Tetrahedron Vol. 45, No. 23, pp. 7387 to 7396, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00
Pergamon Press plc

MIXED CARBOXYLIC - CARBONIC ANHYDRIDE METHOD IN PHOSPHONO PEPTIDE SYNTHESIS

Paweł KAFARSKI and Barbara LEJCZAK

Institute of Organic and Physical Chemistry, Technical University of Wrocław, 50-370 Wrocław, POLAND

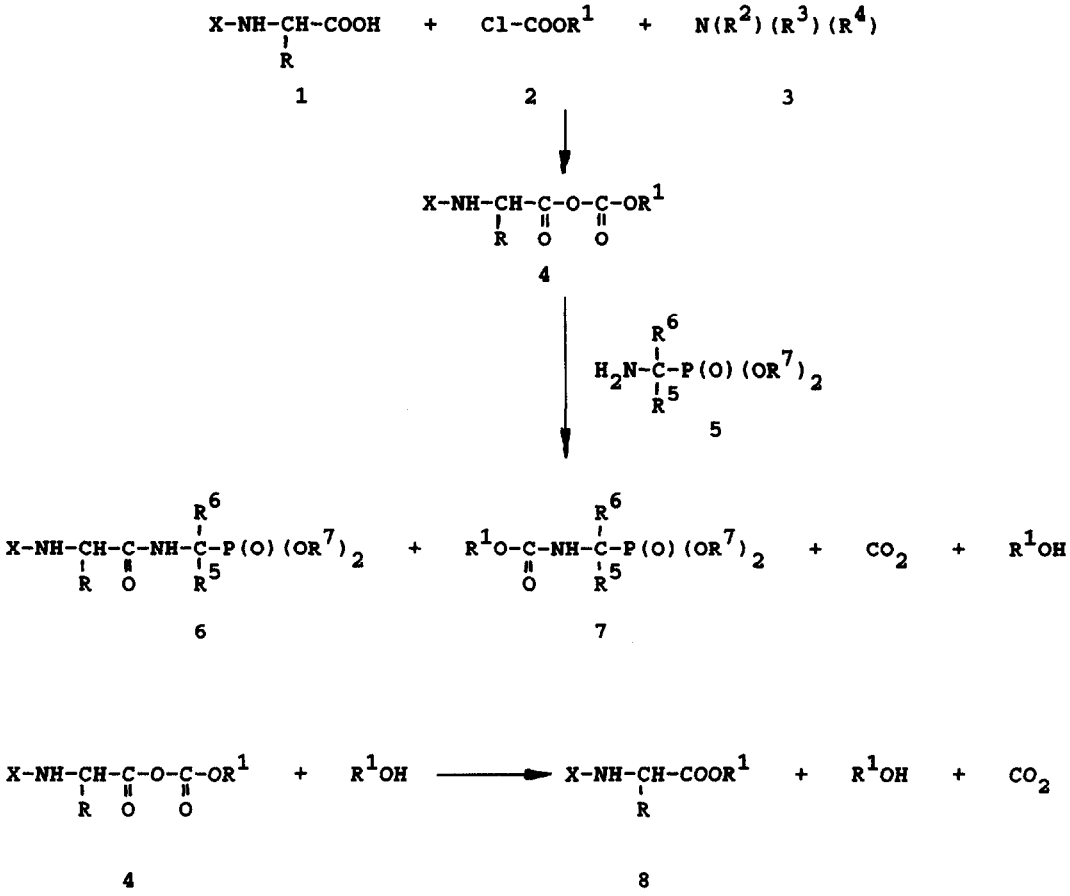
(*Received in UK* 4 *September* 1989)

Abstract - Factors influencing the reaction yields and formation of the side products during coupling of N-carbobenzoxyamino acids with dialkyl or diphenyl aminoalkylphosphonates, by means of mixed anhydride procedure, were evaluated. The recommended procedure, arising from these studies, involves the use of chloroform/ethyl chloroformate/triethylamine system and delicate warming of the reaction mixture during the coupling step.

