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Aggregation of a crown ether-based copper amphiphile as a mimic for the superstructure of hemocyanin

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Inspired by the alkali metal ion-controlled self-assembly of the oxygen-transporting dinuclear copper protein hemocyanin, a novel crown ether amphiphile (**1**) has been designed. A diaza-18-crown-6 moiety (diaza-18-crown-6 = 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane) is appended with an aliphatic C_{16} chain and with a PY2 ligand (PY2 = bis[2-(2-pyridyl)ethyl]amine), resulting in an amphiphile that has independent ligand sets for the coordination of copper ions and alkali metal ions. The copper complex of this amphiphile, $[Cu^{II}(\mathbf{1})](ClO_4)_2$, forms monolayers at the air–water surface. In the presence of certain alkali metal salts, the molecules in the monolayers stretch out and form sandwich complexes (2 : 1 amphiphile : metal ion stoichiometry). This is in contrast to the 1 : 1 complexes which are obtained with amphiphiles that lack the Cu–PY2 part. Electron microscopy experiments reveal that hollow tubules are generated when $[Cu^{II}(\mathbf{1})](ClO_4)_2$ is dispersed in water. The diameters of these tubes range from 45–55 nm, while their length can extend up to 5 µm. The presence of an alkali metal salt leads to vesicular structures with diameters ranging from 180–210 nm. Based on powder X-ray diffraction results, the amphiphile $[Cu^{II}(\mathbf{1})](ClO_4)_2$ is packed in strongly intercalated bilayers in both tubes and vesicles with a layer thickness of 4.8 nm. Cyclic voltammetry shows that the midpoint potential of aqueous dispersions of $[Cu^{II}(\mathbf{1})](ClO_4)_2$, $E_{1/2} = -0.08$ V, undergoes an anodic shift to +0.083 V in the presence of K^+ ions. An *in situ* preparation of $[Cu^{I}(\mathbf{1})](ClO_4)$ did not show binding of molecular oxygen, whereas $[Cu^{II}(\mathbf{1})](ClO_4)_2$ reacted with H_2O_2, leading to degradation of ligand **1**.

Introduction

One of the most intriguing supramolecular structures in nature is the functional aggregate formed by the oxygen-transporting protein hemocyanin.[1] Arthropod hemocyanin molecules are composed of hexamers and multiple hexameric subunits with molecular weights of approximately 75 kDa, each subunit containing one dinuclear copper center for molecular oxygen binding. Molluscan hemocyanin subunits, with molecular weights of 350–450 kDa, contain multiple O_2-binding sites and assemble into rings with apparent 5- and 10-fold symmetry, that further aggregate into immense cylindrical structures.[2] Divalent metal ions have been shown to be essential in the formation of these cylindrical assemblies.[3] In many hemocyanins, oxygen binding to the copper takes place in a highly cooperative way, and positive allosteric effects have been observed under the influence of ions such as H^+, Mg^{2+}, and Cl^-.[4] In a previous communication, we reported on supramolecular models of the O_2-binding site of this protein, and it was shown that the allosteric effects of alkali metals on its oxygen binding can be mimicked to some extent with the help of crown ether-based copper–pyridine complexes.[5a] In this paper we present a study on a crown ether-based amphiphilic copper complex and the effects of alkali metal ions on its aggregation in water.

Bearing in mind the objective of controlling the assembly of copper centers by allosteric effects of cations, we designed an amphiphile (**1**) which contains a ligand set for copper ions and a crown ether ring for alkali metal ion complexation, see Fig. 1. For the former ligand set, a so-called PY2 ligand was chosen (PY2 = bis[2-(2-pyridyl)ethyl]amine), which has been used previously by Karlin and by us to model biological $Cu_2 : O_2$ centers.[5–7] We chose to design a system combining the PY2 set with a ligand for alkali metal ions, rather than for the divalent metal ions of our biological example, because of the difficulty of combining in one molecule two ligand sets for metal binding, of which one would have to be selective for Cu ions, and the other for other divalent metal ions. The diaza-18-crown-6 moiety (diaza-18-crown-6 = 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane) was selected as the crown ether, which also serves as the connector between the transition metal center and the lipophilic C_{16} tail. A carbon chain of this length was expected to be large enough to provide the molecule with the desired amphiphilic properties. In the following, we present the synthesis of this novel amphiphile and report on its aggregation behaviour in water. The effect of copper ions and alkali metal ions on the self-assembling properties of **1** and the reactivity of the copper complex of **1** with O_2 and H_2O_2 will also be discussed.

Results and discussion

Synthesis

Compound **1** was synthesized by stepwise alkylation and acylation of the secondary nitrogen atoms in diaza-18-crown-6. Since initial experiments revealed that acylation followed by alkylation resulted in low yields because diacylated products instead of the desired monoacylated products were obtained, it was decided to reverse the procedure. Amphiphile **1**, therefore, was prepared by first reacting diaza-18-crown-6 with *α*-chloro-*α′*-{bis[2-(2-pyridyl)ethyl]amino}-*p*-xylene (**2**) to yield **3**, which was then reacted with hexadecanoyl chloride (Scheme 1).

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The overall yield of **1** amounted to 42%. Reference compound **4** was prepared *via* treatment of diaza-18-crown-6 with 1-bromohexadecane in butyronitrile.

The Cu(II) complex of **1** was synthesized by dropwise addition of a methanolic solution of $Cu^{II}(ClO_4)_2 \cdot 6H_2O$ to a solution of **1** in the same solvent. Isolation of the complex was achieved by adding Et_2O to the concentrated reaction mixture. Mass spectrometric analysis confirmed the formation of a mononuclear Cu(II) complex, formulated as $[Cu^{II}(\mathbf{1})](ClO_4)_2$.

Aggregation behaviour

Monolayer formation. Monolayer studies revealed that **1** and its Cu(II) complex can be spread as a floating monolayer at the air–water interface (Fig. 2). The surface area–surface pressure isotherms obtained upon compression were rather featureless and their shapes showed small changes with temperature. The head group sizes of the individual molecules in the monolayers were obtained by extrapolating the steepest part of the isotherms near the collapse to zero pressure (not shown). At pH 5.5, a head group size of 115 Å^2 at 21.8 °C and 100 Å^2 at 10 °C was found for **1**, while for **4** this size amounted to 22 Å^2 at 21.8 °C (see Table 1). These numbers suggest that in the case of **4**, the molecules are oriented perpendicular to the water surface, with the crown ether moieties submerged in the subphase. Similar structural proposals have been made for related crown ether amphiphiles, the so-called annelides, which form micellar aggregates upon dispersal in water.[8] In monolayers of **1**, the crown ether moieties are apparently completely extended and oriented parallel to the water surface. Inspection of CPK models gave an area of approximately 100 Å^2 for the diaza crown ether ring in **1**, when this ring is in a D_{3d} conformation.[9] This is consistent with the results in Table 1. In the region of the isotherm just before the collapse, the xylylene spacer and the pyridine ligand set in **1** are likely to point upward in the air, together with the aliphatic tails.

