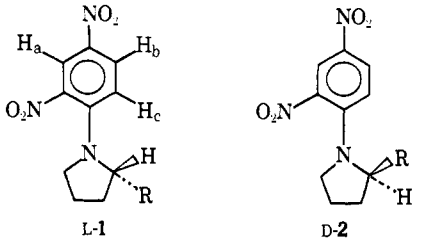
Dissymmetric Recognition of the Helical Sense of Deoxyribonucleic Acid. Evidence for a Right-Handed Helix in Solution[1]

Sir:

In an attempt to determine the helical sense of DNA in solution, we have synthesized the DNP derivatives of L- and D-prolines, **1** and **2**. From molecular model considerations, intercalation of the dinitroaniline ring of the L-proline derivative **1** between base pairs of a right-handed DNA helix is considerably more hindered than the corresponding D-proline derivative **2**. This effect is schematically illustrated in Figure 1. It is noted that intercalation of the L-proline derivative **1** places the carboxyamido side chain, *i.e.*, the –CONHR′ group, in a sterically unfavorable position. Thus, it would be expected that the L-enantiomer **1** will interact with native DNA to a lesser extent than the corresponding D-enantiomer **2**.



$R = CONHCH_2CH_2\overset{+}{N}(CH_3)_2(CH_2)_3\overset{+}{N}(CH_3)_3 \cdot 2Br^-$

The results of the absorption and circular dichroism studies of free and DNA-bound reporter molecules **1** and **2** are given in Table I. The results of the temperature dependent proton magnetic resonance studies are given in Table II, and the low-shear viscometric data are shown in Figure 2.

A number of interesting observations may be made. (1) The D enantiomer shows a large hypochromic effect (42%) and a 5-nm red shift in the 4-nitroaniline transition on binding to DNA, whereas the L enantiomer elicits a 21% hypochromism and a 2-nm red shift[2] (Table I). (2) The circular dichroism spectra of **1** and **2** indicate that there are two optically active transitions, *i.e.*, at 405 and 335 nm. These transitions have been shown to be due to the 2-nitroaniline (405 nm) and the 4-nitroaniline (335 nm) electronic transitions.[2e] It is noted that the D enantiomer undergoes a larger change in the molar ellipticities, $[\theta]$, of the peaks at 405 and 335 nm as compared to the $[\theta]$ of the troughs of the L enantiomer on binding to DNA (Table I). (3) The pmr results shown in Table II are also consistent with a more intimate binding of **2** to DNA as compared to **1**.

Table I. Effect of Salmon Sperm Deoxyribonucleic Acid (ss DNA) on the Absorption and Circular Dichroism Spectra of the L and D Enantiomers **1** and **2**, Respectively[a]

	Absorption Spectra					Circular dichroism							
	H_2O-buffer		ss DNA			H_2O-buffer				ss DNA			
Reporter	λ_{nm}^{max}	ϵ^{max}	λ_{nm}^{max}	ϵ^{max}	% H^b	λ_1	$[\theta]_1 \times 10^{-4}$	λ_2	$[\theta]_2 \times 10^{-4}$	λ_1	$[\theta]_1 \times 10^{-4}$	λ_2	$[\theta]_2 \times 10^{-4}$
L-**1**	374	9,900	376	8200	21	405	−1.22	335	−1.18	405	−1.14	333	−0.94
D-**2**	374	10,100	379	7150	42	405	1.14	335	1.04	390	0.88	330	0.48

[a] At ambient temperature in 0.01 *M* 2-(*N*-morpholino)ethanesulfonic acid (Mes) buffer, pH 6.2 (0.005 *M* in Na^+). Absorption spectra were taken in 10-mm cells using a Cary-15 spectrometer at DNA and reporter concentrations of 2.5×10^{-3} mol of phosphorus/l. and 1×10^{-4} *M*, respectively. Under these conditions, the reporter molecules are fully bound. Circular dichroism spectra were taken in 50-mm cells using a Jasco J-20 spectrometer at DNA and reporter concentrations of 2.7×10^{-3} mol of phosphorus/l. and 9.1×10^{-5} *M*, respectively. [b] Per cent hypochromicity ($\% H = [\epsilon_{H_2O}^{max}/\epsilon_P^{max} - 1)]100$), where $\epsilon_{H_2O}^{max}$ and ϵ_P^{max} are the extinction coefficients in the absence and presence of DNA.

