

Structural aspects of metal complexes with functionalized azamacrocyclic ligands

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Abstract. The systematic study of the structures of metal complexes with mono- and tetra-N-functionalized azamacrocycles carrying carboxylic groups in their side chains shows the importance of the cavity size of the macrocycle, of the nature and length of the side chain and of the ionic radius of the metal ion on the coordination geometry. Applications of such complexes as NMR-contrast agents or to label monoclonal antibodies for tumour diagnosis and therapy are also discussed.

INTRODUCTION

The functionalization of macrocycles allows to modify or introduce new properties into a ligand: it can make it more selective towards a metal ion, it can increase the thermodynamical stability and the kinetical inertness, it can change the solubility and extractability into an organic phase and it can allow to covalently attach the macrocycle to a polymeric support or to a protein (ref.1).

Functionalization can be achieved either using a carbon or a nitrogen atom of the ring as attaching point. The carbon substituted derivatives have the advantage of not influencing the nature of the heteroatomic donor group and are ideal for side chains, which do not coordinate. A pendant chain attached to a nitrogen, on the other side, can be designed so that five or six-membered chelate rings are formed, when the side chain donor group binds to the metal ion, without a too strong deformation of the macrocycle. Of course a combination of both type of functionalization offers all the advantages and allows to fulfil several functions at the same time.

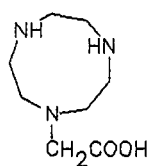
In this paper we shall concentrate on azamacrocycles functionalized with carboxylate side chains, since they have been studied in great detail and a large number of structures have been solved. Of course, what is said in this special instance can easily be transferred to other types of functionalized macrocycles.

MONO-N-FUNCTIONALIZED DERIVATIVES

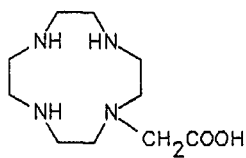
The monofunctionalization of azamacrocycles with a side chain carrying a carboxylate group is relatively simple (ref. 2). An excess of macrocycle over the alkylating agent, generally a 5:1 ratio is used, is sufficient, to ensure that statistically the mono-substituted derivative becomes the main product of the reaction. The separation of the excess of unreacted macrocycle from the mono-substituted compound is easy, since from alkaline solutions the macrocycle can be extracted with an organic solvent such as CHCl_3 or CH_2Cl_2 , whereas the carboxylate substituted derivative remains in

the aqueous phase, from which it can be isolated as hydrochloride. With this general procedure a series of compounds starting from [9]aneN₃, [12]aneN₄ and [14]aneN₄ (ref. 3) have been prepared.

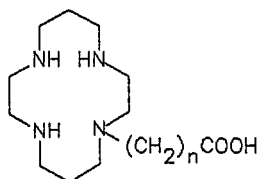
As a first example we shall discuss the mono acetate derivative of [9]aneN₃ (1), the Cu²⁺ complex of which was studied by pH-titrations in solution as well by X-ray diffraction in the solid state (ref. 4). Equilibrium measurements show that in solution the species Cu(L)⁺, Cu(L)₂, Cu(L)OH and Cu₂L₂OH⁺ are formed. From crystallisation experiments at different pH values, at which these species are present, it was possible to obtain crystals of [CuL]ClO₄ and [Cu₂L₂OH]ClO₄ suitable for an X-ray diffraction study.



