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Metalloenzyme Mimics

Conformational Analysis and Binding Properties of a Cavity Containing Porphyrin Catalyst Provided with Urea Functions

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Abstract: Urea-functionalized porphyrin catalysts containing a cavity, which are used for the processive epoxidation of polymers, are thoroughly characterized and their binding properties and other supramolecular features disclosed. Intramolecular coordination of the urea side chains to the metal center of the porphyrin moiety is unveiled through NMR, IR, UV, and fluorescence spectroscopy studies. This intramolecular coordination appears to be essential in order to facilitate catalysis in a pseudo-rotaxane fashion, i.e. by preventing the use of an excess of bulky axial ligands. The current investigation provides information on how to modulate in a dynamic fashion the catalytic activity of supramolecular systems, which is of interest for the design of increasingly efficient processive catalysts.

Introduction

Over the years Nature has served as a continuous source of inspiration for the design of novel molecular systems displaying properties and functions similar to the chemical architectures found in living systems.[1] For instance, catalytic systems containing a metal center and a substrate binding site have been synthesized, mimicking the action of enzymes in terms of rate and selectivity.[2] These man-made systems are relatively simple and in that sense contrast with the complex structural and functional properties of biological molecules, which are thought to be essential to achieve the unique features of biological transformations. In order to increase their level of sophistication an in depth analysis and study of the structural features and supramolecular behavior of the man-made systems are essential.

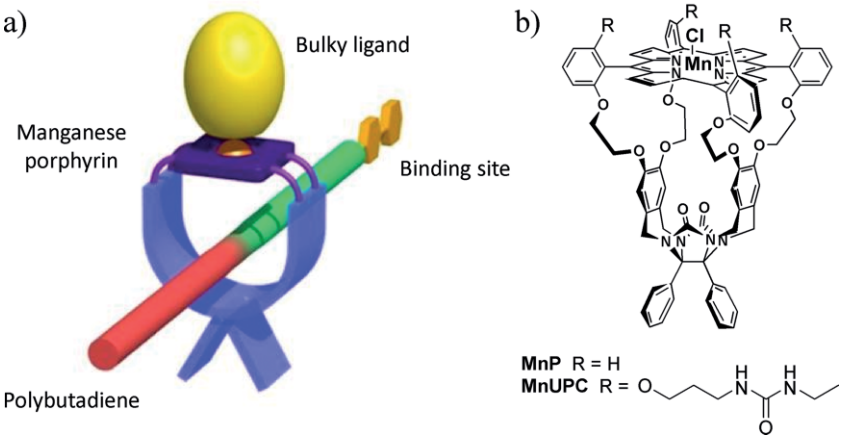

Figure 1. (a) Schematic representation of the cavity-containing manganese porphyrin catalyst, which epoxidizes polybutadiene in a pseudo-rotaxane fashion. (b) Molecular structures of **MnP** and **MnUPC**.[6]

Inspired by naturally occurring processive enzymes, e.g. DNA polymerase III and λ-exonuclease,[3] which move along a DNA chain while modifying it, we designed a toroidal enzyme mimic based on a manganese porphyrin appended to a glycoluril clip.[4] This catalyst was capable of binding to a polymer, i.e. polybutadiene, and move along it, while epoxidizing the dou-

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ble bonds of the polymer chain in a pseudo-rotaxane fashion (Figure 1, a). In order to force the catalytic reaction to take place inside the cavity of the porphyrin catalyst, a large excess of a bulky pyridine ligand was applied. This ligand also prevents decomposition of the catalyst, which has been proposed to take place via the formation of an unreactive μ-oxo bridged Mn^{IV}-porphyrin dimer.[5] In a separate paper[6] we have described a urea-functionalized cavity porphyrin **MnUPC** that turned out to be a better catalyst for oxidizing polybutadiene than **MnP** while it did not require an additional outside axial ligand (Figure 1, b).[6] We anticipated that the urea functions exerted an effect on the catalytic activity of the manganese porphyrin by coordinating to the metal center, just as the bulky pyridine did. In order to further investigate this feature we present here the structural characterization of the free-base and zinc derivatives of **MnUPC** and discuss their host–guest binding properties with nitrogen ligands and viologen guests.

Results and Discussion

Structural Analysis

The free-base derivative of **MnUPC** (designated as **H_2UPC**; see Figure 2) was synthesized as described in our previous paper.[6] **ZnUPC** was obtained in 94 % yield by reacting **H_2UPC** with $Zn(OAc)_2{\cdot}4H_2O$ in a mixture of chloroform and methanol.

The structure of **H_2UPC** was investigated with the help of NMR spectroscopy. All proton resonances could be unambiguously assigned after a COSY experiment. The ^{1}H NMR spectrum of **H_2UPC** in $CDCl_3$ at room temperature reveals the C_{2v} molecular symmetry of the compound since equivalent signals for the protons of the four different alkylurea substituents were observed (Figure 3). Compared to those of the reference compound, 1-ethyl-3-(3-phenoxypropyl)urea (**1**), the resonances of the alkylurea chains were shifted upfield due to the shielding effect of the porphyrin ring current (Table 1). Interestingly, each pair of diastereotopic protons of the alkylurea groups (H-15, 17 and 20; see Figure 3 and Table 1) gives rise to two different resonances suggesting a slow exchange process on the NMR timescale. This feature can possibly be caused by a hindered rotation of the C–O–C bonds, due to their *ortho*-substitution with respect to the porphyrin plane, or by an intramolecular interaction, viz. hydrogen bonding, between the urea groups. The latter explanation, which we propose to be the main reason, is supported by the observation of two NH stretching bands of similar intensities, at 3403 cm^{-1} (free NH) and 3330 cm^{-1} (H-bonding NH), in the FT-IR spectrum of a 1 mM solution of **H_2UPC** in $CHCl_3$.[7] Based on the results of the NMR and FT-IR studies, we conclude that the urea-containing substituents of **H_2UPC** are situated above the porphyrin plane and, most likely, are engaged in intramolecular hydrogen bonding via their urea moieties to generate a pseudo-cavity structure.[8]

The insertion of a zinc ion into **H_2UPC** to give **ZnUPC** caused considerable line broadening and some noticeable differences in the pattern and chemical shift of the signals of the alkylurea groups (Figure 4 and Table 1). The observed downfield shifts of protons H-19 and H-20 imply that the alkylurea groups are not directly located on top of the porphyrin center. The diastereotopic protons H-16 and H-19, which in the case of **H_2UPC** showed two different resonances, appear as one broad signal

