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Reversible and Selective Encapsulation of Dextromethorphan and β-Estradiol Using an Asymmetric Molecular Capsule Assembled via the Weak-Link Approach

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Supporting Information

ABSTRACT: An allosterically regulated, asymmetric receptor featuring a binding cavity large enough to accommodate three-dimensional pharmaceutical guest molecules as opposed to planar, rigid aromatics, was synthesized via the Weak-Link Approach. This architecture is capable of switching between an expanded, flexible "open" configuration and a collapsed, rigid "closed" one. The structure of the molecular receptor can be completely modulated *in situ* through the use of simple ionic effectors, which reversibly control the coordination state of the Pt(II) metal hinges to open and close the molecular receptor. The substantial change in binding cavity size and electrostatic charge between the two configurations is used to explore the capture and release of two guest molecules, dextromethorphan and β-estradiol, which are widely found as pollutants in groundwater.

Biological systems, such as molecular chaperonins[1−3] and G-protein-coupled receptors,[4−6] rely on the reversible binding of bioactive molecules and proteins to guide crucial cellular functions ranging from protein folding to signal transduction. To achieve these tasks, functional subunits of proteins assemble to form nanoscale binding pockets that are often allosterically regulated to reversibly and selectively encapsulate guests through intermolecular interactions such as hydrogen bonding, π−π stacking, and electrostatic forces.[7−10] Chemists have sought to mimic these properties through the design of abiotic systems for applications in the fields of drug delivery,[11−14] sensing,[15−17] and catalysis.[18−23] Despite efforts to build such systems,[24] allosteric regulation of supramolecular constructs remains challenging, and no example of an allosterically regulated molecular receptor capable of selectively encapsulating large, three-dimensional bioactive molecules with switchable selectivity has been reported. Indeed, the field has been primarily restricted to designing receptors capable of differentiating relatively simple, flat aromatic structures.

However, a promising strategy to design allosterically regulated molecular receptors is through the Weak-Link Approach (WLA),[25−29] which is a coordination chemistry-based method for assembling supramolecular systems that may be allosterically regulated using small molecule effectors. This platform has enabled the development of several constructs with applications in sensing,[30] catalysis,[19,31−33] and signal and target amplification.[17,34] Recently, the WLA was used to synthesize a switchable molecular receptor with multiple states that selectively encapsulates small aromatic guests.[35] Although this construct was the first example of a system capable of not only reversibly binding guest molecules and toggling binding selectivity between three different states, the limited size of its binding pocket prevents the encapsulation of larger bioactive molecules. Herein, we report the design and synthesis of the first synthetic biomimetic construct featuring a nanoscale binding pocket capable of selectively encapsulating the pharmaceuticals dextromethorphan and β-estradiol, structures that are found as contaminants in groundwater.[36−39] The selectivity for these molecules is manifested in the ability to change both the size and charge of the binding pocket (Scheme 1).

To realize a structure with a large enough pocket to bind dextromethorphan or β-estradiol, an asymmetric macrocyclic WLA complex was designed. Assembly of such a macrocycle requires the use of two ditopic ligands, which prevents ligand rearrangement to form a nonproductive monometallic complex.[40] In addition, a rigid cavitand is needed to avoid the formation of such a complex.[35] For these reasons, a calix[4]arene with two phosphine alkyl thioether (P,S) moieties (**1**), and a resorcin[4]arene with two N-heterocyclic carbene alkyl thioether (NHC,S) moieties chelated to two Pt(II) centers (**2**) were designed and synthesized.

Under ambient conditions, Pt(II) precursor **2** and P,S ligand **1** react in CH_2Cl_2 to form the open, asymmetric receptor **3** quantitatively (Scheme 1). In this neutral configuration, each Pt(II) center is bound to two chlorides, which unhinge the thioethers from the metal centers, and enable the flexible linkers to expand the internal size of the cavity to 2.3 nm (Figure 1c). By abstracting the molecular effector chloride ions with a Ag(I) salt, the molecular receptor closes to form complex **4**, which has an internal cavity of 1.8 nm (Figure 1d) due to the chelation of the weakly coordinating thioether moieties. Notably, the incorporation of the NHC,S moiety enables the molecular receptor to be fully reversible with respect to the open and closed configurations, a feature not previously achievable, due to the strength of the metal thioether bond when moieties other than carbenes have been used to design molecular receptors via the WLA.[35]

