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A New Heterotopic Allostere With Low Pre-Organization

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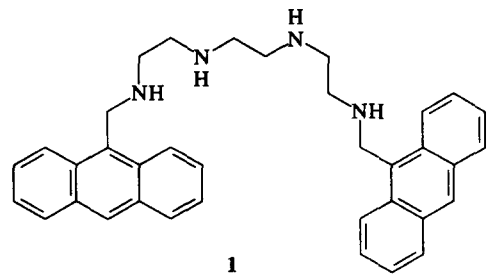
A new molecule based on a branched polyamine is described that exhibits Zn(II) dependence for binding a second, fluorescent substrate in a hydrophobic binding site. © 1997 Elsevier Science Ltd.

A wide variety of tasks that are yet to be approached by synthetic models can be easily accomplished in biosystems using the impetus of the non-covalent bond. How enzymes bind and transform specific substrates has been the central issue that spawned the field of molecular recognition. The recognition event has become and continues to be one of the field's most active areas of research.[1] One important aspect of this field is to answer questions about how molecular conformation can be controlled using these non-covalent bonds[2] as enzymes do in folding or under allosteric regulation.

One non-covalent binding interaction that has been exploited in water is ionophore complexation of metals.[3] This chemistry is dominated by the use of macrocyclic polyethers and polyamines primarily because of the large binding affinities for metal ions (relative to their open-chained counterparts). This results from preorganization (the macrocyclic effect)[4] and complementarity of shape and size to that of the ion. Nonetheless, the template effect[5] on the synthesis of some of these macrocycles suggests that the binding affinity of the open-chain species is strong enough to do useful work on the molecular scale. A few aqueous allosteric or self assembling systems have been designed on this principle.[6] Linear polyamines are well known to bind transition metal ions in aqueous solution. We intend to use linear polyamine based hosts to determine the limits of conformational mobility that will still result in a significant allosteric effect.

Enzymes use non-covalent interactions to organize themselves in aqueous media. It is our intent to use non-covalent interactions to organize molecules in specific conformations that will perform a task under aqueous conditions. We have synthesized a series of terminally modified linear polyamines as described earlier.[7] Upon titration of N1,N4-bis(9-anthryl,ethyl)triethylenetetramine (**1**) with Zn2+ an enhancement of excimer fluorescence is observed.[8] This result is likely attributable to a conformational change for the polyamine, in aqueous solution, resulting in proximity of the aromatic ends of the ligand. (**Figure 1**, derived by CAChe molecular modeling software).



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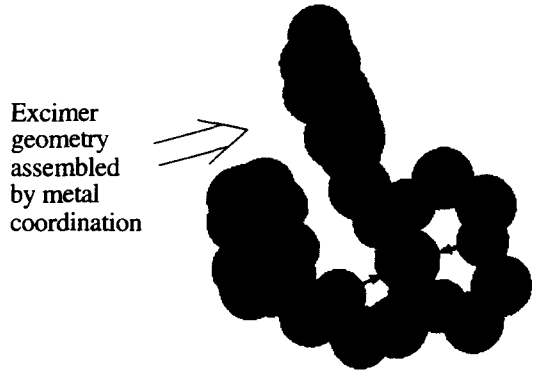
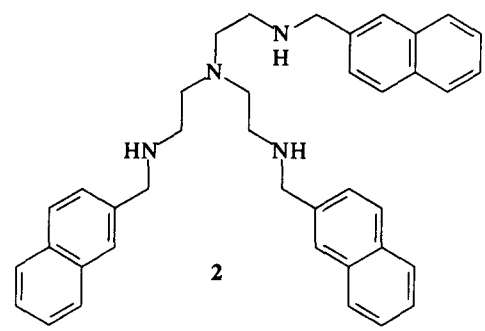


Figure 1

Complexes that regulate biological processes, such as recognition of a natural substrate or of a drug, rely on a combination of electrostatic and lipophilic interactions for their formation. In enzymology it has long been understood that effector molecules binding in zones remote to the active site can have profound effects on the conformation of the protein. These conformational changes can activate or deactivate the enzyme toward action on the substrate. The study of designed molecules that are much less complex than biological hosts (e.g., proteins) can increase understanding of the activities of the more complicated biological macromolecules or provide us with synthetically accessible means of performing similar functions. Remote binding dependence is one such process that has been modeled by synthetic molecules.[9] Except for Cole's,[6a] Nolte's,[6d] and Schneider's[6b,c] systems these models do not display positive heterotopic cooperativity between ionic and hydrophobic binding sites in one molecule.

