Application of Diphenylphosphinic Carboxylic Mixed Anhydrides to Peptide Synthesis.[1]

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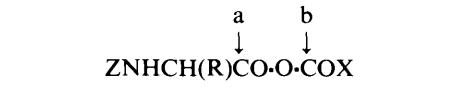
Diphenylphosphinic carboxylic mixed anhydrides formed *in situ* from N_{α}-protected amino acids and diphenylphosphinic chloride have been critically evaluated in peptide synthesis. Wherever possible, 32.4 MHz ^{31}P n.m.r. spectroscopy has been employed to follow the rates of both mixed anhydride formation and aminolysis.

Although the synthesis of peptides by stepwise coupling of suitably protected α-amino acids may be accomplished by a wide variety of methods[2] with the virtual elimination of racemisation,[3] the efficiency of many is impaired by the instability of activated intermediates and the formation of unwanted by-products. In an effort to circumvent these latter, prohibitive considerations the design and practical exploitation of novel reagents for use as improved and more effective mediators of amide bond formation are under constant investigation.

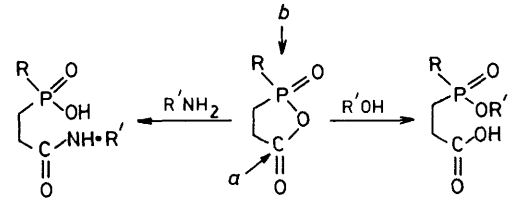
Mixed carboxylic anhydrides[4] of N_{α}-protected amino acids ‡ [*e.g.* (**1**)] have been widely used in peptide synthesis.[5] The group 'X' is chosen so as to ensure that steric and electronic factors favour attack by an amine component (R^1NH_2) at *a* (to give the desired product) rather than at *b* (cause of a decrease in yield and a source of impurities).[6] This type of coupling offers a definite advantage in cases where R^1NH_2 has a limited lifetime, *e.g.* as a dipeptide ester favourably disposed towards cyclisation to a piperazine-2,5-dione, since mixed anhydride couplings are relatively fast and over the past decade or so, since its inception,[7] the repetitive excess mixed anhydride (REMA) method[8] has become increasingly important for the rapid synthesis of pure peptides in excellent yield. However, significant problems can occur when the groups R and R^1 are bulky, for example in couplings involving valyl or isoleucyl residues,[9] where attack at *b* may become competitive from a steric standpoint. Another problem associated with the use of carboxylic mixed anhydrides is that of facile thermal disproportionation[10] [to the symmetrical anhydrides (**2**) and (**3**)] which again leads not only to a decrease in yield but also to the concomitant formation of unwanted by-products. In order to avoid such disproportionation it is necessary to form the anhydrides in solution at low temperatures (*e.g.* −20 °C)[11] and to employ short activation times.[12]

In an attempt to surmount these side reactions it was our intention to devise a new class of mixed anhydrides which would be stable towards disproportionation, react with



nucleophiles specifically by path *a* and also, if possible, be isolable crystalline compounds. One alternative approach is the exploitation of anhydrides derived from organophosphorus acids. The rôle of such reagents in carbonyl activation has been reviewed[13] and continuing developments in this area are periodically collectively chronicled.[14]

Whilst the anhydrides (**4**) derived from *O,O*-dialkyl and *O,O*-diaryl phosphoric acids have previously been employed in peptide synthesis,[15] but have failed to receive widespread acceptance, little or no attention has been paid to the analogous phosphinic–carboxylic mixed anhydrides.



Scheme 1.

Phosphinic Carboxylic Mixed Anhydrides.—The following considerations suggested that phosphinic carboxylic mixed anhydrides may be useful intermediates in peptide synthesis.

(i) The cyclic system (**5**) (Scheme 1) undergoes aminolysis by attack at the carbonyl group (*a*) but alcoholysis occurs by attack at phosphorus (*b*).[16] This change in regiospecificity depending upon the nature of the nucleophile is entirely compatible with the requirements of peptide synthesis.

(ii) Dimethyl phosphorochloridate (**6**) has been shown to be less reactive toward oxygen nucleophiles than dimethylphosphinic chloride (**7**).[17] The rate of solvolysis of (**7**) in absolute alcohol is some 300 times as fast as that of (**6**), which would suggest that phosphinic chlorides should react with carboxylate anions to form mixed anhydrides[18] very much faster than phosphorochloridates. Fast formation of mixed anhydrides is

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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: Adoc = adamantyloxycarbonyl, Bpoc = 1-biphenyl-4-yl-1-methylethoxycarbonyl, Bu^t = t-butyl, DCCI = *N,N'*-dicyclohexylcarbodi-imide, DCM = dichloromethane, DCU = *N,N'*-dicyclohexylurea, DMF = *N,N*-dimethylformamide, Dpp = diphenylphosphinoyl, Dpp-Cl = diphenylphosphinic chloride, HONSu = *N*-hydroxysuccinimide, NMM = *N*-methylmorpholine, OMe = methyl ester, OPh = phenyl ester, Piv-Cl = pivaloyl chloride, THF = tetrahydrofuran, and Z = benzyloxycarbonyl.

an important consideration in coupling reactions and a further advantage in the use of phosphinic carboxylic mixed anhydrides would lie in the elimination of the danger of substitution of the OR^2 groups which occurs in intermediates such as (**4**).[15] In addition, structural variation of phosphinic chlorides would allow a more direct effect of substituents to be transmitted to the subsequent mixed anhydride than modifications of phosphorochloridates where the substituents are further from the crucial phosphorus atom.

$(MeO)_2POCl$ (**6**)

Me_2POCl (**7**)

$MeCO{\cdot}O{\cdot}P(O)Ph_2$ (**8**)

$ZNHCH(R)CO{\cdot}O{\cdot}P(O)Ph_2$ (**9**)

(iii) Finally, there is some evidence to suggest that phosphinic–carboxylic mixed anhydrides are more stable to thermal disproportionation than mixed carboxylic anhydrides. Acetic diphenylphosphinic anhydride (**8**) has been prepared[19] by reaction between acetic anhydride and diphenylphosphinic acid at 60 °C. It is a crystalline compound (m.p. 93—97 °C) which undergoes disproportionation only at elevated temperatures.[19]

From this limited knowledge, together with the ready availability of diphenylphosphinic chloride[20] for the preparation of the mixed anhydrides (**9**), it was decided to investigate the possible exploitation of diphenylphosphinic carboxylic mixed anhydrides in peptide bond forming reactions.[1]

Discussion and Results

Evaluation of the Potential of Intermediates (**9**) via *Model Compounds.*—As a test of relative merits of pivalic and diphenylphosphinic mixed anhydrides, it was decided to form (**10**) which would give a measure of the tendency towards nucleophilic attack by path *b* (Scheme 1). The compound was conveniently prepared by reaction of sodium diphenylphosphinate with pivaloyl chloride in a yield of 77%. The reaction was complete after the mixture had been stirred for 2 h at ambient temperature in THF but was found to be slower in less polar solvents such as ethyl acetate or dichloromethane. It was also found that (**10**) could be isolated as a crystalline compound (70%, m.p. 131—133 °C) by reaction between diphenylphosphinic acid and pivaloyl chloride in the presence of

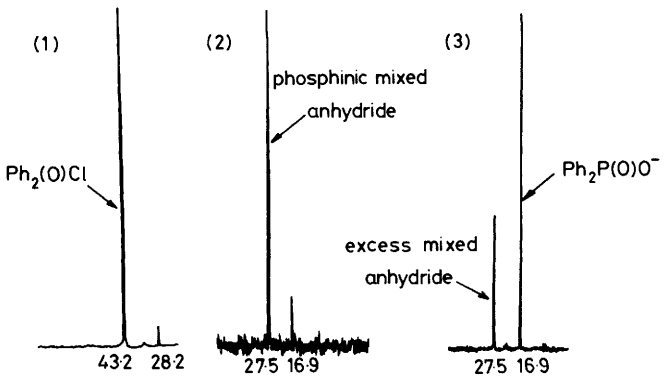
$Bu^tCO{\cdot}O{\cdot}P(O)Ph_2$ (**10**)

$Bu^tCONH(CH_2)_2Ph$ (**11**)

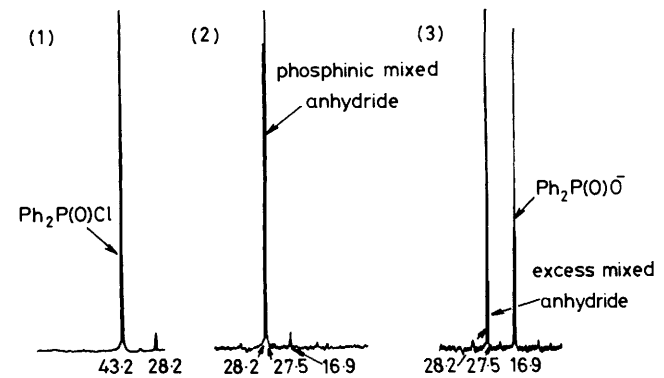
DPPLeu–AlaOMe (**12**)

poly(di-isopropylaminomethylstyrene). The resulting pivalic diphenylphosphinic mixed anhydride proved to be remarkably stable. It could be recrystallised repeatedly [ethyl acetate-light petroleum (b.p. 60—80 °C)] without any evidence of disproportionation and was found to react with 2-phenylethylamine to give (**11**) (m.p. 84—85 °C) exclusively. This is a clear indication of the potential of diphenylphosphinic mixed anhydrides as intermediates in peptide coupling reactions.

