Nucleotide complexes with azoniacyclophanes containing phenyl-, biphenyl- or bipyridyl- units[1]

Kaliappa G. Ragunathan and Hans-Jörg Schneider*

FR Organische Chemie der Universität des Saarlandes, D 66041 Saarbrücken, Germany

Three cyclophanes (1–3) with *p*-phenyl, *p*-biphenyl- and *m*-bipyridyl spacers between diethylenetriamine units are studied by NMR titrations in water (D_2O) with naturally occurring mononucleotides in the form of monophosphates. The biphenyl host 1 shows association constants K (in mol dm^{-3} units) of 2200 with AMP5′, 1270 with the isomeric AMP3′, and smaller constants with G, C and U derivatives. An exception is GMP5′ with 3, which in contrast to most host compounds shows a four-fold stronger binding than AMP5′. Thymidine (TMP5′) is complexed with K = 2050 dm^3 mol^{-1}; the intracavity immersion of the T methyl substituent is visible also in the observed upfield NMR shifts. With the smaller hosts 2 and 3, constants of approximately 100–200 dm^3 mol^{-1} are observed, indicating essentially salt bridge contributions. Structural conclusions are supported by the observed NMR shieldings and by selected molecular mechanics calculations.

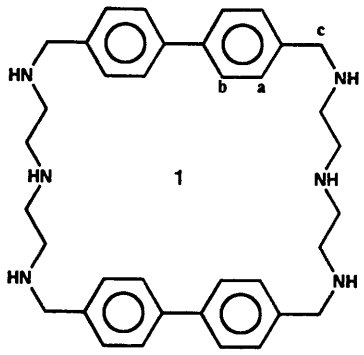
Macrocyclic host compounds can provide efficient complexing agents for nucleotides.[2] The presence of positive charges in the macrocycles can lead to Coulomb interactions with the phosphate anions of usually 5 kJ mol^{-1} per salt bridge. This quantity has been established with over 100 complexes in water, ranging from zinc sulfate to polyamine–DNA associations.[3] In consequence, ion pairing between the highly charged ATP and complementary numerous ammonium centres of suitable hosts alone can yield association constants of up to 10^{11} dm^3 mol^{-1}.[2b] The ion pair contribution, worth *ca.* 10 dm^3 mol^{-1} association constant per bridge, is visible *e.g.* in constants of *ca.* 10^4 dm^3 mol^{-1} reported by Lehn *et al.*[4a] for complexes of a cyclophane with maleate or fumarate; corresponding increases with the number of interacting charges are invariably observed with other macrocyclic hosts.[4,5] Interactions of the nucleobases, mostly with lipophilic parts of the host, will then provide for base selectivity and for additional affinity. With azoniacyclophanes of the Koga-type, containing biphenylmethyl units, the major non-electrostatic driving force is the effect of N^+ host cation on the π-moiety of the guest.[5a] Other cyclophanes, containing larger π-moieties such as naphthalene,[4a] acridine[4b,c] or phenanthridine[4d] in the host will bind nucleobases predominantly by dispersive and/or hydrophobic interactions. A full gain from electrostatic and other contributions is only possible if there is a simultaneous and strain-free match between all kinds of binding sites.[6] It should also be noted, that high selectivity with respect not only to the nucleobases, but even to the isomeric 2′- or 5′-nucleotides does not necessarily require attractive interactions between the base or sugar parts of the guest and the host.[5b]

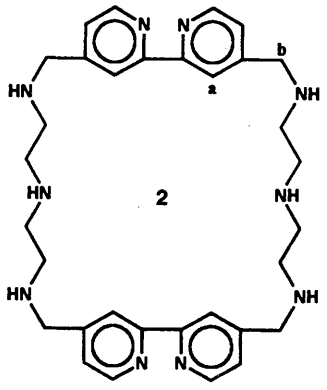
Most supramolecular complexes studied so far invariably show a preference for purines over pyrimidines, and—with few exceptions[4b]—for adenine over guanosine derivatives. The present paper describes host systems **1**–**3** in which the selectivities could change due to different cavity sizes and the presence of smaller, yet flexible, aromatic units in the cyclophanes. The hosts furthermore allow us to study the minimum requirements of phenyl ring contributions for nucleobase stacking. It was also hoped that the known capacity[7] of these hosts to complex metals could be used to provide additional binding by co-complexation to the phosphates. Unfortunately, precipitation of the complexes with increasing amounts of nucleotides in the presence of all the metal ions tried (Eu^{3+}, Zn^{2+} and Cu^{2+}) prevented this use of the metallated cyclophanes.

