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

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Received February 27, 1985

With application of mixed carbonic anhydride and dicyclohexylcarbodiimide–*N*-hydroxysuccinimide peptide bond forming methods culminating in a 2,4,5-trichlorophenol active ester promoted cyclization reaction, synthesis (cf., Scheme I) of *cyclo*-[L-Pro-L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz] was completed. Comparison of this cyclic peptide with the Indian Ocean sea hare cell growth inhibitory constituent dolastatin 3 clearly showed that the natural peptide possesses a closely related structure. The high resolution (400 MHz) ^{1}H NMR and ^{13}C NMR spectra of the synthetic peptides were evaluated in detail and suggested that dolastatin 3 may contain (*R*)-(gln)Thz and/or a modified Pro-Leu-Val sequence.

Some species of *Streptomyces* are adept at biosynthesis of thiazoles from cysteine and the resulting structurally diverse antibiotics (Chart I) range from althiomycin (**1**)[2] to the cyclic peptides nosiheptide (**2**)[2] and thiostrepton.[3] Fortunately, the structure of nosiheptide yielded to an X-ray crystal structure determination[2] and thiotrepton to a combination of spectral and crystal structure methods.[3,4] Thiazole-containing cyclic peptides of animal origin have so far been limited to the cell growth inhibitory marine tunicate (*Lissoclinum patella*)[5,6] and sea hare (*Dolabella auricularia*)[7,8] constituents such as patellamide B (**3**)[6] and dolastatin 3 (**4**).[8] Both sets of marine animal thiazole peptides proved unsuitable for crystal structure analysis and required extensive spectral and degradation studies.[5,8] Interestingly, the tunicate peptides were found relatively abundant and obtained in quantities quite sufficient for complete structural assignments, whereas the opposite was true for the sea hare peptides.

The cyclic peptide dolastatin 3 was one of nine such potentially important cell growth inhibitory (murine P388 lymphocytic leukemia) substances isolated in about 1-mg quantities from the Indian Ocean sea hare *D. auricularia*.[8] Structural investigations while greatly restricted by the 1 mg available did show that the compound was composed of five amino acids in a 1:1:1:1:1 molar ratio. The amino acids were identified as proline, valine, leucine, and two thiazole amino acids, (gly)Thz (**5a**) and (gln)Thz (**5b**). Spectral evidence for the amino acid sequence suggested structure 4 or the reverse order of peptide bonding which was considered less likely[8] and subsequently eliminated by comparison with a specimen obtained by total synthesis.[1] Since chirality of the dolastatin 3 amino acids could not be determined,[9] this aspect of the structural proposal (**4**) limited a direct approach to the establishment or removal of structure **4** by total synthesis. One of the more likely stereoisomers of structure proposal **4** would be an all-L configuration for the Pro-Leu-Val unit and either *R* or *S* for (gln)Thz.[9] The present investigation was undertaken to obtain such a specimen of structure **4** by total synthesis for comparison with dolastatin 3.

The *N*-Boc-(gly)Thz (**5c**)[1] and the *N*-Z-(gln)Thz (**5d**)[10] required for this study were prepared by methods based on the Hantzsch thiazole synthesis. The (gln)Thz prepared via an L-iso-Gln thioamide was racemized during the Hantzsch cyclization step.[10] Although (gln)Thz was resolved by fractional crystallization of its brucine salt, the yield was unattractive. Therefore, it was decided to undertake the synthesis of cyclic peptide **4** using racemic (gln)Thz with the expectation of separating the diastereomeric intermediates or final products and thereby obtaining both (*R*)- and (*S*)-(gln)Thz isomer of peptide **4**. General strategy of the synthesis was based on preparing the protected linear pentapeptide **12** as immediate precursor of cyclic peptide **4**. The decision to use proline as terminal amino acid was based on the resistance of Pro-OMe to racemization during saponification.[11] Pentapeptide **12** was obtained by using the sequence of reactions summarized in Scheme I.

By means of a mixed carbonic anhydride procedure L-Pro-OMe and *N*-Boc(gly)Thz were coupled in good yield. The resultant protected dipeptide **6** was found to exist at room temperature as a ca. 2:1 mixture of s-cis and s-trans forms as a result of restricted rotation about the central amide C–N bond and this complicated interpretation of ^{1}H and ^{13}C NMR spectra. However, spectra obtained at 100 °C in Me_2SO-d_6 were easily interpreted. Dipeptide **6** was deprotected by treatment with trifluoroacetic acid and the corresponding amine was condensed (at −23 °C) with Z-(*R*)- and -(*S*)-(gln)Thz using the mixed carbonic anhydride method. The product, protected tripeptide **8**, was chromatographically homogenous but the diastereomeric composition was confirmed by its ^{13}C NMR spectrum. When protected tripeptide **8** was deprotected by treatment with hydrogen bromide in acetic acid and cou-

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(2) Pascard, C.; Ducruix, A.; Lunel, J.; Prangé, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418–6423.

(3) Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 814–831.

(4) Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 832–845.

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

(9) Recently a degradative method based on a 4 + 2 cycloaddition reaction of singlet oxygen (from triphenyl phosphite ozonide) with the thiazole ring of, e.g., peptide **3**, followed by methanolysis and hydrolysis reactions proved to be effective in ascertaining chirality of such thiazole amino acids. As we (ref 8, footnote 12) considered quite possible for dolastatin 3, the thiazole amino acids of the tunicate *L. patella* peptides examined by the oxygen cycloaddition reaction proved to have the *R* configuration derived from a D-amino acid: Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302–2304.