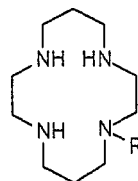
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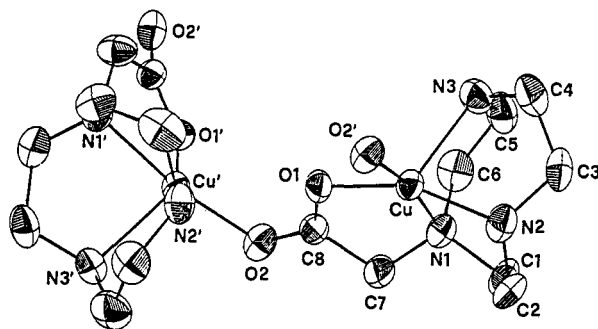
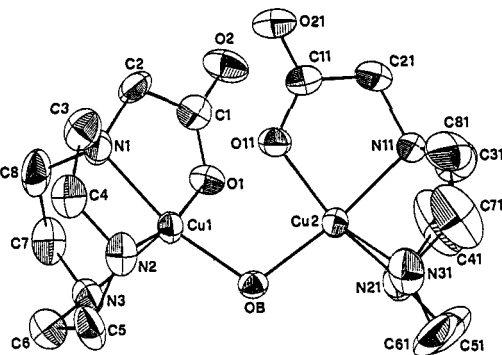
2



3 n = 1, 2, 3

4 R = o-C₆H₄-COOH5 R = p-C₆H₄-COOH

In [CuL]ClO₄ the metal ion is pentacoordinate by the three nitrogens of the macrocycle, by the carboxylate of the same macrocycle and by a second carboxylate oxygen stemming from a different molecule as bridging unit (Figure 1). The same pentacoordinate geometry is also found in the binuclear complex [Cu₂L₂OH]ClO₄. In this complex beside the three nitrogens and the carboxylate from one macrocycle there is an OH⁻ group as bridging ligand which completes the coordination sphere (Figure 2). The Cu-Cu distance is 3.17 Å.

Figure 1. Structure of [CuL]ClO₄ with 1Figure 2. Structure of [Cu₂L₂OH]ClO₄ with 1

In the structure of the Cu²⁺ complex of the monoacetate derivative of [12]aneN₄ (2), obtained at acid pH, at which generally the carboxylic group is protonated, we find that indeed the carboxylic group is not coordinated (ref. 5). The Cu²⁺ is bound by the four nitrogens of the macrocycle and a Cl⁻ ion in a square pyramidal structure and is above the best plane through the four nitrogens by 0.539 Å in the direction of the Cl⁻, the Cu-Cl bond being 2.387 Å (Figure 3).

The most throughout study was run with the 14-membered macrocycle, introducing a series of pendant carboxylic groups with different chain lengths (3, n=1, 2, 3) and structures (4 and 5) (ref. 2). The systematic study of the length of the pendant arm was done to investigate the coordination tendency of the carboxylate of the side chain. In solution this can easily be followed by VIS-

spectrophotometry, since the Cu-chromophore changes, when the carboxylate is displaced from the metal ion by protonation according to Eqn 1.

