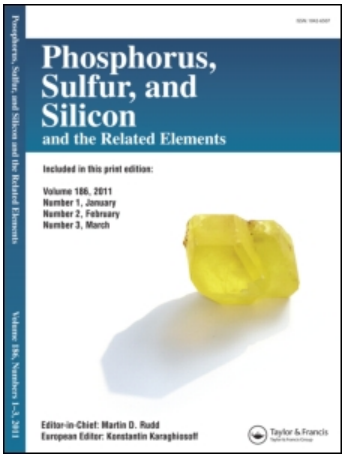

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ORGANOPHOSPHORUS COMPOUNDS AS POTENTIAL FUNGICIDES. PART III.[1] PEPTIDE DERIVATIVES OF α-AMINOPROPANEPHOSPHONIC ACID

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ORGANOPHOSPHORUS COMPOUNDS AS POTENTIAL FUNGICIDES. PART III.[1] PEPTIDE DERIVATIVES OF α-AMINOPROPANEPHOSPHONIC ACID

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Di- and tri-peptides with α-aminopropanephosphonic acid as the acid-terminal residue have been prepared containing *L*-alanyl, *D*-alanyl, *DL*-alanyl, and glycyl groups. Nmr parameters (^1H, ^{13}C, and ^{31}P) are reported for solutions of the peptides in D_2O and are discussed. Diastereoisomeric forms of the *L*-ala-, and *L*-ala-*L*-ala-peptides were separated on a cation exchange-column and it was found in each case that the methine and carbonyl groups of the alanyl residue that is adjacent to the aminophosphonic group exhibit slightly different ^{13}C chemical shifts in each of the two diastereoisomers. Fast-atom bombardment mass spectrometry gave characteristic $[MH]^+$ ions, which usually appeared as the base peak. Fragment ions were formed by the elimination of α-lactam units from the amino-carboxylic acid residues and phosphorous acid from the aminophosphonic acid residue. The *L*-ala-peptides showed similar activity to that of α-aminopropanephosphonic acid when applied as seed-dressings at 400 ppm for the control of *Drechslera* spp. Glycyl peptides were slightly less active but the *D*-ala-peptides had little or no activity.

Key words: Organophosphorus; fungicides; phosphonopeptides; NMR spectroscopy; FAB mass spectrometry.

INTRODUCTION

The role of aminophosphonic acids in biological processes has attracted widespread interest since the discovery of 2-aminoethanephosphonic acid in lipid hydrolysates derived from ciliated protozoa[2] and other natural organisms,[3,4] and the suggestion that aminophosphonic acids may occur in natural protein structures[5] and polypeptide chains.[6] It is now well known that a number of phosphonopeptides are produced by microorganisms.[7] The first example to be isolated, phosphinothricyl-*L*-alanyl-*L*-alanine (**1**), showed activity against gram-positive and gram-negative bacteria and against the fungal pathogens *Botrytis cinerea*, *Piricularia oryzae*, and *Rhizoctonia solani*.[8] The principal interest in synthetic phosphonopeptides has, however, been in their antibacterial activity,[9] especially that of *L*-ala-*L*-ala(P) (alafosfalin, **2**) for which the biochemistry and mode of action have been studied in detail.[10] The fungicidal activity of synthetic phosphonopeptides has attracted less interest; in an earlier investigation, *N*-glycyl-α-aminobenzylphosphonic acid (**3**) was shown to be neither antibacterial nor fungicidal against unspecified organisms at a con-

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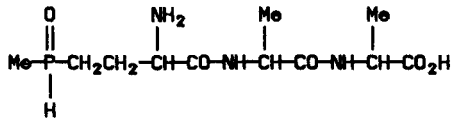

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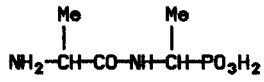

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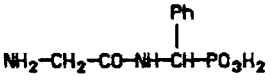

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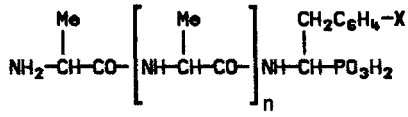
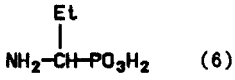


X = 4-F or 4-Me

(4, n = 0); (5, n = 1)



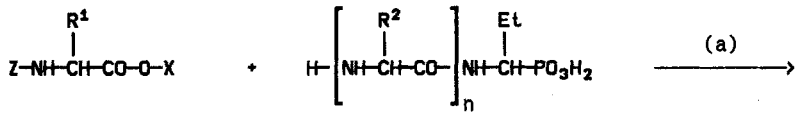
(6)

centration of 10 mg cm^{-3} (10,000 ppm).[11] It has been reported more recently, however, that the *N*-(*L*-ala-) and *N*-(*L*-ala-*L*-ala-) derivatives (**4**, **5**) of 1-amino-2-(4-fluoro- or 4-methylphenyl)ethylphosphonic acid show fungicidal activity that is similar to that of the parent aminophosphonic acids.[12] In view of the activity of α-aminopropanephosphonic acid (**6**) (ampropylfos)[13] when applied as a seed-dressing agent for the control of *Drechslera* spp. and certain other pathogens of cereal crops,[14] we have studied the effects of incorporating this molecule into various peptide structures. In this paper we report the preparation and characterization of a range of such peptides (**7**, **8**) and discuss their activity against *Drechslera* spp.

