Phosphinamides: A New Class of Amino Protecting Groups in Peptide Synthesis[1]

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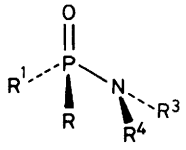
N_α-Diphenylphosphinyl protected α-amino acids have been prepared from the corresponding methyl or benzyl esters using diphenylphosphinic chloride–*N*-methylmorpholine followed by mild alkaline hydrolysis or catalytic hydrogenolysis, respectively. The suitability of these derivatives for use in amide bond forming reactions and their stability during the customary manipulations of peptide synthesis have been exhaustively examined. Acid-catalysed removal of the diphenylphosphinyl group has also been studied, with the aid of 32.4 MHz ^{31}P n.m.r. spectroscopy, and compatability of cleavage with tryptophan and methionine residues—in the absence of scavengers—has been demonstrated by the synthesis of the partially protected C-terminal tetrapeptide of gastrin, $Cl^-H_2{}^+$Trp-Met-Asp(OBu^t)-PheNH$_2$.‡

One of the most successful classes of amino protecting groups is that based on the t-butylurethane which may be cleaved by mild acid. Structure variation gives rise to groups susceptible to deprotection over a wide range of acid conditions. A disadvantage of this type of protection is the formation of relatively stable carbenium ions during the deprotection process which can react with the side-chain functionality of cysteine, methionine, tryptophan, or tyrosine leading to alkylated products.[2] Similarly, removal of the benzyloxycarbonyl[3] and related groups in the presence of methionine residues under the standard conditions employed in peptide synthesis has incurred serious difficulties.[2] For example, the methionine side chain can be demethylated with sodium in liquid ammonia resulting in the formation of homocysteine and attempts at acidolytic cleavage of the protecting group can result in *S*-benzylation; the corresponding sulphonium salt, instead of reverting to a methionine derivative is known to decompose to a *S*-benzylhomocysteine derivative. Furthermore, reports of the interference of methionine with catalytic hydrogenation because of the poisoning effect of the sulphur atom on the catalyst are legion and although extended reaction times can lead to the desired deprotection, some conversion of methionine into α-aminobutyric acid has been reported to occur.[2]

Despite the possible inclusion of additives to prevent poisoning of the catalyst or of scavengers, *e.g.* anisole or thiols, to remove carbenium ions, it was thought desirable to design another series of protecting groups which have the same propensity towards acid cleavage, but which occasion no deleterious side reactions during deprotection. In this respect, surprisingly few amine protecting groups have been developed utilising the well known acid lability of the phosphorus–nitrogen (P–N) bond. Zervas and co-workers[4] have investigated the application of phosphoramides to amino protection but the absence of any real advantages over benzyloxycarbonyl[3] protection, together with untoward nucleophilic displacement at phosphorus under certain circumstances, mean that this work has failed to receive widespread application. Such disadvantages can be circumvented by replacing phosphoric acid-based derivatives with the much more reactive derivatives of thiophosphinic acid[5–7] or phosphinic acid.[1,7]



(1)

a; $R^1 = R^2 = Ph$, $R^3 = R^4 = H$
b; $R^1 = R^2 = Ph$, $R^3 = R^4 = Me$
c; $R^1 = R^2 = Ph$, $R^3 = (CH_2)_2Ph$, $R^4 = Me$

Our approach to this problem has been to utilise the remarkable acid lability of the P–N bond of phosphinamides, *e.g. P,P*-diphenylphosphinamide (**1a**), which appears to be very much dependent on the nature of the substituents on phosphorus,[8] thereby providing scope for design of a range of acid-labile protecting groups[9,10] as in the urethane series. The mechanism of acidolysis of phosphinamides has been the subject of much debate.[11,12] *N*-Protonation, which appears to be the source of the lability of phosphorus amides, is followed by nucleophilic attack on the phosphorus *via* either a dissociative *A*1 mechanism or an associative *A*2 mechanism, depending upon the nature of the substrates. Work by Harger[11] has established that a bimolecular associative reaction operates in most cases. Harger showed that acidolysis of optically active *P,P*-methylphenylphosphinamide (**2**) in solution in methanolic hydrogen chloride occurs *via* a transient, trigonal bipyramidal intermediate (**3**) with inversion of configuration at phosphorus to give (**4**) (Scheme 1), producing no reactive intermediates capable of participating in undesired side reactions with the side chains of cysteine, methionine, tryptophan, or tryosine. This *A*2 cleavage mechanism is fundamentally different from the mechanism of acidolysis of carboxylic amides which is initiated by protonation of the carbonyl oxygen, but is consistent with conclusions drawn from examination of the available *X*-ray

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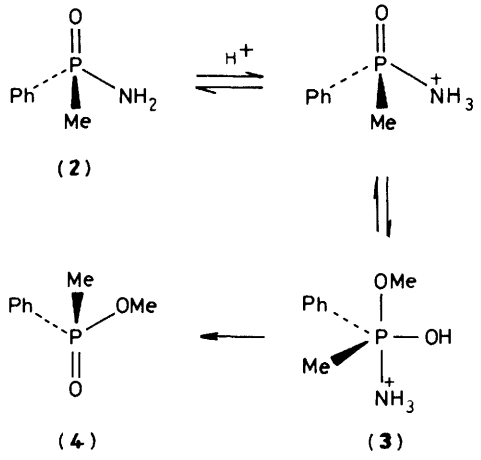
‡ With the exception of glycine, all α-amino acids are of the L-configuration unless otherwise stated and standard abbreviations are used throughout in the formulation of derivatives (IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, 1972, **247**, 977). In addition, the following undefined abbreviations have been used: Boc t-butyloxycarbonyl; Bpoc 2-biphenyl-4-ylisopropyloxycarbonyl; DCCI *N,N'*-dicyclohexylcarbodi-imide; DCHA *N,N*-dicyclohexylamine; DMF *N,N*-dimethylformamide; DppCl diphenylphosphinic chloride; Dpp diphenylphosphinyl; HONSu *N*-hydroxysuccinimide; NMM *N*-methylmorpholine; OBu^t t-butyl ester; OBzl benzyl ester; OEt ethyl ester; OTcp 2,4,5-trichlorophenyl ester; TMG tetramethylguanidine; Triton B benzyltrimethylammonium methoxide; *p*-TSA·H_2O toluene-p-sulphonic acid monohydrate; Z benzyloxycarbonyl.

data on the structures of *N,N*-dimethyl-*P,P*-diphenylphosphinamide (**1b**)[13a] and *N*-methyl-*N*-(2-phenylethyl)-*P,P*-diphenylphosphinamide (**1c**).[13b] In both compounds the geometry of the nitrogen is that of a flattened tetrahedron in which the lone pair of electrons on nitrogen is almost in the N–P–O plane whereas in the amide bond the lone pair on the trigonal nitrogen is orthogonal to the N–C–O plane. The diminished delocalisation of the lone pair of electrons on nitrogen with the P=O bond makes the N-atom of the phosphinic amides much more basic than that of the analogous carboxylic amides.



Scheme 1.

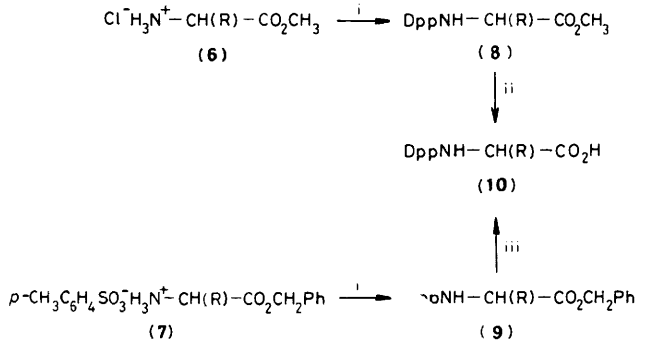
This stereoelectronic difference is reflected in the relative rates of acid hydrolysis of (**1a**) and benzamide ($PhCONH_2$) (**5**). Haake[8a] showed that although (**1a**) and (**5**) are hydrolysed at approximately the same rate in base, (**1a**) is hydrolysed some 10^5 times faster than (**5**) in acid. Bulky substituents on phosphorus lead to slow cleavage in acid. Whereas t-butyl substituted phosphinamides are often stable to acid, di-n-alkylphosphinamides are so labile that they may defy isolation.[8,9] This is best explained in terms of steric crowding in the intermediate (**3**). Due to the ready availability of diphenylphosphinic chloride (DppCl),[8b] N_α-diphenylphosphinylamino acid derivatives (**10**) were prepared for a critical evaluation of the suitability of this class of compounds as amino protecting groups in peptide synthesis.

Results and Discussion

Introduction of the Diphenylphosphinyl (Dpp) Group.—It was not possible to devise satisfactory conditions for the direct introduction of the Dpp group starting from the free α-amino acids. DppCl was found to hydrolyse extremely quickly[14] under aqueous conditions and attempted reaction with α-amino acids at controlled pH led only to diphenylphosphinic acid (DppOH, m.p. 193—195 °C).[15] Reaction in non-aqueous media (alanine 'Triton B' salt or alanine-TMG salt with DppCl) also gave diphenylphosphinic acid on aqueous work-up. These results are consistent with those of Swan[16] who had previously attempted the preparation of the n-butylamide of diphenylphosphinic acid by reaction between DppCl and butylamine in an aqueous medium, but, similarly, was only able to isolate hydrolysis by-products (DppOH, 75%).

Azides are widely used to introduce the important urethane protecting groups Boc[17] and Bpoc[18] and whilst Swan[16] had enjoyed limited success in the formation of *N*-butyl-*P,P*-diphenylphosphinamide (16%) by reaction of diphenylphosphinic azide,[19] $DppN_3$, and n-butylamine together under aqueous conditions [DppOH being the major product (64%) of the reaction], it was our experience that $DppN_3$ would not react successfully with α-amino acids. For example, reaction between alanine and $DppN_3$ in aqueous dioxane at pH 11 yields diphenylphosphinic acid as the only isolable product, a result entirely compatible with Swan's inability to extend his method to the derivatisation of immunoglobins under such conditions.

The reagent BocOTcp has been successfully employed[20] for the introduction of the Boc group and by analogy 2,4,5-trichlorophenyl-*P,P*-diphenylphosphinate, DppOTcp, was prepared. This reagent failed to react with α-amino acids either under the conditions specified by Morley[20] (water–t-butyl alcohol, solvent; triethylamine, base) or in non-aqueous media. In aqueous media slow hydrolysis of the ester predominated. In a similar vein, attempts to prepare the *N*-hydroxysuccinimide ester of diphenylphosphinic acid by condensation using DCCI were unsuccessful and evaluation of the corresponding *p*-nitrophenyl ester as a potential reagent for the introduction of the Dpp group had previously been carried out.[16] It was prepared in the conventional manner (diphenylphosphinic acid and *p*-nitrophenol using DCCI as the condensing agent) but would not react with α-amino acids.



