Molecular recognition of cationic phenothiazinium and phenoxazinium dyes with π-extended 2′-deoxyadenosine nucleotides†

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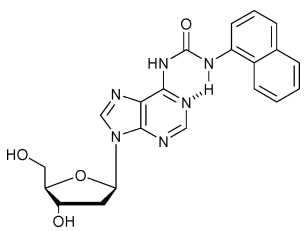
N^6-(N'-Arylcarbamoyl)-2′-deoxyadenosine-*H*-phosphonates displayed molecular recognition towards cationic phenothiazinium and phenoxazinium dyes in aqueous solutions; studies have shown that binding is driven mainly by aromatic interactions and that size and shape-complementarity of the aromatic rings in host and guest provides selectivity.

Non-covalent interactions between aromatic species play a prominent role in molecular recognition phenomena in chemistry, biology and materials sciences.[1,2] Stacking of the aromatic bases is the dominant contribution to the stability of the DNA duplex, and the incorporation of natural and non-natural nucleosides as dangling end residues generally provides an extra stabilization due to the interaction of the aromatic moiety with the neighboring base pair.[3] For example, N^6-(N'-naphthylcarbamoyl)-2′-deoxyadenosine (Scheme 1) as a dangling end residue produced one of the largest stabilities of the DNA duplex.[4] Sugimoto and co-workers suggested that co-planarity of the adenine and the naphthyl group, possibly aided by an intramolecular hydrogen bond, virtually extends the available π surface and enhances the stacking interaction.[4]

For aromatic guest molecules only a few water-soluble host species are available and these are mostly macrocycles.[1,5,6] For instance, receptors derived from charged tetrakis-arylporphyrins have shown selectivity to bind aromatic guests in aqueous solutions.[6] The systematic analysis of these supramolecular porphyrin complexes with ligands containing 6, 10 and 14 π-electrons showed that the association energy is well described by constant free energy increments depending on the number of participating π-electrons.[6a] Although dispersive interactions frequently represent a significant portion of the net attractive effect between two aromatic systems, there is still



Scheme 1

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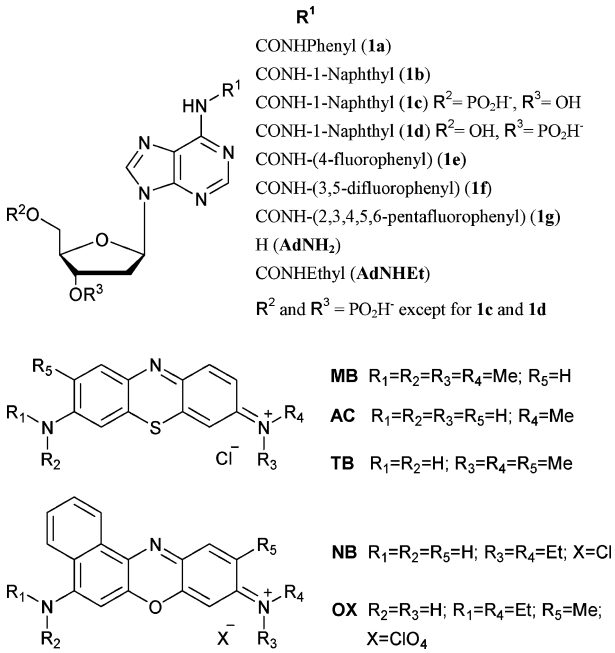
† Electronic supplementary information (ESI) available: Synthetic procedures and characterization data, UV-vis titrations, ^{19}F NMR spectra, modeling, and thermodynamic data. See DOI: 10.1039/b913315a



Scheme 2

controversy as to the corresponding contribution by other effects such as electrostatics and charge-transfer among others.[1a,c,e] Therefore, novel water-soluble aromatic hosts in which, size, shape and electronic properties can be tailored to suit a particular guest are still needed. Compounds like N^6-(N'-arylcarbamoyl)-2′-deoxyadenosine (**1a–g**, Scheme 2) are synthetically accessible and may serve as water-soluble receptors for aromatic species. Using 2′-deoxyadenosine as a scaffold also allows the incorporation of additional recognition sites on the hydroxyl groups and possibly to further elongate the mononucleoside to di- or trinucleotides employing DNA synthetic methodologies.[3,4] We now report on the molecular recognition properties of H-phosphonate nucleotides **1a–g** that were synthesized as triethylammonium salts starting from 2′-deoxyadenosine with excellent yields, offering a simple route to incorporate different aromatic residues (Scheme S1 ESI†). Cationic phenothiazinium and phenoxazinium salts were chosen as the target aromatic guest. Among these are well-known biological indicator probes such as methylene blue (**MB**), azur C (**AC**), toluidine blue (**TB**), Nile blue (**NB**) and oxazine-170 (**OX**) (Scheme 2).[7] They are also used as antitumorals, antibacterials, DNA triplex stabilizers, and antimalarials,[8] and in most cases their biological activities have been related to their π-stacking capability.