(10) Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.* **1985**, *50*, 2323.

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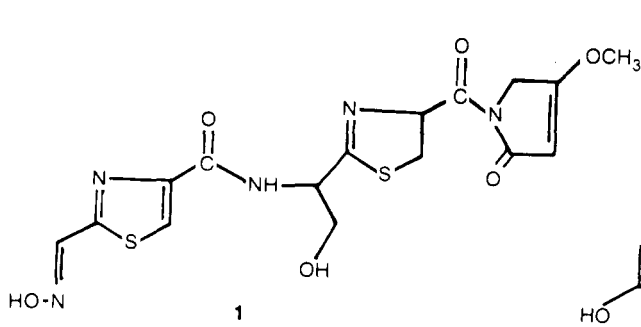
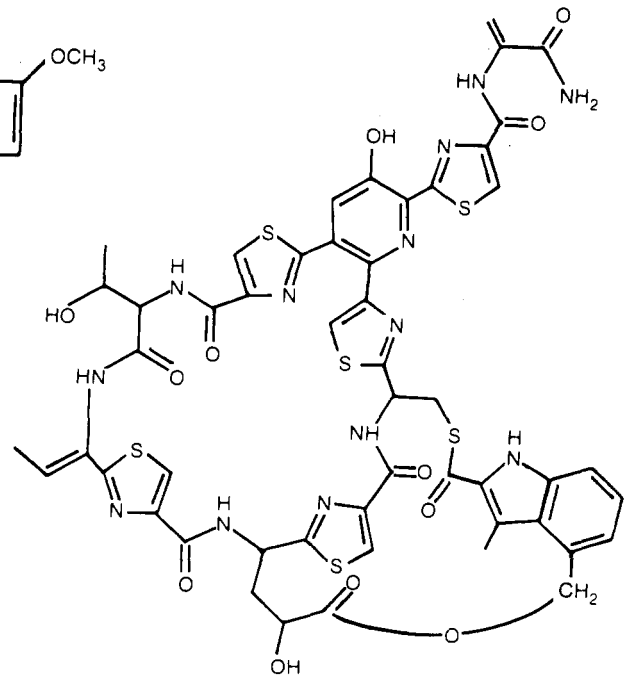
0022-3263/86/1951-4580$01.50/0

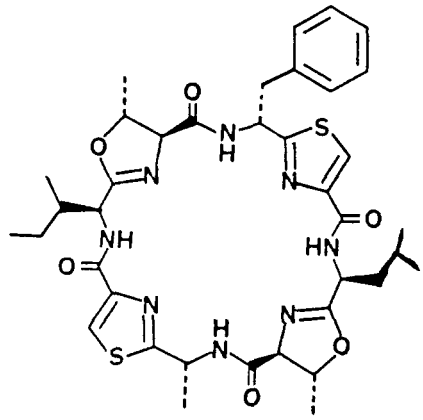
Chart I



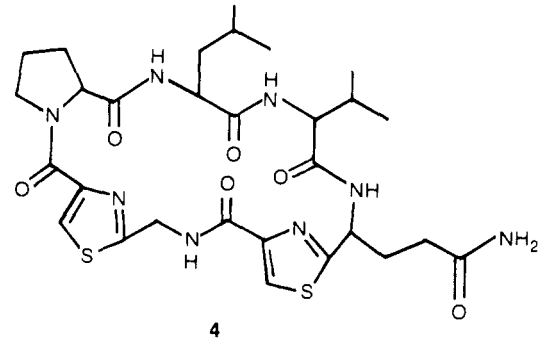
1



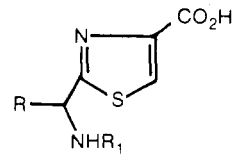
2



3



4



5a, R = R_1 = H

b, R = $CH_2CH_2CONH_2$, R_1 = H

c, R = H, R_1 = Boc-

d, R = $CH_2CH_2CONH_2$, R_1 = Z-

pled with the mixed carbonic anhydride from Boc-L-Val protected tetrapeptide, **10** was obtained in poor yield. Condensation of deprotected tripeptide **9** with Boc-L-Val using dicyclohexylcarbodiimide/*N*-hydroxysuccinimide[11] furnished Boc-L-Val-(*R*)- and -(*S*)-(gln)Thz-(gly)Thz-Pro-OMe (**10**) in excellent yield. A ^{13}C NMR spectrum of peptide **10** substantiated the diastereomeric mixture, but all attempts at separation by careful chromatography over silica gel or activated alumina were unsuccessful.

