(1α,4aα,6β,8aα)-**10b**, 96617-05-9; (±)-**11a** (trans-fused), 96617-06-0; (±)-**11a** (cis-fused), 96647-96-0; (±)-**11b**, 96617-07-1; (±)-**12a**, 96617-08-2; (±)-**12b**, 96617-09-3; (±)-**12c**, 87332-41-0; **13**, 58-22-0; **14**, 25469-53-8; (±)-**15**, 96479-43-5; (±)-**16**, 96647-97-1; (17β)-androsta-3,5-diene-3,7-diol diacetate, 1778-93-4; (6β,17β)-17-(acetyloxy)-6-hydroxyandrost-4-en-3-one, 13096-48-5; (6α,17β)-17-(acetyloxy)-6-hydroxyandrost-4-en-3-one, 13573-36-9; 2-bromo-3,3-diethoxy-1-propene, 17592-40-4; 3-buten-2-one, 78-94-4; 2,5-dimethylcyclohexanone, 932-51-4; methyl 4-oxo-2-pentenoate, 4188-88-9; methyltriphenylphosphonium bromide, 1779-49-3.

Synthesis of the *cyclo*-[(gly)Thz-(*R*)- and *cyclo*-[(gly)Thz-(*S*)-(gln)Thz-L-Val-L-Leu-L-Pro] Isomers of Dolastatin 3[1a]

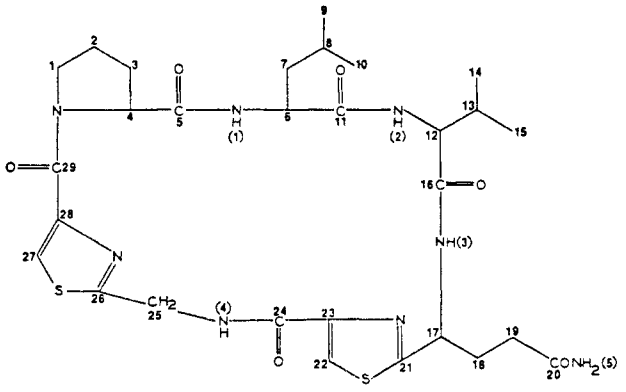
George R. Pettit,* Paul S. Nelson,[1b] and Cedric W. Holzapfel

Cancer Research Institute and Chemistry Department, Arizona State University, Tempe, Arizona 85287

Received August 8, 1984

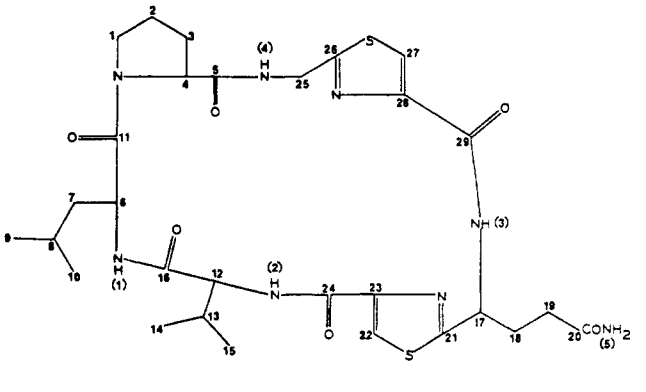
An all-L configuration reverse order of peptide bonding possibility for the cell growth inhibitory (PS system) cyclic peptide dolastatin 3 was eliminated by synthesis of thiazole amino acid containing peptide **2**. By employing a series (Scheme I) of mixed carbonic anhydride (except for **9** → **11** where DCCI–HBT was used) peptide bond forming reactions with *N*-Boc protection and a 2,4,5-trichlorophenol active ester cyclization step, cyclic pentapeptide **2** was obtained as a mixture of diastereomers corresponding to the (*R*)- and (*S*)-(gln)Thz unit. The thiazole amino acid components were synthesized employing a Hantzsch reaction as the key step (cf. Scheme II). Spectral analysis of the individual (*R*)- and (*S*)-(gln)Thz cyclic pentapeptide **2** removed both as structural candidates for dolastatin 3.

The Aplysiomorpha mollusc *Dolabella auricularia* has been found to contain a series of potent cell growth inhibitory (murine P388 lymphocytic leukemia, PS system) peptides designated dolastatins.[2] After an extensive series of isolation studies guided by bioassay (PS system) methods we were able to obtain nine of these potentially important substances in approximately 1-mg amounts. One of these, dolastatin 3, was subjected to detailed spectral and hydrolytic studies. On the basis of only 1 mg, dolastatin 3 was tentatively assigned structure **1**. As a



1, Cyclo[Pro-Leu-Val-(gln)Thz-(gly)Thz]

consequence of the limited supply and lack of crystallinity, two structural ambiguities remained unsettled. One question concerned the possibility of a reverse order of peptide bonding and the second the absolute configuration of the four chiral amino acids. Both possibilities became especially important when we synthesized (summary in preparation) the all-L isomers [with (*R*)- and (*S*)-(gln)Thz] corresponding to structure **1** and found it to be close to but not identical with dolastatin 3. Thus, in order to eliminate or confirm the reverse order of bonding for dolastatin 3 the synthesis of *cyclo*-[(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro] (**2**) was undertaken. Synthesis of all 16 diastereomers of cyclic peptide **2** would require considerable effort. So we decided to concentrate on preparing the diastereomer possessing an all-L configuration in the Val-Leu-Pro segment. The overall synthetic strategy is briefly outlined in Scheme I.



2, Cyclo[(gly)Thz-R and S-(gln)Thz-L-Val-L-Leu-L-Pro]

Reaction of Boc-L-Leu with isobutyl chloroformate and *N*-methylmorpholine followed by L-Pro-OMe gave Boc-L-Leu-L-Pro-OMe (**3**) in 80% yield. Treatment of dipeptide **3** with ethereal hydrogen chloride gave (86%) L-Leu-L-Pro-OMe (**4**)·HCl[4] and this dipeptide was more conveniently prepared in 68% overall yield by eliminating chro-

(1) (a) Antineoplastic Agents and Structural Biochemistry series, contibutions 109 and 24, respectively. For parts 108 and 23, see: Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.*, submitted for publication. (b) Abstracted in part from the Ph.D. dissertation of PSN, submitted to the Graduate School, Arizonia State University, Tempe, May 1983.

