Molecular Complexes of Nucleosides and Nucleotides with a Monomeric Cationic Porphyrin and Some of Its Metal Derivatives

R. F. Pasternack,* E. J. Gibbs,* A. Gaudemer,* A. Antebi, S. Bassner, L. De Poy, D. H. Turner, A. Williams, F. Laplace, M. H. Lansard, C. Merienne, and M. Perree-Fauvet

Contribution from the Departments of Chemistry, Swarthmore College, Swarthmore, Pennsylvania 19081, Goucher College, Towson, Maryland 21204, University of Rochester, Rochester, New York 14627, and Laboratoire de Chemie de Coordination Bioorganique (LA255), Université Paris-Sud, Centre d'Orsay, 91405 Orsay, France. Received June 19, 1985

Abstract: Tetrakis(4-*N*-methylpyridyl)porphine (H₂TMpyP) and a number of its metal derivatives interact extensively with mononucleotides and mononucleosides in aqueous solution. The complexes formed are of a stacking-type involving extensive overlap of the π-systems of the porphyrin and purine or pyrimidine bases. Coulombic attractions help stabilize the complexes but there is no evidence for ligation of the bases to axial sites of the metalloporphyrins. Stability constants determined via NMR and spectrophotometric titrations are larger for purine bases than pyrimidines with a given porphyrin derivative. More dramatic influences on stability result from changing porphyrins. Porphyrins having no axial ligands (e.g., metal-free copper(II), palladium(II), and nickel(II) derivatives) or one axial ligand (Zn(II)) produce much larger interactions with a given nucleotide or nucleoside than do metalloporphyrins having two axial ligands (e.g., Mn(III), Fe(III), or Co(III)). The kinetics of the interaction of H₂TMpyP with 2'-deoxyadenosine 5'-monophosphate (dAMP) were studied via the laser raman temperature-jump method. The measured rate constants are consistent with a simple stacking model for the interaction.

Introduction

It has been shown recently that various cationic, meso-substituted porphyrins strongly complex to nucleic acids in solution.[1-4] Absorption spectral, circular dichroic, and kinetic evidence have been presented which indicate that tetrakis(4-*N*-methylpyridyl)porphine (H₂TMpyP, Figure 1) and its metal derivatives interact differently with GC rich regions of DNA than with AT regions.[3,4] Specifically, porphyrins can intercalate in GC regions whereas at AT regions they either partially intercalate or associate with the nucleic acid in an outside-bound complex. Intercalated porphyrin species are characterized by the following: (i) large bathochromic shifts of the Soret band (≥15 nm), (ii) substantial hypochromicity of the Soret maximum (≥35%), (iii) induced circular dichroism bands in the Soret region having negative ellipticities, and (iv) formation and dissociation half-lives for the porphyrin–DNA complex >3 ms. In contrast, the non-intercalated complexes show the following: (i) small red shifts, usually ≤8 nm, (ii) little hypochromicity or even hyperchromicity of the Soret maxima, (iii) induced CD bands in the Soret region having positive ellipticities, and (iv) formation and dissociation half-lives for the porphyrin–DNA complex <3 ms.

The type of interaction with DNA depends on the nature of the interacting porphyrin.[3,4] Those porphyrins without axial ligands such as the metal-free, copper(II) and nickel(II) derivatives intercalate into DNA; those metalloporphyrins which maintain axial ligands such as Fe^{III}TMpyP, Co^{III}TMpyP, Mn^{III}TMpyP, and Zn^{II}TMpyP do not intercalate presumably because the axial ligands prevent the porphyrin molecular ion from inserting between closely stacked base pairs. Furthermore, the metal-free porphyrin tetrakis(2-*N*-methylpyridyl)porphine (H₂TMpyP-2) for which the barrier to rotation of the peripheral *N*-methyl pyridine is very large also does not intercalate but rather, like the axially liganded metalloporphyrins, shows high specificity for AT regions of DNA.

In the present study we consider the interactions of these porphyrins with the building blocks of nucleic acids: nucleosides and nucleotides. From these investigations we determine which

of the spectral and kinetic features described above are simulated by these simpler fragments and which arise from interactions with the intact polymer. We also determine the chemical forces which are most significant in promoting the associations between nucleotides and porphyrins and from these considerations obtain a more thorough understanding of the factors responsible for the strong binding of porphyrins to DNA.

Materials and Methods

All the porphyrins used in this study were converted to the chloride form with use of a published procedure.[5] The two nonmetalloporphyrins, H₂TMpyP and H₂TMpyP-2, were purchased from Man-Win Coordination Chemicals as the tosylate salts. The copper(II), cobalt(III), manganese(III), iron(III), nickel(II), and zinc(II) derivatives of H₂TMpyP were prepared and purified by literature methods.[4,6-8] The palladium(II) derivative was prepared by refluxing a mixture of Na₂PdCl₄ (25 mg) and H₂TMpyP (Cl⁻ form, 100 mg) in water for an hour. After the mixture was cooled, the metalloporphyrin was precipitated by addition of excess NaI. The precipitate (105 mg; yield 68%) was filtered and washed with cold water. PdTMpyP was obtained in the chloride form by passing an iodide slurry over an ion exchange resin.[5]

Anal. Calcd for C₄₄H₃₆N₈PdI₄·0.5H₂O: C, 40.66; H, 2.87; N, 8.62; I, 39.68; Pd, 8.19. Found: C, 40.68; H, 3.54; N, 8.68; I, 39.23; Pd, 8.18.

Ribonucleosides and ribonucleotides were purchased from Fluka Co.; the deoxyribonucleotide phosphates 2'-deoxyguanosine 5'-monophosphate (dGMP) and deoxythymidine 5'-monophosphate (dTMP) were purchased from P-L Biochemicals in the sodium form. The remaining deoxyribonucleotide phosphates 2'-deoxycytidine 5'-monophosphate (dCMP) and 2'-deoxyadenosine 5'-monophosphate (dAMP) as well as the nucleosides 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA) were purchased from Sigma Chemical Co. All NMR solutions were prepared in deuterated phosphate buffer, pD 7.0 ± 0.1, μ = 0.3 M with 99.8% D₂O from CEA (Saclay). Solutions for spectrophotometric experiments were prepared in phosphate buffer (8 mM), pH 6.9 ± 0.1, μ = 0.2 M.

The ¹H NMR spectra reported here were measured at 23 °C with a Cameca 250 spectrometer or with a 400-MHz spectrometer at the Laboratoire d'Electronique Fondamentale in Orsay, France. Solutions for NMR measurements were typically in the range of 10⁻³ to 10⁻² M in

(1) Fiel, R. J.; Howard, J. C.; Mark, E. H.; Dattagupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093-3118.

(2) Fiel, R. J.; Munson, B. R. *Nucleic Acid Res.* **1980**, *8*, 2835-2842.

(3) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406-2414.

(4) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 5409-5417.

(5) Pasternack, R. F.; Huber, P. R.; Boyd, P.; Engasser, G.; Francesconi, L; Gibbs, E.; Fasella, P.; Venturo, C. G.; Hinds, L. C. *J. Am. Chem. Soc.* **1972**, *94*, 4511-4517.

(6) Pasternack, R. F.; Spiro, E. G.; Teach, M. *J. Inorg. Nucl. Chem.* **1974**, *36*, 599-606.

(7) Pasternack, R. F.; Lee, H.; Malek, P.; Spencer, C. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1865-1870.

(8) Harriman, A.; Porter, G. *J. Chem. Soc., Faraday Trans. 2* **1979**, *75*, 1532-1542.
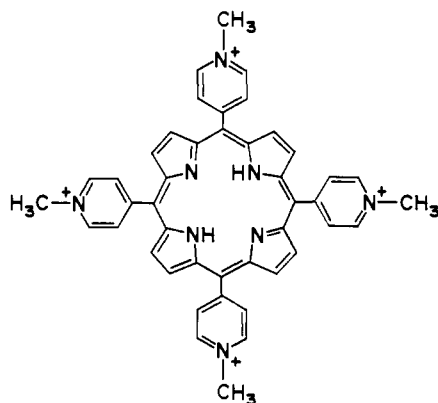
Figure 1. Tetrakis(4-*N*-methylpyridyl)porphine.