Scheme 2. *Reagents and conditions*: i, DppCl–NMM 0 ii, OH^-–H^+; iii, H_2–10% Pd/C

Thus, since it was not feasible to prepare N_α-diphenylphosphinylamino acids directly, these compounds had to be prepared from the corresponding methyl (**6**) or benzyl (**7**) esters using DppCl/NMM, with dichloromethane as the solvent (Scheme 2). The method employed was to add the chloride slowly to a solution of the α-amino acid ester containing two equivalents of base. Although the reaction time was not critical 1.5—2 h was found to give good yields (65—85%) in the cases studied. Ueki and Ikeda have reported[5b] a quantitative yield of DppGlyOEt by reaction between ethyl glycinate and DppCl in the presence of triethylamine, but in none of our reactions could a yield of this order be claimed. The derivatives (**8**) and (**9**) could all be converted into N_α-diphenylphosphinylamino acids. Methyl esters were cleaved by mild alkaline hydrolysis in dioxane–water. Yields were generally 80—85% but DppGlyOH was isolated in only 59% yield, possibly owing to slight solubility of this compound in water. Benzyl esters were cleaved in the conventional manner by catalytic hydrogenolysis, generally in methanol. Yields of 78—91% were obtained except in the case of DppProOBzl, where slow hydrogenation (55% after 72 h) may be attributed to steric factors.* In addition, the Dpp group was found to be stable to the conditions of hydrazinolysis.

* In a separate project it has been observed that benzyl and *p*-nitrobenzyl esters of proline are difficult to cleave under normal conditions of catalytic hydrogenolysis. However, significantly improved yields could be obtained by carrying out the reaction in t-butyl alcohol at a pressure of 150 lb in ^{2} for 48 h.[21] This has not been attempted with DppProOBzl.

Table 1. N_α-Diphenylphosphinyl α-amino acid derivatives [(**8**), (**9**), (**10**)].

Compound	Crystn. solvent	Yield[a] (%)	M.p. (°C)	$[\alpha]_D^{25°}$[b]	T.l.c.[c]		Elemental analysis (%)*			
							C	H	N	P
DppGlyOBzl	EtOH–H_2O	79	117—118		0.67(A)	$C_{21}H_{20}NO_3P$ (365.37)	69.0	5.5	3.8	
							69.0	5.6	3.7	
DppIleOBzl[d]	EtOAc–chex †	75	106—108	−30.7	0.73(A)	$C_{25}H_{28}NO_3P$ (421.28)	71.2	6.7	3.3	7.3
							71.1	6.7	3.6	7.8
DppLeuOBzl	Et_2O–LP ‡	62	100—102	−27.8	0.74(A)	$C_{25}H_{28}NO_3P$ (421.28)	71.2	6.7	3.3	
					0.71(B)		71.0	7.0	3.3	
DppPheOBzl	EtOAc–LP ‡	63	158	−46.1	0.82(C)	$C_{28}H_{26}NO_3P$ (455.50)				
DppProOBzl	Et_2O–hexane	39	80—82	−43.3	0.76(A)	$C_{24}H_{24}NO_3P$ (405.44)	71.1	6.0	3.4	
					0.67(B)		71.2	6.2	3.4	
DppAlaOMe	EtOAc–chex †	76	114—115	−31.6	0.72(A)	$C_{16}H_{18}NO_3P$ (303.30)	63.4	6.0	4.6	10.2
							63.1	6.1	4.4	10.3
DppGlyOMe	Et_2O–chex †	74	110—112		0.60(A)	$C_{15}H_{16}NO_3P$ (289.27)	62.3	5.6	4.8	10.7
							62.3	5.8	5.1	10.7
DppLeuOMe	EtOAc–petroleum ‡	85	102	−31.5	0.66(A)	$C_{19}H_{24}NO_3P$ (345.37)	66.1	7.0	4.1	9.0
							66.1	7.0	4.0	8.9
$(Dpp)_2$LysOMe	EtOAc–Et_2O	71	152—153	−1.3	0.65(C)	$C_{31}H_{34}N_2O_4P$ (560.58)	66.4	6.1	5.0	
							66.2	6.1	4.9	
DppMetOMe	Et_2O	69	93—94	−35.8	0.74(A)	$C_{18}H_{22}NO_3PS$ (363.42)	59.5	6.1	3.8	8.5 (S: 8.8)
							59.8	6.0	3.6	8.7 (S: 8.4)
DppPheOMe	CH_2Cl_2–petroleum ‡	88	156—158	−49.7	0.89(A)	$C_{22}H_{22}NO_3P$ (379.40)	69.6	5.8	3.7	8.2
							69.3	5.8	3.7	7.9
DppTrpOMe	Et_2O	78	153—155	−48.9	0.51(A)	$C_{24}H_{23}N_2O_3P$ (418.44)	68.9	5.5	6.7	7.4
					0.73(D)		69.1	5.3	6.4	7.4
$(Dpp)_2$TyrOMe[e]	EtOAc–Et_2O	57	189—190	−25.2	0.83(A)	$C_{34}H_{31}NO_5P_2$ (595.58)	68.6	5.2	2.4	10.3
							68.5	5.1	2.4	10.3
DppValOMe	EtOAc–chex †	72	119—124	−32.7	0.70(A)	$C_{18}H_{22}NO_3P$ (331.36)	65.3	6.7	4.2	
							65.3	6.9	4.0	
DppAlaOH	EtOAc–chex †	79(S)	152—154	−21.4	0.38(E)	$C_{15}H_{16}NO_3P$ (289.27)	62.3	5.6	4.8	10.7
							62.5	5.5	4.8	10.7
DppGlyOH[f]	EtOAc	59(S)	133—134		0.60(C)	$C_{14}H_{14}NO_3P$ (275.25)	61.1	5.1	5.1	11.3
		87(H)			0.23(E)		61.1	4.8	4.9	11.3
DppIleOH	EtOAc–hexane	78(H)	92	−7.2	0.60(E)	$C_{18}H_{22}NO_3P$ (331.36)	65.3	6.7	4.2	9.4
							65.3	6.6	4.1	9.4
DppIleOH DCHA salt	MeOH–Et_2O	84	129—131	+5.0		$C_{30}H_{45}N_2O_3P$ (512.68)	70.4	9.0	5.5	6.1
							70.3	9.0	5.5	6.1
DppLeuOH	EtOAc–petroleum ‡	88(S)	135—136	−18.6	0.25(A)	$C_{18}H_{22}NO_3P$ (331.36)	65.2	6.7	4.2	9.4
		91(H)			0.65(C)		65.2	7.0	3.9	9.3
DppLys(Z)OH DCHA salt	EtOAc–Et_2O	80(S)	152—154	+13.3		$C_{38}H_{52}N_3O_5P$ (661.83)	69.0	7.9	6.4	
							68.7	8.2	6.4	
DppMetOH	EtOAc	80(S)	142—144	−13.9	0.62(E)	$C_{17}H_{20}NO_3PS$ (349.39)	58.4	5.8	4.0	8.9 (S: 9.2)
							58.4	5.6	3.9	8.9 (S: 9.2)
DppPheOH	EtOAc–LP ‡	87(S)	131—133	−40.1	0.60(C)	$C_{21}H_{20}NO_3P$ (365.37)	69.0	5.5	3.8	8.5
		79(H)			0.30(A)		69.2	5.2	3.9	8.4
DppProOH	MeOH–Et_2O	55(H)	170—173	−17.6	0.69(C)	$C_{17}H_{18}NO_3P$ (315.31)	64.8	5.7	4.4	9.8
					0.43(E)		65.1	5.7	4.5	9.8
DppTrpOH	Et_2O	90(S)	159—162	−60.5	0.42(E)	$C_{23}H_{21}N_2O_3P$ (404.41)	68.0	5.2	6.9	7.7
							68.0	5.3	6.9	7.7

Table 1. (*cont.*)

Compound	Crystn. solvent	Yield[a] (%)	M.p. (°C)	$[\alpha]_D^{25}$ [b]	T.l.c.[c]		Elemental analysis (%)* C	H	N	P
DppTyrOH[e]	MeOH–Et_2O	78(S)	218—220	+9.9		$C_{33}H_{44}N_2O_4P$ (562.70)	70.4	7.7	5.0	5.5
DCHA salt							70.1	7.7	4.9	5.7
DppValOH	Et_2O	82(S)	103—104	−15.2	0.65(C)	$C_{17}H_{20}NO_3P$ (317.33)	64.4	6.4	4.4	
							64.5	6.6	4.4	

* For elemental analyses, the data is presented as follows: calculated figures (upper), found (lower). † chex = cyclohexane. ‡ LP = petroleum (b.p. 40—60 °C); petroleum = petroleum (b.p. 60—80 °C). [a] (S) mild alkaline hydrolysis, (H) catalytic hydrogenolysis. [b] *c* 1 in methanol. [c] For t.l.c. systems and development techniques see general notes of the Experimental section. [d] Considerable difficulties encountered in the crystallisation of $Cl^- H_2{}^+$IleOMe and in the subsequent preparation of DppIleOMe forced us to abandon this approach in favour of the benzyl ester (carried out in part with C. P. Ashton). [e] Carried out in collaboration with S. J. Gurr and A. J. Hamilton. [f] Crystallisation from ethyl acetate–cyclohexane leads to the isolation of DppGlyOH·$\frac{1}{2}C_6H_{12}$ {m.p. 132 °C [Found: C, 64.6; H, 6.8; N, 4.7; P, 9.6. $C_{17}H_{20}NO_3P$ (317.25) requires C, 64.5; H, 6.4; N, 4.4; P, 9.6%]}, substantiated by the appearance in the ^{13}C n.m.r. spectrum of a single signal at δ_P +26.48 p.p.m.

Table 2.[a] Mass spectral[b] and ^1H n.m.r.[b] data of N_α-diphenylphosphinyl α-amino acid derivatives (**8**), (**9**), (**10**)

