Dolastatin H and Isodolastatin H, Potent Cytotoxic Peptides from the Sea Hare *Dolabella auricularia*: Isolation, Stereostructures, and Synthesis

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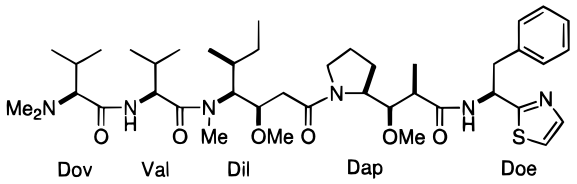
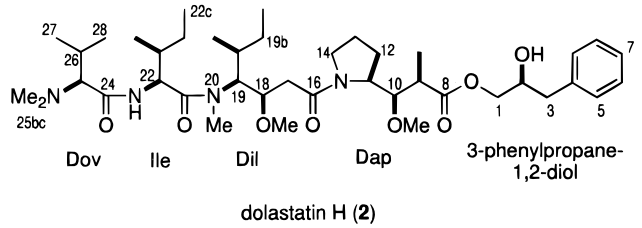
Abstract: A bioassay-directed investigation of the cytotoxic constituents of the Japanese sea hare *Dolabella auricularia* resulted in the isolation of two cytotoxic compounds designated dolastatin H (**2**) and isodolastatin H (**3**) from the wet animals at the yields of 9×10^{-7}%. On the basis of spectroscopic analysis, these compounds were shown to be new peptides that were closely related to dolastatin 10 (**1**) isolated from Western Indian Ocean specimens of this animal. The notable structural feature of these new compounds, **2** and **3**, is that 3-phenylpropane-1,2-diol is attached through the ester linkage to the C-terminus of a tetrapeptide containing unusual amino acids. The absolute stereostructures of **2** and **3** were unambiguously determined by enantioselective total synthesis. A cytotoxicity test for synthetic **2**, **3**, and their C-2 epimers revealed that the stereochemistry of the 3-phenylpropane-1,2-diol moiety on the C-terminus plays an important role in their cytotoxicity. *In vivo* antitumor activity against murine P388 leukemia was evaluated, and it was shown that isodolastatin H (**3**) exhibited antitumor activity a little weaker than that of dolastatin 10 (**1**).

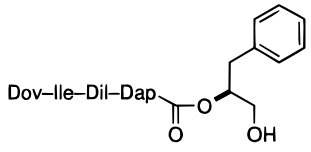
The Western Indian Ocean sea hare *Dolabella auricularia* is known to produce remarkably potent antitumor agents with novel structures that have been referred to as dolastatins.[1] Among these, dolastatin 10 (**1**) is a unique linear peptide containing unusual amino acids and has been shown to possess unprecedented potent antitumor activity.[2] Due to its chemical, biological, and clinical significance, extensive studies on the total synthesis of **1**[3] and the structure−activity relationships of synthetic analogues[3d,4] have been carried out. We recently found that the Japanese specimens of this animal contained new cytotoxic peptides and depsipeptides[5] and other unique metabolites,[6] which have not been isolated from Western Indian Ocean specimens. Further investigation of the cytotoxic constituents of the Japanese sea hare *D. auricularia* has resulted in the isolation of two potent cytotoxic peptides, dolastatin H (**2**)[7] and isodolastatin H (**3**),[7] as very minor constituents. We report here the isolation of these new compounds and their absolute stereostructures, which were determined using the enantioselective synthesis of **2** and **3** and their stereoisomers. Results on the biological evaluation of these synthetic compounds are also described.



dolastatin 10 (**1**)



dolastatin H (**2**)



isodolastatin H (**3**)

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.

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Results and Discussion

Isolation. The MeOH extract of the internal organs of the sea hare *D. auricularia*, collected in Mie Prefecture, Japan, was partitioned between EtOAc and H_2O. The EtOAc-soluble material, which exhibited cytotoxicity against HeLa-S_3 cells with an IC_{50} of 1.2 μg/mL, was further partitioned between 9:1 MeOH/H_2O and hexane. Bioassay-guided separation of the material obtained from the aqueous MeOH portion was performed by repeated chromatography on silica gel [(1) benzene/EtOAc, then EtOAc/MeOH, step gradient; (2) 80:16:4 to 0:80:20 benzene/EtOAc/MeOH, linear gradient] and ODS silica gel [(1) 4:1 MeOH/H_2O, then MeOH; (2) 7:3 MeOH/H_2O to MeOH, linear gradient] to give a cytotoxic fraction (IC_{50} = 0.036 μg/mL). The fraction was further separated by reversed-phase HPLC (5:5 to 6:4 MeCN/H_2O, linear gradient) and subsequently by silica gel TLC (20:7:3 $CHCl_3$/acetone/MeOH) to give a potent cytotoxic fraction (IC_{50} = 0.0038 μg/mL), which contained dolastatin H (**2**) and isodolastatin H (**3**) in a ratio of 1:1. This cytotoxic fraction was then purified by silica gel TLC (12:1 $CHCl_3$/MeOH) and by reversed-phase HPLC (7:3 MeOH/0.01 M NH_4OAc) to afford pure **2** (9×10^{-7}% yield based on wet weight) and **3** (9×10^{-7}%) as a colorless powder. The cytotoxicities of **2** and **3**, while not tested with natural **2** and **3** because of the inadequate amounts of natural samples (0.3 mg each), were demonstrated using synthetic samples of these compounds as described below.

Gross Structures. Dolastatin H (**2**) has a molecular formula of $C_{41}H_{70}N_4O_8$, which was determined by high-resolution FABMS [*m*/*z* 747.5294 (MH^+), Δ + 2.2 mmu] and high-field ^{1}H NMR data (Table 1). The IR spectrum showed bands at 3425, 1730, 1660, 1635, and 1260 cm^{-1} that were assigned to hydroxyl, ester, and amide groups. The ^{1}H NMR data showed the presence of an amide NH group (δ 6.72) and an *N*-methylamide group (δ 2.73), suggesting the peptidic nature of **2**. Resonances in the ^{1}H NMR spectrum were assigned by DQF-COSY, HMBC, and decoupling experiments, as shown in Table 1.[8] Although the ^{13}C NMR spectrum could not be obtained due to the scarcity of the sample, carbon chemical shifts were partially determined by HMBC experiments (J_{CH} = 6 and 8 Hz). These spectroscopic data suggested the presence of five components in **2**:*N*,*N*-dimethylvaline (dolavaline, Dov), isoleucine (Ile), dolaisoleuine (Dil), dolaproine (Dap), and 3-phenylpropane-1,2-diol (Figure 1). Three of them, Dov, Dil, and Dap, are the unusual amino acid units of dolastatin 10 (**1**).[2] It was difficult to prove the presence of the Dil unit, since most proton signals from this unit were extremely broad and no cross-peaks were observed in the COSY spectrum regarding the connectivities between H-19 and H-19a and between H-19a and H-19b. However, the HMBC correlations shown in Figure 1 clarified these connectivities. The HMBC correlation between the oxymethylene protons (H-1) of 3-phenylpropane-1,2-diol and the carbonyl carbon (C-8) of Dap indicates connectivity between these two components, as shown in Figure 1. Although the presence of three carbonyl groups (C-8, C-21, and C-24) was disclosed by HMBC experiments, the molecular formula of **2** suggested the presence of an additional carbonyl group, which must correspond to the C-16 carbonyl group of the Dil portion. Further evidence for the connectivity of these partial structures could not be obtained from either HMBC experiments or NOESY data due to the scarcity of the sample. However, considering that the Dov and Dap units are the N- and C-termini of **2**, respectively, the carbonyl carbon (C-24) of the Dov unit must be bonded to the amino nitrogen (N-23) of Ile and the methine carbon (C-22) of Ile should be connected to the carbonyl carbon (C-21) of the Dil unit. Thus, the gross structure of dolastatin H is unequivocally shown as **2**.

The gross structure of isodolastatin H (**3**) was elucidated by comparison with the spectral data of **2**. The molecular formula of **3** was identical with that of **2**. The ^{1}H NMR data (Table 1) were similar to those for **2** except for the chemical shifts of the oxymethylene (H-1) and oxymethine (H-2) protons: the signals due to H-1 were observed at δ 3.76 and 4.60 and the signal of H-2 was at δ 4.35 in **2**, whereas the signals arising from H-1 were observed at δ 3.80 and 3.90 and the signal of H-2 was at δ 5.54 in **3**. These data suggest that the Dap unit in **3** is esterified by the secondary hydroxyl group of 3-phenylpropane-1,2-diol. Thus, isodolastatin H (**3**) was shown to be a structural isomer of **2** with regard to the 3-phenylpropane-1,2-diol moiety.

