Nucleoside Complexing. A ^{13}C NMR Spectroscopic Investigation of the Metal Binding Sites in 7-Methylguanosine, 7-Methylinosine and some Related New Synthetic Betains

KAZUO SHINOZUKA, KENNETH WILKOWSKI, BARBARA L. HEYL and LUIGI G. MARZILLI*

Department of Chemistry, Emory University, Atlanta, Ga. 30322, U.S.A.

Received October 19, 1984

Abstract

Once deprotonated, both the N1 and O6 positions of 6-oxopurine nucleosides become important metal binding sites. In a continuation of our studies of metal binding to 6-oxopurines alkylated at N7 and deprotonated at N1, we have carried out a ^{13}C NMR spectroscopic study of the binding of various metal species including hard metal species (Sr, Ba, La, Pr), intermediate metal species (Zn, Cd, Pb), and soft metal species (Pt, Hg). A detailed study was not possible with $HgCl_2$ since mercuration occurred readily at C8. The ^{13}C NMR shift patterns for the O6 resonance of 7-methylguanosine, 7-methylinosine, 2-dimethylamino-7,9-dimethylhypoxanthinium betain, 2-diethylamino-7-methyl-9-propylhypoxanthinium betain and ethylamino and 6 thio analogs of the latter betain suggest that metal species of intermediate 'softness' prefer endocyclic N1 binding over exocyclic O6 to a larger extent than they prefer endocyclic N3 binding over exocyclic O2 binding in cytosine derivatives. Most dramatically, the presence of a dialkylamino group *ortho* to the endocyclic binding site does not appear to prevent N binding in the betains whereas such binding is greatly, if not completely, prevented in cytosine derivatives. In particular, the complex *cis*-$[Pt(Me_2SO)_2Cl_2]$ forms a complex with 2-dimethylamino-7,9-dimethyl-hypoxanthinium betain with an upfield shift characteristic of endocyclic N binding. The hard metal salts, $Ba(NO_3)_2$ and $Pr(NO_3)_3$ interacted weakly, if at all, with 2-diethylamino-7-methyl-9-propyl-6-thiopurinium betain whereas the nitrate salts of Zn, Cd and Pb gave pronounced upfield shifts of C6. This result is consistent with coordination at the exocyclic S.

The compound, 2-dimethylamino-9-methylhypoxanthine, was prepared starting from 5-amino-4,6-dihydroxy-2-dimethylaminopyrimidine. This 5-aminopyrimidine derivative and methyl isothiocyanate were converted to N-(4,6-dihydroxy-2-dimethylamino-5-pyrimidinyl)-N′-methylthiourea which was then converted to 2-dimethylamino-6-hydroxy-9-methyl-8-purinethiol with hot hydrochloric acid. Raney nickel desulfurization in a basic solution gave 2-dimethylamino-9-methylhypoxanthine.

The related compounds, 2-ethylamino-9-propyl- and 2-diethylamino-9-propylhypoxanthine, were prepared from 4,6-diamino-2-methylmercapto-5-nitrosopyrimidine. Treatment of this pyrimidine with dimethyl-, ethyl- and diethyl-amine led to the initial intermediate 2-alkyl-amino-4,6-diamino-5-nitrosopyrimidines. Treatment of these pyrimidines with sodium hydrosulfite, formic acid and formamide in one flask gave the mixture of corresponding 2-alkylaminoadenines and 2-alkylamino-6-formamidopurines. The latter compounds were successfully converted to the desired 2-alkylaminoadenines by alkali treatment. Alkylation with alkyl halides at the 9-positions of these adenine derivatives and subsequent deamination with nitrous acid gave 2-dimethylamino-9-methyl-, 2-ethylamino-9-propyl- and 2-diethylamino-9-propylhypoxanthines.

These hypoxanthines were further methylated at their 7-position to give the corresponding hypoxanthinium betains.

2-Diethylamino-7-methyl-9-propyl-6-thiopurinium betain was prepared by the methylation at the 7-position of 2-diethylamino-9-propylhypoxanthine followed by 6-chlorination and, then, by treatment with thiourea.

Introduction

The belief that the anti-tumor properties of some platinum(II) drugs [1] arise from binding to the nucleic acid bases of DNA [2] has stimulated an increase in research into the interactions of metals with nucleic acids [3]. The anti-tumor drug *cis*-$Pt(NH_3)_2Cl_2$ = (cis Pt) and its derivatives interact preferentially with the guanine bases of DNA [4]. Several theories exist to explain the mode

*Author to whom correspondence should be addressed.

0020-1693/85/$3.30

of interaction of the Pt drugs with the guanine bases [4].

One proposal suggests the formation of an intrastrand crosslink between the Pt atom and two guanine bases [4]. A second theory that has been proposed involves the formation of a chelate between the exocyclic oxygen on the guanine and one of the endocyclic nitrogens. One chelate, between N7–O6 [2], could facilitate mispairing upon DNA replication. A second type of chelate could involve N1 and O6 [5]. This could either be a chelate between the N1 and O6 of a single guanine [6], or a bridging structure between two bases, similar to the structure that has been observed in the platinum pyrimidine blues [7].

X-ray structures that support the chelate formation involving the exocyclic O6 are lacking. Gelbert *et al.* reported a tetranuclear copper complex with two inosine monophosphate ligands bound through both N7 and N1 and O6 of each ligand [8]. But in several structures using 7-alkylated guanine derivatives with Pt(II), Marzilli *et al.* found binding of the Pt(II) only to N1 of the purine base [9–12].

Solution studies which lend support to O6 involvement in the binding of metals to nucleosides are also limited. ^{13}C NMR spectroscopic evidence does exist to support the binding of metals to cytidine and some of its derivatives [13]. In these studies binding to the exocyclic O2 of cytidine was observed with hard metal ions like Ba^{2+}. In addition, lanthanide ions, which bind oxygen preferentially, caused large shifts of the C2 resonance. Such large shifts with paramagnetic metal ions are consistent with O2 binding [13]. In a similar study with guanosine, oxygen interaction with Ba^{2+} is also observed when the base triethylamine is present. This result suggests that N1 deprotonation is required for O6 binding of hard metal ions. Soft metal species were shown to bind to N1 after deprotonation had occurred [14]. Abbott and Woods [15] also used ^{13}C NMR spectroscopy to study the interaction of $[Rh(PPh_3)_2(CO)_3]^+$ with guanosine and inosine under neutral and basic conditions. They observed that O6 binding resulted in an increase in the acidity of the N1 proton.

