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# Protons as the Triggers to Regulate Hydrogen-Bonding Receptors

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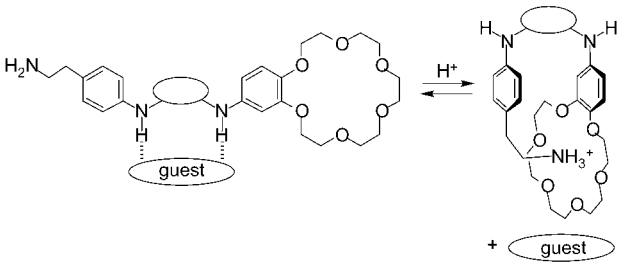
## ABSTRACT

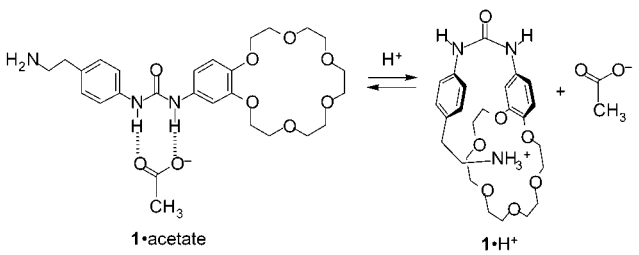



**The protonation of alkylamines in two novel receptors results in intramolecular host–guest associations between the resulting ammonium ions and crown ether macrocycles. These interactions result in conformational changes of the receptors and prevent them from acting as hydrogen bond complements for uracil and carboxylate guest species.**

It is remarkable how the presence or absence of mere hydrogen atoms can influence how molecular systems as complicated as those in Nature operate. It is well accepted, however, that the harnessing of structural changes that accompany variations in the pH of the environment is one of the most versatile methods to regulate the activity of natural receptors.[1] This regulation is often accomplished not by interfering directly with the recognition event but by reversibly protonating a location distant from the active binding site to induce a conformational change in the receptor and to subsequently affect its affinity for its substrate.[2] The induced structural change can either enhance (positive allostery) or diminish (negative allostery) the receptor's binding efficacy.

Despite Nature's frequent use of this regulatory strategy, there are few synthetic systems that have borrowed from its principles.[3] Here we report two novel examples of artificial hydrogen-bonding receptors that use a single proton as a negative allosteric cofactor to inhibit substrate recognition. The operation of the two receptors is designed around the same principle: the protonation of a functional group that is not an integral member of the substrate-specific molecular recognition motif creates a self-complementary species that folds in on itself with the concomitant distortion of the hydrogen bond site. The only difference between the two receptors is in what substrates they target. Receptor **1** presents a two-point donor−donor hydrogen-bonding urea appropriate for binding anionic carboxylate guests (Scheme 1). Receptor **2** possesses the three-point triaminotriazine donor−acceptor−donor site better suited to bind imides (Scheme 2). Both

**Scheme 1**

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(1) Scrimgeour, K. G. *Chemistry and Control of Enzyme Reactions*; Acedemic Press Inc.: New York, 1977.

(2) (a) Schetz, J. A.; Sibley, D. R. *J. Pharmacol. Exp. Ther*. **2000**, *296*, 359. (b) Traynelis, S. F.; Burgess, M. F.; Zheng, F.; Lyuboslavsky, P.; Powers, J. L. *J. Neurosci.* **1998**, *18*, 6163.

