A Simple Polyheterotopic Molecular Receptor Derived from Bispyrazolylmethane Showing Ambivalent Allosteric Cooperation of Zinc(II)

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The preparation of compounds **1–4** as simple systems to display allostery is described. Transport experiments (bulk liquid membrane) of ammonium cations in the presence or absence of ZnI_2 in the receiving and donor phases evidenced strong negative or positive allosteric effects of the metal towards ammonium complexation, which depended on the relative arrangement of crowns with respect to the pyrazole nitrogens.

Supramolecular interactions involving allostery are essential to life.[1] A great deal of enzymatic systems need the concourse of a co-factor to express their activity.[2] Along these lines, numerous efforts are being directed, in the recent literature, towards the construction of relatively simple hosts whose binding to guests could be modulated by an induced self-assembly or allosteric mechanism.[3] This chemistry would lead to such exciting goals as controlled liberation of drugs, of catalysts, of dyes etc. as well as selective transport. To imitate the elegant molecular systems found in nature, these artificial assemblies must typically bear: *i)* a host cavity to enshroud the guest molecule; *ii)* a remote allosteric center to interact with a particular effector; and *iii)* a conformational mechanism to transmit the effector action over the host cavity, and thus remotely regulate the host-guest interaction.

We have designed very simple systems based in crown ethers and heteroaromatic rings as building blocks. Figure 1 schematically depicts the possibilities of such a system. The host cavity may be formed by the co-operation of two crown ethers,[4] which would interact with dicationic species such as alkyl diammonium ions. These crowns are bonded by means of a spacer to the allosteric center, composed of two pyrazole or pyridine rings whose nitrogens (black dots) may interact with a metal, thus playing the effector role. The union between the heteroaromatic rings should act as the conformational transmission belt by which the effector will alter the relative arrangement of the crowns. Thus, simply by placing the crowns in different positions of the heteroaromatic rings, one should be able to get a system with negative (Figure 1, part a) or positive allostery (Figure 1, part b). Although current efforts are directed toward the design of sophisticated host molecules which can bind guests by an induced fit or allosteric mechanism, Figure 1 describes in our believe, one of the simplest imaginable molecular constructions embracing the conditions to be an artificial assembly to display negative or positive allosteric effects.

Following these lines, we reported in a previous communication[5] the true allosteric effect displayed by the molecule in Scheme 1.

The transport rate across bulk membranes of 1,5-diammonium picrate by the host of Scheme 1 was in fact retarded by the presence of Zn^{2+}, whereas methylammonium picrate, which should not need co-operation of the crowns to be transported, was carried at an even higher rate when Zn^{2+} was present.

In this paper we give a full account of our progress in this field, and report on the preparation of the systems listed in Figure 2, in which the crowns are bonded to different positions of the pyrazole rings. We thus expect compound **2** to display a positive allosteric effect, since the arrangement matches that of Figure 1, part b. The negative allostery shown by **4** in our preliminary work agrees with the expectations of Figure 1, part a. Compound **3** may give an ambiguous response, and compound **1**, with only one crown, was prepared as a standard to evaluate the real need of co-operation between the crowns to interact with the diammonium salts.

Synthesis

The preparation of compounds **1–4** may be envisaged as shown in Scheme 2, where ester functions are precursors of the methylcrown groups by means of reduction to the corresponding alcohol and double nucleophilic displacement, firstly of OH by Br, and secondly of Br by 1-aza-18-crown-6. Construction of bispyrazolylmethane can be accomplished by reaction of either ethyl pyrazole-3-carboxylate with methylene iodide, or by nucleophilic substitution of Br in ethyl 1-(bromomethyl)pyrazole-3-carboxylate with the appropriate pyrazolate. The methylene iodide method has been described previously,[6] and allowed the synthesis of ester precursors of compounds **2** and **4**, while the pyrazolate method rendered the parent esters of compounds **1** and **3**.

The reaction of ethyl pyrazole-3-carboxylate with methylene iodide in basic conditions yielded a mixture of diesters

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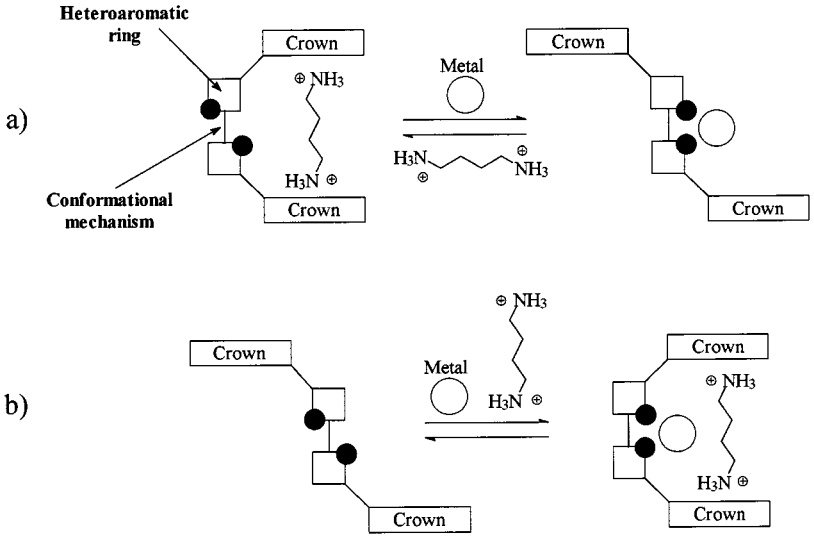
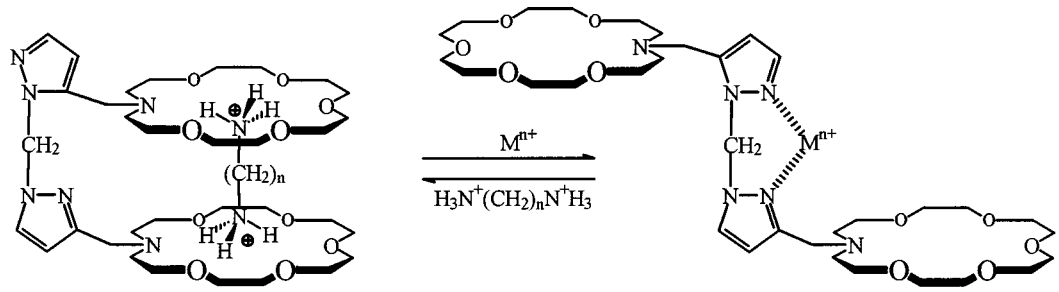
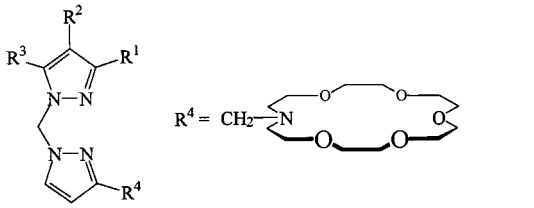


Figure 1. Schematic picture of artificial systems showing negative (A) and positive allostery (B)

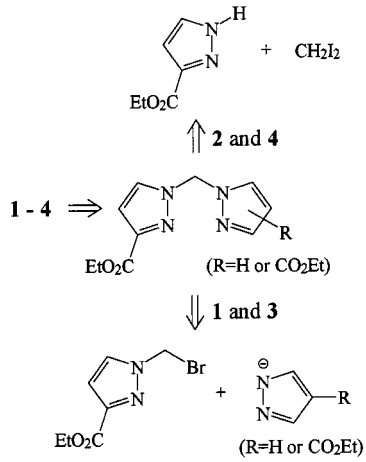


Scheme 1



Compound	R^1	R^2	R^3
3-bpm (**1**)	H	H	H
3,3'-bpm (**2**)	R^4	H	H
3,4'-bpm (**3**)	H	R^4	H
3,5'-bpm (**4**)	H	H	R^4

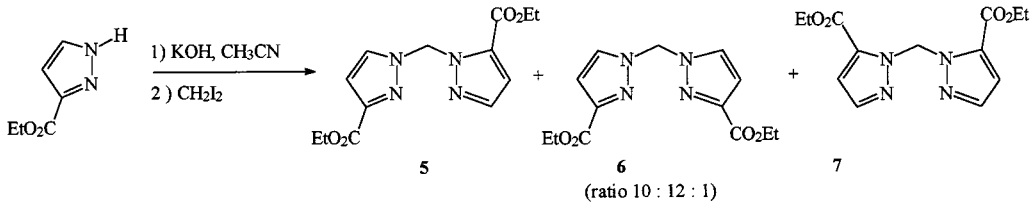
Figure 2. Allosteric systems described in this work