Attempted Isolation of N-*Protected* α-*Amino Acid Diphenylphosphinic Mixed Anhydrides.*—In an effort to further demonstrate the stability of diphenylphosphinic mixed anhydrides for use in peptide synthesis, the anhydride of N_α-diphenylphosphinyl-leucine (DppLeuOH)[21] and diphenylphosphinic acid was prepared by the reaction between the protected α-amino acid and DppCl in the presence of poly(diethylaminomethylstyrene). Evaporation of the solvent after filtration of the base gave a 70% yield of crystalline material (m.p. 120—125 °C) which was stored at 0 °C for 7 days and then allowed to react with alanine methyl ester. A 48% yield of DppLeu-AlaOMe (**12**) (m.p. 168—170 °C) was obtained in this way. However, no effect was made to optimise the yields of this route since initial attempts at peptide formation by *in situ* generation of the mixed anhydride followed by aminolysis met with considerable success (Table 1). In this latter respect, ^{31}P n.m.r. spectroscopy proved central to our investigative work as a non-invasive monitor of experimentation aimed at devising optimum reaction conditions [Figure (a) and (b)].



(a) ^{31}P n.m.r. spectrum of (**1**) DppCl (δ^P +28.2 p.p.m. is contaminating diphenylphosphinic acid); (**2**) 2 min after adding ZValOH/NMM/DppCl; (**3**) 10 min after addition of $TosO^-H_2{}^+$AlaOPh/NMM to (**2**).



(b) Same reaction as (a) demonstrating the effect of undesired partial hydrolysis.

Figure. Representative ^{31}P n.m.r. spectra of diphenylphosphinic chloride–mediated peptide bond formation in the preparation of the protected peptide ZVal-AlaOPh.

32.4 ^{31}P *N.M.R. Spectroscopy.*—Results from the study of the *in situ* formation and subsequent aminolysis of diphenylphosphinic carboxylic mixed anhydrides, with the aid of phosphorus-31 n.m.r. spectroscopy in which dichloromethane was used as the reaction solvent ($CDCl_3$ lock) and all measurements were carried out at 0 °C, indicate the following (Figure a).

(i) Mixed anhydride formation is almost instantaneous. The shift in the single signal attributable to diphenylphosphinic chloride from δ_P +43 ± 1 p.p.m. (lit.,[22] δ_P +42.7 p.p.m.) to that of the diphenylphosphinic mixed anhydrides at δ_P +27 ± 1 p.p.m. occurs completely within the time needed to mix the reactants, place in the spectrometer, and accumulate sufficient scans to build up a representative peak—a maximum of 3 min.

Table 1. Stepwise synthesis of peptides *via* diphenylphosphinic carboxylic mixed anhydrides. (↑ Indicates the point of coupling.)

Peptide*	M.p. (°C)	$[\alpha]_D^{25}$ [a] (°)	Yield (%)[b]	Lit.
ZOrn(Adoc) ↑ GlyOPh	109—110	−8.4	60 (49)	*d*
ZVal ↑ Orn(Adoc)-GlyOPh	138—139	−9.1	64 (64)	*e*
ZTyr(Bu^t) ↑ Orn(Adoc)-GlyOPh	101	−11.8	81	*d*
ZSer(Bu^t) ↑ Leu-GlyOPh	99	−12.6	73 (73)[c]	*d*
ZTyr(Bu^t) ↑ Ser(Bu^t)-Leu-GlyOPh	194—197	−13.2	55	*d*
BpocLeu ↑ Ala-GlyOPh	92—95	−29.1	63 (68)[c]	*d*
ZSer(Bu^t) ↑ AlaOPh	115—116	−28.9	75 (52)	*e*
ZVal ↑ Ser(Bu^t)-AlaOPh	164—165	−23.1	72 (64)	*e*
ZVal ↑ AlaOPh (**14**)	172—173	−40.5	81 (60)	26
ZLeu ↑ Val-AlaOPh (**16**)	184—186	−45.1	85 (65)	26
BpocPro ↑ Trp-LeuOPh	83—85	−42.5	88	
ZAsn ↑ GlyOPh (**17**)	172—174	−6.8	21 (83)	29
ZGln ↑ GlyOPh (**18**)	182	−16.1	75 (61)[c]	26

[a] $c = 1$ in DMF. [b] Yield from PivCl coupling. [c] Yield from isobutyl chloroformate coupling. [d] 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. [e] I. J. Galpin, G. W. Kenner, R. Ramage, and W. D. Thorpe, *Tetrahedron*, 1981, **37**, 3037.

(ii) The acylation reaction is almost instantaneous. The shift in the signal representative of the diphenylphosphinic carboxylic mixed anhydride from δ_P +27 ± 1 p.p.m. to that of the diphenylphosphinate anion, $Ph_2P(O)O^-$, at δ_P +17 ± 2 p.p.m. [lit.,[25] δ_P + 19.5 p.p.m. (in ethylene glycol)] occurs within the time taken to add the amino component (usually as a salt) and base, place in the spectrometer, and accumulate sufficient scans to build up a representative peak—a maximum of 4 min. The use of excess mixed anhydride (5—10%) acts as a useful internal standard. However, a cautionary note should be raised at this point. It has frequently been observed that unless great care is taken with small-scale reactions a limited amount of hydrolysis may occur giving rise to a signal adjacent to that of the mixed anhydride and only some 0.5 p.p.m. further downfield. The precise origin of this peak has not been unequivocally determined but is consistent with the symmetrical anhydride (**13**) arising from reaction between diphenylphosphinic acid and diphenylphosphinic chloride (but not from disproportionation[18]). Fortunately, this does not unduly cloud the overall picture (Figure b) and the rate of the acylation reaction is not significantly affected; such hydrolysis of the chloride will lead, however, to a decrease in the yield of reaction.

Nonetheless, ^{31}P n.m.r. spectroscopy has proved to be an invaluable technique that not only highlights the rapidity with which these reactions proceed but also acts as a powerful tool for the optimisation of the reaction conditions which are outlined below and were employed in the synthesis of a series of model peptides (Table 1).

Synthesis of Model Peptides: Reaction Conditions.—The possible application of diphenylphosphinic carboxylic mixed anhydrides to peptide synthesis was originally evaluated by the stepwise synthesis of small peptide fragments related to the amino acid sequence of an analogue of Hen Egg White lysozyme.[24] In the initial study, the standard conditions used for the pivalic mixed anhydride couplings[25] were employed, *viz.* formation of the mixed anhydride was allowed to take place at −20 °C in dichloromethane or ethyl acetate using NMM as base and the amino component was added after a further 20 min. The reaction mixture was then left to warm to room temperature overnight when work-up gave easily purified products in good yield (Table 1)—usually better than had previously been achieved with pivaloyl chloride. However, in view of the results of our ^{31}P n.m.r. studies (above) and a series of syntheses of the dipeptide ZVal-AlaOPh (**14**)[26] in which activation and acylation times were varied (Table 2), the following general experimental procedure was developed. To a

$Ph_2P(O)\cdot O\cdot P(O)Ph_2$ (**13**)

ZVal–AlaOPh (**14**)

$Br^-H_2{}^+$Val–AlaOPh (**15**)

ZLeu–Val–AlaOPh (**16**)

ZAsn–GlyOPh (**17**)

ZGln–GlyOPh (**18**)

solution of a suitably protected α-amino acid (10 mmol) dissolved in dry, freshly distilled dichloromethane (30—40 ml) cooled to 0 °C were added NMM (10 mmol) and a solution of diphenylphosphinic chloride (10 mmol) in dichloromethane (10 ml) in quick succession; after the mixture had been stirred at low temperature for 10 min, a solution of the amino component (9 mmol) in dry distilled *N,N*-dimethylformamide (10—20 ml) was then added followed by NMM (9 mmol)—where appropriate. The reaction mixture was worked up after being stirred for a further 30 min. There is, of course, the added advantage that in large-scale preparations the diphenylphosphinic acid by-product may be isolated allowing subsequent re-cycling to diphenylphosphinic chloride.[27]

With the exception of asparagine (Table 1) no problems were experienced with unwanted by-products formed during the coupling reactions. The low yield (21%) of the union leading to ZAsn-GlyOPh (**17**) (carried out in *N,N*-dimethylformamide with a 10 min. activation time, triethylamine as base and in the presence of 1 equiv. of pyridine) must be attributed to interference by the asparagine side-chain primary amide.[28] Attempts to increase the yield by varying the activation time were largely ineffective (Table 3) and the synthesis of this dipeptide was finally achieved[29] by the pivalic mixed anhydride method.[25] In this context, it should be noted that no such problems have been encountered in the synthesis of glutamine-containing compounds for example, ZGln-GlyOPh (**18**).

