Isolation and Stereostructures of Dolastatin G and Nordolastatin G, Cytotoxic 35-Membered Cyclodepsipeptides from the Japanese Sea Hare *Dolabella auricularia*

Tsuyoshi Mutou, Takashi Kondo, Makoto Ojika, and Kiyoyuki Yamada*

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

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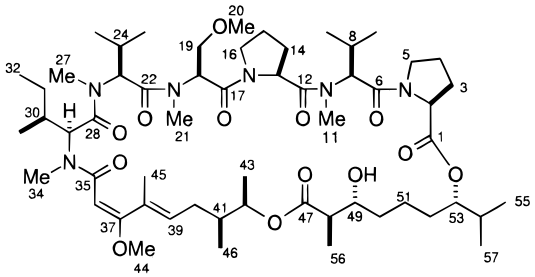
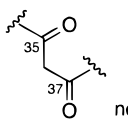
A bioassay-directed fractionation of the cytotoxic constituents of the Japanese sea hare *Dolabella auricularia* resulted in the isolation of two 35-membered depsipeptides dolastatin G (**1**) and nordolastatin G (**2**), which showed cytotoxicity against HeLa S_3 cells with IC_{50} values of 1.0 and 5.3 μg/mL, respectively. The gross structures of these substances were established by spectroscopic analysis including 2D NMR techniques. The absolute stereostructure of **1** was determined by chiral HPLC analysis of amino acid components obtained from acid hydrolysis of **1** and by the enantioselective syntheses of two degradation products arising from polyketide portions. Nordolastatin G (**2**) is a congener that has the same absolute stereochemistry as that of **1**.

The Western Indian Ocean sea hare *Dolabella auricularia* (Aplysiidae) is known to be a rich source of antineoplastic and/or cytostatic peptides such as dolastatins 10 and 15.[1] We have examined the constituents of Japanese specimens of this animal and found several cytotoxic depsipeptides[2] and other unique metabolites.[3] We now report the isolation and structural determination of cytotoxic macrocyclic depsipeptides of mixed peptide−polyketide biogenesis, dolastatin G (**1**) and nordolastatin G (**2**), from the Japanese sea hare *D. auricularia*.





The MeOH extract of the internal organs of the sea hare *D. auricularia* (40 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material, which exhibited strong cytotoxicity against HeLa S_3 cells with an IC_{50} of 1.2 μg/mL, was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc and EtOAc/MeOH, step gradient; ii. 4:1 hexane/acetone to acetone, linear gradient) and ODS silica gel (70% aqueous MeOH to MeOH, linear gradient) successively. Further separation by HPLC (ODS, 60% aqueous MeCN) afforded dolastatin G (**1**) (35 mg) as colorless prisms and a fraction containing nordolastatin G (**2**). The fraction containing **2** was purified by repeated preparative TLC with two solvent systems (2.5:1 $CHCl_3$/acetone and 2:1 benzene/acetone) to afford **2** (0.5 mg) as a colorless powder. Dolastatins G (**1**) and nordolastatin G (**2**) showed cytotoxicity against HeLa S_3 cells with IC_{50} values of 1.0 and 5.3 μg/mL, respectively.

The molecular formula of **1** was established to be $C_{57}H_{96}N_6O_{13}$ by combustion analysis and high-resolution FABMS. ^{1}H and ^{13}C NMR data for **1** are summarized in Table 1, in which all of the protonated carbons were assigned by ^{1}H−^{13}C COSY data. The depsipeptide nature of **1** was indicated by the presence of six nitrogen atoms and nine sp^2 carbon signals in the ester and amide region (δ 166.8−175.5) of the ^{13}C NMR spectrum as well as by the absorption bands at 1735, 1635, and 1460 cm^{-1} in the IR spectrum. DQF-COSY experiments and HMBC data (Table 1) established the presence of six amino acid residues (two prolines, two *N*-methylvalines, *N*,*O*-dimethylserine, and *N*-methylisoleucine) and two hydroxy acid portions (C35−C46 and C47−C57). One of the nine sp^2 carbon signals (δ 170.2) in the ester and amide region was assigned to C37 of the enol ether group by the HMBC correlations (C37/H36, C37/H39, and C37/H44). The location of a hydroxyl group in **1** was determined to be at C49 by the COSY correlation between the oxymethine proton at δ 4.17 (H49) and the D_2O-exchangeable proton at δ 4.23. The low-field chemical shifts of H42 (δ 5.00) and H53 (δ 5.10) suggest that the acyloxy groups are at these positions. The *E* geometry of two olefinic bonds was evidenced by difference NOE data (H36/H44 and H40/H45). The degree of unsaturation in **1** suggests a cyclic nature of **1**. The HMBC correlations shown in Table 1 enabled us to connect the partial structures described above (six amino acids and two polyketide units) and to assign all the carbonyl and quaternary olefinic carbons, establishing the gross structure of **1**.

The molecular formula of the minor congener nordolastatin G (**2**), $C_{56}H_{94}N_6O_{13}$, was determined by high-resolution FABMS. Resonances in the ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the

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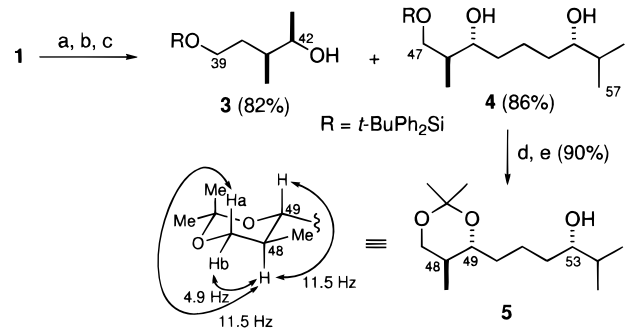
Table 1. NMR Data and HMBC Correlations for Dolastatin G (1) in C_6D_6

position	$^1H^a$	$^{13}C^b$	HMBC^c	position	$^1H^a$	$^{13}C^b$	HMBC^c
1		170.9 s	H-2, 53	29	5.61 d (11.0)	57.0 d	H-30, 33, 34
2	4.65 br d (8.1)	60.3 d	H-3 (1.53)	30	2.39 m	33.3 d	H-29, 32, 33
3	1.53, 1.91 m	31.7 t	H-2	31	1.15, 1.41 m	24.6 t	H-29, 32, 33
4	1.22, 1.45 m	21.9 t	H-2, 3	32	0.90 t (7.3)	11.1 q	
5	3.45 ddd (12.1, 8.8, 8.8)	46.0 t	H-2, 3 (1.91)	33	0.84 d (6.2)	15.9 q	H-29, 31
	3.67 ddd (12.1, 8.8, 2.2)			34	2.85 s	30.9 q	H-29
6		169.2 s	H-7	35		167.9 s	H-29, 34, 36
7	5.18 d (11.0)	58.2 d	H-8, 9, 10, 11	36	4.89 s	93.4 d	
8	2.42 ddq (11.0, 6.6, 6.6)	27.9 d	H-7, 9, 10	37		170.2 s	H-36, 39, 44
9	0.77 d (6.6)	19.0 q	H-7, 8, 10	38		133.2 s	H-36, 45
10	1.04 d (6.6)	18.8 q	H-7, 8, 9	39	5.73 m	130.4 d	H-45
11	3.07 s	29.6 q	H-7	40	1.84 m	31.6 t	H-39, 42
12		171.2 s	H-7, 11, 13		2.00 br dd (12.5, 5.9)		
13	4.68 br d (8.8)	58.4 d	H-14 (1.52)	41	1.73 m	38.5 t	H-40, 42, 43
14	1.52, 1.90 m	27.7 t	H-13	42	5.00 dq (4.4, 6.2)	74.2 d	H-46
15	1.65, 2.37 m	24.2 t	H-13, 14	43	1.11 d (6.2)	14.6 q	H-42
16	3.13 ddd (9.9, 9.9, 7.3)	47.3 t	H-13, 14 (1.90)	44	3.08 s	54.9 q	
	3.80 br t (8.8)			45	1.96 br s	14.9 q	H-39
17		166.8 s	H-18	46	1.12 d (6.6)	13.5 q	H-40 (2.00), 42
18	5.94 dd (11.4, 4.8)	54.8 d	H-19, 21	47		175.5 s	H-42, 48
19	3.62 dd (11.4, 4.8)	68.6 t	H-18, 20	48	2.79 dq (9.5, 7.0)	48.3 d	H-56
	3.92 dd (11.4, 11.4)			49	4.17 m	74.0 d	OH, H-48, 56
20	2.91 s	58.3 q	H-19 (3.92)	50	1.75, 1.82 m	35.7 t	
21	2.90 s	29.8 q	H-18	51	1.43, 2.07 m	23.6 t	
22		170.7 s	H-18, 21, 23	52	1.89, 2.28 m	32.1 t	
23	5.42 d (10.6)	58.9 d	H-24, 25, 26, 27	53	5.10 ddd (7.7, 5.5, 2.2)	78.5 d	H-55, 57
24	2.67 dqq (10.6, 6.6, 6.6)	27.4 d	H-23, 25, 26	54	1.77 m	30.9 d	H-55, 57
25	0.93 d (6.6)	17.7 q	H-23, 24, 26	55	0.83 d (6.6)	19.9 q	H-53, 54, 57
26	1.01 d (6.6)	20.4 q	H-23, 24, 25	56	1.07 d (7.0)	15.2 q	H-48
27	3.31 s	30.8 q	H-23	57	0.85 d (6.6)	14.8 q	H-53, 54, 55
28		170.4 s	H-27, 29	OH	4.23 d (10.9)		