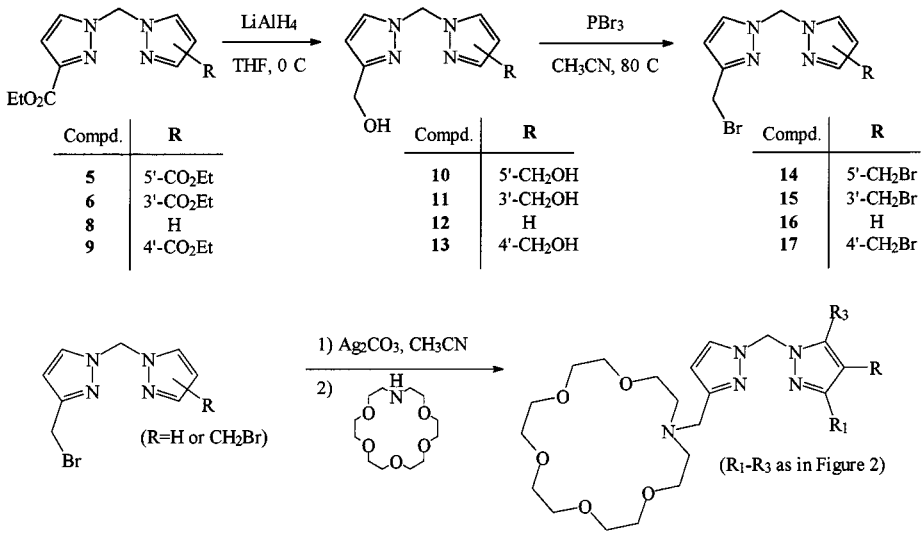
Scheme 2

5–**7** (Scheme 3) in a 10:12:1 ratio, which were easily separated by flash chromatography.

We attempted the synthesis of the monoester precursor of **1** by reaction of a 1:1 mixture of methyl pyrazole-3-carboxylate and pyrazole with methylene iodide under the same conditions, but not surprisingly, a complex mixture was obtained in which the desired monoester **8** was fortunately one of the major products. Although this mixture could be resolved by flash chromatography and all compounds spectroscopically characterized, the very low selectivity of this process made us devise a more efficient synthetic method for **8**, which was easily extended to the preparation of the diester precursor of **3** (Scheme 2). Thus, treatment of ethyl pyrazole-3-carboxylate with 37% aqueous formaldehyde yielded a mixture of ethyl 1-(hydroxymethyl)pyrazole-3- and -5-carboxylate in 25:1 ratio. The OH groups were then cleanly exchanged with PBr_3 to the corresponding ethyl 1-(bromomethyl)pyrazole-3- and -5-carboxylates, which were separated by flash chromatography to yield the ethyl 3-carboxylate in 60% isolated yield. On the other hand, pyrazole or its methyl 4-carboxylate[7] was converted, by reaction with potassium in THF, into the corresponding pyrazolate anion, which displaced Br in ethyl 1-(bromomethyl)-



Scheme 3



Scheme 4

pyrazole-3-carboxylate, affording the corresponding esters in excellent yield. Other milder bases (K_2CO_3, KOH) gave rise to complex reaction mixtures. Reduction of CO_2Et groups with $LiAlH_4$ (yield 70–97%) and substitution of OH groups with Br by reaction with PBr_3 (yield 70–85%) rendered the corresponding bromomethyl derivatives (Scheme 4), which were transformed into compounds **1–4** by direct nucleophilic substitution of Br by 1-aza-18-crown-6 in the presence of Ag_2CO_3 (yield 70–93%).

Alternatively, the dibromo derivative **14** was treated with 2 equiv. of diethylamine to get the bisdiethylamino derivative **18**, which was used as a model of **4** in metal chelation studies (see below).

Table 1. Stability constants by Cram′s method of compounds **1–4** with various alkyldiammonium picrates

Alkyl diammonium picrate	$-\Delta G^0$ [a] 3,3′-bpm (**2**)		3,4′-bpm (**3**)		3,5′-bpm (**4**)		3-bpm (**1**)	K_d(rel)
1,4-	12.1	(12.1)	11.7	(11.7)	11.0	(11.0)	7.7	1.0
1,5-	12.0	(12.1)	11.7	(11.8)	11.3	(11.5)	7.6	0.8
1,6-	12.1	(12.0)	12.1	(12.0)	11.4	(11.3)	7.8	1.2
1,7-	12.9	(12.6)	12.6	(12.3)	11.8	(11.5)	8.0	1.7
1,8-	14.1	(12.9)	13.7	(12.5)	12.9	(11.6)	8.5	8.2
1,9-	14.1	(12.5)	13.9	(12.3)	13.1	(11.5)	8.9	16.0

[a] kcal/mol; values in parentheses are corrected for K_d rel. (see text).

Structure of Diammonium Complexes

Table 1 contains the stability constants measured by Cram's method,[8] using equations for ditopic systems[9] in the case of compounds **2–4**, and assuming the formation of 1:1 complexes for all ligands (see below). The values of $-\Delta G°$ that resulted were similar to those measured in similar systems with well established co-operation between two crowns,[9] and showed that the stability of the complexes varied in the order 3,3′-bpm > 3,4′-bpm > 3,5′-bpm >> 3-bpm, although differences were relatively small except for singly crowned **1**.

Table 1 shows that the complexes with the longest diammonium salt are more stable by ca. 2 kcal/mol but, quite surprisingly, a similar phenomenon was also observed for 3-bpm (**1**), whose non co-operative complexation through one crown (vide supra) should not be affected by the salt chain length. It should be noted that Cram's method is extractive, and thus the calculation of stability constants depends on the knowledge of distribution constant (K_d). Unfortunately, K_d for diammonium salts cannot be measured due to their extremely low solubility in $CHCl_3$, and it is taken as 1 M^{-1} in all cases. However, K_d could be lower and more importantly, it might get higher as the lipophilicity of the diammonium salts increases. Assuming that the vari-

ation of $-\Delta G^\circ$ for 3-bpm (**1**) was only apparent, and depended on the different values of K_d for the different salts, we calculated their relative K_d values that equalize $-\Delta G^\circ$ of **1** with all picrates (last column in Table 1). Using these relative K_d values we recalculated $-\Delta G^\circ$ for all hosts (Table 1; values in parentheses). The resulting $-\Delta G^\circ$ values still show that our hosts displayed some selectivity for the longer salts, and suggest that there is a maximum in their recognition abilities towards the salt of eight carbon atoms in all cases.

The first observation concerning the 1:1 stoichiometry of the complexes formed by compounds **2–4** is relatively trivial: solubility of diammonium picrates in pure $CDCl_3$ is negligible, but the presence of these hosts dissolved ca. 1 equiv. of the diammonium picrate. We have further investigated this matter by UV/Vis in THF (*cf.* Table 2), and ^{1}H-NMR titrations in 9:1 $CDCl_3/CD_3OD$ (*cf.* Figure 3). It has been shown that the tight binding of ammonium to a crown ether causes its picrate counterpart to increase its charge density and thus suffer a bathochromic shift of ca. 30 nm.[10] On the other hand, ^{1}H chemical shift variations may reveal the conformational changes necessary to form the different complexes.

Table 2. Bathochromic shifts observed in the picrate absorption when 1,5- and 1,9-alkyldiammonium cations were bound to compounds **1–4**

	bathochromic shift [nm]	
Host	**1,5-diamm.**	**1,9-diamm.**
1	+7	+10
2	+14	+23
3	+11	+19
4	+5	+4

The singly crowned compound **1**, prepared as reference, exerted a moderate bathochromic displacement of the picrate band (Table 2), and its protons displayed a uniform chemical shift variation up to ca. 0.5 mol of added diammonium (Figure), which is in agreement with the predominant formation of a 2:1 host/guest complex.

The cases of 3,3- and 3,4-bpm are quite straightforward, in that they showed the highest bathochromic shifts in UV/Vis (Table 2), and a uniform variation of chemical shifts (Figure 3) up to ca. 1 mol of guest. Both facts are compatible with the formation of 1:1 complexes. Besides, the higher bathochromic shift suffered in the presence of 1,9-diammonium (Table 2) is in agreement with the higher stability of this complex measured by Cram's method. On the other hand, the observed shielding/deshielding trends of some protons in the ^{1}H-NMR spectra resulted in a coherence in terms of the conformational changes that should be involved. For instance, protons 5 and 5′ were gradually deshielded (Figure 3). Figure 4 shows the predicted most stable conformers (MM+ HyperChem), resulting from the combined rotation of the two C–N bonds of the N–CH_2–N fragment of 3,3′- and 3,4′-dimethylbispyrazolylmethanes, used as models of hosts **2** and **3**, respectively.

