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Construction of supramolecular multi-component assemblies by using allosteric interactions

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ABSTRACT

Supramolecular complexes between two cavity-appended porphyrin hosts and three bifunctional guests are described. The host with a single cavity exclusively forms dimers with the bifunctional guests, while the double cavity host yields tetramers and higher order assemblies. The role of allosteric interactions in the binding and assembly process is highlighted.

1. Introduction

Cooperative interactions are common in nature; they are, for instance, employed to influence the composition and function of hierarchical, self-assembled systems such as the tobacco mosaic virus, and also to transfer information, like in the binding of oxygen to haemoglobin.[1–4] Cooperative interactions also play a critical role in gene transcription; for instance, cyclic AMP has a strong cooperative effect on the binding of the gene transcription regulating cAMP receptor protein (CRP) to DNA.[5] Chemists have recognised that utilising cooperative interactions might also help in the construction of functional nanoscale objects. In the past decades this type of interaction has received increasing attention and a myriad of self-assembled structures that employ cooperative effects have been developed.[6,7]

Cooperative effects have been widely used and studied in the field of supramolecular chemistry and are interesting tools to control the properties of host–guest systems[8] and polymers.[9] Allosterism is a special case of cooperativity,[10] in the sense that a binding event at one site in a multivalent system like a biomolecule[11,12] or synthetic host[13] causes a discrete, reversible alteration in the structure at a remote binding site. Allosteric interactions can be both positive and negative in nature, and can act between a host and identical guests (homotropic allosterism) or between a host and different types of guests (heterotropic allosterism).

In previous research in our group a number of cavity-appended porphyrin hosts that display allosteric binding behaviour have been developed.[14–17] The most extensively studied host molecule is the monocavity-appended zinc porphyrin **ZnMC**, which is depicted in Figure 1 together with its allosteric binding behaviour. It was found that **ZnMC** can bind viologen molecules with high binding constants. The addition of a nitrogen-donor ligand that is too bulky to fit inside the cavity, like 4-*tert*-butyl-pyridine (**tbpy**), results in a positive heterotropic allosteric effect, which is demonstrated by an increase in binding constant between dimethylviologen (**V**) and **ZnMC** (Fig. 1b). Vice versa, an increase in binding constant between **tbpy** and **ZnMC** was observed upon the addition of viologen molecules to a mixture of the host and the axial ligand.

In the double cavity analogue of **ZnMC** and **ZnDC**, the interactions are even more complicated (Fig. 2). For the binding of viologens, this double cavity porphyrin system shows highly negative allosteric binding. The free base host molecule has the ability to bind two dimethylviologen molecules, but the association constant for the second binding (K_a=5×10^4 M^{-1}) is considerably lower than for the first binding event (K_a=7×10^7 M^{-1}). A combination of NMR studies and computational modelling indicated that this lower binding was not due to electronic repulsion between the two guests, but the result of conformational changes in the host molecule.[15]

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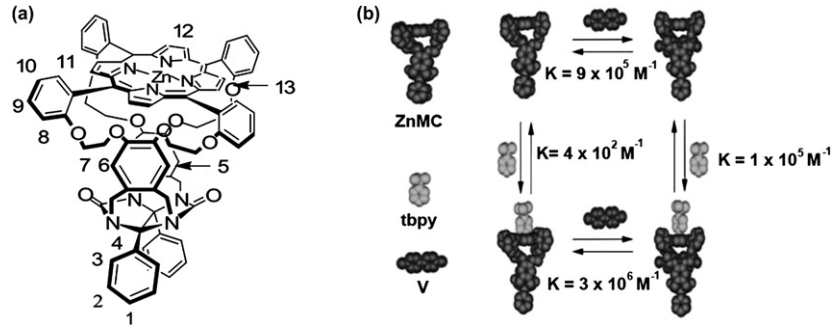

Figure 1. (a) Structure of porphyrin host **ZnMC**, together with the relevant proton numbering. (b) Schematic representation of the allosteric binding properties of this compound.

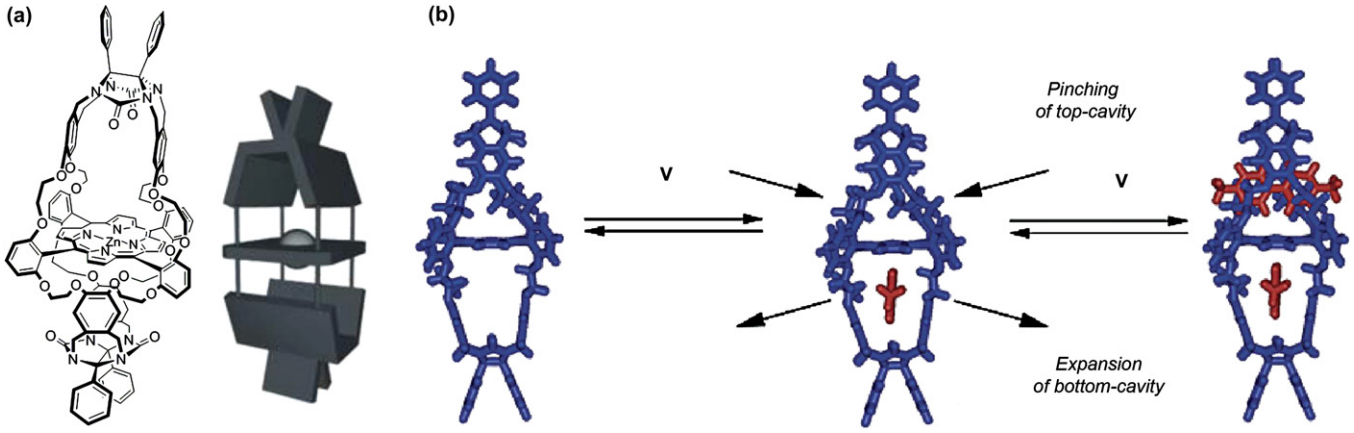

Figure 2. (a) **ZnDC** and its schematic representation. (b) Negative homotropic allosteric binding behaviour of **DC**.

As a continuation of this research we describe here attempts to construct more complex self-assembled allosteric assemblies based on bifunctional viologen and pyridine guests, and both **ZnMC** and **ZnDC** as hosts. The ultimate goal of this research is to generate materials of which both the architecture and the properties can be influenced by allosteric interactions.

