Chiral Complexes of Copper(II), containing Polyether Podands and a Quaternary Ammonium Group, as Potential Receptors and Carriers of α-Amino Acid Anions

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In an attempt to develop a viable metalloreceptor for the recognition of α-amino acid anions a detailed study of proline (Pro) complexation by {(*R*)-7,8,15,18-tetrahydro-5,10-bis(2'-methoxyethoxy-5'-methylphenyl)-7-(*p*-trimethylammoniophenyl)dibenzo[*e,m*]-[1,4,8,11]tetra-azacyclotetradecine-16,17-dionato(1–)-$N^6N^9N^{15}N^{18}$}copper(II) chloride (**1**) in D_2O at pD 11.0, using n.m.r. relaxation techniques, was undertaken. Structural information has been obtained, including the distances between hydrogen or carbon atoms of proline (Pro) and copper ion in the mixed complex where Pro occupies the apical position. Preliminary data on the ability of (**1**) to serve as a phase-transfer carrier for α-amino acid anions (Phe, Leu, Pro, or Hyp) are also reported. The extraction constants were correlated with those found using a routine phase-transfer agent, tetrapentylammonium iodide. Noticeable differences in phase-transfer properties were detected for these reagents.

Transport of amino acids across lipophilic membranes plays an important role in biochemical phenomena.[1] With the advent of supramolecular chemistry, including in its scope artificial transport agent and receptor design,[2] the transport of α-amino acids and their derivatives has attracted considerable interest. Most of the research has been undertaken with amino ester hydrohalides or other salts,[3,4a] *i.e.* complexation and transport were studied under acidic conditions. Asymmetric crown ethers have been successful in chiral recognition under these conditions.[3] At the other end of the pH scale, transport of the anions of α-amino acid N-acyl derivatives[4b] or amino carboxylates as alkali-metal salts has also been studied, and some work has been done on artificial transport of amino acid anions as ammonium salts.[5] However, no examples of chiral recognition under the latter conditions are known.

We have undertaken the task of developing specific receptors based on transition-metal complexes for the recognition and transport of α-amino acid anions, including the recognition of their enantiomers. The difficulty in creating such systems arises from the high chelating ability of the amino acid anions, which usually destroy metal complexes by effective competition for the transition metal. Our premise was that chiral complexes of transition metals and hydrophobic macrocyclic ligands containing strong donor atoms for co-ordination of the metal ion, and also special donor groups capable of dipole–dipole and electrostatic interactions with a weakly co-ordinated amino acid anion, may serve the purpose, as illustrated in Figure 1. A weak apical interaction of the α-amino acid amino group with the metal ion, the positive charge of which is formally neutralized by the negative charge of the macrocyclic ligand, should orient the anion in space. The polyether podands and the positively charged quaternary ammonium group might serve as anchoring sites for the amino group protons and the substrate carboxylate anion. Relative non-rigidity of the podand groups may provide for fast exchange of the substrate.

Here, we examine the properties of [(*R*)-7,8,15,18-tetrahydro-5,10-bis(2'-methoxyethoxy-5'-methylphenyl)-7-(*p*-trimethylammoniophenyl)dibenzo[*e,m*][1,4,8,11]tetra-azacyclotetradecine-16,17-dionato(1–)-$N^6N^9N^{15}N^{18}$]copper(II) chloride (**1**) as a receptor and carrier of α-amino acid anions. Its synthesis was previously described.[6]

