Rates of turbidity changes of polymeric vesicles are appreciably smaller than those for their unpolymerized counterparts (Table I).

Proton and hydroxide ion permeabilities in polymerized vesicles are much slower than those in their unpolymerized analogues. Permeabilities of these ions in dimethyldioctadecylammonium chloride surfactant vesicles are instantaneous.[1] Conversely, hydroxide ion permeates into polymerized surfactant vesicles with half lives ranging from 5–20 min (Table I). Significantly, permeation into completely polymerized vesicles of **5** is slower than that into vesicles of **5** "zipped-up" only on their outer surfaces.

Polymerization of surfactant vesicles provides convenient permeability control and allows the creation of pH gradients in addition to enhancing stabilities. Importantly, beneficial properties (ability to compartmentalize substrates in different microenvironments, presence of high-surface potential, charge density, and phase transition) remain unaffected. Further characterization and exploitation of these and related surfactant vesicles are under active investigation in our laboratories.

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Registry No. 1, 79898-71-8; **1** polymeric, 79899-59-5; **2**, 79898-72-9; **2** polymeric, 79899-60-8; **3**, 79898-73-0; **3** polymeric, 79899-61-9; **4**, 79898-74-1; **4** polymeric, 79899-62-0; **5**, 79898-75-2; **5** polymeric, 79899-64-2; **6**, 79898-76-3; **6** polymeric, 79899-65-3; 7 HCl, 79919-75-8; **8** HCl, 79898-77-4; **9** HCl, 79898-78-5; **10**, 79898-79-6; 10-undecenoyl chloride, 38460-95-6; *N*-methyliminobis[ethanol], 105-59-9; iminobis-[ethanol] HCl, 14426-21-2; lauroyl chloride, 112-16-3; *p*-vinylaniline, 1520-21-4; 11-bromoundecanoyl chloride, 15949-84-5; 2-bromoethanol, 540-51-2; phosphorus oxychloride, 10025-87-3; *N*-(2-sulfoethyl)iminobis[ethanol], 10191-18-1; allyl bromide, 106-95-6; *N,N*-dimethyl-*n*-hexadecylamine, 112-69-6.

Nucleoside Complexing: A ^{13}C NMR Spectroscopic Study of Binding of Metal Ions to Guanosine and Related Nucleosides in Solution. Evidence for O-6 Binding under Basic Conditions

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Abstract: The influence of hard and soft metals on the ^{13}C NMR spectrum of guanosine and inosine under both neutral and basic conditions in Me_2SO has been determined. Several related molecules also studied include 2′-deoxyguanosine, 1-methylguanosine, and N^2,N^2-dimethylguanosine. Under neutral conditions, hard metal species such as $Ba(NO_3)_2$ and $Pr(NO_3)_3$ do not perturb the ^{13}C NMR spectrum of guanosine, but under basic conditions (in the presence of the amine base triethylamine) a considerable change in the ^{13}C NMR spectrum is observed. An analogous effect is observed for N^2,N^2-dimethylguanosine, but is absent for 1-methylguanosine. Under neutral conditions, soft metal species such as $HgCl_2$ bind readily to guanosine, 1-methylguanosine, and N^2,N^2-dimethylguanosine, but in the presence of the amine base triethylamine, $HgCl_2$ binds only to guanosine. Triethylamine alone does not significantly perturb the ^{13}C NMR spectrum of guanosine or its derivatives, and these results suggest that metals do promote deprotonation at N(1). The shifts observed are best interpreted as resulting from the binding of the hard metal species to O(6) of guanosine when N(1) is deprotonated and the binding of the soft metal species to N(1) after deprotonation. The influence of metal ions on the ^{13}C NMR shifts with 1-methylguanosine and N^2,N^2-dimethylguanosine is consistent with these binding site assignments.

Since the discovery of the effectiveness of certain platinum(II) antitumor agents,[1] which are generally believed to function by binding to nucleic acids in the tumor cells,[2] there has been increased research focused at understanding the interactions of metals with nucleic acid derivatives.[3] In particular, the antitumor agent *cis*-$[Pt^{II}(NH_3)_2Cl_2]$ is known to interact with guanine[4] or at least at the GC pair,[5] and may interact with the 6-oxo group promoting deprotonation at N(1).[6-9] Such deprotonation could lead to the mispairing of guanosine with thymine, and eventually cell death.[2] Such mispairing also bears directly on metal mutagenicity. However, virtually all attempts to demonstrate the binding of Pt(II) complexes and many other metal species with O(6) have proved unsuccessful although very weak binding has been demonstrated in the solid state for a few special cases.[6,10-13] One impediment to the definitive evaluation of the importance of such binding in solution is the lack of effective spectroscopic criteria for its assessment.[14]

We believe that N(9)-alkylated guanines (Figure 1; when S = D-ribose the molecule is named guanosine) can be viewed as having three likely metal binding regions. Region K, involving the N(7) site of the five-membered ring, is the *kinetically* favored site since it is not protonated under neutral conditions. Region T, involving the N(1) site, is the *thermodynamically* favored binding site for softer metal species (Hg(II), Pt(II), Pd(II), etc.). However, this site is protonated under neutral conditions and is

