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A NEW REAGENT FOR THE MEDIATION OF AMIDE BOND FORMATION IN PEPTIDE SYNTHESIS.[1]

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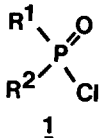
The potential application of 1-oxo-1-chlorophospholane (5) as a novel reagent for the in situ *activation of* N_{α} *-protected amino acids for use in peptide bond forming reactions has been examined. Wherever possible, 32.4MHz* ^{31}P *nuclear magnetic resonance (n.m.r.) spectroscopy*† *was employed to follow both the formation of the intermediate phospholanic-carboxylic mixed anhydride and the subsequent aminolysis reaction.*

We have recently reported[2] the results of our extensive investigations into the relative latent carbonyl activating power of a series of phosphinic chlorides (**1**) from which it was concluded that diphenylphosphinic chloride (**1e**) displays the optimum combination of ease of synthesis from readily available starting materials together with the properties of the corresponding phosphinic-carboxylic mixed anhydride required to fulfil the essential prerequisites[3] for use in peptide synthesis. Although this reagent has



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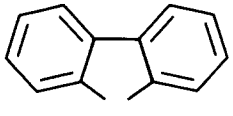
(**1a**) $R^1 = R^2 = PhCH_2$ (**1b**) $R^1 = R^2 = Et$ (**1c**) $R^1 = R^2 = Me$
(**1d**) $R^1 = R^2 =$ n-Bu (**1e**) $R^1 = R^2 = Ph$ (**1f**) $R,^1 R^2 =$
(**1g**) $R^1 = Me$, $R^2 = Ph$ (**1h**) $R^1 = R^2 =$ i-Pr
(**1i**) $R^1 = R^2 =$ i-Bu

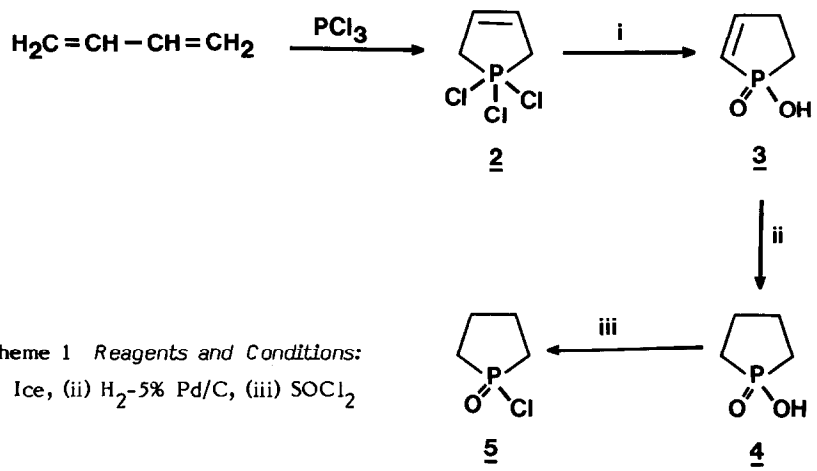


been successfully exploited by us in the construction of a series of synthetically challenging peptides,[3,4] our search continued for a phosphinic chloride with all the advantages of (**1e**) but whose corresponding acid - formed as a byproduct of the acylation reaction - would, ideally, be water soluble, thereby allowing ready removal from the final product mixture. In this latter respect the corresponding diphenylphosphinic acid (DppOH) has occasionally proved troublesome, particularly when the physical properties of the product preclude its removal by conventional means or require the use of convoluted and time-con-

†All ^{31}P n.m.r. spectra were recorded on a Bruker WP80 operating at 32.4MHz. Unless otherwise stated, shifts were measured in $CDCl_3$ relative to external 85% aqueous phosphoric acid assigned as zero.

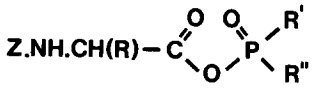
suming purification techniques. The preliminary results presented in this communication indicate 1-oxo-1-chlorophospholane (5) (which, within the widest limits of etymological license, has been given the alphabetism CptCl) to be an expedient choice for our purposes.

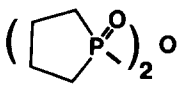
Synthesis of CptCl[5] **(Scheme 1):** Phosphorus trichloride (185mL, 2.1mol), 1,3-butadiene (116g, 2.15mol) and m-dinitrobenzene (2.0g) were placed in a Pyrex glass tube (900mL) and vigorously shaken at ambient temperature with PTFE chips. The desired product, 1,1,1-trichlorophospholene (2), began to form after 7 days and after 60 days the mixture was worked up by careful distillation of all the volatile components using cold traps at -78°C, to condense excess PCl_3, and at -198°C, to condense mainly excess 1,3-butadiene. Complete removal of all volatiles was ensured by immersing the tube in hot water (50-70°C) for approximately one hour to give a brown powder consisting of (2) and polymeric material at which point the reaction tube was closed, allowed to cool to room temperature and filled with dry nitrogen. CAUTIOUS hydrolysis of the contents of the tube, by addition of iced water, gave a red-orange coloured solution which was concentrated to 350-400mL by evaporation under reduced pressure. This concentrated aqueous solution was saturated with sodium chloride and subjected to continuous chloroform extraction which led to the isolation of a pale-yellow, waxy solid (72.8g). In general, yields of 1-oxo-1-hydroxyphospholene (3) obtained in this way from PCl_3 are of the order of 30% [δ_H ($CDCl_3$) 12.2(1H, s, acid OH), 6.9(1H, m), 2.7-2.6(2H,m) and 2.0-1.8(2H,m)]. **(N.B.** The ß,γ-unsaturated isomer was observed as a minor component). After hydrogenation of (3) (95.5g, 0.81mol) in water in the presence of 5% palladium-charcoal catalyst at room temperature and pressure over a period of 24 hours, 220MHz ^1H n.m.r. indicated the reduction to be complete [δ_H ($CDCl_3$) 12.2(1H,s), 2.0-1.6(8H, m); δ_P 78ppm]. Work up gave (4) as a pale-yellow solid (91.4g, 95%) which was dissolved, in small amounts over a period of 45 minutes at room temperature, in thionyl chloride (82.4mL, 1.14mol). The mixture was warmed slowly to 90°C when a vigorous evolution of gas was observed. After heating under nitrogen at 90°C for a further one hour the reactants were allowed to cool overnight and excess $SOCl_2$ was removed under reduced pressure and collected in an external trap to prevent air contacting the crude product. The remaining brown liquid was kept under an oil pump vacuum until a constant weight was recorded and then distilled to give the desired chloride (5) [b.pt. 76-77°C/0.05mm - lit:[5] 137-141°C/12mm (87.4g, 83%); δ_H ($CDCl_3$) 2.3-1.8(8H, m); δ_P +87ppm] as a clear, colourless liquid.[6]



