Kulokekahilide-1, a Cytotoxic Depsipeptide from the Cephalaspidean Mollusk *Philinopsis speciosa*

Junji Kimura, Yuuki Takada, Tomoko Inayoshi, Yoichi Nakao, Gilles Goetz, Wesley Y. Yoshida, and Paul J. Scheuer*

Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, Hawaii 96822-2275, and Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, 6-16-1 Chitosedai, Setagaya, Tokyo 157-8572, Japan

office@gold.chem.hawaii.edu

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The cytotoxic depsipeptide kulokekahilide-1, which contains two unusual amino acids, 4-phenylvaline and 3-amino-2-methylhexanoic acid, was isolated from the cephalaspidean mollusk *Philinopsis speciosa*. Structure elucidation of kulokekahilide-1 was carried out by spectroscopic analysis and chemical degradation. The absolute stereochemistry was determined by Marfey analysis for amino acids and chiral HPLC analysis for hydroxy acids. All four stereoisomers of 4-phenylvaline and 3-amino-2-methylhexanoic acid, which were necessary for Marfey analysis, were synthesized by use of the Heck reaction and Evans's method, respectively. Kulokekahilide-1 showed cytotoxicity against P388 murine leukemia cells with an IC_{50} value of 2.1 μg/mL.

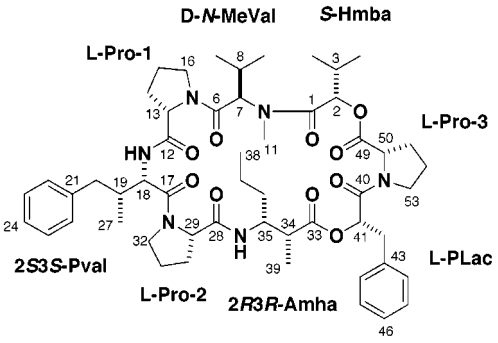
Introduction

The marine mollusk *Philinopsis speciosa* harbors a variety of chemical constituents, which include alkylpyridines,[1] polypropionates,[2] and especially peptides.[3] Its peptidic constituents are, to some extent, similar to those of *Dolabella auricularia* and *Onchidium* sp., which suggests their origin from dietary cyanobacteria.[4] In fact, cyclic peptides lyngbyabellins isolated from the marine cyanobacterium *Lyngbya majuscula* contain 7,7-dichloro-2,2-dimethyl-3-hydroxyoctanoic acid,[5] which is presumed to be a precursor of 2,2-dimethyl-3-hydroxyoctynoic acid in kulolide-1 from *P. speciosa*.[3a,b] Further investigation of the cytotoxic fraction from *P. speciosa* has led to the isolation of a new cyclic bidepsipeptide, kulokekahilide-1 (**1**).[6] Kulokekahilide-1 is related to dolastatin 16 isolated by Pettit *et al.* from *D. auricularia*,[4a] which contains two unusual amino acids: 4-phenylvaline (**2**, dolaphenvaline) and 3-amino-2,4-dimethylpentanoic acid (dolamethylleucine, Dml). Kulokekahilide-1 also involves two unusual amino acids: the same 4-phenylvaline (**2**, Pval) and 3-amino-2-methylhexanoic acid (**3**, Amha) instead of dolamethylleucine. Determination of absolute stereochemistry of unusual amino acids often requires a synthetic approach when they are not commercially available. In the case of dolastatin 16, neither dolaphenvaline nor dolamethylleucine was determined for their absolute stereochemistry.[4a] We have synthesized all four stereoisomers of these two unusual amino acids **2** and **3** and thus were able to determine the absolute stereochemistry of **1**.

In this paper, we describe isolation, structure elucidation, and biological activity of this compound, as well as synthesis of two unusual amino acids, 4-phenylvaline (**2**, dolaphenvaline)[4a] and 3-amino-2-methylhexanoic acid (**3**).

Results and Discussion

The organic extract of *P. speciosa* was evaporated and separated by modified Kupchan procedure[7] to yield



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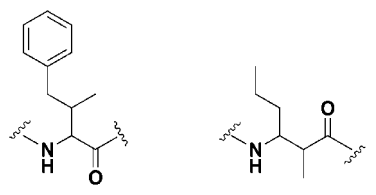

2 **3**

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(6) *Kekahi* means another in Hawaiian, thereby kulokekahilide means another kulolide.

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Table 1. NMR Data of Kulokekahilide-1 (1) in CD_3CN

C	^{13}C	^1H (ppm, mult, Hz)	HMBC	NOESY	C	^{13}C	^1H (ppm, mult, Hz)	HMBC	NOESY
1	170.6				30a	26.1	2.28 m		
2	77.3	5.38 d 2.7	C: 1, 3, 4, 5, 49	H: 5, 11; NH: 35	30b		1.55 m		
3	29.1	2.17 m			31	25.8	1.80 m		H: 32a,b
4	20.5	0.93 d 6.3	C: 2, 3, 5		32a	47.2	3.09 m	C: 31	H: 18, 31
5	16.5	0.97 d 6.7	C: 2, 3, 4		32b		2.96 m	C: 31	H: 18, 19, 31
6	170.4				33	175.1			
7	60.2	5.07 d 11.1	C: 6, 8, 9, 10, 11	H: 11, 16a,b	34	41.7	2.57 qd 7.2, 3.4	C: 33	H: 35
8	26.5	2.24 m	C: 9		35	50.8	3.88 m	C: 34	H: 34, 39
9	19.9	0.85 d 6.5	C: 7, 8, 10		36a	37.4	1.18 m		
10	18.2	0.78 d 6.7	C: 7, 8, 9		36b		1.10 m		
11	30.1	2.99 s	C: 1, 7	H: 7	37a	20.2	1.13 m		
12	173.4				37b		1.04 m		
13	62.4	4.37 dd 9.4, 2.2	C: 12		38	14.3	0.71 t 6.9		
14a	31.3	2.20 m			39	15.1	0.87 d 7.2	C: 33, 34, 35	H: 35; NH: 35
14b		2.01 m			NH		7.55 d 10.1		H: 2, 29, 39
15a	25.6	2.05 m			40	168.5			
15b		1.98 m			41	72.7	5.23 dd 9.0, 5.8	C: 33, 42, 43	H: 44, 48, 50
16a	48.5	3.79 ddd 9.1, 7.9, 2.4		H: 7, 16b; NH: 18	42a	38.3	3.03 dd 13.7, 5.8	C: 40, 41, 43, 44	H: 44, 48, 50
16b		3.44 m		H: 7, 16a	42b		2.96 dd 13.7, 9.0	C: 40, 41, 43, 44	H: 44, 48
17	172.1				43	137.4			
18	52.5	4.92 bd 8.7	C: 17, 19, 20, 27	H: 19, 20a 22, 26, 32a,b	44	130.3	7.29	C: 46, 48	H: 41, 42a,b, 50
19	40.7	1.90 m		H: 18, 22, 26, 32b	45	129.5	7.31	C: 43, 47	
20a	41.8	2.61 dd 13.4, 7.5	C: 18, 19, 21, 22, 26, 27	H: 18, 22, 26	46	128.0	7.25	C: 44, 48	
20b		2.27 dd 13.4, 7.0	C: 18, 19, 21, 22, 26, 27		47	129.5	7.31	C: 43, 45	
21	141.8				48	130.3	7.29	C: 44, 46	H: 41, 42a,b, 50
22	130.5	7.26	C: 24, 26	H: 18, 19, 20a	49	172.2			
23	129.1	7.27	C: 21, 25		50	58.5	4.17 dd 8.7, 7.7	C: 49, 51, 52	H: 41, 44, 48
24	127.0	7.19	C: 22, 26		51a	31.3	2.01 m		
25	129.1	7.27	C: 21, 23		51b		1.92 m		
26	130.5	7.26	C: 22, 24	H: 18, 19, 20a	52a	22.7	2.00 m		
27	14.8	0.62 d 6.7	C: 18	H: 22, 26, 53a; NH: 18	52b		1.83 m		H: 53b
NH		6.70 d 8.9	C: 12	H: 27	53a	47.2	3.48 m		H: 27, 53a
28	171.7				53b		3.26 m		H: 52b, 53a
29	60.0	4.56 d 7.2	C: 17, 28, 30, 31, 32	NH: 35					

hexane, CH_2Cl_2, and aqueous MeOH extracts. The CH_2Cl_2 extract was submitted to two-step ODS flash chromatography, followed by gel filtration, and amino short column chromatography. The fraction containing peptides was further separated by ODS HPLC yielding nine fractions (fractions 1−9). From fractions 6 and 7, kulokekahilide-1 (**1**; 1.4 mg; (1.6×10^{-5})% yield based on wet weight) was obtained after repetitive ODS HPLC.

