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Synthesis of Dolastatin I, a Cytotoxic Cyclic Hexapeptide from the Sea Hare *Dolabella auricularia*

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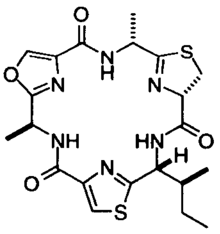
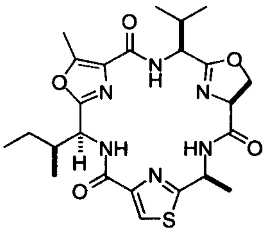
Abstract: Dolastatin I, a cytotoxic cyclic hexapeptide isolated from the Japanese sea hare *Dolabella auricularia*, was enantioselectively synthesized, which confirmed its stereostructure. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Synthesis; Peptides and Polypeptides; Biologically active compounds; Marine metabolites

The sea hare *Dolabella auricularia* is known to be a rich source of bioactive and structurally unique metabolites. Recently, we have isolated two cytotoxic cyclic hexapeptides, dolastatins E (**1**)[1] and I (**2**),[2] from the Japanese specimen of this animal and have determined their stereostructures. In the determination of the stereostructure of dolastatin E (**1**) we have had difficulty because the amino acid components were extremely labile and underwent epimerization during the acidic hydrolysis of **1**, but we have finally established the stereostructure of **1** by its total synthesis.[1b] The stereostructure of dolastatin I (**2**) was also determined by chiral HPLC analysis of the acidic hydrolysates,[2] and it was necessary to confirm the stereostructure of **2** by a synthetic method. We report herein the synthesis of dolastatin I (**2**), which confirmed its absolute stereostructure.



dolastatin E (**1**)



dolastatin I (**2**)

The synthesis of dolastatin I (**2**) was effected in a convergent manner (Scheme 1). The reactive oxazoline ring in **2** was constructed during the very last stage of the synthesis. The precursor **5** was prepared from the oxazole unit **3**, the thiazole unit **4**, *N*-Boc-L-valine, and *N*-Boc-L-serine by condensation reactions and macrocyclization.

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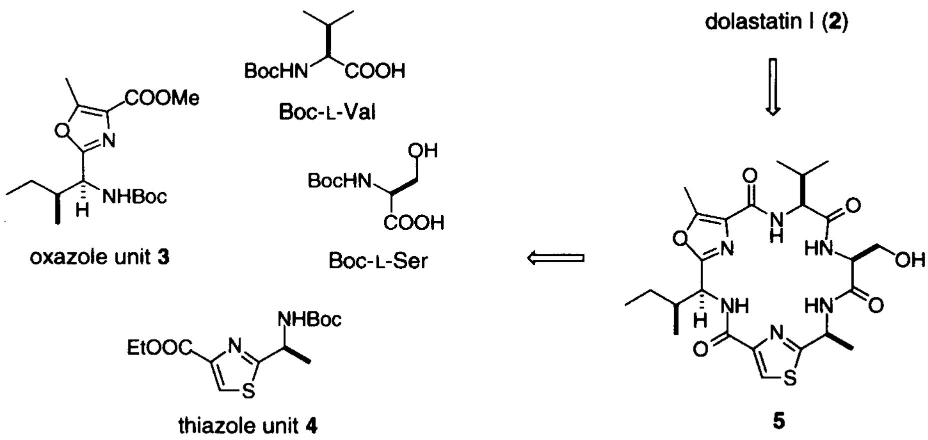
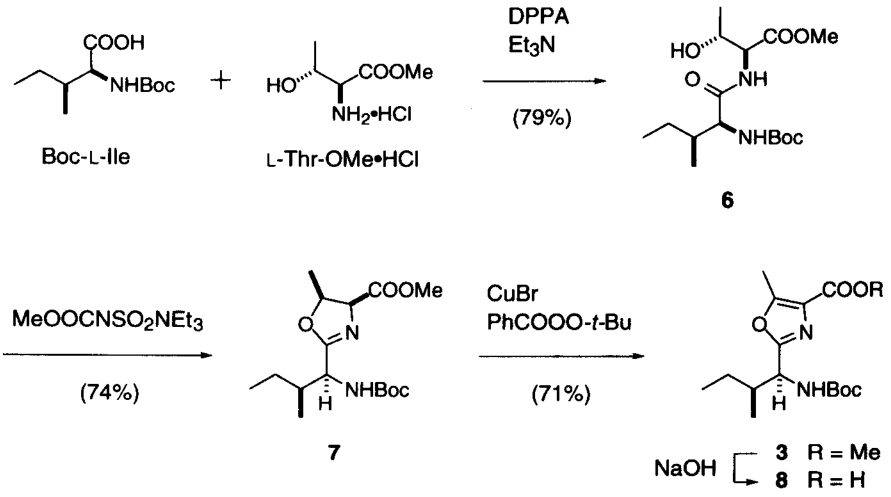
Scheme 1



