Tetrahedron Letters No. 47, pp 4573 - 4576. 0040-4039/79/1115-4573$02.00/0

SYNTHESIS OF OLIGONUCLEOTIDE INHIBITOR OF PROTEIN SYNTHESIS: pppA2'p5'A2'p5'A

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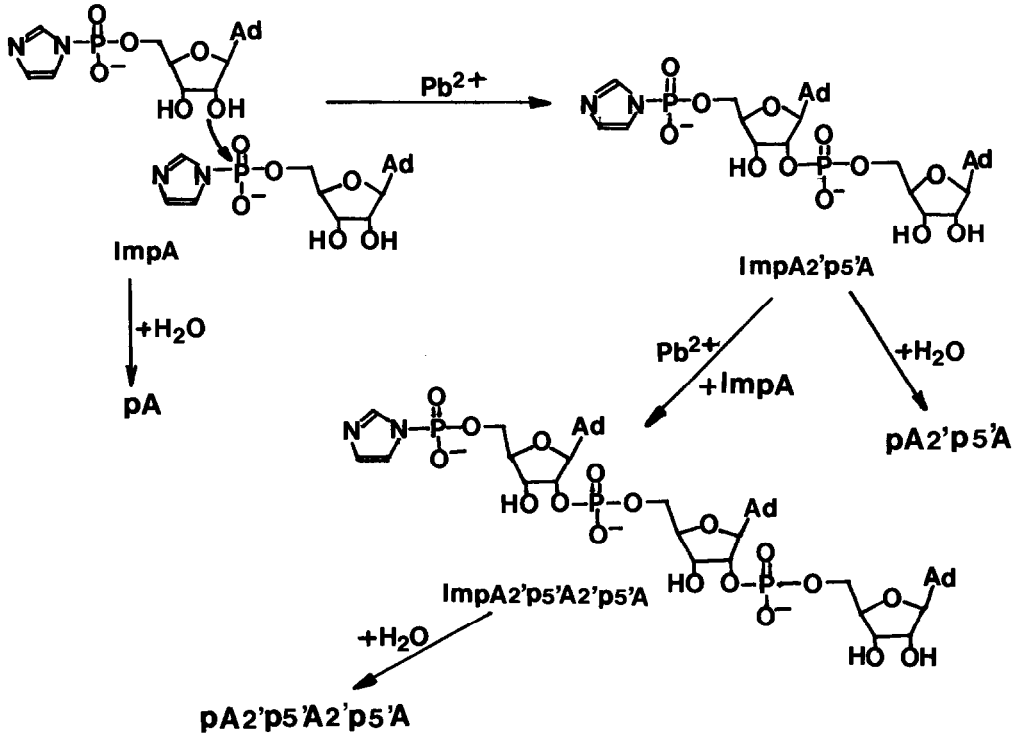
Abstract: Oligoadenylates with 2'-5' linkage were prepared in aqueous solution catalyzed by lead nitrate. Phosphorylation of the resulting triadenylate gave oligonucleotide inhibitor of protein synthesis, pppA2'p5'A2'p5'A.

Recently oligoadenylates with 2'-5' linkage (ppp(A2'p5')$_n$A, n=1,2,3,4) have been insolated from interferon-treated cells or reticulocytes as a first natural occurring 2'-5' linked oligonucleotide.[1-3] The ppp(A2'p5')$_n$A is synthesized from ATP by the interferon-meditated or reticulocyte enzymes with double-stranded RNA. The trimer pppA2'p5'A2'p5'A has been shown to inhibit cell-free protein synthesis, effective at subnanomolar concentration.

