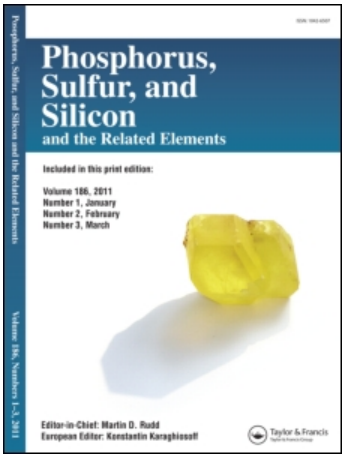

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FAST-ATOM BOMBARDMENT MASS SPECTROMETRY OF PHOSPHONOPEPTIDES

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The fast-atom bombardment mass spectra of phosphonopeptides, having an α-aminoalkanephosphonic acid as the acid-terminal residue, have been recorded using a glycerol matrix and a primary beam of xenon atoms operating at 6–8 kV. The identities of selected ions were confirmed by exact mass measurements. All peptides gave characteristic pseudomolecular ions, $[MH]^+$, which appeared as the base peak in many cases. Protonated dimers, $[2M + H]^+$, higher clusters, and ions resulting from association with glycerol were also observed, particularly in the case of dipeptides. Fragmentation occurred mainly by the elimination of α-amino-carboxylic acid residues, via peptide bond cleavage with hydrogen transfer, and by elimination of phosphorous acid from the aminophosphonic moiety. In addition, evidence was seen in certain cases for amide-bond fission with formation of an acylium ion which subsequently lost carbon monoxide.

Key words: Phosphonopeptides, FAB mass spectrometry.

INTRODUCTION

Phosphonopeptides are of considerable interest as molecules with potential for application in medicine and agriculture.[1] There are numerous references to methods for their preparation[2,3] and to their activity as antibacterial agents,[1,4] plant growth regulators and herbicides,[1,5] fungicides,[3,6] and inhibitors of collagenase,[7] and other peptidases.[8] Fast-atom bombardment mass spectrometry (FAB MS)[9] is an ideal technique for use in the characterization of zwitterionic compounds of this type but its application to phosphonopeptides has hitherto received only limited attention.[3] We now report the principal characteristics of the FAB positive ion mass spectra for a representative range of phosphonopeptides having an α-aminoalkanephosphonic acid unit as the acid-terminal residue.[10]

RESULTS AND DISCUSSION

A number of phosphonodipeptides (**1**–**6**) and phosphono-oligopeptides (**7**–**11**) were prepared by previously described procedures.[3] In each case, fast-atom bom-

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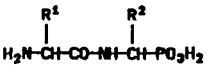
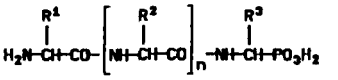


1 (R^1 = H, R^2 = Et)
2 (R^1 = Me, R^2 = Et)
3 (R^1 = Et, R^2 = Et)
4 (R^1 = Me, R^2 = n-C_5H_{11})
5 (R^1 = Me, R^2 = n-C_6H_{13})
6 (R^1 = $PhCH_2$, R^2 = Et)



7 (n = 1, R^1 = H, R^2 = Me, R^3 = Et)
8 (n = 1, R^1 = Me, R^2 = H, R^3 = Et)
9 (n = 1, R^1 = Me, R^2 = Me, R^3 = Et)
10 (n = 2, R^1 = Me, R^2 = Me, R^3 = Et)
11 (n = 3, R^1 = Me, R^2 = Me, R^3 = Et)

bardment mass spectrometry was shown to be a useful aid in their characterization, giving prominent pseudomolecular ions, $[MH]^+$. Cluster ions corresponding to $[2M + H]^+$ etc. and ions associated with a molecule of glycerol were generally recorded for the dipeptides but were less commonly observed for tri- or higher peptides. The principal ions observed in the FAB MS of the di- and oligo-phosphonopeptides are shown in Tables I and II, respectively, and a typical spectrum, obtained for *N*-(*L*-ala-)$_3$-α-aminopropanephosphonic acid (**10**), is shown in Figure 1.

