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PROPOSED STRUCTURE OF THE CYCLIC PEPTIDE DOLASTATIN 3, A POWERFUL CELL GROWTH INHIBITOR, SHOULD BE REVISED![1,2]

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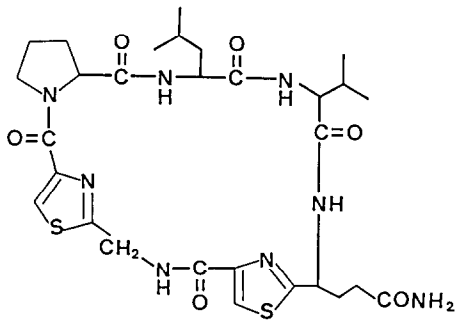
The cyclic peptide proposed for the structure of the powerful cell growth inhibitory dolastatin 3, its diastereomers, and their isomers on the reverse order have been synthesized by the use of diphenyl phosphorazidate(DPPA) and diethyl phosphorocyanidate(DEPC). Their physicochemical and biological properties have shown that the proposed structure is untenable.

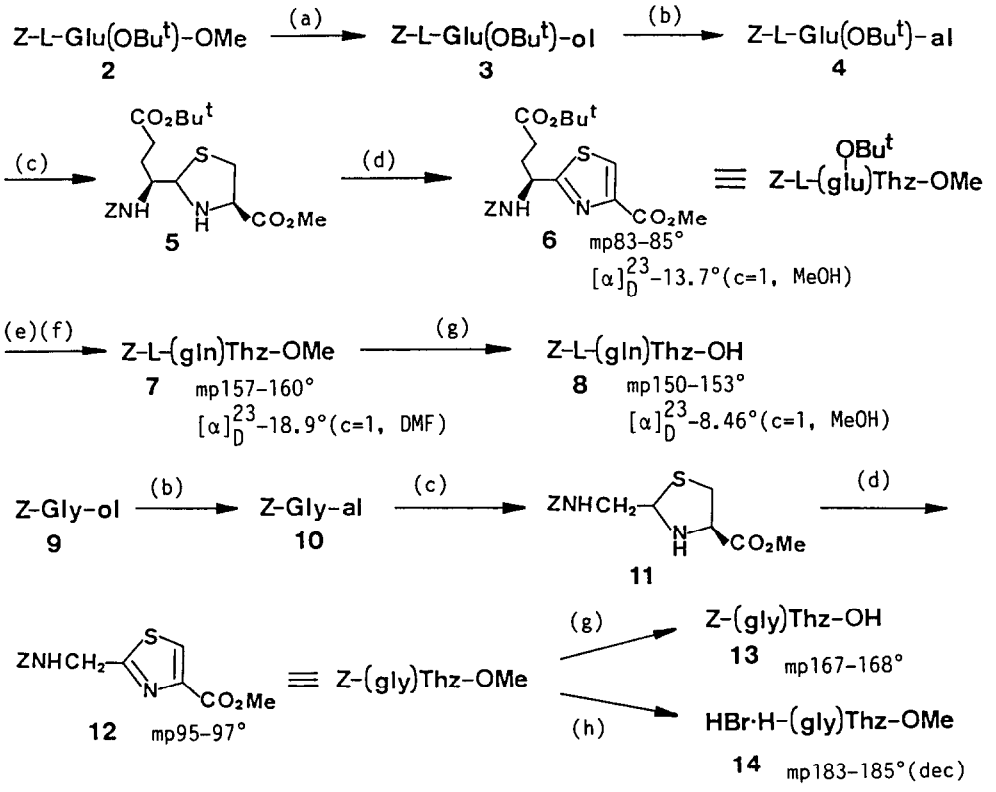
Dolastatin 3,[3] a cyclic peptide[4] exhibiting powerful cell growth inhibitory activity against murine P388 lymphocytic leukemia cells, was isolated by Pettit and co-workers from the Indian Ocean sea hare *Dolabella auricularia*. Its primary structure was proposed[3] as *cyclo*[Pro-Leu-Val-(gly)Thz-(gln)Thz](**1**)[5] containing two unusual thiazole amino acids and the absolute configurations of each amino acid unit were speculated to bear the usual L-configuration on the biosynthetic grounds. However, a series of the cytotoxic cyclic peptides[4a,4b,4e] from marine tunicates has been recently established to possess the thiazole amino acids with unusual D-configuration.[4c,4e]



