Tetrahedron Letters,Vol.26,No.42,pp 5155-5158,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

NEW METHODS AND REAGENTS IN ORGANIC SYNTHESIS. 55.†,[1] TOTAL SYNTHESES OF PATELLAMIDES B AND C, CYTOTOXIC CYCLIC PEPTIDES FROM A TUNICATE 1. THEIR PROPOSED STRUCTURES SHOULD BE CORRECTED.[2]

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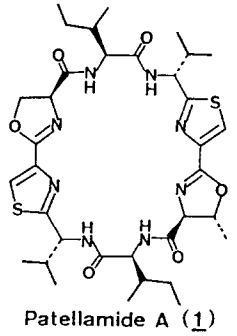
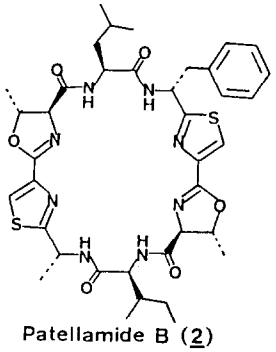
Patellamides B and C, cytotoxic lipophilic cyclic peptides from a marine tunicate, with proposed structures have been synthesized by the use of diphenyl phosphorazidate(DPPA) and diethyl phosphorocyanidate(DEPC). Their physicochemical properties lead to reassign the structures of patellamides B and C on the bonding order.

Lipophilic cyclic peptides from marine organisms constitute a growing class of naturally occurring antineoplastic and/or cytotoxic substances having unique thiazole amino acid structures.[3] As part of a program on peptide synthesis using diphenyl phosphorazidate(DPPA, $(C_6H_5O)_2P(O)N_3$) and diethyl phosphorocyanidate(DEPC, $(C_2H_5O)_2P(O)CN$), we have already revealed by synthesis[4] that the proposed structure of dolastatin 3,[5] a very potent cytotoxic lipophilic cyclic peptide from a mollusc, should be revised.[6] Further, we have succeeded[7] a synthesis of ascidiacyclamide, another cytotoxic cyclic peptide from an ascidian,[8] and clearly established its absolute configuration.

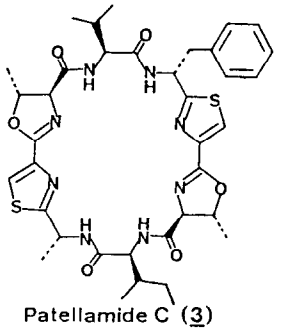
Now we turned our attention to the synthesis of patellamides A, B, and C, cytotoxic lipophilic cyclic peptides isolated from a tunicate Lissoclinum patella. The structures of patellamides A, B, and C have been proposed[9] as 1, 2, and 3, respectively, containing unusual fused thiazole-oxazoline units.



Patellamide A (1)



Patellamide B (2)



Patellamide C (3)

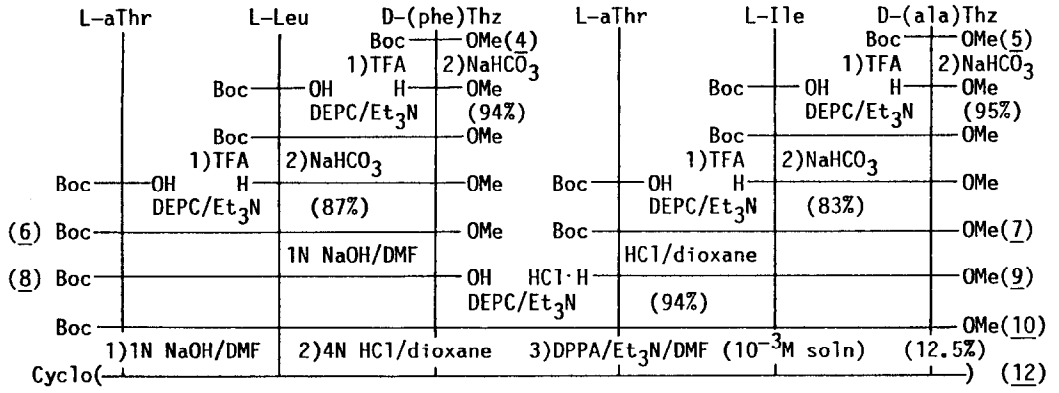
† Dedicated to Professor Shun-ichi Yamada on the occasion of his 70th birthday.

In this and the following[10] papers we wish to describe (1) the syntheses of patellamides B and C with proposed structures, (2) the revision of their structures, and (3) the syntheses of patellamides B and C with the revised structures ; a series of these synthetic studies have unambiguously determined the real structures of patellamides B and C. The key feature of our synthetic strategy is the formation of the trans-oxazoline ring : the use of L-allothreonine and its one-step conversion to the trans-oxazoline part[11] with inversion of configuration after construction of the full peptide skeleton, as shown in Chart 1.

