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Reversal of polyamine selectivity for DNA and RNA by steric hindrance

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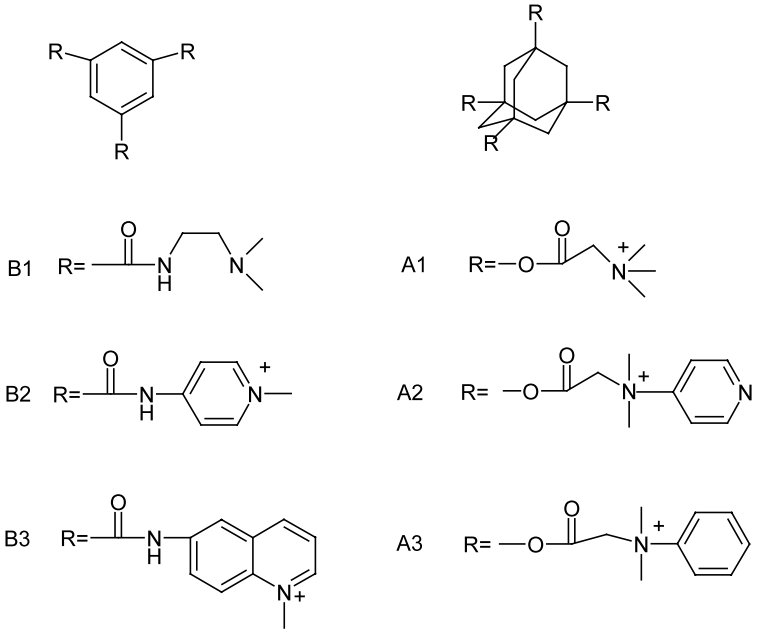
Abstract—The polyamines known until today generally bind better to double-stranded RNA than to DNA, as shown in significantly higher melting point increases with RNA. We report that large and bulky polyamines with three or four positively charged nitrogen centers connected via flexible or rigid linkers to either a benzene or an adamantane core show high affinities and are large enough to exhibit a strong preference for double-stranded DNA (polydApolydT) in comparison to RNA (polyApolyU), which differs by its smaller and deeper groove.

Principles ruling the selectivity of nucleic acid ligands are of vital interest for the design of new agents for diagnostic and therapeutic applications.[1] Most of the reported ligands make use of selective binding e.g. by hydrogen bonding, such as in anti-gene or anti sense strategies,[2] or e.g. by lipophilic interactions in deeper grooves.[3] We wanted to explore the possibility of achieving some selection by steric limitations based on ligands with a particularly large volume. As the majority of natural and synthetic antibiotics directed against double-stranded nucleic acids bind to minor grooves, it was also of interest to develop simple organic compounds which in analogy to peptide helices or proteins may bind to major grooves. Our hope was also based



Scheme 1.

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on recent findings with some macrocyclic polyamines showing unexpected destabilization of folded RNA in contrast to DNA, which can be ascribed to the better fitting of these ligands to the larger major groove in DNA as compared to RNA.[4] However, it is difficult with such cyclophanes to eliminate other factors, such as intracavity inclusion of a nucleobase after the polymer unfolding as a major driving force for the observed effect. Other macrocycles in fact show in most cases a larger stabilization of double-stranded RNA in comparison to DNA, in line with invariable observations with other polyamines.[5,6]

The new ligands shown in Scheme 1 were accessible in usually 35–50% yields from reactions of the benzotricarboxylic acid trichloride (for **B1**, **B2**, **B3**) and the tetrakisbromoacetoxyadamantane (for **A1**, **A2**, **A3**) with the appropriate amines. They all showed satisfactory analytical and spectral data.

The ligands contain three or four positively charged nitrogen atoms connected via flexible or rigid spacers to either a benzene or a adamantane core. According to molecular mechanics calculations (gas phase simulations using MM2 (1991))[7] the distances between the nitrogen centers vary from 8 to 13 Å. These distances are far too large to allow favorable electrostatic interaction with the negatively charged minor grooves; they would also better fit the wider major groove of DNA than to RNA. Fig. 1 illustrates that the 'thickness' of ligand **A2** indeed matches that of a zinc-finger protein helix, which is known to bind to major grooves. We tested the affinity and selectivity of the ligands both with changes ΔT in the melting temperature of double-stranded RNA (polyA–polyU) and DNA (polydA–polydT).

