Allosteric Cooperativity and Template-Directed Synthesis with Stacked Ligands in Porphyrin Nanorings

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ABSTRACT: The link between allosteric cooperativity and template-directed synthesis has been investigated by studying complexes in which two oligopyridine ligands bind inside a zinc porphyrin nanoring in a stacked arrangement. The binding of a 6-porphyrin nanoring to two tridentate ligands (with *s*-triazine or benzene cores) occurs with high negative allosteric cooperativity ($\alpha \approx 10^{-3} - 10^{-4}$). Formation constants for 1:1 and 1:2 complexes were determined by UV-vis-NIR denaturation titration, using pyridine as a competing ligand, and cooperativity factors were confirmed by NMR spectroscopy. The rate constants for formation of the 1:1 and 1:2 complexes are approximately equal and the negative cooperativity can be attributed to faster dissociation of the 1:2 complex. These tridentate ligands are not effective templates for directing the synthesis of the 6-porphyrin nanoring, in keeping with their negative cooperativity of binding. In contrast, the binding of a 12-porphyrin nanoring to two hexadentate ligands occurs with high positive allosteric cooperativity ($\alpha > 40$), and the ligand is an effective Vernier template for directing the synthesis of the 12-porphyrin nanoring. This stacked Vernier template approach creates the product in an open circular conformation, which is advantageous for preparing macrocycles that do not easily adopt a figure-of-eight geometry.

INTRODUCTION

Cooperative phenomena, such as allosteric effects, play a vital role in regulating cellular metabolism.¹ Positive cooperativity can also be exploited in artificial sensors,² drug delivery systems³ and catalysts.⁴ There have been many studies of the origins of cooperativity in multivalent molecular recognition.⁵ A key challenge is to use cooperative thermodynamic behavior to control the kinetics of bond-formation, *i.e.* to translate cooperative binding into catalysis. Here we analyze the allosteric cooperativity in 1:2 complexes consisting of two molecules of a radial oligopyridine ligand stacked inside a zinc porphyrin nanoring, and we explore how cooperative binding can be exploited in template-directed synthesis.

We compare two types of 1:2 complexes (Figure 1): a 6-porphyrin nanoring binding two stacked tridentate ligands, **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂, and a 12-porphyrin nanoring binding two stacked hexadentate ligands, **c-P12**·(**T6e**)₂. The allosteric effect is strongly negative (anti-cooperative) for formation of **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂, but strongly positive for formation of **c-P12**·(**T6e**)₂. This difference in cooperativity is reflected by the fact that **T3** and **T3N** do not act as templates for the formation of **c-P6**, whereas **T6e** is an effective template for the synthesis of **c-P12**.

Classical template-directed synthesis requires a 1:1 complementarity between the size and shape of the template and the product of the reaction.⁶ The formation of Vernier complexes allow two or more template molecules to act cooperatively to direct the formation of a larger product.⁷ Previous examples of Vernier templated cyclizations have involved the formation of strained complexes with figure-of-eight geometries, with one template residing in each loop of the Vernier complex.^{7a-c} Here we demonstrate that stacked radial ligands can also be very effective for Vernier template-directed syn-

thesis. This new Vernier mode allows the product to be synthesized in an open circular conformation, which is advantageous for preparing macrocycles which do not easily adopt a figure-of-eight geometry, such as porphyrin nanorings with bulky sidechains.

RESULTS AND DISCUSSION

Molecular design and modeling. The **T3N** ligand was selected for investigation because its *s*-triphenyltriazine core was anticipated to be flatter than the 1,3,5-triphenylbenzene core of **T3**,⁸ which should allow two **T3N** ligands to stack more easily inside **c-P6**. Computational modeling of the 1:2 complexes **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂ was carried out using density functional theory (DFT, B3LYP/3-21G; *meso*-aryl substituents replaced with hydrogen atoms to reduce computational cost). Both complexes have saddle-shaped geometries with parallel eclipsed triazine or benzene cores, as illustrated for **c-P6**·(**T3N**)₂ in Figure 2a. The two triazine cores in **c-P6**·(**T3N**)₂ (separation: 3.5 Å) are closer than the benzene cores in **c-P6**·(**T3**)₂ (separation: 4.0 Å). The doming of the ligands can be quantified by the angle θ_{dome} between the 4-pyridyl carbon, the Zn center and the centroid of the zinc atoms (Figure 2b); this analysis gives average dome angles of 12.9° and 12.1° for **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂, respectively. The shapes of the complexes are similar and the CH/N substitution has only a subtle effect on the global conformation.

