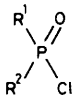
A Kinetic Study of Phosphinic Carboxylic Mixed Anhydrides [1]

Robert Ramage,* Butrus Atrash, David Hopton, and Maxwell J. Parrott
Department of Chemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD

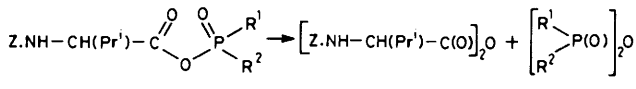
Using 32.4 MHz ^{31}P n.m.r. spectroscopy, disproportionation of a series of phosphinic carboxylic mixed anhydrides derived from protected α- amino acids has been studied both as a function of the substituents at phosphorus and structure of the α-amino acid† being activated. It was found that the rates of disproportionation were insignificant from a preparative aspect compared with aminolysis at 0 °C.

The need for the continual design, development and practical exploitation of novel reagents for use as improved and more efficient mediators of amide bond formation has recently been discussed.[2] Of increasing significance in this area has been the examination of anhydrides derived from organophosphorus acids.[3] The role of such reagents in carboxyl activation has been reviewed[4] and the ever-increasing number of reagents available are periodically collated.[5] Our successful preliminary results in this field of research, in which diphenylphosphinic carboxylic mixed anhydrides formed *in situ* from N_α-protected amino acids and diphenylphosphinic chloride (in the presence of *N*-methylmorpholine) were evaluated for use in peptide synthesis, prompted a kinetic study of the thermal stability and reactivity of a range of phosphinic carboxylic mixed anhydrides derived from a series of *P,P*-disubstituted phosphinic chlorides (**1a**—**h**).



(**1a**) $R^1 = R^2 = PhCH_2$ (**1b**) $R^1 = R^2 = Et$
(**1c**) $R^1 = R^2 = Me$ (**1d**) $R^1 = R^2 = Bu$
(**1e**) $R^1 = R^2 = Ph$ (**1f**) R^1, R^2 = 2,2′-Biphenylylene
(**1g**) $R^1 = R^2 = Bu^i$
(**1h**) $R^1, R^2 = -[CH_2]_4-$

Of particular importance was the development of mixed anhydrides considerably less prone to thermal disproportionation than corresponding carboxylic mixed anhydrides and, also, which would be subject to facile aminolysis in a totally regiospecific manner to afford the desired peptide bond. This combination of properties would allow the application of very reactive mixed anhydrides to solid-phase peptide synthesis, which normally operates at room temperature. Unfortunately, h.p.l.c. could not be used to follow the reactions since (*a*) the reaction could not be quenched, (*b*) low temperatures could not be used during a chromatographic run, and (c) this technique would require the use of aqueous conditions. The only satisfactory method found for following the reaction was ^{31}P nuclear magnetic resonance (n.m.r.) spectroscopy and the second order rate constants derived from the accumulated spectroscopic data at four temperatures were estimated by the method of linear least squares (see below).



2 **3** **4**

		δ_P	δ_P
(**a**)	$R^1 = R^2 = PhCH_2$	44 ± 1	43 ± 1
(**b**)	$R^1 = R^2 = Et$	60 ± 1	57 ± 1
(**c**)	$R^1 = R^2 = Me$	52 ± 1	50 ± 1
(**d**)	$R^1 = R^2 = Bu$	56 ± 1	53 ± 1
(**e**)	$R^1 = R^2 = Ph$	24 ± 1	23 ± 1
(**f**)	R^1, R^2 = 2,2′-Biphenylylene	37 ± 1	36 ± 1
(**g**)	$R^1 = R^2 = Bu^i$	55 ± 1	52 ± 1
(**h**)	$R^1, R^2 = -[CH_2]_4-$	77 ± 1	78 ± 1

Scheme 1. Thermal disproportionation of phosphinic carboxylic mixed anhydrides (**2**) derived from N_α-benzyloxycarbonylvaline

Assessment of the Thermal Disproportionation of Phosphinic Carboxylic Mixed Anhydrides (Scheme 1): General Experimental Procedure.—The phosphinic carboxylic mixed anhydrides (**2**) were prepared as follows. The phosphinic chloride (**1**) (5 mmol) in dry ethyl acetate (20 ml) was slowly added to a stirred mixture of the N_α-amino acid (5 mmol) and *N*-methylmorpholine (5 mmol) in anhydrous ethyl acetate (50 ml) at −20 °C and the mixture was stirred for a further 20 min. The cold solution was filtered, concentrated, and re-filtered (the recovery of *N*-methylmorpholine hydrochloride was virtually quantitative in all cases), and finally evaporated to dryness under reduced pressure; the residue was weighed and the yields found to be in the range 95—99%. All work-up was carried out in the cold as quickly as possible. The purity of the product was evaluated by ^{31}P n.m.r. (all shifts were recorded in ethyl acetate–$CDCl_3$ lock relative to 85% aqueous phosphoric acid) to ensure the absence of phosphinic chloride or the corresponding acid. A 0.2M-solution in ethyl acetate (25 ml) was prepared in the cold, 10% deuteriochloroform and 8% triphenylphosphine (internal standard) added and the resulting mixture placed in a thermostatted bath at 0, 30, 40, and 50 °C and timing was begun. Samples were periodically removed, placed in 5 mm n.m.r. tubes and cooled to −80 °C. The ^{31}P n.m.r. spectra were obtained at −40 °C giving a measure of peak heights and areas for the mixed anhydride, symmetrical anhydride, and Ph_3P. Care was taken to ensure that all samples were examined under identical conditions with low powers and as many data points as possible. Second order rate constants (k_2) were calculated graphically from the disappearance of the asymmetric anhydrides and the appearance of the symmetrical phosphinic

