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THE SOLUTION ORDERING OF ALKALI METAL
SALTS OF GUANYLIC ACIDS

- I. THE EFFECT OF PHOSPHATE POSITION ON THE
SELF-AGGREGATION OF GUANYLIC ACIDS.
- II. ETHIDIUM BINDING TO UNSTRUCTURED AND STRUCTURED
FORMS OF GUANYLIC ACIDS.
- III. A NOVEL PROTON EXCHANGE BETWEEN THE AMINO
PROTONS AND SOLVENT WATER IN GUANYLIC ACIDS.

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1981

G 1157.05

ABSTRACT

THE SOLUTION ORDERING OF ALKALI METAL SALTS OF GUANYLIC ACIDS

- I. THE EFFECT OF PHOSPHATE POSITION ON THE SELF-AGGREGATION OF GUANYLIC ACIDS.
- II. ETHIDIUM BINDING TO UNSTRUCTURED AND STRUCTURED FORMS OF GUANYLIC ACIDS.
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In aqueous solution, the dianion of guanosine-5-mono-phosphate (5'GMP) forms regular ordered structures that are slow to exchange on the ^1H NMR time scale. The most compelling evidence for structure formation is the presence of multiple inequivalent H(8) resonances in the limiting spectra of dialkali metal ion salts in D_2O solution near 0°C . This self-assembly process is dramatically dependent on the nature of the alkali metal counterion, which is believed to direct structure formation through a size-selective coordination mechanism. Such metal ion dependent ordering is unprecedented in other nucleotide systems.

To assess the role of the phosphate group in the solution self-structuring of guanylic acids, guanosine

aggregation was investigated by ^1H NMR. The addition of Na^+ , K^+ , Sr^{+2} , or Tl^{+2} does not affect the H(8) resonance, either in chemical shift or half-width, up to the point of precipitation or gelation. At the gel or precipitation point, all resonances are understandably broadened.

Although guanosine does not form an NMR-observable ordered structure, selective deuteration experiments were carried out to determine if guanosine could incorporate into the 5'GMP structure. Guanosine was found to destabilize the Na_2 5'GMP structure, as determined by the loss of H(8) structure resonance intensities as the concentration of d-H(8) guanosine in a Na_2 5'GMP solution increased. The mechanism of destabilization involves formation of a mixed guanosine-5'GMP soluble gel type of structure. That is, 5'GMP is incorporated into a guanosine gel structure rather than guanosine incorporated into a 5'GMP solution self-structure.

Further evidence for the importance of the phosphate position has been provided by detailed studies of 2'GMP aggregation. The Na^+ , K^+ , Rb^+ , and Cs^+ salts of 2'GMP have been found to form ordered solution structures as evidenced by multiple H(8) resonances in the ^1H NMR and frequency shifts in the carbonyl region of the IR spectra. The order of stability is $\text{K}^+ > \text{Na}^+ \approx \text{Rb}^+ > \text{Cs}^+$. The Li^+ , tetramethylammonium ion (TMA^+), and tetrabutylammonium

ion (TBA^+) salts did not form an NMR-observable self-structure. The stability of the 2'GMP to form self-structures is less than that of the corresponding 5'GMP systems. Higher concentrations of (alkali metal)₂2'GMP and lower temperatures are needed to form the same percent of structure as in the 5'GMP case. The number of 2'GMP structure H(8) environments and their chemical shifts are similar to, but not exactly the same as, the corresponding 5'GMP system.

An attempt was made to vary the concentration of alkali metal by using TMA^+ as a non-inhibiting, non-structure directing ion as in the 5'GMP case. TMA^+ was found to inhibit the structuring process in 2'GMP. The Na_2 2'GMP self-structure formed by adding NaCl to a TMA_2 2'GMP solution is ~5% structured even under conditions where 50% solution self-structure is present in the TMA^+ free system. The K^+ - TMA^+ mixed system forms a self-structure, but the extent of structure formation is inhibited by TMA^+ .

It is postulated that ion pairing between the phosphate and TMA^+ in 2'GMP inhibits the formation of tetramers or the stacking of tetramers for steric reasons. This is verified by mixed TMA^+ - Na^+ , 2'GMP-5'GMP selective deuteration experiments. A destabilization of the 5'GMP solution self-structure which is not observed in the pure Na^+ mixed nucleotide system is observed in this system. Nuclear Overhauser effect (NOE) experiments showed TMA^+

was located near the ribose protons and the amino protons. A position near the amino protons would sterically hinder the formation of tetramers. Previous work has shown that TMA^+ in the $\text{Na}_25'\text{GMP}$ system is located near H(8) away from the H-bonding positions involved in tetramer formation.

The Na^+ and K^+ , 3'GMP and guanosine-5'-diphosphate (5'-GDP) systems are similar to those of guanosine, forming gels in preference to ordered solution structures. The TMA^+ inhibition of structure formation is present in both systems, manifested by a destabilization of gel formation. On addition of K^+ to a $\text{TMA}_23'\text{GMP}$ solution, new H(8) resonances appear. TMA^+ is therefore blocking the formation of large aggregates. New resonances in the H(8) NMR spectrum of $\text{TMA}_25'\text{GDP}$ occur on addition of KCl. The H(8) resonance of both 3'GMP and 5'GDP is broadened on addition of NaCl but no new resonances occur. Both 3'GMP and 5'GMP were found to interact with each other. It is proposed that $\text{TMA}_23'\text{GMP}$ and $\text{Na}_25'\text{GMP}$ form a solution self-structure. Several new resonances occur in the H(8) NMR of 5'GDP in mixed $\text{TMA}_25'\text{GMP}$ -8-deutero- $\text{Na}_25'\text{GMP}$ solutions, indicating mixed solution self-structures of 5'GMP and 5'GDP can occur.

It is clear from the above results that the phosphate group plays a role in the solution ordering of guanylic acids. The structuring ability at pH = 7-8 of the nucleotides is guanosine > 3'GMP > 5'GDP > 5'GMP > 2'GMP,

with guanosine, 3'GMP and 5'GDP preferably forming gels and 5'GMP and 2'GMP forming solution self-structures.

Analyses of NMR spectra taken in $^1\text{H}_2\text{O}$ have led to the assignment of a generalized structure for the solution self-structuring of guanylic acids. One Na^+ solution self-structure is composed of two head-to-head stacked planar tetramers with a Na^+ located on the outer side of the cavity formed by the carbonyl oxygens. A water molecule may be trapped in the octameric cavity formed by the carbonyl oxygens between the plates, hydrogen bonded to an upper and a lower carbonyl oxygen. A water bridge is formed between a phosphate and an adjacent guanylic acid $-\text{NH}_2$. In 5'GMP there are hydrogen bonds formed between the 2'OH of the upper plate and the O(1') of the lower plate. A hydrogen bond is also formed between the 3'OH and the O(5') of the lower plate. A time-averaged NMR value indicates two Na^+ ions are territorially bound to the outer periphery of the structure. Another structure is similar to that described above except the tetramers are stacked head to tail. A different hydrogen bonding scheme is also present. The other alkali metal solution self-structures are believed to be based on the stacking of these two models with the metal ion located in the octameric cavity.

During the investigation of 2'GMP a novel proton exchange was noted for 2'GMP. The exchange is between the amino protons and water. The most interesting aspect of

this exchange is that the integral intensity of the amino protons varies from ~ 1 to 2 relative to H(8) in $\text{Na}_2 2'\text{GMP}$, but is always 2 in $\text{TMA}_2 2'\text{GMP}$. The conditions where the integral intensity is < 2 are consistent with an ordered form of nucleotide being present. It is proposed that the loss of amino proton relative integral intensity is caused by the formation of a water bridge between the phosphate group of an adjacent nucleotide in a tetramer and the amino proton not involved in tetramer formation. This allows for the rapid exchange of the amino protons with solvent water. This water bridge places the nucleotide in the preferred conformation in $5'\text{GMP}$ and $2'\text{GMP}$.

The binding of ethidium was also investigated. Ethidium was found to form a 1:1 and a 2:1 ethidium:nucleotide complex with unstructured $5'\text{GMP}$ and $2'\text{GMP}$. The equilibrium constant for the 1:1 complex in both nucleotides is $\sim 10^4 \text{ M}^{-1}$. For the 1:1 and 1:2 ethidium: $2'\text{GMP}$ species the enthalpies were determined to be $-19.8 \pm 1.0 \text{ Kcal/mol}$ and $0.1 \pm 0.3 \text{ Kcal/mol}$, respectively, and the entropies were $-53.3 \pm 6.6 \text{ eu}$ and $3.9 \pm 1.6 \text{ eu}$, respectively.

Ethidium was also found to bind with structured forms of $5'\text{GMP}$ and $2'\text{GMP}$. Formation of a 2:1 and a 1:1 complex occurs. Binding constants could not be determined, but the NMR shifts suggest the binding constants are 10^3 M^{-1} .

The amino proton exchange reaction in the absence of structure was investigated for the TMA^+ salts of $2'\text{GMP}$ and $5'\text{GMP}$ and the Na^+ salt of $2'\text{GMP}$. The process was found to

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be pseudo first order in amino proton. The exchange reaction has a $\Delta H^\ddagger = +15$ to $+19$ Kcals/mol and a $\Delta S^\ddagger = -4.0$ to $+9.0$ eu.

To My Parents

ACKNOWLEDGMENTS

It is difficult to properly thank everyone who has been of help over the past few years. To those not mentioned, a general thank you is extended. I would like to thank Dr. Harry A. Eick for serving as my second reader. Assistance received from Dr. Tom V. Atkinson on computer operation and programming, which made much of this work possible, is noted.

The encouragement and love of my parents, Frank and Minnie Barr, grandparents and brother, Dennis, has always been a source of guidance for me.

Dr. Thomas J. Pinnavaia has always been a continual source of guidance, wisdom and encouragement. Words cannot express the respect and admiration I hold for him as a research director, teacher and friend. I look forward to continued collaboration with him.

I would also like to thank Dr. Elene Bouhoutsos-Brown, Dr. Richard Farmer, Dr. Emmanuel Schmidt, Mr. Rasik Raythatha, Mr. Walt Cleland, Mr. Steve Christiano, and Dr. Chris Marshall whose collaboration and fruitful discussions were extremely helpful.

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INTRODUCTION

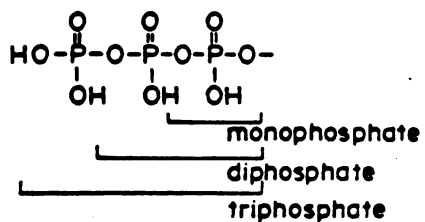
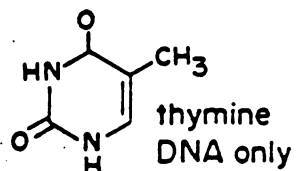
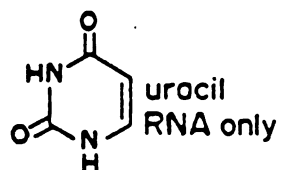
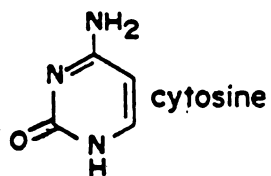
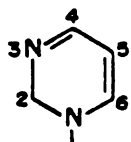
I. General Information on Nucleic Acids

A. Nomenclature

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are chainlike macromolecules that function in the storage and transfer of genetic information. They are major components of all cells, together making up from 5 to 15 percent of their dry weight. The monomeric units of the nucleic acids are the nucleotides. DNA and RNA share a number of chemical and physical properties. In both types of polynucleotides the successive units are covalently linked in identical fashion by phosphodiester bridges formed between the 5'hydroxyl group of one nucleotide and the 3' hydroxyl group of the next. The nucleotides and their derivatives occur in the free form in cells in significant amounts.

The monomeric units of DNA are called deoxyribonucleotides; those of RNA are ribonucleotides. Each nucleotide contains three characteristic components: (1) a nitrogenous heterocyclic base, either a derivative of purine or pyrimidine, (2) a pentose sugar, and (3) a phosphate group (cf. Figure 1). The backbone of both DNA and RNA

Pyrimidines



Purines

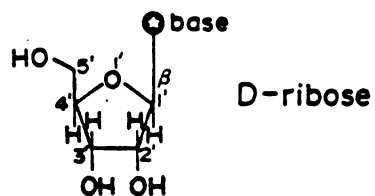
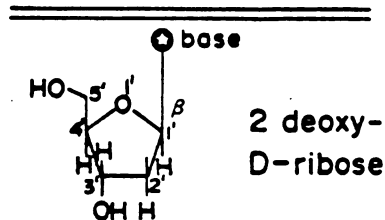
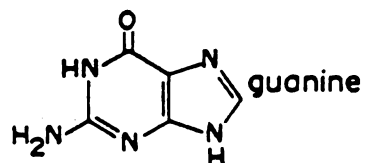
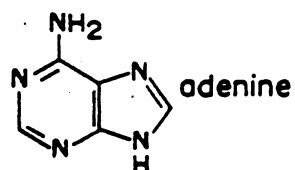
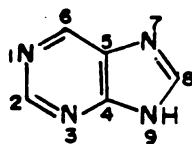


Figure 1. The purine and pyrimidine bases, ribose and deoxyribose, and phosphates of nucleotides. Taken from Reference 49.

consists of alternating phosphate and pentose groups, in which phosphodiester bridges provide the covalent continuity. The purine and pyrimidine bases of the nucleotide units are not present in the backbone structure but constitute distinctive side chains.

Four different deoxyribonucleotides serve as the major components of DNA, differing only in their nitrogenase base components: adenine, guanine, cytosine, and thymine (cf. Figure 1). Similarly, four different ribonucleotides are the major components of RNA: adenine, guanine, cytosine, and uracil (cf. Figure 1). The precise 3-D structures of various pyrimidines and purines have been deduced by X-ray diffraction analysis¹⁻². Pyrimidines are planar molecules, whereas purines are very nearly planar, with a slight pucker. Both have pronounced aromatic character. Free pyrimidine and purine bases are relatively insoluble in water. They are weakly basic compounds that may exist in two or more tautomeric forms depending on the pH. All the purine and pyrimidine bases of nucleic acids absorb light in the ultraviolet region between 250 and 280 nm.

Deoxyribonucleotides contain 2-deoxy-D-ribose as their pentose component whereas ribonucleotides contain D-ribose (cf. Figure 1). Both sugars occur in their furanose forms in the nucleotides. The pentose is joined to the base by a β -N-glycosyl bond between C(1') and N(9) of purine bases or N(1) of pyrimidine bases. The phosphate group of

nucleotides in DNA or RNA is in an ester linkage to the C(5') position. Other nucleotides exist in which the phosphate group is in the 2' or 3' position of the sugar. The 5' isomer is the most abundant in cells. When the phosphate group of a nucleotide is removed by hydrolysis, the structure remaining is called a nucleoside. Thus nucleotides are the phosphates of the corresponding nucleosides. The nucleosides are named after the base which they contain; adenosine, cytidine, guanosine, thymidine, and uridine. In cells many polyphosphates also exist. The major polynucleotides are the 5'diphosphates and 5'triphosphates. Cyclic phosphates can also be found, where a phosphate group is condensed with two of the ribose hydroxy groups, either in the 2' and 3' position or the 3' and 5' position. Phosphates groups can also be found attached to different hydroxy groups (e.g., 5'diphosphate 3'diphosphate guanosine). Polyphosphates serve as energy storage compounds in cells, whereas cyclic nucleotides and phosphates attached to several hydroxy groups serve as metabolic regulators.

B. Structural Information

An almost infinite number of conformations are possible for the nucleotides. The torsion angle between the base and C(1') of the sugar, the ribose conformation, and

the exocyclic C(4')-C(5') bond can all vary. A great deal of interest has been expressed in both theoretical and experimental aspects of the stereochemistry of nucleosides and nucleotides³⁻¹⁰. Two preferred conformations exist about the glycoside bond of purine nucleosides and nucleotides. Rotation about this bond is defined in terms of χ , the angle defined by O(1')-C(1')-N(9)-C(8) as shown in Figure 2. The two common conformations are termed syn ($\chi = 0^\circ \pm 90^\circ$) and anti ($\chi = 180^\circ \pm 90^\circ$). The different conformations in the sugar ring have been discussed extensively by Sundaralingam⁴. The major conformers are shown in Figure 2. In 5' nucleotides, the conformation of the exocyclic C(4')-C(5') bond is specified by the angle ψ , which is defined by the orientation of the C(5')-O(5') bond with respect to the C(4')-O(1') and C(4')-C(3') bonds as shown in Figure 2⁴. The orientation of the phosphate with respect to C(4') is defined by the angle ϕ , and also shown in Figure 2. A number of reviews are available on the stereochemistry of nucleosides and nucleotides¹¹⁻¹³.

C. Base-pairing and Base Stacking

Nucleotide bases can associate with each other by two methods. They can hydrogen bond through $>\text{N-H}$ and $-\text{OH}$ groups, or they can stack vertically via hydrophobic bonding. The former method is well known to account for base pairing in DNA. In addition to Watson and Crick hydrogen bonding,

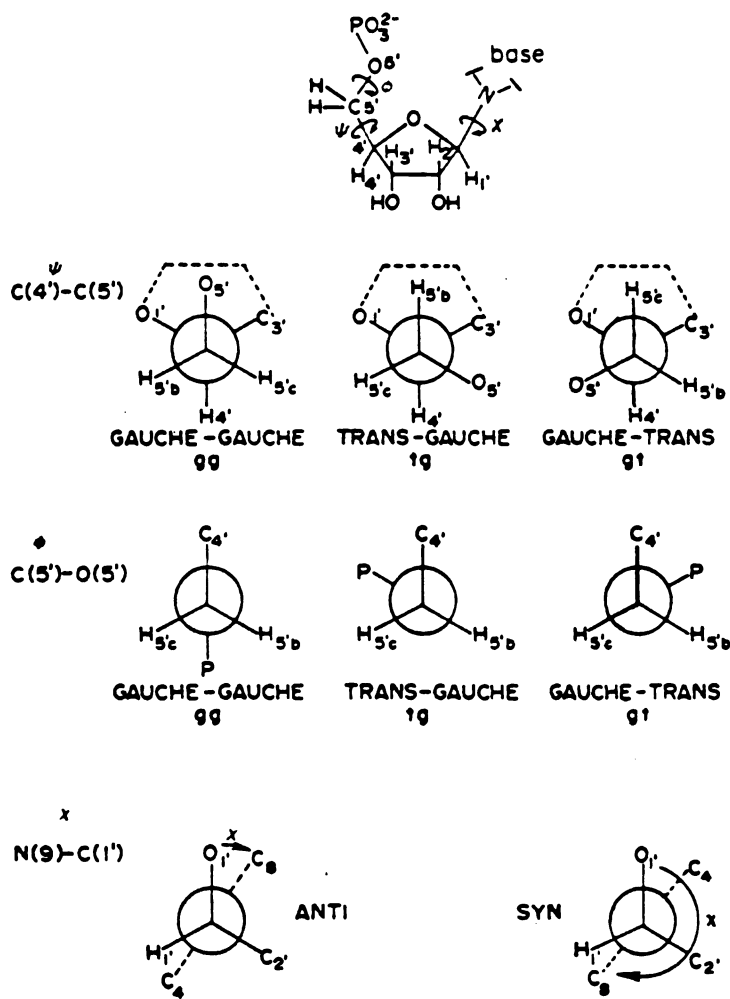


Figure 2. Newman projections showing the preferred nucleotide conformations and their nomenclature. Taken from Reference 49.

which allows for semi-conservative replication of cells, other hydrogen bonding schemes have been found which may be of biological importance. Base pairing has been studied in non-aqueous solutions by infrared spectroscopy¹⁴⁻¹⁷. In both non-aqueous and aqueous solutions, NMR spectroscopy of nucleotides has shown evidence for hydrogen bond pairing that involves the amino and aromatic ring protons¹⁸⁻²¹.

A review of stacking patterns of the bases and derivatives in the crystalline state has been prepared by Bugg²². Base stacking effects have been shown to provide ordered stabilization²³. Gupta and Sasisekharan²⁴ have reported theoretical calculations of base-base stacking in the free base systems. Physical methods such as NMR²⁵⁻²⁹, vapor phase osmometry²⁷⁻³⁰, calorimetry³¹, and fluorescence³²⁻³⁴ have been used to study the stacking phenomenon. Base stacking in purine derivatives occurs to a far lesser extent in non-aqueous solvents than in water. Hanlon³⁵ and Bugg²² argue that polarizable solvents which possess polar substituents, like dimethyl sulphoxide, interact with the polarizable π -electron systems of the purines and inhibit stacking interactions in these solvents. Purine nucleosides have a far greater tendency to form vertical stacks than the corresponding mononucleotides which carry some negative charge on the phosphate moiety²⁶⁻²⁸. Although usually deemed responsible for base stacking, hydrophobic effects are not entirely responsible. The stabilization

effect of base stacking has been described in terms of hydrophobic forces³⁵, special Van der Waals interactions³⁶, π -electron polarizability³⁶, dipole-dipole induced forces³⁷, and electrostatically stabilized ionic complexes³⁶. The association is most likely due to a combination of all these forces.

D. Metal Ion Interactions With Nucleic Acids

A large body of literature related to nucleic acids exists from a wide variety of disciplines due to their multifaceted involvement in vital life processes. The biological functions of nucleic acids often involve the participation of metal ions. As pointed out by Makrigiannis et al.³⁸, it is difficult to investigate metal interactions with DNA directly, so that binding must be studied for each base. The binding of pre-transition and post-transition metals to nucleic acids or their constituents has been very important for the understanding of a large number of biological effects. Multiple coordination sites exist on the nucleotides. Interest in metal binding by nucleotides has been stimulated by the finding that Pt(II)-nucleic acid complexes have significant antineoplastic activity³⁹⁻⁴¹. Several reviews of the literature on metal binding by nucleic acids and their constituents have appeared⁴²⁻⁴⁴. In guanosine the N(7) position, due to its lone pair of electrons, is a favored binding position for many transition

metal ions^{42,45}. At neutral pH N(1) is protonated, however, bonding of metal ions to this position can be so favored as to lower the effective pK_a ⁴⁵. Chelation of metal ions by the N(7)-O(6) or N(1)-O(6) positions of guanosine has been proposed. Shimokawa *et al.*⁴⁶ concluded from ¹H NMR work in DMSO that alkaline earth cations formed more stable complexes with guanosine than the transition metal ions. Marzilli and coworker⁴⁷ found that alkaline earth nitrates do not cause the same spectral changes in the ¹H NMR spectra found in the chloride salts in guanosine solutions. Tetramethylammonium chloride also produced large spectral shifts. Therefore, he concluded that the chloride ion was responsible for the shifts. The order of anions causing shifts, Cl^- , $Br^- > I^- > ClO_4^- > SCN^-$ is the same as the influence of anions on stabilizing DNA. Sohma *et al.*⁴⁸ concluded that both cation and anion effects must be considered. A review of X-ray structures of metal ions with guanosine and its derivatives has been prepared by Brown⁴⁹. Alkali metal-nucleic acid interactions have not been extensively studied for specific coordination binding, even though large amounts of Na^+ and K^+ are present in cells. The alkali metal ions have been regarded as non-specific electrolytes in balancing the negative charge of DNA⁵⁰⁻⁵³.

II. Self-Structuring of Guanylic Acids

A. General Information on Guanylic Acids

Of all the nucleic acid components guanosine has the most complex structure and widest variety of possible hydrogen-bonding interactions⁵⁴. The physio-chemical properties of guanosine-containing regions of nucleic acids are frequently different. Intercalation drugs, such as actinomycin⁵⁵⁻⁵⁷ and ethidium bromide⁵⁸⁻⁶¹, and cancer drugs like $\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2$ ⁶² prefer guanosine rich regions of DNA or RNA. The uniqueness of guanosine had been attributed to multiple degrees of freedom about the glycosidic bond^{63,64}, the different electronic structures of guanosine which results in a completely different orientation of the dipole moment compared to other bases⁶⁵, and multiple protonation sites⁵⁴.

B. Gelation

The self-association of guanylic acids in aqueous solution may be manifested by the formation of anisotropic gels. As early as 1910 Bang⁶⁶ reported the formation of gels in concentrated, acidic guanylic acid solutions. This phenomenon has since been found to occur with several other guanosine derivatives (e.g., 3'GMP, 2'GMP, 8-bromoguanosine, and 2'deoxyguanosine)⁶⁷⁻⁷⁰. Gel formation has

also been observed in methanol and ethanol but the stability of the gels is decreased⁷¹.

In order to obtain insight on the nature of gel formation by guanosine, Guschlbauer⁶⁷ studied gel formation of different derivatives in which the various functional groups had been substituted or changed under standard conditions (0.02M nucleoside, 0.1M KCl). Purine nucleosides which did not contain the guanosine skeleton did not yield gels. These included inosine, xanthosine, adenosine, 2,6-diaminopurine riboside, 2-amino-purine riboside. While the presence of the sugar on the base seems to be necessary, since guanine does not form gels, the nature of the sugar appears to be of little importance. The ribosides, deoxyribosides and arabinosides of guanine form gels. Methylation of N(7) or N(1) inhibited gel formation. Methylation of the amino group is of particular interest, since N₂-methylguanosine formed a gel, while the doubly methylated compound did not. The results indicate that N(7), 6-keto, N(1), and one hydrogen atom of the amino group in position 2 are essential for gel formation. This is in agreement with the stacked planar tetramer model (cf. Figure 3) for the 3'GMP gel structure and the regular helix model (cf. Figure 3) for H₂5'GMP gels reported by Gellert et al.⁷² based on X-ray and optical rotation measurements. A segment of the planar tetramer structure is shown in Figure 4. Gels of guanylic acids have been studied by IR^{73,82,83},

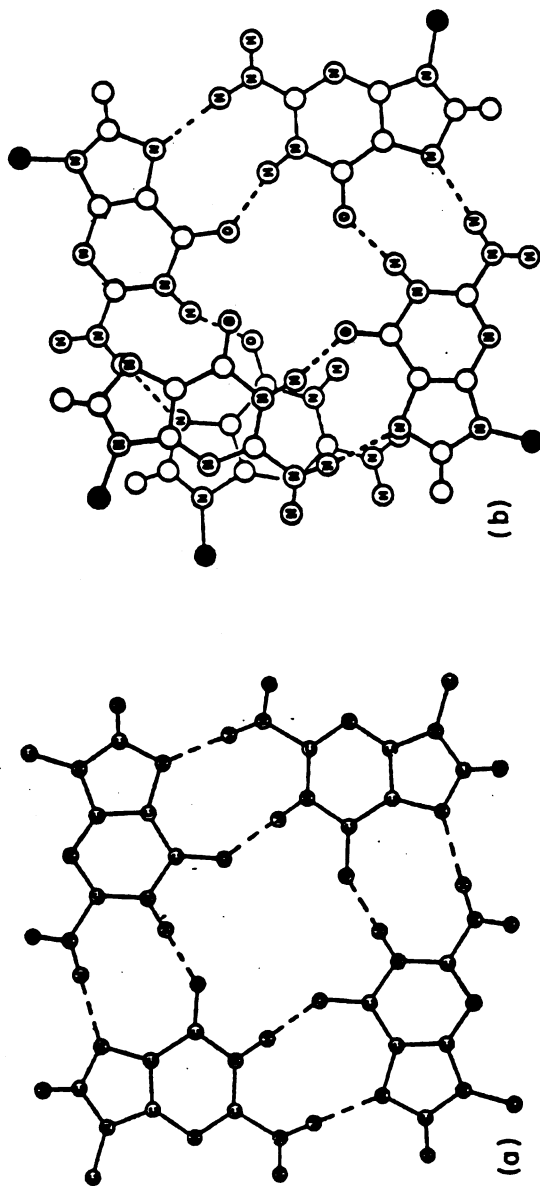


Figure 3. The planar tetramer unit and helix of the guanosine gel structure showing hydrogen bonding between N(1) and O(6) as well as N(2) and N(7). Taken from Reference 2.

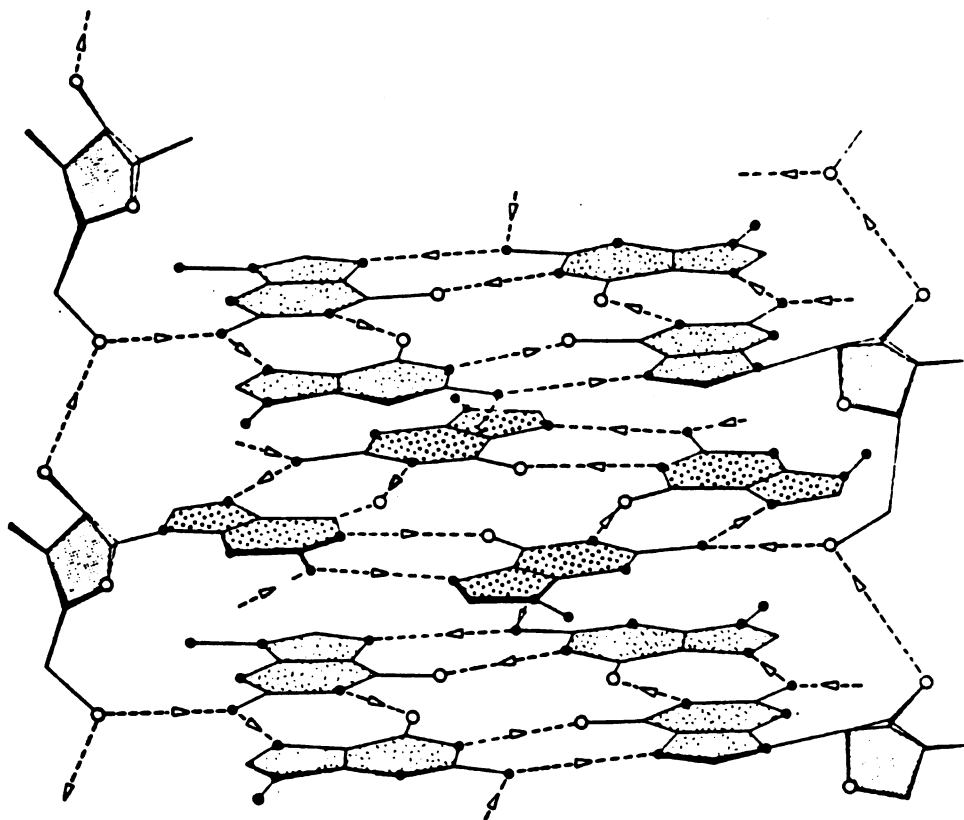


Figure 4. Schematic representation of the tetrameric arrangement of a 3'GMP gel. Taken from Reference 23.

ORD^{67,68,74}, CD^{67-69,71,74}, Raman^{70,84,85}, Calorimetry^{75,86}, and X-ray diffraction^{72,76-81}.

Among the various conditions necessary for gel formation, the nature of the alkali metal or alkaline earth counterion is of great importance⁷¹. However, an adequate structural explanation of the cation dependence of gel formation has not been provided. The order of gel stability for guanosine is $\text{Sr}^{2+} > \text{K}^+ > \text{Na}^+$, $\text{Rb}^+ > \text{Cs}^+$, Li^+ ^{68,71}. The anion dependence of gel formation is much less important⁷¹. Chantot and Guschlbauer have measured the melting temperature, T_m , the temperature at which one-half of the nucleotide disordered. They found only a small difference in the T_m of gels formed with various anions⁶⁸. They attributed the differences in T_m to the "salting out" properties of large anions (e.g., Cl_3CCOO^- , ClO_3^- , I^-). Their results follow the same general trend of the effect of anions on the solubilities and activity coefficients of bases and nucleosides as reported by Robinson and Grant⁸⁷.

C. Solution Self-Structuring

In 1972 Miles and Frazier⁸⁸ reported IR evidence for a regular, ordered structure formation of $\text{Na}_25'\text{GMP}$ which is not associated with gel formation in neutral aqueous solution. The melting transition of the solution structure is more cooperative than the gel melting transition at pH=5,

but the solution structure has a lower melting temperature. Based on IR arguments they postulated that the structure was stacked planar tetramers. Pinnavaia *et al.*⁸⁹ have shown by ^1H NMR that the solution self-structure, which is slow to exchange with unstructured nucleotide on the NMR time scale, can be monitored by the appearance of multiple H(8) resonances. The binding constant for complexation of alkali metal ions by phosphate groups in polyphosphates⁹⁰, adenine nucleotides^{91,92} and DNA⁹³ are known to decrease slightly with increasing metal ion radius. In contrast to this electrostatically determined order, the qualitative stability order for self-assembled 5'GMP salts is $\text{K}^+ > \text{Na}^+$, $\text{Rb}^+ > \text{Cs}^+$, Li^+ , the same as for gel formation⁹⁴. The ^1H NMR spectra of the limiting H(8) lines of the alkali metal salts of 5'GMP in D_2O solution are shown in Figure 5. A size-selective metal complexation mechanism has been postulated to account for the alkali metal ion dependence⁹⁴. This order has been observed when a size-selective mechanism is operative⁹⁵, although it is unprecedented in other nucleotide systems. The O(6) positions of the planar tetramer units define the coordination sites in the Na^+ self-structure and the O(6) positions of two stacked tetramers define an eight-coordinate site for K^+ or Rb^+ . As shown in Figure 6, the cavity of the planar tetramer unit has a diameter of approximately $2.2 \text{ \AA}$ ⁹⁴, a value close to that observed for Na-O bonds⁹⁶. When two tetramers are

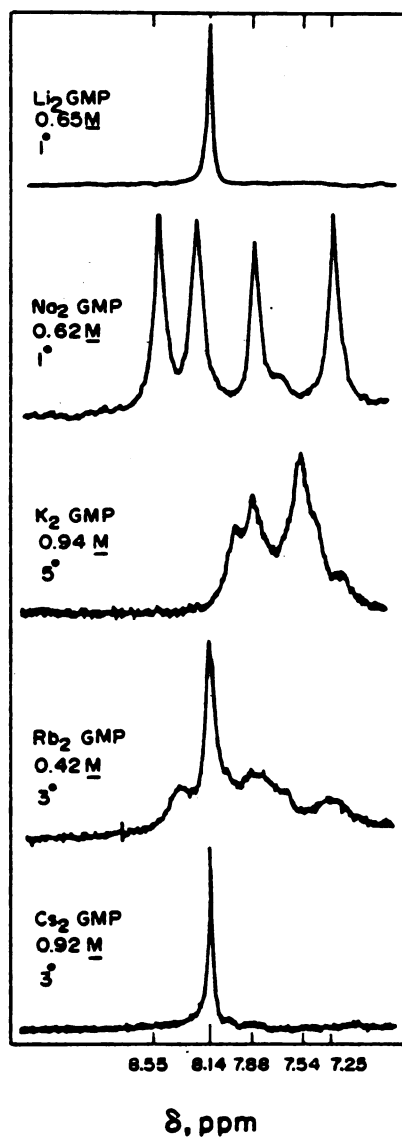


Figure 5. Limiting H(8) resonance lines of the alkali metal salts of 5'GMP in D_2O at high concentration and low temperatures. Taken from Reference 94.

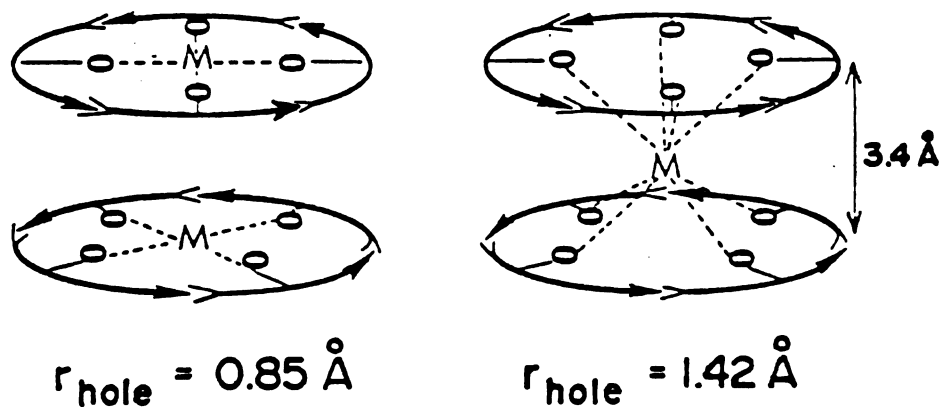


Figure 6. Schematic diagram of the proposed size-selective coordination sites in the structuring of guanylic acids. a) coordination in the center of the tetrameric plate by Na^+ ($r = 0.99 \text{ \AA}$). b) Coordination between the plates by K^+ and Rb^+ ($r = 1.51 \text{ \AA}$ and $1.60 \text{ \AA}$, respectively). Taken from Reference 97.

stacked, a central cavity defined by the eight oxygens is formed. The radius of this cavity, approximately 2.8 Å assuming a 3.4 Å spacing between plates⁹⁴, can accommodate a K^+ or Rb^+ ion. The center of a tetramer unit is too large to coordinate a Li^+ and too small to coordinate Cs^+ properly. The interplatelet distance in an octamer can be increased to accommodate Cs^+ (4.6 Å)⁹⁴, but this reduces the favorable base overlap distance, 3.4 Å, between plates.

The multiple H(8) environments for $Na_2 5'GMP$ have been explained by symmetry arguments⁹⁷. This model study was based on the results of Zimmerman et al.⁸¹ from fiber diffraction studies of $Na_2 5'GMP$. The plates were assumed to have a twist angle of 30° with respect to each other and the ribose assumed to have an anti configuration and C(3') endo conformation. Due to the directionality of the hydrogen bonds, stacking can occur two ways in an octamer. Either the direction of hydrogen bonding can be the same in the two tetramers (referred to as head-to-tail or normal stacking) or it can be in opposite directions (referred to as head-to-head or reversed stacking). This is shown in Figure 7. The head of an arrow signifies the hydrogen bond donors; the tail the hydrogen bond acceptors, and R the ribose phosphate. The results of this study are shown in Table 1 and Table 2.

Models for the solution self-structure of 5'GMP have been proposed by Brown⁴⁹. Those for $Na_2 5'GMP$ and $K_2 5'GMP$

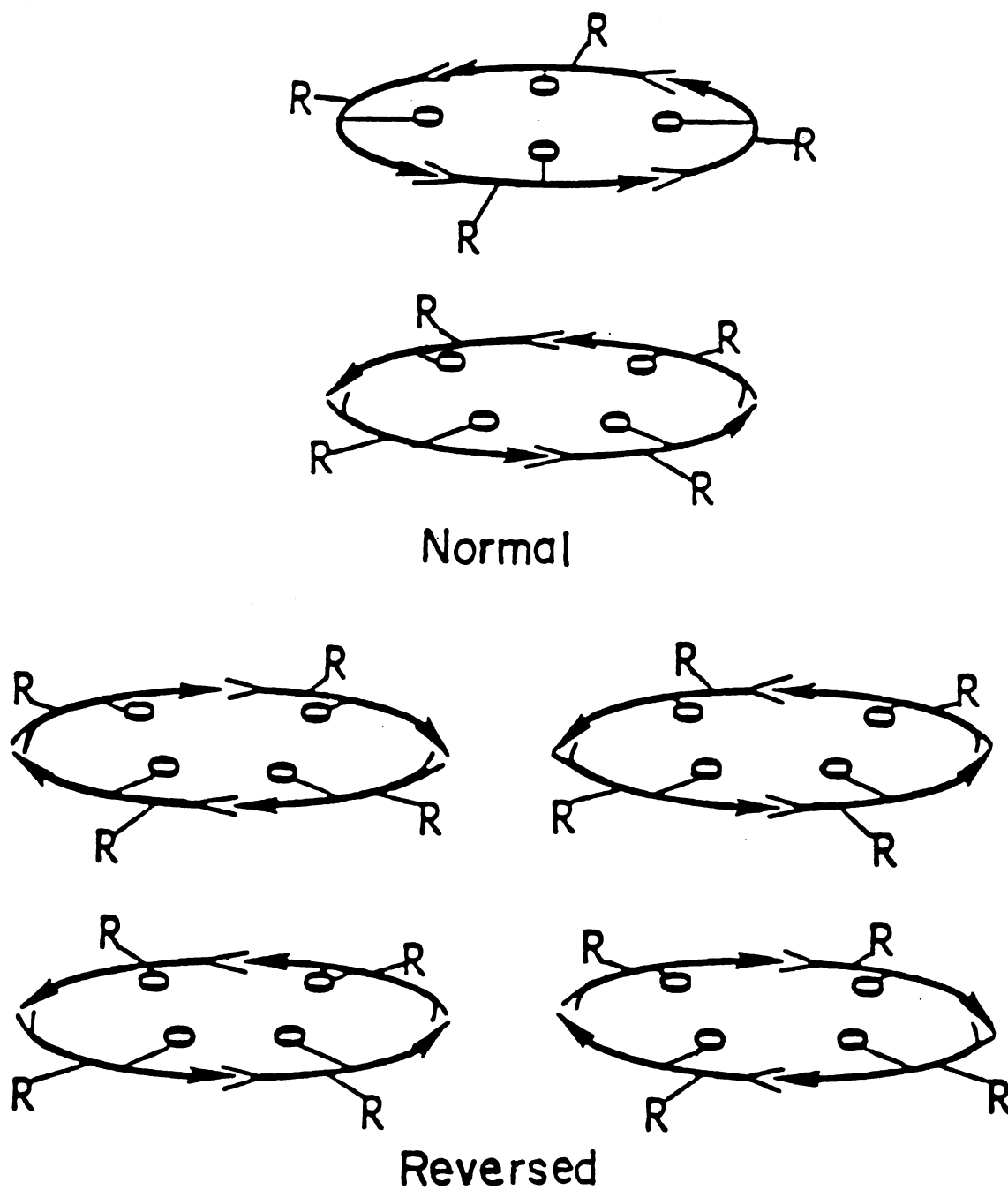


Figure 7. Schematic diagram of a) normal stacking mode and b) the two enantiomeric reversed stacking modes of the octamers of 5'GMP. Taken from Reference 97.

Table 1. Number of Isomers and H(8) Lines Expected for Three Exchange Regions.
Taken from Reference 97.

Conditions	Normal Stacking	Reversed Stacking	Total
Slow twisting with $\theta = +30^\circ$ and $\theta = -30^\circ$ equally preferred. Slow stack separation.	Isomers: 2 H(8) lines: 4	Isomers: 4 H(8) lines: 4	Isomers: 6 H(8) lines: 8
	$C_4(CW, CW, +30^\circ)$	$D_4(CW, CCW, +30^\circ)$	
	$C_4(CW, CW, -30^\circ)$	$D_4(CW, CCW, +60^\circ)$	
		$D_4(CCW, CW, -30^\circ)$	
		$D_4(CCW, CW, -60^\circ)$	
Fast twisting with isomerization of θ . Slow stack separation.	Isomers: 1 H(8) lines: 2	Isomers: 2 H(8) lines: 2	Isomers: 3 H(8) lines: 4
	$C_4(CW, CW, +30^\circ)$ ++	$D_4(CW, CCW, +30^\circ)$ ++	
	$C_4(CW, CW, -30^\circ)$	$D_4(CW, CCW, +60^\circ)$	
		$D_4(CCW, CW, -30^\circ)$ ++	
		$D_4(CCW, CW, -60^\circ)$	
Fast twisting. Fast stack separation.	$C_4(CW, CW, +30^\circ)$ ++	$D_4(CW, CCW, +30^\circ)$ ++	Isomers: 1 H(8) lines: 1
	$C_4(CW, CW, -30^\circ)$	$D_4(CW, CCW, +60^\circ)$ ++ $D_4(CCW, CW, -60^\circ)$	(time averaged with tetramer (monomer))

Table 2. Results of a Model Building Study of the Octameric Forms of 5'GMP.
Taken from Reference 97.

θ	χ	ψ	ϕ	ω	$\Delta\delta_{\text{H}(8)} \text{ (ppm)}$	Ribose Conformations	Hydrogen Bonds
<u>Normal Stacking</u>							
$C_4 \text{ (CW, CW, } +30^\circ)$	20°	60°	181°	69°	0.0	C(3')-endo	Four OH(3') $\rightarrow$ OP bonds
					0.4		
$C_4 \text{ (CW, CW, } -30^\circ)$	20°	60°	181°	69°	0.0	C(3')-endo	Four OH(2') $\rightarrow$ OP bonds
					0.4		
<u>Reversed Stacking</u>							
$D_4 \text{ (CCW, CW, } -30^\circ)$	20°	60°	181°	69°	0.2	C(3')-endo	Eight NH(2') $\rightarrow$ OP bonds
					0.2		
$D_4 \text{ (CCW, CW, } -60^\circ)$	20°	60°	181°	69°	0.2	C(3')-endo	Eight NH(2') $\rightarrow$ OP bonds
					0.2		
<u>Reversed Stacking</u>							
$D_4 \text{ (CW, CCW, } +30^\circ)$	20°	60°	181°	69°	0.2	C(3')-endo	Four OH(2') $\rightarrow$ O(3') bonds
					0.2		
$D_4 \text{ (CW, CCW, } +60^\circ)$	20°	60°	181°	69°	0.2	C(3')-endo	Four OH(2') $\rightarrow$ OP bonds
					0.2		

are shown in Figure 8. The $\text{Na}_2 5'\text{GMP}$ self-structure consists of 2 stacked tetramers with one Na^+ associated with the cavity of each tetramer. There are also four Na^+ ions chelated by interplatelet phosphate oxygens. Na^+ chelation was proposed since an increase in Na^+ concentration first causes an increase in the extent of solution structure, then beyond a certain Na^+ concentration the extent of ordering decreases. Two types of K^+ and Rb^+ structures were found; one at high ratios of $5'\text{GMP}/\text{M}^+$ (>1), termed "simple" structures and the other at low ratios of $5'\text{GMP}/\text{M}^+$ (<1 , $>.5$), termed "complex" structures. For these structures the metal ion was coordinated to 8 carbonyl oxygens between the tetramer units, the simple structure being an octamer and the complex structure being a hexadecamer. Marshall⁹⁷ has determined the structured form of $\text{Na}_2 5'\text{GMP}$ to contain eight nucleotide units and four Na^+ ions. Thus he modified the model by having only two Na^+ ions chelated by interplatelet phosphate oxygens. He also postulates a water molecule in the octameric hole formed by the two plates to separate the two Na^+ ions near the centers of the tetramers. Fisk *et al.*⁹⁸ have measured the T_1 's for the ^{13}C atoms in $\text{Na}_2 5'\text{GMP}$ and have concluded that the most stable species formed in the self-structure is an octamer, formed by the association of two tetrameric plates. They postulate the existence of at least three other structural species present in solution with octamer,

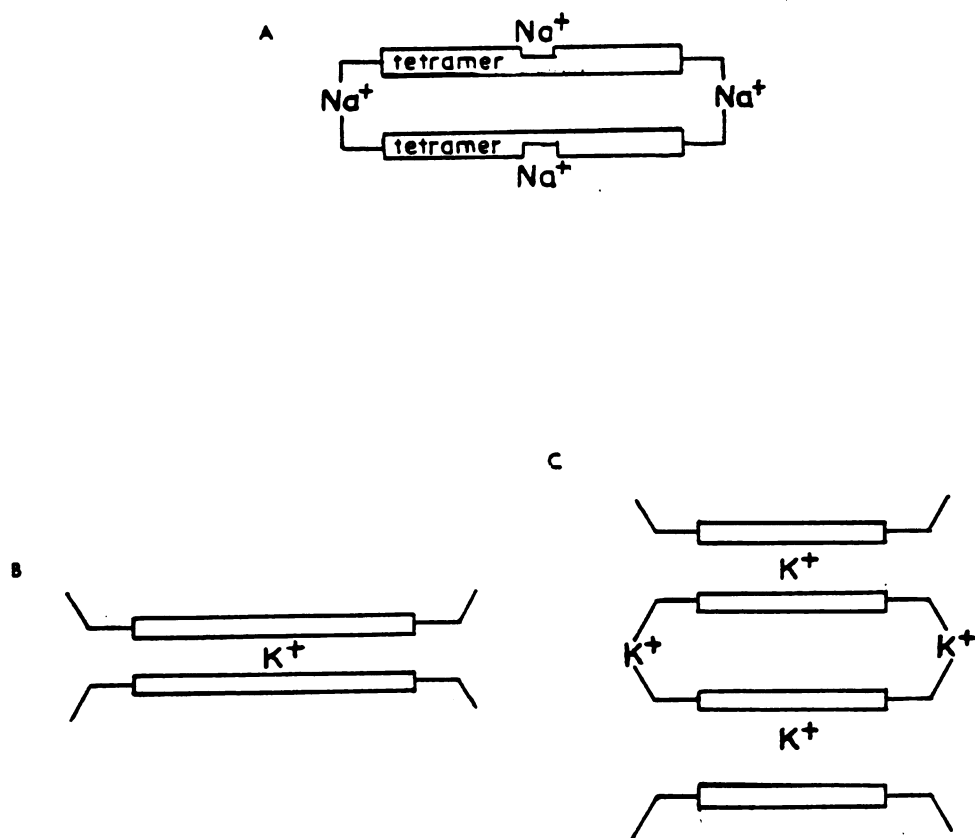


Figure 8. Brown's⁴⁹ proposed models for 5'GMP solution self-structure. a) The Na^+ self-structure; b) the "simple" K^+ self-structure and c) the "complex" K^+ self-structure. (Taken from Reference 49).

probably representing less-stable, stacked complexes.

Laszlo et al.⁹⁹⁻¹⁰⁶ have proposed several models for the $\text{Na}_2 5'\text{GMP}$ and $\text{K}_2 5'\text{GMP}$ self-structure. Their first model, based on ^{23}Na NMR, is a micelle type structure. The structure contained between 12 and 20 monomer units. They then proposed the coordination of Na^+ and K^+ into the central cavity of the tetramer plate. Based on ^1H and ^{31}P NMR evidence they proposed the stacking of tetramer units as shown in Figure 9. In this staircase model, which is stated to represent the two outer H(8) lines, the metal ion is coordinated by the four oxygens in the tetramer unit and a phosphate group from an adjacent tetramer. The metal ion is located in the center of the tetramer unit. They indicate that two of the adjacent phosphates of a tetramer unit point in the opposite direction of the other two phosphates. Rapid exchange occurs as shown in Figure 9. They suggest that the non-equivalent H(8) resonances result from different degrees of stacking of this structure.

In the same paper they also propose a stacked tetramer structure where Na^+ is found either at the center of two stacked tetramers or in the center of each tetramer unit. In a later paper Laszlo explains the more complex K^+ structure by changing the ribose conformation so the number of phosphates pointing "up" and "down" in the octamer staircase unit differ.

Brown⁴⁹ has determined that the tetramethylammonium

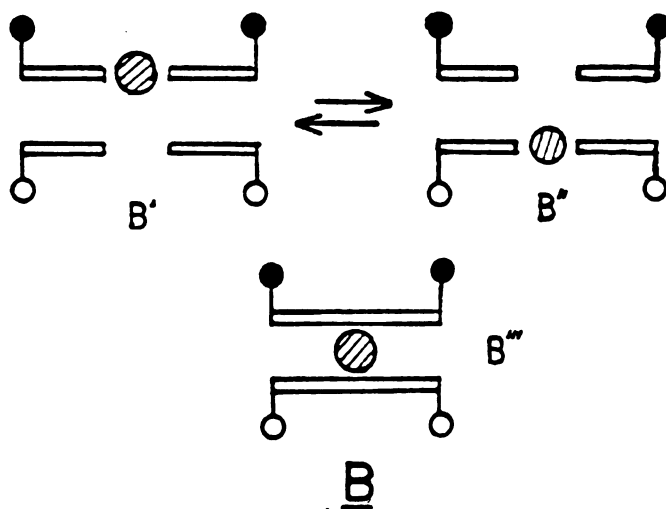
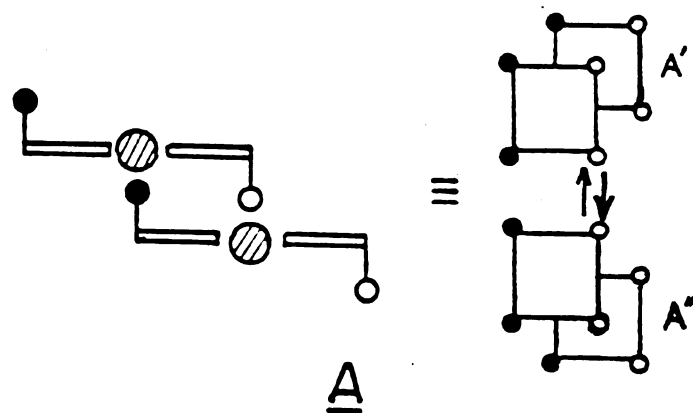


Figure 9. Structure proposed by Laszlo⁹⁹⁻¹⁰⁶ for the solution self-structuring of $\text{Na}_25'\text{GMP}$. Open circles represent phosphate groups pointing down, while closed circles represent phosphate groups pointing up. Na^+ ions are represented by striped circles, A is the staircase model proposed by Laszlo. B is an alternate model proposed by Laszlo.

ion (TMA^+) acts as neither a structure directing nor structure inhibiting ion. This has facilitated the study of 5'GMP self-structure at varying ratios of metal to nucleotide. She has also found that K^+ is the better stabilizing ion and that Na^+ is the better structure director in the self-structuring of 5'GMP. This is based on the observation that the addition of K^+ to high 5'GMP/ Na^+ ratios results in the stabilization of the Na^+ self-structure and that the addition of Na^+ to high ratios of 5'GMP/ K^+ results in the "simple" K^+ self-structure being replaced by a Na^+ type of self-structure. On the other hand, the addition of K^+ to Na_2 5'GMP causes a decrease in the extent of Na_2 5'GMP self-structure and the formation of the K_2 5'GMP self-structure.

Laszlo has investigated the ^{23}Na NMR of Na_2 2'GMP up to 0.45M and found no evidence for solution self-structuring. Brown⁴⁹ has reported a brief investigation of 2'GMP, 3'-GMP, 2'3'cGMP, 3'5'cGMP, and 5'dGMP. She found ^1H NMR evidence for the self-structuring of 2'GMP; the Na^+ salt formed only a very limited structure (<5%), whereas the K^+ salt structured to approximately 70% at high concentration and low temperature.

III. Ethidium Binding to Nucleic Acids

A. General Intercalation

The determination of the structures of nucleic acids has been aided in recent years by the use of intercalation drugs¹⁰⁷. With an intercalation drug a portion of the drug molecule, usually an aromatic ring, is inserted between bases of nucleotides. The intercalation mode of binding was first proposed by Lerman¹⁰⁸ to explain the binding of the aminoacridines to DNA. There is a wide range of compounds in which only a portion of the molecule may intercalate. The intercalation of aromatic amino acids is probably one of the modes involved in protein-nucleic acid recognition¹⁰⁹. Purine and pyrimidine bases interact in living things not only with each other but also with proteins. They bind to the enzymes of nucleic acid and nucleotide metabolism and they serve as a means by which proteins can hold on to many metabolic intermediates and coenzymes.

B. Ethidium Cation General Information

Ethidium bromide (EtdBr; Figure 10) is biologically active, possessing trypanocidal¹¹⁰, antiviral¹¹¹, and antibacterial¹¹¹ properties. EtdBr inhibits DNA synthesis in vivo¹¹²; and in vitro, EtdBr interferes with DNA dependent DNA and RNA polymerases of *Escherichia Coli*^{113,114}. EtdBr-DNA complexes exhibit several properties which are

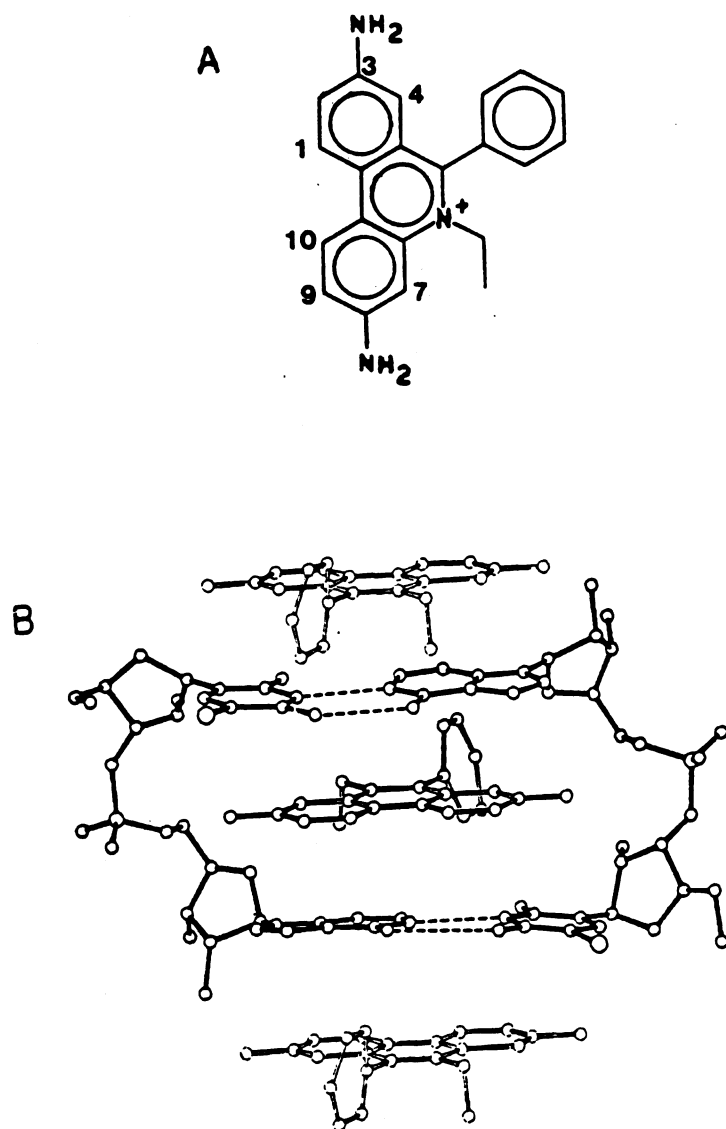


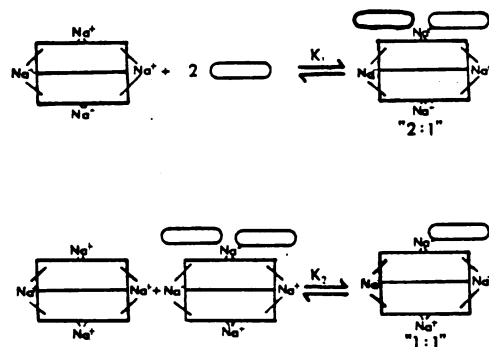
Figure 10. A. The structure of ethidium. B. A schematic diagram showing the intercalation of ethidium in U-A. Taken from Ref. 122.

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indicative of intercalation. These include a diminished sedimentation coefficient¹¹⁵, enhanced intrinsic viscosity¹¹⁵, and a greatly augmented fluorescence^{116,117}. Recently, interest in EtdBr has centered on its special ability to unwind closed circular DNA¹¹⁸⁻¹²¹.

Ethidium binding to DNA, RNA, oligonucleotides and dinucleotides has been studied^{58-61,122-127}. Proton NMR has proven to be a useful tool in studying the interaction of ethidium with short nucleic acid polymers. Krugh et al.^{59,60} and Chan and coworkers¹²⁸ have used dinucleoside monophosphates as a model for intercalation of EtdBr into DNA. When the drug is intercalated, the aromatic protons shift upfield due to ring current effects. The side chain methyl group resonance lies upfield of all other resonances and its change in chemical shift as a function of nucleotide concentration provided stoichiometric information. In all cases it was found that one EtdBr intercalates between two stacked H-bonded base pairs. A preference for binding pyrimidine-purine sequence isomers especially CpG has been determined. To date, no studies have been conducted on the mononucleotides. Studies on the binding to mononucleotides may provide information on the mode of binding and sequence preferences in DNA. Marshall⁹⁷ has investigated the interaction of ethidium cation with the structured forms of Na₂5'GMP and K₂5'GMP⁹⁷. His models are shown in Figure 11. The binding constants of ethidium cation to structured 5'GMP were found to be $>10^3$.

A



B

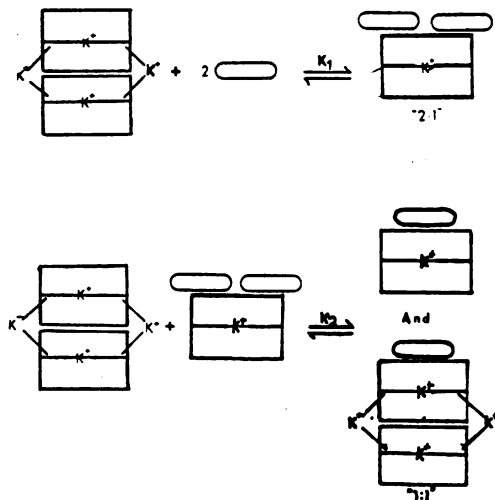


Figure 11. Schematic diagram of binding of ethidium to structured forms of 5'GMP. Ethidium is represented by and the guanylic acid tetramer by . A is Marshall's (97) model for ethidium binding to the $\text{Na}_2 5'\text{GMP}$ self-structure. B is Marshall's (97) model for ethidium binding to the $\text{K}_2 5'\text{GMP}$ self-structure.

IV. Amino Exchange in Mononucleotides

Hydrogen bonding between the monomers of nucleic acids has been the object of numerous investigations¹²⁹⁻¹³⁷. Although interactions occurring in aqueous solvents are more applicable to biological systems, little work has been done on hydrogen bonding of nucleotide bases in water until recently. Proton NMR spectroscopy is ideally suited for differentiation between vertical ring stacking and hydrogen bonding. In most cases, upfield shifts of the ring protons are observed upon stacking of aromatic bases, whereas hydrogen bonding results in downfield shifts for the participating protons. Analysis of ^1H NMR linewidths of the amino proton resonances can give detailed data on the rates of proton exchange between the amino groups and the solvent^{129,138,139}. Spectra taken in $^1\text{H}_2\text{O}$ permit the study of slow exchanging NH, NH_2 , and OH groups.

McConnell and Seawell¹³³ have measured the intrinsic hydrogen-exchange of purine amino protons to determine a mechanism for amino exchange in DNA, specifically the nature and position of the structure motility in the sequence of events that initiate exchange. A space-filling model of the double helix shows one proton of each of the amino pairs is sterically accessible to solvent for exchange in the helical grooves and free from participation in intramolecular hydrogen bonding. In contrast, the second amino proton and the imino protons of guanine and thymine are

much less accessible to solvent molecules because of their location and involvement in hydrogen bonding.

Information on the amount of macromolecular structure, obtained by counting slow-exchanging non-hydrogen bonded amino hydrogens, and the "motility" of the DNA structure derived from exchange rates can be obtained if a quantitative knowledge of the hydrogen exchange rates is known in the absence of structure. The latter exchange rates are not well known for nucleic acids. The first-order rates for amino exchange of the purine nucleotides were determined to be several orders of magnitude slower than rates for amino groups of the aliphatic and aromatic compounds, whose rate constants are diffusion controlled. These low intrinsic rates account for the slow exchange of the non-hydrogen bonded amino protons of the helical grooves of DNA. A second observation which explains the distribution of different kinetic classes of exchangeable interbase protons in DNA was that the pH dependence is not the same for amino proton exchange of adenine and guanine. It is suggested that proton transfer involving the outside amino protons of DNA base nitrogens might precede structural events such as strand separation.

In another paper McConnell¹³⁰ points out that the important mechanism of amino exchange at physiological pH appears to involve the ability of the conjugated purine structure to communicate the effects of ring protonation as a regulatory aspect of proton exchange at the exocyclic

-NH₂. A two-step exchange, in which transfer of the -NH₂ proton to solvent water is preceded by protonation of the basic N-1 proton of the purine, accounts for properties of hydrogen exchange in A-U double helical structures and polymeric G-C^{140,141}. This exchange mechanism would provide a means for communication from solvent to the helix interior with consequent effects on Watson-Crick hydrogen-bonding.

Iwahashi and Kyogoku^{136,137} have used the saturation transfer method of ¹H NMR to elucidate the sites and rates of exchangeable nuclei in nonaqueous solvents. An exchange takes place between the imino proton of 1-cyclohexyl-5-bromo-uracil (BrU) and the amino proton of 9-ethyladenine (9EA), and between the amino proton of 1-methylcytosine (1MC) and the imino proton of 9-ethylguanine (9EG), while the amino proton of 9EG does not exchange at all with the other protons. Experiments at low temperature show that, of the two amino protons of cytosine only the proton directly participating in the hydrogen bond exchanges with the imino proton of 9EG (cf. Figure 12). Activation energies of the proton exchange between some 1-cyclohexyluracil derivatives and 9EA in chloroform were reported. They vary from 7 to 16 kcal/mole.

Although the experiments were carried out on the monomeric systems, there is no reason that the proton exchange cannot occur in the interior of nucleic acids. The exchange is possibly due to the presence of the keto-enol tautomerism.

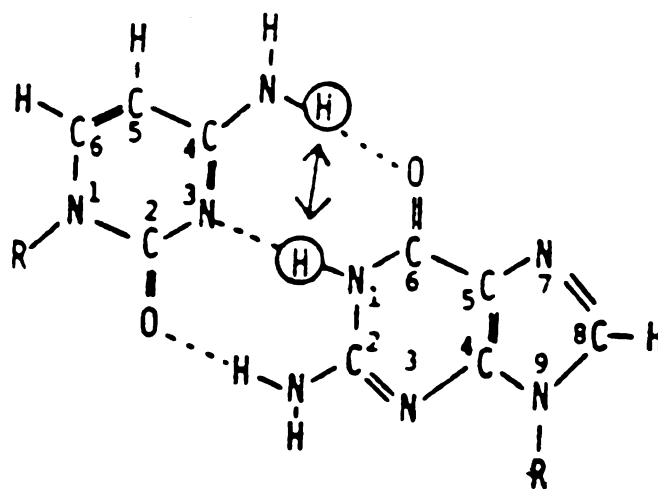


Figure 12. Exchangeable protons in the G-C pair. The circled protons exchange with each other. Taken from Reference 122.

V. Statement of Research Aims

The phosphate group in Brown's model⁴⁹ for 5'GMP self-association plays an important role in stabilizing the solution self-structure. The interplatelet phosphate chelation of Na^+ is a major factor in her model in the stability of the structure. Perturbations in the placement of the phosphate groups should cause an effect on the structure, which may lead to a better understanding of the structure. Therefore the alkali metal dependence of the solution structuring of guanosine, 2'GMP, 3'GMP, and 5'GDP were investigated. Mixed nucleotide experiments were carried out to determine if one nucleotide could be incorporated into the structure of another. An ordering of the structure-forming tendencies of the guanylic acid derivatives was to be determined.

In preliminary work on 2'GMP, a novel proton exchange was noted for unstructured nucleotide. The exchange is between 2-NH_2 and solvent water. The most interesting aspect of this exchange is that the integral intensity of NH_2 varies from ~ 1 to 2 relative to H(8) in $\text{Na}_2 2'\text{GMP}$, but is always 2 in $\text{TMA}_2 2'\text{GMP}$. The conditions where the integral intensity is < 2 are consistent with an ordered form, perhaps tetramers, of nucleotide being present. A detailed analysis of the exchange reaction may lead to the determination of thermodynamic parameters for the equilibrium monomers $\rightleftharpoons$ tetramers.

