Metal-ion Complexation by a New Urea Macrocyclophane

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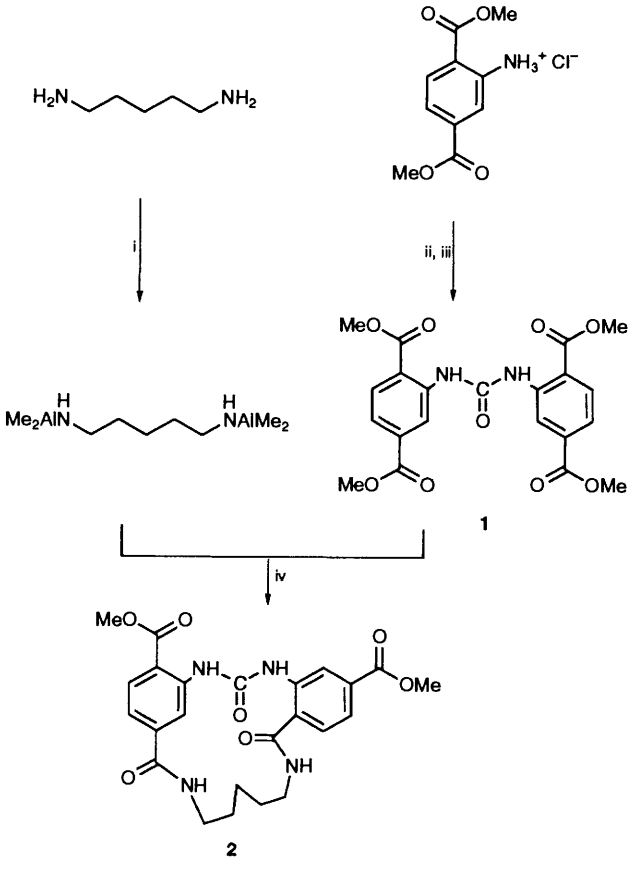
A challenge in the biosensor area is to create artificial receptors capable of reversible binding with biological target analytes. Here we present the design, synthesis and characterization of a fluorescent urea macrocyclophane (**2**) which forms complexes with Zn^{II} and Cd^{II} ions. The metal-binding activity was characterized by fluorescence, FAB and FTIR analysis. The Zn^{II}–**2** complex is a tetrahedral tetracoordinated system involving two macrocyclophane molecules and one Zn^{II} ion.

Our laboratory has been involved for several years in the development of biosensors for the detection and measurement of various biological analytes in complex media.[1] A biosensor results from the association of a biological element capable of the molecular recognition of a target analyte with a transducer providing an electrical signal dependent on the concentration of the analyte. Different types of transducer can be used, either electrochemical, thermal or optical or based on mass variation.[2] For instance, enzymes which exhibit a very high specificity for their substrate can be used in immobilized form on artificial membranes closely associated with a sensitive electrode. The transformation of the substrate by the enzyme involves both molecular recognition and catalytic action. Considering the molecular mechanisms dealing with these two steps has led to the idea that host–guest chemistry can be a useful approach for substrate–receptor biochemistry in the biosensors area. Consequently, the design and construction of artificial receptors has become attractive. Ultimately, the immobilization of such a molecular receptor intimately associated with a transducer would be the first step towards the emergence of a new generation of biomimetic chemical sensors based on artificial molecular receptors.[3]

Our aim is to develop a number of systems capable of reversible binding with metals, amino acids, amines and acids. A possible route for the detection of such metabolites might be the modification of the fluorescence emission of the host after binding with its guest.[4] For such a purpose, we have considered the synthesis of a cyclophane macrocycle. Cyclophanes represent the central class of synthetic hosts in the molecular recognition field.[5]

We chose the urea macrocyclophane shown in Scheme 1 for several reasons. (1) The structure of this molecular receptor is sterically well defined by the presence of conformationally restrictive bonding arrays such as hydrogen bonds and *para*-substituted aromatics. With this approach, we simplify the conformational problem due to the high flexibility of small molecules such as cyclopeptides and consequently, predicting binding properties is less difficult. (2) Powerful dipoles are needed to bind a guest: amide and particularly urea moieties are suitable for this. (3) A water soluble receptor is required for hypothetical use in a chemical sensor designed for biological media. (4) The urea macrocyclophane which is a fluorescent molecule should be useful, owing to the usually high sensitivity of the fluorescent signal detection of complexation.

