Articles

The Dolastatins. 22. Synthesis of Boc-dolaproinyl-dolaphenine and Four Related Chiral Isomers[1]

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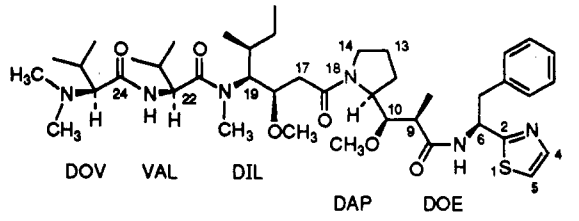
Synthesis of the dolastatin 10 (1) dipeptide segment *N*-(*tert*-butoxycarbonyl)-dolaproinyl-dolaphenine (2, Boc-Dap-Doe) and four chiral isomers (3–5, 13) has been summarized. Formation of the amide bond was readily accomplished employing diethyl cyanophosphonate. The stereochemical assignments for Boc-Dap-Doe (2) and Boc-Dap-(6*R*)-Doe (3) were confirmed by X-ray crystal structure analyses.

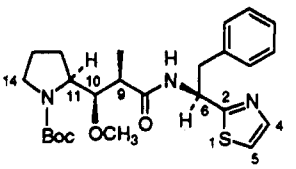
Dolastatin 10 (1), a linear peptide isolated from the Indian Ocean sea hare *Dolabella auricularia*, exhibits a remarkable spectrum of potent antineoplastic activity[2] and is presently in clinical development. A practical synthesis of this potentially useful anticancer drug became exceptionally important in order to provide the clinical supply and open approaches to various structure/activity studies.

Due to the few milligrams of amorphous dolastatin 10 (1) originally isolated and used to elucidate[2a] its structure, the absolute configuration was not determined. Since the peptide contains nine asymmetric centers corresponding to one of 512 possible isomers, the stereochemical determination was challenging and required total synthetic approaches for solution. As originally outlined,[3] our approach to dolastatin 10 involved formation of dolaproinyl-dolaphenine (Dap-Doe) and the tripeptide dolavalyl-valyl-dolaisoleuine (Dov-Val-Dil) and their subsequent coupling to give peptide 1. In this paper our syntheses of *N*-Boc-dolaproinyl-dolaphenine (2) and four chiral isomers (3, 4, 5, 13) are outlined.

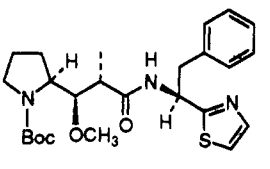
As previously reported,[3,4] the dolaphenine unit of dolastatin 10 was assumed on biosynthetic grounds to have the *S* configuration, and synthesis of this unusual phenylalanine derivative with *N*-(*tert*-butoxycarbonyl)-protection (6) was accomplished by the method we previously described. Analogously, the proline precursor to the dolaproine unit was also assumed to have the *S* configuration. Since the chirality of the side chain in dolaproine



Dolastatin 10
1



Boc-Dap-Doe
2



Boc-(9*S*,10*R*,11*S*)-*iso*-Dap-Doe
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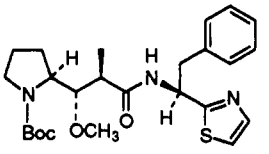

Boc-(9*R*,10*S*,11*S*)-*iso*-Dap-Doe
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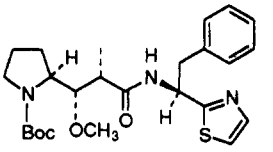
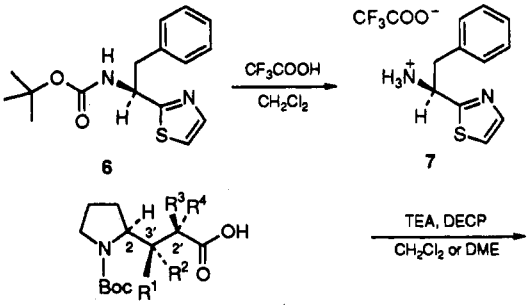


Boc-(9*S*,10*S*,11*S*)-*iso*-Dap-Doe
5

was unknown, all four diastereomeric derivatives (8, 2*R*,3*R*,2′*S*; 9, 2*S*,3*R*,2′*S*; 10, 2*R*,3*S*,2′*S*; 11, 2*S*,3*S*,2′*S*) were synthesized *via* an aldol condensation route followed by



