. Stereoselective methylation of (*R*)-**3** and (*S*)-**3** and removal of the chiral auxiliary afforded alcohols (*R*)-**5** and (*S*)-**5**. The configuration of the stereogenic center in **5** was confirmed by application of the PGME method to the derivatized carboxylic acid (see Supporting Information S2). Elongation of the alkyl chain was achieved by TEMPO oxidation of the primary alcohol in **5** and Horner−Wadsworth−Emmonds reaction of the resulting aldehyde to give α,β-unsaturated esters (*R*)-**6** and (*S*)-**6**. Catalytic hydrogenation of the double bond followed by alkaline hydrolysis of the methyl ester gave desired fatty acids (*R*)-**7** and (*S*)-**7**, respectively.

Meanwhile, synthesis of the peptide moiety commenced with the condensation of known dipeptide **8**[8] with *N*-Boc-L-Val (Scheme 2). The product, tripeptide **9**, was further elongated by sequential condensation with *N*-Boc-L-Gln, *N*-Boc-*N*-Me-D-*allo*-Ile, and *N*-Boc-L-Leu to give hexapeptide **12**. Next, condensation between **12** and fatty acid (*R*)- or (*S*)-**7** afforded intermediates (*R*)-**13** and (*S*)-**13**, respectively. Finally, deprotection of the Boc group in **13** followed by acylation with 4-(4-hydroxyphenyl)butanoic acid gave two isomers of hoshinoamide, (43*R*)-hoshinoamide C (**1**) and (43*S*)-hoshinoamide C (**2**). We compared the NMR data of **1**, **2**, and natural hoshinoamide and found that the data of (43*R*)-hoshinoamide C (**1**) matched those of natural hoshinoamide C (see Supporting Information S52). Therefore, we concluded that the absolute configuration at C-43 in natural hoshinoamide C was *R* and clarified its complete structure as shown as **1**.

We assessed the biological activities of hoshinoamide C (**1**) along with its epimer, **2**. First, we assessed their growth-inhibitory activities against human cancer cells, HeLa and HL60, using the MTT assay. As a result, they did not show any growth-inhibitory activities against human cancer cells when tested at 10 μM.

Next, we evaluated their growth-inhibitory activities against the malarial parasite, *Plasmodium falciparum*, and the parasite responsible for African sleeping sickness, *Trypanosoma brucei rhodesiense*. As shown in Table 2, hoshinoamide C (**1**) and its epimer (**2**) showed moderate toxicities against these organisms. We also found that the configuration at C-43 does not affect the antiparasite activities. The antiparasite activity of **1** was similar to those of hoshinoamides A and B.[5]

In conclusion, we isolated a new hoshinoamide analogue, hoshinoamide C (**1**), from the marine cyanobacterium *C. penicillata*. Its planar structure was determined based on the spectral analyses, and its absolute configuration was clarified on the basis of degradation reactions and total synthesis of the two possible isomers. Comparing with the structures of hoshinoamides A and B, **1** lacks one α-amino acid residue and has the methylation in the unusual long-chain amino acid residue. Although hoshinoamide C (**1**) did not show any cytotoxicity against human cells, it inhibited the growth of the parasites responsible for malaria and African sleeping sickness to the same extent as hoshinoamides A and B. The genus *Caldora* was proposed by Engene et al. in 2015,[9] and several bioactive compounds have been discovered from the genus so far.[5,6,10] Our discovery of hoshinoamide C (**1**) also indicates that the genus *Caldora* has high potential as a source of natural products.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO RT/IR-4200 instrument. All NMR data were recorded on a JEOL JNM-ECX400 spectrometer for ^{1}H (400 MHz) and ^{13}C (100 MHz). ^{1}H NMR chemical shifts (referenced to residual $CHCl_3$ and CHD_2OD observed at δ_H 7.26 and 3.31, respectively) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to $CHCl_3$ and CD_3OD observed at δ_C 77.16 and 49.0) were assigned

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Table 1. NMR Data for Hoshinoamide C (1) in $CDCl_3$

residue	position	δ_C,[a] type	δ_H[b] (J in Hz)	COSY	selected HMBC
Pro-*O*-Me	1	52.4, CH_3	3.70, s		2
	2	172.7, C			
	3	59.4, CH	4.39, dd (8.3, 6.1)	4a, 4b	2
	4a	29.0, CH_2	2.14, m	3, 4b, 5a, 5b	
	4b		1.86, m	3, 4a, 5a, 5b	
	5a	25.3, CH_2	1.97, m	4a, 4b, 5b, 6a, 6b	
	5b		1.78, m	4a, 4b, 5a, 6a, 6b	
	6a	47.2, CH_2	3.37, m	5a, 5b, 6b	
	6b		3.34, m	5a, 5b, 6a	
N-Me-Phe	7	168.6, C			
	8	56.0, CH	5.63, dd (8.3, 7.4)	9a, 9b	7, 16
	9a	35.3, CH_2	3.25, dd (14.1, 7.4)	8, 9b	10, 11, 15
	9b		2.88, dd (14.1, 8.3)	8, 9a	10, 11, 15
	10	136.9, C			
	11	129.6, CH	7.22, m	12	
	12	126.9, CH	7.14, m	11, 13	
	13	128.6, CH	7.22, m	12, 14	
	14	126.9, CH	7.14, m	13, 15	
	15	129.6, CH	7.22, m	14	
	16	31.1, CH_3	3.11, s		8, 17
Val	17	172.0, C			
	18	54.3, CH	4.67, dd (9.0, 6.1)	19, NH	17
	19	30.6, CH	1.75, m	18, 20, 21	
	20	17.2, CH_3	0.63, d (6.7)	19	
	21	19.6, CH_3	0.73, d (6.7)	19	
	NH		7.05, d (9.0)	18	22
Gln	22	171.3, C			
	23	53.3, CH	4.24, dt (7.2, 7.1)	NH, 24	22
	24	27.4, CH_2	2.00, m	23, 25	
	25	31.6, CH_2	2.21, m	24	26
	26	175.1, C			
	NH		7.36, d (7.2)	23	27
N-Me-Ile	27	170.2, C			
	28	61.4, CH	4.71, d (10.8)	29	27, 33
	29	32.1, CH	2.07, m	28, 30a, 30b, 32	
	30a	26.7, CH_2	1.51, m	29, 30b, 31	
	30b		1.10, m	29, 30a, 31	
	31	11.4, CH_3	0.92, t (7.7)	30a, 30b	
	32	15.1, CH_3	0.80, d (6.7)	29	
	33	31.4, CH_3	3.00, s		28, 34
Leu	34	174.4, C			
	35	48.4, CH	4.90, ddd (7.9, 5.5, 5.5)	36a, NH	34
	36a	41.8, CH_2	1.48, m	35, 36b, 37	
	36b		1.37, m	36a, 37	
	37	25.0, CH	1.64, m	36a, 36b, 38, 39	
	38	22.2, CH_3	0.97, d (6.5)	37	
	39	23.3, CH_3	0.93, d (6.7)	37	
	NH		6.68, d (7.9)	35	40
8-amino-4-methyloctanoic acid	40	173.9, C			
	41	34.1, CH_2	2.19, m	42b	
	42a	32.4, CH_2	2.21, m	42b, 43	
	42b		1.64, m	41, 42a, 43	
	43	32.1, CH	1.38, m	42a, 42b, 44a, 44b, 48	
	44a	36.4, CH_2	1.27, m	43, 44b,	
	44b		1.12, m	43, 44a, 45	
	45	24.2, CH_2	1.27, m	44b, 46	
	46	29.7, CH_2	1.40, m	45, 47	
	47	39.4, CH_2	3.18, dt (5.8, 6.3)	46, NH	
	48	19.5, CH_3	0.83, d (6.1)	43	
	NH		5.81, t (5.8)	46	
4-(4-hydroxyphenyl)-butanoic acid	49	173.3, C			

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Table 1. continued

residue	position	δ_C,[a] type	δ_H[b] (J in Hz)	COSY	selected HMBC
	50	35.5, CH_2	2.11, t (7.3)	51	49
	51	27.1, CH_2	1.92, m	50, 52	
	52	34.0, CH_2	2.57, t (7.1)	51	53, 54, 58
	53	132.7, C			
	54	129.7, CH	6.98, d (8.5)	55	52, 56, 58
	55	115.5, CH	6.76, d (8.5)	54	53, 56, 57
	56	154.9, CH			
	57	115.5, CH	6.76, d (8.5)	58	53, 55, 56
	58	129.7, CH	6.98, d (8.5)	57	52, 54, 56

[a]Measured at 100 MHz. [b]Measured at 400 MHz.