† With the exception of glycine, all α-amino acids are of the L-configuration and standard abbreviations are used throughout in the formulation of derivatives (IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, 1972, **247**, 977). In addition, the following, undefined abbreviations have been used: DCM, dichloromethane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulphoxide; DppCl, diphenylphosphinic chloride; Dpp, diphenylphosphinoyl; EtOAc, ethyl acetate; h.p.l.c., high pressure liquid chromatography; NMM, *N*-methylmorpholine; Phg, α-phenylglycine; Ph_3P, triphenylphosphine; Z, benzyloxycarbonyl.

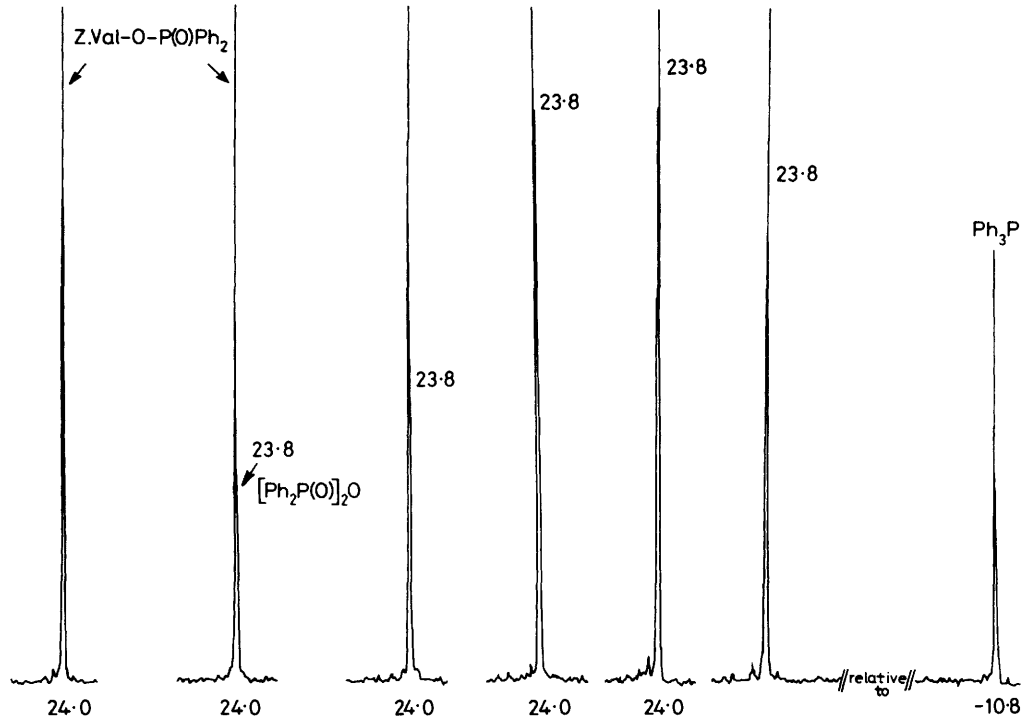


Figure 1. Selected ^{31}P n.m.r. spectra of the disproportionation of Z.Val–O–P(O)Ph_2 at 40 °C

anhydrides using integration values and peak heights normalised with respect to triphenylphosphine. In addition, a solution of each asymmetric anhydride was also mixed with 2-phenylethylamine and the ^{31}P n.m.r. spectrum was recorded. On no occasion was a peak corresponding to the phosphinic amide[6] observed.

Calculation of Kinetic Parameters from ^{31}P N.m.r. Data.— Consider a reaction of the type A + B → Products. If at any time c_A is the concentration of A and c_B is the concentration of B, then, assuming that the reaction is first order with respect to both A and B, the overall order is second and the rate law can be written as equation (1). In the present situation, the reaction is

$$\frac{-dc_A}{dt} = k_2 c_A c_B \quad (1)$$

of the type 2A → M + L, hence the rate law is given by equation (2). Separation of variables gives equation (3) which upon

$$\frac{-dc_A}{dt} = k_2 c_A^2 \quad (2)$$

$$\frac{-dc_A}{c_A^2} = k_2 dt \quad (3)$$

integration gives equation (4). At $t = 0$, $c_A = a$ and $C = 1/a$ and substituting these values into equation (4) gives equation (5). A

$$\frac{1}{c_A} = k_2 t + C \quad (4)$$

$$\frac{1}{c_A} = k_2 t + \frac{1}{a} \quad (5)$$

plot of the reciprocal concentration of A (mixed anhydride) against time should then give a straight line with slope k_2. Since two moles of mixed anhydride disproportionate to give one mole of symmetrical phosphinic anhydride and one mole of symmetrical carboxylic anhydride (Scheme 1), then if x is the symmetrical phosphinic anhydride concentration at time t, the concentration of asymmetrical anhydride would be $(a - 2x)$. Thus, a plot of $1/(a - 2x)$ versus time would give a straight line whose slope is k_2. The remaining problem to be solved is that of equating peak integrals and heights as measured from the spectra with the concentrations of the two anhydrides.

