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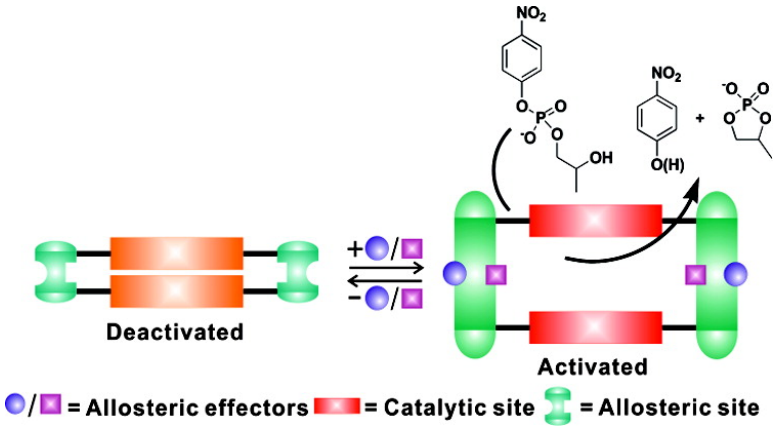

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Allosteric Regulation of Phosphate Diester Transesterification Based upon a Dinuclear Zinc Catalyst Assembled via the Weak-Link Approach

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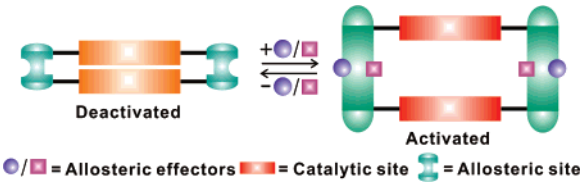
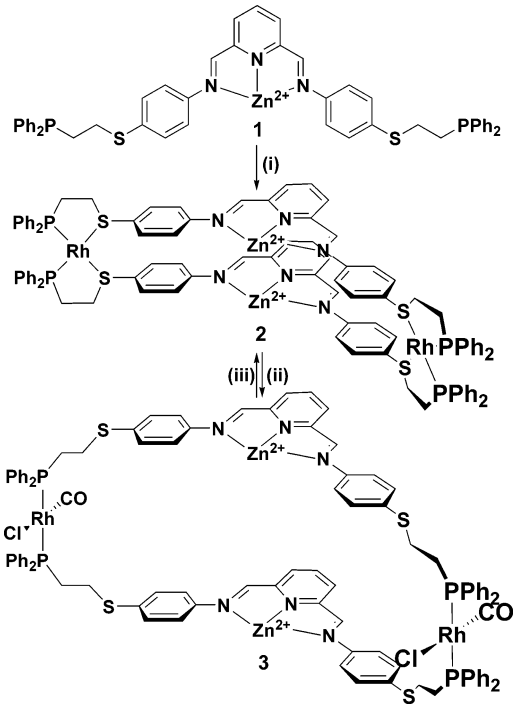
Phosphate diesters are key structural components in nucleic acids, and nature has evolved sophisticated chemical pathways for controlling their hydrolysis in important biomolecules like DNA and RNA. Under neutral or basic conditions in the absence of enzymatic catalysts, these structures are extraordinarily stable, exhibiting half-lives on the >100 year time scale.[1,2] In order to use such structures, nature relies on transition-metal-containing enzymes that facilitate hydrolysis.[2] Many of these enzymes, including P1 nuclease, DNA polymerase I, phospholipase C, and alkaline phosphatase, rely on the synergistic action of two metal centers (typically Zn^{2+}) to promote the hydrolytic cleavage of the phosphate diester bonds.[3] Several research groups have attempted to mimic this process with simple compounds in which two metal centers have been linked by a spacer group.[4] From these studies, it appears that dinuclear complexes are typically more reactive than their mononuclear counterparts. However, although various organometallic systems for phosphate ester hydrolysis have been developed and studied, including unidirectional "turn-on" systems that resemble zinc finger proteins,[4d] complexes that provide completely reversible allosteric regulation do not yet exist.

