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NEW METHODS AND REAGENTS IN ORGANIC SYNTHESIS. 51.†,1 A SYNTHESIS OF ASCIDIACYCLAMIDE, A CYTOTOXIC CYCLIC PEPTIDE FROM ASCIDIAN —— DETERMINATION OF ITS ABSOLUTE CONFIGURATION

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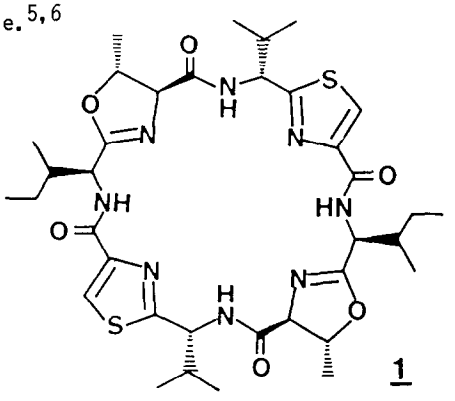
A first synthesis of ascidiacyclamide, a cytotoxic cyclic peptide from ascidian, has been achieved through the cyclodimerization with diphenyl phosphorazidate(DPPA), which has unambiguously determined the absolute configuration of ascidiacyclamide as ***1****.*

Ascidiacyclamide,[2] a cytotoxic cyclic peptide containing thiazole and oxazoline amino acids, was isolated from an unidentified species of ascidian collected in Australia. Although its structure was presented by the Suntory research group, its absolute configuration has remained to be determined. Our interest in peptide synthesis using two organophosphorus reagents, diphenyl phosphorazidate(DPPA, $(C_6H_5O)_2P(O)N_3$) and diethyl phosphorocyanidate(DEPC, $(C_2H_5O)_2P(O)CN$), has now focused on the synthesis of a series of cytotoxic cyclic peptides of marine origin.[2-4] We already succeeded to synthesize the proposed structure of dolastatin 3,[4] a powerful cytotoxic cyclic peptide from a sea hare, revealing that its structure was untenable.[5,6]



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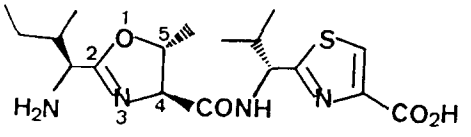

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† Dedicated to Professor Shun-ichi Yamada on the occasion of his 70th birthday.

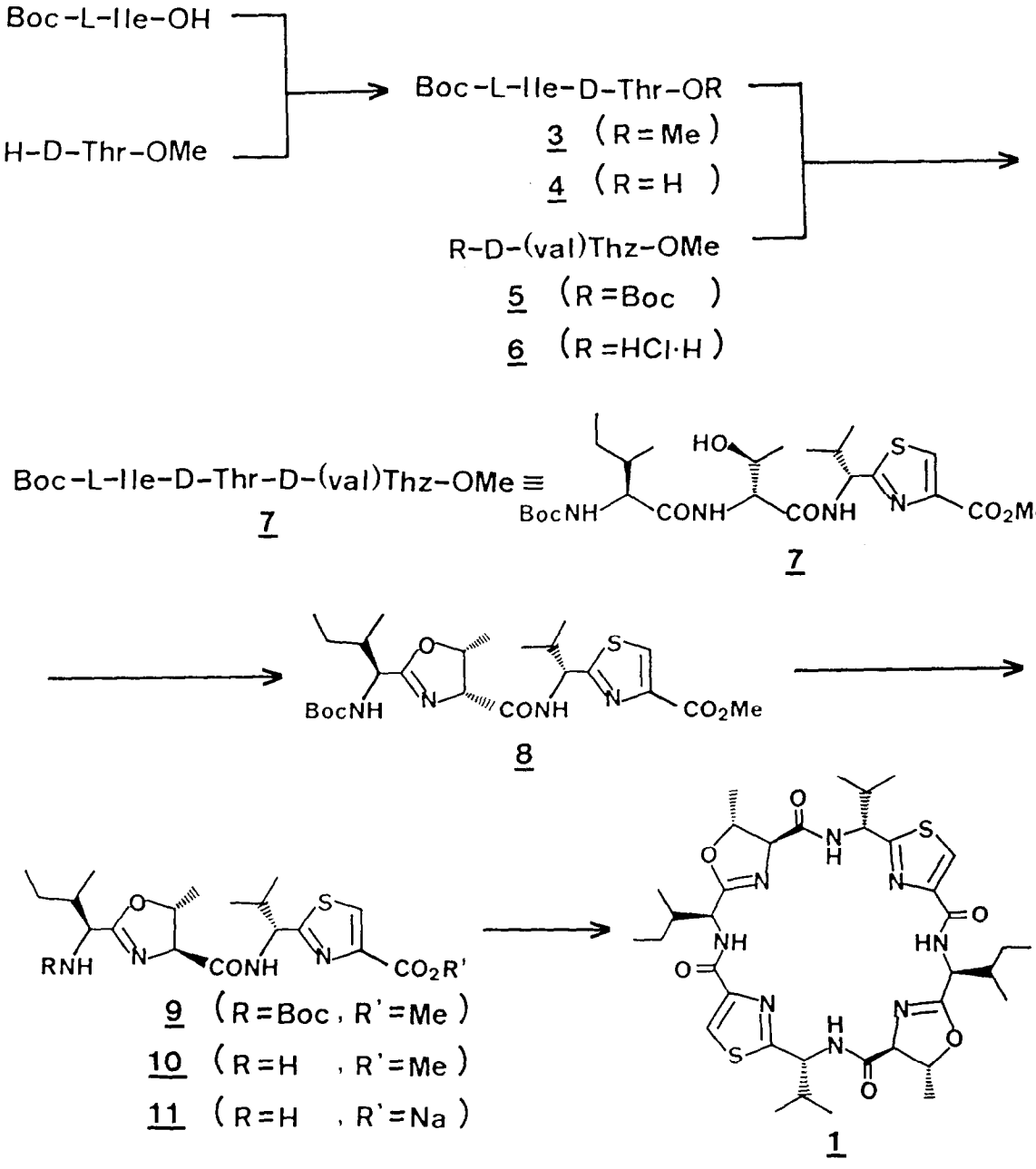
We now wish to report a first synthesis of ascidiacyclamide through the cyclodimerization with DPPA, which has unambiguously established the absolute configuration of this unique dimeric cyclic peptide as 1.