The **T6e** ligand was designed to have a flat hexa-ethynyl benzene core to favor stacking. The 12 *n*-octyl chains are attached away from this core to improve the solubility, while not hindering stacking. The geometry of the **c-P12**·(**T6e**)₂ complex was modeled using the semi-empirical PM6 method (without solubilizing sidechains), because we found it to be too large to converge by DFT. The central benzene units of the two template molecules are closely stacked (separation: 3.7

Å). Surprisingly, the two **T6e** units in the energy-minimized structure have different conformations: one is essentially flat and the other is domed (Figure 2c; green and blue, respectively). However, the calculated energy barrier for interconverting the conformations of the two bound templates, via a more symmetrical transition state, is only about 2.5 kJ mol⁻¹ (see Supporting Information) implying that the ligand environments exchange rapidly under ambient conditions. The average dome angle (averaged over both template units) is $\theta_{\text{dome}} = 6.8^\circ$ and the nanoring has a regular circular geometry. All 12 zinc atoms are essentially in the same plane (root mean square deviation 0.02 Å), which contrasts with the saddle-shaped geometries of **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂ in which the six zinc atoms deviate significantly from planarity (root mean square deviation 1.05 Å and 1.01 Å, respectively).

Figure 1. Structures of **c-P6**·(**T3**)₂, **c-P6**·(**T3N**)₂ and **c-P12**·(**T6e**)₂. Ar = 3,5-bis(trihexylsilyl)phenyl (except where specified otherwise).

Synthesis. The tridentate templates **T3** and **T3N** were prepared using modifications of published procedures.^{9,10,11} The expanded hexadentate template **T6e** was made by a similar route to the fluorinated analogue (see Supporting Information).¹² The porphyrin nanorings **c-P6** and **c-P12** were synthesized as reported previously.^{7,12}

Figure 2. a) Calculated geometry of **c-P6**·(**T3N**)₂ (B3LYP/3-21G). (b) Definition of the dome angle, θ . (c) Calculated geometry of **c-P12**·(**T6e**)₂ (PM6). b) Two orthogonal views of each complex were shown in (a) and (c); aryl and octyl solubilizing groups were replaced by hydrogen to simplify the calculations.

Thermodynamic Cooperativity. The formation constants, K_f , of the 1:2 complexes for **c-P6**·(**T3**)₂, **c-P6**·(**T3N**)₂ and **c-P12**·(**T6e**)₂, in non-coordinating solvents, are all much too high to measure by direct titration ($K_f > 10^{25}$ M⁻²). Furthermore, it takes many weeks to establish equilibrium between 1:1 and 1:2 complexes in these systems (as discussed below), which hampers evaluation of allosteric cooperativity. The solution to both these problems is to add a competing ligand, such as pyridine, which destabilizes the complexes and accelerates ligand exchange. Denaturation titrations, using a denaturant such as pyridine, have proved to be very effective for determining the stabilities of porphyrin oligomer complexes.^{7b-d,13} These titrations can be understood by considering the thermodynamic cycles shown in Figure 3. Formation of the

1:2 complex must occur in two steps, with equilibrium constants $K_{f,1}$ and $K_{f,2}$ (Figure 3a). Each of these binding processes can be reversed by adding pyridine, with denaturation constants $K_{dn,1}$ and $K_{dn,2}$. However if $K_{f,2} \gg K_{f,1}$, the 1:1 complex does not build up to significant concentrations and it can be neglected (Figure 3b).

Figure 3. Thermodynamic cycles for the formation and denaturation of 1:2 complexes, and their denaturation by pyridine. The simple blue arrow represents pyridine and the red circles represent zinc porphyrin sites.

