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SYNTHESIS OF A BICYCLIC DIPEPTIDE WITH THE SHAPE OF β-TURN CENTRAL PART

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Summary: A bicyclic dipeptide derivative with fixed conformation simulating that of the two central amino acid residues in type II' β-turn has been synthesized starting from L-glutamic acid and L-cysteine in short steps.

It is an attractive problem to elucidate the conformation of bioactive peptides on the receptor. However, flexibility of linear peptide conformation makes the problem difficult to solve since stable conformation in solid state or in solution cannot always be the correct answer. A way to approach the problem is use of conformationally restricted analogs.

β-Turn[2] is a common secondary structure of polypeptides which occurs frequently in many of bioactive peptides. So, if peptide conformation could be restricted to β-turn form by replacement of amino acid residues at the part of folding with suitably designed derivatives of amino acids or peptides, they would be powerful tools for investigating the active conformation of such bioactive peptides. A few papers on this line of approach are reported[3-5]. However, there seems to be no report which employed bicyclic dipeptide skeleton for the restriction required in spite of naive nature of the idea.

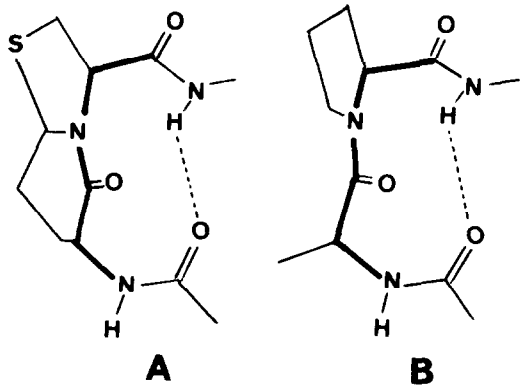
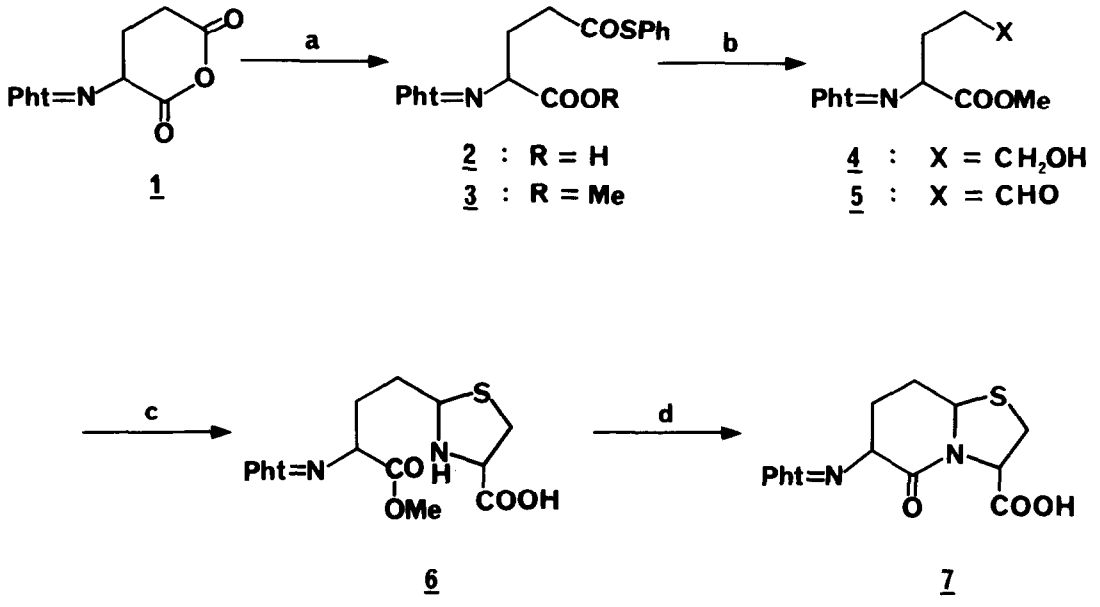


Fig. 1 Sketches of Dreiding models of A: the acylated cyclic dipeptide amide and B: the corresponding part of the GS analog (see text). Dotted lines mean H-bonding. Bold lines indicate the central part of backbone in β-turn, which are fixed in A.

