Dolastatins 24. Synthesis of (−)-dolastatin 10.[1] X-Ray molecular structure of *N,N*-dimethylvalyl-valyl-dolaisoleuine *tert*-butyl ester

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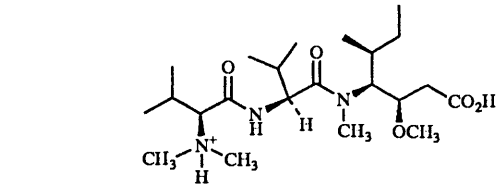
Total synthesis of the extraordinary antineoplastic constituent, dolastatin 10, from the Indian Ocean mollusc *Dolabella auricularia* has been summarized. The final synthetic step involved diethyl cyanophosphonate-mediated coupling of Dov-Val-Dil with Dap-Doe. Improved syntheses of these important precursors has led to a very practical synthesis of natural dolastatin 10. Important details of the HPLC and high-field (500 MHz) NMR characterization techniques employed to confirm the purity of dolastatin 10 have been recorded.

Introduction

Dolastatin 10 **1** is the most potent antineoplastic and tubulin-inhibitory substance known.[2-12] Dolastatin 10 **1** was initially isolated in 1984 and structurally defined by our group in 1987.[13] Importantly, this remarkable Indian Ocean sea hare (*Dolabella auricularia*) peptide was found to contain hitherto unknown amino acid units. Subsequently, we developed an efficient synthetic route to dolastatin 10 that confirmed the structure and absolute configuration[14] of this unique peptide. Since then, a number of partial[15] or total[16] syntheses have appeared.

Dolastatin 10 **1** initially presented a challenging target for synthesis owing to its nine asymmetric centres of then unknown chirality and the urgent need for obtaining a clinical supply. In the latter regard, some 700 tons of the sea hare would have been required and this was clearly untenable for ecological and other reasons. In addition, a practical total synthesis of dolastatin 10 would provide the segments and synthetic analogues needed for elucidation of structure/activity relationships as well as to provide insights into the mechanism of action.

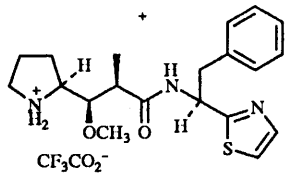
We envisaged preparing peptide **1** as outlined below. By coupling tripeptide unit **2** and dipeptide unit **3**, we planned to avoid some of the downfalls anticipated with a sequential elongation approach. In addition, difficulty in preparation of the dolaphenine (Doe) unit precluded our utilizing a reaction series in which any of this valuable thiazole was forfeited. The absence of a chiral centre next to the carbonyl group in the dolaisoleuine (Dil) unit and the expected ease of its coupling with a proline derivative made this 3 + 2 approach strategically attractive.



