Complexing Properties of Phosphonodipeptides containing 1-Aminoethylphosphonic Acid †

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The acid–base and complexing properties of diastereoisomers of phosphonodipeptides containing glycyl, L-alanyl, L-leucyl and L-phenylalanyl and terminal 1-aminoethylphosphonic acid residues were studied pH-metrically at 25 °C and at an ionic strength of 0.1 mol dm^{-3} (KNO_3). The protonation and complex-formation stability constants with Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} indicate the presence of the same types of complexes and the same tendencies as in the aminomethylphosphonic acid series. In contrast to the common dipeptides, the differences found for diastereoisomers are much higher, probably due to stronger interaction between the hydrophobic and hydrophilic parts of the molecule. Crystal structure determinations confirmed the absolute configurations of *S,S*-$PhCH_2CH(NH_2)$-$CONHCH(Me)PO_3H_2$ and *S,R*-$Pr^iCH_2CH(NH_2)CONHCH(Me)PO_3H_2$. The distances and bond angles found show that the peptide bond is not influenced by the phosphonic group.

The biological activity of a molecule often depends upon its stereochemistry. Therefore, differences in the reactivity and solution properties of diastereoisomers of the common dipeptides have been widely investigated and reviewed.[1] Phosphonodipeptides containing terminal aminoalkylphosphonic acid groups exhibit biological activity and were reviewed by Kafarski *et al.*[2] In contrast to the common dipeptides, only three papers have dealt with their acid–base and complexing properties.[3-5] Attention has been focused on the phosphonodipeptides without a chiral centre[4] or those containing such a centre in the amino acid part of the molecule[3] or on racemic mixtures.[5] The stability constants determined confirmed the formation of protonated, non-protonated and deprotonated complexes with molar ratios of metal (Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}): ligand = 1:1 and, except for zinc, 1:2. Simultaneous deprotonation and co-ordination of the peptide amide bond was observed only in systems with copper.[3-5]

The aim of this study was to extend the number of phosphonodipeptides studied and, for compounds of general formula $NH_2CHRCONHCH(Me)PO_3H_2$ (R = H, Me, Bu^i or CH_2Ph), to investigate their complexing properties with common divalent metals. This series contains glycyl (Gly), L-alanyl (Ala), L-phenylalanyl (Phe), L-leucyl (Leu) and terminal 1-aminoethylphosphonic acid residues and is analogous to that described in the preceding paper.[3] In view of the fact that this terminal phosphonic acid also contains a centre of chirality, the diastereoisomers were separated and attention focused on the differences in the solution properties. To confirm the configuration, the crystal structures of two different diastereoisomers were determined.

Results and Discussion

Synthesis, Thermal Stability and Structure.—The phosphonopeptides were synthesised and diastereoisomers separated according to the procedure described previously.[6] Their hydration and thermal stability were tested by TGA and the results are summarised in Table 1. The compounds are stable up to at least 220 °C [decomposition of *S,R*-$Pr^iCH_2CH(NH_2)CONHCH(Me)PO_3H_2$, *i.e.* *SR*-Leu-Ala-(*P*)] and the stability is a little higher than for the $NH_2CH_2PO_3H_2$ series.[3]

† *Supplementary data available:* see Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1995, Issue 1, pp. xxv–xxx.

The structure determinations described in this paper, including absolute configurations, are the first reported for phosphonodipeptides. The molecule of *S,S*-Phe-Ala-(*P*) with the labelling scheme is shown in Fig. 1, and selected bond distances are listed in Table 2. The peptide bond connects the N(1) and C(3) atoms. Atoms N(1), C(3), O(4) and H(N1) lie in a plane, as in the structures of Gly-Gly-(*P*)[7] and common dipeptides.[8] Bond distances and angles for the peptide bond (see Table 2) are in good agreement with the values found for common dipeptides.[9] No important influence of the phosphonic group on the formation of the peptide bond has been observed, as in the structure of Gly-Gly-(*P*).[7]

The orientation of the hydrophilic groups [$-PO_3H^-$, $-NH_3^+$ and carbonyl atom O(4)] and of the hydrophobic groups (Me and CH_2Ph) towards the 'main' chain C(1)–N(1)–C(3)–C(4) follows from the *S,S* configuration at atoms C(1) and C(2) (see Fig. 1). As will be shown later, this fact explains the difference

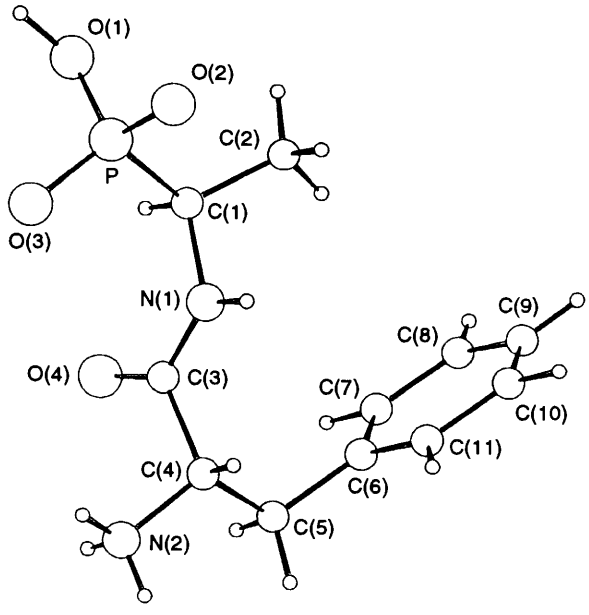


Fig. 1 View of *S,S*-Phe-Ala-(*P*)·$2H_2O$ with the atom numbering scheme

Table 1 Results of TGA in the temperature range 25–280 °C

Compound	Dehydration temperature/°C	Weight loss (%)	Decomposition temperature/°C	Drying temperature/°C	Weight loss (%)
Gly-Ala-(*P*)	80–105	13.54 (1.5 H_2O)	250	110–115	13.41 (1.5 H_2O)
S,S-Ala-Ala-(*P*)	—	—	260	60–70	—
S,R-Ala-Ala-(*P*)	—	—	290	60–70	—
S,S-Leu-Ala-(*P*)	120–130	2.24 (0.3 H_2O)	280	130–135	3.69 (0.5 H_2O)
S,R-Leu-Ala-(*P*)	70–140	13.38 (2.0 H_2O)	220	135–140	13.51 (2.1 H_2O)
S,S-Phe-Ala-(*P*)	85–120	11.58 (2.0 H_2O)	270	120–125	11.61 (2.0 H_2O)
S,R-Phe-Ala-(*P*)	100–170	6.45 (1.0 H_2O)	270	60–70	

Table 2 Selected bond lengths (Å) and angles (°) for *S,S*-Phe-Ala-(*P*)