8: $R^1 = OCH_3$, $R^2 = H$, $R^3 = CH_3$, $R^4 = H$
9: $R^1 = OCH_3$, $R^2 = H$, $R^3 = H$, $R^4 = CH_3$
10: $R^1 = H$, $R^2 = OCH_3$, $R^3 = CH_3$, $R^4 = H$
11: $R^1 = H$, $R^2 = OCH_3$, $R^3 = H$, $R^4 = CH_3$

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(1) Part 287 of our Antineoplastic Agents series. For Part 21 (contribution no. 286), see: Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Hamel, E. *J. Am. Chem. Soc.*, submitted.

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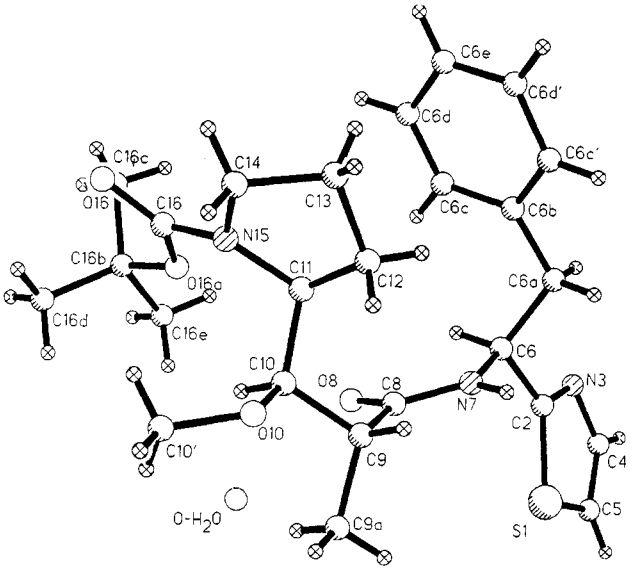


Figure 1. Computer-generated drawing and X-ray numbering system for amide **2** (Boc-Dap-(6*S*)-Doe).

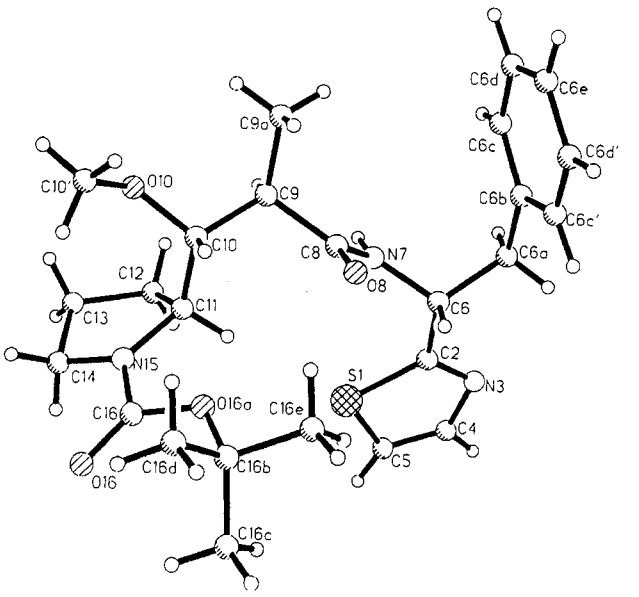


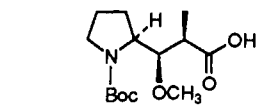
Figure 2. Computer-generated perspective drawing and X-ray numbering system for amide **13** (Boc-Dap-(6*R*)-Doe).

O-methylation. The absolute configuration of each methyl ether was established by NMR and/or X-ray crystal structure analyses.[3,5] While the principal purpose of synthesizing these four diastereoisomers was to define the absolute configuration of dolaproine, the unnatural isomers **9–11** proved very useful for the later syntheses of three dolastatin 10 isomers.[6]

Formation of the dipeptide derivatives **2–5** was readily accomplished using diethyl cyanophosphonate (DECP) as the coupling agent. Thiazole derivative **6** was first deprotected using trifluoroacetic acid. A mixture of the trifluoroacetate salt **7** and each methyl ether (**8–11**) in turn was treated with DECP and triethylamine at 0 °C to afford dipeptides **2**, **3**, **4**, and **5**, respectively. No racemization was observed, and an X-ray crystallographic analysis of amide **2** established that the chirality of the asymmetric centers in the reactants was preserved in the resulting amide (6*S*,9*R*,10*R*,11*S*; see Figure 1).