Recently, our research has focused on utilizing the concept of reversible allosteric regulation[5] in coordination complexes formed via the weak-link approach (WLA).[6] The WLA, which builds upon the growing number of methods for preparing metal-containing macrocyclic complexes,[7] allows one to synthesize structures through coordination chemistry and hemilabile ligands with transition-metal-based regulatory sites that can be modulated through ligand displacement reactions. In this manner, one can turn on or off a catalytic event depending upon the presence or absence of small molecules or elemental anion effectors (Scheme 1). We have utilized this concept to create structures that offer amplification in chemical detection where the effector molecule is the analyte and the catalytic reaction turned on by the effector generates a fluorescent surrogate molecule that provides the signal output associated with recognition.[5] One of the drawbacks of all previously studied systems is that they are not perfect turn-on/turn-off systems. Indeed, the off states often exhibit small but measurable background catalysis. In the context of chemical sensing, this limitation leads to a background signal that raises the lower limit of analyte detection. Moreover, the catalysts studied thus far are typically sluggish, which limits the response time of any chemical detection system based upon them. Therefore, a new allosteric system that can be turned on and completely off with rapid catalytic activity would be a significant advance in this field. Herein, we report a novel tetrametallic system, **2** (Scheme 2), assembled via the WLA that behaves as an efficient and completely reversible allosteric modulator for the hydrolysis of 2-(hydroxypropyl)-*p*-nitrophenyl phosphate (HPNP), a model substrate for RNA.[8] Most importantly, the structural changes induced by small molecule regulators Cl^- and

Scheme 1. Supramolecular Allosteric Regulation via the WLA



Scheme 2. Synthesis of the Supramolecular Allosteric Catalyst[a]



[a] The OAc^- ligands and counterions for the complexes are omitted for clarity. Reagents and solvents: (i) $Rh(norbornadiene)_2BF_4$, CH_2Cl_2; (ii) tetrabutylammonium chloride/CO, CD_2Cl_2; (iii) N_2 bubbling or addition of 2 equiv of $AgBF_4$.

CO transition this system from a catalytically inactive state to a very active structure in a highly reversible fashion.

Complex **2** was targeted with the idea that the effector molecules could be used to control the distance between the Zn sites, which in turn could affect the ability for the structure to catalyze hydrolysis of HPNP (Scheme 2). In order to prepare target complex **2**, we first synthesized hemilabile ligand **1**. Ligand **1** can be made in 93% overall yield from 4-(2-diphenylphosphanylethylthio)phenylamine.[9] Upon introduction of 1 equiv of ligand **1** to 1 equiv of a Rh(I) precursor, target compound **2** assembles in 92% isolated yield. Compound **2** has been characterized in solution by ^1H and $^{31}P\{^1H\}$ NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS), and all data are consistent with the proposed

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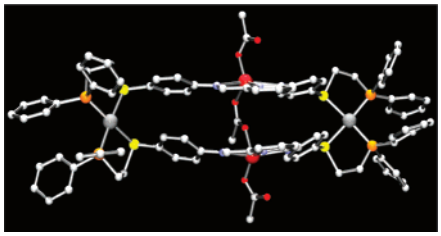

Figure 1. X-ray crystal structure of compound **2**. The crystal was grown from degassed CD_2Cl_2 and pentane. Hydrogen atoms, solvent molecules, and free counterions are omitted for clarity. Color labeling scheme is as follows: Rh (gray), Zn (red), P (orange), S (yellow), O (light red), N (blue), C (light gray).

