

PEPTIDE SYNTHESIS BY USING N-ACYLPHOSPHORAMIDITES

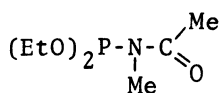
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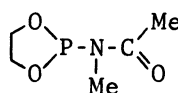
New trivalent phosphorus compounds, N-acetyl-N-methylphosphoramidites were prepared readily in good yield by the reaction of phosphorous chlorides and N-methylacetamide. By using the phosphoramidites as condensing agents, Young's peptide and prolyl-leucyl-glycinamide (MSH-RIF) were synthesized without racemization in high yields under mild conditions.

Many phosphorus compounds have been investigated as condensing agents in peptide synthesis.¹⁾ Recently, it has been emphasized that phosphorus compounds with phosphoryl group such as diphenyl phosphorazidate,²⁾ diethyl phosphorocyanidate,³⁾ diethyl phosphorobromidate,⁴⁾ and bis(2-nitrophenyl) phenylphosphonate⁵⁾ have high activities with little racemization for peptide bond formation. On the other hand, a few trivalent phosphorus compounds such as tetraethyl pyrophosphate⁶⁾ were applied to condensation of amino acids, though appreciable racemization was encountered in the course of the reaction.

In this report, we describe the preparation of new trivalent phosphorus compounds,⁷⁾ diethyl N-acetyl-N-methylphosphoramidite, I, 2-N-acetyl-N-methylamino-1,3,2-dioxaphospholane, II, and the syntheses of Young's dipeptide⁸⁾ and melanocyte stimulating hormone-release-inhibiting factor (MSH-RIF) without racemization by use of these compounds.



(I)



(II)

The trivalent phosphorus compounds I and II were prepared readily in good yield by the reaction of N-methylacetamide with diethyl chlorophosphite and 2-chloro-1,3,2-dioxaphospholane respectively in the presence of triethylamine followed by fractional distillation. In order to determine their structure, I was oxidized with ozone to give the corresponding phosphoric compound. It was found that this compound was spectroscopically identical with diethyl N-acetyl-N-methylphosphoramidate which was separately prepared by the acetylation of diethyl N-methylphosphoramidate according to Matrosov et al.⁹⁾ Therefore, I was identified as diethyl N-acetyl-N-methylphosphoramidite. The results of ^1H , ^{13}C , ^{31}P NMR and IR spectroscopic measurements for I are as follows: ^1H ; 1.27(t,3,J=7.0, $\text{CH}_3\text{CH}_2\text{O}$ -) 2.28(d,3,J=5.6, CH_3CO -) 2.80(s,3, CH_3N -) 3.82(qi,2, $J_{\text{C-C}}=7.0, J_{\text{P}}=9.5, \text{CH}_3\text{CH}_2\text{O}$ -) ^{13}C ; 16.81(d,J=6.1, $\text{CH}_3\text{CH}_2\text{O}$ -) 23.15 and 24.12(s, CH_3CO -) 25.58(s, CH_3N -) 60.55(d,J=18.3, $\text{CH}_3\text{CH}_2\text{O}$ -) 172.94(d,J=27.4, $\text{C}=\text{O}$) ^{31}P ; 139.7 IR; 1670(C=O) 1023(P-O-C) The spectral data also supported the structure described above. Preparation of I is as follows: To a solution of N-methylacetamide 8.8g(0.12mol) and triethylamine 12.1g(0.12mol) in benzene 200ml was added diethyl chlorophosphite 18.8g(0.12mol) with stirring at $-5-0^\circ\text{C}$. After stirring for 1 hr at 0°C , the mixture was filtered and subsequently concentrated in vacuo. The residual oil was distilled under reduced pressure resulting in 13.7g(59%) of II, bp $87-8^\circ\text{C}/1.1\text{mmHg}$. Similarly, II(73%, bp $71-2^\circ\text{C}/0.2\text{mmHg}$) was prepared by the reaction of N-methylacetamide with 2-chloro-1,3,2-dioxaphospholane and was proved to be 2-N-acetyl-N-methylamino-1,3,2-dioxaphospholane.

The resulting phosphoramidites, I, II, were applied to the syntheses of Young's dipeptide and the tripeptide(MSH-RIF). The results of Young test in various solvents with I are summarized in Table 1. In acetonitrile at 35°C , the dipeptide was obtained in the highest yield maintaining the optical purity. In order to increase the yield, the use of a small excess of the phosphoramidites is favorable at 35°C , since racemization is enhanced at an elevated temperature(60°C).

Table 1. Young Test with Phosphoramidite I

Solvent	Temp. ($^\circ\text{C}$)	Time(days)	Yield(%)	$[\alpha]_{\text{D}}^{17}$ *	L-Isomer(%)
Benzene	35	2	30	-23.7	69
DMF	35	2	40	-28.1	82
CH_3CN	35	2	75	-33.6	98
CH_2CN	60	1	90	-21.1	62

* c 2.6 in ethanol

Interestingly, the high yield of III is obtained without racemization in the method shown in Scheme 2 where the carboxylic group of the dipeptide was activated with the phosphoramidite. This suggests possibility of a fragment condensation at any points with the phosphoramidites. In both cases, the desired peptides were easily isolated only by extraction. Use of amino acids salts which are stable and hence easily handled lowered the yield of the peptides.

The typical procedure of the condensation reaction is given below: Carbobenzoxy-propyl leucine 2.18g(6.0mmol), phosphoramidite,II, 1.1g(7.2mmol) and ethyl glycinate 0.62g(6.0mmol) were dissolved in acetonitrile(30ml). After standing the reaction mixture at 30°C for 20 hrs, the solvent was removed in vacuo. The residue was dissolved in CHCl_3 (60ml) and was washed successively with 1.5N HCl(3x60ml), water(60 ml), sat. NaHCO_3 (3x60ml) and water(3x60ml) again. The organic solution was dried over anhydrous sodium sulfate and was evaporated to give a crystalline,III, in the yield of 2.44g(91%); mp 148-50°C, $[\alpha]_D^{17} -81.3$ (c 2.43, ethanol).¹⁰⁾ The protected tripeptide,III, was transformed to MSH-RIF by conventional method.¹¹⁾

This condensation method with the phosphoramidites, which were easily prepared from phosphorous chlorides and amides, has several advantages. Under mild conditions, coupling reaction proceeded smoothly and optically pure products were isolated only by extraction. No racemization was encountered even in chain-elongation starting with the NH_2 -terminal residue.

References

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