$CF_3CO_2^-$ **2**

Dov-Val-Dil•TFA salt

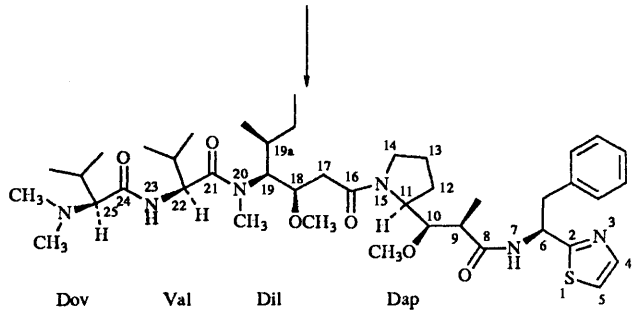
+



$CF_3CO_2^-$

3

Dap-Doe•TFA salt



Dov Val Dil Dap Doe

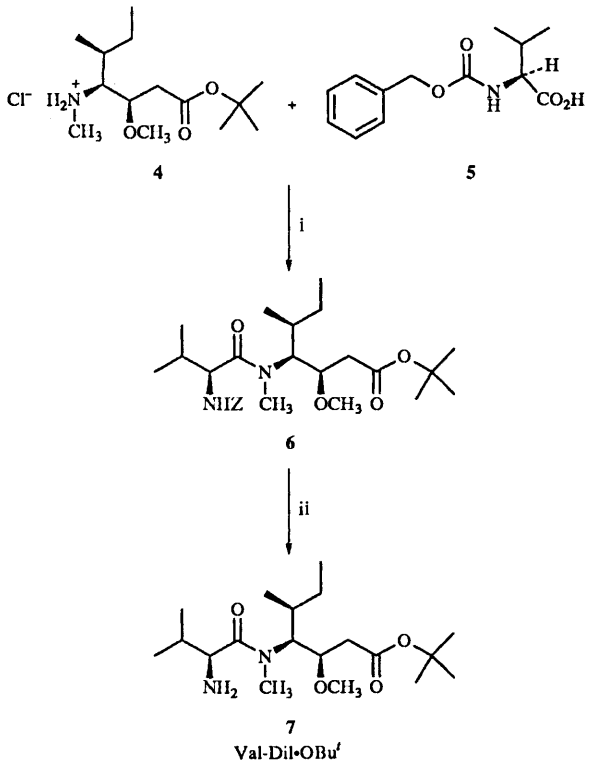
1

Dolastatin 10

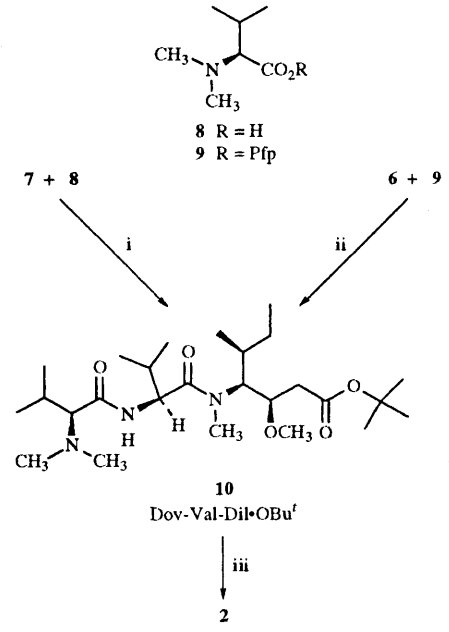
Results and discussions

Syntheses of the dolaproyl-dolaphenine (Dap-Doe) and the Dil components have been described in previous communications in this series.[1,14,17] Hence, synthesis of the tripeptide unit now follows. Dolaisoleuine *tert*-butyl ester hydrochloride [(Dil-OBu^t•HCl, **4**)][17d] was prepared by an aldol sequence from *N*-benzyloxycarbonyl-L-isoleucine. Coupling of amine **4** with commercially available Z-L-valine **5** was accomplished by use of either pivaloyl chloride[18] or tris(dimethylamino)phosphonium bromide hexafluorophosphate (BrOP).[19] Reaction of Z-Val **5** with *N*-methylmorpholine and pivaloyl chloride at −23 °C followed by treatment with amine **4** furnished dipeptide **6** in reasonable yield (73%). However, reaction time was long (74 h) and ^{1}H NMR spectroscopic studies indicated that 5–10% racemization had occurred. A variety of other peptide-bond-forming reagents and techniques were less effective or unworkable. To circumvent this problem, BrOP[19] was used as coupling agent and formation of dipeptide **6** was accomplished at 0 °C in 1 h in 82% yield without racemization. Conversion into amine **7** was realized by use of hydrogen transfer catalysed by 10% palladium on carbon suspended in cyclohexene–methanol.

Coupling of the Val-Dil unit with *N,N*-dimethylvaline (Dov), prepared[20] from L-valine, was next undertaken. Again, two coupling methods were selected from a series of prior



Reagents: i, $(Me_2N)_3P^+BrPF_6^-$, $EtNPr^i_2$; CH_2Cl_2; ii, 5% Pd/C, MeOH, cyclohexene



Reagents: i, $(EtO)_2P(O)CN$, Et_3N, CH_2Cl_2; ii, H_2, 10% Pd/C, 1,4-dioxane; iii, TFA, CH_2Cl_2

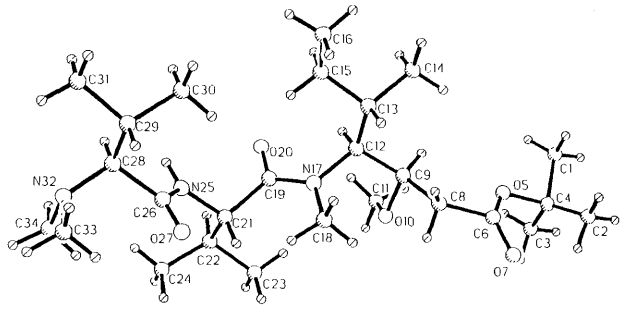


Fig. 1 Computer-generated perspective drawing and X-ray numbering system of the tripeptide derivative Dov-Val-Dil-OBu^t **10**

