various monosaccharides and stabilizes various ammonium–anion salts by the induced-fit mechanism or what may be called *flexible* intramolecular polar microsolvation, in a similar manner as solvent water dissolves various polar solutes. This may also be why noncyclic host **5** works fairly well too. Thus, *versatility* is an important aspect here.[19]

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Registry No. 1, 63681-43-6; **2**, 138093-67-1; **3**, 138093-68-2; **4**, 13274-42-5; **5**, 138093-69-3; **6a**, 138093-70-6; **6b**, 138093-71-7; **10**, 1199-65-1; **11**, 3324-58-1; **12**, 130-40-5; **13**, 4578-31-8.

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Molecular Recognition in Aqueous Micellar Solution: Adenine–Thymine Base-Pairing in SDS Micelles

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Hydrogen bonding is a fundamental force in molecular recognition by biological macromolecules. It is central to nucleic acid base-pairing, yet does not occur significantly between individual nucleotides or nucleic acid bases in aqueous solution.[1] Model systems generally require noncompetitive organic solvents, such as $CDCl_3$, to achieve hydrogen bonding between uncharged receptors and substrates.[2,3] Here, we report that self-assembling molecular receptors, comprising (thyminyloctyl)ammonium groups in sodium dodecyl sulfate (SDS) micelles, bind adenine derivatives by means of hydrogen bonding in aqueous solution.[4]

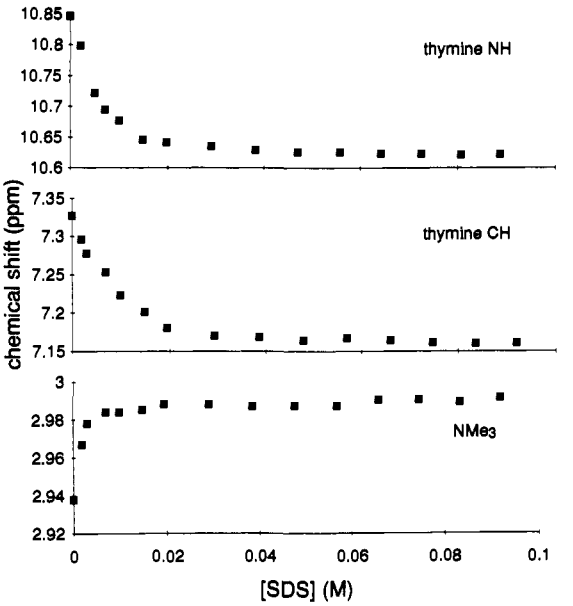
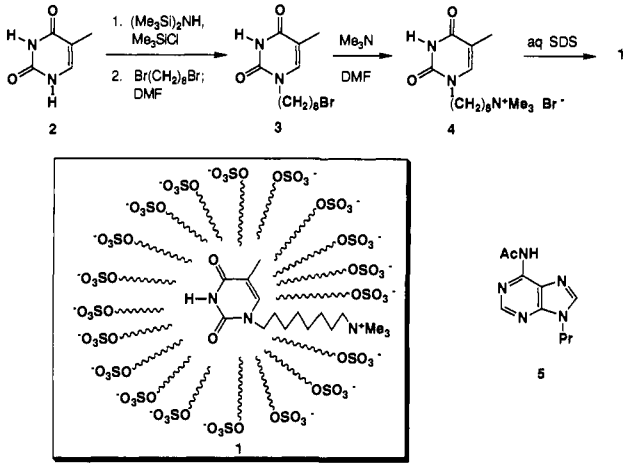


Figure 1. Effect of SDS concentration on chemical shift of protons of thymine **4**. Titrations were performed on a 300-MHz NMR instrument at 22 ± 1 °C by addition of 1 M SDS solution to a 1.0 mM solution of **4** in D_2O (CH protons) or 10% H_2O/D_2O (NH proton, 1.0 mM HOAc added). HOD or H_2O was used as a reference (δ 4.65).

Scheme I



The receptors (represented by structure **1**) were prepared from thymine as shown in Scheme I.[3b,5] ^1H NMR studies indicate that ammonium salt **4**, which is complementary in charge and structure to SDS, readily incorporates in SDS micelles (Figure 1). Increasing the SDS concentration from 0 to 20 mM results in large changes in the spectrum of **4**, suggesting that the environment of **4** changes drastically as the SDS forms micelles (CMC = 8.2 mM).[6] Incorporation is complete above 20 mM SDS. On the

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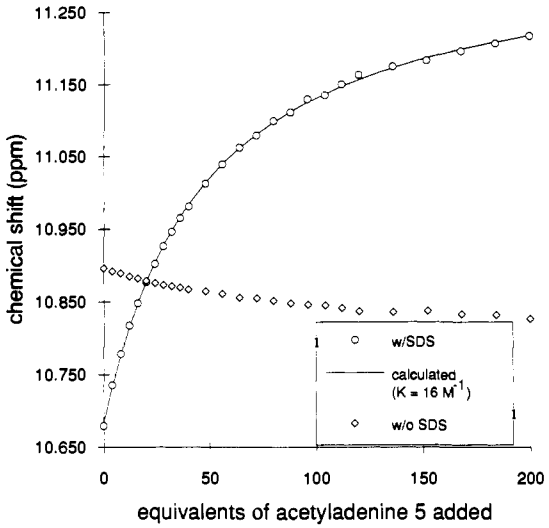

