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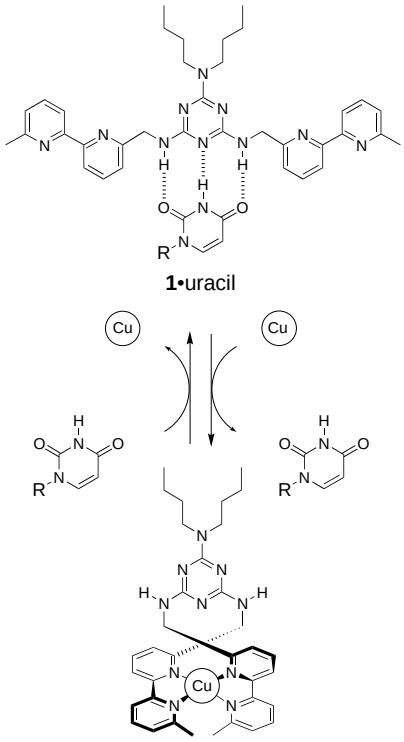


Scheme 1. Exchange of a substituted uracil and Cu^{I} in receptor **1**. R = C_4H_9.

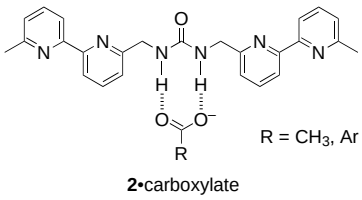
Metal Ions as Allosteric Inhibitors in Hydrogen-Bonding Receptors**

Mohammad H. Al-Sayah and Neil R. Branda*

Although there are many examples of artificial systems that display positive allostery,[1] where the induced conformational change increases the receptor's binding efficacy, there are few reports of simple negative allosteric receptors,[2] where the initial interaction with the cofactor results in a decrease in the receptor's binding affinity for its substrate. Of those that have been described, none, as far as we are aware, involve the destruction of well-ordered multipoint hydrogen-bond surfaces that are specific for biologically interesting guest species.

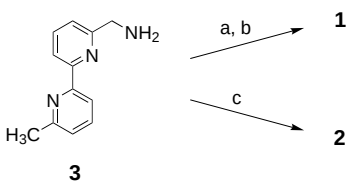
We report here how two novel receptors inhibit substrate binding in the presence of Cu^{I}. Receptor **1** is based on the triaminotriazine scaffold, which presents a donor–acceptor–donor hydrogen-bond surface well suited to act as a host for imide guests such as uracil (Scheme 1) and thymine.[3] When **1** is exposed to the metal ion, the two bipyridine arms swing towards each other and form a metal–ligand coordination compound. It is the Lewis acid that acts as the allosteric inhibitor, as its complexation to the chelating ligands forces two of the triazine's exocyclic C–N bonds to rotate, distorting the hydrogen-bonding surface. When the metal ion is extracted from the coordination pocket, the original hydrogen-bonding surface is reconstructed. This reactivates the receptor.

In a similar fashion, urea **2** presents a donor–donor hydrogen-bond recognition site for carboxylate guests.[4] It can exist in its active, natural form in the absence of Lewis acids, or it can be deactivated upon copper complexation.



Both receptors can be conveniently prepared from the readily available 6-aminomethyl-6′-methyl-2,2′-bipyridine (**3**)[5] as outlined in Scheme 2. All new species were characterized by multinuclear NMR spectroscopy, IR spectroscopy, and mass spectrometry.

The binding efficacy of receptors **1** and **2** to their substrates was evaluated by analyzing the changes that occurred in the



Scheme 2. Reagents and conditions: a) cyanuric chloride, Et_3N, THF, 72%; b) Bu_2NH, THF, 52%; c) 1,1′-carbonyldiimidazole, THF, 75%.

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[**] This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and by Research Corporation (Research Innovation Award).

^{1}H NMR spectra upon titrating solutions of *N*-butyluracil with **1**, and solutions of **2** with tetrabutylammonium acetate or tetrabutylammonium *p*-methylbenzoate. The significant downfield shift ($\Delta\delta > 2$) observed for the imide NH of uracil (2 mM in [D_8]toluene) as it was treated with aliquots of a solution of **1** (30 mM in [D_8]toluene) is indicative of effective hydrogen bonding. A similar downfield shift was observed for the urea NH in **2** (5 mM in [D_6]DMSO) when it was titrated with tetrabutylammonium acetate or tetrabutylammonium *p*-methylbenzoate (100 mM in [D_6]DMSO). Data sets from both experiments correlate well with calculated curves using 1:1 binding models,[6] and give association constants K_a of $600 \pm 10\,M^{-1}$ for **1**, $50 \pm 4\,M^{-1}$ for **2** with tetrabutylammonium acetate, and $55 \pm 2\,M^{-1}$ for **2** with tetrabutylammonium *p*-methylbenzoate. These values are in good agreement with those reported for similar host–guest complexes.[3, 4]