1, Proposed Structure of Dolastatin 3

Our efforts towards the elucidation of a real structure of dolastatin 3 have revealed that the proposed structure **1** assigned to dolastatin 3 is untenable. The synthetic principle for **1** is based on the utilization of our organophosphorus reagents, diphenyl phosphorazidate (DPPA, $(C_6H_5O)_2P(O)N_3$)[6] and diethyl phosphorocyanidate(DEPC, $(C_2H_5O)_2P(O)CN$).[1,6]

The two thiazole amino acids, L-/D-(gln)Thz and (gly)Thz derivatives, were synthesized via oxidation of the thiazolidines **5** and **11**, respectively, as shown in Chart 1. Selective reduction of the L-Glu derivative **2** gave the N-protected β-amino alcohol **3**(91% yield) which was oxidized by the Parikh-Doering method[7] to give the N-protected α-amino aldehyde **4**(79% yield). Condensation of **4** with L-cysteine methyl ester gave a diastereomeric mixture of the thiazolidine **5**(82% yield) which was converted to the thiazole **6**(50% yield) using activated



(a) LiCl(2Mol.eq.), $NaBH_4$(2Mol.eq.), THF-EtOH(1:2) (b) Me_2SO, $Py{\cdot}SO_3$(3eq.), 20°, 10min
(c) H-L-Cys-OMe, PhH, rt (d) activated MnO_2(20Mol.eq.), PhH, 60° (e) CF_3CO_2H, rt
(f) $ClCO_2Et$, Et_3N, THF; conc.NH_4OH (g) NaOH, MeOH (h) 25%HBr-AcOH, rt

Chart 1

manganese dioxide.[8] The γ-amide function was finally introduced to **6** by acidic cleavage of the t-butyl ester group, activation of the γ-carboxylic acid function, followed by ammonolysis to give the thiazole **7**, whose optical purity, after replacement of its carbobenzoxy group with 3,5-dinitrobenzoyl one, was determined to be 98.6% by HPLC using chiral stationary phase.[9] The (gly)Thz derivative was prepared from N-protected glycinol **9** in similar reaction sequences.

To construct the full carbon skeleton of **1**, DPPA and DEPC were used for the chain elongation and cyclization,[10] respectively. On coupling position for cyclization, our choice is coupling between the (gln)Thz and (gly)Thz residues at C- and N-termini,

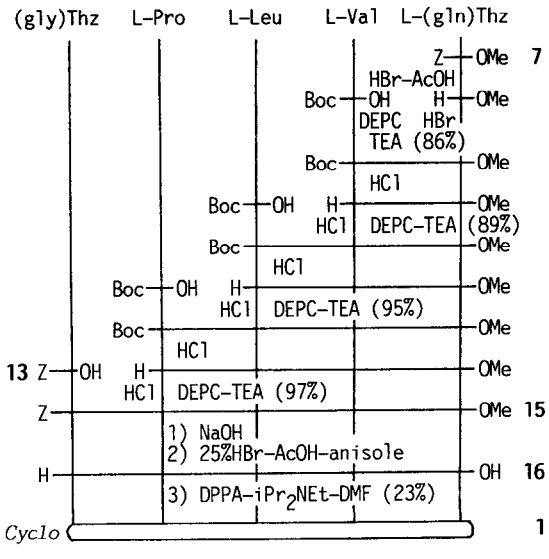


Chart 2

Table 1. Comparisons of Synthetic Cyclic Peptides and Natural Dolastatin 3.[a)]

