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ONE-POT CYCLIZATION OF A PEPTIDE BY THE USE OF (5-NITROPYRIDYL)DIPHENYL PHOSPHINATE: THE SYNTHESIS OF CYCLIC DECAPEPTIDE GRAMICIDIN S

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One-pot synthesis of gramicidin S, cyclic decapeptide, was successfully achieved by treatment of the corresponding linear decapeptide with (5-nitropyridyl)diphenyl phosphinate, a new condensing reagent, in pyridine. Similarly, the phosphinic ester can be successfully employed in the Young test as well as syntheses of dipeptides.

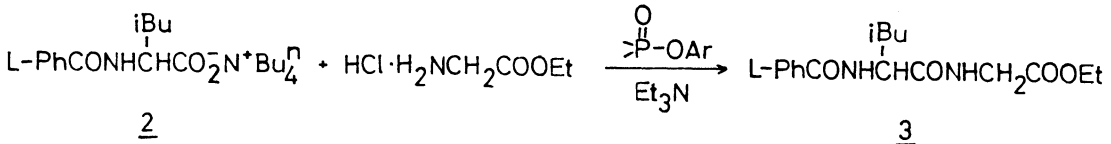
Of various methods for the synthesis of a cyclic peptide, it is pointed out that cyclization reaction is carried out in most cases by employing a pre-activated linear peptide such as a peptide active ester or azide. However, little[1),2)] has been reported concerning one-pot cyclization just starting from non-activated linear peptide. Low yielding of the cyclic peptide by this way may be attributed to an entropically disadvantageous condition in a highly diluted solution with the conventional condensing reagent. In order to overcome the above mentioned problem, we investigated the one-pot cyclization based on our organophosphorus condensing reagent which generally enables the rapid formation of mixed anhydride, an activated peptide, and successive aminolysis.

In our previous papers,[3),4)] it has been shown that the employment of N-protected amino acid (or peptides) as their tetrabutylammonium salts and the use of bis(o-or-p-nitrophenyl)phenyl phosphonate gave successful results in the Young test as well as the syntheses of peptides such as Leucine-enkephalin. However, when the above coupling reagent was used in the Young test, rather low temperature (-10 °C) was required to obtain racemization-free peptides and it also involved a difficulty in removing o-or-p-nitrophenol liberated along with peptide.

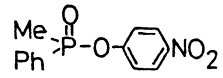
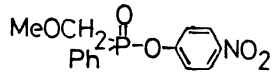
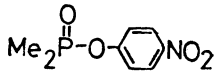
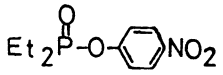
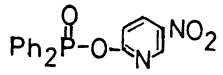
We investigated the exploration of the effective organophosphorus condensing reagent. Various phosphorus compounds were screened by use of the Young test known as the most severe racemization test and results are summarized in Table 1.

It is noted that the reaction by employing (5-nitropyridyl)diphenyl phosphinate(1)[5)] and tetrabutylammonium N-benzoyl-L-leucinate(2) proceeded smoothly under mild conditions (0 °C to room temperature) and the almost pure L-isomer(3) was obtained in excellent yield. In addition, 2-hydroxy-5-nitropyridine formed along with peptide is well soluble in water and is easily separated from the peptide.

Table 1. Results of the Young test using various phosphinic esters



2 3

Phosphorus compound	Solv.	Reaction conditions	Yield %	L-Isomer %	$[\alpha]_D$(t,°C)
	DMF	0 °C,2h then r.t.,3h	91	98	-33.4(24)
	DMF	0 °C,5h then r.t.,overnight	83	98	-33.3(25)
	DMF	0 °C,12h	81	98	-33.5(22)
	DMF	0 °C,2h then r.t., overnight	91	98	-33.4(21)
	DMF	0 °C,30min then r.t., 1.5h	89	98	-33.4(22)
1	DMF	r.t.,2h	89	97	-33.0(23)
	DMF	0 °C,30min then[a] r.t.,1.5h	92	98	-33.3(25)
	DMF	0 °C,30min then[b] r.t., 1.5h	86	98	-33.3(25)
	THF	0 °C,30min then r.t., 1.5h	85	98	-33.4(23)
	CH_3CN	0 °C,30min then r.t.,1.5h	89	98	-33.5(22)

a) One more equivalent of triethylamine was added.
b) N-Bz-Leu-OH was used with triethylamine.

