A Cyclophane Receptor for the Selective Complexation of Adenine Derivatives in Water

M. Carmen Rotger, José F. González, Pablo Ballester, Pere M. Deyà, and Antoni Costa*

Departament de Química, Universitat de les Illes Balears, 07071 Palma de Mallorca, Spain

Received January 25, 1994®

A water-soluble oxacyclophane based upon *p*-xylylenebis[4-hydroxy-3-carboxybenzene] and *p*-xylene units tethered trough an aliphatic chain has been synthesized. The receptor binds selectively to neutral adenine derivatives forming an intracavity 1:1 complex. The interaction energies for the complexes range from 7 to 13 kJ mol^{-1} in alkaline D_2O. The geometry of the complex between **1**(dicarboxylate) and 9-ethyladenine has been mapped out through NMR studies and is also supported by molecular modeling and theoretical AM1 calculations.

Complexation of neutral nucleotide bases has been extensively studied in order to understand their behavior in complex biological systems.[1] Although numerous studies using synthetic receptors in apolar organic solvents have shown that hydrogen bonding[2] alone or combined[3] with aromatic stacking are the most significant binding forces, there have been only few and recent efforts to experimentally measure and determine the role and energetic contribution of the different binding forces for nucleotide bases in water.[4,5] In this work we report the synthesis and characterization of a new water-soluble macrocyclic receptor that features a high selectivity for binding neutral adenine derivatives.

The oxacyclophane **1** is composed of 1,4-dibenzylbenzene and *p*-xylene units tethered through an aliphatic chain. The location of the four aromatic rings in **1** creates a cavity that allows two efficient T-shaped edge to face and two edge offset stacking contacts to take place[6] as is shown schematically in Figure 1.[7a] This structural arrangement provides a unique binding geometry with respect to the cyclophane. The length of the connecting chain in **1** was selected slightly larger than that required to accommodate the adenine ring (ca. 6.6 Å).[7] Modeling studies suggested that receptor **1** could adopt a symmetrical, rhomboid-shaped conformation that produce a cavity (ca. 10 × 7.6 Å) well suited for binding adenine derivatives (see below).

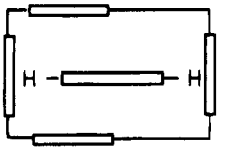


Figure 1. Schematic of complex geometry.

Oxacyclophane **1** was synthesized as shown in Scheme 1. Briefly, condensation of 5-bromosalicylic acid dilithium salt with terephthalaldehyde and further elaboration yielded the dimethyl ester **4a**.[8] Condensation of **4a** with dichloride **5** under high dilution conditions,[9] followed by alkaline hydrolysis, yielded the macrocyclic diacid **1**, in 8% overall yield from **4a**. Cyclophane **1** is insoluble in neutral or acidic water but dissolves easily in alkaline water[10] up to 10^{-2} M.

The cyclic structure of this compound is consistent with the magnitude and sign of the cyclization-induced proton NMR shifts measured on **1** (methyl ester) and the open counterpart **4b** in $CDCl_3$. The observed values (−0.07, −0.05, and 0.03 ppm for H_{26}, H_{39}; H_{25}, H_{40}; and H_{23}, H_{36}, respectively) are within the ranges set by literature precedents.[11,12]

® Abstract published in *Advance ACS Abstracts,* July 1, 1994.

(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1988. *Nucleic Acid-Metal Ion Interactions*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1980.

(2) (a) Zimmerman, S. C. In *Bioorganic Chemistry Frontiers 2*; Dugas, H., Ed.; Springer-Verlag: Berlin, 1991; pp 35–70. (b) Lindsey, J. S.; Kearney, P. C. *J. Am. Chem. Soc.* **1988**, *110*, 6575. (c) Tjivikua, T.; Deslongchamps, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 8408. (d) Jorgensen, W.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008.

(3) (a) Benzing, T.; Wolfe, J.; Rebek, J., Jr. *Science* **1988**, *242*, 266. (b) Askew, B.; Ballester, P.; Buhr, C.; Jeong, K.-S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082. (c) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K.-S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090. (d) Jeong, K.-S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 3327. (e) Zimmerman, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054. (f) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055. (g) Park, T. K.; Schroeder, J.; Rebek, J., Jr. *Tetrahedron* **1991**, *47*, 2507. (h) Goswami, S.; Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 3425. Conn, M. M.; Deslongchamps, G.; de Mendoza, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 3548.