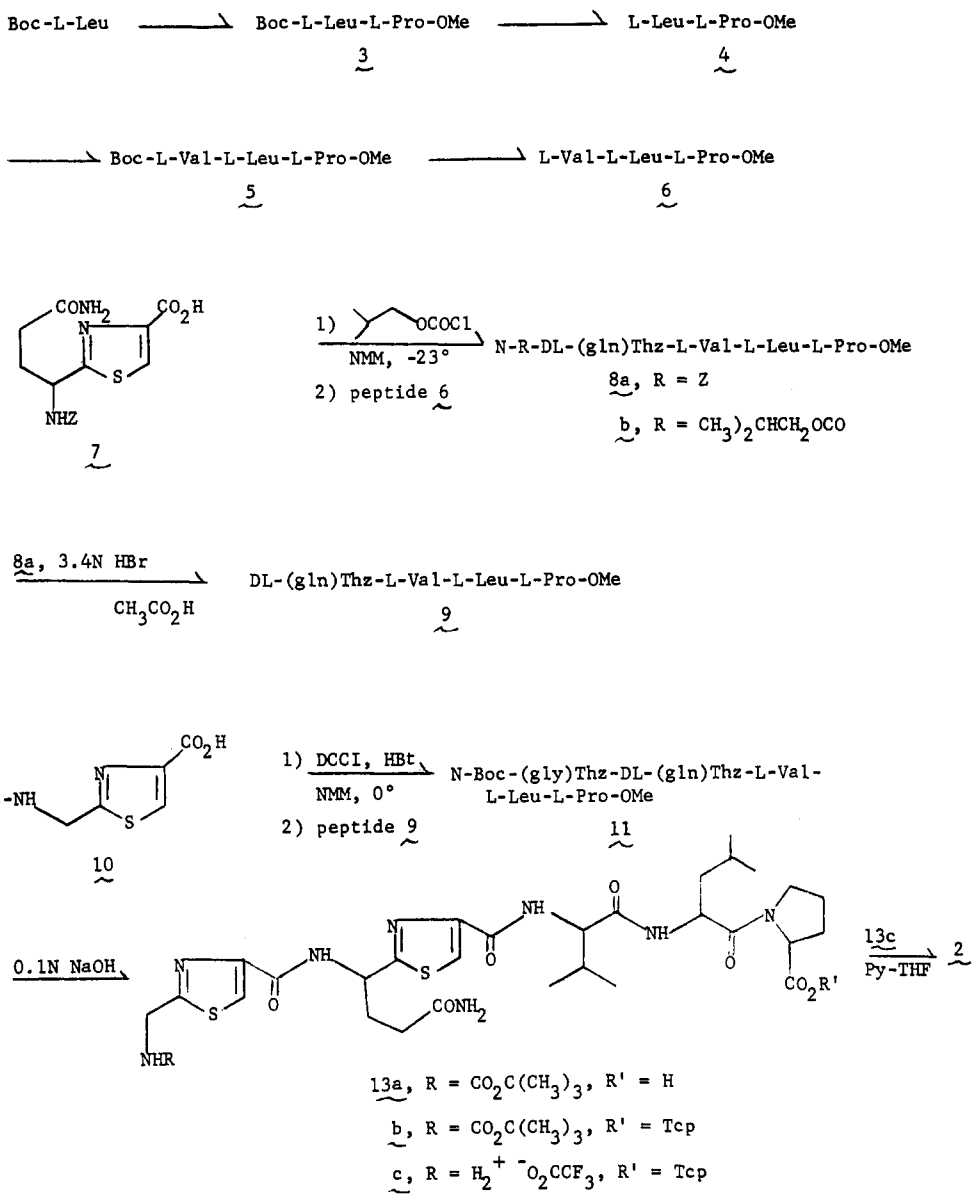
(2) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482–485.

(3) Pettit, G. R.; Kamano, Y.; Brown, P.; Gust, D.; Inoue, M.; Herald, C. L. *J. Am. Chem. Soc.* **1982**, *104*, 905–907.

(4) Starratt, A. N.; Brown, B. E. *Can. J. Chem.* **1977**, *55*, 4238–4242.

0022-3263/85/1950-2654$01.50/0

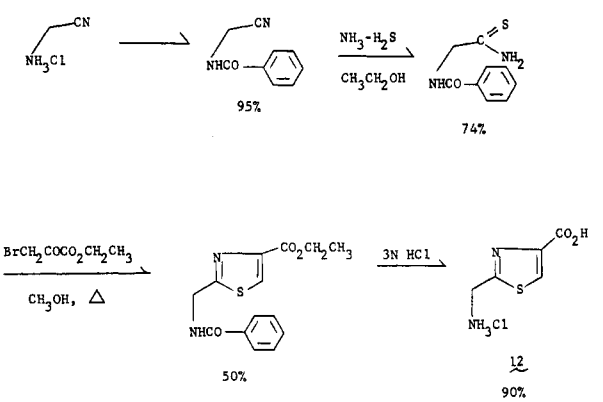
Scheme I



matographic purification of dipeptide **3**. A relatively poor yield (27%) resulted when Boc-L-Leu-L-Pro-OMe was deprotected with trifluoroacetic acid-methylene chloride (1:1) and coupled with the mixed carbonic anhydride derivative of Boc-L-Val to afford tripeptide **5**. Cleavage of the Boc group with dry ethereal hydrogen chloride gave L-Val-L-Leu-Pro-OMe (**6**)·HCl in 98% yield. Condensation of L-Leu-L-Pro-OMe·HCl with *N*-Boc-L-Val by the same method (isobutyl chloroformate, *N*-methylmorpholine, −23 °C) followed by ethereal hydrogen chloride treatment to afford (86% overall yield) tripeptide **5** hydrochloride was found more efficient.

Thiazole amino acids **7** and **12** were synthesized by Hantzsch reaction routes (cf. Scheme II)[1,3,5] and reaction of thiazole **12** with di-*tert*-butyl pyrocarbonate led to *N*-Boc-(gly)Thz (**10**). In the synthesis of thiazole **7** from L-Glu racemization occurred at the Hantzsch synthesis step and chiral integrity was lost.[1] Because of an interest in obtaining both (*R*)- and (*S*)-(gln)Thz isomers of *cyclo*-[(gly)Thz-(gln)Thz-L-Val-L-Leu-L-Pro] the synthesis was attempted with racemic thiazole **7** assuming that the resulting diastereomers might be readily separated. Condensation of *N*-Z-DL-(gln)Thz (**7**) with tripeptide **6** using a mixed carbonic anhydride procedure afforded *N*-Z-DL-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (**8a**) in 94% yield. But separation of the diastereomers at this stage proved to be difficult and only a small quantity (~5 mg) of each diastereomer was finally obtained pure by HPLC for characterization purposes. Next achiral *N*-Boc-(gly)Thz

Scheme II



(5) Cross, D. F. W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E. *J. Chem. Soc.* **1963**, 2143-2150.