experiments and used in the formation of tripeptide. The first involved the coupling of Dov as its pentafluorophenyl ester (Dov-OPfp **9**, prepared by reaction of carboxylic acid **8** with pentafluorophenyl trifluoroacetate in the presence of pyridine).[21] Upon treatment with dipeptide **6**, active ester **9** gave tripeptide **10** in 60% overall yield. Racemization was found to be unpredictable and this problem, combined with difficulty in separating the resultant isomers, led to selection of diethyl cyanophosphonate(DEPC)[22]-mediated coupling of Dov **8** with dipeptide **7** to afford tripeptide **10** in 84% yield. No isomeric products were detected during this mild reaction sequence. The X-ray molecular structure determined for tripeptide **10** is shown in Fig. 1. Treatment of tripeptide **10** with trifluoroacetic acid (TFA) gave the corresponding trifluoroacetate **2**.

Reaction of Boc-Dap-Doe[1,14] with TFA furnished trifluoroacetate **3**, which was coupled with tripeptide **2** in the presence of DEPC to afford dolastatin 10 **1** in 97% yield. The physical and spectroscopic data exhibited by the synthetic product were identical with those of the natural product, and activity exhibited against the murine P388 lymphocytic leukaemia was comparable (ED_{50} 4.8×10^{-6} μg cm^{-3}). When dolastatin 10 was first isolated[13] from *Dolabella auricularia*, considerable difficulty was experienced in determining whether or not the substance was pure and this problem was compounded by our having only a few milligrams of the natural product. Some perception of this problem can be ascertained by the following summary of current HPLC and ^1H NMR interpretations, where dolastatin 10 was found readily to undergo conformational changes with variations in experimental conditions.

Clearly, to assess the purity of synthetic and natural dolastatin 10, very specific chromatographic and NMR techniques are required. Development of useful and reliable chromatographic procedures for the analysis of dolastatin 10 (and the dolastatins in general) became an important objective and has been dealt with in detail in a preceding contribution.[23] Initial HPLC analyses of dolastatin 10 were performed using a reversed-phase (C_8) column with 1% acetic acid in 3:1 acetonitrile–water. Generally, this system resulted in good resolution of dolastatin 10 from its impurities, but results were not always reproducible. In addition, this system failed to resolve a mixture of authentic dolastatin 10 and its diastereoisomer (6*R*)-isodolastatin 10[24] (the *S*-Doe unit replaced by the unnatural *R*-Doe). Resolution of these two diastereoisomers was considered a stringent requirement for a useful dolastatin 10 HPLC procedure. Buffered mobile-phase systems gave more useful results.[23] Addition of phosphate salts (50 mmol dm^{-3} as KH_2PO_4) to a 3:1 methanol–water mobile phase (C_8 column) provided well defined peaks, but had limitations including precipitation of the phosphate buffer as well as poor resolution of the dolastatin 10 and (6*R*)-isodolastatin 10 isomer. However, it did provide the first firm evidence that dolastatin 10 could exist as two different conformers at room temperature.[23] Depending upon the sample's physical history, dolastatin 10 exhibited a retention time of either 3.8 min, 4.6 min, or both, with use of the phosphate-buffered solvent system with a reversed-phase C_8 column.

Other HPLC mobile-phase additives were also examined. Sodium hexane-1-sulfonate (5–10 mmol dm^{-3} $HexSO_3Na$) in a mixture of acetonitrile–propan-2-ol–water resolved dolastatin 10 and (6*R*)-isodolastatin 10 with a small difference in retention times (Δt_R = 0.19 min). However, resolution was not realized when an equimolar mixture of the isomers was chromatographed. Although this resulted in a single broadened peak, the technique did provide the best overall system for the HPLC examination of other diastereoisomeric dolastatins, their synthetic intermediates and impurities. Interestingly, with this HPLC solvent only one dolastatin 10 conformational isomer