It was noted that the integrals and heights of the symmetrical anhydride peaks at 100% conversion were, within experimental error, the same as those of the asymmetrical anhydride at time zero. It was also observed that the line widths of the peaks were similar and it was therefore assumed that the relaxation times of both types of ^{31}P nuclei are similar. Extensive measurements were carried out on spectra representative of the phosphinic carboxylic mixed anhydrides in the presence of triphenylphosphine, with and without trace amounts of [Fe(acac)$_3$], and it was found that the conditions employed for the kinetic measurements were satisfactory. Thus the average sums of both integrals and heights of all spectra within a reaction system were used to calculate the values of a and hence x, c_A and $(a - 2x)$. Arrhenius parameters were also calculated *via* the usual ancillary equations described previously[6] and, as representative examples, the ^{31}P n.m.r. spectra of the disproportionation of Z.Val–O–P(O)Ph_2 at 40 °C (Figure 1), the corresponding graph of $1/c_A$ *versus* time (Figure 2a) and the Arrhenius plot (Figure 2b) are presented.

Results and Discussion

In order to evaluate the effect of the substituents R^1 and R^2 on phosphorus on the rate of disproportionation, it was decided to employ N_α-benzyloxycarbonylvaline (Z.ValOH) as the protected α-amino acid (Scheme 1) because it is sterically hindered, and therefore a case where disproportionation could

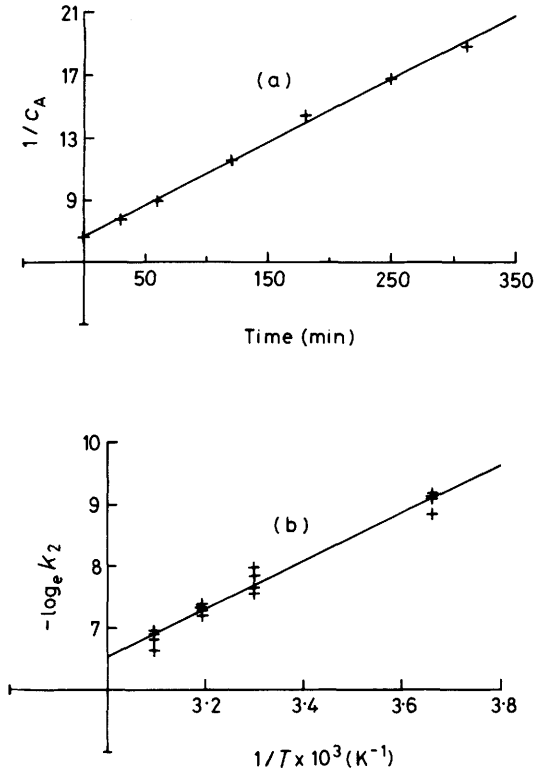


Figure 2.

provide serious competition to amide formation. The chemical shifts of the asymmetrical (**2**) and symmetrical anhydrides (**4**) were separated sufficiently to show two distinct peaks. In the case of the phenyl substituent (*i.e.* $R^1 = R^2 =$ Ph) the difference was smaller but reasonable resolution was obtained (Figure 1a). During the disproportionation of the mixed anhydrides with benzyl and biphenylylene substituents, the symmetrical phosphinic anhydrides precipitated from solution after approximately 50% reaction. At that stage, sampling was stopped. The mixed anhydrides with phenyl, benzyl, and biphenylylene substituents were prepared as white foams whilst the others were isolated as clear, colourless oils. In all cases some initial disproportionation was detected (*ca.* 5%) which must have occurred during work-up. Although this phenomenon, plus the slight inaccuracy in the weight of the mixed phosphinic carboxylic anhydride would be expected to contribute to some error in the values of the rate constants, the second order least mean square analysis produced lines of acceptable precision (Figure 1). The values of the rate constants presented in Table 1, imply that electron-withdrawing substituents on phosphorus, for example phenyl, benzyl, and biphenylylene accelerate the disproportionation reaction relative to alkyl substituents. From these were calculated the activation parameters displayed in Table 2.

The activation entropy for a second order reaction normally[7] has the value -34 J mol^{-1} K^{-1}. The large negative activation entropies indicate that the reaction proceeds *via* a highly ordered transition state. All the mixed anhydrides were regioselectively attacked by 2-phenylethylamine at the carboxyl centre. In no case was a signal in the ^{31}P n.m.r. for the phosphinic amide observed.[6] Regioselectivity of aminolysis is essential for anhydrides of this type to be of use in peptide bond formation. This observation, coupled with the rates determined for the disproportionation reactions, indicate—at this point—that the phosphinic chlorides bearing alkyl substituents exhibit greater potential for our purposes.

The effect of the solvent medium on the disproportionation reaction was investigated by studying the kinetics of the disproportionation of Z.Val–O–P(O)Ph_2 in dimethylformamide—see Tables 3 and 4. Comparison of the rate constants clearly demonstrates that the more polar solvent slows down the disproportionation reaction. A highly ordered transition state would be expected to be favoured by a non-polar solvent and this probably explains why the reaction is slower in dimethylformamide than in ethyl acetate. Dimethylformamide, therefore, is a preferable solvent to ethyl acetate for peptide synthesis utilising the mixed anhydride method for formation of the amide bonds. The increase of the activation entropy from -203 to -135 J mol^{-1} K^{-1} on changing from ethyl acetate to dimethylformamide further demonstrates that the transition state is more ordered in the former solvent.

