Nuclear Magnetic Resonance Study of Ln^{3+} Complexes with Aspartate and Glutamate Residues. Thermodynamic and Structural Analysis

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Binding of aspartate and glutamate residues with the lanthanide cation Yb^{3+} has been studied in D_2O, involving two kinds of peptide backbones: *N*-acetylamino acid methyl esters and cyclic dipeptides. A method is described for the quantitative analysis of the lanthanide-induced shifts (LIS) from which the best model of solution, the binding constants, the induced shifts, and the geometry of the complexes have been determined. Aspartate and glutamate derivatives are found to form both 1:1 and 2:1 complexes (peptide:cation). Backbone flexibility and, to a lesser extent, side-chain length seem to govern the magnitude of the binding constants. Only one oxygen of the carboxylate group is involved in the complex. The $Yb^{3+} \cdots O^-$ distances are found to be different according to the Asp (2.70—2.75 Å) and Glu (2.50—2.55 Å) residues. The predominant conformer has an extended side-chain so no chelation occurs with the peptide backbone.

The complexation of cations by amino acid residues is an important biochemical process, as this interaction can be involved in the transport of cations or in enzymatic activations or inhibitions.

The aim of this study was to define the influence of structural factors on the binding strength of the residues and also to determine the geometry of the complexes in an aqueous medium.

We first focused our attention on the intrinsic complexation of a single residue, aspartate or glutamate, in a dipeptide backbone, without interaction of any other residue or peptide end. Since various types of peptide flexibilities can be found, two different kinds of peptides were selected: (i) rigid diketopiperazines [cyclic dipeptide (**1**), Gly–Asp or Gly–Glu]; and (ii) *N*-acetylamino acid methyl esters (**2**) (where more rotational freedom occurs).

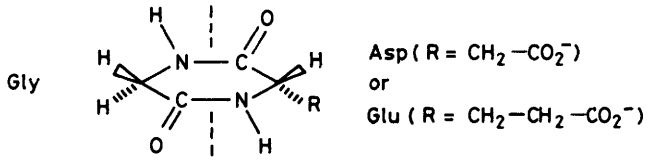
^1H and ^{13}C nuclear magnetic resonance lanthanide-induced shift (LIS) measurements seemed a suitable technique for this investigation.[1-3] As regards biological activity, the possibilities and limits of substituting Ln^{3+} for Ca^{2+} has already been discussed extensively.[3]

Furthermore, the induced shifts are large enough to allow an accurate determination of the binding constants. The Yb^{3+} ion was chosen as a probe because the shifts it induces often have no substantial contact interaction and consequently are of use in the structural elucidation of the complexes.[1,2,4,5] Pr^{3+}, for which the effective axial symmetry of the magnetic susceptibility tensor seems best demonstrated,[6,7] was not convenient because of difficult experimental conditions: its LIS are at low field in water and many ^1H signals overlap with the HDO or other signals, so that detailed thermodynamic analysis becomes very difficult.

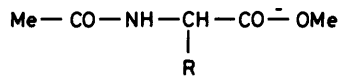
The first part of this paper deals with the quantitative analysis of LIS in order to derive the stoicheiometry of the complexes and their binding constants, and to discuss the results with respect to the backbone and side-chain flexibilities. The structural investigation, carried out from the calculated LIS in the complexes, is reported in the second part.

Experimental

Perchlorate salts of the lanthanides were either of commercial origin (Alfa-Ventron) or prepared by addition of a solution of $Ln_2(SO_4)_3 \cdot 8H_2O$ in D_2O to a solution of $Ba(ClO_4)_2$. The concentrations of the salt solutions were determined through the usual EDTA titration procedures.[8]



(1)



(2)

Peptides were purchased from Interchim and Bachem, except cyclo(Gly–Asp) and cyclo(Gly–Glu), which were synthesized according to the literature.[9,10]

Two series of peptide solutions were prepared, one 0.1M (for the ^{13}C and ^1H spectra) and the other 0.01M (for the ^1H spectra only). It is well known that analysis of data from different concentration ranges gives an improvement in the thermodynamic results. The concentration of the salt was varied up to 0.1M. The precipitation of the lanthanide hydroxides occurs at *ca.* pH 7,[11] but the products of hydrolysis can be formed before.[12] Thus a relatively low pH was selected, but one that was allowed to have $[CO_2^-]/[CO_2H] = 4$ (pH = 4.6 for aspartate derivatives and 5.1 for the glutamates). These pH readings were actual meter readings, *i.e.*, uncorrected for deuterium isotopic effects (pH = pD + 0.4).[13]

N.m.r. spectra were recorded at 27 ± 2 °C on Bruker WH 90 and Varian CFT 20 spectrometers. Most of the peak assignments were carried out according to literature data.[14] There were still a few problems, which were solved in the following way: ^1H signals of $CH_{2\beta}$ and $CH_{2\gamma}$ in Glu residues – proton-selective decoupling experiments at 400 MHz; C_β and C_γ of Glu – pH variations of the chemical shifts; C=O resonances in *N*-Ac-amino acid OMe – proton-selective decoupling experiments on acetyl Me and O–Me; C=O in cyclo(Gly–Asp) – coupled spectrum (at 62.9 MHz).

Owing to the low solubility of cyclo(Gly–Glu), *ca.* 5×10^{-2}M, the C=O resonances in this compound were assigned by analogy with cyclo(Gly–Asp) data.

Proton and carbon-13 chemical shifts are reported in Table 1.

