Conformation, hydrogen bonding and aggregate formation of guanosine 5'-monophosphate and guanosine in dimethylsulfoxide

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ABSTRACT

The tetrabutylammonium salt of guanosine 5'-monophosphate (5'-GMP) dissolves in DMSO- d_6 forming aggregated species which exhibit some properties of reverse micelles. ^1H NOESY experiments show that the 5'-GMP adopts the *syn* conformation about the glycosidic bond. Molecular mechanics calculations reveal a stable structure with this conformation in which the phosphate group and the amino group of the base are in close enough proximity to hydrogen bond. In contrast inosine 5'-monophosphate in DMSO- d_6 , which has no NH_2 group for hydrogen bond stabilization of the *syn* conformation, is shown by NMR to have the *anti* structure. Guanosine in DMSO- d_6 behaves differently from 5'-GMP. Guanosine adopts the *anti* conformation and forms a symmetric dimer via hydrogen bonding between the N3 and NH_2 of the bases.

INTRODUCTION

The solubilization of nucleotides in organic solvents is relevant to the transfer of nucleotidic drugs through cell membranes. This has been accomplished in model systems by using organic cations which can serve as transfer agents for anionic nucleotides, (1–3) assisted by site-specific hydrogen bonding in some cases (4). In this way mononucleotide mono- and triphosphates have been transported between two aqueous layers through a chlorocarbon layer.

A study of the properties of nucleotides in organic solvents permits the characterization of base–base hydrogen bonding without the complication of base stacking, which can lead to the formation of large aggregates. In previous studies of this type, bases or nucleosides have been used, frequently substituted with solubilizing organic groups (5–9). However, this does not allow for possible effects arising from the presence of the phosphate group.

Although the structure has not been definitively proven, the aggregates of the disodium salt of guanosine 5'-monophosphate,

$\text{Na}_2(5'\text{-GMP})$, I, which form in concentrated *aqueous* solution at pH 8, are believed to consist of cyclic tetramers (G-tetrad) of base-base hydrogen-bonded 5'-GMP units (10–16). Recently, a G-tetrad structure has been identified in the guanine-rich telomeric sequences of chromosomes (12,17–22). In these systems the G-tetrads form stacks held together by strong hydrophobic interactions and, in the latter case, also by the phosphodiester backbone.

In an attempt to form and characterize a single, unstacked, hydrogen-bonded species in this system, we have been investigating the solubility of several salts of 5'-GMP in polar organic solvents. We have found that the tetrabutylammonium salt of 5'-GMP, $(\text{TBA})_2(5'\text{-GMP})$, dissolves in dimethylsulfoxide to form aggregates with reverse micellar-like properties (23,24) in which the 5'-GMP has the *syn* conformation about the glycosidic linkage. However, we have not been able to

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determine the hydrogen-bonding scheme in this case. In aqueous solution, 5'-GMP has been reported to contain from 50% *anti* conformer to being 'predominantly' in the *anti* conformation (25–27). A 100% *syn* population in solution has only been observed when the C8 is substituted with a bulky group, such as Br (28,29) or 4-methylaniline (30). The *syn* conformation also occurs in guanosine residues in solutions of Z-DNA/Z-RNA (31–33) and in some telomeric G-tetrad structures (12,34). For comparative purposes, we have also investigated inosine 5'-monophosphate, 5'-IMP, **II**, which is the same as 5'-GMP except it lacks the NH₂ group at the 2 position of the base.

A new dimeric hydrogen-bonded structure has been observed for guanosine in DMSO.

MATERIALS AND METHODS

Materials

Guanosine 5'-monophosphate in the form of the free acid was purchased from Sigma Chemical Co. and used as received. Tetrabutylammonium hydroxide, TBAOH, (Aldrich) was in the form of a 40 % aqueous solution. DMSO-d₆ (99.9 atom %) and D₂O (99.9 atom %) were purchased from Aldrich.

(TBA)₂(5'-GMP) was prepared by titrating 1.0 g of H₂(5'-GMP)·H₂O dissolved in 50 ml H₂O with TBAOH to pH 7.9 using a Fisher Accumet 925 pH meter. The solid (TBA)₂(5'-GMP)·XH₂O, where X = 2–3, was isolated by lyophilization and transferred in a glove bag under an N₂ atmosphere to a storage vial. It is hygroscopic and tenaciously binds H₂O. For the concentration dependent ¹H NMR study, the solid was further dried under vacuum by raising the temperature from 60–90°C over a 4 h period. It appears to lose some H₂O at 70–95°C. The dried sample still contained some H₂O although it was not detectable as a water resonance in the proton NMR, or by a molecular weight determination using UV concentration measurements.