Two principal modes of fragmentation were observed. The first, which is commonly observed in carboxy-peptides for which FAB MS is a well-established method

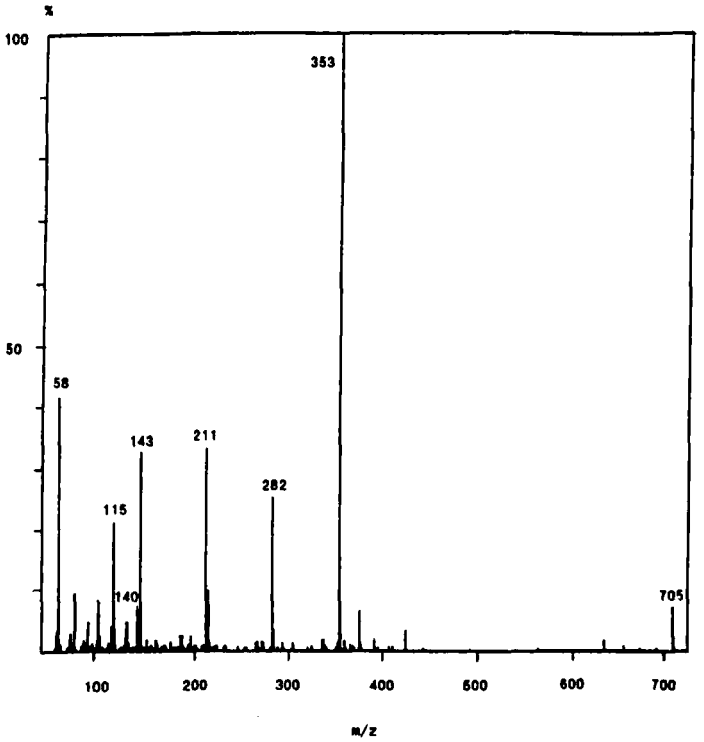
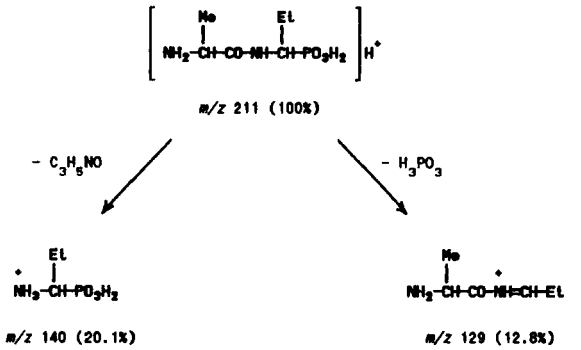


FIGURE 1 FAB mass spectrum (glycerol subtracted) for *N*-(*L*-ala)$_3$NHCH(Et)PO_3H_2 (**10**).

of analysis,[11] involved peptide bond cleavage with hydrogen transfer and the elimination of one or more amino-carboxylic acid units. The fragmentation is illustrated in Scheme I for a typical dipeptide, from which the *N*-terminal amino acid unit is eliminated as a neutral fragment whose structure is uncertain but for which the molecular composition is equivalent to that of an α-lactam (C_2H_3NO in the case of a glycyl residue or C_3H_5NO for an alanyl residue). The second mode of fragmentation, which has previously been observed in the FAB MS of α-aminophosphonic acids,[12] occurred by the elimination of phosphorous acid, H_3PO_3, from the phosphonic moiety by phosphorus-carbon cleavage (Scheme I) (Table I). The latter



SCHEME I

TABLE I

Principal ions in the FAB mass spectra of phosphonodipeptides

Peptide[a]	m/z (%)[b]				
	$[2M + H]^+$	$[2MH - X]^+$	$[MH]^+$	$[MH - X]^+$	$[MH - H_3PO_3]^+$
1	393(15.8)	336(4.0)	197(100)	140(22.8)	115(17.4)[c]
2	421(18.6)	350(4.8)	211(100)	140(20.1)	129(12.8)[d]
3	449(22.0)	-	225(86.0)	140(6.7)	143(10.0)[e]
4	505(17.5)	-	253(100)	182(14.0)	171(15.5)[f]
5	533(15.5)	-	267(79.8)	196(17.6)	185(16.2)[g]
6	573(10.3)	-	287(58.3)	-	205(6.5)[h]