The success of this investigative combination of ^{31}P n.m.r. and synthetic work prompted a thorough kinetic study,[18,30,31] the results of which will be published in full in due course.[18]

Practical Exploitation.—In view of these results it was decided to evaluate further the practical potential of mixed anhydrides of this type. We have recently reported[21] the synthesis of the fully protected C-terminal tetrapeptide of gastrin—DppTrp-Met-Asp(OBu^t)-PheNH$_2$—*via* a combination of

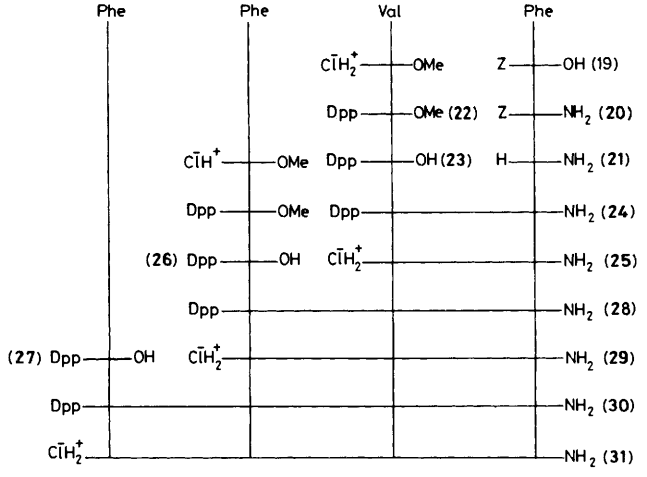
Table 2. Preparation of ZVal-AlaOPh[26] (**14**)

Activating agent	Activation time (min)	Acylation time (min)	Yield (%)
Isobutyl chloroformate	20	Overnight	76
Pivaloyl chloride	20	Overnight	60
Diphenylphosphinic chloride	20	Overnight	80
	20	120	83
	20	30	78
	10	30	80
	5	10	75

Table 3. Effect of varying activation time on the yield of ZAsn-GlyOPh[29] (**17**)

Activation time (min)	Yield (%)
2	31
7	28
10	21
30	24
60	15

N_α-diphenylphosphinyl protection and, the more classical, DCCI/HONSu activation.[32] It was, therefore, our initial intention to repeat this synthesis by employing both diphenylphosphinyl protection and activation to compare the yields obtained. The results of this work are summarised in Table 4. In addition, the tetrapeptide HPhe-Phe-Val-PheNH$_2$ was considered to present an excellent opportunity to examine further the suitability of our Dpp-based synthetic strategy for peptide synthesis together with the effectiveness of our techniques for the selective removal of the protecting group whilst leaving the C-terminal amide unscathed (Scheme 2).



Scheme 2.

Preparation of ZPheOH (**19**) was achieved in excellent yield (see Experimental section) and without any of the problems experienced in earlier attempts[33] where acceptable material could be obtained only after purification by counter current distribution.[35a] This was then converted into the corresponding amide (**20**), again in good yield, by overnight reaction of (**19**) with the ammonium salt of hydroxybenzotriazole ($^+NH_4^-$OBt)[34] in the presence of DCCI. Catalytic hydrogenolysis of (**20**) provided phenylalanine amide (**21**) which was shown to be identical with authentic material purchased from a commercial source. From this point onwards the synthesis proceeded virtually without incident. DppValOH and DppPheOH (twice) were added sequentially to (**21**) *via* the general diphenylphosphinic carboxylic mixed anhydride procedure derived above to give the fully protected di-, tri-, and tetra-peptides in excellent yields (80—90%). The protecting group was removed from these intermediates in the usual way[21] to provide, eventually, $Cl^-H_2{}^+$Phe-Phe-Val-PheNH$_2$.

Conclusion. The results of this investigation illustrate the potential of diphenylphosphinic carboxylic mixed anhydrides for peptide synthesis. Only 5—10% excess of the mixed anhydride is required for optimum results and there are none of the problems associated with undesired opening of the mixed anhydride in couplings involving α-amino acids. In the cases quoted in Tables 1, 2, and 4 the products were easier to isolate in pure form from the syntheses involving the organophosphorus methodology. The exceptional case in Table 1 was the formation of ZAsn-GlyOPh and we would recommend, therefore, the pivaloyl chloride method of activation for asparagine residues. It should be noted that an investigation of the application of this methodology to solid-phase synthesis has recently been reported.[35]

Experimental

The general experimental methods, abbreviations and solvent systems for thin-layer chromatography are those previously described,[21] with the addition of (F) $CHCl_3$:AcOH:MeOH 95:5:3, (G) $CHCl_3$:Pr^{i}OH 15:1 and (H) Bu^{n}OH:AcOH:H_2O 7:2:1. As a postscript to all coupling reactions, the precautionary measure of establishing the absence of any contaminating diphenylphosphinic acid (DppOH—the by-product of these reactions) was taken by employing both thin-layer chromatography [remains at the base line in all systems employed to date (I, UV)] and ^{31}P n.m.r. [δ_P +27 ± 1 p.p.m. (methanol/$CDCl_3$ lock)] techniques. The numbering of the carbon atoms in the aromatic ring of the benzyloxycarbonyl group and phenyl ester are as for phenylalanine in footnote (c) of Table 3 in ref. 21.

N$_\alpha$-Diphenylphosphinoyl-leucylalanine Methyl Ester, DppLeu-AlaOMe (**12**).—*N$_\alpha$*-Diphenylphosphinoyl-leucine[21] (662 mg, 2 mmol) was dissolved in DCM (3 ml), NMM (220 μl, 2 mmol) added and the solution cooled to −20 °C; at this temperature DppCl (473 mg, 2 mmol) in DCM (3 ml) was added in such a way as to maintain the temperature of the reactants at −20 °C. After being stirred for 15 min, the reaction mixture was filtered, the solvent removed by evaporation under reduced pressure, and the remaining semi-crystalline residue triturated with anhydrous diethyl ether. The resultant powder [(720 mg, 70%), m.p. 120—125 °C] was filtered off and stored in a refrigerator for 7 days prior to its use in a coupling reaction. Thus, to a solution of the crystalline mixed phosphinic anhydride (265 mg, 0.5 mmol) dissolved in DCM (2 ml) and cooled to −20 °C were added alanine methyl ester hydrochloride[36] (70 mg, 0.5 mmol)

Table 4. Comparison of yields obtained in the stepwise synthesis of DppTrp-Met-Asp(OBu^t)-PheNH$_2$

Amino acid	Method of activation	Excess (%) of acylating agent	Amine component	Product	Yield (%)
ZAsp(OBu^t)OH	DCCI/HONSu	—	HPheNH$_2$	ZAsp(OBu^t)-PheNH$_2$	70
	Piv-Cl	10			68
	DppCl	10			80
DppMetOH	DCCI/HONSu	—	HAsp(OBu^t)-PheNH$_2$	DppMet-Asp(OBu^t)-PheNH$_2$	73
	DppCl	10			78
DppTrpOH	DCCI/HONSu	—	Cl$^-$H$_2$$^+$Met-Asp(OBu^t)-PheNH$_2$	DppTrp-Met-Asp(OBu^t)-PheNH$_2$	65
	DppCl	10			80

in DMF (3 ml) and NMM (55 μl, 0.5 mmol). The reactants were then allowed to warm to room temperature overnight with vigorous stirring when the solvents were evaporated under reduced pressure to afford a pale yellow oil which was partitioned between ethyl acetate and water. The organic layer was isolated and washed with saturated $NaHCO_3$ (×5), 5% citric acid (×3), water (×2), saturated $NaHCO_3$ (×2), water (×2), and brine (×2) before being dried ($MgSO_4$). Evaporation of the dry solution gave N_α-*diphenylphosphinoyl-leucylalanine methyl ester* as a white powder which was filtered off and recrystallised from diethyl ether–cyclohexane (100 mg, 48%). A repeat preparation of (**12**) following the general procedure for the *in situ* formation of the amide bond given in the text led to a much improved yield of the fully protected dipeptide (84%), m.p. 168—170 °C (Found: C, 63.1; H, 7.3; N, 6.7; P, 7.6. $C_{22}H_{29}N_2O_4P$ requires C, 63.4; H, 7.0; N, 6.7; P, 7.5%); $[\alpha]_D^{24}$ +26.5° (*c* 1 in MeOH); t.l.c.-A R_F 0.71 (UV, I); amino acid analysis Ala$_1$ 0.99, Leu$_1$ 1.01; $\nu_{max.}$ 3 360, 3 250, 3 220 (NH), 1 740 (ester CO), 1 663 (amide CO), 1 550 (CONH), 1 440 (Ph-P), and 1 180 cm^{-1} (PO); $\lambda_{max.}$ 253infl. (ε 789), 258 (1 063), 264 (651) and 271nm (1 020); $\delta_H(CDCl_3)$ 7.80—7.20 (11 H, m, ArH and Ala NH), 4.25 (1 H, m, Ala α-CH), 3.60 (1 H, m, Leu NH—exchanges with D_2O) partially obscured by 3.55 (4 H, s, ester CH_3 and Leu α-CH). 1.55 (2 H, m, Leu β-CH_2), 1.33 (1 H, m, Leu γ-CH), 1.12 (3 H, d, $^3J_{\alpha CH\text{-}\beta CH_3}$ 7.3 Hz, Ala β-CH_3), 0.65 (3 H, d, $^3J_{\gamma CH\text{-}\delta CH_3}$ 6.20 Hz), and 0.55 (3 H, d $^3J_{\gamma CH\text{-}\delta CH_3}$ 6.20 Hz, Leu-δ-CH_3); $\delta_C(CDCl_3)$ 173.15, 172.98 (d, $^3J_{P\text{-}CO}$ 3.4 Hz, Leu CO), 172.39 (Ala CO), 135.28—127.70 (ArC), 52.44 (Leu α-C),[a] 51.65 (ester CH_3),[b] 47.78 (Ala α-C), 43.80, 43.53 (d, $^3J_{P'\text{-}C}$ 5.5 Hz Leu β-C), 23.96 (Leu γ-C), 22.37, 21.91 (Leu δ-C), 16.96 (Ala β-C) [[a,b] assigned on the basis of multiplicity of respective signals in the off-resonance decoupled spectrum]; δ_P(MeOH–$CDCl_3$) 23.45.

