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Allosteric Regulation of the Conformational Dynamics of a Cavitand Receptor

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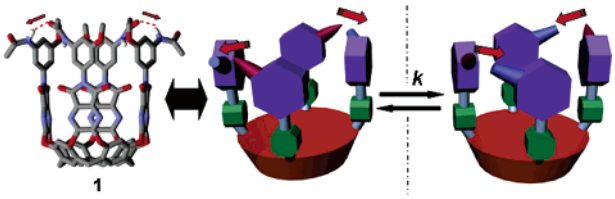
ABSTRACT



Inspired by allostery in nature, we synthesized cavitand 1 and investigated regulation of its conformational dynamics. Quantitative ^{1}H NMR studies have revealed that the rate of the conformational isomerization of 1 can be modulated using the external addition of acid. As 1 maintains its vase-like conformation in an acidic environment, ample opportunities for controlling the kinetics of molecular recognition, and thus reactivity, in this and related receptors have arisen.

The conformational dynamics of biological molecules is of prime importance in regulating their molecular recognition and activity.[1] Mechanisms have been proposed,[1c] and they include propagating conformational changes in addition to stochastic modulation of the binding site entry. As adapted to supramolecular chemistry, this concept will allow the preparation of receptors capable of controlling the kinetics of molecular encapsulation and reactivity in an unnatural setting.[2] The conformational dynamics of artificial receptors has long been recognized as an essential parameter in the thermodynamics of molecular recognition.[3,4] The dynamic nature of receptors[5,6] has been considered in elucidating the mechanisms of molecular encapsulation. In the study presented herein, we report on the preparation and the conformational behavior of the cavitand **1** (Figure 1); *in particular, we directed our efforts toward understanding the allosteric regulation of the kinetics of its conformational change using a strong acid as a stimulus*. It was originally demonstrated by Cram et al. that resorcin[4]arene-based cavitands akin to **1** assume either vase or kite conformations, for which the equilibrium can be set by varying the external conditions.[7] Additionally, Diederich and others have shown that the vase/kite thermodynamic equilibrium can be perturbed if the four pyrazine-based units are to interact with an external source of acid or a transition metal cation.[8]

(1) (a) McCammon, J. A.; Northrup, S. H. *Nature* **1981**, *293*, 316−317. (b) Wlodek, S. T.; Clark, T. W.; Scott, L. R.; McCammon, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9513−9522. (c) Gunasekaran, K.; Buyong, M.; Nussinov, R. *Proteins* **2004**, *53*, 433−443.

(2) (a) Mogck, O.; Pons, M.; Boehmer, V.; Vogt, W. *J. Am. Chem. Soc.* **1997**, *119*, 5706−5712. (b) Beno, B. R.; Sheu, C.; Houk, K. N.; Warmuth, R.; Cram, D. J. *Chem. Commun.* **1998**, *3*, 301−302. (c) Vysotsky, M. O.; Thondorf, I.; Bohmer, V. *Angew. Chem., Int. Ed.* **2000**, *39*, 1264−1267. (d) Yoshizawa, M.; Takeyama, Y.; Kusukawa, T.; Fujita, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1347−1349. (e) Davis, A. V.; Yeh, R. M.; Raymond, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4793−4796. (f) Zhong, Z.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2003**, *42*, 3005−3008. (g) Kaanumalle, L. S.; Gibb, C. L. D.; Gibb, B. C.; Ramamurthy, V. *J. Am. Chem. Soc.* **2004**, *126*, 14366−14367. (h) Davis, J. T.; Kaucher, M. S.; Kotch, F. W.; Iezzi, M. A.; Clover, B. C.; Mullaugh, K. M. *Org. Lett.* **2004**, *6*, 4265−4268. (i) Fiedler, D.; Bergman, R. G.; Raymond, K. N. *Angew. Chem., Int. Ed.* **2004**, *43*, 6748−6751. (j) Purse, B. W.; Gissot, A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2005**, *127*, 11222−11223. (k) Zuccaccia, D.; Pirondini, L.; Pinalli, R.; Dalcanale, E.; Macchioni, A. *J. Am. Chem. Soc.* **2005**, *127*, 7025−7032. (l) Purse, W. P.; Rebek, J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10777−10782. (m) Maslak, V.; Yan, Z.; Xia, S.; Gallucci, J.; Hadad, C. M.; Badjiæ, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 5887−5894. (n) Hooley, R. J.; Van A.; Hillary, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2006**, *128*, 3894−3895.

