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Zinc(II) complexes of phosphonic acid analogues of glutamic acid

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The stoichiometries and stability constants of proton and zinc(II) complexes of two phosphonic acid analogues of glutamic acid and three analogues of pyroglutamic acid (5-oxopyrrolidine-2-carboxylic acid) have been determined potentiometrically at 25 °C and at ionic strength 0.2 mol dm^{-3} (KCl). Complementary studies by means of ^{31}P and ^{13}C NMR spectroscopy were also undertaken and indicated that the analogue obtained by the replacement of the γ-carboxylic group of glutamic acid by a phosphonic acid moiety and the phosphonic acid analogue of pyroglutamic acid exhibit predictable properties when complexing zinc(II) ions. The analogue obtained by the replacement of the α-carboxylic group by a phosphonic acid group underwent chemical transformation upon reaction with zinc(II) ions under neutral and alkaline conditions yielding most probably a cyclic phosphonamidate composed of two ligand molecules. The crystal structures of some analogues of pyroglutamic acid were also determined.

Zinc is an essential element for the occurrence of reactions that are required in the metabolic processes of living organisms. It has become increasingly apparent that zinc-containing enzymes are widely distributed in nature (over 200 enzymes from various sources have been isolated so far) and play important roles in many physiological processes. Most often, the role of zinc is to bind substrate and activate it for the required reaction. In these cases zinc acts as a Lewis acid accepting a lone electron pair from a donor, usually oxygen from C=O or P=O bonds. In some reactions it also acts by complexing a molecule of water, changing significantly its nucleophilicity.

The standard procedure in the development of inhibitors of zinc-containing enzymes is to design analogues of the substrates by incorporating functional groups that can form strong co-ordinate bonds with zinc ion: carboxyalkyls, thiols, hydroxamates or phosphonates.[1] The most successful examples are potent inhibitors of metalloproteases which were obtained by replacing the scissile amide bond of the substrate by phosphonic acid or phosphonamidate groups.[2,3] Despite its complexing properties, tetrahedral phosphonate effectively mimics the transition state of the catalysed hydrolysis reaction. Although less effective than phosphonopeptides containing a phosphonamidate bond, simple aminoalkylphosphonic acids are especially promising because, in contrast to the phosphonopeptides, they are stable in aqueous solutions and their toxicity is exceptionally low.[4]

Phosphonic acid analogues of glutamic acid display interesting biological activities, the possible applications of which range from medicine to agriculture.[4] The aim of this work was to obtain information on the complex-forming ability of analogues of glutamic acid, in which one carboxylic group was replaced by a phosphonic acid moiety, by establishing the compositions and stabilities of the species formed with zinc ion.

Experimental

Materials

Phosphonic acid analogues of glutamic acid were obtained according to previously described procedures.[5] All the compounds were used in their racemic forms.

The purities and exact concentrations of the solutions of the pro-ligands used for potentiometric studies were determined by the Gran method.[6] The concentration of zinc(II) chloride stock solution was standardized by complexometric ethylenedinitrilotetraacetate (edta) titration. Carbonate-free potassium hydroxide (the titrant) was prepared and standardized against a standard potassium hydrogenphthalate solution. The concentration of KOH was *ca.* 0.2 mol dm^{-3}.

Potentiometric measurements

The stability constants of the proton and zinc(II) complexes of the pro-ligands were determined by pH-metric titration of 5 cm^3 samples. The pro-ligand concentration was 4×10^{-3} mol dm^{-3}, and the metal ion:pro-ligand ratio 1:1, 1:2 or 1:4. The ionic strength was adjusted to 0.2 mol dm^{-3} with KCl in each case. The titrations were performed over the range pH 2–11 with KOH solution of known concentration (*ca.* 0.2 mol dm^{-3}). In the case of complexes with zinc(II) reliable titration curves were not obtained at higher pH values because of precipitation of complex or metal hydroxide. All titration solutions were thermostatted at 25 ± 0.1 °C using a constant-temperature water-bath.

The pH was measured with a Radelkis OP-208/1 instrument equipped with a GK-2701 combined electrode using an ABU80 titration unit. The electrode system was calibrated by periodic titrations of HCl solution (5×10^{-2} mol dm^{-3} in KCl) against standard KOH solution. The resulting titration data were used to calculate the standard electrode potentials, E°, and the dissociation constant for water ($pK_w = 13.732$). These values were then used to calculate the hydrogen-ion concentration $[H^+]$ from potential readings.[7] The calculations on the pH-metric data were performed with the aid of the SUPERQUAD computer program.[8] The overall stability constants ($\log \beta_n$) of the complexes were calculated through the refinement of several sets of potentiometric titration data (numbers of experimental data points are given in Tables 1 and 2).

For clarity, the charges of the complexes are omitted in the text and tables. It has to be mentioned, however, that the fully deprotonated form of 2-amino-2-phosphonobutanoic acid [γ-Glu(P)] is L^{3-} while the analogues of pyroglutamates

are L^{2-} or L^{-} (in the case of the *P*-phenylphosphonic derivative).

NMR measurements

Phosphorus and proton NMR spectra were recorded on a Bruker DRX spectrometer and are given in relation to 85% H_3PO_4 and $SiMe_4$ respectively, ^{13}C spectra on a Tesla BS 567A spectrometer at 25.14 MHz, with dioxane as an internal standard (titration experiments) and on a Bruker DRX (75.46 MHz) spectrometer. Reported values are given in relation to $SiMe_4$ and all downfield shifts are denoted as positive. Samples for NMR studies were prepared in deuteriated water with pro-ligand concentrations of 0.02 or 0.1 mol dm^{-3}. A zinc(II) to pro-ligand molar ratio of 1:2 was applied in all cases, using zinc nitrate as a source of Zn^{II}. The pH of the samples was measured using a Radiometer pHM83 instrument equipped with a 2401C combined electrode and given as meter readings, without correction for pD.

Crystallography

Colourless crystals of compounds **4** and **5** were obtained by recrystallization from water and were used for the data collection. The space groups and approximate unit-cell dimensions were determined from rotation and the Weissenberg photographs. The diffraction data were measured on a KUMA KM4 computer-controlled four-circle diffractometer with graphite-monochromated Cu-Kα and Mo-Kα radiation.[9]

Details of the diffraction experiments, crystal data collection and refinement are given for both compounds in Table 4. The structures were solved by direct methods with SHELXS 86[10] and refined on *F* by full-matrix least-squares methods using SHELXL 93[11] with anisotropic thermal parameters for non-hydrogen atoms. At intermediate stages of the refinement the difference maps showed all hydrogen atoms. In the final cycles of refinement hydrogen atom parameters with isotropic thermal parameters were included. During the refinement an extinction correction was applied but no adsorption correction. Scattering factors and real as well as imaginary components of anomalous dispersion were those incorporated in SHELXL 93. The ORTEP[12] package was used to generate molecular drawings.

Atomic coordinates, thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 186/128.

Results

Proton and metal complexes of phosphonic acid analogues of glutamic acid

The pH-metrically determined dissociation constants of the pro-ligands, together with corresponding literature data,[13] are given in Tables 1 (glutamic acid analogues) and 2 (pyroglutamic acid analogues).