Using an aqueous subphase of pH 2 significantly decreased the head group size of the molecules in the monolayers of **1**, *viz.* to 55 Å^2. Apparently, protonation of the pyridine and tertiary amine functions of **1** transforms the initially apolar ligand set into a polar, water-soluble one. At a subphase pH of 10, the isotherm was only slightly different from that at pH 5.5,

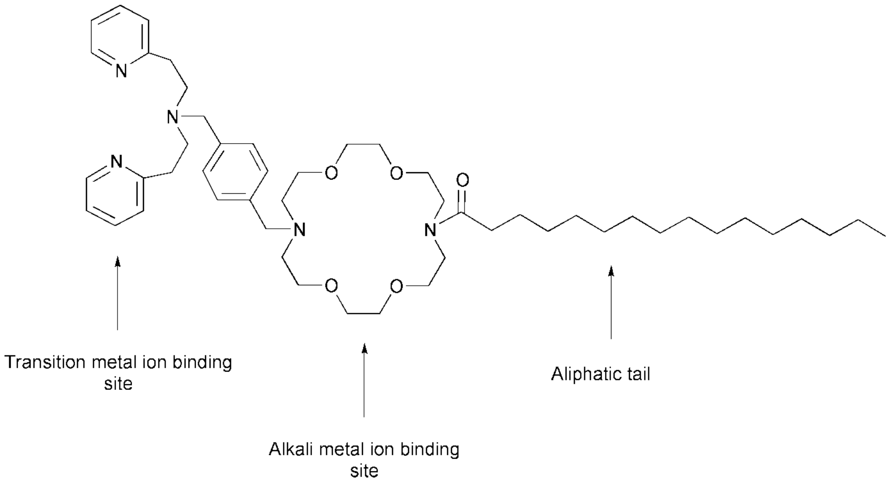
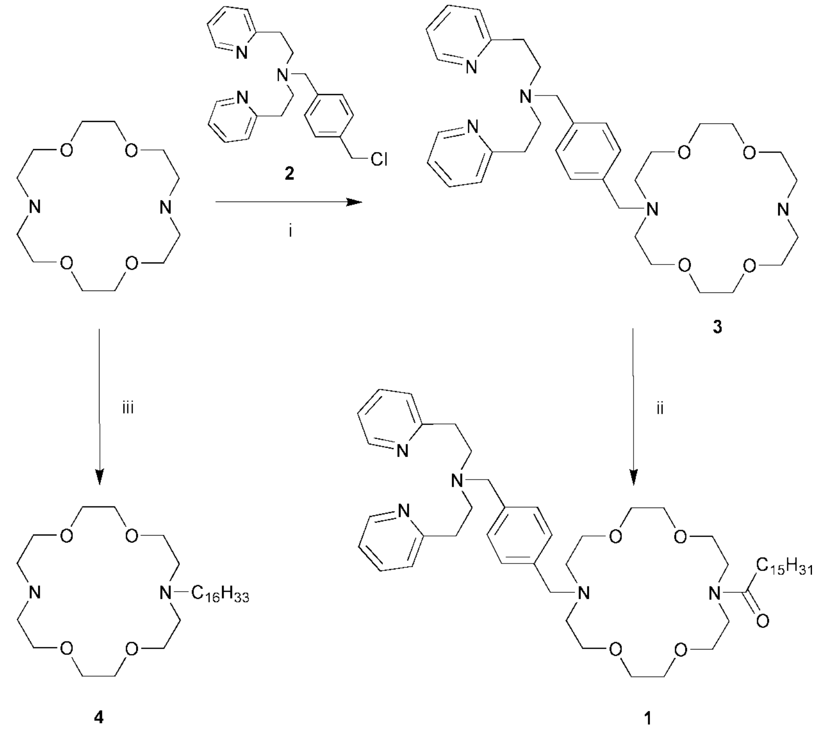


Fig. 1 The different functionalities present within amphiphile **1**.



Scheme 1 Synthesis of amphiphiles **1** and **4**. (i) Cs_2CO_3, KI, acetone 40 °C; (ii) hexadecanoyl chloride, triethylamine, CH_2Cl_2; (iii) 1-bromohexadecane, Na_2CO_3, KI, butyronitrile, reflux (4 days).

Table 1 Molecular areas of amphiphiles in compressed monolayers at the air–water interface[a]

Amphiphile	pH of subphase	Alkali metal ion	Molecular area/Å^2
1	2	—	55
	5.5	—	115
	10	—	115
	5.5	K$^+$	110
	5.5	Rb$^+$	110
[Cu^{II}(**1**)](ClO$_4$)$_2$	2	—	57
	5.5	—	105
	5.5	K$^+$	98
	5.5	Rb$^+$	102
4	5.5	—	22
	5.5	K$^+$	118
	5.5	Rb$^+$	108

[a] T = 21.8 °C.

implying a very similar head group size for the molecules at these two pH values. The orientation of the molecules in compressed monolayers of **1** at pH 5.5 does not appear to be altered by protonation of the tertiary amine sites. It does, however, influence the orientation of non-compressed molecules of **1** at the air–water surface, as can be concluded from the lower lift-off area at pH 5.5 as compared to pH 10.

Monolayer studies on the Cu(II) complex of **1**, [Cu^{II}(**1**)](ClO$_4$)$_2$, were performed by spreading **1** on a 1 mM aqueous Cu(ClO$_4$)$_2$ subphase of pH 5.5. Because of the high affinity of the PY2 ligand for copper ions,[5–7] the formation of the desired complex will take place readily using this procedure. As can be concluded from Fig. 2(b), incorporation of Cu(II) ions in the pyridine ligand set of **1** does not have a large effect on the head group size; only a small increase is found compared to the free ligand. The measured head group area of 105 Å^2 suggests that the crown ether groups are floating on the water surface and still determine the head group size of [Cu^{II}(**1**)](ClO$_4$)$_2$, while there is only a small contribution from the Cu–PY2 unit. At pH 2, a head group area of 57 Å^2 was found, which is similar to the value recorded for the free ligand under these conditions. This result can be explained if it is assumed that no copper ions are bound to the PY2 ligand at high proton concentrations. The respective orientations which we propose for **1**, its copper complex, and **4** in a compressed monolayer under different conditions are summarized in Fig. 3.

Electron microscopy. The aggregation behaviour of **1** and [Cu^{II}(**1**)](ClO$_4$)$_2$ in water was examined by means of transmission electron microscopy (TEM). Inspection of the electron micrographs of aqueous dispersions revealed that **1** does not form well-defined structures (not shown). For [Cu^{II}(**1**)](ClO$_4$)$_2$ tubular aggregates were observed, see Fig. 4.[10] These tubules are probably formed from bilayer structures (*vide infra*) which roll up, as can be seen from the sequence of pictures in Fig. 4(a)–(c). This process of tubule formation has some analogy with the rolling of hand-made cigarettes; the outer ends of the bilayers come together over their full lengths, as is visible in Fig. 4(b). Tubular objects formed *via* such a mechanism will be hollow, which is indeed suggested by the electron micrographs. A scrolling mechanism which starts from one side of the bilayer would yield non-hollow rods or fibers, and is less likely in the present case.[11] The diameter of the relatively short tubule in Fig. 4(a) is 45 ± 3 nm, whereas the large tubule in Fig. 4(d) (total length approximately 5 μm) has a diameter of 54 ± 5 nm. It is not clear whether the larger tubules are formed by the assembly of smaller ones or by the rolling up of very large and slim bilayer structures.

The aggregation of molecules of [Cu^{II}(**1**)](ClO$_4$)$_2$ into hollow tubules bears an interesting resemblance to the aggregation of hemocyanin subunits into hollow cylinders. The shapes of the aggregates are rather similar; the dimensions of the assembled cylinders of hemocyanin are approximately 31 nm × 0.5 μm,[2c] while those of aggregates of [Cu^{II}(**1**)](ClO$_4$)$_2$ are approximately 45 nm × 5 μm. The constituent building blocks are of course completely different in both cases.