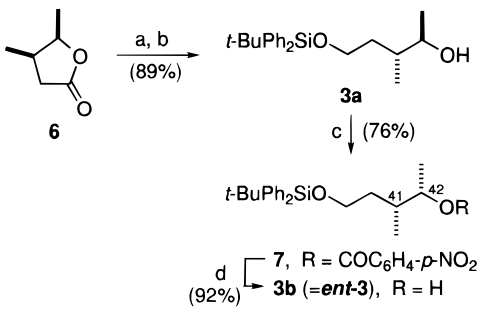
[a] Recorded at 600 MHz. Coupling constants in Hz are in parentheses. [b] Recorded at 100 MHz. [c] Recorded at 400 MHz. Parameters were optimized for $J_{CH} = 8$ Hz.

Scheme 1[a]



[a] Key: (a) O_3, MeOH, −78 °C; (b) $LiAlH_4$, Et_2O, rt; (c) *t*-$BuPh_2SiCl$, imidazole, DMF, 0 °C; (d) Bu_4NF, THF, rt; (e) $(MeO)_2CMe_2$, 10-camphorsulfonic acid (CSA), acetone, rt.

Scheme 2[a]



[a] Key: (a) $LiAlH_4$, THF, 0 °C; (b) *t*-$BuPh_2SiCl$, imidazole, DMF, 0 °C; (c) DEAD, Ph_3P, *p*-$NO_2C_6H_4CO_2H$, benzene, rt; (d) NaOMe, MeOH, rt.

observation of the signals due to the keto group (δ_{C37} 193.8) and a methylene group (δ_{H36} 3.41 and 3.60) in place of those due to the methyl group (δ_{H44} 3.08) of the enol ether part and an olefinic proton (δ_{H36} 4.89) in **1**. Interpretation of these NMR data coupled with the molecular formula indicated that **2** was the 44-nor derivative of **1**.

The absolute stereostructures of **1** and **2** were elucidated as follows. Acid hydrolysis of **1** (6 M HCl at 110 °C) followed by chiral HPLC analysis [CHRALPAK MA-(+)] established the configurations of six amino acids to be all L. The absolute stereochemistry of five asymmetric carbons (C41, C42, C48, C49, and C53) in **1** was determined by the enantioselective syntheses of degradation products of **1**. Ozonolysis of **1** followed by $LiAlH_4$ reduction and selective protection of primary hydroxyl groups with *tert*-butyldiphenylsilyl chloride produced the C39−C43 fragment **3** and the C47−C57 fragment **4** derived from two polyketide portions (Scheme 1). Diol **4** was further converted to hydroxy acetonide **5**. Thus, the relative stereochemistry of C48−C49 was determined to be *anti* from the spin−spin coupling constants $J_{47a,48} = J_{48,49} = 11.5$ Hz in **5**.

To determine the absolute stereochemistry of **3**, we synthesized two possible diastereomeric silyl ethers, **3a** and **3b** (Scheme 2). Reduction of lactone **6**[4] followed by selective protection of the resulting primary hydroxyl group gave silyl ether **3a**. The diastereomer **3b** was prepared by methanolysis of *p*-nitrobenzoate **7**, which was obtained by a modified Mitsunobu reaction[5] of **3a**. On comparison of the spectroscopic data and specific rotations, synthetic **3b**, $[\alpha]^{25}_D$ +6 (*c* 0.12, $CHCl_3$), was found to be identical to natural **3**, $[\alpha]^{30}_D$ −6 (*c* 0.11, $CHCl_3$), except for the sign of specific rotation, establishing the 41*S*,42*R* configurations in **1**.

The absolute stereochemistry of **5** was determined by the syntheses of two possible diastereomeric hydroxy

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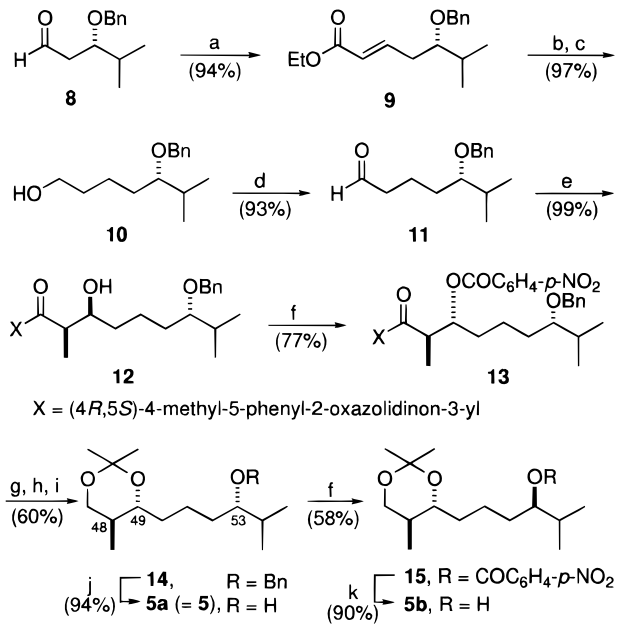
Scheme 3[a]



[a] Key: (a) $(i\text{-PrO})_2P(O)CH_2CO_2Et$, *t*-BuOK, THF, −78 → 0 °C; (b) $NiCl_2$, $NaBH_4$, MeOH, rt; (c) $LiAlH_4$, THF, −78 → 0 °C; (d) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; Et_3N, −78 → 0 °C; (e) (4*R*,5*S*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone, Bu_2BOTf, Et_3N, CH_2Cl_2, −78 → 0 °C; (f) DEAD, Ph_3P, *p*-$NO_2C_6H_4CO_2H$, benzene, rt; (g) LiOH, H_2O_2, aqueous THF, rt; (h) $LiAlH_4$, THF, rt; (i) $(MeO)_2CMe_2$, CSA, acetone, rt; (j) H_2, 20% $Pd(OH)_2$/C, dioxane, 40 °C; (k) NaOMe, MeOH, rt.

