Complexation of Nucleosides, Nucleotides, and Analogs in an Azoniacyclophane. van der Waals and Electrostatic Binding Increments and NMR Shielding Effects[1]

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Abstract: Binding free energies (ΔG) and complexation-induced NMR shifts (CIS) were determined for complexes in water between 16 nucleic acid components—including some analogs—and lipophilic receptor models bearing positively charged nitrogen atoms. The CIS values with up to −1.7 ppm shielding, e.g., on adenine protons, agree with earlier shielding calculations of the corresponding azacyclophane (CP66) naphthalene complex; the shifts on the host protons exerted, for example, by adenine are only about one-fourth of those exerted by naphthalene. All CIS values demonstrate intracavity inclusion for the adenine moiety, whereas the sugar parts, and in particular the pyrimidines, stay outside the CP66 cavity, although MM simulations as well as earlier measurements with related benzene guest molecules indicate that these heterocycles would also be suitable for encapsulation in CP66. The selectivity for adenine derivatives is also seen in binding constants with, for example, 1900 M^{-1} for AMP^{2-} compared to 450 M^{-1} for GMP^{2-}. ΔG values show regular differences between nucleotides and nucleosides as well as between differently charged nucleotides which can be factorized to 5 + 1 kJ mol^{-1} per salt bridge. Comparison of AMP^{2-} binding between CP66 and a structurally similar, cleftlike host with ΔG = 9.5 kJ mol^{-1} shows, numerically, the same hydrophobic cavity effect as completely different guest molecules.

Molecular recognition of nucleobases and their derivatives[2] has recently received much attention in the framework of biomimetic host–guest chemistry.[3] Electrostatic interaction between the phosphates of nucleotides and positively charged nitrogen atoms in polyammonium host compounds leads to binding constants of up to 10^{11} M^{-1} [4a] and can be rationalized with additive increments of $K \approx 10^{-1}$ or $\Delta G = 5 \pm$ kJ mol^{-1} per ion salt or bridge in water as solvent.[5] Base-selective recognition is attainable either by hydrogen bonds to suitably constructed receptors[6-8] or by stacking interactions with π-systems attached to the host compounds.[4a,9,10]

Azoniacyclophanes (CP*nn*) containing diphenylmethane units possess lipophilic cavities[11] which can accommodate nucleobases *inside*, in contrast to the macrocyclic polyammonium systems reported earlier.[4] Investigations with a large variety of substrates have revealed[12] that, for example, naphthalenes with substituents such as $X = SO_3^-$, COO^-, O^-, OPO_3H^- are predominantly bound by hydrophobic interactions, since only one contact ion pair at a time can materialize.[5a] From studies with cyclophanes[13] and other host molecules[14] lacking the ^+N groups as well as with substrates of low polarizability,[15,16] there is clear evidence that the major contribution for the association of aromatic substrates with ^+N receptor parts is the attraction with a dipole induced in the π-moiety of the guest. This van der Waals effect, which dominates in aqueous solvents due to the low polarizability of water, amounts to ~2 kJ mol^{-1} for a single ^+N···arene interactions.[5b] It is known that intercalation into DNA is greatly enhanced by the presence of positive charges in corresponding ligands.[17,18] Studies with a conformationally well-defined synthetic receptor model bearing positively charged centers can also shed light on the interaction mechanisms of DNA with polyamines and histones, which are known to be of great biological importance.[19,20]

As a major tool not only for measuring association constants K but also for providing insight into the intracavity inclusion geometry in aqueous solution, we observe NMR shift changes upon complexation. NMR shielding variation in nucleic acids, their fragments, and their complexes is a valuable method for structural evaluations.[21] In view of the many shielding mechanisms op-

(1) Host–Guest Supramolecular Chemistry. 35. Part 34: Schneider, H.-J.; Schiestel, T.; Zimmermann, P. *J. Am. Chem. Soc.*, preceding article in this issue.

(2) (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1988. (b) Ts'o, P. O. P. *Basic Principles in Nucleic Acid Chemistry*; Academic Press: New York, 1974; Vol. 1.

(3) (a) Lehn, J.-M. *Angew. Chem.* **1988**, *100*, 91; *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89. (b) See ref 2 in the preceding paper[1] and literature cited therein.

(4) For leading references, see: (a) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896. (b) Kimura, E. *Top. Curr. Chem.* **1985**, *128*, 131, 141. (c) Schmidtchen, F. P. *J. Am. Chem. Soc.* **1986**, *132*, 101. (d) Marecek, J. F.; Fischer, P. A.; Burrows, C. J. *Tetrahedron Lett.* **1988**, *29*, 6231 and references cited therein.

