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Positive Homotropic Allosteric Binding of Benzenediols in a Hydrindacene-Based Exoditopic Receptor: Cooperativity in Amide Hydrogen Bonding

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The design of new receptors for molecular recognition that mimic the binding processes in nature[1] has recently attracted considerable interest. Allosteric systems,[1−4] where the first ligand influences the binding strength of the receptor toward subsequent ligands, are of particular importance in many enzymatic systems. The mechanistic understanding and realization of similar processes in artificial receptors have long been challenging targets in chemistry.[2,3] By definition, allosteric systems are classified as positive or negative in terms of cooperativity[2] and are further divided into homotropic and heterotropic systems. In the study of artificial allosteric systems, the positive homotropic type is particularly important in view of the nonlinear amplification of affinities and selectivities toward ligands.[3b] There are a limited number of successful examples,[3,4] as reviewed by Rebek, Jr.[3a] and Shinkai et al.[3b,c]

However, recent studies on proteins[5,6] have suggested that the cooperativity in amide hydrogen bonds[7−10] plays an important role in the stabilization of folded structures such as α-helices and β-sheets,[5,8,9] resulting in extremely high affinities and selectivities in substrate binding to enzymes.[6] Further recent theoretical[7,8] and experimental[9,10] studies of amide clusters are indicative of moderate to strong cooperativity in the formation of one-dimensional chains and 2D-sheets, as well as their ligand binding at the amide groups. There, the origin of cooperativity has been proposed to be the restriction of internal motions (entropic contribution) as well as the inductive polarization (enthalpic contribution) of the amide groups. However, due to the shortage of experimental support,[10] the importance of the latter has not been well established. Herein, we report the complexation properties of newly prepared exoditopic receptors **1**, in which the amide cooperative effect, by polarization, induces positive homotropic allostericity in the binding process with benzenediols.

This novel class of receptors is designed to incorporate the hydrindacene (1,2,3,5,6,7-hexahydro-*s*-indacene) skeleton as the platform, which consists of a rigid aromatic ring and two flexible five-membered alicyclic rings. Functionalization by the introduction of appropriate binding sites such as amide or ester groups on the periphery may offer the skeleton the opportunity to act as a suitable receptor toward various substrates. We expect that the amide groups on the aromatic ring of **1** can function as both the hydrogen-bond donor and the hydrogen-bond acceptor, so that **1** may bind two identical guests such as **2**−**4**, on opposite sides of the molecular plane (Scheme 1). In this way, the novel receptor **1** appears to be an ideal compound to investigate if amide cooperativity can be used to induce homotropic allostericity.

The receptors **1a** and **1b** were readily prepared in three or four steps from 1,4-dibromo-2,3,5,6-tetrakis(bromomethyl)benzene.[11] X-ray structural analyses of **1a** and **1b** revealed that the two amide groups were significantly twisted around the $C_{amide}-C_{aromatic}$ bonds and oriented in opposite directions.[11] In solution, the ^{1}H NMR spectra of **1a** and **1b** exhibit higher symmetry (D_{2h}) due to rotational freedom around the $C_{amide}-C_{aromatic}$ bonds.

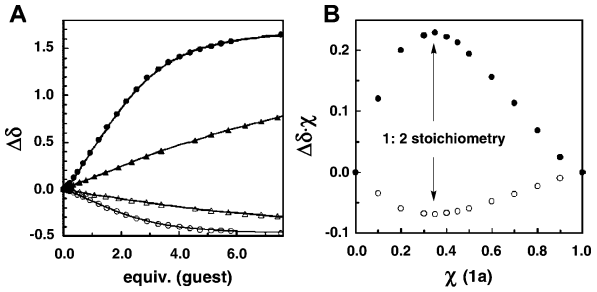
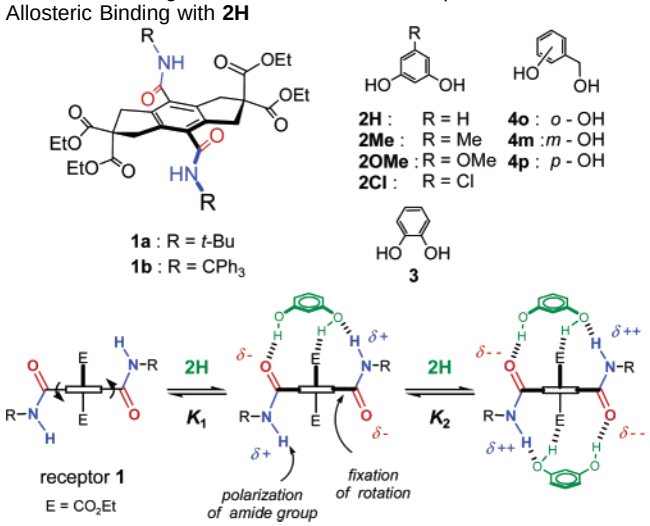


