Molecular Recognition in Micelles: The Roles of Hydrogen Bonding and Hydrophobicity in Adenine–Thymine Base-Pairing in SDS Micelles

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Received March 17, 1993

Abstract: This paper describes a new class of molecular receptors **2** that bind adenine derivatives **3** in aqueous solution by means of hydrogen bonding (base-pairing). The receptors are supramolecular assemblies of thymine groups embedded in micelles, which self-assemble when (thyminylalkyl)ammonium salts **1** [Thy-$(CH_2)_n$-N^+Me_3] are mixed with aqueous sodium dodecyl sulfate (SDS) solutions. NMR titration studies and Job's method indicate that binding occurs with 1:1 adenine–thymine stoichiometry in a fashion consistent with base-pairing within the micelles. In the absence of SDS, base-stacking occurs in preference to base-pairing. Three factors that were anticipated to be most significant in binding were examined: the lipophilicity of the adenine substrate, the position of the thymine group within the micelles, and the role of SDS in binding. To study these factors, the length of the alkyl group on adenine **3** (m = 1, 2, 3, and 4), the length of the alkylammonium chain of thymine **1** (n = 4, 6, 8, and 10), and the concentration of SDS (0–40 mM) were varied. The lipophilicity of the adenines was found to have the greatest effect upon the measured association constant K_{obs}. A binding model is proposed in which adenine **3** first partitions between the bulk aqueous solution and the interior of the micelle and then base-pairs to the thymine group within the micelle. In accordance with this model, a linear relationship is observed between log K_{obs} and log K_{ow} (where K_{ow} is the octanol–water partition coefficient of adenine **3**).

Molecular recognition is central to the study of fundamental intermolecular forces, the mimicry of biological systems, and the development of supramolecular devices.[1,2] Most synthetic molecular receptors fall into two broad classes: those that bind molecules in aqueous solutions and those that bind in relatively noncompetitive organic solvents. The former class includes cyclodextrins[3] and cyclophanes,[4] whereas the latter includes various molecular clefts and cavities.[5] Molecular receptors that bind substrates in water generally achieve binding with the aid of hydrophobic interactions, whereas those that bind in noncompetitive organic solvents often rely heavily upon hydrogen bonding.

Hydrogen bonding generally does not provide significant driving force for molecular recognition in aqueous solution, since the enthalpic benefits of forming hydrogen bonds between the receptor and substrate are offset by the enthalpic cost of breaking hydrogen bonds between these molecules and water.[6,7] For this reason, small molecules that readily hydrogen bond to each other in organic solvents do not do so to any appreciable extent in water.[8] The nucleic acid bases illustrate this point admirably. Individual bases form Watson–Crick and Hoogsteen base-pairs and triplets in organic solvents but stack in water.[9,10]

Few synthetic receptors have been developed to hydrogen bond to substrates in aqueous solution. In 1987, Lhomme reported that adenine and thymine derivatives base-pair in aqueous solution when one of the bases is tethered to a hydrophobic proflavin molecule that serves as a platform upon which base-pairing occurs.[11] Recently, Rebek has shown that adenine derivatives form hydrogen-bonded complexes within water-soluble analogs of Kemp's triacid imide bearing hydrophobic aromatic groups.[12]

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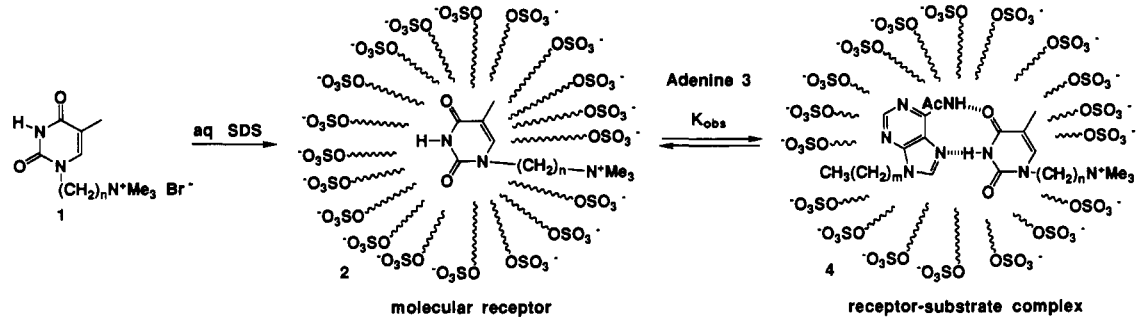
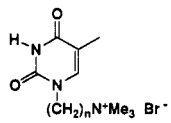
Scheme I



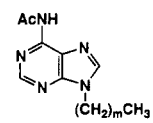
In these systems, hydrophobic interactions act in conjunction with hydrogen bonding to achieve recognition. The interface of a monolayer and water can also provide a suitable environment for hydrogen bonding. Kunitake and co-workers have found that functionalized monolayers bind sugars, nucleic acid bases, and nucleotides at air–water interfaces.[13]

We recently introduced a new strategy to achieve hydrogen bonding in aqueous solution.[14] In this strategy, the hydrogen-bonding groups are incorporated inside micelles to shield the hydrogen bonds from water.[15] We have established that (thyminylalkyl)ammonium groups **1** incorporate into sodium dodecyl sulfate (SDS) micelles to form molecular receptors that bind adenine derivative **3** by means of hydrogen bonding in aqueous solution. Scheme I illustrates the incorporation and binding processes and provides working models for the structures of the molecular receptors **2** and receptor–substrate complexes **4**. NMR studies indicate that the (thyminyloctyl)ammonium derivative **1c** (n = 8) is fully incorporated in the micelles at SDS concentrations of 20 mM or greater. The receptor **2c** (n = 8) binds acetylpropyladenine **3b** (m = 2) by base-pairing with an association constant K_{obs} of 16 M^{-1}. In the absence of SDS, the adenine and thymine groups interact by stacking rather than hydrogen bonding.

In this paper, we report detailed studies of the binding phenomenon. We have examined three factors that we anticipated to be most significant in binding: the lipophilicity of the adenine substrate, the position of the thymine group within the micelles, and the role of SDS in binding. To study these factors, we have varied the length of the alkyl group on adenine (m = 1, 2, 3, and 4), the length of the alkylammonium chain (n = 4, 6, 8, and 10), and the concentration of SDS (0–40 mM). We find that hydrogen bonding and hydrophobic interactions contribute to binding. To explain this observation, we propose a model in which the adenine derivative first partitions between the bulk aqueous phase and the micellar phase and then binds to the thymine derivative within the micelle by means of hydrogen bonding. Furthermore, we establish that the binding process is consistent with a 1:1 binding stoichiometry.



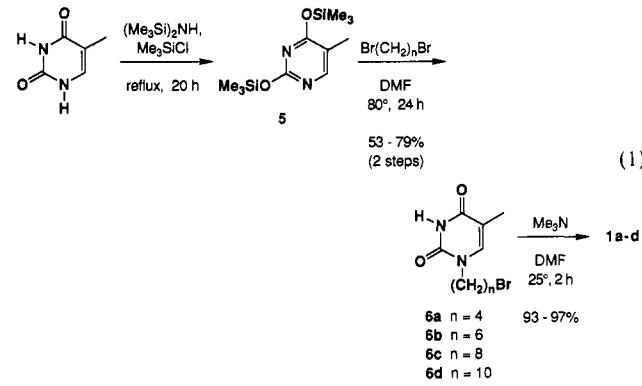
1a n = 4
1b n = 6
1c n = 8
1d n = 10



3a m = 1
3b m = 2
3c m = 3
3d m = 4

Results

Synthesis of (Thyminylalkyl)ammonium Salts 1. (Thyminylalkyl)ammonium salts **1a–d** were prepared from thymine as shown in eq 1. Thymine was converted to its bis(trimethylsilyl) ether derivative **5** by treatment with hexamethyldisilazane and chlorotrimethylsilane.[16] The bis(trimethylsilyl) derivative was then alkylated by treatment with 3 equiv of the appropriate dibromoalkane in DMF to afford the (bromoalkyl)thymine derivative **6**.[16] Treatment of the (bromoalkyl)thymine derivative with trimethylamine in dimethylformamide afforded the ammonium salt **1**.



(1)

NMR Studies of Thymine Derivatives in Aqueous Solution. The ^1H NMR chemical shift of a thymine imino proton provides a sensitive probe of its environment. The NH resonance of a thymine derivative appears substantially farther downfield in hydrogen-bond-accepting solvents than in non-hydrogen-bond-accepting solvents. Similarly, the NH resonance of a base-pairing thymine appears downfield of that of a free thymine. For this reason, we used the chemical shift of the NH group of thymine derivative **1** as an index of its incorporation into micelles and its binding of adenine derivatives.