X-Ray powder diffraction. X-Ray powder diffraction (XRD) experiments on cast films of [Cu^{II}(**1**)](ClO$_4$)$_2$ showed a sharp reflection corresponding to an average intermolecular distance of 48 Å (4.8 nm). According to CPK models, the length of a completely extended molecule of [Cu^{II}(**1**)](ClO$_4$)$_2$ is 42 Å. A head-to-tail orientation of stacked monolayers in the cast films appears unlikely, because in such a packing 6 Å of interlamellar space remains unaccounted for. Moreover, an interlayer distance of this size is rather large for a head-to-tail type arrangement. The most plausible aggregation mode, therefore, is a bilayer. Fig. 5 shows two bilayer assemblies that can be considered for [Cu^{II}(**1**)](ClO$_4$)$_2$. Orientation (a), which involves a strong intercalation[12] of the aliphatic tails without tilt, must be considered as being less likely because it requires the overlap of the polar crown ethers rings with the apolar C$_{16}$ chains.[13]

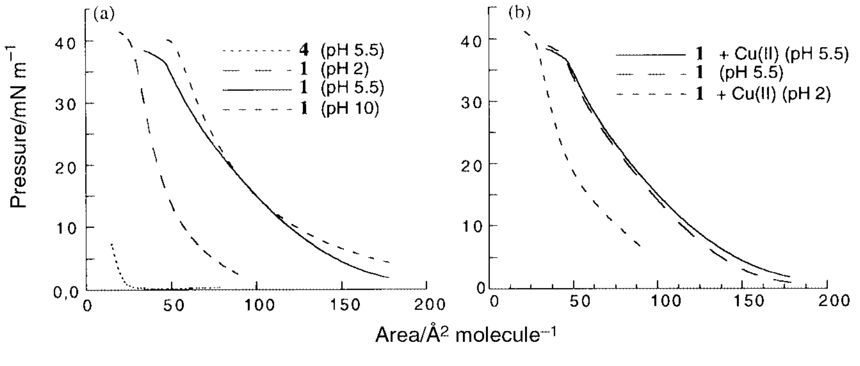


Fig. 2 Selected surface pressure–surface area isotherms of **1**, [Cu^{II}(**1**)](ClO$_4$)$_2$ [Cu^{II}(ClO$_4$)$_2$ concentration in subphase 10^{-3} M] and **4** on water at different pH values (T = 20 °C).

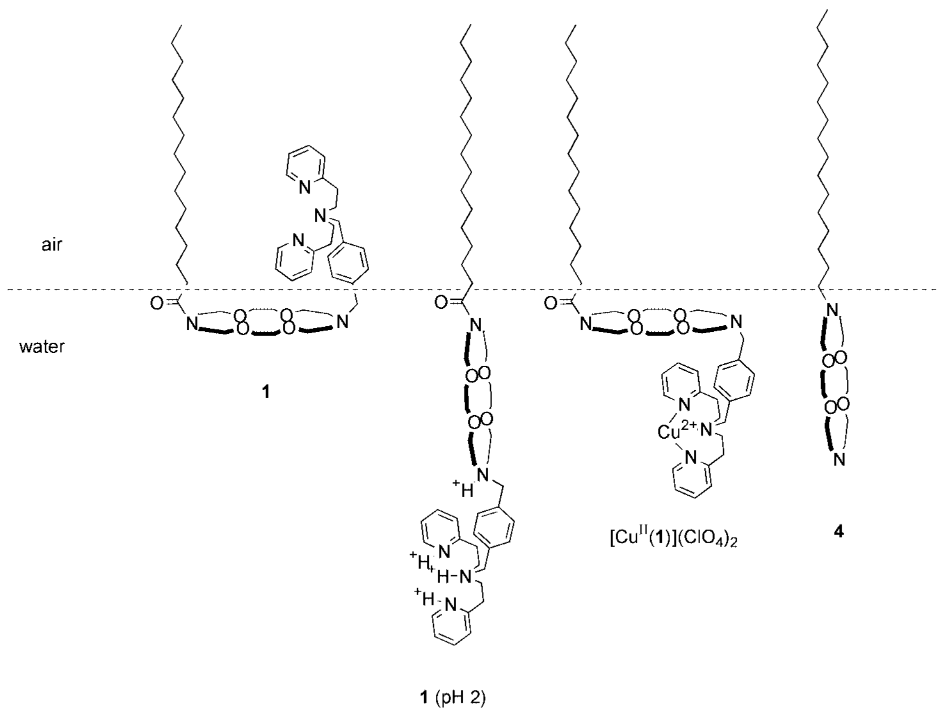


Fig. 3 Orientations of molecules of **1**, [Cu^{II}(**1**)](ClO$_4$)$_2$, and **4** in monolayers at the air–water interface under various conditions.

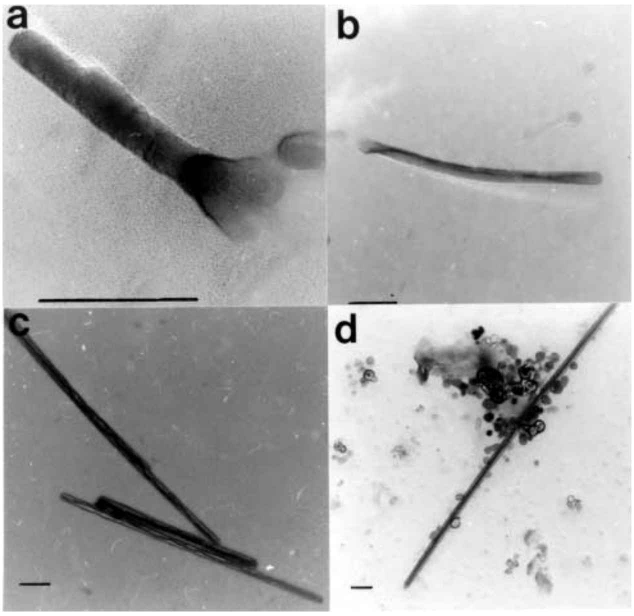


Fig. 4 Electron micrographs of aqueous 0.1% (w/w) dispersions of [Cu^{II}(**1**)](ClO$_4$)$_2$ (Pt shadowing technique). Scale bars represent 200 nm.

Intercalation involving only overlap of the aliphatic tails combined with a tilt of 40° [orientation (b)] can account for the observed periodicity of 48 Å. In this type of bilayer orientation, the copper centers are in close proximity to the tertiary amine functions of the crown ethers of neighbouring amphiphiles, which would allow these nitrogen donors to coordinate to the copper centers. Such coordination would induce a kink in the conformation of the amphiphilic molecules, which in turn could result in closer and more favourable packing. The presence of such a kink would mean that the tilt of the C_{16} chains in the bilayer is less than 40°.

Electrochemistry. The electrochemical characteristics of [Cu^{II}(**1**)](ClO$_4$)$_2$ and its aggregates were studied in acetone and water by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). In acetone solution, in which [Cu^{II}(**1**)](ClO$_4$)$_2$ is assumed not to form aggregates, a reversible reduction was observed by CV at 0.08 V *vs.* Fc/Fc$^+$ (ΔE_p = 110 mV, i_b/i_f = 1.0). This reduction potential is considerably higher than that of a related binuclear Cu(II) triflate complex derived from a bis-PY2 appended diazacrown ether (DPV: $E_{p,red} = -0.14$ V, $W_{1/2}$ = 95 mV).[14] Apparently, the Cu(I) state is more stabilized by **1** than by the dinucleating PY2 ligand, but differences in the counter ion or solvent coordination may also account for the results.