Table 1.—Chemical shifts in D_2O (p.p.m., ref. DSSNa)

		*N*AcAspOMe pH = 4.6		Cyclo(Gly–Asp) pH = 4.6		*N*AcGluOMe pH = 5.1		Cyclo(Gly–Glu) pH = 5.1	
		$\delta_{^1H}$	$\delta_{^{13}C}$	$\delta_{^1H}$	$\delta_{^{13}C}$	$\delta_{^1H}$	$\delta_{^{13}C}$	$\delta_{^1H}$	$\delta_{^{13}C}$
Asp	CO_2^-		179.82		179.32		182.81		182.80
or	CH_{γ}					2.33	35.14	2.34	34.04
Glu	CH_{β_1}	2.72	40.78	2.84	41.83	2.05	29.45	2.19	32.06
	CH_{β_2}			2.71				2.07	
	CH_{α}	4.66	52.98	4.30	54.69	4.41	55.37	4.16	56.77
	C=O		176.57		172.88		177.00		172.66
Gly	C=O				171.20				171.19
	CH_2			4.06	46.73			4.05	46.45
Ester	Me	3.75	55.68			3.75	55.60		
Acetyl	C=O		176.70				177.00		
	Me	2.03	24.41			2.03	24.27		

Table 2. Experimental data

Complex	n^a ^1H	n^a ^{13}C	$\lvert\Delta\delta\rvert_{max.}$(p.p.m.) ^1H	$\lvert\Delta\delta\rvert_{max.}$(p.p.m.) ^{13}C
Yb^{3+} complexes				
cyclo(Gly–Asp)	30	10	6.15	17.80
*N*AcAspOMe	32	9	5.25	15.51
cyclo(Gly–Glu)	39	10	7.53	21.13
*N*AcGluOMe	36	14	7.57	15.69
Eu^{3+} complex				
cyclo(Gly–Asp)	30	11	2.14	42.0

[a] Number of experimental solutions.

Sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) was used as an internal standard.

Thermodynamic Analysis

Method.—In our systems, shift ratios $\Delta\delta_j/\Delta\delta_i$ are fairly constant until $[Yb^{3+}] = 0.1$M, both in dilute and concentrated solutions. Therefore, the extrapolation of these ratios to μ (ionic strength) = 0, as in some works,[15–17] was not necessary here. Furthermore, in other works on histidine[18] or carboxylic acids,[12] no significant effect on the chemical shifts due to ionic strength variations (of the same order of magnitude as in our systems) was found.

Two models of solution can be used, the simplest one involving a single complex PLn^{2+} (model I) and the other one two complexes PLn^{2+} and P_2Ln^+ (model II) as previously shown for some amino acids in D_2O.[19–21]

From these models, some improvements can be made, taking into account, for example, a macroscopic medium effect arising from loose interactions between ClO_4^- and the complexed ligands. So a secondary term, proportional to the salt concentration, could be added either in model I or in model II.

Concerning model II, an average LIS value in the two complexes was taken as a first approximation,[20] $\Delta\delta_{C_i} = \Delta\delta_{C_i(PLn)} = \Delta\delta_{C_i(P_2Ln)}$, but a more sophisticated model could be considered with two different values of $\Delta\delta_{C_i}$.[19,21]

Calculation of the thermodynamic and spectroscopic parameters, using the four most refined models, required sufficient data: in fact we obtained 150—230 LIS for the whole set of 10—13 sites (Table 2).

For more coherent results, all the data from the different magnetic sites were processed simultaneously. In that way, the K_1 and K_2 values are better than when averaging the values obtained separately from each nucleus. This procedure is required particularly when different ranges of peptide concentration are used. Furthermore, to get coherent $\Delta\delta_{C_j}/\Delta\delta_{C_i}$ ratios, the $\Delta\delta_{C_i}$ have to be calculated from the same K_1, K_2 set.

Three considerations now have to be discussed. (i) Each nuclear site (^1H or ^{13}C) can give thermodynamic information if the uncertainty about the frequency measurement, in a given sample, is small enough when compared with the maximum LIS for this probe. In our systems, the precision is better than 1% for all the ^1H and two ^{13}C sites. (ii) The most sensitive data are also the most affected by uncertainties about pH, temperature, concentration, etc. Consequently, when the mixing of data from different nuclei is required, the best way is to normalize, *i.e.*, to divide these data by the maximum LIS of each probe. (iii) In order to calculate the best values of the binding constants in the peptide concentration range 0.01—0.1M, we chose to give about the same weight to the data coming from the two lines of solutions, the dilute and the concentrated ones.

Hence we first processed all of the data, carefully studying the standard deviation for the ^{13}C nuclei for which the spectroscopic precision was the worst. Except for the case of *N*-Ac-Glu-OMe, we finally kept all the data because the standard deviations for the nuclei in question never exceeded twice the mean standard deviation for the whole system.

As the maximum value of LIS for ^1H sites came from 0.01M solutions, a further correction had to be made for the ^{13}C data, for which only 0.1M solutions were available [equation (1)].

$$\Delta\delta_{i(\text{normalized})} = \frac{\Delta\delta_i}{\Delta\delta_{i\ \text{max.(0.1M)}}} \cdot \frac{\Delta\delta(^1H)_{\text{max.(0.1M)}}}{\Delta\delta(^1H)_{\text{max.(0.01M)}}} \quad (1)$$

Results and Discussion.—(a) *Models of solution.* Normalized shift variations were analysed using a least-squares method to determine the best fit for models I, II, and the improved ones. Models involving a secondary effect on the chemical shifts (medium effects or when $\Delta\delta_{C_i,P_2Ln} \neq \Delta\delta_{C_i,PLn}$) give results that are physically meaningless. Probably both secondary effects should be introduced as neither correcting term is dominant. This would imply three spectroscopic parameters for each magnetic site, requiring more ^{13}C data than could be obtained.

As a consequence, the only two models discussed further involve either one or two complexes with, in the latter case, the approximation of an average $\Delta\delta_{C_i}$ in the two complexes. Results are reported in Tables 3 and 4.

The (PLn + P_2Ln) model gives the best normalized standard deviation. A statistical *F*-test[22] was used to corroborate this result. The sum of the squares of deviation (S.S.) associated with model I was taken as the total S.S. and that of model II as the residual S.S. Moreover the number of linear parameters that could approximate to a binding constant in the relationship $\Delta\delta = f(P_0, Ln_0)$ has been chosen as 3 rather than 1. In these conditions the validity of the second model, compared with the first one, was demonstrated (with a confidence level better than 99% for each of the five studied systems).

The confidence level was also calculated independently for each site to make sure that it was not systematically too low for any one of them. This was not the case, even for nuclei far from the ytterbium cation (see Table 4).

