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Polynucleotides. LV.[1] Synthesis and Properties of Dinucleoside Monophosphates derived from Adenine 8,2′-S- and Uracil 6,2′-*O*-Cyclonucleosides. Further Support for the Left-Handed Stacking of Oligonucleotides having High-Anti Base Torsion Angles

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Two sequence isomers of dinucleoside monophosphates, 8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenylyl-(3′—5′)-6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil (A^spU°) (III) and 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluridylyl-(3′—5′)-8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine ($U^\circ pA^s$) (IV), were synthesized by condensation of suitably protected nucleoside and nucleotide units using dicyclohexyl carbodiimide as a condensing reagent. Examination of the UV, CD and NMR spectra of these dimers led us to the conclusion that, whereas compound III did not take a stacked conformation, compound IV took a well-stacked conformation, in which the bases were stacked along a left-handed screw axis. The adoption of this conformation could be interpreted in terms of the high base torsion angles in both nucleoside units.

Keywords——8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine; 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil; torsion angle (χ_{CN}); UV; CD; NMR; DEAE-cellulose column chromatography; paper chromatography

Introduction

We previously showed that homooligonucleotides of 8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine (A^s) (Ia)[3] and 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (A°)[4,5] take conformations in which adenine bases stacked in a left-handed fashion, in contrast to the right-handed helical structure of natural nucleic acids.[6] This is also the case for the complexes between poly(A^s) and homopolynucleotides composed of 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil (U°) (II)[4,7,8] or Laurusin (Formycin B) phosphate.[9] A recent report on the dinucleoside monophosphate,A^spA^s, by high resolution nuclear magnetic nesonance (NMR)[10] and a conformational analysis by computer calculation[11] also supported a lefthanded stacked structure for A^spA^s.

This evidence led to the suggestions that stacking forces together with the charge repulsion between dissociated internucleotide phosphates are governing factors in the stabi-

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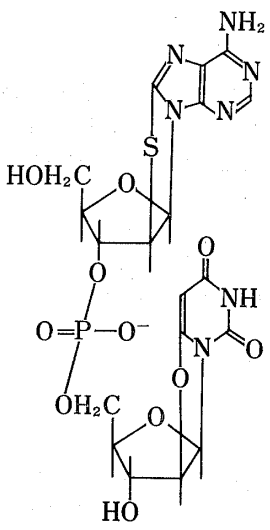
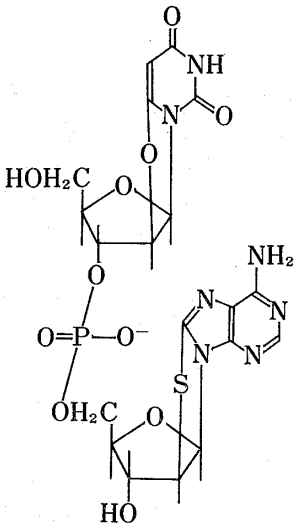


$A^s_pU^0$ (III)



$U^0_pA^s$ (IV)

Chart 1

lization of oligo- or polynucleotide helical structures and that the direction of the stacking (right- or left-handedness) is determined by the base torsion angle. When the base torsion angle (χ_{CN})[12] is in the range of 0—30° (anti region), bases stack in a right-handed fashion and when χ_{CN} is in the range of 110—120° (high-anti region), bases stack in a left-handed fashion. This was further supported by an examination of heterodinucleotides of various *S*-cyclonucleosides having a variety of χ_{CN} values (40—120°) and adenosine.[13] In this paper we report synthetic approaches to hetero-dinucleoside monophosphates consisting of 8,2′-*S*-cycloadenosine (A^s) and 6,2′-O-cyclouridine (U°), 8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenylyl-(3′—5′)-6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil (A^spU°) (III) and its sequence isomer, 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluridylyl-(3′—5′)-8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine (U°pA^s) (IV). Examination of the ultraviolet (UV), circular dichroism (CD) and NMR spectra of these compounds led us to the conclusion that A^spU° (III) does not adopt a stable stacked conformation, whereas U°pA^s does take a stacked conformation stabilized by vertical stacking forces. If we assume left-handed structure for both dinucleotides, these properties of A^spU° and U°pA^s can be rationalized.

Synthesis of A^spU° and U°pA^s

To obtain the 3′-linked components, A^s(Ia) and U° (II) were treated with monomethoxytrityl chloride in pyridine to give 5′-MMTr–A^s (V) and 5′-MMTr–U° (VI) in yields of 71 and 81%, respectively. The structure of these compounds were confirmed by their UV spectral and paper chromatographic properties.