X-ray quality single crystals of **1** · Cu were isolated by slowly diffusing diethyl ether into a solution of the coordination compound in CH_3CN. The crystal structure[7] highlights the metal's role in the negative allosteric process (Figure 1). The

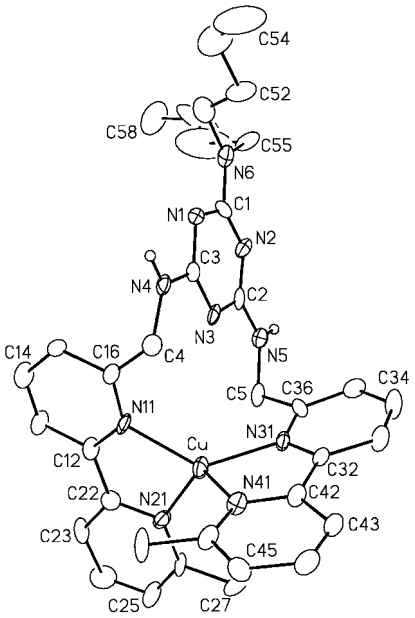

Figure 1. Molecular structure of **1** · Cu in the crystal. The PF_6^- counterion and all hydrogen atoms, except for those on N4 and N5, have been removed for clarity. The thermal ellipsoids are drawn at the 20% probability level.

C2–N5 and C3–N4 bonds must rotate by 180° from their original position in the active hydrogen-bonding receptor in order to form the copper complex. In the crystal structure, the torsional angles C4-N4-C3-N1 and C5-N5-C2-N2 are 175° and 179°, respectively. This reduces the recognition elements to two identical, but weakly bonding, donor–acceptor hydrogen-bond surfaces (N1, H(N4) and N2, H(N5)).

The ability of the metal to shut down the recognition process for both **1** and **2** was evaluated by repeating the titration experiments, but replacing the active receptors with preformed complexes **1** · Cu and **2** · Cu. The solvent was changed to $CD_3CN/CDCl_3$ in the case of **1** · Cu to ensure solubility of all components. The insignificant change ($\Delta\delta < 0.1$) in the chemical shift of the imide NH of uracil upon the addition of a solution of **1** · Cu in $CD_3CN/CDCl_3$ (1/1) signifies the existence of only very weak hydrogen bonds (Figure 2).[8] This small movement of the chemical shifts can be attributed to the association of uracil to the less effective

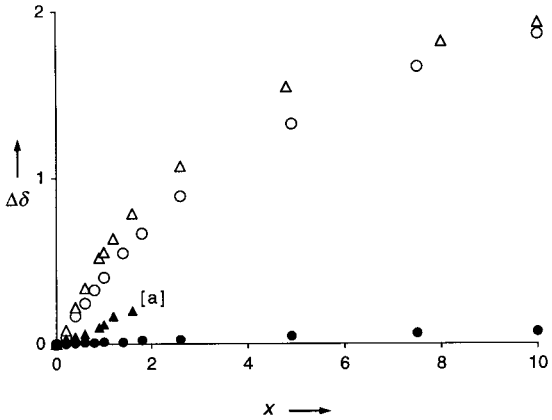

Figure 2. Titration of *N*-butyluracil with **1** (○) and **1** · Cu (●) in $CD_3CN/CDCl_3$ (1/1), and of **2** with tetrabutylammonium acetate (△) and **2** · Cu with tetrabutylammonium acetate (▲) in [D_6]DMSO, as followed by ^{1}H NMR spectroscopy. The observed changes in chemical shift ($\Delta\delta$) are plotted against equivalents of titrant (x). The symbol [a] indicates a peak that broadens into the baseline.

donor–acceptor sites in **1** · Cu. Despite the existence of two of these sites in the coordination compound, the reduced inherent binding affinity of recognition surfaces presenting only two hydrogen bonds couples with the presence of the *N*-butyl groups to prevent the facile approach of the substrate. These factors produce a significantly less effective receptor.

