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Programmed Negative Allostery with Guest-Selected Rotamers Control Anion−Anion Complexes of Stackable Macrocycles

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Supporting Information

ABSTRACT: A new rotamer-based strategy for negative allostery has been used to control host−host interactions and product yield upon anion complexation. Coassembly of anion dimers as guests inside two cyanostar macrocycles drives selection of one rotamer in which all ten steric groups get directed outward to destabilize triply stacked macrocycles. A large entropy penalty (ΔS) is quantified upon anion binding when the multiple dynamic rotamers collapse down to one.

Rotamers are ubiquitous. They are conformational states related by simple rotations about covalent bonds that have found relevance in protein folding,[1] in the rotary motors of both biological[2] and artificial machines,[3,4] and in molecular switches.[5] In supramolecular chemistry, the effects of rotamers have been detailed in foldamers,[6] molecular tweezers,[7] and allosteric macrocycles.[8] With their ability to dynamically respond to input stimuli, however, rotamers have yet to be investigated as a deliberate element of design for controlling molecular self-association and recognition. Rather, rotational isomers are typically avoided given that restricting their rotations produces entropy penalties that impair affinity.[9] We address these issues by investigating the benefits of rotamers as key elements for producing an effect similar to negative allostery. But instead of inhibiting the binding of other guests, we use allostery to inhibit association of more hosts. Here we show how to optimize the yield (aka fidelity)[10] of a new self-assembly system[11−14] involving anion−anion dimers and stacks of cyanostar macrocycles (Figure 1a). These planar macrocycles are exemplary of many others[15−17] used in guest binding,[18,19] coordination chemistry,[20] and soft matter,[21,22] which undergo uncontrolled self-association and thus we offer a new way[19,23] to control stacking in this privileged class of building blocks.[24]

Anion-driven assembly[25−27] is exemplified by the formation of dimers of bisulfate,[11,13] $[HSO_4\cdots HSO_4]^{2-}$, which are cooperatively stabilized by stacks of macrocyclic cyanostars.[27] Yet, uncontrolled mixtures of complexes are formed (Figure 1b) with 3:2 and 2:2 cyanostar:bisulfate stoichiometries possible that differ by the number of stacked macrocycles. We attribute this outcome to uncontrolled π stacking of macrocycles. This idea inspired the design strategy of using guest-selected rotamers to preferentially form a doubly stacked 2:2 assembly over the triply stacked complex.

The design strategy is based on creating a macrocycle with one face open for stacking and the other blocked by sterically bulky groups (Figure 1d). Dynamic rotamers fulfill this requirement. This noncovalent approach bypasses the synthetically challenging covalent alternative of preparing a single rotamer with all five substituents on the same side, like a single atropisomer seen in picket-fence porphyrins.[28] Using dynamic rotamers allows sterically bulky groups to randomly distribute above and below the macrocycle's plane up until the moment an anion is bound. Thereafter, the π-stacked dimer is expected to form by rotation of the steric groups away from the center of the macrocycles. Once directed outward, they would block further stacking. Though use of sterics to control stacking,[29] and the idea that guest binding freezes conformations[30] is not new, this is the first time the two ideas have been unified together in a deliberate design that mimics negative allostery.[31−33]