5′-Phosphates of A^s (VII) and U° (VIII) were synthesized by the methods previously reported from our laboratory[2,8] and they were derivatized to N^6,N^6-3′-O-tribenzoyl-pA^s (IX) and 3′-O-acetyl-pU° (X) in yields of 84 and 55%, respectively. The structures of these compounds were confirmed by their UV spectra and thier properties in paper chromatography and paper electrophoresis. Further support for the structures was obtained by deprotection using methanolic ammonia, which gave rise to the starting materials, pA^s and pU°. Condensation of MMTrA^s(V) and pU°(OAc) (X) to synthesize A^spU° (III) was effected by treating them with a 5-fold molar excess of dicyclohexylcarbodiimide (DCC) in anhydrous pyridine at 31° for 72 hr. After appropriate work-up, the MMTr and acyl groups were removed by

12) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).
13) S. Uesugi, J. Yano, E. Yano, and M. Ikehara, *J. Am. Chem. Soc.*, **99**, 2313 (1977).

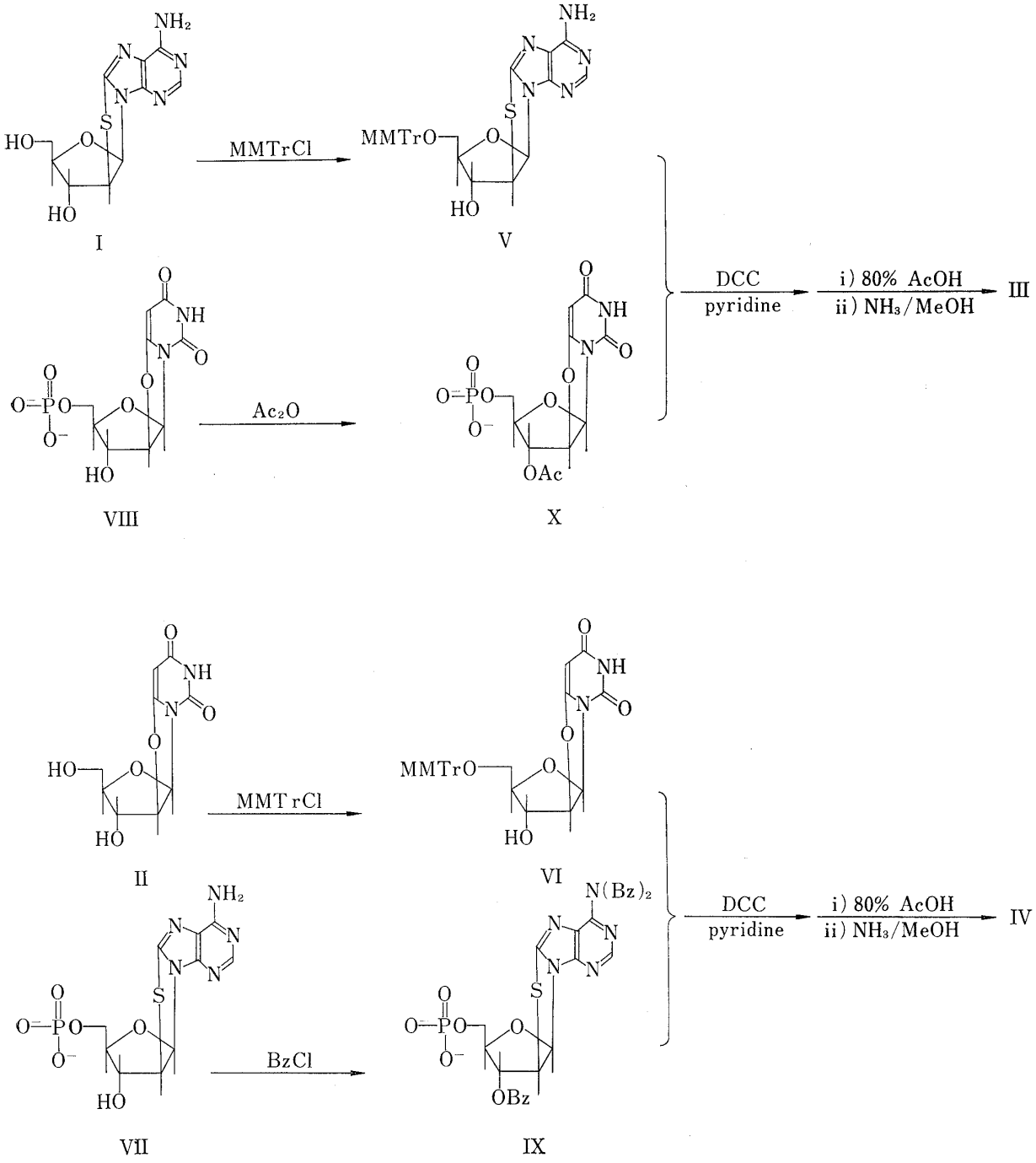


Chart 2

treating the products with 80% acetic acid and methanolic ammonia. Column chromatography on DEAE-cellulose (bicarbonate form) gave $A^{s}pU^{\circ}$ in a yield of 47% (Fig. 1a). The structure of compound III was confirmed by UV, CD and NMR spectroscopy as described later, as well as its chromatographic properties (Table I). This dinucleoside monophosphate was rather resistant to hydrolysis with crude snake venom phosphodiesterase. It was hydrolyzed to the extent of 11% in 24 hr under conditions which caused complete digestion of ApA in 8 hrs. The resulting A^{s} and pU° were identified by paper chromatographic comparison with authentic samples and were present in a ratio of almost 1: 1.