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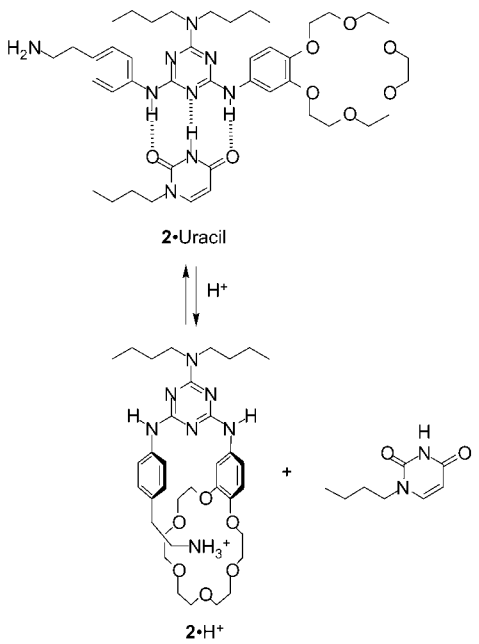
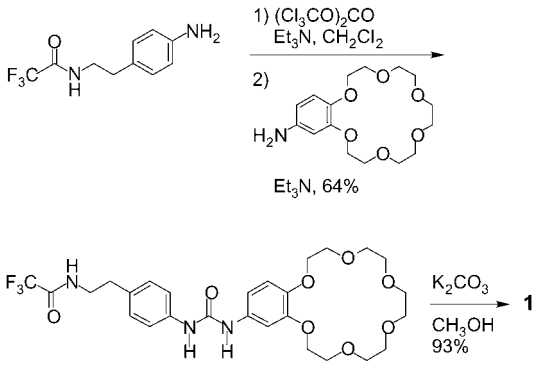
**Scheme 2**




brandish the two components responsible for the allosteric event, a primary amine and a crown ether. In both receptors, 1 equiv of an acid converts the former functional group into an ammonium cation which is a well-tested guest for the crown ether macrocycle. It is the creation of this host−guest pair that induces the conformational change and forces the receptors' C−N bonds to rotate into a nonproductive geometry, thus shutting down the receptors. Deprotonation destroys the ammonium−crown ether partnership and reactivates the receptors.[4]

Urea **1** was prepared in two steps by treating *N*-[2-(4-aminophenyl)ethyl]-2,2,2-trifluoroacetamide[5] with triphosgene, followed by 4-aminobenzo-18-crown-6 as outlined in Scheme 3. The primary amine was deprotected with 10%

**Scheme 3**




$K_2CO_3$/MeOH, affording the final receptor **1** as a white solid. The ammonium form, **1**•$H^+$, was generated as its tetrafluoroborate salt by the careful addition of a methanolic solution of $HBF_4$ (1.2 equiv) to **1**, followed by drying under vacuum.

The intramolecular association between the ammonium group and the crown ether macrocycle in **1**•$H^+$ is easily diagnosed by two-dimensional NMR spectroscopy (TROESY) which shows correlation peaks between the methylene $CH_2$ protons of the ethylamino chain and those of the crown ether. These correlation peaks are absent in the TROESY spectrum of the neutral form of the receptor, **1**, suggesting that protonation forces the receptor to turn in on itself, although the presence of intermolecular association between the cation of one receptor and the macrocycle of another cannot be completely ruled out with only this evidence. However, the electrospray mass spectrum of **1**•$H^+$ exhibits a major peak corresponding to the molecular mass of the monomer **1**•$H^+$ as well a minor peak corresponding to the dimer (**1**•$H^+$)$_2$.

The binding efficacy of receptor **1** to its substrate class was evaluated by analyzing the $^1$H NMR spectral changes that occur upon titrating DMSO-$d_6$ solutions of **1** with tetrabutylammonium (TBA) acetate. The significant downfield shift ($\Delta\delta$ > 2.5 ppm) observed for the signals corresponding to the urea N−H of **1** when it is treated with TBA acetate is indicative of effective hydrogen bonding even in this competitive solvent (Figure 1). After 2−3 equiv of

