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A New Water Soluble Cyclophane Host That Is Organized by Calcium Binding

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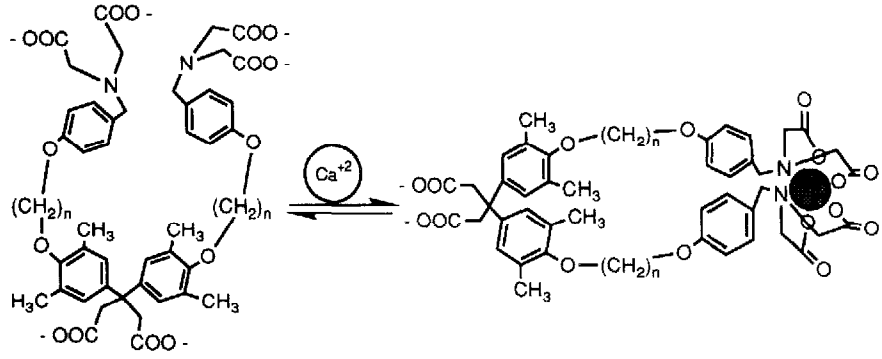
Abstract: Two new water soluble cyclophanes **1a** and **1b** were synthesized. The allosteric regulation of the host-guest interactions was studied by fluorescence spectroscopy.

The allosteric control of binding site conformation is one of the most common regulatory motifs in biology.[2] In recent years several recognition systems have been developed that demonstrate allosteric regulation.[3] We wish to communicate the synthesis and initial studies of a new system that forms a cyclophane-like host when the two terminal aminodicarboxylate groups converge to form the preferred octahedral complex in the presence of Ca^{+2} (scheme 1).

Scheme 1



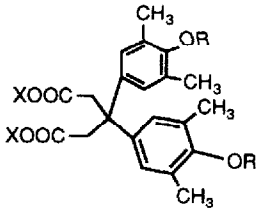
1a n=3
1b n=4

The concept we are interested in developing is the efficient organization of hydrophobic binding sites in aqueous medium. The design chosen for **1**, through CPK model examination, places the cation binding site external to the cyclophane portion of the molecule. Upon cation complexation a binding site is formed that is complementary to aromatic guests so that all the hydrated charges are arranged on the exterior of the cavity. In this manner the two binding sites exist in a symbiotic relationship.

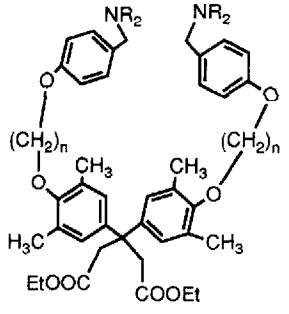
SYNTHESIS OF HOSTS **1a** and **1b**

The synthetic plan revolved around the selective reduction of a nitrile in the presence of an ester to the corresponding benzyl amine. The first new compound synthesized was diiodide **3a** , from the known diphenol **2** via reaction with bromochloropropane (acetone/K_2CO_3) and treatment with NaI in acetone.[4] Dibromide **3b** was synthesized by reaction of **2** with an excess of dibromobutane (acetone/K_2CO_3). The halide was displaced in both compounds with 4-cyanophenol (acetone/K_2CO_3) and the key hydrogenation was most successfully completed with platinum(VI) oxide[5] in EtOH with a few milliliters of chloroform added to give the hydrochlorides of benzyl amines **4a** and **4b**.

The amines were reacted with methyl bromoacetate in the presence of Proton Sponge® to give tetramethyl esters **5a** and **5b**. The esters were saponified (MeOH/NaOH) to give hosts **1a** and **1b**. These acids were isolated as disodium salts as confirmed by FAB mass spectrometry. Model compound **6** was obtained by treatment of **2** with iodomethane in DMF and subsequent saponification[6] of the resulting ester.



2 R=H, X= Et
3a R= $(CH_2)_3I$, X= Et
3b R= $(CH_2)_4Br$, X= Et
6 R= CH_3, X= H



4a n=3, R=H **5a** n=3, R= CH_2COOCH_3
4b n=4, R=H **5b** n=4, R= CH_2COOCH_3

INITIAL BINDING STUDIES

A series of studies were conducted that used 6-(toluidino)-2-naphthalenesulfonic acid (TNS) as a fluorescent probe (Figure 1). It has been demonstrated previously that the fluorescence intensity of TNS is very small in aqueous solution but increases markedly upon enclosure in a hydrophobic cavity.[7] The first experiments with our system involved monitoring the intensity of TNS fluorescence as a function of both calcium and host concentrations; the results for host **1a** are presented in Figure 1. Compound **6**, which cannot assemble a cyclophane type binding site in the presence of calcium ion was used in this study as a control. At pH 9.5, and in the absence of calcium, the observed TNS fluorescence of **1a** and **6** were quite similar (note the small emission at 410 nm due to water Raman scattering). However in 5 mM calcium chloride the fluorescence intensity increased by a factor of 6 for **1a** but remained unchanged for **6**. The increase in TNS fluorescence intensity was reversed upon the addition of a strong calcium chelating agent such as ethylene diamine tetraacetic acid (EDTA). If any components (**1a**, calcium chloride) were absent the TNS fluorescence was negligible.

