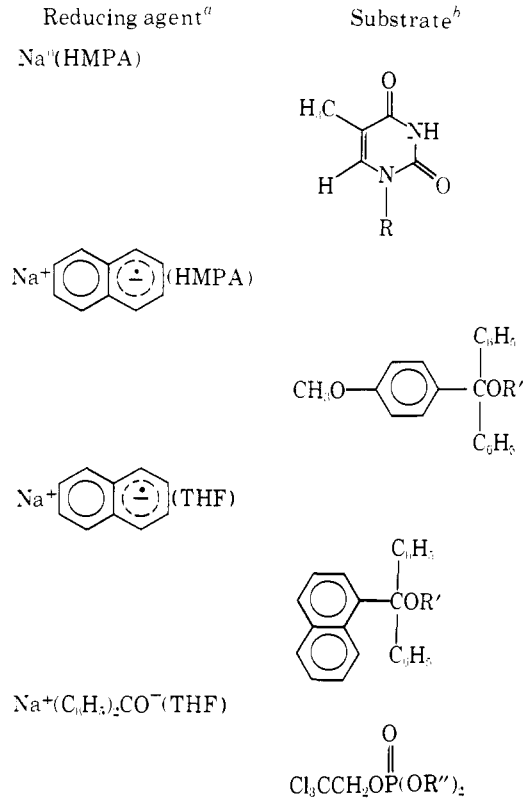
Chart I. Order of Reductive Cleavage of Protecting Groups



[a] HMPA, hexamethylphosphoric triamide; THF, tetrahydrofuran. [b] The thymine ring is reduced; in the other cases the indicated protecting groups are removed.

noted that the relative reactivity of these triarylmethyl derivatives toward radical anions is the reverse of that toward acid.[13]

Further differentiation in deprotecting was observed with the benzophenone radical anion in THF. This agent was relatively unreactive toward the *O*-α-napthyldiphenylmethyl group (only 4% of thymidine was obtained from **1**); but it was sufficiently active to remove the β,β,β-trichloroethyl group from trichloroethylphosphotriester derivatives. Thus **3** in tetrahydrofuran was converted to 5′-*O*-α-naphthyldiphenylmethylthymidylyl[3′-3′]-5′-*O*-α-naphthyldiphenylmethylthymidine, which was isolated in 88% yield. Control experiments showed that the anthracene radical ion converted **3** to thymidyly[3′-3′]thymidine and that the benzophenone radical anion failed to react with **2**.

The relative reactivity of the radical anions toward these protected nucleosides is summarized in Chart I. A given reducing agent reacts efficiently with those substrates lying below it in the chart and reacts very little with those lying above it. It is reasonable to expect that the chart can be expanded to include a number of other reducing agents, protecting groups, and classes of polyfunctional compounds.

References and Notes

(1) This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health, GM10265. We are also pleased to acknowledge helpful discussions with Professor Richard P. Van Duyne concerning electrochemical reductions.
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(10) The relative order of reducing power of the radical ions is indicated by the half-wave potentials for polarographic reduction of the hydrocarbons and the ketone under comparable conditions (dimethylformamide solvent, 0.1 *M* tetraethylammonium iodide supporting electrolyte, standard calomel reference electrode at 25°); naphthalene −2.50, anthracene −1.94, benzophenone −1.72 V. See tables 2-7 and 6-1 in C. K. Mann and K. K. Barnes, "Electrochemical Reactions in Non-aqueous Systems", Marcel Dekker, New York, N.Y., 1970. *Caution:* HMPA has recently been shown to cause cancer in experimental animals.
(11) In contrast, sodium naphthalene in THF is reported to cleave the benzyl group from *N*-benzyluridine without complications; K. D. Phillips and J. P. Horwitz, *J. Org. Chem.*, **40**, 1856 (1975).
(12) The concentration of HMPA is approximately 1 *M*. This reaction is therefore more sensitive to added HMPA than the alkylation reaction investigated by E. J. Panek, *J. Am. Chem. Soc.*, **95**, 8460 (1973).
(13) The half life for hydrolysis of 5′-*O*-*p*-methoxytritylthymidine to thymidine in 80% aqueous acetic acid at 27° is 8.5 min: H. Schaller, G. Weiman, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3821 (1963). Under the same conditions the half life for hydrolysis of **1** is 3.2 hr.