Table 1. ^{1}H NMR Data for Dolastatin H (**2**) and Isodolastatin H (**3**) in C_6D_6[a]

position	**2**	**3**
1	3.76 dd (11.0, 1.9)	3.80 ddd (13.0, 7.0, 6.2)
	4.60 dd (11.0, 9.5)	3.90 ddd (13.0, 7.5, 2.4)
2	4.35 m	5.54 m
3	2.58 dd (13.5, 6.6)	2.77 dd (14.0, 6.2)
	2.84 dd (13.5, 6.6)	2.93 dd (14.0, 7.5)
5, 6, 7	7.05−7.20 m	7.07−7.15
9	2.57 dq (10.3, 7.0)	2.51 dq (10.6, 7.0)
9a	1.30 d (7.0)	1.22 d (7.0)
10	4.29 dd (10.3, 1.5)	4.27 dd (10.6, 1.1)
10a,b	3.27 s	3.27 s[b]
11	4.28 ddd (6.3, 4.2, 1.5)	4.20 m
12	1.57, 1.90 m	1.62, 1.92 m
13	1.21, 1.63 m	1.20, 1.63 m
14	2.84, 3.00 br	2.83, 3.01 br
17	1.91, 2.11 br	1.90, 2.10 br
18	4.12 br	4.13 br
18a,b	3.25 s	3.26 s[b]
19	4.99 br	4.99 br
19a	1.48 br	1.47 br
19b	1.06, 1.41 m	1.07, 1.40 m
19c	0.88 t (7.7)	0.87 t (7.3)
19d	0.93 d (7.0)	0.94 d (7.0)
20a	2.73 s	2.73 s
22	4.99 dd (8.8, 8.1)	4.98 dd (8.8, 7.6)
22a	1.86 m	1.86 m
22b	1.23, 1.71 m	1.22, 1.70 m
22c	0.86 t (7.7)	0.85 t (7.3)
22d	1.06 d (6.6)	1.05 d (6.6)
23	6.72 d (8.8)	6.68 d (8.8)
25	2.30 d (7.0)	2.29 d (7.0)
25b,c	2.19 s	2.19 s
26	2.02 m	2.01 m
27	0.96 d (7.0)	0.96 d (7.0)
28	1.11 d (7.0)	1.11 d (7.0)
OH	5.26 d (3.4)	5.21 dd (7.5, 7.0)

[a] Recorded at 600 MHz. Coupling constants (Hz) are in parentheses. [b] Interchangeable signals.

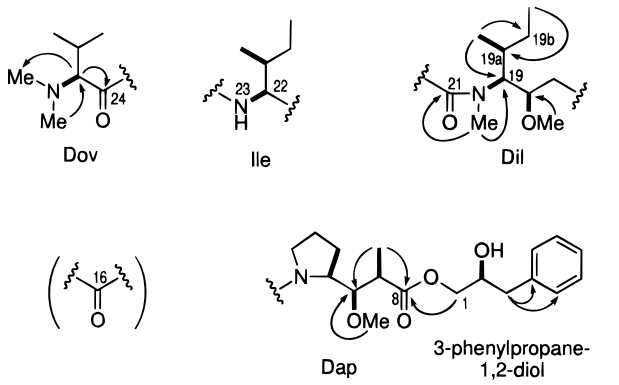
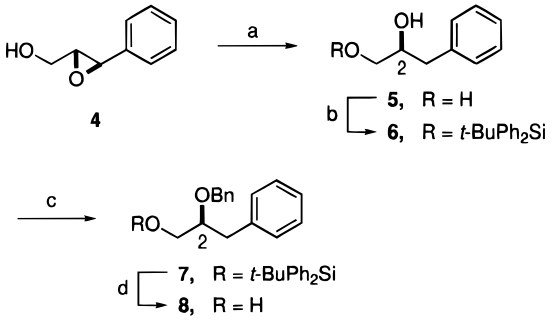


Figure 1. Partial structures of **2** derived from 2D NMR experiments. Important HMBC correlations are shown with arrows.

(8) The numbering adopted in this paper corresponds to that of dolastatin 10 (**1**) (ref 2).

Scheme 1[a]



[a] (a) DIBAL, CH_2Cl_2, hexane, 0 °C. (b) *t*-$BuPh_2SiCl$, imidazole, DMF, rt. (c) BnBr, $LiN(SiMe_3)_2$, DMF, THF, rt. (d) HF, MeCN, H_2O, 0 °C → rt.

Absolute Stereochemistry and Synthesis. The stereochemistries of dolastatin H (**2**) and isodolastatin H (**3**) were determined by the synthesis of the possible stereoisomers, assuming that the stereochemistries of the Dov, Dil, and Dap units were identical to those of dolastatin 10 (**1**) and that isoleucine has an L configuration. Since the absolute stereochemistry of the 3-phenylpropane-1,2-diol unit is unknown, our synthetic targets were (2*S*)-**2** and (2*S*)-**3** and their C2-epimers (2*R*)-**2** and (2*R*)-**3**.

The 3-phenylpropane-1,2-diol unit **8** was prepared from the optically pure epoxy alcohol **4**[9] (Scheme 1). Regioselective reduction[10] of **4** gave (*S*)-3-phenylpropane-1,2-diol (**5**),[11] which was converted to silyl ether **6**. Benzylation of **6** followed by deprotection of the silyl group of the resulting benzyl ether **7** gave the desired primary alcohol **8**. The enantiomer of **8** was also prepared from *ent*-**4** in the same manner. The Dap and Dil units, Boc-Dap (**9**) and Cbz-Dil-O-*t*-Bu (**11**), were synthesized using the methods of Shioiri[3d] and Koga,[3c] respectively.

The first target (2*S*)-**2** was synthesized as follows (Scheme 2). Condensation of primary alcohol **8** and Boc-Dap (**9**) under the Keck conditions[12] gave ester **10**. Deprotection of the Cbz group of Cbz-Dil-O-*t*-Bu (**11**) followed by coupling with Cbz-L-isoleucine using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)[13] gave dipeptide **12**, which was converted to tripeptide **13** by condensation with *N*,*N*-dimethylvaline (Dov)[14] using DEPC.[15] After deprotection, tripeptide **13** and ester **10** were coupled using DEPC to provide tetrapeptide **14**, debenzylation of which yielded (2*S*)-**2**. The epimer (2*R*)-**2** was also synthesized using *ent*-**8** by the same sequence of reactions employed for the synthesis of (2*S*)-**2**. Of the two synthetic stereoisomers, (2*S*)-**2** was found to be identical to natural **2** in all respects, including specific rotation [synthetic **2** $[\alpha]^{28}_D$ −48.0° (*c* 0.061, MeOH); natural **2**, $[\alpha]^{25}_D$ −56° (*c* 0.04, MeOH)], thus establishing the absolute stereostructure of **2**.

The synthesis of (2*S*)-**3** was performed by a sequence similar to that used for the synthesis of (2*S*)-**2** (Scheme 3). Thus, esterification of Boc-Dap (**9**) with silyl ether **6** gave ester **15**. Ester **15** and tripeptide **13** were deprotected, respectively, and condensed to provide tetrapeptide **16**. Removal of the silyl group in **16** gave (2*S*)-**3**. The epimer (2*R*)-**3** was also synthesized using *ent*-**6** in the same manner. Of the two stereoisomers, the spectral data for (2*S*)-**3**, $[\alpha]^{28}_D$ −47.6° (*c* 0.051, MeOH), were identical to those for natural **3**, $[\alpha]^{24}_D$ −47° (*c* 0.04, MeOH), thus establishing the absolute stereostructure of **3**.

Cytotoxicity of Dolastatin H (2), Isodolastatin H (3), and Their C2-Epimers. Since the cytotoxicity of natural **2** and **3** could not be tested, as described above, synthetic **2** and **3**, as well as their epimers C2-*epi*-**2** and C2-*epi*-**3**, were subjected to cytotoxicity testing using the HeLa-S_3 cell line. Both synthetic **2** and **3** showed potent cytotoxicity with IC_{50} values of 0.0022 and 0.0016 μg/mL, respectively, indicating that both natural **2** and **3** are the active principles of the cytotoxic fraction [IC_{50} = 0.0038 μg/mL (see the section on isolation)] prepared from the sea hare *D. auricularia*. It is noteworthy that the C2-epimers of **2** and **3** are much less cytotoxic (IC_{50} = 0.020 and 0.029 μg/mL, respectively) than **2** and **3** themselves. These findings suggest that the cytotoxicity of **2** and **3** is quite sensitive to the C-terminal structures of these peptides. It should be noted that the phenyl group of the C-terminus of **1** was reported to be essential for biological activity in a study of a structure−activity relationships of dolastatin 10 (**1**).[4b]

Evaluation of *in Vivo* Antitumor Activity for Dolastatin H (2) and Isodolastatin H (3). Dolastatin H (**2**) showed acute toxicity against mice at doses above 10 mg/kg by intravenous injection. On the basis of this toxicity test, *in vivo* antitumor activity of **2** and **3** was examined. While no significant activity was shown for dolastatin H (**2**), isodolastatin H (**3**) exhibited antitumor activity with a T/C of 141% at a dose of 6 mg/kg/day against P388 leukemia (intraperitoneal tumor inoculation−intravenous drug administration). This antitumor activity for **3** is a little weaker than that for dolastatin 10 (**1**) (T/C = 155% at 6.5 μg/kg/day against P388 leukemia)[1] and **3** requires a higher concentration than **1**.