In order to test the binding capacities of nucleotides **1a–g** towards the cationic dyes, association constants were

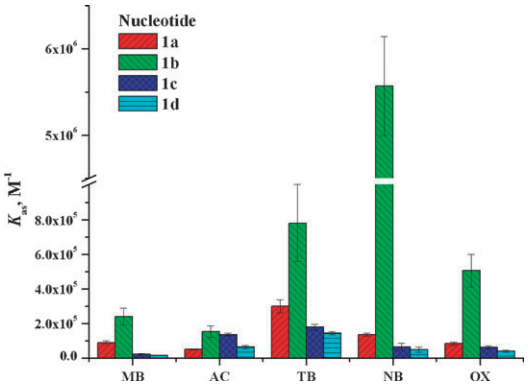


Fig. 1 Comparison of association constants (K_{as}) for nucleotides **1a–d** with phenothiazinium and phenoxazinium dyes. (See ESI†).

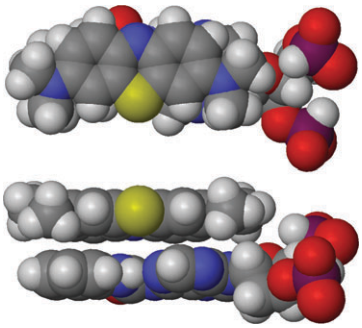


Fig. 2 Equilibrium geometry for complex **1a–MB**. (Bottom) Side view showing co-planarity for adenine and phenyl rings and stacking of the dye. (Top) View from **MB** face showing complementarity of aromatic surfaces. (see ESI†).

determined by UV-vis absorption titrations in MOPS buffer (4-morpholinepropanesulfonic acid, pH 7, 25 °C and 0.010 M NaCl). Qualitative information on the binding modes was obtained by ^{1}H and ^{19}F NMR spectroscopy and molecular modeling. The titrations were monitored by the decay of the charge-transfer bands that regularly appear in the 530–680 nm region for the dyes. In some cases the absorbance decay was concomitant with the emergence of a new red-shifted band featuring an isosbestic point. Nucleotides without further aryl residue such as **AdNH$_2$** and **AdNHEt**, only produced a marginal change in the absorbance within the concentration range used for **1a** or **1b** (Fig. S9 ESI†). Table S1 (ESI†) summarizes the average binding constants obtained from the titration experiments and the data for derivatives **1a–1d** are depicted in Fig. 1.‡ For the phenyl derivative **1a** the binding constants for the complexation of phenothiazinium dyes were in the order of $\sim 10^4$–10^5 M^{-1} following the trend **TB** > **MB** > **AC**. The same tendency was observed for the 1-naphthyl derivative **1b**, with a constant gain in the free binding energy of $\Delta\Delta G^{o} \sim -2.5$ kJ mol^{-1} ongoing from **1a** to **1b**, thus indicating that the size of the aromatic residue is of importance for the binding strength. In order to assess the contribution from ion-pair interactions, monoanionic 1-naphthyl compounds **1c** and **1d**, in which one H-phosphonate at positions 5′ or 3′ is present, were also tested.§ These monoanionic nucleotides reported lower binding energies in comparison to the 3′,5′-bis-*H*-phosphonate salt **1b**. In some cases the difference in energy was approximately 5–6 kJ mol^{-1}, which matches the value expected per salt bridge in aqueous solutions (Table S2 ESI†).[1a,6] Further insight in the ion-pair interaction came from studying the binding dependence on the ionic strength for complex **1b–TB**. The binding constant for this complex decreased concomitant to the increase of saline concentration from 5×10^{-3} to 8×10^{-2} M NaCl giving an overall 7.5-fold drop (Fig. S10 ESI†). This behavior is typical for host–guest complexes in which ion-pair interactions contribute to the overall affinity.[1a,6]

