The Synthesis and Rotational Isomerism of 1-Amino-2-imidazol-4-ylethylphosphonic Acid [Phosphonohistidine, His(P)] and 1-Amino-2-imidazol-2-ylethylphosphonic Acid [Phosphonoisohistidine, Isohis(P)]

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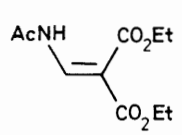
The synthesis of phosphonohistidine [His(P)] and phosphonoisohistidine [Isohis(P)] is described, in each case by a strategy in which the α-aminophosphonic acid grouping is assembled first and the imidazole ring is built last. The key α-aminophosphonic acid building block is phosphonoaspartic acid, protected as the *N*-acetyl phosphonate diethyl ester derivative. The n.m.r. spectra of histidine, isohistidine, phosphonohistidine, and phosphonoisohistidine are analysed at three pH values, using an iterative spin simulation program to confirm results where necessary. The preferred conformations of the four compounds are determined from vicinal H,H and H,P coupling constants. This allows prediction of the conformational differences to be expected in replacing carboxylate by phosphonate groups. In free energy terms, phosphonate appears to exert a larger steric effect than carboxylate by *ca.* 1 kcal mol^{-1}.

The concentration of free serum histidine has been observed to be significantly decreased in patients with rheumatoid arthritis.[1] This decrease is specific for histidine and is not associated with a fall in the serum levels of other free amino acids.[2] The role of endogenous serum histidine and a possible mode of action of the slow acting antirheumatic drug D-penicillamine in man have been combined in a hypothesis based on formation *in vivo* of copper complexes with superoxide dismutase-like activity.[3] We have, therefore, undertaken the synthesis of analogues of histidine for biological evaluation. One aspect of this approach involved the synthesis of analogues and derivatives of α-amino-2-imidazolepropionic acid (isohistidine), and certain of these compounds showed particularly interesting biological properties.[4] In view of these encouraging results we undertook the synthesis of the phosphonic acid analogues of histidine [phosphonohistidine, His(P)] (**16**) and isohistidine [phosphonoisohistidine, Isohis(P)] (**21**) for biological evaluation.

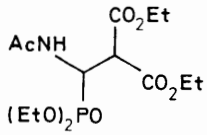
Results and Discussion

Synthesis.—Phosphonohistidine is the only phosphonic acid analogue of the common amino acids whose synthesis has not yet been described.[5] Earlier attempts in these laboratories to prepare phosphonohistidine from imidazole-containing precursors with subsequent incorporation of the α-aminophosphonate grouping have been unsuccessful. For the synthesis of both His(P) (**16**) and Isohis(P) (**21**) we, therefore, decided to adopt a strategy which involved first assembling the α-aminophosphonic acid grouping in a suitably protected form, then elaborating the carbon backbone if necessary and finally building the imidazole ring.

The preparation of phosphonoaspartic acid has already been described,[6] and involved hydrolysis and decarboxylation of the crude protected intermediate (**2**) by heating with concentrated hydrochloric acid. We chose this dicarboxylic ester (**2**), which we were able to isolate in pure form, as our α-aminophosphonic acid building block. This diester (**2**) was hydrolysed by treatment with 1 equiv. of sodium hydroxide to give a solution of pH 7 which was then boiled under reflux to give the monocarboxylic acid (**3**). This was then re-esterified by treatment with ethanol–sulphuric acid to form the monocarboxylic ester (**4**). No conditions were found which gave the desired carboxylic ester (**4**) directly from (**2**).



(**1**)



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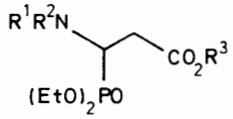
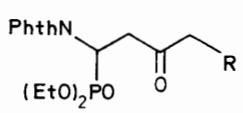
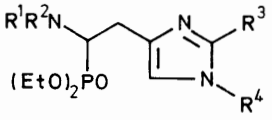
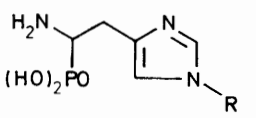




(**3**) R^1 = Ac, R^2 = R^3 = H
(**4**) R^1 = Ac, R^2 = H, R^3 = Et
(**5**) R^1 = R^2 = H, R^3 = Et
(**6**) R^1, R^2 = Phth, R^3 = Et
(**7**) R^1 = 2-$HO_2CC_6H_4CO$, R^2 = R^3 = H
(**8**) R^1, R^2 = Phth,

(**9**) R = Cl
(**10**) R = $N(CH_2Ph)_2$
(**11**) R = $NHCH_2Ph \cdot HCl$





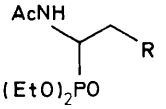
(**12**) R^1, R^2 = Phth, R^3 = SH, R^4 = CH_2Ph
(**13**) R^1, R^2 = Phth, R^3 = H, R^4 = CH_2Ph
(**14**) R^1 = R^2 = R^3 = H, R^4 = CH_2Ph

(**15**) R = CH_2Ph
(**16**) R = H

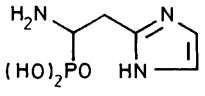
The acetamido group was then cleaved by successive treatment with phosphorus pentachloride–*N*-methylmorpholine, methanol–*N*-methylmorpholine, and water,[7] and the resulting amino group reprotected as the phthalimido derivative by reaction with phthalic anhydride. Hydrolysis of the carboxylic ester group in compound (**6**) with base brought about undesired concomitant ring-opening of the phthalimido group, which was recyclised by treatment with trifluoroacetic anhydride to give the fully protected carboxylic acid intermediate (**8**). This was converted into the corresponding acid chloride, which was treated with diazomethane and then hydrogen chloride to form the chloromethyl ketone (**9**).

This key intermediate (**9**) contained the full backbone of

carbon atoms with the appropriate functionality for building the required imidazole ring.[8] Displacement of the chlorine with dibenzylamine followed by hydrogenolysis of the resulting dibenzylamino group gave the benzylamino ketone (**11**) which readily formed the imidazole (**12**) on treatment with potassium thiocyanate. All that now remained was to remove the mercapto function and the protecting groups to give His(P) (**16**). Treatment with Raney nickel removed the mercapto group, then successive treatment first with hydrazine then with strong hydrochloric acid cleaved the phthalimido and phosphonate ester groups respectively. The *N*-benzyl group was most conveniently removed by hydrogenolysis to give the desired amino acid analogue His(P) (**16**). Cleavage of the *N*-benzyl group could also be brought about with sodium in liquid ammonia but then there were problems in completely removing the resulting sodium ions. The sequence shown which uses volatile reagents and gives either volatile or water insoluble by-products is the most convenient for isolating His(P) (**16**).



