Pergamon

Tetrahedron Vol. 50, No. 44, pp. 12743-12754, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00807-8

The Preparation of Phosphono Peptides Containing a Phosphonamidate Bond

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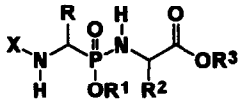
Abstract: The synthesis of phosphono peptides containing phosphonamidate bond by means of phosphorylation of amino acid esters with N-protected aminoalkylphosphonochloridates, is accompanied by the formation of an unidentified side-product. The factors influencing this reaction were studied in some detail.

INTRODUCTION

Replacement of an amide bond in peptides by phosphonamidate moiety results in peptide analogues (phosphono peptides, compounds 1) of significantly different shape, charge distribution and resistance to hydrolysis.[1] These phosphono peptides are among the transition-state analogues which show the best resemblance, both from a steric and electronic point of view, to the transition state of amide bond hydrolysis. Consequently they form an important class of enzyme inhibitors [2] and play an important role in the development of catalytic antibodies with protease-like specificity.[3]



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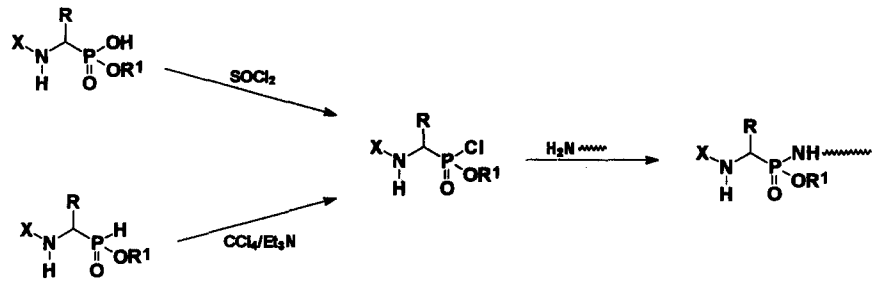
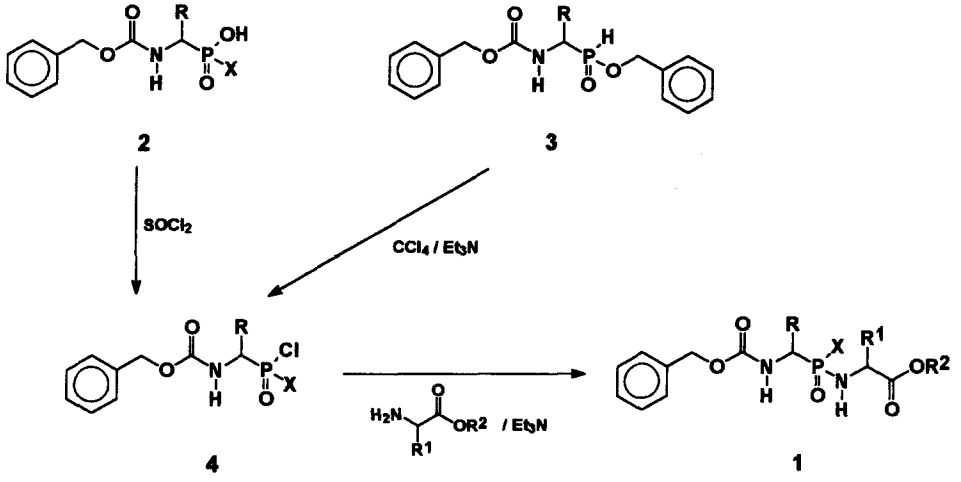
The commonly used method for the synthesis of the peptides 1 begins with *N*-protected aminophosphonate monoesters which are activated by formation of phosphonochloridates and then coupled to the appropriate aminoester or peptide fragment.[3,4] An interesting modification of this procedure involves the *in situ* formation of phosphonochloridates from the corresponding phosphinites in an oxidative Atherton-Todd process. [5]



In this paper we report our studies on the scope and limitations of both methods for the synthesis of phosphono peptides 1 which are due to the formation of an unexpected side-product.

