Z-Ile-Gln-Asn-Cys(Bzl)-ONB. A mixture of 1.38 g (2 mmol) of Boc-Gln-Asn-Cys(Bzl)-ONB and 1.5 ml of anisole was stirred for 30 min with 17.5 ml of trifluoroacetic acid (TFA). The salt was precipitated with Et_2O (250 ml), filtered off, washed with three 30-ml portions of Et_2O, and dried *in vacuo*. The white powder was dissolved in 11 ml of anhydrous DMF and neutralized to pH 7 with *N*-methylmorpholine. Z-Ile-ONp (0.90 g, 2.5 mmol) was added, and the mixture was stirred for 20 hr at room temperature. The slurry was triturated with 2 ml of H_2O and 75 ml of EtOAc. The precipitate was filtered off and washed with 15 ml of EtOAc, 15 ml of EtOH, two 15-ml portions of EtOH–H_2O (1:1), 15 ml of EtOH, and two 15-ml portions of Et_2O. The product was dried *in vacuo:* wt 1.14 g (68%); mp 241–243° dec (lit.[5] mp 240–241.5° dec). This sample was found to be identical by infrared and mixture melting point comparison with the sample prepared earlier.[5]

Tocinoic Acid. A solution of about 0.6 mmol of Na in anhydrous liquid NH_3 (125 ml, freshly distilled from Na) was brought near boiling with stirring, and 65 mg of finely powdered Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB[5] was added. The solution was stirred for 4–5 min and the blue color was maintained throughout by addition of small amounts of Na. The excess Na was destroyed by TFA and the NH_3 removed by evaporation and lyophilization. The residue was dissolved in 150 ml of 0.1% aqueous HOAc. The pH of the solution was adjusted to 8.5 with 2 *N* NH_3, and the sulfhydryl compound was oxidized with 12 ml of 0.01 *N* $K_3Fe(CN)_6$. After 30 min, the pH was adjusted to 5 with dilute HOAc, and 4 ml of Rexyn 203 (Cl^- cycle) (Fisher Scientific Co.) was added to remove ferro- and excess ferricyanide ions. The suspension was stirred for 15 min, the resin was filtered off and washed with three 15-ml portions of 10% HOAc, and the combined filtrates were lyophilized. The residue was dissolved in 6 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (4:1:1:0.4:6.4) and subjected to partition chromatography by the method of Yamashiro[14] on a 2.2 × 61 cm column of Sephadex G-25 (100–200 mesh) at a flow rate of 17 ml/hr. The peptide material was detected by the Folin–Lowry method[23] and that part comprising the major peak at R_f 0.23 was isolated by lyophilization: wt 21.6 mg. This product was dissolved in 3 ml of 0.2 *N* HOAc and further purified by gel filtration on a 2.8 × 65 cm column of Sephadex G-25 (200–270 mesh) equilibrated with 0.2 *N* HOAc. The compound emerged as a single sharp peak at 93% of the total column volume and was isolated by lyophilization: wt 16.2 mg; $[\alpha]^{22}D$ −7.0° (*c* 0.5, 1 *N* HOAc). The peptide gave a single spot on tlc in system A. Amino acid analysis[24] following 24-hr hydrolysis in 6 *N* HCl at 110° gave the following molar ratios: Asp, 1.0; Glu, 1.0; Cys, 2.0; Ile, 1.1; Tyr, 0.87; NH_3, 2.0. *Anal.* Calcd for $C_{30}H_{44}N_8O_{10}S_2$: C, 48.64; H, 5.98; N, 15.13. Found: C, 48.35; H, 6.12; N, 14.95.

Deaminotocinoic Acid. A sample of 155 mg (0.15 mmol) of *S*-benzyl-β-mercaptopropionyl-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB[5] was treated with a solution of excess Na in 200 ml of liquid NH_3 as described previously. The lyophilized product was dissolved in 400 ml of 0.1% HOAc and oxidized at pH 8.3 with 30 ml of 0.01 *N* $K_3Fe(CN)_6$. The ferro- and excess ferricyanide ions were removed with 6 ml of Rexyn 203 (Cl^- cycle), and the resulting solution was lyophilized. The residue was dissolved in 5 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (5:1:1:0.15:7) and subjected to partition chromatography on a 2.9 × 63 cm column of Sephadex G-25 (100–200 mesh). The major peak of peptide material was eluted at R_f 0.40. The product isolated from this peak (40.6 mg) was subjected to gel filtration on a 2.9 × 65 cm column of Sephadex G-25 (200–270 mesh) in 0.2 *N* HOAc and eluted at 100% of column volume: wt 25 mg; $[\alpha]^{22}D$ −57.0° (*c* 0.5, 1 *N* HOAc). The peptide showed a single spot on tlc in system A. Amino acid analysis[24] following 24-hr hydrolysis in 6 *N* HCl gave the following molar ratios: Asp, 1.0; Glu, 1.0; Ile, 1.0; Tyr, 0.85; NH_3, 2.2; Cys, 0.27; mixed disulfide of Cys and β-mercaptopropionic acid, 0.78. *Anal.* Calcd for $C_{30}H_{43}N_7O_{10}S_2 \cdot H_2O$: C, 48.44; H, 6.10; N, 13.18. Found: C, 48.81; N, 5.91; N, 13.06.

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Synthesis and Properties of the Dinucleoside Monophosphate of Adenine 8-Thiocyclonucleoside

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Abstract: The dinucleoside monophosphate (A^spA^s) of 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)-adenine was synthesized; its properties were investigated by ultraviolet absorption, circular dichroism, and proton magnetic resonance. Comparison was made with diadenosine monophosphate (ApA) in these properties. The results indicate that the conformation of A^spA^s has two special characteristics: (1) it is a left-handed stack with considerable base–base overlap, and (2) it is relatively stable against thermal perturbation. Also, A^spA^s is very resistant to both venom and spleen phosphodiesterases and does not form a complex with poly U.

