Hydrophilic oxybathophenanthroline ligands: synthesis and copper(II) complexation

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Received (in Montpellier, France) 26th March 2008, Accepted 8th May 2008
First published as an Advance Article on the web 16th July 2008
DOI: 10.1039/b805136d

Hydrophilic oxybathophenanthroline dendrons (generation 1–3) have been synthesized by treatment of 4,7-bis(4′-hydroxyphenyl)-1,10-phenanthroline with the corresponding bromo-functionalized etheraryl branching units containing triethylene glycol monomethyl end groups. Radiotracer experiments using ^{64}Cu prove the rapid formation of stable copper(II) complexes in aqueous solution. These ^{64}Cu complexes remain unchanged even upon addition of a high excess of glutathione as competing ligand, thus demonstrating the high stability of the formed copper(II) complexes. Electronic and EPR spectroscopy indicate the formation of $[Cu(\mathbf{L})_2(OH_2)_2]^{2+}$ ($\mathbf{L}$ = ligand) complexes in aqueous solution, confirmed by time-resolved laser fluorescence spectroscopy and supported by molecular mechanics modeling.

Introduction

Dendrimer chemistry is a rapidly expanding field for both fundamental studies and applications.[1] The typical behavior of dendritic systems emerges from their compact tree-like molecular structure, which provides an arrangement of inner and outer molecular functionalities, making them useful for applications in medicine, catalytic processes and nanotechnology. The introduction of the structurally reinforced 1,10-phenanthroline unit onto dendritic frameworks leads to unique material properties. Thus, metallodendrimers based on ruthenium(II) complexes with bisphenanthroline ligands, bridged in the 5,6-positions, provide very robust, structurally rigid and well-defined nanoscopic complexes.[2] Dendritic phenanthroline-containing ligands described so far possess hydrophobic skeletons, which can be employed in applications that require an organic environment. Particularly, ruthenium(II) complexes with branched phenanthroline moieties exhibit interesting absorption, luminescence and redox properties in polar organic solvents.[3] Supramolecular self-assembled structures with tuneable coordination geometries could be obtained with amphiphilic dendritic phenanthroline ligands and appropriate metal ions in acetonitrile.[4] Furthermore, a dendrimer with an arylethynyl scaffold with six peripheral phenanthroline units showed significant activity in copper-catalyzed reactions in methanol.[5] Kinetically inert copper(I) complexes with Fréchet-type dendrons, attached adjacent to the metal coordination center (2,9 positions of 1,10-phenanthroline), have been observed in dichloromethane.[6] Recently, we have described hydrophobic oxybathophenanthroline derivatives with attached Fréchet-type dendrons in the 4,7-positions (Fig. 1), which are able to rapidly form stable copper(II) complexes in trichloromethane.[7]

In view of biomedical applications such as the design of novel diagnostic and therapeutic agents, it was of interest to develop the corresponding hydrophilic systems.[8] In this paper, we report the synthesis and the copper(II) complexation of dendritic 4,7-diphenyl-1,10-phenanthroline (dpp) ligands with attached Fréchet-type dendrons, containing triethylene glycol monomethyl ether end groups (Fig. 2).

Results and discussion

Syntheses

The phenanthroline ligand **1** with two peripheral hydroxy groups was obtained in five steps, according to a previously reported procedure.[7] The dendritic precursors **2–4** with terminal hydrophilic ethylene glycol chains and a benzylic bromide functional group (generation 1–3) have also been prepared

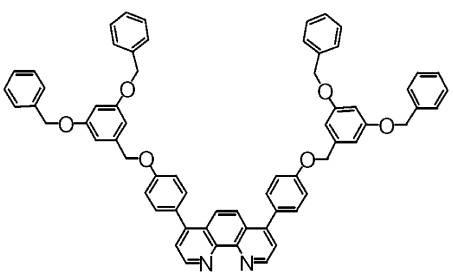

Fig. 1 Hydrophobic dendritic oxybathophenanthroline ligand (generation 1).

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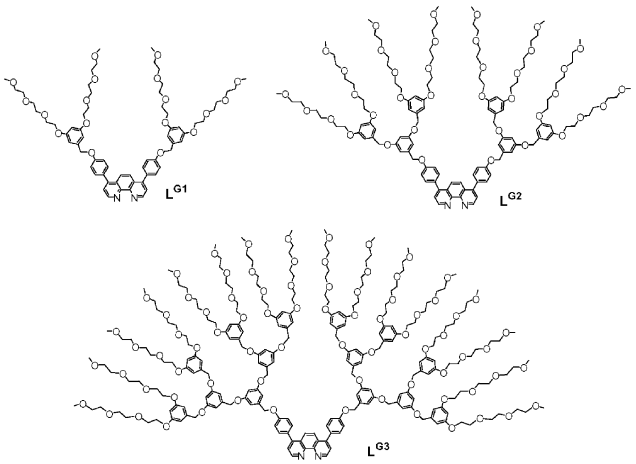
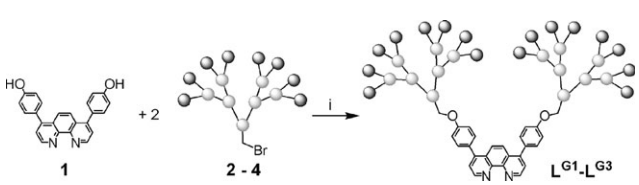