Figure 2. Chemical shift of thymine **4** NH vs number of equivalents of added adenine **5** in the presence (○) and absence (◇) of 20.0 mM SDS. Line corresponds to the best-fit curve calculated for a 1:1 binding isotherm (K = 16.09 M^{-1}, $\delta_{unbound}$ = 10.682 ppm, δ_{bound} = 11.552 ppm). Titrations were performed on a 500-MHz NMR instrument at 22 ± 1 °C. H_2O was used as a reference (δ 4.65).

basis of a reported mean aggregation number of 60 for SDS,[7] the receptor should comprise about 3 molecules of **4** among 60 molecules of SDS at 20 mM SDS and 1.0 mM **4**. In the absence of SDS, ammonium salt **4** does not significantly self-associate in aqueous solution, exhibiting variations in chemical shift of less than 0.01 ppm over a concentration range of 0.5–25 mM.

Receptor **1** binds adenine derivatives in aqueous solution. Binding studies were performed by ^{1}H NMR titration of a solution of 1.0 mM **4**, 20.0 mM SDS, and 1.0 mM AcOH (to reduce the rate of exchange of the thymine NH) in 10% D_2O/H_2O with a solution of 200 mM acetyladenine **5** and 20.0 mM SDS in 10% D_2O/H_2O. A $1\bar{3}\bar{1}$ pulse sequence[8] provided water-suppression and permitted monitoring of the thymine NH resonance.[9] Substantial downfield shifts of the NH resonance occur upon addition of **5** (Figure 2) and are consistent with adenine–thymine base-pairing.[10] In the absence of SDS, smaller upfield shifts occur, indicating that aromatic-stacking interactions predominate.[1,11] Analysis of the micelle data affords an excellent fit to a 1:1 binding model for the association of **4** and **5** and reveals an association constant of 16 M^{-1}.[12,13]

Our data support a model in which the micelles exclude bulk water from the hydrogen-bonding surface of the thymine group, thus providing a microenvironment suitable for binding.[4] The binding constant in aqueous SDS (16 M^{-1}) is smaller than that of **3** and **5** in $CDCl_3$ (37 M^{-1}),[12] suggesting that the thymine group resides in an environment comparable to a polar organic solvent.[14] ^{1}H NMR studies suggest that concentration of **5** inside the micelles may also contribute to binding.[15] Thus, addition of 20 mM SDS to a solution of **5** in 10% D_2O/H_2O results in small (≤0.02 ppm) shifts in the ^{1}H NMR spectrum of **5**.

In summary, we have found that base-pairing of simple adenine and thymine derivatives occurs in micelles. We anticipate that the incorporation of hydrogen-bonding groups into micelles will prove a general strategy for the design of aqueous molecular receptors.

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(11) Analysis of this limited range of data affords an association constant of 4.6 M^{-1}.
(12) Association constants were determined by a nonlinear least-squares fitting of NMR titration data to a 1:1 binding isotherm. Values of K, $\delta_{unbound}$, and δ_{bound} were allowed to vary during the fitting procedure.
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Magnetic Circular Dichroism Spectroscopic Definition of the Intermediate Produced in the Reduction of Dioxygen to Water by Native Laccase

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Laccase, a multicopper oxidase, catalyzes the irreversible 4-electron reduction of dioxygen to water. The enzyme contains a blue (type 1, T1) copper and a trinuclear copper cluster comprised of a normal (type 2, T2) copper and a binuclear (type 3, T3) copper center.[1] Intermediates in the reaction of reduced enzyme with dioxygen have been detected in the native enzyme[2] and in a derivative, T1Hg,[3] where the T1 copper is replaced with redox-inactive Hg^{2+}. The intermediate in T1Hg has been shown to be a 2-electron peroxide intermediate, with the T3 oxidized and T2 reduced.[4] Studies of the intermediate in native laccase have led to proposals that this intermediate is a 3-electron reduced oxygen radical.[2] Evidence for this includes the rapid reappearance of absorption features at 614 and 330 nm, associated with oxidized T1 and T3, respectively, and lack of a T2 EPR signal.[2b] In addition, an EPR signal, attributed to the intermediate, is observed at helium temperature which exhibits a low g value and fast relaxation.[5] ^{17}O line broadening of this signal indicates the direct involvement of oxygen.[5] To elucidate this intermediate's structure we have employed magnetic circular dichroism (MCD) spectroscopy to probe its electronic properties. The appearance of intense MCD C-terms at 364 and 318 nm provides definitive evidence for the intermediate having significant Cu(II) character. In addition, the T3 site, diamagnetic in the resting enzyme due to antiferromagnetic coupling, is paramagnetic in the intermediate. An alternative description is presented for the electronic structure of this intermediate based on the MCD data. MCD spectroscopy[6] is found to be a powerful probe of paramagnetic intermediates in reaction mixtures.

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