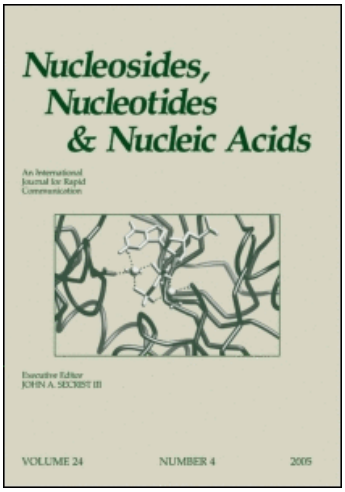

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Synthesis and Properties of Dinucleoside Monophosphates Containing 2-Aminoadenine 8,2'-S- and Uracil 6,2'-O-Cyclonucleosides

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SYNTHESIS AND PROPERTIES OF DINUCLEOSIDE MONOPHOSPHATES CONTAINING 2-AMINOADENINE 8,2'-S- AND URACIL 6,2'-O-CYCLONUCLEOSIDES

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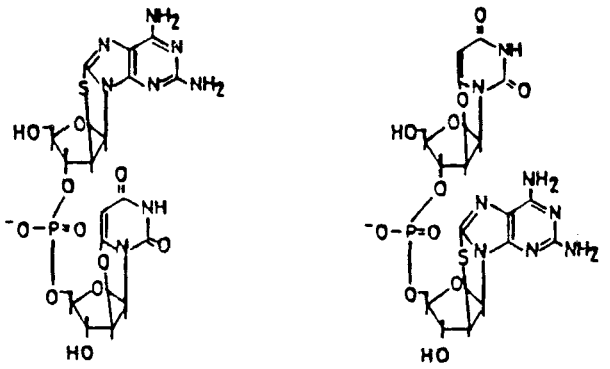
Abstract: Two sequence isomers of dinucleoside monophosphates containing 8,2'-anhydro-2,6-diamino-8-mercapto-9-β-D-arabinofuranosylpurine ($2NH_2A^S$) and 6,2'-anhydro-6-hydroxy-1-β-D-arabinofuranosyluracil (U^O), $2NH_2A^S{}_pU^O$ (**1**) and $U^O{}_p2NH_2A^S$ (**2**) were synthesized by the phosphodiester method. Examination of the UV, CD and NMR spectra of these dimers led us to the conclusion that, whereas compound (**1**) did not take a stacked conformation, compound (**2**) took a well stacked conformation in which the bases were stacked along a left-handed screw axis. Both the dimers formed a complex with ethidium bromide.

It was reported that the oligonucleotides containing cyclonucleosides having a high anti (χ=120°)[1] glycosidic torsion angle have a tendency to take a left-handed helical structure [2,3] in contrast to the right-handed helical structure of natural nucleic acids.

The proton NMR studies on self complementary dinucleoside monophosphates, 8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosyladenylyl-(3'-5')-6,2'-anhydro-6-hydroxy-1-β-D-arabinofuranosyluridine ($A^S{}_pU^O$) and 6,2'-anhydro-6-hydroxy-1-β-D-arabinofuranosyluridylyl-(3'-5')-8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine ($U^O{}_pA^S$) confirmed



$2NH_2A^S{}_pU^O$ (1)

$U^O{}_p2NH_2A^S$ (2)

FIG. 1.

that $U^O{}_pA^S$ takes a stable stacked conformation but $A^S{}_pU^O$ does not [4]. This phenomenon was explained in terms of their tendency to take a left-handed stack and the mode of overlap between the bases in the stack. To investigate further the effects of base sequence on dimer conformation, we attempted to synthesize self complementary dinucleoside monophosphates containing the 8,2'-S-cylonucleoside of 2-aminoadenine (8,2'-anhydro-2,6-diamino-8-mercapto-9-β-D-arabinofuranosylpurine, $2NH_2A^S$)[5] (3) as an analog of adenosine[6] and 6,2'-O-cyclouridine (6,2'-anhydro-6-hydroxy-1-β-D-arabinofuranosyluracil, U^O)[7] (FIG. 1).

Two sequence isomers, $2NH_2A^S{}_pU^O$ (1) and $U^O{}_p2NH_2A^S$ (2), were synthesized by the phosphodiester method with suitably protected nucleoside and nucleotide units. The synthetic routes are shown in FIG. 2. Before the condensation, $2NH_2A^S$ (3) [5] was treated with trimethylchlorosilane in pyridine for protection of the 3'- and 5'- hydroxyl groups in the sugar moiety and then reacted with benzoyl chloride to effect N-benzoylation of two amino groups in the purine base. After treatment with 2 M ammonium hydroxide, the

