Allosteric Guest Binding in Chiral Zirconium(IV) Double Decker Porphyrin Cages

Bruekers, J.P. J.; Hellinghuizen, M.A.; Vanthuyne, N.; Tinnemans, P.; Gilissen, P.J.; Buma, W.J.; Naubron, J.-V.; Crassous, J.; Elemans, J.A.A.W.; Nolte, R.J.M.

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Allosteric Guest Binding in Chiral Zirconium(IV) Double Decker Porphyrin Cages


Chiral zirconium(IV) double cage sandwich complex Zr(1)_2 has been synthesized in one step from porphyrin cage H_21. Zr(1)_2 was obtained as a racemate, which was resolved by HPLC and the enantiomers were isolated in > 99.5% ee. Their absolute configurations were assigned on the basis of X-ray crystallography and circular dichroism spectroscopy. Vibrational circular dichroism (VCD) experiments on the enantiomers of Zr(1)_2 revealed that the chirality around the zirconium center is propagated throughout the whole cage structure. The axial conformational chirality of the double cage complex displayed a VCD fingerprint similar to the one observed previously for a related chiral cage compound with planar and point chirality. Zr(1)_2 shows fluorescence, which is quenched when viologen guests bind in its cavities. The binding of viologen and dihydroxybenzene derivatives in the two cavities of Zr(1)_2 occurs with negative allostery, the cooperativity factors α (= 4 K_a/K_c) being as low as 0.0076 for the binding of N,N'-dimethylviologen. These allosteric effects are attributed to a pinching of the second cavity as a result of guest binding in the first cavity.

Introduction

To mimic the action of porphyrin-containing enzymes, a wealth of synthetic model systems have been constructed in which (metallo)porphyrins are provided with bulky substituents, caps, and straps.[1–5] Many of these model compounds have been employed to study electron transfer processes and the binding of O_2 and CO to their metal centers. For the additional binding and catalytic conversion of substrates, more sophisticated porphyrin architectures, in which specific substrate-recognition functionalities[6–12] or macromyclic receptor cavities such as cyclolocholates,[13] cyclodextrins,[14–16] cyclophanes,[17] calixarenes,[18–20] cages,[21–23] tweezers,[24–25] and boxes[26–29] have been developed. In our group we have designed cage molecules derived from glycoluril and imparted them with a porphyrin roof, e.g. H_21 (Figure 1).[30,31] They feature very strong binding of viologen guests. When a metal center is inserted into the porphyrin, e.g. manganese(III), the cage compound (Mn1) acts as a biomimetic catalyst in the epoxidation of low molecular weight[32,33] and polymeric alkenes, which thread through the cavity of the cage compound.[34–36] Another interesting aspect of the glycoluril-based metalloporphyrin cages is that they display cooperative host-guest binding properties. For instance, binding of a viologen guest in the
cavity of Zn1 is enhanced when a bulky pyridine ligand coordinates to the zinc center at the outside of the cage molecule.\(^{[37,38]}\) As part of a program aimed at the development of biomimetic catalytic systems that can write information on a polymeric chain in a controlled fashion,\(^{[38,40]}\) we intend to develop double porphyrin molecule cages that display allosteric host-guest binding interactions. The idea is that a polyolefin substrate threads through one cavity and is catalytically converted into a poly-epoxide, while the second cavity can bind a cofactor\(^{[41]}\) that controls the catalytic reaction via structural (allosteric) changes in the first cavity. This allosteric control may involve a change in shape, size, or chirality of the first cavity, thereby inducing shape- or stereo-selectivity in the catalytic conversion of the polymer substrate, or changes in its threading speed\(^{[42]}\) and/or direction. Allosteric regulation is widely encountered in natural systems, and many artificial host-guest systems displaying allosteric binding have been investigated in the literature in order to better understand these processes.\(^{[22,43–49]}\)

Along our efforts to develop porphyrin double cage compounds, we previously reported on \(\text{H}_2\text{Zr}(\text{TPP})_{2}\text{Zr}^{2+}\) \((\text{Figure} 1)\) in which a porphyrin molecule is featured to which two glycoluril cages are attached via 8 covalent bonds.\(^{[50,51]}\) This host was found to bind two viologen guests via negative allosteric cooperativity, i.e., the second viologen was bound more than 3 orders of magnitude weaker than the first. However, significant drawbacks for the application of this system in further research towards processive enzyme mimicry are its very cumbersome synthesis and the fact that the compound can only be obtained in extremely low yield (\(\approx 0.5\%\)) in the final step, \(< 1 \text{ mg} \text{ per batch}\). We therefore explored alternative synthesis routes to obtain allosterically binding porphyrin cages more efficiently. In this paper we report the double porphyrin cage derivative \(\text{Zr}(1)_2\) \((\text{Figure} 1)\), in which two \(\text{H}_2\text{Zr}(\text{TPP})\) cages are linked in a single step via coordination to a zirconium(IV) center. The resulting sandwich complex, which is readily obtainable in \(> 100 \text{ mg}\) quantities, has a twisted structure and is chiral. We were able to resolve the racemate and determine the absolute configuration of the enantiomers by X-ray diffraction. \(\text{Zr}(1)_2\) was found to bind viologens and resorcinol derivatives via negative allosteric cooperativity as a result of structural reorganizations throughout the whole double cage complex upon consecutive binding of the guest molecules.