(4) Rotello, V. M.; Viani, A. E.; Deslongchamps, G.; Murray, B. A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 797.

(5) (a) Doig, A. J., Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338. (b) Williams, D. H.; Cox, J. P. L.; Doig, A. J.; Gardner, M.; Gerhard, U.; Kaye, P. T.; Lal, A. L.; Nicholls, I. A.; Salter, C. J.; Mitchell, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 7020. (c) Searle, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 10690. (d) Searle, M. S.; Williams, D. H.; Gerhard, U. *J. Am. Chem. Soc.* **1992**, *114*, 10697.

(6) T-shaped contacts for benzene dimer in water have been suggested by MD calculations to be particularly favorable. See: (a) Linse, P. *J. Am. Chem. Soc.* **1992**, *114*, 4366. (b) Jorgensen, W. L. Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768. For a discussion on favorable geometries for stacking in cyclophane hosts see: Hunter C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.

(7) (a) Diederich, F. In *Cyclophanes*; Stoddart, J. F., Ed.; Royal Society of Chemistry: Cambridge, 1991; pp 85 and 91. (b) Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 362. (c) Diederich, F.; Dick, K.; Griebel, D. *Chem. Ber.* **1985**, *118*, 3588.

(8) Rotger, M. C.; Costa, A.; Saá, J. M. *J. Org. Chem.* **1993**, *58*, 4083.

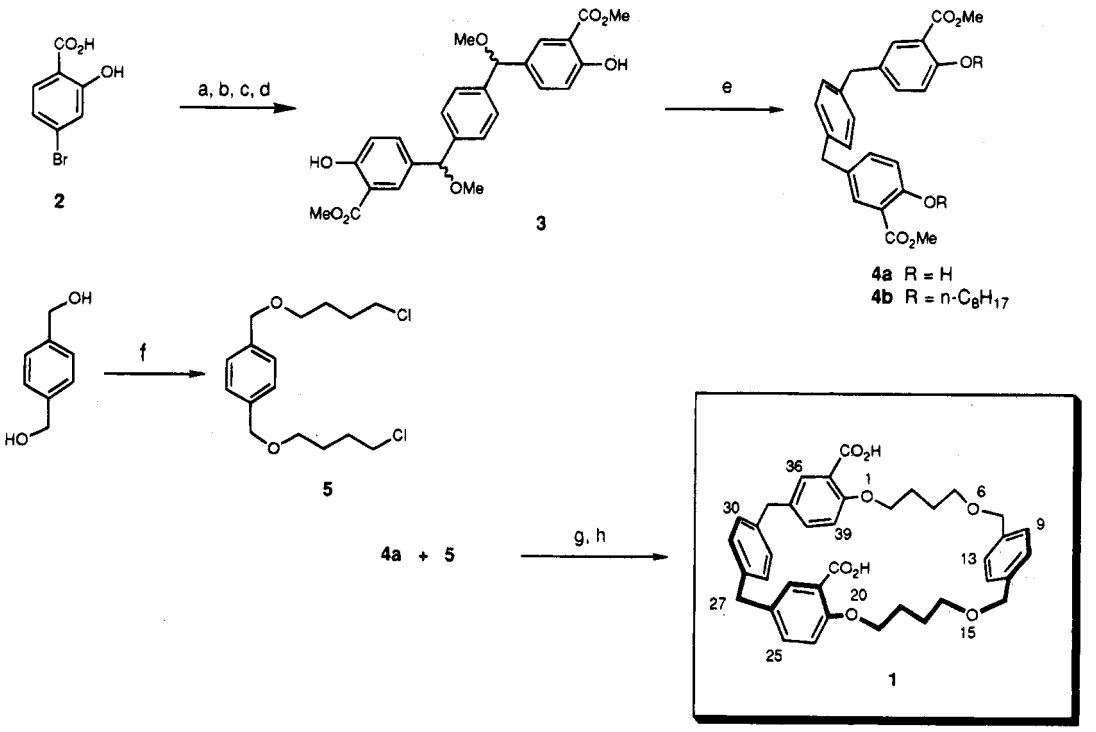
(9) Carcanague, D. R.; Knobler, C. B.; Diederich, F. *J. Am. Chem. Soc.* **1992**, *114*, 1515.