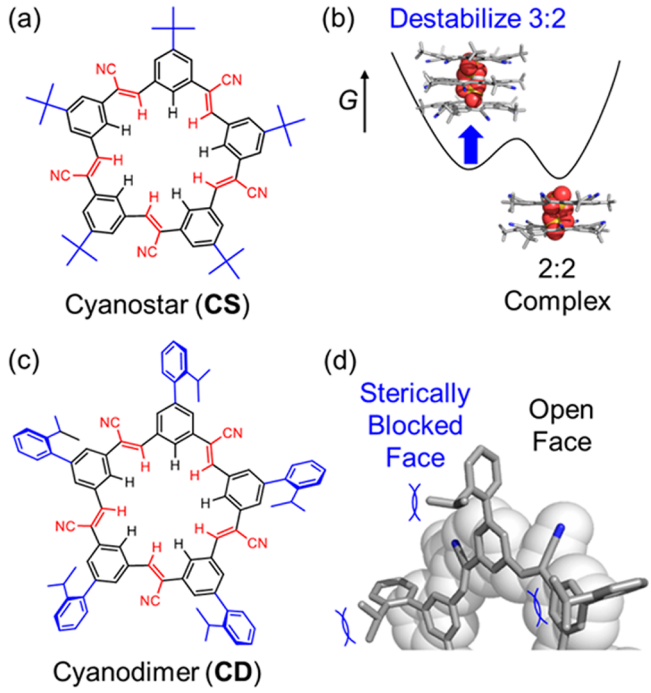

Figure 1. (a) Parent cyanostar forms (b) a mix of 3:2 and 2:2 complexes as visualized on an energy landscape. Block arrow indicates the principle of negative design. (c) New cyanodimer design with substituents (blue) that can rotate to (d) sterically block faces and shut down access to the 3:2 complex.

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Inspired by prior work,[29] as well as experimental and computational estimates of rotational barriers of monosubstituted biaryl compounds,[34] the isopropyl moiety was selected as the bulky substituent for biaryl rotamers. Isopropyl achieved a good balance between steric bulk and rotational kinetics ($k \sim 5 \times 10^5$ s^{-1}, $\Delta G^{\ddagger}$ = 11 kcal mol^{-1} at 300 K).

To estimate how well sterics could control stacking, intermacrocycle distances of various rotamers (Figure 2a–c)

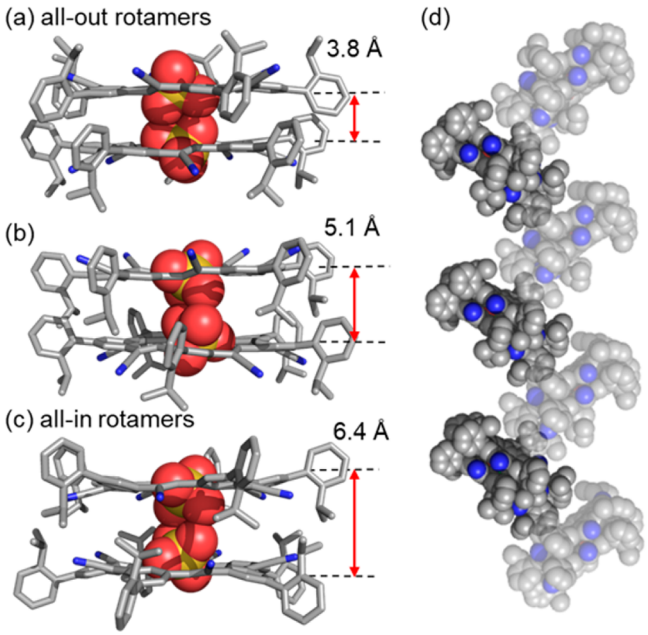


Figure 2. (a–c) Molecular models (MMFF) and interplanar distances of cyanodimer macrocycles with exterior groups in various conformations: all-out, in-out, and all-in. Geometries optimized as complexes of the bisulfate dimer. (d) Preliminary crystal structure showing the herringbone packing of the 2:1 complex between cyanodimer and perchlorate.