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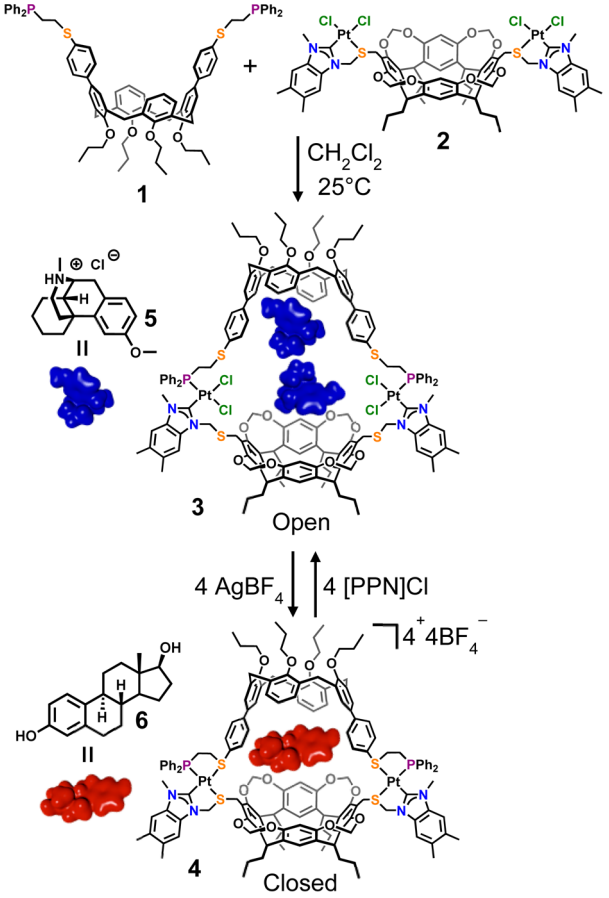
Scheme 1. Formation of Open Dimeric Capsule (3) from Two Cavitand-Based Ligands and Allosteric Control between Open (3) and Closed (4) Configurations That Exhibit Selectivity for Dextromethorphan HCl (5) and β-Estradiol (6), Respectively



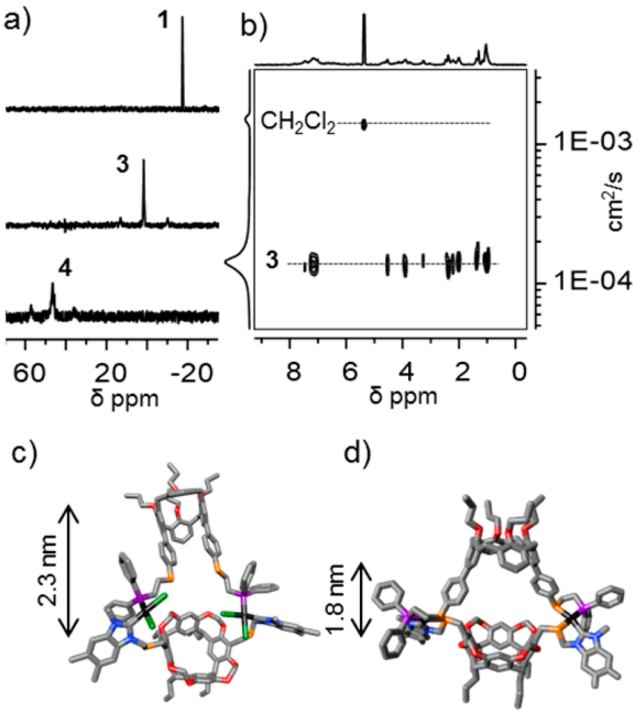


Figure 1. (a) $^{31}P\{^{1}H\}$ NMR spectrum of calix[4]arene ligand **1** in CD_2Cl_2, showing full conversion into open receptor **3** and closed receptor **4**. Both **3** and **4** show satellite signals indicating coordination to Pt(II) metal centers. (b) ^{1}H DOSY NMR showing the quantitative formation of **3** from **1** and **2**. (c) DFT computational model of **3**, showing both cavitand moieties are aligned cofacially to create a cavity size of approximately 2.3 nm. (d) Computational model of **4**, showing the collapse of the binding cavity to approximately 1.8 nm upon removal of Cl^- and coordination of all the thioether moieties to the Pt(II) centers.

The formation of the asymmetric receptor has been confirmed via ^{31}P NMR spectroscopy (Figure 1a). Upon the addition of P,S ligand **1** to Pt(II)-NHC **2**, the ^{31}P NMR spectrum of **1**, which consists of a single resonance at −17 ppm, changes to a spectrum that consists of a single resonance at 3 ppm with characteristic Pt satellites (J_{P-Pt} = 3737 Hz), confirming the binding of **1** to **2** to form open molecular receptor **3**. Abstraction of the chlorides to form closed molecular receptor **4** results in the shift of the resonance to 42 ppm (J_{P-Pt} = 3475 Hz) due to the increase in Lewis acidity of the Pt(II) center.[16] To further confirm the formation of the molecular receptor, ^{1}H diffusion ordered spectroscopy (DOSY) was used to determine the relative hydrodynamic radius (R_H) of Pt(II)-NHC **2** and molecular receptor **3** with respect to P,S ligand **1** (Figure 1b). The aromatic signals from the cavitand cores of each compound were used to determine the ratios of diffusion coefficients due to their presence in all three species. In accordance with the expected increase in size upon formation of molecular receptor **3** from P,S ligand **1** and Pt(II)-NHC **2**, at room temperature, **2** and **3** have a R_H value 1.2 times and 1.9 times greater than **1**, respectively (Figure S1).

