mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and the solvent was removed at reduced pressure. The residue was purified by flash chromatography (1:3 CH_2Cl_2-petroleum ether) to afford 743.3 mg (83%) of **52** as a light yellow solid: mp >244 °C; ^1H NMR δ 8.48 (d, $J_{1,3}$ = $J_{11,13}$ = 1.5, 2 H, H-1, H-13), 7.48-7.41 (m, 5 H, H-3, H-11, H-3', H-4', H-5'), 7.16-7.12 (m, 4 H, H-4, H-10, H-2', H-6'), 2.62 (t, $J_{5,6}$ = 6.6, 4 H, H-6, H-8), 2.43 (t, $J_{5,6}$ = 6.6, 4 H, H-5, H-9); ^{13}C NMR δ 143.9, 138.7, 138.3, 138.1, 137.3, 134.1, 131.6, 130.7, 130.0, 128.7, 127.9, 120.2, 120.1, 104.0, 28.7, 27.5; MS (EI, 70 eV), m/z (relative intensity) 539 (4), 541 (7), 543 (3); MS (FAB, Xe) exact mass calcd for $C_{29}H_{20}Br_2N$ m/z 541.998 75, found 541.992 80.

2,12-Bis(2,7-dimethoxy-9-acridinyl)-7-phenyl-5,6,8,9-tetrahydrobenz-[a,j]anthracene 14-Cyanide (50). To a solution of 105 mg (0.19 mmol) of **52** in 40 mL of THF at -90 °C was added 0.42 mL (0.394 mmol) of n-butyllithium in hexane. The resulting solution was stirred for 0.5 h and was transferred via canula to a solution of 199 mg (0.58 mmol) of **52** in 10 mL of THF at -78 °C. The mixture was stirred for 3 h and was quenched with a saturated aqueous solution of ammonium chloride at -78 °C. The mixture was extracted with CH_2Cl_2 and dried over Na_2SO_4, and the solvent was removed at reduced pressure. The residue was purified by flash chromatography (1:4 EtOAc-CH_2Cl_2) to afford 112 mg (65%) of **50** as a light green powder: ^1H NMR δ 8.25 (d, $J_{1,3}$ = 1.1, 2 H, H-1, H-13), 8.06 (d, $J_{3',4'}$ = 9.4, 4 H, H-4', H-5'), 7.58-7.51 (m, 3 H, H-3'', H-4'', H-5''), 7.48 (d, $J_{3,4}$ = 7.5, 2 H, H-3, H-11), 7.36-7.27 (m, 8 H, H-4, H-10, H-3', H-6', H-2'', H-6''), 6.78 (d, $J_{1',3'}$ = 2.7, 4 H, H-1', H-8'), 2.86 (t, $J_{5,6}$ = 6.5, 4 H, H-6, H-8), 2.61 (t, $J_{5,6}$ = 6.5, 4 H, H-5, H-9); MS (FAB, Xe), m/z 858 (M + H), exact mass calcd for $C_{59}H_{43}N_3O_4$ m/z 858.333 16, found 858.331 50.

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Complexation of Nucleotide Bases by Molecular Tweezers with Active Site Carboxylic Acids: Effects of Microenvironment

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Abstract: In chloroform-d molecular tweezer **1** forms a 1:1 complex (Job plot) with 9-propyladenine (**4**). Changes in the UV-visible absorption spectrum of **1** upon addition of **4** and the changes **1** and **4** induce in each other's ^1H NMR spectrum are consistent with those of a complex comprised of hydrogen bonds and π-stacking interactions. The microenvironment around the carboxylic acid group in **1** markedly alters its complexation behavior relative to a simple carboxylic acid such as butyric acid (Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037-7042). The association constants for the **1-4** and butyric acid-**5** complexes are 25 000 M^{-1} (298 K) and 160 M^{-1} (303 K), respectively. Butyric acid prefers a type 1 hydrogen bonding pattern while **1** adopts a type 7 pattern. The nucleotide base selectivities follow the order G > C > A > U for butyric acid and A > G ≫ C > U for **1**. The presence of protic solvents markedly decreases the strength of the complex between **1** and **4**. Two analogues of **1** have also been studied, molecular tweezer **2** and **3**. Both lack the dimethylamino substituent found in **1**, while **3** has a spacer unit that is fully oxidized. The association constants for the **2-4** and **3-4** complexes are 14 000 and 120 000 M^{-1}, respectively.

Noncovalent interactions are of fundamental importance to all biological processes. This has inspired the study of host-guest chemistry whose goals include the development of artificial enzymes and the understanding of complexation phenomena.[1] While the small, usually nonpeptidic, organic hosts bear little resemblance to natural "receptors" such as enzymes and antibodies, they have several distinct advantages. They provide a more manageable degree of structural complexity. Furthermore, hosts with different functional group orientations, varying degrees of flexibility, and modified electronic properties can be synthesized and compared. Additionally, synthetic receptors are often soluble in several solvents, and because these molecules are constructed of covalent bonds they maintain their structural integrity in a wide range of media. Well-chosen changes in structure and solvent provide invaluable insights into molecular recognition phenomena.