Table 2. Preparation of dipeptides using (5-nitropyridyl) diphenyl phosphinate

Peptide[a]	Reaction Conditions	Yield (%)	Mp(°C) [lit.]	$[\alpha]_D$(temp.,C,solv.) [lit.]	Ref.
Z-Asn-Gly-OEt	0 °C,2h,r.t.,3h	87	185-6.5 [185-7]	-5.4(23,1.0,DMF) [-5.6]	6
Z-Met-Gly-OEt	0 °C,2h,r.t.,3h	93	95-6 [98-9]	-18.0(22,4.0,EtOH) [-17.9]	7
Z-Tyr-Gly-OEt	0 °C,30min,r.t.,1.5h	94	169-70 [170-1]	-23.7(24,50,DMF) [-24.2]	8
Boc-Trp-Gly-OEt	0 °C,30min,r.t.,1.5h	92	112-3 [112-3]	-17.5(22,1.0,DMF) [-18]	9
Z-Ile-His-OMe	0 °C,2h,r.t.,3h	80	182-3 [181-3]	-44.0(23,1.0,MeOH-NH_4Cl(1:1) [-44.7]	10
Z-Phe-Ser-OMe	0 °C,30min,r.t.,1.5h	75	122-4 [125]	-5.8(22,1.0,DMF) [-5.7]	11
Z-Arg(NO_2)-Gly-OEt	0 °C,30min,r.t.,1.5h	91	112-3 [119-20]	-12.3(23,2.0,MeOH) [-15.4]	6

a) Amino acid symbols except Gly and D-Phe denote the L-configuration.

Generality of this method was shown in syntheses of dipeptides including various functional groups in the side chains. The results are summarized in Table 2. All peptides were obtained in good yield and no protection of functional groups in the side chains such as aliphatic and phenolic hydroxy groups, and imidazole and indole rings was demonstrated in the present peptide synthesis.

In the next place, the synthesis of gramicidin S (GS)(4)[12] was carried out by using (5-nitropyridyl)diphenyl phosphinate as a condensing reagent, according to the following scheme.

General procedures for the synthesis of linear peptide, the one-pot cyclization and the synthesis of gramicidin S are as follows:

(I) Linear peptide; A methanol solution of equimolar amounts of an N-protected α-amino acid (or peptide) and tetrabutylammonium hydroxide was subjected to evaporation and the residue was azeotroped with benzene and dried in vacuo. To a stirred DMF (10 ml/mmol) solution of the ammonium salt thus obtained and an α-amino acid ester (or peptide)(1.1 equiv.) was added (5-nitropyridyl)-diphenyl phosphinate (1.1 equiv.) and the mixture was stirred at 0 °C for 30 minutes and then room temperature for 1.5 hrs. After removal of DMF in vacuo, the residue was dissolved in ethyl acetate, and the organic solution was washed successively with saturated sodium hydrogen carbonate, water (twice), N-hydrochloric acid, water (twice), and saturated brine, and then dried ($MgSO_4$). After evaporation of ethyl acetate, the peptide was purified by column chromatography using silica gel.

(II) One-pot cyclization; (5-nitropyridyl)diphenyl phosphinate (5 equiv.) was dissolved in small amount of THF and linear decapeptide in pyridine (3×10^{-3} M reactant in solvent) was added to this condensing reagent at room temperature. The reaction mixture was stirred for 3 hrs and then evaporated in vacuo. The residue was applyed to ion exchange column, IRA-400 (OH^- form), IR-120 (H^+ form) and eluted with MeOH-H_2O (5:1). The effluent was evaporated, and the product was collected by filtration with aid of water. The product was further purified by a column (2 × 110 cm) with Sephadex LH-20 in MeOH. Main fractions were evaporated, and the residual solid was recrystallized from MeOH-ether-petroleum ether.

(III) Synthesis of gramicidin S; diZ-GS(6)[14] obtained was converted to GS·2HCl(7)[15] by hydrogenolysis (H_2/Pd in AcOH for 4 hrs).

It is noted that cyclic peptide is synthesized just starting from free linear peptide, by the present procedure, in such higher yield than the conventional methods.