The calculated equilibrium geometry for compounds **1a–1d** indicated molecular structures with intramolecular H-bonding interactions between the purinic N_1 nitrogen and the ureido hydrogen having an (*E*,*Z*) conformation (see Fig. S11 ESI†); these results are in qualitative agreement with the structures observed for urea-functionalized pyridines, pyrimidines, guanines and 2,6-diaminopurines that display an *anti*/*syn* conformational equilibrium in non-aqueous solutions.[9,10] Generally, conformations with N–H$\cdots$N$_{arom}$ interactions are favored. Assuming that this configuration holds in the complexes, an adequate contact between the aromatic surfaces can be found for the three ring-systems of the phenothiazinium dyes and nucleotides **1a–1d**, as exemplified for **1a–MB** in Fig. 2. To shed light on the mechanism driving the recognition between the aromatic groups, the estimated free energy contribution from salt bridges was subtracted from the observed free binding energies. For complex **1a–MB**, after subtraction of two salt bridges (10 kJ mol^{-1}),[1a,6] the remaining free binding energy was -18.2 kJ mol^{-1}. This value is in excellent agreement with the value previously reported for condensed three ring systems with 14 π-electrons ($\Delta G_{dispersion} = -18.5$ kJ mol^{-1}).[6a] In the case of **1b–MB**, their salt bridge contributions can be estimated more accurately from the $\Delta\Delta G^{o}$ values obtained in the comparison with complexes **1c–MB** (+6 kJ mol^{-1}) and **1d–MB** (+6.7 kJ mol^{-1}) (see Table S2 ESI†). After subtracting these values from $\Delta G^{o} = -30.6$ kJ mol^{-1} (for **1b–MB**) the remaining free binding energy was -17.9 kJ mol^{-1}, which also agrees well with the $\Delta G_{dispersion}$ indicated above. Thus, for complexes in which host and guest aromatic surface fits well, the association energy can be explained from aromatic and ion-pair interactions, underscoring dispersion as the main mechanism in aromatic recognition.

Preliminary thermodynamic data were acquired for **1b–TB** by measuring the binding constants in the temperature range from 5 to 45 °C, and then fitting the data to the van′t Hoff equation (see Fig. S13 ESI†). An enthalpy of $\Delta H^{\circ} = -29.8 \pm 3$ kJ mol^{-1} was thus obtained. For comparison, a value of $\Delta G^{o} = -33.6$ kJ mol^{-1} was measured for **1b–TB** at 298 K, pointing out that the association is enthalpically driven, which further supports the dominance of aryl–aryl interactions.

Fluoroaromatics have become the standard probe to illustrate the relative importance of electrostatics in aromatic interactions.[2d,2e,11] Derivatives containing 4-fluorophenyl (**1e**), 3,5-difluorophenyl (**1f**) and pentafluorophenyl (**1g**) were also examined. As shown in Fig. S14 and Table S1 (ESI†), no clear trend can be delineated from the binding constants; for example, by complexation of **MB** with **1e** and **1f** the binding constants decreased by 2.5-fold in comparison to **1a**, but in other complexes the binding constant remained the same (*i.e.*, **1e–NB**) or even increased by 2.3 and 4-fold for **1f–TB** and **1f–AC**,

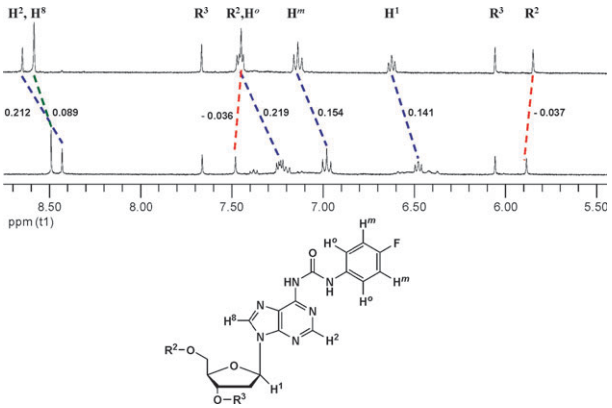


Fig. 3 (Top) ^{1}H NMR spectra of **1e** (1×10^{-3} M in D_2O). (Bottom) Changes upon addition of one molar equivalent of **AC**. Only the region between 5.5 to 9.0 ppm is shown. R^2 and $R^3 = PO_2H^-$.

