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# THE SYNTHESIS AND CONFORMATION IN SOLUTION OF CYCLO[L-PRO-L-LEU-L-(GLN)THZ-(GLY)THZ-L-VAL] (DOLASTATIN 3)

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**Abstract:** Dolastatin 3, a unique cyclic peptide, was synthesised in high yield. Its minimum energy conformation in solution was established by NMR spectroscopy and force field calculations, and is characterised by three intramolecular hydrogen bonds.

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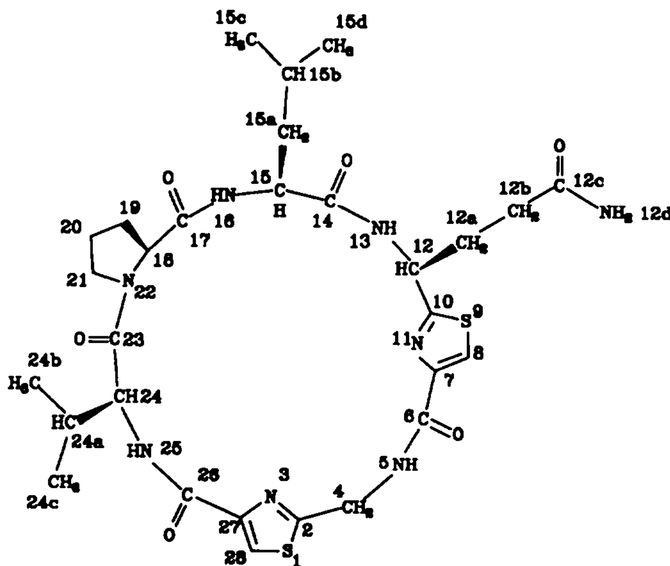
Dolastatin 3, isolated in 1981 by Pettit *et al.*[1] in a 1 mg quantity from 100 kg of wet Indian Ocean sea hare *Dolabella auricularia*, is one of the most active antineoplastic agents known to man (ED = $1 \times 10^{-4}$ to $1 \times 10^{-7}$ $\mu g/m\ell$ against P388 lymphocytic leukemia cells). Pettit proposed a unique cyclic pentapeptide structure containing three common amino acids, Pro, Leu and Val, and two unusual heterocyclic amino acids, the achiral (Gly)Thz and the chiral (Gln)Thz, for dolastatin 3. Ultimate structural and conformational analysis of this natural product, which *inter alia* can lead to a better understanding of the conformational-activity relationships of cyclic peptides, was precluded by the small amount available from nature.

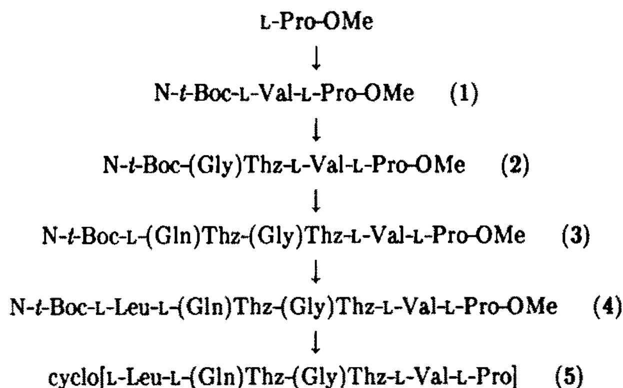


**Dolastatin 3 (5)**

Independent syntheses by three groups showed that the structure proposed by Pettit for dolastatin 3 is incorrect.[2] Significantly, none of the synthetic isomers of dolastatin 3[2,3] show any cell growth inhibition against L1210 murine leukemia cells cultured *in vitro* and no activity was observed in concentrations of 250 μg/mℓ.[2,3] The structure, cyclo[L-Pro-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val] (**5**), which was derived independently by high resolution NMR spectroscopy studies[4] and mass spectroscopic fragmentation studies of dolastatin 3 isomers,[5] was shown by synthesis to be identical to dolastatin 3 (**5**).[4] In this paper we describe the synthesis as well as the most likely conformation in solution of dolastatin 3, the latter based mainly on structural data compiled from NMR spectroscopy.

## RESULTS AND DISCUSSION

**Synthesis:** Optically pure N-*t*-Boc-L-(Gln)Thz-OH was prepared in high yield from the N-*t*-Boc-thiocarboxamide precursor by means of a modified Hantzsch reaction developed in this laboratory.[5] The high yield synthesis of the linear pentapeptide N-*t*-Boc-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe (**4**, Scheme 1) was accomplished by employing diethylphosphoryl cyanide[6] as coupling reagent. The secondary amine Pro-OMe was used as starting material due to its resistance to racemisation during basic hydrolysis.[7] Cyclisation of the linear pentapeptide by pentafluorophenol activation[8] afforded dolastatin 3 (**5**) in a yield of 76%. This method does not require the high dilution and long reaction times normally associated with this type of reaction.[2,3] Efforts to crystallise this compound from a variety of solvents, or as the corresponding triphenylphosphine oxide complexes,[9] were unsuccessful.




