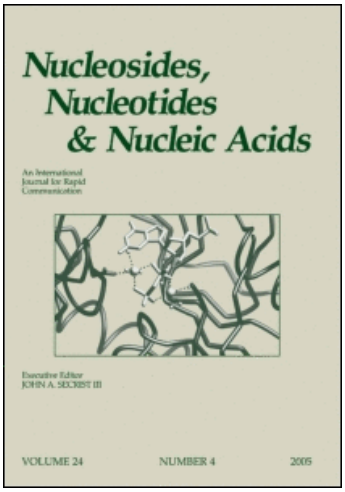

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CHIRAL PHOSPHOROMORPHOLIDATE DERIVATIVES OF OLIGOTHYMIDYLATE AND THEIR HYBRIDIZATION ABILITIES§

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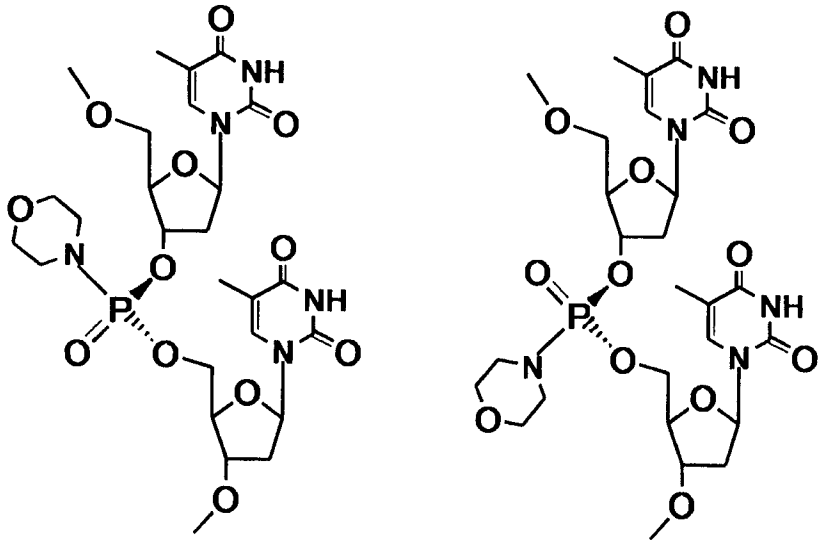
ABSTRACT: *Interactions of diastereomerically pure phosphoromorpholidate analogs of oligothymidylate with poly(dA) were examined. Obvious differences between diastereomers appeared in duplex formation and tentative stereochemical characterizations of them were done.*

INTRODUCTION

Chemically functionalized oligonucleotides can serve as an efficient class of interactive molecules specific for sequences in DNA and RNA[1], and in particular oligodeoxynucleotide analogs with modified internucleotide linkages have been proposed to modify the parent function of oligodeoxynucleotides. This type of analog is highly resistant to degradation by nucleases, which has caused it to be often applied to the modulation of gene expression[2,3]. Recently it has been found that the ability of this type of analog to form a heteroduplex with a complementary sequence was generally dependent on the absolute configuration of the chiral center of the modified phosphorus atom[4-11]. Understanding the nature of this phenomenon has great significance for the enhancement of the specific affinity for the complementary DNA, but has not yet been fully achieved. So far the studies on this problem based on the adequate characterization of the absolute stereochemistry of the modified phosphate have been done only with methanephosphonate[5-8], phosphorothioate[9] and alkyl phosphotriester[10] analogs.

§ This paper is dedicated to Professor Tohru Ueda, the former editor of this journal, who passed away on September 19, 1990.

We have studied the synthesis of oligonucleotide phosphoramidates and recently reported on the synthesis and characterization of diastereomerically pure phosphoromorpholidate analogs[11-14]. As a consecutive study, the chiral effects of the phosphoromorpholidate linkage on the structure and hybrid ability of oligothymidylate was explored.