(5) (a) Schneider, H.-J.; Theis, I. *Angew. Chem.* **1989**, *101*, 757; *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 753. (b) Reference 1.

(6) (a) Rebek, J., Jr. *Science (Washington)* **1987**, *235*, 1478. (b) Rebek, J., Jr. *Angew. Chem.* **1990**, *102*, 261; *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245. (c) Rebek, J., Jr. *Acc. Chem. Res.* **1990**, *23*, 399. (d) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090 and earlier references.

(7) (a) Hamilton, A. D.; Pant, N.; Muehldorf, A. V. *Pure Appl. Chem.* **1988**, *60*, 533. (b) Muehldorf, A. V.; Engen, D. V.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561.

(8) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055.

(9) Zimmermann, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054.

(10) Kim, M.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1987**, 1686.

(11) Odashima, K.; Koga, K. In *Cyclophanes*; Kuhn, P. M., Rosenfeld, S. M., Eds.; Academic Press: New York, 1983; Vol. 2, p 629–677.

(12) Schneider, H.-J.; Blatter, T.; Kramer, R.; Kumar, S.; Schneider, U.; Theis, I. In *Inclusion Phenomena and Molecular Recognition*; Atwood, J., Ed.; Plenum Press: New York, 1990; pp 65–74.

(13) Schneider, H.-J.; Blatter, T. *Angew. Chem.* **1988**, *100*, 1211; *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1163.

(14) Schneider, H.-J.; Blatter, T.; Zimmermann, P. *Angew. Chem.* **1990**, *102*, 1194; *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1161.

(15) (a) Petti, M. A.; Sheppod, T. J.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825. (b) Stauffer, D. A.; Dougherty, D. A. *Tetrahedron Lett.* **1988**, *29*, 6039 and earlier references.

(16) Schneider, H.-J.; Blatter, T.; Simova, S.; Theis, I. *J. Chem. Soc., Chem. Commun.* **1989**, 580.

(17) For recent reviews, see: (a) Neidle, S.; Pearl, L. H.; Skelly, J. V. *Biochem. J.* **1987**, *243*, 1. (b) Neidle, S.; Abraham Z. *CRC Crit. Rev. Biochem.* **1985**, *17*, 73. (c) *Chemistry and Physics of DNA-Ligand Interactions*; Kallenbach, N. R., Ed.; Adenine Press: Schenectady, NY, 1990.

(18) For recent references, see: Wilson, W. D.; Strekowski, L.; Tanious, F. A.; Watson, R. A.; Mokrosz, J. L.; Strekowska, A.; Webster, G. D.; Neidle, S. *J. Am. Chem. Soc.* **1988**, *110*, 8292.

(19) (a) Cohen, S. S. In *Introduction to the Polyamines*; Prentice-Hall: Englewood, NJ, 1971. (b) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1976**, *45*, 285. (c) *Ibid.* **1984**, *53*, 749. (d) Bachrach, U. *Function of Naturally Occurring Polyamines*: Academic Press: New York, 1973.

(20) (a) Feuerstein, B. G.; Basu, H. S.; Marton, L. J. *Adv. Exp. Med. Biol.* **1988**, *250*, 517. (b) Bratek-Wiewiorowska, M. D.; Alejeska, M.; Figlerowicz, M.; Barciszewski, J.; Wiewiorowski, M.; Jaskokolski, M.; Zielenkiewicz, W.; Zielenkiewicz, A.; Kaminski, M. *Pure Appl. Chem.* **1987**, *59*, 407.

(21) For recent reviews on NMR methods for nucleic acid derivatives, see: (a) Shafer, R. H.; Brown, S. C. Reference 17c, p 109. (b) Perkins, S. J. *Biol. Magn. Reson.* **1982**, *4*, 193. (c) Van de Ven, F. J. M.; Frank, J. M.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *178*, 1. (d) Wuethrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986. (e) For leading references on ^1H NMR shift studies of groove binders and intercalators, see: Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Wydra, R. L.; Jones, R. L.; Boykin, D. W.; Strekowski, L. *Anti-Cancer Drug Res.* **1990**, *5*, 31 and references cited therein.