Synthesis of $U^{\circ}pA^{s}$ (IV) was achieved by essentially the same method. $MMTrU^{\circ}$ (VI) and pA^{SBz} (OBz) (IX) were condensed using DCC and $U^{\circ}pA^{s}$ was obtained in a yield of 74%.

Table I. Chromatographic Properties of Various Compounds

	PPC *Rf*				PEP
	A	B	C	D	Rm(pA–A)
A^s	0.56	0.62	0.55	0.66	−0.06
U^o	0.57	0.74	0.52	0.60	0.26
pA^s	0.14	0.25	0.27	0.28	1.00
pU^o	0.15	0.34	0.32	0.27	1.26
A^spU^o	0.24	0.35	0.22	0.36	0.51
U^opA^s	0.28	0.35	0.24	0.34	0.54

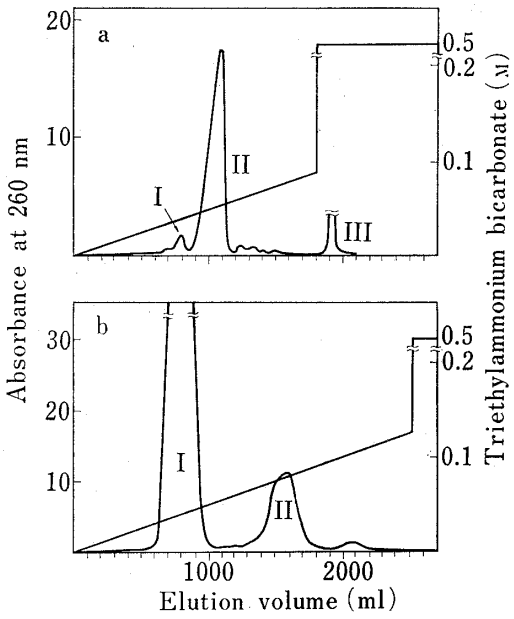


Fig. 1. (a) Chromatography of A^spU^o; (b) Chromatography of U^opA^s

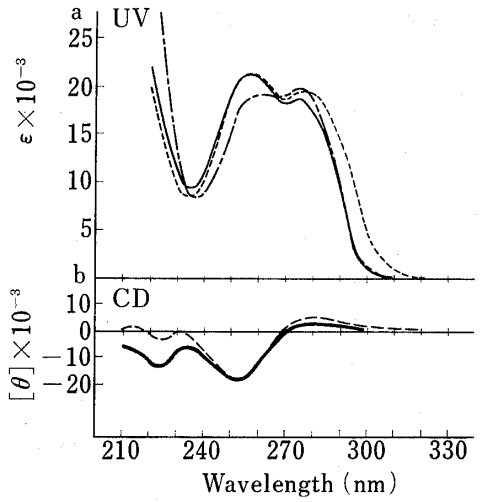


Fig. 2. UV and CD Spectra of A^spU^o and Its Components

——, pH 2, ——, pH 7, – –, pH 12. ——, Ap^sU^o, ----, A^s+pU^o. 1:1

The DEAE-cellulose column chromatography pattern is shown in Fig. 1b. The structure of compound IV was confirmed by its chromatographic properties (Table I) and by UV, CD and NMR spectroscopy as described later. This dimer was also rather resistant to hydrolysis with snake venom phosphodiesterase and it was hydrolyzed to the extent of 19% in 24 hr under the conditions used for A^spU°. The ratio of U° and pA^s was approximately 1:1. It is noteworthy that the elution position of $U^\circ pA^s$ in DEAE-cellulose chromatography is significantly earlier than that of A^spU° under identical conditions. This may be indicative of a more globular conformation of $U^\circ pA^s$ than of A^spU°, which may take an extended form.