Compound	M^+ ion	220 MHz ^1H n.m.r. in $CDCl_3$: Aromatic H's[c]	NH[d]	α-CH	β-CH	γ-CH	δ-CH	$OC'H_2Ph$ or OCH_3	Coupling constants
DppGlyOBzl[e]		8.20—7.30 (15 H, m)	3.30 (1 H, m)	3.15 (2 H, dd)				5.25 (2 H, s)	$^3J_{P,\alpha CH}$ 11.0 Hz, $^3J_{NH,\alpha CH}$ 6.0 Hz
DppIleOBzl	421.1799	7.95—7.30 (15 H, m)	3.90 (1 H, m)	3.90 (1 H, m)	1.80 (1 H, m)	1.15 (2 H, m) 0.90 (3 H, d)	0.78 (3 H, tr)	5.20 (1 H, d) 5.08 (1 H, d)	$^2J_{C'H,C'H}$ 12.0 Hz $^3J_{\beta CH,\gamma CH}$ 8.0 Hz, $^3J_{\gamma CH,\delta CH}$ 8.0 Hz
DppProOBzl[e]		8.10—7.10 (15 H, m)		4.0—4.40 (1 H, m)	2.60—1.80 (4 H, m)		3.50—3.05 (2 H, m)	5.03 (2 H, s)	
DppAlaOMe	303.1030	8.00—7.40 (10 H, m)	4.10—3.70 (2 H, m)		1.45 (3 H, d)			3.58 (3 H, s)	$^3J_{\alpha CH,\beta CH}$ 7.3 Hz
DppGlyOMe	289.0868	7.80—7.05 (10 H, m)	4.25 (1 H, m)	3.45 (2 H, dd)				3.30 (3 H, s)	$^3J_{P,\alpha CH}$ 11.0 Hz, $^3J_{NH,\alpha CH}$ 6.0 Hz
DppLeuOMe	345.1494	7.80—7.20 (10 H, m)	3.35 (1 H, m)	3.60 (1 H, m)	1.40 (2 H, tr)	1.60 (1 H, m)	0.65 (3 H, d) 0.48 (3 H, d)	3.48 (3 H, s)	$^3J_{\alpha CH,\beta CH} = {}^3J_{\beta CH,\gamma CH}$ 7.6 Hz, $^3J_{\gamma CH,\delta CH}$ 6.0 Hz
DppMetOMe	363.1073	7.80—7.20 (10 H, m)	3.40 (1 H, m)	3.75 (1 H, m)	1.80 (2 H, m)	2.35 (2 H, tr)	1.83 (3 H, s)	3.50 (3 H, s)	
DppPheOMe	379.1331	7.90—7.15 (15 H, m)	3.79 (1 H, dd)	4.06 (1 H, q, t)	3.10 (2 H, d)			3.60 (3 H, s)	$^3J_{P,\alpha CH}$ 11.0 Hz, $^3J_{NH,\alpha CH}$ 8.0 Hz $^3J_{\alpha\text{-}CH,\beta CH}$ 6.0 Hz
DppTrpOMe[f]	418.1444	8.20—7.55 (13 H, m), Dpp and Trp 4-H, 7-H, and 2-H	6.25 (1 H, m)	4.15 (1 H, m)	3.50 (2 H, m)	11.30 (1 H, s, indole NH), 7.45 (1 H, tr, Trp 6-H), 7.30 (1 H, tr, Trp 5-H)		3.70 (3 H, s)	$^3J_{4,5} = {}^3J_{5,6} = {}^3J_{6,7}$ 8.5 Hz
$(Dpp)_2$TyrOMe[f]	595.1680	8.00—7.30 (20 H, m)	3.60 (1 H, m)	4.05 (1 H, m)	3.00 (2 H, d)	7.25 (2 H, d, Tyr 2-H, 6-H), 7.05 (2 H, d, Tyr 3-H, 5-H)		3.55 (3 H, s)	$^3J_{o,m}$ 8.5 Hz, $^3J_{\alpha CH,\beta CH}$ 8.0 Hz
DppValOMe	331.1361	8.05—7.35 (10 H, m)	3.60 (1 H, m)	4.20 (1 H, m)	2.05 (1 H, m)	0.95 (6 H, d)		3.70 (3 H, s)	
	$M^+ - 45$							CO_2H[d]	
DppAlaOH		7.90—7.20 (10 H, m)	4.25—3.70 (2 H, m)		1.43 (3 H, d)				$^3J_{\alpha CH,\beta CH}$ 7.3 Hz

Table 2.[a] (*contd.*)

Compound	$M^+ - 45$ ion	220 MHz ^{1}H n.m.r. in $CDCl_3$							
		Aromatic H's[c]	NH[d]	α-CH	β-CH	γ-CH	δ-CH	CO_2H[d]	Coupling constants
DppGlyOH		7.90—7.20 (10 H, m)	3.95 (1 H, m)	3.65 (2 H, dd)				10.20 (1 H, s)	$^3J_{P,\alpha CH}$ 11.0 Hz, $^3J_{NH,\alpha CH}$ 6.0 Hz
DppIleOH	286.1361	8.00—7.30 (10 H, m)	4.10 (1 H, m)	3.70 (1 H, m)	1.80 (1 H, m)	1.50 (1 H, m) 1.15 (1 H, m)	0.75 (3 H, tr),	12.48 (1 H, s)	$^3J_{\beta CH,\gamma CH}$ 8.0 Hz, $^3J_{\gamma CH,\delta CH}$ 8.0 Hz
DppLeuOH	286.1397	8.00—7.40 (10 H, m)	3.85 (1 H, m)	3.60 (1 H, m)	1.60 (2 H, m)	0.90 (3 H, d) (1 H, m)	0.65 (3 H, d)	11.73 (1 H, s)	$^3J_{\gamma CH,\delta CH}$ 6.5 Hz
DppMetOH	304.0954	8.00—7.40 (10 H, m)	4.15 (1 H, m)	3.90 (1 H, m)	2.00 (2 H, m)	2.55 (2 H, tr)	1.90 (3 H, s)	12.60 (1 H, s)	$^3J_{\beta CH,\gamma CH}$ 8.3 Hz
DppPheOH	320.0022	7.90—7.10 (15 H, m)	4.10—3.80 (2 H, m)		3.15 (1 H, ABq)[g] 2.85 (1 H, ABq)[g]			12.63 (1 H, s)	$^2J_{\beta CH,\beta CH}$ 13 Hz, $^3J_{\alpha CH,\beta CH}$ 5.5 Hz ($\delta_{3.15}$), $^3J_{\alpha CH,\beta CH}$ 8.0 Hz ($\delta_{2.85}$)
DppProOH[h]	270.1048	8.05—7.30 (10 H, m)		4.00 (1 H, m)	2.60—1.0 (4 H, m)		3.30—3.05 (2 H, m)		
DppTrpOH[f,h]	359.1228	7.60—6.85 (13 H, m Dpp, 4-H, 7-H, and 2-H)	5.45 (1 H, m)	3.55 (1 H, m)	3.00 (2 H, m)	10.75 (1 H, s, indole NH), 6.80 (1 H, tr, Trp 6-H), 6.70 (1 H, tr, Trp 5-H)		12.50 (1 H, s)	$^3J_{4,5} = {}^3J_{5,6} = {}^3J_{6,7}$ 8.5 Hz
DppTyrOH·DCHA[f,h]		7.90—7.30 (10 H, m)	3.40 (1 H, m)	4.70 (1 H, m)	2.80 (2 H, m)	7.05 (2 H, d, Tyr 2-H, 6-H), 6.60 (2 H, d, Tyr 3-H, 5-H)			$^3J_{o,m}$ 8.5 Hz, 2.00—1.00 (DCHA CH·'s)
DppValOH		7.90—7.20 (10 H, m)	3.90—3.30 (2 H, m)		2.00 (1 H, m)	0.90 (6 H, dd)		11.60 (1 H, s)	

Multiplicity: d, doublet; dd, doublet of doublets; tr, triplet; q, quartet; ABq, AB quartet; s, singlet; m, multiplet. [a] Full characterisation was completed with the data presented in Table 3 and by taking i.r. (thin film; bromoform and hexachlorobutadiene) and u.v. (in spectroscopic grade ethanol) spectra which are not reported here. [b] For machine types and operating conditions see general notes of Experimental section. [c] 'Aromatic H's' represents the protons of the aromatic rings of phenylalanine, the benzyl ester and the diphenylphosphinyl protecting group, see note *f*. [d] Exchange with D_2O. [e] Spectrum recorded at 100 MHz (see note *b*). [f] Aromatic protons of tryptophan and tyrosine are numbered as in note *c* below Table 3. [g] See ref. 46. [h] Spectrum run in $(CD_3)_2SO$.

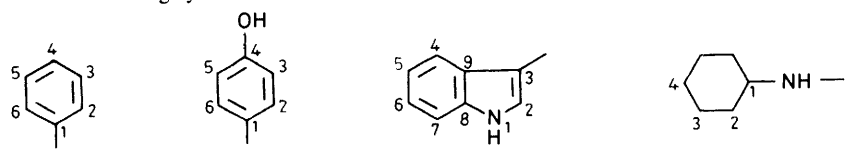
Table 3. ^{13}C N.m.r. spectra ($CDCl_3$) and ^{31}P n.m.r shifts (MeOH–$CDCl_3$) of selected N_α-diphenylphosphinyl N_α-amino acid derivatives (**8**), (**9**), (**10**)[a,b]

Compound	C=O	α-C	β-C	γ-C	δ-C	Others†	$^3J_{P,CO}$/Hz	$^3J_{P,\beta C}$/Hz	^{31}P
DppIleOBzl	172.9*	57.9	39.6*	24.8 (CH_2) 15.1 (CH_3)	11.3	136.6—127.6 (Aromatic) 66.7 (OCH_2Ph)	4.8	3.9	21.6
DppAlaOMe[b]	174.5*	49.1	21.6*			139.8—127.6 (Aromatic) 52.0 (OCH_3)	7.3	4.9	22.2
DppGlyOMe[b]	171.1*	41.2				134.8—127.8 (Aromatic) 51.6 (OCH_3)	8.5		25.3
DppLeuOMe[b]	174.5*	51.9	44.3*	24.2	22.3 21.9	135.8—127.3 (Aromatic) 51.9 (OCH_3)	4.8	4.8	23.1
DppMetOMe[b]	173.7*	51.8	33.1*	29.6	14.4	135.5—127.7 (Aromatic) 52.2 (OCH_3)	6.1	4.9	23.7
DppPheOMe[b]	173.0*	54.5	40.7*			136.0—128.1 (Aromatic, Phe C-2, C-3, C-5, C-6)[c] 137.2 (Phe C-1),[c] 126.7 Phe C-4),[c] 51.7 (OCH_3)	6.1	6.1	21.9
DppTrpOMe[b,d]	173.7*	54.7	30.0*			136.7—128.2 (Aromatic), 136.4 (Trp C-8),[c] 127.5 (Trp C-9),[c] 124.2 (Trp C-2),[c] 121.2 (Trp C-6),[c] 118.5 (Trp C-5),[c] 118.3 (Trp C-4),[c] 111.6 (Trp C-7),[c] 109.7 (Trp C-3),[c] 51.6 (O-CH_3)	3.7	7.3	24.6[d]
$(Dpp)_2$TyrOMe[b]	172.9*	54.5	40.1*			149.9* (Tyr C-4,[c] $^2J_{P,C\text{-}4}$ 8.6 Hz), 135.1—127.4 (Aromatic, Tyr C-1, C-2, C-6[c]), 120.5* (Tyr C-3, C-5,[c] $^3J_{P,C\text{-}3/C\text{-}5}$ 4.9 Hz), 51.9 (OCH_3)	6.1	4.9	29.2 (O–P) 21.9 (N–P)
DppValOMe[e]	173.8*	58.7	32.5*	18.6 17.8		135.9—128.1 (Aromatic), 51.7 (OCH_3)	4.9	4.9	21.9
DppAlaOH	175.5*	49.5	21.7*			134.2—127.5 (Aromatic)	6.4	4.3	24.3
DppGlyOH	172.5*	42.1				133.9—127.3 (Aromatic)	8.5		26.5[d]
DppIleOH	174.1*	58.0	39.3*	24.7 (CH_2) 15.1 (CH_3)	11.43	134.3—127.0 (Aromatic)	3.9	5.2	22.4
DppLeuOH	174.9*	52.0	44.0*	23.9	22.1 21.6	134.3—127.6 (Aromatic)	4.0	7.3	24.3
DppMetOH	174.3*	53.0	34.1*	29.7	15.2	134.0—127.0 (Aromatic)	3.7	6.2	22.2
DppPheOH	174.1	55.6	40.6*			134.8—128.9 (Aromatic, Phe C-3, C-5, C-2, C-6),[c] 137.1 (Phe C-1),[c] 126.8 (Phe C-4)[c]		8.5	22.8
DppProOH[d]	175.0*	59.6[e]	31.6[f]	25.3[f]	47.1[e]	135.9—128.2 (Aromatic)	4.9		27.9[d]
DppTrpOH[d]	174.5*	54.9	30.1*			136.2—127.7 (Aromatic, Trp C-9),[c] 136.4 (Trp C-8),[c] 124.3 (Trp C-2),[c] 121.1 (Trp C-6),[c] 118.6 (Trp C-5, C-4),[c] 111.7 (Trp C-7),[c] 110.2 (Trp C-3)[c]	3.6	4.9	25.1[d]
DppTyrOH[d] DCHA salt	173.5*	54.9	38.0			153.7 (Tyr C-4),[c] 134.6—126.1 (Aromatic, Tyr C-2, C-6, C-1),[c] 112.5 (Tyr C-3, C-5),[c] 50.4 (DCHA C-1),[c] 27.0 (DCHA C-2),[c] 22.8 (DCHA C-4),[c] 22.0 (DCHA C-3)[c]	6.1		23.2

* Doublet due to phosphorus coupling.