(10) was condensed with the diastereomers of tetrapeptide 9 with the prospect that the corresponding diastereomers of N-Boc-(gly)Thz-(R and S)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (11) and/or its cyclic pentapeptide derivative might exhibit greater chromatographic differences. Attempts to condense (mixed carbonic anhydride method, isobutyl chloroformate) N-Boc-(gly)Thz (10) with amine 9 yielded (91%) N-[i-BuOC(=O)]-(R and S)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (8b) instead of the expected N-Boc pentapeptide 11. Since the electron withdrawal effect of the thiazole ring of 10 should enhance electrophilic character of the adjacent carbonyl and the electron releasing expected from the isobutoxy group should lessen electrophilic character of its carbonyl, formation of N-isobutoxy peptide 8b was unexpected and may have resulted from steric constraints. The problem was easily circumvented by promoting the coupling with dicyclohexylcarbodiimide (DCCI). After treating N-Boc-(gly)Thz (10) with hydroxybenzotriazole[6] and dicyclohexylcarbodiimide, reaction with tetrapeptide 9 gave (91% yield) N-Boc-(gly)Thz-DL-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (11). Again it was found best to defer separation of the diastereomers.

Cyclization of pentapeptide 11 was accomplished by the following series of reactions. The methyl ester was hydrolyzed and the resulting carboxylic acid 13a was converted to the corresponding 2,4,5-trichlorophenol ester 13b. The *tert*-butyloxycarbonyl protection was removed with trifluoroacetic acid-methylene chloride (1:1) to give Tfa salt 13c. A solution (high dilution) of the salt was stirred at room temperature in pyridine-tetrahydrofuran (1:5) for 43 h. Subsequent Sephadex LH-20 gel exclusion and silica gel chromatographic separations afforded both diastereomers of *cyclo*-[(gly)Thz-(R and S)-(gln)Thz-L-Val-L-Leu-L-Pro] in 24% overall yield. The diastereomer with the higher R_f value (0.25) using 9:1 methylene chloride–methanol was completely characterized whereas the lower R_f (0.22) diastereomer[7] showed some contamination by high-resolution (400 MHz) ^{1}H NMR spectral analysis.

As anticipated neither diastereomer of cyclic pentapeptide 2 was found identical with an authentic sample of dolastatin 3. Comparative TLC showed similar but not equivalent mobility properties. The ^{1}H and ^{13}C NMR spectra of the two diastereomers and dolastatin 3 demonstrated distinct differences (cf. Tables I and II). Analysis of the mass and nuclear magnetic resonance spectra data obtained from cyclic pentapeptide 2 clearly allowed exclusion of the all-L configuration [and both the (R)- and (S)-(gln)Thz isomers] reverse peptide bonding option for dolastatin 3. The result was a significant step toward eventually ascertaining the detailed structural features of dolastatin 3. In addition a workable synthetic route to this interesting family of cyclic thiazole peptides was devised and valuable spectral and physical data were obtained.

Experimental Section

L-Pro-OMe·HCl, N-Boc-L-Leu, and N-Boc-L-Val were obtained from Sigma Chemical Co. All solvents were redistilled. Solvent extracts of aqueous solution were dried over anhydrous sodium sulfate and concentrated on a rotatory evaporator. Tetrahydrofuran and dimethylformamide were distilled from lithium aluminum hydride and calcium hydride, respectively. Ether refers to diethyl ether. Analtech Silica Gel GF (0.25 mm) plates were used for thin-layer chromatography (TLC) and developed with concentrated sulfuric acid or 1% palladium chloride sprays or by ultraviolet light. Column chromatography was accomplished with silica gel (70–230 mesh) supplied by E. Merck (Darmstadt) or Sephadex LH-20 manufactured by Pharmacia Fine Chemicals, AB, Uppsala, Sweden. Some of the HPLC procedures were conducted by Dr. M. Inoue using an Altex System.

Melting points were observed using a Kofler melting point apparatus. For optical rotation measurements the solute concentration was 1.0 in chloroform. Ultraviolet spectra were measured in methanol solution.

Boc-L-Leu-L-Pro-OMe (3). To a stirred solution (under nitrogen) of Boc-L-Leu (5.15 g, 22.3 mM) in dry tetrahydrofuran (120 mL) cooled to −23 °C (dry ice–carbon tetrachloride) was added N-methylmorpholine (2.45 mL, 22.3 mM) and isobutyl chloroformate (2.89 mL, 22.3 mM). After stirring 15 min at −23 °C, a solution of L-Pro-OMe HCl (3.69 g, 22.3 mM) in dry dimethylformamide (15 mL) was added followed by N-methylmorpholine (2.45 mL, 22.3 mM). After replacement with an ice bath the solution was stirred 16 h (allowing to warm to room temperature) and filtered and the solid rinsed with tetrahydrofuran. The filtrate solvent was evaporated and the residue dissolved in ethyl acetate (325 mL). The organic phase was washed with water (2 × 100 mL), 1% citric acid (2 × 100 mL), 2% sodium bicarbonate (2 × 100 mL), and brine (1 × 100 mL) and dried. After removal of solvent, the resultant clear oil was chromatographed on a column of silica gel (100 g). Elution was conducted with ethyl acetate–methylene chloride by increasing the ratio from 1:9 to 1:3. Fractions with TLC R_f 0.31 (ethyl acetate–methylene chloride, 1:5) were collected and the solvent evaporated to give 6.07 g (80%) of dipeptide 3 as a colorless syrup: $[\alpha]^{25}_D$ −70.6°.

Satisfactory elemental analyses were obtained (see below) for its hydrochloride derivative (4).

L-Leu-L-Pro-OMe (4)·HCl. Method A. To a saturated ethereal solution of dry hydrogen chloride (3 mL) at 0 °C was added Boc-L-Leu-L-Pro-OMe (3, 0.20 g, 0.58 mM) with stirring. After warming to room temperature and stirring for 15 min, ether (30 mL) was added and the resultant precipitate collected. The solid was recrystallized once from methylene chloride–ether to afford 0.14 g (86%) of dipeptide 4 hydrochloride as colorless crystals: mp 186–194 °C dec (lit.[4] mp 176–178 °C); $[\alpha]^{25}_D$ −87.7°; ^{13}C NMR ($CDCl_3$) δ 22.16 (q), 23.20 (q), 24.24 (d), 24.99 (t), 29.05 (t), 39.84 (t), 47.35 (t), 50.79 (d), 52.19 (q), 59.24 (d), 168.88 (s), 172.32 (s); MS (SP-SIMS), m/z 243 $[M - Cl]^+$. Anal. Calcd for $C_{12}H_{23}ClN_2O_3$: C, 51.70; H, 8.32; Cl, 12.72; N, 10.05. Found: C, 51.66; H, 8.19; Cl, 12.52; N, 9.91.