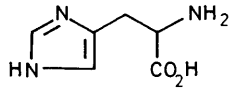
(**17**) R = $CONH_2$
(**18**) R = CN
(**19**) R = C(=NH·HCl)OMe
(**20**) R = C(=NH·HCl)$NHCH_2CH(OEt)_2$



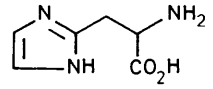
(**21**)

The ester (**4**) was also a suitable α-aminophosphonic acid building block for the synthesis of Isohis(P) (**21**). Treatment of this ester (**4**) with aqueous ammonia gave the corresponding amide (**17**) which was dehydrated to the nitrile (**18**) by heating with acetic anhydride. We found that the addition of a catalytic amount of nickel acetate[9] gave an improved yield of crude nitrile (**18**) which was then easier to purify. The initial product of the dehydration reaction with acetic anhydride was the *N*,*N*-diacetyl derivative of the acetamido compound (**18**). This could be mono-deacetylated by treatment with ammonia, but the by-product acetamide could only be removed completely from the desired acetamido compound (**18**) by column chromatography. However, mono-deacetylation by treatment with ethane-1,2-diamine gave the still basic 2-acetamidoethane-1-amine, so that both it and unchanged reagent could be simply removed by extraction with aqueous acid.* Reaction of the nitrile (**18**) with methanolic hydrogen chloride gave the imino ester hydrochloride (**19**). This imino ester was very sensitive to moisture, forming the corresponding carboxylic ester which was readily detected by n.m.r. Subsequent reaction of the imino ester hydrochloride (**19**) with aminoacetaldehyde diethyl acetal[10] gave the amidine hydrochloride (**20**). This was treated with 6M hydrochloric acid, first at room temperature for 24 h then at 80 °C for 24 h to give, after evaporation, a crude product which by n.m.r. analysis contained no OEt or NHAc groups but did show imidazole protons at δ *ca.* 7.5. The Isohis(P) (**21**) was isolated by absorption onto a cation exchange (H^+) column followed by elution with ammonia and thorough evaporation of the eluate under reduced pressure. Recrystallisation from water unfortunately gave a poor recovery, but sufficient material was obtained for characterisation and *in vitro* biological evaluation.

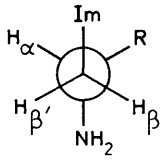
N.m.r. Spectra.—The rotational isomerism of histidine (**22**) has been examined previously and the populations about the C_α–C_β bond determined at various pH values.[11–15] The phosphorus atom in His(P) (**16**) provides an additional handle



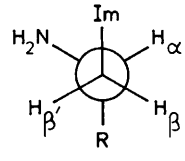
(**22**)



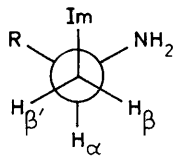
(**23**)



(**Ia**; R = CO_2H)
(**Ib**; R = PO_3H_2)



(**IIa**; R = CO_2H)
(**IIb**; R = PO_3H_2)



(**IIIa**; R = CO_2H)
(**IIIb**; R = PO_3H_2)

particularly for the assignment of the β protons, since three bond H,P coupling constants are subject to the same $\cos^2\varphi$ torsion angle dependence as their H,H counterparts.[16] This was demonstrated effectively in a recent paper analysing the n.m.r. spectra and rotational isomerism of phosphonoaspartic acid.[17] Comparison of the rotamer populations of histidine (**22**), isohistidine (**23**), and their phosphonic acid analogues (**16**) and (**21**) respectively enables the steric requirements of tetrahedral phosphonate to be determined relative to the planar carboxylate group. This has implications for drug design in the synthesis of false amino acids and peptide mimetics containing the phosphonate group.

Chemical shifts and coupling constants for these four compounds are recorded in Tables 1 and 2 respectively. In cases where the three protons α, β, and β′ were closely coupled, assignment was made easier by the fact that the ββ′ coupling $^2J_{HH}$ varied very little in the four compounds and was in the range −14 to −16.5 Hz. Individual assignment of the two β protons required an analysis of the rotameric forms present at each pH, using the well established dependence of $^3J_{HH}$ and $^3J_{HP}$ on $\cos^2\varphi$, where φ is the torsion angle between the C–H and/or C–P bonds.[16] For example, His(P) (**16**) in basic solution shows small and similar $^3J_{HP}$ values of 6.05 and 6.42 Hz suggesting a strong favouring of rotamer (**IIb**), with the C–P bond bisecting the two C–Hβ bonds. (φ in each case = 60°.) It is then clear that the $^3J_{\alpha\beta}$ coupling of 11.4 Hz is assigned to that β proton *trans* to the α proton in (**IIb**). In this way the assignment of the β protons in His(P) (**16**) and Isohis(P) (**21**) is readily achieved. In His (**22**) and Isohis (**23**), though the biasing of the conformation is much less than in the phosphonate derivatives [rotamer (**IIa**) mole fraction 0.52 for His and 0.67 for IsoHis, both in basic solution], the assignment of the β-protons is nevertheless clear, assuming the carboxy group to exert a similar influence to that of the phosphonate albeit to a lesser extent.

Model compounds for calculating the absolute values of the vicinal coupling constants in the four compounds were alanine and the corresponding phosphonate Ala(P)[18] which were examined in acid, neutral, and basic solution to ensure that ionisation did not affect the 3-bond couplings significantly. The results (Table 3) allowed the prediction of $^3J_{HP}$ (*trans*) = 34.6 and $^3J_{HP}$ (*gauche*) = 4.9 Hz, using the procedure of Siatecki and Kozlowski.[17] Similarly a combination of several approaches[19–21] provided the relevant $^3J_{HH}$ values for each rotamer.

The equations finally used to determine rotamer populations were as follows:

^1H $\quad J_{13} = 3.0\,p_I + 13.0\,p_{II} + 2.5\,p_{III}$ (1)

$\quad J_{23} = 13.0\,p_I + 3.0\,p_{II} + 3.3\,p_{III}$ (2)

^{31}P $\quad J_{1P} = 34.6\,p_I + 4.9\,p_{II} + 4.9\,p_{III}$ (3)

* We are grateful to Professor J. E. Baldwin for this suggestion.

$$J_{2P} = 4.9\ p_I + 4.9\ p_{II} + 34.6\ p_{III} \quad (4)$$

$$p_I + p_{II} + p_{III} = 1 \quad (5)$$

A small computer program allowed rapid calculation of p_I, p_{II}, and p_{III} for each possible assignment of the two β protons. The values obtained are recorded in Table 4. The additional conformational information in the two phosphonates provided by the $^3J_{HP}$ values allowed us to discard the assignment which was obviously wrong. The rotamer populations for His(P) (**16**) and Isohis(P) (**21**) determined from $^3J_{HH}$ values on the one hand, and from $^3J_{HP}$ values on the other are in reasonable agreement considering the approximations involved in the calculation.

pK_a Values (Table 5) were obtained by classical titration methods. The imidazole pK_a has values between 6.1 and 6.9, as proven by following chemical shift changes in the ^{1}H n.m.r. spectrum with pH.