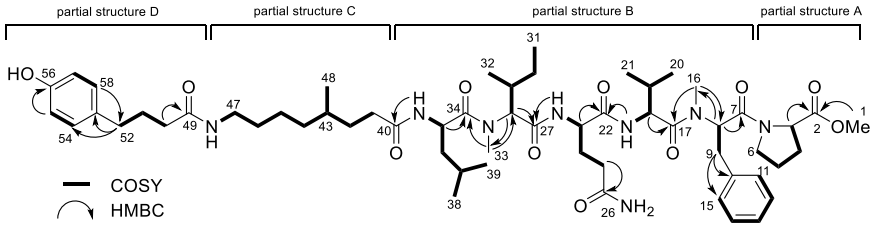
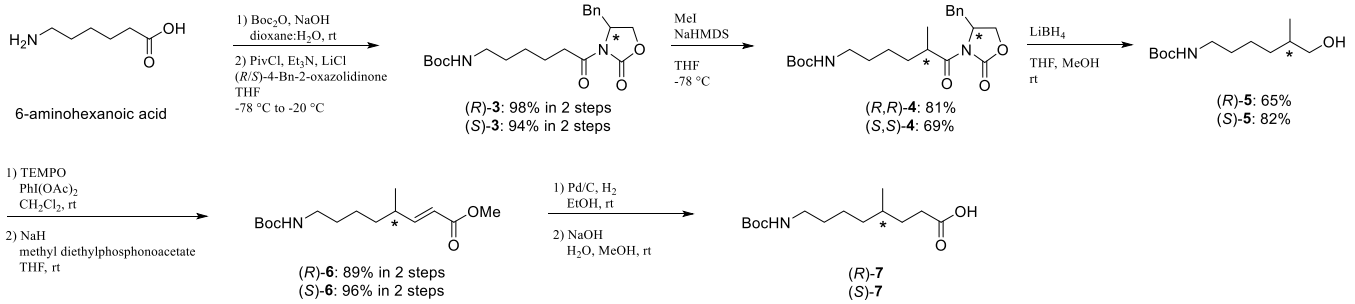
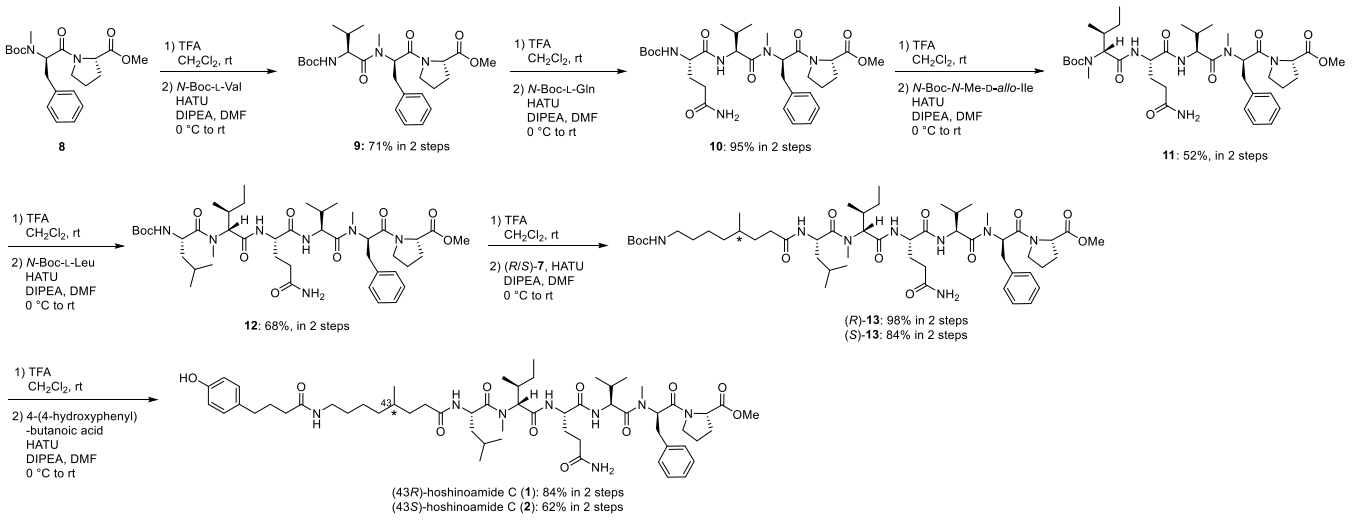


Figure 1. Gross structure of hoshinoamide C (**1**) based on 2D NMR.

Scheme 1. Synthesis of the Two Isomers of *N*-Boc-8-amino-4-methyloctanoic Acids, (*R*)-7 and (*S*)-7



Scheme 2. Synthesis of Two Isomers of Hoshinoamide C, (43*R*)-Hoshinoamide C (1) and (43*S*)-Hoshinoamide C (2)



based on HMBC and HMQC experiments. HRESIMS spectra were obtained on an LCT Premier XE time-of-flight (TOF) mass spectrometer. Chromatographic analyses were performed using an HPLC system consisting of a pump (model PU-2080, JASCO) and a UV detector (model UV-2075, JASCO). All chemicals and solvents used in this study were the best grade and available from a commercial source (Nacalai Tesque). Reactions were monitored by thin-layer chromatography (TLC), and TLC plates were visualized by both UV detection and phosphomolybdic acid solution. Silica gel 60N (Irregular, 63−212 μm) was used for column chromatography unless otherwise noted. All moisture-sensitive reactions were performed under an atmosphere of nitrogen, and the starting materials were azeotropically dried with toluene before use.

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Table 2. Growth-Inhibitory Activities of Hoshinoamides C (1) and Its Epimer (2)

compound	IC_{50} values (μM)	
	Plasmodium falciparum	*Trypanosoma brucei rhodesiense*
hoshinoamide C (**1**, natural)	0.96	2.9
hoshinoamide C (**1**, synthetic)	3.2	3.7
43-*epi*-hoshinoamide C (**2**)	0.87	4.4
atovaquone[a]	0.00096	
pentamidine[a]		0.001

[a]Positive control.

Collection, Extraction, and Isolation. The cyanobacterium *Caldora penicillata* (2000 g, wet weight) was collected at Ikei Island, Okinawa, Japan, in March 2017. The identification of this sample has been reported in our previous paper.[6] The collected cyanobacterium was extracted with MeOH (2 L) for 1 week. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between EtOAc (3 × 0.3 L) and H_2O (0.3 L). The material obtained from the organic layer was partitioned between 90% aqueous MeOH (0.3 L) and hexane (3 × 0.3 L). The aqueous MeOH fraction (674 mg) was separated by column chromatography on ODS (6.7 g) eluted with 40% MeOH, 60% MeOH, 80% MeOH, and MeOH. The fraction (161 mg) eluted with 80% MeOH was subjected to HPLC [Cosmosil $5C_{18}$MS-II (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 80% MeOH] to give a fraction that contained hoshinoamide C (**1**) (9.4 mg, t_R = 35.9 min). This fraction was further purified by HPLC [Cosmosil 5PE-MS (ϕ 20 × 250 mm); flow rate 5 mL/min; detection, UV 215 nm; solvent 80% MeOH] to give hoshinoamide C (**1**, 4.1 mg, t_R = 41.5 min).

*Hoshinoamide C (**1**):* colorless oil; $[\alpha]^{26}_D$ +22 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 279 (3.35) nm; IR (neat) 3315, 2876, 2832, 2876, 1747, 1634, 1541, 1516, 1456, 1269 cm^{-1}; ^1H NMR, ^{13}C NMR, COSY, and HMBC data, Table 1; HRESIMS *m*/*z* 1097.6646 $[M + Na]^+$ (calcd for $C_{58}H_{90}N_8O_{11}Na$, 1097.6627).