Our interest in this area has been with receptors, called "molecular tweezers",[2] which complex aromatic guests through π-sandwiching[3] and, more recently, through π-sandwiching combined with hydrogen bonding.[4,5] These latter receptors were inspired by two quite different areas of research. The first involves the study of bichromophoric molecules that can bind to DNA by bis-intercalation.[6] The second area is the study of protein-DNA recognition.[7] It was proposed several years ago that amino acid side chains might recognize DNA bases and base-pairs through π-stacking and hydrogen-bonding interactions. The most selective

(1) (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009-1112. (b) *Host Guest Complex Chemistry, Macrocycles*; Vögtle, F., Weber, E., Eds.; Springer-Verlag: New York, 1985. (c) Rebek, J., Jr. *Science (Washington, D.C.)*)1987, *235*, 1478-1484. (d) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112. (e) Diederich, F. *Ibid.* **1988**, *27*, 362-386. (f) Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170-177.

(2) Chen, C.-W.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4921-4922.

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(6) Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275-340.

(7) See: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 18 and references therein.

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Table I. ^{1}H NMR Complexation Shifts ($\Delta\delta$) in 9-Propyladenine and Related Compounds Induced by Molecular Tweezer **1** in Chloroform-*d* at 298 K[a]

guest	$\Delta\delta$ (ppm)[b] H-2	NH-6	NMe-6	H-8(7)[c]	CH_2N-9
4	0.17	−0.40		0.42	0.07
8	0.07	−0.31	−0.14	0.34	0.08
9	0.46			0.22	−0.02

[a] Shifts at ca. 40% saturation of guest. [b] Positive values represent upfield shifts. [c] H-8 in **4** and **8**, H-7 in **9**.

binding contacts between proteins and nucleic acids were realized to involve at least two hydrogen bonds, requiring the carboxylic acid group of aspartic or glutamic acid, the amide group of asparagine or glutamine, or the guanidinium group of arginine.[8] Numerous model studies have confirmed the feasibility of these interactions both in solution[9] and in the solid state.[10] While this "direct readout" mechanism has been observed recently in the X-ray structures of repressor-DNA operator complexes,[11] recognition mechanisms that do not require direct hydrogen bonds appear possible.[12]

Using concepts found in these two areas of research, we sought to develop small molecules that could selectively bind mono- or polynucleotides. Other investigators have been similarly motivated and synthetic receptors for each of the four common nucleotide bases have been reported in the past several years.[13] These receptors were designed to hold multiple hydrogen bond donor and acceptor groups, and sometimes a single π-stacking surface, in a spatial arrangement that is complementary to only one nucleotide. The results have been impressive. Most show selectivity in binding nucleotide bases and several form exceptionally stable complexes. By contrast, our approach was an empirical one. We chose to use a single carboxylic acid group, already shown by Lancelot[14] to bind only moderately well to nucleotide bases and with only slight selectivity in chloroform (vide infra). The question to be answered was whether these unassuming properties might be improved by removing the carboxylic acid group from bulk solvent and placing it deep within the cleft of our molecular tweezers.[4] The work described herein has shown the answer to be in the affirmative. Just as the properties of amino acid side chains held in clefts and depressions on the surface of proteins are altered by the microenvironment,[15] so are the carboxylic acid groups in molecular tweezers **1–3**[16] strongly influenced by the surrounding aromatic cleft in their interaction with nucleotide bases.

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(9) See: Lancelot, G.; Mayer, R.; Hélène, C. *Biochim. Biophys. Acta* **1979**, *564*, 181–190 and references therein.
(10) Sasada and co-workers have been very active in this area: Fujita, S.; Takenaka, A.; Sasda, Y. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1707–1712 and references therein.
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(14) Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037–7042. These studies were performed at 303 K.
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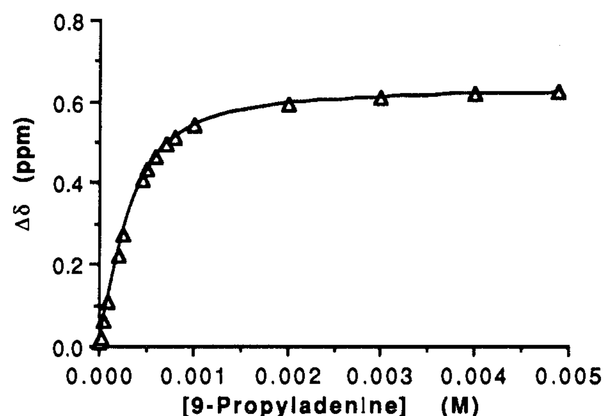
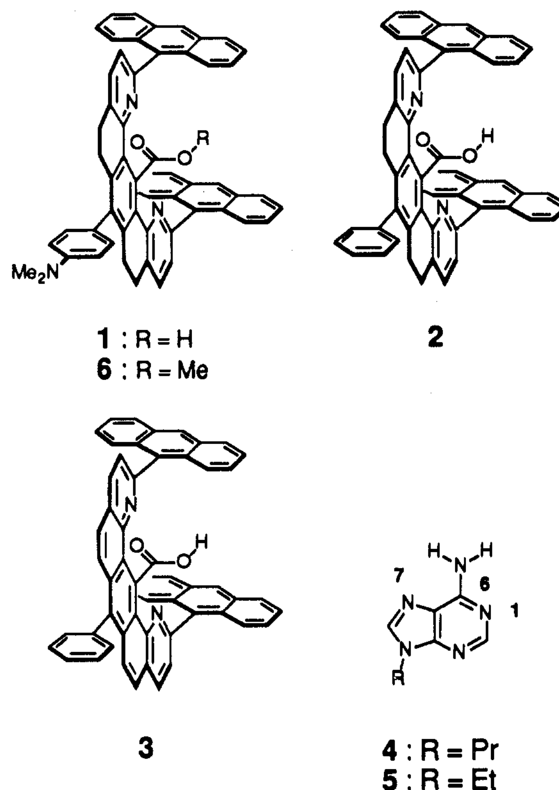


Figure 1. Plot of the upfield chemical shift (^{1}H NMR) of anthracene H-10 resonance in **1** as a function of added 9-propyladenine (**4**).