Examination of an aqueous 0.5% (w/w) dispersion of [Cu^{II}(**1**)](ClO$_4$)$_2$ by CV showed that its resting potential (E_{rest}) was exactly at the Cu(I)/Cu(II) couple. This points to the presence of Cu(II) as well as Cu(I) complexes. The CV ($E_{1/2} = -0.08$ V, ΔE_p = 197 mV, i_b/i_f = 0.64) and DPV data ($E_{p,red} = -0.11$ V, $W_{1/2}$ = 83 mV), both recorded in the oxidative direction, suggest that in the dispersion, multiple quasi-reversible one-electron processes with similar redox properties take place. Given the fact that compound [Cu^{II}(**1**)](ClO$_4$)$_2$ forms aggregates, such multiple one-electron redox processes are not unlikely.

The finding that both Cu(II) and Cu(I) complexes are present in aqueous dispersions of [Cu^{II}(**1**)](ClO$_4$)$_2$ is surprising and implies that during sample handling, reduction has taken place and that the Cu(I) state in the aggregates is considerably stabilized. Although we have observed before that certain Cu(II) complexes, *viz.* complexes based on related pyrazole ligands, can be reduced by alcohols, resulting in the formation of aldehydes and Cu(I) complexes,[15] a similar reduction was not anticipated here for the following reasons: (i) the reduction potentials of the pyridine complexes are approximately 200 mV lower than those of the pyrazole complexes, and (ii) Cu(I)–PY2 complexes generally show a high reactivity towards molecular oxygen to give Cu(II) complexes.[5–7] Golub and Meyerstein have argued that in aqueous solutions at least four tertiary nitrogen donors are needed to provide a Cu(I)-stabilizing coordination environment.[16] In [Cu^{II}(**1**)](ClO$_4$)$_2$ one tertiary amine and two pyridine amine donors coordinate to the metal center. A fourth amine donor could be provided by the tertiary amine function of a crown ether ring of a neighbouring amphiphilic complex. These considerations would support the proposal for additional nitrogen coordination to the copper centers in aggregates of [Cu^{II}(**1**)](ClO$_4$)$_2$, as based on the EM and X-ray diffraction studies (*vide supra*).

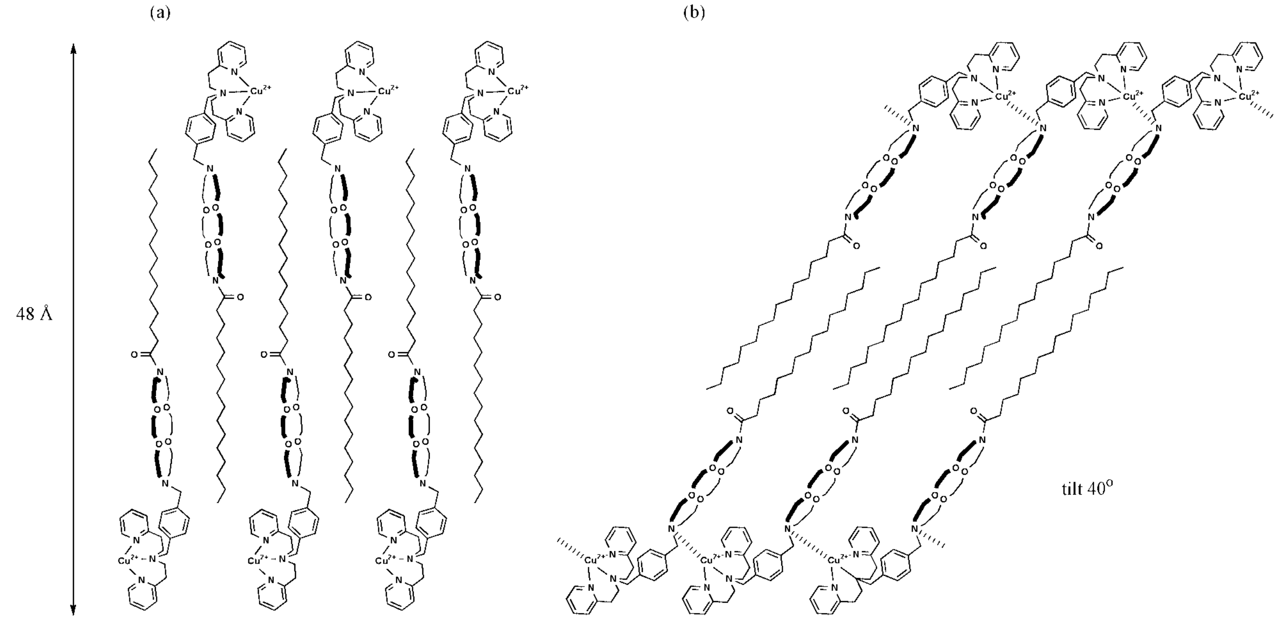


Fig. 5 Possible bilayer structures of [Cu^{II}(**1**)](ClO$_4$)$_2$.

Reactivity with O_2 and H_2O_2

The ultimate goal of our research is to obtain a model which also mimics the functional aspect of hemocyanin, *viz.* reversible oxygen binding, and possibly that of the related enzymes tyrosinase and catechol oxidase, *i.e.* oxygen activation.[5,7] In the literature, few examples of supramolecular aggregates that bind molecular oxygen have been described. Simon and co-workers have prepared amphiphilic cobalt complexes anchored to micellar structures which display this property.[17] More recently, the groups of Tsuchida and Fuhrhop have reported on O_2 binding by vesicles derived from an amphiphilic iron porphyrin.[18] Preliminary studies on the reactivity of the aggregate derived from the Cu(I) complex of **1** towards molecular oxygen were performed by means of UV/Vis experiments. Complex [Cu^I(**1**)](ClO$_4$) was prepared *in situ* in methanol by mixing **1** with a stoichiometric amount of [Cu^I(CH$_3$CN)$_4$]ClO$_4$. A colorless, opalescent dispersion of [Cu^I(**1**)](ClO$_4$) in water was prepared by the methanol injection method. Upon bubbling O_2 through this dispersion no changes in the UV/Vis absorption characteristics were observed, apart from an overall decrease in absorption intensity due to a clearing of the dispersion. Presumably, the Cu(I) state is stabilized in these dispersions (*vide supra*) to such an extent that reaction with O_2 is disfavoured.