Conclusion. New potent cytotoxic peptides, dolastatin H (**2**) and isodolastatin H (**3**), were isolated from Japanese specimens of the sea hare *D. auricularia*. Their structures were deduced and unambiguously established by total synthesis. The cytotoxicities of **2**, **3**, and their C-2 epimers were evaluated and depended largely on the C-terminal stereostructures of these peptides. The present investigation provides the first isolation from the natural source of potent cytotoxic peptides, **2** and **3**, which are closely related to dolastatin 10 (**1**), a well-known potent antitumor agent.[2]

Experimental Section

General. Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-181 polarimeter. UV and IR spectra were recorded on a JASCO UVIDEC-510 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. NMR spectra were recorded on a JEOL JNM EX270 (270 MHz for ^{1}H), a JEOL ALPHA400 (100 MHz for ^{13}C), or a JEOL ALPHA600 (600 MHz for ^{1}H). NMR chemical shifts were referenced to solvent peaks: δ_H 7.26 (residucal $CHCl_3$) for $CDCl_3$, δ_H 7.16 (residual C_6HD_5) and δ_C 128.0 for benzene-d_6. Mass spectra were determined on a JEOL JMS LG2000 spectrometer operating in the FAB mode (*m*-nitrobenzyl alcohol as a matrix). Elemental analyses were performed with a LECO CHN-900 elemental analyzer. Both TLC analysis and preparative TLC were conducted on 0.25 mm E. Merck precoated silica gel 60 F_{254}. Fuji Silysia silica gel BW-820 MH was used for column chromatography unless otherwise noted. Preparative HPLC and medium-pressure liquid chromatography (MPLC) were performed using a JASCO TRI ROTAR pump and JASCO 880 pumps, respectively. Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Organic solvents for anhydrous reactions were distilled from the following drying agents: triethylamine and diisopropylethylamine (CaH_2), DMF (CaH_2 under reduced pressure), and CH_2Cl_2

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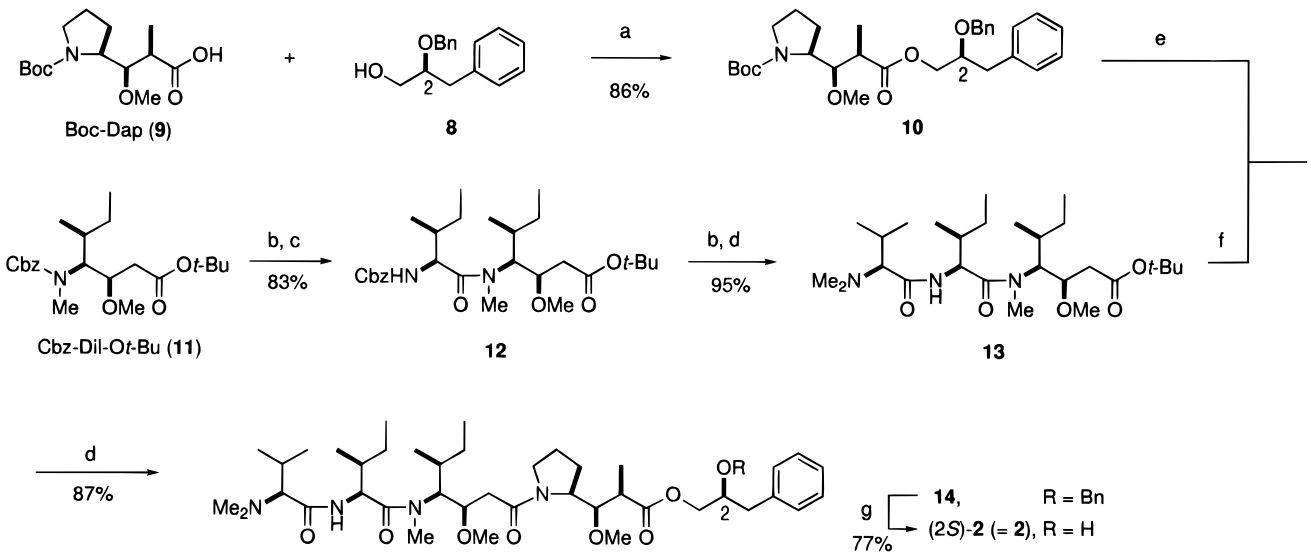
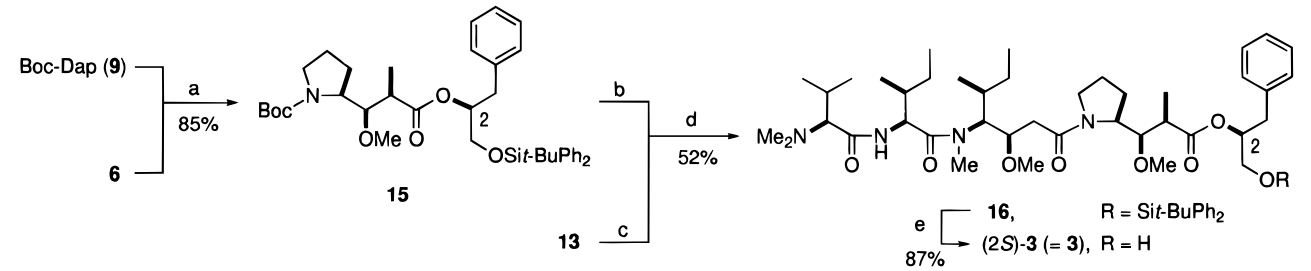
Scheme 2[a]



[a] (a) DCC, 4-(dimethylamino)pyridine (DMAP), 10-camphorsulfonic acid (CSA), CH_2Cl_2, 0 °C. (b) H_2, 10% Pd−C, MeOH, rt. (c) Cbz-Ile, PyBOP, *i*-Pr_2EtN, CH_2Cl_2, 0 °C. (d) DEPC, Et_3N, DMF, 0 °C. (e) CF_3CO_2H, CH_2Cl_2, 0 °C. (f) CF_3CO_2H, CH_2Cl_2, rt. (g) H_2, 10% Pd−C, MeOH, H_2O, AcOH, rt.

Scheme 3[a]



[a] (a) DCC, DMAP, CSA, CH_2Cl_2, 0 °C. (b) CF_3CO_2H, CH_2Cl_2, 0 °C. (c) CF_3CO_2H, CH_2Cl_2, 0 °C → rt. (d) DEPC, Et_3N, DMF, 0 °C. (e) HF, MeCN, H_2O, 0 °C.

(P_2O_5). All moisture-sensitive reactions were performed under an atmosphere of nitrogen. Organic extracts were dried over anhydrous Na_2SO_4.

Isolation of Dolastatin H (2) and Isodolastatin H (3). *D. auricularia* (33 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 April 1993. The specimens, which were stored at −20 °C for 4 months, were divided into the internal organs and thick outer skin, and the former (20 kg, wet wt) were extracted with MeOH (40 L). The methanolic extract was concentrated to ca. 2 L in vacuo and extracted with EtOAc (3 × 2 L). After concentration in vacuo, the EtOAc extract (91.4 g, IC_{50} against HeLa-S_3 cells = 1.2 μg/mL) was dissolved in 9:1 MeOH/H_2O (1 L), and the solution was washed with hexane (2 × 1 L). Evaporation of the aqueous MeOH portion gave a dark brown oil (30.8 g), which was chromatographed on silica gel (590 g), eluting with 1:1 benzene/EtOAc, EtOAc, 95:5, 9:1, and 8:2 EtOAc/MeOH, and MeOH, successively. The fraction (1.46 g, IC_{50} = 0.1 μg/mL) eluted with 9:1 EtOAc/MeOH was subjected to MPLC [Fuji Silysia Micro Bead Silica Gel 4B (65 g), linear gradient from 80:16:4 to 0:80:20 benzene/EtOAc/MeOH, flow rate 6.0 mL/min]. The fraction eluted between 28:58:14 and 18:66:16 benzene/EtOAc/MeOH (371 mg, IC_{50} = 0.044 μg/mL) was subjected to chromatographic filtration through a pad of ODS (Nacalai Tesque Cosmosil 75C_{18}-OPN, 7.2 g), eluting with 4:1 MeOH/H_2O and then MeOH. The fraction eluted with 4:1 MeOH/H_2O (300 mg) was subjected to MPLC [Nomura Chemical Develosil ODS 30/60 (70 g), linear gradient from 7:3 MeOH/H_2O to MeOH, flow rate 5.0 mL/min]. The fraction eluted between 92:8 MeOH/H_2O and MeOH (46 mg, IC_{50} = 0.036 μg/mL) was separated by preparative HPLC [Develosil ODS-10/20 (20 × 250 mm), linear gradient from 5:5 to 6:4 MeCN/H_2O, flow rate 5.0 mL/min, detection at 215 nm] to afford an oil (16 mg, t_R = 50−110 min, IC_{50} = 0.019 μg/mL). This oil was separated by preparative TLC (20:7:3 $CHCl_3$/acetone/MeOH) to give a potent cytotoxic fraction (4.0 mg, R_f = 0.53−0.87, IC_{50} = 0.0038 μg/mL) as an oil. The active fraction was further purified by preparative TLC (12:1 $CHCl_3$/MeOH, R_f = 0.52) followed by preparative HPLC [Develosil ODS-10 (20 × 250 mm), 7:3 MeCN/0.01 M NH_4OAc, flow rate 5.0 mL/min, detection at 215 nm] to give pure **2** (0.3 mg, t_R = 42 min) and **3** (0.3 mg, t_R = 46 min) as colorless powders, respectively.

Dolastatin H (2): R_f = 0.60 (10:1 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ −56° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 208 (ε 23 000) nm; IR ($CHCl_3$) 3425, 1730, 1660, 1635, 1495, 1455, 1260, 1095, 1060 cm^{-1}; ^1H NMR data, see Table 1; HRFABMS calcd for $C_{41}H_{71}N_4O_8$ *m/z* 747.5272 (MH^+), found 747.5294.

