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Supplementary Material Available: Crystal structure determination data for neristatin 1 including coordinates, bond lengths, bond angles, and coefficients (9 pages). Ordering information is given on any current masthead page.

A Molecular Clip with Allosteric Binding Properties

Rint P. Sijbesma and Roeland J. M. Nolte*

Department of Organic Chemistry
University of Nijmegen
Toernooiveld, 6525 ED Nijmegen
The Netherlands

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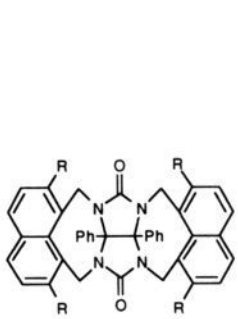
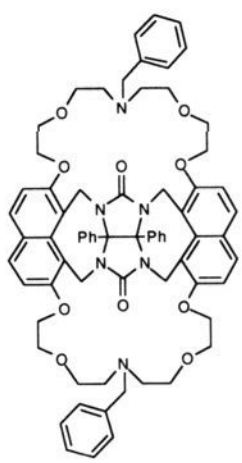
In allosteric receptors the binding of one substrate is influenced by the binding of a second substrate at a remote site. Allosteric effects play an important role in regulating biological processes like oxygen transport and enzyme activity.[1] There are only a few reports in literature on synthetic receptors in which binding is regulated by the allosteric effect. Most of these reports concern hosts with binding sites for metal ions.[2] Very recently, Schneider and Ruf have described a synthetic receptor which shows enhanced binding of aromatic substrates in the presence of Cu^{2+} or Zn^{2+}, due to an allosteric effect.[3] We report herein a molecular receptor (**1**), that can exist in different conformations, one of which is able to bind 1,3-dinitrobenzene by $\pi-\pi$ interactions. Moreover, we describe a bis-crown ether analogue of this receptor (**2**) in which a dinitrobenzene-binding conformation can be induced by addition of a metal salt (Figure 1a).



1a R = H
1b R = OAc
1c R = OMe



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Compound **1b** exists in $CDCl_3$ solution as a mixture of conformers, that interconvert slowly on the NMR time scale. This process could be monitored with a two-dimensional ^1H NMR exchange experiment.[4] Inspection of CPK models and molecular mechanics calculations[5] on **1a** suggest that compounds **1** can exist in three conformations of similar energy, designated as *ss* (syn syn), *sa* (syn anti), and *aa* (anti anti), respectively (Figure 1b). This feature is in accordance with the presence of four AB patterns in the ^1H NMR spectrum for the methylene protons of **1b**, one AB pattern for each of the *aa* and *ss* conformers, and two for the less symmetric *as* conformer.[6] The NMR data show that at 25 °C, 91% of molecules **1b** are in the *as* conformation, 4.7% in the *ss* conformation, and 4.3% in the *aa* conformation. Compound **1c**, of which an even lower proportion of the molecules is present in the *aa* conformation, forms a complex with 1,3-dinitrobenzene (DNB) in $CHCl_3$, as can be concluded from the development of a yellow color upon addition of this substrate. In the ^1H NMR spectrum of **1c**, the intensity of the signals of the *aa* conformer shift and increase in intensity when DNB is added, whereas the signals of the other conformers decrease in intensity.[7] Therefore this receptor has a higher affinity for DNB in its *aa* conformation, leading to an induced fit type of binding mechanism. From a UV titration in $CHCl_3$, the association constant for DNB was calculated to be $K_a = 4.2 \pm 0.4\ M^{-1}$.

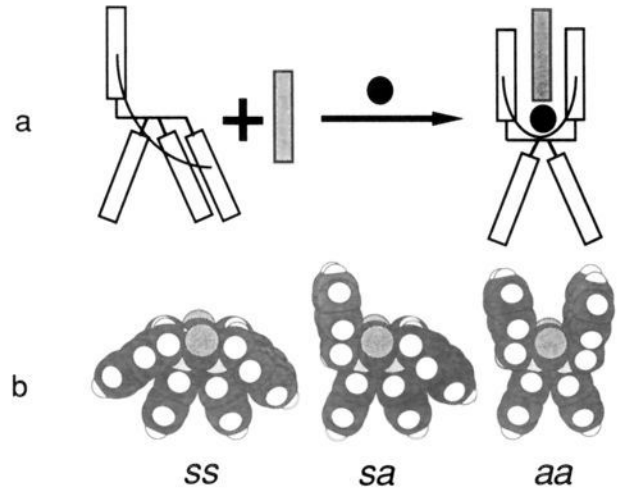

Figure 1. (a) Induced binding of 1,3-dinitrobenzene in **2** by addition of metal ions and (b) modeled structures of the three conformations of **1a**.