The pK_a values obtained allow the prediction of the state of ionisation of the 4 compounds at their 'natural' pH. For histidine (**22**) and isohistidine (**23**) at pH 7.4 and 7.5 respectively, 95% of the molecules are in zwitterionic form with the imidazole unprotonated. For His(P) (**16**) at pH 5.8 *ca.* 92% of the molecules are neutral, with positive charges on the protonated amino and imidazole groups, and the phosphonate as the dianion. For Isohis(P) (**21**) *ca.* 89% of the molecules are in this state.

The rotamer populations determined from vicinal H,H and H,P coupling constants reflect the structural differences which cause the conformational biasing. Rotamer (**II**) with the imidazole *trans* to the carboxylate or phosphonate is preferred in all four compounds at all pH values, though more so in base than in acid solution. It is tempting to interpret the extra population of (**II**) in base as reflecting some attraction between the uncharged NH_2 and imidazole groups, perhaps by intramolecular hydrogen bonding, however unlikely in aqueous solution. The difference between the carboxylates and the phosphonates reflected for instance in the differences in preference for rotamer (**II**), indicates that the *gauche* steric effect of phosphonate is larger than carboxylate by *ca.* 1 kcal mol^{-1}. As the phosphonate is tetrahedral and the carboxylate planar this may be as expected. The 'A' value (axial/equatorial energy difference) for cyclohexane phosphonate dimethyl ester is 1.9 kcal mol^{-1} [22] and for methyl cyclohexanecarboxylate 1.1 kcal mol^{-1}.[23] The difference of 0.8 kcal mol^{-1} is close to the estimate found in this work of 1 kcal mol^{-1} for the acid functions PO_3H_2 and CO_2H. In neutral and basic solution the 'A' values for the anions of these groups may be considerably larger due to preferential solvation. The 'A' value for CO_2^- is 2.2 kcal mol^{-1},[24] suggesting that the value for PO_3H^- would be at least 3 kcal mol^{-1}, explaining to some extent that marked preference for rotamer (**II**) in basic solution.

Table 1. ^{1}H Chemical shifts in D_2O solution

	pH	Hα	Hβ	Hβ′	H2′[b]	H4′[b]	H5′[b]
Histidine	1.7	4.36[a]	3.48	3.45	7.47	8.72	
	7.4	3.99	3.23	3.73	7.10	7.77	
	13.3	3.50	2.97	2.80	6.91	7.65	
Isohistidine	<1	4.62	3.77	3.73		7.47	7.47
	7.5	4.04	3.38	3.24		7.10	7.10
	13.5	3.60	3.11	2.84		7.02	7.02
His(P)	~1	3.68	3.42	3.26	7.45	8.68	
	5.8	3.42	3.37	3.14	7.34	8.46	
	13.2	2.87	3.08	2.60	6.92	7.66	
Isohis(P)	1.1	3.80	3.52	3.63		7.42	7.42
	6.0	3.77	3.64	3.80		7.34	7.34
	12	2.97	3.18	2.73		7.02	7.02

[a] δ Values, referred to internal TSP. [b] Imidazole ring protons.

Table 3. Variation of $J_{\alpha\beta}$ and $J_{\beta P}$ with pH in alanine and phosphonoalanine

	pH	$^3J_{HH}$	$^3J_{HP}$
Alanine	1	7.3	
	~7*	7.3	
	10	7.0	
Ala(P)	1	7.3	15.6
	~6*	7.2	14.8
	10	7.0	14.7

* Natural pH in D_2O solution.

Table 4. Rotamer populations[a] calculated from equations (1)—(4)

Compd.	pH	P_I	P_{II}	P_{III}
His	1.7	0.34	0.37	0.28
	7.4	0.17	0.51	0.32
	13.3	0.20	0.50	0.30
Isohis	<1	0.27	0.60	0.13
	7.5	0.20	0.53	0.27
	13.5	0.17	0.62	0.21
His(P)	~1	0.29 (0.25)[b]	0.61 (0.62)	0.10 (0.13)
	5.8	0.21 (0.17)	0.60 (0.70)	0.19 (0.13)
	13.2	0.01 (0.05)	0.85 (0.91)	0.17 (0.04)
Isohis(P)	1.1	0.38 (0.39)	0.54 (0.44)	0.08 (0.17)
	6.0	0.35 (0.23)	0.43 (0.55)	0.22 (0.22)
	12.0	0.02 (0.04)	0.85 (0.89)	0.13 (0.07)

[a] Calculated from ^{1}H,^{1}H couplings (estimated maximum errors ±20%). [b] Bracketed figures calculated from ^{1}H,^{31}P couplings (estimated maximum errors ±16%).

Table 2. Coupling constants $J(^1H,^1H)$ and $J(^1H,^{31}P)$

Compd.	pH	αβ	αβ′	ββ′	αP	βP	β′P	β2′	β′4′	2′4′
Histidine	1.7	6.57[a]	6.53	−16.00						
	7.4	4.76	7.97	−15.49				0.09		1.22
	13.3	5.12	7.85	−14.63				0.09		1.22
Isohistidine	<1	5.73	8.92	−15.74						
	7.5	5.11	8.11	−15.79						
	13.5	4.75	9.10	−14.73						
His(P)	~1	5.97	9.00	−16.00	−13.67	8.80	12.43	1.00	0.69	1.46
	5.8	5.17	8.92	−15.89	−12.31	8.66	10.00	0.99	0.45	1.28
	13.2	2.91	11.40	−14.97	−11.26	6.05	6.42	1.05	0.50	1.28
Isohis(P)	1.1	6.86	8.31	−16.04	−13.22	16.51	9.97			
	6.0	6.52	7.20	−16.55	−13.02	11.78	11.31			
	12.0	3.25	11.41	−15.01	−11.25	6.17	6.96			

[a] Accurate to ~ ±0.05 Hz, and confirmed by simulation of spectra in second-order cases.

Table 5. Correlation of pK_a values

	His[a]	Isohis	His(P)[b]	Isohis(P)
$pK1$[c]	2.9	2.7	2.9	2.9
$pK2$			4.9	4.6
$pK3$	6.1	6.1	6.9	6.9
$pK4$	9.4	8.9	9.8	9.5

[a] Lit. values 1.8, 5.97, 8.97.[26] [b] From chemical shift variation of 2-H and 5-H, pK values of 6.9 and 10.1 are obtained. [c] Determined on a Radiometer Model D470 and Hewlett-Packard 85B computer.

Differences between the 2- and 4-substituted imidazoles as reflected in rotameric preferences are much smaller than with the carboxylate/phosphonate change. Since the two groups are almost identical sterically, any conformational differences may reflect electrostatic effects which are more difficult to predict, since charge calculations do not take account of the critical role of solvation in dissipating charges.