4-Amino-4-phosphonobutanoic acid [α-Glu(P) **1**] and 2-amino-4-phosphonobutanoic acid [γ-Glu(P) **2**] contain three dissociable protons within the measurable pH range 2–11 (of CO_2H, PO_3H^- and NH_3^+ groups). One of the two phosphonic protons dissociates at around pH 1.0[15] and does not take part in metal co-ordination equilibria. Thus, these protons are not included in Table 1. The analogues of pyroglutamic acid (5-oxopyrrolidine-2-carboxylic acid) (**3** and **4**) contain two dissociable protons of PO_3H_2 within the measurable pH range. For the *P*-phenylphosphinic acid analogue of pyroglutamic acid (**5**) metal complexes were not detected potentiometrically. This most probably is due to the fact that the phosphinic proton does not participate in metal co-ordination. Compounds **4** and **5** represent the analogues of glutamic acid in which the strain caused by introduction of an additional methyl group into γ-Glu(P) results in spontaneous cyclization and quantitative formation of pyroglutamic acid analogues.[5a]

The pH-metric titration curves for the zinc(II)–pro-ligand systems were evaluated by assuming all feasible models. The data obtained from the models that provided the best fit and refined formation constants, together with earlier data for complexes of γ-Glu(P),[13] are shown in Tables 1 and 2. As an

Table 1 Deprotonotation constants (p*K*) and zinc(II) complex stability constants (log β_n) of phosphonic acid analogues of glutamic acid at 25 °C and $I = 0.2$ mol dm^{-3}. Standard deviations are given in parentheses; the ion charges are omitted

		Compound **2**	
	Compound **1**	This work	Ref. 13
$pK(NH_3^+)$	9.99(1)	10.01(1)	10.21
$pK(PO_3H^-)$	5.74	6.81	6.90
$pK(CO_2H)$	4.10	2.52	2.51
log β[Zn(HL)]	—	12.39(2)	13.18
log β[ZnL]	6.51(2)	5.94(1)	6.55
log β[ZnL_2]		10.48(3)	10.72
log K_2		4.54	4.17
log (K_{ZnL}/K_{ZnL_2})		1.40	2.38
p*K*[Zn(HL)]		6.45	6.63
Number of data points		200	

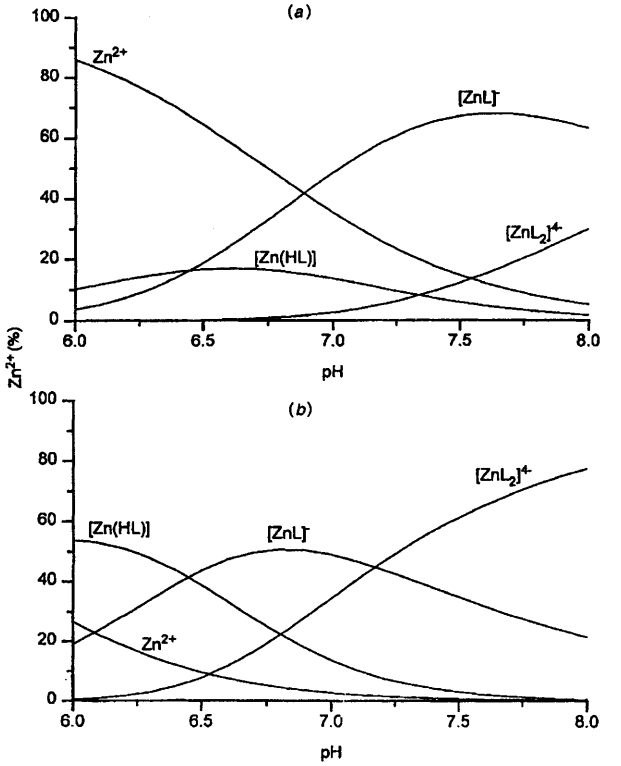


Fig. 1 Species distribution for the zinc(II)–γ-Glu(P) system as a function of pH: (*a*) for the measurement conditions ($c_M = 2.0 \times 10^{-3}$, $c_L = 4.0 \times 10^{-3}$ mol dm^{-3}); (*b*) simulated for conditions used in NMR studies ($c_M = 5.0 \times 10^{-2}$, $c_L = 1.0 \times 10^{-1}$ mol dm^{-3})

illustration, the concentration-distribution curves for the complexes formed in the zinc(II)–γ-Glu(P) and –**3** systems are depicted in Figs. 1 and 2, respectively. The pH-metric titrations followed by calculations (using the COMICS computer program [16]) indicated the existence of three complex species in a solution containing zinc(II) at a concentration of 2×10^{-3} mol dm^{-3} and γ-Glu(P) at a concentration of 4×10^{-3} mol dm^{-3}. Under these conditions the [ZnL] complex dominates at pH 6.5–8.5 reaching a maximum (*ca.* 65%) at pH around 7.5. At higher pH the new species $[ZnL_2]$ appears. Precipitation was observed at pH *ca.*8. As shown in Fig. 1, there is a minor species [Zn(HL)] at around pH 6.5. The p*K* value of this complex corresponds to that of $pK_{PO_3H^-}$ (see Table 1) and suggests aminocarboxylate-type co-ordination.[13]

The data given in Table 1 for α-Glu(P) **1** require some comment. The NMR data clearly show that in alkaline solutions a new phosphorus compound appears. Thus, the data in Table 1 were calculated using only those portions of the potentiometric curves from which this compound is absent. The characteristic feature of this system is the lack of a [Zn(HL)] species within the pH range studied. This may suggest co-ordination of zinc(II) ion by the NH_2 moiety.

Potentiometric studies of the zinc(II)–**3** and –**4** systems clearly showed the existence of three species: [ZnL] and two hydroxo complexes $[ZnLH_{-1}]$ and $[ZnLH_{-2}]$, in the range pH 5.5–8.5 (Fig. 2). Also in this system precipitation was observed around pH 8.5. There is also a possibility that the complexes of the [Zn(HL)] type exist at pH < 4. In these complexes the zinc is bound *via* an oxygen atom of the phosphonate group, while the second oxygen atom is protonated and not co-ordinated. Such a complex could not, however, be detected potentiometrically.

Since the *P*-phenylphosphinic acid **5** has only one dissociable proton which is very acidic (p*K* = 1.6, see Table 2) we assumed hydroxo-species formation as the only base-consuming process in the measurable pH range. This assumption significantly improved the fit between the experimental and the calculated titration data resulting in the parameters given in Table 2.

Table 2 Deprotonotation constants (p*K*) and zinc(II) complex stability constants (log β_n) of analogues of pyroglutamic acids at 25 °C and I = 0.2 mol dm^{-3}. Standard deviations are given in parentheses; the ion charges are omitted

	Compound **3**	Compound **4**	Compound **5**
p*K*(PO_3H^-)	6.39(4)	6.74(2)	
p*K*(PO_3H_2)	1.77	1.51	
p*K*(PO_2H)			1.6[a]
log β[ZnL]	2.09(1)	2.19(4)	—
log β[$ZnLH_{-1}$]	−5.97(5)	−5.45(5)	—
log β[ZnH_{-2}]	−13.96(2)	−14.14(8)	−15.62(3)
log β[ZnL(OH)]	8.97	8.11	
log β[$ZnL(OH)_2$]	13.54	13.36	11.86
log β[$Zn(OH)_2$]			12.22[b]
Number of data points	180	159	200
χ^2[c]	7.95	8.32	7.00
σ[c]	3.82	4.65	2.53

[a] From NMR measurements. [b] Ref. 14. [c] See ref. 8.