Deprotection of tetrapeptide **10** by treatment with trifluoroacetic acid followed by coupling with Boc-L-Leu using the dicyclohexylcarbodiimide/*N*-hydroxysuccinimide method led to Boc-L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (**12**). The ^{13}C NMR spectrum of peptide **12** confirmed the presence of two diastereomers in approximately equal amounts. Again the two components resisted separation even by thin layer chromatography (TLC) or high pressure liquid chromatography (HPLC) on Partisil using several solvent systems. Saponification using sodium hydroxide in dioxane–water followed by acidification provided the carboxylic acid Boc-L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz-L-Pro (**13**) which was treated with trifluoroacetic acid to furnish the trifluoroacetate salt. A solution of L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz-Pro trifluoroacetate in a large volume of dimethylformamide was adjusted to pH 7.5 by careful addition of triethylamine. The solution was cooled (−20 °C) and 2 molar equiv of diphenylphosphoryl azide (DPPA)[12] was added. The reaction was allowed to proceed at 0 °C for 4 days. Careful steric exclusion (Sephadex LH-20) and silica gel chromatography of the product yielded cyclic peptide **16** as a

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Scheme I

L-Pro-OMe → N-Boc(gly)Thz-L-Pro-OMe (6) → (gly)Thz-L-Pro-OMe (7) →

N-Z-R- and S-(gln)Thz-(gly)Thz-L-Pro-OMe (8) → R- and S-(gln)Thz-(gly)Thz-L-Pro-OMe (9) →

Boc-L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro-OMe (10) → L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro-OMe (11) →

Boc-L-Leu-L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro-OMe (12) →

Boc-L-Leu-L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro (13) →

Boc-L-Leu-L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro-OTcp (14) →

L-Leu-L-Val-R- and S-(gln)Thz-(gly)Thz-L-Pro-OTcp (15) →

cyclo[L-Pro-L-Leu-L-Val-R- and S-(gln)Thz-(gly)Thz] (16)

mixture of diastereomers, in a yield of ca. 24%.

An improved yield (ca. 45%) of cyclic peptide **16** was realized by another method based on conversion of Boc-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro to the corresponding 2,4,5-trichlorophenol ester[1,11] (**14**), deprotection by cleavage (**14** → **15**) with trifluoroacetic acid, and treating a dilute solution in tetrahydrofuran of the resulting TFA salt with pyridine. Cyclic peptide **16** was again obtained as a mixture of diastereomers. Analytical HPLC revealed the presence of two diastereomers in an approximate ratio of 2:1, which suggested some stereoselectivity in the cyclization reaction, and this has been observed[13] with certain other peptide cyclization reactions. Also we recently found the cyclization of (gly)Thz-(*R,S*)-(gln)Thz-L-Val-L-Leu-L-Pro to furnish the two diastereomers of *cyclo*-[(gly)Thz-(*R,S*)-(gln)Thz-L-Val-L-Leu-L-Pro] in unequal amounts.[1] The diastereomers were separated by preparative HPLC and the major diastereomer was isolated in a pure state by careful chromatography on silica gel. Neither of the diastereomers corresponding to structure **4** proved to be identical with dolastatin 3. On TLC (silica gel) the synthetic cyclic peptides (**4**) moved with somewhat lower R_f values compared to that of dolastatin 3. The ^{1}H NMR and ^{13}C NMR spectra of both diastereomers (Tables I and II, respectively) differed significantly from those of dolastatin 3.[8] But the overall similarity of the natural and synthetic products was very reassuring and provided good evidence for the general structure (**4**) of dolastatin 3. Especially noteworthy differences were found in the strong upfield shifts of two secondary methyls and the strong downfield shifts of protons on positions 4, 6, and 12 (protons on α-carbons of proline, leucine, and valine) of the two diastereomeric synthetic cyclic peptides compared to the corresponding protons of dolastatin 3. The pronounced NMR differences in the dolastatin 3 Leu and (gln)Thz components compared with the synthetic cyclic peptides (**16**) suggest that one or both of these amino acids may have the D configuration in the natural product. Among less likely possibilities considered, and eliminated by the spectral data, was replacement of the Gln amide by the isomeric oxime group as in the modified (gly)Thz unit of althiomycin (**1**). The preceding ^{1}H and ^{13}C NMR comparison studies of *cyclo*-[L-Pro-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz] and dolastatin 3 primarily suggest differences in chirality and/or the need for an adjustment in the Pro-Leu-Val sequence.

The conformation and biological activity of a cyclic peptide is markedly influenced by the absolute configuration of its component amino acids. Changes in side chains are usually less important in respect to conformation.[14] Therefore, NMR comparisons of dolastatin 3 containing some D-amino acid components with the L counterparts in cyclic peptide **4** should result in substantial differences. Indeed, substitution of D-Phe for L-Phe at position 2 in *cyclo*-[Phe-Phe-Phe-Gly-Gly],[14] D-Leu for L-Leu in *cyclo*-[D-Tyr(Me)-L-Ile-L-Pip-L-Leu],[15] and D- for L-MeAla in the cyclic tetrapeptide tentoxin[16] results in pronounced changes in the NMR spectra. The absolute configuration of dolastatin 3 components can be decisively evaluated when a sufficient quantity of this interesting peptide can be reisolated and that program combined with

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Table I. Cyclic Pentapeptide 4 (Isomer A) ^1H NMR (400 MHz) and ^{13}C NMR Assignments Relative to Tetramethylsilane in Deuteriochloroform Solution