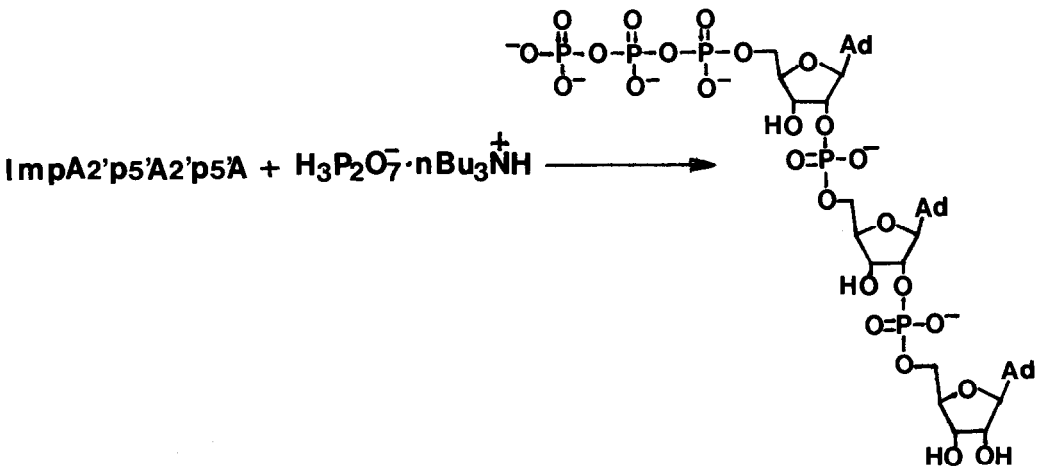
Here we wish to demonstrate a novel and simple synthesis of pppA2'p5'A2'p5'A by using our procedure of oligonucleotide synthesis in aqueous solution. Very recently two papers have appeared concerning the chemical synthesis of 2'-5' linked oligoadenylaytes.[4,5] Our synthetic approach is completely different from these studies. Some years ago we reported that metal ion promotes the synthesis of oligoadenylates in neutral aqueous solution.[6] Particularly, Pb^{2+} ion is effective, and substantial yield of oligoadenylates with 2'-5' linkage is obtained.[7] This reaction was carried out in very small scales from the point of prebiotic chemistry. We modified this reaction for the preparative purpose and lead to the oligonucleotide inhibitor of protein synthesis. The characteristics of our procedure are (1) polymerization in aqueous solution, (2) using no protecting group and (3) very simple and short reaction steps.

An activated intermediate adenosine 5'-phosphorimidazolide (ImpA) was prepared from adenosine 5'-monophosphate (15 mmol) and imidazole (ImH) (150 mmol) by a modification of a published procedure.[8] Triphenylphosphine and 2,2'-dipyridyldisulfide were employed as a condensing agent. ImpA obtained as a sodium salt was practically pure and the yield was over 90%.

The condensation of ImpA was carried out in aqueous solution by using lead nitrate as a catalyst. Thus, to a solution (47.5 ml) of ImpA (2.5 mmol) in 0.2M imidazole buffer (pH 6.5) was added lead nitrate solution (0.25M x 2.5 ml) with stirring. White precipitates were formed. The reaction mixture was kept for 5 days at room temperature with stirring. The mixture was treated with Versenol solution (0.25M x 3 ml) to remove Pb^{2+} ion as a Versenol-Pb^{2+} chelate. The resulting homogeneous solution by this treatment was subjected to a column chromatography on QAE-Sephadex A-25 (HCO_3^- form). The products were eluted with linear gradient of triethylammonium bicarbonate buffer (H_2O-1M $H\overset{+}{N}Et_3HCO_3^-$). The fractions of pA2'p5'A and pA2'p5'A2'p5'A were collected and evaporated under reduced pressure below 30°C. Lyophilization of the residue gave pA2'p5'A and pA2'p5'A2'p5'A as a triethylammonium salt. The isolated yields of pA2'p5'A and pA2'p5'A2'p5'A were 24% and 9% based on UV absorption after allowing the hypochromicity of each oligoadenylate, respectively. A number of other oligoadenylates containing 3'-5' and 2'-5' linkage were also obtained, but each in less than 4% yields. The total yield of these oligoadenylates was 20%. 2'-5' Linkage was preferentially formed in this reaction. The structure of pA2'p5'A and PA2'p5'A2'p5'A was confirmed by selective degradation with enzyme digestion. pA2'p5'A2'p5'A was degradated by snake venom phosphodiesterase completely to adenosine 5'-monophosphate. While pA2'p5'A2'p5'A was insensitive to RNase T_2 or nuclease P_1 which are specific for 3'-5' linkage. Degradation of pA2'p5'A2'p5'A with 1M NaOH solution yielded adenosine, adenosine 2'-(3')-monophosphate and adenosine 5',2'(3')-diphosphate.



