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Complexing properties of diastereoisomers of 1-(L-methionylamino)-ethylphosphonic acid†

Ivan Lukeš,* Leoš Bláha, František Kesner, Jan Rohovec and Petr Hermann

Universita Karlova, Department of Inorganic Chemistry, Hlavova 2030, 138 40 Prague 2, Czech Republic

The acid–base and complexing properties of (*S*,*S*) and (*S*,*R*) diastereoisomers of 1-(methionylamino)ethylphosphonic acid were studied pH-metrically (25 °C, 0.1 mol dm^{-3} KNO_3). The protonation and stability constants for complex formation with Co^{2+}, Ni^{2+} and Zn^{2+} indicate co-ordination only *via* the amine, carbonyl peptide and phosphonic groups and, for Cu^{2+}, also *via* nitrogen of the deprotonated peptide amide group. The presence of the same types of complexes as in phosphonodipeptide series which do not contain sulfur in the amino acid side chain was observed. Similarly, the differences found between diastereoisomers correspond to the interaction of the hydrophobic and/or hydrophilic parts of the molecule. Co-ordination *via* sulfur has not been observed. The species determined by 'out of cell' titrations of the platinum(II) and palladium(II) systems depend on the initial metal : phosphonic acid molar ratio. A 1 : 2 ratio indicates the co-ordination of amine and sulfur in a wide pH region; in the alkaline region, sulfur atoms are replaced by the peptide amide group. A 1 : 1 ratio indicates the formation of dimers with co-ordinated phosphonic groups. The pH-metrically determined species were confirmed by ^{31}P-{^1H} and ^1H NMR titrations of the systems involving Pd^{II} and Pt^{II}.

Phosphonopeptides containing N-terminal aminoalkylphosphonic acids have received considerable attention because of their biological activity.[1] Their synthesis and properties have been reviewed by Kafarski *et al.*[2] The acid–base and complexing properties of phosphonodipeptides were investigated for glycylaminomethylphosphonic [Gly-Gly-(*P*)] and 2-glycylaminoethylphosphonic acids with Cu^{2+}, Ni^{2+} and Co^{2+},[3] and for diastereoisomeric mixtures of phosphonodipeptides containing 1-aminoethylphosphonic acid Ala-(*P*), or 1-amino-3-methylbutylphosphonic acid, Leu-(*P*), with Cu^{2+}.[4] We synthesized two series of phosphonodipeptides containing glycine (Gly), L-alanine (Ala), L-phenylalanine (Phe) and L-leucine (Leu) and aminomethylphosphonic or 1-aminoethylphosphonic acids[5] and determined their protonation and stability constants for complex formation with Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}.[6,7] The protonation constants exhibited the same dependence on the amino acid side chain as for common dipeptides but the differences were not as large. The stability constants indicated the formation of protonated, non-protonated and deprotonated complexes with a metal : ligand molar ratio of 1 : 1 and, except for zinc, the formation of 1 : 2 complexes. Simultaneous deprotonation and co-ordination of the peptide amide bond was confirmed only for Cu^{2+}. The same influence on the size of the amino acid side chain as found for common dipeptides was observed; however, the differences are smaller due to the stronger complexing ability of the phosphonic group. In contrast to the common dipeptides, the differences found for diastereoisomers both in protonation and stability constants[7] were much higher, probably due to the stronger interaction between the hydrophobic parts and/or hydrophilic parts of the molecule.

No papers dealing with complexes of phosphonodipeptides with soft metals have been published, except our preliminary results[8] and a study of Gly-Gly-(*P*) which is part of a parallel paper.[9] However, some systems of aminoalkylphosphonic acids with Pd^{II} were investigated by Matczak-Jon and Wojciechowski[10] and also by Glowacki *et al.*[11] using ^{13}C and ^{31}P NMR spectroscopy. Complexes of Pt^{II} with aminoalkylphosphonic acids have been studied because of their antitumor activity[12] and, in addition, the interaction of cisplatin *cis*-[$Pt(NH_3)_2Cl_2$] with some aminophosphonic acids was investigated by Appleton *et al.*[13]

Platinum(II) and paladium(II) complexes have a very high affinity towards sulfur-containing molecules including common amino acids such as cysteine and methionine.[14] Therefore, the interaction of cisplatin and its analogues with sulfur biomolecules was investigated.[15] This interaction is responsible for a variety of effects, such as deactivation of platinum(II) antitumour complexes, cellular resistance to platinum and toxic side effects such as nephrotoxicity.[16] On the other hand, some papers point to the potential use of {S,O} and {S,N}-donor ligands such as diethyl thiocarbamate[17] and methionine[18] capable of forming five-membered metallocycles to induce release of platinum from protein cysteine residues and thus to reduce the nephrotoxicity mentioned above. Recent results have also suggested that platinum complexes with methionine or methionine-containing peptides may play a role in the biological activity of these complexes and could transport platinum to DNA.[19]

The aim of this study is to investigate the effect of a thioether group in the amino acid side chain on the complexing properties of phosphonodipeptides and compare the results with those for common dipeptides. Therefore, we chose (*S*,*S*) and (*S*,*R*) diastereoisomers of 1-(methionylamino)ethylphosphonic acid Met-Ala-(*P*) with the formula $CH_3SCH_2CH_2C^*H(NH_2)$-$CONHC^*H(CH_3)PO_3H_2$. The complexing properties were studied for both hard transition-metal ions used previously[6,7] and soft metals such as Pt^{II} and Pd^{II}. Investigation of the platinum systems could aid in understanding co-ordination competition among amine, sulfur, peptide amine and phosphoric groups in biological materials.

Results and Discussion

Synthesis, mass spectra and thermal stability

The compound Met-Ala-(*P*) was synthesized by a slight modification of the active ester method using *N*-hydroxybenzotriazole and dicyclohexylcarbodiimide.[5] The synthesis and

† Dedicated to Professor Jaroslav Podlaha, who focused our attention on co-ordination chemistry of the organophosphorus ligands, on the occasion of his 60th birthday.

separation of the diastereoisomers was analogous to that described for a previous series of phosphonodipeptides.[5,20] The purity of the diastereoisomers was checked by ^{31}P and ^{1}H NMR spectroscopy and by HPLC chromatography.[21] The protected diethyl ester of Met-Ala-(*P*), $CH_3SCH_2CH_2CH(Me_3C\text{-}OCONH)CONHCHPO_3(OEt)_2$, was characterised by its electron impact (EI) mass spectrum. The important ions are listed in the Experimental section. The mechanism of fragmentation follows the basic principles of the common peptide mass spectral fragmentation and is similar to that for the previous series of phosphonodipeptides.[5]

The DTA measurements of both diastereoisomers showed that the (*S*,*S*) isomer does not contain water of crystallisation; the (*S*,*R*) isomer is a stable monohydrate up to 60 °C and its dehydration is complete at 125 °C. Both isomers are stable up to 200 °C. Therefore, for preparation of stock solutions, the (*S*,*S*) isomer was dried to constant weight at 60 °C and the (*S*,*R*) isomer at 130 °C and they were used as the anhydrous substances.

