Tetrahedron Letters,Vol.27,No.23,pp 2653-2656,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

TOTAL SYNTHESIS OF ULITHIACYCLAMIDE, A STRONG CYTOTOXIC CYCLIC PEPTIDE FROM MARINE TUNICATES†,[1]

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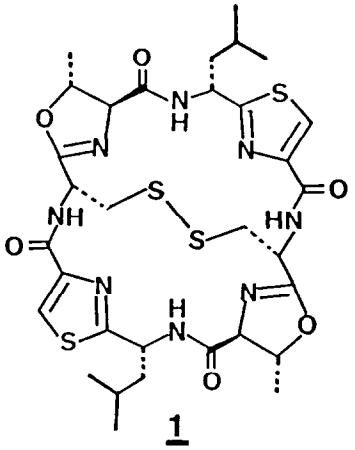
Abstract: Total synthesis of ulithiacyclamide, a strong cytotoxic cyclic peptide from marine tunicates, has been achieved by the use of diphenyl phosphorazidate(DPPA) and diethyl phosphorocyanidate(DEPC). Two different cyclization processes were applied to the construction of this unique cyclic peptide having a disulfide bridge.

Ulithiacyclamide was first isolated from the ascidian *Lissoclinum patella* collected in Western Caroline Islands and its structure was elucidated by Ireland and Scheuer.[2a,c] Endo and co-workers also isolated the peptide from another ascidian.[3] Ulithiacyclamide has a unique dimeric structure as 1 containing a characteristic disulfide bridge in its cyclic backbone. Moreover, the most potent cytotoxicity was reported on ulithiacyclamide among several similar cyclic peptides having thiazole and oxazoline rings.[2b] We have already succeeded the syntheses of some of these cytotoxic cyclic peptides, ascidiacyclamide,[3-5] patellamides[2b,c] A,[6] B,[7,8] and C,[7] 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 peptide coupling reagents. We now wish to report the first total synthesis of ulithiacyclamide(1).



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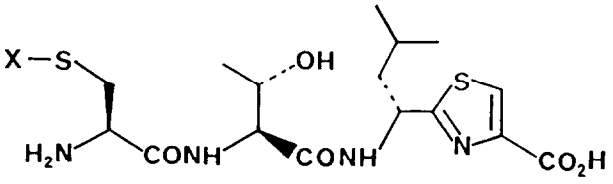


2 (X = Acm)

3 (dimer)

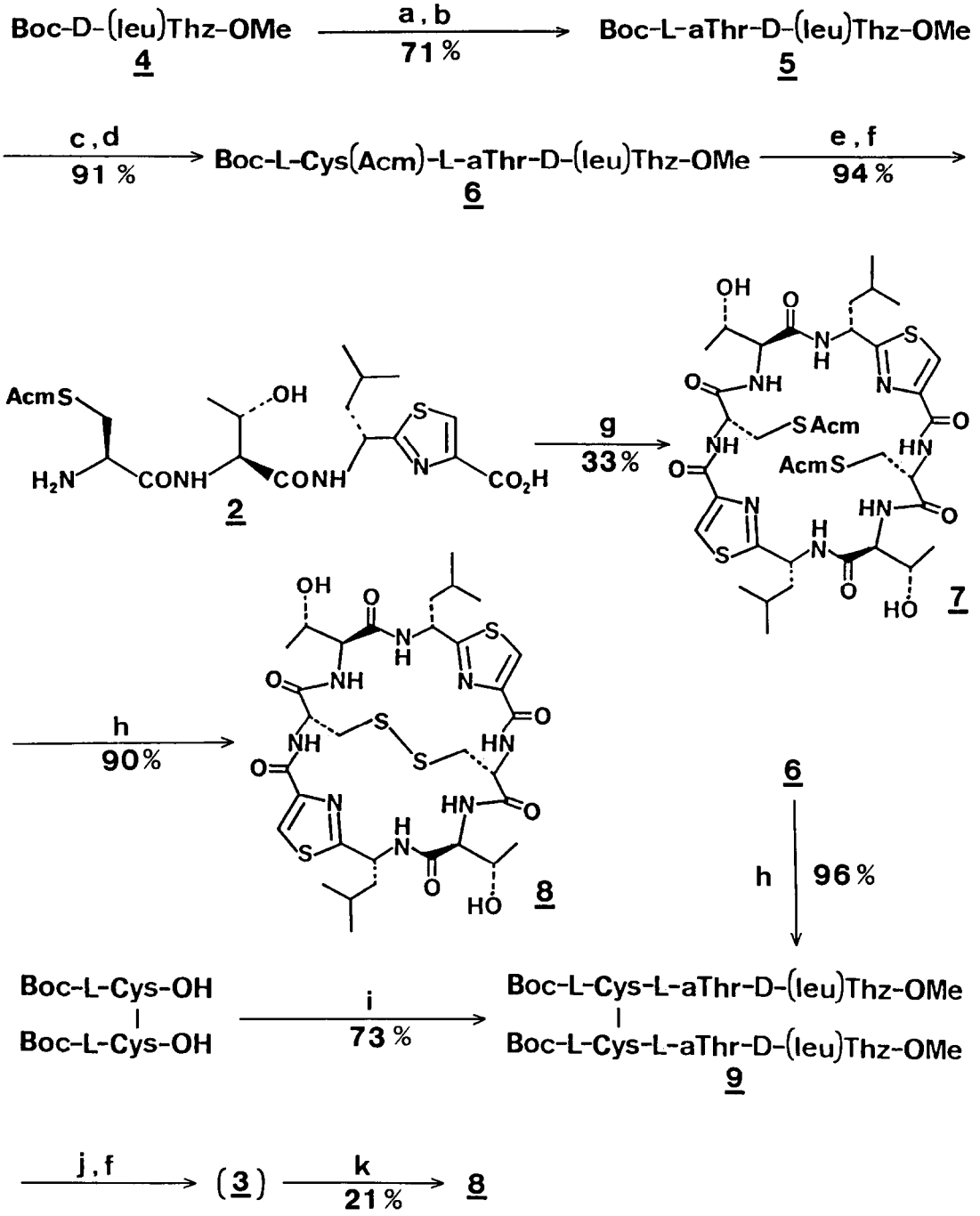
† Dedicated to Professor Shigehiko Sugasawa on the occasion of his 88th birthday.