(10) For other examples of water-soluble cyclophanes bearing carboxylate groups see: (a) Dhaenens, M.; Lacombe, L. Lehn, J.-M.; Vigneron, J.-P. *J. Chem. Soc., Chem. Commun.* **1984**, 1097. (b) Vögtle, F.; Merz, T.; Wirtz, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 221.(c) Schneider, H.-J.; Blatter, T. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1163. (d) McCurdy, A.; Jimenez, L.; Stauffer, D. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 10314 and references cited therein. (e) Méric, R.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 129.

(11) (a) Odashima, K.; Koga, K. In *Cyclophanes II*; Keehn, P. M., Rosenfeld, S. M., Eds.; Academic Press: New York, 1983; p 640. (b) Jarvi, E. T. Whitlock, H. W., Jr.; Jarvi, E. T. *J. Am. Chem. Soc.* **1980**, *102*, 657. (c) Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 3910.

0022-3263/94/1959-4501$04.50/0 © 1994 American Chemical Society

Scheme 1[a]



[a] Key: (a) LiOMe, MeOH, then drying at 60 °C/0.05 mmHg; (b) t-BuLi, THF, −90 °C, then terephthalaldehyde, THF, −40 °C to room temperature; (c) MeOH, TsOH, room temperature; (d) diazomethane, Et_2O (40% based on terephthalaldehyde); (e) H_2, 10% Pd−C, MeOH−$CHCl_3$ (54%); (f) NaH, DMF, 1,4-dichlorobutane, room temperature (50%); (g) Cs_2CO_3, DMF, 90 °C, high dilution (20%); (h) NaOH, H_2O−THF, 80 °C (40%).

Table 1. Association Constants and Limiting Complexation Shifts of Adenine Derivatives with Cyclophane 1[a]

guest	(Δδ) (H_8)	(Δδ) (H_2)	K_a[b] (M^{-1})
purine			<5
adenine	−0.54	−0.29	22 ± 5
9-ethyladenine	−0.55	−0.28	185 ± 15
adenosine[c]	−0.52	−0.48	80 ± 15
N-6-benzyladenosine	−0.50	−0.42	181 ± 20

[a] Measurements in alkaline D_2O (Na_2CO_3/$NaDCO_3$ buffer, at pD = 10.6). [b] Titrations were performed at a constant guest concentration of 0.5−0.7 mM (see Experimental Section for details). [c] Reference 12.

Titrations were performed by addition of a solution of the receptor **1** in alkaline D_2O to a solution of the nucleobase guest in the same solvent. Reverse titrations resulted in characteristic NMR upfield shifts of the H_2 and H_8 protons in all the tested adenine derivatives. The association constants for the 1:1 complexes were obtained by monitoring these shifts (Δδ) by using the HOSTEST II program[30] (see Table 1).

A direct titration of **1** with 9-ethyladenine revealed significant upfield shifts in the aliphatic chain as well as protons of the carboxyl-bearing aromatic rings in the cyclophane.[13] Interestingly, the resonances arising from the two noncarboxyl-substituted aromatic rings were almost unaffected by the presence of 9-ethyladenine as anticipated due to their perpendicular location to the adenine nucleus.

Taken together these results indicate that host **1** interacted with the adenine moiety in a 1:1 intracavity complex[14] in which the residues appended on N_6 and N_9 protruded above and below the cavity.

The geometry of the complex between **1** and 9-ethyladenine in solution was further investigated by steady-state NOE. The intermolecular contacts[15,16] observed between adenine (H_2) and the aliphatic chain of the receptor (H_5 and H_{16}) provide direct evidence of the intracavity complex. On the other hand, the detection of NOE enhancements with similar magnitudes on both noncarboxyl-bearing aromatic rings suggests the existence of different binding modes of complexation. Indeed, by using CPK models several modes of introducing the adenine ring within the cavity can be envisaged. The selectivity of the receptor for adenine is illustrated by the fact that the ^{1}H-NMR spectra of different nucleobases,[17] namely hypoxantine, cytosine, uracil, and thymine, did not show any significant change upon addition of a large excess of the receptor. However, despite the affinity for the adenine nucleus,[18] the complexation of phosphorylated adenine derivatives (AMP^{2-}, ADP^{3-}, and

(12) Adenosine showed an upfield shift of −0.2 ppm for H1′ of the ribose ring. This is in accordance with recently published observations; see ref 14. The signals of other substituents appended to N_6 and N_9 did not change appreciably upon complexation.