It is technically challenging to study the NH group of thymine derivatives in aqueous solution. Since the imino proton is labile, ^1H NMR studies must be performed in H_2O to which a small amount (e.g., 10%) of D_2O has been added to provide a lock signal. Chemical exchange with solvent is too rapid to permit use of presaturation to suppress the solvent peak. Instead, tailored excitation must be used to suppress the water peak;[17] the $1\bar{3}3\bar{1}$ pulse sequence is well-suited for this purpose.[18] Under mildly acidic conditions, the exchange rate of the thymine imino proton is sufficiently slow to permit its observation using this pulse

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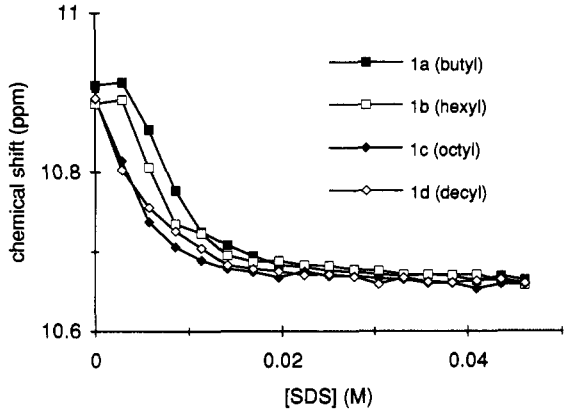
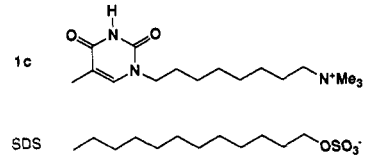


Figure 1. Incorporation of (thyminylalkyl)ammonium salts **1** into SDS micelles: effect of SDS concentration on the chemical shift of NH protons in thymines **1a–d**. Studies were performed on a 500-MHz ^{1}H NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

sequence.[19] Typically, we acidified the thymine solutions with 1.0 mM AcOH to allow observation of this proton.

Incorporation of (Thyminylalkyl)ammonium Salts 1 into SDS Micelles. (Thyminylalkyl)ammonium salts **1** are designed to incorporate into SDS micelles. The trimethylammonium group is complementary in charge to the sulfate head group of SDS, and the alkyl chain and hydrophobic thymine surfaces of **1** are complementary to the dodecyl chain of SDS. Assuming that the alkyl chains adopt extended (*anti*) conformations, the (thyminyloctyl)ammonium derivative **1c** is comparable in length to SDS.

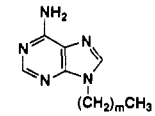


On the basis of these considerations, (thyminylalkyl)ammonium salts may be anticipated to incorporate into micelles as shown in structure **2**. We postulate that receptors **2** consist of two or three molecules of **1** in a micelle composed of about 60 molecules of SDS, since the mean aggregation number of SDS is reported to be 60 and we generally use a solution of 1.0 mM of **1** and 20 or 30 mM of SDS for molecular recognition studies.[20]

^{1}H NMR studies suggest that structure **2** is a reasonable model for the receptors. Upon addition of a solution of SDS to solutions of **1**, large (ca. 0.21 ppm) upfield shifts of the imino resonances occur between 0 and 20 mM, indicating that the environment of the thymine group changes significantly as the SDS forms micelles (Figure 1). The upfield direction of the shifting is consistent with the change to a less polar environment.[21] Only small changes in the chemical shift of this proton are observed at SDS concentrations above 20 mM, suggesting that incorporation is complete above 20 mM SDS. The resonance of the thymine ring proton shifts upfield (0.15 ppm), corroborating the change in environment of the thymine ring. Significant (0.05 ppm) downfield shifting of the trimethylammonium resonance also occurs, indicating that its environment changes as well.

The changes in chemical shift of the NH groups at concentrations of SDS below the critical micelle concentration (cmc = 8.2 mM)[22] suggest that the cationic alkylammonium derivatives **1** facilitate aggregation of the anionic SDS molecules.[23] This observation is consistent with reports that mixtures of anionic and cationic surfactants form micelles well below the cmc of either pure surfactant[24] and that nonsurfactant counterions bearing hydrophobic alkyl chains also lower the cmc of surfactants.[25] The formation of aggregates at low SDS concentration may be attributed to attractive electrostatic interactions between the cationic ammonium group of **1** and the anionic sulfate group of SDS.

Preliminary Studies of Adenine–Thymine Interactions in Aqueous SDS Solution. We chose to initially examine the interactions of 9-alkyladenine derivatives **7** and thymine derivatives **1** in aqueous SDS solution, since the interactions of 9-alkyladenines have been studied extensively in *organic* solvents. The association constant for 9-ethyladenine (**7a**) and 1-cyclohexylthymine in $CDCl_3$ is known to be 130 M^{-1},[26] and numerous strategies have been developed for binding 9-alkyladenine derivatives in chloroform solution.[27]



7a m = 1
7b m = 2
7c m = 3
7d m = 4

We found that SDS has a strong effect upon the interactions of 9-alkyladenines **7** and (thyminylalkyl)ammonium salts **1**. Addition of 9-propyladenine (**7b**) to a solution of (thyminyloctyl)ammonium bromide (**1c**) in 20 mM aqueous SDS solution containing 1.0 mM AcOH (to reduce the rate of exchange of the thymine NH group) results in downfield shifting of the thymine imino group ^{1}H NMR resonance. In the absence of SDS, slight upfield shifting of the thymine resonance occurs. These observations suggest that base-pairing between thymines **1** and adenines **7** occurs in the presence of SDS but that stacking of the adenine and thymine bases occurs in its absence.

Technical difficulties prevent the rigorous study of the interactions of thymines **1** and adenines **7** in aqueous SDS by ^{1}H NMR titration. As increasing quantities of 9-propyladenine are added, the thymine NH resonance becomes very broad. This observation suggests that the 9-propyladenine, which is weakly basic, increases the rate of exchange of the thymine NH group. Attempts to reduce the rate of exchange by buffering the adenine with additional AcOH result in precipitation of 9-propyladenine as its dodecyl sulfate salt. To permit further study of the binding interactions, we substituted the nonbasic N^6-acetyl-9-alkyladenines **3** for the basic 9-alkyladenines **7**.

Comparison of Alkyladenines 7 and N^6-Acetyl-9-alkyladenines 3. In organic solvents, thymine derivatives bind 9-alkyladenines

(19) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; Chapter 2.

(20) Turro, N. J.; Yekta, A. *J. Am. Chem. Soc.* **1978**, *100*, 5951.

(21) Although the NH resonance of SDS-incorporated **1** (ca. δ 10.7) is upfield of free **1** in water (ca. δ 10.9), it is much further downfield than that of thymine derivatives in organic solvents (e.g., δ 8.05 for 1-(8-bromooctyl)thymine (**6c**) in $CDCl_3$). These observations suggest that the thymine group of **1** is hydrated within the micelle.

(22) Mukerjee, P.; Mysels, K. J. *Critical Micelle Concentrations of Aqueous Surfactant Systems*; National Standard Reference Data Series 36; National Bureau of Standards, U.S. Government Printing Office: Washington, DC, 1971; NSRDS-NBS 36.

(23) Although (thyminylalkyl)ammonium salts **1** incorporate into micelles, these compounds do not appear to be surfactants. Aqueous solutions of **1** exhibit none of the foaminess typically associated with surfactants, and at millimolar concentrations, these compounds demonstrate no concentration-dependent changes in ^{1}H NMR spectra characteristic of self-association.

(24) (a) Malliaris, A.; Binana-Limbele, W.; Zana, R. *J. Colloid Interface Sci.* **1986**, *110*, 114. (b) Graciaa, A.; Ghoulam, M. B.; Marion, G.; Lachaise, J. *J. Phys. Chem.* **1989**, *93*, 4167.

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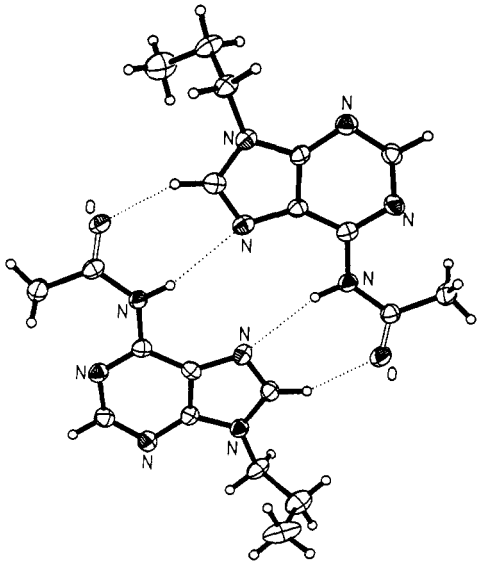
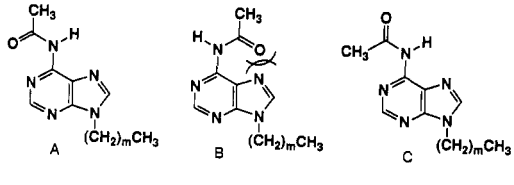


Figure 2. Crystal structure of acetylpropyladenine **3b**.