Methods

DMSO-d₆ and solutions of (TBA)₂(5'-GMP) in DMSO-d₆ were dried with 3A Molecular Sieves. Concentrations were determined on diluted aqueous solutions with a Perkin-Elmer Lambda 19 spectrometer at 252 nm ($\epsilon = 1.37 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$).

The NMR spectra were run on a General Electric QE 300 spectrometer equipped with variable temperature capability; 300.66 MHz for ¹H and 121.71 MHz for ³¹P. TMS was used as an internal reference for DMSO-d₆ solutions and DSS for D₂O solutions. The reference for ³¹P NMR was external conc. H₃PO₄. Line widths and chemical shifts in DMSO-d₆ solutions containing small amounts of added H₂O were determined using the CHRCAP program (spectral deconvolution) of the General Electric QE 300 CHARM software. ¹H NOESY spectra were obtained at mixing times of 125, 250, 300, and 400 ms with a data set size of 512×512 K, a 90° pulse width of 9.0 μ s, 16 acquisitions at each time interval, and a delay time of 2 s.

Molecular calculations were performed using the Tektronix CAChe Scientific system, Version 3.0. (PO Box 500, MS 13-400, Beaverton, OR 97077). The molecular mechanics calculations use an augmented version of Allinger's MM2 force field (35), whereby force field parameters are estimated for cases not explicitly addressed by MM2. The AM1 calculations (36) use the semi-empirical Hamiltonian developed by M.J.S.Dewar without modification. The only constraint placed on the AM1 calculations is the initial geometry and optimization to an energy

minimum or local minimum. Hydrogen bonding between the phosphate and the NH₂ is taken into account in the minimization for the *syn* conformation.

RESULTS AND DISCUSSION

Because it was impossible to remove all of the water of hydration from (TBA)₂(5'-GMP), either by vacuum drying of the solid or drying of the solutions, all of the DMSO solutions contained traces of H₂O. The amount was sufficiently small that no 'free' H₂O was observed in the form of a proton signal in the NMR. Its presence was deduced from the absence of signals from the exchangeable 5'-GMP protons in the NMR. The water is strongly

Figure 1. 300 MHz ¹H NMR (A) 36 mM (TBA)₂(5'-GMP) in DMSO-d₆, internal ref. TMS; (B) 38 mM (TBA)₂(5'-GMP) in D₂O, internal ref. DSS; (C) 40 mM guanosine in DMSO-d₆, internal ref. TMS. α , β , γ , δ refer to the carbons in the C₄H₉ groups in TBA, with α adjacent to the N.

interacting with the solute and must be considered a part of the system; the behavior and properties of the $(\text{TBA})_2(5'\text{-GMP})$ in DMSO are contingent upon the presence of this water.

NMR studies

The ^1H NMR spectra of 36–40 mM solutions of $(\text{TBA})_2(5'\text{-GMP})$ in DMSO-d_6 and in D_2O are very different (Fig. 1 and Table 1). All resonances in DMSO-d_6 are shifted upfield as a result of its decreased degree of hydrogen bonding and dielectric effect compared to that of D_2O . In DMSO-d_6 , the N7, N3, C=O, O4', and phosphate oxygens cannot hydrogen bond to solvent as they can in D_2O . In both solvents the ribose OH protons and the N(1)H protons are not observed in the spectrum. In D_2O this is the result of exchange of the protons with deuterium and in DMSO-d_6 the protons are exchanging with the H_2O and interacting so strongly that the averaged signal is broadened into the baseline. The NH_2 protons in DMSO-d_6 are in intermediate exchange with the trace H_2O and are observed as a somewhat broadened signal. The absence of the N(1)H resonance line precludes our drawing definitive conclusions about the presence and nature of base–base hydrogen bonding in DMSO under these conditions. However, a downfield shift of the NH_2 protons with increasing 5'-GMP concentration indicates the occurrence of intermolecular association via NH_2 hydrogen bonding.