Method B. The peptide bond forming reaction (see above) between Boc-L-Leu (4.88 g, 21.1 mM), isobutyl chloroformate (2.74 mL, 21.1 mM), and Pro-OMe·HCl (3.49 g, 21.1 mM) was repeated. The resulting clear oil (6.7 g) was dissolved in a solution of saturated hydrogen chloride in ethyl ether (75 mL) at 0 °C and the cleavage reaction of method A was repeated. After the mixture was stirred for 10 min at 0 °C and 15 min at room temperature (monitoring by TLC), ether (400 mL) was added. The mixture was allowed to stand at 0 °C for 1 h. After filtration, washing of the solid with ether, and recrystallization from methylene chloride–ether 4.02 g (68%) of the hydrochloride was obtained. The product was found identical[9] with that obtained using method A.

Boc-L-Val-L-Leu-L-Pro-OMe (5). To a stirred solution of Boc-L-Leu-L-Pro-OMe (3, 4.37 g, 12.76 mM) in methylene chloride (22 mL) cooled to 0 °C was added trifluoroacetic acid (22 mL, dropwise). The solution was warmed to ambient temperature and stirred for 40 min. Carbon tetrachloride (25 mL) was added and the solvent removed. Addition of carbon tetrachloride (25 mL) and evaporation was repeated twice more to remove excess trifluoroacetic acid. The resulting thick syrup (L-Leu-L-Pro-OMe·TFA) was placed under a high vacuum for 1 h.

A solution of Boc-L-Val (2.52 g, 11.6 mM) in dry tetrahydrofuran (60 mL, under a nitrogen atmosphere) was cooled to −23 °C (dry ice–carbon tetrachloride) with magnetic stirring and N-methylmorpholine (1.28 mL, 11.6 mM) plus isobutyl chloroformate (1.50 mL, 11.6 mM) was added. The mixture was stirred for 15 min

(6) Pettit, G. R. "Synthetic Peptides"; Elsevier Science Publishers: Amsterdam, 1976; Vol. 4.

(7) The ^{1}H NMR spectra of diastereomer 2 (lower TLC R_f component) of cyclic peptide 2 was not included due to ambiguities arising from a minor impurity.

(8) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.* **1983**, *46*, 917–922.

(9) Identity was determined by comparison of ^{1}H NMR and IR spectra and thin-layer chromatographic mobilities.

at −23 °C and a solution of the above prepared L-Leu-L-Pro-OMe·TFA in dry dimethylformamide (20 mL) was added followed by *N*-methylmorpholine (1.28 mL, 11.6 mM). The mixture (ice-bath cooling) was stirred 16 h while allowing the temperature to warm to ambient. The solution was filtered, solid rinsed with tetrahydrofuran, and the filtrate concentrated to an oil. A solution of the oil in ethyl acetate (300 mL) was washed with water (1 × 100 mL), 1% citric acid (2 × 100 mL), 1% sodium bicarbonate (2 × 100 mL), and brine (1 × 100 mL) and dried. The yellow oil obtained upon removal of solvent was chromatographed on a column of silica gel (100 g). Successive elution with 1:5 (200 mL), 1:2 (500 mL), and 1:1 (500 mL) ethyl acetate–methylene chloride gave a series of fractions where those with R_f 0.30 (ethyl acetate–methylene chloride, 1:2) were combined to afford 2.4 g (27%) of a colorless solid which resisted all attempts at recrystallization: mp 76–79 °C; $[\alpha]^{25}_D$ −73.4°; MS (SP-SIMS), *m/z* 442 $[M + H]^+$. Anal. Calcd for $C_{22}H_{39}N_3O_6$: C, 59.84; H, 8.90; N, 9.52. Found: C, 59.77; H, 8.88; N, 9.59.

L-Val-L-Leu-L-Pro-OMe (6)·HCl. Method A. To a stirred solution of Boc-L-Val-L-Leu-L-Pro-OMe (**5**, 1.67 g, 3.8 mM) in dry ether (40 mL, at 0 °C) was added (with stirring) a saturated ethereal solution of dry hydrogen chloride (20 mL). After 1.5 h at room temperature the solid was collected, rinsed with ether, and recrystallized from ethyl acetate–methanol–pentane to afford 0.40 g of tripeptide **6** hydrochloride. To the filtrate was added saturated ethereal hydrogen chloride (10 mL) followed by pentane until cloudiness was observed. The reaction mixture was allowed to stand overnight at ambient temperature and solid collected, rinsed with ether, and recrystallized once from ethyl acetate–methanol–pentane to yield an additional 0.99 g of colorless solid to provide a total yield of 1.39 g (98%): mp 221–229 °C dec; $[\alpha]^{25}_D$ −81.2°; IR (KBr) 3490 br, 3370, 2990, 1756, 1695, 1658, 1547, 1499, 1477 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88–1.24 (m, 12 H), 1.31–2.56 (m, 8 H), 3.40–4.11 (m, 6 H, contains OMe singlet at 3.74), 4.53 (m, 1 H, asymmetric H), 4.83 (m, 1 H, asymmetric H), 8.01 (d, *J* = 8 Hz, 1 H, NH), 8.21–9.24 (br s, 3 H, NH_3^+); ^{13}C NMR ($CDCl_3$) δ 16.68 (q), 18.91 (q), 22.06 (q), 23.23 (q), 24.60 (d), 25.05 (t), 29.08 (t), 30.35 (d), 40.52 (t), 47.18 (t), 49.59 (d), 52.23 (q), 59.17 (d), 60.05 (d), 167.61 (s), 170.89 (s), 172.71 (s); MS (SP-SIMS), *m/z* 342 $[M - Cl]^+$. Anal. Calcd for $C_{17}H_{32}ClN_3O_4$: C, 54.03; H, 8.54; Cl, 9.38; N, 11.12. Found: C, 53.76; H, 8.61; Cl, 9.57; N, 10.91.

Method B. The reaction between Boc-L-Val (2.34 g, 10.8 mM), isobutyl chloroformate (1.40 mL, 10.8 mM), and L-Leu-L-Pro-OMe (**4**)·HCl (3.00 g, 10.8 mM) was repeated and the solid product (4.53 g) was dissolved in a saturated solution of hydrogen chloride in ether (35 mL) at 0 °C. After stirring for 10 min at 0 °C and 23 min at room temperature (monitoring by TLC), ether (200 mL) was added and the mixture stored at 0 °C for 1.5 h to allow complete precipitation. The solid hydrochloride was washed (ether) to provide 3.52 g (86%, once recrystallized from ethyl acetate–methanol–pentane). The hydrochloride was found identical[9] with the product of method A.

