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Pegylation of 1,4,8,11-tetraazacyclotetradecane (cyclam) and its Cu(II) complexation

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Dedicated to Dr. Hartmut Spies on the occasion of his 65th birthday

Abstract—Two novel star-like cyclam derivatives appended with four amino groups and four PEG-arms have been synthesised. The complex formation of cyclam and the two cyclam derivatives with Cu(II) has been studied by UV–vis and time-resolved laser-induced fluorescence (TRLFS) measurements outlining 1:1 complexes between cyclam ligands and Cu(II). By TRLFS measurements a quench effect of the Cu(II) on the fluorescence of the ligand was also found to determine the complex formation between Cu(II) and the ligands.

Derivatives of the cyclam **1** (1,4,8,11-tetraazacyclotetradecane) and its metal complexes are of considerable interest in bioinorganic chemistry, biology and medicine.[1] Cyclam derivatives form very stable complexes in particular with transition and rare earth metal ions.[2] Cyclam-based metal complexes are attractive for many applications. Thus, selective metal ion separation by solvent extraction and membrane experiments using *N*-benzylated cyclams have been employed.[3] Nanoparticles with appending cyclam chelating units are very efficient sorbents for binding cupric ions.[4] Dendritic modification of the cyclam core by introduction of dimethoxybenzene and naphthyl units give very interesting spectroscopic properties for these dendrimers themselves and their metal complexes.[5] Phenylazomethine dendrimers with a cyclam core have been found being able to form multinuclear hetero-metal complexes.[6] Four-arm oligonucleotide Ni–cyclam complexes can form highly ordered lattices with exceptional structural, electric and photoelectric properties.[7] Recently, it was found that lipophilic cyclams possess anti-tumour activity.[8] Xylyl-bicyclam and their Zn complexes are highly potent and selective in anti-HIV chemotherapy.[9] Furthermore, cyclam derivatives have been studied extensively as possible agents for magnetic resonance imaging,[10] radiodiagnostic imaging[11] and therapeutic radiopharmaceuticals.[12] With regard to the latter application particularly radiocopper-labelled cyclam bioconjugates are of considerable interest.[13] In this context, most recently cross-bridged cyclam derivatives are gaining in importance.[14] Regarding to medical applications metal complexes of cyclam derivatives having a hydrophilic environment appear very attractive. Thus, the introduction of polyethylene glycol (PEG) into pharmaceuticals (Pegylation) is a well-recognised methodology to improve the pharmacokinetics and tumour retention.[15] This paper reports the synthesis of two novel cyclam derivatives with appended four amino groups **2** and four PEG-arms **3** (Scheme 1). The complexation behaviour of these two cyclam-based ligands towards Cu(II) has been characterised by UV–vis and time-resolved laser-induced fluorescence spectroscopy (TRLFS) and was compared with cyclam **1**.

Subsequent modification of the cyclam **1** (Scheme 1) via Michael addition reaction with methyl acrylate (i) and aminolysis of the tetraester-functionalised cyclam

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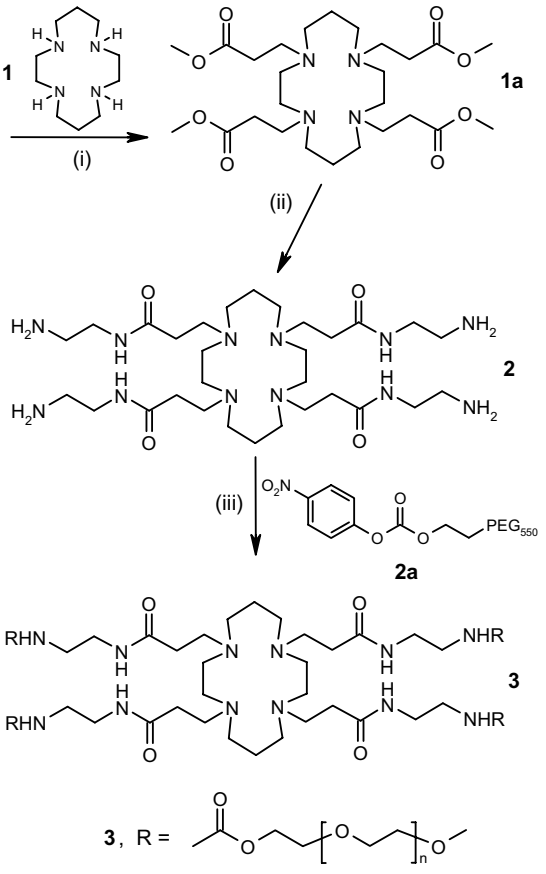

Scheme 1. Synthesis of **2** and **3**. Reagents and conditions: (i) excess methyl acrylate, MeOH, rt, 5 days (99%); (ii) excess 1,2-diaminoethane, MeOH, rt, 3 days in the dark (90%); (iii) **2a**, DMF, 40 °C, 7 days (51%).

