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One-Pot Synthesis of α, γ -Dinucleoside 5'-Triphosphates, $G^{5'}$pppG and $A^{5'}$pppA, Using *S*, *S'*-Bis(4-chlorophenyl) phosphorodithioate

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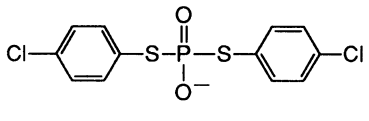
S, *S'*-Bis(4-chlorophenyl) phosphorodithioate was useful for the synthesis of α, γ-dinucleoside 5'-triphosphates, $G^{5'}$pppG and $A^{5'}$pppA starting from the corresponding unprotected nucleoside 5'-phosphates under neutral conditions. $G^{5'}$pppG was used for the synthesis of $m^7G^{5'}$pppG by means of the N^7-methylation of one of two guanine moieties of $G^{5'}$pppG.

Polyphosphate derivatives of nucleoside such as nucleotide coenzymes, the cap structure of mRNAs and diadenosine polyphosphates (Ap_nA) are well known and play important roles in several biological processes. Methods for the synthesis of α, β-dinucleoside 5'-diphosphates have already been well established.[1] However, efficient methods for α, γ -dinucleoside 5'-triphosphates such as the cap structure[2,3] have not yet been established because most of the synthetic methods have involved more than two steps using appropriately protected nucleotides and the triphosphates could not be obtained in large scale due to problems in the separation from the side products. Recently, we have found a simple method for the synthesis of symmetrical α, γ -dinucleoside 5'-triphosphates such as $G^{5'}$pppG and $A^{5'}$pppA in relatively large scale.

S, *S'*-Diphenyl phosphorodithioate has been used effectively for oligonucleotide synthesis by means of the phosphotriester approach.[4] The P-S bonds of the phosphorodithioate can be activated very smoothly by addition of silver ion at room temperature in the presence of water under neutral conditions. Therefore, phenylthio group has been employed as a protecting group in oligonucleotide synthesis and also applied to the substitution reaction with phosphate derivatives to form the pyrophosphates.[5] Since *S*, *S'*-diaryl phosphorodithioate may be regarded as a bifunctional phosphorylating reagent having two arylthio groups which can be activated by silver ion, the symmetrical α, γ-dinucleoside 5'-triphosphate would be obtained in one-pot reaction of *S*, *S'*-diaryl phosphorodithioate with nucleoside 5'-phosphates in the presence of silver nitrate as an activating reagent. In order to find the most suitable arylthio group, several phosphorodithioates were tested, and finally *S*, *S'*-bis (4-chlorophenyl) phosphorodithioate **1**[6] was found to be the most suitable reagent for this purpose.