Taking the two complexes into account not only improved the fit of the data but also increased the validity of the parameters. Besides the binding constants, the chemical shifts of the complexes also depend on the model. In fact, concerning the geometrical analysis, the choice of model would not matter if the LIS values of each site were proportional from one model to the other one, but this was not the case in the systems under study: $\Delta\delta_{C_i}/\Delta\delta_{C_j}$ ratios from the analysis using model I were not in agreement with the experimental $\Delta\delta_{C_i}/\Delta\delta_{C_j}$ values. In contrast, the results obtained from model II fit these experimental ratios well (Table 5). This shows that $\Delta\delta_{C,P_2Ln} = \Delta\delta_{C,PLn}$ is a good approximation of these systems.

(b) *Binding Constants.*—Binding constant values are the average values in the range 0—0.1M for $Yb(ClO_4)_3$. It was assumed that the ionic strength effects would not significantly modify the K_1 ratios for similar compounds.

Two structural factors may affect the K_1 binding constants, the backbone flexibility and, to a lesser extent, the side-chain length: (i) for *N*-acetylamino acid methyl esters, K_1 is twice as large as for cyclic dipeptides (see Table 3). The larger degree of freedom in the first compounds may facilitate their binding. (ii), K_1 is increased by 20—30% for glutamate over the aspartate derivatives. One more CH_2 group may increase the complexation either because of carboxylate basicity or of conformational freedom.

As K_2 is the last supplied parameter, its value can take into account other secondary effects (*cf. Models of solution*); it is then difficult to discuss K_2/K_1 ratios.

In order to test any intrinsic backbone binding, a cyclic inactive dipeptide, cyclo(Gly–Ala), was also investigated. A weak interaction with Dy^{3+}, the lanthanide that induces the largest LIS, was detected, the constant K_1 being less than $1M^{-1}$.

Table 3. Binding constants for Yb^{3+} complexes (M^{-1})

Ligand	Model	K_1	K_2	σ^a
Cyclo(Gly–Asp)	1:1	29		0.0704
	1:1 + 2:1	34	17	0.0273
*N*AcAspOMe	1:1	66		0.0568
	1:1 + 2:1	70	6.1	0.0465
Cyclo(Gly–Glu)	1:1	86		0.0517
	1:1 + 2:1	48	17	0.0354
*N*AcGluOMe	1:1	88		0.0338
	1:1 + 2:1	87	3.4	0.0295

[a] σ For normalized chemical shifts.

Table 4. Induced shifts $\Delta\delta_{C_i}$ (p.p.m.) and normalized standard deviation (σ_i)

		Complex 1:1		Complexes 1:1 + 2:1		Complex 1:1		Complexes 1:1 + 2:1	
		$-\Delta\delta_{C_i}$	σ_i	$-\Delta\delta_{C_i}$	σ_i	$-\Delta\delta_{C_i}$	σ_i	$-\Delta\delta_{C_i}$	σ_i
		Cyclo(Gly–Asp)				Cyclo(Gly–Glu)			
Asp	CO_2^-	36.47	0.063	23.64	0.019	34.53	0.036	27.96	0.025
or	C_γ					23.13	0.052	20.15	0.022
Glu	H_γ					8.01	0.053	8.56	0.035
	C_β	23.71	0.085	15.43	0.036	6.84	0.041	5.67	0.020
	H_{β_1}	8.01	0.072	6.60	0.024	4.57	0.059	4.87	0.037
	H_{β_2}	8.14	0.072	6.70	0.022	4.93	0.057	5.21	0.037
	C_α	7.28	0.083	4.69	0.031	3.64	0.040	3.01	0.021
	H_α	3.97	0.071	3.32	0.022	2.06	0.054	2.20	0.034
	C=O	5.49	0.084	3.53	0.032	2.08	0.040	1.69	0.032[a]
Gly	C=O	2.26	0.061	1.47	0.020	0.70	0.043	0.59	0.059[b]
	CH_2 C	1.60	0.046	1.03	0.033	0.79	0.041	0.65	0.044[b]
	H	0.40	0.061	0.36	0.033	0.45	0.050	0.48	0.037
		*N*AcAspOMe				*N*AcGluOMe			
Asp	CO_2^-	22.83	0.058	19.95	0.030	29.02	0.027	25.88	0.020
or	C_γ					23.28	0.027	19.84	0.031[b]
Glu	H_γ					8.12	0.036	8.02	0.026
	C_β	14.45	0.070	12.64	0.042	5.85	0.032	5.22	0.025
	H_β	5.89	0.041	5.64	0.048[b]	4.82	0.030	4.73	0.029[b]
	C_α	4.45	0.064	3.89	0.035	3.55	0.031	3.16	0.024
	H_α	2.99	0.042	2.89	0.046[b]	2.02	0.036	2.01	0.033
	C=O	2.52	0.073	2.21	0.041	1.76	0.033	1.57	0.022
Methyl ester	C	−0.47	0.123	−0.42	0.095	*c*			
	H	−0.43	0.047	−0.41	0.041[a]	*c*			
Acetyl	C=O	0.88	0.045	0.76	0.040[a]	0.51	0.050	0.48	0.051[b]
	Me C	−0.30	0.099	−0.27	0.067	*c*			
	H	−0.39	0.049	−0.37	0.041	*c*			

[a] Confidence level (model II compared with model I) less than 95%. [b] Confidence level (model II compared with model I) less than 90%. [c] Site removed from analysis (experimental induced chemical shifts ≤ 0.08 p.p.m.).

