Reactivity of side chain functional groups in macrocyclic Cu^{2+} complexes

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Abstract - Using three examples it is shown how the metal ion incorporated in the macrocyclic ring modifies the reactivity of functional groups in the side chain. The first series includes Cu^{2+} promoted hydrolysis reactions of a nitrile **2**, of several esters **3-5**, of a phosphonate ester **6** and of an amide **7**. The mechanism of the hydrolysis of the nitrile and of the phosphonate ester are consistent with an internal OH$^-$ attack onto the functional group, whereas that of the carboxylic ester hydrolysis cannot defenitively be proven. The second example consists in the selective acylation of only one amino group of the bis-functionalized macrocycle **8**, which has two equivalent side chains. Since the metal ion coordinates only one of them, it is possible to selectively benzoylate the other one. The third example shows how mono-N-functionalized macrocyclic metal complexes of **11** can be coupled to amines and proteins. This allows to prepare isotopically marked antibodies, which have diagnostic and therapeutic applications in nuclear medicine.

INTRODUCTION

As the incorporation of side chains into tetraazamacrocycles modifies the properties of the coordinated metal ion (VIS spectra, redox potential,etc.), one might expect that similarly the metal ion also changes the properties of the functional groups of the side chains (basicity, reactivity, etc.). Whereas the first aspect has been studied in detail (ref. 1), much less has been done to investigate the second one, although it implements several interesting applications and can be used to model the reactivity of substrates in metallo enzymes. To exemplify this three specific reactions will be presented and the consequences will be discussed.

Cu^{2+} PROMOTED HYDROLYSIS REACTIONS OF SIDE CHAIN FUNCTIONAL GROUPS. MODELS FOR HYDROLYTIC METALLO ENZYMES

In metallo enzymes with esterase and/or peptidase activity it has been shown that the metal ion acts as a Lewis-acid and polarizes through coordination the functional group which will be cleaved hydrolytically (ref. 2). So for example in carboxypeptidase, a Zn^{2+} metallo enzyme with esterase and

peptidase activity, X-ray structure diffraction studies have shown that in the pseudo-substrate complex with glycyl-tyrosine (gly-tyr) the Zn^{2+} ion, coordinated to the protein through one glutamic acid and two histidine units, also binds to the carbonyl oxygen of the amide group of gly-tyr (ref. 3). It is, however, important to point out that in enzymes other interactions, such as hydrogen bonding, hydrophobic interactions and electrostatic attraction additionally help to fix the substrate into the cavity of the enzyme and thus stabilize the enzyme-substrate complex.

To model such metal ion/substrate interactions we have prepared and studied a series of Cu^{2+} complexes with mono-N-functionalized tetraazamacrocycles, which have in their side chain a group, which can be hydrolysed. These ligands are designed in such a way, that the macrocycle holds the metal ion in close proximity of the reactive group of the side chain, thus allowing to modify its reactivity and influence the hydrolytic cleavage. We have studied compounds, which have a nitrile (2), an ester (3-5), a phosphonate ester (6) and an amide group (7) in their side chain and have found that for the Cu^{2+} complexes the rate of hydrolysis in alkaline aqueous solution follows the order: $7 \lll 6 < 5 < 4 < 3 \lll 2$.
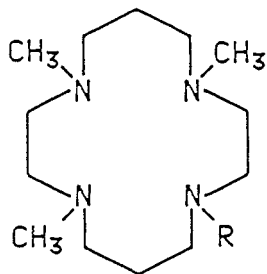
1 R = CH_3
2 R = CH_2-CN
3 R = CH_2-COOMe
4 R = CH_2-COOEt
5 R = CH_2CH_2-COOEt
6 R = CH_2-CH_2-P(O)(OEt)$_2$
7 R = CH_2-CON(Me)$_2$

The amide 7 does not react (or only extremly slowly), whereas the phosphonate diester 6 , the carboxylic acid esters 3-5 and the nitrile 2 are hydrolysed to the phosphonate monoester, to the carboxylic acids and to the amide, respectively (ref. 4). In the case of the nitrile and the phosphonate hydrolysis the pH-profile of the rate and inhibition studies with SCN^- allow to state that the mechanism consists of an internal nucleophilic attack of an axially coordinated OH^- ion onto the functional group. In these complexes the metal ion does not act as a Lewis acid, but as an organizer, whose function is to bring the reactands, i.e. the substrate and the nucleophile, close to each other so that a favourable transition state can occur (ref. 5). In the case of the carboxylic acid esters 3-5 , the mechanism cannot be proven definitively. The results of the linear dependence of the rate constant on [OH^-] can equally well be explained by an external OH^- attack onto the coordinated ester group, as it is observed in the solid state (ref. 4), or by an internal OH^- attack in a hydroxylated species, which only exists in very small amounts even at the highest pH studied. These examples show that the metal ion can play different roles in the hydrolytic processes here studied: it can act as Lewis acid or as an organizer. In both cases one expects an enhanced reactivity.