Isodolastatin H (3): R_f = 0.60 (10:1 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ −47° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 208 (ε 23 000) nm; IR ($CHCl_3$) 3425, 1725, 1660, 1635, 1495, 1455, 1260, 1095, 1060 cm^{-1}; ^1H NMR data, see Table 1; HRFABMS calcd for $C_{41}H_{71}N_4O_8$ *m/z* 747.5272 (MH^+), found 747.5309.

(*S*)-3-Phenylpropane-1,2-diol (5). To a solution of epoxy alcohol **4**[9] (492 mg, 3.28 mmol) in CH_2Cl_2 (7.0 mL) was added a solution of diisobutylaluminum hydride (DIBAL) in hexane (1.0 M, 9.85 mL) over 7 min with stirring at 0 °C. The solution was stirred at 0 °C for 30 min, the reaction was quenched by the addition of ethyl acetate (5.0 mL), and the solution was warmed to ambient temperature. The mixture was diluted with 1 M HCl (30 mL) and ether (30 mL) and stirred at ambient temperature for 30 min. The organic layer was separated, and the aqueous layer was extracted with ether (2 × 50 mL). The organic layer and the extracts were combined, washed with saturated aqueous NaCl (20 mL), dried, and concentrated. The residual oil was purified by column chromatography (1:1 and then 1:2 hexane/EtOAc) to give **5**[11] (307 mg, 62%) as crystals: colorless needles; mp 46−47 °C (hexane/CH_2Cl_2); R_f = 0.19 (1:1 hexane/EtOAc); $[\alpha]^{18}_D$ −35.4° (*c* 1.00, EtOH) [lit.[11a] $[\alpha]^{20}_D$ −36° (*c* 1, EtOH)].

***ent*-5.** Using the same procedure as described for the preparation of **5**, *ent*-**4** was converted to *ent*-**5**[11b]: mp 45−46 °C (hexane/CH_2Cl_2); $[\alpha]^{22}_D$ +29.9° (*c* 1.05, EtOH).

(*S*)-1-(*tert*-Butyldiphenylsiloxy)-2-hydroxy-3-phenylpropane (6). To a solution of alcohol **5** (184 mg, 1.22 mmol) and imidazole (182 mg, 2.68 mmol) in DMF (1.0 mL) was added *tert*-butyldiphenylsilyl chloride (0.35 mL, 1.3 mmol) with stirring at 0 °C. After being stirred at ambient temperature for 20 min, the solution was diluted with 1:1 benzene/EtOAc (50 mL), and the mixture was washed with 10% aqueous citric acid (5 mL), H_2O (5 mL), saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), and saturated aqueous NaCl (5 mL) successively, dried, and concentrated. The residual oil was purified by column chromatography (20:1 and then 10:1 hexane/EtOAc) to give silyl ether **6** (368 mg, 78%) as a colorless oil: $R_f = 0.28$ (10:1 hexane/EtOAc); $[\alpha]^{28}_D$ −0.30° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3580, 1600, 1585, 1495, 1470, 1425, 1115, 1080, 820 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.08 (s, 9 H), 2.43 (d, $J = 4.3$ Hz, 1 H), 2.77 (d, $J = 6.6$ Hz, 2 H), 3.57 (dd, $J = 10.0$, 6.4 Hz, 1 H), 3.67 (dd, $J = 10.0$, 4.0 Hz, 1 H), 3.94 (m, 1 H), 7.12−7.31 (m, 5 H), 7.32−7.48 (m, 6 H), 7.60−7.75 (m, 4 H); FABMS (addition of NaI) *m*/*z* (relative intensity) 413 (MNa^+, 22), 199 (46), 135 (100), 117 (53); HRFABMS calcd for $C_{25}H_{30}O_2SiNa$ *m*/*z* 413.1913 (MNa^+), found 413.1928.

***ent*-6.** Using the same procedure as described for the preparation of **6**, *ent*-**5** was converted to *ent*-**6**: $[\alpha]^{25}_D$ +0.25° (*c* 1.7, $CHCl_3$).

(*S*)-2-(Benzyloxy)-1-(*tert*-butyldiphenylsiloxy)-3-phenylpropane (7). To a solution of silyl ether **6** (1.88 g, 4.82 mmol) and benzyl bromide (2.90 mL, 24.4 mmol) in DMF (7.5 mL) was added a solution of $LiN(SiMe_3)_2$ in THF (1.0 M, 7.20 mL) with stirring at ambient temperature. After being stirred at ambient temperature for 1.5 h, the reaction mixture was cooled to 0 °C and diluted with saturated aqueous NH_4Cl (30 mL). The mixture was extracted with 1:1 hexane/benzene (200 mL, 2 × 100 mL). The combined organic extracts were washed with H_2O (30 mL) and saturated aqueous NaCl (30 mL), dried, and concentrated. The residual oil was purified by flash chromatography twice (Fuji Silysia silica gel FL60D, 90:1 hexane/ether) to give benzyl ether **7** (1.74 g, 75%) as a colorless oil: $R_f = 0.27$ (10:1 hexane/EtOAc); $[\alpha]^{30}_D$ −34.1° (*c* 1.19, $CHCl_3$); IR ($CHCl_3$) 1600, 1585, 1495, 1475, 1455, 1425, 1115, 1080, 820 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.09 (s, 9 H), 2.81 (m, 1 H), 2.98 (m, 1 H), 3.63−3.80 (m, 3 H), 4.36 (d, $J = 11.6$ Hz, 1 H), 4.52 (d, $J = 11.6$ Hz, 1 H), 7.10−7.45 (m, 16 H), 7.63−7.73 (m, 4 H); FABMS (addition of NaI) *m*/*z* (relative intensity) 503 (MNa^+, 54), 197 (57), 135 (100), 105 (45); HRFABMS calcd for $C_{32}H_{36}O_2SiNa$ *m*/*z* 503.2383 (MNa^+), found 503.2410.

***ent*-7.** Using the same procedure as described for the preparation of **7**, *ent*-**6** was converted to *ent*-**7**: $[\alpha]^{25}_D$ +34.8° (*c* 0.74, $CHCl_3$).

(*S*)-2-(Benzyloxy)-1-hydroxy-3-phenylpropane (8). To a solution of benzyl ether **7** (1.73 g, 3.60 mmol) in acetonitrile (60 mL) was added 47% hydrofluoric acid (30 mL, 810 mmol) with stirring at 0 °C. After being stirred at 0 °C for 30 min and at ambient temperature for 2 h, the reaction mixture was poured into a mixture of ice (1000 g) and saturated aqueous $NaHCO_3$ (1000 mL). The mixture was extracted with ether (800 mL, 2 × 400 mL), and the combined organic extracts were washed with saturated aqueous NaCl (200 mL), dried, and concentrated. The residual oil was purified by column chromatography (3:1 hexane/EtOAc) to give **8** (848 mg, 97%) as a colorless oil: $R_f =$ 0.40 (2:1 hexane/EtOAc); $[\alpha]^{29}_D$ −14.5° (*c* 1.77, $CHCl_3$); IR ($CHCl_3$) 3580, 3460 (br), 1600, 1585, 1495, 1455, 1395, 1350, 1100, 1085, 1070, 1040, 1030, 915 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 2.08 (br t, $J =$ 4.6 Hz, 1 H), 2.81 (dd, $J = 13.5$, 6.9 Hz, 1 H), 2.94 (dd, $J = 13.5$, 6.3 Hz, 1 H), 3.49 (m, 1 H), 3.58−3.76 (m, 2 H), 4.49 (d, $J = 11.6$ Hz, 1 H), 4.55 (d, $J = 11.6$ Hz, 1 H), 7.16−7.38 (m, 10 H); FABMS *m*/*z* (relative intensity) 265 (MNa^+, 8), 243 (MH^+, 16), 207 (22), 181 (18), 165 (12), 117 (100); HRFABMS (addition of NaI) calcd for $C_{16}H_{18}O_2$-Na *m*/*z* 265.1205 (MNa^+), found 265.1208.

***ent*-8.** Using the same procedure as described for the preparation of **8**, *ent*-**7** was converted to *ent*-**8**: $[\alpha]^{25}_D$ +13.5° (*c* 0.65, $CHCl_3$).

(*S*)-2-(Benzyloxy)-1-((*N*-(*tert*-butoxycarbonyl)dolaproyl)oxy)-3-phenylpropane (10). DCC (639 mg, 3.10 mmol) was added to a stirred solution of benzyl ether **8** (515 mg, 2.13 mmol), Boc-Dap (**9**)[3d] (732 mg, 2.55 mmol), 4-(dimethylamino)pyridine (DMAP) (62.8 mg, 0.514 mmol), and 10-camphorsulfonic acid (CSA) (60.2 mg, 0.259 mmol) in CH_2Cl_2 (15 mL) at 0 °C, and the mixture was stirred at 0 °C for 12 h. The mixture was filtered through a plug of cotton, and the solid was washed with 1:1 hexane/benzene (15 mL). The filtrate and the washing were combined and concentrated. The residual oil was purified by column chromatography (10:1 hexane/EtOAc) to give ester **10** (940 mg, 86%) as a colorless oil: $R_f = 0.68$ (2:1 hexane/EtOAc); $[\alpha]^{30}_D$ −40.0° (*c* 1.34, $CHCl_3$); IR ($CHCl_3$) 1730, 1680, 1495, 1475, 1455, 1400, 1365, 1245, 1160, 1095, 1025, 900, 865 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.27 (d, $J = 6.9$ Hz, 3 H), 1.47 (s, 9 H), 1.55−1.74 (m, 1 H), 1.74−2.05 (m, 3 H), 2.47−2.63 (m, 1 H), 2.79−2.99 (m, 2 H), 3.13−3.29 (m, 1 H), 3.32−3.62 (m, 1 H), 3.41 (s, 3 H), 3.65−4.18 (m, 5 H), 4.45 (d, $J = 11.6$ Hz, 1 H), 4.56 (br d, $J = 11.6$ Hz, 1 H), 7.16−7.33 (m, 10 H); FABMS *m*/*z* (relative intensity) 534 (MNa^+, 7), 512 (MH^+, 11), 456 (7), 412 (100), 380 (6), 316 (6), 170 (66), 138 (37), 114 (82); HRFABMS (addition of NaI) calcd for $C_{30}H_{41}NO_6Na$ *m*/*z* 534.2831 (MNa^+), found 534.2853.