Table 5. Ratios $\Delta\delta_C/\Delta\delta_{C_{CO_2^-}}$ in the two models of solution and experimental $\Delta\delta_i/\Delta\delta_{CO_2^-}$ in 10^{-1}M peptide solutions

	Cyclo(Gly–Asp)–Yb^{3+}				*N*AcGluOMe–Yb^{3+}		
	$\Delta\delta_C/\Delta\delta_{C_{CO_2^-}}$ model PLn	$\Delta\delta_C/\Delta\delta_{C_{CO_2^-}}$ model PLn + P_2Ln	$\Delta\delta_i/\Delta\delta_{C_{CO_2^-}}$ (experimental 10^{-1}M)		$\Delta\delta_C/\Delta\delta_{C_{CO_2^-}}$ model PLn	$\Delta\delta_C/\Delta\delta_{C_{CO_2^-}}$ model PLn + P_2Ln	$\Delta\delta_i/\Delta\delta_{C_{CO_2^-}}$ (experimental 10^{-1}M)
C_β	0.650	0.653	0.642	C_γ	0.806	0.767	0.769
H_{β_1}	0.220	0.279	0.276	H_γ	0.280	0.310	0.303
H_{β_2}	0.223	0.283	0.278	C_β	0.202	0.202	0.201
C_α	0.200	0.198	0.197	H_β	0.166	0.183	0.172
$H_{\alpha_{Asp}}$	0.109	0.140	0.141	C_α	0.122	0.122	0.121
$C{=}O_{Asp}$	0.150	0.149	0.148	H_α	0.070	0.078	0.079
$C{=}O_{Gly}$	0.062	0.062	0.063	$C{-}O_{Glu}$	0.061	0.061	0.060
$C_{\alpha_{Gly}}$	0.044	0.044	0.044	$C{=}O_{Ac}$	0.018	0.019	0.018
$H_{\alpha_{Gly}}$	0.011	0.015	0.015				

Thus for Asp and Glu derivatives, the intrinsic complexation of the peptide backbone could reasonably be neglected.

We also wondered whether chelation could occur, involving another ligand group apart from the carboxylate. A first indication of this was given by comparison of the cyclo(Gly–Asp) binding constants with Eu^{3+} (K_1 = 132M^{-1}) and Yb^{3+} (34M^{-1}). The larger value observed with Eu^{3+} (a light lanthanide) generally demonstrates that only one ligand group interacts with the cation.[23,24] This feature will be of interest for the subsequent structural analysis.

Structural Analysis

Method.—Structural Analysis. Our aim was to compare the structures of the four 1:1 peptide complexes, looking for some differences that could explain the K_1 values and indicate whether chelation occurs or not.

The paramagnetic induced shifts (*i.e.*, LIS corrected for diamagnetic terms) can be used for a structural elucidation if the contact contribution is small, which is generally the case for Yb^{3+}. Moreover, in order to simplify the analysis, axial symmetry of the magnetic susceptibility tensor is required. Rapidly interconverting states of the complexes[6,25] and/or small values of the non-axial effects[26] should allow the use of an approximation of an axial symmetry.

For some light lanthanides, axial symmetry has been often demonstrated, particularly in the case of Pr^{3+}, while recently Delepierre *et al.*[7] observed strong non-axial effects with Tm^{3+} and Er^{3+}, two heavy lanthanides as is Yb^{3+}. However, in this latter work, the differences in the LIS ratios between Pr^{3+} and Yb^{3+}, involving aquo ions, were not so important. Moreover, other studies have shown the validity of the axial approximation along the lanthanide series[20] or that the behaviour of Yb^{3+} is very close to one of the light lanthanides.[16,27] Under these conditions the axial symmetry assumption seemed a reasonable approximation in order to compare the structures of the four peptide complexes.

Before we describe the procedure for analysing the data, we will first summarize the determination of the diamagnetic term and the choice of the geometric data, *i.e.*, bond angles, interatomic distances, and conformations of the substrates.

(a) Diamagnetic term (Δd_i). We measured, in a few solutions, the chemical shifts induced by Lu^{3+} ($\Delta\delta_i$) in the substrate. As the binding constants for Yb^{3+} and Lu^{3+} have nearly the same values,[3,28,29] the peptide proportions η_1 and η_2 in the 1:1 and the 2:1 complexes were evaluated for each solution using the Yb^{3+} binding constant values, and Δd_i given by equation (2). The average values for different samples are reported in Table 6. The diamagnetic contribution is small for ^1H (less than 0.10 p.p.m.) but significant for ^{13}C especially for those nuclei close to the site of interaction (up to 8 p.p.m. for the carboxylate carbons).

$$\Delta d_i = \frac{\Delta\delta_i}{\eta_1 + \eta_2} \qquad (2)$$

(b) Geometric data. Bond angles and interatomic distances were selected from the literature on crystallographic results or conformational parameter values[30–39] (see Table 7).

The diketopiperazine ring was assumed to be rigid and planar.[35] However, for the *N*-acetylamino acid methyl ester backbone, different conformations have to be considered. For amide and ester groups, *trans* and *cis* planar configurations, respectively, were chosen.[35,40] We assumed a free rotation of the methyl groups, six staggered conformations around the N–C_α bond, and three eclipsed ones around the C_α–C′ axis.

For the side-chains, three staggered conformations around the C_{sp^3}–C_{sp^3} bonds were assumed, but for the $C_{\beta(\text{or }\alpha)}$–$CO_2^-$ axis either staggered or eclipsed conformations could be adopted, according to a bidentate or a monodentate binding of the carboxylate group, respectively. In fact, as the induced chemical shifts of the β-protons in the cyclo(Gly–Asp) complex are different, especially in the case of Eu^{3+} (see Table 6), we first assumed a dissymetrical complexation, *i.e.*, a monodentate one. Therefore three eclipsed conformations around the $C_{\beta(\text{or }\gamma)}$–$CO_2^-$ axis were investigated; however, the staggered conformations were also tested in a few cases.

The final number of conformers was 9 for cyclo(Gly–Asp), 27 for cyclo(Gly–Glu), and 162 for *N*AcAspOMe and *N*AcGluOMe.

For the last compound, conformers from C_α–C′ rotation have not been considered, as the induced chemical shifts of the methyl esters are too small to be included in the analysis.

(c) Data analysis. Figure 1 shows the internal cartesian co-ordinates chosen with the carboxylate in the *x*,*y* plane and a positive *y* co-ordinate for the adjacent carbon.

