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Polynucleotides. LIII.[1] Synthesis and Properties of 2′-Azido-2′-deoxyadenylyl-(3′-5′)-2′-azido-2′-deoxyadenosine

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A dinucleoside monophosphate, 2′-azido-2′-deoxyadenylyl-(3′-5′)-2′-azido-2′-deoxyadenosine (II) was synthesized by condensation of the 5′-monomethoxytrityl (IV) and N^6,3′-O-dibenzoyl-5′-phosphoryl derivatives (III) of 2′-azido-2′-deoxyadenosine using DCC as a condensing reagent.

The ultraviolet absorption properties of compound II were similar to those of ApA. The circular dichroism (CD) spectrum of II was also similar to that of ApA in pattern, but the magnitudes of the CD bands were about a half of those of ApA. ^{1}H nuclear magnetic resonance signals appeared in the expected position for a dimer, and the dimerization shifts were similar to those of ApA. However, the 3′-linked residue of compound II showed a $J_{1'-2'}$ value significantly higher than that of ApA. These results suggest that compound II takes an *anti-anti*, right-handed stacked conformation similar to that of ApA, but with decreased stability or with a significantly different stacking geometry.

Keywords——dinucleoside monophosphate; ApA analog; UV; NMR; CD; right-handed stacking

We are interested in the conformational differences between ribo- and deoxyribonucleic acids, which differ chemically only in the presence or absence of 2′-OH groups. We have previously reported the synthesis of adenosine analogs having *e.g.* azido (I),[3] fluoro,[4] chloro,[3,4] bromo[4] and iodo[4] substituents in the 2′-position and a *ribo*-configuration. ^{1}H nuclear magnetic resonance (NMR) measurements on the 2′-azido analog (I) showed that the coupling constant $J_{1'-2'}$ was 5.9 Hz, which is similar to that of adenosine (6.0 Hz). The circular dichroism (CD) spectrum of compound I was also similar to that of adenosine. These findings suggested that the conformations of these two nucleosides might be very similar. However, when we synthesized a polynucleotide, poly(2′-azido-2′-deoxyadenylic acid[5] [poly(Az)] from the monomer 2′-azido-2′-deoxyadenosine 5′-diphosphate using polynucleotide phosphorylase, the optical properties of this polymer were somewhat different from those of poly(adenylic acid). For example, the hypochromicity was larger, the molecular ellipticity in CD was smaller and the Tm of the complex with poly(uridylic acid) was higher in the case of poly(Az) than poly(A). We, therefore, attempted to synthesize a dinucleoside monophosphate, 2′-azido-2′-deoxyadenylyl-(3′-5′)-2′-azido-2′-deoxyadenosine (AzpAz) (II), since this compound represents the shortest polynucleotide sequence of poly(Az).

For the synthesis of compound II, we adopted the scheme illustrated in Chart 1. Starting from compound I, 5′-phosphorylation with $POCl_3$[6] and subsequent benzoylation gave N^6,3′-O-dibenzoyl-2′-azido-2′-deoxyadenosine 5′-phosphate (III) in an overall yield of 77%. From compound I, we also obtained the 5′-monomethoxytrityl derivative (IV) by the standard procedure in a yield of 81%. DCC was used to condense compounds III and IV; the reactants were kept under strictly anhydrous conditions at room temperature for 7 days. After the

1) Part LII of this series: M. Ikehara, T. Fukui, and N. Kakiuchi, *Nucleic Acids Res.*, **5**, 1877 (1978).
2) Location: *133-1, Yamadakami, Suita, Osaka 565, Japan.*
3) M. Ikehara, T. Maruyama, and H. Miki, *Tetrahedron Lett.*, **1976**, 4485; *idem*, *Tetrahedron*, **34**, 1133 (1978).
4) M. Ikehara and H. Miki, *Chem. Pharm. Bull.* (Tokyo), **26**, 2449 (1978).
5) M. Ikehara, T. Fukui, and N. Kakiuchi, *Nucleic Acids Res.*, **3**, 2089 (1976).
6) M. Yoshikawa, T. Kato, and T. Takenishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505 (1969).

