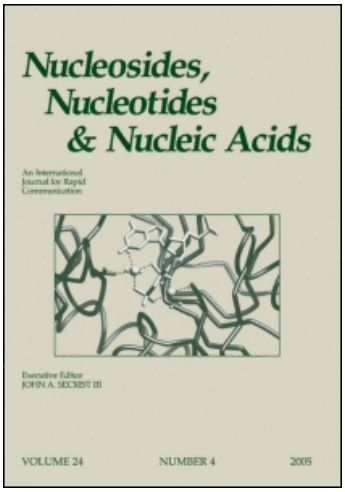

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

NMR Investigations of the Solution Conformations of Deoxynucleotidyl (3'-5') Arabinonucleosides

S. H. Gray[a]; C. F. Ainsworth[b]; C. L. Bell[a]; S. S. Danyluk[b]; M. Maccoss[b]
[a] Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois [b] Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

To cite this Article Gray, S. H. , Ainsworth, C. F. , Bell, C. L. , Danyluk, S. S. and Maccoss, M.(1986) 'NMR Investigations of the Solution Conformations of Deoxynucleotidyl (3'-5') Arabinonucleosides', Nucleosides, Nucleotides and Nucleic Acids, 5: 5, 481 — 491
To link to this Article: DOI: 10.1080/07328318608068690
URL: http://dx.doi.org/10.1080/07328318608068690

NUCLEOSIDES & NUCLEOTIDES, 5(5), 481-491 (1986)

NMR INVESTIGATIONS OF THE SOLUTION CONFORMATIONS OF DEOXYNUCLEOTIDYL(3'-5')ARABINONUCLEOSIDES

S. H. Gray[a,1], C. F. Ainsworth,[b] C. L. Bell,[a]

S. S. Danyluk,[b] and M. MacCoss[b]

[a]Department of Medicinal Chemistry and Pharmacognosy,
College of Pharmacy, Health Sciences Center,
University of Illinois at Chicago,
Chicago, Illinois 60680, and

[b]Division of Biological and Medical Research,
Argonne National Laboratory,
Argonne, Illinois 60439

Abstract: The solution conformations of all eight deoxynucleotidyl(3'-5')arabinonucleosides containing 9-B-D-arabinofuranosyladenine and 1-B-D-arabinonfuranosylcytosine have been analyzed by NMR methods and compared to dinucleoside monophosphates containing the corresponding deoxyriboside units.

The detailed molecular mechanism of action of the inhibition of DNA polymerase by the triphosphates of the arabinonucleosides, 1-B-D-arabinofuranosylcytosine (AraC) and 9-B-D-arabinofuranosyladenine (AraA), remains to be elucidated[2-4]. One possible mechanism[4-6] involves incorporation of the arabinonucleotide into the 3' terminus of a growing DNA chain, providing a poor primer for the incoming deoxynucleoside triphosphate and therefore slowing chain growth. This study describes the solution conformations of all eight possible

 0732-8311/86/0505-0481$3.50/0

dXparaA* and dXparaC* dinucleoside monophosphates and compares their conformations with those of the corresponding dXpdA* (or dC) dimers. The arabino-containing dimers can be considered as the simplest models of a DNA chain after terminal arabinonucleotide incorporation. Evaluation of the solution conformation of these dimers could give valuable insight into how the arabinonucleotides inhibit DNA polymerases.

The ^{1}H-NMR parameters obtained from the computer simulated spectra are shown in Tables 1 and 2. As an example, Figure 1 shows the spectral simulations for dAparaA. Based on these data, a pseudorotational analysis of the deoxynucleotidyl fragment of each dimer was performed with methods developed by Lee et al.[7] and Ezra et al.[8]. The percentage population of ^{3}E-type conformers in the dX portion of the dXparaA (or araC) dimers was calculated. The results, listed in Table 3, indicate that ^{3}E populations range from 26-42%, distinctly higher, in all cases except for dGparaC, than the corresponding fragment of the dXpdA (and dC) dimers[9]. The largest increase in the ^{3}E population occurred in the dimers that contained dC as the 3'-nucleotidyl unit: a 27% change (33% to 42%) for the transition from dCpdC to dCparaC and a 36% change (28% to 38%) for the transition from dCpdA to dCparaA. Interestingly, the smallest increases were observed when purine was the 3'-nucleotidyl unit, implying that the conformational changes may well be sequence dependent. In all cases, except Tp-containing dimers, the % ^{3}E of the dX portion increased upon dimerization, consistent with previous data on deoxydinucleoside monophosphates[9] and ribodinucleoside monophosphates[8].