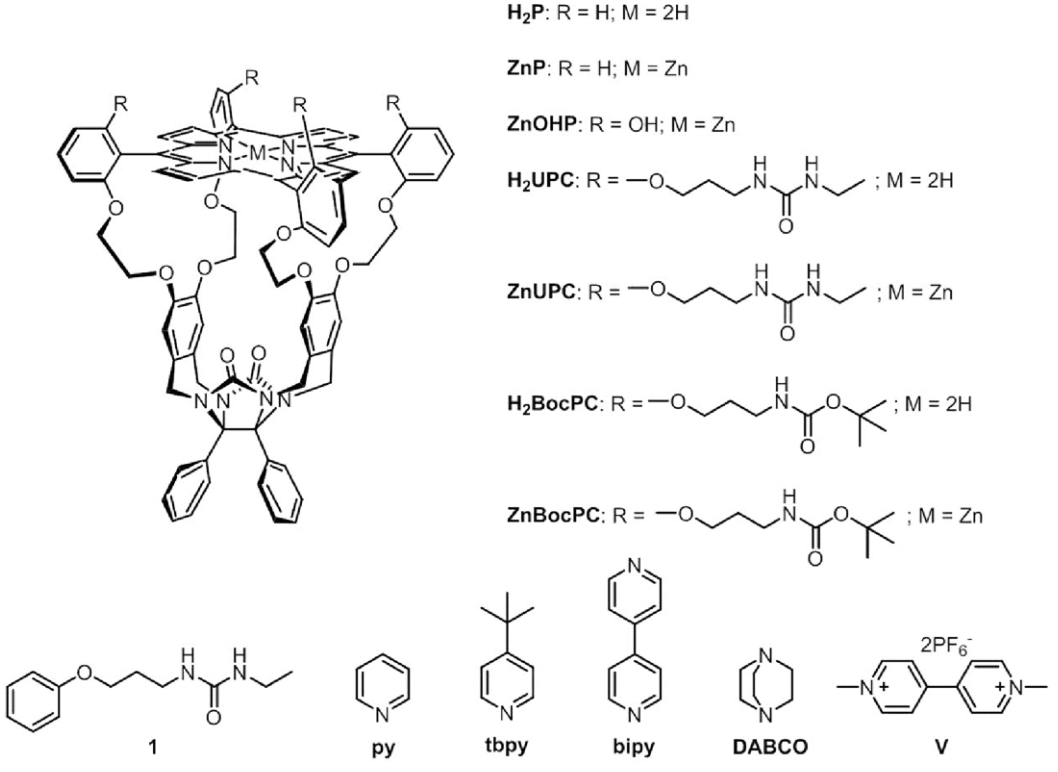

Figure 2. Molecular structures of **H_2P**, **ZnP**, **ZnOHP**, **H_2UPC**, **ZnUPC**, **H_2BocPC**, **ZnBocPC**, model compound **1**, py, tbpy, bipy, DABCO and V.

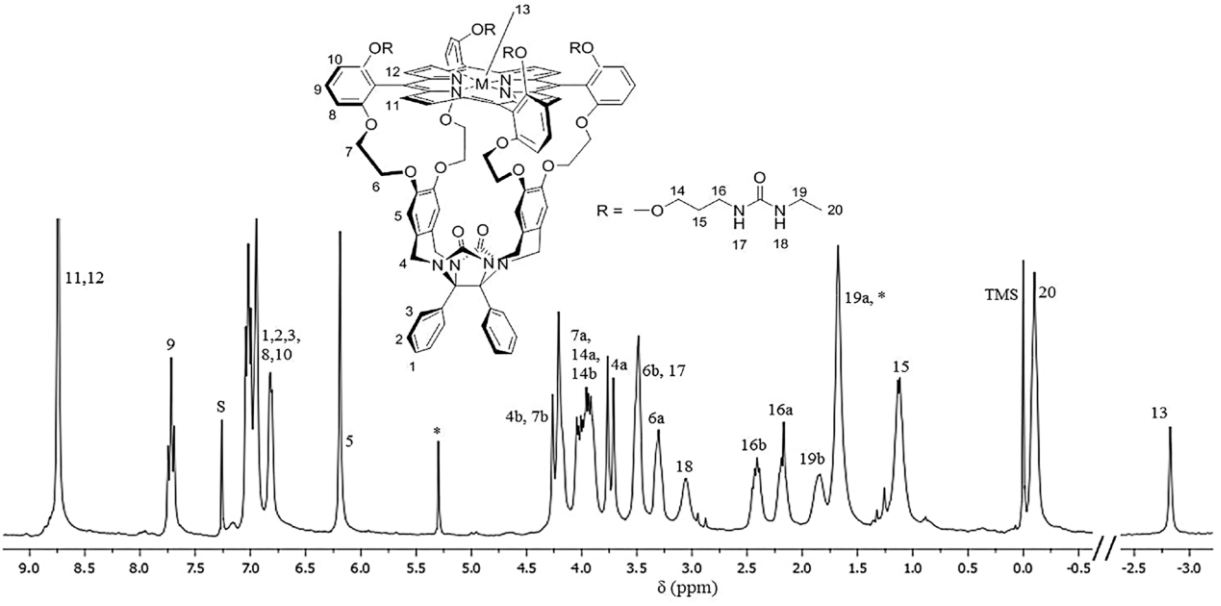

Figure 3. Assignments in the 400 MHz ^{1}H NMR spectrum of **H_2UPC** (M = 2H) in $CDCl_3$; asterisks correspond to solvent signals.

Table 1. Chemical shifts[a] and $\Delta\delta$ values (in ppm) of the 3-(3-ethylureido)-propoxy substituents of compounds **H_2UPC** and **ZnUPC** relative to those of model compound **1**.

	δ **H_2UPC**	δ **ZnUPC**	δ **ZnUPC**[b]	δ **1**	$\Delta\delta$ **H_2UPC**[c]	$\Delta\delta$ **ZnUPC**[d]
H-14a	3.98	3.97	–0.01	4.03	–0.06	–0.06
H-14b	3.94	3.91	–0.02	4.03	–0.10	–0.12
H-15	1.13	1.16	0.04	2.01	–0.88	–0.85
H-16a	2.41	2.24	–0.17	3.38	–0.97	–1.14
H-16b	2.19	2.18	–0.01	3.38	–1.19	–1.21
H-17	3.49	3.48	–0.01	4.51	–1.02	–1.03
H-18	3.05	3.16	0.11	4.24	–1.19	–1.08
H-19a	1.84	2.01	0.18	3.17	–1.33	–1.15
H-19b	1.65	2.01	0.36	3.17	–1.52	–1.15
H-20	–0.10	0.07	0.17	1.10	–1.20	–1.03

[a] A negative $\Delta\delta$ value corresponds to an upfield shift (400 MHz, 298 K, $CDCl_3/CD_3CN$, 1:1, v/v). [b] $\Delta\delta$(**ZnUPC**) = δ(**ZnUPC**) – δ(**H_2UPC**). [c] $\Delta\delta$(**H_2UPC**) = δ(**H_2UPC**) – δ(**1**). [d] $\Delta\delta$(**ZnUPC**) = δ(**ZnUPC**) – δ(**1**). For proton numbering see Figure 3.