It should be noted that, in the majority of the predicted, most-stable conformations, protons 5 and 5′ lay in the shielding region of their geminal pyrazole ring, and that the methyl groups remained very far apart. However, to form a 1:1 complex the two crowns must co-operate in an almost parallel arrangement. Therefore, a simple molecular mechanics calculation suggests that hosts **2** and **3** should suffer an important conformational change around the N–CH_2–N hinge, in order to bind diammonium salts. An example of the predicted conformers appropriate in achieving the aforementioned co-operation of the two crowns is depicted in Figure 5, in which one can observe that protons 5 and 5′ are no longer under the shielding influence of the geminal pyrazole, and should thus be deshielded in agreement with the experimental observation (Figure 3).

The picture of 3,5′-bpm (**4**) was somewhat more intricate. Let us first analyze its UV/Vis behavior. Table 2 shows that **4** displayed the lowest bathochromic shift in the series, even lower than that of 3-bpm (**1**). This observation implies the presence of relatively large amounts of picrates, tightly ion-paired to ammonium in the case of **4** (and therefore a higher presence of ammoniums not bound to crowns), than in the singly crowned **1**. This suggests that the contribution of complexes formed by one molecule of **4** and two diammonium molecules (host/guest stoichiometry 1:2) should be very important in the equilibrium of 3,5′-bpm in THF (B in Figure 6). On the other hand, it may be seen that the variation of chemical shifts in the ^{1}H-NMR titration extends to amounts of added diammonium salt higher than 1 mol, which is only compatible with an important contribution of the aforementioned 1:2 complexes in 9:1 $CDCl_3/CD_3OD$. The initial, nonuniform chemical shift change suggests the coexistence of complexes of various stoichiometries as shown in Figure 6.

Finally, MS analysis (FAB+) of the complexes also supports the formation of 1:1 complexes with 1,9-diammonium salt for hosts **2** and **3**, since their spectra displayed a low intensity (ca. 1%[11]) signal at $m/z = 1086.6$, which corresponds to the 1:1 complex with only one picrate. This peak was not observed in the case of host **4**, whose behavior with diammonium salts has been shown to be much more complex by NMR and UV/Vis spectra.

Transport Measurements

Transport rates across bulk membranes (see experimental section) of diammonium salts of quite different length by hosts **2–4** are listed in Table 3. Host 3-bpm (**1**) is not included in the Table because it transported almost negligible amounts of diammonium salts under the conditions used (see experimental part).

To scrutinize possible allosteric effects, the rate of transport was measured in the presence/absence of Zn^{2+} in both donor and acceptor aqueous phases, because the metal is expected to interact with the available N atoms of the aromatic heterocycles and thus change the conformation and

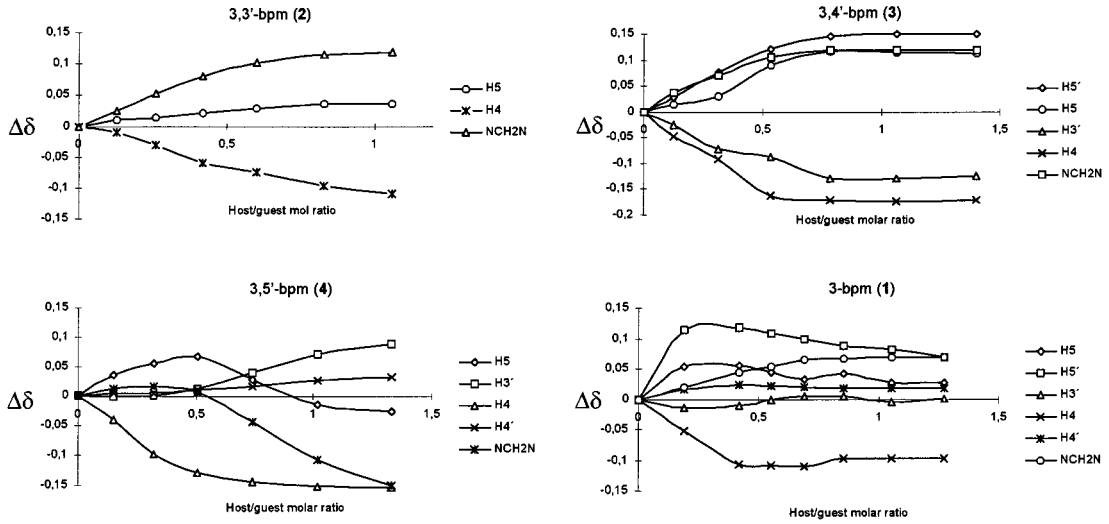


Figure 3. Variation of proton chemical shifts of compounds **1–4** induced by 1,9-nonanediammonium picrate

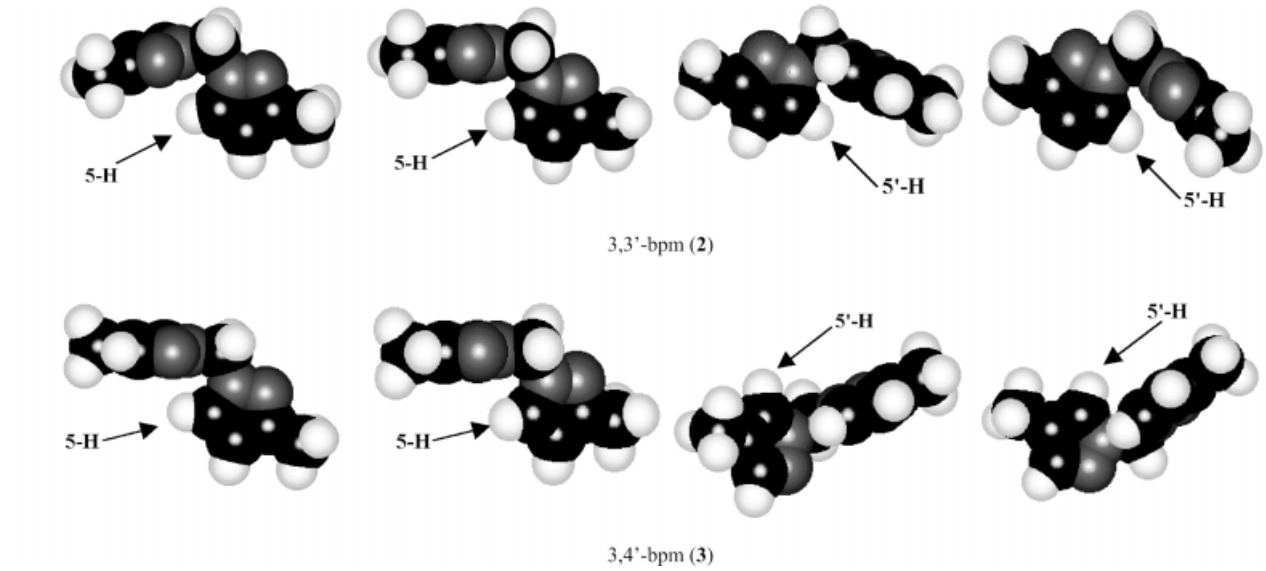


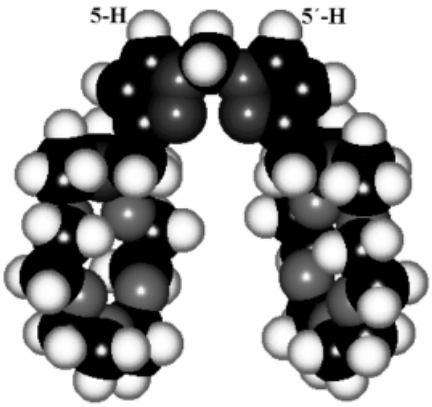
Figure 4. Predicted most stable conformations of model dimethyl compounds (see text) showing the arrangement of protons 5 and 5′ relative to aromatic shielding region.

chelation abilities of the host in a remote manner. In fact, dynamic ^{1}H-NMR studies of the 1:1 mixture of bispyrazolylmethane and ZnI_2 in $CDCl_3$ showed that the metal strongly binds to the pyrazoles, forming a six-membered ring (Scheme 5) with an energy barrier to inversion of 10.5 kcal/mol.