For the construction of ulithiacyclamide(1), we developed two different cyclization processes: the cyclodimerization[4,5] of the S-acetamidomethyl(S-Acm) tripeptide 2 and the double cyclization of the hexapeptide 3 having a disulfide bond. The unstable oxazoline moieties were constructed from L-allothreonine residues in the final step as previously established.[5-7]

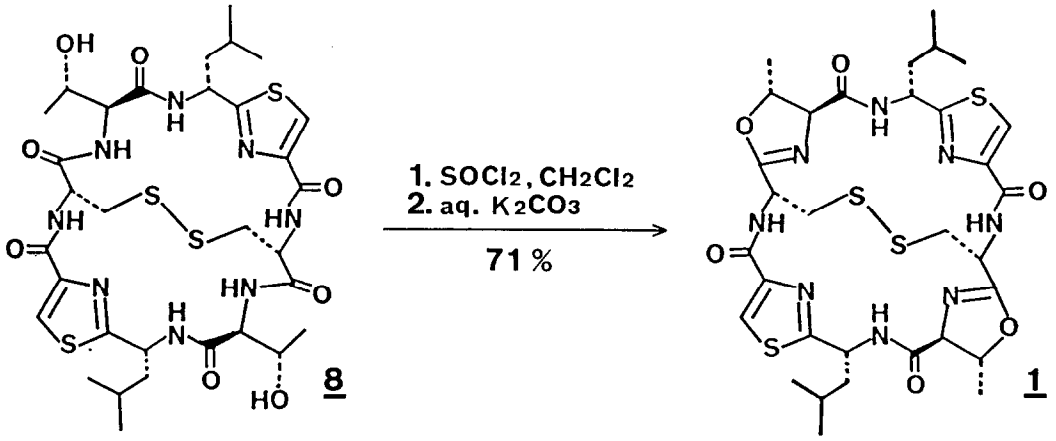
The protected tripeptide, Boc-L-Cys(Acm)-L-aThr-D-(leu)Thz-OMe(6)(mp 120-125°C, $[\alpha]_D^{21}$ +8.8°(c=1, MeOH)), was synthesized stepwisely from the thiazole amino acid derivative, Boc-D-(leu)Thz-OMe[5,9](4)(mp 56-58°C, $[\alpha]_D^{25}$ +22.5°(c=1, MeOH)), via Boc-L-aThr-D-(leu)Thz-OMe(5)(mp 111-113°C, $[\alpha]_D^{25}$ +17.0°(c=1, MeOH)) by the use of DEPC as a coupling reagent. The ester function of 6 was cleaved with sodium hydroxide(1.3eq) in aqueous methanol at room temperature for 4h, then the tert-butoxycarbonyl group was removed with trifluoroacetic acid at room temperature for 1h. After purification on a Dowex 50Wx4 column, the deblocked tripeptide 2 was obtained in 94% overall yield from 6. Cyclodimerization of 2 was achieved with DPPA(1.5eq) and triethylamine(1.5eq) in 5mM dimethylformamide solution at 0-5°C for 2 days, then at room temperature for 1 day. The cyclodimerized product 7(mp 240-242°C(hot plate), $[\alpha]_D^{21}$ +82.0°(c=0.5, DMF)) was obtained in 33% yield as the major product. The disulfide bridge required in ulithiacyclamide(1) was smoothly constructed from the S-Acm functions of 7.[10] Treatment of 7 with iodine(5eq) in 4mM methanol solution at room temperature for 40min afforded the desired cyclic precursor 8(mp 260-263°C(hot plate), $[\alpha]_D^{22}$ +65.0°(c=0.5, DMF)) in 90% yield. This precursor peptide 8 was also synthesized in another route in which the double cyclization process was employed.[5]