RESULTS AND DISCUSSION

Although the two above procedures are commonly used in phosphono peptide synthesis, little is known about the influence of the reaction conditions on the reaction course and yields of the phosphorylation step. In this study monobenzyl 1-(*N*-benzyloxycarbonylamino)alkylphosphonates (compounds **2**, X = $OCH_2C_6H_5$)[6] and 1-(*N*-benzyloxycarbonylamino)(*P*-phenyl)alkylphosphinates (compounds **2**, X = C_6H_5) were converted into their chlorides **4** by reaction with thionyl chloride (Scheme I). The obtained phosphonochloridates were then used for the phosphorylation of amino acid esters.



Scheme I

For comparison, the same reaction was carried out using benzyl 1-(*N*-benzyloxycarbonylamino)alkyl-phosphinites (compounds **3**) as precursors of the phosphonochloridates **4** (see also Scheme I).

Data summarized in Tables 1 and 2 clearly indicate that the use of both methods of activation gave practically the same results.

Table 1. Phosphono peptides **1** obtained from compounds **2**.

N-Terminal substrate			C-terminal substrate	Yield of **1**		Recovered **2**
Compound	**R**	**X**		**Compound**	%	%
2a	CH_3	$OCH_2C_6H_5$	methyl glycinate hydrochloride	**1a**	63	16
2b	C_6H_5	$OCH_2C_6H_5$	methyl glycinate hydrochloride	**1b**	73	14
2b	C_6H_5	$OCH_2C_6H_5$	benzyl *DL*-alaninate hydrochloride	**1d**	49	24
2c	CH_3	C_6H_5	methyl glycinate hydrochloride	**1f**	82	6
2c	CH_3	C_6H_5	benzyl *DL*-alaninate hydrochloride	**1g**	54	44
2d	C_6H_5	C_6H_5	methyl glycinate hydrochloride	**1h**	58	21
2d	C_6H_5	C_6H_5	benzyl *DL*-alaninate	**1i**	59	40
2d	C_6H_5	C_6H_5	benzyl *DL*-alaninate hydrochloride	**1i**	56	41
2e	*iso*butyl	CH_3	methyl glycinate hydrochloride	**1j**	44*	56*

* established on the basis of ^{31}P-n.m.r. spectrum of crude reaction mixture

Reaction yields, however, were quite moderate irrespective of the method used and substantial amounts of compounds **2** were recovered from alkaline extracts of the reaction mixture. Since these compounds were not used as substrates if the Atherton-Todd approach was used, the simple oxidation of phosphinites **3** appeared to be the side-reaction in this case.

It is worth to notify that the yields of reactions starting from benzyl *DL*-alaninate *p*-toluenosulfonate and phosphinic acids **2c** and **2d** were low (around 10% and 30% respectively). We speculate that the reaction generated a mixed phosphinic *p*-toluenesulfonate anhydride as a side-product.

Table 2. Phosphono peptides **1** obtained from compounds **3**.

N-Terminal substrate		C-terminal substrate	Yield of 1		Recovered 2
Compound	**R**		**Compound**	%	%
3a	CH_3	methyl glycinate hydrochloride	**1a**	52	26
3b	C_6H_5	methyl glycinate hydrochloride	**1b**	52 65* 70**	24 30**
3b	C_6H_5	methyl *L*-alaninate	**1c**	48 54**	 46**
3b	C_6H_5	methyl *L*-valinate hydrochloride	**1e**	43 48**	 52**

* from pure recrystallized substrate; reaction was carried out in 0° C

**established on the basis of ^{31}P-n.m.r. spectrum of crude reaction mixture

Examination of the crude reaction mixtures by means of ^{31}P-n.m.r. revealed that the formation of the desired phosphono peptides is always accompanied by the formation of additional products as indicated by the presence of signals shifted down-field. Extraction of the reaction mixtures with sodium hydroxide solution caused disappearance of these signals and compounds **2** were recovered from the alkaline extracts upon acidification.

Formation of side-product

In trying to understand the course of phosphonamidate bond formation we have studied this reactions in same detail using three different substrates. Reaction of (*N*-benzyloxycarbonylamino)benzyl(*P*-phenyl)-phosphinic acid (compound **2d**) with thionyl chloride yielded a single product which was presumed to be corresponding phosphinochloridate (compound **4**, R = X = C_6H_5; δ 35.6 ppm in ^{31}P-n.m.r. spectrum). This compound readily reacted with ethanol, both in the presence and absence of triethylamine, and gave its ethyl ester (compound **5a**) in good yield. Also the reaction with benzyl alcohol afforded the corresponding ester **5b** in nearly quantitative yield.