***N*-Z-(*R* and *S*)-(gln)Thz (7).** To a magnetically stirred solution of *N*-Z-(*R* and *S*)-(gln)Thz-OEt (1.63 g, 4.2 mM)[1] in dimethoxyethane (40 mL)–water (30 mL) was added 1.0 N sodium hydroxide (4.6 mL, 4.6 mM). The mixture was stirred for 1 h at room temperature, extracted with ether (2 × 80 mL), and acidified with 2.6 N hydrochloric acid. A precipitate formed immediately and upon standing for 15 h (at 5 °C); the solid was collected, rinsed with several portions of water, and dried under reduced pressure. Recrystallization from methanol–chloroform–pentane afforded 1.32 g (87%) of crystals melting at 185–186 °C: UV λ_{max} 235 nm (ε 6570). Anal. Calcd for $C_{16}H_{17}N_3O_5S$: C, 52.88; H, 4.72; N, 11.56; S, 8.82. Found: C. 52.67; H, 4.50; N, 11.52; S, 8.55.

***N*-Z-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (8a).** To a stirred (magnet) solution (under nitrogen) of *N*-Z-(*R* and *S*)-(gln)Thz (**7**, 0.85 g, 2.34 mM) in dry tetrahydrofuran (20 mL)–dimethylformamide (4 mL) cooled to −23 °C (dry ice–carbon tetrachloride) was added *N*-methylmorpholine (0.26 mL, 2.34 mM) and isobutyl chloroformate (0.30 mL, 2.34 mM). Before slowly adding a solution of L-Val-L-Leu-L-Pro-OMe (**6**)·HCl (0.97 g, 2.56 mM) in dry dimethylformamide (5 mL) followed by *N*-methylmorpholine (0.26 mL, 2.34 mM), stirring and cooling (−23 °C) were continued 15 min. After warming to 0 °C (ice bath) stirring was continued 18 h while reaching room temperature. The solution was filtered (solid rinsed with tetrahydrofuran), concentrated, dissolved in ethyl acetate (90 mL), and washed with water (1 × 25 mL), 2% citric acid (1 × 25 mL), 2% sodium bicarbonate (1 × 25 mL), and brine (1 × 25 mL). Solvent was removed and the residue was chromatographed on a column of silica gel (60 g). Upon elution with methanol–chloroform (1:19) 1.51 g (94%) of a colorless amorphous solid was obtained which represented a mixture of diastereomers (evidenced by TLC, R_f 0.46 and 0.50, methanol–chloroform, 1:9): UV λ_{max} 231 (ε 11 120); ^{1}H NMR ($CDCl_3$) δ 3.69 (s, OMe), 5.14 (s, CH_2Ph), 7.35 (s, C_6H_5), 8.00 and 8.02 (2s, thiazole protons); ^{13}C NMR ($CDCl_3$) δ 52.2 (q, OMe), 67.2 (t, CH_2Ph), 128.2 and 128.6 (2d, C5 thiazole), 149.5 and 149.6 (2s, C2 thiazole), 160.8 and 160.9 (2s, C4 thiazole). Anal. Calcd for $C_{33}H_{46}N_6O_8S·H_2O$: C, 56.23; H, 6.86; N, 11.92; S, 4.55. Found: C, 56.45; H, 6.62; N, 11.93; S, 4.34.

Approximately 5 mg of each diastereomer was obtained in pure form by HPLC (Altex system) using a methanol–methylene chloride–water gradient (solvent A 1:99:0.05, solvent B 25:75:2.5, 0–30% B: 180 min, 2 mL/min) and the following physical data were recorded.

Diastereomer A: TLC R_f 0.50 (methanol–chloroform, 1:9); mp 102–105 °C; $[\alpha]^{25}_D$ −26.0° (*c* 0.27, $CHCl_3$); IR (KBr) 3320 br, 2980, 1730, 1553, 1550, 1504, 1460, 1253, 1056, 755, 709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95–1.04 (m, 12 H), 1.26 (m, 1 H), 1.48–2.38 (m, 11 H), 3.55–3.87 (m, 5 H, OMe singlet at 3.68), 4.40–4.61 (m, 2 H, asymmetric H), 4.68–5.08 (m, 2 H, asymmetric H), 5.15 (s, 2 H, CH_2Ph), 5.77 and 5.99 (2 br s, 2 H, $CONH_2$), 6.19 (d, *J* = 8 Hz, 1 H, NH), 7.04 (d, *J* = 8 Hz, 1 H, NH), 7.35 (s, 5 H, C_6H_5), 7.83 (d, *J* = 9.5 Hz, 1 H, NH), 8.00 (s, 1 H, thiazole); MS (SP-SIMS), *m/z* 709 $[M + Na]^+$, 687 $[M + H]^+$.

Diastereomer B: TLC R_f 0.46 (methanol–chloroform, 1:9); mp 100–103 °C; $[\alpha]^{25}_D$ +17.0° (*c* 0.17, $CHCl_3$); IR (KBr) 3430 br, 2980, 1730, 1653, 1550, 1503, 1460, 1255, 1056, 760, 709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89–1.04 (m, 12 H), 1.26 (m, 1 H), 1.40–2.64 (m, 11 H), 3.56–3.92 (m, 5 H, OMe singlet at 3.69), 4.42–4.59 (m, 2 H, asymmetric H), 4.70–5.03 (m, 2 H, asymmetric H), 5.14 (s, 2 H, CH_2Ph), 5.67 and 6.04 (2 br s, 2 H, $CONH_2$), 5.91 (d, *J* = 8.4 Hz, 1 H, NH), 7.15 (d, *J* = 8.4 Hz, 1 H, NH), 7.35 (s, 5 H, C_6H_5), 7.85 (d, *J* = 9.7 Hz, 1 H, NH), 8.02 (s, 1 H, thiazole); MS (SP-SIMS), *m/z* 709 $[M + Na]^+$, 687 $[M + H]^+$.