We first expected that the absolute configuration of ascidiacyclamide would be similar to that of ulithiacyclamide, since it was isolated along with ascidiacyclamide from the same ascidian[2] and its absolute stereochemistry was established by Ireland and co-workers.[3a,3c] Thus, the structure of ascidiacyclamide was deduced to be 1 which was composed of L-isoleucine, L-threonine, and a thiazole amino acid, D-(val)Thz.[7] The key feature of our synthetic strategy was the formation of the peptide 2 containing the (4S,5R)-trans-oxazoline ring and the cyclodimerization of 2 using DPPA.

Condensation of tert-butoxycarbonyl-L-isoleucine with D-threonine methyl ester using DEPC and triethylamine in dimethylformamide gave the dipeptide 3 (mp 164-166°, $[\alpha]_D^{24}$ -10.5° (c=1, MeOH)) in 72% yield, which was saponified with sodium hydroxide in aqueous methanol to give the carboxylic acid 4 (mp 168-170° (dec), $[\alpha]_D^{21}$ -22.9° (c=1, MeOH)) in 90% yield. Fragment coupling of 4 with H-D-(val)Thz-OMe·HCl(6), obtained by the deprotection of Boc-D-(val)Thz-OMe[8](5) (mp 121-123°, $[\alpha]_D^{23}$ +27.5° (c=1, MeOH)) with 4N hydrogen chloride-dioxane, was achieved by the DEPC method to furnish the tripeptide 7 (mp 153-154°, $[\alpha]_D^{23}$ +34.0° (c=1, MeOH)) in 71% yield from 5. The cis-oxazoline ring function was cleanly introduced to 7 by its treatment with an excess of thionyl chloride in tetrahydrofuran at 0-5° for 22.5hr, yielding the cis-oxazoline peptide 8 (mp 111-113°, $[\alpha]_D^{23}$ +4.7° (c=1, MeOH)) in 93% yield. Epimerization at C-4 of 8 proceeded with sodium ethoxide in refluxing ethanol for 1hr. Since the partial hydrolysis of the methyl ester function at the C-terminus was observed, the crude product was subjected to methyl esterification with trimethylsilyldiazomethane[9] to give the trans-oxazoline derivative 9 as a colorless oil in 76% yield.[10] The trans configuration of 9 was confirmed by the respective $J_{4,5}$ values[11] at C-4 in the NMR spectra of cis-8 (4.72ppm, $J_{4,5}$=9Hz) and trans-9 (4.20ppm, $J_{4,5}$=7Hz), though the epimerization at the chiral center derived from L-isoleucine remained unclear at this stage.

Since the trans-oxazoline ring in 9 was presumed to be acid-sensitive and easily opened, 9 was subjected to the chemoselective deprotection followed by the cyclodimerization. Thus, treatment of 9 with trimethylsilyl trifluoromethanesulfonate[12] (3eq) in dry dichloromethane at 0° for 1.5hr afforded the N-deprotected derivative 10. Saponification of 10 with sodium hydroxide (1.1eq) in aqueous dimethylformamide at 0° for 1.5hr, followed by drying over molecular sieves 4A produced the sodium salt 11 in dimethylformamide. The solution was diluted with dimethylformamide to a ca. 5mM solution, and treated with DPPA in the presence of potassium hydrogen phosphate at 0-5° for 4 days, followed by concentration in vacuo below 45° to furnish the cyclodimerized product 1 as colorless prisms from benzene (mp 243-245° (hot plate), $[\alpha]_D^{23}$ +162° (c=0.5, $CHCl_3$)) in 27% yield from 9.

The cyclodimerized product 1 thus obtained was completely identified with natural ascidiacyclamide(1, mp 239-239.5°,[13] $[\alpha]_D^{25}$ +164° (c=0.466, $CHCl_3$)) by melting point, mixed melting point,[14] specific rotation, chromatographic behavior, amino acid analysis, IR, ^1H- and ^{13}C-NMR, and high resolution mass spectra. Furthermore, the synthetic ascidiacyclamide showed strong cell growth inhibitory activity against L-1210 murine leukemia cells cultured in vitro.



The above synthesis clearly establishes the absolute stereochemistry of ascidiacyclamide, and DPPA was proven to be useful as a cyclodimerization reagent, though the yield should be improved. Furthermore, transformation of 7 to 9 was revealed by this synthesis to proceed with little or no epimerization at the chiral center derived from L-isoleucine.

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References and Notes

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7. For abbreviation, see the reference 4.
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12. Cf. For the use of trimethylsilyl perchlorate, see H. Vorbrüggen and K. Krolikiewicz, Angew. Chem. Int. Ed. Engl., **14**, 818(1975).
13. Reported[2] mp 139-139.5° of natural ascidiacyclamide was corrected to be 239-239.5°(private communication from Dr. M. Endo). We observed a purified sample of natural ascidiacyclamide showed mp 245-246°.
14. Mixed mp 244-246°.

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