a potential surface with a shallow minimum. The repulsive part of this surface intersects an attractive potential surface and the crossing between the two surfaces is the rate-determining step of the reaction. A positive activation energy clearly corresponds to the crossing point lying at higher energies than the separated reactants. However, if the intersection lies below the separated reactants, an increase in temperature decreases the probability of crossing between surfaces due to the higher average velocity of the collision complex at the crossing point.[16] This is responsible for the negative temperature coefficient found in the reactions with tetramethylethylene.

Acknowledgment. We thank the National Research Council of Canada for financial support.

(16) V. N. Kondrat'ev, "Chemical Kinetics of Gas Reactions," Pergamon, London, 1964, p 217.

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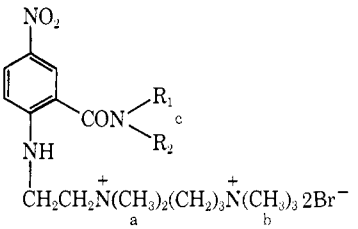
Topography of Nucleic Acid Helices in Solutions. Steric Requirements for Intercalation[1]

Sir:

It is well known that planar molecules such as acridine orange, ethidium bromide, proflavine, and actinomycin may intercalate between base pairs in DNA.[2] This phenomenon leads to an increase in the length of the helix and is usually accompanied by an increase in the viscosity of the solution. In order to delineate the steric requirements for the intercalation process, reporter molecules, I, with increasing size of R substituents were synthesized.[3]



1, $R_1 = R_2 = H$
2, $R_1 = CH_3$; $R_2 = H$
3, $R_1 = R_2 = CH_3$
4, $R_1 = R_2 = CH_2CH_3$

The results of the absorption and the induced circular dichroism studies of free and DNA-bound reporters are given in Table I. The temperature dependent partial proton magnetic resonance spectra are shown in Figure 1, and the results of the low-shear viscometric studies are given in Table II.

A number of interesting observations may be made. (1) The reporter molecules **1** and **2** where $R_1 = R_2 = H$ and $R_1 = H$, $R_2 = CH_3$, respectively, show a strong hypochromic effect (47 and 41%), and a large induced circular dichroism ($[\theta]^t = -6590$ and $-10{,}060$) of the 4-nitroaniline transition on binding to salmon sperm DNA (Table I). (2) Considerable broadening of the pmr signal of the a- and b-methyl protons of **1** and **2** are observed in the DNA complex at 32°.[4] In the case of DNA–**2** complex the pmr signal of the c-methyl protons is completely broadened and indistinguishable from base-line noise at 32 and 58°, indicating a restricted tumbling rate of the 4-nitroaniline ring of **2**. At 90°, where melting of the DNA helix has occurred,[5] sharp resonance lines characteristic of the free reporter molecules are observed for the a-, b-, and c-methyl protons. More rapid tumbling and/or efficient averaging of the chemical-shift environment is indicated. (3) The low-shear viscometric studies show that the intrinsic viscosity of the DNA-bound reporters **1** and **2** relative to free DNA are increased by 0.26 and 0.30, respectively (Table II). The above results, 1, 2, and 3, are strongly suggestive that the 4-nitroaniline ring of reporters **1** and **2** is intercalated between base pairs in DNA. This is

Table I. Effect of Salmon Sperm DNA (ss DNA) on the Absorption and Induced Circular Dichroism Spectra of Reporter Molecules **1–4**[a]

	Absorption spectra					Circular dichroism	
	H_2O–buffer		ss DNA			ss DNA	
Reporter	λ^{max}, nm	ϵ^{max}	λ^{max}, nm	ϵ^{max}	% H[b]	λ^t, nm	$(\theta)^t \times 10^{-3}$
1	373	16,190	389	11,000	47.0	385	−6.59
2	374	14,770	393	10,450	41.0	395	−10.06
3	372	13,650	375	13,540	1.0	360–400	0.34
4	372	12,140	374	11,500	5.6	360–400	0.40

[a] At 25.0 ± 0.2° in 0.01 *M* sodium phosphate buffer, pH 6.40–6.50 (0.01 *M* in Na^+). Spectra were taken in 10-mm cells using Cary 14 and Cary 60 spectrometers. Values of λ^{max}, ϵ^{max}, λ^t, and $(\theta)^t$ in the presence of nucleic acid reported in this table are limiting values, *i.e.*, additional change in spectra is not observed at further excess of nucleic acid. [b] Percentage hypochromicity (% H) = $[\epsilon^{max}_{H_2O}/\epsilon^{max}_{p} - 1.00]100$, where $\epsilon^{max}_{H_2O}$ and ϵ^{max}_{p} are the extinction coefficients in the presence and absence of the polynucleotides.