SCHEME 1

**NMR Studies of Conformation in Solution:** All the $^{1}H$ and $^{13}C$ NMR data of dolastatin 3 (**5**, 400 MHz, $CDCl_3$) are listed in Table 1. Assignment of the $^{1}H$ resonances is based on spin decoupled and COSY experiments, as well as by comparison with the spectra of the shorter synthetic fragments (**1-3**). The $^{13}C$ signals

Table 1: $^{1}H$ and $^{13}C$ NMR data[a] of dolastatin 3 (5)[1b]

| Nuclei | Chemical shifts (δ) $^{13}C^{b}$ | $^{1}H$ | $^{1}H$ multiplicity (Hz)$^{c}$ |
|---|---|---|---|
| 2 | 148.30 s | – | |
| 4 | 40.97 t | 4.65 | 1H, dd, J = 18.1 and 2.4 |
| | | 5.23 | 1H, dd, J = 18.1 and 7.3 |
| 5 | – | 8.73 | 1H, dd, J = 7.3 and 2.4 |
| 6 | 174.30 s | – | |
| 7 | 160.80 s | – | |
| 8 | 124.26 d | 8.07 | 1H, s |
| 10 | 149.00 s | – | |
| 12 | 54.97 d | 5.52 | 1H, ddd, J = 10.9, 9.2 and 4.6 |
| 12a | 29.62 t | 2.13 | 2H, m |
| 12b | 33.27 t | 2.40–2.70 | 2H, m |
| 12c | 171.09 s | – | |
| 12d | – | 5.48 | 1H, br s |
| | – | 6.29 | 1H, br s |
| 13 | – | 7.83 | 1H, d, J = 9.2 |
| 14 | 172.00 s | – | |
| 15 | 48.61 d | 3.83 | 1H, ddd, J = 11.0, 7.0 and 4.0 |
| 15a | 37.71 t | 1.55 | 2H, m |
| 15b | 25.43 d | 2.33 | 1H, m |
| 15c | 18.55 q | 0.90 | 3H, d, J = 6.6 |
| 15d | 19.53 q | 0.95 | 3H, d, J = 6.6 |
| 16 | – | 6.39 | 1H, d, J = 7.0 |
| 17 | 169.40 s | – | |
| 18 | 62.61 d | 4.00 | 1H, t, J = 7.8 |
| 19 | 28.41 t | 2.24 | 2H, m |
| 20 | 25.45 t | 1.94 | 2H, m |
| 21 | 48.26 t | 3.69 | 1H, dt, J = 9.6 and 5.9 |
| | | 3.87 | 1H, ddd, J = 9.6, 7.5 and 3.3 |
| 23 | 170.92 s | – | |
| 24 | 55.62 d | 4.75 | 1H, dd, J = 9.2 and 7.2 |
| 24a | 31.88 d | 2.05 | 1H, m |
| 24b | 21.18 q | 1.04 | 3H, d, J = 6.7 |
| 24c | 23.30 q | 1.15 | 3H, d, J = 6.8 |
| 25 | – | 8.31 | 1H, d, J = 9.2 |
| 26 | 165.77 s | – | |
| 27 | 160.30 s | – | |
| 28 | 123.71 d | 8.05 | 1H, s |

a) Chemical shifts are downfield from internal TMS in $CDCl_3$ at 400 MHz. b) Chemical shift and multiplicity of proton undecoupled carbon signals. c) Relative integral, multiplicity and coupling constants.

were assigned by comparison with reported $^{13}C$ resonances of amino acids[12] complimented by DEPT and HETCORR experiments. With the exception of the chemical shifts of the NH resonances, no notable changes in the $^{1}H$ and $^{13}C$ NMR spectra (chemical shifts, coupling constants) occur when the spectra are recorded in solvents other than $CDCl_3$.

Table 2: $^1$H NMR data[a] for NH protons of dolastatin 3 (5) as functions of solvent and temperature

| Residue | Proton | Chemical shifts ($\delta$) CDC$\ell_3$ | DMSO-$d_6$ | $\Delta\delta^b$ | d$\delta$/dT$^c$ | $^3J_{NH,C^{\alpha}H}$ (Hertz) CDC$\ell_3$ | DMSO-$d_6$ |
|---|---|---|---|---|---|---|---|
| (Gly)Thz | 5 | 8.73 | 8.73 | 0.0 | 0.3 | 2.4, 7.3 | 2.5, 7.5 |
| (Gln)Thz | 12d | 5.48 | 6.67 | +1.19 | 4.5 | – | – |
| | | 6.29 | 7.12 | +0.83 | 5.0 | – | – |
| | 13 | 7.83 | 7.73 | –0.10 | 0.8 | 9.2 | 9.2 |
| Leu | 16 | 6.39 | 8.34 | +1.95 | 3.5 | 7.0 | 6.7 |
| Val | 25 | 8.31 | 8.21 | –0.10 | 0.0 | 9.2 | 8.9 |

a) Downfield from internal TMS. b) Downfield shift in DMSO-$d_6$ relative to CDC$\ell_3$. c) Temperature range: 20 – 100°C (in DMSO-$d_6$, 10°C intervals), d$\delta$/dT = $10^{-3}$ ppm/K.