acetonides, **5a** and **5b** (Scheme 3). The homologation of aldehyde **8**[6] by the Horner−Emmons reaction gave conjugated ester **9**, which was converted to alcohol **10** by sequential reduction. The Swern oxidation of **10** gave aldehyde **11**, which was converted to aldol **12** with 96% de by the Evans aldol reaction.[7] Inversion of the hydroxyl group in **12** afforded *p*-nitrobenzoate **13**, which was converted to acetonide **14** by a sequence of reactions. Debenzylation of **14** afforded hydroxy acetonide **5a**. The diastereomeric hydroxy acetonide **5b** was obtained by inversion at C53 of **5a** followed by methanolysis of the resulting *p*-nitrobenzoate **15**. Of the two synthetic diastereomers of hydroxy acetonide, **5a** and **5b**, the spectral data for **5a**, $[\alpha]^{26}_D$ +20 (*c* 0.10, $CHCl_3$), was identical to that of natural **5**, $[\alpha]^{26}_D$ +19 (*c* 0.07, $CHCl_3$), in all respects, establishing the 48*R*,49*R*,53*S* configurations in **1**. On the basis of these findings the complete stereostructure of dolastatins G was determined as shown in **1**. The absolute stereochemistry of nordolastatin G (**2**) was shown to be identical to that of dolastatin G (**1**), since **1** was converted to **2** on acidic hydrolysis (HCl, aqueous dioxane, rt, 98%).

Experimental Section

General Methods. Melting points are uncorrected. NMR spectra were measured at 270, 400, or 600 MHz for ^1H and 100 MHz or 150 MHz for ^{13}C. *J* values are given in Hz. Both TLC analysis and preparative TLC were conducted on E. Merck precoated silica gel 60 F_{254} (0.25 mm layer thickness). Fuji Silysia silica gel BW-820 MH and E. Merck aluminum oxide 90 (activity II−III) were used for column chromatography unless otherwise noted. Organic solvents for anhydrous reactions were distilled from the following drying agents: THF and ether (Na−benzophenone ketyl), benzene (Na), triethylamine (calcium hydride), DMSO (calcium hydride under reduced pressure), CH_2Cl_2 (P_2O_5), acetone (anhydrous K_2CO_3), and MeOH (Mg). All moisture-sensitive reactions were performed under an atmosphere of nitrogen.

Isolation of Dolastatin G (1) and Nordolastatin G (2). Specimens of *D. auricularia* (40 kg, wet wt) were collected by hand at a depth of 0−1 m off the coast of the Shima Peninsula, Mie Prefecture, Japan, in May 1993 and stored at −20 °C for several months until extraction. The specimens were separated into the internal organs and the thick outer skin, and the former (23 kg) was extracted with MeOH (48 L). This methanolic extract was concentrated in vacuo to *ca.* 1/12 of its volume and extracted with EtOAc (6 × 2 L). The EtOAc extracts were concentrated in vacuo, the residue (135.5 g, IC_{50} against HeLa S_3 cells = 1.2 μg/mL) was dissolved in 9:1 MeOH/H_2O (2 L), and the solution was washed with hexane (2 × 2 L). Evaporation of the aqueous MeOH portion gave a dark brown oil (40.3 g), which was chromatographed on silica gel, eluting with 1:1 toluene/EtOAc, EtOAc, 95:5, 90:10, and 80:20 EtOAc/MeOH, and MeOH, successively. The fraction eluted with 95:5 EtOAc/MeOH (2.36 g) was subjected to silica gel MPLC [Micro Bead Silica Gel 4B (Fuji Silysia, 64 g), linear gradient from 80:20 hexane/acetone to acetone, flow rate 6.0 mL/min]. The fraction eluted between 7:93 and 5:95 hexane/acetone (516 mg) was subjected to reversed-phase MPLC [Develosil ODS 30/60 (Nomura Chemical, 70 g), linear gradient from 70:30 MeOH/H_2O to MeOH, flow rate 5.0 mL/min]. The fraction eluted between 94:6 MeOH/H_2O to MeOH (151 mg) was further separated by preparative HPLC [Develosil ODS 10 column (10 × 250 mm), 60:40 MeCN/H_2O, flow rate 5.0 mL/min] to afford pure dolastatin G (**1**) (35 mg) and a fraction containing nordolastatin G (**2**) (19.9 mg).

The fraction containing **2** (19.9 mg) was chromatographed on alumina (2 g) with 100:0, 98:2, and 95:5 EtOAc/MeOH to afford crude **2** (2.5 mg), which was purified by repeated preparative TLC with two solvent systems (2.5:1 $CHCl_3$/acetone and 2:1 benzene/acetone) to afford pure **2** (0.5 mg) as a colorless amorphous powder. Using the same procedure as described above, the sea hares (146 kg wet wt) collected in 1993 and 1994 were extracted and separated to yield an additional sample of **2** (0.7 mg). Thus, a total amount of **2** obtained from 186 kg of wet animals was 1.2 mg.

Dolastatin G (1): colorless prisms; mp 138−139 °C (hexane/benzene); t_R = 11.0 min [Develosil ODS-HG-5 column (4.6 × 250 mm), 85:15 MeOH/H_2O, flow rate 1.0 mL/min, detection at 215 nm]; R_f = 0.32 (5:1 $CHCl_3$/acetone); $[\alpha]^{25}_D$ −211 (*c* 0.40, MeOH); UV (MeOH) λ_{max} 205 (ε 38 200), 250 nm (9600); IR ($CHCl_3$) 3450 (br), 1735, 1635, 1460, 1195 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRMS (FAB) calcd for $C_{57}H_{97}N_6O_{13}$ (MH^+) 1073.7113, found 1073.7070. Anal. Calcd for $C_{57}H_{96}N_6O_{13}$: C, 63.78; H, 9.01; N, 7.83. Found: C, 63.61; H, 8.92; N, 7.59.

Nordolastatin G (2): colorless amorphous powder; t_R = 17.2 min [Develosil ODS-HG-5 column (4.6 × 250 mm), 80:20 MeOH/H_2O, flow rate 1.0 mL/min, detection at 215 nm]; R_f = 0.23 (5:1 $CHCl_3$/acetone); $[\alpha]^{25}_D$ −183 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 205 (ε 35 900), 225 (sh, 22 700), 291 nm (5000); IR ($CHCl_3$) 3450 (br), 1730, 1635, 1460, 1195, 1100 cm^{-1}; ^1H NMR (600 MHz, C_6D_6) δ 0.75 (d, *J* = 7.0 Hz, 3 H, H-25), 0.76 (d, *J* = 6.2 Hz, 6 H, H-9 and H-33), 0.81 (t, *J* = 7.3 Hz, 3 H, H-32), 0.82 (d, *J* = 7.3 Hz, 3 H, H-46), 0.84 (d, *J* = 7.3 Hz, 6 H, H-55 and H-57), 0.95 (d, *J* = 6.2 Hz, 3 H, H-26), 1.01 (m, 1 H, H-31), 1.01 (d, *J* = 6.6 Hz, 3 H, H-10), 1.07 (d, *J* = 6.4 Hz, 3 H, H-56), 1.08 (d, *J* = 6.4 Hz, 3 H, H-43), 1.21 (m, 2 H, H-4 and H-31), 1.40 (m, 1 H, H-4), 1.44 (m, 1 H, H-51), 1.52 (m, 1 H, H-14), 1.56 (m, 1 H, H-3), 1.62 (m, 1 H, H-15), 1.72 (m, 1 H, H-50), 1.73 (m, 1 H, H-40), 1.74 (m, 2 H, H-41 and H-54), 1.77 (br s, 3 H, H-45), 1.88 (m, 1 H, H-14), 1.90 (m, 3 H, H-3, H-50, and H-52), 2.04 (m, 1 H, H-51), 2.22 (m, 2 H, H-30 and H-40), 2.25 (m, 1 H, H-52), 2.36 (m, 1 H, H-15), 2.40 (m, 1 H, H-8), 2.51 (m, 1 H, H-24), 2.68 (s, 3 H, H-34), 2.70 (m, 1 H, H-48), 2.83 (s, 3 H, H-21), 2.86 (s, 3 H, H-20), 3.06 (s, 3 H, H-11), 3.08 (s, 3 H, H-27), 3.12 (m, 1 H, H-16), 3.41 (d, *J* = 14.3 Hz, 1 H, H-36), 3.45 (m, 1 H, H-5), 3.60 (d, *J* = 14.3 Hz, 1 H, H-36), 3.60 (dd, *J* = 11.4, 4.7 Hz, 1 H, H-19), 3.66 (m, 1 H, H-5), 3.77 (br t, *J* = 8.8 Hz, 1 H, H-16), 3.84 (dd, *J* = 11.4,

