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THE 2-(DIPHENYLPHOSPHINO) ETHYL GROUP (DPPE) AS A NEW CARBOXYL-PROTECTING GROUP IN PEPTIDE CHEMISTRY

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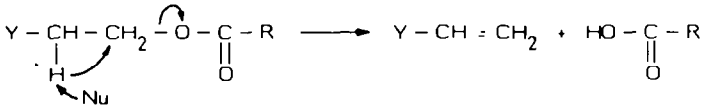
Abstract - The use of the 2-(diphenylphosphino)ethyl group for carboxyl-protection of amino acids or peptides is described. This group is easily introduced by esterification using 2-(diphenylphosphino)ethanol in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine. These Dppe esters are stable under the standard conditions for peptide synthesis. Deprotection is carried out under mild conditions by quaternisation using methyl iodide followed by a β-elimination induced by fluoride ion or potassium carbonate.

Key words - Peptide synthesis; carboxyl-protection; 2-(diphenylphosphino)ethyl group; esterification; β-elimination.

Numerous studies concerning peptide syntheses have focused on the development of protecting groups for use in an orthogonal protection scheme as defined by Merrifield[1]. Some of these protecting groups rely on a β-elimination reaction to bring about deprotection either under mildly basic conditions or by the action of a nucleophile :

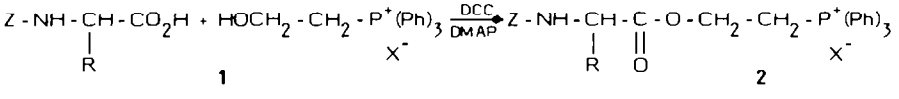


In the case of N-protection (R = NHR') such a reaction leads to an unstable carbamic acid which undergoes decarboxylation to give the free amine, whilst for carboxylprotection the free acid is obtained directly.

The Y group may be a sulfonium ion or a sulfone[2-10], a silicon derivative[11-13] or a phosphonium salt[14-16]. In the last case only protection of the amine function has been described by Kunz using 2-(phosphonio)ethyl groups.

The present work describes the use of the 2-(diphenylphosphino)ethyl group (Dppe) for carboxyl-protection. Deprotection is carried out by quaternisation of the phosphine using methyl iodide followed by a β-elimination induced by fluoride ion or potassium carbonate.

In preliminary experiments two N-protected amino acids were esterified with 2-hydroxyethyltriphenylphosphonium bromide, **1**, in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) :



2a : R=H; 2b : R=CH_3; X = Br^- or $(Ph)_4B^-$

However the resulting esters **2** were found to be extremely labile, with partial β-elimination being observed during recrystallisation or anion exchange. Complete β-elimination is obtained by treatment with 10 % potassium carbonate solution or a solution of potassium fluoride in acetonitrile.

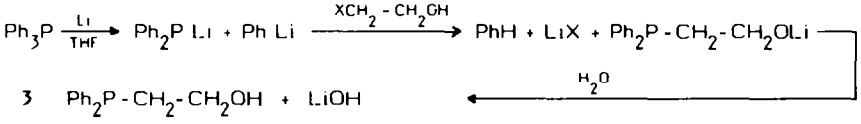
Thus it was decided to use a phosphine which is stable under the normal conditions for peptide syntheses and to induce the β-elimination by quaternisation at the end of the synthesis. Such a system also offers the advantage of being applicable to solid phase synthesis, which is not the case for a charged group.

2-(Diphenylphosphino)ethanol (DppeOH) **3** was chosen as a suitable reagent. Although this coumpound has been previously prepared[17-20] we developed a new simple method of synthesis. This reagent was selected because of the low sensitivity of alkyl diaryl phosphines to atmospheric oxygen.

Synthesis of DppeOH, 3

Among the possible routes to synthetise **3**, Horner and Mentrup[18] reported the electroreduction of benzyl(2-hydroxyethyl)diphenylphosphonium salt. Two additionnal preparations have been described[19-20] making use of diphenylphosphine, a toxic and oxygen sensitive compound.

We will now describe the synthesis of compound **3** in an improved single run procedure from commercially available triphenylphosphine :

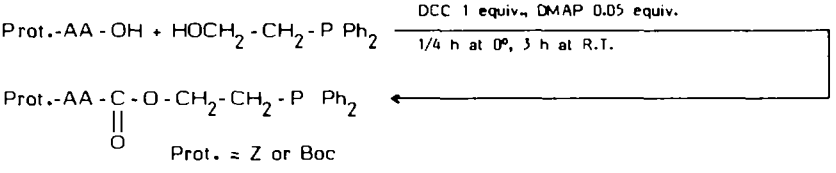


The phenyllithium arising from the cleavage of the triphenylphosphine molecule leads to a 2-halogenoethanol alkoxide (or an ethylene oxide) which prevents the phosphide reacting with the hydroxy group of the 2-halogenoethanol. However, this secondary reaction occurs partially, since the formation of about 10 % of diphenylphosphine is observed.