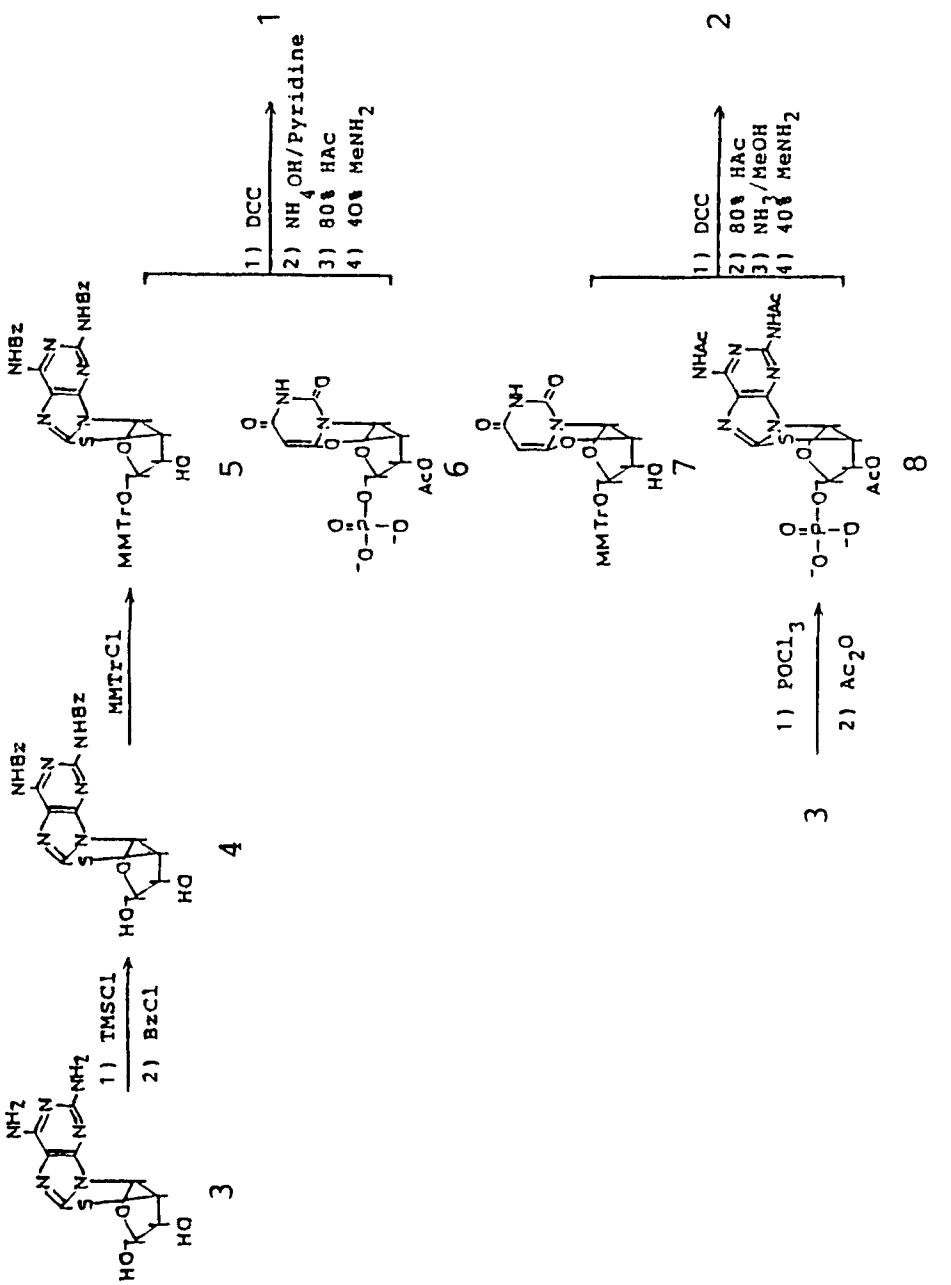


FIG. 2.

desired $\underline{N}^2,\underline{N}^6$-dibenzoyl-$2NH_2A^s$ (**4**) was obtained in a yield of 76 % and then treated with monomethoxytrityl chloride in pyridine-DMSO to give $\underline{N}^2,\underline{N}^6$-dibenzoyl-5'-$\underline{O}$-monomethoxytrityl-$2NH_2A^s$ (**5**) in a yield of 74 %. The condensation of **5** and 3'-$\underline{O}$-acetyl-$_pU^o$ (**6**)[4] was achieved by using dicyclohexylcarbodiimide (DCC) as the condensing reagent at 30° for 72 hr. Deprotection of benzoyl, acetyl and monomethoxytrityl groups was carried out with 28 % aqueous ammonia at 55° for 5 hr and 80 % aqueous acetic acid at 25 ° for 2 hr. Debenzoylation at the $\underline{N}^2$ position was achieved by use of 40% methylamine at 25° for 2 hr. Column chromatography on DE-23 cellulose (bicarbonate form) and further chromatography on Dowex 1X2 (formate form) gave $2NH_2A^s{}_pU^o$ (**1**) in a yield of 28 % (FIG. 3a, 3b). For synthesis of $U^o{}_p2NH_2A^s$ (**2**), $2NH_2A^s$ (**3**) was phosphorylated to $2NH_2A^s$-5'-phosphate ($_p2NH_2A^s$) with phosphorus oxychloride and trimethyl phosphate in a yield of 45 % . The structure was confirmed by ultraviolet (UV) spectra and paper electrophoresis (PEP). Next the 3'-hydroxyl and two amino groups of $_p2NH_2A^s$ were acetylated with acetic anhydride. Condensation of 5'-$\underline{O}$-MMTr-U^o (**7**)[7,8] and $\underline{N}^2,\underline{N}^6$-3'-$\underline{O}$-triAc-$_p2NH_2A^s$ (**8**) to prepare $U^o{}_p2NH_2A^s$ (**2**) was achieved by essentially the same method as described above. The dimer (**2**) was obtained in a yield of 20 % (FIG. 4a, 4b). The structure of these dimers, **1** and **2**, were confirmed by PEP properties, UV, circular dichroism (CD) and NMR spectra as described later. These dinucleoside monophosphates were rather resistant to hydrolysis with crude snake venom phosphodiesterase and hydrolysed to the extent of 3.5 % and 4 % in 14 hr at 37°, under the conditions which caused complete digestion of A_pU and U_pA. The products were identified as the corresponding nucleoside and nucleotide in a 1 : 1 ratio respectively by PEP with authentic samples.