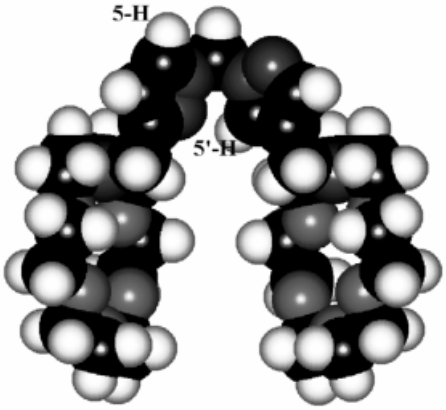
However, this finding does not rule out direct interaction of Zn^{2+} with the crowns, thus competing in a nonallosteric fashion with diammonium salts. Unfortunately, it was not possible to perform the same dynamic experiments on the hosts **2–4** because the spectra were too complex in the observable range of temperatures, probably due to the conformational mobility of the crowns. To avoid this problem we have prepared the bisdiethylamino derivative **18** (Scheme 5) as a useful analog of host **4**, and checked the structure of its Zn and Pd complexes[12] at room temperature, which were made from ZnI_2 and $PdCl_2$, respectively. The methylene protons of one diethylamino group became diastereotopic in the Pd complex (Scheme 5), suggesting a clear interaction between one diethylamino nitrogen and the metal. However, this was not observed in the case of ZnI_2 complex, indicating that the interaction of Zn in **18** with the nitrogen homologous to that of the azacrown in **4**, if any, must be much weaker.

Yet, two simple observations end this discussion concerning direct competition of Zn^{2+} with ammonium salts: *i*) in one case Zn^{2+} increased transport (see host **2** in Table 3); and *ii*) in a previous paper we reported that 3,5′-bpm (**4**) transported 1,5-diammoniumpentane and methylammonium[5] slower and faster, respectively, in the presence of Zn^{2+}. The increase of transport is totally incompatible with a predominant interaction of Zn^{2+} with crowns.

Table 3 shows that all hosts transported the longest diammonium salt ca. three times faster, which is in fair qualitative agreement with the binding constants measured by



3,3'-bpm (**2**)



3,4'-bpm (**3**)

Figure 5. Selected conformations of compounds **2** and **3** with adequate arrangement of crowns to achieve mutual co-operation

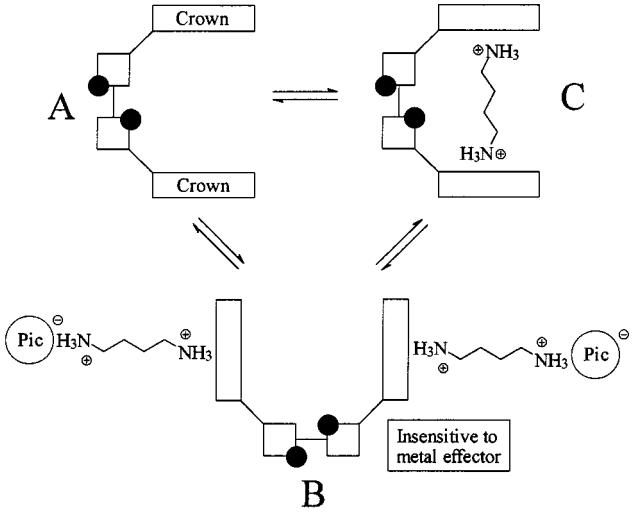


Figure 6. Equilibrium among host **4** and its diammonium complexes of stoichiometries 1:1 (C) and 1:2 (B)

increase and diminution of transport displayed when the metal is present should be the consequence of the different arrangement among the crowns and the pyrazole nitrogens. Therefore, host **2** was revealed as the positive, simple allosteric system we expected (Figure 1, part b), in which the interaction of a simple metal effector sizably reinforced the co-operative behavior of the crowns in embracing the diammonium salt. Host **3**, whose structure did not allow us to clearly predict its allosteric conduct, was found to be a system with moderately negative allostery.

In the case of host **4** some additional remarks are needed. From NMR and UV/Vis data we concluded that the population of 1:2 (host/guest) species in the equilibrium of **4** should be very important (vide supra). This 1:2 complex

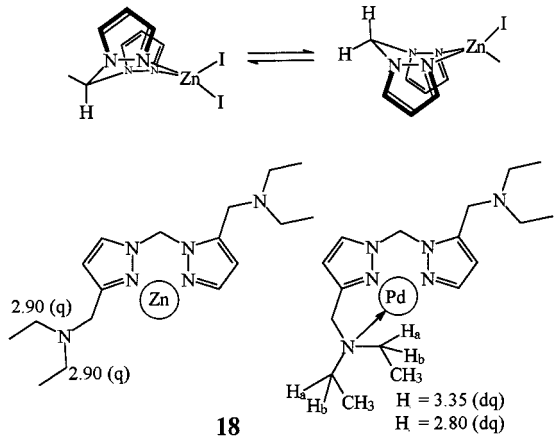
Table 3. Transport rates of diammonium salts by hosts **2–4**

	1,5-diammoniumpentane (%·h^{-1})		1,9-diammoniumnonane (%·h^{-1})	
Host	without metal	with Zn^{2+}	without metal	with Zn^{2+}
3,3′-bpm (**2**)	5.2	5.9	15.4	19.2
3,4′-bpm (**3**)	4.6	4.3	17.8	15.7
3,5′-bpm (**4**)	3.0	2.7	11.8	8.2

Cram's method (Table 1), thus displaying moderate, but estimable recognition abilities towards chain length. More significantly, Table 3 clearly shows that Zn^{2+} affected transport of all studied hosts, the effect being much more substantial in the case of the longest diammonium salt (see ref.[13]). The metal exerted a transport increase of ca. 25% in **2**, whereas **3** and **4** suffered a diminution of ca. 12% and 31%, respectively, in their transport aptitudes when the metal was present.

As we have shown by NMR, UV/Vis, and MS data, hosts **2** and **3** formed complexes of 1:1 stoichiometry with diammonium salts in the absence of a metal. Their respective should not be affected by allostery because each crown acts independently, binding one diammonium cation each (Figure 6). However, 3,5′-bpm displayed the highest negative allosteric effect with Zn^{2+} (31%), which can only be exerted in the 1:1 complex, less abundant than in the case of **2** and **3**, suggesting that the expected, negative effect of the metal (Figure 1, part a) is quite high in the 1:1 complex of **4**.

Preliminary results in the study of analogous hosts derived from bipyridine[14] showed similar metal effects that also depended on the relative arrangement of crowns relative to heteroaromatic nitrogens (a full account of these results will be given elsewhere).



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Scheme 5

Conclusion

The simple rationale (Figure 1) described in this paper allowed us to construct efficient artificial allosteric systems from elemental organic building blocks. Allostery was easily and clearly revealed in straightforward transport experiments. The relative arrangement of the co-operative crowns with respect to the bispyrazolylmethane framework was the key to switching between quite sizable positive or negative allosteric effects. This work should thus be the starting point in developing new molecules, not necessarily much more elaborate, with practical applications in important fields such us controlled drug liberation, tuning of catalysts, selective transport, and switched recognition.

Experimental Section

General: ^{1}H NMR: Bruker AC 300 (300 MHz), Bruker DRX 500 (500 MHz); δ (ppm) = 0 for tetramethylsilane, 7.24 for $CHCl_3$, 3.34 for CH_3OH. Characterization of signal multiplicities: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet br. m = broad multiplet. – ^{13}C NMR: Bruker AC 300 (75 MHz), Bruker DRX 500 (125 MHz); δ = 77.0 for $CDCl_3$, 34.9 for CD_3OD. Assignments of ^{13}C signals were supported by measurements applying DEPT, HMQC and COSY 45 experiments. – High-resolution mass and FAB spectra and were recorded on a VG Autospec spectrometer. – UV/Vis measurements were performed in a Perkin–Elmer spectrophotometer at 380 nm.