7 more strongly than they bind N^6-acetyl-9-alkyladenines **3**. NMR titration experiments indicate that 1-(8-bromooctyl)thymine (**6c**) binds 9-propyladenine (**7b**) in $CDCl_3$ with an association constant of 100 M^{-1}, whereas it binds N^6-acetyl-9-propyladenine (**3b**) with an association constant of 37 M^{-1}.

This difference may arise because fewer binding modes are available to the acetylated adenines. Adenines **7** have two hydrogen-bonding surfaces and can bind 1-alkylthymines in Watson–Crick, Hoogsteen, reversed Watson–Crick, and reversed Hoogsteen modes. Adenines **3** have only one hydrogen-bonding surface. On a pure statistical basis, N^6-acetyl-9-alkyladenines **3** might be expected to bind thymine derivatives with an association constant that is half as large as that of 9-alkyladenines **7**.

We postulate that binding occurs in the Hoogsteen and reverse Hoogsteen modes, since adenines **3** are expected to adopt conformation **A** in preference to conformation **B**. In conformation **B**, there are unfavorable interactions between N_7 of the adenine ring and the lone pairs of electrons of the amide carbonyl group, whereas in conformation **A**, there is a favorable interaction between N_7 of the adenine ring and the amide NH group. This structural model is further supported by the observation that N^6-methyl-9-alkyladenine derivatives adopt the *syn* conformation (analogous to conformation **A**) in solution.[28]



X-ray crystallography supports the postulate that binding occurs in the Hoogsteen mode. Acetylpropyladenine **3b** is found as the Hoogsteen hydrogen-bonded dimer in the solid state (Figure 2). Of particular interest is the observation that the acetamide group of **3b** adopts the *cis*-amide conformation **C** rather than *trans*-amide conformation **A**. This result is surprising, since secondary amides generally adopt a *trans*-conformation.[29] We attribute this conformation to the stabilization of the *cis*-amide in the solid state by intermolecular hydrogen bonding.

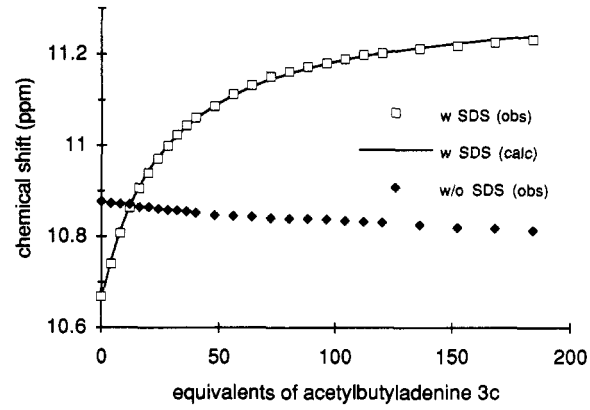


Figure 3. Titration of thymine **1c** with adenine **3c**: chemical shift of the NH group of thymine **1c** vs equivalents of added adenine **3c** in the presence and in the absence of 30.0 mM SDS. The curve is the theoretical 1:1 binding isotherm that best fits the experimental data (K_{obs} = 33.4 M^{-1}, δ_{free} = 10.660 ppm, δ_{bound} = 11.422 ppm). Titrations were performed on a 500-MHz ^1H NMR instrument at 20 ● 3 °C. H_2O or HOD was used as a reference (δ 4.65).

^1H NMR Titrations and Binding Stoichiometry in Aqueous SDS Solution. ^1H NMR titration studies indicate that (thyminylalkyl)ammonium salts **1** and N^6-acetyl-9-alkyladenines **3** base-pair in aqueous SDS solution.[30] Figure 3 illustrates a typical experiment. Addition of aliquots of a solution of 200 mM N^6-acetyl-9-butyladenine (**3c**) and 30 mM SDS in 10% D_2O/H_2O to a solution of 1.0 mM (thyminyloctyl)ammonium bromide **1c**, 1.0 mM AcOH, and 30 mM SDS in 10% D_2O/H_2O results in substantial downfield shifting of the thymine imino resonance. The titration data fit a 1:1 binding isotherm extremely well, suggesting that 1:1 base-pairing is occurring. Analysis of the data by nonlinear least-squares fitting to a 1:1 binding isotherm reveals an association constant of 33 M^{-1}. This result is highly reproducible; repetition of the titration four times generated binding constants of 33 ± 1 M^{-1}.

The surfactant is essential for base-pairing. In the absence of SDS, smaller upfield shifts occur (Figure 3). This result indicates that stacking of the adenine and thymine bases predominates in the absence of SDS.

Job's method provides further evidence for 1:1 binding stoichiometry.[31] When varying fractions of solutions of (thyminylbutyl)ammonium bromide **1a** and acetylpentyladenine **3d** in aqueous SDS solution are mixed, a maximum concentration of adenine–thymine complex forms at equimolar concentrations of **1a** and **3d** (Figure 4). In this experiment, a stock solution of 5.0 mM (thyminylbutyl)ammonium bromide **1a**, 1.0 M AcOH, and 20 mM SDS in 10% D_2O/H_2O and a stock solution of 5.0 mM acetylpentyladenine **3d**, 1.0 mM AcOH, and 20 mM SDS in 10% D_2O/H_2O were mixed in varying proportions. The chemical shift of the imino proton of **1a** was recorded at different mole fractions of **1a**, and the concentration of the **1a·3d** complex was calculated on the basis of this chemical shift. The large magnitudes of the error bars, particularly at high mole fractions of **1a**, arise because of the relatively large uncertainties in measuring small differences in chemical shifts. These uncertainties reflect the difficulty in applying Job's method to a complex with a relatively low association constant.

Although binding is, for the most part, consistent with 1:1 adenine–thymine base-pairing within micelles, deviations from 1:1 binding stoichiometry do occur. These deviations are most readily apparent in the interactions of (*thyminylalkyl*)*ammonium*

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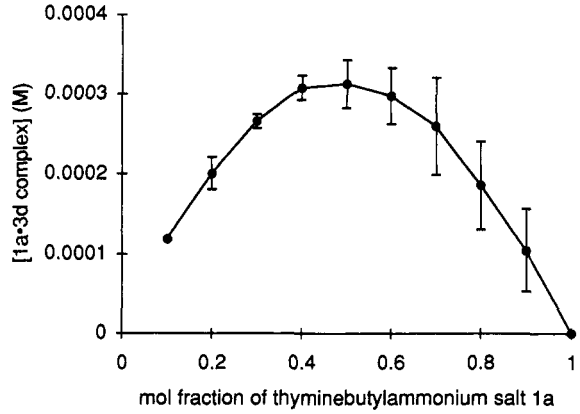

Figure 4. Job plot for thymine **1a** and adenine **3d**: concentration of the **1a·3d** complex as a function of the mole fraction of (thyminylbutyl)ammonium salt **1a** upon mixing varying portions of 5.0 mM solutions of **1a** and **3d** in 20 mM SDS solution. For details, see the Experimental Section. The data points are the average of four independent experiments; the error bars represent standard deviations. Studies were performed on a 500-MHz ^{1}H NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

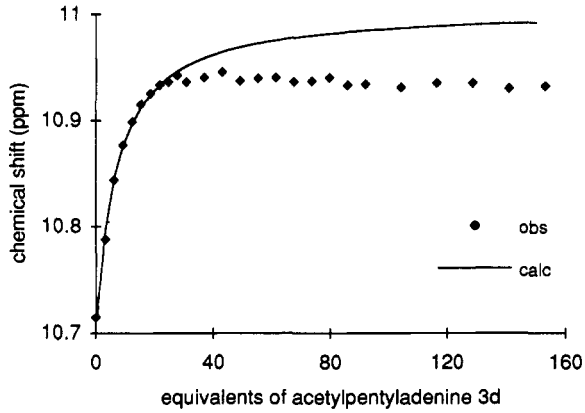

Figure 5. Titration of thymine **1a** with adenine **3d**: chemical shift of the NH group of thymine **1a** vs equivalents of added adenine **3d** at 20.0 mM SDS. The curve is the theoretical 1:1 binding isotherm that best fits the first 10 data points (K_{obs} = 145 M^{-1}, δ_{free} = 10.706 ppm, δ_{bound} = 11.018 ppm). The titration was performed on a 500-MHz ^{1}H NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

salts with shorter chains and *acetylalkyladenines with longer alkyl groups* at *higher concentrations of added adenine.* The titration of (thyminylbutyl)ammonium bromide **1a** with acetylpentyladenine **3d** provides the most extreme example of this behavior (Figure 5). The binding isotherm for this titration exhibits two regions. Between 0 and 30 equiv of added adenine **3d**, large downfield shifts of the imino proton of thymine **1a** occur. In this region, the data fit reasonably well to a 1:1 binding isotherm (K_{obs} = 145 M^{-1}). At higher concentrations of added adenine, no further downfield shifting occurs and the NH resonance of the thymine group actually begins to shift upfield.