2. Results and discussion

2.1. Synthesis

To study allosteric assembly three different bifunctional guests were synthesised, one containing two pyridine moieties (**PyPy**), another one containing two viologen moieties (**VV**) and a third one containing one pyridine and one viologen moiety (**VPy**) (Fig. 3).

Bis-pyridine bifunctional guest **PyPy** was synthesised by a straightforward reaction of suberoyl chloride with 3-amino-pyridine (yield 73% after recrystallisation). An amide bond linker was chosen because it was found to increase the binding of the pyridine guest in **ZnMC** due to the formation of a weak hydrogen bond with one of the carbonyl groups in the cavity of the host.[14]

For the synthesis of diviologen bifunctionalised guest **VV**, 1,10-dibromodecane was reacted with an excess of 4,4′-bipyridine to give an α,ω-bis-bipyridyl-functionalised decane. This compound was then treated with iodomethane, after which anion exchange was carried out by dissolving the compound in a hot aqueous solution of ammonium hexafluorophosphate. Upon cooling, the desired product **VV** precipitated as the tetrakis-hexafluorophosphate salt in a yield of 28%.

Viologen-pyridine bifunctionalised guest **VPy** was prepared from 9-bromo-1-nonanol, which was converted into 9-bromononanoic acid by oxidation with CrO_3 and then into an acid chloride using standard conditions. The latter compound was subsequently reacted with 3-aminopyridine to yield the corresponding amide. In order to prevent cyclisation and oligomerisation, this amide was treated with an excess of mono-methylated bipyridine at room temperature.

2.2. Self-assembled complexes based on ZnMC

The association constants of the complexes between the three guest molecules and the host **ZnMC** were determined by UV–vis and fluorescence spectroscopy (Table 1). All association constants were calculated assuming independent (non-cooperative) binding of the separate binding moieties of the guest molecules.

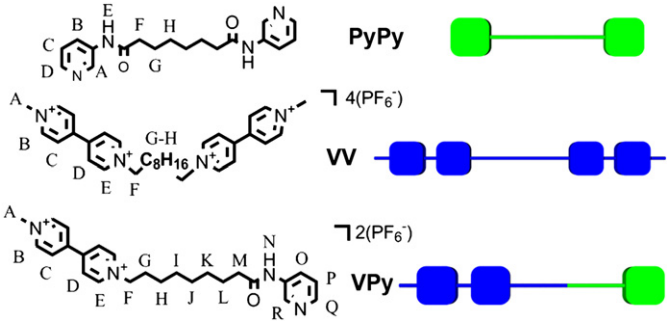

Figure 3. The bifunctional guests used in this study and their proton assignment (left). Schematic representation of the guest molecules (right).

Table 1
Association constants K_a[a] and binding free energies ΔG of complexes of **ZnMC** and different guests

Guest	K_a (M^{-1})	ΔG (kJ mol^{-1})
VV	4.6×10^6	−38
VV[b]	2.2×10^8	−48
PyPy	5×10^5	−33
Vpy	1.0×10^7	−38

[a] Determined by UV–vis spectroscopy, $CHCl_3/CH_3CN$ 1:1 (v/v) at 298 K, [**ZnMC**]=1.5×10^{-6} M. Association constants determined by fluorescence spectroscopy (excitation wavelength=426 nm; emission wavelength (max)=605 nm) were compared with the association constants obtained from UV–vis data. They were found to be similar and are therefore not mentioned. In their calculation it was assumed that the quantum yields of the host and of the host–guest complexes were the same.
[b] Association constant in the presence of 500 equiv **tbpy**.

The association constant obtained for the complex between **VV** and **ZnMC** was approximately five times higher than that of the complex between this host and **V** (K_a=9×10^5 M^{-1}), which is in line with the general observation that viologens equipped with long alkyl chains bind more strongly in **ZnMC**.[16] The accuracy of the fit (Fig. 4) deviated at higher concentrations of the guest suggesting additional binding geometries.

A 40-fold increase in association constant of **VV** was observed upon the addition of an excess **tbpy**. More remarkable, however, was the fact that after the addition of only half an equivalent of **VV** to **ZnMC** the titration curve reached saturation, whereas a full equivalent was needed to reach this level in the absence of **tbpy** (Fig. 4). ^{1}H NMR confirmed the observed allosteric effect in the binding process. A 1:1 stoichiometry was observed for the **VV**–**ZnMC** complex without an axial ligand present, whereas the **VV**–**ZnMC** complex in the presence of 10 equiv of **tbpy** showed a 1:2 stoichiometry. From the NMR spectra the geometry of the 1:2 **VV**/**ZnMC** complex could be derived (Fig. 5). A splitting of the proton signals 5–7 and 11–13 of **ZnMC** into two sets of signals for each proton had occurred, which can be attributed to the non-symmetrical complexation of the viologen guests inside the cavities. Only four doublets were observed for the viologen protons, and since the signals of the host and the **VV** guest are in slow exchange in the NMR spectrum, this observation indicates that two **ZnMC** hosts bind to one **VV** guest. Only very broad signals were found for the free **tbpy** ligand (indicated by an asterisk in Fig. 5), indicating that it is in relatively fast exchange with axially coordinated **tbpy**.

Knowing that both **VV** and **PyPy** are excellent guests for **ZnMC**, the combination of all three host–guest components was attempted. NMR studies on a 2:1:1 molar mixture of **ZnMC**, **VV** and **PyPy** in $CDCl_3/CD_3CN$ 1:1 (v/v) revealed that the discrete self-assembled complex depicted in Figure 6 had formed exclusively. Again, doubling of the signals of protons 5–7 and of 11–13 indicated a strong non-symmetrical complexation of the guest inside the host cavities, whereas the upfield shifts of the signals for the protons of both the viologen and pyridine moieties of both bifunctional guests confirmed the complexation to **ZnMC**.

In the case of the hetero-bifunctional guests **VPy** and **ZnMC**, an association constant of roughly K_a=1.0×10^7 M^{-1} between the viologen moiety and the host could be calculated from the titration curve obtained by UV–vis spectroscopy (Fig. 7). Since the binding affinity of the pyridine is at least two orders of magnitude smaller than that of the viologen, the formation of a 2:1 complex between **ZnMC** and **VPy** in which both ends of the guest molecule are bound to two different hosts was not included in the fitting model. The association constant of the complex with **VPy** is higher than the association constant of the complex with **VV** (K_a=4.6×10^6 M^{-1}), suggesting that the pyridine moiety acts as an allosteric effector.