---

*Abbreviations for dinucleoside monophosphates follow IUPAC-IUB guidelines.

TABLE 1. CHEMICAL SHIFTS[a] OF DEOXYNUCLEOTIDYL(3'-5')ARABINONUCLEOSIDES IN D_2O[b,c]

Nucleotide		8(6)		2(5, Me)		1'		2'		2"		3'		4'		5'		5"	
TparaC[d]	Tp-	7.658	(0.045)	1.874	(0.030)	6.226	(0.024)	2.416	(-0.039)	2.582	(-0.037)	4.775	(0.012)	4.190	(0.002)	3.844	(0.000)	3.794	(0.004)
	-paraC	7.854		6.062		6.235		4.466		-		4.200		4.070		4.217		4.132	
TparaA	Tp-	7.369	(0.008)	1.814	(0.007)	6.013	(-0.025)	1.854	(-0.252)	2.360	(-0.183)	4.662	(-0.051)	4.098	(-0.057)	3.716	(-0.470)	3.685	(-0.016)
	-paraA	8.373		8.173		6.380		4.685		-		4.563		4.128		4.225		4.169	
dAparaC	dAp-	8.280	(0.020)	8.128	(0.058)	6.385	(0.025)	2,868	(-0.014)	2.868	(-0.098)	4.891	(0.019)	4.345	(0.006)	3.895	(-0.004)	3.857	(-0.018)
	-paraC	7.550		5.654		6.092		4.404		-		4.163		4.054		4.289		4.144	
dAparaA	dAp-	8.050	(0.054)	7.897	(-0.061)	6.164	(-0.096)	2.493	(-0.330)	2.709	(-0.262)	4.855	(-0.051)	4.280	(-0.057)	3.793	(-0.02)	3.793	(-0.062)
	-paraA	8.184		7.922		6.183		4.619		-		4.503		4.135		4.326		4.203	
dCparaC	dCp-	7,831	(-0.005)	6.016	(0.009)	6.171	(-0.119)	2.429	(-0.069)	2.610	(-0.042)	4.712	(0.026)	4.205	(0.008)	3.874	(-0.018)	3.801	(-0.010)
	-paraC	7.814		5.971		6.214		4.459		-		4.197		4.065		4.214		4.134	
dCparaA	dCp-	7.561	(-0.022)	5.816	(-0.041)	5.997	(0.024)	1.958	(-0.395)	2.430	(-0.194)	4.600	(-0.052)	4.096	(-0.053)	3.766	(-0.075)	3.706	(-0.071)
		8.393		3.190		6.370		4.673		-		4.522		4.122		4.222		4.157	
dGparaC	dGp-	7.980	(-0,013)	-		6.222	(-0.017)	2.784	(-0.060)	2.788	(-0.011)	4.891	(-0.003)	4.288	(0.005)	3.847	(0.002)	3.817	(0.001)
	-paraC	7.640		5.740		6.189		4.438		-		4.182		4.082		4.280		4.151	
dGparaA[e]	dGp-	8.03	(-0.29)	-		5.98	(-0.10)	2.42	(-0.47)	2.58	(-0.08)	4.82	(-0.05)	f		3.73	(-0.03)	3.73	(-0.02)
	-araA	8.47		8.26		6.32		4.66		-		4.53		f		f		f	