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11.4 Hz, 1 H, H-19), 4.19 (m, 1 H, H-49), 4.20 (m, 1 H, OH), 4.65 (br d, J = 7.7 Hz, 1 H, H-2), 4.67 (br d, J = 8.8 Hz, 1 H, H-13), 4.92 (m, 1 H, H-42), 5.07 (m, 1 H, H-53), 5.16 (d, J = 11.0 Hz, 1 H, H-7), 5.30 (d, J = 10.6 Hz, 1 H, H-23), 5.44 (d, J = 10.6 Hz, 1 H, H-29), 5.90 (dd, J = 11.4, 4.7 Hz, 1 H, H-18), 6.95 (m, 1 H, H-39); ^{13}C NMR (150 MHz, C_6D_6) δ 11.0 (q, C-32), 11.8 (q, C-45), 13.7 (q, C-46), 14.9 (q, C-57), 15.1 (q, C-56), 15.2 (q, C-43), 15.7 (q, C-33), 17.5 (q, C-25), 18.8 (q, C-10), 18.9 (q, C-9), 19.8 (q, C-55), 20.2 (q, C-26), 21.9 (t, C-4), 23.5 (t, C-51), 24.1 (t, C-31), 24.2 (t, C-15), 27.2 (d, C-24), 27.7 (t, C-14), 27.8 (d, C-8), 29.6 (q, C-11), 29.8 (q, C-21), 30.0 (d, C-54), 30.2 (q, C-27), 30.6 (q, C-34), 31.7 (t, C-3), 32.1 (t, C-52), 32.7 (d, C-30), 33.5 (t, C-40), 35.5 (t, C-50), 38.5 (d, C-41), 46.0 (t, C-5), 47.2 (t, C-16), 48.4 (d, C-48), 48.5 (t, C-36), 55.0 (d, C-18), 57.4 (d, C-29), 58.2 (d, C-7), 58.3 (q, C-20), 58.5 (d, C-13), 58.8 (d, C-23), 60.3 (d, C-2), 68.7 (t, C-19), 73.9 (d, C-49), 74.0 (d, C-42), 78.5 (d, C-53), 138.3 (s, C-38), 144.7 (d, C-39), 166.8 (s, C-17), 168.0 (s, C-35), 169.1 (s, C-6), 169.6 (s, C-28), 170.5 (s, C-22), 170.9 (s, C-1), 171.1 (s, C-12), 175.6 (s, C-47), 193.8 (s, C-37); MS (FAB) m/z 1059 (MH^+); HRMS (FAB) calcd for $C_{56}H_{95}N_6O_{13}$ (MH^+) 1059.6956, found 1059.6940.

Degradation of Dolastatin G (1). A stream of ozone gas was passed through a solution of **1** (4.8 mg, 0.0045 mmol) in MeOH (0.5 mL) at −78 °C for 15 min. The solution was then flushed with nitrogen, and dimethyl sulfide (0.025 mL) was added. The mixture was warmed to room temperature and concentrated to give an oil (4.0 mg). To a stirred solution of the oil (4.0 mg) in Et_2O (0.75 mL) at 0 °C was added a 1.0 M solution of lithium aluminum hydride in Et_2O (0.045 mL, 0.045 mmol). The solution was stirred at room temperature for 1.5 h, and then a small amount of ice (*ca.* 1 g) and 0.2 M aqueous HCl (1 mL) were added. After being stirred at room temperature for 30 min, the mixture was extracted with EtOAc (8 × 2 mL). The combined extracts were washed with H_2O (1 mL) and saturated aqueous NaCl (1 mL), dried (Na_2SO_4), and concentrated. The residual oil was dissolved in DMF (0.3 mL), and the solution was cooled to 0 °C. To the solution were added imidazole (13.1 mg, 1.92 mmol) and *tert*-butyldiphenylsilyl chloride (0.01 mL, 0.385 mmol) with stirring. The reaction mixture was stirred at 0 °C for 20 min, and a small amount of ice (*ca.* 1 g) was added. The resulting mixture was stirred at room temperature for 15 min and extracted with EtOAc (5 × 3 mL). The combined extracts were washed with saturated aqueous NaCl (1 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (1 g, step gradient from 8:1 to 2:1 hexane/EtOAc) to give crude **3** (7.1 mg) and crude **4** (2.3 mg). Further purification of crude **3** by column chromatography on silica gel (0.5 g, 5:1 and then 3:1 hexane/Et_2O) to give pure **3** (1.3 mg, 82%) as a colorless oil and further purification of crude **4** by column chromatography on silica gel (0.5 g, 3:1 and then 2:1 hexane/EtOAc) to give pure **4** (1.7 mg, 86%) as a colorless oil.