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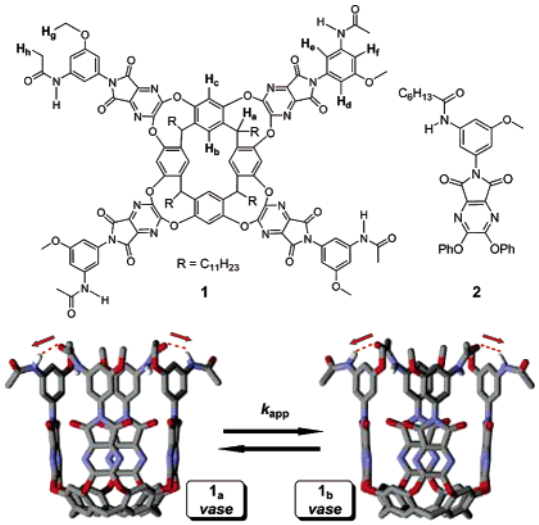

Figure 1. Chemical structures of cavitand **1** and model compound **2**. Energy minimized (AMBER) enantiomeric forms $\mathbf{1_a}$ and $\mathbf{1_b}$ of C_2 symmetrical **1** as vase-like conformers.

Cavitand **1** was designed to contain four identical pyrazine-based "flaps", with each flap having a benzene unit functionalized with an acetamide and a methoxy group (Figure 1). The acetamides were predisposed to act as donors (but may also be acceptors!) and the methoxy groups as acceptors of hydrogen bonding, so that **1** can develop a vase-like geometry with a seam of *intramolecular* hydrogen bonds along its upper rim.[5c] As the vase structure is maintained via hydrogen bonding, the pyrazine units were foreseen to act as basic sites to, upon reacting with an external acid, promote change in the conformational dynamics of **1**.

The preparation of **1** and model compounds **2** and **3** (Figure 3) is described in the Supporting Information. The

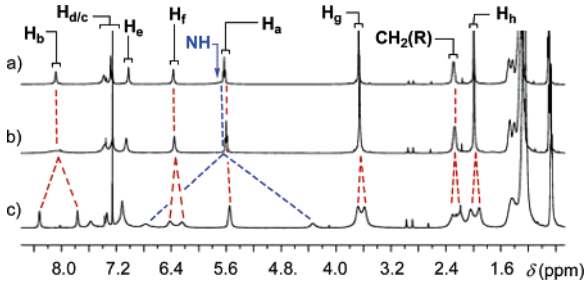

Figure 2. ^{1}H NMR spectra (500 MHz) of **1** (1.50 mM) in dry $CDCl_3$ recorded at (a) 328 K, (b) 300 K, and (c) 258 K.

^{1}H NMR spectrum of **1** at 300 K (Figure 2b) showed a set of broad signals, indicating the existence of either ill-defined intermolecular aggregates or conformational isomers exchanging at intermediate rates on the ^{1}H NMR time scale. Diffusion NMR spectroscopy (HR-DOSY) in combination with vapor pressure osmometry (VPO) measurements dis-

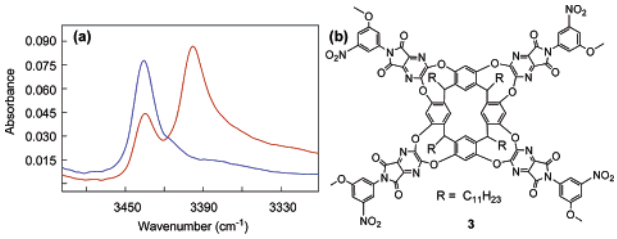

Figure 3. (a) N−H stretching region of the infrared spectra for 5.4 mM $CHCl_3$ solution of **1** (red line) and 11.2 mM $CHCl_3$ solution of **2** (blue line). (b) Chemical structure of model cavitand **3**.