The molecular formula of kulokekahilide-1 (**1**) was determined as $C_{53}H_{74}N_6O_{10}$ on the basis of HR-FABMS [*m*/*z* 955.5550 $(M + H)^+$ (Δ +0.5 mmu)] and NMR spectral analysis (Table 1). NMR analysis revealed that *N*-methylvaline (MeVal), phenyllactic acid (Plac), 2-hydroxy-3-methylbutyric acid (Hmba), and three residues of Pro are present, as well as the two other unusual amino acids **2** and **3**.

The amino acid **2** contains a phenyl group at the γ-position, which was supported by HMBC correlations between H_2-20/C-21 and 22. From CH_2-20, COSY correlations could be traced to the NH bonded to C-18 via CH-19, which was also bearing a methyl group. The α-methine proton of this unit also showed an HMBC[8] correlation to a carbonyl carbon at δ 172.1, thereby completing the substructure of 4-phenylvaline (**2**, Pval).

The last amino acid residue **3** was elucidated as follows. Connecting COSY cross-peaks led to the two spin systems of NH-CH-CH_2-CH_2-CH_3 and CH_3-CH. Although no COSY cross-peak was seen between the two methine protons, HMBC correlations between H-35/C-34 and H_3-39/C-35 confirmed the connectivity between C-34 and 35. Further long-range CH coupling between H_3-39 and carbonyl carbon at δ 175.1 revealed the structure of 3-amino-2-methylhexanoic acid (**3**, Amha).

Sequencing of these units was accomplished by HMBC and NOESY analyses. HMBC correlations revealed three sets of fragments, i.e., Pro3-Hmba-MeVal (H-2/C-49 and H_3-11/C-1), Pro1-Pval-Pro2 (NH-18/C-12 and H-29/C-17), and Amha-Plac (H-41/C-33). NOESY cross-peaks (H-7/H_2-16, H-29/NH-35, H-41/H-50, and H-42a/H-50) connected these substructures to complete the planar structure of **1** (Figure 1). **Synthesis of Unusual Amino Acids 2 and 3.** As the planar structure of **1** was determined, we next embarked on synthesis of all four stereoisomers of **2** and **3**, which are necessary for Marfey analysis.[9] The synthetic approach to **2** involved a Heck reaction of 3,4-dehydrovaline with halobenzene,[10] followed by catalytic hydrogenation. The stereochemistry was determined by NMR spectroscopy and X-ray analy-

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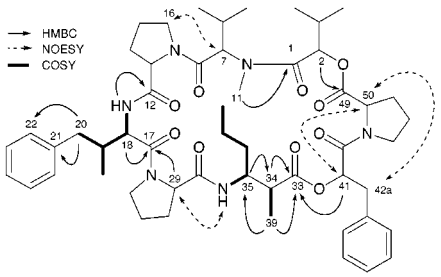


Figure 1. Amino acid sequence of **1**.

sis. However, **3** was diastereoselectively synthesized with optically pure oxazolidinones and aldehydes by use of Evans's method.[11]

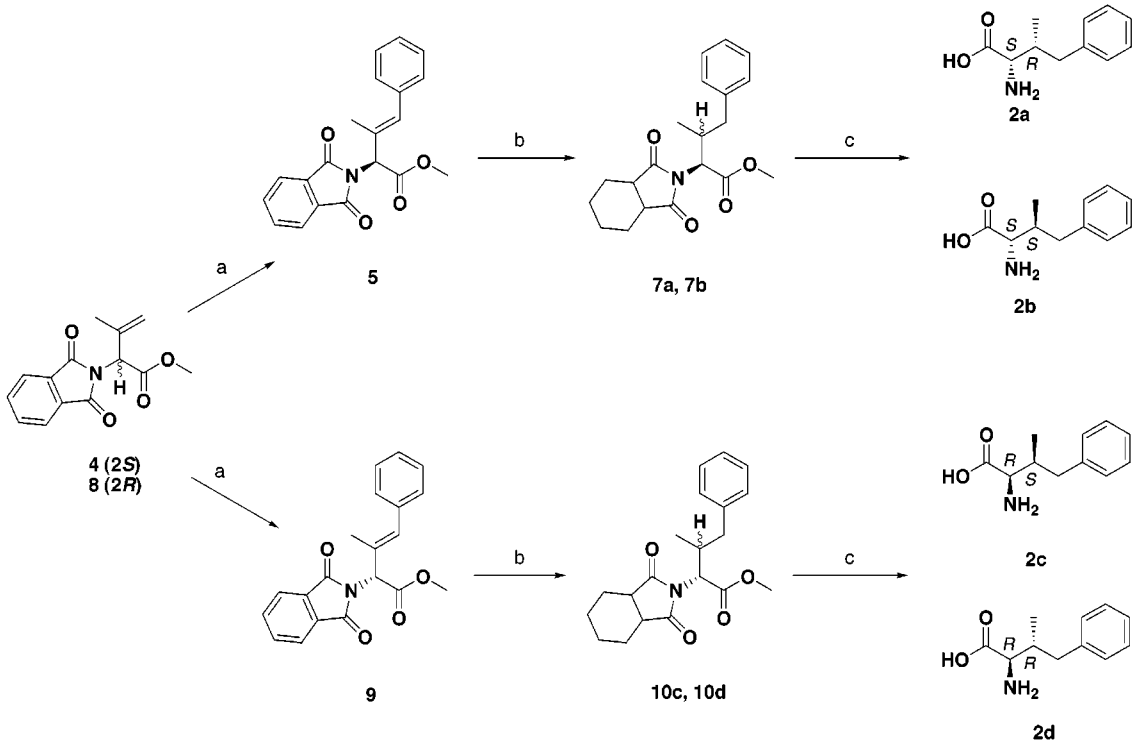
Heck reaction between 3,4-dehydrovaline and halobenzene was applied to the synthesis of 4-phenylvaline. Readily available starting (*S*)- or (*R*)-valine was transformed into 3,4-dehydrovaline derivatives using known procedures.[12]

The reaction of (2*S*)-*N*-phthaloyl-3,4-dehydrovaline methyl ester (**4**) with iodobenzene in the presence of palladium acetate and triethylamine afforded undesired *N*-phthaloyl-2,3-dehydrovaline methyl ester, quantitatively. The product was formed as the result of hydrogen transfer by triethylamine acting as base. The alternative reaction of using silver nitrate instead of triethylamine gave (2*S*)-*N*-phthaloyl-3,4-dehydro-4-phenylvaline methyl ester (**5**) in 51% yield. Catalytic hydrogenation of **5** afforded diastereomeric mixtures of (2*S*)-*N*-phthaloyl-4-phenylvaline methyl ester (**6**) and (2*S*)-*N*-1,2-cyclohexanedicarboxy-4-phenylvaline methyl ester (**7**) in an approximately 1:1 ratio. Generally, hydrogenation of an aromatic ring needs high pressure or Raney Ni catalyst; therefore the formation of compound **7** was not expected.

To our surprise, the diastereomers (**7a** and **7b**) gave better separation in Si-HPLC with 20% ethyl acetate in hexane than the diastereomers **6**, which were barely separated by HPLC. Purified **7a** and **7b** were hydrolyzed by a 2:1 mixture of 6 M hydrochloric acid and acetic acid under reflux to give the corresponding 4-phenylvaline (**2a**, 2*S*3*R*) and **2b** (2*S*3*S*), respectively. Similarly, (2*R*)-*N*-phthaloyl-3,4-dehydrovaline methyl ester (**8**) obtained from D-valine was reacted with iodobenzene in the presence of palladium acetate and silver nitrate to afford (2*R*)-*N*-phthaloyl-3,4-dehydro-4-phenylvaline methyl ester (**9**). Meanwhile, compound **9** was converted into the diastereomers (**10c** and **10d**) of (2*R*)-*N*-cyclohexanedicarboxy-4-phenylvaline methyl ester by complete catalytic hydrogenation. The diastereomers of **10c** and **10d** could also be separated by HPLC. Hydrolysis of **10c** and **10d** afforded the corresponding free 4-phenylvaline (**2c**; 2*R*3*S*) and **2d** (2*R*3*R*). The ^{1}H NMR spectrum of **10c** is superimposable on that of **7a**, and likewise, **10d** has the same spectrum as **7b**; this is due to their enantiomeric relationship (Scheme 1).