The direction of the principal magnetic axis was calculated for each lanthanide position (ρ, ω, λ) taking into account the distances between the ion and the two oxygen atoms, with the weighting factor for each oxygen inversely proportional to the square of its distance to Ln^{3+}. Previous work had shown the validity of such an approximation.[19,41–44]

To locate the lanthanide cation, space was explored between two spheres of radius ρ = 2.30 and 2.90 Å (a reasonable domain)[4,19,20,45] with steps of 0.05 Å for ρ and 20° for ω and λ.

For each point (ρ, ω, λ), the geometric factors of the McConnell and Robertson relationship[46] were calculated for all the magnetic sites *i* and conformers *j* [equation (3)]. A mean-squares method was applied to the paramagnetic terms Δp_i

Table 6. Diamagnetic (Δd_i) and pseudo-contact (Δp_i) terms (p.p.m.). $\Delta p_i = \Delta\delta_{C_i} - \Delta d_i$

a. Ytterbium complexes

			Cyclo(Gly–Asp)		*N*AcAspOMe		Cyclo(Gly–Glu)		*N*AcGluOMe	
			Δd_i	Δp_i	Δd_i	Δp_i	Δd_i	Δp_i	Δd_i	Δp_i
Asp or Glu	CO_2^-		5.15	−28.79	3.95	−23.90	8.12	−36.04	7.39	−33.27
	CH_{2_γ}	C					0.57	−20.25	−0.47	−19.37
		H					0.06	−8.64	0.04	−8.05
	CH_{2_β}	C	0.60	−16.03	0.20	−12.84	−1.07	−4.59	−0.79	−4.43
		H_{β_1}	0.06	−6.66	0.07	−5.71	0.04	−4.92	0.06	−4.79
		H_{β_2}	0.04	−6.74			0.04	−5.26		
	CH_α	C	−0.39	−4.30	−0.48	−3.42	−0.15	−2.85	−0.52	−2.64
		H	0.10	−3.42	0.08	−2.97	0.03	−2.24	0.03	−2.04
	C=O		−0.25	−3.28	−0.18	−2.03	−0.03	−1.66	−0.27	−1.31
Gly	C=O		−0.10	−1.37			0.03	−0.60		
	CH_2	C	−0.04	−0.99			−0.02	−0.63		
		H	0.04	−0.40			0.02	−0.50		
Methyl ester	Me	C			0.28	0.16				
		H			0.01	0.40				
Acetyl	C=O				0.17	−0.60			0.10	−0.58
	Me	C			0.12	0.15				
		H			0.01	0.36				

b. Cyclo(Gly–Asp)–Eu^{3+} complex. Δp_i values

Asp	CO_2^-		55.07	Asp	CH_α	C	−2.22	Gly	C=O		−1.47
	CH_{2_β}	C	−51.58			H	−1.34		CH_2	C	−0.57
		H_{β_1}	−1.76		C=O		−2.33			H	−0.34
		H_{β_2}	−2.30								

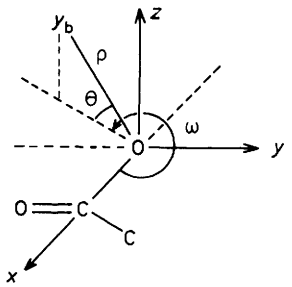


Figure 1. The axis system for the Ln^{3+} complexes.

$$GF_i^j = [(3\cos^2\theta_i - 1)/r_i^3]_j \quad (3)$$

(Table 6) in order to determine the variance for each conformer. Only the lowest variance value was retained, V (ρ, ω, λ) corresponding to the 'best' conformer.

Finally, for each sphere the lanthanide co-ordinates ω and λ were refined, to a resolution of 1°, for all the sub-minima for which $V_{sub.min} < 1.5\ V_{min}$ (V_{min} being the smallest over-all variance of the 13 spheres). Thus the best geometry of the complex (conformer and Ln^{3+} co-ordinates) was determined with a resolution of 1° for ω and λ and 0.05 Å for ρ.

Another analysis was carried out, taking into account two conformers j and l (assuming the co-ordinates of the cation to be the same for both). The variances were then calculated for all the combinations of two conformers j and l with a mean-squares method applied to equation (4), ω_j being the weight of conformer j. For *N*-acetylamino acid methyl esters, on account of the great number of combinations of two conformers (about 13 000), the minimal variance was determined by only exploring the space around the lanthanide positions indicated by the analysis based on a single conformer. In all cases, the validity of the analysis involving two conformers, with respect to the one involving a single conformer, was checked by a statistical *F*-test.[22]

$$\Delta p_i = k \cdot [\omega_j GF_i^j + (1 - \omega_j)GF_i^l] \quad (4)$$

Results and Discussion

Comments should first be made on the comparison of Yb^{3+}- and Eu^{3+}-induced shifts. The regular increase in Yb^{3+} shifts, according to the proximity of the co-ordination site, is not observed for Eu^{3+} shifts; in this case, a positive shift for the carboxylate and a negative one for the adjacent carbon show that these shifts include an important contact contribution and, consequently, these two shifts should be discarded from the analysis of the Eu^{3+} complexes.

Concerning the four ytterbium complexes, the analysis was carried out by first taking into account a single conformer and all the magnetic sites. Results are reported in Table 8. It is worth noting that the $Ln^{3+} \cdots O^-$ distances are 2.70—2.75 Å for the aspartate derivatives and 2.50—2.55 Å for the glutamates.