Since simultaneous introduction in 1951 by Wieland and Bernhard [1], Boissonas [2] and Vaughan [3] mixed anhydrides have been widely used in peptide synthesis. This method appears to be also of choice in phosphono peptide synthesis as it gives the desired peptides with satisfactory yields and practically without racemization.[4] In the present work the influence of reaction conditions on the yields of coupling step, as well as on the purity of phosphono peptides formed from dialkyl or diphenyl esters of aminoalkylphosphonic acids was studied in some detail.

The mixed anhydride method of coupling, if used in phosphono peptide synthesis, involves the reaction of protected amino acid 1 with an alkyl chloroformate 2 in the presence of tertiary amine 3. The formed anhydride 4 is then reacted with an amine nucleophile 5, which is diethyl [5] or diphenyl

aminoalkylphosphonate,[6] yielding the protected peptide 6. Three side-reactions may accompany the coupling reaction. An additional acylation product, urethane 7, may be formed either by aminolysis of the carbonyl of carbonate moiety of the anhydride 4 or by aminolysis of unconsumed chloroformate 2. Alcohol liberated as a by-product may react with the activated acid 4 to give the corresponding ester 8.

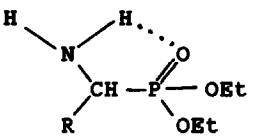


The amounts of undesired side-products can be controlled, at least to some extent, by proper selection of the mixed anhydride reagent, tertiary amine and solvent. In classical peptide chemistry conditions for minimizing the formation of side-products have been defined and protocols for

obtaining maximum coupling yields have been recommended.

Although the mixed anhydride procedure is commonly used in phosphono peptide synthesis [4] little is known about the influence of the reaction conditions on the reaction course and yields of the coupling step.

Diethyl aminoalkylphosphonates 5 (R^7= C_2H_5) undergo acylation less readily than the corresponding esters of amino acids. This was attributed by Gilmore and McBride [11] to the protection of amino group of the aminophosphonate by the formation of intramolecular hydrogen bonding with the phosphonate moiety:



The lower reactivity of diethyl aminoalkylphosphonates is probably the cause, why we have never observed the formation of the side product, urethane 7, during the coupling of N-protected amino acids with these esters by means of the mixed anhydride procedure.

The results of the determination of the influence of the solvent, tertiary amine and acylating reagent on the yield of coupling of N-carbobenzoxy-L-leucine 1 [X = $C_6H_5CH_2OCO$; R = $(CH_3)_2CHCH_2$] with diethyl [1-amino-2-(4-methoxyphenyl)]ethylphosphonate 5 (R^5= H; R^6= p-$CH_3OC_6H_4$; R^7= C_2H_5), the chosen model reaction, are presented in Table 1. If taking into account only chemical yields, the data in Table 1 clearly show that tetrahydrofuran seems to be the best solvent when using chloroformate in the presence of triethylamine. However, the solvent effect does not seem to be very critical in this reaction, while the convenience of the work-up procedure favours the use of chloroform. Couplings carried out in dichloromethane showed that there is no difference if ethyl or isobutyl chloroformates were used, while the use of N-hydroxybenzotriazole, a recently recommended additive [12] gave, surprisingly, lower yields of the reaction. Among five of the tertiary amines studied, only triethylamine and N-methylmorpholine gave satisfactory results. Triethylamine is not recommended for the classical procedures if carried out in halogenated hydrocarbons [13,14] because its use decreases the rate of formation of anhydride and thus increases the yield of urethane. We have carried out the formation of anhydride step for extended period of time (over 20 min.) and

have never observed the appearance of urethane. Thus, in phosphono peptide synthesis triethylamine is as good an agent as N-methylmorpholine, usually recommended for mixed anhydride procedure.

Table 1. Formation of diethyl [1-(N-benzyloxycarbonyl-L-leucylamino)-2-(4-metoxyphenyl)]ethylphosphonate in the reaction of N-carbobenzoxy-L-leucine and diethyl [1-amino-2-(4-methoxyphenyl)]ethylphosphonate at various reaction conditions.