The $^1$H and $^{13}$C NMR spectra of dolastatin 3 (5) in CDC$\ell_3$ and DMSO-$d_6$ contain only one set of signals (Table 1). There is strong differentiation between the separate amino acid NH signal temperature gradients (Table 2). The diastereotopicity of the geminal methylene protons of (Gly)Thz (H-4) is reflected by differences in their chemical shifts and coupling constants (see Table 1). The side chain methylene protons of the Leu and (Gln)Thz moieties also display their diastereotopicity with large differences in vicinal coupling constants with the respective $C^{\alpha}$ protons. On the basis of the NMR data, summarised in Tables 1 and 2, it may be concluded that dolastatin 3 fulfills all the empirical rules[10] for conformational homogeneity. However, the possibility cannot be excluded that at equilibrium one conformation is so dominant that the effect of other conformations on spectroscopic parameters is negligible. Nevertheless, NMR spectroscopy may be utilised for conformational studies in both instances.

Useful information concerning the conformational rigidity of peptides can be extracted from their circular dichroism (CD) spectra, since the CD curve is a sensitive function of the relative orientation of chromofores and neighbouring groups.[11] The CD spectrum of a methanolic solution of dolastatin 3 (5, 0.220 mg/ml) at room temperature is given in Figure 1. This CD plot displays a weak positive Cotton effect at 258 nm and a strong negative Cotton effect at 230 nm ($\Delta\epsilon_{max}$ = –19.10). This spectrum is indicative of a rigid peptide backbone.[11]

The $^{13}$C NMR resonances of $C^{\alpha}$(C-18), $C^{\beta}$(C-19) and $C^{\gamma}$(C-20) of Pro (Table 1) provide strong evidence[12] in favour of the *trans* configuration of the Pro peptide bond (i.e. the amide bond between Pro and Val). This result is substantiated by strong NOE effects and strong negative cross correlation ROE effects between H-24a and both H-21 protons. These effects will be highly unlikely in the case of a *cis* Pro peptide bond. This result is significant, especially in view of the strong conformation directing properties of Pro,[10] as well as the fact that cyclic pentapeptides are the smallest cyclic peptides in which all amide linkages can have the unstrained *trans* configuration.[10] This led to the following postulates *viz.*: (i) all the peptide groups are *trans* planar and (ii) the thiazolyl carboxamide moieties are planar due to the resonance effect. This plana-

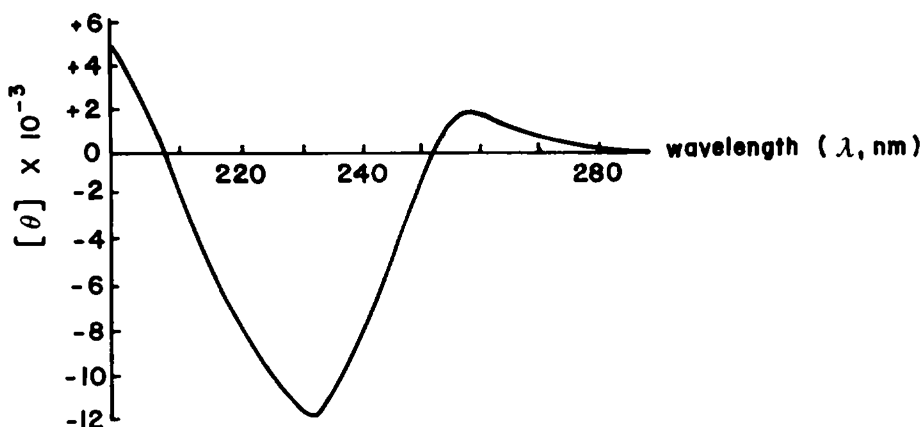



Figure 1: The CD spectrum of a methanolic solution of dolastatin 3 (5)

rity has been observed in the crystal structures of the related cyclic peptides nosiheptide[13] and cyclo[L-Pro-L-Leu-L-Val-(Gly)Thz-(Gly)Thz].[14]

The involvement of amide protons in intramolecular hydrogen bonding[15] was established on the basis of $^{1}H$ NMR. The chemical shifts of amide protons, available for intermolecular hydrogen bonding, are much more dependent on solvent and temperature than their intramolecularly hydrogen bonded counter parts.[10] The NH proton of Leu and the $NH_2$ protons of (Gln)Thz (Table 2) are thus identified as protons exposed to the solvent.[10] In contrast with the other amide protons, these protons exchange rapidly with deuterium at room temperature as well. The behaviour of the NH protons of (Gln)Thz, (Gly)Thz and Val are typical of protons that are intramolecularly hydrogen bonded.[10,15]

A Dreiding model of the peptide backbone of dolastatin 3 (5) was constructed compatible with the abovementioned results and a set of selfconsistent $\phi$ torsion angles (CO-NH-$C^{\alpha}$HR-CO)[10] calculated from $J_{NH,C^{\alpha}H}$ values by means of modified Karplus equations[16] (Equations 1 and 2, see Experimental). The effects of electronegative influences of substituents on $C^{\alpha}$ were taken into consideration using Equations 3 and 4[16] (see Experimental). Although it has been proposed that Pro usually exists as two fast equilibrating Ramachandran conformations,[17] exact analysis later showed that a greater degree of *pseudo*-rotation must be taken into account.[18] With this in mind we derived the $\phi$ torsion angle of Pro, along with all the $\psi$ torsion angles (NH-$C^{\alpha}$HR-CO-NH), from the Dreiding model corresponding to our proposed minimum energy conformation of dolastatin 3.