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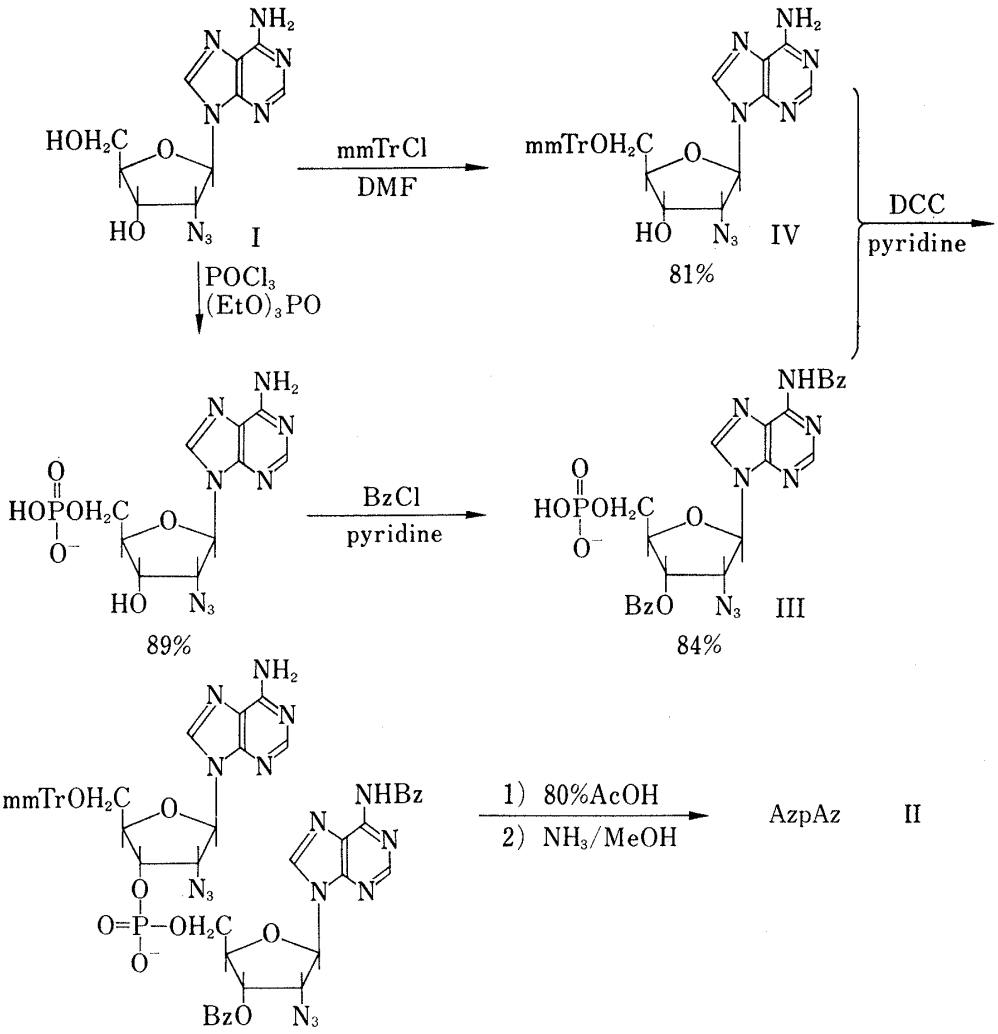