**Results and Discussion**

**Synthesis, resolution, and crystallographic characterization**

Double cage compound \(\text{Zr}(1)_2\) was synthesized in 33% yield and in substantial quantities (up to 160 mg per batch) by stirring the free base porphyrin cage compound \(\text{H}_2\text{Zr}(\text{TPP})_{2}\text{Zr}^{2+}\) with freshly prepared \(\text{Zr}(\text{Et}_3\text{N})_4\) in refluxing toluene. The same approach had previously been used by Kim et al. to prepare \(\text{Zr}(\text{TPP})_{2}\) \((\text{TPP} = \text{tetakis-meso-phenyl porphyrin}),\ i.e. \) the double decker porphyrin complex without glycoluril-based cages.\(^{[52]}\) Since the environment around the zirconium(IV) center becomes chiral upon complexation of the two \(C_2\)-symmetric porphyrin cages, the product is obtained as a racemic mixture of (M)- and (P)-enantiomers \((\text{Figure} 2)\). With the help of chiral HPLC we were able to resolve these enantiomers, which were obtained with ee-values of \(\approx 99.5\%\). In theory, the enantiomers can interconvert via a 90° rotation of one of the cage molecules relative to the other. However, \(\text{Zr}(1)_2\) turned out to be very stable against thermal racemization, as refluxing a solution of the pure enantiomer \((\pm)-\text{Zr}(1)_2\) in toluene for 18 h did not result in any changes in its CD spectrum \((\text{Figure} 512)\). This result is in line with previous reports on other chiral zirconium porphyrin sandwich complexes, which were also inert to thermal racemization.\(^{[53]}\)

The absolute configurations of the enantiomers could be assigned on the basis of the X-ray structure of the compound that eluted second from the column during the purification \((\text{CCDC} 2023416),\ i.e. \((\pm)-\text{Zr}(1)_2\) which turned out to correspond to the \((M)\)-isomer \((\text{Figure} 3)\). The obtained crystals of \((\pm)-\text{Zr}(1)_2\) were of insufficient quality to perform an absolute structure determination. The unit cell of the crystal contains two molecules of \((\pm)-\text{Zr}(1)_2\). The overall coordination geometry around the zirconium center is square antiprismatic. The porphyrin cages of these two molecules are rotated relative to each other by angles of 47° in one double cage molecule in the
The solubility of (±)-Zr(1)\textsubscript{2} in most common solvents and solvent mixtures turned out to be quite low, i.e. <1 mg/mL, except in dichloromethane, where the compound showed a relatively high solubility of \(\approx5\) mg/mL. We rationalized that in the poor solvents (±)-Zr(1)\textsubscript{2} was prone to aggregation. To further investigate the ability of (±)-Zr(1)\textsubscript{2} to self-assemble, droplets of solutions of racemic and enantiopure Zr(1)\textsubscript{2} in 1-phenyloctane (c \(\approx 10^{-4}\) M) were brought onto a freshly cleaved surface of highly oriented pyrolytic graphite (HOPG) in a scanning tunneling microscope (STM) operating at a solid/liquid interface. The pure enantiomers failed to form stable monolayers, but for the racemate extended domains of self-assembled lamellar arrays, containing near-rectangular features with dimensions close to that of a (±)-Zr(1)\textsubscript{2} molecule were observed (Figure 4A). The slightly oblique unit cell appears to contain two molecules of (±)-Zr(1)\textsubscript{2} and is defined by vectors with dimensions \(a = 3.5 \pm 0.2\) nm and \(b = 2.4 \pm 0.15\) nm, under an angle of 93°±2°. Although the near-rectangular features contain sub-molecular features, the structure of (±)-Zr(1)\textsubscript{2} cannot be directly identified in them. This is not surprising, given the fact that the adsorbed double cages are large 3D architectures of which the aromatic and aliphatic parts are superimposed from the point of view of the STM probe. Nevertheless, two types of roughly equally sized near-rectangular features can be discerned, differing in brightness, which alternate along each of the lamellar arrays. The observed difference in brightness may be caused by (i) the alternating adsorption of the (M)-isomer and the (P)-isomer of (±)-Zr(1)\textsubscript{2}, (ii) two different adsorption geometries of the double cage molecules (i.e., independent of the enantiomorph), or (iii) a combination of these two possibilities. Unfortunately, the complex appearance of the rectangular features prohibited identification of either the isomer identity or the cage orientation. A molecular model was constructed assuming the possibility that both enantiomers of Zr(1)\textsubscript{2} adsorb on the surface, i.e. as a 2D racemate (Figure 4B). Within a lamellar array, the 2D assembly of (±)-Zr(1)\textsubscript{2} (the two enantiomers are indicated in red and blue, respectively) is stabilized by intermolecular \(\pi-\pi\) stacking interactions between the meso-phenyl rings of adjacent porphyrin planes, while between the lamellae the phenyl rings of the glycoluril frameworks of the double cages are within Van der Waals contact distance. As mentioned above, a stable monolayer of the racemate formed readily at the solid/liquid interface, while multiple attempts to construct and image stable monolayers of the pure enantiomers failed. This might imply that the presence of both enantiomers of the double cage compound is required for the formation of a stable layer. However, since layer formation at a solid/liquid interface can be quite unpredictable and is dependent on a variety of conditions, such a conclusion may be premature and requires additional research.

