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SYNTHESIS AND PROPERTIES OF OLIGOADENYLIC ACIDS CONTAINING 2'-5' PHOSPHORAMIDE LINKAGE

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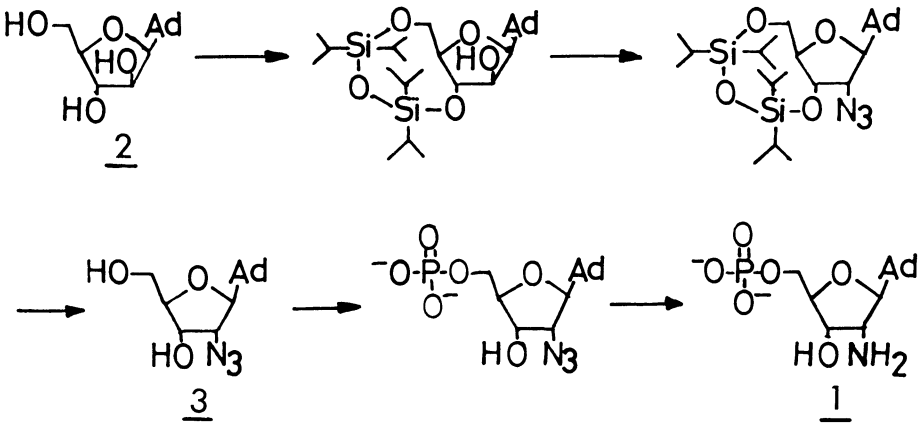
Oligoadenylic acids containing 2'-5' phosphoramide linkage were prepared by poly (U) template-directed condensation. Some of their properties are described.

Oligoadenylic acids containing 2'-5' phosphodiester linkage (abbreviated as 2-5 A) are synthesized in interferon-treated cell and have been implicated to be directly correlated with the interferon's antiviral activity.[1-3] Several analogs of 2-5 A have been prepared to evaluate the crucial region involved in the activity of 2-5 A and to explore the potential antiviral agents.[4-7] The adenine base and 2'-5' internucleotide linkage have been found to be important for the activity of 2-5 A.[4] However, native 2-5 A is rapidly degraded by cellular phosphodiesterase to lose its activity.[8] These observations prompted us to prepare phosphoramide linked 2-5 A analogs which are expected to be resistant to the degradation. The synthesis and the hydrolytic stability of 3'-5' phosphoramide (3'-(O-P-NH)-5') linked oligonucleotides were described by Letsinger and Mungall.[9] In this paper, the author wishes to report the synthesis and some properties of 2-5 A analogs containing one or two phosphoramidic (2'-(NH-P-O)-5') bonds.

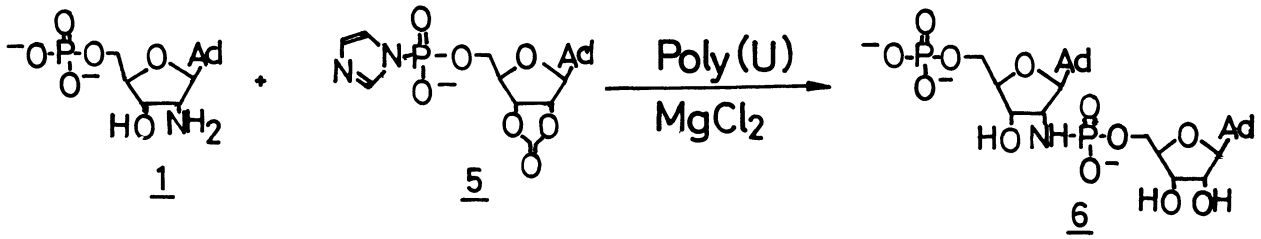
Chemical synthesis of oligonucleotides is accomplished in general by the phosphotriester approach using various bolcking groups. Contrary to the general method for the oligonucleotide synthesis, we employed template-directed condensation on poly (U)[10] for the key step of the phosphoramidic bond formation, where no additional blocking group was used and the reaction proceeded in aqueous solution.