Occasionally, thiazole **6** (Boc-Doe) was obtained as a partial racemate from the manganese dioxide catalyzed thiazolidine dehydrogenation step.[4] When a sample of the Doe racemate with $[\alpha]^{25}_{D}$ −3.7° (natural Doe at the same concentration showed $[\alpha]^{25}_{D}$ −23.3°) was treated with trifluoroacetic acid and the product coupled (DECP) with methyl ether **8** the result was a mixture (2:3) of two amides separable by column chromatography. The major diastereoisomer (and more polar of the two) proved to be identical to amide **2** which possessed the natural dolastatin 10 asymmetry. The less polar amide (**13**) was shown by X-ray crystal structure analysis to have the *R* configuration at the dolaphenine chiral center (Figure 2). Retention of configuration at the dipeptide chiral centers derived from dolaproine **8** (2*R*,3*R*,2′*S*) and (*R*)-dolaphenine during formation of amide **13** (9*R*,10*R*,11*S*) confirmed the lack of any significant racemization during the coupling reaction.

The four amides (**2–5**) of known stereochemistry were each coupled with Dov-Val-Dil.[3] The peptide which incorporated amide **2** proved to be identical with natural dolastatin 10 (**1**). Thus, the stereochemistry of the chiral



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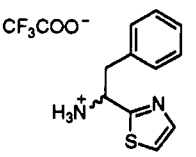

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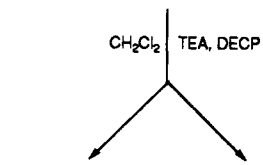
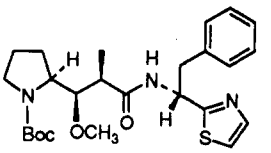


Boc-Dap-Doe
2



Boc-Dap-(6*R*)-*iso*-Doe
13

centers in the dolaphenine and dolaproine units of dolastatin 10 were found to be 6*S*,9*R*,10*R*,11*S*.[3] After the absolute configuration of dolastatin 10 (**1**) was established by our first total synthesis[3] several other synthetic approaches to dolastatin 10 appeared.[7–10]

Synthesis of Dap-(6*R*)-Doe (**13**) allowed solution of a long-standing problem involving our attempts to crystallize dolastatin 10 or a chiral isomer with comparable anticancer activity. Conversion[1] of amide **13** to (6*R*)-isodolastatin 10 gave the first appropriate isomer that could be induced to crystallize and yield to X-ray crystal structure determination and subsequent molecular modeling.[1] The experi-

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ments described here should allow convenient syntheses of the remaining four diastereoisomeric amides (containing (*S*)-Doe) based on (*R*)-proline for biological evaluations and conversion to isodolastatins.

Experimental Section

All reagents were employed as obtained from Sigma–Aldrich Chemical Co., and solvents were redistilled. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Evaporation of solvents was performed under reduced pressure on a rotary evaporator at 40 °C. Thin-layer chromatography was carried out with silica gel GHLF Uniplates (Analtech, Inc.). Both ambient column chromatography (Kieselgel 60, <0.063 and 0.063–0.200 mm) and "flash" chromatography (Kieselgel 60, 0.040–0.063 mm) were performed using silica gel supplied by E. Merck (Darmstadt).

Melting points are uncorrected and were determined on a Kofler-type microscope hot-stage apparatus. Optical rotation measurements were recorded using a Perkin-Elmer 241 polarimeter. Nuclear magnetic resonance spectra were recorded with tetramethylsilane as internal standard with Varian Gemini 300 MHz (CHE-88-131109) and Bruker AM 400 instruments; chemical shifts are recorded in ppm (δ). The EIMS mass spectra were recorded with a FINNIGAN-MAT 312 instrument (70 eV). The X-ray data collections were accomplished with an Enraf-Nonius CAD4 or Siemens P3/V difractometer.