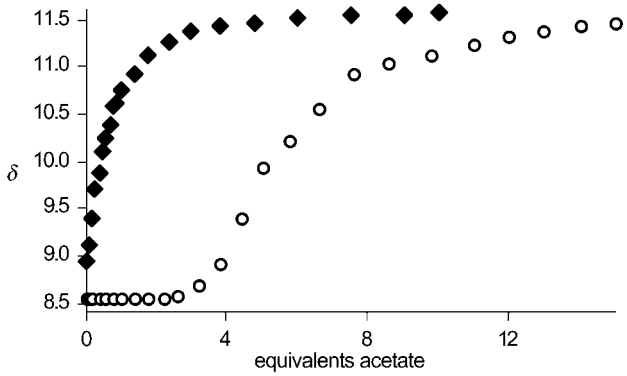



**Figure 1.** Observed chemical shifts ($\delta$) of the urea N−H resonances in the $^1$H NMR spectra of receptor **1** when titrated with aliquots of TBA acetate (◆) and when **1**•$H^+$ is titrated with TBA acetate (○) in DMSO-$d_6$. [**1**] and [**1**•$H^+$] = 2 × $10^{-3}$ M, [TBA acetate] = 2 × $10^{-2}$ M.

acetate have been added, the neutral form of the receptor reaches saturation. Data sets from all titration experiments correlate well with the curves calculated using a 1:1 binding model[6] and give an average association constant ($K_a$) value

(3) (a) Cordova, E.; Bissell, R. A.; Spencer, N.; Ashton, P.; Stoddart, J. F.; Kaifer, A. E. *J. Org. Chem.* **1993**, *58*, 6550. (b) Martinez-Diaz, M.-V.; Spencer, N.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1904. (c) Nakatsuji, Y.; Kobayashi, H.; Okahara, M. *J. Chem. Soc., Chem. Commun.* **1983**, 801.

(4) We have previously shown how metal ions can be used as allosteric cofactors to bring about similar structural changes: (a) Al-Sayah, M. H.; Branda, N. R. *Chem. Commun.* **2002**, 178. (b) Al-Sayah, M. H.; Branda, N. R. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 945.

(5) Desaubry, L.; Johnson, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 123.

(6) The $^1$H NMR titration data were analyzed using Christopher A. Hunter's 1:1 complexation model program (Department of Chemistry, University of Sheffield, UK).

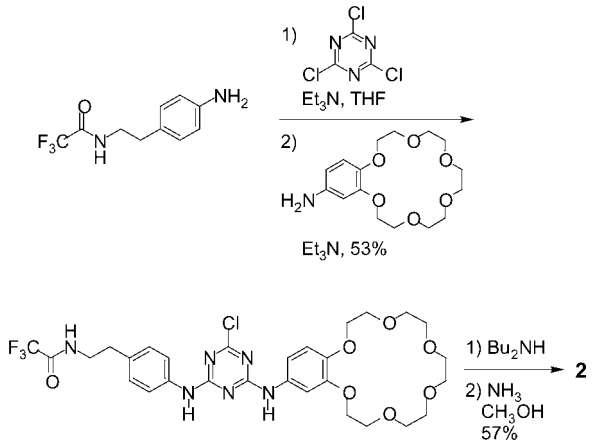
of 4400 ± 1100 $M^{-1}$ over three experiments. This value is large when compared to those reported for the complexation between alkylurea hosts and carboxylate guests which are typically in the range of 50−100 $M^{-1}$ in this solvent.[7,8] We attribute this discrepancy to the fact that the reported values of $K_a$ are for dialkylureas which possess significantly less acidic N−H protons than their diaryl counterparts. The greater acidity of these hydrogen bond donor groups in receptor **1** explain the enhanced binding observed in our studies. Hamilton invokes a similar explanation to account for the increase in the binding of carboxylate guests by the more acidic thiourea receptors (p$K_a$ ∼ 21) than the urea analogues (p$K_a$ ∼ 27).[8] Receptor **1** must, therefore, be treated as a diphenylurea derivative (p$K_a$ = 19.5)[9a] instead of a diethylurea derivative (p$K_a$ = 26).[9b] As a consequence, the urea in **1** will act as a better hydrogen bond donor and the larger value of $K_a$ is not unexpected.[8]

Repeating the titration experiments but replacing the active receptor with the preformed complex **1**·$H^+$ generated data that could not be fit to any of the binding models. The insignificant shifting ($\Delta\delta$ < 0.1 ppm) of the resonances corresponding to the N−H protons upon the addition of a DMSO-$d_6$ solution TBA acetate signifies that the two components only associate to a very small extent, at least in the early phase of the titration experiment. However, as the experiment progresses and 2−3 equiv of TBA acetate are added to the solution, the receptor seems to be reactivated, as evident by the sudden downfield shifting of the N−H signal. Clearly the hydrogen-bonding recognition site for the acetate anion is reconstructed in response to the excess guest species.