All of these studies have shown that ^{13}C NMR spectroscopy can be an effective method to probe metal interactions with nucleic acids. We are interested in the use of ^{13}C NMR shifts to define weak metal binding sites in nucleobases. In this report, ^{13}C NMR spectroscopy was used to study the metal binding properties of 7-methylinosine (7-MeIno), 7-methylguanosine (7-MeGuo). The related betain analogs, 2-dimethylamino-7,9-dimethylhypoxanthinium betain (DMA-7,9-DMH), 2-diethylamino-7-methyl-9-propylhypoxanthinium betain (DEA-7-M-9-PH), and 2-ethylamino-7-methyl-9-propylhypoxanthinium betain (EA-7-M-9-PH) with metal species will be reported and compared. These 6-oxopurine derivatives have N7 blocked by a methyl group, so that N1 is deprotonated at neutral conditions, making N1 a favorable site for electrophilic attack by metal ions and the use of a base for deprotonation unnecessary.

Initially, it was thought that the dimethylamino group would sterically block metal binding at N1, thus favoring only O6 binding, as seen in the similar N_4,N_4-dimethyl-1-methylcytosine (DMC) [13]. Evidence will be presented that shows that even with a diethylamino group present, N1 binding is still possible in DEA-7-M-9-PH. This unexpected result prompted us to prepare 2-diethylamino-7-methyl-9-propyl-6-thiopurinium betain (DEA-7-M-9-PT) where the hard 6-oxo group is replaced by the soft 6-thio group.

Experimental

Materials

The nucleosides, 7-methylinosine and 7-methylguanosine, were obtained from Vega Biochemicals. Thiourea (99+% gold label grade), methyl isothiocyanate, 1-bromopropane and methyl p-toluenesulfonate were all obtained from Aldrich. The metal salts supplied by Fisher were $Ba(NO_3)_2$, $Pb(NO_3)_2$, $La(NO_3)_3 \cdot 6H_2O$ and $Zn(NO_3)_2 \cdot 6H_2O$. The metal salts $Pr(NO_3)_3 \cdot 5H_2O$ and $Cd(NO_3)_2 \cdot 4H_2O$ were from Alfa. The compound, *cis*-$[Pt(Me_2SO)_2Cl_2]$, was prepared by a literature method [16].

NMR Studies

^1H NMR spectra were obtained on either a Nicolet 360-NB spectrometer operating at 360 MHz or a Varian EM-390 spectrometer operating at 90 MHz. Spectra obtained in DMSO-d_6 solution were referenced to tetramethylsilane, while spectra obtained in D_2O solution were referenced to 3-(trimethylsilyl)-propionic acid, sodium salt. ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrometer operating at 20 MHz or an IBM WP200SY spectrometer operating at 50.327 MHz. DMSO-d_6 was used as the solvent with tetramethylsilane as an internal standard. The ligand concentration was 0.1 M, except as noted.

Synthesis

2-Ethylamino-7-methyl-9-propylhypoxanthinium Betain

4,6-Diamino-2-methylmercapto-5-nitrosopyrimidine [17] (5 g) was heated at reflux in ethylamine (150 ml, 20% aqueous solution) for 20 min with vigorous stirring. The reaction mixture was filtered while hot to remove some insoluble matter and the filtrate was cooled in an ice-bath. The tan-colored *4,6-diamino-2-ethylamino-5-nitrosopyrimidine* was collected and recrystallized from water (yield = 82%), m.p. 193–195 °C. *Anal.* Calcd for $C_6H_{10}N_6O$·

H_2O: C, 35.99; H, 6.04; N, 41.98. Found: C, 36.01; H, 6.09; N, 41.86%.

The material (5 g) was suspended in a mixture of formic acid (8 ml, 90%) and formamide (50 ml). To this suspension was added sodium hydrosulfite (0.0006 to 0.02 mol) in very small portions at ~100–110 °C with stirring. After the addition was complete, the solution was stirred at the same temperature for another 10 min; the temperature was then raised to ~190–200 °C and maintained for 30 min. The cooled reaction mixture was poured into cold water (200 ml) and the deposited crystals were collected. The crystals were treated with 1 N NaOH (70 ml) at ~50–60 °C for 1 h then the pH of the solution was adjusted to ~8–9 with dil. HCl. The resulting *2-ethylaminoadenine* was collected and recrystallized from water (yield = 57%) m.p. >228 °C (decomp.). ^{1}H NMR (DMSO-d_6): 7.70, S, H-8; 6.58, br, NH_2; 6.05, t, *J*6, NH; 3.29, quin, *J*6, CH_2 of Et; 1.12, t, *J*6, CH_3 of Et. (For these data and subsequent NMR data, shifts are in ppm and couplings are designated as J6, which is 6 Hz). *Anal.* Calcd for $C_7H_{10}N_6$: C, 47.18; H, 5.66; N, 47.17. Found: C, 47.29; H, 5.43; N, 47.06%.

This material (2.5 g) was added to the suspension of NaH (0.4 g) in dry dimethylacetamide (70 ml) and stirred 1 h under an atmosphere of N_2. 1-Bromopropane (2.1 g) was added dropwise and the mixture was stirred at 50–55 °C for 12 h. After evaporation of the solution to dryness, the residue was triturated with cold water. *2-Ethylamino-9-propyladenine* was recrystallized from water (yield = 85%), m.p. 163–164 °C. ^{1}H NMR (DMSO-d_6): 7.73, s, H-8; 6.62, br, NH_2; 6.18, t, *J*7, NH; 3.96, t, *J*7, CH_2 of Pr; 3.32, quin, *J*7, CH_2 of Et; 1.77, m, *J*7, CH_2 of Pr; 1.12, t, *J*7, CH_3 of Et; 0.84, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{10}H_{16}N_6$: C, 54.52; H, 7.32; N, 38.16. Found: C, 54.41; H, 7.38; N, 38.07%.