Starting from the dimethylaminoterephtalate (DMAT), which is fluorescent, the tetramethyl 2,2′-ureylenediterephthalate **1** was readily synthesized. Cyclisation with pentane-1,5-diamine using trimethyl aluminium under high dilution conditions led to the urea macrocyclophane **2** (Scheme 1). The macrocycle was characterized by ^1H NMR and FAB mass spectra. It bears two methyl ester groups which may be utilized for a further functionalization. The length of the pentane bridge is of crucial importance since it is too short to give rise to a symmetrical macrocycle which should result from bridging of both the *o*-methyl or *m*-methyl ester groups of the ureylenediterephthalate but it is just the right size to permit cyclisation after rotation about the Ar–NH bonds. Conversely, a butane bridge would be too short to lead to the formation of the urea macrocyclophane. Thus, the urea macrocyclophane obtained after pentanediamine cyclisation is an asymmetrical molecule. This is illustrated by the six NMR signals of the aromatic protons which were assigned by NOESY. Another



Scheme 1

† Fax (33) 72 44 28 34.

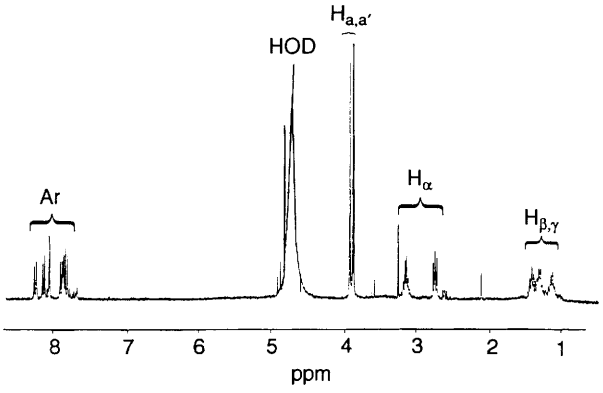


Fig. 1 300 MHz ^{1}H NMR spectrum (D_2O; c = 2 mmol dm^{-3}) of the 13,21-bis(methoxycarbonyl)-1,9,17-trioxo-2,8,16,18-tetraaza[9.3]-metaorthocyclophane

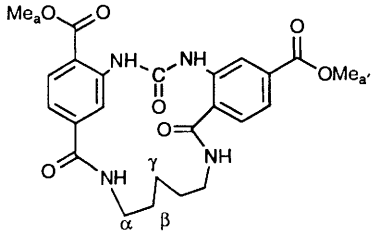


illustration of the asymmetry of **2** is represented in the 300 MHz ^{1}H NMR spectrum (Fig. 1) by the protons of the α- and β-methylene groups of the pentane bridge which are not equivalent on both sides of the γ-methylene group especially for both α-methylene groups. Molecular mechanics calculations led us to the following hypothesis taking into account this behaviour. If we consider the tension of the macrocycle, the amide bonds should not be equivalent, one might be in the *cis* conformation, the other in the *trans* conformation and this would lead to different chemical shifts of the neighbouring methylene protons as exhibited by the spectrum. Indeed, direct proof confirming the non-equivalence of the amide bonds is necessary to verify this hypothesis.