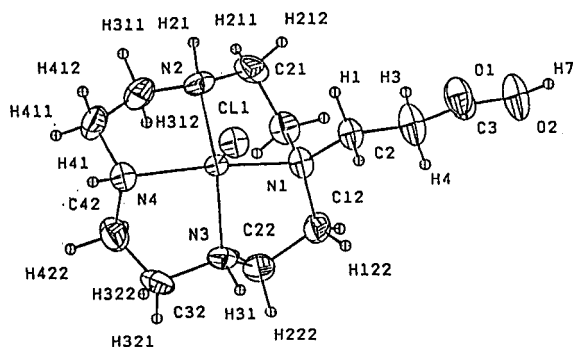


Figure 3. Structure of $[\text{CuL}]\text{Cl}$ with 2

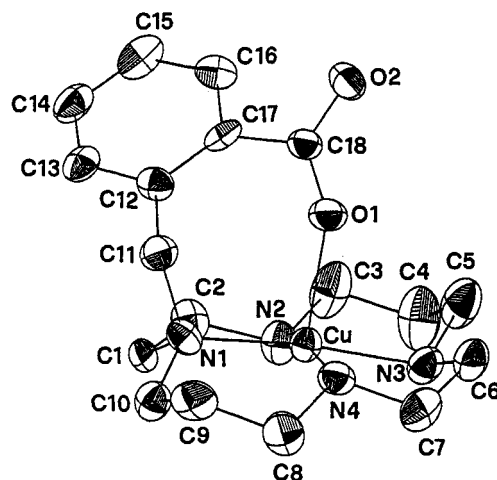


Figure 4. Structure of $[\text{CuL}]\text{ClO}_4$ with 4

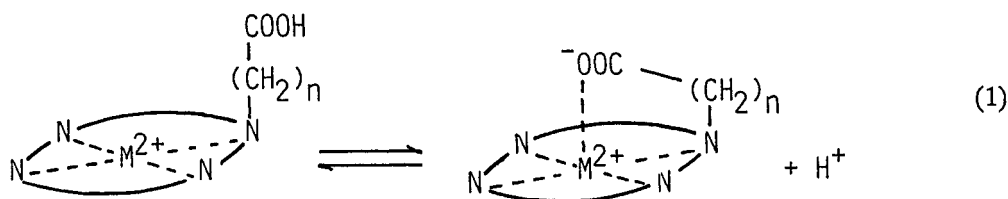


Table 1 shows that in acidic solution the spectra of the complexes with 3 closely resemble each other and that of $[\text{14}] \text{aneN}_4$. When the pH is shifted to neutral the acetate (3, $n=1$) and propionate (3, $n=2$) derivatives undergo a spectral change, the absorption maximum being shifted to longer wavelengths. This indicates that after deprotonation of the carboxylic group, the carboxylate binds to the metal ion. For the Cu^{2+} complex of the derivative with the butyric acid side chain (3, $n=3$) no spectral change is observed by changing the pH, so that we must infer that in this case depro-

Table 1. Absorption maxima (in nm) and molar absorptivities (in $\text{M}^{-1}\text{cm}^{-1}$) of the Cu^{2+} complexes with $[\text{14}] \text{aneN}_4$, 3 ($n=1, 2, 3$), 4 and 5 in acidic and neutral solution

	$[\text{14}] \text{aneN}_4$	3, $n=1$	3, $n=2$	3, $n=3$	4	5
acidic	510 (125)	514 (97)	512 (113)	506 (119)	516 (80)	516 (98)
neutral	510 (123)	547 (98)	533 (123)	508 (124)	544 (123)	514 (94)

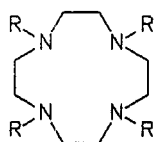
nation does not automatically imply coordination of the side chain donor group. In the acetate and propionate derivatives a five- and six-membered chelate ring, respectively, is formed, and it is well known that these are the most stable ones. A seven-membered chelate would develop in the case of 3, $n=3$ and this is not ideal for coordination.

However, when the more rigid *o*-toluic acid side chain (4) is introduced, coordination of the carboxylate to give a seven membered ring is observed in solution (Table 1). Two complexes of this ligand, which correspond to the protonated and deprotonated species, were isolated as solids (ref. 6). Within the limits of experimental error, the bond lengths and angles in the two complexes are

similar. In both cases the macrocycle is in the *trans-III* configuration (ref. 7). The Cu^{2+} is square pyramidally coordinated by four nearly coplanar nitrogens and an axial oxygen (Figure 4). The distance of the metal ion to the best N_4 -plane is 0.18 and 0.11 Å, respectively. The fact that the axial O atom originates from a carboxylate and from a carboxylic group has only little effect on the coordination geometry of the Cu^{2+} . The only significant difference between the two Cu^{2+} complexes is observed in the carboxylate and carboxylic group. In the carboxylate the two C-O bonds are similar to each other, whereas in the carboxylic group they are distinctly different, the shorter being that of coordinated oxygen.

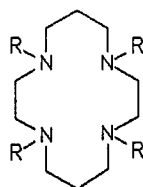
TETRA-N-SUBSTITUTED DERIVATIVES

The synthesis of these compounds is simple since with an excess of alkylating agent each nitrogen of the macrocycle can be substituted (ref. 8). Thus compounds such as 6, 7 and 8 can be prepared.