The ^{1}H NMR spectrum of **7a** was similar to that of **7b** except for two sets of signals. While one doublet of doublets was observed at δ 3.15 (J = 13.3, 3.8 Hz, Hb-4) and one doublet at δ 0.76 (CH_3-3) in **7a**, the chemical shifts of the corresponding protons in **7b** were δ 2.67 (J = 14.6, 6.7 Hz, Hb-4) and 1.00 (CH_3-3). These results suggested that Hb-4 in **7a** and CH_3-3 in **7b** were deshielded by the carboxyl group. Irradiation of δ 2.28 (Ha-4) increased intensity of the peaks at δ 7.17−7.32 (H-6, H-10), 4.55 (H-2), and 0.76 (CH_3-3). Similarly, NOEs between Ha-4/H-14 and H-2/CH_3-3 in **7b** were observed (Figure 2). These coupling constants and NOEs were reasonably explained by a Dreiding model; thus crystallized **7a** seems to be the 2*S*3*R* isomer, whereas the oily **7b** is the 2*S*3*S* isomer.

Scheme 1[a]



[a] (a) Idobenzene, $AgNO_3$, $Pd(OAc)_2$, MeCN; (b) H_2, PtO_2, EtOH; (c) 6 M HCl−AcOH (2:1), 120 °C.

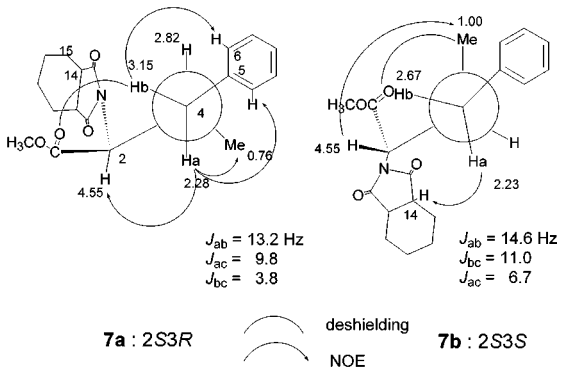


Figure 2. NOE correlations of **7a** and **7b**.

Auspiciously, **7a** and **10c** crystallized from ethanol, and we could perform single-crystal X-ray analysis in order to confirm the absolute configurations. Crystallographic data, data collection, and reduction parameters are summarized in Supporting Information. The molecular structures of **7a** and **10c** are shown in Figure 3. Dihedral angles (χ) of H(3)−C(3)−C(4)−Ha(4) and H(3)−C(3)−C(4)−Hb(4) for **7a** are 193° and 76°. The intramolecular nonbonded distances of H(2)−Ha(4) and H(2)−Hb(4) are 2.61 and 2.52 Å, respectively. Similarly, Ha(4)−H(6) and Hb(4)−H(10) are 2.52 and 2.38 Å, respectively. Analyses of their crystal structures well agreed with NMR spectral data. Also, the *cis* relationship between H-14 and H-19 in **7a** was confirmed.

The synthetic strategy for all four stereoisomers of **3** relied on the reaction developed by Evans et al.,[13] which was recently applied by Riguera et al.[14] The enol borinate of optically pure *N*-propionyl oxazolidinones and aldehydes was diastereoselectively coupled to obtain enantiomerically pure aldols, whose hydroxyl group was converted to an amino group by an S_N2 reaction (Scheme 2).

Reaction of *N*-propionyl oxazolidinone (**11a**) with *n*-butanal gives the aldol **12** with 2*S*3*R* configuration, whereas the reaction of oxazolidinone **11b** with the same aldehyde provides aldol **13** with 2*R*3*S* configuration. Compounds **12** and **13** were obtained optically pure since no other diastereomers were detected in the ^{1}H NMR spectrum. Tosylation in pyridine afforded intermediates **14** and **15** with yields of 70% and 68%, respectively. Reaction with sodium or tetramethylguanidium azide provided the azido derivatives **16** and **17** with inversion of configuration at C-3. Hydrogenation over palladium−charcoal provided 3-amino-2-methylhexanoic acid (**3c**, 2*S*3*S*; **3d**, 2*R*3*R*).

To prepare the two other stereoisomers (**3a**, 2*S*3*R*; **3b**, 2*R*3*S*), inversion of one of the chiral centers of both **12** and **13** was needed. We decided to invert the configuration at C-3 and tried a modified Mitsunobu reaction,[15] which unfortunately did not work. Our next strategy was to invert the configuration at C-3 of tosylate **14** and **15** using bromine, which successfully yielded the desired **18** (2*S*3*R*) and **19** (2*R*3*S*). To avoid decreasing yield, azido **20** and **21** were partially purified before being used for the synthesis of **3a** and **3b**. Thus, all four theoretically possible isomers of 4-phenylvaline and 3-amino-2-methylhexanoic acid were successfully prepared.

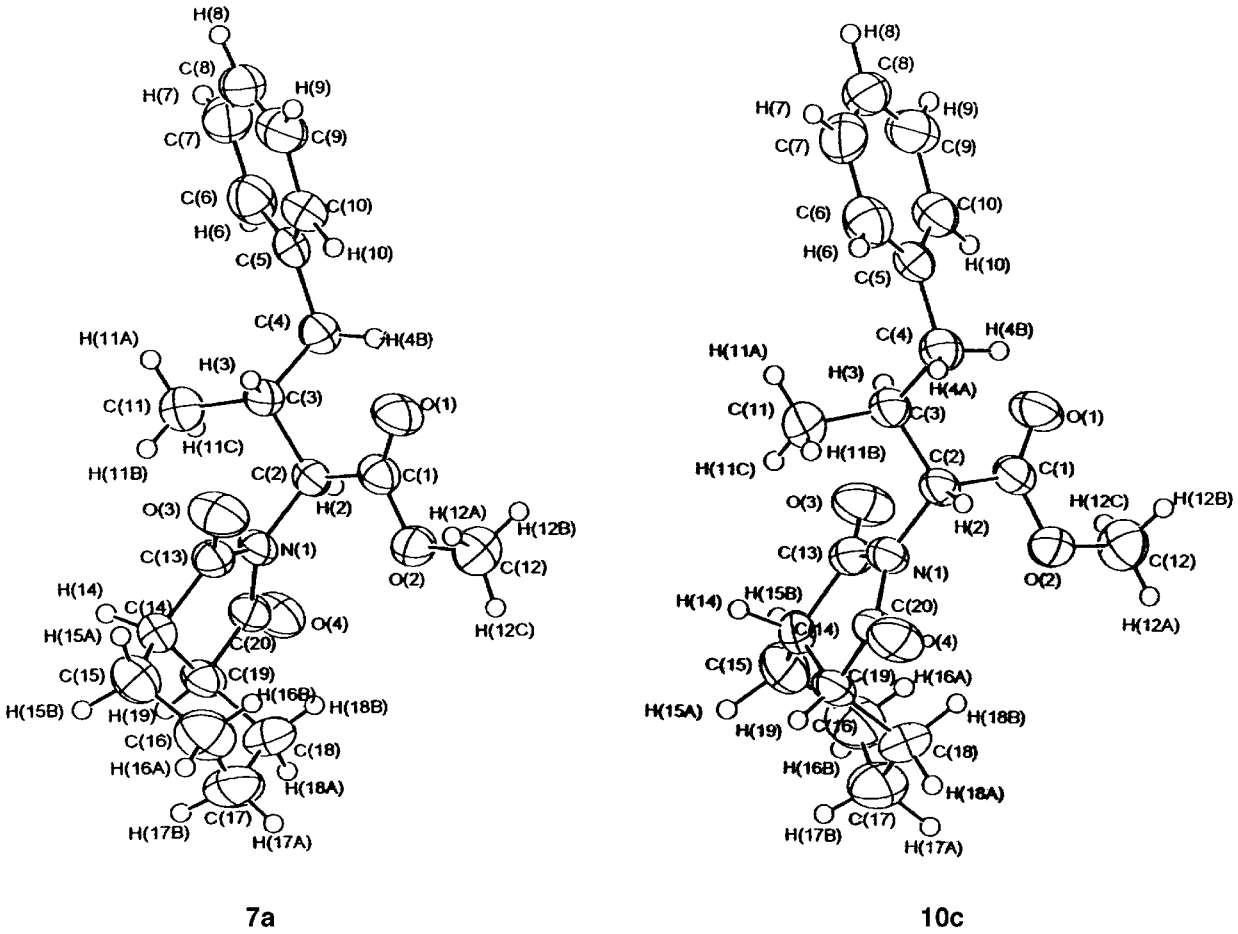
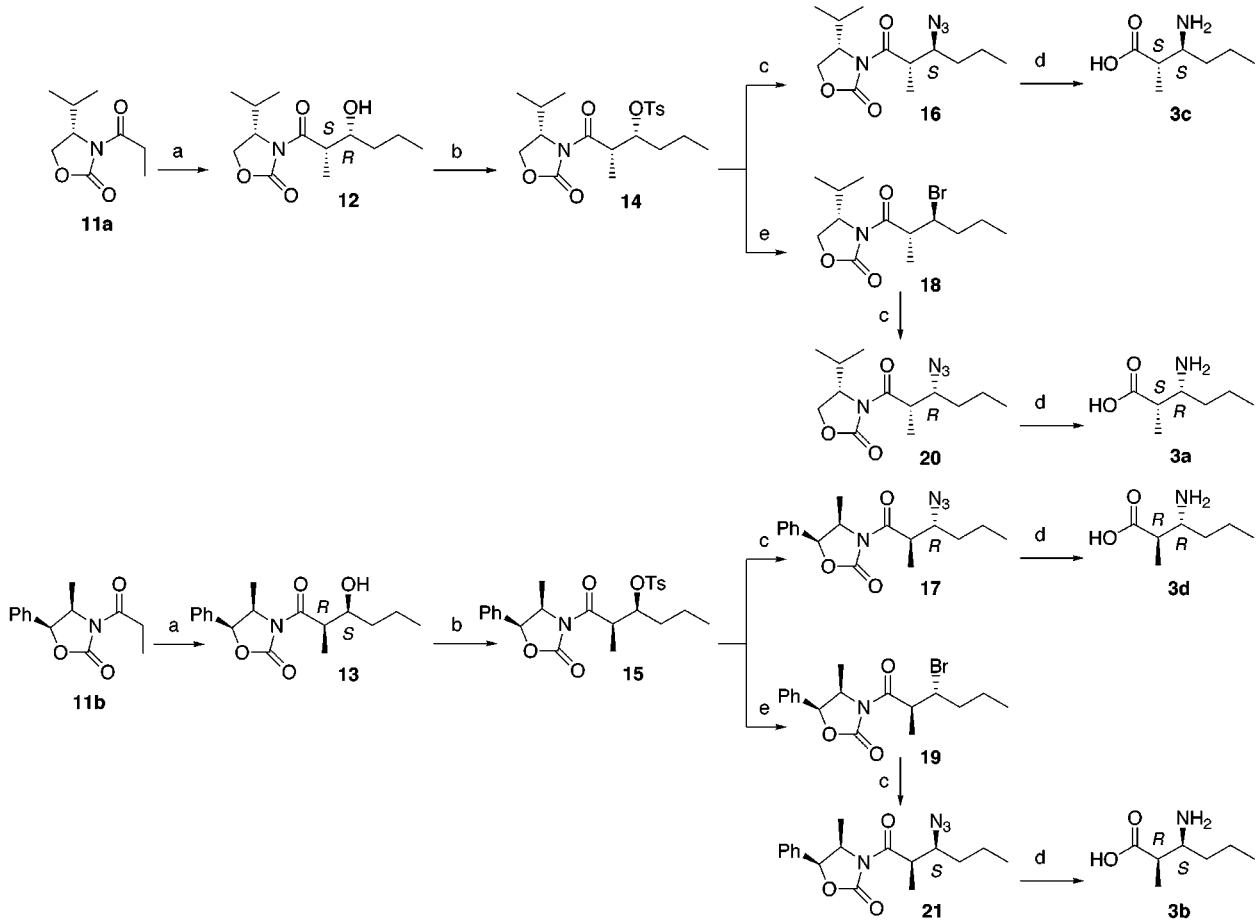