The oxazole unit **3** was synthesized from *N*-Boc-L-isoleucine and L-threonine methyl ester hydrochloride (Scheme 2). The condensation of these two amino acid components provided the dipeptide **6**,[3] which was cyclized with Burgess reagent[4] to give the oxazoline **7** (74%). Although the oxidation of the oxazoline **7** with NiO_2 or by a selenoxide elimination sequence provided the oxazole unit **3** only in poor yields, the oxidation was effected under radical conditions (PhCOOO-*t*-Bu, CuBr)[5] in 71% yield. Basic hydrolysis using NaOH in aqueous methanol gave the carboxylic acid **8**.

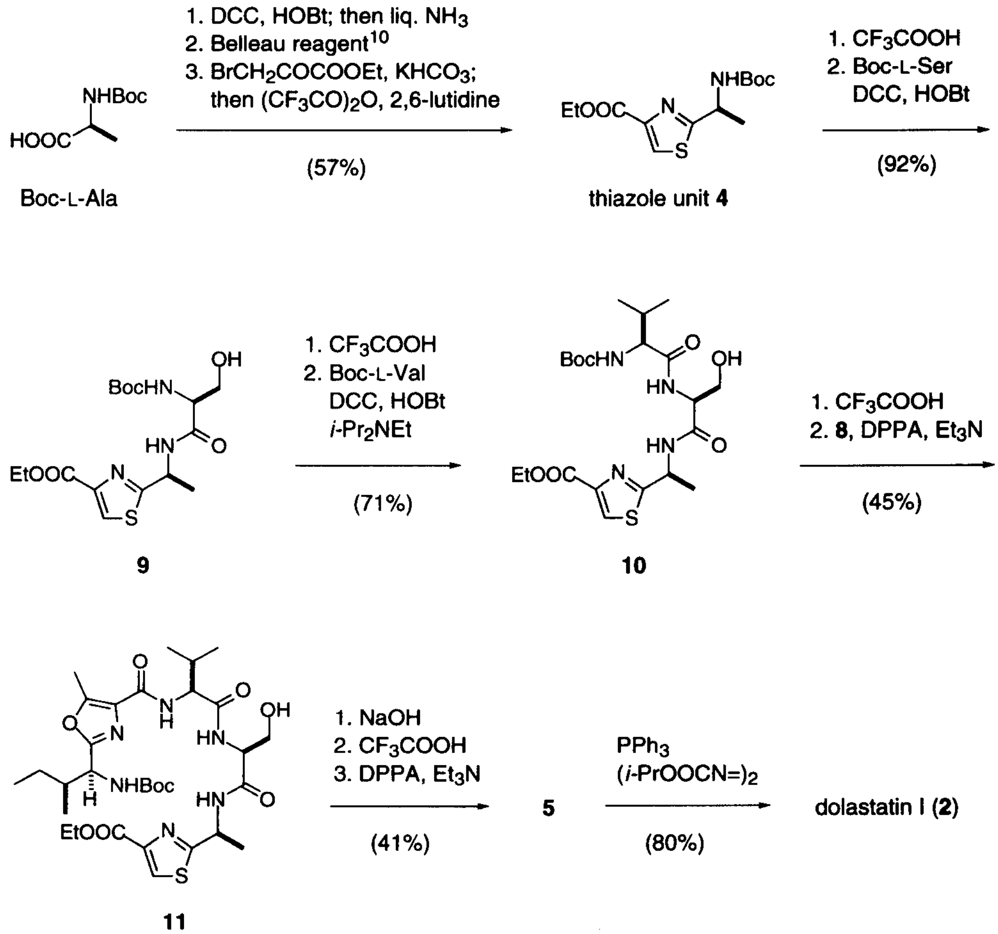
Scheme 2



The thiazole unit **4**[6] was prepared from *N*-Boc-L-alanine using a modified Hantzsch thiazole formation[7] (Scheme 3). Removal of the Boc group in the thiazole unit **4** and subsequent condensation with *N*-Boc-L-serine using diphenylphosphoryl azide (DPPA)[8] afforded the tripeptide **9** in 74% yield along with a phosphate ester of **9** (ca. 20%). The use of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt)[9] as coupling reagents improved the yield of the tripeptide **9** to 92%. The tripeptide **9** was transformed into the tetrapeptide **10** by the same sequence of reactions as for the conversion of **4** into **9** (71%). An amine prepared from the tetrapeptide **10** and the carboxylic acid **8** were condensed with DPPA to give the hexapeptide **11** (45%), which includes all the amino acid components in dolastatin I (**2**). The Boc and ethyl ester groups in the hexapeptide **11** were removed by the standard procedures, and the resulting amino acid was cyclized with DPPA under high-dilution conditions to give the cyclic peptide **5** in 41% yield. Finally, dehydration of the cyclic peptide **5** under Mitsunobu conditions gave dolastatin I (**2**) in 80% yield. Synthetic dolastatin I (**2**) was found to be identical with the natural one **2** by comparison of their spectral data.

In summary, we synthesized dolastatin I (**2**), a cytotoxic cyclic hexapeptide from the Japanese sea hare *D. auricularia*. This result confirmed its previously reported stereostructure.[2]

Scheme 3



EXPERIMENTAL

General. Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO FT/IR-230 spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) or a JEOL JNM-A400 (400 MHz). NMR chemical shifts were referenced to the TMS peak (δ_H 0). Mass spectra were determined on a JEOL JMS-LG2000 spectrometer operating in the FAB mode (*m*-nitrobenzyl alcohol as the matrix). Both the 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 TOSOH CCPM-II pump and a JASCO 880 pump, respectively. Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Organic solvents for the anhydrous reactions were purified by standard procedures. All moisture-sensitive reactions were performed under an atmosphere of nitrogen. Organic extracts were dried over anhydrous Na_2SO_4.

Boc-Ile-Thr-OMe (6). Thionyl chloride (4.3 mL, 60.0 mmol) was slowly added to MeOH (17 mL) at −10 °C. After stirring for 10 min, L-threonine (2.02 g, 16.9 mmol) was added to the solution. The mixture was stirred at room temperature for 48 h and concentrated to give L-threonine methyl ester hydrochloride as an oil (3.13 g).

The methyl ester hydrochloride thus prepared (1.65 g) and *N*-Boc-L-isoleucine (2.33 g, 9.72 mmol) were dissolved in DMF (14 mL) and cooled to 0 °C. To the solution were added diphenylphosphoryl azide (DPPA) (2.50 mL, 11.6 mmol) and Et_3N (3.3 mL, 23.6 mmol). After stirring at 0 °C for 5 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (20 mL) and extracted with EtOAc (50 mL, 5 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried, and concentrated. The residual oil was purified by column chromatography (1:1 to 1:2 hexane/EtOAc) to give the dipeptide **6** (2.44 g, 79% from *N*-Boc-L-isoleucine) as a colorless amorphous powder: $R_f = 0.51$ (1:3 hexane/EtOAc); $[\alpha]^{29}_D$ −14.9° (*c* 1.32, $CHCl_3$); IR ($CHCl_3$) 3430 (br), 1745, 1680, 1500, 1370, 1165 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92 (t, $J = 7.2$ Hz, 3 H), 0.96 (d, $J = 6.6$ Hz, 3 H), 1.19 (m, 1 H), 1.21 (d, $J = 6.6$ Hz, 3 H), 1.43 (s, 9 H), 1.49 (m, 1 H), 2.05 (m, 1 H), 3.10 (br s, 1 H), 3.77 (s, 3 H), 3.98 (dd, $J = 8.9$, 7.2 Hz, 1 H), 4.36 (qd, $J =$ 6.6, 6.3 Hz, 1 H), 4.62 (dd, $J = 8.9$, 6.3 Hz, 1 H), 5.17 (br d, $J = 8.9$ Hz, 1 H), 6.84 (br d, $J = 8.9$ Hz, 1 H); FABMS *m/z* (relative intensity) 369 (MNa$^+$, 12), 347 (MH$^+$, 67), 291 (100).