(13) Limiting complexation-induced shift values (ppm): −0.48 for $H_{26,39}$; −0.42 for $H_{2,19}$; −0.2 for $H_{5,16}$. Undetermined upfield shifts were also noticed for $H_{25,40}$ and the middle methylene hydrogens of the chain. The rest of the spectra remained essentially unchanged.

(14) (a) Schneider, H.-J.; Blatter, T.; Palm, B.; Pfingstag, U.; Rüdiger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704. (b) See ref 7a; p 99.

(15) Neuhaus, D.; Williamson, M. In *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 1989.

(16) NOEs measured at 300 MHz on a sample containing a mixture 3:1 of 9-ethyladenine and receptor, by NOE difference spectroscopy, were 1.2% on H_2 of 9-ethyladenine by saturating the methylenic protons $H_{5,16}$ of the receptor and 0.9% and 0.8% on both noncarboxyl-bearing aromatic rings of the receptor by saturating the methylenic protons of 9-ethyladenine. The reported percentages were calculated by direct integration of the difference spectrum assigning an integration value of 100% to the saturated signal.

(17) For binding of nucleobases and nucleosides other than adenine derivatives in water see Kobayashi, K.; Asakawa, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10307.

ATP^{4-}) was not detected. This behavior can be explained on the basis of electrostatic repulsion between the phosphate groups of the nucleotide and the carboxylate residues of the host. At this point, one might be tempted to account for the observed selectivities simply using the fact that adenine offers more lipophilic surface area than other nucleobases.[19]

In order to obtain three-dimensional structures accounting for the observed NMR effects and to gain insight on the intermolecular forces involved in the complexation, we performed a computational study of the complex between 9-ethyladenine and **1**. 9-Ethyladenine was chosen as target guest for this exploration because it is the simplest adenine derivative featuring a notable association constant.

Initial geometries for cyclophane **1** were located through a Monte Carlo search of conformational space (see Experimental Section). Among the lowest energy conformations generated by the conformational search, two were selected, one having the two carboxylate groups oriented on the same side and the other having them on opposite sides. Then, the geometries of the two selected forms were reoptimized at the semiempirical SCF-MO AM1 level. The energy difference calculated between the fully minimized conformers was 2.5 kcal/mol, with the cisoid form being of lower energy.

Taking the above-calculated cisoid form as the starting geometry of **1**, four independent geometries of the complex were generated by docking a previously AM1-minimized structure of 9-ethyladenine within the host cavity. In two of these structures the amino group of the adenine is pointing toward the carboxylate residues of the macrocycle. Each geometry was, in turn, fully optimized at the AM1 level using the recently described COSMO[20] continuum model for solvation[21] and the eigenvector following method of geometry optimization.[22]

The observation of the final minimized structures revealed that in two cases the 9-ethyladenine remained inside the cavity while in the two other cases the adenine was pulled out of the cavity. It is worthwhile to note that the two successful binding geometries obtained this way arise from starting geometries having the amino group of the adenine on the same side as the carboxylate groups. Due to the limited starting geometries of the complex these results, at best, would suggest that the interaction of the amino group with the carboxylates might also be of importance for complexation to take place. This would offer a complementary explanation for the observed selectivity toward the adenine nucleus.

The structures of both complexes[23] (Figure 2a,b) are in good agreement with both shielding and NOE effects described above. Inspection of the complex in Figure 2a, shows that H_2 of 9-ethyladenine is close to H_5 (2.97 Å) and H_{16} (2.43 Å) protons. In Figure 2b, the methylenic protons of the ethyl group in 9-ethyladenine are located relatively close to aromatic H_{30}, H_{32} and H_{29}, H_{33} protons accounting for the detected NOEs.