Figure 2. Plot of NMR chemical shifts vs. porphyrin concentration for the meta (H_a), ortho (H_b), and methyl (H_c) protons of the *p-N*-methylpyridyl rings.

porphyrin and nucleotide or nucleoside. Optical spectra and spectrophotometric titrations were conducted with a Varian 2290 UV/vis spectrophotometer. Relaxation experiments were carried out with a DIALOG joule-heating temperature-jump apparatus[9] and a laser raman temperature-jump apparatus described elsewhere.[10] The former instrument has a heating time of about 1 μs while the latter instrument's heating time is approximately 5 ns. Circular dichroism experiments were conducted on a Jasco Model J-40A spectropolarimeter equipped with Exocal and Endocal temperature regulators.

Results

A. State of Aggregation of Reactant Species. Evidence has been presented that with the exception of the iron(III) derivative,[7] the porphyrins employed here are monomeric under the conditions of these experiments.[5,6,11,12] A recent report has noted changes in the fluorescence spectrum of H_2TMpyP as a function of concentration, ionic strength, solvent, and temperature which the authors interpret as arising from aggregation of this porphyrin.[13] These fluorescence features have been reproduced in our laboratory but, as Kano et al. state, the data do not lend themselves to a detailed analysis. However, if we adopt the interpretation of Kano et al., an equilibrium constant for dimerization can be estimated as $K_D \geq 10^8$ M^{-1} at *an ionic strength approaching zero*. This value is larger than that obtained for any other metal-free porphyrin with the possible exception of protoporphyrin IX[14] and is, for example, at least two orders of magnitude larger than the value obtained for the tetraethylenediamine derivative of protoporphyrin IX (PPIX(en)$_4$) at μ = 0.1 M.[15] The aggregation of porphyrins is enhanced with increasing ionic strength. Porphyrin derivatives which have shown to be extensively dimerized at μ = 0.1 M are monomeric in the absence of added salt.[5] Described below are the results of several experiments which when taken together mount a strong (but not definitive) case against the aggregation model.

(i) Protoporphyrin IX which is the only known porphyrin for which $K_D > 10^8$ M^{-1} undergoes higher aggregation with increasing concentration so that by a concentration of 4 μM it is purported to "micellize".[14] In sharp contrast to this behavior Kano et al.[13] agree with our earlier results[5,11] that for the concentration range $10^{-7} \leq [H_2TMPyP] \leq 10^{-4}$, there is no change in the aggregation state of the porphyrin. We considered this state to be a monomer; Kano et al. propose it to be a dimer. Foster[16] has considered the aggregation of this porphyrin up to 60 mM using an NMR
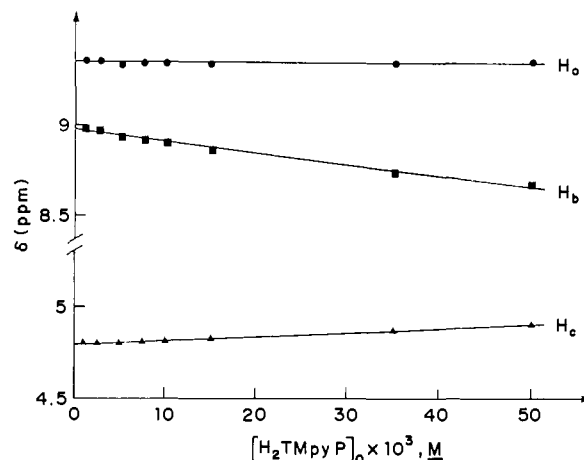
technique, but the results of his experiments are somewhat ambiguous because of the presence in the solution of tosylate anion which can participate in a stacking-type interaction with the porphyrin. Still Foster concludes that the porphyrin remains monomeric up through 60 mM. We have repeated these NMR experiments using chloride as the gegenion and considered the aggregation tendencies from 1 to 50 mM at μ = 0.3 M. The analysis involves the method of Dimicoli and Helene[17] for the determination of a dimerization equilibrium constant, K_D.

If δ_1 is the chemical shift of the proton in the monomer and δ_2 the chemical shift of the same proton measured at various concentrations of porphyrin, P_0, it can be shown that

$$\left(\frac{\Delta}{P_0}\right)^{1/2} = \left(\frac{2K_D}{\Delta_2}\right)^{1/2}(\Delta_2 - \Delta) \qquad (1)$$

where $\Delta_2 = \delta_1 - \delta_2$, $\Delta = \delta_1 - \delta$, and δ_1 is obtained from the curve $\delta = f(|P|)$; it is the value of δ at infinite dilution. In the case of H_2TMpyP, no significant variation of δ is observed below 10^{-2} M (cf. Figure 2). As seen in Figure 2, H_a, the meta protons of the *p-N*-methylpyridyl rings, show no variation of the chemical shift while H_b, the ortho protons, show a slight shift to high field (0.3 ppm between 10^{-3} and 5 $\times$ 10^{-2} M). H_c shows the methyl proton shift to lower field (0.1 ppm between 10^{-3} and 5 $\times$ 10^{-2} M). Using the values for H_b for which the effect is maximized, a plot of $(\Delta/P_0)^{1/2}$ vs. Δ is linear leading to δ_2 = 7.86 ppm and K_D = 5.4 M^{-1}. Thus, through visible absoprtion and NMR experiments it can be concluded that a single, very small value of an autoassociation constant is obtained in solutions varying in concentration from 0.1 μM to 50 mM. If we were to accept the interpretation of Kano et al., we would have to conclude that H_2TMpyP is totally dimerized by 0.1 μM but does not form a higher aggregate through at least five orders of concentration range until 10 mM, in marked contrast to what is observed for protoporphyrin IX.

(ii) Kano et al. report that 0.1 M SDS totally converts "dimeric" H_2TMpyP to the monomer form. We have conducted experiments in which we determined that an SDS concentration of 0.4 mM produces the same fluorescence and absorption spectra as does 0.1 M detergent. By contrast, PPIX(en)$_4$ which like H_2TMpyP is a 4$^+$ ion under these conditions and whose aggregation constant is at least 2–3 orders of magnitude smaller than that estimated for H_2TMpyP (from a dimerization model) is only partially converted to monomer by 2 mM SDS. Figure 3 shows the Soret spectra of the two porphyrins for comparison both in 0.1 M NaCl and in a solution of the same ionic strength containing 2 mM SDS. Notice that for PPIX(en)$_4$, unlike H_2TMpyP, the spectrum of the aggregated species is broad and ill-defined in the absence of SDS. In 2 mM SDS, a clear maximum develops at 398 nm but a

(9) Rigler, R.; Rabl, C.; Jovin, T. M. *Rev. Sci. Instrum.* **1974**, *45*, 580–588.

(10) Turner, D. H. "Techniques in Chemistry Series"; Bernasconi, C., Ed.; Wiley-Interscience: New York; in press.

(11) Pasternack, R. F. *Ann. N.Y. Acad. Sci.* **1973**, *206*, 614–630.

(12) Pasternack, R. F.; Francesconi, L.; Raff, D.; Spiro, E. *Inorg. Chem.* **1973**, *12*, 2606–2611.

(13) Kano, K.; Miyake, T.; Uomoto, K.; Sato, T.; Ogawa, T.; Hashimoto, S. *Chem. Lett.* **1983**, 1867–1870.

(14) Brown, S. B.; Shillock, M.; Jones, P. *Biochem. J.* **1976**, *153*, 279–285.

(15) Das, R. R.; Pasternack, R. F.; Plane, R. A. *J. Am. Chem. Soc.* **1970**, *92*, 3312–3316.