To the solution of this adenine derivative (1.1 g in 40 ml of 10% H_2SO_4) was added dropwise, with stirring, a solution of $NaNO_2$ (15 ml, 20%) at room temperature. After the addition was complete, the mixture was stirred for another hour then neutralized with conc. NH_4OH to precipitate the product. Recrystallization from hot water gave bright yellow *2-(N-nitrosoethylamino)-9-propylhypoxanthine* (yield = 91%), m.p. >195 °C (decomp.). ^{1}H NMR (DMSO-d_6): 8.10, s, H-8; 4.11, quar, *J*7, CH_2 of Et and CH_2 of Pr; 1.83, m, *J*7, CH_2 of Pr; 1.08, t, *J*7, CH_3 of Et; 0.87, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{10}H_{14}N_6O_2$: C, 47.99; H, 5.64; N, 23.58. Found: C, 48.08; H, 5.69; N, 33.47%.

Brief treatment of 0.2 g of material with sodium hydrosulfite (0.5 to 1 g) in hot water (60–80 °C) gave a crude product. Recrystallization from H_2O gave the desired *2-ethylamino-9-propylhypoxanthine* (yield = 87%), m.p. 243–245 °C. ^{1}H NMR (DMSO-d_6): 7.73, s, H-8; 6.89, t, *J*7, NH; 3.93, t, *J*7, CH_2 of Pr; 3.30, quin, *J*7, CH_2 of Et; 1.75, m, *J*7, CH_2 of Pr; 1.16, t, *J*7, CH_3 of Et; 0.85, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.41; H, 6.80; N, 31.42%.

The above hypoxanthine (0.5 g) and dimethylsulfate (0.35 g) in dry dimethylacetamide (20 ml) were heated at ~125–135 °C for 1 h. After cooling, conc. NH_4OH was added to adjust the pH to 9–10; then an excess of diethylether was added. The resulting precipitate was collected and dissolved in 5 N NH_4OH (50 ml). This solution was stirred at room temperature overnight. Evaporation of this solution gave a residue. Recrystallization of the residue gave *2-ethylamino-7-methyl-9-propylhypoxanthinium betain* (yield = 79%). However, the elemental analysis indicated that the compound is half-protonated. Several attempts to obtain fully deprotonated hypoxanthinium betain were not successful. M.p. 215–216 °C. ^{1}H NMR (DMSO-d_6): 9.90, s, H-8; 7.62 t, *J*7, NH; 4.33, t, *J*7, CH_2 of Pr; 4.03, s, Me-7; 3.35, quin, *J*7, CH_2 of Et; 1.85, m, *J*7, CH_2 of Pr; 1.15, t, *J*7, CH_3 of Et; 0.92, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{11}H_{17.5}N_5O \cdot 0.5CH_3SO_4 \cdot 0.5H_2O$: C, 45.99; H, 6.71; N, 23.32. Found: C, 45.71; H, 6.58; N, 23.21%.

2-Diethyalmino-7-methyl-9-propylhypoxanthinium Betain

The first intermediate, *4,6-diamino-2-diethylamino-5-nitrosopyrimidine,* was prepared as its 2-ethylamino analog but using diethylamine (20% aqueous solution). This compound was recrystallized from water (yield = 78%), m.p. 170–171 °C. ^{1}H NMR (DMSO-d_6 360 MHz): 3.35, s, NH_2(2); 2.84, quar, *J*5, CH_2 of Et; 1.13, t, *J*5, CH_3 of Et. *Anal.* Calcd for $C_8H_{14}N_6O$: C, 45.70; H, 6.71; N, 39.98. Found: C, 45.61; H, 6.73; N, 39.94%.

This nitrosopyrimidine (10.5 g) was suspended in a mixture of formic acid (8 ml, 90%) and formamide (50 ml) then treated with sodium hydrosulfite (0.0006 to 0.02 mol) in a similar manner as its 2-ethyl analog. The resulting solution was heated to ~170–180 °C for 20 min. The cooled reaction mixture was poured into cold water (300 ml). The resulting *2-diethylamino-6-formamidopurine* was recrystallized from water (yield = 25%), m.p. >225 °C (decomp.). ^{1}H NMR (DMSO-d_6): 9.86, br, COH; 7.97, s, H-8; 3.61, quar, *J*5, CH_2 of Et; 1.17, t, *J*5, CH_3 of Et. *Anal.* Calcd for $C_{10}H_{14}N_6O$: C, 51.27; H, 6.02; N, 35.88. Found: C, 51.20; H, 6.01; N, 35.94%.

The filtrate of the foregoing reaction was treated with dil. NH_4OH to pH ~8 to 9 and the resulting *2-diethylaminoadenine* was collected. Brief treatment of the above 6-formamidopurine with 1 N NaOH (60 °C 1 h) also gave the same adenine, in almost quantitative yield. The total yield of this

adenine from the nitrosopyrimidine was 86%, m.p. 207–208 °C. ^{1}H NMR (DMSO-d_6): 7.83, s, H-8; 6.50, br, NH_2; 3.55, quar, *J*5, CH_2 of Et; 1.11, t, *J*6, CH_3 of Et. *Anal.* Calcd for $C_9H_{14}N_6$: C, 52.41; H, 6.84; N, 40.73. Found: C, 52.46; H, 6.85; N, 40.70%.

This substituted adenine (5 g) was dissolved in an ethanol solution of NaOH (1.4 g/60 ml). To this solution was added dropwise 1-bromopropane (2.6 ml) and the solution was stirred at 60–65 °C for 2 h then at room temperature overnight. Evaporation and recrystallization of the residue from water gave *2-diethylamino-9-propyladenine* (yield = 62%), m.p. 206–207 °C. ^{1}H NMR (DMSO-d_6): 7.66, s, H-8; 6.53, br, NH_2; 3.94, t, *J*6, CH_2 of Pr; 3.57, quar, *J*6, CH_2 of Et; 1.77, m, *J*6, CH_2 of Pr; 1.11, t, *J*6, CH_3 of Et; 0.83, t, *J*6, CH_3 of Pr. *Anal.* Calcd for $C_{12}H_{20}N_6$: C, 58.04; H, 8.12; N, 33.85. Found: C, 57.97; H, 8.22; N, 33.76%.

The above material was converted to *2-diethylamino-9-propylhypoxanthine* by the method used for the 2-ethylamino analog. The product was recrystallized from water (yield = 73%), m.p. 244–246 °C. ^{1}H NMR (DMSO-d_6): 7.66, s, H-8; 3.91, t, *J*7, CH_2 of Pr; 3.52, quar, *J*6, CH_2 of Et; 1.73, m, *J*7, CH_2 of Pr; 1.11, t, *J*6, CH_3 of Et; 0.82, t. *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{12}H_{19}N_5O$: C, 57.81; H, 7.68; N, 28.09. Found: C, 57.83; H, 7.70; N, 28.02%.