Transport Measurements: They were performed in a stoppered, thermostated, cylindrical cell, provided with a magnetic stirrer. Receiving and donor aqueous phases (deionized water) were separated by a glass wall, and no physical contact was allowed between them except through the organic phase. The empty cell was first kept at 30 °C for 10 min and then 3 mL of a stock solution of hosts **1–4** in $CHCl_3$ and 27 mL of $CHCl_3$ were added. Final concentration of host was 10^{-4} M. The donor phase containing the alkyldiammonium picrate (10 mL; $5\cdot10^{-4}$ M) was then placed in the right side of the cell. The system was allowed to equilibrate for 30 min. The acceptor phase (10 mL) was then placed in the left side of the cell. In the case of the experiments run in the presence of metal, 50 μL of a $2\cdot10^{-2}$ solution of ZnI_2 in deionized water was added to each aqueous phase. Immediately after the acceptor phase was placed in the cell, an aliquot (75 μL) was extracted from each aqueous phase and that of the donor phase was made-up to 5 mL with CH_3CN. UV/Vis measurement of this solution marked time zero and 0% of transport. At regular time intervals spanning up to six hours, thirteen fractions of 75 μL were extracted from both aqueous phases in order to keep their volumes constant. The fractions from the acceptor phase were discarded and those of the donor phase were taken up to 5 mL with CH_3CN, and UV/Vis spectrum measured. Therefore, transport was measured as a decrease of the picrate concentration in the donor phase. Data of Table 3 have been obtained by linear regression of the plot between time and the measured absorbance referred as a percentage of that at time zero.

Reduction of Esters. – General Method: $LiAlH_4$ (1.3 equiv. for the monoester and 2.5 equiv. for diesters) was slowly added in portions to 1 equiv. of mono or diester in THF at 0 °C, the reaction mixture was stirred at that temperature for 1 h and allowed to reach room temp. for an additional hour. The reaction mixture was then quenched by sequential addition of *n* mL of water, *n* mL of 15% NaOH and 3*n* mL of water, where *n* is the number of g of $LiAlH_4$ used. The precipitated solid was filtered off and the mother liquors evaporated to dryness.

Bromination of (Hydroxymethyl)pyrazoles. – General Method: PBr_3 (1.5 equiv. for the monohydroxymethyl derivative and 2.5 for the dihydroxymethyl derivative) was slowly added to a refluxing solution of the alcohol in CH_3CN. The reaction was followed by TLC until the alcohol was no longer observed. The reaction mixture was then allowed to reach room temp., the solvent was removed in vacuo, and the resulting solid was treated with a small portion of saturated $NaCO_3H$ solution. The aqueous layer was extracted several times with CH_2Cl_2, the organic layers were washed with a portion of water, dried over $MgSO_4$, and the solvent removed in vacuo to afford the corresponding bromomethyl derivative as a white solid, which was used without further purification unless otherwise indicated.

Substitution of Bromine Atom by 1-Aza-18-crown-6. – General Method: A solution of the corresponding bromo derivative in CH_3CN was slowly added over a period of 2–3 h to a refluxing solution of 1-aza-18-crown-6 and Ag_2CO_3 in CH_3CN. The reaction was monitored by TLC and when the bromo derivative was no longer observed, the mixture was allowed to reach room temp. The solid was filtered off and the solvent removed affording a dark oil, which was re-dissolved in the minimum amount of ethyl ether and the flask stoppered. The solution was carefully cooled down in a liquid nitrogen bath and allowed to slowly warm up. The resulting solid was rapidly filtered off when the solution was still very cold and discarded. The desired product was thus obtained from the mother liquors as a yellow hygroscopic oil by evaporation in vacuo.

Ethyl 1-(Bromomethyl)pyrazole-3-carboxylate: To a suspension of 1.13 g (8 mmol) of ethyl pyrazole-3-carboxylate in 30 mL of water, 1.25 mL of 37% formaldehyde (15.4 mmol) was added. The mixture was stirred until the solution was clear (ca. 15 min) and extracted with CH_2Cl_2 (3 × 30 mL). Usual workup of the organic extracts yielded a mixture of 1-hydroxymethylated ethyl pyrazole-3- and -5-carboxylate, of which the 3-derivative was the major product. This mixture was then heated under reflux in 225 mL of CH_3CN. Once the reflux was established, 1.13 mL (12.1 mmol) of PBr_3 was added. After 90 min at 80 °C, the reaction mixture was filtered and the solvent removed. The solid residue was treated with 25 mL of

CH_2Cl_2 and 50 mL of saturated solution of Na_2CO_3. The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL), the combined organic extracts were washed with water (2 × 50 mL), and usual workup of the organic layer yielded a white solid that was a mixture of the 1-bromomethylated ethyl pyrazole-3- and -5-carboxylates. The two regioisomers were separated by flash chromatography on silica-gel (hexane/AcOEt, 2:1). Yield: 65%. – ^{1}H NMR ($CDCl_3$): δ = 7.69 (d, 1 H, *J* = 2.2 Hz, Pz*5-H*), 6.90 (d, 1 H, *J* = 2.2 Hz, Pz*4-H*), 5.98 (s, 2 H, NCH_2Br), 4.40 (q, 2 H, *J* = 7.5 Hz), 1.40 (t, 3 H, *J* = 7.5 Hz). – ^{13}C NMR ($CDCl_3$): δ = 161.3 (*C*O), 145.5 (*C-3*), 131.8 (*C-5*), 110.6 (*C-4*), 60.9 (N*C*H$_2$Br), 44.2 (*C*H$_2$), 14.0 (*C*H$_3$). – MS (EI); *m/z* (%): 234 (17) [M^+ + 2], 232 (16) [M^+], 188 (12) [M^+ + 2-OEt], 186 (12) [M^+ – OEt], 153 (100) [M^+ – Br]. – $C_7H_9N_2O_2Br$ (233.1): calcd.: C 36.07, H 3.89, N 12.02; found: C 36.32, H 3.82, N 11.92. [The ^{1}H NMR spectrum of **ethyl 1-(bromomethyl)pyrazole-5-carboxylate** is given for comparison: ^{1}H NMR ($CDCl_3$): δ = 7.65 (d, 1 H, *J* = 2.1 Hz, Pz*5-H*), 6.95 (d, 1 H, *J* = 2.1 Hz, Pz*4-H*), 6.41 (s, 2 H, NCH_2Br), 4.40 (q, 2 H, *J* = 7 Hz), 1.40 (t, 3 H, *J* = 7 Hz).].

Diethyl 1,1′-Methylenebis(1*H*-pyrazole)-3,5′-dicarboxylate and Diethyl 1,1′-Methylenebis(1*H*-pyrazole)-3,3′-dicarboxylate (5 and 6): To a solution of ethyl pyrazole-3-carboxylate (6 g, 42.8 mmol) in 300 mL of CH_3CN, was added 2.9 g (50 mmol) of KOH, and the reaction mixture was heated under reflux for 30 min. To this was then added 5.9 g (22 mmol) of CH_2I_2, and the reflux was maintained for two additional hours. The mixture was allowed to cool at room temp., the precipitate filtered and the solvent evaporated in vacuo. The resulting white solid was dissolved in 100 mL of CH_2Cl_2 and washed with water (5 × 200 mL). Usual workup of the organic layer rendered a mixture of the three possible isomers (Scheme 3), which were separated by flash chromatography on silica-gel (hexane/AcOEt, 1:1). – **Compound 5:** M.p. 140–142 °C. – Yield: 32%. – ^{1}H NMR ($CDCl_3$): δ = 7.75 (d, 2 H, *J* = 2.4 Hz, Pz*5-H*), 6.85 (d, 2 H, *J* = 2.4 Hz, Pz*4-H*), 6.45 (s, 2 H, NCH_2N), 4.45 (q, 4 H, *J* = 8 Hz), 1.4 (t, 6 H, *J* = 8 Hz). – ^{13}C NMR ($CDCl_3$): δ = 161.5 (*C*O), 145 (*C-3*), 131.3 (*C-5*), 109.9 (*C-4*), 65.9 (N*C*H$_2$N), 60.9 (O*C*H$_2$), 14.1 (*C*H$_3$). – **Compound 6:** M.p. 131–132 °C. – Yield: 29%. – ^{1}H NMR ($CDCl_3$): δ = 7.70 (d, 1 H, *J* = 1.9 Hz, Pz*5′-H*), 7.60 (d, 1 H, *J* = 2.3 Hz, Pz*5-H*), 6.90 (d, 1 H, *J* = 1.9 Hz, Pz*4-H′*), 6.85 (s, 2 H, NCH_2N), 6.80 (d, 1 H, *J* = 2.3 Hz, Pz*4-H*), 4.40 (q, 4 H, *J* = 7.5 Hz), 1.40 (t, 4 H, *J* = 7.5 Hz). – ^{13}C NMR ($CDCl_3$): δ = 161.8 (*C*O), 160.0 (*C*O), 144.4 (*C-3*), 139.9 (*C-3′*), 132.4 (*C-5′*), 130.9 (*C-5*), 112.4 (*C-4′*)109.4 (*C-4*), 64.0 (N*C*H$_2$N), 61.4 (*C*H$_2$O), 60.7 (*C*H$_2$O), 14.0 (*C*H$_3$), 13.9 (*C*H$_3$).