RESULTS AND DISCUSSION

Preparations of Phosphonopeptides

The incorporation of aminophosphonic acids into synthetic peptides was first investigated by Yamauchi *et al.*[15] who found that derivatives in which the aminophosphonic unit constitutes the acid-terminal group could be prepared by conventional methods, such as reaction with an *N*-protected α-amino-carboxylic acid in the presence of dicyclohexylcarbodiimide. Coupling of the phosphonic group to form a phosphonamide link was less straightforward, requiring activation of the phosphonic group by its conversion to phosphonochloridate. Numerous methods for the preparation of phosphonopeptides, usually with an acid-terminal phosphonic group[16–18] but also in certain cases containing phosphonamide bonds,[19] were subsequently reported. In our present studies we have prepared a range of derivatives (**8**) containing α-aminopropanephosphonic acid as the acid-terminal residue. Two types of reaction were used (Scheme I), the more successful being that in which the free aminophosphonic acid (**7**, $n = 0$), or lower peptide thereof (**7**, $n = 1$), was coupled to the *N*-hydroxysuccinimide ester of an *N*-protected α-amino-car-



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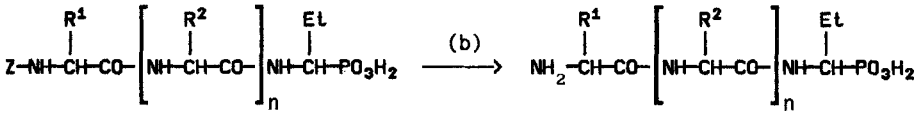

(8)

Z = $PhCH_2O_2C-$

X =  (a): $NaHCO_3$ (Method A) or Et_3N (Method B) in ethanol or DMF.

X = $-CO_2Bu^i$ (a): Et_3N in ethanol (Method C).

(b): (i) HBr/HOAc; (ii) propylene oxide.

SCHEME I

boxylic acid in the presence of sodium bicarbonate (Method A) or triethylamine (Method B).[17] The second procedure, which gave somewhat lower yields in those cases in which comparison was made, involved interaction of α-aminopropanephosphonic acid or its peptide, in the presence of triethylamine in aqueous ethanol, with a mixed carboxylic-carbonic anhydride which has been prepared *in situ* from the *N*-protected α-amino-carboxylic acid and isobutyl chloroformate (Method C).[17] Yields obtained by the first procedure, after deprotection using hydrogen bromide in glacial acetic acid and treatment with propylene oxide,[17,18] were in most cases within the range 50–70% after recrystallization. Use of a dialkyl ester of the aminophosphonic acid[18] was not found to be necessary.[20] The products were obtained as white powders or crystalline solids, which sometimes retained some water of crystallization after prolonged drying *in vacuo* at 60°C.

Characterization of Phosphonopeptides

Characterization was carried out by the combined use of microanalysis, nmr spectroscopy, and FAB mass spectrometry. In addition, optical rotations were measured where appropriate but the results for any one product were found to be dependent to some extent on the method of preparation, as also were melting points. One possible cause may be the occurrence of partial racemization during synthesis, the extent depending on the experimental procedure used.[21]

Separation of Diastereoisomers[22]

The diastereoisomeric forms of (1*RS*)-1-(*L*-alanylamino)propanephosphonic acid (**8**, R^1 = Me, n = 0) have been resolved previously by chromatography on Dowex 50W-X8(H^+) cation-exchange resin[18] and the same procedure was employed in the present work. U.v. spectroscopy proved, however, to be a more sensitive means of monitoring the eluted fractions than the use of ninhydrin. The first isomer to be eluted was assumed[18] to be the (*S*,*S*)-isomer, i.e., (1*S*)-1-(*L*-alanylamino)propanephosphonic acid, followed by the (*S*,*R*)-isomer, (1*R*)-(1-*L*-alanylamino)propanephosphonic acid. These assignments had been made previously[18] on the basis of comparison with other related systems. In the present studies, specific optical rotations of $[\alpha]_{578}^{25}$ +88 and −46° were obtained for the (*S*,*S*)- and (*S*,*R*)-isomers, respectively, these values being similar, but not identical, to those reported previously ($[\alpha]_{578}^{25}$ +75 and −53°, respectively).[18] We also employed the same cation-exchange column for separation of the diastereomers of (1*RS*)-1-(*L*-alanyl-*L*-alanylamino)propanephosphonic acid (**8**, R^1 = R^2 = Me, n = 1) (not previously studied) and obtained two major fractions with specific optical rotations of $[\alpha]_{578}^{25}$ −52° (eluted first) and $[\alpha]_{578}^{25}$ −14.3° (eluted second). These fractions are tentatively assigned, on the basis of ^{13}C nmr spectroscopy (see below), as the diastereoisomers containing the α-aminopropanephosphonic acid residue in the (*R*)- and (*S*)-configurations, respectively.