Thus two heterodimers consisting of A^s and U° were obtained for the first time and subjected to physical studies in order to investigate the effects of base sequence on the conformation of dinucleoside monophosphates having high-anti nucleosides.

UV Absorption Properties of the Dimers

UV absorption spectra of A^spU° (III) taken at pH 2,7 and 12 are shown in Fig. 2a. Under neutral conditions the curve had two maxima at 256 nm and 274.5 nm, showing that compound III consists of A^s ($\lambda_{max}^{pH\,7}$ 276 nm) and U° ($\lambda_{max}^{pH\,7}$ 250 nm). The $\varepsilon(P)_{256}$ value obtained by phosphate analysis of III was 22000, which corresponds to a hypochromicity of 11% with respect to the calculated sum of ε values of A^s and pU° at this wavelength.

When the hypochromicity values were obtained across the whole wavelength region, 220—300 nm, they varied greatly between 0% at 240 nm and 27% at 285 nm, as shown in Fig. 3. This suggests that the interaction of bases to cause hypochromicity may occur preferentially at the A^s residue and only slightly, if at all, at the U° residue. Therefore, an intermolecular interaction of the A^s residue of $A^spU°$ may be responsible for this hypochromic effect. It was previously found that A^spA^s,[3] had a very large hypochromicity, and the interaction of two A^s residues was shown to be strong, as indicated by a thermal melting temperature (*Tm*) of above 80°.

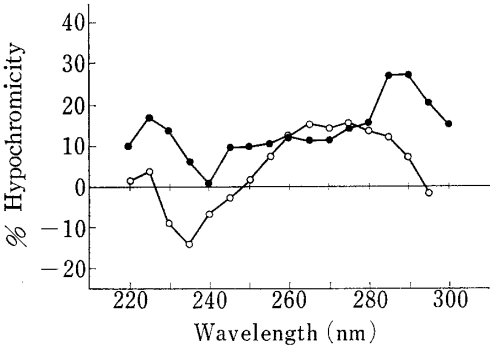


Fig. 3. Hypochromicity of $A^spU°$ (—●—) and $U°pA^s$ (—○—)

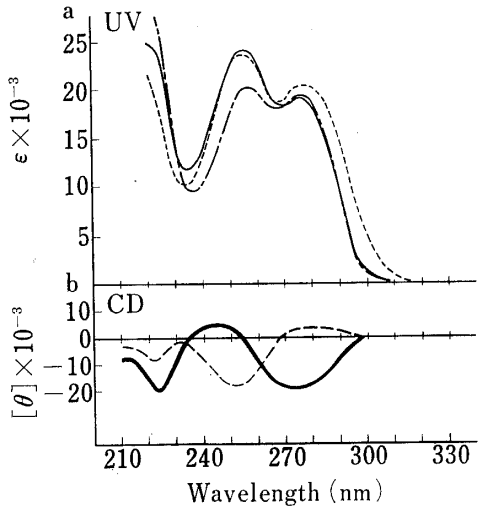


Fig. 4. UV and CD Spectra of $U°pA^s$ and Its Components

----, pH 2, ——, pH 7, – – –, pH 12.
——, $U°pA^s$, ----, $U°+pA^s$ 1:1.