The excimer observed for (**1**) in the presence of Zn^{2+} implies that this extent of conformational control is possible in order to form a second hydrophobic binding cavity. With this strategy in mind we have prepared (**2, TNMTAEA**) as a potential heterotopic allostere dependent on metal ions in solution for formation of the hydrophobic binding cleft. The host can be synthesized in two steps using a reductive amination sequence reported previously.[10]



2

In order to test for cooperative binding we employed the well established fluorescent probe 6-(p-toluidino)-2-naphthalenesulfonic acid (TNS). The fluorescence intensity of TNS is known to be dependent on the polarity of its environment.[6ac,11] Solutions were prepared in 0.05 **M** pH 10.5 CAPS buffer containing various combinations of host (**2**) (25 μ**M**), TNS (2.0 μ**M**) and the non-quenching metal ion Zn^{2+} (25 μ**M**). Samples were excited at 360 nm and emission was measured over the range including λ_{max} for TNS in non-aqueous solution (**Figure 2**). The TNS, when in solution with host and Zn^{2+} shows a fluorescence increase of approximately 25-fold relative to the fluorescence of TNS in buffer solution. This indicates that the TNS resides in a hydrophobic region formed by the host in the presence of Zn^{2+} ion. Interestingly, the TNS, when in solution with host but NOT Zn^{2+} shows a small (~5-fold) increase in fluorescence relative to the fluorescence of TNS in buffer solution. The increase in TNS fluorescence with host and Zn^{2+} *vs* that with host only is approximately 3-fold. An equilibrium between a TNS binding state and a TNS non-binding state for the host system can explain these obervations. In aqueous solution the TNS binding state may form to a small extent

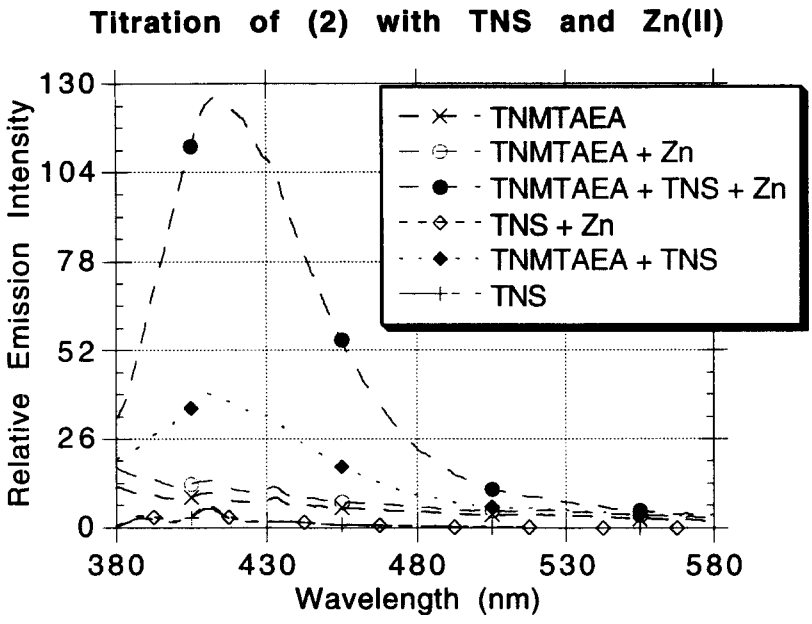


Figure 2

because of the potential hydrophobic interactions between naphthalene units in the same host molecule. The addition of the Zn^{2+} ion favors the secondary binding state (**Figure 3** derived by CAChe molecular modeling software) as a result of the primary binding interaction between the ion and the polyamine.

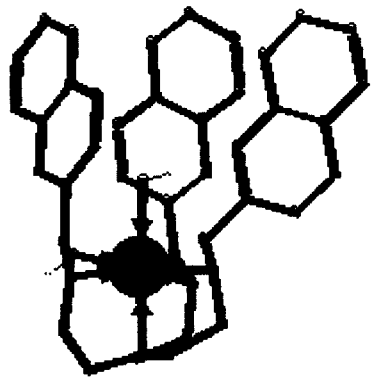


Figure 3

We are currently concentrating on the synthesis of a series of hosts with similar paradigms of geometry with the intent of undergoing the same type of study. The potential for inducing stronger affinity or a more hydrophobic secondary binding site with other metal ions will be examined as will the possibility of decreasing affinity for the fluorescence probe by favoring the non-binding state upon alternative metal ion complexation.

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