To a stirred solution of **4** (1.6 mg, 0.0036 mmol) in THF (0.3 mL) at 0 °C was added a 1.0 M solution of tetrabutylammonium fluoride in THF (0.02 mL, 0.02 mmol). The solution was stirred at room temperature for 15 min and diluted with saturated aqueous NH_4Cl (1 mL). The mixture was extracted with EtOAc (5 × 2 mL). The combined extracts were washed with saturated aqueous NaCl (1 mL), dried (Na_2SO_4), and concentrated. The residual oil was dissolved in acetone (0.4 mL), and 2,2-dimethoxypropane (0.1 mL, 0.81 mmol) and 10-camphorsulfonic acid (2.1 mg, 0.0086 mmol) were added. The mixture was stirred at room temperature for 30 min, diluted with saturated aqueous $NaHCO_3$ (1 mL), and extracted with EtOAc (4 × 2 mL). The combined extracts were washed with saturated aqueous NaCl (1 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (1 g, step gradient from 5:1 to 1:1 hexane/EtOAc) to give **5** (0.8 mg, 90%) as a colorless oil. (2*R*,3*S*)-5-(*tert*-Butyldiphenylsiloxy)-3-methyl-2-pentanol (**3**): colorless oil; R_f = 0.47 (4:1 hexane/EtOAc); $[\alpha]^{30}_D$ −6 (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) 3400 (br), 1475, 1425, 1115, 1085, 900 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 6.8 Hz, 3 H), 1.05 (s, 9 H), 1.15 (d, J = 6.3 Hz, 3 H), 1.53 (m, 1 H), 1.63−1.73 (m, 2 H), 2.37 (br s, 1 H), 3.63 (m, 1 H), 3.67 (ddd, J = 10.3, 7.3, 4.9 Hz, 1 H), 3.76 (ddd, J = 10.3, 4.9, 4.9 Hz, 1 H), 7.38−7.45 (m, 6 H), 7.65−7.70 (m, 4 H); MS (FAB) m/z 357 (MH^+); HRMS (FAB) calcd for $C_{22}H_{33}O_2Si$ (MH^+) 357.2250, found 357.2263. (2*S*,3*R*,7*S*)-1-(*tert*-Butyldiphenylsiloxy)-2,8-dimethyl-3,7-nonanediol (**4**): colorless oil; R_f = 0.13 (4:1 hexane/EtOAc); $[\alpha]^{26}_D$ +8 (*c* 0.14, $CHCl_3$); IR ($CHCl_3$) 3470 (br), 1425, 1110, 1055 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81 (d, J = 7.3 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.92 (d, J = 6.9 Hz, 3 H), 1.05 (s, 9 H), 1.35−1.84 (m, 10 H), 3.40 (m, 1 H), 3.62 (dd, J = 10.2, 7.6 Hz, 1 H), 3.65 (m, 1 H), 3.76 (dd, J = 10.2, 4.0 Hz, 1 H), 7.36−7.48 (m, 6 H), 7.64−7.70 (m, 4 H); MS (FAB) m/z 443 (MH^+); HRMS (FAB) calcd for $C_{27}H_{43}O_3Si$ (MH^+) 443.2982, found 443.2998. (4*R*,5*S*)-4-[(*S*)-4-Hydroxy-5-methylhexyl]-2,2,5-trimethyl-1,3-dioxane (**5**): colorless oil; R_f = 0.35 (3:1 hexane/acetone); $[\alpha]^{26}_D$ +19 (*c* 0.07, $CHCl_3$); IR ($CHCl_3$) 3610, 3460 (br), 1460, 1390, 1370, 1200, 1060 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6) δ 0.41 (d, J = 6.3 Hz, 3 H), 0.84 (d, J = 6.9 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 1.32 (s, 3 H), 1.54 (s, 3 H), 1.24−1.66 (m, 8 H), 1.70−1.82 (m, 1 H), 3.18 (br ddd, J = 8.3, 4.3, 4.3 Hz, 1 H), 3.30 (dd, J = 11.5, 11.5 Hz, 1 H), 3.32 (m, 1 H), 3.59 (dd, J = 11.5, 4.9 Hz, 1 H); MS (FAB) m/z 245 (MH^+); HRMS (FAB) calcd for $C_{14}H_{29}O_3$ (MH^+) 245.2117, found 245.2104.

(2*R*,3*R*)-5-(*tert*-Butyldiphenylsiloxy)-3-methyl-2-pentanol (3a). To a stirred solution of (3*R*,4*R*)-3,4-dimethyl-4-butanolide (**6**)[4] (22.6 mg, 0.198 mmol) in THF (1.0 mL) at 0 °C was added a 1.0 M solution of lithium aluminum hydride in THF (0.4 mL, 0.4 mmol) dropwise. The solution was stirred at room temperature for 1.5 h, and then NaF (0.15 g) and a mixture of 9:1 THF/H_2O (2 mL) were added. After being stirred at room temperature for 30 min, the mixture was filtered through a pad of Celite. The residue was washed with THF (20 mL), and the filtrate and the washings were combined and concentrated. The residual oil was dissolved in DMF (1.0 mL), and the solution was cooled to 0 °C. To the stirred solution were added imidazole (42 mg, 0.617 mmol) and *tert*-butyldiphenylsilyl chloride (0.06 mL, 0.231 mmol). The reaction mixture was stirred at 0 °C for 30 min, and a small amount of ice (*ca.* 3 g) was added. The resulting mixture was stirred at room temperature for 15 min and extracted with EtOAc (8 × 3 mL). The combined extracts were washed with saturated aqueous NaCl (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (5 g, step gradient from 5:1 to 3:1 hexane/Et_2O) to give silyl ether **3a** (63.1 mg, 89%) as a colorless oil: R_f = 0.47 (4:1 hexane/EtOAc); $[\alpha]^{30}_D$ +8 (*c* 0.12, $CHCl_3$); IR ($CHCl_3$) 3400 (br), 1470, 1430, 1110, 995 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 6.8 Hz, 3 H), 1.05 (s, 9 H), 1.15 (d, J = 6.3 Hz, 3 H), 1.42 (m, 1 H), 1.67−1.78 (m, 2 H), 2.20 (d, J = 4.9 Hz, 1 H), 3.68 (ddd, J = 10.2, 7.3, 4.9 Hz, 1 H), 3.76 (ddd, J = 10.2, 5.4, 5.4 Hz, 1 H), 3.77 (m, 1 H), 7.36−7.46 (m, 6 H), 7.64−7.70 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (q), 19.1 (s), 19.8 (q), 26.8 (q, 3 C), 35.5 (t), 37.1 (d), 62.4 (t), 70.7 (d), 127.6 (d, 4 C), 129.6 (d, 2 C), 133.5 (s, 2 C), 135.5 (d, 4 C); MS (FAB) m/z 357 (MH^+); HRMS (FAB) calcd for $C_{22}H_{33}O_2Si$ (MH^+) 357.2250, found 357.2246.

(2*S*,3*R*)-5-(*tert*-Butyldiphenylsiloxy)-3-methyl-2-pentyl 4-Nitrobenzoate (7). To a stirred solution of silyl ether **3a** (28.5 mg, 0.08 mmol), triphenylphosphine (148 mg, 0.564 mmol), and *p*-nitrobenzoic acid (93 mg, 0.556 mmol) in benzene (2.0 mL) was added a 1.0 M solution of diethyl azodicarboxylate in benzene (0.56 mL, 0.56 mmol) at room temperature. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated. The residual oil was purified twice by column chromatography [(1) silica gel 5 g, step gradient from 7:1 to 1:1 hexane/benzene; (2) silica gel 5 g, step gradient from 20:1 to 10:1 hexane/Et_2O] to give *p*-nitrobenzoate **7** (34.5 mg, 76%) as a colorless oil: R_f = 0.49 (5:1 hexane/Et_2O); $[\alpha]^{25}_D$ +26.0 (*c* 1.11, $CHCl_3$); IR ($CHCl_3$) 1720, 1610, 1530, 1350, 1280, 1105 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.95 (d, J = 6.8 Hz, 3 H), 1.05 (s, 9 H), 1.30 (d, J = 6.3 Hz, 3 H), 1.38 (m, 1 H), 1.83 (m, 1 H), 2.04 (m, 1 H), 3.66−3.83 (m, 2 H), 5.10 (dq, J = 6.3, 6.3 Hz, 1 H), 7.32−7.47 (m, 6 H), 7.62−7.69 (m, 4 H), 8.16 (m, 2H), 8.26 (m, 2 H); MS (FAB) m/z 528 (MNa^+); HRMS (FAB) calcd for $C_{29}H_{35}NO_5SiNa$ (MNa^+) 528.2182, found 528.2174.

Synthesis of Silyl Ether 3b (= *ent*-3). To a stirred solution of *p*-nitrobenzoate **7** (33.2 mg, 0.065 mmol) in MeOH

(0.5 mL) at 0 °C was added a 0.5 M solution of NaOMe in MeOH (0.15 mL, 0.75 mmol). The mixture was stirred at 0 °C for 30 min and at room temperature for 4.5 h. After addition of ion-exchange resin (Amberlite IRC−50, H^+ form, 120 mg), the mixture was stirred at room temperature for 20 min and then poured on a pad of the same resin (160 mg). The resin was washed with MeOH (15 mL), and the filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on silica gel (2 g, step gradient from 4:1 to 2:1 hexane/Et_2O) to give **3b** (21.6 mg, 92%) as a colorless oil: $R_f = 0.47$ (4:1 hexane/EtOAc); $[\alpha]^{25}_D$ +6 (*c* 0.12, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8 (q), 19.1 (s), 20.1 (q), 26.8 (q, 3 C), 35.5 (t), 37.9 (d), 62.2 (t), 71.7 (d), 127.7 (d, 4 C), 129.7 (d, 2 C), 133.5 (s, 2 C), 135.6 (d, 4 C); MS (FAB) *m/z* 357 (MH^+); HRMS (FAB) calcd for $C_{22}H_{33}O_2Si$ (MH^+) 357.2250, found 357.2273. IR and ^1H NMR (400 MHz, $CDCl_3$) spectra were identical with those of the C39−C43 fragment **3**.