Results and Discussion

Complexation of 9-Propyladenine. The self-association of **1** was studied over a wide concentration range. From 10^{-6} to 10^{-4} M chloroform, the absorption spectra of **1** obeyed Beer's law. From 10^{-4} to 10^{-2} M chloroform-*d*, the ^{1}H NMR chemical shifts of the anthracene rings in **1** were found to change by <0.05 ppm. Carboxylic acids **2** and **3** were also found to obey Beer's law up to 3×10^{-5} M, the highest concentration investigated by UV–vis spectroscopy. Thus, the carboxylic acids in **1**, **2**, and **3** do not



dimerize to an appreciable extent under the conditions employed for the binding studies ([**1**] $\approx 10^{-4}$ M, [**2**, **3**] $\approx 5 \times 10^{-6}$ M). The self-association constant for 9-ethyladenine (**5**) in chloroform-*d* has been reported to be very small (K_{dimer} = 3.1 M^{-1}), so dimerization of **4** and its analogues can be neglected.[17]

Standard titration experiments were monitored by both UV-visible and ^{1}H NMR spectroscopy. When a chloroform solution of **1** was titrated with 9-propyladenine (**4**) a nonoverlapping absorption of the anthracene chromophores (λ_{max} = 386 nm) was found to exhibit a metachromic shift indicative of π-stacking. It

(17) Kyogoku, Y.; Lord, R. C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250–257.

Table II. Association Constants (K_{assoc}) and ^{1}H NMR Complexation Shifts ($\Delta\delta_{max}$) Obtained from the Titration of **1** and **6** with 9-Propyladenine and Related Compounds at 298 K[a]

host	guest	solvent	$\Delta\delta_{max}$[b] H-10	H-4	H-2	K_{assoc}, M^{-1}	$-\Delta G°_{298}$[c]
1	4	$CDCl_3$	0.62	0.53	−21[d]	25000	6.0
6	4					<5	<1.0
1	7		0.59			580	3.8
1	8		0.65			30000	6.1
1	9		0.77			11000	5.5
1	4	10% v/v $CD_3OD/CDCl_3$	0.54			520	3.7
1	4	50% v/v $CD_3OD/CDCl_3$	0.51			430	3.6

[a] For methods used see text and the Experimental Section. [b] For the protons of **1**. H-4 and H-10 of the anthracene group and H-2 of the (dimethylamino)phenyl group. In ppm unless indicated. Positive values represent upfield chemical shifts. [c] kcal mol^{-1}. [d] Hertz (Hz).

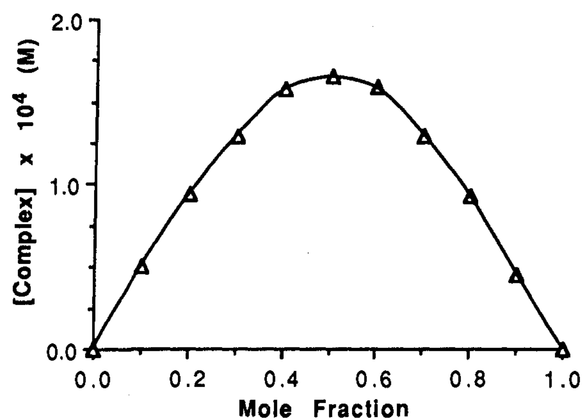


Figure 2. Plot (Job) of [**1·4**] versus the mole fraction [**1**]/([**1**] + [**4**]) at constant [**1**] + [**4**].

was also found that in chloroform-*d* **1** and 9-propyladenine (**4**) altered each others ^{1}H NMR spectrum in a manner consistent with the formation of a complex in rapid exchange with its components. The direction and magnitude of the shifts support a complex containing both π-stacking and hydrogen bonding. Thus, in the presence of 0.4 equiv of **1**, a 1 mM solution of **4** (ca. 40% bound at 298 K) has an H-6 resonance which is shifted *downfield* by 0.40 ppm, while resonances for H-2, H-8, and 9-CH_2 are shifted *upfield* by 0.17, 0.42, and 0.07 ppm, respectively (Table I). Downfield shifts are expected for a proton involved in a hydrogen bond, while upfield shifts are generally seen in aromatic rings that are π-stacked.

Only a few of the resonances of **1** were sufficiently well-resolved to monitor for an entire ^{1}H NMR titration experiment. A typical binding isotherm is seen in Figure 1, this particular case showing the upfield shift of the anthracene H-10 resonance of **1** as a function of added **4**. Similar plots were constructed for the anthracene H-4 and the (dimethylamino)phenyl H-2 resonances, the shift in the latter case being downfield. Acid **1** bound **4** very strongly and 99% saturation could be obtained. The binding constants and complexation shifts were obtained by curve fitting the titration data as described previously and can be found in Table II.[4] The method of Higuchi has also been used and found in all cases to give association constants that agree within 15% of those obtained by curve fitting.[4,18] The main advantage of this method is the linear plot. However, neither method affords accurate values for high association constants (>10^4 M^{-1}) when working at convenient concentrations for ^{1}H NMR. This problem is remedied either by working at lower concentrations or by using UV–visible spectroscopy (vide infra).