The effect of varying the side-chain group on the α-amino acid component on the disproportionation reaction was investigated by studying the kinetics of the disproportionation of a series of diphenylphosphinic amino acid anhydrides. Values for the rate constants and activation parameters are shown in Tables 5 and 6. The values of the rate constants reveal that the bulky isopropyl side-chain group of valine had an accelerating effect on the rate of reaction. However, the remainder of the values show little difference in terms of steric factors. It is surprising that the mixed anhydride derived from α-phenylglycine disproportionated so fast and it should be noted that the symmetrical carboxylic anhydride started to separate out after approximately 50% reaction. This was the only symmetrical carboxylic anhydride to precipitate out of solution. The activation parameters again reveal a low activation energy and a large negative activation entropy. One of the objectives of the present study was to develop mixed anhydrides which would be sufficiently thermally stable for application to solid phase peptide synthesis. In order to assess the effect of phosphinic carboxylic mixed anhydrides in this respect Table 6 shows the time required for 10% disproportionation at 0 °C whilst keeping the organophosphorus moiety constant. From the data in Table 6 it can be seen that the mixed anhydride of Z.ValOH is relatively unstable. Comparison of these data with that found in Table 2 shows the remarkable stabilising effect of certain substituents on phosphorus. Thus by a combination of the substituent effects in Tables 2 and 6 we would suggest that the five-membered ring phosphinic carboxylic mixed anhydrides have adequate thermal stability for use in solid phase methodology.

Kinetics of Coupling Reactions: General Experimental Procedure.—It was considered necessary to determine the rates of coupling reactions of phosphinic carboxylic mixed anhydrides for comparison with those obtained for disproportionation as these are essentially competing reactions in peptide synthesis. Typically, experiments were carried out as follows. N_α-Benzyloxycarbonylvaline (0.2 g, 0.8 mmol) was stirred with *N*-methylmorpholine (0.08 μl, 0.8 mmol) in dry ethyl acetate (50 ml) at -20 °C for 5 min. A solution of diphenylphosphinic chloride[2] (0.19 g, 0.8 mmol) in dry ethyl acetate (20 ml) was added dropwise to the solution and stirring continued for 20 min. After filtration, the reaction solvent was removed under reduced pressure to leave a white foam (0.35 g, 0.77 mmol). The mixed anhydride thus isolated was dissolved in cold, dry ethyl acetate (10 ml) and Me_2SO (1 ml)–$(CD_3)_2SO$ (1 ml) containing Ph_3P (0.3 g) to give a concentration of 0.064 mmol ml^{-1}. Similarly, glycine benzyl ester toluene-*p*-sulphonate[12] (0.26 g, 0.77 mmol) was dissolved in Me_2SO (1 ml)–$(CD_3)_2SO$ (1 ml) to give a solution of concentration 0.064 mmol ml^{-1}.

The purity of the mixed anhydride was assessed by ^{31}P n.m.r.

Table 1. Rate constants (1 mol^{-1} s^{-1} × 10^5) for the disproportionation of Z.valine phosphinic acid mixed anhydride.

Amino acid	Substituent R^1, R^2	0 °C	30 °C	40 °C	50 °C
(2a) Z.ValOH	$PhCH_2$	20.4	41.9	163.0	277.0
(2b) Z.ValOH	Et	1.7	4.6	10.8	22.8
(2c) Z.ValOH	Me	1.4	7.4	15.2	21.2
(2d) Z.ValOH	Bu	0.7	5.6	17.0	20.1
(2e) Z.ValOH	Ph	11.7	43.0	67.0	109.0
(2f) Z.ValOH	2,2′-Biphenylylene	7.7	96.7	128.2	240.3
(2g) Z.ValOH	Bu^i	2.3	6.4	—	17.1
(2h) Z.ValOH	$-[CH_2]_4-$	0.3	1.3	2.4	3.6

Table 2. Energy (kJ mol^{-1}), entropy (J^{-1} mol^{-1} K^{-1}), free energy (kJ mol^{-1}) of activation and time (min) for 10% reaction at 0 °C for a 1M-solution, for the disproportionation of Z.valine phosphinic acid anhydrides.

Amino acid	Substituent R^1, R^2	E	ΔS	ΔG	Time for 10% reaction at 0 °C
(2a) Z.ValOH	$PhCH_2$	38 ± 2	−176 ± 6	91 ± 8	9 ± 1
(2b) Z.ValOH	Et	36 ± 3	−205 ± 10	97 ± 13	130 ± 18
(2c) Z.ValOH	Me	41 ± 2	−188 ± 4	97 ± 5	138 ± 7
(2d) Z.ValOH	Bu	51 ± 3	−156 ± 10	97 ± 12	240 ± 31
(2e) Z.ValOH	Ph	32 ± 1	−203 ± 5	93 ± 6	17 ± 1
(2f) Z.ValOH	2,2′-Biphenylylene	51 ± 2	−137 ± 8	92 ± 10	23 ± 2
(2g) Z.ValOH	Bu^i	30 ± 2	−232 ± 7	97 ± 10	85 ± 9
(2h) Z.ValOH	$-[CH_2]_4-$	46 ± 7	−181 ± 24	100 ± 29	400 ± 10