Compound **2** is an analogue of **1**, in which the naphthalene moieties are bridged by two aza crown ether rings. Compounds similar to **2** are strong binders of alkali-metal ions.[8] Like **1b** and **1c**, **2** exists predominantly in the *sa* conformation. In this conformation as well as in the *ss* form, the crown ether parts of the molecule cannot effectively bind an alkali-metal ion due to interference by the glycoluril part of the molecule. CPK models show, however, that in the *aa* conformation each of the crown ether moieties can wrap nicely around an ion. Addition of a potassium salt to a solution of **2** leads to an increase in intensity of the ^1H NMR signals of the *aa* conformation. When a solution of **2** in $CDCl_3$/DMSO-d_6 (3:1 v/v) was titrated with potassium picrate, initially 2.08 potassium ions were required to bring one molecule of **2** in the *aa* conformer. This indicates that **2** binds potassium ions in a 1:2 stoechiometry.

Since the *aa* conformer of **2** is expected to display the highest affinity for 1,3-dinitrobenzene, we were tempted to assess the ability of metal ions to induce substrate binding by an allosteric

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(6) Peaks in the spectrum could be assigned on the basis of the fact that in the *ss* and *sa* conformers the protons of the phenyl groups undergo an upfield shift due to the proximity of the naphthalene rings and on the fact that the signals from the nonequivalent sides of the *sa* conformer have the same intensity.
(7) From the observed induced shifts on host and guest protons we conclude that DNB is bound with its NO_2 groups pointing away from the cavity.
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mechanism. To this end 2 mM solutions of **2** in $CHCl_3$/DMSO (9:1 v/v) were titrated with DNB both in the absence and in the presence of 8 mM KSCN. The absorbances of the solutions were monitored at 415 nm during the titrations. Fitting of the data gave association constants of 1.2 ± 0.3 M^{-1} and 7.2 ± 1 M^{-1} for the salt-free and the potassium-containing solutions, respectively. These results show that binding of DNB is stronger by a factor of 6 in the presence of potassium ions. This enhancement is caused by the conversion of **2** into the *aa* conformer and not by an ionic strength effect, as we verified in a control experiment with 2,7-dimethoxynaphthalene and DNB.

The magnitude of the allosteric effect depends on the solvent mixture used. We observed by UV-vis that in $CHCl_3$/DMSO (3:1 v/v) the increase in DNB binding by KSCN is only 1.7 (K_a without KSCN, 0.3 ± 0.15 M^{-1}; K_a in the presence of 4 equiv KSCN, 0.5 ± 0.12 M^{-1}). An approximately 2-fold increase was calculated from ^1H NMR experiments, measuring the induced shifts on DNB. Further experiments are in progress.

Synthesis of the First Large Annulene Fused to Cyclopentadienide. A Comparison of the Effective Aromaticity of Cyclopentadienide Anion with Benzene

Reginald H. Mitchell,* Nasr A. Khalifa, and Thomas W. Dingle

Department of Chemistry, University of Victoria Box 3055, Victoria, British Columbia V8W 3P6, Canada

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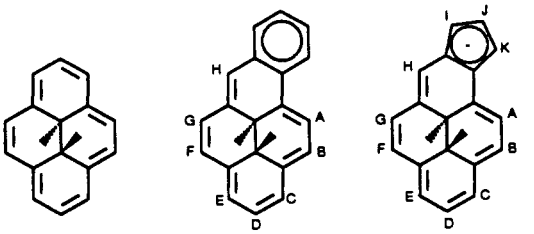
While the cyclopentadienide anion (Cp^-) is the prototype and best known of the charged aromatic species, very few experimental comparisons of its aromaticity with that of benzene are available. We here present evidence that the effective aromaticity of Cp^- is substantially less than that of benzene, contrary to most calculations.