Inspection of this Dreiding model showed that intramolecular N-H hydrogen bonds over the centre of the ring cannot be accomodated since transannular hydrogen bonds would require substituting a *trans* for a *cis* Pro peptide bond. This is also true for cyclo[L-Pro-L-Leu-L-Val-(Gly)Thz-(Gly)Thz], where Schmidt *et al.*[14] proved by X-ray analysis that the Pro peptide bond is indeed in the *cis* configuration and this compound

thus able to accomodate two central transannular N-H hydrogen bonds, one from Leu–NH to a Thz endocyclic nitrogen atom and the other from a (Gly)Thz-NH to the Leu-CO. It is interesting to note that Bernier *et al.*[19] in a study of the conformation in solution of this dolastatin 3 analogue, which adopts a definite preferred conformation both in the crystal and solution,[14] did not speculate on the significance of the Pro peptide bond configuration. Not surprisingly, the intramolecular hydrogen bond assignments by this group were shown to be incorrect by Schmidt *et al.*[14] The presence of a *trans* Pro peptide bond in dolastatin 3 (5), therefore, precludes transannular hydrogen bonds over the centre of the ring. Further inspection of conformational isomers of dolastatin 3 showed that in the presence of a *trans* Pro peptide bond the following hydrogen bonds can be simultaneously accomodated in dolastatin 3: (i) Val-NH with the endocyclic N-atom of (Gly)Thz, (ii) (Gly)Thz with the endocyclic N-atom of (Gln)Thz and (iii) (Gln)Thz-NH with the carbonyl of Pro. The latter hydrogen bond leads to the formation of an inverse $\gamma$ loop[10] between Pro and (Gln)Thz. This result is also substantiated by a definite NOE effect between the $C^{\alpha}$-H of Pro and the NH of Leu.[20]

**Refinement of the Conformation of Dolastatin 3 (*5*) by Molecular Force Field Calculations**: The relative orientations of the side chains were tentatively established to be in accordance with NOE and ROE effects as well as $^1$H-$^1$H coupling constants. Standard bond lengths and angles[14] were used in addition to the Dreiding model deduced torsion angles for the construction of the model to be computed by the SYBYL molecular force field calculating program.[21] Due to the limitations inherent in this type of force field calculations it was appreciated that the refined conformation will only represent a realistic minimum energy conformation if this starting conformation (Dreiding model) is close to the real preferred conformation. The influence of solvent on the conformation of the solute was not taken into consideration, since the molecular calculations were performed for a model *in vacuo*. The SYBYL molecular force field calculating program yielded, as expected, a conformation not too different from the starting conformation. This conformation, therefore, remains consistent with the NMR data of dolastatin 3 in solution. The complete set of torsion angles ($\phi$, $\psi$ and $\omega$) for the peptide backbone of the proposed minimum energy conformation in solution of dolastatin 3 is listed in Table 3.

**Table 3**: The peptide backbone refined torsion angles of the minimum energy conformation of dolastatin 3

| Angle | Amino acid residue | | | | |
|---|---|---|---|---|---|
| type[a] | Val | Leu | (Gln)Thz | (Gly)Thz | Pro |
| $\phi$ | −118 | −66 | −161 | −143 | −52 |
| $\psi$ | −83 | +101 | −61 | −60 | +139 |
| $\omega$ | +175 | −158 | +179 | +170 | +178 |

a) Two additional angles: $N_{(gln)thz}CC(O)N_{val} = 0^\circ$ and $N_{(gly)thz}CC(O)N_{(gln)thz} = 0^\circ$.

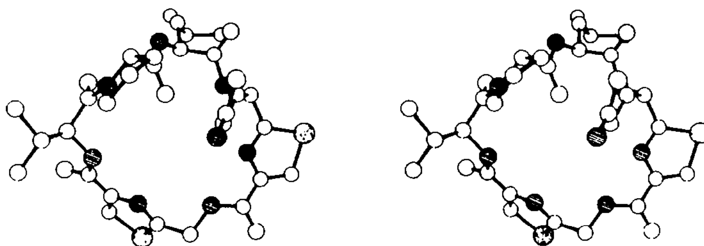


Figure 2: ORTEP stereoscopic projection of the minimum energy conformation of dolastatin 3 (5)