N$_\alpha$-*Benzyloxycarbonylvalylalanine Phenyl Ester*, ZVal-Ala-OPh (**14**).—N_α-Benzyloxycarbonylvaline[37] (30.15 g, 0.12 mol) was dissolved in DCM (200 ml) and cooled to 0 °C at which temperature NMM (13.2 ml, 0.12 mol) and a solution of DppCl (28.38 g, 0.12 mol) in DCM (200 ml) were added in quick succession. After an activation period of 15 min a precooled solution of alanine phenyl ester tosylate[38] (33.74 g, 0.1 mol) in dry, distilled DMF (80 ml) was added immediately followed by NMM (11 ml, 0.1 mol); the resulting mixture was stirred for a further 30 min before being worked up as described for (**12**). Evaporation of solvent under reduced pressure from the final dried solution afforded a white powder which was recrystallised from ethyl acetate with light petroleum (b.p. 60—80 °C) to give pure N_α-benzyloxycarbonylvalylalanine phenyl ester (38.73 g, 81%), m.p. 172—173 °C (lit.,[26] 173—174 °C) (Found: C, 66.0; H, 6.7; N, 6.9. Calc. for $C_{22}H_{26}N_2O_5$ C, 66.3; H, 6.6; N, 7.0%); $[\alpha]_D^{26}$ −45.1° (*c* 1 in DMF) [lit.,[26] $[\alpha]_D^{30}$ −42.1° (*c* 1 in DMF)]; t.l.c.-D R_F 0.75 (I, UV); amino acid analysis Ala$_1$ 1.02, Val$_1$ 1.00; $\nu_{max.}$ 3 300 (NH), 1 740 (ester CO), 1 690 (amide CO), 1 540 (CONH) and 1 180 cm^{-1} (C-OPh); $\lambda_{max.}$ 251 (ε 356), 257 (428), 260infl. (356), 262 (330) and 266 nm (228); $\delta_H(CDCl_3)$ 7.11—6.71 (10 H, m, ArH), 6.51 (1 H, br s, Ala NH), 5.28 (1 H, br, Val NH—exchanges with D_2O), 4.80 (2 H, s, Z-CH_2), 4.41 (1 H, m, Ala α-CH), 3.71 (1 H, m, Val α-CH), 1.71 (1 H, m, Val β-CH), 1.22 (3 H, d, $^3J_{\alpha CH\text{-}\beta CH_3}$ 7.5 Hz, Ala β-CH_3), 0.70 (3 H, d, $^3J_{\beta CH\text{-}\gamma CH}$ 5.0 Hz), and 0.65 (3 H, d, $^3J_{\beta CH\text{-}\gamma CH_3}$ 5.0 Hz, Val γ-CH_3); $\delta_C(CDCl_3)$ 171.23 (Val and Ala CO), 156.46 (urethane CO), 150.51 (ester C-l), 136.24 (Z C-l), 129.37 (ester C-3, C-5), 128.40 (Z C-3, C-5), 128.04 (Z C-4), 127.85 (Z C-2, C-6), 125.97 (ester C-4), 121.11 (ester C-2, C-6), 66.94 (Z-CH_2), 60.32 (Val α-C), 48.11 (Ala α-C), 31.05 (Val β-C), 18.90 (Val γ-C), and 17.69 (Val γ-C and Ala β-C); *m/z* 305.15 (M^+ −93, 15.14%), ZVal-NHCHCH(CH$_3$)$_2$C$^+$=O), 234.11 [M^+ −164, ZNHCHCH(CH$_3$)$_2$C$^+$=O], 206.12 [M^+ −192, 4.53%, Z$^+$N(H)=CHCH(CH$_3$)$_2$], 169.09 (M^+ −229, 18.73%, $C_8H_{13}N_2O_2$),[39] 162.13 [M −236, 15.09%, PhCH$_2$$^+$N(H)=CHCH(CH$_3$)$_2$], 107.05 ($M^+$ −291, 17.30%, PhCH$_2$O$^+$), 94.05 (M^+ −304, 15.43%, PhO$^+$H), and 91.05 (M^+ −307, 100.00%, PhCH$_2$$^+$).

Valylalanine Phenyl Ester Hydrobromide, Br$^-$H$_2$$^+$Val-AlaOPh, (**15**).— ZVal-AlaOPh (**14**) (7.39 g, 18.5 mmol) was dissolved in 45% HBr–glacial acetic acid (12 ml) and the resulting clear, pale yellow solution was stirred under anhydrous conditions at room temperature for 2.5 h when *valylalanine phenyl ester hydrobromide* was precipitated by the addition of anhydrous diethyl ether as white crystals which were filtered off and dried. Purification of (**15**) was achieved by recrystallisation from propan-2-ol with anhydrous diethyl ether (5.89 g, 92%), m.p. 185—187 °C (Found: C, 48.4; H, 6.1; Br, 23.1; N, 8.2. $C_{14}H_{21}BrN_2O_3$ requires C, 48.7; H, 6.1; Br, 22.8; N, 8.1%); $[\alpha]_D^{24}$ −11.2° (*c* 1 in methanol); t.l.c.-H R_F 0.75 (I, N); amino acid analysis Ala$_1$ 1.00, Val$_1$ 0.97; $\nu_{max.}$ 3 400—2 350, 1 980 ($^+$NH$_3$), 3 230 (NH), 1 740 (ester CO), 1 690 (amide CO), and 1 190 cm^{-1} (C–OPh); $\lambda_{max.}$ 252inf. (ε 188), 255infl. (204), 257 (213), 260infl. (185), and 264nm (160); $\delta_H(D_2O)$ 6.91—6.55 (5 H, m, ArH), 4.27 (m, obscured by HOD peak, Ala α-CH), 3.51 (1 H, d, $^3J_{\alpha CH\text{-}\beta CH}$ 6.0 Hz, Val α-CH), 1.84 (1H, m, Val β-CH), 1.17 (3 H, d, $^3J_{\alpha CH\text{-}\beta CH_3}$ 7.5 Hz, Ala β-CH_3), and 0.60 (6 H, m, Val γ-CH_3); δ_C[$(CD_3)_2SO$] 171.14 (Val C=O),[a] 168.23 (Ala C=O),[b] 150.62 (ester C-1), 129.85 (ester C-3, C-5), 126.26 (ester C-4), 121.65 (ester C-2, C-6), 57.15 (Val α-C), 48.34 (Ala α-C), 29.94 (Val β-C), 18.22 and 17.86 (Val γ-C), and 16.58 (Ala β-C), [[a,b] not unequivocally assigned and may be reversed]; *m/z* 171.11 (M^+ 174, 12.03%, HVal-Ala.C$^+$=O), 143.12 (M^+ −202, 4.74%, HVal-$^+$N(H)=CHCH$_3$), 94.04 (M^+ −251, 42.41%, PhO$^+$H), and 72.08 [M^+ −273, 100.00%, H$_2$$^+$N=CHCH(CH$_3$)$_2$].

N$_\alpha$-*Benzyloxycarbonyl-leucylvalylalanine Phenyl Ester*, ZLeu-Val-AlaOPh (**16**).—N_α-Benzyloxycarbonyl-leucine (ZLeuOH) was liberated from its dicyclohexylammonium salt[40] (25.76 g, 58 mmol) by suspension of the latter in ethyl acetate (250 ml) and vigorous shaking of the suspension with an equal volume of saturated aqueous citric acid; the organic solution was then separated and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a colourless oil (ZLeuOH, 16g >100%) which was taken up in DCM (100 ml) and cooled to 0 °C. NMM

(6.38 ml, 58 mmol) and a solution of DppCl (13.72 g, 58 mmol) in DCM (70 ml) were added and the reactants stirred for 15 min after which time (**15**) (17.94 g, 52 mmol) was added followed by NMM (5.72 ml, 52 mmol). The resulting mixture was stirred for a further 30 min when DCM was removed *in vacuo* and saturated aqueous $NaHCO_3$ was added to the remaining DMF solution. The white solid which separated was filtered off, washed as described for (**12**), and dried. Reprecipitation from DMF with water gave pure N_α-benzyloxycarbonyl-leucylvalyl-alanine phenyl ester as a finely divided white powder which was filtered off and dried (23.4 g, 87%), m.p. 184—186 °C (lit.,[26] 186 °C) (Found: C, 65.4; H, 7.5; N, 7.9. Calc. for $C_{28}H_{37}N_3O_6$ C, 65.7; H, 7.3; N, 8.2%); $[\alpha]_D^{20}$ −41.3° (*c* 1 in DMF) [lit.,[26] $[\alpha]_D^{26}$ −45.1° (*c* 1 in DMF)]; t.l.c.-A R_F 0.95 (I, UV); amino acid analysis Ala_1 1.02, Leu_1 1.00, Val_1 1.10; $\nu_{max.}$ 3 300 (NH), 1 760 (ester CO), 1 690 (amide CO), 1 640 (urethane CO), 1 540 (CONH), and 1 200 cm^{-1} (C-OPh); $\lambda_{max.}$ 251 (ε 356), 257 (450), 262 (350), and 266infl. nm (234); $\delta_H[(CD_3)_2SO]$ 9.10 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 6.9 Hz, Ala NH), 8.15 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.4 Hz, Val NH), 7.93 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.5 Hz, Leu NH), 7.90—7.45 (10 H, m, ArH), 5.45 (2 H, s, Z-CH_2), 4.85 (1 H, m, Leu α-CH), 4.70 (1 H, m, Val α-CH), 4.55 (1 H, m, Ala α-CH), 2.40 (1 H, m, Leu γ-CH), 2.05 (1 H, br m, Val β-CH), 1.85 (d $^3J_{\alpha CH\text{-}\beta CH_3}$ 7.5 Hz) superimposed on 1.95—1.80 (m, 5 H—Ala β-CH_3 and Leu β-CH_2), 1.35—1.10 (12 H, m, Val γ-CH_3 and Leu δ-CH_3); δ_C $[(CD_3)_2SO]$ 172.30 (Leu CO), 171.39 (Val CO),[a] 171.20 (Ala CO),[b] 156.02 (urethane CO), 150.68 (ester C-1), 137.20 (Z C-1), 129.60 (ester C-3, C-5), 128.39 (Z C-3, C-5), 127.78 and 127.66 (Z C-2, C-4, C-6), 125.96 (ester C-4), 121.59 (ester C-2, C-6), 65.47 (Z-CH_2), 56.91 (Val α-C), 53.51 (Leu α-C), 48.10 (Ala α-C), 40.63 (Leu β-C), 31.10 (Val β-C), 24.24 (Leu γ-C), 22.96 and 21.38 (Leu δ-C), 18.95 and 17.92 (Val γ-C), and 16.40 (Ala β-C) [[a,b]These assignments may be reversed]; *m/z* 418.23 (M^+ −93, 2.9%, ZLeu-Val-AlaC$^+$=O), 347.20 (M^+ −164, 2.5%, ZLeu-ValC$^+$=O), 319.20 [M^+ −192, 0.9%, ZLeu$^+$N(H)=CHCH$(CH_3)_2$], 248.13 (M^+ −263, 2.9%, ZLeuC$^+$=O), 220.3 [M^+ −291, 4.4%, Z$^+$ N(H)=$CHCH_2CH(CH_3)_2$], 94.01 (M^+ −417, 15.2%, PhO$^+$H), 91.05 (M^+ −420, 100.00%, $PhCH_2^+$), 86.10 [M^+ −425, 2.9%, H_2N^+=$CHCH_2CH(CH_3)_2$], and 72.08 [M^+ −439, 10.9%, H_2N^+=CHCH−$(CH_3)_2$].