(16) Foster, N. *J. Magn. Reson.* **1984**, *56*, 140–143.

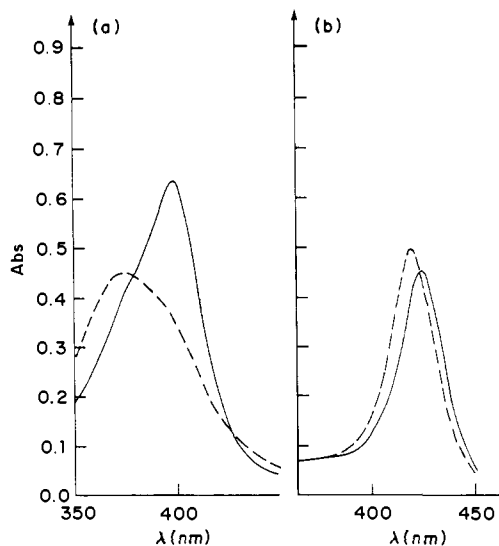(17) Dimicoli, J. C.; Helene, C. *J. Am. Chem. Soc.* **1973**, *95*, 1036–1044.

Figure 3. Spectra of (a) PPIX(en)$_4$ and (b) H$_2$TMpyP in the Soret region at 25 °C, μ = 0.1 M: (---) no SDS added, (—) in the presence of 2 mM SDS.

shoulder remains at the maximum of the aggregate at 373 nm. There is also the commonly observed hypochromicity of the PPIX(en)$_4$ aggregate when compared to the monomer spectrum.[5] In contrast, for H$_2$TMpyP there is no significant narrowing or absorbance increase of the Soret band in 2 mM SDS and there is some hyperchromicity of the Soret band as monomers are converted to "aggregates".

(iii) We have shown previously,[5,12] that the forward rate constant for porphyrin dimerization remains relatively constant at $k_D \sim 10^8$ M^{-1} s^{-1}. Therefore we would conclude using the estimated K_D that the rate constant for monomerization of H$_2$TMpyP would be $k_{-D} < 1$ s^{-1}. We have attempted stopped-flow experiments in which we mix 10^{-5}–10^{-6} M H$_2$TMpyP with varying SDS concentrations as low as 0.4 mM. In all cases, the spectral conversion is too fast to measure; i.e., $t_{1/2} < 1.5$ ms. Thus SDS would have to catalyze the dissociation of H$_2$TMpyP aggregates by about 10^8 to account for these kinetic results. Whereas there is no theoretical reason why this could not occur, the influence of SDS on the rate of disaggregation of PPIX(en)$_4$ or the μ-oxo dimer of FeTMpyP is far more modest.[4,18]

(iv) Kano et al. infer that measured differences in the fluorescence lifetime between H$_2$TMpyP[13,19] and H$_2$TPPS, *meso*-tetra(4-sulfonatophenyl)porphine,[20] reflect that the former porphyrin is aggregated, while the latter porphyrin is not. In fact, considerable evidence exists that both H$_2$TPPS$_3$ and H$_2$TPPS$_4$ dimerize.[5,21] Under the conditions of the experiments described[20] ([H$_2$TPPS$_3$] = 25 μM, pH 8, 0.05 M phosphate buffer), the porphyrin is expected to be extensively aggregated. Therefore, if as Kano et al. suggest a correlation between fluorescence lifetimes and state of aggregation can be made, the conclusion to be drawn is that H$_2$TMpyP is monomeric. Kano et al.[20] noted that whereas 9,10-anthraquinone-2,6-disulfonate (AQDS^{2-}) efficiently quenches the fluorescence of H$_2$TPPS$_3$$^{3-}$, methyl viologen (MV^{2+}) has virtually no influence on the fluorescence of H$_2$TMpyP^{4+}. This result can be interpreted as indicating that the anionic porphyrin shows greater tendency to participate in stacking-type interactions than does this cationic porphyrin, as suggested by us earlier.[5,11]

(v) The analysis (Rose–Drago method[22]) of the experiments on the interaction of porphyrins with deoxyribonucleotides to be described below is very sensitive to changes in aggregation of the
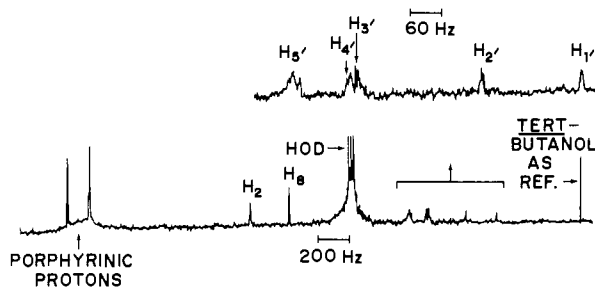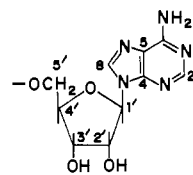
(18) Gibbs, E. J. Ph.D. Dissertation, **1982**, pp 75–81.
(19) Kalyanasundaram, K. *Inorg. Chem.* **1984**, *23*, 2453–2459.
(20) Kano, K.; Sato, T.; Yamada, S.; Ogawa, T. *J. Phys. Chem.* **1983**, *87*, 566–569.
(21) Hambright, P.; Gore, T.; Burton, M. *Inorg. Chem.* **1976**, *15*, 2314–2315.
(22) Rose, N. J.; Drago, R. S. *J. Am. Chem. Soc.* **1959**, *81*, 6138–6145.

Figure 4. 400-MHz ^{1}H NMR spectrum of ADP in the presence of H$_2$TMpyP; [ADP]$_0$ = [H$_2$TMpyP]$_0$ = 10^{-2} M.

reactant species. If H$_2$TMpyP is dimeric as Kano et al. suggest, our results show no indication of a change in aggregation of this porphyrin as it interacts with purine and pyrimidine bases in a stacking-type interaction (vide infra). It might be anticipated that since these interactions are similar to that proposed for porphyrin dimerization the equilibria would be antagonistic especially if one considers that H$_2$TMpyP shows little if any tendency to form higher aggregates. Therefore, perturbation of the porphyrin dimerization equilibrium by the mononucleotides should present a complication in the data-fitting analysis. None is observed.

On the basis of these various considerations, it can be reasoned that H$_2$TMpyP *either* forms a unique type of dimer or is monomeric in solution. To account for the experimental results, the H$_2$TMpyP "dimer" would have to be characterized by an unprecedentedly large stability constant with little or no tendency to add another porphyrin monomer unit. Furthermore, the "dimer" would have to remain intact as it enters into stacking interactions with nucleosides and nucleotides. In short, it would be required that none of the experiments to be described below (which have been conducted over four orders of magnitude of porphyrin concentration) perturb the monomer–dimer equilibrium. The absence of any quantitative data to support the dimerization model as well as the unique properties required for the dimer lead us to conclude that H$_2$TMpyP exists as a monomer in solution up to about 40 mM, as suggested earlier.[5]

Aggregation of nucleotides has also been considered, and it is generally concluded that guanosine 5'-monophosphate has a greater tendency to aggregate than do other nucleotides studied.[23] However, under concentration conditions employed in these experiments 5'-GMP is monomeric in solution.[24] As stated earlier, the Rose–Drago analysis used to determine stability constants from the spectrophotometric titration data would have exposed any monomer–dimer equilibration coupled to the porphyrin/nucleotide interactions. We therefore proceed with our results and their analysis in terms of porphyrin monomers interacting with nucleotide monomers to form complexes. This is the minimum model needed to account for our spectral, NMR, and kinetic results.

