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Nucleotides. VI. Syntheses and spectral properties of some deazaadenylyl-deazaadenosines (dinucleoside monophosphates with unusual CD spectrum) and closely related dinucleoside monophosphates.

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ABSTRACT

Nine dinucleoside phosphates containing 1-deaza-(^{1}A) and 3-deazaadenosine (^{3}A) were prepared. Hypochromicity and CD spectra of these dimers were determined. It was found that varying degrees of base-stacking are operative with these oligonucleotides and their CD spectra fall into three classes. The first class CD spectra which are more or less similar in profile to those of adenylyl-(3'-5')-adenosine includes the CD spectra of ^{1}A2'p5'A, ^{1}A3'p5'A, ^{3}A2'p5'A and ^{3}A3'p5'A. The second class includes the CD spectra of A2'p5'^{1}A and A3'p5'^{1}A whose characteristic is that the positive Cotton band appears in the range of 280-310 nm. The third type CD spectra has the characteristics that the negative Cotton band appears in the longer wavelength region and the CD spectra are similar in profile to those of L-adenylyl-(3'-5')-L-adenosine which has the "left-handed helical" conformation. The CD spectra of A2'p5'^{3}A, A3'p5'^{3}A and ^{3}A3'p5'A belong to this class. Another salient observation emerging from the CD-determination is that ^{3}A3'p5'^{3}A has the spectrum quite different from that of poly 3-deazaadenylic acid.

INTRODUCTION

Circular dichroism spectra have been extensively employed to provide valuable information about the conformation (in solution) of biologically interesting substances. These include adenylyl-(3'-5')-adenosine (A3'p5'A)[2], oligo- and polyadenylic acids[3], polydeazaadenylic acids [4-6], L-adenylyl-(3'-5')-L-adenosine [7] (L-A3'p5'A), and dinuleoside monophosphates, containing 8,2'-anhydroadenosines [viz., 8,2'-anhydro-8-thio-9-β-D-arabinofuranosyladenine phosphoryl-(3'-5')-8,2'-anhydro-8-thio-9-β-D-arabinofuranosyladenine (A^S3'p5'A^S) [8] and 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine phosphoryl-(3'-5')-8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (A^O3'p5'A^O) [9]]. The latter three were thus shown to adopt left-handed helical conformation [7-9]. It has been also shown primarily on the basis of spectral comparisons between A3'p5'A and polyadenylic acid (neutral form), that the former (the simplest adenylic polymer) adopts a conformation similar to that of the corresponding

polymer and is very like the beginning of a poly A helix [2].

We have for some time been interested in factors which exert the influence in determination of the "handedness" of the screw axis of these important polymers. During the course of our investigation along this line, we determined the hypochromicity and the CD spectra of some dinucleoside monophosphates containing deazaadenosines. Comparison of their CD spectra has led us to conclude that adenylyl-3-deazaadenosines (A2'p5'^{3}A and A3'p5'^{3}A) and 3-deazaadenylyl-(3'-5')-3-deazaadenosine(^{3}A3'p5'^{3}A), in particular, might adopt unusual conformation.

MATERIALS AND METHODS.

Uv spectra were recorded on a Hitachi ESP 3T recording spectrophotometer. CD spectra were recorded on a JASCO spectropolarimeter. Measurements were made in 10-mm cells at each maximum and at a concentration of 0.5-1.5 O.D./ml, dissolved in 0.01 M phosphate buffer (pH 8.37) of ionic strength of 0.1 M K^+ at room temperature. Molar ellipticity [θ] and molar extinction coefficient (ε) are presented in terms of per-residue basis. Hypochromicity was determined at pH 8.37 and at the ionic strength of 0.1 M K^+ at room temperature, according to a reported procedure [10]. Paper chromatography (PPC) was performed on Toyo filter paper No. 51A using the following solvent systems: solvent S_1, isoPrOH-cNH_4OH-H_2O (7:1:2); S_2, saturated $(NH_4)_2SO_4$-1 M AcONa-isoPrOH (80:18:2); S_3, nBuOH, saturated with H_2O; S_4, EtOH-0.5 N AcONa (5:2). Paper electrophoresis was performed for 1.5 hr at 1000 vol. on the above filter paper with triethyl-ammonium bicarbonate (0.05 M, pH 8). Ribonuclease M [EC 2.7.7.17][11] was a gift from Dr. Masachika Irie of Hoshi College of Pharmacy. Snake venom phosphodiesterase (PDase) was purchased from Sigma Chemical Co. Adenylyl-(3'-5')-adenosine was a gift from Dr. Kazunobu Miura, Hokkaido University.

The structures of dinucleoside monophosphates reported were confirmed by enzymatic digestion both with venom phosphodiesterase and with RNase M by the procedure described below. Their purities were checked by paper chromatography (in the solvent systems: S_1, S_2, and S_4) and by uv spectra.