Self-assembly at a surface

The solubility of (±)-Zr(1)\textsubscript{2} in most common solvents and solvent mixtures turned out to be quite low, i.e. <1 mg/mL, except in dichloromethane, where the compound showed a relatively high solubility of \(\approx5\) mg/mL. We rationalized that in the poor solvents (±)-Zr(1)\textsubscript{2} was prone to aggregation. To further investigate the ability of (±)-Zr(1)\textsubscript{2} to self-assemble, droplets of solutions of racemic and enantiopure Zr(1)\textsubscript{2} in 1-phenyloctane (c \(\approx 10^{-4}\) M) were brought onto a freshly cleaved surface of highly oriented pyrolytic graphite (HOPG) in a scanning tunneling microscope (STM) operating at a solid/liquid interface. The pure enantiomers failed to form stable monolayers, but for the racemate extended domains of self-assembled lamellar arrays, containing near-rectangular features with dimensions close to that of a (±)-Zr(1)\textsubscript{2} molecule were observed (Figure 4A). The slightly oblique unit cell appears to contain two molecules of (±)-Zr(1)\textsubscript{2} and is defined by vectors with dimensions \(a = 3.5 \pm 0.2\) nm and \(b = 2.4 \pm 0.15\) nm, under an angle of 93°±2°. Although the near-rectangular features contain sub-molecular features, the structure of (±)-Zr(1)\textsubscript{2} cannot be directly identified in them. This is not surprising, given the fact that the adsorbed double cages are large 3D architectures of which the aromatic and aliphatic parts are superimposed from the point of view of the STM probe. Nevertheless, two types of roughly equally sized near-rectangular features can be discerned, differing in brightness, which alternate along each of the lamellar arrays. The observed difference in brightness may be caused by (i) the alternating adsorption of the (M)-isomer and the (P)-isomer of (±)-Zr(1)\textsubscript{2}, (ii) two different adsorption geometries of the double cage molecules (i.e., independent of the enantiomorph), or (iii) a combination of these two possibilities. Unfortunately, the complex appearance of the rectangular features prohibited identification of either the isomer identity or the cage orientation. A molecular model was constructed assuming the possibility that both enantiomers of Zr(1)\textsubscript{2} adsorb on the surface, i.e. as a 2D racemate (Figure 4B). Within a lamellar array, the 2D assembly of (±)-Zr(1)\textsubscript{2} (the two enantiomers are indicated in red and blue, respectively) is stabilized by intermolecular \(\pi-\pi\) stacking interactions between the meso-phenyl rings of adjacent porphyrin planes, while between the lamellae the phenyl rings of the glycoluril frameworks of the double cages are within Van der Waals contact distance. As mentioned above, a stable monolayer of the racemate formed readily at the solid/liquid interface, while multiple attempts to construct and image stable monolayers of the pure enantiomers failed. This might imply that the presence of both enantiomers of the double cage compound is required for the formation of a stable layer. However, since layer formation at a solid/liquid interface can be quite unpredictable and is dependent on a variety of conditions, such a conclusion may be premature and requires additional research.

NMR characterization

The structure of (±)-Zr(1)\textsubscript{2} in solution was investigated by means of 1D and 2D NMR spectroscopy, which allowed the assignment of all proton and carbon signals. Compared to the \(^1\text{H}\) NMR spectrum of H\(_2\)1, the spectrum of (±)-Zr(1)\textsubscript{2} (Figure 5) is more complex, since asymmetry is induced into this compound upon coordination of the two cages to the zirconium(IV) center. This induced asymmetry causes a doubling of nearly all proton signals of the cages, making the protons located at opposite cage ‘quadrants’ (I and III, and II and IV, see Figure 5) chemically equivalent. In addition to this doubling of signals, the majority of them shifted upfield compared to the signals in H\(_2\)1 (Table S1). The porphyrin β-pyrrrole protons were assigned based on the observed shifts of their signals upon the inclusion of viologen guests G1 inside the cavities (vide infra): the signals...
of protons H-3, H-4, H-13, and H-14, which are located directly above the large cavity openings in which the guest binds, experience significant downfield shifts upon binding, while the signals of protons H-8, H-9, H-18, and H-19 barely shift (see Figure 11 and Table 3). The two sets of protons display ROE contacts with different meso-phenyl ortho-protons H-22 of the porphyrin, which allowed the assignment of each quadrant of the porphyrin cage. Compared to their resonances in H$_2$1, the signals of protons H-22 of (±)-Zr(1)$_2$ are shifted significantly downfield (+1.63 and +1.45 ppm compared to H$_2$1) as a result of their location in the deshielding zone of the close-by porphyrin of the second cage in the sandwich complex. The distortion in the porphyrin planes results in a decrease in their aromaticity, causing an upfield shift of the β-pyrrole signals compared to H$_2$1. Simultaneously, the X-ray structure shows that the meso-phenyl rings are slightly tilted with respect to the porphyrin plane, which is likely the result of the distortion of the latter plane, combined with the steric hindrance imposed by the other, nearby porphyrin ring. This tilting in turn seems to cause the oxyethylene protons H-27 and H-28 to rotate to the inside of the cage and into the shielding zone of the porphyrin, resulting in strong upfield shifts (up to +1.32 ppm) of their proton signals in the NMR spectrum compared to those of H$_2$1.