Diastereomers of oligothymidylate phosphoromorpholidate

EXPERIMENTAL

Polydeoxyadenylic acid (poly(dA)) was purchased from Pharmacia LKB Biotechnology. Thymidine and 5'-*O*-dimethoxytritylthymidine loaded silica gel support (T-resin) was purchased from Dojindo Laboratories. High-performance liquid chromatography(HPLC) was carried out on DAISO GEL ST-120-5-ODS(6.0×150mm) at 35°C using Shimadzu LC-6A chromatographic system. Dithymidine monophosphate, heptathymidylic acid ($(dT)_7$) and undeca-thymidylic acid($(dT)_{11}$) were synthesized by a solid phase phosphoramidite method. UV spectra were measured by Shimadzu MPS-2000 spectrophotometer. Circular dichroism (CD) spectra were measured by JASCO J-720 spectropolarimeter. Diastereomers of dithymidine phosphoromorpholidate were synthesized from diastereomerically pure 5'-*O*-dimethoxytrityldithymidine phosphoromorpholidate (DMTrTp(mor)T isomer I, isomer II) according to the procedure described elsewhere (Tp(mor)T : T2-I from isomer I, T2-II from isomer II, see TABLE I)[11].

All of the diastereomerically pure phosphoromorpholidate analogs of oligothymidylate were synthesized using DMTrTp(mor)T isomer I and isomer II on T-resin according to the

TABLE I : Preparation of Oligothymidylate Analogs Possessing Chirally Defined Phosphoromorpholidate Linkages.

Compound	Designation	isomer	Yield	HPLC retention time[a)]
			%	min
T2-I	Tp(mor)T	I		16.1
T2-II	Tp(mor)T	II		15.9
T7-I	$(Tp(mor)Tp)_3T$	I	77	18.5
T7-II	$(Tp(mor)Tp)_3T$	II	73	19.0
T11-I(1)	$(Tp(mor)Tp)(Tp)_8T$	I	64	15.5
T11-II(1)	$(Tp(mor)Tp)(Tp)_8T$	II	43	15.3
T11-I(1C)	$(Tp)_5(Tp(mor)Tp)(Tp)_3T$	I	67	14.4
T11-II(1C)	$(Tp)_5(Tp(mor)Tp)(Tp)_3T$	II	53	14.2
T11-I(3)	$(Tp(mor)Tp)_3(Tp)_4T$	I	48	18.1
T11-II(3)	$(Tp(mor)Tp)_3(Tp)_4T$	II	64	17.8
T11-I(5)	$(Tp(mor)Tp)_5T$	I	54	21.3
T11-II(5)	$(Tp(mor)Tp)_5T$	II	53	21.3

a) column : DAISO GEL ST-120-5ODS 6.0 × 150 mm, eluent : CH_3CN gradient in 0.1M triethylammonium acetate buffer.

method described in our previous report($(Tp)_k(Tp(mor)Tp)_l(Tp)_mT$: T7-I, T11-I(1), T11-I(1C), T11-I(3), T11-I(5) from isomer I ; T7-II, T11-II(1), T11-II(1C), T11-II(3), T11-I(5), T11-II(5) from isomer II, see TABLE I)[14]. Synthesized oligothymidylate derivatives possessing a 5'-*O*-trityl group were purified by reversed phase HPLC. After detritylation with 80% acetic acid the products were again purified by reversed phase HPLC. To confirm the length of these products, they were analyzed by HPLC after removal of the morpholine moieties by a published procedure[15].

Most of solutions for measurements of UV spectra were prepared by using a buffer containing 0.01M sodium phosphate and 0.15 M NaCl, adjusted to pH 7.2. Solutions for measurements of CD spectra were prepared by using a buffer containing 0.01M sodium phosphate and 5M NaCl, adjusted to pH 7.2. Concentrations of oligothymidylate analogs were determined based on the absorbance at 266 nm and ε value for oligothymidylic acid(8.7×10^3 $M^{-1}cm^{-1}$)[16]. Poly(dA) concentration was determined based on the absorbance at 257 nm and the published ε value(8.6×10^3 $M^{-1}cm^{-1}$)[17]. The measurements of UV spectra were carried out at an equal nucleotide concentration of either oligothymidylate analogs and poly(dA), 2×10^{-5}M. The measurements of CD spectra were carried out at the thymine nucleotide concentration of 1×10^{-4}M.