In conclusion, replacing carboxylate by phosphonate in amino acids or at the C-terminus of a peptide provides a more polar end group, for the extra steric requirements of such a group may well negate any extra binding to positively charged groups on the receptor protein. Introducing isohistidine as a replacement for histidine, on the other hand is a more subtle change which may well modify enzyme/substrate or enzyme/inhibitor interactions.

Biological Activity.—Both His(P) (**16**) and Isohis(P) (**21**) showed superoxide dismutase-like activity *in vitro*, but showed no interesting *in vivo* anti-rheumatic activity.

Experimental

^1H N.m.r. spectra were determined on Bruker WM-300 and AM-400 spectrometers. Detailed analyses were carried out on spectra determined for acid (pH *ca.* 1), neutral (pH 5—7), and alkaline (pH > 10) solutions (*ca.* 0.15M) of histidine, isohistidine, and their corresponding phosphonic acids at 313 K to improve resolution. ^1H Spectra were analysed, by first-order methods where appropriate, and using the Bruker iterative program PANIC in the more closely coupled systems to simulate the spectra. The chemical shifts were determined to ± 0.1 Hz and the coupling constants to ± 0.05 Hz except where stated. Chemical shifts are referred to internal TSP (3-trimethylsilyl propionate). The pK_a values for the four compounds were determined by classical titration methods. In one case [His(P)], the pK_a values were confirmed by titration using the n.m.r. chemical shifts to obtain the curves.

Thin layer chromatograms (t.l.c.) were run on Merck silica gel F-254 plates in the solvent systems (A) H_2O–CH_3CN 1:4; (B) EtOAc; (C) MeOH–CH_2Cl_2 1:9; (D) BuOH–EtOH–H_2O–880 NH_3 3:3:2:1; (E) Pr^iOH–EtOAc 2:3.

Diethyl [*Acetamido*(*diethoxyphosphinoyl*)*methyl*]*malonate* (**2**).[6]—A mixture of diethyl acetamidomethylenemalonate (229 g, 1.0 mol) (**1**) and freshly distilled diethyl phosphite (151.8 g, 1.1 mol) was stirred and heated to 60 °C. The heating bath was then removed while ethanolic sodium ethoxide (1M; 5 ml) was added in 1 ml portions, whereupon an exothermic reaction took place, the reaction temperature rising to *ca.* 105 °C. The reaction mixture was stirred and heated at 100 °C for 1.5 h and then cooled to below 30 °C. The mixture was boiled under reflux with ether (400 ml) until a clear solution was formed, then this solution was filtered and the filtrate cooled at 0 °C. The precipitated pale yellow crystals of the *dicarboxylic ester* (**2**), were filtered off and dried (308.9 g, 84%), m.p. 63—65 °C (Found: C, 46.1; H, 7.0; N, 4.0; P, 8.1. $C_{14}H_{26}NO_8P$ requires C, 45.8; H, 7.1; N, 3.8; P, 8.4%);* $\delta(CDCl_3)$ 1.3 (12 H, m, CH_3CH_2O), 2.03 (3 H, d, CH_3CONH), 3.9 [1 H, m, $CHCH(CO_2Et)_2$], 4.2 (8 H, m, CH_3CH_2O), 5.18 [1 H, m, P(O)CHCH], and 7.02 (1 H, d, NH).

3-Acetamido-3-(*diethoxyphosphinoyl*)*propionic Acid* (**3**).—The malonate diester (**2**) (184 g, 0.5 mol) was dissolved in aqueous sodium hydroxide (1M; 500 ml) and the solution kept at room temperature for 24 h. The solution, now pH *ca.* 7, was boiled under reflux for 3 h, then passed down a column of Duolite C225 (H^+ form) (600 ml wet resin) and the column eluted with water until the eluate pH was *ca.* 4. The eluates were combined and evaporated under reduced pressure. The colourless syrup obtained was dissolved in ethanol (400 ml) and the solution evaporated once more. Trituration of the residual colourless gum with ether (500 ml) gave crystals (126.7 g, 95%), m.p. 108—115 °C. Two recrystallisations from methyl ethyl ketone gave the pure *carboxylic acid* (**3**), m.p. 130—132 °C (Found: C, 40.2; H, 6.75; N, 5.3; P, 11.5. $C_9H_{18}NO_6P$ requires C, 40.45; H, 6.8; N, 5.2; P, 11.6%); $\delta(D_2O)$ 1.31 (6 H, t, CH_3CH_2O), 2.0 (3 H, d, CH_3CONH), 2.7 and 2.91 (2 H, m, $CHCH_2CO_2H$), 4.2 (4 H, m, CH_3CH_2O), and 4.78 [1 H, m, P(O)CHCH$_2$].

Ethyl 3-Acetamido-3-(*diethoxyphosphinoyl*)*propionate* (**4**).—The crude acid (**3**) (78.5 g, 294 mmol) was added to a solution of concentrated sulphuric acid (1.6 g) in ethanol (630 ml), and the mixture stirred and boiled under reflux for 4.5 h; the solid dissolved during the first hour. The solution was cooled to room temperature and 10% aqueous potassium hydrogen carbonate added to adjust the pH to 7—8. The mixture was evaporated at 40 °C under reduced pressure and the residue partitioned between dichloromethane (300 ml) and saturated aqueous potassium hydrogen carbonate (300 ml). The aqueous phase was extracted with more dichloromethane (2 × 150 ml), and the extracts were combined and evaporated at 30 °C under reduced pressure to give a mobile colourless syrup (48.0 g, 55%) which slowly crystallised with time. Sublimation at 160 °C/0.1 mmHg gave the pure *carboxylic ester* (**4**) as low-melting deliquescent crystals (Found: C, 43.05; H, 7.8; N, 4.6; H_2O, 4.0. Calculated to dryness C, 44.8; H, 7.7; N, 4.8. $C_{11}H_{22}NO_6P$ requires C, 44.75; H, 7.5; N, 4.7%); $\delta(CDCl_3)$ 1.30 (9 H, m, CH_3CH_2O), 2.0 (3 H, s, CH_3CONH), 2.75 (2 H, m, $CHCH_2CO_2Et$), 4.18 (6 H, m, CH_3CH_2O), 4.9 [1 H, m, P(O)CHCH$_2$], and 6.4 (1 H, d, NH).