In conclusion, the oxacyclophane **1** forms intracavity complexes with neutral adenine derivatives. Experimental evidence as well as theoretical calculations support the existence of several geometries for these complexes. The analysis of the K_a values indicates that the hydrophobic effect is the main driving force for binding. Thus, the presence of lipophilic substituents at N_6 and/or N_9 of adenine also contribute to the overall binding.[24] In addition, in the present work we have also demonstrated the feasibility of modeling the complexes using semiempirical methods[25] with solvation treatment.

Experimental Section

General. All commercially available compounds (Aldrich) were used without further purification. $CDCl_3$ (99.8% D) and D_2O (99.8% D, Euriso-top) were used from freshly opened vials. Flash chromatography was performed on Merck Silica Gel 60 (230–400 mesh) according to the method of Still.[26] Melting points were taken on a capillary melting point apparatus and are uncorrected. Low-resolution EI mass spectra were recorded at 70 eV ionizing energy. ^{13}C NMR spectra were obtained on a 75-MHz spectrometer in $CDCl_3$ or D_2O. ^1H NMR spectra in D_2O were recorded on a Bruker AMX-300 spectrometer equipped with a reverse broad-band probe. Chemical shifts are reported as parts per million (δ) relative to sodium 3-(trimethylsilyl)-1-propanesulfonate as external standard for D_2O. Peak assignments were made on the basis of monodimensional ^1H NMR experiments. Steady-state NOE experiments were made using the NOEMUL[27] pulse program as implemented in the Bruker software (eight frequencies per multiplet, D20 = 0.02 s; L4 = 300 cycles, DL0 = 62 db) total saturation time 4.8 s). Eight transients were acquired with the decoupler on resonance followed by eight reference transients. Typically 1000–2000 transients were collected.

Titrations. NMR titrations were performed at 300 MHz in a 5-mm-o.d. NMR tube at ambient temperature (approximately 21 °C). Stock solutions were prepared in alkaline D_2O (Na_2CO_3/$NaDCO_3$ buffer, at pD = 10.6). In "reverse" titrations 500 μL of nucleobase (0.5–0.7 mM) was treated with aliquots of a solution containing both receptor **1** (4 mM) and the same nucleobase (0.5–0.7 mM), and changes in the chemical shifts of H_2 and H_8 of the adenine derivative were monitored. In the experimental conditions described above, the guest concentration is kept constant and the concentration of adenine dimers is negligible.[28] For the "forward" titration, **1** (0.4 mM) was titrated with a solution of 9-ethyladenine (4 mM) prepared as above. Residual hydroxyl protons of D_2O were partially eliminated by presaturation.[29]

(18) Assuming that $K_a < 5$ for noninteracting nucleobases, the selectivity for the adenine nucleus in terms of $\Delta\Delta G$ range from 6 to 9 kJ mol^{-1}.

(19) See ref 1, p 135 and ref 14a.

(20) COSMO (conductor-like screening model) evaluates the solvent screening energy for a cavity based on the solvent accessible surfaces and for a charge distribution derived from a distributed multipole analysis: Klamt, A.; Schuurmann, G. *J. Chem. Soc., Perkin. Trans. 2* **1993**, 799.

(21) Inclusion of solvation effects in our calculations are necessary at this point, since in gas-phase calculations the amino group of adenine collapsed with one of the two carboxylate groups of **1**. Of course, this option considerably increases the cpu computer time, mainly with large molecules as in the present case; i.e., starting from the separately AM1-optimized geometry for both **1** and 9-ethyladenine, the AM1(COSMO) calculation of a complex geometry takes 3 days and 21 h of cpu time on an Alpha AXP DEC3000/500 Digital workstation.

(22) Minimization of the energy with eigenvector following (EF) mode must be carefully controlled since, in some cases, important conformational changes in the cyclophane ring are induced by introducing the 9-ethyladenine within the cavity. To assure a successful optimization the Hessian must have the correct structure during the calculation.

(23) AM1(COSMO) heats of formation (kcal/mol) of the involved species are the following: −510.65 for **1**, 63.20 for 9-ethyladenine, −424.87 for the complex shown in Figure 2a, and −420.86 for the complex in Figure 2b.

(24) Nowick, J. S.; Chen, J. S.; Noronha, G. *J. Am. Chem. Soc.* **1993**, *115*, 7636.

(25) Topiol, S.; Talbot, G. *J. Am. Chem. Soc.* **1990**, *112*, 8734.

