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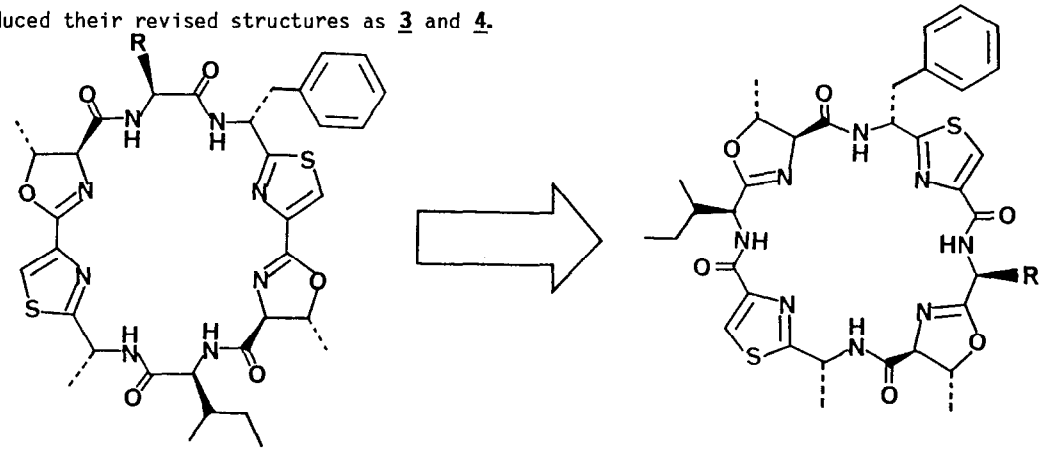
NEW METHODS AND REAGENTS IN ORGANIC SYNTHESIS. 56.†,[1] TOTAL SYNTHESES OF PATELLAMIDES B AND C, CYTOTOXIC CYCLIC PEPTIDES FROM A TUNICATE 2. THEIR REAL STRUCTURES HAVE BEEN DETERMINED BY THEIR SYNTHESES.[2]

Yasumasa Hamada,* Makoto Shibata, and Takayuki Shioiri*

Faculty of Pharmaceutical Sciences, Nagoya City University,
Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

The proposed structures of patellamides B and C, cytotoxic lipophilic cyclic peptides from a marine tunicate, have been revised on the basis of the spectral data of their partial hydrolysates, and their revised structures have been synthetically confirmed by the use of diphenyl phosphorazidate(DPPA) and diethyl phosphorocyanidate(DEPC).

In the preceding paper[1] we have reported that the syntheses of patellamides B and C, cytotoxic lipophilic cyclic peptides isolated from a tunicate Lissoclinum patella, having the proposed structures[3] 1 and 2 were achieved by the use of diphenyl phosphorazidate(DPPA, $(C_6H_5O)_2P(O)N_3$) and diethyl phosphorocyanidate(DEPC, $(C_2H_5O)_2P(O)CN$) as coupling reagents. However, the synthetic patellamides B and C were not identical with the natural ones and we deduced their revised structures as 3 and 4.



1, R=$(CH_3)_2CHCH_2$
2, R=$(CH_3)_2CH$

3 R=$(CH_3)_2CHCH_2$
4 R=$(CH_3)_2CH$

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

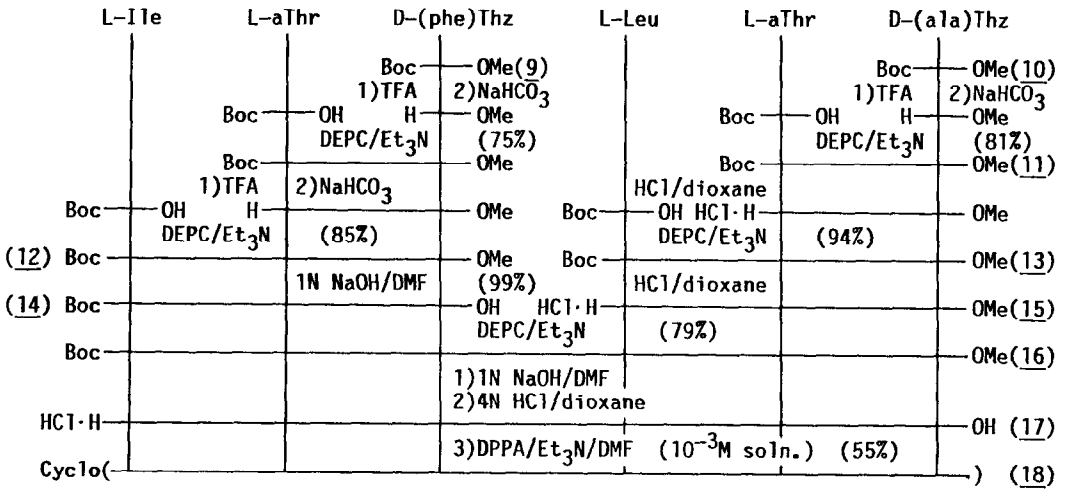
In this paper we wish to describe (1) the conclusive evidence on the revised structures (3 and 4) of patellamides B and C by spectral comparisons of a partially hydrolysate tripeptide from natural patellamide B[3a] with synthetic tripeptides, and (2) the total syntheses of patellamides B and C with revised structures 3 and 4. Complete identity of the synthetic peptides with the natural ones have unambiguously determined the real structures of patellamides B (3) and C (4).