Solvent	Chloroformate	Tertiary amine	Reaction yield [%]
DMF	ClCOOEt	Et_3N	78.5
THF	ClCOOEt	Et_3N	100
$CHCl_3$	ClCOOEt	Et_3N	90
$CHCl_3$	ClCOOEt	NMM [a]	90
$CHCl_3$	ClCOOEt	DMAP	37
$CHCl_3$	ClCOOEt	DBN	34
$CHCl_3$	ClCOOEt	DBU	52
CH_2Cl_2	ClCOOEt	Et_3N	78.5
CH_2Cl_2	$ClCOOBu^i$	Et_3N	71
CH_2Cl_2	ClCOOEt/HOBt	Et_3N	64
CH_2Cl_2	$ClCOOBu^i$/HOBt	Et_3N	64

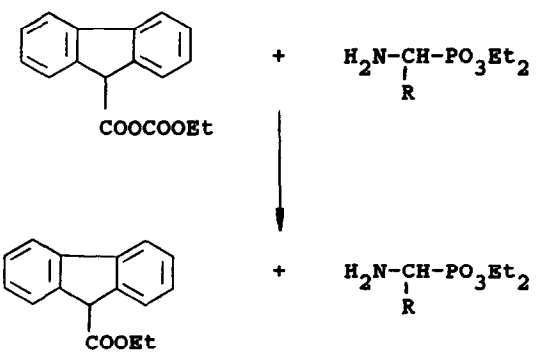
[a] Abbreviations: NMM, N-methylmorpholine; DMAP, 4-(dimethylamino)pyridine; DBN, diazabicyclo [4.3.0] nonane; DBU, diazabicyclo [5.4.0] undecane; HOBt, N-hydroxybenzotriazole

Also the results of acylation of diethyl (1-amino-1-methyl)ethylphosphonate 5 (R^5= R^6= CH_3; R^7= C_2H_5) with N-carbobenzoxy-L-leucine, presented in Table 2, indicate that the influence of solvent on the reaction yield does not seem to be of critical value. Obviously the acylation of this branched aminoester resulted in lower yields of reaction if compared with those observed when [1-amino-2-(4-methoxyphenyl)]ethylphosphonate was used as substrate. However, the careful warming of the reaction mixture (up to the boiling point) during the coupling step usually results in significant increase of the yield. As indicated in Table 3 in some cases the effect of warming on the coupling yield is quite dramatic.

Table 2. Yields of coupling of diethyl (1-amino-1-methyl)ethylphosphonate with N-carbobenzoxy-L-leucine in various solvents.

Solvent	Chloroformate/amine	Yield [%]	$[\alpha]_{20}^{578}$ (c 1,methanol) [°]
CH_2Cl_2	$ClCOOEt/Et_3N$	40	-31
$CHCl_3$		50	-28
THF		53	-29
DMF		40	-28

However, the elevation of the temperature increases the possibility of the reaction between mixed anhydride 4 and ethanol liberated in this reaction. This is indeed the case if using bulky acylating agents. The extreme case concerns acylation of dialkylaminoalkylphosphonates with fluoren-9-ylcarboxylic acid. When the procedure involving warming of the mixed anhydride-amino ester solution was applied ethyl fluoren-9-ylcarboxylate was found to be exclusively the reaction product.



Since the long activation times (20 - 30 min) and heating of the reaction mixture were used, an additional source of the formation of alkyl esters of blocked amino acids may be the cyclization of the mixed anhydride 4 yielding 2-alkoxy-5(4H)-oxazolone 9 [13] accompanied by release of the alkoxy group as the alcohol.

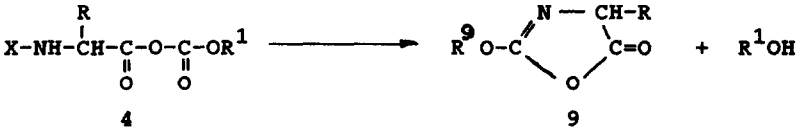
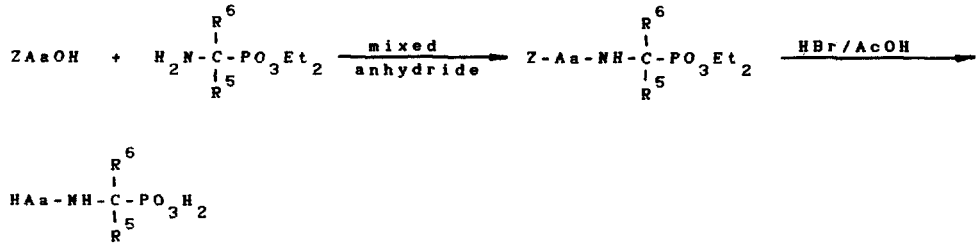