The importance of the torsion angle (ϕ_{cn})—which defines the geometrical relationship between the base and the furanose—to the nucleoside conformation has been generally recognized.[2-4] The influence of the

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torsion angle of the individual nucleoside unit on the conformation of dinucleotides, oligonucleotides, etc., has not been sufficiently investigated. During the past 5 years, one of our laboratories has prepared a series of purine cyclonucleosides in which the torsion angle is fixed by a covalent linkage between the base and the furanose.[5] The conformation of these monopurine cyclonucleosides has been studied and reported previously.[6,7] The study of the dinucleotide and oligonucleotides which contain these cyclonucleosides having various fixed torsion angles as the monomeric units will be important in elucidating the influence of the torsion angles on the polynucleotide conformation.

For this purpose, considering the position of phosphodiester linkage, the strength of base stacking, and chemical stability, 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine[8] (8,2′-*S*-cycloadenosine, or A^s) is the most suitable nucleoside. In this paper, the synthesis of A^spA^s, the dinucleoside monophosphate of 8,2′-*S*-cycloadenosine, and the studies on its ultraviolet absorption, circular dichroism, and proton magnetic resonance are presented.[9] Its conformation and properties are compared in detail with those of ApA, the dinucleoside monophosphate of adenosine.

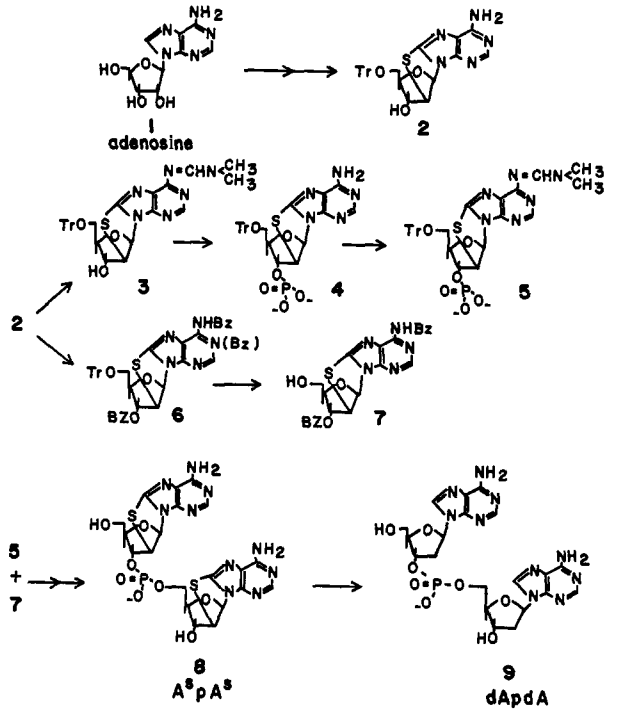
The studies from uv absorbance, circular dichroism, and proton magnetic resonances all indicate that the conformation of this dicyclonucleoside monophosphate, A^spA^s, is a left-handed stack with considerable base–base overlap, and the stack is relatively stable against thermal perturbation. The facts that A^spA^s does not form a complex with poly U under appropriate conditions and that A^spA^s is very resistant to both venom and spleen phosphodiesterases support the above conclusion. Probable explanations for the conformation of A^spA^s are discussed.

Results and Discussion

Synthesis of the Dinucleoside Monophosphate, A^spA^s. The synthesis of the dinucleoside monophosphate of 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)-adenine is outlined in Scheme I. It was accomplished by condensation of the protected 3′-monophosphate derivative **5** with the protected nucleoside **7** bearing a free 5′-hydroxyl group.

5′-*O*-Trityl-8,2′-*S*-cycloadenosine (**2**) was prepared by the method of Ikehara and Kaneko[10] starting from adenosine **1**. Nucleoside **2** was treated with dimethylformamide dimethyl acetal[11] in dimethylformamide at room temperature for protection of the 6-amino group of adenine. This reagent is useful for specific protection of the exocyclic amino group of the bases in

Scheme I. Synthetic Scheme for A^spA^s and Its Conversion to dApdA



nucleosides[12] and nucleotides.[13] The nucleoside **3** thus obtained gave uv absorption characteristics of N^6-dimethylaminomethylene derivatives, having a λ_{max}^{EtOH} at 320 nm. *S*-Cycloadenosine, on the other hand, has a λ_{max} at 276 nm. Compound **3** also has a higher R_f value on thin layer than that of starting material **2**. Compound **3** was condensed with 2-cyanoethyl phosphate in anhydrous pyridine with DCC, followed by removal of the cyanoethyl group by treatment with methanolic ammonia, and the deblocking of the amino group. The resulting nucleotide **4** had a relative mobility (relative to 5′-AMP) of 0.86 on paper electrophoresis and gave positive results in color developing tests for trityl and phosphate groups. Compound **5** was obtained by protection of the 6-amino group using the same conditions described above. It had a $\lambda_{max}^{H_2O}$ at 319 nm and a relative mobility of 0.83 on paper electrophoresis.

The nucleoside **2** was benzoylated by treatment with benzoic anhydride in pyridine at 80–85° for 35 hr. After work-up, the mixture of dibenzoyl and tribenzoyl derivatives was obtained. Both have a $\lambda_{max}^{EtOH,\ H^+}$ at 301–302 nm, showing the presence of a benzoyl group at the N-6 position. The mixture was detritylated by heating in an aqueous 80% acetic acid solution at 100° for 30 min to give **7**, which contains a free 5′-hydroxyl group. Trityl alcohol was removed by extraction with hot ether after evaporation of the solvent. The nucleoside **7** gave a negative color test for the trityl groups, and showed a single spot on thin-layer chromatography. The uv spectrum showed the presence of the N^6-benzoyl group. The overall yield of **7** from **2** was 74%.