The treatment of this hypoxanthine with dimethylsulfate as above gave *2-diethylamino-7-methyl-9-propylhypoxanthinium betain.* However, the product was extracted with chloroform from NH_4OH solution and recrystallized from acetonitrile (yield = 66%), m.p. >244 °C (decomp.). ^{1}H NMR (DMSO-d_6): 8.79, s, H-8; 4.00, t, *J*7, CH_2 of Pr; 3.98, s, Me-7; 3.49, quar, *J*7, CH_2 of Et; 1.82, m, *J*7, CH_2 of Pr; 1.04, t, *J*7, CH_3 of Et; 0.85, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{13}H_{21}N_5O$: C, 59.29; H, 8.04; N, 26.60. Found: C, 59.02; H, 8.05; N, 26.46%.

2-Diethylamino-7-methyl-9-propyl-6-thiopurinium Betain

The mixture of 2-diethylamino-9-propylhypoxanthine (2.9 g) and methyl *p*-toluenesulfonate (2.8 g) in dry dimethylacetamide (50 ml) was heated at 125–130 °C for 1 h. Evaporation and treatment of the residue with THF (100 ml) gave white crystals of, presumably, *2-diethylamino-7-methyl-9-propylhypoxanthinium-p-toluenesulfonate* (yield = 81%). This compound was used in the next step without further purification.

The above material (4.5 g) was heated at reflux in phosphoryl chloride (70 ml) for 2 h. After evaporation, the resulting gum was dissolved in cold ethanol (70 ml) and thiourea (3.1 g) was added to this solution. The mixture was heated at reflux for 2 h then chilled in an ice-bath. Dry ammonia gas was bubbled through this chilled solution for 30 min. This procedure caused the precipitation of an inorganic salt. The mixture was left at room temperature overnight. After removal of the salt, the filtrate was evaporated and the resulting residue was dissolved in chloroform (50 ml). The chloroform layer was washed with very dilute NH_4OH (30 ml × 2) in a separatory funnel. Evaporation and treatment of the residue with benzene gave *2-diethylamino-7-methyl-9-propyl-6-thiopurinium betain* (yield = 74%) which was recrystallized from benzene, m.p. 198–200 °C. ^{1}H NMR (DMSO-d_6): 9.06, s, H-8; 4.39, s, Me-7; 4.09, t, *J*7, CH_2 of Pr; 3.58, quar, *J*7, CH_2 of Et; 1.83, m, *J*7, CH_2 of Pr; 1.12, t, *J*7, CH_3 of Et; 0.90, t, *J*7, CH_3 of Pr. *Anal.* Calcd for $C_{13}H_{21}N_5S$: C, 55.88; H, 7.58; N, 25.07; S, 11.47. Found: C, 55.82; H, 7.61; N, 24.98; S, 11.54%.

2-Dimethylamino-7,9-dimethylhypoxanthinium Betain

4,6-Dihydroxy-2-dimethylaminopyrimidine [18] (12.2 g) was nitrosated in the usual manner to give *4,6-dihydroxy-2-dimethylamino-5-nitrosopyrimidine* (yield = 93%). The crude material (13.5 g) was reduced with sodium hydrosulfite to give *5-amino-4,6-dihydroxy-2-dimethylaminopyrimidine* (yield ~67%). This compound was used immediately in the next step since it became highly colored upon prolonged exposure to air.

The compound (~8 g) was dissolved in 1 N NaOH (160 ml). To this solution was added methyl isothiocyanate (~6 ml) at 60 to 70 °C and the solution was stirred at this temperature for 4 h. The color of the reaction mixture changed from blue to orange during the reaction. After cooling, the solution was acidified with acetic acid and allowed to stand at room temperature for at least 6 h. The resulting *N-(4,6-dihydroxy-2-dimethylamino-5-pyrimidinyl)-N'-methylthiourea* (yield ~61%) was collected. It was used immediately in the next step since it became highly colored upon prolonged exposure to air.

The above urea (~7 g) was heated at 70–80 °C in conc. HCl (100 ml) for 5 h with stirring. After cooling, the mixture was diluted with water (100 ml) and *2-dimethylamino-6-hydroxy-9-methyl-8-purinethiol* (~80%) was collected and washed with water. This compound was used in the next step without further purification or characterization.

The above material (5 g) was dissolved in 1 N NaOH (100 ml) and Raney nickel (20 g wet weight) was added to this solution. The mixture was heated at reflux overnight with stirring. After removal of Raney nickel by filtration, the filtrate was neutralized with acetic acid. The resulting *2-dimethylamino-9-methylhypoxanthine* (yield = 91%) was

recrystallized from water, m.p. >238 °C (decomp.). ^{1}H NMR (DMSO-d_6): 7.65, s, H-8; 3.56, s, Me-9; 3.10, s, N(Me)$_2$. This compound was identical to that prepared in this laboratory by a slightly different method [19]. It was also prepared in good yield by methylation of 2-dimethylaminoadenine [20] with methyl iodide and subsequent deamination of the resulting 2-dimethylamino-9-methyladenine [21] with nitrous acid.

The above material was converted to *2-dimethylamino-7,9-dimethylhypoxanthinium betain* (yield = 84%) by the method used above. However, the product was precipitated from NH_4OH solution with an excess of acetone. The compound was recrystallized from n-propanol, m.p. >272 °C. (decomp.). ^{1}H NMR (D_2O): 4.03, s, Me-7; 3.57, s, Me-9; 3.09, s, N(Me)$_2$. *Anal.* Calcd for $C_9H_{13}N_5O \cdot 1.5H_2O$: C, 46.13; H, 6.88; N, 29.91. Found: C, 45.80; H, 6.53; N, 29.85%.

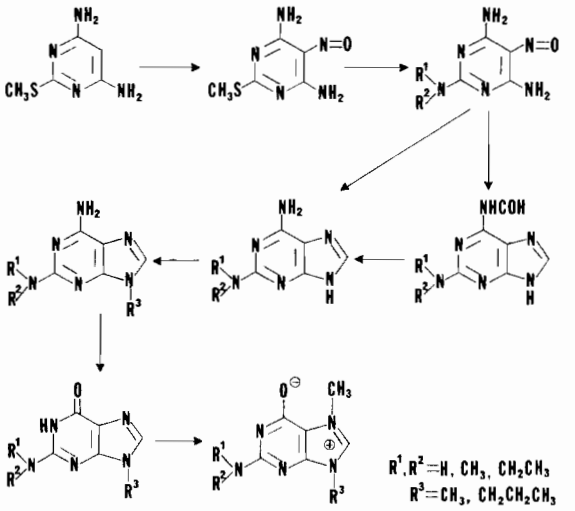
Results

This section is organized as follows. First, we discuss the synthetic strategy utilized in the synthesis of the betains. Next, we consider the methods of assigning structures and spectra. Third, we present our spectroscopic data.