^1H NMR Spectroscopy

The ^1H nmr spectra of the peptides show close similarities to those of the aminophosphonic[1] and amino carboxylic[23] acids from which they are derived. The methyl triplet of the aminopropanephosphonic residue is slightly upfield (*ca.* 0.90 ppm) compared to that for the parent acid (*ca.* 1.1 ppm),[1] whilst the methyl protons of the alanine residues give rise to doublets (overlapping if more than one is present) in the region of 1.5 ppm. Half of the aminophosphonic CH_2 multiplet is hidden beneath the alanine methyl signals but in peptides containing other amino acid residues (e.g., glycyl) the complex CH_2 multiplet[1] can be seen separately. The CH multiplets of both the aminopropanephosphonic and alanine residues overlap in the region 3.6–4.5 ppm and in glycine-containing peptides, the CH_2 signal is further superimposed on this multiplet. No differences between the diastereomeric forms of the phosphonopeptides could be seen at 60 MHz.

^{13}C NMR Spectroscopy

The broad-band proton-decoupled ^{13}C nmr spectra for solutions of the peptides in D_2O are, like the proton spectra, similar to those for the component aminophosphonic[1] and amino carboxylic[23] acids. Methyl signals for the aminopropanephosphonic residue appear as doublets at 13.3–13.4 ppm ($^3J_{PC}$ 13–14 Hz) whilst the alanine methyl groups give rise to singlets at 19–20 ppm. Methylene protons of the aminopropanephosphonic group resonate in the region 25–26 ppm, whilst the CH_2 group of glycine appears further downfield at 46.6 ppm in the glycyl dipeptide (**8**, R^1 = H, n = 0) and 49.6 ppm in the glycyl-*L*-alanyl tripeptide (**8**, R^1 = H, R^2 = Me, n = 1). As in the parent aminophosphonic acid, the carbon atom adjacent to

phosphorus in the peptides appears as a doublet at 53.0–53.4 ppm, with characteristically large one-bond coupling ($^1J_{PC}$ *ca.* 147–148 Hz).

The signals arising from the methine carbon atoms of the alanyl residues are of particular interest in that they provide a means for differentiating the diastereoisomeric forms of the di- and tri-peptides. As shown in Table I, two distinct signals can be observed for the methine carbon atoms of alanine in the *L*-alanyl dipeptide (**8**, R^1 = Me, n = 0). In the diastereoisomer having the aminophosphonic residue with the (*S*)-configuration, the chemical shift (δ_c 52.5) is at slightly lower field than that with the (*R*)-configuration (δ_c 52.2).[24] Similarly, in the case of the *L*-ala-*L*-ala-tripeptide (**8**, R^1 = R^2 = Me, n = 1), the three observed signals (Table II) can be correlated with those of the separate diastereoisomers. The common signal at 51.8 ppm is assumed to be due to the terminal alanyl group (more remote from the phosphonic group), whilst those at 53.1 and 52.8 are tentatively assigned to

TABLE I

^{13}C nmr chemical shifts of alanyl CH and carbonyl groups in diastereomeric dipeptides (**7**, R^2 = Me)[a]

Compound[b]	δ/ppm (alanyl CH)		δ/ppm (C=O)	
L-ala-(*RS*)-APPA (9)	52.2	52.5	173.6[c]	
L-ala-(*S*)-APPA (10)		52.5		173.7[d]
L-ala-(*R*)-APPA (11)	52.2		173.5[e]	

[a] Solvent D_2O. [b] APPA = α-aminopropanephosphonic acid (**6**). [c] Unresolved multiplet. [d] $^3J_{PCNC}$ 4.9 Hz. [e] $^3J_{PCNC}$ 4.3 Hz.