Figure 3. Molecular structures for **7a** and **10c**.

Scheme 2[a]



[a] (a) $(n\text{Bu})_2$BOTf, $i\text{Pr}_2$EtN, CH_2Cl_2, 0 °C and then 1-butanal, −78 °C; (b) *p*-TsCl, Py; (c) tetramethyl-guanidium azide, CH_2Cl_2 or NaN_3, 15-crown-5, DMF; (d) H_2O_2, LiOH then HCl, and H_2, Pd−C, AcOH−H_2O; (e) LiBr, acetone reflux.

Absolute Stereochemistry of 1. The absolute stereochemistry of the proteinogenic amino acids was determined by Marfey analysis as D-MeVal and three L-Pro. The stereochemistry of 4-phenylvaline was also determined as 2*S*3*R* by Marfey analysis by using all four stereoisomers synthesized as above. All four synthetic Amha were also used in Marfey analysis, which showed 2*R*3*R*-Amha is in **1**. The absolute stereochemistry of the hydroxy acids was determined by chiral HPLC analysis as L-phenyllactic acid and *S*-2-hydroxy-3-methylbutyric acid.

Biological Activity. Although dolastatin 16 showed marked cytotoxicity against several cancer cell lines,[4a] cytotoxicity of kulokekahilide-1 (**1**) against P388 was only moderate (IC_{50} 2.1 μg/mL).

Experimental Section

General Procedures. UV spectra were measured with a diode array spectrophotometer. Optical rotations were measured on a digital spectropolarimeter. IR spectra were recorded using a KBr disk. Melting points were determined in open capillary tubes. NMR spectra were recorded at 500 or 300 MHz for ^{1}H and 125 and 75 MHz for ^{13}C. Glycerol was used as matrix for FAB-MS measurements.

Isolation. *Philinopsis speciosa* (300 animals, 9.0 kg wet weight) collected on midsummer nights in 1994 at Shark's Cove, Pupukea, O'ahu, were extracted with EtOH (3 × 3 L) and $CHCl_3$/MeOH (1:1, 3 L). The combined extracts were concentrated and extracted with $CHCl_3$. The aqueous layer was further extracted with *n*-BuOH, and the *n*-BuOH extract was combined with the $CHCl_3$ layer. The combined organic layers were evaporated to dryness and separated by the modified Kupchan procedure to yield hexane, CH_2Cl_2, and aqueous MeOH extracts. The CH_2Cl_2 extract was evaporated to dryness and submitted to two-step ODS flash chromatography (first with aqueous MeOH as solvent, second with aqueous MeCN), followed by gel filtration (Sephadex LH-20, MeOH) and amino short column chromatography [Lichroprep NH_2, ϕ 1.5 × 3.5 cm, $CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (7:3:0.5), and MeOH]. The $CHCl_3$/MeOH (9:1) fraction was separated by ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (7:3)], giving nine fractions (fractions 1−9). Fractions 6 and 7 were separated by the same scheme [COSMOSIL 5C_{18}-AR, 2-PrOH/H_2O (1:1)], and fractions containing identical peaks were combined. The combined fraction was purified by repetitive ODS HPLC [COSMOSIL 5C_{18}-AR, with MeCN/H_2O (65:35) and then with 2-PrOH/H_2O (47.5:52.5)], yielding kulokekahilide-1 (**1**; 1.4 mg; 1.6×10^{-5} % yield based on wet weight). **Kulokekahilide-1 (1):** colorless amorphous solid; $[\alpha]_D$ +22° (*c* 0.07, MeOH); UV (MeOH) 235 nm (ϵ 3,200), 205 (ϵ 14,000); HR-FABMS (M + H)$^+$ *m*/*z* 955.5550 (calcd for $C_{53}H_{75}N_6O_{10}$ 955.5545).

***N*-Phthaloyl-2,3-dehydrovaline Methyl Ester.** (2*S*)-*N*-Phthaloyl-3,4-dehydrovaline methyl ester (**4**) was obtained

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from (*S*)-valine by the method of Easton et al.[16] Iodobenzene (204 mg, 1.0 mmol), triethylamine (152 mg, 1.5 mmol), and palladium acetate (2.3 mg, 0.01 mmol) were added with stirring to a solution of **4** (329 mg, 1.3 mmol) in MeCN (1 mL) under nitrogen. After reflux for 8 h, the reaction mixture was hydrolyzed with water (20 mL) and extracted with ether (20 mL × 2). The organic layer was dried over sodium sulfate, which was removed by filtration. The solvent was evaporated under reduced pressure, and the oily residue was identified to be undesired *N*-phthaloyl-2,3-dehydrovaline by NMR analysis. ^{1}H NMR ($CDCl_3$) δ 1.83, 2.39 (s, CH_3-3), 3.64 (s, -OCH_3), 7.75, 7.88 (m, phthaloyl-H). The product was not further investigated.