B. NMR Experiments with Ribonucleotides. The NMR spectra of adenosine (A), adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), and adenosine 5'-triphosphate (ATP) are well resolved, giving signals for the ribose protons which do not superimpose and are easily identified. In the presence of the anionic porphyrins *meso*-tetra(4-carboxyphenyl)porphine (H$_2$T-CPP) or *meso*-tetra(4-sulfonatophenyl)porphine (H$_2$TPPS) no significant changes are observed in the NMR spectra, suggesting
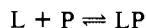
(23) Fisk, C. L.; Becker, E. D.; Miles, H. T.; Pinnavaia, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 3307–3314.
(24) Mantooth, D.; Georghiou, S. *Photochem. Photobiol.* **1983**, *38*, 623–626.

Table I. Formation Constants K_1 of Porphyrin Complexes with Nucleosides and Nucleotides (μ = 0.3 M; pD = 7.0; t = 23 °C; $K_1 \times$ 10^{-3})

porphyrin/base	H$_2$TMpyP	ZnTMpyP	NiTMpyP	PdTMpyP
A	0.64			
AMP	1.4			
ADP	2.9	3.7	5.0	12.
ATP	2.9			
G	0.77			
U	0.20			
UMP	0.22	0.41	0.68	0.97
CMP	0.14			

that adenosine and its phosphoric esters do not form strong complexes with anionic porphyrins. In contrast, in the presence of H$_2$TMpyP upfield shifts are observed for all protons, the largest being observed for adenine H$_2$ and H$_8$ protons and for H$_1'$ (Figure 4). These large differences suggest a well-defined geometry for the complex between the nucleosides (and nucleotides) and H$_2$TMpyP (and its metal derivatives) in which the purine or pyrimidine base sits above the center of the macrocycle and the phosphate group(s) when present lie close to the positively charged pyridinium groups. The large induced shifts make these protons useful probes for the evaluation of association constants which are determined with Bouquant's method.[25] The major reaction occurring in solution is

$$L + P \rightleftharpoons LP$$

where L is the nucleoside or nucleotide and P is the porphyrin. If Δ^i is the induced chemical shift of the ith proton and Δ^i_{LP} the chemical shift of the same proton in the complex LP (intrinsic chemical shift), the relationship between the complexation constant K_1 and Δ^i and Δ^i_{LP} is

$$\frac{\Delta^i}{\Delta^i_{LP}} = K_1 L_0 \left(1 - \frac{\Delta^i}{\Delta^i_{LP}}\right)\left(\frac{P_0}{L_0} - \frac{\Delta^i}{\Delta^i_{LP}}\right) \quad (2)$$

where L_0 and P_0 are the initial concentrations of nucleoside (or nucleotide) and porphyrin, respectively. If these concentrations are equal $L_0 = P_0 = C_0$, then the above relationship simplifies to

$$\Delta^i = \Delta^i_{LP} - (\Delta^i_{LP}/K_1)^{1/2}(\Delta^i/C_0)^{1/2} \quad (3)$$

A plot of Δ^i vs. $(\Delta^i/C_0)^{1/2}$ is predicted to be linear with a slope equal to $-(\Delta^i_{LP}/K_1)^{1/2}$ and an intercept equal to Δ^i_{LP}. This method proves quite suitable for measuring the formation constants K_1 and the chemical shifts Δ^i_{LP} when the saturation factor s lies between 0.2 and 0.8. Since $s = \Delta^i/\Delta^i_{LP} = (2K_1C_0 + 1 - (4K_1C_0 + 1))^{1/2}/2K_1C_0$, this corresponds to values of K_1C_0 ranging between 0.3 and 20. Typical curves of Δ^i vs. $(\Delta^i/C_0)^{1/2}$ obtained for A and ATP are shown in Figure 5; the values of K_1 obtained from such plots are shown in Table I. The K_1 values are given to $\pm10\%$.

At 23 °C in neutral aqueous solution, the formation constants vary from 0.64 × 10^3 M^{-1} for the complex between A and H$_2$TMpyP to 2.9 × 10^3 M^{-1} for the ATP–H$_2$TMpyP complex. These results indicate that two types of interactions contribute to the stability of the complex: (1) hydrophobic and/or van der Waals' interactions between the nucleoside and the porphyrin ring and (ii) electrostatic attractions between the phosphate groups and the cationic porphyrin. It would appear that the van der Waals'/hydrophobic types of interactions are by far the more significant in determining the stabilities of these porphyrin complexes, the value of K_1 for AMP being only a factor of 2 greater than for the nucleoside adenosine. The same variations in Δ^i_{LP} are observed for all four complexes which suggest that they all have approximately the same average geometry.

In order to determine the influence of temperature, ionic strength, and pD on the induced chemical shifts we have measured the spectra of equimolar mixtures of AMP or ADP with H$_2$TMpyP under various conditions. We observed the following:

(i) The constant K_1 is ionic strength dependent. An increase in μ produces a decrease of K_1 with no significant changes in the
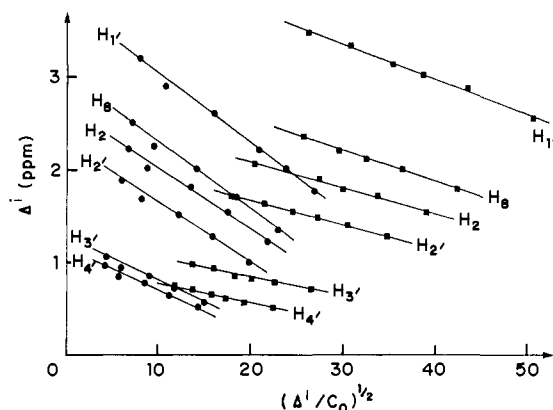

Figure 5. Complex formation between H$_2$TMpyP and A ($\bullet$) and H$_2$TMpyP and ATP ($\blacksquare$). Analysis of NMR data according to eq 3 for the base protons and the sugar protons of A and ATP; 5 × 10^{-2} M > [A]$_0$ > 2.5 × 10^{-3} M; 5 × 10^{-3} M > [ATP]$_0$ > 5 × 10^{-4} M.

intrinsic chemical shift, Δ^i_{LP}. An analogous effect has been previously observed by us in that we have reported the equilibrium constant of H$_2$TMpyP–nucleic acid complexes decreases with increasing ionic strength.[3,4,26]

(ii) K_1 varies with pD. In the AMP–H$_2$TMpyP complex, the induced chemical shift of H$_8$ decreases regularly with increasing pD while a maximum is observed at pD = 7.5 for all other protons. An analysis of these trends with acid ionization tendencies of H$_2$TMpyP and the nucleotide has not been attempted.

(iii) K_1 values decrease regularly as the temperature is increased whereas the intrinsic chemical shifts Δ^i_{LP} are not affected by temperature which strongly suggests that the geometry of the complex is temperature independent. We determine from these data that for the ADP–H$_2$TMpyP association $\Delta H°$ = -38 ± 1 kJ/mol and $\Delta S°$ = -47 ± 3 J/(mol·deg).

Equilibrium constants K_1 have also been determined for the complexes formed between H$_2$TMpyP and other nucleosides (guanosine (G) and uridine (U)) or nucleotides (uridine 5'-monophosphate (UMP) and cytidine 5'-monophosphate (CMP)). A comparison of the constants obtained for these compounds with those obtained for adenosine or AMP (Table I) shows that (i) purine derivatives give somewhat stronger associations than pyrimidine derivatives (this same trend will be seen more graphically in the presentation of our results with deoxyribonucleotides) and (ii) within each family of nucleic acid derivatives changes in the base substituents (G vs. A or UMP vs. CMP) have no great influence on the constants.

The same pattern in the intrinsic chemical shifts Δ^i_{LP} is observed in all cases; i.e., the base protons and H$_{1'}$ are much more shielded than other protons suggesting comparable geometry for all complexes.

Finally, we have considered the interactions of three diamagnetic metal derivatives of the water-soluble porphyrin, ZnTMpyP, NiTMpyP, and PdTMpyP with ADP and UMP. The results are summarized in Table I. We observe that the stability constants are somewhat larger for the three metal derivatives and the presence of the metal causes no change in the geometry of the complexes as can be seen from the intrinsic chemical shifts.