TABLE II

^{13}C nmr chemical shifts for alanyl CH and carbonyl groups in diastereomeric tripeptides (**8**, R^1 = R^2 = Me, n = 1)[a]

Compound[b]	δ/ppm (alanyl CH)			δ/ppm (C=O)			
L-ala-*L*-ala-(*RS*)-APPA (15)	51.8	52.8	53.1	173.4	173.5	177.2[c]	177.4[d]
L-ala-*L*-ala-(*S*)-APPA (17)	51.8		53.1		173.6		177.4
L-ala-*L*-ala-(*R*)-APPA (16)	51.8	52.8		173.5		177.3	

[a] Solvent D_2O. [b] APPA ◻ α-aminopropanephosphonic acid (**6**). [c] $^3J_{PCNC}$ 5.5 Hz. [d] $^3J_{PCNC}$ 4.9 Hz.

the central *L*-alanyl groups in the diastereoisomers having aminophosphonic residues with the (*S*)- and (*R*)-configuration, respectively.[25]

The carbonyl carbon atoms of the *L*-alanyl residues adjacent to the aminophosphonic group in the diastereoisomeric forms of the di- and tri-peptides also exhibit slightly different chemical shifts. The signals are relatively weak and are complicated by coupling to phosphorus ($^3J_{PCNC}$ 4–6 Hz). Distinct doublets were, however, recognized for the carbonyl groups in the separate diastereoisomers of the dipeptide (**8**, R^1 = Me, n = 0) and, as for the methine carbon atoms, the chemical shift in the case of the *L*-ala-(*S*)-dipeptide (173.7 ppm) was seen to be at slightly lower field than that for the *L*-ala-(*R*)-isomer (173.5 ppm). In the *L*-ala-*L*-ala-tripeptide (**8**, R^1 = R^2 = Me, n = 1), signals at 177.4 and 173.6 ppm are assigned, on the same basis, to the carbonyl carbon atoms of the central and terminal *L*-alanine residues, respectively, of the tripeptide which has the aminophosphonic group in the (*S*)-configuration, and the signals at 177.3 and 173.5 ppm to the corresponding carbon atoms in the *L*-ala-*L*-ala-(*R*)-tripeptide.[25] All four signals are clearly seen in the mixture of diastereoisomers.

^{31}P *NMR Spectroscopy*

The ^{31}P chemical shift for all peptides in D_2O was in the range 18.2–18.5 ppm. Whereas α-aminoalkanephosphonic acids exhibit a chemical shift of 13.5–14.5 ppm in D_2O, those of the ω-aminoalkanephosphonic acids vary with chain length, moving downfield from 11 ppm for aminomethanephosphonic acid to 26.7 ppm for ω-aminohexanephosphonic acid.[1] This difference may possibly be associated with changes in intramolecular hydrogen-bonding between the protonated amino group and the phosphonic acid group as the chain length increases.[26] In the peptides, the α-amino group has become part of an amide linkage and is much less likely to be protonated than is the terminal amino group. The fact that the ^{31}P chemical shift of the peptides is almost constant, irrespective of chain length, suggests that hydrogen-bonding interaction between the terminal amino and phosphonic groupings is of little significance in these compounds.

Fast Atom Bombardment Mass Spectrometry[27]

The positive-ion fast atom bombardment mass spectra of all peptides gave characteristic pseudomolecular ions, $[MH]^+$, which in most cases appeared as the base peak. In addition, prominent ions were commonly observed corresponding to $[2M + H]^+$, etc. and to various aggregates involving the matrix (glycerol). Characteristic fragmentations occurred by the loss of α-lactam units (C_3H_5NO from *N*-terminal alanyl residues or C_2H_3NO from *N*-terminal glycyl residues), and the elimination of H_3PO_3 from the phosphonic acid group.[1,28] Fragmentation patterns were confirmed in selected cases by exact mass measurements and linked scan techniques.

Fungicidal Activity

Peptides were examined for activity as seed-dressing agents by the "osmos" test, using seeds (Tellus corn) infected with *Drechslera teres*, *Drechslera graminae*, and

Drechslera avenae. The method has previously been used for the assessment of activity in simple aminoalkanephosphonic acids[1] and is described elsewhere.[29]

The results showed that the *L*-ala-dipeptide (**9**) and the *L*-ala-*L*-ala-tripeptide (**15**) have similar levels of activity to that of the parent aminophosphonic acid (ampropylfos, **6**) at 400 ppm, giving 90–100% control of *D. teres* or *D. avenae*, and 60–80% control of *D. graminae*. Tests of the separate diastereoisomeric forms of the *L*-ala-dipeptide (**10**, **11**) against the three organisms, and of the *L*-ala-*L*-ala-tripeptide (**16**, **17**) against *D. teres*, showed both diastereoisomers to be active in each case. The glycyl dipeptide (**14**) and *L*-ala-glycyl tripeptide (**20**) were slightly less active than the *L*-alanyl analogues, giving 70–80% control of *D. teres* at 400 ppm, but peptides with a *D*-alanyl residue attached to the aminophosphonic acid group, viz. the *D*-ala-dipeptide (**13**), and the *D*-ala-*D*-ala- (**18**) and *L*-ala-*D*-ala- (**19**) tripeptides, showed little or no activity. The activity of the *DL*-alanyl dipeptide (**12**) was not significantly less than that of the *L*-alanyl compound (**9**) but is presumably due to the *L*-alanyl component only.[30]