2'-Amino-2'-deoxyadenosine-5'-phosphate 1 was chosen as a component of the



phosphoramide linked 2-5 A analogs. The compound 1 was prepared from arabinofuranosyladenine 2 by the modification of the method of Robins.[11] Tetraisopropyldisiloxane-1,3-diyl (TIPDSi) was used for the protection of 3',5'-hydroxyl group of 2.[12] Activation of the 2'-hydroxyl group with trifluoromethanesulfonyl chloride, subsequent substitution with LiN_3[13] and removal of the 3',5'-TIPDSi group with tetra-n-butylammonium fluoride gave 2'-azide-2'-deoxyadenosine 3. The yield of 3 was 58% from 2. Phosphorylation of 3 with $POCl_3$ in triethyl phosphate[14] and subsequent hydrogenation on Pd-carbon gave 1 in 63% yield.

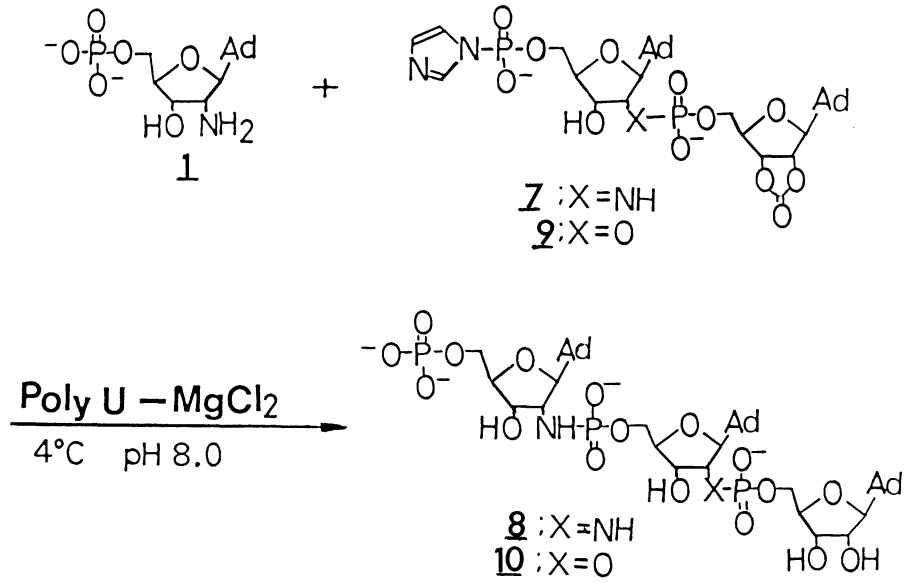
Adenosine-5'-phosphate 4 was activated with N,N'-carbonyldiimidazole (CDI) to yield adenosine-5'-phosphoimidazolide-2',3'-cyclic carbonate 5.[15] Template-directed condensation of 1 (15 μmol) with 5 (10 μmol) was carried out in the presence of poly (U) (50 μmol) and $MgCl_2$ (100 μmol) in aqueous solution (0.1 M N-methylimidazole buffer, pH 8.0) at 4 °C for 5 d. The reaction mixture was treated with EDTA solution and passed through a Sephadex G-50 column to separate poly (U). The low molecular weight fraction was subjected to column chromatography on QAE-Sephadex A-25 and eluted with a linear gradient of triethylammonium bicarbonate buffer (0.25 M-0.5 M). The phosphoramide linked dimer 6 was obtained in 59% yield, along with the monomers, 1 and 4. The carbonyl residue was eliminated from 2',3'-diol during evaporation of triethylammonium bicarbonate buffer, as it is easily removed under mild alkaline conditions. When the condensation reaction was carried out in the absence of poly (U) or at room temperature, the yield of 6 was very low and the pyrophosphate formation took place in a small amount.



1 5 6

The dimer 6 was allowed to react with CDI to activate 5'-phosphoryl group giving 7, which was condensed with 1 in the presence of poly (U) template and $MgCl_2$ in aqueous solution (pH 8.0) at 4 °C for 5 d. Treatment of the reaction mixture with EDTA solution and separation of poly (U) from the solution by Sephadex G-50 column chromatography gave low molecular weight compounds which were subjected to a column of QAE-Sephadex A-25 and eluted with a linear gradient of triethylammonium bicarbonate buffer (0.25 M-0.5 M). The 2'-5' linked triadenylic acid 8 containing two phosphoramidic bonds was obtained in 36% yield.

