A New, Aqueous ^{1}H NMR Shift Reagent Based on Host−Guest Molecular Recognition Principles for Organic Compound Structural Analysis: Non-covalent $\pi-\pi$ and Hydrophobic Interactions Using a Supramolecular Host, $[Cp^*Rh(2'\text{-deoxyadenosine})]_3(OTf)_3$

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We have discovered that the supramolecular host $[Cp^*Rh(2'\text{-deoxyadenosine})]_3(OTf)_3$ (**1**, $Cp^* = \eta^5\text{-}C_5Me_5$, $OTf = CF_3SO_3^-$) has utility as a new, aqueous ^{1}H NMR shift reagent, via a host−guest molecular recognition process that occurs by non-covalent $\pi-\pi$ and hydrophobic interactions, with a wide variety of H_2O-soluble organic substrates. These organic compound guests that we present, to illustrate the utility of host **1** as a novel, aqueous ^{1}H NMR shift reagent, encompass examples such as aromatic carboxylic acids, phenylacetic acid (**G1**), 1-naphthoic acid (**G2**), and 2-naphthoic acid (**G3**), an aliphatic carboxylic acid, cyclohexylacetic acid (**G4**), as well as biological compounds, a di- and a tetrapeptide containing terminal L-tryptophan (Trp) or L-phenylalanine (Phe) groups, L-Trp-L-Phe (**G5**) and L-Trp-L-Met-L-Asp-L-Phe amide (**G6**) in the pH range 5−10. A discussion of the molecular recognition parameters that effect the ^{1}H NMR shifts of the organic guests and a comparison with the water-soluble lanthanide shift reagents (LSRs) will be presented to demonstrate the usefulness of this aqueous molecular receptor as an aid for organic compound structural analysis.

Introduction

The pioneering studies of Reuben et al.,[2], Horrocks et al.,[3] and others[4−6] on aqueous NMR lanthanide shift reagents (LSRs) clearly showed the utility of these paramagnetic metal complexes (for example, Eu^{3+}, Pr^{3+}, and Yb^{3+} complexes) over a wide pH range for structural analysis. These LSRs work on a mechanism whereby the Lewis-acidic metal ions preferentially bind to a heteroatom (Lewis-basic sites), providing a contact or pseudocontact shift of the protons in proximity of the magnetic anisotropy of the LSRs. However, the resolution of the NMR signals can be poor sometimes, since line-broadening can occur as the concentration of the LSRs is increased, and other problems are involved in using these LSRs, such as the presence of higher than 1:1 complexes, contact shifts, hydrolytic stability, and nonaxial symmetry.[3] Added to this factor, a considerable amount of substrate is needed for the LSRs technique, since they are normally used as very dilute solutions to prevent the line-broadening phenomena mentioned above.

Therefore, it was of interest to provide an alternative concept for mechanisms associated with aqueous NMR shift reagents for organic compound structural analysis. The molecular recognition concept, using aqueous supramolecular hosts, such as cyclodextrins,[7] cyclophanes,[8] and calixarenes,[9] has been concerned with the binding of relatively hydrophobic molecules as guests, including those of a biological origin such as amino acids,[10] steroids,[11] and nucleotides.[12]

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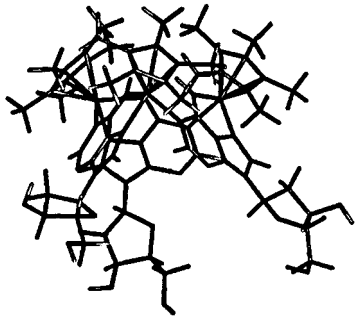
These host−guest complexes generally engender upfield chemical shifts for guest protons that are inside the host via non-covalent interactions. Moreover, Aoyama et al. introduced calix[4]resorarene derivatives as aqueous, chiral NMR shift reagents for aromatic guests.[9a] In that paper, no mention of an optimal pH range was reported nor were aliphatic guests or biologically important molecules a focus, while the association constants (K_a) for the aromatic guests were relatively small.