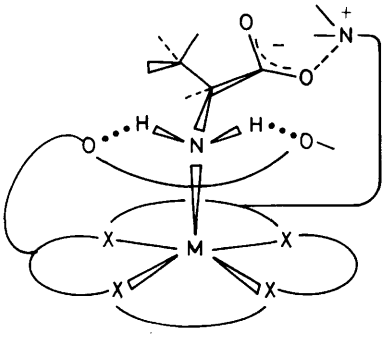
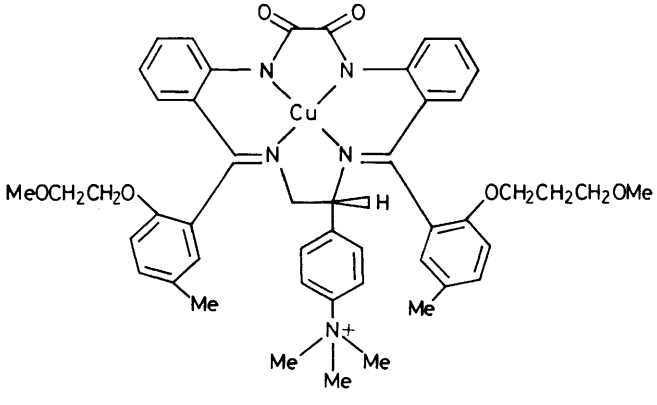


Figure 1. Schematic illustration of the ideal three-point attachment of the anion of an amino acid to a metal complex incorporating podands and a quaternary ammonium group

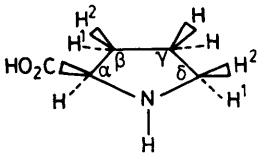


(**1**)

Results and Discussion

Complexation of Organic Substances by Complex (**1**) *in Water as Revealed by* ^{1}H *N.M.R. Experiments.*—Co-ordination of a substrate to a paramagnetic ion of a transition metal can often

Table 1. Spin–lattice relaxation times of the protons of Pro in the presence of complex (**1**)*



T	N_S/N_P	α	β_1	$\gamma + \beta_2$	δ_2	δ_1
T_2	0	2.53	2.20	2.15	1.93	1.84
T_2	5×10^{-4}	0.18	0.47	0.57	0.22	0.21
T_1	0	3.44	1.91	2.77	2.22	2.02
T_1	10^{-4}	0.92	1.66	1.96	1.37	0.93
T_1	5×10^{-4}	0.20	0.88	1.07	0.48	0.26
T_1	10^{-3}	0.11/0.12	0.65/0.63	0.79/0.77	0.29/0.31	0.16/0.16
T_1	5×10^{-3}	0.08/0.08	0.54/0.47	0.68/0.57	0.22/0.21	0.12/0.10

* T = 295 K. Standard deviations ±5% for both L- and D-proline.

Table 2. Changes in the spin–lattice relaxation rates of proline protons, caused by complex (**1**)*

N_S/N_P	α	β_1	$\gamma + \beta_2$	δ_2	δ_1
10^{-4}	0.80	0.08	0.15	0.28	0.58
5×10^{-4}	4.64	0.61	0.58	1.63	2.35
10^{-3}	8.48/8.16	1.03/1.08	0.91/0.94	3.02/2.83	5.92/5.86
5×10^{-3}	11.76/12.53	1.34/1.63	1.12/1.39	4.12/4.36	8.20/9.41

* Calculated using the values from Table 1. Standard deviations ±5% for both L- and D-proline.

be determined and the geometry of the complex established by studying the relaxation rates of the substrate nuclei.[7] The relaxation rates of ^1H and ^{13}C nuclei (and the linewidths of the signals) are determined by the magnetic energy exchange with their magnetic environment. Usually, in diamagnetic liquids the transfer of energy occurs at a slow rate, and the relaxation rate of the nuclei is slow. Paramagnetic complex (**1**), as any paramagnetic molecule, is a strong magnet capable of inducing fast relaxation of neighbouring nuclei. It survives in a 0.6 mol dm^{-3} solution of L- or D-proline (Pro) at pH 11.0 for at least 3 weeks, as revealed by its electronic spectra. Unchanged (**1**) can also be extracted with $CHCl_3$ from solution, and, finally, the ^1H relaxation rates of Pro are the same, whether measured 30 min after adding (**1**) to the solution or a week later.