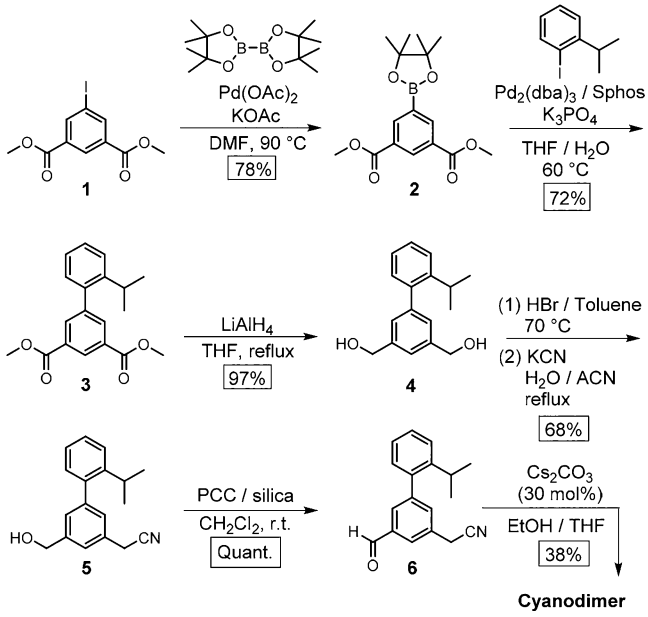
were determined using molecular mechanics. The closest interplanar contact (3.8 Å, Figure 2a) is achieved when all of the exterior isopropyl groups on both macrocycles are facing outward. Larger distances are seen with inward facing substituents (Figures 2b,c).

Cyanodimer was prepared (Scheme 1) using a modification of the synthesis of the cyanosolo macrocycle in order to enhance modularity.[29] Macrocyclization proceeded in a one-pot Knoevenagel cyclocondensation from aldehyde monomer in a 38% yield.

We first verified that stacking is suppressed in the absence of the anionic guest as a result of random distributions of up–down rotamers. Consistently, NMR and UV–vis spectra (Figures S1 and S2) show no change with concentration (1 μM to 10 mM, dichloromethane) in contrast to the parent cyanostar.[13]

We confirmed that anionic guests trigger formation of a π–π seam. A preliminary crystal structure of the 2:1 complex (Figure 2d, see Supporting Information for details), which was formed around perchlorate in lieu of bisulfate, confirms the dimer of macrocycles is accessible. Our crystal data lacks high-resolution (beyond 1.5 Å), which could not be mitigated. This is a known pathology for cyanostar structures that display high levels of disorder[11–13,27,29,35–39] from large, solvent accessible areas and whole-molecule disorder. Though unit cell, space group and

Scheme 1. Synthesis of Cyanodimer



packing are unambiguously established, the structure cannot be fully determined and refined on account of a lack of data.

Once dimerized, the macrocycles stop further stacking as shown by the herringbone packing differing from slip-stack packing observed for complexes with parent cyanostar.[27,35] Though the isopropyl groups are disordered (Figure S22), most isopropyl sites are pointing away from the macrocycle seam. At least one isopropyl site is not. In solution, we also see the 2:1 stoichiometry around ClO_4^- with 0.5 equiv of added anion (CD_2Cl_2, Figure S11). These findings are consistent with anion-driven selection of all-out rotamers.

Next, we undertook studies to verify that anion binding turned off the triple stack. Consistently, titrations with bisulfate in chloroform as monitored by ^{1}H NMR spectroscopy (Figure 3a) show conversion of free macrocycle into 2:2 complex with exclusion of the 3:2 complex across 0–1 equiv. By comparison, the parent cyanostar shows the 3:2 complex as well as its corresponding OH resonances at ~13 ppm for the hydrogen-bonded dimers of bisulfate in the 0.2–0.9 equiv region (red boxes, Figure 3b). For cyanodimer, we also see a resonance for the hydrogen-bonded dimer but at ~14 ppm, which is characteristic of the 2:2 complex.[11] The ^{1}H NMR titration confirms that guest-selected rotamers control stacking with exclusive formation of the 2:2 complex.

We switched to dichloromethane to provide a more challenging environment for the negative allostery on account of the fact that this solvent is known to enhance formation of the 3:2 complex.[13] Though we see that the same control feature is expressed, it is not as perfect, e.g., the 3:2 emerges at 0.2–0.6 equiv (Figure S4). Nevertheless, the 3:2 complex is completely converted into the 2:2 by 1.0 equiv fulfilling the requirement[10] for high fidelity. With the parent cyanostar, however, the 3:2 persisted in solution out to 4.0 equiv (Figure S4). This suppression of the 3:2 complex was also verified by mass spectrometry (Figure S6).