Potentiometry

The titration procedure for the systems with Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} was discussed in our previous papers.[6,7] The kinetics of reaction of the complexes of Pt^{II} and Pd^{II} is very slow, and equilibrium was achieved in the test systems studied after a prolonged period of time. Examination of the UV spectra for the palladium(II) systems and pH values for platinum(II) systems indicated that the former attained equilibrium after 2–3 h, and the latter after 7 d. In the acidic region equilibrium was usually attained more rapidly, while in the alkaline region it was necessary to wait a longer time. Therefore, it was necessary to use the 'out of cell' method. Determination of the stability constants for complex formation with Pt^{II} and Pd^{II} is also complicated by the known instability of $[Pt(H_2O)_4]^{2+}$ and $[Pd(H_2O)_4]^{2+}$ ions in aqueous solutions.[22] Therefore, we used the chloro-complexes $K_2[PdCl_4]$ and $K_2[PtCl_4]$ instead of the appropriate cations and the stability constants of these complexes were included in the calculation of the stability constants of Met-Ala-(*P*) complexes. Several papers[23–25] deal with the stability constants of the chloro-complexes of Pd^{II} and Pt^{II}. In the light of the ionic strength used, we used the respective stability constants log K_{1-4} 4.94, 4.0, 2.92 and 2.1 according to Elding[23] for the platinum(II) systems and 4.58, 3.55, 2.42 and 1.13 according to Kragten[24] for the palladium(II) systems. The influence of chlorohydroxo complexes is discussed in the preceding paper.[9] As in the platinum(II) system with Gly-(*P*) or Gly-Gly-(*P*), we observed no influence of the stability constants for $[PtCl_{4-n}(OH)_n]^{2-}$, $n = 1$–3, species on chemical models of the systems investigated.

The protonation and stability constants of the systems studied are listed in Tables 1 and 3. Calibration of the glass electrode in a wide pH region from 1.7 to 12 by the method described in the Experimental section permitted determination of the log β values with a standard deviation of ±0.01. This points to the good reproducibility and precision of the measurements under our experimental conditions. However, the real accuracy could be lower and according to ref. 26 we estimate it to be within ±0.05 log unit. The accuracy of the stability constants for formation of the complexes of Pt^{II} and Pd^{II} is lower due to the method used and is also influenced by the stability constants of the chloro-complexes taken from the literature.

As in previous series,[6,7] the log β_{011} value corresponds to protonation of the amine group, log β_{012} to protonation of PO_3^{2-} and log β_{013} to protonation of the protonated phosphonic group PO_3H^-. The protonation sequence was confirmed by ^{31}P-{^{1}H} NMR titration (see below). It is evident from Table 1 that corresponding diastereoisomers exhibit large differences in their protonation constants. These differences are virtually the same as for the diastereoisomers of Leu-Ala-(*P*) or 1-(phenylalanylamino)ethyl phosphonic acid, Phe-Ala-(*P*), and correspond to the interaction of hydrophobic substituents on one side of the molecule and hydrophilic parts on the other, *i.e.* the (*S*,*S*) isomer. The differences in the acid–base properties are also illustrated in Fig. 1, from which it is clear that the abundance of the HA^- form is higher for the (*S*,*S*) than for the (*S*,*R*) isomer. The H_2A form is not as strongly affected by the intramolecular interaction as the HA^- form and the abundance of both H_2A diastereoisomers is virtually the same. Thus, it seems that the interaction between NH_3^+ and PO_3^{2-} or hydrophobic substituents in HA^- is greater than between those polar and/or non-polar groups in H_2A.

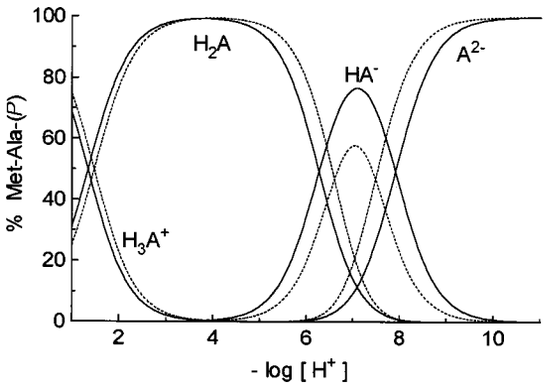

Fig. 1 Distribution diagrams of (*S*,*S*)- (full line) and (*S*,*R*)-Met-Ala-(*P*) (dotted line) as a function of $-\log[H^+]$

The stability constants determined for Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} are listed in Table 1 and differences in the complexing properties of (*S*,*S*)- and (*S*,*R*)-Met-Ala-(*P*) with these transition metals are illustrated in Figs. 2 and 3. In the acid region the protonated complexes $[M(HA)]^+$ are formed with Cu^{2+}, Ni^{2+} and Co^{2+}. The derived constants log $K_{M(HA)}$ and $pK_{M(HA)}{}^{-H}$ for processes (1) and (2) are listed in Table 2. The $pK_{M(HA)}{}^{-H}$ values confirm the protonation of the phosphonic group and the log $K_{M(HA)}$ values reflect the differences in stability between the diastereoisomers without the contribution from the protonation constant of the phosphonic groups. The log $K_{M(HA)}$ values for (*S*,*S*) diastereoisomers are higher and the amount of these species in solution is greater and similar to the values found for the diastereoisomers of Leu-Ala-(*P*) and Phe-Ala-(*P*).[7] Therefore, the same means of co-ordination of the ligand to the metal *via* the amine and carbonyl group and possibly *via* the protonated phosphonic group as in the previous series is assumed.[6,7]

$$M^{2+} + HA^- \longrightarrow [M(HA)]^+; \quad \log K_{M(HA)} = \log \beta_{111} - pK_2 \quad (1)$$

$$[M(HA)]^+ \longrightarrow [MA] + H^+; \quad pK_{M(HA)}{}^{-H} = \log \beta_{111} - \log \beta_{110} \quad (2)$$

All the ions studied form neutral, non-protonated [MA] species that are predominant in Ni^{2+}, Co^{2+} and Zn^{2+} systems. The log β_{110} values also lie in the same region as the values for Leu-Ala-(*P*) and Phe-Ala-(*P*),[7] while the values for (*S*,*S*) isomers are higher than for (*S*,*R*) isomers and exhibit the same dependence on the transition metal. The co-ordination sphere would be formed only by the amine, carbonyl and phosphonic groups as in the previous series. The abundance of the [CuA] complex is low and is obscured by the dominant species $[CuAH_{-1}]^-$, and the log β_{11-1} value is higher for the (*S*,*R*) isomer (see Fig. 2). The log β_{11-1} values of both isomers and the derived constants $pK_1{}^{-1}$ for process (3) are again very similar to the appropriate values

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

Table 1 Protonation constants of the (*S*,*S*) and (*S*,*R*) diastereoisomers of Met-Ala-(*P*) and stability constants β_{pqr} of their complexes at 25 °C and $I = 0.1$ mol dm^{-3}

Ion	*p*	*q*	*r*	(*S*,*S*)-Met-Ala-(*P*)	(*S*,*R*)-Met-Ala-(P)
H^+	0	1	1	7.91	7.48(1)
	0	1	2	14.18(1)	14.08(1)
	0	1	3	15.52(1)	15.55(1)
Cu^{2+}	1	1	1	11.67(2)	11.78(1)
	1	1	0	6.53(1)	6.58(1)
	1	1	−1	1.568(7)	1.933(5)
	1	1	−2	−7.50(4)	−6.67(3)
	1	2	0	11.93(7)	12.18(4)
	1	2	−1	4.63(5)	5.08(3)
Ni^{2+}	1	1	1	10.06(2)	10.01(2)
	1	1	0	3.923(7)	3.685(7)
	1	1	−1	−5.02(4)	−4.81(2)
	1	2	0	7.07(2)	6.19(2)
Co^{2+}	1	1	1	9.88(1)	9.36(4)
	1	1	0	3.152(6)	2.73(1)
	1	1	−1	−5.477(8)	−5.78(4)
	1	2	0	5.49(2)	—
Zn^{2+}	1	1	0	4.16(6)	3.85(1)
	1	2	0	—	6.5(1)