Ethyl 3-Amino-3-(*diethoxyphosphinoyl*)*propionate* (**5**).—A solution of the acetamido compound (**4**) (29.5 g, 0.1 mol) and *N*-methylmorpholine (24.2 g, 0.24 mol) in dry carbon tetrachloride (280 ml) was cooled in ice–salt and stirred while a solution of phosphorus pentachloride (25.0 g, 0.12 mol) in dry carbon tetrachloride (280 ml) was added during 30 min, the reaction temperature being kept at −10 °C. The resulting yellow mixture was stirred for 1 h at 0—5 °C, then recooled in ice–salt, and treated with a solution of *N*-methylmorpholine (24.2 g, 0.24 mol) in methanol (140 ml) at < 0 °C. After being stirred for a further 2 h at 0—5 °C, the mixture was diluted with water (340 ml) and transferred to a separating funnel. The aqueous layer was separated and concentrated under reduced pressure until *ca.* 150 ml of distillate had been collected. The residue was extracted with dichloromethane (2 × 150 ml) and then cooled in ice and neutralised to pH 8 by addition of 2M aqueous sodium hydroxide. The solution was extracted again with dichloromethane (4 × 150 ml), the dried (Na_2SO_4) extracts evaporated under reduced pressure, and the residual

* This compound with first obtained crystalline by Dr. P. J. Machin of these laboratories.

syrup dissolved in ether (100 ml). The ether solution was filtered from a little insoluble material then evaporated under reduced pressure to give the *carboxylic ester* (**5**) (21.0 g, 83%) as a yellow syrup, R_F 0.65 (system A, ninhydrin spray); δ($CDCl_3$) 1.20 (3 H, t, $CO_2CH_2CH_3$), 1.26 [6 H, t, $PO(OCH_2CH_3)_2$], 1.72 (2 H, br s, NH_2), 2.43 and 2.73 (2 H, dm, $CHCH_2CO_2Et$), 3.48 [1 H, m, $P(O)CHCH_2$], 4.10 (2 H, q, $CO_2CH_2CH_3$), and 4.12 [4 H, q, $PO(OCH_2CH_3)_2$].

Ethyl 3-(Diethoxyphosphinoyl)-3-phthalimidopropionate (**6**).—A solution of the amino compound (**5**) (21.0 g, 83 mmol) and phthalic anhydride (13.4 g, 91 mmol) in toluene (400 ml) was boiled under reflux for 3 h. Water (1.5 ml) which formed during the reaction was collected using a Dean and Stark trap. The solution was cooled, washed successively with 2M hydrochloric acid (2 × 200 ml), 1M aqueous sodium carbonate (2 × 200 ml), and water (200 ml), and dried (Na_2SO_4). Evaporation of the toluene solution under reduced pressure gave crude phthalimido compound (**6**) (24.3 g, 76%) as a yellow syrup. A sample was chromatographed on silica gel 60 using ethyl acetate–hexane (70:30) as the eluting solvent. The purified product was obtained as a colourless syrup, R_F 0.60 (system B, u.v. absorption); δ($CDCl_3$) 1.17 (3 H, t, $CO_2CH_2CH_3$), 1.36 [6 H, dt, $PO(OCH_2CH_3)_2$], 3.07 and 3.64 (2 H, dm, $CHCH_2CO_2Et$), 4.09 (2 H, m, $CO_2CH_2CH_3$), 4.22 [4 H, m, $PO(OCH_2CH_3)_2$], 5.12 [1 H, m, $P(O)CHCH_2$], and 7.77 and 7.87 (4 H, dm, ArH).

3-(2-*Carboxybenzamido*)-3-(*diethoxyphosphinoyl*)*propionic Acid* (**7**).—Aqueous sodium hydroxide (1M; 124 ml, 124 mmol) was added to a stirred solution of the crude phthalimido compound (**6**) (22.7 g, 59 mmol) in ethanol (7 ml). After 1 h a clear solution was obtained which was kept at room temperature overnight. The solution was concentrated to remove ethanol and then passed down a column containing Duolite C225 cation exchange resin (300 ml of wet resin, H^+ form). The column was eluted with water and the acidic eluate collected and evaporated under reduced pressure. The residue was dissolved in ethanol (300 ml) and evaporated and then dissolved in dichloromethane (300 ml) and evaporated again to give a crisp foam (21.4 g, 97%). This product could be crystallised from ethanol to give white crystals of the 3-(2-*carboxybenzamido*) *compound* (**7**) *ethanol solvate*, m.p. 93—98 °C, R_F 0.38 (system A, u.v. absorption) (Found: C, 48.5; H, 6.25; N, 3.25; P, 7.6. $C_{15}H_{20}NO_8P \cdot C_2H_6O$ requires C, 48.7; H, 6.25; N, 3.3; P, 7.4%); δ($CDCl_3$) 1.19 (3 H, t, CH_3CH_2OH), 1.32 [6 H, dt, $PO(OCH_2CH_3)_2$], 2.85 (2 H, m, $CHCH_2CO_2H$), 3.67 (2 H, q, CH_3CH_2OH), 4.17 [4 H, m, $PO(OCH_2CH_3)_2$], 5.04 [1 H, m, $P(O)CHCH_2$], 7.5 (4 H, m, ArH), 7.84 (1 H, d, NH), and 8.49 (3 H, br s, $2CO_2H$ and CH_3CH_2OH).

3-(*Diethoxyphosphinoyl*)-3-*phthalimidopropionic Acid* (**8**).—The crude 3-(2-carboxybenzamido) compound (**7**) (21.3 g, 57 mmol) was dissolved in trifluoroacetic anhydride (42 ml) with ice cooling. The solution was kept at room temperature overnight and then evaporated to dryness under reduced pressure. The residue was treated with water (200 ml) and the mixture stirred for 15 min; it was then extracted with dichloromethane (200 ml, 100 ml). The combined extracts were washed with water (100 ml), dried (Na_2SO_4), and evaporated under reduced pressure to give a syrup (20.1 g) which crystallised. Recrystallisation from ethyl acetate gave the *phthalimido compound* (**8**) (12.3 g, 61%), m.p. 153.5 – 157.5 °C. A second recrystallisation from ethyl acetate gave material, m.p. 155—158 °C, R_F 0.45 (system C, u.v. absorption) (Found: C, 51.0; H, 5.2; N, 4.0; P, 8.5. $C_{15}H_{18}NO_7P$ requires C, 50.7; H, 5.1; N, 3.9; P, 8.7%); δ($CDCl_3$) 1.3 [6 H, dt, $PO(OCH_2CH_3)$], 3.05 and 3.56 (2 H, dm, $CHCH_2CO_2H$), 4.16 [4 H, m, $PO(OCH_2CH_3)_2$], 5.05 [1 H, m, $P(O)CHCH_2$], 7.77 (4 H, 2m, ArH), and 8.5 (1 H, br s, CO_2H).