At saturation (>10 equiv of TBA acetate), the $^1$H NMR spectrum shows that the chemical shift of the N−H signal and the aromatic region of **1**·$H^+$ are similar to that of the neutral receptor **1**, also in the presence of excess guest. This implies that a host−guest complex (**1**·$H^+$)(TBA acetate)$_n$ has adopted a similar geometry to that of (**1**)(TBA acetate) where the ammonium is solvated by the excess acetate instead of forming an intramolecular interaction with the macrocycle. This does not, however, rule out the presence of dimers and oligomers at lower concentrations of acetate. In fact, the electrospray mass spectrometry studies of the protonated form (**1**·$H^+$)(TBA acetate)$_n$ showed peaks corresponding to both the monomer and the dimer in a 9:1 ratio. When more TBA acetate was added, this ratio remained unchanged even in the presence of up to 20 equiv of acetate.

The protonated form of the triazine receptor, **2**·$H^+$, did not show this substrate competition. Receptor **2** was prepared as outlined in Scheme 4 starting with cyanuric chloride and the same two amines as were used to prepare urea **1**. The ammonium form **2**·$H^+$ was prepared as its tetrafluoroborate salt by using $HBF_4$ in an identical fashion as already described for **1**·$H^+$.

**Scheme 4**




The association constant for the binding of receptor **2** to imide guests was estimated by titrating $CD_2Cl_2$ solutions of *n*-butyluracil with the receptor while monitoring the signals for the protons of the guest species in the $^1$H NMR spectra. In this case, the signal for the N−H proton of *N*-butyluracil could not be followed because it consistently disappeared after about 0.5 equiv of the receptor was added, although the significant downfield shifting ($\Delta\delta$ > 2 ppm) that occurred before the signal completely vanished clearly indicates effective host−guest association through hydrogen bonding. The disappearance of the N−H signal can be explained by the occurrence of rapid proton exchange between the N−H of the uracil substrate and the alkylamine of receptor **2** as they are expected to be compatible in an acid−base reaction. (The acidities of the ethylammonium cation (p$K_a$ = 10.8) and *N*-pentyluracil (p$K_a$ = 9.98) are similar.)[10] This argument is supported by a simple experiment. When 1 equiv of *n*-butylamine was added to a $CD_2Cl_2$ solution of *N*-butyluracil, the signal corresponding to the N−H proton of the latter species immediately disappeared. The presence of *n*-butylamine, however, had no observable effect on the chemical shifts of the methine C−H protons of *N*-butyluracil. The latter observation is significant because the small ($\Delta\delta$ ∼ 0.1 ppm) but still meaningful downfield shifting of signals corresponding to the C−H protons of the uracil provide a means to measure the association constant which was estimated to be 570 ± 100 $M^{-1}$ as an average of three runs (Figure 2). The data fit well to a 1:1 binding model.

This association constant corresponds well with that measured for the binding of *N*-butyluracil to diphenyltriazine ($K_a$ = 487 and 508 $M^{-1}$ when the signals for the N−H and C−H protons were followed, respectively). The fact that the signals for the N−H could be monitored throughout this last titration experiment supports our claim that the N−H in *N*-butyluracil is exchanging with the $NH_2$ of receptor **2** in an acid−base reaction. The association constant for the protonated form of the receptor, **2**·$H^+$, could only be estimated to be below 10 $M^{-1}$ due to the shallow curve which

(7) Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072.
(8) Fan, E.; Arman, A. V.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369.
(9) (a) Bordwell, F. G.; Algrim, D. J.; Harrelson, J. A., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 5903. (b) Valter, B.; Terekhova, M. I.; Petrov, E. S.; Stehlicek, J.; Sebenda, J. *Collect. Czech. Chem. Commun.* **1985**, *50*, 840.
(10) Hine, J.; Hwang, J. S.; Balasubramanian, V. *J. Org. Chem.* **1988**, *53*, 5145.