N_α-*Benzyloxycarbonylasparaginylglycine Phenyl Ester*, ZAsn-GlyOPh (**17**).—A solution of glycine phenyl ester hydrobromide[38] (2.32 g, 10 mmol) in DMF (20 ml) was added to the diphenylphosphinic mixed anhydride formed between the reaction of N_α-benzyloxycarbonylasparagine[41] (2.66 g, 10 mmol), triethylamine (1.39 ml, 10 mmol), pyridine (0.81 ml, 10 mmol), and DppCl (2.36 g, 10 mmol) in DMF (15 ml) at −20 °C. This was immediately followed by the addition of triethylamine (1.39 ml, 10 mmol) and the resultant mixture was stirred overnight at ambient temperature. The reactants were poured into water and the solid thus precipitated was filtered off and washed successively with 5% aqueous $NaHCO_3$, 1M-acetic acid, water and diethyl ether. Reprecipitation of the dried powder from DMF with water afforded pure N_α-benzyloxycarbonylasparaginylglycine phenyl ester (838 mg, 21%), m.p. 172—174 °C (lit.,[29] 172—174 °C); $[\alpha]_D^{25}$ −6.8° (*c* 1 in DMF) [lit.,[29] $[\alpha]_D^{25}$ −6.8° (*c* 1 in DMF)]. Repetition of this reaction using Piv-Cl (10 mmol) to form the mixed anhydride gave (**17**) in 64% yield (2.55 g), m.p. 173—174 °C; $[\alpha]_D^{25}$ −7.0° (*c* 1 in DMF). The analytical data by which the purity of this compound was assessed has previously been reported.[29]

N_α-*Benzyloxycarbonylglutaminylglycine Phenyl Ester*, ZGln-GlyOPh (**18**).—To a stirred suspension of finely powdered N_α-benzyloxycarbonylglutamine[42] (9.63 g, 34.4 mmol), in DCM (40 ml) cooled to 0 °C were added pyridine (2.79 ml, 34.4 mmol), NMM (3.78 ml, 34.4 mmol) and a solution of DppCl (8.14 g, 34.4 mmol) in DCM (40 ml) and the reactants stirred for 15 min after which time $Br^-H_2{}^+$GlyOPh[38] (6.39 g, 27.52 mmol) in DMF (25 ml) was added followed by NMM (3.03 ml, 27.52 mmol). The resulting mixture was stirred for a further 30 min when the DCM was removed under reduced pressure and the desired product precipitated from the remaining DMF solution by the addition of saturated aqueous $NaHCO_3$. An oil initially separated which readily solidified to give the crude fully protected dipeptide as a pale yellow powder which was filtered off, washed as described for (**12**), and dried. Reprecipitation from DMF with water afforded pure N_α-benzyloxycarbonyl-glutaminylglycine phenyl ester (7.17 g, 75%), m.p. 182—184 °C (lit.,[26] 182 °C) (Found: C), 60.9; H, 5.8; N, 10.5. Calc. for $C_{21}H_{23}N_3O_6$: C, 61.0; H, 5.6; N, 10.2%); $[\alpha]_D^{23}$ −16.1° (*c* 1 in DMF) [lit.,[26] $[\alpha]_D^{27}$ −16.1° (*c* 1 in DMF)]; t.l.c.-B R_F 0.66, t.l.c.-B R_F 0.42 (I, UV); amino acid analysis Glu_1 1.01, Gly_1 1.00; $\nu_{max.}$ 3 425, 3 325, 3 300, 3 200 (NH), 1 760 (ester CO), 1 690 (amide CO), 1 660 (amide CO), 1 640 (urethane CO), 1 550 (CONH), and 1 210 cm^{-1} (CO–OPh); $\lambda_{max.}$ 251 (ε 358), 257 (434), 260infl. (367), 262 (345), and 266 infl nm (241); $\delta_H[(CD_3)_2SO]$ 8.40 (1 H, tr, $^3J_{NH\text{-}\alpha CH}$ 6.0 Hz, Gly NH), 7.45 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 7.5 Hz, Gln NH), 7.35—6.95 [11 H, m, ArH and (1) Gln $CONH_2$], 6.70 [1 H, s, (1) Gln $CONH_2$], 4.90 (2 H, s, Z-CH_2), 4.10—3.85 (3 H, m, Gln α-CH and Gly α-CH_2), 2.10 (2 H, tr, $^3J_{\beta CH_2\text{-}\gamma CH_2}$ 8.0 Hz, Gln γ-CH_2), 1.85 (1 H, m) and 1.70 (1 H, m) Gln β-CH_2; $\delta_C[(CD_3)_2SO]$ 174.18 (Gln δ-CO), 172.84 (Gln CO), 168.90 (Gly CO), 156.20 (urethane CO), 150.62 (ester C-1), 137.20 (Z C-l), 129.73 (ester C-3, C-5), 127.84 (Z C-2, C-4, C-6), 126.14 (ester C-4), 121.77 (ester C-2, C-6), 65.71 (Z CH_2), 54.54 (Gln α-C), 41.24 (Gly α-C), 31.52 (Gln γ-C), 27.82 (Gln β-C); *m/z* 320.12 (M^+ −93, 3.76%, ZGln-GlyC$^+$=O), 235.11 [M^+ −178, 3.28%, Z$^+$N(H)=CH-$(CH_2)_2$-$CONH_2$], 120.08 [M^+ −293, 4.76%, $PhCH_2$- $^+$N(H)=CH_2], 94.04 (M^+ −319, 100.00%. PhO$^+$H), 91.03 (M^+ −322, 84.72%, $PhCH_2^+$).

N_α-*Benzyloxycarbonylphenylalanine*, ZPheOH (**19**).—Benzyl chloroformate (32 ml) and 4M-aqueous sodium hydroxide (80 ml) were added simultaneously over a period of 45 min to a vigorously stirred solution of phenylalanine (33 g, 0.2 mol) dissolved in 4M-aqueous sodium hydroxide (70 ml)/1M-aqueous sodium hydrogen carbonate (200 ml) precooled to 0 °C. The mixture was then allowed to warm to room temperature and stirred for a further 18 h. After extraction of the reaction liquors with diethyl ether (2 × 500 ml), the aqueous solution was added dropwise and very slowly to a vigorously stirred mixture of 4M-hydrochloric acid (500 ml) and ethyl acetate (750 ml). The aqueous phase was subsequently isolated, extracted with ethyl acetate (3 × 100 ml), and the organic phases combined. These in turn were washed with water (3 × 100 ml) and brine (2 × 100 ml) before being dried ($MgSO_4$). Evaporation of the solvent from the dried solution gave a clear, colourless oil which, upon trituration with light petroleum (b.p. 60—80 °C), gave N_α-benzyloxycarbonylphenylalanine as a finely divided white powder. This was filtered off, dried, and recrystallised from ethyl acetate with light petroleum (b.p. 60—80 °C) to afford pure (**19**) (45 g, 75%), m.p. 88 °C (lit.,[33] 87 °C) (Found: C, 68.4; H, 5.7; N, 4.9. Calc. for $C_{17}H_{17}NO_4$: C, 68.2; H, 5.7; N, 4.7%); $[\alpha]_D^{24}$ +5.2° (*c*2 in EtOAc) [lit.,[33] $[\alpha]_D^{18}$ +5.3° (*c* 6.6 in AcOH)]; t.l.c.-F R_F 0.46 (UV, I); $\nu_{max.}$ 3 500—2 350 (acid OH) (3 420 NH), and 1 710 cm^{-1} (acid and urethane CO); $\lambda_{max.}$ 242infl. (ε 161), 247 (222), 251 (307), 257 (391), 260infl. (294), 263 (309), and 267nm (194); $\delta_H(CDCl_3)$ 10.45 (1 H, br s, CO_2H), 7.35—7.10 (10 H, m, aromatic H), 5.46 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 7.8 Hz, Phe NH—exchanges with D_2O), 5.10 (2 H, s, Z-CH_2), 4.70 (1 H, m, Phe α-CH—collapses to tr on D_2O exchange), 3.12 (2 H, m—overlapping ABq centred at 3.20 and 3.05, Phe β-CH_2); $\delta_C(CDCl_3)$ 175.40 (Phe CO), 156.00 (urethane CO), 135.60 (Z,

Phe C-1), 129.12—126.87 (m, Z, Phe C-2–C-6), 66.98 (Z-CH_2), 54.55 (Phe α-C), and 37.49 (Phe β-C); *m/z* 254.117 (M^+ −45, 0.14%, $Z^+NH{=}CHCH_2Ph$), 120.080 (M^+ −179, 2.94%, $H_2N^+{=}CHCH_2Ph$), and 91.055 (M^+ −208, 100.00%, $PhCH_2^+$).