The urea macrocyclophane is readily soluble in water, methanol and ethanol. It is slightly soluble in dichloromethane and chloroform. Its critical aggregation concentration (CAC) in water was above 20 mmol dm^{-3}, as determined by ^{1}H NMR spectroscopy. Consequently, all our complexation experiments in water were conducted at a macrocycle concentration close to 2 mmol dm^{-3}. In these conditions, 15–20% of macrocycle are in the dimer form, even at very low concentration (0.1 mmol dm^{-3}, as determined by 500 MHz ^{1}H NMR spectroscopy). Compared with the NMR spectrum of the monomer, the dimer spectrum is characterized by the slight high-field shift of the proton signals of one of the two α-methylene groups of the pentane bridge. There is also a slight high field shift of some of the aromatic proton signals. The FAB mass spectrum confirmed the formation of dimer.

As a consequence of the monomer and dimer forms coexisting in water solution, the urea macrocyclophane gives a relatively broad fluorescence emission spectrum. At alkaline pH the intermolecular excimer exhibits the characteristic red (bathochromic) shift of the emission band and, from the low degree of shifting ($\Delta\nu \approx 760$ cm^{-1}) it may be concluded that the binding energy in the singlet excimer is low[6,7] and consequently that the intermolecular interactions are weak. Based on the approach developed by Staab *et al.*,[8] this would also indicate a relatively large distance between the two monomers.

This dimer formation led us to the idea that this macrocycle might undergo complexation with metal ions and particularly with transition-metal ions. Owing to the biological interest in the zinc(II) ion we focused our experiments on the Zn^{II}–urea macrocyclophane complex. Cd^{II} was also compared with Zn^{II} because Cd^{II} is often used in place of Zn^{II} in NMR studies of metal complexation in which metal binding modes in Cd-substituted metalloproteins that normally contain Zn are investigated.[9] Experiments were conducted using the fluorescence properties of the molecule. Upon addition of metal chlorides at neutral pH, an appreciable quenching of the urea macrocyclophane fluorescence was observed. The magnitude of this quenching was dependent on the nature of the metal and varied with the amount of metal ion added. Two sets of typical experimental data obtained at pH 7.5, with the Zn^{II} in a buffered solution of 0.1 mmol dm^{-3} urea macrocyclophane, are shown in Fig. 2. The Cd^{II} fluorescence decrease at 391 nm was weak, < 4%. Similar results were obtained in water. It must be stressed that when the Zn^{II} and Cd^{II} chlorides, which are easily hydrolysed, were added at a concentration above 0.05 mmol dm^{-3}, partial precipitation of polymeric metal hydroxides (in water) or hydroxy phosphates (in phosphate buffer) occurred. The amount of Cd^{II}–macrocycle complex which could be obtained was too low to be detected in the FAB mass spectrum. The Zn^{II}–macrocycle complex could be detected by FAB mass spectrometry by the presence of a peak due to $[(M)_2Zn^{2+}Cl^-]^+$ (with M = the urea macrocyclophane). As seen in Fig. 2(*b*), upon complexation, a red shift of the fluorescence emission band was observed. Concurrently, the ^{1}H NMR spectrum of the Zn^{II}–macrocycle complex indicates a slight increase in the amount of the dimer form, at the beginning of the addition of $ZnCl_2$ to a 2 mmol dm^{-3} aqueous solution of the macrocycle. However, in contrast with the fluorescence method using concentrations in the range 10^{-1} mmol dm^{-3}, for NMR, a concentration close to 2 mmol dm^{-3} was necessary for titration, but at this concentration the method is rapidly limited by the partial precipitation of zinc hydroxide. Complexation of various metal ions by the macrocycle over a wide pH range is currently under investigation.

With the aim of knowing more about the mode of complexation of zinc with the urea macrocyclophane, the Zn^{II}–macrocycle complex was studied by FTIR spectroscopy in the form of KBr pellets. The carbonyl stretch frequency of the amides and urea groups of the free urea macrocycle is 1674 cm^{-1}. Upon complexation with the Zn^{II} ion the frequency is lowered by 41 cm^{-1}, implying coordination through oxygen,[10] which is the standard mode of complexation of transition-metal ions to amide and urea groups. This is attributed to the higher basicity of the oxygen lone pair compared with the nitrogen site.[11] With these data, we conclude that the zinc(II)–urea macrocyclophane complex is a tetrahedral tetracoordinated system: three of the coordinations of the zinc ion involve urea and amide carbonyl oxygens of two macrocycles and the fourth implies a chloride ion.