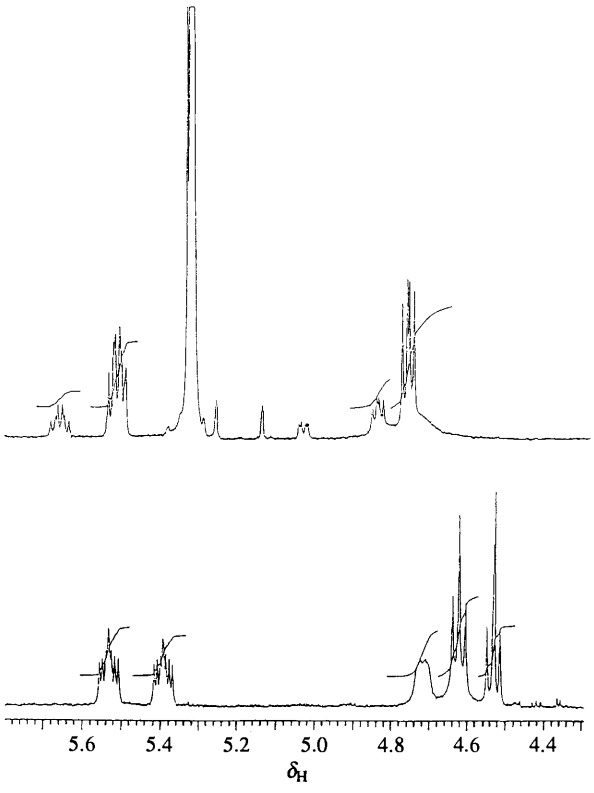


Fig. 2 Effect of solvent on conformer populations. C^{α}-H region of the ^1H NMR spectrum of dolastatin 10 (500 MHz). Top spectrum acquired in CD_2Cl_2, bottom in [$^2H_6$]DMSO.

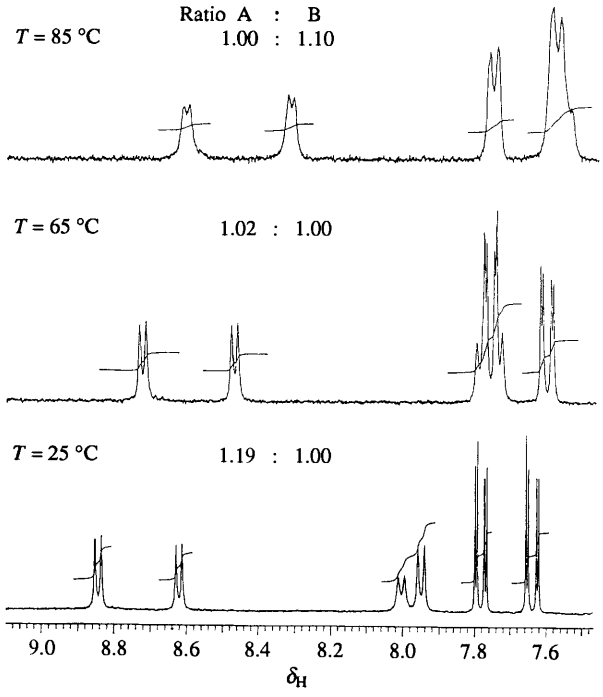


Fig. 3 Temperature dependence of conformer populations. Comparison of the amide signals of conformers A and B (500 MHz; [$^2H_6$]DMSO).

was observed. This medium apparently allows for rapid equilibration to a single, stable conformer. Although this HPLC system greatly enhanced analysis of dolastatin 10 samples, it must be coupled with other methods such as optical rotation and careful interpretation of high-field ^1H and ^{13}C NMR spectra to allow accurate determination of overall sample purity.

Initial NMR studies of dolastatin 10 suggested that this unusual peptide was a mixture of two conformers as indicated by doubling of nearly every signal in the spectra. That the ratio of doubled signals varied with solvent and temperature served to confirm this fact. Spectra obtained in solvents with high relative permittivities [*e.g.*, dimethyl sulfoxide (DMSO), acetone or acetonitrile] showed nearly equal conformer populations, whereas use of solvents with low relative permittivities (*e.g.*, dichloromethane or chloroform) resulted in the greatest difference in conformer populations (Fig. 2). The spectrum obtained in DMSO gave a good separation of the conformer signals. Additionally, temperature dependence studies in DMSO indicated that the ratio of signals varied with temperature (Fig. 3). More recently, the conformational isomerism in dolastatin 10 was shown to arise from *cis–trans* isomerism along the Dil-Dap peptide bond and was further supported by a detailed molecular modelling/NMR analysis of the isomeric (6*R*)-isodolastatin 10.[24]