(26) Still, W. C.; Hahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(27) Kins, M.;Sanders, J. K. M. *J. Magn. Reson.* **1984**, *56*, 518.

(28) Assuming that $K_a = 13\ M^{-1}$ (see ref 4) for the self-association of 9-ethyladenine, for a total concentration of 0.5 mM, the calculated concentration of dimeric adenine is 0.6% of the total (free + dimeric) 9-ethyladenine (0.5 mM) and is therefore negligible.

(29) Hore, P. J. *Methods Enzymol.* **1989**, *176*, 3

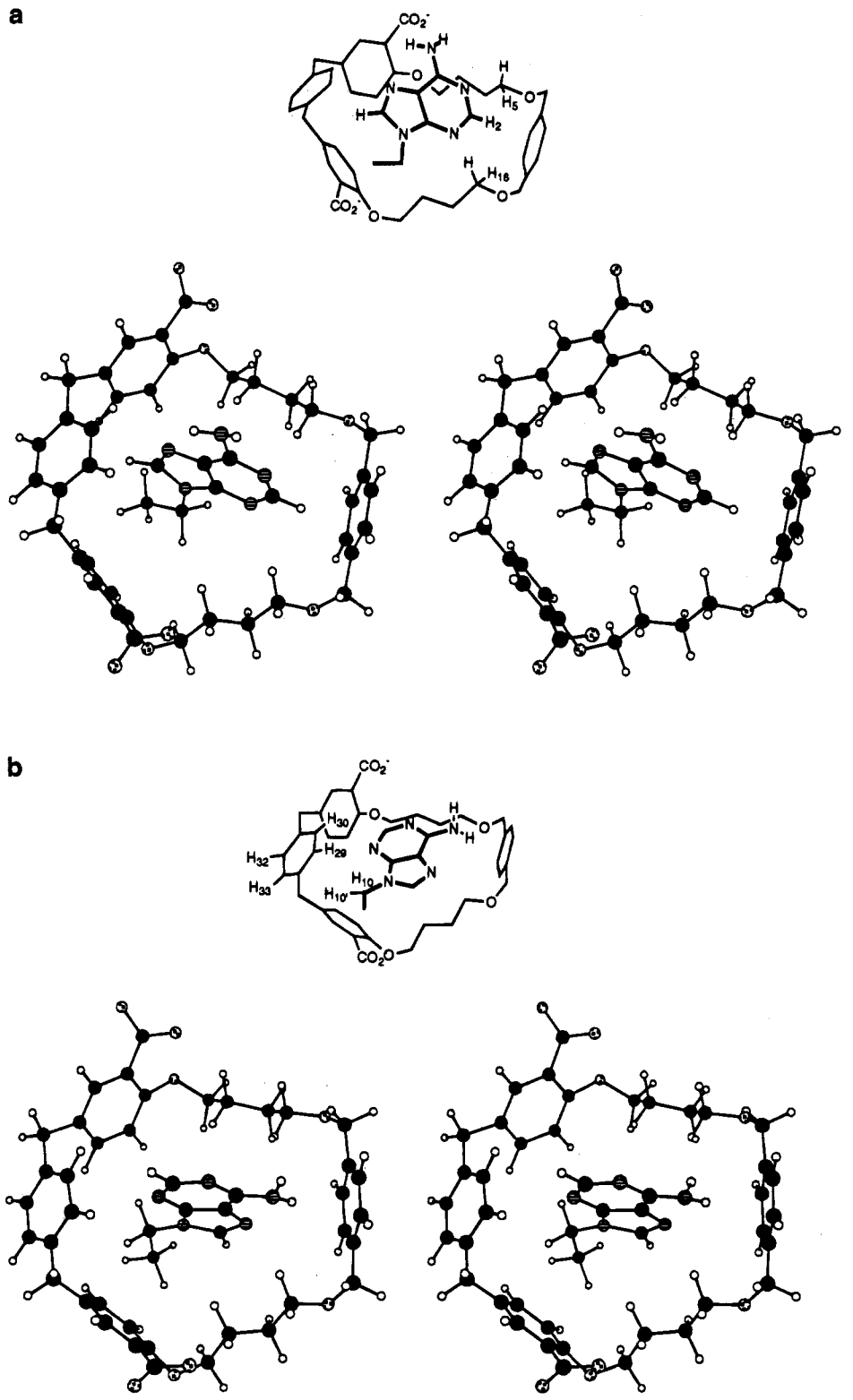


Figure 2. Stereoviews of the calculated AM1-optimized geometries for the 1:1 complex between **1** and 9-ethyladenine. Some selected distances are given: (a) H_2 to H_{16} 2.43 Å, H_2 to H_5 2.97 Å; (b) H_{10} to H_{30} 4.71 Å, H_{10} to H_{32} 4.70 Å, $H_{10'}$ to H_{29} 4.10 Å, $H_{10'}$ to H_{33} 4.26 Å.

The HOSTEST II program[30] was used to estimate association constants, along with the limiting chemical shifts of each of the monitored protons at saturation degrees ranging from 20 to 50%. The reported values are the average of the association constants obtained for H_2 and H_8 of the adenine nucleus in three independent titrations.

Molecular Modeling and Theoretical Calculations. All molecular modeling was performed on a Silicon Graphics Personal Iris workstation (4D/35) using MacroModel 3.1X.[31] The Monte Carlo[32] random-search method for finding the low-energy conformations for **1**(dicarboxylate) was run in the MacroModel version of the MM2[33] force field with GB/SA[34]

(30) Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H. J., Dürr, H., Eds.; VCH: Weinheim, 1991; p 123.

(31) MacroModel 3.1X: Still, W. C. Columbia University.

(32) Chang, G.; Guida, W. C.; Still, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

solvation treatment in water. Automatic setup, standard cutoffs, and a distance constraint of 7–14 Å between C_{14} and C_{27} to avoid total collapse of the receptor cavity were used. The 700 structures generated during the conformational search were minimized to yield 450 unique conformers. Twenty-six structures were within 3 kcal/mol of the lowest energy conformer; among them, the two structures of minimum energy having cis and trans orientations of the carboxylate groups and featuring an open cavity enough to fit the adenine nucleus were selected as starting point in semiempirical calculations.