Table 3. Rate constants (1 mol^{-1} s^{-1} × 10^5) for the disproportionation of Z.Val–O–P(O)Ph_2 in (a) EtOAc–$CDCl_3$ and (b) DMF–$CDCl_3$ (9:1)

Reaction	0 °C	30 °C	40 °C	50 °C
(a)	11.7	43.0	67.2	108.8
(b)	—	19.0	40.4	70.3

Table 4. Energy (kJ mol^{-1}), entropy (J $mol^{-1}K^{-1}$), free energy (kJ mol^{-1}) of activation and time (min) for 10% reaction at 0 °C for a 1M-solution, for the disproportionation of Z.Val–O–P(O)Ph_2 in (a) EtOAc–$CDCl_3$ and (b) DMF–$CDCl_3$ (9:1).

Reaction	E	ΔS	ΔG	Time for 10% reaction
(a)	32 ± 1	−203 ± 5	93 ± 6	16 ± 1
(b)	55 ± 6	−135 ± 20	95 ± 25	109 ± 30

of the solution (400 μl) at −20 °C. The reaction was initiated by adding an equivalent volume (400 μl) of the cold (−20 °C) glycine benzyl ester toluene-*p*-sulphonate solution and stirring was started; equivalent amounts were reacted to facilitate the kinetic calculations. The progress of the reaction was monitored by following the disappearence of the mixed (asymmetric) anhydride, considering both peak integral values and peak heights normalised with respect to triphenylphosphine. Second order rate constants were determined in the same way as for the disproportionation rate constants. The values are displayed in Table 7.

The data for the reactions (*a*) and (*c*) clearly show that replacement of hydrogen on the side-chain of the α-amino acid component on the mixed anhydride by an isopropyl group slows down the reaction three-fold. Thus, the aminolysis reaction is sensitive to steric hindrance at the carbonyl of the asymmetric anhydride and the data from reactions (a) and (e) indicate that steric effects on the carbonyl component of the mixed anhydride are more important in determining the rate of reaction than in the free amine component. Comparison of reactions (a) and (b) indicates that a change of solvent, from ethyl acetate to dimethylformamide, halved the reaction rate,

Table 5. Rate constants (1 mol^{-1} s^{-1} × 10^5) for the disproportionation of diphenylphosphinic amino acid mixed anhydrides.

Amino acid	Substituent R^1, R^2	0 °C	30 °C	40 °C	50 °C
Z.GlyOH[8]	Ph	3.3	15.5	49.2	138.0
Z.AlaOH[9]	Ph	1.4	22.0	62.4	113.0
Z.ValOH[8]	Ph	11.7	43.0	67.3	108.8
Z.LeuOH[10]	Ph	0.5	7.4	18.1	40.0
Z.PheOH[2]	Ph	1.4	6.7	26.0	75.4
Z.PhgOH[11]	Ph	—	52.2	135.0	248.0

whereas the comparison of reactions (c) and (d) shows that the same change of solvent doubles the reaction rate. Reactions (e) and (f) have almost the same rate. Thus, a full understanding of these solvent effects is not possible due to the presence of the extremely polar dimethyl sulphoxide or, more importantly, to the fact that this reaction is very fast. It could therefore be said that both dimethylformamide and ethyl acetate have almost the same effect on the rate of reaction. However, dimethylformamide was shown to produce a retarding effect on the disproportionation reaction of mixed phosphinic carboxylic anhydrides. Overall, therefore, dimethylformamide appears to be the preferred solvent for stepwise peptide synthesis by this method, provided care is taken to avoid undesired side reactions of the phosphinic chlorides with dimethylformamide.

Kemp[13] has reported the rate constants for 41 aminolyses of N_α-protected amino acid *p*-nitrophenyl esters with α-amino acid ethyl or t-butyl esters in dimethylformamide at 30 °C. The results demonstrated that the steric effect on the coupling rate *via* the *p*-nitrophenyl active ester method is comparable to the phosphinic carboxylic mixed anhydride method. However, the actual values of the rate constants show that coupling *via* the mixed anhydride is many times faster considering that these values are for reactions carried out at 30 °C whilst our values were determined at −20 °C. It can also be concluded from the results of Kemp that the steric effect at the carbonyl of the *p*-nitrophenyl ester on the coupling rate is more important than that on the free amine component.

A comparison of the coupling rates with those of mixed

Table 6. Energy (kJ mol^{-1}), entropy (J^{-1} $mol^{-1}K^{-1}$), free energy (kJ mol^{-1}) of activation and time (min) for 10% reaction at 0 °C for a 1M-solution, for the disproportionation of diphenylphosphinic amino acid anhydrides.