The ^1H NMR spectra of 40 mM solutions of $(\text{TBA})_2(5'\text{-GMP})$ and guanosine in DMSO-d_6 are also quite different, although it is expected that they should be quite similar (Fig. 1). For one thing, all of the protons in the guanosine molecule, including the exchangeable ones, are observed, even though this solution also contains H_2O as shown by the resonance at 3.33 ppm. The guanosine assignments are based on the literature (37). The line widths in guanosine are narrow and the multiplets are well resolved. In contrast, in $(\text{TBA})_2(5'\text{-GMP})$ at concentrations above 40 mM all the resonances are broadened to such an extent that none of the multiplets are resolved. The TBA^+ ion lines are also broadened to some extent. The broadening is thought to be due to intermolecular aggregation of the 5'-GMP because the lines narrow and the multiplets become partially resolved as the concentration is decreased to 5 mM.

Some of the protons of guanosine exhibit significant chemical shift differences from those of $(\text{TBA})_2(5'\text{-GMP})$ in DMSO-d_6 (Table 1). The largest difference occurs for the H8 proton. It is located at 7.93 ppm in guanosine and is shifted 0.29 ppm upfield to 7.65 ppm in $(\text{TBA})_2(5'\text{-GMP})$ and the direction of the

shift is unusual. Typically, as in D_2O solution, the H8 in 5'-GMP is *downfield* from that of guanosine; for example $\Delta\delta = 0.126$ for 5'-GMP–1-methylguanosine (38). The downfield shift is ascribed to deshielding of the H8 proton through space by the phosphate group when 5'-GMP has an *anti* conformation with respect to the glycosidic bond (38). The H5',5' and H3' protons have the next largest chemical shift differences between the two molecules. The protons most affected, the relative magnitude of the $\Delta\delta$ values, and the direction of the chemical shift changes are consistent with, but not proof of, a *syn* conformation about the glycosidic torsion angle in $(\text{TBA})_2(5'\text{-GMP})$ and an *anti* conformation in guanosine.

^1H NOESY data provide strong evidence for a *syn* conformation in a $(\text{TBA})_2(5'\text{-GMP})$ solution in DMSO-d_6 . The 2-D NOESY of a 60 mM solution at $t_{\text{mix}} = 300$ ms (Fig. 2a) reveals off-diagonal peaks between the H8 with both the H1' and H2'. Increasing t_{mix} to 400 ms shows additional H8 interactions with the H3',H4' and H5',5' protons in order of decreasing intensity, indicating that these off-diagonal peaks are the result

Figure 2. ^1H NOESY of 60 mM $(\text{TBA})_2(5'\text{-GMP})$ in DMSO-d_6 ; (a) $t_{\text{mix}} = 250$ ms; (b) a slice through the NOESY H8 cross peaks.

Table 1. ^1H Chemical shifts

	40 mM $(\text{TBA})_2(5'\text{-GMP})$ in D_2O	36 mM $(\text{TBA})_2(5'\text{-GMP})$ in DMSO-d_6	40 mM GUANOSINE in DMSO-d_6	40 mM $(\text{TBA})_2(5'\text{-IMP})$ in DMSO-d_6
N(1)H			10.634 ppm	
H8	8.197 ppm	7.646 ppm	7.933	7.887 ppm
H2				7.792
NH_2		6.676	6.456	
H1'	5.908	5.659	5.693	5.767
OH2'			5.387	
OH3'			5.115	
OH5'			5.035	
H2'	4.758	4.388	4.392	4.577
H3'	4.484	4.203	4.081	4.228
H4'	4.309	3.912	3.866	3.943
H5',5''	3.987	3.750	3.564	3.731

of secondary transfer through scalar coupling. A slice through the H8 and its off-diagonal peaks (Fig. 2b) shows that the H1' and H2' cross peaks have similar intensities and probably both arise from primary polarization transfer from the H8. Volume integration of these cross peaks gave an intensity ratio of H1':H2' = 1.00:1.34, resulting in a distance ratio of H8-H1':H8-H2' = 1.05:1.00. For the H8 to be nearly equidistant between the two protons the H1' and H2' must be in the same residue. If the H1' and H2' were in different molecules within an aggregate one would expect the distances to the H8 to be quite different. The distance ratio obtained leads to an estimated glycosidic torsion angle (39a), χ_{CN} , of -10° (*syn*). This is an unusual torsion angle for the *syn* conformation in ribonucleotides, (39b) but it places the NH₂ group near the phosphate, allowing for hydrogen bonding between them (39c) and a stabilization of the *syn* structure, I(b). (40)

An alternate interpretation of the above NOESY data is that there is a 1:1 mixture of *syn* and *anti* conformers. However, a comparison of the δ values of NH₂ (Table I) shows that in (TBA)₂(5'-GMP) δ_{NH_2} is 0.22 ppm downfield of that in guanosine, consistent with NH₂-OPO₃ hydrogen bonding in (TBA)₂(5'-GMP).

