Studies on the Unique RNA Duplex Destabilization by an Azoniacyclophane – NMR Titrations with Mono- and Oligonucleotides of the RNA and DNA Types

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The mechanism of the unique destabilization of a folded RNA by the azoniacyclophane **CP66** has been investigated by complexation studies with mono- and short single-stranded oligonucleotides as models. For the mononucleotides, NMR titrations revealed only a slight difference of binding free energy ΔG between RNA-type nucleotides compared to DNA analogues, with $\Delta\Delta G$ values of up to 1.3 kJ/mol. The complexation-induced NMR shifts (CIS values) both in the ligand and in the cyclophane were also similar for deoxyribo- and ribonucleotides, suggesting similar inclusion geometries in both series. Similar observations had been made earlier on the CIS effects of denatured single-strand polymers. Titrations with dinucleotides showed a slight preference for ribose derivatives, with $\Delta G = -11.2$ kJ/mol for ApA and –13.2 kJ/mol for ApG, and –9.9 kJ/mol for dApdA. With the dinucleotides and with selected tri- and tetranucleotides, the NMR shielding effects indicated stronger binding to adenine amongst the nucleobases, with a preference for the 5′-end position where available. In view of the small affinity decrease from ribose to deoxyribose derivatives, possible differences in the geometric disposition of the cyclophane towards the major groove of the duplexes were analysed with a simplified point charge model. The phosphates in the RNA groove indeed matched the cationic ammonium centres of the ligand less well than did those in the larger B-DNA groove. The conclusion from these results is that the destabilization of duplex RNA relative to that of duplex DNA is caused at least in part by the differences in interaction between **CP66** and the duplex species and not the single-stranded species.

Introduction

Therapeutic targeting of RNA is not as well developed as that of DNA and proteins, and the many structures and functions of RNA suggest that it is an under-utilized target. Rational design of drugs that target RNA is in its initial stages[1–7] and has been largely focused around the aminoglycoside class of compounds.[1,4,8] Few compounds that selectively interact with RNA through intercalation or minor groove interactions have been identified,[9] although compounds that selectively interact with DNA through these modes are well developed.[10–19] Aminoglycosides have been shown to target the RNA major groove, and a number of these compounds are valuable antimicrobial therapeutics.[5,20–23] The development of synthetic agents that selectively interact with RNA is thus of considerable interest both for possible medicinal uses and for their contribution to understanding of RNA interaction mechanisms. Cationic cyclophanes are attractive agents as semi-rigid scaffolds for construction of agents that can target the RNA grooves. Cationic libraries based on a macrocyclic piperazinyl polyazacyclophane scaffold with various functionalities have been synthesized and shown to exhibit potent activity in disrupting the TAR-Tat complex.[24] Libraries with a 13-membered ring were most active, whereas libraries with a 15-membered scaffold were inactive. Libraries with a large variety of side chains have been synthesized and tested for inhibition of TAR-Tat complexation, and compounds displaying IC_{50} values of 80 and 90 nM have been generated by this method.

Azoniacyclophanes of the **CP*nn*** type (Figure 1), which feature conformationally rather rigid structures, have been shown to complex nucleosides and nucleotides with efficiencies depending on the number *n* of methylene groups separating the diphenylmethane units.[25] The most favourable interaction energetics with the bases are shown by **CP66**, the cyclophane with $n = 6$. Binding comparison of the complexation free energies, ΔG, of mononucleosides and mononucleotides with **CP*nn*** compounds suggests that electrostatic attraction between the ammonium centres of the cyclophanes and the nucleotide phosphate is responsible for a large part of the ΔG. An additional driving force stems from cation-π interactions of the **CP*nn*** ammonium centres

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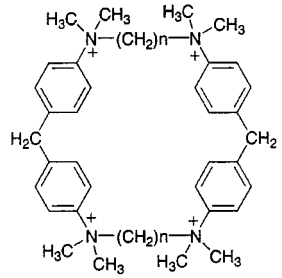

Figure 1. Structure of **CP66**

on the aromatic ligand moieties.[25] We have previously reported that cyclophanes, depending on the *n* value, can either stabilize or destabilize RNA duplexes. When $n = 3$ (**CP33**), a large stabilization of RNA is obtained, with $n = 4$ (**CP44**) one observes a smaller, yet still noticeable stabilization of RNA, but a particularly large one of DNA. However, **CP66** can cause complete denaturation of folded RNA polymers.[26] In sharp contrast, only stabilization of the DNA double helix is observed for all *n* values. Upfield NMR shifts of the **CP*nn*** methylene protons to similar extents occur upon interaction of **CP66**, but not of **CP44**, with unfolded DNA or RNA, and on complexation with mononucleotides, which attests to inclusion of nucleobases within the **CP66** cavity. With double-stranded DNA there are no significant upfield shifts or signal broadenings of the cyclophane protons, in line with the absence of intercalation and therefore with groove binding. The aim of this investigation was to analyse the possible mechanism of the unique selective destabilization of folded RNA with **CP66** and to lay the grounds for possible further improvement in such selective destabilization. The intriguing difference between the activities against RNA and against DNA was investigated by measuring complexations with alternative 2-oxy- and 2-deoxynucleotides and oligonucleotides.