Amino acid	Substituent R^1, R^2	E	ΔS	ΔG	Time for 10% reaction at 0 °C
Z.GlyOH[8]	Ph	52 ± 4	−139 ± 14	94 ± 17	74 ± 3
Z.AlaOH[9]	Ph	66 ± 2	−96 ± 81	95 ± 11	143 ± 17
Z.ValOH[6]	Ph	32 ± 1	−203 ± 5	93 ± 6	16 ± 1
Z.LeuOH[10]	Ph	64 ± 3	−112 ± 8	97 ± 11	390 ± 44
Z.PheOH[2]	Ph	56 ± 1	−134 ± 15	96 ± 18	170 ± 33
Z.PhgOH[11]	Ph	64 ± 5	−97 ± 15	93 ± 21	56 ± 13

Table 7. Rate constants (1 mol^{-1} s^{-1} × 10^3) for the coupling reaction Z.A–O–P(O)Ph_2 + H–B.OCH_2Ph ⟶ Z.A–B.OCH_2Ph + $Ph_2P(O)OH$ (at −20 °C)

Reaction	A	B	Solvent System*	k_2
(a)	Val	Gly	EtOAc–DMSO	9.3
(b)	Val	Gly	DMF–DMSO	4.8
(c)	Val	Val	EtOAc–DMSO	3.5
(d)	Val	Val	DMF–DMSO	7.4
(e)	Gly	Val	EtOAc–DMSO	23.4
(f)	Gly	Val	DMF–DMSO	20.2

* 5:1 by volume

anhydride disproportionation shows that all the disubstituted phosphinic reagents examined could be successfully employed for coupling *via* the mixed anhydride method with little effect of competing side reactions on the yield because, significantly, the coupling reactions take place many times faster than disproportionation. Ultimately, therefore, the choice of phosphinic chloride for routine use in the stepwise construction of peptides or peptide fragments lies in the consideration of other factors *viz*: (*a*) cost and availability of starting materials, (*b*) ease of synthesis, (*c*) stability, (*d*) ease of handling, (*e*) ready removal of the by-product of coupling reactions, and (*f*) yield and purity of products. Taking these into consideration, we would propose diphenylphosphinic chloride[2] (**1e**) and 1-chlorophospholane 1-oxide[14] (**1h**) as the optimum choices for use in the synthesis of peptides *via* the phosphinic carboxylic mixed anhydride method.

Practical Exploitation.—The data presented results from an investigation of the *P,P*-disubstituted phosphinic chloride-mediated activation of N_α-benzyloxycarbonyl amino acids at − 20 °C and in the earliest practical exploitations[15] of this work this was the reaction temperature employed. Since that time, we have demonstrated the exceedingly rapid formation of diphenylphosphinic carboxylic mixed anhydrides at 0 °C in the presence of *N*-methylmorpholine (dichloromethane was chosen as the reaction solvent to overcome solubility problems encountered in this work) together with an equally rapid rate of aminolysis to give a variety of peptides in good yield.[2] This experimentation has also been extended to an examination of 1-chlorophospholane 1-oxide[14] and will be the subject of future reports. From this work, it was concluded that peptides could be produced in acceptable yield if reactions were carried out with activation and acylation times of 3 min each (see the Experimental section). To test this hypothesis the sterically hindered[16] peptides DppLeu-LeuOMe and DppIle-ValOMe were initially synthesized in yields of 80% and 70% respectively. This would appear to indicate that longer acylation times (10—15 min) are required for more sterically hindered, activated α-amino acids—consistent with the assertion that aminolysis is sensitive to steric effects at the carbonyl of the mixed anhydride.

Conclusion.—It has been demonstrated that all the *P,P*-disubstituted phosphinic chlorides (**1a**—**h**) examined, exhibit potential in the field of amide formation *via* phosphinic carboxylic mixed anhydride intermediates, the rates of disproportionation being insignificant from a preparative viewpoint compared with desired aminolysis. On balance, diphenylphosphinic chloride and 1-chlorophospholane 1-oxide are considered to be the optimum choices for routine use in peptide synthesis.

Experimental

The general experimental methods and abbreviations used in this work are those previously reported.[17] Thin-layer chromatography was carried out on glass plates coated with silica gel 60GF-254 (Merck) in the solvent system chloroform–methanol (9:1). Visualisation of the compounds was achieved by u.v. absorption at 254 nm (u.v.) and exposure to iodine vapour (I).[18] Phosphorus-31 magnetic resonance spectra (^{31}P n.m.r.) were recorded on a Bruker WP80 operating at 32.4 MHz using solutions made up in the solvent(s) indicated. All chemical shift values δ(p.p.m.) were measured relative to external 85% aqueous phosphoric acid assigned as zero and are given as positive for low-field shifts. The preparation and characterisation of phosphinic chlorides (**1a**—**h**) have been described previously.[6,14]

2,2′-*Biphenylylenephosphinic Anhydride.* (**4f**).—This compound precipitated out of solution during the thermal disproportionation of N_α-benzyloxycarbonylvaline 2,2′-biphenylylenephosphinic mixed anhydride, as a crystalline solid which was filtered off, washed with cold EtOAc, and dried, m.p. 264 °C (Found: C, 69.6; H, 3.6; P, 14.8. $C_{24}H_{16}O_3P_2$ requires C, 69.5; H, 3.9; P, 14.6%); $\nu_{max.}$ 1 430 (Ar–P), 1 250 (P=O), and 970 cm^{-1} (P–O–P); $\delta_P(CDCl_3)$ 36.5.