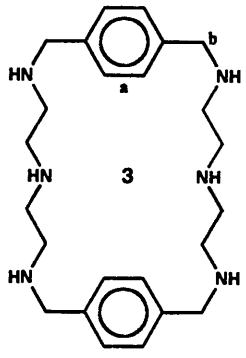
Results and discussion

The cyclophanes **1** and **3** (Scheme 1) were prepared according to literature[8] procedures and used as their hexahydrochloride salts. Cyclophane **2** (Scheme 1) was prepared using 4,4′-diformyl-2,2′-bipyridine and triethylenetriamine. At the pH used (6.0) **1**–**3** exist—as do related structures[4a,7]—in the tetracationic form. Titration of the nucleotides to the cyclophanes in D_2O using known protocols[5a] gives rise to characteristic changes in the NMR spectrum of the host cyclophanes. The complexation constants K, along with the complexation induced shift (CIS) values (shifts induced at 100% complexation, from non-linear curve fitting) for different nucleotides with the cyclophanes **1**, **2** and **3** are given in Table 1 and the corresponding ΔG values of free energy of complexation in Table 2.

The cyclophane **1** shows higher sensitivity and selectivity with all nucleotides than the cyclophanes **2** or **3**. This is in line with the larger cavity as observed in computer-aided molecular modelling studies with the CHARMm forcefield.[9] Fig. 1 illustrates that host **1** accommodates a nucleotide with almost perfect stacking interactions between the aromatic moieties of host and guest. The NMR shifts indeed suggest a strong interaction between the parallel biphenyl and the adenine moiety in particular for the cyclophane **1**. The biphenyl proton resonances are shifted upfield, and so are the H-2 and H-8 protons of the adenine base in AMP5′ (Table3). These NMR spectral changes result from the anisotropy effects of the aromatic units,[10] and agree with the stacking—as opposed to edge-to-face—orientations shown in Fig. 1. A comparable structure where a guanine base stacks between the aromatic moieties of the cyclophane with the help of metal ion has been characterized[10] by X-ray crystallography. The aliphatic proton resonances of the host are shifted downfield, which could be due to the electrostatic interaction between the positively charged ammonium groups of the cyclophane and the anionic phosphate group of AMP5′, as observed[11a] with macrocyclic receptors for ATP or ADP. Alternatively, or in addition, these protons may be in the deshielding cone of the aromatic units. The ^{31}P NMR resonance of AMP5′ is shifted downfield (CIS + 0.54 ppm) in the presence of the cyclophane **1**; this could be due to electrostatic interaction with the phosphate group. Similar, but larger downfield shifts for terminal P atoms were observed[11] with ATP or ADP binding to polyammonium macrocycles; the P atom of AMP was reported[11] to show smaller shifts when complexed by the same macrocycles.







Scheme 1 Structure of cyclophanes **1**, **2** and **3**

Molecular modelling indicates (Fig. 1) three electrostatic interactions between the phosphate group and the polyammonium alkyl chain of the cyclophane **1**. The protons of the sugar unit, except the anomeric proton, show small changes in resonance position upon complexation. This is expected from the hydrophilic nature of this group, which will reside largely outside of the lipophilic cavity of **1**.

Cyclophane **1** shows a lower complexation constant with AMP3′ compared to that of AMP5′, and amongst the pyrimidine nucleotides a surprisingly large binding constant with TMP5′. GMP5′ has the lowest complexation constant among the nucleotides studied, even though the area of contact for stacking interactions is comparable to that with AMP5′. The proton resonances of all the nucleotide bases studied show large upfield shifts implying stacking interactions with the biphenylic units of the host. The CIS values of the biphenyl groups are larger for purine nucleotides (Table 1) compared to those for the pyrimidine nucleotides, again in line with the deeper immersion of the purine nucleotides into the cavity. The methyl group of the thymidine base shows a large upfield shift upon complexation, implying that this group, due to its hydrophobic nature, resides inside the cavity. In all complexes with **1** only the anomeric proton of the sugar part show a strong upfield shift, suggesting the location of this proton in the shielding cone of the aromatic rings.