UV Absorption and CD Spectral Properties of the Dimers

The UV and CD spectra of $2NH_2A^s{}_pU^o$ (**1**) and $U^o{}_p2NH_2A^s$ (**2**) are shown in FIG. 5 and 6. The dimer **1**, which consists of $2NH_2A^s$ (λ_{max}^{pH7} 264, 292 nm) and U^o (λ_{max}^{pH7} 251 nm), shows two

FIG. 3. a. Chromatography of $2NH_2A^S{}_pU^O$ (1) on a Column (1.5 x 38 cm) of DE-23 Cellulose (Bicarbonate form)

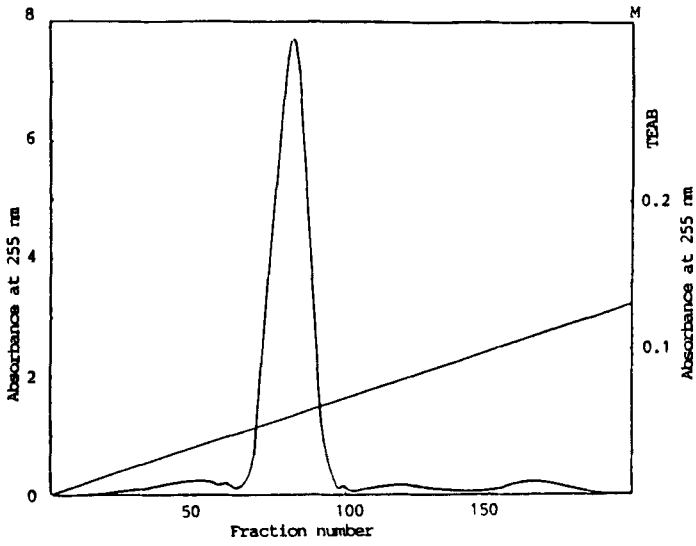


FIG. 3. b. Chromatography of $2NH_2A^S{}_pU^O$ (1) on a Column (1.5 x 54 cm) of Dowex 1x2 (Formate form)

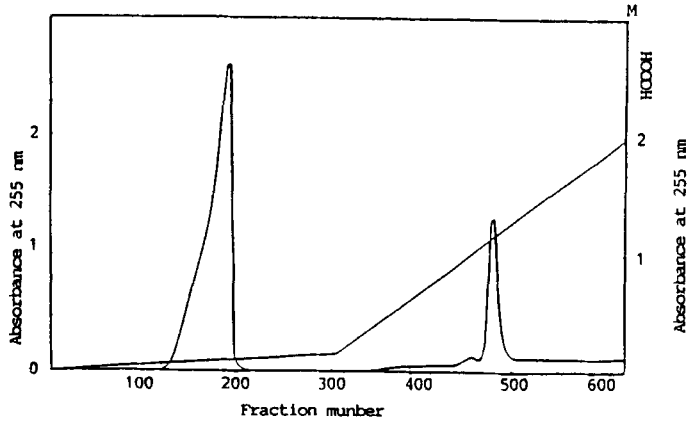


FIG. 4. a. Chromatography of $U^O{}_p2NH_2A^S$ (2) on Column (1.5 x 42 cm) of DE-23 Cellulose (Bicarbonate form)

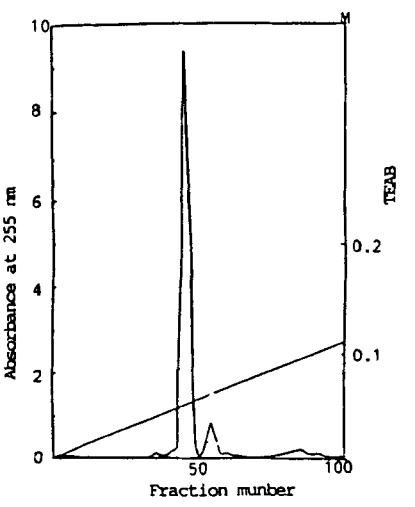
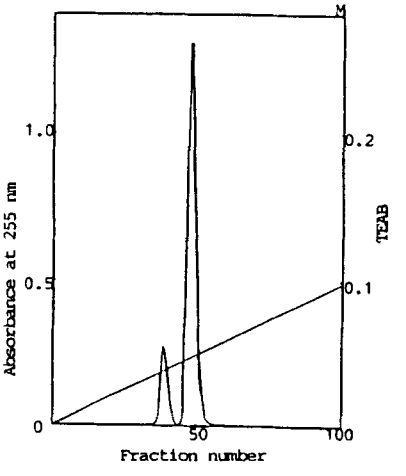


FIG. 4. b. Chromatography of $U^O{}_p2NH_2A^S$ (2) on Column (1.5 x 42 cm) of DE-23 Cellulose (Bicarbonate form)



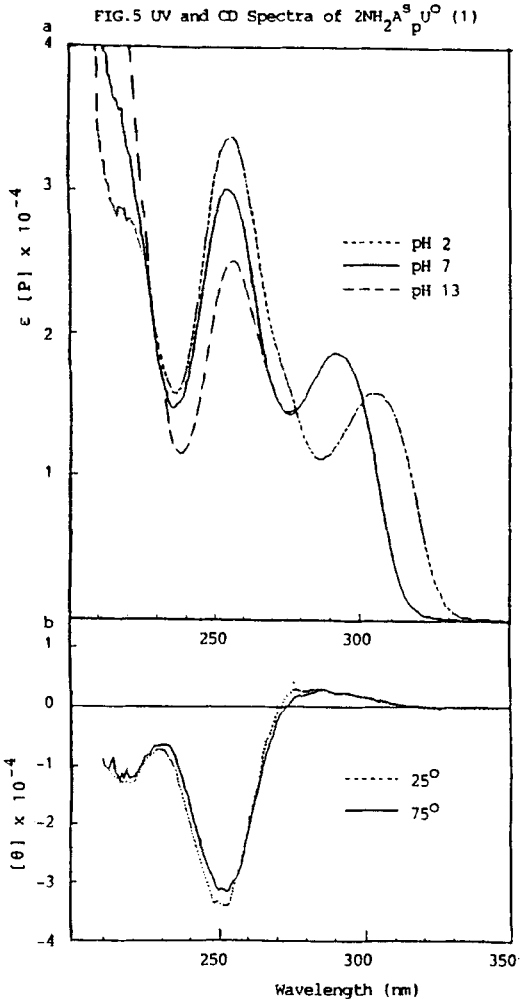


FIG.5 UV and CD Spectra of $2NH_2A^spU^o$ (1)