The pyridinium salts of **5** and **7** were rendered anhydrous by repeated evaporation with anhydrous pyridine and were condensed using DCC in anhydrous pyridine. All protecting groups were removed by suc-

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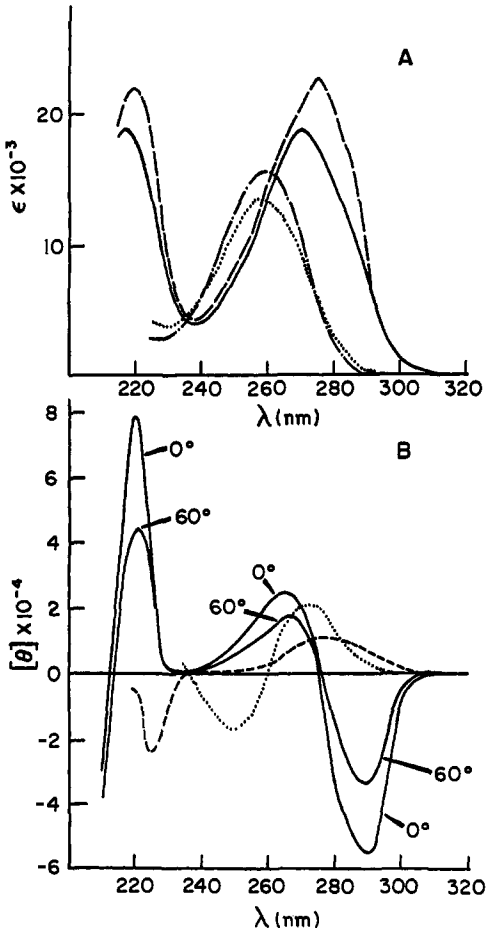

Figure 1. (A) Uv absorption spectra of A^spA^s (—), pA^s (– – –), ApA (· · ·), and pA (–·–) in 0.01 *M* phosphate buffer, 0.1 *M* KF, pH 7.5, room temperature. (B) CD spectra of A^spA^s at 0 and 60° (—), pA^s at room temperature (– – –), and ApA at 25° (· · ·); 0.01 *M* phosphate buffer; pH 7.0; ε and [θ] are calculated per nucleoside residue.

cessive treatment with methanolic ammonia and with aqueous 80% acetic acid. The final aqueous solution was applied on a column of DEAE-cellulose (bicarbonate form). Linear gradient elution with 0.01–0.08 *M* triethylammonium bicarbonate buffer, pH 7.5, gave a major peak around 0.025 *M* buffer concentration, which, however, gave two spots on paper chromatography. The material was further fractionated on a column of Dowex (formate form) resin. Elution with 0.025 *N* formic acid gave two completely separated peaks. The second peak, which was the major component, contained the desired A^spA^s (**8**) in pure form.

A^spA^s thus obtained was desulfurized with Raney nickel in ethanol–methyl Cellosolve to afford 2′-deoxyadenylyl-(3′–5′)-2′-deoxyadenosine (dApdA, **9**), whose uv absorption spectrum and paper chromatographic values were identical with authentic material. Thus, a dideoxyribonucleoside monophosphate was synthesized starting from ribonucleoside for the first time.

In addition to chromatographic and electrophoretic evidence, the dimeric structure of A^spA^s was also confirmed by phosphorus analysis, uv absorption, CD, and pmr data, which are described in the following sections. A^spA^s is extremely resistant to spleen and venom phosphodiesterase. It can be only partially hydrolyzed in the presence of a very large amount of snake venom phosphodiesterase.

Ultraviolet Absorption and Circular Dichroism Studies. Uv absorption and CD spectra of pA^s and A^spA^s, together with those of pA and ApA, are shown in Figure 1. The uv absorption of A^spA^s at neutral pH has its λ_{max} at 271 nm, a 5-nm hyposochromic shift compared to the monomer pA^s, which has a λ_{max} at 276 nm. The extent of this hypsochromic shift from monomer to dimer is substantially larger than that (~1 nm) observed for pA to ApA and is even larger than that (3-nm blue shift) observed for pA to poly A (λ_{max} 256 nm). Upon protonation, the λ_{max} of A^spA^s is located at 276.5 nm, very close to that (λ_{max} 278 nm) of the monomeric pA^s in acidic solution. Protonated ApA also has a λ_{max} (257 nm)[14] identical with that (λ_{max} 257 nm) of protonated pA. It is known that upon protonation, the stacking of the bases in the dimer is sharply reduced due to charge repulsion.[14]

The ε(p) values at λ_{max} of A^spA^s are 4.20×10^4 and 3.80×10^4 at pH 1 and 7, respectively. These values are about twice as large as those of the monomer pA^s (2.15×10^4 at pH 1 and 2.25×10^4 at pH 7), thus confirming the presence of two bases per phosphorus atom in the dimer. On a molar basis, the hypochromicity of A^spA^s calculated from ϵ_{max} of the dimer *vs.* monomer is about 15% (Figure 1A). This value is definitely higher than that of the $A_{3'}p_{5'}A$ (12%),[15] but is similar to that of $A_{2'}p_{5'}A$ (15.5–16%), and is less than that of $A_{5'}p_{5'}A$ (22%).[15] In summary, the uv absorption data on A^spA^s certainly indicate a strong interaction between the two bases in the dimer.