Experimental

All solvents employed were redistilled prior to use and stored over appropriate drying agents. Solvent extracts of aqueous solutions were dried over sodium sulfate. Amino acids were obtained from Sigma-Aldrich Chemical Co. Column chromatography was performed on Kieselgel 60 (0.063–0.200 mm) and flash chromatography was performed on Kieselgel 60 (0.040–0.063 mm), each supplied by E. Merck, Darmstadt, Germany. Analytical TLC was performed on silica gel GHLF Uniplates (Analtech, Inc.). Mps were measured on a Kofler-type hot-stage apparatus and are uncorrected. $[\alpha]_D$-Values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Mattson 2020 Galaxy Series FT-IR. NMR spectra were recorded on either a Bruker AM 400 or a Varian Gemini 300 MHz instrument and are referenced to tetramethylsilane. *J*-Values are given in Hz. Low-resolution mass spectra were determined on a Finnigan-Mat 312 instrument. High-resolution mass spectra were obtained by the Midwest Center for Mass Spectrometry at the University of Nebraska. Combustion analyses were performed by Spang Microanalytical Laboratories, Eagle Harbor, MI. X-Ray data collection was performed on an Enraf-Nonius CAD4 diffractometer.

[3*R*-(3*R,4*S**,5*S**)]-3-Methoxy-5-methyl-4-[*N*,3-dimethyl-1-2-(phenylmethoxycarboxamido)butanamido]heptanoic acid 1,1-dimethylethyl ester 6, Z-Val-Dil-OBu^t**

Method (a). To a cooled (0 °C) solution of Z-L-valine **5** (1.01 g, 4.02 mmol) in anhydrous dichloromethane (10 cm^3 under nitrogen) were added sequentially *N*,*N*-diisopropylethylamine (1.90 cm^3, 10.95 mmol), BrOP[19] (1.55 g, 4.02 mmol) and Dil *tert*-butyl ester hydrochloride[17d] **4** (1.08 g, 3.65 mmol). The mixture was warmed to room temperature and stirred for 1 h. Removal of solvent under reduced pressure followed by silica gel column chromatography [eluent (9:1) hexane–acetone] furnished dipeptide **6** as a viscous oil (1.48 g, 82%).

Method (b). To a cold (−23 °C) solution of Z-L-valine **5** (4.73 g, 18.82 mmol) in anhydrous chloroform (150 cm^3 under nitrogen) was added *N*-methylmorpholine (3.81 g, 37.70 mmol) followed in 10 min by pivaloyl chloride (2.27 g, 18.82 mmol). The mixture was stirred at −23 °C for 3 h and ester **4** (3.70 g, 9.40 mmol) was added. After 2 h at −23 °C the reaction volume was reduced to 50 cm^3 by being swept with nitrogen, and stirring was continued for 74 h at ambient temperature. The mixture was dissolved in chloroform (180 cm^3) and washed successively with 10% aq. citric acid (50 cm^3), saturated aq. sodium hydrogen carbonate (50 cm^3) and water (50 cm^3). The solvent was removed under reduced pressure. Chromatography of the residue on a column of silica gel [eluent (4:1) hexane–acetone] afforded *dipeptide* **6** as a viscous oil (3.38 g, 73%); $[\alpha]_D^{25}$ −4.65 (*c* 3.14, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3300, 2934, 1726, 1640, 1522, 1507, 1499, 1369 and 1153; δ_C($CDCl_3$) 173.17 (CO),

171.08 (CO), 156.52 (CO), 136.53 (qC), 128.48 (2 × CH), 128.02 (CH), 127.85 (2 × CH), 80.86 (qC), 78.16 (CH), 66.76 (PhCH_2O), 62.44 (CH), 57.87 (OMe), 56.04 (CH), 38.50 (CH_2CO), 33.30 (NMe), 31.12 (CH), 28.08 (CMe_3), 25.85 (CH_2), 20.08 (2 × Me), 17.04 (Me), 15.84 (Me) and 10.95 (Me); δ_H($CDCl_3$) 7.36–7.25 (5 H, m, Ph), 5.48 (1 H, d, *J* 9.0, Val-NH), 5.08 (2 H, s, PhCH_2O), 4.72 (1 H, m, Dil-NCH), 4.48 (1 H, dd, *J* 5.6 and 9.9, Val-α-CH), 3.85 (1 H, m, OCH), 3.30 (3 H, s, OMe), 2.92 (3 H, s, NMe), 2.44–2.22 (2 H, m, CH_2CO), 1.95 (1 H, m, CH), 1.70–1.55 (1 H, m), 1.42 (9 H, s, CMe_3), 1.40–1.25 (2 H, m, CH_2), 0.97 (3 H, d, *J* 6.8, Me), 0.92 (3 H, d, *J* 6.7, Me), 0.88 (3 H, d, *J* 6.7, Me) and 0.80 (3 H, t, *J* 7.4, Me); EIMS *m/z* 492 (M^+), 419, 333, 276, 190, 162, 146 and 100 (100%) [Found: (HR-FAB) *m/z*, 493.3278 $(M + H)^+$. $C_{27}H_{45}N_2O_6$ requires *m/z*, 493.3278].