derivative **1a** with excess of 1,2-diaminoethane (ii) led smoothly to the tetraamino-functionalised cyclam derivative **2**. The final conversion of **2** with pegylated carbonate ester **2a**[16] (iii) gave the star-shaped cyclam derivative **3** with four PEG-arms. Compound **3** was found to exhibit good solubility in polar organic solvents and water. Since ligands **1**–**3** are quite soluble in methanol, first complexation experiments have been performed in methanolic solution by UV–vis spectroscopy. Deep coloured solutions have been obtained immediately after addition of Cu(II) triflate to cyclam ligands **1**–**3** dissolved in MeOH indicating the complex formation (visible absorption spectra for **1**–**3** shown in Figure in Supplementary Material).

Both a red-shift of absorption bands and an increase of extinction coefficients, shown in Table 1, have been observed for [Cu ⊂ **2**] $(CF_3SO_3)_2$ (blue) and [Cu ⊂ **3**] $(CF_3SO_3)_2$ (green) in comparison to the pink Cu(II) complex of cyclam **1**.[14a,17] Visible absorption spectra have been recorded both as function of time (each 20 min for 10 h) and ligand concentration. In the case of cyclam **1** and the pegylated derivative **3**, the Cu(II) complexes were immediately formed, and the spectra remained stable for the whole time. It is worthy to mention that despite of the polyethylene glycol shell of ligand **3** the complexation reaction with Cu(II) is so rapid. For comparison, Ni(II) metallisation completely disappears in the case of a PEG-tagged cyclam ligand.[18] Unlike to **1** and **3**, the binding of Cu(II) in the cyclam core of ligand **2** needs approximately 1 h resulting in a decrease of absorbance (Figure in Supplementary Material). This finding is in agreement with the formation kinetics of Cu(II) with derivatives having pendant primary amino groups on the cyclam core. This behaviour can be explained on the way that the metal ion is bound by the primary amino groups in the initial complexation step. These intermediates show consistently higher absorptivities than the final products where the Cu(II) is bound into the core.[19]

Titration of Cu(II) to the MeOH solutions of cyclams **1**–**3** (pre-equilibrated in the case of **2**) gave the maximum of absorbance at a ratio of 1 between metal to ligand indicating 1:1 complex formation for all ligands investigated. This result was corroborated by fluorescence measurements. From theory it can be expected that the fluorescence emitted by the non-complexed ligand is decreased due to complex formation with metal ions. This effect is well known as static quench effect. Under normal conditions (room temperature) the formed complex does not show any fluorescence properties.

The data of the fluorescence intensity of the non-complexed ligand at different Cu(II)–ligand ratios were used in order to obtain information about the stability of the formed complexes. The total concentration of the ligand was kept constant at 5.0×10^{-5} M. The added Cu concentration ranged from 0 to 1.0×10^{-4} M. All measurements were carried out in MeOH.

The fluorescence spectra of the free ligand **1** and of a solution, when the Cu–cyclam **1** complex (equimolar ratio of metal to ligand) is formed, were recorded (Figure in Supplementary Material). However, a relatively weak fluorescence emission of the free ligand was found in both cases. Due to this low fluorescence intensity a relatively high uncertainty in the determination of the stoichiometry and the derived formation constant has been found (see Table 2).

Table 1. UV–vis data of Cu(II) complexes with cyclam ligands **1**–**3** in MeOH solution at 22 ± 1 °C

Complex	λ_{max}/nm	ε_{max}/dm^3 mol^{-1} cm^{-1}
[Cu ⊂ **1**] $(CF_3SO_3)_2$	506	93
[Cu ⊂ **2**] $(CF_3SO_3)_2$	664	200
[Cu ⊂ **3**] $(CF_3SO_3)_2$	710	192

Table 2. Results of fluorescence measurements for the complex formation with Cu(II) in MeOH solution at 22 ± 1 °C

Ligand	Stoichiometry	Formation constant
1	0.64 ± 0.40	4.61 ± 0.21
2	1.01 ± 0.05	4.86 ± 0.02
3	0.94 ± 0.03	4.99 ± 0.03

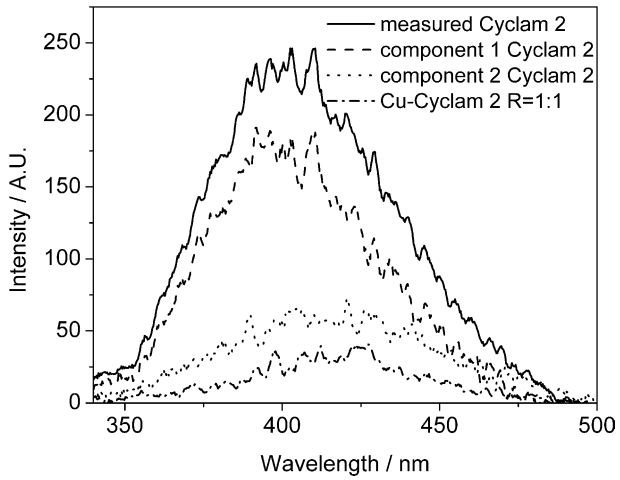

Figure 1. Fluorescence spectra of the cyclam ligand **2** with and without Cu(II) in MeOH solution (R = ratio).