Results

Complexation with 2-Deoxymononucleotides

NMR titrations with 2-deoxymononucleotides and **CP66** yielded association constants very similar to those of the ribose analogues (Table 1). The observed complexation-induced NMR shift (CIS) values for the nucleotides (Table 1), as well as for the host **CP66** (Table 2), were also close for the ribose and the deoxyribose nucleotides (see Tables 1 and 2 and ref.[25]). Distinct upfield shifts were exerted by adenine on the central and 3-H protons of the **CP66** methylene chain, located above the centre of the nucleobase anisotropy cone. Upfield shifts also occurred, although to a lesser extent, during interaction with guanine, while the smaller pyrimidine bases − cytosine and thymine − induced almost no CIS. Conversely, the purine nucleobase protons closely approach the shielding cones of the **CP66** biphenylmethane units and their signals were shifted upfield by 0.41 ppm to $\delta = 0.57$, with much smaller effects on the cytosine and thymine. The signals of the ribose anomeric protons showed appreciable upfield shifts, suggesting that they were in the shielding cone of the **CP66** aryl function, although it is possible that part of the shift variation of the signals of the sugar protons was due to complexation-induced changes of the torsion angles within the furanose ring. However, the spacings of the sugar signals, which agreed with literature data, changed by < 1 Hz during the titrations. By compar-

Table 2. Shift effects of nucleotides on **CP66**

Nucleotide[a]	H_o	H_m	CH_2	CH_3	2-H	3-H	4-H
$dAMP^{2-}$	−0.01	0.07	−0.01	−0.08	−0.03	−0.14	−0.06
$dCMP^{2-}$	−0.01	0.01	−0.02	−0.01	< 0.005	< 0.005	−0.06
$dGMP^{2-}$	0.03	0.01	−0.01	−0.06	−0.02	−0.09	−0.1
$dTMP^{2-}$	−0.01	< 0.005	−0.01	0.02	< 0.005	< 0.005	−0.01

[a] Calculated from association constants from Table 1 with known concentrations, as described in the text, see Figure 5. All shifts are in ppm relative to uncomplexed material; negative signs indicate upfield shift changes.

Table 1. NMR titrations of deoxynucleotides with **CP66**

Guest		2-H	5-H	6-H	8-H	CH_3	1′-H[a]	2′-H[a]	H3′[a]	ΔG_{av} (deoxy)	ΔG_{av} [b] (ribo)
$dAMP^{2-}$	CIS[c]	−0.55			−0.57		−0.23	−0.16			
	10^{-3} *K*[d]	2.6			2.5		2.5	2.5		−19.4	−18.7
$dCMP^{2-}$	CIS		−0.07	−0.09			−0.06	−0.08			
	10^{-3} *K*		1.6	1.6			1.6	1.6		−18.3	−17
$dGMP^{2-}$	CIS				−0.41		−0.18	−0.08	−0.13		
	10^{-3} *K*	3.5					2.1	2.8	2.3	−19.7	−19.6
$dTMP^{2-}$	CIS			−0.04			−0.05	−0.06			
	10^{-3} *K*			2.2			1.5	1.7		−18.6	−17.6

[a] Signals of other protons of deoxyribose were overlapped by signals of water or host protons. [b] Taken from ref.[25] and this paper. [c] Measured in D_2O at pD = +8.4 ± 0.02, all shifts are in ppm relative to uncomplexed material, negative sign indicates upfield shift changes. [d] Given in L/mol at 300 ± 1 K, error ±10%.

ison with the values for the coupling constants given by Lee,[27,28] one can conclude that there were no major conformational changes during titration. Preliminary docking experiments showed that both AMP^{2-} and $dAMP^{2-}$ could be accommodated in the **CP66** cavity with nearly the same geometry.

Complexation with Dinucleotides

Studies with ApA and dApdA

Figure 2 shows the chemical shift changes of ApA and dApdA at increasing concentrations of **CP66**. In all cases we were only able to observe one averaged signal for both complexed and uncomplexed nucleotide, due to fast exchange on the NMR timescale. Assignments and conformation of the uncomplexed dinucleotides have been described in earlier reports.[29−32] The signals at $\delta \approx 8.31$ in both the ApA and dApdA spectra each correspond to 8-H of the adenosine base of the pA nucleotide (3′-adenosine). While the signals at $\delta \approx 8.24$ for ApA and $\delta \approx 8.06$ for dApdA correspond to 8-H of the Ap nucleotide (5′-adenosine), the 2-H protons of adenine bases usually resonate at higher field than 8-H protons. Complexation could be monitored until approximately 70% of completion for calculation of the equilibrium constants (see Table 3).

Table 3. NMR titrations of dinucleotides with **CP66**

Nucleotide	Proton[a]	δ_0	10^{-3} K[b]	CIS[c]	ΔG_{av}
ApA	8-H (Ap)	7.58	0.21	−0.74	−14.5
	2-H (Ap)	7.57	0.2	−0.67	
dApdA	8-H (dAp)	7.74	0.11	−0.32	−12.8
	2-H (dAp)	7.83	0.11	−0.3	
ApG	8-H (Ap)	7.19	0.2	−1.06	−14.4
	2-H (Ap)	7.26	0.2	−0.93	

[a] Signals of other protons were overlapped by signals of water or host protons. [b] Given in L/mol at 328 ± 1 K, error in K is K ±10%. [c] Measured in D_2O at pD = 7.0 ± 0.01 (phosphate buffer), all shifts are in ppm relative to uncomplexed material, negative sign indicates upfield shift changes.