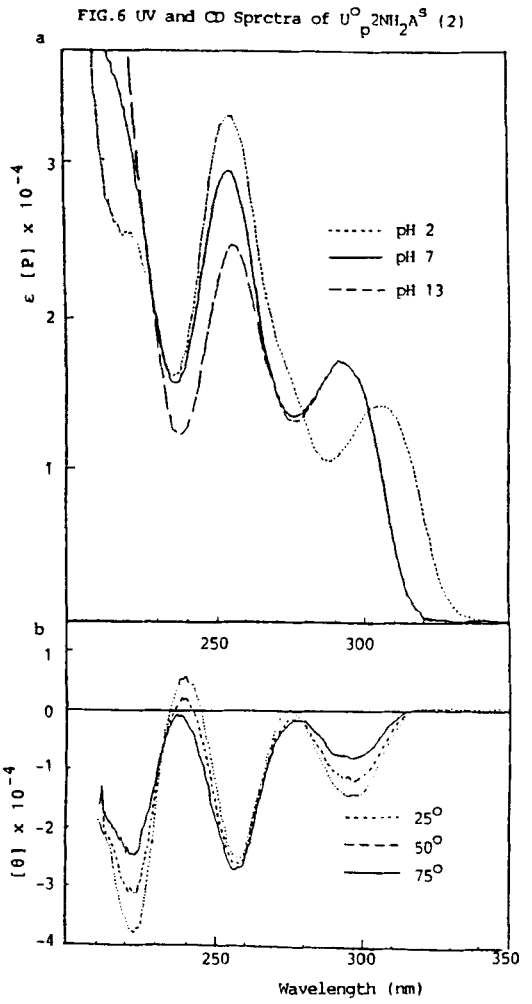


FIG.6 UV and CD Sprctra of $U^op2NH_2A^s$ (2)

maxima at 255 nm and 292 nm at neutrality (FIG. 5a). The ε(P) 256 value obtained by phosphorus analysis of **1** was 30500. The sequence isomer **2** shows a similar UV absorption spectrum (FIG. 6a) with maxima at 256 nm and 292 nm at neutrality. The ε(P)256 value of **2** was 29000.

The CD spectra of $2NH_2A^spU^o$ (**1**) at 25° and 75° are presented in FIG. 5b. The curve has two peaks at 282 nm and 232 nm with [θ] values of $+2.5 \times 10^{-3}$ and -7.5×10^{-3}, respectively, and two troughs at 255 nm and 222 nm with [θ]

values of -3.45×10^{-4} and [θ] -1.35×10^{-4}, respectively. This curve is almost superimposable with the curve obtained from a solution containing $2NH_2A^S$ (3) and $_pU^O$ in a 1 : 1 ratio. This suggests that $2NH_2A^S$ (3) and U^O residues in the $2NH_2A^S{}_pU^O$ (1) molecule do not interact as strongly as in the case of $A^S{}_pA^S$ [9]. The CD spectrum of $U^O{}_p2NH_2A^S$ (2) at 25° is presented in FIG. 6b (dotted line). The curve has three troughs at 297 nm , 259 nm and 224 nm with [θ] values of -1.45×10^{-4}, -2.5×10^{-4} and -3.8×10^{-4}, respectively, and two peaks at 275 nm and 240 nm with [θ] values of 0 and 5×10^{-3}, respectively. This curve is completly different from that of $2NH_2A^S{}_pU^O$ (1). This result suggests that even at 25°, base residues in $U^O{}_p2NH_2A^S$ (2) interact to cause splitting of Cotton bands in a (-)-(+) fashion from the long wavelength region in contrast to the (+)-(-) splitting in the CD spectrum of A_pA. This type of (-)-(+) splitting has previously been assigned to a left-handed stacking conformation in the case of $U^O{}_pA^S$ [4]. Therefore, it is assumed that $U^O{}_p2NH_2A^S$ (2) takes conformation with bases stacked in a left-handed fashion.

PMR Spectra of the Dimers

The PMR spectral data of the dimers are presented in TABLE 1 and 2. The singlet peaks at 5.36 ppm and 5.02 ppm are assigned to the H-5 resonances for U^O in the dimers 1 and 2, respectively. For the dimer 1, the doublet peaks at 6.53 ppm and 5.57 ppm are assigned as the H-1' and H-2' resonances of $_pU^O$ residue. Similarly, for the dimer 2, the doublet peaks at 6.21 ppm and 5.37 ppm are assigned to the H-1' and H-2' resonances of $U^O{}_p$ residue. From CD spectra, the dimer 2 is assumed to take a left-handed stack as $U^O{}_pA^S$[4]. In this conformation, H-5, H-1' and H-2' of the $U^O{}_p$ residue are expected to be shielded by the ring current of the pyrimidine part of the $2NH_2A$. Comparison of the corresponding chemical shifts for the dimer and monomers reveals that this is the case. Thus H-5, H-1' and H-2' of the U^O in $U^O{}_p2AcNHA^S$ shows upfield shifts of 4.91 ppm, 6.10 ppm and 5.15 ppm, respectively, with respect to those for

TABLE 1 ^{1}H-NMR Data for $2NH_2A^s{}_pU^o$ (1) and $U^o{}_p2NH_2A^s$ (2)