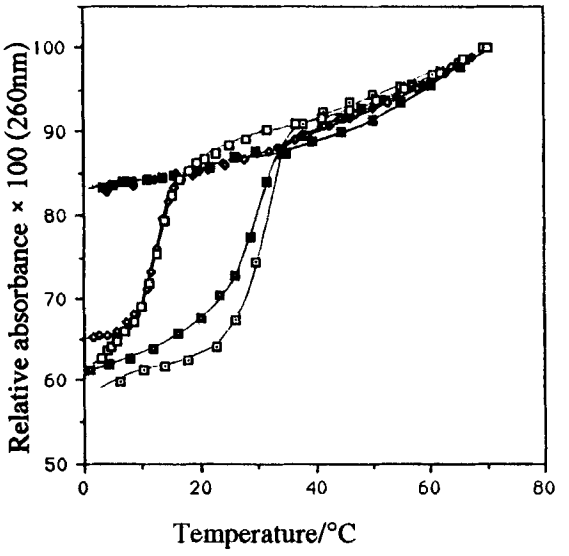


FIG.1. UV melting curves for the duplex of T7-I (-◆-), T7-II(-◇-), T11-I(5)(-■-), T11-II(5)(-▪-), (dT)7(-□-) or (dT)11(-▫-) with poly(dA) in 10mM sodium phosphate buffer (pH 7.2) containing 150mM NaCl.

RESULTS AND DISCUSSION

Interactions of heptathymidylate analogs and undecathymidylate analogs possessing chirally defined phosphoromorpholidate linkages on the defined sites with poly(dA) were investigated by temperature-dependent UV spectra in a pH 7.2 aqueous buffer.

Duplexes between Poly(dA) and Oligothymidylate Analogs Possessing Phosphoromorpholidate Linkages and Phosphodiester Linkages in Alternating Manner. FIG.1 shows the UV melting curves for the duplexes between poly(dA) and oligothymidylate analogs : T7-I, T7-II, T11-I(5) and T11-II(5). T7-I and T11-I(5), which are of isomer I class, did not show melting transition in this range of temperature. T7-II and T11-II(5), which are of isomer II class, showed the melting curves which have roughly the same profile as their parent oligonucleotide. TABLE II summarizes the melting temperature values(Tm) of the duplex of T11-I(5), T11-II(5) or undecathymidylic acid with poly(dA), derived from their UV melting curves at various ionic strengths. T11-I(5) did not show a melting transition in this range of salt concentration. T11-II(5) showed melting curves at the lowest salt concentration of these experiments and had the same tendency as undecathymidylic acid in the dependency of Tm on ionic strength.

TABLE II : Melting Temperatures (°C) of the Duplex of T11-I(5), T11-II(5) or $(dT)_{11}$ with Poly(dA)[a)]

sodium ion concn / M	0.01	0.025	0.16	1
T11-I(5)		n.d.[b)]	n.d.[b)]	n.d.[b)]
T11-II(5)	16	19	28	37
$(dT)_{11}$	12	15	30	43

a) 10mM sodium phosphate buffers (pH 7.2) were used. b) n.d. indicates that melting temperature was not detected.

FIG.2 indicates the UV mixing curves of T11-I(5), T11-II(5) or undecathymidylic acid with poly(dA) at 10 °C. The absorbance values(260 nm) of T11-I(5) and poly(dA) were almost alike for all dT/dA ratios. T11-II(5) and undecathymidylic acid showed roughly the same mixing curves with poly(dA), which had an absorbance minimum when thymidine concentration was even with that of deoxyadenosine.

These results reveal that the apparent hybridization ability of the phosphoromorpholidate analogs of isomer II is comparable to that of their parent oligothymidylates over a range of salt concentration, and that the phosphoromorpholidate analogs of isomer I do not have an intrinsic ability to hybridize with poly(dA) under the same conditions. These results are consistent with our previous results on duplex structures, derived from temperature-dependent CD spectra of T11-I(5), T11-II(5) and undecathymidylic acid with poly(dA)[14].

Duplexes between Poly(dA) and Undecathymidylate Analogs Containing Different Content of Phosphoromorpholidate Linkages. FIG.3 shows the UV melting curves for the duplexes between poly(dA) and undecathymidylate analogs of isomer I class : T11-I(1), T11-I(1C), T11-I(3) and T11-I(5). There seemed to be a tendency of Tm to decrease, accompanying the increase in the content of phophoromorpholidate linkage of isomer I. FIG.4 shows the UV melting curves for the duplexes between poly(dA) and the analogs of isomer II class : T11-II(1), T11-II(1C), T11-II(3) and T11-II(5). These have provided almost the same Tm as that of undecathymidylic acid. No significant difference seemed to be detected between the analogs possessing a phosphoromorpholidate linkage at different sites (T11-I(1), T11-I(1C), T11-II(1), T11-II(1C)).