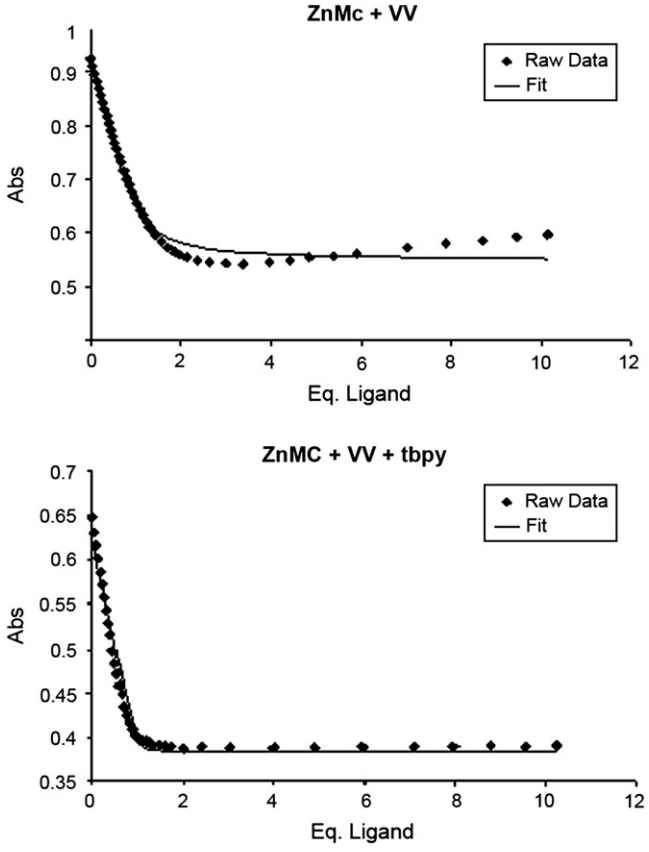

Figure 4. Titration curves for the binding of **VV** in **ZnMC** obtained by UV–vis spectroscopy in $CHCl_3/CH_3CN$ 1:1 (v/v) in the absence of **tbpy** (top) and in the presence of 500 equiv of **tbpy** (bottom).

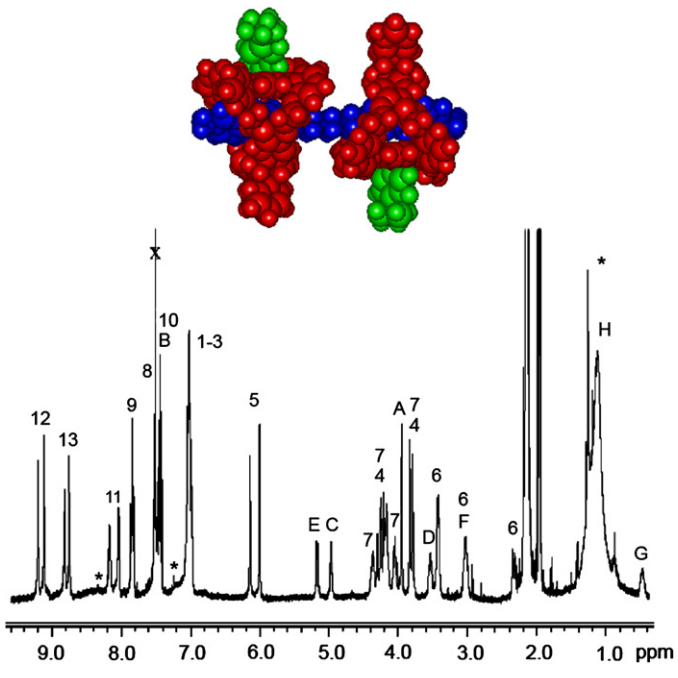

Figure 5. Computer modelled structure of the 1:2 assembly of **VV** and **ZnMC** in the presence of **tbpy** (top, please note that the **tbpy** is in fast exchange). ^{1}H NMR spectrum (400.15 MHz, $CDCl_3/CD_3CN$ 1:1 (v/v), 298 K, [**ZnMC**]=2.90×10^{-3} M) in a 1:2:10 molar **VV**/**ZnMC**/**tbpy** mixture (bottom). The proton signals of **tbpy** are indicated by an asterisk. For proton numbering see Figures 1 and 3. See Supplementary data for the 2D NMR spectra.

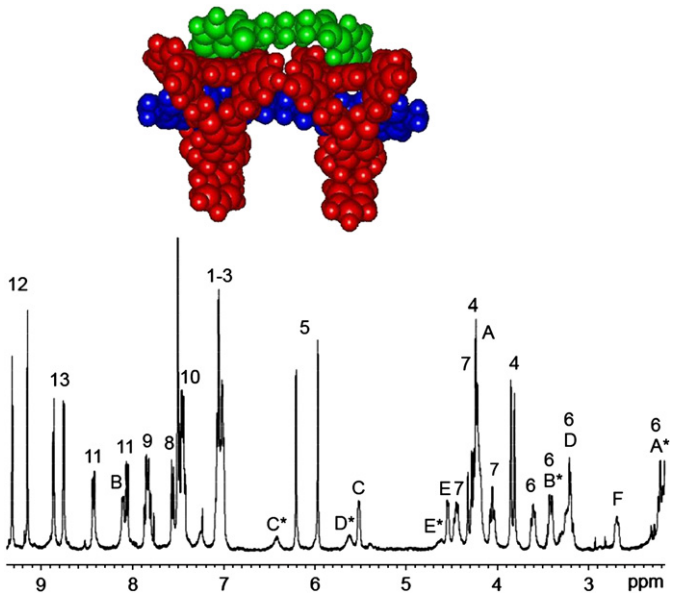

Figure 6. Computer modelled structure of the 2:1:1 assembly of **ZnMC**, **VV** and **PyPy** (top). ^{1}H NMR spectrum (400.15 MHz, $CDCl_3/CD_3CN$ 1:1 (v/v), 298 K, [**ZnMC**]=2.16×10^{-3} M) in a 1:2:1 molar **VV**/**ZnMC**/**PyPy** mixture (bottom). The proton signals of **PyPy** are indicated by an asterisk. For proton numbering see Figures 1 and 3. See Supplementary data for the 2D NMR spectra.