Fig. 2 Constitutions of the investigated hydrophilic dendritic oxybathophenanthroline ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$.

following synthetic routes described in literature.[9] The dendritic diphenylphenanthroline ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ were synthesized as shown in Scheme 1. One equivalent of 4,7-bis(4′-hydroxyphenyl)-1,10-phenanthroline **1** was dissolved in DMF and deprotonated by treatment with sodium hydride. Addition of two equiv. of the corresponding dendritic benzyl bromides **2–4**, followed by purification on silica gel, gave the products in medium yields as colorless viscous oils in all cases. $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ are well soluble in polar solvents such as water and methanol and can also easily be dissolved in more apolar solvents such as CH_2Cl_2, $CHCl_3$, or THF. The structures of all dendritic ligands could be readily deduced from ^1H and ^{13}C NMR spectra as well as from mass spectrometric analysis (see Experimental section).

Radiotracer experiments with ^{64}Cu

Phenanthroline derivatives are able to form strong complexes with copper(II). In particular, water-soluble phenanthroline ligands are attractive candidates for the development of copper radiopharmaceuticals.[10] With the increasing dissemination of copper radionuclides, the use of radiocopper complexes for imaging and therapy is an emerging field.[11] In this perspective, highly thermodynamically and kinetically stable copper(II) complexes are required. Based on these requirements preliminary radiolabeling experiments of the dendritic diphenylphenanthroline ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ with ^{64}Cu have been performed. Initially, the complex formation was studied by thin-layer chromatography using C18 TLC plates. The R_f-values of free copper ($^{64}CuCl_2$, $R_f = 0$) and the dendritic ^{64}Cu-dpp complexes ($R_f = 0.17$–0.20) are well-separated. The copper complexation by the ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ is very efficient and rapid. Radio



Scheme 1 Preparation of the hydrophilic dpp-ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$. *Reagents and conditions*: (i) NaH (60% in paraffin), rt, 1 d, DMF (white spheres represent the ether aryl branching units, grey spheres represent triethylene glycol monoethyl ether end groups).

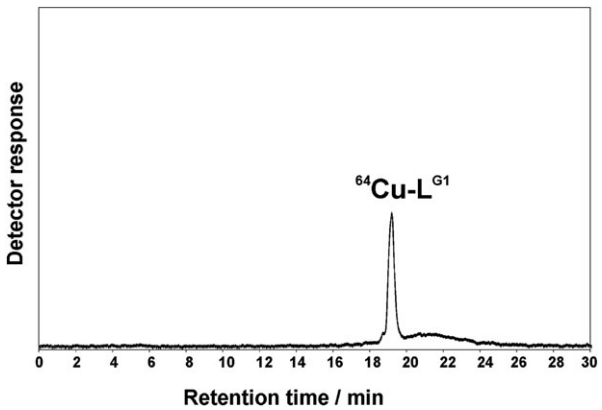


Fig. 3 HPLC trace of ^{64}Cu-$\mathbf{L^{G1}}$ at 12 h in the presence of glutathione: $c_{ligand} = 1$ μM, $c_{glutathione} = 5$ mM, pH = 5.4 (MES/NaOH buffer).

copper(II) is completely bound with very low ligand concentration (1 μM) in aqueous buffer solution (pH range from 5.4 to 7.4) within 1 min at room temperature. The qualitative stability of the radioactive copper(II)–dpp complexes was deduced from challenge experiments using glutathione as competing ligand.[12] To get reliable results, TLC measurements are unsuitable. It was not possible to clearly distinguish radiocopper complexes of glutathione and dpp-ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G2}}$ (^{64}Cu-glutathione, R_f = 0–0.15). Therefore, Radio-HPLC experiments were used to obtain well-separated R_t-values ($^{64}CuCl_2$, $R_t = 2.8$ min; ^{64}Cu-glutathione, $R_t = 7.6$ min; ^{64}Cu-$\mathbf{L^{G1}}$ = 19.7 min; ^{64}Cu-$\mathbf{L^{G2}}$ = 21.1 min; ^{64}Cu-$\mathbf{L^{G3}}$ = 23.8 min). After full complexation, the ^{64}Cu-dpp complexes were reacted with glutathione for 12 h at room temperature. The radiochromatogram shown in Fig. 3 illustrates the elution profile of ^{64}Cu-$\mathbf{L^{G1}}$. In the presence of even a large excess of the competing ligand glutathione (5 mM *vs.* 1 μM), there was no evidence of transchelation (^{64}Cu-glutathione, $R_t = 7.6$ min) and demetalation ($^{64}CuCl_2$, R_t = 2.8 min), indicative for a high stability of the ^{64}Cu-dpp complexes formed.