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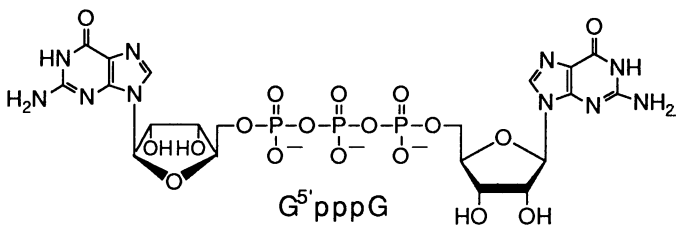
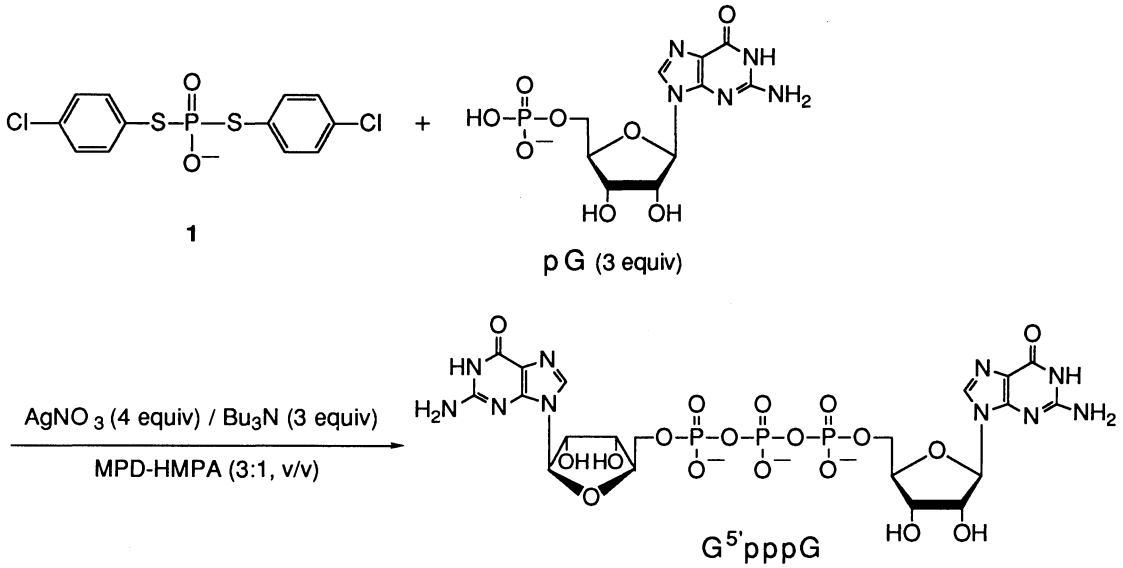


$G^{5'}$pppG

On the other hand, it is known that unprotected guanosine 5'-phosphate (pG) is poorly soluble in aprotic solvents so that reproducible results have not been expected. To overcome this problem, we have examined a lot of aprotic polar solvents. After several screenings, pG was found to be dissolved in a mixture of 1-methylpyrrolidone (MPD)-HMPA(3:1, v/v) and α, γ-diguanosine 5'-triphosphate ($G^{5'}$pppG) could be synthesized as shown in Scheme 1.



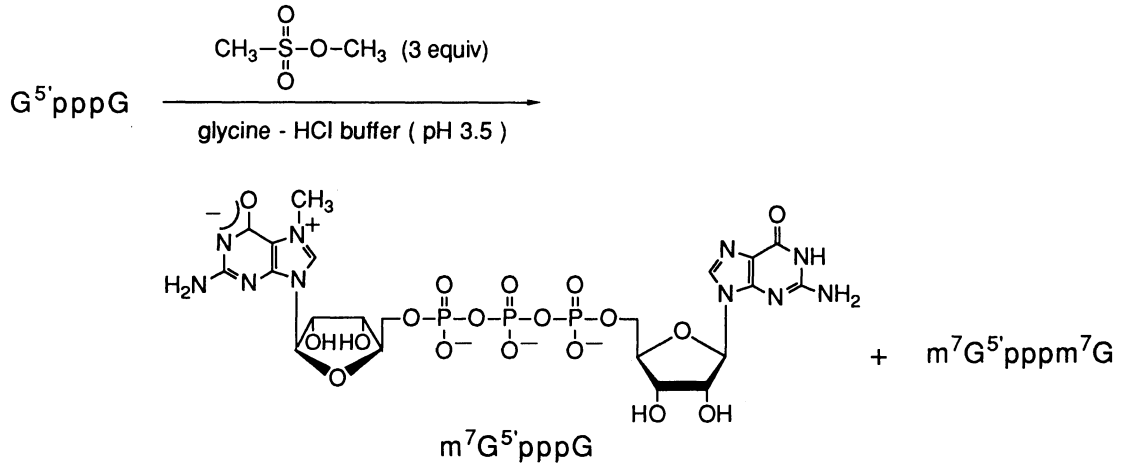
Scheme 1.