Interpretation of the observed base shielding variations was less straightforward for the dinucleotides than for the mononucleotides,[25] since insertion in the cyclophane cavity might be accompanied by changes in the orientation of the nucleobases within the oligonucleotides. It is known that such oligonucleotides are stacked in helical conformations at low temperature, but that little energy is required to alter or disrupt stacking.[28−30] Small complexation-induced changes of the anisotropy cones between the nucleobases may thus have an effect on the CIS values. This makes it

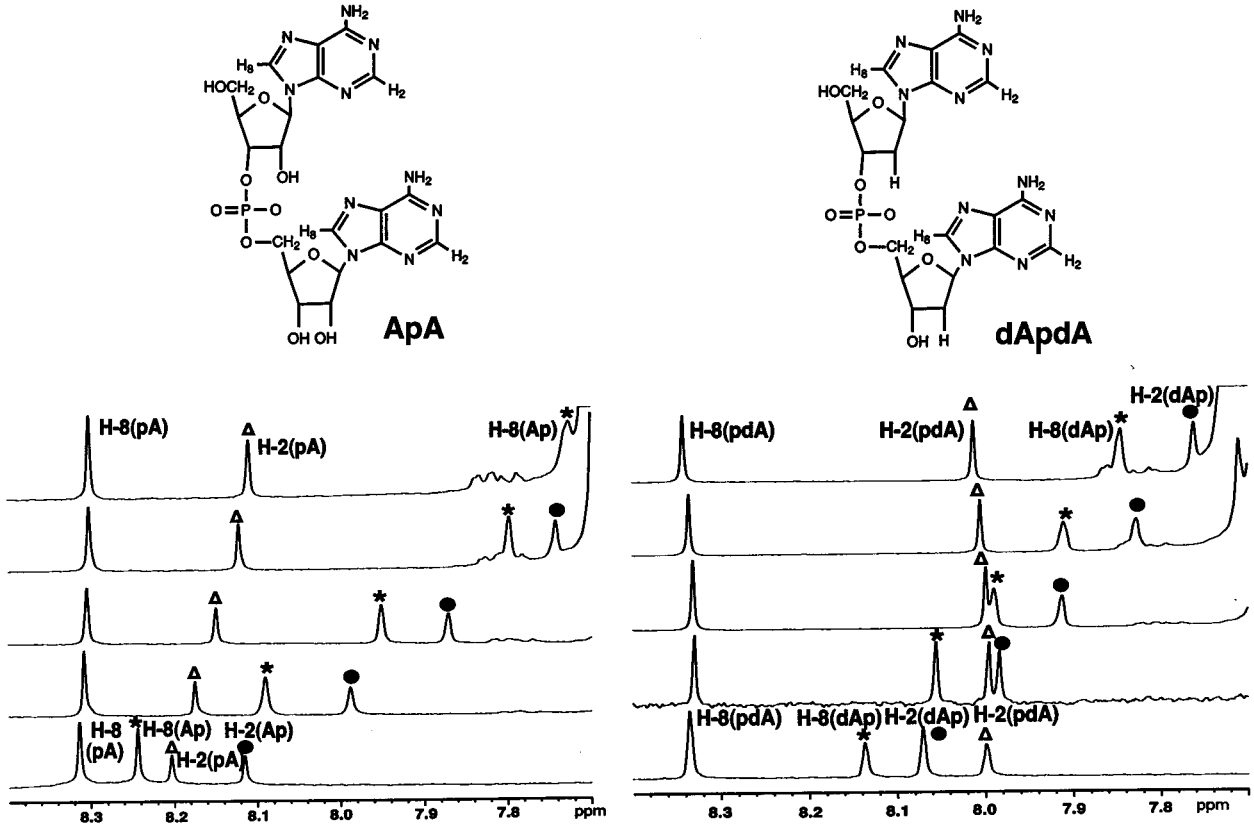

Figure 2. Shift changes of dinucleotides ApA and dApdA upon addition of **CP66** in D_2O buffer, pD = 7.4 (pH = 7.0; 20 mM phosphate buffer), at 323 K; ApA titration: 1 mM ApA, **CP66** concentrations titrations curves from bottom to top: 0, 2, 4, 8, 12 mM; dApdA titration: 1 mM dApdA, **CP66** concentrations from bottom to top: 0, 4, 8, 12, 20 mM

difficult to separate intra- from intermolecular shielding effects between host and guest. Additionally, it has been shown that relatively small displacements in the position of a guest relative to the host can result in shift changes greater than 0.5 ppm.[27] Nevertheless, several conclusions relating to the preferred binding modes can be drawn from the observed shifts.

It is clear that **CP66** mostly binds to the dAp moiety of the dinucleotide, since the corresponding pdA signals shifted only slightly downfield upon complexation. However, the ApA ribodinucleotide showed a complexation pattern with **CP66** different to that of dApdA at a similar degree of complexation (Figure 2). The signal of the 2-H proton of pA shifted upfield as the degree of complexation increased, although, as with the deoxy dimer, the shift was smaller than for the signals of protons of the Ap nucleotide. The 8-H signal of pA shifted very slightly upfield. From this one can conclude that the complex geometry differs at least slightly from that with dApdA. The major conclusion, however, is that **CP66** binds the less-hindered 5′-adenine with similar equilibrium constants in both the ribo and the deoxyribo systems.