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Table I. NMR Shifts (δ_0), Complexation-Induced Shifts (CIS), and Equilibrium Constants K of Adenine Derivatives with the Azoniacyclophane CP66[a]

cmpd		H-8	H-2	H-1′	H-2′	H-3′	H-4′	H-5′
adenine	δ_0	8.22	8.27					
	CIS	−1.72	−1.54					
	$K_A \times 10^{-3}$	0.04	0.04					
adenosine	δ_0	8.37	8.29	6.11	4.79	4.48	4.34	3.92
	CIS	−1.68	−1.61	−0.54	−0.04	−0.01	0.00	−0.01
	$K_A \times 10^{-3}$	0.05	0.05	0.05	*b*	*b*	*b*	*b*
AMP^{2-}	δ_0	8.68	8.31	6.19		4.56	4.41	4.05
	CIS	−0.71	−0.77	−0.25	*c*	−0.04	−0.04	−0.02
	$K_A \times 10^{-3}$	1.88	1.92	1.90		*b*	*b*	*b*
ADP^{3-}	δ_0	8.60	8.31	6.19	4.80	4.69	4.42	4.28
	CIS	−1.08	−0.98	−0.35	−0.07	−0.06	−0.02	−0.02
	$K_A \times 10^{-3}$	11.59	15.71	11.65	*b*	*b*	*b*	*b*
ATP^{4-}	δ_0	8.61	8.31	6.19		4.79	4.45	4.29
	CIS	−0.92	−1.26	−0.38	*c*	−0.28	−0.01	−0.02
	$K_A \times 10^{-3}$	*b*	32.10	41.52		*b*	*b*	*b*

[a] Measured at 300 ± 5 K in D_2O; all shifts in ppm (±0.005). K is in mol^{-1} (±10%), unless noted otherwise. CIS from nonlinear least-squares fits where single K values are given and in other cases from single measurements at selected concentrations, calculated with independently obtained K values (see text). [b] CIS and K values not accessible due to too small shifts or to masked signals. [c] Signal masked by HDO peak.

Table II. NMR Shifts (δ_0), Complexation-Induced Shifts (CIS),[d] and Equilibrium Constants (K) of Selected Nucleosides (G, U, C) and Nucleotides[a]

cmpd		H-5	H-6	H-8	CH_3	H-1′	H-2′	H-3′	H-4′	H-5′	K_{av}
G	δ_0			7.96		5.87	4.69	4.37	4.20	*b*	
	CIS			−1.28		−0.58	−0.29	−0.20	−0.07		
	K			*b*		10	10	*b*	*b*		10
GMP^{2-}	δ_0			8.26		5.98	*c*	4.54	4.36	4.03	
	CIS			−0.72		−0.23		−0.05	−0.03	∼0.00	
	$K \times 10^{-3}$			0.41		0.49		*b*	*b*	*b*	0.45
U	δ_0	7.82	5.86			5.83	4.30	4.17	4.07	*b*	
	CIS	−0.11	−0.10			−0.28	−0.10	−0.06	−0.06		
	K	*b*	12			*b*	9	10	*b*		10
UMP^{2-}	δ_0	6.04	8.20			6.05	4.48	4.41	4.31	4.04	
	CIS	−0.04	−0.07			−0.03	−0.02	−0.01	−0.01	0.00	
	$K \times 10^{-3}$	0.75	*b*			0.93	0.73	*b*	*b*	*b*	0.80
C	δ_0	6.00	7.78			5.85	4.26	4.15	4.08	*b*	
	CIS	−0.18	−0.09			−0.09	−0.09	−0.01	*b*		
	K	17	*b*			*b*	17	*b*	*b*		17
CMP^{2-}	δ_0	6.19	8.17			6.06	4.40	4.40	4.29	4.05	
	CIS	−0.06	−0.06			−0.05	−0.02	−0.02	−0.02	−0.015	
	$K \times 10^{-3}$	0.995	0.87			1.03	*b*	*b*	*b*	*b*	0.93
TMP^{2-}	δ_0		7.86		1.98	6.39	*c*	4.63	4.18	4.00	
	CIS		−0.03		−0.06	−0.07		−0.01	−0.04	−0.00	
	$K \times 10^3$		*b*		1.25	1.18		*b*	*b*	*b*	1.21

[a-c] See footnotes to Table I; *G* = guanosine, *U* = uridine, C = cytidine, and corresponding 5′-monophosphates. TMP = thymidine 5′-phosphate. [d] All CIS values upfield.

erating here,[21] which even for simple complexes with azoniacyclophanes of the type CP66 require at least explicit calculations of aromatic ring current[22] *and* electric field effects,[23] one of the incentives for the present investigation was to obtain better models both for NMR screening effects of aromatic units and positively charged nitrogen atoms *on* nucleosides, nucleotides, and their derivatives as well as for the corresponding effect *of* these on surrounding host protons. NOE measurements[24] with complexes such as CP66·ATP were not possible as unfavorable correlation times (particularly in water, and at the high magnetic fields needed) as well as the fast averaging of protons feeling larger NOEs lowers the observable effects to ≤0.5%, even with the use of spin-lock techniques such as ROESY.[24]