The 1:1 stoichiometry of the complex between **1** and 9-propyladenine was demonstrated with the method of continuous

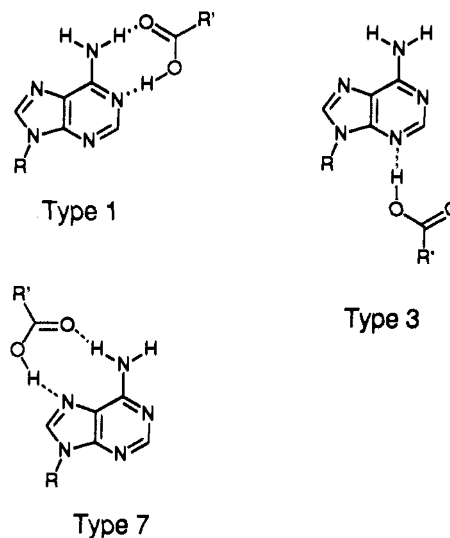


Figure 3. Schematic representation of possible hydrogen-bonded complexes between a 9-alkyladenine and a carboxylic acid. See ref 14.

variations. As seen in Figure 2, the Job plot has a maximum at a mole fraction of 0.5 which is indicative of a 1:1 complex.[18b,19] These data, combined with changes seen in the ^{1}H NMR and UV–visible spectra and the high stability of the complex, indicate the formation of an inclusion complex comprised of hydrogen bonding and π-stacking forces as represented by structures **10** and **11**. The importance of hydrogen bonding in the process is evidenced by the fact that ester **6** does not bind 9-propyladenine (K_{assoc} < 5 M^{-1}). This result also rules out an external, π-stacked complex.

In order to determine the role played by the aromatic cleft in receptors **1–3** an appropriate standard is needed for comparison. Butyric acid was chosen because its complexation of nucleotide bases has been studied thoroughly by Lancelot.[14] Furthermore, the association constant for the complex of butyric acid and **5** is nearly identical with the value obtained for the complex between **4** and an analogue of **1** lacking anthracene rings.[20] Given that butyric acid is a suitable model, the importance of the aromatic cleft in **1** is immediately evident: the association constants for **1·4** and butyric acid·**5** complexes are 25 000 and 160 M^{-1}, respectively. Thus, the aromatic cleft in **1** contributes ca. 3 kcal mol^{-1} to the complex stability.

In addition to increasing its stability, the aromatic cleft to **1** also alters the hydrogen-bonding geometry between the carboxylic acid group and the adenine guest. With butyric acid Lancelot has reported three types of hydrogen-bonded complexes with 9-ethyladenine (**5**) in chloroform (Figure 3).[14] Of these, type 1 ($-\Delta G°$ = 2.9 kcal mol^{-1}) is the strongest, followed by type 7 ($-\Delta G°$ = 2.2 kcal mol^{-1}), and then type 3 ($-\Delta G°$ = 1.0 kcal mol^{-1}). Presented below are data which indicate that the carboxylic acid in **1** also forms two hydrogen bonds to the adenine guest, but that its microenvironment results in an altered preference for a type 7 complex.

Direct methods (X-ray, NOESY, etc.) have not yet allowed determination of the **1·4** complex geometry. However, the orientation of the adenine nucleus within the binding cleft can be inferred from the binding results with nucleotide bases **7-9**. Receptor **1** shows a large decrease in affinity for *N,N*-dimethyl-9-ethyladenine (**7**), $-\Delta\Delta G°_{298}$ = 2.2 kcal mol^{-1}, demonstrating the importance of the exocyclic amino group and thus implicating the double hydrogen bonding provided in type 1 and type 7 complexes. Strikingly, *N*-methyl-9-ethyladenine (**8**) binds to **1** as strongly as does 9-propyladenine (**4**). The methyl group on N-6 prefers to orient toward N-1 (syn) favoring a type 7

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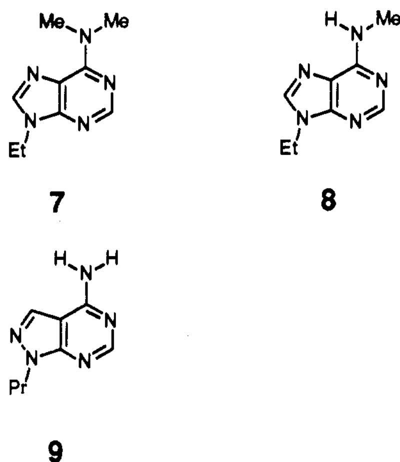
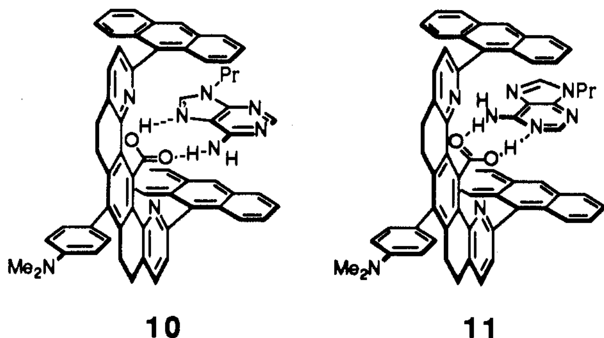
(19) Job, P. *Ann. Chim. Ser. 10* **1928**, *9*, 113–134.