Determination of the Absolute Configuration of Hoshinoamide C (1). Hoshinoamide C (**1**, 0.1 mg) was treated with 9 M HCl (100 μL) for 24 h at 110 °C. The hydrolyzed product was evaporated to dryness and could be separated into each component [conditions for HPLC separation: column, Cosmosil $5C_{18}$-PAQ (ϕ 4.6 × 250 mm); flow rate, 1.0 mL/min; detection at 215 nm; solvent H_2O; retention times (min) of components; Pro (3.3), Val (3.4), Leu (4.9), *N*-Me-Ile (5.2), *N*-Me-Phe (13.1); conditions for HPLC separation: column, Cosmosil $5C_{18}$-PAQ (ϕ 4.6 × 250 mm); flow rate, 1.0 mL/min; detection at 215 nm; solvent 0.1% aqueous TFA; retention time (min) of component; Glu (3.4)].

Each amino acid except for *N*-Me-Ile was dissolved in H_2O (40 μL) and analyzed by chiral-phase HPLC, and the retention times were compared to those of authentic standards [DAICEL CHIRALPAK MA(+) (ϕ 4.6 × 50 mm); flow rate 1 mL/min; detection, UV 254 nm; solvent 2.0 mM $CuSO_4$, 2.0 mM $CuSO_4$–MeCN = 95/5, 2.0 mM $CuSO_4$–MeCN = 90/10]. With 2.0 mM $CuSO_4$ as a solvent, the retention times of Pro, Val, and Glu matched those of the authentic standards of L-Pro (5.0 min; D-Pro, 2.6 min), L-Val (6.3 min; D-Val, 3.3 min), and L-Glu (15.7 min; D-Glu, 9.6 min). With 2.0 mM $CuSO_4$–MeCN = 95/5 as a solvent, the retention time of Leu matched that of the authentic standard of L-Leu (7.6 min; D-Leu, 4.0 min). With 2.0 mM $CuSO_4$–MeCN = 90/10 as a solvent, the retention time of *N*-Me-Phe was matched that of the authentic standard of *N*-Me-D-Phe (6.2 min; *N*-Me-L-Phe, 6.6 min).

Regarding *N*-Me-Ile, we used Marfey's method to clarify its absolute configuration. To *N*-Me-Ile were added a 0.1% L-FDLA acetone solution (100 μL) and 1 M $NaHCO_3$ (25 μL). The mixture was heated at 80 °C for 3 min, cooled to room temperature (rt), and neutralized with 1 M HCl. The product was analyzed by HPLC, and the retention time was compared with those of the authentic standards [Cosmosil PBr (ϕ 4.6 × 250 mm); flow rate 1 mL/min; detection, UV 340 nm; solvent MeCN/H_2O–TFA = 55/45/0.1] The retention time of *N*-Me-Ile-L-DLA from the hydrolysate of **1** was matched with that of *N*-Me-D-*allo*-Ile-L-DLA (t_R = 18.7 min; *N*-Me-L-Ile-L-DLA, 14.0 min; *N*-Me-L-Ile-D-DLA, 17.1 min; *N*-Me-D-*allo*-Ile-D-DLA, 14.9 min).

Total Synthetic Procedures. *tert-Butyl (R)-(6-(4-Benzyl-2-oxooxazolidin-3-yl)-6-oxohexyl)carbamate ((R)-**3**).* To a stirred solution of 6-aminohexanoic acid (1.00 g, 7.62 mmol) in dioxane (15 mL) and H_2O (7 mL) were added NaOH (380 mg, 9.5 mmol) and Boc_2O (1.98 g, 9.16 mmol). After stirring at rt for 6 h, the reaction was quenched with 1 M HCl (20 mL), and the mixture was extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated to give crude *N*-Boc-6-aminohexanoic acid. To a solution of the crude *N*-Boc-6-aminohexanoic acid in THF (13 mL) cooled at −78 °C were added Et_3N (2.44 mL, 17.5 mmol) and PivCl (1.33 mL, 12.9 mmol) dropwise, and the mixture was stirred at the same temperature for 30 min. To this stirred suspension was added a solution of (*R*)-4-benzyloxazolidin-2-one (1.98 g, 11.2 mmol) and LiCl (510 mg, 12.0 mmol) in THF (13 mL) dropwise. After stirring at −20 °C for 3 h, the reaction was quenched with saturated aqueous NH_4Cl (30 mL), and the mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (120 g, hexane–EtOAc, 3:1) to give oxazolidinone (*R*)-**3** (2.92 g, 98%) as a colorless oil: $[\alpha]^{28}_D$ −27.9 (*c* 1.00, $CHCl_3$); IR (neat) 3382, 2932, 1781, 1700, 1172 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.26 (m, 3H), 7.23–7.19 (m, 2H), 4.67 (m, 1H), 4.54 (brs, 1H, NH), 4.20 (dd, *J* = 9.3, 7.8 Hz, 1H), 4.17 (dd, *J* = 9.3, 2.9 Hz, 1H), 3.30 (dd, *J* = 13.4, 2.9 Hz, 1H), 3.19–3.09 (m, 2H), 2.97 (dt, *J* = 17.1, 7.3 Hz, 1H), 2.90 (dt, *J* = 17.1, 7.3 Hz, 1H), 2.76 (dd, *J* = 13.4, 9.3 Hz, 1H), 1.76–1.66 (m, 2H), 1.57–1.47 (m, 2H), 1.44 (s, 9H), 1.45–1.36 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 156.0, 153.5, 135.3, 129.5 (2C), 129.0 (2C), 127.4, 79.1, 66.2, 55.2, 40.4, 37.9, 35.4, 29.9, 28.5 (3C), 26.3, 23.9; HRMS (ESI-TOF) *m*/*z* 413.2069 $[M + Na]^+$ (calcd for $C_{21}H_{30}N_2O_5Na$ 413.2052).

*tert-Butyl (S)-(6-(4-Benzyl-2-oxooxazolidin-3-yl)-6-oxohexyl)-carbamate ((S)-**3**).* To a stirred solution of 6-aminohexanoic acid (1.03 g, 7.85 mmol) in dioxane (15 mL) and H_2O (7 mL) were added NaOH (380 mg, 9.5 mmol) and Boc_2O (1.90 g, 8.71 mmol). After stirring at rt for 6 h, the reaction was quenched with 1 M HCl (20 mL), and the mixture was extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated to give crude *N*-Boc-6-aminohexanoic acid. To a solution of the crude *N*-Boc-6-aminohexanoic acid in THF (13 mL) cooled at −78 °C were added Et_3N (2.44 mL, 17.5 mmol) and PivCl (1.33 mL, 12.9 mmol) dropwise, and the mixture was stirred at the same temperature for 30 min. To this stirred suspension was added a solution of (*S*)-4-benzyloxazolidin-2-one (1.98 g, 11.2 mmol) and LiCl (510 mg, 12.0 mmol) in THF (13 mL) dropwise. After stirring at −20 °C for 3 h, the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (120 g, hexane–EtOAc, 3:1) to give oxazolidinone (*S*)-**3** (2.80 g, 94%) as a colorless oil: $[\alpha]^{28}_D$ +33.0 (*c* 1.00, $CHCl_3$); IR (neat) 3382, 2933, 1782, 1702, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.26 (m, 3H), 7.23–7.19 (m, 2H), 4.67 (m, 1H), 4.53 (brs, 1H, NH), 4.21 (dd, *J* = 9.3, 7.8 Hz, 1H), 4.17 (dd, *J* = 9.3, 2.9 Hz, 1H), 3.30 (dd, *J* = 13.4, 2.9 Hz, 1H), 3.19–3.09 (m, 2H), 2.97 (dt, *J* = 17.1, 7.3 Hz, 1H), 2.90 (dt, *J* = 17.1, 7.3 Hz, 1H), 2.76 (dd, *J* = 13.4, 9.3 Hz, 1H), 1.75–1.66 (m, 2H), 1.57–1.47 (m, 2H), 1.44 (s, 9H), 1.45–1.36 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 156.1, 153.5, 135.3, 129.5 (2C), 129.0 (2C), 127.4, 79.1, 66.3, 55.2, 40.4, 38.0, 35.5, 29.9, 28.5 (3C), 26.3, 23.9; HRMS (ESI-TOF) *m*/*z* 413.2059 $[M + Na]^+$ (calcd for $C_{21}H_{30}N_2O_5Na$ 413.2052).