The flattening and upfield shifting at high concentrations of added **3d** suggest that the adenine groups undergo stacking, as well as base-pairing, with the thymine group. The stacking occurs to a greater extent at higher concentrations of added adenine and is most easily observed as the base-pairing interactions approach saturation. In addition, stacking interactions within the micelles occur to a greater extent with the more lipophilic adenine derivatives (e.g., **3d**), since there are greater intramicellar concentrations of these derivatives (vide infra).

For these reasons, the association constants measured by these NMR titrations must be interpreted with caution. For the titration of **1a** with **3d**, the case that exhibited the worst fit to a 1:1 binding isotherm, values of K_{obs} ranging from 100 to 300 M^{-1} were

Table I. Effect of Adenine Chain Length upon K_{obs} for the Titration of Thymines **1a** and **1c** with Adenines **3** at 30 mM SDS

adenine	**1a** K_{obs} (M^{-1})	**1c** K_{obs} (M^{-1})
3a (ethyl)	15	8.8
3b (propyl)	31	16
3c (butyl)	92	33
3d (pentyl)	145	49

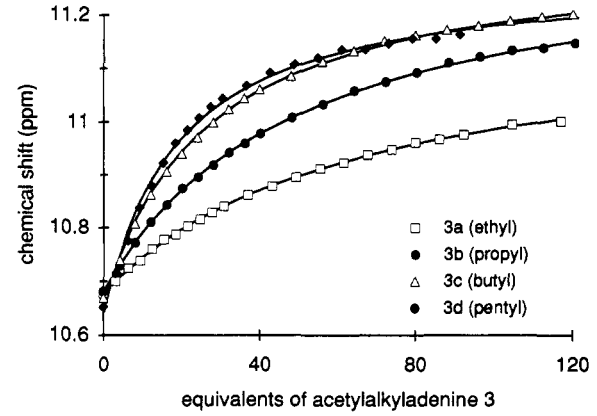

Figure 6. Effect of adenine chain length upon binding: chemical shift of the NH group of (thyminyloctyl)ammonium salt **1c** vs equivalents of added acetylalkyladenines **3a–d** at 30.0 mM SDS. The curves are the theoretical 1:1 binding isotherms that best fit the experimental data (**3a**: K_{obs} = 8.8 M^{-1}, δ_{free} = 10.678 ppm, δ_{bound} = 11.565 ppm. **3b**: K_{obs} = 15.8 M^{-1}, δ_{free} = 10.679 ppm, δ_{bound} = 11.548 ppm. **3c**: K_{obs} = 33.4 M^{-1}, δ_{free} = 10.660 ppm, δ_{bound} = 11.422 ppm. **3d**: K_{obs} = 48.8 M^{-1}, δ_{free} = 10.635 ppm, δ_{bound} = 11.367 ppm). Titrations were performed on a 500-MHz NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

calculated by analyzing either part or all of the data presented in Figure 5. The flattening of the binding isotherm results in an artificially elevated value of K_{obs} when the entire data set is analyzed by this method. Analysis of the first 10 data points provides a more reasonable estimate of K_{obs} (145 M^{-1}), which we consider reliable to within about 50%.

In contrast to the large deviations described above, most cases studied exhibited excellent fit to a 1:1 binding isotherm. The titration of **1c** and **3c** (Figure 3) is typical. The data fit a 1:1 binding isotherm extremely well and show no flattening at high concentrations of added adenine. Analysis of the first 10 data points gives a value of K_{obs} of 27 M^{-1}, which is close to the value of 32 M^{-1} that is obtained by analyzing the entire data set. On the basis of this analysis, we estimate most of the values of K_{obs} that we report in this paper to be reliable to within about 20%.

Effect of Adenine Chain Length upon Binding. The length of the alkyl chain of adenine derivative **3** has a strong effect upon the observed binding constant K_{obs}. Comparison of the ethyl, propyl, butyl, and pentyl derivatives **3a–d** indicates that adenine derivatives bearing longer alkyl chains are bound with larger association constants. Titration of (thyminylbutyl)ammonium salt **1a** with adenines **3a–d** revealed association constants of 15, 31, 92, and 145 M^{-1}, respectively (Table I). A similar trend is observed when (thyminyloctyl)ammonium salt **1c** is titrated with this series of adenines (Table I).

Figure 6 provides a graphical comparison of the binding isotherms of (thyminylalkyl)ammonium salt **1c** and adenines **3**. The steeper binding isotherms of adenines with longer alkyl chains reflect the larger association constants of these compounds. The isotherms converge to similar chemical shifts for the complexes of **1c** and adenines **3a–d**. By nonlinear least-squares fitting of the titration data to 1:1 binding isotherms, we calculated chemical shifts of the thymine imino proton to be δ 11.57 and 11.55, respectively, in the **1c·3a** and **1c·3b** complexes. The chemical

Table II. Effect of (Thyminylalkyl)ammonium Chain Length upon K_{obs} for the Titration of (Thyminylalkyl)ammonium Salts **1** with Adenine **3b** at 30 mM SDS

thymine	K_{obs} (M^{-1})
1a (butylammonium)	31
1b (hexylammonium)	19
1c (octylammonium)	16
1d (decylammonium)	14

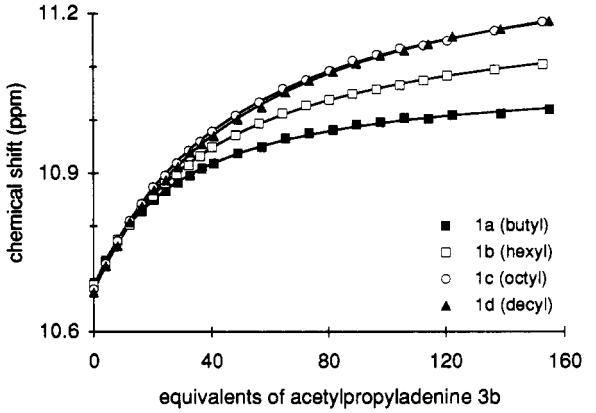

Figure 7. Effect of alkylammonium chain length upon binding: chemical shift of the NH groups of thymines **1a–d** vs equivalents of added acetylpropyladenine **3b** at 30.0 mM SDS. The curves are the theoretical 1:1 binding isotherms that best fit the experimental data (**1a**: K_{obs} = 31.3 M^{-1}, δ_{free} = 10.687 ppm, δ_{bound} = 11.133 ppm. **1b**: K_{obs} = 19.2 M^{-1}, δ_{free} = 10.686 ppm, δ_{bound} = 11.357 ppm. **1c**: K_{obs} = 15.8 M^{-1}, δ_{free} = 10.679 ppm, δ_{bound} = 11.548 ppm. **1d**: K_{obs} = 13.9 M^{-1}, δ_{free} = 10.676 ppm, δ_{bound} = 11.600 ppm). Titrations were performed on a 500-MHz NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

shifts for the complexed thymine are calculated to be slightly lower in the **1c·3c** and **1c·3d** complexes (δ 11.42 and 11.38, respectively). The smaller downfield shifts calculated in the latter case may reflect the deviations from a 1:1 binding isotherm described above. When only the first 10 data points are used to calculate the chemical shifts of the NH groups in the **1c·3c** and **1c·3d** complexes, values of about δ 11.5 are obtained.

Effect of Alkylammonium Chain Length upon Binding. Whereas varying the chain length of adenines **3** results in large differences in K_{obs}, varying the chain length of thymines **1** generates much smaller changes in K_{obs}. Titration of butyl-, hexyl-, octyl-, and decylammonium salts **1a–d** with acetylpropyladenine **3b** reveals association constants of 31–14 M^{-1} (Table II). As is shown in Figure 7, the butyl- and hexylammonium salts approach saturation at lower concentrations of added **3b** than the octyl- and decylammonium salts. By nonlinear least-squares fitting of the titration data to 1:1 binding isotherms, we calculated chemical shifts of the thymine imino proton to be δ 11.55 and 11.60, respectively, in the **1c·3b** and **1d·3b** complexes. The chemical shifts for the complexed thymine are calculated to be substantially lower for the **1a·3b** and **1b·3b** complexes (δ 11.13 and 11.36, respectively). These differences in association constants and chemical shifts may result from complexation occurring within different regions of the micelles for the different alkylammonium chain lengths.