Molecular orbital calculations were carried out at the AM1[35] level using the MOPAC 93 program package[36] running on Digital Alpha AXP DEC3000/500 workstations. The initial AM1 geometries were reoptimized at the semiempirical level using the recently described COSMO continuum model for solvation.[20] Minimum energy structures were located using the eigenvector following method[22] (EF) and constructing the full Hessian matrix in the initial step (option HESS = 1).

Synthesis. *p*-Xylylenebis[4-hydroxy-3-(methoxycarbonyl)benzene] (4a) was prepared as recently described.[8]

Bis(4-chlorobutyl)-1,4-xylylene diether (5). 1,4-Benzenedimethanol (3.0 g, 22 mmol) in dry DMF (45 mL) was slowly added at 0 °C to a suspension of oil-free sodium hydride (3.5 g, 116 mmol) in dry DMF (45 mL). After the mixture was stirred under argon at room temperature for 1 h, 1,4-dichlorobutane (14.4 g, 113 mmol) was added. The resulting suspension was stirred at room temperature for 12 h and then quenched by pouring the mixture into ice–water (200 mL). The crude was acidified with 1 N HCl and partitioned between water and Et_2O. The organic layer was decanted, washed with several portions of water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude concentrate was purified by bulb-to-bulb distillation to give 3.5 g (50%) of pure diether **5** as a colorless oil that solidified on standing: bp 190–3 °C (0.002 mmHg); IR (KBr) 2930, 2850, 1125, 1090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.31 (s, 4H), 4.49 (s, 4H), 3.56 (t, 4H, J = 6.5 Hz), 3.49 (t, 4H, J = 6.0 Hz), 1.88 (m, 4H), 1.76 (m, 4H); EIMS (*m/z*) 318 (0.8 M^+), 211 (9), 161 (5), 139 (6), 121 (7), 104 (27), 93 (32), 91 (100). Anal. Calcd for $C_{16}H_{24}O_2Cl_2$: C, 60.35; H, 7.60. Found: C, 60.37; H, 7.56.