 Electrochemical properties

The redox properties of (±)-Zr(1)$_2$ were investigated by means of cyclic voltammetry, and compared to the properties of other zirconiumporphyrin sandwich complexes and the non-metalated parent porphyrin cage H$_2$1 (Figure 6, Table 1). In CH$_2$Cl$_2$, both (±)-Zr(1)$_2$ and H$_2$1 display two reversible oxidations and one reversible reduction of the porphyrin ligand. For both (±)-

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_1$</th>
<th>$E_2$</th>
<th>$E_3$</th>
<th>$E_4$</th>
<th>$\Delta E_{23}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$1</td>
<td>0.82</td>
<td>0.39</td>
<td>-1.79</td>
<td>–</td>
<td>2.18</td>
</tr>
<tr>
<td>(±)-Zr(1)$_2$</td>
<td>0.36</td>
<td>-0.10</td>
<td>-1.96</td>
<td>–</td>
<td>1.86</td>
</tr>
<tr>
<td>TPP</td>
<td>0.82</td>
<td>0.52</td>
<td>-1.64</td>
<td>-1.93</td>
<td>2.16</td>
</tr>
<tr>
<td>Zr(TPP)$_2$</td>
<td>0.55</td>
<td>0.12</td>
<td>-1.73</td>
<td>-2.09</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Table 1. Redox potentials (in [V]) of H$_2$1 and (±)-Zr(1)$_2$, and for comparison those of TPP$^{35}$ and Zr(TPP)$_2$$^{36,37}$.$^{38-40}$ Solvent/supporting electrolyte: CH$_2$Cl$_2$/NBu$_4$PF$_6$ referenced to ferrocene ($E_{1/2}$ = 0.497 mV). $\Delta E_{23}$ is the difference between the potentials for the first oxidation and the first reduction.
Zr(1)₂ and H₂1, no second reduction was observed due to the limits of the combination of solvent and electrolyte. Compared to H₂1, (±)-Zr(1)₂ is easier to oxidize and more difficult to reduce, which indicates that both the HOMO and the LUMO of (±)-Zr(1)₂ are higher in energy than those of H₂1. The same trend is visible for TPP and Zr(TPP).[54] This increase in energy observed for the porphyrins in zirconium(IV) sandwich complexes might be explained by the distortion in the porphyrin plane (visible in the crystal structure in Figure 3), which causes a reduction of the aromaticity of the porphyrin. Decreased oxidation potentials have been found before for other sandwich complexes and their corresponding monoporphyrinates.[54,57] For Zr(TPP)₂ it was hypothesized that the energy rise of the HOMO is the result of π-π stacking interactions between the porphyrin planes.[54]

Compound (±)-Zr(1)₂ is easier to oxidize and more difficult to reduce compared to Zr(TPP)₂, which is likely the result of the more electron-rich porphyrin because of the alkoy substituents at the meso-phenyl groups. A similar redox behaviour was reported for a porphyrin with meso-o-methoxyphenyl substituents (T(oo-OMe)PP), of which the oxidation potentials are ~0.1 V lower than those of TPP.[51] Another consequence of the presence of the cage moieties is that the double cage sandwich complex is less flexible than the unfunctionalized sandwich complex, which may induce more strain in the porphyrin rings, causing their aromaticity to be reduced.

**Chiroptical properties**

Compared to the porphyrin Soret band in the UV-vis spectrum of H₂1 in CH₂Cl₂, the Soret band of (±)-Zr(1)₃ is blue-shifted by ~20 nm to 398 nm (Figure 7A). This increase in energy of the porphyrin π-π* transition is likely the result of the decrease in conjugation due to the distortion of the porphyrin planes. The mirror-like ECD and VCD spectra (Figure 7B and Figure 8B, respectively) of the two compounds separated by chiral HPLC revealed that they are enantiomers, as expected. The shape of the Soret band is correlated to a strong exciton coupling between the two porphyrin planes. The ECD spectra display a right-handed couplet at 398 nm for the first eluted enantiomer (+)-Zr(1)₃, which corresponds to a clockwise orientation of the porphyrin planes, and thus this enantiomer is the (P)-isomer.[62] The second eluted enantiomer, (−)-Zr(1)₃, displays a left-handed couplet at 398 nm, which is in agreement with an anti-clockwise orientation (M) of the porphyrin rings, which in turn corresponds to the X-ray crystallography data. The Q bands around 500 and 550 nm are also weakly CD-active, and other strong CD signals are present in the high-energy region between 250 and 350 nm.

The VCD spectra of the enantiomers (+)-Zr(1)₃ and (−)-Zr(1)₃ in CD₂Cl₂ displayed many vibrations of the receptor-parts of the molecules, implying that the chiral environment around the zirconium(IV) center is propagated to the entire cage structure (Figure 8A–B). Strikingly, these sandwich complexes display VCD fingerprints that are very similar to the recently reported single porphyrin cage compound H₂3 (Figure 1), in which the chirality was introduced into the molecule in a completely different manner, i.e. by substituting each of the xylylene sidewalls with a nitro-group.[63] The VCD spectra of (+)-Zr(1)₃ and S₃⁻(R,R)-H₂3 both exhibit four characteristic positive bands at 1253, 1285, 1330/1336 and 1375 cm⁻¹, and a negative-posititive couplet at 1705–1728 cm⁻¹ (Figure 8C–D) and are rather intense (dissymmetry factors g₀, around 1.2 × 10⁻³). The positive band at 1336 cm⁻¹ for S₃⁻(R,R)-H₂3 is dominated by vibrational modes of deformations of the porphyrin part. In (±)-Zr(1)₃ the formation of bonds with the zirconium(IV) center leads to a red shift of this band of 6 cm⁻¹. The characteristics of this band (sign, shape and intensity) can be related to the concave geometry adopted by the porphyrin in both molecules S₃⁻(R,R)-H₂3 and (±)-Zr(1)₃. The complexation of the cage with zirconium(IV) significantly emphasizes this concavity. Similarly, the sign and intensity of the couplet, which results from the mixing of the two C=O stretching modes, depends on the relative asymmetry of these functional groups in the most abundant conformations of S₃⁻(R,R)-H₂3 and (±)-Zr(1)₃.[64,65] This phenomenon represents a rare example where the VCD spectra reflect a molecule with conformational chirality (atropisomerism) and it confirms the usefulness of VCD spectroscopy for characterizing porphyrin derivatives, an aspect that was not emphasized before.