P–O(2)	1.494(2)	C(2)–C(1)	1.529(4)
P–O(3)	1.506(2)	C(5)–C(6)	1.507(4)
P–O(1)	1.561(2)	C(5)–C(4)	1.535(4)
P–C(1)	1.827(2)	C(6)–C(11)	1.373(5)
C(3)–O(4)	1.227(3)	C(6)–C(7)	1.382(5)
C(3)–N(1)	1.318(3)	C(7)–C(8)	1.381(6)
C(3)–C(4)	1.526(3)	C(8)–C(9)	1.344(7)
N(1)–C(1)	1.460(3)	C(9)–C(10)	1.390(7)
N(2)–C(4)	1.483(3)	C(10)–C(11)	1.388(6)
Hydrogen bonds			
N(2)···O(4)	2.822(5)	N(2)···O(5)	2.823(6)
O(2)···O(6^{I})	2.791(6)	O(1)···O(3^{IV})	2.517(6)
O(3)···O(5^{II})	2.710(6)	O(1)···N(2^{IV})	2.758(6)
O(4)···O(6^{III})	2.859(7)		
O(2)–P–O(3)	114.04(10)	C(3)–N(1)–C(1)	123.4(2)
O(2)–P–O(1)	112.27(11)	C(6)–C(5)–C(4)	112.2(2)
O(3)–P–O(1)	111.56(11)	N(1)–C(1)–C(2)	109.4(2)
O(2)–P–C(1)	109.29(11)	N(1)–C(1)–P	110.1(2)
O(3)–P–C(1)	108.64(11)	C(2)–C(1)–P	113.0(2)
O(1)–P–C(1)	100.05(10)	N(2)–C(4)–C(3)	109.9(2)
O(4)–C(3)–N(1)	125.6(2)	N(2)–C(4)–C(5)	110.9(2)
O(4)–C(3)–C(4)	119.4(2)	C(3)–C(4)–C(5)	109.5(2)
N(1)–C(3)–C(4)	114.9(2)		

Symmetry relations: I $1 + x, -1 + y, z$; II $-\frac{1}{2} + x, -\frac{1}{2} - y, 1 - z$; III $x, -1 + y, z$; IV $\frac{1}{2} - x, -\frac{1}{2} - y, 1 - z$.

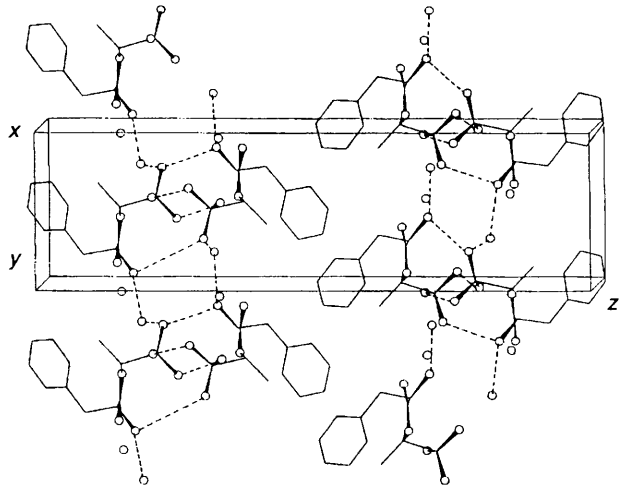


Fig. 2 Crystal packing of *S,S*-Phe-Ala-(*P*)·2H_2O. The shortest hydrogen bonds are denoted by dashed lines

found in the solution properties of the *S,S* and *S,R* diastereoisomers. The hydrophilic and hydrophobic interactions in the isolated molecule of *S,S*-Phe-Ala-(*P*) can be illustrated by the non-bonding distances P···N(2) [5.607(3)], P···O(4) [3.932(3)], N(2)···O(4) [2.822(6) Å] and by the distance of atom C(2) from the plane of the phenyl moiety [4.021(1) Å]. This orientation affects the crystal packing (Fig. 2). Methyl and benzyl groups of different molecules interact and form hydrophobic layers in contrast to the hydrophilic layers formed by phosphonic and amine groups together with H_2O molecules. In the hydrophilic layer the molecules are connected by a complex network of hydrogen bonds. The shortest one links atom O(1) and O(3) from the next molecule $(x + \frac{1}{2}, -y - \frac{1}{2}, -z + 1)$ with a distance of 2.517(6) Å.

The co-ordination around the P atom differs from a regular tetrahedron. The P–O(1) bond length differs from P–O(2) and P–O(3) by about 0.06 Å due to the protonation of O(1). On the other hand, the geometry of the phosphonic group corresponds to that found in aminoalkylphosphonic acids.[10] The aminophosphonic acids as well as Gly-Gly-(*P*) form zwitterion structures containing $-NH_3^+$ and $-PO_3H^-$ groups. We looked for the position of the next acid proton, but only found a broad diffuse maximum between the atoms N(2) and O(4) in the Fourier-difference map. This vague position is probably caused by the strong interaction of the proton with both atoms.

The structure of *S,R*-Leu-Ala-(*P*) in the crystalline state consists of two crystallographically independent molecules differing only negligibly in their geometry and of four water molecules. The molecules with the labelling scheme are shown in Fig. 3 and bond distances and angles are listed in Table 3. The geometry of the peptide bond is similar to that observed in *S,S*-Phe-Ala-(*P*). The orientation of the hydrophobic and hydrophilic parts of the molecule towards the C(1)–N(1)–C(3)–C(4) chain is in accord with the *S,R* configuration at the C(1) and C(4) atoms (see Fig. 3). The amine and atom O(4) lie on the same side of the 'main' chain [torsion angle O(4)–C(3)–C(4)–N(2) −30.9(8) and −52.7(4)° for molecules A and B, respectively] and the phosphonic group is on the opposite side. In contrast to *S,S*-Phe-Ala-(*P*), hydrophobic intermolecular interactions are not possible in *S,R*-Leu-Ala-(*P*). Molecule A is connected by the intramolecular interaction of the phosphonic group to two molecules of water and through them to the phosphonic group of molecule B, as shown in Fig. 3. The hydrogen bond lengths are in the range 2.56(2)–2.90(3) Å. The crystal packing is based on the connection of two independent molecules of *S,R*-Leu-Ala-(*P*) (Fig. 4).