Addition of a small amount of hydrogen peroxide to the aqueous dispersion of [Cu^{II}(**1**)](ClO$_4$)$_2$ resulted in the development of an absorption band at 380 nm, which gradually faded within a few minutes. Although it was not possible to determine the absorption coefficients for this transient species, due to the turbidity of the dispersion, these spectral changes might point to the formation of intermediate hydroperoxo copper complexes.[19] Attempts to recover ligand **1** from the reaction mixtures failed. According to mass spectrometric analysis, the starting ligand was not recovered; instead, a molecular peak was observed with a *m*/*z* ratio corresponding to the loss of a $CH_2N(CH_2CH_2Py)_2$ fragment. This is probably the result of oxidation of the ligand by analogy to the other substrate-appended ligand oxidations that have been reported recently,[20] although the reaction outcome is different from that obtained for related pyridine complexes, in which case benzaldehydes are formed upon oxygenation.[5c,6f]

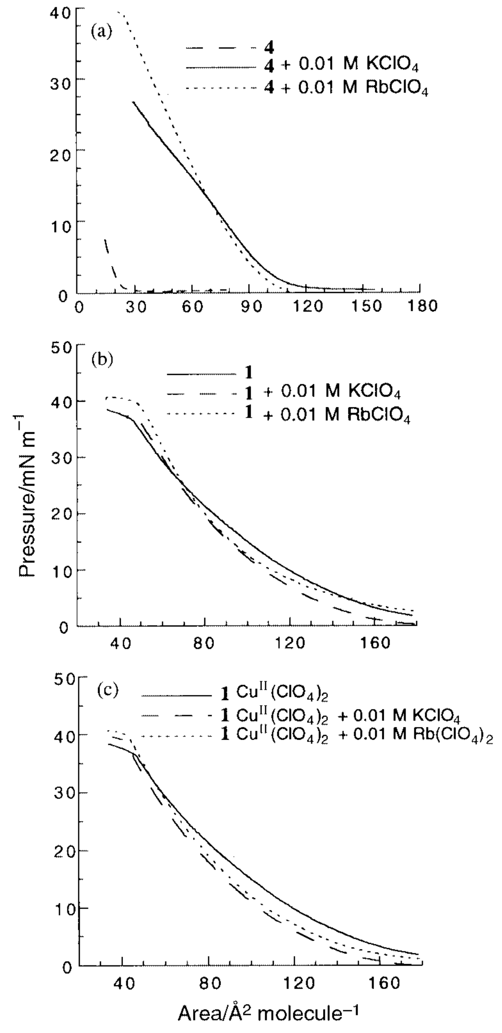


Fig. 6 Surface pressure–surface area isotherms of (a) **4**, (b) **1**, and (c) [Cu^{II}(**1**)](ClO$_4$)$_2$ on water and on aqueous 0.01 M KClO$_4$ and RbClO$_4$ (pH 5.5 and T = 20 °C).

Influence of alkali metal ions

Monolayer formation. The effects of alkali metal ions on the aggregation behaviour of amphiphile **1** and its Cu(II) complex were initially studied on monolayers of these compounds, and were compared with the behaviour of model compound **4** under the same conditions. Surface area–surface pressure isotherms were recorded on aqueous subphases containing 0.01 M KClO$_4$

and 0.01 M $RbClO_4$ and on pure water (see Fig. 6 and Table 1).

The head group size in monolayers of **4** (22 Å^2 on water) increased considerably when K^+ or Rb^+ ions were added, *i.e.* to 118 and 101 Å^2, respectively [Fig. 6(a)]. The difference in head group size appears to be determined by the kind of complex that is formed between the crown ether amphiphile and these metal ions. It has been shown before that upon compressing crown ether amphiphiles on an aqueous subphase containing alkali metal salts, so-called sandwich complexes (crown : metal ion = 2 : 1) are initially generated.[21] When all crown ether sites are occupied in 2 : 1 complexes, and the monolayer is further compressed, 1 : 1 complexes can be formed, provided the crown ether has the proper size to fit the metal ion. Rb^+ ions (ionic radius 1.48 Å) are too big to fit inside an 18-membered crown ether ring and therefore bind in a 2 : 1 host–guest fashion, whereas K^+ ions (ionic radius 1.33 Å) fit perfectly in 18-membered crown ether rings and yield 1 : 1 host–guest complexes. The average head group size in a 2 : 1 complex is smaller than in a 1 : 1 complex. In addition, the Coulombic repulsion between 2 : 1 complexes organized in a monolayer is expected to be smaller than between 1 : 1 complexes, resulting in a smaller head group area. These considerations explain the observed differences in head group size for **4** in the presence of K^+ and Rb^+ ions.

For amphiphile **1**, the effect of alkali metal ions on the architecture of the monolayers [Fig. 6(b)] was found to be more subtle than for **4**. One of the reasons is the expected lower affinity of the crown ether rings in **1** for metal ions, due to the fact that one of its amine functions is acylated. K^+ and Rb^+ ions appeared to have almost no effect on the head group size (in all cases *ca.* 110 Å^2, see Table 1). In the previous section, it was proposed that in the absence of alkali metal ions, molecules of **1** form a monolayer in which the crown ether rings float on the water surface and the C_{16}-tails, together with the pyridine ligands, point into the air. With such an orientation it is hard to imagine that sandwich complexes can easily be formed. It is, therefore, assumed that both Rb^+ and K^+ yield weak 1 : 1 complexes and no sandwich complexes. The fact that on pure water and on a subphase containing K^+ ions a higher lift-off area is observed than on a subphase containing Rb^+ ions indicates that in the latter case, the metal ion–crown ether interaction is weaker.

Alkali metal ions did have some effects on monolayers of the copper perchlorate complex $[Cu^{II}(\mathbf{1})](ClO_4)_2$. The alkali metal ions had only small effects on the pressure and molecular area at the collapse, but very significant effects on the pressure during compression and on the molecular area as derived from the slope of the isotherm [Fig. 6(c)]. The head group size decreased from 105 Å^2 on pure water to 98 and 102 Å^2 in the presence of K^+ and Rb^+, respectively, implying a change in the packing of the molecules. Without alkali metal ions present, $[Cu^{II}(\mathbf{1})](ClO_4)_2$ adopts a conformation in which its crown ether ring lies flat on the air–water interface (*vide supra*). Upon complexation of a metal ion, this orientation apparently changes into one in which this ring is positioned in a more perpendicular fashion with respect to the air–water interface. The head group size of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in the presence of K^+ is much smaller than that observed for **1** itself in combination with this ion, whereas for Rb^+ a more similar size is found. These findings can be rationalized by assuming that with Rb^+ as well as with K^+ sandwich complexes are formed, see Fig. 7. Apparently, the packing constraints in the monolayers are such that 2 : 1 host–guest complexes are thermodynamically more stable than their corresponding 1 : 1 complexes. An additional reason might be that the formation of 1 : 1 complexes leads to very unfavourable charge repulsions.

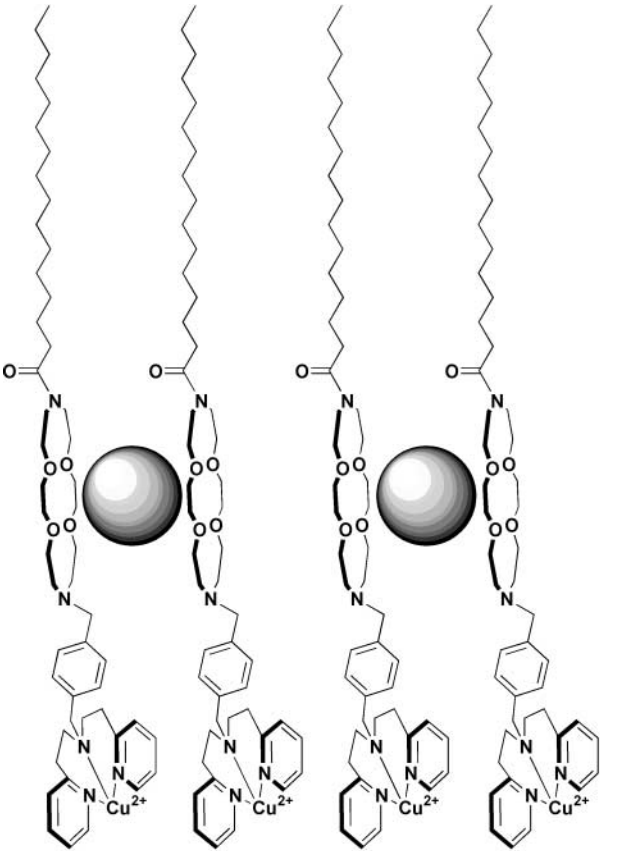


Fig. 7 Orientation of molecules of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in monolayers in the presence of K^+ or Rb^+ ions.