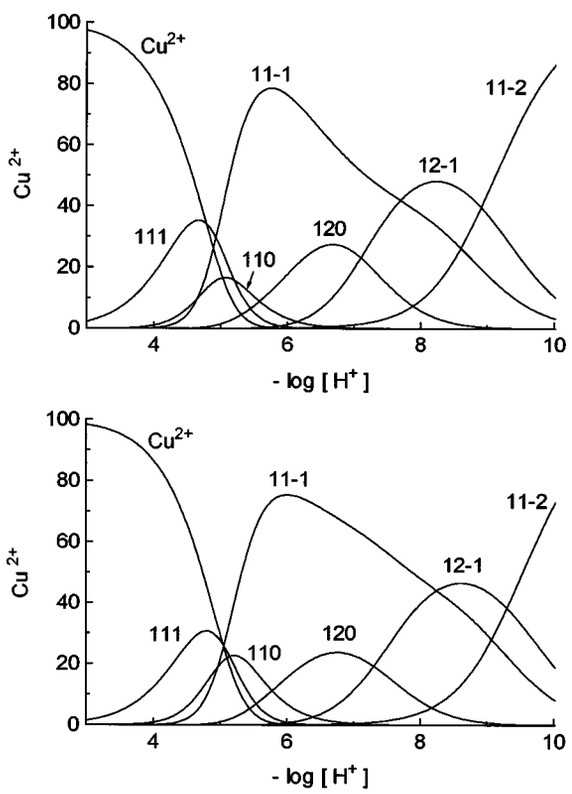

Fig. 2 Distribution diagrams of complexes formed in the Cu^{2+}–(*S*,*R*)-Met-Ala-(*P*) (upper) and –(*S*,*S*)-Met-Ala-(*P*) (lower) systems as a function of $-\log[H^+]$ ($c_{Cu} = 0.0025$, $c_{H_2A} = 0.005$ mol dm^{-3})

for the previous series and, therefore, the same means of co-ordination through amine, peptide amide and phosphonic groups is assumed. The pK_1^{-1} values for the Co^{2+}, Ni^{2+} and Zn^{2+} systems lie in the alkaline region and correspond to the pK_1^{-2} of process (4) for the $[CuAH_{-2}]^{2-}$ species. These deproton-

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

ation reactions correspond, as in the previous series, to deprotonation of a co-ordinated water molecule.

In addition to the 1:1 complexes, the metals form complexes with a 1:2 metal:ligand ratio. The $[MA_2]^{2-}$ species were found for Cu^{2+}, Ni^{2+}, Co^{2+} and in part for Zn^{2+}. The log β_{120} values

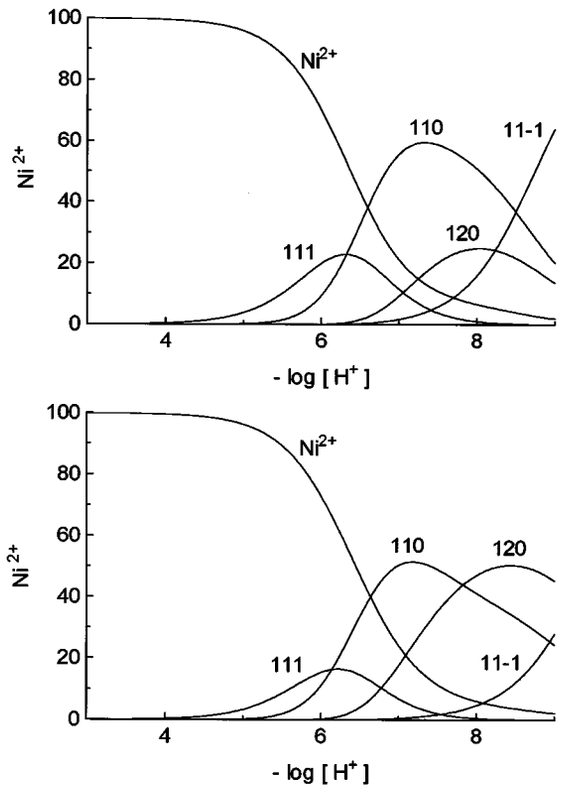

Fig. 3 Distribution diagrams of complexes formed in the Ni^{2+}–(*S*,*R*)-Met-Ala-(*P*) (upper) and –(*S*,*S*)-Met-Ala-(*P*) (lower) systems as a function of $-\log[H^+]$ ($c_{Ni} = 0.0025$, $c_{H_2A} = 0.005$ mol dm^{-3})

and the derived constants log K_2 for process (5) or log(K_1/K_2)

$$[MA] + A^{2-} \longrightarrow [MA_2]^{2-}; \log K_2 = \log \beta_{120} - \log \beta_{110} \quad (5)$$

are similar to those for the previous series. Copper also forms the $[CuA_2H_{-1}]^{3-}$ species. The derived constants pK_2^{-1} and log K_{2A} for processes (6) and (7) are in the same range as for the analogous systems of Phe-Ala-(*P*) and Leu-Ala-(*P*).

$$[MA_2]^{2-} \longrightarrow [MA_2H_{-1}]^{3-} + H^+; \quad pK_2^{-1} = \log \beta_{120} - \log \beta_{12-1} \quad (6)$$

$$[MAH_{-1}]^- + A^{2-} \longrightarrow [MA_2H_{-1}]^{3-}; \quad \log K_{2A} = \log \beta_{12-1} - \log \beta_{11-1} \quad (7)$$

There are several possible ways in which Met-Ala-(*P*) could co-ordinate Ni^{2+}, Co^{2+} and Zn^{2+} in the 1:2 complexes. According to Kiss *et al.*[4] and the results in our previous papers[6,7] we assume co-ordination to the metal *via* the amine, carbonyl and phosphonate groups and to Cu^{2+} only through amine and carbonyl groups. Co-ordination is expected to occur in $[CuA_2H_{-1}]^{3-}$ through two amine groups, one carbonyl group and one peptide amide group.

All the stability constants and the derived constants determined point to the fact that the complexing properties of both diastereoisomers are very similar to those of phosphonodipeptides with a similarly large side chain such as Phe-Ala-(*P*) and Leu-Ala-(*P*).[6,7] Therefore, any interaction of the transition metal with sulfur in the side chain is improbable and the methionyl side chain exhibits only hydrophobic interactions. The results correspond to those for common dipeptides containing methionine. Except in a low-temperature investigation,[27] the sulfur atom is not co-ordinated in analogous complexes.[28] Only Sóvágó and Petocz[29] considered a weak axial interaction with the central metal ion.

The complexing properties of (*S*,*S*)- and (*S*,*R*)-Met-Ala-(*P*) for Pt^{II} and Pd^{II} are completely different. In the Experimental section the procedure for titrations of solutions with metal to phosphonic acid molar ratios of 1:1 and 1:2 is mentioned.