In a similar procedure, 5'-phosphoryl-adenylyl(2'-5')adenosine[16] was condensed with 1 in the presence of poly (U) and $MgCl_2$, after activation of the 5'-phosphoryl group with CDI. A similar workup and purification of the reaction mixture gave the 2'-5' linked trimer 10 containing one phosphoramidic bond in 49% yield. The homogeneity of the resulting 2'-5' linked oligomers were checked by TLC and HPLC.



The phosphoramide linked oligomers, 6, 8, and 10 were characterized by 400 MHz NMR in D_2O (Table 1). Most cases, adenine protons of 6, 8, and 10 showed lower-field shift compared to those of the corresponding 2'-5' phosphodiester linked oligomers.[17] The NMR data suggest that base-stacking of the phosphoramide linked oligomers is weaker than that of the 2'-5' phosphodiester linked oligomers, considering the effect of the ring current magnetic anisotropy.

Table 1. H^1 NMR[a] of 1, 6, 8, and 10 in D_2O (pD 6.7)

	$pA^{2'}_{NH_2}$ (1)	$pA^{2'}_{NH}pA$ (6)	$pA^{2'}_{NH}pA^{2'}_{NH}pA$ (8)	$pA^{2'}_{NH}pA^{2'}pA$ (10)
1'-H, d	6.17	5.98 6.00	5.71 5.85 5.92	5.86 5.90 5.91
($J_{1,2}$/Hz)	(7.0)	(2.2) (6.7)	(5.9) (2.7) (6.3)	(3.4) (1.2) (4.2)
Adenine proton	8.26	8.04 (2H)	7.94 8.00 8.01	7.94 7.97 8.00
H-2 and H-8	8.61	8.23 8.49	8.16 8.22 8.31	8.01 8.16 8.31

a) Chemical shifts are given in δ. TSP-d_4 was used as an internal standard.

Figures 1 and 2 show CD spectra of the oligomers, 6, 8, and 10, along with the corresponding 2'-5' phosphodiester linked dimer and trimer. CD spectra also indicate that phosphoramidic bond decreases the stacking effect of the 2'-5' oligoadenylic acids. The CD spectrum of 6, a negative band in the long wavelength region and a positive one in the short wavelength around 270 nm, is rather similar to that of the left handed helical dinucleotides.[18] These results suggest that the phosphoramide linked oligomers have different conformation from the phosphodiester linked oligomers.

The trimer 10 was degraded with snake venom phosphodiesterase to give 4 and 6 in nearly 1:1 ratio, while 6 and 8 were insensitive to the enzyme. They were cleaved by treatment with 0.5 M HCl.

The biological activity of the oligomers will be published in due course.

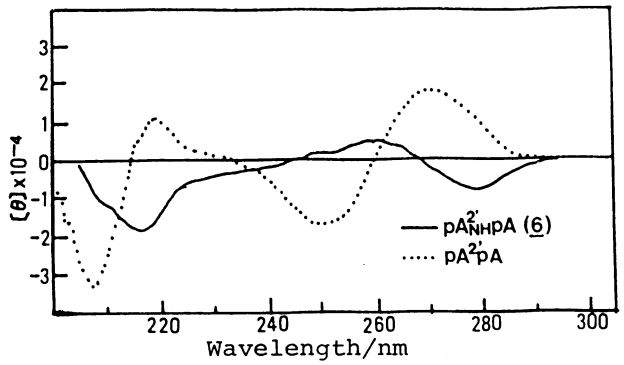


Fig. 1. CD spectra of the 2'-5' linked dimers in 0.1 M phosphate buffer (pH 6.7) at 25 °C.

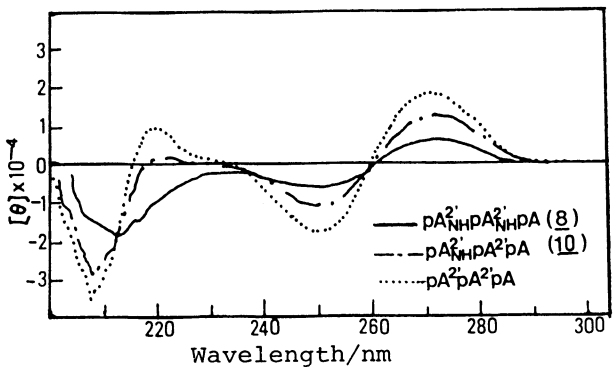


Fig. 2. CD spectra of the 2'-5' linked trimers in 0.1 M phosphate buffer (pH 6.7) at 25 °C.

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