5a: R = C_2H_5
5b: R = $CH_2C_6H_5$

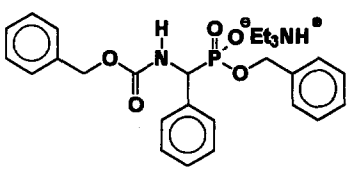
Reaction of this phosphinochloridate with benzyl *DL*-alaninate hydrochloride appeared to be quite capricious yielding the mixtures of products consisting of two major components: (1) four enantiomeric pairs of the peptide **1i** (one pair of δ 37.7 plus 37.2 and the second of δ 28.9 plus 28.6 ppm in 2:8 molar ratio; up to 60%), and (2) two products (δ 21.9 and 21.5 ppm in 2:8 molar ratio; up to 50%) which disappeared upon alkaline extraction. Addition of triethylamine to the chloroformic solution (*N*-benzyloxycarbonylamino)-benzyl(*P*-phenyl)phosphinochloridate also gave the two products (δ 22.7 and 22.2 ppm in 2:8 molar ratio). Extraction of this mixture with sodium hydroxide solution yielded the disappearance of both ^{31}P-n.m.r. signals and the substrate (compound **2d**) was recovered quantitatively upon acidification.

Benzyl (*N*-benzyloxycarbonylamino)benzylphosphonate (compound **2b**) gave corresponding phosphonochloridate (compound **4**, R = C_6H_5, X = $OCH_2C_6H_5$; δ 34.2 and 34.3 ppm in 2:3 molar ratio). Addition of triethylamine to the solution of this chloride in chloroform yielded quite complex mixture of organophosphorus compounds. The side-product observed during phosphonamidate bond formation predominates in this mixture (δ 15.3 ppm). Also benzyl (*N*-benzyloxycarbonylamino)benzylphoshinite (compound **3b**) gave complex mixture of products when treated with triethylamine in carbon tetrachloride solution. Also one product predominates here (δ 17.2 ppm). ^{31}P-n.m.r. spectrum taken from equivalent mixture of both mixtures revealed that the both observed shifts correspond to the same compound .

The described above experiments clearly indicate that the formation of phosphonamidate bond is accompanied by the formation of side-product. Addition of water to the reaction mixture suggests that it may be salt of monoester **2b** (compound **6**) formed in the reaction of phosphonochloridate with traces of water present in the reaction mixture since this product is formed in 95% while the yield of peptide **1b** decreased to 5%. Also addition of the equimolar quantity of the salt **6** to the reaction mixture resulted in change of peptide **1b** to dead-end product ratio from 70:30 to 33:67. This seems to be somewhat unlikely because all the reagents were carefully dried before use and the reaction when carried out under argon gave identical result. Moreover, the amino group is believed react with phosphonochloridates preferentially to hydroxylic moiety.[7] Thus the possibility of formation of other product should be also taken into consideration. We suggest that it might be

compound 7. Formation of similar product containing pyridine moiety has been reported by Yamazaki.[8] This suggestion is additionally supported by the fact that the yield of side-product is not dependent on the molar concentration of substrates and thus on the volume of solvent used.

As seen from Table 2 the decrease of Atherton-Todd reaction temperature to 0° C resulted in moderate rise of the yield of phosphono peptide with simultaneous decrease of side-product yield.



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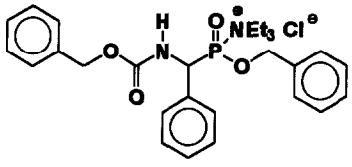

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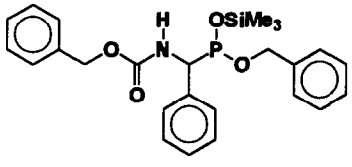
Sampson and Bartlett reported the use of silylated phosphinite **8** the in Atherton-Todd reaction.[5a] The use of this method did not, however, prevent the formation of the side-product and thus this approach has no advantage over the simple reaction used in this work.