The ¹H NMR of (TBA)₂(5'-IMP) was also investigated since this molecule does not have an amino group to hydrogen bond with the phosphate in a *syn* conformation. The spectrum (Fig. 3 and Table I) is similar to that of (TBA)₂(5'-GMP) in that the exchangeable protons are not observed, and the lines are highly broadened. At a t_{mix} of 150 ms the ¹H NOESY spectra showed a weak off-diagonal signal between H8 and H2' as the only dipolar interaction of H8. This interaction increased in intensity as the mixing time was increased to 400 ms. At 400 ms an extremely weak interaction between H8 and H1', very much less intense than that of H8-H2', was also observed. This would indicate that the predominant conformation of 5'-IMP under these conditions is *anti*. It seems apparent that some type of aggregation or reversed micelle formation is occurring in the 5'-IMP system also.

Figure 3. ¹H NMR of 41 mM (TBA)₂(5'-IMP) in DMSO-d₆, internal ref. TMS.

It was hoped that the ³¹P NMR spectra would provide evidence for the *syn* conformation in (TBA)₂(5'-GMP) in DMSO-d₆. Although hydrogen bonding is not known to appreciably affect the ³¹P chemical shift, an NH₂-phosphate interaction could induce a change in the C5'-O5' torsion angle and/or the O-P-O bond angles which do affect δ (41). A large difference in the ³¹P chemical shift is observed between 40 mM (TBA)₂(5'-GMP) solutions in DMSO-d₆ and D₂O: $\delta_{\text{DMSO}} = 1.932$ ppm; $\delta_{\text{D}_2\text{O}} = 4.309$ ppm. The ³¹P chemical shifts for (TBA)₂(5'-IMP), which we have shown has the *anti* conformation under the same conditions, are: δ_{DMSO} (40 mM) = 1.029 ppm; $\delta_{\text{D}_2\text{O}}$ (39 mM) = 4.426 ppm. Since a similar large chemical shift change is observed for both 5'-GMP and 5'-IMP it is concluded that this is just a solvent effect.

Conformation and dimer formation of guanosine

In contrast to (TBA)₂(5'-GMP), the ¹H NOESY data show clearly that guanosine (40 mM) has an *anti* conformation in DMSO-d₆ (spectrum not shown), as has been found in other solution studies (42,43) and in the solid state (44). Several NOEs build up and decay as the mixing time is varied from 150 to 400 ms (Table II). At $t_{\text{mix}} = 150$ ms there is a weak cross peak between the H8 and the H5'-OH and H5',5". This is no longer observed at $t_{\text{mix}} = 400$ ms, but rather an NOE between H8 and H2' is building up. These are all indicative of an *anti* structure. In addition strong interactions are observed between the NH₂ and both the H1' and H2'-OH. It was first thought that this was another indication of an *anti* conformation, but the distances between the protons in that conformation are too great to produce such a strong NOE. An alternative explanation which is more consistent with the data is the formation of a symmetric dimer in which the two guanosines are held together by two N3...H-N-H hydrogen bonds, III (R=ribose). In the dimer

Table II. Dipolar interactions in guanosine in DMSO-d₆

Concentration = 40 mM		
t_{mix}	Interactions	
150 ms	a) H8 - 5'OH H8 - H5',5"	weak weak
	b) NH ₂ - H1' NH ₂ - 2'OH	strong strong
	c) N(1)H - H ₂ O	
	d) 2', 3', 5'OH - H2', 3', 4', 5', 5" some of these are secondary transfer	
300 ms	a) H8 - H ₂ O	
	b) NH ₂ - H2'	very weak
	c) N(1)H - H ₂ O	
	d) 2', 3', 5'OH - H2', 3', 4', 5', 5" some of these are secondary transfer	
400 ms	a) H8 - H2'	weak
	b) NH ₂ - H ₂ O	
	c) N(1)H - H ₂ O	
	d) 2', 3', 5'OH - H2', 3', 4', 5', 5" some of these are secondary transfer	

the non-hydrogen bonded NH₂ proton is in very close proximity to both the H1' and H2'OH and would produce the NOE's observed. Such a hydrogen bonding scheme is not commonly found for guanine nucleosides or nucleotides, but has been observed, for example, in Na⁺ and NH₄⁺ salts of 2'-deoxycytidylyl-(3'-5')-2'-deoxyguanosine (45,46).