struct assnmt no.	chem shift, ppm ^{13}C	chem shift, ppm ^1H	^1H, mult (J, Hz)
1	47.4	3.67	1 H, m
		3.86	1 H, m
2	24.8	1.8–2.1	2 H, m
3	29.3	1.8–2.1	2 H, m
4	60.8	4.13	1 H, t (J = 8.7)
5	169.8		
6	51.0	4.38	1 H, m
7	43.1	1.8–2.1	1 H, m
8	24.8	1.55	1 H, m
9	23.2	0.48	3 H, d (J = 6.5)
10	21.6	0.32	3 H, d (J = 6.5)
11	174.4		
12	59.7	5.62	1 H, dd (J = 7.5, 2.1)
13	30.9	1.8–2.1	1 H, m
14	18.1	1.0	3 H, d (J = 6.5)
15	19.7	0.95	3 H, d (J = 6.5)
16	170.8		
17	52.1	5.25	1 H, m
18	29.3	2.54	2 H, m
19	31.9	2.25	2 H, m
20	167.2		
21	149.6		
22	129.7	8.23	1 H, s
23	161.2		
24	174.7		
25	40.3	5.25	1 H, dd (J = 15.3)
		4.65	1 H, dd (J = 15.5)
26	148.3		
27	124.3	8.10	1 H, s
28	160.0		
29	171.7		
(1)		7.18	1 H, d (J = 7.5)
(2)		7.70	1 H, d (J = 8)
(3)		7.91	1 H, d (J = 8.5)
(4)		9.28	1 H, t (J = 5)
(5)		6.27	1 H, br s
		5.84	1 H, br s

Table II. Cyclic Pentapeptide 4 (Isomer B) ^1H NMR (400 MHz) and ^{13}C NMR Assignments Relative to Tetramethylsilane in Deuteriochloroform Solution

struct assnmt no.	chem shift, ppm ^{13}C	chem shift, ppm ^1H	^1H, mult (J, Hz)
1	47.7	3.58	1 H, m
		3.91	1 H, m
2	24.5	1.8–2.1	2 H, m
3	28.2	1.8–2.1	2 H, m
4	60.1	4.23	1 H, t (J = 6.6)
5	167.5		
6	51.3	4.60	1 H, m
7	40.9	1.90	1 H, m
8	24.5	1.53	1 H, m
9	22.3	0.63	1 H, d (J = 6.5)
10	21.9	0.45	1 H, d (J = 7.2)
11	174.3		
12	60.0	5.65	1 H, dd (J = 7.0, 2.1)
13	30.9	1.8–2.1	1 H, m
14	18.6	0.96	3 H, d (J = 6.5)
15	19.8	0.94	3 H, d (J = 6.5)
16	169.5		
17	51.5	5.20	1 H, m
18	28.2	2.35	2 H, m
19	29.7	2.35	2 H, m
20	166.6		
21	148.5		
22	130.3	8.22	1 H, s
23	161.2		
24	174.5		
25	37.7	5.20	1 H, m
		4.65	1 H, dd (J = 15.0, 3.0)
26	148.0		
27	124.3	8.05	1 H, s
28	160.4		
29	171.5		
(1)		7.53	1 H, d (J = 6.7)
(2)		7.60	1 H, d (J = 6.8)
(3)		8.44	1 H, d (J = 9.0)
(4)		9.25	1 H, t (J = 3.0)
(5)		6.29	1 H, br s
		6.16	1 H, br s

additional synthetic investigations of other possible amino acid sequences are presently underway.

Experimental Section

The amino acid derivatives L-Pro-OMe·HCl, Boc-L-Leu, and Boc-L-Val were obtained from Sigma Chemical Co. All solvents were redistilled and solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Solvents were removed under reduced pressure on a Buchii rotatory evaporator. The ether designation refers to diethyl ether. Tetrahydrofuran and dimethylformamide were distilled from lithium aluminum hydride and calcium hydride, respectively. Analtech silica gel GF (0.25 mm) plates were used for thin layer chromatography (TLC) and developed with either concentrated sulfuric acid or a 1% palladium chloride spray. Materials used for column chromatography were E. Merck (Darmstadt) silica gel (70–230 mesh) or Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Melting points were observed on a Kofler melting point apparatus. Optical rotation measurements were recorded on a Perkin-Elmer 241 polarimeter. The ultraviolet spectra were obtained in methanol on a Hewlett-Packard 8450A UV/vis spectrophotometer. A Perkin-Elmer 299 infrared spectrophotometer was employed for infrared measurements in potassium bromide. Tetramethylsilane was used as an internal standard for nuclear magnetic resonance measurements (deuteriochloroform solution) determined with Varian XL-100, Bruker WH-90, or Bruker WH-400 instruments. The EI and solution phase secondary ion (SP-SIMS)[17] mass spectra were recorded with a Mat 312 instrument, but accurate mass determinations were obtained with a micromass 7070E or a Finigan Mat 8200 double focussing mass spectrometer. Elemental analyses were provided by the Microanalytical Laboratory, CSIR, Pretoria, Republic of S. Africa.