Table 3. **The influence of warming during the coupling step in phosphono peptide synthesis:**



Structure			Yield [%] Method	M.p., dec. [°C]	$[\alpha]_{20}^{578}$ (c 1, MeOH), [°C]
Aa	R^5	R^6			
Pro	H	CH_3	28/A 40.5/B	277-82	- 45
Gly	H	CH_3CH_2	8/A 66/B	269-72	-
Ala	H	CH_3CH_2	48/A 80/B	272-80	+ 18
Ala	CH_3	CH_3	38/A 81/B	238-40	+ 73
Val	CH_3	CH_3	26/A 71.5/B	236-38	+ 91
Leu	CH_3	CH_3	32/A 71.5/B	248-53	+ 82
Phe	CH_3	CH_3	21/A 33/B	246-49	+ 113
Val	CH_3	CH_3CH_2	48/A 80/B	236-39	+ 52
Val	CH_3	$(CH_3)_2CH$	31/A 79/B	213-19	+ 51

For the preparative procedure see Experimental; Method A, without heating during the coupling step; Method B, with heating.

This should give rise to the epimerization in the product B and would exert

8. Coupling of the anhydride 4, formed from N-carbobenzoxy-L-leucine after 5, 10, 15, 20 and 30 min. of activation, with diethyl [1-amino-2-(4-methoxyphenyl)]ethylphosphonate had not resulted in the changes of specific rotation of the formed phosphono peptide 6, indicating that the formation of cyclic product 9 is not significant in this case or that it did not react with diethyl aminophosphonate. This essential aspect of phosphono peptide synthesis requires, however, more detailed studies.

Coupling of diphenyl 1-aminoalkylphosphonates with N-blocked amino acids is an attractive alternative in phosphono peptide synthesis.[6] The course of the reaction between N-carbobenzoxy-L-leucine and diphenyl [amino(4-methoxy phenyl)]methylphosphonate 1 (R^5= H; R^6= p-$CH_3OC_6H_4$; R^7= C_6H_5) was examined in four solvents. The yields of coupling step and formation of the side-product, ethyl N-carbobenzoxy-L-leucinate, are presented in Table 4. It is seen from Table 4 that when DMF was used as the solvent the only product was ethyl N-carbobenzoxy-L-leucinate, while in methylene chloride, chloroform and tetrahydrofuran the desired peptide was predominantly formed. It is worth noting that diphenyl amino(4-methoxyphenyl)methylpho sphonate is less reactive because of steric hindrance. Thus, the formation of the side-product is not surprising.

Table 4. Effect of the solvent on yields of coupling and ethyl N-carbobenzo xy-L-leucinate formation in the syntheses starting from diphenyl [amino(4-methoxyphenyl)]methylphosphonate and N-carbobenzoxy-L-leu cine. Couplings were carried out using ethyl chloroformate/trie-thylamine system.

Solvent	Yields [%]	
	peptide 6	side-product 8
CH_2Cl_2	56	17
$CHCl_3$	65	28
THF	67	24
DMF	0	68

The studies presented in this paper indicate that the effects of the components of mixed-anhydride reaction influence the course and yields of coupling step in a manner somewhat different from the observed in classical peptide synthesis. The recommended procedure involves the use of

chloroform/ethyl chloroformate/triethylamine system and careful warming of the reaction mixture during the coupling step.

EXPERIMENTAL

DIETHYL [1-(N-BENZYLOXYCARBONYL-L-LEUCYLAMINO)-2-(4-METHOXYPHENYL)]ETHYL-PHOSPHONATE.