RNase M digestion: reaction mixture contained in 0.1 ml: 50 mmol of NaOAc (pH 5.0), 20-40 µg of RNase M and ca. 10 O.D. units of a test sample and was incubated for 12-24 hr at room temperature. Products were separated by PPC or paper electrophoresis. The structure was determined by the comparison of their uv and mobilities with those of a respective authentic sample.

PDase digestion: reaction mixture contained in 0.1 ml: 50 mmol of triethylammonium bicarbonate (pH 8), 20 µg of PDase, and ca. 10 O.D. units of a

test sample. Separation and the structural determination of products were carried out by the same procedure described above.

Synthesis of dinucleoside monophosphates.

N^6-Dimethylaminomethylene-5'-O-dimethoxytrityl-1-deazaadenosine (3). To a solution of 1-deazaadenosine[12] (1) (1.186 g, 1.699 mmol) in DMF (2 ml) was added dimethylformamide dimethylacetal[13] (0.4 ml) and the mixture was kept at room temperature for 2 days and then concentrated to dryness. The residue was dissolved in pyridine (5 ml), containing dimethoxytrityl chloride (DMTrCl, 536 mg, 1.57 mmol). The mixture was allowed to stand at room temperature for 7 days. The solvent was then evaporated to dryness in vacuo. The residue was dissolved in chloroform (20 ml) which was poured into ice and water (25 ml). The whole was extracted with chloroform (20 ml x 2). The organic layer was separated, dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was dissolved in chloroform (10 ml) which was added with stirring into a large proportion of n-hexane. The precipitate was collected by centrifugation and dried (P_2O_5). Yield, 379 mg (87%). Rf-value in PPC: 0.81 (S_1); 0.87 (S_3).

N^6-Dimethylaminomethylene-5'-O-dimethoxytrityl-3-deazaadenosine (4). This compound was prepared analogously from 3-deazaadenosine[14] (2). Yield was 87%. UV: λ_{max}(H_2O) 324 nm; λ_{max}(pH 1) 324; λ_{max}(pH 13) 302. Rf in PPC: 0.87 (S_1); 0.77 (S_3).

1-Deazaadenylyl-(2'-5')-adenosine [^{1}A2'p5'A (6)] and 1-deazaadenylyl-(3'-5')-adenosine [^{1}A3'p5'A (7)]. To a solution of (3) (240 mg, 0.395 mmol) and $O^{2'}$, $O^{3'}$, N^6-triacetyladenosine 5'-phosphate [(5), pyridinium salt, 0.593 mmol] in pyridine (4 ml) was added 2,4,6-triisopropylbenzenesulfonyl cholride (TPS, 1.19 mmol). The solution was kept at room temperature for 20 hr. There was then added water (3 ml) and tri-n-butylamine (1.2 ml). The solid which had deposited on addition of water (30 ml) was filtered off. The filtrate was washed with ether (50 ml x 10). The dried (P_2O_5) residue was treated with 5% acetic acid (10 ml) for 20 hr at room temperature. The solution was concentrated to dryness and the residue was dissolved in methanolic ammonia (50 ml). The solution was allowed to stand at room temperature for 19 hr. The solution was then concentrated to dryness and the residue was dissolved in a small amount of water which was applied to a Dowex (formate, 48 x 1.8) column. The column was washed with a linear gradient of 2 l. of water and 2 l. of 0.5 M formic acid. Each 20-ml fraction was collected. Fractions (No. 68-100) and fractions (No. 104-170) were separately pooled. The former afforded after lyophilization (6), yield being $A_{260\ nm}$ 614 units, whereas the latter afforded (7), yield being $A_{260\ nm}$ 429 units.

3-Deazaadenylyl-(2'-5')-adenosine [^{3}A2'p5'A (8) and 3-Deazaadenylyl-(3'-5')-adenosine [^{3}A3'p5'A (9)]. These dimers were prepared from (4) (140 mg, 0.23 mmol) and (5) (0.345 mmol) by a similar procedure as in the case of (6) and (7). The deblocked nucleotides were applied to a Dowex (formate, 54 cm x 2.8) column which was washed with a linear gradient of 2 l. of water and 2 l. of 0.008 M formic acid. Each 20-ml fraction was collected. Fractions (No. 49-78) and fractions (No. 82-120) were separately pooled. The former afforded after lyophilization (8), yield being $A_{260\ nm}$ 417 units, whereas the latter afforded after the same procedure (9), yield being $A_{260\ nm}$ 368 units.

N^{6}-Dimethylaminomethylene-2',3'-O-ethoxymethylene-1-deazaadenosine (11). 2',3'-O-Ethoxymethylene-1-deazaadenosine (296 mg, 0.885 mmol) which was prepared from N^{6}-acetyl-1-deazaadenosine [12b] by a reported method [15] reacted with dimethylformamide dimethylacetal (0.8 ml) at room temperature for 4 days. The solution was evaporated to dryness. The dried (P_2O_5) residue weighed 382 mg. This was used for the subsequent reaction without further purification. UV: λ_{max}(H_2O) 287 nm; λ_{max}(pH 1) 283; λ_{max}(pH 13) 303. Rf-values in PPC: 0.67 (S_1); 0.87 (S_3).