Table 2 Derived constants for Cu^{2+}, Ni^{2+} and Co^{2+} systems with the (*S*,*S*) and (*S*,*R*) diastereoisomers of Met-Ala-(*P*)

Ion	Isomer	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+}	*S*,*S*	5.41	5.14	4.96	9.07	5.40	1.13	7.30	3.06
	S,*R*	5.17	5.20	4.65	8.60	5.60	0.98	7.10	3.15
Ni^{2+}	*S*,*S*	3.80	6.14	8.94		3.15	0.77		
	S,*R*	3.40	6.32	8.50		2.50	1.19		
Co^{2+}	*S*,*S*	3.62	6.73	8.63		2.34	0.81		
	S,*R*	2.75	6.63	8.51		—	—		

Table 3 Stability constants β_{pqrs} of the (*S*,*S*) and (*S*,*R*) diastereoisomers of Met-Ala-(*P*) with Pt^{II} and Pd^{II} at 25 °C and $I = 0.1$ mol dm^{-3}

					$M^{2+}:A^{2-}$			
					(*S*,*R*)-Met-Ala-(*P*)		(*S*,*S*)-Met-Ala-(*P*)	
Ion	*p*	*q*	*r*	*s*	1:1	1:2	1:1	1:2
Pd^{II}	1	1	1	2	24.14(2)	—	24.01(3)	—
	1	1	0	1	17.67(5)	—	17.87(4)	—
	1	1	−1	0	11.20(3)	—	10.76(4)	—
	1	1	−2	0	0.45(4)	—	−0.01(5)	—
	1	2	2	0	—	34.99(2)	—	34.96(2)
	1	2	1	0	—	28.68(3)	—	28.74(2)
	1	2	0	0	—	21.48(3)	—	21.60(2)
	1	2	−1	0	—	12.66(4)	—	12.51(4)
	1	2	−2	0	—	2.45(4)	—	2.48(4)
Pt^{II}	1	1	1	2	25.72(4)	—	25.72(5)	—
	1	1	0	1	19.01(5)	—	18.81(7)	—
	1	1	−1	0	10.19(6)	—	9.79(8)	—
	1	1	−2	0	1.88(7)	—	1.41(8)	—
	1	2	2	0	—	36.51(2)	—	36.68(4)
	1	2	1	0	—	30.13(3)	—	30.47(4)
	1	2	0	0	—	22.89(3)	—	23.14(4)
	1	2	−1	0	—	14.29(3)	—	14.58(5)
	1	2	−2	0	—	4.62(4)	—	4.78(6)

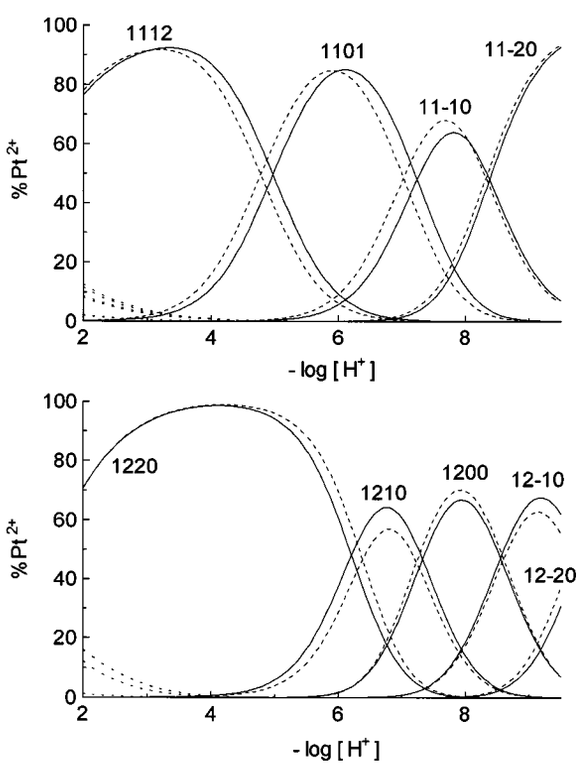

Fig. 4 Distribution diagrams of the complexes formed in Pt^{2+}–(*S*,*S*)-(full line) and –(*S*,*R*)-Met-Ala-(*P*) (dashed line) as a function of $-\log[H^+]$ [$c_{Pt} = 0.005$, $c_{H_2A} = 0.005$ (upper), 0.01 mol dm^{-3} (lower)]. Dotted lines (low abundance, region pH 2–4) correspond to $[PtCl_{4-n}(H_2O)_n]^{n-2}$ species.

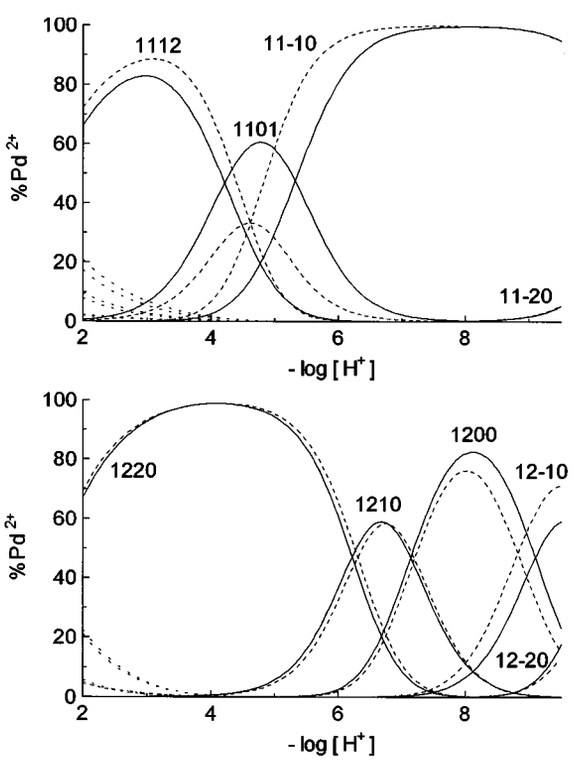

Fig. 5 Distribution diagrams of the complexes formed in Pd^{2+}–(*S*,*S*)-(full line) and –(*S*,*R*)-Met-Ala-(*P*) (dashed line) as a function of $-\log[H^+]$ [$c_{Pd} = 0.005$, $c_{H_2A} = 0.005$ (upper), 0.01 mol dm^{-3} (lower)]. Dotted lines (low abundance, region pH 2–4) correspond to $[PdCl_{4-n}(H_2O)_n]^{n-2}$ species.

However, when the titration data of both ratios were calculated together the $[M(HA)Cl_2]^-$ (1112) and $[M(HA)_2]$ (1220) species were correlated and the distribution diagrams found did not correspond to the abundance of the species observed in the ^{31}P NMR spectra, in contrast to the other species (see below). For this reason the titrations at 1:1 and 1:2 molar ratios were cal-

Table 4 Derived constants for complexes of the (*S*,*R*) and (*S*,*R*) diastereoisomers of Met-Ala-(*P*) with Pd^{II} and Pt^{II}

Ion	Isomer	$pK_{M(HA)}$*	pK_1^{-1}	pK_1^{-2}	$pK_{M(HA)_2}$	$pK_{MA(HA)}$	pK_2^{-1}	pK_2^{-2}
Pd^{II}	*S*,*S*	6.14	7.11	10.77	6.22	7.14	9.09	10.03
	S,*R*	6.47	6.47	10.75	6.31	7.20	8.22	10.21
Pt^{II}	*S*,*S*	6.91	9.02	8.38	6.21	7.33	8.56	9.80
	S,*R*	6.71	8.82	8.31	6.38	7.24	8.60	9.67

* Contribution of substitution of Cl^- by H_2O is not included.