CD spectra of the monomer pA^s (Figure 1B) show a broad and strongly positive peak around 280 nm, a small positive region at 250 nm, and a negative band at 225 nm. The uv and CD spectra both suggest that there are at least two transitions in the 280-nm region. The pA^s CD spectrum is quite different from the pA or other naturally occurring purine nucleotides or nucleosides, which normally have a dominant negative CD region around 260 nm, and a small positive CD band at 280 nm.[16–18] The significance of this observation has been fully discussed elsewhere.[7]

The CD spectrum of A^spA^s at neutral pH has a strong negative band around 290 nm, and two positive bands around 265 and 222 nm. The spectra at 0 and 60° are shown in Figure 1B. The spectrum at 30° was published earlier in a preliminary communication.[9] The Cotton effect in the CD spectrum of A^spA^s is much larger than in those of pA^s and ApA. As shown in Figure 1B, the CD spectrum of A^spA^s is more or less opposite to that of ApA. For example, the [θ] values of the extrema in the long-wavelength region are about -4×10^4 (285 nm) and 2×10^4 (272 nm), respectively, for A^spA^s and ApA at room temperature.

Theoretical studies on the CD spectrum of ApA in comparison with its monomer have indicated conclusively that the stacking interaction between the base chromophores gives rise to splitting of the energy level of each transition and splitting of the CD spectrum.[19,20]

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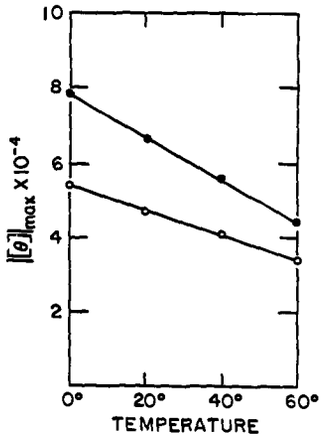

Figure 2. Effect of temperature on $[\theta]$ of $A^sp A^s$ at 290 (Δ, minimum) and at 220 nm (●, maximum), in 0.01 *M* phosphate (pH 7.5), 0.1 *M* KF.

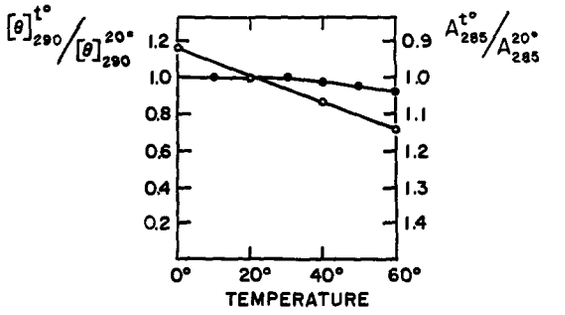

Figure 3. Comparison of temperature effects on CD (○) *vs.* uv absorption (●) of $A^sp A^s$. The observed values ($[\theta]$ for CD and A for uv absorption) are normalized to that measured at 20°: $[\theta]_{pA^st°}/[\theta]_{A^spA^s20°}$ at 290 nm = −0.15; $A_{pA^st°}/A_{A^spA^s20°}$ at 285 nm = 1.35.

As shown in Figure 1B, the CD spectrum of ApA, which is very different from that of the monomer, has two extrema centered around 260 nm. The two peaks are opposite in sign but nearly equal in magnitude. Since the CD peak at long wavelength is positive, the splitting pattern of ApA can be termed as "positive splitting." The sign and magnitude of the splitting are related to the handedness of the stack and the angle of the transition moments in a simple dipole approximation.[15,19] Calculations based on this approach as well as on the approach of monopole approximation[20] indicated that the CD spectrum of ApA can be explained on the basis of a right-handed stack, with a geometry similar to that of a DNA helix.[19] The conformation of ApA as a right-handed stack has been unambiguously confirmed by pmr studies.[15,21,22]

In contrast to that of ApA, the spectrum of $A^sp A^s$ is a "negative splitting" pattern centered at 275 nm which is near its absorption maximum (Figure 1B). The CD spectrum of $A^sp A^s$ is substantially larger in magnitude than that of ApA as mentioned above, but is less symmetrical (or less conservative[23]). However, it has a positive band at about the 220-nm region, similar to that of ApA.[24] Based on the discussion in the preceding paragraph, the "negative splitting" pattern of $A^sp A^s$ would suggest that the conformation of $A^sp A^s$ is a left-handed stack. However, the uv absorption spectrum of pA^s (Figure 1A) clearly suggests that there are at least two transitions in the region 265–285 nm. Therefore, the CD spectrum of $A^sp A^s$ could have a complex origin and may not be easily interpreted. The CD spectrum of $A^sp A^s$, nevertheless, clearly indicates a substantial interaction between the base chromophores.

In Figure 2, the temperature dependencies of $[\theta]$ at two extrema are shown. The temperature dependencies of the CD and that of the uv absorbance are compared in Figure 3. When temperature is raised over the range 0–60°, the magnitude of the extrema decreases linearly. The decrease in the absolute value of $[\theta]_{290\,nm}$ and $[\theta]_{220\,nm}$ is 38 and 40%, respectively. For ApA, the $[\theta]$ value of the first positive extrema also decreases linearly over the same temperature range, but by more than 66% (Kondo and Tazawa). Even in the case of poly A, the decrease is about 60% under a comparable condition.[25] Therefore, the CD of $A^sp A^s$ is much less sensitive to the temperature perturbation than ApA and poly A.

Proton Magnetic Resonance Studies. The pmr spectra of $A^sp A^s$ (sodium salt, 0.05 *M*) and of pA^s (disodium salt, 0.1 *M*) in D_2O were shown in the previous communication.[9] The singlets around 8.00 ppm (TMS, capillary) and the doublets around 6.5–7.0 ppm are the resonance lines of H-2 and H-1′, respectively. However, in the case of the $A^sp A^s$, the two H-2 resonances, and the two H-1′ resonances cannot be assigned unambiguously to either of the nucleosidyl subunits in the dimer, *i.e.*, the A^sp or the pA^s residue.