(2*S*)-*N*-Phthaloyl-4-phenyl-3,4-dehydrovaline (5). The reaction using silver nitrate instead of triethylamine proceeded to give desired product **5**. Iodobenzene (408 mg, 2.0 mmol), palladium acetate (190 mg, 0.85 mmol), and silver nitrate (340 mg, 2.0 mmol) were added with stirring to a solution of **4** (442 mg, 1.7 mmol) in MeCN (4 mL) under nitrogen. After 6 h at 50 °C, the reaction mixture was hydrolyzed with water (20 mL) and extracted with ether (20 mL × 2). The organic extract was separated by PLC (EtOAc/benzene 1:14) to give **5** in 51% yield (colorless oil). ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H, CH_3-3), 3.81 (s, 3H, −OCH_3), 5.51 (s, 1H, H-2), 6.63 (s, 1H, H-4), 7.30 (m, 5H, aromatic-H), 7.75, 7.89 (m, 4H, phthaloyl-H); ^{13}C NMR ($CDCl_3$) δ 16.2 (q, C-11), 52.8 (q, C-12), 59.4 (d, C-2), 123.6 (d, C-15, C-18), 127.0, 128.1, 129.1 (d, C− 6, C-7, C-8, C-9, C-10), 131.5 (s, C-3), 131.8 (s, C-14, C-19) 131.9 (d, C-4), 134.2 (d, C-16, C-17), 136.6 (s, C-5), 167.3 (s, C-13, C-20), 168.4 (s, C-1).

(2*S*,3*R*)- and (2*S*,3S)-*N*-1,2-cyclohexanedicarboxy-4-phenylvaline Methyl Esters (7a,b). Compound **5** (293 mg) was hydrogenated in the presence of platinum oxide (60 mg) in ethanol (7 mL). After removal of the catalyst, the reaction mixture was separated by PLC by use of ethyl acetate and benzene in a ratio of 1:14. The products were mixtures of *N*-phthaloyl-4-phenylvaline methyl ester (**6**, 144 mg, 50%) and the byproducts (**7**, 112 mg, 38%), which were unexpectedly formed by hydrogenation of the phthaloyl group. Part of byproducts **7** was purified by HPLC to afford **7a** and **7b** in a 1:1 ratio, but the diastereomer **6** could not be separated. **Compound 6 (diastereomeric mixture):** ^{1}H NMR ($CDCl_3$) δ 0.79, 1.00 (d, CH_3-3), 2.24, 2.31 (dd, Ha −4), 2.88 (m, H-3, and one diastereomer Hb-4), 3.12 (dd, the other Hb-4), 4.71, 4.72 (d, H-2), 7.12 (m, aromatic-H), 7.55, 7.70, 7.80 (m, phthaloyl-H). **Compound 7a (2*S*,3*R*):** crystallized from EtOH, mp 68−70 °C; LREIMS *m*/*z* 343 [M^+, $C_{20}H_{25}NO_4$]; HREIMS *m*/*z* 312.1563 [M − OCH_3]$^+$ (calcd for $C_{19}H_{22}NO_3$ 312.1594); ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3H, *J* = 6.8 Hz, CH_3-3), 1.46 (m, 4H, H-16, H-17), 1.87 (m, 4H,H-15, H-18), 2.28 (dd, 1H, *J* = 13.2, 9.8 Hz, Ha-4) 2.81 (m, 1H, H-3), 2.93 (m, 2H, H-14, H-19), 3.15 (dd, 1H, *J* = 13.3, 3.8 Hz, Hb-4), 3.73 (s, 3H, -OCH_3), 4.55 (d, 1H, *J* = 7.9 Hz, H-2), 7.17−7.32 (m, 5H, Ar−H); ^{13}C NMR ($CDCl_3$) δ 16.1 (q, C-11), 22.2 (t, C-16, C-17), 24.0, 24.4 (t, C-15, C-18), 35.6 (d, C-3), 40.3, 40.1 (d, C-14, C-19), 41.6 (t, C-4), 52.9 (q, C-12), 56.3 (d, C −2), 126.6, 128.8, 129.8 (d, C− 6, C-7, C-8, C-9, C-10), 140.4 (s, C-5), 169.6 (s, C-1), 179.5, 179.6 (s, C-13, C-20). **Compound 7b (2*S*,3*S*):** colorless oil, LREIMS *m*/*z* 343 [M^+, $C_{20}H_{25}NO_4$]; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, 3H, *J* = 6.7 Hz, CH_3-3), 1.44 (m, 4H, H-16, H-17), 1.83 (m, 4H, H-15, H-18), 2.23 (dd, 1H, *J* = 14.6, 11.0 Hz, Ha-4), 2.67 (dd, 1H, *J* = 14.6, 6.7 Hz, Hb- 4), 2.82 (m, 3H, H-3, H-14, H-19), 3.72 (s, 3H, -OCH_3), 4.55 (d, 1H, *J* = 7.5 Hz, H-2), 7.13−7.29 (m, 5H, Ar-H).

(2*R*,3*S*)- and (2*R*,3*R*)-*N*-Cyclohexanedicarboxy-4-phenylvaline Methyl Ester (10c,d). Similarly, (2*R*)-*N*-phthaloyl-3,4-dehydro-4-phenylvaline methyl ester (**9**) obtained from D-valine was completely hydrogenated by use of PtO_2. Compounds **10c** and **10d** were obtained in 34% and 27% yields, respectively, and *N*-cyclohexylcarboxy-4-cyclohexylvaline was also obtained in 22% yield. Compound **10c** was recrystallized from EtOH (mp 74−76 °C). **Compound 10c (2*R*,3*S*):** ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3H, *J* = 6.9 Hz, CH_3-3), 1.46 (m, 4H, H-16, H-17), 1.86 (m, 4H,H-15, H-18), 2.28 (dd, 1H, *J* = 13.2, 9.9 Hz, Ha-4) 2.82 (m, 1H, H-3), 2.93 (m, 2H, H-14, H-19), 3.16 (dd, 1H, *J* = 13.2, 4.0 Hz, Hb-4), 3.73 (s, 3H, −OCH_3), 4.55 (d, 1H, *J* = 7.6 Hz, H-2), 7.17−7.32 (m, 5H, Ar-H). Anal. Calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 69.54; H, 7.33; N, 3.99. **Compound 10d (2*R*,3*R*):** ^{1}H NMR ($CDCl_3$) δ 1.00 (d, 3H, *J* = 6.8 Hz, CH_3-3), 1.43 (m, 4H, H-16, H-17), 1.82 (m, 4H, H-15, H-18), 2.23 (dd, 1H, *J* = 14.7, 11.0 Hz, Ha-4), 2.68 (dd, 1H, *J* = 14.5, 6.9 Hz, Hb- 4), 2.82 (m, 3H, H-3, H-14, H-19), 3.72 (s, 3H, -OCH_3), 4.55 (d, 1H, *J* = 7.3 Hz, H-2), 7.13−7.29 (m, 5H, Ar-H). ***N*-Cyclohexylcarboxy-4-cyclohexylvaline (diastereomeric mixture):** colorless oil, ^{1}H NMR ($CDCl_3$) δ 0.86, 1.00 (d, CH3−3), 2.58 (m, H-2), 2.90 (m, H-3, H-14, H-19), 3.72 (s, -OCH_3), 4.41 and 4.43 (d, H-2). This product was not further investigated.

Isolation of 4-Phenylvaline. (2*S*,3*R*)-4-Phenylvaline (2a). Compound **7a** (18.7 mg) was treated with 6 M hydrochloric and glacial acetic acids (2:1, 1 mL) at 120 °C for 8 h. The reaction mixture was evaporated by reduced pressure, and the residue was applied to ODS-HPLC using MeOH/H2O (30:70). Compounds **2a** was obtained in 42% yield: $[\alpha]_D$ +25° (*c* 0.11, H_2O); mp 210−220 °C (dec); HRFABMS *m*/*z* 194.1182 [M + H]$^+$ (calcd for $C_{11}H_{16}NO_2$ 194.1181); ^{1}H NMR (D_2O) δ 0.80 (d, *J* = 6.7 Hz, CH3−3), 2.42 (m, H-3), 2.51 (dd, *J* = 13.3, 9.2 Hz, H-4a), 2.71 (dd, *J* = 12.9, 5.7 Hz, H-4b), 3.56 (d, *J* = 1.5 Hz, H-2), 7.19−7.31 (Ar-H); ^{13}C NMR (D_2O) δ 13.3 (q, C-11), 36.2 (d, C-3), 38.7 (t, C-4), 58.7 (d, C-2), 126.7, 128.9, 129.2 (d, C-6, C-7, C-8, C-9, C-10), 139.7 (s, C-5), 174.2 (s, C-1).