Cyclic Peptide	Mp(Rec.Solv.)	$[\alpha]_D^{21-24}$(c=0.1, MeOH)	^{1}H-NMR(TMS/$CDCl_3$)[b)]
Cyclo[Pro-Leu-Val-(gln)Thz-(gly)Thz]			
(A) L-L-L-L[c)]	188-194°(aq. EtOH)	+71.6°	8.05, 8.22
(B) L-L-L-D	182-189°(CH_2Cl_2-Et_2O)	+147.9°	8.10, 8.13
(C) L-L-D-L	165-180°(CH_2Cl_2-Et_2O)	-68.3°	8.04, 8.09
(D) L-D-L-L	208-212°(EtOH-EtOAc)	+57.0°	8.04, 8.12
(E) D-L-L-L	168-172°(EtOH-EtOAc)	-60.8°	8.08, 8.23
(F) L-L-D-D	160-175°($CHCl_3$)	+28.8°	8.00, 8.08
(G) L-D-L-D	305-310°(EtOH-EtOAc)	+94.3°	7.96, 8.17
(H) D-L-L-D	243-245°(MeOH-$CHCl_3$)	+43.8°	8.02
Cyclo[(gly)Thz-(gln)Thz-Val-Leu-Pro]			
(I) L-L-L-L	170-174°(CH_2Cl_2-Et_2O)	-80.3°	8.11, 8.16
(J) D-L-L-L	184-190°(CH_2Cl_2-Et_2O)	+50.5°	8.05, 8.14
(K) L-D-L-L	175-188°(EtOH-EtOAc)	-127.2°	8.08
(L) L-L-D-L	166-185°(EtOH-EtOAc)	-96.4°	8.09, 8.21
(M) L-L-L-D	206-225°(EtOH-EtOAc)	+19.9°	8.14, 8.17
(N) D-D-L-L	176-184°(EtOH-EtOAc)	+49.3°	8.05, 8.13
(O) D-L-D-L	148-155°($CHCl_3$)	+51.1°	8.06, 8.20
(P) D-L-L-D	167-174°(EtOH-EtOAc)	+63.7°	8.05, 8.11
Natural dolastatin 3[d)]	133-137°(CH_2Cl_2-MeOH)	-35.5°[e)]	8.07, 8.082[f)]

a) All of the synthetic peptides showed M^+ peaks at m/z 660 on their EI mass spectra. b) H-4 Protons of two thiazole rings. c) Proposed structure. High-resolution MS m/z: Calcd. for $C_{29}H_{40}N_8O_6S_2$: 660.2515. Found: 660.2469. UV λ_{max}^{MeOH}nm: 209(ε17400), 242(ε17100). d) High-resolution MS m/z: Found: 660.2767. e) (c=0.09, MeOH) f) 400MHz ^{1}H-NMR.

respectively. The protected pentapeptide **15** was stepwisely synthesized starting from the thiazole **7** by the DEPC method in an overall yield of 70.5%, as shown in Chart 2. Saponification of **15** followed by treatment with hydrogen bromide in acetic acid containing anisole gave the linear precursor **16** of dolastatin 3 with the proposed structure. Final cyclization of **16** was conducted with DPPA in a 1-mM solution of dimethylformamide at pH7.5 in the presence of N,N-diisopropylethylamine. The reaction mixture was stirred at 5°C for 40hr and at room temperature for 22hr, and concentrated *in vacuo* at 60°C for 2hr. The residue was purified by silica gel column chromatography and recrystallized from aqueous ethanol to give the crystalline cyclic peptide **1** in an overall yield of 16.2% from **7**. Unfortunately, the melting point, the optical rotation, and IR, UV, ^{1}H- and ^{13}C-NMR spectra of the synthetic cyclic peptide 1 differed substantially from those reported for dolastatin 3.[3] Therefore, the

structure **1** assigned to dolastatin 3 is untenable.[11]

For the possible structures bearing D-amino acid residues and reverse order of bonding, the diastereomers, and their isomers on reverse order of **1** except their enantiomers were analogously prepared from (gly)Thz and (gln)Thz derivatives, respectively. However, the fifteen isomers of 1 thus obtained showed apparent differences in melting points, optical rotations, and spectral data, as shown in Table 1.

Furthermore, all of the synthetic cyclic peptides were examined for cell growth inhibitory activity against L-1210 murine leukemia cells cultured in vitro and no activities were observed in concentration of 250μg/ml.

Above results show that the structure **1** assigned to dolastatin 3, containing the planar structure, requires revision.

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5) Abbreviations of thiazole amino acids are in accordance with Pettit's recommendation.[3]
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11) Professor G. R. Pettit of Arizona State University also recently synthesized dolastatin 3 with the proposed structure and its isomer on reverse order and reached the same non-identical conclusions as ours(Private communication from Professor Pettit).

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