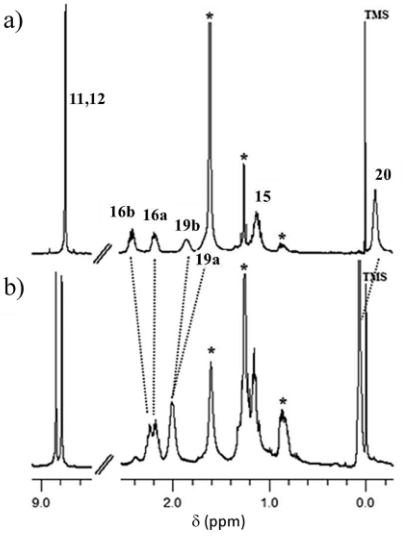

Figure 4. (a) Selected regions in the ^{1}H NMR spectra of free-base porphyrin **H_2UPC** and (b) of its zinc derivative **ZnUPC**. The most important changes are indicated by dashed lines; (400 MHz, 298 K, $CDCl_3$; asterisks correspond to solvent signals – water, and *n*-heptane which remained after precipitation). See Figure 3 for proton numbering.

at room temperature as well as at 238 K. This is indicative of a process with a large entropy of activation and characteristic of a flexible system that switches between one or more strictly defined conformations.[9] An intense band in the free NH stretching region (3391 cm^{-1}) and a weaker band in the hydrogen-bonded NH stretching region (3330 cm^{-1}), which is visible in the FT-IR spectrum of **ZnUPC** in $CHCl_3$, reveal that the intramolecular hydrogen bonding between the alkylurea tails is still present, just as in **H_2UPC**.

In the literature, several examples of metal porphyrin complexes displaying a coordination bond between oxygen-based functionalities within the molecule itself and the metal center have been reported.[10] We previously proposed that the carbonyl groups of the urea moieties in **ZnUPC** could also act as axial ligands.[6] Evidence for such an intramolecular coordination was obtained from the UV/Vis spectrum of **ZnUPC** in $CHCl_3/CH_3CN$ (1:1 v/v), which showed a somewhat red-shifted Soret band ($\Delta\lambda$ = 1–1.5 nm) compared to this band in the UV/Vis spectra of its analogues **ZnP** and **ZnOHP** where such an interaction is absent.

To get more insight in this possible intramolecular coordination, the precursor porphyrin cages of **H_2UPC** and its zinc derivative (designated as **H_2BocPC** and **ZnBocPC**, respectively) were also studied. Compared to **ZnUPC**, the intramolecular hydrogen bonding possibilities in **ZnBocPC** should be reduced due to the absence of the urea functions and the presence of the bulky *tert*-butyloxycarbonyl (Boc) groups, but the intramolecular coordination of the carbonyl group to the zinc metal ion should still be possible. The ^{1}H NMR spectrum of **H_2BocPC** in $CDCl_3$

is practically identical to that of **H_2UPC**, except for the signal corresponding to the Boc group (Figure 5, a). Upon insertion of zinc, the spectrum showed an overall broadening and a higher number of resonances (Figure 5, b). Notably, the signal of only one of the Boc groups displayed a large upfield shift ($\Delta\delta$ = –1.74 ppm), which is proposed to be the effect of the intramolecular coordination of a carbonyl group to the zinc metal ion. In the intramolecular bound state, which slowly exchanges with the unbound state, the methyl protons of the Boc group are non-equivalent and as a result one of these groups is substantially shielded. Moreover, this coordination also causes a deformation of the whole cavity scaffold, as many resonances corresponding to protons of the glycoluril-based cavity appeared split or broadened (Figure 5). This is in agreement with the distortion of the whole structure as predicted by molecular modelling (Figure 6).

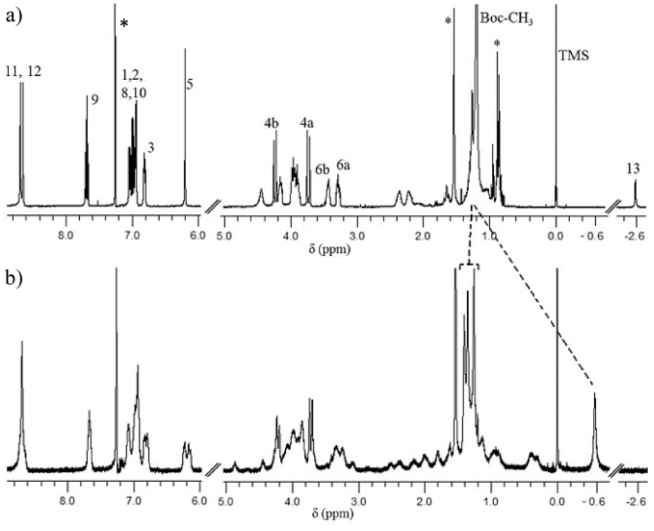

Figure 5. (a) ^{1}H NMR spectrum of **H_2BocPC**. (b) Idem, of **ZnBocPC** (400 MHz, 298 K, $CDCl_3$). The most important changes are indicated by dashed lines; asterisks correspond to solvent signals – water and *n*-heptane which remained after precipitation. See Figure 3 for proton numbering.

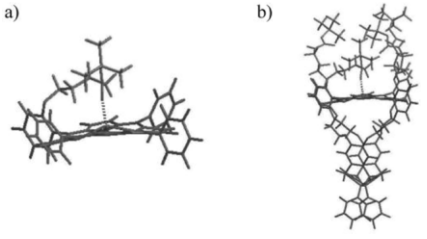

Figure 6. (a) Molecular modelling image of a porphyrin in which one Boc-protected aminoalkyloxy chain is coordinated to the zinc center (the remaining three substituents and the glycoluril-based cavity are not shown for clarity reasons). (b) Molecular modelling of **ZnBocPC** showing the coordination of the carbonyl group of one of the chains to the zinc center and the resulting distortion of the porphyrin cavity scaffold.

Further evidence for the intramolecular coordination of the carbonyl moiety was obtained from a ^{1}H NMR titration of **ZnBocPC** in $CDCl_3$ with a competing pyridine (py) ligand (Figure 7).