2-(Aminomethyl)thiazole-4-carboxylic Acid Hydrochloride [(gly)Thz·HCl, 12]. A mixture of ethyl 2-(benzamidomethyl)thiazole-4-carboxylate (2.14 g)[5] and 5.5 N hydrochloric acid (25 mL) was stirred and heated under nitrogen at reflux for 16 h. The solution was allowed to cool and stored at 0 °C for 3 h. The mass of colorless needles that crystallized from the solution was collected and washed with ether (150 mL). A TLC showed that the ether-soluble portion consisted of pure benzoic acid. The aqueous filtrate was extracted with ether (2 × 50 mL) and concentrated to dryness and the residue crystallized from methanol–ether. Both crops of ether-insoluble crystals (1.16 and 0.13 g) weighed a total of 1.29 g (90%) and were shown to be (gly)-Thz·HCl (**12**): mp 268–270 °C dec (lit.[5] mp 269–270.5 °C); MS (SP-SIMS) (glycerol), *m/z* 159 [free base + H]$^+$.

***N*-Boc-(gly)Thz (10). Method A.** To 1.0 N sodium hydroxide (52 mL, 52 mM) was added (gly)Thz·HCl (**12**, 5.0 g, 25.7 mM) and dimethoxyethane (50 mL). The mixture was cooled (ice bath), di-*tert*-butyl pyrocarbonate (5.6 g, 25.7 mM) in dimethoxyethane (10 mL) was slowly added to 0 °C, and stirring was continued for 30 min at 0 °C and 1 h at ambient temperature. After acidification to pH 2 with 2.7 N hydrochloric acid followed by filtration the solution was extracted with ethyl acetate (2 × 80 mL). The combined extracts were dried and solvent was removed in vacuo to yield a colorless solid. Recrystallization from methanol–ether–pentane furnished 2.3 g (35%) of colorless crystals melting at 185–186 °C: UV λ_{max} 235 nm (ε 6570); IR (KBr) 3470, 3390, 3135, 1740, 1720, 1693, 1540, 1523, 1430, 1378, 1292, 1243, 1175, 1120, 1057, 940, 927, 780, 754, 620 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.43 (s, 9 H, CMe_3), 4.46 (d, *J* = 6, 2 H, CH_2), 7.74–8.01 (m, 1 H, NH), 8.43 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 28.1 (q),[10] 41.9 (t), 78.6 (s), 128.4 (d), 146.8 (s), 155.7 (s), 162.0 (s), 171.4 (s); MS (SP-SIMS), *m/z* 281 $[M + Na]^+$, 259 $[M + H]^+$. Anal. Calcd for $C_{10}H_{14}N_2O_4S$: C, 46.50; H, 5.46; N, 10.85; S, 12.41. Found: C, 46.39; H, 5.48; N, 10.85; S, 12.33.

Method B. To (gly)Thz·HCl (**12**, 1.16 g, 6 mM) suspended in dioxane (12 mL) was added 1 N sodium hydroxide (12 mL). The stirred mixture was cooled to 0 °C and a solution of di-

Table I. High-Resolution (400 MHz, $CDCl_3$ Solution) ^1H NMR Comparison of Dolastatin 3 (1) and *cyclo*-[(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro] (2)

	dolastatin 3[3]		cyclic peptide 2 (diastereomer 1)[7]	
position	chem shift, ppm	multiplicity (*J*, Hz)	chem shift, ppm	multiplicity (*J*, Hz)
1	3.69	1 H, m	3.42	1 H, m
	3.85	1 H, m	3.61	1 H, m
2	1.9–2.3	2 H, m	1.56–1.98	2 H, m
3	1.9–2.3	2 H, m	1.56–1.98	2 H, m
4	3.98	1 H, t (*J* = 7.9)	4.45	1 H, t (*J* = 8.7)
6	3.85	1 H, m	4.17	1 H, m
7	2.14	1 H, dd	1.56–1.98	1 H, m
8	1.53	1 H, m	1.23–1.45	1 H, m
9	0.957	3 H, d (*J* = 6.6)	1.011	3 H, d (*J* = 6.4)
10	0.905	3 H, d (*J* = 6.6)	0.995	3 H, d (*J* = 6.4)
12	4.76	1 H, dd (*J* = 7.4, 9.2)	4.35	1 H, m
13	2.06	1 H, m	1.23–1.45	1 H, m
14	1.048	3 H, d (*J* = 6.6)	0.767	3 H, d (*J* = 6.4)
15	1.161	3 H, d (*J* = 6.8)	0.860	3 H, d (*J* = 6.4)
17	5.54	1 H, ddd (*J* = 9.0, 10.6, 4.2)	5.54	1 H, dd (*J* = 7.5, 9.5)
18	2.54	2 H, m	2.45	2 H, m
19	2.30	2 H, m	2.15	2 H, m
22	8.082	1H	8.138	1 H
25	5.249	1 H, dd (*J* = 7.3, 18.1)	4.94	1 H, dd (*J* = 5, 14.2)
	4.661	1 H, dd (*J* = 2.2, 18.3)	4.50	14, dd (*J* = 6.5, 14.6)
27	8.070	1 H	8.083	1 H
(1)	5.99	1 H d (*J* = 6.8)	7.31	1 H, d (*J* = 5.2)
(2)	8.32	1 H, d (*J* = 9.3)	7.93	1 H, d (*J* = 9.0)
(3)	7.86	1 H, d (*J* = 9.0)	8.71	1 H, d (*J* = 9.2)
(4)	8.76	1 H, dd (*J* = 5.4, 1–2)	9.40	1 H, m
(5)	6.31	1 H, br s	6.36	1 H, br s
	5.42	1 H, br s	5.57	1 H, br s

tert-butyl pyrocarbonate (1.44 g, 6.6 mM) in dioxane (2 mL) was added (dropwise). After 3 h of stirring at 0 °C and 1 h at room temperature the mixture was acidified to pH 2 with dilute hydrochloric acid and extracted with chloroform (2 × 50 mL). The chloroform extract was dried and concentrated in vacuo. The residue (1.4 g, 75%, pure by TLC) was recrystallized from methanol–ether to afford *N*-Boc-2-(aminoethyl)thiazole-4-carboxylic acid[9] melting at mp 188–189 °C.