Chart 1

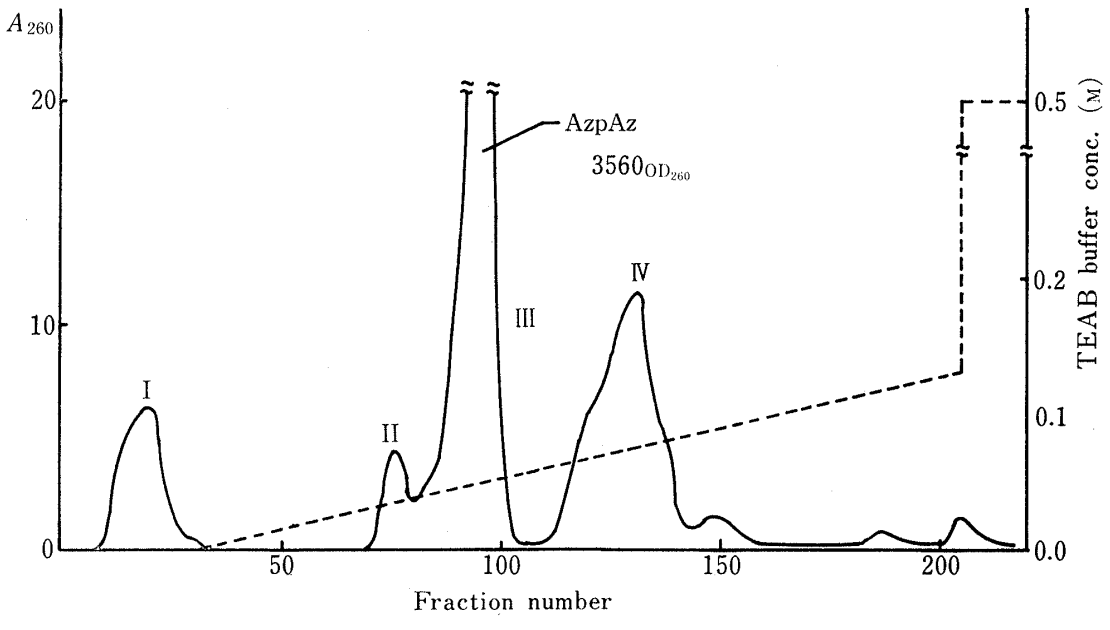

Fig. 1. DE23 Cellulose Column Chromatography (Bicarbonate)

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usual work-up, the product was purified by chromatography on a column of DEAE-cellulose. The elution pattern is shown in Fig. 1. The dimer (II) was obtained in peak III, which contained 3,506 A_{258} units of the desired product in a yield of 19%. A pyrophosphate intermediate was obtained from peak IV and it was recovered as pAz after decomposition by treatment with acetic anhydride and pyridine, in a yield of 17%. The reason for this