Molecular modelling studies on a 1:1 complex between **ZnMC** and **VPy** revealed that there is a too high energy penalty for adopting a conformation in which both binding moieties of the guest bind to the same host molecule, i.e., the viologen moiety of **VPy** inside the cavity of **ZnMC** and the pyridine moiety to the porphyrin zinc ion on the outside of the host, despite the increased effective molarity.

According to ^{1}H NMR spectroscopy, the addition of 1 equiv of **VPy** to **ZnMC** in $CDCl_3/CD_3CN$ 1:1 (v/v) resulted in the full complexation of the viologen moiety inside the cavity of the host and the full axial binding of pyridine to the zinc ion. The complexation of a non-symmetrical viologen inside the cavity, in combination with the axial complexation of a non-symmetrical pyridine on top of the host, evidenced by an upfield shift of the signals of the pyridine protons, causes an extreme level of dissymmetry in **ZnMC**, which was indicated by the splitting of the NMR signals of protons 5–7, 9–11 and 13 (Fig. 7). The dissymmetric conformation of the complex was also revealed by an unequal upfield shift of the signals of the protons of the two respective pyridine groups in the viologen moiety of **VPy**. The formation of the proposed 2:2 assembly was confirmed by COSY and NOESY 2D NMR studies.

Apparently, upon complexation with **ZnMC**, the equally sized spacers of the bifunctional guests favour the formation of discrete assemblies over supramolecular oligomers or polymers. To investigate whether a mismatch with regard to the alkyl spacers between a **VV** guest and **PyPy** guest in combination with **ZnMC** would disfavour the self-assembly of the components into a discrete complex and favour the formation of polymeric structures, a fourth bifunctional guest was synthesised, containing two viologen moieties, separated by an alkyl spacer of 16 CH_2-units (**V–C_{16}–V**). The addition of half an equivalent of **V–C_{16}–V** and half an equivalent of **PyPy** to a solution of **ZnMC** in $CDCl_3/CD_3CN$ 1:1 (v/v) ([**ZnMC**]=2×10^{-3} M) also resulted in the formation of a discrete

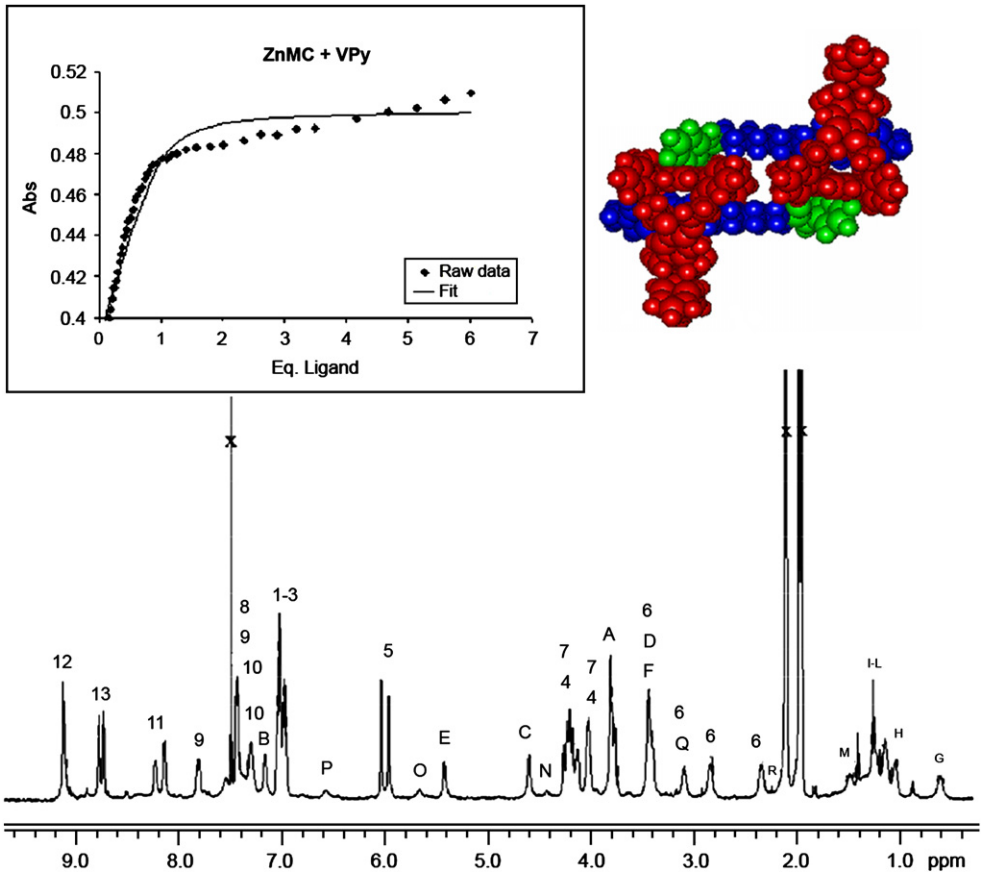

Figure 7. Titration curve for the binding of **VPy** in **ZnMC** as measured by UV–vis spectroscopy in $CHCl_3/CH_3CN$ 1:1 (v/v) (top left). Computer modelled structure of the 2:2 assembly of **ZnMC** and **VPy** (top right). ^{1}H NMR spectrum (400.15 MHz, $CDCl_3/CD_3CN$ 1:1 (v/v), 298 K, [**ZnMC**]=1.06×10^{-3} M) in a 1:1 mixture of **ZnMC** and **VPy** (bottom). For proton numbering see Figures 1 and 3. See Supplementary data for the 2D NMR spectra.

self-assembled 2:1:1 complex. The formation of supramolecular polymers apparently is entropically not favoured when compared to the formation of discrete host–guest complexes, which is probably the result of the increased effective molarity.[6]

2.3. Self-assembled complexes containing ZnDC

In a second series of experiments the formation of self-assembled allosteric complexes of **ZnDC** was studied. Since the two cavities of **ZnDC** are oriented orthogonally to each other, the combination of **VPy** with **ZnDC** or **VV** and **PyPy** with **ZnDC** cannot easily lead to complexes of small size, hence the formation of larger assemblies was expected.