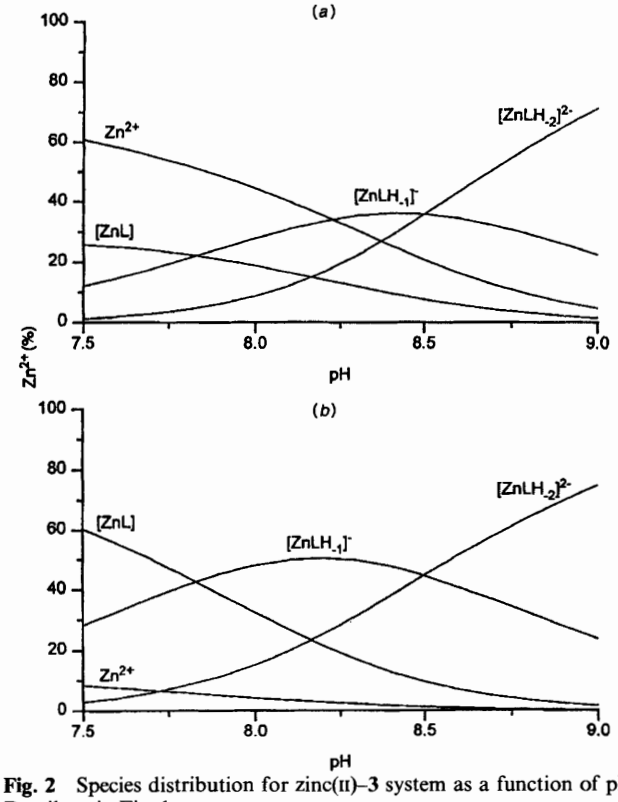


Fig. 2 Species distribution for zinc(II)–**3** system as a function of pH. Details as in Fig. 1

NMR studies

In order to obtain supporting evidence for our interpretation of the potentiometric data, as well as to provide some information about the zinc(II) binding sites, we undertook NMR studies of the zinc complexes with phosphonic acid analogues of glutamic acid. It should be noted, however, that the concentrations used in these experiments were 5- or 25-fold higher than those in the potentiometric studies. The ^{31}P and ^{13}C proton-decoupled spectra were measured as a function of pH for each pro-ligand alone and in solutions containing zinc(II) ions. The measurements in solutions containing zinc(II) ions were limited to a narrow range of pH (between 7 and 9.5) because of precipitation between pH 4 and 7. The precipitate redissolved when pH > 7, probably as a result of the formation of a highly soluble ionic species. Finally, at pH > 9.5 release of the ligand (as indicated by narrowing of the ^{31}P NMR line) with simultaneous formation of a fine suspension of hydroxide or hydroxo complexes was observed. The results of the NMR studies are summarized in Table 3.

The ^{31}P chemical shifts for α-Glu(P) and its zinc(II) complexes as a function of pH are shown in Fig. 3. The changes observed for the pro-ligand are generally consistent with results obtained from potentiometric studies. The largest shift (Δδ =

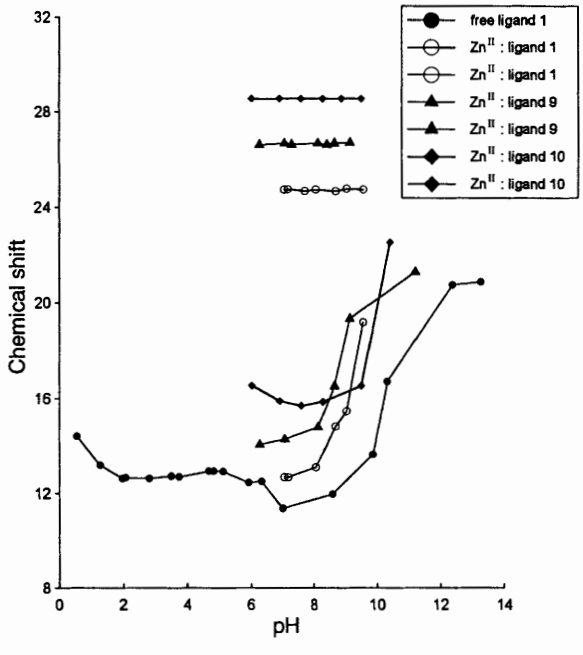


Fig. 3 Phosphorus-31 NMR chemical shifts as a function of pH for α-Glu(P) **1** and Zn^{II}–α-Glu(P) solutions (1:2 molar ratio). Similar shifts for the complexes formed with compounds **9** and **10** are also shown

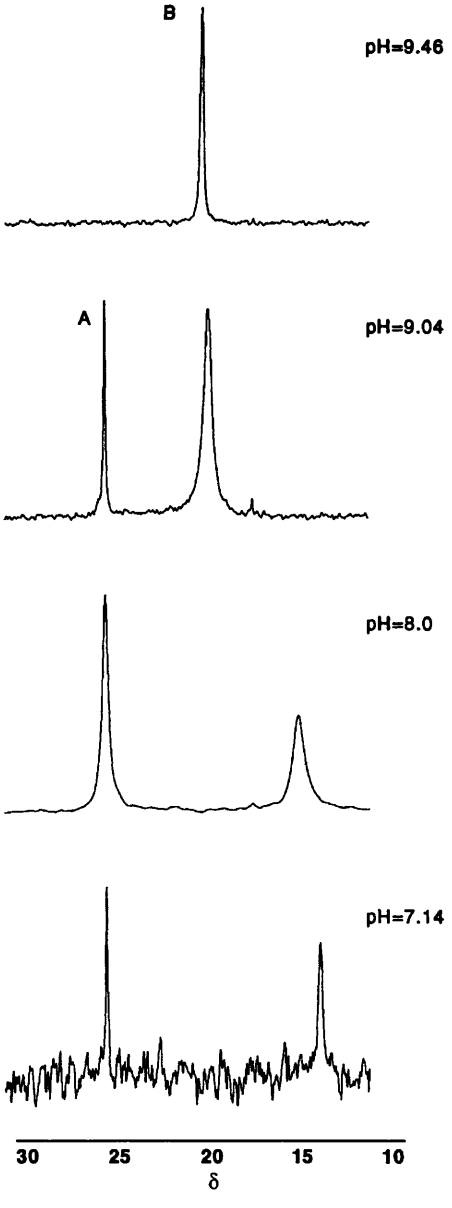


Fig. 4 The ^{31}P NMR spectra for Zn^{II}–α-Glu(P) solutions (1:2 molar ratio) *versus* pH

9.5 ppm) corresponds to deprotonation of the amino group and suggests the existence of an electrostatic interaction or/and hydrogen bonding between the phosphonate and amino groups. This feature is common for aminophosphonic acids possessing amino and phosphonic moieties bound in a conformationally labile molecule.[17]

Surprisingly, in the presence of zinc ions a pair of signals was observed (Fig. 4). The first (A), sharp and shifted downfield, remained almost intact *versus* pH. The second (B), broader and appearing at higher field, moved gradually downfield when the pH was increased. Integration of both signals showed that the total share of species A never exceeded 40%. The second peak may result from the existence of a slow pro-ligand–complex exchange process. If so the ratio of both species should vary upon temperature changes. As seen from Fig. 5 an increase in temperature did not result in a change in chemical shifts of the species nor in their ratio. The only effect was an apparent narrowing of the upfield signal. The above results seem to indicate that at least two different species exist in equilibrium in solution. These equilibria did not undergo any appreciable change upon dilution to the concentrations used in the potentiometric studies. Also there was no influence of the type of counter ion (NO_3^- *versus* Cl^-) on the equilibrium.