N_α-*Benzyloxycarbonyl-α-phenylglycinic Anhydride.*—This compound precipitated out of solution during the thermal disproportionation of N_α-benzyloxycarbonyl-α-phenylglycine diphenylphosphinic mixed anhydride as a white crystalline solid which was filtered off, washed with cold ethyl acetate, and dried, m.p. 165 °C (Found: C, 69.8; H, 5.0; N, 5.0. $C_{32}H_{28}N_2O_7$ requires C, 69.5; H, 5.1; N, 5.1%).

N_α-*Diphenylphosphinoyl-leucyl-leucine Methyl Ester*, DppLeu^1-Leu^{2}OMe.—N_α-Diphenylphosphinoyl leucine[17] (3.32 g, 10 mmol) was dissolved in DCM (50 ml) and cooled to 0 °C at which temperature NMM (1.1 ml, 10 mmol) and a solution of DppCl (2.36 g, 10 mmol) in DCM (20 ml) were added in quick succession. After an activation period of 3 min a pre-cooled solution of leucine methyl ester hydrochloride[19] (1.64 g, 9 mmol) in dry, distilled DMF (8 ml) was added immediately followed by NMM (1.0 ml, 9 mmol) and the

resulting mixture stirred for a further 5 min before evaporation to dryness under reduced pressure. The resulting oil was partitioned between EtOAc and water (100 ml), and the isolated organic phase was washed with saturated $NaHCO_3$ (4 × 40 ml), 5% citric acid (4 × 40 ml), water (2 × 30 ml), and saturated NaCl solution (2 × 30 ml) before drying ($MgSO_4$). Removal of solvent from the organic solution gave pure N_α-*diphenylphosphinoyl-leucyl-leucine methyl ester* as a white powder which was collected under anhydrous diethyl ether, filtered and dried (3.3 g, 80%), m.p. 164—166 °C (Found: C, 65.5; H, 7.7; N, 6.1; P, 6.7. $C_{25}H_{35}N_2O_4P$ requires C, 65.7; H, 7.8; N, 5.8; P, 6.8%); t.l.c.-R_F 0.68 (u.v., I); $[\alpha]_D^{25}$ − 27.4°(*c*, 1 in MeOH); $\nu_{max.}$ 3 240 (N–H), 1 740 (ester CO), 1 660 (amide C=O), 1 560 (CONH), 1 440 (Ar–P), and 1 195 cm^{-1} (PO); $\lambda_{max.}$ 252 infl. (832), 259 infl. (1 062), 264 (1 231), and 270 nm (917); $\delta_H(CDCl_3)$ 7.9—7.3 (11 H, m, ArH and Leu^2NH), 4.5 (1 H, m, $Leu^2\alpha$–CH), 3.8—3.6 (2 H, m, $Leu^1\alpha$-CH, Leu^1-NH) obscured by 3.7 (3 H, s, ester Me), 1.8—1.4 (6 H, m, Leu^1 and Leu^2 β-CH_2, γ-CH), and 0.9—0.7 (12 H, m, Leu^1 and Leu^2 δ-Me); $\delta_C(CDCl_3)$ 173.3 (Leu^2 CO), 173.2, 172.9 (d, $^3J_{P-C}$ 6.3 Hz, Leu^1 CO), 135.0—127.5 (m, Ar), 52.8, 51.6 (Leu,[1] Leu^2 α-C), 50.8 (ester Me), 43.9, 43.6 (d, $^3J_{P-C}$ 5.3 Hz, Leu^1 β-C), 40.5 (Leu^2 B-C), 24.4, 24.1 (Leu,[1] Leu^2 γ-C), 22.4, 22.3, 21.9, and 21.7 (Leu,[1] Leu^2 δ-C); δ_P(MeOH–$CDCl_3$) 25.2.

N_α-*Diphenylphosphinoylisoleucylvaline Methyl Ester* DppIle-ValOMe.—N_α-Diphenylphosphinoylisoleucine[17] (1.66 g, 5 mmol) was dissolved in DCM (20 ml) and cooled to 0 °C at which temperature NMM [4.58 ml of a solution of 3.0 ml in DCM (25 ml); 5 mmol] and a solution of DppCl (1.18 g, 5 mmol) in DCM (10 ml) were added in quick succession. After an activation period of 3 minutes a pre-cooled solution of valine methyl ester hydrochloride[20] (0.75 g, 4.5 mmol) in DMF (5 ml) was added, immediately followed by NMM (4.22 ml of above solution). The resulting mixture was stirred for a further 5 min before being worked up as described for DppLeu-LeuOMe to give pure N_α-*diphenylphosphinoylisoleucylvaline methyl ester* as a white powder which was collected under petroleum (b.p. 60—80 °C), filtered off and dried (1.4 g, 70%) m.p. 194 °C (Found: C, 64.5; H, 7.5; N, 6.2; P, 7.1. $C_{24}H_{33}N_2O_4P$ requires C, 64.8; H, 7.5; N, 6.3; P, 7.0%); $[\alpha]_D^{25}$ − 33.1° (*c*, 1 in MeOH); $\delta_H(CDCl_3)$ 7.9—7.3 (11 H, m, ArH and Val-NH), 4.41 (1 H, m, Val α-CH), 3.8 (1 H, m, Ile-NH, exchanges with D_2O), and 3.6 (1 H, m, Ile α-CH) both obscured by 3.7 (3 H, s, ester Me), 2.1 (1 H, m, Val β-CH), 1.9 (1 H, m) and 1.5 (1 H, m, Ile-γCH_2), 1.1 (1 H, m, Ile β-CH), and 0.9—0.7 (12 H, m, Ile γ-Me, δ-Me, Val γ-Me); $\delta_C(CDCl_3)$ 172.6, 172.4 (d, $^3J_{P-CO}$ 3.3 Hz, Ile CO), 171.8 (Val CO), 135.6—127.8 (m, Ar), 58.8 (Val α-C),[a] 57.5 (Ile α-C).[b] 51.5 (ester C), 38.8, 38.6 (d, $^3J_{PC}$ 4.1 Hz, Ile β-C), 30.3 (Val β-C), 24.5 (Ile γ-CH_2), 18.6, 17.9 (Val γ-C), 15.1 (Ile γ-Me), 11.2 (Ile δ-C); δ_P(MeOH–$CDCl_3$) 24.8.