Table II. Chemical Shifts (ppm) from the Internal Standard Sodium 2,2-Dimethyl-2-silapentanesulfonate (DSS) and Line Width in Hz at Half-Height ($\Delta\nu_{1/2}$) of Free and DNA-Bound Reporter Molecules, **1** and **2**, at Various Temperatures[a]

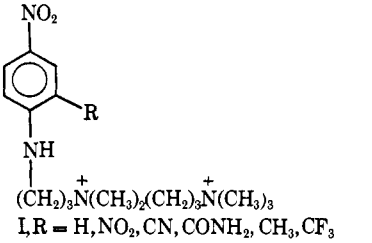
Reporter	Temp, °C	Chemical shift (δ)[b] H_a	H_b	H_c	$H_d + H_e$
1	39	873 d_1	829 q	707 d_2	318 (1.5)
1	90	870 d_1	829 q	708 d_2	318 (1.5)
DNA-**1**	39	821 (7)	*c*	*c*	319 (3.0)
DNA-**1**	50	823 (4)	789 (10)	682 (20)	318 (2.5)
2	39	873 d_1	829 q	707 d_2	318 (1.5)
2	90	870 d_1	829 q	708 d_2	318 (1.5)
DNA-**2**	39	809 (8)	*c*	*c*	319 (5)
DNA-**2**	50	809 (5)	766 (15)	*c*	318 (3)

[a] Sonicated low molecular weight ss DNA was used at 0.16 mol of phosphorus/l. in D_2O. The concentration of reporter molecule was 0.02 *M*. Spectra were taken on a Varian XL-100. It should be noted that the pmr spectrum of DNA in the temperature range of 20–70° is completely broadened and indistinguishable from base-line noise.[2] [b] The chemical shift, δ (Hz), and the multiplicity of the pmr signal are given as follows: d_1, doublet with $^1J_{H_a-H_b} = 2.5$ Hz; d_2, doublet with $^2J_{H_b-H_c} = 9.3$ Hz; q, doublet of doublets with $^1J = 2.5$ and $^2J = 9.3$ Hz. [c] Proton signal is indistinguishable from base-line noise.

A preliminary report of this work was presented at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972.

(1) Topography of Nucleic Acid Helices in Solutions. XXV. For the previous paper in this series, see E. Gabbay, R. DeStefano, and K. Sanford, *Biochem. Biophys. Res. Commun.*, **46**, 155 (1972).

(2) Extensive work from this laboratory has shown that the 4-nitroaniline ring of the reporter molecule I intercalates between base pairs of DNA. A hypochromic effect, approximately 25–65%, and an induced circular dichroism are observed for the (330–400 nm) 4-nitroaniline transition of I upon binding to DNA. In addition, an increase in the intrinsic viscosity of DNA as well as a restricted tumbling of the 4-nitroaniline ring (evidenced by total line broadening and upfield chemical shift of the proton magnetic resonance signals of the aromatic protons of I) are observed. These results are completely consistent with the intercalation model. See (a) E. J. Gabbay and A. DePaolis, *J. Amer. Chem. Soc.*, **93**, 562 (1971); (b) E. J. Gabbay and B. L. Gaffney, *J. Macromol. Sci., Chem.*, **4** (6), 1315 (1970); (c) E. J. Gabbay, B. L. Gaffney, and R. Glaser, *Ann. N. Y. Acad. Sci.*, 171, 810 (1970); (d) F. Passero, E. J. Gabbay, B. L. Gaffney, and T. Kurucsev *Macromolecules*, 158 (1970); (e) E. J. Gabbay, *J. Amer. Chem. Soc.*, **91**, 5136 (1969).