Synthesis of patellamide B with the proposed structure **2**, which was reported to have the most potent cytotoxicity among three congeners,[9a] was started from Boc-D-(phe)Thz-OMe[12,13] (**4**)(mp 103-105°, $[\alpha]_D^{22}$ -8.8°(c=1, MeOH)) and Boc-D-(ala)Thz-OMe[12,13] (**5**)(mp 101-103°, $[\alpha]_D^{23}$ +28.5°(c=0.5, MeOH)). Stepwise coupling of Boc-L-Leu-OH and Boc-L-aThr-OH to **4** was carried out by the use of trifluoroacetic acid(TFA) and DEPC-Et_3N as deprotecting and coupling reagents, respectively, to give the tripeptide **6** (mp 102-105°, $[\alpha]_D^{22.5}$ -33.3°(c=0.27, MeOH)). The tripeptide **7** (mp 167-169°, $[\alpha]_D^{22.5}$ -12.4°(c=0.5, MeOH)) was similarly prepared from **5** as shown in Chart 1. After saponification of **6** with sodium hydroxide in dimethylformamide and removal of the Boc group from **7** with 4N hydrogen chloride in dioxane, the fragment coupling of the resulting **8** and **9** smoothly proceeded by the DEPC method to give the hexapeptide **10** (mp 213-217°(dec), $[\alpha]_D^{22.5}$ +13.2°(c=0.28, DMF)) in 94% yield from **7**. Saponification of **10** with sodium hydroxide in dimethylformamide at 0°for 3h followed by removal of the Boc group with 4N hydrogen chloride in dioxane at room temperature for 2h afforded the hexapeptide hydrochloride **11**, which was subjected to cyclization with DPPA (2 equiv.) and triethylamine (2 equiv.) in a 1-mM solution of dimethylformamide at 4° for 70h and at room temperature for 46h. The resulting mixture was concentrated in vacuo below 50° for 4h to afford the cyclic precursor **12** (mp 254-256°) in 12.5% yield. Final introduction of two trans-oxazoline functions was achieved by treatment of **12** with an excess of thionyl chloride at 4° for 53h, yielding cleanly patellamide B with the proposed structure **2** in 94% yield. Its structure was well supported by its IR, NMR, and high resolution mass (M^+ peak (m/z): Calcd. for $C_{38}H_{48}N_8O_6S_2$, 776.31383. Found, 776.31356) as well as its amino acid analysis (Leu 1, Ile 0.81, Thr 1.94). As shown in Table, however, the ^{1}H-NMR spectral data and the specific rotation of the synthetic peptide **2** substantially differ from those[9a] for natural patellamide B. Furthermore, their IR spectra and thin layer chromatographic behavior are completely different from each other, clearly indicating that the proposed structure **2** of patellamide B is untenable.

Next, our attention was turned to the synthesis of patellamide C with the proposed structure **3** which only differs from patellamide B (**2**) by one amino acid residue : L-valine in place of L-leucine. Thus, Boc-D-(phe)Thz-OMe (**4**) was stepwisely coupled with Boc-L-Val-OH (87%) and Boc-L-aThr-OH (74%) by the same procedure as in the synthesis of patellamide B (**2**). The resulting Boc-L-aThr-L-Val-D-(phe)Thz-OMe (mp 187-189°, $[\alpha]_D^{22.5}$ -28.5°(c=0.5, MeOH)) was saponified with sodium hydroxide in dimethylformamide and coupled with the tripeptide **9** using the DEPC method to give Boc-L-aThr-L-Val-D-(phe)Thz-L-aThr-L-Ile-D-(ala)Thz-OMe (mp 211-214°(dec), $[\alpha]_D^{22.5}$ +23.9°(c=0.22, DMF)) in 88% yield. Stepwise deprotection at its C- and then N-terminals followed by cyclization using the DPPA method similar to above afforded the cyclic precursor (mp 163-166°) in 47% yield, which was treated with thionyl chloride in

Chart 1. Synthesis of Patellamide B with Proposed Structure 2.