Results

The measurements were performed at pH ranges where only one protonation/deprotonation stage of the free substrates could be assumed on the basis of known pK values.[25] Complexation with the lipophilic as well as with the positively charged cavity of CP66 is expected to lead to a higher acidity of the encapsulated substrates, for which reason the pH values were selected so that as little acid/base concentration change as possible was maintained. Only in the case of GMP^{2-} and UMP^{2-} is it possible that at the measuring pH (8.0) not only the phosphate (pK 6.3–6.4) but to some degree also the ring "amide" function (pK = 9.5) is deprotonated, which would enhance the complexation constants by additional Coulombic interaction. The titration curves observed with CP66, however, did not indicate any deviations from single 1:1 complexes with only one protonation form of the substrates. Self-association of the substrates[26] by stacking interactions is negligible at the concentrations employed, as is self-association of the cyclophane, which was visible by the absence of any concentration-dependent chemical shifts of the components.

Complexation constants K and complexation-induced shifts (CIS values at 100% complexation) for nucleotides, nucleosides, and—as far as solubility in water permitted this—also nucleobases and/or analogs (Tables I–V, Charts I and II) were determined as described earlier.[5b] ^1H NMR titration curves usually gave satisfactory or excellent fits for 1:1 complexes (if $K > 10^2$ M^{-1}

(22) For calculations on ring current and other effects on NMR shifts with nucleobases see, ref 21b.

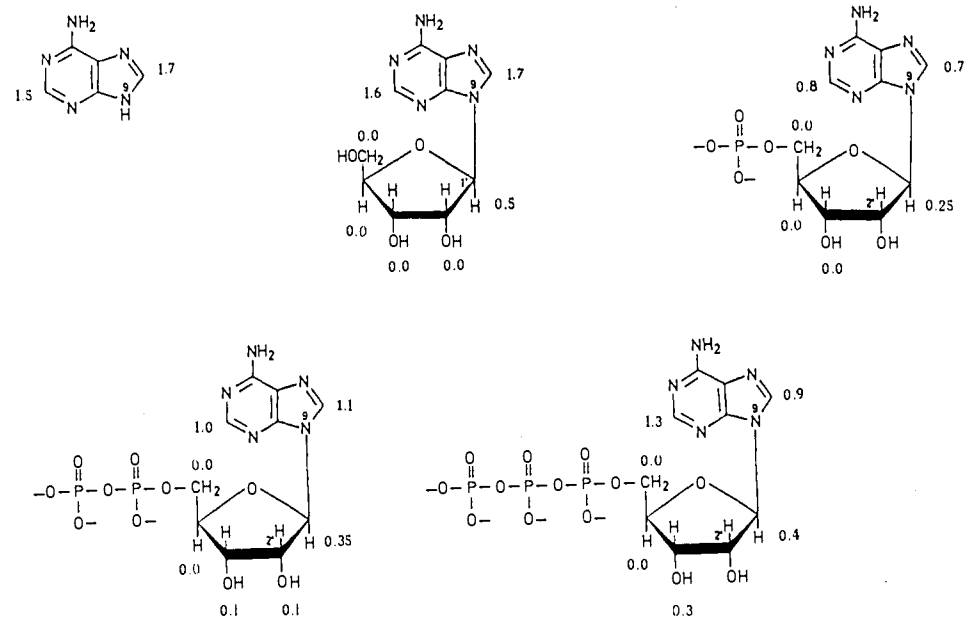
(23) (a) Schneider, H.-J.; Pöhlmann, J. *Biorg. Chem.* **1987**, *15*, 183. (b) See also: Schneider, H.-J.; Buchheit, U.; Becker, N.; Schmidt, G.; Siehl, U. *J. Am. Chem. Soc.* **1985**, *107*, 7827.

(24) Neuhaus, D.; Williamson, N. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; Verlag Chemie: New York, 1989.

(25) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Batterworth: London, 1965.

(26) Ts'o, P. O. P. Reference 2b, p 537 ff.

Chart I. Host Structures (CP66, V) and Adenosine Derivatives with ^1H NMR Shifts Induced by Complexation with CP66[a]



[a] CIS in ppm calculated for 100% complexation. CIS values are rounded; if not indicated, they were masked. See Table I.