Inspection of molecular models revealed that the backbone skeleton of compound 7 has almost superimposable conformation on that of D-Ala-L-Pro residues in [D-Ala$^{4,4'}$]-gramicidin S (GS), which is known to be type II' β-turn[6]. So, we attempted to synthesize the bicyclic dipeptide structure and succeeded to prepare the N-phthalyl derivative 7 by the route shown in Scheme 1.

N-phthalyl-L-glutamic anhydride (1) was reacted with thiophenol in the presence of dicyclohexylamine (DCHA) using dioxane as the solvent to give the DCHA-salt (85% yield), from which 2, (mp 190-193° $[\alpha]_D^{23}$



Scheme 1. Reagents - a: (i) PhSH, DCHA, (ii) CH_2N_2, b: (i) Raney-Ni reflux in EtOH, (ii) PCC in CH_2Cl_2, c: L-cysteine HCl·AcONa in aq. EtOH, d: heating 10 h at 70°C.

-36.0°, c 1.0, DMF) was obtained quantitatively. Compound 2 was then converted to methyl ester 3 (oil) by treatment with diazomethane in a mixture of ethyl acetate and ether (99% yield). Raney-nickel reduction of 3, followed by oxidation with pyridinium chlorochromate (PCC) in methylene chloride[7] afforded aldehyde 5 (oil; 3 to 5, 34% yield). Reaction of 5 with L-cysteine hydrochloride in aqueous ethanol containing sodium acetate yielded thiazolidine compound 6 as white precipitates. Without purification 6 was converted to 7 by heating at 70°C overnight with loss of methanol (5 to 7, 51% yield). Crude 7 was purified by chromatography (silica gel/$CHCl_3$-MeOH-AcOH) and recrystallization from methanol. Overall yield from 1 was 14.7%, which would be improved by optimization of the reaction conditions. Physical properties of 7 are as follows: mp 266-268° (dec); $[\alpha]_D^{26}$ -249° (c 1.0, DMF); TLC Rf=0.23 (solvent $CHCl_3$:MeOH:AcOH=91:8:1); UV λ_{max} (ε) 297 nm (sh.1770), 291 (1910), 237 (sh.8760), 217 (49200); IR(KBr) ν_{max} cm^{-1} 3280 (carboxyl), 2930 (CH-stretching), 2560 (carboxyl), 1780, 1760, 1720, 1650 (4 carbonyls); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.8-2.6 (4H, br.m.), 3.0-3.6 (2H, d.q.), 4.6-5.6 (3H, m.), 7.89 (4H, s.); ^{13}C-NMR (DMSO-d_6) δ ppm: 25, 28, 31, 49, 61, 62, 123, 131, 135, 164, 167, 171. These spectral data support the structure of 7 together with the results of elemental analysis ($C_{16}H_{14}N_2O_5S$ requires C: 55.48, H: 4.07, N: 8.09, S: 9.25%; Found C: 55.22, H: 4.06, N: 8.22, S: 9.26%)

Compound 7 has three asymmetric carbons, two of which derives from α-carbons of L-glutamic acid and L-cysteine of known configuration. But the third one formed by thiazolidine cyclization is unknown.

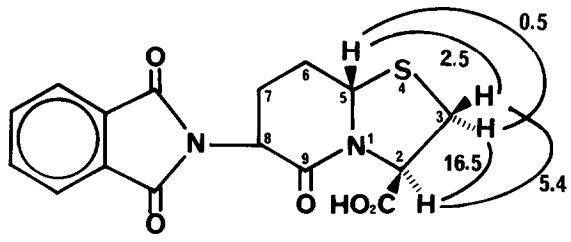


Fig. 2 Results of NOE experiments. The numbers on the arc indicate percent increment of the peak height when irradiated the nuclei connected with the arc line.

Results of NOE measurements are summarized in Fig. 2, from which the bridge-head hydrogen was assigned to have cis configuration to 3β-hydrogen(H) that is farther located than 3α-H from 2α-H of known configuration. Coupling constants, $J_{2\alpha,3\alpha}$ and $J_{2\alpha,3\beta}$, were determined by decoupling experiment to be 7.91 and 5.15 Hz, respectively. It indicates that the C2-carbon is puckered a little up from the mean plane in the thiazolidine ring. The lactam ring was suggested to have some flexibility from the fact that the proton signals on C-6 and C-7 (δ 1.8-2.6 ppm) of ^{1}H-NMR were broad and became sharper on heating. It is noteworthy that a minor spot was detected by TLC (Rf Ø.17 in the same solvent system) of recrystallization mother liquor of 7 since it suggests formation of the minor diastereomer having 5α-H configuration during thiazolidine cyclization.