The serious line broadening of the upfield signal B observed at room temperature reveals that it may correspond to a mixture of free pro-ligand and its complex(es) which are rather slowly exchanging. This relatively slow exchange rate which results in ^{31}P NMR broadening seems to be a common feature of all 'fast equilibria' (*i.e.* those having one signal) systems discussed in this paper. The gradual downfield of shifts of the signals upon increasing pH reflects the deprotonation of both the free pro-ligand and complex(es) amino groups.

The profiles of the ^{13}C NMR chemical shifts for α-Glu(P) and its complexes (1:2 zinc to pro-ligand molar ratio) are shown in Fig. 6 using the same scale for each carbon atom. Subsequent deprotonation of α-Glu(P) resulted in a downfield shift of all the carbon resonance signals. The shifts of the C_α and C_β signals were affected mainly by deprotonation of the phosphonic and carboxylic groups, whereas shifts of C_0 and C_γ reflect the changes arising from deprotonations of all functional groups. The spectra of zinc(II)–α-Glu(P) solutions contain two sets of signals strongly supporting the existence of additional ligand in the system.

It is well established that zinc complexation leads to upfield shifts of the signals when compared to those of the deprotonated form of the free pro-ligand, thus yielding a similar effect to that of protonation. The value of the observed shifts depends on two effects: (*i*) a change in electronegativity of the particular donor group upon metal co-ordination which influences mainly the carbon atom in neighbouring position (C_α inductive effect); (*ii*) a steric effect influencing carbons which are not directly bound to the given substituent (C_γ and C_δ atoms). In the case of the pH-independent set of ^{13}C signals the largest changes in chemical shifts were observed for C_α and C_γ. Thus, $\Delta\delta$ (−1.82 ppm) found for C_α might reflect an electronegativity decrease due to the contribution of zinc binding *via* the phosphonate moiety. However, the appreciable shift observed for C_γ seems to reflect either the deprotonated state of the amino group or the involvement of both the amino and phosphonate groups of the ligand in some structural management upon zinc binding.

The second set of resonances was pH-dependent, as was observed in the case of the ^{31}P NMR spectra. The only information regarding the structure of the complex can be derived from the changes in chemical shifts of C_γ ($\Delta\delta$ = 1.34 ppm) and C_0 ($\Delta\delta$ = 0.52 ppm) and seems to confirm stepwise deprotonation of a free amino group.

Proton NMR spectra obtained for compound **1** in the presence of zinc ion clearly show the existence of two $C_\alpha H$ multiplets (Table 3) corresponding to the pH-dependent and -independent systems discussed above. Thus, in Zn^{II}–α-Glu(P) solution at a measurable pH range of 7–9.5 an apparent equilibrium between the zinc(II) complexes of two different ligands exists. One system consists of α-Glu(P) which, most probably, binds zinc in monodentate manner and contains a free unbound amino group (the pH-dependent set). The new ligand forms upon zinc ion-promoted reaction of α-Glu(P) in slightly alkaline solution.

In the case of γ-Glu(P) the ^{31}P and ^{13}C NMR spectra confirm the results of potentiometric studies (Fig. 7). The ^{31}P signal shifted gradually upfield upon deprotonation of both acidic groups (PO_3H^- and CO_2H), whereas removal of a proton from the H_2L^- form of the ligand is accompanied by a downfield shift of $\Delta\delta$ = 1.7 ppm (Table 3). This value is, however, smaller than those usually recorded for α-aminophosphonic acids and, although the ligand seems to retain a cyclic conformation in solution, may reflect a larger distance between the amino and phosphonate groups.

The Zn^{II}–γ-Glu(P) system is a rapidly exchanging one which results in a good correlation between NMR and potentiometric measurements. The chemical shifts for the $[ZnL_2]$ complex (Table 3) have been assigned directly from spectra under conditions of 90–95% complex formation. Both ^{31}P and ^{13}C

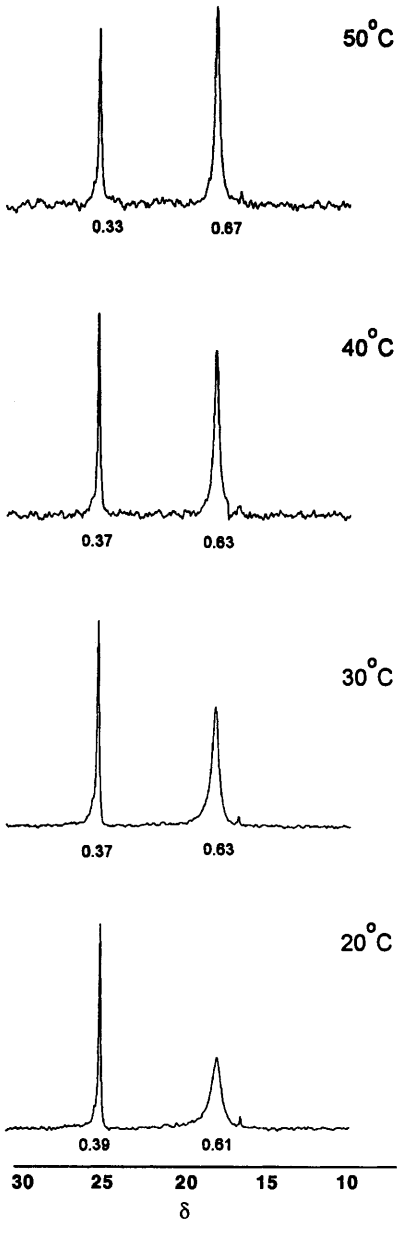


Fig. 5 The ^{31}P NMR spectra for Zn^{II}–α-Glu(P) solutions (1 : 2 molar ratio) *versus* temperature (at pH 8.85)

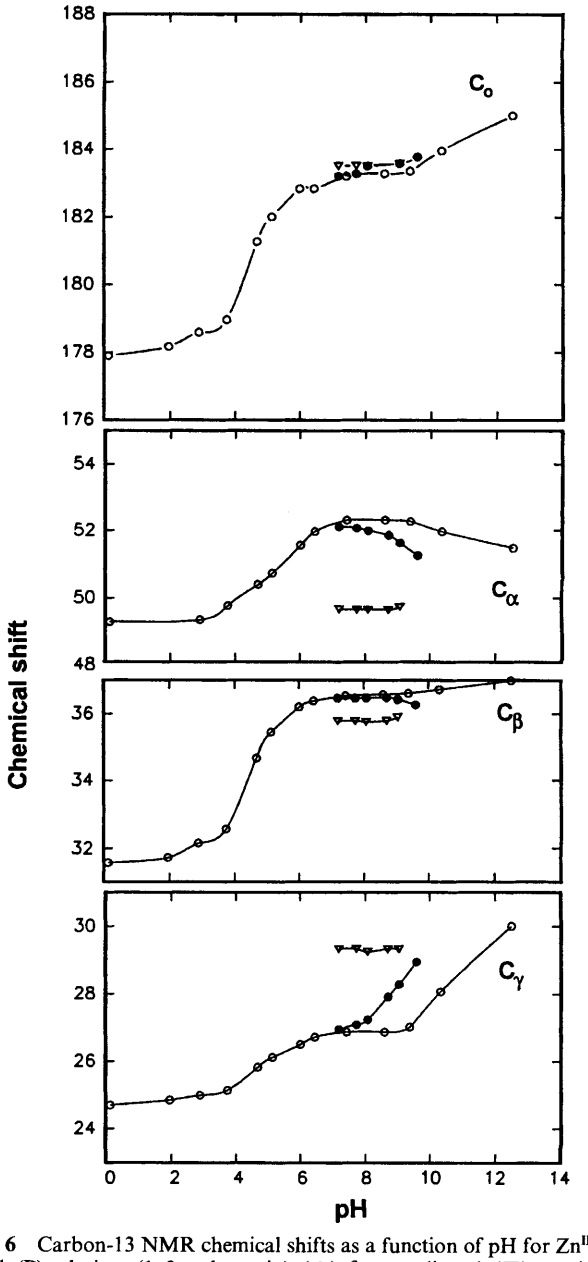


Fig. 6 Carbon-13 NMR chemical shifts as a function of pH for Zn^{II}–α-Glu(P) solutions (1 : 2 molar ratio). (○), free pro-ligand; (▽), species A; (●), species B