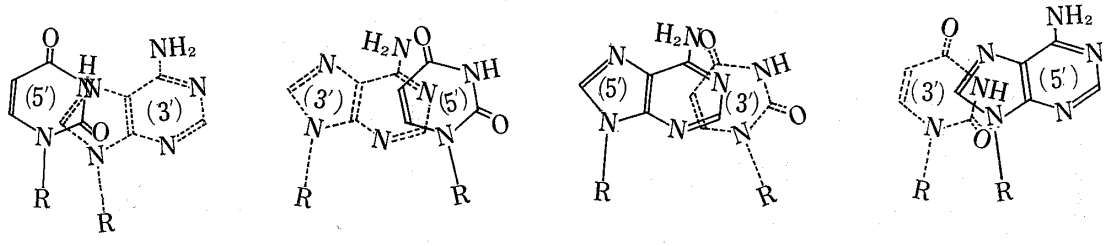
The sequence isomer $U°pA^s$ (IV) showed a similar UV absorption spectrum (Fig. 4a) with maxima at 254.5 nm and 275.5 nm at neutrality. Shifts of these maxima at pH 2 were also quite similar, but at pH 12 the second maximum of $U°pA^s$ did not show any increase, as observed with $A^spU°$. The $\varepsilon(P)_{256}$ value of $U°pA^s$ at pH 7 was 24,100, showing only a small hypochromicity (*ca.* 4%). However, as shown in Fig. 3, the hypochromicity (15%) at 270 nm exceeded that of $A^spU°$ (11%). Moreover, great differences between the hypochromicities of the two compounds were observed around the second maxima at 275—300 nm and in the region of 220—250 nm. In the former region the hypochromicity of $U°pA^s$ decreased to near 0 in contrast to that of $A^spU°$, which rose to 27%. This suggests that intermolecular interaction of A^s residues may be almost absent in the case of $U°pA^s$ and that intramolecular interaction between U° and A^s may be more favorable in this molecule. In the short wavelength region of the hypochromicity curve (Fig. 3) a hyperchromic effect was even observed for $U°pA^s$.

CD Spectra of the Dimers

The CD spectrum of $A^spU°$ at 13° is presented in Fig. 2b. The curve has three peaks at 282, 230 and 215 nm and two troughs at 253 and 223 nm. The amplitudes of these maxima are rather small, except in the case of the trough at 253 nm (−19100). This curve is almost snperimposable on a curve (dotted line) obtained by measurement of a solution containing A^s and pU° in a 1: 1 ratio. This suggests that A^s and U° residues in the $A^spU°$ molecule may not interact as strongly as in the case of A^spA^s.[3] In the latter case, larger splitting bands appeared at 290 nm ($[\theta]=-4\times10^4$) and 265 nm $[\theta]=1.8\times10^4$).

When the CD spectrum of $U°pA^s$ was taken at 22°, the curve presented in Fig. 4b (solid

line) was obtained. The curve has two troughs at 271 nm and 224 nm having $[\theta]$ values of −20000 and −19000, respectively, and a peak at 245 nm having $[\theta]$ of 4700. This curve is completely different from that obtained by measuring a solution containing U° and pA^s in a 1:1 ratio (Fig. 4b, dotted line). This suggests that even at 22°, base residues in the U°pA^s molecule interact to cause splitting of Cotton bands in a (−)-(+) fashion from the long wavelength region in contrast to the (+)-(−)splitting in the CD of ApA. This type of (−)-(+) splitting has previously been assigned to a left-handed stacking conformation in the case of A^{s}pA^s and A^s oligomers.[3] Therefore, in the case of U°pA^s a conformation having bases stacked in a left-handed fashion may be assumed. This is the first case in which A^s and U° residues have been shown to stack well.



a b c d

Fig. 5. Schematic Representation of Possible Conformations of A^{s}pU° [(a) left-handed stack; (b) right-handed stack] and U°pA^s [(c) left-handed stack; (d) right-handed stack]

The reason why A^{s}pU° did not show any tendency for the coupling of base transition moments and U°pA^s did may be interpreted in terms of the mode of stacking. If we assume a left-handed stacking of A^s and U° residues in both A^{s}pU° (III) and U°pA^s (IV) molecules (Fig. 5a and 5c), the former case shows an overlap between the imidazole part of adenine and the uracil ring. By contrast, in U°pA^s the uracil ring overlaps with the pyrimidine portion of the A^s residue and a stronger electronic interaction might be expected. However, if we assume a right-handed stacking for these molecules (Fig. 5b and 5d), this order must be reversed and would not be consistent with the UV and CD observations.