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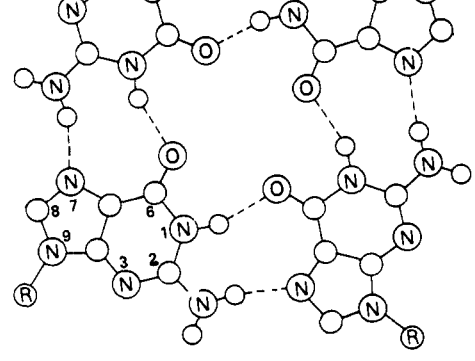
Self-Assembled 5′-Guanosine Monophosphate. Nuclear Magnetic Resonance Evidence for a Regular, Ordered Structure and Slow Chemical Exchange

Sir:

Among all of the nucleic acid components, guanosine monophosphate (GMP) possesses a unique ability to undergo spontaneous formation of a regular, ordered structure in aqueous solution. This property is manifested in part by the formation of anisotropic acid gels[1-6] (pH ~5) in which the bases are hydrogen-bonded to form continuous helixes (5′-isomer)[7] or planar tetramer units which stack in a helical array (3′-isomer).[1] The 5′-isomer also forms an ordered structure in neutral solution (pH 7-8),[8] but it is distinguished from the acid structure by lack of gel formation, different ir properties, a more cooperative melting profile, and a lower transition temperature. It has been proposed on the basis of ir and chemical evidence[8] that the neutral structure consists of helically arranged stacks of planar tetramer units (**1**) formed by incorporating a hydrogen-bonding scheme similar to that found for the acid gels (positions N(1) and N(2) as donors, O(6) and N(7) as acceptors).



We now wish to report NMR evidence for a regular, ordered structure and slow chemical exchange for self-assem-

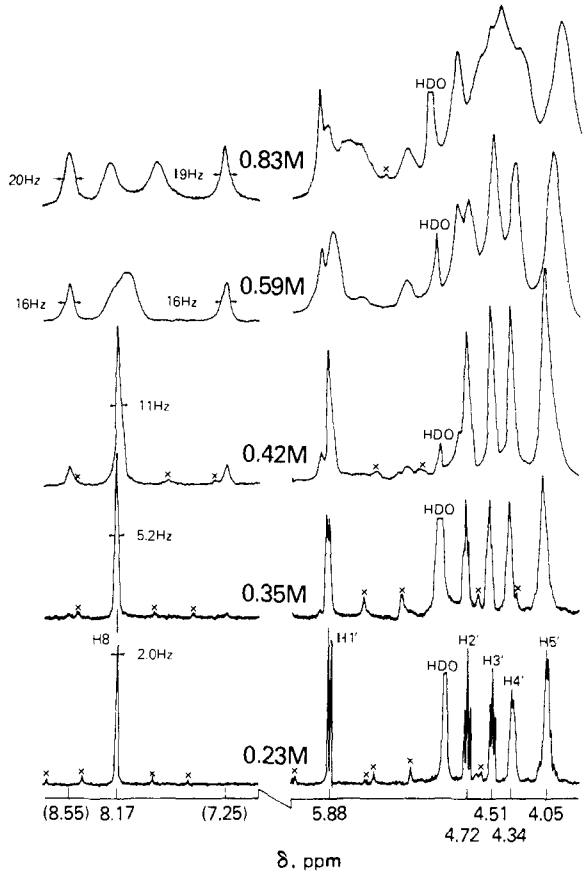


Figure 1. Proton NMR spectra (220 HMz) of Na_2[5′-GMP] in D_2O solutions at 19 ± 1°. The chemical shifts are relative to TSP as internal standard and apply only to the 0.23 *M* solution. The values in parentheses are the concentration-independent shifts of the highest and lowest field H(8) lines that appear in the remaining spectra. Line widths at half-maximum amplitude are provided for selected H(8) resonances. The positions of spinning side bands are marked "×".

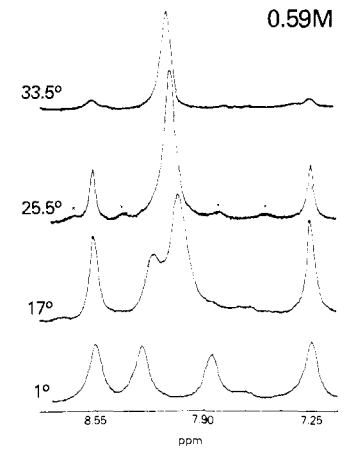


Figure 2. Temperature dependence of the H(8) resonances of 5′-GMP in D_2O at a concentration of 0.59 *M*.