More recently, we reported on molecular recognition studies of aromatic and aliphatic amino acids, as well as aromatic and aliphatic carboxylic acid guests with a bioorganometallic, supramolecular host, [Cp*Rh(2′-deoxyadenosine)]$_3$(OTf)$_3$ (**1**, Cp* = η^5-C_5Me_5, OTf = $CF_3SO_3^-$), in aqueous solution at pH 7.2.[13] More importantly, in those studies, the non-covalent hydrophobic interactions were found to be fully operational in aqueous solution by solvophobic forces that enhanced host−guest complexation, along with the equally important non-covalent $\pi-\pi$ interactions.

During the course of carrying out the above-mentioned studies, we found that host **1** might be useful as a water-soluble ^{1}H NMR shift reagent for guest compounds, since the recognition process resulted in the diamagnetic anisotropic upfield shifts of those protons of the guest compounds located inside the molecular receptor; the extent of the selective shifts depended upon the orientation of the protons on the guest molecules in proximity to the inner adenine shell.



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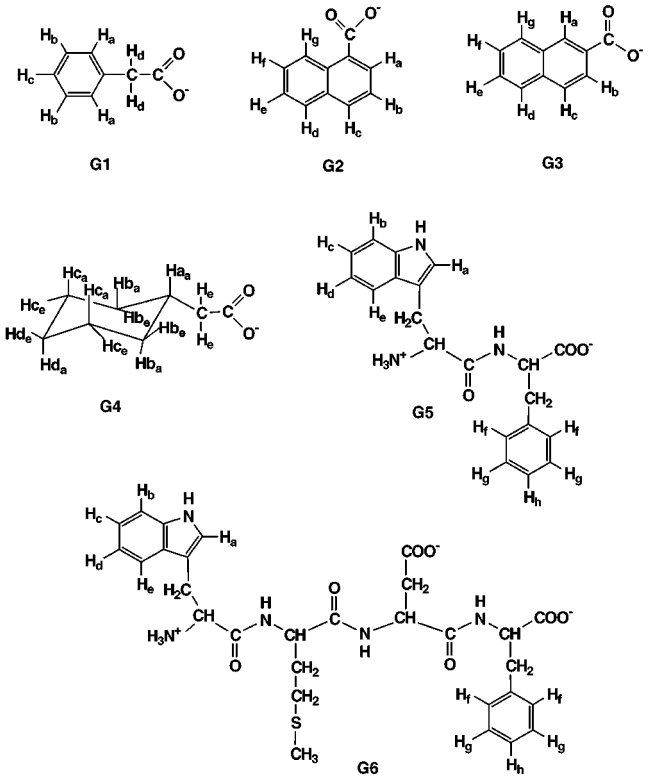
It was intriguing to extend these above-mentioned aqueous molecular recognition studies with host **1** to its utility as an aqueous NMR shift reagent, since **1** should be extremely useful for the simplification of complex NMR spectra of H_2O-soluble guest substrates in a pH range of 5−10. In this paper, we will discuss the new H_2O-soluble shift reagent, **1**, in the ^{1}H NMR spectra of guests that encompass aromatic and aliphatic carboxylic acids, **G1**, **G2**, **G3**, and **G4**, as well as peptides containing terminal L-tryptophan (Trp) or L-phenylalanine (Phe) groups, L-Trp-L-Phe (**G5**) and L-Trp-L-Met-L-Asp-L-Phe amide (**G6**). The host−guest chemistry that allows first-order ^{1}H NMR spectra for representative guests, **G1**−**G6** (Chart 1), occurred via non-covalent $\pi-\pi$ and hydrophobic interactions.[13]

Chart 1. Guests



Results and Discussion

Description of Supramolecular Host 1. From the X-ray crystal structure of an analogue, [Cp*Rh(9-methyladenine)]$_3$(OTf)$_3$, **2**, host **1**, as demonstrated in the Dreiding model above, has a triangular domelike cavity, with three Cp* groups stretching out from the top of the dome, three 2′-deoxyribose groups pointing to the bottom, three adenine planes forming the surrounding shell, and three Rh atoms embedded on the top of the dome.[14] The distance between the adjacent ribose groups at the bottom of the dome is ∼7.5 Å, with a depth of this molecular receptor being ∼4 Å. We had found earlier that this particular analogue, host **1**, was the most effective structure for the molecular recognition of a variety of guests in aqueous solution.[13] As stated, we realized that host **1** had all the attributes for an aqueous shift reagent; that is, it can be readily synthesized in 95% yield, it has high solubility in water (870 mg/mL at 23 °C), it is exceptionally stable for weeks in aqueous solution in a pH range of 5−10, and it causes appreciable upfield shifts of protons in proximity to the inner shell of this molecular receptor.[13] The ^{1}H NMR spectrum for host **1** is provided to show the areas of the aromatic and aliphatic regions that are not encumbered by the signals of **1** (Chart 2).