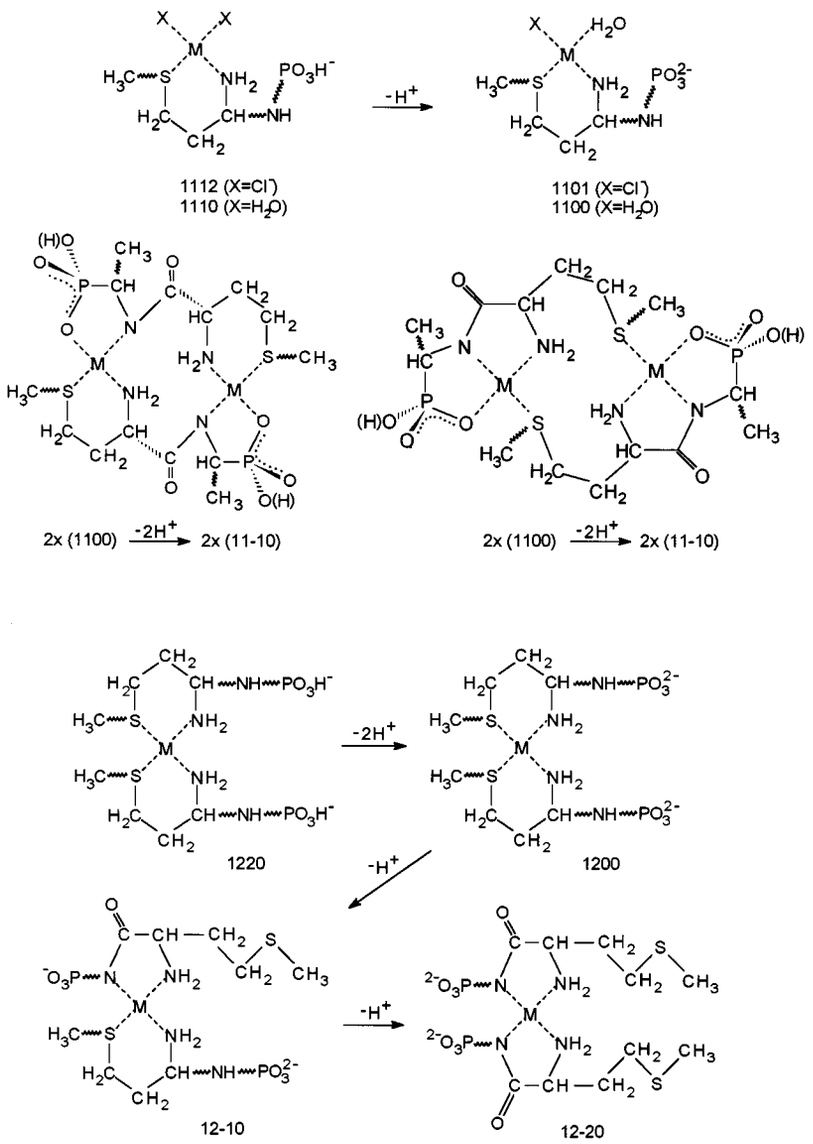

Fig. 6 Tentative structures of the species found in the platinum(II) and palladium(II) systems. Only *cis* isomers are shown; the probability of the *trans* isomer is the same.

culated separately and the results are listed in Table 3 and the distribution diagrams are shown in Figs. 4 and 5. From Table 3 it can be seen that the 1:1 species were found only in the solutions containing metal and phosphonic acid in a molar ratio of 1:1, and the 1:2 species were found in the solutions with a molar ratio of 1:2. Selected derived constants are listed in Table 4. For the 1:1 titration of both metals the formation of the protonated complexes $[M(HA)Cl_2]^-$ (1112) in the strongly acidic region was observed. Co-ordination *via* sulfur, amine and two chlorides is assumed, as depicted in Fig. 6. The phosphonic group is free and protonated. Deprotonation in the neutral region leads to the formation of the 1101 species $[MA(Cl)]^-$. The values of $\log \beta_{1112} - \log \beta_{1101}$ [analogous to process (2)] for all the systems correspond to the pK_2 of the free diastereoisomers of Met-Ala-(*P*), the δ_P changes are the same as for free Met-Ala-(*P*) (see below) and they thus confirm the deprotonation of the phosphonic group in this step. The values are not significantly influenced by substitution of chloride by water in the co-ordination sphere. Consequently, the next deprotonation steps yield the 11–10 species which dominates in the palladium(II) systems and, in the alkaline region, the 11–20 species. The pK_1^{-1} values [process (3)] correspond to the deprotonation and co-ordination of the peptideamide group. These values for the palladium(II) systems are very close to the $pK_{M(AH)}^{-H}$ value, similar to values found for copper(II) systems,[6,7] and point to the fact that, in both such systems, the deprotonations of the amide and phosphonic groups partially overlap. In the next step the pK_1^{-2} values [process (4)] correspond to substitution of the thioether or phosphonic groups by hydroxide. Compared to Pd^{II}, in systems containing Pt^{II} this

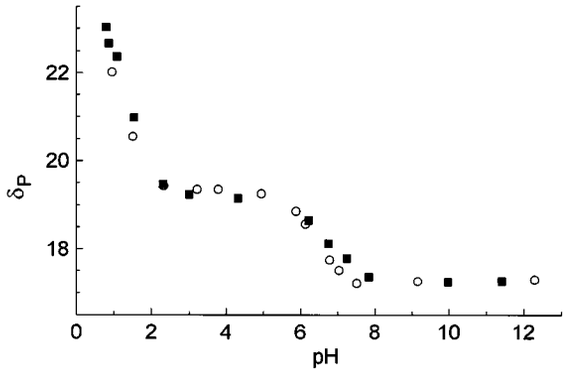

Fig. 7 Plot of δ_P *vs.* $-\log[H^+]$ for the phosphonic group of (*S*,*S*)- (○) and (*S*,*R*)-Met-Ala-(*P*) (■)

substitution occurs at lower pH and is partly connected with deprotonation of the peptide amide group.

The 1:2 titrations of both metals point to the formation of the predominant protonated species 1220 $[M(HA)_2]$ in the acidic region and co-ordination *via* the sulfur and amine groups is assumed (see Fig. 6). In the next steps the formation of monoprotonated species 1210 and deprotonated 1200 species correspond to the deprotonation of the phosphonic groups. The $pK_{M(HA)_2}^{-H}$ and $pK_{MA(HA)}^{-H}$ for processes (8) and (9)

$$[M(HA)_2] \longrightarrow [MA(HA)]^- + H^+; \quad pK_{M(HA)_2}^{-H} = \log \beta_{1220} - \log \beta_{1210} \quad (8)$$

$$[MA(HA)]^- \longrightarrow [MA_2]^{2-} + H^+; \quad pK_{MA(HA)}^{-H} = \log \beta_{1210} - \log \beta_{1200} \quad (9)$$

indicate that the second deprotonations occur at higher pH values than in free Met-Ala-(*P*). Formation of the 12–10 and 12–20 species should be connected with substitution of a sulfur by a simultaneously deprotonated peptide amide group.