***N*-[*i*-BuOC(=O)]-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (8b).** A solution of *N*-Z-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (**8a**, 103 mg, 0.16 mM) in 3.4 N hydrogen bromide–acetic acid (1.3 mL) was stirred for 75 min at room temperature. Anhydrous ether (5 mL) was added (immediate precipitation) and the solution was filtered under argon. The hydrobromide was washed with dry ether (5 mL) and stored under argon. To a cold (−23 °C) solution of *N*-Boc-(gly)Thz (**10**, 50 mg, 0.19 mM) in dry tetrahydrofuran (3 mL) was added (with stirring) *N*-methylmorpholine (0.021 mL, 0.19 mM) and isobutyl chloroformate (0.025 mL, 0.19 mM) under argon. After 15 min at −23 °C, a solution of the hydrobromide in a dry dimethylformamide (2 mL) was slowly added followed by *N*-methylmorpholine (0.021 mL, 0.19 mM). The mixture was stirred 2 h at 0 °C and 20 h at room temperature. Ethyl acetate (40 mL) was added and the organic phase was washed with water (1 × 15 mL), 2% citric acid (1 × 15 mL), 2% sodium bicarbonate (1 × 15 mL), and brine (1 × 15 mL). After evaporation of solvent, the residue was chromatographed on a column of silica gel (30 g) and the product eluted with chloroform–methanol (a gradient progressing from 49:1 to 24:1) to give 89 mg (91%) of tetrapeptide diastereomers **8b** as a colorless amorphous solid: ^1H NMR ($CDCl_3$) δ 0.86–1.13 (m, 18 H, 6 Me), 3.72 (s, 3 H, OMe), 3.92 (d, *J* = 7 Hz, OCH_2), 8.12 and 8.14 (2s, 1 H, thiazole); MS (SP-SIMS) (glycerol–silver tetrafluoroborate),[8] *m*/*z* 759 $[M + Ag^{107}]^+$, 761 $[M + Ag^{109}]^+$, as very intense molecular ions. Anal. Calcd for $C_{30}H_{48}N_6O_8S \cdot 0.1H_2O$: C, 55.04; H, 7.42; N, 12.84; S, 4.90. Found: C, 54.64; H, 7.33; N, 12.58; S, 5.45.

***N*-Boc-(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (11).** A solution of *N*-Z-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (**8a**, 105 mg, 0.15 mM) in 3.4 N hydrogen bromide–acetic acid (1.3 mL) was stirred for 70 min at room temperature. Anhydrous ether (5 mL) as added (immediate precipitation), the solution was filtered under argon, and the resultant hydrobromide of **9** was rinsed with anhydrous ether (5-mL portions) and stored under argon.

To a stirred solution (under argon, cooled to 0 °C) of *N*-Boc-(gly)Thz (**10**, 50 mg, 0.19 mM) in dry tetrahydrofuran (3 mL) was added hydroxybenzotriazole (60 mg, 0.39 mM) and dicyclohexylcarbodiimide (40 mg, 0.19 mM). The solution was stirred for 1 h at 0 °C and 1 h at room temperature and recooled to 0 °C, and the hydrobromide of **9** in dry dimethylformamide (3 mL) was slowly added followed by *N*-methylmorpholine (0.02 mL, 0.19 mM). Before adding ethyl acetate (40 mL) the mixture was stirred 16 h as it warmed to room temperature. The ethyl acetate solution was washed successively with water (1 × 15 mL), 2% citric acid (1 × 15 mL), 2% sodium bicarbonate, and brine (1 × 15 mL). Solvent was removed, the residue was chromatographed on a narrow column of silica gel (30 g), and elution with 3:97 (200 mL) and 5:95 (500 mL) methanol–chloroform was used to obtain a series of fractions with TLC R_f 0.37 (methanol–chloroform, 1:9), yield 0.11 g (91%) of the colorless amorphous diastereomers of pentapeptide 11: $[\alpha]^{25}_D$ −33.6° (CH_3OH); UV λ_{max} 234 nm (ε 22 720); ^1H NMR ($CDCl_3$) δ 0.81–1.14 (m, 4 Me), 1.46 and 1.47 (2s, CMe_3), 3.70 (s, 3 H, OMe), 8.090, 8.087, 8.071, and 8.061 (4s, thiazole protons); ^{13}C NMR ($CDCl_3$) δ 123.9, 124.0, 124.4, 124.6 (4d, C5 thiazole), 148.8, 148.9, 149.2, 149.4 (4s, C2 thiazole), 160.8,[10] 161.0, 161.2 (3s, C4 thiazole); MS (SP-SIMS), *m*/*z* 815 $[M + Na]^+$, 793 $[M + H]^+$. Anal. Calcd for $C_{35}H_{52}N_8O_9S \cdot H_2O$: C, 51.84; H, 6.71; N, 13.82; S, 7.92. Found: C, 51.61; H, 6.79; N, 13.58; S, 7.47.

***cyclo*-[(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro][11,12] (2).** To a stirred 0.1 N aqueous sodium hydroxide solution (3.0 mL) was added *N*-Boc-(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro-OMe (**11**, 102 mg, 0.13 mM) and enough 1,4-dioxane to complete solution. After stirring for 7 h at room temperature the mixture was neutralized with 0.1 N hydrochloric acid and extracted with chloroform (3 × 15 mL). The combined extract was dried and the solvent evaporated. The residue **13a** was

(10) Carbon resonances superimposed as evidenced by area integration.

(11) For a review of *cyclo*-peptide syntheses, refer to: Pettit, G. R. In "Synthetic Peptides"; Elsevier Scientific: Amsterdam, 1982; Vol. 6 and 1–4, especially Vol. 4. An important study of racemization in peptide cyclization reactions was recently summarized by: Ji, A.; Bodanszky, M. *Int. J. Peptide Protein Res.* **1983**, *22*, 590–596.

(12) Over a year following completion of this study Drs. T. Shioiri and Y. Hamada thoughtfully informed us in a private communication (Aug 13, 1983) that they had synthesized this reverse isomer (**2**) at Nagoya City University in the all-L configuration and it exhibited $[\alpha]^{27}_D$ −134° (*c* 0.05, CH_3OH).

Table II. ^{13}C NMR ($CDCl_3$ Solution) Comparison of Dolastatin 3 (1) and *cyclo*-[(gly)Thz-(*R* and *S*)-(gln)Thz-L-Val-L-Leu-L-Pro] (2)

		cyclic peptide 2	
position	dolastatin 3	diastereomer 1	diastereomer 2
1	48.3	50.7	47.0
2	25.5	24.7	23.5
3	29.7	32.3	31.3
4	62.6	60.9	60.9
5	169.5	170.2	170.5
6	48.6	51.3	51.6
7	41.0	46.1	42.2
8	25.5	29.6	25.0
9	23.3	23.4	22.1
10	21.2	22.0	21.3
11	171.9	172.9	172.5
12	55.7	59.7	56.8
13	31.8	34.6	32.9
14	18.6	18.9	17.5
15	19.6	18.9	18.7
16	171.1	170.6	170.7
17	55.0	58.4	51.6
18	29.7	32.9	32.2
19	33.3	34.6	33.3
20	165.8	166.5	167.5
21	149.1	148.3	148.7
22	124.4	126.3	124.7
23	161.0	161.8	162.6
24	174.8	174.1	174.0
25	37.7	41.8	39.7
26	148.3	148.0	148.6
27	123.8	124.4	124.5
28	160.2	160.2	160.1
29	171.2	170.7	170.8

dissolved in dry dimethoxyethane (3 mL) and treated with 2,4,5-trichlorophenol (39 mg, 0.19 mM) and dicyclohexylcarbodiimide (40 mg, 0.19 mM) at 0 °C. After stirring (under argon) for 0.5 h at 0 °C and 3 h at room temperature the solvent was removed and the residue chromatographed on a column of silica gel (35 g). Fractions eluted with methanol–chloroform (1:19) led to 109 mg of 2,4,5-trichlorophenol ester **13b** (TLC R_f 0.41, methanol–chloroform, 1:9) as a light yellow oil: ^1H NMR ($CDCl_3$) δ 1.47 (s, 9 H, CMe_3), 7.35, 7.58 (2s, 2 H, OTcp), 8.18, 8.22 (2s, 2 H, thiazole).