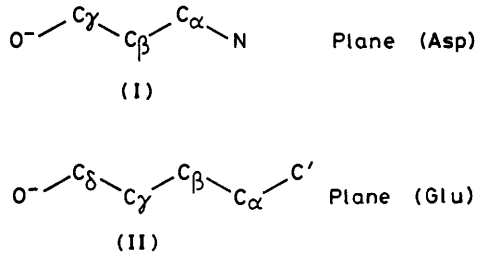
The values are consistent with a monodentate complexation of the carboxylate group and an extended structure of the best conformers [(I) and (II)] (see also Figure 2). So no chelation occurs with the peptide backbone. However, this analysis leads to rather large values of R (0.08—0.14), R being the Willcott agreement factor[47] used to check the validity in such a structural analysis [equation (5)]. Moreover, an important

$$R = \left[\frac{\Sigma(\Delta p_i^{exp} - \Delta p_i^{calc})^2}{\Sigma(\Delta p_i^{exp})^2}\right]^{\frac{1}{2}} \quad (5)$$

Table 7. Bond angles (°) and bond lengths (Å)

Diketopiperazine ring		Mean value
C_α–C′	1.509;[a] 1.519[b]	1.52
C_α–N	1.452;[a] 1.460[b]	1.46
C′–N	1.334;[a] 1.329[b]	1.33
$\widehat{NC'C_\alpha}$	118.9;[a] 118.6[b]	119
$\widehat{C_\alpha NC'}$	126.0;[a] 127.9[b]	127
$\widehat{C'C_\alpha N}$	115.1;[a] 113.4[b]	114
C_α–C_β	1.532–1.545;[c] 1.551[b] 1.544–1.548;[d] 1.548[e] 1.542[f]	1.54
$\widehat{C'C_\alpha C_\beta}$	107.6–108.0;[d] 112.4[e] 113.1[f]	110
Peptide group		
N–C_α	1.47[30]	1.47
N–C′	1.32[30]	1.32
C′=O	1.24[30]	1.24
$\widehat{H_3CC'N}$	117[30]	117
$\widehat{C_\alpha NC'}$	123[30]	123
Ester group		
C_α–C′	1.53[30,35]	1.53
C′–O	1.36;[30] 1.34[35]	1.35
O–Me	1.45;[30] 1.47[35]	1.45
$\widehat{C_\alpha C'O}$	115;[30] 111[37]	113
$\widehat{C'OMe}$	108;[36] 106[37]	107
Asp or Glu		
C_β–C_γ	1.53[30]	1.53
$\widehat{C_\alpha C_\beta C_\gamma}$	115[30]	115
$\widehat{HC_\beta C_\gamma}$	107;[30] 109[38]	108
C–O	1.25[37,39]	1.25
C–CO	1.52[39]	1.52
$\widehat{CCO}$	118–119;[39] 117[37]	118

[a] Cyclo(Gly–Gly).[31] [b] Cyclo(D-Ala–L-Ala).[32] [c] Cyclo(Ala–Ala).[32] [d] Cyclo(Thr–His).[33] [e] Cyclo(Gly–Tyr).[34] [f] Cyclo(Ser–Tyr).[34]



systematic deviation is observed for the carbon adjacent to the carboxylate group (from 1.3 to 4.6 p.p.m.).

At first sight and without dismissing the pseudo-contact model, these discrepancies could arise from the presence of a second conformer or from some staggered conformations around the carboxylate group. In fact, the results of the two-conformer analysis, involving the four ytterbium complexes, were unsatisfying: large deviations for the carbon adjacent to the carboxylate were still present and the improvement in the *R* factors was too small with respect to the loss of one degree of freedom. In addition, calculations for the staggered conformations, in the cyclo(Gly–Asp)–Yb^{3+} system, led to slightly larger *R* values than for the eclipsed ones. Moreover, the deviation for C_β was not reduced. Thus, we had to take into consideration contact shifts, at least for nuclei close to the lanthanide ion. The removal from the analysis of the induced shifts of the carboxylate carbon and of the adjacent one (as in the case of europium complex) then gave acceptable *R* values (4.3—5.5%, see Table 9). It should be observed that this procedure does not change the main features of the structure of the ytterbium complexes, *i.e.*, $Ln^{3+} \cdots O^-$ distances, monodentate complexation, and extended side-chains [see Table 9 and Figures 3 and (2*a* and *c*)].

Two slight differences should be noted, the ω angles (most of them being now *ca.* 180°) and the *N*-acetylamino acid methyl ester conformers, which differ from those previously determined only by a C_α–N rotation (a rotation defined by nuclei far from the site of interaction and thus not accurately positioned).

These structural results could have been strengthened by relaxation measurements but this was not technically possible

Table 8. Structural results for the Yb^{3+} complexes. One-conformer analysis involving all the n.m.r. ^1H and ^{13}C sites

	Cyclo(Gly–Asp)	*N*AcAspOMe	Cyclo(Gly–Glu)	*N*AcGluOMe
ρ/Å	2.70	2.75	2.50	2.55
ω/°	122	117	142	138
λ/°	25	−26	4	0
R/%	10.8	8.1	13.6	14.0
		φ − 90°[a]		φ + 90°
		ψ − 60°[a]		

[a] φ and ψ defined following the IUPAC–IUB conventions (*Biochemistry*, 1970, **9**, 3471).

Table 9. Structural results for the Yb^{3+} complexes: one-conformer analysis after removal of the carboxylate and adjacent group ^{13}C

	Cyclo(Gly–Asp)	*N*AcAspOMe	Cyclo(Gly–Glu)	*N*AcGluOMe
ρ/Å	2.70	2.70	2.55	2.50
ω/°	185	153	182	177
λ/°	−35	−46	−29	−12
R/%	5.3	4.3	5.5	5.0
		φ − 150°		φ − 90°
		ψ − 60°		