The discovery by Brown⁴⁹ that TMA^+ acts as neither a

structure directing nor inhibiting ion has facilitated the study of 5'GMP self-assembly at varying ratios of metal to nucleotide and has allowed the determination of the stoichiometry of the nucleotide in the Na_2 5'GMP self-structure. It was found that TMA^+ interferes with structure formation in 2'GMP and 3'GMP. Investigations were carried out to determine the mode of this interaction.

Marshall⁹⁷ found that the drug, Ethidium Bromide formed a 1:1 and a 2:1 complex with structured 5'GMP. The binding of EtdBr to unstructured and structured 2'GMP and 5'GMP was investigated. Proton NMR spectra of nucleotide solutions in $^1\text{H}_2\text{O}$ were studied to determine if structural information could be obtained from the resonances and intensities of the exchangeable protons.

EXPERIMENTAL

I. Materials, Techniques, and Preparations

Guanosine monohydrate and disodium 5'-guanosine monophosphate were purchased from Calbiochem, Inc. Disodium 2'-guanosine monophosphate was purchased from P and L Laboratories or Sigma Chemical Co. All other nucleotides were purchased from Sigma Chemical Co. Inosine 5'-monophosphate was obtained as the free acid, while 5'-guanosine diphosphate and 3'-guanosine phosphate were obtained as the disodium salts. The nucleoside and nucleotides were used without further purification. The desired metal ion forms of free acid nucleotides were obtained by titrating dilute solutions ($\sim 0.01M$) with the appropriate metal hydroxide to $pH=7.8$. Metal ion exchange of the other nucleotides was accomplished using Dowex 50W-X8 (100-200 mesh) cation exchange resin obtained from the Dow Chemical Co. The resin had a cation exchange capacity of 1.7 meq/ml. The resin was prepared by the following washing sequence: (1) 10% HCl, (2) H_2O and EDTA, (3) 10% NaOH, (4) H_2O , (5) 1:1 H_2O-CH_3OH , (6) CH_3OH , (7) 1:1 $CH_3OH-CH_2Cl_2$, (8) CH_2Cl_2 , (9) 1:1 $CH_3OH-CH_2Cl_2$, (10) CH_3OH , (11) 1:1 H_2O-CH_3OH , (12) 10% HCl, (13) H_2O and EDTA, (14) 1N desired metal hydroxide, and (15) washed to neutrality with H_2O .

A dilute solution ($<0.1M$) of the nucleotide was passed through two columns each containing a twenty-fold excess of resin to ensure complete exchange. The flow rate was approximately 20 ml/hr. The exchanged nucleotide was then lyophilized to dryness. All of the water used in these studies was purified by passing distilled water through an Illinois Water Treatment Co. purification system to remove paramagnetic ion impurities and organic residues.

The metal hydroxides were used without further purification. NaOH and KOH were purchased from Mallinckrodt, RbOH and CsOH from Apache Chemicals, Inc., tetramethylammonium hydroxide pentahydrate and tetrabutylammonium hydroxide (25% in methanol) from Matheson, Coleman and Bell, tetraethylammonium hydroxide (20% in water) from Aldrich Chemical Co. and, LiOH and $TlOH \cdot H_2O$ from Alfa-Ventron. The metal chlorides were also used without further purification. LiCl, NaCl, and KCl were purchased from Mallinckrodt. RbCl and CsCl were ultrapure quality from Alfa-Ventron. $CaCl_2$ was obtained from Matheson, Coleman and Bell. Tetramethylammonium chloride and tetrabutylammonium iodide was purchased from Aldrich Chemical Co. Tetraethylammonium perchlorate, purchased from Eastman Chemicals, was recrystallized twice from water. $SrCl_2$ was purchased from J. T. Baker Chemical Co. $TlNO_3$ was obtained by titrating $TlOH \cdot H_2O$ with HNO_3 to pH=7 and drying. Metal salts were dried by heating and storing in a desiccator. Ethidium

bromide, purchased from Sigma Chemical Co., was lyophilized twice from 99.5% D_2O to remove ethanol and to replace water of crystallization with D_2O .

NMR samples in D_2O were prepared from the nucleoside or desired metal nucleotide lyophilized twice from a 100 fold weight excess of 99.5% D_2O purchased from Norell Co. or 99.75% D_2O obtained from KOR Isotopes. The nucleoside was also studied in d-6 DMSO, 99% D, purchased from Strohler Isotope Chemicals, and dried over 4 Å molecular sieves. Unless otherwise noted 8 mM sodium or tetramethylammonium 2,2,3,3,- d_4 -3 trimethylsilylpropionate (TSP), purchased from Dohme Canada Limited, was used as an internal reference. Cation exchange of TSP was accomplished by the same method as the nucleotides. An external reference and lock were used to obtain NMR spectra in ^1H_2O . The nucleotide dissolved in ^1H_2O was placed in a 5 mm tube. A capillary NMR tube containing 8 mM NaTSP used as an external reference, D_2O used as lock, and 1.0M NaCl to prevent freezing at low temperatures was placed in the 5 mm NMR tube containing the sample. To complex paramagnetic impurities 2 mM ethylenediaminetetraacetic acid (EDTA) purchased from Mallinckrodt as the free acid was included in the samples. Some samples were recorded without the addition of EDTA. These samples exhibited the same linewidths and chemical shifts as those containing EDTA. All solutions used for the NMR studies were passed through a 0.65 μm millipore

filter obtained from Millipore Corp. Samples were run in 5 mm NMR tubes of good quality. The concentration of the solutions was determined by ultraviolet absorption spectroscopy. The pH of the solutions was determined by an Ingold Electrodes Inc. micro-combination electrode (Wilma Glass Co.) and a Fisher model 370 pH meter. The pD of a solution was taken as $\text{pH} + 0.4$ ¹⁴². NMR samples used for T_1 measurements were degassed by bubbling N_2 gas through the sample. This was not critical since T_1 's were always $\ll 60$ sec.¹⁴³. Sample height was 10-12 mm to limit diffusion out of the receiver coils. The receiver coils were 10 mm in length. A teflon vortex plug was used to prevent vortexing. Integrations were accomplished with a K & E Planimeter. Baseline corrections were accomplished by the 5 parameter baseline correction routine of DISNMR. Zero filling of NMR spectra was used when possible. In the zero filling technique zeros are added to the end of a fully decayed free induction decay (FID). On transforming the spectrum, the point to point resolution is increased. This technique allows samples with short T_1 's to be accumulated in a shorter time period without loss of point to point resolution.

II. Instrumentation

A. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained on either a Bruker WH-180 interfaced to a Nicolet 1180 computer with 16K of memory or a Bruker WM-250 interfaced to an Aspect 2000 computer with 32K of memory. Both were operated in the Fourier Transform mode. Probe temperature was maintained to $\pm 1^\circ\text{C}$ by a Bruker temperature control unit coupled to a probe-mounted thermocouple. The unit was calibrated by measuring the chemical shift differences between the methanol proton resonances and applying the Van Geet equations¹⁴⁴. Nuclear Overhauser Effect (NOE) and T_1 experiments were performed on the Bruker WM-250 using a 5 mm ^1H probe. Calculation of T_1 's was accomplished by the T_1 calculation routine of DISNMR version 801015, Bruker Instruments, Inc. (1981). The pulse sequences used for NOE and T_1 data collection are given in Appendix 1. Chemical shifts are accurate to ± 0.005 ppm.

B. Ultraviolet/Visible Absorption Spectroscopy (UV/Vis)

Concentrations of all nucleoside, nucleotide and EtdBr solutions were determined by UV/Vis absorption by dilution of aliquots either on a Beckman DU spectrometer updated with a Gilford photometer 252 or a Cary 219. Solution concentrations were determined using Beer's Law. The molar absorptivity (ϵ) at the absorption maximum are listed in Table 3

Table 3. Molar Absorptivities Used to Determine Concentration¹⁴⁵.

Compound	(M ⁻¹ cm ⁻¹)	λ_{max} (nm)
5'GMP	13,700	252
2'GMP	13,300	253
3'GMP	13,300	253
Guanosine	14,598	253
5'GDP	13,700	253
5'IMP	12,700	249
Ethidium	5,450	480

for the guanylic acids and EtdBr. It was assumed that the molar absorptivity was independent of metal ion and that the absorbances were additive for mixed solutions.

C. Infrared Spectroscopy (IR)

IR spectra were obtained on a Perkin-Elmer 283B infrared spectrophotometer. Matched and sealed CaF₂ cells of approximately 15 μm pathlength were used. The cells were modified to be contained in a water jacket to enable temperature control. Water jacket temperature was controlled by a Forma Scientific model 2095 bath and circulator. The temperature of the solution was determined using a copper-constantan thermocouple mounted on the cell window.

D. Computation

i. KINFIT - Non-linear least square fitting was accomplished on a Control Data Corporation, Cyber 170 model 750 computer using the KINFIT program developed by Dye¹⁴⁶. This method uses Wentworth's¹⁴⁷ minimization procedure. Equations used for individual programs are given in Appendix 1.

ii. LORENT - Amino exchange rates were estimated by LORENT (T. V. Atkinson, Department of Chemistry, Michigan State University) a Fortran program running under RSX11M on a PDP 11/40. This program calculates theoretical NMR line shapes for the case of general two site exchange. The equations used are those given by Rogers and Woodbrey¹⁴⁸.

iii. RESOL - Determination of line width in NMR spectra was accomplished with DISNMR (Bruker Instruments, version, 1981) on the Aspect 2000 for single non-overlapping resonances, and with RESOL (T. V. Atkinson, Department of Chemistry, Michigan State University), a Fortran program running under RSX11M on a PDP 11/40 for overlapping resonances. RESOL is a non-linear least squares fitting program which attempts to find the set of a given number of Lorentzian lines which best fits the experimental spectrum. This program uses LMSTR1¹⁵⁰, a subroutine for non-linear

least squares.

The digitized spectra were transferred from the ASPECT 2000 to the PDP11 via a multiple step process. The first step involved transfer over an asynchronous serial link with the program BEAM (Bruker Instruments, version, 1980) handling the ASPECT end of the link and the program CDC running under RT11 handling the PDP11 end. The transfer from RT11 to RSX11M was accomplished using the utility FILEX.

III. Synthesis

A. Selective H(8) Deuteration

Selective H(8) deuteration of a guanylic acid was accomplished by refluxing a dilute solution of nucleotide ($<0.01\text{M}$) in D_2O for 1-2 hrs. and lyophilizing to dryness. If a residual H(8) resonance was observed in the ^1H NMR spectra the procedure was repeated¹⁵⁰.

B. 8-Bromoguanosine

8-Bromoguanosine was synthesized by the method of Holms and Robins¹⁵¹. Guanosine (0.5 g) was suspended in 50 ml H_2O and saturated bromine water was added dropwise with vigorous stirring. The solution was allowed to become white before adding another drop. When the solution remained light yellow no more bromine was added and the

solution was allowed to stir 30 minutes. The solution was rotovaporated to 5 ml, filtered cold, and washed with cold water. The precipitate was recrystallized from water twice. Purity was determined by the loss of H(8) intensity in the NMR spectrum.

RESULTS

I. Guanosine

A. The Solution Self-Structure of Guanosine

Nuclear magnetic resonance spectroscopy (NMR) is an excellent method for the study of the self-association of guanylic acids. The most compelling evidence for ordered structure formation is the presence of multiple inequivalent H(8) resonances. The resonance in non-structured solutions is a singlet positioned downfield from the other resonances, thus making it facile to monitor. Solution self-structure manifests itself in the formation of new H(8) resonances, showing that the formation of structure is slow on the NMR time scale. Broadening is due to processes fast on the NMR time scale, such as base stacking and possibly tetramer formation. Ordered structure in this work will refer to species which are slow to exchange on the NMR time scale and/or show evidence for structure formation in the IR absorption region. Monomeric or "disordered" nucleotide refers to monomeric nucleotide and all weakly aggregated species which are in rapid (NMR time frame) equilibrium with it and exhibit little or no IR frequency perturbation.

To access the role of the phosphate group in the

solution self-structuring of guanylic acids, guanosine aggregation was investigated by ^1H NMR. The addition of NaI, KI, SrCl_2 , and TlNO_3 to a saturated guanosine solution (0.02 M) at 0°C does not affect the H(8) resonance, neither in chemical shift nor half-width, up to the point of precipitation or gelation. At the gel or precipitation point, all resonances are understandably broadened due to the increased viscosity of the medium. To increase the concentration of nucleoside in solution, DMSO-d_6 was used as the solvent. The results were similar except that the nucleoside always precipitated from solution instead of gelling. The addition of small amounts of water (1%) to saturated salt solutions of guanosine in DMSO resulted in instant gelation of the solution. Thus, water is important in guanylic self-structuring. Guschlbauer⁷¹ has reported that water is the best solvent for gel formation. Methanol and ethanol can be used but the stability of the gel is greatly reduced. No evidence for solution structuring was found in the ^1H NMR of mixed $\text{DMSO-d}_6/\text{D}_2\text{O}$ salt solutions of guanosine.

To determine the potential importance of the glycosidic bond torsion angle in guanosine gelation, 8-bromoguanosine was investigated. Guanosine is preferentially in the anti conformation^{68,79}, while 8-bromoguanosine is in the syn conformation^{68,79}. Since H(8) is absent in 8-bromoguanosine, the H(1') region of the NMR spectrum was monitored. The

results were the same as with guanosine. The spectra of salt solutions of concentrated 8-bromoguanosine in D_2O , $DMSO-d_6/D_2O$ mixtures and $DMSO-d_6$ showed no evidence for solution self-structuring.

B. Incorporation of Guanosine into the Na_25' GMP Solution Self-Structure

Although guanosine does not form an NMR-observable ordered structure, selective deuteration experiments were carried out to determine if guanosine could incorporate into the Na_25' GMP structure. Two experiments were performed: (1) guanosine was added to an 8-d Na_25' GMP (8-deutero-disodium guanosine-5'-monophosphate) solution and (2) 8-d guanosine was added to an Na_25' GMP solution. These procedures permit the examination of the H(8) resonance of each guanylic acid individually, because the deuterated nucleotides are not NMR observable.

Figure 13 shows the results of the addition of 8-d guanosine to a structured 0.32M Na_25' GMP solution. There is a relative intensity loss of the α and δ resonances and an upfield shift of the β resonance¹⁵² (cf. Figure 13), all indicative of a decrease in the amount of structure. The H(1') resonance is sharpened, along with a decrease in the small upfield structure H(1') resonance. There is a linear decrease in structure with addition of 8-d guanosine (cf. Table 4). The percent structure present in

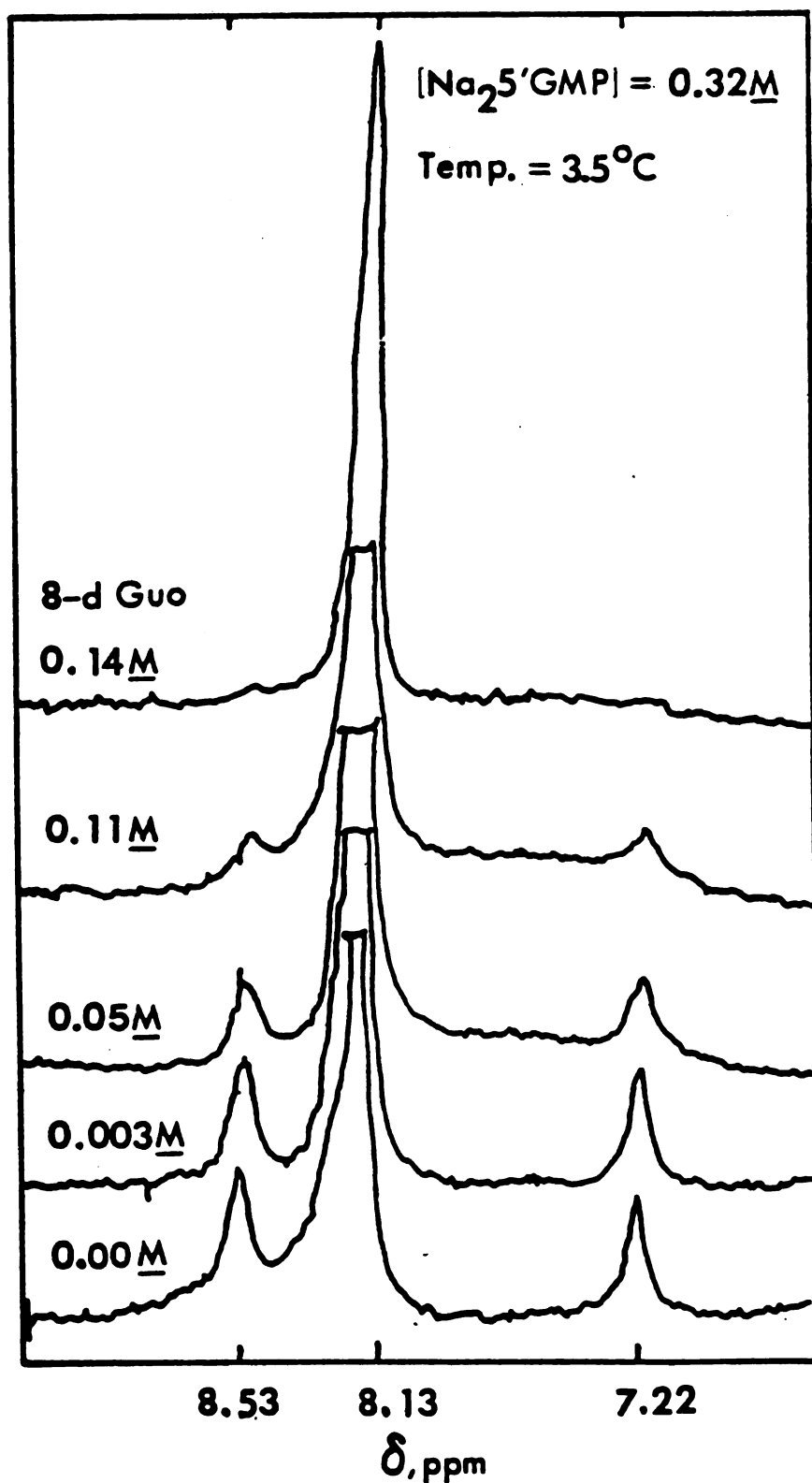


Figure 13. The effect on the H(8) region of the NMR spectrum of 0.32M $\text{Na}_25'\text{GMP}$ at 3.5°C on the addition of 8-d guanosine. All solutions were homogeneous and not gels.

Table 4. The Effect on the Solution Self-Structure of 0.32M Na₂5'GMP on Addition of 8-d Guanosine at 3.5°C.

[8-d Guo] Added	% Structure	
	Absolute ^a	Relative ^b
0.142M	0.0	0.0
0.107M	10.1	30.0
0.085M	17.8	52.0
0.071M	16.3	48.0
0.047M	24.3	72.0
0.024M	26.5	78.0
0.003M	33.2	98.0
0.00M	33.9	100.0

^aThe absolute percent structure was determined by integrating the resonances and using the following equation:

$$3\left(\frac{\alpha+\delta}{2}\right)/(\alpha+\chi+\delta).$$

^bThe relative percent structure is:

[absolute % structure without 8-d guanosine added-absolute % structure with 8-d guanosine added/absolute % structure without 8-d guanosine added].

solution was determined by assuming the β resonance was equal in intensity to both the α and δ resonances. The equivalence of the α , β , and δ resonances has been continually observed under all conditions in all work done to date on the $\text{Na}_2\text{5'GMP}$ self-structure^{49,97}.

Solutions of 0.32M $\text{Na}_2\text{5'GMP}$ containing $>0.04\text{M}$ 8-d guanosine have a broad resonance ($\nu_{1/2} \sim 125$ Hz) in the H(8) region, which is not present in an equivalent $\text{Na}_2\text{5'GMP}$ solution, as shown in Figure 14. The broad resonance increases in intensity with increasing 8-d guanosine concentration. We assign this resonance (shaded resonance in Figure 14) to a large slow tumbling structure that incorporates 5'GMP and 8-d guanosine. The broad resonance accounts for 35% of the observable 5'GMP H(8) integral intensity in a 0.32M $\text{Na}_2\text{5'GMP}$ solution which is 0.11M in 8-d guanosine at 3.5°C . In addition, a decrease in the integral intensity of H(8) is observed as 8-d guanosine is added to a 0.32M $\text{Na}_2\text{5'GMP}$ solution. This loss is attributed to a larger slow-tumbling structure that incorporates 5'GMP and 8-d guanosine, whose half-height is so broad that it is not observable. In a solution containing 0.11M 8-d guanosine and 0.32M $\text{Na}_2\text{5'GMP}$ the loss of integral intensity of H(8) accounts for 30% of the total H(8) intensity.

It is postulated that large, soluble aggregates are formed that contain 5'GMP and 8-d guanosine. These polymeric structures exist in various sizes. The smaller

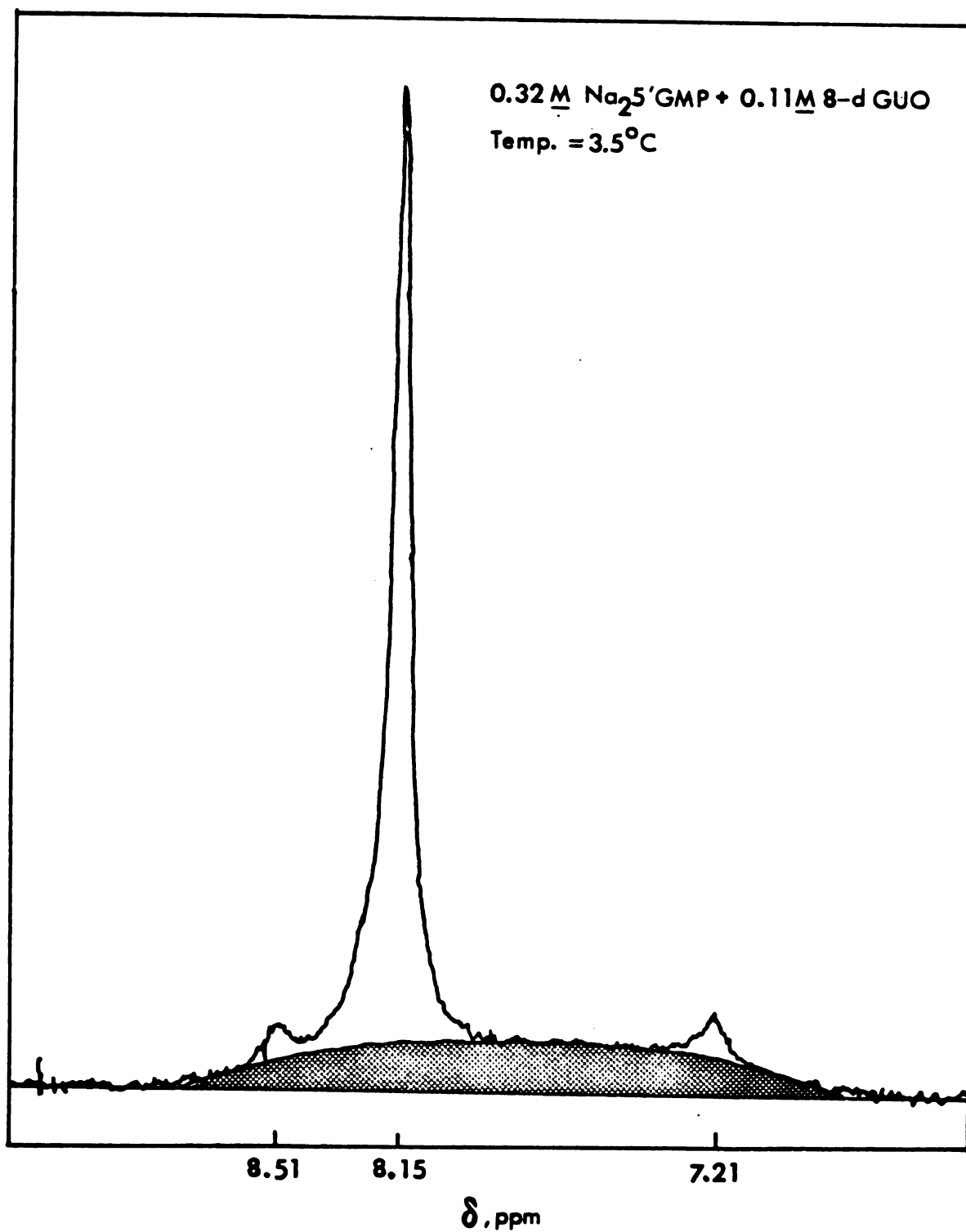


Figure 14. The H(8) NMR spectrum of a 0.32M $\text{Na}_2\text{5'GMP}$ and 0.11M 8-d guanosine solution at 3.5°C. Note the very broad resonance present (shaded).

polymeric units give rise to the observable, broad H(8) resonance. The resonance of the larger polymeric structures is broadened into the baseline and is therefore not observable, causing a decrease in H(8) integral intensity.

The expected complimentary broad H(8) resonance of guanosine was not observable when guanosine was added to a 0.32M 8-d Na₂5'GMP solution at 3°C. The guanosine H(8) resonance is assumed to be broadened into the baseline, due to the guanosine being present in large slow-tumbling structures. The Na₂5'GMP structure was observable in the ribose region of the spectra. No attempt was made to determine the amount of Na₂5'GMP structure present, but the ribose regions of the spectra were quantitatively similar to the corresponding 8-d guanosine/Na₂5'GMP solutions.

The solubility of guanosine was greatly enhanced in the mixed system. Guanosine is soluble to 0.02M in H₂O at 3°C, whereas in a 0.32M Na₂5'GMP solution its solubility is increased to greater than 0.14M. Increasing the guanosine concentration beyond 0.14M causes the solution to gel.

Guanosine has a greater "structure-forming tendency" than 5'GMP. In all the mixed guanosine and 5'GMP solutions, guanosine was in large aggregates that were NMR unobservable. The samples with larger concentrations of guanosine (>0.01M) should have been gels. Gelation was most likely inhibited by phosphate repulsion of 5'GMP units, which were incorporated in the guanosine self-structure.

The loss of 5'GMP solution self-structure cannot be attributed to the removal of Na^+ of solution by incorporation into the guanosine gel-structure. Assuming there is one Na^+ per tetramer unit in the guanosine gel-structure, only a 5% decrease of Na^+ concentration would occur in the most concentrated sample. This decrease in $[\text{Na}^+]$ concentration could not account for the total loss of Na_2 5'GMP self-structure that is found.

II. The Solution Self-Structuring of 2'GMP

The alkali metal salts of 2'GMP are a better system for study than guanosine. They are more soluble in H_2O and do not form gels at neutral pH, regardless of the nature of the alkali metal ion. Figure 15 shows the limiting H(8) resonance lines of the alkali metal salts of 2'GMP in concentrated D_2O solutions, except for the spectrum of 1.22M Na_2 2'GMP which was taken in H_2O , near the freezing point of the solvent. It is immediately clear that the Na^+ , K^+ , and Rb^+ salts form slowly exchanging order structures with multiple H(8) environments, whereas Li^+ and Cs^+ provide little or no evidence for structure formation. In dilute solution ($\sim 0.02M$), all of the salts exhibit a single sharp H(8) resonance at 7.98 ppm at 23°C characteristic of disordered 2'GMP. An increase in the nucleotide concentration or an increase in temperature causes a downfield shift of the H(8) resonance. Figure 16 and Figure 17 show the effect of temperature and concentration, respectively, on the chemical shift of H(8) of various salts of 2'GMP.

A. The Li^+ Salt of 2'GMP

As seen in Figure 15, a 0.98M Li_2 2'GMP sample at -2°C shows no evidence of structure formation. The H(8) resonance is broadened but no new structure resonances are

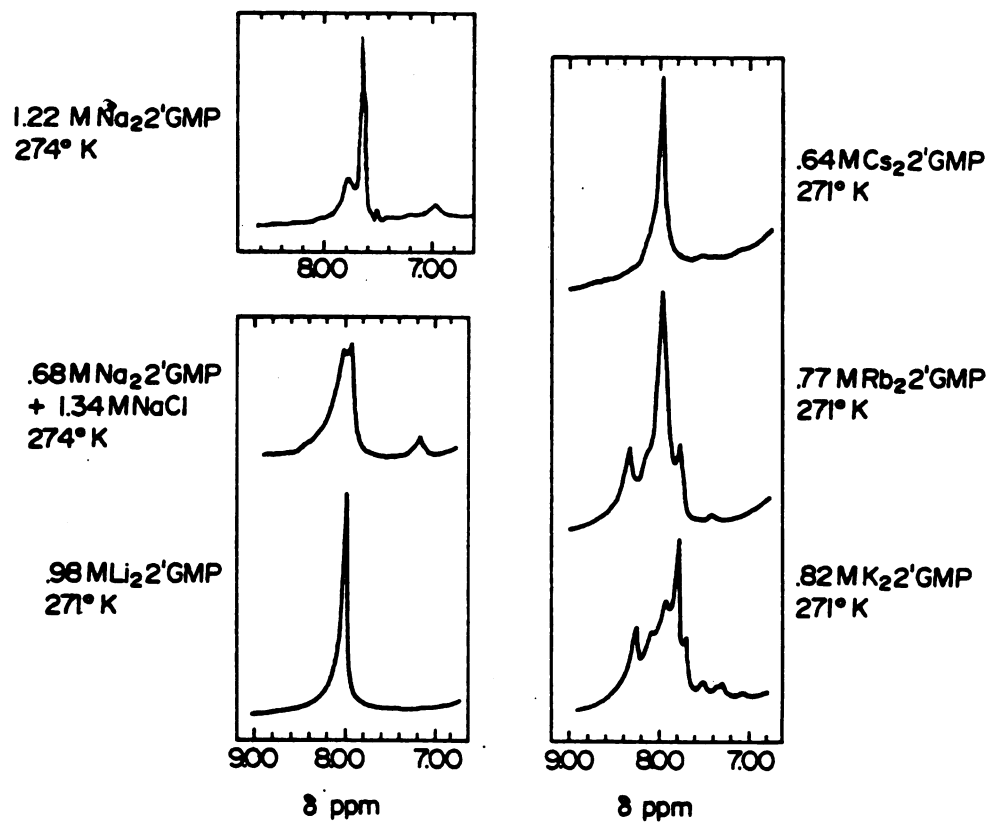


Figure 15. The limiting H(8) resonance lines of alkali metals salts of 2'GMP. Spectra were recorded in D_2O solutions, except for the spectrum of 1.22 M $\text{Na}_2 2'\text{GMP}$ which was recorded in $^1\text{H}_2\text{O}$.

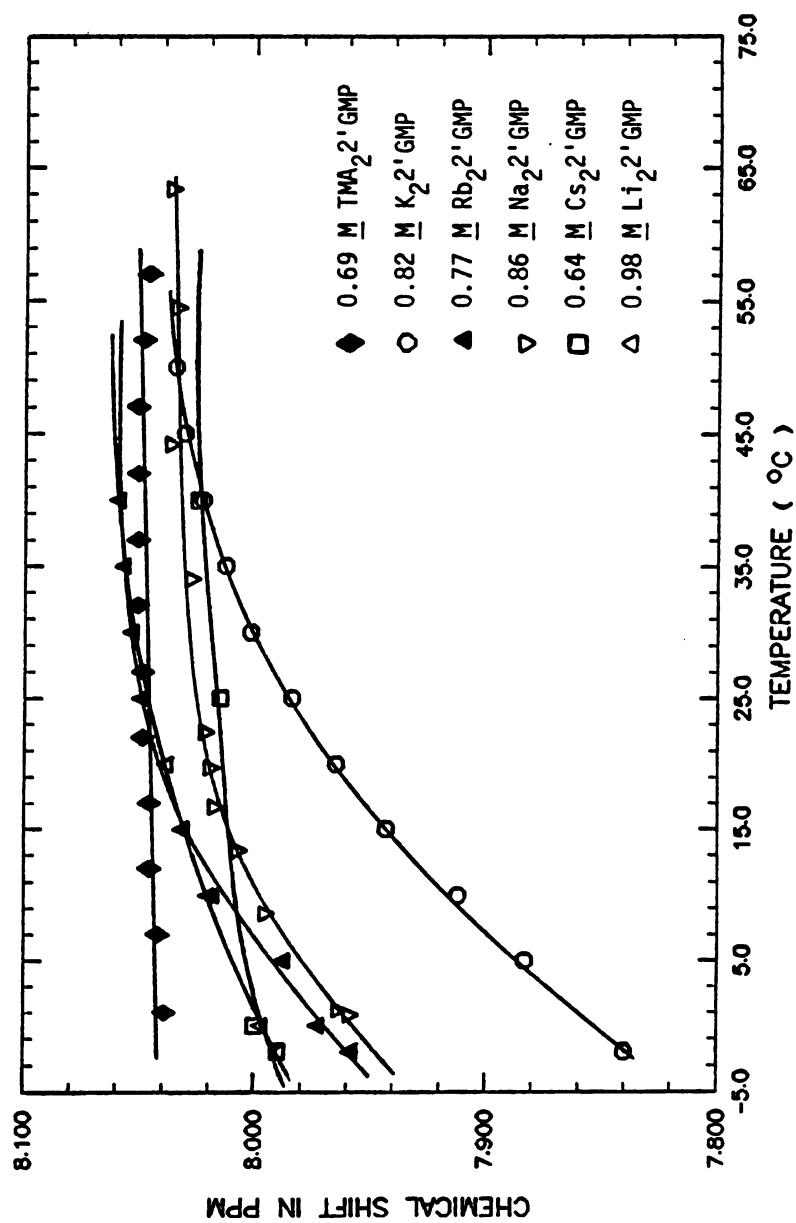


Figure 16. The H(8) chemical shift of 2'GMP versus temperature.

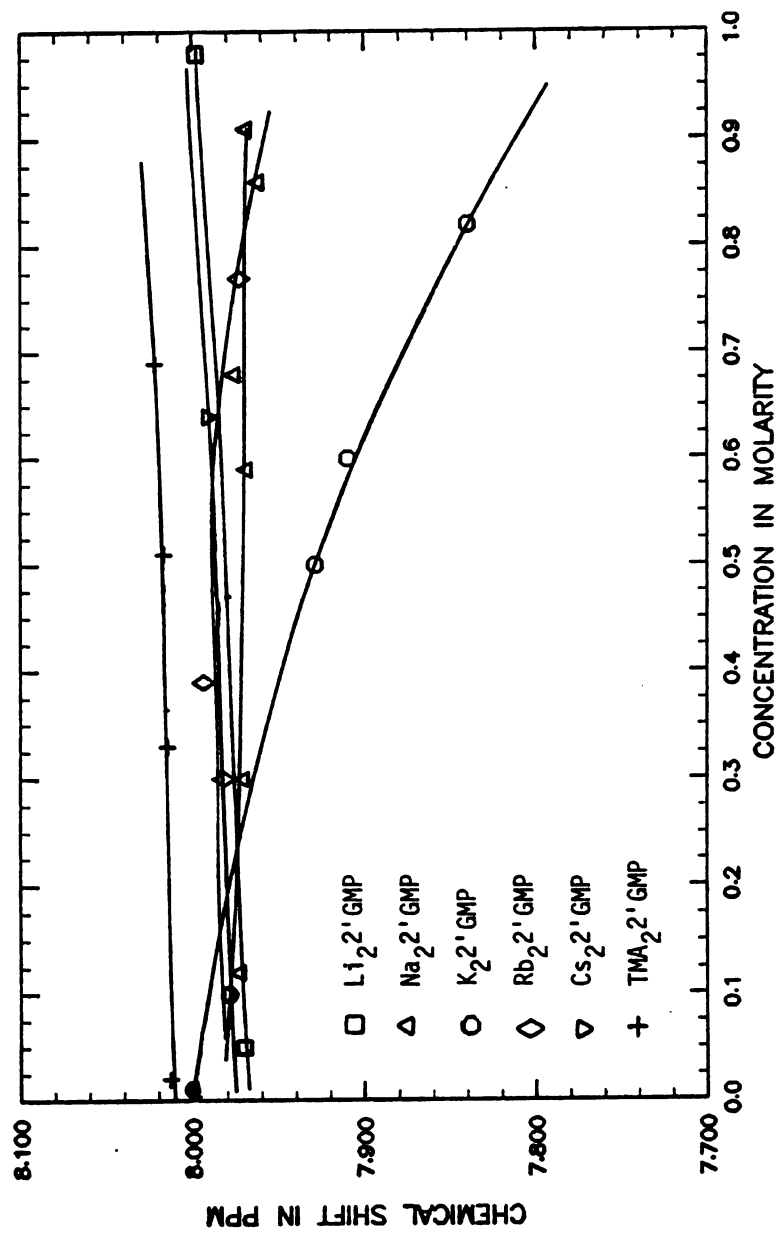


Figure 17. The H(8) chemical shift of selected salts of 2'GMP versus concentration at 1°C.

evident. The width at half-height for the H(8) line at 2°C increases from 3.7 Hz at 0.05M to 16.0 Hz at 0.98M. No corrections were made for the change in viscosity. An increase in temperature also causes a narrowing of the H(8) resonance. Increasing the temperature of a 0.98M Li₂2'GMP sample from -2°C ($\nu_{1/2}$ = 16.0 Hz) to 40°C ($\nu_{1/2}$ = 6.7 Hz) causes a decrease of 9.3 Hz.

The carbonyl stretching region of the infrared spectrum of 0.98M Li₂2'GMP, Figure 18, does not show any frequency shifts or intensity changes over the temperature range 3°-50°C that could be attributed to formation of an ordered structure. A discussion of the expected changes in the infrared spectra on structure formation is presented in the section on the Na⁺ self-structure of 2'GMP.

B. The Na⁺ Salt of 2'GMP. Two new resonances appear in the H(8) region of a 0.70M Na₂2'GMP solution at 0°C, one downfield at 8.51 ppm and one upfield at 7.24 ppm of the monomer (7.94 ppm). In a 0.91M solution at 2°C, as shown in Figure 19, the structure resonances account for less than 5% of the total nucleotide concentration. This spectrum is indicative of what will be referred to as the simple structure. As the concentration of Na₂2'GMP or Na⁺ is increased, the downfield resonance disappears and a new resonance appears at 7.99 ppm (cf. Figure 15). A

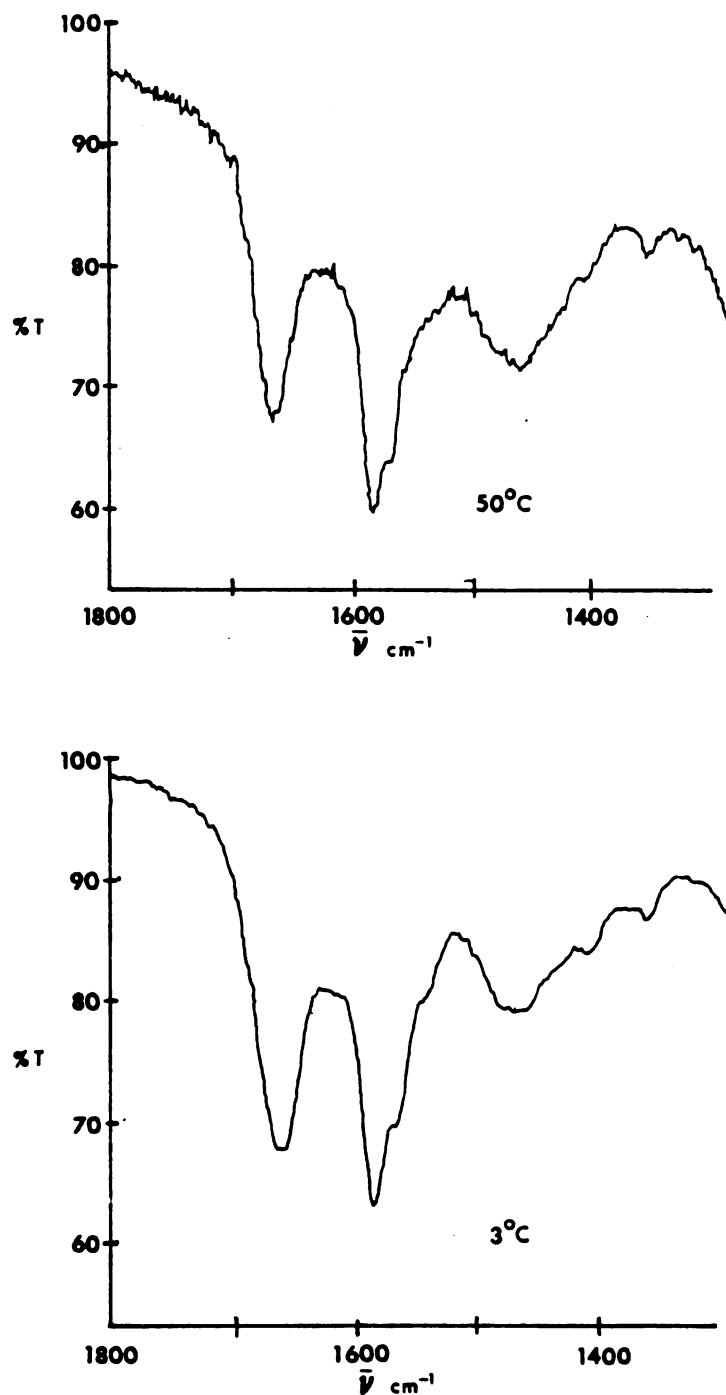


Figure 18. The infrared spectra of 0.98M $\text{Li}_22'\text{GMP}$ in D_2O at 50°C and 3°C. Neither these spectra nor the ^1H NMR spectra of this solution shows evidence for structure formation at either temperature.

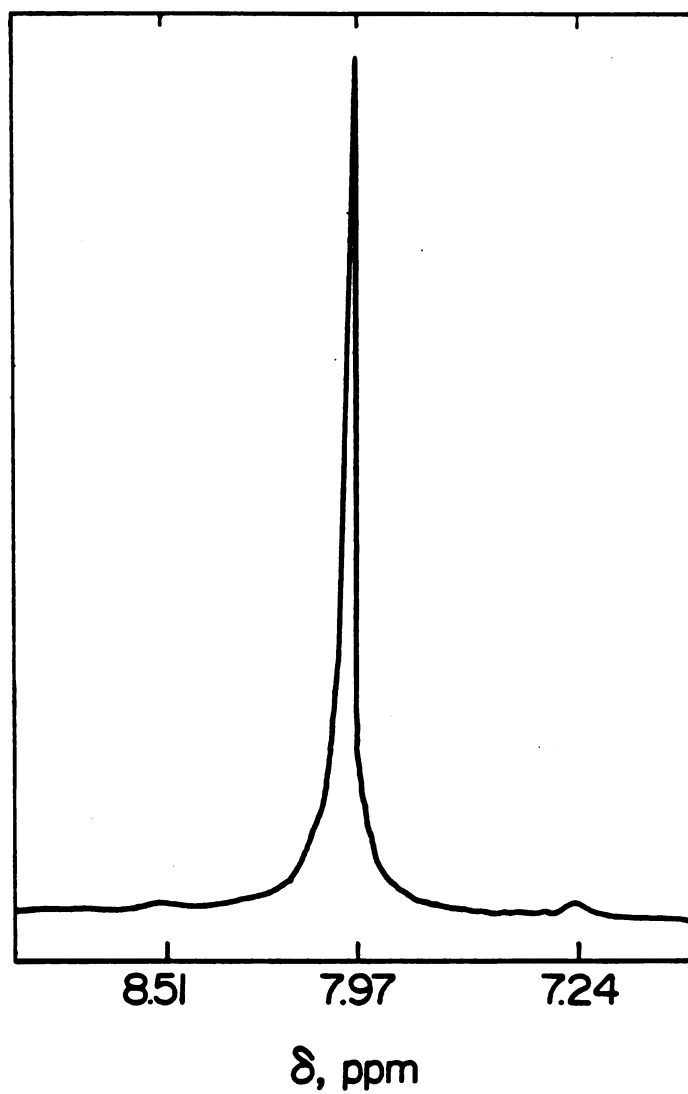


Figure 19. The H(8) NMR lines of 0.91 M Na₂2'GMP in D₂O at 2°C. The two weak lines at 8.51 ppm and 7.24 ppm are indicative of the simple Na₂2'GMP self-structure.

small shoulder is also present upfield of the monomer. The upfield structure H(8) resonance increases in intensity but at a slower rate than the one at 7.99 ppm. As shown in Figure 20, the addition of NaCl to a 0.69M $\text{Na}_2\text{-}2'\text{GMP}$ solution at 1°C, which shows no evidence of structuring in absence of added Na^+ , causes the 7.22 ppm resonance, but not the downfield H(8) structure resonance to grow in. The structure line shifts upfield with increasing Na^+ concentration. Computer simulation (cf Results IIH), of the spectra reveals the presence of a small broad resonance at 7.94 ppm. The structure resonance at 7.99 ppm appears and increases in intensity with increasing Na^+ concentration. The $\text{Na}_2\text{-}2'\text{GMP}$ solution at the limiting condition of solubility and low temperature is approximately 60% structured. This form of structure will be referred to as the extended structure. The extent of structure formation is considerably less than in the corresponding $\text{Na}_2\text{-}5'\text{GMP}$ system, which would be ~80% structured under the same conditions. The number and chemical shifts of the structure resonances are similar to those of the $\text{Na}_2\text{-}5'\text{-GMP}$ structure, but the intensities are not equivalent. The $\text{Na}_2\text{-}5'\text{GMP}$ structure resonances always appear to be of equal intensity. Figure 21 shows the melting profile of the H(8) region of a 0.69M $\text{Na}_2\text{-}2'\text{GMP}$ solution 1.32M in NaCl. The downfield structure resonance decreases in intensity while shifting upfield and merging with the

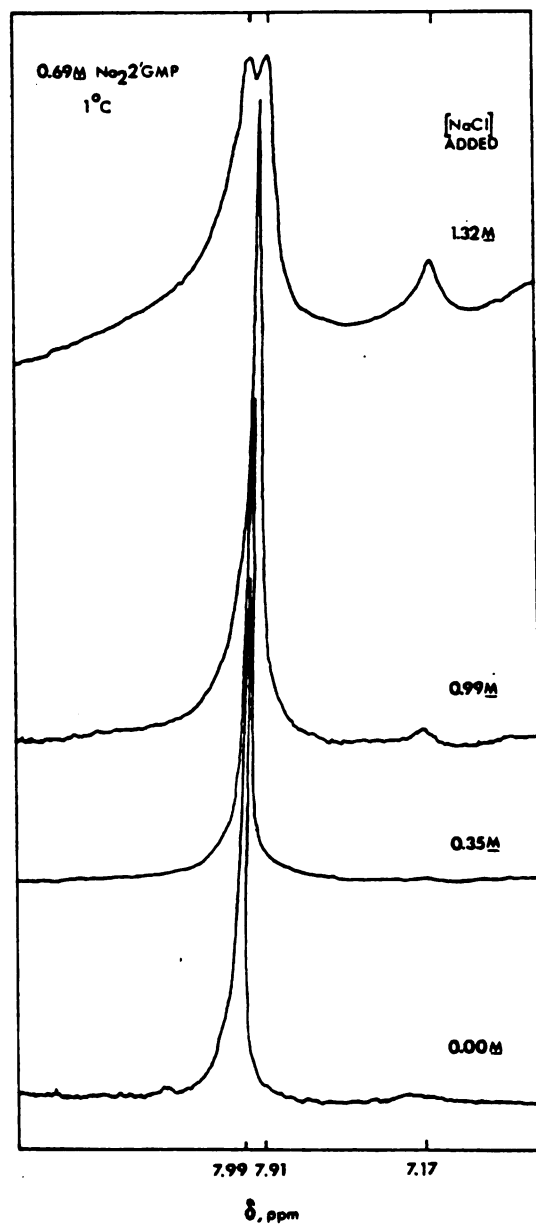


Figure 20. The change in the NMR spectra of H(8) upon addition of NaCl to a 0.69M Na₂2'GMP solution at 1°C.

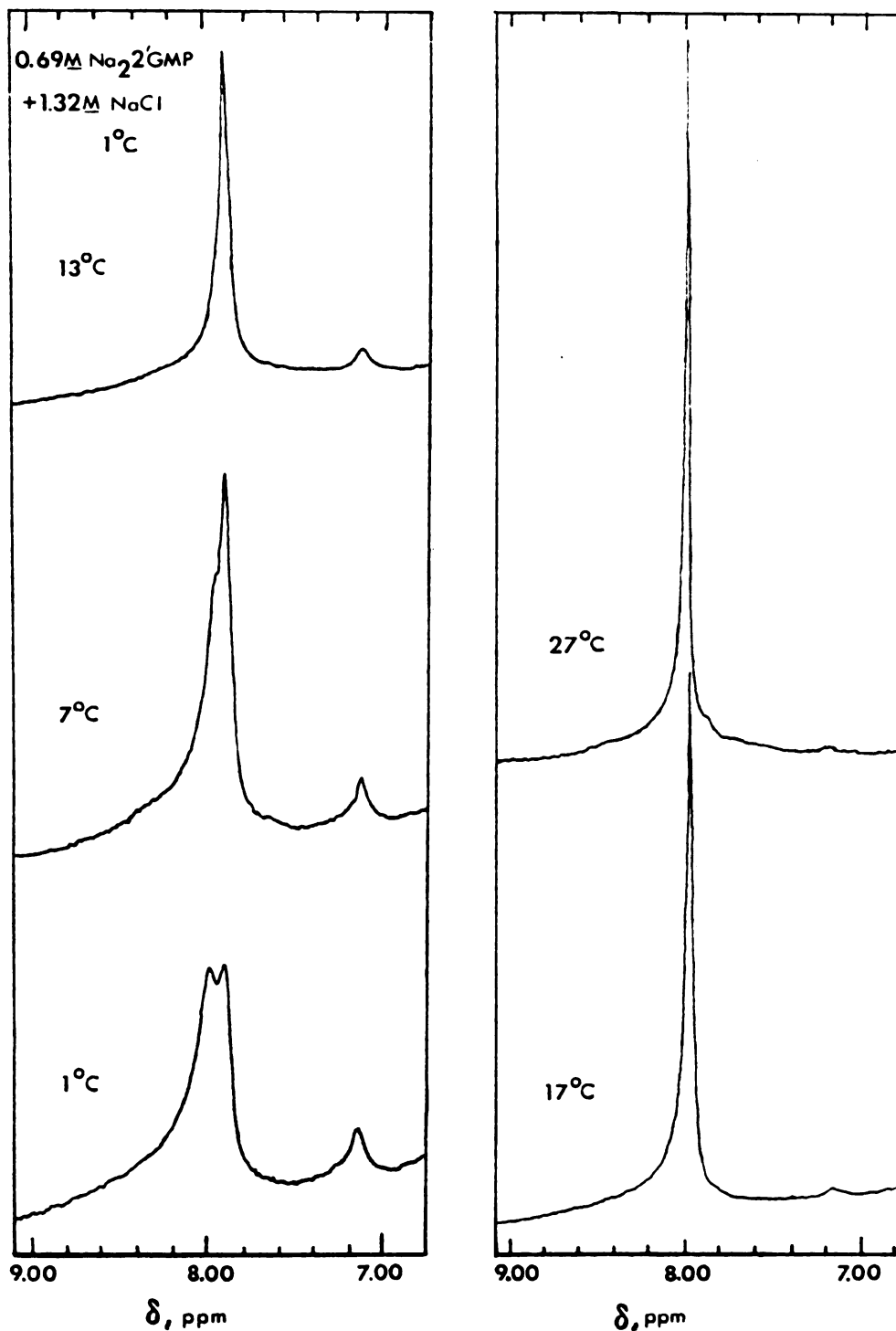


Figure 21. The temperature dependence of the H(8) NMR resonances of a 0.69M $\text{Na}_22'\text{GMP}$ and 1.32M NaCl solution.

monomer resonance. The upfield structure resonance decreases in intensity and shifts from 7.17 ppm at 1°C to 7.21 ppm at 27°C.

Figure 22 shows the spectrum of 1.22M $\text{Na}_2\text{2'GMP}$ in $^1\text{H}_2\text{O}$ at 1°C. Under these conditions, the amine and imide protons are observable. The imide proton N(1)H is assigned to 3 resonances, at 11.41 ppm, 10.98 ppm, and a very broad resonance at 9.53 ppm. The total area of the 3 resonances is less than one proton intensity per total 2'GMP. A non-structured solution at the same temperature does not have the imide proton resonance present, presumably due to rapid exchange with solvent. The imide proton in the ordered structure is presumably involved in intermolecular hydrogen bonding, causing the exchange with solvent to be slow on the NMR time scale. The integral intensity of NH(1) is greater than that of the structure H(8) resonances. The intensity of the resonances is discussed in detail in Results VI.

The three lines labelled B are attributed to H(8). The resonances at 7.75 ppm and 6.95 ppm are solution self-structure resonances. Monomer and structures which exchange with monomer fast on the NMR time scale give rise to the resonance at 7.48 ppm. The amino proton resonance is labelled C. The integral intensity of the amino proton resonance is approximately one. This discrepancy is discussed in Results VIB. The lines labelled D at 5.77 ppm is a solution self-structure resonance of H(1'). The other

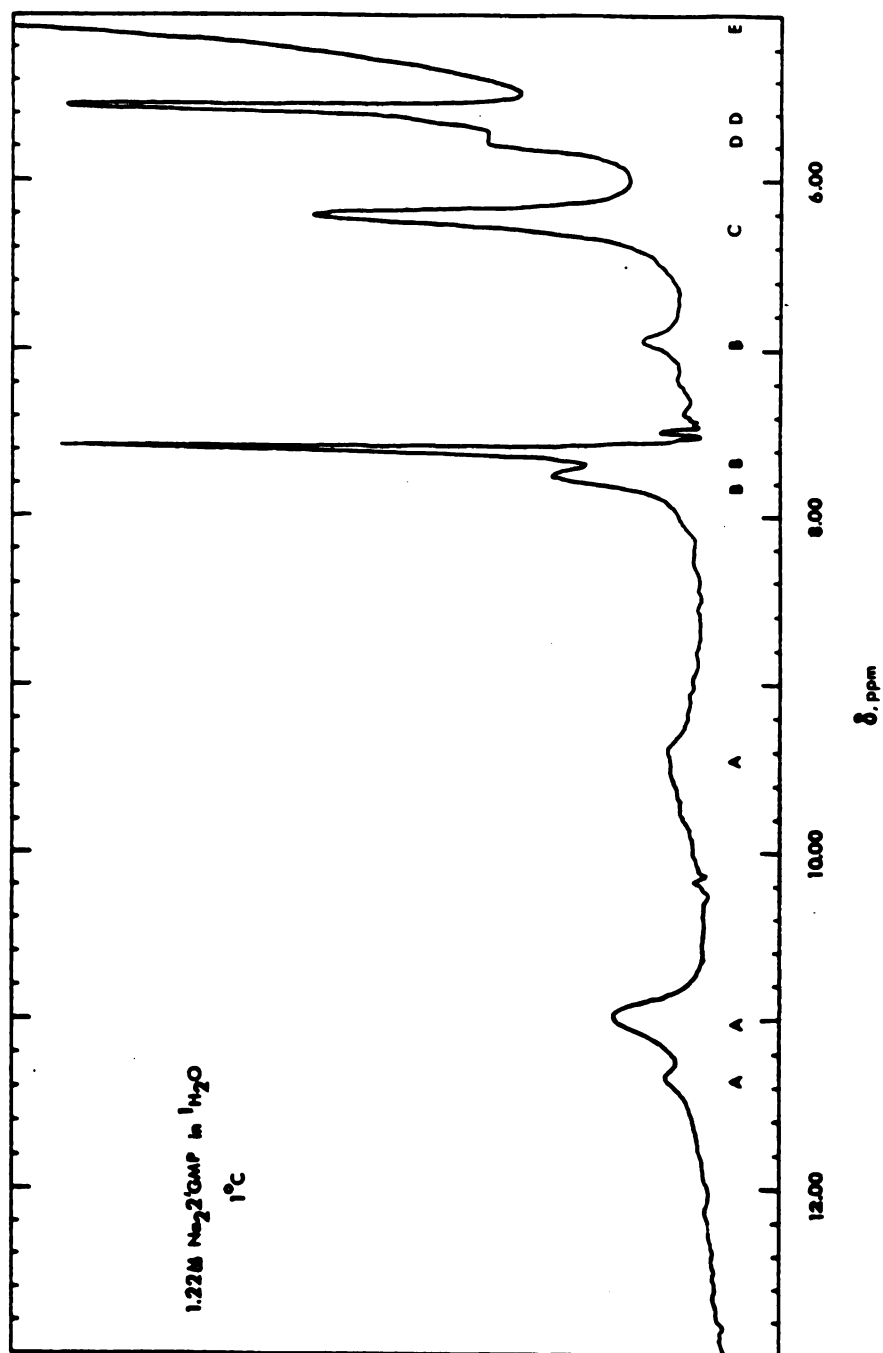


Figure 22. The ^1H NMR spectrum of 1.22M $\text{Na}_2\text{2'GMP}$ in $^1\text{H}_2\text{O}$ at 1°C from 5.00 ppm to 13.00 ppm. The A resonances are attributed to structured N(1)H , the B resonances to H(8) , C to the amino protons, the D resonances to H(1') and E to H_2O .

D resonance at 5.59 ppm is attributed to H(1') of monomeric and fast equilibria structures. The water proton resonance is labeled E.

Perturbations in the carbonyl region of the infrared spectrum similar to those found in the self-structuring of 5'GMP, occur upon ordered structuring of 2'GMP. Figure 23 shows the infrared spectra of disordered 0.30M Na_2 2'GMP at 3°C and 52°C. These spectra are similar to those reported by Miles and Frazier⁸⁸ and Marshall⁹⁷ for disordered Na_2 5'GMP. The absorptions of interest are the carbonyl stretching vibration at 1665 cm^{-1} , the C(4) = C(5) stretching vibration at 1592 cm^{-1} , and the shoulder at 1570 cm^{-1} assigned to a normal mode which involves to a small extent motion of the carbonyl oxygen. The band assignments have been discussed extensively in the literature^{54,83,88}.

Figures 24 and 25 show the infrared spectra of ordered solutions of 0.95M Na_2 2'GMP and 0.71M Na_2 2'GMP containing 1.1M NaCl. Both solutions exhibit H(8) lines for ordered forms in the ^1H NMR spectra; the 0.95M Na_2 2'GMP sample having the simple structure (~5% total structure) and the 0.71M Na_2 2'GMP sample containing 1.1M NaCl the extended structure (~60% total structure). At 2°C, both samples have a new absorption occurring at 1540 cm^{-1} and assigned to a C(8) = N(7) ring vibration and attributed to structure formation. When the sample is heated to 52°C, the 1540 cm^{-1} band decreases in intensity and becomes a small

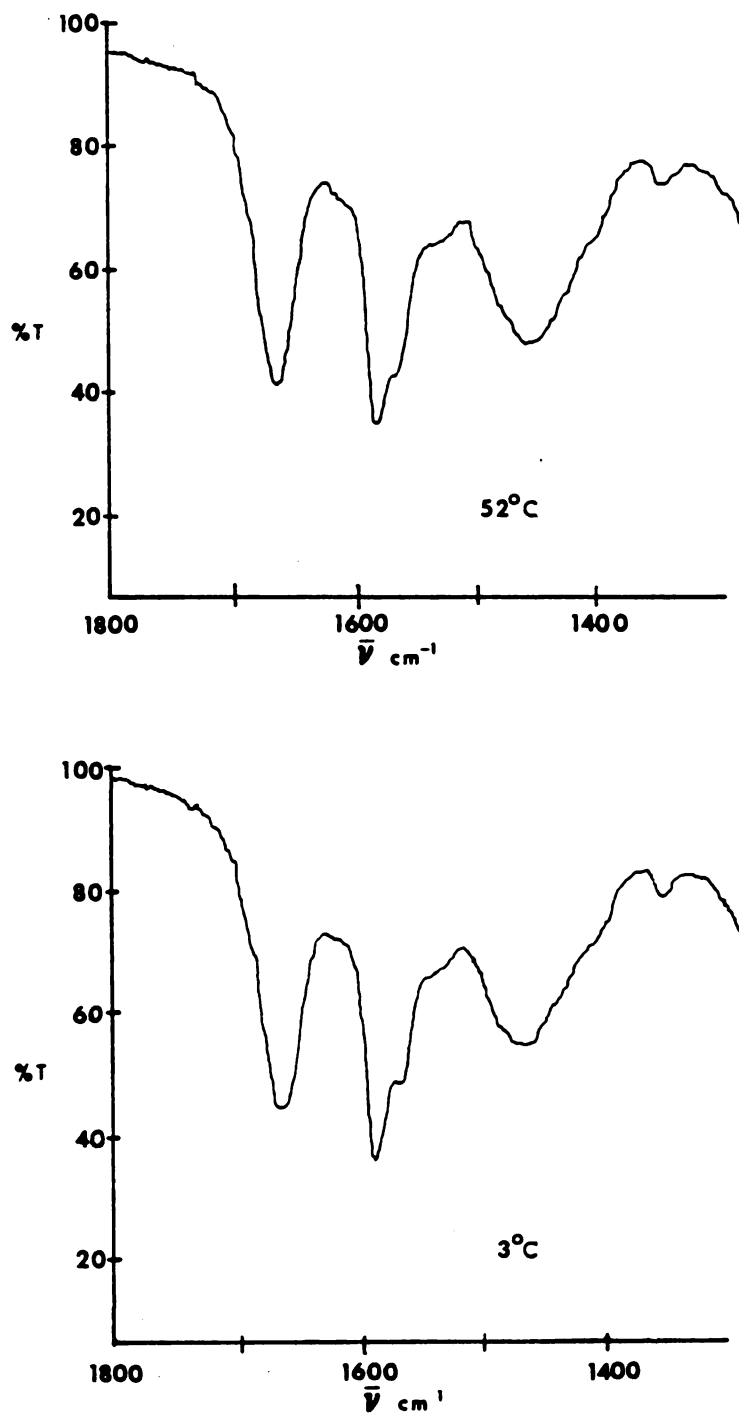


Figure 23. The infrared spectra of 0.30M Na₂2'GMP in D₂O at 3°C and 52°C. Neither of these spectra nor the ¹H NMR spectra of these solutions shows evidence for structure formation at both temperatures.

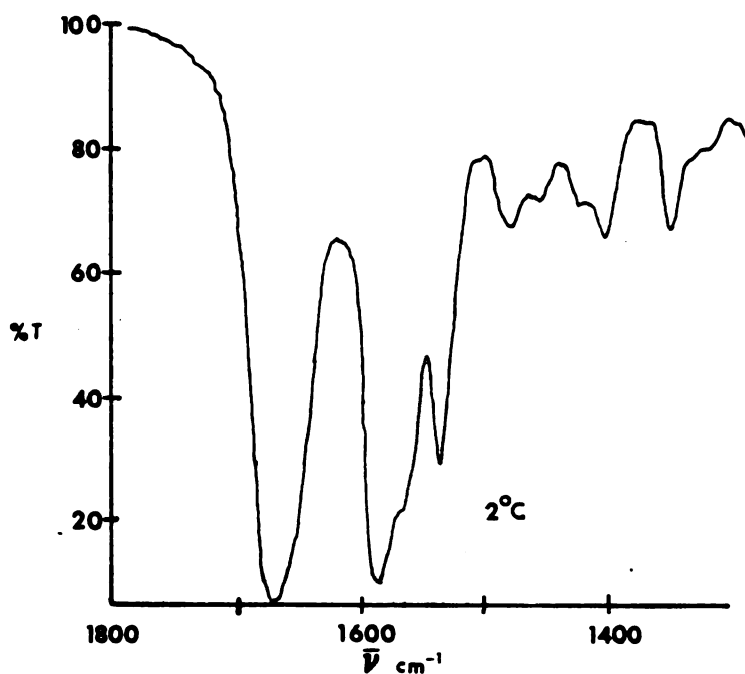
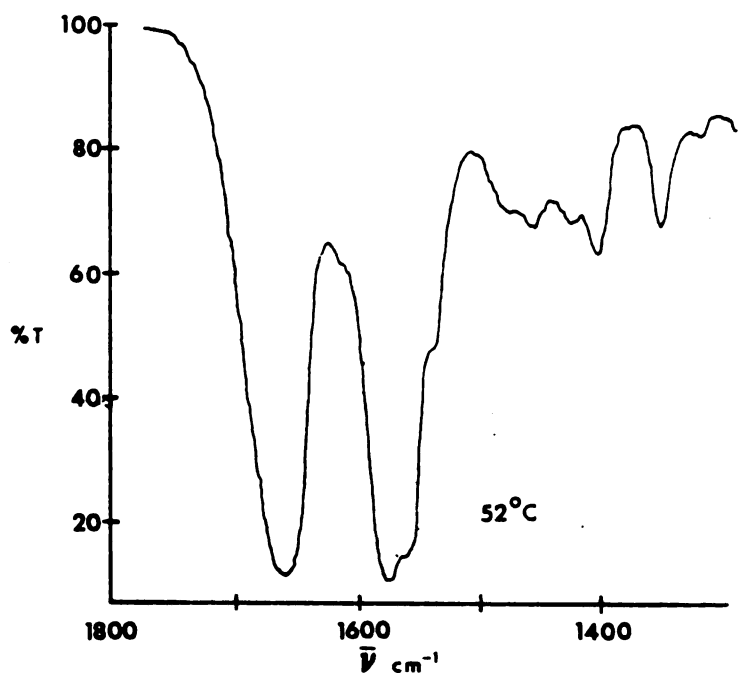


Figure 24. The infrared spectra of 0.95M $\text{Na}_22'\text{GMP}$ in D_2O at 2°C and 52°C . Both the $\text{H}(8)$ NMR spectrum and the IR spectrum at 52°C show no evidence of structure formation; while at 2°C the $\text{H}(8)$ NMR spectrum is characteristic of a simple $\text{Na}_22'\text{-GMP}$ solution structure. The structure resonances account for $<5\%$ of the $\text{H}(8)$ resonance. The IR spectrum at 2°C shows perturbations which have been attributed to solution self-structure.