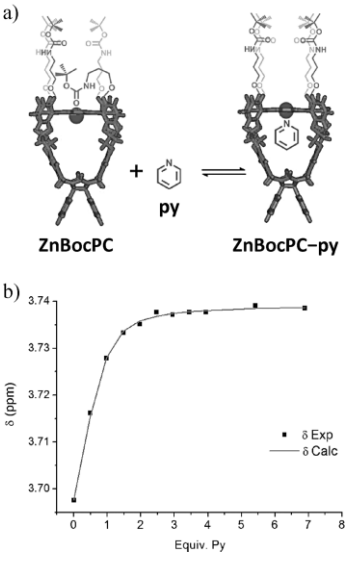

Figure 7. (a) Schematic representation of the competitive binding of pyridine to **ZnBocPC**. (b) The titration curve of **ZnBocPC** (displaying the shift of proton 4a) with py as recorded by ^{1}H NMR (400 MHz, 298 K, $CDCl_3$).

This ligand binds inside the cavity and coordinates to the Zn center.[11] Upon this binding the coordination of the carbonyl moiety is proposed to be weakened. Indeed, the NMR spectra show that upon increasing amounts of pyridine the Boc groups become on average less shielded and the signal at δ = –0.6 ppm shifts downfield (Figure 8). In the presence of an excess of pyridine, the spectrum of the **ZnBocPC**–py complex is very similar to that of the free base analogue **H_2BocPC**, except for the shifts observed for the signals of the glycoluril framework, the cavity side-wall and the crown ethers, which change because of the coordination of pyridine to the zinc metal inside the cavity. The calculated apparent association constant of the **ZnBocPC**–py complex is about two orders of magnitude weaker than that of the **ZnP**–py complex ($K_{py,app}$ = 5.7 × 10^3 M^{-1} vs. $K_{py,app}$ = 1.1 × 10^5 M^{-1} for **ZnP**–py),[11] which is due to the above mentioned competition between the intramolecular zinc–carbonyl coordination and the coordination of the pyridine ligand (Figure 7).

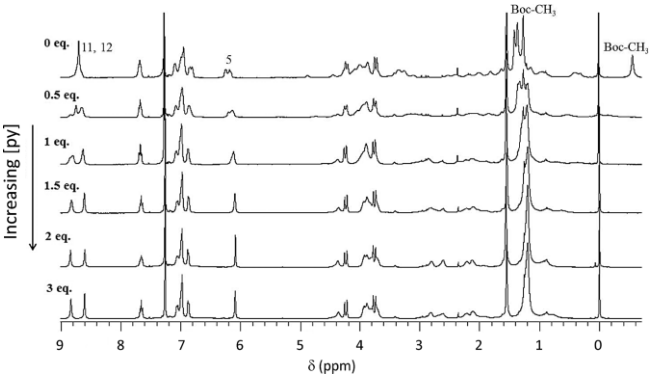

Figure 8. From top to bottom: ^{1}H NMR spectra of **ZnBocPC** at increasing concentrations of pyridine (400 MHz, 298 K, $CDCl_3$). See Figure 3 for proton numbering.

Binding Properties

Binding of Monodentate Ligands

A number of ^{1}H NMR titrations were carried out in CD_3Cl/CD_3CN (1:1 v/v) to investigate the binding of pyridine derivatives to host **ZnUPC**. Upon the addition of pyridine, the broad signals in the ^{1}H NMR spectrum of **ZnUPC** sharpened (Figure 9), indicating a replacement of the proposed intramolecular zinc–urea oxygen coordination by the intermolecular coordination of the pyridine ligand. The upfield shifts of the signals of cavity sidewall protons H-5 and crown ether protons H-6 reveal that this complexation predominantly took place inside the cavity.[10c,11]

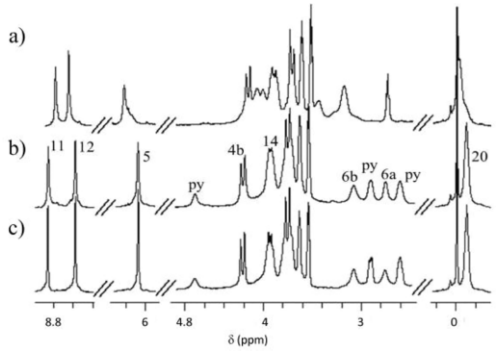

Figure 9. ^{1}H NMR spectra obtained upon the addition of increasing amounts of pyridine to **ZnUPC** [from (a) to (c); 0, 0.5, and 1 equiv. of py; 400 MHz, 298 K, $CDCl_3/CD_3CN$, 1:1, v/v]. See Figure 3 for proton numbering.

On the contrary, the bulky 4-*tert*-butylpyridine (tbpy) ligand is too large to fit inside the cavity and as a result its coordination to the zinc ion can only take place on the outside.[11] The association constant of tbpy with **ZnUPC** is relatively low, as expected, and even lower than the value obtained for **ZnP** in the same solvent mixture (K_a = 110 M^{-1} for **ZnUPC** and K_a = 400 M^{-1} for **ZnP**, Table 2, entries 5 and 6). These low values are, in general, due to the absence of additional stabilizing interactions by the cavity[11] and, in the particular case of **ZnUPC**, due to the steric interactions with the alkylurea substituents on the "top face" of the porphyrin and the competition of the tbpy ligand with the intramolecular zinc–oxygen coordination of the urea carbonyl groups.

Binding of Bidentate Ligands

The addition of linear bidentate ligands to **ZnUPC** was investigated in order to study the possibility of self-assembly of the porphyrin host into dimeric species.[12] Two molecules of **ZnUPC** should be able to dimerize head to head, at least in principle, by forming hydrogen bonds between the urea functions, and this process may be facilitated by the addition of a bidentate nitrogen base that bridges the two zinc centers. Molecular modelling studies suggested that 4,4′-bipyridine (bipy) is the best ligand to bridge the distance between two **ZnUPC** hosts. Surprisingly, the ^{1}H NMR spectra of **ZnUPC** in the presence and in the absence of 0.5 equiv. of bipy in $CDCl_3/CD_3CN$ (1:1, v/v) were not significantly different, which suggests that dimer formation does not occur (Figure 10, a–b). Apparently the urea-containing substituents prefer to form intramolecular hydrogen bonds. The fact that no bipy signals are visible in the NMR spectrum may indicate that the ligand binds weakly inside the cavity of the **ZnUPC** host,[13] via π–π interactions and is in rapid equilibrium with free bipy in solution. This explanation was confirmed by the addition of dimethylviologen (V) to the solution, which strongly binds in the cavity of the host (see Table 2, entries 1 and 9) resulting in the displacement of bound bipy and the appearance of non-bound bipy proton signals (δ = 8.68 and 7.61 ppm) as well as a sharpening of the signals (Figure 10, c). The further addition of bipy to the sample only resulted in an increase in the intensity of the uncomplexed bipy proton signals. In a separate experiment the UV/Vis spectrum of a ca. 10^{-6} M **ZnUPC** solution in $CHCl_3/CH_3CN$ (1:1, v/v) in the presence of 1.5 equiv. V was measured. The latter compound was added because it may increase the binding of a N-donor ligand to the porphyrin host by allosteric interactions (vide infra).[14] The UV/Vis spectrum only showed a very small red shift (<0.2 nm) of the Soret band upon the addition of a large excess of bipy (> 10,000 equiv.), confirming that virtually no metal–bipy coordination takes place on the "top face" of **ZnUPC**. These observations further support the idea that in **ZnUPC** one