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Table I. Effects of Nitrogen Bases on the ^{13}C NMR Spectra of Guanosine and Related Nucleosides (0.2 M)[a]

nucleoside	nitrogen base	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′	CH_3
guanosine	none	153.57	151.23	116.57	156.71	135.54	86.28	70.28	73.62	85.12	61.67	
guanosine	$(C_2H_5)_3N$, 0.4 M	153.96	151.32	116.76	157.25	135.56	86.53	70.42	73.75	85.25	61.40	
guanosine	$(HOC_2H_4)NH_2$, 0.8 M	155.72	151.33	116.93	159.95	135.35	86.92	70.49	73.56	85.39	61.51	
deoxyguanosine	none	153.59	150.81	116.60	156.73	135.25	82.56	c	70.68	87.52	61.65	
deoxyguanosine	$(C_2H_5)_3N$, 0.4 M	153.87	150.89	116.74	157.05	135.21	82.69	c	70.78	87.61	61.75	
inosine	none	145.82	148.16	124.39	156.51	138.69	87.44	70.24	74.07	85.57	61.22	
inosine	$(C_2H_5)_3N$, 0.4 M	146.12	148.25	124.48	157.04	138.67	87.63	70.34	74.14	85.67	61.32	
inosine	$(HOC_2H_4)NH_2$, 0.8 M	149.60	148.74	124.60	162.39	138.01	88.15	70.66	73.72	85.99	61.67	
1-methylguo	none	154.22	149.41	115.67	156.41	135.69	86.06	70.34	73.62	85.11	61.36	28.01
1-methylguo	$(C_2H_5)_3N$, 0.4 M	154.25	149.41	115.67	156.44	135.70	86.03	70.37	73.68	85.13	61.37	28.03
1-methylguo	$(C_2H_5)_3N$, 0.3 M	154.24	149.44	115.74	156.44	135.69	86.11	70.39	73.71	85.16	61.41	28.03
N^2,N^2-DMG[b]	none	152.97	150.77	116.03	157.31	136.36	86.72	70.36	73.33	85.05	61.47	37.66
N^2,N^2-DMG[b]	$(C_2H_5)_3N$, 0.3 M	153.15	150.83	116.13	157.55	136.35	86.82	70.41	73.41	85.08	61.52	37.41
N^2,N^2-DMG[b]	$(OHC_2H_4)NH_2$, 0.6 M	156.27	151.19	116.73	161.52	136.10	87.12	70.53	73.17	85.08	61.65	37.40
N^2,N^2-DMG[b]	$(C_2H_5)_3N$, 0.4 M	153.22	150.85	116.18	157.60	136.37	86.89	70.44	73.45	85.12	61.54	37.45

[a] Me_2SO-d_6, 32 °C, shifts in ppm relative to Me_4Si. [b] N^2,N^2-Dimethylguanosine, 0.1 M. [c] The peak is masked by the methyl resonances of Me_2SO-d_6.

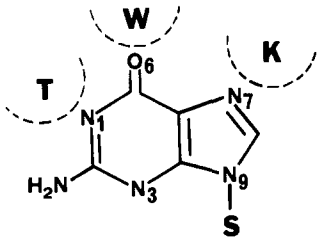

Figure 1. Potential metal binding regions for N(9)-alkylated guanines or N(9)-alkylated hypoxanthines (where the two amino groups are replaced by H).

therefore less accessible for inert metal species such as those containing Pt(II). The W region, involving the O(6) binding site, is expected to be a *weak* binding site and to interact primarily with very hard metal species or in conjunction with N(7) or N(1) in a chelate binding mode.

Binding at O(6) will be enhanced if N(1) is deprotonated. Deprotonation is facilitated in more acidic nucleosides (for example, inosine, where the NH_2 group of guanosine is replaced by H) or by metal binding at N(1). When a metal is bound to N(1), the charge in the ring is not neutralized as well (or electrons are not withdrawn from the ring as well) as in the neutral species, where N(1) is protonated. The O(6) is therefore a better binding site. Recently, a solid N(1) metalated/O(6) metalated species was reported by Bau.[12]

In previous ^{13}C NMR work, we have found that in the solvent Me_2SO, metal ion–nucleoside binding is enhanced.[15,16] This enhancement probably results from the lower degree of solvation of the nucleoside lone pairs of electrons by Me_2SO than by H_2O, which can donate H bonds. Nevertheless, metal binding sites to nucleosides are similar in Me_2SO and H_2O, except that binding sites which require deprotonation are not usually observed for Me_2SO.[14] By adding the amine base triethylamine, we have been able to observe binding at sites which are normally protonated at or near neutrality. Thus, the major difference between complex formation in H_2O and Me_2SO is eliminated, allowing us to investigate weak or transient interactions which involve deprotonation.

As part of a continuing program designed to examine the influence of metal salts and complexes on the ^{13}C NMR spectra of the common nucleosides, we have examined metal binding to guanosine, inosine, and some relevant guanosine derivatives. In this report we present evidence for the binding of metal species to O(6) and show that metals do facilitate deprotonation at N(1), when the metal interacts with O(6).

Experimental Section

Materials. The following were from Fisher: $HgCl_2$, $Ba(NO_3)_2$, $La(NO_3)_3\cdot 6H_2O$, $Pb(NO_3)_2$, $Cr(NO_3)_3\cdot 9H_2O$. Alfa supplied $Pr(NO_3)_3\cdot 5H_2O$ and $Lu(ClO_4)_3\cdot 6H_2O$, Merck supplied $Ba(NO_3)_2$ and $Zn(NO_3)_2$, and Mallinckrodt supplied $HgCl_2$. The $Lu(NO_3)_3\cdot 5H_2O$ came from ROC/RIC. Nucleosides 2′-deoxyguanosine, guanosine, 1-methylguanosine, and inosine were from Sigma. Vega Fox supplied the N^2,N^2-dimethylguanosine, and the triethylamine (99%) and the Me_2SO-d_6 (99.5 atom % D) were from Aldrich.

cis-[Dichlorobis(dimethyl sulfoxide)platinum(II)]. This derivative was prepared as described in the literature[17] and was kindly provided by Dr. Purush Chalilpoyil.