Methyl (*S*,*S*,*S*)-2-[1-(*tert*-butoxycarbonylamino)-2-methylbutyl]-5-methyloxazoline-4-carboxylate (7). To a solution of the dipeptide **6** (242 mg, 0.700 mmol) in THF (10 mL) was added Burgess reagent (189 mg, 0.791 mmol), and the mixture was refluxed for 5 h. The reaction mixture was concentrated, and the residual oil was purified by column chromatography (3:1 to 2:1 hexane/EtOAc) followed by MPLC [Nomura Chemical Develosil Lop 60 (21 g), 1:1 hexane/EtOAc, flow rate 3.0 mL/min] to give the oxazoline **7** (170 mg, 74%) as a colorless oil: $R_f = 0.50$ (1:1 hexane/EtOAc); $[\alpha]^{30}_D$ +20.4° (*c* 1.09, $CHCl_3$); IR ($CHCl_3$) 3440, 1750, 1710, 1660, 1500, 1370, 1170, 1045 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.94 (t, $J = 7.2$ Hz, 3 H), 0.99 (d, $J = 6.9$ Hz, 3 H), 1.20 (m, 1 H), 1.29 (d, $J = 6.3$ Hz, 3 H), 1.43 (s, 9 H), 1.53 (m, 1 H), 1.88 (m, 1 H), 3.75 (s, 3 H), 4.36 (ddd, $J = 8.9$, 3.6, 1.3 Hz, 1 H), 4.79 (dd, $J = 10.2$, 1.3 Hz, 1 H), 4.95 (dq, $J = 10.2$, 6.3 Hz, 1 H), 5.23 (br d, $J = 8.9$ Hz, 1 H); FABMS *m/z* (relative intensity) 351 (MNa$^+$, 60), 329 (MH$^+$, 97), 273 (100); HRFABMS calcd for $C_{16}H_{29}N_2O_5$ *m/z* 329.2076 (MH$^+$), found 329.2085.

Methyl (*S*,*S*)-2-[1-(*tert*-butoxycarbonylamino)-2-methylbutyl]-5-methyloxazole-4-carboxylate (3). *tert*-Butyl perbenzoate (0.2 mL, 1.05 mmol) was added to a boiling solution of the oxazoline **7** (228 mg, 0.693 mmol) and CuBr (113 mg, 0.777 mmol) in benzene (4.7 mL) over 1 h, then the mixture was further refluxed for 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (6 mL) and extracted with ether (2 x 7 mL). The combined organic layers were washed with H_2O (2 x 7 mL) and saturated aqueous NaCl (4 mL), dried, and concentrated. The residual oil was purified by column chromatography (10:1 benzene/EtOAc) to give the oxazole unit **3** (161 mg, 71%) as a colorless oil: $R_f = 0.27$ (5:1 benzene/EtOAc); $[\alpha]^{29}_D$ −38.7° (*c* 1.07, $CHCl_3$); IR ($CHCl_3$) 3440, 1715, 1620, 1500, 1370, 1165, 1100 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.9 Hz, 3 H), 0.91 (t, *J* = 7.2 Hz, 3 H), 1.18 (m, 1 H), 1.43 (s, 9 H), 1.46 (m, 1 H), 1.91 (m, 1 H), 2.61 (s, 3 H), 3.90 (s, 3 H), 4.79 (dd, *J* = 8.9, 6.3 Hz, 1 H), 5.27 (br d, *J* = 8.9 Hz, 1 H); FABMS *m/z* (relative intensity) 349 (MNa^+, 27), 327 (MH^+, 44), 271 (100); HRFABMS calcd for $C_{16}H_{27}N_2O_5$ *m/z* 327.1920 (MH^+), found 327.1908.

Ethyl (*S*)-2-[1-(*tert*-butoxycarbonylamino)ethyl]thiazole-4-carboxylate (4). A solution of *N*-Boc-L-alanine (2.00 g, 10.6 mmol) and HOBt (1.43 g, 10.6 mmol) in CH_2Cl_2 (120 mL) was cooled to 0 °C. To the solution was added DCC (2.76 g, 13.4 mmol), then the mixture was stirred at room temperature for 1 h. After the mixture was cooled to 0 °C, liquid NH_3 (3 mL) in CH_2Cl_2 (12 mL) was added. The mixture was stirred at 0 °C for 1 h and filtered, and the solid was washed with CH_2Cl_2. The filtrate and the washings were combined and concentrated. The residue was purified by column chromatography (3:1 to 1:1 CH_2Cl_2/acetone) to give 2-(*tert*-butoxycarbonylamino)propionamide (1.94 g, 97%) as colorless needles: $R_f =$ 0.17 (3:1 CH_2Cl_2/acetone); m.p. 127–128 °C (hexane/EtOAc). $[\alpha]^{29}_D$ −38.2° (*c* 1.11, $CHCl_3$); IR ($CHCl_3$) 3435, 3340, 1690, 1590, 1500, 1450, 1390, 1370, 1240, 1160, 1060 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.38 (d, *J* = 6.9 Hz, 3 H), 1.45 (s, 9 H), 4.19 (m, 1 H), 5.02 (br m, 1 H), 5.55 (br m, 1 H), 6.19 (br m, 1 H); FABMS *m/z* (relative intensity) 211 (MNa^+, 11), 189 (MH^+, 63), 133 (100).