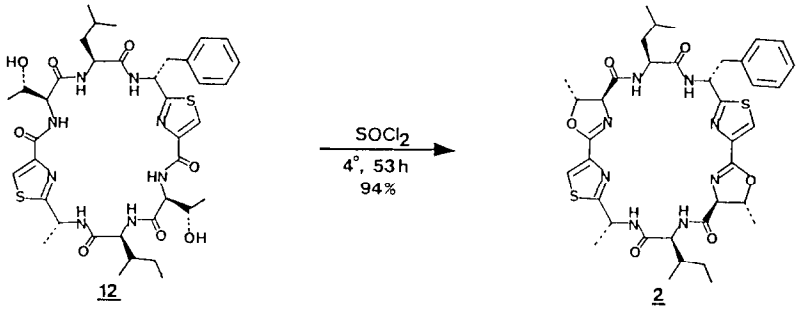


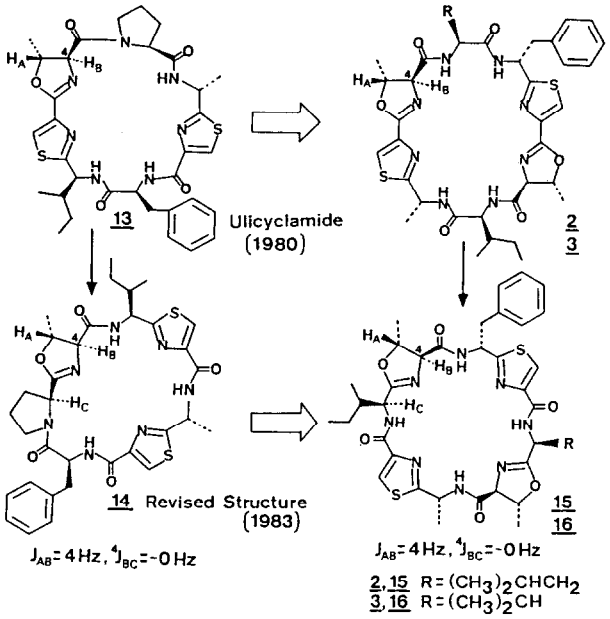
Table. Comparisons of Synthetic and Natural Patellamides B and C

	$[\alpha]_D$ (CH_2Cl_2)	^{1}H-NMR (270MHz) Oxazoline Methyl		Oxazoline H-4		Thiazole H-5	
Patellamide B							
Synthetic(Proposed structure)	+69.9°(c=0.34)	1.62	1.64	4.31(7.9Hz)	4.47(7.9Hz)	7.88	7.92
Natural	+29.4°(c=0.34)[a]	1.45	1.47	4.29(4Hz)	4.38(4Hz)	7.39	7.49
Patellamide C							
Synthetic(Proposed structure)	+47.2°(c=0.365)	1.65	1.67	4.41(8Hz)	4.51(8Hz)	7.89	7.97[b]
Natural	+19° (c=0.21)	1.41	1.44	4.26(4Hz)	4.36(4Hz)	7.44	7.50

a)Purified sample: +50.6°(c=0.19 CH_2Cl_2). b)100MHz

tetrahydrofuran at 4° for 48h to give patellamide C with the proposed structure **3** in 74% yield. Again, the physicochemical data of the synthetic patellamide C support its structure but are completely different from those reported[9a] for natural one, as shown in Table.

At the early stage of the structural determination, the similarities between the spectra of patellamides and ulicyclamide[14a](**13**) isolated from the same tunicate were noted. Especially, the absence of homoallylic coupling at C-4 protons of the oxazoline parts in each peptide led to assign the fused thiazole-oxazoline structures. Subsquently, however, the structure of ulicyclamide **13** has been revised[14b] as **14** with 2-(1'-aminoalkyl)oxazoline moiety. Therefore, the structures of patellamides should be also reassigned on the basis of the similarities with the revised structure **14** of ulicyclamide.



As shown in Table, proton signals on both thiazole and oxazoline rings of our synthetic patellamides B and C are observed at lower fields than those of natural peptides. This suggests the absence of the fused thiazole-oxazoline structures for natural patellamides. Furthermore, a detailed inspection of the evidences[9] used for the originally assigned structures has led us to deduce that the structures of patellamides B and C should be reassigned as **15** and **16**, respectively, having the reverse order of amino acid residues and 2-(1'-amino-alkyl)oxazoline structures. This has been verified by the synthesis as described in the following paper.[10]

Interestingly, the synthetic patellamides B (**2**) and C (**3**) with proposed structures showed potent cytotoxicity against L-1210 murine leukemia cells cultured in vitro similarly to natural patellamides B and C.

References and Notes

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10. Y. Hamada, M. Shibata, and T. Shioiri, Tetrahedron Lett., submitted (the following paper).
11. cf. D.F. Elliott, J. Chem. Soc., 62 (1950)
12. The thiazole amino acid derivatives **4** and **5** were prepared from Boc-D-Phe-OH and Boc-D-Ala-OH, respectively, in 5 steps according to the method described in our previous paper.[4]
13. For abbreviation of the thiazole amino acid derivatives, see the reference 5.
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(Received in Japan 9 July 1985)