The host−guest properties of molecular receptors **3** and **4**, which were evaluated in the context of dextromethorphan HCl (**5**) and β-estradiol (**6**) were found to be highly dependent upon the coordination state of the Pt(II) center. Molecular receptor **3** is neutral and has a large internal cavity with increased flexibility imparted by the uncoordinated thioether linkages, making it a suitable host for cationic guests, which may engage in cation−π interactions between the electron-rich cavitands of the molecular receptor and an electron-deficient moiety, such as the ammonium group on guest **5**.[41−43] In contrast, molecular receptor **4** has a smaller, more rigid cavity with higher local electrostatic charge and is expected to repel cationic guests while facilitating C−H/π interactions with neutral hydrophobic guests like **6**.[13,44]

To study the host−guest properties of the molecular receptors, ^{1}H NMR was used to determine the binding stoichiometries and affinities of guests **5** and **6** for **3** and **4**. First, Job plots of guest **5** with the open and closed molecular receptors, **3** and **4**, respectively (Figure 2a) were generated. Due to the expanded size of the internal cavity in the open state, molecular receptor **3** binds guests **5** in a 1:2 ratio, whereas closed molecular receptor **4** does not bind guest **5**. This is likely due to two factors: (1) the increased size of the cavity in open molecular receptor **3** facilitates guest binding compared to closed receptor **4**, and (2) the charge repulsion that inhibits binding between closed receptor **4** and guest **5** does not exist between open receptor **3** and guest **5**.

The binding affinity of **5** for molecular receptor **3** was determined via ^{1}H NMR titration in CD_2Cl_2 (Figure S7). The binding curve was fitted to a 2:1 binding model based upon the resulting stoichiometry obtained by the Job plot (K_{a11} = 70 ± 5 M^{-1}, K_{a12} is 6 ± 2 M^{-1}). The magnitude of the affinity of the first binding event suggests that the main driving force in the

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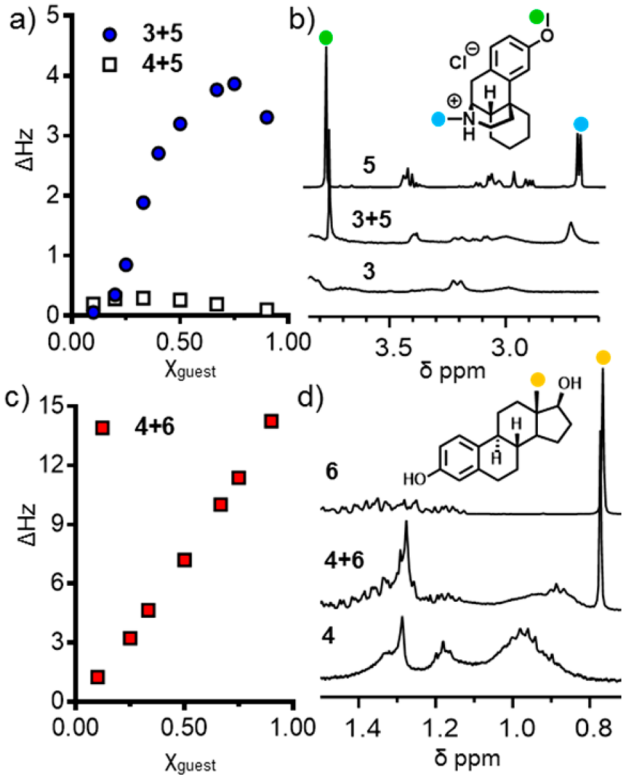

Figure 2. (a) ^{1}H NMR resonance shift response of receptors **3** and **4** to increasing mole fractions of guest **5**. Only in the open configuration of **5** is a host–guest complex observed. (b) ^{1}H NMR spectra showing chemical shift changes upon the addition 1 equiv of **5** to molecular receptor **3**. (c) ^{1}H NMR resonance shift response of receptor **4** to increasing mole fractions of guest **6**. (d) ^{1}H NMR spectra showing the spectral changes when **1** equiv of guest **6** is added to receptor **4**.

formation of the host–guest complex between molecular receptor **3** and guest **5** is a cation–π interaction between the electron-rich aromatic moieties of the cavitand receptors and the electron deficient cationic ammonium moiety of the guest molecule. Additionally, since guest **5** is cationic, the relatively weak affinity of the second binding event can be attributed to electrostatic repulsion between the two guest molecules.