Adenylyl-(2'-5')-1-deazaadenosine [A2'p5'^{1}A (12)] and Adenylyl-(3'-5')-1-deazaadenosine [A3'p5'^{1}A (13)]. To a solution of $O^{5'}$, N^{6}-diacetyladenosine 2',3'-cyclic phosphate [16] [tri-n-butylammonium salt (10), 0.6 mmol] in pyridine (6 ml) was added TPS (1.79 mmol). After a 10-min period, (11) (135 mg, 0.37 mmol) was added. The mixture was allowed to stand at room temperature for 4 days. After usual work-up including deblocking procedure [see preparation of (6) and (7)] the crude products were applied to a Dowex (formate, 48 cm x 1.8) column. The column was washed with a linear gradient of 2 l. of water and 2 l. of 0.05 M formic acid. Each 20-ml fraction was collected. Fractions (No. 68-100) and fractions (No. 104-170) were separately pooled. The former afforded after lyophilization (12), yield being $A_{263\ nm}$ 913 units, whereas the latter afforded after the same procedure (13), yield being $A_{263\ nm}$ 913 units.

N^{6}-Dimethylaminomethylene-2',3'-O-ethoxymethylene-3-deazaadenosine (14). To a solution of 3-deazaadenosine[14] [(2), 545 mg, 205 mmol] in DMF and ethyl orthoformate (2 ml) was added a solution of HCl in DMF (12.6 M, 0.15 ml). The solution was stirred for 3.5 hr at room temperature. The neutralized solution (with triethylamine) was concentrated after filtration to dryness. The residue was subjected to column (32 cm x 2.6) chromatography on silica (100 g). The column was washed with AcOEt-MeOH (7:1 to 4:1). Fraction containing 2',3'-O-ethoxymethylene-3-deazaadenosine was pooled. After work-up, the product

was obtained as a glass. Yield, 584 mg (93%). The product (285 mg, 0.852 mmol) was dissolved in DMF (2 ml) and dimethylformamide dimethylacetal (0.8 ml). The solution was kept at room temperature for 2 days, after which period the solution was concentrated to dryness. The dried (P_2O_5) residue weighed 350 mg.

Adenylyl-(2'-5')-3-deazaadenosine [A2'p5'^{3}A (15)] and Adenylyl-(3'-5')-3-deazaadenosine [A3'p5'^{3}A (16)]. These dimers were prepared from (10) (79 mmol) and (14) (210 mg, 0.581 mmol) by the similar procedure as described above. Each isomer was separated by a Dowex (formate, 60 cm x 2.2) column. The column was washed with a linear gradient of 2 l. of water and 2 l. of 0.006 M formic acid. Each 20-ml fraction was collected. Fractions (No. 46-82) and fractions (No. 84-120) were separately pooled. The former gave after lyophilization (15), yield being $A_{263\ nm}$ 787 units, while the latter afforded (16), yield being $A_{263\ nm}$ 938 units.

3-Deazaadenylyl-(3'-5')-3-deazaadenosine [^{3}A3'p5'^{3}A (18)]. This dimer was prepared enzymatically from (2) and 3-deazaadenosine 2',3'-cyclic phosphate (17)[15] with RNase M. Details of the preparation will be reported elswhere[17].

Reaction conditions and yields obtained in the dinucleoside monophosphate synthesis are summarized in Table I.

Table I. Preparation of Dinucleoside monophosphates. Reaction conditions and yields.

Nucleoside (mmol)	Nucleotide (mmol)	TPS (mmol)	Reaction Time (hr)	Yield of dinucleoside monophosphate
(3) 0.395	(5) 0.593	1.19	20	(6) 614 (7) 429 A_{260} units
(4) 0.230	(5) 0.345	0.694	12	(8) 417 (9) 368
(11) 0.374	(10) 0.600	1.79	4	(12) 913 (13) 904 A_{263} units
(14) 0.581	(10) 0.790	2.37	12	(15) 787 (16) 938

Rf-values (in paper chromatography) of the dimers synthesized are summarized in Table II.