To a stirred solution of the amide (1.69 g, 9.00 mmol) in THF (85 mL) was added Belleau reagent at 0 °C, and the mixture was stirred at room temperature for 35 min. The reaction mixture was poured into a mixture of ice (85 g) and saturated aqueous $NaHCO_3$ (85 mL), and extracted with ether (3 x 100 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried, and concentrated. The residual oil was purified by column chromatography (5:1 to 1:1 benzene/EtOAc) to give 2-(*tert*-butoxycarbonylamino)propionyl thioamide (1.81 g, 99%) as colorless columns: R_f = 0.33 (2:1 benzene/EtOAc); m.p. 85–87 °C (hexane/ether); $[\alpha]^{29}_D$ −70.1° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3440, 3200, 1695, 1500, 1370, 1240, 1160, 1060 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.44 (s, 9 H), 1.48 (d, *J* = 6.9 Hz, 3 H), 4.53 (dq, *J* = 6.9, 6.9 Hz, 1 H), 5.25 (br d, *J* = 6.9 Hz, 1 H), 7.56 (br m, 1 H), 7.92 (br m, 1 H); FABMS *m/z* (relative intensity) 205 (MH^+, 35), 149 (100).

To a solution of the thioamide (905 mg, 4.43 mmol) in DME (7.2 mL) at −15 °C was added $KHCO_3$ (3.55 g, 35.5 mmol). After vigorous stirring at −15 °C for 5 min, ethyl bromopyruvate (1.65 mL, 13.1 mmol) was added. The mixture was stirred for 1 min, and a solution of $(CF_3CO)_2O$ (2.5 mL, 17.6 mmol) and 2,6-lutidine (4.4 mL, 37.7 mmol) in DME (4.5 mL) was added. After stirring at −15 °C for 3 h, the reaction mixture was poured into H_2O (35 mL) and extracted with $CHCl_3$ (3 x 35 mL). The combined organic layers were dried and concentrated. The residue was purified by column chromatography (8:1 benzene/EtOAc) to give the thiazole unit **4** (790 mg, 59%) as colorless needles: $R_f = 0.43$ (3:1 benzene/EtOAc); m.p. 86.5–87.5 °C (hexane/ether); $[\alpha]^{29}_D$ −42.1° (*c* 0.989, $CHCl_3$); IR ($CHCl_3$) 3440, 3120, 1715, 1500, 1370, 1240, 1165, 1100 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3 H), 1.45 (s, 9 H), 1.62 (d, *J* = 7.2 Hz, 3 H), 4.42 (q, *J* = 7.2 Hz, 2 H), 5.10 (m, 1 H), 5.13 (br m, 1 H), 8.08 (s, 1 H); FABMS *m/z* (relative intensity) 323 (MNa^+, 26), 301 (MH^+, 65), 245 (100).

Boc-Ser-Ala-thiazole-COOEt (9). A solution of the thiazole unit **4** (303 mg, 1.01 mmol) in CH_2Cl_2 (6.5 mL) and CF_3COOH (6.5 mL) was stirred at 0 °C for 3 h and concentrated. The residual oil was purified by column chromatography on alumina [E. Merck aluminum oxide 90 (Activity II-III), 10:1 $CHCl_3$/EtOH] to give the corresponding amine as an oil. The amine, *N*-Boc-L-serine (294 mg, 1.43 mmol), and HOBt (192 mg, 1.42 mmol) were dissolved in DMF (2.7 mL) and cooled to 0 °C. A solution of DCC (326 mg, 1.57 mmol) in DMF (0.5 mL) was added, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The reaction mixture was filtered, and the solid was washed with a small amount of DMF. The filtrate and the washings were combined and concentrated. The residue was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ (4 mL). The aqueous $NaHCO_3$ layer was extracted with EtOAc (4 × 4 mL), and the organic layer and the EtOAc extracts were combined and successively washed with 2 M aqueous citric acid (4 mL), saturated aqueous $NaHCO_3$ (4 mL), and H_2O (4 mL). The organic layer was dried and concentrated, and the residual oil was purified by column chromatography (1:2 to 1:5 hexane/EtOAc) to give the tripeptide **9** (361 mg, 92% from the thiazole unit **4**) as a colorless oil: R_f = 0.36 (1:5 hexane/EtOAc); $[\alpha]^{30}_D$ –51.6° (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) 3425, 3400 (br), 1715, 1680, 1490, 1370, 1240, 1160, 1100 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.2 Hz, 3 H), 1.45 (s, 9 H), 1.65 (d, *J* = 6.9 Hz, 3 H), 3.66 (ddd, *J* = 10.9, 6.9, 6.9 Hz, 1 H), 3.95 (m, 1 H), 4.05 (m, 1 H), 4.24 (m, 1 H), 4.39 (q, *J* = 7.2 Hz, 2 H), 5.44 (dq, *J* = 7.2, 6.9 Hz, 1 H), 5.50 (br s, 1 H), 7.06 (br d, *J* = 7.2 Hz, 1 H), 8.10 (s, 1 H); FABMS *m/z* (relative intensity) 410 (MNa^+, 68), 388 (MH^+, 100); HRFABMS calcd for $C_{16}H_{26}N_3O_6S$ *m/z* 388.1542 (MH^+), found 388.1546.