Compound		Chemical shift (ppm)									
		5	1'	2'	3'	4'	5'	5"	CH_3	Bz	
$2NH_2A^s{}_pU^o$	$2NH_2A^s$-		6.56	5.12	4.82	4.38	3.80	3.67			
	-U^o	5.36	6.53	5.57	4.76	4.49	4.12	4.02			
$U^o{}_p2NH_2A^s$	U^o-	5.02	6.21	5.37	4.65	4.26	3.52	3.44			
	-$2NH_2A^s$		6.41	4.93	4.84	4.64	4.18	4.13			
$2BzNHA^s{}_pU^o$	$2BzNHA^s$-		6.58	5.05	4.81	4.31	3.73	3.58		9.80 7.59 7.48	
	-U^o	5.21	6.39	5.38	4.64	4.38	3.94	3.81			
$U^o{}_p2AcNHA^s$	U^o-	4.91	6.10	5.15	4.35	4.21	3.45	3.34			
	-$2AcNHA^s$		6.62	5.01	4.94	4.37	4.15	4.01	2.21		
$2NH_2A^s{}_p$			6.53	5.13	4.74	4.35	3.73	3.58			
$_p2NH_2A^s$			6.51	4.96	4.68	4.37	3.86				
$U^o{}_p$		5.24	6.53	5.61	4.75	4.47	3.94	3.62			
$_pU^o$		5.28	6.43	5.44	4.42	4.41	3.79	3.76			

the corresponding monomer. On the other hand, the dimer 1 shows no significant shielding of the protons examined.

Absorption and CD Spectra of the Ethidium bromide-Dimer Complexes

Ethidium bromide is known to bind specifically to double helixes of nucleic acids. It is also known to stabilize base pairing between dinucleoside monophosphates by intercalation [10]. Cyclonucleotide oligomers, which can form a left-handed double helix, show sequence dependence in ethidium bromide binding opposite to that of natural oligo nucleotides which form a right-handed helix[11]. Absorption spectra in the visible region were recorded before and after mixing of ethidium bromide with the dimer solutions (FIG.

TABLE 2 Coupling constants for $2NH_2A^S{}_pU^O$ (1) and $U^O{}_p2NH_2A^S$ (2)

Compound		Coupling constant (Hz) 1'2'	2'3'	3'4'	4'5'	4'5"	5'5"	3'P	4'P	5'P	5"P
$2NH_2A^S{}_pU^O$	$2NH_2A^S$-	7.08	2.93	4.63	4.03	5.98	-12.58				
	$-U^O$	5.65	1.46	3.66	5.31	5.13	-11.96		1.71	3.60	5.13
$U^O{}_p2NHA^S$	U^O-	5.68	6.73	3.12	3.85	4.77	-12.83	7.6			
	$-2NH_2A^S$	6.97	3.67	3.85	4.95	4.77	-11.91		1.47	2.75	4.37
$2BzNHA^S{}_pU^O$	$2BzNHA^S$-	6.94	0.90	2.92	3.65	5.83	-12.80	0.73			
	$-U^O$	5.83	3.12	4.73	5.48	5.48	-12.03			4.62	6.57
$U^O{}_p2AcNHA^S$	U^O-	5.49	0.5	3.12	4.49	5.87	-12.55	7.62			
	$-2AcNHA^S$	6.78	3.30	3.85	4.95	5.04	-12.28		1.47	3.48	3.20
$2NH_2A^S{}_p$		6.84	1.95	2.20	4.40	6.83	-12.45				
$_p2NH_2A^S$		6.59-6.60	2.57	3.30	5.86	6.05	-11.36			5.96	6.14
$U^O{}_p$		5.49									
$_pU^O$		5.48	0.73	2.56	5.85	5.85					

7). Both the dimers exhibit a change in spectral properties after mixing with ethidium bromide, suggesting formation of a complex with ethidium bromide. The ethidium bromide-1 complex shows a negative CD band in 320-350 nm region as shown in FIG. 8. On the ethidium bromide-2 complex, a relatively small positive band is observed in this region. These bands must be induced by interactions of the phenanthridinium ring of ethidium bromide with the bases of the dimer because ethidium bromide itself has no optical activity. The spectral changes in both the visible and CD spectra for 1 are larger than those for 2 suggesting stronger intercalative interactions for 1. Those results for 1 and 2 are similar to those for the corresponding dimers $A^S{}_pU^O$ and $U^O{}_pA^S$ 10).