Proton n.m.r. spectra of L- or D-Pro in D_2O solution[8] (0.58 mol dm^{-3}, pH 11) have a signal at δ 3.01, attributed to α-H of

ordination sphere of (**1**), the measured relaxation rates can be regarded as constituting essentially the weighted averages of two environments: the bulk solution and the bound state. In the case of a large excess of proline over complex (**1**) and a supposed complete saturation of the co-ordination site, the relaxation rate is described by equation (1).

$$\frac{1}{T_{1M}} = \frac{N_p}{N_s q}\left(\frac{1}{T_1} - \frac{1}{T_{1F}}\right) \quad (1)$$

In equation (1) $1/T_1$ and $1/T_F$ are the observed relaxation rates in the presence and the absence of complex (**1**) in solution, N_p is the molar concentration of Pro, and N_s that of (**1**), and q is the Pro/(**1**) ratio in the guest–host complex being formed, which we assumed to be equal to 1:1, taking into account the usually observed square-pyramidal type of copper(II) complex with strong ligands in the main co-ordination plane.[9] From the data in Table 2, T_{1M} has a constant value in the range $N_s/N_p = 10^{-4}$—10^{-3}, which supports the assumption of a fast exchange of Pro in the co-ordination sphere of (**1**). When the host to guest ratio becomes greater than 5×10^{-3}:1, T_{1M} begins to change, indicating the appearance of some uncomplexed (**1**) in solution. This result reflects a low binding constant of Pro to complex (**1**).

The T_{1M} values allow detailed characterisation of the complex formed by (**1**) and Pro. The distances of a nucleus from a paramagnetic site are related to T_{1M} *via* the Solomon–Blombergen equations[7] (2) and (3). The first terms in both

$$\frac{1}{T_{1M}} = \frac{2}{15}\cdot\frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right) + \frac{2}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \quad (2)$$

$$\frac{1}{T_{2M}} = \frac{1}{15}\cdot\frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_S^2\tau_c^2}\right) + \frac{1}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2} + \tau_e\right) \quad (3)$$

equations arise from dipole–dipole interactions between the electron spin, S, and the nuclear spin, I, whereas the second term is a hyperfine contact component.*

Direct measurements of the spin–lattice relaxation rate ($1/T_{1M}$) of a nucleus allow an estimation of r, provided there is no significant hyperfine term contribution. Estimation of τ_e [equation (4)] is crucial for this task. The spin relaxation time

Table 3. Distances (Å) between the protons of Pro and Cu^{II} of (1) in the mixed complex

N_S/N_P	α	β_1	$\gamma + \beta_2$	δ_2	δ_1
10^{-4}	3.38 ± 0.25	4.96 ± 0.35	4.46 ± 0.35	4.02 ± 0.3	3.56 + 0.25
5×10^{-4}	3.29 ± 0.25	4.62 ± 0.35	4.66 ± 0.35	2.92 ± 0.3	3.49 ± 0.25
10^{-3}	3.37 ± 0.25	4.71 ± 0.35	4.82 ± 0.35	4.01 ± 0.3	3.56 ± 0.25
5×10^{-3}	4.10 ± 0.3	5.76 ± 0.45	5.91 ± 0.45	4.89 ± 0.4	4.30 ± 0.35

Table 4. Spin–lattice relaxation time of the protons of propylamine, propanoic acid, and butanoic acid in the presence of complex (1); $N_S/N_P = 5 \times 10^{-4}$

	α	β	γ
Propylamine	5.51	5.60	5.43
Propylamine: (1)	0.62	1.15	2.03
Propanoic acid	5.00	6.17	—
Propanoic acid: (1)	3.23	3.77	—
Butanoic acid	2.61	2.73	2.82
Butanoic acid: (1)	2.04	2.16	2.27

Table 5. Spin–lattice relaxation times of the ^{13}C nuclei in the presence of complex (1), and ^{13}C–Cu distances in the mixed complex *