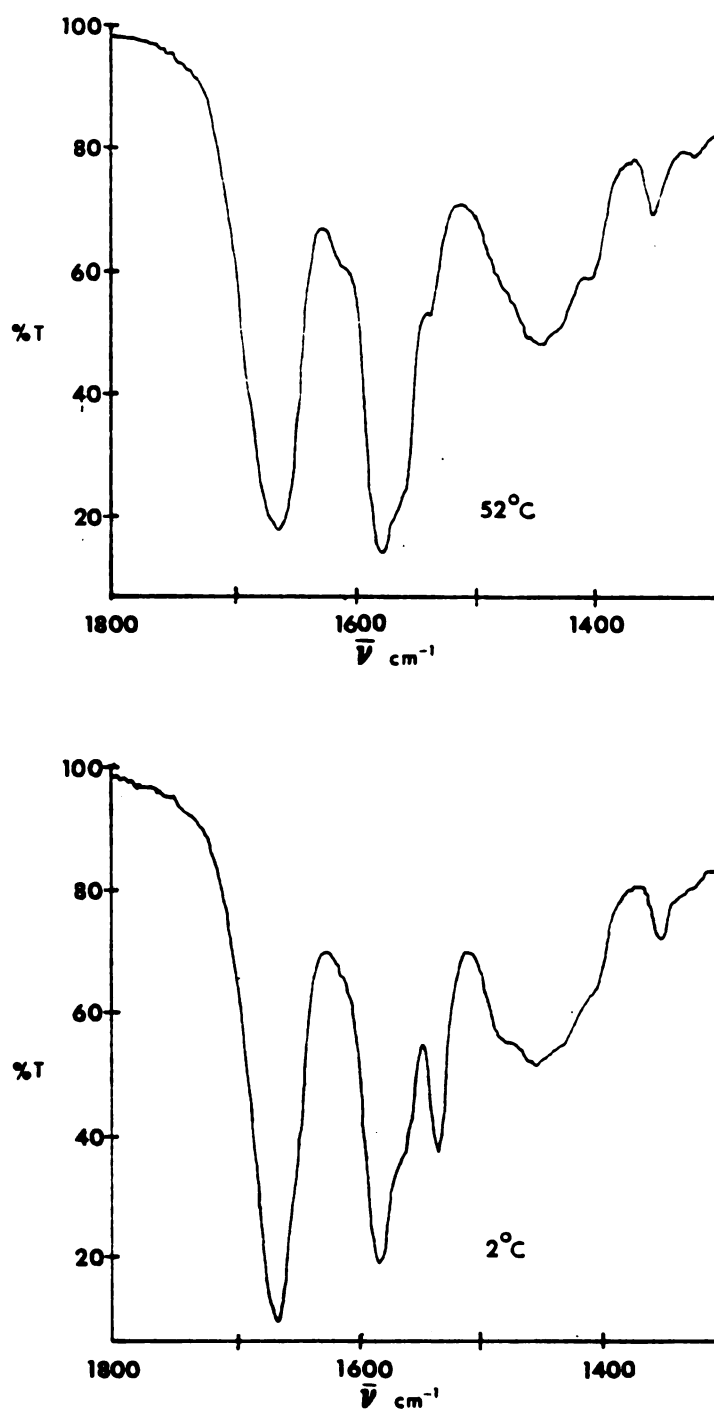


Figure 25. The infrared spectra of 0.71M Na₂2'GMP containing 1.1M NaCl in D₂O at 2°C and 52°C. Both the H(8) NMR spectrum and the spectrum of this sample at 52°C show no evidence for structure formation. At 2°C the H(8) NMR structure resonances account for ~50% of the total H(8) intensity. The IR spectrum at 2°C shows perturbation characteristic of structure formation.

shoulder on the broad overlapping bands at 1584 cm^{-1} and 1565 cm^{-1} . The intensity of the absorption at 1590 cm^{-1} is decreased upon structure formation, becoming less intense than the line at 1669 cm^{-1} .

The absorbances do not seem to be correlated with the % structure present. The %T of the 1540 cm^{-1} absorbance is approximately the same in the $0.95\text{M Na}_22'\text{GMP}$ solution at 2°C , which is $\sim 5\%$ structured as determined by ^1H NMR of the H(8) resonance, and in $0.71\text{M Na}_22'\text{GMP}$ containing 1.1M NaCl at 2°C , which is $\sim 50\%$ structured.

C. The K^+ Salt of $2'\text{GMP}$

Figure 26 shows the H(8) NMR spectra of $\text{K}_22'\text{GMP}$ at -2°C as the concentration of $\text{K}_22'\text{GMP}$ is increased. A $0.10\text{M K}_22'\text{GMP}$ solution at -2°C has a single H(8) resonance at 7.97 ppm with a width at half-height of 4.3 Hz . When the concentration of $\text{K}_22'\text{GMP}$ is increased to 0.50M , several new resonances appear with chemical shifts of 8.29 ppm , 8.06 ppm , 7.93 ppm (monomer), 7.72 ppm , 7.51 ppm and 7.30 ppm . Increasing the concentration to 0.82M causes the appearance of two new resonances, for a total of at least seven solution self-structure resonances. A $0.82\text{M K}_22'\text{-GMP}$ solution at -2°C is $\sim 90\%$ structured. The extent of ordered structure is much greater than in $\text{Na}_22'\text{GMP}$, but is less than in a comparable $\text{K}_25'\text{GMP}$ solution. There is no

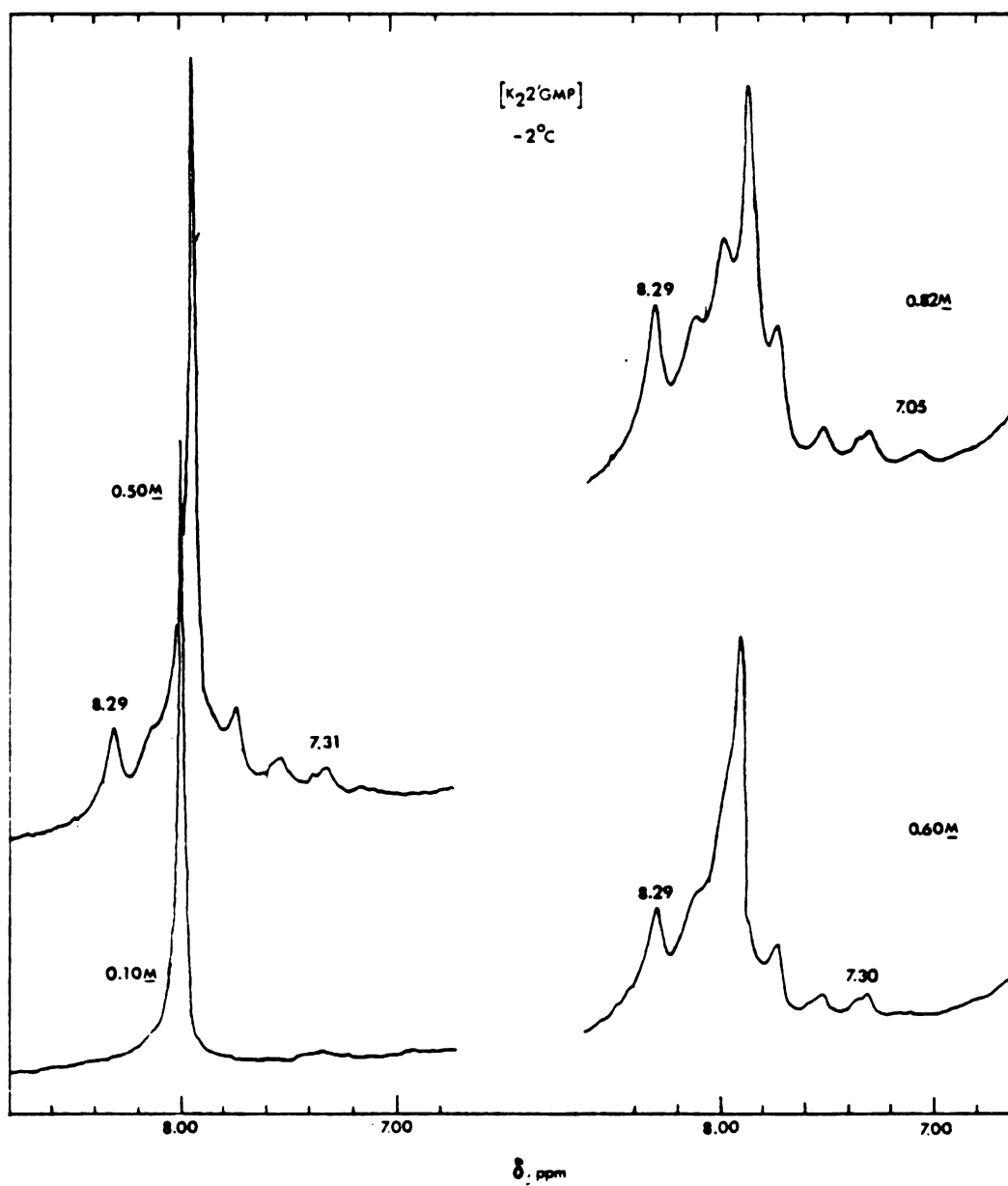


Figure 26. The concentration dependence of the H(8) NMR spectrum of $K_22'GMP$ at $-2^\circ C$.

correlation between the number and chemical shifts of the K_22' GMP and K_25' GMP structure resonances. In K_25' GMP, all structure resonances occur upfield of the monomer, whereas in K_22' GMP structure resonances occur both upfield and downfield of the monomer.

The temperature dependence of the H(8) region of 0.82M K_22' GMP is presented in Figure 27. The resonances due to ordered nucleotide do not decrease in intensity at the same rate. The resonances shift toward the monomer resonance while decreasing in intensity. At 50°C, only the monomer resonance is observed.

Figures 28 and 29 show the carbonyl stretching region of 0.50M K_22' GMP and 0.82M K_22' GMP. The same perturbations are present as in the ordered Na_22' GMP system. In the 0.50M K_22' GMP sample at 3°C (~20% ordered by NMR), the 1587 cm^{-1} absorption is less intense than the one at 1668 cm^{-1} , and the absorption at 1536 cm^{-1} is present, both indicative of structure formation. At 51°C these perturbations are no longer present. These changes are even more pronounced in 0.82M K_22' GMP at 2°C. At 50°C, the spectrum is indicative of a disordered system. The melting profiles of 0.82M K_22' GMP by infrared and ^1H NMR are compared in Figure 30 using the change of absorbance at 1568 cm^{-1} and the percent structure determined by integration of the H(8) region of the ^1H NMR spectra. Both methods show the melting temperature, T_m , defined as the temperature at

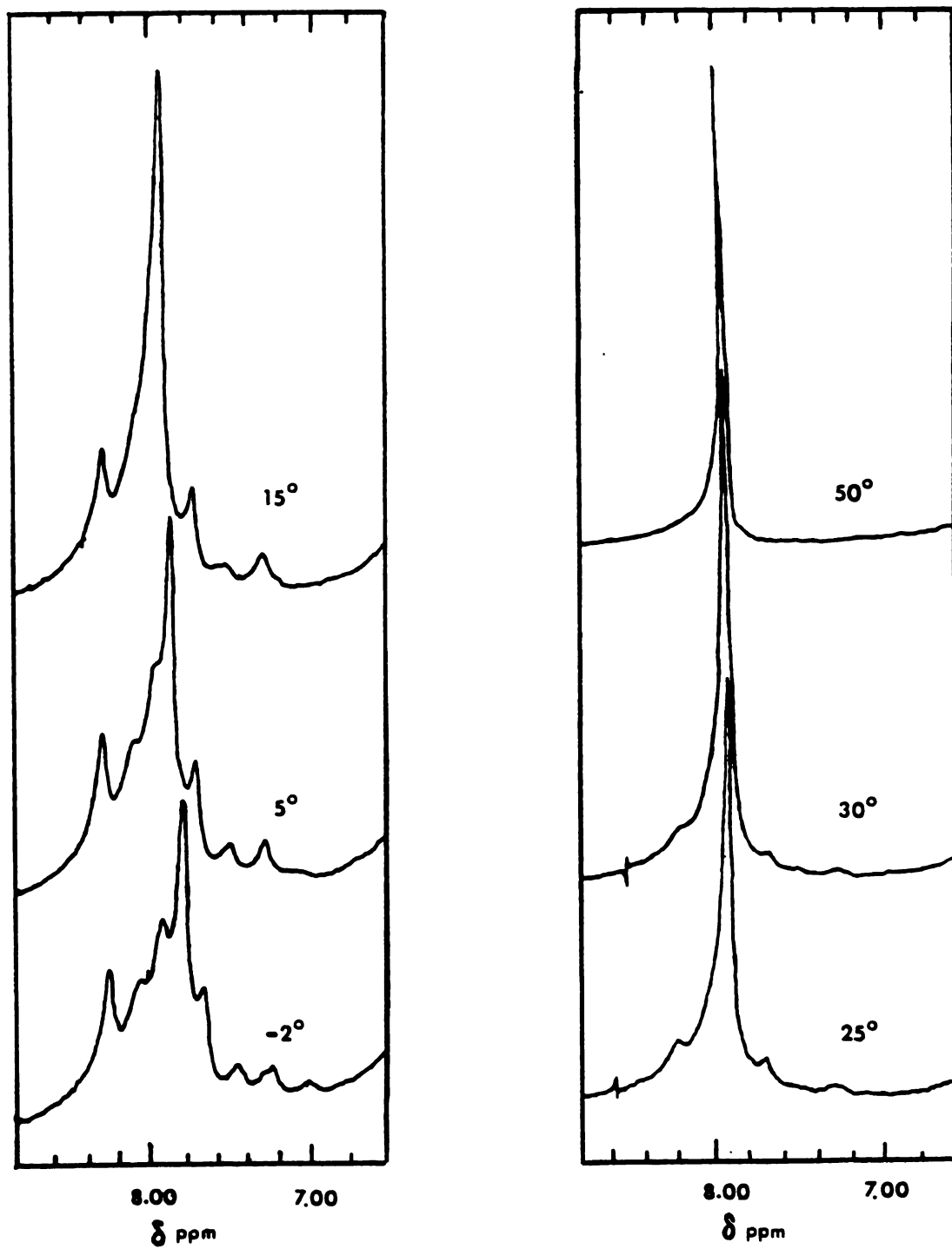


Figure 27. The temperature dependence of the H(8) NMR spectrum of 0.82M K_22' GMP.

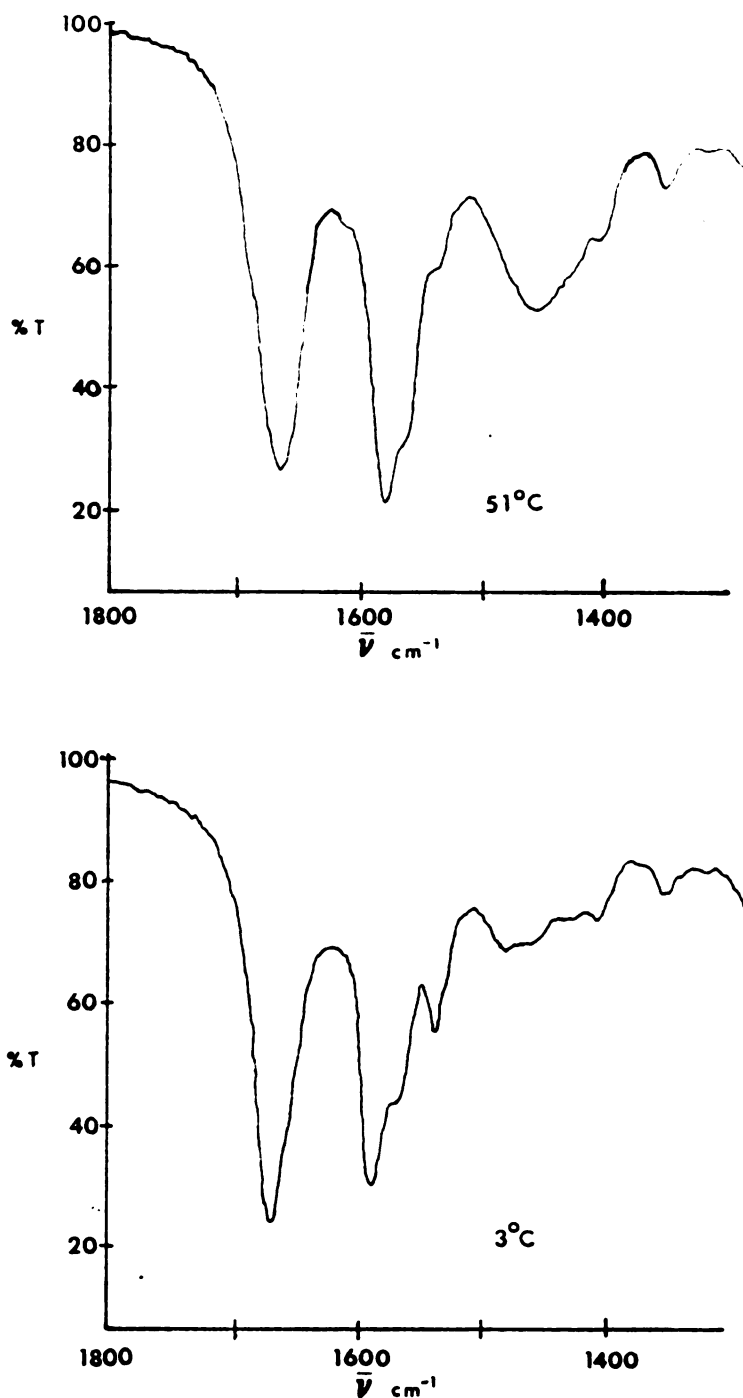


Figure 28. The infrared spectra of 0.50M K₂2'GMP in D₂O at 51°C and 3°C. The H(8) NMR spectrum and IR spectrum at 51°C show no evidence for structure formation; at 3°C the sample is ~30% structured as determined by proton NMR. The IR spectrum show perturbations characteristic of structure formation.

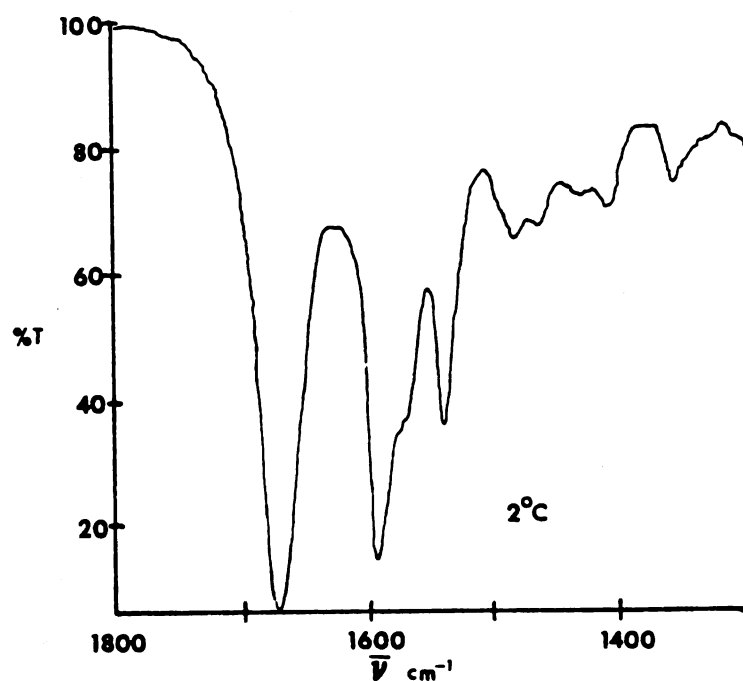
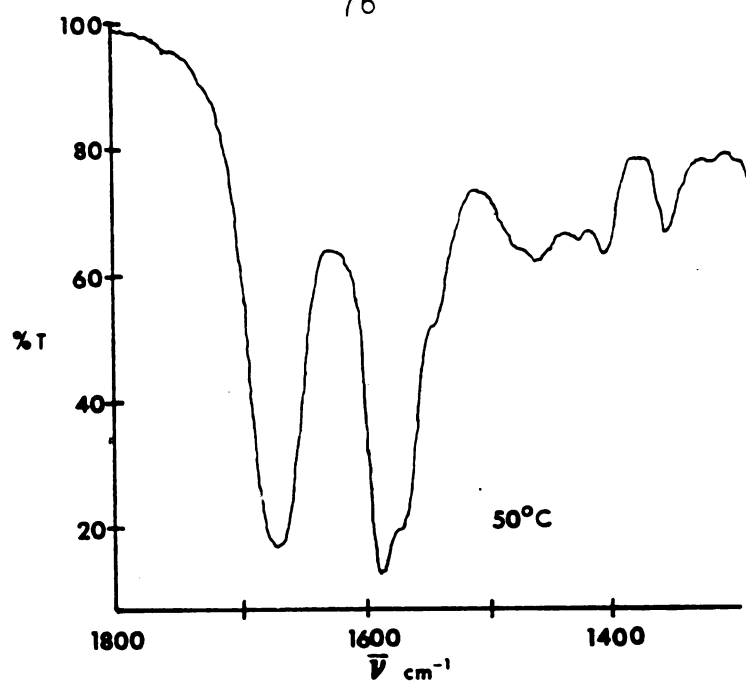


Figure 29. The infrared spectra of 0.82M $\text{K}_22'\text{GMP}$ in D_2O at 50°C and 2°C. At 2°C this sample is ~80% structured, determined by the H(8) NMR resonance. The IR spectrum shows perturbation characteristic of structure formation. There is no structure present at 50°C determined by proton NMR or by IR.

DETERMINATION OF THE MELTING TEMPERATURE
BY IR AND NMR FOR 0.82M $K_22'GMP$

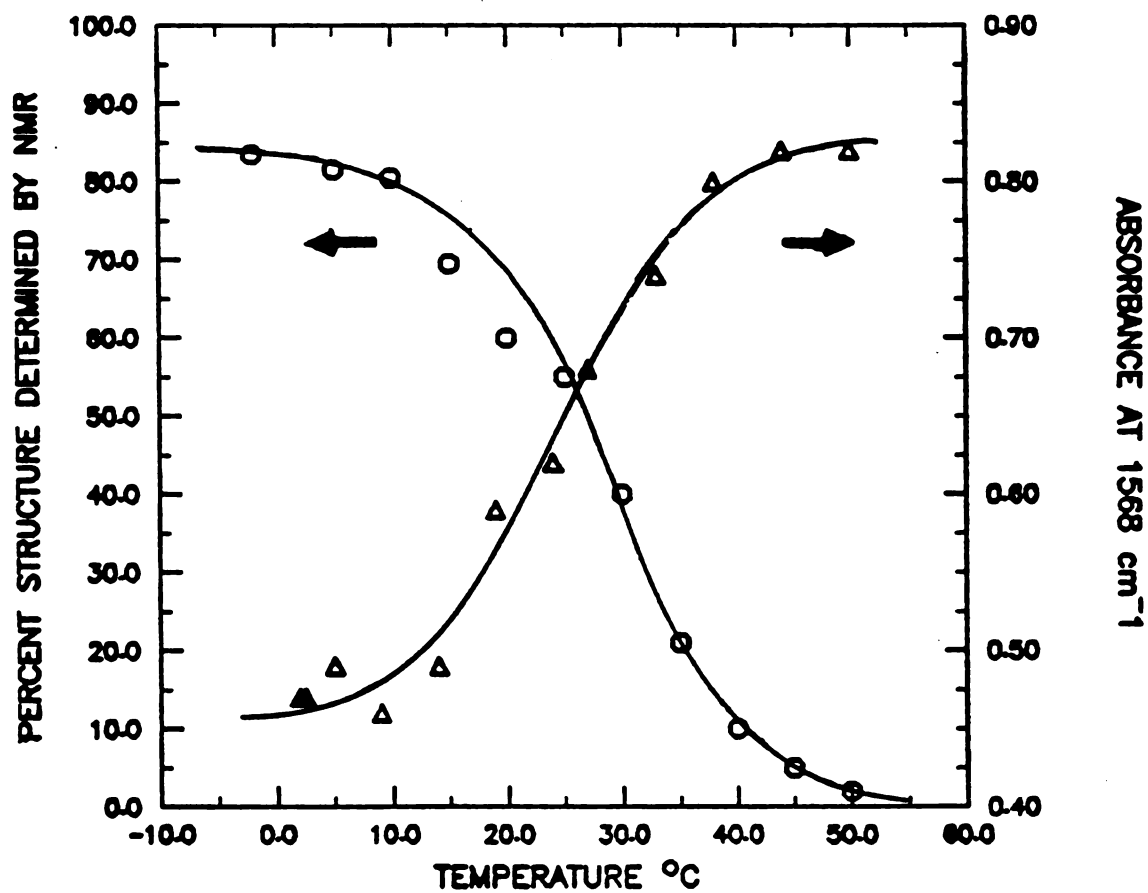


Figure 30. Comparison of melting profiles of 0.82M $K_22'GMP$ in D_2O by IR and ^1H NMR.

which half of the structure remains, to be in the range of 25°-30°C. This is evidence that the same phenomenon is being observed in ^1H NMR and infrared spectroscopy. The degree of cooperativity is defined by the temperature range over which the nucleotide goes from structured to unstructured form. The smaller the temperature range, the greater the cooperativity in structure formation. This corresponds to a large positive value of ΔH upon solution "melting" of structure. The absorbance at $\nu 1530\text{ cm}^{-1}$ does not seem to be correlated with structure formation, although it is always present in structured solutions. Table 5 lists the temperature dependence for the absorbances of 0.82M K_22' -GMP IR bands. The absorbance bands at 1590 cm^{-1} and 1568 cm^{-1} correlate to the amount of solution self-structure present in solutions.

D. The Rb^+ Salt of $2'\text{GMP}$

The Rb^+ salt of $2'\text{GMP}$ at low temperature and high concentration has four structure resonances, two upfield and two downfield of the monomer over the range of 7.4 to 8.4 ppm. Figure 31 shows the effect of concentration on the H(8) resonance of $\text{Rb}_22'\text{GMP}$. A single ($\nu_{1/2} = 16\text{ Hz}$) resonance is observed in a 0.30M $\text{Rb}_22'\text{GMP}$ at -2°C . When the concentration is increased to 0.39M $\text{Rb}_22'\text{GMP}$, the resonance broadens to $\nu_{1/2} = 32.5\text{ Hz}$. At 0.77M $\text{Rb}_22'\text{GMP}$, four structure resonances are present with chemical shifts of

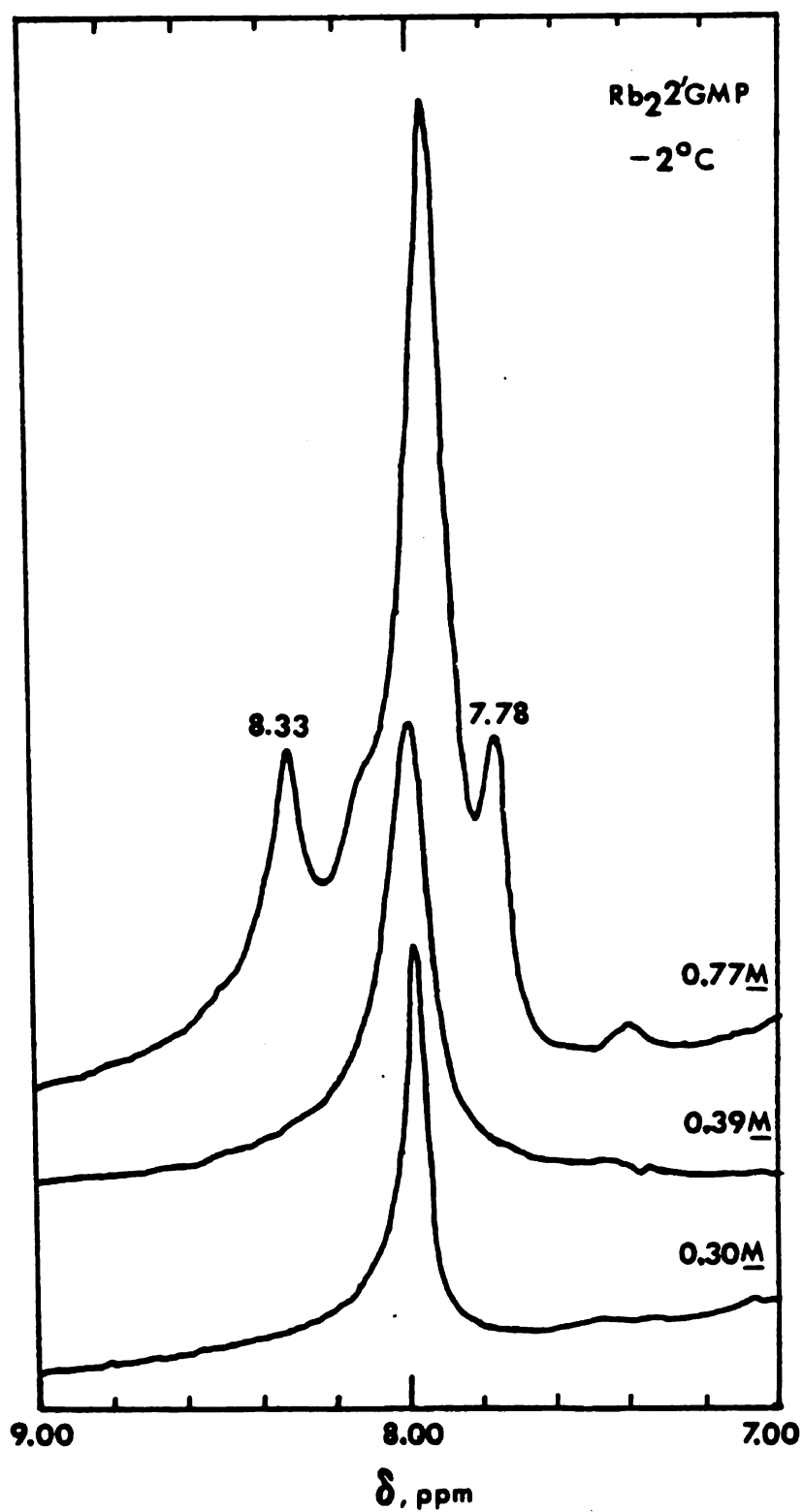


Figure 31. The concentration dependence of the H(8) NMR spectra of $\text{Rb}_2 2'\text{GMP}$ at -2°C .

Table 5. The Temperature Dependence for the Absorbances of Various 0.82M $K_2^{2'}GMP$ IR Bands.

Temp. ($^{\circ}C$)	Abs. at 1590 cm^{-1}	Abs. at 1540 cm^{-1}	Abs. at 1568 cm^{-1}
2.5	0.74	0.37	0.47
5	0.73	0.36	0.49
9	0.67	0.35	0.46
14	0.67	0.35	0.49
19	0.81	0.33	0.59
24	0.84	0.34	0.62
27	0.91	0.36	0.68
33	0.97	0.34	0.74
38	1.00	0.32	0.80
44	1.04	0.31	0.82
50	1.05	0.30	0.82

8.33 ppm, 8.13 ppm (shoulder of monomer), 7.78 ppm (monomer), and 7.41 ppm. The monomer resonance is very broad ($\nu_{1/2} = 33.5$ Hz) and is shifted upfield to 7.96 ppm. A 0.77M $\text{Rb}_2\text{'GMP}$ solution at 2°C is approximately 40% structured.

The temperature dependence of the H(8) resonance of 0.77M $\text{Rb}_2\text{'GMP}$ is presented in Figure 32. The small upfield structure resonance decreases in intensity as the temperature is increased and is no longer present at 15°C. The other structure resonance decreases slightly in intensity and merges with the monomer resonance as the temperature is increased. This merging of the lines may be due in part to the structures exchanging in the intermediate range with monomer, while the concentration of the ordered form is decreasing as the temperature is increased. The monomer resonance remains broad even at 35°C ($\nu_{1/2} = 35$ Hz).

The infrared spectra for 0.30M $\text{Rb}_2\text{'GMP}$ and 0.77M $\text{Rb}_2\text{'GMP}$ are shown in Figures 33 and 34, respectively. In agreement with ^1H NMR data, the spectrum of this sample at 3°C shows no evidence of structure formation. The 0.77M $\text{Rb}_2\text{'GMP}$ spectra at 3°C has the same perturbations as discussed before for structure formation. At 50°C, the spectrum is indicative of an unstructured system, in agreement with NMR data.

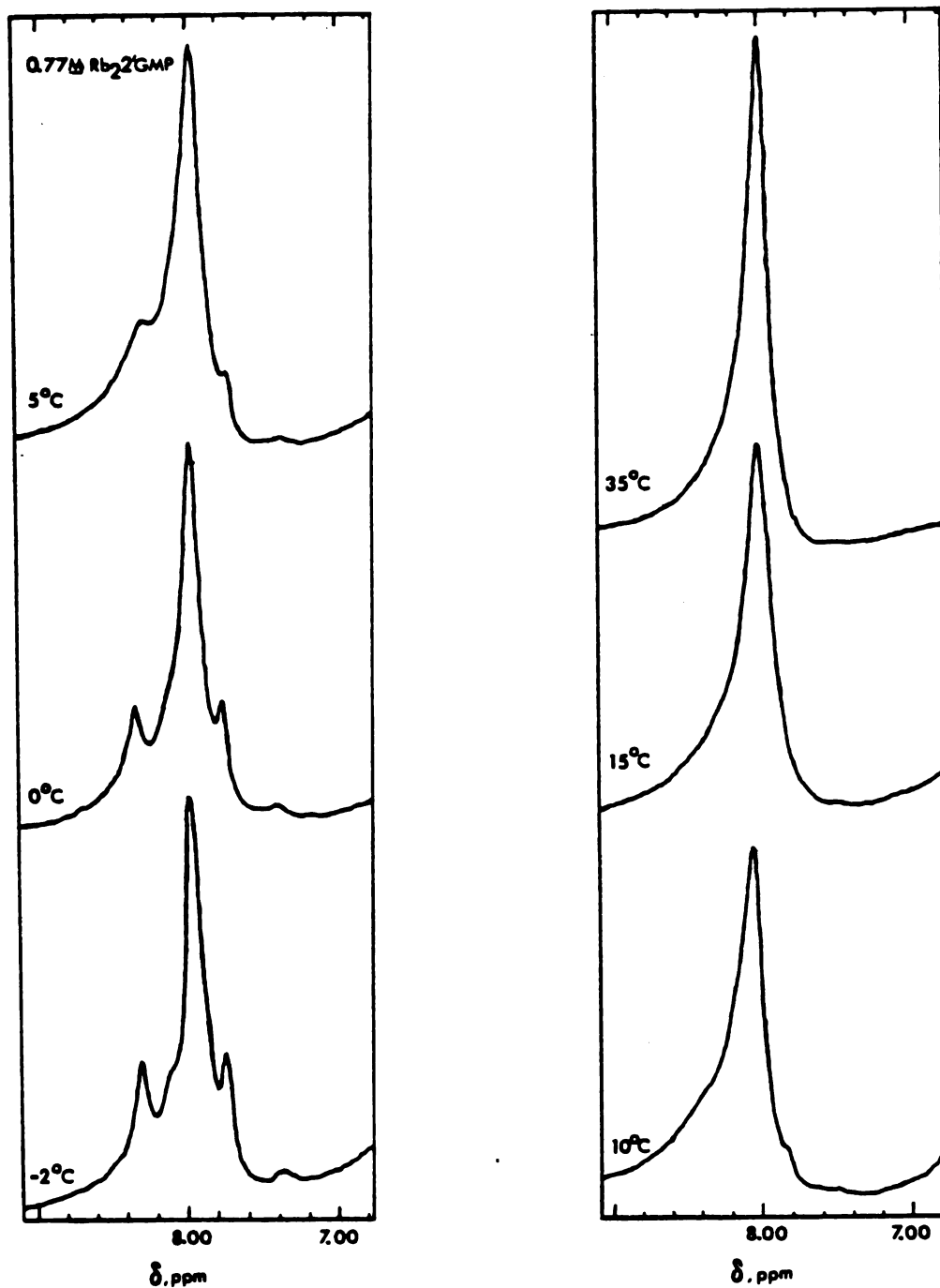


Figure 32. The temperature dependence of the H(8) NMR spectrum of 0.77M Rb₂2'GMP.

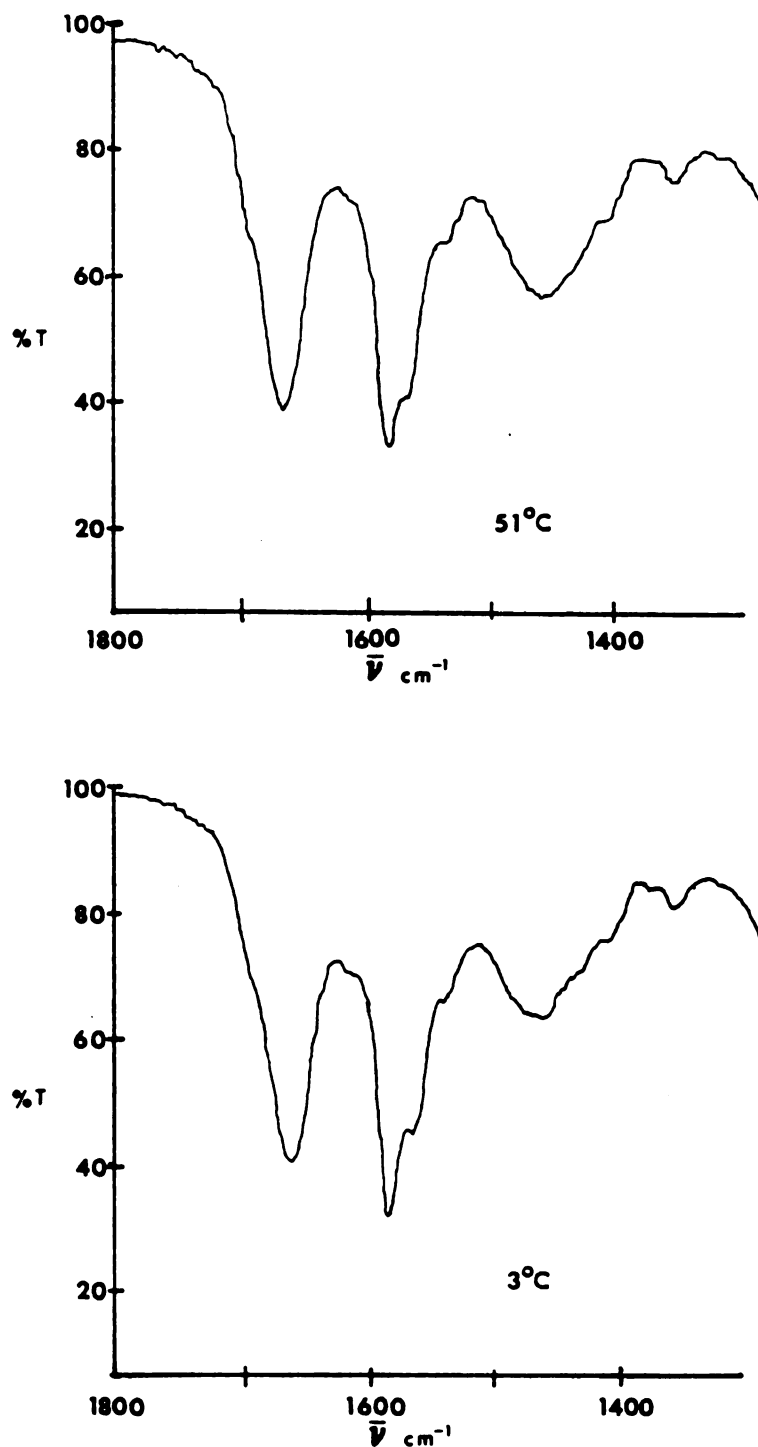


Figure 33. The infrared spectra of 0.30M $\text{Rb}_22'\text{GMP}$ in D_2O at 51°C and 3°C. Neither these spectra nor the H(8) NMR resonance shows evidence of structure formation at either temperature.

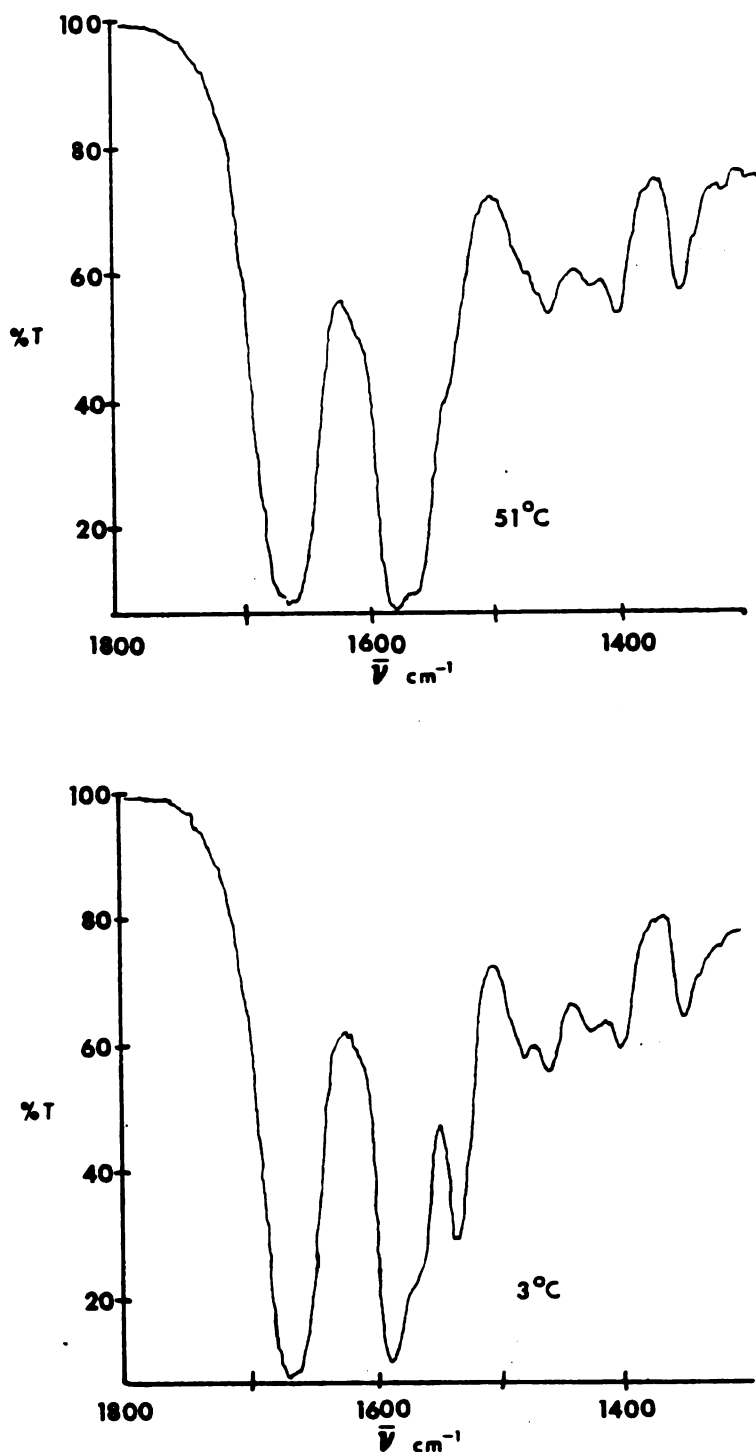


Figure 34. The infrared spectra of 0.77M Rb₂2'GMP in D₂O at 50°C and 3°C. At 50°C neither the H(8) resonance nor the IR spectrum shows evidence for structure formation, while at 3°C the H(8) structure resonances account for ~40% of the total H(8) resonance. The IR spectrum at 3°C shows perturbations characteristic of structure formation.

E. The Cs^+ Salt of 2'GMP

The cesium salt of 2'GMP has only a slight tendency for self-association. A 0.64M $\text{Cs}_2\text{2'GMP}$ solution at -2°C has a small H(8) resonance at 7.55 ppm (cf. Figure 15). The structure resonance accounts for $\sim 3\%$ of the total nucleotide. The H(8) resonance is broad even at high temperature as seen in Figure 35. The infrared spectrum of 0.64M $\text{Cs}_2\text{2'GMP}$ is shown in Figure 36. No evidence for ordered structuring is present. The band at 1587 cm^{-1} is more intense than the one at 1665 cm^{-1} and no peak is present at 1536 cm^{-1} .

F. Tetramethylammonium Ion Destabilization of the 2'GMP Self-Structure

The discovery by Brown⁴⁹ that the tetramethylammonium ion (TMA^+) acts as neither a structure directing nor inhibiting ion has facilitated the study of 5'GMP self-structure at varying ratios of metal to nucleotide. Experiments were conducted to determine if TMA^+ is such a "non-interfering" ion for 2'GMP. Solutions of $\text{TMA}_2\text{2'GMP}$ have a single sharp resonance at 8.04 ppm even at high concentration ($>1.0\text{M}$) and low temperature (1°C). The addition of $\text{NaCl}_{(\text{s})}$ to 0.68M $\text{TMA}_2\text{2'GMP}$ at 0°C causes a broadening of the H(8) resonance and only a small structure resonance at 7.38 ppm at 2.2M NaCl (cf. Figure 37).

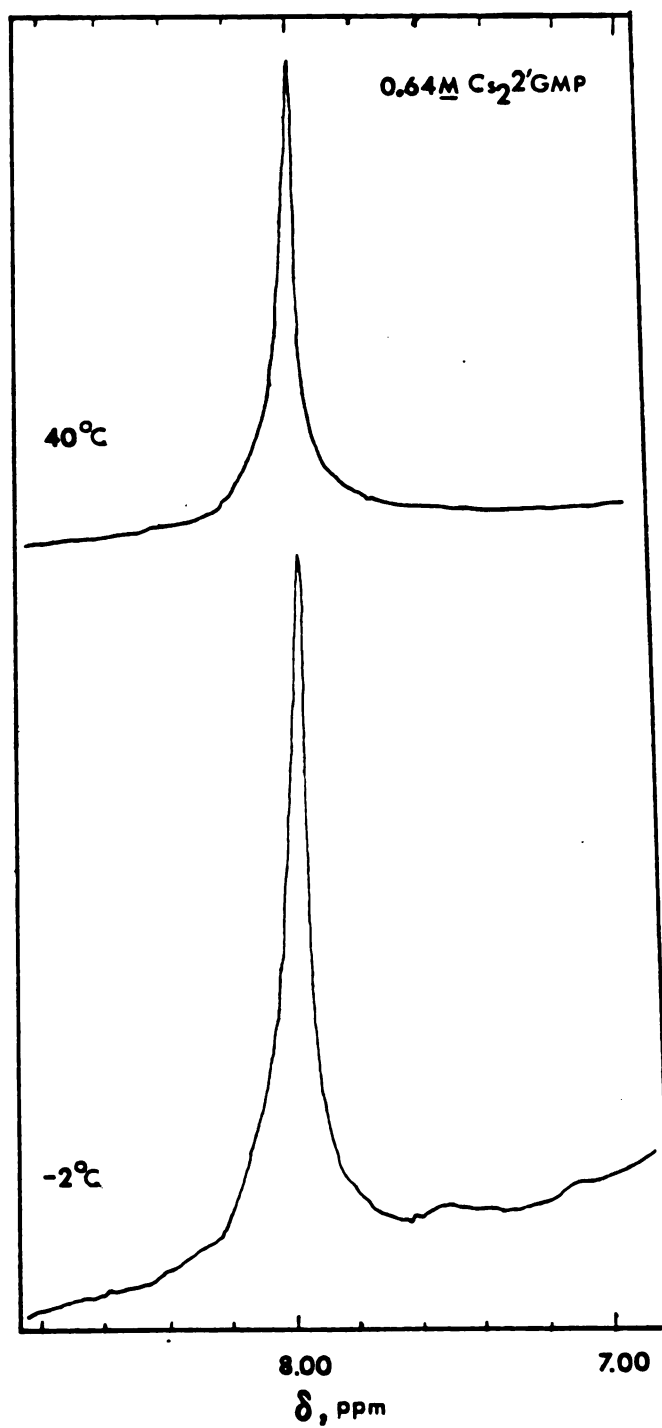


Figure 35. The temperature dependence of the H(8) NMR spectrum of 0.64M Cs₂2'GMP in D₂O.

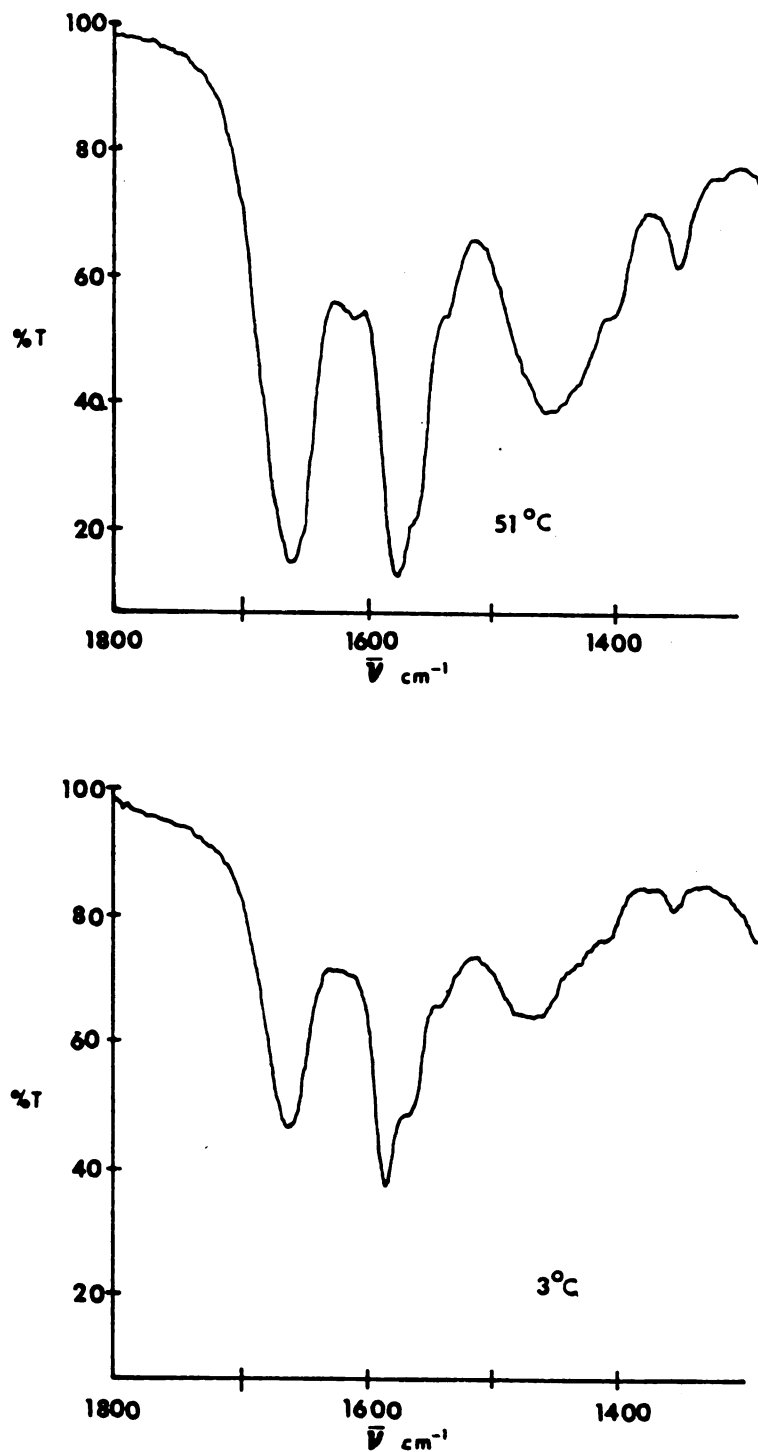


Figure 36. The infrared spectra of 0.64M $\text{Cs}_22'\text{GMP}$ in D_2O at 51°C and 3°C. Neither the $\text{H}(8)$ NMR spectrum nor the IR spectrum at 51°C shows evidence of structure formation, while at 3°C the sample is ~5% structured by proton NMR. The IR spectrum at 3°C shows perturbations characteristic of structure formations.

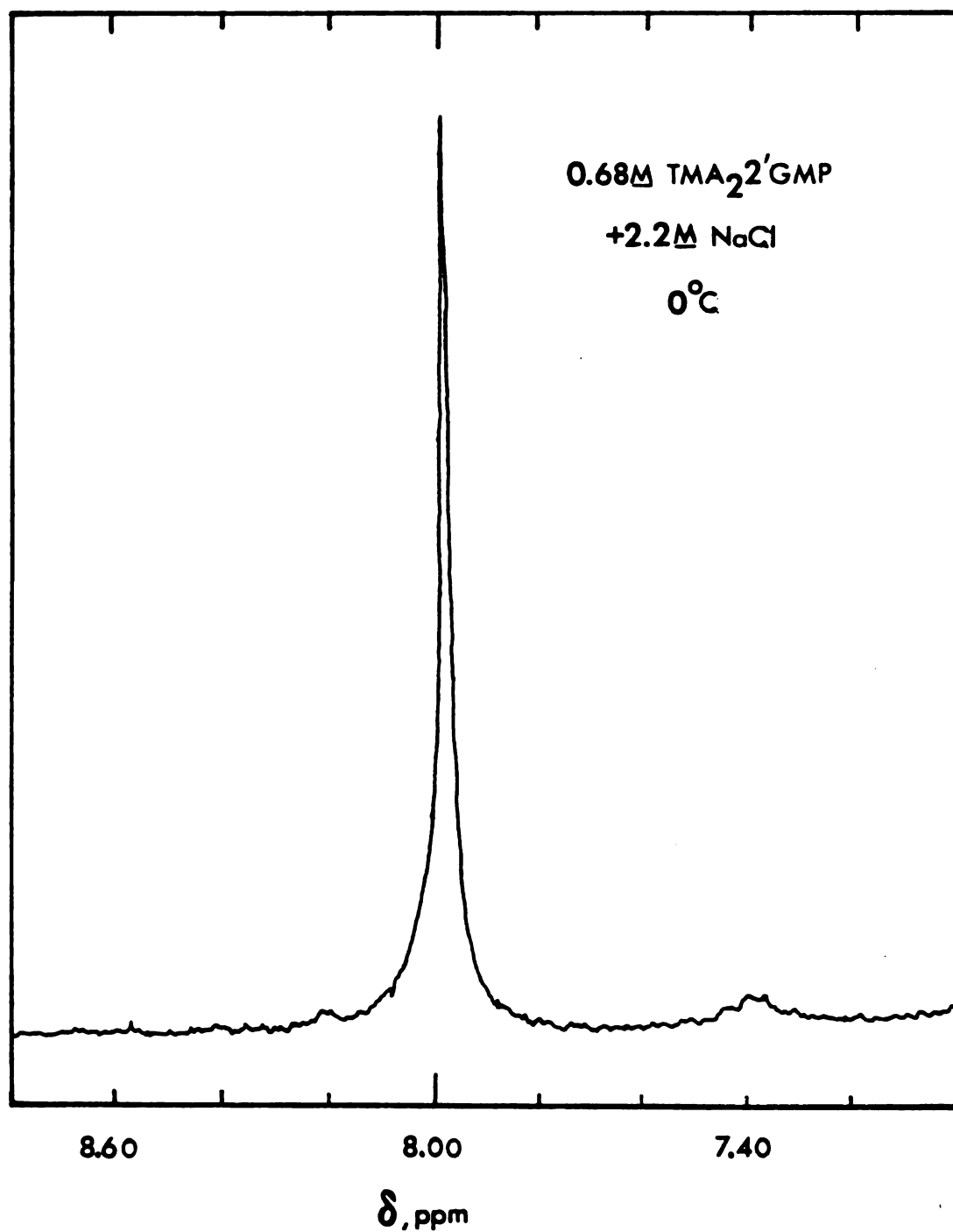


Figure 37. The H(8) region of a 0.68M TMA₂2'GMP and 2.2M NaCl solution at 0°C. This solution in absence of TMA⁺ would be approximately 50% structured.

This solution in absence of TMA^+ is approximately 50% structured.

Figure 38 shows the results of a 0.71M $\text{Na}_2\text{'GMP}$ solution made 1.1M in NaCl and 1.36M in TMAcI at -3°C . The solution in absence of TMA^+ is approximately 60% structured. The resonances in the TMA^+ -free system are very broad. The structure resonance at 7.99 ppm and the monomer at 7.92 ppm are of the same intensity and overlap making the resonances appear as one. Upon addition of $\text{TMAcI}_{(s)}$ there is an immediate loss of all structure resonances and the sharpening of the monomer resonance ($\nu_{1/2} = 23 \text{ Hz}$ to $\nu_{1/2} = 4.0 \text{ Hz}$).

The addition of $\text{KCl}_{(s)}$ to 0.69M $\text{TMA}_2\text{'GMP}$ does show some structure formation but the amount is greatly decreased relative to the TMA^+ -free system (cf. Figure 39). A 0.60M $\text{K}_2\text{'GMP}$ solution is 80% structured at 0°C while a 0.69M $\text{TMA}_2\text{'GMP}$ solution with 1.08M KCl added is 30% structured at 0°C (cf. Figures 39 and 40). Figure 40 shows the effect of making a 0.60M $\text{K}_2\text{'GMP}$ 1.33M in TMAcI at -3°C . There is a drastic change in the spectrum. All structure resonances disappear immediately and the monomer line is sharpened. Similar results were obtained with Rb^+ and Cs^+ salts. The addition of TMAcI to solutions of $\text{Rb}_2\text{'GMP}$ and $\text{Cs}_2\text{'GMP}$ caused the loss of all structure resonances present and a sharpening of H(8). No self-structure resonances were found when RbCl or CsCl were added to $\text{TMA}_2\text{'GMP}$.

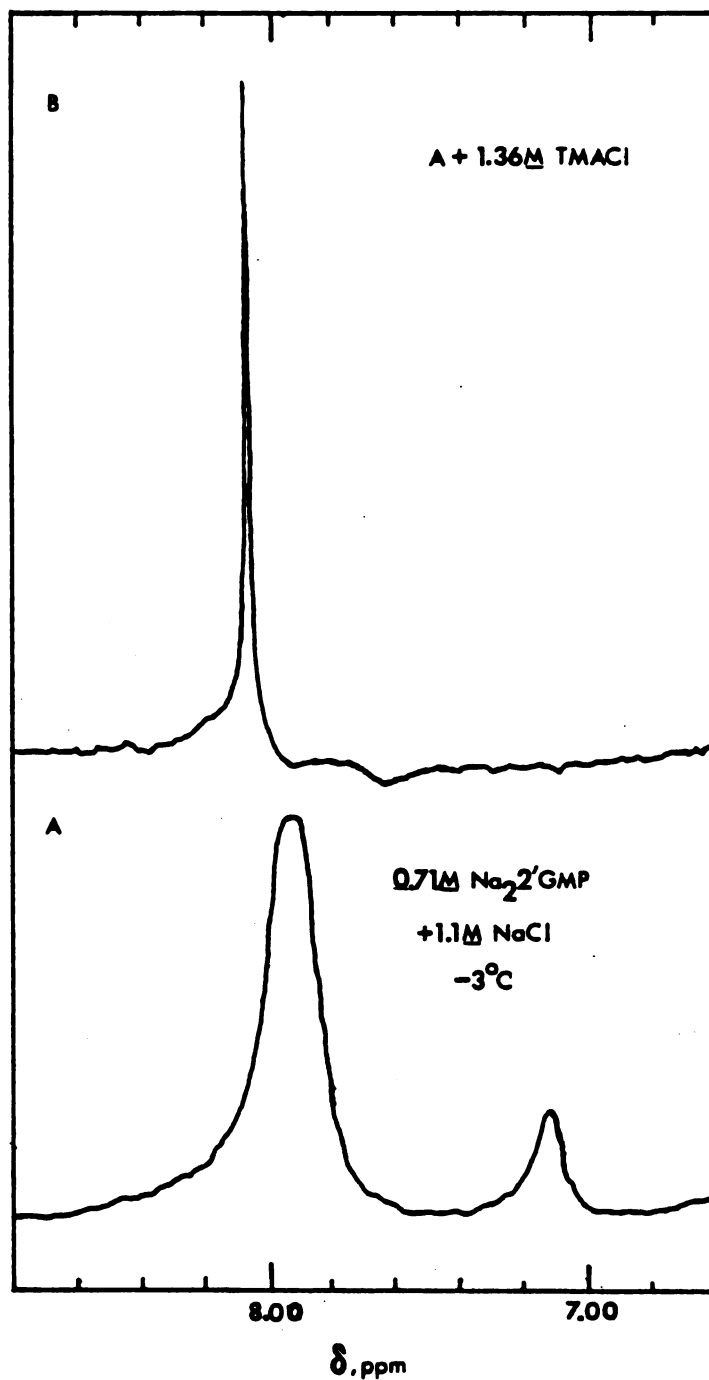


Figure 38. The effect on the H(8) NMR spectrum on addition of TMACl(s) to a 0.71M Na₂2'GMP solution containing 1.1M NaCl at -3°C. B illustrates the H(8) resonance of the sample made 1.36M in TMACl. A illustrates the H(8) resonances of the sample in absence of TMA⁺.

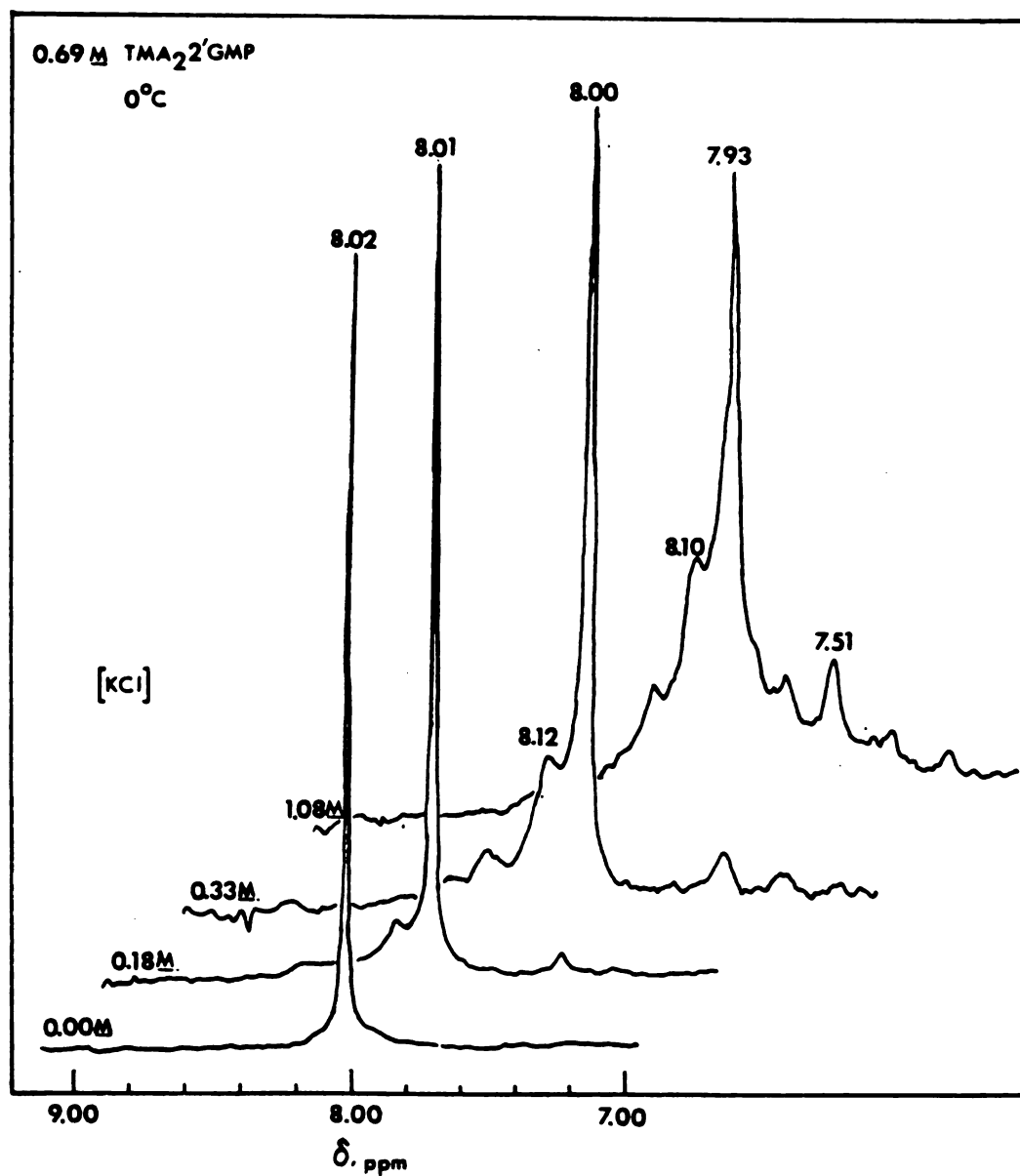


Figure 39. The change in the proton NMR spectra of 0.69M TMA₂2'GMP upon addition of KCl at 0°C.

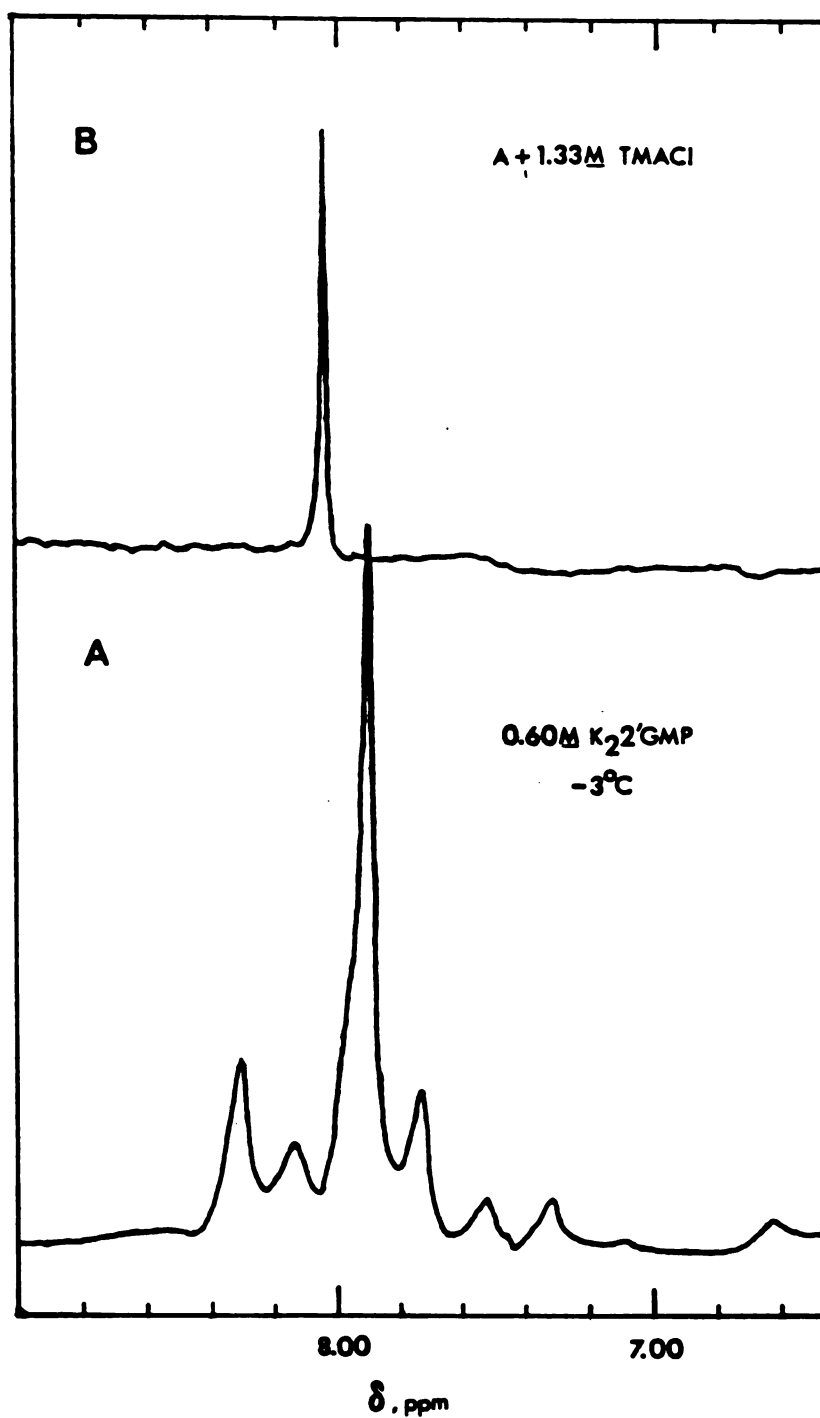


Figure 40. Effect on the H(8) NMR spectrum on making 0.60M $K_22'GMP$ 1.33M in $TMACl(s)$ at $-3^\circ C$. A is the H(8) NMR spectrum in absence of TMA^+ . B is the H(8) spectrum after making the solution 1.33M in $TMACl(s)$.