Clearly, Chart 2 shows that the aromatic proton region, from 6.0 to 8.5 ppm, has only the 2′-deoxyribose H1′ proton and the adenine H2 proton as possible interference signals, but the concentrations of host **1** that are needed to provide the upfield shifts for a first order spectrum were found to be much less than the guest concentrations used. In addition, the aliphatic region from 0.5 to 2.2 ppm has the Cp* signal as the only

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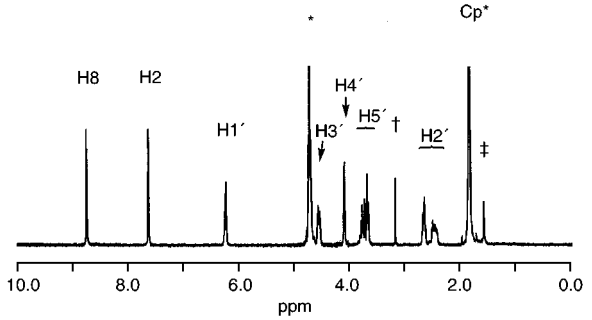
Chart 2. 500 MHz ^{1}H NMR Spectrum of Host 1: (*) Water, (†) NMe_4OH as an Internal Reference, (‡) $[Cp^*Rh(H_2O)_3](OTf)_2$



interfering ^{1}H NMR signal. With these minor interfering ^{1}H NMR signals documented, we will now demonstrate the utility of host **1** as an aqueous shift reagent with six guest substrates. It is also important to note that $(CH_3)_4NOH{\cdot}5H_2O$ in D_2O was used as the internal reference with the methyl proton resonance set at 3.180 ppm, since the most common commercially available H_2O-soluble internal references, 2,2-dimethyl-2-silapentane-5-sulfonate, sodium salt (DSS) or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP), which are considerably more hydrophobic than $(CH_3)_4NOH{\cdot}5H_2O$, act as guests with host **1**, and thus, the signal for the methyl protons of DSS or TSP are slightly shifted upfield.

Aromatic Carboxylic Guests with Aqueous Shift Reagent 1. The first guest we studied, **G1**, provided indications of the usefulness of host **1** as a novel, aqueous shift reagent at pH 7.0. As we increased the concentration of host **1** from 0.0 to 0.8 equiv, the aromatic protons, **a**−**c** (Chart 1), are shifted upfield 0.1 (7.19 ppm), 0.33 (7.05 ppm), and 0.49 ppm (6.8 ppm), respectively, rendering a first-order NMR spectrum for **G1** (the H_d protons at 3.49 ppm were not affected by host **1**). The estimated association constant (K_a) for the host−guest complex, **G1**−host **1**, was found to be 710 M^{-1} by an NMR method (this K_a value allowed a calculated free energy of complexation, $\Delta G° = -3.9$ kcal/mol), and the molecular recognition process, in this case, is dominated by noncovalent $\pi-\pi$ interactions for this aromatic substrate.

The next aromatic carboxylic guests we studied were the 1- and 2-naphthoic acids, **G2** and **G3**. In these two examples, we observed that their 500/270 MHz ^{1}H NMR spectra were not first-order (Figure 1A and 2A). In addition, the orientation of the 1-substituted naphthoic acid in host **1** is decidedly different than the 2-substituted isomer via the complexation-induced chemical shifts (CICS) and reflects the fact that the hydrophilic carboxylate groups are in the water phase, not in the molecular receptor. For example, the **G2** protons, **b**−**f**, are shifted an average 0.44 ppm upfield, with proton **e** the most upfield shifted (0.61 ppm), while with **G3**, protons **d**−**g**, have an average upfield shift of 0.57 ppm, with protons **e** and **f** the most upfield shifted at 0.78 and 0.67 ppm, respectively. The estimated association constants, K_a, for **G2** and **G3** demonstrate that the 2-isomer had a slightly larger value of 1040 M^{-1} ($\Delta G° = -5.7$ kcal/mol) versus 862 M^{-1} ($\Delta G° = -4.7$ kcal/mol) for the 1-isomer and possibly reflect the steric consequences of each positional isomer ($\Delta(\Delta G°) = \sim 1.0$ kcal/mol). We have also conducted 2D NMR (H−H COSY) experiments to unequivocally assign the proton chemical shifts of all guest as demonstrated with **G2** (Figure 3). *It is interesting to note*