NMR Studies. The ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrometer operating at 20 MHz (1.8682 T) in the Fourier transform mode at a temperature of 32 °C and using a 10-mm probe. Me_2SO-d_6 containing Me_4Si as an internal standard was used as the solvent and provided the internal deuterium lock. Typically a total of 4000–6000 transients were required in 8K of memory with a pulse width of 14 μs, a pulse delay of 3.4 s, and broad-band proton decoupling. The 4000-Hz spectral width used results in an acquisition time of 0.909 s and in digital resolution no better than 0.97 Hz ~0.05 ppm. The chemical shifts reported are reproducible to ±0.06 ppm. For high concentrations of lanthanide ions, as many as 18 000 transients were needed to achieve a good signal-to-noise ratio.

The ^{13}C NMR assignments were made according to the literature.[18] Samples were prepared by dissolving the desired quantities of nucleoside and metal species in 2 mL of Me_2SO-d_6. For those samples run under basic conditions, ~100 μL of triethylamine was then introduced into the NMR tube, giving a concentration of 0.4 M.

General Results

The metal species we have studied can be crudely divided into two classes—O- and N-binding metals. Since of the alkaline earth ions Ba^{2+} salts have the greatest effects on ^{13}C NMR spectra,[15,16] we have primarily employed $Ba(NO_3)_2$ as a prototype of the typically O-bonding metal species. We have utilized $HgCl_2$ because of its known affinity for nitrogen, particularly endocyclic ring nitrogens of nucleosides,[15] as a typical N-binding metal species. We have also examined the effect of these metal ions under both neutral and basic conditions. The base ethanolamine was found to be strong enough to cause deprotonation, and significant perturbations on the ^{13}C NMR spectra of the various nucleosides were seen. But the amine base triethylamine was found to cause little perturbation on the ^{13}C NMR spectra (Table I). However, in the triethylamine–Me_2SO solution several metal ion–nucleoside interactions of a type not previously reported were observed. Additionally, several examples of binding not observed in Me_2SO but known to occur in water were also seen.

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Table II. Comparative Effects of Various Salts on the ^{13}C NMR Shifts of Guanosine (0.2 M)[a]

salt	concn, M	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′
	0	153.58	151.22	116.60	156.71	135.52	86.36	70.28	73.62	85.11	61.32
$Ba(NO_3)_2$	0.7	153.58	151.24	116.46	156.95	135.79	86.37	70.38	73.60	85.23	61.34
$La(NO_3)_3$	0.5	153.60	151.33	116.39	156.87	135.88	86.33	70.38	73.58	85.18	61.40
$Lu(NO_3)_3$	0.5	153.60	151.32	116.39	156.83	135.86	86.29	70.36	73.54	85.28	61.39
$Pr(NO_3)_3$	0.5	153.69	151.42	116.35	157.01	136.04	86.42	70.39	73.58	85.38	61.46
$Pb(NO_3)_2$	0.7	153.60	151.24	116.50	156.82	135.67	86.29	70.27	73.59	85.15	61.27
$Zn(NO_3)_2$	0.7	153.86	151.22	114.61	156.93	137.48	86.75	70.39	74.03	85.59	61.19
$HgCl_2$	0.7	154.26	149.99	113.94	155.67	137.15	87.47	70.08	74.11	85.55	60.87
$Pt(Me_2SO)_2Cl_2$	0.2	154.10	149.22	113.00	154.75	138.84	87.58	69.83	73.98	85.55	60.84

[a] See footnote for Table I.

Table III. Comparative Effects of Various Salts on the ^{13}C NMR Incremental Chemical Shifts of Guanosine (0.2 M) in the Presence and Absence of Nitrogen Bases[a]

salt	nitrogen base	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′
	$(C_2H_5)_3N$, 0.4 M	−0.39	−0.09	−0.19	−0.54	−0.02	−0.25	−0.14	−0.13	−0.13	0.27
	$(HOC_2H_4)NH_2$, 0.8 M	−2.15	−0.10	−0.36	−3.24	0.19	−0.64	−0.21	0.06	−0.27	0.16
$Ba(NO_3)_2$, 0.7 M	none	−0.01	−0.01	0.11	−0.24	−0.25	−0.09	−0.10	0.02	−0.11	0.33
$Ba(NO_3)_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−0.66	0.06	0.07	−1.37	−0.10	−0.10	−0.10	0.23	−0.13	−0.07
$Ba(NO_3)_2$, 0.7 M	$(HOC_2H_4)NH_2$, 0.8 M	−2.57	0.23	−0.41	−5.03	0.01	−0.59	−0.28	0.28	−0.36	−0.19
$HgCl_2$, 0.7 M	none	−0.69	1.24	2.63	+1.04	−1.61	−1.19	0.20	−0.49	−0.43	0.80
$HgCl_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−3.90	0.48	1.78	−2.75	−1.04	−0.74	0.01	0.13	−0.35	0.09
$HgCl_2$, 0.7 M	$(HOC_2H_4)NH_2$, 0.8 M	−2.37	0.06	0.78	−0.86	−0.82	−0.03	0.07	0.15	−0.02	0.08
$Zn(NO_3)_2$, 0.7 M	none	−0.29	0.01	1.96	−0.22	−1.94	−0.47	−0.11	−0.41	−0.47	0.48
$Zn(NO_3)_2$, 0.2 M	$(C_2H_5)_3N$, 0.4 M	−0.64	−0.16	0.64	−1.03	−0.24	−0.08	0.06	0.13	−0.09	0.02
$Pr(NO_3)_3$, 0.5 M	none	−0.12	−0.19	0.22	−0.30	−0.50	−0.14	−0.11	0.04	−0.26	0.21
$Pr(NO_3)_3$, 0.03 M	$(C_2H_5)_3N$, 0.4 M	−1.11	−1.20	−2.68	3.24	−0.56	−0.30	−0.22	−0.19	−0.16	−0.12
$Pb(NO_3)_2$, 0.7 M	none	−0.03	−0.01	0.07	−0.11	−0.13	−0.01	0.01	0.03	−0.03	0.40
$Pb(NO_3)_2$, 0.3 M	$(C_2H_5)_3N$, 0.4 M	−1.31	−0.39	−1.19	−3.87	−0.33	−0.27	−0.25	−0.00	−0.28	−0.16