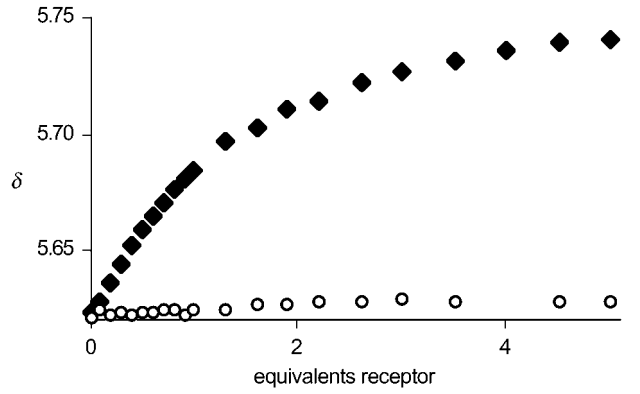



**Figure 2.** Observed chemical shift ($\delta$) of the C−H signal of *N*-butyluracil in the $^1$H NMR spectra when titrated with aliquots of receptor **2** (◆) and when titrated with **2**·H$^+$ (○) in $CD_2Cl_2$. [*N*-butyluracil] = $2 \times 10^{-3}$ M, [**2**] and [**2**·H$^+$] = $2 \times 10^{-2}$ M.

could not be fit to any binding model (Figure 2). The small movement of the C−H resonance of *N*-butyluracil ($\Delta\delta$ < 0.01 ppm) can be attributed to the hydrogen bonding of *N*-butyluracil to the substantially less effective donor−acceptor hydrogen bond sites in **2**·H$^+$. Despite the existence of two of these sites in the protonated compound, the reduced inherent binding affinity of recognition surfaces, presenting only two hydrogen bonds couples with the fact that these sites are projecting into the bulky *N*-butyl groups and prevents the effective docking of the imide substrate. It is the combination of these factors that are responsible for the substantially reduced efficacy of the protonated receptor.

The addition of excess *N*-butyluracil to receptor **2**·H$^+$ did not result in any increase in the extent of the changes in the $^1$H NMR spectra as was seen when **1**·H$^+$ was treated with excess TBA acetate. Clearly, **2**·H$^+$ is not reactivated by the presence of its substrate. We attribute this absence of substrate competition to three factors. First, because $CD_2Cl_2$ does not have the same ability to solvate ions as does DMSO, the unimolecular complexation equilibrium will lie heavily toward the intramolecularly associated ammonium ion and the crown ether. Second, the *N*-butyluracil is a neutral substrate and, therefore, cannot effectively solvate the ammonium ion even when it is in excess. Finally, the rotational barrier imposed on the two exocyclic C−N bonds on the triaminotriazine heterocycle in structures such as receptor **2** is smaller than that of the two C−N bonds in urea derivatives (such as found in receptor **1**). In fact, urea derivatives most often prefer to exist in their *trans-trans* conformations.[11] This is a geometry that is satisfied in the neutral form of the receptor (**1**) and not in its protonated state (**1**·H$^+$). The other conformations of ureas exhibit little, if any, affinity for carboxylate guests.

In conclusion, urea **1** and triazine **2** represent novel examples of proton-regulated receptors that are only active at high pH. The conformational changes that accompany the lowering of the pH govern the activity of the receptors.

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**Supporting Information Available:** Experimental procedures for the preparation of compounds **1** and **2** and $^1$H NMR spectra for **1**, (**1**·H$^+$), **2**, and (**2**·H$^+$). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Haushalter, A.; Lau, J.; Roberts, J. D. *J. Am. Chem. Soc.* **1996**, *118*, 8891.