The assignment of the two H-1′ (6.92 and 6.50 ppm at 30°) was carried out by an approach based on the specific broadening effect of Mn^{2+} upon binding. The relaxation effect of the paramagnetic Mn^{2+} ion is extremely distance dependent. The resonance of the proton very nearest to the Mn^{2+} bound to the phosphate group will be specifically broadened. This approach was first used by Chan and Nelson[22] for the assignment of the H-8 proton and was later adopted by Fang, *et al.*,[26] for the assignment of two H-1′ protons in the deoxydiadenosine monophosphate, dApdA. Thus, in the presence of dilute Mn^{2+} ($\sim 2 \times 10^{-4}$ *M* for 0.01 *M* $A^sp A^s$), one of the H-1′ resonances of $A^sp A^s$ (6.50 ppm) was specifically broadened. Based on the results from the study on dApdA, we conclude that the broadened resonance belongs to the H-1′ of the 3′-nucleotidyl unit (A^sp). This proton is closer to the Mn^{2+} bound to the phosphate group than the H-1′ of the 5′-nucleotidyl unit (pA^s). Therefore, the H-1′ at the higher field (6.50 ppm) is assigned to the A^sp residue while the H-1′ at the lower field (6.92 ppm) is assigned to the pA^s residue.

The relative field positions of the two H-1′ protons cannot be derived from a right-handed stack, but are expected for a dimer in a left-handed stack. As shown

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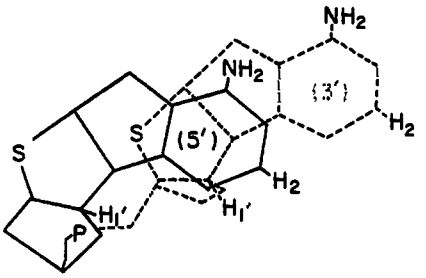

Figure 4. Schematic presentation of A^spA^s: solid line is 5′-linked nucleoside and dotted line shows 3′-linked nucleoside.

from the model of A^spA^s in a left-handed stack (Figure 4), the H-1′ of the A^sp residue is more shielded by the ring current of the base of the neighboring pA^s residue than is the H-1′ of the pA^s residue by the neighboring A^sp residue. Therefore, the pmr and CD data discussed in the previous section both support the left-handed stack for the conformation of A^spA^s. Based on this model, the H-2 of the 5′-nucleotidyl unit should be more shielded by the neighboring base than the H-2 of the 3′-nucleotidyl unit. Thus the H-2 at the high-field position (8.19 ppm) is assigned to the pA^s residue and the H-2 at the low-field position (8.56 ppm) is assigned to the pA^s residue.

In Table I, the chemical shifts of the H-2 and H-1′ of

Table I. Chemical Shifts (δ)[a] and Dimerization Shifts ($\Delta\delta_D$)[b] of H-2 and H-1′ Protons of A^spA^s at Various Temperatures in D_2O

		H-1′		H-2	
T, °C		3′	5′	5′	3′
7	A^spA^s	6.36	6.79	8.08	8.49
	pA^s	7.01	7.01	8.48	8.48
	$\Delta\delta_D$	0.65	0.22	0.40	0.01
30	A^spA^s	6.50	6.92	8.19	8.56
	pA^s	7.09	7.09	8.56	8.56
	$\Delta\delta_D$	0.59	0.17	0.37	0.00
60	A^spA^s	6.72	7.09	8.36	8.71
	pA^s	7.22	7.22	8.70	8.70
	$\Delta\delta_D$	0.50	0.13	0.34	0.01

[a] Relative to TMS, capillary; extrapolated to infinite dilution. [b] $\Delta\rho_D = \delta_{A^spA^s} - \delta_{pA^s}$.

A^spA^s extrapolated to infinite dilution at 7, 30, and 60° are listed. The comparison between the δ values of A^spA^s and the δ values of the monomeric pA^s has also been made and is expressed as the dimerization shift, $\Delta\delta = (\delta_{pA^s} - \delta_{A^spA^s})$, which is a measure of the shielding (or deshielding) influence of the neighboring unit in the dimer. The values of $\Delta\delta_D$ of A^spA^s (Table I) are large compared to those of ApA.[13] For instance, at 30° the $\Delta\delta_D$ for H-1′ of A^spA^s are 0.59 and 0.17 ppm for the A^sp and pA^s residues, respectively. The corresponding values in ApA are 0.26 ppm (Ap) and 0.15 ppm (pA). The shielding pattern of H-2 is also different. $\Delta\delta_D$ for H-2 of A^spA^s is 0 ppm (A^sp) and 0.37 ppm (pA^s) as compared to that of 0.075 (Ap) and 0.215 ppm (pA) for ApA. These differences reflect the differences in the geometry of the stack of A^spA^s (Figure 4) compared to that of ApA published earlier.[15] Interestingly, the values of $\Delta\delta_D$ of A^spA^s are far less sensitive to the temperature perturbation than those of ApA. In ApA, $\Delta\delta_D$ of H-1′ (Ap) and H-2 (Ap) decrease by 33 and 58%, respectively, over the temperature range of 4–60°, while in A^spA^s, $\Delta\delta_D$ of H-1′ (A^sp) and H-2 (pA^s) decreases only by 23 and 15%, respectively, over the temperature range of 7–60°. This low sensitivity of A^spA^s toward temperature variation has been found in the CD and uv hyperchromicity studies as described in the previous section.

