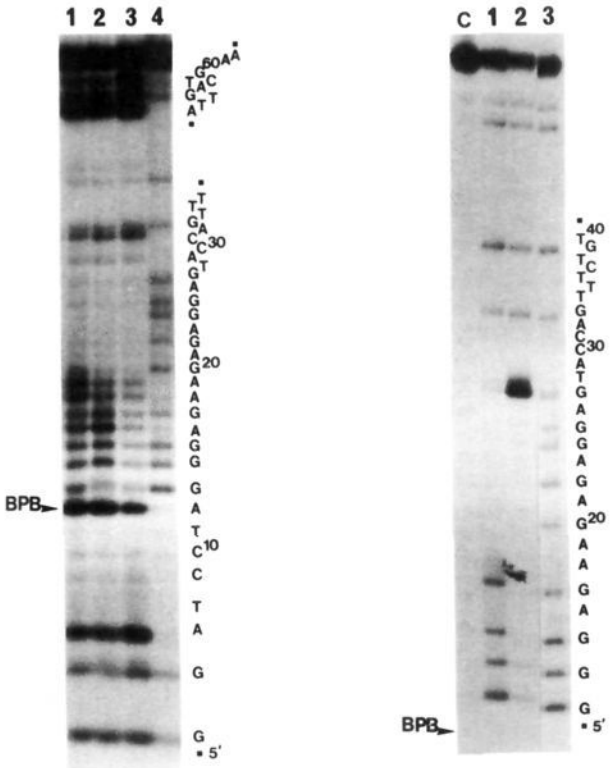

Figure 2. Autoradiograms of polyacrylamide gel electrophoresis of the double hairpin complex. The ^{32}P 5′ end-labeled 69-mer (5×10^{-8} M) was preincubated for 30 min at 15 °C in a 50 mM sodium acetate buffer, pH 6.0, containing 10 mM $MgCl_2$, in the absence or in the presence of the desired oligomer. Left (a): Following preincubation the samples were reacted for 90 min at 25 °C with diethyl pyrocarbonate (10% final concentration). After addition of 1 vol of 5 mM EDTA, the mixture was extracted with ethyl ether and the DNA was ethanol precipitated. The modified DNA was then treated (30 min at 90 °C) with 1 M piperidine, precipitated twice by ethanol, solubilized in 80% formamide containing marker dyes (bromophenol blue (BPB) and xylene cyanol), and analyzed on a 16% polyacrylamide gel containing 7 M urea in TBE buffer. Lane 1: 69-mer. Lane 2: 69-mer + 6×10^{-5} M 10-mer. Lane 3: 69-mer + 5×10^{-6} M 26-mer. Lane 4 shows a G reaction of the 69-mer whose sequence is written to the right. Right (b): Following preincubation the samples were incubated for 20 min at 25 °C in the presence of 0.5% dimethyl sulfate, treated with piperidine, and analyzed as described above. Lane 1: 69-mer + 6×10^{-5} M 10-mer. Lane 2: 69-mer + 2×10^{-5} M 26-mer. Lane 3: 69-mer. Lane C corresponds to the 69-mer treated with piperidine without any other previous treatment. The 69-mer sequence is given to the right of the panel.

detected with the 10-mer, suggesting a particular geometry of this region in the 26-mer/69-mer complex, due to the binding of the 5′ part of the 26-mer.

The formation of the complex depicted in Figure 1 was also monitored from the antisense standpoint. The reactivity of T's to potassium permanganate was determined for the 26-mer either in the presence or in the absence of the 69-mer target. A reduced sensitivity was observed from T(15) up to the 3′ end of the oligomer (not shown), in the oligonucleotide mixture compared to the 26-mer alone. No modification of the reactivity was observed either for T(6) or for T(1). As A(12) was not protected by the 26-mer from reaction with DEPC (Figure 2b), this suggested that this TA·T triplet is not formed in the 26-mer/69-mer hybrid.