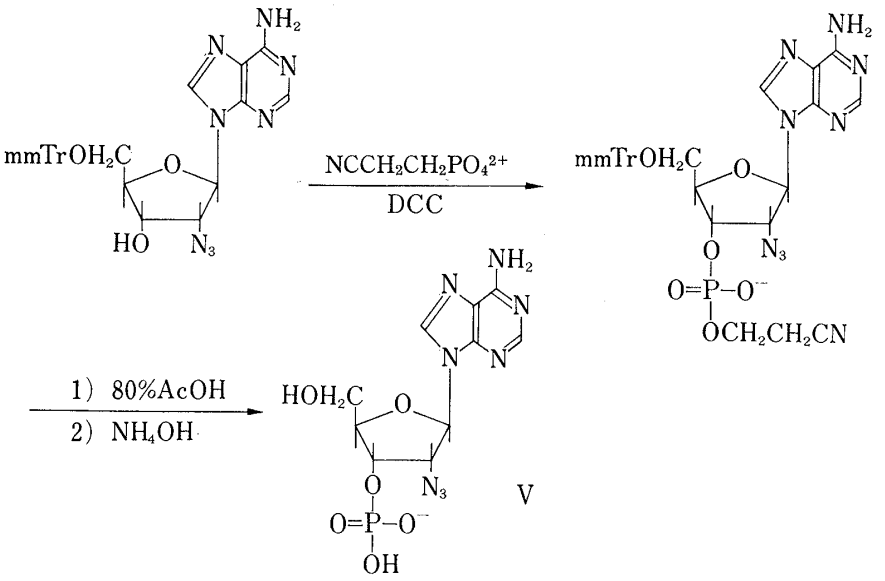

Chart 2

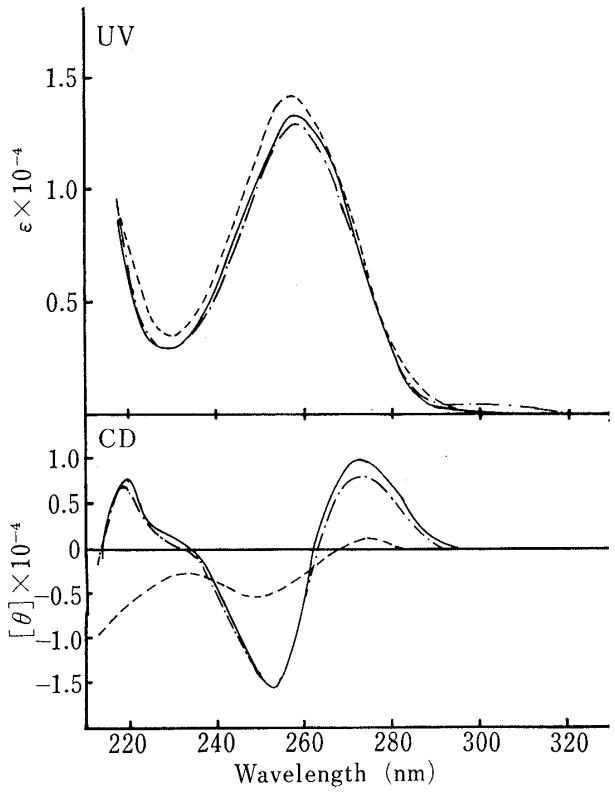

Fig. 2

———, pH 7; – – – –, pH 2; —·—·—, pH 12.

relatively low yield in condensation might be steric interaction between the azido group in the position-2′ and the 3′-OH.

2′-Azido-2′-deoxyadenosine 3′-phosphate (V), which is required for NMR signal assignment, was synthesized by phosphorylation of IV using β-cyanoethyl phosphate and DCC followed by removal of protecting groups.

The ultraviolet (UV) absorption properties of the dimer (II) are very similar to those of ApA,[7] having a maximum at 258 nm at neutrality and an ε value of 26,500 at pH 7 (Fig. 2). This corresponds to a hypochromicity of 12%, which is the same as that of ApA. These observations suggest that compound II has an ordered structure stabilized by stacking of adenine bases, similar to that of ApA. Under acidic conditions the hypochromicity was reduced to only 7%, presumably due to destruction of the ordered structure. The CD spectrum of AzpAz (II) is shown in Fig. 2. At pH 7 and 12, compound II showed characteristic curves, with two

7) M.M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, 13, 54 (1965).

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peaks at 273 nm and 220 nm, a shoulder at 235 nm, and a trough at 252 nm. When the pH was brought to 2, a simple curve having two maxima at 275 and 234 nm and a minimum at 245 nm was observed. These observations suggest the presence of a stacked ordered form for II at neutrality as well as at pH 12, but an unstacked protonated form at pH 2. Though the CD pattern at pH 7 is very similar to that of ApA, the magnitudes of $[\theta]$ values of these spectra are about a half of those reported for ApA.[7]

Table I. PMR Data

Compounds	Conc. (M)	pD	Chemical shift H-8	Chemical shift H-2	Chemical shift H-1	$J_{H_1'-H_2'}$
3′-AzMP	0.1	5.5	8.75	8.57	6.55	6.4
3′-AMP	0.1	5.4	8.66	8.44	6.43	6.0
5′-AzMP	0.1	5.5	8.88	8.61	6.53	5.0
5′-AMP	0.1	5.4	8.78	8.47	6.44	5.5
AzpAz Azp-	0.1	7.5	8.70	8.38	6.33	6.0
-pAz			8.57	8.49	6.38	4.2
ApA Ap-	0.1	7.5	8.64	8.27	6.23	4.0
-pA			8.53	8.39	6.35	4.6
$\Delta\delta$ AzpAz Azp-			0.05	0.19	0.22	
-pAz			0.31	0.12	0.15	
ApA Ap-			0.02	0.17	0.20	
-pA			0.25	0.08	0.09	