[a] Computer simulated shifts; data for dXparaA (or araC) taken from ref. 10.
[b] pD = 6.5 - 7.5; concentration = 0.01 to 0.015M; temp = 20±2°C
[c] Values in parentheses represent shift differences from the corresponding dXpdA (or dC) dimer; i.e. difference in $\delta=\delta_{dXpdA\ (or\ dC)} - \delta_{dXparaA(or\ araC)}$ for the corresponding deoxyribose portion only. A negative value refers to a downfield shift of the dXparaA (or araC) relative to the chemical shift value for corresponding dXpdA(or dC) dimers. The chemical shift values for dXpdA(or dC) dimers were taken from reference 9.
[d] Spectrum analyzed at 360 MHz.
[e] Spectrum not simulated; shifts estimated directly from spectrum.
[f] Shifts not shown because of inaccuracy in measurement.

TABLE 2. COUPLING CONSTANTS[a] FOR THE DEOXYNUCLEOTIDYL(3'-5')ARABINONUCLEOSIDES IN D_2O[b] AS COMPARED TO THE CORRESPONDING DEOXYNUCLEOTIDYL(3'-5') DEOXYNUCLEOSIDES[c]

Nucleotide		1'2'		1'2"		2'2"		2'3'		2"3'		3'4'		4'5'		4'5"		5'5"		3'P		4'P		5'P		5"P		(Me)5 6	
TparaC	Tp-	7.3	(7.8)[d]	6.2	(6.1)	-14.1	(-14.2)	6.3	(6.4)[d]	3.5	(3.1)	3.3	(2.9)	3.5	(3.3)	4.3	(5.0)	-12.5	(-12.6)	7.0	(7.4)	---		---		---		1.1	
	-paraC	5.6	(6.5)[d]	---		---		5.6	(4.0)[d]	---		6.3	(3.5)	2.7	(2.4)	4.8	(3.6)	-11.7	(-11.6)	---		-1.6	(3.0)	5.1	(4.6)	4.8	(4.4)	7.6	(8.0)
TparaA	Tp-	8.0	(8.2)[d]	5.9	(6.2)	-14.1	(-14.5)	5.9	(6.2)[d]	2.8	(2.4)	3.0	(2.6)	3.6	(4.2)	4.0	(4.2)	-12.3	(-12.8)	6.2	(7.8)	---		---		---		1.0	
	-paraC	6.5	(6.4)[d]	---		---		7.7	(4.2)[d]	---		7.9	(3.9)	2.2	(2.5)	3.7	2.5	-11.6	(-11.8)	---		2.1	(3.0)	4.9	(3.6)	3.8	(3.6)	7.6	