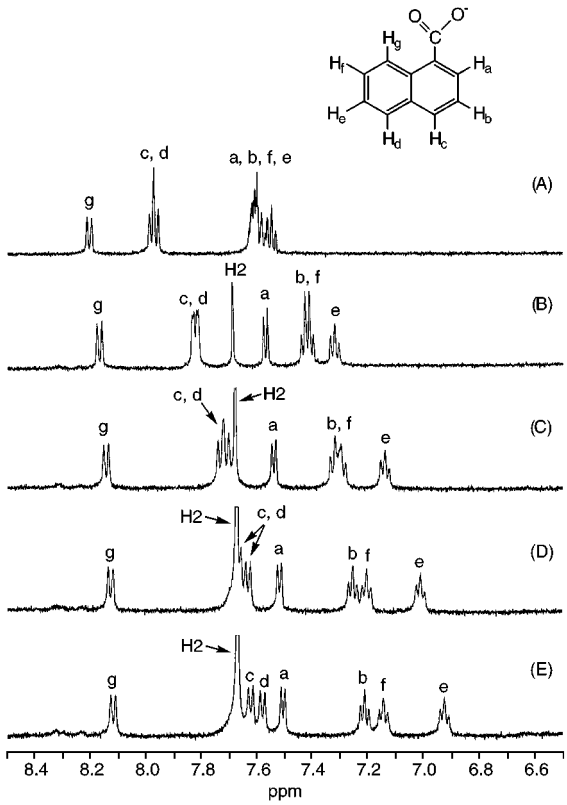


Figure 1. 500 MHz ^{1}H NMR spectra of **G2** with varying equivalents of **1**: (A) 0 equiv; (B) 0.25 equiv; (C) 0.5 equiv; (D) 0.75 equiv; (E) 1.0 equiv at pH 7.0.

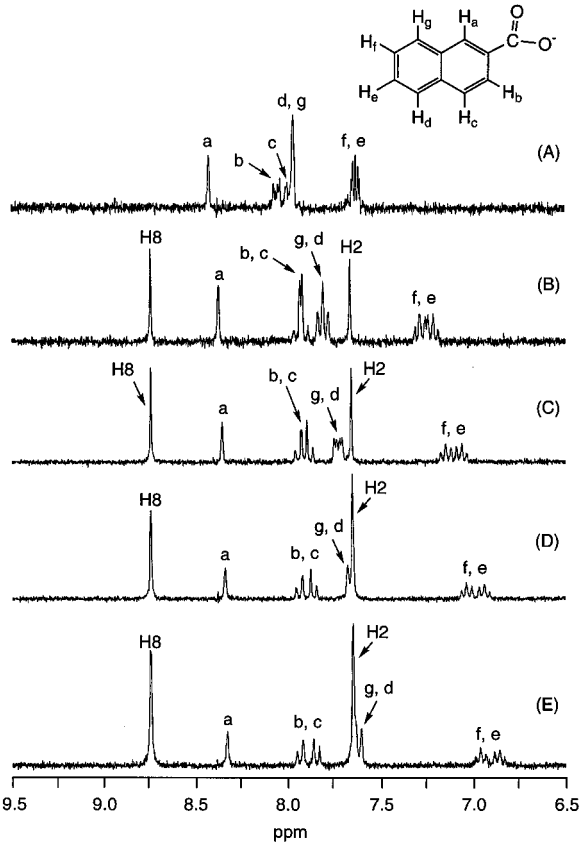


Figure 2. 270 MHz ^{1}H NMR spectra of **G3** with varying equivalents of **1**: (A) 0 equiv; (B) 0.25 equiv; (C) 0.5 equiv; (D) 0.75 equiv; (E) 1.0 equiv at pH 7.0.