Figure 1 shows the fluorescence spectra for the cyclam ligand **2** as function of the Cu(II) concentration. For the solution without any metal a relatively high intensity was found. This spectrum was deconvoluted into its components. The maximum of the fluorescence emission was shifted to the blue region compared to the spectra of the two other ligands due to the high intensity of one component. The other component shows the same fluorescence properties as observed in the solutions with the formed complex.

Therefore only this part of the spectrum was used for the determination of the complex formation. Again a quenching of the fluorescence due to the complex formation was found. At the moment an explanation for the reason of the two components cannot be given.

The fluorescence spectra of the cyclam ligand **3** as function of the Cu(II) concentration are shown in Figure 2. In the same way as found for ligands **1** and **2** a decrease of the fluorescence intensity due to the complex formation has been observed.

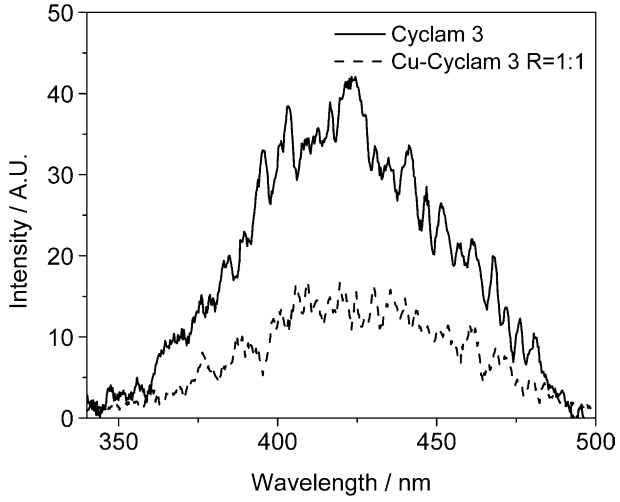

Figure 2. Fluorescence spectra of the cyclam ligand **3** with and without Cu(II) in MeOH solution (R = ratio).

As described the fluorescence intensity decreases for all ligands investigated with increasing Cu(II) concentration caused by the decreasing concentration of the non-complexed ligand. The formed complex does not show any fluorescence properties. Examining this behaviour (static quenching of fluorescence due to complex formation with heavy metals) one will be able to calculate the complex formation constants.[20] Exemplary, the results for the cyclam **2** are outlined as Stern–Volmer plot (Figure in Supplementary Material). The slope of the straight line represents the formation constant for the reaction. It was found to be $\log K = 4.86 \pm 0.02$.

Using the calculated concentrations of the species in solution a validation of the complex formation reaction can be performed. This is shown for the complexation of Cu(II) with ligand **2** (Figure in Supplementary Material). From the slope (1.0 ± 0.05) clearly a 1:1 complex reaction was obtained. The intersection in this case represents the logarithm of the formation constant. It was found to be $\log K = 4.85 \pm 0.26$. This is in good agreement with the results of the Stern–Volmer calculation. The results for the complex stoichiometry and the averaged data for the formation constants of all three ligands are summarised in Table 2. The derived formation constants of the Cu complexes increase slightly in the order **1** < **2** < **3**. In the complexes under investigation the metal ion is bound into the cyclam core. Hence, the order of stability may be interpreted by both an increasing shielding effect of the metal ion and additional interaction of potential ligand atoms from pendant arms.

Cu(II) complexes with cyclam **1** are extremely stable in vivo.[13a] Hence, particularly complexes of Cu(II) with the water-soluble pegylated cyclam ligand **3** have a great potential to be applied in medicine.

In conclusion, we have investigated the complexation of Cu(II) with two novel star-shaped cyclam ligands. Clean 1:1 complex formation was found. Rapid attainment of complexation was determined in the case of cyclam and the pegylated ligand **3**, but not for **2** possessing four external amino groups. Studies of the fluorescence of cyclam ligands **1**–**3** and their Cu-complexes have shown a quench effect of the Cu(II) on the fluorescence of the bound ligand. From the decrease of the fluorescence intensity the formation constants were derived. The formation constants increase slightly in the order **1** < **2** < **3**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.03.051. Spectroscopic data and experimental details for the compounds **1**, **2** and **3** and additional Figures for UV–vis and fluorescence spectroscopy and plots of complex formation for **2**.

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