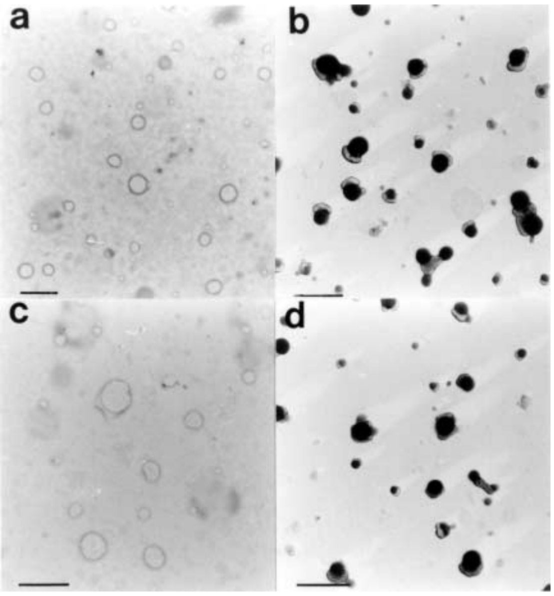


Fig. 8 Electron micrographs of 0.1% (w/w) dispersions of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in (a), (b) aqueous 10 mM $KClO_4$ solution and in (c), (d) aqueous 10 mM $RbClO_4$ solution; (a), (c) negative staining, (b), (d) Pt shadowing technique. Scale bars represent 500 nm.

Electron microscopy. Upon dispersal of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in aqueous 10 mM solutions of $KClO_4$ or $RbClO_4$, milky, opalescent solutions were obtained. TEM analysis revealed that in both cases vesicles were present (see Fig. 8). To a small extent, tubular structures were also found (not shown). The diameters of the vesicles were 212 ± 27 nm in the case of K^+ and 179 ± 35 nm in the case of Rb^+. XRD experiments on cast films of the vesicles prepared with K^+ ions revealed an average intermolecular distance of 48 Å. This periodicity is exactly the same as the periodicity found for the tubular structures which are formed in the absence of alkali metal ions. Apparently, the packing parameters are very similar for both types of

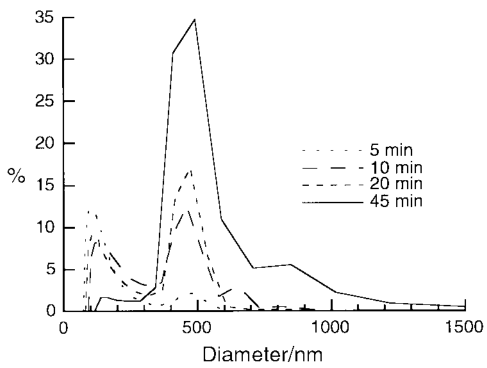


Fig. 9 Change in vesicle size distribution as a function of time in 0.1% (w/w) dispersions of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in aqueous 10 mM $KClO_4$ solution.

aggregates. The presence of alkali metal ions, which induce the formation of sandwich complexes, possibly leads to a different curvature of the bilayer, giving rise to vesicles instead of tubes. Alternatively, the coordination of the copper centers to the tertiary amines in the crown ether rings of neighbouring amphiphile molecules may become less favourable due to the formation of the crown ether–alkali metal ion complexes. This in turn may lead to a less intercalated bilayer structure with more curvature and, hence, to vesicle formation.

Closer examination of Fig. 8(a)–(d) shows that the vesicles are covered with 'blebs', which could point to a change in the aggregate size over time. Indeed, inspection of samples of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ after seven weeks of storage at 4 °C revealed that the average vesicle diameter had increased over time (5–10% for vesicles containing K^+ and 15–20% for vesicles containing Rb^+). Dynamic light scattering experiments on hand-shaken vesicle samples containing K^+ ions showed that a strong increase in vesicle diameter actually took place within the first hour after sample preparation, *viz.* from 90–130 to 380–540 Å, see Fig. 9.

We propose that the increase in vesicle diameter is the result of vesicle fusion. There may be an analogy between the fusion of these vesicles and the fusion of natural[22] and synthetic[23] phospholipid vesicles under the influence of Ca^{2+} ions. The fusion of the latter vesicles is induced by dehydration of the amphiphile head groups upon coordination of Ca^{2+}. This disturbs to some extent the local structure of the bilayer, which in turn induces the formation of an intervesicular complex and ultimately vesicle fusion. In the present case, coordination of alkali metal ions to the crown ether parts of the amphiphilic molecules of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in a similar way may result in a fusion process. Intervesicular complexes generated by sharing amphiphilic molecules between vesicles *via* metal ion sandwich complexes may be considered as an intermediate stage in the fusion process.

Electrochemistry. Aqueous dispersions of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in 0.1 M $KClO_4$ showed an anodically shifted reduction potential and an increase of the ΔE_p value in the CV ($E_{1/2} = 0.083$ V, $\Delta E_p = 360$ mV, $i_b/i_f = 0.84$) compared to dispersions in the absence of K^+ ions. The resting potential remained on the Cu(II)/Cu(I) couple, which again implies the presence of mixtures of Cu(II) and Cu(I) complexes. From monolayer and EM studies, it was concluded that K^+ ions are complexed in the crown ether part of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in the form of sandwich-like complexes, which probably prevents the crown ether amine nitrogens from coordinating to the copper centers. The fact that the Cu(I) state is nevertheless found to be more stabilized in the presence of K^+ ions points to a different driving force for the Cu(I) stabilization in this case. The incorporation of K^+ ions probably results in a considerable charge accumulation, which might induce the preference for the Cu(I) over the Cu(II) state. It is necessary that $[Cu^{II}(\mathbf{1})](ClO_4)_2$ is aggregated for K^+ ions to have this effect of charge accumulation, as addition of $KClO_4$ to $[Cu^{II}(\mathbf{1})](ClO_4)_2$ in acetone solution, where it is presumably not aggregated, did not change its electrochemical characteristics (*vide supra*). By which mechanism the copper centers are reduced is not clear at this point. In the above, we ascribed the lack of reactivity of $[Cu^{I}(\mathbf{1})](ClO_4)$ dispersions toward O_2 to the stability of the Cu(I) state in these aggregates. The fact that the Cu(I) state is even more stabilized in the presence of K^+ ions made us decide not to study the reactivity of $[Cu^{I}(\mathbf{1})](ClO_4)_2$ towards O_2 in the presence of these ions.