Interaction of A^spA^s with Poly U. A^spA^s (0.5×10^{-4} M per A^s residue) and poly U (1×10^{-4} M per U residue) were mixed at a ratio of 1:2 (A:U) in 0.02 M $MgCl_2$ and 0.02 M Tris (pH 7.5). After incubation at 0°, the uv absorbances of the solution at 260 and 271 nm were recorded from −4 to 20°. The observed uv absorbance of the solution over this temperature range is identical with the arithmetic sum of the uv absorbances of the two separate solutions of A^spA^s and poly U (a helix–coil transition was observed, which was that of poly U). This result indicates that there is no complex formation between A^spA^s and poly U. CD studies of various mixtures of poly U and A^spA^s in varying ratios of Mg^{2+}–Tris buffer at low temperature (the mixing experiment) also indicate that poly U does not form a complex with A^spA^s.

This situation is not surprising, perhaps, since the conformation of A^spA^s is a left-handed stack which is stable against thermal perturbation. The dinucleoside monophosphate of L-adenosine, L-ApA, was previously reported to have a left-handed stack conformation.[24] This is the mirror image of the right-handed conformation of D-ApA. Although L-ApA·2poly U complex has been found, the CD spectrum of the complex indicates that the L-ApA assumes a *right-handed* conformation in the triple-stranded dimer·2 polymer complex. This observation implies that L-ApA (or its optical isomer, D-ApA) can assume both a left-handed stack as well as a right-handed stack in solution, although the left-handed conformation is predominant for L-ApA (the opposite is true for D-ApA). The relatively rigid conformation of A^spA^s as indicated by its thermal stability may prevent a dynamic conversion of A^spA^s from a left-handed stack to a right-handed stack which is required for the formation of a complex with poly U. The large magnitude of the CD curve (Figure 1B) of A^spA^s *vs.* that of ApA may also reflect the low percentage of the minor conformation of opposite handedness (right-handed stack for A^spA^s and left-handed stack for ApA) existing in a solution of A^spA^s. In addition, the torsion angle (ϕ_{cn}) between the base and the furanose ring of the nucleoside is around −108° for 8,2′-*S*-cycloadenosine, but about −10° for adenosine in crystalline form.[4] This large difference in the torsion angle may be another reason why A^spA^s does not form a complex with poly U.

Conclusion

As discussed above, the conformation of the dicyclonucleoside monophosphate, A^spA^s, has two special characteristics: (1) it is a left-handed stack with considerable base–base overlap; (2) it is relatively stable against thermal perturbation. In fact, A^spA^s is the first confirmed example of a left-handed stack in the dimer of the β-D-nucleoside series, while the left-handed stack of L-ApA reported earlier comes from the β-L-nucleoside series.[24] This finding provides us with ad-

ditional information about the forces which govern the handedness of the helical turns in single-stranded polynucleotides.

Strictly speaking, the handedness of the helical conformation of the oligo- or polynucleotide chain is defined by the configuration of the furanose–phosphodiester backbone only, and is not defined by the arrangement of the bases. In the case of dinucleoside monophosphate, it contains only one unit (or one link) of the furanose–phosphodiester backbone; therefore, it does not contain sufficient information to define the handedness of the helical turn based solely on the configuration of the backbone. Thus, in this situation, the arrangement of the bases is used for the definition of the handedness of the screw axis. The plane of the rotation is the same as the plane of the bases, and the angle in rotation is the one formed between the geometrical principal axis of the two bases in a homo dimer. If the axis of the stack is advancing upward from the plane of the bases and is simultaneously rotating counterclockwise in following the direction of the backbone, then the stack is termed as right-handed. If the conformation of the dimer is extended to the trimer, oligomer, and polymer in a repetitive fashion, the turn of the sugar–phosphate backbone should follow the turn of the bases; therefore, the definition given by the arrangement of the bases is the same as that given by the backbone.

The conformation of A^spA^s depicted in Figure 4 is a left-handed stack, in which the 5′-nucleosidyl unit is rotating upward in a clockwise fashion. This conformational model is supported by all the uv, CD, and pmr data: a large percentage of hypochromicity and a large hypsochromic shift from monomer to dimer, a large amplitude with a negative splitting pattern in CD spectrum, a large difference in shielding of H-2 and H-1′ located in the A^sp residue *vs.* the same protons located in the pA^s residue in the pmr spectrum. Extension of the dimer model in Figure 4 to a polymer in a repetitive fashion will lead to the formation of a left-handed helix with bases located inside and the phosphate group outside the helix. This difference of handedness between the conformation of A^spA^s and that of ApA is attributable to the difference of ϕ_{cn} which is $-108°$ for the cyclonucleoside and about $-10°$ [4] for the adenosine in crystal form. In order to maintain a maximal base–base overlap and a minimal stereochemical hindrance in the backbone, the 5′-nucleosidyl unit rotates upward in a clockwise fashion instead of a counterclockwise fashion in A^spA^s. Examination of the Corey–Pauling–Koltun models reveals that the change of the configuration of the 3′-OH–phosphate–5′-OH linkage for the stack of ApA to the stack of A^spA^s is rather small. The major difference is that the rotation of the 5′-nucleotidyl unit is changed from a counterclockwise fashion to a clockwise fashion in order to accommodate the change of the ϕ_{cn} angle and to maintain maximal base–base overlap.

The exact reason for the thermal stability of the conformation of A^spA^s is still open to conjecture. The absence of the oscillation around the torsional angle could be one reason. The second covalent linkage between the base and the furanose in a cyclonucleoside removes a degree of freedom and flexibility. Therefore, from the standpoint of entropy consideration—and perhaps even enthalpy consideration—the conformation of A^spA^s may be more stable than that of ApA.

Recently we have synthesized the oligomers of 8,2′-*S*-cycloadenosine, which revealed essentially the same properties as those of A^spA^s. This will be reported elsewhere. Also, in order to learn the effect of the sulfur atom, the synthesis of the dimer of 8,2′-*O*-cycloadenosine is now in progress.