Denaturation titrations were carried out by titrating a large excess of pyridine (py, concentration 0–1 M) into solutions of the 1:2 complexes (concentration $\sim 1 \mu\text{M}$, in chloroform¹⁴ or toluene at 298 K), while monitoring the UV-vis-NIR spectrum (Figure 4). The denaturation titrations of **c-P12**·(**T6e**)₂ are highly isosbestic (Figure 4a), indicating a two-state equilibrium from **c-P12**·(**T6e**)₂ to **c-P12**·(py)₁₂ without formation of significant concentrations of the 1:1 complex **c-P12**·(**T6e**)·(py)₆. The spectral change is dramatic: **c-P12**·(**T6e**)₂ exhibits a sharp intense peak at 890 nm, reflecting its rigid π -conjugated conformation, whereas **c-P12**·(py)₁₂ gives a broad peak at 820 nm, as it is more disordered and less conjugated. The denaturation titrations of **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂ (Figure 4c,e and SI Section 3.7) are not isosbestic, indicating that there are more than two nanoring complexes in the equilibrium. The spectral change is much smaller than in the case of **c-P12**·(**T6e**)₂, because the conformation of **c-P6** is less sensitive to its coordination state.

The denaturation of **c-P12**·(**T6e**)₂ was analyzed using a simple all-or-nothing model,¹³ described by equation 1 and 2,

$$K_{dn} = \frac{[\text{c-P12} \cdot (\text{py})_{12}] \cdot [\text{T6e}]^2}{[\text{c-P12} \cdot (\text{T6e})_2] \cdot [\text{py}]^{12}} \quad (1)$$

$$K_f = \frac{K_{py}^{12}}{K_{dn}} \quad (2)$$

where K_{py} is the binding constant of pyridine to a zinc porphyrin unit (see SI Section 3.6; $K_{py} = 8.4 \times 10^3$ and $1.3 \times 10^4 \text{ M}^{-1}$ in CDCl_3 and toluene, respectively, at 298 K). The experimental data fit well to this binding isotherm (Figure 4b), confirming that the system is essentially a two-state equilibrium with no significant population of the 1:1 complex (Figure 3b). The formation constants are $\log K_f = 49.1 \pm 0.3$ in chloroform

and $\log K_f = 50.7 \pm 0.4$ in toluene (Table 1). The highly positive cooperativity of the denaturation of **c-P12**·(**T6e**)₂ is illustrated by the Hill plot (insert in Figure 4b). Simulations show that the Hill coefficient n_H is expected to be sensitive to the number of coordination sites in the complex, N , and that the best fit is obtained with $N = 12$. The observed Hill coefficient ($n_H = 5.9$) is higher than that reported in most natural¹⁵ and synthetic¹⁶ allosteric systems. The higher cooperativity of this denaturation process is a direct expression of the high allosteric and chelate cooperativity involved in forming this 1:2 complex.^{5e}

Figure 4. UV-vis-NIR denaturation titrations of stacked template complexes (all in CDCl_3 at 298 K). a,c,e) the spectra with the complexes in thick black lines and the end spectra (of the pyridine-saturated rings) in red; b,d,f) the experimental (black squares) and calculated (red lines) binding isotherms (insert: Hill plot with simulated curves for 10, 12 and 14 binding sites). $[\text{c-P12}] = 0.25 \mu\text{M}$, $[\text{c-P6}]_{\text{T3}} = 0.79 \mu\text{M}$, $[\text{c-P6}]_{\text{T3N}} = 1.3 \mu\text{M}$.

The denaturation titrations of **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂ are biphasic, as seen from the rise and fall of absorption at selected wavelengths (Figure 4c-f and SI Section 3.7), which indicates formation of an intermediate with one bound template. The data were fitted to a two-step binding model (Figure 3a) by global nonlinear regression of the entire set of spectra using the ReactLab Equilibria software (Jplus Consulting). The simulated curves for two-step denaturation fit the experimental data, as shown in Figure 4d,f. The values of $K_{f,1}$ and $K_{f,2}$ (Table 1) were used to calculate speciation curves for the denaturation process (Figure 5), which illustrate that a high mole fraction of the 1:1 complex is formed during denaturation of **c-P6**·(**T3**)₂ and **c-P6**·(**T3N**)₂.