Information about the lipophilicity was obtained from the measurement of the distribution of the dendritic copper(II)-dpp complexes in the water/1-otanol system. Fig. 4 shows the distribution ratio log D_{Cu} as a function of pH.[13] As expected, the ^{64}Cu complexes of $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ are rather hydrophilic. With increasing generation of the covalently linked dendritic Fréchet-type branches the higher number of ethylene glycol groups causes a decreasing lipophilicity in the order ^{64}Cu-$\mathbf{L^{G1}}$ ($\log P = -0.02$) > ^{64}Cu-$\mathbf{L^{G2}}$ ($\log P = -1.09$) > ^{64}Cu-$\mathbf{L^{G3}}$ ($\log P = -1.67$). Therefore, the observed lipophilicity data with the ^{64}Cu complexes of the corresponding dendritic diphenylphenanthroline ligands are in the typical range of approved radiopharmaceuticals.[10b,14]

Geometry of dendritic copper(II) complexes

The complexation behavior and coordination geometry of the dendritic ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ have been examined by UV/Vis titration and EPR experiments as well as by molecular mechanics modeling. The UV/Vis titration of the ligands with $Cu(NO_3)_2$ shows the establishment of a 1 : 1 and a 1 : 2 (Cu^{2+} : ligand) species. Addition of more ligand did not affect the absorption, thus there is no indication for 1 : 3 complexes.

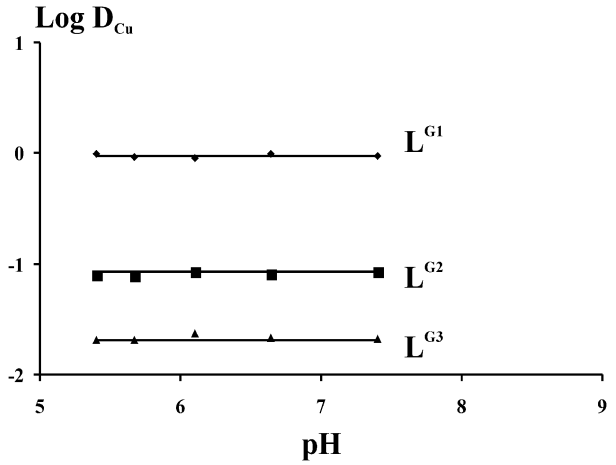


Fig. 4 Distribution of ^{64}Cu complexes of dpp ligands in the system buffer/1-octanol: c_{ligand} = 1 μM, pH = 5.4–7.4 (MES/NaOH buffer).

This is expected since phenanthroline (phen) is known to preferably form 1 : 2 complexes with Cu(II).[15] The electronic spectra show a broad transition at 711 nm for $\mathbf{L^{G1}}$, 721 nm for $\mathbf{L^{G2}}$ and 724 nm for $\mathbf{L^{G3}}$, due to transitions from d_{xy}, d_{xz} and d_{yz}-type orbitals of the copper(II) center. $[Cu(dpp)_2(OH_2)_2](X)_2$ (X = Cl^-, Br^-, ClO_4^-) has transitions at 714–740 nm in nitromethane, depending on the counter anion.[16] Therefore, it was concluded that $[Cu(\mathbf{L^{Gn}})_2(OH_2)_2]^{2+}$ complexes are formed upon addition of Cu^{2+} in aqueous solution.

The assignment of these transitions to CuN_4O_n (n = 0, 1, 2) chromophores is supported by the EPR spectra of the same solutions at 120 K. Fig. 5 shows the spectra of the complexes with $\mathbf{L^{G2}}$ at different Cu : L ratios (1 : 1, 1 : 2, 1 : 3). The analysis of the spin Hamiltonian (for the 1 : 2 complexes; $g_x = g_y = 2.065$, $g_z = 2.370$, $A_x = A_y = 11 \times 10^{-4}$ cm^{-1}, $A_z = 135 \times 10^{-4}$ cm^{-1}) are similar to those of $[Cu(phen)_2(OH_2)_2]^{2+}$.[17]