These results of TMA^+ with the dialkali metal salts of 2'GMP indicate that TMA^+ is not a structure directing ion, but is a structure inhibiting ion.

The destabilization of self-structure can be observed in the IR spectra. Figure 41 shows the IR spectra of 0.69M TMA_2 2'GMP at 51°C and 3°C. The strong absorbance at approximately 1500 cm^{-1} and the broad absorbance at approximately 1470 cm^{-1} are due to the TMA^+ ion. Figure 42 shows the IR spectra of 0.69M TMA_2 2'GMP made 1.1M in NaCl at 51°C and 3°C. There is no evidence of solution self-structure in agreement with proton NMR data.

The effect of TMA^+ on the chemical shift of the H(8) resonance is shown in Figures 16 and 43. In Figure 16 observed H(8) chemical shift versus temperature for 0.69M TMA_2 2'GMP and 0.86M Na_2 2'GMP are shown. As the temperature is lowered there is an upfield shift of the H(8) resonance which is much more pronounced for the Na_2 2'GMP system. The addition of $\text{NaCl}_{(s)}$ to a 0.69M TMA_2 2'GMP solution also has an effect on the H(8) resonance. A plot of concentration of NaCl versus the chemical shift of H(8) at 5°C and 0.5°C is given in Figure 43. There is a much greater effect of Na^+ ion concentration at low temperature. This suggests that there are at least two forms of nucleotide exchanging rapidly on the NMR time scale. Based on other results in this work, we propose the rapidly exchanging structure is a tetramer unit.

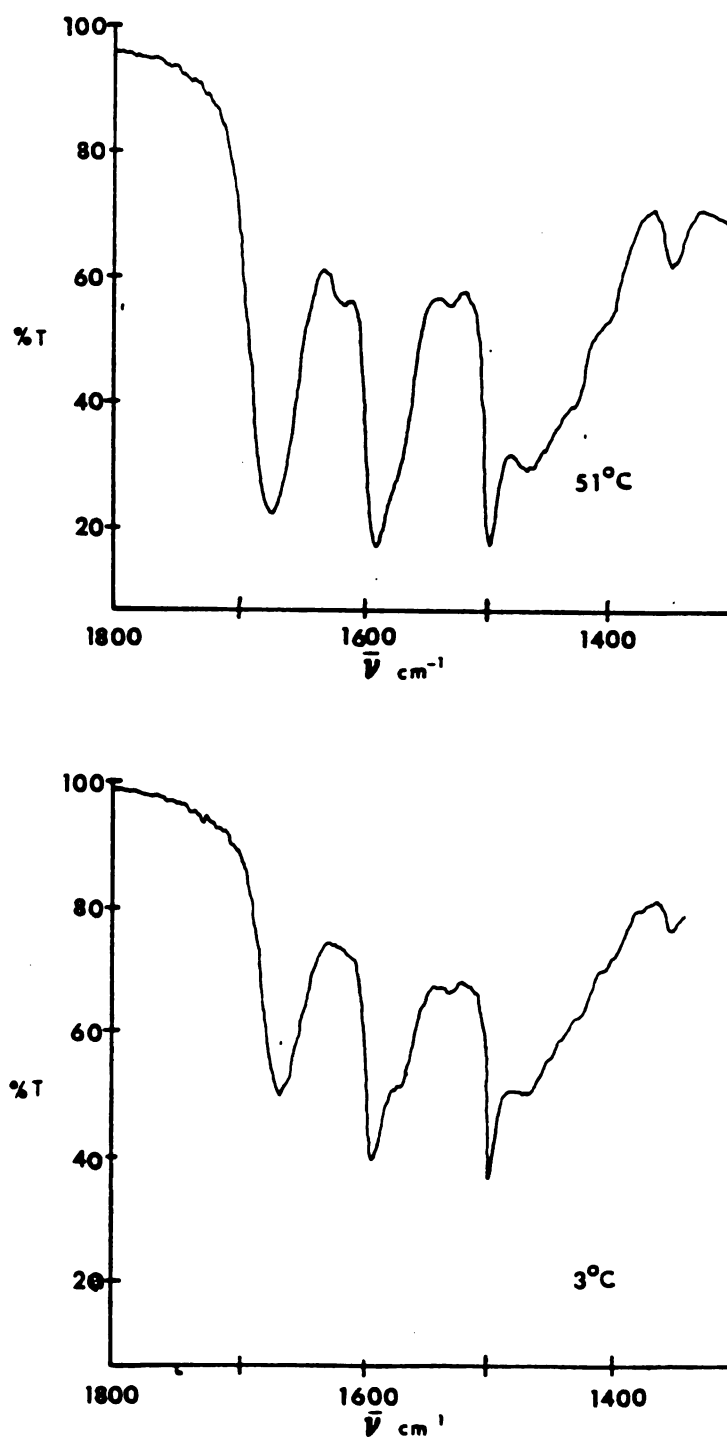


Figure 41. The infrared spectra of 0.69M TMA₂'GMP at 51°C and 3°C NMR studies of this solution at comparable temperatures show no evidence for structure formation.

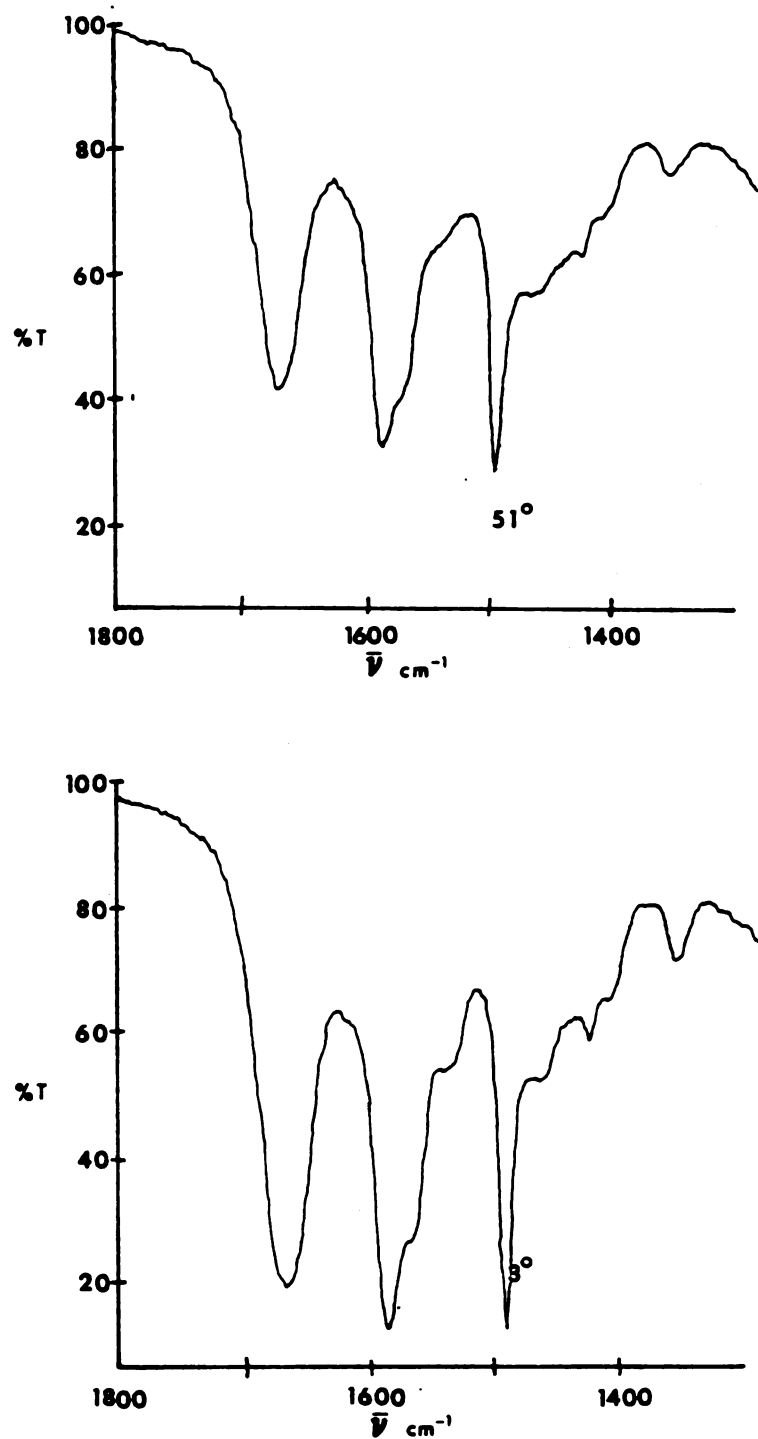


Figure 42. The infrared spectra of a 0.69M TMA_{22'}GMP solution made 1.1M in NaCl at 51°C and 3°C. NMR studies of this solution at comparable temperatures show no evidence for structure formation.

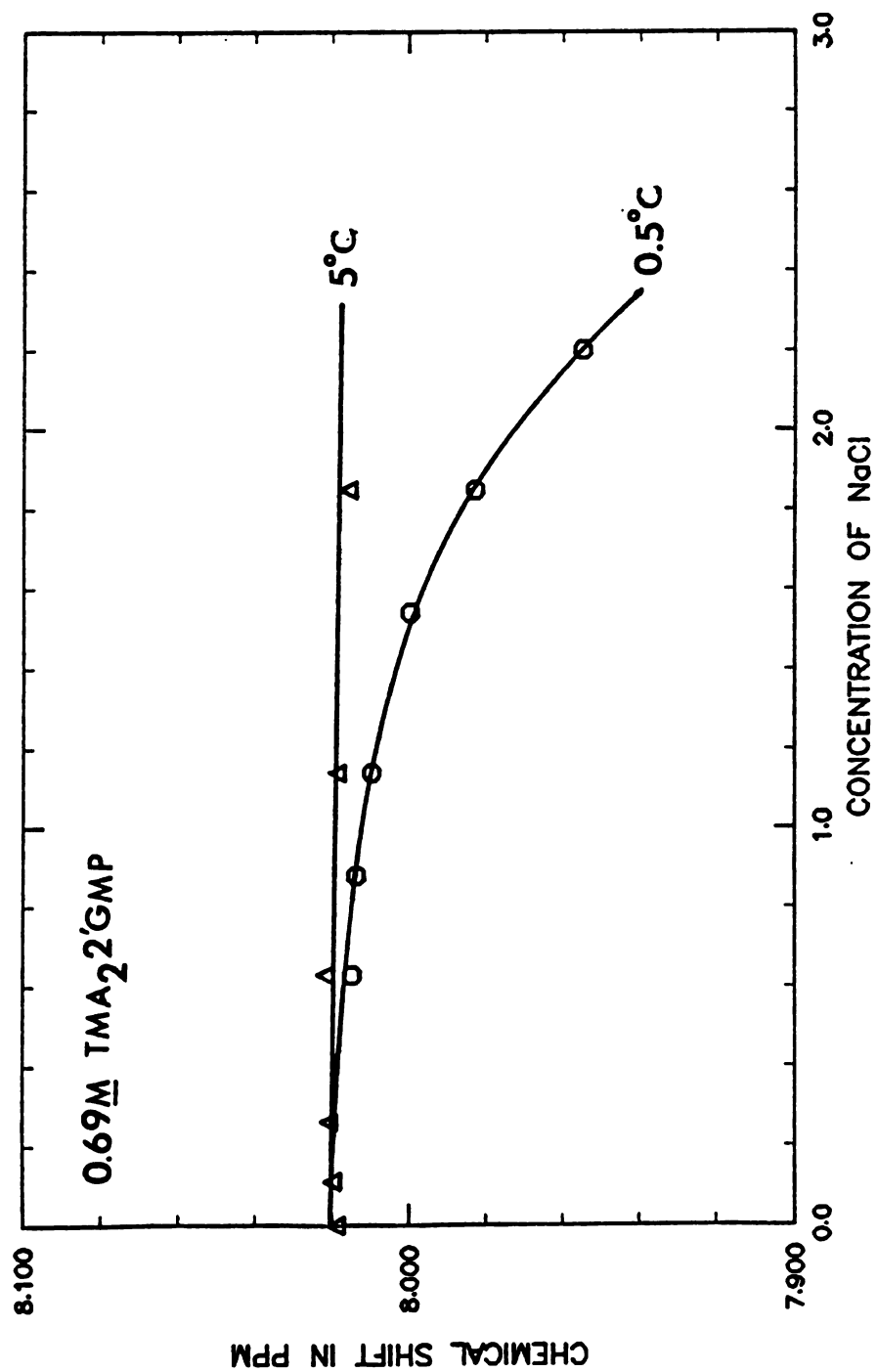


Figure 43. The chemical shift of H(8) versus the concentration of NaCl in 0.69M TMA₂'GMP at 5°C and 0.5°C.

In order to determine if the TMA^+ destabilization of 2'GMP self-structuring is due to a steric or an ion pairing effect, tetraethylammonium (TEA^+) and tetrabutylammonium (TBA^+) ions were investigated. The addition of $\text{NaCl}_{(s)}$ to 0.89M TEA_2 2'GMP caused a broadening of the H(8) resonance but no structure resonances were present. At 1.10M NaCl a precipitate formed in the solution. Similar results were obtained with the addition of KCl (at 1.17M KCl precipitation occurred).

Figure 44 shows the results of adding TEAP (tetraethylammonium perchlorate) to a 0.82M K_2 2'GMP solution at 0°C. The solution was made 0.08M in TEAP. The TEAP was not all soluble, so the ^1H NMR spectrum was taken of the filtered liquid. The final concentration of 2'GMP was determined to be 0.60M, that of TEA^+ was determined by NMR to be $\sim 0.01\text{M}$. The solution containing TEAP has a spectrum similar to a TEAP free system (cf. Figure 40), but the extent of structure formation is decreased by the presence of TEA^+ .

Solutions of TBA_2 2'GMP were very viscous and had a light green color. The H(8) resonance of a 0.14M TBA_2 2'GMP solution was a very broad singlet. The addition of NaCl or KCl caused a non-miscible oil to form in the solution. Thus, no information could be obtained on the TBA^+ system.

Nuclear Overhauser experiments were performed to determine if the tetraalkylammonium ion was located in a position where it would block the tetramer unit hydrogen

Figure 44. The effect on the H(8) NMR spectrum of 0.82M K_22' GMP at 0°C on making the solution 0.08M in TEAP. B is the H(8) region of 0.82 M K_22' -GMP at 0°C. A is the H(8) region after the solution is made 0.08M. A precipitate formed which was removed by filtration. The concentration of 2'GMP was determined to be 0.60M and the concentration of TEAP was approximately 0.01M, determined by NMR.

bonding positions. The Nuclear Overhauser effect (NOE) has been used to determine conformations of nucleotides¹⁵⁴⁻¹⁵⁶. When a strong radiofrequency field is applied at the frequency of the one resonance, the integral intensity of another resonance of the same nuclei may be reduced, to yield a negative intermolecular Nuclear Overhauser effect. This NOE can originate either from exchange modulation of scalar coupling or it can be the result of dipole-dipole interactions¹⁵⁷. Since the tetraalkylammonium ion protons are not capable of rapid exchange, a dipole-dipole mechanism must be invoked. In spin systems which relax primarily through dipole-dipole interactions, such as all proton systems, the magnitude of the Overhauser effect has a $1/r^6$ dependence¹⁵⁸ on the spatial separation of the observed and irradiated spins. Because of this, the NOE has found application in studies of molecular conformation and structure. Brown⁴⁹ has reported that TMA^+ is located near the H(8) of the $\text{Na}_2\text{5'GMP}$ self-structure(s) represented by the two outer H(8) lines in the proton NMR spectrum.

A 0.80M $\text{TMA}_2\text{2'GMP}$ solution at 25°C in $^1\text{H}_2\text{O}$ showed only a slight decrease in H(5') when a gated decoupling experiment was conducted with the TMA^+ resonance irradiated. Small differences in spectra taken in $^1\text{H}_2\text{O}$ are hard to quantize due to the dynamic range problems which occur.

The results of a gated decoupling experiment on a 0.89M $\text{TEA}_2\text{2'GMP}$ solution at 27°C are given in Table 6.

Table 6. Results of a Gated Decoupling Experiment on
0.89M TEA₂2'GMP at 27°C.

Decoupler Power Applied	TEA ⁺ Reson. Irrad.	H(8)	NH ₂	$\frac{I_o - I_d^a}{I_o} \times 100$			
				H(1')	H(3')	H(4')	H(5')
0.4W	-CH ₃	-20	11	2	-18	-2	2
0.1W	-CH ₂ -	-30	15	8	-18	8	3
0.4W	-CH ₂ -	-6	22	-10	-7	2	10
1.1W	-CH ₂ -	-15	42	4	0	15	30

^aI_o is the integral intensity with the decoupler power off
and I_d is the integral intensity with the decoupler on.

There is an NOE on the residual NH₂ resonance (solution contained 70% D₂O and 30% ¹H₂O) and the H(5') resonance. The only way TEA⁺ can be located near both H(5') and -NH₂ is for 2'GMP to be in the syn conformation. The NOE is a time averaged effect, so the NOE can be attributed to a TEA⁺ residing near the NH₂ group for at least some time and near H(5') for some time. The TEA⁺ molecule can therefore prevent the formation of tetramers by sterically blocking the -NH₂ group.

Table 7 shows the results of irradiating the TMA⁺ resonance in a gated decoupling experiment on a 0.71M Na₂2'GMP solution 1.10M in NaCl and 1.36M in TMAcI at -3°C. There is a large decrease in H(5') and a smaller

Table 7. Results of a Gated Decoupling Experiment in Which the TMA⁺ Resonance of a 0.71M Na₂2'GMP Solution 1.10M in NaCl and 1.36M in TMACl was Irradiated at -3°C.

Decoupler Power Applied	H(8)	H(1')	$\frac{I_o - I_d^a}{I_o} \times 100$ H(3')	H(4')	H(5')
0.1W	-5	2	8	5	8
0.4W	10	3	4	6	13
1.1W	2	14	23	31	46

^aI_o is the integral intensity with the decoupler power off and I_d is the integral intensity with the decoupler on.

decrease in H(1'), H(3') and H(4'). Figure 45 shows the effect on the proton NMR spectrum in the H(3') to H(5) region on irradiation of the TMA⁺ resonance.

TMA⁺ in this case is located near H(5') as in the other cases. It also spends some time near the other ribose protons, especially H(4'). This is consistent with the other cases.

G. Mixed Nucleotide Experiments

1. Mixed 2'GMP - 5'GMP - Two experiments were performed to determine if 2'GMP could incorporate into the Na₂5'GMP solution self-structure or vice-versa; (1) TMA₂2'-GMP was added to an 0.68M 8-d Na₂5'GMP solution and

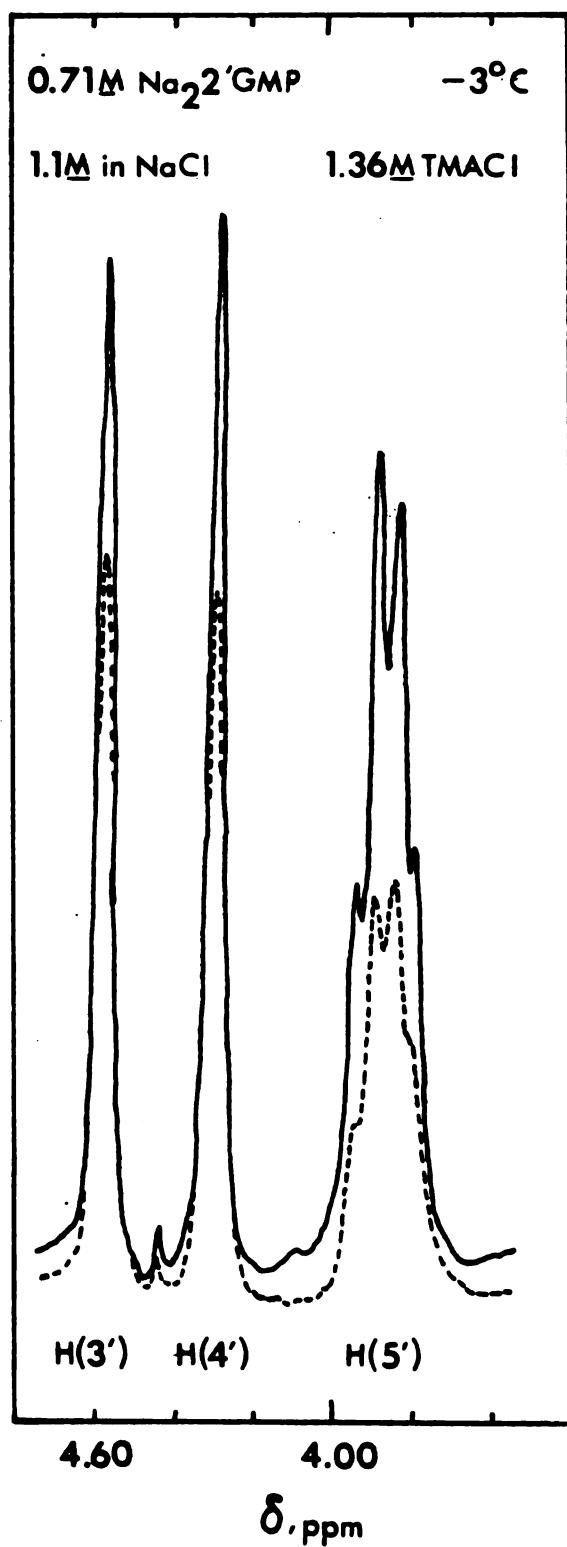


Figure 45. The effect on the H(3'), H(4') and H(5') resonances when the TMA⁺ resonance is irradiated in a gated decoupling experiment on a solution of 0.71M Na₂2'GMP, 1.10M NaCl, and 1.36M TMACl at -3°C.

(2) 8-d TMA₂2'GMP was added to an 0.68M Na₂5'GMP solution. Figure 46 shows the effect of adding 8-d TMA₂2'GMP to 0.68M Na₂5'GMP solutions at 5°C. There is a relative intensity loss of the α and δ resonances and an upfield shift of the γ resonance, both indicative of a decrease in structure. The loss of structure is also seen in the ribose region of the spectra. At 0.67M 8-d TMA₂2'GMP no structure is present. The loss of structure can also be monitored by IR. The absorbance at 1585 cm⁻¹ increases in intensity and becomes more intense than that at 1665 cm⁻¹. It is interesting to note that the 2'GMP-NH₂ resonance shifts downfield on addition of more TMA₂2'GMP. The addition of TMA₂2'GMP to a 0.32M 8-d Na₂5'GMP solution causes only slight broadening of the H(8) resonance of 2'GMP.

Since TMA⁺ destabilizes the 2'GMP self-structure and high concentrations of Na⁺ are needed for 2'GMP self-structuring, solutions containing Na₂2'GMP, Na₂5'GMP, and NaCl to keep the concentration of Na⁺ constant, were investigated. The addition of large amounts of 8-d Na₂2'GMP (up to 0.90M) to 0.37M Na₂5'GMP at 5°C with the Na⁺ concentration held constant at 2.5M by addition of NaCl caused a small decrease (<10%) in the amount of Na₂5'GMP structure present. Thus, Na₂2'GMP does not inhibit 5'GMP ordering but TMA₂2'GMP does inhibit 5'GMP ordering.

In the complementary experiment, the addition of Na₂2'GMP to 0.90M in 0.37M 8-d Na₂5'GMP at 3°C, yielded

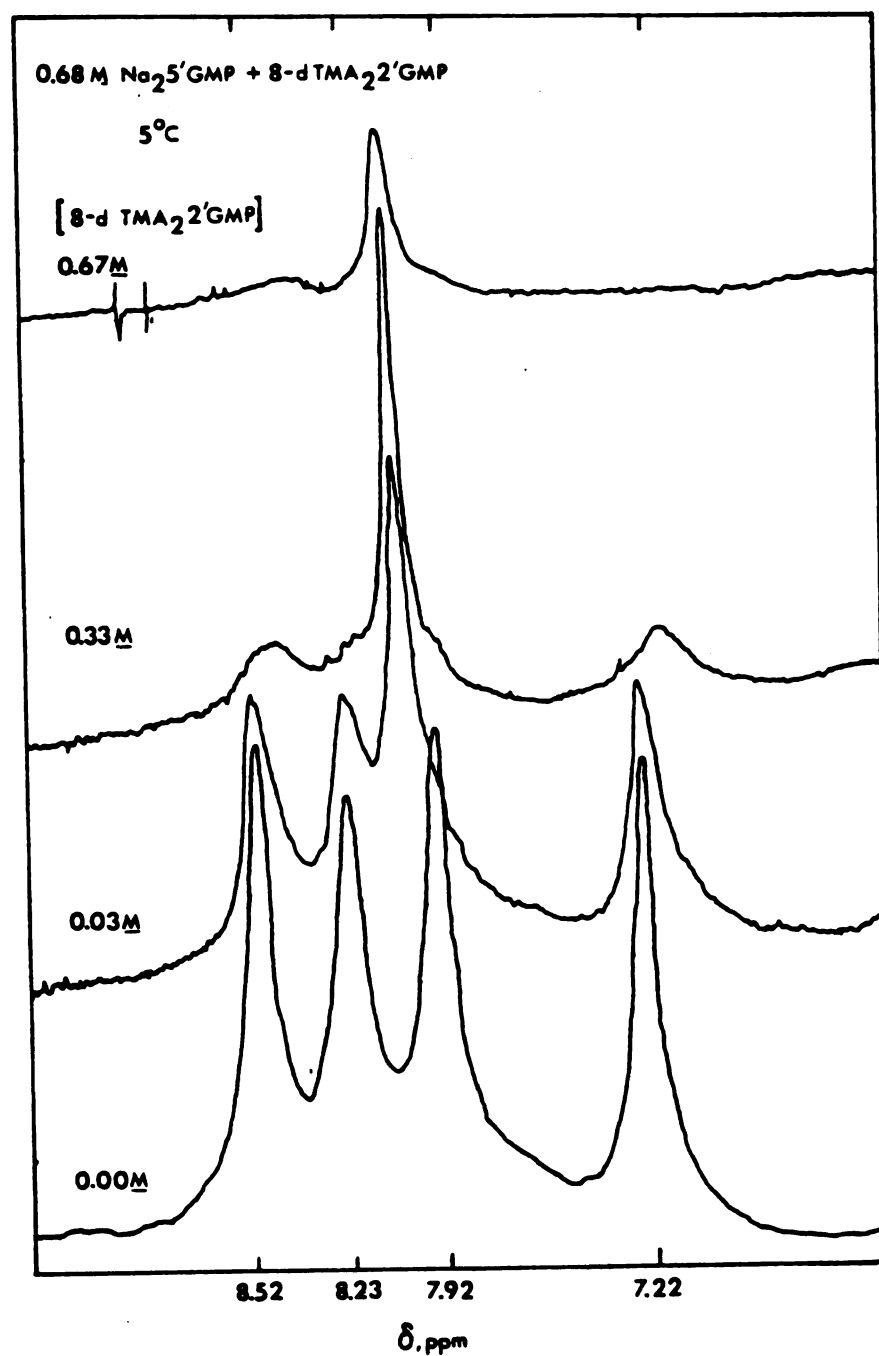


Figure 46. The effect of 8-d TMA₂'GMP on the H(8) NMR spectrum of 0.68M Na₂5'GMP at 5°C. The spectra are not drawn to scale. There is no major integral intensity loss.

only one broad ($\nu_{1/2} = 23$ Hz) 2'GMP H(8) resonance. The increased broadening may be due to an increase in the viscosity or interaction fast on the NMR time scale with 5'GMP.

ii. Mixed 2'GMP - Guanosine Systems - Only one H(8) resonance is observed at 8.00 ppm when guanosine is added to 0.69M 8-d TMA₂2'GMP at 1°C. This observation implies no long-lived structure is formed. The H(8) resonance is broad ($\nu_{1/2} = 25$ Hz), possibly due to interactions between the nucleoside and nucleotide. One would not expect solution self-structure to occur in this sample, since no structure directing metal is present. A solution 0.03M in guanosine and 0.69M in 8-d Na₂2'GMP shows the formation of structure in the NMR spectrum. Two new resonances at 8.52 ppm and 7.70 ppm and possibly a shoulder on the down-field side of the monomer (cf. Figure 47) appear. This spectrum is similar to that of Na₂5'GMP which evidences self-structure. Thus, guanosine and Na₂2'GMP form a solution self-structure under conditions where neither individually forms a self-structure.

H. Stoichiometry of the Na₂2'GMP Solution Self-Structure

In order to determine the nucleotide stoichiometry for the Na₂2'GMP self-structure, the concentration of monomer and structure need to be determined at various concentrations of nucleotide. Equilibrium (1) is assumed to

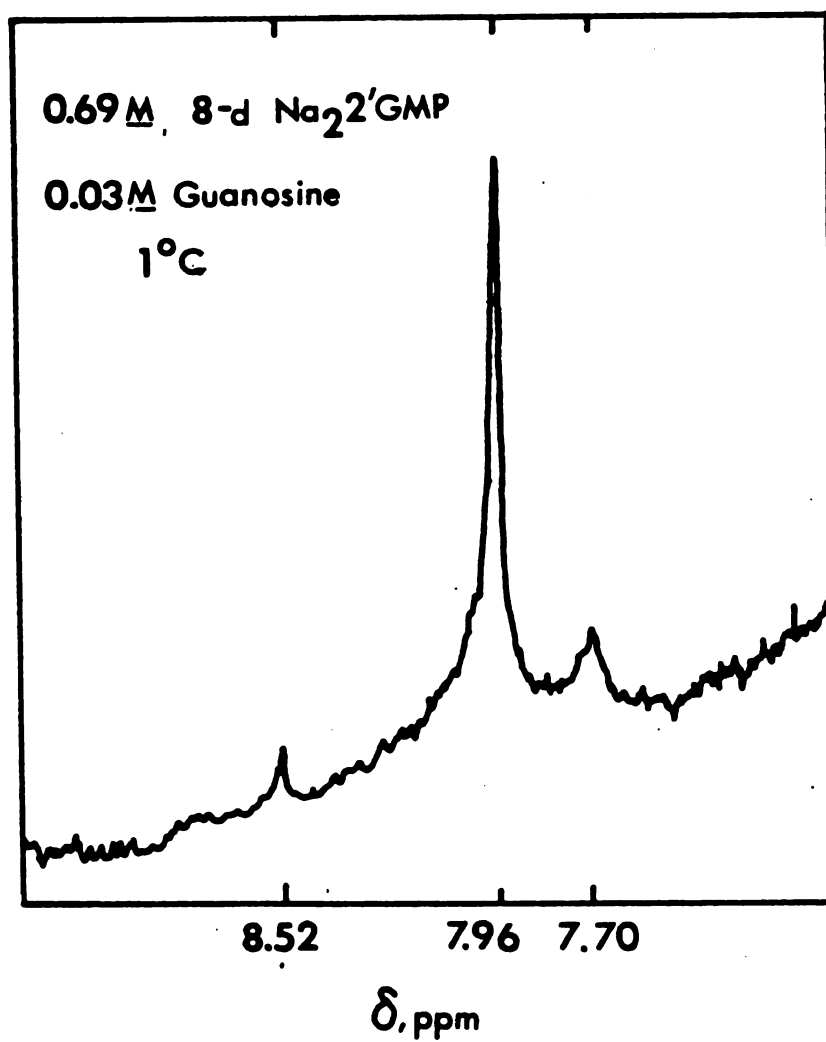


Figure 47. The H(8) spectrum of a 0.69M 8-d Na₂2'GMP and 0.03M guanosine solution at 1°C.

represent the self-structuring phenomenon.



If $[\text{Na}^+]$ is constrained such that $[\text{Na}^+] \gg [\text{Na}_x(2'\text{GMP})_n^{(2n-x)-}]$, then $[\text{Na}^+]^x$ is approximately constant. The equilibrium constant can then be expressed as:

$$K' + K[\text{Na}^+]^x = \frac{[\text{Na}_x(2'\text{GMP})_n^{(2n-x)-}]}{[2'\text{GMP}^-]^n} \quad (2)$$

Proton NMR spectra were recorded for solutions of 2'GMP in the concentration range 0.40M to 0.18M while holding the Na^+ concentration constant at 3.0M by addition of NaCl(s). The H(8) region of the NMR spectra of 0.40M and 0.18M $\text{Na}_22'\text{GMP}$ made 3.0M in Na^+ by the addition of NaCl(s) at 0°C are given in Figure 48. The H(8) region is composed of four resonances. The monomer resonance at 7.98 ppm, a shoulder at 8.01 ppm which is a structure resonance, and a small resonance(s) at 7.18 ppm which is a structure resonance. The NMR resonance at 7.18 ppm often seems to be composed of two overlapping resonances as seen in Figure 48. It is not known if the peak is composed of 1 or 2 resonances. The third structure resonance is a small shoulder at 7.94 ppm which is barely detected as a

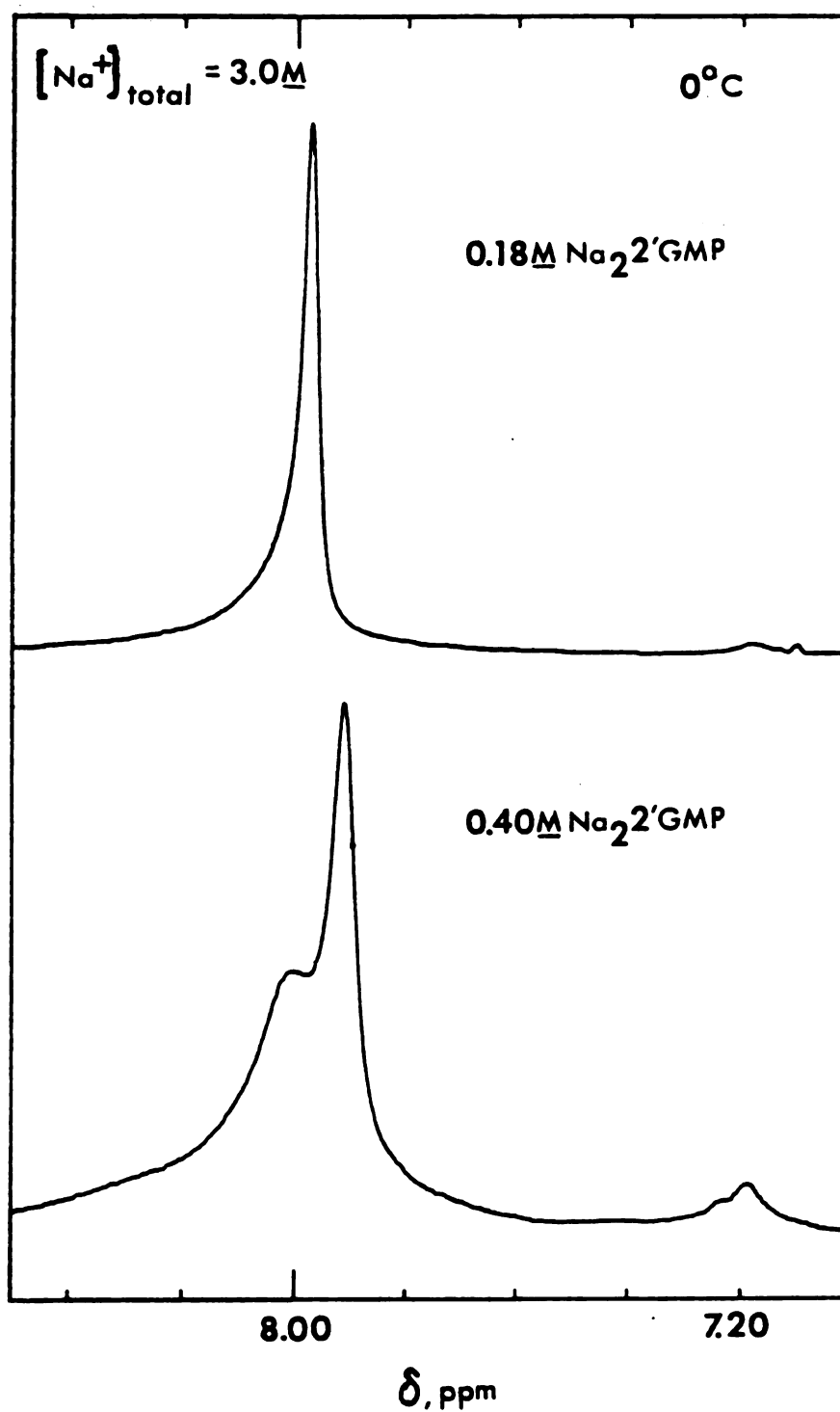


Figure 48. The H(8) region of 0.18M Na₂2'GMP and 0.40M Na₂2'GMP at 0°C. The $[Na^+]$ for both samples was made 3.0M by the addition of NaCl.

shoulder on the 7.93 ppm resonance. Computer simulation of the spectrum, as described below, shows that the resonance at 7.94 ppm is indeed present. A good fit of the H(8) peak shape cannot be obtained if this resonance is not included. The concentrations of 2'GMP and $\text{Na}_x(2'\text{GMP})_n^{-(2n-x)-}$ can therefore be determined from the integration of the H(8) proton NMR spectra. Note that on integration of the structure resonances, $n[\text{Na}_x(2'\text{GMP})_n^{-(2n-x)-}]$ is measured.

The program RESOL (Atkinson) was used to resolve the overlapped Lorentzian peaks. Digitized spectra were transferred on floppy disks from the Aspect 2000 on the Bruker WM250 to the PDP-11 minicomputer as described in Appendix 2. Given initial estimates of peak height and peak position, RESOL determines the best fit of the resonance peak height and peak position using a nonlinear least squares fit and determines the area of each resonance. An example of a computer resolved spectrum is given in Figure 49. The computer fitted results, listed in Table 8 were not accurate enough to attempt a calculation of the stoichiometry of the nucleotide. The lowfield set of overlapping H(8) lines could not be accurately resolved with RESOL due to small changes in relative intensities between spectra.

To determine the Na^+ stoichiometry, it is necessary to hold the 2'GMP concentration constant while the Na^+

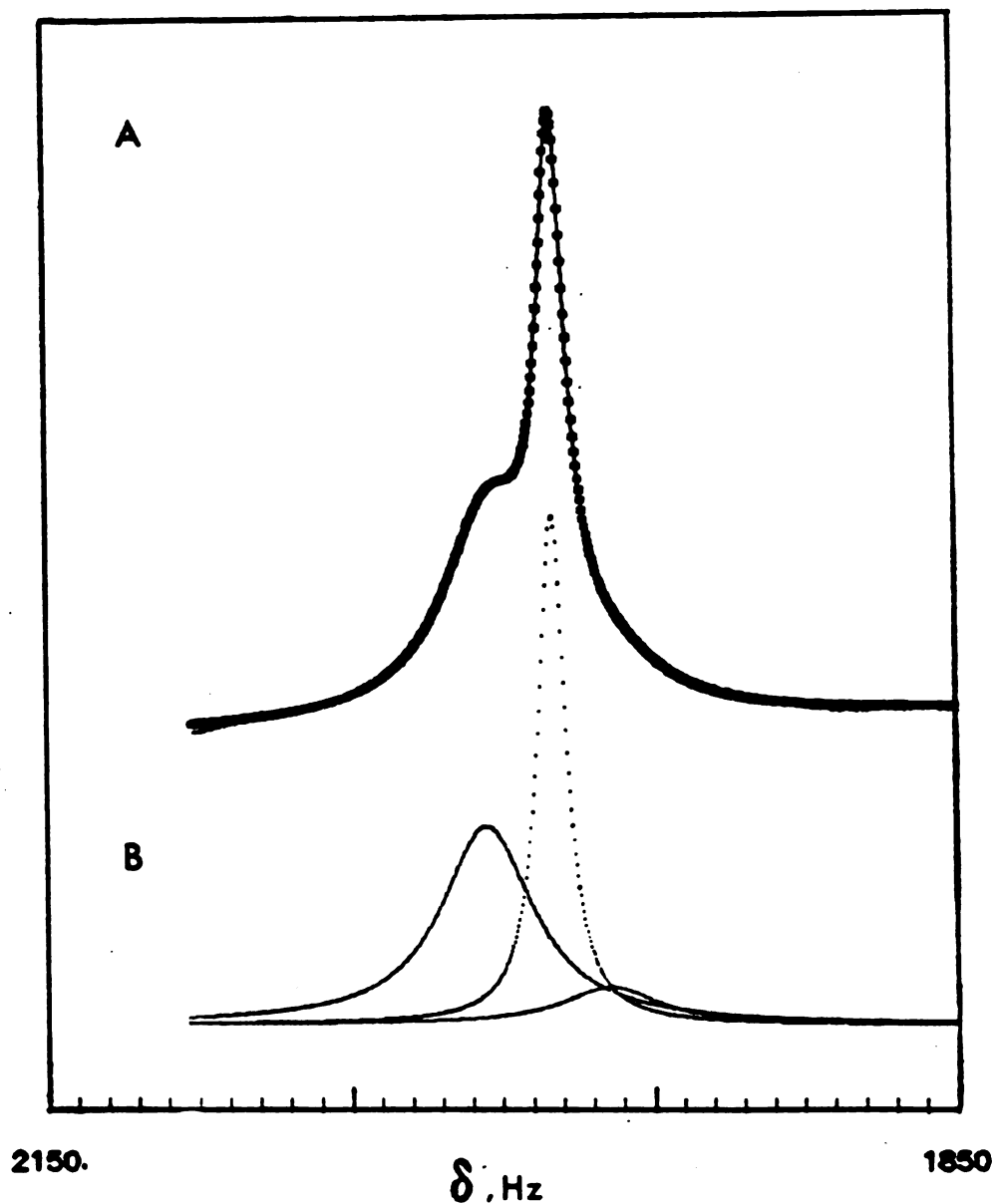


Figure 49. The computer deconvolution of the lowfield resonances of the H(8) NMR resonances for 0.38M $\text{Na}_2\text{2'GMP}$ made 3.0M in Na^+ by the addition of NaCl . The solid line in A is the observed low-field H(8) spectrum. The x's are the computed values. B shows the three deconvoluted resonances.

Table 8. Deconvolution of the Lowfield Set of H(8) Resonances for Na₂2'GMP in the Presence of 3.0M Na⁺.

Line 1				
[2'GMP]	Position ^a	% Area ^b	$\nu_{1/2}$ ^a	Amplitudes ^c
0.38	2003.7	31	15.8	71.1
0.36	2008.1	51	18.8	92.9
0.34	2008.8	43	16.3	96.7
0.32	2009.7	58	19.2	107.4
0.30	2006.1	57	18.2	150.3
0.27	2011.1	56	18.4	78.0
0.23	2014.7	55	20.9	113.7
0.21	2023.4	49	14.9	64.8
0.20	2022.6	48	14.6	64.9
0.18	2027.1	40	12.4	71.2
Line 2 (Monomer)				
0.38	1977.0	51	8.7	217.7
0.36	1986.5	39	5.5	237.1
0.34	1987.5	49	6.1	299.0
0.32	1991.3	35	4.6	271.1
0.30	1990.8	34	4.4	371.7
0.27	1996.3	42	4.1	260.0
0.23	2002.4	45	5.3	366.4
0.21	2007.1	51	4.4	227.2
0.20	2008.6	52	3.8	265.3
0.18	2013.9	60	3.6	370.8
Line 3 ^d				
0.38	1986.2	18	45.4	14.2
0.36	1967.7	10	20.0	17.6
0.34	1959.3	8	19.0	15.6
0.32	1974.6	7	10.7	21.7
0.30	1966.2	8	20.4	19.7
0.27	1987.6	2	20.4	2.7
0.23	-----	<2%	----	---
0.21	-----	<2%	----	---
0.20	-----	<2%	----	---
0.18	-----	<2%	----	---

Table 8. Continued.

^ain Hz.

^bDetermined by integration of deconvoluted spectra.

^cIn arbitrary units for each sample.

^dErrors are estimated to be position ± 2 Hz, % Area $\pm 10\%$,
 $\nu_{1/2}$ ± 3 Hz, and amplitude $\pm 5\%$.

concentration is varied. This was accomplished for Na_25' -GMP aggregation by adding TMA_25' GMP solutions to Na_25' -GMP solutions. This is not possible for $2'$ GMP since TMA^+ was found to interfere with $2'$ GMP aggregation. The experiment might have been accomplished by adding $\text{NaCl}_{(s)}$ to a Na_22' GMP solution, but this experiment was not attempted. It is unlikely that the solution will gel since $2'$ GMP does not form gels at neutral pH, but precipitation may be a problem. The stoichiometry of the Na_22' GMP solution self-structure therefore could not be determined.

III. $3'$ GMP Self-Structuring

A. Alkali Metal Salts

Only a single H(8) resonance is observed for solutions of Na_23' GMP or K_23' GMP in D_2O at low temperatures. As the concentration is increased the resonance broadens but remains a singlet until the solution gels. At the point of gelation all resonances are understandably broadened. No evidence for solution self-structuring is found in the ^1H NMR spectra. An attempt was made to observe a Na_23' GMP self-structure by adding NaCl to a solution of TMA_23' GMP. TMA_23' GMP does not gel at high concentrations but the solution becomes very viscous. Only a single H(8) resonance at 8.05 ppm is observed in 0.43M TMA_23' GMP at 1°C . The addition of aliquots of 2.0M NaCl to a 0.43M

TMA₂3'GMP solution at 1°C caused a broadening of H(8) (cf. Figure 50). At 0.37M TMA₂3'GMP and 0.29M NaCl the solution gelled. Therefore no evidence of solution self-structuring was obtained. As in 2'GMP, TMA⁺ is inhibiting the solution self-structure of 3'GMP. If TMA⁺ did not affect 3'GMP aggregation, the solution should have gelled at a much lower concentration of Na⁺.

Figure 51 shows the spectra of a 0.43M TMA₂3'GMP solution on addition of a 2.0M KCl solution. At 0.03M KCl the H(8) resonance is broadened. As the concentration of KCl in the solution is increased to 0.10M, two new resonances appear as shoulders of the monomer resonance. The structure resonances grow in intensity as the concentration of KCl is increased. At 0.27M KCl, a new intense structure resonance appears at 8.26 ppm. The melting profile for this sample is shown in Figure 52. There was no time dependence on the appearance of the resonance at 8.26 ppm.

Since gels are known to be less stable in ethanol⁷¹, a concentrated solution of Na₂3'GMP in 1-deutero-ethanol was investigated. If the solution self-structure is a restricted gel structure, then weakening the gel may allow solution self-structure to be stable. The nucleotide in ethanol showed no solution self-structure present. This may have been due to the low solubility (0.02M). A mixed solvent experiment was attempted. A 0.04M Na₂3'GMP

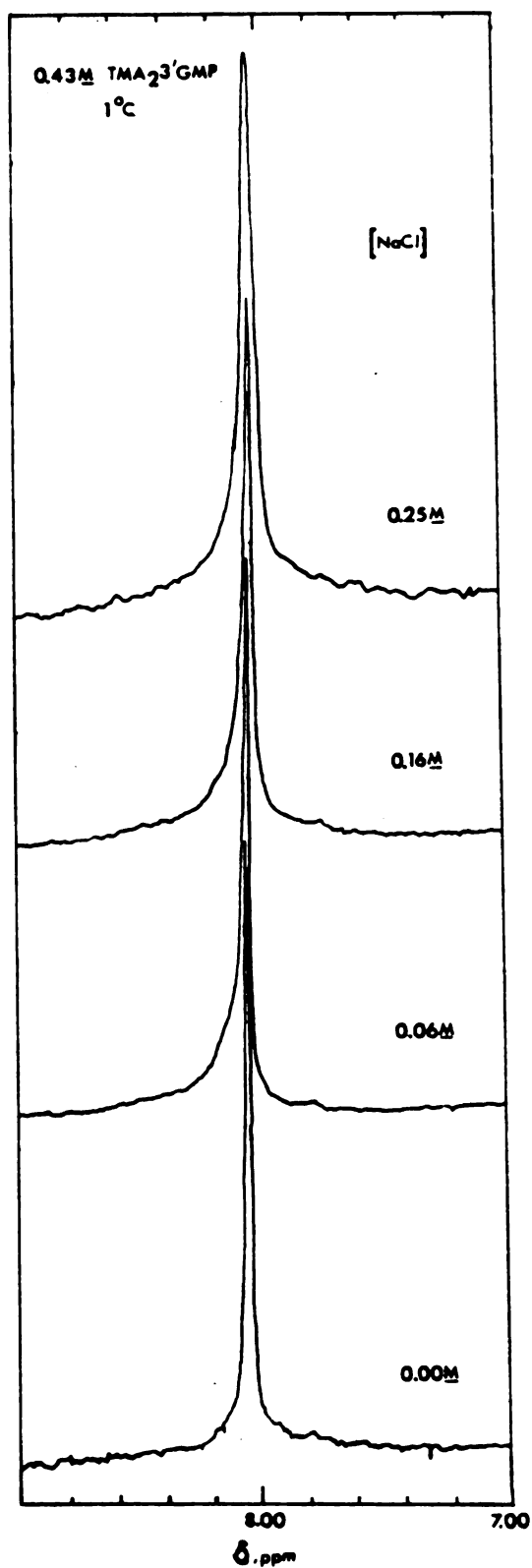


Figure 50. The effect on the NMR spectrum of the H(8) region of 0.43M TMA_{23'}GMP on addition of NaCl(s).

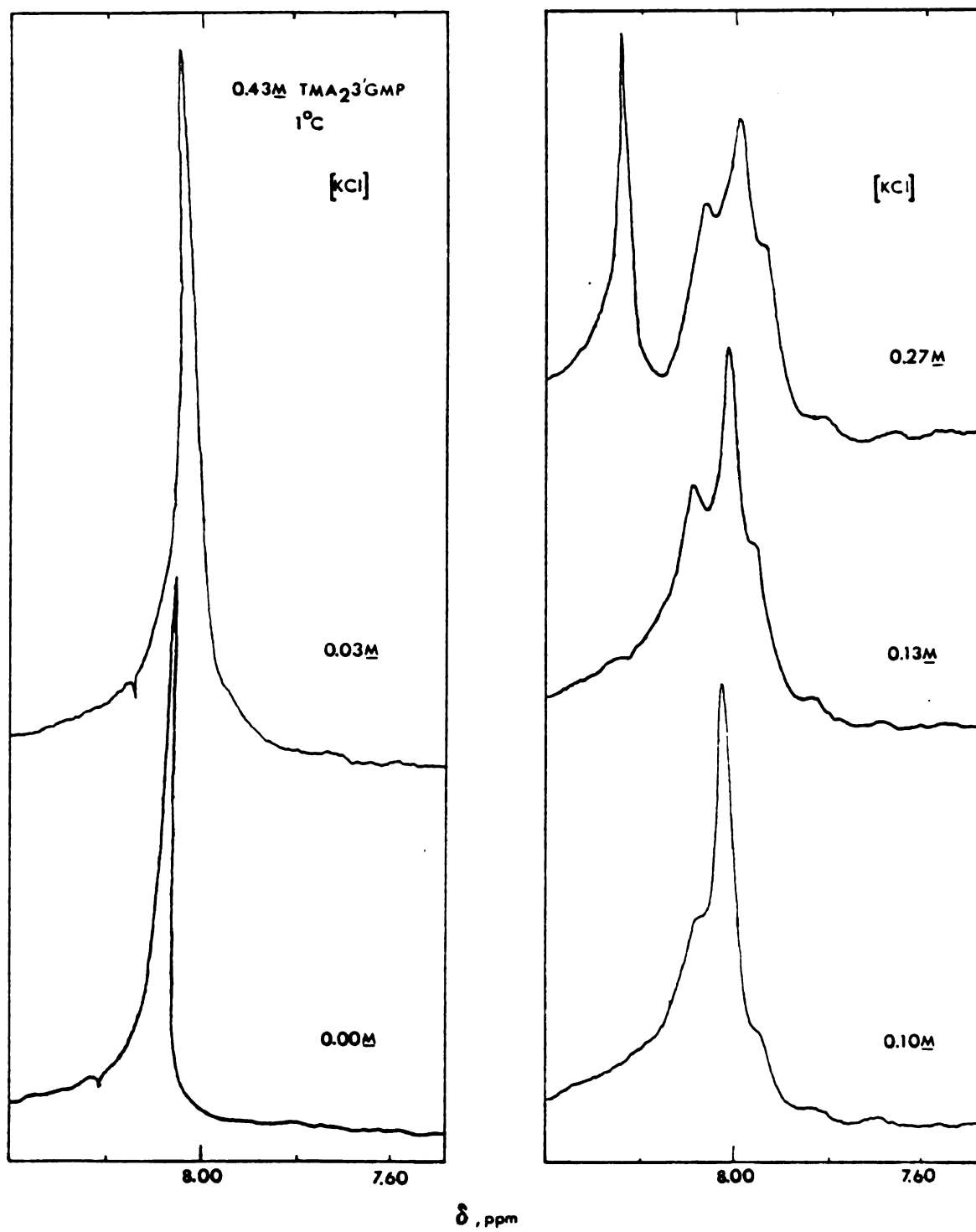


Figure 51. The effect on the NMR spectrum of the H(8) region on addition of KCl to 0.43M TMA₂3'GMP.

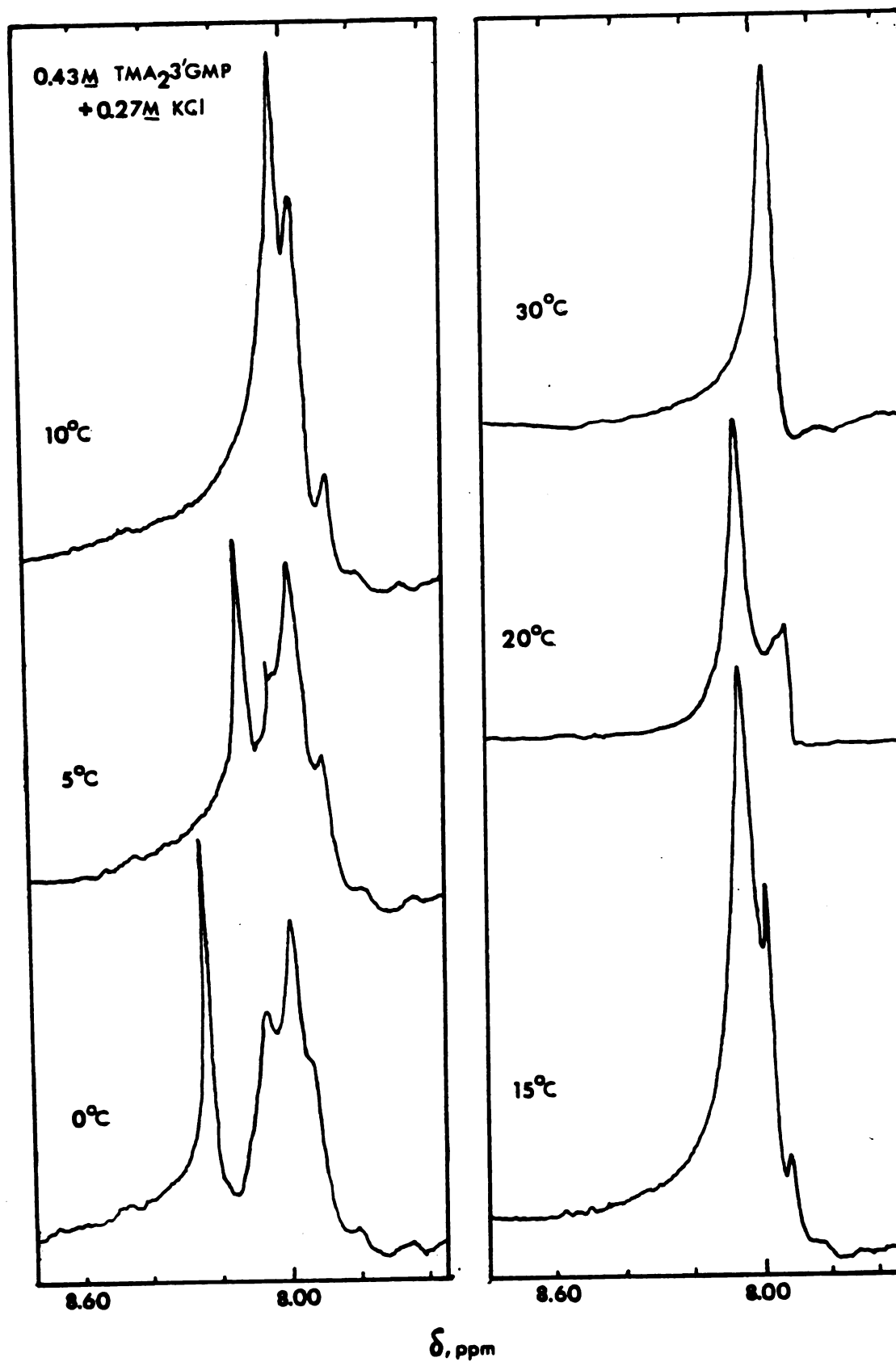


Figure 52. The temperature dependence of the H(8) region of a 0.43M TMA_{23'}GMP solution 0.27M in KCl.

solution in 50/50 v/v of $D_2O/EtOD$ showed no evidence of solution self-structure.

B. Mixed Nucleotide Systems

Two experiments were performed to determine if 3'GMP could incorporate into the Na_25' GMP solution self-structure; (1) TMA_23' GMP was added to an $0.68M$ 8-d Na_25' GMP solution and (2) 8-d TMA_23' GMP was added to a $0.68M$ Na_25' GMP solution. Figure 53 shows the effect of adding 8-d TMA_23' -GMP to a $0.68M$ Na_25' GMP solution at $7^\circ C$. There is a relative intensity loss of the α and δ resonances and an upfield shift of the β resonance, both indicative of a decrease in structure. The loss of structure is not as drastic as that seen in the 2'GMP case. A shoulder is present on the peak which increases with increasing concentration of 3'GMP. These samples contain TMA^+ as a counterion, which has been shown to destabilize the gel self-structure of 3'GMP.

The complimentary experiment, the addition of TMA_23' -GMP to $0.67M$ 8-d Na_25' GMP, is shown in Figure 54. In this experiment the H(8) resonance of 3'GMP is observed. Even at very low concentrations of added 3'GMP a new H(8) resonance which occurs at 7.50 ppm and accounts for 30% of the total H(8) intensity is present. At higher concentrations all resonances are broadened. The broadening was assumed to be caused by an increase in viscosity.

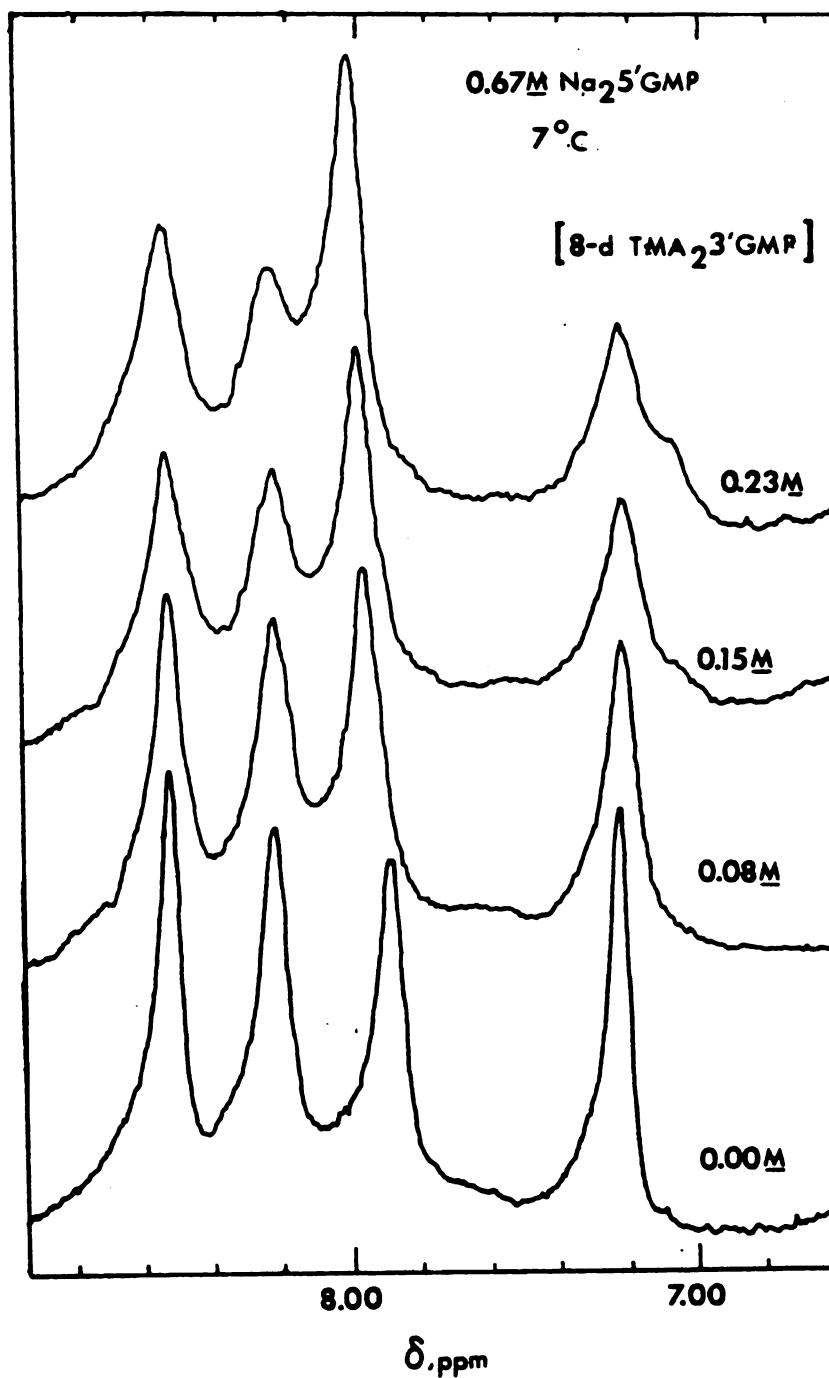


Figure 53. The effect of 8-d TMA₂3'GMP on the H(8) NMR spectrum of 0.67M Na₂5'GMP at 7°C.

0.67M 8-d Na₂5'GMP + TMA₂3'GMP

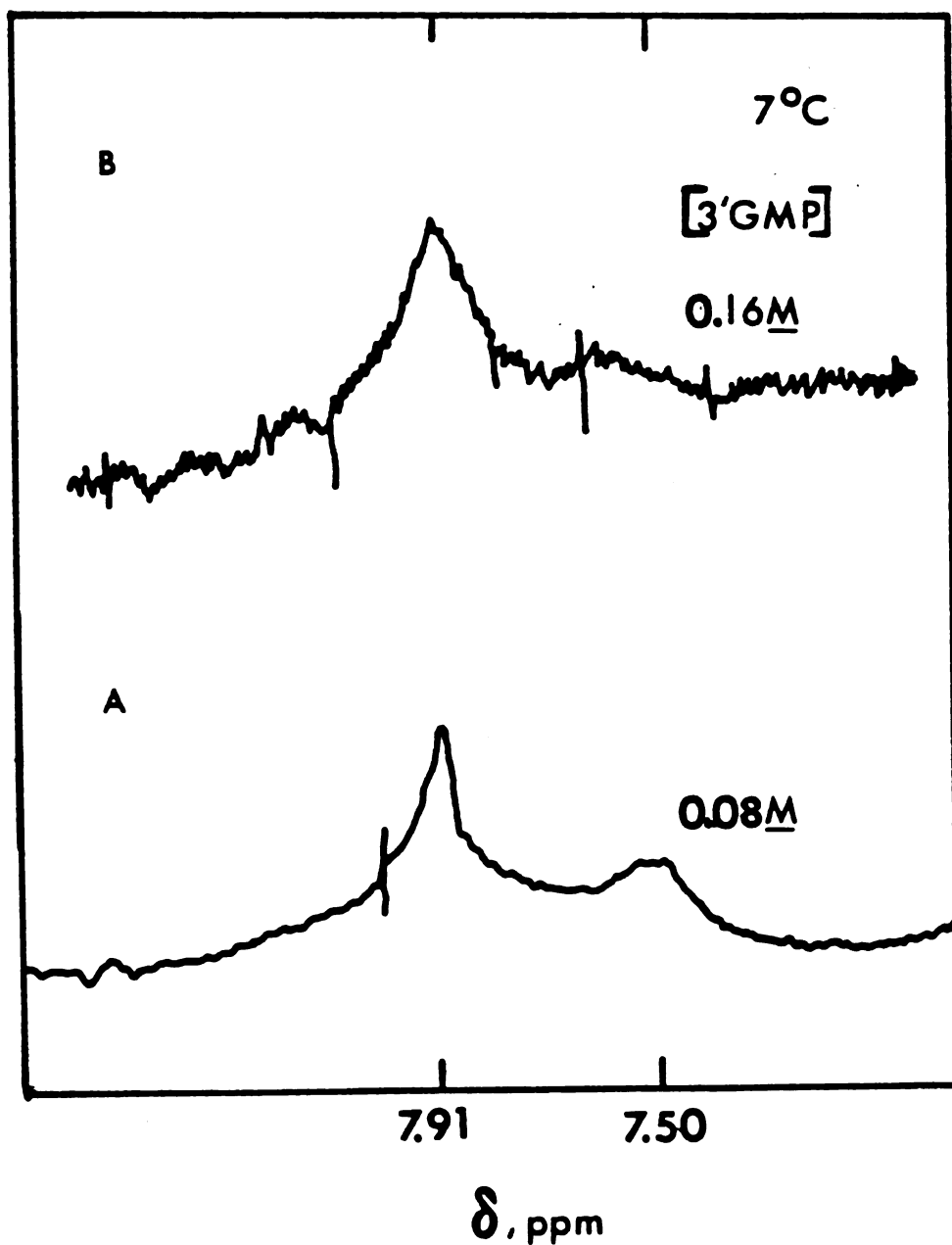


Figure 54. The H(8) NMR spectra of 0.67M 8-d Na₂5'GMP and two concentrations of TMA₂3'GMP at 7°C. A is the H(8) NMR spectrum of a 0.67M 8-d Na₂5'GMP and 0.08M TMA₂3'GMP solution. B is the NMR spectrum of a 0.67M 8-d Na₂5'GMP and 0.16M TMA₂3'GMP solution.