Table 1. Formation constants and cooperativity factors (α) for stacked complexes in chloroform or toluene^a

complex	solvent	$\log K_f$	α
c-P12·(T6e)₂	CDCl ₃	$\log K_f = 49.1 \pm 0.3^b$	$> 40^c$
	PhMe	$\log K_f = 50.7 \pm 0.4^b$	$> 40^c$
c-P6·(T3)₂	CDCl ₃	$\log K_{f,1} = 15.4 \pm 0.1$ $\log K_{f,2} = 13.7 \pm 0.1$	0.04 ± 0.01
	PhMe	$\log K_{f,1} = 16.5 \pm 0.1$ $\log K_{f,2} = 12.9 \pm 0.1$	$(4.9 \pm 1.7) \times 10^{-4}$
c-P6·(T3N)₂	CDCl ₃	$\log K_{f,1} = 16.2 \pm 0.1$ $\log K_{f,2} = 12.9 \pm 0.1$	$(1.3 \pm 0.3) \times 10^{-3}$
	PhMe	$\log K_{f,1} = 16.6 \pm 0.1$ $\log K_{f,2} = 12.3 \pm 0.1$	$(1.0 \pm 0.4) \times 10^{-4}$

^aAll UV-vis-NIR titrations were carried out at 298 K using THS porphyrin nanorings; Ar = 3,5-bis(trihexylsilyl)phenyl. ^bFor **c-P12·(T6e)₂**, it is only possible to measure the product $K_f = K_{f,1}K_{f,2}$. ^cA lower limit of α was calculated assuming that the mole fraction of the 1:1 complex is less than 10% (see main text and SI Section 3.8).

Figure 5. Speciation curves for denaturation of (a) **c-P12·(T6e)₂**, (b) **c-P6·(T3)₂** and (c) **c-P6·(T3N)₂** calculated from the binding constants in CDCl₃ (Table 1), for a total porphyrin nanoring concentration of 1 μ M. θ is the mole-fraction of the porphyrin nanoring in each species.

The cooperativity factor α is a measure of allosteric cooperativity⁵ and it can be calculated from the ratio of the intrinsic

statistically corrected binding constants $K_{chem,2}$ and $K_{chem,1}$ (equation 3) for the two sequential binding processes. Statistically corrected binding constants are calculated by dividing the measured K_f values by their statistical factors K_{σ} , which are calculated from symmetry numbers (Table 2 and SI Section 3.5).¹⁷

$$\alpha = \frac{K_{chem,2}}{K_{chem,1}} = \frac{K_{f,2}}{K_{f,1}} \cdot \frac{K_{\sigma,1}}{K_{\sigma,2}} \quad (3)$$

Table 2. Statistical factors.^a

equilibrium	statistical factor
c-P6 + T3 $\rightarrow$ c-P6·T3	$K_{\sigma,1} = 96$
c-P6·T3 + T3 $\rightarrow$ c-P6·(T3)₂	$K_{\sigma,2} = 48$
c-P12 + T6e $\rightarrow$ c-P12·T6e	$K_{\sigma,1} = 1536$
c-P12·T6e + T6e $\rightarrow$ c-P12·(T6e)₂	$K_{\sigma,2} = 768$

^asee SI Section 3.5 for calculations of statistical factors.

Values of α for **c-P6/T3** and **c-P6/T3N** (Table 1) are all far below unity, reflecting negative cooperativity, and α is about an order of magnitude smaller in toluene than in chloroform. In the case of **c-P12/T6e**, no intermediate 1:1 complex was detected in the UV-vis-NIR titration, which makes it impossible to determine α . However, a lower limit for the value of α can be estimated by assuming that the peak mole-fraction of **c-P12·(T6e)** in the denaturation titration is less than 10%, which implies that $\alpha > 40$ (*vide infra*).

¹H NMR spectroscopy was used to check the results from the UV-vis-NIR titrations. The unbound nanorings, 1:1 and 1:2 complexes have distinctive signals, which makes it possible to determine α directly from a single ¹H NMR spectrum, by measuring the template-redistribution equilibrium constant K_{eq} (Figure 6, equation 4).

Figure 6. The template-redistribution equilibrium of **c-P6/T3**.

$$K_{eq} = \frac{[c-P6·(T3)_2][c-P6]}{[c-P6·T3]^2} = \frac{K_{f,2}}{K_{f,1}} \alpha = \alpha \frac{K_{\sigma,2}}{K_{\sigma,1}} \quad (4)$$