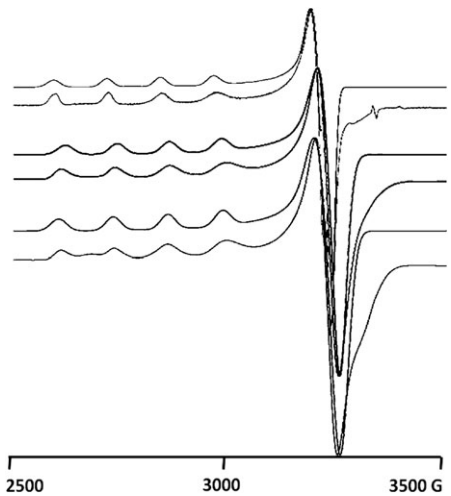


Fig. 5 Experimental (spectra 2, 4, 6 from top) and computed (spectra 1, 3, 5 from top) EPR spectra of Cu(II) complexes with $\mathbf{L^{G2}}$ at different Cu : ligand ratios (from top to bottom: 1 : 1, 1 : 2, 1 : 3); $g_\perp$, $g_\parallel$, $A_\perp$, $A_\parallel$ values: 2.080, 2.400, 6×10^{-4} cm^{-1}, 130×10^{-4} cm^{-1} (1 : 1); 2.065, 2.370, 11×10^{-4} cm^{-1}, 135×10^{-4} cm^{-1} (1 : 2) and 2.065, 2.380, 11×10^{-4} cm^{-1}, 138×10^{-4} cm^{-1} (1 : 3).

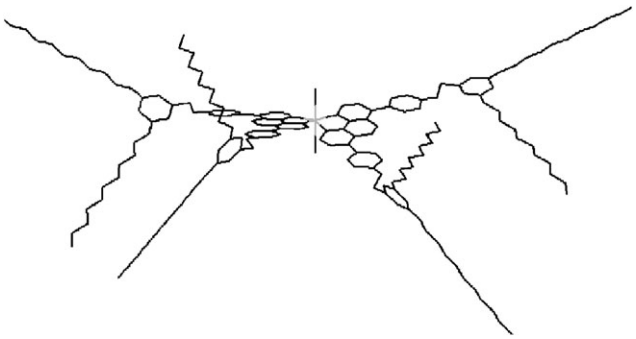


Fig. 6 Strain energy minimized structure of $[Cu(\mathbf{L^{G1}})_2(OH_2)_2]$, using the MOMEC97 program and force field.[18,19]

In agreement with the electronic spectra, the EPR spectra do not change after addition of more than two equivalents of ligand to the copper(II) salts (see Fig. 5).

The conclusion, that the assembled Cu(II) complexes have the composition $[Cu(\mathbf{L^{Gn}})_2(OH_2)_2]^{2+}$ is further supported by strain energy minimized structures, using the MOMEC program[18] and force field.[19] An optimized structure is shown in Fig. 6 (for clarity, only the Cu(II) complex with $\mathbf{L^{G1}}$ is shown; the coordination geometry does not change with the higher generations, and is also largely unchanged for various possible conformational minima). The Cu–N distances (*ca.* 1.97 Å) and a tetrahedral twist of the CuN_4 chromophores (*ca.* 40°) are, as expected, very similar to those reported for other $[Cu(phen)_2(OH_2)_2]^{2+}$-type structures.[20]

Luminescence properties

The fluorescence properties of the ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ were characterized by time-resolved fluorescence spectroscopy. The fluorescence spectra 50 ps after excitation (λ_{exc} = 266 nm) are given in Fig. 7.

Compared to 1,10-phenanthroline, the three dendritic ligands $\mathbf{L^{G1}}$–$\mathbf{L^{G3}}$ show a shift to higher wavelengths, and with higher ligand generations a slight hypsochromic shift is observed. The emission maxima and the decay time of the ligands investigated are summarized in Table 1. The spectra cannot be fitted by a single gaussian waveform. With respect to the fluorescence lifetime, more complex features have been noticed for the dpp-ligands than for 1,10-phenanthroline. This may be due to the appended phenyl rings adjacent to the fluorescence center. Energy transfer between the fluorescence center and the phenyl rings may lead to multiple fluorescence lifetimes. In all cases, the fluorescence around 400 nm has the highest intensity.

Addition of copper(II) to an aqueous solution of the appropriate ligands leads to a decrease of fluorescence intensity. Thus, the complex stability of the copper(II) complexes with the dpp-ligands can be determined with time-resolved laser-induced fluorescence.

The determination of the stability constants exploits the luminescence properties of the non-complexed ligand, and free metal ion and the complex do not emit. This is well known as a static quench effect.[21] With this assumption, the species concentration can be calculated from the fluorescence intensities of the metal-free ligand as a function of metal

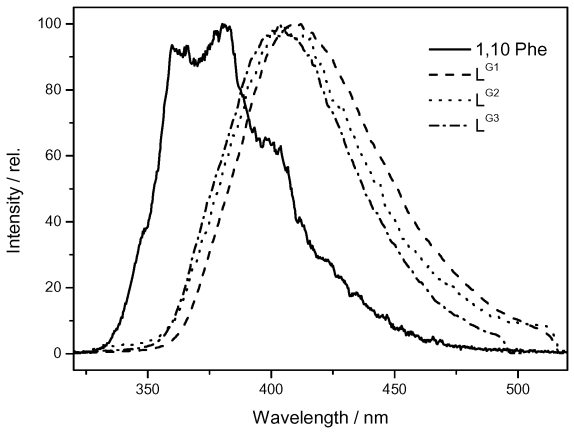


Fig. 7 Time-resolved fluorescence spectra of the dpp-ligands and 1,10-phenanthroline, c_{ligand} = 10 μM in water.