From 2-chloro or 2-bromoethanol, this method leads to overall yield of 60-65 %.

Synthesis of Dppe esters.

Although being a primary alcohol DppeOH is unusually unreactive since it is not possible to esterify N-protected amino acids with this reagent in presence of DCC. This agrees with the results of Turner and Soloway[19] who made the corresponding alkoxide react with acid chlorides in order to effect esterification. However, esterification of N-protected amino acids with DppeOH can be performed under very mild conditions by the action of DCC in the presence of catalytic quantities of DMAP[21-23] :



Compounds **4-10** (Table 1) are thus obtained as oils (except for Z-Gly-ODppe) which are relatively insensitive to atmospheric oxygen, oxidation being detected only after several days.

Table 1 - Dppe esters of N-protected amino acids and N-deprotection.

N°	Compound	$[\alpha]_D^{20}$*	% Yield of N-deprotection Méthod A	Method B	Method C
4	Z-Gly-ODppe	-	93	-	-
5	Boc-Gly-ODppe	-	90	75	90
6	Z-Ala-ODppe	- 7.1	90	-	-
7	Boc-Ala-ODppe	- 13	90	70	80
8	Z-Phe-ODppe	- 14.5	85	-	-
9	Boc-Val-ODppe	+ 91	80	70	85
10	Boc-Leu-ODppe	+ 43.5	85	75	80

*) concentration 2 in dichloromethane
Dppe = $(Ph)_2P\text{-}CH_2\text{-}CH_2$-

Method A : action of HBr/AcOH (24); Method B : BF_3/Et_2O (25);
Method C : CF_3CO_2H (26).

Peptide chain extension

N-deprotection of compounds **4-10** is carried out using standard methods (Table 1). The resulting compounds are then coupled with various N-protected amino acids in the presence of DCC and 1-hydroxybenzotriazole (HOBt) to produce compounds **11-23**; these dipeptide Dppe esters exist as non-cristallisable oils and are characterised by NMR : the presence of characteristic signals of the Dppe group (doublet of triplets at 4.2-4.3 ppm and triplet at 2.35-2.40 ppm) as well as those of the various amino acids.

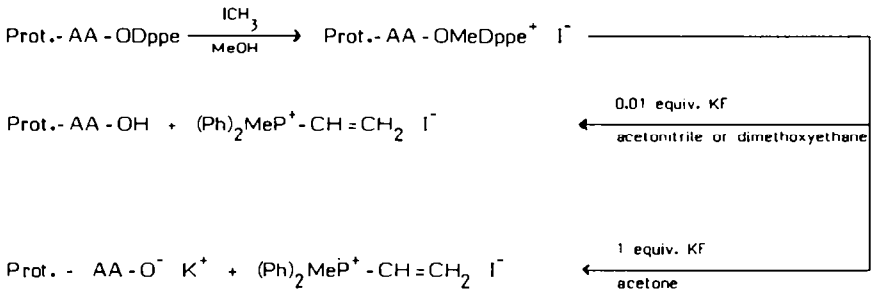
11 Z-Ala-Gly-ODppe
12 Boc-Ala-Gly-ODppe
13 Z-Phe-Gly-ODppe
14 Boc-Leu-Gly-ODppe
15 Z-Gly-Ala-ODppe
16 Z-Phe-Ala-ODppe
17 Boc-Leu-Ala-ODppe
18 Z-Gly-Phe-ODppe
19 Z-Ala-Phe-ODppe
20 Z-Gly-Val-ODppe
21 Boc-Leu-Val-ODppe
22 Z-Gly-Leu-ODppe
23 Boc-Ala-Leu-ODppe

Deprotection of the acid function.

The Dppe esters are quaternised using methyl iodide in methanol to give methyldiphenylphosphonioethyl esters in 90-100 % crude isolated yield. These can be obtained as solids but recristallysation results in considerable loss, probably due to a partial β-elimination.

Complete deprotection is effected by the action of fluoride ion; catalytic quantities of KF in acetonitrile or dimethoxyethane produce methyldiphenylvinylphosphonium iodide and the amino acid (or peptide); however, due to separation difficulties, it is found preferable to use one equivalent of KF, in acetone which leads to the insoluble potassium salt of the amino acid (or peptide), whilst the phosphonium salt remains in solution.

Deprotection is also possible by treating methyldiphenylphosphonioethyl ester in dimethoxyethane or acetonitrile with K_2CO_3 10 % in water.