(1) This is related (as XXII) to other articles on Topography of Nucleic Acids in Solutions. For part XXI, see E. J. Gabbay, B. Gaffney, and R. Glaser, *Ann. N. Y. Acad. Sci.*, **171**, 810 (1970).

(2) (a) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961); (b) D. S. Drummond, N. J. Pritchard, V. F. W. Simpson-Gildemeister, and A. R. Peacocke, *Biopolymers*, **4**, 971 (1966); (c) G. Cohen and H. Eisenberg, *ibid.*, **8**, 45 (1969); (d) W. Müller and D. M. Crothers, *J. Mol. Biol.*, **35**, 251 (1968).

(3) The reporter molecules **1–4** were prepared according to the procedure of E. J. Gabbay (*J. Amer. Chem. Soc.*, **91**, 5136 (1969)) and analyzed by ir, pmr, uv, and elemental analysis.

(4) If the rate of molecular tumbling of molecules in solutions is lower than the typical Larmor frequencies W_0 (of the order of 10^8–10^9 radians sec^{-1} for protons in the conventional magnetic field), then T_2, the transverse relaxation time, is considerably diminished, leading to substantial line broadening of the proton signal (see O. Jardetsky and C. D. Jardetsky in "Methods of Biochemical Analysis," D. Glick, Ed., Interscience, New York, N. Y., 1962). This situation is obtained if the proton is contained in a rigid macromolecule, *e.g.*, DNA, or if the proton is contained in a slowly tumbling small molecule bound to a macromolecule.

(5) Evidence for the melting of DNA is seen in Figure 1 by the appearance of the thymine methyl peaks at 1.5–2.0 ppm [C. C. McDonald, W. D. Phillips, and J. Lazar, *J. Amer. Chem. Soc.*, **89**, 4166 (1967)].