† 'Aromatic' represents the C atoms of the aromatic rings of the diphenylphosphinyl group and the benzyl ester and consists of a series of peaks which have not been individually assigned.

[a] For operating conditions see general notes of the Experimental section. [b] Where necessary, the distinction between the α-C signal and that of the methyl carbon of the ester was made with the aid of an off-resonance decoupled spectrum. [c] Numbering of carbon atoms in the ring systems is as follows:



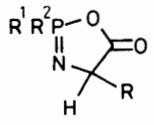
Phenylalanine Tyrosine Tryptophan[h] Dicyclohexylamine

[d] Run in $(CD_3)_2SO$. [e] *cis* and *trans* Isomers. [f] Two peaks probably due to *cis*/*trans* isomers but ^{31}P coupling may be present. [g] See Table 2, footnote *a*. [h] See R. E. London, *Org. Magn. Reson.*, 1981, **17**, 134.

The fully protected methyl (**8**) and benzyl (**9**) esters as well as the *N*-protected α-amino acids (**10**) prepared during the course of this work—together with the data used in their characterisation—are presented in Tables 1—3.

Racemisation.[22]—An important constraint in the development of amino protecting groups for use in peptide synthesis is the ability of the protective group to preserve the chirality of derivatives during condensation reactions. Such loss of chirality can occur by 2 mechanisms, (i) direct base abstraction of the α-CH hydrogen atom or (ii) *via* formation of an oxazol-5(4*H*)-one intermediate (**11**). From the stereoelectronic considerations mentioned above, the enolic form of the phosphorus analogue of an oxazolone (**12**) would not be expected to enjoy stabilisation through π-delocalisation and, therefore, with the appropriate choice of base, racemisation during activation of Dpp-amino acids was not expected to be a problem.

(**11**)



(**12**)

To confirm this, DppLeu-AlaOBzl was prepared using standard coupling techniques (Table 4) and the dipetide, after deblocking, was applied to a JEOL JLC-5AH amino acid analyser using the elution conditions specified by Manning and Moore.[23a] The following condensation methods were employed: pivalic mixed anhydride,[24] (ii) DCCI,[25] and (iii) DCCI–HONSu[26] and, by using *N*-methylmorpholine as base, less than 1% racemisation was found in each case. The reactions were carried out using DppLeuOH prepared from both the methyl ester and benzyl ester and a parallel series of experiments was carried out using ZLeuOH. The full results are set out in Table 4.

Although racemisation was observed in each case, the results for ZLeuOH and DppLeuOH are practically the same so it may be concluded that the Dpp protecting group behaves in the same way as in coupling reactions [which forms oxazol-5(4*H*)-one intermediates only under very forcing conditions[27]] and does not cause anything more than negligible degrees of racemisation.

An additional, related problem in this area is the epimerisation of Ile to allo-Ile. To confirm that no such conversion occurs with Dpp-protection, DppIle-GlyOBu^t was prepared (DCCI[25]) and the neutral product was shown to contain no allo-Ile by amino acid analysis[23b] (see general notes, Experimental section; Ile, 0.98, Gly, 1.00; DCCI[25] 90%; m.p. 92—94 °C).

Conditions for Cleavage.—As is well known, the rate of acid cleavage of phosphinamide groups depends critically upon the nature of the substituents on phosphorus. In order to gain some information concerning the rate and conditions of cleavage at room temperature of the diphenylphosphinyl group, N_α-diphenylphosphinyl-2-phenylethylamine, DppNHCH$_2$CH$_2$Ph[9,28] (**13**) was prepared by reaction between diphenylphosphinic chloride and 2-phenylethylamine in the presence of triethylamine, and acid cleavage of this compound studied.

95% Trifluoroacetic acid (TFA) gave complete cleavage in *ca.* 10 min, but the product was ninhydrin negative on thin-layer chromatography (t.l.c.) except when heated strongly. A similar result was obtained using TFA–dichloromethane (1 : 1), complete cleavage having occurred after 40 min. Standard conditions for removal of the very acid-labile Bpoc[18] protecting group were tried. 80% Aqueous acetic acid resulted in incomplete cleavage after 72 h whereas the slightly more acidic acetic acid–formic acid–water (7:1:2) mixture—which has been preferred to 80% aqueous acetic acid for Bpoc deprotection—gave complete cleavage after 24 h indicating that Dpp is slightly more acid labile than Boc.[17] In an attempt to confirm this finding, and at the same time demonstrate the compatability of Dpp protection with protecting groups derived from t-butyl alcohol, N_α-diphenylphosphinylglycylglycine t-butyl ester, DppGly-GlyOBu^t, was synthesised (m.p. 88—90 °C; DCCI[25] method) and found to undergo selective cleavage of the Dpp group in 15% TFA–$CDCl_3$ using n.m.r. as the probe. The n.m.r. spectrum of this compound shows a doublet ($^3J_{NH,H}$ 6 Hz) at δ_H 3.95 p.p.m. attributable to $CH_2CO_2Bu^t$ and a double doublet ($^3J_{P,H}$ 11 Hz, $^3J_{NH,H}$ 6 Hz)

Table 4.*

Amino component	Condensation method	% Racemisation
DppLeuOH	Pivalic mixed anhydride	1.0
ZLeuOH†	Pivalic mixed anhydride	1.0
DppLeuOH	DCCI	0.7
ZLeuOH	DCCI	0.8
DppLeuOH	DCCI–HONSu	0.7
ZLeuOH	DCCI–HONSu	0.7

* Coupling to TosO$^-$H$_2$N$^+$AlaOBzl (prepared following the general procedure in ref. 44), 1 mmol scale, in dichloromethane using *N*-methylmorpholine as base in each case. Elution conditions:[23a] short column pH 3.28; 50 ml h^{-1}.

† E. Klieger, E. Schroder, and H. Gibian, *Liebigs Ann. Chem.*, 1961, **640**, 157.

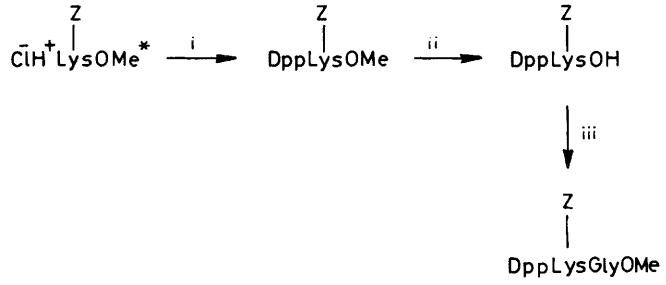
Table 5.

Starting material	Product	Conditions	Time taken for complete deprotection
DppNH(CH$_2$)$_2$Ph (**13**)	Cl$^-$H$_3$N$^+$(CH$_2$)$_2$Ph (**14**)	95% TFA	10 min
(**13**)	(**14**)	TFA–CH_2Cl_2 (1:1)	40 min
(**13**)	(**14**)	80% aqueous CH_3CO_2H	Incomplete cleavage after 72 h
(**13**)	(**14**)	CH_3CO_2H–HCO_2H–H_2O (7:1:2)	24 h
DppGly-GlyOBu^t	X$^-$H$_2$N$^+$Gly-GlyOBu^t	15% TFA–$CDCl_3$	2 h*
DppLys(Z)-GlyOMe	Cl$^-$H$_2$N$^+$Lys(Z)-GlyOMe	1M-HCl in dioxane–water (2:1)	3 h
(**13**)	(**14**)	HCl in methanol	See text

* As determined by n.m.r.

attributable to DppNHCH_2 at δ_H 3.70 p.p.m. The double doublet collapsed to a doublet ($^3J_{P,H}$ 11 Hz) with D_2O, indicating rapid exchange of the relatively acidic phosphinamide hydrogen. Cleavage of the Dpp group in 15% TFA in $CDCl_3$ was observed by following collapse of the doublet at δ_H 3.70 to a *singlet* at δ_H 3.86 p.p.m. The cleavage was 90% complete after 1 h and complete as judged by n.m.r. spectroscopy after 2 h.

In order to demonstrate the applicability of the diphenylphosphinyl protecting group for N_α-protection when side chain protecting groups are based on benzyl alcohol, the synthesis and selective deprotection of DppLys(Z)-GlyOMe (**15**) was undertaken (Scheme 3).



Scheme 3. *Reagents and conditions:* i, DppCl–NMM 0 °C; ii, OH^-–H^+, DCHA salt (80%, 152—154 °C); iii, pivaloyl chloride–$Cl^-H_2^+$GlyOMe†–NMM

* J. Coggins, R. Demayo, and N. L. Benoitin, *Can. J. Chem.*, 1970, **48**, 385.

† For example, K. H. Deimer, 'Houben-Weyl's Methoden der Organischen Chemie,' ed. E. Wunsch, G. Thieme, Stuttgart 1974, vol. 15, part 1, p. 325.

The protected dipeptide (**15**) was prepared in low yield (28%) using the pivalic mixed anhydride procedure[24] (m.p. 112—113 °C; amino acid analysis, Lys_1 0.98; Gly_1 1.02; t.l.c.-A R_F 0.54). 1M-HCl in dioxane–water (2:1) was found to bring about complete, selective cleavage of the diphenylphosphinyl group within 3 h. Simultaneous formation of diphenylphosphinic acid could pose a problem in such a deprotection in that its removal may complicate the process of crystallising the product. However, the real significance of this aspect of our work is that it demonstrates the potential use of this type of approach in solid-phase synthesis, where isolation of the peptide is not a problem.[29] These results are summarised in Table 5. 80% Trifluoroacetic acid, 0.4M-HCl in 90% trifluoroethanol (TFE), and toluene-*p*-sulphonic acid monohydrate (2 equiv.) in water–methanol all proved to be satisfactory deprotection conditions for peptide derivatives, the choice depending upon the ease of separation of the product from Ph_2PO_2H or Ph_2PO_2R, where R is derived from the alcoholic co-solvent. The results of the work carried out by Harger[11] together with this preliminary evaluation of the rate and conditions of cleavage of the Dpp group from (**13**) prompted a kinetic study[9,10] of substituent effects on the acidic solvolysis of the series, $R_2P(O)NHCH_2$-CH_2Ph—from which is taken Table 6.