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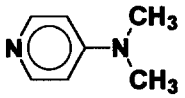
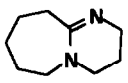
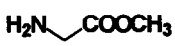
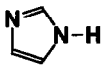
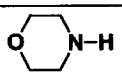
The influence of the amine on the course of Atherton-Todd reaction was also studied. Results presented in Table 3 clearly indicate that in all the cases the oxidation of phosphinite **3b** is the main reaction. It is also worth to notify that the use of morpholine (secondary amine) gave the mixed stereoisomers of morpholyl phosphonamide as predominant reaction product.

Chiral phosphinites* 3b *as substrates

The reaction of both enantiomers of phosphinite **3b** and methyl L-valinate showed the formation of two stereoisomers of **1e** in each case (Table 4). We were unable, however, to separate them neither chromatographically nor by recrystallization. Reaction of racemic **3b** with methyl L-valinate gave, as expected, a mixture of four products in molar ratio which is simple superposition of products obtained from enantiomers of **3b**.

Table 3. Influence of amine on the course of Atherton-Todd reaction

Amine	Products	
	Yield (%)	d in ^{31}P-n.m.r.
	peptide - 56% side-product- 44%	28.8 and 27.3 ppm (62:38 molar ratio) 16.2 and 16.6 ppm (58:42 molar ratio)
	complicated mixture of products	
	peptide - 65% side-product- 35%	26.3 ppm* 13.8 ppm*
	side-product - 100%	16.2 ppm
	no reaction	
K_2CO_3	no reaction	
	phosphonamide - 90% side-product - 10%	25.5 and 26.1 ppm (1:1 molar ratio) 16.8 ppm

* taken at 80 MHz apparatus

EXPERIMENTAL

Materials and Methods. Unless otherwise stated, materials were obtained from commercial suppliers and used without purification. Ethanol- and water-free chloroform was obtained by passing the solvent through activated alumina followed by its distillation from phosphorus pentoxide. Triethylamine was distilled and stored over potassium hydroxide.

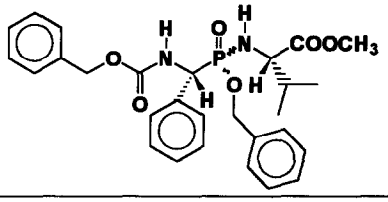
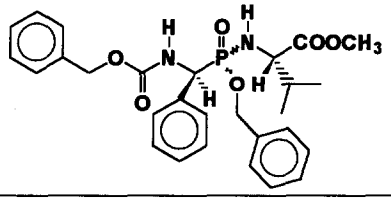
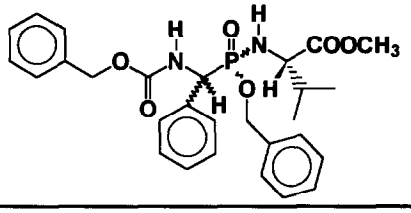
Benzyl (*N*-benzyloxycarbonylamino)alkylphosphonates (compounds 2) and -phosphinites (compounds 3), as well as (*N*-benzyloxycarbonylamino)alkylphosphinic acids, were obtained by the described procedures.[6]

Melting points were taken on Mettler FP5 apparatus and were uncorrected. Infrared spectra were recorded on a Perkin Elmer 377 spectrometer. ^{1}H-n.m.r. spectra were recorded on Varian EM360 (60MHz) and Bruker 250 (250 MHz) spectrometers. ^{31}P-n.m.r. spectra were obtained with use of broad-band ^{1}H decoupling on Bruker WP80DS (80 MHz) and Bruker 250 (250 MHz) spectrometers; chemical shifts are reported as ppm relative to 85% H_3PO_4 (sealed capillary). Microanalyses (C, H, N) were performed either by Service de

Microanalyse du C.N.R.S. or by Instrumental Analysis Unit of the Institute of Organic Chemistry, Biochemistry and Biotechnology.

Even if not indicated, the structures of all the compounds were supported by their H^1-n.m.r., ^{31}P-n.m.r., as well as infrared spectra.