(20) Wu, W. Unpublished results. It has also been found that butyric acid has a similar pK_a value to the analogue of **1** lacking anthracene rings in a mixed aqueous–organic solvent: Zeng, Z. Unpublished results.



complex for this species.[21] This result provides strong evidence for the formation of complex **10**. However, the presence of some



complex **11** (type 1 hydrogen bonding) cannot be ruled out for two reasons. First, the syn orientation of the methyl group in **8** is preferred over the anti by a ratio of only 95:5.[21] Thus, the methyl group could coincidentally contribute enough binding energy to offset the energy cost of binding the anti form. Second, adenine analogue **9** cannot bind to **1** with a type 7 geometry yet a very strong complex still forms (Table II). Nonetheless, complex **10** appears to be the best description of **1·4**, since **1** induces shifts in the ^{1}H NMR spectrum of **4** that are similar to those seen in **8**, but very different from those seen in **9** (Table I).[22] In complex **10** there is more π–π overlap between the adenine and the anthracene rings than in complex **11** wherein the imidazolyl ring of the guest projects into the solvent.

Complexation in Chloroform-Containing Protic Solvents. During the complexation studies between **1** and **4** it was observed that the use of undried chloroform-*d* noticeably depressed the association constants. Thus, in "wet" chloroform-*d* the association constant for **1·4** was ca. 10 000 M^{-1}. Although this corresponds to a $\Delta\Delta G°_{298}$ of only 0.5 kcal mol^{-1}, which is near the limit of experimental error, the result does raise concern about the validity of comparing association constants obtained under conditions assumed to be identical. Even during the normal handling of dried $CDCl_3$ solutions, sufficient water was absorbed to be observable by ^{1}H NMR. The effect of protic solvents was investigated more quantitatively through the use of CD_3OD–$CDCl_3$ mixtures. As seen in Table II, both 10% and 50% v/v CD_3OD–$CDCl_3$ reduced the stability of the **1·4** complex by ca. 2.3 kcal mol^{-1} (298 K) as compared to pure chloroform-*d*.

Complexation of Nucleoside Bases. Each of the four common nucleic acid bases has an adjacent donor and acceptor site that can form two hydrogen bonds with a carboxylic acid.[7] For example, butyric acid in chloroform binds 2-(dimethylamino)-6-hydroxypurine, 1-cyclohexylcytosine, 9-ethyladenine, and 1-cyclohexyluracyl with free energies of complexation of 3.9, 3.4, 3.0, and 2.6 kcal mol^{-1}, respectively.[14] Thus, as a recognition element, a carboxylic acid group would not be predicted to afford high selectivity in nucleotide base complexation. It was found, however, that the microenvironment of the carboxylic acid group in **1** substantially altered its base selectivity leading to a satisfactory degree of discrimination.

Table III. Association Constants (K_{assoc}) and ^{1}H NMR Complexation Shift (H-10 $\Delta\delta_{max}$) for the Complexation of Tri-*O*-acetyl Nucleosides in Chloroform-*d* by **1** at 298 K[a]

tri-*O*-acetyl nucleoside	$\Delta\delta_{max}$ (H-10, ppm)	K_{assoc}, M^{-1}	$-\Delta G°_{298}$[c]	$\Delta\Delta G°$[c]
adenosine (**12**)	0.55	15500	5.7	
guanosine[b] (**13**)	0.61	2800	4.7	1.0
cytidine (**14**)	0.27	213	3.2	2.5
uridine (**15**)	0.26	132	2.9	2.8

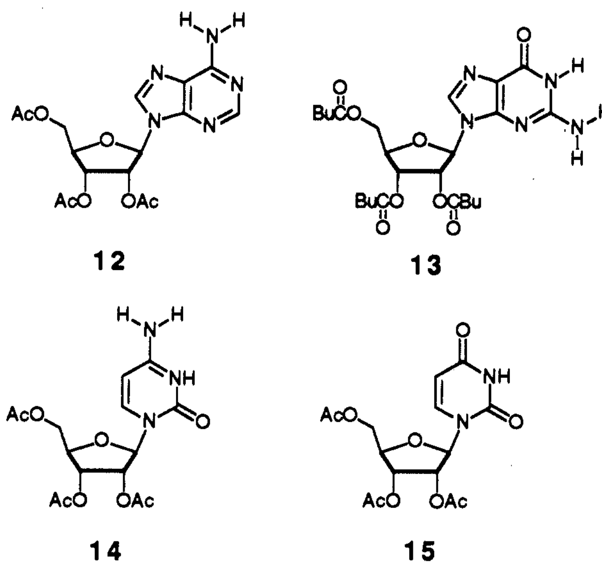
[a] For methods used see text and the Experimental Section. [b] Tri-*O*-pentanoyl was used due to solubility difficulties. [c] kcal mol^{-1}.

Table IV. Complexation Data from the UV–Visible Titration of **2** and **3** with 9-Propyladenine (**4**) in Chloroform-*d* at 298 K[a]

compd	λ_{max}, nm	$\Delta\epsilon$	$-\Delta G°$, kcal mol^{-1}	K_{assoc}, M^{-1}
16				<10[b]
2	386	2110	5.7	14000[c]
3	406	2160	6.9	120000[c]

[a] For methods used see text and the Experimental Section. [b] On the basis of negligible shift in ^{1}H NMR of anthracene protons of **16** (4×10^{-4} M) in the presence of 5×10^{-3} M **4**. [c] On the basis of triplicate runs, the values are good to ±15% for **2** and ±20% for **3**.