counted the possibility of *intermolecular* aggregation. The observed diffusion coefficients of **1** remained almost a constant value of $(5 \pm 2) \times 10^{-6}$ cm^2 s^{-1} for concentrations of 0.7−6.9 mM of **1** in $CDCl_3$.[9] The VPO measurements, 3.0−8.9 mM $CDCl_3$ solutions of **1**, showed that the observed molecular weight ($M_w^{obs} = 1210 \pm 120$) is about 50% lower than the value calculated for the monomeric **1** ($M_w = 2339$). The discrepancy is significant; however, additional experiments with model compounds were consistent in showing nonideal behavior of this class of molecules.[10] The ^{1}H NMR spectrum of **1** at 328 K revealed a set of well-defined resonances corresponding to a molecule with average C_4 symmetry (Figure 2a). At 258 K, however, each resonance was clearly split into two signals with equal intensities, so

(3) (a) Gutsche, C. D.; Bauer, L. J. *Tetrahedron Lett.* **1981**, *22*, 4763−4766. (b) Groenen, L. C.; Van Loon, J. D.; Verboom, W.; Harkema, S.; Casnati, A.; Ungaro, R.; Pochini, A.; Ugozzoli, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1991**, *113*, 2385−2392. (c) Blixt, J.; Detellier, C. *J. Am. Chem. Soc.* **1994**, *116*, 11957−11960. (d) Ikeda, A.; Shinkai, S. *Chem. Rev.* **1997**, *97*, 1713−1734. (e) Ma, S.; Rudkevich, D. M.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **1999**, *38*, 2600−2602. (f) Jasat, A.; Sherman, J. C. *Chem. Rev.* **1999**, *99*, 931−967. (g) Naumann, C.; Patrick, B. O.; Sherman, J. C. *Tetrahedron* **2002**, *4*, 787−798. (h) Gibb, C. L. D.; Li, X.; Gibb, B. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4857−4862. (i) Kang, S.-W.; Castro, P. P.; Zhao, G.; Nunez, J. E.; Godinez, C. E.; Gutierrez-Tunstad, L. M. *J. Org. Chem.* **2006**, *71*, 1240−1243.

(4) Cram, D. J. *Science* **1988**, *240*, 760−767.

(5) (a) Houk, K. N.; Nakamura, K.; Sheu, C.; Keating, A. E. *Science* **1996**, *273*, 627−629. (b) Rudkevich, D. M.; Hilmersson, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 9911−9912. (c) Rudkevich, D. M.; Hilmersson, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 12216−12225. (d) Wang, X.; Houk, K. N. *Org. Lett.* **1999**, *1*, 591−594. (e) Palmer, L. C.; Rebek, J., Jr. *Org. Biomol. Chem.* **2004**, *2*, 3051−3059.

(6) (a) Hiraoka, S.; Harano, K.; Shiro, M.; Shionoya, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 2727−2731. (b) Davis, A. V.; Raymond, K. N. *J. Am. Chem. Soc.* **2005**, *127*, 7912−7919. (c) Zuccaccia, D.; Pirondini, L.; Pinalli, R.; Dalcanale, E.; Macchioni, A. *J. Am. Chem. Soc.* **2005**, *127*, 7025−7032. (d) Davis, A. V.; Fiedler, D.; Seeber, G.; Zahl, A.; Van E. R.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 1324−1333.

(7) (a) Moran, J. R.; Karbach, S.; Cram, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 5826−5828. (b) Moran, J. R.; Ericson, J. L.; Dalcanale, E.; Bryant, J. A.; Knobler, C. B.; Cram, D. J. *J. Am. Chem. Soc.* **1991**, *113*, 5707−5714. (c) Soncini, P.; Bonsignore, S.; Dalcanale, E.; Ugozzoli, E. *J. Org. Chem.* **1992**, *57*, 4608−4612.