Conservation of chirality.

DMAP has been used sometimes in solid phase peptide synthesis to fix the first amino acid into the resin[27-32]. The possibility of some racemization in the esterification of N-protected amino acids by DppeOH in presence of DCC/DMAP must be taken in account. Benoiton and al.[33] have already shown that DMAP favours the cyclisation of the amino acids to oxazolone leading to such a racemization. However the same authors[34] have shown that significant racemization may occur with this reagent especially when used in stoichiometric quantities.

However with catalytic amounts (less than 10 mole-percent) of DMAP, introduced after all the other reagents, esterifications occur with negligible racemization.

Thus it was decided to check for racemization in both the DppeOH esterifications and the deprotections by β-elimination. Specific optical rotations were first measured for the N-protected amino acids (or peptides) obtained by deprotection of the Dppe esters **6-23**; the observed values agree, within experimental error, with those reported in the literature.

Following literature reports[35-40], NMR is also used since it allows to distinguish generally between the two diastereoisomers of a dipeptide. The Dppe esters of dipeptides **11-23** are deprotected by β-elimination and reesterified by diazomethane; these methyl esters are studied by 200 Mz H^1 NMR following the Castro and al.[40] method; the methyl ester signals of the two enantiomers of racemic dipeptides are separated generally with more than 3Hz by using acetone as solvent. The methyl esters corresponding to compounds **11-23** do not exhibit the signal OCH_3 of the D,L isomer proving therefore that notable racemization does not occur during the esterification step by DppeOH and the following β-elimination reaction. The sensitivity of this method lies between 2 or 3 %.

Conclusion

Our results demonstrate the usefulness of the Dppe group as a protecting agent for the acidic function in peptide synthesis : easy introduction, stability towards N-deprotection and coupling reagents, elimination under mild and specific conditions.

Experimental

NMR spectra were recorded with a Varian T60 spectrometer. Optical rotations are measured using a Perkin Elmer 141 polarimeter. Commercially available N-protected amino acids are used directly. All reactions involving the Dppe group are carried out in the presence of air, although the products are stored under nitrogen.

2-Hydroxyethyl-triphenyl phosphonium bromide 1

Sodium hydroxyde (4 g) is added to a suspension of triphenylvinylphosphonium bromide (8 g; 25mmol) in water (150ml) and the mixture is stirred at room temperature for four hours. The resulting homogeneous solution is neutralised with hydrobromic acid and extracted with chloroform. The organic layer is dried over sodium sulfate and concentrated under vacuum. The residue is recristallised from ethanol-ether mixture to give 2-hydroxyethyl-triphenylphosphonium bromide (6.6 g; 85%) m.p. 212-213° (lit.[41] m.p. 212°).

2-(Triphenylphosphonio)ethyl esters 2

DCC (12 mmol) and DMAP (0.2 mmol) are added to a solution of 1 (4 g; 10 mmol), kept at 0° and of the N-protected amino acid (10 mmol) in dichloromethane (20ml). The mixture is stirred for three hours at 0° and left to stand for twelve hours at this temperature. After removal of DCU by repeated filtrations, the solvent is evaporated to give compounds 2 as oils. They are identified by their NMR spectra and by the corresponding tetraphenylborates obtained by anion exchange with sodium tetraphenylborate.

2a : Z - Gly - O - CH_2 - CH_2 - P^+ $(Ph)_3$ $(Ph)_4$ B^- m.p. 138-140°

Analysis : $C_{54}H_{49}BNO_4P$ C% Calc. : 79.36; Fd. : 79.38
H% Calc. : 6.00; Fd. : 6.10

2b : Z - Ala - O - CH_2 - CH_2 - P^+ $(Ph)_3$ $(Ph)_4$ B^- m.p. 160-162°

Analysis : $C_{55}H_{51}BNO_4P$ C% Calc. : 79.47; Fd. : 79.52
H% Calc. : 6.14; Fd. : 6.22

2-(Dyphenylphosphino)ethanol (DppeOH), 3

The reaction, before hydrolysis, is carried out in an atmosphere of dry oxygen-free nitrogen. Dry tetrahydrofuran (250 ml) is slowly added to a stirred mixture of triphenylphosphine (26.6 g; 0.1mole) and finely cut lithium wire (3.5 g; 0.5 g-atom) kept at - 10°; stirring is continued for 2 h at room temperature. In order to eliminate the excess of lithium metal, the solution is transfered to another flask under nitrogen. 0.1 mole of 2-chloroethanol (or 2-bromoethanol) is slowly added to the cooled solution (- 10°). The mixture is then allowed to reach room temperature and stirring is continued for a further 3 h. The solution is then hydrolysed, the organic layer separated and the aqueous layer extracted with tetrahydrofuran; the combined organic layers are dried and evaporated in vacuum. The residue is laid on a 10 cm silicagel column and rapidly eluted with 250 ml of chloroform-ethylacetate mixture (70-30). The concentrated oil is then chromatographied on a Prep LC/System 500 Waters with two silica-gel columns using chloroform-ethylacetate mixture (90-10) as eluent, affording, in order of increasing polarity, diphenylphosphine (10-12%) and (2-hydroxyethyl) diphenylphosphine (60-65%).