6 R = CH_2COOH

7 R = *o*- C_6H_4 -COOH



8 R = CH_2COOH

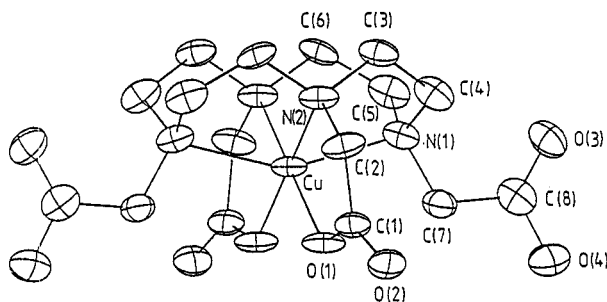


Figure 5. Structure of CuLH_2 with 6

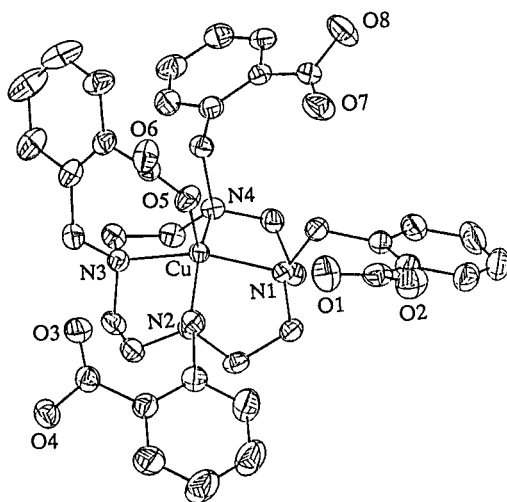


Figure 6. Structure of $[\text{CuL}]\text{ClO}_4$ with 7

The most well studied compound is DOTA (6), for which structures of different metal complexes are available. In the 1:1 complexes with Cu^{2+} , Ni^{2+} (ref. 9) and Zn^{2+} (ref. 10) the same *cis*-octahedral geometry with a folded macrocycle in the *cis-I* configuration is observed (Figure 5). The metal ion is coordinated by the four nitrogens of the macrocycle and two carboxylate oxygens of the side chains. The other two acid groups are not bound and point away from the metal ion, which being hexacoordinated is coordinatively saturated. The two nitrogens and the two oxygens from the acetate groups attached to these nitrogens form a plane in which the metal ion situated. The other two nitrogens are axially bound. The $\text{N}_{\text{ax}}\text{-M-N}_{\text{ax}}$ angle, which in all complexes is between 154° and 159° degree, is distinctly smaller than the angle of 180° expected for an octahedron. This is probably due to the strain inherent in the 12-membered macrocycle, which does not allow the nitrogens to occupy the ideal position. Interesting is also the comparison between the structure of the Cu^{2+} complex and those of the two other metal ions. The difference in the axial and equatorial N-bonds is relatively small for Ni^{2+} and Zn^{2+} , whereas it is pronounced for Cu^{2+} , because of the Jahn-Teller distortion in the d^9 -ion.

In the complex with **7** the Cu^{2+} is pentacoordinated by the four nitrogens from the macrocyclic ring and one carboxylate from a side chain (ref. 11). The geometry is square pyramidal, the four nitrogens forming a nearly perfect plane ($\pm 0.019 \text{ \AA}$) and the Cu^{2+} being 0.49 \AA out of this plane (Figure 6). The Cu-O bond with 2.08 \AA is relatively short for an axial bond. In this ligand the carboxylate oxygen is not completely free to take up any position, since it is part of a relatively rigid side chain, which determines the optimal distance between the Cu^{2+} and the oxygen atom.