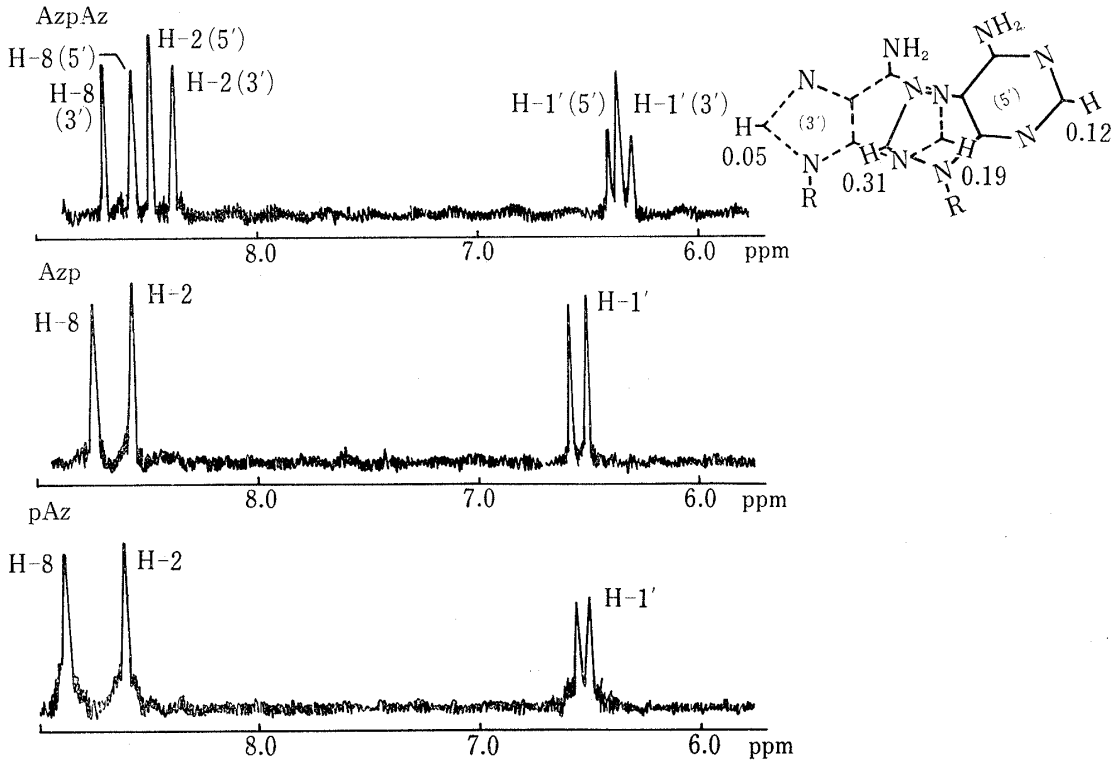

Fig. 3

^{1}H NMR data for compound II and its component nucleotides are summarized in Fig. 3 and Table I, together with relevant data for adenosine phosphates. Assignment of the proton signals were made by analogy with the published data for adenine nucleotides and ApA.[8]

8) N.S. Kondo, H.M. Holmes, L.M. Stempel, and P.O.P. Ts'o, *Biochemistry*, **9**, 3479 (1970).

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Two H-8 signals of AzpAz were assigned by the deuterium exchange method (90° for 30 min at pD 7.4).[9] In the mononucleotides, the chemical shift values of H-8, H-2 and H-1′, protons and coupling constants, $J_{1'-2'}$ are similar to those of adenosine 3′- and 5′-phosphates. AzpAz shows a dimerization shift, $\Delta\delta$,[8] similar to that of ApA on each proton. Therefore, AzpAz may also take an *anti-anti* right-handed stacking conformation, as assumed for ApA (Fig. 3). It should be noted that $J_{1'-2'}$ of the Azp residue in AzpAz is only slightly smaller than that of monomer Azp, whereas $J_{1'-2'}$ is reduced by 2 Hz in the Ap residue in ApA with respect to that in a stacked *ribo*-dimer. Both residues take a 3′-*endo* sugar puckering conformation in preference to a 2′-*endo* conformation and, therefore, show smaller $J_{1'-2'}$ values than the component monomers.[8]

In the case of the 3′-linked residue, a 3′-*endo* conformation reduces steric repulsion between 2′-OH and the 5′-linked residue. In the case of AzpAz, the high $J_{1'-2'}$ value of the Azp residue suggests a relatively high content of 2′-*endo* conformation, which is not favorable for stacking. There could be some interaction between the 2′-azido group and 3′-phosphate group which inhibits the Azp residue from adopting a more 3′-*endo* conformation.

From these optical and NMR data, it may be concluded that AzpAz (II) takes an *anti-anti*, right-handed stacked conformation similar to that of ApA but with decreased stability or with a significantly different stacking geometry. Poly(Az) shows a CD spectrum similar to that of ApA in pattern but with much smaller magnitude (about two-thirds).[5] Therefore, AzpAz makes a good model for poly(Az), as ApA does for poly(A).