[a] Me_2SO-d_6; 32 °C; the incremental chemical shifts are defined as the difference between the chemical shift of ligand and nitrogen base and the observed shift; negative shifts downfield. Shifts in ppm relative to Me_4Si.

The results section will be divided according to two main classes of metal species, oxygen-binding and nitrogen-binding species. For each class the nucleosides examined will be treated in separate subsections, and the results for each nucleoside will be further divided according to basicity of the medium. The nucleosides guanosine and 2′-deoxyguanosine will be examined first in each class. These sections will be followed by the guanosine derivatives studied, which have proved to be useful in clarifying the conclusions we have drawn concerning the metal binding to the guanine base of guanosine. Results on the metal binding of inosine will then be presented and related to the guanosine results where possible. In the following discussion, positive changes in shifts correspond to upfield shifts.

Prior to the presentation of the results on nucleosides, metal binding to the added amine base needs to be discussed. The ^{13}C NMR shifts of CH_2 and CH_3 of triethylamine (0.4 M) in Me_2SO were measured and determined to be 45.69 ppm for CH_2 and 11.62 ppm for CH_3. In the presence of 0.7 M $Ba(NO_3)_2$, the observed triethylamine shifts were 45.65 ppm for CH_2 and 11.62 ppm for CH_3. Thus, there seems to be no evidence from the ^{13}C NMR spectrum of triethylamine that $Ba(NO_3)_2$ forms a complex with this base. But in the presence of 0.7 M $HgCl_2$ the observed triethylamine shifts were 46.49 ppm for CH_2 and 9.50 ppm for CH_3. These large perturbations on the ^{13}C NMR spectrum of triethylamine caused by $HgCl_2$ suggest that $HgCl_2$ does bind to the triethylamine, since the alternative possibility that the triethylamine is being protonated is precluded by a different pattern of shifts associated with protonation. The ^{13}C NMR shifts for a solution 0.4 M in triethylamine and 0.2 M in *p*-toluenesulfonic acid are 45.75 and 9.79 ppm, respectively.

Oxygen-Binding Metal Species. Guanosine. Recently it was claimed that ^{13}C NMR evidence was obtained, indicating that alkaline earth cations interact with guanosine.[19] We have found no substantiating evidence to support this contention. The addition of nitrate salts of alkaline earth, lanthanide, and divalent lead causes only minor perturbations to the ^{13}C NMR spectrum of guanosine (Tables II and III). For example, the largest shifts we have observed for guanosine on the addition of $Ba(NO_3)_2$ are −0.25 ppm for C(8) and −0.24 ppm for C(6) (Table III). Furthermore, the Raman difference spectra for guanosine show very little effect for $Ba(NO_3)_2$ as compared to other metal species which definitely bind to guanosine (Figure 2).

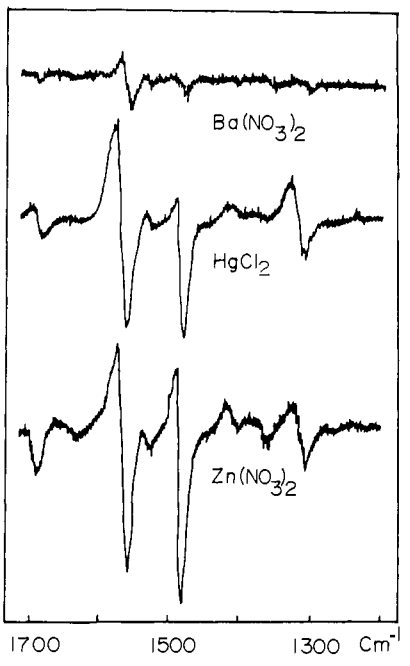

Figure 2. Raman difference spectra obtained as described previously[16] for guanosine (0.7 M) in Me_2SO-d_6 (in the absence of added base) for the inorganic species indicated (0.7 M).

In the $Ba(NO_3)_2$ experiment, the ^{13}C NMR signal of the solvent shifted 0.33 ppm, and if the solvent is used as a reference instead of Me_4Si, the C(8) shift appears to be −0.58 ppm. Thus, the previous use of the solvent signal as a standard[19] is obviously wrong.