C2-*epi*-10. Using the same procedure as described for the preparation of **10**, *ent*-**8** was coupled with **9** to give C2-*epi*-**10** in 75% yield: colorless oil; $R_f = 0.68$ (2:1 hexane/EtOAc); $[\alpha]^{30}_D$ −20.9° (*c* 0.86, $CHCl_3$); IR ($CHCl_3$) 1730, 1680, 1495, 1475, 1455, 1400, 1365, 1245, 1160, 1095, 1025, 900, 865 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.25 (d, $J = 6.3$ Hz, 3 H), 1.46 (s, 9 H), 1.56−2.04 (m, 4 H), 2.46−2.66 (m, 1 H), 2.76−2.98 (m, 2 H), 3.14−3.29 (m, 1 H), 3.40−3.62 (m, 1 H), 3.41 (s, 3 H), 3.65−4.36 (m, 5 H), 4.41−4.66 (m, 2 H), 7.16−7.34 (m, 10 H); FABMS *m*/*z* (relative intensity) 534 (MNa^+, 6), 512 (MH^+, 9), 456 (7), 412 (100), 170 (70), 138 (26), 114 (86); HRFABMS calcd for $C_{30}H_{41}NO_6Na$ *m*/*z* 534.2831 (MNa^+), found 534.2838.

Cbz-Ile-Dil-O-*t*-Bu (12). A mixture of Cbz-Dil-O-*t*-Bu (**11**)[3c] (518 mg, 1.32 mmol) and 10% Pd on carbon (134 mg) in MeOH (6.6 mL) was stirred at ambient temperature under 1 atm of H_2 gas for 70 min. The reaction mixture was filtered through a membrane filter (pore size 0.50 μm), and the catalyst was washed with MeOH (15 mL). The filtrate and the washing were combined and concentrated. The residue and Cbz-L-isoleucine (525 mg, 1.98 mmol) were dissolved in CH_2Cl_2 (5.0 mL), and the solution was cooled to 0 °C. To the solution were added (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)[13] (1.04 g, 2.01 mmol) and diisopropylethylamine (0.70 mL, 4.0 mmol) with stirring. The mixture was slowly warmed to ambient temperature over 11 h with stirring and further stirred for 6.5 h at ambient temperature. The mixture was diluted with 1:3 benzene/EtOAc (200 mL) and washed with 10% aqueous citric acid (20 mL), H_2O (20 mL), saturated aqueous $NaHCO_3$ (20 mL), H_2O (20 mL), and saturated aqueous NaCl (20 mL) successively, dried, and concentrated. The residual oil was purified repeatedly by column chromatography [(1) silica gel, 9:1 and then 8:1 hexane/EtOAc, (2) flash chromatography using silica gel FL60D, step gradient from 20:1 to 10:1 hexane/EtOAc, (3) Cosmosil 75C_{18}-OPN, 85:15 and then 9:1 MeOH/H_2O, (4) Cosmosil 75C_{18}-OPN, 4:1 MeOH/H_2O, (5) silica gel, 3:1 hexane/EtOAc] to give dipeptide **12** (550 mg, 83%) as a colorless oil: $R_f = 0.71$ (2:1 hexane/EtOAc); $[\alpha]^{30}_D$ −8.75° (*c* 1.30, $CHCl_3$); IR ($CHCl_3$) 3430, 1715, 1640, 1505, 1455, 1410, 1365, 1295, 1235, 1155, 1095, 1040, 1025, 840 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 5:1) δ 0.83 (t, $J = 7.3$ Hz, 3 H), 0.88 (t, $J = 7.4$ Hz, 3 H), 0.95−1.17 (m, 2 H), 0.96 (t, $J = 6.9$ Hz, 3 H), 0.98 (t, $J = 7.6$ Hz, 3 H), 1.26−1.82 (m, 4 H), 1.44 (s, 1.5 H), 1.45 (s, 7.5 Hz), 2.30 (dd, $J =$ 15.5, 8.9 Hz, 1 H), 2.41 (dd, $J = 15.8$, 3.0 Hz, 0.17 H), 2.44 (dd, $J =$ 15.5, 2.6 Hz, 0.83 H), 2.76 (s, 0.5 H), 2.97 (s, 2.5 H), 3.33 (s, 0.5 H), 3.34 (s, 2.5 H), 3.68 (dd, $J = 9.2$, 5.0 Hz, 0.17 H), 3.88 (m, 0.83 H), 3.98 (m, 0.17 H), 4.52 (dd, $J = 9.2$, 6.6 Hz, 0.83 H), 4.57−4.84 (m, 1 H), 5.09 (s, 2 H), 5.44 (d, $J = 9.2$ Hz, 0.83 H), 5.60 (d, $J = 9.2$ Hz, 0.17 H), 7.28−7.40 (m, 5 H); FABMS *m*/*z* (relative intensity) 529 (MNa^+, 2), 507 (MH^+, 22), 451 (41), 419 (26), 311 (11), 204 (28), 186 (15), 176 (21), 146 (12), 100 (100); HRFABMS (addition of NaI) calcd for $C_{28}H_{46}N_2O_6Na$ *m*/*z* 529.3254 (MNa^+), found 529.3275.

Dov-Ile-Dil-O-*t*-Bu (13). A mixture of dipeptide **12** (354 mg, 0.700 mmol) and 10% Pd on carbon (70 mg) in MeOH (3.5 mL) was stirred at ambient temperature under 1 atm of H_2 gas for 30 min. The reaction mixture was filtered through a membrane filter (pore size 0.50 μm), and the catalyst was washed with MeOH (8 mL). The filtrate and washings were combined and concentrated. The residue and *N*,*N*-dimethyl-L-valine[14] (125 mg, 0.866 mmol) were dissolved in DMF (2.0 mL), and the solution was cooled to 0 °C. To the solution were added DEPC (0.13 mL, 0.86 mmol) and triethylamine (0.12 mL, 0.86 mmol) with stirring. After being stirred at 0 °C for 2 h, the mixture was diluted with 1:2 benzene/EtOAc (100 mL) and extracted with 10% aqueous citric acid (10 mL). The aqueous extract was made basic (ca. pH 9) with $NaHCO_3$ and extracted with $CHCl_3$ (3 × 80 mL). The extracts were combined, washed with saturated aqueous NaCl (20 mL), dried,

and concentrated. The residual oil was chromatographed on ODS (Cosmosil 75C_{18}-OPN, 4:1 MeOH/H_2O) to give **13** (331 mg, 95%) as crystals: colorless prisms; mp 58−59 °C (pentane); R_f = 0.50 (2:1 benzene/acetone); $[\alpha]^{27}_D$ −43.4° (*c* 0.58, MeOH); IR ($CHCl_3$) 3430, 3370 (br), 1720, 1660, 1635, 1500, 1460, 1410, 1370, 1300, 1240, 1150, 1095, 1035, 840 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 15:1) δ 0.80 (t, *J* = 7.4 Hz, 3 H), 0.89 (t, *J* = 6.9 Hz, 3 H), 0.90 (d, *J* = 6.6 Hz, 3 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 0.95−1.42 (m, 3 H), 0.99 (d, *J* = 6.6 Hz, 3 H), 1.00 (d, *J* = 6.6 Hz, 3 H), 1.44 (s, 0.56 H), 1.46 (s, 8.44 H), 1.52−1.85 (m, 3 H), 2.07 (dqq, *J* = 6.0, 6.0, 6.0 Hz, 1 H), 2.20−2.37 (m, 1 H) [for major rotamer: 2.29 (dd, *J* = 15.5, 9.6 Hz, 0.94 H)], 2.24 (s, 5.63 H), 2.27 (s, 0.37 H), 2.38−2.51 (m, 1 H) [for major rotamer: 2.45 (dd, *J* = 15.5, 2.3 Hz, 0.94 H)], 2.42 (d, *J* = 6.6 Hz, 1 H), 2.75 (s, 0.19 H), 3.01 (s, 2.81 H), 3.34 (s, 2.81 H), 3.35 (s, 0.19 H), 3.68 (dd, *J* = 10.1, 5.0 Hz, 0.83 H), 3.88 (m, 0.94 H), 3.96 (m, 0.06 H), 4.75 (m, 0.94 H), 4.81 (dd, *J* = 9.2, 7.3 Hz, 0.94 H), 5.09 (dd, *J* = 9.2, 3.7 Hz, 0.06 H), 6.81 (d, *J* = 9.2 Hz, 0.94 H), 7.04 (d, *J* = 9.2 Hz, 0.06 H); FABMS *m*/*z* (relative intensity) 500 (MH^+, 12), 444 (3), 241 (2), 204 (3), 169 (2), 100 (100). Anal. Calcd for $C_{27}H_{53}N_3O_5$: C, 64.89; H, 10.69; N, 8.41. Found: C, 64.72; H, 10.67; N, 8.36.