Observation of the 3:2 complex with cyanodimer was a surprise. Molecular models shows that the triple stack with the least steric pressure (Figure S7) involves a central macrocycle with three up and two down isopropyl units sandwiched by two

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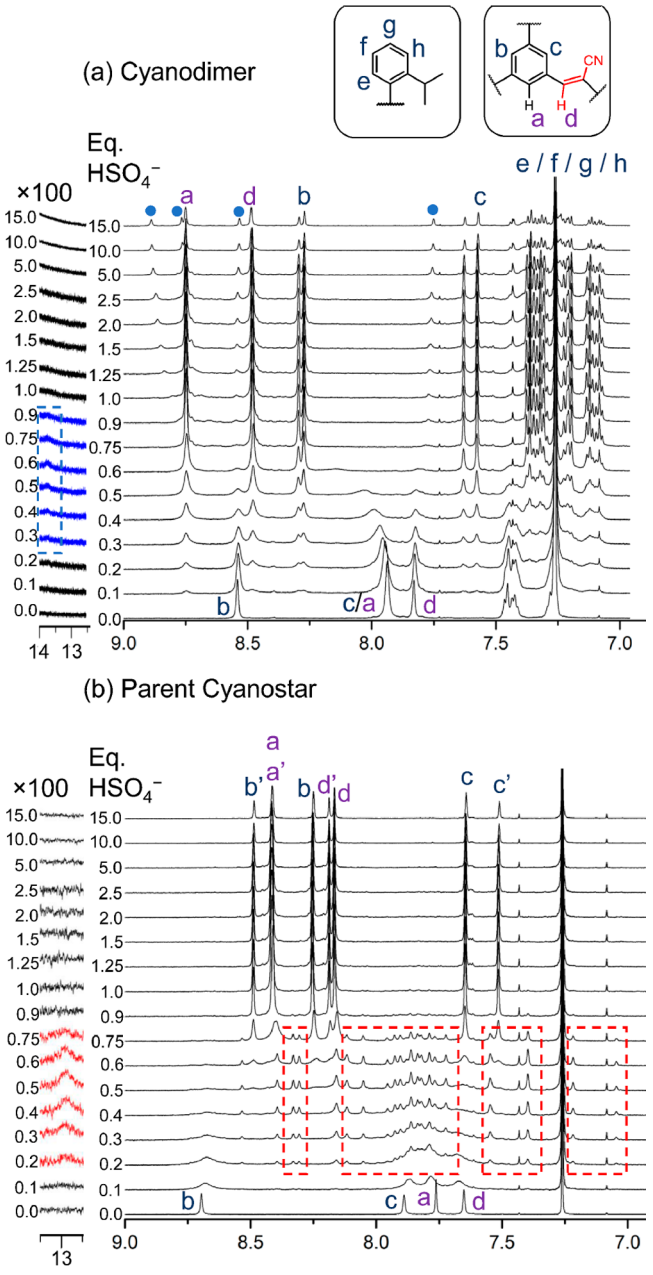

Figure 3. ^{1}H NMR titration of (a) cyanodimer and (b) the parent cyanostar with tetrabutylammonium bisulfate ($TBAHSO_4$, 1 mM, $CDCl_3$, 298 K, 600 MHz). The signature for the 3:2 complex is indicated by dashed red boxes. The signature for the 2:2 complex bisulfate −OH peak is indicated by a dashed blue box. The signatures assigned to the 1:1 complex with cyanodimer are indicated by blue dots.

all-out rotamers. With two partially congested seams, intermacrocycle distances (3.9 Å) are consistent with weakened $\pi-\pi$ stacking that would favor 2:2 over 3:2, as observed. The fact that the triple stack forms at all shows that the steric repulsions inhibiting formation of a tight seam can be overcome by interactions between the triple stack and the bisulfate dimer dianion.