Studies with ApG and GpA

To determine possible sequence selectivity of **CP66**, complexation between **CP66** and ApG and GpA was also investigated. Shift changes of ApG and GpA upon titration with **CP66** are shown in Figure 3. Assignments for the peaks of the uncomplexed dinucleotides were taken from the literature.[29−32] The signals of both bases of ApG appeared at lower field than those of GpA. Spectra of the complex with ApG showed that **CP66** bound selectively to the adenine part (Ap), as with ApA. The CIS values for the aromatic protons of Ap were large and upfield, whereas the 8-H (pG) peak moved slightly downfield. In contrast, all proton signals of GpA moved upfield. While the signals of the adenine protons (8-H and 2-H) of ApG showed large shifts, those for A in GpA were relatively small. On the other hand, the signal of the guanine 8-H proton in GpA showed CIS values higher than those of pG 8-H in ApG, although those values were smaller in absolute magnitude than the values for the 8-H adenine proton in ApG. The 8-H proton of G in ApG showed deshielding, locating this proton outside the cavity of **CP66** (Figure 4). The chemical shift values suggested that both purines of GpA were potential sites for complex formation, while the fitted curves did not display any detectable deviations from a 1:1 stoichiometry. It thus appears that either the guanosine or the adenine unit was complexed with similar strength. The association constants, *K*, of ApA and ApG are similar (Table 3), in line with constants observed for AMP and GMP, which are also similar (Table 1).

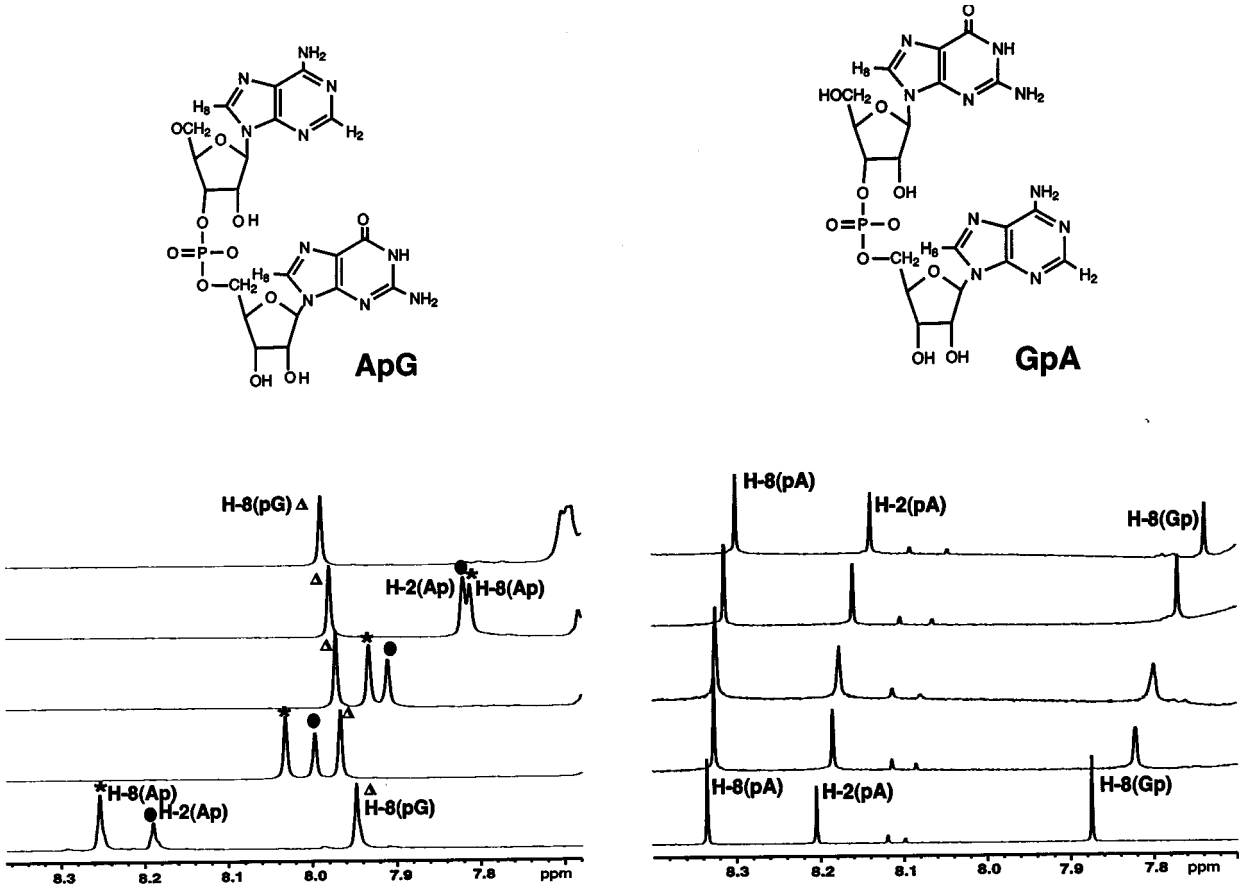

Figure 3. Shift changes of dinucleotides ApG and GpA upon addition of **CP66** (conditions as described in Figure 2); ApG titration: 1 mM ApG, **CP66** concentrations from bottom to top 0, 2, 3, 4, 5 mM; GpA titration: 1 mM GpA, **CP66** concentrations from bottom to top 0, 2, 3, 5, 8 mM