This and subsequent extensive investigations[30] into the deprotection of a variety of phosphinamide peptide derivatives has led to the conclusion that a six-fold excess of methanolic HCl and a reaction temperature of 35—40 °C represent the optimum reaction conditions required to bring about rapid and efficient cleavage. Furthermore, in order to minimise exposure of the peptide to acid, the progress of all deprotections was initially followed using 32.4 MHz ^{31}P n.m.r. spectroscopy as a non-invasive monitor. Typically, 0.3 mmol of the protected peptide in methanol ($CDCl_3$ lock) showed a ^{31}P resonance* at δ_P +24 (±1) p.p.m. which, on treatment with six equivalents of hydrogen chloride in methanol, disappeared giving rise to a new signal at δ_P +35 (±1) p.p.m. representative of the by-product of deprotection, methyl diphenylphosphinate [DppOMe (lit.,[31] δ_P +32.2 p.p.m. in dichloromethane)]; reaction times were typically between 30 and 35 min with work-up leading to isolation of the deprotected peptide methyl ester hydrochlorides in yields of between 85 and 98%.

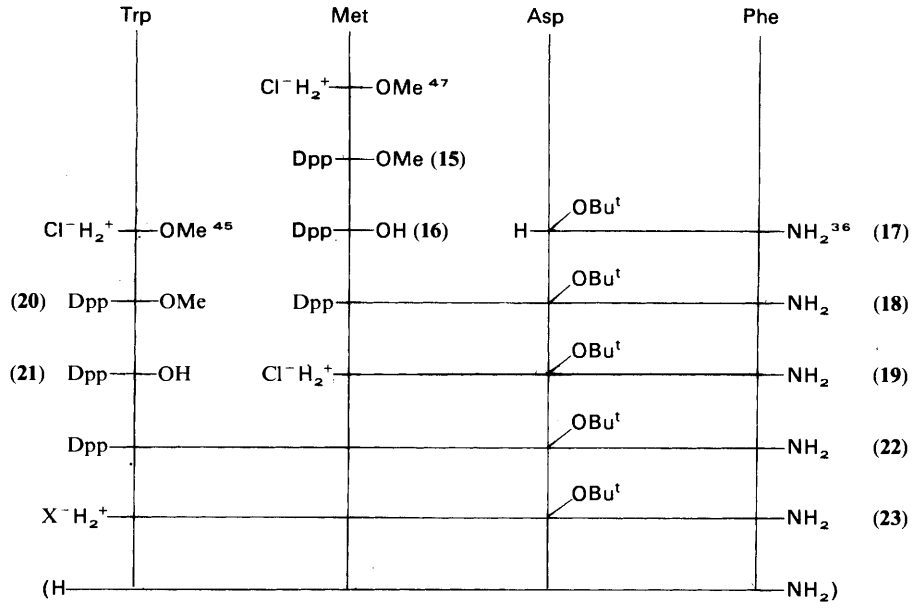
Table 6. The rate constants ($s^{-1} \times 10^5$) and half-lives (min) for the acid-catalysed methanolysis

$$Ph_2P(O)NH(CH_2)_2Ph \xrightarrow{HCl\text{–}MeOH} Ph_2P(O)OMe + Cl^-H_3N^+(CH_2)_2Ph$$

25 °C		30 °C		37 °C		45 °C	
k	$T_{\frac{1}{2}}$	k	$T_{\frac{1}{2}}$	k	$T_{\frac{1}{2}}$	k	$T_{\frac{1}{2}}$
41.0	28	53.6	21	68.7	17	94.7	12

It was considered that HCl–MeOH would be (and has since proven to be[30]) an ideal reagent for the routine cleavage of the Dpp group from peptides whose C-terminus was protected as the methyl ester. However, it was deemed of paramount importance to develop and critically evaluate alternative conditions of cleavage for application to those situations where the use of HCl–MeOH would be deleterious, *e.g.* where there may be a risk of esterifying peptide amides or the possibility of transesterification where such as the phenyl ester[32] is the chosen C-terminus protecting group. With this in mind, together with the desire to test the compatibility of Dpp cleavage with Trp and Met residues in the absence of scavengers and with protecting groups derived from t-butyl alcohol it was decided to synthesise the partially protected C-terminal tetrapeptide of gastrin (**23**) (Scheme 4). This synthesis has been attempted[33,34] using analogues having Nps (*o*-nitrophenylsulphenyl)[35] or Bpoc[18] N_α-protection. Removal of the Nps group from tryptophan-containing peptides must be performed with care. Cleavage from NpsTrp-Met-Asp(OBu^t)-PheNH_2 with anhydrous HCl has been shown[33a] to give the corresponding tetrapeptide hydrochloride but the product retained the *o*-nitrophenylsulphenyl group through intermediate sulphenium ion attack at position 2 of the indole ring of tryptophan. Morley,[33b] therefore, introduced the use of thioglycolic acid to act as both scavenger and acid for this deprotection leading to the corresponding tetrapeptide amide thioglycolate. Use of Bpoc[34] led to the formation of intractable product mixtures from which pure tri-[BpocMet-Asp(OBu^t)-PheNH_2] and tetra-[BpocTrp-Met-Asp(OBu^t)-PheNH_2] peptides could not be isolated. Thus, N_α-diphenylphosphinylmethionine (**16**) was coupled to the free amine (**17**)[36] *via* both the pivalic mixed anhydride procedure[24] and the DCCI/HONSu method[26] to give the protected tripeptide DppMet-Asp(OBu^t)-PheNH_2 (**18**) in yields of 70 and 73% respectively. Selective cleavage of the Dpp group from (**18**) in the presence of the Asp(OBu^t) residue proved difficult and it was apparent that the Dpp group is not as readily cleaved in acid as Bpoc or Nps. The CIBA-GEIGY method[37] for selective removal of the trityl group in the presence of t-butyl alcohol derived protecting groups could not be employed in this instance. This method involves titration of protected peptides at pH 3—4 in 90% 2,2,2-trifluoroethanol with cessation of acid uptake indicating complete cleavage. Under these conditions no cleavage of the Dpp group from the protected tripeptide occurred. Optimum conditions for deprotection were found to be 0.25M-HCl in 2,2,2-trifluoroethanol–water (9:1) at ambient temperature. A six-fold stoicheiometric excess of acid was employed so as to maintain the acid concentration during the reaction. A 75% yield of

* Measured relative to external 85% aqueous phosphoric acid assigned as zero.



Scheme 4.

$Cl^-H_2^+$Met-Asp(OBu^t)-PheNH$_2$ (**19**) was obtained after a reaction time of 6 h by crystallisation of the product from the reaction medium with anhydrous diethyl ether. Selective deprotection using 0.25M-HCl in methanol–water (9:1) gave (**19**) in 62% yield. The DCCI–HONSu[26] mediated coupling of DppTrpOH (**21**) with (**19**) in DMF gave the fully protected tetrapeptide amide (**22**) in 65% yield. Selective deprotection of the Dpp group again required careful control. The initial conditions devised were to use 2 equivalents of toluene-*p*-sulphonic acid monohydrate in methanol from which the product was isolated in 74% yield. Concurrently, the opportunity was again taken to assess alternative reagents for this selective deprotection. Hydrogen chloride in isopropyl alcohol, aqueous 4M-hydrochloric acid, and hydrogen chloride in 2,2,2-trifluoroethanol all proved effective but required prolonged reaction times ranging from 1 to 6 h. One viable alternative was the use of 6 equivalents of HCl–MeOH which reacted smoothly and cleanly to bring about complete removal of the N_α-protecting group from (**22**) within 2.5 h at 0 °C (as monitored by ^{31}P n.m.r., see Figure) leading to the isolation of (**23**; X = Cl) in a yield of 78%. The data by which this compound was fully characterised is presented in the Experimental section with the exception of the ^{1}H and ^{13}C n.m.r. spectra which showed excellent agreement with those presented for the fully protected tetrapeptide amide (**22**)—those peaks attributed to the aromatic rings of the protecting group now being absent. Furthermore, it should be noted that cleavage conditions for Boc (80% aqueous trifluoroacetic acid, 4 h, 0 °C) led to complete deprotection of the peptide (**22**) to give HTrp-Met-Asp-PheNH$_2$.

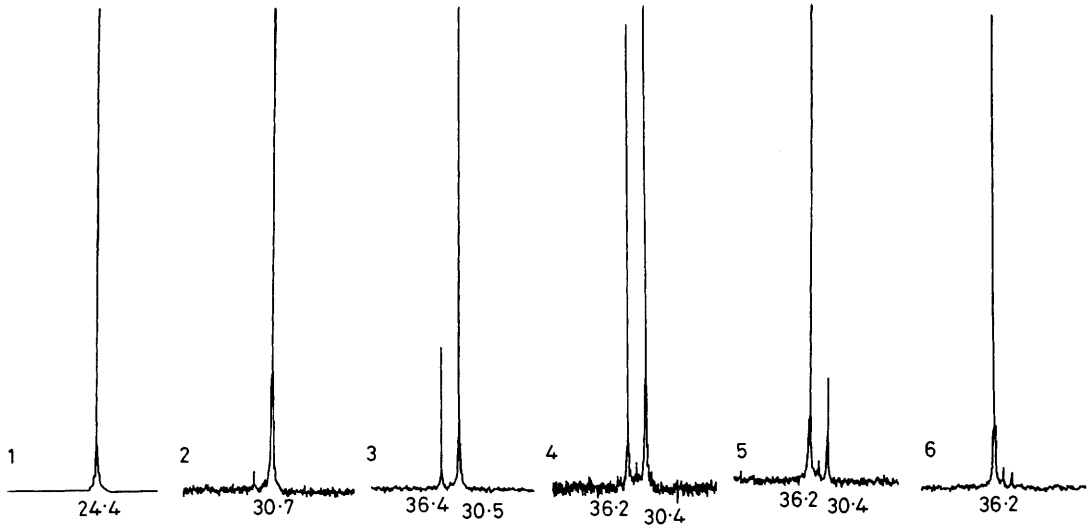
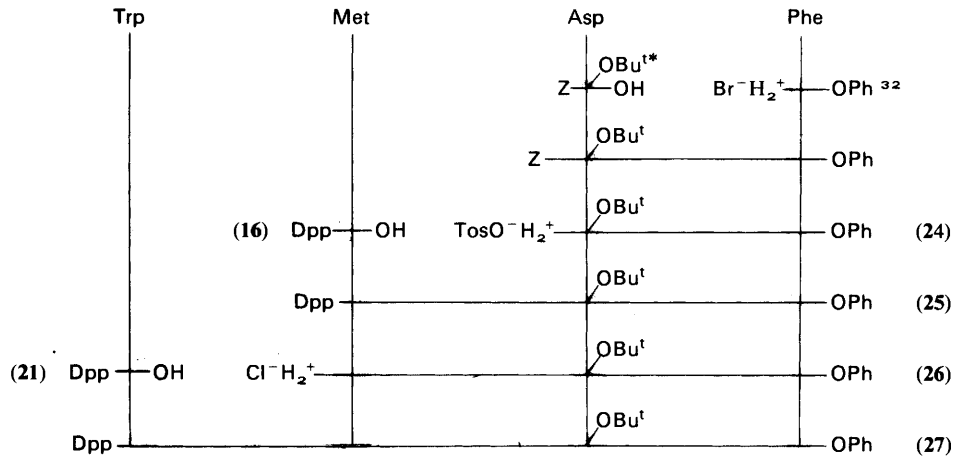


Figure. A timed series of ^{31}P n.m.r. spectra of the acid-catalysed methanolysis of DppTrp-Met-Asp(OBu^t)-PheNH$_2$ (**22**). (1) δ_P (**22**) 24.4 p.p.m.; (2) t = 2 min after addition of HCl–MeOH, peak broadens and shifts to δ_P 30.7 p.p.m. [believed to be due to protonated (**22**)]; (3) t = 20 min δ_P 36.4 p.p.m. [methyl diphenylphosphinate]; (4) t = 60 min; (5) t = 120 min; (**6**) t = 150 min.