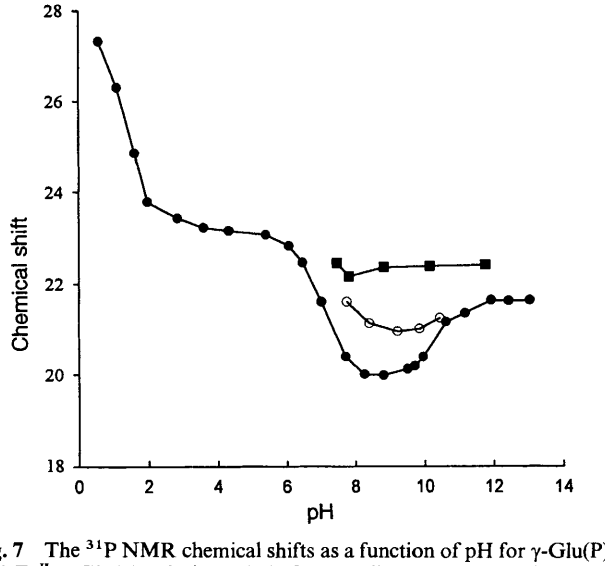


Fig. 7 The ^{31}P NMR chemical shifts as a function of pH for γ-Glu(P) and Zn^{II}–γ-Glu(P) solutions. (●), free pro-ligand; (○), metal to pro-ligand molar ratio 1 : 2; (■), molar ratio 1 : 4

NMR spectra clearly indicate that the carboxylate group is involved in metal chelation in this case. This is not surprising since five-membered chelate rings containing NH_2 and CO_2^- are generally more stable than those containing phosphonate moieties (NH_2, PO_3^{2-}). As seen from Table 3 the cyclic chelate formation produces Δδ 0.64 ppm in the ^{31}P NMR spectrum and −2.25, −1.72, −0.42 and −4.4 ppm, for C_α, C_β, C_γ and C_0 respectively, in the ^{13}C NMR spectra. The J_{PC} coupling constant for the $[ZnL_2]$ complex is 131.5 Hz.

The ^{31}P chemical shift profiles of phosphonic acid analogues of pyroglutamic acid and species formed upon complexation to zinc(II) ions are given in Fig. 8. The zinc(II)–pyroglutamate analogue systems are rapidly exchanging as indicated by the presence of only one peak for each ligand in the phosphorus spectra. The data seem to confirm a weak complexation of zinc(II) *via* the PO_3^{2-} group of compounds **3** and **4** and lack of metal co-ordination for **5**, since only slight downfield shifts respective to the free pro-ligands were found in the ^{31}P NMR spectra. This is not surprising since literature data clearly indicate that L-pyroglutamic acid binds zinc, sodium, calcium and lithium exclusively through carboxylate oxygen atoms, forming tetrahedral complexes.[18,19]

Zinc(II)–α-Glu(P)–6-amino-6-phosphonohexanoic acid 9. Some other amino acids used in this study also react with zinc(II) ions in a manner similar to that observed for α-Glu(P) (see Fig. 3). In order to find out if the newly formed compound is of dimeric nature and to study it in some detail, we undertook ^{31}P and ^{13}C NMR studies on a ternary system composed of zinc(II), α-Glu(P) and 6-amino-6-phosphonohexanoic acid **9**. The latter component was found to exhibit similar properties to α-Glu(P) in the presence of zinc(II) ions.

The ^{13}C NMR spectrum recorded upon mixing equivalent volumes of the 1:2 binary systems Zn^{II}–α-Glu(P) and Zn^{II}–**9** appears to be a superposition of the corresponding spectra obtained for the binary components (Fig. 9). However, the ^{31}P NMR spectrum of this system shows the appearance of two additional low-field resonances closely related to the parent signals (Fig. 10). The possibility of the presence of ^{31}P–^{31}P coupling in this system was ruled out by both changing the mutual pro-ligand ratio and by the fact that no cross-peaks were recorded in ^{31}P–^{31}P correlation spectra. This implies that additional species form in this system and that they are in equilibria with other species.

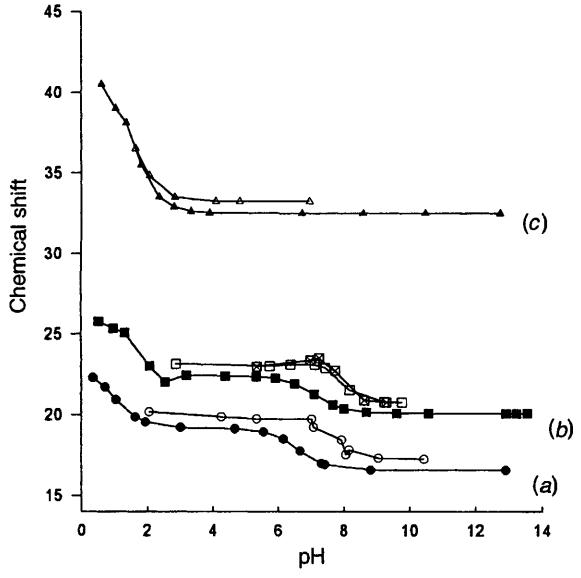


Fig. 8 The ^{13}P NMR chemical shifts as a function of pH for: (*a*) compound **3** and its analogues, (*b*) **4** and (*c*) **5** and their solutions with Zn^{II} in 1:2 (○,□,△) and 1:1 (⊠) molar ratios

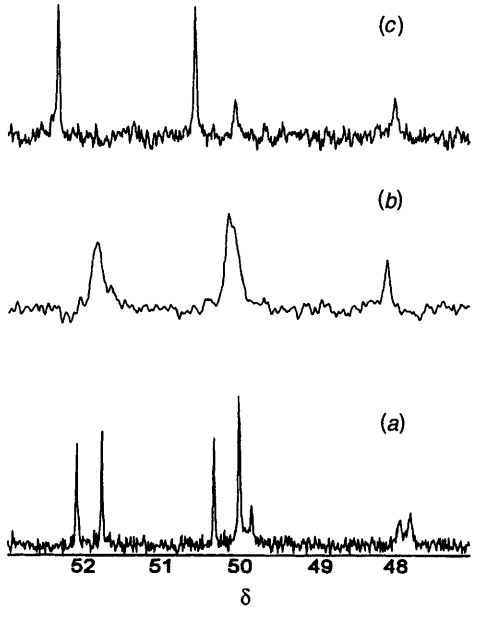


Fig. 9 The ^{13}C NMR spectra (C_α region) for (*a*) the Zn^{II}–α-Glu(P)–**9** (1:1:1) ternary system at pH 7.37 and for its binary components, (*b*) Zn^{II}–α-Glu(P) (1:2), solution at pH 7.08, and (*c*) Zn^{II}–**9** (1:2), solution at pH 7.42

Crystal structures

The molecular structures and atom numbering of compounds **4** and **5** are given in Figs. 11 and 12 respectively. Selected bond distances and angles are given in Table 5. Similarly, as described previously for compound **3**,[20] both compounds occur in the unionized form. Although the pyroglutamic acid analogue **3** crystallized in the non-centrosymmetric space group $Pca2_1$ the molecular structures of all the analogues of pyroglutamic acid (**3**–**5**) are quite similar. The geometry of the phosphonic and phosphinic acid groups does not differ from those of other unionized phosphonic and phosphinic acid derivatives.[21] Only in the case of compound **5** the P–O bond length of 1.515(1) Å is shorter than the usually observed value of 1.542(2) Å. The shortening of the P–O bond may be attributed to hydrogen-bond formation where the O(2) oxygen atom is the donor of a very short hydrogen bond of the type O(2)–H(2)···O(3w) 2.420(2) Å.