Experimental[10]

2′-Azido-2′-deoxyadenosine 5′-Phosphate——Phosphoryl chloride (0.19 ml, 2 mmol) was added to triethyl phosphate (5 ml) and the solution was cooled to 0°. 2′-Azido-2′-deoxyadenosine (292 mg, 1 mmol) was added to the solution and the mixture was stirred at 0°. Paper electrophoresis after 2.5 hr showed completion of the reaction. The mixture was poured into ice-water (*ca.* 300 ml) and applied to a column (1.7×9 cm) of charcoal. After washing with water (2 l), the column was eluted with 50% EtOH (500 ml) containing 2% conc. NH_4OH. The nucleotide was obtained in a yield of 13730 A_{259} units (91% on the basis of 15100). The solvent was evaporated off, then the residue was dissolved in water (40 ml) and applied to a column (1.0×13 cm) of Dowex 1×8 (formate form, 10 ml). Fractions of 280 drops were collected. Fractions No. 22—43 were pooled and evaporated. pAz was obtained in a yield of 13460 A_{258} units (89%). Az (430 A_{258} units, 3%) was recovered from fractions No. 1—10. PEP: *Rf*(A) 0.21, *Rf*(B) 0.31, *Rf*(C) 0.52, *Rf*(D) 0.53. PEP: R_{pA-A} 0.97. (P): 15100. Digestion of this sample with snake venom 5′-nucleotidase gave Az and inorganic phosphate as confirmed by PEP and PPC.

N⁶,3′-O-Dibenzoyl-2′-azido-2′-deoxyadenosine 5′-Phosphate (III)——2′-Azido-2′-deoxyadenosine 5′-phosphate (0.89 mmol) obtained as described above was rendered anhydrous by adding pyridine then removing it by evaporation, and was then dissolved in pyridine (15 ml). Benzoyl chloride (2.3 ml) was added to the solution at 0° and the mixture was kept at room temperature for 1 hr. The mixture was added dropwise to ice-water (50 ml) and the nucleotide was extracted with $CHCl_3$ (50 ml). After washing with water, chloroform was removed by evaporation and the residue was rendered anhydrous by adding pyridine and evaporating it off. The residue was removed by evaporation *in vacuo*, remaining traces of acetic acid were evaporated off with water, and the residue was treated with 50% aqueous pyridine (15 ml) at room temperature for 2 hr. The solvent was evaporated off and the residue was rendered anhydrous by adding pyridine and evaporating it off several times. The residue was dissolved in a small amount of anhydrous pyridine and precipitated in ether (*ca.* 250 ml). N⁶,3′-O-Dibenzoyl-pAz was obtained in a yield of 495 mg (0.75 mmol, 17000 A_{280} units, 84%). PPC: *Rf*(D) 0.83. PEP: R_{A-pA} 0.48.

9) P.O.P. Ts'o, N.S. Kondo, M.P. Schweizer, and D.P. Hollis, *Biochemistry*, **8**, 997 (1969).

10) UV absorption spectra were taken with a Hitachi EPS-3T or 124 spectrophotometer. CD spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. NMR spectra were taken with a Hitachi R-22 spectrometer operated at 90 MHz using tetramethylsilane as an external standard. Paper chromatography (PPC) was performed on Toyo filter paper No. 51A in the following solvent systems: A, isopropanol–conc. NH_4OH–water (7:1:2); B, ethanol–1 M NH_4OAc (7:3); C, *n*-butanol–acetic acid–water (5:2:3); D, *n*-butanol–conc. NH_4OH–water (6:1:3). Paper electrophoresis (PEP) was performed on Toyo filter paper No. 51A in a 0.05 M triethylammonium bicarbonate buffer (pH 7.5) at 900 V/40 cm. m.p.'s were measured on a Yanagimoto hot plate and are uncorrected.

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5′-O-Monomethoxytrityl-2′-azido-2′-deoxyadenosine (IV)——2′-Azido-2′-deoxyadenosine (293 mg, 1 mmol) was rendered anhydrous by adding pyridine then removing it by evaporation, and dissolved in DMF (5 ml). Monomethoxytrityl chloride (377 mg, 1.2 equiv.) was added with stirring. The solution was kept at room temperature for 2 days in the dark. After checking completion of the reaction by TLC ($CHCl_3$–EtOH, 8: 1), the mixture was poured into ice-water (25 ml) containing 2% conc. NH_4OH. Precipitates were collected by filtration, washed with water, and dissolved in $CHCl_3$ (30 ml). The $CHCl_3$ solution was washed with water (20 ml), and the solvent was evaporated off *in vacuo*. The residue was rendered anhydrous by adding pyridine then removing it by evaporation. It was then dissolved in AcOEt (3 ml) and precipitated in *n*-hexane (*ca.* 200 ml). Yield was 456 mg (0.81 mmol, 81%). *Anal.* Calcd. for $C_{30}H_{28}N_8O_4$: C, 63.58; H, 5.34; N, 19.78. Found: C, 63.24; H, 5.16; N, 19.43.