Table 2. Association constants K_a (M^{-1}) and Gibbs binding free energies ΔG_a (kJ mol^{-1}) for the complexation of **ZnUPC** and **ZnP** with various ligands L or guests G in the absence and presence of varying concentrations of ligands L or guests G at 298 K. All measurements were performed in duplicate or triplicate in a mixture of $CHCl_3/CH_3CN$ (1:1, v/v) in the case of the fluorescence and UV/Vis measurements, and $CDCl_3/CD_3CN$ (1:1, v/v) in the case of the ^{1}H NMR measurements. Estimated errors in K_a: 20 %.

Entry	Host	L or G fixed	L or G varied	Without G K_a	ΔG_a	With G K_a	ΔG_a	$\Delta\Delta G_a$[a]	^GAM_L[b]
1[c]	**ZnUPC**	V	tbpy (500 equiv.)	2.2×10^7	–42	2.3×10^7	–42	0	1
2[c][f]	**ZnP**	V	tbpy (500 equiv.)	9×10^5	–34	3×10^6	–37	–3	3
3[c]	**ZnUPC**	V	dabco (500 equiv.)	2.2×10^7	–42	5.3×10^7	–44	–2	2
4[c]	**ZnP**	V	dabco (10 equiv.)	9×10^5	–34	2×10^6	–36	–2	2
5[d]	**ZnUPC**	tbpy	V (1.5 equiv.)	1.1×10^2	–12	2.2×10^2	–13	–1	2
6[d]	**ZnP**	tbpy	V (1.5 equiv.)	4×10^2	–15	1×10^5	–29	–14	250
7	**ZnUPC**	dabco	V (10 equiv.)	6.2×10^3[d]	–22	2.3×10^4[e]	–25	–3	4
8[f]	**ZnP**	dabco	V (10 equiv.)	5.0×10^4[d]	–27	4.0×10^5[e]	–32	–5	8
9[c]	**H_2UPC**	V	–	2.0×10^6	–36	n.d.	n.d.	n.d.	n.d.
10[c,g]	**H_2P**	V	–	6.0×10^5	–33	n.d.	n.d.	n.d.	n.d.

[a] $\Delta\Delta G_a$ = (ΔG_a for L with G) – (ΔG_a for L without G). [b] Allosteric magnification defined as ^GAM_L = (K_a of L with G)/(K_a of L without G). [c] Measured by fluorescence titration. [d] Measured by ^{1}H NMR titration. [e] Measured by UV/Vis titration. [f] Values taken from ref.[14] [g] Values taken from ref.[11]

of the urea carbonyl groups coordinates to the zinc center, very effectively competing with the binding of a ligand.

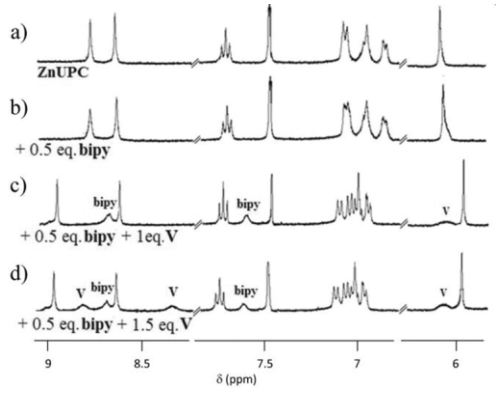

Figure 10. Downfield region of the ^{1}H NMR spectra of **ZnUPC** (a), after the addition of 0.5 equiv. of bipy (b), and subsequently 1 equiv. (c) and 1.5 equiv. (d) of V; [400 MHz, 298 K, $CDCl_3/CD_3CN$ (1:1), v/v].

To further investigate any possible dimerization of **ZnUPC**, a smaller but more basic bidentate ligand than bipy, i.e., 1,4-diazabicyclo[2.2.2]octane (DABCO), was tried as a linker between the zinc centers. In contrast to our previous studies with **ZnP** and other examples in the literature in which the addition of only half an equivalent of DABCO leads to the formation of zinc porphyrin dimers,[14,15] **ZnUPC** did not exhibit any dimerisation behavior, as was also confirmed by a 1D-^{1}H NMR and a 2D-DOSY NMR measurement. Furthermore, upfield shifted DABCO signals at around –5 ppm, which characterize the formation of zinc-porphyrin–DABCO–zinc-porphyrin sandwich complexes,[16] were not observed (vide infra).

Binding of Dimethylviologen and Cooperative Binding Effects

As already mentioned above, dimethylviologen (V) is a guest that strongly binds in the cavity of **ZnUPC**. To further study this binding, a ^{1}H NMR titration experiment with **ZnUPC** and V in $CDCl_3/CD_3CN$ (1:1 v/v) was carried out. The side-wall protons H-5 displayed an upfield complexation induced shift (Figure 11), revealing that V is clamped in between the cavity side-walls in an edge-to-face geometry with respect to the porphyrin plane, which is the same binding geometry as in the complex of V with **ZnP**.[14,17] Based on reported examples of porphyrin receptors functionalized at their *meso*-positions with diarylurea moieties, which have high association constants with V substrates ($K_a \approx 10^6$ M^{-1}),[18] the possibility of having a dipole-cation interaction between the urea groups and the viologen moiety should also be taken into account. ^{1}H NMR experiments, however, did not indicate any relevant interaction between **ZnUPC** and a second V molecule.

The formation of the complex between **ZnUPC** and V can also be monitored by fluorescence spectroscopy, since the binding of the guest inside the cavity leads to quenching of the fluorescence emitted by the porphyrin. It was observed that V binds strongly to **ZnUPC** in an 1:1 host-guest ratio with an association constant of $K_a = 2.2 \times 10^7$ M^{-1}, which is an order of magnitude higher than that for the binding of V in the non-modified host **ZnP** ($K_a = 9.0 \times 10^5$ M^{-1}) (Table 2, entries 1 and 2).