N-Benzyloxycarbonyl-L-leucine (2.65 g, 0.01 mole) was dissolved in dry solvent (50 ml) containing appropriate amine (0.01 mole) and cooled to -5°C. Ethyl chloroformate (1.0 ml, 0.011 mole) was then added and the mixture was kept at -5°C for 30 min. A solution of diethyl 1-amino-2-(4-methoxyphenyl)ethylphosphonate oxalate (3.77 g, 0.01 mole) in 30 ml of the same solvent containing corresponding amine (0.02 mole) was then added and the mixture allowed to stand overnight. The resulting solution was then evaporated to dryness (if THF or DMF used as solvents), dissolved in chloroform (or extracted directly if chloroform or methylene chloride were used) and washed succesively with: water (30 ml), 5% hydrochloric acid solution (2x30 ml), water (30 ml), saturated sodium bicarbonate solution (30 ml), water (30 ml) and brine (30 ml) and dried over anhydrous sodium sulfate. Solvent was then removed in vacuo yielding the desired product as a dense oil.

IR (film): ν = 3250(NH); 1700, 1645(CO); 1520(NH); 1210(PO); 1010(POC) cm^{-1}

^{1}H-NMR ($CDCl_3$,TMS): δ = 0.80 and 0.87 (d,J=6.0Hz,3H each,$CHCH_3$); 1.27 (t, J=7.0Hz, 6H,2xCH_2CH_3); 1.1-1.7 (m, 3H, CH_2CHCH_3); 2.75-3.3 (m, 2H, $PCHCH_2$); 3.68 and 3.72 (s, 1.5H each,OCH_3); 3.9-4.4 (m, J=J_{PH}=7.0Hz, 5H, 2x$POCH_2$, CHCO); 4.4-5.2 (m, 1H, CHP); 5.03 (bs, 2H, CH_2OCO); 5.95 (bt, J=J_{PH}=8.5Hz, 1H, NHCHP); 6.78 (d, J=8.0Hz, 2 aromatic protons); 7.16 and 7.18 (d, J=8.01Hz, 1H each, aromatic proton); 7.29 (s, 5H, C_6H_5); 7.75 and 8.08 (d, J=9.5 Hz, 0.5 H each,NHCHCO) ppm.

$[\alpha]_{20}^{578}$= -27° (c 1, in methanol)

Elemental analyses: calcd. for $C_{27}H_{39}N_2O_7P$ (534.6): 5.80% P and 5.24% N
found: 5.86% P and 5.09% N.

DIETHYL [1-(N-CARBOBENZOXY-L-LEUCYLAMINO)-1-METHYL]ETHYLPHPOSPHONATE.

The compound was synthesized according to the above given procedure. The product was obtained as an oil.

IR (film): ν = 3295(NH); 1720, 1680(CO); 1535(NH); 1210(PO). 1055, 1020(POC) cm^{-1}.

^{1}H-NMR ($CDCl_3$,TMS): δ = 0.90 (d, J=6.0Hz,6H,2x$CHCH_3$); 1.28 (t, J=7.5Hz, 6H, 2xCH_2CH_3); 1.4-1.75 (m, J_{PH}=16.0Hz, 2xCH_3CP, CH_3CHCH_2); 3.0-3.45 (m, 1H,

NCHCO); 4.11 and 4.13 (q-q, J=J_{PH}=7.5Hz, 2H each,OCH_2); 5.09 (s,2H, CH_2CO); 5.75 (d, J_{PH}=9.0Hz, 1H, NHCP); 6.56 (d, J=5.5Hz, 1H, NHCHCO); 7.33 5s, 5H, C_6H_5) ppm.

Elemental analyses calcd. for $C_{21}H_{35}N_2O_6P$ (442.3): 7.00% P and 6.33% N
found: 6.70% P and 5.99% N

COUPLING OF DIPHENYL [AMINO-(4-METHOXYPHENYL)]METHYLPHOSPHONATE WITH N-CARBOBENZOXY-L-LEUCINE.

The coupling step was carried out as described above yielding the mixture of the desired diphenyl [N-carbobenzoxy-L-leucylamino(4-methoxyphenyl)]methylphosphonate and ethyl N-carbobenzoxy-L-leucinate. The composition of the mixtures was studied by means of NMR and elemental analysis.

PHOPHONO PEPTIDES - THE RECOMMENDED PROCEDURE.