Table 4. Stereoisomers of **1e**

Phosphono peptide	P-n.m.r. [ppm] ($CDCl_3$/85% H_3PO_4)
	26.3 and 26.9 (3:2 molar ratio)
	26.2 and 26.9 ppm (2:3 molar ratio)
	26.2: 26.3 and 26.9 ppm (2:3:5 molar ratio)

General Procedure for the Preparation of Phosphono Peptides 1. Phosphonic acid monoester (compounds **2a-2b**; 5 mmol) or phosphinic acid (compounds **2c-2d**; 5 mmol) was suspended in dry chloroform (50 ml) and thionyl chloride was added (0.6 ml; 7.5 mmol). The mixture was stirred for 2 h at room temperature and heated at 60° C (bath temperature) for additional 2 h. The volatile components of the reaction mixture were evaporated under reduced pressure, oily residue was redissolved in chloroform and evaporated again in order to remove hydrogen chloride and unreacted thionyl chloride. This step was repeated three times using dry chloroform. The resulting phosphonochloridate was dissolved in chloroform (30 ml) and added dropwise to the mixture of amino acid ester hydrochloride (5 mmol) and triethylamine (2.0 ml; 13.5 mmol) in chloroform cooling in an ice bath. The resulting solution was left for two days at room temperature and then washed successively with: 1M sodium hydroxide solution (40 ml, unreacted substrate was recovered from this extract upon acidification), water (3x30

ml), 5% hydrochloric acid (30 ml), water (30 ml) and brine (30 ml). The chloroform layer was dried over anhydrous sodium sulphate. Removal of drying agent and solvent afforded crude peptides of satisfactory purity.

***Peptide* 1a.** Crude product was obtained as a mixture of two enantiomeric pairs in 1:1 molar ratio (^{31}P-n.m.r. - δ 31.2 and 30.0 ppm). Recrystallization from hexane did not change this ratio.
M.p. 102° C; IR (KBr): 3325 (amidate NH), 3245 (phosphonamidate NH), 1755, 1715 and 1685 (CO), 1245 and 1205 (PO) cm^{-1}; ^{1}H($CDCl_3$, 60 MHz): 1.38 (dd, J=7.5Hz, J_{PH}=17.0Hz, 3H, CH_3), 3.66 and 3.69 (s, s, 1.5H each, $COOCH_3$), 3.5-4.65 (m, 4H, NCHP, NCH_2COO, PNH), 5.05 (d, J=7.5Hz, 2H, $POCH_2$), 5.08 (s, 2H, CH_2OCO), 5.72 and 6.13 (bd, J=9.0Hz, 0.5H each, NHCO), 7.35 (s, 10H, 2xC_6H_5) ppm; Anal.: calcd. for $C_{20}H_{25}N_2O_6P$: C, 57.14; H, 6.00; N, 6.66; found: C, 57.02; H, 5.87; N, 6.51.

***Peptide* 1b.** Recrystallized from chloroform-hexane mixture gave mixture of two enantiomeric pairs in 1:1 molar ratio (^{31}P-n.m.r. - δ 26.5 and 26.0 ppm).
M.p. 109° C; IR (KBr): 3360, 3290 and 3210 (NH), 1740 and 1690 (CO), 1560 (dNH), 1260 (PO); ^{1}H($CDCl_3$, 60MHz): 3.4-3.8 (m, 2H, NCH_2COO), 3.65 (s, 3H, $COOCH_3$), 4.8-5.2 (m, 2H, $POCH_2$), 5.10 (s, 2H, CH_2OCO), 5.20 (dd, J=9.0Hz, J_{PH}=20.0Hz, 1H, NCHP), 6.2-6.8 (m, 2H, 2xNH), 7.2-7.7 (m, 15H, 3xC_6H_5); Anal.: calcd. for $C_{25}H_{27}N_2O_6P$: C, 62.24; H, 5.64; N, 5.81; found: C, 62.19; H, 5.79; N, 5.90.