The co-ordination around the P atom, including bond distances, angles and protonation of one O atom, is the same as in *S,S*-Phe-Ala-(*P*). One proton of phosphonic acid was not found and we did not find any other maximum in the Fourier map for two hydrogen atoms at N(2A) or N(2B) of the amine groups. It is difficult to decide whether this is because of the high *R* value or if the proton is bonded to the water molecules. The high value of *R* is caused by two factors. The first was the poor quality of the crystals obtained, the second the disorder of the isopropyl group in molecule A. An

Table 3 Bond lengths (Å) and angles (°) for *S,R*-Leu-Ala-(*P*)

Molecule A		Molecule B	
P(1A)–O(1A)	1.569(5)	P(1B)–O(1B)	1.571(5)
P(1A)–O(2A)	1.488(5)	P(1B)–O(2B)	1.507(5)
P(1A)–O(3A)	1.507(5)	P(1B)–O(3B)	1.473(6)
P(1A)–C(1A)	1.832(8)	P(1B)–C(1B)	1.814(7)
C(1A)–N(1A)	1.466(9)	C(1B)–N(1B)	1.450(9)
C(1A)–C(2A)	1.531(12)	C(1B)–C(2B)	1.509(13)
N(1A)–C(3A)	1.313(9)	N(1B)–C(3B)	1.306(9)
C(3A)–O(4A)	1.221(8)	C(3B)–O(4B)	1.247(9)
C(3A)–C(4A)	1.533(10)	C(3B)–C(4B)	1.534(10)
C(4A)–N(2A)	1.473(9)	C(4B)–N(2B)	1.488(10)
C(4A)–C(5A)	1.53(1)*	C(4B)–C(5B)	1.510(12)
C(5A)–C(6A)	1.34(2)*	C(5B)–C(6B)	1.53(2)
C(6A)–C(7A)	1.59(2)*	C(6B)–C(7B)	1.44(2)
C(6A)–C(8A)	1.51(2)*	C(6B)–C(8B)	1.54(2)
O(1A)–P(1A)–C(1A)	102.4(3)	O(1B)–P(1B)–C(1B)	102.1(3)
O(2A)–P(1A)–O(3A)	115.0(3)	O(2B)–P(1B)–O(3B)	116.4(4)
O(2A)–P(1A)–O(1A)	111.3(4)	O(2B)–P(1B)–O(1B)	105.8(3)
O(3A)–P(1A)–O(1A)	109.4(3)	O(3B)–P(1B)–O(1B)	112.2(4)
O(2A)–P(1A)–C(1A)	110.7(3)	O(2B)–P(1B)–C(1B)	111.3(3)
N(1A)–C(1A)–C(2A)	111.3(6)	N(1B)–C(1B)–C(2B)	113.0(7)
N(1A)–C(1A)–P(1A)	110.6(5)	N(1B)–C(1B)–P(1B)	110.7(5)
C(2A)–C(1A)–P(1A)	113.1(5)	C(2B)–C(1B)–P(1B)	114.4(6)
C(3A)–N(1A)–C(1A)	121.7(6)	C(3B)–N(1B)–C(1B)	121.8(6)
O(4A)–C(3A)–N(1A)	124.2(7)	O(4B)–C(3B)–N(1B)	123.9(7)
O(4A)–C(3A)–C(4A)	121.1(7)	O(4B)–C(3B)–C(4B)	117.2(6)
N(1A)–C(3A)–C(4A)	114.8(6)	N(1B)–C(3B)–C(4B)	118.8(6)
N(2A)–C(4A)–C(5A)	110.8(6)*	N(2B)–C(4B)–C(5B)	111.2(6)
N(2A)–C(4A)–C(3A)	108.4(6)	N(2B)–C(4B)–C(3B)	106.9(6)
C(5A)–C(4A)–C(3A)	110.3(6)*	C(5B)–C(4B)–C(3B)	111.0(6)
Hydrogen bonds			
N(2A)···O(4A)	2.76(2)	O(6)···O(8)	2.78(3)
N(2B)···O(4B)	2.81(1)	O(5)···O(6^{II})	2.85(4)
O(2A)···O(6)	2.87(3)	O(3A)···O(1B^{II})	2.58(2)
O(1A)···O(5)	2.56(2)	O(3A)···N(2A^{III})	2.88(3)
O(3B)···O(5)	2.67(2)	O(2A)···N(2B^{IV})	2.70(2)
O(3B)···O(6)	2.68(2)	N(2A)···O(2B^{V})	2.81(3)
N(2B)···O(7^{I})	2.88(2)	N(2B)···O(2B^{VI})	2.81(2)
O(7)···O(8)	2.74(3)		

Symmetry relations: I $\frac{3}{2}+x, \frac{1}{2}-y, -z$; II $-1-x, \frac{1}{2}+y, \frac{3}{2}-z$; III $\frac{1}{2}-x, -y, \frac{1}{2}+z$; IV $\frac{3}{2}+x, -\frac{1}{2}-y, -z$; V $\frac{1}{2}+x, -\frac{1}{2}-y, -z$; VI $\frac{1}{2}-x, 1-y, -\frac{1}{2}+z$.

* Values of σ are only estimated; length or angle involves atoms in fixed positions.

Table 4 Protonation constants of the phosphonodipeptides

Peptide	log β_1	log β_2	log β_3
Gly-Ala-(*P*)	8.259(3)	14.726(4)	15.932(8)
S,S-Ala-Ala-(*P*)	8.290(4)	14.586(7)	15.91(1)
S,R-Ala-Ala-(*P*)	8.023(5)	14.564(6)	16.18(1)
S,S-Leu-Ala-(*P*)	8.219(3)	14.405(5)	15.62(1)
S,R-Leu-Ala-(*P*)	7.814(3)	14.490(4)	16.038(7)
S,S-Phe-Ala-(*P*)	7.915(3)	14.243(4)	15.507(7)
S,R-Phe-Ala-(*P*)	7.357(5)	13.912(5)	15.40(1)

Dissociation constants of analogous common dipeptides[1]

	pK_1	pK_2
Gly-L-Ala	3.12	8.15
L-Ala-L-Ala	3.30	8.17
L-Ala-D-Ala	3.12	8.30
L-Leu-L-Ala	3.36	8.08
L-Leu-D-Ala	2.98	8.17

adequate model was not found for the behaviour of this group and atoms C(5A), C(6A), C(7A) and C(8A) were fixed in the positions obtained from the Fourier-difference map.

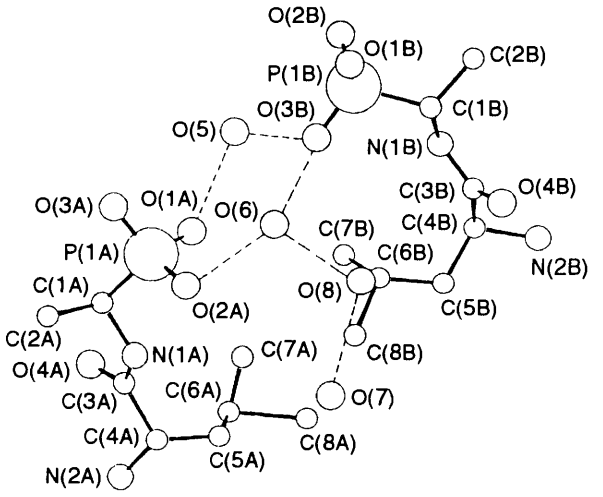


Fig. 3 Perspective view of *S,R*-Leu-Ala-(*P*)·2H_2O dimer with the atom numbering scheme