Solutions of the template and the nanoring in CDCl₃ were mixed in an NMR tube, in 1:1 mole ratio, together with a small amount of *d*₅-pyridine to catalyze ligand exchange, and the mixture was allowed to equilibrate at 298 K until no further changes were observed. In the case of **c-P12/T6e**, only the 1:2 complex **c-P12·(T6e)₂** and the empty nanoring **c-P12** were observed, with no detectable amount of the 1:1 complex **c-P12·T6e**. We would expect to have detected this 1:1 complex if its mole fraction had exceeded 10%, so this experiment confirms that $\alpha > 40$. In the case of **c-P6/T3**, the equilibrated solution contained 10.1% **c-P6·(T3)₂**, 79.6% **c-P6·T3**, and 10.3% **c-P6** (SI Section 4), which indicates that $K_{eq} = 0.016 \pm 0.006$; $\alpha = 0.03 \pm 0.01$. This is consistent with the results from the UV-vis-NIR titrations (Table 1), $\alpha = 0.04 \pm 0.01$. In the case of **c-P6/T3N**, only the 1:1 complex **c-P6·T3N** was observed, with no detectable amount of the unbound nanorings and 1:2 complex. If we again assume a 10% detection thresh-

old, this implies that $\alpha < 0.02$, which is consistent with the results from the UV-vis-NIR titrations (Table 1), $\alpha = 0.0013$.

Kinetics. The coordination of **T3** or **T3N** to **c-P6** is essentially irreversible, in noncoordinating solvents on the time-scale of a few days at room temperature, and thus the distribution of 1:1 and 1:2 complexes is under kinetic control. When solutions of **T3** and **c-P6** in CDCl_3 are mixed, in 1:1 mole ratio, the composition of the resulting mixture depends on the mixing rate, as illustrated by the ^1H NMR spectra in Figure 7. If the solutions are mixed slowly, the result is mostly **c-P6**·(**T3**)₂ and **c-P6**, because nanorings bind two templates in regions of high local template concentration, before the solutions have completely mixed. In contrast, if **T3** is added dropwise under vigorous stirring, the mixture is statistical, i.e. the ratio of **c-P6** : **c-P6**·**T3** : **c-P6**·(**T3**)₂ is 1:2:1, which implies that the rate constants for complexation of **T3** to **c-P6** and **c-P6**·**T3** are almost equal. Both these mixing protocols give mixtures that are far from thermodynamic equilibrium; as mentioned above the equilibrium ratio of **c-P6** : **c-P6**·**T3** : **c-P6**·(**T3**)₂ is 1:8:1. We monitored the equilibration of a 1:1 mixture of **c-P6** and **c-P6**·(**T3**)₂ over time in CDCl_3 at 298 K by NMR spectroscopy (Figure 8). These equilibration experiments were carried out at a range of concentrations, and we found that the

Figure 7. ^1H NMR spectra of 1:1 mixtures of **c-P6** and **T3**, mixed under different conditions. a) **T3** was added with slow mixing, b) **T3** was added in a dilute solution with vigorous stirring. CDCl_3 , 298 K, 500 MHz; spectra recorded approximately 15 minutes after mixing.

Figure 8. Evolution in the concentrations of **c-P6**·(**T3**)₂ (black), **c-P6**·**T3** (grey), and **c-P6** (red) from integration of ^1H NMR spectra of the mixture (298 K, CDCl_3). The data points are fitted to an exponential decay with an apparent first order rate constant of $k = 3.2 \times 10^{-7} \text{ s}^{-1}$.

initial rate of consumption of **c-P6**·(**T3**)₂ is independent of the concentration of **c-P6**, which implies that the exchange mechanism is dissociative. The rate-determining step is the unimolecular dissociation of a **T3** molecule from **c-P6**·(**T3**)₂ (Figure 9) and at low concentrations of **c-P6**·**T3** the rate of reaction is given by equation 5.

Figure 9. Mechanism of the template-redistribution equilibrium shown in Figure 6.

$$\frac{d[\text{c-P6} \cdot (\text{T3})_2]}{dt} = -\frac{k_1 k_{-2} [\text{c-P6} \cdot (\text{T3})_2] [\text{c-P6}]}{k_1 [\text{c-P6}] + k_2 [\text{c-P6} \cdot \text{T3}]} \quad (5)$$