Figure 1. (A) NMR titration curves for **1a** with **2H** (● at amide proton (NH), ○ at five-membered ring proton (H_a) of **1a**) and **1a** with **3** (▲ at NH, △ at H_a of **1a**) in $CDCl_3$ at 25 °C. Also shown are the calculated binding isotherms (—) obtained by nonlinear regression assuming 1:2 allosteric binding. (B) Job plot for complexation of **1a** with **2H** in $CDCl_3$. χ = mol fraction of **1a**.

Scheme 1. (Top) Exoditopic Receptor **1** and Guests **2**, **3**, **4**; (Bottom) Schematic Representations of the 1:2 Complexes Formed Indicating the Mechanism of Homotropic Positive Allosteric Binding with **2H**



The complexation of receptors **1a** and **1b** with aromatic diols **2**−**4** was investigated by ^{1}H NMR spectroscopy in $CDCl_3$. Under these conditions, no indication of self-association of **1** was observed. The guest bindings were evidenced by a significant downfield shift of the receptor amide protons (NH) and moderate upfield shift of the CH_2 resonances on the five-membered rings (H_a) (Figures 1 and S1[11]). The 1:2 stoichiometry of receptor−guest complexes was confirmed by a Job plot. The binding constants were determined on the basis of titration experiments. Cooperativity in the guest-binding of **1a** and **1b** was indicated by the titration isotherms with a sigmoidal curvature,[2] which were analyzed by nonlinear regression

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Table 1. Microscopic Association Constants K (M^{-1}) and Cooperativity Parameters K_2/K_1 for the Complexation of Receptors **1a** and **1b** with Guests **2**,[a] **3**,[b] and **4**[b] in $CDCl_3$ at 298 K

complex	K_1	K_2	K_2/K_1	complex	K_1	K_2	K_2/K_1
1a·(**2H**)$_2$	116	2800	24	**1a**·(**4m**)$_2$	40	120	3
1a·(**2Me**)$_2$	92	2400	26	**1a**·(**4o**)$_2$	13	12	~1
1a·(**2OMe**)$_2$	100	3000	30	**1b**·(**2H**)$_2$	43	520	12
1a·(**2Cl**)$_2$	152	5000	33	**1b**·(**3**)$_2$	49	112	~3
1a·(**3**)$_2$	50	120	~3	**1b**·(**4m**)$_2$	18	68	4

[a] Estimated errors are within 15%. [b] Estimated errors are within 30%.

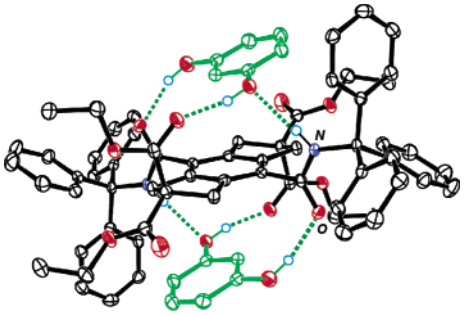

Figure 2. X-ray crystal structure of complex **1b**·(**2H**)$_2$. ORTEP representation with 50% probability. Hydrogen atoms not involved in hydrogen bonds are omitted for clarity.

using a nonstatistical 1:2 binding model (Table 1). The microscopic binding constants K_1 and K_2 are 116 and 2800 M^{-1}, respectively, for 1:2 complexation of **1a** with resorcinol **2H**. The large value for the ratio K_2/K_1 clearly indicates the positive homotropic allosteric nature[2−4] of this complexation. High allostericity ($K_2/K_1 = 12$) was observed again for the complexation of **1b** and **2H**, although the bulkiness of the trityl groups in **1b** leads to a decrease in K_1 and K_2. Similarly, large values for K_2/K_1 (26−33) were observed for the combination of **1a** and 5-substituted resorcinols (**2Me**, **2OMe**, **2Cl**).