2′-Azido-2′-deoxyadenylyl-(3′-5′)-2′-azido-2′-deoxyadenosine (II)——5′-O-Monomethoxytrityl-Az (10600 A_{262} units, 0.62 mmol) and dibenzoyl-pAz (17000 A_{280}, 0.77 mmol, 1.2 equiv.) were dissolved in 50% aqueous pyridine (*ca.* 40 ml) and passed through a column (1.0×5.9 cm) of Dowex 50×2 (pyridinium form, 46 ml). After elution with 50% aqueous pyridine (*ca.* 25 ml), the eluent was evaporated and Dowex 50×2 (pyridinium form, *ca.* 0.6 ml) was added. The mixture was rendered anhydrous by adding pyridine then removing it by evaporation several times. The residue was dissolved in pyridine (3 ml) and DCC (1.53 g, 7.7 mmol) was added. The reaction mixture was kept at room temperature with exclusion of moisture for 7 days in the dark. The reaction was quenched by addition of 50% aqueous pyridine (30 ml) and pyridine (15 ml). The solution was kept at room temperature overnight. Dicyclohexylurea was removed by filtration, the filtrate was evaporated *in vacuo*, and traces of pyridine were removed by coevaporation with added toluene. The residue was dissolved in AcOH (52 ml) followed by addition of water (8 ml) and kept at room temperature for 2 days. The solid material was filtered off and the filtrate was evaporated *in vacuo*. The residue was treated successively with water and pyridine, which were each removed by evaporation. After addition of 9 N methanolic ammonia, the solution was kept at 30° for 24 hr. The solvent was evaporated off, then the residue was dissolved in pyridine and water, and extracted with $CHCl_3$ and ether. The aqueous solution was evaporated and the residue was dissolved in water (*ca.* 150 ml). The solution was then applied to a column (1.5×39 cm) of DE-23 (99 ml). After washing with water, the column was eluted with a linear gradient formed from water (2 l) and 0.2 M triethylammonium bicarbonate buffer (2 l). Fractions of 15 ml were collected. The elution pattern is shown in Fig. 2. By PCP and PEP, it was found that peak III contained the desired product, AzpAz (II). Fractions 89—99 were pooled and evaporated. The yield was 3560 A_{258} units (0.12 mmol, 19%). PPC: *Rf*(A) 0.57, *Rf*(B) 0.53, *Rf*(C) 0.60, *Rf*(D) 0.79. PEP: R_{pA-A} 0.27. UV: $\lambda_{max}^{pH\,7}$ 258 nm (ε 26500), $\lambda_{max}^{pH\,2}$ 257 nm (ε 28200), $\lambda_{max}^{pH\,12}$ 258 nm (ε 25800). CD and NMR data are discussed in the text (Fig. 1 and Table I).

pAz was obtained from the fraction corresponding to peak IV (No. 114—140) after acetic anhydride–pyridine treatment and Dowex 1×2 (formate form) column chromatography.

Enzymic Digestion of AzpAz——A reaction mixture (55 μl) containing AzpAz ($3A_{259}$ units), 0.05 M triethylammonium bicarbonate buffer (pH 7.5, 50 μl) and snake venom phosphodiesterase[11] (5 mg/ml, 5 μl) was incubated at 37° for 5 hr. After quenching the reaction at 100° for 2 min the digest was subjected to PEP. The absorptions corresponding to Az and pAz was measured after extraction of the appropriate spots with water. The ratio of Az: pAz was 1.00: 1.01. The hypochromicity of the incubation mixture was 12% at pH 7.0. The CD curve after digestion changed from that of AzpAz to that of the monomers.

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11) Purchased from Worthington Biochem. Corp.

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