Scheme 5. See text for details of synthetic transformations based on the fully protected tetrapeptide (**27**)

* E. Schroder and H. Klieger, *Liebigs Ann. Chem.*, 1964, **673**, 208.

Table 7. Study of deprotection of (**25**) to $X^-H_2^+$Met-Asp(OBu^t)-PheOPh

	Reagent(s)	Conditions	Result
(1)	80% Aqueous CH_3CO_2H	4 h at room temp. and 2 h at 60 °C	No reaction Incomplete cleavage
(2)	1 equiv. *p*-TSA·H_2O in ethyl acetate	45 h at room temp.	Incomplete cleavage
(3)	As (2) but after 45 h a second equiv. of *p*-TSA·H_2O was added	Further 20 h at room temp.	TosO$^-H_2^+$Met-Asp(OBu^t)-PheOPh + TosO$^-H_2^+$Met-Asp-PheOPh
(4)	2M-HCl in dioxane–water (2:1)	3.5 h at room temp.	Cl$^-H_2^+$Met-Asp(OBu^t)-PheOPh (68%)
(5)	0.7M-HCl in dioxane–water (15:2)	6 h at room temp.	Cl$^-H_2^+$Met-Asp(OBu^t)-PheOPh (67%)
(6)	1.5M-HCl in dioxane-water (3:1)	6 h at room temp.	Cl$^-H_2^+$Met-Asp(OBu^t)-PheOPh + Cl$^-H_2^+$Met-Asp-PheOPh

Removal of the Dpp Group in the Presence of a Phenyl Ester.— There has been considerable interest in the physiological action of modifications of the C-terminal fragment of the gastrin molecule. It would be especially useful to prepare fragments of gastrin in which the C-terminal primary amide group is replaced by a carboxy group, since the metabolism of gastrin is believed to start by deamidation at the C-terminus.[38] A supply of the C-terminal free acid might allow antibodies specific to this initial metabolism product to be produced. In this respect the synthesis of a C-terminal tetrapeptide fragment of gastrin with phenyl ester protection for the phenylalanine carboxy group is of great interest. This protecting group is especially suitable since it can be removed under mild racemisation-free conditions[32] or, alternatively, it may be converted into the amide with ammonia. The phenyl ester is preferred to the methyl ester since alkaline hydrolysis of the latter may lead to partial racemisation of the phenylalanine residue.

DppMet-Asp(OBu^t)-PheOPh (**25**) and DppTrp-Met-Asp-(OBu^t)-PheOPh (**27**) were successfully synthesised (Scheme 5) whereas considerable difficulty had been encountered previously[33,34] in analogues having Bpoc or Nps *N*-protection. Hydrogenolysis of the fully protected dipeptide ZAsp(OBu^t)-PheOPh[34] at room temperature and pressure in the presence of toluene-*p*-sulphonic acid monohydrate gave TosO$^-H_2^+$ Asp(OBu^t)-PheOPh (**24**) in 82% yield. The coupling between DppMetOH (**16**) and (**24**) was effected in 74% yield *via* the pivalic mixed anhydride, the fast coupling method being preferred so as to avoid possible cyclisation of the dipeptide phenyl ester to a 2,5-diketopiperazine. Conditions for the deprotection of (**25**) were studied extensively and are summarised in Table 7.

The original conditions for the cleavage of Bpoc (80% aqueous acetic acid) were not suitable for cleavage of the rather more stable diphenylphosphinyl group. After 4 h there was only very slight reaction evident by t.l.c., and even after warming to 60 °C for 2 h the cleavage reaction was incomplete. Treatment of (**25**) with 1 equivalent of toluene-*p*-sulphonic acid monohydrate in ethyl acetate for 45 h at ambient temperature gave incomplete cleavage of the Dpp group. When a further equivalent of acid was added and reaction continued for a further 20 h the solution was found to contain mostly the desired product TosO$^-H_2^+$Met-Asp(OBu^t)-PheOPh but also significant quantities of starting material and a second fluorescamine positive species, presumed to result from partial cleavage of the t-butyl group. The most convenient conditions for removal of the Dpp group in this instance are the use of 2M-HCl in dioxane–water (2:1) at ambient temperature. A 68% yield of Cl$^-H_2^+$Met-Asp(OBu^t)-PheOPh (**26**) was obtained after 3.5 h by direct crystallisation of the product from the reaction mixture. In another reaction, a 67% yield was obtained using 0.7M-HCl in dioxane–water (15:2) for 6 h. 1.5M-HCl in dioxane–water (3:1) gave a yield of only 34% after a reaction time of 6 h owing to partial cleavage of the aspartyl t-butyl ester. In these reactions the work-up procedure is critical since evaporation of the solvent can result in the peptide being exposed to high concentrations of acid for a short time, with the resultant possibility of cleavage of the t-butyl group.

A pivalic mixed anhydride coupling with DppTrpOH (**21**) gave the fully protected tetrapeptide DppTrp-Met-Asp(OBu^t)-PheOPh (**27**) in 52% yield. Cleavage of the N_α-protecting group in the presence of Asp(OBu^t) once again required careful experimentation (the results of our efforts to develop a range of

phosphinamide protecting groups of varying degrees of acid lability will be presented in due course) and the preferred conditions were found to be 2 equivalents of toluene-*p*-sulphonic acid monohydrate, this time in isopropyl alcohol or DMF since methanol would be likely to effect transesterification of the phenyl ester. Deprotection was essentially complete after 6 h as judged by t.l.c. Furthermore, it was possible to cleave the phenyl ester using standard conditions[32] [pH 10.5, hydrogen peroxide catalysis in dioxane–DMF–water (4:1:1)] to give DppTrp-Met-Asp(OBu^t)-PheOH (**28**) in 77% yield.

Treatment of (**27**) with ammonia in dichloromethane gave (**22**) as an amorphous powder in 90% yield. This is a most convenient route to analogues of gastrin with C-terminal substituted amide[39] or, indeed, of peptide amides in general.

Experimental

All α-amino acids were purchased from the Sigma Chemical Co. Ltd. and were used as supplied. Melting points were taken in an open capillary tube on an electrically heated Buchi 510 melting point apparatus and are uncorrected. Optical rotations were measured on a Thorn NPL 243 automatic polarimeter using a 10 mm cell. Thin layer chromatography was carried out on glass plates coated with silica gel 60GF-254 (Merck) in the following systems: (A) $CHCl_3$–MeOH (9:1), (B) CH_3CN–H_2O (9:1), (C) $CHCl_3$–MeOH (1% AcOH) (6:1), (D) $CHCl_3$–Pr^{i}OH (6:1), (E) EtOAc–Pyr–AcOH–H_2O (120:20:6:11). (In all the systems employed, any contaminating DppOH remained at the base line.) Visualisation of the compounds was achieved by a suitable combination of the following methods: iodine vapour (I),[40] u.v. absorption at 254 nm (UV), ninhydrin (N) or fluorescamine (F)[41] for peptides with free *N*-amino groups and Ehrlich's reagent (E)[42] for tryptophan. High-performance liquid chromatography (h.p.l.c.): a Waters HPLC system was employed (Waters Associates, Cheshire), 2 × 6000A pumps, Waters 6 U6K injection system, 660 gradient former, Waters μ-Bondapak C-18 analytical column and a Waters UV detector (model 441). Isocratic elution [CH_3CN (90%)–H_2O (9.9%)–TFA(0.1%) at 2 ml min^{-1}] of samples from the column was monitored, unless otherwise stated, for u.v. absorbance at 254 nm [retention times (R_t) are given in minutes]. Amino acid analyses were carried out on a JEOL JLC-5AH amino acid analyser with electrical integration of the peak areas following sealed tube hydrolysis with constant boiling hydrochloric acid at 110 °C for 24 h. Theoretical amino acid ratios are shown as a subscript following the amino acid in question. I.r. spectra were measured using the bromoform mull technique on a Perkin-Elmer 197 double beam spectrometer. U.v. spectra were measured in spectroscopic grade ethanol on a Cary Unicam 118X spectrometer. High-resolution mass spectra (h.r.m.s.) were measured on a Kratos MS45 machine. ^{1}H N.m.r. spectra were, unless otherwise stated, recorded on a Perkin-Elmer R34 machine operating at 220 MHz. Samples were dissolved in the deuteriated solvents indicated and tetramethylsilane (TMS) was used as an external standard reference. Carbon-13 magnetic resonance spectra (^{13}C n.m.r.) were measured on a Bruker WP80 machine operating at 20.1 MHz as solutions in the solvent indicated. All chemical shifts, δ (p.p.m.), were measured relative to TMS assigned at zero. Phosphorus-31 magnetic resonance spectra (^{31}P n.m.r.) were recorded on a Bruker WP 80 machine operating at 32.4 MHz using solutions made up in the solvent(s) indicated. All chemical shift, δ (p.p.m.), values were measured relative to external 85% aqueous phosphoric acid assigned as zero. The classification which has been used throughout this work to assign unambiguously the positions of the various ring systems is as given in note *c* of Table 3 (multiplicity: s singlet, d doublet, tr triplet, q quartet, m multiplet, dd double doublet, ABq AB quartet). A Radiometer (Copenhagen) autotitrator was used for the maintenance of constant pH. All solvents used in this work are listed below and were distilled immediately before use from the drying agent given in parentheses: methanol (Mg–I_2), diethyl ether (sodium wire), dichloromethane (calcium hydride), ethyl acetate (anhydrous potassium carbonate), *N*,*N*-dimethylformamide (calcium hydride). Petroleum (60—80) refers to that fraction which boils between 60—80 °C whilst light petroleum refers to that fraction which boils between 40—60 °C. Evaporations were under reduced pressure and carried out at the minimum possible temperature; DMF and other high-boiling solvents were removed at 20 °C under an oil pump vacuum.

The preparations given are typical (rather than those giving maximum yield) and are not necessarily on the largest scale studied. Amino acid methyl ester hydrochlorides were prepared by the thionyl chloride–methanol procedure[43] and the benzyl ester toluene-*p*-sulphonates by the method of Zervas.[44] The phrase 'washed as usual' refers to the following sequence: saturated $NaHCO_3$ (×5), 5% citric acid (×3), water (×2), saturated $NaHCO_3$ (×2), water (×2), and saturated sodium chloride (×2).