Diethyl 1,1′-Methylenebis(1*H*-pyrazole)-3,4′-dicarboxylate (9): A solution of 100 mg (0.71 mmol) of ethyl pyrazole-4-carboxylate and 28 mg (0.71 at g) of potassium in 25 mL of THF was heated under reflux until the metal was dissolved. The mixture was allowed to reach room temp., and 166 mg (0.71 mmol) of ethyl 1-(bromomethyl)pyrazole-3-carboxylate was then added in several portions during 30 min. The reaction was heated at 70 °C for 3 h, the solvent was removed and the residue was dissolved in 30 mL of water and 30 mL of CH_2Cl_2. Usual workup of the organic layer afforded a white solid which was used without further purification. M.p. 105–106 °C. – Yield: 83%. – ^{1}H NMR ($CDCl_3$): δ = 8.20 (s, 1 H, Pz*5′-H*), 7.95 (s, 1 H, Pz*3′-H*), 7.72 (d, 1 H, *J* = 2.3 Hz, Pz*5-H*), 6.84 (d, 1 H, *J* = 2.3 Hz, Pz*4-H*), 6.37 (s, 2 H, NCH_2N), 4.40 (q, 2 H, *J* = 8 Hz), 4.30 (q, 2 H, *J* = 8 Hz), 1.31–1.50 (m, 6 H). – ^{13}C NMR ($CDCl_3$): δ = 162.3 (*C*O), 161.7 (*C*O), 145.3 (*C-3*), 142.4 (*C-3′*), 133.3 (*C-5′*), 131.2 (*C-5*), 117.0 (*C-4′*), 110.3 (*C-4*), 65.8 (N*C*H$_2$N), 61.3 (*C*H$_2$), 60.4 (*C*H$_2$), 14.3 (*C*H$_3$). – MS (EI); *m/z* (%): 292 (40) [M^+], 263 (25) [M^+ – Et], 219 (41) [M^+ – CO_2Et]. – $C_{13}H_{16}N_4O_4$ (292.3): calcd.: C 53.40, H 5.52, N 19.17; found: C 53.33, H 5.22, N 19.56.

3,5′-Dihydroxymethyl-1,1′-methylenebis(1*H*-pyrazole) (10): It was obtained from 1 g (3.4 mmol) of the corresponding diester **5** by reduction with 0.29 g (7.5 mmol) of $LiAlH_4$ in 75 mL of THF, following the general method described above. The reaction afforded a colorless oil which solidified overnight and did not need further purification. Yield: 97%. – M.p. 136–137 °C. – ^{1}H NMR (CD_3OD): δ = 7.70 (d, 1 H, *J* = 2.2 Hz, Pz*5-H*), 7.45 (d, 1 H, *J* = 1.9 Hz, Pz*3′-H*), 6.40 (s, 2 H, NCH_2N), 6.33 (d, 1 H, *J* = 2.2 Hz, Pz*4-H*), 6.28 (d, 1 H, *J* = 1.9 Hz, Pz*4-H′*), 4.80 (s, 2 H, Pz′CH_2OH), 4.60 (s, 2 H, PzCH_2OH), 4.50 (s broad, 2 H, O*H*). – ^{13}C NMR (CD_3OD): δ = 155.4 (*C-3*), 144.7 (*C-5′*), 140.9 (*C-3′*), 132.8 (*C-5*), 107.8 (*C-4′*), 106.3 (*C-4*), 63.3 (N*C*H$_2$N), 55.1 (Pz*C*H$_2$OH), 58.8 (Pz′*C*H$_2$OH).

3,3′-Dihydroxymethyl-1,1′-methylenebis(1*H*-pyrazole) (11): It was obtained from 0.63 g (2.2 mmol) of the corresponding diester **6** by reduction with 0.21 g (5.5 mmol) of $LiAlH_4$ in 40 mL of THF, following the general method described above. The reaction afforded a white solid which was used without further purification. Yield: 71%. – M.p. 137–138 °C. – ^{1}H NMR (CD_3OD): δ = 7.80 (d, 2 H, *J* = 2.1 Hz, Pz*5-H*), 6.38 (d, 2 H, *J* = 2.1 Hz, Pz*3-H*), 6.30 (s, 2 H, NCH_2N), 4.55 (s, 4 H, PzCH_2OH). – ^{13}C NMR (CD_3OD): δ (ppm) = 155.0 (*C-3*), 132.8 (*C-5*), 106.4 (*C-4*), 65.4 (N*C*H$_2$N), 58.8 (*C*H$_2$OH).

3,4′-Dihydroxymethyl-1,1′-methylenebis(1*H*-pyrazole) (13). It was obtained from 1 g (3.4 mmol) of the corresponding diester **9** by reduction with 0.35 g (9.3 mmol) of $LiAlH_4$ in 75 mL of THF, following the general method described above. The reaction afforded a colorless oil which was used without further purification. Yield 89%. – ^{1}H NMR: δ = 7.50 (s, 1 H, Pz*3′-H*), 6.70 (s, 1 H, Pz*5′-H*), 6.67 (d, 1 H, *J* = 2.5 Hz, Pz*5-H*), 6.30 (d, 1 H, *J* = 2.5 Hz, Pz*4-H*), 6.20 (s, 2 H, NCH_2N), 4.60 (s, 2 H, PzCH_2OH), 4.50 (s, 2 H, Pz′CH_2OH), 4.20 (s broad, 2 H, CH$_2$O*H*). – ^{13}C NMR (CD_3OD): δ = 173.2 (*C-3*), 159.6 (*C-3′* and *C-5′*), 150.5 (*C-5*), 148.0 (*C-4′*), 125.1 (*C-4*), 84.5 (N*C*H$_2$N), 78.1 (Pz*C*H$_2$OH), 75.1 (Pz′*C*H$_2$OH).

3-Hydroxymethyl-1,1′-methylenebis(1*H*-pyrazole) (12): It was obtained from 1 g (3.4 mmol) of the corresponding monoester **8** by reduction with 0.13 g (3.3 mmol) of $LiAlH_4$ in 40 mL of THF, following the general method described above. The reaction afforded a white solid which was recrystallized from toluene. Yield: 68%. – M.p. 53–54 °C. – ^{1}H NMR (CD_3OD): δ = 7.65 (d, 1 H, *J* = 2.2 Hz, Pz*5-H*), 7.62 (d, 1 H, *J* = 2.3 Hz, Pz*5′-H*), 7.44 (d, 1 H, *J* = 1.92 Hz, Pz*3′-H*), 6.20–6.30 (m, 2 H, Pz*4-H* and Pz*4′-H*), 6.23 (s, 2 H, NCH_2N), 4.70 (s, 2 H, PzCH_2OH). – ^{13}C NMR (CD_3OD): δ = 156.0 (*C-3*), 139.8 (*C-3′*), 129.8 (*C-5*), 128.7 (*C-5′*), 106.1 (*C-4′*), 104.6 (*C-4*), 64.0 (N*C*H$_2$N), 57.7 (*C*H$_2$).

3,3′-Dibromomethyl-1,1′-methylenebis(1*H*-pyrazole) (14): It was obtained from 0.68 g (3.3 mmol) of the corresponding diol **10** and 2.2 g (8.3 mmol) of PBr_3 in 50 mL of CH_3CN. The reaction was complete after 2 h of heating at reflux. Yield 71%. – M.p. 160–165 °C. – ^{1}H NMR (CD_3OD): δ = 7.60 (d, 1 H, *J* = 2.1 Hz, Pz*5-H*), 7.45 (d, 1 H, *J* = 1.8 Hz, Pz*3′-H*), 6.35 (s, 2 H, NCH_2N), 6.31 (d, 1 H, *J* = 2.1 Hz, Pz*4-H*), 6.30 (d, 1 H, *J* = 1.8 Hz, Pz*4′-H*), 4.79 (s, 2 H, PzCH_2Br), 4.45 (s, 2 H, Pz′CH_2Br). – ^{13}C NMR (CD_3OD): δ = 150.0 (*C-3*), 139.7 (*C-3′*), 138.7 (*C-5′*), 131.1 (*C-5*), 108.3 (*C-4′*), 107.0 (*C-4*), 62.2 (N*C*H$_2$N), 24.6 (Pz*C*H$_2$Br), 19.9 (Pz′*C*H$_2$Br).