2-*O*-Benzyldolastatin H (14). To a solution of tripeptide **13** (607 mg, 1.22 mmol) in CH_2Cl_2 (3.0 mL) was added CF_3CO_2H (3.0 mL) with stirring at 0 °C, and the solution was stirred at ambient temperature for 1.5 h to give a solution (solution A). To a solution of ester **10** (650 mg, 1.27 mmol) in CH_2Cl_2 (3.0 mL) was added CF_3CO_2H (3.0 mL) with stirring at 0 °C, and the solution was stirred at 0 °C for 40 min to give a solution (solution B). The solutions A and B were combined and concentrated to give an oil, which was dissolved in DMF (5.0 mL). To this solution were added DEPC (0.25 mL, 1.5 mmol) and triethylamine (0.95 mL, 6.8 mmol) with stirring at 0 °C. After being stirred at 0 °C for 2 h, the mixture was diluted with benzene (200 mL) and H_2O (50 mL), and the aqueous layer was made basic (ca. pH 11) with Na_2CO_3. The organic layer was separated, and the aqueous layer was extracted with benzene (2 × 100 mL). The organic layers were combined, washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried, and concentrated. The residual oil was purified by flash chromatography (silica gel FL60D, step gradient from 2:1 to 0:1 hexane/EtOAc) followed by column chromatography on alumina [E. Merck aluminum oxide 90 (Activity II-III), step gradient from 3:1 to 1:1 hexane/EtOAc] to give **14** (888 mg, 87%) as a colorless oil; R_f = 0.51 (9:1 $CHCl_3$/MeOH); $[\alpha]^{28}_D$ −49.5° (*c* 0.79, MeOH); IR ($CHCl_3$) 3430, 3370 (br), 1730, 1655, 1635, 1495, 1455, 1425, 1415, 1240, 1165, 1095 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 4:1) δ 0.81 (t, *J* = 7.3 Hz, 3 H), 0.86 (t, *J* = 7.3 Hz, 3 H), 0.90 (d, *J* = 6.9 Hz, 3 H), 0.90−1.23 (m, 2 H), 0.96 (d, *J* = 6.3 Hz, 3 H), 0.99 (d, *J* = 6.3 Hz, 3 H), 1.00 (d, *J* = 6.9 Hz, 3 H), 1.23−1.44 (m, 1 H), 1.29 (d, *J* = 6.9 Hz, 2.4 H), 1.30 (d, *J* = 6.9 Hz, 0.6 H), 1.50−1.88 (m, 5 H), 1.89−2.66 (m, 7 H), 2.24 (s, 4.8 H), 2.25 (s, 1.2 H), 2.90 (d, *J* = 6.6 Hz, 2 H), 3.02 (s, 2.4 H), 3.09 (s, 0.6 H), 3.26−3.52 (m, 2 H), 3.29 (s, 2.4 H), 3.32 (s, 0.6 H), 3.39 (s, 2.4 H), 3.40 (s, 0.6 H), 3.68−5.08 (m, 10 H), 6.84 (d, *J* = 9.2 Hz, 1 H), 7.15−7.34 (m, 10 H); FABMS *m*/*z* (relative intensity) 859 (MNa^+, 2), 837 (MH^+, 5), 805 (2), 241 (3), 170 (2), 117 (3), 100 (100); HRFABMS (addition of NaI) calcd for $C_{48}H_{76}N_4O_8Na$ *m*/*z* 859.5561 (MNa^+), found 859.5569.

C2-*epi*-14. Using the same procedure as described for the preparation of **14**, C2-*epi*-**10** was coupled with **13** to give C2-*epi*-**14** in 89% yield: colorless oil; R_f = 0.51 (9:1 $CHCl_3$/MeOH); $[\alpha]^{26}_D$ −39.8° (*c* 0.904, MeOH); IR ($CHCl_3$) 3430, 3370 (br), 1730, 1655, 1635, 1495, 1455, 1425, 1415, 1240, 1165, 1095 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 4:1) δ 0.76−1.22 (m, 20 H), 1.24−1.43 (m, 1 H), 1.27 (d, *J* = 6.9 Hz, 0.6 H), 1.28 (d, *J* = 6.9 Hz, 2.4 H), 1.50−1.85 (m, 5 H), 1.85−2.68 (m, 7 H), 2.24 (s, 4.8 H), 2.25 (s, 1.2 H), 2.68−2.97 (m, 2 H), 3.02 (s, 2.4 H), 3.08 (s, 0.6 H), 3.23−3.52 (m, 2 H), 3.29 (s, 2.4 H), 3.32 (s, 0.6 H), 3.39 (s, 2.4 H), 3.41 (s, 0.6 H), 3.60−5.09 (m, 10 H), 6.86 (d, *J* = 9.2 Hz, 1 H), 7.15−7.34 (m, 10 H); FABMS *m*/*z* (relative intensity) 837 (MH^+, 5), 100 (100); HRFABMS calcd for $C_{48}H_{76}N_4O_8Na$ *m*/*z* 859.5561 (MNa^+), found 859.5549.

(2*S*)-2 [= Dolastatin H (2)]. A mixture of tetrapeptide **14** (451 mg, 0.540 mmol) and 10% Pd on carbon (101 mg) in 12:5:3.5 MeOH/H_2O/AcOH (20.5 mL) was stirred at ambient temperature under 1 atm of H_2 gas for 1 h. The reaction mixture was filtered through a membrane filter (pore size 0.50 μm), and the catalyst was washed with MeOH (5 mL). The filtrate and washings were combined, and to the resulting solution was added 10% Pd on carbon (197 mg). The mixture was stirred for 75 min under the same conditions as described above. Since the hydrogenolysis reaction of **14** was slow, the catalyst was further added to the mixture three times at intervals [weight of the catalyst and time for stirring: (1) 109 mg for 140 min, (2) 152 mg for 17 h 40 min, (3) 120 mg for 3 h 45 min]. The reaction mixture was filtered through a membrane filter (pore size 0.50 μm), and the catalyst was washed with MeOH (20 mL). The combined filtrate and washings were concentrated, and the residual oil was chromatographed on alumina [aluminum oxide 90 (activity II-III), 2:1 and then 1:1 hexane/EtOAc] to provide (2*S*)-**2** (308 mg, 77%) as a colorless powder: $[\alpha]^{28}_D$ −48.0° (*c* 0.061, MeOH); ^{13}C NMR (100 MHz, C_6D_6) δ 11.0 (q, 2 C, C-19c and C-22c), 14.9 (q, C-9a), 15.6 (q, C-22d), 16.1 (q, C-19d), 18.4 (q, C-27), 20.3 (q, C-28), 24.3 (t, C-12), 25.0 (t, C-13), 25.0 (t, C-22b), 26.1 (t, C-19b), 27.9 (d, C-26), 32.0 (q, C-20a), 33.4 (d, C-19a), 37.7 (d, C-22a), 38.0 (t, C-17), 40.1 (t, C-3), 42.7 (q, 2 C, C-25bc), 45.5 (d, C-9), 47.9 (t, C-14), 53.1 (d, C-22), 57.0 (d, C-19), 57.7 (q, C-18ab), 60.3 (d, C-11), 61.1 (q, C-10ab), 69.5 (t, C-1), 70.2 (d, C-2), 76.2 (d, C-25), 78.8 (d, C-18), 81.6 (d, C-10), 126.4 (d, C-7), 128.5 (d, 2 C, C-6), 129.8 (d, 2 C, C-5), 138.9 (s, C-4), 170.7 (s, C-16), 171.1 (s, C-24), 173.4 (s, C-8), 174.0 (s, C-21). Anal. Calcd for $C_{41}H_{70}N_4O_8$: C, 65.92; H, 9.44; N, 7.50. Found: C, 65.80; H, 9.59; N, 7.45.