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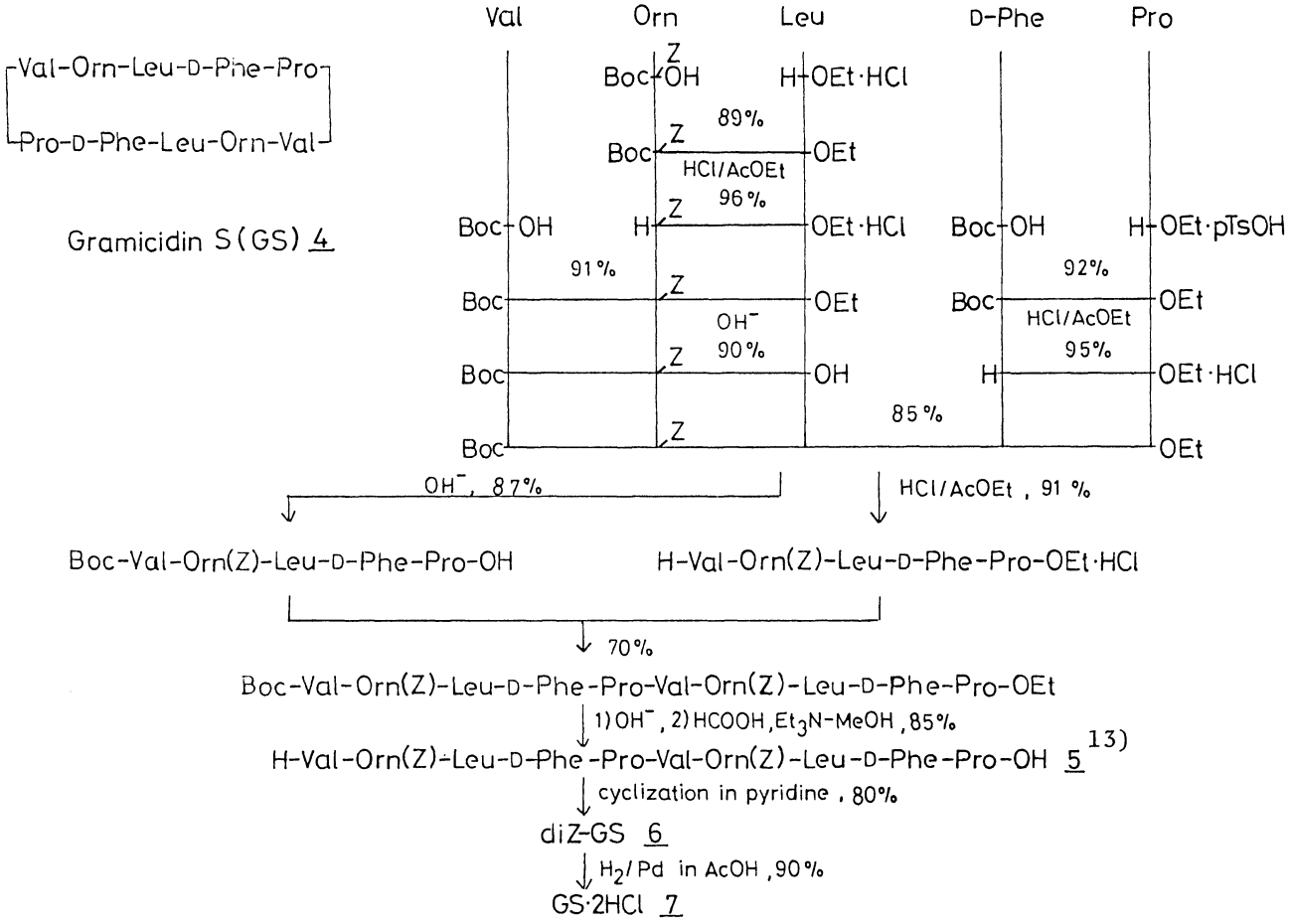
4) Y. Watanabe, N. Morito, K. Kamekawa, and T. Mukaiyama, Chem. Lett., 1981, 65.
5) This phosphinic ester was prepared from diphenylphosphinyl chloride and 2-hydroxy-5-nitropyridine in the presence of triethylamine. mp 125-127 °C; Found: C, 60.30; H, 3.69; N, 8.31, Calcd. for $C_{17}H_{13}O_4N_2P$: C, 60.01; H, 3.85; N, 8.23.

Scheme. Synthesis of Gramicidin S



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15) The product was isolated by the use of a silica gel 60 pre-packed column ($CHCl_3$:MeOH:aq NH_4OH=24:6:1) and then recrysted from EtOH-1NHCl, mp 274-276 °C [lit. (ref 16), 274-276 °C]; $[\alpha]_D^{23}$-270° (c 0.1, EtOH)[lit. (ref 16) $[\alpha]_D^{20}$-271° (c 0.1, EtOH)]. Found: C, 48.12; H, 8.49; N, 11.09, Calcd. for $C_{60}H_{92}N_{12}O_{10}\cdot 2\,HCl\cdot 16H_2O$: C, 47.96; H, 8.45; N, 11.19. Amino acid analysis (after acidic hydrolysis): Val, 0.99; Orn, 1.01; Leu, 1.01; D-Phe, 0.99; Pro, 0.98.

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