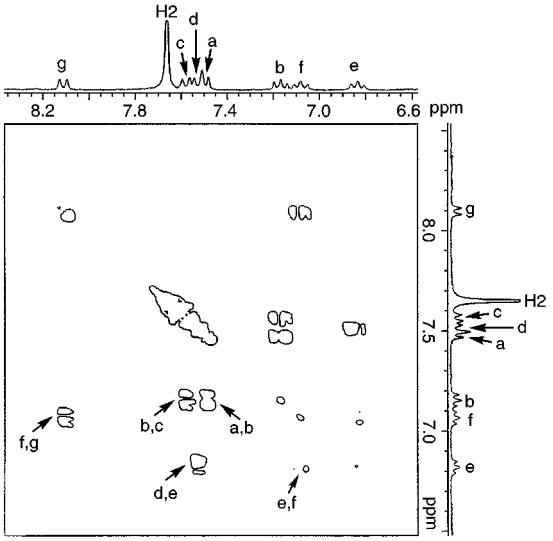
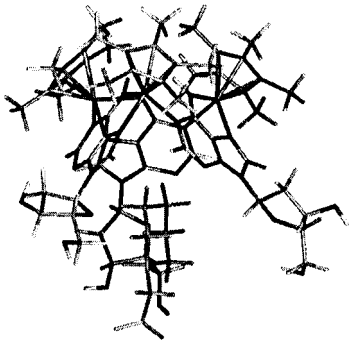


Figure 3. 270 MHz H−H COSY NMR spectrum of **G2** with 1.0 equiv of **1** at pH 7.0.

*that for **G2** all seven aromatic protons were separated and it further demonstates how the aromatic rings are orientated in the molecular receptor so as to not include the hydrophilic carboxylate group.*

An Aliphatic Carboxylic Acid Guest with Aqueous Shift Reagent 1. The aliphatic guest **G4** was used as an example to demonstrate that axial and equatorial protons on nonfluxional cyclohexane rings can be easily identified via their coupling constants ($H_{ax}-H_{ax}$ coupling $\gg H_{ax}-H_{eq}$ coupling) and separated on the basis of the orientation of the H_c and H_d axial and equatorial protons in host **1**. The ^{1}H NMR spectrum showed the dramatic upfield shifts of $H_{c(eq)}$ and $H_{d(eq)}$ protons (0.3 (1.46 ppm) and 0.53 (1.30 ppm), respectively), which then allows separation of all axial and equatorial protons, except $H_{a(ax)}$ from $H_{b(eq)}$ protons, to occur. The largest upfield shift was found for $H_{d(eq)}$ (0.53 ppm, see Chart 1 for the proton designations), reflecting its prominent role in the host−guest process; that is, it is deeply embedded in receptor **1** and experiences the maximum diamagnetic anisotropic effect afforded by the adenine inner shell (host−guest **3**).



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One note on the possible limitation of host **1** as a shift reagent was found to occur with multiple cyclohexane ring systems such as a steroidal structure, as epitomized with deoxycholic acid. Unfortunately, we observed no upfield chemical shifts for the A ring of deoxycholic acid, and from previous molecular recognition studies, we speculate that this is a consequence of a severe steric effect that inhibits host−guest complexation.[13a]