(2*S*,3*S*)-4-Phenylvaline (2b). Similarly, **2b** was obtained by hydrolysis of **7b** in 54% yield: $[\alpha]_D$ +9° (*c* 0.12, H_2O); mp 185−187 °C (dec); ^{1}H NMR (D_2O) δ 0.81 (d, *J* = 6.8 Hz, CH_3-3), 2.25 (m, H-3), 2.41 (dd, *J* = 13.3, 10.1 Hz, H-4a), 2.74 (dd, *J* = 13.0, 4.6 Hz, H-4b), 3.61 (d, *J* = 1.5 Hz, H-2), 7.17−7.29 (Ar−H); ^{13}C NMR (D_2O) δ 14.4 (q, C-11), 36.5 (d, C-3), 38.0 (t, C-4), 59.3 (d, C-2), 126.6, 128.7, 129.4 (d, C-6, C-7, C-8, C-9, C-10), 140.0 (s, C-5), 173.8 (s, C-1).

(2*R*,3*S*)-4-phenylvaline (2c). Compound **10c** was hydrolyzed with 6 M hydrochloric and glacial acids to afford **2c**: $[\alpha]_D$ −34° (*c* 0.11, H_2O); mp 235−239 °C (dec); ^{1}H NMR (D_2O) δ 0.80 (d, *J* = 6.7 Hz, CH_3-3), 2.42 (m, H-3), 2.51 (dd, *J* = 13.3, 9.2 Hz, H-4a), 2.71 (dd, *J* = 12.9, 5.7 Hz, H-4b), 3.56 (d, *J* = 1.5 Hz, H-2), 7.19−7.31 (Ar−H); ^{13}C NMR (D_2O) δ 13.3 (q, C-11), 36.2 (d, C-3), 38.7 (t, C-4), 58.7 (d, C-2), 126.7, 128.9, 129.2 (d, C-6, C-7, C-8, C-9, C-10), 139.7 (s, C-5), 174.2 (s, C-1).

(2*R*,3*R*)-4-phenylvaline (2d). Using the above method for hydrolysis, **2d** was obtained from **10d**: $[\alpha]_D$ −13° (*c* 0.11, H_2O); mp 193−195 °C (dec, recrystallized from aqueous EtOH); IR (KBr) 3431, 3032, 2978, 2933, 1591, 1512, 1402, 1335, 733, 702 cm^{-1}; ^{1}H NMR (D_2O) δ 0.81 (d, *J* = 6.8 Hz, CH_3-3), 2.25 (m, H-3), 2.41 (dd, *J* = 13.3, 10.1 Hz, H-4a), 2.74 (dd, *J* = 13.0, 4.6 Hz, H-4b), 3.61 (d, *J* = 1.5 Hz, H-2), 7.17−7.29 (Ar-H); ^{13}C NMR (D_2O) δ 14.4 (q, C-11), 36.5 (d, C-3), 38.0 (t, C-4), 59.3 (d, C-2), 126.6, 128.7, 129.4 (d, C-6, C-7, C-8, C-9, C-10), 140.0 (s, C-5), 173.8 (s, C-1). Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.86; H, 7.76; N, 7.12.

(4*S*,2′*S*,3′*R*)-3-(3′-Hydroxy-2′-methylpentyl)-4-isopropyl-2-oxazolidinone (12). A stirred solution of *N*-propionyl oxazolidinone **11a** (1.0 g, 5.4 mmol) in dry CH_2Cl_2 (15 mL) under argon was treated with 1 M dibutylboron triflate (6.0 mL, 6.0 mmol, 1.1 equiv in CH_2Cl_2) and diisopropylmethylamine (1.15 mL, 6.5 mmol, 1.2 equiv) at 0 °C. After 30 min, the reaction mixture was cooled to −78 °C, and *n*-butanal (0.55 mL, 6.0 mmol, 1.1 equiv) was added dropwise. The resulting mixture was stirred at −78 °C for 30 min and then 90 min at room temperature. The reaction was quenched by addition of pH 7 aqueous phosphate buffer (10 mL) and oxidized with 30% hydrogen peroxide/methanol (1:1, 20 mL). The resulting solution was stirred at 0 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was freeze-dried. The residue was dissolved in water (30 mL) and extracted with EtOAc (25 mL × 3). The combined organic layer was washed with 5% $NaHCO_3$ (25 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by flash chromatography (EtOAc/hexane 25:75), and the aldol **12** was

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obtained as colorless oil (1.17 g, 4.55 mmol, 84%): $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$); HREIMS *m/z* 239.1525 [$M^+ - H_2O$] (calcd for $C_{13}H_{21}NO_3$ 239.1522); IR (neat) 3426, 2960, 1780, 1749, 1686, 1386, 1203 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (d, 3H, *J* = 7.1 Hz, H-7), 0.90 (d, 3H, *J* = 7.1 Hz, H-8), 0.92 (t, 3H, *J* = 5.9 Hz, H-6′), 1.23 (d, 3H, *J* = 6.6 Hz, H-7), 1.27−1.40 (m, 2H, H-5′), 1.41−1.58 (m, 2H, H-4′), 2.33 (dsept, 1H, *J* = 4.0, 7.1 Hz, H-6), 2.92 (s, br, 1H, OH), 3.74 (dq, 1H, *J* = 2.5, 7.1 Hz, H-2′), 3.93 (m, 1H, H-3′), 4.20 (dd, 1H, *J* = 3.0, 9.1 Hz, H-5a), 4.27 (t, 1H, *J* = 3.0, 9.1 Hz, H-5b), 4.46 (dt, 1H, *J* = 3.5, 8.1 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 10.6, 13.9, 14.5, 17.8, 19.1, 28.2, 35.8, 41.9, 58.1, 63.2, 70.8, 153.4, 177.8.

(4*R*,5*S*,2′*R*,3′*S*)-3-(3′-Hydroxy-2′-methylpentyl)-5-phenyl-4-methyl-2-oxazolidinone (13). Using the method described for the preparation of **12**, *N*-propionyloxazolidinone **11b** (1.0 mL, 5.0 mmol) was treated with 1 M dibutylboron triflate (5.5 mL, 5.5 mmol, 1.1 equiv in CH_2Cl_2) and diisopropylethylamine (1.1 mL, 6.0 mmol, 1.2 equiv). The resulting enol borinate was allowed to react with *n*-butanal (0.5 mL, 5.5 mmol, 1.1 equiv). After workup and purification, aldol **13** was obtained as a colorless oil (1.31 g, 4.32 mmol, 86%): $[\alpha]_D$ +1.7° (*c* 2.0, $CHCl_3$); HREIMS *m/z* 287.1523 [$M - H_2O$]$^+$ (calcd for $C_{17}H_{21}NO_3$ 287.1516); IR (neat) 3418, 1779, 1646, 1456, 1345, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (d, 3H, *J* = 6.6 Hz, 6-H), 0.95 (t, 3H, *J* = 7.1 Hz, H-6′), 1.24 (d, 3H, *J* = 6.8 Hz, H-7′), 1.34−1.44 (m, 2H, H-5′), 1.45−1.61 (m, 2H, H-4′), 3.77 (ddq, 1H, *J* = 1.2, 2.7, 7.1 Hz, H-2′), 3.97 (m, 1H, H-3′), 4.80 (dq, 1H, *J* = 6.1, 7.1 Hz, H-4), 5.69 (d, 1H, *J* = 7.1 Hz, H-5), 7.29−7.46 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 10.1, 13.9, 14.1, 19.0, 35.9, 42.1, 54.5, 71.1, 78.7, 125.4 (2C), 128.4 (2C), 128.5, 133.0, 152.5, 176.9.