The metal-induced allosteric inhibition is also effective when the coordination compound **1** · Cu is prepared in situ, again diagnosed by the relative position of the chemical shift of the imide NH in the ^{1}H NMR spectra. The addition of one equivalent of **1** to a solution of *N*-butyluracil in $CD_3CN/CDCl_3$ (1/1) produces an immediate downfield shift of the signal for this hydrogen atom ($\Delta\delta = 0.4$). This signal returns to its original position when $[Cu(CH_3CN)_4](PF_6)$ is added directly to the NMR sample. In the aromatic region of the spectrum, peaks that correspond to those for the isolated **1** · Cu complex in this solvent replace all original signals. Subsequent addition of an excess of the stronger coordinating ligand neocuproine (2,9-dimethyl-1,10-phenanthroline, dmph)[1a, 9] results in the immediate disappearance of these signals and the regeneration of those corresponding to the hydrogen-bonded **1** · uracil complex.

A similar trend is observed for the NH protons of preformed **2** · Cu when this complex is titrated with a solution of tetrabutylammonium acetate in [D_6]DMSO (Figure 2). In this case, signals for the urea NH could not be followed throughout the entire concentration range of the titration, as they quickly broadened to become indistinguishable from the baseline. The observed trend up to the addition of 1.6 equiv of substrate, however, proves successful metal-induced allosteric inhibition. Another complication was apparent when more than one equivalent of the carboxylate was added. At this molar ratio, signals corresponding to the hydrogen-bonded

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2 · acetate[10] complex began to appear and became significantly more intense as the concentration of tetrabutylammonium acetate was increased. This observation illustrates a competition between the complexation of Cu^{I} to the bipyridine ligands and the binding of acetate for the hydrogen-bond surface in **2**.

The addition of 0.5 equiv of tetrabutylammonium acetate to a solution of **2** in [D_6]DMSO produces a small but significant downfield shift for the urea NH proton ($\Delta\delta =$ 0.2), indicative of hydrogen bonding. When 1 molar equiv of [$Cu(CH_3CN)_4$](PF_6) is added directly to the NMR sample, **2** · Cu is immediately generated at the expense of **2** · acetate, and the signal for the urea NH returns to the original position for **2**. Unlike in the case of **1**, the NH signal does not shift significantly downfield when the sample is treated with an excess of neocuproine. This can be attributed to the preference of the acetate ion to act as a counterion for the harder copper cation and not the softer tetrabutylammonium cation. This favorable ion pairing effectively prevents the acetate from acting as a guest for receptor **2**. This phenomenon was also observed for samples of the noncoordinating *N*,*N*′-dimethylurea when they were treated under identical conditions.

Experimental Section

1: Receptor **1** was prepared in two steps from **3**, cyanuric chloride, and di-*n*-butylamine following an adapted procedure of Whitesides et al.[11] (37% overall yield): m.p. 88–90 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 8.23 (t, $J = 7.8$ Hz, 4H), 7.62–7.73 (m, 4H), 7.26 (d, $J = 7.8$ Hz, 2H), 7.11 (d, $J = 7.5$ Hz, 2H), 5.99 (br s, 2H), 4.74 (d, $J = 5.7$ Hz, 4H), 3.42 (t, $J = 7.5$ Hz, 4H), 2.60 (s, 6H), 1.47 (br s, 4H), 1.23 (br s, 4H), 0.83 (br s, 6H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta =$ 157.9, 155.8, 155.5, 137.4, 137.1, 124.3, 123.3, 121.2, 119.4, 118.4, 46.8, 46.2, 30.1, 24.7, 20.2, 14.0 (16 of 18 carbon atoms); MS-EI: *m*/*z*: 602.36 [M^+].

2: A solution of **3** (0.12 g, 0.6 mmol) in dry THF (40 mL) was added dropwise to a solution of 1,1′-carbonyldiimidazole (0.049 g, 0.3 mmol) under argon and stirred overnight. Evaporation and trituration of the residue in ethyl acetate and then in water afforded **2** as a white solid (95 mg, 75%): m.p. 240–242 °C (decomp.); ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta =$ 8.23 (t, $J = 7.2$ Hz, 4H), 7.85 (t, $J = 7.8$ Hz, 2H), 7.79 (t, $J = 7.8$ Hz, 2H), 7.33 (d, $J = 7.5$ Hz, 2H), 7.28 (d, $J = 7.5$ Hz, 2H), 6.89 (t, $J = 3.0$ Hz, 2H), 4.43 (d, $J = 6.0$ Hz, 4H), 2.60 (s, 6H); ^{13}C NMR (100.5 MHz, [D_6]DMSO): $\delta =$ 189.2, 159.3, 158.2, 157.4, 154.5, 137.6, 137.3, 123.4, 121.0, 118.4, 117,.6 45.1, 24.2; MS (ES, positive ion): *m*/*z*: 424.19 [M^+].