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Table IV. Comparative Effects of Various Salts on the ^{13}C NMR Incremental Chemical Shifts of 1-Methylguanosine (0.2 M) in the Presence and Absence of Nitrogen Bases[a]

salt	nitrogen base	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′	CH_3
	$(C_2H_5)_3N$, 0.3 M	−0.02	−0.03	−0.07	−0.03	0.00	−0.05	−0.05	−0.09	−0.05	−0.05	−0.02
	$(C_2H_5)_3N$, 0.4 M	−0.03	0.00	0.00	−0.03	−0.01	0.03	−0.03	−0.06	−0.02	−0.01	−0.02
$Ba(NO_3)_2$, 0.7 M	none	0.06	0.10	0.07	−0.21	−0.23	−0.05	−0.06	0.07	−0.11	−0.03	0.01
$Ba(NO_3)_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	0.05	0.09	0.01	−0.23	−0.25	−0.19	−0.08	0.04	−0.12	−0.06	−0.01
$HgCl_2$, 0.7 M	none	−0.54	1.25	2.60	0.85	−1.88	−1.12	0.17	−0.49	−0.45	0.44	−0.23
$HgCl_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−0.23	0.65	1.42	0.40	−0.98	−0.53	0.10	−0.09	−0.25	0.20	−0.16
$Pr(NO_3)_3$, 0.5 M	none	−0.06	−0.12	0.30	−0.09	−0.43	−0.20	0.02	0.02	0.04	−0.09	−0.01
$Pr(NO_3)_3$, 0.5 M	$(C_2H_5)_3$, 0.3 M	−0.05	−0.22	0.17	−0.22	−0.70	−0.99	−1.31	*b*	−0.75	−0.29	0.00
$Zn(NO_3)_2$, 0.5 M	none	−0.19	0.33	1.57	0.00	−1.65	−0.55	0.01	−0.24	−0.32	−0.03	−0.26
$Zn(NO_3)_2$, 0.5 M	$(C_2H_5)_3$, 0.3 M	−0.15	0.21	*b*	−0.06	−0.11	0.16	*b*	0.01	*b*	0.01	−0.27

[a] See footnote for Table III. [b] No peak assignments registered.

Table V. Comparative Effects of Various Salts on the ^{13}C NMR Incremental Chemical Shifts of N^2,N^2-Dimethylguanosine (0.1 M) in the Presence and Absence of Nitrogen Bases[a]

salt	nitrogen base	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′	CH_3
	$(C_2H_5)_3N$, 0.3 M	−0.18	−0.06	−0.10	−0.24	0.01	−0.10	−0.05	−0.08	−0.03	−0.05	0.25
	$(C_2H_5)_3N$, 0.4 M	−0.25	−0.08	−0.15	−0.44	−0.01	−0.17	−0.08	−0.12	−0.07	−0.07	0.21
	$(OHC_2H_4)NH_2$, 0.6 M	−3.30	−0.42	−0.70	−4.21	0.20	−0.40	−0.16	−0.16	−0.03	−0.18	0.26
$Ba(NO_3)_2$, 0.7 M	none	0.15	−0.02	0.17	0.26	0.35	−0.06	−0.03	0.05	−0.08	0.01	0.05
$Ba(NO_3)_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−2.03	−0.15	−0.11	−2.88	0.16	−0.16	−0.08	0.24	0.04	−0.11	−0.17
$HgCl_2$, 0.4 M	none	−2.26	1.19	2.36	0.81	−1.51	−0.95	0.11	−0.35	−0.46	0.33	−0.16
$HgCl_2$, 0.4 M	$(C_2H_5)_3N$, 0.4 M	−0.31	0.02	0.34	−0.31	−0.19	−0.02	0.07	0.15	0.01	0.09	−0.25
$Pr(NO_3)_3$, 0.5 M	none	0.03	−0.13	0.15	−0.04	−0.24	−0.19	−0.03	0.07	−0.09	−0.06	0.05
$Pr(NO_3)_3$, 0.002 M	$(C_2H_5)_3N$, 0.3 M	−2.04	−1.14	−1.87	*b*	−0.85	−0.32	−0.16	−0.12	−0.11	−0.09	*c*

[a] See footnote for Table III. [b] No peak assignment registered. [c] The peak is masked by the methyl resonances of Me_2SO-d_6.

Table VI. Comparative Effects of $Ba(NO_3)_2$ and $HgCl_2$ on the ^{13}C NMR Chemical Shifts of Inosine (2 M) in the Presence and Absence of Nitrogen Bases[a]

salt	nitrogen base	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)′	C(3)′	C(2)′	C(4)′	C(5)′
	$(C_2H_5)_3N$, 0.4 M	−0.30	−0.09	−0.09	−0.53	0.02	−0.19	−0.10	−0.07	−0.10	−0.10
	$(HOC_2H_4)NH_2$, 0.8 M	−3.78	−0.58	−0.21	−5.88	0.68	−0.71	−0.42	0.35	−0.42	−0.45
$Ba(NO_3)_2$, 0.7 M	none	−0.15	0.03	0.09	−0.06	−0.17	0.04	−0.09	0.02	−0.10	−0.14
$Ba(NO_3)_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−1.32	−0.08	0.13	−2.05	0.02	−0.09	−0.18	0.21	−0.15	−0.13
$HgCl_2$, 0.7 M	none	−0.88	0.66	1.27	0.37	−0.67	−0.57	0.19	−0.20	−0.11	0.27
$HgCl_2$, 0.7 M	$(C_2H_5)_3N$, 0.4 M	−5.69	0.18	0.37	−2.58	−0.24	−0.26	0.00	0.25	−0.07	−0.02

[a] See footnote *a* for Table III.