Ethyl (*S*)-5-(Benzyloxy)-6-methyl-2-heptenoate (9). To a stirred solution of diisopropyl [(ethoxycarbonyl)methyl]-phosphonate (1.8 mL, 7.6 mmol) in THF (3 mL, 2 × 1 mL rinse) at 0 °C was added potassium *tert*-butoxide (670 mg, 6.0 mmol), and the resulting solution was stirred at 0 °C for 1 h. The solution was cooled to −78 °C, and a solution of (*S*)-3-(benzyloxy)-4-methylpentanal (**8**)[6] (360 mg, 1.75 mmol) in THF (5 mL) was added. After being stirred at −78 °C for 1 h and at 0 °C for 1 h, the reaction mixture was diluted with Et_2O (20 mL), saturated aqueous NH_4Cl (20 mL), and H_2O (20 mL), successively. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer and the extracts were combined, washed with saturated aqueous NaCl (25 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (30 g, 12:1 hexane/Et_2O) to give conjugated ester **9** (355 mg, 94%) as a colorless oil: $R_f = 0.58$ (5:1 hexane/EtOAc); $[\alpha]^{26}_D$ −0.5 (*c* 0.49, $CHCl_3$); IR ($CHCl_3$) 1710, 1660, 1470, 1460, 1370 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 3 H), 0.95 (d, J = 6.9 Hz, 3 H), 1.29 (t, J = 6.9 Hz, 3 H), 1.88 (dqq, J = 5.9, 6.9, 6.9 Hz, 1 H), 2.42 (ddd, J = 7.3, 5.6, 1.6 Hz, 2 H), 3.27 (ddd, J = 5.9, 5.6, 5.6 Hz, 1 H), 4.18 (q, J = 6.9 Hz, 2 H), 4.49 (d, J = 11.5 Hz, 1 H), 4.55 (d, J = 11.5 Hz, 1 H), 5.88 (dt, J = 15.5, 1.6 Hz, 1 H), 7.01 (dt, J = 15.5, 7.3 Hz, 1 H), 7.25−7.35 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2 (q), 18.0 (q), 18.1 (q), 31.0 (d), 33.5 (t), 60.0 (t), 71.8 (t), 82.9 (d), 123.0 (d), 127.4 (d), 127.6 (d), 128.2 (d), 138.4 (s), 146.0 (d), 166.2 (s); MS (EI) *m/z* (relative intensity) 276 (M^+, 2), 163 (15), 91 (100); HRMS (EI) calcd for $C_{17}H_{24}O_3$ (M^+) 276.1725, found 276.1717.

(*S*)-5-(Benzyloxy)-6-methylheptanol (10). To a stirred solution of conjugated ester **9** (440 mg, 1.59 mmol) in MeOH (16 mL) at 0 °C were added nickel(II) chloride (34.7 mg, 0.27 mmol) and sodium borohydride (184 mg, 4.86 mmol). After being stirred at room temperature for 2 h, the mixture was diluted with CH_2Cl_2 (15 mL), 6 M aqueous HCl (1 mL), and H_2O (6 mL), successively. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer and the extracts were combined, washed with saturated aqueous $NaHCO_3$ (15 mL), H_2O (15 mL), and saturated aqueous NaCl (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was dissolved in THF (5.4 mL). To the solution cooled at −78 °C was added a 1.0 M solution of lithium aluminum hydride in THF (3.2 mL, 3.2 mmol). The mixture was stirred at −78 °C for 3.5 h and at room temperature for 30 min, and then NaF (0.65 g) and a 9:1 mixture of THF and H_2O (20 mL) were added. After being stirred at room temperature for 1 h, the mixture was filtered through a pad of Celite. The residue was washed with THF (40 mL), and the filtrate and the washings were combined and concentrated. The residual oil was purified twice by column chromatography [(1) silica gel 14 g, 2:1 hexane/Et_2O; (2) silica gel 14 g, 2.5:1 and then 2:1 hexane/Et_2O] to give alcohol **10** (362 mg, 97%) as a colorless oil: $R_f = 0.14$ (5:1 hexane/EtOAc); $[\alpha]^{26}_D$ −16.9 (*c* 0.91, $CHCl_3$); IR ($CHCl_3$) 3620, 3450 (br), 1600, 1500, 1460, 1390, 1370, 1350 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 7.2 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 1.34−1.60 (m, 7 H), 1.93 (dqq, J = 4.8, 7.2, 6.8 Hz, 1 H), 3.16 (m, 1 H), 3.62 (m, 2 H), 4.48 (d, J = 11.6 Hz, 1 H), 4.54 (d, J = 11.6 Hz, 1 H), 7.24−7.36 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.7 (q), 18.2 (q), 21.7 (t), 29.7 (t), 30.3 (d), 32.6 (t), 62.2 (t), 71.6 (t), 84.1 (d), 127.2 (d), 127.6 (d, 2 C), 128.1 (d, 2 C), 138.8 (s); MS (EI) *m/z* (relative intensity) 236 (M^+, 6), 193 (6), 128 (3), 91 (100); HRMS (EI) calcd for $C_{15}H_{24}O_2$ (M^+) 236.1776, found 236.1778.

(*S*)-5-(Benzyloxy)-6-methylheptanal (11). To a stirred solution of oxalyl chloride (0.08 mL, 0.92 mmol) in CH_2Cl_2 (2.0 mL) at −78 °C was added a 2.1 M solution of DMSO in CH_2Cl_2 (0.84 mL, 1.76 mmol) dropwise. The resulting solution was stirred at −78 °C for 7 min, and a solution of alcohol **10** (105 mg, 0.445 mmol) in CH_2Cl_2 (1.5 mL, 2 × 0.75 mL rinse) was added dropwise. The mixture was stirred at −78 °C for 10 min, and triethylamine (0.64 mL, 4.59 mmol) was added. The resulting mixture was stirred at −78 °C for 10 min and at 0 °C for 15 min. H_2O (8 mL) was added, and the mixture was extracted with 4:1 benzene/Et_2O (3 × 15 mL). The combined extracts were washed with saturated aqueous NaCl (8 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (3 g, 8:1 and then 6:1 hexane/Et_2O) to give aldehyde **11** (97 mg, 93%) as a colorless oil: $R_f = 0.49$ (5:1 hexane/EtOAc); $[\alpha]^{26}_D$ −20.4 (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) 2730, 1720, 1600, 1500, 1455, 1390, 1365 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 6.6 Hz, 3 H), 0.93 (d, J = 5.9 Hz, 3 H), 1.44−1.87 (m, 4 H), 1.95 (dqq, J = 5.3, 6.6, 5.9 Hz, 1 H), 2.41 (m, 2 H), 3.16 (ddd, J = 5.9, 5.9, 5.3 Hz, 1 H), 4.47 (d, J = 11.5 Hz, 1 H), 4.55 (d, J = 11.5 Hz, 1 H), 7.24−7.36 (m, 5 H), 9.74 (t, J = 1.7 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6 (q), 18.2 (t), 18.4 (q), 29.4 (t), 30.2 (d), 43.9 (t), 71.6 (t), 83.7 (d), 127.4 (d), 127.7 (d, 2 C), 128.2 (d, 2 C), 138.9 (s), 202.5 (d); MS (EI) *m/z* (relative intensity) 234 (M^+, 1), 191 (9), 128 (8), 91 (100); HRMS (EI) calcd for $C_{15}H_{22}O_2$ (M^+) 234.1620, found 234.1593.