N_α-*Benzyloxycarbonylphenylalanine Amide*, $ZPheNH_2$ (**20**).—A solution of DCCI (5.5 g, 26.67 mmol) in dry distilled DCM (35 ml) was added to a vigorously stirred clear, colourless solution of (**19**) (7.25 g, 25.25 mmol) and $^+NH_4{}^-OBt$ [34] (3.69 g) in DCM (75 ml) in such a way as to ensure that the temperature of the reaction mixture remained at 0 °C. This was allowed to warm to room temperature overnight when glacial acetic acid (2 ml) was added. After the mixture had been stirred for a further 1 h at ambient temperature DCU was filtered off and the resulting pale yellow organic solution was washed with 1M-$NaHCO_3$, water, and brine (3 × 50 ml) before being dried ($MgSO_4$). Evaporation of the reaction solvent under reduced pressure gave a pale yellow powder which was recrystallised from hot methanol to afford pure N_α-benzyloxycarbonylphenylalanine amide as long white needles. These were filtered off, washed with anhydrous diethyl ether, and dried (6.0 g, 83%), m.p. 164 °C (lit.,[43] 164—165 °C) (Found: C, 68.2; H, 6.2; N, 9.4. Calc. for $C_{17}H_{18}N_2O_3$ C, 68.4; H, 6.1; N, 9.4%); $[\alpha]_D^{25}$ −6.3° (*c* 1 in MeOH [lit.,[43] $[\alpha]_D^{25}$ −6.8° (*c* 1 in MeOH); t.l.c.-G R_F 0.48 (UV, I); $\nu_{max.}$ 3 425, 3 325, 3 275, 3 200 (NH), 1 700 (urethane CO), 1 660 (amide CO), and 1 540 cm^{-1} (CONH); $\lambda_{max.}$ 241 (ε 161), 246 (218), 251 (302), 257 (384), 260infl. (287), 263 (304), and 267nm (191); $\delta_H[(CD_3)_2SO]$ 7.47 (1 H, s) and 7.08 (1 H, s, $CONH_2$), 7.38 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.0 Hz, Phe NH), 7.35—7.12 (10 H, m, ArH), 4.95 (2 H, s, Z-CH_2), 4.22 (1 H, m, Phe α-CH), 3.00 (1 H, ABq, $^2J_{\beta CH\text{-}\beta CH}$ 13.5 Hz, $^3J_{\alpha CH\text{-}\beta CH}$ 5.0 Hz) and 2.75 (1 H, ABq, $^2J_{\beta CH\text{-}\beta CH}$ 13.5 Hz, $^3J_{\alpha CH\text{-}\beta CH}$ 9.0 Hz, Phe β-CH_2); $\delta_C[(CD_3)_2SO]$ 173.65 (Phe CO), 156.03 (urethane CO), 138.47 (Phe C-1), 137.26 (Z C-1), 129.33 (Phe C-3, C-5), 128.40 (Z C-3, C-5), 128.18 (Phe C-2, C-6), 127.80 (Z C-4), 127.58 (Z C-2, C-6), 126.37 (Phe C-4), 65.31 (ZCH_2), 56.12 (Phe α-C), and 37.57 (Phe β-C); *m/z* 298.13 (M^+, 0.50%), 254.11 (M^+ −44, 35.36%, $Z^+NH{=}CHCH_2Ph$), 210.12 (M^+ −88, 49.83%, $PhCH_2{}^+NH{=}CHCH_2Ph$), 120.081 (M^+ −178, 1.53%, $H_2N^+{=}CHCH_2Ph$), and 91.053 (M^+ −207, 100.00%, $PhCH_2^+$).

Phenylalanine Amide, $HPheNH_2$ (**21**).—A solution of (**20**) (4.42 g, 14.83 mmol) in dry, distilled DMF (35 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 under reduced pressure to yeild phenylalanine amide (**21**) as a white powder which was collected under anhydrous diethyl ether, filtered off, and dried (2.33 g, 96%), m.p. 90—91 °C (lit.,[44] 91—92 °C) (Found: C, 65.7; H, 7.2; N, 16.9. Calc. for $C_9H_{12}N_2O$; C, 65.8; H, 7.4; N, 17.1%); $\nu_{max.}$ 3 450, 3 360, 3 300 (NH), 1 680 (amide CO) and 1 550 cm^{-1} (CONH); $\delta_H[(CD_3)_2SO]$ 7.15 (1 H, s) and 6.82 (1 H, s, $CONH_2$), 7.00—6.87 (5 H, m, ArH), 3.15 (1 H, m, Phe α-CH), 2.72 (1 H, ABq $^2J_{\beta CH\text{-}\beta CH}$ 13.5 Hz, $^3J_{\alpha CH\text{-}\beta CH}$ 5.0 Hz) and 2.38 (1 H, ABq $^2J_{\beta CH\text{-}\beta CH}$ 13.5 Hz, $^3J_{\alpha CH\text{-}\beta CH}$ 8.5 Hz, Phe β-CH_2), and 1.43 (2 H, s, Phe NH_2); $\delta_C[(CD_3)_2SO]$ 177.34 (Phe CO), 139.14 (Phe C-1), 129.54 (Phe C-2, C-6), 128.33 (Phe C-3, C-5), 126.32 (Phe C-4), 56.42 (Phe α-C), and 41.30 (Ph β-C).

N_α-*Diphenylphosphinoylvaline Methyl Ester*, DppValOMe (**22**).—To a vigorously stirred solution of valine methyl ester hydrochloride [45] (33.5 g, 0.2 mol) in DCM (300 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 DCM (90 ml)—in such a way as to maintain the temperature of the reaction mixture at 0 °C. After the mixture had been stirred for 2 h the reaction solvent was removed under reduced pressure to afford a colourless oil which was partitioned between ethyl acetate and water. The organic layer was isolated and washed as described for (**12**) before being dried ($MgSO_4$). Evaporation of the dry solution under reduced pressure gave N_α-*diphenylphosphinoylvaline methyl ester* as a white powder which was filtered off and recrystallised from ethyl acetate with hexane (52.0 g, 78%); $\nu_{max.}$ 3 180 (NH), 1 740 (ester CO), 1 440 (Ph–P), and 1 180 cm^{-1} (PO); $\lambda_{max.}$ 254infl. (ε 805), 259 (1 125), 264 (1 466), and 271nm (1 180); the remainder of the analytical data by which this compound was fully characterised has previously been reported.[21]

N_α-*Diphenylphosphinoylvaline*, DppValOH (**23**).—To a solution of (**22**) (14.5 g, 44 mmol) dissolved in 1,4-dioxane (100 ml) was added 1M-sodium hydroxide (49 ml, 49 mmol) and the resulting mixture stirred at room temperature until thin-layer chromatography [t.l.c.-A, follow disappearance of (**22**), R_F 0.70] indicated saponification to be complete. The dioxane was removed under reduced pressure and acidification to pH 3 of the residual aqueous solution with 5% citric acid caused 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 being dried ($MgSO_4$). Evaporation to dryness under reduced pressure gave a clear, colourless oil which solidified under diethyl ether after several days to afford (**23**) (11.57 g, 80%). Subsequent analysis indicated the product to contain one molecule of crystallisation of dioxane, *i.e.* to be N_α-*diphenylphosphinoylvaline*·$C_4H_8O_2$, m.p. 102 °C (Found: C, 62.6; H, 7.3; N, 3.5; P, 7.7. $C_{21}H_{28}NO_5P$ requires C, 62.2; H, 7.0; N, 3.5; P, 7.6%); $\nu_{max.}$ 3 325 (NH), 3 200—2 100 (acid OH), 1 720 (ester CO), 1 440 (Ph–P) and 1 180 cm^{-1} (PO); $\lambda_{max.}$ 254 (ε 750), 259 (1 050), 265 (1 400), and 271nm (1 100); $\delta_C(CDCl_3)$ 174.13 (d, $^3J_{P'\text{-}CO}$ 2.7 Hz, Val CO), 134.26—127.98 (aromatic C), 66.74 (dioxane), 58.89 (Val α-C), 31.97 (d, $^3J_{P'\text{-}C}$ 4.0 Hz, Val β-C), 18.74 and 17.43 (Val γ-C); $\delta_P(CDCl_3)$ +25.18; *m/z* 317.120 (M^+, 1.05%), 274.063 (M^+ −43, 14.06%, $Dpp^+NH{=}CHCO_2H$), 272.119 [M^+ −45, 51.20%, $Dpp^+NH{=}CHCH(CH_3)_2$], 217.043 (M^+ −100, 100.00%, $Ph_2\overset{+}{P}{=}O$), 72.083 [M^+ −245, 7.48%, $H_2N{\triangleq}CHCH(CH_3)_2$]; the remainder of the analytical data by which this compound was fully characterised has previously been reported.[21]