First it was investigated whether **ZnDC** displayed allosteric binding behaviour upon the complexation of both a pyridine and a viologen guest. To this end, the association constant of the complex between **ZnDC** and pyridine (**Py**) was determined in the presence and absence of 1 equiv of **V**. In the absence of **V** the association constant of the complex in $CHCl_3/CH_3CN$ 4:1 (v/v) was measured to be $K_a=2.2\times10^4\ M^{-1}$, which is somewhat lower than the association constant of the complex between **Py** and **ZnMC** ($K_a=7.5\times10^4\ M^{-1}$).[18] The association constant between **Py** and **ZnDC** in the presence of 1 equiv of **V** increased fivefold to $K_a=1.1\times10^5\ M^{-1}$, which indicates a significant positive heterotropic allosteric binding behaviour (Fig. 8). This, in combination with the negative homotropic binding of **V**, makes **ZnDC** an ideal host for the formation of higher molecular weight self-assembled complexes using the bifunctional guests shown in Figure 3.

The binding affinities of the three different bifunctional guests for **ZnDC** were measured, again assuming independent (non-cooperative) binding between the two binding moieties of the guests (Table 2). The bifunctional guest **VV** gave an association constant of $K_a=1\times10^8\ M^{-1}$, almost two orders of magnitude higher than the association constant measured for **ZnMC** ($K_a=4.6\times10^6\ M^{-1}$). In fitting the binding data it was assumed that only [3]-pseudo-rotaxanes were formed, because it is expected that **ZnDC** displays similar negative allosteric binding behaviour upon the binding of two viologen moieties as its free base analogue **DC**. This assumption may not completely reflect reality; however, a reasonable fit was obtained (Fig. 9).

The titration was repeated in the presence of 1 equiv of **PyPy** to evaluate whether allosteric binding occurred. A 70-fold increase in binding constant of **VV** proved that this was indeed the case (Fig. 9). In the presence of **PyPy** already half an equivalent of **VV** was enough to completely quench the fluorescence of the zinc porphyrin in the titration experiment followed by fluorescence spectroscopy, whereas in absence of **PyPy** a whole equivalent was required. The deviation in the latter stage of the titration from the fitting curve seems to indicate that several different self-assembled structures are formed. The formation of non-discrete self-assembled oligomers, together with the possible formation of [4]-pseudo-rotaxanes, are the most obvious possibilities. Further studies are currently in progress to investigate the structural distribution in detail.

Similar to what was observed for the **ZnMC** upon the addition of only half an equivalent of **VPy** to **ZnDC** complete binding and

Table 2
Association constants K_a[a] and binding free energies ΔG for the complexation of **ZnDC** with different guests

Guest	K_a (M^{-1})	ΔG (kJ mol^{-1})
PyPy	4×10^5	−32
VV	1×10^8	−46
VV[b]	7×10^9	−56
VPy	1×10^6; 1×10^8	−34; −46

[a] Determined by UV–vis spectroscopy, $CHCl_3/CH_3CN$ 1:1 (v/v) at 298 K, [**ZnDC**]=2.6×10^{-6} M. Association constants determined by fluorescence spectroscopy (excitation wavelength=426 nm; emission wavelength (max)=605 nm) were compared with the association constants obtained from UV–vis data. They were found to be similar and are therefore not mentioned. In their calculation it was assumed that the quantum yields of the host and of the host–guest complexes were the same.

[b] Association constant in the presence of 1 equiv **PyPy**.

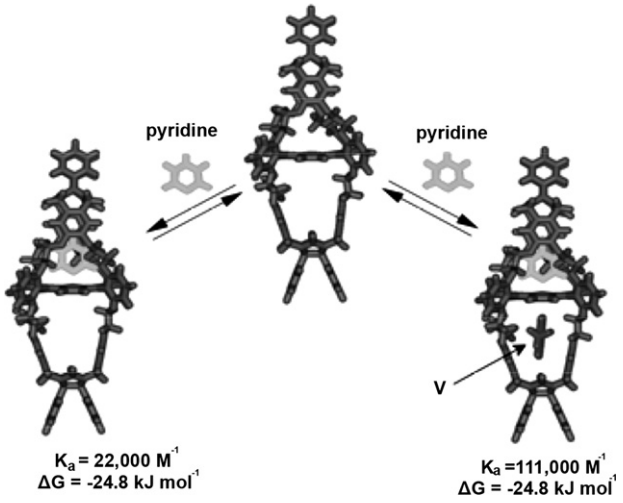

Figure 8. Positive heterotropic allosteric binding behaviour of **ZnDC**.

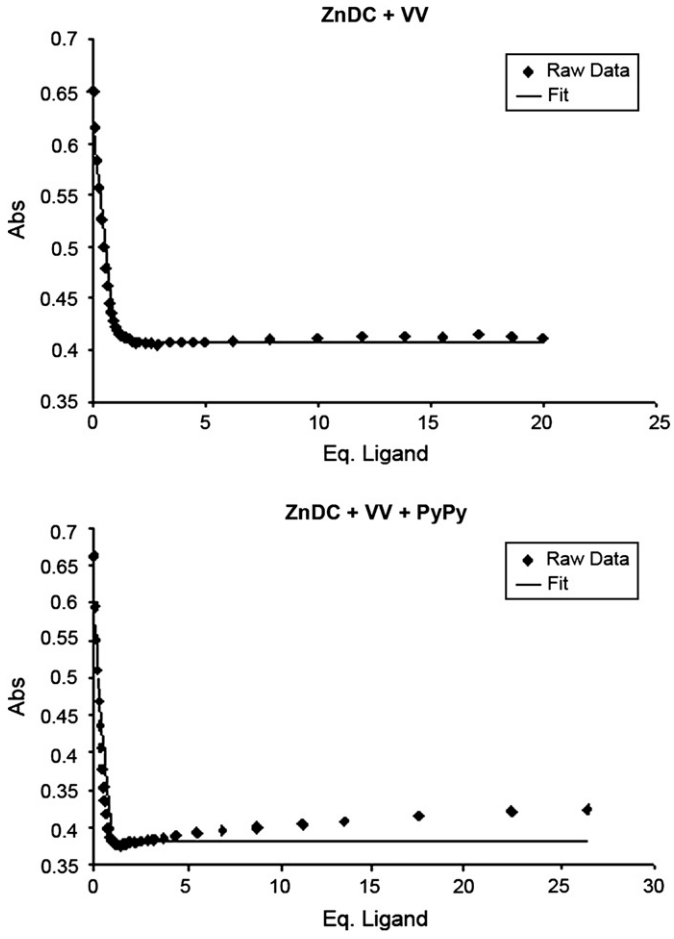

Figure 9. Titration curves for the binding of **VV** in **ZnDC** obtained by UV–vis spectroscopy in $CHCl_3/CH_3CN$ 1:1 (v/v) in the absence of **PyPy** (top) and in the presence of 1 equiv of **PyPy** (bottom).