SELECTIVE ACYLATION OF A BIFUNCTIONALIZED
TETRAAZAMACROCYCLE. MODEL FOR SELECTIVE MODIFICATIONS

It has always been a challenge for chemists to perform reactions in such a way, that only one of several reactive groups will be modified. In general this can be achieved either by protecting the groups, which should not react, or by choosing reaction conditions and reagents specific enough for one functional group only.

The Cu^{2+} complex with **8** gives in aqueous solution a series of pH-dependent equilibria, according to which a high pH one amino group of the side chain is axially coordinated to the metal ion, whereas the second one is not (Figure 1). This is a direct consequence of the trans-I (RSRS) configuration, which
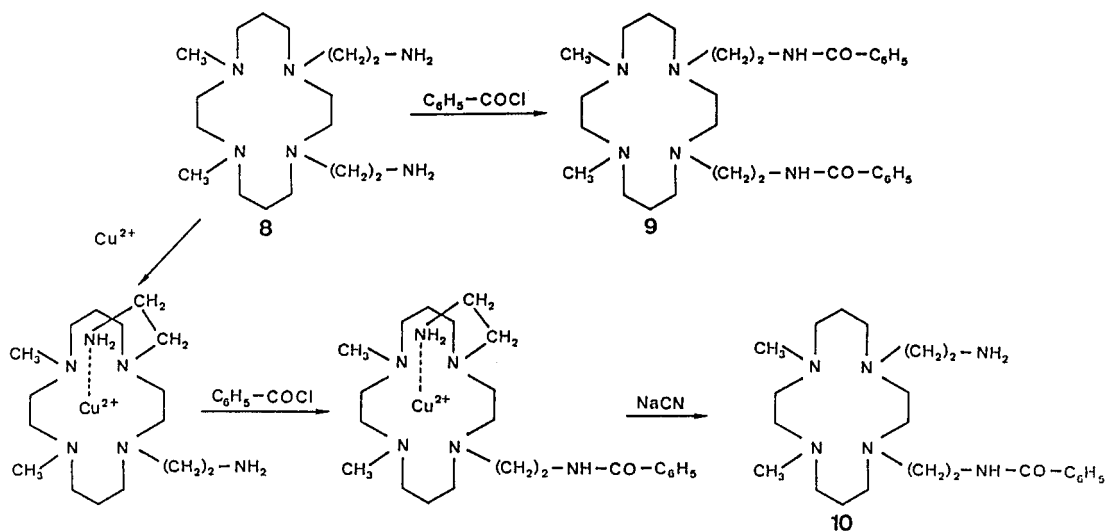

Figure 1. Benzoylation of 8 and its Cu^{2+} complex

is often found in metal complexes of macrocycles with tertiary amino groups, such as **1** (ref. 6). Because of this configuration the substituents at the four nitrogens point to the same side of the N_4-plane, so that only one amino group can coordinate to the metal ion.

These observations induced us to investigate whether in this system a selective acylation would be possible. If one reacts the free macrocycle **8** with benzoyl chloride one obtains as expected the bis-amide **9**. However, if one first complexes the ligand **8** by Cu^{2+} and then reacts it with benzoyl chloride at alkaline pH one isolates, after remotion of the Cu^{2+} ion, the mono-amide **10** (Figure 1). Thus starting from a ligand with two equivalent amino groups one can block one of them by coordination, so that only the non-coordinated amino function is selectively benzoylated. This is an interesting example of the protecting role a metal ion plays in such ligands and of how a metal ion can change the reactivity of the functional group in the side chain.