The marked difference between the effects of *L*- and *D*-alanyl residues when attached to the aminophosphonic group is of interest. It has previously been shown that the tripeptide, *L*-*m*-fluorophenylalanyl-*L*-alanyl-*L*-alanine, has much higher fungitoxicity towards *Pythium ultimatum* than either the dipeptide, *L*-*m*-fluorophenylalanyl-*L*-alanine, or the parent amino-acid, *L*-*m*-fluoro-phenylalanine and it was suggested that the tripeptide acts as an effective carrier for the delivery of *m*-fluorophenylalanine into the fungal cell.[31] It is also of interest to note that *L*-α-aminoethanephosphonic acid shows virtually no antibacterial activity unless combined in a suitable di- or higher peptide (e.g., the *L*-alanyl dipeptide, 2) to aid transport into the bacterial cell.[9] In contrast, α-aminopropanephosphonic acid is fungicidal whether or not it is combined in an *L*-alanyl peptide structure. This different behaviour may be due to a difference in the mode of action.

EXPERIMENTAL

Starting Materials. *N*-Hydroxysuccinimide was prepared from succinic anhydride and hydroxylamine hydrochloride in the presence of sodium hydroxide.[32] α-Aminopropanephosphonic acid,[1] *N*-carbobenzoxy derivatives[33] of glycine and alanine, and their *N*-hydroxysuccinimide esters,[34] were prepared by the described procedures; other starting materials were obtained commercially.

Spectroscopy and Instrumentation. ^{1}H nmr spectra were recorded at 60 MHz on a Perkin-Elmer R12B spectrometer or at 80 MHz on a Bruker WP-80 spectrometer. ^{13}C and ^{31}P nmr spectra were recorded on the Bruker WP-80 instrument, operating at 20.12 and 32.395 MHz respectively. ^{1}H and ^{13}C chemical shifts were determined for solutions in D_2O and are relative to sodium trimethylsilylpropionate (tsp). ^{31}P chemical shifts (also determined in D_2O) are relative to 85% phosphoric acid. Positive ion fast-atom bombardment mass spectra were obtained using a glycerol matrix on a VG Micromass ZAB-1F instrument, with a primary beam of xenon atoms operating at 8 kV. (In the spectral data, G = glycerol). Optical rotations were measured at 578 nm, using an Optical Activity Ltd. photoelectric polarimeter, type AA-10.

Preparations of Peptides. Peptides were prepared as described,[17] (a) by the interaction in ethanol or DMF of the *N*-hydroxysuccinimide esters of *N*-carbobenzoxy-amino-acids with α-aminopropanephosphonic acid (or a lower peptide derivative thereof), using either sodium bicarbonate (Method A) or triethylamine (Method B) as base, and (b) by the mixed carboxylic-carbonic anhydride method (Method C),[17] in which the carbobenzoxy derivative of an amino acid was allowed to react with isobutyl chloroformate in triethylamine/toluene (−8°C) and the so-formed anhydride was treated with the aminophosphonic acid and triethylamine in aqueous ethanol. The initial products (*N*-protected peptides) were

concentrated under reduced pressure, diluted with water, extracted with dichloromethane to remove non-polar impurities, acidified (to pH 2) with dilute hydrochloric acid and, after further extraction with dichloromethane, purified on a column of freshly regenerated (acid cycle) cation-exchange resin [Dowex 50 W-X 8(H^+), 16-40 mesh]. Deprotection was carried out by the use of hydrogen bromide in glacial acetic acid at room temperature[17,18] and the free peptides were liberated by treatment with propylene oxide.[1] Peptides were recrystallized from aqueous acetone and dried *in vacuo* at 60°C to give the following.[35]

(1R,S)-1-(L-alanylamino)propanephosphonic acid (**9**). (**8**, R^1 = Me, n = 0) as the monohydrate (3.66 g, 56% by method A in ethanol), m.p. 270–274°C (lit.[18] m.p. 271–275°C) (Found: C, 32.1; H, 7.1; N, 12.2. Calc. for $C_6H_{15}N_2O_4P.H_2O$: C, 31.6; H, 7.5; N, 12.3%); $[\alpha]_{578}^{30}$ +14° (c = 1% in water) (lit.[18] +18°); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.31 Hz), 1.57 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.99 Hz), 1.18–2.13 (2H, br m, CH_2), 3.72–4.25 (2H, br m, CH); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3CH$), 25.7 (s, $\underline{C}H_2$), 52.2 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 53.4 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.6 (m, C=O); δ_P (D_2O) 18.2; FAB ms: *m/z* (%) 421 ($2MH^+$, 18.6), 350 ([2MH − $C_3H_5NO]^+$, 4.8), 303 ([MH + G]$^+$, 3.8), 232 ([MH + G − $C_3H_5NO]^+$, 2.3), 211 (MH^+, 100), 140 ([MH − $C_3H_5NO]^+$, 20.1), 129 ([MH − $H_3PO_3]^+$, 12.8). The unrecrystallized compound was separated into diastereoisomers (**10**, **11**) (see below).