Experimental

Synthesis of Tetramethyl 2,2'-Ureylenediterephthalate (**1**).—Diphosgene (4.8 g, 23.5 mmol) was added to a solution of dimethyl aminoterephtalate hydrochloride (1.5 g, 6.1 mmol) in dry dioxane (30 cm^3) and stirred at 60 °C for 10 h. The solvent and excess of diphosgene were evaporated off under reduced pressure. The crude product was redissolved in dry dioxane (30 cm^3) and dry dimethyl aminoterephtalate (DMAT) (1.7 g, 8.13 mmol) was added. The mixture was stirred at 80 °C and compound **1** began to precipitate after 20 min. The reaction was allowed to continue for 2 h. The substituted urea obtained was filtered off and washed with dioxane. Evaporation of the residual solvent under reduced pressure gave a white solid

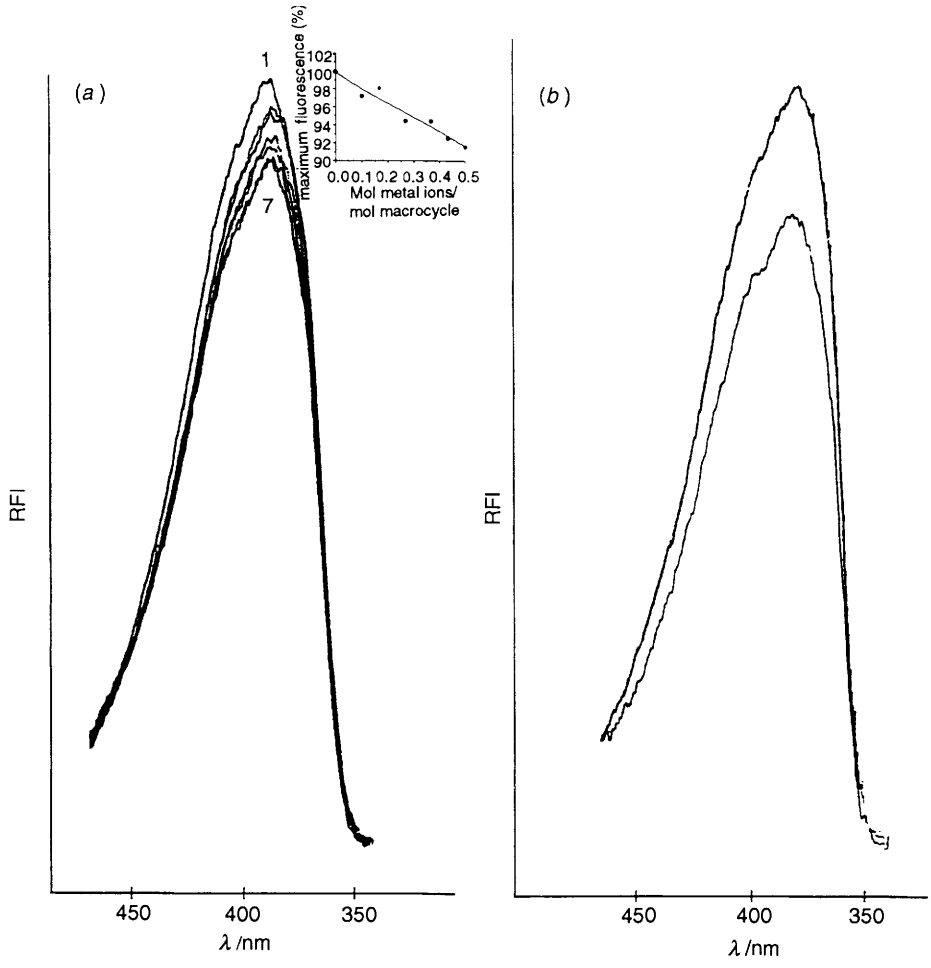


Fig. 2 (*a*) Electronic emission spectra in 0.05 mol dm^{-3} pH 7.5 phosphate buffer of the urea macrocyclophane in the absence (spectrum 1) and in the presence of Zn^{II} (spectra 2–7). [**2**] is 0.1 mmol dm^{-3}; λ_{exc} = 332 nm. From spectra 2–7 the Zn^{II} concentrations are 10, 17, 27, 37, 44 and 50 μmol dm^{-3}, respectively. Inset: corresponding decrease of the fluorescence emission intensity as a function of the Zn^{II}/**2** molar ratio; λ_{em} = 391 nm. (*b*) Electronic emission spectrum of the urea macrocyclophane in the presence of 0.3 mmol dm^{-3} Zn^{II} (Zn^{II}/**2** molar ratio = 3), compared with the free **2** emission spectrum (upper spectrum). RFI = relative fluorescence intensity.

(1.26 g, 46%) (Found: C, 56.05; H, 4.6; N, 6.25; O, 31.35. Calc. for $C_{21}H_{20}N_2O_9$: C, 56.76; H, 4.54; N, 6.30; O, 32.4%); δ(300 MHz; $CDCl_3$) 10.80 (2 H, s, NH), 9.20 (2 H, d, $J_{3,5}$ = 2 Hz, ArH^3), 8.10 (2 H, d, $J_{6,5}$ = 8.5 Hz, ArH^6), 7.69 (2 H, dd, $J_{5,6}$ = 8, $J_{5,3}$ = 2 Hz, ArH^5), 3.99 (6 H, s, OCH_3) and 3.95 (6 H, s, OCH_3); m.p. 234–235 °C.