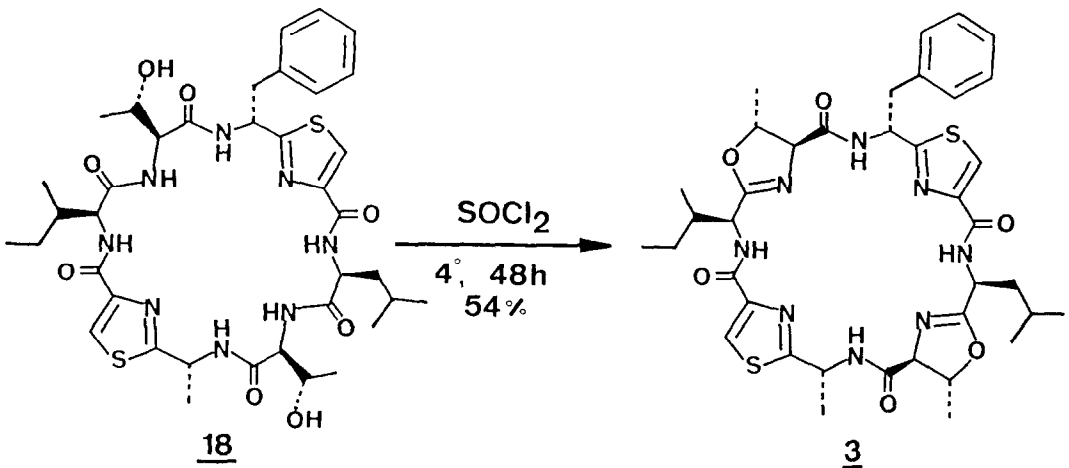
In their paper[3a] on structural determinations for patellamides, Ireland and coworkers reported that partial hydrolysis of natural patellamide B with 5% sulfuric acid in methanol followed by derivatization with (i) Ac_2O/pyridine, (ii) 1% KOH/MeOH, and then (iii) CH_2N_2 yielded a pair of tripeptides, Ac-L-Thr-L-Leu-D-(phe)Thz-OMe (5) and Ac-L-Thr-L-Ile-D-(ala)Thz-OMe (6). However, the tripeptides with alternative linkage, Ac-L-Thr-D-(phe)Thz-L-Leu-OMe (7) and Ac-L-Thr-D-(ala)Thz-L-Ile-OMe (8), would be possible candidates on the basis of our previous considerations.[1] Thus, the tripeptides 5 and 7 were prepared in a stepwise manner by the use of DEPC and triethylamine from Boc-D-(phe)Thz-OMe[1] (9) and H-L-Leu-OMe·HCl, respectively.

The EI mass spectrum of the synthetic tripeptide 5 having the reported structure[3a] showed a completely different fragmentation pattern (m/z: 518(M^+), 409, 257, 239, 211, 171) from that reported[3a] by Ireland and coworkers (m/z: 518(M^+), 459, 299, 284, 116). On the contrary, the mass spectrum of the alternative tripeptide 7 was virtually identical with the reported one. The ^{1}H-NMR spectra of 7 and the hydrolysate from natural patellamide B also coincided with each other while the spectrum of 5 was not identical. Accordingly, the tripeptides 5 and 6 should be read as 7 and 8, respectively. Through recombination of 7 and 8, we have concluded that the structure of patellamide B should be revised as 3 with 2-(1'-aminoalkyl)oxazoline structures, and the structure of patellamide C should be analogously reassigned as 4.

Using the same strategy[1] employed for the synthesis of patellamide B with the proposed structure 1, patellamide B with the revised structure 3 was synthesized as followed. The two tripeptides 12 (mp 175-176°, $[\alpha]_D^{21.5}$ -12.7°(c=0.5, MeOH)) and 13 (mp 155-156°, $[\alpha]_D^{21.5}$ -12.8°(c=0.5, MeOH)) were prepared in a stepwise manner by the DEPC method starting from the thiazole amino acids[1] 9 and 10, respectively. After respective deprotection of 12 and 13, the fragment condensation of the resulting 14 and 15 by the DEPC method afforded the hexapeptide 16 (mp 225-230°(dec), $[\alpha]_D^{22.5}$ +41.0°(c=0.18, DMF)) in 79% yield from 12. Saponification of 16 with sodium hydroxide in dimethylformamide at 0° for 2h followed by deprotection at N-terminal with 4N hydrogen chloride in dioxane at room temperature for 2h afforded the hexapeptide hydrochloride 17, which was directly subjected to cyclization with DPPA (1.2 equiv.) and triethylamine (2.4 equiv.) in dimethylformamide at 4° for 72h and at room temperature for 24h. The resulting mixture was concentrated in vacuo below 50° for 2.5h to furnish the cyclic precursor 18 (mp >300°, $[\alpha]_D^{21.5}$ -20.4°(c=0.18, DMF)) in 55% yield from 15. DEPC was also effective in place of DPPA for the cyclization reaction to give 18 in 44% yield under similar reaction conditions. Treatment of 18 with an excess of thionyl chloride at 4° for 48h afforded patellamide B with the revised structure 3 as a colorless powder in 54% yield, mp 139-141°, $[\alpha]_D^{22.5}$ +50.5°(c=0.19, CH_2Cl_2).