Table II. Rf-values in paper chromatography

Compound	S_1	S_2	S_4	Compound	S_1	S_2	S_4
(6)	0.23	0.04	0.24	(13)	0.30	0.12	0.24
(7)	0.27	0.04	0.25	(15)	0.20	0.11	0.15
(8)	0.15	0.12	0.16	(16)	0.21	0.11	0.17
(9)	0.23	0.15	0.32	(18)	0.26	0.25	0.21
(12)	0.23	0.11	0.22				

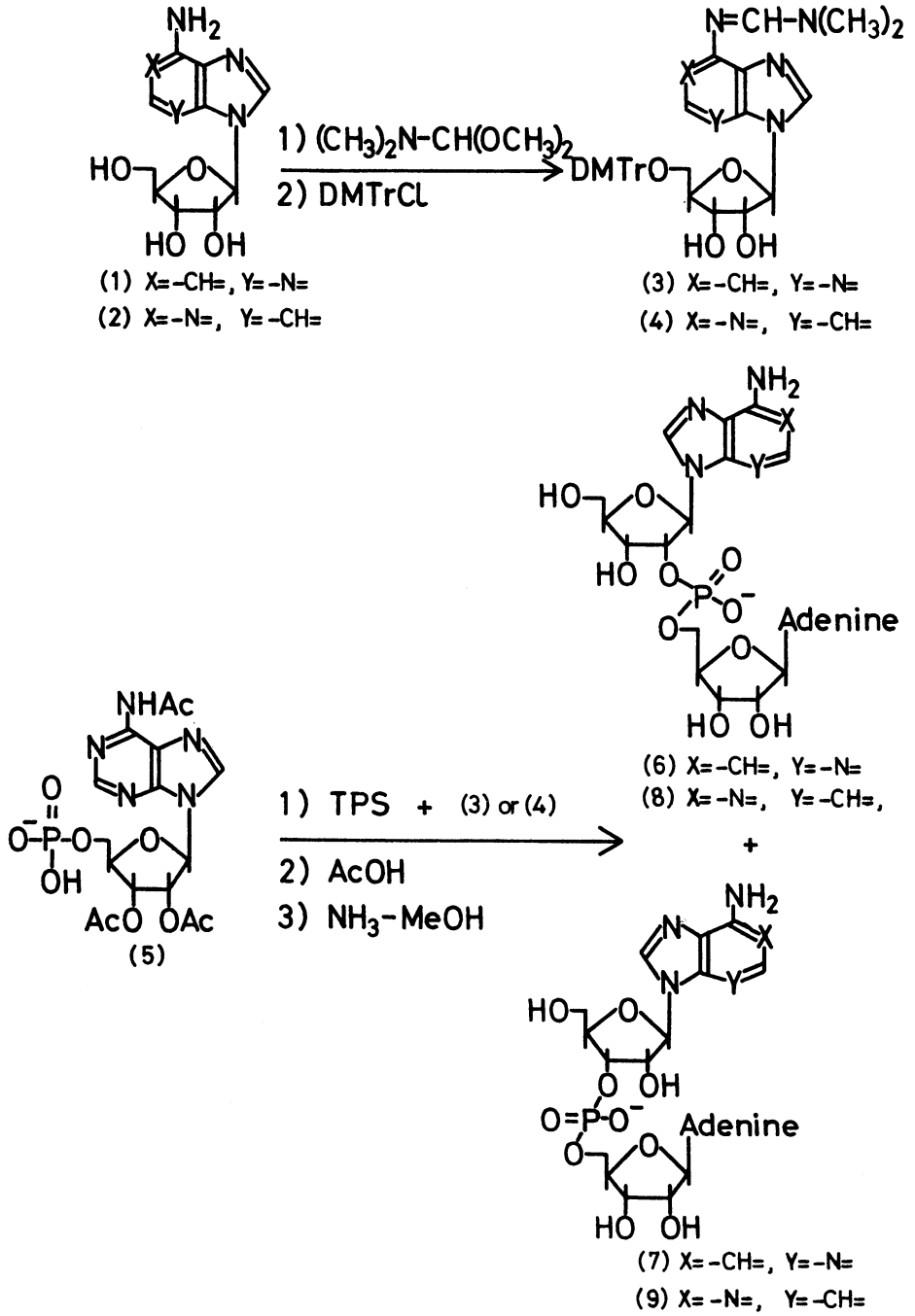
RESULTS AND DISCUSSION

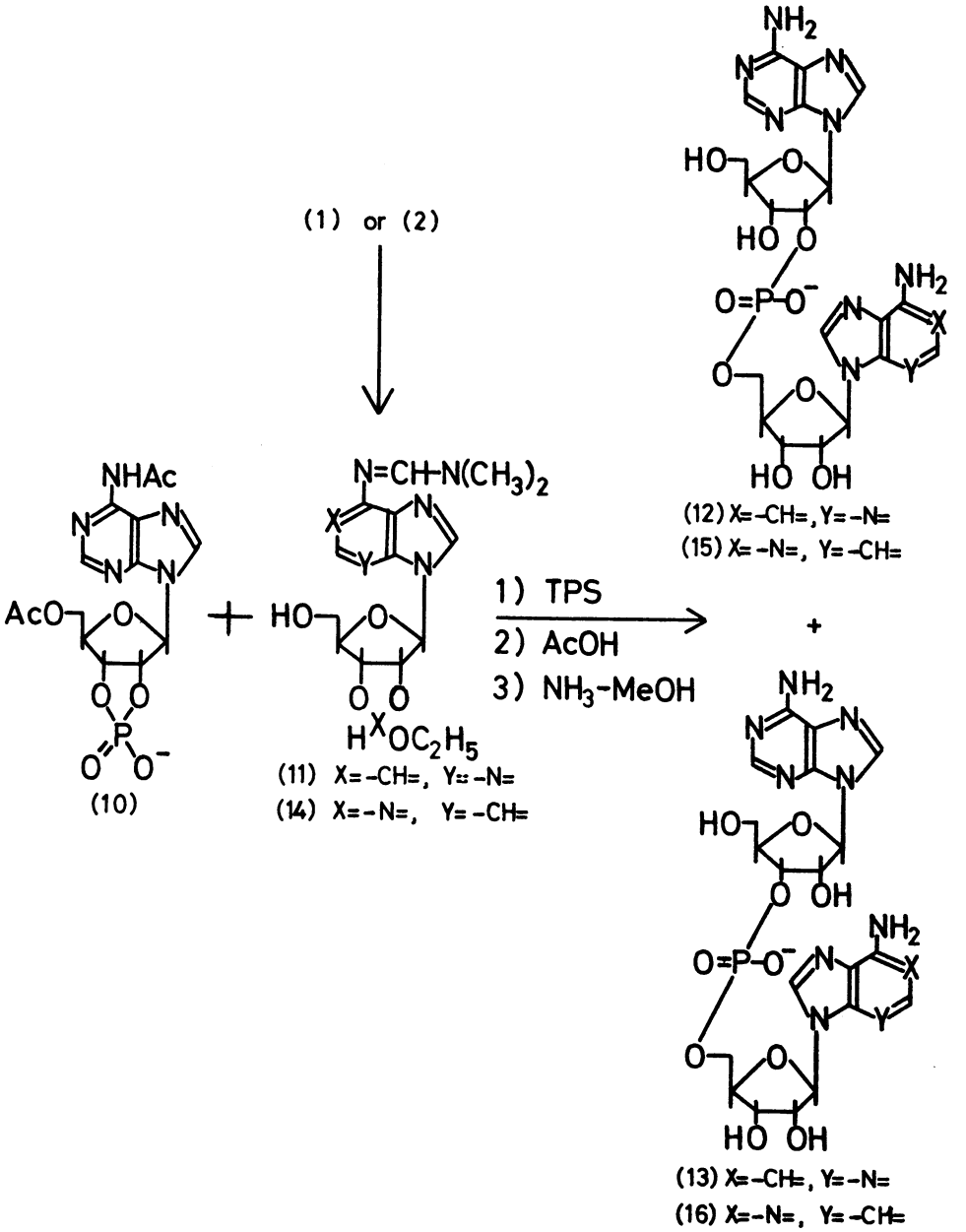
Synthesis.

Preparation of deazaadenylyladenosine [(6), (7), (8), and (9)]. These dimers were prepared according to a reported method [16], with some modifications. Our preliminary experiments showed that during synthesis of nucleotides containing deazaadenosines, both 5'-hydroxyl and 6-amino groups have to be protected and besides that even the acetyl group is too stable on de-blocking. We therefore adopted more "alkali-labile" dimethylaminomethylene protection which was originally introduced into oligonucleotide synthesis by Czechoslovak chemists [18]. 1-Deazaadenosine (1) [12] reacted smoothly with dimethylformamide dimethylacetal [13] to give N^6-dimethylaminomethylene 1-deazaadenosine[19, 20] which in turn was treated with dimethoxytrityl chloride (DMTr chloride) to afford N^6-dimethylaminomethylene 5'-O-dimethoxytrityl 1-deazaadenosine (3) in excellent yield. N^6-Dimethylaminomethylene 5'-O-dimethoxytrityl 3-deazaadenosine (4) was analogously prepared from 3-deazaadenosine (2) [14].

Treatment of (3) with $O^{2'}$, $O^{3'}$, N^6-triacetyladenosine 5'-phosphate (5) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) afforded the corresponding blocked dinucleoside monophosphate which was then deblocked by the conventional procedure. Separation of the isomers so obtained was achieved by a Dowex column. Dinucleoside monophosphates [(8) and (9)] were analogously prepared from (4) and (5).