dAparaC	dAp-	6.6[e]	(7.4)	6.6[e]	(6.1)	-14.0	(-14.0)	5.9	(6.0)[d]	3.6	(3.0)	2.9	(2.8)	3.0	(3.2)	3.6	(3.6)	-13.0	(-12.8)	5.4	(6.6)	---		---		---		---		
	-paraC	5.6	(6.6)	---		---		5.7	(4.6)[d]	---		6.8	(4.0)	2.1	(3.6)	4.5	(4.0)	-11.7	(-11.4)	---		-1.6	(4.0)	5.1	(4.0)	4.8	(4.0)	7.5	(7.7)
dAparaA	dAp-	8.1	(8.7)	5.7	(5.5)	-13.9	(-14.0)	5.6	(5.6)	2.6	(2.3)	2.8	(2.4)	3.4	(3.3)	2.9	(3.3)	-12.0	(-12.0)	5.0	(5.6)	---		---		---		---		
	-paraA	6.2	(6.7)	---		---		7.4				2.7	(4.0)	2.0	(2.5)	2.7	(2.8)	-12.2	(-12.0)			2.0	(2.7)	4.1	(3.8)	2.7	(3.6)	---	
dCparaC	dCp-	6.35	(7.8)[d]	6.25	(5.8)	-14.0	(-13.6)	6.5	(6.6)[d]	4.85	(3.5)	4.5	(3.6)	3.1	(3.6)	4.6	(3.6)	-12.7	(-12.2)	6.9	(7.0)	---		---		---	(7.6)	6.6	
	-paraC	5.6	(7.0)[d]	---		---		5.8	(4.2)[d]	---		6.7	(3.5)	2.6	(3.2)	4.6	(3.2)	-11.7	(-11.8)	---		2.2	(2.0)	4.8	(4.4)	4.6	(4.8)	7.6	(6.7)
dCparaA	dCp-	6.6	(8.1)[d]	6.0	(6.3)	-13.7	(-14.5)	6.5	(5.8)[d]	4.3	(3.0)	4.1	(3.0)	3.1	(3.4)	4.2	(4.7)	-12.7	(-12.6)	6.6	(7.0)	---		---		---		7.6	(7.1)
	-paraA	6.5	(6.4)[d]	---		---		7.5	(4.1)[d]	---		8.1	(3.9)	2.2	(2.5)	2.9	(2.5)	-11.9	(-11.8)	---		2.3	(3.0)	4.8	(3.6)	3.2	(3.6)	---	
dGparaC	dGp-	6.7[e]	(7.0)[d]	6.7[e]	(6.5)	-14.0	(-13.8)	6.0	(6.6)[d]	3.5	(6.5)	3.0	(3.4)	3.8	(3.4)	4.5	(4.0)	-12.6	(-13.0)	6.0	(7.0)	---		---		---		---		
	-paraC	5.7	(6.6)[d]	---		---		5.4	(3.4)[d]	---		6.9	(4.0)	2.1	(3.0)	4.1	(3.6)	-11.8	(-12.0)	---		2.0	(1.0)	4.5	(4.0)	5.2	(4.0)	7.6	(7.5)
dGparaA	dGp-	8.3	(8.4)[d]	5.9	(6.3)	-13.8	(-14.2)	5.4	(5.4)[d]	1.5	(2.8)	1.7	(3.6)	3.0	(3.4)	2.5	(3.6)	-12.0	(-12.8)	5.8	(7.0)	---		---		---		---		
	-paraA	6.36	(6.4)[d]	---		---		7.3	(4.1)[d]	---		7.6	(4.0)	2.1	(2.8)	2.7	(2.8)	-11.8	(-11.8)	---		2.6	(1.0)	4.6	(3.6)	3.4	(3.6)	---	