***N*-(*tert*-Butoxycarbonyl)-dolaproinyl-dolaphenine (2). Part A. Synthesis.** A solution of *N*-(*tert*-butoxycarbonyl)-dolaphenine (**6**, 2 g) in dichloromethane (25 mL) was cooled to 0 °C under argon, and trifluoroacetic acid (25 mL) was added. The mixture was stirred at 0 °C for 1 h. The solvent was removed *in vacuo* to yield a residue which was dissolved in toluene (20 mL) and this process repeated once more to yield an oil which was dried under high vacuum for 2 h. Diethyl ether (50 mL) was added, and the mixture was stirred for 15 min. The crystals of trifluoroacetate **7** that separated were collected by filtration and washed with diethyl ether (2.05 g, 98%): ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 3.2 Hz, 1 H,), 7.31–7.27 (m, 4 H), 7.30–7.10 (m, 2 H, aromatic), 4.95 (dd, J = 8.5, 6.2 Hz, 1 H, H-6), 3.50 (dd, J = 13.6, 8.7 Hz, 1 H, *H*CH), 3.30 (dd, J = 13.6, 8.7 Hz, 1 H, *H*CH).

Before the dropwise addition of triethylamine (1.46 mL, 10.5 mmol) followed by diethyl cyanophosphonate (0.82 mL, 5.4 mmol), a solution of *N*-(*tert*-butoxycarbonyl)-dolaproine (**8**, 1.34 g, 4.7 mmol) and 2-phenyl-1-(*S*)-(2′-thiazolyl)ethylamine trifluoroacetate (**7**, 1.5 g, 4.7 mmol) in dichloromethane (30 mL, distilled from calcium hydride) was stirred under argon and cooled to 0 °C. The reaction mixture was stirred for 3 h. The solvent was removed (*in vacuo* at room temperature) to yield an oil which was separated by column chromatography: eluant, hexane–acetone (3:1). The oil obtained was covered with a small amount of hexane–acetone and retained for 16 h. The crystals which formed were suspended in diethyl ether and collected by filtration. Recrystallization from diethyl ether yielded pure amide **2** (1.68 g, 76%): mp 135–136 °C; R_f 0.31 (3:1 hexane–acetone); $[\alpha]^{25}_D$ −69.3° (*c* 2.3, $CHCl_3$); IR (neat) 3294, 2974, 2931, 2876, 1693, 1653, 1539, 1456, 1400, 1168, 1105 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 3.2 Hz, 1 H, H-4 thiazole), 7.21 (d, J = 3.2 Hz, 1 H, H-5 thiazole), 7.30–7.00 (m, 5 H, aromatic), 7.02, 6.35 (m, NH), 5.57 (m, 1 H, NHC*H*), 3.85–3.75 (m, 1H), 3.60 (m, 1 H,), 3.48 (m, 1 H), 3.38–3.17 (m, 2 H), 3.32 (s, 3 H), 3.13 (m, 1 H), 2.35–2.24 (m, 1H), 1.80–1.50 (m, 4 H), 1.44 (s, 9 H), 1.11 (d, J = 6 Hz, 3H); EIMS m/z 473 (M^+), 441 ($M - CH_3OH$), 289, 260, 170, 114, and 70 (100) (see ref 3). Anal. Calcd for $C_{25}H_{35}N_3O_4S\cdot0.5H_2O$: C, 62.11; H, 7.45; N, 8.69. Found: C, 61.84; H, 7.61; N, 8.49.

Part B. X-ray Crystal Structure Determination. A colorless crystal (0.48 × 0.36 × 0.36 mm) of amide **2**, from acetone–hexane, was used for X-ray structure determination. Crystal data: $C_{25}H_{35}N_3O_4S\cdot H_2O$, orthorhombic space group $P2_12_12_1$, with a = 11.471(5) Å, b = 14.090(6) Å, c = 16.221(4) Å, V = 2621.6 Å^3, λ (Cu Kα) = 1.54184 Å, ρ_o = 1.20 g cm^{-3}, ρ_c = 1.21 g cm^{-3} for Z = 4, and FW = 491.65. All reflections corresponding to a quadrant of data, with $2\theta \leq 130°$, were measured at reduced temperature (−65 °C) on an Enraf-Nonius CAD4 diffractometer using the $\omega/2\theta$ scan technique. Immediately after the intensity measurement of each reflection, the Friedel pair was also collected (whenever possible). A total of 5139 reflections were obtained, of which 4514 (containing Friedels) were unique and not systematically absent. After Lorentz and polarization corrections, linear decay and empirical absorption corrections (based on a series of ψ-scans)[11] were made, and a total of 4272 unique reflections ($I_o > 3\sigma(I_o)$) was employed in the structure determination and refinement. Direct methods were used in the structure determination using SHELXS-86.[12] All non-hydrogen atoms of the main molecule plus one oxygen atom from a molecule of water were located on the first run using the default settings. Refinement was performed with MolEN.[13] The hydrogen atom coordinates were calculated at optimum positions and included (with fixed isotropic thermal parameters) in the final stages of full-matrix least-squares anisotropic refinement but were restrained to ride on the atom to which they were bonded. Anomalous dispersion effects[14] were included in F_c; the values for f' and f'' were those of Cromer.[15] The final cycle of refinement yielded standard crystallographic residuals of R = 0.073 and R_w = 0.066 (a secondary extinction correction was also applied in the refinement).