(3*R,4*S**,5*S**)-4-[*N*,*N*-Dimethyl-L-valyl-(*N*-methyl-L-valin-amido)]-3-methoxy-5-methylheptanoic acid *tert*-butyl ester 10 (Dov-Val-Dil-OBu^t)**

Method (a). To a solution of the dipeptide **6** (4.25 g, 8.64 mmol) in anhydrous methanol (10 cm^3 under nitrogen) was added cyclohexene (10 cm^3) followed by 5% palladium on carbon (4.20 g). The mixture was heated to reflux and maintained at that temperature for 6 min. The solution was cooled and quickly filtered through Celite 454. Removal of solvent from the filtrate under reduced pressure afforded dipeptide **7** as an oil which was used as follows.

To a solution of *N*,*N*-dimethyl-L-valine **8** (1.50 g, 10.37 mmol)[20] in dichloromethane (10 cm^3; under nitrogen, cooled to 0 °C) was added triethylamine (1.44 cm^3, 10.37 mmol) followed by DEPC (1.58 cm^3, 10.37 mmol). Dipeptide **7** was added and the mixture was allowed to warm to room temperature and was stirred for an additional 1 h. Removal of solvent under reduced pressure yielded a residue, which was chromatographed [silica gel column; eluent (9:1) hexane–acetone] to afford tripeptide **10** as a crystalline solid (3.53 g, 84%).

Method (b). A solution of *N*,*N*-dimethyl-L-valine **8** (6.10 g, 42.00 mmol) in warm pyridine (17 cm^3 under argon) was cooled to 0 °C and pentafluorophenyl trifluoroacetate (17.50 g, 62.50 mmol) was then added. The mixture was warmed to room temperature and stirred for 45 min. Ethyl acetate (400 cm^3) was added and the solution was washed sequentially with saturated aq. sodium hydrogen carbonate (2 × 50cm^3) and water (50 cm^3). Removal of solvent under reduced pressure gave a residue, which was purified by column chromatography on silica gel [eluent (6:1) hexane–acetone] to afford *N*,*N*-dimethyl-L-valine pentafluorophenyl ester **9** (Dov-Pfp) as an oil (9.54 g, 73%).

A portion (1.40 g, 4.50 mmol) of ester **9** was added to a solution of dipeptide **6** (0.89 g, 1.70 mmol) in anhydrous 1,4-dioxane (30 cm^3), followed by 10% palladium on carbon (1.0 g). The mixture was subjected to hydrogenolysis for 24 h at ambient temperature. Removal of the catalyst by filtration, and concentration of the filtrate, yielded a residue, which was chromatographed [silica gel column: eluent (4:1) hexane–acetone] to afford *tripeptide* **10** as a crystalline solid (0.50 g, 60%), mp 99–100 °C; $[\alpha]_D^{25}$ −47.6 (*c* 2.10, MeOH); ν_{max}(neat)/cm^{-1} 3296, 2964, 2876, 1732, 1622, 1367 and 1153; δ_C($CDCl_3$) 173.37 (CO), 171.71 (CO), 171.17 (CO), 80.87 (qC), 78.10 (Dil-OCH), 76.48 (Dov-α-CH), 62.34 (Dil-NCH), 57.92 (OMe), 53.70 (Val-α-CH), 42.86 (NMe_2), 38.61 (CH_2CO), 33.25 (NMe), 30.87 (CH), 28.06 (CMe_3), 27.65 (2 × CH), 25.92 (CH_2), 20.13 (Me), 19.73 (Me), 18.06 (Me), 17.57 (Me), 15.72 (Me) and 11.02 (Me); δ_H($CDCl_3$) 6.85 (1 H, d, *J* 8.8, Val-NH), 4.80–4.65 (2 H, br m, Val-α-CH, NCH), 3.85 (1 H, br m, OCH), 3.31 (3 H, s, OMe), 2.96 (3 H, s, NMe), 2.45–2.40 (2 H, m, CH_2CO), 2.28 (1 H, d, *J* 9.3, CH), 2.22 (6 H, s, NMe_2), 2.15–1.95 (2 H, m, 2 × CH), 1.75–1.55 (1 H, m, CH), 1.41 (9 H, s, CMe_3), 1.40–1.20 (2 H, m, CH_2), 0.98 (3 H, d, *J* 6.5, CH_3), 0.96 (3 H, d, *J* 6.1, CH_3), 0.92 (3 H, d, *J* 6.7, Me), 0.90 (3 H, d, *J* 7.6, Me), 0.87 (3 H, d, *J* 7.1, Me) and 0.76 (3 H, t, *J* 7.3, Me) [Found: EIMS *m/z*, 485 (M^+), 442, 412, 227, 199, 155 and 100 (100%). [(HR-FAB) *m/z*, 486.3906 $(M + H)^+$. $C_{26}H_{52}N_3O_5$ requires *m/z*, 486.391] (Found: C, 64.6; H, 10.9; N, 8.5. $C_{26}H_{51}N_3O_5$ requires C, 64.3; H, 10.6; N, 8.65%).