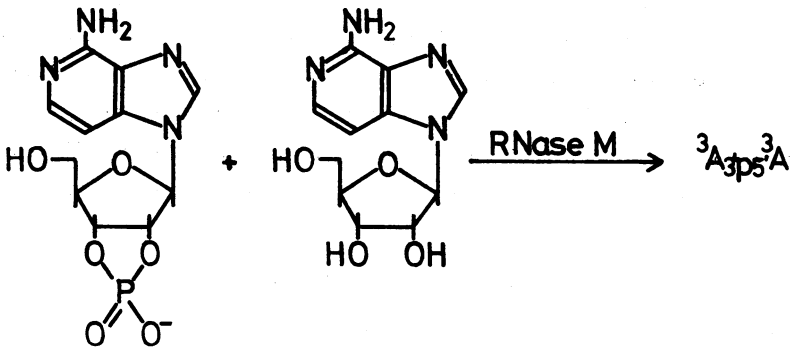
Preparation of adenylyldeazaadenosines [(12), (13), (15), and (16)]. These dimers were prepared essentially according to Michelson's method [21]. Because of the same reasons as discussed above, we again adopted dimethylaminomethylene protection for the amino group of deazaadenosines. 2',3'-O-Ethoxymethylene 1-deazaadenosine which was prepared from 1-deazaadenosine (1)


NH2
HO
HO OH
1) (CH3)2N-CH(OCH3)2
2) DMTrCl
N=CH-N(CH3)2
DMTrO
HO OH
(1) X=-CH=, Y=-N=
(2) X=-N=, Y=-CH=
(3) X=-CH=, Y=-N=
(4) X=-N=, Y=-CH=
NHAc
O
O-P-O
OH
AcO OAc
(5)
1) TPS + (3) or (4)
2) AcOH
3) NH3-MeOH
Adenine
(6) X=-CH=, Y=-N=
(8) X=-N=, Y=-CH=,
+
O=P-O
(7) X=-CH=, Y=-N=
(9) X=-N=, Y=-CH=



and ethyl orthoformate was treated with dimethylformamide dimethylacetal in DMF. After work-up, chromatographically homogeneous product (11) could be obtained whose uv maximum [λ_{max} (pH 13) 303 nm] in basic medium was shifted

to the longer wave-length by ca. 41 nm [18-20], as compared to that of (1). The reaction of (11) with $O^{5'}$, N^6-diacetyladenosine 2',3'-cyclic phosphate [16] (10) in the presence of TPS, followed by two consecutive deblocking procedures afforded, after separation, a pair of isomers [(12) and (13)]. The structural confirmation of products was based on enzymatic digestion with RNase M [11] and venom phosphodiesterase. The purity was checked by paper chromatography in three different solvent systems. Dinucleoside monophosphates [(15) and (16)] were prepared analogously from 3-deazaadenosine (2) in three steps.



Preparation of 3-deazaadenylyl-(3'-5')-3-deazaadenosine [^{3}A3'p5'^{3}A (18)]. Some unrewarded attempts have been made to prepare ^{3}A3'p5'^{3}A starting from (2), according to an analogous route (see the preparation of ^{1}A3'p5'A). Owing to the relatively more basic (nucleophilic) nature of the 6-amino group of 3-deazaadenosine (pKa 6.8) [12], 3-deazaadenosine derivative suitable for a building block of the dimers (e.g., $O^{2'}$,$O^{3'}$,N^6-tribenzoyl-3-deazaadenosine) was not readily available by the conventional procedure. We thus adopted an enzymatic (RNase M) preparation that has been quite recently discovered in our laboratories [16].

Incubation of (17) and (2) (molar ratio being 1:6.2) in the presence of RNase M (at pH 5.0 at -5° for 5 days) afforded the 3-deazaadenylyl-(3'-5')-3-deazaadenosine (18) which was easily separated and purified by preparative paper electrophoresis. Even though no attempts to optimize the conditions have been done, the yield of (18) amounted to 14%, on the basis of the 2',3'-cyclic phosphate (17).

Hypochromicity. Hypochromicity of the dinucleoside monophosphates [(6)-(9), (12), (13), (15), (16), and (18)] was determined at the ionic strength of 0.1 M K^+ according to a reported procedure [10]. In the present case, however, in order to ensure the comparison of the neutral species of each sample the determination was carried out at pH 8.37, because 3-deazaadenosine (2) is comparatively basic (pKa 6.80) [12, 22], Results obtained are given in Table III, where in each series [(2'-5') or (3'-5')] the dinucleoside monophosphates are arranged in the decreasing order of the hypochromicity values.

The first point of interest is that as pointed out earlier by Michelson[21] and Ts'o[10], among a pair of positional isomers, the 2'-5'-isomer has a larger hypochromicity than the 3'-5'-isomer. The second point of interest is that among each series, varying degrees of hypochromicity (19% to 10% in the case of the 2'-5' series and 13% to 4% in the other series) have been observed. This can be taken as a reflection of varying degrees of base-stacking that is operating in each member. It is worthy of note that A2'p5'A or A3'p5'A is located in the middle of each sequence (Table III). In cases where an ade-

Table III. Hypochromicity values of dinucleoside monophosphates

Compound		Hypochromicity (%)	λ_{max} nm	$\varepsilon \times 10^{-4}$
A2'p5'^{1}A	(12)	19	262	1.1
^{1}A2'p5'A	(6)	15	263	1.1
A2'p5'A		15	260	(1.29)*
A2'p5'^{3}A	(15)	13	262	1.0
^{3}A2'p5'A	(8)	10	265	1.1
A3'p5'^{1}A	(13)	15	263	1.1
^{1}A3'p5'A	(7)	13	263	1.2
A3'p5'A		13	260	(1.36)*
^{3}A3'p5'^{3}A	(18)	8	265	0.94
A3'p5'^{3}A	(16)	8	263	1.1
^{3}A3'p5'A	(9)	4	265	1.1

* Extinction coefficients per nucleoside-residue basis of A2'p5'A and A3'p5'A are taken from ref. 21.