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*tert-Butyl ((R)-6-((R)-4-Benzyl-2-oxooxazolidin-3-yl)-5-methyl-6-oxohexyl)carbamate ((R,R)-**4**).* To a stirred solution of oxazoidinone (*R*)-**3** (2.84 g, 7.27 mmol) in THF (25 mL) cooled at −78 °C were added a 1.0 M solution of NaHMDS in THF (9 mL, 9 mmol) and MeI (0.61 mL, 9.8 mmol) dropwise. After stirring at −78 °C for 17 h, the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and the mixture was extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (120 g, hexane−EtOAc, 4:1) to give α-methylated compound (*R*,*R*)-**4** (2.39 g, 81%) as a colorless oil: $[\alpha]^{28}_D$ −40.4 (*c* 1.00, $CHCl_3$); IR (neat) 3383, 2976, 2933, 1780, 1698, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36−7.27 (m, 3H), 7.23−7.19 (m, 2H), 4.67 (m, 1H), 4.52 (brs, 1H, NH), 4.25−4.15 (m, 2H), 3.70 (m, 1H), 3.26 (dd, *J* = 13.4, 3.4 Hz, 1H), 3.14−3.07 (m, 2H), 2.76 (dd, *J* = 13.4, 9.3 Hz, 1H), 1.76 (m, 1H), 1.43 (s, 9H), 1.54−1−38 (m, 3H), 1.37−1.28 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 156.0, 153.1, 135.3, 129.5 (2C), 129.0 (2C), 127.4, 79.0, 66.1, 55.3, 40.4, 37.9, 37.7, 33.0, 30.1, 28.5 (3C), 24.5, 17.4; HRMS (ESI-TOF) *m*/*z* 427.2189 $[M + Na]^+$ (calcd for $C_{22}H_{32}N_2O_5Na$ 427.2209).

*tert-Butyl ((S)-6-((S)-4-Benzyl-2-oxooxazolidin-3-yl)-5-methyl-6-oxohexyl)carbamate ((S,S)-**4**).* To a stirred solution of oxazolidinone (*S*)-**3** (2.02 g, 5.17 mmol) in THF (16 mL) were added NaHMDS (12 mL, 12 mmol, 1.0 M in THF) and MeI (0.40 mL, 6.4 mmol) dropwise at −78 °C. After stirring at the same temperature for 17 h, the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (120 g, hexane−EtOAc, 4:1) to give α-methylated compound (*S*,*S*)-**4** (1.44 g, 69%) as a colorless oil: $[\alpha]^{28}_D$ +40.6 (*c* 1.00, $CHCl_3$); IR (neat) 3384, 2976, 2933, 1781, 1699, 1174 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36−7.27 (m, 3H), 7.23−7.19 (m, 2H), 4.67 (m, 1H), 4.52 (brs, 1H, NH), 4.25−4.15 (m, 2H), 3.70 (m, 1H), 3.26 (dd, *J* = 13.4, 3.4 Hz, 1H), 3.14−3.07 (m, 2H), 2.76 (dd, *J* = 13.4, 9.3 Hz, 1H), 1.76 (m, 1H), 1.43 (s, 9H), 1.54−1.38 (m, 3H), 1.37−1.28 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 156.0, 153.1, 135.3, 129.5 (2C), 129.0 (2C), 127.3, 79.0, 66.1, 55.3, 40.4, 38.1, 37.9, 33.0, 30.0, 28.4 (3C), 24.4, 17.4; HRMS (ESI-TOF) *m*/*z* 427.2216 $[M + Na]^+$ (calcd for $C_{22}H_{32}N_2O_5Na$ 427.2209).

*tert-Butyl (R)-(6-Hydroxy-5-methylhexyl)carbamate ((R)-**5**).* To a stirred solution of α-methylated compound (*R*,*R*)-**4** (2.09 g, 5.17 mmol) in THF (19 mL) and MeOH (1 mL) cooled at 0 °C was added a 4 M solution of $LiBH_4$ in THF (2.9 mL, 12 mmol). After stirring at rt for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (20 mL) and the mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc, 2:1) to give alcohol (*R*)-**5** (773 mg, 65%) as a colorless oil: $[\alpha]^{28}_D$ −79.3 (*c* 1.00, $CHCl_3$); IR (neat) 3349, 2975, 2931, 2869, 1689, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.53 (brs, 1H, NH), 3.48 (dd, *J* = 10.4, 6.1 Hz, 1H), 3.43 (dd, *J* = 10.4, 6.3 Hz, 1H), 3.18−3.06 (m, 2H), 1.64−1.53 (m, 2H), 1.51−1.23 (m, 4H), 1.44 (s, 9H), 1.10 (m, 1H), 0.90 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 79.0, 67.6, 40.3, 35.5, 32.6, 30.3, 28.4 (3C), 23.9, 16.5; IR (neat); 3349, 2975, 2931, 2869, 1689, 1173; HRMS (ESI-TOF) *m*/*z* 232.1906 $[M + H]^+$ (calcd for $C_{12}H_{26}NO_3$ 232.1913).

*tert-Butyl (S)-(6-Hydroxy-5-methylhexyl)carbamate ((S)-**5**).* To a stirred solution of α-methylated compound (*S*,*S*)-**4** (1.34 g, 3.31 mmol) in THF (11 mL) and MeOH (1 mL) cooled at 0 °C was added $LiBH_4$ (1.6 mL, 6.4 mmol, 4 M in THF). After stirring at rt for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc, 2:1) to give alcohol (*S*)-**5** (629 mg, 82%) as a colorless oil: $[\alpha]^{28}_D$ +72.3 (*c* 1.00, $CHCl_3$); IR (neat) 3346, 2975, 2931, 2869, 1689, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.52 (brs, 1H, NH), 3.48 (dd, *J* = 10.4, 6.1 Hz, 1H), 3.43 (dd, *J* = 10.4, 6.3 Hz, 1H), 3.17−3.06 (m, 2H), 1.64−1.53 (m, 2H), 1.51−1.23 (m, 4H), 1.44 (s, 9H), 1.10 (m, 1H), 0.91 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 79.1, 67.8, 40.3, 35.6, 32.6, 30.3, 28.4 (3C), 23.9, 16.6; HRMS (ESI-TOF) *m*/*z* 232.1912 $[M + H]^+$ (calcd for $C_{12}H_{26}NO_3$ 232.1913).

*Methyl (R,E)-8-((tert-Butoxycarbonyl)amino)-4-methyloct-2-enoate ((R)-**6**).* To a stirred solution of alcohol (*R*)-**5** (43.0 mg, 0.186 mmol) in CH_2Cl_2 (1 mL) were added TEMPO (7 mg, 0.045 mmol) and $PhI(OAc)_2$ (133 mg, 0.413 mmol). After stirring at rt for 1 h, the reaction mixture was diluted with saturated aqueous $Na_2S_2O_3$ (5 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (8 g, hexane−EtOAc, 5:1) to give aldehyde (35.5 mg, 83%) as a colorless oil. To a suspension of NaH (12 mg, 0.30 mmol, 60% oil suspension) in THF (1 mL) cooled at 0 °C was added methyl diethylphosphonoacetate (0.056 mL, 0.31 mmol), and the mixture was stirred at the same temperature for 40 min. To this stirred suspension was added a solution of the aldehyde (34.5 mg, 0.150 mmol) in THF (1 mL) dropwise. After stirring at rt for 20 min, the reaction mixture was diluted with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc, 4:1) to give conjugated ester (*R*)-**6** (39.3 mg, 89%) as a colorless oil: $[\alpha]^{28}_D$ −17.6 (*c* 1.00, $CHCl_3$); IR (neat) 3372, 2974, 2932, 2862, 1719, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.84 (dd, *J* = 15.6, 7.8 Hz, 1H), 5.77 (dd, *J* = 15.6, 1.0 Hz, 1H), 4.49 (brs, 1H, NH), 3.72 (s, 3H), 3.14−3.05 (m, 2H), 2.29 (m, 1H), 1.50−1.33 (m, 4H), 1.44 (s, 9H), 1.33−1.24 (m, 2H), 1.04 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 156.1, 154.7, 119.5, 79.1, 51.5, 40.5, 36.6, 35.7, 30.2, 28.5 (3C), 24.5, 19.5; HRMS (ESI-TOF) *m*/*z* 308.1830 $[M + Na]^+$ (calcd for $C_{15}H_{27}NO_4Na$ 308.1838).