Effect of SDS Concentration upon Binding. In the earlier sections of this paper, we established that (thyminyloctyl)-ammonium salts incorporate into SDS micelles and that SDS is essential for base-pairing. In this section, we examine the effect of SDS concentration on binding.

We have titrated (thyminylbutyl)ammonium salt **1a** with acetylpropyladenine **3b** at 0, 5, 10, 20, 30, and 40 mM SDS (Figure 8, Table III). Below the cmc of SDS (8.2 mM[22]), upfield shifting of the thymine imino proton occurs as adenine **3b** is added, indicating that aromatic stacking occurs and that there is no

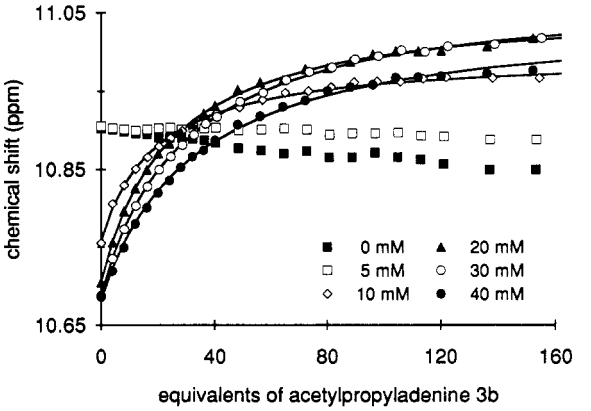

Figure 8. Effect of SDS concentration upon binding: chemical shift of the NH group of (thyminylbutyl)ammonium salt **1a** vs equivalents of added acetylpropyladenine **3b** at varying (0–40 mM) SDS concentrations. The curves are the theoretical 1:1 binding isotherms that best fit the experimental data (10 mM: K_{obs} = 50.2 M^{-1}, δ_{free} = 10.757 ppm, δ_{bound} = 11.021 ppm. 20 mM: K_{obs} = 38.7 M^{-1}, δ_{free} = 10.704 ppm, δ_{bound} = 11.110 ppm. 30 mM: K_{obs} = 31.3 M^{-1}, δ_{free} = 10.687 ppm, δ_{bound} = 11.133 ppm. 40 mM: K_{obs} = 26.8 M^{-1}, δ_{free} = 10.681 ppm, δ_{bound} = 11.119 ppm). Titrations were performed on a 500-MHz NMR instrument at 20 ± 3 °C. H_2O or HOD was used as a reference (δ 4.65).

Table III. Effect of SDS Concentration upon K_{obs} for the Titration of Thymine **1a** with Adenine **3b**

SDS (mM)	K_{obs} (M^{-1})
0	—
5	—
10	50
20	39
30	31
40	27

significant base-pairing. At concentrations of SDS of 10 mM or greater, downfield shifts occur in a fashion consistent with 1:1 binding. Between 10 and 40 mM, association constants of 50–27 M^{-1} and small differences in the shapes of the binding isotherms are observed. The smaller shifts, the flatter binding isotherm, and the larger association constant at 10 mM may reflect incomplete incorporation of **1a** into the micelles. The small differences in binding isotherms and association constants for 20, 30, and 40 mM SDS may reflect small differences in micellar structure or intermicellar interactions at varying SDS concentration.[32] In summary, these studies establish that significant adenine–thymine base-pairing only occurs above the cmc of SDS.

Discussion

Of the factors that we have examined (adenine chain length, thymine chain length, and SDS concentration), the length of the alkyl group of acetylalkyladenine **3** exerts the greatest effect on K_{obs}. The correlation between increasing hydrophobicity of the adenine derivative and increasing association constant suggests that the adenine derivative first partitions between the bulk aqueous solution and the micellar phase and then the thymine group binds the intramicellar adenine.[33] Scheme II illustrates this model.

In this model, the concentration of intramicellar adenine depends on the micelle–water partition coefficient K_{part} and the concentration of adenine in the bulk aqueous phase. The value of K_{part} depends exclusively on the lipophilicity of adenine **3**. Within the micelles, (thyminylalkyl)ammonium salt **1** binds

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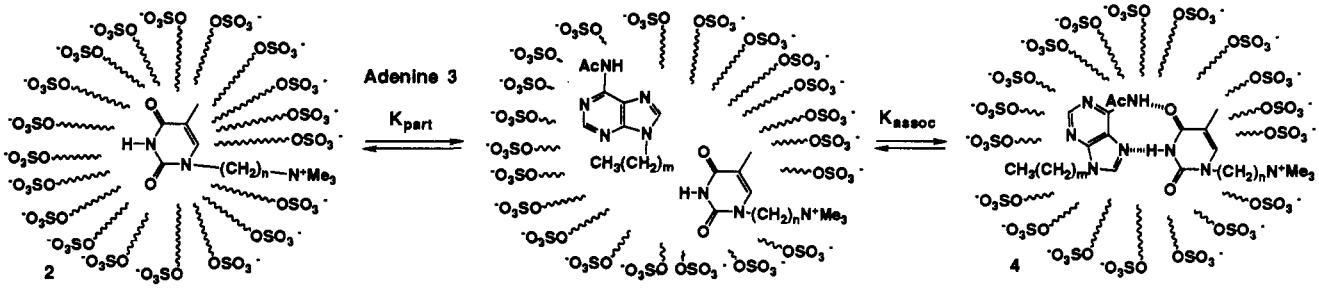
Scheme II



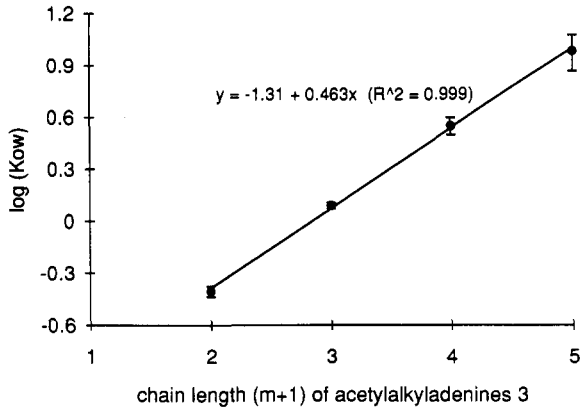


Figure 9. Effect of the length of the alkyl chains of adenines **3** on the octanol–water partition coefficients (K_{ow}). The data points are the average of four independent experiments; the error bars represent standard deviations. The line is generated by linear least-squares treatment of the data.

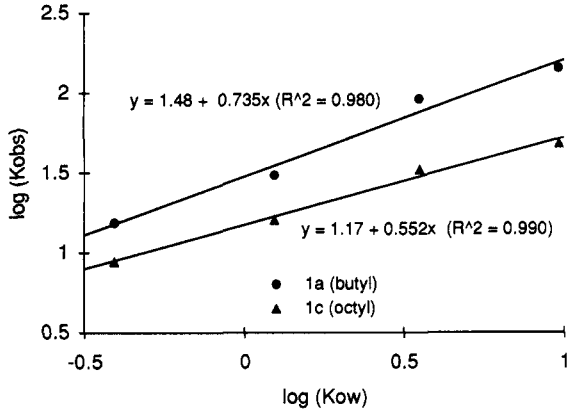


Figure 10. Relationship between log K_{obs} and log K_{ow} for (thyminylalkyl)ammonium salts **1a** and **1c**. The lines are generated by linear least-squares treatment of the data.

intramicellar adenine by means of hydrogen bonding (base-pairing) with an association constant, K_{assoc}. The value of K_{assoc} depends on the inherent affinity of thymine derivatives for acetylalkyladenines, any localization of the adenine or thymine groups within different regions of the micelle's interior, and any differences in the tendency of adenine and thymine to base-pair within different regions of the micelle's interior. The intramicellar association constant K_{assoc} should be independent of the alkyl chain of adenine **3**.

On the basis of this model, the measured association constant K_{obs} will be the product of the micelle–water partition coefficient K_{part} and the intramicellar association constant K_{assoc} (eq 2). (Since

$$K_{obs} = K_{part}K_{assoc} \quad (2)$$

K_{obs} is determined on the basis of the overall concentration of added adenine, this treatment assumes that the micelles do not significantly diminish the concentration of adenine in the bulk aqueous solution.) For a given (thyminylalkyl)ammonium salt and SDS concentration, the measured association constant K_{obs} should vary linearly with the micelle–water partition coefficient of adenine **3**.