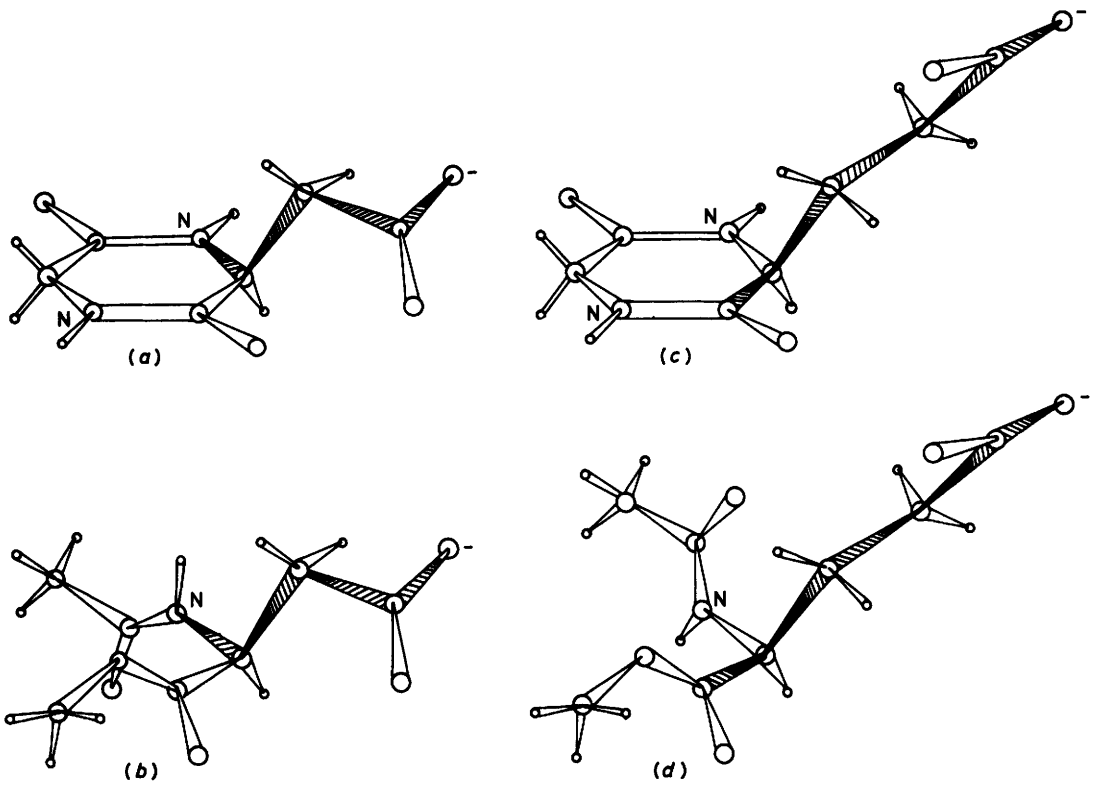


Figure 2. Conformations of the ligand in the Yb^{3+} complexes. (*a*) Cyclo(Gly–Asp); (*b*) *N*AcAspOMe; (*c*) Cyclo(Gly–Glu); and (*d*) *N*AcGluOMe. (*b*) and (*d*), One-conformer analysis involving all the n.m.r. ^1H and ^{13}C sites

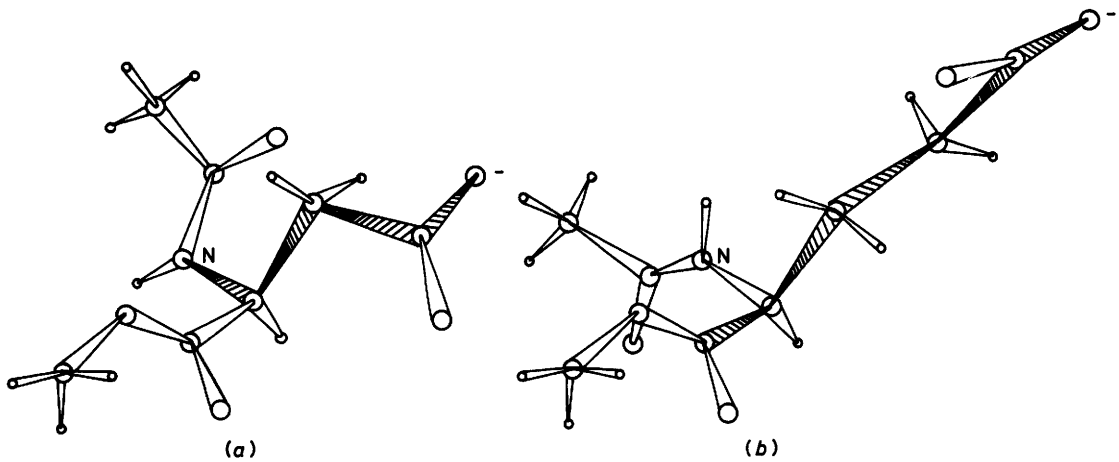


Figure 3. Conformations of the ligand in the Yb^{3+} complexes (one-conformer analysis after removal of the carboxylate and adjacent group ^{13}C). (*a*) *N*AcAspOMe; (*b*) *N*AcGluOMe.

for ^{13}C nuclei in 0.1M peptide solution; moreover, too few ^1H nuclei were available to discriminate the structures of the complexes just from the T_1 measurements. Finally, broadening effects were in agreement with the order of the Yb^{3+} distance as deduced from the analysis of the LIS.

On considering the thermodynamic results, it is worth noting that the $Yb^{3+} \cdots O^-$ distances, shorter in glutamate derivatives than in the aspartates, are consistent with the K_1 binding constant values, which are larger by 20—30% in the case of the glutamate complexes. These results are probably a consequence of the carboxylate basicity, unless some other interaction with the peptide backbone is taking place. In fact, whatever residue or peptide backbone is involved, the predominant conformer has an extended side-chain and, consequently, no chelation occurs with the peptide backbone. The fact that K_1 for the *N*-acetylamino acid methyl esters is roughly twice that for the cyclodipeptides could arise from the larger degree of conformational freedom of the non-cyclic backbones, which are not involved in the complexation.

Finally, with the europium complex, it seems that the contact contribution is so large that the analysis based on pseudo-contact shifts fails, even when removing more than two induced shifts. Thus, to obtain structural information about this complex without specifying a parameter (lanthanide co-ordinates or substrate conformation, for example) does not seem possible.

Conclusions

Aspartate and glutamate side-chains form 1:1 and 2:1 complexes with lanthanide ions in an aqueous medium. A model assuming an average LIS value for the two complexes yields a good fit of the data, the normalized standard deviation being less than 5%

This study suggests that two factors affect the binding constants: the peptide backbone flexibility and, to a lesser extent, the side-chain length.

With Yb^{3+} as a probe, the main features of the structure of the ytterbium complexes can be obtained, using all the proton and the ^{13}C data, but removal of the two carbons closest to the lanthanide allows a refinement of the results.

In the four compounds, the complexation of the carboxylate is monodentate, the $Yb^{3+} \cdots O^-$ distances being shorter in the Glu derivatives (2.50—2.55 Å) than in the Asp (2.70—2.75 Å). With the predominant conformation of the side-chain being extended, no chelation occurs with the peptide backbone.