Diphenylphosphine : physical characteristics and spectral properties (^{31}P-NMR, H-NMR) were identical in all respects with those given in the literature[42-43].

(2-Hydroxyethyl)diphenylphosphine : ^{31}P NMR : at 32,37 MHz on a Bruker WP 80 with noise decoupling ($CDCl_3$, H_3PO_4 as external reference) : δ = 23.71 ppm.

Esterification of N-protected amino acids with DppeOH

A solution containing DppeOH (10mmol), the N-protected amino acid (10mmol) and DMAP (0.5mmol) in dichloromethane (30ml) is cooled to 0° and DCC (10mmol) added. The mixture is stirred for 15 mn at 0° then three hours at room temperature. The solution is cooled to 0° and repeatedly filtered until complete removal of DCU. The filtrate is washed three times with 10 ml of water, dried over sodium sulfate and concentrated under reduced pressure. The residual oil is rapidly run through a short (10 cm) column of silica using dichloromethane as eluent. Concentration of the dichloromethane solution gives the Dppe esters, **4-10**, in yields ranging from 85-95%.

They are oils, except for Boc-Gly-ODppe which is obtained as a solid (m.p. 64-65°; Anal. calc. for $C_{21}H_{26}O_4NP$: C 65.14, H 6.71. Found : C 65.12, H 6.86).

N-Deprotection of compounds 4-10

The N-deprotection of compounds 4-10 (methods A, B or C) leads to the corresponding aminoester salt. Then it is dissolved in dichloromethane, washed three times with water after adding 2 equivalents of N-methylmorpholine, dried over sodium sulfate and concentrated under reduced pressure. The Dppe aminoacid esters are thus obtained as viscous oils. Whose NMR spectra do not exhibit signals corresponding to Z or Boc groups.

Peptide chain extension

To a mixture of the preceding Dppe esters (10 mmol), N-protected amino acid (10 mmol) and HOBt (10 mmol) in dichloromethane (20 ml) maintained at 0°, are added 10 mmol of DCC. The mixture is stirred for 15 mn at 0° and left to stand 3 h at room temperature. After removal of DCU, the filtrate is concentrated under reduced pressure, the residual oil is diluted in ethyl acetate and left to stand 3 h at 0°. The solution after filtration is concentrated under reduced pressure. Dichloromethane is added to the residue and the solution is washed three times with water. The organic layer is dried over sodium sulfate and rapidly passed through a short (10 cm) silica column. The non crystallisable compounds **11-23** thus obtained are characterized by NMR spectroscopy.

Quaternisation of Dppe esters

Two equivalents of methyl iodide are added to the Dppe ester in 5 to 10 times its volume of anhydrous methanol and the mixture which is protected from the moisture stirred for 8 to 10 h at room temperature. The solvant is then removed under reduced pressure to give the methyl diphenylphosphonium iodide in quantitative yield as an oil which is difficult to crystallise. These compounds can be obtained as solids by treating the methanolic reaction mixture with a large volume of ether and stirring the solution continuously for several hours; under these conditions, the yield is about 50 %.

β-Elimination with KF in acetone

The methiodide of the Dppe ester is dissolved in 10 times its volume of anhydrous acetone and one equivalent of KF is added. The mixture is stirred for 5 or 6 hours at room temperature and the precipitated potassium salt of the amino acid (or peptide) filtered and washed several times with acetone. It is then dissolved in water and acidified to pH 1 with hydrochloric acid. The solution is extracted several times with dichloromethane and the extracts then dried over sodium sulfate and concentrated under reduced pressure. The residue is recrystallised according to conditions given in the literature. Yields range from 80 to 85 %.

β-Elimination with K_2CO_3 in acetonitrile

The methiodide of the Dppe ester is dissolved in 10 times its volume of acetonitrile and 1.5 equivalent of K_2CO_3 10 % water is added. The mixture is stirred for 30 mn at room temerature followed by solvent evaporation under reduced pressure. The residue is dissolved in water and extracted with dichloromethane to remove the hydroxyethyldiphenylmethylphosphonium iodide. The aqueous layer is acidified to pH 1 and treated as described above.

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