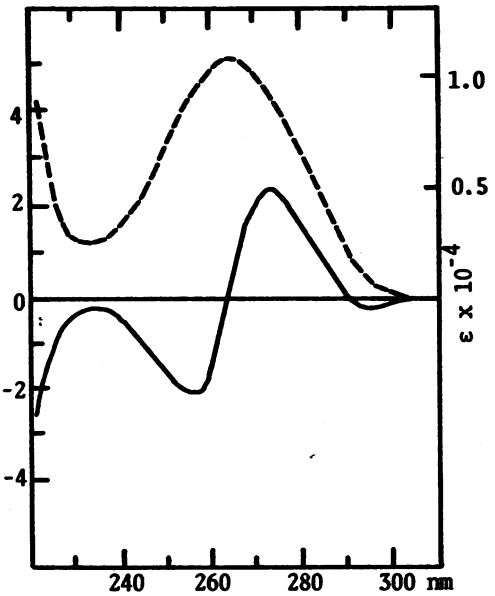


Fig. I. UV and CD spectra of A3'p5'A (pH 8.37).

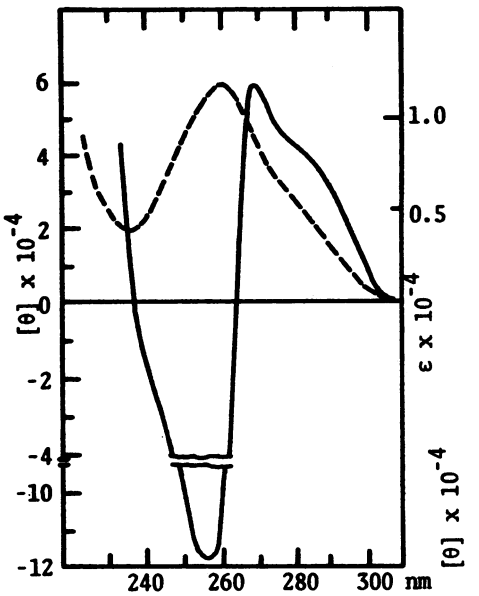


Fig. II. UV and CD spectra of ^{1}A3'p5'A (pH 8.37).

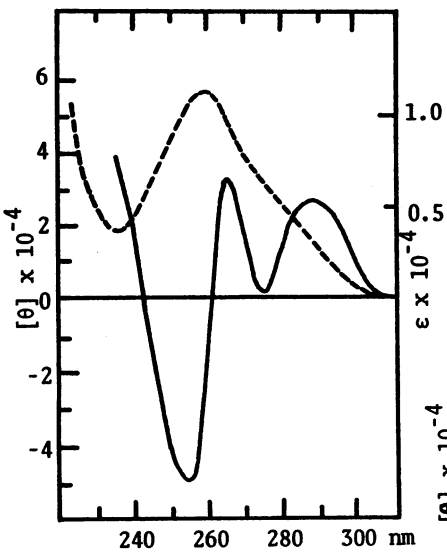


Fig. III. UV and CD spectra of A3'p5'^{1}A (pH 8.37).

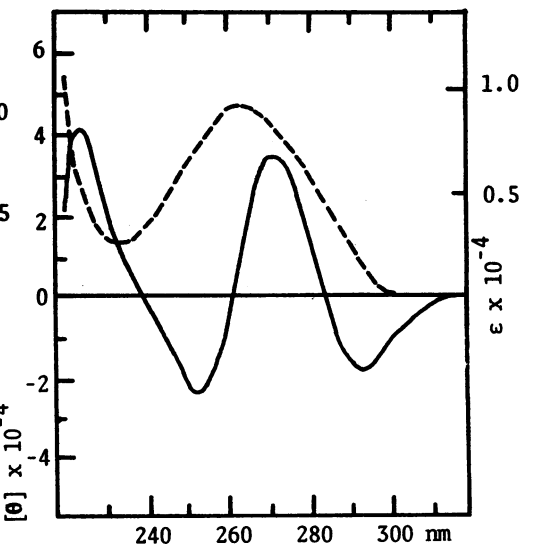


Fig. IV. UV and CD spectra of ^{3}A3'p5'^{3}A (pH 8.37).

nosine residue of ApAs [23] is replaced by 1-deazaadenosine, higher (at least equal) hypochromicity results. On the other hand in cases where one of ApAs is replaced by 3-deazaadenosine lesser (at most equal) hypochromicity results. One extreme case is A2'p5'^{1}A whose hypochromicity value amounts to 19%, whereas another extreme case is ^{3}A3'p5'A, its hypochromicity value being only 4%. Even in the latter case, however, it was shown that a considerable base-stacking is still operative, because a well-defined CD spectra could be observed (vide infra).