[a] Coupling constants are accurate to ± 0.2 Hz
[b] Solution conditions are the same as in Table I.
[c] Values in parentheses are for the corresponding dXpdA (or dC) couplings, taken from Ref. 9.
[d] These values are actually $J_{1'2''}$ and $J_{2''3'}$ because $H_{2''}$ in the deoxy dimers corresponds to $H_{2'}$ in the arabino dimers
[e] Virtual coupling exists for these resonances, and only $J_{1'2'} + J_{1'2''}$ is available when $\delta 2' \equiv \delta 2''$
[f] Values are taken from spectral expansions.

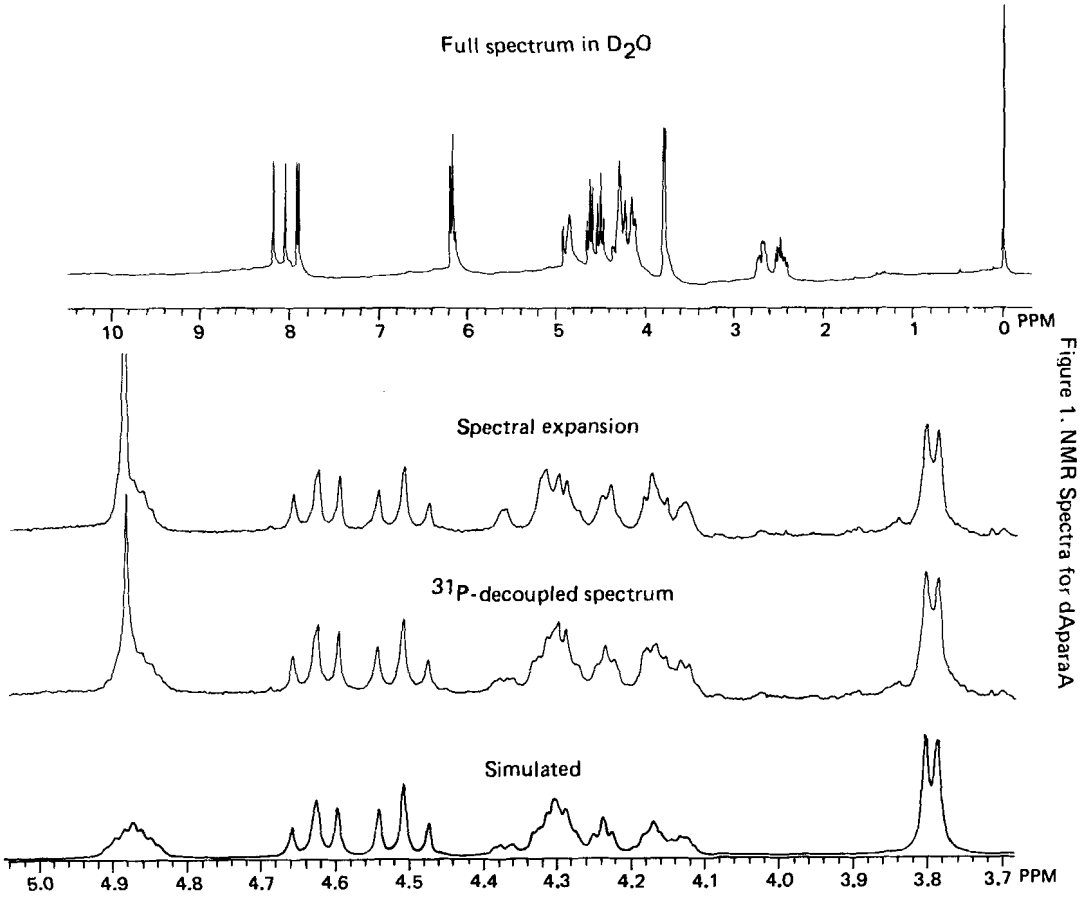

Figure 1. NMR Spectra for dAparaA

A similar analysis of the arabinosyl portion of each dimer is not straightforward because of the configurational differences of the arabinose sugar and the electronegativity effects of the 2'-hydroxyl[13-15,17]. However, Table 2 indicates that $J_{2'3'}$ for the arabino portion of the dXparaA (or araC) dimers is much greater than the corresponding $J_{2'3'}$ for the 3'-terminus of the dXpdA (or dC) dimers with the differences ranging from 1.1 Hz (a 24% increase) for dAparaC to 3.2 Hz (a 78% increase) for dGparaA. These differences are greater

TABLE 3. STACKING PROPERTIES OF THE DEOXYNUCLEOTIDYL(3'-5')ARABINONUCLEOSIDES IN SOLUTION

Nucleotide		^{3}E Deoxyribose Ring[a] (%^{3}E)[c]			Backbone[b] K_{eq}^{e}	%γ^+	%β^t	θPH
			Dimer	Monomer[d]				
TparaC	Tp-	(27)	31	33	0.44	61	--	±40°
	-paraC		--		----	64	72	---
TparaA	Tp-	(24)	28	33	0.38	63	--	±44°
	-paraA		--		----	80	78	---
dAparaC	dAp-	(26)	27	23	0.37	73	--	±47°
	-paraC		--		----	71	72	---
dAparaA	dAp-	(22)	26	23	0.35	76	--	±49°
	-paraA		--		----	93	89	---
dCparaC	dCp-	(33)	42	35	0.72	62	--	±42°
	-paraC		--		----	69	75	---
dCparaA	dCp-	(28)	38	35	0.61	64	--	±43°
	-paraA		--		----	89	82	---
dGparaC[f]	dGp-	(31)	28	26	0.39	56	--	±45°
	-paraC		--		----	75	74	---

[a]Computer by using $J_{1'2'} + J_{3'4'} = 10.8$ Hz for 3'-nucleotidyl unit

[b]Rotomer equations used: $\gamma^+ = [13.7 - (J_{4'5'} + J_{4'5''})]/9.7$;
$\beta^t = [25 - (5'P + 5''P)]/20.8$

In this paper the α-ζ notation for backbone torsion angles is used[11].