Because of the difficulties in measuring micelle–water partition coefficients,[34] we have chosen to correlate K_{obs} with the octanol–water partition coefficient K_{ow}[35] rather than the micelle–water partition coefficient K_{part}. Micelle–water partition coefficients are known to correlate well with octanol–water partition coefficients K_{ow}; log K_{part} is linearly related to log K_{ow} (eq 3).[36] Combining eqs 2 and 3 generates eq 4. (The term c is a constant that combines a and log K_{assoc}.) Thus, the model predicts that log K_{obs} will be linearly related to log K_{ow}.

$$\log K_{part} = a + b\log K_{ow} \quad (3)$$

$$\log K_{obs} = b\log K_{ow} + c \quad (4)$$

We have measured K_{ow} for adenines **3a–d** by octanol–water partition experiments.[37] For this series of compounds, we obtained values of 0.39, 1.24, 3.5, and 9.6, respectively. As is expected for a series of homologs, log K_{ow} is linearly related to the length of the acetylalkyladenine chain (Figure 9). The slope (0.46) correlates well with the Hansch π_{CH_2} value of 0.50.[35]

The association constants K_{obs} correlate remarkably well with the octanol–water partition coefficients K_{ow}. As predicted by eq 4, log K_{obs} varies linearly with log K_{ow}. Figure 10 illustrates this relationship for the titrations of (thyminylbutyl)ammonium salts **1a** and **1c** with adenines **3a–d**. (Thyminylbutyl)ammonium salt **1a** generates a straight line with slope b = 0.73 and intercept c = 1.48. (Thyminyloctyl)ammonium salt **1c** generates a straight line with slope b = 0.55 and intercept c = 1.17.[38]

The slopes are consistent with the known relationships between octanol–water partition coefficients and micelle–water partition coefficients (eq 3). For a large group of organic compounds, including aliphatic halocarbons, aliphatic alcohols, aliphatic amides, aromatic hydrocarbons, phenols, aromatic halocarbons, halogenated phenols, and aliphatic hydrocarbons, best-fit values of a = 0.32 and b = 0.83 were reported.[36] Although no data are available for the micelle–water partition coefficients of adenine derivatives **3**, we speculate that the micelle–water partition coefficients of these compounds should be most similar to those reported for phenols (a = 0.59, b = 0.75) or halogenated phenols (a = 0.76, b = 0.66), since both phenols and adenines have hydrophobic, hydrogen-bond-donating, and hydrogen-bond-accepting functionality.[36] These reported values of b (ca. 0.7) are similar to those determined for **1a** and **1c** (0.73 and 0.55).[38]

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(38) Because of the uncertainties associated with measuring K_{obs}, we ascribe no significance to the difference between the slopes of these lines.

Summary and Outlook. In this study, we have prepared a series of supramolecular receptors consisting of (thyminylalkyl)ammonium salts incorporated in SDS micelles and we have found that the receptors bind adenine derivatives by adenine–thymine base-pairing. Two major factors have been identified as contributing to binding: hydrogen bonding between the adenine and thymine groups and hydrophobic interactions between the adenine substrate and the micelle. The receptors have been found to bind adenine derivatives that are more hydrophobic with a larger binding constant. We have proposed a model to explain this behavior, in which the adenine substrate partitions between the aqueous and micellar phases and is bound within the micelle.

The development of molecular receptors in which multiple subunits self-assemble is an ideal strategy for creating complex molecular devices. The present system contains two types of components, SDS molecules and thymine groups, that function together. The SDS creates a hydrophobic microenvironment, and the thymine groups recognize the adenine molecules. This strategy can be extended to permit the development of molecular devices that achieve even greater levels of complexity. We are currently extending this strategy to the recognition and transport of nucleotides and will report our findings in due course.

Experimental Section

Materials. Reagents and solvents were used without further purification except as indicated below. Dichloromethane and pyridine were distilled from calcium hydride. Diethyl ether was distilled from sodium and benzophenone. Anhydrous DMF was purchased from Aldrich. High-purity sodium dodecyl sulfate was purchased from Mallinckrodt (GenAR grade).

Instrumentation. IR spectra were obtained on a Mattson Galaxy 5000 FTIR spectrometer. ^{1}H NMR spectra were measured on a Bruker AC-300 (300 MHz) or a General Electric GN-500 (500 MHz) NMR spectrometer. UV spectra were obtained on a Shimadzu UV160U UV–visible spectrophotometer.

Representative Procedure for the Preparation of 1-(Bromoalkyl)-thymines 6.[16] A 50-mL, two-necked, round-bottomed flask equipped with a condenser fitted with a nitrogen inlet adapter, a glass stopper, and a magnetic stirring bar was charged with thymine (3.1 g, 0.025 mol), 1,1,1,3,3,3-hexamethyldisilazane (16 mL, 0.076 mol), and chlorotrimethylsilane (1.6 mL, 0.012 mol). The mixture was heated at reflux for 20 h, during which time NH_4Cl collected in the condenser. The solution was then concentrated by rotary evaporation to afford thymine bis(trimethylsilyl) ether **5** as a colorless oil. A 50-mL, round-bottomed flask equipped with a condenser and fitted with a nitrogen inlet adapter was charged with the entire portion of **5**, 10 mL of DMF, and 0.075 mol of the appropriate 1,*n*-dibromoalkane. The resulting solution was heated at 80 °C for 24 h. Ice water (150 mL) was then added, and the mixture was stirred for 30 min and then extracted with two 200-mL portions of CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield a yellow oil. The oil was triturated with ca. 15 mL of pentane at −20 °C to afford a yellow solid. Recrystallization from $CHCl_3$ and pentane or hexanes afforded (bromoalkyl)thymine **6** as white crystals.

1-(4-Bromobutyl)thymine (6a): yield 53%; mp 133–135 °C; IR (5% in $CHCl_3$) 3398, 3178, 3043, 2953, 2934, 2843, 1690, 1468, 1438, 1386, 1287, 1191, 1117, 1041, 899, 868, 660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (br s, 1 H), 6.99 (s, 1 H), 3.74 (t, J = 6.8 Hz, 2 H), 3.45 (t, J = 6.0 Hz, 2 H), 1.94 (s, 3 H), 1.90–1.88 (m, 4 H); HRMS m/e calcd for $C_9H_{14}N_2O_2{}^{79}Br$ [M + H]$^+$ 261.0239, found 261.0221. Anal. Calcd for $C_9H_{13}N_2O_2Br$: C, 41.40; H, 5.02; N, 10.73. Found: C, 41.32; H, 4.88; N, 10.56.

1-(6-Bromohexyl)thymine (6b): yield 76%; mp 112–113.5 °C; IR (5% in $CHCl_3$) 3401, 3178, 2945, 2863, 1707, 1686, 1468, 1392, 1360, 1262, 1241, 1121, 904, 660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (br s, 1 H), 6.98 (s, 1 H), 3.70 (t, J = 6.6 Hz, 2 H), 3.42 (t, J = 6.7 Hz, 2 H), 1.93 (s, 3 H), 1.90–1.83 (m, 2 H), 1.75–1.66 (m, 2 H), 1.55–1.45 (m, 2 H), 1.41–1.33 (m, 2 H); HRMS m/e calcd for $C_{11}H_{18}N_2O_2{}^{79}Br$ [M + H]$^+$ 289.0552, found 289.0549.

1-(8-Bromooctyl)thymine (6c): yield 53%; mp 96–96.5 °C; IR (5% in $CHCl_3$) 3397, 3183, 2936, 2861, 1699, 1683, 1468, 1440, 1387, 1355, 1273, 1116, 900, 865, 663 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (br s, 1 H), 6.97 (s, 1 H), 3.68 (t, J = 7.4 Hz, 2 H), 3.41 (t, J = 6.8 Hz, 2 H), 1.93 (s, 3 H), 1.88–1.82 (m, 2 H), 1.70–1.66 (m, 2 H), 1.46–1.40 (m, 2 H), 1.34 (br s, 6 H); HRMS m/e calcd for $C_{13}H_{22}N_2O_2{}^{79}Br$ [M + H]$^+$ 317.0865, found 317.0860. Anal. Calcd for $C_{13}H_{21}N_2O_2Br$: C, 49.22; H, 6.67; N, 8.83. Found: C, 49.00; H, 6.48; N, 8.79.

1-(10-Bromodecyl)thymine (6d): yield 79%; mp 92–94 °C; IR (5% in $CHCl_3$) 3404, 3183, 2935, 2865, 1700, 1679, 1474, 1438, 1388, 1361, 1247, 1107, 902, 870, 645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (br s, 1 H), 6.98 (s, 1 H), 3.68 (t, J = 7.4 Hz, 2 H), 3.41 (t, J = 6.8 Hz, 2 H), 1.93 (s, 3 H), 1.90–1.80 (m, 2 H), 1.70–1.65 (m, 2 H), 1.42–1.29 (m, 2 H), 1.29 (apparent s, 10 H); HRMS m/e calcd for $C_{15}H_{26}N_2O_2{}^{79}Br$ [M + H]$^+$ 345.1178, found 345.1168.