1-*Chloro*-4-*diethoxyphosphinoyl*-4-*phthalimidobutan*-2-*one* (**9**).—A suspension of the carboxylic acid (**8**) (30 g, 84.5 mmol) in toluene (150 ml) was stirred and cooled in ice while a solution of phosphorus pentachloride (21.1 g, 102 mmol) in toluene (180 ml) was added during 15 min, the reaction temperature being kept below 10 °C. The resulting solution was stored at room temperature overnight and then evaporated to dryness under reduced pressure in a carefully dried rotary evaporator to yield the corresponding acid chloride (31.6 g) as a syrup. This was dissolved in anhydrous ether (300 ml) and the solution added gradually to a stirred solution of ethanol-free diazomethane (7.86 g, 187 mmol) in ether (634 ml) (prepared from *N*-methyl-*N*-nitrosotoluene-4-sulphonamide) with cooling in ice–salt. The solution was stirred at < 0 °C for 1 h and then stored at room temperature overnight. The solution was again cooled in ice–salt while it was saturated with dry hydrogen chloride gas. The mixture was then filtered and the filtrate evaporated to dryness under reduced pressure. The residual syrup was dried azeotropically with toluene (400 ml) and then dissolved in warm ether (150 ml) and the solution filtered. On cooling (finally to −20 °C), the filtrate deposited crystals of the *chloro ketone* (**9**) (26.2 g, 80%), m.p. 70—73 °C. A sample recrystallised from ether had m.p. 72.5—74.5 °C, R_F 0.50 (system B, u.v. absorption) (Found: C, 49.6; H, 5.0; Cl, 9.2; N, 3.7; P, 8.0. $C_{16}H_{19}ClNO_6P$ requires C, 49.6; H, 4.9; Cl, 9.1; N, 3.6; P, 8.0%; δ($CDCl_3$) 1.33 [6 H, dt, $PO(OCH_2CH_3)_2$], 3.40 and 3.98 (2 H, dm, $CHCH_2CO$), 4.21 [6 H, m, $PO(OCH_2CH_3)_2$ and $COCH_2Cl$], 5.16 [1 H, m, $P(O)CHCH_2$], and 7.79 (4 H, dm, ArH).

1-*Dibenzylamino*-4-*diethoxyphosphinoyl*-4-*phthalimidobutan*-2-*one* (**10**).—A solution of dibenzylamine (55.0 g, 279 mmol) in acetonitrile (250 ml) was added during 10 min to a stirred solution of the chloro ketone (**9**) (51.5 g, 133 mmol) in acetonitrile (250 ml). The yellow solution was stirred at room temperature for 20 h, during which time a precipitate of dibenzylamine hydrochloride separated. The mixture was evaporated to dryness under reduced pressure and the residue shaken with a mixture of water (500 ml) and toluene (500 ml). The mixture was filtered and the aqueous layer separated and extracted again with toluene (100 ml). The combined toluene extracts were washed with water (100 ml), dried (Na_2SO_4), and evaporated under reduced pressure to yield a syrup which crystallised on trituration with light petroleum (b.p. 60—80 °C; 100 ml). The product was filtered off, washed with light petroleum, and dried *in vacuo* to give the *dibenzylamino compound* (**10**) (70.3 g, 96%), m.p. 78—82.5 °C. A sample recrystallised twice from diethyl ether had m.p. 83—85.5 °C, R_F 0.70 (system B, u.v. absorption) (Found: C, 65.7; H, 6.1; N, 5.2; P, 5.6. $C_{30}H_{33}N_2O_6P$ requires C, 65.7; H, 6.1; N, 5.1; P, 5.65%); δ($CDCl_3$) 1.31 [6 H, dt, $PO(OCH_2CH_3)_2$], 3.20, and 3.78 (2 H, dm, $CHCH_2CO$), 3.23 (2 H, s, $COCH_2N$), 3.63 [4 H, q, $N(CH_2Ph)_2$], 4.17 [4 H, m, $PO(OCH_2CH_3)_2$], 5.12 [1 H, m, $P(O)CHCH_2$], 7.3 [10 H, m, $(CH_2Ph)_2$], and 7.76 (4 H, dm, Phth).

1-*Benzylamino*-4-*diethoxyphosphinoyl*-4-*phthalimidobutan*-2-*one Hydrochloride* (**11**).—A solution of the dibenzylamino compound (**10**) (68.0 g, 124 mmol) in 1M ethanolic hydrogen chloride (1 500 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% Pd–C catalyst (5.0 g) until uptake of hydrogen ceased. The catalyst was filtered off and the pale yellow filtrate evaporated to dryness under reduced pressure to give the crude hydrochloride (**11**) (61.4 g) as a crisp foam.

Diethyl [2-(1-Benzyl-2-mercaptoimidazol-4-yl)-1-phthali-

midoethyl]*phosphonate* (**12**).—A solution of potassium thiocyanate (17.5 g, 180 mmol) in ethanol (450 ml) was added at room temperature to a stirred solution of the crude hydrochloride (**11**) (61.0 g, 120 mmol) in ethanol (450 ml), to give a red suspension. The red mixture was stirred and boiled under reflux for 2 h during which time it turned yellow. It was evaporated then to dryness under reduced pressure and the residue partitioned between 1M hydrochloric acid (500 ml) and dichloromethane (500 ml, 250 ml). The combined extracts were washed with water (250 ml), dried (Na_2SO_4), and evaporated under reduced pressure. The residue (60.5 g) was crystallised from ethanol (480 ml) to give the *imidazole* (**12**) (46.8 g, 76%), m.p. 169—171 °C. A sample recrystallised from ethanol had m.p. 171.5—173 °C, R_F 0.42 (system **B**, u.v. absorption) (Found: C, 57.5; H, 5.2; N, 8.5; P, 6.2; S, 6.2. $C_{24}H_{26}N_3O_5PS$ requires C, 57.7; H, 5.25; N, 8.4; P, 6.2; S, 6.4%); δ($CDCl_3$) 1.33 [6 H, dt, $PO(OCH_2CH_3)_2$], 3.32 and 3.78 (2 H, dm, $CHCH_2$), 4.28 [4 H, m, $PO(OCH_2CH_3)_2$], 5.07 (2 H, d, NCH_2Ph), 5.10 [1 H, m, $P(O)CHCH_2$], 6.25 (1 H, s, Imid. CH), 7.1 (5 H, m, CH_2Ph), 7.76 (4 H, m, Phth), and 11.62 (1 H, br s, SH).

Diethyl [2-(1-*Benzylimidazol-4-yl*)-1-*phthalimidoethyl*]*phosphonate* (**13**).—A solution of the thiol (**12**) (32 g, 64 mmol) in ethanol (600 ml) was stirred with W-5 Raney nickel[25] (75 g of ethanol-wet solid) and boiled under reflux for 3 h. If the reaction was incomplete at this stage, as shown by t.l.c. (system C, u.v. absorption), further portions of Raney nickel were added and refluxing continued until no remaining thiol could be detected. The nickel was removed by filtration through 'Hyflo' and the filtrate evaporated to dryness. The residual syrup was recrystallised from toluene to give the *title compound* (**13**) (19.8 g, 66%), m.p. 126—127 °C (Found: C, 61.9; H, 5.6; N, 8.75; P, 6.5. $C_{24}H_{26}N_3O_5P$ requires C, 61.7; H, 5.6; N, 9.0; P, 6.6%); δ($CDCl_3$) 1.34 [6 H, dt, $PO(OCH_2CH_3)_2$], 3.27 and 3.75 (2 H, dm, $CHCH_2$), 4.23 [4 H, m, $PO(OCH_2CH_3)_2$], 4.95 (2 H, s, CH_2Ph), 5.05 [1 H, m, $P(O)CHCH_2$], 6.60 (1 H, s, Imid. 4-H), 6.89 and 7.16 (2 H, and 3 H, dm, CH_2Ph), 7.34 (1 H, s, Imid. 2-H), and 7.72 (4 H, dm, Phth).