3,3′-Dibromomethyl-1,1′-methylenebis(1*H*-pyrazole) (15): It was obtained from 0.67 g (3.2 mmol) of the corresponding diol **11** and

2.2 g (8.3 mmol) of PBr_3 in 50 mL of CH_3CN. The reaction was complete after 1 h of heating at reflux. Yield: 84%. – M.p. 177–178 °C. – ^{1}H NMR (CD_3OD): δ = 7.60 (d, 2 H, *J* = 2.2 Hz, Pz*5-H*), 6.35 (d, 2 H, *J* = 2.2 Hz, Pz*3-H*), 6.20 (s, 2 H, NCH_2N), 4.55 (s, 4 H, PzCH_2Br). – ^{13}C NMR (CD_3OD): δ = 150.4 (*C-3*), 131.2 (*C-5*), 107.4 (*C-4*), 65.2 (N*C*H_2N), 24.4 (*C*H_2).

3,4′-Dibromomethyl-1,1′-methylenbis(1*H*-pyrazole) (17): It was obtained from 0.69 g (3.3 mmol) of the corresponding diol **13** and 2.2 g (8.3 mmol) of PBr_3 in 50 mL of CH_3CN. The reaction was complete after 2.5 h of heating at reflux. The white solid was recrystallized from toluene. Yield: 67%. – M.p. 124–126 °C. – ^{1}H NMR (CD_3OD): δ = 7.69 (s, 1 H, Pz*5′-H*), 7.58 (d, 1 H, *J* = 2.5 Hz, Pz*5-H*), 7.56 (s, 1 H, Pz*3′-H*), 6.35 (d, 1 H, *J* = 2.5 Hz, Pz*4-H*), 6.20 (s, 2 H, NCH_2N), 4.45 (s, 2 H, PzCH_2Br), 4.40 (s, 2 H, Pz′CH_2Br). – ^{13}C NMR (CD_3OD): δ = 150.4 (*C-3*), 140.9 (*C-3′*), 139.7 (*C-5′*), 131.6 (*C-5*), 130.2 (*C-4′*), 107.1 (*C-4*), 64.6 (N*C*H_2N), 24.0 (Pz*C*H_2Br), 22.0 (Pz′*C*H_2Br)

3-Bromomethyl-1,1′-methylenebis(1*H*-pyrazole) (16): It was obtained from 0.37 g (2.2 mmol) of the corresponding alcohol **12** and 0.9 g (3.2 mmol) of PBr_3 in 30 mL of CH_3CN. The reaction was complete after 2 h of heating at reflux. Yield: 85%. – M.p. 112–113 °C. – ^{1}H NMR (CD_3OD): δ = 7.65 (d, 1 H, *J* = 2.2 Hz, Pz*5-H*), 7.59 (d, 1 H, *J* = 2.3 Hz, Pz*3′-H*), 7.55 (d, 1 H, *J* = 1.8 Hz, Pz*5′-H*), 6.33 (d, 1 H, *J* = 2.2 Hz, Pz*4-H*), 6.30 (dd, *J* = 1.8 and 2.3 Hz, 1 H, Pz*4′-H*). 6.25 (s, 2 H, NCH_2N), 4.45 (s, 2 H, PzCH_2Br). – ^{13}C NMR (CD_3OD): δ = 150.6 (*C-3*), 141.4 (*C-3′*), 129.8 (*C-5*), 119.4 (*C-5′*), 107.9 (*C-4*), 97.6 (*C-4′*), 59.9 (N*C*H_2N), 24.6 (Pz*C*H_2Br).

3,5′-Bis[(4,7,10,13,16-pentaoxa-1-azacyclooctadecyl)methyl]-1,1′-methylenebis(1*H*-pyrazole) (4): This compound was obtained from 0.8 g (3 mmol) of 1-aza-18-crown-6 and 1 g (3.5 mmol) of Ag_2CO_3 in 90 mL of CH_3CN and 0.5 g (1.5 mmol) of the corresponding dibromo derivative **14** in 60 mL of CH_3CN. The reaction was complete after 6 h of heating at reflux. Yield 86%. – ^{1}H NMR (CD_3OD): δ = 7.62 (d, 1 H, *J* = 2.7 Hz, 1 H, Pz*5-H*), 7.30 (d, 1 H, *J* = 1.7 Hz, Pz*3′-H*), 6.47 (s, 2 H, NCH_2N), 6.10 (d, 1 H, *J* = 2.7 Hz, Pz*4-H*), 6.04 (d, *J* = 1.7 Hz, 1 H, Pz*4′-H*), 3.79 (s, 2 H, Pz′CH_2N), 3.40–3.60 (m, 42 H, OCH_2 and PzCH_2N), 2.68 (t, *J* = 7 Hz, 8 H, OCH$_2$CH_2N). – ^{13}C NMR (CD_3OD): δ =152.1 (*C-3*), 142.0 (*C-5′*), 141.2 (*C-3′*), 132.7 (*C-5*), 109.5 (*C-4′*), 108.6 (*C-4*), 69.8–70.1 (O*C*H$_2$), 64.3 (N*C*H$_2$N), 62.1 (Pz′*C*H$_2$N), 60.3 (PzCH$_2$N), 55.0 (N*C*H$_2$CH$_2$O); 50.0 (N*C*H$_2$CH$_2$O). – MS (FAB+); observed *m*/*z*: 699.4292; calculated 699.4293.

3,3′-Bis[(4,7,10,13,16-pentaoxa-1-azacyclooctadecyl)methyl]-1,1′-methylenebis(1*H*-pyrazole) (2): It was obtained from 0.8 g (3 mmol) of 1-aza-18-crown-6 and 1 g (3.5 mmol) of Ag_2CO_3 in 200 mL of CH_3CN and 0.5 g (1.5 mmol) of the corresponding dibromo derivative **15** in 60 mL of CH_3CN. The reaction was complete after 5 h of heating at reflux. Yield: 93%. – ^{1}H NMR (CD_3OD): δ = 7.51 (d, 2 H, *J* = 2.1 Hz, Pz*5-H*), 6.22 (d, 2 H, *J* = 2.1 Hz, Pz*4-H*), 6.18 (s, 2 H, NCH_2N), 3.70 (s, 4 H, PzCH_2NCH$_2$), 3.60–3.80 (m, 40 H, OCH_2), 2.80 (t, *J* = 7 Hz, 8 H, PzCH$_2$NCH_2). – ^{13}C NMR (CD_3OD): δ = 51.3 (*C-5*), 130.0 (*C-3*), 107.0 (*C-4*), 70.5–69.3 (O*C*H$_2$), 64.9 (N*C*H$_2$N), 53.4 N*C*H$_2$CH$_2$O), 48.9 (PzCH$_2$N). MS (FAB+); observed *m*/*z*: 699.4295; calculated 699.4293.

3,4′-Bis[(4,7,10,13,16-pentaoxa-1-azacyclooctadecyl)methyl]-1,1′-methylenebis(1*H*-pyrazole) (3): It was obtained from 0.7 g (2.7 mmol) of 1-aza-18-crown-6 and 0.8 g (3 mmol) of Ag_2CO_3 in 150 mL of CH_3CN and 0.4 g (1.4 mmol) of the corresponding dibromo derivative **17** in 15 mL of CH_3CN. The reaction was complete after 5 h of heating at reflux. Yield: 70%. – ^{1}H NMR (CD_3OD): δ = 7.53 (s, 1 H, Pz*5′-H*), 7.52 (d, 1 H, *J* = 1.9 Hz, Pz*5-H*), 7.44 (s, 1 H, Pz*3-H*), 6.24 (d, *J* = 1.9 Hz, 1 H, Pz*4-H*), 6.18 (s, 2 H, NCH_2N), 3.72 (s, 2 H, PzCH_2N), 3.55–3.70 (m, 40 H, OCH_2), 2.80 (t, *J* = 7 Hz, 4 H, PzCH$_2$NCH_2), 2.70 (t, *J* = 7 Hz, 4 H, Pz′CH$_2$NCH_2). – ^{13}C NMR (CD_3OD): δ = 150.9 (*C-3*), 140.8 (*C-3′*), 129.9 (*C-5*), 128.9 (*C-5′*), 118.9 (*C-4′*), 106.8 (*C-4*), 70.2–69.8 (O*C*H$_2$), 64.6 (N*C*H$_2$N), 53.1 (N*C*H$_2$CH$_2$O), 52.8 (N*C*H$_2$CH$_2$O′), 52.0 (PzCH$_2$N), 48.6 (Pz′*C*H$_2$N). – MS (FAB+); observed *m*/*z*: 699.4298; calculated 699.4293.