A comparison of the structures of the Cu^{2+} complex with **6** and **7** shows that although in both cases the macrocycle is folded in the *cis-I* configuration, in the complex with **6** two carboxylates bind to the metal thus giving a *cis*-octahedral geometry, whereas in the complex with **7** only one carboxylate is coordinated in a square-pyramidal structure. This might stem from the different sterical requirements of the two types of side chains and their reciprocal interactions.

The structures of the lanthanide complexes with DOTA are completely different from those just discussed. Because of the larger ionic radius and the higher coordination number the ligand can bind with all eight donor groups (ref. 12). For Gd^{3+} a capped antiprism, in which the four nitrogens, the four carboxylates of the side chains and an additional water molecule are coordinated, was found. Beside the 1:1 species a 2:1 Cu^{2+} complex with DOTA was also studied (ref. 13). The structure consists of a sheet, in which two well defined structural units are connected. One of these consists of a Cu^{2+} species exactly similar to the complex $\text{Cu}(\text{LH}_2)$ discussed above. The Cu^{2+} is coordinated by four nitrogens and two carboxylates in a *cis*-octahedral geometry. Each of the two carboxylic chains, which are not involved in this unit and point away, is used to built up a two Cu^{2+} /four carboxylate moiety, similar to that found in $\text{Cu}_2(\text{acetate})_4$. So four carboxylate chains coming from four different macrocyclic units converge to bind two Cu^{2+} ions.

The structures of the Cu^{2+} and Zn^{2+} complexes of TETA (**8**) show a *trans*-octahedral geometry, four nitrogens and two carboxylates being coordinated (ref. 14). The other two carboxylate are not used for coordinative bonds and point away from the metal ion (Figure 7). In contrast to the structures of the complexes with DOTA, the metal ions sits in the centre of the macrocycle, which assumes the *trans-III* configuration. The large 14-membered ring is able to encompass the metal ion and does not need to fold as the 12-membered ring does.

In contrast to the binuclear complex with DOTA described above, the complex Cu_2TETA has two Cu^{2+} in a similar environment (ref. 13). Each Cu^{2+} is coordinated outside of the ring by two nitrogens, two carboxylates coming from the side chains attached to those nitrogens and an additional O donor, which is either a water molecule or a bridging oxygen.

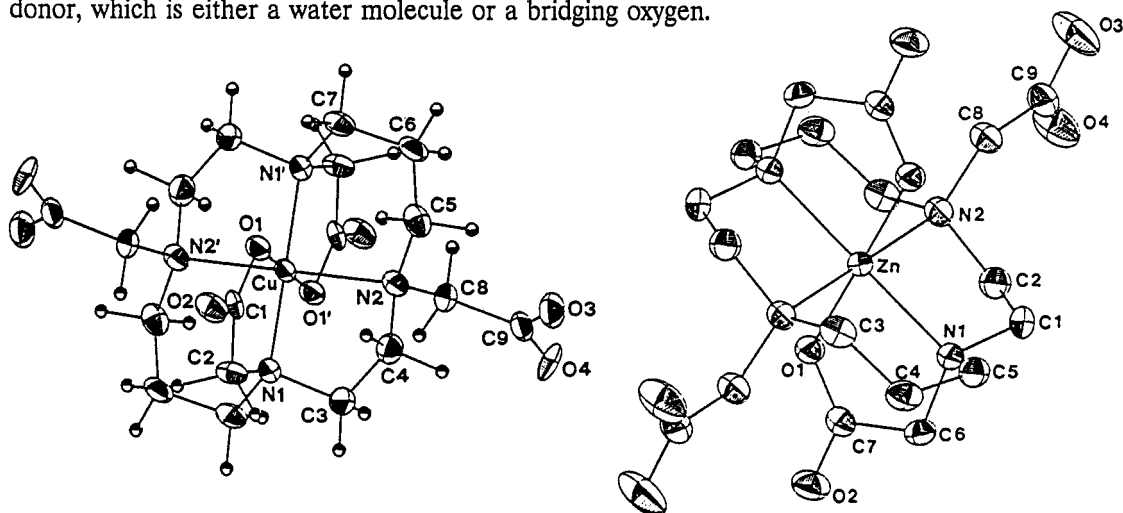


Figure 7. Structures of $\text{Ba}[\text{CuL}]$ and $\text{Zn}(\text{LH}_2)$ with **8**

APPLICATIONS

Because of the high thermodynamical stability and the kinetical inertness metal complexes of functionalized macrocycles have a high potential for medical applications, since they do not dissociate under physiological conditions.