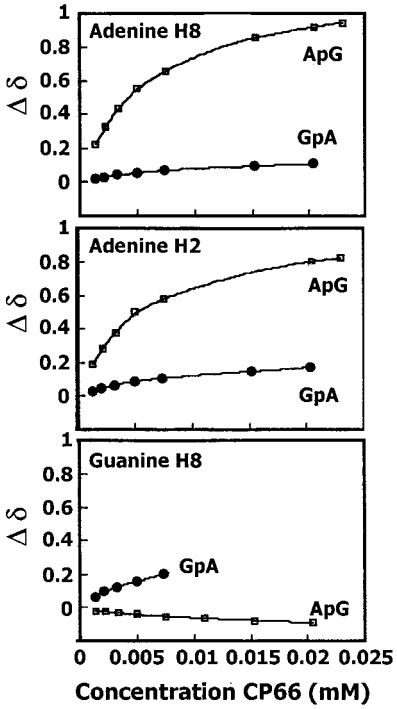

Figure 4. NMR titration isotherms for the 8-H and 2-H protons of the adenine base and 8-H of guanine of the dinucleotides ApG and GpA with **CP66** (conditions are described in Figure 2)

In addition to the CIS values of the nucleotides, we calculated the shift changes of the **CP66** protons effected by the nucleotides. Determination of CIS values for a host present in excess is difficult, but can be done by linear extrapolation from the first data of the titration, if the association constant is known. Plots of chemical shifts vs. the degree of complexation of **CP66** (Figure 5) yielded straight lines with slopes equal to the CIS of the different host **CP66** protons. Surprisingly, the CIS values for **CP66** (Table 4) with all dinucleotides exceeded the values obtained from measurements with mononucleotides (Table 2). It was thus apparent that the complexes with dinucleotides adopted conformations in which one of the two nucleobases came almost face-to-face with the aromatic rings of the cyclophane. Preliminary docking experiments (not shown) indicated that conformations with the dinucleotide embracing either the methylene chain or one of the phenyl rings of the **CP66** host were possible. As mentioned above, we were not able to obtain evidence for the existence of these isomeric complexes by observation of separated signals, due to fast interconversion between the two isomers.

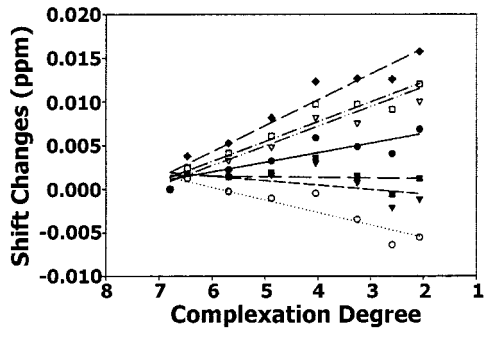

Figure 5. Plots of the **CP66** proton shifts versus degree of complexation of **CP66**; explanation see text; for assignment see CIS values in Table 2 (conditions are described in Figure 2)

Table 4. Calculated shift effects of nucleotides on **CP66**

Nucleotide	H_o	H_m	H_2C	CH_2[a]	$^+NCH_3$	CH_2[b]	CH_2[c]
ApG	−0.953	−0.554	−0.82	−1.197	−0.886	−1.374	−1.596
GpA	−0.89	−0.683	−0.391	−1.143	−1.039	−1.157	−1.232
dApdA	−1.116	−0.883	−1.596	−1.741	–	−1.795	−1.868
ApA	−0.109	−0.142	−0.051	−0.227	−0.007	−0.226	−0.296
ApApC	−0.364	−0.182	−0.044	−0.813	−0.89	−0.995	−1.316

[a] Calculated from association constants from Table 3 with known concentrations, all shifts are in ppm relative to uncomplexed material, negative signs indicate upfield shift changes.

Complexes with Larger Oligonucleotides

The sequence and positional selective binding of **CP66** to RNA was also examined with the trinucleotide ApApC and the tetranucleotide CpApApC, which each have the same ApA subunit as described above. Proton NMR spectra of the aromatic region of ApApC with increasing amounts of added **CP66** are shown in Figure 6. The signals of the trinucleotide at different temperatures were assigned by Lee and Tinoco.[27] At 55 °C, signals of both 8-H adenine protons had the same chemical shift. At higher temperatures, however, the peaks corresponding to the pA base appeared at higher chemical shift than those belonging to Ap, while at temperatures below 55 °C, the signals of Ap had higher chemical shifts. When **CP66** was added to the solution at increasing concentrations, only the peaks corresponding to the protons of Ap moved significantly upfield. Some slight upfield chemical shifts for the 2-H (pA) proton, similar to the shifts observed in ApA for the 2-H (pA) proton, were also observed. The peaks corresponding to the 5-H and 6-H protons of cytosine did not undergo any significant change. One can conclude that **CP66** was binding primarily to the Ap base through an insertion complex of the Ap base of ApApC probably very similar to that formed with ApA. Similar results were obtained for base selectivity in binding of the analogous deoxytrimer (not shown). The signals of the protons of the adenine bases shifted to a larger extent than those of the cytosine. The shift changes of the signal of the cytosine 5-H proton were only about one tenth those of the purine protons. In contrast to the results for the ribonucleotide, the differences between the two purine bases were smaller, especially for the 2-H protons. The overall shift changes of the signals of the 8-H protons were smaller than those of the 2-H protons, but the differences between them were larger. From these results one can conclude a reduced preference for the 5′-adenine in the case of the deoxynucleotide. Although the association isotherms with