α β γ δ ε ζ
P-O5'-C5'-C4'-C3'-O3'-P

The usual Klyne-Prelog convention for torsion angle notation is adhere to also.[12]

θPH refers to the dihedral angle P-O3'-C3'-H3' and is calculated as in Ref. 9.

[c]Figures in parentheses are the %^{3}E for the dX portion of the corresponding dXpdA (or dC) dimers, taken from Ref. 9.

[d]Data taken from Ref. 9.

[e]$K_{eq} = {}^2E \rightleftarrows {}^3E$, $X_N/X_S = K_{eq}$

[f]Data for dGparaA not shown; spectrum was not simulated.

than those expected for electronegativity changes alone. Such increases in $J_{2'3'}$ are consistent, qualitatively, with a greater value of ^3E for the arabino portion of the dXparaA (or araC) dimers than for corresponding dXpdA (or dC) dimers. Such increases in % ^3E values in dimers have been associated with increases in the amount of base stacking[9,18], although Doornbos et al.[17] has shown that in oligomers containing araA alone, "mixed-stacked states" (stacks containing some combination of N-type (^3E) and S-type (^2E) sugar puckers), are common.

The dXparaA (or araC) dimers exhibited an average ΘPH value of ±44° (Table 3), somewhat higher than that found for the corresponding dXpdA (or dC) dimers[9]. This indicates that the intranucleotidyl phosphate backbone conformation is more loosely held in the araA- or araC- containing dimers. Furthermore, the βγ bonds showed conformational proclivities for $\beta^t\gamma^+$ domains, indicating that significant stacking interactions occur in the araA-containing dimers. The lower values for the corresponding araC-containing dimers indicated less stacking interaction while retaining a clear preference for N-type sugar pucker[17].

Examination of the chemical shift differences between the sugar protons of the dX portion of the dXparaA (or araC) dimers and those of the corresponding dXpdA (or dC) dimers (see Table 1) reveals interesting trends. In particular, the dXparaA dimers show large downfield shifts (0.08 to 0.47 ppm) for H2′ and H2″ of the dX portion relative to the corresponding resonances in the dXpdA series, while the shift changes for the other protons are small. To examine this observation more closely, the ^{1}H-NMR spectra of the dimer dAparaA was examined over a range of temperatures and concentrations and the shift of H2′ of the dA portion was

carefully monitored. As the temperature increased to about 40°C the resonance moved upfield. Above 40°C the shift was reversed, and a downfield trend was observed. These data are in contrast to the other resonances in the molecule (and the trend usually seen for most dimers and trimers[18,19]), which show a downfield trend as the temperature is increased.

Consideration of all of these facts led to the conclusion that the conformational equilibrium in the dXparaA (or araC) dimers is perturbed from that usually associated with the corresponding dXpdA (or dC) dimers. This perturbation may be due (i) to an increased contribution of the "loop-stack" (see Ref. 16 for a description of this conformational possibility, designated stack II in Ref. 16, which would place the 2'-hydroxyl of the arabinose moiety in close proximity to the H2′ and H2″ of the dX moiety and could account for the selective deshielding of these resonances); (ii) to increased contributions of other, less-stacked conformational forms, or (iii) to a combination of "mixed-stacked states"[17]. An unequivocal resolution of this structural problem awaits a detailed pseudorotational analysis of the arabino portion of the dimers[13-14] and a complementary CD study of the type described by Altona and co-workers[13-15,17]. Such a study was outside the scope of this work, but the observation is significant: a change in the normal conformational properties of a growing DNA chain would undoubtedly lead to a slowing of chain elongation upon the addition of an arabinoside at the 3′-terminus. Whether such changes would occur on the enzyme surface is open to conjecture, but the general observations detailed herein are consistent with the biochemical observations[5,6].