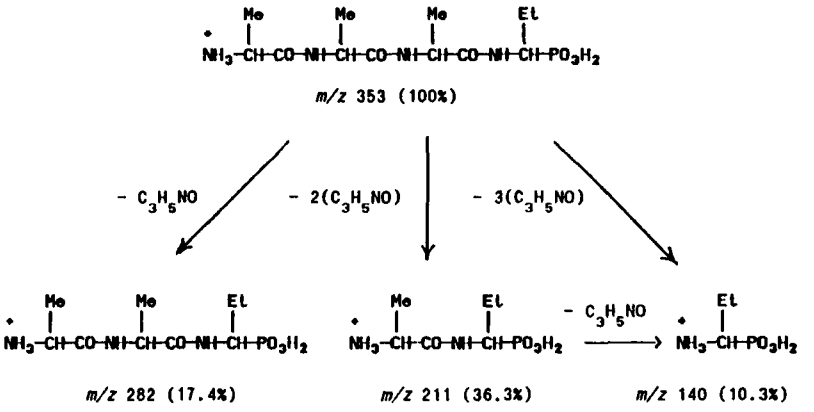
[a] Alanyl residues have the *L*-configuration; all other aminocarboxylic and aminophosphonic residues are racemic. [b] X = C_2H_3NO (when R^1 = H) or C_3H_5NO (when R^1 = Me) ; G = glycerol. [c] Also *m/z* 289 ($[MH + G]^+$, 12.3). [d] Also *m/z* 303 ($[MH + G]^+$, 3.8); *m/z* 232 ($[MH + G - X]^+$, 2.3). [e] Also *m/z* 897 ($[4M + H]^+$, 0.7); *m/z* 673 ($[3M + H]^+$, 2.3); *m/z* 58 ($EtCH{=}NH_2^+$, 100). [f] Also *m/z* 100 (n-$C_5H_{11}CH{=}NH_2^+$, 83.5). [g] Also *m/z* 114 (n-$C_6H_{13}CH{=}NH_2^+$, 100). [h] Also *m/z* 120 ($PhCH{=}NH_2^+$, 100); *m/z* 91 ($C_7H_7^+$, 13).

process, however, was observed only for the protonated dipeptides or the protonated α-aminoalkanephosphonic acid fragment. Fragmentation of the higher peptides appeared to occur preferentially by cleavage of carboxy-peptide bonds (Scheme II) to yield sequence information (Table II). In the example illustrated (Scheme II), it was shown by linked scanning that the protonated α-aminoalkanephosphonic acid fragment (m/z 140) is a product-ion of m/z 211.

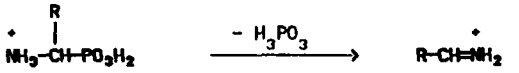
Elimination of phosphorous acid from the protonated α-aminoalkanephosphonic acid fragment, derived from either di- or oligo-peptides, generally gave rise to one of the more intense peaks in the spectrum, assigned to the iminium ion, $RCH{=}NH_2^+$ (Scheme III). However, the elimination of metaphosphoric acid, HPO_3, an alternative fragmentation observed in the case of certain α-aminophosphonic acids,[12] was not detected in the present investigations.

In the case of *N*-(gly-*L*-ala)-α-aminopropanephosphonic acid (**7**), a series of ions at m/z 631, 568, 489 and m/z 350, 421, and 279, were assigned to fragments derived from $[3M + H]^+$ and $[2M + H]^+$, respectively, by the loss of a number of amino-carboxylic acid residues (see Table II). Examples of the elimination of amino-carboxylic residues from $[2M + H]^+$ and from $[MH + G]^+$ were observed in other cases also (Tables I and II).

A further fragmentation, observed for the *N*-(*L*-ala-)$_2$-, *N*-(*L*-ala-)$_3$-, and *N*-(*L*-ala-)$_4$-derivatives of α-aminopropanephosphonic acid (**9**, **10**, and **11**, respectively) (Table II), involved peptide bond cleavage with the formation of an acylium ion, which itself fragmented further by the loss of carbon monoxide (Scheme IV). This fragmentation pathway has been recognized in the FAB MS of carboxy-peptides.[11] In addition, *N*-(Phe-)-α-aminopropanephosphonic acid (**6**), underwent fragmen-