The absolute stereochemistry of amide **2** was readily assigned due to the pronounced anomalous dispersion effects caused by the sulfur atom. The correct absolute stereochemistry, as depicted in Figure 1,[16] was determined by refinement of the absolute structure index, η, as described by Rogers.[17] The absolute structure index refined to +1.08 (esd 0.08) when initially set to either +1 or −1 for the model shown. On the other hand, refinement of η with starter values of +1 or −1 for the enantiomeric image caused η to refine to −1.08 (esd 0.08). In addition, larger R values were observed for refinement of the mirror image of Figure 1, e.g., R = 0.081, R_w = 0.079.[18] As a consequence, the absolute stereochemistry for the four chiral centers of amide **2** using the numbering shown in Figure 1 are as follows: 6*S*,9*R*,-10*R*,11*S*.

***N*-Boc-(9*S*,10*R*,11S)-*iso*-dolaproinyldolaphenine (3).** The preceeding experiment (*cf.*, **2**) was repeated using trifluoroacetate **7** (95 mg, 0.3 mmol), *N*-Boc-(2*S*,3′*R*,2′*S*)-dolaproine (**9**, 82 mg, 0.3 mmol), dimethoxyethane (3 mL), triethylamine (0.13 mL, 0.93 mmol), and diethyl cyanophosphonate (0.068 mL, 0.45 mmol). In this example, the mixture was stirred under argon for 1 h at 0 °C and for 1 h at room temperature. Solvent was removed *in vacuo*, and the residue was separated by flash column chromatography (eluant: 7:3 hexane–ethyl acetate) to afford amide **3** (106 mg, 78%) as a powder from hexane–ethyl acetate: mp 115–117 °C; $[\alpha]^{30}_D$ −98° (*c* 0.7, $CHCl_3$); IR (NaCl) 3305, 2974, 2933, 1691, 1667, 1534, 1498, 1454, 1401, 1366, 1166, 1115 cm^{-1}; ^{1}H NMR (DMSO-d_6 at 110 °C) δ 8.08 (brd, J = 8.2 Hz, 1 H, NH), 7.69 (d, J = 3.2 Hz, 1 H), 7.50 (d, J = 3.2 Hz, 1H), 7.23–7.12 (m, 5 H, aromatic), 5.40 (ddd, J = 14.4, 9.3, 5.2 Hz, 1 H), 3.80 (dd, J = 9.8, 1.9 Hz, 1 H), 3.75 (m, 1 H), 3.39 (m, 2 H), 3.12 (dd, J = 16.7, 7.3 Hz, 1 H), 3.11 (s, 3 H), 3.08 (dd, J = 16.7, 7.3 Hz), 2.27 (dq, J = 9.7, 6.8 Hz, 1 H), 1.87 (m, 2 H), 1.78 (m, 1 H), 1.64 (m, 1 H, CH), 1.40 (s, 9 H), 0.78 (d, J = 6.9 Hz, 3 H); HRFAB MS m/z 474.2427 $[M + H]^+$, calcd 474.2428 for $C_{25}H_{36}N_3O_4S$ (see ref 3).