PMR Spectra of the Dimers

The PMR spectral data are presented in Table II. The spectra of the monomers were measured at pD 5.5 to minimize the secondary phosphate dissociation. Assignments of the proton signals in the monomers were made mainly according to the published data for cycloadenosine nucleotides[3] and cyclouridine.[14] In the case of the dimers, H-2 of A^s residues and H-5 of U° residues can be easily assigned because they appear as singlet peaks at around 8.4—8.5 ppm and 5.5—5.7 ppm, respectively. H-2′ of U° residues can also be easily assigned to a doublet (—pU°) or double doublet (U°p—) at around 5.8—5.9 ppm, distinct from the H-1′ signals of the U° and A^s residues. The H-1′ signals were assigned by decoupling experiments. Thus, in the case of U°pA^s, the doublet peaks at 6.60 ppm was specifically decoupled by irradiation of the H-2′ signal of the U°p residue. Similarly, in the case of A^{s}pU°, the doublet at 6.89 ppm was assigned to H-1′ of the pU° residue. Examination of Corey-Pauling-Koltun (CPK) molecular models showed that the dimerization shift data for U°pA^s (Table II) are consistent with a left-handed stack (Fig. 5c), as proposed on the basis of CD studies, but not with a right-handed stack (Fig. 5d). Thus, H-5, H-1′ and H-2′ of the U°p residue are shielded by the ring current of the pyrimidine part of the adenine ring. H-2 of the pA^s residue, which is over the uracil ring, is not shielded but this is due to the much smaller ring

14) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **33**, 3593 (1968).

Table II. Proton Chemical Shifts (δ, ppm),[a] Coupling Constants ($J_{H-1', H-2'}$, Hz, in parentheses) and Dimerization Shifts ($\Delta\delta$, ppm)[b] of the Dinucleoside Monophosphates and Related Compounds

Compound[c]										
A^spU_o	8.38	−0.04	6.99 (7)	−0.07	5.72	−0.01	6.89	0.03	5.93	−0.04
A^sp	8.34		6.92 (6.5)							
pU^o					5.71		6.92 (6)		5.89 (6)	
U^opA^s	8.47	−0.01	6.98 (6.5)	0.03	5.50	0.21	6.60 (5)	0.37	5.76 (5)	0.31
U^op					5.71		6.97 (5.5)		6.07 (5.5)	
pA^s	8.46		7.01 (7)							

a) Chemical shifts are given relative to external TMS.
b) $\Delta\delta=\delta$ (monomer)-δ (dimer).
c) 0.2 M solution in D_2O (sodium salt, pD 7.5 for the dimers and pD 5.5 for the monomers).

current intensity of the uracil ring compared with the adenine ring.[15] The same phenomenon is observed for H-2 of ApU, which takes a right-handed stacking conformation.[6] On the other hand, A^spU^o shows no significant shielding of the protons examined. This result does not necessarily imply that A^spU^o does not take a stacked conformation. Examination with CPK models showed that a right-handed stack (Fig. 5b) can be excluded because the H-5 of the pU^o residue is not shielded, but a left-handed stack (Fig. 5a) cannot be excluded by PMR results alone.

Conformation analysis of U^opA^s will be performed in more detail by X-ray crystallography and the results will be reported elesewhere.

Experimental

General Procedures——UV absorption spectra were recorded on a Hitachi 124 spectrophotometer. CD spectra were recorded on a Jasco ORD/UV-5 spectropolarimeter equipped with a CD attachment. The molar ellipticity, $[\theta]$, and molar extinction coefficient, ε, are presented as the per residue values. Paper chromatography was performed by the descending technique on Whatman No. 1 paper using the following solvent systems: solvent A, 2-propanol–conc. ammonia–water -7: 1: 2 v/v); solvent B, ethanol–1 M ammonium acetate, pH 7.5 (7: 3 v/v); solvent C, 1-butanol–acetic acid–water (5: 2: 3 v/v); solvent D, 1-propanol–conc. ammonia–water (55: 10: 35 v/v). Paper electrophoresis was performed for 1 hr at a voltage of 35 V/cm on Toyo filter paper No. 51A using 0.05 M triethylammonium bicarbonate buffer (pH 7.5).

PMR Measurements——An aqueous solution of each compound (0.08 mmol) was neutralized with 1 N NaOH and passed through columns of Dowex 50 (Na^+) and Chelex 100 resins successively. The lyophilized compound was then dissolved in D_2O and the pD was adjusted with NaOD or DCl to 7.5 (for the dimers) or 5.5 (for the monomers). After lyophilization from D_2O (three times), the compound was finally dissolved in D_2O (99.8% 2H, 0.4 ml). PMR spectra were recorded with a Hitachi R-22 spectrometer (90 MHz; ambient probe temperature, 34°). Chemical shifts were measured from an external tetramethylsilane (TMS) capillary.