***Peptide* 1d.** Recrystallization of the crude product from the mixture of chloroform and hexane yielded mixture of three isomers in 2:1:1 molar ratio (^{31}P-n.m.r - δ 25.5, 25.7 and 25.9).
M.p. 87° C; IR (KBr): 3290 and 3210 (NH), 1745 and 1700 (CO), 1545 (dNH), 1245 (PO); ^{1}H($CDCl_3$, 60MHz): 0.96, 1.18 and 1.29 (major isomer) (d, d, d, J=7.0Hz, 3H together, CH_3), 3.1-4.2 (m, 2H, PNH, NCHCOO), 5.01 (d, J=8.0Hz, 2H, $POCH_2$), 5.08 (bs, 4H, $COOCH_2$ and CH_2OCO), 4.8-5.4 (m, 1H, NCHP), 6.4-6.6 (m, 1H, CONH), 7.30 and 7.34 (bs, bs, 20H together, 4xC_5H_5); Anal.: calcd. for $C_{31}H_{33}N_2O_6P$: C, 66.42; H, 5.93; N, 5.00; found: C, 66.13; H, 6.09; N, 5.23.

***Peptide* 1f.** Crude product was purified by boiling in hexane for 0.5 h. Mixture of two enantiomeric pairs in 2:8 molecular ratio (^{31}P-n.m.r. - δ 35.1 and 35.5) was obtained in this manner.
M.p. 123° C; IR (KBr): 3360, 3250 and 3200 (NH), 1750, 1730, 1705 and 1700 (CO), 1545 (dNH), 1265 and 1250 (PO); ^{1}H($CDCl_3$, 250MHz): 1.17 (minor isomers, dd, J=7.4Hz, J_{PH}=15.8Hz, 0.6H, CH_3), 1.31 (major isomers, dd, J=7.4Hz, J_{PH}=15.0Hz, 2.4H, CH_3), 3.63 (minor isomers, s, 0.6H, $COOCH_3$), 3.69 (major isomers, s, 2.4H, $COOCH_3$), 3.4-3.8 (m, 2H, NCH_2COO), 4.1-4.4 (m, 1.8H, NCHP of major isomers and PNH), 4.4-4.55 (minor isomers, m, 0.2H, NCHP), 5.03 and 5.08 (AB system, J_{AB}=14.0Hz, 2H together, CH_2OCO), 5.25 (major isomers, d, J=8.8Hz, 0.8H, CONH), 5.57 (minor isomers, d, J=8.8Hz, 0.2H, CONH), 7.25-7.85 (m, 10H, 2xC_6H_5); Anal.: calcd. for $C_{19}H_{23}N_2O_5P$: C, 58.46; H, 4.91; N, 7.18; found: C, 58.12; H, 4.93; N, 7.28.

***Peptide* 1g.** Crude reaction product, being a mixture of four enantiomeric pairs of 1:1:3:5 molar ratio (^{31}P-n.m.r. - δ 40.0, 39.3, 34.2, and 33.7) and some impurities, was recrystallized from ethyl acetate-hexane yielding two enantiomeric pairs of 4:6 molar ratio (^{31}P-n.m.r. - δ 34.7 and 34.0).
M.p. 104° C; IR (KBr) 3300, 3250 and 3185 (NH), 1740 and 1690 (CO), 1555 (dNH), 1255 (PO); ^{1}H($CDCl_3$, 60MHz): 1.05-1.55 (m, J=7.5Hz, J_{PH}=13.5Hz, 6H, 2xCH_3), 3.6-4.5 (m, 3H, NCHP, NCHCOO, PNH), 5.00,

5.06 and 5.13 (s, s, s, 4H together, $COOCH_2$, CH_2OCO), 5.45 (bd, J=10.0Hz, CONH), 7.1-8.0 (m, 15H, $3xC_6H_5$); Anal.: calcd for $C_{26}H_{29}N_2O_5P$: C, 64.99; H, 6.08; N, 5.83; found: C, 65.12; H, 5.89; N, 6.07.