X-Ray molecular structure determination of tripeptide 10

A substandard crystal (0.30 × 0.40 × 0.14 mm) of tripeptide **10**, grown from hexane solution, was used in the data collection (Enraf-Nonius CAD4 diffractometer). Crystal data: $C_{26}H_{51}N_3O_5$, triclinic, space group *P*1, with a = 11.253(2), b = 12.210(2), c = 12.375(2) Å, α = 91.88(1)°, β = 114.73(1)°, γ = 95.11(1)°, V = 1533.78 Å^3, λ(Cu-Kα) = 1.541 84 Å, ρ_o = 1.045 g cm^{-3}, ρ_c = 1.052 g cm^{-3} for Z = 2 and M = 485.71. All reflections corresponding to a complete hemisphere, with $2\theta \leqslant 140°$, were measured at 26 ± 1 °C using the $\omega/2\theta$ scan technique. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 5638 unique reflections remained, of which 3932 were considered observed [$I_o > 3\sigma(I_o)$]. These were used in the subsequent structure determination and refinement. Linear decay and empirical absorption corrections (based on a series of psi-scans)[25] were used to refine the data. Direct methods were used in the structure determination using SHELXS-86.[26] The major portions of both independent molecules of tripeptide **10** were eventually located *via* repeated recycling using the TEXP function of SHELXS-86. Considerable difficulty was encountered in assigning coordinates for the *tert*-butoxycarbonyl portions of the molecule, but these were eventually assigned using difference Fourier maps. Refinement was performed with CRYSTALS.[27] Owing to the apparent disorder in the *tert*-butoxycarbonyl regions, refinement proceeded with difficulty. Restraints were eventually placed on the *tert*-butoxycarbonyl portions of the molecules to assist the refinement process. 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 not refined. Anomalous dispersion effects were included in the calculation of F_c. The final cycle of refinement yielded standard crystallographic residuals of R = 0.106 and R_w = 0.094. Although the final distances and angles were of mediocre quality, the absolute stereochemical assignments of the five chiral centres of tripeptide **10** could be ascertained. Based upon the known absolute stereochemistry of L-valine, the five chiral centres of tripeptide **10** were assigned, using the numbering shown in Fig. 1,† as follows: 9*R*,12*S*,13*S*, 21*S*,28*S*, corresponding to 18*R*,19*S*,19a*S*,22*S*,25*S*, respectively, in dolastatin 10.‡[1]

(2*R,3*R**)-3-Methoxy-2-methyl-*N*-[(*S*)-2-phenyl-1-(thiazol-2-yl)ethyl]-3-[(*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate salt 3 (Dap-Doe TFA)**

A solution of Boc-Dap-Doe[1,14] (1.6 g, 3.38 mmol) in dichloromethane (20 cm^3) was cooled to 0 °C under nitrogen. TFA acid (20 cm^3) was added and the mixture was stirred for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in toluene. The toluene was removed (reduced pressure) and this operation was repeated. The residue was purified by crystallization from diethyl ether to afford *dipeptide-TFA salt* **3** as a solid (1.6 g, 97%), mp 160.5–162.5 °C; $[\alpha]_D^{25}$ −53.8 (*c* 0.29, CH_3OH); δ_H([$^2H_6$]DMSO) 9.05 (1 H, m), 8.88 (1 H, d, *J* 8.6), 7.95 (1 H, m), 7.79 (1 H, d, *J* 3.3), 7.66 (1 H,

† Preparation of Fig. 1 was done with 'SHELXTL-PLUS', G. Sheldrick, Siemens Analytical X-Ray Instruments, Inc., Madison, WI 53719, USA.