As seen in Table III, tri-*O*-acetyladenosine (**12**) binds nearly as tightly to **1** ($-\Delta G°_{298}$ = 5.7 kcal mol^{-1}) as does **4**. Due to the low solubility of guanosine its tri-*O*-pentanoyl derivative (**13**) was used and found to bind only slightly less strongly ($-\Delta G°_{298}$ = 4.7 kcal mol^{-1}) than did **12**. The pyrimidine nucleosides **14** and **15**

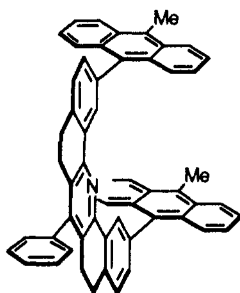


were complexed by **1** with a significantly lower affinity. The nucleotide base preference shown by butyric acid is small in the order G > C > A > U, while **1** prefers A > G ≫ C > U. Thus, **1** can be classified as a purine-selective receptor. This selectivity most likely originates in an enhanced stacking interaction with the purines, although steric hindrance may also play a role. Space-filling models show that double hydrogen bonding of the carboxylic acid in **1** to cytidine **14** or uridine **15** would require partial inclusion of the ribose in the binding cleft.

Comparative Binding Analysis of 2 and 3 with 4. Receptor **2**, an analogue of **1**, also formed a complex with 9-propyladenine (**4**) that was in rapid exchange with its components on the ^{1}H NMR time scale. However, the fully oxidized receptor **3** exhibited quite different behavior. Its resonances due to the anthracene moieties became extremely broad upon addition of a solution of

(21) Engel, J. D.; von Hippel, P. H. *Biochemistry* **1974**, *13*, 4143–4158.
(22) (a) These results have been confirmed independently by computational methods: ref 22b. (b) Blake, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1990**, *112*, 7269–7278. The authors thank Prof. W. L. Jorgensen for making their results available prior to publication.

4, indicating slow exchange on the ^{1}H NMR time scale. As a result of this observation and the strength of the complexation, the binding constants were determined with UV-visible spectroscopy (Table IV). Receptor **2** is identical with **1** except for the absence of the dimethylamino substituent on the pendant phenyl group. This change is not expected to alter the binding and it was found that the association constant for the **2·4** complex was only slightly smaller than that for the **1·4** complex. This small difference might be attributed to experimental error, but it may in fact reflect the greater accuracy of the UV-visible spectroscopy method or the different conditions under which the measurements were made. Concentrations almost two orders of magnitude lower than in the ^{1}H NMR studies were employed for the spectrophotometric method.



16

The association constant for the **3·4** complex (K_{assoc} = 120 000 M^{-1}) is exceptionally high. The 1.2 kcal mol^{-1} increase in stability over the **2·4** complex probably reflects the slightly smaller inter-anthracene distance and the greatly reduced twist in the spacer unit of **3**.[16] With butyric acid as a standard, it can be concluded that the aromatic cleft in **3** contributes ca. 4.0 kcal mol^{-1} to the stability of the **3·4** complex, a value which agrees well with that obtained computationally.[22b] What is the origin of this contribution? Anthracene is a highly polarizable chromophore while adenine is moderately polarizable.[23] Thus, it is reasonable to assume that dispersion and polarization forces will favor inclusion of the adenine nucleus in the cleft of the molecular tweezer.[24] However, previous studies with molecular tweezers lacking hydrogen-bonding functionality showed that complex stabilities of 3–4 kcal mol^{-1} could be achieved only with very π-deficient guests such as 2,4,5,7-tetranitrofluorenone.[3] Indeed, an aromatic cleft alone is insufficient to cause complexation of adenine. Dianthryl molecular tweezer **16**, available from another study, shows no affinity for **4** in chloroform-*d* (K_{assoc} < 10 M^{-1}).

That the anthracene rings in receptors **1**, **2**, and **3** contribute to complex stability only in concert with a hydrogen-bonding interaction demonstrates a cooperative effect. This cooperativity may be of a traditional nature where multiple host-guest contacts share the cost of the translational and rotational entropy loss upon complexation.[25] Alternatively, the anthracene rings may work together to desolvate the aromatic cleft and/or the carboxylic acid. However, we have reported evidence that solvent is not excluded from the clefts of related receptors.[3c] With the receptors described herein, Jorgensen has shown with computational techniques that 1 or 2 solvent molecules reside in the cleft of **3** in chloroform solution.[22b] This important result shows that binding is not driven by the filling of a naked cleft. In fact, the poor complexation by **6**, and perhaps by inference **16**, has been attributed to the inability of the adenine guest to displace bound solvent molecules in the absence of hydrogen bonding.[22b] Another interesting possibility is that the hydrogen-bonding capacity of the carboxylic acid group is enhanced by partial desolvation.

Conclusions

Relative to butyric acid, the aromatic cleft in molecular tweezer **1** increases the association constant for purines by ca. 3 kcal mol^{-1}. This produces a satisfactory level of nucleotide base selectivity (A > G ≫ C > U) that is not found with butyric acid (G > C > A > U). Thus, **1** is a purine-selective receptor. The aromatic cleft in **1** also alters the hydrogen-bonding geometry of the carboxylic acid–adenine interaction from type 1 as seen in butyric acid to type 7 as represented by complex **10**. This alteration most likely reflects an enhanced π-stacking interaction when the long axis of the adenine nucleus and the long axis of the anthracene groups in **1** are aligned.