SCHEME II



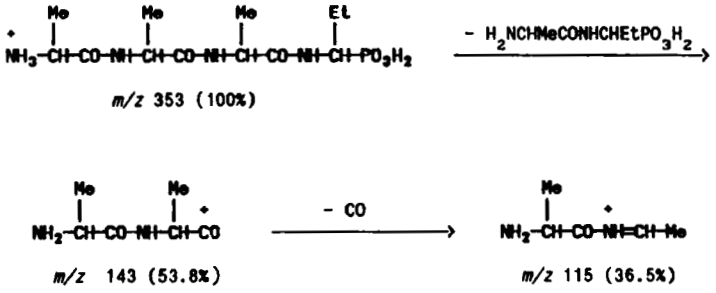
SCHEME III

TABLE II

Principal ions in the FAB mass spectra of phosphono-oligopeptides

Peptide[a]	m/z (%)[b]				
	$[MH]^+$	$[MH - X]^+$	$[MH - X - Y]^+$	$[C_6H_{11}N_2O_2]^+$	$[C_5H_{11}N_2O]^+$
7	268(7.0)	211(100)	140(50.7)	-	-[c]
8	268(15.4)	197(15.5)	140(9.2)	-	115(57.2)
9	282(100)	211(21.4)	140(10.7)	143(12.8)	115(58.4)[d]
10	353(100)	282(26.0)	211(35.0)	143(34.5)	115(22.0)[e]
11	424(12.0)	353(86.7)	282(100)	143(60.0)	115(41.0)[f]

[a] Alanyl residues have the *L*-configuration; aminophosphonic residues are (*RS*). [b] X = C_2H_3NO (when R^1 = H) or C_3H_5NO (when R^1 = Me); Y = C_2H_3NO (when R^2 = H) or C_3H_5NO (when R^2 = Me); G = glycerol; $[C_6H_{11}N_2O_2]^+$ = [ala-ala-]$^+$; $[C_5H_{11}N_2O]^+$ is assigned the structure $[NH_2CH_2CONH{=}CHEt]^+$ in the case of the *L*-ala-gly-peptide (8) (i.e. MH - X - H_3PO_3]$^+$, but in other cases is considered to be formed by the loss of CO from [ala-ala-]$^+$, i.e. $[NH_2CHMeCONH{=}CHMe]^+$. [c] Also *m/z* 631 ([3MH - 3X]$^+$, 2); *m/z* 568 ([3MH - 3X - Y]$^+$, 3); *m/z* 489 ([3MH - 3X - 2Y]$^+$, 3); *m/z* 478 ([2MH - X]$^+$, 2.0); *m/z* 421 ([2MH - 2X]$^+$, 18); *m/z* 350 ([2MH - 2X - Y]$^+$, 24); *m/z* 279 ([2MH - 2X - 2Y]$^+$, 17); *m/z* 129 ([MH - X - H_3PO_3]$^+$, 9); *m/z* 58 ([EtCH=NH_2]$^+$, 69). [d] Also *m/z* 374 ([MH + G]$^+$, 4.1). [e] Also *m/z* 705 ([2M + H]$^+$, 7.5); *m/z* 140 ([MH - X - 2Y]$^+$, 8.0); *m/z* 58 ([EtCH=NH_2]$^+$, 41.5). [f] Also *m/z* 211 ([MH - X - 2Y]$^+$, 68.0); *m/z* 140 ([MH - X - 3Y]$^+$, 11.5); *m/z* 58 ([EtCH=NH_2]$^+$, 90.0).



SCHEME IV

tation to give the iminium ion, $PhCH{=}NH_2^+$ (m/z 120) as the base peak, and the $C_7H_7^+$ ion at m/z 91, with a relative intensity of 13%.

In summary, FAB MS provides an excellent means for the analysis and structure determination of phosphonopeptides, giving prominent pseudomolecular ions which are useful for the confirmation of molecular weight, and characteristic fragment ions which parallel those observed in the mass spectrometry of carboxy-peptides[11] and α-aminoalkanephosphonic acids.[12]

EXPERIMENTAL

Starting Materials

α-Aminopropane- and α-aminohexane-phosphonic acids were prepared as described.[12b] An analogous procedure gave α-aminoheptanephosphonic acid, m.p. 272–273°C, δ_H (D_2O/D_2SO_4) 0.86 (3H, t, CH_3, $^3J_{HH}$ 6.0 Hz), 1.33 (8H, br m, CH_2) 1.62–2.12 (2H, br m, $C\underline{H}_2CH$), 3.20–3.53 (1H, m, CH); δ_C (D_2O/D_2SO_4) 16.0 (s, CH_3), 24.5 (s, CH_2), 27.6 (d, $\underline{C}H_2CH_2CHP$, $^3J_{PC}$ 8.6 Hz), 30.3 (s, CH_2), 30.6 (s, CH_2). 33.2 (s, CH_2), 50.7 (d, CHP, $^1J_{PC}$ 152.0 Hz); δ_P (D_2O/D_2SO_4) 14.4. Other reagents were obtained commercially.