The carbonyl C=O double bonds [1.247(2) and 1.233(2) Å in compounds **4** and **5** respectively] are significantly longer than the standard length [1.215(5) Å].[22] It is in a good agreement,

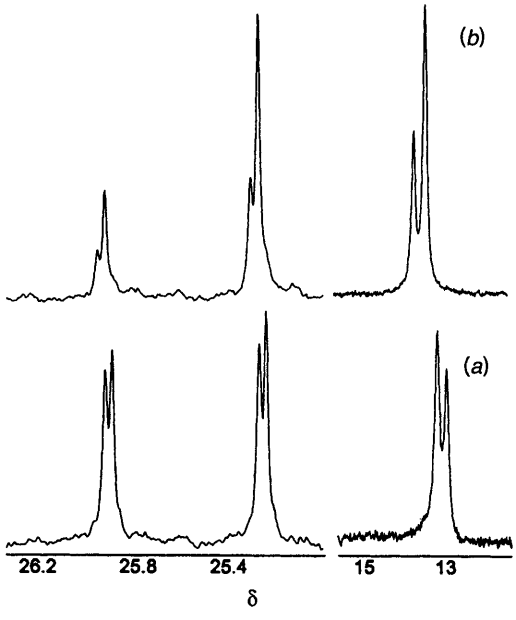


Fig. 10 The ^{31}P NMR spectra for the Zn^{II}–α-Glu(P)–**9** ternary system: (*a*) 1:1:1 molar ratio, pH 7.37, (*b*) 1:2:1 molar ratio, pH 7.08

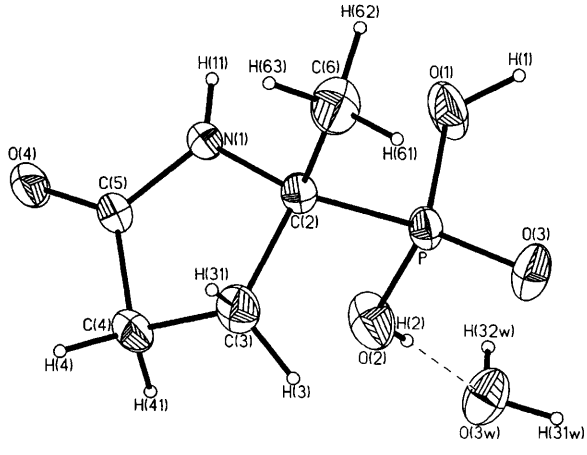


Fig. 11 Crystal structure of 2-methyl-5-oxopyrrolidin-5-ylphosphonic acid **4**. Displacement ellipsoids are shown at the 35% probability level

Table 3 Carbon-13, ^{31}P NMR and ^{1}H chemical shifts for α-Glu(P) and γ-Glu(P) and their complexes with zinc(II) ions (measured values)

	δ					
Species	C_α	C_β	C_γ	C_0	P	HC_α
α-Glu(P)						
H_3L	49.52 (142.7) *	31.37 (7.5)	25.00	178.18	12.63	3.30
H_2L^-	50.74 (140.8)	35.49 (9.4)	26.12	182.01	12.92	3.28
HL^{2-}	52.31 (133.4)	36.54 (9.4)	26.87	183.21	11.37	3.20
L^{3-}	51.48 (137.1)	36.99 (13.1)	30.01	185.00	20.88	2.60
Species A	49.66 (157.7)	35.79 (9.4)	29.34	183.51	24.75	2.79
B						
(protonated)	52.11 (137.1)	36.47 (9.4)	26.95	183.21	12.68	2.98
(deprotonated)	51.63 (137.1)	36.43 (9.4)	28.29	183.73	19.20	2.79
γ-Glu(P)						
H_3L	54.84 (16.9)	25.60 (3.8)	24.48 (135.2)	173.43	23.42	
H_2L^-	56.38 (15.0)	26.20 (3.8)	25.04 (133.3)	175.37	23.14	
HL^{2-}	57.01 (13.1)	29.78 (3.8)	26.76 (129.6)	175.55	19.98	
L^{3-}	58.69 (16.8)	31.50 (3.8)	26.69 (129.6)	184.70	21.64	
$[ZnL_2]$	56.14 (7.5)	28.29 (3.8)	26.27 (131.5)	180.30	22.16	

* J_{PC} values (Hz) in parentheses.

Table 4 Summary of crystal data, data collection and refinement conditions for compounds **4** and **5**[a]

	4	**5**
	$C_5H_{10}NO_4P{\cdot}H_2O$	$C_{11}H_{14}NO_3P{\cdot}H_2O$
M	197.13	257.22
Space group	*C*2/*c*	$P2_1/n$
No. reflections (2θ/°)	25 (20–36)	25 (16–27°)
a/Å	21.006(4)	10.208(2)
b/Å	5.904(1)	7.535(2)
c/Å	14.353(3)	16.536(3)
β/°	98.22(3)	103.66(3)
U/Å^3	1761.8(6)	1235.9(5)
Z	8	4
D/g cm^{-3}	1.486(1)	1.382
D_m/g cm^{-3} [b]	1.49(1)	1.38(1)
F(000)	832	544
Scan	ω/2θ	ω/2θ
Radiation (λ/Å)	Cu-Kα (1.5418)	Mo-Kα (0.710 69)
Crystal dimensions/mm	0.3 × 0.3 × 0.35	0.4 × 0.3 × 0.35
Decay of standards (%)	2	1.8
Reflections measured	2893	3071
2θ Range/°	8.0–162.0	4.0–55.0
h,*k*,*l* Ranges	0–26, 0 to −7, −18 to 18	0–13, 0–9, −21 to 20
Reflections observed [$F_o \geqslant 4\sigma(F)$]	1628	2085
μ/mm^{-1}	2.74	0.23
Extinction correction	0.0188(8)	0.003(2)
No. parameters varied	158	219
Weights (*a*,*b*,*f*) [c]	0.0463, 1.40, $\frac{1}{3}$	0.0524, 0.37, $\frac{1}{3}$
Goodness of fit	1.105	1.064
$R1 = \Sigma(\|F_o\| - \|F_c\|)/\|F_o\|)$	0.0372	0.0314
$wR2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{\frac{1}{2}}$	0.0987	0.0839
Largest feature final difference map/e Å^{-3}	0.36, −0.30	0.31, −0.25

[a] Details in common: monoclinic; 293(2) K; ω–2θ scans; three standard reflections every 100; Lorentz polarization correction. [b] By flotation in chlorobenzene–carbon tetrachloride. [c] $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ where $P = [f{\cdot}\max(0 \text{ or } F_o^2) + (1 - f){\cdot}F_c^2)]$.

within experimental accuracy, with the bond length expected when an oxygen atom takes part in hydrogen-bond formation.[23] Thus, the carbonyl oxygen atom is an acceptor of two hydrogen bonds in compound **4** and an acceptor of one in **5** (see Table 6). The torsional angles (Table 7) indicate that the pyrrolidone rings, as a whole, are non-planar in all the pyroglutamic acid analogues. The conformation of the rings is, therefore, intermediate between an envelope and a half-chair. The ring-puckering parameters[24] are: $Q = 0.189(2)$ Å and $\Phi = 65.2(6)°$, **4**; $Q = 0.199(2)$ Å and $\Phi = 260.7(5)°$, **5**; and $Q = 0.105(5)$ Å and $\Phi = 98(2)°$, **3**.