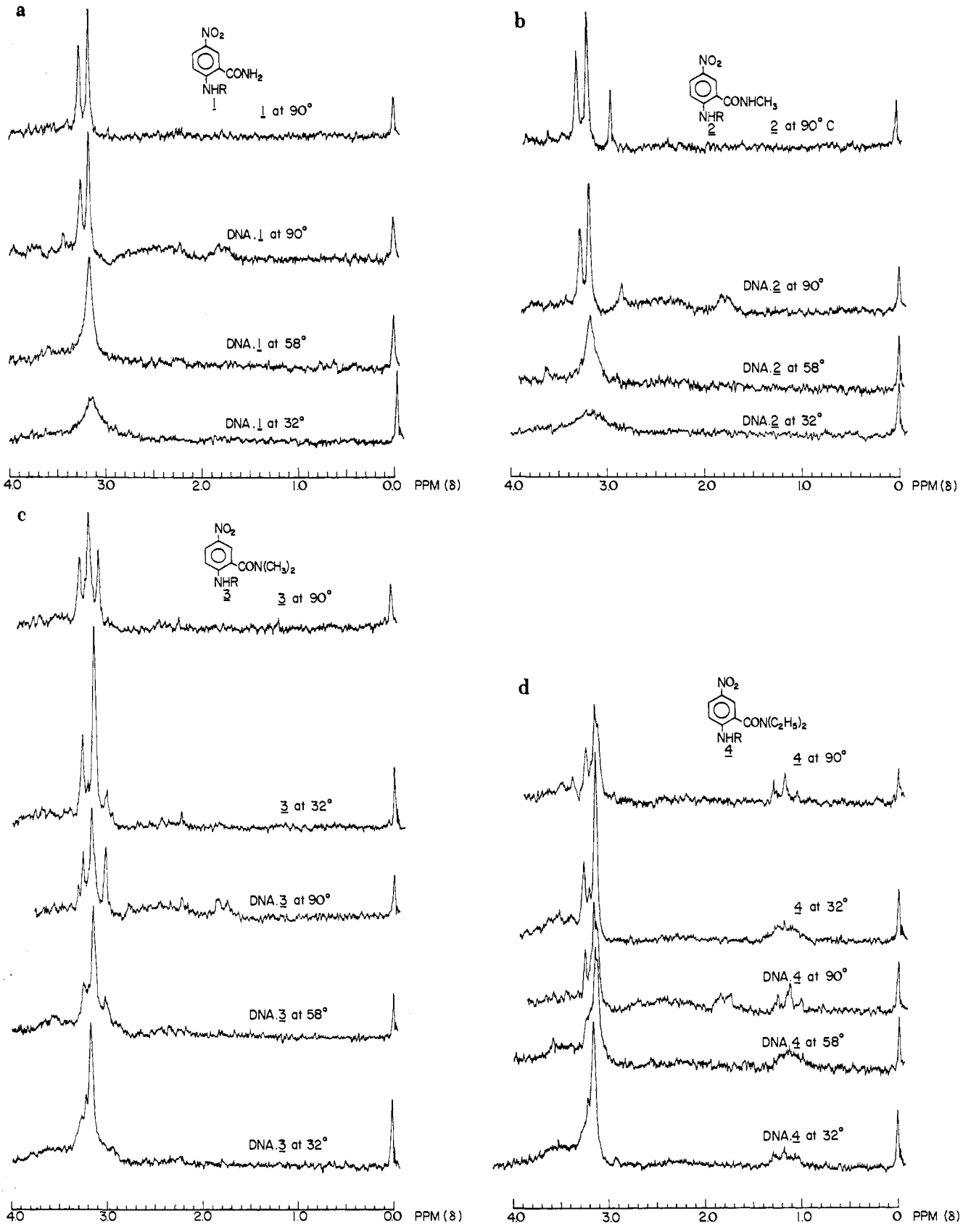


Figure 1. The temperature-dependent partial pmr spectra of free and DNA-bound reporter molecules: (a) reporter **1**; (b) reporter **2**; (c) reporter **3**; (d) reporter **4**. Spectra were taken on a Varian A-60A. Sonicated low molecular weight ss DNA was used at 0.16 mol of P/l. in D_2O in 10^{-4} sodium phosphate buffer (pD 7.0 ± 0.2). The concentration of the reporter molecule was 0.02 *M*.

in contrast to the behavior of reporter molecules **3** and **4** with *N*,*N*-dimethyl- and *N*,*N*-diethyl-substituted 2-carboxyamido groups, respectively. For example, the latter reporters show little or no effect on the absorption

Table II. Relative Intrinsic Viscosity of DNA–Reporter Complexes to Free DNA[a,b]

Reporter	$(\eta)/(\eta_0)$
1	1.26
2	1.30
3	0.97
4	0.97
$(CH_3)_3N^+(CH_2)_3N^+(CH_3)_3$	0.98

[a] The intrinsic viscosities of free DNA, (η_0), and the DNA–reporter complex, (η), were calculated using the equation $\eta_{sp}/c = (\eta) + k'(\eta)^2c$ and assuming that the value of the Huggins constant, k', is the same for DNA and the DNA–reporter complex. This assumption is justifiable and has been recently shown by P. D. Ross and R. L. Scruggs [*Biopolymers*, **2**, 79 (1964)] to apply for DNA and DNA–dye complexes under similar ionic strength conditions. [b] Viscosity measurements were carried out using 1.13×10^{-3} *M* in poise/liter of DNA and 1.0×10^{-4} *M* in reporter molecule in 0.025 *M* sodium phosphate buffer (pH 6.50) at 25° using a Zimm viscometer. Under these conditions, the reporter molecules are fully bound to DNA as evidenced by spectrophotometric titration studies.

and induced circular dichroism of the 4-nitroaniline transition on binding to DNA (Table I). In addition, the pmr signals of the a-, b-, and c-alkyl protons of **3** and **4** are clearly discernible in the presence of DNA at 32° with only a slight broadening and an upfield chemical shift.[6] In line with these results, the intrinsic viscosity of the DNA-bound reporters **3** and **4** is slightly decreased (Table II). A similar decrease of the intrinsic viscosity of DNA is observed with the diammonium salt **5**, $(CH_3)_3N^+(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$. The mode of binding of **3** and **4** to DNA seems to be external electrostatic binding to the phosphodiester chain with the 4-nitroaniline ring freely tumbling in solution. Thus, intercalation between base pairs of DNA by **3** and **4** is inhibited presumably due to either (a) the absence of an H-bond donor or (b) steric hindrance by the nonplanar *N,N*-dialkylcarboxamido substituent of **3** and **4**. We favor the latter alternative since the requirement for a hydrogen bond donor at the 2 position of the 4-nitroaniline ring is not essential for the intercalation process. For example, it has been shown that the unsubstituted, the 2-cyano-, and the 2-nitro-substituted 4-nitroaniline labeled diamines intercalate between base pairs in DNA as evidenced by the absorption, induced circular dichroism, proton magnetic resonance, and viscosity studies.[7] It should be noted that the results of the preliminary binding studies of these reporters to DNA show that the reporter molecules **1** and **2** are bound approximately 10–15-fold more strongly than **3** and **4**.[8] This is in line with the proposed binding modes of these molecules described above. Further work along this area is in progress.