One additional behavior differing from the parent cyanostar was the emergence of a new species beyond 1 equiv attributed to the 1:1 complex. At saturation (~15 equiv of bisulfate, $CDCl_3$, Figure 3), this species has a signature matching the 1:1 complex (Figure S3) that forms between bisulfate and the cyanosolo macrocycle.[29]

The 1:1 complex is observed to a greater extent in chloroform (34:100 for the ratio of 1:1 to 2:2 at 15 equiv of added bisulfate) than in dichloromethane (7:100). This finding is consistent with greater ion pairing in the less polar solvent as seen elsewhere.[11,13,36,40−43] Thus, pairing of the TBA^+ cation with the 1:1 complex likely plays a role in the difference between cyanostar and cyanodimer.

Observation of a 1:1 complex also indicates a loss in cooperativity for the 2:2 complex with cyanodimer. This outcome is attributed to a loss of entropy[9] (Figure 4a) on

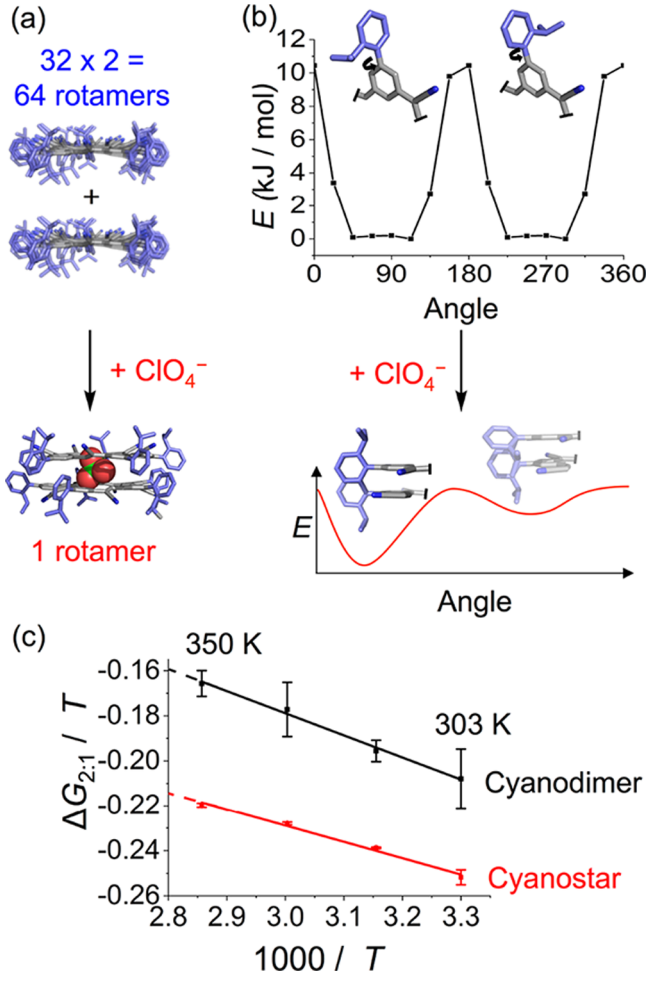

Figure 4. (a) Loss in conformational entropy upon complexation with perchlorate. As a mimic for entropy losses upon macrocycle dimerization with a bisulfate dimer. (b) Model of loss in torsional entropy upon ClO_4^- binding that is based on the potential energy surface calculated (B3LYP/6-31G*) using one biphenyl compound. Binding selects one rotamer and narrows the rotational energy well. (c) Van't Hoff plots for cyanodimer (black) and cyanostar (red) for 2:1 complex formation with $TBAClO_4$ in dichloroethane (CH_2ClCH_2Cl).

account of the restricted rotations of the biphenyl groups in the 2:2 complex. By contrast, the *tert*-butyl groups in cyanostar's complexes have been seen to rotate freely in its dimer stacks.[44]

We considered two approaches to calculate the entropy loss from restricting biphenyl rotations. The isopropyl groups were not considered as they largely retain mobility. The first calculation involves collapse of 32 rotational isomers in a

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cyanodimer to one in the complex (57 J mol^{-1} K^{-1}, Figures 4a, Supporting Information). The second relies on Whitesides' method of calculating torsional entropy.[45] A biphenyl model was used to plot potential energy versus dihedral angle (Figure 4b) to predict torsional entropy loss (123 J mol^{-1} K^{-1}, Supporting Information S8). Though both show significant penalties, the discrepancy between them impacts their usefulness for providing a deeper understanding of the recognition phenomenon[11,41,42,46−48] and for their use in the accurate computer-aided design[49] of receptors.