Mass Spectrometry

Positive ion fast-atom bombardment mass spectra were obtained on a VG ZAB-1F spectrometer fitted with a saddle-field ion source (Ion Tech Ltd) operated with a primary beam of 8 kV (1 mA) xenon atoms, or a Kratos MS30 spectrometer fitted with a capillaritron direct insertion probe FAB gun and target (Phrasor Scientific Inc.) operated at 6 kV (40 μA). Samples were prepared in a glycerol matrix (in the spectral data, G = glycerol) and the spectra were recorded at a scan rate of 10 s/decade. Accurate masses were measured by peak matching at a resolution of 10,000 (10% valley).

Preparations of Peptides

Dipeptides (**1**, **2**) and tripeptides (**8**, **9**) were prepared from 1-(*RS*)-α-aminopropanephosphonic acid and the corresponding amino-carboxylic acids (glycine and/or *L*-alanine) as described.[3] The following new peptides were prepared from the free α-aminoalkanephosphonic acids by the *N*-hydroxysuccinimide ester method in the presence of either sodium bicarbonate (Method A) or triethylamine (Method B),[3] or by the mixed carboxylic-carbonic anhydride procedure (Method C).[3] Nmr and other analytical data were obtained as described.[3]

(1R,S)-1-(DL-butyrinylamino)propanephosphonic acid (**3**). (1.2 g, 30% by method B in ethanol), m.p. 260–262°C (Found: C, 37.3; H, 7.5; N, 13.4. $C_{12}H_{19}N_2O_4P \cdot H_2O$ requires: C, 37.5; H, 7.6; N, 12.5%); δ_H (D_2O) 0.93 (3H, t, $C\underline{H}_3CH_2CHNH_2$, $^3J_{HCCH}$ 7.21 Hz), 1.03 (3H, t, $C\underline{H}_3CH_2CHP$, $^3J_{HCCH}$ 6.84 Hz), 1.41–2.16 (2H, br m, $C\underline{H}_2CHP$), 1.90 (2H, q, $C\underline{H}_2CH_3$), 3.67–4.09 (2H, br m, CH); δ_C (D_2O) 11.4 (s, CH_3), 13.6 (d, $\underline{C}H_3$, $^3J_{PC}$ 13.4 Hz), 25.7 (s, $\underline{C}H_2CHP$), 27.2 (s, CH_2), 53.5 (d, CHP, $^1J_{PC}$ 147.7 Hz), 57.9 (s, CH), 172.8 (br s, C=O); δ_P (D_2O) 17.9.

(1R,S)-1-(L-alanylamino)hexanephosphonic acid (**4**). (0.6 g, 29% by method B in ethanol), m.p. 228–230°C (Found: MH^+, m/z 253.1315. $C_9H_{22}N_2O_4P$ requires: m/z 253.1313), δ_H 0.87 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HH}$ 4.4 Hz), 1.28 (8H, br m, CH_2); 1.48 (3H, d, $C\underline{H}_3CH$, $^3J_{HH}$ 6.84 Hz), 3.65–4.23 (2H, br m, CH).

(1R,S)-1-(L-alanylamino)heptanephosphonic acid (**5**). (0.57 g, 80% by method A in ethanol), m.p. 278–279°C (Found: MH^+ m/z 267.1479. $C_{10}H_{24}N_2O_4P$ requires: m/z 267.1474), $[\alpha]_{578}^{30}$ +2.6 (c = 2% in water); δ_H 0.92 (3H, br t, $C\underline{H}_3CH_2$), 1.30 (8H, br m, CH_2), 1.48 (3H, d, $C\underline{H}_3CH$, $^3J_{HH}$ 7.32 Hz), 1.48–2.25 (2H, br m, $C\underline{H}_2CH_3$), 3.83–4.25 (2H, br m, CH); ^{13}C (D_2O) 16.3 (s, $\underline{C}H_3CH_2$), 19.2 (s, $\underline{C}H_3CH$), 19.7 (s, $\underline{C}H_3CH$), 24.9 (s, CH_2), 33.9 (d, CH_2), 51.8 (d, CHP, $^1J_{PC}$ 147.1 Hz), 52.3 (s, $CH_3\underline{C}H$), 53.7 (s, $CH_3\underline{C}H$), 173.2 (m, C=O); ^{31}P (D_2O) 18.2.

