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SYNTHESIS OF A PROTEIN BIOSYNTHESIS INHIBITOR, 5'-TRI-PHOSPHORYLADENYLYL-(2'-5')-ADENYLYL-(2'-5')-ADENOSINE

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A protein biosynthesis inhibitor, 5'-triphosphoryladenylyl-(2'-5')-adenylyl-(2'-5')-adenosine was synthesized by polymerization of N^6-benzoyl-3'-O-(o-nitrobenzyl)adenosine 5'-phosphate follwed by reaction with pyrophosphate using 1,1'-carbonyldiimidazole.

Recently an inhibitor of protein biosynthesis in interferon-treated cells has been found and characterized as 5'-triphosphoryladenylyl-(2'-5')-adenylyl-(2'-5')-adenosine ($pppA^{2'}p^{5'}A^{2'}p^{5'}A$)[1] (I). In this paper we wish to communicate a method of chemical synthesis of the compound I.

N^6-Benzoyl-3'-O-(o-nitrobenzyl)adenosine (II) [m.p. 218-221°, NMR: δ 4.22 (br. s, 2H, 3'- and 4'-OH), 5.08 (d, 2H, Ar-CH_2)] was obtained by crystallization from the mixture of 2'- and 3'-o-nitrobenzyl derivatives, which were synthesized by benzylation of N^6-benzoyl-5'-monomethoxytrityladenosine[2] with o-nitrophenyldiazomethane.[3] The compound II was phosphorylated with $POCl_3$ according to a standard procedure[4] and 5'-monophosphate (III) (paper electrophoresis, RpA 0.98) was obtained by chromatography on a DEAE-Sephadex A-25 column in a yield of 64%.

Compound III was then polymerized using dicyclohexyl carbodiimide as condensing reagent. After 15 days at room temperature, the reaction mixture was worked up as usual and applied to a column of DEAE-cellulose (bicarbonate form) and eluted with a linear gradient of triethylammonium bicarbonate buffer (0-0.5M, total 10 l.). The trimer (IV) was obtained in a yield of 7.7% together

with dimer (9.8%), tetramer (6.3%) and higher oligomers (13.2%). For analysis a small amount of samples were irradiated with UV (λ over 280 nm, for 1hr) to remove o-nitrobenzyl groups. Each product is characterized separately by comparison of Rf in paper chromatography before and after bacterial alkaline phosphatase treatment[5], UV absorption properties, hypochromicity and ε values as tabulated in Table I.