The minimum energy conformation of dolastatin 3 (5) in solution (Figure 1) takes on a saddle shape with turning points at the positions between the two Thz units and at the inverse $\gamma$ loop in which Leu occupies the middle position. The torsion angles of this inverse $\gamma$ loop is in accordance with the reported values of an inverse $\gamma$ turn.[22] The two endocyclic N atoms of the two Thz units are elevated to a point just above the ring. Pro takes on an *endo* or up conformation[23] and is slightly bent over the ring. The Val side chain is directed to a point above but away from the ring, whereas the (Gln)Thz side chain is directed to a point directly above the ring. All the NH hydrogens of the peptide bonds, except that of Leu, are directed to a point in the middle of the ring. It is interesting to note that the two planar Thz units form a torsion angle of $-143^{\circ}$ around the CH-NH bond of (Gly)Thz. It follows from this discussion that the conformation of dolastatin 3 in solution clearly differs in quite a few respects from that obtained by Schmidt *et al.*[14] of a biologically inactive dolastatin 3 analogue.

## CONCLUSION

An efficient synthesis of dolastatin 3 (5) provided sufficient material for a study of its conformation in solution. The conformational homogeneity of dolastatin 3, which may contribute significantly to its unique biological activity, is propably due to the presence of two directly linked planar thiazolyl carboxamide systems which together with a *trans* Pro peptide bond, and the intramolecular hydrogen bonds, markedly reduce the conformational mobility of the peptide backbone. The proposed preferred conformation of dolastatin 3 in solution is, however, based on limited structural information gleaned from NMR-measurements. Final proof of the conformation of dolastatin 3, albeit in static form, will only follow from X-ray analysis of this compound.

## EXPERIMENTAL

Modified Karplus equations (Equations 1 and 2)[16] were used to calculate the $\phi$ torsion angles of the peptide backbone. The effect of electronegative influences of substituents on $C^{\alpha}$ were taken into consideration using Equations 3 and 4.[16]

$$^{3}J_{NH,C^{\alpha}H} = 9.8\cos^2\theta - 1.1\cos\theta + 0.4\sin^2\theta \qquad \text{Equation 1}$$

$$\Sigma(^{3}J_{NH,C^{\alpha}H_2}) = -9.4\cos^2\phi - 1.1\cos\phi + 14.9 \qquad \text{Equation 2}$$

$$^{3}J_{NH,C^{\alpha}H} = 1.09\ J_{obs} \qquad \text{Equation 3}$$

$$\Sigma(^{3}J_{NH,C^{\alpha}H_2}) = 1.04\ \Sigma(J_{obs}) \qquad \text{Equation 4}$$

Where: $\theta$ is the dihedral angle H-N-$C^{\alpha}$-H; $\phi = \theta + 60^{\circ}$ for *S*-configurated amino acids; Equations 1 and 2 are applicable to glycine residues.

The Boc-L-Leu, L-Pro-OMe.HCℓ and Boc-L-Val were used as received from Sigma Chemical Co. 1,2-Dimethoxyethane and dioxane were distilled from sodium and dimethylformamide was distilled from calcium hydride. Whatman silica gel KSF (250 $\mu$) plates were used as stationary phase for thin layer chromatography (TLC). Silica gel (70-230 mesh, E. Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals) was used as stationary phase for column chromatography. Nuclear magnetic resonance spectra were recorded on a Varian VXR-400 or Bruker WP-500 instruments using tetramethylsilane (TMS) as internal standard. Overlapping signals in the $^{13}C$ spectra are indicated with an asterisk. Infrared spectra were recorded in chloroform solutions. Mass spectra were obtained with a Varian MAT-212/55-188 mass spectrometer.

**N-*t*-Boc-L-Val-L-Pro-OMe (*1*)**

To a stirred solution of L-proline methyl ester hydrochloride (4.00 g, 24.2 mmol) and N-*t*-Boc-L-valine (5.29 g, 24.3 mmol) in 1,2-dimethoxyethane at $0^{\circ}C$ were added triethylamine (7.07 mℓ, 50.8 mmol) and diethylphosphoryl cyanide (4.03 mℓ, 26.6 mmol). After 1 h at $0^{\circ}C$ and 4 h at room temperature the reaction mixture was diluted with ethyl acetate (250 mℓ) and washed successively with 5% hydrochloric acid (1 x 50 mℓ), aqueous sodium hydrogencarbonate (1 x 50 mℓ) and with saturated brine (1 x 50 mℓ). Removal of the solvent *in vacuo* and chromatography of the residue furnished N-*t*-Boc-L-Val-L-Pro-OMe (1) as a colourless syrup[24] (7.29 g, 92%); $[\alpha]_D^{25}$ −70.0° (*c* 4.0, chloroform); MS(EI) accurate mass m/z 328.1984 ($M^{+}$; calc. 328.1998 for $C_{16}H_{28}N_2O_5$); $\nu_{max}$ 3420, 1740, 1700 and 1640 $cm^{-1}$; $^{1}H$ NMR[24] ($CDCl_3$) $\delta$ 0.91 (3H, d, J = 6.6 Hz, $CH_3CH$), 1.00 (3H, d, J = 6.8 Hz, $CH_3CH$), 1.40 (9H, s, $(CH_3)_3C$), 1.85-2.30 (4H, m), 2.00 (1H, m, $(CH_3)_2CH$), 3.55-3.80 (2H, m, $NCH_2$), 3.69 (3H, s, $OCH_3$), 4.25 (1H, dd, J = 9.2 and 6.3 Hz, NCHCON), 4.50 (1H, m, $NCHCOOCH_3$), 5.18 (1H, d, J = 9.2 Hz, NHCO); $^{13}C$ NMR ($CDCl_3$) $\delta$ 17.33 (q), 19.24 (q),