Conclusions

We have presented the aggregation behaviour of a new type of amphiphile (**1**), in which a binding site for hard, alkali metal ions is combined with a ligand set for copper complexation. A combination of monolayer and electron microscopy studies reveals that the incorporation of a copper ion in **1** leads to a better packing of the molecules in the monolayers and to the formation of tubular structures with lengths up to several microns. Binding of alkali metal ions in the crown ether rings of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ organized in monolayers leads to the formation of sandwich-type complexes, both with K^+ and with Rb^+ ions, and the transformation of the tubules into vesicles. These vesicles have a strong tendency to fuse. Electrochemical experiments reveal that the Cu(I) state is stabilized in the aggregates of $[Cu^{I}(\mathbf{1})](ClO_4)_2$, either because the ligand stabilizes this state or because the Cu(II) state is disfavoured due to charge accumulation. Incorporation of K^+ ions leads to a further stabilization of the Cu(I) state. Preliminary reactivity studies show that the Cu(I) aggregates are unreactive towards O_2, whereas the Cu(II)-based aggregates are capable of generating a reactive intermediate when treated with H_2O_2.

It is remarkable that the alkali metal ions are observed to exert such significant effects on the monolayer behaviour and assembly of the amphiphiles, as the affinity of the crown ethers for these ions in water is relatively low.[24] The fact that different metals have different effects which can be rationalized on the basis of the known ionic radii and the proposed binding stoichiometries proves that the effects observed are genuinely metal-induced. It is worth noting that the crown ether moieties are in a relatively hydrophobic environment in the proposed packing of intercalated bilayers in Fig. 5 and 7.

The idea of bringing the above-mentioned functionalities together within one amphiphilic molecule was inspired by the fascinating properties of the oxygen transport protein hemocyanin. We have shown that a synthetic molecule, *viz.* $[Cu^{II}(\mathbf{1})](ClO_4)_2$, can display some of these properties, *e.g.* upon dispersal in water it forms tubular structures with shapes and dimensions very similar to the cylindrical aggregates formed by the hemocyanin subunits. In addition, the aggregation behaviour of $[Cu^{II}(\mathbf{1})](ClO_4)_2$ changes upon addition of alkali metal salts just like that of hemocyanin.

We conclude that we have been able to mimic some aspects of the supramolecular aggregation chemistry of hemocyanin with the crown ether-based PY2-appended amphiphilic copper complex presented here, but that the design will have to be modified for modeling of the oxygen binding. Substitution of the pyridines for other copper-complexing ligands, leading to copper complexes with a more favourable redox potential in an aqueous environment, can be considered. The design of amphiphiles which form bilayers, but have the copper center situated in the hydrophobic interior rather than at the interface with water is another option. Studies directed toward the construction of functional supramolecular assemblies which bind and subsequently activate molecular oxygen for reaction with saturated and unsaturated hydrocarbon substrates are currently in progress.

Experimental

General

Bis[2-(2-pyridyl)ethyl]amine was synthesized *via* a published procedure.[25] Diaza 18-crown-6 and all other chemicals were purchased commercially. Solvents were dried prior to use when needed. Benzene, $\mathrm{CH_2Cl_2}$, and $\mathrm{CHCl_3}$ were distilled from calcium hydride, while diethyl ether and THF were distilled from sodium–benzophenone. Other solvents used were of analytical grade quality. ^{1}H-NMR spectra were recorded on a Bruker WH-200 machine at room temperature. Chemical shifts are presented relative to TMS as an internal standard. Mass spectra (FAB) were recorded on a VG 7070E using nitrobenzyl alcohol as the matrix. Elemental analyses were carried out on a Carlo Erba EA 1108 instrument. Particle size distributions were determined at 25 °C by dynamic light scattering using a Malvern Autosizer IIc apparatus at an angle of 90°. Flash chromatography was performed over silica gel (60 H) purchased from Merck or over neutral alumina purchased from Acros. Prior to use, neutral alumina was activated to activity III according to the Brockman scale.[26] TLC analyses were performed on glass silica-coated 60 $\mathrm{F_{254}}$ plates or on glass alumina-coated 150 $\mathrm{F_{254}}$ Type T plates from Merck.

Syntheses

α-Chloro-α′-{bis[2-(2-pyridyl)ethyl]amino}-*p*-xylene (2). To a solution of bis[2-(2-pyridyl)ethyl]amine (1.5 g, 6.61 mmol) in THF–benzene (1 : 1, v/v, 50 mL) was added dropwise, while stirring, a solution of *α*,*α*′-dichloro-*p*-xylene (4.63 g, 26.43 mmol) and triethylamine (667 mg, 6.61 mmol) in THF–benzene (1 : 1, v/v, 75 mL). The reaction mixture was stirred at 40 °C for two days. After removal of the solvent *in vacuo*, the residue was subjected to flash chromatography (silica). Using diethyl ether as the first eluent resulted in the removal of the excess of dichloroxylene. Upon subsequent elution with an EtOAc–MeOH mixture (5 : 1, v/v), pure compound **2** was recovered as a yellowish oil. Yield: 1.0 g (41%). ^{1}H-NMR (200 MHz, $\mathrm{CDCl_3}$): δ 8.49 (m, J = 0.93, 4.92, 2H; PyH), 7.54 (m, J = 1.87, 7.65, 2H; PyH), 7.05 (m, 4H; PyH), 7.14, 7.23 (2d, ^3J = 8.1 Hz, 4H; ArH), 4.59 (s, 2H; $\mathrm{ClC}H_2\mathrm{Ar}$), 3.69 (s, 2H; $\mathrm{NC}H_2\mathrm{Ar}$), 2.93 (s, 8H; $\mathrm{NC}H_2\mathrm{C}H_2\mathrm{Py}$).

α-{*N*-1,10-Diaza-4,7,13,16-tetraoxacyclooctadecane}-α′-{bis-[2-(2-pyridyl)ethyl]amino}-*p*-xylene (3). A solution of **2** (100 mg, 0.27 mmol) in acetone (10 mL) was added dropwise at 40 °C to a stirred solution of 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane (71.5 mg, 0.27 mmol) in acetone (20 mL), which also contained $\mathrm{Cs_2CO_3}$ (89 mg, 0.27 mmol) and some solid KI (*ca.* 5 mg). The resulting mixture was stirred at that temperature for 3 days. After removal of the solvent *in vacuo* and column chromatography (alumina, $\mathrm{CHCl_3}$–MeOH 92 : 8, v/v) of the resulting residue, 475 mg (65%) of the oily product **3** was obtained. ^{1}H-NMR (200 MHz, $\mathrm{CDCl_3}$): δ 8.0–8.5 (m, J = 4.6, 2H; PyH), 7.51 (m, J = 7.7 Hz, 2H; PyH), 7.04–7.20 (m, 8H; ArH, PyH), 3.58–3.67 (m, 20H; $\mathrm{C}H_2\mathrm{O}$, $\mathrm{NC}H_2\mathrm{Ar}$), 2.92 (s, 8H; $\mathrm{NC}H_2\mathrm{C}H_2\mathrm{Py}$), 2.80 (m, 8H; $\mathrm{C}H_2\mathrm{N}$), 2.60 (br, 1H; NH); MS (FAB) *m*/*z* (%): 630 (3) [M + K$^+$], 614 (53) [M + Na$^+$], 592 (58) [M + K$^+$]; $\mathrm{C_{34}H_{49}N_5O_4{\cdot}CHCl_3}$ (741.6): cal. C 59.11, H 7.09, N 9.85; found C 59.28, H 7.07, N 9.69%.