In order to confirm the ability of the bicyclic dipeptide skeleton to restrict the peptide conformation containing it, 7 was derived to 1Ø according to Scheme 2 in analytical scale. Compound 1Ø showed the CD spectrum (in MeOH) closely similar to that of Dnp-Gly-D-Ala-L-Pro-Gly-pNA which is known to take

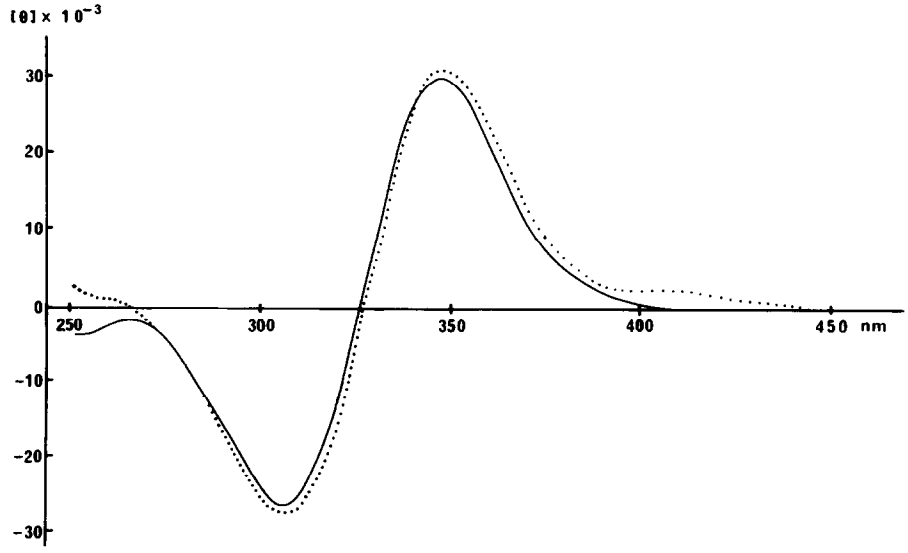
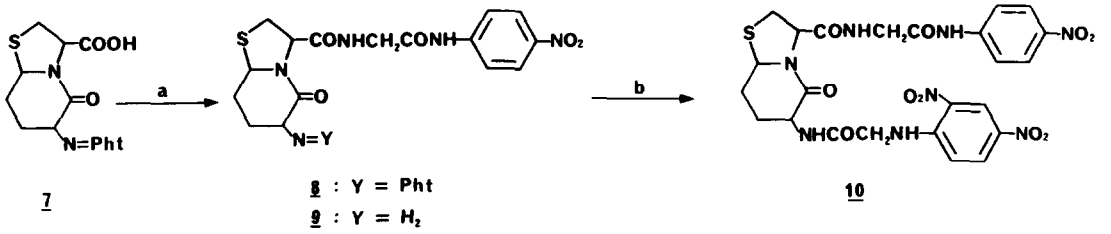


Fig. 3 CD spectra of the Dnp-peptide p-nitroanilides: solid line, compound 10; dotted line, Dnp-Gly-D-Ala-L-Pro-Gly-pNA



7

8 : Y = Pht
9 : Y = H

10

Scheme 2. Reagents: a, (i) Gly-pNA, DPPA, NEt_3 in DMF[8]; (ii) $N_2H_4 \cdot H_2O$, AcOH in MeOH. b, Dnp-Gly, DPPA, NEt_3 in DMF[8]

the conformation of type II' β-turn in high population[9] (Fig. 4). The result indicates that 10 also takes the same type of turn conformation. Considering from the semi-rigid structure of 7 skeleton, it cannot take other conformation except minor change in the lactam ring which would not affect overall conformation of the molecule. Therefore, the skeleton of 7 seems to be useful as a β-turn compelling building block for synthesizing analogs of bioactive peptides with restricted conformation to the desired turn form. Attempts to incorporate the unit into analogs of some bioactive peptides are in progress for elucidation of their active conformation.

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REFERENCES and NOTES

1. Abbreviations used are as follows. Dnp: 2,4-Dinitrophenyl, pNA: p-nitroanilide, DPPA: Diphenylphosphoryl azide, Pht: phthalyl
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