fluorescence quenching was observed. By assuming that only the viologen moiety would be engaged in binding, no adequate fit was obtained, however, when it was assumed that both ends could bind, two association constants were obtained, viz. $K_a(1)=1\times10^6\ M^{-1}$ and $K_a(2)=1\times10^8\ M^{-1}$ (Fig. 10). These values might be the association constants for pyridine and viologen binding to **ZnDC**, respectively, which corresponds to the association constants found for the two other bifunctional guests (Table 2 entries 1 and 2). This conclusion would suggest that in this case the pyridine moiety does not act as an allosteric effector. A possible reason for this may be that there are other factors that influence the allosteric binding process, for example, geometric or steric factors.

To obtain more information about the geometry of the formed complex, ^{1}H NMR experiments were carried out on a 1:1 mixture of **ZnDC** and **VPy** in $CDCl_3/CD_3CN$ 1:1 (v/v). In contrast to all previous experiments, a very broad NMR spectrum was obtained in which no clear structure picture could be observed. When the host and guest were dissolved in a $CDCl_3/CD_3CN$ 19:1 (v/v) mixture, the resolution was increased, but still only the separate regions of the **ZnDC** could be assigned. Numerous different signals were observed indicating that a variety of complexes are present in solution. According to the isotope pattern of the peak corresponding to a mass of [**ZnDC**–**VPy**-$PF_6]_n^{n+}$ in a high resolution ESI MS spectrum of the 1:1 mixture, at least four molecules of each component were present.

As an alternative investigative tool a diffusion-ordered NMR (DOSY NMR) spectrum of the 1:1 mixture of **ZnDC** and **VPy** was recorded in $CDCl_3/CD_3CN$ 1:1 (v/v) applying a 10^{-3} M concentration for **ZnDC**. Somewhat surprisingly, the DOSY NMR spectrum suggested that the solution contained only one complex with a well-defined diffusion coefficient. Using the Einstein–Stokes equation it was possible to estimate an approximate radius of this complex, which amounted to 193 Å (see Section 4). The measured value suggests the formation of a complex that contains many host and guest molecules. However, given the strong allosteric behaviour of the **ZnDC**, an equally defined structure is expected as seen for the **ZnMC** complexes. All the above data can be explained by a defined 4:4 tetrameric complex (with four different diastereomeric structures, Fig. 10), although supramolecular polymers cannot be excluded.

3. Conclusions

An array of bifunctional guests have been synthesised containing either two viologen or two pyridine moieties, or one viologen and one pyridine moiety. In combination with **ZnMC** these guests were shown to form discrete self-assembled complexes in solution, which according to UV–vis and fluorescence titrations displayed allosteric interactions. In order to increase the size and complexity of the assemblies, the bifunctional guests were combined with the double cavity host **ZnDC**. The binding behaviour of this host towards the bifunctional guests proved to be complex and suggests formation of allosterically driven tetrameric assemblies. The combination of allosterism and self-assembly as demonstrated elegantly by nature, opens genuine possibilities for the controlled construction of hierarchical architectures. The present studies are just a first step in this direction.

4. Experimental

4.1. Methods and materials

Acetonitrile-d_3 was distilled over CaH_2 and chloroform-*d* was distilled over $CaCl_2$ prior to use. **ZnMC**[18] and **ZnDC**[15] were synthesised according to the literature procedures. NMR spectra were obtained on Bruker DPX 300, Varian Unity Inova 400 and Bruker DRX 500 instruments at 298 K. All NMR studies of host–guest complexes were performed in freshly prepared $CDCl_3/CD_3CN$ 1:1 (v/v) mixtures at 1×10^{-3} M concentrations. All the ratios that are mentioned with regard to mixtures of hosts and guests are molar ratios. UV–vis spectra were measured on a Varian Cary 50 UV–vis spectrophotometer and MALDI-TOF MS spectra were recorded on a Bruker Biflex III spectrometer. ESI mass spectra were measured on a Jeol Accu-TOF-CS. IR spectra were recorded on a ATI Matson Genesis Series FTIR spectrometer with an ATR cell. Fluorescence titrations were performed on a Perkin–Elmer Luminescence Ls50B spectrometer. UV–vis and fluorescence titration experiments and the calculation of binding constants were carried out following the standard methods reported earlier by our group assuming independent binding of the separate binding moieties of the guest molecules.[15,17]

Diffusion rate values in DOSY NMR studies were obtained using data analysis in Mestre-C 4.7.0.0. The diffusion rate that was used for the calculation of the radius was an average of the diffusion rates obtained for at least six different peaks in the DOSY NMR spectra to minimise errors. The radius was calculated from the diffusion rate, with the assumption that the viscosity of a 1:1 (v/v) mixture of $CDCl_3$ and CD_3CN is the average (0.4435×10^{-3} kg m^{-1} s^{-1}) of the viscosity values of the two individual solvents, using the rearranged Einstein–Stokes equation:

$$r = kT/6\pi D\eta$$

in which r=radius (m), k=Boltzmann's constant (J K^{-1}), T=temperature (K), D=diffusion rate (m^2 s^{-1}) and η=viscosity of the medium (kg m^{-1} s^{-1}).