For the synthesis of purinium betains, two approaches were used to prepare the key 2-alkylamino-9-alkylhypoxanthines intermediates.

The first approach applied Koppel and Robins' general method [22] for the synthesis of 9-alkylguanine. In this method, the 2-alkylamino group is introduced during the formation of the pyrimidine ring by the reaction of N,N-dialkylguanidine hydrochloride with diethyl malonate. The 9-alkyl group was introduced during the formation of the purine ring by the reaction of 5-amino-2-dialkylamino-4,6-dihydroxypyrimidine with alkyl isothiocyanate. However we found that this approach was not sufficiently versatile. It requires several different N-alkylguanidine derivatives; except for the N,N-dimethyl analog these derivatives are either difficult to prepare or very expensive. The reactions of alkyl isothiocyanates are inconvenient. Furthermore, this method gives unstable intermediates in several steps. The alkyl isothiocyanates commercially available are expensive and limited in number.

In the second method, the 2-alkylamino group was introduced after the formation of the pyrimidine ring by the reaction of 4,6-diamino-2-methylmercapto-5-nitrosopyrimidine with alkylamines. The 9-alkyl group was introduced after the formation of the purine ring by the reaction of 2-alkylaminoadenines with alkyl halides. 4,6-Diamino-2-methylmercapto-5-nitrosopyrimidine is highly reactive with a variety of alkylamines, which are easily obtained from commercial sources. Also alkylations of 2-alkylamino adenines were easily achieved with com-



mon alkylhalides, such as methyl iodide or 1-bromopropane, in satisfactory yields. Thus, although more separate steps are required, this latter approach is superior.

The ^{13}C NMR asignments were made by comparison with literature values for inosine [23], guanosine [23], and 7-MeGuo [24]. For the 7-MeIno ligand, the C2 and C6 resonances were differentiated by a proton non-decoupled experiment since the C2 resonance would be split by its attached proton. For the synthetic betains, the assignment of C6 was made by the use of Pr^{3+}. The lanthanides have a known preference for oxygen and, for the betain ligands, should have the greatest effect on the C6 resonance. For example, at low concentrations of Pr^{3+} the C6 resonance of DMA-7,9-DMH is shifted drastically (~7.5 ppm) and, at high Pr^{3+} concentration, the C6 resonance disappears completely. This behavior is similar to that obtained with guanosine [14].

The betain type structure of the ligands studied here make the H-8 proton acidic due to the electron deficient imidazole ring [25]. Nitrate salts were used because it has been shown that Cl^- has the ability to hydrogen bond to the bases and sugars of the nucleosides [13, 26].

The spectroscopic data are assembled as follows. In Table I we present the standard spectral shift values we used for each ligand studied. In subsequent tables, we give the shift differences for each resonance and specify the metal species and the concentration employed. From these two types of data, the actual shift values observed on addition of metal salts can be calculated. Since the nature of the experiments was such as to generate a considerable amount of data involving signals unaffected

TABLE I. ^{13}C NMR Spectra (PPM) of Betains Used in This Study.[a]

Betain	C2	C4	C5	C6	C8	7CH_3
7-MeIno	156.78	146.97	114.22	162.77	135.67	35.25
7-MeGuo	162.90	149.25	108.68	163.08	132.46	35.02
DMA-7,9-DMH	162.22	149.92	107.64	162.37	133.60	34.48
DEA-7-M-9-PH	161.16	149.89	107.84	162.87	132.63	34.54
EA-7-M-9-PH[b]	158.31	149.79	107.39	159.09	136.01	35.07
DEA-7-M-9-PT	158.05	146.67	117.62	178.90	135.85	36.27

[a]All ligands were at 0.1 M except 7-MeIno which was at 0.2 M. DMSO-d_6, 32 °C. For DMA-7,9-DMH; $(CH_3)_2$, 36.85; 9-CH_3, 29.97. For DEA-7-M-9-PH: $PrCH_2$, 45.19, 21.72, CH_3, 10.69, $EtCH_2$, 40.90, CH_3, 13.42. For DEA-7-M-9-PT: These values are 45.36, 21.65, 10.71, 41.08, 13.24. For EA-7-M-9-H: These values are 45.75, 21.71, 10.61, 35.51, 14.35. [b]This compound is half protonated.

TABLE II. Comparative Shifts of the ^{13}C NMR Resonances of 7-MeIno.[a]

Salt	[M]	C2	C4	C5	C6	C8	7CH_3
CF_3CO_2H	XS	6.64	1.07	−1.36	9.74	−3.35	−0.77
$Sr(NO_3)_2$	0.40	0.36	−0.19	0.24	−0.48	−0.83	−0.10
$Ba(NO_3)_2$	0.40	0.32	−0.09	0.17	−0.46	−0.66	−0.09
$La(NO_3)_3$	0.50	1.11	−0.40	0.55	−0.64	−2.02	0.18
$Pr(NO_3)_3$	0.01	0.20	0.00	−0.56	1.81	−0.28	0.00
$Zn(NO_3)_2$	0.40	0.72	0.24	−0.53	3.23	−2.17	−0.44
$Pb(NO_3)_2$	0.40	2.36	0.09	−1.45	1.33	−2.23	−0.31
$Cd(NO_3)_2$	0.40	0.47	−0.05	−0.23	2.72	−2.07	−0.40
cis-[$Pt(Me_2SO)$(7-MeIno)Cl_2]	0.20	−1.95	1.90	0.44	4.73	−2.06	−0.48

[a]− denotes downfield shift, + denotes upfield shift.

TABLE III. Comparative shifts of the ^{13}C NMR Resonances of 7-MeGuo.

Salt	M	C2	C4	C5	C6	C8	7CH_3
$Ba(NO_3)_2$	0.04	0.63	−0.45	0.79	−1.02	−1.21	−0.06
$Pr(NO_3)_3$	0.003	0.27	−0.11	0.34	0.83	−0.66	−0.05
$Zn(NO_3)_2$	0.2	2.94	−0.80	1.56	3.12	−3.09	−0.45
$Pb(NO_3)_2$	0.4	3.58	−0.37	−0.44	2.08	−3.11	−0.36

or minimally affected by complex formation, the sugar resonances have been omitted.