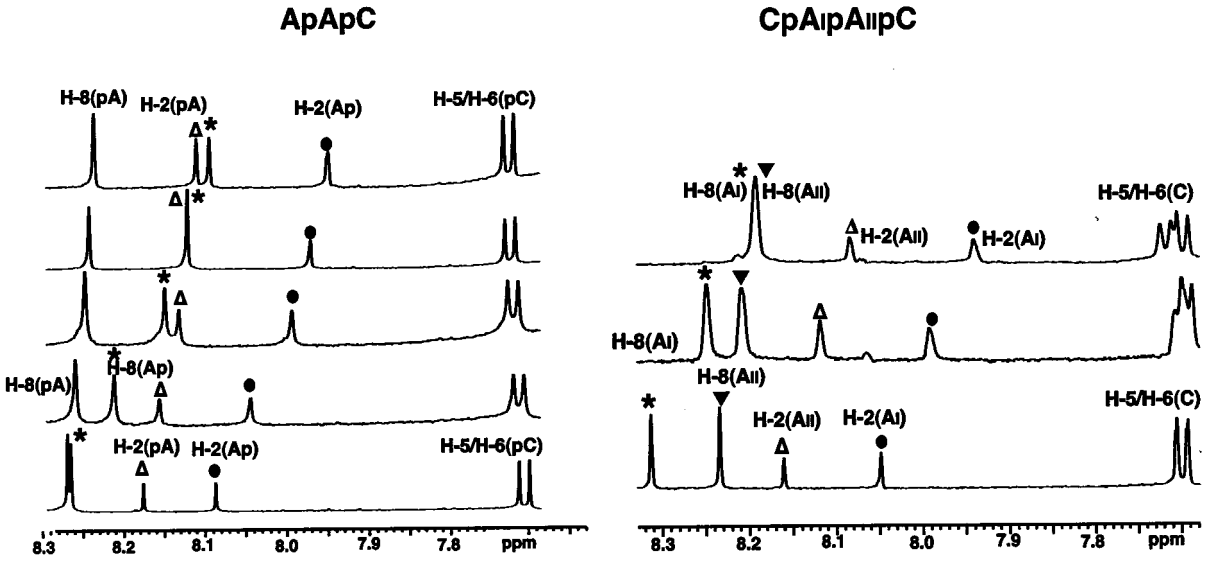

Figure 6. Shift changes of tri- and tetranucleotides upon addition of **CP66** (conditions are described in Figure 2); ApApC titration: 1 mM ApApC, from bottom to top **CP66** concentrations 0, 0.5, 1, 1.5, 2 mM; CpApApC titration: 0.5 mM CpApApC, from bottom to top **CP66** concentrations 0, 0.25, 0.5 mM

these trinucleotides could not be evaluated with the same accuracy as for the dinucleotides, the titration experiments qualitatively suggested a binding constant in the same range as those obtained for ApA and ApG.

An NMR titration of CpApApC with **CP66** under the same conditions as for the dinucleotides is also shown in Figure 6. Assignment of the peaks was performed by comparing the chemical shifts with the trinucleotides ApApC and UpApA reported by Lee and Tinoco.[27,28] Spectra of CpApApC showed upfield shifts for the signals of all protons of both adenine bases on titration with **CP66**, although the chemical shifts for signals of the first Ap base were again larger than those of the second Ap base. Chemical shift changes for the signals of both pyrimidine bases were small. The K values obtained from fitting different protons on the A bases were similar (Table 5), corresponding to an average ΔG value of 13.0 kJ/mol, similar to the results with other nucleotides. Titration experiments with the analogous deoxy tetramer (not shown) yielded upfield NMR shifts for the purine bases (up to −0.35 ppm), indicating preferred inclusion of these nucleobases in the **CP66** cavity, whereas the shift of the signals of the pyrimidine protons were distinctively smaller and downfield.

Table 5. NMR titrations of CpApApC with **CP66**

Proton	δ_0 [a]	10^{-3} K[b] [L/mol]	CIS[c]
2AH8	8.05	0.17	−0.176
3AH8	7.962	0.28	−0.177
2AH2	7.757	0.23	−0.241
3AH2	7.671	0.17	−0.162
1CH6	7.469	0.28	0.027

[a] Signals of protons not mentioned were either overlapped with **CP66** signals or poorly resolved, signals of ribose protons in particular. [b] At 303 K in D_2O at pD = +7.4 ± 0.02 (phosphate buffer). [c] CIS = δ(100% complexation) − δ_0; other shifts are relative to TMS, negative signs indicate upfield shift changes.