Representative Procedure for the Preparation of (Thyminylalkyl)ammonium Salts 1. An ice-cooled, 50-mL, two-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a gas inlet tube, and a magnetic stirring bar was charged with 7.66 mmol of 1-(bromoalkyl)thymine **6** and 10 mL of DMF. Trimethylamine was introduced via the gas inlet tube until ca. 10 mL (0.11 mol) had condensed. The ice bath was then removed, and the solution was stirred for 2 h. Diethyl ether (ca. 30 mL) was added, and the resulting white suspension was transferred via a cannula to a filter funnel capped with a rubber septum. The precipitate was washed with three 10-mL portions of diethyl ether and dried in vacuo to yield ammonium salt **1** as a white solid.

(4-(1-Thyminyl)butyl)trimethylammonium bromide (1a): yield 97%; mp 198–200 °C; IR (KBr) 3451, 3172, 3102, 3039, 2969, 2843, 1715, 1664, 1486, 1436, 1366, 1296, 1258, 1220, 979, 929, 859, 758 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 7.33 (s, 1 H), 3.66 (t, J = 6.4 Hz, 2 H), 3.23–3.17 (m, 2 H), 2.95 (s, 9 H), 1.71 (s, 3 H), 1.71–1.56 (m, 4 H); HRMS m/e calcd for $C_{12}H_{22}N_3O_2$ [M − Br]$^+$ 240.1712, found 240.1719.

(6-(1-Thyminyl)hexyl)trimethylammonium bromide (1b): yield 97%; mp 225–227 °C; IR (KBr) 3446, 3153, 3047, 2966, 2941, 2866, 1697, 1676, 1473, 1448, 1398, 1286, 1136, 955, 918, 817, 787 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 7.35 (s, 1 H), 3.62 (t, J = 7.1 Hz, 2 H), 3.19–3.15 (m, 2 H), 2.96 (s, 9 H), 1.74 (s, 3 H), 1.65–1.54 (m, 4 H), 1.26–1.24 (m, 4 H); HRMS m/e calcd for $C_{14}H_{26}N_3O_2$ [M − Br]$^+$ 268.2025, found 268.2035.

(8-(1-Thyminyl)octyl)trimethylammonium bromide (1c): yield 93%; mp 256–257 °C; IR (KBr) 3435, 3154, 3088, 3006, 2933, 2857, 1703, 1657, 1482, 1430, 1394, 1375, 1349, 1338, 1274, 1264, 1252, 1221, 1155, 1103, 971, 914, 570 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 7.77 (s, 1 H), 3.58 (t, J = 7.1 Hz, 2 H), 3.15–3.11 (m, 2 H), 2.93 (s, 9 H), 1.71 (s, 3 H), 1.64–1.57 (m, 2 H), 1.54–1.48 (m, 2 H), 1.18 (apparent s, 8 H); HRMS m/e calcd for $C_{16}H_{30}N_3O_2$ [M − Br]$^+$ 296.2338, found 296.2331.

(10-(1-Thyminyl)decyl)trimethylammonium bromide (1d): yield 95%; mp 187–189 °C; IR (KBr) 3440, 3159, 3028, 2934, 2860, 1711, 1667, 1480, 1430, 1367, 1299, 1230, 961, 911, 768 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 7.35 (s, 1 H), 3.60 (t, J = 7.0 Hz, 2 H), 3.18–3.12 (m, 2 H), 2.95 (s, 9 H), 1.73 (s, 3 H), 1.65–1.60 (m, 2 H), 1.55–1.50 (m, 2 H), 1.19–1.15 (m, 12 H); HRMS m/e calcd for $C_{18}H_{34}N_3O_2$ [M − Br]$^+$ 324.2651, found 324.2653.

Representative Procedure for the Preparation of 9-Alkyladenines 7.[39] A 250-mL, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a glass stopper, a rubber septum, and a magnetic stirring bar was charged with adenine (13.9 g, 0.103 mol) and NaH (2.88 g, 0.120 mol). Anhydrous DMF (180 mL) was added by syringe, and the mixture was stirred for 1 h to form a white suspension. The appropriate 1-bromoalkane (0.120 mol) was added by syringe over 5 min, and the reaction mixture was stirred for 16 h. The resulting pale yellow solution was filtered, the precipitate was washed with ca. 5 mL of CH_2Cl_2, and the filtrate was concentrated by rotary evaporation to afford a yellow solid. Recrystallization from CH_3OH afforded 9-alkyladenine **7** as a white solid.

9-Ethyladenine (7a): yield 30%; mp 184–186 °C; IR (5% in $CHCl_3$) 3526, 3493, 3345, 3273, 3212, 2997, 2975, 1631, 1592, 1509, 1482, 1421, 1250, 1228, 964 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 7.83 (s, 1 H), 5.92 (br s, 2 H), 4.27 (q, J = 7.3 Hz, 2 H), 1.55 (t, J = 7.3 Hz, 3 H); HRMS m/e calcd for $C_7H_9N_5$ 163.0858, found 163.0852.

9-Propyladenine (7b): yield 17%; mp 173–174 °C; IR (5% in $CHCl_3$) 3525, 3491, 3422, 3320, 3264, 3178, 2979, 2882, 1641, 1595, 1510, 1476, 1414, 1306, 1089, 1004, 902, 862, 651 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 7.80 (s, 1 H), 5.78 (s, 2 H), 4.17 (t, J = 7.1 Hz, 2 H), 1.98–1.90 (m, 2 H), 0.98 (t, J = 7.2 Hz, 3 H); HRMS m/e calcd for $C_8H_{11}N_5$ 177.1014, found 177.1006.

(39) Carraway, K. L.; Huang, P. C.; Scott, T. G. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; Vol. 1, pp 3–5.

9-Butyladenine (7c): yield 13%; mp 130–132 °C; IR (5% in $CHCl_3$) 3530, 3486, 3420, 3178, 2974, 2880, 1641, 1597, 1421, 1360, 1305, 1250, 1200, 1096, 1008, 964, 909, 864, 655 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 7.80 (s, 1 H), 5.90 (br s, 2 H), 4.20 (t, J = 7.2 Hz, 2 H), 1.94–1.84 (m, 2 H), 1.44–1.32 (m, 2 H), 0.97 (t, J = 7.4 Hz, 3 H); HRMS m/e calcd for $C_9H_{13}N_5$ 191.1171, found 191.1174.

9-Pentyladenine (7d): yield 47%; mp 125–127 °C; IR (5% in $CHCl_3$) 3526, 3486, 3411, 3325, 3262, 3175, 2969, 2940, 2865, 1635, 1594, 1513, 1479, 1422, 1359, 1330, 1313, 1250, 1112, 1094, 1008, 859, 652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 7.80 (s, 1 H), 5.82 (br s, 2 H), 4.20 (t, J = 7.2 Hz, 2 H), 1.97–1.88 (m, 2 H), 1.40–1.30 (m, 4 H), 0.90 (t, J = 6.8 Hz, 3 H); HRMS m/e calcd for $C_{10}H_{15}N_5$ 205.1327, found 205.1339.

Representative Procedure for the Preparation of N^6-Acetyl-9-alkyladenines 3. A 250-mL, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a glass stopper, a rubber septum, and a magnetic stirring bar was charged with 3.39 mmol of the appropriate 9-alkyladenine **7**, 80 mL of CH_2Cl_2, and pyridine (0.301 mL, 3.72 mmol). Acetyl chloride (0.265 mL, 3.73 mmol) was added by syringe over 2 min. The resulting solution was stirred at room temperature for 2 h and then extracted with 20 mL of saturated $NaHCO_3$ solution. The aqueous phase was separated and extracted with 20 mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield a yellow oil. Column chromatography on silica gel (elution with 5% CH_3OH in $CHCl_3$) afforded acetylalkyladenine **3** as a white solid.

N^6-Acetyl-9-ethyladenine (3a): yield 16%; mp 122–123 °C; IR (5% in $CHCl_3$) 3411, 3377, 3271, 3199, 3126, 3004, 2881, 1728, 1704, 1609, 1587, 1526, 1470, 1403, 1375, 1353, 1303, 1163, 1091, 1041, 1013, 962 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.76 (br s, 1 H), 8.70 (s, 1 H), 8.02 (s, 1 H), 4.33 (q, J = 7.3 Hz, 2 H), 2.64 (s, 3 H), 1.58 (t, J = 7.3 Hz, 3 H); HRMS m/e calcd for $C_9H_{11}N_5O$ $[M + H]^+$ 206.1042, found 206.1024.