Experimental Section

General Procedures. Ultraviolet absorption spectra were recorded on a Hitachi EPS-3T recording spectrophotometer. In melting curve experiments, measurements were made either by a Shimadzu AQV-50 digital spectrophotometer equipped with a thermojacketed cell, or by a Cary 15 uv spectrophotmeter equipped with a thermal-controlled cell compartment. Circular dichroism spectra were recorded on a JASCO ORD-UV-5 spectropolarimeter equipped with a thermojacketed cell. Measurements were made in 10-mm cells at a concentration of 1–2 OD/ml at λ_{max}. The instrument was calibrated with an aqueous solution of *d*-10-camphorsulfonic acid. The molecular ellipticity [θ] and molar extinction coefficient (ϵ) are presented in terms of the per residue value.

Proton magnetic resonance spectra were recorded on a Varian HA-100 spectrometer equipped with a Varian C-1024 computer of average transients. Temperature readings were calibrated with ethylene glycol and methanol standards from Varian. Thin-layer chromatography was performed on a silica gel plate with Merck Kieselgel HF254. Paper chromatography was performed on Toyo filter paper No. 51A using the following solvent systems: solvent A, isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2); solvent B, *n*-butyl alcohol–acetic acid–water (5:2:3); solvent C, *n*-propyl alcohol–concentrated ammonium hydroxide–water (55:10:35); solvent D, ethanol–1 *M* ammonium acetate (7:3). Paper electrophoresis was performed for 1 hr with a voltage gradient of 35 V/cm on Toyo filter paper No. 51A with triethylammonium bicarbonate buffer (0.05 *M*, pH 7.5). The results are presented as relative mobility to pA and A, where those of pA and A are 1.0 and 0, respectively.

Spleen and venom phosphodiesterase were purchased from Worthington Biochemical Corp.

Synthetic Procedures. N^6,$O^{3'}$-Dibenzoyl-8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine (7). Benzoic anhydride (300 mg) was added to 100 mg (0.19 mmol) of 5′-*O*-trityl-8,2′-anhydro-9-(β-D-arabinofuranosyl)adenine[4] (**2**) dissolved in pyridine (6 ml), and the resulting solution was heated at 80–85° for 35 hr. After cooling to room temperature, the reaction mixture was poured dropwise into ice-cooled aqueous sodium bicarbonate solution (0.4 g of $NaHCO_3$/40 ml of solution) with stirring. The aqueous layer was extracted with three 15-ml portions of chloroform and the combined chloroform solutions were dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the filtrate was evaporated to dryness. The resulting white residue was dissolved in 80% aqueous acetic acid (30 ml) and after heating at 100° for 13 min, the solvent was removed *in vacuo* at 30° to yield 167 mg of pale yellow powder. After drying over phosphorus pentoxide at room temperature, this pale yellow powder was refluxed with anhydrous ether for 15 min and the ether decanted from the solid material. This procedure was repeated three times and, after drying the remaining residue, there was obtained 68 mg (74% yield) of the desired product **7**. This product gave a single spot on thin-layer chromatography and gave a negative result in the color test for trityl group: uv $\lambda_{max}^{H^+}$ 301, 237 nm; $\lambda_{max}^{70\%\ EtOH}$ 301, 237 nm; $\lambda_{max}^{OH^-}$ 315 nm. The R_f values of **7** and **2** on thin-layer chromatography (silica gel) were 0.44 and 0.13, respectively, in $CHCl_3$–EtOH (37:3, v/v).

5′-*O*-Trityl-N^6-dimethylaminomethylene-8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine (3). 5′-*O*-Trityl-8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine (**2**) (168 mg, 0.32 mmol) and dimethylformamide dimethy lacetal[5] (0.18 ml) were dissolved in dimethylformamide (3 ml) and the resulting solution was left under anhydrous conditions for 23 hr at room temperature. The reaction mixture was then poured dropwise into ice–water with stirring. After an additional 3 hr of stirring, the solid which precipitated was removed by filtration and dried over phosphorus pentoxide to give a quantitative yield (185 mg) of the product **3**: mp 144–145°; uv $\lambda_{max}^{H^+}$ 305, 343 nm; $\lambda_{max}^{H_2O}$ 251 nm; $\lambda_{max}^{OH^-}$ 320, 251 nm. R_f values of **3** and **2** on thin-layer chromatography

(silica gel) were 0.43 and 0.34, respectively, in $CHCl_3$–EtOH (7:1, v/v).

5′-*O*-Trityl-N^6-dimethylaminomethylene-9-(β-D-arabinofuranosyl)-adenine 3′-Monophosphate (5). A mixture of 5′-*O*-trityl-N^6-dimethylaminomethylene-9-(β-D-arabinofuranosyl)adenine (**3**) (190 mg, 0.33 mmol) and 2-cyanoethyl phosphate (pyridinium salt, 3 equiv) was rendered anhydrous by repeated evaporation with anhydrous pyridine. This anhydrous mixture was then dissolved in 10 ml of dry pyridine and 1.2 g of DCC was added. After standing at 45° for 18 hr, pyridine–water (3:1, 30 ml) was added and the solution filtered to remove dicyclohexylurea. The filtrate was extracted with three 40-ml portions of *n*-hexane. The aqueous pyridine layer was kept at room temperature for 25 hr and then evaporated to dryness *in vacuo*. Methanol–concentrated ammonia (1:1, 25 ml) was added to the residue, and the resulting solution was kept at 45° for 10 hr and then evaporated *in vacuo* at room temperature, and water (30 ml) was added. The precipitated solid was collected by centrifugation, dried over phosphorus pentoxide at room temperature for 10 hr, and dissolved in dimethylformamide (2 ml). After addition of dimethylformamide dimethyl acetal (0.2 ml), the solution was kept at 25° for 20 hr and evaporated *in vacuo* at room temperature. The residue was dissolved in 50% aqueous pyridine and the solution was passed through a column of Dowex 50-X2 (pyridinium form) resin. The column was washed with 50% aqueous pyridine and the eluate was concentrated to 2 ml volume to which ether (50 ml) was added. The precipitated product **5** was dried over phosphorus pentoxide under reduced pressure: uv $\lambda_{max}^{H^+}$ 305, 343 nm; $\lambda_{max}^{H_2O}$ 319, 241 nm; $\lambda_{max}^{OH^-}$ 319, 241 nm. The paper electrophoretic mobility was 0.83 relative to pA and A.