To obtain information on the manner of allosteric binding, the solid-state complex **1b**·(**2H**)$_2$ was isolated by crystallization from a chloroform/ether solution and analyzed by X-ray diffraction (Figure 2). Two molecules of **2H** are presented on opposite sides of the receptor molecular plane with identical binding geometry. No direct interaction between guests was observed. One of the two hydroxyl groups in **2H** is doubly hydrogen bonded so that it is clipped between the amide NH and the ester carbonyl (N−H···O−H···O═C). The other hydroxyl group is hydrogen bonded to amide carbonyl only. The face-to-face overlap of aromatic nuclei (dihedral angle, 20.1°) observed in this crystal is in accord with the upfield shifts for the corresponding protons in the ^{1}H NMR spectrum in solution.

Other than resorcinol **2**, **1a** and **1b** form complexes with catechol **3** and 3-hydroxybenzyl alcohol **4m**, although the cooperativity is weak ($K_2/K_1 \approx 4$). No meaningful associations were observed for **1a** and **b** with **4p**, hydroquinone, or phenol. The large selectivity observed for the complexation toward aromatic diols containing a similar functionality suggests that it is the degree of cooperativity (K_2/K_1) as well as the affinity of individual functionalities that are important for the recognition properties of **1**. These results show that obviously positive homotropic allosteric systems can be utilized to attain high guest selectivity and guest affinity, which cannot be attained by conventional 1:1-type guest binding.[3b]

The observed positive allostericity for **1**···**2** can be attributed to the rotational restriction around the amide bond (ΔS), the induced polarization in the electronic structure of the amide (ΔH), or both (Scheme 1). Preliminary titration experiments at several temperatures for **1a**···**2Me** revealed that the degree of allostericity (K_2/K_1) depends on the temperature[3a] ($\Delta\Delta H_{2-1} = -4.6 \pm 0.7$ kJ mol^{-1}, $\Delta\Delta S_{2-1} = +11.9 \pm 2.4$ J mol^{-1} K^{-1}), indicating the importance of the contribution of the enthalpy term.

Although the polarization effect in amide hydrogen bonding has been previously proposed,[7−10] the experimental verification has scarcely been reported.[9,10] In our system, the polarization of the amide groups by hydrogen bonding was investigated in detail by the analysis of a series of crystal structures of **1b**.[11] The C−N bond length of the amide group is 1.342(2) Å in **1b**·(**2H**)$_2$, much shorter than the value of 1.366(3) Å in guest free **1b**. On the contrary, the C═O bond length of the amide group is 1.232(2) Å in **1b**·(**2H**)$_2$, much longer than the value of free **1b** (1.217(3) Å).[7d] An examination of amide geometry in **1b**·(acetone)$_2$ (C−N 1.343(3) and C═O 1.227(3) Å)[11] showed that the C═O bond is significantly longer than in free **1b**, although only the amide NH part is involved in the hydrogen bonding with the acetone carbonyls. This result indicates that the amide groups are polarized prior to the involvement of the N−H and C═O in hydrogen bonding. Consequently, this induced polarization effect must enhance the subsequent association to another side, resulting in positive cooperativity in the amide group.

In summary, the exoditopic artificial receptor **1** based on the hydrindacene platform provides the first clear evidence that the cooperativity in amide hydrogen bonding by polarization plays a part in the positive homotropic allosteric binding property with benzenediols.

Supporting Information Available: Synthetic procedures and characterization data for all compounds, details of binding studies (PDF), and X-ray crystallographic data of **1a**, **1b**, **1b**·(**2H**)$_2$, **1b**·(**3**)$_2$, and **1b**·acetone (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) See Supporting Information.

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