*Methyl (S,E)-8-((tert-Butoxycarbonyl)amino)-4-methyloct-2-enoate ((S)-**6**).* To a stirred solution of alcohol (*S*)-**5** (338 mg, 1.46 mmol) in CH_2Cl_2 (8 mL) were added TEMPO (57 mg, 0.36 mmol) and $PhI(OAc)_2$ (1.05 g, 3.26 mmol). After stirring at rt for 1 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ (15 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine (40 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc, 5:1) to give aldehyde (321 mg, 96%) as a colorless oil. To a suspension of NaH (111 mg, 2.7 mmol, 60% oil suspension) in THF (5 mL) at 0 °C was added methyl diethylphosphonoacetate (0.52 mL, 2.8 mmol), and the mixture was stirred at the same temperature for 40 min. To this stirred suspension was added a solution of the aldehyde (321 mg, 1.40 mmol) in THF (5 mL) dropwise. After stirring at rt for 20 min, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine (40 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc, 4:1) to give conjugated ester (*S*)-**6** (387 mg, 97%) as a colorless oil: $[\alpha]^{28}_D$ +17.4 (*c* 1.00, $CHCl_3$); IR (neat) 3372, 2974, 2932, 2862, 1720, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.84 (dd, *J* = 15.6, 7.8 Hz, 1H), 5.77 (dd, *J* = 15.6, 1.0 Hz, 1H), 4.49 (brs, 1H, NH), 3.73 (s, 3H), 3.14−3.05 (m, 2H), 2.29 (m, 1H), 1.50−1.33 (m, 4H), 1.44 (s, 9H), 1.33−1.24 (m, 2H), 1.04 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 156.0, 154.7, 119.4, 79.1, 51.5, 40.5, 36.6, 35.7, 30.2, 28.5 (3C), 24.4, 19.5; HRMS (ESI-TOF) *m*/*z* 308.1824 $[M + Na]^+$ (calcd for $C_{15}H_{27}NO_4Na$ 308.1838).

*(R)-8-((tert-Butoxycarbonyl)amino)-4-methyloctanoic Acid ((R)-**7**).* To a solution of conjugated ester (*R*)-**6** (39.3 mg, 0.138 mmol) in EtOH (1 mL) was added 5% Pd/C (53% H_2O content, 4 mg). The reaction mixture was vigorously stirred under a hydrogen atmosphere at rt for 5 h. The reaction mixture was filtered and concentrated to give crude ester. To a stirred solution of the crude ester in MeOH (11 mL) was added 2 M aqueous NaOH (0.5 mL, 1 mmol). After stirring at rt for 2 h, the reaction mixture was acidified with 1 M HCl (5 mL)

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and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated to give crude carboxylic acid (*R*)-**7** (28.7 mg). This product was used in the next reaction without purification.

*(S)-8-((tert-Butoxycarbonyl)amino)-4-methyloctanoic Acid ((S)-**7**).* To a solution of conjugated ester (*S*)-**6** (339 mg, 1.19 mmol) in EtOH (10 mL) was added 5% Pd/C (53% H_2O content, 30 mg). The reaction mixture was vigorously stirred under a hydrogen atmosphere at rt for 5 h. The reaction mixture was filtered and concentrated to give a crude ester. To a stirred solution of the crude ester in MeOH (10 mL) was added 2 M aqueous NaOH (5 mL, 10 mmol). After stirring at rt for 2 h, the reaction mixture was acidified with 1 M HCl (20 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (80 mL), dried (Na_2SO_4), and concentrated to give crude carboxylic acid (*S*)-**7** (209 mg). This product was used in the next reaction without purification.

*Methyl N-((tert-Butoxycarbonyl)-L-valyl)-N-methyl-D-phenylalanyl-L-prolinate (**9**).* To a stirred solution of known dipeptide **8** (719 mg, 1.84 mmol) in CH_2Cl_2 (15 mL) was added TFA (7.5 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and Boc-L-valine (760 mg, 3.50 mmol) in DMF (1.2 mL) cooled at 0 °C were added DIPEA (0.7 mL, 3.7 mmol) and HATU (1.41 g, 3.71 mmol). After stirring at rt for 9 h, the reaction mixture was diluted with EtOAc (20 mL), washed with 10% aqueous citric acid (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine, dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, $CHCl_3$−MeOH, 30:1) to give tripeptide **9** (641 mg, 71% in 2 steps) as a colorless amorphous solid. The ratio of major and minor rotamers is 5:1: $[\alpha]^{28}_D$ +57.0 (*c* 1.00, $CHCl_3$); IR (neat) 2974, 1747, 1693, 1653, 1171 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.29−7.13 (m, 5H), 5.73 (dd, *J* = 8.2, 6.8 Hz, 1H), 5.06 (brm, 1H, NH), 4.45 (dd, *J* = 8.6, 6.8 Hz, 1H), 4.39 (dd, *J* = 9.7, 4.5 Hz, 1 H), 3.75 (s, 3H), 3.48 (m, 1H), 3.34 (m, 1H), 3.29 (dd, *J* = 14.5, 6.8 Hz, 1H), 3.08 (s, 3H), 2.89 (dd, *J* = 14.5, 8.6 Hz, 1H), 2.24 (m, 1H), 1.98−1.79 (m, 2H), 1.62 (m, 1H), 1.45 (m, 1H), 1.41 (s, 9H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.53 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 172.5, 172.3, 168.5, 156.2, 137.2, 129.7 (2C), 128.4 (2C), 126.7, 79.6, 59.5, 56.0, 55.2, 52.3, 47.0, 35.1, 30.6, 30.4, 29.0, 28.4 (3C), 25.5, 19.9, 16.6; HRMS (ESI-TOF) *m*/*z* 512.2753 $[M + Na]^+$ (calcd for $C_{26}H_{39}N_3O_6Na$ 512.2737).

*Methyl N-(tert-Butoxycarbonyl)-L-glutaminyl-L-valyl-N-methyl-D-phenylalanyl-L-prolinate (**10**).* To a stirred solution of tripeptide **9** (620 mg, 1.27 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and Boc-L-glutamine (559 mg, 2.27 mmol) in DMF (0.90 mL) were added DIPEA (0.90 mL, 5.2 mmol) and HATU (1.00 g, 2.63 mmol) at 0 °C. After stirring at rt for 7 h, the reaction mixture was diluted with EtOAc (20 mL), washed with 10% aqueous citric acid (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine, dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, $CHCl_3$−EtOAc−MeOH, 20:20:1) to give tetrapeptide **10** (745 mg, 95% in 2 steps) as a colorless amorphous solid; the ratio of major and minor rotamers is 6:1; $[\alpha]^{28}_D$ +14.0 (*c* 1.00, $CHCl_3$); IR (neat) 3319, 2974, 1743, 1637, 1453, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.28−7.13 (m, 5H), 7.00 (brm, 1H, NH), 6.50 (brs, 1H, NH), 5.64 (m, 1H), 5.64 (brm, 1H, NH), 5.41 (brm, 1H, NH), 4.70 (dd, *J* = 8.6, 5.4 Hz, 1H), 4.41 (dd, *J* = 8.2, 5.9 Hz, 1H), 4.11 (m, 1H), 3.72 (s, 3H), 3.40−3.32 (m, 2H), 3.25 (dd, *J* = 14.5, 6.8 Hz, 1H), 3.14 (s, 3H), 2.91 (dd, *J* = 14.5, 9.1 Hz, 1H), 2.37−2.11 (m, 3H), 2.01−1.74 (m, 6H), 1.42 (s, 9H), 0.77 (d, *J* = 6.3 Hz, 3H), 0.58 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.4, 172.7, 172.1, 171.9, 168.5, 155.8, 136.9, 129.5 (2C), 128.5 (2C), 126.8, 80.0, 59.3, 56.0, 54.2, 53.6, 52.3, 47.0, 35.1, 31.7, 31.0, 30.5, 29.0, 28.9, 28.4 (3C), 25.3, 19.6, 17.1; HRMS (ESI-TOF) *m*/*z* 640.3303 $[M + Na]^+$ (calcd for $C_{31}H_{47}N_5O_8Na$ 640.3322).