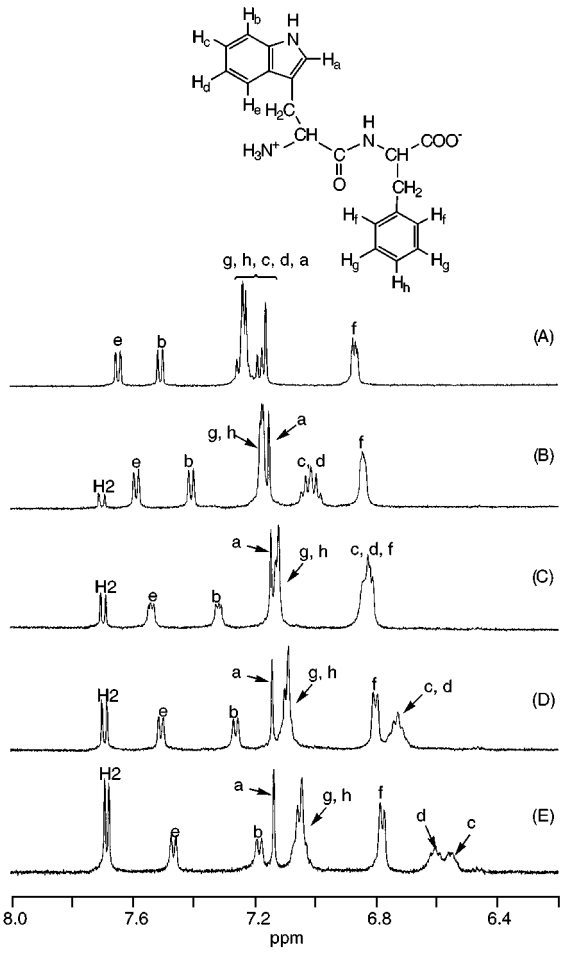
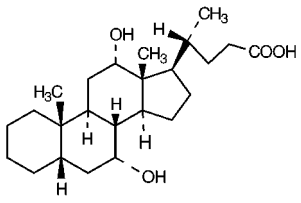


Figure 4. 500 MHz ^{1}H NMR spectra of **G5** with varying equivalents of **1**: (A) 0 equiv; (B) 0.1 equiv; (C) 0.2 equiv; (D) 0.3 equiv; (E) 0.4 equiv at pH 9.4.



deoxycholic acid

Biological Guests, Di- and Tetrapeptides with 1. Another focus of our aqueous shift reagent studies was the analysis of biologically relevant guests such as di- and tetrapeptides, as a function of pH. The examples we chose were the dipeptide L-Trp-L-Phe, **G5**, and the tetrapeptide L-Trp-L-Met-L-Asp-L-Phe amide, **G6**. We preface these results with a study on the competition between L-Trp and L-Phe for host **1**, since we wanted to ascertain the individual effect of each amino acid as it pertains to their ^{1}H NMR spectrum and, thus, the association constant, K_a, of the di- or tetrapeptide. We found that at pH 7.0 the K_a value of L-Trp (~607 M^{-1}) was very similar in the presence or absence of L-Phe (~456 M^{-1}) and vice versa; both compete effectively for host **1**. Figure 4 clearly shows that at 500 MHz and pH 9.4 (**G5** is not soluble in D_2O at pH 7.0; at pH 9.4 the

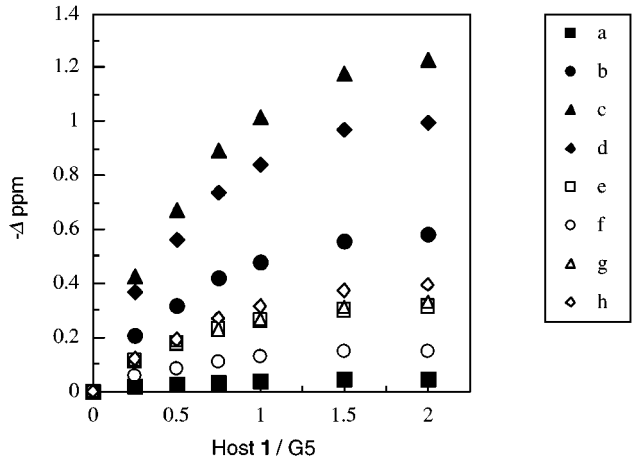


Figure 5. ^{1}H NMR titration for **G5** for aromatic protons **a**−**h** (see proton designation in Chart 1).