	α	β	γ	δ	CO_2^-
T_{1F}(s)	6.43	5.02	6.81	5.29	100
$1/T_{1F}(s^{-1})$	0.155	0.199	0.147	0.189	0.01
T_1(s)	2.55	3.90	4.68	2.55	15
$1/T_1(s^{-1})$	0.393	0.256	0.214	0.393	0.067
$\Delta 1/T_1(s^{-1})$	0.238	0.057	0.067	0.204	0.057
r(Å)	3.1	4.0	3.7	3.2	4.0

* $N_S/N_P = 5 \times 10^{-4}$.

$$\frac{1}{\tau_c} = \frac{1}{\tau_s} + \frac{1}{\tau_M} + \frac{1}{\tau_R} \qquad (6)$$

In the case of copper(II) ions,[7] the correlation time of the complex, τ_R, is usually the smallest of the three components in the equation and thus dominates the value of τ_C. We have taken (from ref. 7*a*) the value of τ_C as 10^{-10} and $\omega_I\tau_C \ll 0.1$, assuming similarity in size of our system and the one in ref. 7*a*. There are linear dependences of $\ln(1/T_{1M})^{-1}$ *vs.* T in the region 295—253 K in the absence and the presence of complex (1). This result supports[7] our assumption regarding the value of $\omega_I\tau_C$. The term C in equation (5) is equal[7] to 539 (for protons) and 340 (for ^{13}C nuclei).

The proton–copper(II) internuclear distances calculated from equation (5) are presented in Table 3. As can be seen from the data, up to the limit of 5×10^{-3} in host/guest ratio the distances are independent of the concentration of complex (1) and have the same values within the limits of the experimental errors.

The data show the orientation of the associated Pro to be chemioselective, with the amino acid nitrogen atom occupying the apical position in the complex. This is strongly supported by the n.m.r. relaxation studies of propylamine, butanoic and propanoic acids in the presence of (1). The data summarized in Table 4 show the relaxation rates of all the protons in the acids to be similarly influenced by complex (1), and, thus, the orientations of their hydrophobic chains are chaotic in the mixed complex (if any such complex exists). On the other hand, relaxation rates of the α-protons of propylamine are most strongly influenced by (1), whereas its β,γ-protons are influenced to a lesser extent. Clearly, it is the nitrogen atom of propylamine that is the closest to the copper atom. Figure 2 shows the schematic representation of the binding of Pro to (1), drawn on the basis of the distances taken from Table 3. Carbon-

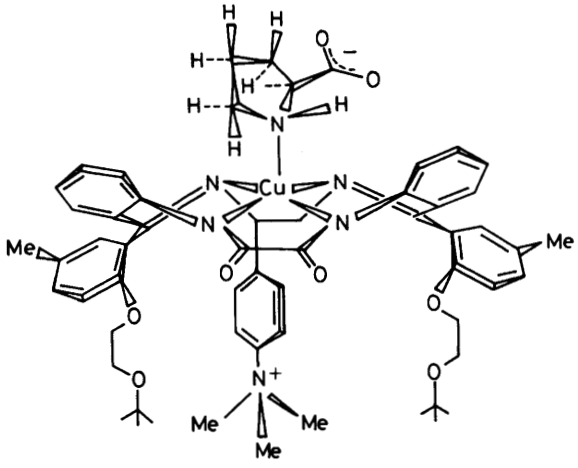


Figure 2. Complex of (1) with the anion of Pro, drawn in accordance with T_{1M} measurements of its protons and ^{13}C nuclei in the presence of (1). The *cis*-α,*cis*-β isomer of (1) has been arbitrarily selected for the drawing

13 spin–lattice relaxation studies (Table 5) support the representation; the calculated distances of the Pro carbon atoms from the paramagnetic atom are also included in the table. There is a preference for an envelope conformation of Pro in the complex, with C_β, C_γ, and C-carboxy atoms turned away from the co-ordination plane of the complex. The distance between the Pro nitrogen atom and the metal ion is within 2.2—2.6 Å. Significantly, there is no detectable difference in relaxation rates between the L and D enantiomers of Pro in the presence of complex (1) (Table 1).