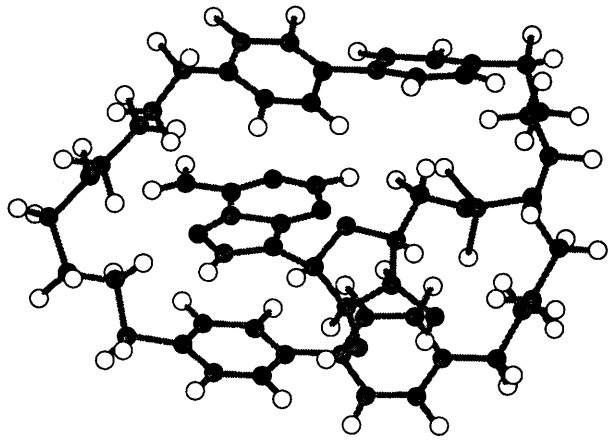


Fig. 1 QUANTA/CHARMm energy minimized structure of the complex **1** × AMP5′

The cyclophanes **2** and **3** show weaker binding constants compared to **1** (Table 1); they were only titrated with the stronger binding AMP5′ and GMP5′. The CIS values induced on the bipyridyl protons of **2** are also smaller compared to those of the aromatic protons of **1**, when AMP5′ is complexed. The proton at the 3 position shows a maximum CIS value, whereas the resonance of the protons in the 5 and 6 positions are barely affected. AMP5′ and GMP5′ have almost the same binding constants, in contrast to that observed with **1**. Molecular modelling indicates that the cavities of **2** and **3** are indeed too small for the stacking and immersion of a nucleobase.

In cyclophane **3** the aromatic protons show a strong upfield shift of the resonances upon complexation with AMP5′ and GMP5′, indicating a strong interaction between the purine bases and the aromatic nuclei of the cyclophane. Cyclophane **3** exhibits an unusual preference and large CIS values for GMP5′ over AMP5′, with an association constant (Table 1) comparable to that observed with **1**. However, the shifts of the aromatic protons of the cyclophane **3** upon adding a 26-fold excess of the pyrimidine nucleotides are negligible, implying a different binding mode than stacking as the major driving force. This implies a weak interaction between the pyrimidine bases and the host aromatic unit, with accordingly low complexation constants (< 50 $dm^3\ mol^{-1}$). In contrast to the observed downfield shift of the alkyl chain protons in **1**, the alkyl chain protons of **3** are shifted upfield upon complexation of AMP5′ and GMP5′. The ^{31}P NMR of GMP5′ in the presence of cyclophane **3** shows a downfield shift (CIS + 1.12 ppm), indicating a phosphate group interaction[11] with polyammonium groups of the cyclophane **3**.

Experimental

Preparation of hexahydrochloride salts of the cyclophanes 1 and 3

Cyclophanes **1** and **3** were prepared following literature procedures.[8] The hexahydrochloride salts of **1** and **3** were obtained by passing HCl through methanol solutions of the cyclophanes, with the hydrochloride salts precipitating out as white solids. The solids were filtered off, and washed with methanol. The hexahydrochloride salt of **1** was recrystallized from hot water at pH 1; that of **3** was recrystallized from water at pH 1. δ_H(400 MHz, D_2O, $SiMe_4$ in CCl_4 external reference): For **1**: 2.864 (8 H, t, $-NHCH_2-$), 3.078 (8 H, t, $-NHCH_2-$). 4.049 (8 H, t; -NHCH2-biphenyl), 7.227 (8 H, d, biphenyl), and 7.425 (8 H, t, biphenyl). For **3**: 2.831 (8 H, t, $-NHCH_2-$), 3.093 (8 H, t,

Table 1 Complexation constants K ($dm^3\ mol^{-1}$) of different nucleotides with cyclophanes **1**, **2** and **3**. The CIS values, in ppm, are from nonlinear least-squares fit at the observed *K* values

Cyclophane	Nucleotide	Protons observed						
		a		b		c		
		K	CIS	K	CIS	K	CIS	K^a
1	AMP5′	2600	−0.45	1463	−0.18	2572	−0.13	2211
	AMP3′	1470	−0.41	1100	−0.19	1250	−0.10	1273
	GMP5′	447	−0.25	657	−0.15	609	−0.10	571
	dGMP5′	440	−0.36	525	−0.21	667	−0.15	544
	TMP5′		*d*	2050	+0.06	1670[c]	−0.04	2050
	dCMP5′		*d*	907	+0.04	533	−0.05	720
	dUMP5′		*e*	837	+0.04	1190	−0.03	1013
2[b]	AMP5′	115	−0.43	304	+0.10			209
	GMP5′	225	−0.14	310	+0.28			267
3[b]	AMP5′	143	−0.48	179	−0.28			161
	GMP5′	540	−0.57		*d*			540