Table III. NMR Shifts (CIS Values) on CP66 Induced by Complexation with Nucleotides[a]

	H-2	H-3	H-4	CH_3	H-5[c]	H-m[c]	H-o[c]
A[b]	−0.23	−0.38	−0.47	−0.06	−0.02	0.16	−0.03
AMP^{2-}	−0.10	−0.20	−0.25	−0.02	<0.005	0.09	<0.005
ADP^{3-}	−0.10	−0.16	−0.22	−0.02	−0.04	0.09	−0.02
ATP^{4-}	−0.05	−0.11	−0.13	−0.02	<0.005	0.07	−0.02
GMP^{2-}	−0.10	−0.15	−0.15	−0.05	−0.05	<0.005	−0.05
TMP^{2-}	−0.01	−0.01	−0.004	−0.01	+0.01	−0.01	−0.03
CMP^{2-}	−0.004	−0.004	−0.008	−0.01	−0.02	−0.03	−0.03
UMP^{2-}	−0.003	−0.004	−0.005	−0.009	−0.01	−0.008	−0.009

[a] From measurements at 300 ± 5 K in D_2O at complexation degrees of around 70%, unless noted otherwise. CIS (for 100% complexation) calculated based on equilibrium constants K from Tables I and II; shifts in ppm (±0.01 ppm). [b] Adenosine (for comparison, from 29% complexation measurement). [c] H-5: PhCH_2 signal; H-m: aromatic signal meta to N; H-o: ortho position.

and/or CIS >0.2 ppm, and solubility sufficient to reach >40% complexation degree) as well as agreement between single constants K evaluated from as many different signals as possible. CIS values of the nucleobase derivatives *on* the receptor CP66 (Table III) were determined by single NMR measurements on the basis of known equilibrium constants K obtained from the reverse titrations (Tables I and II), using mostly concentrations which lead to ≥70% complexation of the receptor CP66.

The observed CIS values provide clear evidence of intracavity inclusion of the *adenine* moiety into CP66 (Chart I) with up to −1.7 ppm shielding. The values come close to the shieldings calculated earlier with aromatic ring current and N^+ electric field effects for full inclusion of naphthalene in an (idealized) CP66 structure.[23a] Preliminary force field calculations with CHARMm[27]/QUANTA show the purine fully immersed in the CP66 cavity (Figure 1c) with negligible distortions even of torsional angles ($\Delta\phi < 2°$) and with ATP^{4-}, e.g., a slight withdrawal of the purine base out of the cavity as a consequence of the need to obtain an optimum contact here for the Coulombic interaction between the phosphate residue and the positive charges at the macrocycle.[28] This effect is clearly seen in the diminished CIS

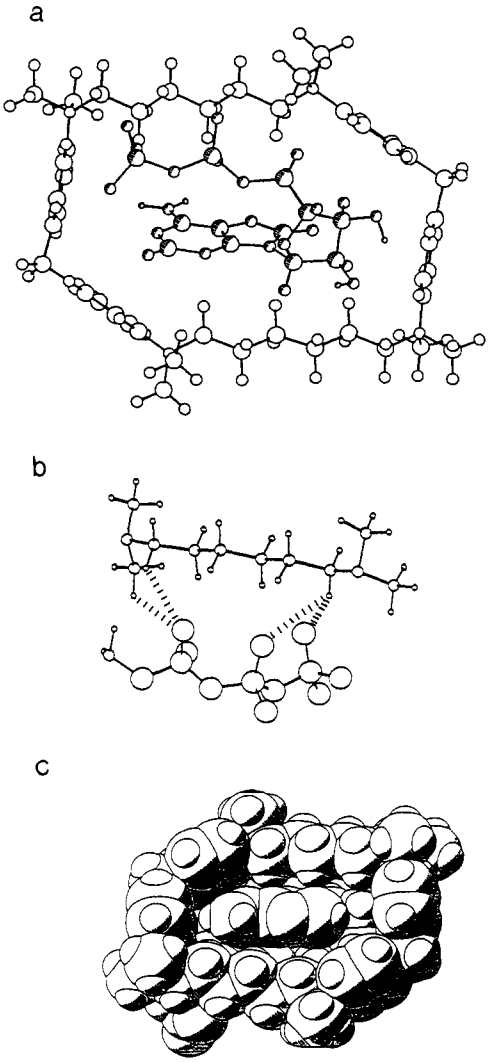
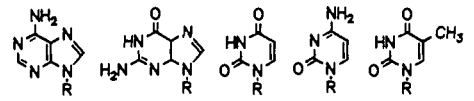


Figure 1. Simulations for the complex CP66·ATP^{4-} (QUANTA/CHARMm): (a) top view; (b) cut out showing four salt bridges between oxygen atoms from ATP^{4-} and hydrogen atoms from CP66 N^+CH fragments (with $d_{H^+\cdots O} \leq 3$ Å); (c) CPK bottom view.