Scheme 1 *Reagents and Conditions:* (i) Ice, (ii) H_2-5% Pd/C, (iii) $SOCl_2$

Application to Peptide Synthesis: 32.4MHz ^{31}P n.m.r. was employed to study the stability of the phosphinic-carboxylic mixed anhydrides [**6a:** δ_P ($CH_2Cl_2/CDCl_3$) +76ppm] derived from the reaction between CptCl with Z.ValOH and Z.GlyOH - in the presence of N-methylmorpholine (NMM) - with respect to thermal decomposition and disproportionation to the symmetrical anhydrides (**7**) and [**8**, δ_P($CH_2Cl_2/CDCl_3$) +79ppm] as well as the rate of the subsequent acylation reactions with various amino components. The results of this work implied that the anhydrides (**6a**) compare favourably with the recently described[2,3] diphenylphosphinic-derived mixed anhydrides (**6b**) and encouraged us to evaluate the potential of (**5**) further by synthesising a series of model peptides (Table 1). Consideration of the yields obtained by following the general procedure given previously[3] leads us to conclude that although anhydrides (**6a**) are less reactive than (**6b**) towards aminolysis they can lead to the formation of peptides in yields of the





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(a) R', R" = 

(b) R', R" = Ph

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8

Gly R = H; Val R = $CH(CH_3)_2$

order of 80-90% if employed in the following way, as exemplified by the synthesis of Z.Gly-GlyOMe (the original preparation of which - *via* the diphenylphosphinic-carboxylic mixed anhydride method[3] - was marred by the contaminating presence of DppOH, not readily removable by the usual routine workup procedures): Finely powdered $Cl^-H_2^+GlyOMe$[7] (1.1g, 8.7mmol) [or dissolved in DMF (10mL)] was added to a vigorously stirred solution of Z.GlyOH[8] (2.0g, 9.6mmol), NMM (1.06mL, 9.6mmol) and CptCl (1.3g, 9.6mmol) in dichloromethane (20mL) cooled to 0°C, followed by NMM (0.96mL, 8.7mmol). After 10 minutes a further 10-15% excess of preformed phospholanic-carboxylic mixed anhydride was added and 25 minutes later the product mixture was worked up by evaporation of the reaction solvent under reduced pressure to give a pale-yellow oil which was partitioned between ethyl acetate and water. The organic phase was isolated and washed with saturated $NaHCO_3$ (x3), 5% citric acid (x3), water (x5), and saturated NaCl (x2) before drying over anhydrous magnesium sulphate. Evaporation of the dry solution gave Z.Gly-GlyOMe as a white powder which was collected under petrol (40-60), filtered and dried (2.14g, 88%) m.pt. 67-68°C (lit:[9] 66.5- 67.5°C).

Conclusion: We have found the cyclic chloride (CptCl, **5**) to be an efficient promoter of amide bond formation through phospholanic-carboxylic mixed anhydrides that are remarkably stable at 0°C. In addition, it was discovered that the corresponding cyclic phosphinic acid was readily removed from the product acylation mixture to provide peptides of high quality in excellent yield requiring the minimum of purification. The results of the use of this reagent in solid phase synthesis, in solution synthesis, and in fragment coupling reactions will be reported in due course.

	Yield		M.pt.	δ_P	$[\alpha]_D^{25}$ (a)
Z.Gly↑GlyOMe	88%	-	67-68°C	-	-
DppLeu↑LeuOMe	85%	-	164°C	25.3[c]	-27.4°
DppIle↑ValOMe	63%	-	195°C	24.8[c]	-33.1°
DppAla↑GlyOMe[10]	89%	79% [b]	131°C	23.8	-40.4°
DppPhe↑Ala-GlyOMe	-	74% [b]	210°C	23.1	-66.5°
DppIle↑Phe-Ala-GlyOMe	-	72% [b]	250°C	23.8	-69.5°
DppLeu↑Ile-Phe-Ala-GlyOMe	82%	75% [b]	255°C (dec.)	22.0	-59.3°[d]

[a] c=1, methanol [b] By procedure given in reference 3 [c] MeOH/$CDCl_3$ [d] c=1, chloroform

Table 1: Use of phospholanic-carboxylic mixed anydrides in the stepwise synthesis of a series of model peptides (↑ indicates the point of coupling).

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