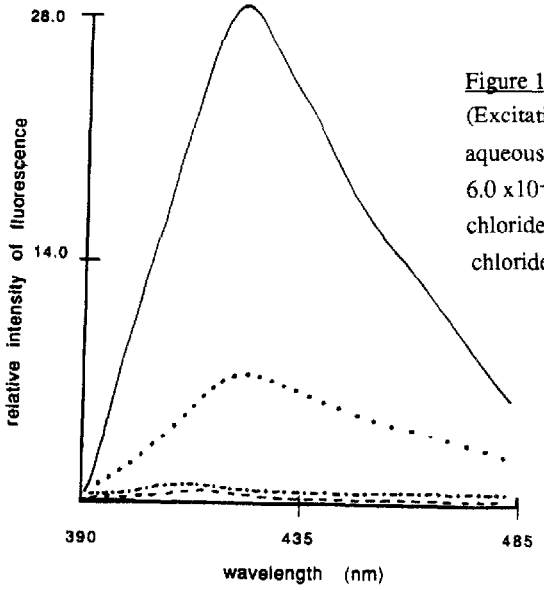


Figure 1: Fluorescence spectrum of 2.0 x 10^{-6} M TNS (Excitation λ=360 nm,Emission λ = 420 nm) in a pH 9.5 aqueous solution upon the addition of 6.0 x10^{-5} M of **1a** (-----), 6.0 x10^{-5} M **6** (-·-·-), 6.0 x10^{-5} M of **1a** with 5 mM calcium chloride (·····), and 6.0 x10^{-5} M of **1b** with 10 mM calcium chloride (——).

We believe that the observed changes in fluorescence intensity are due to the formation of an inclusion complex that occurs when **1a** binds calcium at the distal end of the molecule and forms a hydrophobic cavity. However, the observed changes in TNS fluorescence intensity in the presence of **1a** are not large when compared to other cyclophane hosts that are known to completely enclose TNS[8], indicating that the cavity of **1a**·Ca^{+2} is too small for TNS. If this is true then **1b** (n =4) should show an increased affinity for TNS due to the larger cavity size of this host. As predicted, the fluorescence intensity is markedly higher upon addition of **1b**·Ca^{+2}. This spectrum is included in figure 1 and shows a fourfold increase in intensity compared to the emission in the presence of **1a**·Ca^{+2}. The spectrum shows both an increased fluorescent quantum yield and a substantial blue shift in the emission spectra of TNS from λ = 500 nm in pure water to λ = 420 nm. These results agree with the premise that the **1b**·Ca^{+2} complex forms a larger cavity that more completely encloses the TNS molecule. The large increase in TNS fluorescence intensity upon the introduction of calcium demonstrates a large degree of cooperativity. Unfortunately, the limited solubility of the complexes precluded NMR titration studies[9] that would enable us to unambiguously determine the structure of the host **1b**·Ca^{+2}·TNS complex.

A Benesi-Hildebrand[10] determination of the TNS binding constant in 10 mM calcium chloride was completed for **1b** giving a value of K_a = 5.0 x 10^3 M^{-1}. The plot was linear throughout the entire region of the titration, but we feel there is a large uncertainty in these results due to contributions from multiple equilibria. We began this study with the assumption that the calcium affinity of **1b** would be very large (K_a >10^8), and that under under our experimental conditions all the host molecules would be organized through calcium binding. We found instead that the TNS fluorescence intensity is dependent upon the concentrations of Ca^{+2} and **1a** until we reached 5 mM calcium chloride. When the same experiment was performed using **1b** the saturation concentration of calcium chloride was found to be 10 mM. It appears that both hosts have modest

calcium affinity, but the smaller system **1a** is a slightly better calcium binder. We have not corrected these results for any contribution from the TNS binding component of the equilibrium, so the differences in calcium affinity could be somewhat larger than these results indicate.

At high calcium chloride concentration (> 20mM) the fluorescence intensity of TNS begins to decrease. We postulate that at very high calcium ion concentration a different mode of calcium binding, possibly a bis-calcium complex, begins to become significant and the percentage of molecules that are organized into a hydrophobic binding site decreases.[11]

We are presently working on methods to determine binding constants for both calcium and TNS in the presence and absence of the other, comparing the effectiveness of different cations as mediators, and constructing modified hosts with increased solubility. We feel the results communicated here are a good first step towards the development of a new class of cation mediated hosts.

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References and Notes

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9. Solubility was determined by right angle scattering at λ= 290 nm. The experiments were done at pH 9.5 and at 5 mM calcium chloride on a Spex Fluorolog 1680 spectrofluorimeter. The intensity, indicating aggregation, began to become significant at 1.1 X 10^{-4} M for both hosts. Without calcium present, both hosts show very good solubility, and the presence of TNS did not change the light scattering results.
10. Benesi, H; Hildebrand, J.H. J. Am. Chem. Soc., **1949**, 71, 2703. The titration was conducted at 298 ± 0.2 °K : [Ca+2] = 10 mM, [TNS] = 0.002 mM, [**1b**] = 0.0056, 0.020, 0.031, 0.052, 0.070, 0.082, and 0.103 mM in pH 9.5 tris buffer.
11. An alternative explanation suggested by the referee is intersystem crossing at heavy ion concentration leading to a fluorescence quenching mechanism.

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