(4*S*,2′*S*,3′*R*)-3-[3′-(4″-Toluenesulfonyl)-2′-methylpentyl]-4-isopropyl-2-oxazolidinone (14). A stirred solution of aldol **12** (642 mg, 2.5 mmol) in dry pyridine (5 mL) under argon was treated at 0 °C with *p*-toluenesulfonyl chloride (525 mg, 2.75 mmol, 1.1 equiv). After 70 h at room temperature, the reaction mixture was poured over a chilled 1 M HCl solution (75 mL). After extraction with $CHCl_3$ (20 mL × 3), the combined organic layer was washed with brine (20 mL × 2), dried over $MgSO_4$, and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by flash chromatography (EtOAc/hexane 10:90), and the tosylate **14** was obtained as colorless oil (720 mg, 1.75 mmol, 70%): $[\alpha]_D$ +77° (*c* 1.0, $CHCl_3$); HREIMS *m/z* 411.1707 [M]$^+$ (calcd for $C_{20}H_{29}NO_6S$ 411.1708); IR (neat) 2964, 2876, 1777, 1704, 1463, 1356, 1366, 1208, 1189, 1176, 1096, 911, 888 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, 3H, *J* = 7.3 Hz, H-6′), 0.88 (d, 3H, *J* = 6.8 Hz, H-8), 0.92 (d, 3H, *J* = 6.8 Hz, H-7), 1.16 (d, 3H, *J* = 6.8 Hz, CH_3-2), 1.20−1.35 (m, 2H, H-5′), 1.53−1.61 (m, 2H, H-4′), 2.39 (dsept, 1H, *J* = 3.6, 7.1 Hz, H-6), 2.44 (s, 3H, Ar−CH_3), 4.01 (dt, 1H, *J* = 4.1, 6.8 Hz, H-2′), 4.21 (dd, 1H, *J* = 2.2, 8.5 Hz, Ha-5), 4.34 (dd, 1H, $J_1 = J_2$ = 8.4 Hz, Hb-5), 4.40−4.45 (m, 1H, H-4), 5.03 (dt, 1H, *J* = 3.9, 6.6 Hz, H-3′), 7.72 (d, 2H, *J* = 8.3 Hz, Ar−H), 7.77 (d, 2H, *J* = 8.3 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 10.3, 13.7, 14.8, 18.0, 18.2, 21.6, 28.5, 34.9, 41.6, 59.3, 63.8, 81.8, 121.8 (2C), 129.6 (2C), 134.1, 144.6, 154.4, 172.8.

(4*R*,5*S*,2′*R*,3′*S*)-3-{3′-[(4″-Toluenesulfonyl)oxy]-2′-methylpentyl}-5-phenyl-4-methyl-2-oxazolidinone (15). Using the method described for the preparation of **14**, aldol **13** (540 mg, 1.8 mmol) was treated with *p*-toluenesulfonyl chloride (380 mg, 2.0 mmol, 1.1 equiv) in dry pyridine (5 mL) and yielded tosylate **15** as a colorless oil (560 mg, 1.22 mmol, 68%): $[\alpha]_D$ −29° (*c* 2.0, $CHCl_3$); HREIMS *m/z* 287.1581 [M − TsOH]$^+$ (calcd for $C_{17}H_{21}NO_3$ 287.1516); IR (neat) 2964, 2935, 2876, 1777, 1705, 1456, 1365, 1350, 1176, 910, 888 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 7.3 Hz, H-6′), 0.92 (d, 3H, *J* = 6.6 Hz, H-6), 1.17 (d, 3H, *J* = 7.1 Hz, H-7′), 1.23−1.38 (m, 2H, H-5′), 1.58−1.65 (m, 2H, H-4′), 2.45 (s, 3H, Ar-CH_3), 4.02 (dq, 1H, *J* = 3.4, 7.0 Hz. H-2′), 4.75 (dq, 1H, *J* = 6.8 Hz, H-4), 5.10 (dt, 1H, *J* = 3.2, 6.6 Hz, H-3′), 5.78 (d, 1H, *J* = 7.1 Hz, H-5), 7.30−7.44 (m, 7H, Ar−H), 7.80 (d, 2H, *J* = 8.1 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 9.4, 13.7, 14.4, 18.4, 21.7, 34.9, 41.6, 55.6, 79.4, 81.9, 125.6 (2C), 127.8 (2C), 128.6 (3C), 129.7 (2C), 133.3, 134.1, 144.7, 153.4, 172.5.

(4*S*,2′*S*,3′*S*)-3-(3′-Azido-2′-methylpentyl)-4-isopropyl-2-oxazolidinone (16). To a solution of tosylate **14** (207 mg, 0.50 mmol) in DMF (2 mL) under nitrogen was added sodium azide (98 mg, 1.5 mmol) and 15-crown-5 (10 mg, 0.1 equiv). The solution was stirred at 70 °C for 4.5 h, allowed to cool to room temperature, then quenched with water (15 mL), and extracted with EtOAc (10 mL × 4). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by PLC (EtOAc/hexane 1:3), and azide **16** was obtained as a colorless oil (77 mg, 0.27 mmol, 54%): $[\alpha]_D$ +114° (*c* 1.0, $CHCl_3$); HREIMS *m/z* 254.1641 [$M - N_2$]$^+$ (calcd for $C_{13}H_{22}N_2O_3$ 254.1625); IR (neat) 2963, 2936, 2876, 2103, 1782, 1698, 1456, 1398, 1456, 1386, 1246, 1204 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (d, 3H, *J* = 6.8 Hz, H-7), 0.93 (d, 3H, *J* = 6.6 Hz, H-8), 0.95 (t, 3H, *J* = 6.7 Hz, H-6′), 1.20 (d, 3H, *J* = 6.8 Hz, H-7′), 1.36−1.53 (m, 2H, H-5′), 1.53−1.70 (m, 2H, H-4′), 2.37 (m, 1H, H-6), 3.67 (m, 1H, H-3′), 3.86 (dq, 1H, *J* = 7.1, 9.3 Hz, H-2′), 4.22 (dd, 1H, *J* = 2.6, 9.2 Hz, Ha-5), 4.31 (dd, 1H, *J* = 8.3, 9.0, Hz, Hb-5), 4.49 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.7, 15.0, 17.9, 18.9, 28.4, 33.4, 42.1, 58.4, 63.4, 64.4, 153.5, 174.8.

(4*R*,5*S*,2′*R*,3′*R*)-3-(3′-Azido-2′-methylpentyl)-5-phenyl-4-methyl-2-oxazolidinone (17). Using the method described for the preparation of **16**, tosylate **15** (146 mg, 0.32 mmol) in CH_2Cl_2 (2 mL) was treated with tetramethyl guanidinium azide (63 mg, 0.40 mmol, 1.25 equiv)[17] in refluxing CH_2Cl_2 (2 mL) and yielded the azide **17** as a colorless oil (32 mg, 0.10 mmol, 30%): $[\alpha]_D$ +7.0° (*c* 1.0, $CHCl_3$); HREIMS *m/z* 302.1650 [$M - N_2$]$^+$ (calcd for $C_{17}H_{22}N_2O_3$ 302.1625); IR (neat) 2957, 2928, 2875, 2101, 1783, 1694, 1455, 1344, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (d, 3H, *J* = 7.6 Hz, H-6), 0.98 (t, 3H, *J* = 6.8 Hz, H-6′), 1.21 (d, 3H, *J* = 6.8 Hz, H-7′), 1.38−1.53 (m, 2H, H-5′), 1.54−0.171 (m, 2H, H-4′), 3.71 (dt, 1H, *J* = 2.7, 8.8 Hz, H-3′), 3.84 (dq, 1H, *J* = 6.8, 9.3 Hz, H-2′), 4.83 (dq, 1H, *J* = 6.6, 6.8 Hz, H-4), 5.72 (d, 1H, *J* = 7.1 Hz, H-5), 7.30−7.46 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.3, 14.6, 18.9, 33.4, 42.3, 55.0, 64.6, 79.0, 125.6 (2C), 128.8 (2C), 128.8, 133.1, 152.6, 174.7.

(2*S*,3*S*)-3-Amino-2-methylhexanoic Acid (3c). A stirred solution of **16** (20 mg, 0.071 mmol) in THF/H_2O (3:1, 1 mL), cooled to 0 °C, was treated with 28 μL (0.28 mmol, 4 equiv) of 30% H_2O_2 followed by 6 mg (0.14 mmol, 2 equiv) of solid LiOH H_2O. After stirring at 0 °C for 30 min, the reaction mixture was treated with a solution of 210 μL of 1.5 M Na_2SO_3, followed by 700 μL of 0.5 M $NaHCO_3$. Following removal of THF under nitrogen, the residue was diluted to 10 mL with H_2O and extracted with CH_2Cl_2 (6 mL × 4). The aqueous layer was acidified to pH 1−2 with 5 M HCl and extracted with EtOAc (13 mL × 4). The organic layer was combined, dried over Na_2SO_4, and dried under nitrogen, yielding azido acid as colorless oil. Then, the crude azido acid was hydrogenated by use of 10% Pd−C (10 mg) in AcOH/H_2O (3:1, 5 mL). After removal of the catalyst, the reaction contents were applied to ODS-HPLC using MeOH/H_2O/TFA (20:80:0.05). Compound **3c** was obtained as colorless oil (7.6 mg, 0.052 mmol, 74%): $[\alpha]_D$ +0.5° (*c* 0.19, H_2O); IR (KBr) 3435, 2968, 2885, 1676, 1464, 1196, 1140 cm^{-1}; ^1H NMR (D_2O) δ 0.87 (t, *J* = 7.3 Hz, H-6), 1.23 (d, *J* = 7.3 Hz, CH_3-2), 1.32, 1.38 (apparently sextet, but *J* was unclear, H-5a, H-5b), 1.61 (apparently quartet, *J* was unclear, H-4a, H-4b), 2.85 (apparently quintet, but *J* was unclear, H-2), 3.48 (q, *J* = 6.2, 12.6 Hz, H-3); ^{13}C NMR (D_2O) δ 13.0 (q, CH_3-2), 13.2 (q, C-6), 18.0 (t, C-5), 32.3 (t, C-4), 41.4 (d, C-2), 53.3 (d, C-3), 178.7 (s, C-1). Anal. Calcd for $C_7H_{15}NO_2 \cdot CF_3COOH \cdot H_2O$: C, 38.99; H, 6.45; N, 5.05. Found: C, 38.95; H, 6.41; N, 5.57.