In previous papers we reported on the effect of V as an allosteric effector on the binding of tbpy to **ZnP**.[14] This guest was shown to have a significant positive allosteric effect, with also the viologen guest binding being two orders of magnitude larger in the presence of the ligand.[19] Analogous experiments were performed with **ZnUPC** as the host. Titrations of **ZnUPC** with tbpy in $CDCl_3/CD_3CN$ (1:1 v/v) in the absence and presence of 1.5 equiv. of V gave, within experimental error, similar association constants, i.e. K_a (**ZnUPC**–tbpy complex) = 1.1×10^2 M^{-1} and K_a (**ZnUPC**–tbpy–V complex) = 2.2×10^2 M^{-1} (Table 2, entry 5), revealing that there is no cooperative effect of V on the binding of tbpy to **ZnUPC**. This result can be explained by the fact that the urea carbonyl groups coordinate to the zinc center of the porphyrin and hence determine the binding profile of the host. Hence, the presence or absence of tbpy has little or no effect on the host-guest binding of viologen.

In separate experiments the binding affinity of V for **H_2UPC** was compared with the binding affinity for its analogue **H_2P** in order to evaluate what conformational effect the introduction of the urea groups has on the binding of V in the cavity of the former porphyrin host. The association constant of the **H_2UPC**–V complex was 3.3 times higher than that of the **H_2P**–V complex (entries 9 and 10), corresponding to a difference in binding energy of $\Delta\Delta G = 3$ kJ mol^{-1}. A possible explanation for the observed difference might be the presence of the intramolecular hydrogen bonding network between the urea moieties, which may lead to torsion of the *meso*-phenyl groups of the porphyrin and hence to a conformational change in the recep-

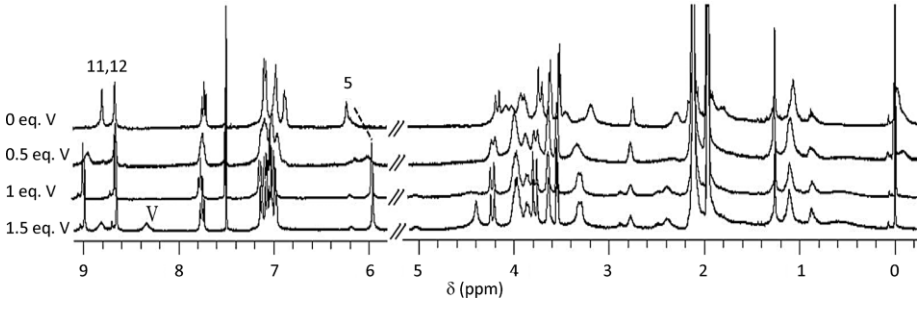

Figure 11. From top to bottom: ^{1}H NMR spectra of **ZnUPC** in the absence and in the presence of increasing amounts of guest V (400 MHz, 298 K, $CDCl_3/CD_3CN$ 1:1, v/v). See Figure 3 for proton numbering.

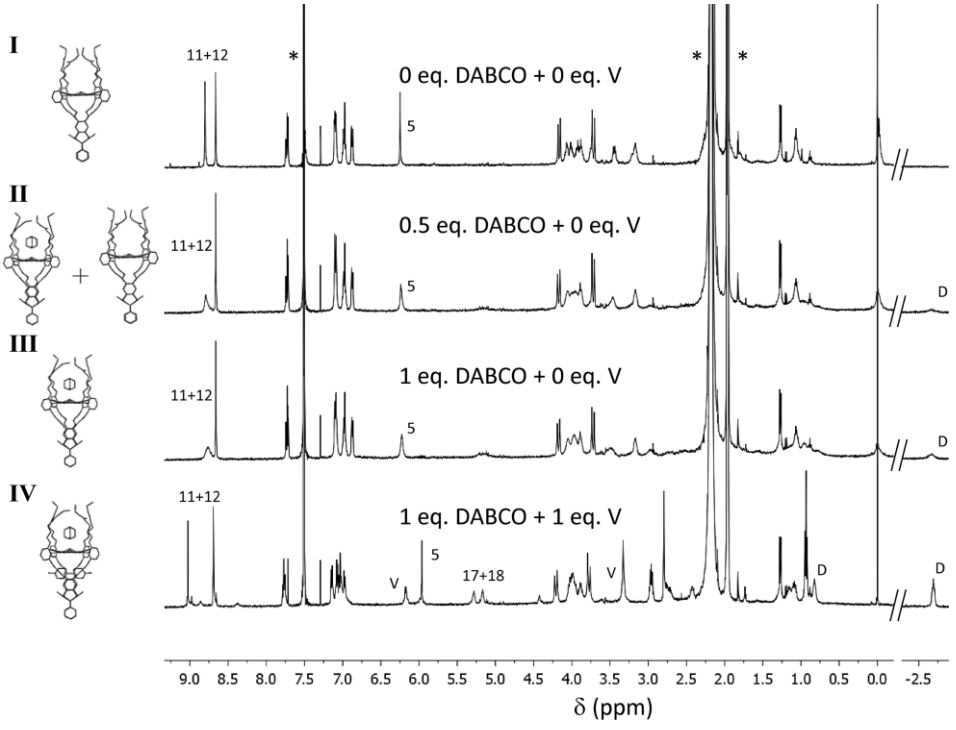

Figure 12. ^{1}H NMR spectra of: (I) free **ZnUPC**, (II) and (III) **ZnUPC** in the presence of 0.5 equiv. and 1 equiv. of DABCO, respectively, and (IV) after the subsequent addition of 1 equiv. of V (500 MHz, 298 K, $CDCl_3/CD_3CN$, 1:1, v/v). See Figure 3 for proton numbering; "V" and "D" indicate proton assignments of V and DABCO, respectively.

tor part of the molecule, which is attached to the porphyrin, leading to a higher binding constant.

As mentioned above the addition of the bidentate ligand DABCO did not facilitate the formation of head-to-head dimers from **ZnUPC**. We decided to investigate if the presence of V in the cavity would change this and induce a heterotropic cooperative effect on the coordination of DABCO leading to a subsequent dimerization of **ZnUPC**. Just as observed for the ternary complex **ZnP**–V–DABCO,[14] the porphyrin proton resonances of **ZnUPC** bound and unbound to DABCO indicated a process of fast exchange on the NMR timescale, which changed into a slow exchange process when V was added (Figure 12). This indicates that V has a positive cooperative effect on the binding event between **ZnUPC** and DABCO. The allosteric magnification, defined as $^{G}AM_{L}$ = (K_a of complex with DABCO in the presence of V)/(K_a of complex with DABCO in the absence of V), amounted to 4, and $\Delta\Delta G_a$ = –3 kJ mol^{-1} (Table 2, entry 7). Molecular modelling calculations for the **ZnUPC**–V–DABCO complex revealed that the alkylurea substituents can wrap themselves around the zinc-coordinated DABCO molecule, in such a way that the second nitrogen atom of DABCO interacts with the urea groups via hydrogen bonding. According to this molecular modelling the DABCO molecule has the appropriate dimensions to fit inside the pseudo-cavity formed by the alkylurea groups on top of **ZnUPC**. 2D-NOESY NMR measurements, however, could not provide evidence for this proposed geometry because the four alkylurea groups are in rapid dynamic exchange. The ^{1}H NMR spectrum obtained when **ZnUPC**, V, and DABCO were mixed in a 1:1:1 ratio, indicated a more symmetric spectrum, suggesting that a well-defined species was predominantly present in solution (Figure 12, IV).