The third point of interest is that a notable difference in hypochromicity was observed among a pair of sequence-isomers [viz, (7) and (13); (8) and (15); (9) and (16)].

Circular dichroism and conformation. CD spectra of these dinucleoside monophosphates were determined in 0.01 M phosphate buffer (pH 8.37) [22] and in the ionic strength of 0.1 M K^{+} at ca. 25°. The spectra determined are able to be classified into three types according to the difference in the gross apperance of the bands. Each representative spectrum is shown in Figs II through IV, together with the spectrum of A3'p5'A (Fig. I). Circular dichroic parameters of all the above dinucleoside monophosphates are summarized in Table IV. A comparison of the CD spectra leads to the following observations. (I) CD spectra of (6), (7), (8), and (9) are similar to each other and are more or less similar to the spectrum of A3'p5'A ("ApA-type" of CD bands). The second class includes the CD spectra of (12) and (13) whose characteristic is that the positive Cotton band appears in the range of 280-310 nm. A characteristic of the third class CD spectra is that the Cotton band appears in the range of 280-310 nm, but the sign of the bands is negative. The CD spectra of (15), (16), and (18) belong to this class, which has this feature (the negative Cotton band in the longer wave-length region) in common with the CD spectrum of LApA[7], $A^{S}pA^{S}$ or $A^{O}pA^{O}$ [9] having all the left-handed helical structure. This suggests that the dinucleoside monophosphates [(15), (16), and (18), in particular] whose CD spectra belong to the third class might have the conformation similar to LApA[7]. Yet, difinite answers to the question concerning the handedness of the screw axis of the helical structure of these dimers must await further investigation from a variety of approaches, i.e., nmr analysis.

It is also of interest to note that an exceptionally large magnitude (-11.3 x 10^{4}) could be observed with the CD band of ^{1}A3'p5'A (7) (Table IV). [24]

Finally the most salient observation which deserves comment is that CD spectra of ^{3}Ap^{3}A which were determined at both pH 5.92 and pH 6.80 (Table V) in addition to pH 8.37 were found to be all quite different from those of

poly 3-deazaadenylic acid[5], suggesting that the former (^{3}Ap^{3}A) adopts a conformation quite different from that of the latter. 3-Deazaadenylyl-(3'-5')-3-deazaadenosine (18) may be not the beginning of a polydeazaadenylic acid helix.

Table IV. Circular dichroic parameters of dinucleoside monophosphates, measured in 0.01 M phosphate buffer (pH 8.37) of the ionic strength of 0.1 M K^{+} at ca.25°

Compound	nm	[θ] x 10^{-4}
^{1}A2'p5'A (6)	282 (p)*	+6.3
	255 (t)**	-3.8
^{1}A3'p5'A (7)	270 (p)	+5.9
	255 (t)	-11.9
A2'p5'^{1}A (12)	291 (p)	+2.9
	275 (t)	0
	266 (p)	+3.8
	255 (t)	
A3'p5'^{1}A (13)	288 (p)	+2.7
	275 (t)	+0.1
	267 (p)	+3.3
	255 (t)	-4.9
A3'p5'A	270 (p)	+3.5
	251 (t)	-4.1
A2'p5'^{3}A (15)	320 (p)	+0.4
	287 (t)	-1.5
	268 (p)	+1.0
	253 (t)	-2.2
^{3}A2'p5'A (8)	297 (t)	-0.4
	275 (p)	+4.5
	253 (t)	-1.7
	243 (p)	+0.3
^{3}A3'p5'A (9)	294 (t)	-0.2
	273 (p)	+2.4
	256 (t)	-2.1
	234 (p)	-0.2
^{3}A3'p5'^{3}A(18)	294 (t)	-1.8
	272 (p)	+3.5
	254 (t)	-2.4
	225 (p)	+4.1

*(p): peak; **(t): trough.

Table V. Cicular dichroic parameters of $^{3}A3'p5'^{3}A$, measured in 0.01 M phosphate buffer (pH 6.80 and 5.92) of the ionic strength of 0.1 M K^{+} at ca.25°

Compound	nm	[θ] x 10^{-4}
$^{3}A3'p5'^{3}A$ (18)	298 (t)*	-1.9
pH 6.80	271 (p)**	+5.3
	249 (t)	-1.3
	225 (p)	+2.6
$^{3}A3'p5'^{3}A$ (18)	297 (t)	-2.4
pH 5.92	269 (p)	+5.0
	255 (t)	-0.7
	225 (p)	+3.4

* (p): peak; ** (t): trough.

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23 ApAs refers to adenylyl-(3'-5')-adenosine, its positional isomer or both.
24 We have no reasonable explanation to this observation.