Boc-Val-Ser-Ala-thiazole-COOEt (10). A solution of the tripeptide **9** (53.3 mg, 0.137 mmol) in CH_2Cl_2 (5.0 mL) and CF_3COOH (5.0 mL) was stirred at 0 °C for 1 h and concentrated. The residual oil, *N*-Boc-L-valine (37.2 mg, 0.171 mmol), and HOBt (23.5 mg, 0.173 mmol) were dissolved in DMF (0.4 mL) and cooled to 0 °C. To the solution were added (i-Pr)$_2$NEt (0.03 mL, 0.168 mmol) and a solution of DCC (38.5 mg, 0.186 mmol) in DMF (0.1 mL). This mixture was stirred at 0 °C for 1 h and then at room temperature for 40 min. The reaction mixture was filtered, and the solid was washed with a small amount of DMF. The filtrate and the washings were combined and concentrated. The residual oil was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ (1 mL). The aqueous $NaHCO_3$ layer was extracted with EtOAc (3 × 1 mL). The organic layer and the EtOAc extracts were combined and successively washed with 2 M aqueous citric acid (1 mL), saturated aqueous $NaHCO_3$ (1 mL) and H_2O (1 mL). The organic layer was dried and concentrated, and the residual oil was purified by column chromatography (3:2 to 1:1 hexane/acetone) to give the tetrapeptide **10** (47.6 mg, 71%) as colorless plates: R_f = 0.41 (1:1 hexane/acetone); m.p. 97–98.5 °C (hexane/EtOAc); $[\alpha]^{28}_D$ –43.6° (*c* 1.01, $CHCl_3$); IR ($CHCl_3$) 3415, 3320 (br), 3120, 1715, 1670, 1500, 1370, 1220, 1160, 1100 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92 (d, *J* = 6.9 Hz, 3 H), 0.99 (d, *J* = 6.9 Hz, 3 H), 1.39 (t, *J* = 7.2 Hz, 3 H), 1.44 (s, 9 H), 1.67 (d, *J* = 7.2 Hz, 3 H), 2.19 (m, 1 H), 3.67 (ddd, *J* = 10.9, 7.2, 7.2 Hz, 1 H), 3.85 (m, 1 H), 3.97 (dd, *J* = 12.5, 6.9 Hz, 1 H), 4.11 (ddd, *J* = 7.2, 6.9, 6.9 Hz, 1 H), 4.39 (q, *J* = 7.2 Hz, 2 H), 4.55 (ddd, *J* = 10.9, 6.9, 6.9 Hz, 1 H), 5.02 (br d, *J* = 6.9 Hz, 1 H), 5.42 (dq, *J* = 7.2, 7.2 Hz, 1 H), 6.94 (br d, *J* = 7.2 Hz, 1 H), 7.28 (br d, *J* = 6.9 Hz, 1 H), 8.09 (s, 1 H); FABMS *m/z* (relative intensity) 509 (MNa^+, 58), 487 (MH^+, 100); HRFABMS calcd for $C_{21}H_{35}N_4O_7S$ *m/z* 487.2227 (MH^+), found 487.2206.