There is extensive hydrogen bonding in the crystal structures of compounds **4** and **5**, with all potential donor and acceptor atoms participating. In **4** three phosphonic acid groups are bridged by one water molecule *via* hydrogen bonds of lengths of 2.496(2), 2.723(2) and 2.721(2) Å. The water molecule is an acceptor of the shortest hydrogen bond formed with a phosphonic group [2.496(2) Å] and is a donor to the O(3) oxygen atom. Furthermore, the molecules are linked to each other by strong hydrogen bonds between phosphonic groups (donors) and the carbonyl groups (acceptor) [2.539(2) Å], as well as by weak hydrogen bonds between the N(1) and carbonyl O(4) atoms [2.909(2) Å]. In the structure of compound **5** there are three short O–H • • • O hydrogen bonds, which involve the water molecules acting as donors [O(3w) • • • O(4) 2.588(2) Å, and O(3w) • • • O(3) 2.594(2) Å] and acceptor [O(3w) • • • O(2) 2.420(2) Å]. The slightly longer hydrogen bond [2.849(2) Å] occurs between atoms N(1) and O(4). These intermolecular hydrogen bonds are similar to those found in crystals of compound **3**.

Discussion

Phosphonic and carboxylic groups differ substantially in many respects including size (the phosphonic is considerably larger), shape (flat carboxylic *versus* tetrahedral phosphonic), basicity and electron-releasing effects. Consequently replacement of one of the carboxylic groups of glutamic acid by a phosphonic acid moiety leads to a family of compounds of interesting chemical and biological properties.

Our studies clearly indicated that the analogue obtained by the replacement of the γ-carboxylic group of glutamic acid by a phosphonic acid moiety [compound **2**, γ-Glu(P)] and the analogues of pyroglutamic acid (**3**–**5**) exhibit predictable properties upon reaction with zinc(II) ions. Quite unexpectedly, however, the third analogue [**1**, α-Glu(P)] underwent chemical transformations upon reaction with zinc(II) ions under neutral and slightly alkaline conditions. As seen from the ^{31}P and ^{13}C NMR spectra, two chemical species are present in solutions containing α-Glu(P) and zinc(II) nitrate, in an apparent

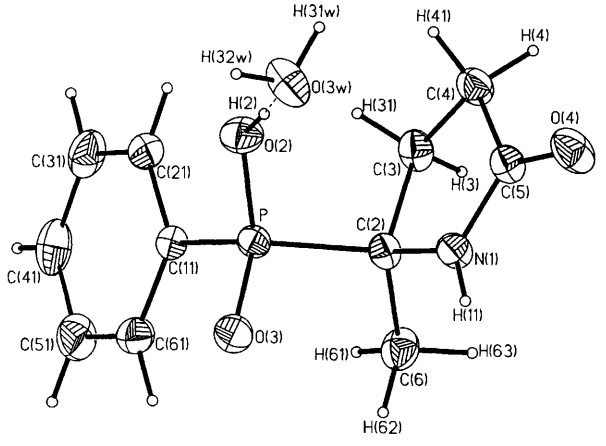


Fig. 12 Crystal structure of (2-methyl-5-oxopyrrolidin-5-yl)phenylphosphinic acid **5**. Displacement ellipsoids are shown at the 35% probability level

Table 5 Selected bond lengths (Å) and selected angles (°) for compounds **4** and **5**

Compound **4**		Compound **5**	
P–O(1)	1.542(2)	P–C(11)	1.797(2)
P–O(2)	1.542(2)	P–O(2)	1.515(1)
P–O(3)	1.484(1)	P–O(3)	1.496(1)
P–C(2)	1.823(2)	P–C(2)	1.838(2)
O(4)–C(5)	1.247(2)	O(4)–C(5)	1.233(2)
N(1)–C(5)	1.319(2)	N(1)–C(5)	1.325(2)
N(1)–C(2)	1.459(2)	N(1)–C(2)	1.466(2)
C(2)–C(6)	1.521(3)	C(2)–C(6)	1.519(2)
C(2)–C(3)	1.551(2)	C(2)–C(3)	1.546(2)
C(3)–C(4)	1.525(3)	C(3)–C(4)	1.523(2)
C(4)–C(5)	1.498(2)	C(4)–C(5)	1.499(2)
		C–C(phenyl)	1.363(3)–1.389(3)
O(3)–P–O(2)	114.8(1)	O(3)–P–O(2)	117.3(1)
O(3)–P–O(1)	113.8(1)	O(3)–P–C(11)	110.5(1)
O(2)–P–O(1)	107.7(1)	O(2)–P–C(11)	105.8(1)
O(3)–P–C(2)	112.7(1)	O(3)–P–C(2)	108.5(1)
O(2)–P–C(2)	102.2(1)	O(2)–P–C(2)	107.6(1)
O(1)–P–C(2)	104.5(1)	C(11)–P–C(2)	106.6(1)
C(5)–N(1)–C(2)	115.4(1)	C(5)–N(1)–C(2)	115.5(1)
N(1)–C(2)–C(6)	110.9(2)	N(1)–C(2)–C(6)	110.4(1)
N(1)–C(2)–C(3)	101.9(1)	N(1)–C(2)–C(3)	101.8(1)
C(6)–C(2)–C(3)	113.3(2)	C(6)–C(2)–C(3)	113.8(1)
C(4)–C(3)–C(2)	105.2(2)	C(4)–C(3)–C(2)	105.7(1)
C(5)–C(4)–C(3)	104.6(1)	C(5)–C(4)–C(3)	104.0(1)
N(1)–C(5)–C(4)	109.2(2)	N(1)–C(5)–C(4)	109.0(1)
N(1)–C(2)–P	108.1(1)	N(1)–C(2)–P	108.1(1)
C(6)–C(2)–P	110.7(1)	C(6)–C(2)–P	111.4(1)
C(3)–C(2)–P	111.6(1)	C(3)–C(2)–P	110.8(1)
O(4)–C(5)–N(1)	123.9(2)	O(4)–C(5)–N(1)	124.7(2)
O(4)–C(5)–C(4)	126.9(2)	O(4)–C(5)–C(4)	126.3(2)

Table 6 Hydrogen bond lengths (Å) and angles (°) for compounds **4** and **5**

D–H···A	D···A	D–H	H···A	D–H···A
Compound **4**[a]				
O(2)–H(2)···O(3w)	2.496(2)	0.70(4)	1.80(4)	175(4)
O(1)–H(1)···O(4^{I})	2.539(2)	0.88(4)	1.66(4)	171(4)
O(3w)–H(31w)···O(3^{II})	2.723(2)	0.83(4)	1.90(4)	171(4)
O(3w)–H(32w)···O(3^{III})	2.721(2)	0.83(4)	1.90(4)	173(3)
N(1)–H(11)···O(4^{IV})	2.909(2)	0.74(3)	2.18(3)	168(3)
Compound **5**[b]				
O(2)–H(2)···O(3w)	2.420(2)	1.09(4)	1.33(4)	177(3)
O(3w)–H(31w)···O(4^{I})	2.588(2)	0.83(3)	1.76(3)	171(3)
O(3w)–H(32w)···O(3^{II})	2.594(2)	0.87(4)	1.73(3)	172(3)
N(1)–H(11)···O(3^{III})	2.849(2)	0.81(2)	2.07(2)	160(2)

[a] Symmetry codes: I $x, -y, \frac{1}{2}+z$; II $\frac{1}{2}-x, -\frac{1}{2}+y, \frac{3}{2}-z$; III $x, -1+y, z$; IV $1-x, -y, 1-z$. [b] Symmetry code: I $\frac{3}{2}-x, \frac{1}{2}+y, \frac{3}{2}-z$; II $1-x, 1-y, 1-z$; III $1-x, -y, 1-z$.