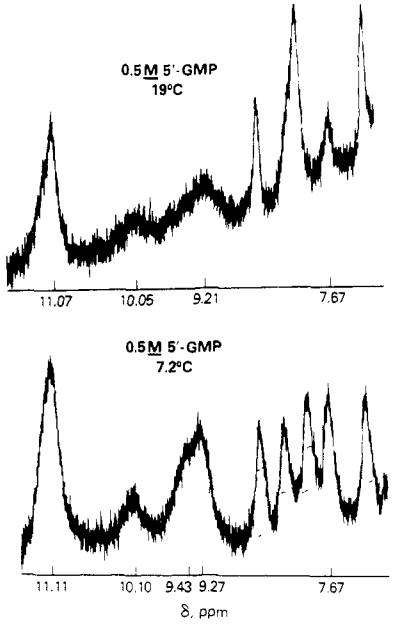


Figure 3. The 220-MHz spectra of 0.5 *M* 5′-GMP in H_2O. Frequencies are given for the exchangeable NH protons, and the H(8) protons are indicated by shading. The ratio of area of signals of the exchangeable to H(8) protons is roughly 1.5. We have not yet obtained satisfactory spectra at higher field because of interference from spinning side bands of water.

bled 5′-GMP in neutral solution. The latter property is unprecedented in the aqueous solution chemistry of base-paired mononucleotides. All previously reported complexes between purine and pyrimidine monomers, including mixed complexes in which the bases are complementary, undergo rapid chemical exchange and give time-averaged NMR spectra.[9a-11]

Proton NMR spectra of Na_2[5′-GMP] at various concentrations in D_2O solution at 19 ± 1° are shown in Figure 1. The lines observed at 0.23 *M* are in accord with those previously reported for the disordered nucleotide in dilute aqueous solution.[9,12-15] However, as the concentration is increased to 0.42 *M*, the lines broaden, spin-spin coupling between ribose protons is obscured, and two new H(8) lines grow out of the base line at 7.25 and 8.55 ppm. At 0.59 *M* a third H(8) line appears as a shoulder on the low-field side of the central resonance. Though broadened by slow molecular tumbling, four H(8) lines are well resolved at 0.83 *M*. Similar changes in the H(8) region are observed with decreasing temperature (see Figure 2).

The assignment of the H(8) lines is verified by a decrease in their absolute intensities after exchange with D_2O under conditions where the H(8) protons but not the ribose protons are replaced by deuterium.[16] We assign those low-field lines which are observed in H_2O but not D_2O (Figure 3) to N-bonded protons, the lowest two (11.1 and 10.10 ppm) very probably to N1-H. Exchange of N1-H of unassociated G is too rapid in H_2O to permit observation of an NMR line (see, for example, ref 9, 17), but N1-H is readily observed in hydrogen bonded complexes (see, for example, ref 18-20 and other papers there cited). Observation of these lines under conditions of complex formation (but not at temperatures or nucleotide concentrations at which the ordered form does not exist) thus provides experimental evidence for hydrogen bonding of N1-H protons in the 5′-GMP ordered structure described in this report.

The second-highest field H(8) resonance, in contrast to the other three H(8) lines, shifts to lower field with increasing temperature and decreasing concentration (Figures 1 and 2), indicating rapid exchange and time averaging of two or more classes of protons. This observation may reflect formation of nonregular stacked aggregates,[9,13] possibly in equilibrium with a rapidly exchanging class of protons in the regular structure. The remaining H(8) lines, however, arise because of the distinctly different association phenomenon, which results in formation of a regular structure in slow exchange equilibrium with unassociated monomers or stacked aggregates.

The changes in the intensities of the new H(8) lines with concentration and temperature are paralleled by changes in double bond stretching vibrations which are sensitive to

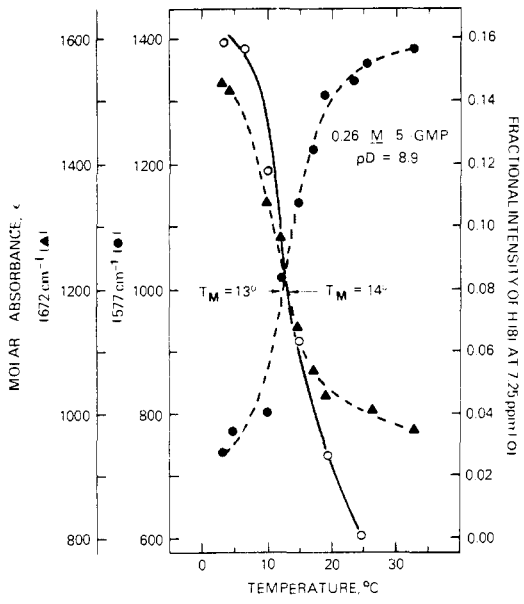


Figure 4. Melting curves for self-structured 5′-GMP in D_2O. Solid curve was obtained by integration of H(8) NMR lines; dashed lines were obtained by ir methods.[8]