Groove Matching as Origin of the Different Behaviour of RNA and DNA

The difference between monoribonucleotide inclusion and deoxyribonucleotide inclusion in the **CP66** cavity is probably too small to explain the observed destabilization of RNA in comparison with the stabilization of DNA duplexes. The geometric conditions for ion-pairing of cyclophane cationic amines with DNA phosphates are depicted in Figure 7. The distances were modelled with idealized duplex conformations and cyclophane structures, which were optimized with a force field (see Exp. Sect.). Only the wide groove of B-DNA is able to accommodate **CP66** with optimum contact between the cyclophane ammonium and the DNA phosphate centres. The importance of this geometric match for stabilization of nucleic acids is emphasized by the results for **CP44**, a cyclophane smaller than **CP66**. The highest melting point (Tm) increase for the cyclophanes in this series was observed for duplex DNA upon complexation with **CP44**.[26] Consistently with the mechanism outlined above for selective stabilization of the DNA duplex, the distances in **CP44** gives an even better fit in the major groove of DNA than for **CP66** (Figure 7). In contrast, the RNA phosphate distances are too small to allow optimum contacts between all nitrogen centres of **CP66** and the phosphate. It appears that, for duplex RNA, the intracavity inclusion mechanism of the single-stranded nucleotides prevails with this larger cyclophane and accounts for the destabilizing influence of **CP66** on the duplex and the shift of the Tm to lower temperature.

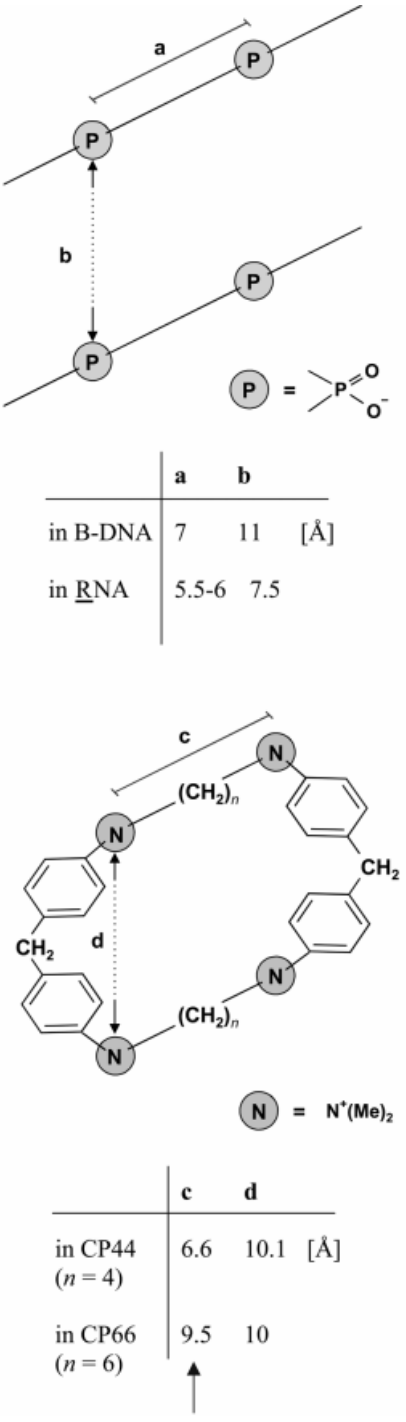

Figure 7. Geometric fitting of cyclophanes **CP*nn*** to major grooves of B-DNA or A-RNA; charges represented by the cationic and anionic centres

Conclusions

NMR titrations of several nucleotides with the azoniacyclophane **CP66** have clearly shown that this host has a distinct preference for purine bases over pyrimidine bases. In addition, it is evident that there is also a preference of this host for the purines at the 5′-end. Comparison of ribonucleotides with analogous deoxynucleotides has shown that the dramatic differences between RNA and DNA helical stabilities in the presence of the cyclophane **CP66** cannot be due to differences in association constants between 2-deoxyribo- and ribonucleotides. An alternative, or additional, explanation for the stabilization of double-stranded DNA is based on a satisfactory fit of the cyclophanes into the wider groove of DNA. The smaller RNA groove makes the intracavity inclusion of a nucleobase upon base flipping preferred, resulting in destabilization of the RNA helix.

Experimental Section

Substances: Adenylyl-(3′,5′)-adenosine (ApA), 2′-deoxyadenylyl-(3′,5′)-2′-deoxyadenosine (dApdA), guanylyl-(3′,5′)-adenosine (GpA), adenylyl-(3′,5′)-guanosine (ApG) and ApApC were purchased from Sigma, the tetraribonucleotide CpApApC from Cruachem, the deoxynucleotides from Fluka, deoxydi-, tetra- and octanucleotides from Pharmacia. All nucleotides were pure enough for NMR experiments. The azoniacyclophane **CP66** was prepared as described earlier.[26] After repetitive recrystallizations and chromatographic purifications of all intermediates, the purity of the final tetracation was higher than that of older samples. This was confirmed by TLC (Al_2O_3/88% acetonitrile + 12% acetic acid). With this solvent system it was possible to achieve separation on TLC plates for the first time.

NMR spectroscopy: NMR experiments were performed with Varian Plus 500 or 600 MHz or Bruker DRX 500 spectrometers in 5-mm tubes. The experiments were carried out at temperatures as indicated in the captions of the figures in D_2O, at pD = 7.4 ± 0.01 (0.02 M phosphate buffer, 10^{-5} M EDTA, 10^{-5} mol/L EDTA) and with TMS as external reference. Titrations with ApA, dApda, GpA, ApG and ApApC were carried out as described earlier.[26]

MM Calculations: Quanta/CHARMm[33] 4.0 with CHARMm 22 and an SGI INDY R4000/100. Waterbox simulations were done with TIP3-type water. The size was chosen to ensure a distance of at least two water molecules to the (periodic) boundaries.