The affinities of molecular tweezers **2** and **3** for 9-propyladenine (**4**) were compared. The structure of **2** is nearly identical with that of **1**, while **3** has a spacer unit that is fully oxidized. This change results in a more rigid receptor, with a smaller inter-anthracene distance and a greatly reduced twist in its spacer unit. Thus, the structure of the aromatic cleft in **3** is close to approaching optimization for complexation of nucleotide bases in this system. Indeed, it was found that **3** complexed **4** with an association constant of 120 000 M^{-1}, as compared to K_{assoc} = 14 000 M^{-1} for the **2·4** complex. The ca. 4 kcal mol^{-1} increase in stability of the **3·4** complex relative to the butyric acid·**5** complex further underscores the remarkable role played by the aromatic cleft. Does this energy come from the attractive forces of dispersion and polarization? Not entirely, since molecular tweezer **16**, which has an aromatic cleft but no acid group, fails to bind 9-propyladenine. Clearly cooperativity between the aromatic cleft and the carboxylic acid group is required. The origin of the cooperativity has not been established, but it may be of the entropic variety often invoked when multisite interactions are involved. Alternatively the aromatic cleft may partially desolvate the carboxylic acid group.

The issue of cooperativity suggests the design of new receptors with additional contacts to the adenine nucleus. This is possible because (1) only three out of the five possible hydrogen bond donor and acceptor sites of 9-propyladenine are occupied in complexes **10** and **11** and (2) the flexibility of the synthesis of **3** should allow incorporation of additional groups. The full enthalpic worth of these additional contacts might be gained since the entropic price has been paid. Thus, work is in progress toward new receptors that might harvest an even greater fraction of the intrinsic binding energy available in the adenine nucleus.

Another challenge is to increase binding affinity in protic solvents. It was shown that the stability of the **1·4** complex dropped from K_{assoc} = 25 000 M^{-1} to K_{assoc} = 430 M^{-1} as the solvent was changed from $CDCl_3$ to 1:1 $CDCl_3/CD_3OD$. Of course, this decrease reflects the fact that protic solvents form hydrogen bonds to both receptor and substrate and that some of these bonds must be broken for complexation to occur. While the same must occur in aqueous media, water has an advantage over methanol–chloroform in that it combines a particularly low polarizability with highly cohesive properties, both of which will enhance π-stacking interactions.[26] In addition to testing this proposal, development of a water-soluble version of **2** or **3** would allow for binding studies with polynucleotides.

Experimental Section

General. All compounds used in this study had correct elemental analyses and had spectroscopic properties that were in accord with the assigned structures. Molecular tweezers **1–3** and **6** were described previously.[16] 9-Propyladenine (**4**) and 2′,3′,5′-triacetylcytidine (**14**) were generous gifts of Professor Nelson J. Leonard. 6-(Dimethylamino)-9-ethylpurine (**7**) was prepared according to the method of Robins[27] and 6-(methylamino)-9-ethylpurine (**8**) was prepared according to the method of Itaya.[28] 2′,3′,5′-Triacetyladenosine, 4-aminopyrazolo[3,4-*d*]pyrimi-

(23) Miller, K. J.; Savchik, J. A. *J. Am. Chem. Soc.* **1979**, *101*, 7206–7213.

(24) Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768–4774. As noted in this article, the geometry of the anthracene–adenine interaction in our system is favored since it is face-to-face stacked and slightly offset.

(25) In a similar system, this type of cooperativity has been attributed to a "spatio-temporal hypothesis": Sheridan, R. E.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 7120–7121.

(26) Diederich, F.; Smithrud, D. B. *J. Am. Chem. Soc.* **1990**, *112*, 339–343.

(27) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379–6382.

(28) Itaya, T.; Matsumoto, H.; Ogawa, K. *Chem. Pharm. Bull.* **1980**, *28*, 1920–1924.

dine, and 2′,3′,5′-triacetyluridine were purchased from the Sigma Chemical Co. Immediately prior to use chloroform was washed with water, dried over K_2CO_2, refluxed with P_2O_5, distilled, and passed through a column of activated alumina.

^1H and ^{13}C NMR spectra of chloroform-*d* solutions were recorded on a General Electric QE-300 instrument at 300 MHz unless specified otherwise. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference, and coupling constants are reported in hertz (Hz). Mass spectra were obtained on a Finnigan AT CH-5 or MAT-731 spectrometer. Elemental analyses were performed at the University of Illinois School of Chemical Sciences. The ^1H NMR titrations were monitored by a General Electric GN 500-MHz instrument. The UV-visible titrations were monitored on either a Shimadzu 160U or a Hewlett Packard 8451A diode array spectrophotometer. In both cases the temperature of the samples was maintained at 298 ± 1 K.

4-Amino-7-propylpyrazolo[3,4-*d*]pyrimidine (9). With use of the procedure of Itaya et al.[28] 405 mg of 4-aminopyrazolo[3,4-d]pyrimidine gave 84 mg (15%) of **9** as colorless needles (from ethyl acetate): mp 164-165 °C; ^1H NMR δ 8.40 (s, 1 H, H-2), 7.92 (s, 1 H, H-9), 5.50 (br s, 2 H, NH), 4.38 (t, J = 7.1, 2 H, NCH_2), 1.96 (m, 2 H, CH_2), 0.93 (t, J = 7.4, 3 H, CH_3); ^{13}C NMR (500 MHz) δ 157.46, 155.61, 153.32, 130.15, 100.52, 48.84, 23.01, 11.16; MS (EI, 70 eV), m/z 177 (M^+, 51), 149 (64), 148 (100). Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.09; H, 6.25; N, 39.21.