pA2'p5'A2'p5'A was further phosphorylated to the corresponding 5'-triphosphate with pyrophosphate using N,N'-carbonyldiimidazole as a condensing agent. Anhydrous triethylammonium salt of pA2'p5'A2'p5'A (0.2 mmol) was dissolved in dry DMF (5 ml) containing triethylamine (0.2 ml) and tri-n-octylamine [$N(Oct)_3$] (0.1 ml). N,N'-Carbonyldiimidazole (1 mmol) was added, and the mixture was kept for 1 h at room temperature. After the completion of 5'-terminal phosphorimidazolide bond formation, 65 µl of methanol was added to degradate excess N,N'-carbonyldiimidazole. Tri-n-butylammonium pyrophosphate (2 mmol) in dry DMF (4.5 ml) was added and the reaction mixture was stirred for 1 day at room temperature. White precipitates were removed by filtration and the solution was concentrated under reduced pressure. The residue was dissolved in water and washed with methylene chloride to remove DMF and amines. The aqueous solution was applied to a column chromatography on QAE-Sephadex A-25 (HCO_3^- form). The elution was conducted by linear gradient of triethylammonium bicarbonate buffer (0.25M-0.75M). Evaporation and lyophilization of the main fractions gave pppA2'p5'A2'p5'A as a triethyl ammonium salt in 49% yield. The corresponding 5'-diphosphate ppA2'p5'A2'p'A was also obtained in 15% yield as a by-product. The 5'-diphosphate has recently been shown to be obtained biologically with 5'-triphosphate and have a similar biological activity as the 5'-triphosphate.[4]



P P PA2'p5'A2'p5'A

The pppA2'p5'A2'p5'A and ppA2'p5'A2'p5'A thus synthesized were degradated by alkaline phosphatase to A2'p5'A2'p5'A.

The procedure described here provides a simple, short and versatile synthetic route to 2'-5' linked homooligoribonucleotides which can be easily converted to the biologically active 5'-triphosphate. The synthesis of 2'-5' linked oligonucleotides with other base such as uridine is also possible by this procedure.[10]

References and Notes

1 I. M. Kerr and R. E. Brown, Proc. Nat. Acad. Sci. USA, 75, 256 (1978).
2 L. A. Ball and C. N. White, ibid., 75, 1167 (1978).
3 A. Schmidt, A. Zilverstein, L. Shulman, P. Federman, H. Berssi and M. Revel, FEBS Letters, 95, 257 (1978).
4 E. M. Martin, N. J. M. Birdsall, R. E. Brown and I. M. Kerr, Eur. J. Biochem., 95, 295 (1979).
5 K. M. Ogilvie and N. Y. Theriault, Tetrahedron Letters, 2111 (1979).
6 H. Sawai and L. E. Orgel, J. Amer. Chem. Soc., 97, 3532 (1975).
7 H. Sawai, ibid., 98, 7037 (1976).
8 R. Lohrmann and L. E. Orgel, Tetrahedron, 34, 853 (1978).
9 D. E. Hoard and D. G. Otto, J. Amer. Chem. Soc., 87, 1785 (1965).
10 H. Sawai, Nucleic Acids Research, S3, 113 (1977).
11 Abbreviations: ATP, adenosine 5'-triphosphate; pA, adenosine 5'-phosphate; ImpA, adenosine 5'-phosphorimidazolide; ImH, imidazole; pA2'p5'A, 5'-phosphoadenylyl-[2'→5']-adenylyl-[2'→5']-adenosine; Ad, adenine; DMF, dimethylformamide; Versenol, N-hydroxyethylethylenediaminetriacetic acid.

(Received in Japan 16 August 1979)