This equation can be simplified as we know that $k_1 \approx k_2$, from the statistical kinetic ratio of **c-P6** : **c-P6**·**T3** : **c-P6**·(**T3**)₂ under fast mixing. The value of the dissociation rate constant ($k_{-2} = 3.0 \pm 0.4 \times 10^{-7} \text{ s}^{-1}$ at 298 K in CDCl_3) was determined from the initial rate of dissociation of **c-P6**·(**T3**)₂ (equation 5, Figure 8, $t < 7$ days). We already know both the equilibrium constants ($K_{f,1} = k_1/k_{-1} = 2.5 \pm 0.6 \times 10^{15} \text{ M}^{-1}$; $K_{f,2} = k_2/k_{-2} = 5.0 \pm 1.1 \times 10^{13} \text{ M}^{-1}$; Table 1), so all the rate constants can be calculated: $k_1 \approx k_2 = 1.5 \pm 0.4 \times 10^7 \text{ s}^{-1}$ and $k_{-1} = 6.0 \pm 2.0 \times 10^{-9} \text{ s}^{-1}$. Although the bimolecular association rate constants k_1 and k_2 are fast, they are well below the diffusion controlled limit. According to the Stokes-Einstein equation, the diffusion constant under these conditions is $D \approx 3.4 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, which agrees well with diffusion constants for similar molecules determined by DOSY NMR experiments.¹⁸ This diffusion constant corresponds to a diffusion-limited rate constant of $1.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, which is about a thousand times greater than k_1 and k_2 (see SI Section 4.2). The values of k_1 and k_2 are similar to bimolecular association constants reported previously for zinc porphyrin complexes.¹⁹

Vernier template-directed synthesis. After elucidating the allosteric cooperativity in stacked template complexes, we investigated these systems for template-directed synthesis. As a proof of concept, we tested the palladium-catalyzed oxidative coupling of the linear porphyrin dimers **I-P2** in the presence of **T3** or **T3N**, in comparison with **T6**, which is known to be an effective template for **c-P6**.^{6b,20} We also investigated the coupling of a linear tetramer **I-P4** in the presence of **T6e** and compared it to the coupling in the presence of the **T6**, which is a Vernier template for **c-P12**.^{7a,7c,17b} The structures of **I-P2**, **I-P4** and **T6** are shown in Figure 10 and the proposed template-directed syntheses are summarized in Figure 11. All these reactions were tested using versions of **I-P2** and **I-P4** with *tert*-butyl and trihexylsilyl (THS) solubilizing groups because the larger THS sidechains have a significant impact on the conformational behavior of these oligomers.

Figure 10. Structure of the **T6** template and linear porphyrin oligomers.

Figure 11. Stacked Vernier template approaches to the synthesis of (a) **c-P6** and (b) **c-P12**, compared with (c) a non-stacked Vernier template synthesis of **c-P12**. Scheme (a) was not found to be viable. Scheme (b) is most effective with $R = \text{THS}$. Scheme (c) is most effective with $R = t\text{-Bu}$, where R is defined in Figure 10.

Template-directed coupling reactions were evaluated by analyzing reaction mixtures by gel permeation chromatography (GPC). The GPC traces (Figures 12 and 13) were acquired by monitoring the absorption at wavelengths at which all of the products have essentially the same molar absorption coefficient per porphyrin unit (466 nm for the experiments starting with **I-P2** and 470 nm for the experiments starting with **I-P4**; see SI Section 5). Calibrated integration of these GPC traces was used to determine analytical yields.

When **I-P2**_{THS} is coupled in the presence of **T3** or **T3N**, the outcome of the reaction is essentially unaffected by the presence of the template and the product is just a mixture of long linear oligomers (Figure 12). A very similar product distribution is obtained without any template (see SI Section 5). In the presence of **T6** however, the formation of **c-P6**_{THS} is efficient (76%). The results are the same with **I-P2**_{t-Bu}: both **T3** and **T3N** give almost exclusively long linear oligomers, whereas **T6** gives a clean formation of **c-P6**_{t-Bu} (63%).

Figure 12. Analytical GPC traces from stacked-template reactions with porphyrin dimer with THS and *t*-Bu sidechains. The GPC traces were measured using two JIAGEL-H columns in series (3H-A then 4H-A), with THF:1% pyridine eluent and a 1 mL/min flow rate. Detection at 466 nm.