Acknowledgements

We thank the S.E.R.C. for a financial award (D. H.) and the Department of Chemistry of the University of Manchester Institute of Science and Technology for the provision of a Research Studentship (B. A.). We are grateful to Dr. H. Coates of Albright and Wilson Ltd. for many helpful discussions and to the company for supplementary finance (B. A.) and supplies of dichlorophenylphosphine. We also thank The Royal Society for an equipment grant.

References

1 R. Ramage, B. Atrash, and M. J. Parrott, in ACS Symposium Series No. 171 (Phosphorus Chemistry), eds. L. D. Quin and J. Verkade, American Chemical Society, Washington D.C. 1981, 199; R. Ramage, C. P. Ashton, B. Atrash, D. Hopton, and M. J. Parrott, in 'Peptides 1982—Proc. 17th Europ. Pept. Sympm.', eds. K. Bláha and P. Malon, Walter de Gruyter, Berlin 1983, 157.
2 R. Ramage, D. Hopton, M. J. Parrott, R. S. Richardson, G. W. Kenner, and G. A. Moore, *J. Chem. Soc., Perkin Trans 1*, 1985, 461.
3 J. Cabre and A. L. Palomo, *Synthesis*, 1984, 413; M. Ueki, Y. Kobayashi, and S. Ikeda, in 'Peptide Chemistry 1982', ed. S. Sakakibara, Prot. Res. Foundation (Osaka) 1983, 25; M. Ueki and T. Inazu, *Chem. Lett.*, 1982, 45; J. I. G. Cadogan, I. Gosney, O. Randles, S. Yaslak, and R. P. Ambler, *J. Chem. Soc., Chem. Commun.*, 1982, 298; T. Mukaiyama, K. Kamewaka, and Y. Watanabe, *Chem. Lett.*, 1981, 1367.
4 R. Ramage in 'Organophosphorus Reagents in Organic Synthesis,' ed. J. I. G. Cadogan, Academic Press, New York 1979, 511.
5 'Amino Acids, Peptides and Proteins,' Specialist Periodical Reports, The Royal Society of Chemistry, London.
6 R. Ramage, B. Atrash, D. Hopton, and M. J. Parrott, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1217.
7 A. Frost and R. Pearson, 'Kinetics and Mechanism in Organic Chemistry,' Wiley, New York, 1953, 147.
8 J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids,' volume II, Wiley, New York, 1961, 891.
9 C. S. Pande, J. Rudick, and R. Walter, *J. Org. Chem.*, 1970, **35**, 1440.
10 E. Klieger, E. Schroder, and H. Gibian, *Justus Liebigs Ann. Chem.*, 1961, **640**, 157.
11 H. Wissman, B. Schoelkens, E. Lindner, and R. Geiger, *Hoppe-Seyler's Z. Physiol. Chem.*, 1974, **355**, 1083.
12 L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, 1957, **22**, 1515.
13 D. S. Kemp, S. L. H. Choong, and J. Pekaar, *J. Org. Chem.*, 1974, **39**, 3841.
14 R. Ramage, C. P. Ashton, D. Hopton, and M. J. Parrott, *Tetrahedron Lett.*, 1984.
15 A. G. Jackson, G. W. Kenner, G. A. Moore, R. Ramage, and W. D. Thorpe, *Tetrahedron Lett.*, 1976, 3627; I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage, B. Singh, and R. G. Tyson, *Tetrahedron*, 1979, **35**, 2779; I. J. Galpin, G. W. Kenner, R. Ramage, and W. D. Thorpe, *Tetrahedron*, 1981, **37**, 3037; I. J. Galpin, D. Hudson, A. G. Jackson, G. W. Kenner, and R. Ramage, *Tetrahedron*, 1980, **36**, 2255.
16 M. Bodanszky and J. C. Talle, *Int. J. Pept. Prot. Res.*, 1977, **10**, 380.
17 R. Ramage, D. Hopton, M. J. Parrott, G. W. Kenner, and G. A. Moore, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1357.
18 G. C. Barrett, *Nature (London)*, 1962, **194**, 1171.
19 E. Pietrzick, H. Kalbacher, and W. Voelter, *Justus Liebigs Ann. Chem.*, 1977, 609 and references therein.
20 R. A. Boissonnas, S. Gutman, R. L. Hugenin, P. A. Jacquenod, and E. Sandrin, *Helv. Chim. Acta*, 1958, **41**, 1867.

[a,b] Not unambiguously assigned and may be reversed.

Received 9th October 1984; *Paper* 4/1691