Most probably, Pro co-ordinates to complex (1) on the side opposite to the anilinium substituent of the ethylenediamine moiety (Figure 2), because the other side is sterically shielded by the protons of the substituent, although co-ordination on the same side cannot be ruled out, especially in non-aqueous solutions where electrostatic interactions may play a major role.

The spin–lattice relaxation rate of water is influenced by the addition of (1). For instance, the relaxation time of water (mole ratio of $^2H_2O/H_2O = 9:1$) in a 0.58 mol dm^{-3} solution of Pro is equal to 10 s. Addition of complex (1) ($N_P/N_S = 5 \times 10^{-4}$) changes the value to 5.5 s. Addition of the same amount of (1) to pure water ($^2H_2O/H_2O = 9:1$) gives a value of T_1 of 6.7 s. This means that the value of T_1 for water decreases upon addition of Pro to (1); in other words, some of the water molecules are brought together with Pro into the vicinity of the copper(II) ion.

Complex (1) *as a Carrier of α-Amino Acid Anions.*—The amino acids were extracted into $CHCl_3$ in the anionic form with complex (1). No discrimination between the enantiomers of the amino acids was observed. Neutral, zwitterionic forms of amino acids cannot be extracted, as was shown by special experiments. Partition experiments were conducted at pH 9.15 to avoid coextraction of the hydroxide ion, which is likely to occur at higher pH. The conditional extraction constants E^1_{QA} of phenylalanine (Phe), leucine (Leu), proline, and hydroxyproline

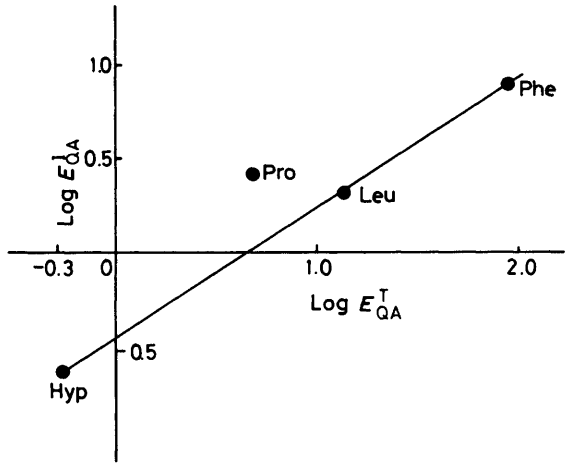


Figure 3. Correlation of the extraction constants of the anions of Hyp, Pro, Leu, and Phe with complex (1) (log E^{I}_{QA}) and tetrapentylammonium iodide (log E^{T}_{QA}). The point for the most basic Pro is not included

(Hyp) were correlated with the literature data[5a] (E^{T}_{QA}) on the extraction of these amino acids into methylene chloride with tetrapentylammonium iodide. The latter set of extraction constants reflects only the relative hydrophobic properties of the amino acid side chain,[5a] because there are no specific interactions between the tetrapentylammonium cation and the anions. Any deviation from a unit slope of the correlation between the two sets of data is meaningful, since it testifies to the existence of specific interactions between complex (1) and the amino acid anions in either water or an organic solvent. The observed correlation is, at best, modest ($\rho = 0.95$) and the data show a significant levelling effect of (1) upon the extraction of the amino acid anions, the slope of the straight line being 0.66. When only three amino acids with a narrow range pK_a (9.15—9.74[11]) were used the correlation was improved ($\rho = 0.999$); the point for Pro, the most basic amino acid[11] (pK_a = 10.64), noticeably deviated from this three-point correlation (Figure 3). Specific effects connected with apical co-ordination and podand-group participation may play a part in the observed effects.