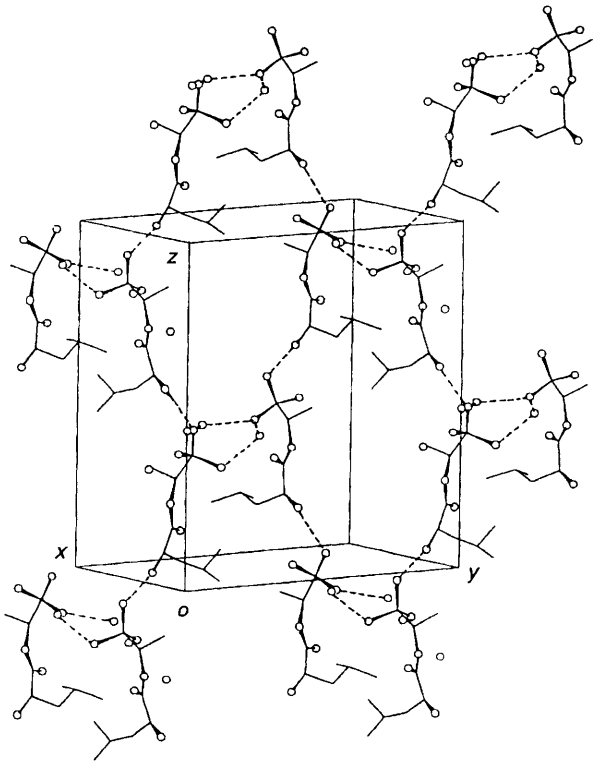


Fig. 4 Crystal packing of *S,R*-Leu-Ala-(*P*)·2H_2O showing the shortest hydrogen bonds

Potentiometry.—The preparation of the stock solution, the titration procedure and the accuracy of the constants calculated were as in the preceding paper.[3] The drying temperatures are listed in Table 1.

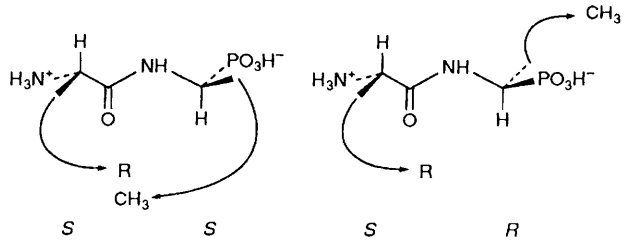
The protonation constants determined for the phosphonodipeptides are given in Table 4 together with those of common dipeptides. Despite the uncertainty regarding the proton position in solid *S,R*-Leu-Ala-(*P*), we also assume amine protonation in solution for the *S,R* diastereoisomers and thus the log β_3 corresponds to protonation of the amine group. The log β_2 values correspond to protonation of the phosphonic group $-PO_3^{2-}$ and log β_1 to protonation of $-PO_3H^-$. The same dependence on the size of the side chain as in the Gly-(*P*) series[3] is observed in this series.

It is evident from Table 4 that corresponding diastereoisomers

Table 5 Complex-formation stability constants of the phosphonodipeptides with divalent ions at 25 °C and I = 0.1 mol dm^{-3}; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$

Ion	Peptide	log β_{111}	log β_{110}	log β_{11-1}	log β_{11-2}	log β_{120}	logβ_{12-1}
Cu^{2+}	Gly-Ala-(*P*)	12.32(1)	6.86(1)	1.864(7)	−6.62(5)	12.60(7)	5.37(4)
	S,S-Ala-Ala-(*P*)	11.80(2)	6.50(1)	1.506(6)	−7.67(3)	11.92(9)	4.24(6)
	S,R-Ala-Ala-(*P*)	11.97(2)	6.47(3)	1.814(7)	−6.99(3)	12.30(8)	4.83(5)
	S,S-Leu-Ala-(*P*)	11.46(3)	6.35(1)	1.314(6)	−7.40(3)	11.60(9)	4.16(6)
	S,R-Leu-Ala-(*P*)	11.55(4)	6.62(2)	1.823(8)	−7.20(3)	—	4.21(9)
	S,S-Phe-Ala-(*P*)	11.44(3)	6.36(1)	1.233(7)	−7.34(5)	11.83(6)	4.45(6)
	S,R-Phe-Ala-(*P*)	11.20(3)	6.57(1)	1.713(6)	−6.93(5)	11.80(7)	4.64(5)
Ni^{2+}	Gly-Ala-(*P*)	10.64(1)	4.625(3)	−4.14(1)	—	7.963(9)	—
	S,S-Ala-Ala-(*P*)	9.53(8)*	3.956(4)	−4.48(2)	—	7.17(2)	—
	S,R-Ala-Ala-(*P*)	9.86(3)	3.767(3)	−4.89(1)	—	6.50(1)	−2.69(8)
	S,S-Leu-Ala-(*P*)	—	3.724(6)	−4.90(2)	—	7.00(1)	—
	S,R-Leu-Ala-(*P*)	9.60(3)	3.599(3)	−5.03(1)	—	5.92(1)	—
	S,S-Phe-Ala-(*P*)	9.60(3)	3.689(3)	−4.71(2)	—	6.69(1)	—
	S,R-Phe-Ala-(*P*)	9.42(3)	3.341(5)	−4.92(1)	—	5.55(4)	—
Co^{2+}	Gly-Ala-(*P*)	9.91(3)	3.633(4)	−5.38(2)	—	6.11(2)	—
	S,S-Ala-Ala-(*P*)	—	3.013(7)	−5.92(2)	—	—	—
	S,R-Ala-Ala-(*P*)	—	2.566(7)	−6.23(1)	—	—	—
	S,S-Leu-Ala-(*P*)	—	2.834(9)	−5.68(2)	—	—	—
	S,R-Leu-Ala-(*P*)	—	2.736(4)	−5.80(1)	—	—	—
	S,S-Phe-Ala-(*P*)	9.26(4)	2.843(6)	−5.832(6)	—	4.92(4)	—
	S,R-Phe-Ala-(*P*)	9.23(1)	2.525(3)	−6.084(7)	—	—	—
Zn^{2+}	*S,S*-Ala-Ala-(*P*)	—	4.22(3)	—	—	—	—
	S,R-Ala-Ala-(*P*)	—	4.24(1)	−4.26(5)	—	—	—
	S,R-Leu-Ala-(*P*)	—	3.761(9)	—	—	—	—

* Low abundance.



Scheme 1

exhibit large differences in log β, larger than those of the diastereoisomers of the common dipeptides. In both types of dipeptides, the zwitterion form with only one H^+ proton is the more stable, with hydrophilic substituents on one side of the molecule and hydrophobic ones on the other, *i.e.* the *S,S* isomers shown in Scheme 1. The difference in the acid–base properties is illustrated in Fig. 5 where the distribution of both diastereoisomers of Phe-Ala-(*P*) is depicted. The stabilisation is highest for the HA^- form of the phosphonodipeptides where the abundance of the *S,S* isomer is higher than that of the *S,R* isomer.

The H_2A forms are not as influenced by the intermolecular interaction. The abundance of both diastereoisomers is virtually the same, but the pH region is slightly shifted.