α-{*N*-[(*N*′-1-Hexadecanoyl)-1,10-diaza-4,7,13,16-tetraoxacyclooctadecane]}-α′-[bis[2-(2-pyridyl)ethyl]amino]-*p*-xylene (1). A solution of hexadecanoyl chloride (60.4 mg, 0.22 mmol) in $\mathrm{CH_2Cl_2}$ (10 mL) was added dropwise to **3** (110 mg, 0.20 mmol) and triethylamine (40 mg, 0.40 mmol) dissolved in $\mathrm{CH_2Cl_2}$ (20 mL). The resulting mixture was stirred for two days at ambient temperature. Removal of the solvent followed by purification of the residue by column chromatography (alumina, $\mathrm{CHCl_3}$–MeOH 98 : 2, v/v) yielded 129 mg (64%) of compound **1** as a viscous oil. ^{1}H-NMR (200 MHz, $\mathrm{CDCl_3}$): δ 8.4–8.6 (m, J = 1.0, 5.2, 2H; PyH), 7.56 (m, J = 2.0, 7.4 Hz, 2H; PyH), 7.0–7.3 (m, 8H; ArH, PyH), 3.5–3.7 [m, 26H; $\mathrm{C(O)NC}H_2$, $\mathrm{C(O)C}H_2$, $\mathrm{NC}H_2\mathrm{Ar}$, $\mathrm{C}H_2\mathrm{O}$], 2.92 (s, 8H; $\mathrm{NC}H_2\mathrm{C}H_2\mathrm{Py}$), 2.7–2.9 (m, 4H; $\mathrm{NC}H_2$), 2.3–2.5 [m, 2H; $\mathrm{C(O)C}H_2\mathrm{CH_2}$], 1.6–1.9 [m, 2H; $\mathrm{C(O)CH_2C}H_2$], 1.25 [s (br), 26H; $\mathrm{C}H_2$ (alkyl)], 0.88 [t (br), 3H; $\mathrm{C}H_3$]; MS (FAB) *m*/*z* (%): 852 (7) [M + Na$^+$], 830 (5) [M + H$^+$]; $\mathrm{C_{50}H_{79}O_5N_5}$ (829.6): cal. C 72.16, H 9.81, N 8.42: found C 72.47, H 9.88, N 8.11%.

$\mathbf{[Cu^{II}(1)](ClO_4)_2}$. To a stirred solution of **1** (50 mg, 0.064 mmol) in MeOH (10 mL) was added $\mathrm{Cu^{II}(ClO_4)_2{\cdot}6H_2O}$ (24 mg, 0.064 mmol) dissolved in MeOH (1 mL). The resulting green solution was quickly refluxed by heating it with a heat gun, after which part of the solvent was removed *in vacuo*. The desired product was isolated after precipitation with $\mathrm{Et_2O}$ and was dried *in vacuo* to give a viscous green oil. MS (FAB) *m*/*z* (%): 992 (3) [M$^+$ − $\mathrm{ClO_4}$], 892 (100) [M$^+$ − 2$\mathrm{ClO_4}$]. No reproducible analysis could be obtained for this compound.

Mono-*N*-hexadecyl-1,10-diaza-4,7,13,16-tetraoxacyclooctadecane (4). This compound was synthesized *via* a procedure developed by Gokel *et al.* for the dodecyl analogue.[27]

A mixture of 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane (1 g, 3.81 mmol), 1-bromohexadecane (948 mg, 3.07 mmol), $\mathrm{Na_2CO_3}$ (5.8 g, 54.8 mmol), and KI (22 mg, 0.12 mmol) in butyronitrile (30 mL) was refluxed for 4 days. Filtration followed by concentration of the filtrate *in vacuo* yielded a yellow syrup, which was subjected to column chromatography (alumina, gradient $\mathrm{CH_2Cl_2}$–^{i}PrOH 99.5 : 0.5–95 : 5, v/v). This procedure yielded 526 mg (28%) of compound **1**. ^{1}H-NMR (200 MHz, $\mathrm{CDCl_3}$): δ 3.53 (m, 16H; $\mathrm{C}H_2\mathrm{O}$, $\mathrm{NCH_2C}H_2\mathrm{O}$), 2.8 (t, ^3J = 5.7, 8H; $\mathrm{NC}H_2\mathrm{CH_2O}$), 2.5 [t (br), 2H; $\mathrm{NC}H_2$ (alkyl)], 1.25 [s, 28H; $\mathrm{C}H_2$ (alkyl)], 0.88 (t, ^3J = 6.7 Hz, 3H; $\mathrm{C}H_3$); MS EI *m*/*z*: 485 [M − 1]$^+$. No reproducible analysis could be obtained for this compound.

Electron microscopy

Samples were prepared *via* the methanol injection method. Typically, 1 mg of amphiphile was dissolved in as little methanol as possible. This solution was then injected into 100 µL of water at 65 °C. Samples used for the TEM experiments were diluted ten times. Finally, the suspensions were sonicated in a thermostatted ultrasonic bath at 65 °C for 1 h.

Grids used in EM experiments were prepared as outlined below. One drop of a 0.1% suspension of amphiphile was gently transferred to a carbon/Formvar-covered copper grid. After approx. 1 min, the solution was drained with filter paper. The sample was then dried in air for 16 h. Samples which were stained by the negative staining technique were immersed directly after removal of the solvent in a freshly prepared solution of uranyl acetate (1% in water). Platinum shadowing was performed in a high vacuum chamber. The shadowing angle was controlled by placing the sample in a tilted position relative to a tungsten wire, through which a current was passed and which was coiled with platinum wire. After these preparations, the grids were examined on a Philips TEM 201 Microscope (60 kV).

Monolayer experiments

Surface pressure–surface area isotherms were recorded on a lab-built thermostatted Teflon trough (30 × 15 cm) placed in a laminar flow cabin. Ultrapure water, used in the subphase, was obtained by filtration through a SERALPUR PRO 90C system. Between 100–300 µL of a chloroform solution containing the compound to be examined, was gently transferred on top of the subphase with a Hamilton syringe. At this point, care was taken to equilibrate the system for at least 10 min. The surface pressure was recorded using Wilhelmy plates mounted on a Trans-Tek transducer (Ellington, CT, USA). The rate of compression was approx. 20 cm^2 min^{-1}.

X-Ray powder diffraction (XRD)

Samples were prepared by transferring a drop of a 1% suspension (*vide supra*) to a silicon single crystal wafer and subsequent evacuation to 0.1 Torr. XRD measurements were carried out on a Philips X-ray powder diffractometer which was optimized for low-angle experiments.[28] Measurements were carried out using a long fine-focus X-ray tube (Cu-Kα) with generator settings of 40 kV and 40 mA. The goniometer was equipped with a variable divergence and variable anti-scatter slits. The receiving slit was set at 1 mm. The detector was of the Peltier cooled Si/Li type. Specimens were measured in a chamber in which the relative humidity could be controlled by a humidifying instrument flushed with He gas. During the measurements, the relative humidity was kept at 50%.

Electrochemistry

CV and DPV experiments were performed under an inert atmosphere, using an EG & G Princeton Applied Research Model 273 electrochemistry system and analyzed using Model 270 Electrochemical Analysis Software 200. A conventional three electrode set-up was used, with platinum working and counter electrodes, and a SCE reference electrode. For the experiments in acetone, 0.1 M $[Et_4N]PF_6$ was used as supporting electrolyte. Potentials are given against the reversible Fc/Fc^+ couple. In water, 0.1 M $[Et_4N]Cl$ and $FcCOOH/FcCOOH^+$ were used, respectively. For experiments involving K^+ ions, 0.1 M $KClO_4$ was used as supporting electrolyte.

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