Therefore, there is an interaction between 3'GMP and 5'GMP which causes 3'GMP to be in at least two slowly exchanging environments.

IV. 5'GDP Self-Structuring

A. Alkali Metal Salts

Only a single H(8) resonance is observed for solutions of $\text{Na}_2\text{5'GDP}$ or $\text{K}_2\text{5'GDP}$ in D_2O at low temperatures. As the concentration is increased the resonance broadens but remains a singlet until the solution gels. At the point of gelation all resonances are understandably broadened. An attempt was made to observe a $\text{Na}_2\text{5'GDP}$ self-structure by adding NaCl to a solution of $\text{TMA}_2\text{5'GDP}$. $\text{TMA}_2\text{5'GDP}$ is very viscous and is almost a gel at high concentrations and low temperature. Only a single resonance at 8.12 ppm is observed in 0.11M $\text{TMA}_2\text{5'GDP}$ at 0°C. The addition of $\text{NaCl}_{(s)}$ to a 0.11M $\text{TMA}_2\text{5'GDP}$ solution at 0°C causes line broadening of H(8) but no appearance of new resonances. There is also an upfield shift of the H(8) resonance. At 0.45M NaCl, the H(8) resonance is present at 8.09 ppm. As in 2'GMP and 3'GMP, TMA^+ is affecting the solution self-structure. If TMA^+ did not affect structure formation, the solution would have gelled at a much lower concentration of Na^+ . A 0.1M $\text{Na}_2\text{5'GDP}$ at 0°C would be a gel.

Figure 55 shows the spectra of a 0.11M $\text{TMA}_2\text{5'GDP}$

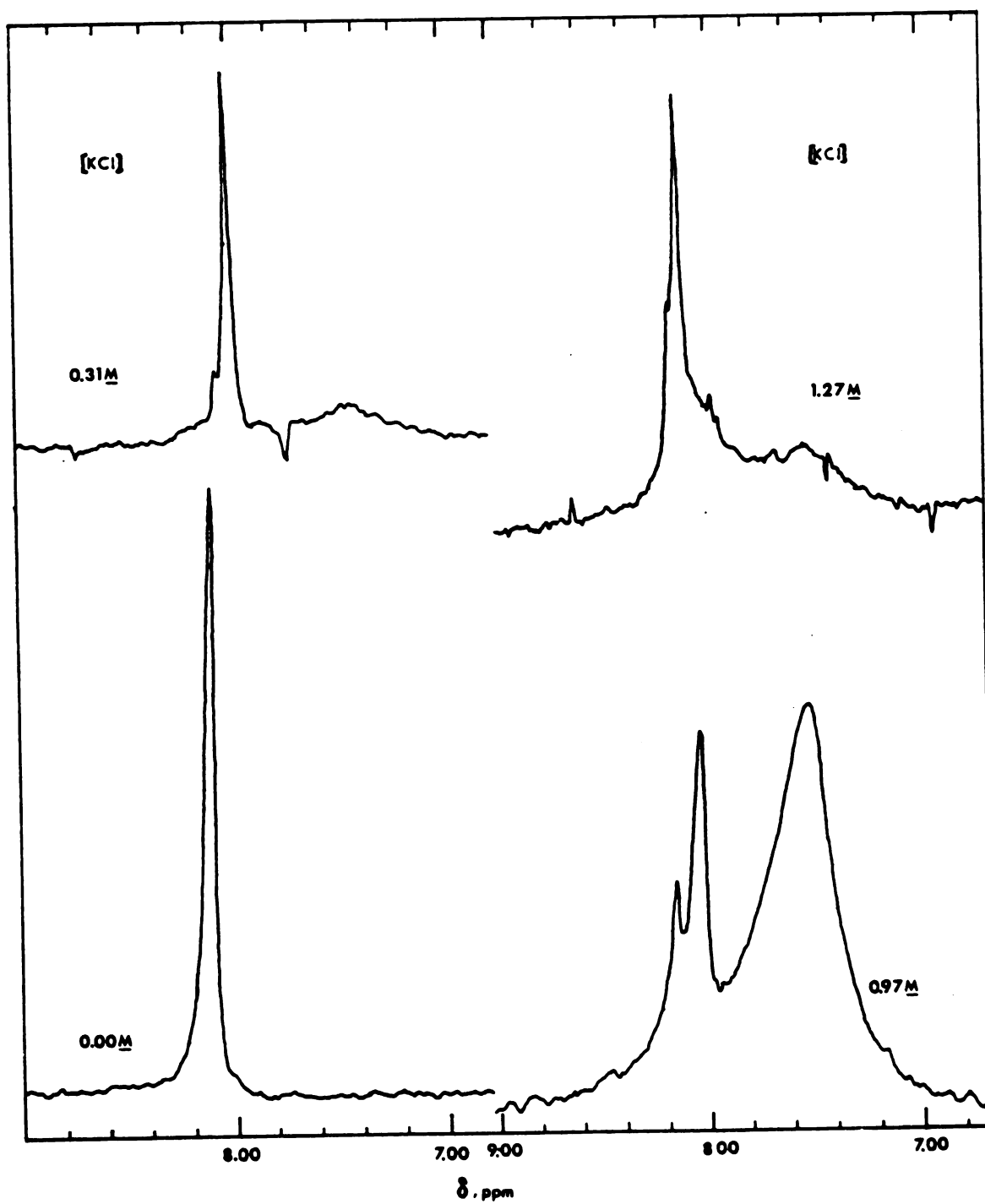


Figure 55. The H(8) ^1H NMR spectra of 0.11M TMA_{25'}GDP containing KCl. The 1.27M sample contained a precipitate when removed from the NMR spectrometer.

solution at 0°C on addition of $\text{KCl}_{(s)}$. At 0.31M KCl , the monomer resonance shifts upfield to 8.04 ppm and a new resonance appears as a shoulder downfield of the monomer. A small broad resonance is present at 7.41 ppm. Increasing the concentration of KCl to 0.97M causes the two new resonances to increase in intensity, so the solution is approximately 80% structured. Increasing the concentration to 1.27M KCl causes the formation of a precipitate and a decrease in the extent of solution self-structure. The decrease in the amount of structure can be attributed to the loss of nucleotide in solution caused by the precipitate. Figure 56 shows the melting profile of a 0.11M $\text{TMA}_25'\text{GDP}$ solution 0.97M in KCl .

B. Mixed Nucleotide Experiments

To determine if 5'GDP could incorporate into the $\text{Na}_25'\text{-GMP}$ solution self-structure, solutions containing 0.32M 8-d $\text{Na}_25'\text{GMP}$ and $\text{TMA}_25'\text{GDP}$ were investigated. The proton NMR spectra of two solutions at 5°C are shown in Figure 57. Several new resonances are present. $\text{TMA}_25'\text{GDP}$, as described in the previous section, does not form a solution self-structure in the presence of Na^+ . Therefore it is believed that 5'GDP has incorporated into the $\text{Na}_25'\text{GMP}$ self-structure. If there were incorporation of 5'GDP into the $\text{Na}_25'\text{GMP}$ solution self-structure, the H(8) region of 5'GMP should be affected. The complimentary experiment,

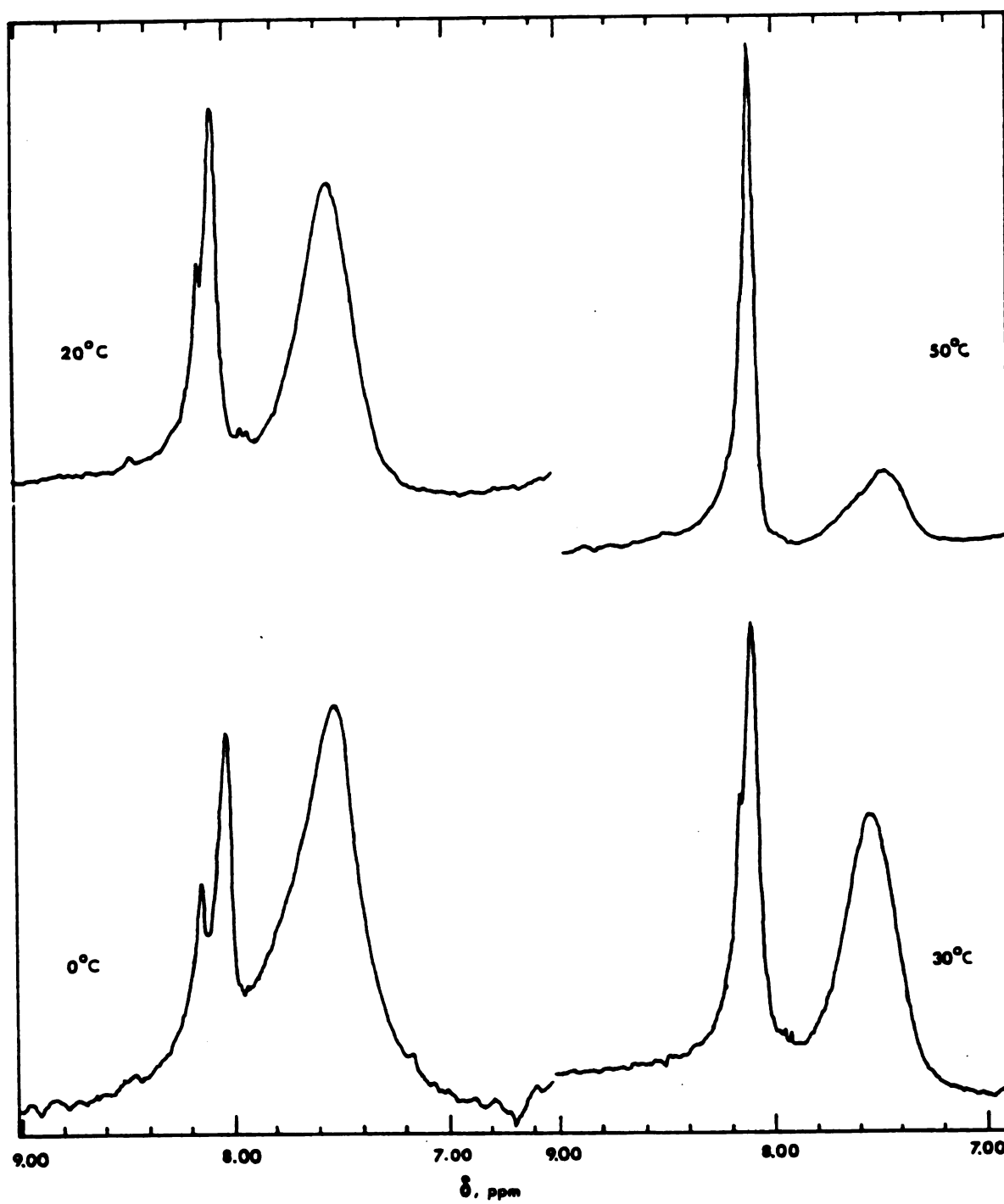


Figure 56. The melting profile of a 0.11M $\text{TMA}_{25}'\text{GMP}$ solution made 1.27M in KCl.

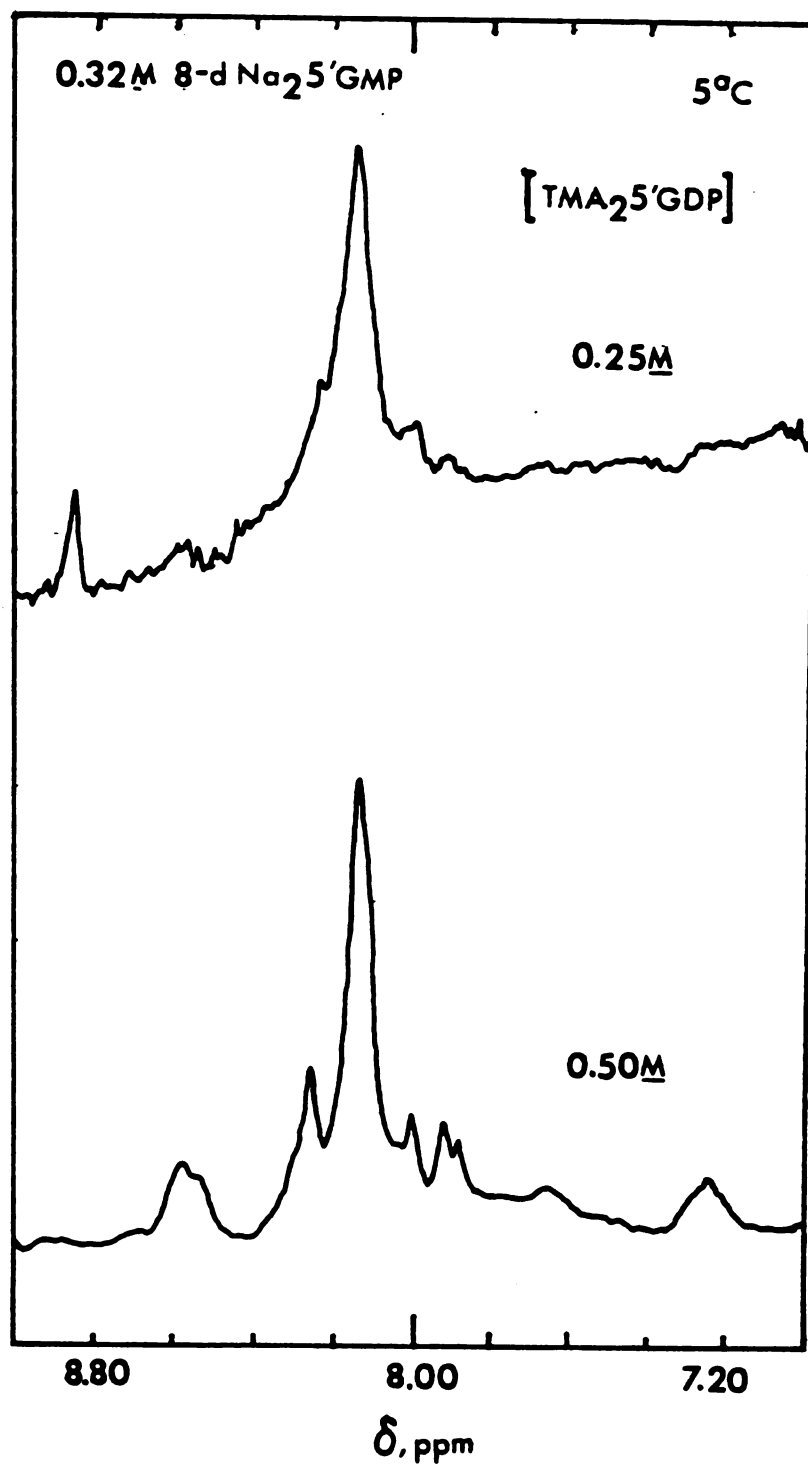


Figure 57. The H(8) NMR spectrum of a 0.32M 8-d Na₂5'-GMP solution containing TMA₂5'GDP at 5°C.

8-d TMA₂5'GDP + Na₂5'GMP, could not be done because deuteration of H(3) would cause the hydrolysis of 5'GDP to 5'GMP.

V. Ethidium Binding to Guanylic Acids

A. Binding of Ethidium to Unstructured Nucleotide

The binding of ethidium (Etd) to 2'GMP and 5'GMP was investigated. Ethidium was found to interact with both unstructured and structured guanylic acids. Figure 58 shows the change in the NMR spectrum of 0.02M Etd as TMA₂2'GMP is added to the solution. The lower spectrum represents the spectrum of the "free" ethidium bromide. The chemical shifts are in agreement with the values reported by Kreishman and coworkers¹²⁸. They assigned the resonances as follows (see Figure 10 for numbering sequence): para- and meta- protons of the phenyl group between 7.80 ppm and 7.70 ppm, H(1) and H(10) at 7.69 ppm and 7.65 ppm respectively; the orthoproton of the phenyl group, H(2), H(9), and H(4) between 7.20 ppm and 7.00 ppm; H(7) at 6.26 ppm; methylene resonance at 4.40 ppm; and the methyl resonance at 1.40 ppm. The splittings of the methyl resonance and methylene resonance are incompletely resolved at conditions where the drug is stacked, either with itself or with the nucleotide.

Kreishman and coworkers¹²⁸ and Marshall⁹⁷ have reported

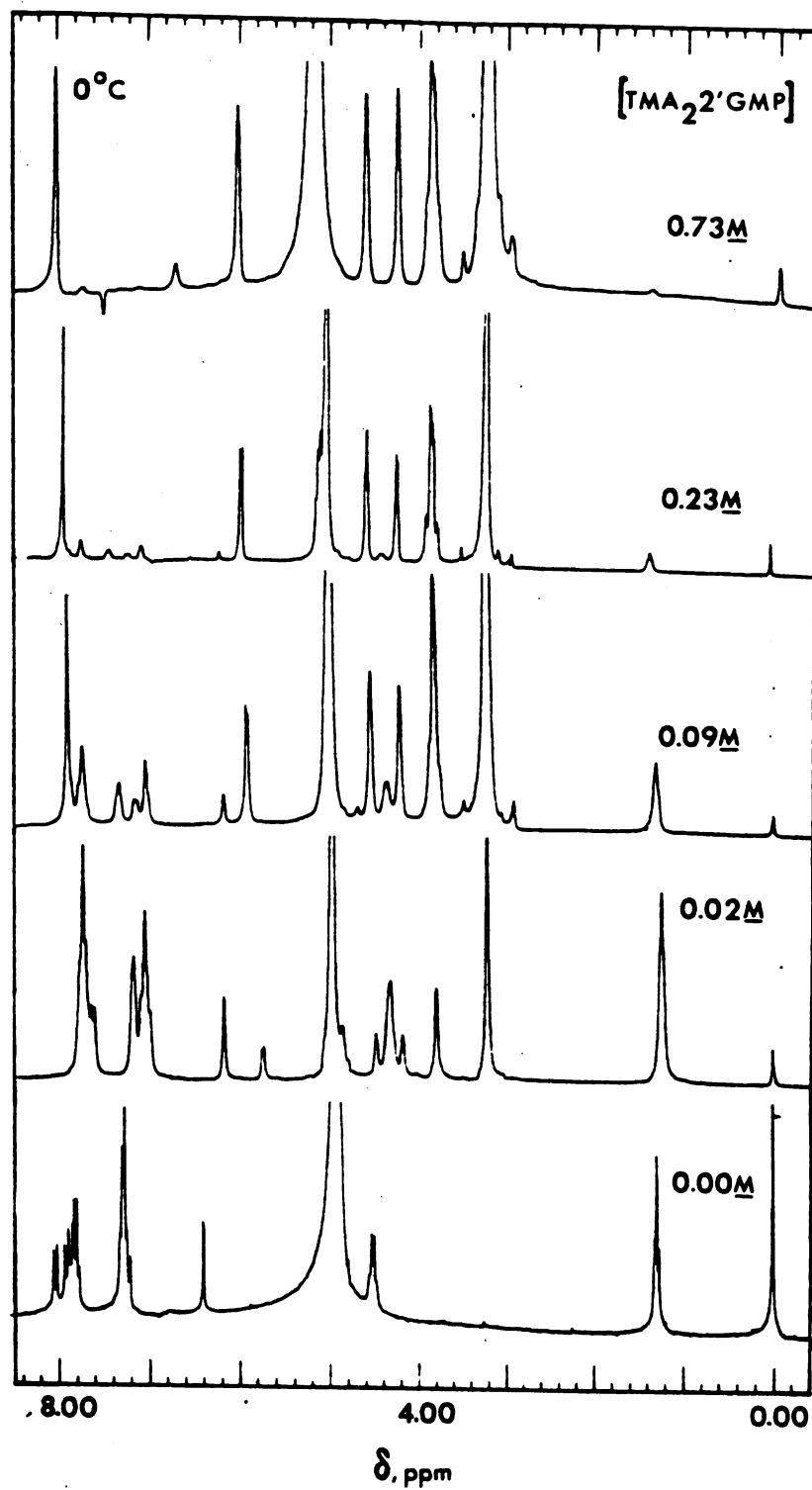


Figure 58. The effect on the proton NMR spectrum with the addition of $\text{TMA}_22'\text{GMP}$ at 0.020M ethidium bromide at 0°C.

a general upfield movement of the aromatic resonances along with considerable broadening as the drug becomes bound to nucleotide. The H(1) and H(10) lines of ethidium were observed to shift upfield by ~ 0.4 ppm, H(2), H(4) and H(9) shift upfield to 6.60 ppm to 7.20 ppm, and H(7) shifts to a position under the nucleotide's H(1') resonance at ~ 5.90 ppm. The chemical shift of the phenyl group protons exhibits little change on binding to the nucleotide. The shifts in the ethidium methyl resonance are a good indicator of binding to nucleotide. The methyl resonance shows relatively large shifts with stacking interactions, and the resonance is in a region of the ^1H NMR spectrum where no other resonances are present. The chemical shift of the methyl ethidium resonances as a function of temperature for 0.02M ethidium bromide is given in Figure 59.

Figure 60 shows the chemical shift of the methyl resonance of ethidium as a function of the $\text{TMA}_22'\text{GMP/Etd}$ ratio at several temperatures. Similar shifts occur in the ethidium methyl resonances when $\text{TMA}_25'\text{GMP}$ is added to solutions containing ethidium (cf. Figure 62). Under these conditions no IR and NMR evidence exists for an ordered solution self-structure in either nucleotide system. The Etd methyl chemical shift in both the $\text{TMA}_22'\text{GMP}$ and the $\text{TMA}_25'\text{GMP}$ solutions moves upfield as the ratio of nucleotide/Etd is increased to 1:1; then the lines move downfield when the ratio exceeds 1:1. This implies the formation of

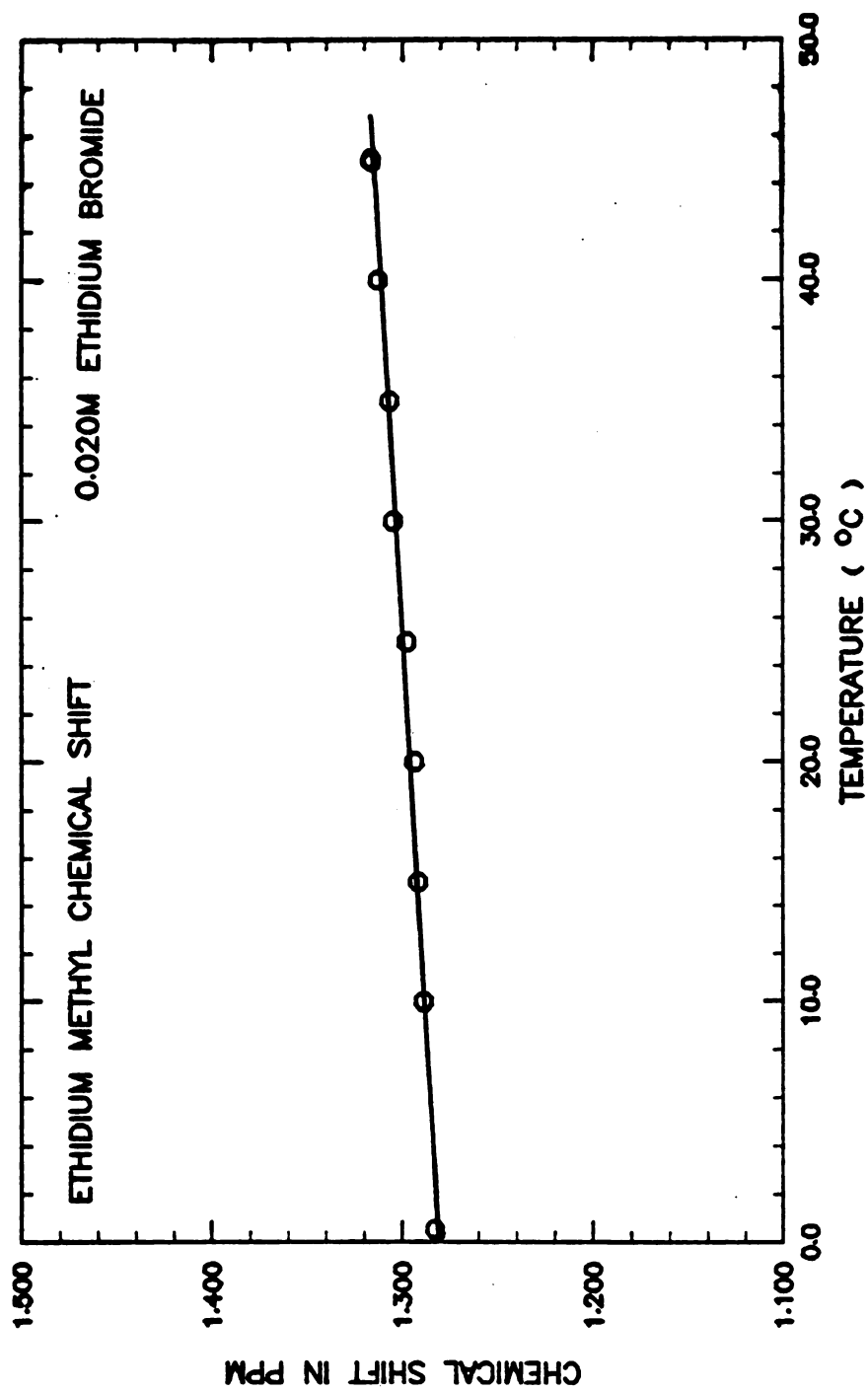


Figure 59. The temperature dependence of the methyl chemical shift of 0.020M ethidium bromide.

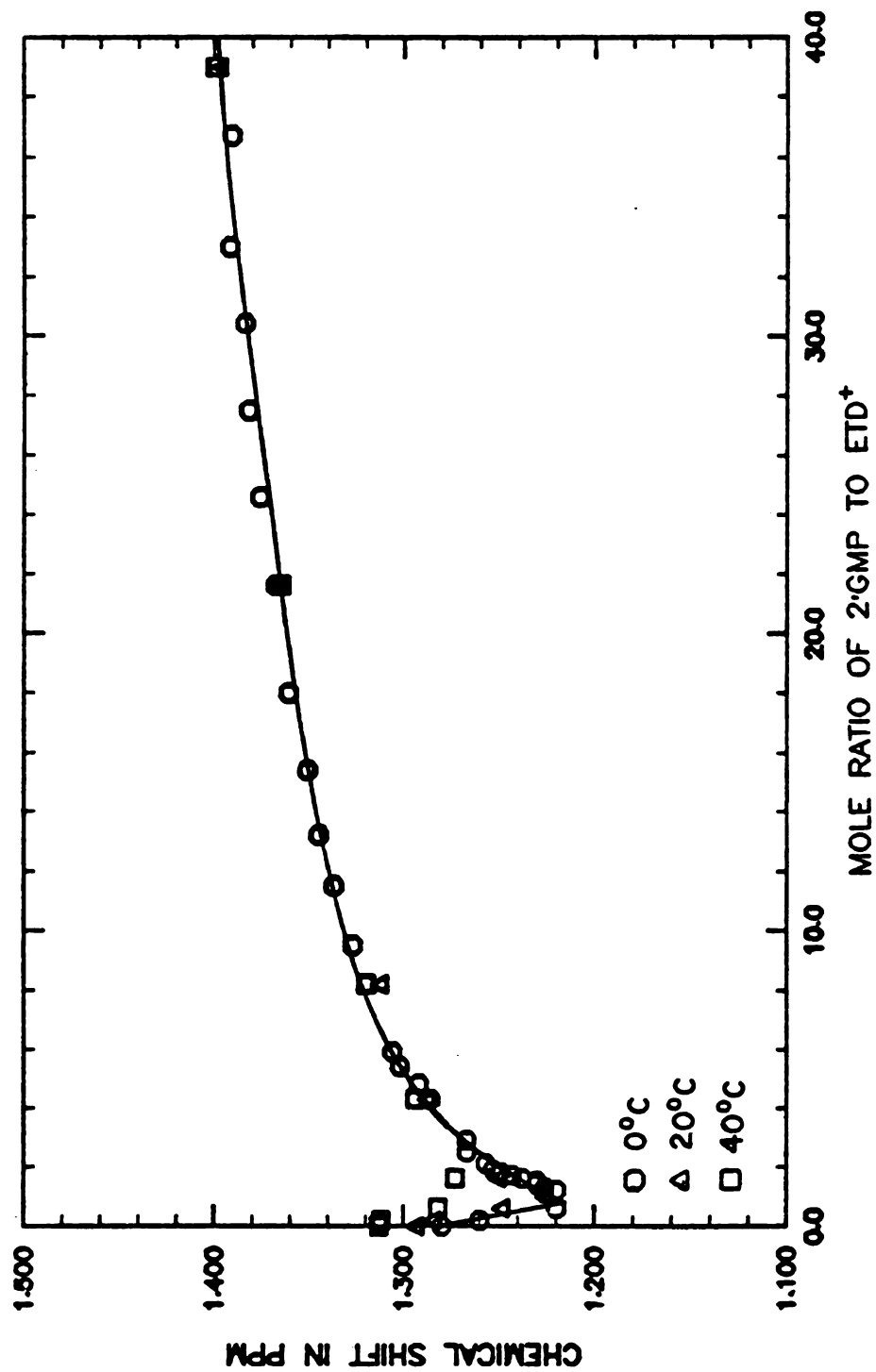
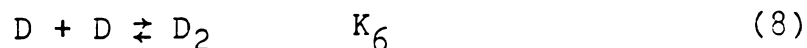
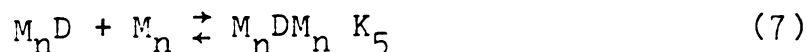
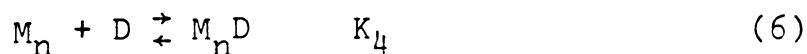
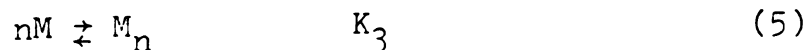


Figure 60. The chemical shift of the ethidium methyl resonance as a function of the mole ratio of TMA₂'GMP to ethidium at various temperatures.

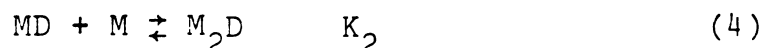
a strong 1:1 complex and a weaker 2:1 nucleotide/Etd complex. Other resonances in the spectrum show that there is an interaction between the nucleotide and ethidium. There is a downfield shift of the H(8) nucleotide resonance and the H(1') resonances of both nucleotides with increasing nucleotide/Etd ratio (cf. Figure 63).

A possible set of equilibria which can occur when ethidium binds to unstructured nucleotide are expressed as shown below, where M symbolizes disordered nucleotide and D symbolizes ethidium.



If one assumes that only the magnetic anisotropy of nearest neighbors is important in determining the chemical shift of the ethidium bound to nucleotide, then the equilibria

given by (5) and (6) are equivalent to equilibria (3) and (4). This assumption appears quite reasonable since ring current effects at twice the intermolecular distance of the conjugated ring systems (assumed to be approximately 3.4Å) will be quite small. At the concentrations of ethidium used, the dimerization reaction, defined by K_6 , can be neglected^{97,159}. Consequently, it should be possible to fit the binding of Etd to the simple reactions



Since the exchange between the three drug sites is fast on the NMR time scale, only one population-averaged resonance will be observed. The frequency of this resonance is given by

$$\delta_{\text{obs}}^D = \delta_D^D x_D + \delta_{MD}^D x_{MD} + \delta_{M_2D}^D x_{M_2D} \quad (9)$$

where δ_D^D , δ_{MD}^D and $\delta_{M_2D}^D$ are the chemical shifts of ethidium in the three environments and x_D , x_{MD} , x_{M_2D} are the relative populations for these species. The superscript D designates the shift is one of ethidium. The total concentration of the ethidium, C_D^t , and of the nucleotide, C_M^t , can be expressed as

$$C_D^t = [D] + [MD] + [M_2D] \quad (10)$$

$$C_M^t = [M] + [MD] + 2[M_2D] \quad (11)$$

by combining equations (3) and (4) with the expressions for the concentration equilibrium constants;

$$K_1 = \frac{[MD]}{[M][D]} \quad (12)$$

$$K_2 = \frac{[M_2D]}{[MD][M]} \quad (13)$$

we obtain from Equations (10), (12), and (13):

$$[D] = C_D^t / \{1 + K_1[M] + K_1K_2[M]^2\} \quad (14)$$

and from Equations (11), (12), and (13) we obtain:

$$2K_1K_2[D][M]^2 + (1 + K_1[D])[M] - C_M^t = 0 \quad (15)$$

Solving Equation (15) for [M],

$$[M] = \{-(1 + K_1[D]) \pm \{(1 + K_1[D])^2 + 8K_1K_2[D]C_M^t\}^{1/2}\} / 4K_1K_2[D] \quad (16)$$

where only the positive roots are allowed.

The relative mole fractions of the ethidium species are given by the equations:

$$x_D = [D]/C_D^t \quad (17)$$

$$x_{MD} = K_1 [D][M]/C_D^t \quad (18)$$

$$x_{M_2D} = K_1 K_2 [D][M]^2/C_D^t \quad (19)$$

The inclusion of activity coefficients would be extremely difficult, due to the very high concentrations used. Ionic strength varied from 0.04M to 3.0M. Therefore all calculations are effected with concentration equilibrium constants.

The set of equations was solved by an iteration technique in the KINFIT program. Starting with initial estimates of the free drug concentration, K_1 and K_2 , the computer program obtained a value of $[M]$ from Equation (16) and then used this value to calculate a more accurate estimate of $[D]$ from Equation (14). This procedure was repeated until convergence occurred. The final values of $[M]$ and $[D]$ were used to calculate relative mole fractions from Equations (17) - (19). The values of K_1 and K_2 were obtained by fitting the calculated δ^D values to the values obtained experimentally. Each nonlinear least-squares iteration used updated and internally consistent values of $[M]$ and of $[D]$. The chemical shift of free drug, δ_D^D , used in the calculations was that of 0.020M ethidium at the given temperature (cf. Figure 59). The KINFIT program used is given in Appendix 1.

The best computer fits of the equilibrium constants for the binding of ethidium to TMA₂2'GMP and TMA₂5'GMP are given in Table 9.

Table 9. The Best Computer Fits of the Equilibrium Constants for the Binding of Ethidium to TMA₂2'-GMP and TMA₂5'GMP.

	TMA ₂ 2'GMP (0°C)	TMA ₂ 5'GMP (5°C)
K ₁	(1.56±0.1)×10 ⁴ M ⁻¹	(1.12±0.22)×10 ⁴ M ⁻¹
K ₂	6.25±0.45M ⁻¹	18.1±11.5M ⁻¹
δ _{MD} ^D	1.229±0.002 ppm	1.271±0.022 ppm
δ _{M₂D} ^D	1.429±0.004 ppm	1.412±0.017 ppm

Figure 61 and Figure 62 compare the calculated and the experimental chemical shift data of ethidium binding to TMA₂2'GMP and TMA₂5'GMP, respectively.

Similar results should be obtained if the ¹H NMR resonances of the nucleotide are used. The H(8) resonance of the nucleotide is in the region of the aromatic protons of ethidium and at several concentrations is overlapped by the ethidium resonances. The H(1') resonance is in a region where it can be observed under all conditions, and is

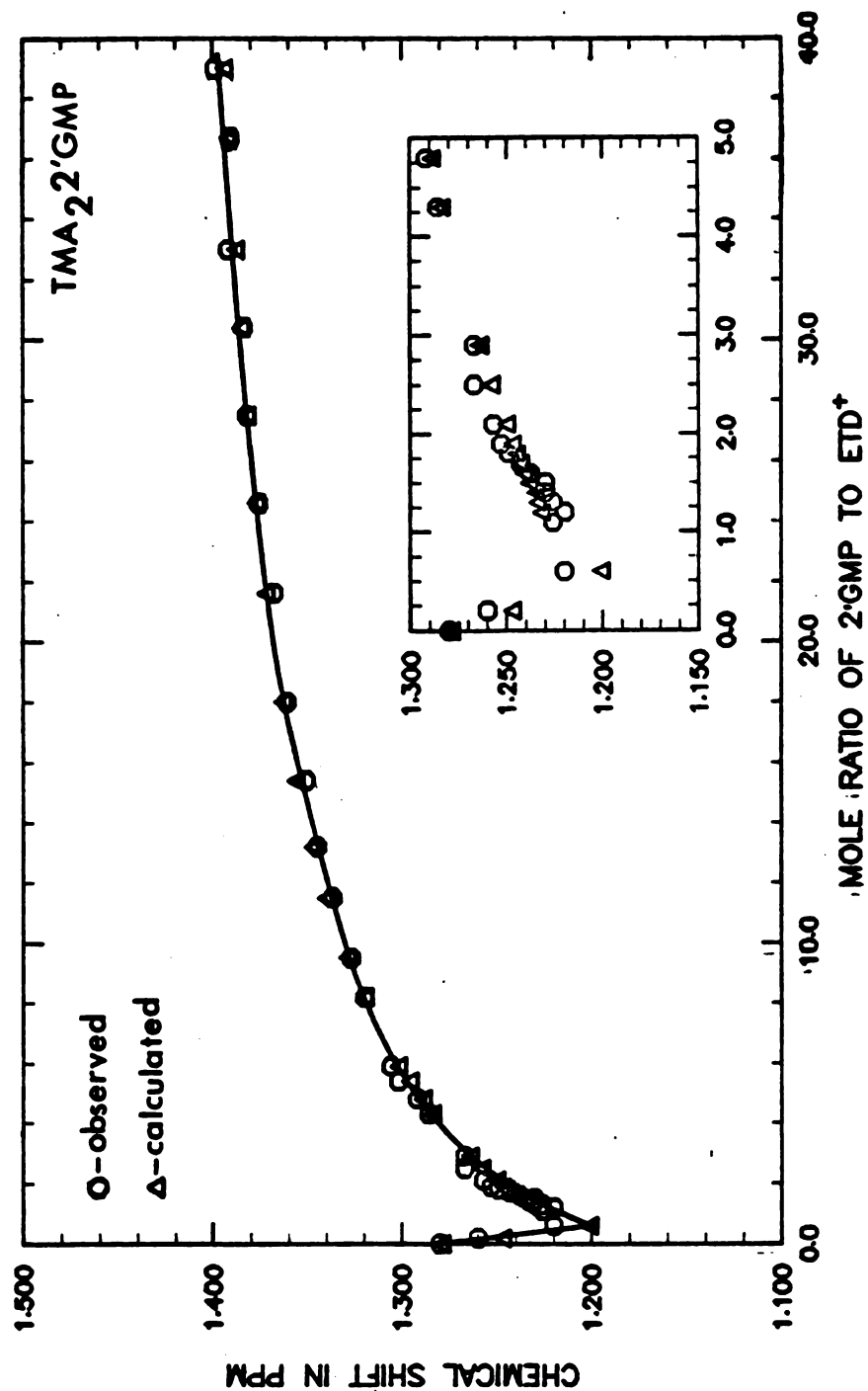


Figure 61. The best computer fit of the ethidium methyl resonance to the binding of ethidium to TMA₂2'GMP at 0°C. The concentration of ethidium is 0.020M.

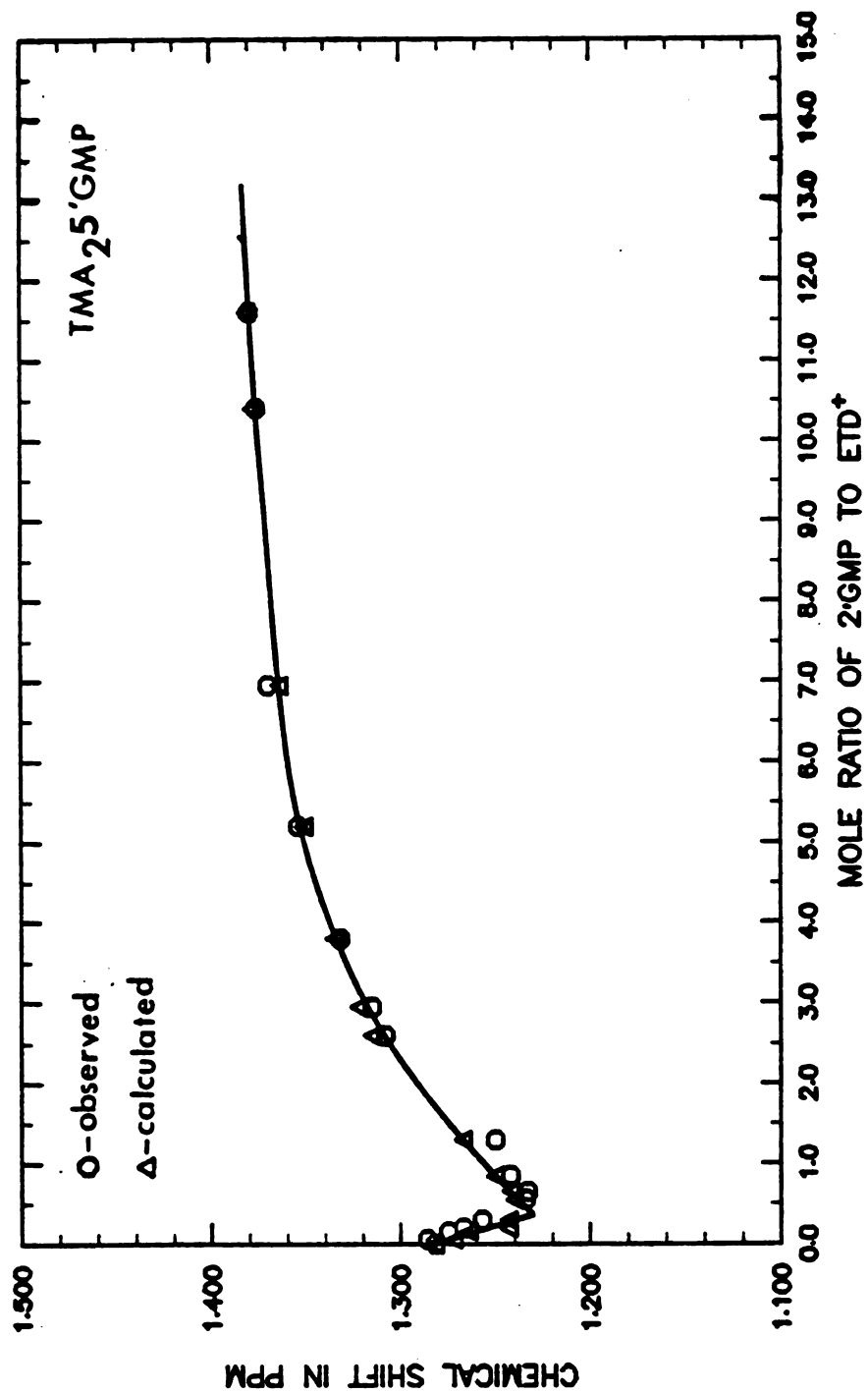


Figure 62. The best computer fit of the ethidium methyl resonance to the binding of ethidium to TMA_{25'}GMP at 5°C. The concentration of ethidium is 0.020M.

therefore the most suited for quantitative investigation. The results showed that the nucleotide H(1') chemical shifts and equilibrium constants were coupled so that the equations could not be solved. Setting K_1 and K_2 equal to the values in Table 9 yields the results in Table 10.

Table 10. The Best Computer Fit of the H(1') Resonance of 2'GMP to the Binding of Ethidium to TMA₂2'GMP.

$$K_3 = 1.0 \pm 1.0 \text{ M}^{-1}$$

$$\delta_{M_2D}^M = 6.074 \pm 0.013 \text{ ppm}$$

$$\delta_{MD}^M = 5.687 \pm 0.007 \text{ ppm}$$

$$\delta_M^M = 5.629 \pm 0.050 \text{ ppm}$$

The calculated and the experimental chemical shift data are shown in Figure 63.

The value for K_3 , $1.0 \pm 1.0 \text{ M}^{-1}$, is very close to that reported by Hélène and coworker¹⁶⁰, $2.2 \pm 0.2 \text{ M}^{-1}$ for purine base stacking in aqueous solution. He used the isodesmic approach to determine a stacking equilibrium constant from ¹H NMR data. A better fit may be obtained by using the isodesmic approach in our fit. In the isodesmic model the stacking constants are equal. The KINFIT program cannot

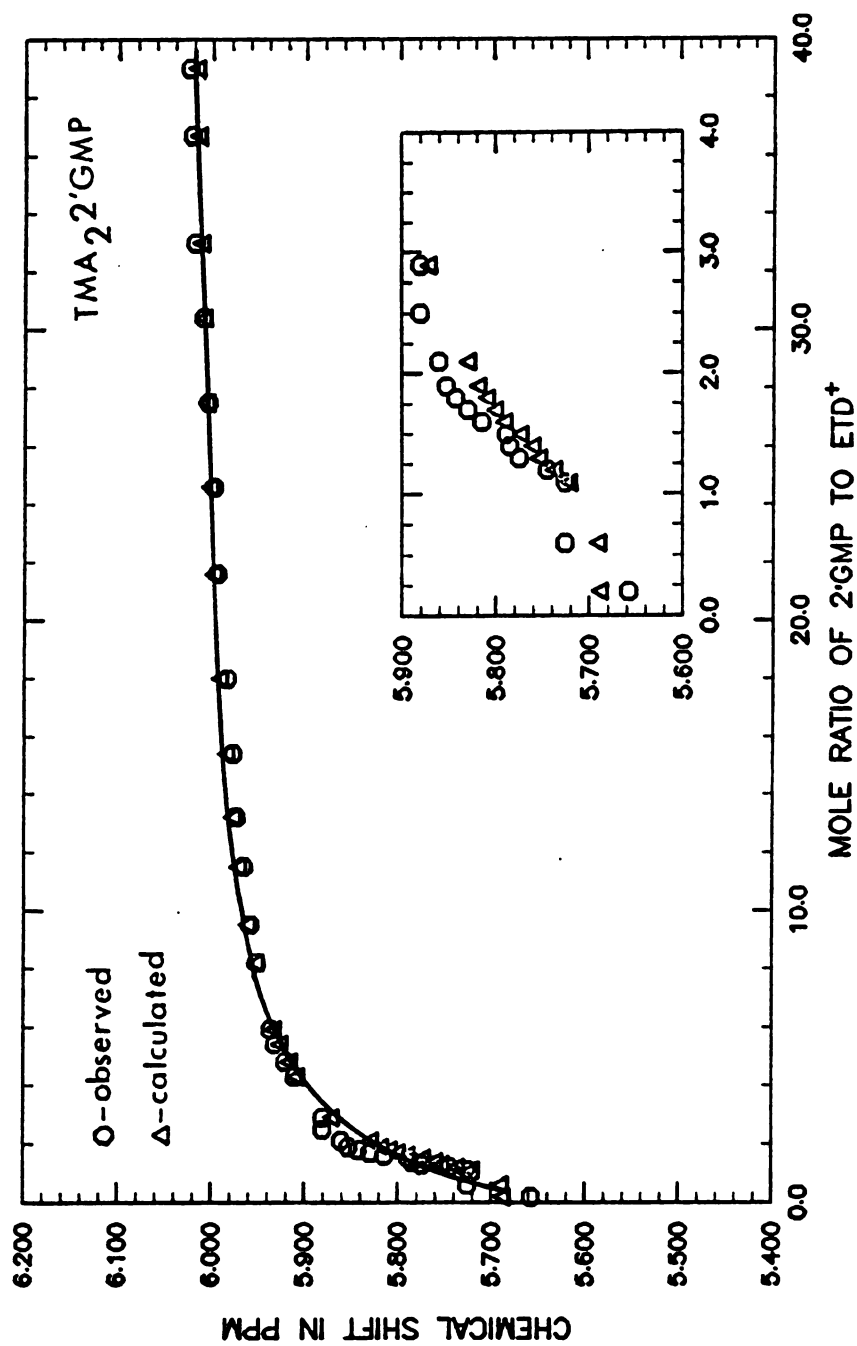


Figure 63. The best computer fit of the H(1') resonance of the binding of ethidium to TMA₂'GMP at 0°C. The concentration of ethidium is 0.020M.

solve these equations due to the correlation between variables. Therefore no further attempt was made to fit the ^1H NMR resonances of the nucleotide.

The value of K_3 of $\geq 1.0 \text{ M}^{-1}$ implies that the fit of the drug resonance is questionable. The $[\text{M}]$ used in the calculations is greater than the actual concentration of free monomer. A simplified explanation is that the stacking of nucleotide with itself reduces the number of nucleotide faces that the drug can bind to. Therefore it is important to note that the values of K_1 and K_2 calculated did not take into account the stacking of nucleotide with itself.

The chemical shifts of the ethidium-TMA₂2'GMP system were studied as a function of temperature to determine the enthalpies of reaction. From the Gibbs-Helmholtz equation the following equations are derived:

$$K_1 = K_1^{\text{ref}} \exp\{(-\Delta H_1/R)(\frac{1}{T_1} - \frac{1}{T_{\text{ref}}})\} \quad (20)$$

$$K_2 = K_2^{\text{ref}} \exp\{(-\Delta H_2/R)(\frac{1}{T_1} - \frac{1}{T_{\text{ref}}})\} \quad (21)$$

The results obtained are listed in Table 11. Substitution into Equation (22) yields,

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (22)$$

the following thermodynamic parameters:

Table 11. The Best Computer Fit of the Thermodynamic Parameters of Ethidium Binding to TMA₂2'GMP.

$$\Delta H_1 = -19.78 \pm 0.96 \text{ Kcal/mole}$$

$$\Delta H_2 = 0.07 \pm 0.26 \text{ Kcal/mole}$$

$$\delta_{MD}^M = 1.232 \pm 0.002 \text{ ppm}$$

$$\delta_{M_2D}^D = 1.428 \pm 0.002 \text{ ppm}$$

$$\Delta S_1 = -53.3 \pm 6.6 \text{ eu}$$

$$\Delta S_2 = 3.9 \pm 1.6 \text{ eu}$$

$$\Delta G_1^\circ(273^\circ\text{K}) = -5.2 \pm 0.8 \text{ Kcal/mole}$$

$$\Delta G_2^\circ(273^\circ\text{K}) = -0.99 \pm 0.04 \text{ Kcal/mole}$$

These thermodynamic parameters are concentration derived. No attempt was made to include activity coefficients due to the large concentrations and ionic strength ranges. It should also be noted that this procedure assumes δ_{MD}^M and $\delta_{M_2D}^D$ are temperature independent, a good assumption over the temperature range studied.

B. Ethidium Bromide Binding to Structured Forms of Guanylic Acids

The chemical shift of the methyl resonance of ethidium cation as a function of mole fraction of $\text{Na}_2 2'\text{GMP}$ added is shown in Figure 64. All solutions had a $[\text{Na}^+]/[2'\text{GMP}]$ ratio of 3.28, obtained by the addition of $\text{NaCl}_{(s)}$ to allow structure formation to occur at a lower concentration of nucleotide. The plot can be divided into four regions, region 1 from a $2'\text{GMP}/\text{Etd}$ ratio of 0-1; region 2 from 1-11, region 3 from 11-30, and region 4, from 30-40. The chemical shift of the methyl resonance of ethidium as a function of mole fraction of $\text{Na}_2 5'\text{GMP}$ (cf. Figure 65), $\text{K}_2 2'\text{GMP}$ (cf. Figure 66), and $\text{K}_2 5'\text{GMP}$ (cf. Figure 67) show the same behavior, but at different ratios of guanylic acid to ethidium. Region 1 and 2 can be attributed to drug binding to un-ordered nucleotide and region 3 and 4 can be attributed to drug binding to structured nucleotide. The onset of structure formation coincides with the downfield shift at $2'\text{GMP}/\text{Etd} > 10$. The addition of ethidium to a structured solution of $\text{Na}_2 2'\text{GMP}$ causes a small increase in the amount of self-structure present (cf. Figure 68). The increase in concentration of solution self-structure for $\text{Na}_2 5'\text{GMP}$ on the addition of ethidium has been reported by Marshall⁹⁷. As seen in Figure 64 and Figure 66, increasing the temperature of both a $\text{Na}_2 2'\text{GMP}$ solution to which $\text{NaCl}_{(s)}$ is added so a ratio of $[\text{Na}^+]/[2'\text{GMP}] = 3.28$ is obtained, and a

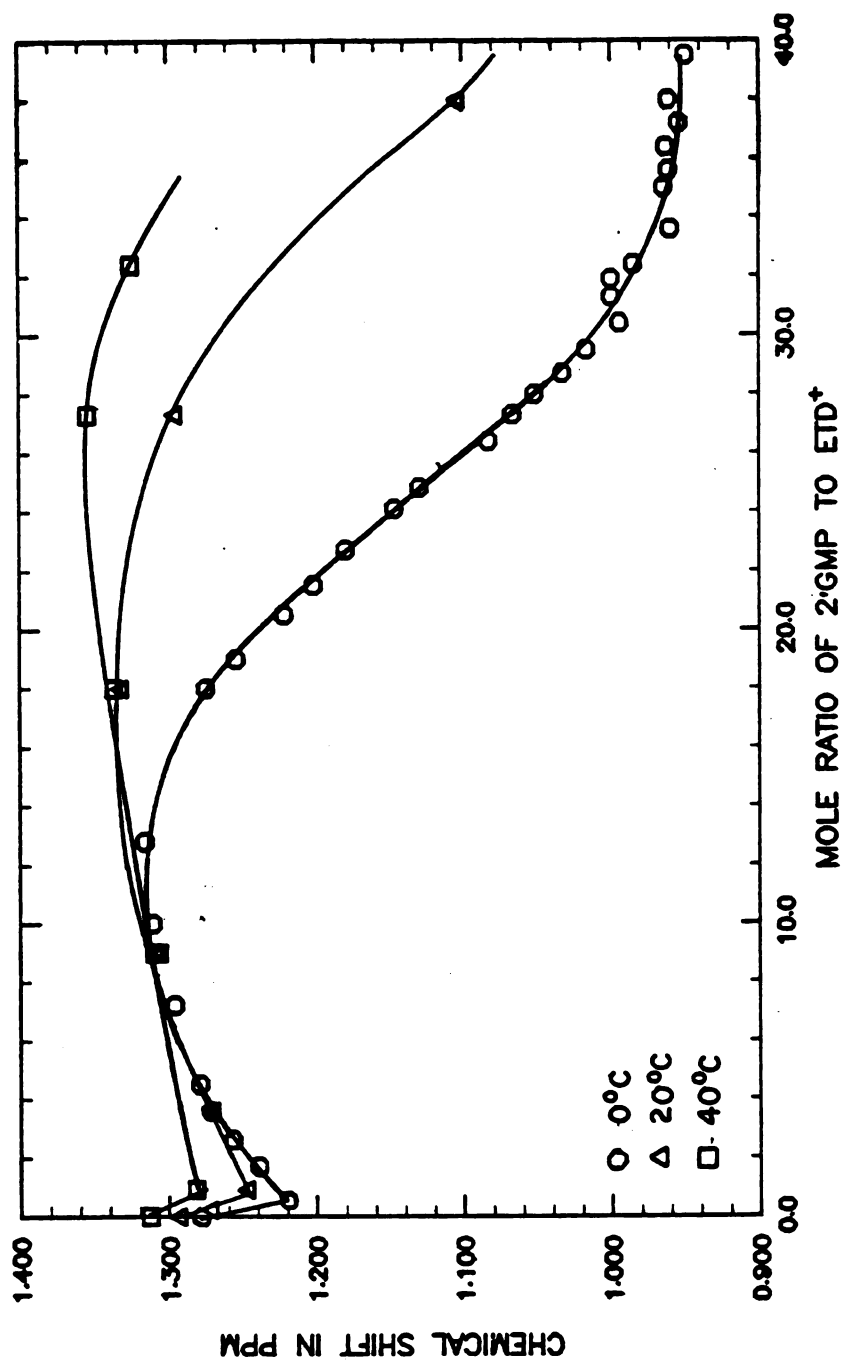


Figure 64. The ethidium methyl chemical shift versus the mole ratio of Na₂2'GMP to ethidium at various temperatures. The solution was made to have a mole ratio of Na⁺/2'GMP of 3.28 by the addition of NaCl.

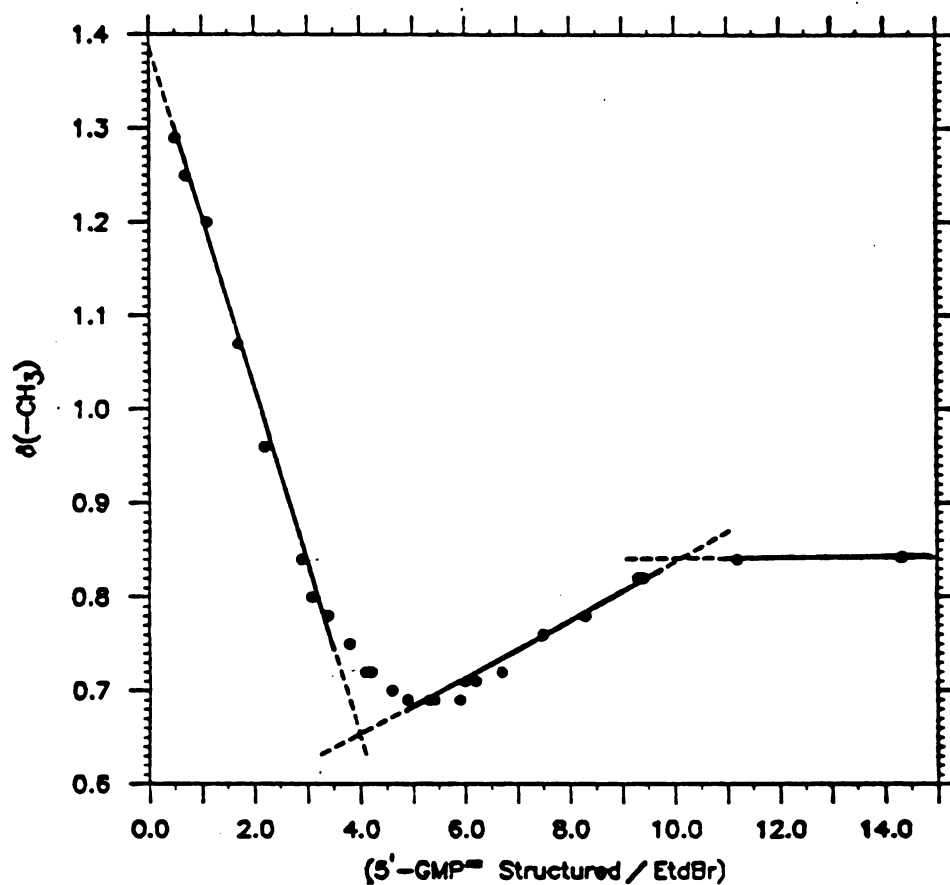


Figure 65. Plot of the chemical shift of the EtdBr methyl resonance as a function of the structured Na_25' -GMP/Drug ratio. Extrapolation of the linear portions of the curve included. Taken from Reference 97.

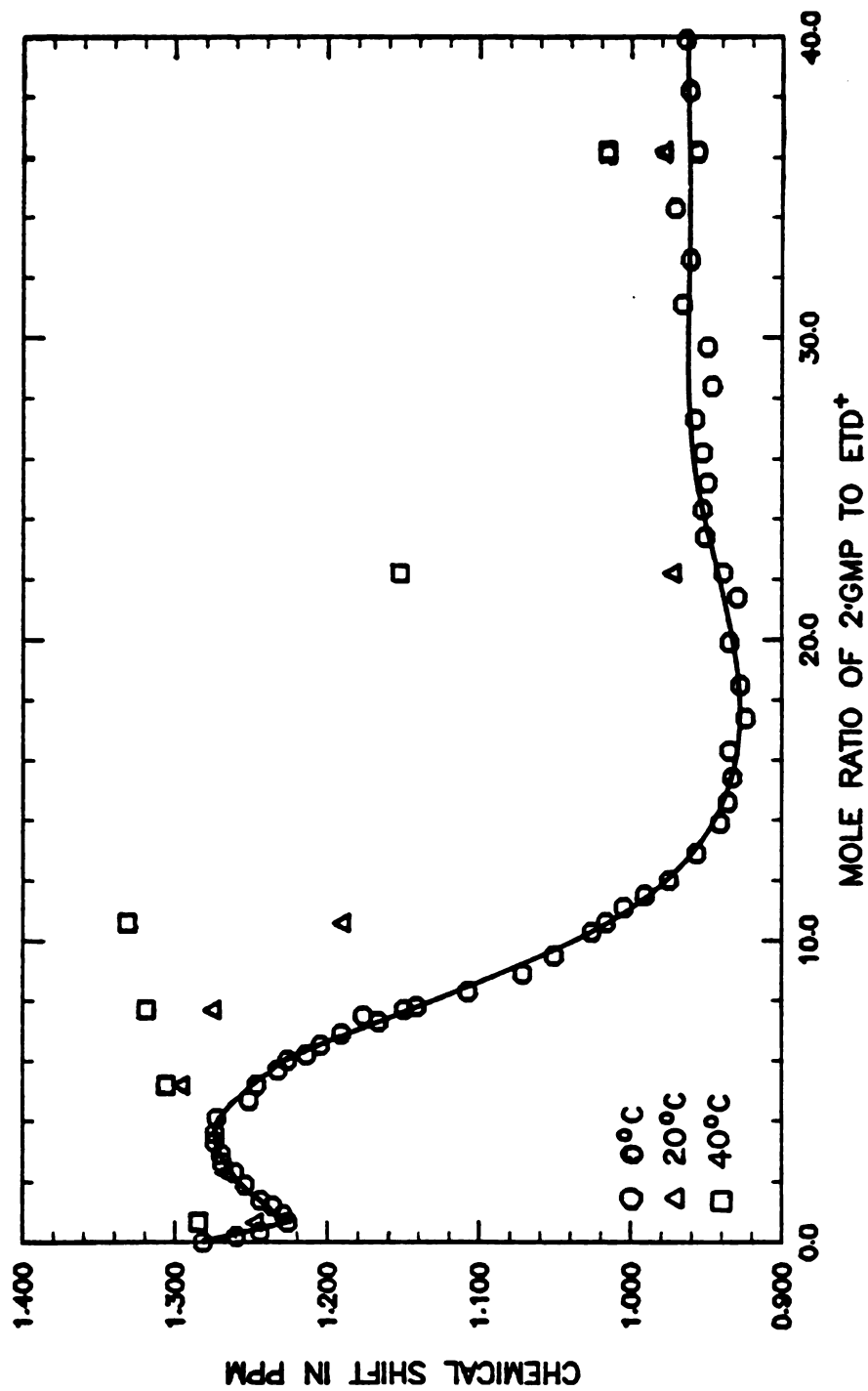


Figure 66. The ethidium methyl chemical shift versus the mole fraction of K₂2'GMP to ethidium at various temperatures. The ethidium concentration was 0.020M.

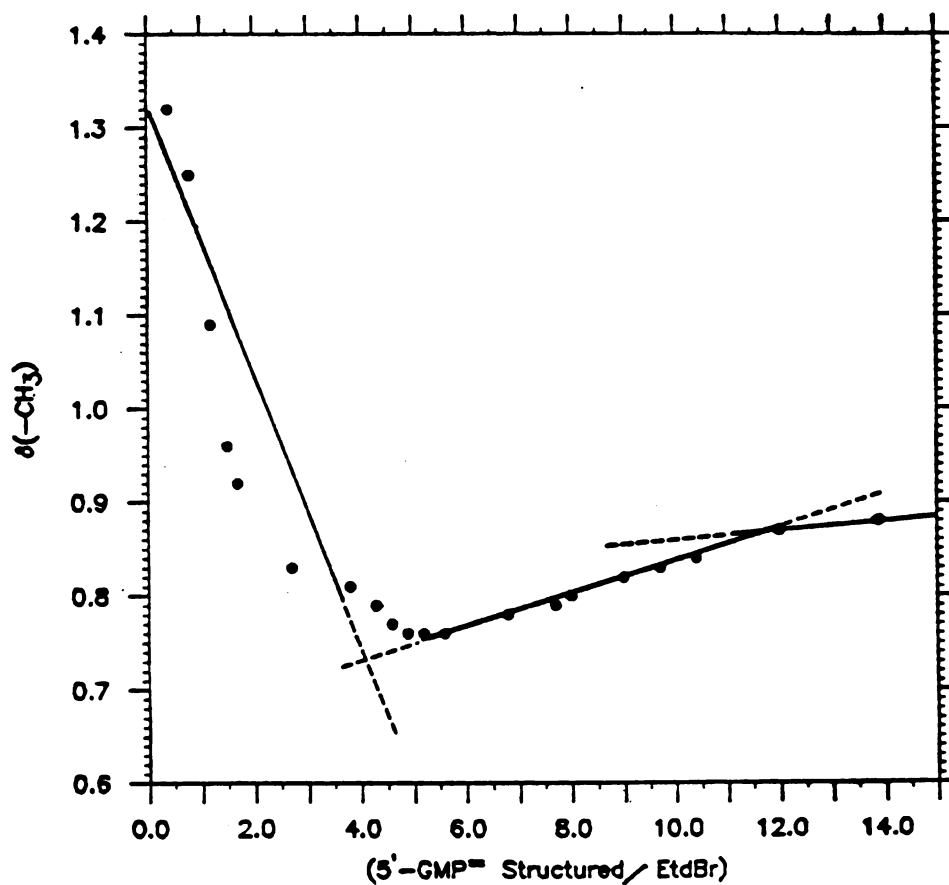


Figure 67. Plot of the chemical shift of the EtdBr methyl resonance as a function of the structured K_25' -GMP/Drug ratio. Extrapolation of the linear portions of the curve included from Reference 97.