Active ester **13b** (109 mg) in methylene chloride (1 mL) at 0 °C was treated with trifluoroacetic acid (1 mL). The reaction mixture was immediately warmed to room temperature and stirred for 1 h. Carbon tetrachloride (25 mL) was added and the solvent evaporated. Carbon tetrachloride (25 mL) was again added and evaporated and the residue subjected to high vacuum for 1 h. To the residue was added pyridine–tetrahydrofuran (1:5, 350 mL) and the solution was stirred for 43 h at room temperature. Solvent was removed and the crude mixture was initially separated by Sephadex LH-20 gel exclusion chromatography (methanol elution) to give 75 mg (90% crude yield) of two major components (TLC R_f 0.22 and 0.25, methanol–methylene chloride, 1:9) as a clear oil. The partially purified product was chromatographed on a long slender column of silica gel (35 g). Elution with methanol–chloroform (4:96) yielded 20 mg (24% overall) of the two diastereomers corresponding to cyclic pentapeptide **2** as amorphous solids: 9 mg of the R_f 0.25 component (diastereomer 1) and 11 mg of the R_f 0.22 component (diastereomer 2).

Diastereomer 1: TLC R_f 0.25 (methanol–methylene chloride, 1:9); mp 190–194 °C; $[\alpha]^{25}_D$ −67.5° (CH_3OH); UV λ_{max} 229, 253 nm (ϵ 7560, 7440); IR (KBr) 3405, 3125 br, 2980, 1649 br, 1563, 1550, 1503, 1456, 1353, 1320, 1294, 1248, 1173, 1065, 762 cm^{-1}; MS (EI) exact mass, m/z 548.1729 (calcd 548.1750 for $C_{23}H_{30}N_7O_5S_2$), 435.1051 (calcd 435.1035 for $C_{18}H_{21}N_5O_4S_2$), 407.1065 (calcd 407.1086 for $C_{17}H_{21}N_5O_3S_2$), 266.0951 (calcd 266.0963 for $C_{12}H_{16}N_3O_2S$), 195.0234 (calcd 195.0228 for $C_8H_7N_2O_2S$); MS (EI), m/z (relative intensity) 660 (M^+, 26), 617 (10), 604 (33), 574 (24), 548 (39), 520 (5), 476 (3), 435 (43), 407 (62), 361 (9), 309 (8), 294 (25), 266 (90), 236 (18), 209 (24), 195 (100), 178 (38), 167 (46), 152 (49), 112 (85); MS (SP-SIMS) (glycerol matrix), m/z (relative intensity) 661 ($M + H^+$, 100), 633 (16), 549 (20), 521 (70), 435 (10), 423 (30), 406 (30), 284 (14), 269 (32), 213 (24). The ^1H and ^{13}C NMR spectra are presented in Tables I and II. Anal. Calcd for $C_{29}H_{40}N_8O_6S_2 \cdot 2.7H_2O$: C, 49.14; H, 6.40; N, 15.81; S, 9.05. Found: C, 49.34; H, 6.39; N, 15.31; S, 9.43.

Diastereomer 2: TLC R_f 0.22 (methanol–methylene chloride, 1:9); the high-resolution (400 MHz) ^1H NMR ($CDCl_3$) spectrum showed traces of an impurity so only the δ 8.07 and 8.15 (2s, thiazole protons) signals were recorded here; the ^{13}C NMR spectrum is summarized in Table II and the MS (EI and SP-SIMS) m/z showed 660 M^+ and 661 $[M + H]^+$.

In the TLC system 10:1:0.1 methylene chloride–methanol–water as mobile phase on silica gel natural[3] dolastatin 3 exhibits R_f 0.27. By comparison diastereomers 1 and 2 are slightly more polar and lead to R_f 0.24.

Acknowledgment. The very necessary financial assistance for this research was contributed by Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, Eleanor W. Libby, the Waddell Foundation (David Ware), The Upjohn Co., the Flinn Foundation, and the Robert B. Dalton Endowment Funds. This investigation was also supported by PHS Grant CA-16049-08A2, awarded by the National Cancer Institute, DHHS, and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723). C.W.H. received financial support from the South African Council for Scientific and Industrial Research.

Registry No. **1**, 80387-90-2; (*S*)-**2**, 92619-33-5; (*R*)-**2**, 92619-34-6; **3**, 68624-06-6; **4**·HCl, 5879-26-5; **5**, 96363-13-2; **6**·HCl, 96363-14-3; (*R*)-**7**, 96363-15-4; (*S*)-**7**, 95716-10-2; (*R*)-**8a**, 96394-07-9; (*S*)-**8a**, 96443-75-3; (*R*)-**8b**, 96394-08-0; (*S*)-**8b**, 96443-76-4; (*R*)-**9**·HBr, 96394-09-1; (*S*)-**9**·HBr, 96443-77-5; **10**, 71904-80-8; (*R*)-**11**, 96443-78-6; (*S*)-**11**, 96363-16-5; **12**, 63628-60-4; (*R*)-**13a**, 96363-17-6; (*S*)-**13a**, 96442-77-2; (*R*)-**13b**, 96363-18-7; (*S*)-**13b**, 96442-78-3; (*R*)-**13c**, 96479-26-4; (*S*)-**13c**, 96394-11-5; Boc-L-Leu, 13139-15-6; L-Pro-OMe·HCl, 2133-40-6; L-Leu-L-Pro-OMe·TFA, 96363-19-8; Boc-L-Val, 13734-41-3; *N*-Z-(*R*)-(gln)Thz-OEt, 96442-79-4; *N*-Z-(*S*)-(gln)Thz-OEt, 96442-80-7; 2,4,5-trichlorophenol, 95-95-4; ethyl 2-(benzamidomethyl)thiazole-4-carboxylate, 88219-00-5.