The picture is completely different with the larger template **T6e** (Figure 13). Coupling of **I-P4**_{THS} in the presence of **T6e** gives a high yield of **c-P12**_{THS} (30%). For comparison, the same experiment with **T6** gave a lower yield of **c-P12**_{THS} (18%, which is close to the reported isolated yield of 14%)^{18b} and the main product was **c-P8**_{THS} (34%), which must be formed via the caterpillar-track complex.²¹

Coupling of **I-P4**_{t-Bu} in the presence of **T6e** gave **c-P12**_{t-Bu} in lower yield (21%), together with larger rings such as **c-P24**_{t-Bu}, which may be formed via a large double Vernier complex. The same coupling in the presence of **T6**, gave **c-P12**_{t-Bu} in a high yield (39%), as reported previously.^{7a}

Comparison of the results from the reactions of **I-P4**_{THS} and **I-P4**_{t-Bu} highlights the ease with which *t*-Bu oligomers form twisted Vernier complexes. The crystal structure of this figure-of-eight complex^{7c} shows that the *t*-Bu substituents interact closely with the pyrrole nitrogen atoms on the faces of the porphyrin π -system in **c-P12**·(**T6**)₂, and this interaction is expected to become repulsive when *t*-Bu is changed to THS, which explains why **T6e** is a better template than **T6** for forming **c-P12**_{THS}.

Figure 13. Analytical GPC traces from stacked-template reactions with porphyrin tetramer with THS and *t*-Bu sidechains. The GPC traces were measured using two JIAGEL-H columns in series (3H-A then 4H-A), with THF:1% pyridine eluent and a 1 mL/min flow rate. Detection at 470 nm.

CONCLUSION

This work illustrates how allosteric cooperativity can be difficult to predict. We anticipated that stacking interactions between the planar *s*-triphenyltriazine cores would lead to positive cooperativity in the formation of **c-P6·(T3N)₂**. In reality, repulsion between the **T3N** units forces **c-P6·(T3N)₂** into a distorted saddle geometry, resulting in negative cooperativity. The 1:2 complex is stable ($\log K_{f2} = 12.9$ in CDCl_3), but it is less stable than would be expected from the 1:1 complex ($\log K_{f1} = 16.2$ in CDCl_3). The cooperativity for **c-P6·(T3N)₂** is slightly more negative than for **c-P6·(T3)₂**, which may reflect the fact that **T3N** is smaller than **T3** (centroid-N distances 9.3 Å and 10.0 Å, respectively). We measured all the association constants in both CDCl_3 and toluene (Table 1). Zinc porphyrin pyridine interactions are generally stronger in toluene, reflecting its lower dielectric constant. The formation of **c-P6·(T3)₂** and **c-P6·(T3N)₂** is more negatively cooperative in toluene, but the behavior is similar in both solvents. Addition of **T3** to **c-P6**, in the absence of pyridine, provides an interesting example of self-assembly away from thermodynamic equilibrium. The system takes many weeks to equilibrate and the composition of the reaction mixture depends on the mixing rate during the first few seconds of mixing.

The negative allosteric cooperativity for formation of **c-P6·(T3)₂** and **c-P6·(T3N)₂** correlates with the failure of **T3** and **T3N** to act as Vernier templates for directing the synthesis of **c-P6**. Conversely, formation of **c-P12·(T6e)₂** displays high positive cooperativity ($\alpha > 40$) and **T6e** is an effective Vernier template for synthesis of **c-P12**. Previously, we reported that the smaller hexadentate template **T6** is also a Vernier template

for synthesis of **c-P12**, and that the formation of **c-P12·(T6)₂** exhibits positive allosteric cooperativity,^{7a,c} which supports the link between allosteric cooperativity and Vernier template effects. It is interesting that the sidechains ($R = t\text{-Bu}$ or THS, Figure 10) have a strong influence on the efficiency of Vernier templating: **T6** is most effective with $R = t\text{-Bu}$, whereas **T6e** is most effective when $R = \text{THS}$. This difference is explained by the short contacts between the *t*-Bu groups and the faces of the porphyrins in the crystal structure of **c-P12·(T6)₂**, which suggest there will be steric clash when $R = \text{THS}$. Vernier template-directed synthesis using stacked templates should be a useful general strategy for creating shape-persistent macrocycles that do not easily fold into figure-of-eight geometries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.xxxx.

Synthetic procedures and characterization data; assignment of NMR spectra; calculation of statistical factors; UV-vis-NIR titrations; kinetic NMR experiments; stacked template-directed synthesis (PDF)

Calculated molecular coordinates (ZIP).

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Notes

The authors declare no competing financial interest.

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