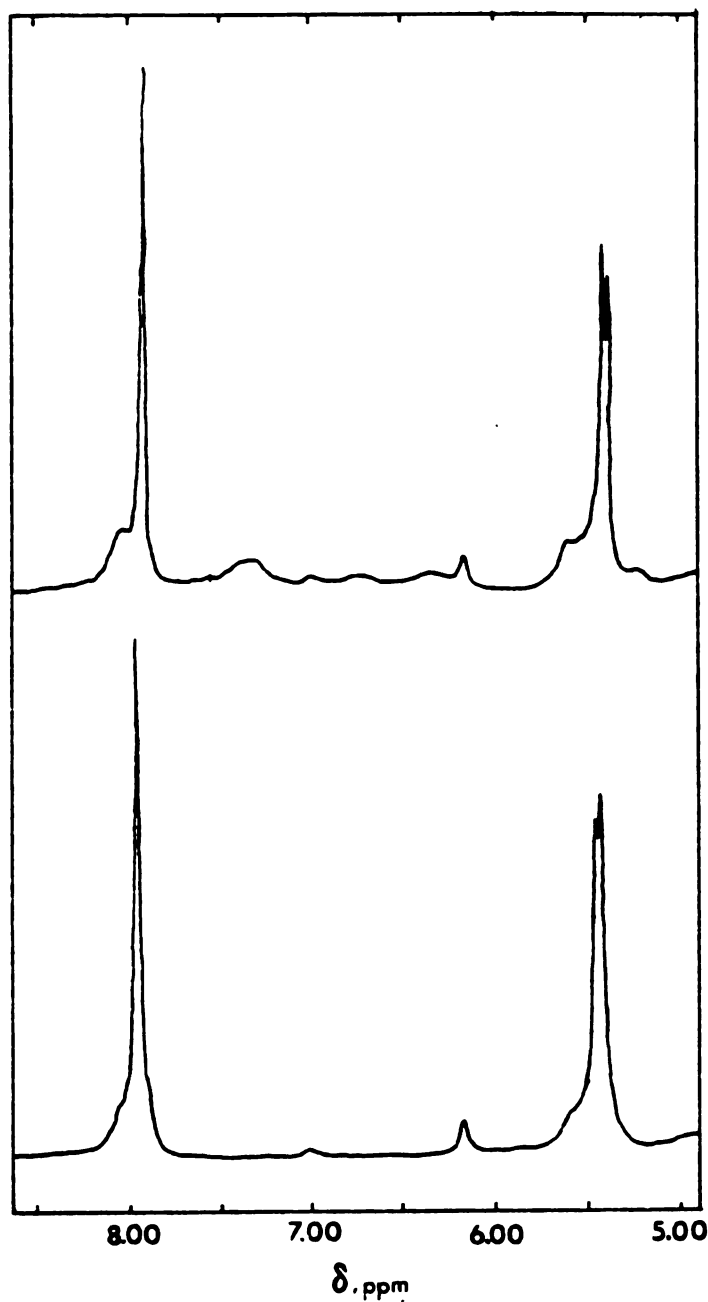
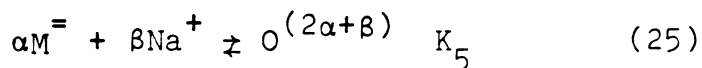
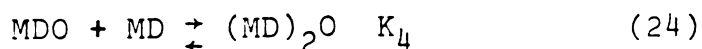


Figure 68. The effect on the solution self-structure of 0.72M $\text{Na}_2 2'\text{GMP}$ upon making the solution 0.020M in ethidium bromide at 0°C . The nucleotide had a ratio of $\text{Na}^+/2'\text{GMP}$ of 3.28 accomplished by the addition of NaCl. Figure A is the NMR spectrum of ethidium absent system. Figure B is the NMR spectrum on making the solution 0.020M in ethidium. Note the increase in the H(8) and H(1') solution self-structure resonances.

K_2 2'GMP solution causes a decrease in the concentration of self-structure. This decrease in solution self-structure causes a general downfield shift of the ethidium methyl resonance. At temperatures where the solution self-structure is melted out, the ethidium methyl resonance as a function of the ratio of guanylic acid/ethidium is the same as for a non-structured TMA guanylic acid solution.

The binding of drug to ordered nucleotide in the presence of disordered nucleotide can be described as



where O is the structured form of nucleotide, α is the number of monomers and β is the number of Na^+ ions in the structured unit.

This model of drug binding to structured solutions of guanylic acids was chosen because of the large equilibrium constant of the 1:1 unstructured nucleotide drug complex (cf. Table 9). Species such as DO and D_2O may exist, but

they are assumed to be minor species. The concentration of monomer present is always very large compared to that of structured nucleotide. The values for α and β were taken as 8 and 4, respectively, based on the similarities between the $\text{Na}_2 2'\text{GMP}$ and $\text{Na}_2 5'\text{GMP}$ self-structures. Most evidence to date suggests a stoichiometry of Na_4M_8 for the solution self structure^{97,98}.

The total concentration of ethidium can be expressed by,

$$c_D^t = [D] + [MD] + [M_2D] + [MDO] + 2[(MD)_2O] \quad (26)$$

Substitution of the equilibrium expressions of Equations (3) + (4) find (23) - (25) yields;

$$\begin{aligned} c_D^t = [D] + K_1[M][D] + K_1K_2[M]^2[D] + K_1K_3K_5[M][M]^\alpha[Na^+]^\beta[D] \\ + 2K_1^2K_3K_4K_5[M]^2[M]^\alpha[Na^+]^\beta[D]^2 \end{aligned} \quad (27)$$

The total concentration of nucleotide can be expressed by

$$\begin{aligned} c_M^t = [M] + [MD] + 2[M_2D] + (\alpha+1)[MDO] + (\alpha+2)[(MD)_2O] \\ + \alpha[O] \end{aligned} \quad (28)$$

Substitution of the equilibrium expressions of Equations (3)

+ (4) and (23) - (25) yields;

$$\begin{aligned}
 c_M^t &= [M] + K_1[M][D] + 2K_1K_2[M]^2[D] + ((\alpha+1)K_1K_3K_5) \\
 &([M][M]^\alpha[Na^+]^\beta[D]) + (\alpha+2)K_1^2K_3K_4K_5[M]^2[D]^2[M]^\alpha[Na^+]^\beta \\
 &+ \alpha K_5[M]^\alpha[Na^+]^\beta
 \end{aligned} \tag{29}$$

Since the reactions are fast on the NMR time scale the observed chemical shift can be expressed as,

$$\delta_{obs}^D = x_D \delta_D^D + x_{MD} \delta_{MD}^D + x_{M_2D} \delta_{M_2D}^D + x_{MDO} \delta_{MDO}^D + x_{(MD)_2O} \delta_{(MD)_2O}^D \tag{30}$$

The mole fractions of the species containing drug can be expressed through the use of Equations (31) - (35) as;

$$x_D = [D]/c_D^t \tag{31}$$

$$x_{MD} = K_1[M][D]/c_D^t \tag{32}$$

$$x_{M_2D} = K_1K_2[M]^2[D]/c_D^t \tag{33}$$

$$x_{MDO} = K_1K_3K_5[M][M]^\alpha[Na^+]^\beta[D]/c_D^t \tag{34}$$

$$x_{(MD)_2O} = 2K_1^2K_3K_4K_5[M]^2[M]^\alpha[Na^+]^\beta[D]^2/c_D^t \tag{35}$$

The mass balance equations (27) - (29) are solved for [D] and [M], by using the same procedure as was used in the TMA₂2'GMP case with ethidium binding. In order to solve for [M]^α, it is set equal to [M_{est}]^α, an estimated value of [M] which is updated with each new value of [M] calculated. The [Na⁺] is arbitrarily set equal to [Na⁺]_{total}/3.0 and updated on each iteration with the correct equation for [Na⁺],

$$[\text{Na}^+] = ([\text{Na}^+]_{\text{T}} - \beta([\text{O}] + [\text{DMO}] + [(\text{MD})_2\text{O}]))^\beta \quad (36)$$

The values of K_1 , K_2 , $\delta_{\text{MD}}^{\text{D}}$, and $\delta_{\text{M}_2\text{D}}^{\text{D}}$ were assumed equal to those calculated for TMA₂2'GMP - ethidium binding.

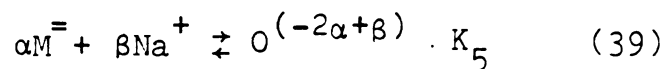
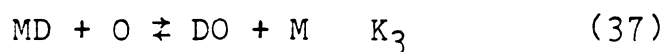
The results of the best computer fit are presented in Table 12.

Table 12. The Best Computer Fit of the Binding Constants for the Binding of Ethidium to Na₂2'GMP Self-Structures.

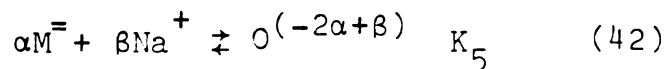
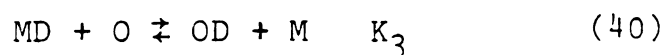
K_3	$= (1.91 \pm 0.10) \times 10^4 \text{ M}^{-1}$
K_4	$= (5.8 \pm 0.6) \times 10^2 \text{ M}^{-1}$
K_5	$= 0.122 \pm 0.007 \text{ M}^{-11}$
$\delta_{\text{MDO}}^{\text{D}}$	$= 0.935 \pm 0.006 \text{ ppm}$
$\delta_{(\text{MD})_2\text{O}}^{\text{D}}$	$= 1.200 \pm 0.020 \text{ ppm}$

As described previously these are concentration equilibrium constants. The best computer fit chemical shifts and the experimental chemical shifts are plotted in Figure 69.

Other models which were tested for ethidium binding to ordered 2'GMP are given below:



and



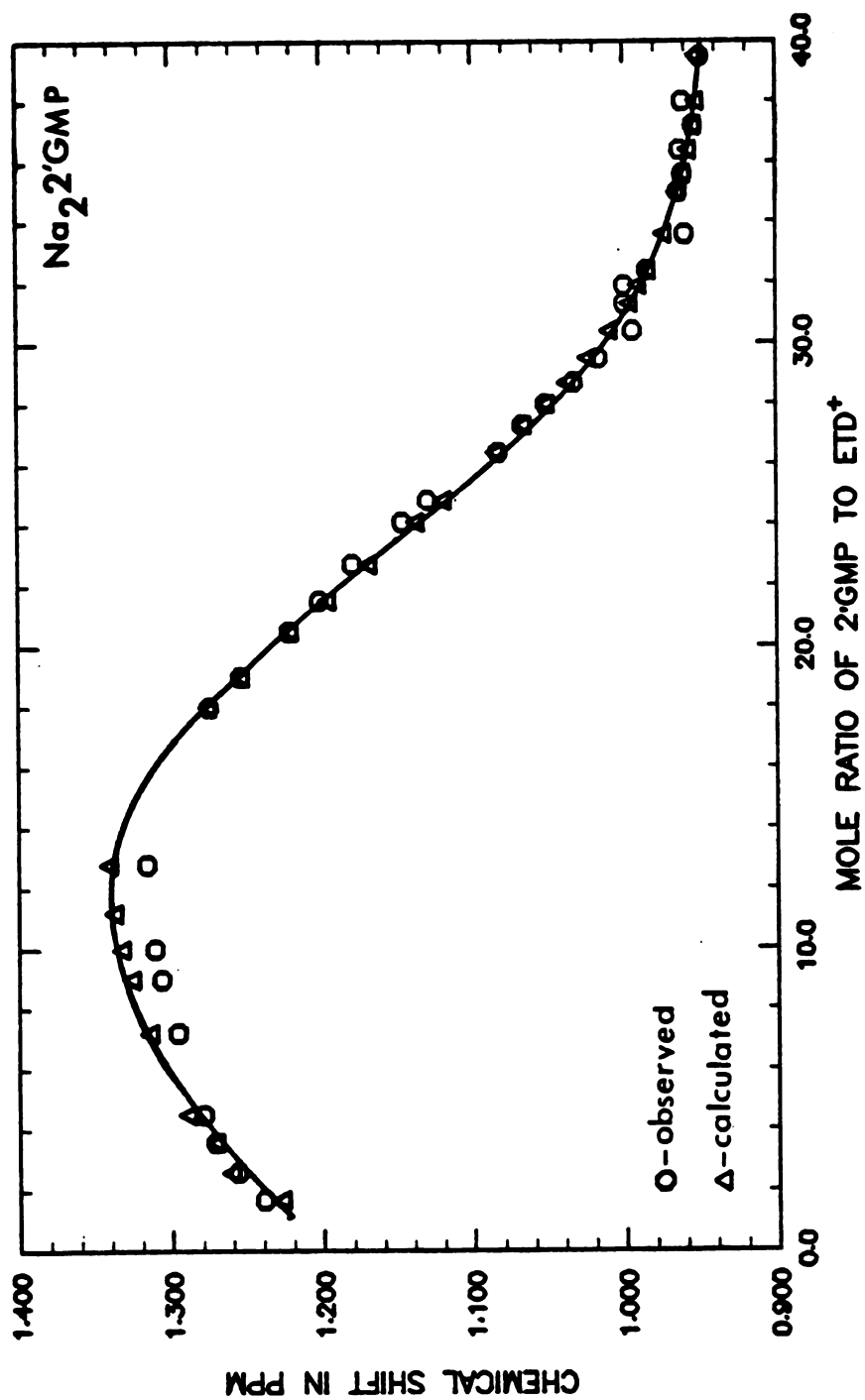


Figure 69. The best computer fit of the ethidium methyl resonance to the binding of ethidium to Na₂2'-GMP at 0°C. The Na⁺/2'-GMP ratio was made 3.28 by the addition of NaCl.

The values of α and β were again taken as 8 and 4, respectively. A computer fit could not be obtained for either model. Calculated chemical shift values were diverging from the extrapolated values and the calculated concentrations of species did not correspond to the integral intensities determined by NMR.

A satisfactory computer fit to any model has not been obtained for the binding of ethidium to K_22' GMP. Marshall's⁹⁷ model of ethidium binding to solution self-structured Na_25' GMP and K_25' GMP does not take into account the binding of ethidium to monomeric $5'$ GMP. The work presented here for the binding of ethidium to TMA_25' GMP proves that binding to monomeric $5'$ GMP is not negligible. No satisfactory fits were obtained for several models of drug binding to structured forms of $5'$ GMP in the presence of unstructured forms of $5'$ GMP.

VI. NMR Spectra of Guanylic Acids in ^1H_2O

A. NMR Spectra in ^1H_2O

A wealth of information can be obtained from NMR spectra of guanylic acids taken in ^1H_2O solutions. Protons which exchange with solvent water can be observed if the exchange is slow. Under conditions of structure formation, the imide protons can be observed. The NH region shows a unique multiline pattern which can be used in conjunction

with the H(8) lines to create a picture of the self-structure formed. By comparing the number of hydrogen bonded NH protons with the number of structured H(8) protons, insight into the number of hydrogen bonds per structured nucleotide should be attainable. At about 6.3 ppm in $^1\text{H}_2\text{O}$ at 0°C , the GMP^- monomer is known to exhibit an amino proton signal which shifts to about 6.5 ppm on hydrogen bonding with $5'\text{GMP}^{134}$. The hydroxide protons have also been observed in nucleotide solutions in $^1\text{H}_2\text{O}^{131}$. Problems occur in distinguishing between NH and OH protons. Chemical shifts of OH protons usually occur less than 6.0 ppm downfield from TSP; they have, however, been reported to have shifts as large as 7.2 ppm in cyclic nucleotide systems¹³¹.

Iwahaski and Kyogoku^{136,137} have used the saturation transfer technique to elucidate the sites and rates of exchangeable nuclei in nonaqueous solvents. In this technique a strong radiofrequency is applied continuously to the frequency of one resonance during the experiment. If this irradiated nuclei exchanges with another, the magnetization is also transferred, causing a decrease in integrated intensity. Saturation transfer experiments were performed on a 0.62M $\text{Na}_25'\text{GMP}$ solution in $^1\text{H}_2\text{O}$ at 2°C . The proton NMR spectrum of 0.62M $\text{Na}_25'\text{GMP}$ downfield from 5.0 ppm in $^1\text{H}_2\text{O}$ at 2°C is shown in Figure 70. The results of the saturation transfer experiment are given in Table 13. The resonances are labeled according to Figure 70.

Table 13. Percent Decrease in Signal Intensity on Irradiation of Other Proton Signals of a 0.62M Na₂S₂O₅ Solution in D₂O at 2°C.

$$\frac{I_0 - I_d}{I_0} \times 100^a$$

Resonance Irradiated	Imide Region ^b			H(8) Region ^b						
	NH(1)	NH(2)	NH(3)	NH(4)	α	β	γ	δ	ϵ	NH ₂
NH(1)	--	44	13	-3	7	0	-7	-14	19	22
NH(2)	50	--	12	10	-9	-7	-10	1	0	-4
H(8) α	20	8	4	5	--	10	-30	4	-10	2
H(8) β	-4	11	-18	-20	14	--	59	16	9	7
H(8) γ	6	6	5	-12	-8	62	--	-11	-14	-5
H(8) δ	2	-24	-21	-20	9	19	22	--	10	9
H(8) ϵ	2	-13	31	7	20	10	-1	5	--	5
NH ₂	17	-16	-5	10	-9	-15	-29	1	0	--
H ₂ O	100	71	91	91	82	27	32	86	71	87

^a I_0 is the integral intensity with the decoupler off and I_d is the integral intensity with the decoupler on.

^b Labeling scheme for the various resonances as defined in Figure 60.

^c The errors in intensity loss are estimated to be ± 15 .

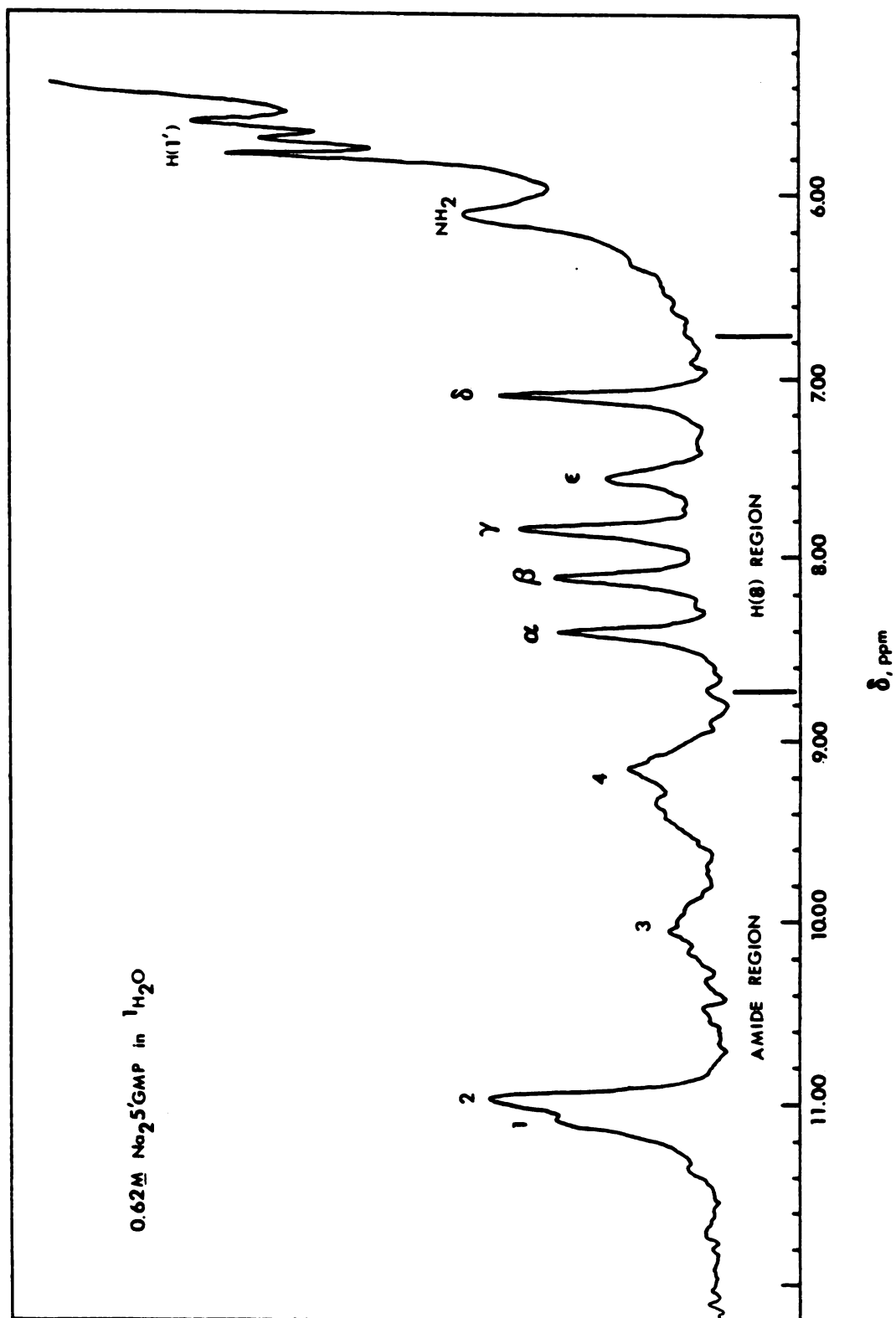


Figure 70. The proton NMR spectrum of 0.62M Na₂5'GMP in ¹H₂O at 2°C downfield from 5.00 ppm.

Four resonances are present downfield from 9.00 ppm which are not present in samples prepared in D_2O . These resonances are attributed to N(1)H protons. Three of the resonances are similar in chemical shift and intensity to the N(1)H protons of $Na_2 2'GMP$ shown in Figure 22. A new resonance is present in the H(8) region of the spectrum (7.55 ppm, ϵ). This resonance at 7.55 ppm is sometimes present in spectra taken in D_2O , but the intensity is greatly decreased. Fisk and coworkers⁹⁸ have attributed the resonance which is seen in D_2O to a H(8) proton of a less stable solution self-structure H(8).

Since the ϵ resonance is similar in chemical shift to the 2'OH resonances which have been observed hydrogen bonded to H_2O in cyclic nucleotides¹³¹, this resonance at 7.55 ppm may be due to the 2'OH or 3'OH group. Based on the probable difference in relaxation rates between an amino and a hydroxyl proton, a relaxation experiment was done by Brown⁴⁹. Inversion recovery T_1 experiments revealed that very small differences exist between T_1 values for all resonances in this region, and thus the results were inconclusive. As shown in Figure 71, a 0.43M 8-d $Na_2 5'GMP$ solution in ^1H_2O at 2°C does contain the resonance at 7.55 ppm. The resonance is therefore not exclusively an H(8) resonance. The other four resonances between 7.00 ppm and 9.00 ppm are exclusively H(8) resonances. They have the same chemical shifts and intensities as do solutions run

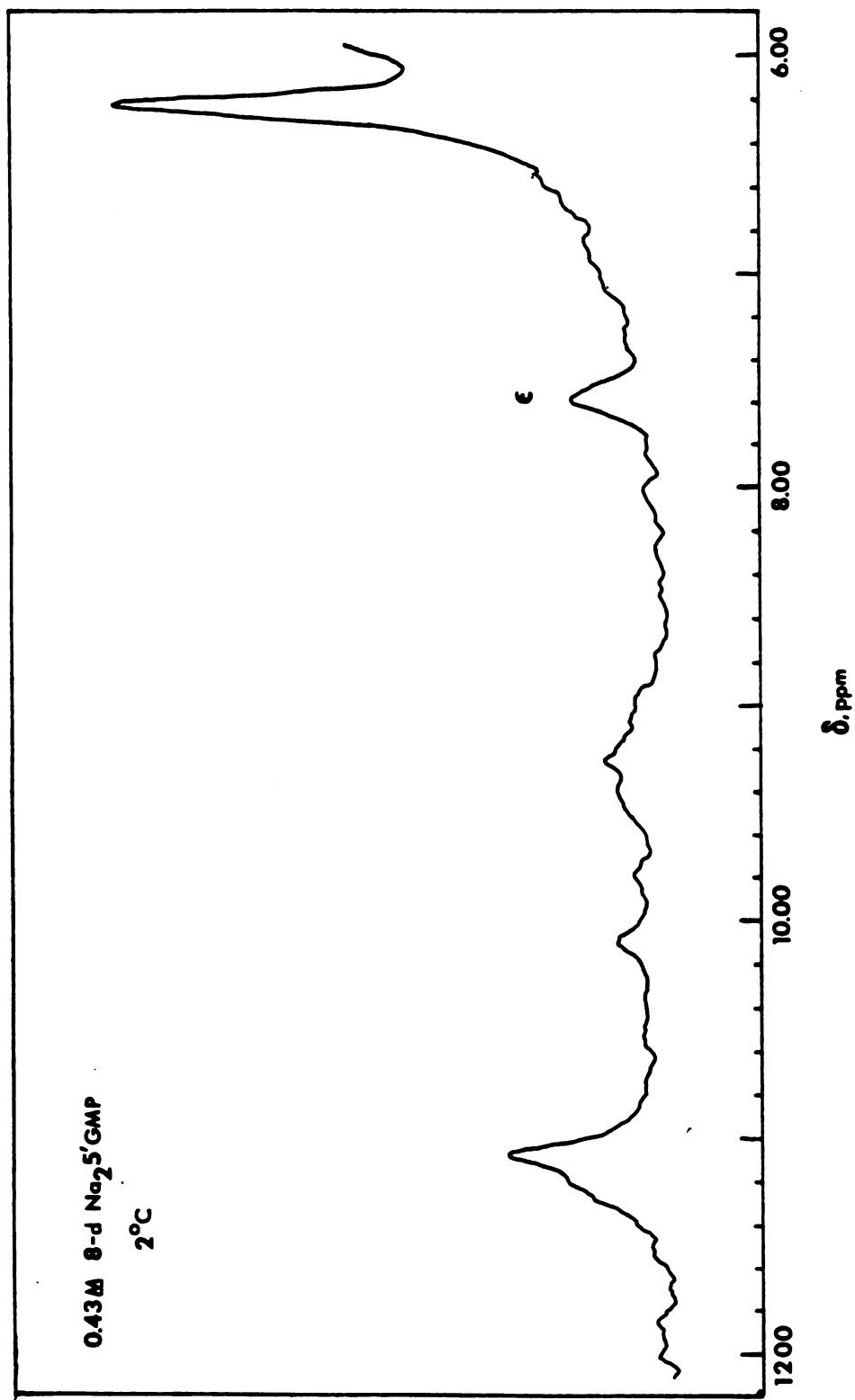


Figure 71. The proton NMR spectrum downfield of 6.00 ppm of a 0.43M 8-d Na₂ 5'GMP solution in ¹H₂O at 2°C.

in D_2O .

The 5'GMP resonance at 6.10 ppm is also present in 2'GMP. For both nucleotides the resonance is absent in D_2O solutions. This line is attributed to the amino protons. The addition of ^1H_2O to a 2'GMP solution causes the 6.10 ppm resonance to increase in intensity. After a TMA₂2'GMP sample is heated to 90°C to allow exchange of H(8) to occur with the mixed D_2O - ^1H_2O solvent, the integral intensity of the resonance relative to the H(8) line intensities is approximately 2. Repeating the addition of ^1H_2O to the sample and heating yields a relative integral intensity of 2 for the amino proton. The resonance is positioned in the region of the nucleotide $-NH_2$ groups. The resonance is not observed in mixed $^1H_2O/D_2O$ solutions of Na₂'(3') Inosine monophosphate (IMP; IMP is identical to guanosine except the NH_2 group is absent). The novel characteristics of this resonance in the presence of alkali metal ions are described in detail in the following section.

As shown in Table 13, the irradiation of the ^1H_2O resonance in 0.62M Na₂5'GMP in ^1H_2O causes a decrease in the intensity of all resonances downfield from 6.00 ppm. Very large intensity decreases occur for the amino protons line at 6.10 ppm, for all of the resonances downfield from 9.00 ppm, and the resonance at 7.55 ppm. All of these lines are not present in D_2O solutions. Therefore, the decrease in intensity of these lines upon irradiation of the H_2O

resonance can be attributed to rapid exchange with the solvent.

The outer H(8) resonances (i.e., the resonance at 8.22 ppm and 7.12 ppm) are also greatly decreased in intensity, when the H₂O line is irradiated. These H(8) protons cannot be exchanging with H₂O protons at this temperature. Therefore, this decrease in the intensity upon irradiation of the water resonances is attributed to a negative Nuclear Overhauser effect. Water must therefore be located very close to the H(8) proton in these structures, more so than in the monomer and the other structure which gives rise to the H(8) resonance at 8.10 ppm. As mentioned previously, water is necessary for self-structure formation.

Returning to the data in Table 13, we may note that the irradiation of NH(1) causes a 44% decrease in the intensity of the NH(2) resonance, but little change occurs in the intensity of the NH(3) or NH(4) resonances. Irradiation of NH(2) causes a 50% decrease in NH(1) and a small change in the intensities of NH(3) and NH(4) resonances. This implies the structures giving rise to NH(1) and NH(2) are exchanging faster with each other than they are exchanging with the structures giving rise to NH(3) and NH(4). An alternate explanation is the band width of the decoupler is too broad and is irradiating both resonances to some extent giving rise to the results obtained. Irradiation of the resonance at 7.88 ppm (i.e., H(8)γ) causes a large decrease in

the structured H(8) β resonance and a small effect on the other H(8) resonances. Irradiation of H(8) β also causes a large decrease in the H(8) γ intensity with little effect on the other H(8) resonances. This suggests that the structure giving rise to the β resonance exchanges faster with monomer (i.e., the structure giving H(8) γ) than to the structure giving rise to the α and δ resonances. The faster exchange of the structure which gives rise to the β resonance and the monomer is also suggested by the merging of the β and γ resonances which give rise to the χ resonance at conditions where structure is present in lower concentrations. Irradiation of H(8) δ causes a loss of 31% in the intensity of the NH(3) resonance, which suggests that the H(8) δ proton is near the NH(3) proton. This implies they are in the same structure.

Table 14 and Table 15 give the integral intensities in arbitrary units of the resonances downfield of the H₂O resonance for several concentrations of Na₂5'GMP at 2°C and Na₂2'GMP at 1°C, respectively. The arbitrary units are not the same for each concentration. For both nucleotides, as the concentration is increased there is an increase in the intensity of H(8) lines assigned to solution self-structures and in the intensities of the amide proton lines. The decrease in the relative intensity of the amino proton line with increasing concentration is discussed in the next section.

Table 14. Integral Intensity of Resonances in Arbitrary Units^a Downfield from the ¹H₂O Resonance of 5'GMP Solutions in ¹H₂O at 2°C.

Conc. of Na ₂ 5'GMP	NH(1) ^b	NH(2)	NH(3)	NH(4)	H(8)α	H(8)β	H(8)γ	H(8)δ	H(8)ε	NH ₂	H(1')	Total H(8)	NH ₂ H(1')	NH ₂ H(8)	% Struc. ^d
0.62M	62.7	67.7	34.7	83.7	41.0	41.3	48.7	37.3	42.0	e	e	173.0	----	----	72
0.47M	35.6	48.4	24.2	63.8	28.7	31.0	114	31.7	30.7	146	156	204.4	0.94	0.71	44
0.32M	11.2	17.5	----	----	9.9	9.9	60.5	8.5	9.5	92.5	75	89.8	1.23	1.03	33
0.28M	5.7	17.3	----	----	10.6	7.4	66.0	10.1	6.1	100.5	78.5	90.1	1.28	1.07	27
0.24M	2.0	3.0	----	----	3.0	----	63.0	2.0	5.0	99.0	63.5	71.0	1.56	1.39	17
0.16M	---	---	----	----	---	---	59.5	---	---	103.0	56.2	59.5	1.83	1.72	<3
0.12M	---	---	----	----	---	---	30.5	---	---	49.5	29.0	30.5	1.71	1.62	0
TMA 5'GMP															
0.30M	---	---	----	----	---	---	25	---	---	49	24	25	2.0	2.0	0

^aUnits are arbitrary and are not the same for different concentrations.

^bSee Figure 70 for nomenclature of resonances.

^cThe sum of the integral intensities of the α, β, γ and δ resonances.

^dDetermined from H(8) integration.

^eThe resonance was too distorted by the ¹H₂O resonance for accurate integration.

^fErrors are estimated to be ±10%.

Table 15. Integral Intensity of Resonances in Arbitrary Units^a Downfield from the ¹H₂O Resonance of 2'GMP at 1°C.

Conc. of Na ₂ 2'GMP	NH(1) ^b	NH(2)	NH(3)	H(8)1	H(8)2	H(8)3	NH ₂	H(1')	Total H(8)	NH ₂ H(1')	NH ₂ ^c H(8) _T	% Struc. ^d
1.22M	24.0	49.0	38.0	50.3	129.0	10.0	e	117	189.3	-----	-----	31.9%
1.81M	8.2	23.4	21.2	36.3	23.7	7.0	53.0	e	67.0	-----	.79	64.6%
0.86M	-----	-----	-----	-----	63.0	-----	99.5	57.5	63.0	1.73	1.58	0.0
0.70M	-----	-----	-----	-----	32.0	-----	58.0	30.0	32.0	1.93	1.81	0.0
0.39M	-----	-----	-----	-----	72.0	-----	142.0	64.0	72.0	2.21	1.97	0.0
0.15M	-----	-----	-----	-----	33.0	-----	66.0	35.0	33.0	1.89	2.00	0.0
TMA ₂ 2'GMP												
0.69M												
1°C	-----	-----	-----	-----	35.0	-----	71.0	36.0	35.0	1.97	2.02	0.0

^aUnits are arbitrary and are not the same for different concentrations.

^bSee Figure 22 for nomenclature of resonances.

^cThe sum of the integral intensities of the α, β, γ and δ resonances.

^dDetermined from H(8) integration.

^eThe resonance was too distorted by the ¹H₂O resonance for accurate integrations.

^fErrors are estimated to be ±10%.

The resonance attributed to the amide proton in both nucleotides should have an integral intensity equal to the integral intensity of the H(8) structure resonances. The NH resonances are only in the slow exchange region when involved in structure formation. Solutions which do not contain self-structure do not have an NH line(s). The results in Table 16 show that the amide resonances are of larger integral intensity for both nucleotides than the structured H(8) resonances. Therefore, some of the intensity of the resonances is due to another exchanging proton or to another structure(s) which is different from that giving rise to the H(8) structure lines.

The spectra of 1.55M K_22' GMP in ^1H_2O at 1°C and 27°C are shown in Figure 72. The imide resonance(s) are down-field from 8.50 ppm at 1°C, the resonance(s) is very broad. The H(8) resonances are located between 6.70 ppm and 8.40 ppm. At 27°C the spectrum is much more resolved due to a decrease in viscosity. There are several imide resonances present at both temperatures. At 1°C the large imide resonance at 10.7 ppm is most likely composed of several broad resonances. The imide proton resonance is different from that of the sodium system as shown in Figure 70.

B. Amino Proton Exchange

Figure 73 and Figure 74 show the intensities of the amino proton lines relative to the H(8) line intensities

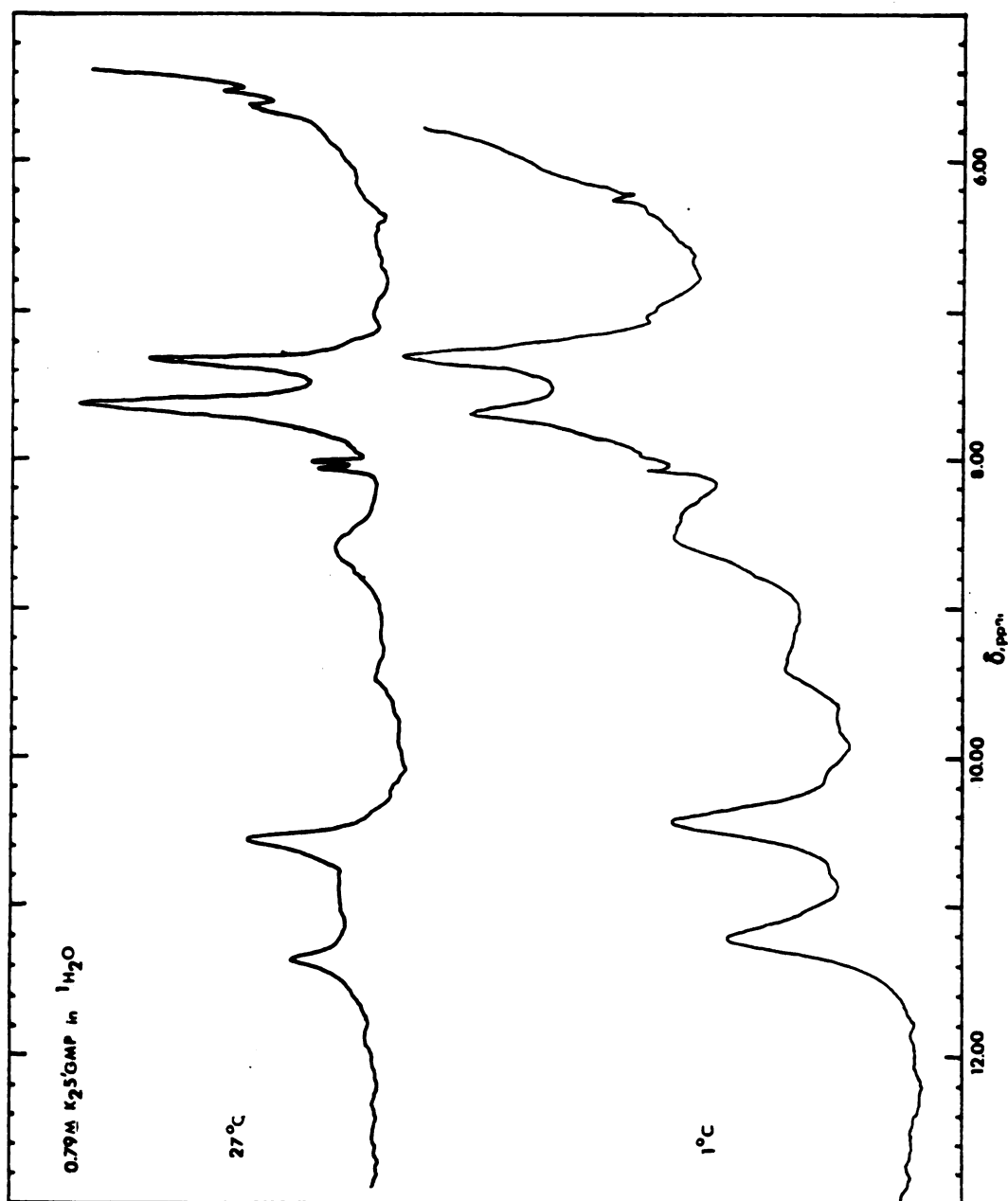


Figure 72. The proton NMR spectra of 1.55M K₂5'GMP in ²H₂O downfield from 5.0 ppm at 1°C and 27°C.

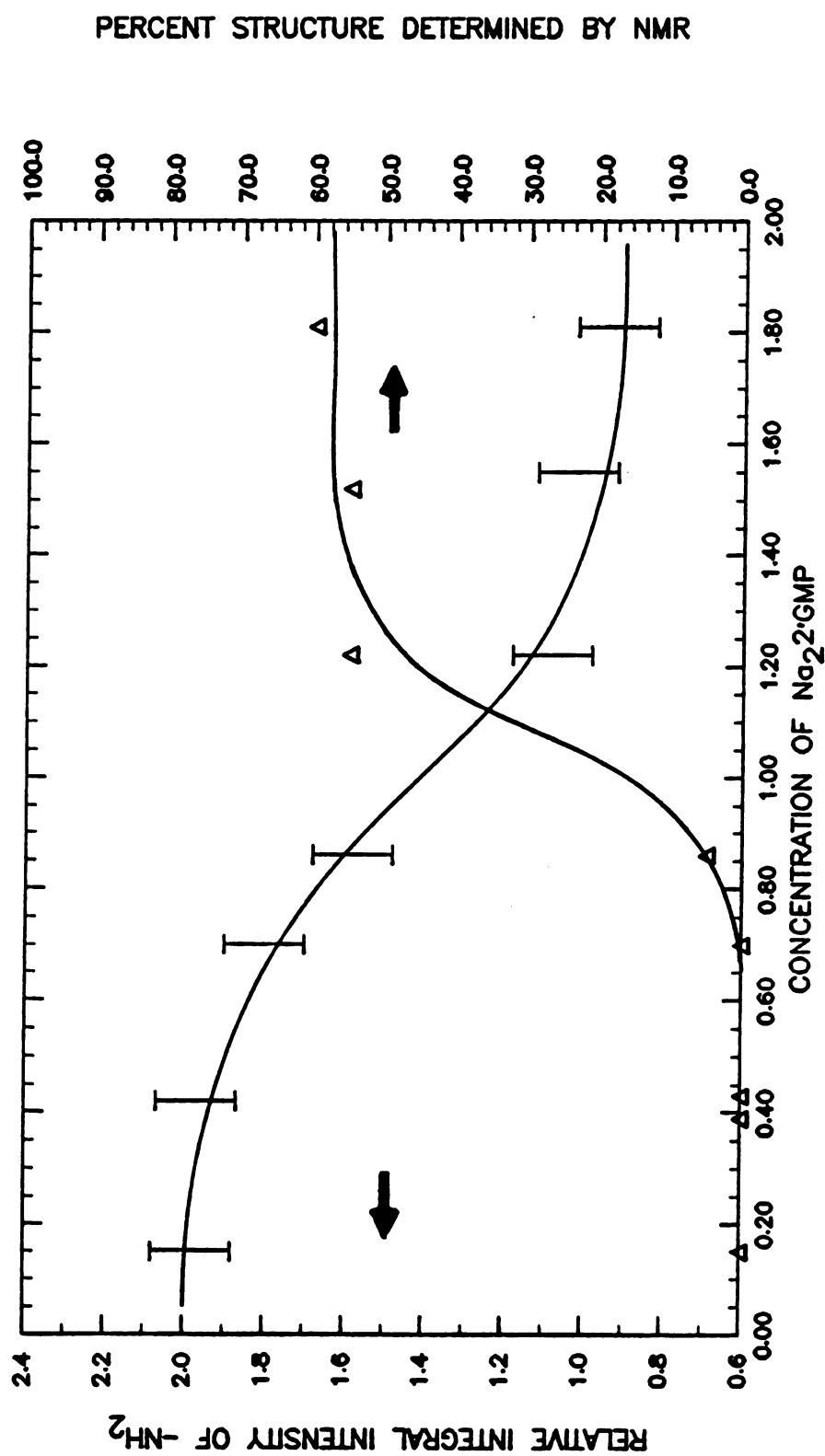


Figure 73. The relative integral intensity of the amino resonance and the % solution self-structure determined by H(8) NMR versus the concentration of $\text{Na}_2\text{2'GMP}$ at 1°C .

PERCENT STRUCTURE DETERMINED BY NMR

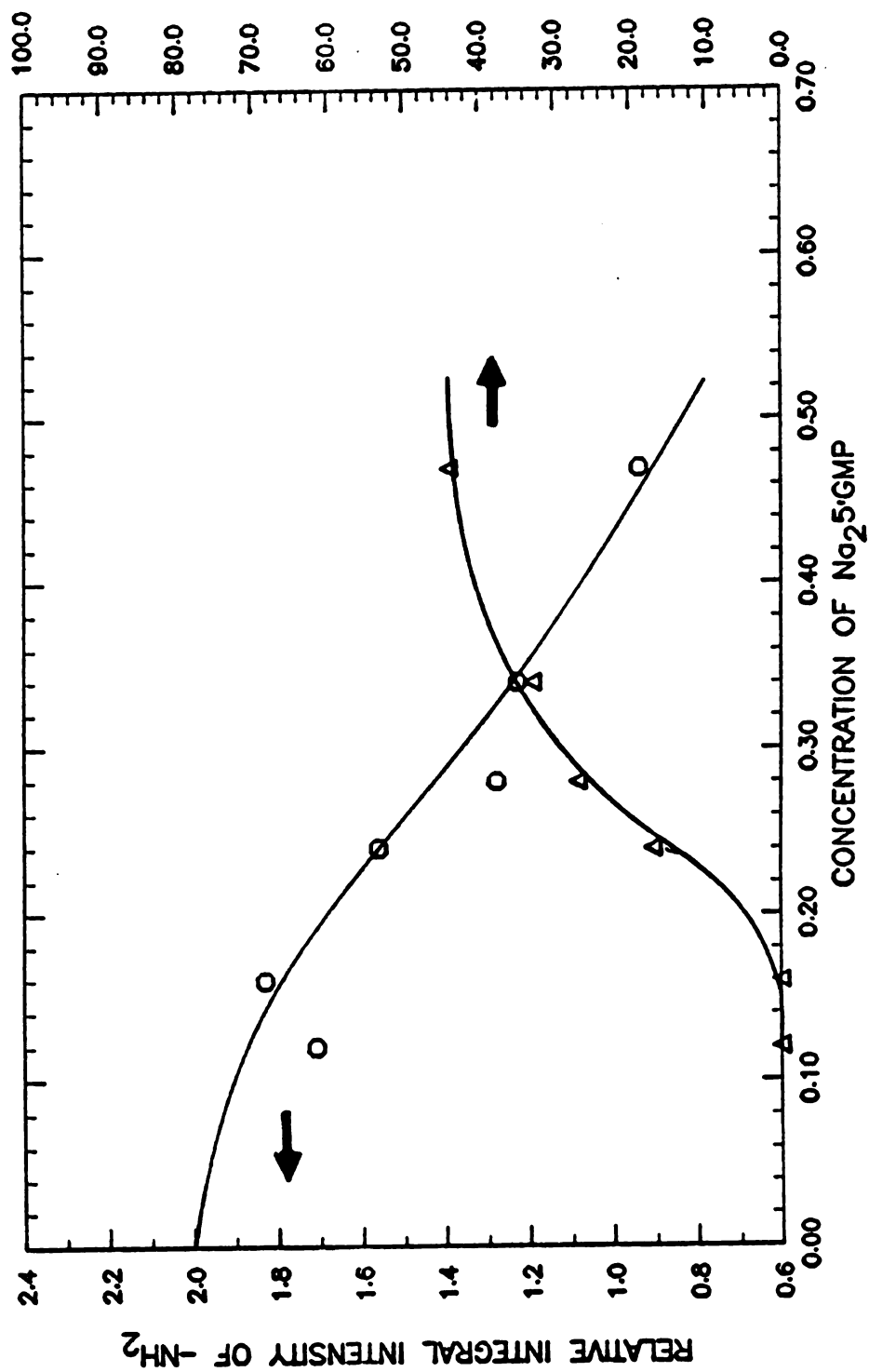


Figure 74. The relative integral intensity of the amino resonance and the % solution self-structure determined by H(8) NMR versus the concentration of $\text{Na}_2 5'\text{GMP}$ at 2°C .

versus concentration for $\text{Na}_22'\text{GMP}$ at 1°C and $\text{Na}_25'\text{GMP}$ at 2°C , respectively. The amount of solution self-structure for each nucleotide is also plotted in the figures. The relative integral intensity varies from 2 to approximately 1 in both $\text{Na}_22'\text{GMP}$ and $\text{Na}_25'\text{GMP}$. Although the amino proton intensity loss parallels structure formation, the onset of amino proton loss occurs at a lower concentration of nucleotide than the onset of structure formation. The relative amino proton intensity approaches a minimum of approximately 1, which in the case of $\text{Na}_25'\text{GMP}$ occurs at about 35% solution self-structure. As shown in Figure 75, heating a 1.81M $\text{Na}_22'\text{GMP}$ solution causes the integral intensity to increase from 1.0 at 0°C to 2.0 at 40°C . The $2'\text{GMP}$ solution self-structure is totally melted out at 27°C as determined by the H(8) resonances. The amino proton relative integral intensity is always found to be 2 in $\text{TMA}_22'\text{GMP}$ or in $\text{TMA}_25'\text{GMP}$ (cf. Table 14 and Table 15).

The amino proton loss can be attributed to the formation of a hydrogen bond, giving rise to a new resonance downfield of the non-hydrogen bonded resonances. Therefore, the proton exchanging with solvent in the H(8) region, H(8) ϵ , occurring at 7.58 ppm in the ordered $\text{Na}_25'\text{GMP}$ solution self-structure or the intensity which cannot be attributed to the imide proton can be a resonance of a hydrogen-bonded amino proton. The relative integral intensities of the ϵ resonance and the integral intensity downfield of

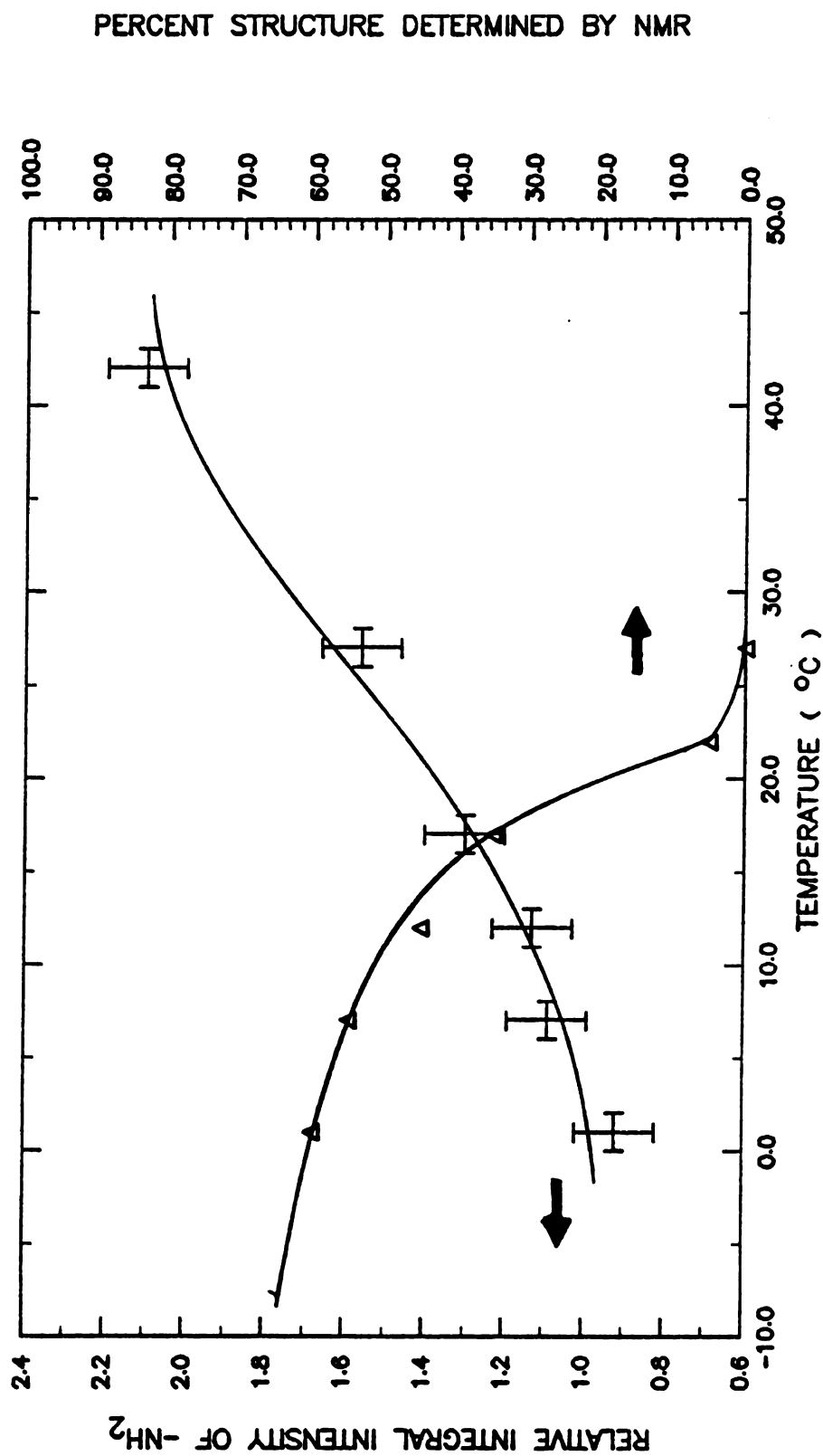


Figure 75. The relative integral intensity of the amino resonance and the % solution self-structure determined by H(8) NMR for 1.81M Na₂'GMP as a function of temperature.

9.0 ppm not attributed to an imide proton resonance are given in Table 16. Neither is correlated with the loss of amino proton intensity loss. The hydrogen-bonded amino proton may occur upfield of the amino group. It would not be seen due to the large water resonance. It is unlikely that the hydrogen-bonded amino resonance would shift upfield since hydrogen bonding usually causes a downfield shift.

It is likely that the same type of phenomenon is occurring with $\text{Na}_2 2'\text{GMP}$. Therefore the hydrogen-bonded amino resonance should occur in both systems. The resonance at 7.58 ppm, $\text{H}(8)\epsilon$, does not occur in $\text{Na}_2 2'\text{GMP}$. This is added proof that the ϵ resonance is not an amino resonance. The resonance is too far upfield for an imide proton, thus it must be a hydroxyl proton hydrogen-bonded in the $\text{Na}_2 5'\text{GMP}$ solution self-structure. It does not occur in the $\text{Na}_2 2'\text{GMP}$ self-structure, because the conformation of the nucleotide is different. This additional hydrogen bond should give the $\text{Na}_2 5'\text{GMP}$ solution self-structure more stability and should account for its larger percent of self-structure at any given set of conditions.

Table 16. A Comparison of the Integral Intensities^a Obtained by Proton NMR of the Amide Protons, H(8), ϵ , and the Amino Protons of Various Nucleotides.

Nucleotide	Total NH Integral Intensity	Integral Intensity of Struct. H(8) ^b	[Total NH] H(8) Struct.	([Total NH]- H(8) Struct.]) H(8) Total ^c	NH ₂ H(8) Total	ϵ H(8) Total	% Struc. ^d
0.62M Na ₂ 5'GMP	248.8	123.4	124.5	0.72		0.22	72
0.47M Na ₂ 5'GMP	172.0	90.4	81.6	0.40	0.71	0.16	44
0.34M Na ₂ 5'GMP	28.7	29.3	-0.6	-0.01	1.03	0.09	33
0.28M Na ₂ 5'GMP	23.0	24.1	-1.1	-0.01	1.07	0.11	27
0.24M Na ₂ 5'GMP	5.0	12.0	-7.0	-0.10	1.39	0.03	17
0.16M Na ₂ 5'GMP	0.0	0.0	0.0	0.00	1.73	0.00	<5
0.12M Na ₂ 5'GMP	0.0	0.0	0.0	0.0	1.62	0.0	<2
1.81M Na ₂ 2'GMP	52.8	43.3	9.5	0.05	0.79	-----	64.6
1.22M Na ₂ 2'GMP	111.0	60.3	50.7	0.76	-----	-----	31.9
0.86M Na ₂ 2'GMP	0.0	0.0	0.0	0.00	1.58	-----	<5
0.70M Na ₂ 2'GMP	0.0	0.0	0.0	0.00	1.81	-----	<2
0.39M Na ₂ 2'GMP	0.0	0.0	0.0	0.00	1.97	-----	0.0
0.15M Na ₂ 2'GMP	0.0	0.0	0.0	0.00	2.00	-----	0.0

^aUnits are arbitrary and are not the same for different concentrations.

^bSum of the α , β and δ resonances in 5'GMP or the sum of the H(8)1 and H(8)2 resonances in 2'GMP.

^cSum of the H(8) structured resonances and the unstructured resonances.

^dDetermined from H(8) integration.

^eErrors are estimated to be $\pm 10\%$.

VII. Amino Proton Exchange With Solvent Water

The unusual properties of the guanylic acid amino protons led us to measure the rate of exchange of the amino protons of Na^+ and TMA^+ salts of 2'GMP and 5'GMP with solvent water. The chemical shift and width at half-height, $\nu_{1/2}$, were measured for the amino proton resonance and the water resonance of several guanylic acid solutions in $^1\text{H}_2\text{O}$ as a function of temperature. The program LORENT, given in Appendix 2, was used to calculate the two-site exchange rate of the amino protons by the method of Rogers and Woodbrey¹⁴⁸. This program uses the chemical shift separation between the two exchanging nuclei and the values of $\nu_{1/2}$ to estimate the rate of exchange. The program requires the chemical shift difference for the proton in sites A and B in absence of exchange, NUAX, and the width at half height in the absence of exchange, $\nu_{1/2\text{abex}}$. NUAX and $\nu_{1/2\text{abex}}$ were estimated by extrapolating into the region of fast exchange the chemical shifts and $\nu_{1/2}$ of the NH_2 protons line in the low temperature region where the amino proton exchange is slow. Figure 76 shows a typical example of this extrapolation. This plot is for the amino protons of 0.91M Na_2 2'GMP. The open squares symbolize the amino proton chemical shift and the closed squares represent the $\nu_{1/2}$. The chemical shift is extrapolated from the low temperature region to give the

Figure 76. A typical example of the graphic determination of the amino chemical shift in the absence of exchange and the width at half-height in the absence of exchange. The closed squares represent the observed $\nu_{1/2}$ and the open squares the observed chemical shift of 0.91M $\text{Na}_2^{2'}\text{GMP}$ as a function of temperature. The straight lines are the extrapolation from the low temperature region to give the chemical shift of the amino protons and $\nu_{1/2}$ in the absence of exchange.

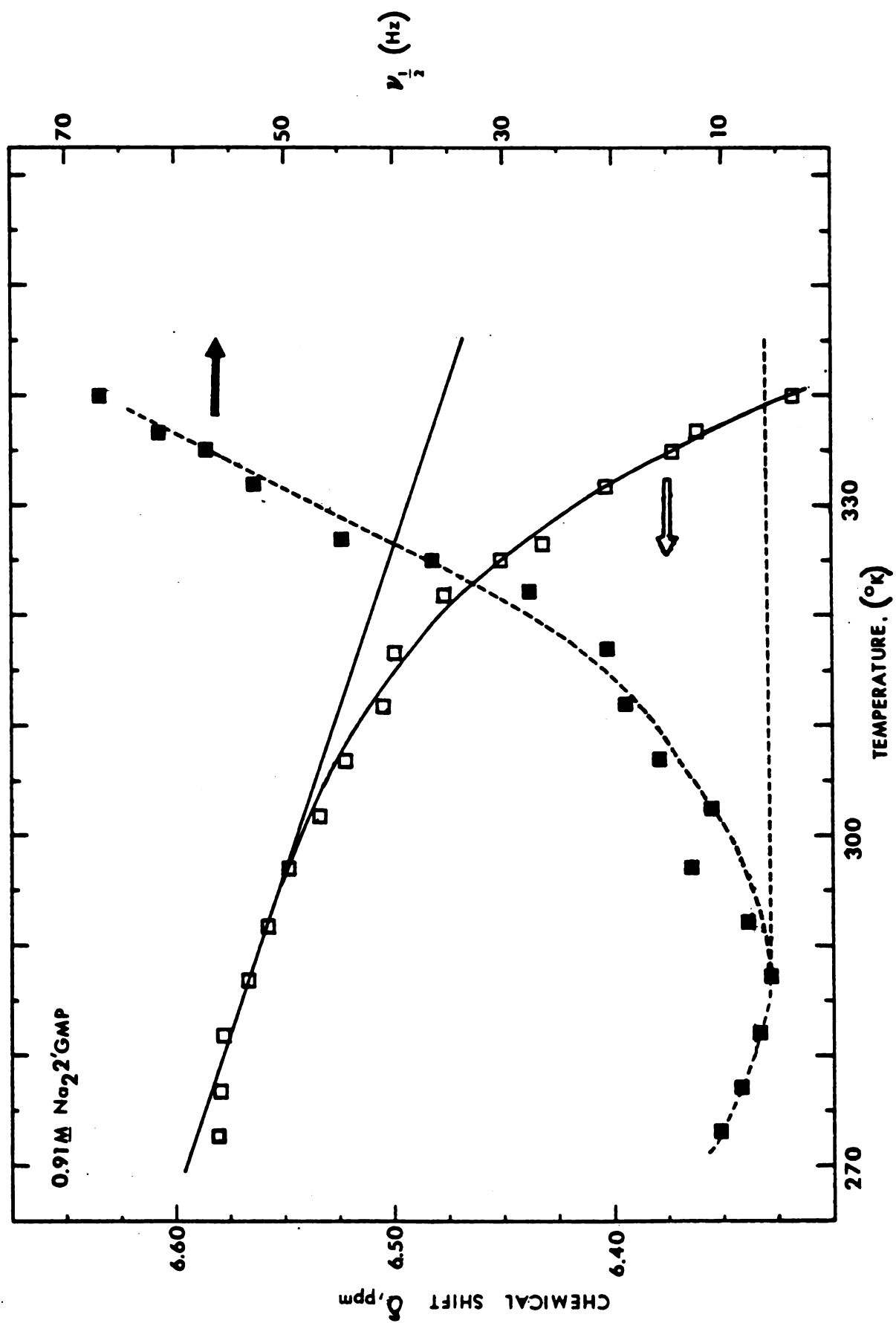


Figure 76

chemical shift of the amino protons in the absence of exchange. Similarly, the $\nu_{1/2}$ is extrapolated from below 290°K to give $\nu_{1/2abex}$. The decrease in $\nu_{1/2}$ as the temperature is increased from 270°K to 290°K is attributed to a decrease in the viscosity of the solution.

The concentration of water is much greater ($\geq 100X$) than that of the amino protons, thus the water resonance was assumed not to be affected by the exchange process. Thus the chemical shift in the absence of exchange and $\nu_{1/2abex}$ were assumed to be equal to the observed chemical shift and the observed half-width, respectively. The population of the amino protons and water protons was calculated with the assumption that aqueous solutions are 55.0M in H_2O .

The equations used in program LORENT are given in Appendix 2. For a given value of τ , a mean lifetime parameter, the program calculates the theoretical NMR lineshape for the case of general two site exchange. Various values of τ are tried until the observed NMR spectrum is matched.

The mean lifetimes of protons on site A ($-NH_2$) and site B (H_2O), τ_A and τ_B , respectively, must be equal to the quantity

$$\tau = \frac{\tau_A \tau_B}{\tau_A + \tau_B} \quad (43)$$

Substituting into (43) relationship (44), where P_A and P_B

are the mole fractions of -NH_2 and H_2O , respectively, and realizing that

$$\tau_B P_A = \tau_A P_B \quad (44)$$

$P_A + P_B = 1$, one obtains

$$\tau = P_B \tau_A \quad (45)$$

The pseudo first order rate constant for exchange, k_a , is given by

$$k_a = \frac{1}{\tau_A} \quad (46)$$

substituting (45) into (46) gives

$$k_a = \frac{P_B}{\tau} \quad (47)$$

The results are tabulated in Tables 17. to 22. The errors generated in k_a for estimated systematic uncertainties in the experimental input parameters are given in Table 23. Thus, the errors in k_a propagated by systematic errors in the experimental data are less than 6%.

Measuring k_a as a function of temperature allows the determination of activation parameters. According to the Arrhenius Theory

Table 17. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 0.30M TMA₂5'GMP.

$$P_A = 0.0054; \quad P_B = 0.9946$$

Temp °K	$\delta_{\text{NH}_2}^{\text{obs}}$ ^b	$\delta_{\text{NH}_2}^{\text{Abex}^c}$	$\delta_{\text{H}_2\text{O}}^{\text{obs}}$ ^b	$\nu_{1/2}^{\text{obs}}$ ^d	$\nu_{1/2\text{NH}_2}^{\text{Abex}^e}$	$\nu_{1/2\text{H}_2\text{O}}$	τ	k_a
270	6.463	6.463	5.106	13.1	13.1	13.0		
273	6.462	6.463	5.085	13.6	13.1	11.7		
278	6.458	6.463	5.048	15.5	13.1	10.8		
283	6.453	6.463	5.008	17.6	13.1	10.2		
288	6.445	6.463	4.963	22.0	13.1	9.5	0.042	23.7
293	6.437	6.463	4.920	31.0	13.1	9.0	0.021	47.3
298	6.430	6.463	4.877	40.6	13.1	8.6	0.0135	73.7
303	6.410	6.463	4.829	42.5	13.1	6.8	0.0125	79.6
308	6.313	6.463	4.779	78.1	13.1	7.8	0.0061	163.0
313	6.336	6.463	4.751	95.7	13.1	5.8	0.0048	207.2

^a P_A is the mole fraction of amino protons, P_B is the mole fraction of water protons.

^bThe observed chemical shift. The error in chemical shift is ± 0.005 ppm.

^cThe extrapolated estimate of the amino proton chemical shift in the absence of exchange. Errors are estimated to be ± 0.052 ppm.

^dThe observed chemical shift in Hz. The error is estimated to be ± 1 Hz.

^eThe extrapolated estimate of the half-width in Hz. The error is estimated to be ± 2 Hz.

^fThe mean lifetime parameter in sec. Errors are estimated to be $\pm 6\%$.

^gThe pseudo first order rate constant for exchange in sec. Errors are estimated to be $\pm 6\%$.

Table 18. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 0.61M $\text{Na}_2^{5'}\text{GMP}$.

$$P_A = 0.0110 \quad P_B = 0.9890$$

Temp °K	$\delta_{\text{NH}_2}^b$	$\delta_{\text{NH}_2}^c$	$\delta_{\text{H}_2\text{O}}^b$	$\nu_{1/2}^{\text{obs}^d}$	$\nu_{1/2\text{NH}_2}^{\text{Abex}^e}$	$\nu_{1/2\text{H}_2\text{O}}$	τ	k_a
273	6.093	6.093	4.936	30.7	30.0	9.7		
278	6.124	6.111	4.901	35.5	30.0	10.1		
283	6.134	6.134	4.864	42.7	30.0	9.5		
288	6.149	6.149	4.839	38.4	30.0	9.2		
293	6.156	6.165	4.796	50.9	30.0	8.8		
298	6.161	6.182	4.763	66.2	30.0	8.5		
303	6.163	6.198	4.733	84.4	30.0	8.3	0.0074	133.7
308	6.169	6.215	4.720	95.7	30.0	8.6	0.0063	157.0
313	6.145	6.230	4.676	101.6	30.0	8.0	0.0057	173.5
318	6.137	6.245	4.648	130.9	30.0	7.5	0.00435	227.4
323	6.075	6.261	4.609	140.6	30.0	7.4	0.0040	247.3

^a P_A is the mole fraction of amino protons, P_B is the mole fraction of water protons.

^b The observed chemical shift. The error in chemical shift is ± 0.005 ppm.

^c The extrapolated estimate of the amino proton chemical shift in the absence of exchange. Errors are estimated to be ± 0.052 ppm.

^d The observed chemical shift in Hz. The error is estimated to be ± 1 Hz.

^e The extrapolated estimate of the half-width in Hz. The error is estimated to be ± 2 Hz.

^f The mean lifetime parameter in sec. Errors are estimated to be $\pm 6\%$.

^g The pseudo first order rate constant for exchange in sec. Errors are estimated to be $\pm 6\%$.

Table 19. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 0.80M TMA₂'GMP.

$$P_A = 0.0143 \quad P_B = 0.9857$$

Temp °K	$\delta_{\text{NH}_2}^{\text{obs}}$	$\delta_{\text{NH}_2}^{\text{Abex}}$	$\delta_{\text{H}_2\text{O}}^{\text{obs}}$	$\nu_{1/2}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{Abex}}$	$\nu_{1/2\text{H}_2\text{O}}$	τ	k_a
274	6.724	6.724	4.611	15.9	15.9	26.0		
280	6.709	6.709	4.614	16.1	16.1	25.7		
285	6.700	6.700	4.616	16.4	16.4	24.1		
290	6.682	6.682	4.613	17.8	16.8	22.0		
295	6.670	6.670	4.614	21.5	17.1	16.7		
300	6.656	6.656	4.616	27.2	17.4	13.0	0.035	28.2
305	6.639	6.645	4.614	36.8	17.8	11.3	0.0179	55.1
310	6.624	6.632	4.614	44.7	18.1	10.8	0.0120	82.1
315	6.607	6.619	4.613	56.6	18.4	10.4	0.0087	113.3
320	6.557	6.607	4.614	79.3	18.8	10.2	0.0057	172.9
325	6.471	6.594	4.616	188.0	19.1	9.6	0.00262	376.2
330	6.395	6.581	4.609	232.2	19.4	9.1	0.002486	396.5

^aThis datum was rejected due to large deviations from other data.
See Footnotes to Table 17.