From the above results, it could be understood that the individual modified linkage of isomer I affected duplex stability around it, and speculated that it prevented thymine bases from forming base pairings with poly(dA) in the vicinity of it, based upon the complete

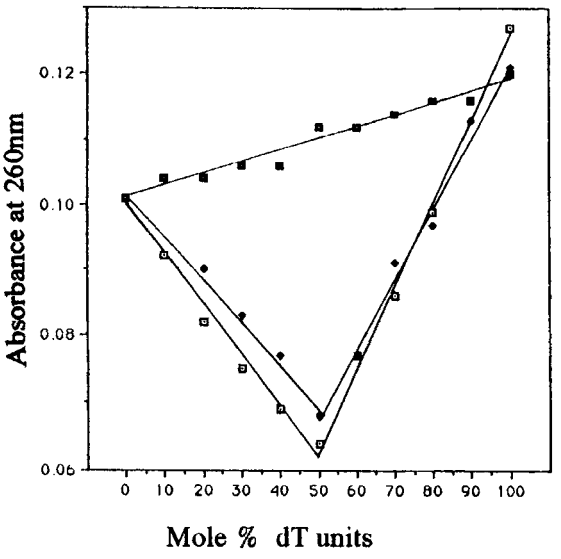


FIG.2. UV mixing curves for T11-I(5)(-■-), T11-II(5)(-◆-) or $(dT)_{11}$(-⊡-) with poly(dA) in 10mM sodiumphosphate buffer (pH 7.2) containing 150mM NaCl at 10°C.

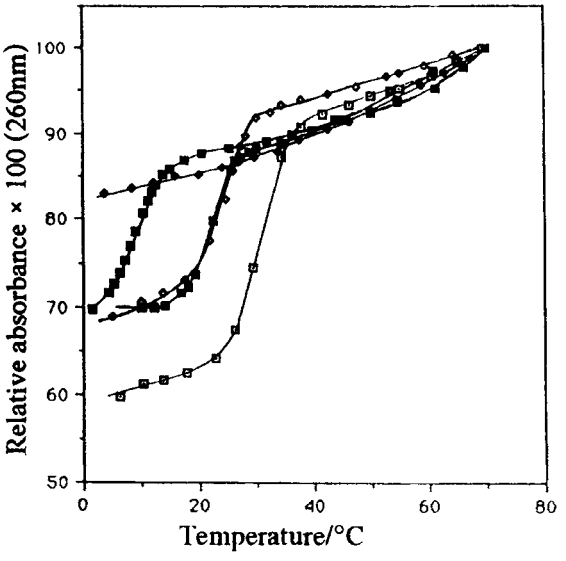


FIG.3. UV melting curves for the duplex of T11-I(1)(-◇-),T11-I(1C)(-■-),T11-I(3)(-■-), T11-I(5) (-◆-) or $(dT)_{11}$(-⊡-) with poly(dA) in 10mM sodium phosphate buffer (pH 7.2) containing ing 150mM NaCl.

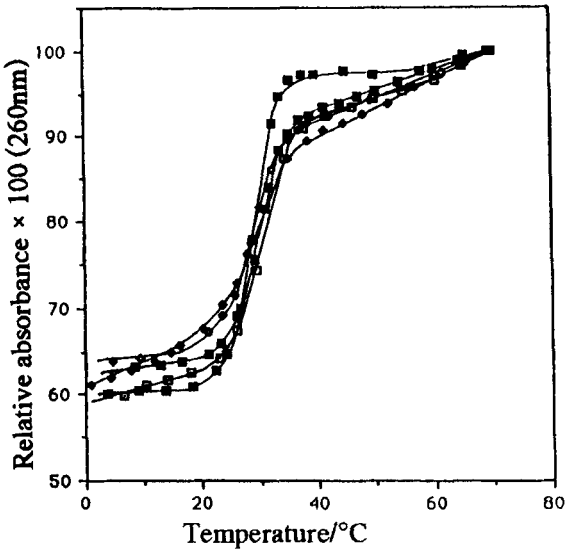


FIG.4. UV melting curves for the duplex of T11-II(1) (-◇-), T11-II(1C)(-■-), T11-(3)(-▣-),T11-II(5)(-◆-) or $(dT)_{11}$(-⊡-) with poly(dA) in 10mM sodium phosphate buffer (pH 7.2) containing 150mM NaCl.

lack of hybridization ability of T11-I(5). As to the analogs of isomer II, it could be concluded that no negative effects were seen.