1 · Cu and **2** · Cu: The Cu^{I} complexes were prepared by stirring a mixture of **1** or **2** (1.03 equiv), [$Cu(CH_3CN)_4$](PF_6) (1 equiv), and CH_3CN for 1 h under argon. Filtration and evaporation afforded the copper complexes as red solids in quantitative yield.

1 · Cu: ^{1}H NMR (300 MHz, CD_3CN): $\delta = 8.270$ (d, $J = 7.8$ Hz, 2H), 8.233 (d, $J = 8.1$ Hz, 2H), 8.057 (t, $J = 8.1$ Hz, 2H), 8.025 (t, $J = 7.8$ Hz, 2H), 7.619 (d, $J = 7.8$ Hz, 2H), 7.446 (d, $J = 7.5$ Hz, 2H), 6.127 (br s, 2H), 4.657 (d, $J = 5.7$, 1H), 4.607 (d, $J = 5.7$ Hz, 1H), 4.090 (d, $J = 6.3$ Hz, 1H), 4.040 (d, $J =$ 6.3 Hz, 1H), 3.511–3.415 (m, 2H), 3.269–3.171 (m, 2H), 2.104 (s, 6H), 1.476–1.377 (m, 4H), 1.253–1.154 (m, 4H), 0.878 9t, $J = 7.2$ Hz, 6H); ^{13}C NMR (100.5 MHz, CD_3CN): δ(17 of 18 carbon atoms) = 167.0, 162.0, 158.5, 152.6, 151.9, 139.5, 139.3, 126.8, 125.4, 121.5, 120.4, 49.6, 46.6, 30.7, 24.9, 20.8, 14.2; UV/Vis (CH_3CN): $\lambda_{max}(\varepsilon)$: 455 nm (3900); MS (ES, positive ion): *m*/*z*: 665.29 [M^+].

2 · Cu: ^{1}H NMR (300 MHz, CD_3CN): $\delta = 8.278$ (d, $J = 7.8$ Hz, 2H), 8.220 (d, $J = 8.1$ Hz, 2H), 8.110 (br s, 2H), 7.997 (t, $J = 7.8$ Hz, 2H), 7.677 (br s, 2H), 7.448 (d, $J = 7.8$ Hz, 2H), 6.308 (br s, 2H), 4.318 (br s, 2H), 3893 (br s, 2H), 2.077 (s, 6H); ^{13}C NMR (100.5 MHz, CD_3CN): $\delta = 159.9$, 158.9, 158.6, 152.7, 152.3, 140.2, 139.5, 127.0, 124.8, 121.9, 120.7, 47.4, 25.2; UV/Vis (CH_3CN): $\lambda_{max}(\varepsilon)$: 448 nm (3900); MS (ES, positive ion): *m*/*z*: 487.13 [M^+].

Received: July 26, 1999 [Z13779]

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[6] The data were analyzed by nonlinear regression using Craig Wilcox's HOSTEST program (University of Pittsburgh, Pittsburgh, PA, USA). All titrations were performed in triplicate.

[7] Crystal data for **1** · Cu ($C_{35}H_{46}CuF_6N_{10}OP$): $M_r = 831.33$, triclinic, space group $P\bar{1}$, $a = 14.278(2)$, $b = 15.202(2)$, $c = 20.190(3)$ Å, $\alpha = 70.922(4)$, $\beta = 78.889(4)$, $\gamma = 69.360(3)^\circ$, $V = 3862.5(10)$ Å^3, $Z = 4$, $\rho_{calcd} =$ 1.430 g cm^{-1}, $\mu = 0.679$ mm^{-1}, crystal dimensions $0.37 \times 0.14 \times$ 0.02 mm, $T = 193$ K; of 14674 unique reflections, 2803 were observed ($F_o^2 \geq 2\sigma(F_o^2)$], $R_1 = 0.0763$, $wR_2 = 0.1869$, $S = 0.753$, largest difference peak and hole 0.558 and -0.584 e Å^{-3}, Bruker P4/RA/SMART 1000 CCD diffractometer, graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda =$ 0.71073 Å). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-133769. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[8] Titration experiments of *N*-butyluracil with **1** were repeated in $CD_3CN/CDCl_3$ (1/1), giving $K_a = 50$ M^{-1}.

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