Upon irradiation of the Soret band of (±)-Zr(1)₃, an intense band at 658 nm in the emission spectrum appeared (Figure S9). This emission was determined to be fluorescence based on lifetime measurements (Figure S10). About 60% of the fluorescence was quenched when oxygen was bubbled through the sample for 3 minutes (Figure S9).
Host-guest binding studies

To investigate the binding strength between Zr(1)₂ and methyl viologen (N,N-dimethyl-4,4'-bipyridinium dihexafluorophosphate) (G1), and to study cooperative effects between the binding of the first and second guest, a fluorescence titration was carried out (Figure 9A–C, Table 2). As expected, the addition of increasing amounts of G1 to a solution of (+)-Zr(1)₂ at 3.0 μM in CH₂Cl₂/CH₃CN, 1:1 (v/v) caused quenching of the fluorescence (a solvent mixture was employed since neither the host nor the guest are sufficiently soluble in a single solvent). Fitting of the titration data to a 1:2 host-guest stoichiometry revealed that the second viologen guest is bound almost 3 orders of magnitude weaker than the first one (K₁ = 1.1 × 10⁷ M⁻¹, K₂ = 1.9 × 10⁴ M⁻¹), indicating a strongly negative cooperative effect with an α-value (4K₂/K₁) of 0.0076. It should be noted that the first part of the titration curve could not be fitted completely, leading to somewhat higher errors. Interestingly, the binding of the viologen guest resulted not only in the fluorescence quenching of the adjacent porphyrin, but also in a partly fluorescence quenching of the porphyrin on the opposite side of the zirconium center. This can be concluded from the fact that the fluorescence at 1 equivalent of added G1 is not 50% but 35%, i.e. 65% is quenched (Figure 9C). This means that also the second porphyrin is within the quenching distance of the guest. The host-guest binding properties were also evaluated by UV-vis titration experiments (Figure 10 and Fig-
ure S13, Tables S13–19), which confirmed the 1:2 host-guest complex formation with negative cooperativity. Taking into account the larger errors in the fits of the fluorescence titrations, rather similar values were obtained for the association constants measured by fluorescence and by UV-Vis titrations (K₁ = 2.1 × 10⁶ M⁻¹, K₂ = 5.0 × 10⁵ M⁻¹, α-value = 0.0096). During the first part of the UV-vis titration (in the presence of 0 to ~2 equivalents of G1) a clear isosbestic point was observed at 400 nm (Figure 10A), suggesting the conversion of the free host to its 1:1 host-guest complex without the significant formation of a 1:2 complex. When the amounts of guest were increased, the isosbestic point at 400 nm disappeared while a new one, albeit less prominent, appeared at ~398 nm (Figure 10B). The latter isosbestic point may indicate the conversion of the 1:1 complex to the 1:2 complex. The host-guest binding could also be monitored by CD spectroscopy (Figures S11–13, Tables S10–12). Complexation between (±)-Zr(1), and G1 induced the emergence of a new CD signal between 350 and 390 nm, but due to a poor signal to noise ratio the determination of the K-values in a titration experiment turned out to be rather unreliable.

The ¹H NMR spectrum of the 1:2 complex between (±)-Zr(1), and G1 in CD₃Cl/CD₃CN 1:1 (v/v) indicated a similar binding geometry of the viologen guests as in the case of H₁,₂. The downfield complexation induced shifts (CIS) of the signals of β-pyrrole protons H-3, H-4, H-13, H-14 and the upfield shifts of the cavity side-wall protons H-30 upon the binding of G1 (Figure 11, Table 3) indicate that the guests bind with their aromatic rings perpendicular to the porphyrin and parallel to the xylylene side-walls. Remarkably, in contrast to the considerable upfield shifts that were observed for the proton signals of the ethyleneoxy spacers upon the binding of G1 in H₁,₂,²¹ for (±)-Zr(1),₂ significant downfield shifts were observed for several of the signals of protons H-27 upon binding of the guests. We attribute these shifts to a structural reorganization of the host in which the ethyleneoxy spacers move out of the shielding zone of the porphyrin by rotating outwards, thereby generating space in the cavities to accommodate the large violagen guests (induced-fit). All proton signals of the guests were very broad, which is attributed to fast exchange between bound and unbound species. Compared to the complexation of

Table 2. Association constants (K) and binding free energies (ΔG) for complexes of porphyrin cage compounds with various guests. The cooperativity factor is defined as α = 4 K₂/K₁. ΔΔGbound is defined as ΔG₂−ΔG₁.