Table 20. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 0.15M $\text{Na}_2\text{P}_2\text{O}_7$.

$$P_A = 0.00272 \quad P_B = 0.99728$$

Temp °K	$\delta_{\text{NH}_2}^{\text{obs}}$	$\delta_{\text{NH}_2}^{\text{Abex}}$	$\delta_{\text{H}_2\text{O}}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{Abex}}$	$\nu_{1/2\text{H}_2\text{O}}$	τ^f	k_a
273	6.532	6.532	5.055	6.8	6.8	7.6		
278	6.517	6.517	5.012	7.7	6.8	7.1		
283	6.500	6.551	4.976	7.2	6.8	6.9		
288	6.476	6.489	4.921	7.4	6.8	5.7		
293	6.455	6.477	4.873	8.2	6.8	8.1		
298	6.433	6.463	4.826	9.8	6.8	7.9		
303	6.411	6.448	4.777	14.7	6.8	7.6	0.045	22.2
308	6.392	6.434	4.730	16.9	6.8	7.0	0.0343	29.1
313	6.379	6.420	4.705	23.0	6.8	7.7	0.022	45.3
318	6.384	6.406	4.684	30.5	6.8	10.2	0.016	62.3 ^a
323	6.337	6.392	4.598	62.0	6.8	9.9	0.0078	127.9 ^a
326	6.346	6.386	4.586	73.1	6.8	6.0	0.0060	166.2 ^a
329	6.372	6.375	4.590	58.5	6.8	7.5	0.0075	133.0

^aThis datum was rejected due to large deviations from other data.
See Footnotes to Table 17.

Table 21. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 0.91M $\text{Na}_2^{2'}\text{GMP}$.

$$P_A = 0.00763 \quad P_B = 0.992$$

Temp $^{\circ}\text{K}$	$\delta_{\text{NH}_2}^{\text{obs}}$	$\delta_{\text{NH}_2}^{\text{Abex}}$	$\delta_{\text{H}_2\text{O}}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{Abex}}$	$\nu_{1/2\text{H}_2\text{O}}$	τ	k_a
273	6.591	6.591	5.100	7.2	7.0	7.8		
275	6.587	6.587	5.087	7.4	7.0	8.6		
280	6.578	6.578	5.055	7.6	7.1	10.8		
285	6.570	6.570	5.024	8.1	7.3	13.0		
290	6.562	6.562	4.988	8.8	7.4	15.1		
295	6.552	6.553	4.955	9.7	7.6	17.2		
300	6.542	6.545	4.921	11.0	7.7	19.3	0.110	9.02
305	6.531	6.537	4.888	13.2	7.85	21.5	0.067	14.81
310	6.517	6.528	4.855	16.3	8.0	21.1	0.044	22.55
315	6.500	6.519	4.821	20.8	8.1	18.8	0.028	35.4
320	6.479	6.511	4.787	27.6	8.3	16.5	0.0186	53.3
325	6.452	6.503	4.754	36.9	8.4	14.1	0.0125	79.4
330	6.415	6.494	4.721	47.3	8.6	11.9	0.0092	107.9
335	6.374	6.456	4.696	57.2	8.7	9.6	0.0073	136.0
340	6.319	6.477	4.654	67.8	8.8	7.3	0.0052	191.0

See Footnotes to Table 17.

Table 22. NMR Lineshape Parameters and First Order Amino Proton Exchange Rate Constants for 1.81M Na₂GMP.

$$P_A = 0.0210 \quad P_B = 0.9780$$

Temp. °K	$\delta_{\text{NH}_2}^{\text{obs}}$	$\delta_{\text{NH}_2}^{\text{Abex}}$	$\delta_{\text{H}_2\text{O}}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{obs}}$	$\nu_{1/2\text{NH}_2}^{\text{Abex}}$	$\nu_{1/2\text{H}_2\text{O}}$	τ	k_a
271	6.275	6.275	4.853	33.4	33.4	29.1		
274	6.270	6.270	4.857	35.4	35.4	26.8		
280	6.261	6.261	4.803	26.6	26.6	25.7		
285	6.243	6.243	4.748	24.4	24.4	23.2		
290	6.234	6.234	4.715	22.1	22.1	28.3		
295	6.222	6.222	4.715	21.0	21.0	32.6		
300	6.212	6.212	4.662	23.8	20.0	26.0		
305	6.205	6.205	4.633	23.2	20.0	22.4		
310	6.193	6.193	4.607	30.0	20.0	16.2		
315	6.183	6.183	4.582	34.0	20.0	11.5	0.025	39.1
317	6.175	6.175	4.555	48.1	20.0	19.7	0.0125	78.2
320	6.170	6.170	4.546	48.7	20.0	16.3	0.0120	81.5
323	6.158	6.158	4.519	72.5	20.0	16.5	0.0069	141.7
325	6.116	6.155	4.513	87.2	20.0	13.3	0.0054	181.1
330	6.116	6.126	4.474	99.1	20.0	11.5	0.0046	212.6
335	6.112	6.111	4.440	107.5	20.0	10.5	0.0042	232.9
340	6.088	6.075	4.406	130.2	20.0	10.2	0.0034	287.7

See Footnotes to Table 17.

Table 23. The Estimated Systematic Uncertainties in the Experimental Input Parameters of LORENT.

Parameter	Uncertainty	Error in k_a
P_B	$\pm 1.0M$	$\pm 0.5\%$
NUAX	± 13.0 Hz	$\pm 2.5\%$
$\nu_{1/2abex}$	± 3.0 Hz	$\pm 2.5\%$

$$\ln k = \ln A - \frac{E_a}{RT} \quad (48)$$

where k is the rate constant, A , the pre-exponential factor, and E_a the activation energy. A linear regression analysis of the dependence of $\ln k$ on $1/T$ was used to determine E_a and A . The entropy of activation was calculated by Equation (49).

$$\Delta S^\ddagger = R \ln A - R \left[1 + \ln \left(\frac{k_B T}{h} \right) \right] \quad (49)$$

The results are tabulated in Table 24.

The enthalpy of activation, $\Delta H^\ddagger$, and the entropy of activation, $\Delta S^\ddagger$, can be estimated by the Transition State Theory,

$$k = \frac{k_B T}{h} e^{\Delta S^\ddagger / R} e^{-\Delta H^\ddagger / RT} \quad (50)$$

rearranging,

$$\ln k + \ln\left(\frac{h}{k_B T}\right) = \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT} \quad (51)$$

Equation (51) was programmed into KINFIT (Appendix 1) and $\Delta H^\ddagger$ and $\Delta S^\ddagger$ determined. The Gibbs free energy of activation, $\Delta G^\ddagger$, was calculated by Equation (52).

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (52)$$

The results are given in Table 24.

The presence of solution self-structure in 0.61M Na₂5'GMP made the data not suitable for accurate measurements. Therefore, activation parameters were not calculated for this solution. The data collected on the other nucleotide systems used in the calculation of activation parameters were in the temperature range where the solution self-structure is not present.

Table 24. Estimates of the Activation Parameters for the Amino Proton Exchange in Various Nucleotides.

Nucleotide	pH	Arrhenius Theory			Transition State Theory			
		k_a 25°C sec ⁻¹	E_a kcal/mole	$\ln A$	$\Delta S^\ddagger$ eu	$\Delta H^\ddagger$ kcal/mole	$\Delta S^\ddagger$ eu	$\Delta G_{298}^\ddagger$ kcal/mole
0.30M TMA ₂ 5'GMP	9.30	6.6±3.0	15.0±1.4	29.5±2.3	-1.9±4.6	14.4±1.4	-1.9±4.7	13.8±2.8
0.80M TMA ₂ 2'GMP	8.10	3.0±16.0	19.1±1.3	35.3±2.1	9.6±4.2	18.5±1.3	9.6±4.2	15.6±2.6
0.15M Na ₂ 2'GMP	7.07	7.7±7.0	19.9±2.1	35.7±3.4	10.3±6.9	19.3±2.1	10.3±6.9	16.2±3.9
0.91M Na ₂ 2'GMP	7.12	8.2±3.2	15.4±0.4	28.1±0.6	-4.7±1.2	14.8±0.4	4.7±1.3	16.2±2.0
1.81M Na ₂ 2'GMP	8.11	4.6±6.5	15.4 2.6	28.7 4.0	-3.5±1.1	14.7±2.6	-3.7±8.0	15.8±4.8

DISCUSSION

Guanylic acids are chemically different from other nucleic acids. For example, enzymatic reactions frequently fail or proceed by a different course when guanosine is involved. A number of systems which demonstrate the unusual behavior of guanosine derivatives have been reviewed¹⁸⁰. The studies presented here on the ordered aggregation of guanylic acids may answer some questions regarding some of these peculiarities. Guanylic acid solutions are a very complex system. The number of possible species in solution is large; monomers, stacked monomers, monomers hydrogen-bonded to each other, to tetramers, and to stacked tetramers. The requirement of high concentration of nucleotide for solution self-structure formation makes it impossible to use activity coefficients in calculations. Thus, the uncorrected concentrations must be used. Proton NMR is the best method of study, although the high concentrations involved cause problems here also. The viscosity of these solutions becomes a factor in detailed studies which are affected by the relaxation times of the solutions. The high concentration and high viscosity do allow the easy accumulation of spectra because of the relatively broad line widths. Even with the unfavorable conditions

of high concentrations, high ionic strength, and high viscosity, proton NMR is still the best method of study of the solution self-structuring. Since the exchange of solution self-structures with monomer is slow on the NMR time scale, non-equivalent environments can be observed for some protons in the NMR spectrum under conditions where structure is present.

The study of guanylic acid solution self-structures is also complicated by exchange fast on the NMR time scale between certain types of aggregated units. Base stacking and tetramer formation are reported to occur in guanylic acid solutions with concentrations of 0.02M ⁷¹. NMR allows the distinction of two types of aggregates, regular, ordered self-structures and disordered structures. The ordered solution self-structures exchange slowly and give rise to new lines in the NMR spectrum, while the disordered structures exchange rapidly and are time averaged with the monomer resonance. Other methods such as IR are able to distinguish between ordered and disordered aggregation.

I. Tetramethylammonium Ion as a Counterion

The effect of TMA^+ on nucleic acid structure and stability has been investigated recently¹⁶¹. The stability of DNA double helix strands has been found to increase when TMA^+ ion pairs to 5'GMP but, the ion is neither a structure directing nor structure inhibiting ion. However, the

present work shows that TMA^+ is not an "inert" ion in the aggregation of other guanosine nucleotides. Gels are not formed at neutral pH by the tetraalkylammonium salts of 3'GMP, 5'GDP, 5'dGMP, guanosine, and 5'GMP (pH=5) even at high concentration ($>0.50\text{M}$) and low temperature (0°C).

The addition of alkali metal ions to the ditetraalkylammonium salts of 3'GMP and 5'GDP causes gels to form at neutral pH but the concentration of alkali metal ion needed for gel structure formation is increased (cf. Results III and Results IV). The aggregation of 2'GMP solution self-structures in the presence of alkali metal ions is greatly decreased in the presence of TMA^+ as described in Results II.F. Larger tetraalkylammonium salts also show the same effect (cf. Results II.F). Therefore, tetraalkylammonium salts interact with 2'GMP, 3'GMP, and 5'GDP in a mode which inhibits the self-aggregation.

Since the inhibition of ordered structures by TMA^+ does not occur for 5'GMP, the inhibition which occurs for the other nucleotides is most likely a steric effect. The alkylammonium ions can ion pair to the phosphate group of the guanylic acid and sterically block the positions needed for aggregation. If a steric effect is operating, TEA^+ would have a larger destabilizing effect than TMA^+ . This assumes that the ion pairing to the phosphates is approximately the same. Solubility problems do not allow the comparison of TMA^+ and TEA^+ to be made experimentally. NOE

experiments (cf. Results II.F) show that some of the TMA^+ and TEA^+ ions reside near H(5') and possibly near the amino group. This observation is in contrast to those of Brown⁴⁹ who found that TMA^+ resides near H(8) in structural forms. Obstructing the NH_2 group would result in the blocking of tetramer formation. The nucleotide conformation may also cause the tetraalkylammonium ions to block the stacking of tetramers.

Figure 16 shows that the H(8) chemical shift of TMA_22' -GMP is relatively invariant with temperature while the chemical shifts of Na_22' GMP and K_22' GMP vary greatly with temperature even under conditions where no solution self-structure is present. The same relationship occurs with chemical shift vs. concentration of nucleotide (cf. Figure 17). TMA^+ is therefore interfering with structures which are exchanging fast on the NMR time scale.

These results from TMA^+ -guanylic acid studies are significant. Guanylic acid substitution and phosphorylation reactions, among others, often have lower yields than the analogous reactions of other nucleotides. This unusual decrease in reactivity could be due, in part, to the formation of hydrogen-bonded aggregates, such as stacked tetramers, in solution. Using a TMA^+ exchanged guanylic acid system to replace alkali metals may cause a significant increase in product yield. The use of TMA^+ in reaction systems may also affect the product distribution. Another

important use of TMA^+ as a counterion for guanylic acids is in the elimination of the formation of large ordered aggregates in solution. In some reactions of guanylic acids it may not be desirable to conduct the reaction under conditions complicated by gel formation if an alkali metal ion is present. Replacing the alkali metal ion with TMA^+ would eliminate the problem of gel formation.

II. Base Stacking Interactions

Despite a large body of literature, the base stacking phenomena of nucleic acids are not well understood^{22,163-165}. Interactions involving base-stacking could play an important role in the selective recognition of individual nucleotides by macromolecules such as proteins and enzymes^{166,167}. Proton NMR has proven the most informative method of investigation. Most investigations assume hydrogen bonding between nucleic acid bases does not take place in water. This is not reasonable at least for guanosine derivatives. Guanosine derivatives form gels, extensively hydrogen bonded structures, at concentrations as low as 0.01M ⁷⁴. Most evidence suggests base stacking proceeds far beyond the dimer stage. Data are usually analyzed by the isodesmic approach¹⁶⁰ which assumes the stacking phenomenon is non-cooperative. The self-stacking tendency decreases within the series adenosine > guanosine > inosine > cytidine ~ uridine¹⁶⁵. Sukhorukov and coworkers

determined that $\text{Na}^+ > \text{Li}^+ > \text{K}^+$ in stabilizing the stacking phenomenon¹⁶⁴.

Neurohr and Manisch¹⁶³ have reported the isodesmic stacking constant for 5'GMP at 30°C as 1.3M^{-1} . They emphasize the hydrophobic nature of this phenomenon since base stacking only occurs in water. Classical hydrophobic interactions are characterized by positive enthalpy and positive entropy changes¹⁶³. The base stacking self-association of nucleic acids, however, is accomplished by negative enthalpy and entropy changes, as determined by calorimetric measurements of several systems¹⁶⁸⁻¹⁷⁰ and by proton NMR¹⁶³. Moreover, the hydrophobic interaction is a rather unspecific phenomenon, which cannot account for the observed high degree of ordered structure formation.

Two different geometries have been proposed for the base stacking of 5'AMP, differing in the placement of the ribosyl substituents relative to each other. Ts'o and coworkers^{23,27} and Evans and Sarma¹⁷¹ have proposed a head-to-head arrangement, which places the ribosyl moieties on the same side of the stack. More recent results^{154,172,173}, based on Nuclear Overhauser effect studies and ^1H spin-lattice relaxation measurements, favor a head-to-tail arrangement, where the ribosyl moieties are placed on opposite sides of the stack while retaining the same stacking pattern. Neurohr and Mantach¹⁶³ conclude that the geometry of interacting adenosine nucleotides can be a combination of both head to head and head to tail stacking.

Individual nucleotide molecules are in rapid motion and base-stacking is a very fast process.

III. Tetramer Formation

Cation dependent aggregation processes that occur in guanylic acid-alkali metal ion systems cannot be directly observed by the appearance of new H(8) NMR resonances because of fast chemical exchange between the aggregates and the monomeric nucleotide. For example, with 2'GMP, H(8) upfield shifts occur with increasing concentration (cf. Figure 17) when the counterion is Na^+ , K^+ , or Rb^+ . The extent of the shift is greatest for K^+ (cf. Figure 17) salt. In contrast the H(8) resonance of the TMA^+ salt shows no concentration or temperature dependence (cf. Figure 16 and Figure 17). Thus a cation dependent process similar to that observed for regular ordered structure formation is operating, but the aggregated species which is formed is in fast equilibrium with monomer. We propose that this structure in fast equilibrium with monomers is a tetramer. The metal ion size dependence may be the result of metal binding in the cavity formed by the four carbonyl oxygens of the tetramer unit. The alkali metal ion binding increases the stability of the tetramer unit. Tetramethylammonium ion would bind weakly, if at all, to the carbonyl oxygen cavity. Tetramers are not stable in the absence of alkali metal ions. The increase in

half-width of the H(8) resonance (cf. Results II and Ref. 49) is also explained by the formation of tetramers. Increasing the concentration of a dialkali metal 2'GMP solution causes larger line broadening than in a tetraalkylammonium 2'GMP solution even in the absence of solution self-structure. A base stacking interaction is unlikely since the chemical shift of H(8) is alkali metal dependent (cf. Figure 16 and Figure 17). Tetramers have been postulated previously for alkali metal solutions of guanylic acids. Based on hypochromicity measurements Chantot and Guschlbauer⁷¹ have proposed that tetramers are present in a 0.003M 3'GMP solution at pH=4 and at 0°C containing 0.25M CH₃COOK. They propose that gel formation occurs by the stacking of tetramers. Other nucleotides were reported to form tetramers at concentrations lower than 0.01M. Lee and Chan¹⁷⁴ have calculated a tetramer formation constant of $2.5 \pm 0.5 \times 10^7 \text{ M}^{-3}$ and a tetramer stacking constant of $40 \pm 10 \text{ M}^{-1}$ in the case of 5'GMP, pD range 3-6. These results were based on the concentration and temperature dependence of the H(8) NMR chemical shift and line width of several guanylic acids at various pD's. The results of Lee and Chan are suspect since EDTA was found to significantly affect the H(8) half-width of the samples¹⁸¹.

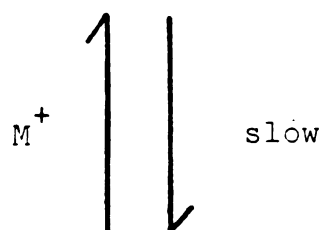
The loss of amino proton relative integral intensity for 2'GMP and 5'GMP (cf. Figure 73 and Figure 74) may be due to the formation of tetramers. The amino proton

intensity loss for both nucleotides occurs under conditions which are favorable for tetramer formation. When the alkali metal ions are replaced by TMA^+ , as in $\text{TMA}_2 2'\text{GMP}$ and $\text{TMA}_2 5'\text{-GMP}$, the amino proton relative integral intensity is two under all conditions, even at high concentrations and low temperature. Tetramethylammonium ion is not expected to stabilize the formation of tetramers due to its relatively large size and low charge density. The onset of amino proton relative integral intensity loss occurs before the onset of solution self-structure formation, which is consistent with the formation of tetramers. A critical concentration of tetramers must be attained before solution self-structuring (stacking of tetramer units) can occur.

In the case of 0.70M $\text{Na}_2 2'\text{GMP}$ at 1°C , there is a relative integral intensity loss of approximately 0.2 protons (cf. Figure 73) before solution structure-formation occurs. If we assume one amino proton per $2'\text{GMP}$ unit in the tetramer is involved in the intensity loss, the concentration of tetramer present would be 0.036M . If the intensity of both amino protons is lost in the formation of tetramers the concentration of tetramers would be 0.018M . The mechanism we propose for the amino proton intensity loss is shown in Figure 77. Monomers, which undergo slow amino proton exchange with water are in fast equilibrium with tetramers. The tetramers undergo very fast exchange of amino protons with solvent water. Tetramers are in slow

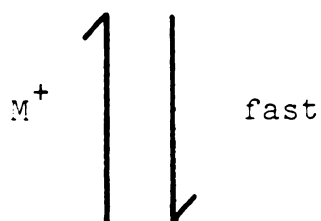
Solution
Self-Structure

Protons exchange between NH_2 -solvent may be fast



Tetramer

Very fast NH_2 -solvent exchange



$\text{M} = \text{Na}^+$

Monomer

Slow NH_2 -solvent exchange

Figure 77. The proposed mechanism for the loss in amino proton NMR intensity for $\text{Na}_2 2'\text{GMP}$ and $\text{Na}_2 5'\text{-GMP}$. We expect this mechanism to occur with other alkali metal structure directing ions.

equilibrium with ordered self-structures, which may also have a very large amino proton exchange rate with water. The very fast amino proton exchange causes the amino proton intensity loss. It is unclear if the relative amino proton integral intensity loss approaches a limit of one proton lost per nucleotide. Solubility limitations do not permit investigation of solutions at concentrations where the integral intensity loss could be significantly less than one proton intensity.

If both amino protons of the nucleotide are exchanging in a tetramer unit, the amino proton exchange with solvent water must be much faster than the formation of tetramers by monomer. If only one of the amino protons in a tetramer unit is exchanging, it is unclear which of the two amino protons is exchanging fast. An investigation of N_2 -methyl-5'GMP would resolve the question. The amino proton involved in the hydrogen bonding of the tetramer would be replaced with a methyl group. If the hydrogen-bonded amino proton is exchanging the amino proton intensity should decrease greatly at high concentration and low temperature. If the bonded amino proton not involved in the hydrogen bonding of the tetramer is exchanging the integral intensity should not decrease on increasing the concentration and decreasing the temperature.

Two points must be made clear: (1) the amino proton intensity loss in absence of solution self-structure is

not accompanied by the appearance of a new line elsewhere in the spectrum. Thus it must be concluded that fast exchange is occurring with solvent, (2) exchange is being catalyzed (i.e., exchange is much faster than with TMA^+ salts) by the structure directing alkali metal ions. Thus the need for postulating a self-structure which is in fast equilibrium with monomer and for which solvent amino exchange is very fast.

The proposed structure for the exchange of the amino proton with solvent water in a tetramer unit is shown in Figure 78. A water bridge is formed between the amino proton not involved in inter-base hydrogen-bonding formation and the phosphates of an adjacent nucleotide in the same tetramer unit. The amino proton is exchanging rapidly with the bridged water molecule and replaced with the hydrogen of another water molecule. Figure 79 is a photograph of a CPK model showing the structure proposed.

This model places 5'GMP in an anti conformation with $\chi = 60^\circ$. The conformation about $\text{C}(5')\text{-O}(5')$ is gg and about $\text{C}(4')\text{-C}(5')$ is gg. This is the preferred conformation of 5'GMP in aqueous solution^{11,182}. The amino proton exchange in this model is extremely dependent on the phosphate position. But because 2'GMP prefers a different conformation, building a model of the tetramer unit in 2'GMP shows that the phosphate is able to form a water bridge with the amino proton. The 2'GMP conformation in the water bridged

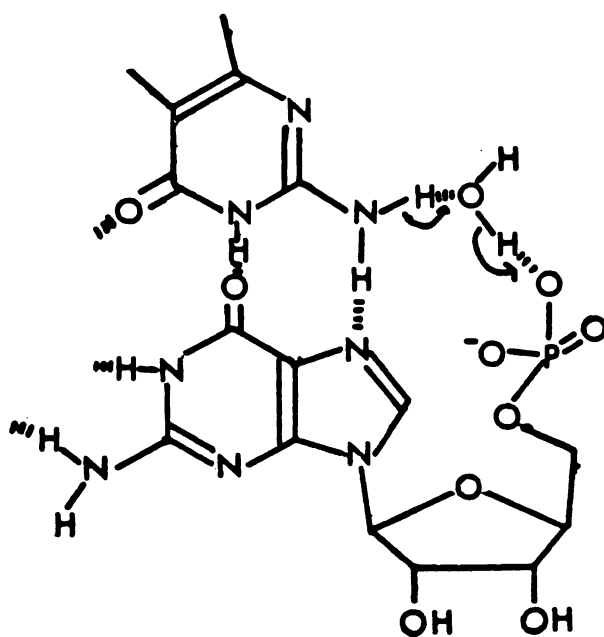


Figure 78. A segment of the proposed tetrameric structure for the exchange of the amino proton of $\text{Na}_2 2'\text{-GMP}$ and $\text{Na}_2 5'\text{-GMP}$ with solvent water. This structure has χ for $\text{Na}_2 5'\text{-GMP}$ and $\text{Na}_2 2'\text{-GMP}$ at 60° and 150° , respectively.



Figure 79. A photograph of a CPK model of the proposed structure for the exchange of the amino proton of $\text{Na}_2\text{5'GMP}$. The arrow points to the water bridge formed between the phosphate group of one nucleotide and the amino proton not involved in the hydrogen bonding of the tetramer unit of an adjacent nucleotide.

tetramer is syn with $\chi = 150^\circ$. This is the preferred conformation¹⁷⁵.

There is precedence for the occurrence of a water bridge with a phosphate group in a cyclic nucleotide system. Bolton and Kearns have proposed that a water bridge occurs between the phosphate and 2'OH group in cyclic nucleotides¹³¹. McConnell¹⁷⁶ has reported evidence that adenylic acid-NH₂ proton exchange at neutral pH occurs almost exclusively by a two step mechanism in which transfer of the -NH₂ proton to solvent water is preceded by protonation of the basic endocyclic nitrogen of the purine N(1). This mechanism is proposed to occur in the other nucleic acids¹³⁰. In the solution self-structure and in tetramers the N(1) is protonated, with the N(1) involved in a hydrogen bond.

Other evidence that a tetramer unit is present in guanylic acid solutions before the onset of solution self-structure is the occurrence of an IR band at 1530-1540 cm⁻¹. The band is always present when an NMR observable solution self-structure is observed but the band does not behave as if it is associated exclusively with the ordered self-structure, as can be seen in Table 5. A large increase in the extent of ordered solution structure does not cause a large increase in the IR band. This absorbance may arise from the formation of tetramers. The formation of tetramers, therefore, occurs before the onset of ordered self-structure. Although one might expect an increase in the

absorbance of this band with increasing concentration of monomer, the band intensity in fact remains constant even over the concentration range from 0.95 M $\text{Na}_2\text{2'GMP}$ (<5% structure) to 0.71 M $\text{Na}_2\text{2'GMP}$ made 1.1 M in NaCl (~50% structure) (cf. Figure 24 and Figure 25). Apparently, a critical concentration of tetramer is retained over the entire concentration range. A small concentration of tetramer would be consistent with a highly cooperative aggregation process.

IV. Self-Structuring Tendencies of Guanylic Acids

A. Effect of the Phosphate Position on Self-Structuring

At pH = 7-8 we can separate the guanylic acids into two groups, those that form gels and those that form solution self-structures. The nucleotides that form gels (3'-GMP and 5'GDP) have a greater tendency to self-structure than those that form only solution soluble, ordered self-structures (2'GMP, 5'GMP). The concentration of nucleotide needed to cause gelation at constant temperature and metal ion concentrations can be taken as an indication of the tendency for the nucleotide to self-structure. The order guanosine > 3'GMP > 5'GDP > 5'dGMP is obtained⁶⁷ using this system, when Na^+ is the structure directing cation. The amount of solution self-structure formed under a given set of conditions by 5'GMP and 2'GMP indicates that

the former nucleotide has a greater tendency to aggregate.

Evidence to date suggests that the solution self-structure is formed by the stacking of tetramer units. Gels have been shown by X-ray fiber diffraction studies to have either a stacked tetramer arrangement or a helical arrangement⁷². The tetrameric arrangement of the bases and the ribose is the same in all of the guanylic acid derivatives. The differences in structure forming tendencies for different GMP isomers must therefore be attributed to the position of the phosphate group.

The phosphate group can stabilize, destabilize, or not affect the self structuring depending on its position. The phosphate group can stabilize structures by forming hydrogen bonds between tetrameric plates. A destabilizing effect can occur if the build-up of negative charge on the phosphate groups causes a repulsion between plates. This destabilization effect is seen in $\text{Na}_2\text{5'GMP}$. At $\text{pH} = 5$, where 5'GMP forms a helical gel structure⁷² the phosphate group is a monoanion. As the pH is raised to 7-8, the phosphate group becomes dianionic, and the gel structure is destroyed and replaced with a solution self-structure. The increased negative charge causes smaller structures to be stable.

One can rationalize the structure forming tendencies of the guanylic acids as follows: guanosine, containing no phosphate, has the highest structure forming tendency.

The phosphate repulsion must always be greater than its possible stabilizing effect. The differences in ordering tendency for the other nucleotides arise from differences in the phosphate group position. The stability of ordered structures will depend on phosphate-phosphate distances and the ability of the phosphates to form interplatelet hydrogen bonds.

B. Experiments on Mixed Nucleotide Solutions

It is expected that in systems with two GMP nucleotides tetramers would be formed of compositions $[GMP(1)]_n[GMP(2)]_{4-n}$ where $n = 0,1,2,3,4$. The bases are the same in all of the guanylic acids. The hydrogen bonding scheme of the mixed nucleotide tetramers should be the same as in the pure systems. The ribose and the phosphate group are not capable of sterically blocking the hydrogen-bonding sites. Solution self-structure would then be composed of two or more stacked mixed tetramers. The systems with two nucleotides should give rise to more H(8) environments than a homogeneous system. If there is incorporation of one nucleotide unit into the regular ordered structure of another, new ^1H resonances should appear in the NMR spectrum. Since there is not much difference between the H(8) environments of different guanylic acid self-structures, new peaks may not be resolved, but there should be an increase in the amount of structure because the addition

of the second nucleotide will cause an increase in the total nucleotide concentration which should force the equilibrium toward self-structure.

In the case of a guanosine- $\text{Na}_2\text{5'GMP}$ system, 5'GMP was incorporated into the guanosine self-structure (cf. Results I.B). Guanosine has a greater "structure-forming tendency" than 5'GMP. In all the samples containing guanosine and 5'GMP, guanosine is in large aggregates that are NMR unobservable. The samples with concentrations of guanosine $>0.01\text{M}$ would have gelled in the absence of 5'GMP. Gelation is most likely inhibited by the 5'GMP which is incorporated into the guanosine gel structure. The repulsion between adjacent phosphates causes the formation of aggregates which are smaller than those needed to form gels. The addition of the negatively charged group into the gel structure, also causes the large increase in the solubility of guanosine. The model proposed for the guanosine-5'GMP structure is given in Figure 80. The guanosine unit is symbolized as a curved arrow, M^+ is a structure directing metal ion, and the solid line extending from a guanosine unit is a phosphate group. Thus those guanosine units with a solid line represent 5'GMP units.

All of the guanosine present in the guanosine-5'GMP solutions is present in these aggregates. The aggregations are of various sizes. Thus, in the H(8) NMR region a broad 5'GMP resonance is present which arises from the

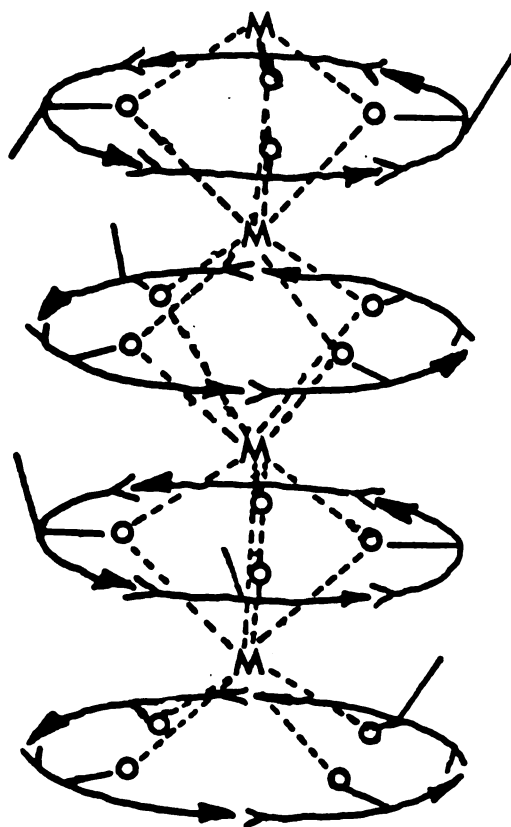


Figure 80. The proposed structure for a mixed guanosine and $\text{Na}_25'\text{GMP}$ self-structure. The guanosine unit is symbolized as a curved arrow, M^+ is a structure directing metal ion, and the solid line extending from a guanosine unit is a phosphate group.

smaller structures and an intensity loss occurs in the H(8) line due to incorporation of 5'GMP into guanosine units which are larger and NMR unobservable.

The results of a $\text{TMA}_22'\text{GMP}-\text{Na}_25'\text{GMP}$ experiment are dramatically different from those obtained from a $\text{Na}_22'\text{GMP}-\text{Na}_25'\text{GMP}$ experiment. In the $\text{TMA}_22'\text{GMP}-\text{Na}_25'\text{GMP}$ system there is a large decrease in the $\text{Na}_25'\text{GMP}$ solution self-structure (cf. Figure 46) while in the $\text{Na}_22'\text{GMP}-\text{Na}_25'\text{GMP}$ system there is only a small decrease in the $\text{Na}_25'\text{GMP}$ self-structure (cf. Results II.G.). There is a structure forming interaction between 2'GMP and 5'GMP as evidenced by these results and the fact that TMA^+ does not affect the $\text{Na}_25'\text{GMP}$ solution self-structure⁴⁹. The results can be explained by the model shown in Figure 81. When mixed, the two nucleotides, 2'GMP and 5'GMP, interact both by stacking interactions and hydrogen bonding. Mixed nucleotide tetramers can be formed by 5'GMP and 2'GMP. When TMA^+ is present in solution, the TMA^+ blocks the formation of mixed nucleotide tetramers by inhibiting the 2'GMP from completing a tetramer unit. This is consistent with the results of TMA^+ inhibition of the 2'GMP self-structure.

Therefore the decrease in the extent of ordered 5'GMP self-structure which occurs upon addition of $\text{TMA}_22'\text{GMP}$ is due to the inhibition of the formation of mixed nucleotide tetramer units by TMA^+ . In the TMA^+ free 5'GMP-2'GMP system, mixed nucleotide tetramers form but stacking of

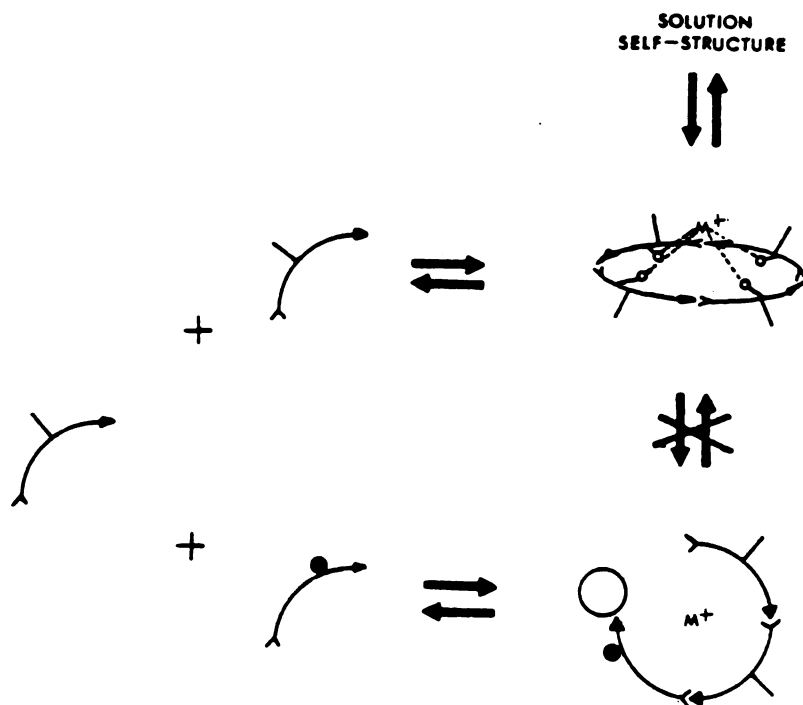


Figure 81. The proposed model for the destabilization of $\text{Na}_25'\text{GMP}$ solution self-structure in the presence of $\text{TMA}_22'\text{GMP}$. The arrow symbolizes a guanosine unit, the solid line extending from a guanosine unit is a 5' phosphate, an open circle extending from a guanosine unit is a 2' phosphate, and the solid curve is a TMA^+ .

mixed tetramers to form solution self-structures is not stable, which accounts for the line broadening of the H(8) resonance in 2'GMP and the decrease in solution self-structure for 5'GMP.

The addition of guanosine to an unstructured $\text{Na}_2\text{2'GMP}$ solution causes the guanosine H(8) NMR region to be very similar to that evidenced by $\text{Na}_2\text{5'GMP}$ self-structure (cf. Figure 47). Thus, the 2'nucleotide and the nucleoside, which form no ordered solution self-structures by themselves under similar conditions, form a solution self-structure when mixed, though guanosine would form an ordered gel structure. A model similar to Figure 77 is proposed. The gel structure of guanosine is destabilized by the formation of mixed nucleotide-nucleoside tetramers. The 2' phosphate group, by the action of its dinegative charge, causes the polymerization of tetramers to be limited to small units.

In 3'GMP- $\text{Na}_2\text{5'GMP}$ experiments, TMA^+ was shown (cf. Results III) to inhibit structure formation by 3'GMP. There is also a small loss in the $\text{Na}_2\text{5'GMP}$ self-structure. We postulate a mechanism similar to that proposed for the $\text{TMA}_2\text{2'GMP-Na}_2\text{5'GMP}$ system (cf. Figure 81) in which the TMA^+ is blocking the formation of tetramers. Since the decrease in the amount of solution self-structure of 5'-GMP is not as dramatic as in the $\text{TMA}_2\text{2'GMP-Na}_2\text{5'GMP}$ system, the inhibition must not be as strong in the $\text{TMA}_2\text{3'GMP-Na}_2\text{5'GMP}$

system. A new H(8) resonance is observed at 7.50 ppm in the proton NMR spectrum of the $\text{TMA}_2\text{3'GMP-8-dNa}_2\text{5'GMP}$ experiment (cf. Figure 54). If the same mechanism is occurring in the 2'GMP-5'GMP system, a new resonance should also be observed in the 2'GMP H(8) region. No such resonance is observed (cf. Results II). This discrepancy is explainable if the 2'GMP units in the 2'GMP-5'GMP aggregates exchange rapidly with 2'GMP monomer. In the 3'GMP-5'GMP system, the 3'GMP units in the mixed aggregates may exchange slowly with 3'GMP monomer, thus giving rise to the 3'GMP H(8) resonance at 8.50 ppm. The 2'GMP-5'GMP mixed aggregate resonance may also be coincidental with the 2'-GMP monomer resonance.

As in the 3'GMP-5'GMP experiment, TMA^+ was present in the mixed $\text{Na}_2\text{5'GMP-TMA}_2\text{5'GDP}$ study. TMA^+ has been shown here to inhibit self-structure formation by 5'GDP (cf. Results IV). As shown in Figure 57, several new 5'GDP H(8) resonances are present in a $\text{Na}_2\text{5'GMP-TMA}_2\text{5'GDP}$ solution. As shown in results IV.A, $\text{Na}_2\text{5'GDP}$ does not form an NMR observable solution self-structure, though it does form a gel structure. Mixed nucleotide tetramers of 5'GMP and 5'GDP must form, then stack to yield solution self-structures which give rise to the new 5'GDP H(8) resonances. The phosphate of 5'GDP in the aggregated units may be oriented outward toward the solvent, thereby minimizing the repulsion between phosphates. The larger size of the

dianion diphosphate allows its negative charge to be dispersed over more oxygen atoms, and leads to smaller repulsions relative to 5'GMP. The large number of new H(8) resonances observed for the mixed 5'GDP-5'GMP aggregation suggests that a larger number of structures is formed in this solution than in an ordered 5'GMP system or in any of the other mixed nucleotide-5'GMP systems discussed previously.

V. The Best Model for Ordered 5'GMP

A. The Assignment of Resonances

The study of the exchangeable protons in guanylic acids has provided valuable information on the solution self-structuring of guanylic acids. The amino proton intensity loss with regards to tetramer formation has already been discussed (cf. Discussion III). The ϵ resonance (cf. Figure 70) of $\text{Na}_25'\text{GMP}$ can be assigned to a hydroxyl proton, though this argument is not unequivocal. If correct this would mean that the 2'OH or the 3'OH of 5'GMP is involved in the stabilization of the $\text{Na}_25'\text{GMP}$ solution self-structure. At least three imide resonances are present in both 2'GMP and 5'GMP, and these suggest the presence of more than one type of structure. The chemical shift differences arise from the magnetic anisotropy in the tetramer units. Analysis of the symmetry

of the stacked tetramer model shows that the possible number of different imide resonances for different twist angles are 1, 2, or 4. The evidence to date suggests that at least two solution self-structures are present in $\text{Na}_2 5'\text{-GMP}$ ⁴⁹. The "extra" integral intensity of the imine resonance in the structured solutions of $5'\text{GMP}$ may be due to a hydrogen bond between a phosphate group and a hydroxyl group (cf. Results VI). Alternatively the "extra" intensity may be assigned to amino protons which are slow to exchange with solvent (cf. Results VI).

The decrease in the outer H(8) lines of solution self-structured $\text{Na}_2 5'\text{GMP}$ (i.e., α and δ lines) in saturation transfer and NOE experiments where the water resonance is irradiated implies that water must be located very close to the H(8) proton in these structures (cf. Result VI), more so than in the monomeric nucleotide and the other structure(s) which gives rise to the H(8) β resonance. It is believed that the outer H(8) lines arise from the same structure. In addition to the NOE results, Pinnavaia⁸⁹ has shown that the addition of Mn^{+2} to a 0.60M $\text{Na}_2 5'\text{GMP}$ solution at 7°C causes the monomer and the β resonance to paramagnetically broaden until at $9.1 \times 10^{-4}\text{M}$ Mn^{+2} , the β and δ resonances are no longer visible. The outer lines are only slightly broadened. The outer H(8) lines also have similar T_1 and T_2 values⁸⁹, and they grow into the spectrum at equal rates when NaCl is added to

TMA₂5'GMP⁴⁹.

The results of the saturation transfer experiments (Result VI) suggest the structure giving rise to the β resonance exchanges faster with monomer than the structure giving rise to the α and δ resonances. The tetramers must separate faster in the base stacking pattern which gives rise to the β resonance due to differences in the base stacking stability or the hydrogen bonding.

These data lead us to suggest the reaction scheme shown in Figure 82 for the structuring of Na₂5'GMP.

B. Other Models That Have Been Proposed

Laszlo and coworkers⁹⁹⁻¹⁰⁶ have proposed several different structures for self-assembled Na₂5'GMP and K₂5'GMP, based on ¹H, ³¹P, ²³Na, and ³⁹K NMR results. One of his early models is the staircase model shown in Figure 9. There are several discrepancies in the staircase model. These workers propose the same coordination sites and identical arrangements to account for the Na⁺ structure and the K⁺ structure. This postulate seems unreasonable since the Na⁺ and K⁺ structures behave differently under similar conditions⁴⁹. In rationalizing the multi-line H(8) environments in the K⁺ systems, Laszlo changes the number of phosphates pointing "up" and "down" in an octamer staircase unit. To achieve these "up" or "down" orientations of the ribose, a conformation other than anti must be

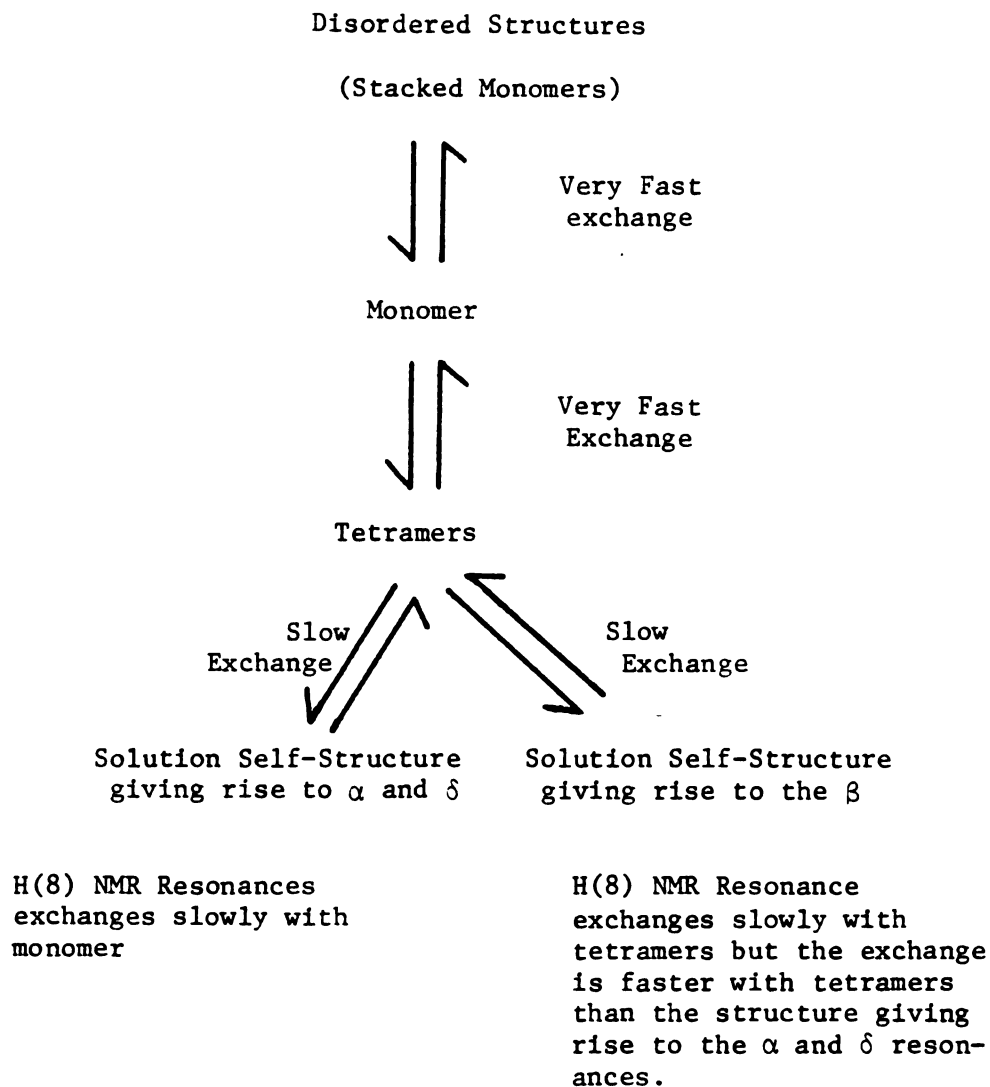


Figure 82. The proposed reaction scheme for the structuring of $\text{Na}_2 5'\text{GMP}$.

generated about the glycosidic bond. If Sundarlingham and Westhof¹⁰ "rigid" nucleotide concept is correct, there are definite preferred conformations of nucleotides. Laszlo's model implies the conformation of nucleotide is dependent on the alkali metal counterion. Most importantly, Laszlo's model ignores the precedent for the tetramer stacking pattern observed by X-ray fiber diffraction studies^{22, 72}. The staircase model totally ignores the favorable base overlap of a stacked tetramer model.

Models for the solution self-structure of 5'GMP have been proposed by Brown⁴⁹. Those for $\text{Na}_2\text{5'GMP}$ and $\text{K}_2\text{5'GMP}$ are shown in Figure 8. The $\text{Na}_2\text{5'GMP}$ self-structure consists of 2 stacked tetramers with one Na^+ associated with the cavity of each tetramer. There are also four Na^+ ions chelated by interplatelet phosphate oxygens.

The K^+ and Rb^+ structures have the metal ion coordinated to 8 carbonyl oxygens between the tetramer units, a simple structure being an octamer and a more complex structure being a hexadecamer. Marshall⁹⁷ has determined that the structured form of $\text{Na}_2\text{5'GMP}$ contains eight nucleotide units and four Na^+ ions. Thus he modified Brown's model by having only two Na^+ ions chelated by interplatelet phosphate oxygens. He also postulates a water molecule in the octameric hole formed by the two plates to separate the two Na^+ ions near the centers of the tetramers. Fisk and coworkers⁹⁸ have measured the T_1 's for the ^{13}C atoms in

$\text{Na}_25'\text{GMP}$ and have concluded that the most stable species formed in the self-structure is an octamer, formed by the association of two tetrameric plates. She postulates that at least three other less stable, stacked complexes are present in solution with octamer.

C. The Model Proposed for the $\text{Na}_25'\text{GMP}$ Solution Self-Structure

The model we propose for the solution self-structure of $\text{Na}_25'\text{GMP}$ which gives rise to the α and δ lines is similar to that of Brown⁴⁹ and Marshall⁹⁷. The structure is composed of two stacked tetramer plates, each having a five coordinate Na^+ in the tetramer cavity with a water molecule at the fifth coordination site of each Na^+ . A time averaged number of two Na^+ are territorially bound (solvent separated ion pairing) to the outer sides of the structure. A water molecule may be present in the octameric cavity between the two tetrameric plates, hydrogen bonded to the carbonyl oxygens. The two tetrameric plates are in a head to head stacking arrangement with a twist angle of 30° .

The ribose has a gg conformation about both $\text{C}(5')\text{-O}(5')$ and $\text{C}(4')\text{-C}(5')$ which corresponds to C-2'-endo. The glycosidic bond is anti with $\chi = 60^\circ$, the preferred conformation for $5'\text{GMP}$ ^{11,182}. This conformation allows a water bridge between a phosphate group and the amino group

of an adjacent nucleotide in the same plate, which catalyzes the amino proton exchange with solvent accounting for the intensity loss discussed earlier (cf Discussion III). Intramolecular hydrogen bonds can be formed between the 2'OH and O(1') of adjacent plates and between the 3'OH and the phosphate oxygen of adjacent plates or between 2'OH and O(1') of adjacent plates and between the 3'OH and a phosphate oxygen which is bonded to the C(5') of an adjacent plate. The imide proton in this conformation is in two non-equivalent sites, which from ring current maps of guanylic acids (cf Figure 83) should be ~ 0.2 ppm different in chemical shift. We therefore assign NH(1) and NH(2) to these imide protons. A schematic representation of this structure is illustrated in Figure 84. Pictures of a CPK model of this structure are shown in Figures 85 and Figure 86.

Other evidence which supports this structure is the NOE experiments of Brown⁴⁹. Placing a TMA^+ near a phosphate group in this model also places it near an H(8), thus when the TMA^+ resonance is irradiated a negative Nuclear Overhauser effect results in an intensity loss in the H(8) resonance as seen by Brown. Another observation by Brown⁴⁹ was the unequal loss of intensity of the outer H(8) lines on irradiation of the HDO resonance. This model places a water molecule, that involved in the phosphate-amino water bridge, close to H(8). The H(8)'s of the upper and lower

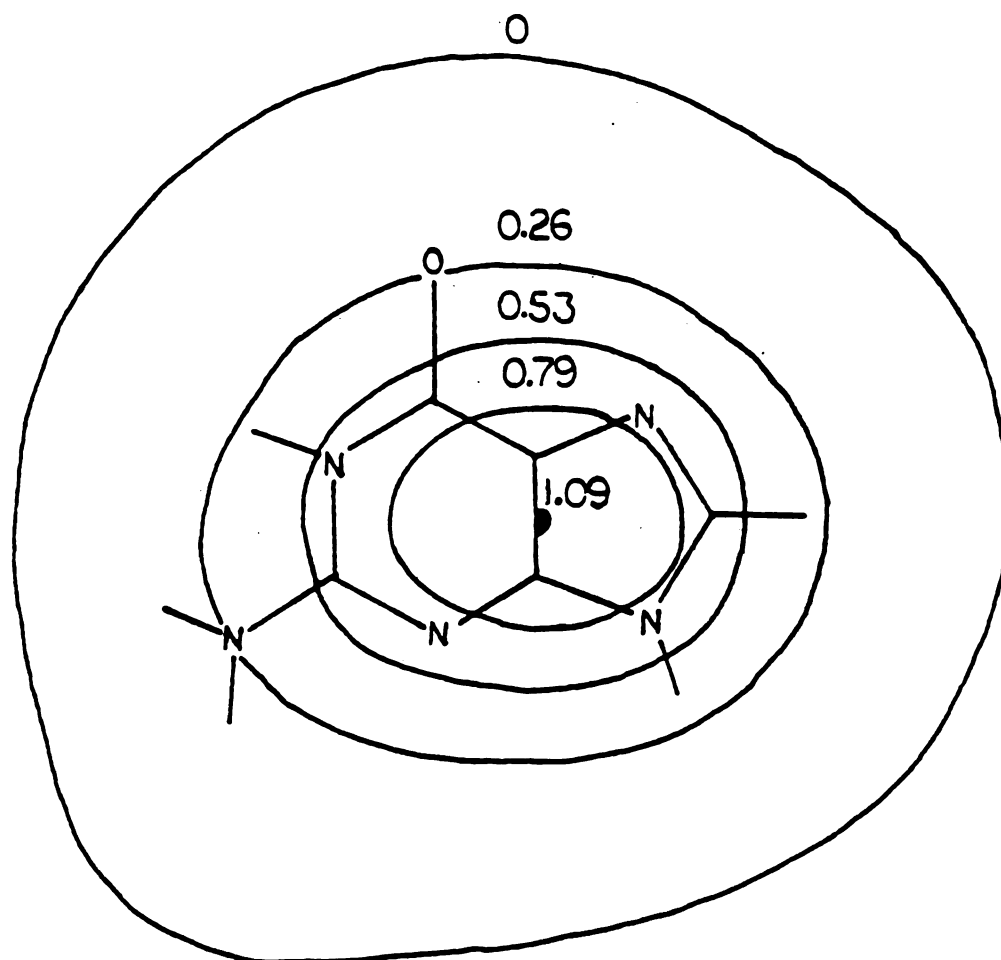


Figure 83. Expected upfield shift (in ppm) of a nucleus 3.5 Å above or below the aromatic plane due to the ring current and diamagnetic susceptibility effects. Taken from Reference 97.

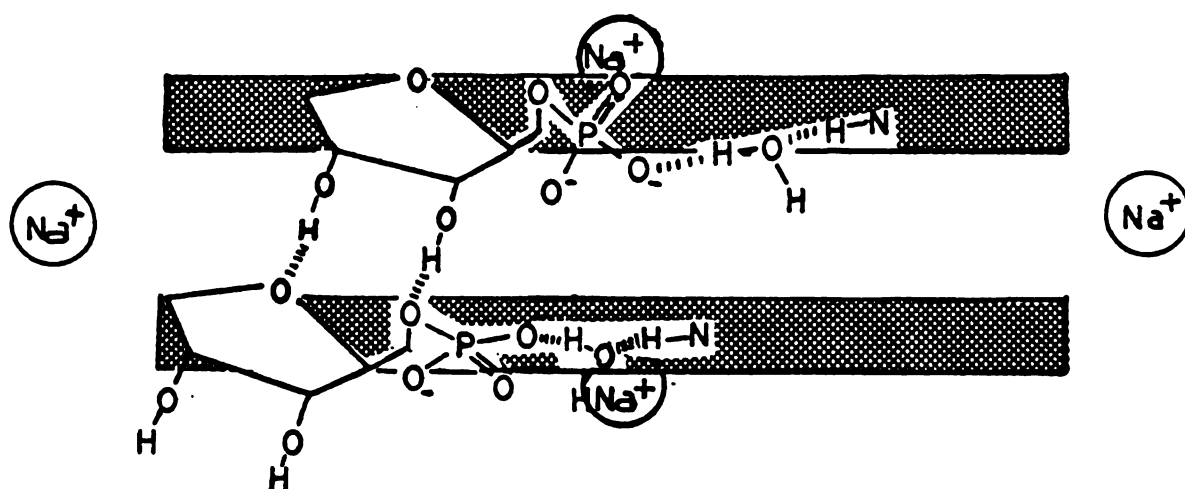


Figure 84. The proposed model for the solution self-structure which gives rise to the α and δ H(8) resonances in $\text{Na}_2 5'\text{GMP}$. The shaded rectangles represent a tetramer unit.

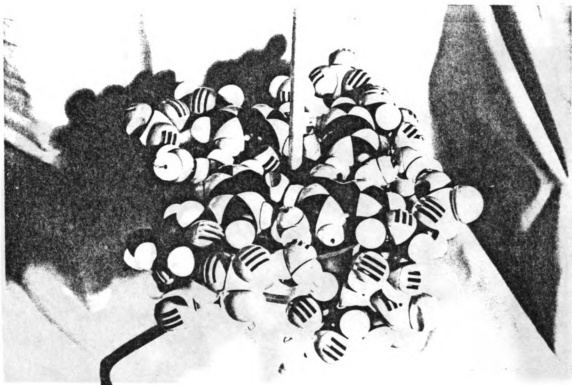


Figure 85. A photograph of a CPK model of the proposed model for the solution self-structure which gives rise to the α and δ H(8) resonances in $\text{Na}_2 5'\text{GMP}$

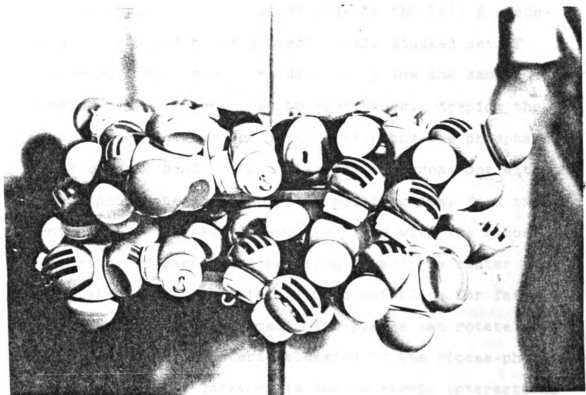


Figure 86. A photograph of a CPK model of the proposed model for the solution self-structure which gives rise to the α and δ H(δ) resonances in $\text{Na}_25'\text{GMP}$.

plates are not equivalent. The H(8)'s of the lower plate are located near the 2'OH of the upper plate, a location which would lead to a larger negative Nuclear Overhauser effect when the HDO resonance is irradiated, since the 2'OH and H₂O are most likely in rapid equilibrium.

The structure which gives rise to the H(8) β resonance is proposed to be a head to tail stacked set of tetramers. This structure does not allow the same hydrogen bonding scheme as the head to head stack. Keeping the water bridge between the amino proton and the phosphate, there are two head to tail stacking patterns, one with the ribose phosphates of each plate pointing away from the center of the plates (I) and the other with the ribose phosphates of each plate pointing toward the center of the plates (II). Structure I does not allow for favorable hydrogen bonds to be formed. The plates can rotate freely because there is no steric blocking by the ribose-phosphate. Structure (II) is unfavorable due to steric interactions (e.g., phosphate-phosphate repulsion) if a plate to plate distance of 3.4 Å is to be maintained. Thus, the H(8) β resonance may arise from a head to tail stacking pattern I. Since the plates are free to rotate only a time averaged signal will be observed for the H(8) resonance.

Some of the structures in the more complicated K⁺ solution self-structure are proposed to have a similar hydrogen bonding scheme as the head to head and head to

tail models proposed for $\text{Na}_25'\text{GMP}$. A K^+ is believed to be in the octameric hole between two tetrameric plates. Other structures are believed to arise from stacking of this structure unit as shown in Figure 87.

The other nucleotides are believed to have solution self-structures similar to that proposed for $5'\text{GMP}$. The phosphate and hydroxyl groups are no longer in the same position but assume a conformation which gives the best hydrogen-bonding scheme.

VI. Ethidium Binding to Unstructured and Structured Forms of $2'\text{GMP}$ and $5'\text{GMP}$

Ethidium bromide was found to bind to both structured and unstructured guanylic acids (cf. Results V). The interactions of ethidium with guanylic acids cause changes in the proton NMR spectra of the ethidium similar to those found for ethidium intercalation in oligonucleotides. Ethidium has been shown to form a 1:1 and a 2:1 complex with both unstructured $5'\text{GMP}$ and $2'\text{GMP}$ (cf. Results V.A). The equilibrium constants for the formation of the 1:1 complex for both $5'\text{GMP}$ and $2'\text{GMP}$ is very large, $>10^4 \text{ M}^{-1}$. The equilibrium constant for the 2:1 (nucleotide:ethidium complex) is $10\text{-}20\text{M}^{-1}$ for both nucleotides (cf. Results V.A). The only other equilibrium constant reported for the binding of ethidium to a nucleotide is that of ethidium binding to $2'\text{deoxyadenosine}$ by ^1H NMR by Jordan and

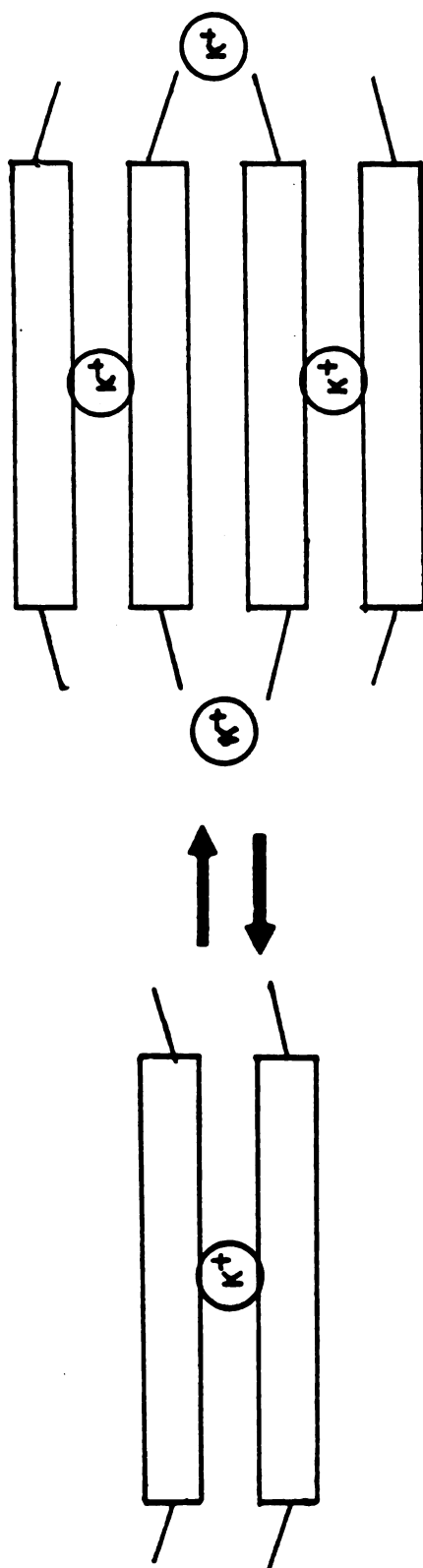


Figure 87. The proposed model for the K^+ solution self-structure of 2'GMP and 5'GMP. The rectangles represent a tetramer unit and the line extending from the tetramer a ribose-phosphate.

coworkers¹⁷⁷. The $K_{\text{association}}$ value they determined is $3.0 \times 10^2 \text{ M}^{-1}$. It is not unexpected that the $K_{\text{association}}$ of ethidium binding to guanylic acids is larger than that to adenylic acids since ethidium has been found to prefer guanosine regions of oligonucleotides and DNA.

In addition to the equilibrium constants for ethidium binding to unstructured 2'GMP and 5'GMP, we have measured the ΔH and ΔS of the formation of both the 1:1 and the 2:1 unstructured ethidium complexes, to our knowledge the first determination of thermodynamic parameters between this intercalator and a mononucleotide. The values for 1:1 complex are $\Delta H_1 = -19.78 \pm 0.96 \text{ Kcal/mole}$ and $\Delta S_1 = -53.3 \pm 6.6 \text{ eu}$, for the 2:1 complex they are $\Delta H_2 = 0.07 \pm 0.26 \text{ Kcal/mole}$ and $\Delta S_2 = 3.9 \pm 1.6 \text{ eu}$. The ΔH_1 is in agreement with the theoretically calculated value of $\Delta H = -12.3$ to -24 Kcal/mole for minihelices¹²⁶, and the values of Sturgill¹⁸³, $\Delta H = -20 \pm 1 \text{ Kcal/mole}$ and $T\Delta S = -4 \text{ Kcal/mole}$ based on several calorimetric experiments for Etd intercalated into tRNA^{Phe} under conditions of incomplete saturation. LePecq and Paoletti¹¹⁶ have found that both ΔH and ΔS are positive at low ionic strength, but both are negative at high ionic strength for the binding of ethidium bromide to DNA at different sodium ion concentration.

Extremely large ethidium methyl resonance shifts are observed when ethidium is added to a guanylic acid which

contains solution self-structure. In the case of $\text{Na}_25'\text{-GMP}$ ⁹⁷ and $\text{Na}_22'\text{GMP}$ (cf. Results V.B) the amount of solution self-structure was increased upon addition of ethidium. Marshall⁹⁷ has reported a decrease in the amount of $\text{K}_25'\text{-GMP}$ solution self-structure on addition of ethidium. This datum is suspect because integration of the H(8) region is difficult due to the overlapping of several resonances. The binding of ethidium to tetramers which may be present in solution cannot be neglected. No evidence to date is available to determine if ethidium binds to tetramers present in solution. It seems likely that if ethidium binds to stacked tetramer it would bind to tetramer also. It is possible that tetramers containing bound drug go on to stack because of the lower net negative charge. Marshall's association constants for the binding of ethidium to structured forms of $\text{Na}_25'\text{GMP}$ and $\text{K}_25'\text{GMP}$ were determined using the model given by Equations (53) and (54).



His data are suspect, since the model totally ignores ethidium to monomer binding, which has been shown in this work not to be neglectable (cf. Results VI). We reject this model and substitute for it that given by Equations

(3) - (4) and (23) - (25). A satisfactory fit was obtained with this model for the binding of ethidium to $\text{Na}_2\text{-2'GMP}$. The equilibrium constants for the formation of a 1:1 and 2:1 ethidium nucleotide complex were $1.9 \pm 0.1 \times 10^4 \text{ M}^{-1}$ and $5.8 \pm 0.6 \times 10^2 \text{ M}^{-1}$, respectively.

A satisfactory fit was not obtained for ethidium binding to $\text{K}_2\text{2'GMP}$, $\text{Na}_2\text{5'GMP}$, and $\text{K}_2\text{5'GMP}$, probably due to the large number of variables present. It is clear from the dependence of the ethidium methyl chemical shift on the nucleotide/ethidium mole ratio (cf. Figures 64 to 67) that the order of $K_{\text{association}}$ for ethidium to solution self-structure is $\text{K}_2\text{5'GMP} > \text{Na}_2\text{5'GMP} \geq \text{K}_2\text{2'GMP} > \text{Na}_2\text{2'GMP}$. A fit to the model represented by Equations (3) - (4) and (23) - (25) may be obtained if the integrated concentrations of structure are used as a known variable, to eliminate some unknown variables from the equations used in the KINFIT program.