8,2′-Anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine-3′-phosphoryl-(3′–5′)-8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)-adenine (8 or A^spA^s). A mixture of compound **5** (pyridinium salt, 1800 OD at 319 mμ, *ca.* 0.09 mmol) and compound **7** (60 mg, *ca.* 0.1 mmol) and Dowex 50-X2 (pyridinium form) resin (0.5 ml) was rendered anhydrous by repeated evaporation with anhydrous pyridine and finally pyridine (2 ml) and DCC (246 mg, 1.2 mmol) were added. After standing at 20° for 11 days, 50% aqueous pyridine was added to the reaction mixture. The precipitated solid was removed by filtration and the filtrate was extracted with three 20-ml portions of *n*-hexane. The aqueous pyridine layer was kept at 20° for 36 hr and then evaporated. The residue was dissolved in methanol saturated with ammonia at 0° (30 ml), kept at 30–33° for 33 hr, and then evaporated. The residue was dissolved in 80% aqueous acetic acid (30 ml), heated for 12 min at 100°, and then evaporated *in vacuo*. The residue was dissolved in water and applied to the top of a DEAE-cellulose (bicarbonate form) column (36 cm × 2 cm). After washing with water, elution was carried out using a linear salt gradient (1.5 l. of 0.001 *M* triethylammonium bicarbonate buffer, pH 7.5, in the mixing vessel and an equal volume of 0.08 *M* solution of the same buffer in the reservoir). The fractions corresponding to the major uv absorbing peak (fractions 61–77, 16 ml each) were collected, concentrated to a small volume, and desalted by repeated addition and evaporation of water. The final aqueous solution was then applied to a Dowex 1-X2 (formate form) resin column (20 cm × 1 cm) and after washing with water elution was carried out using 0.025 *N* formic acid. The fractions corresponding to the major uv absorbing peak (fractions 55–100, 16 ml each) were collected, evaporated at room temperature under vacuum to approximately 50-ml volume, and lyophilized to afford 38 mg (*ca.* 70% yield) of the desired product **8**: uv λ_{max}^{pH1} 276.5 nm (ϵ 4.2 × 10^4),[22] λ_{max}^{pH7} 271 nm (ϵ 3.8 × 10^4), λ_{max}^{pH13} 271.5 nm (ϵ 3.58 × 10^4). The R_f values of **8** on paper chromatograms were as follows: solvent A (0.23, pA 0.12), solvent B (0.27, pA 0.24), solvent C (0.47, pA 0.45), solvent D (0.27, pA 0.24). The paper electrophoretic mobility was 0.30 relative to pA and A. Pmr data are shown in Table I and CD data are shown in Figures 1 and 3.

2′-Deoxyadenylyl-(3′–5′)-2′-deoxyadenosine (9). The dinucleoside monophosphate **8** (1 mg, 1.6 μmol) was suspended in ethanol (0.5 ml) and methyl Cellosolve (0.5 ml), and a small amount of Sponge nickel was added and heated at 100° for 7 hr. The mixture was cooled and then centrifuged. The supernatant and washings were chromatographed on paper in solvent D to isolate the product, which was identical with dApdA synthesized by known methods[27] on paper chromatography and paper electrophoresis: uv $\lambda_{max}^{H^+}$ 257.5 nm, $\lambda_{max}^{H_2O}$ 258 nm, $\lambda_{max}^{OH^-}$ 258 nm. The R_f values on paper chromatograms were as follows: solvent B (0.37, authentic dApdA 0.37), solvent D (0.40, authentic dApdA 0.41).

Enzymatic Studies. A. Snake Venom Phosphodiesterase. (1) Purified Enzyme. A solution (200 μl) containing substrate **8** (10 OD at λ_{max}), 1 *M* ammonium carbonate (40 μl) and enzyme (1 mg/ml, 40 μl) was incubated at 37° for 14 hr. Examination of the incubated solution by paper electrophoresis revealed that **8** was not hydrolyzed. Under similar conditions, thymidylyl-(3′–5′)-thymidine (TpT) was hydrolyzed to an extent of 95% with 20 μl of enzyme solution in 2 hr.

(2) Crude Snake Venom. A solution (230 μl) containing substrate **8** (5 OD at λ_{max}), 1 *M* ammonium carbonate (40 μl) and crude snake venom (20 mg/ml, 100 μl) was incubated at 37° and examined by paper electrophoresis. Hydrolysis of **8** occurred to an extent of 6.4 and 17.6% in 5 and 18 hr, respectively, to give A^s due to the presence of 5′-nucleotidase in the crude snake venom preparation. Under similar conditions TpT was hydrolyzed to an extent of 97% with 10 μl of venom solution in 25 min.

B. Spleen Phosphodiesterase. A solution (200 μl) containing substrate **8** (10 OD at λ_{max}), 1 *M* ammonium acette buffer pH 5.7 (40 μl), and enzyme (20 units/ml, 40 μl) was incubated at 37° for 14 hr. Paper electrophoretic examination of the incubated solution revealed that **8** was completely resistant to hydrolysis in the presence of spleen phosphodiesterase. Under similar conditions, TpT was hydrolyzed completely with 20 μl of enzyme solution in 2 hr.

(27) S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Amer. Chem. Soc.*, **87**, 2988 (1965).