The question then arises whether or not the shifts of the order of 0.3 ppm as observed for $Ba(NO_3)_2$ + guanosine can be considered significant in view of the major solvent changes which must accompany the addition of the salt. One way to test this point would be to examine the influence of the paramagnetic lanthanide ions which would cause a somewhat comparable change in solvent structures, which have a coordination chemistry somewhat analogous to that of the alkaline earth cations, and which can induce large paramagnetic shifts upon coordination. We have performed such experiments and find that 0.5 M $La(NO_3)_3$ induces a maximal shift of −0.36 ppm in the C(8) resonance and 0.21 ppm in the C(5) resonance. The results with $Lu(NO_3)_3$ were almost identical. The analogous experiment with $Pr(NO_3)_3$ leads to shifts of −0.48, −0.30, and 0.25 ppm respectively for C(8), C(6), and C(5) (shifts of other resonances were smaller). Corrected chemical shifts, adjusted for the diamagnetic $La(NO_3)_3$, are even less, being only −0.12, −0.14, and 0.04 ppm, respectively.

When similar experiments with 0.7 M $Ba(NO_3)_2$ were performed in the presence of 0.4 M triethylamine, a significant downfield shift of −1.37 ppm is found for C(6) (Tables III and S1). C(2) shifted −0.66 ppm, and the other shifts were considerably smaller. For the nitrate salt of the paramagnetic lanthanide ion Pr^{3+}, the C(6) resonance was broadened, and at a Pr concentration >0.1 M, the C(6) resonance could not be detected above the noise. Note that in the presence of base and a relatively low concentration of $Pr(NO_3)_3$ (0.03 M), C(6) shifts by over 3 ppm (Table III). Also the effect of $Pb(NO_3)_2$ now becomes quite dramatic with a maximal influence on C(6) which changes by −3.87 ppm.

2′-Deoxyguanosine. Analogous effects were seen for 2′-deoxyguanosine. Under neutral conditions, the hard metal species $Ba(NO_3)_2$ did not significantly perturb the ^{13}C NMR chemical shifts, but in the presence of "basic" Me_2SO a significant perturbation was found. In this case C(6) shifted −1.32 ppm, C(2) shifted −0.62 ppm, and the other shifts were considerably smaller (Tables S2 and S3).

Several possible explanations for these effects were considered. A metal–triethylamine complex might have a high affinity for neutral guanosine and could bind directly to N(7), the most common metal binding site. However, no significant perturbations were found for C(5) or C(8). To further explore the observed effects, the guanosine derivatives 1-methylguanosine and N^2,N^2-dimethylguanosine were studied.

1-Methylguanosine. 1-Methylguanosine has an N(7) coordination chemistry similar to that of neutral guanosine, but from ^{13}C NMR experiments with this nucleoside there was no evidence for Ba^{2+} binding in either neutral or basic Me_2SO, in contrast to our findings for guanosine, which has a deprotonation site at N(1). Under neutral conditions the largest perturbations were only −0.23 ppm for C(8) and −0.21 ppm for C(6). The shifts were only slightly modified under basic conditions; C(8) shifted −0.25 ppm and C(6) shifted −0.23 ppm (Table IV, S4, and S5).

N^2,N^2-Dimethylguanosine. For N^2,N^2-dimethylguanosine in which N(1) is sterically blocked by the bulky dimethylamino group at C(2), no significant perturbations were seen when $Ba(NO_3)_2$ was added at neutral conditions. However, a substantial shift of −2.66 ppm was seen for C(6) upon the addition of the triethylamine (Table V). This shift is larger than that observed for guanosine, and this may be due to an increased acidity for N(1)H in N^2,N^2-dimethylguanosine over the parent guanosine, perhaps resulting from a slight tilt of the dimethylamino group relative to the purine.[20]

Inosine. Under neutral conditions negligible shifts were seen in the presence of $Ba(NO_3)_2$ (Table VI). The largest perturbation was only −0.17 ppm for C(8). N(1)H of inosine should be more acidic than N(1)H of guanosine,[21,22] and under basic conditions one might expect a larger downfield shift for C(6) in inosine than for C(6) in guanosine. In fact, in the presence of triethylamine, a large downfield shift for C(6) of −2.05 ppm was observed. A significant downfield shift for C(2) of −1.32 ppm was also seen (Table VI).

Nitrogen-Binding Metal Species. Guanosine. The softer metal species should have a higher affinity for the endocyclic ring nitrogens, and under neutral conditions perturbations in the ^{13}C NMR spectra were found. The largest shifts found for $Zn(NO_3)_2$ are 1.96 ppm for C(5) and −1.94 ppm for C(8), and those for $HgCl_2$ are 2.63 ppm for C(5) and −1.61 ppm for C(8) (in agreement with previous work[23]). Even larger shifts for C(5) and C(8) are found for *cis*-$[Pt(Me_2SO)_2Cl_2]$. These shifts suggest that the soft metal species are binding in the vicinity of N(7). In the presence of $HgCl_2$, the shifts for C(2) and C(6) are −0.69 and +1.04 ppm, respectively. It is interesting to note that C(1)′ shifts −1.19 ppm.

We have examined the stability of the complex formed between $HgCl_2$ and guanosine. For all C resonances which exhibit an adequate shift to be treated meaningfully, the value for K ranges from 1.0 to 3.2 M^{-1} (Table S8). This wide spread of values led us to suspect that the Cl^- interaction with the NH groups might be influencing the results.