*Methyl N,N-(tert-Butoxycarbonyl)-N-methyl-D-alloisoleucyl-L-glutaminyl-L-valyl-N-methyl-D-phenylalanyl-L-prolinate (**11**).* To a stirred solution of tetrapeptide **10** (440 mg, 0.713 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and crude Boc-D-*allo*-isoleucine (256 mg) in DMF (0.48 mL) cooled at 0 °C were added DIPEA (0.50 mL, 3.0 mmol) and HATU (542 mg, 1.43 mmol). After stirring at rt for 15 h, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (15 g, $CHCl_3$−EtOAc−MeOH, 20:20:1) to give pentapeptide **11** (275 mg, 52% in 2 steps) as a colorless amorphous solid. The ratio of major and minor rotamers is 6:3:1: $[\alpha]^{28}_D$ +22.2 (*c* 1.00, $CHCl_3$); IR (neat) 3317, 2966, 1745, 1642, 1443, 1156 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.27−7.12 (m, 5H), 7.06 (brm, 1H, NH), 6.96 (m, 1H, NH), 6.57 (brs, 1H, NH), 5.68 (brs, 1H, NH), 5.62 (dd, *J* = 7.8, 7.3 Hz, 1H), 4.67 (m, 1H), 4.40 (dd, *J* = 8.3, 5.9 Hz, 1H), 4.34 (d, *J* = 7.3 Hz, 1H), 4.16 (m, 1H), 3.72 (s, 3H), 3.41 (m, 1H), 3.34 (m, 1H), 3.23 (dd, *J* = 14.2, 6.8 Hz, 1H), 3.10 (s, 3H), 2.92 (dd, *J* = 14.2, 8.8 Hz, 1H), 2.75 (s, 3H), 2.30−2.10 (m, 3H), 2.07−1.73 (m, 6H), 1.67 (m, 1H), 1.45 (s, 9H), 1.42 (m, 1H), 1.03 (m, 1H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H), 0.57 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.8, 173.0, 172.2, 171.2, 171.2, 168.9, 156.9, 136.9, 129.4 (2C), 128.5 (2C), 126.8, 80.4, 63.0, 59.2, 56.1, 54.3, 52.5, 52.4, 47.0, 34.9, 32.5, 31.5, 31.2, 30.4, 29.7, 28.8, 28.4 (4C), 26.2, 25.1, 19.4, 17.0, 14.6, 11.3; HRMS (ESI-TOF) *m*/*z* 767.4299 $[M + Na]^+$ (calcd for $C_{38}H_{60}N_6O_9Na$ 767.4319).

*Methyl N,N-((tert-Butoxycarbonyl)-L-leucyl)-N-methyl-D-alloisoleucyl-L-glutaminyl-L-valyl-N-methyl-D-phenylalanyl-L-prolinate (**12**).* To a stirred solution of pentapeptide **11** (154 mg, 0.206 mmol) in CH_2Cl_2 (6 mL) was added TFA (3 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and Boc-L-leucine·H_2O (97.6 mg, 0.422 mmol) in DMF (0.18 mL) cooled at 0 °C were added DIPEA (0.18 mL, 1.0 mmol) and HATU (168 mg, 0.441 mmol). After stirring at rt 14 h, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine, dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (15 g, $CHCl_3$−EtOAc−MeOH, 15:15:1) to give hexapeptide **12** (120 mg, 68% in 2 steps) as a colorless amorphous solid. The ratio of major and minor rotamers is 6:3:1: $[\alpha]^{28}_D$ +17.3 (*c* 1.00, $CHCl_3$); IR (neat) 3310, 2961, 1636, 1525, 1454, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.39 (brd, *J* = 7.3 Hz, 1H, NH), 7.26−7.10 (m, 5H), 7.13 (brm, 1H, NH), 6.55 (brs, 1H, NH), 6.36 (brs, 1H, NH), 5.92 (brd, *J* = 8.2 Hz, 1H, NH), 5.63 (m, 1H), 4.65−4.57 (m, 2H), 4.53 (d, *J* = 10.9 Hz, 1H), 4.39 (dd, *J* = 8.2, 5.9 Hz, 1H), 4.31 (m, 1H), 3.71 (s, 3H), 3.38−3.29 (m, 2H), 3.21 (dd, *J* = 14.5, 6.8 Hz, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.91 (dd, *J* = 14.5, 9.1 Hz, 1H), 2.31−1.61 (m, 9H), 1.60−1.33 (m, 4H), 1.39 (s, 9H), 1.07 (m, 1H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.93 (m, 1H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.90 (t, *J* = 7.7 Hz, 3H), 0.78 (d, *J* = 6.3 Hz, 3H), 0.62 (d, *J* = 6.3 Hz, 3H), 0.61 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.4, 174.5, 172.9, 172.0, 171.2, 170.5, 168.7, 156.0, 136.8, 129.1 (2C), 128.5 (2C), 126.9, 79.7, 62.1, 59.4, 55.9, 54.3, 52.9, 52.5, 49.8, 47.1, 42.3, 35.1, 32.4, 31.1, 31.0, 30.5, 29.9, 29.0, 28.5 (4C), 26.4, 25.4, 24.9, 23.4, 22.1, 19.3, 17.3, 15.0, 11.4; HRMS (ESI-TOF) *m*/*z* 880.5187 $[M + Na]^+$ (calcd for $C_{44}H_{71}N_7O_{10}Na$ 880.5160).

*Methyl N,N-(((R)-8-((tert-Butoxycarbonyl)amino)-4-methyloctanoyl)-L-leucyl)-N-methyl-D-alloisoleucyl-L-glutaminyl-L-valyl-N-methyl-D-phenylalanyl-L-prolinate ((R)-**13**).* To a stirred solution of hexapeptide **12** (16.5 mg, 19.2 μmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and carboxylic acid (*R*)-**7** (16.9 mg, 61.9 μmol) in DMF (0.020 mL) cooled at 0 °C were added DIPEA (0.015 mL, 86 μmol)

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and HATU (20.0 mg, 52.6 μmol). After stirring at rt for 10 h, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC ($CHCl_3$−EtOAc−MeOH, 5:5:1) to give peptide (*R*)-**13** (19.3 mg, 98% in 2 steps) as a colorless amorphous solid. The ratio of major and minor rotamers is 6:1: $[\alpha]^{28}_D$ +270 (*c* 0.37, $CHCl_3$); IR (neat) 3318, 2960, 2929, 1633 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.40 (brm, 1H, NH), 7.26−7.12 (m, 5H), 6.91 (brm, 1H, NH), 6.61 (brs, 1H, NH), 6.41 (brm, 1H, NH), 5.73 (brm, 1H, NH), 5.63 (dd, *J* = 7.7, 7.7 Hz, 1H), 4.95 (m, 1H), 4.72−4.62 (m, 2H), 4.39 (dd, *J* = 8.2, 6.3 Hz, 1H), 4.27 (m, 1H), 3.71 (s, 3H), 3.40−3.30 (m, 2H), 3.26 (dd, *J* = 14.0, 6.8 Hz, 1H), 3.13−3.03 (m, 2H), 3.10 (s, 3H), 2.99 (s, 3H), 2.88 (dd, *J* = 14.0, 8.6 Hz, 1H), 2.31−2.12 (m, 6H), 2.11−1.92 (m, 5H), 1.92−1.58 (m, 6H), 1.53−1.35 (m, 4H), 1.44 (s, 9H), 1.33−1.20 (m, 3H), 1.17−1.02 (m, 2H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.7 Hz, 3H), 0.84 (d, *J* = 5.9 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.73 (d, *J* = 6.8 Hz, 3H), 0.61 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.0, 174.3, 173.5, 172.6, 171.8, 171.0, 170.1, 168.5, 156.1, 136.9, 129.5 (2C), 128.5 (2C), 126.8, 79.1, 61.3, 59.3, 55.9, 54.1, 53.2, 52.3, 48.2, 47.1, 42.1, 40.7, 36.4, 35.2, 34.2, 32.5, 32.5, 32.3, 31.6, 31.3, 31.0, 30.5, 29.8, 29.0, 28.5 (3C), 27.4, 26.7, 25.3, 24.9, 24.2, 23.3, 22.1, 19.6, 19.4, 17.1, 15.0, 11.3; HRMS (ESI-TOF) *m*/*z* 1035.6502 $[M + Na]^+$ (calcd for $C_{53}H_{88}N_8O_{11}Na$ 1035.6470).