<table>
<thead>
<tr>
<th>Guest</th>
<th>Host</th>
<th>K₁ [M⁻¹]</th>
<th>ΔG₁ [kJ mol⁻¹]</th>
<th>K₂ [M⁻¹]</th>
<th>ΔG₂ [kJ mol⁻¹]</th>
<th>ΔΔGbound [kJ mol⁻¹]</th>
<th>α</th>
</tr>
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<tbody>
<tr>
<td>G1</td>
<td>H₂</td>
<td>7.0 × 10⁵</td>
<td>-43</td>
<td>5.0 × 10⁶</td>
<td>-28</td>
<td>15</td>
<td>0.003</td>
</tr>
<tr>
<td>(+)-Zr(1),₂</td>
<td>1.11 ± 0.42 × 10⁷</td>
<td>-40.2 ± 0.9</td>
<td>1.87 ± 0.20 × 10⁷</td>
<td>-24.4 ± 0.3</td>
<td>15.8 ± 1.2</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>(-)-Zr(1),₂</td>
<td>2.11 ± 0.47 × 10⁷</td>
<td>-36.2 ± 0.5</td>
<td>5.00 ± 1.08 × 10⁷</td>
<td>-21.1 ± 0.6</td>
<td>15.1 ± 1.3</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>H₂</td>
<td>3.73 ± 0.84 × 10⁷</td>
<td>-14.7 ± 0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(+)-Zr(1),₂</td>
<td>4.10 ± 1.06 × 10⁷</td>
<td>-20.6 ± 0.7</td>
<td>5.45 ± 1.97 × 10⁷</td>
<td>-15.5 ± 0.8</td>
<td>5.1 ± 1.5</td>
<td>0.03 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>(-)-Zr(1),₂</td>
<td>9.80 ± 3.30 × 10⁷</td>
<td>-17.0 ± 1.0</td>
<td>4.44 ± 1.60 × 10⁷</td>
<td>-12.1 ± 1.2</td>
<td>5.1 ± 2.2</td>
<td>0.58 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>G3R</td>
<td>G₁H₂</td>
<td>1.24 ± 0.20 × 10⁸</td>
<td>17.7 ± 0.4</td>
<td>1.78 ± 0.48 × 10⁷</td>
<td>-12.8 ± 0.7</td>
<td>4.9 ± 1.1</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td>G3S</td>
<td>G₁H₂</td>
<td>1.10 ± 0.26 × 10⁸</td>
<td>-17.3 ± 0.6</td>
<td>1.58 ± 0.53 × 10⁷</td>
<td>-12.4 ± 0.9</td>
<td>4.9 ± 1.5</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td>(+)-Zr(1),₂</td>
<td>1.19 ± 0.24 × 10⁸</td>
<td>-17.5 ± 0.5</td>
<td>1.68 ± 0.57 × 10⁷</td>
<td>-12.6 ± 0.9</td>
<td>4.9 ± 1.4</td>
<td>0.56 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>


Figure 10. UV-vis spectra during the titration of (±)-Zr(1), with G1 in CHCl₃/CH₃CN (1:1, v/v) with host concentration remaining constant at 2.0 μM and G1 ranging from 0 to 1370 equivalents. Isosbestic points are indicated by the black dotted circles. (A) Spectra taken in the first part of the titration (0–2 equiv. of G1 present) showing a clear isosbestic point due to the conversion of free host to a 1:1 complex. (B) Spectra taken in the second part of the titration (2–1370 equiv. of G1 present) showing a second isosbestic point, suggesting the transition of the 1:1 to the 1:2 complex. The arrows indicate the spectral changes upon the addition of increasing amounts of G1. (C) Full set of UV-vis spectra of the titration.
Figure 11. $^1$H NMR spectra (400 MHz) of (A) (±)-Zr(1)$_2$ and (B) its 1:2 host-guest complex with G1 in CD$_2$Cl/CD$_3$CN (1:1, v/v); shifts of selected proton signals are indicated with dashed lines; see Figure SC for atom labeling. The asterisks indicate residual solvent peaks.

Figure 12. $^1$H NMR spectra (500 MHz, CD$_2$Cl/CD$_3$CN (1:1, v/v), 245 K) of the region of the signals of side-wall protons H-30 of solutions containing (A) (±)-Zr(1)$_2$ (B) a 1:1 mixture of (±)-Zr(1)$_2$ and G1, and (C) a 1:2 mixture of (±)-Zr(1)$_2$ and G1. \( [\pm\text{-Zr(1)}_2] = 0.24 \text{ mM} \). See supporting information Figure S29 for the full spectra. The signal indicated by the asterisk is a $^1$H satellite of the CDCl$_3$ solvent peak.

G1 in H$_2$1,[31] the first guest in (±)-Zr(1)$_2$, is bound significantly stronger, and the second one significantly weaker. The difference in the Gibbs free energy of binding of G1 in the two cavities of (±)-Zr(1)$_2$ (Δ\(\Delta\)G$_{allo}$) is 15.1 kJ mol$^{-1}$

In an effort to confirm the large difference in the association constants \( K_1 \) and \( K_2 \) of solutions of (±)-Zr(1)$_2$, and of this host in the presence of one and two equivalents of G1 were investigated by $^1$H NMR spectroscopy at 245 K. This low temperature was chosen in order to induce slow host-guest exchange in the complexes. The spectra of (±)-Zr(1)$_2$ and of the 1:2 host-guest mixture were recorded in the room temperature spectra, while the spectrum of the 1:1 host-guest mixture showed a doubling of many of the proton signals (Figure 12 and Figure S29). In particular the doubling of the number of side-wall proton signals H-30 is illustrative (Figure 12B) and can be attributed to symmetry breaking within the host, which can be expected when only one single G1 guest binds in one of the cavities of (±)-Zr(1)$_2$. As a result, the two cavities are no longer equivalent, resulting in different chemical shifts for previously identical protons. The four signals of the side-wall protons are located at positions that are distinctly different from the positions of the side-wall proton signals in the free host and the 1:2 host-guest complex. This is further evidence for the presence of predominantly a 1:1 complex when one equivalent of G1 is added. The upfield shift of the signals of proton H-30 indicates that also in the 1:1 complex the guest binds with its aromatic rings parallel to the xylene side-walls.[2] The addition of the second equivalent of guest restores the symmetry of the host. Unfortunately, due to the poor solubility of (±)-Zr(1)$_2$ (0.24 mM) at low temperature we were unable to employ 2D NMR techniques to further characterize the precise geometry of the 1:1 complex.