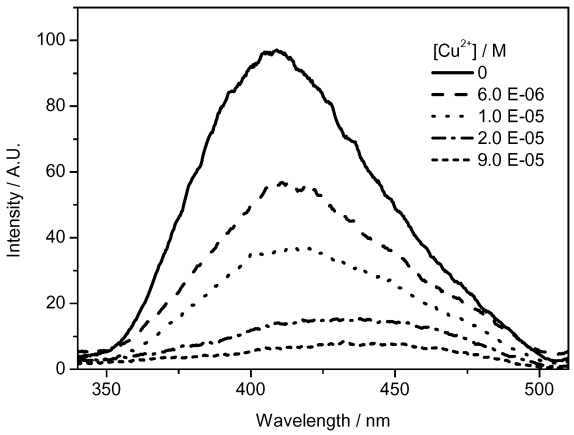


Fig. 8 Fluorescence intensity of $\mathbf{L^{G3}}$ as function of Cu(II) concentration, c_{ligand} = 30 μM in 0.1 M $NaClO_4$.

Table 1 Fluorescence properties of phen and dendritic dpp-ligands

Ligand	Emission maxima (λ_{max})/nm	Decay time/ps
phen	359, 375, 395, 411, 430	1170 ± 23
$\mathbf{L^{G1}}$	396, 422, 465	150 ± 5
		670 ± 13
		2450 ± 41
$\mathbf{L^{G2}}$	392, 415, 447	403 ± 20
		1710 ± 100
$\mathbf{L^{G3}}$	398, 428	554 ± 10
		2100 ± 30

concentration. The fluorescence lifetime was also determined in all solutions to establish dynamic quench effects.

The stability of the copper(II) complex with 1,10-phenanthroline in 0.1 M $NaClO_4$ and water–MeOH (1 : 1) was determined for comparison. The formation of a $Cu(L)_2$ complex was established by slope analysis (slope = 1.94 ± 0.23), and the formation constant was determined to be $\log \beta_{12}$ = 13.86 ± 0.12, compared to $\log \beta_{12}$ = 13.7 in 0.1 M $NaNO_3$ and $\log \beta_{12}$ = 16.14 in 0.1 M KNO_3, both in dioxane–water (1 : 1).[22] Therefore, time-resolved fluorescence spectroscopy is well-suited to determine reliable formation constants for Cu(II)-phenanthroline complexes.

The fluorescence intensity for $\mathbf{L^{G1}}$ and $\mathbf{L^{G2}}$ decreases with increasing copper concentration. The fluorescence disappears at metal : ligand ratios of about 1 : 4 and 1 : 3. However, for $\mathbf{L^{G1}}$ and $\mathbf{L^{G2}}$ it was not possible to determine the complex stoichiometry and the stability constants. This may be due to dynamic hydration/dehydration effects of the triethylene glycol monomethyl ether end groups adjacent to the fluorescence center. Increasing the distance of these arms from the chromophore results in a decrease of the influence on the fluorescence by energy transfer from the excited state of the chromophore to the increasing number of phenyl rings. Hence, the estimation of the stability was possible for the copper(II) complex formed with $\mathbf{L^{G3}}$. The fluorescence spectra of $\mathbf{L^{G3}}$ as a function of the total copper concentration are shown in Fig. 8.

There was found a small residual fluorescence intensity at higher metal concentration, which did not change with ongoing increase of the metal concentration (*cf.* Fig. 9).

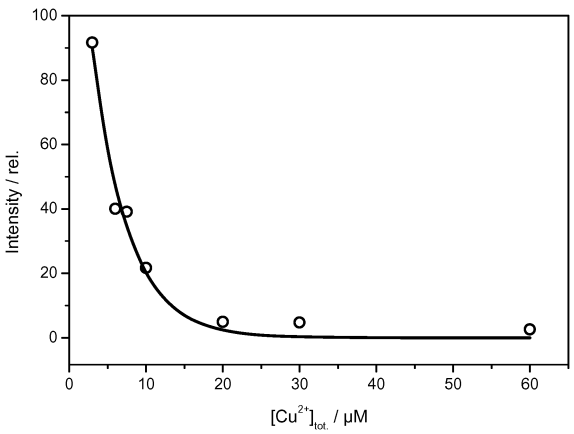


Fig. 9 Fluorescence intensity of $\mathbf{L^{G3}}$ as function of Cu(II) : $\mathbf{L^{G3}}$ ratio, c_{ligand} = 30 μM in 0.1 M $NaClO_4$.