N_α-*Diphenylphosphinoylvalylphenylalanine Amide*, DppValPheNH$_2$ (**24**).—To a stirred solution of DppValOH·$C_4H_8O_2$ (**23**) (4.05 g, 10 mmol) in DCM (40 ml) cooled to 0 °C were added NMM (1.1 ml, 10 mmol) and a solution of DppCl (2.36 g, 10 mmol) in DCM (10 ml). After the mixture had been stirred for 15 min, a solution of (**21**) (1.48 g, 9 mmol) in DMF (12 ml) was added and the whole then stirred for a further 40 min. The solvents were then removed under reduced pressure to yield an oil which was partitioned between ethyl acetate and water. The organic phase was isolated, washed as described for (**22**), and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave N_α-*diphenylphosphinoylvalylphenylalanine amide* as a white powder which was collected under light petroleum (b.p. 60—80 °C) filtered off and dried. The product (**24**) thus prepared could be used without further purification (3.33 g, 80%), m.p. 233 °C (decomp.) (Found: C, 67.5; H, 6.5; N, 9.0; P, 6.4. $C_{26}H_{30}N_3O_3P$ requires C, 67.3; H, 6.5; N, 9.0; P, 6.7%); $[\alpha]_D^{20}$ −64.9° (*c* 1 in MeOH); t.l.c.-A R_F 0.48 (UV, I); amino acid analysis Phe_1 1.00, Val_1 1.00; $\nu_{max.}$ 3.375, 3 255, 3 200 (NH), 1 680, 1 640 (amide CO), 1 550 (CONH), 1 440 (Ph–P), and 1 180 cm^{-1} (PO); $\lambda_{max.}$ 253 (ε 942), 254infl. (938), 258 (1 300), 264 (1 600), and 271nm (1 200); $\delta_H[(CD_3)_2SO]$ 8.50—7.50 (18 H, m, ArH, Phe NH and Phe $CONH_2$), 5.65 (1 H, m, Val NH—exchanges with D_2O), 4.75 (1 H, m, Phe α-CH), 3.80 (1 H, m, Val α-CH), 3.20—2.90 (2 H, m—overlapping ABq—Phe β-CH_2), 2.00 (1 H, m, Val β-CH), 1.00 (3 H, d $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0

Hz) and 0.60 (3 H, d, $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0 Hz, Val γ-CH_3); δ_C[$(CD_3)_2SO$] 173.37 (Phe C=O),[a] 172.01 (Val C=O),[b] 138.13—126.17 (m, aromatic C), 61.63 (Val α-C), 53.64 (Phe α-C), 31.67 (d, $^3J_{P\text{-}C}$ 8.5 Hz, Val β-C), 19.09 and 18.79 (Val γ-C)— Phe β-C resonance is obliterated by the strong solvent peaks [[a,b]these assignments may be reversed]; δ_P(MeOH/$CDCl_3$) +24.83; *m/z* 463.20 (M^+, 30.39%), 419.19 [M^+ −44, 61.95%, DppVal-$^+$N(H)=$CHCH_2Ph$], 272.12 [M^+ −191, 86.56%, Dpp$^+$N(H)=$CHCH(CH_3)_2$], 217.04 [M^+ −246, 100.00%, $Ph_2P(O)O^+$], 201.05 (M^+ —262, 98.48%, Ph_2P^+O), 91.06 (M^+ −372, 25.13%, $PhCH_2^+$), and 72.08 [M^+ −391, 32.84%, H_2N^+=$CHCH(CH_3)_2$].

Valylphenylalanine Amide Hydrochloride, $Cl^-H_2{}^+$Val-Phe-NH_2, (**25**).—Methanol (1.3 ml) and $CDCl_3$ (lock, 0.4 ml) were added to (**24**) (1 g, 2 mmol) contained in a 10-mm n.m.r. tube and the resulting solution was cooled to 0 °C. In order that the reaction time could be kept to a minimum, thereby limiting both exposure of the peptide to acid and the possibility of esterification of the C-terminal amide, the deprotection was monitored by ^{31}P n.m.r.: δ_P +24.83 p.p.m. Injection of 10M-methanolic HCl (1.3 ml, 12.9 mmol) caused a broadening of the ^{31}P signal together with a shift in its position to δ_P +29.05 p.p.m. (believed to be due to the protonated dipeptide amide[21]). The progress of the cleavage reaction was measured by following the disappearance of this peak in conjunction with the appearance of a new signal at δ_P +34.86 p.p.m. representative of the by-product of deprotection, methyl diphenylphosphinate. In this way, the reaction was adjudged to be complete within 100 min when the reactants were poured into vigorously stirred, cold anhydrous diethyl ether (300 ml). After the mixture had been stirred at low temperature for a further 30 min *valylphenylalanine amide hydrochloride* was filtered off, washed with cold, anhydrous diethyl ether, and dried (570 mg, 90%), m.p. 218—221 °C (Found: C, 55.9; H, 7.30; Cl, 11.6 N, 13.8. $C_{14}H_{22}ClN_3O_2$ requires C, 56.1; H, 7.4; N, 14.0; Cl, 11.8%); $[\alpha]_D^{20}$ +24.15° (*c* 1 in methanol); t.l.c.-H R_F 0.63 (weak UV, N); amino acid analysis Phe_1 1.00, Val_1 1.00; $\nu_{max.}$ 3 500—2 400 ($^+NH_3$), 3 430 (NH), 1 680, 1 665 (amide CO) and 1 560 cm^{-1} (CONH); $\lambda_{max.}$ 246 (ε 106), 251 (136), 257 (162), 263 (121), and 268infl. nm (95); δ_H[$(CD_3)_2SO$] 9.00 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.0 Hz, Phe NH), 8.50 (3 H, br s, Val $^+NH_3$), 7.85 (1 H, s, (1) Phe $CONH_2$), 7.52—7.30 [6 H, m, ArH and (1) Phe $CONH_2$], 4.60 (1 H, m, Phe α-CH), 3.70 (1 H, d, Val α-CH), 3.35—2.98 (2 H, m—overlapping ABq, Phe β-CH_2), 2.30 (1 H, m, Val β-CH), 1.00 (6 H, m—overlapping d—Val γ-CH_3); δ_C[$(CD_3)_2SO$] 175.53 (Phe CO), 169.65 (Val CO), 136.82 (Phe C-1), 129.89 (Phe C-2, C-6), 129.44 (Phe C-3, C-5), 127.90 (Phe C-4), 58.91 (Val α-C), 55.65 (Phe α-C), 37.51 (Phe β-C), 30.64 (Val β-C), and 18.39 and 17.36 (Val γ-C).

N_α-*Diphenylphosphinoylphenylalanine Methyl Ester*, DppPhe-OMe (**26**).—To a suspension of phenylalanine methyl ester hydrochloride[46] (64.7 g; 0.3M) in DCM (350 ml) at 0 °C were added NMM (66 ml; 0.6M) and a solution of DppCl (70.95 g; 0.3M) in DCM (200 ml); the reaction mixture was then stirred for 2 h. Removal of the reaction solvent under reduced pressure gave a pale yellow solid which was suspended in saturated aqueous $NaHCO_3$ (200 ml) and stirred vigorously for 30 min. The solid was then filtered off, washed thoroughly with water, and dried. Recrystallisation from DCM with light petroleum (b.p. 60—80 °C) afforded pure N_α-*diphenylphosphinoylphenylalanine methyl ester* as a mass of long white needles which were filtered off and dried (100 g, 88%), $\nu_{max.}$ 3 350, 3 150 (NH), 1 740 (ester CO), 1 440 (Ph–P), and 1 802 cm^{-1} (PO); $\lambda_{max.}$ 254infl. (ε 884), 259 (1 270), 265 (1 564), and 272nm (1 160); *m/z* 379.14 (M^+, 1.86%), 320.12 [M^+ −59, 14.49%, Dpp$^+$N(H)=$CHCH_2Ph$], 288.08 [M^+ −91, 44.07%, Dpp$^+$N(H)=$CHCO_2Me$], 201.05 (M^+ −178, 100.00%, Ph_2P^+=O), 120.08 (M^+ −259, 15.30%, H_2N^+=$CHCH_2Ph$), and 88.04 (M^+ −291, 1.32%, H_2N^+=$CHCO_2Me$); the remainder of the analytical data by which the purity of this compound was assessed have previously been reported.[21]

N_α-*Diphenylphosphinoylphenylalanine*, DppPheOH (**27**).—Sodium hydroxide (2M; 19 ml) was added to a suspension of (**26**) in 1,4-dioxane (50 ml) and the resulting solution stirred at room temperature for 40 min when thin-layer chromatography [t.l.c.-A, follow disappearance of (**26**), R_F 0.78] indicated saponification to be complete. The reaction was worked up as described for (**23**). Concentration of the final, dried organic solution by evaporation of the solvent under reduced pressure and addition of light petroleum (b.p. 60—80 °C) led to the formation of a white gum which readily solidified at room temperature to give pure N_α-*diphenylphosphinoylphenylalanine* which was filtered off, washed with light petroleum (b.p. 60—80 °C) and dried (10.04 g, 88%), $\nu_{max.}$ 3 365 (NH), 3 300—2 100 (acid OH), 1 730 (acid CO), 1 440 (Ph–P), and 1 160 cm^{-1} (PO); $\lambda_{max.}$ 254infl. (ε 898), 259 (1 273), 265 (1 355), and 272nm (1 152); *m/z* 365.12 (M^+, 8.18%), 320.12 [M^+ −45, 18.17%, Dpp$^+$N(H)=CH·CH_2Ph], 217.04 [M^+ −148, 100.00%, $Ph_2P(O)O^+$], 201.05 (M^+ —164, 70.70%, Ph_2P^+=O), 120.08 (M^+ −245, 11.88%, H_2N^+=$CHCH_2Ph$), and 91.05 (M^+ −274, 74.10%, $PhCH_2^+$); the remainder of the analytical data by which the purity of this compound was assessed have previously been reported.[21]