C. Spectrophotometric Titrations with Deoxyribonucleotides. We have studied the association reactions of deoxyribonucleotides with porphyrins through spectrophotometric titrations in the Soret region. Experiments were conducted to yield both absolute values of absorbance and differences in absorbance. Table II summarizes the spectral properties of the various porphyrin–deoxynucleotide complexes obtained from absolute measurements. For the latter type of experiment, solutions containing equal concentrations of porphyrin were placed in the sample and reference compartments but the deoxyribonucleotide ligand was added only to the sample

(25) Bouquant, J.; Chuche, J. *Tetrahedron Lett.* **1972**, *23*, 2337–2340.
(26) Pasternack, R. F.; Antebi, A.; Ehrlich, B.; Sidney, D.; Gibbs, E. J.; Bassner, S. L.; De Poy, L. M. *J. Mol. Catal.* **1984**, *23*, 235–242.

Table II. Spectral Properties of Porphyrin–Deoxyribonucleotides and Related Species[a]

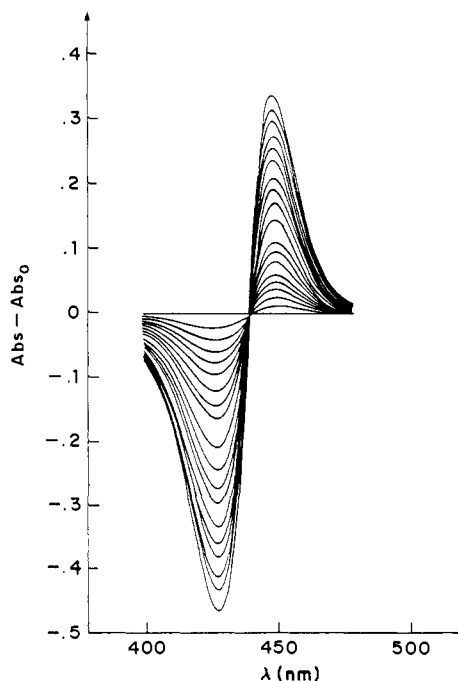| porphyrin	buffer λ_{max}	dG λ_{max}	% H	dGMP λ_{max}	% H	dAMP λ_{max}	% H	dCMP λ_{max}	% H	dTMP λ_{max}	% H	poly(dG-dC) λ_{max}	% H
H$_2$TMpyP	422	427	18	429	23	431	20	425	6	428	9	444	41
CuTMpyP	424	429	14	431	23	430	11	427	6	428	7	440	35
NiTMpyP	417/442	423	33	426	38	423	31	419	17	420	19	434	46
FeTMpyP	424	423	8	425	3	428	3	427	−3	423	0	424	−2
CoTMpyP	436	435	−1	436	2	436	0	436	0	436	0	434	6
MnTMpyP	463	463	1	463	−5	463	2	463	1	463	−5	463	5
ZnTMpyP	437	439	9	442	6	444	5	439	4	439	−4	437	4
H$_2$TMpyP-2	414			414	13	415	12	414	4	414	4	414	−3

[a] % H = percent hypochromicity.

Figure 6. Spectra at 25 °C of ZnTMpyP in varying dGMP concentrations. [ZnTMpyP]$_0$ = 9.4 μM, 0.1 mM $\leq$ [dGMp]$_0$ $\leq$ 44 mM. In this experiment ZnTMpyP was placed in the reference beam and ZnTMpyP + dGMP in the sample beam.

Figure 7. A plot of $1/K_1$ vs. $\Delta\epsilon_1$ at 451 nm from the SIMPLEX routine.

side leading to direct measurements of Abs − Abs$_0$, the difference in absorbance of a porphyrin solution in the presence and absence of the ligand. An example of a complete titration is shown in Figure 6 for ZnTMpyP with dGMP. The presence of an isobestic point on the base line suggests that there are two chromophores in equilibrium in the solutions, the free porphyrin and a single deoxyribonucleotide associated complex. These data were analyzed by the application of a general minimization routine, SIMPLEX.[27] In each case a fit of the data was attempted by assuming a 1:1 complex and applying the equation

$$\text{Abs} - \text{Abs}_0 = \frac{K_1 L_0 \Delta\epsilon_1}{1 + K_1 L_0} P_0 \qquad (4)$$

where $\Delta\epsilon_1 = \epsilon_1 - \epsilon_f$, the molar absorptivities of the 1:1 complex and the free porphyrin, respectively, and as earlier P_0 is the total porphyrin concentration and L_0 is the total ligand concentration. In these spectrophotometric titration experiments, $L_0 \gg P_0$. These data were analyzed at two wavelengths, one near the maximum for the free porphyrin and the other near the absorption maximum of the complex. The analysis of the data shown in Figure 6 yields K_1 = 750 and 560 M^{-1} at 427 and 451 nm, respectively. We take the average $\langle K_1 \rangle$ = 650 $\pm$ 100 M^{-1}. A variation of eq 4 leads to a measure of the internal agreement of the data at a given wavelength:

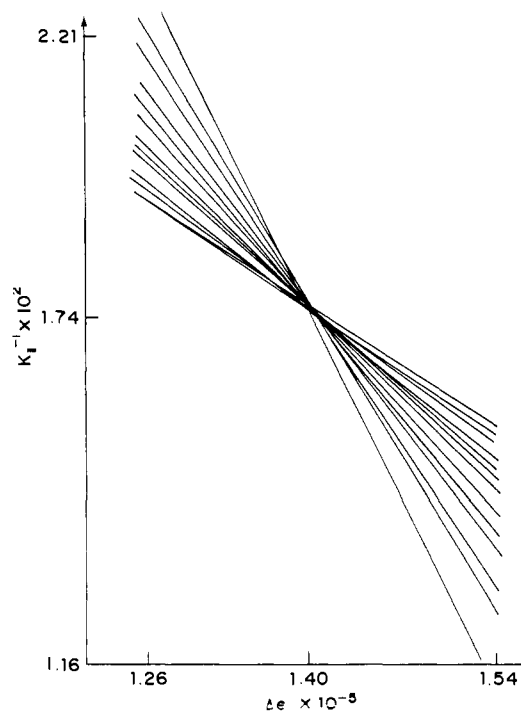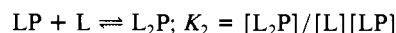$$1/K_1 = L_0 \left(\frac{P_0 \Delta\epsilon_1}{\text{Abs} - \text{Abs}_0} \right) - 1 \qquad (5)$$

Equation 5 is applied by systematically varying $\Delta\epsilon_1$ and calculating values of $1/K_1$. Each data point, therefore, generates a curve and the various curves should, in theory, intersect at a point. The results for this experiment at 451 nm are shown in Figure 7; the maximum deviation in this determination of K_1 is less than 1%.