In the presence of triethylamine, $HgCl_2$ binds in the vicinity of N(1), and appreciable downfield shifts for C(2) of −3.90 ppm and C(6) of −2.75 ppm are seen (Table III). The shifts for C(5) decreased to 1.78 ppm and for C(8) to −1.04 ppm. The shift for C(1)′ decreased to −0.74 ppm. The influence of $Zn(NO_3)_2$ also suggests a change toward a greater interaction with the pyrimidine ring; note the decreased effect on C(1)′.

2′-Deoxyguanosine. Analogous results are seen for 2′-deoxyguanosine (Table S3). Under neutral conditions in the presence of $HgCl_2$, C(5) shifts 2.76 ppm and C(8) shifts −1.75 ppm. In the presence of triethylamine, C(2) shifts −4.07 ppm and C(6) shifts −3.04 ppm. The change in shifts for C(5) and C(8) decreases to 2.13 and −1.17 ppm, respectively. It is also interesting to note that under neutral conditions, in the presence of $HgCl_2$, C(1)′ shifts −1.38 ppm and under basic conditions −0.94 ppm.

1-Methylguanosine. To further explore the observed effects for guanosine, we examined the effects of $HgCl_2$ with 1-methylguanosine (Tables IV, S4, and S5). Under neutral conditions the largest shifts found for $Zn(NO_3)_2$ are 1.57 ppm for C(5) and −1.65 ppm for C(8), and those for $HgCl_2$ are 2.60 ppm for C(5) and −1.88 ppm for C(8). It is interesting to note that C(1)′ shifted −1.12 ppm. Evaluation of the stability of 1-methylguanosine (which lacks the most acidic H of guanosine) with $HgCl_2$ gave more consistent values with $K = 3.0 \pm 0.5$ M^{-1} (Table S9). The similar patterns of the ^{13}C NMR shifts induced by $HgCl_2$ in both guanosine and 1-methylguanosine suggest that binding to N(1) is not important for guanosine under neutral conditions.

In the presence of triethylamine and $HgCl_2$ decreased shifts for C(5) of 1.42 ppm and for C(8) of −0.98 ppm are seen. C(1′) shifts −0.53 ppm. In contrast to guanosine which has a deprotonation site at N(1), no substantial perturbations for C(2) and C(6) are seen.

N^2,N^2-Dimethylguanosine. Under neutral conditions substantial shifts of 2.36 ppm for C(5) and −1.51 ppm for C(8) are seen, indicating N(7) binding of $HgCl_2$ (Table V). It is interesting to note that C(1)′ shifted −0.95 ppm. With the N(1) position effectively blocked in N^2,N^2-dimethylguanosine, $HgCl_2$ cannot bind to N(1) under basic conditions. This result is reflected by the ^{13}C NMR results. The largest perturbation is only 0.34 ppm for C(5). In fact, since no good coordination site for $HgCl_2$ is available on these two guanosine derivatives, the $HgCl_2$ binds preferentially to triethylamine. This binding appears to dominate over N(7) coordination.

Inosine. Under neutral conditions in the presence of $HgCl_2$, the ^{13}C NMR perturbations for inosine are smaller than those for guanosine, but the shifts are of a similar pattern. C(5) shifts 1.27 ppm, and C(8) shifts −0.67 ppm. C(2) also shifts −0.88 ppm, and C(4) shifts 0.66 ppm. C(1)′ shifts −0.57 ppm.

In the presence of triethylamine, C(2) shifts substantially downfield by 5.69 ppm, and C(6) shifts downfield by 2.58 ppm, suggesting metal binding at N(1). The shift for C(1)′ decreases to −0.26 ppm.

Discussion

Clear patterns emerge from the results presented above for the oxygen-binding metal species. These metal species interact very weakly, if at all, with guanosine and the other 6-oxopurine nucleosides used in this study, unless a base is present. Under such conditions, complexes are formed. The shifts observed indicate that the major perturbations are occurring in the six-membered ring. Of the possible binding regions, these changes are most easily rationalized as being caused by metal binding at O(6) with deprotonation at N(1).

The evidence for N(1) deprotonation appears to us to be very strong. The effect disappears for 1-methylguanosine. The stronger base 2-aminoethanol causes larger shifts than triethylamine. The same pattern of shifts is found for inosine which lacks the 2-amino group.

The evidence for O(6) binding is also strong. The shifts at C(6) are usually most pronounced. However, since deprotonation accompanies binding, this large shift at C(6) reflects primarily the effects of N(1) deprotonation as can be seen from the results in Table III for the addition of base in the absence of metal ions. The proton usually has the biggest perturbing effects on the ^{13}C NMR spectra of nucleosides, and the pattern of shifts for the diamagnetic metal species mainly reflects the effect of deprotonation. (Note that in Table III, the effect of the base is allowed for in the data presented for solutions containing both base and metal species.) However the effect of $Pr(NO_3)_3$ is primarily due to metal binding.

It could be argued that the metal is binding at N(1) and N(7) or at a combination of N(1), O(6), and N(7), O(6) or that several species coexist in solution.

The N(1) and N(7) binding possibilities with no O(6) involvement seems highly unlikely for several reasons. First, the typical N-binding metal species cause smaller perturbations than the O-binding metal species in the presence of base (except for the effect of $HgCl_2$ on C(2); see below). Specific further arguments can be made against binding at N(1) and at N(7).

