

THE SYNTHESIS OF α,γ -DINUCLEOSIDE TRIPHOSPHATES.

THE CONFRONTED NUCLEOTIDE STRUCTURE FOUND AT THE 5'-TERMINUS
OF EUKARYOTE MESSENGER RIBONUCLEIC ACID

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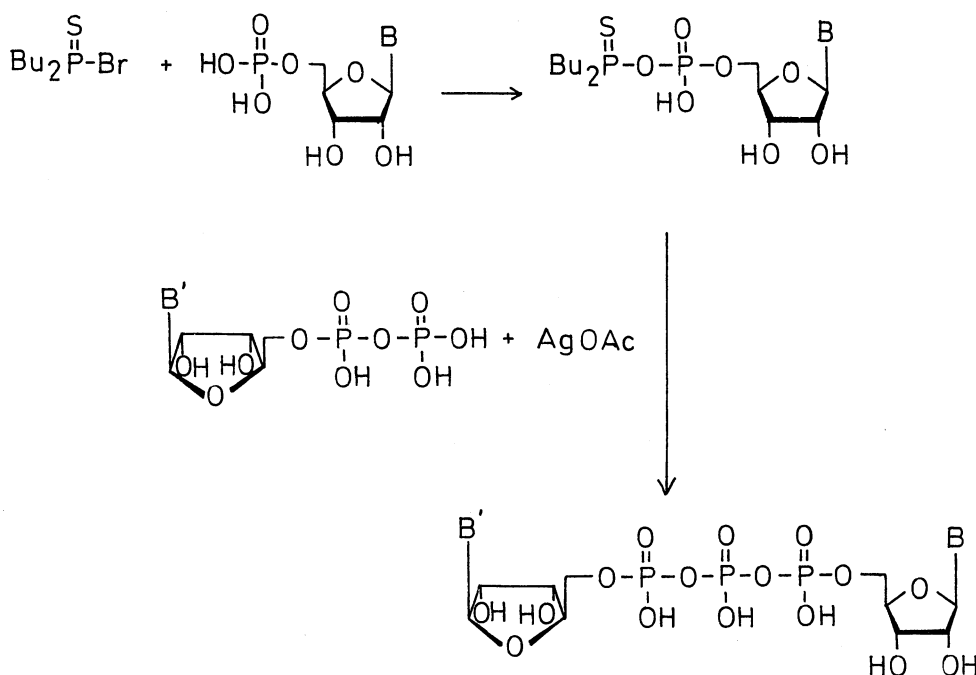
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α,γ -Dinucleoside triphosphates showing the recently discovered modified structure at the 5'-terminus of eukaryote mRNA were synthesized by use of a new method employing di-n-butylphosphinothioyl bromide.

In succession to the discovery of the modified structure at the 5'-terminus of mRNA of the double-stranded RNA containing virus, cytoplasmic polyhedrosis virus,¹⁾ and reovirus,²⁾ and of the double-stranded DNA containing virus, vaccinia virus,^{3,4)} the similar modified structure has been found in several kinds of viral mRNA and cellular mRNA of eukaryote organisms.⁵⁻¹¹⁾ The modified structure consists of the blocking of the terminal phosphates by 7-methylguanylic acid and of methylation of ribose moiety in the first nucleotide of mRNA, although in some cases the latter ribose methylation is not detected. Thus the modified structure is expressed generally as $m^7G^5'pppN(m)pNpNp\dots$ In order to investigate the biological properties of the mRNA, chemical synthesis of the unusual 5'-terminal structure of mRNA would be helpful. Although several methods for the synthesis of α,β -disubstituted diphosphates are available, little attention has been paid to the synthesis of α,γ -disubstituted triphosphates,¹²⁾ especially, no example for the synthesis of unsymmetrical α,γ -disubstituted triphosphates has yet been reported.

We now wish to report a general method for the synthesis of α,γ -disubstituted triphosphates by means of di-n-butylphosphinothioyl bromide which has been developed recently in this laboratory.¹³⁾ In this experiment, α,γ -dinucleoside triphosphates,

such as m^7G^5' pppA, G^5' pppA, G^5' pppG, and m^7G^5' pppG were synthesized as described in the following.



B = adenine, guanine

B' = guanine, 7-methylguanine

For example, adenosine 5'-phosphoric di-n-butylphosphinothioic anhydride (0.1 mmol) prepared from di-n-butylphosphinothiyl bromide (0.2 mmol) and adenosine 5'-phosphate (0.1 mmol), and bis(tri-n-butylammonium) salt of guanosine 5'-diphosphate (0.2 mmol) were mixed and dissolved in dry pyridine. To the solution silver acetate (1 mmol) was added at once. After the mixture was stirred at room temperature for 1 day, dry formamide (0.5 ml) was added and the stirring was further continued for 1 day. Then, an aqueous solution saturated with sodium chloride (1 ml) was added. Precipitate was filtered by using Celite. The filtrate was concentrated to small volume and applied to a filter paper (Toyo Roshi No. 51A). The compound, G^5' pppA, was separated and obtained in 40% yield by paper

electrophoresis using acetic acid - morpholine buffer at pH 3.5.

In a similar manner, $m^7G^{5'}$ pppA, $G^{5'}$ pppG, and $m^7G^{5'}$ pppG were obtained in 30-40% yields. In the above reactions, side-products were the nucleoside 5'-di- and 5'-mono-phosphates. Properties of the synthesized compounds are listed in Table 1.

The structures of these compounds were confirmed by degradation to the corresponding nucleotides with snake venom phosphodiesterase and by comparison with the authentic samples which were prepared by a modification of the procedure of Michelson.¹⁴⁾

Table 1 Chromatographic and Spectral Properties of the α,γ -dinucleoside triphosphates ($N^{5'}$ pppN')

Starting materials	Product	E.P. ^{a)}	Spectral data (in H ₂ O at pH 7)		
			$\lambda_{\text{max}}^{\text{H}_2\text{O}}$	$\epsilon (10^{-4})^{\text{b)}$	$\lambda_{\text{min}}^{\text{H}_2\text{O}}$
pA + ppG	$G^{5'}$ pppA	1.44	255	(2.24)	232
pG + ppG	$G^{5'}$ pppG	1.55	253	(1.37)	225
pp m^7G + pG	$m^7G^{5'}$ pppG	1.00	254	(1.82)	227
pp m^7G + pA	$m^7G^{5'}$ pppA	0.71	259	(2.25)	232

a) E.P. = Electrophoretic mobility relative to guanosine 5'-phosphate. Paper electrophoresis was carried out with an apparatus similar to that by Markham and Smith.¹⁵⁾ The buffer solution used was acetic acid-morpholine at pH 3.5.

b) The α,γ -dinucleoside triphosphates were degraded completely to the corresponding nucleotides by means of snake venom phosphodiesterase (Boehringer Mannheim). The ϵ values were calculated based on the absorbance of mononucleotides after elution of the spot from the paper chromatogram.

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