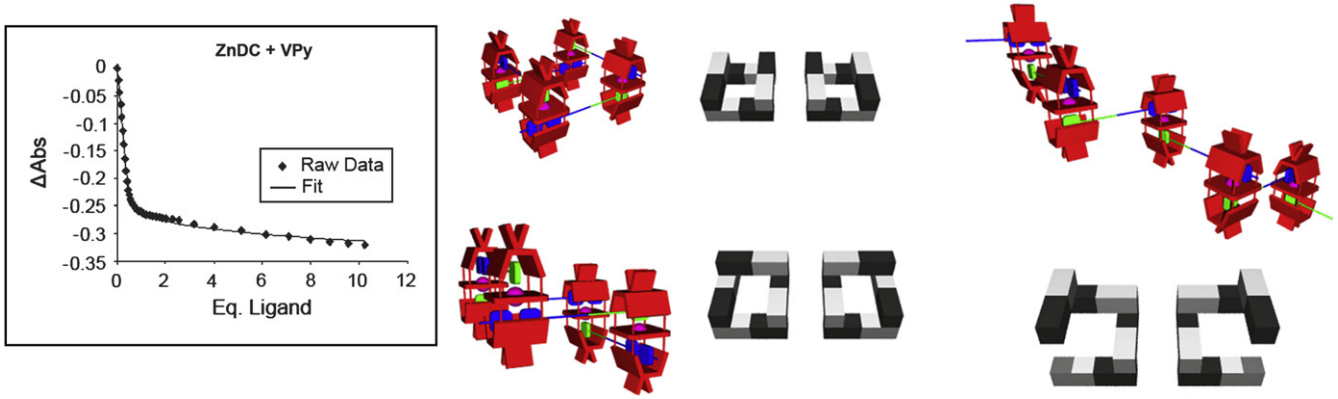

Figure 10. Titration curve for the binding of **VPy** in **ZnDC** obtained by UV–vis spectroscopy in $CHCl_3/CH_3CN$ 1:1 (v/v) (left) and schematic representation of possible self-assembled allosteric complexes in which **ZnDC** and **VPy** are present in a 1:1 ratio (right).

4.2. Synthesis of PyPy

To triethylamine (3.3 mL, 24 mmol) and 3-aminopyridine (1.6 g, 17 mmol) in CH_2Cl_2 (40 mL) was added dropwise at 0 °C a solution of suberoyl chloride (2.1 g, 9.8 mmol) in CH_2Cl_2 (3 mL). This mixture was stirred at 0 °C for 1 h and subsequently at room temperature for 2 h. It was then poured into water and a white solid was isolated via filtration, which was dissolved in a small volume of DMSO and this solution was again poured into water. After filtration, the resulting solid was recrystallised from boiling MeOH and by allowing Et_2O to diffuse into a saturated MeOH solution of the compound yielding a white solid (1.5 g, 6.4 mmol, 73%). ^{1}H NMR (300 MHz, $CD_3CN/CDCl_3$ 1:1 (v/v)): δ=8.64 (s, 2H), 8.26 (d, ^3J=3.9 Hz, 2H), 8.19 (s, 2H), 8.08 (d, ^3J=8.3 Hz, 2H), 7.22 (dd, ^3J=8.3, ^3J=3.9 Hz, 2H), 2.37 (t, ^3J=7.2 Hz, 4H), 1.73 (m, 4H), 1.43 (m, 4H); ^{13}C{^{1}H} NMR (50 MHz, CDOD): δ=174.6, 144.6, 141.3, 137.1, 128.5, 124.9, 37.3, 29.6, 26.1; IR (solid) 1670, 1535, 1417, 703 cm^{-1}; UV–vis (MeOH) λ/nm (log ε/M^{-1} cm^{-1}): 240 (4.3), 278 (3.8); HRESIMS *m*/*z* 327.1820, calcd for $C_{18}H_{23}N_4O_2$ [M]$^+$ 327.1821, 349.1650, calcd for $C_{18}H_{23}N_4O_2Na$ [M+Na]$^+$ 349.1640.

4.3. Synthesis of VV

A mixture of 1,10-dibromodecane (1.0 g, 3.3 mmol) and 4,4′-bipyridine (7.4 g, 47 mmol) in CH_3CN (50 mL) was refluxed overnight under nitrogen. After cooling, the resulting precipitate was filtered off and washed with Et_2O and CH_3CN. The residue was dissolved in water and after addition of a saturated aqueous NH_4PF_6 solution a precipitate was formed, which was filtered off, washed with water and dried in vacuo. A solution of the resulting white solid (0.99 g) and iodomethane (0.33 mL, 5.3 mmol) in CH_3CN (35 mL) was refluxed under nitrogen for 64 h. The precipitate was filtered off and dissolved in water, and this solution was added slowly to an aqueous saturated solution of NH_4PF_6 resulting in the formation of a precipitate. The suspension was heated until the entire solid redissolved again. Upon cooling, a white solid was formed, which was filtered off, washed with water and recrystallised by the allowing Et_2O to diffuse into a saturated CH_3CN solution of the compound yielding the title compound as a white solid (0.45 g, 0.4 mmol, 28%), dp=250 °C. ^{1}H NMR (400.15 MHz, CD_3CN): δ=8.85 (m, 8H), 8.37 (m, 8H), 4.60 (t, ^3J=7.6 Hz, 4H), 4.40 (s, 6H), 2.00 (m, 4H), 1.37 (m, 12H); ^{13}C{^{1}H} NMR (50 MHz, CD_3CN): δ=150.8, 150.6, 147.4, 146.5, 140.0, 63.1, 49.6, 32.0, 29.9, 29.6, 26.6; IR (solid) 819, 560 cm^{-1}; HRESIMS *m*/*z* 1085.1888, calcd for $C_{32}H_{42}F_{24}N_4NaP_4$ [M+Na]$^+$ 1085.1874, 917.2334, calcd for $C_{32}H_{42}F_{18}N_4P_3$ [M−PF_6]$^+$ 917.2334; UV–vis (CH_3CN) λ/nm (log ε/M^{-1} cm^{-1}): 265 (4.6).