(2*R*)-2 [= 2-*epi*-Dolastatin H]. Using the same procedure as described for the preparation of (2*S*)-**2**, C2-*epi*-**14** was converted to (2*R*)-**2** in 73% yield: colorless powder; R_f = 0.35 (2:1 benzene/acetone); $[\alpha]^{26}_D$ −52.1° (*c* 0.065, MeOH); IR ($CHCl_3$) 3430 (br), 1730, 1665, 1635, 1495, 1455, 1410, 1245, 1095, 1060 cm^{-1}; ^1H NMR (600 MHz, C_6D_6) δ 0.86 (t, *J* = 7.5 Hz, 3 H, H-22c), 0.89 (t, *J* = 7.5 Hz, 3 H, H-19c), 0.97 (d, *J* = 6.8 Hz, 6 H, H-27 and H-19d), 1.04 (d, *J* = 6.6 Hz, 3 H, H-22d), 1.06 (m, 1 H, H-19b), 1.12 (d, *J* = 6.6 Hz, 3 H, H-28), 1.23 (m, 2 H, H-13 and H-19b), 1.34 (d, *J* = 7.0 Hz, 3 H, H-9a), 1.43 (m, 1 H, H-19b), 1.47 (m, 1 H, H-19a), 1.59 (m, 1 H, H-12), 1.66 (m, 1 H, H-13), 1.71 (m, 1 H, H-22b), 1.87 (m, 2 H, H-12 and H-22a), 1.91 (m, 1 H, H-17), 2.02 (dqq, *J* = 7.0, 6.8, 6.6 Hz, 1 H, H-26), 2.10 (br, 1 H, H-17), 2.20 (s, 6 H, H-25bc), 2.30 (d, *J* = 7.0 Hz, 1 H, H-25), 2.58 (dq, *J* = 10.6, 7.0 Hz, 1 H, H-9), 2.72 (s, 3 H, H-20a), 2.78 (dd, *J* = 13.7, 5.5 Hz, 1 H, H-3), 2.83 (br, 1 H, H-14), 3.01 (br, 1 H, H-14), 3.03 (dd, *J* = 13.7, 8.1 Hz, 1 H, H-3), 3.26 (s, 3 H, H-10ab), 3.29 (s, 3 H, H-18ab), 3.68 (dd, *J* = 11.0, 6.4 Hz, 1 H, H-1), 4.14 (m, 1 H, H-2), 4.14 (br, 1 H, H-18), 4.18 (m, 1 H, H-11), 4.27 (dd, *J* = 10.6, 1.1 Hz, 1 H, H-10), 4.82 (dd, *J* = 11.0, 2.2 Hz, 1 H, H-1), 4.98 (br, 1 H, H-22), 5.00 (br, 1 H, H-19), 5.21 (d, *J* = 6.2 Hz, 1 H, OH), 6.72 (d, *J* = 8.8 Hz, 1 H, NH), 7.11 (m, 1 H, H-7), 7.20 (m, 2 H, H-6), 7.31 (m, 2 H, H-5); ^{13}C NMR (100 MHz, C_6D_6) δ 11.0 (q, C-19c), 11.1 (q, C-22c), 14.7 (q, 9a), 15.6 (q, C-22d), 16.1 (q, C-19d), 18.3 (q, C-27), 20.4 (q, C-28), 24.3 (t, C-12), 24.9 (t, C-22b), 25.0 (t, C-13), 26.1 (t, C-19b), 27.9 (d, C-26), 32.0 (q, C-20a), 33.4 (d, C-19a), 37.7 (d, C-22a), 38.0 (t, C-17), 40.3 (t, C-3), 42.8 (q, 2 C, C-25bc), 45.6 (d, C-9), 47.9 (t, C-14), 53.1 (d, C-22), 57.1 (d, C-19), 57.9 (q, C-18ab), 60.2 (d, C-11), 61.1 (q, C-10ab), 69.1 (t, C-1), 70.8 (d, C-2), 76.3 (d, C-25), 78.8 (d, C-18), 81.7 (d, C-10), 126.4 (d, C-7), 128.5 (d, 2 C, C-6), 129.9 (d, 2 C, C-5), 139.3 (s, C-4), 170.3 (s, C-16), 171.1 (s, C-24), 173.4 (s, C-8), 173.9 (s, C-21); FABMS *m*/*z* (relative intensity) 769 (MNa^+, 3), 747 (MH^+, 5), 100 (100). Anal. Calcd for $C_{41}H_{70}N_4O_8$: C, 65.92; H, 9.44; N, 7.50. Found: C, 65.79; H, 9.70; N, 7.56.

(*S*)-2-((*N*-(*tert*-Butoxycarbonyl)dolaproyl)oxy)-1-(*tert*-butyldiphenylsiloxy)-3-phenylpropane (15). DCC (480 mg, 2.33 mmol) was added to a solution of silyl ether **6** (756 mg, 1.94 mmol), Boc-Dap (**9**)[3d] (672 mg, 2.34 mmol), DMAP (118 mg, 0.966 mmol), and 10-camphorsulfonic acid (135 mg, 0.581 mmol) in CH_2Cl_2 (20 mL) with stirring at 0 °C, and the mixture was stirred at 0 °C for 11 h. The mixture was filtered through a plug of cotton, and the solid was washed with 1:1 hexane/benzene (30 mL). The filtrate and washings were combined and concentrated. The residual oil was purified by column chromatography (step gradient from 15:1 to 8:1 hexane/EtOAc) to give ester **15** (1.09 g, 85%) as a colorless oil: R_f = 0.40 (5:1 hexane/EtOAc); $[\alpha]^{30}_D$ −41.9° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 1725, 1680, 1600, 1590, 1500, 1475, 1455, 1430, 1400, 1395, 1365, 1255, 1160, 1115, 820 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.07 (s, 9 H), 1.13−1.22 (m, 3 H), 1.47 (s, 9 H), 1.55−1.74 (m, 2 H), 1.74−1.97 (m, 2 H), 2.30−2.54 (m, 1 H), 2.82−2.99 (m, 1 H), 3.05 (dd, *J* = 13.9, 5.9 Hz, 1 H), 3.12−3.26

(m, 1 H), 3.30 (s, 3 H), 3.34−3.90 (m, 5 H), 5.16−5.32 (m, 1 H), 7.13−7.30 (m, 5 H), 7.31−7.47 (m, 6 H), 7.61−7.76 (m, 4 H); FABMS (addition of NaI) *m*/*z* (relative intensity) 682 (MNa^+, 16), 660 (MH^+, 4), 560 (49), 239 (15), 197 (37), 170 (36), 135 (100), 114 (40); HRFABMS calcd for $C_{39}H_{53}NO_6SiNa$ *m*/*z* 682.3539 (MNa^+), found 682.3519.

C2-*epi*-15. Using the same procedure as described for the preparation of **15**, *ent*-**6** was coupled with **9** to give C2-*epi*-**15** in 77% yield: colorless oil; R_f = 0.40 (5:1 hexane/EtOAc); $[\alpha]^{30}_D$ −8.71° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1725, 1680, 1600, 1590, 1500, 1475, 1455, 1430, 1400, 1395, 1365, 1255, 1160, 1115, 820 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.06 (s, 9 H), 1.07−1.15 (m, 3 H), 1.46 (s, 9 H), 1.53−1.96 (m, 4 H), 2.34−2.52 (m, 1 H), 2.85−3.05 (m, 2 H), 3.13−3.25 (m, 1 H), 3.34 (s, 3 H), 3.46−3.95 (m, 5 H), 5.14−5.25 (m, 1 H), 7.12−7.48 (m, 11 H), 7.55−7.74 (m, 4 H); FABMS *m*/*z* (relative intensity) 660 (MH^+, 11), 560 (93), 239 (17), 199 (39), 170 (65), 135 (100), 114 (70); HRFABMS calcd for $C_{39}H_{54}NO_6Si$ *m*/*z* 660.3720 (MH^+), found 660.3741.

1-*O*-(*tert*-Butyldiphenylsilyl)isodolastatin H (16). To a solution of tripeptide **13** (506 mg, 1.01 mmol) in CH_2Cl_2 (5.0 mL) was added CF_3CO_2H (5.0 mL) with stirring at 0 °C, and the solution was stirred at 0 °C for 1.5 h and at ambient temperature for 30 min to give a solution (solution A). To a solution of ester **15** (637 mg, 0.967 mmol) in CH_2Cl_2 (5.0 mL) was added CF_3CO_2H (5.0 mL) with stirring at 0 °C, and the solution was stirred at 0 °C for 70 min to give a solution (solution B). The solutions A and B were combined and concentrated to give an oil, which was dissolved in DMF (3.0 mL). To this solution were added triethylamine (0.40 mL, 2.9 mmol) and DEPC (0.17 mL, 1.0 mmol) with stirring at 0 °C. After being stirred at 0 °C for 10 h, triethylamine (0.40 mL, 2.9 mmol) was added and the mixture was stirred at 0 °C for 70 min. The mixture was diluted with $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (20 mL), and the aqueous layer was made basic (ca. pH 10) with Na_2CO_3. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 100 mL). The organic layers were combined, washed with saturated aqueous NaCl (20 mL), dried, and concentrated. The residual oil was purified by column chromatography [(1) silica gel, step gradient from 30:30:1 to 10:10:1 hexane/EtOAc/MeOH); (2) Sephadex LH-20, 1:1 CH_2Cl_2/MeOH, twice] to give **16** (491 mg, 52%) as a colorless oil; R_f = 0.65 (2:1 benzene/acetone); $[\alpha]^{27}_D$ −45.9° (*c* 0.32, MeOH); IR ($CHCl_3$) 3430, 3360 (br), 1725, 1655, 1635, 1500, 1455, 1415, 1260, 1165, 1095 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 3:1) δ 0.77−1.22 (m, 20 H), 1.06 (s, 6.8 H), 1.08 (s, 2.2 H), 1.19 (d, *J* = 6.9 Hz, 0.7 H), 1.20 (d, *J* = 6.9 Hz, 2.3 H), 1.27−1.47 (m, 1 H), 1.50−2.17 (m, 8 H), 2.20−2.54 (m, 4 H), 2.25 (s, 4.5 H), 2.27 (s, 1.5 H), 2.82−3.14 (m, 2 H), 3.02 (s, 2.3 H), 3.08 (s, 0.7 H), 3.19−3.50 (m, 2 H), 3.29 (s, 4.6 H), 3.32 (s, 0.7 H), 3.35 (s, 0.7 H), 3.63−4.30 (m, 5 H), 4.64−5.11 (m, 2 H), 5.13−5.34 (m, 1 H), 6.87 (d, *J* = 9.2 Hz, 1 H), 7.12−7.30 (m, 5 H), 7.31−7.49 (m, 6 H), 7.60−7.72 (m, 4 H); FABMS *m*/*z* (relative intensity) 985 (MH^+, 5), 669 (13), 431 (41), 401 (15), 343 (100), 327 (47), 311 (75), 242 (34), 163 (36), 133 (48), 100 (65); HRFABMS (addition of NaI) calcd for $C_{57}H_{88}N_4O_8SiNa$ *m*/*z* 1007.6270 (MNa^+), found 1007.6300.