Condensation of Boc-L-cystine with the hydrochloride of H-L-aThr-D-(leu)Thz-OMe, prepared from the dipeptide 5 by deprotection with 4N hydrogen chloride in dioxane, gave the hexapeptide 9(mp 211-212.5°C(dec), $[\alpha]_D^{24}$ -34.2°(c=1, MeOH)) in 73% yield by the use of the DPPA method. DPPA was more efficient than DEPC for this coupling. Alternatively, the hexapeptide 9 was obtained in 96% yield from the tripeptide 6 by treatment with iodine in methanol[10] at room temperature for 30min. Deprotection of the hexapeptide 9 was carried out first at the C-termini with sodium hydroxide(3eq) in aqeous dimethylformamide at 0-5°C, then at the N-termini with trifluoroacetic acid at room temperature. After treatment on a Dowex 50Wx4 column, the resulting crude hexapeptide 3 was subjected to cyclization without further purification. Double cyclization of 3 with DPPA(4eq) and triethylamine(2eq) in 2mM dimethylformamide solution at 0-5°C for 5 days was intramolecularly realized to give the precursor peptide 8 as the major product in 21% overall yield from 9.

Finally, the oxazoline rings were introduced to 8 by its treatment with a large excess of thionyl chloride in 7mM methylene chloride solution at 0-5°C for 17h. Ulithiacyclamide(1) was obtained in 71% yield as a colorless powder from diethyl ether-hexane (mp 160-163°C(hot plate), $[\alpha]_D^{23}$ +119°(c=0.14, CH_2Cl_2)). Identity of the synthetic sample with the natural one[3](mp 154-158°C(hot plate), $[\alpha]_D^{23}$ +120°(c=0.135, CH_2Cl_2)) was rigorously established by comparisons of their spectra(IR, ^1H- and ^{13}C-NMR, low and high resolution Mass) as well as TLC behavior.



(a) CF_3CO_2H, then aq. $NaHCO_3$; (b) Boc-L-aThr-OH, DEPC, Et_3N, DMF; (c) 4N HCl-dioxane; (d) Boc-L-Cys(Acm)-OH, DEPC, Et_3N,DMF; (e) NaOH, aq. MeOH; (f) CF_3CO_2H, then ion exchange resin(Dowex 50Wx4); (g) DPPA, Et_3N, DMF(5mM soln); (h) I_2, MeOH; (i) HCl·H-L-aThr-D-(leu)Thz-OMe, DPPA, Et_3N, DMF; (j) NaOH, aq. DMF; (k) DPPA, Et_3N, DMF(2mM soln).



Acknowledgement Partial financial supports of this research by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, and Culture, Japan (No. 60470151(to our group), No. 59340034(to Prof. T. Shiba at Osaka University), and No. 60307009(to Prof. Y. Kanaoka at Hokkaido University)), the Japan Research Foundation for Optically Active Compounds, the Tokyo Biochemical Research Foundation, and the Ishida Foundation are gratefully acknowledged. We are grateful to Dr. M. Endo of Suntory Institute for Biomedical Research for gifts of natural ulithiacyclamide and its spectral data.

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8. For another synthesis of patellamide B, see U. Schmidt and H. Griesser, Tetrahedron Lett., **27**, 163 (1986).
9. The thiazole amino acid derivative **4** was prepared from Boc-D-Leu-OH in 5 steps in 62% overall yield[5] according to the method developed by us: Y. Hamada, K. Kohda, and T. Shioiri, Tetrahedron Lett., 25, 5413 (1984).
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11. Ireland and Scheuer[2a] have reported that ulithiacyclamide(**1**) is a colorless oil, $[\alpha]_D^{25}$ +62.4°(c=2.9, CH_2Cl_2).

(Received in Japan 13 March 1986)