References

1 F. Inagaki and T. Miyazawa, *Prog. Nucl. Magn. Res. Spectrosc.*, 1980, **14**, part 2.
2 J. Reuben, *Prog. Nucl. Magn. Res. Spectrosc.*, 1973, **9**, part 1.
3 E. Nieboer, *Struct. Bonding (Berlin)*, 1975, **22**, 1.
4 A. D. Sherry and E. Pascual, *J. Am. Chem. Soc.*, 1977, **99**, 5871.
5 R. E. Davis and M. R. Wilcott III, 'Nuclear Magnetic Resonance, Shift Reagents,' ed. R. E. Sievers, Academic Press, New York, 1973, p. 143.
6 B. A. Levine and R. J. P. Williams, *Proc. R. Soc. London, Ser. A*, 1975, **345**, 5.
7 M. Delepierre, C. M. Dobson, and S. L. Menear, *J. Chem. Soc., Dalton Trans.*, 1981, 678.
8 'Comprehensive Analytical Chemistry,' eds. C. L. Wilson and D. W. Wilson, Elsevier, Amsterdam, 1960, vol. I B, p. 356.
9 G. R. Brubaker and N. Y. Sakkab, *Bioinorg. Chem.*, 1974, **3**, 243.
10 M. R. Harnden, *J. Chem Soc. C*, 1967, 2341.
11 A. Glasel, *Prog. Inorg. Chem.*, 1973, **18**, 383.
12 A. *D.* Sherry, C. Yoshida, E. R. Birnbaum, and D. W. Darnall, *J. Am. Chem. Soc.*, 1973, **95**, 3011.
13 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.
14 K. Wuthrich, 'NMR in Biological Research: Peptides and Proteins,' North-Holland, Amsterdam, 1976, pp. 42, 170.
15 C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Nature (London)*, 1971, **232**, 236.
16 C. D. Barry, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J. Chem. Soc., Dalton Trans.*, 1974, 1765.
17 C. F. G. C. Geraldes and M. H. Mendonca-Dias, *J. Magn. Reson.*, 1981, **45**, 394.
18 D. H. Sachs, A. N. Schechter, and J. S. Cohen, *J. Biol, Chem.*, 1971, **246**, 6576.
19 B. T. Pennington and J. R. Cavanaugh, *J. Magn. Reson.*, 1978, **29**, 483.
20 G. A. Elgavish and J. Reuben, *J. Magn. Reson.*, 1981, **42**, 242.
21 J. Mossoyan, M. Asso, and D. Benlian, *J. Magn. Reson.*, 1982, **46**, 289.
22 C. J. Brookes, I. G. Betteley, and S. M. Loxston, 'Mathematics and Statistics for Chemists,' John Wiley, New York, 1966, p. 365.
23 M. Cefola, A. S. Tompa, A. V. Celiano, and P. S. Gentile, *Inorg. Chem.*, 1962, **1**, 290.
24 G. R. Choppin and W. F. Strazik, *Inorg. Chem.*, 1965, **4**, 1250.
25 W. DeW. Horrocks Jr., J. P. Sipe III, and D. Sudnick, 'Nuclear Magnetic Resonance, Shift Reagents,' ed. R. E. Sievers, Academic Press, New York, 1973, p. 53.
26 J. W. M. De Boer, P. J. D. Sakkers, C. W. Hilbers, and E. De Boer, *J. Magn. Reson.*, 1977, **25**, 455.
27 A. D. Sherry, C. A. Stark, J. R. Ascenso, and C. F. G. C. Geraldes, *J. Chem. Soc., Dalton Trans.*, 1981, 2078.
28 G. R. Choppin and A. J. Graffeo, *Inorg. Chem.*, 1965, **4**, 1254.
29 R. S. Kolat and J. E. Powell, *Inorg. Chem.*, 1962, **1**, 293.
30 T. S. Ooi, R. A. Scott, G. Vandekooi, and H. A. Scheraga, *J. Chem. Phys.*, 1967, **46**, 4410.
31 R. Degeilh and R. E. Marsh, *Acta Crystallogr.*, 1959, **12**, 1007.
32 E. Sletten, *J. Am. Chem. Soc.*, 1970, **92**, 172.
33 M. Cotrait, M. Ptak, B. Busetta, and A. Heitz, *J. Am. Chem. Soc.*, 1976, **98**, 1073.
34 C. F. Lin and L. E. Webb, *J. Am. Chem. Soc.*, 1973, **95**, 6803.
35 B. Pullman and A. Pullman, *Adv. Protein Chem.*, 1974, **28**, 347.
36 A. C. Hopkinson, K. Yates, and I. G. Csizmadia, *J. Chem. Phys.*, 1970, **52**, 1784.
37 P. W. Borthwick, *Acta Crystallogr., Sect. B*, 1980, **36**, 628.
38 J. L. Derissen, H. J. Endeman, and A. F. Peerdeman, *Acta Crystallogr., Sect. B*, 1968, **24**, 1349.
39 H. Einspahr and C. E. Bugg, *Acta Crystallogr., Sect. B*, 1981, **37**, 1044.
40 R. M. Moravie, Thesis, University of Paris-6, France, 1975.
41 G. Hawkes, D. Leibfritz, D. W. Roberts, and J. D. Roberts, *J. Am. Chem. Soc.*, 1973, **95**, 1659.
42 D. J. Chadwick and D. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1974, 1202.
43 B. A. Levine, J. M. Thornton, and R. J. P. Williams, *J. Chem. Soc., Chem. Commun.*, 1974, 669.
44 C. M. Dobson, L. O. Ford, S. E. Summers, and R. J. P. Williams, *J. Chem. Soc., Faraday Trans. 2*, 1975, **71**, 1145.
45 S. P. Sinha, *Struct. Bonding (Berlin)*, 1976, **25**, 69.
46 H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, 1958, **29**, 1361.
47 M. R. Willcott III, R. E. Lenkinski, and R. E. Davis, *J. Am. Chem. Soc.*, 1972, **94**, 1742.

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