N^6-Acetyl-9-propyladenine (3b): yield 30%; mp 122–123 °C; IR (5% in $CHCl_3$) 3411, 3373, 3276, 3243, 3200, 3129, 3005, 2978, 2945, 2886, 1727, 1705, 1613, 1597, 1526, 1467, 1407, 1380, 1353, 1304, 1104, 1050, 866 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.23 (br s, 1 H), 8.71 (s, 1 H), 8.06 (s, 1 H), 4.24 (t, J = 7.2 Hz, 2 H), 2.64 (s, 3 H), 2.03–1.90 (m, 2 H), 0.98 (t, J = 7.4 Hz, 3 H); HRMS m/e calcd for $C_{10}H_{13}N_5O$ $[M + H]^+$ 220.1198, found 220.1199. Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.94. Found: C, 54.83; H, 5.74; N, 31.68.

N^6-Acetyl-9-butyladenine (3c): yield 34%; mp 84–84.5 °C; IR (5% in $CHCl_3$) 3409, 3381, 3277, 3200, 3128, 3007, 2968, 2880, 1729, 1702, 1613, 1586, 1465, 1404, 1377, 1349, 1300, 1233 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.73 (br s, 1 H), 8.70 (s, 1 H), 7.97 (s, 1 H), 4.27 (t, J = 7.3 Hz, 2 H), 2.63 (s, 3 H), 1.96–1.86 (m, 2 H), 1.45–1.32 (m, 2 H), 0.97 (t, J = 7.3 Hz, 3 H); HRMS m/e calcd for $C_{11}H_{15}N_5O$ $[M + H]^+$ 234.1355, found 234.1359. Anal. Calcd for $C_{11}H_{15}N_5O$: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.38; H, 6.20; N, 29.82.

N^6-Acetyl-9-pentyladenine (3d): yield 53%; mp 62.5–64 °C; IR (5% in $CHCl_3$) 3409, 3378, 3245, 3192, 3033, 2964, 2938, 1729, 1702, 1618, 1591, 1470, 1385, 1300, 1242, 1162, 1109, 1045, 1014 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.87 (br s, 1 H), 8.71 (s, 1 H), 8.00 (s, 1 H), 4.26 (t, J = 7.2 Hz, 2 H), 2.63 (s, 3 H), 1.97–1.88 (m, 2 H), 1.41–1.31 (m, 4 H), 0.90 (t, J = 6.8 Hz, 3 H); HRMS m/e calcd for $C_{12}H_{17}N_5O$ $[M + H]^+$ 248.1511, found 248.1511.

^1H NMR Studies in Aqueous Solution. Incorporation and binding studies were performed on a GN-500 (500 MHz) NMR spectrometer. A $1\bar{3}3\bar{1}$ pulse sequence provided ca. a 1000-fold suppression of the water peak and permitted facile monitoring of the thymine NH resonance.[18] Parameters were selected to achieve maximum excitation of the thymine NH resonance (ca. 10.65 ppm). Typically, 128 scans were collected, and the data was then Fourier transformed with a line-broadening factor of 1.0 to improve the signal-to-noise ratio. A mixture of 10% D_2O and 90% H_2O (v/v) was used as a solvent. The solution was acidified with acetic acid (1.0 mM, ca. pH 4) to reduce the rate of exchange of the thymine NH proton. All studies were performed at ambient temperature (20 ± 3 °C). H_2O or HOD was used as a reference (δ 4.650).

Incorporation of (Thyminylalkyl)ammonium Salts 1 into SDS Micelles. A 500-μL portion of a solution of 1.00 mM (thyminylalkyl)ammonium salt **1** and 1.00 mM AcOH in 10% D_2O/H_2O was placed in a 5-mm NMR tube, and the ^1H NMR spectrum was recorded at 500 MHz using a $1\bar{3}3\bar{1}$ pulse sequence.[18] Seventeen 1.8-μL aliquots of a 0.80 M solution of sodium dodecyl sulfate in 10% D_2O/H_2O were added to the NMR tube, and the spectrum was recorded after the addition of each aliquot.

^1H NMR Titrations (Representative Procedure).[30] A 500-μL portion of a solution of 1.00 mM (thyminylalkyl)ammonium salt **1**, 1.00 mM AcOH, and 30.0 mM sodium dodecyl sulfate in 10% D_2O/H_2O was placed in a 5-mm NMR tube, and the ^1H NMR spectrum was recorded at 500 MHz using a $1\bar{3}3\bar{1}$ pulse sequence.[18] Aliquots of a solution of acetylalkyladenine **3** (200 mM **3a**, **3b**, or **3c** or 153 mM **3d**[40]) in a solution of 30.0 mM sodium dodecyl sulfate in 10% D_2O/H_2O were added to the NMR tube, and the spectrum was recorded after the addition of each aliquot. Typically, 20–25 aliquots of acetylalkyladenine **3** were added as follows: 10 10-μL aliquots, 10 20-μL aliquots, and 5 40-μL aliquots.

The titration data were analyzed by nonlinear least-squares fitting of the data to the 1:1 binding isotherm: $\delta_{obs} = \delta_1 + (\delta_{1\cdot3} - \delta_1)(([\mathbf{1}]_{tot} + [\mathbf{3}]_{tot} + 1/K_{obs}) - (([\mathbf{1}]_{tot} + [\mathbf{3}]_{tot} + 1/K_{obs})^2 - 4[\mathbf{1}]_{tot}[\mathbf{3}]_{tot})^{1/2})/(2[\mathbf{1}]_{tot})$, where δ_{obs} = observed chemical shift, δ_1 = chemical shift of the NH group of **1**, $\delta_{1\cdot3}$ = chemical shift of the NH group of **1** in the **1·3** complex, $[\mathbf{1}]_{tot}$ = the total concentration of **1** in solution, $[\mathbf{3}]_{tot}$ = the total concentration of **3** in solution, and K_{obs} = the equilibrium constant for formation of the **1·3** complex. The quantities δ_1, $\delta_{1\cdot3}$, and K_{obs} were allowed to vary during the fitting procedure, the quantity δ_{obs} was measured during the titration, and the quantities $[\mathbf{1}]_{tot}$ and $[\mathbf{3}]_{tot}$ were calculated on the basis of volumes and concentrations of the solutions used in the titration.

Job Plot.[31] Two stock solutions were prepared as follows. Solution A: 5.0 mM of **1a**, 1.0 mM of AcOH, and 20.0 mM of sodium dodecyl sulfate in 10% D_2O/H_2O. Solution B: 5.0 mM of **3d**, 1.0 mM of AcOH, and 20.0 mM of sodium dodecyl sulfate in 10% D_2O/H_2O. Ten 5-mm NMR tubes were filled with solutions A and B in the following volume ratios: 50:450, 100:400, 150:350, 200:300, 250:250, 300:200, 350:150, 400:100, 450:50, and 500:0 μL. ^1H NMR spectra were recorded at 500 MHz using a $1\bar{3}3\bar{1}$ pulse sequence.[18] The concentration of the **1a·3d** complex was estimated as follows: $[\mathbf{1a}\cdot\mathbf{3d}] = ([\mathbf{1a}]_{tot})(\delta_{obs} - \delta_{1a})/(\delta_{1a\cdot3d} - \delta_{1a})$, where $[\mathbf{1a}]_{tot}$ = the total concentration of **1a** in solution, δ_{obs} = observed chemical shift, δ_{1a} = chemical shift of the NH group of **1a**, and $\delta_{1a\cdot3d}$ = chemical shift of the NH group of **1a** in the **1a·3d** complex (estimated as 11.018 ppm on the basis of NMR titration data shown in Figure 5). The experiment was repeated in quadruplicate to ensure accuracy.

Octanol–Water Partition Coefficients. The octanol–water partition coefficients of acetylalkyladenines **3** were determined using the method of Hansch and co-workers.[37] In a typical experiment, a 10.0-mL portion of a 2×10^{-5} M solution of **3** in water-saturated octanol was shaken with 10.0 mL of octanol-saturated water in a 60-mL separatory funnel for 5–10 min. The octanol layer was separated and then centrifuged to remove suspended water. Before and after extraction, the concentration of acetylalkyladenine **3** in the octanol solution was determined spectrophotometrically (λ = 274 nm). The partition coefficient was calculated on the basis of the spectrophotometrically determined concentrations. Octanol–water partition coefficients were measured in quadruplicate to ensure accuracy.

Acknowledgment. We thank Dr. Joseph W. Ziller for obtaining the X-ray crystal structure of **3b**. Grant support for this work was provided by the following: the National Science Foundation (CHE-9118885); the Donors of The Petroleum Research Fund, administered by the American Chemical Society (ACS-PRF# 25240-G1); the University of California Cancer Research Coordinating Committee; and the UCI Committee on Research, J.S.N. thanks the following agencies for support in the form of awards: The Camille and Henry Dreyfus Foundation (New Faculty Award), The American Cancer Society (Junior Faculty Research Award), and The National Science Foundation (Young Investigator Award).

(40) The limited solubility of acetylpentyladenine **3d** necessitated the use of 153 mM solutions of this compound.