The data were further analyzed for the formation of a 1:2 complex, i.e.,

$$\text{LP} + \text{L} \rightleftharpoons \text{L}_2\text{P}; \quad K_2 = [\text{L}_2\text{P}]/[\text{L}][\text{LP}]$$

The equation used in order to consider a second complex is

$$\text{Abs} - \text{Abs}_0 = \left(\frac{K_1 L_0 \Delta\epsilon_1 + K_1 K_2 L_0^2 \Delta\epsilon_2}{1 + K_1 L_0 + K_1 K_2 L_0^2} \right) P_0 \qquad (6)$$

The results obtained for K_1 and $\Delta\epsilon_1$ were employed in the determination of K_2 and $\Delta\epsilon_2$. These latter values were then assumed and values of K_1 and $\Delta\epsilon_1$ recalculated, etc., until a self-consistent set of parameters were obtained. The results of these calculations are shown in Table III. The ratio K_1/K_2 is greater than ten, indicating that the 1:2 complex is not a major form under the conditions of these experiments. The CuTMpyP–dGMP and H$_2$TMpyP–dAMP system were investigated at several temperatures over a 20-deg range. We determined that for the formation of the 1:1 dAMP–H$_2$TMpyP complex $\Delta H°$ = −28 kJ/mol and $\Delta S°$ = −34 J/(mol·deg) while for the 1:1 dGMP–CuTMpyP complex $\Delta H°$ = −52 kJ/mol and $\Delta S°$ = −110 J/(mol·deg). The NMR method was applied to the H$_2$TMpyP–dA and H$_2$TMpyP–dAMP systems for comparison. The intrinsic chemical shifts were very similar to those determined for the ribonucleotides, suggesting a similar structure for the final complex. The porphyrin
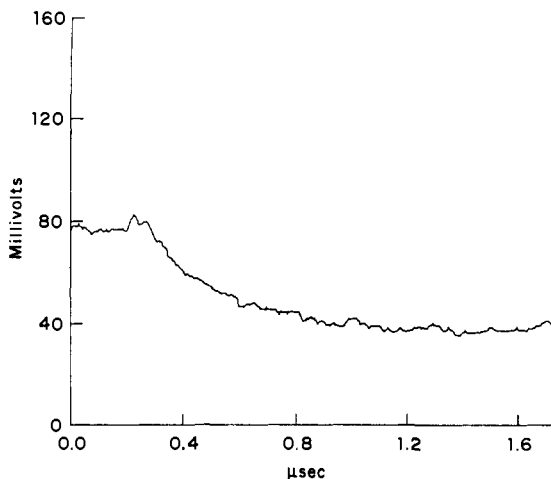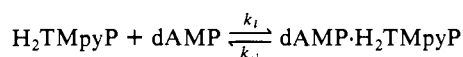
8184 *J. Am. Chem. Soc., Vol. 107, No. 26, 1985*

Pasternack et al.

Figure 8. Relaxation effect obtained for a H$_2$TMpyP–dAMP solution at μ = 0.2 M, pH 6.8, 23 °C, λ 430 nm. [H$_2$TMpyP]$_0$ = 42 μM, [dAMP]$_0$ = 830 μM, τ = 302 ns.

Figure 9. Plot of $1/\tau$ vs. [dAMP]$_0$ as in eq 8 yielding k_1 = 1.9 × 10^9 M^{-1} s^{-1} and k_{-1} = 1.9 × 10^6 s^{-1}.

concentration conditions for these experiments were from 1 to 10 mM as compared to <10 μM in the spectrophotometric titrations and no attempt was made to include a bis complex in the analysis of the NMR experiments. The values of K_1 determined from NMR are 0.51 × 10^3 M^{-1} for dA and 1.5 × 10^3 M^{-1} for dAMP. This compares well to the spectrophotometric value of K_1 of 1.1 × 10^3 M^{-1} for the H$_2$TMpyP–dAMP system at the same temperature.

D. Kinetics of Deoxyribonucleotide–Porphyrin Reaction. Standard joule-heating temperature-jump experiments were attempted for the various deoxyribonucleotide–porphyrin systems, and although color changes were observed concomitant with the 4-deg temperature rise, in all cases the relaxation time for these optical effects were within the heating time of the instrument (τ < 1 μs). The laser Raman temperature-jump technique which heats solutions in about 5 ns was applied to the dAMP–H$_2$TMpyP system. Relaxation effects were obtained (cf. Figure 8) at wavelengths near the Soret maxima of the free porphyrin (422 nm) and of the complex (430 nm). The relaxation times for a given solution are independent of wavelength but, as expected, the observed color changes are in opposite directions. For the reaction

$$\text{H}_2\text{TMpyP} + \text{dAMP} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{dAMP·H}_2\text{TMpyP}$$

standard mathematical methods for analyzing relaxation data lead to the expression

$$^1\!/_\tau = k_1 (\overline{[\text{dAMP}]} + \overline{[\text{H}_2\text{TMpyP}]}) + k_{-1} \tag{7}$$

where the horizontal bars indicate equilibrium concentrations. For all of the experiments conducted here [dAMP]$_0$ ≫ [H$_2$TMpyP]$_0$ and therefore 7 may be written as

$$1/\tau = k_1[\text{dAMP}]_0 + k_{-1} \tag{8}$$

A plot of $1/\tau$ vs. [dAMP]$_0$ is shown in Figure 9; the slope of the line is k_1 = 1.9 × 10^9 M^{-1} s^{-1} and the intercept is k_{-1} = 1.9 × 10^6 s^{-1}. The ratio gives an independent determination of K_1 = 1.0 × 10^3 M^{-1} in excellent agreement with the value determined via spectrophotometric titrations (cf. Table III).

Discussion

That H$_2$TMpyP and several of its metal derivatives form complexes with mononucleotides in aqueous solution has now been established with a variety of techniques. The stability constants obtained for these systems depend only slightly on the structure of the nucleic acid derivative. For example, addition of one or more phosphates at the C-5' of adenosine results in a somewhat enhanced stability but leaves the stability constants of uridine derivatives virtually unchanged. We conclude that electrostatic
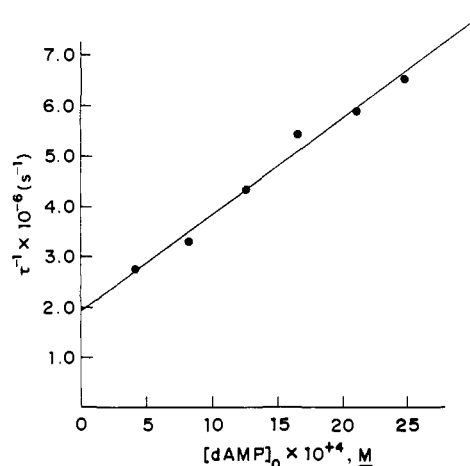
interactions are not of prime importance in the formation of these complexes. For a given porphyrin derivative stronger complexes form with purines than pyrimidines although the differences are again small, about a factor of 3 or 4 for deoxyribonucleotides and about an order of magnitude for ribonucleotides. Similar trends were seen in the binding of ethidium to deoxynucleotides[24] although for this latter ion the respective stability constants are smaller by an order of magnitude than those obtained for H$_2$TMpyP. Much more dramatic influences on stability are seen when we consider changes in porphyrin structure. Whereas stability constants are comparable for a given nucleotide with the metal-free, copper(II), nickel(II), zinc(II), and palladium(II) derivatives, virtually no interactions are observed for the iron(III), cobalt(III), nor manganese(III) derivatives. From these results we conclude that coordination of mononucleotides to axial sites is not playing a major role in the formation of these complexes. Under the conditions of these experiments, all of the Fe(III), Co(III), and Mn(III) porphyrins are six-coordinate with axial sites occupied by water molecules and/or hydroxyl groups. Both FeTMpyP and CoTMpyP (and especially the latter) are capable of forming strong complexes with axial ligands such as pyridine or imidazole by displacing water or hydroxy ligands.[28-30] For these two metalloporphyrins changing the identify of the axial ligand leads to major spectral changes, particularly in the Soret region. Such spectral changes are not oberved for these two metallo derivatives nor for the Mn(III) species with any of the mononucleotides. Thus there appears to be little tendency for potential metal coordinating sites on the mononucleotides to bind at the axial sites of these metals. Large spectral changes are observed for the nonmetallo and Cu(II) derivatives which have no axial chemistry. We have previously shown that like H$_2$TMpyP CuTMpyP is best thought of as a square coplanar complex of copper(II) in which there is virtually no tendency for the metal site to become coordinated to an axial ligand.[12] It appears that the presence of axial ligands prevents complex formation between the porphyrin and the mononucleotide. The Zn(II) porphyrin species is an interesting case to consider here. It behaves much like the nonaxially liganded H$_2$TMpyP and CuTMpyP. Yet the metal site in ZnTMpyP is five coordinate with the zinc out of the porphyrin plane and attached to a water molecule. This geometry still leaves the distal side of the ZnTMpyP available for reaction

(27) Copyrighted by J. P. Chandler, Department of Physics, University of Indiana, Bloomington, IN, 1965. The routine was further expanded by T. Needham, Department of Chemistry, University of Illinois, Urbana, IL.