Structure of Dithymidine Phosphoromorpholidate. Relative to the above insight on the effects of modified linkages, structures in the vicinity of such a linkage were investigated by temperature-dependent CD spectra of dithymidine phophoromorpholidate (T2-I, T2-II) in a pH 7.2 aqueous buffer.

FIG.5 indicates the four families of temperature-dependent CD spectra for T2-I, T2-II, thymidine and dithymidine monophosphate. All spectra showed positive Cotton effects around 260 nm, which could be interpreted as showing that they have the natural orientation of thymine base to deoxyribose : *anti* - conformation[18-20]. T2-I exhibited no significant CD spectral change in this temperature range, which was analogous to CD spectra of thymine. T2-II and dithymidine monophosphate showed relatively similar CD spectra with a tendency of molar ellipticity [Θ] at λmax to decrease upon heating the solutions.

Inspection of the dependency of CD spectra of a single stranded dinucleoside monophosphate on temperature provides information on the stacking interaction between the adjacent bases[21,22]. As shown in the CD spectra of dithymidine monophosphate, the

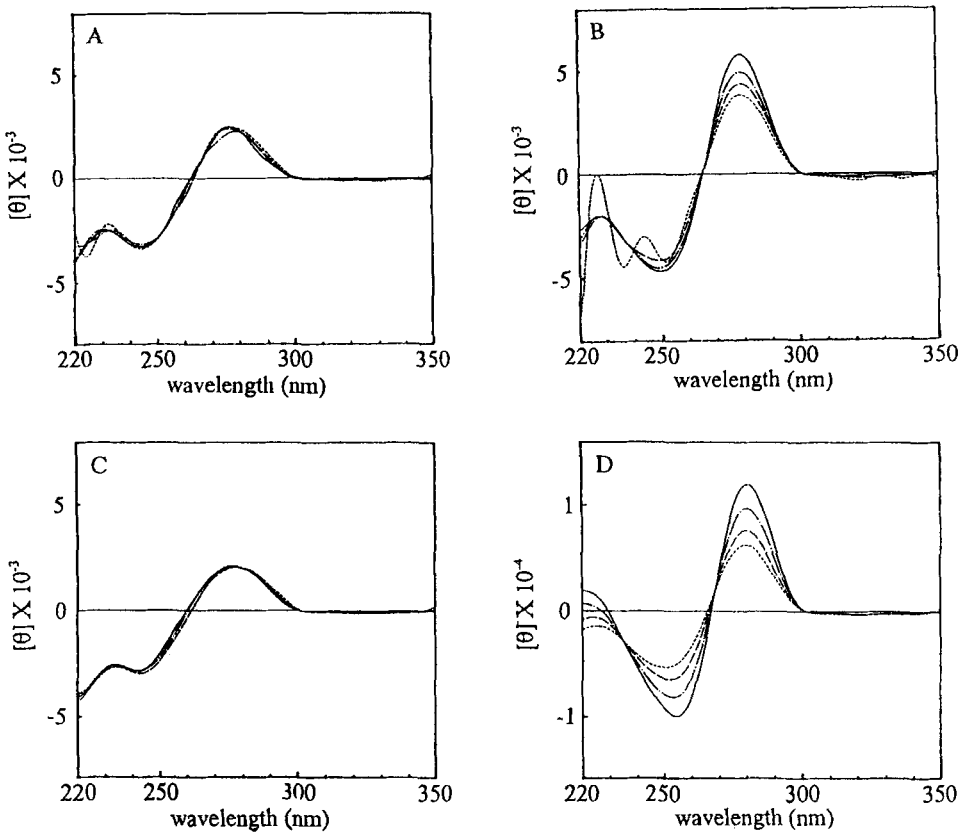


FIG.5. Temperature-dependent CD spectra of T2-I(A), T2-II(B), thymidine(C) and dithymidine monophosphate(D) in 10mM phosphate buffer (pH 7.2) containing 5M NaCl. —— , -10°C ; —·—, 20°C ; — — , 50°C ; - - - -, 80°C.

decrease of [Θ] at λmax accompanying the rise of temperature reflects the thermal destabilization of adjacent base stacking. In this sense T2-II probably maintains a naturally base-stacked conformation of dithymidine monophosphate, although the relatively small change of [Θ] suggests weak base stacking. From this insight it is tentatively thought that the morpholine moiety of isomer II is relatively easy to orient outward into the surroundings of stacked bases, which results in a favorable orientation upon duplex formation. To the contrary T2-I was revealed to have no intramolecular base stacking interaction. This indicates that the morpholine moiety of isomer I brings a serious hindrance to a base-stacked conformation. Based on this results it can be speculated that the phosphoromorpholidate linkage of isomer I in oligothymidylate prevents the adjacent

bases from forming stacked conformations and consequently stable base pairings cannot be formed. This speculation agrees with the observed duplex stability of the isomer I class.