24.96 (t), 28.31 (q)*, 29.00 (t), 31.31 (d), 47.08 (t), 52.12 (q), 56.77 (d), 58.74 (d), 79.41 (s), 155.84 (s), 171.15 (s), 172.44 (s).

**N-*t*-Boc-(Gly)Thz-L-Val-L-Pro-OMe (*2*)**

To a stirred solution of N-*t*-Boc-L-Val-L-Pro-OMe (1, 3.93 g, 12.0 mmol) in dry dichloromethane (20 mℓ) at 0°C, trifluoroacetic acid (20 mℓ) was added dropwise. After 1 h at 0°C and 1 h at room temperature the reaction mixture was diluted with dry carbon tetrachloride (100 mℓ) and the solvents removed *in vacuo*. The residue, a colourless syrup, was dried *in vacuo* for 12 h. The crude dipeptide trifluoroacetate and N-*t*-Boc-(Gly)Thz[5] (3.09 g, 12.0 mmol) were dissolved in 1,2-dimethoxyethane (9 mℓ). The solution was cooled to 0°C and triethylamine (3.66 mℓ, 26.3 mmol) and diethylphosphoryl cyanide (2.00 mℓ, 13.2 mmol) were added dropwise. After 1 h at 0°C the mixture was allowed to warm to room temperature. After 4 h the mixture was diluted with ethyl acetate (100 mℓ) and successively washed with 5% hydrochloric acid (1 x 50 mℓ), aqueous sodium hydrogencarbonate (1 x 50 mℓ) and saturated brine (1 x 50 mℓ). The organic phase was dried (anhydrous sodium sulphate) and the solvent removed *in vacuo*. Chromatography of the residue on a column of silica gel (250 g) in 3:7 hexane-ethyl acetate furnished *N-t-Boc-(Gly)Thz-L-Val-L-Pro-OMe* (2) (5.09 g, 91%) as a colourless syrup; $[\alpha]_D^{25}$ −39.8° (*c* 2.44, chloroform), MS(EI) accurate mass m/z 468.2029 ($M^+$, calc. 468.2043 for $C_{21}H_{32}N_4O_6S$); $\nu_{max}$ 3440, 3380, 1740, 1720 and 1640 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.98 (3H, d, J = 6.6 Hz, $CH_3CH$), 1.06 (3H, d, J = 6.6 Hz, $CH_3CH$), 1.43 (9H, s, $(CH_3)_3C$), 1.88-2.30 (4H, m), 2.00 (1H, m, $(CH_3)_2CH$), 3.69 and 3.85 (2x 1H, 2x m, $CH_2NCO$), 3.70 (3H, s, $OCH_3$), 4.51 (1H, m, $NCHCOOCH_3$), 4.52 (2H, m, $CH_2NHCO$), 4.72 (1H, dd, J = 9.2 and 7.1 Hz, CHNHCO), 5.40 (1H, br t, NH-Boc), 7.90 (1H, d, J = 9.2 Hz, CHNHCO), 7.95 (1H, s, thiazole proton); $^{13}C$ NMR ($CDCl_3$) $\delta$ 17.93 (q), 19.24 (q), 24.99 (t), 28.29 (q)*, 29.05 (t), 31.57 (d), 42.24 (t), 47.30 (t), 52.18 (q), 55.55 (d), 58.87 (d), 80.10 (s), 123.72 (d), 149.33 (s), 155.52 (s), 160.82 (s), 169.0 (s), 170.49 (s), 172.41 (s).