I, R = H, NO_2, CN, $CONH_2$, CH_3, CF_3

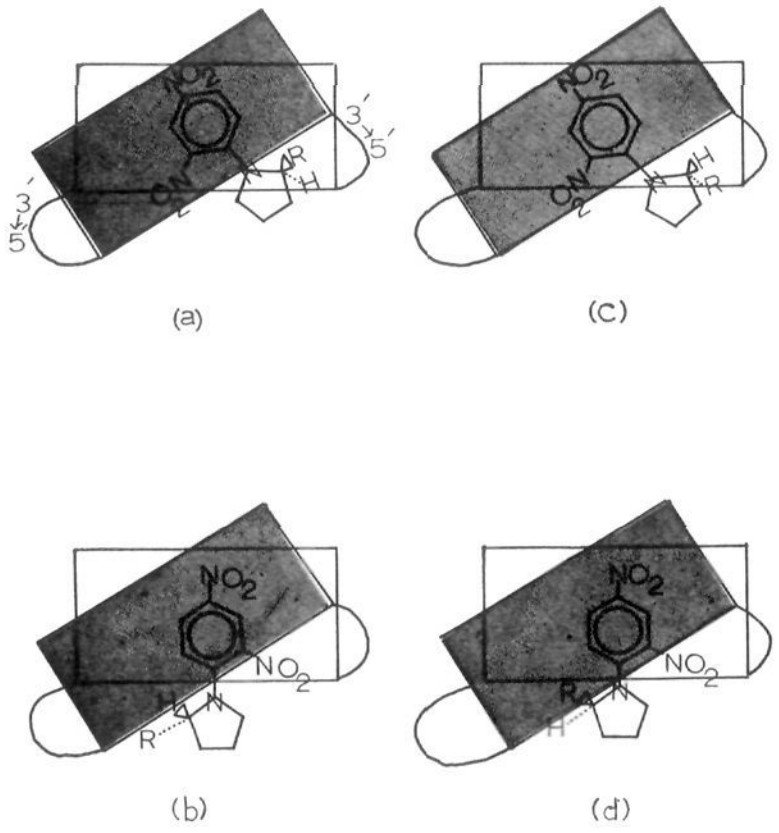


Figure 1. Schematic illustration (top view) of the possible intercalated complexes of the D-prolyl derivative **2** (a and b) and L-prolyl derivative **1** (c and d) between base pairs of a right-handed DNA helix. The direction of the $3' \rightarrow 5'$ sugar phosphate chain with respect to the base pairs at the top (shaded) and bottom is indicated.