General Procedure 1: *Preparation of* N_α*-Diphenylphosphinyl Amino Acid Methyl* (**8**)*/Benzyl* (**9**) *Esters.*—To a vigorously stirred suspension of the methyl ester hydrochloride (**6**) [or clear, colourless solution of the benzyl ester toluene-*p*-sulphonate (**7**)] (0.2 mol) in dichloromethane (500 ml) at 0 °C were added NMM (43.5 ml, 0.4 mol) and a clear, colourless solution of DppCl (47.4 g, 0.2 mol) in dichloromethane (90 ml) in such a way as to maintain the temperature of the reaction mixture at 0 °C. The reactants were then stirred for 1½—2 h during which time the temperature rose to *ca.* 15 °C. Removal of the reaction solvent left a pale yellow oil which was partitioned between ethyl acetate and water. The organic phase was washed as usual before drying over anhydrous magnesium sulphate. Following evaporation of the solution, the desired product was crystallised from the residue with the appropriate crystallisation solvent(s) (Table 1); yield 65—80%.

General Procedure 2: *Preparation of* N_α*-Diphenylphosphinyl Amino Acids* (**10**) *by Mild Alkaline Hydrolysis of* (**8**).—The N_α-diphenylphosphinyl amino acid methyl ester (**8**) (75 mmol) was suspended in freshly distilled 1,4-dioxane (175 ml) and 2M-sodium hydroxide (43 ml) added. After the mixture had been stirred for 5 min at room temperature a clear, colourless solution resulted (it was occasionally necessary to add a few drops of methanol to complete dissolution) which was stirred at room temperature until thin layer chromatography (t.l.c.-A) showed the saponification to be complete. Dioxane was removed and the aqueous solution was acidified to pH 3—4 with saturated citric acid causing a white gum to separate which was extracted with ethyl acetate (3 × 100 ml). The combined organic phase was washed with water (6 × 100 ml) and brine (2 × 75 ml) before drying over anhydrous $MgSO_4$. Evaporation of the solution gave crude (**10**) which was (re)crystallised from the appropriate solvent(s) (Table 1); yield 80—90%.

General Procedure 3: *Preparation of* N_α*-Diphenylphosphinyl Amino Acids* (**10**) *by Catalytic Hydrogenolysis of* (**9**).—A solution of the benzyl ester (5 mmol) in methanol (20 ml) was hydrogenolysed at room temperature and pressure over 10% palladium on charcoal catalyst for 18 h. The catalyst was removed by filtration through Celite and the solvent evaporated to yield a clear, colourless oil which was crystallised from the appropriate solvent(s) (Table 1); yield 75—85%.

N_α*-Diphenylphosphinylmethionine Methyl Ester*, DppMet-OMe (**15**)^1.—$Cl^-H_2{}^+$MetOMe[44] (24.95 g, 125 mmol), NMM

(27.5 ml, 250 mmol) and DppCl (29.55 g, 125 mmol) were allowed to react together as described under general procedure 1. Evaporation of the final, dry solution gave a clear, colourless oil which was taken up in the minimum volume of anhydrous diethyl ether. The white crystalline compound which separated from the ethereal solution overnight at room temperature was collected by filtration and dried (31.0 g, 69%); $\nu_{max.}$ 3 150 (NH), 1 730 (C=O), 1 440 (PhP), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 253infl. (ε 805), 258 (1 119), 264 (1 466), and 271 nm (1 176); the other analytical data by which this compound was fully characterised are given in Tables 1—3.

N_α-*Diphenylphosphinylmethionine*, DppMetOH (**16**)[1].—The reaction of DppMetOMe (**15**) (17.68 g, 48.7 mmol) with 2M-sodium hydroxide (25 ml) in 1,4-dioxane (200 ml) over 60 min as described in general procedure 2 led to the isolation of (**16**) as fine white crystals (14.0 g, 80%), $\nu_{max.}$ 3 400—2 340 (acid OH), 3 300 (NH), 1 710 (C=O), 1 440 (PhP), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 254infl. (ε 819), 259 (1 150), 265 (1 500), and 271 nm (1 200); the other analytical data by which this compound was full characterised are given in Tables 1—3.

N_α-*Diphenylphosphinylmethionyl*(β-*t-butyl*)*aspartylphenylalanine Phenyl Ester*, DppMet-Asp(OBu^t)-PheOPh (**25**).—DppMetOH (**16**) (384 mg, 1.2 mmol) was dissolved in dichloromethane (5 ml) and NMM (0.13 ml, 1.2 mmol) added. The solution was cooled to −15 °C and pivaloyl chloride (0.132 g, 1.1 mmol) added dropwise. After the mixture had been stirred for 7 min $TosO^-H_2^+$Asp(OBu^t)-PheOPh[34] (584 mg, 1 mmol) in dichloromethane (3 ml) was added followed by NMM (0.11 ml, 1 mmol); stirring was then continued for 24 h. Dichloromethane was removed and the residue partitioned between ethyl acetate and water; the organic phase was washed as usual before being dried over Na_2SO_4. Evaporation of the solvent gave N_α-*diphenylphosphinylmethionyl*(β-*t-butyl*)*aspartylphenylalanine phenyl ester* (**25**) which was crystallised from ethyl acetate with petroleum (60—80) (660 mg, 74%), m.p. 171—173 °C (Found: C, 64.5; H, 6.4; N, 5.6. $C_{40}H_{46}N_3O_7PS$ requires C, 64.6; H, 6.2; N, 5.6%); $[\alpha]_D^{24}$ −78° (*c* 1 in DMF): t.l.c.-B R_F 0.69 (I, UV); amino acid analysis Met_1 1.00, Asp_1 0.99, Phe_1 1.01.

Methionyl(β-*t-butyl*)*aspartylphenylalanine Phenyl Ester Hydrochloride*, $Cl^-H_2^+$Met-Asp(OBu^t)-PheOPh (**26**).—Aqueous 6M-HCl (2 ml) was added to a solution of (**25**) (743 mg, 1 mmol) in 1,4-dioxane (4 ml). The progress of the reaction was monitored by t.l.c. (t.l.c.-C) using fluorescamine to demonstrate free NH_2 and u.v. absorption to show starting material. Optimal cleavage was observed after 3 h of stirring. The solution was then evaporated to dryness and the solid product triturated with anhydrous diethyl ether. Recrystallisation from propan-2-ol gave *methionyl*(β-*t-butyl*)*aspartylphenylalanine phenyl ester hydrochloride* (**26**) (391 mg, 68%), m.p. 175—177 °C (Found: C, 57.3; H, 6.6; N, 7.0. $C_{28}H_{38}ClN_3O_6S$ requires C, 58.0; H, 6.6; N, 7.2%); $[\alpha]_D^{25}$ −49.0° (*c* 1 in DMF); t.l.c.-E R_F 0.60 (I, UV, N, F); amino acid analysis Met_1 0.97, Asp_1 0.99, Phe_1 1.04.

N_α-*Diphenylphosphinyltryptophan Methyl Ester*, DppTrpOMe (**20**).[1]—$Cl^-H_2^+$TrpOMe[45] (23.5 g, 98 mmol), NMM (21.7 ml, 197 mmol) and DppCl (23.3 g, 98 mmol) were allowed to react together as described under general procedure 1. Evaporation of the final dry solution gave a white foam which readily crystallised overnight at room temperature under anhydrous diethyl ether. The white powder thus formed was filtered off and dried (31.8 g, 80%), $\nu_{max.}$ 3 400, 3 350 (NH), 1 730 (C=O), 1 440 (PhP), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 259infl. (ε 4 720), 265 (6 000), 272 (6 650), 277 (5 950), 281 (6 100), and 289 nm (5 270); the other analytical data by which this compound was characterised are given in Tables 1—3.

N_α-*Diphenylphosphinyltryptophan*,DppTrpOH (**21**)[1].—The reaction of DppTrpOMe (**20**) (17.2 g, 41 mmol) with 2M-sodium hydroxide (21 ml) in 1,4-dioxane (68 ml)–methanol (3 ml) as described in general procedure 2 led to the isolation of (**21**) as a white foam (13.8 g, 86%) which readily solidified overnight at room temperature under anhydrous diethyl ether, $\nu_{max.}$ 3 400 (NH), 3 200—2 000 (acid OH), 1 710 (C=O), 1 440 (PhP), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 259infl. (ε 4 720), 265 (6 000), 272 (6 650), 277 (5 950), 281 (6 100), and 289 nm (5 270); the other analytical data by which this compound was fully characterised are given in Tables 1—3.

N_α-*Diphenylphosphinyltryptophanylmethionyl*(β-*t-butyl*)*aspartylphenylalanine Phenyl Ester*, DppTrp-Met-Asp(OBu^t)-PheOPh (**27**).—DppTrpOH (**21**) (202 mg, 0.5 mmol) was dissolved in DMF (0.5 ml)–dichloromethane (2.5 ml) in a darkened flask under nitrogen. The solution was cooled to −15 °C and NMM (55 μl, 0.5 mmol) and pivaloyl chloride (60 mg, 0.5 mmol) added. After 15 min (**26**) (290 mg, 0.5 mmol) was added followed by NMM (55 μl, 0.5 mmol). The mixture was stirred for 24 h and then evaporated, partitioned between ethyl acetate and water, and washed and dried as usual. Removal of solvent gave N_α-*diphenylphosphinyltryptophanylmethionyl*(β-*t-butyl*)*aspartylphenylalanine phenyl ester* (**27**) which was recrystallised from propan-2-ol with anhydrous diethyl ether (484 mg, 52%), m.p. 187—188 °C (Found: C, 65.6; H, 6.4; N, 7.4. $C_{51}H_{56}N_5O_8PS$ requires C, 65.9; H, 6.1; N, 7.5%); t.l.c.-E R_F 0.77; amino acid analysis Trp_1 1.02, Met_1 1.14, Asp_1 1.00, Phe_1 0.99.

N_α-*Diphenylphosphinyltryptophanylmethionyl*(β-*t-butyl*)*aspartylphenylalanine*, DppTrp-Met-Asp(OBu^t)-PheOH (**28**).—Water (0.5 ml) was added to a solution of (**27**) (465 mg, 0.5 mmol) in 1,4-dioxane (2 ml)/DMF (0.5 ml) and the pH adjusted to 10.5 with 0.1M-sodium hydroxide. Dimethyl sulphide (3 g) was added followed by hydrogen peroxide solution (100 v/v; 1 equiv.). 0.1M-Sodium hydroxide was then added whilst the pH was maintained at 10.5. Alkali uptake ceased after 30 min (6.2 ml, 124%). The pH was adjusted to 7 with saturated citric acid solution, the solvent evaporated, and the residue taken up in water. The pH was further adjusted to 4 and the product extracted into ethyl acetate (3 × 15 ml). The combined extracts were washed with water, dried over anhydrous $MgSO_4$, and evaporated to yield N_α-*diphenylphosphinyltryptophanylmethionyl*(β-*t-butyl*)*aspartylphenylalanine* (**28**) which was purified by recrystallisation from methanol with water (320 mg, 77%), m.p. 204—205 °C (Found: C, 63.4; H, 6.2; N, 8.2. $C_{42}H_{52}N_5O_8PS$ requires C, 63.3; H, 6.1; N, 8.2%); t.l.c.-E R_F 0.57; amino acid analysis Trp_1 1.00, Met_1 0.96, Asp_1 1.03, Phe_1 1.00.