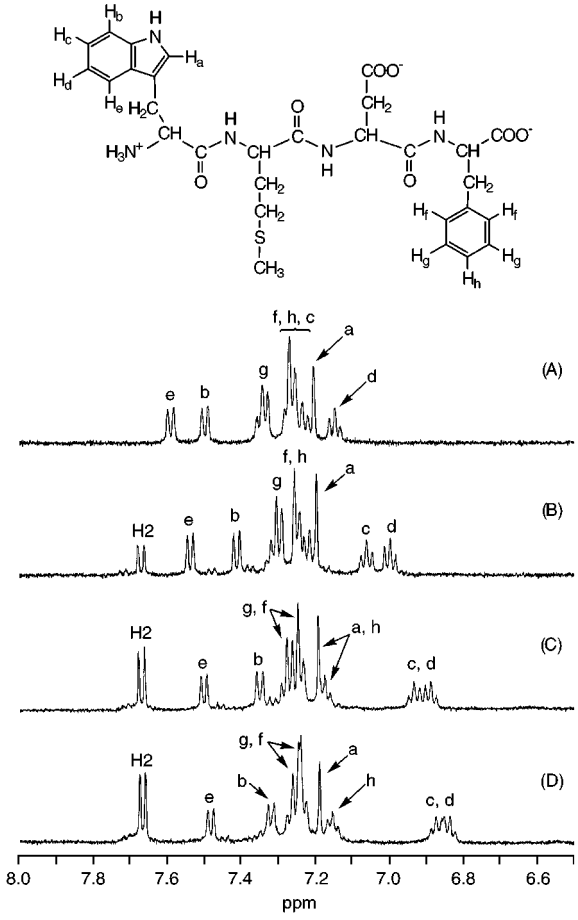


Figure 6. 500 ^{1}H NMR spectra of **G6** with varying equivalents of **1**: (A) 0 equiv; (B) 0.1 equiv; (C) 0.2 equiv; (D) 0.3 equiv at pH 9.4.

NH_3^+ group is deprotonated) the ^{1}H NMR spectrum of **G5** is far from first-order (spectrum A) with protons **a**, **c**, **d**, **h**, and **g** being a particular problem for complete structural analysis. As increasing amounts of host **1** are added, the spectrum is almost completely resolved; however, protons **g** and **h** on the L-Phe portion are still slightly overlapping.

An NMR titration experiment with varying ratios of **1**/**G5** is shown to clearly demonstrate the CICS values for the aromatic protons **a**−**h** (Figure 5). As can be seen, both the L-Trp protons, **a**−**e**, and the L-Phe protons, **f**−**h**, saturate at a 1:1 host−guest complex ratio (**1**/**G5** = 2), reflecting very similar K_a values for both amino acids with host **1**.[9]

Similar experiments (Figure 6) with the sleep-inducing tetrapeptide **G6** show resolution of protons **c**, **f**, and **h** (spectrum A) at pH 9.4 (**G6** is not soluble in D_2O at pH 7.0) upon increasing the concentration of host **1** and almost total identification of all 10 aromatic protons. Since host **1** has no effect on the aliphatic region (∼1−4.5 ppm) of **G6**, this can be readily resolved by ^{1}H NMR spectroscopy without the presence of **1**.

Conclusions

We believe that we have demonstrated that the molecular recognition process can be very effective in helping to elucidate complex organic compound structures utilizing ^{1}H NMR spectroscopy in conjunction with host **1**. In contrast to the aqueous lanthanide shift reagents, host **1** is not limited by line-broadening of protons at saturation concentrations nor by hydrolytic problems at high pH. Also, 1:1 host−guest complexes are readily formed with host **1** and various water-soluble guests (Chart 1). Moreover, with this molecular recognition process, the binding to various heteroatoms, e.g., −OH, −NH, and −SH, does not occur (the hydrophilic groups remain in the aqueous phase) and, therefore, provides a more global mechanism to influence the difficult structural assignments of overlapping protons of both aromatic and aliphatic guests; that is, more of the guest molecule experiences the magnetic anisotropic effect of the inner adenine receptor site. However, we must also point out that severe steric effects can limit this molecular recognition process as previously observed,[13a] and shown in this study with a steroidal guest, deoxycholic acid.

The comparison of host **1** to the calixarene bowls[9] as aqueous shift reagents relates to the very facile procedures for the preparation of various Cp*Rh cyclic trimer derivatives and their pronounced K_a values. The calixarene synthetic procedures are multistep, while host **1** or its derivatives are synthesized in one flask in aqueous solution in almost quantitative yields. More importantly, the K_a values for the reported calixarene−aromatic guest complexes are very small, which may possibly limit there future usefulness as aqueous shift reagents.