(28) Pasternack, R. F.; Cobb, M. A.; Sutin, N. *Inorg. Chem.* **1975**, *14*, 866–873.

(29) Pasternack, R. F.; Gillies, B. S.; Stahlbush, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2613–2619.

(30) Thompson, A. N.; Krishnamurthy, M. *Inorg. Chem. Acta* **1979**, *34*, 145–150.

(31) Mauzerall, D. *Biochemistry* **1965**, *4*, 1801–1810.

(32) Shelnutt, J. A. *J. Phys. Chem.* **1984**, *88*, 6121–6127.

Table III. Equilibrium Constants for the Association of Porphyrins with Deoxyribonucleotides ($\mu = 0.2$ M; pH 6.9; $t = 25$ °C)

	dGMP		dAMP		dCMP		dTMP	
porphyrin	$K_1 \times 10^{-3}$	K_2	$K_1 \times 10^{-3}$	K_2	$K_1 \times 10^{-3}$	K_2	$K_1 \times 10^{-3}$	K_2
H$_2$TMpyP	0.75	6	1.0	30	0.40	2	0.22	3
CuTMpyP	1.7	150	1.8	<1	1.0	10	0.60	<1
NiTMpyP	1.7	130	1.2		0.20		0.53	18
ZnTMpyP	0.65	4	0.90	1	0.30	2	0.29	4
H$_2$TMpyP-2			0.80	<1				

with mononucleotides. Thus, what appears to be essential for the formation of complexes with mononucleotides is the availability of at least one side of the porphyrin plane without interference from an axial ligand. All these observations together suggest a stacking-type interaction between porphyrins and mononucleotides similar to that observed between methyl viologen, adenine, or caffeine with uroporphyrin and its metal derivatives.[31,33] The intrinsic chemical shifts Δ^i_{LP} reported here support this conclusion. The large shieldings observed in all cases for H$_{1'}$ and the heterocyclic base protons compared to other protons strongly suggest that the associations between the porphyrins and nucleotides (or nucleosides) involve primarily π–π interactions between the aromatic rings. That H$_{1'}$ shows a greater Δ^i_{LP} value than either H$_2$ or H$_8$ can be explained by assuming a roughly parallel orientation of the base and porphyrin rings with an "anti" conformation about the glycosidic bond. Molecular models show that such an arrangement minimizes steric interactions between the ribose ring and its substituent as well as the porphyrin ring. This arrangement serves to bring H$_{1'}$ closest to the porphyrin ring. To complete this discussion, the NiTMpyP derivative should be considered. NiTMpyP exists in solution as an equilibrium mixture of four- and six-coordinate nickel.[6] However, on interaction with nucleic acids and mononucleotides the equilibrium is shifted toward the four-coordinate form with binding occurring only to the non-axially coordinate species in which π–π overlap can be maximized.[3,4]

NMR experiments indicate that adenosine and its phosphoric esters do not form strong complexes with anionic porphyrins such as H$_2$TCPP or H$_2$TPPS. With the mono-, di-, and triphosphates it is to be expected that Coulombic barriers will play a major role in this lack of interaction, both porphyrins have a formal 4– charge. No such barrier exists for adenosine and yet it too fails to form a complex with these anionic porphyrins. However, it should be recalled that H$_2$TPPS and H$_2$TCPP are aggregated under the conditions of these experiments.[5,21] The formation of a complex would require either (i) a dimer–nucleoside interaction which would be expected to have a very small stability constant (cf. values of K_2 in Table III for an estimate for the formation of a three-tier stack) or (ii) a replacement of a monomer unit by the nucleoside: P$_2$ + L $\rightleftharpoons$ LP + P. However, the dimerization equilibrium constants for H$_2$TCPP and H$_2$TPPS are $\sim 10^5$ M^{-1} and the monomerization would not be driven under the conditions of these experiments. The failure to form a complex in these systems further supports our conclusion that, unlike H$_2$TCPP and H$_2$TPPS, H$_2$TMpyP exists in solution as a monomer under the experimental conditions used here.

The kinetic results obtained for dAMP with H$_2$TMpyP lend further support to a simple stacking model for the interaction. The observation of a single relaxation time and the excellent agreement between the kinetically and spectrophotometrically determined equilibrium constants indicate the reaction is a simple bimolecular association. Moreover, the forward rate constant of 1.9×10^9 M^{-1} s^{-1} is too large to allow for dissociation of aggregates, but is similar to the values of 2.4×10^9 M^{-1} s^{-1} and 1.1×10^9 M^{-1} s^{-1} obtained for dimerization of thionine and proflavin, respectively.[33] Since stacking of thionine and proflavin involves association of two cations, whereas stacking of dAMP and H$_2$TMpyP involves association of a cation with an anion, the

similarity in rates suggests partial control by the rate of desolvation.[33,34]

Thus, even when free in solution without the structural constraints imposed by being part of a helical polymer, nucleotides interact with porphyrins primarily through a van der Waals's type stacking interaction. We expect that site (as contrasted to territorial) binding to DNA will involve similar interactions.[35] The absorption spectral shifts for bound porphyrins relative to free chromophores are in the same direction for the mononucleotides as for DNA but some interesting differences should be noted. The position of the Soret band for all the nucleotide complexes of H$_2$TMpyP, CuTMpyP, and NiTMpyP are closer to what is obtained for poly(dA-dT) than for poly(dG-dC), but the degree of hypochromicity with the purines is more similar to those obtained with poly(dG-dC).[3] We believe that the degree of hypochromicity reflects the overlap of the π systems, and since these three porphyrins intercalate, the overlap is comparable in the intact poly(dG-dC) duplex as for individual nucleotide units. Similarly, the hypochromicity for these porphyrins is greater for purine mononucleotides than for poly(dA-dT), supporting our earlier conclusion that for this latter duplex, while there is some base involvement, the mode of interaction is not intercalation.

The position of the Soret maximum in intercalated duplex complexes appears to involve delocalized wave mechanical effects and thus a duplex may be required to simulate the position of the Soret band observed for poly(dG-dC). Even in the presence of complementary dinucleotides or mixtures of mononucleotides, none of the porphyrin complexes shows any CD spectrum and so it is most unlikely that the porphyrin serves as a template for duplex formation. However, it should prove useful to determine the minimum number of GC base pairs needed in an oligonucleotide to simulate the poly(dG-dC) spectrum. This dependence of spectral position on duplex involvement is not peculiar to porphyrins. For ethidium ion, the spectral band at about 470 nm shifts 12 nm for mononucleotides compared to 40 nm for DNA.[24]

As may be seen for Tables II and III, the hypochromicity induced in the Soret band of H$_2$TMpyP-2 by purine monophosphates is considerable and the stability of the complex formed with dAMP is comparable to that formed by H$_2$TMpyP. Yet these two porphyrins show very different reactivities with polynucleotide duplexes. H$_2$TMpyP intercalates in poly(dG-dC) with a 22 nm spectral shift and 41% hypochromicity; H$_2$TMpyP-2 shows no spectral shift with poly(dG-dC) and shows a 3% hyperchromicity, characteristic of territorial binders.[3] It has been noted through examination of molecular models that the presence of the N-methyl group in the ortho position of the pyridyl substituent could interfere with sugar residues or a portion of the purine base.[36] Thus even if the poly(dG-dC) duplex is sufficiently flexible to permit H$_2$TMpyP-2 to pass through its interior, there may be a stereochemical barrier to the closing of the duplex required to keep the porphyrin from dissociating. This dramatic difference in stabilities of the ortho and para derivatives with poly(dG-dC) is a consequence of the existence of an intact duplex. For the mononucleotides with their freedom to orient in solution, this stereochemical barrier does not exist and stability constants are similar for the ortho and para derivatives of the porphyrin.