N-Boc-(gly)Thz-L-Pro-OMe (6). To a suspension of *N*-Boc-2-(aminomethyl)thiazole-4-carboxylic acid hydrochloride (**5c**, 2.59 g, 10 mmol)[1] in tetrahydrofuran (40 mL) was added *N*-methylmorpholine (1.1 mL, 10 mmol), and a clear solution was obtained. Upon cooling to −23 °C isobutyl chloroformate was added (dropwise) over a period of 1 min to the stirred solution and some precipitate separated. The mixture was stirred at −23 °C for 20 min. A solution of L-proline methyl ester hydrochloride (1.655 g, 10 mmol) and *N*-methylmorpholine (1.1 mL, 10 mmol) was added over 2 min. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The precipitate was collected and washed with tetrahydrofuran (40 mL). After concentrating the filtrate in vacuo ethyl acetate (250 mL) was added. The organic phase was washed with water (2 × 50 mL), 5% citric acid (2 × 50 mL), 2% sodium bicarbonate (2 × 50 mL), and brine (1 × 50 mL) and dried. Removal of solvent furnished a colorless gum (3.6 g) which was chromatographed on a column of silica gel in hexane–ethyl acetate mixtures. The fraction eluted with ethyl acetate provided *N*-Boc-(gly)Thz-Pro-OMe (**6**) as a colorless foam (0.332 g, 90%): $[\alpha]^{25}_D$ −44° (*c* 2, CH_3OH); MS(EI) exact mass, *m/z* 369.1351 (M^+, calcd 369.1358 for $C_{16}H_{23}N_3O_5S$); IR ν_{max} 3320 (br), 3220, 1735, 1720, 1630, and 1510 cm^{-1}; ^1H NMR (Me_2SO-d_6, 100 °C) δ 1.45 (s, 9 H, $(CH_3)_3C$), 3.67 (s, 3 H, $COOCH_3$), 3.85 (br t, J = 6.5 Hz, 2 H, -CH_2CH_2NCO-), 4.52 (d, J = 6 Hz, 2 H, Thz CH_2NH-), 5.16 (dd, J = 4 and 8 Hz, 1 H, -CHNHCO), 7.3 (br t, J = 6 Hz, -CH_2NH-), 8.13 (s, 1 H, thiazole CH); ^{13}C NMR (Me_2SO-d_6, 100 °C) δ 23.4 (t), 28.4 (q, 3×), 30.5 (t), 42.5 (t), 48.2 (t), 52.2 (q), 60.75 (d), 80.2 (s), 126.2 (d), 150.3 (s), 155.7 (s), 168.4 (s), 161.3 (s), 173.2 (s).

(gly)Thz-L-Pro-OMe (7). A solution of *N*-Boc-(gly)Thz-L-Pro-OMe (1.55 g, 4.2 mmol) in 9 mL of dry methylene chloride

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

was placed in an ice bath. Trifluoroacetic acid (9 mL) was added dropwise to the stirred solution over a period of 1 min and the colorless solution was stirred at 0 °C for 12 min. The ice bath was removed and the solution stirred at room temperature for 40 min. The reaction mixture was diluted with carbon tetrachloride (35 mL) and the solvent removed at 50 °C. The resulting colorless syrup could not be induced to crystallize. A solution of the *trifluoroacetate salt* in chloroform (100 mL) was washed with 5% sodium bicarbonate (1 × 50 mL), and the aqueous phase was extracted with chloroform (2 × 70 mL). The chloroform solutions were combined, dried, and evaporated in vacuo to give the free amine, **(gly)Thz-L-Pro-OMe**, as a colorless oil (1.07 g, 95.5%) which resisted attempts at crystallization. A TLC (silica gel–ethyl acetate) analysis confirmed the purity. A solution of the amine in ether (at 0 °C) was treated with excess hydrogen bromide–acetic acid. The amorphous precipitate was collected and the **(gly)Thz-L-Pro-OMe·HBr** was found to be extremely hygroscopic. Recrystallization from acetic acid–ether and drying in vacuo at 60 °C afforded crystals melting at 212–214 °C dec; $[\alpha]^{25}_D$ −48° (*c* 1, CH_3OH); MS (SP-SIMS)[17] (glycerol matrix), *m/z* 270 [free base M + H]$^+$. Anal. Calcd for $C_{11}H_{16}N_3O_3SBr$: C, 37.70; H, 4.61; N, 12.00; Br, 22.82. Found: C, 37.44; H, 4.48; N, 11.76; Br, 23.06.

***N*-Z-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (8).** To a solution of *N*-Z-(*R*)- and -(*S*)-(gln)Thz (**5d**)[10] (0.363 g, 1 mmol) in tetrahydrofuran (8 mL, dry)–dimethylformamide (1 mL, dry) was added *N*-methylmorpholine (0.115 mL, 1.05 mmol), and the mixture was cooled to −23 °C. On addition of isobutyl chloroformate (0.135 mL, 1.04 mmol) an immediate colorless precipitate formed. The mixture was stirred at −25 °C for 30 min and then a solution of amine **7** was added which was prepared as follows: 0.388 g (1.05 mmol) of the corresponding hydrobromide in dimethylformamide (1 mL)–tetrahydrofuran (3 mL) was treated with 1 molar equiv (0.115 mL) of *N*-methylmorpholine. After addition the mixture was kept at −23 °C for 10 min and then allowed to warm to room temperature. After 12 h the solution was diluted with ethyl acetate (50 mL), washed with 5% citric acid (2 × 60 mL) and aqueous sodium bicarbonate (2 × 60 mL), and dried, and solvent was removed. The residue (0.64 g, colorless gum) was chromatographed on a column of silica gel in 9:1 ethyl acetate–methanol to afford ***N*-Z-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (8)** (0.40 g, 65%) as a colorless amorphous solid. Final purification was achieved by precipitation from ethyl acetate using a slow addition of hexane and drying in vacuo at 60 °C: mp 106–108 °C; $[\alpha]^{25}_D$ −31.7° (*c* 2.5, CH_3OH); MS(EI) exact mass, *m/z* 614.1629 (M$^+$, calcd 614.1617 for $C_{27}H_{30}N_6O_7S_2$); UV λ_{max} 239 nm (ϵ 8020); ^{1}H NMR (Me_2SO-d_6, 100 °C) δ 3.68 (s, 3 H, $COOCH_3$), 3.77 (t, *J* = 6 Hz, 2 H, -CH_2NCO), 4.82 (d, *J* = 6 Hz, 2 H, Thz-CH_2NH-), 4.9–5.1 (m, 2 H, 2 × -*C*HNHCO), 5.18 (s, 2 H, PhCH_2), 6.7 (br s, 2 H, -CONH_2), 7.43 (br s, 5 H, Ph), 7.95 (d, *J* = 8 Hz, 1 H, -N*H*CH-), 8.15 (s, 1 H), 8.25 (s, 1 H, 2 × thiazole CH), 8.85 (br t, *J* = 6 Hz, ThzCH$_2$N*H*-).