N-Carbobenzoxyamino acid (0.01 mole) was dissolved in 30 ml of dry chloroform and cooled to -5°C. Then triethylamine (1.5 ml, 0.01 mole) was added followed by addition of ethyl chloroformate (1.0 ml, 0.011 mole). The mixture was then kept at -5°C for 20-30 min and the solution of diethyl or diphenyl aminoalkylphosphonate (0.01 mole) in dry chloroform (30 ml) was added. The mixture was allowed to warm up to room temperature and then was slowly (15 min.) warmed up to boiling. After cooling 90 ml of chloroform was added and the solution washed succesively with: water (30 ml), 5% hydrochloric acid solution (2x30 ml), water (30 ml), saturated sodium bicarbonate solution (30 ml), water (30 ml) and brine (30 ml) and dried over anhydrous sodium sulfate. Then the solvent was removed under reduced pressure and the oily residue worked-up according to the following procedures:

METHOD A (if diethyl aminophosphonates were used as substrates)[5]: The oily product was dissolved in 30 ml of 45% hydrogen bromide in glacial acetic acid solution and kept at room temperature overnight. Then the volatile components of the reaction mixture were removed under reduced pressure, residue dissolved in water (30 ml) and extracted with diethyl ether (2x20 ml) in order to remove benzyl bromide. Water was removed in vacuo and the oily phosphono peptide hydrobromide dissolved in ethyl alcohol (50 ml). Addition of pyridine (up to pH about 6) or propylene oxide resulted in the slow precipitation of the desired product.

METHOD B (designed for deprotection of peptides prepared from diphenyl aminoalkylphosphonates)[6]: The oily N-protected phosphono peptide diphenyl ester was dissolved in 30 ml of methanol and 8.0 g of potassium fluoride monohydrate and 20 mg of 18-crown-6 were added. The mixture was then heated up to the boiling point and allowed to stand overnight. The solvent was

removed under reduced pressure and the residue suspended in ethyl acetate (50 ml) and extracted with water (40 ml) and brine (30 ml) and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded crude N-protected phosphono peptide dimethyl ester which was worked-up as in Method A.

ACKNOWLEDGEMENTS: This study was supported by CPBR 03.13 Programme.

REFERENCES

1. Wieland, T.; Bernhard, H. *Justus Liebigs Ann. Chem.* 1951, *572,* 190-194.
2. Boissonas, R. A. *Helv. Chim. Acta* 1951, *34,* 874-879.
3. Vaughan, J. R., Jr. *J. Am. Chem. Soc.* 1951, *73,* 3547.
4. Kafarski, P.; Lejczak, B.; Mastalerz, P. *Phopshonopeptides - synthesis and biological activity; Beitr. Wirkstofforsch.* Vol. 25; 1985.
5. Kafarski, P.; Lejczak, B.; Mastalerz, P.; Szewczyk, J.; Wasielewski, C. *Can. J. Chem.* 1982, *60,* 3081-3084.
6. Lejczak, B.; Kafarski, P.; Szewczyk, J. *Synthesis* 1982, 412-414.
7. Meienhofer, J. In: *The Peptides,* Vol.1., E.Gross & J. Meienhofer Eds., Academic Press, New York 1979, p. 263.
8. Anderson, G.W. In: *Peptides: chemistry and biochemistry,* B. Weinstein & S. Lande Eds., Marcel Dekker, New York, 1970, p. 255.
9. Wieland, T.; Fuesel, J.; Konz , W. *Justus Liebigs Ann. Chem.* 1969, *722,* 197-209.
10. Wieland, T.; Faulstisch, H.; Fahrenholz, F. *Justus Liebigs Ann. Chem.* 1971, *743,* 77-82.
11. Gilmore, W. F.; McBride, M. A. *J. Pharm. Sci.* 1972, 1087-1089.
12. Prasad, K. U.; Iqbal, M. A.; Urry, D. W. *Int. J. Pept. Prot. Res.* 1985, *25,*408-413.
13. Chen, F. M. F.; Bentoin, N. L; *Can. J. Chem.* 1987, *65,* 619-25.
14. Chen, F. M. F.; Lee, Y.; Steinhauser, R.;Bentoin, N. L. *Can. J. Chem.* 1987, *65,* 613-618.