From the above study we conclude that a stable complex can be formed, between a DNA hairpin structure and a complementary oligonucleotide, through the formation of both Watson–Crick and Hoogsteen hydrogen bonds with the homopurine target sequence. Preliminary data indicate that such complexes might be formed with RNA targets. These structures might be efficient at inhibiting either translation of mRNA or reverse transcription of viral RNA.

Molecular Recognition in Water: New Receptors for Adenine Derivatives

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Individual base pairs between nucleic acid components are generally not observed in water, since the entropic price of bimolecular association is generally not paid by the newly formed intermolecular hydrogen bonds. Accordingly, such interactions are studied in contexts where other forces are involved, and interpretation is difficult.[1,2] We have initiated a program to evaluate these forces in water and we report here our preliminary results.

We use the imides **1**, related to those used in organic solvents,[3] as the complement to adenine. These hydrophilic analogs of Kemp's triacid were prepared as shown in Scheme I. Alkylation of the trianion of triester **2** with chloromethyl benzyl ether gave the all-cis tribenzyloxy trimethyl ester **3**[4] after recrystallization from formic acid. Saponification yielded the triacid **4**, which provided the anhydride **5a** upon dehydration. Ammonolysis of **5a** yielded the imide acid **5b**, which was reacted with $SOCl_2$ to form the imide acid chloride **6**. Coupling of imide acid chloride **6** with aromatic amines gave the protected J-shaped receptors **7**. Debenzylation of these systems was quantitative using HBr(g) in formic acid, providing the water-soluble receptors **1**.

The solubility of these receptors ranged from 15 mM for the anilide **1a** (Table I) to 0.2 mM for the anthracyl derivative **1e** at 10 °C.[5] These systems were titrated with 9-ethyladenine (**8**) to obtain the association constants. In 9:1 H_2O/D_2O, the use of binomial solvent suppression[6] at 10 °C[7] permitted monitoring of the exchangeable protons. The imide peak moved consistently downfield from 10.6 ppm to a limiting value of ~13 ppm upon addition of 9-ethyladenine, behavior that confirms hydrogen-bonded base pairing as shown in Scheme II. The association constant was obtained from this movement using a nonlinear least-squares fit to the 1:1 binding isotherm, with allowance for guest dimerization.[8] Scheme II shows only the Watson–Crick mode of base pairing; not shown are the Hoogsteen, reverse Watson–Crick, and reverse Hoogsteen base pairs. The association constants reported in Table I represent the sum of all four binding modes.

Hydrophobic surface contacts are revealed to be the most significant contributors to binding. The phenyl-substituted receptor

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(2) For examples of base pairing in nonhomogeneous aqueous systems, see: Nowick, J.; Chen, S. *J. Am. Chem. Soc.* **1992**, *114*, 1107. Kurihara, K.; Ohto, K.; Honda, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 5077.

(3) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K.-S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090. For other adenine receptors, see: Goswami, S.; Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 3425. Zimmerman, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054. Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055. Claude, S.; Lehn, J.-M.; Schmidt, F.; Vigneron, J.-P. *J. Chem. Soc., Chem. Commun.* **1991**, 1182.

(4) All new compounds have been characterized by infrared, NMR, and low- and high-resolution mass spectrometry.

(5) Solubilities were determined by lyophilization of saturated solutions of the hosts.

(6) Hore, P. *J. Magn. Reson.* **1983**, *54*, 539. Hore, P. *J. Magn. Reson.* **1983**, *55*, 283.

(7) At 20 °C, the imide peak broadened excessively after one or two additions of 9-ethyladenine.

(8) The dimerization constant for 9-ethyladenine was measured as 13.8 M^{-1} under the titration conditions.

(9) Dilution studies of the receptors **1a–e** show that no host dimerization or aggregation occurs at the concentrations used for this study.