The association constants for the binding of G1 in (±)-Zr(1)$_2$ shown in Table 2 are quite similar to those found for the binding of the same guest in our previously reported double porphyrin cage compound H$_2$2 (Figure 1), in which the two receptor cavities are separated by a single porphyrin.[34] For the latter compound, binding of a viologen in the first cavity resulted in a pinching of the second, empty cavity, thereby causing an allosteric effect which made the cavity an inferior host for the second viologen. A similar allosteric mechanism may be operative in the case of (±)-Zr(1)$_2$ since the two receptor cavities are structurally connected via their coordination to the zirconium center.

To investigate this possibility, we calculated the structures of the free host (→)-Zr(1)$_2$ and its 1:1 and 1:2 host-guest

Table 3. $^1$H NMR chemical shifts ($\delta$, ppm) and complexation induced shifts [CIS, $\delta$, ppm] of selected proton signals of (±)-Zr(1)$_2$ and its 1:2 host-guest complexes with G1 and G2; see Figure SC for atom labeling.

<table>
<thead>
<tr>
<th>Proton</th>
<th>(±)-Zr(1)$_2$</th>
<th>(±)-Zr(1)$_2$-[G1]</th>
<th>CIS</th>
<th>(±)-Zr(1)$_2$-[G2]</th>
<th>CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta$</td>
<td>$\delta$</td>
<td>$\delta$</td>
<td>$\delta$</td>
<td>$\delta$</td>
<td>$\delta$</td>
</tr>
<tr>
<td>3,4,13,14</td>
<td>8.02, 7.76</td>
<td>8.32, 8.26</td>
<td>+0.30, +0.50</td>
<td>7.97, 7.71</td>
<td>8.08, 7.89</td>
</tr>
<tr>
<td>27a</td>
<td>3.26, 3.09</td>
<td>3.46, 3.15</td>
<td>+0.20, +0.06</td>
<td>3.34, 3.14</td>
<td>2.83, 2.62</td>
</tr>
<tr>
<td>27b</td>
<td>2.67</td>
<td>2.57, 3.33</td>
<td>−0.10, +0.66</td>
<td>2.77, 2.67</td>
<td>2.22, 2.05</td>
</tr>
<tr>
<td>28a</td>
<td>2.82, 2.34</td>
<td>2.91, 2.44</td>
<td>+0.09, +0.10</td>
<td>2.91, 2.43</td>
<td>2.25, 2.07</td>
</tr>
<tr>
<td>28b</td>
<td>2.26</td>
<td>2.39, 2.20</td>
<td>−0.13, −0.06</td>
<td>2.41, 2.23</td>
<td>2.07, 1.60</td>
</tr>
<tr>
<td>30</td>
<td>5.99, 5.91</td>
<td>5.60, 5.81</td>
<td>−0.39, −0.10</td>
<td>6.01, 5.87</td>
<td>5.94, 5.91</td>
</tr>
</tbody>
</table>

[a] In CD$_2$Cl$_2$/CD$_3$CN 1:1 (v/v). [b] In CD$_2$Cl$_2$. [c] Not used in the fitting due to the difficulty to distinguish signals during the titration.
complexes with G1 by molecular modelling (Spartan '14™, semi-empirical, PM3). The calculated energy-minimized structures (Figure 13) show significant variations in the cavity widths of (−)−Zr(1)2, depending on the presence or absence of guest molecules. As an indication of these widths, the distances between protons H-30 of the two cavities at opposite xylylene side-walls are summarized in Table 4. The calculations reveal that when the first viologen guest binds in cavity 1, the latter widens when compared to its width in the absence of the guest. At the same time, the width of cavity 2 decreases. When the second viologen guest binds, cavity 2 retains its narrow size, while cavity 1 further narrows. The observation that the second guest needs to bind in a relatively narrow cavity is in line with the observed negative cooperativity of guest binding. Since this narrowing is apparently caused by a structural rearrangement throughout the whole double cage complex, induced by the binding of the first guest, this negative cooperativity can indeed be attributed to allostery.

In addition to viologens, resorcinol (1,3-dihydroxybenzene) derivatives are also suitable guests for the porphyrin cage compounds.[81] They generally bind by means of hydrogen bonding interactions between their hydroxyl groups and the urea carbonyl oxygen atoms of the host, while their aromatic rings interact via π-π stacking with the xylylene side-walls of the host and with the porphyrin plane.[66] The binding of resorcinol-derived guest molecules G2, G3R, and G3S in the cavities of (±)−Zr(1)2 was investigated with the help of 1H NMR titrations. The titration curves for the binding of G2 in (±)−Zr(1)2 could only be fitted to a binding equilibrium assuming the formation of a 1:2 host-guest complex, and provided K1 and K2 values which are summarized in Table 2. The CI5-values obtained from the titrations (Table 3) indicate that the guests bind in the cavities of (±)−Zr(1)2 with their aromatic surface parallel to the xylylene side-walls, thereby strongly shifting upfield the signals of particularly the ethyleneoxy protons H-27 and H-28. These upfield shifts indicate that, in contrast to what was observed for the binding of G1, the binding of the smaller guest G2 does not lead to a significant movement of the ethyleneoxy spacers of (±)−Zr(1)2 out of the porphyrin shielding zone, and hence suggest that the host requires no or much less rearrangement during binding than in the case of G1. The proton signals of the guest were again broad due to fast exchange between bound and unbound species. Interestingly, the first G2 guest was found to bind an order of magnitude stronger in (±)−Zr(1)2 than in H1. This enhanced binding is attributed to the stretching of the cavities of the double cage complex. Since the aromatic ring of a resorcinol derivative bound in a glycoluril-based clip molecule is clamped in a position slightly above the xylylene side-walls of the cavity,[66] this stretching and distortion of the cavities of Zr(1)2 along the z-axis, together with the distortion of the porphyrin plane, provides more space for the guest compared to the binding in H1. For the binding of the two G2 guests, an α-value of 0.53 was observed, which is a much less prominent negative binding cooperativity than was found for the binding of G1. Molecular modeling calculations of the complexes of (−)−Zr(1)2 with G2 (Figure S46) reveal that a similar, but less pronounced, allosteric effect is operative as in the complexes of the host with G1. Upon binding the first G2 guest in cavity 1, the latter expands somewhat compared to the previously empty cavity, but significantly less than when it binds G1 (Table 4). Simultaneously, cavity 2 narrows, thus disfavoring the binding of the second G2 guest. Subsequent binding of the second guest widens cavity 2, while the width of cavity 1 remains nearly unaffected. The latter observation is different from what was found for the complexes of (−)−Zr(1)2 with G1, where the binding of the second guest leads to a more overall rearrangement of both cavity spaces. This requirement for more extensive rearrangement of the host is in line with the observed much more pronounced negative allosteric cooperativity for the binding of G1 in (±)−Zr(1)2.