As in the analogous series of common dipeptides,[1] an influence of the hydrophobic side chain on the differences in the pK_a values of both diastereoisomers is evident. The differences increase in the series Me < CH_2Pr^i < CH_2Ph. This influence is notable for H_2A species and very strong for HA^- species. It is consistent with the fact that in this form the *S,S* isomers are entrapped better than *S,R* isomers in the non-polar reversed phase in HPLC.[11]

The crystal structures show a strong interaction of the phosphonic group with water molecules. However, it is difficult to define the influence of the hydration sphere on the conformation of phosphonodipeptides, except that the hydration should decrease the hydrophobic interactions between the methyl group of the phosphonic part and the side chain of the amino acid part of the phosphonodipeptide.

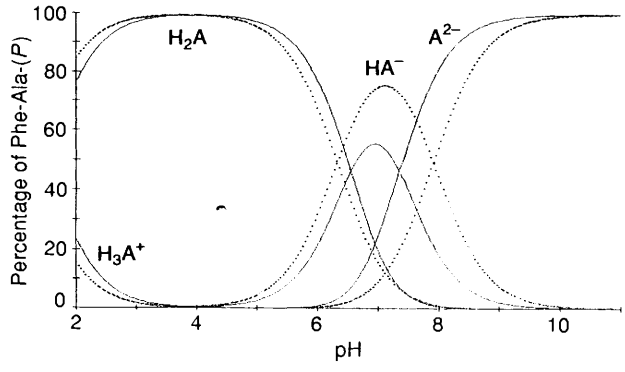


Fig. 5 Distribution diagrams of *S,R*- (full line) and *S,S*-Phe-Ala-(*P*) (dotted line) as a function of pH

The complex-formation constants determined are listed in Table 5. This set of values reflects the same types of species and the same dependence on the transition metal or on the size of the side chain as in the Gly-(*P*) series.[3] On the other hand, large differences were found in the complexing properties of the diastereoisomers, larger than in analogous systems of common dipeptides. These differences are illustrated in Figs. 6 and 7, where the systems of Cu^{2+} and Ni^{2+} with *S,S*-Phe-Ala-(*P*) and *S,R*-Phe-Ala-(*P*) are shown, and in Tables 6 and 7 for the derived constants of phosphonodipeptide and common dipeptide complexes [reactions (1)–(6)].

In the acidic region the protonated complexes $[M(HA)]^+$ are formed for all the systems with Cu^{2+}, and some are formed with Ni^{2+} and Co^{2+}. Co-ordination of the ligand to the metal through the amine and carbonyl groups and possibly through the protonated phosphonic group as in the previous series is assumed.[3] The derived constants $pK_{M(HA)}^{-H}$ and log $K_{M(HA)}$ for processes (1) and (2) are listed in Table 6. The log $K_{M(HA)}^{-H}$ values confirm the protonation of phosphonic group as for the Gly-(*P*) series[3] and the log $K_{M(HA)}$ values reflect the differences in stability between diastereoisomers without the contribution from the protonation constant of the phosphonic groups. The

$$[M(HA)]^+ \longrightarrow [MA] + H^+$$
$$pK_{M(HA)}{}^{-H} = \log \beta_{111} - \log \beta_{110} \quad (1)$$

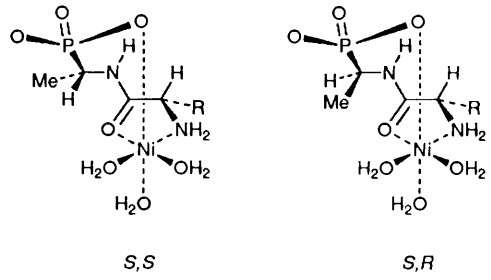
$$M^{2+} + HA^- \longrightarrow [M(HA)]^+$$
$$\log K_{M(HA)} = \log \beta_{111} - pK_2 \quad (2)$$

$\log K_{M(HA)}$ values again decrease with the size of the side chain. In the Ni^{2+} and Co^{2+} systems both diastereoisomeric $[M(HA)]^+$ species were found only for Phe-Ala-(*P*). The log $K_{M(HA)}$ values for *S,S* diastereoisomers are higher as is the amount of these species in solution. As in the zwitterion form of the free phosphonodipeptides, this higher stability would correspond to a hydrophobic interaction. The different stability of the diastereoisomers is consistent with the suggested co-ordination of the monoprotonated phosphonic group because, for co-ordination through the amine and carbonyl peptide groups alone, no influence on the stability should be observed. This means of co-ordination is shown in Scheme 2 for co-ordinated phosphonic groups. The phosphonic group can occupy both axial and equatorial positions but the axial position is more probable due to rigidity of the peptide bond. From this scheme it is clear that the hydrophobic interaction increases the stability of the *S,S* diastereoisomers.

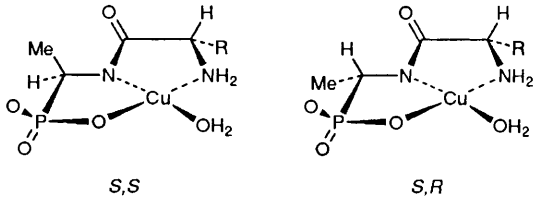
All the studied ions form neutral, non-protonated [MA] species that are the predominant species in systems with Ni^{2+}, Co^{2+} and Zn^{2+}. In contrast to the common dipeptide systems (Table 7, log K_1 values), the phosphonodipeptides exhibit higher values of log β_{110} for the *S,S* diastereoisomers as well as for the protonated complexes. Thus they have similar co-ordination to that shown in Scheme 2 through the amine, carbonyl and, in this case, the deprotonated phosphonic groups.

The systems with Cu^{2+} are different. The abundance of the [CuA] species is low and is overlapped by the dominant species $[CuAH_{-1}]^-$, and the log β_{11-1} values are higher for the *S,R* isomers. Higher log β_{110} values were also observed for these isomers. We thus assume a different means of co-ordination than for the nickel, cobalt and zinc systems. Deprotonation of the amide peptide bond in the $[Cu(HA)]^+$ species and co-ordination through the amine and amide groups is likely. In the next step, deprotonation of the phosphonic group starts and formation of the dominant $[CuAH_{-1}]^-$ species occurs. The means of deprotonation of $[Cu(HA)]^+$ was discussed for the Gly-(*P*) series.[3] On the basis of the different complexing properties of the diastereoisomers found in the Ala-(*P*) series, the proposed mechanism is more probable than those starting with deprotonation of the phosphonic group. The suggested stereochemistry of the $[CuAH_{-1}]^-$ species is shown in Scheme 3.