***N*-Boc-(9*R*,10*S*,11*S*)-*iso*-dolaproinyl-dolaphenine (4).** The experimental conditions employed for obtaining amide **3** were

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(16) Preparation of Figures 1 and 2, data collection, and refinement were done with SHELXTL-PLUS, G. Sheldrick, Siemens Analytical X-Ray Instruments, Inc., Madison, WI 53719.
(17) Rogers, D. *Acta Crystallogr.* **1981**, *A37*, 734.
(18) The author has deposited tables of bond distances, bond angles, atomic coordinates, and anisotropic thermal parameters for dipeptides **2** and **13** with the Cambridge Crystallographic Data Centre. These tables can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

repeated using trifluoroacetate 7 (88 mg, 0.26 mmol) and *N*-Boc-(2*R*,3*S*,2′*S*)-dolaproine (10, 29.5 mg, 0.10 mmol). Here the eluant was 4:1 hexane–ethyl acetate, and amide 4 (27 mg, 55%) was obtained as a viscous oil: $[\alpha]^{28}_D$ −46° (*c* 0.5, $CHCl_3$); IR (NaCl) 3270, 2975, 2927, 2880, 1683, 1662, 1531, 1497, 1454, 1394, 1367, 1170, 1115, 1094 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.07 (brs, 1 H, NH), 7.69 (d, *J* = 3.2 Hz), 7.44 (d, *J* = 3.2 Hz, 1 H), 7.24–7.10 (m, 5 H), 5.46 (ddd, *J* = 10.7, 8.4, 6.1 Hz, 1 H) 3.96 (m, 1 H), 3.44 (t, *J* = 5.7 Hz, 1 H), 3.37 (dd *J* = 14.0, 6.3 Hz, 1 H), 3.34 (m, 1 H), 3.27 (s, 3 H), 3.18 (dd, *J* = 14.0, 8.4 Hz, 1 H), 3.15 (m, 1H), 2.50 (m, 1 H), 1.81 (m, 2 H), 1.72 (m, 2 H), 1.41 (s, 9 H), 0.98 (d, *J* = 7.0 Hz, 3 H); HRFAB MS *m/z* 474.2425 $[M + H]^+$, calcd 474.2428 for $C_{25}H_{38}N_3O_4S$ (see ref 3).

***N*-Boc-(9*S*,10*S*,11*S*)-*iso*-dolaproinyl-dolaphenine (5).** The procedure used to obtain amide 4 was repeated (7:3 ratio for the hexane–ethyl acetate eluting solvent) employing trifluoroacetate 7 (100 mg, 0.31 mmol) and Boc-(2*S*,3*S*,2′*S*)-dolaproine (11, 53 mg, 0.18 mmol) to obtain amide 5 as a glass (60 mg, 69%): $[\alpha]^{28}_D$ −55° (*c* 0.5, $CHCl_3$); IR (NaCl) 3305, 2974, 2931, 1690, 1534, 1498, 1479, 1454, 1392, 1367, 1170, 1115, 1094 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.91 (brs, 1 H), 7.71 (d, *J* = 3.4 Hz, 1 H), 7.50 (d, *J* = 3.4 Hz, 1 H), 7.24 (m, 4 H), 7.18 (m, 1 H), 5.43 (m, 1 H), 4.01 (m, 1 H), 3.50 (dd, *J* = 8.3, 4.5 Hz, 1 H), 3.45 (m, 1 H), 3.38 (dd, *J* = 14.5, 5.8 Hz, 1 H, CH-Ph), 3.21 (m, 1 H), 3.18 (m, 1 H), 3.18 (s, 3 H), 2.50 (m, 1 H), 1.78 (m, 4 H), 1.44 (s, 9 H), 1.00 (d, *J* = 6.9 Hz, 3 H); HRFAB MS *m/z* 474.2430 $[M + H]^+$, calcd 474.2428 for $C_{25}H_{38}N_3O_4S$ (see ref 3).