We have also shown that structurally complex and biologically important guests, i.e., peptides, amino acids, etc., can be readily analyzed by ^{1}H NMR spectroscopy from pH 5 to 10 without host degradation. The powerful nature of host **1** for organic structural analysis, via the molecular recognition process, is that both non-covalent $\pi-\pi$ and hydrophobic interactions are possible, while again, pointing out that severe steric effects can limit this process. Finally, we are hopeful that other workers will extend our aqueous shift reagent results with host **1** to various difficult-to-solve structural problems with a variety of water-soluble organic compounds of biological interest.

Experimental Section

Material and Methods. The synthetic procedure for $[Cp^*Rh(2'\text{-deoxyadenosine})]_3(OTf)_3$, **1**, was previously reported.[13a] All guest molecules, **G1**−**6** (highest purity available), were purchased from either Aldrich or Sigma Chemical Co. and used as received. The 400 MHz ^{1}H NMR spectra were recorded on a Bruker AM 400 spectrometer located in the Department of

Chemistry, University of California, Berkeley, CA. The 500 and 270 MHz ^{1}H NMR spectra were recorded on an JEOL JNM-LA 500 or a JNM-EX 270 spectrometer, respectively, located in the Institute for Molecular Science, Okazaki, Japan.

NMR Sample Preparation for Host−Guest Experiments. A $(CH_3)_4NOH\cdot 5H_2O$ solution (60 mM) in D_2O was used as the internal reference with the methyl proton resonance set at 3.180 ppm. A pH 7.0 buffer solution (10 mM) was prepared with $Na_2HPO_4\cdot 12H_2O$ (35.8 mg, 0.1 mmol), KH_2PO_4 (13.6 mg, 0.1 mmol), and D_2O (10 mL); a pH 9.4 buffer solution (20 mM), with $K_2B_4O_7\cdot 4H_2O$ (61.0 mg, 0.2 mmol) and D_2O (10 mL). A typical NMR sample preparation ([host **1**]:[guest **G1**−**6**] ratios = 1:1) is described as follows: Host **1** (20.6 mg, 0.01 mmol) and an appropriate amount of guest molecules (0.01 mmol), in a 5-mm NMR tube, were dissolved in 1.0 mL of D_2O. To this was added 50 μL of pH 7.0 or 9.4 buffer solution and 10 μL of the internal reference.

Association Constants and Free Energies of the Host−Guest Complexation. The association constants (K_a) of host−guest complexation were measured by using a standard NMR method,[15] while the free energies of the host−guest complexation, $\Delta G°$ ($\Delta G° = -RT \ln K_a$), were calculated using the K_a values determined by the above-mentioned NMR technique. Therefore, the association constants (K_a) for host−guest complexes (HG) in dilute solution ([host]:[guest] ratios are 1:10 to 30 in the presence of a constant concentration of the host) from ^{1}H NMR chemical shift data were determined as follows:

$$\text{host} + \text{guest} \rightleftharpoons \text{HG} \tag{1}$$

$$K_a = \frac{[HG]}{[H][G]} = \frac{[HG]}{([H]_0 - [HG])([G]_0 - [HG])} \tag{2}$$

where [H], [G], and [HG] are the concentration of the host, guest,and host−guest complex in the solution, respectively. $[H]_0$ and $[G]_0$ are the concentration of provided host and guest, respectively.

For the dilute solution in which $[G]_0 \gg [H]_0$,

$$[G]_0 - [HG] \approx [G]_0 \tag{3}$$

From eqs 2 and 3,

$$K_a = \frac{[HG]}{([H]_0 - [HG])[G]_0} \tag{4}$$

The relationship between the concentration and the chemical shift difference (Δ) of the guest in the dilute solution is:

$$\frac{[HG]}{[G]_0} = \frac{\Delta}{\Delta_{max}} \tag{5}$$

where Δ_{max} is the chemical shift difference for the guest in the pure host−guest complex solution (Δ_{max} is not measurable). From eqs 4 and 5 we obtain:

$$[G]_0 = \Delta_{max}[H]_0\frac{1}{\Delta} - \frac{1}{K_a} \tag{6}$$

Thus, the plots of $[G]_0$ vs $1/\Delta$ give K_a.

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