Diethyl [1-*Amino*-2-(1-*benzylimidazol*-4-*yl*)*ethyl*]*phosphonate* (**14**).—A solution of the phthalimido compound (**13**) (25.7 g, 55 mmol) in ethanol (120 ml) was stirred at room temperature while a solution of hydrazine hydrate (4.21 g, 84 mmol) in ethanol (120 ml) was added. The solution was boiled under reflux for 3 h and then evaporated to dryness under reduced pressure. The residue was treated with 1M hydrochloric acid (250 ml), and the phthalohydrazide filtered off and washed with water (2 × 25 ml). The combined filtrates were extracted with dichloromethane (2 × 100 ml) and then adjusted to pH 8 by addition of 2M aqueous sodium hydroxide. The solution was then extracted with dichloromethane (150 ml, 2 × 100 ml) and the combined extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give the crude amino compound (**14**) as a syrup (17.3 g, 93%).

[1-*Amino*-2-(1-*benzylimidazol*-4-*yl*)*ethyl*]*phosphonic Acid* (**15**).—A solution of the crude phosphonate diester (**14**) (25.5 g, 76 mmol) in 6M hydrochloric acid (255 ml) was stirred and boiled under reflux for 20 h. The solution was evaporated to dryness under reduced pressure and the residue treated with water (250 ml) and evaporated again. The residue was redissolved in water (50 ml) and the solution adjusted to pH 6 by addition of ammonia (*d* 0.880) and refrigerated for 2 h. The crystalline solid which separated was filtered off, washed with ice-cold water (25 ml), and dried to give the *phosphonic acid* (**15**) (14.8 g), m.p. 257—258 °C (decomp.). A second crop (2.5 g) was obtained by concentration of the filtrate followed by readjustment of the pH to 6; total yield 17.3 g (82%). A sample recrystallised from water had m.p. 258.5—260 °C (decomp.), R_F 0.38 (system D, ninhydrin spray) (Found: C, 46.1; H, 6.4; N, 13.6; P, 10.0; H_2O, 9.5. Calc. to dryness: C, 50.9; H, 5.9; N, 15.0; P, 11.0. $C_{12}H_{16}N_3O_3P$ requires C, 51.25; H, 5.7; N, 14.9; P, 11.0%); δ(D_2O/DCl) 3.2 and 3.4 (2 H, dm, $CHCH_2$), 3.66 [1 H, m, $P(O)CHCH_2$], 5.40 (2 H, s, CH_2Ph), 7.47 (6 H, m, CH_2Ph and Imid. 4-H), and 8.80 (1 H, d, Imid. 2-H).

1-*Amino*-2-*imidazol*-4-*ylethylphosphonic Acid* (*Phosphonohistidine*) (**16**).—A solution of the *N*-benzyl compound (**15**) (20.7 g, 74 mmol) in water (1 500 ml) containing ammonia (*d* 0.880) (4 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% Pd–C (10 g) until t.l.c. (system D, ninhydrin spray) showed that no *N*-benzyl compound (**15**) remained (*ca.* 40 h). The catalyst was removed by filtration and the colourless filtrate evaporated to dryness. The residual solid foam (13 g) was recrystallised from water (45 ml) to give the *phosphonohistidine* (**16**) as colourless crystals (7.1 g, after drying at 60 °C *in vacuo* followed by overnight equilibration by exposure to the atmosphere), m.p. 255—256 °C (efferv.) (Found: C, 26.5; H, 6.35; N, 18.6; P, 13.6; H_2O, 15.6. $C_5H_{10}N_3O_3P{\cdot}2H_2O$ requires C, 26.4; H, 6.2; N, 18.5; P, 13.6; H_2O, 15.9%). A second crop of product (3.7 g) was obtained by concentration of the recrystallisation mother liquors, total yield 10.8 (64%).

3-*Acetamido*-3-(*diethoxyphosphinoyl*)*propionamide* (**17**).—The ethyl ester (**4**) (111 g, 376 mmol) was dissolved in ammonia (*d* 0.880; 900 ml) and the solution was stored at room temperature for 24 h. It was then evaporated to dryness under reduced pressure at 40 °C and the residue dissolved in ethanol (250 ml); the solution was once more evaporated to dryness. The residual oil was dissolved in acetone (500 ml), seeded, and the mixture stored in the refrigerator for 24 h. The precipitated 3-*acetamido compound* (**17**) was filtered off, washed with acetone (200 ml), and light petroleum (b.p. 40—60 °C, 2 × 200 ml) and then dried (84.1 g, 84%), m.p. 141—147 °C, R_F 0.2 (system E, Cl_2/KI visualisation). Recrystallisation from butanol gave material with m.p. 159—161 °C (Found: C, 40.6; H, 7.1; N, 10.5; P, 11.3. $C_9H_{19}N_2O_5P$ requires C, 40.6; H, 7.2; N, 10.5; P, 11.6%); δ (100 MHz, D_2O) 1.30 (6 H, t, CH_3CH_2O), 2.0 (3 H, d, CH_3CONH), 2.7 (2 H, m, $CHCH_2CONH_2$), 4.2 (4 H, m, CH_3CH_2O), and 4.8 [1 H, m, $P(O)CHCH_2$].

3-*Acetamido*-3-*diethoxyphosphinoylpropionitrile* (**18**).—A solution of the amide (**17**) (28.7 g, 108 mmol) and nickel acetate tetrahydrate (0.29 g, 1.16 mmol) in acetic anhydride[10] (250 ml) was stirred at 160 °C (bath temperature) under reflux for 3 h. The solution was cooled and evaporated to dryness under reduced pressure. The residual oil was dissolved in water (2 × 150 ml) and the solution evaporated to dryness once more. The pale brown syrup was dissolved in water (50 ml), ethane-1,2-diamine (6.5 g, 108 mmol) added, and the strongly alkaline solution was stored for 2 h at room temperature. The solution was extracted with dichloromethane (4 × 50 ml) and the combined extracts were washed with 4M HCl (40 ml), dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give the *nitrile* (**18**) (14.3 g, 53%), R_F 0.7 (system A, u.v. absorption). Recrystallisation from toluene gave material with m.p. 91—92 °C (Found: C, 43.4; H, 6.8; N, 11.2; P, 12.3. $C_9H_{17}N_2O_4P$ requires C, 43.55; H, 6.9; N, 11.3; P, 12.5%); δ($CDCl_3$) 1.37 (6 H, dt, CH_3CH_2O), 2.08 (3 H, d, CH_3CONH), 2.85 (2 H, m, $CHCH_2CN$), 3.20 (4 H, m, CH_3CH_2O), 3.73 [1 H, m, $P(O)CHCH_2$], and 7.75 (1 H, d, NH).