*Methyl N,N-(((S)-8-((tert-Butoxycarbonyl)amino)-4-methyloctanoyl)-L-leucyl)-N-methyl-D-alloisoleucyl-L-lutaminyl-L-valyl-N-methyl-D-phenylalanyl-L-prolinate ((S)-**13**).* To a stirred solution of hexapeptide **12** (14.3 mg, 16.7 μmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and carboxylic acid (*S*)-**7** (14.4 mg, 52.7 μmol) in DMF (0.03 mL) cooled at 0 °C were added DIPEA (8.7 μL, 50 μmol) and HATU (20.2 mg, 53.1 μmol). After stirring at rt for 9 h, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC ($CHCl_3$−EtOAc−MeOH, 5:5:1) to give peptide (*S*)-**13** (14.2 mg, 84% in 2 steps) as a colorless amorphous solid. The ratio of major and minor rotamers is 6:1: $[\alpha]^{28}_D$ +12.5 (*c* 1.00, $CHCl_3$); IR (neat) 3320, 2961, 2931, 2872, 1632 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.39 (brd, *J* = 6.8 Hz, 1H, NH), 7.33−7.08 (m, 5H), 7.00 (brd, *J* = 8.3 Hz, 1H, NH), 6.65 (brd, *J* = 6.8 Hz, 1H, NH), 6.49 (brm, 1H, NH), 5.80 (brm, 1H, NH), 5.63 (dd, *J* = 7.8, 7.8 Hz, 1H), 4.94 (dd, *J* = 13.4, 6.8 Hz, 1H), 4.70−4.61 (m, 2H), 4.39 (dd, *J* = 8.3, 6.3 Hz, 1H), 4.28 (dd, *J* = 13.4, 6.8 Hz, 1H), 3.71 (s, 3H), 3.40−3.30 (m, 2H), 3.26 (dd, *J* = 14.2, 7.3 Hz, 1H), 3.13−3.03 (m, 2H), 3.10 (s, 3H), 2.99 (s, 3H), 2.87 (dd, *J* = 14.2, 8.3 Hz, 1H), 2.31−2.10 (m, 6H), 2.10−1.69 (m, 11H), 1.68−1.55 (m, 2H), 1.53−1.40 (m, 2H), 1.43 (s, 9H), 1.33−1.20 (m, 3H), 1.17−1.02 (m, 2H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.8, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.72 (d, *J* = 6.8 Hz, 3H), 0.62 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.1, 174.3, 173.6, 172.6, 171.9, 171.1, 170.1, 168.5, 156.3, 137.0, 129.6 (2C), 128.5 (2C), 126.8, 79.2, 61.4, 59.4, 56.0, 54.1, 53.3, 52.4, 48.2, 47.1, 42.1, 40.7, 36.5, 35.3, 34.3, 32.6, 32.5, 32.4, 31.6, 31.3, 31.0, 30.6, 30.4, 29.0, 28.6 (3C), 27.5, 26.7, 25.4, 25.0, 24.2, 23.4, 22.2, 19.6, 19.4, 17.3, 15.0, 11.4; HRMS (ESI-TOF) *m*/*z* 1013.6671 $[M + H]^+$ (calcd for $C_{53}H_{89}N_8O_{11}$ 1013.6651).

*(43R)-Hoshinoamide C (**1**).* To a stirred solution of peptide (*R*)-**13** (12.4 mg, 12.2 μmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and 4-(4-hydoroxyphenyl)butanoic acid (6.6 mg, 36.6 μmol) in DMF (0.02 mL) cooled at 0 °C were added DIPEA (0.01 mL, 57 μmol) and HATU (14.1 mg, 37.1 μmol). After stirring at rt for 12 h, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by HPLC (Cosmosil PBr (ϕ 20 × 250 mm), MeOH−H_2O, 95:5, flow rate 5 mL/min, detection UV 215 nm) to give (43*R*)-hoshinoamide C (**1**) (11 mg, t_R = 43.1 min 84% in 2 steps) as a colorless oil. The ratio of major and minor rotamers is 8:1: $[\alpha]^{28}_D$ +4.6 (*c* 1.00, $CHCl_3$); IR (neat) 3312, 2960, 2931, 1632 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.40 (brd, *J* = 7.3 Hz, 1H, NH), 7.26−7.10 (m, 5H), 7.07 (brd, *J* = 8.5 Hz, 1H, NH), 6.98 (d, *J* = 8.2 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 2H), 6.73 (brd, *J* = 7.7 Hz, 1H, NH), 6.59 (brs, 1H, NH), 5.82 (t, *J* = 5.9 Hz, 1H, NH), 5.72 (brs, 1H, NH), 5.62 (dd, *J* = 7.7, 7.7 Hz, 1H), 4.89 (m, 1H), 4.71 (d, *J* = 10.4 Hz, 1H), 4.66 (dd, *J* = 8.6, 6.3 Hz, 1H), 4.39 (dd, *J* = 8.2, 5.9 Hz, 1H), 4.23 (dt, *J* = 6.8, 6.8 Hz, 1H), 3.70 (s, 3H), 3.43−3.29 (m, 2H), 3.26 (dd, *J* = 14.0, 6.8 Hz, 1H), 3.23−3.13 (m, 2H), 3.11 (s, 3H), 3.00 (s, 3H), 2.88 (dd, *J* = 14.0, 8.6 Hz, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 2.25−2.15 (m, 3H), 2.11 (t, *J* = 7.3 Hz, 2H), 2.11 (m, 1H), 2.06−1.70 (m, 9H), 1.69−1.58 (m, 2H), 1.57−1.44 (m, 2H), 1.43−1.34 (m, 4H), 1.32−1.20 (m, 5H), 1.17−1.03 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 5.9 Hz, 3H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.72 (d, *J* = 6.8 Hz, 3H), 0.63 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.3, 174.5, 174.0, 173.3, 172.8, 172.0, 171.4, 170.2, 168.7, 154.9, 136.9, 132.7, 129.7 (2C), 129.6 (2C), 128.6 (2C), 126.9, 115.5 (2C), 61.4, 59.4, 56.0, 54.3, 53.4, 52.4, 48.4, 47.2, 41.8, 39.4, 36.4, 35.5, 35.3, 34.1, 34.0, 32.5, 32.4, 32.1, 31.7, 31.4, 31.1, 30.6, 29.7, 29.0, 27.3, 27.2, 26.7, 25.3, 25.0, 24.2, 23.3, 22.2, 19.6, 19.5, 17.3, 15.0, 11.4; HRMS (ESI-TOF) *m*/*z* 1097.6596 $[M + Na]^+$ (calcd for $C_{58}H_{90}N_8O_{11}Na$ 1097.6627).

*(43S)-Hoshinoamide C (**2**).* To a stirred solution of peptide (*S*)-**13** (10.9 mg, 10.1 μmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of crude amine·TFA and 4-(4-hydoroxyphenyl)butanoic acid (5.5 mg, 30.5 μmol) in DMF (0.01 mL) cooled at 0 °C were added DIPEA (0.01 mL, 57 μmol) and HATU (12.1 mg, 31.8 μmol). After stirring for 8 h at rt, the reaction mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine, dried (Na_2SO_4), and concentrated. The residual oil was purified by HPLC (Cosmosil PBr (ϕ20 × 250 mm), MeOH−H_2O, 95:5, flow rate 5 mL/min, detection UV 215 nm) to give (43*S*)-hoshinoamide C (**2**) (6.8 mg, t_R = 42.3 min, 62% in 2 steps) as a colorless oil. The ratio of major and minor rotamers is 5:1: $[\alpha]^{28}_D$ +21.0 (*c* 1.00, $CHCl_3$); IR (neat) 3316, 2960, 2932, 1632 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.26−7.10 (m, 5H), 6.97 (d, *J* = 8.2 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 2H), 6.71 (brd, *J* = 7.3 Hz, 1H, NH), 6.62 (brs, 1H, NH), 5.90 (t, *J* = 5.7 Hz, 1H, NH), 5.73 (brs, 1H, NH), 5.63 (dd, *J* = 7.7, 7.7 Hz, 1H), 4.90 (m, 1H), 4.73 (d, *J* = 10.4 Hz, 1H), 4.66 (d, *J* = 5.9 Hz, 1H), 4.38 (dd, *J* = 8.2, 6.3 Hz, 1H), 4.24 (dt, *J* = 6.8, 6.8 Hz, 1H), 3.69 (s, 3H), 3.40−3.30 (m, 2H), 3.26 (dd, *J* = 14.3, 6.8 Hz, 1H), 3.23−3.13 (m, 2H), 3.11 (s, 3H), 3.01 (s, 3H), 2.87 (dd, *J* = 14.3, 8.2 Hz, 1H), 2.56 (t, *J* = 7.0 Hz, 2H), 2.23−2.15 (m, 3H), 2.11 (t, *J* = 7.3 Hz, 2H), 2.11 (m, 1H), 2.06−1.71 (m, 9H), 1.68−1.58 (m, 2H), 1.56−1.45 (m, 2H), 1.44−1.17 (m, 9H), 1.13−1.11 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.3 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.72 (d, *J* = 6.8 Hz, 3H), 0.63 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.2, 174.5, 173.9, 173.3, 172.6, 172.0, 171.3, 170.2, 168.6, 155.0, 136.9, 132.7, 129.7 (2C), 129.6 (2C), 128.5 (2C), 126.9, 115.5 (2C), 61.4, 59.4, 56.1, 54.3, 53.4, 52.4, 48.4, 47.1, 41.7, 39.4, 36.5, 35.6, 35.3, 34.1, 34.1, 32.5 (2C), 32.2, 31.7, 31.5, 31.1, 30.6, 29.7, 29.0, 27.4, 27.2, 26.7, 25.3, 25.0, 24.2, 23.3, 22.2, 19.5 (2C), 17.3, 15.1, 11.4; HRMS (ESI-TOF) *m*/*z* 1097.6589 $[M + Na]^+$ (calcd for $C_{58}H_{90}N_8O_{11}Na$ 1097.6627).