Boc-Ile-oxazole-Val-Ser-Ala-thiazole-COOEt (11). A solution of the oxazole unit **3** (46.1 mg, 0.141 mmol) in MeOH (0.5 mL) and 2 M aqueous NaOH (0.18 mL, 0.36 mmol) was stirred at room temperature for 1 h. To the mixture was added 2 M aqueous NaOH (0.07 mL, 0.14 mmol), and the mixture was further stirred at room temperature for 30 min. To the reaction mixture was added 5% aqueous $KHSO_4$ (1.5 mL) to adjust the pH to 3-4. The mixture was then extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (4 mL), dried, and concentrated to give the carboxylic acid **8**.

A solution of the tetrapeptide **10** (49.5 mg, 0.101 mmol) in CH_2Cl_2 (1.0 mL) and CF_3COOH (1.0 mL) was stirred at 0 °C for 1 h and concentrated to give the corresponding amine trifluoroacetic acid salt as an oil. The amine trifluoroacetic acid salt and carboxylic acid **8** were dissolved in DMF (0.1 mL) and cooled to 0 °C. To the solution were added Et_3N (0.05 mL, 0.358 mmol) and DPPA (0.03 mL, 0.135 mmol), and the mixture was then stirred at 0 °C for 5.5 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL), and then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (4 mL), dried, and concentrated. The residual oil was purified by column chromatography (1:10 hexane/EtOAc to EtOAc) to give the hexapeptide **11** (30.8 mg, 45% from the tetrapeptide **10**) as a colorless oil: $R_f = 0.38$ (1:1 hexane/acetone); $[\alpha]^{29}_D$ –45.1° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3405, 3320, 3120, 1715, 1665, 1500, 1370, 1240, 1160 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (d, *J* = 6.9 Hz, 3 H), 0.93 (t, *J* = 7.2 Hz, 3 H), 1.02 (d, *J* = 6.9 Hz, 3 H), 1.03 (d, *J* = 6.6 Hz, 3 H), 1.19 (m, 2 H), 1.38 (t, *J* = 7.2 Hz, 3 H), 1.46 (s, 9 H), 1.66 (d, *J* = 6.6 Hz, 3 H), 1.91 (m, 1 H), 2.28 (m, 1 H), 2.58 (s, 3 H), 3.68 (ddd, *J* = 10.9, 6.9, 6.6 Hz, 1 H), 3.90 (dd, *J* = 6.6, 6.6 Hz, 1 H), 4.10 (ddd, *J* = 10.9, 6.6, 6.3 Hz, 1 H), 4.32 (dd, *J* = 7.6, 6.3 Hz, 1 H), 4.39 (q, *J* = 7.2 Hz, 2 H), 4.57 (ddd, *J* = 9.2, 6.9, 6.3 Hz, 1 H), 4.76 (dd, *J* = 9.2, 6.9 Hz, 1 H), 5.12 (br d, *J* = 9.2 Hz, 1 H), 5.43 (dq, *J* = 7.2, 6.6 Hz, 1 H), 6.92 (br d, *J* = 7.2 Hz, 1 H), 7.24 (br d, *J* = 9.2 Hz, 1 H), 7.34 (br d, *J* = 7.6 Hz, 1 H), 8.09 (s, 1 H); FABMS *m/z* (relative intensity) 703 (MNa^+, 38), 681 (MH^+, 28), 310 (100); HRFABMS calcd for $C_{31}H_{49}N_6O_9S$ *m/z* 681.3281 (MH^+), found 681.3278.