Scheme 3 indicates clearly that the hydrophobic interaction for the *S,R* isomers is more efficient and thus the log β_{11-1}



Scheme 2



Scheme 3

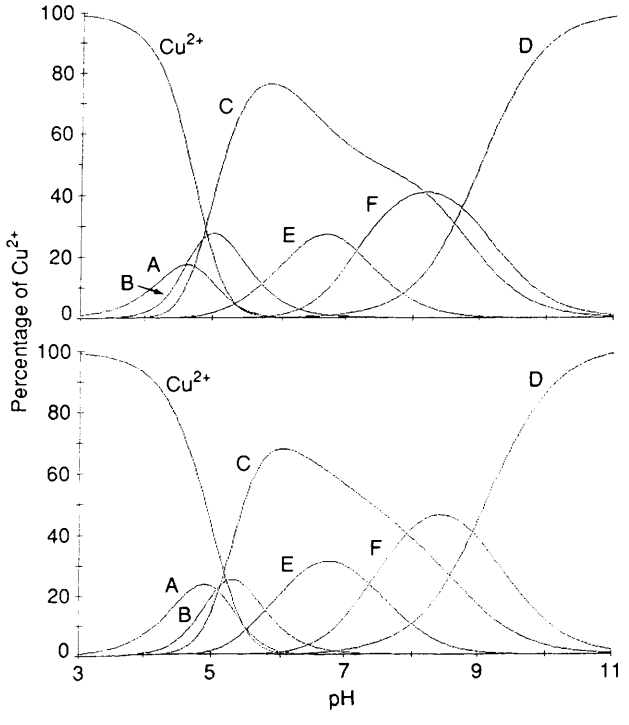


Fig. 6 Distribution diagrams of the complexes formed in the Cu^{2+}–*S,R*-Phe-Ala-(*P*) (upper) and Cu^{2+}–*S,S*-Phe-Ala-(*P*) (lower) systems as a function of pH (c_{Cu} = 0.0025, c_L = 0.005 mol dm^{-3}). Species: A = $[Cu(HA)]^+$, B = [CuA], C = $[CuAH_{-1}]^-$, D = $[CuAH_{-2}]^{2-}$, E = $[CuA_2]^{2-}$ and F = $[CuA_2H_{-1}]^{3-}$

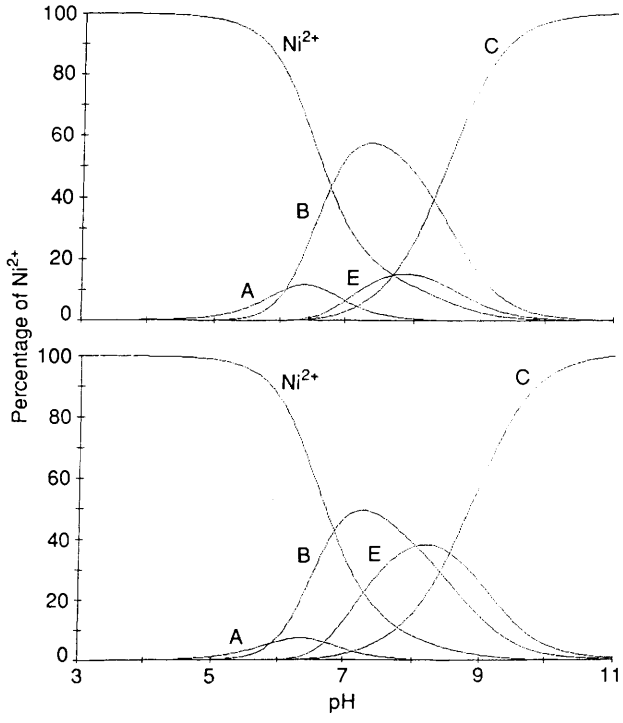


Fig. 7 Distribution diagrams of the complexes formed in the Ni^{2+}–*S,R*-Phe-Ala-(*P*) (upper) and Ni^{2+}–*S,S*-Phe-Ala-(*P*) (lower) systems as a function of pH (c_{Ni} = 0.0025, c_L = 0.005 mol dm^{-3}). Species: A = $[Ni(HA)]^+$, B = [NiA], C = $[NiAH_{-1}]^-$ and E = $[NiA_2]^{2-}$

Table 6 Derived constants of the phosphonodipeptides

Ion	Peptide	$\log K_{M(HA)}$	$pK_{M(HA)}{}^{-H}$	$pK_1{}^1$	$pK_1{}^2$	$\log K_2$	$\log(K_1/K_2)$	$pK_2{}^{-1}$	$\log K_{2A}$
Cu^{2+}	Gly-Ala-(*P*)	5.85	5.46	5.00	8.48	5.74	1.12	7.23	3.51
	S,S-Ala-Ala-(*P*)	5.50	5.30	4.99	9.18	5.42	1.08	7.68	2.73
	S,R-Ala-Ala-(*P*)	5.43	5.50	4.66	8.80	5.83	0.64	7.47	3.02
	S,S-Leu-Ala-(*P*)	5.27	5.11	5.04	8.71	5.25	1.10	7.44	2.85
	S,R-Leu-Ala-(*P*)	4.87	4.93	4.80	9.02	–	—	—	2.39
	S,S-Phe-Ala-(*P*)	5.11	5.08	5.13	8.57	5.47	0.89	7.38	3.22
	S,R-Phe-Ala-(*P*)	4.64	4.63	4.86	8.64	5.23	1.34	7.16	2.93
		$\log K_{M(HA)}$	$pK_{M(HA)}{}^{-H}$	$pK_1{}^1$	$\log K_2$	$\log(K_1/K_2)$	$pK_2{}^{-1}$		
Ni^{2+}	Gly-Ala-(*P*)	4.17	6.01	8.77	3.33	1.30	—		
	S,S-Ala-Ala-(*P*)	– –	—	8.44	3.21	0.75	—		
	S,R-Ala-Ala-(*P*)	3.32	6.09	8.66	2.73	1.04	9.19		
	S,S-Leu-Ala-(*P*)	– –	—	8.62	3.24	0.44	—		
	S,R-Leu-Ala-(*P*)	2.92	6.00	8.63	2.32	1.28	—		
	S,S-Phe-Ala-(*P*)	3.27	5.91	8.40	3.00	0.69	—		
	S,R-Phe-Ala-(*P*)	2.86	6.08	8.26	2.21	1.13	—		
Co^{2+}	Gly-Ala-(*P*)	3.44	6.28	9.01	2.48	1.15	—		
	S,S-Ala-Ala-(*P*)	—	—	8.93	—	—	—		
	S,R-Ala-Ala-(*P*)	—	—	8.80	–	—	—		
	S,S-Leu-Ala-(*P*)	—	—	8.51	—	—	—		
	S,R-Leu-Ala-(*P*)	–	—	8.54	–	—	—		
	S,S-Phe-Ala-(*P*)	2.93	6.42	8.67	2.08	0.76	—		
	S,R-Phe-Ala-(*P*)	2.67	6.70	8.61	—	—	—		

Table 7 Derived complex-formation stability constants of the common dipeptides from the literature[1]