Cell Growth Analysis. HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM (Nissui) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HL60 cells were cultured at 37 °C with 5% CO_2 in RPMI (Nissui) supplemented with 10% heat-inactivated FBS,

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100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa cells were seeded at 5×10^3 cells/well in 96-well plates (Iwaki) and cultured overnight. HL60 cells were seeded at 2×10^4 cells/well in 96-well plates. Various concentrations of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by the MTT assay.

Cultivation of *P. falciparum*. *P. falciparum* 3D7 strain was cultured in fresh type A human erythrocytes (obtained from Japanese Red Cross Society) suspended at 2% hematocrit in RPMI-1640 medium (Gibco, ThermoFisher Scientific) supplemented with 2 g/L sodium bicarbonate, 50 mg/L hypoxanthine (Sigma), 10 μg/mL gentamicin sulfate, and 0.5% (w/v) AlbumaxII. Parasite cultures were incubated under a gas mixture of 5% CO_2, 5% O_2, and 90% N_2 at 37 °C and maintained in asynchronous form between 0.1% and 4% parasitemia. Thin blood smears fixed in 100% MeOH and stained with 10% Giemsa for 10−15 min were prepared to monitor parasite growth.

***Plasmodium falciparum* Growth Inhibition Assay.** Asynchronous asexual parasites were synchronized using 5% (w/v) D-sorbitol. The level of parasitemia was determined by light microscopy. The stock culture was diluted with complete RPMI medium and 50% RBC (red blood cells) to a starting 2% hematocrit and 0.3% parasitemia. Compounds and drug controls were dissolved in DMSO prior to being assayed. One hundred microliter cultures containing different concentration of the compounds were dispensed into 96-well plates. The maximum concentration of DMSO in each well was 0.5%. The assay plates were then incubated at 37 °C for 72 h in a 5% CO_2, 90% N_2, and 5% O_2 atmosphere. After 72 h plates were frozen at −30 °C overnight to lyse the red blood cells. Plates were thawed at rt for at least 1 h before starting the assay. The LDH assay was performed as previously described[11] with some modification. Briefly, a solution to assess parasites' LDH activity containing 50 mM sodium L-lactate, 0.25% Triton X-100, 100 mM Tris-HCl (pH 8.0), 50 μM APAD, 240 μM NBT, and 1 U/mL diaphorase was prepared. Immediately, 150 μL of the solution was dispensed into the plates. and plates were shaken to ensure mixing. After 30 min of incubation at rt in the dark, absorbance was measured at 650 nm using a SpectraMax Paradigm multimode microplate reader (Molecular Devices).

***In Vitro* Antitrypansomal Assay.** The *Trypanosoma brucei rhodesiense* strain IL-1501[12] was cultured at 37 °C under a humidified 5% CO_2 atmosphere in HMI-9 medium[13] supplemented with 10% heat-inactivated FBS. For *in vitro* studies, compounds were dissolved in DMSO and diluted in culture medium prior to being assayed. The maximum DMSO concentration in the *in vitro* assays was 1%. The compounds were tested in an AlamarBlue serial drug dilution assay[14] to determine the 50% inhibitory concentrations (IC_{50}). Serial drug dilutions were prepared in 96-well microtiter plates containing the culture medium, and wells were inoculated with 4.0×10^4 cells/mL *T. b. rhodesiense* IL-1501 parasites. Cultures were incubated for 69 h at 37 °C under a humidified 5% CO_2 atmosphere. After this time, 10 μL of resazurin (12.5 mg of resazurin [Sigma] dissolved in 100 mL of phosphate-buffered saline) was added to each well. The plates were incubated for an additional 3 h. The plates were read in a SpectraMax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01209.

NMR spectra for hoshinoamide C (**1**) and synthetic compounds; HPLC chromatograms for determination of the absolute configurations (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Linington, R. G.; Clark, B. R.; Trimble, E. E.; Almanza, A.; Ureña, L.-D.; Kyle, D. E.; Gerwick, W. H. *J. Nat. Prod.* **2009**, *72*, 14−17.

(2) Sanchez, L. M.; Lopez, D.; Vesely, B. A.; Togna, G. D.; Gerwick, W. H.; Kyle, D. E.; Linington, R. G. *J. Med. Chem.* **2010**, *53*, 4187−4197.

(3) Kurisawa, N.; Iwasaki, A.; Jeelani, G.; Nozaki, T.; Suenaga, K. *J. Nat. Prod.* **2020**, *83*, 1684−1690.

(4) Iwasaki, K.; Iwasaki, A.; Sumimoto, S.; Matsubara, T.; Sato, T.; Nozaki, T.; Saito-Nakano, Y.; Suenaga, K. *J. Nat. Prod.* **2020**, *83*, 481−488.

(5) Iwasaki, A.; Tadenuma, T.; Sumimoto, S.; Shiota, I.; Matsubara, T.; Saito-Nakano, Y.; Nozaki, T.; Sato, T.; Suenaga, K. *J. Nat. Prod.* **2018**, *81*, 2545−2552.

(6) Shiota, I.; Iwasaki, A.; Sumimoto, S.; Tomoda, H.; Suenaga, K. *Tetrahedron Lett.* **2018**, *59*, 1261−1263.

(7) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(8) Ma, Z.; Song, N.; Li, C.; Zhang, W.; Wang, P.; Li, Y. *J. Pept. Sci.* **2008**, *14*, 1139−1147.

(9) Engene, N.; Tronholm, A.; Salvador-Reyes, L. A.; Luesch, H.; Paul, V. J. *J. Phycol.* **2015**, *51*, 670−681.

(10) (a) Gunasekera, S. P.; Imperial, L.; Garst, C.; Ratnayake, R.; Dang, L. H.; Paul, V. J.; Luesch, H. *J. Nat. Prod.* **2016**, *79*, 1867−1871. (b) Zhang, C.; Naman, C. B.; Engene, N.; Gerwick, W. H. *Mar.*

Drugs **2017**, *15*, 121−131. (c) Ozaki, K.; Iwasaki, A.; Suenaga, K.; Teruya, T. *Tetrahedron* **2019**, *75*, 3382−3386.
(11) Gamo, F. J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J. L.; Vanderwall, D. E.; Green, D. V.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305−310.
(12) Kuboki, N.; Inoue, N.; Sakurai, T.; Cello, F. D.; Grab, D. J.; Suzuki, H.; Sugimoto, C.; Igarashi, I. *J. Clin. Microbiol.* **2003**, *41*, 5517−5524.
(13) Räz, B.; Iten, M.; Grether-Bühler, Y.; Kaminsky, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139−147.
(14) Huber, W.; Koella, J. C. *Acta Trop.* **1993**, *55*, 257−261.

https://dx.doi.org/10.1021/acs.jnatprod.0c01209
J. Nat. Prod. 2021, 84, 126−135