Synthesis of the 13,21-*Bis*(*methoxycarbonyl*)-1,9,17-*trioxo*-2,8,16,18-*tetraaza*[9.3]*metaorthocyclophane* (**2**).—Trimethylaluminium (1.12 cm^3 of a 2.0 mol dm^{-3} solution in hexanes; 2.24 mmol) was added under nitrogen to 10 cm^3 of dry chloroform. The mixture was cooled with salt–ice at −10 °C and 134 mm^3 (1.12 mmol) of diaminopentane-1,5-diamine were slowly added with a syringe through a septum. The mixture was stirred for 20 min at −10 °C and then 45 min at room temperature. After addition of 160 cm^3 of dry chloroform, the mixture was cooled to 0 °C and a solution of **1** (250 mg, 0.562 mmol) in chloroform (30 cm^3) was rapidly added. The reaction mixture was stirred under nitrogen for 12 h at 0 °C then 24 h at 60 °C. After this time the chloroform was evaporated off under vacuum. The product was redissolved in methanol and by-products were filtered off. The filtrate was dried under vacuum and redissolved in water. Insoluble by-products were removed again and after evaporation of the water, the product was dried under vacuum and flash-chromatographed on a silica column with 80:20 CH_2Cl_2–CH_3OH as the eluent to afford 80 mg (0.166 mmol, 30%) of a white solid.* R_f (silica gel; 20% MeOH in CH_2Cl_2) 0.5; δ(300 MHz; D_2O, 2.3 × 10^{-3} mol dm^{-3}): 8.27–7.81 (6 H, ArH), 3.98 and 3.93 (6 H, s, OCH_3), 3.33–2.66 (4 H, m, H_α AB systems) and 1.54–1.23 (6 H, m, H_β + H_γ); m.p. > 340 °C (decomp.); *m*/*z* (FAB) 483 [(*M*H)$^+$], 505 [(*M*, Na)$^+$], 965 [(2*M*H)$^+$] and 987 [(2*M*, Na)$^+$] (Found: (2MH)$^+$, 965.3768. Calc. for (2*M*H), 965.3681].

* Microanalysis of compound **2** was unsatisfactory. The purity of the product was determined by TLC.

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