(4*R*,5*S*)-3-[(2*R*,3*S*,7*S*)-7-(Benzyloxy)-2,8-dimethyl-3-hydroxynonanoyl]-4-methyl-5-phenyl-2-oxazolidinone (12). To a stirred solution of (4*R*,5*S*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone (195 mg, 0.836 mmol) in CH_2Cl_2 (1.7 mL) at 0 °C were added a 1.0 M solution of dibutylboron triflate in CH_2Cl_2 (0.92 mL, 0.92 mmol) and triethylamine (0.16 mL, 1.15 mmol), successively. The reaction mixture was stirred at 0 °C for 30 min and cooled to −78 °C. A solution of aldehyde **11** (96 mg, 0.41 mmol) in CH_2Cl_2 (1.0 mL, 2 × 0.75 mL rinse) was added, and the reaction mixture was stirred at −78 °C for 1.5 h and at 0 °C for 15 min. The mixture was diluted with 0.5 M phosphate buffer (pH 7) (1 mL). MeOH (9 mL) and 30% aqueous H_2O_2 (1 mL) were added, and the mixture was stirred at 0 °C for 1 h. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer and the extracts were combined, washed with 5% aqueous $NaHCO_3$ (15 mL) and saturated aqueous NaCl (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, 3:1 and then 2.5:1 hexane/Et_2O) to give aldol **12** (189 mg, 99%) as a colorless oil. The diastereomeric ratio of the product was shown to be 98:2 by ^{13}C NMR analysis. **12**: $R_f = 0.16$ (5:1 hexane/EtOAc); $[\alpha]^{25}_D$ +2.5 (*c* 1.06, $CHCl_3$); IR ($CHCl_3$) 3540 (br), 1780, 1690, 1605, 1500, 1460, 1380, 1365, 1345, 1240, 1200 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 1.23 (d, J = 6.8 Hz, 3 H), 1.38−1.60 (m, 6 H), 1.94 (dqq, J = 5.3, 6.8, 6.8 Hz, 1 H), 2.86 (br s, 1 H), 3.18 (m, 1 H), 3.76 (dq, J = 2.9, 6.8 Hz, 1 H), 3.96 (m, 1 H), 4.50 (d, J = 11.7 Hz, 1 H), 4.54 (d, J = 11.7 Hz, 1 H), 4.79 (dq, J = 7.3, 6.8 Hz, 1 H), 5.67 (d, J = 7.3 Hz, 1 H), 7.24−7.44 (m, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.3 (q), 14.2 (q), 17.8 (q), 18.3 (q), 22.3 (t), 30.0 (t), 30.4 (d), 34.1 (t), 42.2 (d), 54.6 (d), 71.4 (d), 71.7 (t), 78.7 (d), 84.1 (d), 125.5 (d, 2 C), 127.2 (d), 127.6 (d, 4 C), 128.1 (d, 2 C), 128.6 (d), 133.0 (s), 139.0 (s), 152.5 (s), 177.1 (s); MS (FAB) *m/z* 490 (MNa^+), 468 (MH^+); HRMS (FAB) calcd for $C_{28}H_{38}NO_5$ (MH^+) 468.2750, found 468.2781.

(4*R*,5*S*)-3-[(2*R*,3*S*,7*S*)-7-(Benzyloxy)-2,8-dimethyl-3-[(4-nitrobenzoyl)oxy]nonanoyl]-4-methyl-5-phenyl-2-oxazolidinone (13). To a stirred solution of aldol **12** (188 mg, 0.402 mmol), triphenylphosphine (737 mg, 2.8 mmol), and *p*-nitrobenzoic acid (468 mg, 2.8 mmol) in benzene (8.0 mL) was added diethyl azodicarboxylate (0.44 mL, 2.8 mmol) at room temperature. After being stirred at room temperature for 33

h, the reaction mixture was concentrated. The residual oil was purified twice by column chromatography [(1) silica gel 30 g, step gradient from 15:1 to 8:1 hexane/acetone; (2) silica gel FL60D (Fuji Silysia) 30 g, step gradient from 20:1 to 13:1 hexane/acetone] to give *p*-nitrobenzoate **13** (190 mg, 77%) as a colorless oil: $R_f = 0.30$ (4:1 hexane/acetone); $[\alpha]^{25}_D$ −24.7 (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) 1780, 1725, 1700, 1605, 1530, 1460, 1385, 1345, 1275 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (d, $J = 6.6$ Hz, 3 H), 0.88 (d, $J = 6.6$ Hz, 3 H), 0.91 (d, $J = 6.3$ Hz, 3 H), 1.28 (d, $J = 6.9$ Hz, 3 H), 1.36−1.98 (m, 7 H), 3.13 (m, 1 H), 4.28 (dq, $J = 8.3$, 6.9 Hz, 1 H), 4.38 (d, $J = 11.5$ Hz, 1 H), 4.49 (d, $J = 11.5$ Hz, 1 H), 4.65 (dq, $J = 7.3$, 6.6 Hz, 1 H), 5.42 (d, $J = 7.3$ Hz, 1 H), 5.53 (ddd, $J = 8.3$, 8.3, 3.3 Hz, 1 H), 7.20−7.44 (m, 10 H), 8.19 (br d, $J = 6.9$ Hz, 2 H), 8.26 (br d, $J = 6.9$ Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0 (q), 14.3 (q), 17.7 (q), 18.4 (q), 21.1 (t), 29.9 (t), 30.4 (d), 31.7 (t), 41.4 (d), 54.9 (d), 71.7 (t), 76.2 (d), 78.8 (d), 83.9 (d), 123.5 (d, 2 C), 125.5 (d, 2 C), 127.3 (d), 127.5 (d, 2 C), 128.2 (d, 2 C), 128.7 (d, 2 C), 128.8 (d), 130.7 (d, 2 C), 132.9 (s), 135.5 (s), 138.9 (s), 150.5 (s), 152.6 (s), 163.7 (s), 173.8 (s); MS (FAB) *m/z* 617 (MH^+); HRMS (FAB) calcd for $C_{35}H_{41}N_2O_8$ (MH^+) 617.2863, found 617.2887.

(4*R*,5*S*)-4-[(*S*)-4-(Benzyloxy)-5-methylhexyl]-2,2,5-trimethyl-1,3-dioxane (14). To a stirred solution of *p*-nitrobenzoate **13** (9.6 mg, 0.015 mmol) in 4:1 THF/H_2O (0.5 mL) at 0 °C were added 30% aqueous H_2O_2 (0.03 mL, 0.26 mmol) and 2.5 M aqueous LiOH (0.035 mL, 0.09 mmol). After the reaction mixture was stirred at 0 °C for 2.5 h and at room temperature for 15 h, 1.5 M aqueous Na_2SO_3 (0.2 mL) was added. The resulting mixture was acidified (*ca.* pH 1) with 6 M aqueous HCl and extracted with CH_2Cl_2 (5 × 6 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a crude carboxylic acid (8.8 mg). To a stirred solution of the crude carboxylic acid (8.8 mg) in THF (0.4 mL) at 0 °C was added a 1.0 M solution of lithium aluminum hydride in THF (0.12 mL, 0.12 mmol) dropwise. The solution was stirred at room temperature for 1.3 h, and then NaF (25 mg) and a mixture of 9:1 THF/H_2O (1.5 mL) were added. After being stirred at room temperature for 30 min, the mixture was filtered through a pad of Celite. The residue was washed with THF (10 mL), and the filtrate and the washings were combined and concentrated. The residual oil was dissolved in acetone (1.0 mL), and then 2,2-dimethoxypropane (0.5 mL, 4.0 mmol) and 10-camphorsulfonic acid (2.2 mg, 0.01 mmol) were added. The mixture was stirred at room temperature for 3 h, diluted with saturated aqueous $NaHCO_3$ (1.5 mL), and extracted with Et_2O (3 × 5 mL). The combined extracts were washed with saturated aqueous NaCl (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (1 g, 10:1 hexane/Et_2O) and preparative TLC (25:1 hexane/acetone) to give acetonide **14** (3.0 mg, 60%) as a colorless oil: $R_f = 0.49$ (4:1 hexane/acetone); $[\alpha]^{25}_D$ +13 (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) 1605, 1500, 1460, 1385, 1370, 1270, 910, 700 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.72 (d, $J = 6.6$ Hz, 3 H), 0.92 (d, $J = 6.9$ Hz, 3 H), 0.93 (d, $J = 6.6$ Hz, 3 H), 1.38 (s, 3 H), 1.42 (s, 3 H), 1.28−1.71 (m, 7 H), 1.91 (dqq, $J = 5.3$, 6.9, 6.6 Hz, 1 H), 3.15 (ddd, $J = 5.9$, 5.3, 5.3 Hz, 1 H), 3.43 (m, 1 H), 3.48 (dd, $J = 11.6$, 11.2 Hz, 1 H), 3.68 (dd, $J = 11.6$, 5.3 Hz, 1 H), 4.51 (s, 2 H), 7.22−7.38 (m, 5 H); MS (EI) *m/z* (relative intensity) 334 (M^+, 3), 319 (8), 291 (7), 276 (11), 91 (100); HRMS (EI) calcd for $C_{21}H_{34}O_3$ (M^+) 334.2508, found 334.2518.