(1R,S)-1-(DL-alanylamino)propanephosphonic acid (**12**). (**8**, R^1 = Me, n = 0) (1.85 g, 66% by method B in ethanol), m.p. 260–262°C (Found: MH^+, *m/z* 211.0855. $C_6H_{16}N_2O_4P$ requires: *m/z* 211.0848), δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.32 Hz), 1.56 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.63–2.25 (2H, br m, $C\underline{H}_2CH$), 3.72–4.27 (2H, m, CH); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$ $^3J_{PC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3CH$), 25.7 (s, $\underline{C}H_2CH$), 52.2 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 53.3 (d, CHP, $^1J_{PC}$ 147.7 Hz), 173.7 (m, C=O); δ_P (D_2O) 18.4. Method C gave a similar product (1.1. g, 31%).

(1R,S)-1-(D-alanylamino)propanephosphonic acid (**13**). (**8**, R^1 = Me, n = 0) as the dihydrate (2.66 g, 63% by method B in ethanol), m.p. 247–248°C (Found: C, 30.0; H, 6.9; N, 11.4. $C_6H_{15}N_2O_4P.2H_2O$ requires: C, 29.3; H, 7.7; N, 11.4%); $[\alpha]_{578}^{25}$ −16° (c = 1% in water); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.1 Hz), 1.56 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.8 Hz), 1.7–2.2 (2H, br m, CH_2), 3.73–4.15 (2H, br m, CH); δ_C (D_2O) 13.4 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.7 (s, CH_3), 25.8 (s, CH_2), 52.3 (s, $\underline{C}HCH_3$), 52.6 (s, $\underline{C}HCH_3$), 53.3 (d, CHP, $^1J_{PC}$ 147.7 Hz), 173.6 (s, C=O); δ_P (D_2O) 18.5; FAB ms: *m/z* (%) 421 ($2MH^+$, 26.6), 350 ([2MH − $C_3H_5NO]^+$, 6.1), 211 (MH^+, 100), 140 ([MH − $C_3H_5NO]^+$, 26.8), 129 (13.2).

(1R,S)-1-(glycylamino)propanephosphonic acid (**14**). (**8**, R^1 = H, n = 0) as the monohydrate (3.2 g, 81.6% by method B in DMF), m.p. 254–255°C (lit.[18] 269–272°C) (Found: C, 28.7; H, 6.6; N, 13.3; MH^+ *m/z* 197.0695. Calc. for $C_5H_{13}N_2O_4P.H_2O$: C, 28.4; H, 7.0; N, 13.1%. Calc. for $C_5H_{14}N_2O_4P$: *m/z* 197.0691), δ_H (D_2O) 0.90 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.0 Hz), 1.55–2.2 (2H, br m, $C\underline{H}_2CH_3$), 3.93 (2H, s, $C\underline{H}_2NH_2$); δ_C (D_2O) 13.4 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 25.8 (s, $\underline{C}H_2CH_3$), 43.6 (s, $\underline{C}H_2NH_2$), 53.4 (d, CHP, $^1J_{PC}$, 147.7 Hz), 169.8 (d, C=O, $^3J_{PCNC}$ 4.9 Hz); δ_P (D_2O) 18.3; FAB ms: *m/z* (%) 393 ($2MH^+$, 15.8). 336 ([2MH − $C_2H_3NO]^+$, 4.0), 289 ([MH + G]$^+$, 12.3), 197 (MH^+, 100), 140 ([MH − $C_2H_3NO]^+$, 22.8), 115 ([MH − $H_3PO_3]^+$, 17.4).