1,6,15,20-Tetraoxa[7.7.1.1]paracyclophane-22,37-dicarboxylic Acid (1). A solution of dimethyl ester **4a** (606 mg, 1.6 mmol) in 70 mL of dry DMF and a solution of dichloride **5** (508 mg, 1.6 mmol) in 70 mL of dry DMF were simultaneously added dropwise during 4 h to a hot suspension of finely grounded Cs_2CO_3 in DMF (140 mL). The mixture was stirred at 90 °C for 24 h, cooled to room temperature, and concentrated in vacuo. The residue was partitioned between water and Et_2O, the organic layer was decanted, washed with water (2 × 20 mL) and brine (20 mL), and dried over Na_2SO_4. Purification by column chromatography on silica gel (AcOEt:hexane, 55/45 v/v) gave 217 mg (21%) of **1** (dimethyl ester) as a white solid: ^1H NMR (300 MHz, $CDCl_3$, ppm) 7.62 (d, 2H, J = 2.3 Hz), 7.23 (s, 4H), 7.14 (dd, 2H, J = 8.5, 2.3 Hz), 7.06 (s, 4H), 6.80 (d, 2H, J = 8.5 Hz), 4.46 (s, 4H), 4.05 (t, 4H, J = 6.3 Hz), 3.89 (s, 4H), 3.85 (s, 6H), 3.46 (t, 4H, J = 6.1 Hz), 1.85 (m, 4H), 1.76 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 166.9, 156.8, 139.0, 137.8, 133.6, 133.1, 131.8, 128.8, 127.4, 120.1, 113.7, 72.5, 69.6, 68.5, 51.9, 40.5, 29.7, 25.9, 25.7.

The solid obtained above was dissolved in THF (2 mL) and treated with aqueous NaOH (3 M, 4 mL). The mixture was heated at 80 °C for 12 h. After being cooled at 0 °C, the mixture was acidified with 6 N HCl, extracted with Et_2O (3 × 30 mL), washed with brine, and dried over Na_2SO_4 to afford 110 mg (54%) of cyclophane **1** as a white solid after recrystallization from EtOH/H_2O: mp 173–4 °C; IR (KBr) 3455, 1738, 1691 ,1610, 1494, 1428, 1239, 1085 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.04 (d, 2H, J = 2.3 Hz), 7.26 (obscured by residual $CHCl_3$), 7.22 (s, 4H), 7.06 (s, 4H), 6.88 (d, 2H, J = 8.5 Hz), 4.49 (s, 4H), 4.27 (t, 4H, J = 6.7 Hz), 3.89 (s, 4H), 3.48 (t, 4H, J = 6.0 Hz), 1.97 (m, 4H), 1.75 (m, 4H); ^1H NMR (300 MHz, D_2O, pD = 10.4, ppm) 7.17 (d, 2H, J = 2.3 Hz), 7.15 (s, 4H), 6.96 (dd, 2H, J = 8.3, 2.3 Hz), 7.04 (s, 4H), 6.76 (d, 2H, J = 8.4 Hz), 4.25 (s, 4H), 3.93 (t, 4H, J = 5.8 Hz), 3.78 (s, 4H,), 3.40 (t, 4H, J = 6.0 Hz), 1.66–1.56 (m, 8H). Anal. Calcd for $C_{38}H_{40}O_8$: C, 73.04; H, 6.46. Found: C, 72.77; H, 6.42.

Acknowledgment. We thank Professor Craig S. Wilcox for making the HOSTEST II program available to us. Time allocation for SCF-MO calculations, performed with Alpha AXP workstations, was generously provided by the Centre de Càlcul de la Universitat de les Illes Balears and by the Physics Department of UIB. We are indebted to Professor Henry S. Rzepa for making this version of MOPAC 93 available to us. We thank the DGICYT (Projects PB90-0040 and PB92-0251) for financial support. One of us (M.C.R) thanks the Ministry of Education for a fellowship.

Supplementary Material Available: Tables of Cartesian coordinates for 1(dicarboxylate) and complexes between **1** and 9-ethyladenine and NOE difference spectra (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(33) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.
(34) Still, W. C.; Tempczyk, R. C.; Hawley, R. C.; Hendricson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
(35) Dewar, M. J. S.; Zoebisch, E. G.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(36) Stewart, J. J. P. MOPAC 93, Fujitsu Limited, Tokyo, Japan (1993). Available from Quantum Chemistry Program Exchange. University of Indiana, Bloomington, IN.