COUPLING OF A MONO-N-FUNCTIONALIZED MACROCYCLIC Cu^{2+}
COMPLEX WITH AMINES AND PROTEINS. MODEL FOR PREPARING
ISOTOPICALLY LABELLED ANTIBODIES

There is a growing interest in nuclear medicine to use macrocyclic metal
complexes to label antibodies (ref. 7). Since in the Cu^{2+} complex of **11** the
carboxylate group does not interact with the metal ion (ref. 8), it was
interesting to see whether one could couple the carboxylic function with the
amino group of a second component. For the coupling of carboxylic acids with
amines there are many preparative methods, which have been used in peptide
synthesis (ref. 9). We have choosen the cyanomethylester method and the
coupling in presence of carbodiimid derivatives with two relatively simple
amines (Figure 2). Both syntheses give the corresponding amides in moderate
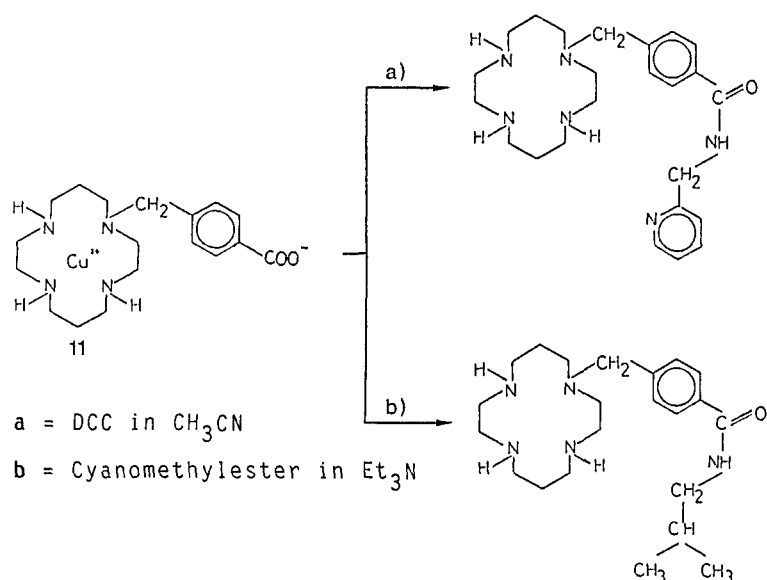yields of 30-45 %. The success of these reactions is due on one side to the

a = DCC in CH$_3$CN

b = Cyanomethylester in Et$_3$N

Figure 2. Coupling of Cu(**11**) with amines

fact that the amino groups of the macrocycle are protected through coor-
dination with Cu^{2+} ion and on the other side by the fact that the carboxylic
group can be activated and brought to reaction.

After having studied these simple reactions we also have tried to couple the
Cu^{2+} complex of **11** with bovine serum albumin, which has about 60 lysine
residues, bearing an amino group in their side chains. The reaction was run
in phosphate buffer (pH 6.5) using the water soluble N-ethyl-N'-(3-diethyl-
aminopropyl)-carbodiimid hydrochloride as coupling reagent. The product, a
pale pink modified albumin, was isolated by chromatography on Sephadex G25
and subseqent lyophilization. Depending on the reaction time and on the
excess of acylating agent over albumin between 10 to 30 Cu^{2+}-macrocyclic units
could be attached to the protein.

A similar experiment with the ^{64}Cu-labelled complex and the antibody b12 was also performed. The ^{64}Cu^{2+} was first incorported into the macrocycle in acetate buffer at pH 4. Then the carboxyl group was activated by preparing the N-hydroxy-succinimid ester and this was coupled with about 1 mg of the antibody b12 in phosphate buffer at pH = 6.7. The purification through a Sephadex G50 column with 0.1 M phosphate buffer as eluent gave a protein fraction, the radioactivity of which indicated that 1.4-1.5 macrocyclic Cu^{2+} complexes per protein were attached. To check whether the chemical modification had changed the activity of the antibody, a cell assay was run, which showed that more than 95 % of the original activity was still present.

From these experiments ligand **11** seems to be an ideal reagent, which after incorporation of a radioactive metal ion, can be used to prepare isotopically labeled antibodies, which can find applications in nuclear medicine for diagnostic as well as for therapeutic purposes.

CONCLUSIONS

The three examples of reactivity of side chain functional groups in the Cu^{2+} complexes of tetraazamacrocycles cover a large spectrum of possibilities offered by such systems: hydrolysis of esters and nitriles, selective modifications and coupling to proteins. These applications are a direct consequence of the many advantages macrocycles have over other ligands: strong binding of metal ions, kinetically inert coordinative bonds even with labile metal ions, stereochemical control of the reactands, which are brought close to each other, and good chances to correlate structure and reactivity.

Acknowledgement

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