Tables II and III present comparative shifts for the 7-methylated nucleosides, 7-MeIno and 7-MeGuo, respectively. Tables IV and V contain data for the 7-MeGuo analogs where the ribose sugar at the 9 position and the amino group at the 2 position have been replaced by 9-methyl and 2-dimethylamino (DMA-7,9-DMH) groups or by 9-propyl and 2-diethylamino groups (DEA-7-M-9-PH), respectively. Table VI contains data for an analog similar to that in Table V, except that the 6-oxo group has been replaced by a 6-thio group (DEA-7-M-9-PT).

Discussion

This work arose from a continuation of our interest in the involvement of the 6-oxo group of purines in complexation to metal species, as described in the Introduction. In previous studies of metal binding to nucleosides and nucleoside analogs we were able to assign binding sites by evaluation of the direction of ^{13}C shifts on complex formation (− downfield or + upfield) in combination with the use of the following: (a) a bulky substituent adjacent to the presumed endocyclic metal binding site, (b), substitution of a 'hard' oxo exocyclic group by a

TABLE IV. Comparative Shifts of the ^{13}C NMR Resonances of DMA-7,9-DMH.[a]

Salt	[M]	C2	C4	C5	C6	C8	$(CH_3)_2$	$7CH_3$	$9CH_3$
H^+	XS	8.13	0.66	1.68	8.28	−5.46	−0.76	−0.66	−0.76
$Ba(NO_3)_2$	0.4	0.21	−0.15	0.58	−0.79	−1.02	−0.17	−0.13	−0.07
$La(NO_3)_3$	0.2	0.71	−0.43	1.07	−0.32	−2.36	−0.27	−0.43	−0.27
$Pr(NO_3)_3$	0.05	−0.05	−1.24	−3.80	−7.44	−1.59	−0.42	−0.23	−0.42
$Zn(NO_3)_2$	0.4	2.45	−1.09	2.12	0.51	−4.37	−0.55	−0.59	−0.55
$Ph(NO_3)_2$	0.4	3.03	−0.33	−0.09	−0.13	−3.85	−0.39	−0.46	−0.39
$Cd(NO_3)_2$	0.4	2.53	−0.90	1.89	−0.52	−4.25	−0.59	−0.56	−0.57
$Pt(DMSO)(DMA\text{-}7,9\text{-}DMH)Cl_2$	0.1	8.55	−0.11	0.79	1.93	−1.37	−0.22	−0.17	−0.22

[a]– denotes downfield shift, + denotes upfield shift.

TABLE V. Comparative Shifts of the ^{13}C NMR Resonances of DEA-7-M-9-PH.[a]

Salt	M	C2	C4	C5	C6	C8	$CH_2(Pr)$	$CH_2(Et)$	$7CH_3$	$CH_2(Pr)$	$CH_3(Et)$	$CH_3(Pr)$
$Ba(NO_3)_2$	0.4	0.26	−0.22	0.34	−0.52	−1.15	−0.12	−0.11	−0.19	0.02	0.07	0.03
$Pr(NO_3)_3$	0.05	−0.23	−1.34	−3.82	−9.19	−1.89	−0.45	−0.06	−0.28	−0.20	0.12	−0.14
$Zn(NO_3)_2$	0.4	2.91	−0.95	2.05	1.33	−4.78	−0.70	−0.47	−0.65	0.12	0.49	0.11
$Pb(NO_3)_2$	0.4	3.07	−0.45	−0.36	−0.39	−4.33	−0.54	−0.79	−0.58	0.07	0.51	0.03
$Cd(NO_3)_2$	0.4	2.14	−1.04	1.91	−0.13	−4.52	−0.69	−0.46	−0.60	0.08	0.35	0.05

[a]– denotes downfield shift, + denotes upfield shift.

TABLE VI. Comparative Shifts of the ^{13}C NMR Resonances of DEA-7-M-9-PT.

Salt[a]	C2	C4	C5	C6	C8	$CH_2(Pr)$	$CH_2(Et)$	$7CH_3$	$CH_2(Pr)$	$CH_3(Et)$	$CH_3(Pr)$
$Ba(NO_3)_2$	0.05	0.10	0.15	0.39	−0.11	0.08	0.09	0.13	0.09	0.06	0.12
$Pr(NO_3)_3$	0.06	−0.04	0.11	0.45	−0.32	0.03	−0.04	0.10	0.09	0.05	0.09
$Zn(NO_3)_2$	0.41	−1.79	1.71	12.29	−4.00	−0.47	−0.12	−0.24	0.17	0.33	0.08
$Pb(NO_3)_2$	2.21	−1.33	−0.40	10.61	−4.50	−0.51	−1.00	−0.54	0.15	0.47	0.03
$Cd(NO_3)_2$	0.53	−1.70	1.58	10.94	−4.35	−0.55	−0.43	−0.32	0.16	0.41	0.09

[a]All 0.4 M.

'soft' thio exocyclic group, (c), utilization of paramagnetic shifts caused by Ln^{3+} ions, and (d), comparative studies of very hard and very soft metal species, as well as hydrogen ion binding.

Platination of N7 of guanine derivatives causes the N1H to increase in acidity by ~2 p*K*a units [5]. The endocyclic N at position 1 could then become further involved in complex formation and deprotonation could, in fact, be facilitated by complex formation at either N1 or O6. Thus, we undertook an investigation of the metal binding of 7-methylated 6-oxopurine nucleosides which have accessible N1 deprotonated species. To provide further substantiation of our binding assignments, we also prepared several synthetic analogs which will be discussed in detail below.

7-MeIno

The data for 7-MeIno were presented in Table II. On protonation, which probably involves N1, C2 and C6 shifts very far upfield >5 ppm whereas C8 shifts downfield by 3.35 ppm, even though this carbon is not in the ring undergoing protonation. This finding with regard to C8 is a general one and we find that the addition of all electrophiles always shifts C8 downfield and, except for the weakest electrophiles, the shift is appreciable. Thus, although C8 shifts in these betain type compounds are useful

in confirming that an interaction does occur, this resonance is not useful in assigning binding sites.