Ion	Peptide	$\log K_1$	$pK_1{}^1$	$pK_1{}^2$	$\log K_2{}^{-1}$		
Cu^{2+}	Gly-L-Ala	5.78	4.14	9.42	3.15		
	L-Ala-D-Ala	5.71	3.96	—	—		
	L-Ala-L-Ala	5.35	3.56	9.48	2.96		
	L-Leu-D-Ala	5.56	4.18	—	—		
	L-Leu-D-Ala	5.62	3.77	–	—		
		$\log K_1$	$\log K_2$	$\log(K_1/K_2)$	$pK_1{}^1$	$pK_2{}^1$	
Ni^{2+}	Gly-L-Ala	4.23	3.37	0.86	– –	8.8	
	L-Ala-D-Ala	3.90	3.02	0.88	9.06	– –	
	L-Ala-L-Ala	4.14	2.88	1.26	8.67	—	
	L-Leu-D-Ala	3.34	2.81	0.53	9.20	–	
	L-Leu-L-Ala	3.36	2.61	0.75	8.92	– –	
Co^{2+}	Gly-L-Ala	3.10	2.58	0.52			
	L-Ala-L-Ala	2.58	1.84	0.74			

values found for those isomers are higher and $pK_1{}^1$ are lower than those for the *S,S* isomers. The deprotonation and simultaneous co-ordination of the peptide amide bond only for Cu^{2+} ions corresponds to the differences in the derived constants $pK_1{}^{-1}$ and $pK_1{}^2$ for the processes (3) and (4) which

$$[MA] \longrightarrow [MAH_{-1}]^- + H^+ \qquad pK_1{}^1 = \log \beta_{110} - \log \beta_{11-1} \quad (3)$$

$$[MAH_{-1}]^- \longrightarrow [MAH_{-2}]^{2-} + H^+ \qquad pK_1{}^2 = \log \beta_{11-1} - \log \beta_{11-2} \quad (4)$$

are listed in Table 6. The $pK_1{}^1$ values of the Cu^{2+} systems correspond to the acidic region and are lower for the *S,R* isomers, thus process (3) occurs at lower pH and easily. In contrast to $pK_1{}^1$ for the Cu^{2+} systems, $pK_1{}^1$ for the other ions and $pK_1{}^2$ for Cu^{2+} correspond to the alkaline region and this deprotonation should correspond, as in the Gly-(*P*) series,[3] to deprotonation of a co-ordinated water molecule. A comparison of the derived constants $pK_1{}^{-1}$ and $pK_1{}^2$ for the common dipeptides and for phosphonodipeptides (Tables 6 and 7) reveals the same influence of the phosphonic group as was discussed for the Gly-(*P*) series.[3] No influence of the side chain was observed.

In addition to the 1:1 complexes, Cu^{2+}, Ni^{2+} and in part Co^{2+} form complexes with a 1:2 ratio of metal:ligand. The values of $\log \beta_{120}$ or the derived constants for processes (5) and (6) or $\log(K_1/K_2)$ of the $[CuA_2]^{2-}$ diastereoisomers do not show any trends.

$$[MA_2]^{2-} \longrightarrow [MA_2H_{-1}]^{3-} + H^+ \qquad pK_2{}^1 = \log \beta_{120} - \log \beta_{12-1} \quad (5)$$

$$[MAH_{-1}]^- + A^{2-} \longrightarrow [MA_2H_{-1}]^{3-} \qquad \log K_{2A} = \log \beta_{12-1} - \log \beta_{11-1} \quad (6)$$

As in the previous series[3] of Cu^{2+} systems, we assume equatorial co-ordination of two amine and probably two carbonyl groups of the two ligands. The phosphonic groups are probably not co-ordinated. Deprotonation of the $[CuA_2]^{2-}$ species leads to the formation of $[CuA_2H_{-1}]^{3-}$. For this complex we assume tridentate co-ordination of one molecule of the ligand through the amine, peptide amide and phosphonic groups. The second molecule would be co-ordinated only through the amine, and probably, similar to common dipeptides, axially through the carbonyl group. This mode of co-ordination is the same as for the $[CuAH_{-1}]^-$ species, but the molecule of water is replaced by the amine of the second ligand. Table 5 shows higher values of $\log \beta_{12-1}$ and lower

values of pK_2 [1] for the *S,R* isomers and hence stabilisation due to the above-mentioned hydrophobic interaction.

For the series of $[NiA_2]^{2-}$ and $[CoA_2]^{2-}$ species, there are large differences in the complexing properties of the diastereoisomers. For the Ni^{2+} systems the values of log β_{120} for the *S,S* isomers are much higher than for the *S,R* isomers, and the log(K_1/K_2) values are lower (Tables 5 and 6). In the Co^{2+} systems only one species, $[CoA_2]^{2-}$ [H_2A = *S,S*-Phe-Ala-(*P*)], was found. Owing to the hydrophobic interaction in [NiA] for the *S,S* diastereoisomers, a similar mode of co-ordination is assumed for the 1:2 complexes, *i.e.* equatorially through the amine and carbonyl groups of both ligands and axially through the phosphonic groups. The amine and carbonyl groups form two five-membered rings and the carbonyl and phosphonic groups would form two seven-membered rings. The peptide bond would not be exactly planar. For this co-ordination arrangement, hydrophobic interaction should be possible for *S,S* isomers.

Conclusion

The crystal structure determinations of *S,S*-Phe-Ala-(*P*) and *S,R*-Leu-Ala-(*P*) confirmed the absolute configurations of both diastereoisomers. The bond distances and angles of the peptide moiety are comparable to those of common dipeptides, *i.e.* they are not affected by the phosphonic group. The values of the dissociation and complex-formation constants exhibit the same dependence on the side chain as was observed in the Gly-(*P*) series.[3] A comparison of the acid–base and complexing properties of these diastereoisomers with the analogous series of common dipeptides again reveals the same trends, but the differences are greater, probably due to stronger interaction of the hydrophilic and/or hydrophobic parts of the present molecules.

Experimental

Crystallography.—Crystal data for S,S-Phe-Ala-(*P*)·$2H_2O$. $C_{11}H_{21}N_2O_6P$, M = 308.27, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 7.566(1), b = 7.924(1), c = 25.930(3) Å, U = 1554.6(3) Å^3 (by least-squares refinement of the diffractometer angles for 15 automatically centred reflections in the range 2θ 6–29°, 293(2) K, λ(Cu-Kα) = 1.571 80 Å, D_m = 1.32(2) g cm^{-3}, D_c = 1.317 g cm^{-3}, Z = 4, F(000) = 656, colourless transparent prismatic crystals, dimensions 0.2 × 0.25 × 0.45 mm, μ(Cu-Kα) = 1.81 mm^{-1}.