In contrast to systems with hard transition-metal ions, differences in the complexing properties of the diastereoisomers are negligible, except for the Pd^{II}–Met-Ala-(*P*) system. The pK_1^{-1}, pK_2^{-1} and pK_2^{-2} values, which are connected with deprotonation of the peptide amide group, indicate that deprotonation occurs in a higher region than for Gly-Gly-(*P*)[9] due to competition with the thioether group.[14]

NMR spectroscopy

The potentiometrically determined dissociation constants of both diastereoisomers correspond to the δ_P *vs.* pH variation (Fig. 7). The upfield shifts of δ_P in the regions pH 0.5–2 and 5–7 are consistent with the dissociation constants of the phosphonic group. The same shape of the δ_P *vs.* pD plot was observed for deprotonation of a phosphorus group in aminoalkyl-phosphonic[13,30] and -phosphinic[31] acids. Deprotonation of the nitrogen atom in aminoalkyl-phosphonic and -phosphinic acids is usually connected with a downfield δ_P shift due to interaction between the amine and phosphoric groups in the molecule.[13,30,31] However, this chemical shift has not been observed for Met-Ala-(*P*) and thus the distance between the amine and phosphonic groups in the dipeptide is too long for any interaction between these groups.

To confirm the species found by potentiometry, ^{31}P-{^1H} and ^1H NMR spectra were measured for the palladium(II) equilibrated system as a dependence on pD. The concentration of the samples was approximately 15 times higher than for potentiometry. Nevertheless, we can compare the two methods because the differences in the stability constants observed for the platinum(II) system with Gly-(*P*) as a dependence on the concentration in this concentration region were small.[9] The ^{31}P-{^1H} NMR spectra are depicted in Fig. 8 for metal:phosphonic acid molar ratios of 1:1 and 1:2. The low-intensity peaks in the

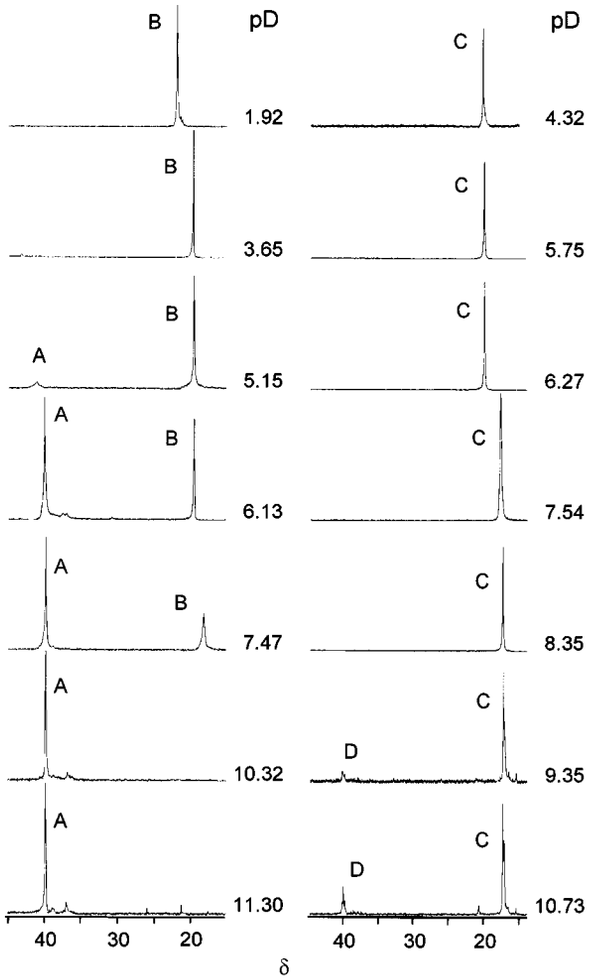

Fig. 8 The ^{31}P-{^1H} NMR spectra of Pd^{II}–Met-Ala-(*P*) as a function of pD: left, (*S*,*R*)-Met-Ala-(*P*), M:H_2A = 1:1 (c_{Pd} = 0.15, c_{H_2A} = 0.15 mol dm^{-3}); right, (*S*,*S*)-Met-Ala-(*P*), M:H_2A = 1:2 (c_{Pd} = 0.075, c_{H_2A} = 0.15 mol dm^{-3}). Peaks A belong to dimers 2200 (2 × (1100)] and their deprotonation and formation of 22–20 [2 × (11–10)], B to species 1112 and 1102 after deprotonation of the phosphonic group, C to deprotonation of the phosphonic group in 1220 and formation of 1201 and 1200 and consequently deprotonation of the peptide amide group and formation of 12–10 and 12–20 and D probably to a complex of Pd^{II} with Ala-(*P*) after hydrolysis of the peptide bond

spectra at the 1:2 ratio above pD 9.5 are caused by partial decomposition of Met-Ala-(*P*). The decomposition was connected with a change of colour and unpleasant smell of the samples and was not observed in the diluted solution used for potentiometry. The spectra contain two regions, *i.e.* around δ 20 corresponding to the non-co-ordinated phosphonic group and around δ 40 which, according to refs. 9 and 13, corresponds to the formation of a five-membered ring with the co-ordinated phosphonic group.

The ^{31}P-{^1H} NMR spectra at 1:2 molar ratio are very simple with only one peak C (Fig. 8) that corresponds to an unco-ordinated phosphonic group. As for methionine and its dipeptides with common amino acids,[32] we assume co-ordination only *via* sulfur and amine in the acidic region, and the 1220, 1210, 1200, 12–10 and 12–20 species (Fig. 6) found by potentiometry correspond to deprotonation of both phosphonic groups and consequently of both peptide amide groups which replace the sulfurs in the co-ordination sphere. The change of δ_P from δ 19.6 at pD 6.27 to δ 18.1 at pD 7.47 (Fig. 8) is virtually the same as for the free dipeptide and thus confirm the deprotonation of the phosphonic groups and formation of the 1210 and 1200 species, *i.e.* $[MA(HA)]^-$ and $[MA_2]^{2-}$. No great changes in the shift in the ^{31}P-{^1H} NMR spectrum that would correspond to the replacement of the thioether group by the peptide amide moiety were observed. The peak D above pD

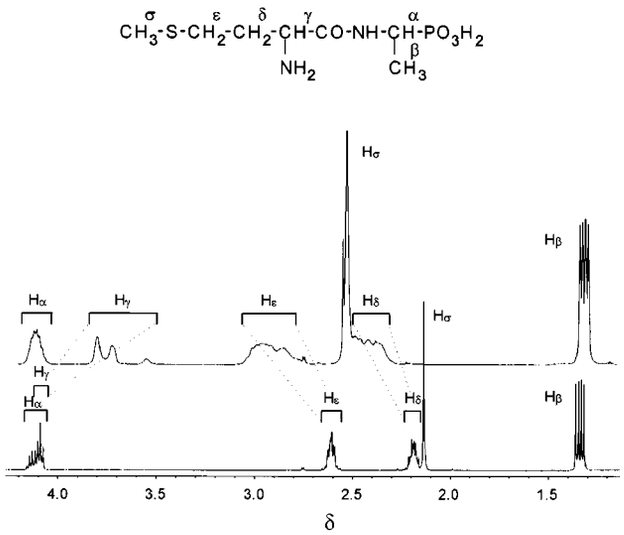

Fig. 9 The ^{1}H NMR spectra of free (*S*,*R*)-Met-Ala-(*P*) (lower) and a mixture of its complexes with Pd^{II} at pD 4.32 ($c_{Pd} = 0.075$, $c_{H_2A} = 0.15$ mol dm^{-3})

9 corresponds to co-ordination of Ala-(P) after decomposition of the peptides mentioned above. The structure proposed and a deprotonation scheme are given in Fig. 6.