From the fluorescence intensities the concentration of the non-complexed ligand $\mathbf{L^{G3}}$ was calculated and the stability data were determined, assuming a 1 : 2 (metal : ligand) complex stoichiometry. Slope analysis confirms the formation of $Cu(\mathbf{L^{G3}})_2$ (slope = 1.96). The resulting stability constant is $\log \beta_2$ = 12.27 ± 0.13. It follows that the stability of copper(II)-phenanthroline complexes is only slightly influenced by the dendritic modification. On the other hand, with higher generation the fluorescence lifetime of the ligands is less affected by copper(II) addition pointing to an increased shielding effect on the metal center.

Conclusions

A new series of fractal ligands, derived from 4,7-diphenyl-1,10-phenanthroline with first to third generation poly(aryl ether) branching units and triethylene glycol monomethyl ether end groups have been prepared. These water-soluble oxybathophenanthroline-derived dendritic ligands were explored by radiotracer experiments using ^{64}Cu, and have shown spontaneous formation of stable copper(II) complexes in aqueous solution. Electronic and EPR spectroscopy suggest

the formation of $[Cu(\mathbf{L}^{\mathbf{G1–G3}})_2(OH_2)_2]^{2+}$ species. This is underscored by molecular modeling and time-resolved laser fluorescence experiments. The latter revealed that the stability of the copper(II) complexes is nearly independent of the dendritic modification of the ligands. However, with higher generation of the fractal structure the metal center is better shielded from the environment. The synthetic approach allows the fine-tuning of the solubility by introduction of appropriate methoxypolyethoxy groups. Moreover, the mild conditions of synthesis offers the possibility to introduce and covalently link biomolecules such as sugars and peptides as surface groups, hence paving the way to produce new ligands with a potential in pharmaceutical targeting. That makes this type of phenanthroline ligands attractive for the development of new (radio)-diagnostically and (radio)therapeutically relevant metal complexes as well as hydrophilic DNA intercalating agents.

Experimental

Materials and methods

All starting materials were purchased from commercial sources and used without further purification. The dpp-ligand[7] **1** and the dendritic bromides[9] **2–4** have been prepared according to literature procedures. The solvents were dried using standard techniques. Reactions were monitored by thin-layer chromatography using TLC plates pre-coated with silica gel $60F_{254}$ (Merck) and compounds detected by UV-light (254 nm). Column chromatography was carried out using silica gel (Merck 15101). ^1H and ^{13}C NMR spectra were recorded using a AM 400 MHz Bruker instrument; the solvent signal was used for internal calibration. FAB mass spectra were recorded using a Concept 1H from Kratos Analytical Ltd., Manchester, GB. ^{64}Cu was produced on the PET cyclotron "Cyclone 18/9" of the FZ Dresden-Rossendorf by the $^{64}Ni(p,n) \rightarrow {}^{64}Cu$ nuclear reaction. The specific activity was >370 MBq μg^{-1}.

Syntheses

General procedure for the preparation of the dendritic ligands $\mathbf{L}^{\mathbf{G1}}$–$\mathbf{L}^{\mathbf{G3}}$. To a suspension of 4,7-bis(4′-hydroxyphenyl)-1,10-phenanthroline[7] **1** (0.1 mmol) in dry DMF (10 mL) was added NaH (0.22 mmol, 60% in paraffin). The reaction mixture turned to red. After 5 min a solution of the corresponding bromides[9] **2–4** (0.21 mmol) in dry DMF (5 mL) was added and the suspension stirred for 1 d at rt. The residual NaH was deactivated by slow addition of water at 0 °C. CH_2Cl_2 (30 mL) was added, the suspension neutralized with aq. HCl (2 M), and the aqueous phase extracted several times with CH_2Cl_2. The collected organic phase was washed with conc. aq. $NaHCO_3$, water, dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified as outlined in the following text.