(8) (a) Skinner, P. J.; Cheetham, A. G.; Beeby, A.; Gramlich, V.; Diederich, F. *Helv. Chim. Acta* **2001**, *84*, 2146−2153. (b) Azov, V. A.; Diederich, F.; Lill, Y.; Hecht, B. *Helv. Chim. Acta* **2003**, *86*, 2149−2155. (c) Azov, V. A.; Jaun, B.; Diederich, F. *Helv. Chim. Acta* **2004**, *87*, 449−462. (d) Frei, M.; Marotti, F.; Diederich, F. *Chem. Commun.* **2004**, *12*, 1362−1363. (e) Lagugne-Labarthet, F.; An, Y. Q.; Yu, T.; Shen, Y. R.; Dalcanale, E.; Shenoy, D. K. *Langmuir* **2005**, *21*, 7066−7070. (f) Azov, V. A.; Schlegel, A.; Diederich, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 4635−4638.

(9) For a review, see: Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 520−554.

(10) For further discussion, see Supporting Information.

that the ^{1}H NMR spectrum of **1** corresponded to a molecule with average C_2 symmetry (Figure 2c). Clearly, dynamic conformational changes occurred in **1** as the temperature of its $CDCl_3$ solution was varied.[11] With the help of 2D ROESY and EXSY NMR spectroscopic methods, we assigned all of the signals in the ^{1}H NMR spectrum of **1**, as shown in Figure 2. The resonance for the methine proton $\mathbf{H_a}$ is positioned at 5.6 ppm and shifted insignificantly with temperature ($\Delta\delta/\Delta T = 1.4 \times 10^{-3}$ ppm/K), indicating the dominance of the vase conformer at all temperatures.[7] With this information in hand, we contemplated that the geometry of the low-temperature C_2 symmetrical **1** incorporates two aromatic flaps which can interact in pairs to form hydrogen bonds via acetamide groups, thereby leaving all of the methoxy units free and uncomplexed ($\mathbf{1_{a/b}}$ in Figure 1).[12] In this way, two amide protons in **1** stay hydrogen bonded, and two remain free. This mode of intramolecular association superseded our originally anticipated and, at least on paper, reasonable mode of interaction with the full occupancy of all hydrogen bonding sites. In the ^{1}H NMR spectrum of **1** (Figure 1c), two N−H singlets were positioned at 6.77 and 4.34 ppm, and according to the proposed structure would correspond to a pair of hydrogen bonded and free N−H protons, respectively. Inspection of a CPK molecular model of C_2 symmetrical **1** revealed that the "free" N−H protons are in fact positioned above the neighboring benzene rings and are possibly involved in the polar π interactions (N−H$\cdots\pi$) (Figure 1). These N−H protons are thus each in the shielding zone of the neighboring benzene, which rationalizes their high-field resonance at 4.34 ppm.

The results of infrared spectroscopic analysis of **1** provided additional support for its C_2 symmetrical geometry (Figure 3a). The FT-IR spectrum of **1** revealed two concentration independent N−H stretching vibrations at 3435 and 3398 cm^{-1}. The model compound **2**, however, revealed a concentration independent N−H band at 3436 cm^{-1}; this vibration can be ascribed to the free N−H, due to the low propensity of **2** for intermolecular association. Accordingly, the 3435 cm^{-1} vibrational band in **1**, observed at a nearly identical frequency, is consistent with the free N−H. The low wavenumber peak at 3398 cm^{-1} would thus be congruent with the hydrogen bonded N−H groups.

An incremental addition of a strong hydrogen bond acceptor DMSO-d_6 to a $CDCl_3$ solution of **1** promoted a complete vase to kite conformational change (Figure 4a), as indicated by the methine $\mathbf{H_a}$ resonance shift, 5.6 to 3.3 ppm.[7] Cavitand **3** incorporates nitro instead of acetamide groups, and it is incapable of hydrogen bonding (Figure 3b). **3** mostly assumed a vase conformation in $CDCl_3$, methine proton $\delta = 4.50$ ppm,[7] which proved to be rather insensitive to the presence of DMSO-d_6 (Figure 4a). Interestingly, incremental addition of trifluoroacetic acid (TFA) to a $CDCl_3$ solution