Boc-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (10). A solution of *N*-Z-(*R*)- and -(*S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (**8**, 2.0 g) in tetrahydrofuran (7 mL) was cooled to 0 °C and treated with 2 N hydrogen bromide in acetic acid (14 mL) for 15 min at 0 °C) and at room temperature for 40 min. The reaction mixture was again cooled at 0 °C and diluted with ether (50 mL). The precipitate was collected, washed with ether, and dried to yield 2.1 g of **(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (9) hydrobromide** melting at 239–241 °C dec: $[\alpha]^{25}_D$ −37.5° (*c* 1.0, CH_3OH); MS (SP-SIMS, glycerol matrix),[17] *m/z* 481 [free base M + H]$^+$. To a stirred solution of *N*-Boc-L-Val (1.12 g, 5.17 mmol) in dry tetrahydrofuran (60 mL, under argon at 0 °C) was added *N*-hydroxysuccinimide (573 mg, 5.17 mmol) and dicyclohexylcarbodiimide (1.06 g, 5.17 mmol). The solution was stirred for 1 h at 0 °C and 2 h at room temperature, and cooled to 0 °C. Peptide **9** hydrobromide in dry dimethylformamide (30 mL) was slowly added followed by *N*-methylmorpholine (0.44 mL, 4 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 16 h. Ethyl acetate (500 mL) was added, the organic phase washed with water (1 × 100 mL), 2% citric acid (1 × 100 mL), and aqueous sodium bicarbonate (1 × 100 mL), and dried. The ethyl acetate was removed and the residue (1.4 g) chromatographed on a column of silica gel. Elution with acetone furnished **Boc-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (10)** (2.03 g, 75%) as a colorless amorphous solid; pure by TLC (R_f 0.61, in acetone). After precipitation from ethyl acetate solution with ether and drying in vacuo at 65 °C, peptide **10** melted at 144–146 °C: $[\alpha]^{25}_D$ −26.2° (*c* 1, CH_3OH); MS (EI) exact mass, *m/z* 679.2441 (M$^+$, calcd 679.2458 for $C_{29}H_{41}N_7O_8S_2$); UV λ_{max} 240 nm (ϵ 7800); ^{1}H NMR (Me_2SO-d_6, 100 °C) δ 0.93 (d, *J* = 7 Hz, 3 H, CH_3CH), 0.95 (d, *J* = 7 Hz, 3 H, CH_3CH), 1.43 (s, 9 H, $(CH_3)_3C$), 3.66 (s, 3 H, $COOCH_3$), 3.95 (m, 2 H, -CH_2NCO-), 4.83 (t, *J* = 6 Hz, ThzCH_2NH), 4.9–5.2 (m, 3 H, 3 × -*C*HNHCO), 6.17 (br d, *J* = 8 Hz, 1 H, -CHN*H*CO), 6.61 (br s, 2 H, $CONH_2$), 8.12 (s, 1 H), 8.23 (s, 1 H, 2 × thiazole CH), 8.33 (br d, *J* = 8 Hz, -CHN*H*CO), 8.72 (br t, *J* = 6 Hz, CH_2N*H*CO).

L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (11) Hydrobromide. To a stirred solution of protected tetrapeptide **10** (1.02 g, 1.5 mmol) in dry methylene chloride (20 mL, cooled to 0 °C) was added trifluoroacetic acid (20 mL dropwise). The solution was allowed to warm to room temperature and stirred for 1 h, and solvent was removed at 40 °C. Carbon tetrachloride (40 mL) was added and the solvent again removed. The resulting thick syrup was dissolved in a minimum of methylene chloride (10 mL) and treated with 5 mL of 3 N hydrogen bromide in acetic acid at 0 °C. Ether (60 mL) was slowly added and the precipitated white solid collected by filtration and washed with ether to afford **L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (11) hydrobromide** which was dried in vacuo at 65 °C: mp 178–181 °C dec; $[\alpha]^{25}_D$ −23.8° (*c* 2, CH_3OH); MS (SP-SIMS, glycerol matrix),[17] *m/z* 580 [free base M + H]$^+$. Anal. Calcd for $C_{24}H_{34}N_7O_6S_2Br$: C, 43.64; H, 5.19; N, 14.84; Br, 12.10. Found: C, 43.35; H, 5.04; N, 14.61; Br, 12.42.