The ^{1}H NMR spectra of free Met-Ala-(*P*) and of Met-Ala-(*P*) with Pd^{II} at a metal : phosphonic acid molar ratio of 1 : 2 at pD 4.32 are shown in Fig. 9. The spectrum with Pd^{II} is rather complicated like the other spectra of the system or at a molar ratio of 1 : 1. This is probably caused by the formation of a mixture of isomers due to sulfur co-ordination and/or different conformations of the ligand and/or *cis*,*trans* isomers. Nevertheless, the changes in the chemical shifts of the hydrogen atoms on the γ-, δ-, ε- and σ-carbon atoms confirm the co-ordination *via* the thioether and amine groups. In the alkaline region the ^{1}H NMR spectra at a molar ratio of 1 : 2 are even less discernible; however, the shifts of ^{1}H on the α and β atoms as well as the shifts of the thioether part of the molecule correspond to the amine–peptideamide co-ordination assumed from the potentiometry and from the ^{31}P-{^{1}H} NMR shifts.

The ^{31}P-{^{1}H} NMR spectra at the 1 : 1 molar ratio in the acidic region point to the formation of species with an uncoordinated phosphonic group (peak B, Fig. 8). The ligand is bonded to the metal *via* the thioether and amine groups and together with chloride ions as is assumed from potentiometric results for the 1112 species $[M(HA)Cl_2]^-$. Comparison of the δ_P *vs.* pD plot for free (*S*,*R*)-Met-Ala-(*P*) with those of the 1 : 1 systems indicates that the shift from δ 20 at pD 1.92 to δ 18 at pD 3.65 corresponds to deprotonation of the phosphonic group. The shift at about δ 40 (peak A, Fig. 8) in neutral and alkaline solutions corresponds to the co-ordination of phosphonic and peptide amide groups and the formation of five-membered rings. Therefore, species 11–10 exhibits co-ordination *via* the peptide amide and phosphonic groups and also *via* the thioether and amine groups because the formation of hydroxo complexes was not observed. (Hydroxo-complex formation should be observed potentiometrically in the next deprotonation step.) However, this means of co-ordination is impossible because of the geometry of Met-Ala-(*P*) if we assume only one molecule of the ligand is bonded to one metal atom. Thus, the formation of dimers is proposed similarly to analogous systems with methionylglycine, Met-Gly,[32] and the structure of the 11–10 (or better 22–20) species is as shown in Fig. 6. The 1101 $[MA(Cl)]^-$ species was found potentiometrically as a transient between the 1112 and 11–10 (22–20) species. This stoichiometry could correspond to several different structures from 1112 after deprotonation of the phosphonic group and substitution of a chloride by a water molecule, maybe through dimers bridged by chlorides to the dimer of 11-10 (22-20) with the protonated phosphonic group. In the ^{31}P-{^{1}H} NMR spectra only two peaks correspond to the monomeric species and the dimer and thus different structural motifs are excluded. Therefore, we assume that structure of the dimer corresponds to two motifs as shown in Fig. 6. Notably, changes in the ^{31}P-{^{1}H} shift between pD 6.13 and 7.47 (Fig. 5) correspond to deprotonation of the phosphonic group and from this point of view the dimer structure with bonded peptide amide and phosphonic moieties and also with a protonated phosphonic group seems to be the most probable. The formation of the dimer could not be determined potentiometrically because, as was mentioned above, the data at both molar ratios could not be calculated together.

So far there have been no reports dealing with the systems involving Pt^{II} or Pd^{II} with methionylalanine, Met-Ala. However, results found for systems with other dipeptides containing methionine[32,33] again point to similarity of the complexing properties. This similarity between Met-Ala-(*P*) and Met-aa (aa = amino acid) is high due to the same co-ordination sphere formed by the Met part of the dipeptide. If we compare the structural motif of cisplatin and its relatives with the motifs found for Pt^{II} in Met-Ala-(*P*) complexes, we can see the similarity of cisplatin and $MACl_2$ having co-ordinated sulfur, amine and two chlorides. The phosphonic chain of this species may assist in passage through the cell membrane, and especially, the phosphonic group would make it possible to anchor the species on the surface of bones or other biological material. From this point of view, further study would be useful.

Conclusion

A comparison of the acid–base and complexing properties of Met-Ala-(*P*) with those of phosphonodipeptides which do not contain sulfur in the amino acid side chain for Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} (refs. 6 and 7) points to the formation of the same species and the stability constant values determined are virtually the same as for systems with Leu-Ala-(*P*) and Phe-Ala-(*P*). Similarly, the differences found between the diastereoisomers correspond to the interaction of the hydrophobic parts and/or hydrophilic parts of the molecule. Co-ordination *via* sulfur has not been observed. On the other hand, the thioether group exhibits a very high affinity for Pt^{II} and Pd^{II} and species with co-ordinated sulfur and amine are predominant in the acidic and neutral pH regions. Replacement of the thioether by the peptideamide group was observed only in the alkaline region and for formation of complexes at a metal : ligand molar ratio of 1 : 2. Co-ordination of the phosphonic group was found only in dimers in systems with a molar ratio of 1 : 1.

Experimental

Chemicals and synthesis

The compounds Me_3COCO-L-Met and *N*-hydroxybenztriazole were obtained from Léčiva (Prague). Solvents were dried by the standard procedure. The compound Ph_3CNH-CH(Me)$PO(OEt)_2$ was prepared by the usual procedure.[5] Thin-layer chromatography (TLC) was run on Silufol silica gel sheets (Kavalier) in the following solvent mixtures: A, $CHCl_3$–ethyl acetate (1 : 1); B, $CHCl_3$–ethyl acetate (1 : 3); C, ethyl acetate; D, ethyl acetate–NEt_3 (10 : 1); and E, Pr^iOH–concentrated NH_3–water (7 : 3 : 3); ninhydrin detection was used.

(*S*,*S*)- and (*S*,*R*)-Met-Ala-(*P*). The compound Ph_3CNH-CH(Me)$PO(OEt)_2$ (10.59 g, 0.025 mol) was dissolved in a hot solution of 1 mol dm^{-3} HCl in dry ethanol (100 cm^3) and refluxed for 15 min under dry nitrogen. The solution was quickly cooled and the solvent removed under vacuum. The residue was triply distilled with dry 1,4-dioxane (30 cm^3) to remove the excess of HCl. Trityl chloride was extracted three