The formation of the pentameric complex V–**ZnUPC**–DABCO–**ZnUPC**–V could not be detected in solution, in contrast to the situation when **ZnP** was used as a host.[14] In the case of **ZnUPC** the absence of the pentameric complex was concluded from the fact that two different chemical shifts were observed for the DABCO methylene protons, at 0.83 and –2.67 ppm, respectively, and no signal corresponding to a possible sandwich complex at ca. –5 ppm.

Conclusions

Previously we reported that **MnUPC** is a better epoxidation catalyst than **MnP** and that this compound, in contrast to the latter one, requires no axial pyridine ligand for activation.[6] We tentatively explained this feature from the fact that the carbonyl groups of the urea functions of **MnUPC** were coordinated to the metal center making that no additional axial ligand is needed. All the studies on the zinc derivative **ZnUPC** presented in this paper, confirm this explanation. They clearly show that the urea functions on the porphyrin roof of this compound bind to the metal center and hence can take over the role of a pyridine ligand as the metal-activating species for catalysis. This conclusion is further supported by the NMR measurements and binding studies of the precursor of **ZnUPC**, **ZnBocPC**, which also possesses carbonyl groups located on the porphyrin roof that can coordinate to the zinc center.

Host **ZnUPC** can thus be regarded as a double "picket fence" porphyrin, with one side shielded by a guest binding cavity and the other side protected by a pseudo-cavity formed by metal binding urea groups that are linked by hydrogen bonds. This feature makes the manganese analogue of **ZnUPC** an ideal processive catalyst for the epoxidation of polymer substrates, as confirmed by our previous studies.

Experimental Section

General: Dichloromethane and acetonitrile were distilled from CaH_2 under atmospheric pressure. DMF (after stirring for 7 days with BaO) was distilled under reduced pressure. K_2CO_3 was dried in an oven (150 °C). All other chemicals were commercial products and used as received. Flash column chromatography was performed using silica gel (0.035–0.075 mm) purchased from Acros or Merck. TLC analyses were performed on silica 60 F_{254} coated glass either from Merck or Acros. Molecular modeling calculations were performed with the use of Spartan® (equilibrium geometry determination by molecular mechanics using MMFF).

Syntheses

Zn-Tetrakis{*o*-3-[(*tert*-butoxycarbonyl)amino]propoxy}porphyrin Clip (ZnBocPC): To a degassed solution of cavity porphyrin **H_2BocPC** (3.2 mg, 1.6 μmol) in a mixture of $CHCl_3$ and MeOH (2:1, v/v; 2 mL) was added $Zn(OAc)_2{\cdot}2H_2O$ (5 mg, 2.3 μmol). The mixture was excluded from light and refluxed under nitrogen for 3 h. After cooling, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 (5 mL). The organic layer was washed with water (2 × 5 mL) and concentrated in vacuo. After purification by preparative TLC (silica, toluene/EtOAc/MeOH, 10:5:1, v/v/v) and filtration through a short plug of silica ($CHCl_3$/MeOH, 98:2, v/v), the product was dissolved in a minimal amount of CH_2Cl_2 and this solution was added dropwise to stirred *n*-heptane to yield, after centrifugation and drying of the product under vacuum, 3.3 mg (99 %) of zinc derivative **ZnBocPC**. ^{1}H NMR (400 MHz, $CDCl_3$); the non-symmetric conformation of the molecule gives rise to a high multiplicity of the signals and therefore an accurate assignment of the spectrum was not fully possible (see Figure 3 for numbering and Figure 5, b): δ = 8.68 (s, 8 H, pyrrole-β*H*, $H_{11,12}$), 7.75–7.62 (m, 4 H, ArH_9), 7.12–7.02 (m, 8 H, Ar$H_{8,10}$), 7.02–6.86 (m, 6 H, Ar$H_{1,2}$), 6.88–6.76 (m, 4 H, ArH_3), 6.30–6.12 (m, 4 H, ArH_5), 4.86 + 4.43 (br., 2 H, N*H*Boc), 4.21 (d, *J* = 15.8 Hz, 4 H, ArCH_2N *out*, H_{4b}), 4.2–0.2 (several multiplets, 42 H, porphOCH_2 + porphOCH_2 + OCH_2CH$_2$ + ArOCH_2 + N*H*Boc + CH_2NHCO + CH$_2$CH_2CH, $H_{7b,7a,14,6,17,16,15}$), 3.72 (d, *J* = 15.8 Hz, 4 H, ArCH_2N *in*, H_{4a}), 1.40 + 1.35 + 1.25 (s, 27 H, CH_3, H_{18}), –0.57 (s, 9 H, CH_3, H_{18}) ppm. MALDI-ToF MS: *m*/*z* calculated for $C_{116}H_{122}N_{12}O_{22}Zn$ (2098.8), found 2098.3 ([M$^+$]). HR-MS [ESI$^+$ (*m*/*z*)] calcd. For [$C_{116}H_{122}N_{12}O_{22}Zn$ + 2Na]$^+$ = 2144.78834; found = 2144.79889 (|Δ| = 4.92 ppm).