**N-*t*-Boc-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe (*3*)**

Trifluoroacetic acid (4 mℓ) was added dropwise to a stirred solution of N-*t*-Boc-(Gly)Thz-L-Val-L-Pro-OMe (2) (500 mg, 1.07 mmol) in dry dichloromethane at 0°C. After 1 h at 0°C and 1 h at room temperature the reaction mixture was diluted with carbon tetrachloride (20 mℓ) and the solvents removed *in vacuo* to furnish the tripeptide trifluoroacetate as a colourless syrup. The product was dried under vacuum for 24 h and dissolved in dry dimethylformamide (3 mℓ). N-*t*-Boc-L-(Gln)Thz[5] (350 mg, 1.06 mmol) was added to the solution. The mixture was cooled to 0°C and triethylamine (0.33 mℓ, 2.4 mmol) and diethylphosphoryl cyanide (0.18 mℓ, 1.2 mmol) was added dropwise over a period of 0.5 h. After 1 h at 0°C and 6 h at room temperature the solvents were removed *in vacuo*. The residue was chromatographed on Sephadex LH-20 in 2:1 chloroform-methanol to furnish *N-t-Boc-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe* (3) (674 mg, 93%), mp 129-130°C (from ethyl acetate-hexane); $[\alpha]_D^{26}$ −70.6° (*c* 1.3, chloroform); MS(EI) m/z 679 ($M^+$); $\nu_{max}$ 3400, 1740, 1665, 1640 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.01 (3H, d, J = 6.8 Hz, $CH_3CH$), 1.08 (3H, d, J = 6.8 Hz, $CH_3CH$), 1.41 (9H, s, $(CH_3)_3C$), 1.80-2.40 (9H, m), 3.68 and 3.79 (2 x 1H, 2 x m, $CH_2NCO$), 3.72 (3H, s, $OCH_3$), 4.55 (1H, m, $NCHCOOCH_3$), 4.76 (1H, dd, J = 9.3 and 7.1 Hz, CHNH), 4.77 and 4.95 (1H, dd, J = 16.5 and 5.2 Hz and 1H, dd, J = 16.5 and 6.1 Hz, respectively, $CH_2NHCO$), 4.94 (1H, m, $CH_2CHNH$), 5.55

and 6.36 (2 x 1H, 2 x br s, $CONH_2$), 6.26 (1H, d, J = 8.1 Hz, NH-Boc), 7.88 (1H, d, J = 9.5 Hz, CHNH), 8.03 and 8.04 (2 x 1H, 2 x s, thiazole protons), 8.33 (1H, dd, J = 6.1 and 5.2 Hz, $CH_2NH$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 18.02 (q), 19.22 (q), 24.88 (t), 28.30 (q)*, 29.08 (t), 30.18 (t), 31.75 (t), 31.89 (d), 40.67 (t), 47.48 (t), 52.31 (q), 52.50 (d), 55.42 (d), 58.70 (d), 80.17 (s), 123.75 (d), 124.23 (d), 148.97 (s), 149.05 (s), 155.80 (s), 160.53 (s), 161.01 (s), 166.82 (s), 170.76 (s), 172.48 (s), 173.21 (s), 174.89 (s); Found: C, 51.34; H, 6.18; N, 14.30. Calcd. for $C_{29}H_{41}N_7O_8S_2$: C, 51.23; H, 6.09; N, 14.44.

**N-*t*-Boc-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe (*4*)**

N-*t*-Boc-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe (3) (531 mg, 781 μmol) was dissolved in trifluoroacetic acid (4 mℓ) at 0°C. After 15 min at 0°C the mixture was allowed to warm to room temperature. After 45 min at ambient temperature the reaction mixture was diluted with carbon tetrachloride (40 mℓ) and the solvents removed *in vacuo*. The residual tetrapeptide trifluoroacetate, a colourless glass, was dried under vacuum for 24 h and dissolved in dimethylformamide (8 mℓ). N-*t*-Boc-L-Leu (217 mg, 938 μmol) was added and the solution cooled to 0°C. To this solution triethylamine (0.26 mℓ, 1.9 mmol) and diethylphosphoryl cyanide (0.14 mℓ, 920 μmol) were added dropwise. After 1 h at 0°C and 4 h at room temperature the solvents were removed *in vacuo*. The residue was chromatographed on Sephadex LH-20 in 2:1 chloroform-methanol. The main fraction was further purified by chromatography on a silica column (50 g) with 7% methanol in ethyl acetate to furnish *N-t-Boc-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val-L-Pro-OMe* (4) (533 mg, 86%) as an amorphous white powder. It had mp 125-126°C (from ethyl acetate-hexane); $[\alpha]_D^{26}$ −75.0° (*c* 3.7, chloroform); MS(EI) m/z 792 ($M^+$); $\nu_{max}$ 3400, 1740, 1660, 1640 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.80 (6H, d, J = 5.4 Hz, $(CH_3)_2CH$), 0.98 (3H, d, J = 6.8 Hz, $CH_3CH$), 1.06 (3H, d, J = 6.6 Hz, $CH_3CH$), 1.36 (9H, s, $(CH_3)_3C$), 1.53 (2H, m), 1.80-2.40 (4H, m), 2.00 (1H, m, $(CH_3)_2CH$), 2.14 (1H, m, $(CH_3)_2CH$), 2.20 (2H, m), 2.30 (2H, m), 3.68 and 3.89 (2 x 1H, 2 x m, $CH_2NCO$), 3.69 (3H, s, $OCH_3$), 4.22 (1H, m, CHNHCO), 4.55 (1H, m, $NCHCOOCH_3$), 4.78 (1H, dd, J = 9.3 and 7.7 Hz, CHCHNH), 4.87 (2H, d, J = 5.7 Hz, $CH_2NHCO$), 5.21 (1H, m, $CH_2CHNH$), 5.31 (1H, d, J = 7.65 Hz, NH-Boc), 5.82 and 6.60 (2 x 1H, 2 x br s, $CONH_2$), 7.90 (1H, d, J = 9.3 Hz, CHNH), 8.01 and 8.03 (2 x 1H, 2 x s, thiazole protons), 8.11 (1H, d, J = 7.9 Hz, CHNHCO), 8.43 (1H, br t, J = 5.7 Hz, $CH_2NH$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 18.08 (q), 19.18 (q), 21.90 (q), 22.79 (q), 24.63 (d), 24.92 (t), 28.30 (q)*, 29.12 (t), 30.21 (t), 31.68 (t), 31.74 (d), 40.82 (t), 41.78 (t), 47.54 (t), 51.00 (d), 52.27 (q), 52.51 (d), 55.53 (d), 58.99 (d), 80.10 (s), 123.90 (d), 124.34 (d), 148.92 (s)*, 155.72 (s), 160.65 (s), 161.12 (s), 167.34 (s), 170.78 (s), 172.28 (s), 172.57 (s), 173.47 (s), 175.00 (s); Found: C, 53.08; H, 6.74; N, 14.27; S, 8.21. Calc. for $C_{35}H_{52}N_8O_9S_2$: C, 53.00; H, 6.62; N, 14.13; S, 8.09.