In a final set of experiments we explored the potential of the enantiopure hosts (+)−Zr(1)2 and (−)−Zr(1)2 to enantioselectively bind chiral resorcinol derivatives G3R and G3S (Table 2). Within experimental error no chiral discrimination was found: all possible host-guest combinations displayed similar K1.

Table 4. Distances [Å] between side-wall protons H-30 calculated by molecular modeling of (−)−Zr(1)2 and its 1:1 and 1:2 complexes with G1 and G2. See Figure 11 for atom labeling.

<table>
<thead>
<tr>
<th>Host or host-guest complex</th>
<th>Cavity 1 H-30 (I)–H-30 (II)</th>
<th>Cavity 1 H-30 (III)–H-30 (IV)</th>
<th>Cavity 2 H-30 (I)–H-30 (II)</th>
<th>Cavity 2 H-30 (III)–H-30 (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)−Zr(1)2</td>
<td>6.73</td>
<td>6.93</td>
<td>6.49</td>
<td>6.76</td>
</tr>
<tr>
<td>(−)−Zr(1)2−G1</td>
<td>7.63</td>
<td>6.98</td>
<td>6.55</td>
<td>6.55</td>
</tr>
<tr>
<td>(−)−Zr(1)2−(G1)2</td>
<td>6.73</td>
<td>6.71</td>
<td>6.49</td>
<td>6.38</td>
</tr>
<tr>
<td>(−)−Zr(1)2−G2</td>
<td>6.91</td>
<td>6.94</td>
<td>6.54</td>
<td>6.38</td>
</tr>
<tr>
<td>(−)−Zr(1)2−(G2)2</td>
<td>6.85</td>
<td>6.95</td>
<td>6.92</td>
<td>7.07</td>
</tr>
</tbody>
</table>
and $K_2$-values, which were all weaker than those observed for the binding of G2 in (±)-Zr(1)$_2$. The lower binding affinities for guests G3 are ascribed to their bulky 5-substituents, which have more steric interactions with the porphyrin roof of the hosts. The CI5-values obtained from the NMR titrations again show strong shielding of the ethylenoxy protons H-27 and H-28 of the hosts upon guest binding, indicating that the host-guest binding geometries are similar as those in the complex between (±)-Zr(1)$_2$ and G2 (Table S32). Because of their size, the ethoxycarbonyl substituents of G3R and G3S are probably directed to one of the cavity portals, which causes the methyl groups connected to the chiral center to point to one particular side of the cavity (Figure S14). As a result, one set of ethylenoxy proton signals shifts more upfield than the other set. This effect is mirrored in the diasteromeric complexes and similar in the enantiomeric host-guest complexes. Despite the relatively weak binding, still negative cooperativity was observed, with $\alpha$-values in the same range as those found for the host-guest complexes between (±)-Zr(1)$_2$ and G2.

Conclusion

Double cage sandwich complex Zr(1)$_2$ was synthesized in a single step from H$_3$- in a yield of 33%. Its enantiomers have been resolved with excellent enantiomeric excesses and their absolute configurations could be assigned based on the crystal structure of (-)-Zr(1)$_2$ and were confirmed by circular dichroism spectroscopy. With the help of STM, the double cage complexes could be imaged at the sub-molecular level in a self-assembled monolayer of the racemate on a graphite surface, which was not possible with either of the pure enantiomers. VCD experiments of the enantiomers of Zr(1)$_2$ revealed that the chirality around the zirconium center is propagated throughout the whole cage structures. The VCD fingerprints are rather similar to the ones previously reported for the enantiomers of the whole cage structures. Future research will be directed towards providing Zr(1)$_2$ with a catalytic functionality, however as the zirconium center of Zr(1)$_2$ only serves as a non-catalytic coordination site, we intend to provide the xylylene side-walls of the double cage complex with oxidation catalysts (e.g. Mn-porphyrins or Mn-salens). These catalysts can modify a polymeric substrate that threads through one of the cages, while the properties of the substrate can be allosterically controlled, e.g. in terms of binding strength, threading speed, etc., via binding of an allosteric effector in the second cage.

Deposition Number 2023416 (for (-)-Zr(1)$_2$) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Allostery · Cooperativity · Host-guest chemistry · Porphyrin · Zirconium