Molecular modeling of 5'-GMP

Molecular modeling was done to see how the geometry optimized structure compared to that determined by experimental NMR measurements. The CAChe system was used to perform AM1 and molecular mechanics calculations on 5'-GMP. To take hydrogen bonding between the NH₂ and phosphate groups into account for the MM2 calculations, the starting molecule was initially constrained to a *syn* conformation by forming a 'weak'

bond (1/5 the strength of a normal O—H bond) between the phosphate oxygen and H—N(2)H and minimizing the energy. Since the 'weak' bond is still in the vicinity of 5–8 times stronger than a hydrogen bond of this type is likely to be, the 'weak' bond was broken and the structure re-minimized using AM1 (Fig. 4 and Table III). The newly calculated structure displayed small changes in the torsion angles and nonbonding distances, but remained in the *syn* conformation, indicating a local minimum at this conformation. It is very difficult with a molecular system this large to systematically analyze for a global minimum. However, when the same calculations were performed with the 5'-GMP in an *anti* conformation, a local minimum was also observed and it was found to be 4.1 kcal mol⁻¹ higher in energy than the minimum for the *syn* conformation.

The *syn* structure is that of a very compact and relatively flat molecule. The bond distance between the NH₂ and phosphate oxygen (N—O) of 3.148 Å is rather long for a hydrogen bond, but still close enough to indicate weak hydrogen bonding. The ribose torsion angles show that the C3' atom is out of the plane of the five-membered ring with the other four atoms nearly planar. This is a C3'-*endo* sugar pucker. Experimentally measured by the H1'—H2' coupling constant, the ribose pucker is approximately 50% C3'-*endo* and 50% C2'-*endo* over the concentration range 5–40 mM. However, at 50 mM the percent C3'-*endo* has increased to 67%. The H2' through H5'5" proton resonances are broadened so that it is not possible to obtain experimental P—O5'—C5'—C4' and H5"5'—C4'—H4' torsion angles from the coupling constants to compare with those from the AM1 calculations. The calculated H8—H1' and H8—H2' distances put the H8 proton considerably closer to the H1' than the H2' with a glycosidic torsion angle of 51.0°. Although this is a more typical *syn* torsion angle for a ribonucleotide, it differs appreciably from the structure indicated by the experimental NOESY NMR data, which suggest that the H8 is nearly equidistant from the two ribose protons. However, no solvent effects or intermolecular interactions were included in the minimization. The significance of the calculations is that they show there is a low energy structure for 5'-GMP which has the *syn* conformation and which would allow phosphate to NH₂ hydrogen bonding.

Reverse micellar properties

The broad lines observed in the ¹H NMR of (TBA)₂(5'-GMP) in DMSO-d₆ are typical of guanine nucleotides that are self-associated via base stacking in aqueous solution. Since base stacking is not observed in organic solvents and since the base and ribose are the most favorable interaction sites with DMSO, it was postulated that a reverse micellar type of species might be forming. One property of a reverse micelle is a decrease in the H₂O line width in the ¹H NMR as the H₂O concentration is increased (24,47,48). As stated earlier, in dried DMSO solutions of (TBA)₂(5'-GMP) there is no resonance due to HOD/H₂O, but its presence in trace amounts is deduced from absence of the N(1)H and OH and the broadening of the NH₂ resonances. Therefore all of the water must be associated with the 5'-GMP moieties, and undoubtedly the TBA⁺ ion also.

In order to observe the water proton resonances without obtaining a very intense signal, water was added in the form of D₂O (0.1 atom %H) and the proton of HOD was observed. In dried, pure DMSO-d₆ the H₂O resonance is found at 3.35 ppm and the line width at half height is constant at 2.5 ± 0.6 Hz over

Table III. Pertinent AM 1 calculated distances and angles in (TBA)₂(5'-GMP)

Bond Distances		
H8 - H1'	2.540 Å	
H8 - H2'	3.661 Å	
N(H ₂) - O(P)	3.148 Å	
(P)O—H(N2)	2.240 Å	
Torsion Angles		
C4-N9-C1'-O4'	51.0°	<i>syn</i>
C2'-C1'-O4'-C4'	-0.769°	C3'- <i>endo</i>
C3'-C2'-C1'-O4'	-21.65°	
H5'-C5'-C4'-H4'	318.6°	<i>trans-gauche</i>
P-O5'-C5'-C4'	172.0°	<i>gauche'-gauche'</i>
Hydrogen Bond Angle		
(P)O—H-N2	165.1 Å