To a mixture of **1** (0.2 mmol), guanosine 5'-phosphate (0.6 mmol, 3 equiv) and tributylamine (0.6 mmol, 3 equiv) in MPD-HMPA (3:1, v/v, 8 ml) was added a solution of silver nitrate (0.8 mmol, 4 equiv) in dry pyridine (3.5 ml) at 0 ℃ for 5 h and the solution was stirred at room temperature for 2 h. Silver 4-chlorobenzenethiolate precipitated. According to monitoring with HPLC,[7] $G^{5'}$pppG was formed almost quantitatively. After addition of water, precipitate was filtered off and the filtrate was washed with chloroform. Excess amount of silver ion was removed as silver sulfide by bubbling hydrogen sulfide through the aqueous solution containing $G^{5'}$pppG. It was concentrated and applied to DEAE Sephadex A-25 column chromatography. $G^{5'}$pppG was obtained in 71% yield (117 mg).[8] Although 4-chlorophenylthio group of **1** could also be activated in MPD without addition of HMPA, a small amount of α, β-diguanosine 5'-diphosphate ($G^{5'}$ppG) and guanosine 5'-diphosphate (ppG) was formed. Therefore, the mixed solvent of MPD and HMPA was remarkably effective and essential to avoid the formation of side products for the preparation of $G^{5'}$pppG.

In a similar manner, $A^{5'}$pppA was obtained in 80% yield (135 mg)[9] based on **1** after the purification as described above.

As an application to the synthesis of the cap structure, one of two guanine moieties of $G^{5'}$pppG was methylated to yield $m^7G^{5'}$pppG. Since $G^{5'}$pppG was poorly soluble in polar organic solvents, the N^7-methylation was carried out in a buffer solution.

$G^{5'}$pppG (114 mg, 0.14 mmol) was treated with 3 equiv of methyl methanesulfonate (35 μl, 0.41 mmol) in 1.0 mol dm^{-3} glycine-HCl buffer (pH 3.5, 3.6 ml) at room temperature for 4 d. After the methylation, $m^7G^{5'}$pppG was obtained (Scheme 2). But it was contaminated by a certain amount of permethylated $m^7G^{5'}$pppm^{7}G[10] and unreacted $G^{5'}$pppG (Fig.1).[11] $m^7G^{5'}$pppG was purified by DEAE Sephadex A-25 column chromatography and obtained in 37% yield (43 mg).[12]



Scheme 2.

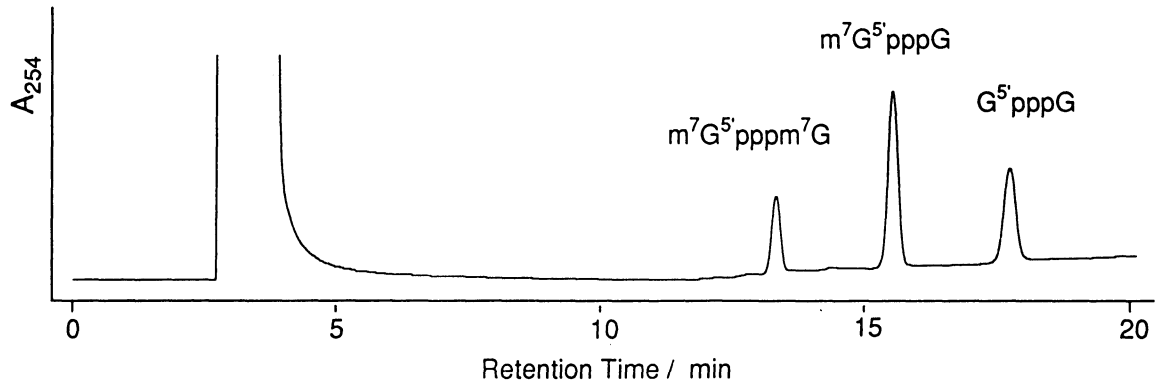


Fig.1. Profile of the anion exchange HPLC (Partisil 10 SAX, Whatman) of the reaction mixture.