To explain the data with N(7) binding one needs to suggest that metal ions bound at N(7) can induce a sufficient perturbation in the electron distribution of the pyrimidine ring such that deprotonation at N(1) is facilitated. However, this possibility seems remote because hard metal species such as $Ba(NO_3)_2$ are much weaker electrophiles than $HgCl_2$, and $HgCl_2$ does not promote deprotonation of N^2,N^2-dimethylguanosine. The binding of another strong electrophilic metal center, Pt(II), at N(7) is known not to induce a much greater N(1) acidity.[24] Additionally, the paramagnetic $Pr(NO_3)_3$ causes rather a small shift at C(8) but learge shifts at C(6) and C(5).

Similarly, specific arguments can be addressed against strong N(1) metal binding by the harder metal species. The N(1) vicinity of a deprotonated species would have a geometry similar to cytosine, which has an exocyclic oxo group and an exocyclic amino group on either side of the potential endocyclic nitrogen donor

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N(3). We recently showed that when the amino group of cytosine has been replaced by a dimethylamino group, N(3) is blocked by the sterically bulky methyl groups, and metals cannot bind strongly to the endocyclic nitrogen donor.[16] However, since a considerable ^{13}C NMR shift for C(6) was still seen for N^2,N^2-dimethylguanosine under basic conditions in the presence of $Ba(NO_3)_2$, a strong N(1) interaction seems unlikely.

For the metal species $Zn(NO_3)_2$, both O and N binding are possible. In the absence of base, this metal species causes a significant perturbation of the Raman spectrum of guanosine (Figure 2). The C(8) resonance shifts appreciably. The pattern of ^{13}C shifts (Table III) and the resemblance of the Raman difference spectrum to that induced by $HgCl_2$ (Figure 2) both suggest N(7) binding. Of particular note is the shift of C(1)′ which we feel tends to indicate N(7) binding. On addition of base, deprotonation occurs leading to a pattern of shifts characteristic of the harder metal species. Note that for 1-methylguanosine, addition of base eliminates the binding.

For $HgCl_2$, the results strongly suggest a change from N(7) to N(1) binding on addition of base. In the absence of base, C(8), C(5), and C(1)′ are shifted the most compared to resonances for guanosine itself and compared to effects of other metals. This same pattern is found for *cis*-$[Pt(Me_2SO)_2Cl_2]$. When N(1) is blocked, as in 1-methylguanosine (Table IV) or N^2,N^2-dimethylguanosine (Table V), the same pattern is observed for $HgCl_2$. There is then little doubt that N(7) binding is occurring.

On addition of base, there is a pronounced decrease in the shifts characteristic of N(7) binding in guanosine. The C(1)′, C(8), and C(5) shifts are diminished (Table III). However, the C(2) shift is greatly increased. We feel this indicates a shift from N(7) binding to N(1) binding. For the N(1)-blocked nucleosides 1-methylguanosine (Table IV) and N^2,N^2-dimethylguanosine (Table V), the shifts characteristic of N(7) binding diminish, but no shifts characteristic of N(1) binding occur. This is because the amine base forms a strong complex with $HgCl_2$ diminishing binding to the nucleoside.

The last possibilities involve N(1)–O(6) chelation and N(7)–O(6) chelation. These possibilities are more difficult to rule out. The affinity of N^2,N^2-dimethylguanosine for the hard metal ions argues against much N(1) involvement, but some involvement cannot be excluded. Binding at N(7), even as part of a chelate, is not indicated in any of the data and seems most unlikely. It does seem clear that O(6) metal binding can facilitate deprotonation, and therefore this is a chemically feasible means for base mispairing.

In this report, we have concentrated our discussion on 6-oxopurine nucleosides. We believe the "basic" Me_2SO conditions used here may afford the opportunity to more fully utilize Me_2SO to investigate weak interactions. In studies on uridine (Table S11), we find $HgCl_2$ binds to N(3) as is found in H_2O whereas under neutral conditions, no binding is observed. Additionally, $Ba(NO_3)_2$ has a small effect on the shifts of uridine base resonances only in the presence of base. With $HgCl_2$, adenosine and cytidine also exhibit different patterns of shifts in the presence and absence of base, but preliminary studies indicate rather complex processes are probably occurring, and the investigation was not pursued. However, the solvent system may be of value in studies not involving metal species or in studies of other classes of biomolecules.[25]

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Registry No. Guanosine, 118-00-3; 2′-deoxyguanosine, 961-07-9; inosine, 58-63-9; 1-methylguanosine, 2140-65-0; N^2,N^2-dimethylguanosine, 2140-67-2; $Ba(NO_3)_2$, 10022-31-8; $La(NO_3)_3$, 10099-59-9; $Lu(NO_3)_3$, 10099-67-9; $Pr(NO_3)_3$, 10361-80-5; $Pb(NO_3)_2$, 10099-74-8; $Zn(NO_3)_2$, 7779-88-6; $HgCl_2$, 7487-94-7; *cis*-$Pb(Me_2SO)_2Cl_2$, 39336-39-5.

Supplementary Material Available: Tables S1–11, comparing the effects of various salts on the ^{13}C NMR chemical shifts of inosine, guanosine, and some relevant guanosine derivatives in the presence and absence of nitrogen bases (12 pages). Ordering information is given on any current msthead page.

(25) Other observations worthy of brief mention follow. The 2′-deoxyguanosine data are consistent with the binding sites discussed here. When the heterocyclic base is not a good metal binder (such as 1-methylguanosine (Table IV) and uridine (Table S11)) and under basic conditions with $Pr(NO_3)_3$, there are shifts in the ribose resonances suggestive of interaction with the hydroxyl or deprotonated hydroxyl groups.