(1R,S)-1-(L-alanyl-L-alanylamino)propanephosphonic acid (**15**). (**8**, R^1 = R^2 = Me, n = 1) as a hydrate (0.95 g, 65.6% by method A in ethanol), m.p. 245°C (Found: C, 33.2; H, 6.9; N, 11.9. $C_9H_{20}N_3O_5P.2.5\ H_2O$ requires: C, 33.1; H, 7.7; N, 12.9%); $[\alpha]_{578}^{30}$ −31.0° (c = 1% in water); δ_H (D_2O) 0.89 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.0 Hz), 1.49 (6H, m, $C\underline{H}_3CH$), 1.75–2.1 (2H, br m, CH_2), 3.68–4.14 (1H, br m, $C\underline{H}CH_2$), 4.39 (2H, q, $C\underline{H}CH_3$, $^3J_{HCCH}$ 8.1 Hz); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.4 (s, $\underline{C}H_3CH$), 19.8 (s, $\underline{C}H_3CH$), 26.0 (s, CH_2), 51.8 (s, $\underline{C}HCH_3$), 52.8 (s, $\underline{C}HCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 147.7 Hz), 53.1 (s, $\underline{C}HCH_3$), 173.4 (s, C=O), 173.5 (s, C=O), 177.2 (d, C=O, $^3J_{PCNC}$ 5.5 Hz), 177.4, (d, C=O, $^3J_{PCNC}$ 4.9 Hz); δ_P (D_2O) 18.3; FAB ms: *m/z* (%) 374 ([MH + G]$^+$, 4.1), 282 (MH^+, 100), 211 ([MH − $C_3H_5NO]^+$, 21.4), 143 ($C_6H_{11}N_2O_2^+$, 12.8), 140 ([MH − 2.$C_3H_5NO]^+$, 10.7), 115 ($C_5H_{11}N_2O^+$, 58.4). The unrecrystallized compound was separated into diastereoisomers (**16**, **17**) (see below).

(1R,S)-1-(D-alanyl-D-alanylamino)propanephosphonic acid (**18**). (**8**, R^1 = R^2 = Me, n = 1) (0.3 g, 37% by method B in ethanol), m.p. 242–244°C; $[\alpha]_{578}^{30}$ +18.2° (c = 12.5% in water); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 6.3 Hz), 1.37–1.6 (6H, m, $C\underline{H}_3CH$), 1.65–2.2 (2H, m, CH_2), 3.63–4.25 (3H, br m, CH); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.4 (s, $\underline{C}H_3CH$), 19.8 (s, $\underline{C}H_3CH$), 26.0 (s, CH_2), 51.9 (s, $\underline{C}HCH_3$), 53.0 (s, $\underline{C}HCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 147.7 Hz), 173.5 (s, C=O), 177.3 (d, C=O, $^3J_{PCNC}$ 3.7 Hz); δ_P (D_2O) 18.3.

(1R,S)-1-(L-alanyl-D-alanylamino)propanephosphonic acid (**19**). (**8**, $R^1 = R^2 = Me$, n = 1) (0.84 g, 33% by method B in ethanol), m.p. 264–265°C (Found: MH^+, *m/z* 282.12156. $C_9H_{21}N_3O_5P$ requires: *m/z* 282.12186), $[\alpha]_{578}^{25}$ +37.5° (c = 1% in water); δ_H (D_2O) 0.94 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.1 Hz), 1.53 (6H, m, $C\underline{H}_3CH$), 1.71–2.05 (2H, m, $C\underline{H}_2CH_3$), 3.75–4.6 (3H, br m, CH); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.4 (s, $\underline{C}H_3CH$), 19.9 (s, $\underline{C}H_3CH$), 26.0 (s, CH_2), 52.1 (s, $\underline{C}HCH_3$), 53.0 (s, $\underline{C}HCH_3$), 53.1 (d, CHP, $^1J_{PC}$ 148.3 Hz), 173.7 (s, C=O), 177.3 (d, C=O, $^3J_{PCNC}$ 6.1 Hz); δ_P (D_2O) 18.3; FAB ms: *m/z* (%) 563 ($2MH^+$, 11.8), 282 (MH^+, 100), 211 ([MH − C_3H_5NO]$^+$, 26.4), 143 ($C_6H_{11}N_2O_2^+$, 18.3), 140 ([MH − $2C_3H_5NO$]$^+$, 7.4), 115 ($C_5H_{11}N_2O^+$, 16.7).

(1R,S)-1-(L-alanylglycylamino)propanephosphonic acid (**20**). (**8**, R^1 = Me, R^2 = H, n = 1) (1.28 g, 72% by method B in ethanol), m.p. 255–256°C (Found: MH^+, *m/z* 268.1067. $C_8H_{19}N_3O_5P$ requires: *m/z* 268.1062), $[\alpha]_{578}^{30}$ +16° (c = 1% in water), δ_H (D_2O) 0.91 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.3 Hz), 1.57 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 7.3 Hz), 1.67–2.06 (2H, br m, $C\underline{H}_2CH_3$), 3.65–4.22 (2H, br m, CH), 4.03 (2H, s, CH_2); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 14.0 Hz), 19.3 (s, $\underline{C}H_3CH$), 25.9 (s, $\underline{C}H_2CH_3$), 45.5 (s, CH_2), 52.2 (s, $\underline{C}HCH_3$), 53.2 (d, CHP, $^1J_{PC}$ 145.3 Hz), 171.5–174.4 (m, C=O); δ_P (D_2O) 18.4; FAB ms: *m/z* (%) 390 (11.8), 290 (16.8), 268 (MH^+, 15.4), 197 ([MH − C_3H_5NO]$^+$, 15.5), 140 ([MH − $C_5H_9N_2O_2$]$^+$, 9.2), 115 ($C_5H_{11}N_2O^+$, 57.2), 102 (100).