Table 1 ^{19}F NMR complexation induced shifts ($\Delta\delta$) in titrations of nucleotides **1e**, **1f** and **1g** with phenothiazinium dyes[a]

Dye	1e	1f	1g[b]
MB	−6.9	+0.472	+0.174, +0.128, +0.037
AC	−3.16	+0.216	+0.081, +0.067, +0.026
TB	−0.553	+0.171	+0.252, +0.207, +0.132

[a] $\Delta\delta$ measured in deuterated water at 25 °C using KF as reference. [b] *ortho*, *meta* and *para* F positions in **1g**, respectively.

respectively. As depicted in Fig. 3, the ^{1}H NMR spectra of the nucleotide **1e** in the regions corresponding to the purine (H^2, H^8) and the 4-fluorophenyl (H^o, H^m) protons showed upfield-shifts in the presence of one molar equivalent of the dye **AC**, thus indicating that its aromatic surface stacks over both rings of the nucleotide. Likewise the anomeric proton (H^1) showed the same tendency, supporting a preference of the dye to stack over one of the faces of the nucleotide. It is worth noting that only the 5′-*H*-phosphonate was shifted towards lower fields.

Upon addition of phenothiazine dyes the ^{19}F NMR spectra of **1e–1g** also changed. The induced shifts at 100% complexation (CIS, $\Delta\delta$) are summarized in Table 1. The **MB** dye produced large CIS values in the presence of **1e** and **1f**, but these changes occurred in opposite directions displacing the *para*-F substituent in **1e** upfield, while the 3,5-*meta*-F in **1f** were shifted downfield. This indicates that the overlap of **MB** relative to the substituted phenyl group in these complexes differs as suggested by molecular modeling (see Fig. S17 ESI†). The results with nucleotide **1g** indicated that the pentafluorophenyl ring does not lie on the same plane as the adenine group due to steric hindrance of the *ortho*-F substituents and the urea carbonyl group. Plausible complexes with **1g** gave interesting cleft-type structures (See Fig. S18 ESI†). The small downfield displacements measured for complexes between **1g** and phenothiazine dyes (Table 1) are in agreement with this model structure.

In conclusion, our results have shown that extending the π-surface of 2′-deoxyadenosine 3′,5′-*H*-phosphonate nucleotides leads to flat aromatic based receptors that are suitable to recognize selectively aromatic species in aqueous solutions. Association constants can be explained by free energy increments from aromatic and ion-pairing interactions. In fluorophenyl derivatives polarity seems to be less important than size and shape-complementarity. These findings indicate that dispersion interactions contribute more significantly to aromatic recognition in aqueous solution. Exploration of further host derivatives and guest species is underway.

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Notes and references

‡ Binding constants were obtained from triplicate experiments and data averaged from at least five wavelengths. Non-linear fit to a 1 : 1 binding model in which concentrations of host and guest are of similar magnitude was used. See H.-J. Schneider and A. K. Yatsimirsky, *Principles and Methods in Supramolecular Chemistry*, Wiley, 2000, p. 142.

§ 1-Naphthylcarbamoyl-2′-deoxyadenosine nucleoside containing free 5′,3′-OH groups is not soluble enough to perform titration experiments in buffer aqueous solutions.

1 (*a*) H.-J. Schneider, *Angew. Chem., Int. Ed.*, 2009, **48**, 3924; (*b*) J.-H. Ryu, D.-J. Hong and M. Lee, *Chem. Commun.*, 2008, 1043; (*c*) E. A. Meyer, R. K. Castellano and F. Diederich, *Angew. Chem., Int. Ed.*, 2003, **42**, 1210; (*d*) M. L. Waters, *Curr. Opin. Chem. Biol.*, 2002, **6**, 736; (*e*) C. A. Hunter, K. R. Lawson, J. Perkins and C. J. Urch, *J. Chem. Soc., Perkin Trans. 2*, 2001, 651; (*f*) S. K. Burley and G. A. Petsko, *Science*, 1985, **229**, 23.