1,10-Phenanthroline(4,7)-phenyl(4′):{1-oxa-3-(phenyl-3″,5″-diylpropyl(3″,5″)}G1/2x:(1-oxa-4-oxa-7-oxa-10-oxaundecane)$_4$-cascadane ($\mathbf{L}^{\mathbf{G1}}$)[23]. Prepared from **1** (70.0 mg, 0.19 mmol), and dendritic bromide **2** (199.8 mg, 0.40 mmol). Gradient column chromatography (SiO_2, CH_2Cl_2–MeOH, 70 : 1 to 10 : 1) yielded $\mathbf{L}^{\mathbf{G1}}$ (158.0 mg, 69%) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 3.30 (s, 12 H; CH_3), 3.46 (m, 8 H; CH_2), 3.60 (m, 16 H; CH_2), 3.66 (m, 8 H; CH_2), 3.78 (t, J = 5 Hz, 8 H; CH_2), 4.07 (t, J = 5 Hz, 8 H; CH_2), 5.01 (s, 4 H; CH_2), 6.40 (t, J = 2 Hz, 2 H; H_{ar}), 6.47 (d, J = 2 Hz, 2 H; H_{ar}), 6.58 (d, J = 2 Hz, 4 H; H_{ar}), 7.04 (AA′ part of the AA′BB′ system, 4 H; H_{ar}), 7.39 (BB′ part of the AA′BB′ system, 4 H; H_{ar}), 7.48 (d, J = 4 Hz, 2 H; H_{ar}), 7.82 (s, 2 H; H_{ar}), 9.12 (d, J = 5 Hz, 2 H; H_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 59.0 (CH_3), 67.6, 69.7, 70.0, 70.5, 70.6, 70.8, 71.9 (OCH_2), 101.1, 106.1, 115.1, 123.5, 124.0, 126.5, 130.5, 131.1, 139.1, 146.9, 148.1, 149.7, 159.0, 160.2 (C_{ar}) ppm; MS (FAB, NBA): m/z (%): 1193.5 ($[M + H]^+$, 62), 663.4 (100), 648.4 (85).

1,10-Phenanthroline(4,7)-phenyl(4′):{1-oxa-3-(phenyl-3″,5″-diylpropyl(3″,5″)}G1,G2/2x,4x:(1-oxa-4-oxa-7-oxa-10-oxaundecane)$_8$-cascadane ($\mathbf{L}^{\mathbf{G2}}$). Prepared from **1** (35.0 mg, 96 μmol), and dendritic bromide **3** (208.2 mg, 0.20 mmol). Gradient column chromatography (SiO_2, CH_2Cl_2/MeOH, 70 : 1 to 10 : 1) yielded $\mathbf{L}^{\mathbf{G2}}$ (146.0 mg, 67%) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 3.30 (s, 24 H; CH_3), 3.48 (m, 16 H; CH_2), 3.58 (m, 32 H; CH_2), 3.66 (m, 16 H; CH_2), 3.79 (t, J = 5 Hz, 16 H; CH_2), 4.05 (t, J = 5 Hz, 16 H; CH_2), 4.92 (s, 8 H; CH_2), 5.04 (s, 4 H; CH_2), 6.40 (t, J = 2 Hz, 6 H; H_{ar}), 6.47 (d, J = 2 Hz, 4 H; H_{ar}), 6.58 (d, J = 2 Hz, 8 H; H_{ar}), 7.09 (AA′ part of the AA′BB′ system, 4 H; H_{ar}), 7.32 (BB′ part of the AA′BB′ system, 4 H; H_{ar}), 7.51 (d, J = 5 Hz, 2 H; H_{ar}), 7.88 (s, 2 H; H_{ar}), 9.14 (d, J = 5 Hz, 2 H; H_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 59.0 (CH_3), 67.5, 69.7, 70.0, 70.1, 70.5, 70.6, 70.8, 71.9 (OCH_2), 101.2, 101.6, 106.1, 106.4, 115.1, 123.5, 124.0, 126.5, 130.6, 131.0, 139.0, 139.2, 147.0, 148.1, 149.7, 159.1, 160.1, 160.2 (C_{ar}) ppm; MS (FAB, NBA): m/z (%): 2267.1 ($[M + H]^+$, 64), 1010.5 (58), 307.1 (100).

1,10-Phenanthroline(4,7)-phenyl(4′):{1-oxa-3-(phenyl-3″,5″-diyl)propyl(3″,5″)}G1,G2,G3/2x,4x,8x:(1-oxa-4-oxa-7-oxa-10-oxa-undecane)$_{16}$cascadane ($\mathbf{L}^{\mathbf{G3}}$). Prepared from **1** (41.2 mg, 0.11 mmol), and dendritic bromide **4** (500.0 mg, 0.24 mmol). Gradient column chromatography (SiO_2, CH_2Cl_2/MeOH, 70 : 1 to 10 : 1) yielded $\mathbf{L}^{\mathbf{G3}}$ (209.8 mg, 42%) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 3.28 (s, 48 H; CH_3), 3.42 (t, J = 5 Hz, 32 H; CH_2), 3.53–3.66 (m, 96 H; CH_2), 3.74 (t, J = 5 Hz, 32 H; CH_2), 4.03 (t, J = 5 Hz, 32 H; CH_2), 4.86 (s, 16 H; CH_2), 4.92 (s, 8 H; CH_2), 5.04 (s, 4 H; CH_2), 6.36 (t, J = 2 Hz, 8 H; H_{ar}), 6.47 (t, J = 2 Hz, 4 H; H_{ar}), 6.50 (d, J = 2 Hz, 16 H; H_{ar}), 6.53 (t, J = 2 Hz, 2 H; H_{ar}), 6.60 (d, J = 2 Hz, 8 H; H_{ar}), 6.66 (d, J = 2 Hz, 4 H; H_{ar}), 7.06 (AA′ part of the AA′BB′ system, 4 H; H_{ar}), 7.39 (BB′ part of the AA′BB′ system, 4 H; H_{ar}), 7.48 (d, J = 5 Hz, 2 H; H_{ar}), 7.85 (s, 2 H; H_{ar}), 9.14 (d, J = 5 Hz, 2 H; H_{ar}) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 59.0 (CH_3), 67.5, 69.7, 70.0, 70.1, 70.6, 70.71, 70.75, 70.8, 71.9 (OCH_2), 101.2, 101.5, 101.6, 106.2, 106.46, 106.53, 115.1, 123.6, 124.0, 126.5, 130.6, 131.0, 139.1, 139.17, 139.21, 147.0, 148.1, 149.7, 159.1, 160.10, 160.13, 160.2 (C_{ar}) ppm; MS (FAB, NBA): m/z (%): 4413.2 ($[M + H]^+$, 64), 1010.5 (58), 307.1 (100).