(2*R*,3*R*)-3-Amino-2-methylhexanoic Acid (3d). Using the same method employed in the preparation of **3c**, **3d** was obtained from **17** in 95% yield: $[\alpha]_D$ −3.1° (*c* 0.23, H_2O); HRFABMS *m/z* 146.1184 [M + H]$^+$ (calcd for $C_7H_{16}NO_2$ 146.1181); ^1H NMR (D_2O) δ 0.87 (t, *J* = 7.3 Hz, H-6), 1.23 (d, *J* = 7.3 Hz, CH_3-2), 1.32, 1.38 (apparently sextet, but *J* was unclear, H-5a, H-5b), 1.61 (apparently quartet, *J* was unclear,

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H-4a, H-4b), 2.85 (apparently quintet, but *J* was unclear, H-2), 3.48 (q, $J = 6.2, 12.6$ Hz, H-3); ^{13}C NMR (D_2O) δ 13.0 (q, CH_3-2), 13.2 (q, C-6), 18.0 (t, C-5), 32.3 (t, C-4), 41.4 (d, C-2), 53.3 (d, C-3), 178.7 (s, C-1).

(4*S*,2′*S*,3′*S*)-3-(3′-Bromo-2′-methylpentyl)-4-isopropyl-2-oxazolidinone (18).[18] To a solution of the tosylate **14** (300 mg, 0.73 mmol) in dry acetone (2.5 mL) was added anhydrous lithium bromide (300 mg, 3.4 mmol, 4.7 equiv) at room temperature under argon. After 4 h reflux, the reaction mixture was allowed to cool to room temperature, and water was added (15 mL). The resulting mixture was extracted with EtOAc (10 mL × 4) and CH_2Cl_2 (10 mL × 1), the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by flash chromatography (EtOAc/hexane 5:95), and bromide **18** was obtained as a colorless oil (170 mg, 0.50 mmol, 75%): $[\alpha]_D$ +138° (*c* 1.0, $CHCl_3$); HREIMS *m*/*z* 319.0782 $[M]^+$ (calcd for $C_{13}H_{22}NO_3{}^{79}Br$ 319.0777); IR (neat) 2963, 2935,-1875, 1782, 1700, 1464, 1386, 1300, 1251, 1204 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, 3H $J = 6.8$ Hz, H-7), 0.88 (d, 3H, $J = 7.1$ Hz, H-8), 1.17 (t, 3H, $J = 7.3$ Hz, H-6′), 1.38−1.49 (m, 1H, 5′-H), 1.55−1.76 (m, 2H, H-4′, H-5′), 1.78−1.90 (m, 1H, H-4′), 2.32 (dsept, 1H, $J = 4.0, 7.1$ Hz, H-6), 4.18 (dd, 1H, $J = 2.8, 9.2$ Hz, Ha-5), 4.27 (t, 1H, $J = 8.7$ Hz, Hb-5), 4.22−4.40 (m, 2H, H-2′, H-3′), 4.44 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 13.3, 14.7, 16.1, 17.8, 20.1, 28.4, 36.4, 45.0, 56.8, 58.5, 63.4, 153.5, 174.5.

(4*R*,5*S*,2′*R*,3′*R*)-3-(3′-Bromo-2′-methylpentyl)-5-phenyl-4-methyl-2-oxazolidinone (19). Using the method described for the preparation of **18**, tosylate **15** (676 mg, 1.47 mmol) was treated with lithium bromide (640 mg, 7.4 mmol, 5 equiv) in refluxing acetone (5.0 mL) and yielded bromide **19** as a colorless oil (377 mg, 1.03 mmol, 70%): $[\alpha]_D$ −40° (*c* 1.0, $CHCl_3$); HREIMS *m*/*z* 367.0875 $[M]^+$ (calcd for $C_{17}H_{22}NO_3{}^{79}$-Br 367.0777); IR (neat) 2960, 2936, 2876, 1783, 1699, 1456, 1386, 1343, 1196 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3H, $J = 6.6$ Hz, H-6), 0.91 (t, 3H, $J = 7.2$ Hz, H-6′), 1.26 (d, 3H, $J = 6.4$ Hz, H-7′), 1.42−1.48 (m, 1H, H-5′), 1.60−1.81 (m, 2H, H-4′, H-5′), 1.82−1.96 (m, 1H, H-4′), 4.32 (m, 1H, H-3′), 4.35 (m, 1H, H-2′), 4.81 (dq, 1H, $J = 6.8, 6.9$ Hz, H-4), 5.70 (d, 1H, $J = 7.1$ Hz, H-5), 7.30−7.45 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 13.3, 14.2, 15.8, 20.1, 36.5, 45.4, 55.0, 57.1, 79.0, 125.5 (2C), 128.6 (2C), 128.7, 133.1, 152.6, 174.3.

(2*S*,3*R*)-3-Amino-2-methylhexanoic Acid (3a). To a solution of bromide **18** (98 mg, 0.30 mmol) in DMF (1.5 mL) under nitrogen was added sodium azide (59 mg, 0.90 mmol) and 15-crown-5 (6 mg, 0.1 equiv). After stirring at 70 °C for 3 h, the solution was quenched with water (15 mL) and extracted with EtOAc (10 mL × 4). The extract was roughly separated by PLC (EtOAc/hexane 1:4) to afford crude azido-2′-methylpentyl)-4-isopropyl-2-oxazolidinone (**20**, 58 mg) as a yellow oil. Using the above hydrolysis and hydrogenation methods for preparation of **3c**, a stirred solution of crude **20** in THF/H_2O was treated with H_2O_2 followed by LiOH H_2O. Then, the crude azido acid was hydrogenated by the use of 10% Pd−C in AcOH/H_2O. Compound **3a** was purified by ODS-HPLC using MeOH/H_2O/TFA (20:80:0.05) to give a colorless oil (6.6 mg, 0.046 mmol, 62%): $[\alpha]_D$ +7.4° (*c* 0.13, H_2O); ^{1}H NMR (D_2O) δ 0.87 (t, $J = 7.2$ Hz, H-6), 1.18 (d, $J = 7.3$ Hz, CH_3-2), 1.32, 1.37 (m, H-5a, H-5b), 1.50−1.65 (m, H-4a, H-4b), 2.84−2.89 (m, H-2), 3.52 (m, H-3);^{13}C NMR (D_2O) δ 11.1 (q, C-6), 12.9 (q, CH_3-2), 18.3 (t, C-5), 31.7 (t, C-4), 40.8 (d, C-2), 52.6 (d, C-3), 178.4 (s, C-1). Anal. Calcd for $C_7H_{15}NO_2{\cdot}CF_3COOH{\cdot}H_2O$: C, 38.99; H, 6.45; N, 5.05. Found: C, 38.38; H, 6.09; N, 5.04.

(2*R*,3*S*)-Amino-2-methylhexanoic Acid (3b). Azidation of **19** by use of sodium azide afforded **21**, and subsequent hydrolysis followed by hydrogenation of **21** (the same preparation for **3a**) gave **3b** quantitatively: $[\alpha]_D$ −9.7° (*c* 0.15, H_2O); ^{1}H NMR (D_2O) δ 0.87 (t, $J = 7.2$ Hz, H-6), 1.18 (d, $J = 7.3$ Hz, CH_3-2), 1.32, 1.37 (m, H-5a, H-5b), 1.50−1.65 (m, H-4a, H-4b), 2.84−2.89 (m, H-2), 3.52 (m, H-3);^{13}C NMR (D_2O) δ 11.1 (q, C-6), 12.9 (q, CH_3-2), 18.3 (t, C-5), 31.7 (t, C-4), 40.8 (d, C-2), 52.6 (d, C-3), 178.4 (s, C-1).

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Supporting Information Available: Tables of crystal data for **7a** and **10c**, Marfey analysis of amino acids, chiral HPLC analysis of α-hydroxy acids, and NMR spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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