Acknowledgment. This work was supported by Grants GM17503 and GM18653 from the U. S. Public Health Service and GB-16044 from the National Science Foundation.

(6) It should be noted that the alkyl protons of the *N,N*-dialkylcarboxamido group of **3** and **4** are not equivalent at 32° due to the restricted rotation about the amide group. At 90°, however, coalescence of the pmr signals is obtained (Figure 1c and d).

(7) (a) F. Passero, E. Gabbay, B. L. Gaffney, and T. Kurucsev, *Macromolecules*, **3**, 158 (1970); (b) E. Gabbay, *J. Amer. Chem. Soc.*, **91**, 5136 (1969); (c) B. L Gaffney, Ph.D. Thesis, Rutgers, 1969.

(8) Binding data were obtained by competitive binding studies using a cation exchange resin technique: A. DePaolis, Ph.D. Thesis, Rutgers, 1971.

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Some Novel Reactions of Vinyl Isocyanide with Organophosphorus and Organoarsenic Compounds

Sir:

Recently the base-catalyzed addition of phosphorus–hydrogen bonds across the vinyl double bonds of vinylphosphines to give polytertiary phosphines with PCH_2CH_2P units was reported.[1] This communication reports base-catalyzed additions of phosphorus–hydrogen bonds to vinyl isocyanide[2] which provide routes not only to a phosphine isocyanide with a PCH_2CH_2NC unit but also to the first derivative of the 3-azaphosphole heterocyclic system.

Reaction of diphenylphosphine with excess vinyl isocyanide in boiling benzene for ~20 hr in the presence of ~10% potassium *tert*-butoxide catalyst resulted in addition of the phosphorus–hydrogen bond across the vinyl double bond to give a 53% yield of slightly yellow viscous liquid, $(C_6H_5)_2PCH_2CH_2NC$: bp 139–142° (0.05 mm) (*Anal.* Calcd for $C_{15}H_{14}NP$: C, 75.4; H, 5.9; N, 5.9; P, 12.9; mol wt, 239. Found: C, 75.1, 75.7; H, 6.2, 5.7; N, 5.6, 5.3; P, 13.1; mol wt, 238 (osmometer in benzene solution)). The infrared spectrum of neat $(C_6H_5)_2PCH_2CH_2NC$ exhibited a strong ν_{CN} frequency at 2154 cm^{-1} confirming the presence of an isocyanide group. The proton nmr spectrum of $(C_6H_5)_2PCH_2CH_2NC$ in $CDCl_3$ exhibited the usual aromatic proton resonance at τ 2.74 as well as two resonances at 6.72 (quartet, 8-Hz separation) and 7.66 (triplet, 8-Hz separation) arising from the nonequivalent methylene groups. The mass spectrum of $(C_6H_5)_2PCH_2CH_2NC$ did not exhibit a molecular ion. Instead the highest *m/e* ion was $(C_6H_5)_2PC_2H_3^+$ formed by elimination of HCN from the molecular ion. Other ions frequently found in the mass spectra of $(C_6H_5)_2PR$ derivatives[3,4] were also observed (*e.g.*, $(C_6H_5)_2PH^+$, $C_{12}H_8P^+$, $C_{12}H_{10}^+$, $C_{12}H_8^+$, $C_8H_7P^+$, $C_8H_6P^+$, $C_6H_6P^+$, $C_6H_5P^+$, and $C_6H_4P^+$). The arsenic analog $(C_6H_5)_2AsCH_2CH_2NC$, a liquid, bp 153° (2 mm), was similarly prepared in 31% yield from diphenylarsine and excess vinyl isocyanide and characterized by elemental analyses, molecular weight determination, and infrared and proton nmr spectra.

The compounds $(C_6H_5)_2ECH_2CH_2NC$ (E = P or As) are the first known compounds containing both an isocyanide group and a tertiary phosphorus or arsenic atom. All of these functionalities coordinate with transition metals to form extensive series of com-

(1) R. B. King and P. N. Kapoor, *J. Amer. Chem. Soc.*, **91**, 5191 (1969).

(2) D. S. Matteson and R. A. Bailey, *ibid.*, **90**, 3761 (1968).

(3) D. H. Williams, R. S. Ward, and R. G. Cooks, *ibid.*, **90**, 966 (1968).

(4) R. Colton and Q. H. Porter, *Aust. J. Chem.*, **21**, 2215 (1968).