N_α-*Diphenylphosphinylmethionyl*(β-*t-butyl*)*aspartylphenylalanine Amide*, DppMet-Asp(OBu^t)-PheNH$_2$ (**18**).—DppMetOH (**16**) (768 mg, 2.2 mmol) was dissolved in dichloromethane (3 ml) and HAsp(OBu^t)-PheNH$_2$[36] (**17**) (737 mg, 2.2 mmol) was added. The solution was cooled to 0 °C and HONSu (506 mg, 4.4 mmol) and DCCI (453 mg, 2.2 mmol) were added. After the mixture had been stirred at low temperature for 2 h the reactants were allowed to warm to ambient temperature overnight. The reaction mixture was filtered and evaporated, the residue taken up in ethyl acetate, and the solution washed as usual. After drying ($MgSO_4$) of the solution, the solvent was removed and the residue recrystallised from methanol and anhydrous diethyl ether to afford N_α-*diphenylphosphinylmethionyl*(β-*t-butyl*)*aspartylphenylalanine amide* (**18**) (1.07 g,

73%), m.p. 210—211 °C (Found: C, 61.2; H, 6.7; N, 8.4; P, 4.8; S, 4.7. $C_{34}H_{43}N_4O_6PS$ requires C, 61.2; H, 6.5; N, 8.4; P, 4.6; S, 4.6%); $[\alpha]_D^{25}$ −104.3° (*c* 1 in MeOH); $[\alpha]_D^{25}$ −110.7° (*c* 1 in DMF); t.l.c.-D R_F 0.53, t.l.c.-C R_F 0.58; amino acid analysis Met_1 1.00, Asp_1 1.00, Phe_1 1.00; $\nu_{max.}$ 3 400, 3 275, 3 175 (NH), 1 720 (ester C=O), 1 690, 1 640 (amide C=O), 1 440 (Ph–P), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 254infl. (ε 1 060), 258 (1 440), 264 (1 750), and 271 nm (1 310); $\delta_H(CDCl_3)$ 8.80 (1 H, d, ^3J 8.5 Hz, Asp NH)[a], 8.13 (1 H, d, ^3J 8.5 Hz, Phe NH)[b], 7.90—7.05 (16 H, m, aromatic H's (1) $CONH_2$), 6.65 (1 H, s, (1) $CONH_2$), 4.65 (3 H, m, Asp and Phe α-CH Met NH), 3.67 (1 H, m, Met α-CH), 3.27 (1 H, ABq, ^2J 13.5 Hz, ^3J 5.5 Hz), and 3.12 (1 H, ABq, ^2J 13.5 Hz, ^3J 8.0 Hz, Phe β-CH_2);[46] 2.60 (4 H, m, Asp β-CH_2 and Met γ-CH_2); 2.20—1.90 (2 H, m, Met β-CH_2) obscured by 2.04 (3 H, s, Met S-CH_3); 1.35 [9 H, s, $C(CH_3)_3$] [(a,b) these have not been unambiguously assigned and may be reversed]; $\delta_C[(CD_3)_2SO]$ 173.39 (Met, Phe CO), 170.27 (Asp γ-CO)[c], 169.44 (Asp CO)[d], 138.53—126.33 (m, aromatic C's), 80.59 [$C(CH_3)_3$], 54.91 (Met and Phe α-C), 50.47 (Asp α-C), 33.10 (Met β-C), 29.64 (Met γ-C); 27.70 [$C(CH_3)_3$], 14.39 (Met S-CH_3), Asp and Phe β-C's obliterated by the strong $(CD_3)_2SO$ signals [(c,d) these have not been unambiguously assigned and may be reversed]; δ_P (MeOH–$CDCl_3$) +21.89 p.p.m.

N_α-Diphenylphosphinyltryptophanylmethionyl(β-t-butyl)aspartylphenylalanine Amide, DppTrp-Met-Asp(OBu^t)-$PheNH_2$ (**22**).—Aqueous 4M-HCl (3.0 ml) was added to a solution of (**18**) (1.32 g, 2 mmol) in 2,2,2-trifluoroethanol (27 ml). The mixture was then stirred at room temperature for 5 h when t.l.c. and ^{31}P n.m.r. spectroscopy indicated optimal cleavage had occurred. Evaporation of the solution to dryness gave an oil which was taken up in methanol and $Cl^-H_2^+$Met-Asp(OBu^t)-$PheNH_2$ (**19**) was precipitated by the addition of anhydrous diethyl ether as a white solid which was filtered off and dried (700 mg, 70%), m.p. 197—198 °C (Found: C, 52.4; H, 6.9; N, 11.3; S, 6.5. $C_{22}H_{35}ClN_4O_5S$ requires C, 52.5; H, 7.0; N, 11.1; S, 6.4%); $[\alpha]_D^{26}$ −12.8° (*c* 1 in DMF); amino acid analysis Met_1 0.98, Asp_1 1.00, Phe_1 1.02.

DppTrpOH (**21**) (404 mg, 1 mmol) and (**19**) (592 mg, 1 mmol) were dissolved in DMF (5 ml). The solution was cooled to 0 °C and HONSu (230 mg, 2 mmol), NMM (0.11 ml, 1 mmol) and DCCI (206 mg, 1 mmol) were added successively. The reaction was stirred for 48 h in a darkened flask under nitrogen and then filtered, diluted with ethyl acetate, and washed as usual. After drying (Na_2SO_4) the solvent was removed and the oily residue triturated with chloroform. Reprecipitation of the white crystals thus obtained from acetonitrile with petroleum (60—80) gave *N_α-diphenylphosphinyltryptophanylmethionyl(β-t-butyl)aspartylphenylalanine amide* (**22**) (554 mg, 65%).

N_α-Diphenylphosphinyltryptophanylmethionyl(β-t-butyl)aspartylphenylalanine mide, DppTrp-Met-Asp(OBu^t)-$PheNH_2$ (**22**) *by Ammonolysis of* DppTrp-Met-Asp(OBu^t)-PheOPh (**27**).—Ammonia was bubbled through a solution of (**27**) (186 mg, 0.2 mmol) in dichloromethane (2 ml) at 0 °C. After 23 h the solvent was removed and the residue triturated with anhydrous diethyl ether to afford (**22**) (152 mg, 90%).

The two samples were identical as assessed by the following analytical data: m.p. 192—194 °C (Found: C, 63.5; H, 6.4; N, 9.8; P, 3.6; S, 3.6. $C_{45}H_{53}N_6O_7PS$ requires C, 63.4; H, 6.3; N, 9.8; P, 3.6; S, 3.8%); $[\alpha]_D^{25}$ −78.7° (*c* 1 in MeOH); $[\alpha]_D^{25}$ −45.4° (*c* 1 in DMF); t.l.c.-E R_F 0.67; h.p.l.c. R_t 2.5; amino acid analysis Trp_1 1.11, Met_1 1.04, Asp_1 0.99, Phe_1 0.98; $\nu_{max.}$ 3 450, 3 300 (NH), 1 730 (ester C=O), 1 680, 1 650 (amide C=O), 1 550 (CONH), 1 440 (PhP), and 1 180 cm^{-1} (P=O); $\lambda_{max.}$(EtOH) 259infl. (ε 5 680), 265 (7 070), 271 (7 690), 278infl. (6 330), 280 (7 030), and 289 nm (6 080); $\delta_H[(CD_3)_2SO]$ 11.00 (1 H, s, indole NH), 9.10 (1 H, d, ^3J 8.0 Hz, Met NH), 8.30 (1 H, d, ^3J 8.0 Hz, Asp NH), 7.80—7.10 (21 H, m, aromatic H except Trp 6-H, 5-H, Phe NH, and (2) $CONH_2$), 7.02 (1 H, tr, ^3J 8.5 Hz, Trp 6-H), 6.95 (1 H, tr ^3J 8.5 Hz, Trp 5-H), 5.60 (1 H, m, Trp NH), 4.55 (1 H, m, Asp α-CH), 4.40—4.20 (2 H, m, Phe and Trp α-CH), 3.70 (1 H, m, Met α-CH), 3.30—2.56 (6 H, m, Phe Asp and Trp β-CH_2), 2.40 (2 H, m, Met γ-CH_2), 2.05—1.80 (m, Met β-CH_2) obscured by 1.95 (s, Met S-CH_3), and 1.40 [9 H, s, $C(CH_3)_3$]; $\delta_C[(CD_3)_2SO]$ 174.79 (Trp CO), 172.78, 172.05 (Phe, Met CO), 170.48 (Asp γ-CO)[a], 169.56 (Asp CO)[b], 137.92—126.08 (aromatic C, Phe C-1—C-6, Trp C-9, C-8), 124.38 (Trp C-2), 121.04 (Trp C-6), 118.43 (Trp C-4, C-5), 111.51 (Trp C-7), 109.81 (Trp C-3), 80.53 [$C(CH_3)_3$], 57.3 (Trp α-C), 54.54 (Phe α-C), 53.81 (Met α-C), 50.77 (Asp α-C), 30.49 (Met β-C), 29.34 (Met γ-C), 27.8 [$C(CH_3)_3$], 14.6 (Met S-CH_3), Trp Asp Phe β-C obliterated by strong $[(CD_3)_2SO]$ signals; δ_P (MeOH–$CDCl_3$) +24.0 p.p.m.

Tryptophanylmethionyl(β-t-butyl)aspartylphenylalanine Amide Hydrochloride, $Cl^-H_2^+$Trp-Met-Asp(OBu^t)-$PheNH_2$ (**23**).—A 1.5M-solution of hydrogen chloride in methanol (3.5 ml, 0.53 mmol) chilled to 0 °C was added to DppTrp-Met-Asp(OBu^t)-$PheNH_2$ (**22**) (750 mg, 0.9 mmol) and the progress of the ensuing deprotection was monitored by ^{31}P n.m.r. spectroscopy [$CDCl_3$ lock (0.4 ml) see Figure]. After 90 min white crystals began to precipitate out of solution and 1 h later optimum cleavage was considered to have occurred. The reaction mixture was poured into vigorously stirred cold, anhydrous diethyl ether (200 ml) and after stirring for a further 1 h at low temperature, *tryptophanylmethionyl(β-t-butyl)-aspartylphenylalanine amide hydrochloride* (**23**; X = Cl) was filtered off, washed with cold diethyl ether, and dried (470 mg, 78%), m.p. 198—200 °C (Found: C, 57.3; H, 6.6; N, 12.1; S, 4.6. $C_{33}H_{45}ClN_6O_6S$ requires C, 57.5; H, 6.6; N, 12.2; S, 4.6%); $[\alpha]_D^{25}$ −30.0° (*c* 1 in DMF); amino acid analysis Trp_1 0.92, Met_1 0.97, Asp_1 1.01, Phe_1 1.02; $\nu_{max.}$ 3 650—2 450 (NH_3^+, NH), 1 730 (ester C=O), 1 670, 1 660, and 1 640 (amide C=O).

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