4.4. Synthesis of VPy

4.4.1. Synthesis of 1-methyl-4-(4′-pyridyl)-pyridinium hexafluorophosphate

A solution of 4,4′-bipyridine (1.2 g, 7.7 mmol) and methyl iodide (0.57 mL, 9 mmol) in CH_2Cl_2 (5 mL) was refluxed overnight under nitrogen. The resulting precipitate was filtered off, redissolved in MeOH, precipitated by the addition of Et_2O, filtered off and washed with EtOH. To an aqueous solution of the resulting solid, a saturated aqueous NH_4PF_6 solution was added, which resulted in the formation of a precipitate (1.8 g, 5.7 mmol, 74%) that was filtered off. Physical properties were in agreement with those previously reported.[19]

4.4.2. Synthesis of 9-bromo-(N-3-pyridyl)nonamide

To a solution of chromium oxide (3.4 g, 35 mmol) in water (5 mL) at 0 °C was added dropwise concentrated sulfuric acid (3 mL) and water (10 mL). This solution was slowly added at −5 °C to a solution of 9-bromononanol (5.4 g, 24.2 mmol) in acetone (250 mL). After stirring at room temperature for 2 h a green precipitate had formed, which was filtered off and discarded. The filtrate was concentrated and a solution of the resulting solid in Et_2O was washed with water (3×). The organic layer was dried ($MgSO_4$), filtered and evaporated, resulting in a solid that was purified by column chromatography (silica; hexane/ethyl acetate 20:1 (v/v)), yielding a white solid. A mixture of this solid (0.41 g, 1.7 mmol) and thionyl chloride (2 mL) was stirred overnight, after which the excess liquid was evaporated until an oil remained that was dissolved in Et_2O (5 mL). This solution was added dropwise to an ice-cold solution of 3-aminopyridine (0.16 g, 1.7 mmol) in Et_2O (15 mL). After warming to room temperature, the mixture was stirred overnight under nitrogen. The resulting suspension was filtered and the residue dissolved in water, after which $NaHCO_3$ was added until pH 8 was reached. The product was extracted with Et_2O, the organic layer was washed with a saturated aqueous $NaHCO_3$ solution, dried with $MgSO_4$, filtered and evaporated, resulting in a white solid (0.4 g, 1.3 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.51 (d, ^3J=2.1 Hz, 1H), 8.31 (d, ^3J=4.2 Hz, 1H), 8.16 (d, ^3J=8.1 Hz, 1H), 7.35 (s, 1H), 7.24 (m, 1H), 3.39 (t, ^3J=6.9 Hz, 2H), 2.39 (t, ^3J=7.5 Hz, 2H), 1.86 (m, 2H), 1.82 (m, 2H), 1.35 (m, 8H).

*4.4.3. Synthesis of **VPy***

A solution of 9-bromo-(*N*-3-pyridyl)nonamide (0.1 g, 0.32 mmol) and 1-methyl-4-(4′-pyridyl)-pyridinium hexafluorophosphate (0.5 g, 1.58 mmol) in CH_3CN (500 mL) was stirred for 2 weeks. The precipitate was filtered off and washed CH_3CN (3×), dissolved in water and this solution was added dropwise to a saturated aqueous NH_4PF_6 solution. The resulting precipitate was isolated via centrifugation and recrystallised by allowing $CHCl_3$ to diffuse into a saturated CH_3CN solution of the compound yielding a white solid (8 mg, 0.01 mmol, 4%). ^{1}H NMR (400.15 MHz, CD_3CN): δ=8.85 (m, 4H), 8.70 (s, 1H), 8.35 (m, 5H), 8.24 (d, ^3J=6.6 Hz, 1H), 8.00 (d, ^3J=11 Hz, 1H), 7.30 (dd, J=6.6, ^3J=11 Hz, 1H) 4.60 (t, ^3J=10 Hz, 2H), 4.39 (s, 3H), 2.34 (t, ^3J=9.6 Hz, 2H), 2.02 (m, 2H), 1.65 (m, 2H), 1.40 (m, 8H); ^{13}C{^{1}H} NMR (50 MHz, CD_3CN): δ=171.6, 145.0, 143.6, 140.2, 126.7, 126.3, 126.2, 123.2, 77.7, 61.6, 36.0, 30.4, 28.2, 28.1, 27.8, 24.9, 24.4 (not all peaks could be assigned due to broadening); IR (solid) 1674, 1536, 820, 555 cm^{-1}; HRESIMS *m*/*z* 717.1799, calcd for $C_{25}H_{32}F_{12}N_4ONaP_2$ [M+Na]$^+$ 717.1757, 549.2241, calcd for $C_{25}H_{32}F_6N_4OP$ [M−PF_6]$^+$ 549.2217.

4.5. Synthesis of V–C_{16}–V

To a suspension of 1,16-dihydroxyhexadecane (1.0 g, 3.8 mmol) in Et_2O (20 mL) was added dropwise PBr_3 (0.24 mL, 2.5 mmol), after which the suspension was refluxed for 5 h, during which the solid dissolved. After cooling, the solution was poured into water (50 mL) and this suspension was extracted with CH_2Cl_2 (3×). The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated. The resulting solid was recrystallised from boiling EtOH to yield 1,16-dibromohexadecane. A mixture of 1,16-dibromohexadecane (0.556 g, 1.44 mmol) and 4,4′-bipyridine (2.25 g, 14.4 mmol) in DMF (25 mL) was stirred at 90 °C for 3 days under nitrogen. After cooling, the resulting precipitate was filtered off and washed with Et_2O and CH_3CN. The filtrate was evaporated and the residue was dissolved in DMSO. After the addition of a saturated aqueous NH_4PF_6 solution a precipitate was formed, which was filtered off, washed with water and dried in vacuo. A solution of the resulting white solid (65 mg, 0.12 mmol) and iodomethane (1 mL, 16 mmol) was refluxed in CH_3CN (15 mL) for 14 h under nitrogen. The precipitate was filtered off and added to a saturated aqueous NH_4PF_6 solution. This mixture was heated to 100 °C (3×) after which a white precipitate was formed upon cooling. This solid was filtered off, washed with water and Et_2O, and dried in vacuo

resulting in a white solid (30%). ^{1}H NMR (400.15 MHz, $CDCl_3/CD_3CN$ 1:1 (v/v)): δ=8.86 (m, 8H), 8.38 (m, 8H), 4.59 (t, ^3J=7.6 Hz, 4H), 4.41 (s, 6H), 2.10 (m, 4H), 1.35 (m, 24H); $^{13}C\{^1H\}$ NMR (50 MHz, CD_3CN): δ=149.9, 149.6, 146.5, 145.5, 127.2, 126.8, 62.1, 48.6, 31.0, 29.5, 29.4, 29.3, 29.1, 28.7, 25.6; IR (solid) 836, 555 cm^{-1}; HRESIMS m/z 1001.3276, calcd for $C_{38}H_{54}F_{18}N_4P_3$ $[M-PF_6]^+$ 1001.3273.

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Supplementary data

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