(27) See, for example: (a) Brooks, C. L.; Karplus, M. *Methods Enzymol.* **1986**, *127*, 369. (b) Brünger, A. T.; Karplus, M. *Acc. Chem. Res.* **1991**, *24*, 54.

(28) Similar effects are seen in complexes of CP66 and with and without acidic side groups: Theis, I. Dissertation, Universität des Saarlandes, Saarbrücken, Germany, 1989.

Chart II. Structures of Nucleosides and Nucleotides with Free Complexation Energies (kJ mol^{-1}) with CP66



R=	A	G	U	C	T
Ribose	10	~6.5	~6	~7	–
Ribose–OPO_2^{2-}	19.3	15.9	17.3	18.3	17.6

values for the purine signals of the adenosine phosphates compared to the electroneutral nucleoside and adenine itself. The observed ribose signals show the sugar units to reside largely outside the receptor cavity, as expected in view of their hydrophilic nature.

An important result from the NMR titrations is the selectivity of CP66 for adenine derivatives, which is indicated not only by the equilibrium constants (being, for example, ~5 times higher for adenosine than for guanosine) but also by the small CIS values both on the other nucleobases (Table II) as well as on CP66 (Table III). Although all nucleobases would fit well into this cavity and CP66 does lead to intracavity inclusion of many aromatic substrates of similar shape and hydrophilicity (such as phenols, naphthols, related amines, etc.[28]), adenine is exceptional. We tentatively ascribe this to the higher polarizability[29] of this amino-substituted purine, as several lines of evidence[3d,5b,30] point to induced dipoles in aromatic moieties as the major driving force for lipophilic inclusion of aromatic substrates in cavities of macrocycles such as CP66. To the extent the electrostatic interactions increase, the selectivity for adenine necessarily drops down, for example, from $\Delta\Delta G > 3$ kJ mol^{-1} for the nucleosides to $\Delta\Delta G > 1$ kJ mol^{-1} for the nucleotides.

There is a remarkable analogy to the dissection of free complexation energies ΔG with CP66 into electrostatic and lipophilic contributions (ΔG_{Es} and ΔG_{Li}) with substituted naphthalenes[3d,31] and adenines, showing ΔG_{Li} to dominate by ~2:3 for substrates bearing one negative charge (e.g., naphthalenesulfonic-, -phosphoric, or -carboxylic acids).[31] It should be stressed that this major van der Waals effect of charge polarizations is at present not taken care of in the available force fields, which would therefore predict encapsulation of all other nucleobases in the CP66 cavity.

The NMR shifts induced *on* the host CP66 are significant only with adenine and to a lesser degree with guanidine derivatives (Table III), both again in general agreement with the reverse CIS and K values (Tables I and II). The pyrimidine derivatives obviously form only loose associations without taking advantage of the lipophilic CP66 cavity. The CIS values of the adenine compounds on the CP66 protons (Table III) are similar in sign (shielding) and sequence ($CIS_{H\text{-}4} > CIS_{H\text{-}3} > CIS_{CH\text{-}2} > CIS_{CH_3}$, etc.) as typical values found for CP66 encapsulated naphthalene derivatives.[3d]

The *Coulombic interactions* present in the nucleotides must be compared to our earlier analysis of the electrostatic contributions of salt bridges.[3d,5] It is gratifying that (a) the free complexation energy differences between nucleoside and the corresponding diphosphates remains always nearly constant and (b) amounts to the expected value of $2 \times 5 = 10$ kJ mol^{-1} (Tables I and II, Chart II). Furthermore, the increase from mono- to di- and then to triphosphates where the complexation energy difference has been measured (Table I) is again of the expected magnitude (adenosine 9.6, AMP^{2-} 18.7, ADP^{3-} 23.8, ATP^{4-} 26.4 kJ mol^{-1}, respectively) with 4.7 kJ mol^{-1} per salt bridge—except the ADP^{3-} to ADP^{4-} value of only 2.6 kJ mol^{-1}: this is the consequence of the dimunition of van der Waals binding by the withdrawal of the nucleobase out of the cavity discussed above

(29) Papadopolous, M. G.; Waite, J. *THEOCHEM* **1988**, *47*, 189.
(30) See particularly footnotes 10, 13, and 19 in ref 5b.
(31) (a) Schneider, H.-J.; Philippi, K.; Pöhlman, J. *Angew. Chem.* **1984**, *96*, 907; *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 908. (b) See also ref 5a.