Syntex $P2_1$ diffractometer, θ–2θ scan, graphite-monochromated Cu-Kα radiation, 4941 reflections measured (h − 8 to 8, k − 8 to 8, l − 28 to 28; $2\theta_{max}$ = 114.5°), 2127 independent reflections [R(int) = 0.0479], 2075 with $F_o > 4\sigma(F_o)$, no absorption correction. The structure was solved by direct methods (SHELX 86):[12] atomic coordinates, thermal parameters (anisotropic for non-hydrogen atoms), the scale factor, secondary isotropic extinction coefficient and Flack parameter x[13] were refined simultaneously by full-matrix least squares (SHELXL 93).[14] The refinement converged at $R1$ = 0.0422 for all the data, $wR2$ = 0.0955, goodness of fit on F^2, S = 1.131, absolute structure parameter x = 0.01(3). Corresponding values for refinement with the inverted absolute structure were $R1$ = 0.0513, $wR2$ = 0.1254, S = 1.043 and x = 0.98(4). The largest residual peak and hole, 0.288 and −0.500 e Å^{-3}, were found for the correct absolute structure. The weighting scheme $w = 1/[\sigma^2(F_o)^2 + (xP)^2 + yP]$ was used where $P = (F_o^2 + 2F_c^2)/3$ and x,y were 0.0678, 0.0737; isotropic type I extinction correction with Lorentz distribution (SHELXL 93),[14] g = 0.0219(14). Atomic coordinates are given in Table 8.

Crystal data for S,R-Leu-Ala-(*P*)·$2H_2O$. $C_8H_{23}N_2O_6P$, M = 274.26, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 9.558(2), b = 16.964(8), c = 17.717(5) Å, U = 2875(2) Å^3 (by least-squares refinement of diffractometer angles for 15 automatically centred reflections in the range 2θ 6–28°), λ(Cu-Kα) = 1.571 80 Å, D_c = 1.268 g cm^{-3}, D_m = 1.26(1) g cm^{-3}, Z = 8 (two crystallographically independent units), F(000) = 1184, colourless transparent crystals, dimensions 0.1 × 0.25 × 0.5 mm, μ(Cu-Kα) = 1.89 mm^{-1}.

Diffractometer, scans and radiation as above. 7679 Reflections measured (h −10 to 10, k −18 to 18, l −19 to 19; $2\theta_{max}$ = 114.8°), 3929 independent reflections [R(int) = 0.0657], 3458 with $F_o > 4\sigma(F_o)$, no absorption correction. The structure was solved by direct methods (SHELX 86);[12] atomic coordinates, thermal parameters [anisotropic for non-hydrogen and isotropic for C(7A), C(8A) and hydrogen atoms], the scale

Table 8 Atomic coordinates (× 10^4) for *S,S*-Phe-Ala-(*P*)

Atom	x	y	z
P	613(1)	369(1)	7 933(1)
O(1)	393(3)	−1 549(2)	8 054(1)
O(2)	2 479(2)	821(2)	7 805(1)
C(3)	−2 078(3)	3 598(3)	8 530(1)
O(3)	−718(2)	961(2)	7 541(1)
O(4)	−3 402(2)	2 718(3)	8 575(1)
N(1)	−439(3)	3 041(3)	8 504(1)
N(2)	−3 736(3)	6 035(3)	8 193(1)
C(2)	1 518(5)	1 097(4)	8 958(1)
C(5)	−2 577(4)	6 125(4)	9 089(1)
C(1)	36(3)	1 264(3)	8 560(1)
C(4)	−2 262(3)	5 516(3)	8 535(1)
C(6)	−1 033(4)	5 745(4)	9 438(1)
C(7)	−1 196(5)	4 610(5)	9 839(1)
C(8)	240(7)	4 274(6)	10 151(1)
C(9)	1 809(7)	5 021(7)	10 067(2)
C(10)	2 030(6)	6 148(6)	9 662(2)
C(11)	577(6)	6 495(5)	9 353(1)
O(5)	−6 996(3)	4 342(3)	8 095(1)
O(6)	−4 621(4)	9 412(3)	8 319(1)

Table 9 Atomic coordinates (× 10^4) for *S,R*-Leu-Ala-(*P*)

Atom	x	y	z
P(1A)	1821(2)	945(1)	3651(1)
O(3A)	1344(5)	791(4)	4448(3)
O(2A)	3354(5)	1064(4)	3560(3)
O(1A)	971(6)	1653(4)	3314(3)
C(1A)	1219(7)	113(5)	3076(4)
C(2A)	1523(11)	−689(6)	3436(5)
N(1A)	1801(6)	162(4)	2311(3)
C(3A)	1074(7)	−52(5)	1717(4)
O(4A)	−149(5)	−259(4)	1738(3)
C(4A)	1889(10)	−41(5)	971(4)
N(2A)	1318(6)	−659(4)	476(3)
C(5A)	1769	771	597
C(6A)	1483	1524	767
C(7A)	2817	1883	359
C(8A)	267	2094	734
P(1B)	2744(2)	4351(1)	4883(1)
O(3B)	2827(7)	3600(3)	4468(4)
O(2B)	1309(5)	4605(4)	5137(3)
O(1B)	3678(5)	4351(4)	5613(3)
C(1B)	3574(7)	5106(5)	4312(4)
C(2B)	3452(12)	5929(6)	4628(6)
N(1B)	3122(5)	5050(4)	3533(3)
C(3B)	3998(7)	5131(5)	2972(4)
O(4B)	5258(5)	5302(5)	3051(3)
C(4B)	3481(7)	4971(5)	2167(4)
N(2B)	3916(6)	5657(4)	1699(3)
C(5B)	4085(10)	4212(6)	1864(5)
C(6B)	3660(14)	3463(6)	2289(6)
C(7B)	2174(18)	3308(8)	2262(9)
C(8B)	4537(22)	2760(8)	2016(9)
O(5)	434(7)	2826(4)	4213(4)
O(6)	4595(7)	2413(4)	4257(5)
O(7)	7977(8)	1844(5)	2778(5)
O(8)	7283(16)	2852(11)	3949(13)

factor, secondary isotropic extinction coefficient and Flack parameter x were refined simultaneously by full-matrix least squares (SHELX 93).[14] The refinement converged at $R1$ = 0.1072 for all data, $wR2$ = 0.2744, goodness of fit on F^2, S = 1.036, absolute structure parameter x = 0.02(6). Corresponding values for the inverted absolute structure were $R1$ = 0.1204, $wR2$ = 0.2946, S = 1.455 and x = 0.98(6). The largest peak and hole, 1.133 and −0.468 e Å^{-3}, were found for the corrected absolute structure. The weighting scheme defined above was used with x and y = 0.2162 and 2.1705, respectively. Isotropic type I extinction correction with Lorentz distribution (SHELXL 93)[14] was used, g = 0.0032(6). Atomic coordinates are given in Table 9.

Additional material available from the Cambridge Crystallographic Data Centre comprises H-atom coordinates, thermal parameters and remaining bond lengths and angles.

TGA Measurements.—These were carried out on a TG-750 Stanton Redcroft instrument in the temperature range 25–280 °C in the presence of air.

Preparation of the Phosphonodipeptides and Chemicals.—The phosphonodipeptides were prepared and their diastereoisomers separated according to the procedure described previously.[6] The stock solutions were prepared as for the aminomethylphosphonic acid series.[3]

Potentiometric Titrations.—The titration procedure, calibration method and calculation were described in the preceding paper.[3]

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