C2-*epi*-16. Using the same procedure as described for the preparation of **16**, C2-*epi*-**15** was coupled with **13** to give C2-*epi*-**16** in 60% yield: colorless oil; R_f = 0.65 (2:1 benzene/acetone); $[\alpha]^{27}_D$ −29.9° (*c* 0.91, MeOH); IR ($CHCl_3$) 3430, 3360 (br), 1725, 1655, 1635, 1500, 1455, 1415, 1260, 1165, 1095 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) (rotamer ratio 3:1) δ 0.75−1.24 (m, 20 H), 1.05 (s, 6.8 H), 1.07 (s, 2.2 H), 1.19 (d, *J* = 6.9 Hz, 3 H), 1.26−1.44 (m, 1 H), 1.48−2.16 (m, 8 H), 2.20−2.63 (m, 4 H), 2.24 (s, 4.5 H), 2.26 (s, 1.5 H), 2.80−3.08 (m, 2 H), 3.02 (s, 2.3 H), 3.10 (s, 0.7 H), 3.24−3.48 (m, 2 H), 3.28 (s, 2.3 H), 3.31 (s, 2.3 H), 3.32 (s, 0.7 H), 3.33 (s, 0.7 H), 3.60−4.22 (m, 5 H), 4.62−4.96 (m, 2 H), 5.11−5.26 (m, 1 H), 6.74−6.92 (m, 1 H), 7.12−7.47 (m, 11 H), 7.56−7.70 (m, 4 H); FABMS *m*/*z* (relative intensity) 985 (MH^+, 8), 100 (100); HRFABMS calcd for $C_{57}H_{89}N_4O_8Si$ *m*/*z* 985.6450 (MH^+), found 985.6468.

(2*S*)-3 [= Isodolastatin H (3)]. To a solution of tetrapeptide **16** (417 mg, 0.424 mmol) in acetonitrile (4.2 mL) was added 47% hydrofluoric acid (7.5 mL, 240 mmol) with stirring at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was poured into a mixture of ice (25 g) and saturated aqueous $NaHCO_3$ (25 mL), and the resulting mixture was made basic (ca. pH 11) with Na_2CO_3 and extracted with $CHCl_3$ (100 mL, 2 × 50 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried, and concentrated. The residual oil was purified by column chromatography (10:10:1 hexane/EtOAc/MeOH) to provide (2*S*)-**3** (274 mg, 87%) as crystals: colorless prisms; mp 84−85 °C (hexane/CH_2Cl_2); $[\alpha]^{28}_D$ −47.6° (*c* 0.051, MeOH); ^{13}C NMR (100 MHz, C_6D_6) δ 10.9 (q, C-19c), 11.0 (q, C-22c), 14.8 (q, C-9a), 15.6 (q, C-22d), 16.1 (q, C-19d), 18.4 (q, C-27), 20.3 (q, C-28), 24.3 (t, C-12), 25.0 (t, C-13), 25.0 (t, C-22b), 26.1 (t, C-19b), 27.9 (d, C-26), 32.0 (q, C-20a), 33.2 (d, C-19a), 37.6 (d, C-22a), 37.1 (t, C-3), 37.9 (t, C-17), 42.7 (q, 2 C, C-25bc), 46.2 (d, C-9), 48.0 (t, C-14), 53.1 (d, C-22), 56.9 (d, C-19), 57.8 (q, C-18ab), 60.2 (d, C-11), 61.2 (q, C-10ab), 63.4 (t, C-1), 76.1 (d, C-25), 76.4 (d, C-2), 79.0 (d, C-18), 81.8 (d, C-10), 126.6 (d, C-7), 128.5 (d, 2 C, C-6), 129.8 (d, 2 C, C-5), 137.7 (s, C-4), 170.7 (s, C-16), 171.1 (s, C-24), 172.9 (s, C-8), 174.0 (s, C-21). Anal. Calcd for $C_{41}H_{70}N_4O_8$: C, 65.92; H, 9.44; N, 7.50. Found: C, 65.71; H, 9.43; N, 7.45.

(2*R*)-3 [= 2-*epi*-Isodolastatin H]. Using the same procedure as described for the preparation of (2*S*)-**3**, C2-*epi*-**16** was converted to (2*R*)-**3** in 70% yield: colorless powder; R_f = 0.55 (3:3:1 hexane/EtOAc/MeOH); $[\alpha]^{26}_D$ −53.8° (*c* 0.052, MeOH); IR ($CHCl_3$) 3430 (br), 1725, 1665, 1635, 1495, 1455, 1410, 1095 cm^{-1}; ^1H NMR (600 MHz, C_6D_6) δ 0.88 (t, *J* = 7.3 Hz, 6 H, H-19c and H-22c), 0.97 (d, *J* = 7.0 Hz, 3 H, H-27), 0.98 (d, *J* = 6.6 Hz, 3 H, H-19d), 1.07 (m, 1 H, H-19b), 1.08 (d, *J* = 7.0 Hz, 3 H, H-22d), 1.12 (d, *J* = 6.6 Hz, 3 H, H-28), 1.25 (m, 1 H, H-22b), 1.30 (d, *J* = 7.0 Hz, 3 H, H-9a), 1.37 (m, 1 H, H-13), 1.43 (m, 1 H, H-19b), 1.57 (br, 1 H, H-19a), 1.60 (m, 1 H, H-12), 1.74 (m, 1 H, H-22b), 1.77 (m, 1 H, H-13), 1.89 (m, 1 H, H-22a), 1.91 (m, 1 H, H-12), 2.04 (dqq, *J* = 7.0, 7.0, 7.0 Hz, 1 H, H-26), 2.21 (s, 6 H, H-25bc), 2.22 (br, 2 H, H-17), 2.33 (d, *J* = 7.0 Hz, 1 H, H-25), 2.56 (dq, *J* = 8.6, 7.0 Hz, 1 H, H-9), 2.78 (s, 3 H, H-20a), 2.92 (br, 1 H, H-14), 3.01 (dd, *J* = 13.5, 7.7 Hz, 1 H, H-3), 3.07 (dd, *J* = 13.5, 6.6 Hz, 1 H, H-3), 3.10 (br, 1 H, H-14), 3.20 (s, 3 H, H-10ab), 3.30 (s, 3 H, H-18ab), 3.64 (dd, *J* = 12.6, 4.9 Hz, 1 H, H-1), 3.92 (dd, *J* = 12.6, 2.4 Hz, 1 H, H-1), 4.10 (dd, *J* = 8.6, 2.0 Hz, 1 H, H-10), 4.16 (br, 1 H, H-18), 4.57 (ddd, *J* = 8.6, 4.0, 2.0 Hz, 1 H, H-11), 5.00 (br, 1 H, H-19), 5.02 (br, 1 H, H-22), 5.24 (m, 1 H, H-2), 6.79 (d, *J* = 9.2 Hz, 1 H, NH), 7.04 (m, 1 H, H-7), 7.12 (m, 2 H, H-6), 7.22 (m, 2 H, H-5); ^{13}C NMR (100 MHz, C_6D_6) δ 11.0 (q, C-19c or C-22c), 11.1 (q, C-22c or C-19c), 13.1 (q, C-9a), 15.7 (q, C-22d), 16.2 (q, C-19d), 18.4 (q, C-27), 20.3 (q, C-28), 24.7 (t, C-12), 25.1 (t, C-13), 24.9 (t, C-22b), 26.1 (t, C-19b), 27.9 (d, C-26), 31.8 (q, C-20a), 33.2 (d, C-19a), 36.6 (t, C-3), 37.6 (d, C-22a), 38.1 (t, C-17), 42.7 (q, 2 C, C-25bc), 43.4 (d, C-9), 47.8 (t, C-14), 53.1 (d, C-22), 57.4 (d, C-19), 57.9 (q, C-18ab), 60.6 (d, C-11), 60.8 (q, C-10ab), 62.5 (t, C-1), 76.3 (d, C-25), 77.2 (d, C-2), 79.2 (d, C-18), 82.0 (d, C-10), 126.7 (d, C-7), 128.7 (d, 2 C, C-6), 129.9 (d, 2 C, C-5), 137.9 (s, C-4), 170.3 (s, C-16), 171.1 (s, C-24), 173.8 (s, C-21), 174.0 (s, C-8); FABMS *m*/*z* (relative intensity) 747 (MH^+, 20), 100 (100). Anal. Calcd for $C_{41}H_{70}N_4O_8$: C, 65.92; H, 9.44; N, 7.50. Found: C, 65.80; H, 9.58; N, 7.50.

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Supporting Information Available: ^1H NMR spectra of natural **2** and **3**; ^1H and ^{13}C NMR spectra of synthetic **2**, **3**, C2-*epi*-**2**, and C2-*epi*-**3** (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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