2 For model systems with rotameric or conformational flexible molecules: (*a*) F. Cozzi, M. Cinquini, R. Annunziata, T. Dwyer and J. S. Siegel, *J. Am. Chem. Soc.*, 1992, **114**, 5729; (*b*) E. Kim, S. Paliwal and C. S. Wilcox, *J. Am. Chem. Soc.*, 1998, **120**, 11192; (*c*) S. L. Mckay, B. Haptonstall and S. H. Gellman, *J. Am. Chem. Soc.*, 2001, **123**, 1244; (*d*) M. J. Rashkin and M. L. Waters, *J. Am. Chem. Soc.*, 2002, **124**, 1860; (*e*) B. W. Gung, X. W. Xue and Y. Zou, *J. Org. Chem.*, 2007, **72**, 2469; (*f*) W. R. Carroll, P. Pellechia and K. D. Shimizu, *Org. Lett.*, 2008, **10**, 3547.

3 (*a*) K. M. Guckian, B. A. Schweitzer, R. X. F. Ren, C. J. Sheils, D. C. Tahmassebi and E. T. Kool, *J. Am. Chem. Soc.*, 2000, **122**, 2213; (*b*) J. Isaksson and J. Chattopadhyaya, *Biochemistry*, 2005, **44**, 5390.

4 S. Nakano, Y. Uotani, S. Nakashima, Y. Anno, M. Fujii and N. Sugimoto, *J. Am. Chem. Soc.*, 2003, **125**, 8086.

5 (*a*) D. B. Smithrud, T. B. Wyman and F. Diederich, *J. Am. Chem. Soc.*, 1991, **113**, 5420; (*b*) C. Jasper, T. Schrader, J. Panitzky and F.-G. Klarner, *Angew. Chem., Int. Ed.*, 2002, **41**, 1355; (*c*) M. S. Cubberley and B. L. Iverson, *J. Am. Chem. Soc.*, 2001, **123**, 7560.

6 (*a*) H.-J. Schneider and M. Xue, *J. Org. Chem.*, 1994, **59**, 7464; (*b*) T. Liu and H.-J. Schneider, *Angew. Chem., Int. Ed.*, 2002, **41**, 1368; (*c*) M. Sirish and H.-J. Schneider, *J. Am. Chem. Soc.*, 2000, **122**, 5881.

7 (*a*) P. Mukerjee and A. K. Ghosh, *J. Am. Chem. Soc.*, 1970, **92**, 6419; (*b*) E. M. Tuite and J. M. Kelly, *Biopolymers*, 1995, **35**, 419.

8 (*a*) J. L. Vennerstrom, M. T. Makler, C. K. Angerhofer and J. A. Williams, *Antimicrob. Agents Chemother.*, 1995, **39**, 2671; (*b*) J. Jose and K. Burgess, *Tetrahedron*, 2006, **62**, 11021; (*c*) G. Song, F. Xing, X. Qu, J. B. Chaires and J. Ren, *J. Med. Chem.*, 2005, **48**, 3471; (*d*) K. Takasu, T. Shimogama, C. Satoh, M. Kaiser, R. Brun and M. Ihara, *J. Med. Chem.*, 2007, **50**, 2281.

9 (*a*) C.-H. Chien, M.-K. Leung, J.-K. Su, G.-H. Li, Y.-H. Liu and Y. Wang, *J. Org. Chem.*, 2004, **69**, 1866; (*b*) S. Rashdan, M. E. Light and J. Kilburn, *Chem. Commun.*, 2006, 4578.

10 (*a*) A. M. Martin, R. S. Butler, I. Ghiviriga, R. E. Giessert, K. A. Abboud and R. K. Castellano, *Chem. Commun.*, 2006, 4413; (*b*) K. Miyata, A. Kobori, R. Tamamushi, A. Ohkubo, H. Taguchi, K. Seio and M. Sekine, *Eur. J. Org. Chem.*, 2006, 3626; (*c*) H. C. Ong and S. C. Zimmerman, *Org. Lett.*, 2006, **8**, 1589.

11 (*a*) J. S. Lai, J. Qu and E. T. Kool, *Angew. Chem., Int. Ed.*, 2003, **42**, 5973; (*b*) S. L. Cockroft, J. Perkins, C. Zonta, H. Adams, S. E. Spey, C. M. R. Low, J. G. Vinter, K. R. Lawson, C. J. Urchc and C. A. Hunter, *Org. Biomol. Chem.*, 2007, **5**, 1062; (*c*) S. Pérez-Casas, J. Hernández-Trujillo and M. Costas, *J. Phys. Chem. B*, 2003, **107**, 4167; (*d*) K. Frey and S. A. Woski, *Chem. Commun.*, 2002, 2206.