Pyridinium N^6,N^6-3′-*O*-Tribenzoyl-8,2′-*S*-cycloadenosine 5′-Phosphate (IX)——Pyridinium 8,2′-S-cycloadenosine 5′-phosphate (VII, 1 mmol) was suspended in pyridine (15 ml) and treated with benzoyl chloride (2.5 ml). The mixture was shaken well to give a homogeneous solution. After 2 hr, the solution was cooled in an ice bath and saturated aqueous $NaHCO_3$ solution (30 ml) was added. The product was extracted with $CHCl_3$ (30 ml) and the $CHCl_3$ layer was washed with water (30 ml × 3). The $CHCl_3$ solution was evaporated to dryness. The residue was rendered anhydrous by co-evaporation with added pyridine and treated with acetic anhydride (15 ml)–pyridine (15 ml) mixture at room temperature overnight. The solvent was removed *in vacuo* and methanol (20 ml) was added to the residue cooled in an ice bath. After a while, the

15) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **27**, 87—95 (1970).

solvent was evaporated off and the residue was dissolved in 50% aqueous pyridine (40 ml). The solution was kept at room temperature overnight. After removal of the solvent, the residue was rendered anhydrous by co-evaporation several times with added pyridine, and finally dissolved in pyridine (3 ml) and added dropwise to ether (170 ml) with stirring. The precipitates were collected by centrifugation. Further material was recovered from the supernatant by the same procedure. The yield was 806 mg (84%); UV: $\lambda_{max}^{pH\,2}$ 233, 305 nm; $\lambda_{max}^{pH\,7}$ 233.5, 300.5 nm; $\lambda_{max}^{pH\,12}$ 300.5 nm. Paper chromatography: *Rf* (C) 0.79 (A^s 0.66, pA^s 0.36); Paper electrophoresis: R_{pA^s} 0.71.

8,2′-*S*-Cycloadenylyl-(3′—5′)-6,2′-*O*-cyclouridine, $A^spU°$ (III)——A mixture of pyridinium 3′-*O*-acetyl-6,2′-*O*-cyclouridine 5′-phosphate[7] (X) (5050 A_{252} units, 0.295 mmol) and 5′-*O*-monomethoxytrityl-8,2′-*S*-cycloadenosine[13] (V) (166 mg, 0.3 mmol) was rendered anhydrous by repeated co-evaporation with added pyridine. The residue was dissolved in anhydrous pyridine (3.5 ml) and DCC (309 mg, 1.5 mmol) was added. The mixture was kept at 31° for 3 days, then 50% aqueous pyridine was added. After 7 hr dicyclohexylurea was removed by filtration. The filtrate was extracted with *n*-pentane (40 ml × 2) and kept at room temperature overnight. Filtration and extraction were repeated and the solvent was evaporated to dryness. After removal of residual pyridine by co-evaporation with toluene, 80% aqueous acetic acid (40 ml) was added. The resulting solution was kept at room temperature for 3 hr. The solvent was removed *in vacuo* and the residual acetic acid was removed by co-evaporation with *n*-butanol–water (1: 1, vol/vol). The residue was rendered anhydrous by co-evaporation with added pyridine and treated with methanolic ammonia (saturated at 0°) overnight at 31°. The volatile materials were removed by evaporation. Water (40 ml) and ether (50 ml) were added to the residue and the mixture was shaken well. The aqueous layer was extracted with ether (50 ml × 3) and filtered to remove insoluble material. The filtrate was diluted to 300 ml and applied to a column (1.8 × 45 cm) of DEAE-cellulose (bicarbonate form). After washing with water, elution was carried out using a linear gradient of triethylammonium bicarbonate buffer, pH 7.5 (0—0.2 M, total 4 l). Fractions of 15 ml were collected at 15 min intervals. The chromatogram is shown in Fig. 1a. From peak II, 2920 A_{260} units of $A^spU°$ were obtained (yield 47%). Desalting was carried out by repeated co-evaporation with water. Samples for various analyses were purified by paper electrophoresis. The properties in paper chromatography and electrophoresis are summarized in Table I. UV: $\lambda_{max}^{pH\,2}$ 256.5 nm (ε 21900), 276.5 nm (ε 20200); $\lambda_{max}^{pH\,7}$ 256 nm (ε 22000), 274.5 nm (ε 19300); $\lambda_{max}^{pH\,12}$ 258.5 nm (ε 19900), 274.5 nm (ε 20500). The UV and CD spectra are shown in Fig. 2. The PMR data are presented in Table II.