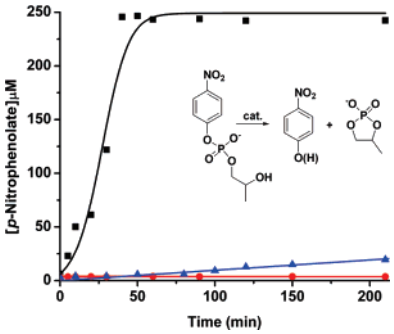

Figure 2. Product (*p*-nitrophenolate) concentration versus time for condensed **2** (●) and open **3** (■) macrocyclic compounds. A control experiment with compound **1** (▲) was carried out under the same reaction conditions. Reactions were monitored by UV−vis spectroscopy.

highly symmetric structural formulation. The $^{31}P\{^1H\}$ NMR spectrum exhibits a single doublet at 65.6 ppm with the diagnostic J_{Rh-P} coupling constant of 160 Hz.[5,6]

The solid-state structure of compound **2** has been determined by a single-crystal X-ray diffraction study (Figure 1). The crystal structure shows that the Rh(I) centers in **2** exhibit square planar geometries with S−Rh−S and P−Rh−P angles of 90.07(1) and 98.78(1)°, respectively. The Zn···Zn distance is 4.514 Å, and the Rh···Rh distance is 16.530 Å. The proximity of the Zn atoms precludes the possibility of an intramolecularly catalyzed HPNP hydrolysis reaction. In addition, the X-ray structure of **2** shows that an acetate counteranion bridges the two zinc atoms in a μ_2-fashion, further inhibiting the ability for the HPNP to bind to the active site, which will inhibit intermolecular catalysis.

When 2 equiv of Cl^- and CO (1 atm) is added to a CD_2Cl_2 solution of compound **2**, open complex **3** rapidly forms.[6c] Macrocycle **3** was characterized in solution by ^1H and $^{31}P\{^1H\}$ NMR spectroscopy and ESI-MS (see Supporting Information).

The catalytic properties of ligand **1**, closed compound **2**, and open compound **3** were evaluated in the context of HPNP hydrolysis. Aliquots of HPNP dissolved in a solution of methanol/water (3:1) and CH_2Cl_2 solutions of each catalyst (0.5 mol %) were added to reaction vessels. The formation of the hydrolysis product, *p*-nitrophenolate, was studied by UV−vis spectroscopy (λ_{max} of *p*-nitrophenolate = 392 nm) as a function of time (Figure 2).[10] There are several notable observations made from this study. The first is that, although ligand **1** exhibits slow but measurable catalytic hydrolysis activity, closed compound **2**, which is assembled from ligand **1**, is completely inactive under identical conditions. Second, open complex **3** is extremely active and capable of quantitatively hydrolyzing all of the HPNP substrate in less than 40 min.[11] Significantly, we demonstrated that one could start with complex **2** and add *n*-Bu_4NCl under CO atmosphere to trigger the reaction to form **3** with subsequent hydrolysis in situ (see Supporting Information for details). Simply bubbling N_2 into the solution results in the reformation of **2** and the generation of an inactive catalyst.

In summary, this work presents the synthesis and characterization of an allosteric catalyst for the cleavage of HPNP. This structure can be efficiently interconverted between the on and totally off states through the use of small molecule regulators that change the size of the macrocycle and the accessibility to the active binuclear Zn site. Interestingly and unexpectedly, the bridging acetate in the closed binuclear complex **2** seems to prohibit the intermolecular catalytic pathway, thus resulting in no background catalytic activity (in the off state) and making it the first system capable of completely reversible allosteric control. In addition, the system is the first prepared via the WLA to work in pseudo-aqueous conditions (mixed solvent), a requisite for transitioning such systems to functional analyte detection strategies that take advantage of catalytic amplification.[5] The attractive feature of identifying an allosteric system that can be turned from the completely off to on state with good turnover and catalytic rates is that it can become a central amplification motif used for many future systems where the regulatory sites are designed to recognize different analytes with comparable amplification capabilities.

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Supporting Information Available: Experimental procedures, spectral data for all new compounds, catalytic reaction monitoring procedures (PDF), and X-ray data for **2** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The rest of the product was observed as *p*-nitrophenol by UV−vis spectroscopy after completion of the reaction (see Supporting Information for details).

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