CONCLUDING REMARK

In this study it could be concluded that the isomeric perturbations due to the introduction of chiral phosphoromorpholidate linkages into oligothymidylate result in two phenomena : 1) an unnatural single-stranded state unable to be transformed into hybridization with a complementary sequence, 2) apparently natural duplex formation. At present it is not possible to discuss the exact features of the effects of phosphoromorpholidate linkages without further stereochemical study. As a consecutive study of this exploratory characterization, further stereochemistry of oligonucleotide phosphoromorpholidates based on NOE-derived assignment of the absolute configuration at phosphorus(R_p for phosphoromorpholidate linkage of isomer I and S_p for that of isomer II)[11], including the comparison with other analogs such as methanephosphonate, is under current investigation.

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REFERENCES

(1) Sigman,D.S. and Chen,C.B. *Annu.Rev.Biochem.* **1990**, 59, 207-236.
(2) Zon,G. *Pharmaceutical Research* **1988**, 5, 539-549.
(3) Uhlmann,E. and Peyman,A. *Chemical Reviews* **1990**, 90, 543-584.
(4) Letsinger,R.L., Bach,S.A. and Eadie,J.S. *Nucleic Acids Res.* **1986**, 14, 3487-3499.
(5) Miller,P.S., Dreon,N., Pulford,S.M. and McParland,K.B. *J.Biol.Chem.* **1980**, 255,9659-9665.
(6) Miller,P.S., Annan,N.D., McParland,K.B. and Pulford,S.M. *Biochemistry* **1982**, 21, 2507-2512.
(7) Bower,M., Summers,M.F., Powell,C., Shinozuka,K., Regan,J.B., Zon,G. and Wilson,W.D. *Nucleic Acids Res.* **1987**, 15,4915-4930.
(8) Lesnikowski,Z.J., Jaworska,M. and Stec,W.J. *ibid.* **1990**, 18, 2109-2115.
(9) LaPlanche,L.A., James,T.L., Powell,C., Wilson,W.D., Uznanski,B., Stec,W.J., Summers,M.F. and Zon,G. *ibid.* **1986**, 14,9081-9093.

(10)Summers,M.F., Powell,C., Egan,W., Byrd,R.A., Wilson,W.D. and Zon,G. *ibid.* **1986**, 14, 7421-7435.

(11)Ozaki,H., Yamana,K. and Shimidzu,T. *Tetrahedron Lett.* **1989**, 30, 5899-5902.

(12)Ozaki,H., Kitamura,M., Yamana,K. and Shimidzu,T. *Nucleic Acids Res.*, Symp.Ser. **1989**, 21, 35-36.

(13)Kitamura,M., Ozaki,H., Yamana,K. and Shimidzu,T. *ibid.* **1990**, 30, 65-66.

(14)Ozaki,H., Kitamura,M., Yamana,K., Murakami,A. and Shimidzu,T. *Bull.Chem.Soc.Jpn.* **1990**, 63, 1929-1936.

(15)Froehler,B.C., Ng,P. and Matteucci,M.D. *Nucleic Acids Res.* **1988**, 16, 4831-4839.

(16)Cassani,G.R. and Bollum,F.J. *Biochemistry* **1969**, 8, 3928.

(17)Chamberlin,M.J. *Fed.Proc.* **1965**, 24, 1446.

(18)Yang,J.T., Samejima,T. and Sarker,P.K. *Biopolymers* **1966**, 4, 623.

(19)Emerson,T.R., Swan,R.T. and Ulbricht,T.L.V. *Biochemistry* **1967**, 6, 843.

(20)Miles,D.W., Robins,M.J., Winkely,M.W. and Eyring,H. *J.Am.Chem.Soc.* **1969**, 91, 831.

(21)Brahms,J., Maurizot,J.C. and Michelson,A.M. *J.Mol.Biol.* **1967**, 25, 481.

(22)Johnson,W.C., Itzkowitz,M.S. and Tinoco,I.,Jr. *Biopolymers* **1972**, 11,225.

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