Diethyl (1-*Acetamido*-3-*imino*-3-*methoxypropyl*)*phosphonate Hydrochloride* (**19**).—All the reagents used in this preparation were carefully dried before use, and moisture was excluded

throughout. The nitrile (**18**) (2.0 g, 8 mmol) was dissolved in dichloromethane (20 ml), methanol (0.32 g, 10 mmol) added and the solution cooled in ice–salt and saturated with hydrogen chloride. The mixture was cooled thus for 1 h and then stored in the refrigerator overnight. The solution was evaporated to dryness under reduced pressure (oil-pump) and the residue redissolved in dichloromethane (20 ml) and re-evaporated to dryness to leave the imino ester hydrochloride (**19**) as a white foam (2.7 g) still containing a little residual dichloromethane.

Diethyl [1-*Acetamido*-3-(2,2-*diethoxyethylamino*)-3-*iminopropyl*]*phosphonate Hydrochloride* (**20**).—The crude imino ester hydrochloride (**19**) (2.7 g, 8 mmol) was dissolved in dry methanol (20 ml) and a solution of aminoacetaldehyde diethyl acetal (1.07 g, 8 mmol) in dry methanol (20 ml) added. After storage at room temperature over the weekend, the solution was evaporated to dryness under reduced pressure to leave the crude amidine hydrochloride (**20**) as an oily solid (3.5 g) containing a little methanol.

1-*Amino*-2-*imidazol*-2-*ylethylphosphonic Acid* (*Phosphonoisohistidine*) (**21**).—The crude amidine hydrochloride (**20**) (3.5 g) was dissolved in 6M hydrochloric acid (45 ml) and stored at room temperature for 24 h. An n.m.r. spectrum of a sample evaporated to dryness showed *ca.* 92% loss of ethoxy groups. The reaction mixture was then heated at 80 °C for 24 h. An n.m.r. spectrum of a sample evaporated to dryness now showed almost complete loss of ethoxy and acetyl groups and the presence of an imidazole peak at δ 7.5. The reaction mixture was evaporated to dryness under reduced pressure, the residue dissolved in water (30 ml), and the solution applied to a column of Duolite C225 cation exchange resin (12 ml, H^+ form). Elution with water (100 ml) and evaporation of the eluate to dryness gave a dark brown gum (1.17 g), the n.m.r. spectrum of which showed only a small peak at δ 7.5. The column was then eluted with 1M ammonia (30 ml), 3M ammonia (70 ml), and 5M ammonia (50 ml). The combined eluates were evaporated to dryness under reduced pressure to leave a brown gum (0.84 g), the n.m.r. of which was consistent with the desired product.

This product was combined with material from earlier preparations (total 1.92 g) and crystallised from water (9 ml). The precipitated crystals were filtered off, washed with ice-cold water (2 × 1 ml), and dried. The almost white hygroscopic *phosphonoisohistidine* (**21**) was allowed to equilibrate in the air overnight: yield 0.27 g, m.p. 160 °C (softened and decomp.), R_F 0.6 (cellulose, BuOH–H_2O–AcOH 5:3:2, ninhydrin spray) (Found: C, 26.4; H, 6.3; N, 18.4; H_2O, 16.0%. Calc. to dryness C, 31.4; H, 5.4; N, 21.9. $C_5H_{10}N_3O_3P$ requires C, 31.4; H, 5.3; N, 22.0%).

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References

1 D. A. Gerber, *J. Clin. Invest.*, 1975, **55**, 1164; D. A. Gerber and M. G. Gerber, *J. Chronic Dis.*, 1977, **30**, 115.
2 D. A. Gerber, *J. Rheumatol.*, 1975, **2**, 384.
3 C. H. Cashin, E. J. Lewis, and T. Burden, *British J. Rheumatol.*, 1985, **24**, 137; N. A. Roberts and B. P. Tong, *Drug Des. Delivery*, 1986, **1**, 113.
4 European Patent Publication No. 118,787A/1984.
5 For references, and the first synthesis of Thr(P), see E. K. Baylis, C. D. Campbell, and J. G. Dingwall, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2845.
6 M. Soroka and P. Mastalerz. *Rocz. Chem.*, 1976, **50**, 661.
7 G. R. Fosker, K. D. Hardy, J. H. C. Nayler, P. Seggery, and E. R. Stove, *J. Chem. Soc. C*, 1971, 1917.
8 'Comprehensive Heterocyclic Chemistry,' A. R. Katritzky and C. W. Rees (eds.), Pergamon Press, Oxford, 1984, vol. 5, p. 473.
9 Ger. Offen. 2,847,625/1980.
10 'Comprehensive Heterocyclic Chemistry,' A. R. Katritzky and C. W. Rees (eds.), Pergamon Press, Oxford, 1984, vol. 5, p. 464.
11 R. J. Weinkam and E. C. Jorgensen, *J. Am. Chem. Soc.*, 1973, **95**, 6084.
12 J. R. Cavanaugh, *J. Am. Chem. Soc.*, 1970, **92**, 1488.
13 K. G. R. Pachler, *Spectrochim. Acta*, 1964, **20**, 581.
14 R. B. Martin and R. Mathur, *J. Am. Chem. Soc.*, 1965, **87**, 1065.
15 J. J. M. Rowe, J. Hinton, and K. L. Rowe, *Chem. Rev.*, 1970, **70**, 1.
16 M. Karplus, *J. Chem. Phys.*, 1959, **30**, 11.
17 Z. Siatecki and H. Kozlowski, *Org. Mag. Res.*, 1981, **17**, 172.
18 F. R. Atherton, C. H. Hassall, and R. W. Lambert, *J. Med. Chem.*, 1986, **29**, 29.
19 J. Feeney, *J. Magn. Res.*, 1976, **21**, 473.
20 R. J. Abraham, P. Loftus, and W. A. Thomas, *Tetrahedron*, 1977, **33**, 1227.
21 R. J. Abraham and G. Gatti, *J. Chem. Soc. B*, 1969, 961.
22 J. Thiem and B. Meyer, *Tetrahedron Lett.*, 1977, 3573.
23 E. L. Eliel, *Angew. Chem. Int. Edn.*, 1965, **4**, 761.
24 J. Dale, 'Stereochemistry and Conformational Analysis,' Verlag Chemie, New York-Weinheim, 1978, p. 154.
25 *Org. Synth.*, Coll. vol. 3, p. 176.
26 'The Merck Index,' ed. M. Windholz, Merck and Co. Inc., Rahway, N.J., 10th edn., 1983, p. 4621.

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