3-[(4,7,10,13,16-Pentaoxa-1-azacyclooctadecyl)methyl]-1,1′-methylenebis(1*H*-pyrazole) (1): It was obtained from 0.5 g (1.7 mmol) of 1-aza-18-crown-6 and 0.5 g (1.8 mmol) of Ag_2CO_3 in 150 mL of CH_3CN and 0.4 g (1.7 mmol) of the monobromo derivative **16** in 30 mL of CH_3CN. The reaction was complete after 8 h of heating at reflux. Yield: 86%. – ^{1}H NMR (CD_3OD): δ = 7.63 (s, 1 H, *J* = 2.2 Hz, Pz*5-H*), 7.56 (d, 1 H, *J* = 2.1 Hz, Pz*5′-H*), 7.53 (d, 1 H, *J* = 1.6, Pz*3′-H*), 6.25–6.28 (m, 2 H, Pz*4-H* and Pz*4′-H*). 6.23 (s, 2 H, NCH_2N), 3.73 (s, 2 H, PzCH_2N), 3.46–3.64 (m, 26 H, OCH_2), 2.79 (t, 4 H, *J* = 6 Hz, PzCH$_2$NCH_2). – ^{13}C NMR (CD_3OD): δ = 151.7 (*C-3*), 140.4 (*C-3′*), 130.2 (*C-5*), 129.3 (*C-5′*), 107.2 (*C-4*), 106.8 (*C-4′*), 70.0–69.0 (O*C*H$_2$), 65.6 (N*C*H$_2$N), 53.5 (PzCH$_2$N), 52.5 (N*C*H$_2$CH$_2$O). – MS (FAB+); observed *m*/*z*: 424.2348; calculated 424.2260. – $C_{20}H_{33}N_5O_5{\cdot}H_2O$ (441.5): calcd.: C 54.41, H 7.99, N 15.86; found: C 54.47, H 7.71, N 15.57.

3,5′-Bis(diethylaminomethyl)-1,1′-methylenebis(1*H*-pyrazole) (18): To a solution of 0.51 mg (7 mmol) of diethylamine and 1 g (9.5 mmol) of Na_2CO_3 in 30 mL of CH_3CN warmed at 40 °C for 45 min, was added dropwise for 90 min, 0.2 g (0.6 mmol) of **14** in 20 mL of CH_3CN. The mixture was heated under reflux for 3 h, and an additional 0.36 g (5 mmol) of diethylamine was added. The reaction mixture was heated under reflux overnight, and the solvent was removed in vacuo. The solid residue was treated with 50 mL of water and 50 mL of CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic extracts were worked up as usual. The resulting solid was purified by flash chromatography on silica-gel, affording **18** as a yellow oil. Yield: 51%. ^{1}H NMR ($CDCl_3$): δ = 7.55 (d, 1 H, *J* = 2 Hz, Pz*3′-H*), 7.45 (d, 1 H, *J* = 1.8 Hz, Pz*5-H*), 6.45 (s, 2 H, NCH_2N), 6.18 (d, 1 H, *J* = 1.8 Hz, Pz*4-H*), 6.12 (d, 1 H, *J* = 2 Hz, Pz*4′-H*), 3.75 (s, 2 H, PzCH_2N(Et)$_2$), 3.65 (s, 2 H, Pz′CH_2N(Et)$_2$), 2.50 (q, 8 H, *J* = 7.5 Hz, NCH_2CH$_3$), 1.10 (t, 12 H, *J* = 7.5 Hz, NCH$_2$CH_3). – ^{13}C NMR ($CDCl_3$): δ = 150.0 (*C-3*), 141.4 (*C-5′*), 140.0 (*C-3′*), 130.3 (*C-5*), 108.1 (*C-4′*), 107.3 (*C-4*), 63.1 (N*C*H$_2$N), 49.5 (Pz′*C*H$_2$N), 48.5 (Pz*C*H$_2$N), 47.0 (*C*H$_2$), 46.9 (*C*H$_2$), 12.0 (*C*H$_3$), 9.7 (*C*H$_3$). – MS (EI); *m*/*z* (%): 318 (11) [M$^+$].

Zn Complex of 18: A solution of 20 mg (0.06 mmol) of diamine **18** and 20 mg (0.06 mmol) of ZnI_2 in 3 mL of CH_3CN was heated under reflux for 1 h. The solvent was then removed and the resulting solid was used directly, without further purification. – ^{1}H NMR ($CDCl_3$): δ = 7.68 (s, 1 H, Pz*5-H*), 7.55 (d, 1 H, *J* = 2.1 Hz, Pz*3′-H*), 6.66 (s, 2 H, NCH_2N), 6.31 (d, 1 H, *J* = 2.1 Hz, Pz*4-H*), 6.22 (s, 1 H, Pz*4′-H*), 3.83 (s, 2 H, PzCH_2N), 3.75 (s, 2 H, Pz′CH_2N), 2.90 (q, 4 H, *J* = 7 Hz, CH_2), 2.60 (q, 4 H, *J* = 7 Hz, CH_2′), 1.15 (t, 6 H, *J* = 7 Hz, CH_3), 1.00 (t, 6 H, *J* = 7 Hz, CH_3′). – ^{13}C NMR ($CDCl_3$): δ = 141.7 (C-5′); 141.3 (C-3′); 133.9 (C-5); 108.4 (C-4′); 105.6 (C-4); 62.0 (NCH$_2$N); 50.6 (Pz′CH$_2$N); 48.8 (PzCH$_2$N); 46.0 (NCH$_2$); 10.0 (CH$_3$).

Pd Complex of 18: A solution of 20 mg (0.06 mmol) of diamine **18** and 10 mg (0.06 mmol) of $PdCl_2$ in 3 mL of methanol was stirred for 24 h. The mixture was filtered and the solvent removed affording a dense brownish oil, which was used without further purification. – ^{1}H NMR ($CDCl_3$): δ = 8.20 (d, 1 H, *J* = 2.7 Hz, Pz*5-*

H), 8.14 (d, *J* = 2.3 Hz, 1 H, Pz*3′-H*), 6.61 (s, 2 H, NCH_2N), 6.60 (d, 1 H, *J* = 2.7 Hz, Pz*4-H*), 6.55 (d, 1 H, *J* = 2.3 Hz, Pz*4′-H*), 4.10 (s, 2 H, PzCH_2N), 3.85 (s, 2 H, Pz′CH_2N), 3.04–2.81 (m, 4 H, NCH_2CH$_3$), 2.60 (q, 4 H, *J* = 7 Hz, CH$_2$), 1.65 (t, 6 H, *J* = 7 Hz, CH_3); 1.00 (t, 6 H, *J* = 7 Hz, CH_3). – ^{13}C NMR (CDCl$_3$): δ = 158.3 (*C-3*), 149.5 (*C-5′*), 147.5 (*C-3′*), 136.6 (*C-5*), 109.9 (*C-4′*), 105.7 (*C-4*), 62.8 (NCH$_2$N), 60.1 (Pz′CH$_2$N), 59.6 (PzCH$_2$N), 48.0 (NCH$_2$), 10.0 (CH$_3$).

Acknowledgments

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[11] The most intense peaks corresponded to the protonated azacrown (*m*/*z* = 264.2) and to the uncomplexed protonated hosts (*m*/*z* = 699.4), showing that under the FAB+ conditions, even the uncomplexed hosts are relatively unstable.

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[13] In the process of revision of the paper, one of the referees objected that since ammonium transport was indirectly measured by the pass of its picrate counterion, Zn^{2+} could constitute another vector for picrate transport, thus adulterating our results. Had this phenomenon really taken place, the observed positive and negative allostery would be lower and 'higher', respectively, than alleged. To definitely answer this question, we ran a control experiment, in which we placed Zn^{2+} in both donor and acceptor phases as usual (see experimental section), and checked the possible transport of picrate using 1,5-pentanediammonium picrate in the donor phase, and bispyrazolylmethane in the organic phase, whose NMR data clearly indicated that it binds Zn^{2+} in CDCl$_3$. In a much longer time than that used for our transport experiments, the UV/Vis spectra of the acceptor phase ('without dilution') did not reveal the presence of even traces of picrate. Concentration of the acceptor phase and extraction of the residue with CD$_3$OD did not show the singlet at 8.8 ppm of the picrate nor any signal belonging to the amine.

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