We believe that the basic structure of complex (1) may be further developed. For example, atropoisomers of (1) could be separated, podands substituted with crown ether structures, and, instead of the phenyl group bearing the quaternary ammonium group, another flexible positively charged group introduced. Modulation of the acceptor properties of the central metal ion may be achieved if electron-withdrawing groups are introduced into the molecule. Eventually, a system capable of three-point attachment of amino acid anions may be created and a way for chiral recognition of amino acid anions opened. Design of receptors that are specific for a particular type of α-amino acids could also be envisaged.

Experimental

The stock solution of complex (1) was prepared by dissolving the complex in a volumetric flask (usually 15 cm^3) containing deuterium oxide. A syringe (10 cm^3) was used to insert aliquots of (1) into a volumetric flask containing 0.58 mol dm^{-3} Pro solution in D_2O. The samples had been carefully degassed using freeze-pump-thaw cycles before the n.m.r. experiments were commenced.

N.m.r. experiments were conducted using a Bruker WP-200 CXP-200 spectrometer. To measure T_1, a standard inversion-recovery pulse sequence (180°–τ–90°) was used; T_2 was measured using the Carr–Purcell–Meiboom–Gill spin-echo method [90°–(t–180°–t)$_n$; t = 1 ms]. To measure relaxation times, 11—14 different values of the variable parameters (τ or n) were used, chosen in accordance with roughly estimated T_1 and T_2. The continuity of the 90° pulse was 4.1 μs for ^1H and 8.3—8.6 μs for ^{13}C. The sample temperature was controlled with the temperature block of the spectrometer, and the experiments were usually commenced after the sample had been kept for 30 min in the block. The T_1 and T_2 data were calculated using a three-parameter fitting procedure taken from the DISNMR program.

Chemicals and Reagents.—Amino acids were purchased from Reanal and used without further purification. Chloroform was purified in the usual manner. The NaOH solution was prepared by dissolving metallic sodium under Ar in water from which CO_2 was removed.

Determination of Partition Ratios.—To an aqueous phase (10 cm^3) containing known amounts of a mixture of Pro, Hyp, Leu, and Phe (4.6×10^{-3} mol dm^{-3} each) brought to pH 9.5 was added $CHCl_3$ (10 cm^3) containing (1) (4.6×10^{-3} mol dm^{-3}). The mixture was stirred for 3 h, after which the organic and the water layers were separated and the concentration of (1) in each was determined spectrophotometrically. The amino acids in the aqueous solutions were determined after purification on Dowex 50 using g.l.c.[12] The organic layer was diluted six-fold with another portion of $CHCl_3$, and washed several times (3×15 cm^3) with 0.05 mol dm^{-3} HCl aqueous solution to remove amino acids. An aliquot of valine (Val) solution was added to the acidic aqueous extracts, and the solution was evaporated. The residue was purified on Dowex 50 (H^+ form), and quantitative and enantiomeric analysis of the amino acids was carried out using g.l.c.[12] In this way the total concentration of each α-amino acid in the organic layer was determined.

The observed extraction constant was calculated using equation (7) where $[QA]_{OP}$ is the total concentration of a

$$E^{I}_{QA} = [QA]_{OP}/[Q^+]_{WP}[A^-]_{WP} \quad (7)$$

particular α-amino acid in the organic solution, presumed to exist as an ion pair with complex (1), $[Q^+]_{WP}$ is the total concentration of (1) in aqueous solution, and $[A^-]_{WP}$ is the concentration of the anion of the amino acid in aqueous solution, calculated from equation (8) where $[A]_T$ is the total

$$[A^-]_{WP} = \frac{K_a([A^-]_T - [QA]_{OP})}{K_a + a_{H^+}} \quad (8)$$

concentration of the α-amino acid in aqueous solution before the partition experiment, K_a is the ionization constant of the amino acid, and a_{H^+} is the activity of hydrogen ions in the aqueous solution.

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