Figure 4. Structure of (TBA)₂(5'-GMP) as determined by an AM1 minimization using the CAChe system and starting with a *syn* conformation.

the range 0.0–0.6 mole fraction D_2O . Similarly, in $DMSO-d_6$ solutions of guanosine with no added H_2O or D_2O , the H_2O chemical shift is 3.3–3.4 ppm and the line width is 2 Hz, varying just slightly with the sample. For $(TBA)_2(5'-GMP)$, a 40 mM solution in $DMSO-d_6$ was mixed with a 40 mM solution in D_2O to give the desired mole fraction of D_2O while keeping the $(TBA)_2(5'-GMP)$ concentration constant. As X_{D_2O} increases from 0.0 to 0.038, a new, very broad line due to water appears at 3.66 ppm (Fig. 5a). With continued increase in the H_2O content of the solution, the resonance initially shifts upfield to 3.62 ppm, narrows and then shifts downfield with continued line narrowing. In 100% D_2O the HOD resonance is located at 4.794 ppm, a typical value for aqueous 5'-GMP solutions. The

slight initial upfield shift occurs as a result of H_2O interaction with the DMSO solvent in addition to the $(TBA)_2(5'-GMP)$ and is an averaged signal. The downfield shift begins when there is sufficient water present so that H_2O-H_2O interactions can occur. The observed line narrowing (Fig. 5b) does not prove that reverse micelles are forming because this phenomenon might be observed in any ternary system where there is a strong solute- H_2O interaction, but it is consistent with reverse micellar behavior.

A concentration-dependent 1H NMR study of $(TBA)_2(5'-GMP)$ in $DMSO-d_6$ was carried out between 5–50 mM in hopes of determining an apparent critical micelle concentration (cmc). Cmc's are typically observed for micelles, but are especially difficult to obtain and not necessarily observed for reverse micelles (48). The chemical shifts of the H8, H1', H4', H5', and H5'' were essentially invariant, changing less than 0.02 ppm over the concentration range. Only the H2' and H3' showed a small, but significant change; 0.105 and 0.046 ppm, respectively (Fig. 6). There does appear to be somewhat of a break in the δ vs. concentration plot between 20–25 mM. At present it is uncertain whether or not this break represents a cmc; it might be a reflection of a change in the ribose conformation. The concentration study does reveal that the nonexchangeable proton resonances narrow to some extent with decreasing concentration, indicating that less intermolecular interactions are occurring at lower concentrations. This becomes especially noticeable at 20 mM and below.

CONCLUSIONS

It has been found that $(TBA)_2(5'-GMP)$ is soluble in DMSO and that this is a ternary system which includes a small amount of water. $(TBA)_2(5'-GMP)$ forms aggregates which exhibit some of the properties of reverse micelles. There is evidence that the 5'-GMP has a predominantly *syn* conformation under these conditions which could be stabilized by amino to phosphate hydrogen bonding. We propose that the TBA ions, phosphate, and ribose OH protons are in or near the center of the micellar-type aggregate along with the H_2O with which they interact. The guanine base is hydrophobic and is interacting with the DMSO at the outer edge of the aggregate. The solubility of

Figure 5. 1H NMR of 38 mM $(TBA)_2(5'-GMP)$ in $DMSO-d_6$ with added D_2O . (a) mole fraction of D_2O : 0.00, 0.04, 0.07, 0.14, 0.20. (b) line width of HOD signal as a function of added D_2O (0.1 atom% H).

Figure 6. 1H chemical shifts of H2' and H3' as a function of $(TBA)_2(5'-GMP)$ concentration; internal ref. TMS.

(TBA)₂(5'-GMP) in polar organic phases which can permeate through lipophilic substances indicate that this might serve as a method for transporting nucleotidic drugs, such as the AIDS drug dideoxyinosine, through cell membranes. The reverse micellar properties of the system might also be utilized to carry hydrophilic drugs or substances in the aqueous interior of the micelle into or through hydrophobic media. Experiments are planned to investigate the solubility and properties of TBA and other salts of the mononucleotides in other organic phases and to delineate the nature of reverse micellar-type structures.

Guanosine behaves differently from 5'-GMP in DMSO and exists as a symmetric dimer in which the N3 and NH₂ of one guanosine are hydrogen bonded to those of another.

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