***N*-Boc-Dolaproinyl-(6*R*)-dolaphenine (13). Part A. Synthesis.** Partially racemized thiazole salt 12 (3.0 g, 9.4 mmol) and dolaproine (8, 2.68 g, 9.4 mmol) were condensed as described for preparation of amide 2. Removal of solvent (*in vacuo*) yielded an oil which was fractionated (1:1:2 hexane–dichloromethane–ethyl acetate as eluant) by silica gel column chromatography. First eluted was (6*R*)-amide 13 which crystallized from diethyl ether–hexane (1.14 g, 26%): mp 132-133 °C; R_f 0.41 (column solvent); $[\alpha]^{25}_D$ −24.6° (*c* 0.48, CH_3OH); IR (thin film), 3308, 2974, 2934, 2878, 1690, 1661, 1530, 1454, 1400, 1366, 1169, and 1105 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 7.71 (d, *J* = 3.3 Hz, 1 H), 7.19 (d, *J* = 3.3 Hz, 1 H), 7.19–7.02 (m, 5 H), 7.01 and 6.40 (m, 1 H, NH), 5.70 (m, 1 H), 5.59 (m, 1 H), 3.87 and 3.71 (m, 1 H), 3.64 and 3.54 (m, 1 H), 3.45 (m, 1 H), 3.33 (s, 3 H), 3.38–3.19 (m, 2 H), 3.16 (m, 1 H), 2.31 and 2.25 (m, 1 H), 1.85–1.40 (m, 4 H), 1.42 (s, 9 H), 1.18 (d, *J* = 6.1 Hz, 3H), 1.11 (d, *J* = 6.1 Hz, 3 H). Anal. Calcd for $C_{25}H_{35}N_3SO_4$: C, 63.40; H, 7.44; N, 8.87; S, 6.77. Found: C, 63.49; H, 7.42; N, 8.84; S, 6.57.

Further elution (same solvent) of the column yielded *N*-Boc-dolaproinyl-dolaphenine (2, 1.84 g crystallized from diethyl ether, 41%).[3]

Part B. X-ray Crystal Structure Determination.[18] A colorless crystal (0.06 × 0.36 × 0.32 mm) of amide 13, from acetone–heptane, was utilized for structure elucidation. Crystal data: $C_{25}H_{35}N_3O_4S$, orthorhombic space group $P2_12_12_1$, with *a* = 8.817(3) Å, *b* = 17.260(9) Å, *c* = 17.544(6) Å, *V* = 2670(2) Å^3, λ (MoKα) = 0.71073 Å, ρ_o = 1.173 g cm^{-3}, ρ_c = 1.178 g cm^{-3} for *Z* = 4, F W = 473.6. All reflections corresponding to an octant of data, with 2θ ≤ 50°, were measured at room temperature (25 °C) using the ω scan technique on a Siemens P3/V diffractometer. Immediately following the intensity measurement of each reflection, and whenever possible, its Friedel opposite was also collected. A total of 5426 reflections were collected, of which 4725 (containing Friedels) were unique and not systematically absent. After Lorentz and polarization corrections, linear decay and empirical absorbtion adjustments (based on a series of ψ-scans)[11] were made, and a total of 1993 reflections ($F_o > 3\sigma(F_o)$) was used in the structure determination and refinement. Direct methods solution and subsequent full-matrix least-squares refinement was done using the SHELXTL PLUS software package.[16] All non-hydrogen atoms were located on the first pass using the default settings of SHELX. The hydrogen atom coordinates were calculated at optimum positions and included (with fixed isotropic thermal parameters) in the final stages of full-matrix least-squares anisotropic refinement, but they were restrained to the respective bonding atom. The final cycle of refinement yielded standard crystallographic residuals of $R = 0.0846$, $R_w = 0.0496$, $wR = 0.0380$ for observed data (a secondary extinction correction was also applied in the refinement). The absolute stereochemistry of amide 13 could not be assigned directly utilizing the anomalous dispersion effects caused by the sulfur atom. Thus, refinement of the absolute structure index, η, described by Rogers,[17] for the model shown in Figure 2, resulted in η refining to +0.58 (esd 0.47) when initially set to either +1 or −1. On the other hand, refinement of η with starter values of +1 or −1 for the enantiomeric image caused η to refine to −0.58 (esd 0.47). The results tended to favor the absolute stereochemistry shown for amide 13 but were not conclusive. In addition, identical *R* values were observed for refinement of the mirror image of Figure 2, e.g., $R = 0.0846$, $R_w = 0.0496$. Consequently, the absolute stereochemistry for the four chiral centers of amide 13 was assigned by relating the known absolute stereochemistry at the chiral center adjacent to the pyrrolidyl N atom (i.e., C11 is known to have the *S* stereochemical configuration) to the remaining chiral centers. As such, the assignment of the chiral centers (using the X-ray numbering shown in Figure 2: is as follows: 6*R*,9*R*,10*R*,11*S*.

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