Returning to the C2 and C6 resonances, we note that these carbons are *ortho* to the endocyclic N undergoing protonation and that in the related cytosine ring system such large upfield shifts on protonation are also observed [13].

Treatment of 7-MeIno with hard metal salts ($Ba(NO_3)_2$, $La(NO_3)_3$) causes the oxo bearing carbon (C6) to shift downfield. Likewise, the oxo bearing carbon (C2) in the pyrimidine nucleoside, cytidine (Cyd), shifts downfield when such salts are added [13]. The other carbon *ortho* to the basic ring N, which lacks an oxo substituent, is shifted upfield by these salts. A similar result is found for Cyd [13].

The paramagnetic metal salt, $Pr(NO_3)_3$, for both 7-MeIno and Cyd [13] causes a large shift in the resonance for the oxo bearing carbon and a comparatively small shift for the other *ortho* carbon. The shifts are upfield in all cases.

For nitrate salts of metal ions with intermediate softness but with nevertheless reasonable N binding ability (Zn, Cd, Pb) the similarities between 7-MeIno and Cyd at first inspection may seem to disappear. Thus, Cd and Pb salts induce the same shift changes in Cyd as do the nitrate salts of the harder metal ions [13]. On the other hand, $Zn(NO_3)_2$ induces an upfield shift of the resonances of the oxo-bearing C2 [13]. By contrast, the nitrate salts of Cd, Pb *and* Zn cause upfield shifts of resonance of the oxo-bearing C6. As with Cyd [13], for the metal species discussed thus far (*i.e.* hard or intermediate), the resonance of the non-oxo bearing C shifts upfield. However, as we now note here and will reinforce below, this shift pattern still appears to reflect a preference for N over O. Thus, for C6 we see the pattern of increasing upfield shifts of Pb < Cd < Zn. If we accept our previous evidence, which is quite strong, that this order reflects the increasing preference of its metal for N interaction over O interaction, then the pattern of shifts of the oxo bearing C in Cyd [13], −1.16 ppm, −0.22 ppm and +0.24 ppm for Pb, Cd and Zn, respectively, reflects the same trend as for 7-MeIno, with perhaps a greater preference for exocyclic O binding for Cyd. Turning now to the resonance of the other *ortho* carbon, we note its shift direction is relatively insensitive to any apparent trend in N *vs.* O binding. For Cyd, the shift of this resonance is not much influenced by the nature of the cation. For 7-MeIno, the resonance shifts are more varied.

Addition of very soft metal species such as $HgCl_2$ or $AgNO_3$ was very valuable in previous studies. Unfortunately, these metal species caused precipitation and we also observed facile mercuration at C8. The soft Pt compound, *cis*-$Pt(DMSO)_2Cl_2$ allowed us to evaluate a soft metal species. Pt is known to bind to N1 and we have previously confirmed that *cis*-$Pt(DMSO)_2Cl_2$ forms complexes with 7-MeIno where N1 is bound [27]. We note that C6 shifts upfield, characteristic of N1 binding. Rather surprisingly, C2 shifts downfield. This pattern is contrary to previous findings with Cyd, where protonation and platination shift resonances in the *same* direction (upfield) [13].

For 7-MeIno, we tentatively speculate that the C2 resonance is less sensitive to metal binding than the corresponding C4 resonance of Cyd and that N1 metallation may cause either a small upfield shift as for Zn or, indeed, a downfield shift as for Pt. It is noteworthy that some metal species which appear to relatively favor O binding can cause appreciable upfield shifts of C2. Note the data for Pb and La in Table II.

7-MeGuo

Commercial preparations of this expensive material appear to be partially protonated and we have had great difficulty in securing fully deprotonated samples. Therefore, only limited data have been obtained (Table III). Again, binding at O6 causes a downfield shift of the C6 resonance. From C6, the binding affinity for N1 compared to O6 appears to be in the usual order: Ba < Pb < Zn. Again, the C2 resonance shifts upfield regardless of the binding preference of the salt, and we suspect that when a C2 N substituent is present, the C2 resonance is more sensitive to N binding.

DMA-7,9-DMH

One of the types of evidence that proved to be very useful in evaluating binding sites in Cyd involved replacing the relatively small *ortho* amino group with the bulkier dimethylamino group in DMC. This substitution changed the pattern of shifts for the resonance of C2, the oxo-bearing carbon, such that all metal species examined induced downfield shifts. Consequently, we prepared DMA-7,9-DMH and examined the effects of H^+ and metal salts on its ^{13}C NMR spectrum (Table IV).

As might be expected, $Pr(NO_3)_3$ caused large shifts in the resonance of C6. However, these shifts were to low field in contrast to our findings with 7-MeIno and 7-MeGuo. However, such a change was also observed for DMC compared to Cyd [13]. We *speculate* here that a possible explanation for this effect is that the position of Pr with respect to the ligand or the molecular axis of the Pr complex has changed (*i.e.* the symmetry of the averaged susceptibility tensor) [28]. To test this idea, we prepared EA-7-M-9-PH and found that the only significant shifts on addition of $Pr(NO_3)_3$ (0.002 M) were 0.20, 0.21 and 0.14 for C2, C6 and C8, respectively. It is also of some interest that whereas the

C6 resonance was broadened so severely by $Pr(NO_3)_3$ as to preclude the use of concentrations >0.01 M. Broadening was much less severe for DMA-7,9-DMH and DEA-7-M-9-PH (see below).

For the hard metal salts (Ba, La) the C6 resonance shifts downfield consistent with O6 binding. For the intermediate metal salts, the uniformly consistent upfield shifts are no longer observed. For Cd and Pb, downfield shifts of the C6 resonance are observed as for DMC [13]. However, $Zn(NO_3)_2$ still leads to upfield (+) shift, albeit relatively small compared to 7-MeIno or 7-MeGuo. This finding reinforces the conclusion reached above concerning the greater preference of metals to bind to N over O in these betains compared to cytosine derivatives. Furthermore, *cis*-$Pt(DMSO)_2Cl_2$ *does* form a complex with DMA-7,9-DMH. This complex formation leads to an upfield shift of the C6 resonance, consistent with N1 binding.

As found above, the C2 resonance is relatively uninformative. It shifts upfield except in the case of the paramagnetic $Pr(NO_3)_3$. Similarly, except for C6, the shifts of all the other resonances tend to be influenced in the same direction by all of the metal ions employed and no pattern is evident in the exceptions.