FIG.7 Absorption spectra of Dimers + Ethidium bromide

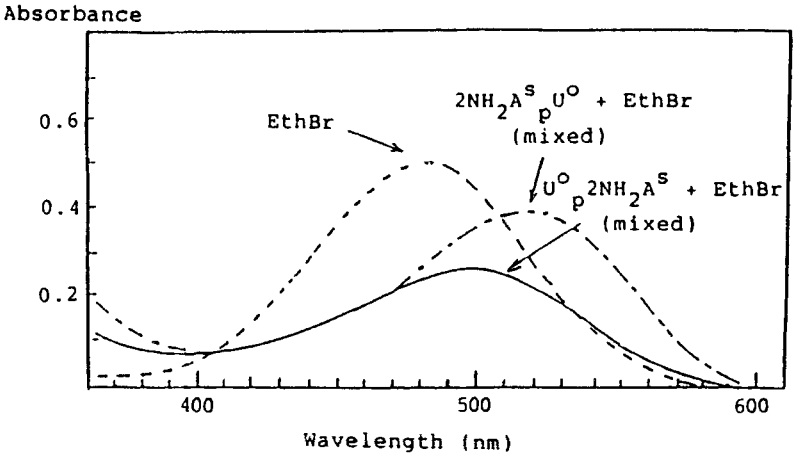
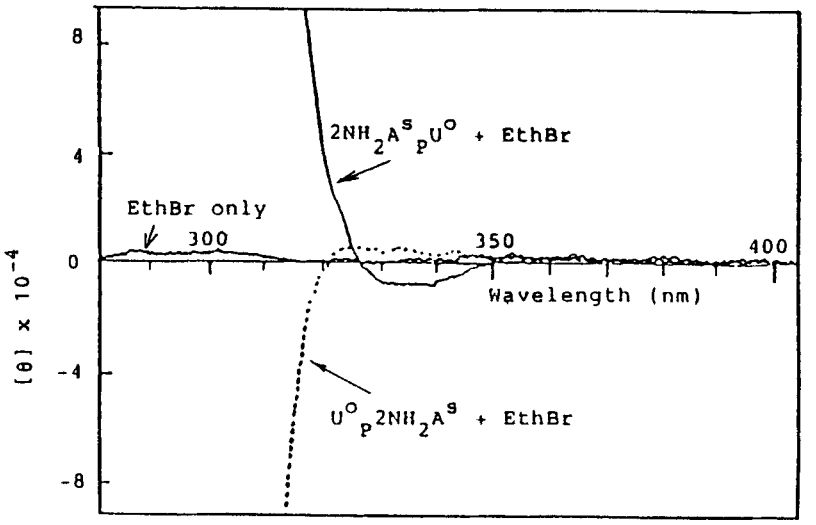


FIG.8 CD SPectra of Dimers + Ethdium bromide



Dimer 1.5 mM, EthBr 0.15 mM
0.1 M NaCl 10mM NaCaCo buffer (pH 7.0)

EXPERIMENTAL

General procedures -- UV absorption spectra were recorded on a Hitachi 340 spectrometer. NMR spectra were recorded on a Bruker WH-270 spectrometer (270 MHz) at ambient probe temperature of 25°C. D_2O was used as the solvent. Paper electrophoresis (PEP) was performed for 1 hr at a voltage of 900V/40 cm on Toyo filter paper No. 51A using 0.05M triethylammonium bicarbonate (TEAB) buffer (pH 7.5). Paper chromatography (PC) was performed on Toyo filter paper No. 51A using the following solvent systems: A, H_2O adjusted to pH 10 with NH_4OH; B, n-BuOH-H_2O (86 : 14 v/v); C, isopropanol-conc. ammonia-water (7:1:2 v/v/v); D, ethanol-1 M ammonium acetate pH 7.5 (7:3 v/v); E, ammonium sulfate-water-isopropanol (66:100:2 w/v/v).

N^2,N^6-Dibenzoyl-2-NH_2A^S (4)

$2NH_2A^S$ (3)(0.27 g, 0.9 mmol) was dried three times by evaporation with pyridine and suspended in 4.5 ml of dry pyridine. Trimethylchlorosilane (0.58 ml, 4.5 mmol) was added to the solution. After the mixture was stirred for 2 hr, benzoyl chloride (1.04 ml, 9 mmol) was added and the reaction mixture was maintained at room temperature for 2 hr. The mixture was then cooled in an ice bath and 1.35 ml of water was added. After 5 min, 2.5 ml of 28 % aqueous ammonia was added and the mixture was stirred at room temperature for 30 min. The mixture was then evaporated to almost dryness and the residue was dissolved in 30 ml of chloroform and washed with 75 ml of saturated $NaHCO_3$. Amorphous product was separated by filtration and recrystallized from 50 % EtOH and purified by column chromatography on a silica gel column (Merck 7734 60H) (4.5 x 8 cm). Elution was performed with CH_2Cl_2-MeOH (19:1). The product (**4**) was obtained as colorless needles 0.234g (76 %). mp. 209-214°C, UV: λ_{max}^{MeOH} nm (ε) 237 (23100), 250 (23100), 284 (sh) (14600), 314 (18100). $\lambda_{max}^{MeOH(OH^-)}$ 276 (16300), 318 (14300). $\lambda_{max}^{MeOH(H^+)}$ 252 (25400), 304 (18500), 328 (sh) (17000). Anal. Calcd. for $C_{24}H_{20}N_6O_5S \cdot 1/2\ H_2O$: C, 56.13; H,

4.12; N,16.36. Found. C, 56.50; H, 3.99; N, 16.39. PC: Rf(A) 0.07, Rf(B) 0.85, Rf(C) 0.90. TLC: CH_2Cl_2 - MeOH (9 : 1) Rf 0.45.

$\underline{N}^2$,$\underline{N}^6$-Dibenzoyl-5'-$\underline{O}$-monomethoxytrityl-2-NH_2A^S ($\underline{N}^2$,$\underline{N}^6$-diBz-5'-MMTr-$2NH_2A^S$, 5)

The $\underline{N}$-benzoyl derivative (**4**) (197 mg, 0.394 mmol) was dried three times by evaporation with pyridine and dissolved in 0.75 ml of DMSO and 0.60 ml of dry pyridine. Monomethoxytrityl chloride (146 mg, 0.47 mmol) was added to the mixture and stirred at room temperature for 6 days. After TLC (CH_2Cl_2 - MeOH, 19 : 1 v/v) showed complete reaction, the reaction mixture was cooled in an ice bath and 1.5 ml of 0.1 M TEAB buffer (pH 7.5) was added to stop the reaction. 5 ml of 0.1 M TEAB buffer and 5 ml of chloroform were added. The aqueous layer was washed three times with 20 ml of chloroform. The organic layers were combined and evaporated to dryness and the residue was purified by chromatography on a silica gel column (Merck 7734 60H) (4.5 x 8 cm) using CH_2Cl_2 - MeOH (19 : 1). **5** was obtained in a yield of 74 % (0.223 g) as colorless needles by recrystallization from ethyl acetate and ethanol. mp. 237-239°C, UV: λ_{max}^{MeOH} nm (ε), 235 (43500), 283 (19300), 314 (22700). Anal. Calcd. for $C_{44}H_{36}N_6O_6S \cdot C_2H_5OH$: Calcd. C, 67.14; H, 5.15; N, 10.21. Found; C, 66.90; H, 5.13; N, 10.08. PC: Rf (B) 0.93, Rf (C) 0.92. TLC: CH_2Cl_2 - MeOH (19 : 1) Rf 0.41.