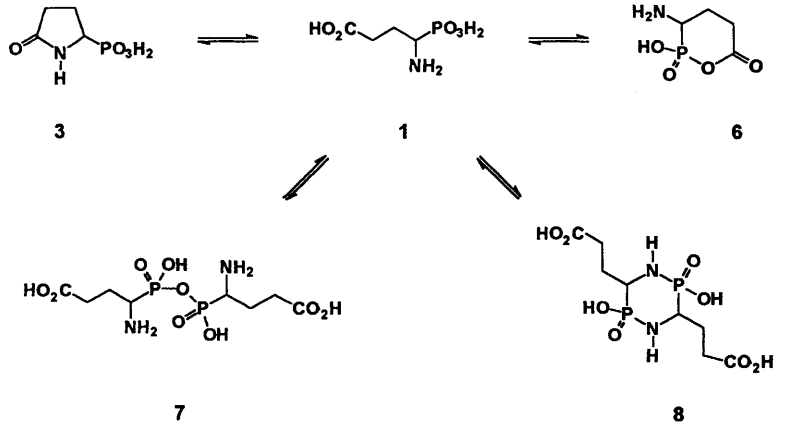


Table 7 Selected torsion angles (°) for compounds **4** and **5**

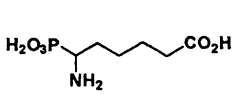
Compound **4**	
O(1)–P–C(2)–N(1)	50.8(1)
O(2)–P–C(2)–N(1)	−61.4(1)
O(3)–P–C(2)–N(1)	174.8(1)
P–C(2)–C(3)–C(4)	−96.7(2)
C(5)–N(1)–C(2)–P	103.7(2)
N(1)–C(2)–C(3)–C(4)	18.4(2)
C(2)–C(3)–C(4)–C(5)	−17.2(2)
C(3)–C(4)–C(5)–N(1)	9.4(2)
C(4)–C(5)–N(1)–C(2)	3.2(2)
C(5)–N(1)–C(2)–C(3)	−13.9(2)
C(6)–C(2)–C(3)–C(4)	137.6(2)
C(2)–N(1)–C(5)–O(4)	−176.1(2)
Compound **5**	
C(11)–P–C(2)–N(1)	−179.9(1)
O(2)–P–C(2)–N(1)	66.9(1)
O(3)–P–C(2)–N(1)	−60.9(1)
P–C(2)–C(3)–C(4)	97.1(1)
C(5)–N(1)–C(2)–P	−107.5(1)
N(1)–C(2)–C(3)–C(4)	−17.6(2)
C(2)–C(3)–C(4)–C(5)	19.9(2)
C(3)–C(4)–C(5)–N(1)	−14.8(2)
C(4)–C(5)–N(1)–C(2)	3.5(2)
C(5)–N(1)–C(2)–C(3)	9.2(2)
C(5)–N(1)–C(2)–C(6)	130.4(2)
C(6)–C(2)–C(3)–C(4)	−136.4(2)
C(2)–N(1)–C(5)–O(4)	−177.3(2)

equilibrium which strongly depends on the pH (see Figs. 3 and 4).

Several compounds may be formed upon reaction of zinc(II) ion with **1** under neutral or slightly basic solutions. The most obvious seems to be the pyroglutamic analogue **3**.[5] This has been ruled out by direct determination of its complex-forming abilities and the complexes differ substantially from that found in the zinc(II)–α-Glu(P) system.

Substantial evidence has lately been provided for a mechanism of intramolecular hydrolysis of phosphonate esters *via* cyclic phosphonic–carboxylic acid anhydrides and imides.[25] The formation of a mixed carboxylic-phosphoric anhydride is also a standard step in many enzymatic reactions,[26] in which an unreactive carboxylic group is activated for nucleophilic displacement. Thus, we speculated that the unknown compound might be such an anhydride (compound **6**) which remains stable as its zinc(II) complex in solution. In order to check this hypothesis NMR studies on the complex-forming abilities of compounds **9** and **10** with zinc(II) were undertaken. In both cases the formation of the new compound (species A) in alkaline solutions was found (Fig. 3). The formation of cyclic anhydrides in the case of compounds **9** and **10** is unlikely. For **9** a seven-membered ring would be formed, while the strain induced by the cyclic structure of **10** would result in chiral differentiation between four possible six-membered cyclic anhydrides. Thus, easy formation of anhydrides from *cis* isomers of the substrate with lack of cyclization upon reaction of the *trans* isomer of **10** were expected. This should result in more than one phosphorus product detectable in NMR spectra but none was observed. Additionally we have considered the possibility of formation of such an anhydride by studying the NMR spectra of zinc(II) complexes with 3-phosphonopropanoic acid **11**, which should form a five-membered cyclic anhydride. The presence of the only one signal in the ^{31}P NMR spectra derived from this substrate in alkaline solution and precipitation at a pH about 5.5–9 clearly indicated the lack of the cyclic anhydride. All the above findings seem to rule out the formation of compound **6**.

Formation of pyrophosphonates has been suggested as a side reaction during the acylation of aminophosphonates[27] and the



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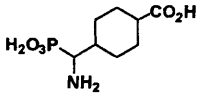

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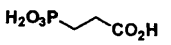

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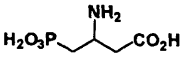

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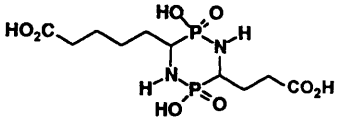

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synthesis of phosphonopeptides containing a phosphonamidate bond.[28] Thus the possibility of the formation of pyrophosphonate **7** should also be taken into consideration. However this is unlikely because no formation of additional product was observed in alkaline solutions of zinc(II) and compound **12**. This compound may be considered as obtained by shifting the amino group of Glu(P) to the β position. Additionally the studies with the ternary system zinc(II)–α-Glu(P) **1** and compound **9** apparently show the lack of the formation of a mixed pyrophosphonate because there is no phosphorus–phosphorus coupling for the new species arising in solution (Fig. 10).

The last possibility is the formation of the cyclic phosphonamidate **8** of structure similar to those of diketopiperazines produced from amino acids. This is strongly supported by the fact that the ^{31}P chemical shift observed for the new compound is characteristic of phosphonamidates.[28,29] Also the studies carried out on the ternary system show the appearance of a new species (Fig. 10) in the solutions which may be attributed to stereoisomers of the cyclic compound **13**.

Acknowledgements

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