In the closed state (**4**), the molecular receptor was found to selectively encapsulate β-estradiol (**6**) and not dextromethorphan. The binding of β-estradiol to molecular receptor **4** can be monitored by the ^{1}H NMR shifts of host **4** in the presence of guest **6** as a function of mole fraction of guest **6** (Figure 2c,d). A ^{1}H NMR Job plot was used to determine the 1:1 binding stoichiometry between host **4** and guest **6**. The binding of one molecule of β-estradiol per molecular receptor correlates with the reduced size of the closed molecular receptor (**4**) compared to the open molecular receptor (**3**). Because β-estradiol is a rather rigid and long steroid (1.16 nm),[45] there is only enough space for one molecule in the smaller binding cavity of host **4**. A ^{1}H NMR titration experiment was used to determine the binding affinity of β-estradiol to host **4**, which was calculated to be 44 ± 6 M^{-1} (Figure S8). The binding of **4** to **6** in methanol is likely driven by two interactions: (1) hydrophobic interactions between the core of **6** and the rigid hydrophobic binding cavity of molecular receptor **4**, and (2) C–H/π interactions between the aliphatic core of **6** and the aromatic rings in host **4**.

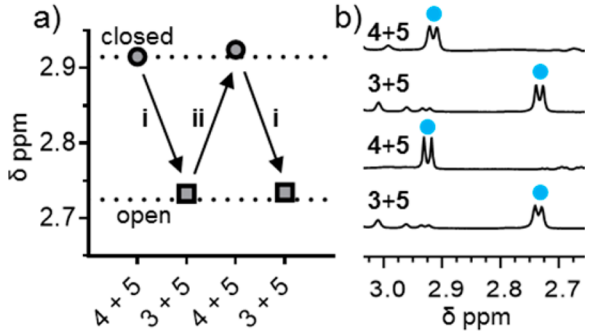

Figure 3. (a) *In situ* reversibility between the closed and open configurations, demonstrating the release and complexation of guest **5** from the molecular receptor. Formation of **3** from **4** was achieved by addition of 4 equiv. [PPN]Cl (i) followed by abstraction of Cl^- using $AgBF_4$ (ii). (b) ^{1}H NMR spectrum of N–CH_3 signal in **5** upon switching between open and closed configurations in CD_2Cl_2.

Finally, the quantitative and reversible encapsulation of guest **5** was demonstrated in situ through the addition and subsequent removal of Cl^- from the coordination sphere at the Pt(II) centers (Figure 3). Starting with a 1:1 solution of open molecular receptor **3** and guest **5**, four equivalents of silver tetrafluoroborate ($AgBF_4$) were used to remove Cl^- and close receptor **3** to form receptor **4**. The ^{1}H NMR spectrum of the solution shows a shift in the *N*-methyl proton signal of guest **5** from 2.77 to 2.92 ppm, indicating that guest **5** is no longer complexed with the molecular receptor. Upon addition of four equivalents of bis(triphenylphosphine)iminium chloride (PPNCl), the closed receptor **4** is opened to form receptor **3**, reforming the host–guest complex between guest **5** and receptor **3**. This process can be repeated, illustrating the robust reversibility of the WLA-assembled molecular receptor.

In conclusion, we have synthesized the first biomimetic molecular receptor with an allosterically regulated nanoscale binding cavity capable of encapsulating large bioactive molecules. This work demonstrates that complex asymmetric macrocyclic assemblies can be synthesized via the Weak-Link Approach in a stepwise fashion by utilizing two distinct ditopic hemilabile ligands, one rigid cavitand that coordinates to d^8 metals much more strongly than the other more flexible cavitand. In addition to enabling the synthesis of a molecular receptor large enough to accommodate three-dimensional guest molecules, the WLA also provides the ability to allosterically regulate such structures *in situ*. By modulating the coordination environment of the Pt(II) metal center, the molecular receptor is transformed from a rigid, cationic configuration to a flexible, neutral configuration, enabling the switching of the binding selectivity and the encapsulation of large bioactive molecules reversibly. This advance in the synthesis of supramolecular host–guest properties opens avenues to the design of systems that are capable of selectively binding and releasing three-dimensional guest molecules, a feature that could enable sensing and purification of chemically complex biomolecules.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b10027.

Synthetic procedures, spectral data, titration studies, Job plots, and computational models (PDF)

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Notes

The authors declare no competing financial interest.

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