2-NH_2A^S-5'-Phosphate ($_p2NH_2A^S$)

To an ice-cold stirred solution of redistilled trimethyl phosphate (10 ml) and phosphorus oxychloride (1 ml) was added dry $2NH_2A^S$ (**3**) (0.296 g, mmol). Progress of the reaction was monitored by PEP. The sample was neutralized with 0.1 M aqueous ammonia and applied to a paper. After being stirred for 5 hr in an ice bath, the reaction mixture was poured into 15 ml of ice-water. The solution was applied to a column of activated charcoal (1.2 x 22.5 cm). The column was washed with water to neutrality and eluted with 50 % EtOH containing 2 % aqueous ammonia. After evaporation of the solvent, the nucleotidic material (1120

A_{262}, 92 %) was obtained. The solution was adjusted to pH 8 with aqueous ammonia and applied to a column (1.5 x 13 cm) of Dowex 1x2 (formate form), after washing with water (2 l), elution was carried out by linear gradient of formic acid (0 - 0.1 M, total 4 l). Appropriate fraction were collected and evaporated. The amount of nucleotide was estimated by absorbance at 264 nm (5546 A_{264}, 45.4 %). PC: Rf (C) 0.02, Rf(D) 0.08, Rf (E) 0.18. PEP: R_{AMP} 0.99. UV: $\lambda_{max}^{50\% \text{ EtOH}}$ nm 216, 262, 291. $\lambda_{max}^{H^+}$ 222, 262, 305. $\lambda_{max}^{OH^-}$ 262, 291.

Pyridinum $\underline{N}^2$,$\underline{N}^6$-3'-$\underline{O}$-triacetyl-2-NH_2A^S-5'-phosphate (8)

$_P2NH_2A^S$ (free) (5540 A_{264}, 0.45 mmole) was suspended in water and the solution was adjusted with 1 M ammonia to pH 7.2. The solution was evaporated to dryness. The residue was dissolved in 10 % aqueous pyridine (10 ml) and passed through a column (1 x 5 cm) of pyridinium Dowex 50Wx2 (100 - 200 mesh) cation-exchange resin. The column was washed with 10 % pyridine (40 ml) and 50 % pyridine (30 ml). The eluent and washings were combined and evaporated to dryness at 30°. The residue was coevaporated with pyridine three times. The anhydrous compound was treated with acetic anhydride (0.85 ml) in pyridine (2.6 ml) for 18 hr at 25° with stirring and the solvent was evaporated. The residue was dissolved in 50 % pyridine with cooling in an ice bath and the solution was concentrated after standing for 2 hr at room temperature. The residue was rendered anhydrous by coevaporation with pyridine and the anhydrous pyridine solution was added to ether with vigorous stirring. The precipitate was collected by centrifugation and washed with ether three times. The yield was nearly quantitative. UV: λ_{max}^{H2O} nm 237, 273, 305.5. $\lambda_{max}^{H^+}$ 216, 241, 287, 323. $\lambda_{max}^{HO^-}$ 253, 305. PC: Rf (C) 0.17, Rf (D) 0.44, Rf (E) 0.46, Rf (F) 0.09., PEP: R_{AMP} 0.82.

8,2'-S-Cyclo-2-aminoadenylyl-(3'-5')-6,2'-O-cyclouridine ($2NH_2A^S{}_pU^O$, 1)

A mixture of the nucleotide (6) (54 mg, 0.12 mmol) and $\underline{N}^2$,$\underline{N}^6$-diBz-5'-MMTr-$2NH_2A^S$ (5) (93mg, 0.12 mmol) was rendered anhydrous by repeated coevaporation with pyridine. The

residue was dissolved in anhydrous pyridine (1.4 ml) and DCC (124 mg 0.6 mmol) was added. The mixture was kept for 3 days at 30°, then 50 % aqueous pyridine was added. After 7 hr at 30° dicyclohexylurea was removed by filtration. The filtrate was extracted with n-pentane (30 ml x 2). After concentration of the solution, the residue was dissolved in 6 ml of pyridine and treated with 30 ml of 28 % aqueous ammonia at 25° for 18 hr and at 55° for 5 hr. The volatile materials were removed by evaporation and the residue was treated with 80 % acetic acid (8 ml) for 2 hr at 25°. The solvent was evaporated and the residue was treated with 40 % methylamine (2 ml) for 2 hr at 25°. After evaporation of the solvent, the residue was dissolved in water (40 ml) and extracted with ether (50 ml x 2). The aqueous solution was applied to a column (1.5 x 38 cm) of DE-23 cellulose (bicarbonate form). The column was washed with water (2 l) and eluted with a linear gradient of TEAB buffer (pH 7.5) (0 - 0.2 M, total 4 l). Fractions of 13 ml were collected at 10 min intervals (FIG. 3a). 1403 A_{255} Units of the dimer was eluted at 0.05 M TEAB buffer and further purified by chromatography on Dowex 1x2 column (formate form) (1.5 x 53 cm). The column was eluted with a linear gradient of formic acid (0 - 0.15 M, total 4 l). $2NH_2A^s{}_pU^o$ (1) (958 A_{255} units) was eluted at 0.08 M formic acid. UV: λ_{max}^{pH7} nm 255, 292. λ_{max}^{pH2} 256, 304. λ_{max}^{pH12} 256, 292. PC: Rf(D) 0.09, Rf (E) 0.04. PEP: R_{UMP} 0.59. $\underline{N}^2$-$BzNHA^s{}_pU^o$ was eluted in a yield of 251 A_{255} units at 1.2 M formic acid as shown in FIG. 3b. UV: λ_{max}^{H2O} nm 230, 280. $\lambda_{max}^{H^+}$ 245, 290. $\lambda_{max}^{OH^-}$ 253, 283. PC: Rf (C) 0.01, Rf(D) 0.35: PEP: R_{UMP} 0.52.