EXPERIMENTAL SECTION

Synthesis of all eight dXparaA and dXparaC dimers has been reported elsewhere[10,20,21]. ^{1}H-NMR spectra were

recorded in the Fourier Transform mode on a Varian HR-220 spectrometer equipped with a Nicolet FT accessory and a 20K data system. Unless otherwise stated, spectra were measured on samples with a concentration of 0.01-0.015 M at 20±2°C, and chemical shifts are reported relative to sodium-3-(trimethylsilyl)-proprionate-2,2,3,3-d_4 (TSP). Computer simulation of the spectra was achieved by using a Nicolet 1090 computer and the ITRCAL simulation programs giving accuracies of ±0.1 Hz for coupling constants and ±0.005 ppm for chemical shifts.

ACKNOWLEDGEMENT. Funding for this work was provided by the U. S. Department of Energy under Contract W-31-109-ENG-38.

These authors wish to thank Marlene P. Linker for the preparation of this manuscript.

REFERENCES

1. Address correspondence to this author at Monsanto, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

2. Fox, R.M., Reddy, R.G.Y.; Gouliau, M. J. Clin. Invest. 1972, 51, 33a-37a.

3. Schrecker, A.W.; Smith, R.G.; Gallo, R.C. Cancer Res. 1974, 34, 286-292.

4. Cozzarelli, N.R. Ann. Rev. Biochem. 1977, 46, 647-648.

5. Tsanglee, M.Y.W.; Byrnes, J.J.; Downey, K.M.; So, A.G. Biochemistry. 1980, 19, 215-218.

6. Majn, D.D.; Egan, E.M.; Herrick, D.J.; Kufe, D.W. Biochem. Pharmacol. 1982, 31, 2937-2940.

7. Lee, D.-H.; Ezra, F.S.; Kondo, N.S.; Sarma, R.H.; Danyluk, S.S. Biochemistry. 1976, 15, 3627-3639.

8. Ezra, F.S.; Lee, C.-H.; Kondo, N.S.; Danyluk, S.S.; Sarma, R.H. Biochemistry. 1977, 16, 1977-1978.

9. Cheng, D.M.; Sarma, R.H. J. Amer. Chem. Soc. 1977, 99, 7333-7348.

10. Gray, S.H., Ainsworth, C.L., Bell, C.L., Danyluk, S.S. and MacCoss, M. Nucleosides and Nucleotides 1983, 2, 435-452.

11. IUPAC-IUB Joint Commission on Biochemical Nomenclature. Document 14.2 Eur. J. Biochem. 1983, 131, 9-15.

12. Klyne, W.; Prelog, V. Experientia 1960, 16, 521-523.

13. DeLeeuw, H.P.M.; Haasnoot, C.A.G.; Altona, C. Israel J. Chem. 1980, 20, 108-126.

14. Haasnoot, C.A.G.; DeLeeuw, F.A.A.M.; DeLeeuw, H.T.M.: and Altona, C. Org. Mag. Res. 1981, 15, 43-52.

15. Haasnoot, C.A.G., DeLeeuw, F.A.A.M.; Altona, C. Tetrahedron. 1980, 36, 2783-2792.

16. Kondo, N.S.; Danyluk, S.S. Biochemistry. 1976, 15, 756-768.

17. Doornbos, J.; Barasent, J.-L.; Lazrek, H.; Imbach, J.-L.; van Westrenen, J.; Visser, G.M.; van Boom, J.H.; Altona, C. Nucleic Acids Res. 1983, 11, 4583-4600.

18. Ts'O, P.O.P.; Kondo, N.S.; Schwezer, M.P.; Hollis, D.P. Biochemistry, 1969, 8, 997-1029.

19. Olsthoorn, C.S.M.; Bostelaas, L.J.; VanBoom, J.H.; Altona, C. Eur. J. Biochem. 1980, 112, 95-110.

20. Wechter, W.J. J. Med. Chem. 1967, 10, 762-778.

21. Gray, S.H. Ph.D. Thesis, University of Illinois at Chicago, May 1982.

Received November 25, 1985.