‡ *Supplementary material available:* Tables of bond distances, bond angles, atomic coordinates and anisotropic thermal parameters are available for structure **10**. See *Instructions for Authors*, in the January issue.

d, J 3.3), 7.34–7.19 (5 H, m), 5.42 (1 H, m), 3.50–3.32 (2 H, m), 3.36 (3 H, s), 3.15–2.90 (4 H, m), 2.45–2.35 (1 H, m), 1.95–1.40 (4 H, m) and 1.14 (3 H, d, J 6.9) (Found: C, 54.2; H, 5.9; N, 8.4. $C_{22}H_{28}F_3N_3O_4S$ requires C, 54.2; H, 5.7; N, 8.6%).

(3*R,4*S**,5*S**)-4-[*N*,*N*-Dimethyl-L-valyl-(*N*-methyl-L-valin-amido)]-3-methoxy-5-methylheptanoic acid trifluoroacetate salt 2 (Dov-Val-Dil TFA)**

A solution of tripeptide **10** (1.65 g, 3.38 mmol) in dichloromethane (20 cm^3) was treated with TFA (20 cm^3) as described above for dipeptide **3** to afford *tripeptide-TFA salt* **2** (1.77 g, 94%) as crystals from diethyl ether, mp 150–151.5 °C; $[\alpha]_D^{25}$ −24.0 (c 0.47, CH_3OH); $\delta_H([^2H_6]DMSO)$ 12.27 (1 H, br s, Me_2N^+H), 9.58 (1 H, br s, CO_2H), 8.92 (1 H, d, J 8.2, NH), 4.64 (1 H, m), 4.55 (1 H, t, J 8.6), 3.70 (1 H, m), 3.40 (1 H, m), 3.23 (3 H, s, OMe), 2.97 (3 H, s, NMe), 2.77 (3 H, s, Dov-NMe), 2.74 (3 H, s, Dov-NMe), 2.58 (1 H, dd, J 2.0 and 14.9, HCHCO), 2.30–2.20 (2 H, m, CH_2), 2.18 (1 H, dd, J 9.0 and 15.9, HCHCO), 2.00 (1 H, m, CH), 1.85 (1 H, m, CH), 1.65 (1 H, m, CH), 0.95–0.81 (15 H, 5 × d, J 6.5, 5 × Me), 0.75 (3 H, t, J 7.4, Me) (Found: C, 53.1; H, 8.0; N, 7.5. $C_{24}H_{44}F_3N_3O_7$ requires C, 53.0; H, 8.2; N, 7.7%).

(2*R,3*R**)-3-(1-{4-[*N*,*N*-Dimethyl-L-valyl-(*N*-methyl-L-valin-amido)]-(3*R**,4*S**,5*S**)-3-methoxy-5-methylheptanoyl}(*S*)pyrrolidin-2-yl)-3-methoxy-2-methyl-*N*-[(*S*)-2-phenyl-1-(thiazol-2-yl)ethyl]propanamide 1 (dolastatin 10)**

Dipeptide-TFA salt **3** (0.30 g, 0.63 mmol) and tripeptide-TFA salt **2** (0.29 g, 0.63 mmol) were combined in 1,2-dimethoxyethane (2 cm^3) under nitrogen. The solution was cooled to 0 °C and triethylamine (0.44 cm^3, 3.13 mmol) was added followed by DEPC (0.15 cm^3, 0.94 mmol). The mixture was stirred at 0 °C for 1 h and then for 2 h at ambient temperature. Removal of solvent under reduced pressure followed by silica gel column chromatography [eluent (1:1) hexane–acetone] furnished dolastatin 10[1] as a clear viscous oil (0.50 g, 97%) which solidified under reduced pressure, mp 104–106 °C; $[\alpha]_D^{25}$ −57.0 (c 0.026, CH_3OH). The synthetic dolastatin 10 exhibited TLC, HPLC, IR, ^1H and ^{13}C NMR and MS spectroscopic data identical with those of the natural product[13] (Found: C, 63.8; H, 9.0; N, 10.4. $C_{42}H_{68}N_6O_6S\cdot\frac{1}{2}H_2O$ requires C, 63.6; H, 8.7; N, 10.6%).

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