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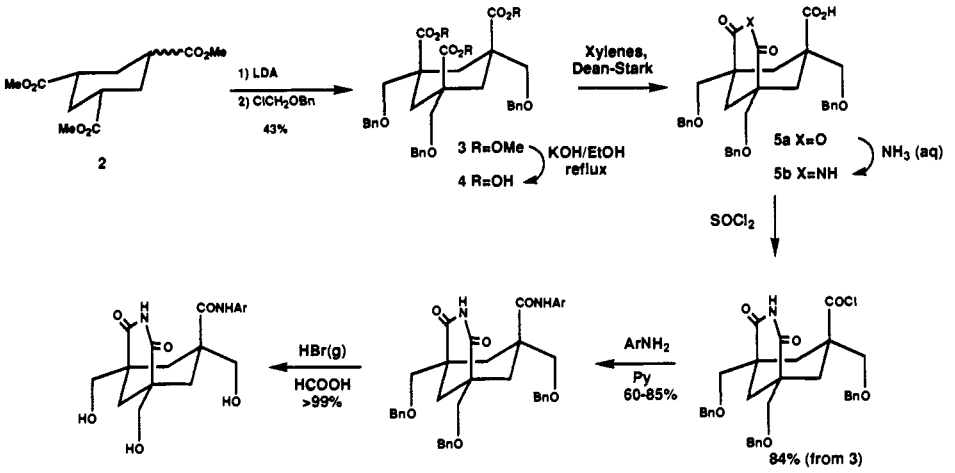
Scheme I



Scheme II

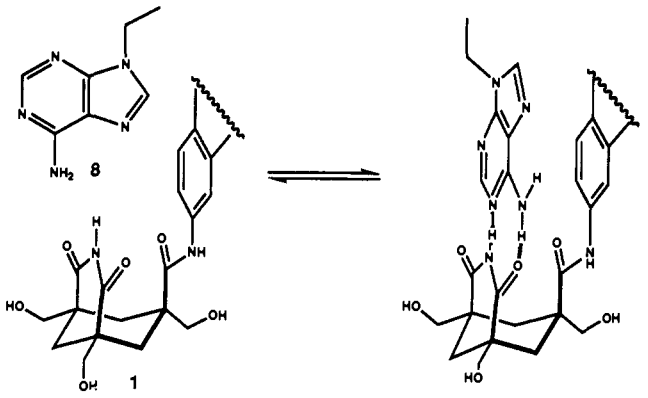
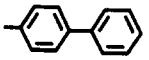
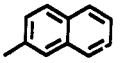
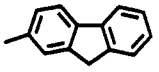
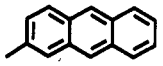


Table I. Solubilities of Hosts 1 in Water and Association Constants in Water with 9-Ethyladenine[a]

	Ar	solubility (mM)	K_a (M^{-1})
1a		15	2
1b		6	15
1c		1.2	29
1d		0.8	50
1e		0.2	70

[a] The solutions were buffered to a constant pH of 6.0 using 10 mM cacodylic acid/sodium cacodylate buffer (ionic strength 50 mM). NMR data were obtained at 283 K. Titrations were performed at a constant host concentration of 0.8 mM, except for **1e** where the concentration was 0.15 mM.[9]

1a has little overlap with the purine nucleus, and it provides a binding constant of 2 M^{-1}. Extension of the hydrophobic surface to the naphthyl host **1c** increases the association constant to 29 M^{-1}. This corresponds to a free energy change (ΔG) of −1.5 kcal/mol. While the relationship of surface area to hydrophobic binding is a matter of some uncertainty, our current results appear consistent with the values suggested by Honig.[10] The quantification of the smaller hydrogen-bonding contribution is the subject of current investigations.

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Mechanism-Based Inactivation of Galactose Oxidase: Evidence for a Radical Mechanism

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Recently, substrate and protein radicals have been recognized as important intermediates in biological reactions.[1] Galactose oxidase (GOase) catalyzes the two-electron oxidation of primary alcohols with O_2 to produce aldehydes and H_2O_2.[2] GOase has two one-electron redox centers at the active site. GOase can exist in two stable forms: a one-electron-reduced inactive form and an oxidized active form.[3] Spectroscopic data show that the active form has Cu(II)[4] and another, non-metal, redox center at the active

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