Boc-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (12) and Boc-L-Leu-L-Val-(*R*)- and -(*S*)-(gln)Thz-(gly)Thz-L-Pro (13). A stirred solution of *N*-Boc-L-Leu (0.323 g, 1.4 mmol) in dry tetrahydrofuran (20 mL, under argon at 0 °C) was treated with *N*-hydroxysuccinimide (0.14 g, 1.4 mmol) and dicyclohexylcarbodiimide (0.247 g, 1.4 mmol). The solution was stirred at 0 °C for 1 h and allowed to warm to room temperature for 3 h, and the solution was filtered to remove dicyclohexylurea. After cooling to 0 °C a solution of L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe·HBr (0.660 g, 1 mmol) in tetrahydrofuran (12 mL)–dimethylformamide (2.5 mL) containing *N*-methylmorpholine (0.15 mL) was added dropwise, over a period of 5 min. The mixture was stirred at room temperature for 16 h, chloroform (250 mL) was added, and the organic phase was washed with water (1 × 50 mL), 2% citric acid (2 × 50 mL), and aqueous sodium bicarbonate (1 × 25 mL) and dried. The chloroform was removed and the residue (0.85 g) chromatographed on a column of silica gel. Elution with 94:6 chloroform–ethanol led to **Boc-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro-OMe (12)** (0.52 g, 67%) as a colorless amorphous solid. Precipitation from chloroform with ether and drying at 65 °C *(in vacuo)* gave a pure specimen: mp 114–117 °C; $[\alpha]^{25}_D$ −50.6° (*c* 1, CH_3OH); MS (EI) exact mass, *m/z* 792.3266 (M$^+$, calcd 792.3298 for $C_{35}H_{52}N_8O_9S_2$); UV λ_{max} 240 nm (ϵ 7840); ^{1}H NMR (Me_2SO-d_6, 100 °C) δ 0.8–1.0 (m, 12 H, 4 × CH_3CH), 1.42 (s, 9 H, $(CH_3)_3C$), 3.65 (s, 3 H, $COOCH_3$), 4.0 (m, 2 H, -CH_2NCO), 4.65–4.83 (m, 2 H, 2 × *C*HNHCO), 4.85 (d, *J* = 6 Hz, 2 H, ThzCH_2NH), 4.95–5.2 (m, 2 H, 2 × *C*HNHCO), 6.70 (br s, 2 H, $CONH_2$), 6.85 (br d, *J* = 6 Hz, 1 H, CHN*H*CO), 7.35 (br d, *J* = 8 Hz, 1 H, -CHN*H*CO), 8.13 (s, 1 H), 8.23 (s, 1 H, 2 × thiazole CH), 8.80 (br t, *J* = 6 Hz, 1 H, ThzCH$_2$N*H*).

Pentapeptide methyl ester **12** (0.396 g, 0.5 mmol) in dioxane (5 mL) was treated with 5.5 mL of 0.1 N sodium hydroxide at room temperature. After 2 h the reaction mixture was diluted with ether (50 mL) and water (5 mL). The aqueous phase was acidified to pH 2 with 3 N hydrochloric acid and extracted with chloroform (2 × 50 mL). The chloroform extract was washed with water (2 × 10 mL), dried, and concentrated to afford **Boc-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz-L-Pro (13)** as a colorless powder (0.34 g, 86%). Precipitation from a minimum of methanol with ether and drying in vacuo at 65 °C yielded a pure sample: mp 130–132 °C; $[\alpha]^{25}_D$ −51.1° (*c* 1.5, CH_3OH); MS(EI) exact mass, *m/z* 778.3134 (M$^+$, calcd 778.3142 for $C_{34}H_{50}N_8O_9S_2$).

***cyclo*-[L-Pro-L-Leu-L-Val-(*R,S*)-(gln)Thz-(gly)Thz] (16).** To a solution of Boc pentapeptide **13** (0.117 g, 0.15 mmol) in dry dimethylformamide (2.5 mL) at room temperature was added 2,4,5-trichlorophenol (0.394 mg, 2 mmol).[1] The solution was cooled at 0 °C and dicyclohexylcarbodiimide (0.412 g, 2 mmol) was added.

The reaction mixture was left for 1 h at 0 °C and 16 h at room temperature. Solvent was removed and the residue treated with chloroform (10 mL). The soluble portion was separated from insoluble dicyclohexylurea and chromatographed on a column silica gel. Elution with 94:6 chloroform-methanol furnished the main product **Boc-L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz-Pro-2,4,6-trichlorophenol ester** (**14**, 97 mg, 68%) as a colorless glass: homogenous by TLC (R_f 0.52, in 9:1 methylene chloride–methanol); MS (SP-SIMS, sodium iodide in sulfolane),[17] m/z 979 $[M + Na]^+$.

A solution of trichlorophenol ester **14** (86 mg, 0.09 mmol) in methylene chloride (1 mL, at 0 °C) was treated with trifluoroacetic acid (1 mL). The mixture was allowed to warm to room temperature, the solvent was removed in vacuo, and the residue was dissolved in 200 mL of freshly distilled tetrahydrofuran. Pyridine (40 mL, freshly distilled from sodium hydroxide) was slowly added with stirring. The reaction mixture was left at room temperature for 12 h, the solvent was removed, and the residue in methanol was chromatographed on a column of Sephadex LH-20 (100 g). The main fraction (51 mg) eluted from the column was chromatographed on a column of silica gel (25 g). The major component (22 mg, 45%) was eluted by 94:6 chloroform–methanol and appeared to be homogenous by TLC (R_f 0.22, 10:1:0.1 chloroform–methanol–water). However, high pressure liquid chromatographic separation of a sample (20 mg) using a Whatman Magnum 9 Partisil column (9.4 mm × 50 cm) and elution with a linear gradient of two solvent mixtures viz. 99:1:0.05 to 75:25:2.2 methylene chloride–methanol–water furnished (together with mixtures) the two diastereomers of ***cyclo*-[L-Pro-L-Leu-L-Val-(*R*,*S*)-(gln)Thz-(gly)Thz]** in order of increasing polarity:

Isomer A (7.5 mg),[18] a colorless amorphous powder: mp 183–186 °C; $[\alpha]^{25}_D$ +52° (*c* 0.5, CH_3OH); MS (EI) exact mass, m/z 660.2547 (M^+, calcd 660.2512 for $C_{29}H_{40}N_8O_6S_2$); 421.1105 (M^+, calcd 421.1117 for $C_{17}H_{21}N_6O_3S_2$); 404.0832 (M^+, calcd 404.0852 for $C_{17}H_{18}N_5O_3S_2$); 335.0281 (M^+, calcd 335.0273 for $C_{13}H_{11}N_4O_3S_2$); MS (SP-SIMS) (glycerol matrix), m/z (relative intensity) 661 ($M + H^+$, 55), 546 (12), 547 (10), 562 (23), 450 (25), 421 (75), 404 (79), 349 (27), 335 (63), 311 (32), 279 (37), 212 (100); UV λ_{max} 244 nm (ϵ 8250); IR ν_{max} 3420, 3250 br, 3070, 1660 br, 1552, 1501, 1462 cm^{-1}; MS (EI), m/z (relative intensity) 660 (M^+, 9), 617 (3), 546 (4), 421 (10), 404 (11), 347 (6), 335 (8), 313 (5), 248 (25), 220 (14), 205 (15), 195 (10).

Isomer B (3 mg),[18] a colorless amorphous powder: mp 157–170 °C; $[\alpha]^{25}_D$ +154° (*c* 0.5, CH_3OH); UV λ_{max} 242 nm (ϵ 8070); MS (EI) exact mass, m/z 660.2552 (M^+, calcd 660.2512 for $C_{29}H_{40}N_8O_6S_2$).

A remaining microgram amount of natural dolastatin 3[8] was compared with isomers A and B by TLC on silica gel employing elution with 10:1:0.1 methylene chloride–methanol–water. The R_f of dolastatin 3 was 0.27 compared with the slightly more polar isomers (**16**) at R_f 0.22.

Acknowledgment. With appreciation we thank for financial support Mary Dell Pritzlaff, the Olin Foundation (S.T. and A.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 Fund; this investigation was 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. **5c**, 104619-52-5; (*R*)-**5d**, 96363-15-4; (*S*)-**5d**, 95716-10-2; **6**, 104619-53-6; **7**, 92506-92-8; **7**·TFA, 104619-54-7; **7**·HBr, 104619-55-8; (*R*)-**8**, 104641-91-0; (*S*)-**8**, 104619-56-9; (*R*)-**9**·HBr, 104619-57-0; (*S*)-**9**·HBr, 104619-58-1; (*R*)-**10**, 104619-59-2; (*S*)-**10**, 104712-82-5; (*R*)-**11**·TFA, 104619-61-6; (*S*)-**11**·TFA, 104712-84-7; (*R*)-**11**·HBr, 104712-85-8; (*S*)-**11**·HBr, 104757-49-5; (*R*)-**12**, 104712-86-9; (*S*)-**12**, 92506-94-0; (*R*)-**13**, 104619-62-7; (*S*)-**13**, 104712-87-0; (*R*)-**14**, 104619-63-8; (*S*)-**14**, 104712-88-1; (*R*)-**15**·TFA, 104619-65-0; (*S*)-**15**·TFA, 104712-90-5; (*R*)-**16**, 92691-34-4; (*S*)-**16**, 103882-99-1; H-Pro-OMe·HCl, 2133-40-6; Boc-Val-OH, 13734-41-3; Boc-Leu-OH, 13139-15-6; dolastatin 3, 80387-90-2.

(18) In a parallel study of dolastatin 3 isomers employing synthetic strategies different than those outlined here, Drs. W. Wierenga, R. Kelley, and colleagues of the Upjohn Co. also obtained isomer 4 and a series of related cyclic peptides. Nearly a year following synthesis of this dolastatin 3 isomer, Drs. T. Shioiri and Y. Hamada (see also footnote 12 of ref 1) informed (private communication, August 13, 1983) us that they have succeeded in preparing cyclic peptide **16** in the all-L configuration and found $[\alpha]^{22}_D$ +71.6° (*c* 0.1, CH_3OH). On the basis of this specific rotation our isomer A may correspond to the all-L configuration [(*S*)-(gln)Thz]. And isomer B would correspond to the (*R*)-(gln)Thz epimer. More recently Drs. P. Fischer and U. Schmidt (Institute of Organic Chemistry, University of Stuttgart) thoughtfully let us know (private communication, June 7 and 12, 1984) that they have also completed syntheses of a series of dolastatin 3 isomers including substance 4 of this manuscript. While our contribution was in preparation, preliminary communications of these studies were reported: Schmidt, U.; Utz, R. *Angew. Chem.* **1984**, *96*, 725–726. Hamada, Y.; Kohda, K.; Shioiri, T. *Tetrahedron Lett.* **1984**, *25*, 5303–5306. **Note Added in Proof**: Meanwhile, the structure of patellamide B has been corrected to **3** by Hamada et al. (Hamada, Y.; Shibata, M.; Shioiri, T. *Tetrahedron Lett.* **1985**, *26*, 5159).