N_α-*Diphenylphosphinoylphenylalanylvalylphenylalanine Amide*, DppPhe^2-Val^3-Phe^4NH_2 (**28**).—To a solution of (**27**) (4.75 g, 13 mmol) dissolved in DCM (20 ml) cooled to 0 °C were added NMM (1.43 ml, 13 mmol) and a solution of DppCl (3.1 g, 13 mmol) in DCM (30 ml) in such a way as to maintain the temperature of the reactants at 0 °C. After an activation period of 15 min a solution of (**25**) (3.0 g, 10 mmol) in DMF (15 ml) was added followed by NMM (1.10 ml 10 mmol) and the whole stirred for a further 30 min; the reaction mixture was then worked up as for (**12**). Evaporation of the solvent from the final, dried solution gave a white semi-solid which was triturated with anhydrous diethyl ether, filtered off, and dried. Recrystallisation from methanol with anhydrous diethyl ether afforded pure N_α-*diphenylphosphinoylphenylalanylvalylphenylalanine amide* (5.08 g, 83%), m.p. 220 °C (decomp.) (Found: C, 68.9; H, 6.1; N, 9.0; P, 5.0. $C_{35}H_{39}N_4O_4P$ requires C, 68.8; H, 6.4; N, 9.2; P, 5.0%); $[\alpha]_D^{20}$ −80.9° (*c* 1 in methanol); t.l.c.-A R_F 0.50 (UV, I); amino acid analysis Phe_2 2.07, Val_1 1.00; $\nu_{max.}$ 3 380, 3 280, 3 200, 3 150 (NH), 1 660, 1 640, 1 630 (amide CO), 1 535 (CONH), 1 435 (Ph–P), and 1 190 cm^{-1} (PO); $\lambda_{max.}$ 252infl. (ε 1 166), 258 (1 614), 264 (1 858) and 271nm (1 256); δ_H[$(CD_3)_2SO$/$CDCl_3$] 8.70 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.4 Hz, Phe^{4}NH),[a] 8.10 (1 H, d, $^3J_{NH\text{-}\alpha CH}$ 8.0 Hz, Val^{3}NH),[b] 7.80—7.00 (22 H, m, ArH and Phe^4 $CONH_2$), 6.30 (1 H, m, Phe^{2}NH—exchanges with D_2O), 4.50 (2 H, m, Phe^2 and Phe^4 α-CH), 3.90 (1 H, m, Val^3 α-CH),[b] 3.40—2.80 (4 H, m, Phe^2 and Phe^4 β-CH_2), 1.90 (1 H, m, Val^3 β-CH), 0.70 (3 H, $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0 Hz) and 0.57 (3 H, $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0 Hz, Val^3 γ-CH_3) [[a,b]assigned on the basis of spin decoupling experiments]; δ_C[$(CD_3)_2SO$] 174.59, 174.29 (Phe^4, Val^3 CO), 170.95 (Phe_2 CO), 138.71 −126.04 (m, aromatic C), 59.78 (Val^3 α-C), 58.59 (Ph^2 α-C), 54.90 (Phe^4 α-C); 36.43 (Phe^2 and Phe^4 β-C), 29.06 (Val^3 β-C), and 18.93, 16.96 (Val^3 δ-C); δ_P(MeOH–$CDCl_3$) 22.43.

N_α-*Diphenylphosphinoylphenylalanylphenylalanylvalylphenylalanine Amide*, DppPhe^1-Phe^2-Val^3-Phe^4NH_2 (**30**).—Treatment of a solution of the tripeptide (**28**) (3.05 g, 5.0 mmol) in methanol (4 ml), with 10M-methanolic HCl (3.0 ml, 30 mmol) at 0 °C over a period of 95 min followed by the work-up procedure given for (**25**) afforded *phenylalanylvalylphenylalanine amide*

hydrochloride (**29**) as a white powder which was used without further purification (2.12 g, 90%), m.p. 210 °C (decomp.) (Found: C, 60.5; H, 7.2; N, 11.7. $C_{23}H_{31}N_4O_3Cl\cdot\frac{1}{2}H_2O$ requires C, 60.5; H, 7.1; N, 12.2%); $[\alpha]_D^{20}$ −36.4° (*c* 1 in methanol); t.l.c.-H R_F 0.66 (N); amino acid analysis Phe_2 2.07, Val_1 1.00. To a solution of (**27**) (1.83 g, 5.0 mmol) dissolved in DCM (15 ml) cooled to 0 °C were added NMM (0.55 ml, 5.0 mmol) and a solution of DppCl (1.18 g, 5.0 mmol) in DCM (15 ml). After an activation period of 15 min a solution of (**29**) (2.0 g, 4.5 mmol) in DMF (15 ml) was added followed by NMM (0.50 ml, 4.5 mmol); after being stirred for a further 30 min the reaction mixture was worked up as described for (**28**). Recrystallisation from methanol with anhydrous diethyl ether afforded pure *N_α-diphenylphosphinoylphenylalanylphenylalanylvalylphenylalanine amide* (1.95 g, 80%), m.p. 220 °C (decomp.) (Found: C, 70.0; H, 6.5; N, 9.1. $C_{44}H_{48}N_5O_5P$ requires C, 69.7; H, 6.4; N, 9.2%); $[\alpha]_D^{20}$ −70.65° (*c* 1 in methanol); t.l.c.-A R_F 0.53 (UV, I); amino acid analysis Phe_3 2.99, Val_1 1.01; $\nu_{max.}$ 3 390, 3 270, 3 200, 3 050 (NH), 1 680, 1 665, 1 640 (amide CO), 1 540 (CONH), 1 440 (Ph–P), and 1 150 cm^{-1} (PO); $\lambda_{max.}$ 252 (ε 1 300), 258 (1 727), 264 (2 136) and 271nm (1 207); δ_H 8.65 (1 H, d, Phe^4 NH),[a,b,c] 8.40 (1 H, d, Val^3 NH),[a,b,c] 8.23 (1 H, d, Phe^2 NH),[a,b,c] 8.00—7.23 (27 H, m, ArH and Phe^4 $CONH_2$), 6.20 (1 H, m, Phe^1 NH—exchanges with D_2O), 4.75 (2 H, m, Phe^2 and Phe^{44} α-CH), 4.25 (1 H, m, Val^3 α-CH), 3.65 (1 H, m, Phe^1 α-CH), 3.50—2.90 (6 H, m, Phe^1, Phe^2, and Phe^4 β-CH_2), 2.20 (1 H, m, Val^3 β-CH), 1.00 (3 H, d, $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0 Hz), and 0.85 (3 H, d, $^3J_{\beta CH\text{-}\gamma CH_3}$ 6.0 Hz, Val^3 γ-CH_3) [[a] poorly resolved doublets assigned on the basis of [b] spin-decoupling experiments and [c] comparison with spectra of other compounds in this series]; δ_C[$(CD_3)_2SO$] 172.88, 171.06, 170.70 (Phe^1, Phe^2, Val^3, and Phe^4 CO), 138.12—126.32 (m, aromatic C), 56.69 (Val^3 α-C), 57.65 (Phe^2 α-C), 54.40 (Phe^1 α-C),[a] 53.84 (Phe^4 α-C), 30.08 (Val^3 β-C), 19.09, 18.74 (Val γ-C)—Phe^1, Phe^2, and Phe^4 β-C obscured by strong solvent peaks [[a,b] these signals have not been unambiguously assigned and may be reversed]; δ_P(MeOH/$CDCl_3$) +22.54.

Phenylalanylphenylalanylvalylphenylalanine Amide Hydrochloride, $Cl^-H_2{}^+Phe^1$-Phe^2-Val^3-Phe^4NH_2 (**31**).—By employing the ^{31}P n.m.r.-based experimental procedure given for (**25**), *phenylalanylphenylalanylvalylphenylalanine amide hydrochloride* was isolated from the deprotection of (**30**) with methanolic HCl as a fibrous white powder which could be adequately purified by reprecipitation from DMF with anhydrous diethyl ether (1.06 g, 90%), m.p. 215 °C (Found: C, 63.0; H, 6.8; N, 11.3. $C_{32}H_{40}N_5O_4\cdot H_2O$ requires C, 62.8; H, 6.6; N, 11.4%); $[\alpha]_D^{20}$ −25.4° (*c* 1 in methanol); t.l.c.-H R_F 0.71 (N); amino acid analysis Phe_3 3.02, Val_1 0.97; $\nu_{max.}$ 3 650—2 350 ($^+NH_3$), 1 680, 1 665 (amide CO) and 1 550cm^{-1} (CONH); δ_H[$(CD_3)_2SO$–$CDCl_3$] 8.90 (1 H, d, Phe^4NH),[a,b,c] 8.25 (1 H, d, Val^3),[a,b,c] 8.10 (1 H, d, Phe^2NH),[a,b,c] 7.40—7.05 (17 H, m, $Phe,^1$ Phe^2, and Phe^4 aromatic H and Phe^4 $CONH_2$), 4.65 (1 H, m, Phe_2 α-CH), 4.50 (1 H, m, Phe^4 α-CH), 4.15 (1 H, m, Val^3 α-CH), 4.00 (1 H, m, Phe^1 α-CH), 3.40—2.80 (6 H, m, Phe^1, Phe^2, and Phe^4βCH_2), 2.00 (1 H, m, Val^3 β-CH), and 0.80 (6 H, m, Val^3γCH_3) [[a] poorly resolved doublets assigned on the basis of [b] spin-decoupling experiments and [c] comparison with spectra of other compounds in this series.]; δ_C[$(CD_3)_2SO/CDCl_3$] 172.85, 170.64, 170.44, 167.88 (Phe^1, Phe^2, Val^3, and Phe^4 CO), 137.65—127.12 (aromatic C), 58.4 (Val^3 α-C), 54.36, 53.78, 53.21 (Phe^1, Phe^2, and Phe^4 α-C), 30.54 (Val^3 β-C), and 19.10 and 18.26 (Val^3 γ-C). [Phe^1, Phe^2, and Phe^4 β-C obscured by strong solvent peaks].

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