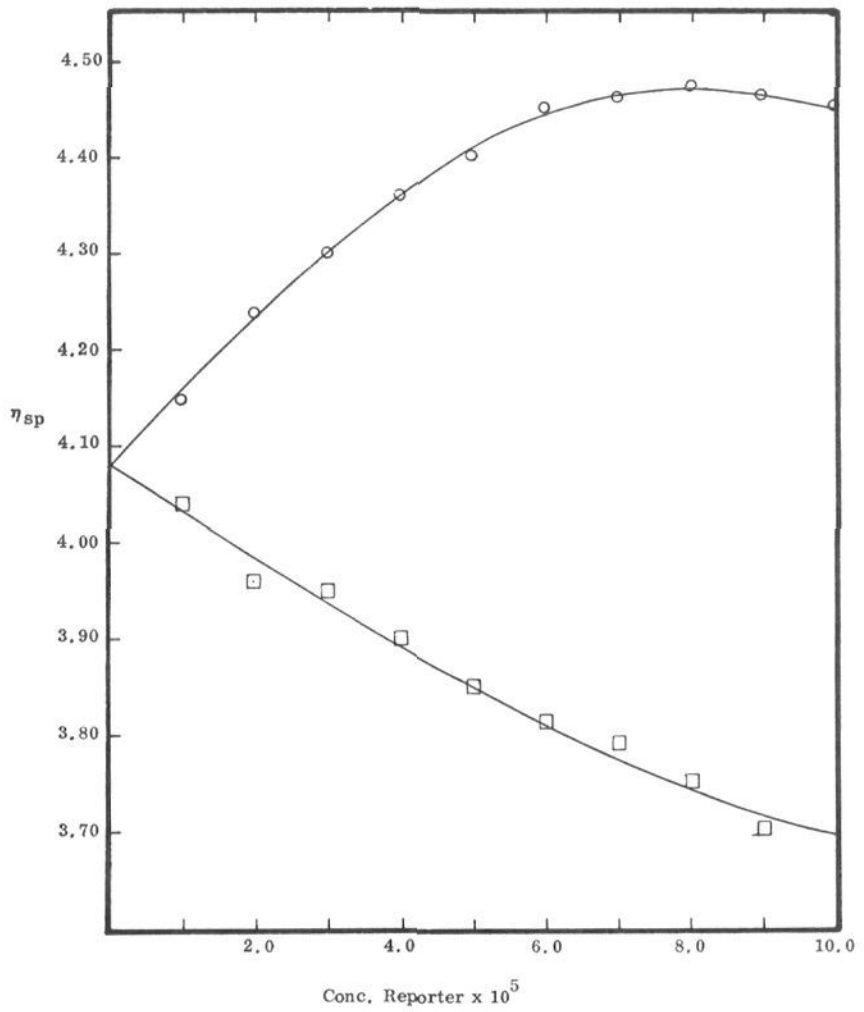


Figure 2. The effect of L- and D-prolyl derivatives **1** and **2** on the specific viscosity of DNA (□—□, L-prolyl; ○—○, D-prolyl derivative). Viscosity measurements were carried out using 6.3×10^{-4} mol of phosphorus/l. in 0.01 *M* 2-(*N*-morpholino)ethanesulfonic acid buffer at 37.5° using the low-shear Zimm viscometer.

For example, the pmr signal of the H_a proton of **2** is upfield shifted by 64 Hz on binding to DNA at 39° and also at 50°. In contrast, the pmr signal of the H_a proton of **1** is upfield shifted by 52 Hz at 39° and 50 Hz at 50° on binding to DNA. Moreover, the entire pmr spectrum of the DNA–**2** complex shows that the pmr signals of **2** are more broadened than the corresponding pmr signals observed for the DNA–**1** complex. The results are indicative that the dinitroaniline ring of **2** experiences greater shielding (*i.e.*, *via* ring current anisotropy) and restricted tumbling than **1**. (4) It is noted from the viscometric titration studies shown in Figure 2 that the specific viscosity, η_{sp}, of the DNA solution increases with increasing concentrations of the D enantiomer and levels off at a base pair to reporter concentration of 4.0. The results are consistent with an intercalation model. In contrast, the L enantiomer causes a lowering of the specific viscosity of the DNA solution which indicates a decrease in the effective length of the helix in the DNA–**1** complex. The results are consistent with a model whereby intercalation of the dinitroaniline ring of **1** between base pairs of DNA results in a distortion of the helical rod, *i.e.*, bending at the point of intercalation. This effect might be expected from examination of molecular framework models of the DNA–L-prolyl derivative complex (Figure 1).

Binding studies were also carried out on the DNA–**1** and **2** complexes. The D enantiomer gave a binding constant of 1.18×10^5 and a maximum number of strong binding sites of one molecule per 4.55 base pairs. A lower binding constant is observed for the DNA–**1** complex, $K = 6.5 \pm 0.3 \times 10^4$, and a maximum number of strong binding sites of one molecule per 5.42 base pairs.[3]

(3) Scatchard type plots were carried according to the modified spectrophotometric procedure of R. W. Hyman and N. Davidson, *Biochim. Biophys. Acta*, **228**, 38 (1971).

In summary, the dissymmetric recognition of the helical sense of native DNA *in solution* has been accomplished *via* the interaction specificities of the DNP derivatives of L- and D-prolines **1** and **2**. The results of the absorption, circular dichroism, viscosity, pmr, and binding studies are consistent with a right-handed helical structure for native DNA in solution, in agreement with the Watson–Crick–Wilkins model.

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Transition Metal Complex Specificity and Substituent Effects in the Transition Metal Complex Promoted Rearrangement of Phenyl-Substituted Bicyclo[1.1.0]butanes[1]

Sir:

Of the various mechanistic proposals which have been presented for the transition metal complex promoted rearrangements of derivatives of bicyclo[1.1.0]butane,

(1) Paper XXVIII on The Chemistry of Bent Bonds. For the previous papers in this series, see P. G. Gassman and F. J. Williams, *J. Chem. Soc., Chem. Commun.*, 80 (1972); P. G. Gassman and T. Nakai, *J. Amer. Chem. Soc.*, **93**, 5897 (1971).