Radiotracer experiments

Dpp-ligands were labelled with ^{64}Cu using $^{64}CuCl_2$. To 200 µl of the ligand solution (1 µM ligand in 0.05 M 2-[*N*-morpholino]-ethanesulfonic acid (MES)–NaOH buffer, pH = 5.4–7.4) 250 kBq of $^{64}CuCl_2$ dissolved in buffer was added. Formation kinetics and labelling yields of the copper complexes were assayed using reverse phase C18 TLC plates developed in acetonitrile (0.1% TFA)–water (0.1% TFA) = 4 : 1. Radio-TLC chromatograms were scanned using a Radioisotope Thin Layer Analyzer (Rita Star, raytest). HPLC runs were performed on a Knauer WellChrom system consisting of an interface box, a HPLC-pump K-1001, a Solvent Organizer K-1500, an UV-detector U-2501 and a home-made γ-ray detector (well-type, NaI(Tl) crystal). Eluent A: acetonitrile containing 0.1% trifluoroacetic acid, eluent B: water containing 0.1% trifluoroacetic acid. Column: Jupiter 4 µ C18 90 Å (Phenomenex), 250 mm × 4.6 mm; gradient elution: 10 to 70% A in 20 min, 70 to 100% A in 25 min, 1 mL min^{-1}. Information about the lipophilicity of the ^{64}Cu complexes of the dendritic ligands has been obtained by distribution measurements in water/1-octanol systems. The experiments were performed with 1 µM solution of the ligands in buffered solution (0.05 M MES/NaOH, pH = 5.4–7.4). The aqueous solution was spiked with $^{64}CuCl_2$. The distribution experiments were carried out at 25 ± 1 °C in microcentrifuge tubes (2 cm^3) by means of mechanical shaking. The phase ratio $V_{(1\text{-octanol})}$: $V_{(aq)}$ was 1 : 1 (0.5 cm^3 each). The shaking time was chosen as 30 min. After extraction, all samples were centrifuged and the phases separated. The copper concentration in both phases was determined radiometrically using γ-radiation [^{64}Cu, NaI(Tl) scintillation automatic gamma counter 1480, Wizard 3″, PerkinElmer]. The results are means of three independent experiments.

UV/Vis and EPR spectroscopy

UV/Vis spectra were measured on a double beam Jasco Cary instrument in a thermostated sample holder. Titration experiments were done in aqueous solution at 20 °C in a standard quartz cell. To 2 ml of a 0.1 mM $Cu(NO_3)_2$ solution were added 1–5 equivalents of ligand in 0.02 ml portions of a 1 mM ligand solution. After each step 0.2 ml of the sample were diluted in 1 : 1 ratio with DMF and frozen in liquid nitrogen for the EPR measurement. EPR spectra were recorded on a Bruker Elexsys E500 spectrometer at 120 K. Spectral parameters were determined by simulation using the program XSophe.[24]

Time-resolved laser-induced fluorescence spectroscopy (TRLFS)

For time resolved fluorescence measurements the samples were excited with laser pulses of 130 femtoseonds pulse duration and 1 kHz repetition rate. For generation of the laserpulses an oscillator (Millenia pumped Tsunami, Spectra Physics) – amplifire (Merlin pumped Spitfire, Spectra Physics) system was used. The excitation wavelength was set to be 266 nm (3rd harmonic of the 800 nm Spitfire output). The energy of the laser pulses was ~150 µJ. The emitted fluorescence was measured in a right angle setup using a combination of a spectrograph (2300i, Acton Research) and an intensified CCD camera system (PicoStar HR, LaVision). The spectra were recorded in time domain mode. The gate width of the intensifier was set to be 2 ns and the stepwidth between the single spectra were 25 ps. For details of the used system see also ref. 25.

Acknowledgements

This work is part of D31 COST action "Organising Non-Covalent Chemical Systems with Selected Functions". The authors are indebted to Ms K. Landrock for their careful experimental assistance.

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