DEA-7-M-9-PH

Since the DMA group did not seem to prevent N1 binding but did induce a pattern of shift changes on addition of the intermediate metal species more indicative of O6 binding, we prepared DEA-7-M-9-PH and examined the effects of addition of metal salts (Table V). The differences between the effects of salts on DMA-7.9-DMH and DEA-7-M-9-PH are typically small to negligible. Perhaps, for the relatively large Pb cation, a greater affinity for O is evident in the shift patterns but, if anything, as much evidence exists that, compared to DMA, the DEA group promotes N1 binding. For example, the effect of $Zn(NO_3)_2$ on the C6 resonance produces a larger upfield shift for DEA-7-M-9-PH (1.33 ppm) than for DMA-7,9-DMH (0.51 ppm). This could result from an increased basicity of N1 induced by the DEA group.

The contrasting behavior between DMC [13] and DMA-7,9-DMH or DEA-7-M-9-PH suggests that the dialkylamino group is readily displaced from an in-plane orientation. However, we are not aware of any data relating to this point.

DEA-7-M-9-PT

The effects of various metal salts on the resonances of this material were given in Table VI. Replacement of the hard oxo group with the soft thio group at position 6 minimizes or eliminates complex formation with the hard metal salts $Ba(NO_3)_2$ and $Pr(NO_3)_3$. For example, *0.4 M* Pr^{3+} (in contrast to ~0.05 M for the oxo analogs) produces only a 0.45 ppm shift in C6. Perhaps the small changes can be attributed to some N interaction or to other factors such as change in ionic strength, *etc.* The very small changes in the C8 and C2 resonances suggest that no complex formation occurs. In contrast, the intermediate metal salts (Zn, Pb, Cd) all behave similarly and cause large shifts in the C6 resonance and relatively small shifts in the C2 resonance. This result is consistent with binding to the 6 thio group.

Conclusions

It appears that compared to cytosine-based ring systems, metal species prefer endocyclic ring N binding over exocyclic oxo group binding in betain systems. The strength of the interaction at N is so large that even very bulky substituents, such as a diethylamino group, *ortho* to the endocyclic N, still do not prevent endocyclic ring binding. 7-MeGuo is present in some tRNA's but the potential biological role of metal binding is not known [29].

Acknowledgements

We are grateful for the support of NIH (grant GM29222), we also thank Johnson-Matthey for a loan of K_2PtCl_4.

References

1 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature (London), 222,* 385 (1969).
2 B. Rosenberg, *Biochemie, 60,* 859 (1978).
3 J. J. Roberts, *Adv. Inorg. Biochem., 3,* 273 (1981).
4 M. P. Hacker, E. B. Douple and I. H. Krakoff, 'Platinum Coordination Complexes in Cancer Chemotherapy' (eds.), Martinus Nishoff, Boston, Mass., 1984.
5 G. Y. Chu, S. Mansy, R. E. Duncan and R. S. Tobais, *J. Am. Chem. Soc., 100,* 593 (1978).
6 J. K. Barton, D. J. Szalda, H. N. Rabinowitz, J. V. Waszczak and S. J. Lippard, *J. Am. Chem. Soc., 101,* 1434 (1979).
7 J. K. Barton and S. J. Lippard, in T. Spiro, (ed.), 'Nucleic Acid-Metal Interactions', New York, 1980, Chap. 2.
8 R. W. Gelbert, B. E. Fisher and R. Bau, *J. Am. Chem. Soc., 102,* 7812 (1980).
9 L. G. Marzilli, K. Wilkowski, C. C. Chiang and T. J. Kistenmacher, *J. Am. Chem. Soc., 98,* 8371 (1976).
10 T. J. Kistenmacher, K. Wilkowski, B. de Castro, C. C. Chiang and L. G. Marzilli, *Biophy. Biochem. Res. Comm., 91,* 1521 (1979).
11 B. de Castro, C. C. Chiang, K. Wilkowski, L. G. Marzilli and T. J. Kistenmacher, *Inorg. Chem., 20,* 1835 (1981).
12 T. J. Kistenmacher, B. de Castro, K. Wilkowski and L. G. Marzilli, *J. Inorg. Biochem., 16,* 33 (1981).
13 (a) L. G. Marzilli, R. C. Stewart, C. P. Van Vuuren, B. de Castro and J. P. Caradonna, *J. Am. Chem. Soc., 100,* 3967 (1978);

(b) L. G. Marzilli, B. de Castro, J. P. Caradonna, R. C. Stewart and C. P. Van Vuuren, *J. Am. Chem. Soc., 102,* 916 (1980).
14 L. G. Marzilli, B. de Castro and C. Solorzano, *J. Am. Chem. Soc., 104,* 461 (1982).
15 D. W. Abbott and C. Woods, *Inorg. Chem., 22,* 597 (1983).
16 J. H. Price, A. N. Williamson, R. F. Schram and B. B. Wayland, *Inorg. Chem., 11,* 1280 (1972).
17 D. Söll and W. Pfleiderer, *Chem. Ber., 96,* 2977 (1963).
18 W. R. Boon, *J. Chem. Soc.,* 1532 (1952).
19 C. Solorzano, *Ph.D. Thesis,* Johns Hopkins Univ., 1981.
20 K. J. M. Andrews, N. Anand, A. R. Todd and A. Topham, *J. Chem. Soc.,* 2490 (1949).
21 E. C. Taylor, G. P. Beardsley and Y. Maki, *J. Organomet. Chem., 36,* 3211 (1971).
22 H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc., 80,* 2751 (1958).
23 A. J. Jones, D. M. Grant, M. W. Winkley and R. K. Robins, *J. Am. Chem. Soc., 92,* 4079 (1970).
24 R. A. Komoroski and A. Allerhand, *Biochemistry, 13,* 369 (1974).
25 P. O. P. Ts'O, N. S. Kondo, R. K. Robins and A. D. Broom, *J. Am. Chem. Soc., 91,* 5625 (1969).
26 C. H. Chiang and L. G. Marzilli, *J. Am. Chem. Soc., 96,* 3656 (1974).
27 M. D. Reily, K. Wilkowski, K. Shinozuka and L. G. Marzilli, *Inorg. Chem., 24,* 37 (1985).
28 C. F. G. C. Geraldes and J. R. Ascenso, *J. Chem. Soc. Dalton Trans.,* 267 (1984).
29 See, for example, *Prog. Nucl. Acid Res. Mol. Biol., 24,* (1980).