[a] *K* average association constants, determined in D_2O, derived after omitting *K* values with error values larger than ±7%. [b] Signal overlap, K values are below 100 $dm^3\ mol^{-1}$. [c] Error ±13%. [d] Signal overlap. [e] CIS < 0.02 ppm.

Table 2 Complexation free energies ΔG (kJ mol^{-1}) in D_2O for the complexation of nucleotides by the cyclophanes **1**, **2** and **3**

Cyclophane	AMP5′	AMP3′	GMP5′	dGMP5′	TMP5′	dUMP5′	dCMP5′
1	19.48	17.68	15.70	15.58	18.88	17.11	16.27
2	13.21		13.82				
3	12.57		15.58				

Table 3 CIS values (ppm) of nucleotide protons upon complexation by the cyclophane **1**[a]

Nucleotide	H-8	H-5	H-6	H-2	H-1	CH-3
AMP5′	−0.51			−1.62	−0.43	
AMP3′	−0.56			−0.56	−0.52	
GMP5′	−0.24				−0.63	
TMP5′			−0.70		−0.55	−1.51
dUMP		*b*	−0.12		−0.48	
dCMP		*b*	−0.96		−0.50	

[a] From single NMR measurements on the basis of known equilibrium constants obtained from the reverse titrations; in D_2O. [b] Overlapped by host signal.

$-NHCH_2-$), 4.109 (4 H, s, $-NHCH_2-$phenyl), and 7.362 (8 H, s, phenyl).

Preparation of cyclophane 2

The cyclophane **2** was prepared from 4,4′-diformyl-2,2′-bipyridine[12] and triethylenetriamine. Triethylenetriamine (1 mmol) in acetonitrile (50 cm^3) was added dropwise to a solution of 4,4′-diformyl-2,2′-bipyridine (1 mmol) in acetonitrile (50 cm^3) over a period of 4 h, and the solution was stirred for 24 h. Then the solution was filtered and the solvent removed at low pressure. The resulting Schiff base was reduced by refluxing with excess $NaBH_4$ in methanol for 6 h. The methanol was removed and the residue was dissolved in water. The aqueous solutions were extracted with chloroform, the organic layer was dried with anhydrous Na_2SO_4 and filtered. The solution on removal of the solvent gave **2** as an oil which was dissolved in chloroform. Diethyl ether was added slowly to precipitate **2** as a solid (185 mg, 65%) mp 160–162 °C, δ_H(400 MHz, $CDCl_3$, $SiMe_4$ internal reference) 2.77 (16 H, M, $-NHCH_2CH_2NHCH_2-CH_2NH-$), 3.864 (8 H, s, $-NHCH_2-$bipyridyl), 7.155 (4 H, dd, bipyridyl), 8.325 (4 H, s, bipyridyl) and 8.365 (4 H, d, bipyridyl); $\delta_{^{13}C}$(100 MHz, $CDCl_3$) 48.87, 49.07, 52.84 (aliphatic carbons), 120.21, 123.03, 149.2, 150.58, 156.21 (bipyridyl); *m/z* 568 (M^+), requires 568.74.

NMR Experiments

The NMR titrations were carried out as described[5a] earlier using a Bruker 400 MHz instrument, in D_2O at pD 6.4. The cyclophanes precipitated in phosphate buffer; hence the titrations were carried out without any buffer and the pD was adjusted with DCl or NaOD. ^{31}P NMR experiments were carried out with a Bruker 500 MHz DRX instrument.

Table 4 Complexation induced ^{31}P NMR shifts of nucleotides[a]

Cyclophane	Nucleotide	CIS (ppm)
1	AMP5′	+0.54
2	GMP5′	+0.79
3	GMP5′	+1.12

[a] Measured in D_2O, calculated for 100% complexation (with known *K* values).

Molecular modelling

Studies were carried out on an IRIS workstation with QANTA/CHARMm[9] (gas-phase calculations) using steepest descend minimization.

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