VII. Amino Proton Exchange in the Absence of Solution Self-Structure

Analysis of proton NMR line widths of the amino proton resonances can give detailed data on the rates of proton exchange between the amino protons and the solvent. In water, the amino proton resonances are broad and sensitive to temperature; at higher temperatures they broaden rapidly and become unobservable above $50^\circ\text{-}60^\circ\text{C}$. The observed rate

constants differ from those for "normal" amino groups and reinforce the argument that a significant amount of imide character may account for the relatively slow proton exchange rates. For imides, it is well established that proton exchange with water occurs at slower rates than maximum (diffusional) because imide pK's lie outside the range in which hydronium-hydroxyl catalysis is efficient¹⁷⁹.

It is obvious that the reaction is pseudo first order in amino proton. The k_a at 25°C is the same within the limits of error for a 12-fold increase in concentration (cf. Table 24) of $\text{Na}_22'\text{GMP}$. The reaction rates and activation parameters are the same for $\text{TMA}_25'\text{GMP}$, $\text{Na}_22'\text{GMP}$ and TMA^+ inhibited $\text{TMA}_22'\text{GMP}$. The reaction rates and activation parameters are independent of nucleotide, concentration of nucleotide, or counterion. These results are consistent with the work of McConnell and coworkers^{130,133}. The mechanism of amino proton exchange in guanylic acids has been discussed extensively in the literature^{130,133}.

CONCLUSIONS

Self-association is a general property of guanylic acids which do not have the N(7), O(6) acceptor positions and N(1), NH₂ donor positions blocked for hydrogen bonding. A size selective metal ion coordination is involved in the self-structuring process. The ordering of the structure directing ability of the metal ions is $K^+ > Rb^+ \geq Na^+ > Cs^+ > Li^+ \geq TMA^+$. The position and charge of the phosphate play an important role in the stability and type of structures formed. Thus solution self-structures are small soluble fragments, probably involving limited stacking of tetramer units. The size of the stacked tetramers are limited by interplate phosphate repulsions. The phosphates also help stabilize structures by interplatlet hydrogen bonding. The order of structure forming tendencies of guanylic acids is guanosine > 3'GMP > 5'GDP > 5'GMP (pH=5) > 5'GMP (pH=7-8) > 2'GMP. The nucleotides are also able to interact with each other forming hydrogen bonded mixed species in solution.

The tetramethylammonium ion was found to be a non-structure directing, structure inhibiting ion for 2'GMP, 3'GMP, and 5'GDP. Since TMA⁺ is a non-structure directing, non-structure inhibiting ion in 5'GMP, TMA⁺ most likely

is sterically blocking the formation or stacking of tetramers in 2'GMP, 3'GMP, and 5'GDP. In some reactions of guanylic acids it may not be desirable to conduct the reaction under conditions complicated by gel formation if an alkali metal ion is present. Replacing the alkali metal ion with TMA^+ would eliminate the problem of gel formation and solution self-structuring.

A catalyzed amino proton exchange reaction occurs in $\text{Na}_2\text{2'GMP}$ and $\text{Na}_2\text{5'GMP}$, which may be due to the formation of tetramers. A mechanism involving a water bridge between a phosphate of an adjacent nucleotide and the amino proton not involved in tetramer formation has been postulated. A solution structure must be involved as the catalyst in the amino exchange reaction because the amino protons of $\text{Na}_2\text{2'GMP}$ were found to exchange at a slow rate at temperatures where no solution self-structure is present. The fast exchange of the amino proton(s) occurs before the onset of structure formation. It is not known if this amino proton exchange is important in biological systems.

Ethidium was found to form a 1:1 and a 2:1 complex with both structured and unstructured 2'GMP and 5'GMP with association constants $>10^3$. The large equilibrium constants are consistent with ethidium preferring to intercalate in guanosine rich regions of DNA and RNA. Equilibrium constants for the ethidium binding to solution self-structured $\text{Na}_2\text{2'GMP}$ were determined assuming a stoichiometry of $\text{Na}_4\text{-(2'GMP)}_8$. Equilibrium constants could not be determined

with other stoichiometries. This supports the postulate that $\text{Na}_22'\text{GMP}$ solution self-structure is an octamer unit.

APPENDIX 1

PROGRAMS USED ON THE ASPECT 2000
ON THE BRUKER WM250 AND
PROGRAMS USED IN KINFIT

1. Programs Used on the Aspect 2000 on the Bruker WM250

1.1. The Gated Decoupling Program

To produce a spectrum with normal coupling pattern but containing the NOE effect the following program was used on the Bruker-WM250

```
1  ZE
2  HG
3  D1
4  D0
5  D2
6  GO=2
7  EXIT
```

Define 02, the decoupler frequency, D1 and D2 delays before executing AU. Typical values used are

DP = 8H-25H (0.1 W-1.1W)

D1 = 3-5 sec.

D2 = 0.002 sec.

1.2. Inversion-Recovery T_1 Program

To obtain estimates of T_1 the following Inversion-recovery T_1 program was used

```

1  ZE
2  D1
3  P1
4  VD
5  GO=2
6  WR NAME
7  IF NAME
8  IN=1
9  EXIT

```

Define $D1=5 \times T_1$, $P1=180^\circ$, $PW=90^\circ$, VD list as desired, and set NE=no. of experiments. When transforming, do the largest FID first and set AI=1 before typing FT. Use PK for phasing the other spectra. Typical values used on the 5 mm proton probe are

```

P1=9.0 μsec
PW=4.5 μsec.
VD=0.01 - 1.0 sec.

```

1.3. KINFIT Programs

1.3.1. KINFIT Programs Used in Ethidium Binding to Guanylic Acids

The following programs are the equations used for ethidium binding to guanylic acids. This method of determining equilibrium constants is based on the method of Popov and coworkers¹⁸⁴.

1.3.1.1. Determination of the equilibrium constants for the formation of a 1:1 and a 2:1 complex between unstructured nucleotide and ethidium. pp (237) - (239).

1.3.1.2. Determination of ΔH and ΔS for the formation of a 1:1 and a 2:1 complex between unstructured guanylic acids and ethidium. pp (240) - (243).

1.3.1.3. Determination of the equilibrium constants for the formation of a 1:1 and a 2:1 complex between structured guanylic acids and ethidium. pp (244) - (247).

1.3.2. Calculation of Activation Parameters for Amino Proton Exchange with Solvent

1.3.2.1. Determination of E_a and A for amino proton exchange with solvent. pp (248) - (250).

1.3.2.2. Determination of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ for amino proton exchange with solvent. pp (251) - (253).

[illegible]


```

TEMP=(1/CONSTS(JDAT,7))-CONSTS(JDAT,5)
EXP1=(-U(1)/CONSTS(JDAT,6))*TEMP
PK1=CONSTS(JDAT,3)*EXP(EXP1)
EXP2=(-U(2)/CONSTS(JDAT,6))*TEMP
PK2=CONSTS(JDAT,4)*EXP(EXP2)
B1=1.0*(PK1+CALCD)
B2=8.0*(PK1+PK2+CALCD+XX(1))
B3=4.0*(PK1+PK2+CALCD)
HR=(B1+2)*B2
IF (HR.LE.0.0) GO TO 300
CALCM=(-B1+SQRT((B1+2)*B2))/B3
200 CONTINUE
D1=1.0*(PK1+CALCM)+(PK1+PK2*(CALCM+2))
CALCD=CONSTS(JDAT,1)/D1
H1=1.0*(PK1+CALCD)
R2=9.0*(PK1+PK2+CALCD+XX(1))
R3=4.0*(PK1+PK2+CALCD)
BH=(B1+2)*B2
IF (BH.LE.0.0) GO TO 300
CALCN=(-B1+SQRT((B1+2)*B2))/B3
RATIO=ARS(CALCM-CALCN)
CALCM=CALCN
IF (RATIO.GT.0.00001) GO TO 200
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C AD=THE MOLE FRACTION OF FREE DRUG
C AMD= THE MOLE FRACTION OF THE 1/1 COMPLEX
C AMD= THE MOLE FRACTION OF THE 2/1 COMPLEX
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AD=CALCD/CONSTS(JDAT,1)
AMD=AD*PK1+CALCM
AMD=AMD*PK2+CALCM
CALC=(CONSTS(JDAT,2)*AD)+(U(3)*AMD)+(U(4)*AMMD)
IF (IMETH.NE.-1) GO TO 35
RETURN
35 CONTINUE
RESID=CALC-XX(2)
IF (ITS.EQ.NOPT) ITS=0
IF (ITS.EQ.0) CALCD=0.0001
RETURN
300 RESID=1.0E5
CALCD=0.0001
RETURN
3 CONTINUE
4 RETURN
5 CONTINUE
IF (IMETH.NE.-1) GO TO 20
RETURN
EQN4
EQN4
EQN4
EQN4
EQN4
EQN4
EQN4
EQN4
EQN4
EQN4

```


[[[PNC 2116125 073181 10 BARR, RICHARD
HAL BANNER, R BARR, NA226MP, 273, EQ CONST.
RETURN, KINFT4, LGO.
HAL, L=DYE, KINFT4=KINFT4.
FTN, B=LGO.
L04D, KINFT4.
LGC.
LECR

```

SUBROUTINE EQN
COMMON KOUNT,ITAPE,JTAPE,IWT,LAP,XINCR,NOPT,NOVAR,NOUNK,X,U,ITMAX,
1:IX,TEST,I,AV,RESID,IAR,EPS,ITYP,XX,RXTYP,DX1I,FOP,FO,FU,P,ZL,TO,E
2:IGVAL,XST,I,DI,L,M,JJJ,Y,DY,VECT,NCST,CONST,NDAT,JDAT,MOPT,LOPT,
3:YY,CONST
COMMON/FREDT/IMETH
COMMON/POINT/KOPT,JOPT,XXX
DIMENSION X(4,300),U(20),WTX(4,300),XX(4),FOP(300),FO(300),FU(300)
1,P(20,21),VECT(20,21),ZL(300),TO(20),EIGVAL(20),XST(300),Y(10),
2DY(10),CONSTS(50,16),NCST(50),ISMIN(50),RXTYP(50),DX1I(50),IRX(50)
3,MOPT(50),LOPT(50),YYV(50),CONST(16),XXX(15)
GO TO (2,3,4,5,1,7,8,9,10,11,12,13) ITYP
1 CONTINUE
ITAPE=50
JTAPE=51
RETURN
7 CONTINUE
NOUNK=5
NOVAR=2
RETURN
8 CALCM=0.3
RETURN
2 CONTINUE
XX(1)=CMT THE ANALYTICLA CONCENTRATIP OF NUCLEOTIDE
XX(2)=DGBS THE OBSERVED CHEMICAL SHIFT OF THE DRUG
U(1)=K3
U(2)=K4
U(3)=K5
U(4)=DMD00 THE DRUG CHEMICAL SHIFT OF THE U/U COMPLEX
U(5)=DMD20 THE DRUG SHIFT OF THE 2/1 OCT-DRUG COMPLEX
CONST(1)=K1
CONST(2)=K2
CONST(3)=D000 THE CHEMICAL SHIFT OF THE FREE DRUG
CONST(4)=D000 THE DRUG CHEMICAL SHIFT OF THE U/U MONO-DRUG COMPLEX
CONST(5)=DMD00 THE DRUG CHEMICAL SHIF TOF THE 2/1 MONO-DRUG COMPLEX
CONST(6)= THE ANALYTICAL CONCENTRATION OF DRUG
ALPHA= THE NUMBER OF NUCLEOTIDES IN A STRUCTURED UNIT
BETA= THE NUMBER OF METAL IONS IN A STRUCTURED UNIT
GAMMA= THE RATIO OF METAL IONS TO NUCLEOTIDE (TOTAL)

```



```

200 ALPHA=8.0
    BETA=4.0
    GAMMA=3.266
    CONC=((GAMMA*XX(1))*RETA)/3.0
    OCT=CALCM**ALPHA
    D1=1.0*(CONST(1)*CALCM)+(CONST(2)*CONST(1)*(CALCM**2))
    D2=CONST(1)*U(1)*U(3)*CALCM*OCT*CONC
    D3=2.0*(CONST(1)**2)*U(1)*U(2)*U(3)*(CALCM**2)*OCT*CONC
    D4=D1+D2
    D5=(D4**2)+(4.0*D3*CONST(6))
    IF(D5.LE.0.0) GO TO 300
    CALCD=(-D4+(SQRT(D5)))/(2.0*D3)
    CONTINUE
    CALM1=1.0+(CONST(1)*CALCD)
    CALM2=(ALPHA+1)*CONST(1)*U(1)*U(3)*OCT*CONC*CALCD
    CALM3=CALM1+CALM2
    CALM4=2.0*CONST(1)*CONST(2)*CALCD
    CALCMA=OCT*CONC*(CALCD**2)
    CALM5=(ALPHA+2)*CONST(1)**2*U(1)*U(3)*U(2)*CALCMA
    CALM6=CALM4+CALM5
    CALO=ALPHA*U(3)*OCT*CONC
    CALM7=(CALM3**2)+(4.0*CALM6*(XX(1)-CALO))
    IF(CALM7.LE.0.0) GO TO 300
    CALCM=(-CALM3+(SQRT(CALM7)))/(2.0*CALM6)
    OCT=CALCM**ALPHA
    CONTINUE
    D1=1.0*(CONST(1)*CALCM)+(CONST(2)*CONST(1)*(CALCM**2))
    D2=CONST(1)*U(1)*U(3)*CALCM*OCT*CONC
    D3=2.0*(CONST(1)**2)*U(1)*U(2)*U(3)*(CALCM**2)*OCT*CONC
    D4=D1+D2
    D5=(D4**2)+(4.0*D3*CONST(6))
    IF(D5.LE.0.0) GO TO 300
    CALCN=(-D4+(SQRT(D5)))/(2.0*D3)
    RATIO=ABS(CALCD-CALCN)

```

210


```

AD=CALCD/CONST(6)
AMD=CALCM*CONST(1)*AD
AMMD=CONST(2)*CALCM*AMD
AMD20=2.0*CONST(1)*U(2)*CALCM+CALCD*AMD0
CALC1=(AD*CONST(3))+(AMD*CONST(4))+(AMMD*CONST(5))
CALC2=(AMD0*U(4))+(AMD20*U(5))
CALC=CALC1+CALC2
IF(IMETH.NE.-1) GO TO 35
RETURN
35 CONTINUE
RETURN
RESID= CALC-XX(2)
RETURN
300 CONTINUE
RESID=1.0E5
CALCM=0.1
RETURN
3 CONTINUE
RETURN
4 CONTINUE
RETURN
5 CONTINUE
IF(IMETH.NE.-1) GO TO 20
RETURN
20 CONTINUE
RETURN
9 CONTINUE
RETURN
10 CONTINUE
RETURN
11 CONTINUE
RETURN
12 CONTINUE
RETURN
13 CONTINUE
RETURN
END

```

*FCR	32	100	6	0.0001
CALCULATION OF THE EQUILIBRIUM CONSTANT FOR NA22CMP WITH ETDBR				
	1.6E046.25	1.279	1.429	0.020
	1.9E04	5.2E020.1	1.192	
	37	100	6	0.0001

[illegible]

[illegible]

```

*EOF
0.30M TMA25GMP 100
0.03472 0.00001 3.16 0.1
0.003356 0.00001 4.30 0.1
0.003247 0.00001 5.09 0.1
0.61M NA25GMP 100
0.00330 0.00001 4.90 0.1
0.003195 0.00001 5.16 0.1
0.003096 0.00001 5.51 0.1
0.15M NA22GMP 100
0.003247 0.00001 3.37 0.1
0.003145 0.00001 4.13 0.1
0.003067 0.00001 5.11 0.1
0.003413 0.00001 1.16 0.1
0.80M TMA22GMP 100
0.003226 0.00001 4.41 0.1
0.003125 0.00001 5.15 0.1
0.003390 0.00001 2.51 0.1
0.003279 0.00001 4.01 0.1
1.01M NA22GMP 100
0.003125 0.00001 4.40 0.1
0.003077 0.00001 5.20 0.1
0.003030 0.00001 5.36 0.1
0.002941 0.00001 5.66 0.1
0.91M NA22GMP 100
0.003333 0.00001 2.2 0.1
0.003226 0.00001 3.12 0.1
0.003125 0.00001 3.98 0.1
0.003030 0.00001 4.68 0.1
0.002941 0.00001 5.25 0.1

1 0.00001 0.00001 3.86 0.1
0.003413 0.00001 4.38 0.1
0.003300 0.00001 5.33 0.1
0.003195 0.00001
1 0.00001 0.00001 5.06 0.1
0.003247 0.00001 5.43 0.1
0.003145 0.00001
1 0.00001 0.00001 3.81 0.1
0.003195 0.00001 4.85 0.1
0.003096 0.00001 4.89 0.1
0.003040 0.00001
1 0.00001 0.00001 4.73 0.1
0.003175 0.00001 5.93 0.1
0.003077 0.00001 5.98 0.1
0.003030 0.00001
1 0.00001 0.00001 4.95 0.1
0.003026 0.00001 5.34 0.1
0.003058 0.00001 5.45 0.1
0.002985 0.00001
1 0.00001 0.00001 2.7 0.1
0.003279 0.00001 3.57 0.1
0.003175 0.00001 4.37 0.1
0.003077 0.00001 4.91 0.1
0.002985 0.00001

```


0.30M TMA25GMP							
6 100							
0.003472 0.00001	3:16	0:1	2 0.0001	0.00001	3:86	0:1	
0.003356 0.00001	4:30	0:1	0.003300 0.00001	0.00001	4:38	0:1	
0.003247 0.00001	5:09	0:1	0.003195 0.00001	0.00001	5:33	0:1	
0.61M NA25GMP							
5 100							
0.00330 0.00001	4:90	0:1	2 0.0001	0.00001	5:06	0:1	
0.003195 0.00001	5:16	0:1	0.003247 0.00001	0.00001	5:43	0:1	
0.003096 0.00001	5:51	0:1	0.003145 0.00001	0.00001			
0.15M NA22GMP							
7 100							
0.003247 0.00001	3:37	0:1	2 0.0001	0.00001	3:81	0:1	
0.003145 0.00001	4:13	0:1	0.003195 0.00001	0.00001	4:85	0:1	
0.003057 0.00001	5:11	0:1	0.003096 0.00001	0.00001	4:89	0:1	
0.003413 0.00001	1:16	0:1	0.003040 0.00001	0.00001			
0.40M TMA22GMP							
7 100							
0.003226 0.00001	4:41	0:1	2 0.0001	0.00001	4:73	0:1	
0.003125 0.00001	5:15	0:1	0.003175 0.00001	0.00001	5:93	0:1	
0.003393 0.00001	2:51	0:1	0.003077 0.00001	0.00001	5:98	0:1	
0.003279 0.00001	4:01	0:1	0.003050 0.00001	0.00001			
1.81M NA22GMP							
7 100							
0.003125 0.00001	4:40	0:1	2 0.0001	0.00001	4:95	0:1	
0.003077 0.00001	5:20	0:1	0.003096 0.00001	0.00001	4:34	0:1	
0.003030 0.00001	5:36	0:1	0.003058 0.00001	0.00001	5:45	0:1	
0.002941 0.00001	5:66	0:1	0.002985 0.00001	0.00001			
0.91M NA22GMP							
9 100							
0.003353 0.00001	2:22	0:1	2 0.0001	0.00001	2:7	0:1	
0.003226 0.00001	3:12	0:1	0.003279 0.00001	0.00001	3:57	0:1	
0.003125 0.00001	3:98	0:1	0.003175 0.00001	0.00001	4:37	0:1	
0.003030 0.00001	4:68	0:1	0.003077 0.00001	0.00001	4:91	0:1	
0.002941 0.00001	5:25	0:1	0.002985 0.00001	0.00001			

APPENDIX 2

AMINO PROTON EXCHANGE THEORY AND
THE PROGRAM LORENT

2.1. Amino Proton Exchange Theory

The following is taken from References 148 and 185.

Consider a two proton exchange in which there are sites with different local fields giving a resonance with two components A and B shifted by $+\delta\omega/2$ and $-\delta\omega/2$ from their average angular frequency. The relative intensities of these components are directly proportional to the proton fractions P_A and P_B contributing to each component.

The process in question interchanges protons between sites A and B, so if the protons N_A and N_B at each site are labeled N_A^* and N_B^* at some instant

$$dN_A^*/dt = -k_A N_A^* \quad (A2-1)$$

$$dN_B^*/dt = -k_B N_B^* , \quad (A2-2)$$

where $k_A P_A = k_B P_B$. The average lifetime of protons at each site is therefore

$$\tau_A = 1/k_A = \tau/P_B \quad \text{and} \quad \tau_B = 1/k_B = \tau/P_A ,$$

where $\tau = \tau_A \tau_B / (\tau_A + \tau_B)$. When averaged over the proton interchange, the total magnetization M is that given by (A2-3)

$$M = \frac{i\omega_1 M_0 [(\tau_A + \tau_B) + \tau_A \tau_B (\alpha_A P_B + \alpha_B P_A)]}{(1 + \alpha_A \tau_A)(1 + \alpha_B \tau_B) - 1}, \quad (A2-3)$$

where ω_1 is the applied rf field, M_0 is the static nuclear magnetization at thermal equilibrium, and $\alpha_A = (1/T_2) - i(\Delta\omega + \delta\omega/2)$ and $\alpha_B = (1/T_2) - i(\Delta\omega - \delta\omega/2)$. This assumes the resonance absorption is plotted at a constant static magnetic field as a function of the different $\Delta\omega$ between the applied radiofrequency and the frequency at the center of the two resonance components. In the absence of exchange effects or overlap, the width of each component at half-maximum is $2/T_2$; this includes all contributions to the line width such as field inhomogeneities and implies a Lorentzian line shape, $\delta\omega$ is the separation of the components assuming no exchange and no overlap of the components. The expansion of Equation (A2-3) with retention of only the imaginary part v , gives

$$v = \frac{\omega_1 M_0 [(1 + \tau/T_2)P + QR]}{P^2 + R^2} \quad (A2-4)$$

where

$$P = \tau[(1/T_2)^2 - (\Delta\omega)^2 + (\delta\omega/2)^2] + 1/T_2$$

$$Q = \tau[\Delta\omega - (\delta\omega/2)(P_A - P_B)]$$

$$R = \Delta\omega[1 + (2\tau/T_2)] + (\delta\omega/2)(P_A - P_B)$$

2.2. The Program LORENT

This program pp (258) - (263) calculates theoretical NMR line shapes for the case of general two site exchange. The equations used are those given in 2.1.

TITLE: LORENT.FTN - GENERATE AND PLOT NMR LINES FOR GENERAL
TWO SITE EXCHANGE

AUTHOR: T V ATKINSON
DEPARTMENT OF CHEMISTRY
MICHIGAN STATE UNIVERSITY
EAST LANSING, MI 48824

DATE: 23-APR-80

THIS PROGRAM CALCULATES THEORETICAL NMR LINE SHAPES FOR THE CASE
OF GENERAL TWO SITE EXCHANGE. THE EQUATIONS USED ARE THOSE
GIVEN IN ROGERS AND WOODBREY, J. PHYS. CHEM., 66, 540 (1962).

THIS PROGRAM WAS ADAPTED FROM THE DECEMBER 1969 PROGRAM OF
D. A. CASE. THIS VERSION ALLOWS GRAPHICS VIA THE MSU VECTOR
PACKAGE.

PARAMETER DICTIONARY (INPUT WILL BE PROMPTED)

IDENT A STRING OF UP TO 72 CHARACTERS OF IDENTIFYING INFO.

XMARO THE DISTANCE(CM) BETWEEN THE EDGE OF THE PLOT AND THE
LEFT SIDE DATA AREA

XLNGTH THE HORIZONTAL DIMENSION(CM) OF THE DATA PLOTTING AREA

YMARO THE DISTANCE(CM) BETWEEN THE BOTTOM OF THE PLOT AND THE
BOTTOM OF THE DATA AREA

VLNGTH THE VERTICAL DIMENSION(CM) OF THE DATA PLOTTING AREA

NPNTS NUMBER OF POINTS TO BE CALCULATED

OMEGAL FREQUENCY(HZ) OF THE LEFT SIDE OF THE DATA PLOTTING AREA

HERTZ/CM NUMBER OF HERTZ PER CM(HORIZONTAL)

AMPLIT HEIGHT(CM) OF PEAK (RELATIVE TO BASE LINE AT OMEGAL)

NUAX DISTANCE(HZ) BETWEEN TWO PEAKS

T2A, T2B TRANSVERSE RELAXATION TIMES FOR THE DOWN FIELD AND UP
FIELD COMPONENTS RESPECTIVELY

PA, PB POPULATIONS OF THE DOWNFIELD AND UPFIELD COMPONENTS

TAU LIFETIME, OR THE INVERSE OF TWICE THE RATE CONSTANT

THIS IS AN INTERACTIVE PROGRAM FOR SIMULATING NMR SPECTRA. UPON
ENTRY THE INPUT OF THE ABOVE PARAMETERS IS PROMPTED AND PERFORMED.
THE LAST PROMPT IS "TAU, OPTION: " AND IS ACTUALLY THE MAIN
CONTROL POINT OF THE PROGRAM. THIS PROMPT LEADS TO THE INPUT OF

```

C      FOUR VARIABLES ON ONE LINE.  THE VARIABLES ARE DESCRIBED BELOW
C
C      TAU      REAL      SEE ABOVE
C
C      OPTION   INTEGER   CONTROL OF OPERATION
C                      =0 GENERATE THE CURRENT SIMULATION AND PLOT
C                        ON GRAPHIC DEVICE IDDEV
C                      =1 GO TO PARAMETER INPUT PHASE
C                      =2 DUMP PARAMETERS ON LOGICAL UNIT LUNOUT
C                      =3 DUMP PARAMETERS AND SIMULATED OMEGA'S AND
C                        AMPLITUDES ON LOGICAL UNIT LUNOUT
C
C      IDDEV    INTEGER   MSU GRAPHIC DEVICE NUMBER
C                      =1 TEKTRONIX
C                      =2 VERSATEC
C                      =3 PRINTRONIX
C
C      LUNOUT   INTEGER   FORTRAN LOGICAL UNIT FOR OUTPUT
C                      =0,5 OUTPUT TO TERMINAL TI:
C                      OTHERWISE TO FORNNN.DAT WHERE NNN=LUNOUT

```

```

C-----
C      INPUT FOR TAU AND THE SIMULATION PARAMETERS ALLOWS FREE FORMAT
C      IN THAT A NULL ARGUMENT (" . . . . ." OR <CR> BEFORE ALL
C      PARAMETERS HAVE BEEN INPUT) CAUSES THE LAST VALUE FOR THAT
C      PARAMETER TO BE USED.
C-----

```

```

C      REAL*4 HMAX,K1,K2,LL,NUA,NUAX,NUA2,INTOCH
C      INTEGER*2 OPTION
C      BYTE IDENT(72),STRNG(72),ITIME(10),IDATE(8)
C      DIMENSION OMEGA (600),HERTZ(600),AMP(600)
C      DIMENSION PHYS(8)
C      DATA XMARG,YMARG,XLNQTH, YLNQTH/1.,1.,15.,12./
C      DATA INTOCM/2.54/
C      DATA BUFFN/600./
C      DATA IDDEV/1/
C      EQUIVALENCE (PHYS(1),XMARG),(PHYS(2),YMARG),(PHYS(3),XLNQTH)
C      EQUIVALENCE (PHYS(4),YLNQTH)
C
C      LUNTI = 5
C      NMRD = 1
C      NMWR = 2
C      TWOPI = 2.0*3.14159
C      WRITE (LUNTI,8531)

```

```

C-----
C      INPUT PARAMETER VALUES
C-----

```

```

50      WRITE (LUNTI,8499)
        READ (LUNTI,8002) L1,STRNG
        IF (L1.EQ.0) GO TO 52
        DO 51 I=1,L1
51      IDENT(I) = STRNG(I)
        LIDENT = L1
52      CONTINUE
        WRITE (LUNTI,8500)

```

```

DO 15 M=1, NPNTS
OMEGAX = OMEGAX + K2
OMEGA(M) = TWOPI*OMEGAX
HERTZ(M) = OMEGAX
C
C   CALCULATE AMPLITUDES
C
P = TAU*(P1-OMEGA(M)**2+NUA2)+ P2
Q = TAU*(OMEGA(M)-Q1)
R = OMEGA(M)*R1+R2
AMP(M) = (P*A1+Q*R)/(P**2+R**2)
15  CONTINUE
C
C   CALCULATE AMPMAX
C
AMPMAX = AMP(1)
ICMAX = 1
DO 16 IC=2, NPNTS
IF (AMPMAX.GT.AMP(IC)) GO TO 16
22  AMPMAX = AMP(IC)
ICMAX = IC
16  CONTINUE
HMAX = AMPMAX/2.
C
C   FIND HALFWIDTH
C
ICFRST = 1
DO 110 I=1, NPNTS
IF (HMAX.GE.AMP(I)) GO TO 110
ICFRST = I
GO TO 112
110  CONTINUE
112  ICLAST = ICMAX
DO 113 I=ICMAX, NPNTS
IF (HMAX.LE.AMP(I)) GO TO 113
ICLAST = I
GO TO 114
113  CONTINUE
ICLAST = ICFRST
114  CONTINUE
C
C-----
C
C   PRINT PARAMETERS
C
C-----
C
CALL TIME (ITIME)
CALL DATE(IDATE)
WRITE (LUN, 8531)
WRITE (LUN, 8530) IDATE, ITIME
WRITE (LUN, 8519) IDENT
WRITE (LUN, 8520) XMARG, YMARG
WRITE (LUN, 8521) XLNGTH, YLNGTH
WRITE (LUN, 8522) NPNTS
WRITE (LUN, 8524) OMEGAL, OMEGCM
WRITE (LUN, 8525) AMPLIT
WRITE (LUN, 8526) NUAX
WRITE (LUN, 8527) T2A, T2B, PA, PB
WRITE (LUN, 8529) TAU
WRITE (LUN, 17) AMPMAX, HMAX
WRITE (LUN, 8528) HERTZ(ICMAX), HERTZ(ICLAST)-HERTZ(ICFRST)

```



```

READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1-L2-1.GT.0) XMARG = R1
IF (L2.GT.0) YMARG = R2

WRITE (LUNTI,8501)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1-L2-1.GT.0) XLNGTH = R1
IF (L2.GT.0) YLNGTH = R2

WRITE (LUNTI,8502)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1.GT.0) NPNTS = AMIN1 (ABS(R1),BUFFN)

WRITE (LUNTI,8504)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1-L2-1.GT.0) OMEGAL = R1
IF (L2.GT.0) OMEGCM = R2
IF (R2.EQ.0.0) OMEGCM = 20.

WRITE (LUNTI,8505)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1.GT.0) AMPLIT = R1

WRITE (LUNTI,8506)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1.GT.0) NUAX = R1

WRITE (LUNTI,8507)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1-L2-1.GT.0) T2A = R1
IF (L2-L3-1.GT.0) T2B = R2
IF (L3-L4-1.GT.0) PA = R3
IF (L4.GT.0) PB = R4
NUA = NUAX*3.1415927

-----
CONTROL POINT AND TAU INPUT
-----

100 WRITE (LUNTI,8508)
READ (LUNTI,8000,END=5) L1,R1,L2,R2,L3,R3,L4,R4
IF (L1-L2-1.GT.0) TAU = R1
OPTION = R2
IDDEV = R3
LUN = R4
IF (LUN.EQ.0) LUN = 5
IF (IDDEV.LE.0) IDDEV = 1
IF (OPTION.EQ.1) GO TO 50

CYCLE THRU OMEGA VALUES

K2 = OMEGCM*XLNGTH/NPNTS
OMEGAX = OMEGAL - K2
P1 = 1. / (T2A*T2B)
P2 = PB/T2B + PA/T2A
NUA2 = NUA*NUA
Q1 = NUA*(PA-PB)
R1 = 1. +TAU*(1.0/T2A+1.0/T2B)
R2 = TAU*NUA*(1. /T2B-1. /T2A)+NUA*(PA-PB)
A1 = 1. + TAU*(PB/T2A + PA/T2B)

```

```

C
C-----
C
C      PLOT SPECTRUM
C-----
C
      IF (OPTION.NE.0) GO TO 200
      PHYS(1) = XMARG/INTOCM
      PHYS(2) = XLNGTH/INTOCM
      PHYS(3) = YMARG/INTOCM
      PHYS(4) = YLNGTH/INTOCM
      PHYS(5) = HERTZ(1)
      PHYS(6) = HERTZ(NPNTS)
      PHYS(7) = 0.0
      PHYS(8) = YLNGTH*AMPMAX/AMPLIT
      CALL PLOT (HERTZ,AMP,NPNTS,PHYS,IDDEV,1)
      GO TO 100

C
C-----
C
C      PRINT SPECTRA
C-----
C
200    IF (OPTION.NE.3) GO TO 100
        LIMIT = NPNTS/3
        DO 19 ID=1,LIMIT
            LIMIT1 = ID + LIMIT
            LIMIT2 = LIMIT1 + LIMIT
            WRITE (LUN,20) HERTZ(ID),AMP(ID),HERTZ(LIMIT1),AMP(LIMIT1),
               1 HERTZ(LIMIT2),AMP(LIMIT2)
19      CONTINUE
13      CONTINUE
        GO TO 100
5        WRITE (LUN,21)

C
C-----
C
C      FORMATS
C-----
C
8000    FORMAT (G,F15.0,G,F15.0,G,F15.0,G,F15.0)
8001    FORMAT (F15.6,3I8)
8002    FORMAT (G,72A1)

C
8499    FORMAT (///'IDENT: ')
8500    FORMAT ('$XMARG(CM),YMARG(CM): ')
8501    FORMAT ('$XLNGTH(CM),YLNGTH(CM): ')
8502    FORMAT ('$NPNTS: ')
8504    FORMAT ('$OMEGA-LEFT(HZ),HERTZ/CM: ')
8503    FORMAT ('$AMPLITUDE(CM): ')
8506    FORMAT ('$NUAX: ')
8507    FORMAT ('$T2A,T2B,PA,PB: ')
8508    FORMAT ('$TAU,OPTION: ')
8519    FORMAT (' IDENT: ',72A1)
8520    FORMAT (' XMARG(CM),YMARG(CM): ',2F8.3)
8521    FORMAT (' XLNGTH(CM),YLNGTH(CM): ',2F8.3)
8522    FORMAT (' NPNTS: ',I8)
8524    FORMAT (' OMEGA-LEFT(HZ),HERTZ/CM: ',2E15.5)
8525    FORMAT (' AMPLITUDE(CM): ',F8.3)

```

```
8526  FORMAT ( ' NUAX. ', E15. 6)
8527  FORMAT ( ' T2A, T2B, PA, PB: ', 4E15. 6)
8528  FORMAT ( ' LOCATION OF MAX, HALF-WIDTH: ', 2E15. 6)
8529  FORMAT ( ' TAU: ', E15. 6)
8530  FORMAT (10A1, 8A1)
8531  FORMAT ( ' LORENT VERSION 24-APR-80')
17    FORMAT (3F15. 9)
20    FORMAT (3(5X, F8. 3, F15. 9))
21    FORMAT (19H1FUNCTION COMPLETED)
      END
```

APPENDIX 3

ADDITIONAL DATA AND SPECTRA

3.1. T₁ measurements on various 2'GMP solutions

Measurements of T₁'s were performed by using the inversion recovery method (1.1.2.). The estimates of T₁'s are listed below.

<u>0.05M Li₂2'GMP 27°C</u>	
<u>Resonance</u>	<u>T₁</u>
H(8)	1.01±0.52
H(1')	1.28±0.44

0.71M Na₂2'GMP + 1.1M NaCl
+ 1.36M TMAc1 -3°C

<u>Resonance</u>	<u>T₁</u>
H(8)	0.731±0.509
H(1')	0.967±0.502
HDO	0.505±0.506
TMA ⁺	1.652±0.503
H(4')	0.461±0.507
H(5')	0.244±0.504

<u>0.82M K₂2'GMP -3°C</u>	
<u>Resonance</u>	<u>T₁</u>
8.108	0.474±0.530
7.808	0.418±0.510
7.802	0.389±0.536
7.685	0.381±0.520
7.332	0.510±0.531
7.128	0.549±0.526
7.120	0.537±0.531

<u>0.30M Rb₂2'GMP 27°C</u>	
8.003	0.166±.87
6.017	0.532±.577
5.996	0.480±0.739
5.133	0.372±0.656
5.109	0.463±0.660
0.000	1.698±0.427

3.2. Transfer of Digitized Spectra from the Aspect 2000 to Another Device

The method of transferring data from the Aspect 2000 on the Bruker WM250 to floppy disks is given on pp. (267) - (270).

3.3. Other Spectra

TRANSFER OF DATA FROM THE ASPECT 2000 COMPUTER ON THE
BRUKER WM 250 NMR TO ANOTHER DEVICE

R.G. Barr
T.V. Atkinson
November 14, 1980

The Aspect 2000 computer on the Bruker WM 250 NMR has been interfaced to a portable PDP 11/03 computer via an asynchronous serial link. This allows data to be transmitted to the PDP 11/03 and stored on the latter's floppy disk. This data can then be easily transported to any other of our computers. Fortran programs can be used to analyze the digitized data (The Aspect is programable but uses Pascal). The Aspect and the larger PDP-11 in CEMCOMGRAF may be linked directly in the future.

The NMR data may be stored on more portable floppy disks or mathematically manipulated on the PDP-11 or other computers. For example, peak resolution, integration and spectral simulation can be carried out. It should be noted that the Bruker DISNMR program can do single peak resolution and the Bruker PANIC program can do spectral simulation. The PDP-11 can use the data for overlapping peak resolution [RESOLVE, TVAtkinson] and integration. It can also be used to calculate exchange rates for the general two site exchange, program LORENT, T.V. Atkinson, 23-Apr-80. The equations used are those given in Rogers and Woodney J. Phys. Chem., 66, 540 (1962).

It will also be possible to send data to be printed on the line printer. This will be useful with the DISNMR program to print out computer fitted T_1 calculations and peak resolution. The PANIC program can also plot out the simulated spectra.

TECHNICAL DESCRIPTION of the serial link

The band rate is set at 4800. The band rate selector switch is located on board 14 of the Aspect. The switch can be changed without removal of the board.

The switch near the front of the Aspect is channel A and the rear one channel B.

<u>Dial setting</u>	<u>Band rate</u>	<u>Dial setting</u>	<u>Band rate</u>
1		6	2400
2	150	7	4800
3	300	8	9600
4	600	9	18200
5	1200	10	38400

Schematics can be found in the "Aspect-2000 Computer Manual Sept., 1979 Fred Rymka.

Spectra are transmitted and accepted as series of numbers representing the actual memory contents of the system as ASC II coded decimal, signed integers. Each transmission starts with the "Begin of text" - character Ctrl-B, followed by the word "BOT" and a carriage return. After each decimal number a CR is transmitted. The end of the transmission is announced with "EOT" and CR. "Line Feeds" are inserted after each "carriage return" by the receiving program. Any block size may be used (1 word....32K words).

The Aspect end of the link is tended by the program BICON. The BICON prompt is >. The command list is:

- EX Return to calling program (i.e. FTNMR Program)
EX may not be used if "BICON" was started at its starting address using the computer's switch register but only after a "USER-FUNCTION-CALL" (.U2)
- MO Call Disk Monitor. This command must not be used unless either the "DIABLO" or the "FLOPPY" disk monitor is ready in core: Else disaster may result!

Data Block Commands:

- .ZE Clear Data Block defined by .SI, .ST
- .SI Size of block, in words (not in 1K-units!)
- .ST Start of block. In words starting at begin of data memory
- .SH Number of times data are divided by 2 before transmission to the other CPU. This may be useful if it is desired to feed data to a 16-bit system in single precision. etc. Note that it is not possible to multiply data before transmission and the bruker system cannot accept data larger than its 20-bit word length permits.

Decimal-Integer Transfer Commands - These commands use the .SI/.ST limits

.HP Punch data on high speed punch
 .PT Punch data on TTY punch (=write them onto paper)
 .HR Read data from high speed reader
 .RT Read data from TTY reader (or from keyboard)
 .RC Receive data via the RS-232 interface
 .XM Transmit data via the RS-232 interface

During data output, the limits .SI/.ST are used. During data input, the first data word is deposited at .ST and no more than SI words are accepted from the other device. If loading less than SI words, the "E"-character must appear at the end of the data set.

The memory of the Aspect is in the following format.

32K spectra

0	24000	64000	100000	124000
Program memory		Real Spectra	Imaginary Spectra	Parameters

16K spectra

0	24000	40000	44000	64000
Program memory		Real Spectra	Parameters	Imaginary Spectra

To determine the address of a data point place the cursor on the data point and use the E command (see Bruker Aspect 2000 manual). This number is decimal and must be converted to octal and added (octal) to 24000. The spectra must be in the form wanted when transmitted. (e.g. transformed and phased). With the spectra in memory call BICON and transmit portion of spectra wanted.

The PDP 11/03 end of the link is tended by the Program NCDC which runs under the operating system RT11. If the PDP 11/03 of CEM 838 is being used the floppy disks used to store data must be formatted with a physical interleaving of one sector between sequential numbered sectors. The PDP 11/03 is initialized as per the CEM 838 experiment RT1. Once RT 11 is running and a properly initialized floppy is in drive DX1:, RUN NCDC. The answer to the first question is "carriage return". At this time the 11/03 is listening to the Aspect. Type a "]" and NCDC will go into

command mode. Enter a "GLCL" request the flow of information from the serial link into a file. NCDC will then prompt your entering a file name to receive the information. Next type an "R CR " to return to listening mode. At the end of a transfer, go to command mode, type "G CR ". When a file specification is requested, type a " CR ". This procedure is necessary to close the data file properly. (More information can be found in the documentation file for the program CDC.)

Programs and more information on data transfer can be obtained from NMR services.

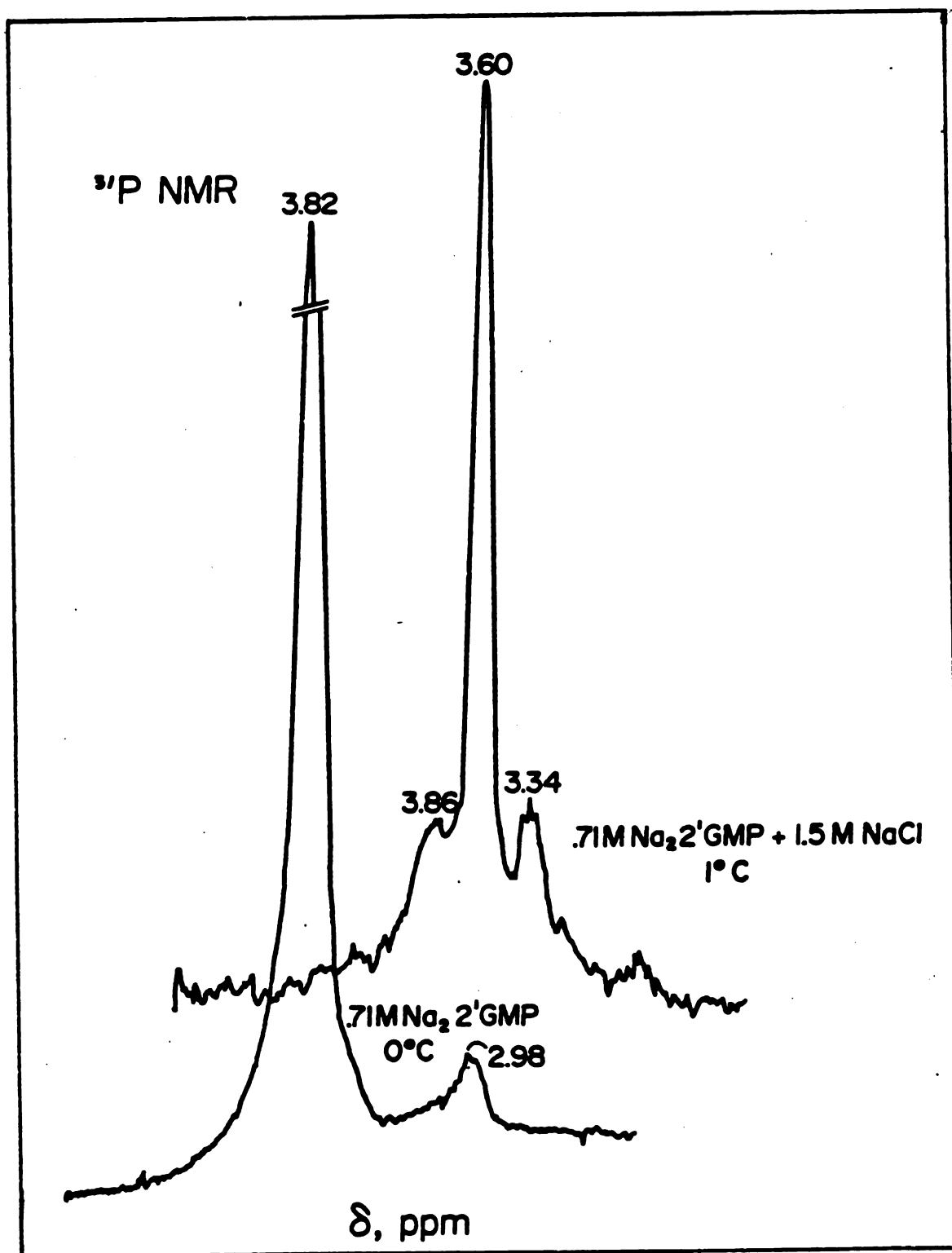


Figure 88. The ^{31}P NMR spectra of $0.71\text{M Na}_2 2'\text{GMP}$ at 0°C and $0.71\text{M Na}_2 2'\text{GMP}$ 1.5M in NaCl at 1°C .

REFERENCES

REFERENCES

1. D. Voet and A. Rich, Progr. Nucl. Acid Res. Mol. Biol., 10, 183 (1970).
2. P. O. P. Ts'o, "Basic Principles in Nucleic Acid Chemistry", P. O. P. Ts'o, Ed., Academic Press, New York, Vol. 1, 1974, pp. 455-457.
3. J. Donohue and K. Trueblood, J. Mol. Biol., 2, 363 (1960).
4. M. Sundaralingham, Biopolymers, 7, 821 (1969).
5. R. H. Sarma, C. H. Lee, F. E. Evans, N. Yathindra, and M. Sundaralingam, J. Am. Chem. Soc., 96, 7337 (1974).
6. C. Altona and M. Sundaralingam, J. Am. Chem. Soc., 95, 2333 (1972).
7. F. S. Ezra, C-H Lee, N. S. Kondo, S. S. Danyluk, and R. H. Sarma, Biochemistry, 16, 1977 (1977).
8. S. Kang, Jer. Sym. Quant. Chem. Biochem., V, 271, (1973).
9. B. Pullman & H. Berthod, Jer. Sym. Quant, Chem. Biochem., V, 209, (1973).
10. M. Sundaralingam and E. Westhof, Int. Jour. Quan. Chem. Quant. Bio. Sym., 6, 115 (1979).
11. D. B. Davies, Prog. NMR Spec., 12, 135 (1978).
12. P. O. P. Ts'o, Basic Principles in Nucleic Acid Chemistry. P. O. P. Ts'o Ed. Academic Press, New York, Vol. 1 (1974), pp. 471-488.
13. M. Sundaralingam, Proc. Jer. Sym. Quan. Chem. Bioc., 4, 44 (1972).
14. J. Pitha, R. N. Jones, and P. Pithora, Can. J. Chem., 44, 1045 (1966).
15. Y. Kyogoku, R. Lord, and A. Rich, Science, 154, 513 (1966).

16. E. Kuchler and J. Derkosch, Z. Naturforsch., 21, 209 (1966).
17. R. M. Hamlin Jr., R. C. Lord, and A. Rich, Science, 148, 1734 (1965).
18. R. R. Shoup, H. T. Miles, and E. D. Becker, Biochem. Biophys. Res. Comm., 2, 194 (1966).
19. L. Katz and S. Penman, J. Mol. Biol., 15, 220 (1966).
20. S. M. Wang and N. C. Li, J. Am. Chem. Soc., 90, 5069 (1968).
21. M. Raszka and N. O. Kaplan, Proc. Nat. Acad. Sci. U.S.A., 69, 2025 (1972).
22. F. E. Bugg, V. Thewalt and R. E. Marsh, Biochem. Biophys. Res. Comm., 33, 486 (1968).
23. P. O. P. Ts'o, "Basic Principles in Nucleic Acid Chemistry", P. O. P. Ts'o Ed., Academic Press, New York, 1974, Vol. 1, pp. 526-562.
24. G. Gupta and V. Sasisekharan, Nucleic Acid Research, 5, 1639 (1978).
25. S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, J. Am. Chem. Soc., 86, 4182 (1964).
26. M. P. Schweizer, S. I. Chan and P. O. P. Ts'o, J. Am. Chem. Soc., 87, 5241 (1965).
27. A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, J. Am. Chem. Soc., 89, 3612 (1967).
28. M. P. Schweizer, A. D. Broom, P. O. P. Ts'o and D.P. Hollis, J. Am. Chem. Soc., 90, 1042 (1968).
29. F. E. Evans, C. H. Lee, and R. H. Sarma, Biochem. Biophys. Res. Commun., 63, 106 (1975).
30. P. O. P. Ts'o and S. I. Chan, J. Am. Chem. Soc., 86, 4176, (1964).
31. H. Klump, Berichte, 80, 121 (1976).
32. A. Bierzynski, H. Kozłowska and K. L. Wierzchowski, Biophys. Chem., 6, 213 (1976).
33. A. Bierzynski, H. Kozłowska and K. L. Wierzchowski, Biophys. Chem., 6, 223 (1976).

34. A. Bierzynski, H. Kozłowska, K. L. Wierzchowski and Z. Proba, Biophys. Chem., 6, 231 (1976).
35. S. Hanlon, Biochem. Biophys. Res. Commun., 23, 861 (1966).
36. W. Schimmack, H. Sapper, and W. Lohmann, Biophys. Struct. Mechanism, 1, 311 (1975).
37. E. Plesiewicz, E. Stepien, K. Drystyna and K. L. Wierzchowski, Nucl. Acids Res., 3, 1295 (1976).
38. G. Makrigiannis, P. Papagiannakopoulos, and T. Theophanides, Inorg. Chim. Acta., 46, 263 (1980).
39. S. J. Lippard, Acc. Chem. Res., 11, 211 (1978).
40. M. H. Grant, K. C. Wu, W. R. Bauer, S. J. Lippard, Biochem., 15, 4339 (1976).
41. A. T. M. Marcelis, C. G. VanKralinaen, and J. Reedejk, Journal of Inorg. Biochem., 13, 213 (1980).
42. H. Pezzano and F. Podo, Chem. Rev., 80, 365 (1980).
43. G. L. Eichorn et al. in "Metal Ions in Biological Systems", S. K. Dhar, Ed. Plenum Press, New York, 1973, pp. 43-46.
44. L. G. Marzilli, Progress in Inorganic Chemistry, 23, 255 (1977).
45. G. L. Eichorn, Inorganic Biochemistry, 2, 1210 (1973).
46. S. Shimokawa, H. Fukui, J. Sohma and K. Hotta, J. Am. Chem. Soc., 95, 1777 (1973).
47. Ch. H. Chang and L. G. Marzilli, J. Am. Chem. Soc., 96, 3656 (1974).
48. T. Yokono, S. Shimokawa and J. Sohma, J. Am. Chem. Soc., 97, 3827 (1975).
49. E. Bouhoutsas-Brown, Ph.D. Thesis, Michigan State University, East Lansing, Michigan, 1980.
50. G. S. Manning, Biopolymers, 11, 937 (1972).
51. M. T. Record, C. F. Anderson, T. M. Lohman, Quarterly Review of Biophysics, 11, 103 (1978).
52. G. S. Manning, Acc. Chem. Res., 12, 443 (1979).

53. R. E. Harrington, Biopolymers, 19, 449 (1980).
54. H. T. Miles, F. B. Howard, and J. Frazier, Science, 142, 1458 (1963).
55. C. Davanloo and D. M. Crothers, Biochemistry, 15, 4433 (1976).
56. H. M. Sobell, Prog. Nucleic Acid Res. Mol. Biol., 13, 153 (1973).
57. H. Lackner, Angew. Chem. Int. Ed. Engl., 14, 375 (1975).
58. R. V. Kastrup, M. A. Young, T. R. Krugh, Biochem., 17, 4855 (1978).
59. T. R. Krugh and C. A. Reirhardt, J. Mol. Biol., 97, 133 (1975).
60. T. R. Krugh, F. M. Wittlin, S. R. Cramer, Biopolymers, 14, 197 (1975).
61. T. R. Reinhardt and T. R. Krugh, Biochem., 17, 4845 (1978).
62. J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lallemand, D. Mansey, J. Am. Chem. Soc., 102, 5565 (1980).
63. A. E. V. Haschemeyer and A. Rich, J. Mol. Biol., 27, 369 (1967).
64. F. Jordan and A. Pullman, Theor. Chim. Acta., 9, 242 (1968).
65. E. D. Bergman and H. Weiler-Feilchenfeld, Proc. Jer. Sym. Quan. Chem. Bioc. 4, 21 (1972).
66. I. Bang, Bioch. Z., 26, 293 (1910).
67. J. F. Chantot, M. T. Sarocchi and W. Guschelbauer, Biochimie, 53, 347 (1971).
68. J. F. Chantot and W. Guschlbauer, FEBS Letters, 4, 173 (1969).
69. J. F. Chantot, T. Haertle, and W. Guschlbauer, Biochimie, 56, 501 (1974).
70. J. M. Delabar, and W. Guschlbauer, Biopolymers, 18, 2073 (1979).

71. J. F. Chantot and W. Guschlbauer, Jer. Sym. Quant. Chem. Biochem., IV, 205 (1972).
72. M. Gellert, M. N. Lepsett, and D. R. Davies, Proc. Nat. Acad. Sci., USA, 48, 2013 (1962).
73. J. P. Leicknam, C. Chauvelier, J. F. Chantot, and W. Guschlbauer, Biophys. Chem., 1, 134 (1973).
74. W. Guschlbauer, J. F. Chantot, M.-Th. Sarocchi and D. Thiele, Studia Biophysica, Berlin, Band 24/25, 29-36 (1970).
75. J. F. Chantot, Archives of Biochemistry and Biophysics, 153, 374 (1972).
76. P. Tougaard, J. F. Chantot and W. Guschlbauer, Biochim. et Biophys. Acta., 308, 9 (1973).
77. S. B. Zimmerman, J. Mol. Biol., 106, 663 (1976).
78. R. B. Hcmer and S. F. Mason, Chem. Comm., 11, 332 (1966).
79. F. B. Howard, J. Frazier and H. T. Miles, Jour. Biol. Chem., 247, 6733 (1972).
80. J. Iball, C. H. Morgan, H. R. Wilson, Nature, 199, 688 (1963).
81. S. B. Zimmerman, G. H. Cohen, and D. R. Davies, J. Mol. Biol., 92, 171 (1975).
82. H. T. Miles and J. Frazier, Biochim. Biophys. Acta., 79, 216 (1964).
83. F. B. Howard and H. T. Miles, J. Biol. Chem., 240, 801 (1965).
84. E. W. Small and W. Peticolas, Biopolymers, 10, 1377 (1971).
85. V. Gramlich, H. Klump, R. Herbeck, and E. D. Schmid, FEBS Lett., 69, 15 (1976).
86. C. Becker and T. Ackerman, Ber. Bunsenge Phys. Chem., 77, 203 (1973).
87. P. L. Robinson and M. E. Grant, J. Biol. Chem., 241, 4030 (1966).
88. H. T. Miles and J. Frazier, Biochem. Biophys. Res. Comm., 49, 199 (1972).

89. T. J. Pinnavaia, H. T. Miles, and E. D. Becker, J. Am. Chem. Soc., 97, 7198 (1975).
90. V. P. Strauss and P. D. Ross, J. Am. Chem. Soc., 81, 5295 (1959).
91. J. Botts, A. Chasin, and H. L. Young, Biochem., 4, 1788 (1965).
92. R. M. Smith and R. A. Alberty, J. Phys. Chem., 60, 180 (1956).
93. P. D. Ross and R. L. Scruggs, Biopolymers, 2, 79 (1964).
94. T. J. Pinnavaia, C. L. Marshall, C. M. Mettler, C. L. Fisk, H. T. Miles, E. D. Becker, J. Am. Chem. Soc., 100, 3625 (1978).
95. G. A. Melson (Ed.), "Coordination Chemistry of Macrocyclic Compounds", Plenum Press, New York (1979).
96. I. L. Karle, Biochem., 13, 2155 (1974).
97. C. L. Marshall, Ph.D. Thesis, Michigan State University, East Lansing, MI (1980).
98. C. L. Fisk, E. D. Becker, H. T. Miles and T. J. Pinnavaia, manuscript submitted.
99. A. Paris and P. Laszlo, ACS Symp. Ser., 34, 418 (1976).
100. P. Laszlo and A. Paris, C. R. Acad. Sc. Paris, t. 286, Series D, 717 (1978).
101. C. Detellier, A. Paris, and P. Laszlo, C. R. Acad. Sc. Paris, t. 286, Series D, 781 (1978).
102. M. Borzo and P. Laszlo, C. R. Acad. Sc. Paris, t. 287, Series C, 475 (1978).
103. A. Delville, C. Detellier, and P. Laszlo, J. Mag. Res., 33, 301 (1979).
104. C. Detellier and P. Laszlo, Helvetica Chimica Acta., 62, 1559 (1979).
105. M. Borzo, C. Detellier, P. Laszlo, and A. Paris, J. Am. Chem. Soc., 103, 1124 (1980).
106. C. Detellier and P. Laszlo, J. Am. Chem. Soc., 102, 1135 (1980).

107. E. F. Gale, E. F. Cundliff, P. E. Reynolds, M. H. Richmonds, and M. J. Waring, "The Molecular Biology of Antibiotic Action", Wiley, New York, 1972.
108. L. S. Lerman, J. Mol. Biol., 3, 18 (1961).
109. T. R. Krugh and M. E. Nuss in "Biological Applications of Magnetic Resonance," R. G. Shulman, Ed., Academic Press, New York, 1979, pp. 113-175.
110. B. A. Newton in "Advances in Chemotherapy", A. Goldin and F. Hawking, Eds., Academic Press, New York, Vol. 1, 1964, p. 35
111. L. Dickinson, D. H. Chantrell, G. W. Inkley, and M. J. Thompson, Brit. J. Pharmacol., 8, 139 (1953).
112. J. F. Henderson, Cancer Res., 23, 491 (1963).
113. W. H. Elliot, Biochem. Jour., 86, 562 (1963).
114. M. J. Waring, Biochim Biophys. Acta., 87, 358 (1964).
115. J. B. LeBecqand and C. Paolette, C. R. Acad. Sci. Paris, 260, 7033 (1965).
116. J. B. LePecq and C. Paoletti, J. Mol. Biol., 27, 87, (1967).
117. V. W. F. Burns, Arch. Biochem. Biophys., 133, 420 (1969).
118. J. Vinograd and W. R. Bauer, J. Mol. Biol., 33, 141 (1968).
119. J. Vinograd and W. R. Bauer, J. Mol. Biol., 54, 281 (1970).
120. J. B. LePecq in "Methods of Biochemical Analysis", D. Glick, Ed., Wiley Interscience, New York, Vol. 20, pp. 42-86 (1971).
121. L. P. G. Wakelin and M. J. Waring, Mol. Pharm., 9, 544 (1974).
122. C. C. Tsai, S. C. Jain, and H. M. Sobell, Proc. Nat. Acad. Sci. USA, 72, 628 (1975).
123. C. G. Reinhardt and T. R. Krugh, Biochem., 16, 2890 (1977).
124. C. R. Jones, P. H. Bolton, and D. R. Kearns, Biochem., 17, 601 (1978).

125. M. J. Waring, J. Mol. Biol., 13, 269 (1965).
126. R. L. Ornstein and R. Rein, Biopolymers, 18, 2821 (1979).
127. D. J. Patel, Acc. Chem. Res., 12, 118 (1979).
128. G. P. Kreishman, S. I. Chan, and W. Bauer, J. Mol. Bio., 61, 45 (1971).
129. M. Raszka, Biochemistry, 13, 4616, (1974).
130. B. B. McConnell, Biochem., 17, 3168 (1978).
131. P. H. Bolton and D. R. Kearns, J. Am. Chem. Soc., 101, 479 (1979).
132. M. Raszka and N. O. Kaplar, Proc. Nat. Acad. Sci. USA, 71, 4546 (1974).
133. B. McConnell and P. C. Seawell, Biochem., 11, 4382 (1972).
134. M. Raszka and N. O. Kaplan, Proc. Nat. Acad. Sci. USA, 69, 205 (1972).
135. T. H. Marshall and E. Grunwald, J. Am. Chem. Soc., 91, 4541 (1969).
136. H. Iwahashi and Y. Kyogoku, J. Am. Chem. Soc., 102, 2913 (1980).
137. H. Iwagasaki and Y. Kyogoku, Jer. Sym. Quan. Chem. Bio., 11, 17 (1978).
138. G. Binsch and H. Kessler, Angew. Chem. 19, 411 (1980).
139. S. Szymanski, M. Witanowski and A. Gryff-Keller, Annual Reports on NMR Spec., 8, 228 (1978).
140. H. Teitlebaum and S. W. Englander, J. Mol. Biol., 92, 79 (1975).
141. H. Teitlebaum and S. W. Englander, J. Mol. Biol., 92, 55 (1975).
142. M. J. Lade and G. J. Thomas, Jr., Biochem., 18, 3839 (1979).
143. M. L. Martin, G. J. Martin, J.-J. Delpuech, "Practical NMR Spectroscopy", M. L. Martin, Ed., Heyden, Philadelphia, 1980, pp. 145-147.

144. A. L. VanGeet, Anal. Chem., 42, 679 (1970).
145. P. L. Biochemicals, Inc., Circular OR-10.
146. J. L. Dye and V. A. Nicely, J. Chem. Educ., 48, 443 (1971).
147. W. E. Wentworth, J. Chem. Educ., 42, 96 (1965).
148. M. T. Rogers and J. C. Woodbrey, J. Phys. Chem., 66, 540 (1962).
149. More, Garbow, and Hillstrom, Users Guide for MINPACK-1, ANL-80-74, Argonne National Laboratory.
150. G. J. Thomas, Jr., and J. Livramento, Biochem., 14, 5210 (1975).
151. R. E. Holmes and R. K. Robins, J. Am. Chem. Soc., 86, 1242 (1964).
152. The H(8) resonances of Na₂5'GMP solution are labeled α , β , γ , δ and χ . α represents the resonance at 8.55, β the resonance at $\sim$ 8.00, γ the monomer resonance, δ the resonance at 7.25 ppm. When the β and δ lines overlap the resulting line is represented as χ .
153. J. L. Dimicoli and C. Hélène, J. Am. Chem. Soc., 95, 1036 (1973).
154. M. Guéron, C. Chachaty and Tran-Dinh Sen, Annals. New York Academy of Sciences, 222, 307 (1973).
155. R. E. Schirmer, J. H. Noggle, J. P. Davis, and P. A. Hart, J. Am. Chem. Soc., 92, 3266 (1970).
156. A. P. Zens, T. A. Bryson, R. B. Dunlap, R. R. Fisher, and P. D. Ellis, J. Am. Chem. Soc., 98, 7559 (1976).
157. J. D. Glickson, S. L. Gordon, T. P. Pitrer, D. G. Agresti, and R. Walter, Biochem., 15, 5721 (1976).
158. S. J. Leach, G. Nemethy and H. A. Scheraga, Biochem. and Biophys. Res. Commun., 75, 207 (1977).
159. F. Jordan, S. Nishikawa, and P. Hemmes, J. Am. Chem. Soc., 102, 3913 (1980).
160. J.-L. Dimicoli and C. Hélène, J. Am. Chem. Soc., 95, 1036 (1973).

161. L. A. Marky, D. Patel, and K. J. Breslauer, Biochem., 20, 1427 (1981).
162. W. Guschlbauer, Jer. Sym. Quan. Chem. Biochem. IV, 297 (1972).
163. K. J. Neurohr and H. H. Manisch, Can. J. Chem., 57, 1986 (1979).
164. B. I. Sukhorukov, I. Ya. Gukovsky, A. I. Petrov, A. S. Gukovskaya, A. A. Mayevsky, and N. M. Gusenkova, Inter. Jour. of Quar. Chem., 17, 339 (1980).
165. K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs, and H. Sigel, J. Am. Chem. Soc., 103, 247 (1981).
166. I. Lascu, T. Barzuv, N. G. Ty, L. D. Ngoc, O. Barzu, and H. H. Mantsch, Biochim. Biophys. Acta., 482, 251 (1977).
167. O. Barzu, R. Tilinca, D. Porutiv, V. Gorun, G. Jebeleanu, L. G. Ngoc, M. Kezdi, I. Guia, and H. H. Mantsch, Arch. Biochem. Biophys. 182, 42 (1977).
168. S. J. Gill, M. Downing and G. F. Sheats, Biochem. 6, 272 (1967).
169. E. L. Farguhar, M. Downing, and S. J. Gill, Biochem. 7, 1224 (1968).
170. M. G. Marenchic and J. M. Sturtevant, J. Phys. Chem., 77, 544 (1973).
171. F. E. Evans and R. H. Sarma, Biopolymers, 13, 2117 (1974).
172. A. P. Zens, T. A. Bryson, R. B. Dunlap, R. R. Fisher, and P. D. Ellis, J. Am. Chem. Soc., 98, 7559 (1976).
173. G. C. Y. Lee and S. I. Chan, J. Amer. Chem. Soc., 94, 3218 (1972).
174. D. B. Davies and S. S. Danyluk, Biochem., 14, 543 (1975).
175. M. Gueron, C. Chachaty, and T. D. Son, Ann. New York Acad. Sci., 222, 307 (1978).
176. B. McConnell, Biochem. 13, 4516 (1974).

- 177. F. Jordan, S. Nishikawa, and P. Hemmes, J. Am. Chem. Soc. 102, 3913 (1980).
- 178. R. R. shoup, H. T. Miles, and E. D. Becker, J. Phys. Chem. 76, 64 (1972).
- 179. M. Eigen, Angew. Chem. Int. Ed. Engl. 3, 1 (1964).
- 180. R. Shapiro, Prog. Nucl. Acid Res. 8, 73 (1968).
- 181. Y. P. Wong, J. Am. Chem. Soc., 95, 3511 (1973).
- 182. M. Sundaralingam, Int. J. Quant. Biol. Symp., 1, 81 (1974).
- 183. T. W. Sturgill, Biopolymers, 17, 1793 (1977).
- 184. E. Mei, J. L. Dye, and A. Popov, J. Am. Chem. Soc., 99, 5308 (1977).
- 185. H. S. Gutowsky and C. H. Holm, J. Chem. Phys. 25, 1228 (1956).