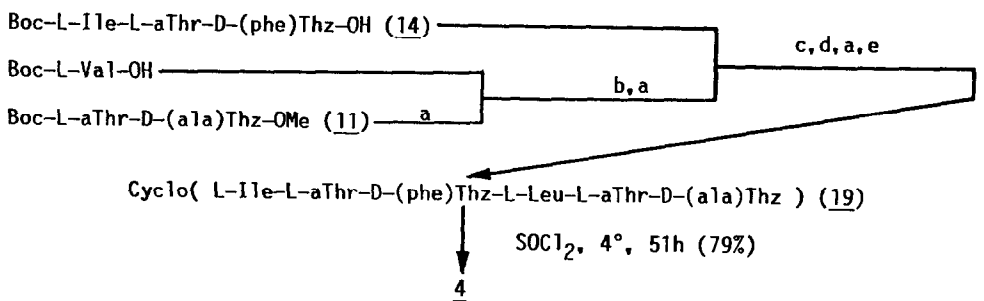
Chart 1. Synthesis of patellamide B with the Revised Structure 3





18 3

Chart 2. Synthesis of Patellamide C with the Revised Structure 4



(a)4N HCl/dioxane; (b)DEPC/TEA/DMF (92%); (c)DEPC/TEA/DMF (82%)

(d)1N NaOH/DMF; (e)DPPA/TEA/DMF ($2X10^{-3}$M soln.) (44%)

The synthetic peptide 3 thus obtained was virtually identical with natural patellamide B[3] (mp 133-138°, $[\alpha]_D^{22.5}$ +50.6°(c=0.19, CH_2Cl_2))[4] by IR, ^{1}H-NMR(270MHz), ^{13}C-NMR, and mass spectral analyses as well as chromatographic mobility on silica gel. Furthermore, both of synthetic and natural patellamide B exhibited entirely parallel activities against L-1210 murine leukemia cells cultured in vitro.

Patellamide C with the revised structure 4 was analogously synthesized starting from 14, Boc-L-Val-OH, and 11, as shown in Chart 2. Both stepwise elongation with DEPC and cyclization with DPPA were smoothly carried out to give the cyclic precursor 19 (mp 286-288°(dec), $[\alpha]_D^{21.5}$ -29.4°(c=0.1, DMF)). Final construction of the oxazoline rings was achieved with thionyl chloride at 0° for 51h, giving patellamide C with the revised structure 4 in 79% yield. Physicochemical properties and biological activity of the synthetic patellamide C (mp 148-150°, $[\alpha]_D^{21.5}$ +32.0°(c=0.21, CH_2Cl_2)) were in good agreement with those reported[3a] for natural patellamide C.[5]

The results described above have clearly established the real structures of patellamides B and C as 3 and 4, respectively, and confirmed the successful outcome of our synthetic effort.

Additional synthetic work on structural elucidation for patellamide A[3a] is now actively in progress in our laboratories.

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References and Notes

1. For Part 55, see Y. Hamada, M. Shibata, and T. Shioiri, Tetrahedron Lett., submitted (the preceding paper).
2. Presented in part (a) at the 105th Annual Meeting of the Pharmaceutical Society of Japan in Kanazawa on April 3, 1985, Abstracts p.588, and (b) at the 50th Spring Annual Meeting of the Chemical Society of Japan in Tokyo on April 1, 1985, Abstracts p.1660.
3. (a) C.M. Ireland, A.R. Durso,Jr., R.A. Newman, and M.P. Hacker, J. Org. Chem., **47**, 1807 (1982), (b) J.E. Biskupiak and C.M. Ireland, J. Org. Chem., **48**, 2302 (1983).
4. These physical data have been obtained from a purified sample of natural patellamide B by means of reprecipitation with ethyl acetate-hexane. No melting point is recorded in the literature,[3a] and the reported specific rotation is $[\alpha]_D$ +29.4°(c=0.34, CH_2Cl_2).
5. No melting point is recorded in the literature,[3a] and the reported specific rotation is $[\alpha]_D$ +19°(c=0.21, CH_2Cl_2). Discrepancy of the specific rotation with ours will be presumably solved by availability of a purified sample of natural patellamide C.

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