***Peptide* 1h.** Crude product was of satisfactory purity and was obtained as a mixture of two enatiomeric pairs of 4:6 molar ratio (^{31}P-n.m.r. - δ 31.2 and 30.0).
M.p. 170° C; IR (KBr): 3360, 3270 and 3245 (NH), 1745, 1725 and 1690 (CO), 1555 and 1520 (dNH), 1260 and 1240 (PO); ^{1}H($CDCl_3$, 60 MHz): 3.39 (d, J=7.5Hz, 2H, NCH_2COO), 3.67 and 3.69 (s, s, 3H together, $COOCH_3$), 4.99 and 5.10 (s, s, 2H together, CH_2OCO), 5.20 (dd, J=9.5Hz, J_{PH}=16.0Hz, 1H, NCHP), 6.0-6.7 (m, 2H, 2xNH), 7.0-8.0 (m, 15H, $3xC_6H_5$); Anal.: calcd. for $C_{24}H_{25}N_2O_5P$: C, 63.71; H, 4.88; N, 5.43; found: C, 63.29; H, 5.03; N, 5.83.
***Peptide* 1i.** Crude product forms as a mixture of four enantiomeric pairs of 2 (^{31}P-n.m.r. - δ 37.7 and 37.2) : 8 (^{31}P-n.m.r. - δ 28.9 and 28.6) molar ratio and some impurities. Recrystallization from the mixture of chlorofom and hexane gave two fractions.
First fraction: 24% of yield; two enantiomeric pairs of 8:2 molar ratio (^{31}P-n.m.r. - δ 28.3 and 28.0); m.p. 172° C; IR (KBr): 3210 and 3145 (NH), 1745 and 1715 (CO), 1555 (dNH), 1255 (PO); ^{1}H($CDCl_3$, 60 MHz): 1.16 (d, J=7.0Hz, 3H, CH_3), 3.2-3.85 (m, 1H, NCHCOO), 4.95 (s, 2H, $COOCH_2$), 5.05 (s, 2H, CH_2OCO), 6.15-6.6 (m, 2H, 2xNH), 7.1-8.4 (m, 20H $4xC_6H_5$); Anal.: calcd. for $C_{31}H_{31}N_2O_5P$: C, 68.63; H, 5.76; N, 5.16; found: C, 68.33; H, 5.79; N, 5.33.
Second fraction: 18% of yield, mixture of four enantiomers in 5:1:2:2 molar ratio (^{31}P-n.m.r. - δ 37.7, 37.2, 29.0 and 28.6) containing 20% of impurity (δ 32.0).

General Procedure for the Preparation of Phosphono Peptides 1 by the Atherton-Todd Approach. Benzyl ester 3 (1 mmol) and amino acid ester hydrochloride (1 mmol) were suspended in carbon tetrachloride (20 ml) and triethylamine (0.3 ml; 2.25 mmol) was added. The mixture was stirred for 24 h at room temperature and then the volatile components of the reaction mixture were removed under reduced pressure. The residue was dissolved in dry chloroform and worked-up analogously as in the general procedure given above.

***Peptide* 1a.** Crude reaction product was recrystallized from the mixture of chloroform and hexane a mixture of two enantiomeric pairs in 1:1 molar ratio (^{31}P-n.m.r. - δ 31.2 and 30.0 ppm); m.p. 101° C.

***Peptide* 1b.** Crude product was purified by means of column chromatography (on Merck silica 60 using ethyl acetate-hexane (7:3 v/v) as eluent. This gave a mixture of two pairs of enantiomers in 1:1 molar ratio (^{31}P-n.m.r. - δ 27.1 and 26.6 ppm). M.p. 109° C.

***Peptide* 1c.** Crude reaction mixture was purified chromatographically (Merck silica 60, ethyl acetate-hexane 65:35 mixture as eluent). This gave mixture of isomers of 2:4:1 molecular ratio (^{31}P-n.m.r. - δ 25.6, 25.9 and 26.2). M.p. 124° C; ^{1}H($CDCl_3$, 250MHz): 0.99, 1.24 and 1.30 (d, d, d, J=7.0Hz, 3H together, CH_3), 3.05-3.50 (m, 1H, NCHCOO), 3.61, 3.63, 3.66 and 3.68 (s, s, s, s, 3H together, $COOCH_3$), 3.75-3.85 (m, 1H, PNH), 4.80-5.05 (m, 2H, $POCH_2$), 5.03, 5.05, 5.06 and 5.08 (s, s, s, s, 2H together, CH_2OCO), 5.11-5.29 (m, 1H, NCHP), 5.98 and 6.29 (m, 1H together, CONH), 7.10-7.45 (m, 15H, C_6H_5); Anal.: calcd. for $C_{26}H_{29}N_2O_6P$: C, 62.90; H, 5.85; N, 5.65; found: C, 62.90; H, 5.97; N, 5.70.