6,2′-*O*-Cyclouridylyl-(3′—5′)-8,2′-*S*-cycloadenosine, $U°pA^s$ (IV)——A mixture of 5′-*O*-monomethoxytrityl-6,2′-*O*-cyclouridine[7] (VI) (479 mg, 0.93 mmol) and tribenzoyl-pA^s (IX) (764 mg, 0.91 mmol) was rendered anhydrous by repeated co-evaporation with added pyridine. The residue was dissolved in pyridine (11 ml) and DCC (944 mg, 5 equiv.) was added. The mixture was stirred for a while and kept at 32° for 4 days, then 50% aqueous pyridine (50 ml) was added and the mixture was kept 32° overnight. The dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residual pyridine was removed by co-evaporation with toluene and the residue was treated with 80% aqueous acetic acid (60 ml) at 33° for 3 hr. The solvent was evaporated off, *n*-butanol–water (1: 1, vol/vol) was added and the solution was again evaporated down *in vacuo*. After co-evaporation with added pyridine, the residue was treated with methanolic ammonia (50 ml) at 34° overnight. The volatile materials were evaporated off and the residue was dissolved in 30% aqueous pyridine. The solution was extracted with ether and the aqueous layer was evaporated to dryness. The residue was dissolved in water (1 l) and applied to a column (1.8 × 50 cm) of DEAE-cellulose (bicarbonate form). After washing with water, elution was carried out using a linear gradient of triethylammonium bicarbonate buffer (pH 7.5) (0—0.2 M, total 4 l). Fractions of 18 ml were collected at 15 min intervals. The chromatogram is shown in Fig. 1b. From peak I, 15040A_{260} units of $U°pA^s$ were obtained (yield 74%). Desalting was carried out by repeated co-evaporation with added water. Samples for various analyses were purified by paper electrophoresis. The properties in paper chromatography and electrophoresis are summarized in Table II. UV: $\lambda_{max}^{pH\,2}$ 255 nm (ε 23800), 277 nm (ε 20500); $\lambda_{max}^{pH\,7}$ 254.5 nm (ε 24100) 275.5 nm (ε 19200); $\lambda_{max}^{pH\,12}$ 256 nm (ε 20300), 276 nm (ε 19400).

6,2′-*O*-Cyclouridine 3′-Phosphate, U°p——A mixture of pyridinium β-cyanoethylphosphate (1.5 mmol) and 5′-*O*-monomethoxytrityl-6,2′-*O*-cyclouridine (463 mg, 0.9 mmol) was rendered anhydrous by co-evaporation with added pyridine. The residue was dissolved in anhydrous pyridine (10 ml) and DCC (928 mg, 4.5 mmol) was added. The mixture was kept at 34° for 22 hr, then 50% aqueous pyridine was added and after standing overnight at room temperature, the precipitate of dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness and the residue was treated with 80% aqueous acetic acid at 50° for 1 hr. The solvent was evaporated off and the residue was treated with conc. ammonium hydroxide at 55° for 1 hr. The volatile materials were evaporated off, and 30% aqueous pyridine was added. The mixture was extracted with *n*-hexane (50 ml × 2). The aqueous layer was concentrated to half the original volume *in vacuo* and diluted to 200 ml with water. The solution was applied to a column (1.8 × 24 cm) of Dowex 1 × 2 (carbonate form). After washing with water (1.5 l), stepwise elution was carried out with 0.05, 0.1 and 0.2 M HCOOH. (8063 A_{252} units, 62%). Fractions containing U°P were pooled and concentrated *in vacuo*. The residue was dissolved in water and lyophilized to give a crystalline compound. UV: $\lambda_{max}^{pH\,2}$ 252.5 nm (ε 14400); $\lambda_{max}^{pH\,7}$ 253 nm (ε 15400); $\lambda_{max}^{pH\,12}$ 254 nm (ε 10700). The properties in paper chromatography and paper electrophoresis were very similar to those of pU°.

Hydrolysis of the Dimers with Snake Venom Phosphodiesterase——Dimer samples (10 A_{max} units) were incubated with snake venom phosphodiesterase (0.2 mg/ml) in 0.25 M ammonium carbonate buffer at 37°. The products were analyzed by paper electrophoresis. Under these conditions ApA was hydrolyzed completely in 8 hr. A^spU° was hydrolyzed to the extent of 11% in 24 hr to give approximately equal amounts of A^s and pU°. $U^\circ pA^s$ was hydrolyzed to the extent of 19% in 24 hr to give approximately equal amounts of U° and pA^s, as estimated from the UV absorptions.