Table IV. NMR Shifts, CIS, and K Values of Complexes between CP66 and Some Nucleobases or Analogs[a,b]

		H-2	H-3	H-4	H-5	H-6	H-8	K_{av}
pyridine	δ_0	8.58	7.52	7.94	7.52	8.58		
	CIS	–0.65	–0.57	–0.50	–0.57	–0.65		
	K	12.8	*b*	11.3	*b*	12.8		12.0
pyrimidine	δ_0	9.19		8.86	7.65	8.86		
	CIS[c]							
	K[c,d]	1.7		1.2	1.0	1.2		~1.3
uracil	δ_0				5.85	7.57		
	CIS[c]							
	K[c,e]				29	7		~20
purine	δ_0	9.00				9.19	8.64	
	CIS	–0.94				–0.20	–0.90	
	K	12.3				17.9	12.4	14.2

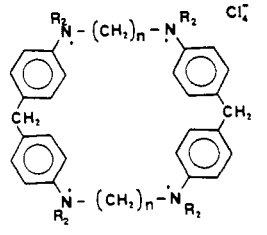
[a,b] See footnotes to Table I (for adenine see Table I). [c] Too low K values and solubilities prevent titration. [d] K estimation based on uniform CIS = 0.7 ppm (taken from pyridine). [e] Maximal K value estimated from CIS values taken from the CMP^{2-} titration (Table II) (for H-5 and H-6: 0.1 ppm).

Table V. NMR Shifts (δ_0), CIS, and K Values of AMP^{2-} and GMP^{2-} Complexed by the "Cleft" Compound V[a]

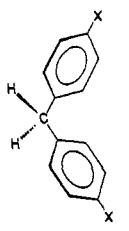
	H-2	H-8	H-1′	H-2′	H-3′	H-4′
			AMP^{2-} Complex			
δ_0	8.189	8.564	6.083	*c*	4.464	4.314
CIS	–0.09	–0.02	–0.08		–0.02	–0.03
K	47	*b*	49		*b*	80
$\Delta G°$	9.5		–9.6			10.8
			GMP^{2-} Complex			
δ_0		8.168	5.892	*c*	4.458	4.286
CIS		–0.03	–0.09		–0.03	–0.04
K		370[d]	75[d]		120[d]	
$\Delta G°$		14.6	10.7		11.8	*b*

[a-c] See footnotes to Table I; δ_0 for H-5′, 3.95. [d] Error ±40%.

for reaching sufficient Couloumbic contacts for the phosphate unit. Molecular modeling secures the geometric boundaries for sufficient salt bridges in the complexes: CHARMm simulations show for CP66·ATP at least four phosphate oxygen atoms approaching CH protons in the α-position to the CP66-N^+ within ≤3.0 Å (Figure 1b, dotted lines). These N^+CH atoms are known from MO calculations[32] to carry most of the positive charge in such ammonium ions. The results add another six examples to the already ~40 ion pairs demonstrating the validity of using an additive binding increment of 5 ± 1 kJ mol^{-1} per salt bridge.



CP66: R=Me, n=6



V: X = $^+NMe_3 \cdot Cl^-$

Finally, complexation of selected nucleotides with the open, cleft-shaped receptor model V was investigated, which in principle could access, for example, DNA both at the groove and by intercalation. We have shown earlier that V indeed binds even electroneutral aromatic substrates (and other well-polarizable substrates such as diiodomethane) in water, if the polarized unit is positioned properly in relation to the inducing N^+ pole with $\Delta G \approx 2$–3 kJ mol^{-1} per N^+ π-interaction.[14] Binding of neutral nucleosides to V was bearly measurable, although NMR titration with A, C, U, and G showed binding with $K \approx 1$ M^{-1} and CIS values from –0.1 to –0.2 ppm. This weak binding is not unexpected, as the nucleobases are not arranged for giving a chelate effect by double action of the N^+ units as in our earlier cases,[14]

(32) See 5b, particularly Table III*.

Chart III. Differences in Energy of Complexation ΔG (kJ mol^{-1}) between Cavity Compound CP66 and Semiopened Compound V

		in CP66	Diff.	in Cleft V	ΔG
(1)	Diiodomethane	7.5	5.8	1.7	kJ mol^{-1}
(2)	Benzene	10.0	7.3	2.7	
(3)	Di-(4-aminophenyl)-methane	14.5	7.6	6.9	
(4)	" - amine	14.5	8.5	6.0	
(5)	A M P $^{2-}$	19.3	9.5	9.8	
(6)	(G M P $^{2-}$ *)	(15.9	3.9	12.0)	