***Peptide* 1e.** Crude reaction mixture was purified chromatographically (Merck silica 60, ethyl acetate-hexane 55:45 mixture as eluent). This gave mixture of isomers of 2:3:5 molecular ratio (see discussion in Results and Discussion Section). M.p. 114° C; ^{1}H($CDCl_3$, 250MHz): 0.71-0.92 (8xd, J=7.0Hz, 3H together, CH_3), 1.83-1.99 (m, 1H, $CHCH_3$), 3.11-3.50 (m, 1H, NCHCOO), 3.58, 3.648 and 3.654 (s, s, s, 3H together, $COOCH_3$), 3.69-3.88 (m, 1H, PNH), 4.67-5.02 (m, 2H, $POCH_2$), 5.04, 5.05, 5.07 and 5.08 (s, s, s, s, 2H together, CH_2OCO), 5.10-5.25 (m, 1H, NCHP), 6.02 (dd, J=4.4Hz, J_{PH}=9.0Hz, 0.3H, CONH), 6.26 (dd, J=4.2Hz, J_{PH}=9.6Hz, 0.7H, CONH), 7.06-7.43 (m, 15H, 3xC_6H_5); Anal.: Calcd. for $C_{28}H_{33}N_2O_6P$: C, 64.12; H, 6.30; N, 5.34; found: C, 64.07; H, 6.56; N, 5.39.

Preparation of Alkyl (*N*-Benzyloxycarbonylamino)benzyl(*P*-phenyl)phosphinates 5. To the solution of phosphinochloridate (2.5 mmol), obtained as described in general procedure for the synthesis of the peptides, solution of alcohol (2.5 mmol) and triethylamine (1.0 ml; 6.7 mmol) in chloroform (15 ml) was added dropwise cooling the mixture in ice bath. The resulting solution was stirred for 2 h in bath and additional 2 h at room temperature. Then it was washed successively with: water (30 ml), 5% hydrochloric acid (30 ml), water (30 ml), saturated sodium bicarbonate solution (30 ml), water (30 ml) and brine (30 ml) and dried over anhydrous sodium sulphate. Filtration of the drying agent and removal of solvent yielded the desired esters of analytical purity.

***Ester* 5a.** Yield 89%, as a mixture of two enantiomeric pairs in 8:2 molar ratio (^{31}P-n.m.r. - δ 37.1 and 38.4); m.p. 164° C; IR (KBr): 3200 (NH), 1715 (CO), 1555 (dNH), 1250 (PO), 1050 and 1035 (POC); ^{1}H($CDCl_3$; 60MHz): 1.03 (t, J=7.0Hz, 3H, CH_3), 3.78 (dq, J=J_{PH}=7.0Hz, 1H, $POCH_2$), 4.87 and 4.95 (AB system, J_{AB}=12.0Hz, 2H, CH_2OCO), 5.27 (dd, J=10.0Hz, J_{PH}=16.0Hz, 1H, NCHP), 6.0-8.5 (m, 16H, 3xC_6H_5, NH); Anal.: calcd. for $C_{23}H_{24}NO_4P$: C, 67.47; H, 5.91; N, 3.42; found: C, 67.14; H, 5.94; N, 3.11.

***Ester* 5b.** Yield 92%, as a mixture of enantiomeric pairs of 4:6 molar ratio (P-n.m.r. - δ 37.4 and 37.1 in DMSO); m.p. 176° C; IR (KBr): 3215 (NH), 1710 (CO), 1545 (dNH), 1250 (PO), 1025 (POC); ^{1}H($CDCl_3$, 60MHz): 4.4-5.5 (m, 6H, CH_2OCO, $POCH_2$, NCHP, NH), 7.0-8.0 (m, 20H, 4xC_6H_5); Anal.: calcd. for $C_{28}H_{26}NO_4P$: C, 67.47; H, 5.91; N, 3.42; found: C, 67.56; H, 6.09; N, 3.48.

ACKNOWLEDGEMENT

Paweł Kafarski thanks Centre Nationale de la Recherche Scientifique for a post-doctoral position. This work was supported in part by *Komitet Badań Naukowych*, grant no. 2.0569.91.01

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(*Received in UK* 8 *August* 1994; *revised* 12 *September* 1994; *accepted* 16 *September* 1994)