In conclusion, it is noteworthy that the symmetrical α, γ -dinucleoside 5'-triphosphate, $G^{5'}$pppG and $A^{5'}$pppA were prepared in high yields by use of **1** in one-pot reaction without employing any protecting groups on the base and sugar moieties of nucleotides. The reaction proceeded at ordinary temperature under neutral conditions. In addition, $G^{5'}$pppG was methylated with methyl methanesulfonate to form the cap structure, $m^7G^{5'}$pppG which is of great value as a primer for the enzymatic synthesis of the capped RNAs.

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7) $G^{5'}$pppG was analyzed by using HPLC under the following conditions; column, Whatman Partisil 10 SAX (25 cm); flow rate, 1 ml/min; buffer, a linear gradient of 5 mM KH_2PO_4 (20% CH_3CN, pH 4.1) to 0.5 M KH_2PO_4 (20% CH_3CN, pH 4.5) for 30 min.

8) NMR data of $G^{5'}$pppG: ^1H NMR (D_2O, 270.05 MHz); δ 8.02 (s, 2H), 5.79 (d, 4.9 Hz, 2H), 4.61 (t, 5.0 Hz, 2H), 4.45 (t, 4.3 Hz, 2H), 4.17-4.35 (m, 6H). ^{31}P NMR (D_2O, 109.25 MHz); δ -10.84 (d, 19.4 Hz, P_α and P_γ), -22.42 (t, 19.4 Hz, P_β), ^{31}P-chemical shifts were given relative to 85% H_3PO_4 as an external standard.

9) NMR data of $A^{5'}$pppA: ^1H NMR (D_2O, 270.05 MHz); δ 8.27 (s, 2H), 8.04 (s, 2H), 5.93 (d, 4.6 Hz, 2H), 4.55(t, 4.9 Hz, 2H), 4.44 (t, 4.3 Hz, 2H), 4.20-4.36 (m, 6H). ^{31}P NMR (D_2O, 109.25 MHz); δ -10.84 (d, 19.4 Hz, P_α and P_γ), -22.32 (t, 19.4 Hz, P_β), ^{31}P-chemical shifts were given relative to 85% H_3PO_4 as an external standard.

10) NMR data of $m^7G^{5'}$pppm^{7}G: ^1H NMR (D_2O, 270.05 MHz); δ 5.87 (d, 3.6 Hz, 2H), 4.49 (t, 4.1 Hz, 2H), 4.36 (t, 5.0 Hz, 2H), 4.20-4.35 (m, 4H), 4.05-4.17 (m, 2H), 3.98 (s, 6H). ^{31}P NMR (D_2O, 109.25 MHz); δ -10.94 (d, 20.8 Hz, P_α and P_γ), -22.46 (t, 20.2 Hz, P_β), ^{31}P-chemical shifts were given relative to 85% H_3PO_4 as an external standard.

11) $G^{5'}$pppG, $m^7G^{5'}$pppG and $m^7G^{5'}$pppm^{7}G were analyzed by using HPLC under the following conditions; column, Whatman Partisil 10 SAX (25 cm); flow rate, 1 ml/min; buffer, a linear gradient of 5 mM KH_2PO_4 (20% CH_3CN, pH 4.1) to 0.5 M KH_2PO_4 (20% CH_3CN, pH 4.5) for 20 min.

12) NMR data of $m^7G^{5'}$pppG: ^1H NMR (D_2O, 270.05 MHz); δ 7.99 (s, 1H), 5.88 (d, 3.3 Hz, 1H), 5.78 (d, 6.3 Hz, 1H), 4.65 (t, 5.6 Hz, 1H), 4.52 (t, 3.6 Hz, 1H), 4.20-4.48 (m, 8H), 4.03 (s, 3H). ^{31}P NMR (D_2O, 109.25 MHz); δ -10.93 (d, 18.4 Hz, P_α and P_γ), -22.50 (bs, P_β), ^{31}P-chemical shifts were given relative to 85% H_3PO_4 as an external standard.

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