When resonance energies of aromatic molecules are calculated, the reference compound used is a (hypothetical) polyene with the same number of atoms. Thus in a comparison of Cp^- with benzene, the two reference compounds are different. A comparison of resonance energies can therefore be somewhat misleading. For example, both Aihara[1] and Trinajstic[2] calculate that Cp^- has a greater resonance energy than benzene. Only Dewar,[3] using MINDO/3, has suggested that the aromatic stabilization energy (ASE) of Cp^- is about half that of benzene, although he points out that his estimate may be too low. Two more recent calculations of indices of aromaticity suggest that Cp^- might be somewhat less aromatic than benzene.[4,5] The only experimental evidence, so far, comes from Bordwell's[6] measurements of acidity of Cp in DMSO, from which he estimates the ASE of Cp^- to be about 24-27 kcal/mol, similar to that calculated for benzene. We have found that aromaticity can be probed by examination of the annelation effects from fusion of the aromatic molecule in question on to a [14]annulene, dimethyldihydropyrene (**1**).[7,8] Thus, Cp^- can be directly compared to benzene by comparison of the two



1 2 3

fused annulenes **2** and **3**. The more aromatic species will suppress the macrocyclic ring current to a greater extent and will result in a less shielded chemical shift for the internal methyl protons of **2** or **3**, resulting in less deshielded distant protons, H_D, in more alternating bond orders, and hence, in different vicinal coupling constants in the macrocyclic ring of **2** or **3**. No hypothetical reference molecules are required, and the measurements are simple, although in a charged species the effect of the charge will have to be considered. To date, however, no large-ring annulenes have ever had a Cp^- fused on to them; thus the stability of such a system was unknown.

Hopf's[9] route to the indenophane anions looked most promising and was adapted for the synthesis of **3** as shown in Scheme I. The green cyclopentadiene, **6**, mp 114-116 °C, showed its internal methyl protons at δ -4.15 and -4.16, indicating no disturbance of the ring current of **1**. Since **6** was not very stable, it was chromatographed immediately prior to its conversion to **3**, which was achieved by using KH in dry d_8-THF. The resulting red solution of **3** gave the ^1H NMR data given in Table I. The spectrum was also run in the presence of [2.2.2]cryptand to simulate a cation-free anion; however, changes in chemical shift were all small (see Table I). Immediately apparent is the large difference between the chemical shift of the methyl protons of **3** and the benzo derivative **2**. *If* the methyl protons are not much affected by the charge, this would clearly indicate Cp^- to have an aromaticity substantially less effective than that of benzene! As will be explained below, we believe that the effect of the charge on the methyl protons of free 3^- is small, giving a shielding of about 0.4 ppm, and that the chemical shift of the methyl protons which should be used for comparison with **2** is thus about -2.8 ppm (-3.2 + 0.4). Two specific approaches and one more general approach yield this result. π-SCF calculations on free 3^- indicate that about 40% of the charge is delocalized over the macrocyclic ring. From the atom charge densities, corrected chemical shifts for the external protons A-H can easily be calculated from known[10] relationships, and these are given as the corrected values in Table I. The value for H_D can then be used to predict the chemical shift for the internal methyl protons using the correlation[11] $\delta_{Me} = 16.94 - 2.60\delta_H$ and yields a value of $\delta_{Me} = -2.6$ *corrected for delocalized charge*. This correction of about 0.6 ppm is the same as would be calculated on the basis of the difference in chemical shift of H_a between 7^{2-} and 7^{2+}, which is observed[12] to be 2.8 ppm, where there is on average a difference of 0.3 unit charge per atom between these species. In 3^- there is only 0.07 unit charge (maximum) per atom. Similarly, the difference in chemical shift between H_a of the neutral [14]annulene[13] **8** and the [14]annulenyl anion[14] **9** (δ -1.13 and -0.84, respectively) is also small, as is the difference in chemical shift[15] of H_a (0.4 ppm) in **10** and **11**. On the basis of molecular mechanics calculations, both have H_a placed

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