(33) Dewey, T. G.; Wilson, P. S.; Turner, D. H. *J. Am. Chem. Soc.* **1978**, *100*, 4550–4554.

(34) Dewey, T. G.; Raymond, D. A.; Turner, D. H. *J. Am. Chem. Soc.* **1979**, *101*, 5822–5826.

(35) Friedman, R. A. G.; Manning, G. S. *Biopolymers* **1984**, *23*, 2671–2714.

(36) Sobell, H. M., private communication.

Supplementary Material Available: Tables of Δ^i_{LP} for all NMR experiments, results for the ionic strength and temperature dependences of K_1 and Δ^i_{LP}, and the conditions for the temperature-jump experiments (6 pages). Ordering information is given on any current masthead page.

Diastereo- and Enantioselective Aldehyde Addition Reactions of 2-Allyl-1,3,2-dioxaborolane-4,5-dicarboxylic Esters, a Useful Class of Tartrate Ester Modified Allylboronates[1]

William R. Roush,[*2] **Alan E. Walts, and Lee K. Hoong**

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Received May 17, 1985

Abstract: The preparation and aldehyde addition reactions of the title compounds (**1a–c**) are described. These tartrate ester based reagents are the most highly enantioselective group of allylboronate esters reported to date. Reagent **1b** prepared from diisopropyl tartrate reacts with achiral aldehydes to give homoallylic alcohols in good yield and high enantioselectivity (71–87% ee). Interestingly, the greatest selectivity is obtained with α-branched aldehydes such as pivaldehyde (82% ee) and cyclohexanecarboxaldehyde (87% ee). These reagents also exhibit useful levels of matched and mismatched diastereoselection in reactions with chiral aldehydes. For example, the reaction of glyceraldehyde acetonide (**4**) and **1b** is selective either for erythro alcohol **6** (96:4) or the threo diastereomer **7** (92:8) depending on the chirality of **1b** and the reaction solvent. The asymmetric induction in these reactions appears to originate from a novel stereoelectronic effect involving n/n repulsive interactions between the aldehydic oxygen atom and an ester carbonyl in the disfavored transition state B.

The reactions of allyl and crotylmetal compounds with aldehydes have been extensively investigated in recent years.[3] Although excellent stereochemical control has been achieved in the additions of several classes of reagents (e.g., B, Sn, Si, Cr, Ti, etc.) to achiral aldehydes,[3a] a general solution to the problem of controlling facial selectivity in reactions with chiral aldehydes has not been found.[3,4] As an extension of our studies of allylic boronate aldehyde addition reactions,[5] we have turned to double-asymmetric synthesis[6] as a possible solution to this problem.[7] We are pleased therefore to report the preparation of a new class of tartrate ester based allylboronates (**1a–c**) that exhibit useful levels of matched and mismatched diastereoselection in reactions with chiral aldehydes.[8,9] In addition, reagent **1b** reacts with achiral
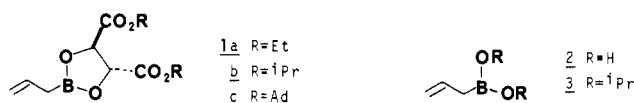
aldehydes to give homoallylic alcohols in good yield and with high enantioselectivity (71–87% ee). This compound is the most highly enantioselective allylboronate reported to date, is very easily prepared in either enantiomeric series, and consequently is well-suited for use as a reagent in organic synthesis.

The preferred method (A) for synthesis of **1a–c** involves esterification of allylboronic acid (**2**)[10] with the appropriate tartrate ester.[11] Although crude **1a–c** can be used directly in aldehyde addition reactions (the only significant impurity is residual tartrate ester, typically 0.3–0.5 equiv), we have found that tartrate-free reagents generally give superior results especially in reactions with achiral aldehydes and therefore recommend that the crude boronic ester be purified by distillation. By using this procedure, ≥97% pure **1b** has been prepared in 78% yield from DIPT (54% yield based on the allylmagnesium bromide used to prepare **2**). This reagent is sensitive to moisture but can be stored under an inert

(1) Portions of this work are described in: Walts, A. E. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, 1985.

(2) Fellow of the Alfred P. Sloan Foundation, 1982–1986.

(3) (a) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 555. (b) Reetz, M. T. *Ibid.* **1984**, *23*, 556. (c) McGarvey, G. J.; Kimura, M.; Oh, T.; Williams, J. M. *J. Carbohydr. Chem.* **1984**, *3*, 125.

(4) For recent stereochemical studies, see: (a) Hoffmann, R. W.; Weidmann, U. *Chem. Ber.* **1985**, *118*, 3966. (b) Keck, G. E.; Boden, E. P. *Tetrahedron Lett.* **1984**, *25*, 729. (f) Yamamoto, Y.; Yatagai, H.; Ishihara, Y.; Maeda, N.; Maruyama, K. *Tetrahedron* **1984**, *40*, 2239. (g) Lewis, M. D.; Kishi, Y. *Tetrahedron Lett.* **1982**, *23*, 2343. (h) Buse, C. T.; Heathcock, C. H. *Tetrahedron Lett.* **1978**, 1685.

(5) (a) Roush, W. R.; Adam, M. A.; Harris, D. J. *J. Org. Chem.* **1985**, *50*, 2000. (b) Roush, W. R.; Walts, A. E. *Tetrahedron Lett.* **1985**, *26*, 3427.

(6) Masamune, S.; Choy, W.; Peterson, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1.

(7) Reactions of chiral aldehydes with allylic boronates prepared from *endo*-3-phenyl-*exo*-2,3-bornanediol have been studied by Hoffman and co-workers: (a) Hoffmann, R. W.; Zeiss, H.-J.; Ladner, W.; Tabche, S. *Chem. Ber.* **1982**, *115*, 2357. (b) Hoffmann, R. W.; Endesfelder, A.; Zeiss, H.-J. *Carbohydr. Res.* **1983**, *123*, 320. For reactions of these reagents with achiral aldehydes, see: (c) Herold, T.; Schrott, U.; Hoffmann, R. W.; Schnelle, G.; Ladner, W.; Steinbach, K. *Chem. Ber.* **1981**, *114*, 359. (d) Hoffmann, R. W.; Herold, T. *Chem. Ber.* **1981**, *114*, 375.

(8) Use of tartrate esters as chiral auxiliaries in the asymmetric reactions of allenyl boronic acid has been reported: Haruta, R.; Ishiguro, M.; Ikeda, N.; Yamamoto, H. *J. Am. Chem. Soc.* **1982**, *104*, 7667.

(9) For leading references to several other classes of chiral allylmetal compounds, see: (a) Midland, M. M.; Preston, S. B. *J. Am. Chem. Soc.* **1982**, *104*, 2330. (b) Jadhav, P. K.; Jadhav, P. K. *Ibid.* **1983**, *105*, 2092. (c) Brown, H. C.; Jadhav, P. K. *J. Org. Chem.* **1984**, *49*, 4089. (d) Hayashi, T.; Konishi, M.; Kumada, M. *J. Org. Chem.* **1983**, *48*, 281. (e) Jephcote, V. J.; Pratt, A. J.; Thomas, E. J. *J. Chem. Soc., Chem. Commun.* **1984**, 800. (f) Hoffmann, R. W.; Landmann, B. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 437. (g) Roder, H.; Helmchen, G.; Peters, E.-M.; Peters, K.; von Schnering, H.-G. *Ibid.* **1984**, *23*, 898.

(10) Matteson, D. S.; Majumdar, D. *Organometallics* **1983**, *2*, 230.

(11) L-(+)-Diethyl tartrate, and L-(+)- and D-(−)-diisopropyl tartrate were obtained from Aldrich Chemical Co. L-(+)-Diadamantyl tartrate was synthesized from tartaric acid by using methods developed by Prof. K. B. Sharpless.