6,2'-O-Cyclouridylyl-(3'-5')-8,2'-S-cyclo-2-aminoadenosine ($U^o{}_p2NH_2A^s$, 2)

A mixture of the nucleotide (8) (25.6 mg, 0.044 mmole) and 5'-MMTrU^o (7) [6,7] (22.6 mg, 0.044 mmole) was rendered anhydrous by repeated coevaporation with added pyridine. The residue was dissolved in dry pyridine (10.8 ml) and DCC (45.3 mg, 0.22 mmole) was added. The mixture was stirred for overnight at 25 ° and kept at 30° for 4 days. 50%

Aqueous pyridine (2.5 ml) was added and the mixture was kept at 30° overnight. The dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness. The residual pyridine was removed by coevaporation with toluene and the residue was treated with 15 N methanolic ammonia (3 ml) at 30 ° overnight. The volatile materials were removed by evaporation and the residue was treated with 80 % acetic acid (3 ml) for 3 hr at 30 °. The solvent was evaporated and the residue was extracted with ether and the aqueous layer was evaporated to dryness. The residue was dissolved in water (30 ml) and applied to a column (1.5 x 40 cm) of DE-23 (bicarbonate form). After washing with water (2 l), elution was carried out using a linear gradient of TEAB buffer (pH 7.5) (0 - 0.2 M, total 4 l). Fractions of 14 ml were collected at 11 min intervals. The chromatogram is shown in FIG. 4a. From peak 1, 468 A_{255} units of $U^{o}_{p}2AcNHA^{s}$ was obtained. The desired dinucleotide (2) was obtained only in a yield of 2.5 %. 313 A_{285} units of the $\underline{N}^2$-acetylated dinucleotide was deacetylated with 40 % methylamine (3 ml) for 2.5 hr at 5°. Methanol (6 ml) was added to the mixture and evaporated to dryness. The residue was dissolved in water and applied to a column (1.5 x 37 cm) of DE-23 (bicarbonate form). After washing with water (300 ml), elution was carried out using a linear gradient of TEAB buffer (pH 7.5) (0 - 0.2 M, total 3 l)(FIG. 4b). Fraction 14.5 ml were collected at 11 min intervals. $U^{o}_{p}2NH_2A^{s}$ (2) (255 A_{255} units) was eluted at 0.05 M TEAB buffer. UV: λ^{pH7}_{max}nm 256, 292. λ^{pH2}_{max} 256, 306, λ^{pH12}_{max}256, 292. PC: Rf (D) 0.12, Rf (E) 0.07, PEP: R_{UMP} 0.59. $U^{o}_{p}2AcNHA^{s}$ (51 A_{255}) was eluted at 0.04 M of TEAB buffer. UV: λ^{H2O}_{max} nm 255, 285. PC: RF (C) 0.01, Rf (D) 0.33, Rf(E) 0.05. PEP: R_{UMP} 0.06.

2-NH_2A^s-3'-Phosphate, ($2NH_2A^s_p$)

A mixture of pyridinium β-cyanoethyl phosphate (0.4 mmole) and N^2,N^6-diBz-5'-MMTr-$2NH_2A^s$ (5) (200 mg 0.25 mmol) was rendered anhydrous by coevaporation with added pyridine. The residue was dissolved in anhydrous pyridine (2.5 ml) and DCC (500 mg, 2.5 mmol) was added. The mixture was kept at

25° for 4 days and then 50% aqueous pyridine was added. After standing overnight at 25°, the precipitated 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 1.5 hr. The volatile materials were evaporated off and 30 % aquous pyridine was added. The mixture was extracted with n-hexane. The aqueous layer was concentrated to dryness. The residue was treated with 40 % methylamine (5 ml) at 25 ° for 4 hr. Methanol (20 ml) was added to the mixture and evaporated to dryness and diluted to 50 ml with water. The solution was applied to a column (1.5 x 10 cm) of Dowex 1x2 (formate form). After washing with water (1 l), elution was carried out using a linear gradient of formic acid (0 - 0.5 M, total 4 l). At 0.1 M formic acid, 1400 A_{265} units of $2NH_2A^S{}_p$ was obtained in a yield of 35 %. UV: λ_{max}^{H2O} nm 263, 292. nm. PEP: R_{UMP} 0.89.

Hydrolysis of the Dimers with Snake Venom phosphodiesterase

The dimer samples (5 A_{max} units) were incubated with 5 µl of crude snake venom phosphodiesterase (1 mg/ml) in 0.05 M TEAB buffer ($_pH$ 7.5) (195 µl) at 37 ° for 14 hr. The products were analyzed by PEP. Under these conditions U_pA and A_pU were completely hydrolyzed. Dimer **1** was hydrolyzed to the extent of 3.5 % giving approximately equal amounts of $2NH_2A^S$ and $_pU^O$. Dimer **2** was hydrolyzed to the extent of 4 % giving approximately equal amounts of U^O and $_p2NH_2A^S$, as estimated from UV absorbance.

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