Zn-Tetrakis[3-(3-ethylureido)propoxy]porphyrin (ZnUPC): This compound was synthesized following the same procedure as described for **ZnBocPC**. Starting from porphyrin clip **H_2UPC**, (20.8 mg, 10.8 μmol) and $Zn(OAc)_2{\cdot}2H_2O$ (20 mg, 92 μmol) in $CHCl_3$/MeOH (2:1 v/v, 2 mL), 20.1 mg (94 %) of **ZnUPC** was obtained after purification by column chromatography (silica, MeOH/CH_2Cl_2, 3:97, v/v) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$; see Figure 3 for numbering): δ = 8.85 (s, 4 H, pyrrole-β*H*, H_{11}): δ = 8.79 (s, 4 H, pyrrole-β*H*, H_{12}), 7.71 (t, *J* = 8.3 Hz, 4 H, ArH_9), 7.13–7.00 (m, 8 H, Ar$H_{8,10}$), 7.00–6.90 (m, 6 H, Ar$H_{1,2}$), 6.85–6.75 (m, 4 H, ArH_3), 6.18 (s, 4 H, ArH_5), 4.22 (d, *J* = 15.7 Hz, 4 H, ArCH_2N *out*, H_{4b}), 4.24–4.11 (m, 4 H, porphOCH_2, H_{7b}), 4.10–3.86 (m, 12 H, porphOCH_2 + OCH_2CH$_2$, $H_{7a,14}$), 3.73 (d, *J* = 15.7 Hz, 4 H, ArCH_2N *in*, H_{4a}), 3.55–3.40 (m, 8 H, ArOCH_2, N*H*, $H_{6b,17}$), 3.38–3.19 (m, 4 H, ArOCH_2, H_{6a}), 3.16 (br. s, 4 H, N*H*, H_{18}), 2.34–2.08 (m, 8 H, CH_2NHCO, H_{16}), 2.10–1.94 (m, 8 H, NHCH_2CH$_3$, H_{19}), 1.24–1.08 (m, 8 H, CH$_2$CH_2CH$_2$, H_{15}), 0.12–0.02 (m, 12 H, CH_3, H_{20}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.88, 159.74, 158.04, 157.11, 150.23, 150.03, 146.72, 133.74, 131.06, 130.89, 130.21, 130.09, 128.72, 128.61, 128.22, 121.79, 115.86, 111.98, 105.91, 105.84, 84.92, 77.36, 67.41, 67.17, 44.50, 36.78, 33.95, 29.46, 14.81 ppm. MALDI-ToF MS: *m*/*z* calculated for $C_{108}H_{110}N_{16}O_{18}Zn$: 1982.8, found 1982.8 ([M$^+$]). UV/Vis [$CHCl_3$/CH_3CN, 1:1 v/v; λ_{max}/nm, (log ε/M^{-1} cm^{-1})]: 428 (5.6), 560 (4.2), 599 (3.1). IR (neat): $\tilde{\nu}$ = 3400, 3343, 1700, 1641, 1564, 1515, 1458, 1247, 1108. HR-MS [ESI$^+$ (*m*/*z*)] calcd. For [$C_{108}H_{110}N_{16}O_{18}Zn$ + 2Na]$^+$ = 2028.7207; found = 2028.72456 (|Δ| = 1.24 ppm).

1-Ethyl-3-(3-phenoxypropyl)urea (1): To a solution of phenol (0.25 g, 2.66 mmol) in dry DMF (2 mL) was added K_2CO_3 (0.6 g, 4.3 mmol). After stirring the solution for 30 min under a nitrogen atmosphere, 3-bromo-1-*tert*-butoxycarbonylpropylamine (0.65 g, 2.73 mmol) was added. The mixture was stirred overnight at 60 °C. After cooling, the solvent was evaporated and the residue dissolved in dichloromethane. This solution was washed with water (3 ×), and the organic phase was separated, dried with Na_2SO_4, filtered and the solvents evaporated to dryness to yield 0.57 g (63 %) of *tert*-butyl 3-phenoxypropylcarbamate as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.25 (m, 2 H, ArH_3), 6.95 (tt, *J* = 7.4, 1.0 Hz, 1 H, ArH_4), 6.89 (dd, *J* = 8.8, 1.0 Hz, 2 H, ArH_2), 4.76 (br. s, 1 H, N*H*CO), 4.02 (t, *J* = 6.0 Hz, 2 H, OCH_2), 3.37–3.30 (m, 2 H, CH_2NH), 2.02–1.94 (m, 2 H, CH$_2$CH_2CH$_2$), 1.44 (s, 9 H, CH_3) ppm.

To *tert*-butyl 3-phenoxypropylcarbamate (0.42 g, 1.67 mmol) was added a saturated solution of HCl in ethyl acetate (10 mL). After stirring for 4 h, the white precipitate formed was filtered off and dried under vacuum to yield 0.31 g (98 %) of 3-phenoxypropan-1-ammonium chloride as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.23 (dd, *J* = 8.8, 7.4 Hz, 1 H, ArH_4), 6.88–6.81 (m, 4 H, ArH_2 + ArH_3), 4.12 (t, *J* = 5.80 Hz, 2 H, OCH_2), 3.16 (t, *J* = 7.3 Hz, 2 H, CH_2NH), 2.24–2.08 (m, 2 H, CH$_2$CH_2CH$_2$) ppm.

To 3-phenoxypropan-1-ammonium chloride (175 mg, 0.93 mmol), dry dichloromethane (5 mL) and triethylamine (0.15 mL, 1.08 mmol) were added. When the compound became soluble, ethyl isocyanate (88 μL, 1.12 mmol) was added and the mixture was stirred overnight at room temperature under a nitrogen atmosphere. Dichloromethane (10 mL) was added to the solution and washed with water (3 × 25 mL). The organic phase was separated, dried with $MgSO_4$, filtered and the solvents evaporated to dryness to yield 200 mg (0.90 mmol) of **1** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31–7.24 (m, 2 H, ArH_3), 6.92 (tt, *J* = 7.3, 1.0 Hz, 1 H, ArH_4), 6.88 (dd, *J* = 8.7, 1.0 Hz, 2 H, ArH_2), 4.51 (br. s, 1 H, CH$_2$N*H*), 4.25 (br. s, 1 H, N*H*CH$_2$CH$_3$), 4.03 (t, *J* = 5.8 Hz, 2 H, OCH_2), 3.41 (t, *J* = 6.5 Hz, 2 H, CH_2NH), 3.19 (q, *J* = 7.2 Hz, 2 H, NHCH_2CH$_3$), 2.04–1.94 (m, 2 H, CH$_2$CH_2CH$_2$), 1.10 (t, *J* = 7.2 Hz, 3 H, NHCH$_2$CH_3) ppm. IR (neat): $\tilde{\nu}$ = 3391, 3304, 1678, 1640, 1607, 1586, 1564, 1542, 1498, 1239 cm^{-1}.

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Keywords: Supramolecular chemistry · Rotaxanes · Metalloenzyme mimics · Porphyrinoids · Viologens · Manganese

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[19] It is proposed that a structural rearrangement of the host and/or electrostatic interactions occur upon binding of V and these alterations are responsible for the observed allosteric effect.

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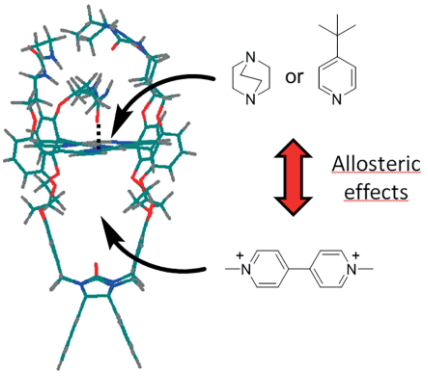
Metalloenzyme Mimics



Diphenylglycoluril-based metalloporphyrin cavities functionalized with alkylurea chains show intramolecular coordination of one of these chains to the metalloporphyrin. Its impact on the binding of viologen guests inside the cavity and coordination of axial ligands at the outside is described, and allosteric binding effects are revealed.

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