2′,3′,5′-Tripentanoylguanosine (13). With use of the procedure of Matsuda,[29] 6.38 g of guanosine gave 5.95 g (56%) of compound **13** as a white solid (ether trituration): mp 237-238 °C; ^1H NMR δ 12.07 (br s, 1 H, H-5), 7.65 (s, 1 H, H-8), 6.08 (br s, 2 H, 6-NH_2), 5.97 (d, $J_{1',2'}$ = 5.3, 1 H, H-1′), 5.87 (m, 1 H, H-2′ or H-3′), 5.70 (m, 1 H, H-2′ or H-3′), 4.40 (m, 3 H, H-4′, H-5′), 2.36 (m, 6 H, CH_2), 1.66 (m, 6 H, CH_2), 1.37 (m, 6 H, CH_2), 0.92 (m, 9 H, CH_3); ^{13}C NMR (500 MHz) δ 173.41, 172.39, 172.13, 159.09, 153.90, 151.37, 136.42, 117.41, 86.15, 80.15, 72.59, 70.48, 62.95, 33.69, 33.55, 33.39, 26.76, 26.66, 22.15, 22.06, 13.64, 13.58; MS (EI, 70 eV), *m/z* 535 (M^+, 1) 85 (100). Anal. Calcd for $C_{25}H_{37}N_5O_8$: C, 56.06; H, 6.96; N, 13.08. Found: C, 56.02; H, 6.96; N, 13.10.

Job Plot. The stoichiometry of the complex between **1** and **4** was determined by Job's method.[18b,19] Stock solutions 5 × 10^{-4} M in **1** and **4** in $CDCl_3$ were prepared. In eleven separate NMR tubes portions of the two solutions were added such that their ratio changed from 0 to 1 while maintaining a total volume of 500 μL. A ^1H NMR spectrum was taken for each tube and the change in chemical shift of the anthracene H-10 resonance of **1** was used to calculate the complex concentration (taking $\Delta\delta_{max}$ = 0.62; see Table II). The complex concentration was plotted against the mole fraction of **1** (Figure 2).

^1H NMR Titrations. For a specific example, the titration of **1** with **7** is described here. A 0.025 M solution of **1** and a 0.050 M solution of **7** in $CDCl_3$ were prepared. In 13 separate NMR tubes 10 μL of the solution of **1** and 0, 1, 2, 5, 10, 20, 30, 40, 60, 80, 100, 200, and 400 μL of the solution of **7** were added, respectively. The total volume in each NMR tube was increased to 500 μL by adding $CDCl_3$. ^1H NMR spectra were taken for each tube and Δδ values were calculated by substracting the chemical shift of interest in the spectrum of the mixtures (δ_x) from the appropriate resonance in the spectrum of pure **1** (δ_0). Thus, a titration curve of Δδ vs [G_0] could be plotted. In each case calculation of association constants used data up to 80-90% of saturation.

UV-Visible Titrations. A chloroform solution ca. 0.025 M in 9-propyladenine (**4**) was prepared and its exact concentration determined by its absorbance at λ_{max} = 262 nm (ε = 1.15 × 10^{-4}) following a 600-fold dilution. A chloroform solution ca. 4 × 10^{-5} M in **2** was prepared in a 1-cm UV cuvette. For **3** the solution was ca. 4 × 10^{-6} M and it was prepared in a 10-cm UV cell. The exact concentrations were determined by UV-vis with λ_{max} = 386 nm (ε = 1.96 × 10^4) for **2** and λ_{max} = 406 nm (ε = 1.62 × 10^4) for **3**. Small aliquots (ca. 10 μL) of the solution of **4** were added to the receptor solution until the absorbance at λ_{max} = 386 nm (**2**) or λ_{max} = 406 nm (**3**) no longer decreased. The concentration of **4** was thus varied as follows: for **2**, [**4**] ≈ 5-30 × 10^{-5} M, and for **3**, [**4**] ≈ 5-60 × 10^{-6} M. Data from ca. 20-30% to 80-90% saturation were used in the calculation of K_{assoc} and Δε.

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(29) Matsuda, A.; Shinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. *Synthesis* **1986**, 385-386.

Convergent Functional Groups. 10. Molecular Recognition of Neutral Substrates

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Abstract: In this paper synthetic molecular clefts with functional groups complementary to adenines, diketopiperazines, and barbiturates are described. Lactams and imides are compared for hydrogen-bonding affinites toward each other and to the heterocycles mentioned above. Titrations in $CDCl_3$ using NMR show association constants vary by factors of 10^4 for adenines, 10^2 for diketopiperazines, and 10 for barbiturates with the new receptors. Enantioselective recognition of *cyclo*-L-Leu-Leu is observed, corresponding to $\Delta\Delta G$ = 2.7 kcal/mol. The relative strengths of hydrogen-bonding arrays are interpreted in terms of secondary interactions such as defined in the following paper in this issue by Jorgensen and Severance.

Introduction

How does one choose the optimal complement to functional groups in a given structure? Patterns of hydrogen bond donors and acceptors are easily and intuitively visualized, but what other factors, less visible, contribute to the intermolecular forces between functional groups? In previous disclosures from these laboratories, we have shown that cleft-like shapes offer a number of advantages for the study of molecular recognition.[1] Here their abilities to probe subtle effects in hydrogen bonding are described particularly in the context of the questions posed above. Their capacities to act as synthetic receptors for neutral, biorelevant targets is further developed.

The cleft-like structures are readily made from Kemp's triacid[2] **1**. The U-shaped relationship between any two carboxyl functions in this subunit permits the construction of molecules which fold back upon themselves, and, in conjunction with suitable spacer

(1) For a recent review, see: Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245-255.

(2) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140. Commercially available from the Aldrich Chemical Co. For a convenient synthesis, see: Rebek, J., Jr.; Askew, B.; Killoran, M.; Nemeth, D.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, *109*, 2426-2431.

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