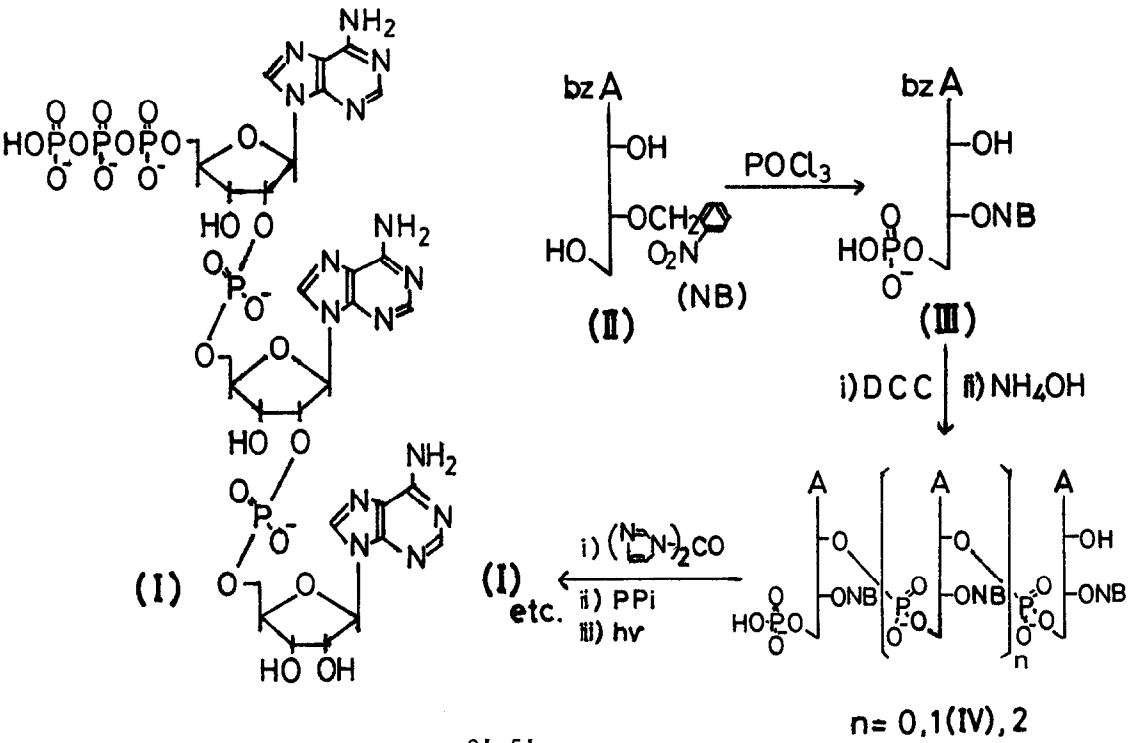

Table I Properties of $pA(^{2'}p^{5'}A)_n$

	Rf[a]	Rf_{pA}[a]	R_{A-pA}[b]	A/pA[c]	H(%)[d]	ε[e]
$pA^{2'}p^{5'}A$	0.34	0.79	1.03			
$A^{2'}p^{5'}A$	0.52	1.20		1/1.10	16.1	12900
$pA(^{2'}p^{5'}A)_2$	0.21	0.50	1.08			
$A(^{2'}p^{5'}A)_2$	0.41	0.95		1/2.08	19.5	12400
$pA(^{2'}p^{5'}A)_3$	0.14	0.33	1.13			
$A(^{2'}p^{5'}A)_3$	0.28	0.65		1/3.13		

(Table I continued)

a. Solvent : n-propanol-conc. ammonia-water (55 : 10 : 35); b. Relative mobility in paper electrophoresis at pH 7.5 as A assumed to be Rm 0.0 and pA as 1.0; c. Ratio of A and pA after venom phosphodiester treatment and paper electrophoresis; d. hypochromicity estimated by alkaline hydrolysis; e. ξ value calculated from ε of pA assumed to be 15400.

Compound IV (triethylammonium salt) was then converted to pyridinium salt by passing through a Dowex 1x2 column (pyridinium form) and dried thoroughly by coevaporation with anhydrous pyridine several times. The residual IV was dissolved in DMF and allowed to react with 1,1'-carbonyldiimidazole[6] (10 equiv.) at room temperature for 14 hrs. After decomposition of unreacted reagent with MeOH, a solution of tri-n-butylammonium pyrophosphate (10 equiv.) in DMF was added and the mixture was kept at room temperature for 24 hrs. After removal of precipitate (probably imidazolium phosphate) by filtration, DMF was evaporated off in vacuo. The residue was dissolved in water and irradiated with UV light over wavelength of 280 nm for 3 hrs. Compound I was isolated by successive paper chromatography and paper electrophoresis in a yield of 40%. The di- and tetramer were obtained by the essentially same method. Properties of these products are summarized in Table II.

Table II Properties of $pppA(^{2'}p^{5'}A)_n$

	Rf[a]	(R_{pA})	Rf[b]	(R_{pA})	Rf[c]	(R_{pA})	(R_{Ap})	R_{A-pA}[d]	ε	H(%)[e]
$pppA^{2'}p^{5'}A$	0.18	(0.58)	0.22	(1.05)	0.16	(0.52)	(0.46)	1.25	12900	16.1
$pppA(^{2'}p^{5'}A)_2$	0.11	(0.35)	0.09	(0.43)	0.10	(0.33)	(0.29)	1.34	11500	25.3
$pppA(^{2'}p^{5'}A)_3$	0.07	(0.23	0.03	(0.14)	0.06	(0.18)	(0.17)	1.40	104000	32.4

a. Solvent, n-propanol-conc. ammonia-water (55 : 10 :35); b. sat. $(NH_4)_2SO_4$ 600g in 0.1M sodium phosphate buffer (pH 6.8) + n-propanol (20 ml); c. n-propanol-conc. ammonia-water (60 : 10 : 30); d. R_{A-pA} stands for relative mobility in paper electrophoresis (pH 7.5) assumed A as Rf 0.0 and pA as 1.0; e. hypochromicity as calculated from alkaline hydrolysis.

Thus the compound I, pppA$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A and related compounds were proved to be identical to natural protein inhibitors obtained previously.[1)]

References

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