Cyclic Peptide (5). A solution of the hexapeptide **11** (26.6 mg, 0.0391 mmol) in MeOH (0.3 mL) and 2 M aqueous NaOH (0.08 mL, 0.16 mmol) was stirred at room temperature for 1 h. To the reaction mixture was added 5% aqueous $KHSO_4$ (0.5 mL) to adjust the pH to 2-3. The mixture was then extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with saturated aqueous NaCl (2 mL), dried, and concentrated. The residual oil was dissolved in CH_2Cl_2 (1.0 mL) and CF_3CO_2H (1.0 mL) at 0 °C, and the solution was stirred at 0 °C for 1 h and concentrated. The residual oil was dissolved in DMF (39 mL), and to the solution were added Et_3N (0.03 mL, 0.215 mmol) and DPPA (0.025 mL, 0.112 mmol) at 0 °C. After the mixture was stirred at 0 °C for 40 h, the reaction mixture was diluted with 1:1 benzene/EtOAc (60 mL) and washed with H_2O (2 × 40 mL). The combined washings were extracted with 1:1 benzene/EtOAc (2 × 40 mL). The organic layer and benzene/EtOAc extracts were combined, washed with saturated aqueous NaCl (14 mL), dried, and concentrated. The residual oil was purified by column chromatography (1:1 to 1:2 hexane/acetone) and subsequently by HPLC [Nomura Chemical Develosil ODS-HG-5 (20 × 250 mm), 65:35 $MeOH/H_2O$, flow rate 5.0 mL/min, detection at 254 nm] to give the cyclic peptide **5** (8.6 mg, 41%) as a colorless powder: $R_f = 0.90$ (10:5:1 $CHCl_3/MeOH/H_2O$); $[\alpha]^{28}_D$ –145° (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) 3430, 3400 (br), 3120, 1670, 1640, 1540, 1500 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.98 (d, *J* = 6.9 Hz, 3 H), 1.01 (t, *J* = 7.2 Hz, 3 H), 1.04 (d, *J* = 6.9 Hz, 3 H), 1.12 (d, *J* = 6.9 Hz, 3 H), 1.30 (m, 1 H), 1.62 (d, *J* = 6.9 Hz, 3 H), 1.65 (m, 1 H), 2.09 (m, 1 H), 2.36 (m, 1 H), 2.63 (s, 3 H), 3.01 (dd, *J* = 7.5, 5.9 Hz, 1 H), 3.82 (ddd, *J* = 11.5, 7.5, 3.9 Hz, 1 H), 4.26 (ddd, *J* = 11.5, 5.9, 3.3 Hz, 1 H), 4.36 (ddd, *J* = 7.5, 3.9, 3.3 Hz, 1 H), 4.63 (dd, *J* = 9.9, 5.6 Hz, 1 H), 5.12 (dd, J = 7.5, 5.6 Hz, 1 H), 5.36 (dq, J = 6.9, 6.9 Hz, 1 H), 6.72 (br d, J = 7.5 Hz, 1 H), 7.51 (br d, J = 9.9 Hz, 1 H), 7.63 (br d, J = 7.5 Hz, 1 H), 8.03 (s, 1 H), 8.39 (br d, J = 6.9 Hz, 1 H); FABMS *m/z* (relative intensity) 557 (MNa^+, 18), 535 (MH^+, 100); HRFABMS calcd for $C_{24}H_{35}N_6O_6S$ *m/z* 535.2338 (MH^+), found 535.2349.

Dolastatin I (2). To a solution of the cyclic peptide **5** (3.5 mg, 0.00655 mmol) and PPh_3 (6.5 mg, 0.0247 mmol) in THF (0.3 mL) was added diisopropyl azodicarboxylate (0.005 mL, 0.0249 mmol) at 0 °C. This mixture was stirred at 0 °C for 30 min and concentrated. The residual oil was purified by column chromatography (3:1 hexane/acetone) followed by preparative TLC (1:1 hexane/acetone) to give dolastatin I (**2**, 2.7 mg, 80%) as a colorless powder: R_f = 0.51 (1:1 hexane/acetone); $[\alpha]^{31}{}_D$ $-50°$ (c 0.044, $CHCl_3$); IR ($CHCl_3$) 3390, 3120, 1675, 1540, 1525, 1515, 1490, 1370 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, J = 6.8 Hz, 3 H), 0.94 (d, J = 6.8 Hz, 3 H), 1.03 (t, J = 7.3 Hz, 3 H), 1.07 (d, J = 6.8 Hz, 3 H), 1.35 (m, 1 H), 1.60 (d, J = 6.8 Hz, 3 H), 1.65 (m, 1 H), 2.26 (m, 1 H), 2.41 (qqd, J =6.8, 6.8, 2.9 Hz, 1 H), 2.66 (s, 3 H), 4.56 (dd, J = 8.2, 7.3 Hz, 1 H), 4.66 (ddd, J = 10.7, 8.2, 2.0 Hz, 1 H), 4.73 (dd, J = 10.7, 7.3 Hz, 1 H), 4.82 (ddd, J = 8.7, 2.9, 2.0 Hz, 1 H), 5.13 (dd, J = 6.8, 4.4 Hz, 1 H), 5.39 (dq, J = 7.3, 6.8 Hz, 1 H), 7.93 (br d, J = 9.0 Hz, 1 H), 8.06 (s, 1 H), 8.09 (br d, J = 7.3 Hz, 1 H), 8.62 (br d, J = 6.8 Hz, 1 H); FABMS *m/z* (relative intensity) 539 (MNa^+, 9), 517 (MH^+, 100); HRFABMS calcd for $C_{24}H_{33}N_6O_5S$ *m/z* 517.2233 (MH^+), found 517.2233.

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