Experimental determination of thermodynamic signatures requires accurate estimates of all equilibrium constants.[11] For this reason, we selected a simpler system derived from perchlorate,[27] which has only two coupled equilibria rather than the four seen with bisulfate.[11] Entropy losses upon macrocycle dimerization with ClO_4^- approximate those with bisulfate. The equilibria involved with the macrocycles (**MC**; as either cyanodimer or cyanostar) are as follows:

$$\mathbf{MC} + ClO_4^- \rightleftharpoons MC{\cdot}ClO_4^- \quad \Delta G_{1:1}$$

$$2\mathbf{MC} + ClO_4^- \rightleftharpoons MC_2{\cdot}ClO_4^- \quad \Delta G_{2:1}$$

The parent cyanostar forms a 2:1 perchlorate complex with large cooperativity[16] and this complex is stable even with excess anion. With cyanodimer, however, after the 2:1 complex forms in high fidelity at 0.5 equiv it is followed by formation of a 1:1 complex (∼5 equiv, Figure S11) indicative of a reduction in cooperativity. This behavior matches bisulfate and is consistent with the 2:1 perchlorate complex serving as a reasonable approximation of the 2:2 bisulfate complex.

The binding thermodynamics were established from van't Hoff plots (Figure 4c, 303−350 K). The overall stability of cyanostar's 2:1 complex with perchlorate differs very little with temperature ($\Delta G_{2:1}$ = 76.0 ± 0.3 kJ mol^{-1}; 303−350 K) generating a small entropic effect on binding ($\Delta S_{2:1}$ = 12 ± 16 J mol^{-1} K^{-1}). The enthalpy of binding for cyanostar is $\Delta H_{2:1}$ = −72 ± 5 kJ mol^{-1}.

Consistent with our idea, cyanodimer has a large entropic penalty to 2:1 binding of −115 ± 18 J mol^{-1} K^{-1}. This value matches the torsional entropy (−123 J mol^{-1} K^{-1}). Thus, cyanodimer's cooperativity is reduced by entropic costs of restricting rotations of 10 biphenyl units and impeding their ±40° rocking (Figure 4b). Finally, the enthalpy of ClO_4^- binding with cyanodimer is $\Delta H_{2:1}$ = −98 ± 4 kJ mol^{-1}

We expect entropy penalties of forming a 2:1 complex with ClO_4^- matches formation of a 2:2 complex with bisulfate; both reactions freeze ten biphenyl rotamers. For the formation of the 3:2 complex, however, one additional macrocycle is attached to the 2:2 complex by freezing out an additional five biphenyl rotamers. Thus, the entropy penalty for the 3:2 complex is expected to be 50% greater that of the 2:2 complex. This entropy cost likely works together with the designed steric effects. Thus, a more accurate formulation of the design principle employed herein is of steric destabilization and enhanced entropic destabilization of the 3:2 species relative to the entropic destabilization of the 2:2 complexes that form around a bisulfate dimer.

In conclusion, a new strategy for negative allostery involves guest-selected rotamers capable of stabilizing π-stacked dimers over multimers to select 2:2 bisulfate complexes with high fidelity. The design mechanism produces lower overall stability of π-stacked dimers in the 2:2 complex originating from losses in torsional entropy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b02993.

Experimental procedures, compound characterization and spectra, NMR titrations, UV−vis titrations, mass spectrometry, and entropy calculations (PDF)

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Notes

The authors declare no competing financial interest.

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