**Cyclo[L-Pro-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val], dolastatin 3 (*5*)**

The N-*t*-Boc pentapeptide methyl ester (4) (250 mg, 315 μmol) in dioxane (1.5 mℓ) and water (1.5 mℓ) was treated with 1N sodium hydroxide (315 μℓ) at room temperature for 3 h. The reaction mixture was diluted with diethyl ether (5 mℓ). The aqueous phase was collected, acidified to pH = 2 with ice cold 3N hydrochloric acid and extracted with chloroform (3 x 8 mℓ). The combined extracts were dried (anhydrous sodium sulphate) and the solvent removed *in vacuo* to furnish the N-*t*-Boc pentapeptide carboxylate (230 mg, 94%). After precipitation from ethanol with diethyl ether it had $[\alpha]_D^{22}$ −37.7° (*c* 1.64, methanol). This compound

(200 mg, 257 μmol) in dry 1,2-dimethoxyethane (3 mℓ) and dry dimethylformamide (1 mℓ) was treated with pentafluorophenol (47.2 mg, 0.256 mmol) and N,N'-dicyclohexylcarbodiimide (52.8 mg, 256 μmol) at –20°C under nitrogen. The mixture was allowed to warm to room temperature. After 12 h the mixture was filtered to remove dicyclohexylurea. The solvents were removed *in vacuo* and the residue chromatographed on Sephadex LH-20 in chloroform to furnish the pentafluorophenyl ester (210 mg, 87%) as a colourless oil with $R_f$ = 0.42 (7% methanol in ethyl acetate).

This pentafluorophenyl ester (114 mg, 121 μmol) was treated with trifluoroacetic acid (6 mℓ) for 0.5 h at room temperature. Dilution of the reaction mixture with carbon tetrachloride (20 mℓ) and removal of the solvents *in vacuo* furnished the pentapeptide ester trifluoroacetate as a colourless glass. After drying under vacuum for 2 h the material was dissolved in dioxane (10 mℓ). This solution was added by motor driven syringe over a period of 1 h into a vigorously stirred solution of 4-pyrrolidinopyridine (17.7 mg, 0.120 mmol) in *t*-butanol (11 mℓ) and dioxane (225 mℓ) at 95°C. After 2.5 h at 95°C the mixture was cooled, filtered through celite and the solvents removed *in vacuo*. The residue was successively chromatographed on Sephadex LH-20 with chloroform and on silica with 7% methanol in chloroform to furnish cyclo[L-Pro-L-Leu-L-(Gln)Thz-(Gly)Thz-L-Val] (dolastatin 3) (5)[25] (60 mg, 75%) as an amorphous white powder. After precipitation from 1:1 ethanol-ethyl acetate with hexane it had mp 187-188°C (Lit.[1] 133-137°C); $[\alpha]_D^{25}$ –45.5° (*c* 0.8, methanol, Lit.[1] –35.5, methanol) and –53.3° (*c* 0.94, chloroform); MS(EI) m/z 660 ($M^+$); MS (SP-SIMS) (glycerol), accurate mass m/z 661.2576 ($[M+H]^+$; calc. 661.2591 for $C_{29}H_{41}N_8O_6S_2$; $\nu_{max}$ 3427, 3379, 3330, 3090, 3020, 1670, 1629, 1544, 1501, 1494, 1445, 1065 $cm^{-1}$ Lit.[1b]; Found: C, 52.82; H, 6.30; N, 16.80; S, 9.55. Calc. for $C_{29}H_{40}N_8O_6S_2$: C, 52.70; H, 6.11; N, 16.96; S, 9.70.

## NOTES AND REFERENCES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25. Identical to natural (–)-dolastatin 3 (5) by $^{1}H$ NMR (400 MHz), SP-HRSIMS, TLC (four different systems) and PS leukemia growth inhibition.[4] The differences between the specific rotations and melting points of the natural and synthetic dolastatin 3 are due to the greater purity of the latter as shown for example by HPLC analysis.