A first application in the field of magnetic resonance imaging has been described for $\text{Gd}(\text{DOTA})^-$ as contrast agent (ref. 15). The compound, the structure of which was described above, exhibits a high stability constant (ref. 16) and a extremely slow acid induced dissociation kinetics (ref. 17). Interesting is also the observation that $\text{Gd}(\text{DOTA})^-$ has a very low toxicity, in contrast to that of the single components. $\text{Gd}(\text{DOTA})^-$ is a powerful NMR-contrast agent, due to the high magnetic moment and the fact that one water molecule can exchange very fast. $\text{Gd}(\text{DOTA})^-$, injected into the blood in relatively high concentrations, remains as a charged species in the blood and is excreted through the kidney. In the case of the brain tumour, when the blood-brain barrier is destroyed, the Gd^{3+} complex, however, can penetrate the tumour and change the relaxation rate of the water there included. In NMR imaging this can clearly be seen and the tumour can thus be diagnosed.

The second field of applications is the labelling of monoclonal antibodies for diagnostic and therapeutic purposes (ref. 18). The example stems from our work on monofunctionalized macrocycles and shows an application of ^{64}Cu or ^{67}Cu as radionuclides. The schematic description of the process is given in *Figure 8*.

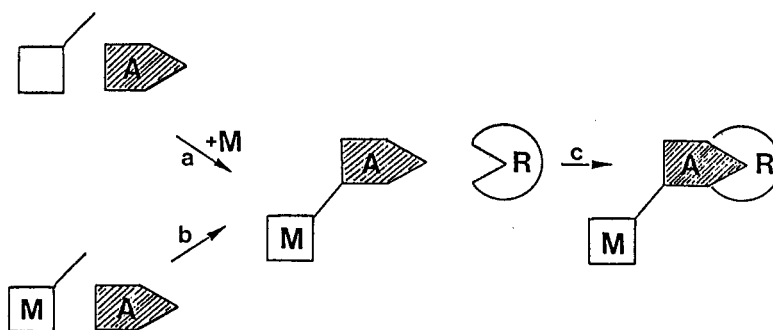


Figure 8. Antibody labelling with a macrocyclic metal complex: a) postlabelling; b) prelabelling and c) antibody/receptor complex formation.

An antibody A is coupled with a macrocyclic ligand or its metal complex to give a chemically modified protein. If the metal ion M is a radionuclide this allows to follow the antibody and to observe its fixation as an antibody-receptor complex in cancerogenic cells.

Synthetical and structural studies on mono-N-substituted derivatives with a pendant carboxylic function have shown that the compound with the p-toluilic group **5** has the greatest potential for an application, since the side chain does not interact with the metal ion. Model reactions with amines have shown that the carboxylic group can be activated either with dicyclohexylcarbodiimide or through a cyanomethylester to form amides in a well known reaction used for peptide condensation (ref. 19). In this case the Cu^{2+} plays the role of a protecting group for the secondary nitrogens of the macrocycle and thus prevents the formation of amides by an intra- or intermolecular reaction. The same reaction applied to bovine serum albumin as a test protein gave a product, which depending on the reaction time and the amount of alkylating agent contains up to 30 Cu-macrocylic units covalently attached to the protein. Similar experiments with the antibody b12 and ab35 have shown that it is possible to attach the Cu^{2+} macrocylic units to these antibodies without loss of activity and use them *in vivo* (ref. 20).

Acknowledgement

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