times with dry diethyl ether (30 cm^3). Oily diethyl aminoethylphosphonate hydrochloride was dissolved in dry tetrahydrofuran (thf) (60 cm^3) and neutralised with dry NEt_3 (1.88 cm^3, 0.026 mol). The solution of the free ester was cooled and filtered into the cooled solution of the active ester Me_3COCO-L-Met (6.23 g, 0.025 mol), *N*-hydroxybenzotriazole (4.1 g, 0.03 mol) and dicyclohexylcarbodiimide (6.20 g, 0.03 mol) in dry thf (70 cm^3); activation for 40 min at −5 °C. The mixture was stirred for 2 h at −5 °C and 20 h at room temperature. The solution of the crude protected peptide was filtered, the filtrate was evaporated and chromatographed on a silica gel column using gradient elution with solvent mixtures A, B and C. The fractions containing $PhCH_2OCO$-Met-$NHCH(Me)PO_3Et_2$ were combined, evaporated and chromatography on silica gel was carried out with mixture D. After removing solvents, the pure protected dipeptide was crystallised from the oil (m.p. 63–65 °C) and characterised by low-resolution EI mass spectrometry: *m/z* 412 (18, M^+), 338 (36), 282 (67), 104 (34), 100 (68), 61 (40), 57 (74), 56 (47) and 44 (100%). The product was dissolved in 30% HBr–acetic acid (25 cm^3) and stirred overnight at room temperature. The solvent was removed under vacuum and the residue triply distilled with dry 1,4-dioxane (20 cm^3) to remove HBr. Then it was dissolved in water, decolourised by charcoal and loaded on Dowex 50 (H^+). The resin was eluted with water to remove acids and the free peptide was eluted with 8% NH_3. A white crystalline solid (4.42 g, 69%) was obtained after removing ammonia and crystallisation from water–acetone. The diastereoisomeric mixture was separated on Dowex 50 (H^+) by the usual procedure.[20] Pure isomers were crystallized from water–acetone. (*S*)-Met-(*R*)-Ala-(*P*)·H_2O: m.p. 228–230 °C (lit.,[34] 245–247 °C); $[\alpha]_D^{20} = -5.8$ (*c* = 1, water) (lit.[34] −7.1) [Found (Calc.): C, 30.9 (30.8); H, 10.1 (10.2); N, 6.72 (6.96)%]; ^{1}H NMR (D_2O, 500 MHz) δ 1.33 (dd, 3 H, C*H*$_3$CHP, $^3J_{HH} = 7.3$, $^3J_{HP} = 15.0$), 2.14 (s, 3 H, SCH_3), 2.15–2.28 (m, 2 H, CH_2CH_2CH), 2.62–2.77 (m, 2 H, SCH_2CH_2), 4.11 (t, 1 H, C*H*CH_2, $^3J_{HH} = 6.7$) and 4.08 (dq, 1 H, PC*H*CH_3, $^3J_{HH} = 7.3$, $^2J_{HP} = 14.6$ Hz). (*S*)-Met-(*S*)-Ala-(*P*): m.p. 277–279 °C; $[\alpha]_D^{20} = 69.9$ (*c* = 1, water) [Found (Calc.): C, 33.1 (32.8); H, 10.9 (10.9); N, 6.71 (6.69)%]; ^{1}H NMR (D_2O, 500 Mz) δ 1.33 (dd, 3 H, C*H*$_3$CHP, $^3J_{HH} = 7.3$, $^3J_{HP} = 15.1$), 2.14 (s, 3 H, SCH_3), 2.14–2.24 (m, 2 H, CH_2CH_2CH), 2.57–2.66 (m, 2 H, SCH_2CH_2), 4.09 (t, 1 H, C*H*CH_2, $^3J_{HH} = 6.7$) and 4.12 (dq, 1 H, PC*H*CH_3, $^3J_{HH} = 7.3$, $^2J_{HP} = 14.6$ Hz).

Potentiometric titrations

The stock solutions of Pt^{II} and Pd^{II} were prepared from $K_2[PtCl_4]$ and $K_2[PdCl_4]$; those of Cu^{II}, Ni^{II}, Co^{II} and Zn^{II} were acidified solutions (pH ≈ 2) of the corresponding nitrates recrystallised from aqueous solutions. Nitric acid was prepared by passing potassium nitrate through a Dowex 50W column in the H^+ form because of traces of NO and NO_2 in the concentrated acid. The contents of Pd and Pt in the solutions were determined gravimetrically after reduction with sodium formate to the metals; the contents of the other metals were determined by titration with ethylenedinitrilotetraacetate solution and excess of nitric acid was determined by pH-metric acid–base titration.

Potentiometric measurements of the Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} systems were carried out using a PHM 84 pH-meter, ABU 80 automatic burette and a GK 2401 B combined electrode (Radiometer) in a glass vessel (10 cm^3) thermostatted at 25 ± 0.1 °C at an ionic strength of I (KNO_3) = 0.1 mol dm^{-3}. An inert atmosphere was ensured by constant passage of argon saturated with the vapour of the solvent. The initial solution volume was 5 cm^3 and the concentration of Met-Ala-(*P*) was 0.005 mol dm^{-3} for determination of the protonation constants and of the stability constants. The metal : phosphonic acid ratio was 1 : 1, 1 : 2 and 1 : 5 and titrations were carried out in the region pH 3–10 or till the formation of a precipitate. Potentiometric measurements of the free peptides were carried out in the range pH 1.7–12. The total number of data points was more than 200. Calibration was carried out by titration of 0.05 mol dm^{-3} HNO_3 with 0.17 mol dm^{-3} KOH in 0.1 mol dm^{-3} KNO_3 in the region pH 1.8–12.0, with the pH-meter yielding *E* values.

The protonation and stability constants β_{pqr} are concentration constants defined by $[M_pH_qA_r]/[M]^p[H]^q[A]^r$. The constants were refined by our program[35] which minimises the criterion of the generalised least-squares method. The program includes the calibration function $E = E_\circ - S(-\log [H^+]) + j_a[H^+] + j_b(K_w/[H^+])$ where the additive term $E_\circ$ contains the standard potentials of the electrodes used and contributions of inert ions to the liquid-junction potential, *S* corresponds to the Nernstian slope, the value of which should be close to the theoretical value, and $j_a[H^+]$ and $j_b[OH^-]$ are the contributions of the H^+ and OH^- ions to the liquid-junction potential. It is clear that j_a and j_b cause deviation from a linear dependence between *E* and $-\log [H^+]$ only in strong acid or strong base. The procedure was tested by the 'glycine test'.[36]

Titration of the palladium(II) and platinum(II) systems was carried out in the range pH 2–9.5 by the 'out of cell' method described in preceding paper.[9] The initial volume was 1 cm^3 and the concentration of the metal was 0.005 mol dm^{-3}. The metal : phosphonic acid molar ratios were 1 : 1 and 1 : 2 and each titration was carried out at least twice. The total number of data points was over 100 for each ratio. The temperature, ionic strength and means of calibration were as described above. Attainment of equilibrium in palladium(II) systems was checked by UV/VIS spectroscopy. Changes in the spectra were observed in the acidic region over 60 min and in the alkaline region over 180 min. Therefore, titrations of the palladium(II) systems were carried out after 3 d when we were sure that they had reached equilibrium. The platinum(II) systems was checked potentiometrically and some changes in the pH values were even observed after 6 d. Titrations in these systems were usually carried out after 10–12 d. The stability constants β_{pqrs} are concentration constants defined as $[M_pA_qH_rCl_s]/[M]^p[A]^q[H]^r[Cl]^s$.

NMR spectra

The ^{1}H NMR spectra used for characterisation of both diastereoisomers were measured using a Varian Unity-500 instrument at 500 MHz and spectra for the titration were run on a Varian XL-200 instrument (200 MHz), both at 25 °C and with sodium 4,4-dimethyl-4-silapentanesulfonate as the internal standard. The ^{31}P-{^{1}H} NMR titration measurements were carried out using a Varian XL-200 instrument at 81 MHz and with 85% H_3PO_4 as the external standard. The concentration of Met-Ala-(*P*) was 0.15 mol dm^{-3} in D_2O. The solutions were prepared by dissolving the dipeptide in D_2O, neutralising with KOD to the pD values estimated and adding the appropriate amount of solid $K_2[PdCl_4]$. The acid–base titration of both diastereoisomers was made in water.

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