Synthesis of Hydroxy Acetonide 5a (= 5). A mixture of acetonide **14** (5.5 mg, 0.0165 mmol) and 20% $Pd(OH)_2$ on carbon (2.0 mg) in dioxane (0.8 mL) was stirred under an atmosphere of hydrogen at 40 °C for 1.8 h. The mixture was filtered through a pad of Celite, and the residue was washed with EtOAc (10 mL). The filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on silica gel (1 g, 2.5:1 hexane/Et_2O) to give **5a** (3.8 mg, 94%) as a colorless oil: $R_f = 0.35$ (3:1 hexane/acetone); $[\alpha]^{26}_D$ +20 (*c* 0.10, $CHCl_3$); MS (FAB) *m/z* 245 (MH^+); HRMS (FAB) calcd for $C_{14}H_{29}O_3$ (MH^+) 245.2117, found 245.2098. IR and ^1H NMR (400 MHz, C_6D_6) spectra were identical with those of **5**.

(4*R*,5*S*)-4-[(*R*)-4-[(4-Nitrobenzoyl)oxy]-5-methylhexyl]-2,2,5-trimethyl-1,3-dioxane (15). To a stirred solution of **5a** (3.0 mg, 0.012 mmol), triphenylphosphine (24.3 mg, 0.092 mmol), and *p*-nitrobenzoic acid (15.1 mg, 0.090 mmol) in benzene (0.4 mL) was added a 0.64 M solution of diethyl azodicarboxylate in benzene (0.14 mL, 0.089 mmol) at room temperature. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated. The residual oil was purified by column chromatography on silica gel (2 g, 15:1 and then 5:1 hexane/Et_2O) and preparative TLC (30:1 benzene/acetone) to give *p*-nitrobenzoate **15** (2.8 mg, 58%) as a colorless oil: $R_f = 0.28$ (10:1 hexane/acetone); $[\alpha]^{25}_D$ +35 (*c* 0.15, $CHCl_3$); IR ($CHCl_3$) 1720, 1610, 1530, 1460, 1365, 1280, 1120, 1100 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.70 (d, $J = 6.6$ Hz, 3 H), 0.97 (d, $J = 6.6$ Hz, 3 H), 0.98 (d, $J = 6.6$ Hz, 3 H), 1.33 (s, 3 H), 1.39 (s, 3 H), 1.30−1.80 (m, 7 H), 2.00 (ddd, $J = 6.6$, 6.6, 5.0 Hz, 1 H), 3.41 (m, 1 H), 3.46 (dd, $J = 11.5$, 11.2 Hz, 1 H), 3.65 (dd, $J = 11.5$, 5.3 Hz, 1 H), 5.04 (ddd, $J = 7.6$, 5.0, 5.0 Hz, 1 H), 8.21 (br d, $J = 8.9$ Hz, 2 H), 8.29 (br d, $J = 8.9$ Hz, 2 H); MS (FAB) *m/z* 394 (MH^+); HRMS (FAB) calcd for $C_{21}H_{32}NO_6$ (MH^+) 394.2230, found 394.2224.

Synthesis of Hydroxy Acetonide 5b. To a stirred solution of *p*-nitrobenzoate **15** (2.5 mg, 0.006 mmol) in MeOH (0.8 mL) at 0 °C was added a 1.0 M solution of NaOMe in MeOH (0.2 mL, 0.2 mmol). The mixture was stirred at room temperature for 4.5 h. After addition of ion-exchange resin (Amberlite IRC-50, H^+ form, 500 mg), the mixture was stirred at room temperature for 1 min and then poured on a pad of the same resin (1 g). The resin was washed with MeOH (15 mL), and the filtrate and the washings were combined and concentrated. The residual oil was purified twice by column chromatography [(1) silica gel 1 g, 8:1 and then 1.5:1 hexane/Et_2O; (2) silica gel 1 g, 1.5:1 hexane/Et_2O] to give **5b** (1.4 mg, 90%) as a colorless oil: $R_f = 0.35$ (3:1 hexane/acetone); $[\alpha]^{25}_D$ +53 (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) 3610, 3460 (br), 1460, 1390, 1370, 1200, 1060 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.41 (d, $J = 6.8$ Hz, 3 H), 0.84 (d, $J = 6.8$ Hz, 3 H), 0.87 (d, $J = 6.8$ Hz, 3 H), 1.00 (br s, 1 H), 1.32 (s, 3 H), 1.55 (s, 3 H), 1.26−1.66 (m, 8 H), 3.17 (br ddd, $J = 7.3$, 4.9, 4.9 Hz, 1 H), 3.29 (m, 1 H), 3.29 (dd, $J = 11.7$, 11.2 Hz, 1 H), 3.59 (dd, $J = 11.7$, 5.3 Hz, 1 H); MS (FAB) *m/z* 245 (MH^+); HRMS (FAB) calcd for $C_{14}H_{29}O_3$ (MH^+) 245.2117, found 245.2105.

Conversion of Dolastatin G (1) into Nordolastatin G (2). To a stirred solution of **1** (9.1 mg, 0.008 mmol) in dioxane (0.6 mL) at room temperature was added 2 M aqueous HCl (0.2 mL). The mixture was stirred at room temperature for 50 min and then poured into saturated aqueous $NaHCO_3$ (2 mL) at 0 °C. The resulting mixture was extracted with CH_2Cl_2 (3 × 3 mL). The combined extracts were washed with saturated aqueous NaCl (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (1 g, 4:1 and then 3:1 benzene/acetone) to give **2** (8.7 mg, 98%) as a colorless amorphous powder: $R_f = 0.23$ (5:1 $CHCl_3$/acetone); $[\alpha]^{27}_D$ −176 (*c* 0.09, $CHCl_3$); HRMS (FAB) calcd for $C_{56}H_{95}N_6O_{13}$ (MH^+) 1059.6956, found 1059.6960. The IR and ^1H NMR (600 MHz, C_6D_6) spectra were identical with those of natural **2**.

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Supporting Information Available: 1D and 2D NMR spectra and HPLC traces of **1** and **2**; table of ^1H and ^{13}C NMR assignments for **2**; ^1H NMR spectra of **3**, **3a**,**b**, **4**, **5**, **5a**,**b**, **7**, and **9**−**15** (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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