*) (GMP^{2-} only partially encapsulated)

and double interaction in fact would require the build up of dipoles of opposite sign in the heterocycles. Complexations of V with nucleotides such as AMP^{2-} and GMP^{2-}, however, could be evaluated with sufficient accuracy (Table V) and give, as we believe for the first time, experimental numbers for the hydrophobic contribution to the binding of nucleotides with a closed cavity compared to a structurally similar semi-open receptor shape (V). The complexation ΔG values between CP66 (Tables I and II) and V (Table V) differ for AMP^{2-} by 9.5 and for GMP^{2-} by 3.5 kJ mol^{-1}. The latter compound is shown by the CIS values (see above) to be encapsulated in the CP66 cavity to a much lesser degree and therefore is less sensitive to the hydrophobic cavity effect. AMP^{2-}, however, with ΔG = 9.5 kJ mol^{-1}, shows within ±1 kJ mol^{-1} the *same* ΔG difference as we observed with four other substrates of totally different nature[14] (Chart III).

Conclusions

We have shown that electrostatic contributions to nucleotide binding can be separated from other effects and are quantified on the basis of constant salt bridge increments. van der Waals contributions show selectivity for adenine in terms of binding constants and binding geometry. The latter is characterized by inclusion of the nucleobase vis-a-vis of positively charged nitrogen and sheds light on DNA intercalation mechanisms. In contrast to predictions from molecular modeling and experiments with benzene compounds, pyrimidine derivatives are not encapsulated. The observed NMR shifts are valuable tools for structural investigations of nucleic acid derivatives in solution. Comparison of binding in structurally related open and closed hosts lends further support to our earlier conclusions[14] that the solvophobic effect of cavity formation on the binding energy of quite different substrates can be tentatively factorized simply by considering the number of hydrogen-disruptions of the intracavity water molecules.

Experimental Details

NMR titrations, evaluation of equilibrium constants and CIS values, and molecular modeling studies were performed as described before.[5b,33] The substrates were used as commercially available without further purification; CP66 was prepared as described earlier.[34]

Bis[4-(trimethylammonio)phenyl]methane (V) was prepared by alkylation of commercially available bis[4-(dimethylamino)phenyl]methane with methyl iodide in methanol.[35]

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(33) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442.
(34) Schneider, H.-J.; Philippi, K. *Chem. Ber.* **1984**, *117*, 3056. Schneider, H.-J.; Busch, R. *Chem. Ber.* **1986**, *119*, 747.
(35) Organikum, VEB Deutscher Verlag der Wissenschaften, Berlin, 1981, p 260.

Single-Electron Transfer in Aromatic Nucleophilic Substitution on Dinitrobenzonitriles

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Abstract: Reaction of OH^- with 3,5-dinitrobenzonitrile in water or water–DMSO gives a mixture of unproductive 2- and 4-Meisenheimer complexes that equilibriate and eventually form 3,5-dinitrobenzamide and finally the benzoate ion. The corresponding reaction of 2,4-dinitrobenzonitrile gives the 5-Meisenheimer complex and then a mixture of 2,4-dinitrobenzamide and 2,4-dinitrophenoxide ion. The ratio amide:phenoxide ion increases with increasing [OH^-]. These reactions appear to involve formation of charge-transfer complexes of the radical anion of the substrate and $^{\bullet}OH$ which collapse to give Meisenheimer complexes and final products. The rate constants of the various reaction steps can be estimated by simulation based on relaxation theory, which also fits the product mixture from 2,4-dinitrobenzonitrile. This reaction scheme is consistent with observations of exchange of arene hydrogen and of extensive broadening of ^1H NMR signals of the substrates during reaction.

Reactions of hydroxide or alkoxide ions with 3,5-dinitrobenzonitrile (**1**) in polar solvents give mixtures of Meisenheimer complexes. The 4-complex forms and then equilibriates with the more stable 2-complex.[2-6] Fyfe and co-workers used flow NMR spectroscopy to show unambiguously that the 2-complex predominates in the equilibrium mixture. This difference in stability is predicted by qualitative models of electronic effects and by molecular orbital calculations.[7]

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(2) Millot, F.; Terrier, F. *Bull. Soc. Chim. Fr.* **1974**, 1823.
(3) Foreman, M. I.; Foster, R. *Can. J. Chem.* **1969**, *47*, 729.
(4) Fyfe, C. A.; Cocivera, M.; Damji, S. *J. Am. Chem. Soc.* **1975**, *97*, 5707.
(5) Crampton, M. R.; Khan, H. A. *J. Chem. Soc., Perkin Trans. 2* **1973**, 710.
(6) Fendler, E. J.; Fendler, J. H.; Arthur, N. L.; Griffin, C. E. *J. Org. Chem.* **1972**, *37*, 812.

0002-7863/92/1514-7708$03.00/0