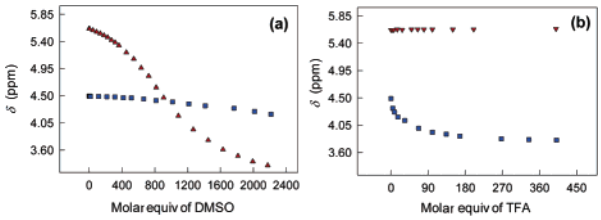

Figure 4. ^{1}H NMR chemical shifts for the methine $\mathbf{H_a}$ proton in **1** (▲, 2.84 mM) and **3** (■, 1.20 mM) in dry $CDCl_3$ as a function of molar equivalents of (a) DMSO-d_6, and (b) trifluoroacetic acid (TFA), incrementally added to their solutions.

of **1** did not affect the vase/kite conformational balance; that is, the equilibrium remained heavily weighted toward the vase conformer (Figure 4b). On the contrary, addition of TFA to a $CDCl_3$ solution of **3** (Figure 4b) affected its vase/kite equilibrium, favoring the kite conformer.[8] The results of the titration experiments corroborated our contention that intramolecular hydrogen bonding is indeed present in **1**, even when this cavitand is dissolved in $CDCl_3$ containing a great excess of TFA.[13] This noncovalent interaction reinforced the vase conformer, which proved to be (a) highly receptive to the presence of a strong hydrogen bond acceptor solvent (DMSO), and (b) thermodynamically stable in the presence of a great excess of TFA.

Since **1** retained the vase-like structure in an acidic environment, we investigated the effect that acid would have on its conformational dynamics. C_2 symmetrical **1** is inherently chiral and exists as a pair of enantiomers, $\mathbf{1_a}$ and $\mathbf{1_b}$ (Figure 1). The molecule flutters between two enantiomeric forms, as the hydrogen bonding pattern oscillates from the clockwise to the counterclockwise arrangement; this interconversion leads to the exchange of ^{1}H NMR signals of the proton nuclei experiencing different chemical environments.[14] Dynamic NMR methods, 2D EXSY or the classic total band-shape analysis, provide quantitative data about the kinetics of chemical exchange processes.[14] The resonance for the $\mathbf{H_b}$ proton in **1** was, at a high temperature, observed as a singlet at 8.11 ppm (Figure 2a). This proton, residing in two chemically different environments in $\mathbf{1_a}$ and $\mathbf{1_b}$, exchanged its positions slowly on the ^{1}H NMR time scale at low temperature, which resulted in the appearance of two distinct singlets at 8.32 and 7.77 ppm (Figure 2c). From the temperature dependence of $\mathbf{H_b}$'s resonance line shape, we calculated the apparent first-order rate constant k_{app} for the $\mathbf{1_a}/\mathbf{1_b}$ interconversion.[10] Subsequently, we titrated a $CDCl_3$ solution of **1** with TFA, and upon each increment of the added acid, variable temperature ^{1}H NMR spectra were acquired and the kinetic parameters evaluated. Outstandingly, the apparent rate constant, k_{app}, for the $\mathbf{1_a}/\mathbf{1_b}$ interconversion

(11) Hindered rotation about the (O)C−N bond in the acetamide group of **1**, in the examined range of temperatures, was discounted by the fact that the conformational isomerism was not observed in parallel experiments with the model compound **2**.

(12) Preliminary computational studies (MM, PM3) have suggested other plausible C_2 symmetrical conformations. The proposed geometry is in agreement with the available experimental results.

(13) In principle, TFA may compete for hydrogen bonds. ^{1}H NMR chemical shifts for NH protons in $\mathbf{1_{a/b}}$ ($\delta = 6.77$ and 4.34 ppm; 253 K, $CDCl_3$) changed, but not dramatically, upon addition of TFA ($\delta = 6.43$ and 4.45 ppm; 253 K, $CDCl_3$). Rather low affinity of TFA for amides in **1** is thus evident.