Separation of Diastereoisomeric Forms of Peptides.[18]

(1R,S)-1-(L-alanylamino)propanephosphonic acid (**9**). (**8**, R^1 = Me, n = 0). A sample of unrecrystallized dipeptide (1.0 g) was dissolved in the minimum of water and placed on a column (20 cm × 2.5 cm) of freshly regenerated (acid cycle) cation-exchange resin [Dowex 50W-X8(H^+), 100–200 mesh]. Elution was carried out with water and the eluant was monitored by u.v. absorption (190–230 nm). A series of fractions (each 16 cm^3) was collected.

Fractions 45–70 gave the *S,S*-dipeptide (**10**) (0.45 g), m.p. 281°C, $[\alpha]_{578}^{25}$ +88° (c = 1% in water) (lit.[18] +75°); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.10 Hz), 1.40–2.20 (2H, br m, CH_2), 1.60 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 7.02 Hz), 3.73–4.10 (1H, m, $C\underline{H}CH_3$), 3.98–4.10 (1H, q, $C\underline{H}CH_3$, $^3J_{HCCH}$ 7.02 Hz); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3CH$), 25.8 (s, CH_2), 52.5 (s, $\underline{C}HCH_3$), 53.3 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.7 (d, C=O, $^3J_{PCNC}$ 4.9 Hz); δ_P (D_2O) 18.0.

Fractions 70–100 gave the *S,R*-dipeptide (**11**) (0.35 g), m.p. 265°C, $[\alpha]_{578}^{25}$ −46° (c = 1% in water) (lit.[18] −53°); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.06 Hz), 1.56 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.63–2.25 (2H, br m, CH_2), 3.72–4.27 (2H, br m, 2 × CH); δ_C (D_2O) 13.4 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 19.6 (s, $\underline{C}H_3CH$), 25.8 (s, CH_2), 52.2 (s, $\underline{C}HCH_3$), 53.4 (d, $\underline{C}HP$, $^1J_{PC}$ 148.9 Hz), 173.5 (d, C=O, $^3J_{PCNC}$ 4.3 Hz); δ_P (D_2O) 18.8.

(1R,S)-1-(L-alanyl-L-alanylamino)propanephosphonic acid (**15**). (**8**, $R^1 = R^2 = Me$, n = 1). A sample of unrecrystallized tripeptide (0.8 g) was dissolved in the minimum of water and placed on a column (20 cm × 2.5 cm) of freshly regenerated (acid cycle) cation-exchange resin [Dowex 50W-X8(H^+), 100–200 mesh]. Elution was carried out with water and the eluent was monitored by u.v. absorption (190–230 nm). A series of fractions (each 16 cm^3) was collected. Fractions 35–55 gave the first diastereoisomer (**16**) (tentatively assigned as the *S,S,R*-tripeptide—see Discussion) (0.18 g), m.p. 258–260°C, $[\alpha]_{578}^{25}$ −52.0°C (c = 4% in water); δ_H (D_2O) 0.92 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.32 Hz), 1.43 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.56 (3H, d, $C\underline{H}_3CHNH_2$, $^3J_{HCCH}$ 6.84 Hz), 1.60–2.25 (2H, m, CH_2), 3.67–4.57 (3H, br m, CH); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$, 13.4 Hz), 19.4 (s, $\underline{C}H_3CH$), 25.9 (s, CH_2), 51.8 (s, $CH_3\underline{C}HNH_2$), 52.8 (s, $\underline{C}HCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 147.7 Hz), 173.5 (s, C=O), 177.3 (br s, $\underline{C}OCHNHP$); δ_P (D_2O) 18.3.

Fractions 60–70 gave the second diastereoisomer (**17**) (tentatively assigned as the *S,S,S*-tripeptide—see Discussion) (0.16 g), m.p. 261–262°C, $[\alpha]_{578}^{25}$ −14.3°C (c = 4% in water); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$, 12.2 Hz), 19.4 (s, $\underline{C}H_3CH$), 26.1 (s, CH_2), 52.2 (s, $CH_3\underline{C}HNH_2$), 53.0 (d, CHP, $^1J_{PC}$ 147.1 Hz), 53.1 (s, $\underline{C}HCH_3$), 173.6 (s, C=O), 177.4 (br s, $\underline{C}OCHNHP$); δ_P (D_2O) 18.3.

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