base pair formation in ordered structures.[8] A comparison of ir and NMR melting curves is provided in Figure 4. Similarities in the cooperativity and T_m values indicate that the two spectroscopic methods are monitoring the same process. Both methods show that the ordered form is based upon interbase hydrogen bonding. Bonding scheme **1** is strongly favored by the formation of two hydrogen bonds per base residue. A search for possible alternative schemes suggests that none is plausible.[8,21] Assuming that **1** is in fact the elementary unit formed by interbase hydrogen bondings, we conclude that the H(8) nonequivalence is due to limited head to tail stacking of tetramer units.[22] It is reasonable to presume that the stacking of tetramers will terminate abruptly because of increased electrostatic repulsion for each added tetramer unit, each with eight negative charges.

Under all conditions investigated, the intensities of the highest (7.25 ppm) and lowest (8.55 ppm) field lines are equal within experimental uncertainty (±3%) Moreover, their chemical shifts are essentially independent of temperature and concentration, and their spin-lattice relaxation times (T_1) are nearly equal (1.96 ± 0.05 and 1.90 ± 0.03 sec, respectively, at 0.59 *M* and 19°). These results are consistent with the two lines representing different H(8) environments within an array of stacked plates (e.g., a dodecamer). It is not certain, however, whether the third-highest field line represents a third environment in a single dodecamer. Unlike the lines at 7.25 and 8.55 ppm, it exhibits appreciable low-field shifts with decreasing temperature. Moreover, its T_1 is relatively short and more nearly equal to that of the second-highest field H(8) line (0.75 ± 0.01 and 0.81 ± 0.02 sec, respectively at 0.59 *M* and 19°). T_1 remains small even in the presence of EDTA as complexing agent for trace amounts of paramagnetic ion impurities. We have observed that addition of 10^{-3} *M* Mn^{2+} or Cu^{2+} preferentially shortens the relaxation time of the third highest field line relative to the lines at 7.25 and 8.55 ppm.

Whatever the precise details of structure may be, the evidence for base pairing and stacking in a regular ordered structure and slow chemical exchange nonetheless is clear. The large upfield shift of 0.95 ppm for the highest-field line relative to disordered 5′-GMP in dilute solution (8.20 ppm at 0.05 *M*) can be attributed to diamagnetic ring currents of overlapping bases. The downfield shifts of the other two self-structure lines are presumably due to electric field effects caused by a doubly ionized phosphate group in the vicinity of these H(8) protons. From the theory of NMR exchange broadening[23] the observed line widths indicate that $k_{25°}$ for exchange of the highest and lowest field H(8) environments is <60 sec^{-1}, and from transition state theory $\Delta G^{\ddagger}_{25°}$ is thus >15.0 kcal/mol.

Finally, we note that the isoshielding contours calculated by Giessner-Prettre and Pullman[24] for the ring currents in guanine do not appear to account quantitatively for the 0.95 ppm upfield shift of the highest-field H(8) line. Whether we assume a dodecamer or an octamer with twist angles between stacked plates in the range 0–45°, the largest upfield H(8) shifts calculated on the basis of the theoretical contours are not greater than ~0.6 ppm. It would appear that magnitudes of the isoshielding contours are underestimated by theory. It may be relevant that Arter et al.[19] found that H(8) protons of G experience a considerably larger shift than expected for the transition from RNA-11 helix to random coil.

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(22) The presence of four nearly equally intense H(8) lines in the limiting NMR spectrum at 0.59 *M* and 1° (cf., Figure 2) could be interpreted in terms of a hexadecamer in which there is head to tail stacking of four planar tetramer units. However, we find this structural interpretation unattractive as it requires the tetramer unit responsible for the second-highest field resonance to have the same chemical shift as the disordered nucleotide at all concentrations studied. Coincidence in chemical shifts could arise because of rapid chemical exchange, but there appears to be no facile mechanism which would allow the disordered nucleotide to preferentially exchange with one tetramer unit in the hexadecamer without interchanging environments of the remaining three layers.
(23) See, for example, J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance", McGraw-Hill, New York, N.Y., 1959, p 221.
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