(14) (a) Jesson, J. P.; Meakin, P. *Acc. Chem. Res.* **1973**, *6*, 269−275. (b) Perrin, C. L.; Dwyer, T. J. *Chem. Rev.* **1990**, *90*, 935−967.

was discovered to be highly affected by the presence of exogenous acid (Figure 5). *The more acidic the solution of*

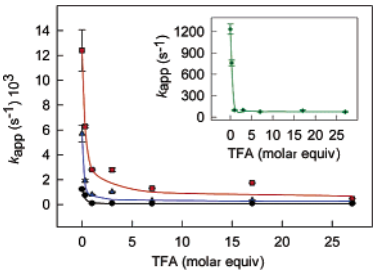

Figure 5. Apparent first-order rate constants, k_{app}, for the $\mathbf{1_{a/b}}$ interconversion in $CDCl_3$ as a function of trifluoroacetic acid (TFA) at 318 K (■), 308 K (▲), 300 K (●), and 288 K (▼, inset).

***1** in $CDCl_3$, the slower the observed interconversion of the enantiomers!* This allosteric effect, expressed by an acid, is more pronounced at higher than at lower temperatures. TFA is a strong acid ($pK_a = -0.25$) that is known to protonate pyrazine nitrogen atoms ($pK_a = 0.36$)[15] of cavitands analogous to **1**.[8] On the basis of that, it is reasonable to assume that the [**1**···TFA] interaction played a role in augmenting the activation energy barrier for the $\mathbf{1_a}/\mathbf{1_b}$ interconversion.

Activation parameters for the $\mathbf{1_a}/\mathbf{1_b}$ interconversion were calculated from an Eyring plot (Table 1). As a result of the inherent correlation that arises between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, as both are computed from the same data set and usually masked by dominant statistical compensation patterns,[16] we refrain from conducting any in-depth analysis. However, for the transition state of the rate-limiting step in the $\mathbf{1_a}/\mathbf{1_b}$ interconversion, both the activation enthalpy and entropy are positive regardless of whether the acid is present in the system. The positive value of $\Delta H^{\ddagger}$ (14−23 kcal/mol) points to the absence of intramolecular hydrogen bonding interactions. That is to say, for $\mathbf{1_a}$ to switch into $\mathbf{1_b}$ the intramolecular hydrogen bonds need to be broken. The positive $\Delta S^{\ddagger}$ (2−30 cal/molK) refers to a disordered transition state, which is consistent with **1** lacking hydrogen bonds.

Table 1. Activation Parameters for $\mathbf{1_{a/b}}$ Interconversion in $CDCl_3$, with and without Trifluoroacetic Acid (TFA)

TFA (molar equiv)	$\Delta H^{\ddagger}$ (kcal/mol)[a]	$\Delta S^{\ddagger}$ (cal/molK)[a]	$\Delta G^{\ddagger}$ (kcal/mol)[b]
0	17 ± 1	9 ± 1	13.3 ± 0.2
0.3	14 ± 1	2.6 ± 0.1	13.6 ± 0.2
1.0	17 ± 2	11 ± 1	14.8 ± 0.2
3.0	23 ± 2	30 ± 3	14.8 ± 0.2
7.0	20 ± 2	18 ± 3	15.0 ± 0.2
17.0	22 ± 2	26 ± 3	15.0 ± 0.2

[a] Calculated from an Eyring plot of $\log(k_{app}/T)$ versus $1/T$, over the temperature range of 258−328 K. [b] Calculated directly from the Eyring equation using k_{app} at 300 K.

In summary, we discovered that the conformational dynamics of our newly designed cavitand **1** can, in principle, be operated at multiple levels using an exogenous source of acid. To obtain more insight into the mechanism of the $\mathbf{1_{a/b}}$ interconversion, we are currently utilizing computational chemistry approaches to understand the dynamics in this system. The allosteric regulation of the kinetics of molecular recognition in **1** and similar receptors is also under experimental investigation in our laboratory.

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Supporting Information Available: Detailed descriptions of experimental methods and synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Brown, H. C. In *Determination of Organic Structures by Physical Methods*; Braude, E. A., Nachod, F. C., Eds.; Academic Press: New York, 1955.

(16) (a) Krug, R. R.; Hunter, W. G.; Grieger, R. A. *Nature* **1976**, *261*, 566−567. (b) McBane, G. C. *J. Chem. Educ.* **1998**, *75*, 919−922.