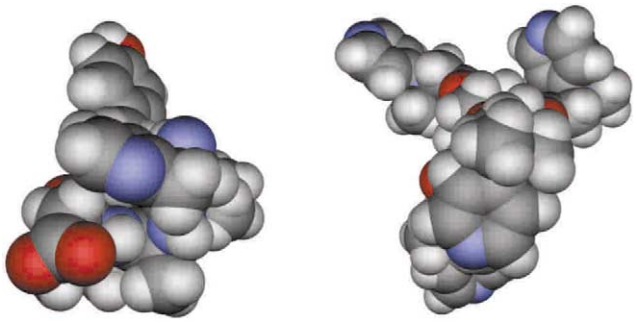

Figure 1. Comparison of thickness of ligand **A2** (right side) with that of a zinc-finger protein helix (view from top).

As it was hoped, the larger ligands indeed show the opposite behavior of known polyamines, with considerably higher stabilization of DNA than of RNA (Table 1). The differences are most dramatic with the adamantane derivatives **A1**–**A3** and the benzene compound **B3**, with a $\Delta\Delta T$ of up to 30°C in favor of DNA. It should be noted that with other polyamines the opposite preference for RNA was observed ($\Delta\Delta T$ up to 30°C).[6b] With the larger and more isotropic adamantane derivatives we see much larger affinities to DNA than to RNA; with **A2** one observes even a small destabilization of double-stranded RNA.

In most cases the affinities as reflected by the ΔT values are smaller than those observed with other polyamines with the same number of positive charges,[6b] particularly with the adamantane derivatives **A1**–**A3**. This can be understood by the impossibility of achieving optimal ion pairing contacts with all the nitrogen centers in the bulky ligands, in contrast to flexible smaller polyamines. Such differences between the known and the new polyamines become larger with increasing ligand to nucleic acid ratio r, suggesting changes of binding modes with r.

Table 1. ΔT_m[a] values (°C) of **B1**, **B2**, **B3**, **A1**, **A2**, **A3** with polydA–polydT and polyA–polyU

Ligand (number of positive charges)	r[b]	ΔT_m (°C) PolydA.PolydT	ΔT_m (°C) PolyA.PolyU
B1 (+3)	0.1	13.3	6.5
	0.2	17.5	9.0
	0.3	19.3	12.0
B2 (+3)	0.1	6.9/19.4	24.5
	0.2	23.4	30.0
	0.3	28.3	30.2
B3 (+3)	0.1	13.5	3.0
	0.2	27.8	3.6
	0.3	34.2	3.9
A1 (+4)	0.1	21.7	10.6
	0.2	26.1	16.3
	0.3	27.6	19.3
A2 (+4)	0.1	14.4	−2.0
	0.2	19.2	−3.2
	0.3	22.7	−4.2
A3 (+4)	0.1	8.5	2.3
	0.2	12.0	4.9
	0.3	15.7	5.9

[a] Conditions: 0.01 M MES buffer, pH 6.25, $I = 0.01$ M, error in $\Delta T_m = \pm 0.5$°C.
[b] r, molar ratio of ligand/nucleic acid phosphate.

Stabilizing effects of aryl groups in ligands with pyridinium and quinolinium units could also be due to intercalation into double-stranded nucleic acids. Preliminary ^{1}H NMR measurements, however, showed only small line width increases of up to 10 Hz, and shift changes of up to only 0.02 ppm, whereas much larger effects are typical even with weak intercalators such a tryptophane-containing peptides, quinine etc.[8] Systematic comparisons[9] with series of ligands containing phenyl- or naphthyl-size aromatic parts have established that, for ligands such as **B2**, **B3**, **A2**, **A3**, no intercalation can be expected.

In conclusion, the steric size of simple synthetic ligands can serve as a basis to direct synthetic ligands to differentiate grooves of folded nucleic acids. Connections of such units, e.g. to oligonucleotides could then be used to achieve base selectivity; combinations with other units can be envisaged to lead to cleaving or alkylating antitumor agents with modified selectivities. With less isotropic ligand shapes one can hope to find selectivity for anisotropic RNA loops, hairpins, etc. by steric control.

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