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[1] S. J. Sucheck, A. L. Wong, K. M. Koeller, D. D. Boehr, K.-A. Draker, P. Sears, G. J. Wright, C.-H. Wong, *J. Am. Chem. Soc.* **2000**, *122*, 5230.
[2] K. Michael, Y. Tor, *Chem. Eur. J.* **1998**, *4*, 2091.
[3] K. Li, M. Fernandez-Saiz, C. T. Rigl, A. Kumar, K. G. Ragunathan, A.W. McConnaughie, D. W. Boykin, H.-J. Schneider, W. D. Wilson, *Bioorg. Med. Chem.* **1997**, *5*, 1157.
[4] T. Hermann, E. Westhof, *J. Med. Chem.* **1999**, *42*, 1250.
[5] T. Hermann, W. Westhof, *Curr. Opin. Biotech.* **1998**, *8*, 278.
[6] M. Afshar, C. D. Prescott, G. Varani, *Curr. Opin. Biotech.* **1999**, *10*, 59.
[7] C. Chow, F. M. Bogdan, *Chem. Rev.* **1997**, *97*, 1489.
[8] C.-H. Wong, M. Hendrix, D. D. Manning, C. Rosenbohm, W.A. Greenberg, *J. Am. Chem. Soc.* **1998**, *120*, 8319.

[9] K. Li, T. M. Davis, C. Bailly, A. Kumar, D. W. Boykin, W. D. Wilson, *Biochemistry* **2001**, *40*, 1150.

[10] S. Mazur, F. A. Tanious, D. Ding, A. Kumar, D. W. Boykin, I. J. Simpson, S. Neidle, W. D. Wilson, *J. Mol. Biol.* **2000**, *300*, 321.

[11] D. E. Wemmer, P. B. Dervan, *Curr. Opin. Chem. Biol.* **1997**, *3*, 355.

[12] D. E. Wemmer, *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 439.

[13] L. Wang, C. Bailly, A. Kumar, D. Ding, M. Bajic, D. W. Boykin, W. D. Wilson, *Proc. Natl. Acad. Sci.* **2000**, *97*, 12.

[14] W. D. Wilson, in *Nucleic Acids in Chemistry and Biology*, 2nd ed. (Eds.: G. M. Blackburn, M. J. Gait), Oxford University Press, New York, **1996**, p. 329.

[15] W. D. Wilson, in *Comprehensive Natural Products Chemistry* (Ed.: E. T. Kool), Elsevier Science Ltd., Oxford, **1999**, vol. 7, p. 427.

[16] B. H. Geierstanger, D. E. Wemmer, *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 463.

[17] P. B. Dervan, R. W. Burli, *Curr. Opin. Chem. Biol.* **1999**, *6*, 688.

[18] C. Bailly, J. B. Chaires, *Bioconj. Chem.* **1998**, *9*, 513.

[19] C. Bailly, in *Advances in DNA Sequence Specific Agents* (Ed.: M. Palumbo), JAI Press Inc., Greenwich, CT, **1998**, vol. 3, p. 97.

[20] S. Wang, P. W. Huber, M. Cui, A. J. Czarnik, H.-Y. Mei, *Biochemistry* **1998**, *37*, 5549.

[21] H.-Y. Mei, A. J. Galan, N. J. Halim, D. P. Mack, J. W. Moreland, K. B. Sanders, H. N. Truong, A. J. Czarnik, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2755.

[22] M. L. Zapp, S. Stern, M. R. Green, *Cell* **1993**, *74*, 969.

[23] H. Umezawa, I. R. Hooper (Eds.), in *Aminoglycoside Antibiotics*, Springer-Verlag, New York, Heidelberg, **1982**.

[24] B. D. Haly, P. D. Cook, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2345.

[25] [25a] H.-J. Schneider, T. Blatter, B. Palm, U. Pfingstag, V. Rüdiger, I. Theis, *J. Am. Chem. Soc.* **1992**, *114*, 7704. [25b] H.-J. Schneider, T. Blatter, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1207; *Angew. Chem.* **1992**, *104*, 1244.

[26] M. Fernandez-Saiz, H.-J. Schneider, J. Sartorius, W. D. Wilson, *J. Am. Chem. Soc.* **1996**, *118*, 4739.

[27] C. H. Lee, *Eur. J. Biochem.* **1983**, *137*, 347.

[28] C. H. Lee, I. J. Tinoco, *Biophys. Chem.* **1980**, 283.

[29] C. Lee, F. E. Evans, R. H. Sarma, *FEBS Lett.* **1975**, *51*, 73.

[30] S. I. Chan, J. H. Nelson, *J. Am. Chem. Soc.* **1969**, *91*, 168.

[31] F. E. Evans, C. Lee, R. H. Sarma, *Biochem. Biophys. Res. Commun.* **1975**, *63*, 106.

[32] P. O. P. Tso, N. S. Kondo, M. P. Schneizer, D. P. Hollis, *Biochem.* **1969**, *8*, 997.

[33] A.T. Brünger; M. Karplus, *Acc. Chem. Res.* **1991**, *24*, 54, and references cited therein.

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