(1R,S)-1-(DL-phenylalanylamino)propanephosphonic acid (**6**). As the monohydrate (1.44 g, 46% by method B in DMF), m.p. 243–244°C (Found: C, 47.1; H, 6.6; N, 9.6. $C_{12}H_{19}N_2O_4P \cdot H_2O$ requires: C,

47.4; H, 6.9; N, 9.2%); δ_C (D_2O/D_2SO_4) 13.0 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 17.7 Hz), 24.5 (s, $\underline{C}H_2CH_3$), 39.7 (s, $\underline{C}H_2Ph$), 52.0 (d, CHP, $^1J_{PC}$ 152.6 Hz), 57.6 (s, $\underline{C}HNH_2$), 58.0 (s, $CHNH_2$), 131.1 (s, Ar—C_4), 132.3–132.6 (m, Ar—C_2, C_3, C_5, C_6), 136.2 (s, Ar—C_1), 136.4 (s, Ar—C_1), 171.9–172.5 (m, C=O); δ_P (D_2O) 17.9.

(1R,S)-1-(glycyl-L-alanylamino)propanephosphonic acid (**7**). (0.8 g, 41% by method C), m.p. 253–255°C, $[\alpha]_{578}^{25}$ +6.6° (c = 2% in water), δ_H (D_2O) 0.90 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 7.3 Hz), 1.43 (3H, d, $CH_3\underline{C}H$, $^3J_{HCCH}$ 7.8 Hz), 1.52–2.0 (2H, br m, CH_2), 3.70–4.50 (2H, br m, CH), 3.87 (2H, s, $C\underline{H}_2NH_2$); δ_C (D_2O) 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 13.4 Hz), 20.0 (s, $\underline{C}H_3CH$), 26.0 (s, CH_2), 49.6 (s, CH_2NH_2), 52.2 (s, $\underline{C}HCH_3$), 53.2 (d, CHP, $^1J_{PC}$, 144.7 Hz), 169.9 (s, C=O), 177.5 (s, C=O); δ_P (D_2O) 18.3.

(1R,S)-1-(L-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid (**10**). (0.6 g, 48% by method A in ethanol), m.p. 239–241°C (Found: MH^+, m/z 353.15981. $C_{12}H_{26}N_4O_6P$ requires: m/z 353.15901), $[\alpha]_{578}^{25}$ −54.0° (c = 0.5% in water); δ_H (D_2O/D_2SO_4) 0.93 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 6.8 Hz), 1.13–1.34 (9H, m, $C\underline{H}_3CH$), 1.34–1.75 (2H, m, CH_2), 3.78–4.25 (4H, br m, CH); δ_C (D_2O) 13.4 (d, $\underline{C}H_3CH_2$, $^3J_{PC}$ 12.8 Hz), 19.5 (br s, $\underline{C}H_3CH$), 25.9 (s, CH_2), 51.9 (s, $\underline{C}HCH_3$), 52.7 (s, $\underline{C}HCH_3$), 53.0 (s, $\underline{C}HCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 146.5 Hz), 177.4 (m, C=O); δ_P (D_2O) 18.4.

(1R,S)-1-(L-alanyl-L-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid (**11**). (1.0 g, 67% by method A in ethanol), m.p. 258–260°C (Found: MH^+, m/z 424.1971. $C_{15}H_{31}N_5O_7P$ requires: m/z 424.1961), $[\alpha]_{578}^{25}$ −35.6° (c = 4% in 0.5 M NaOH); δ_H (D_2O) 0.90 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 6.8 Hz), 1.42 (6H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.8 Hz), 1.55 (6H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.8 Hz), 1.65–2.25 (2H, br m, CH_2), 3.52–4.5 (5H, 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), 51.9 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 52.8 (s, $\underline{C}HCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 147.7 Hz), 173.6 (s, C=O), 177.4 (br s, C=O); δ_P (D_2O) 18.3.

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