Pergamon

Tetrahedron Letters, Vol. 35, No. 7, pp. 1063-1066, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(93)E0406-A

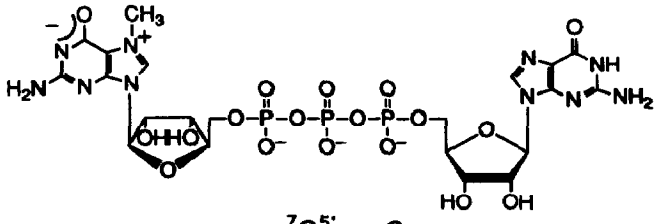
One-pot reaction for the synthesis of $m^7G^{5'}pppG$ and $m^7G^{5'}pppA$ by using an activatable bifunctional phosphorylating reagent

Koichiro Fukuoka, Fuminori Suda, Ryo Suzuki, Hiroshi Takaku†, Masahide Ishikawa and Tsujiaki Hata*

Department of Life Chemistry, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 227, Japan
†Laboratory of Bioorganic Chemistry, Department of Industrial Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275, Japan

Abstract: A new bifunctional phosphorylating reagent 1 was prepared and employed for the syhthesis of the cap structure, $m^7G^{5'}pppG$ and $m^7G^{5'}pppA$ in large scale without using any protecting groups starting from the corresponding nucleoside-5'-phosphates.

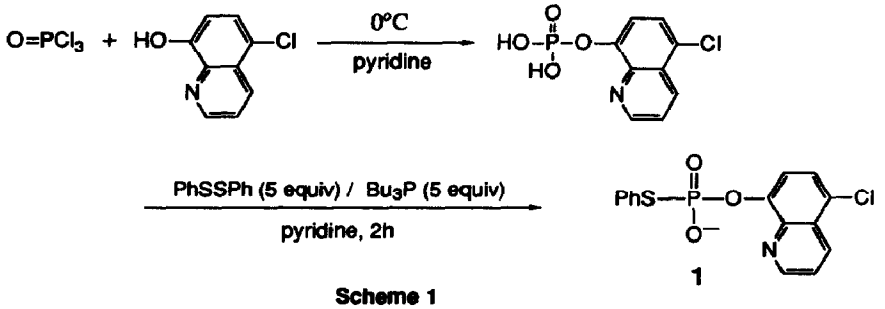
It is well known that eukaryotic mRNAs have the cap structure at their 5'-terminus, which acts as important role in translation. The cap structure, $m^7G^{5'}pppG$ can be applied as the primer to the synthesis of the capped RNAs[1]. Therefore, the synthesis of $m^7G^{5'}pppG$ would be useful for the study on translation. However, the synthesis of a large amount of $m^7G^{5'}pppG$ could not be expected to the separation procedure from the enzymatically degraded products of mRNAs. To extend the study on translation, a convenient chemical method for the synthesis of the cap structure, especially, $m^7G^{5'}pppG$ in large scale should be explored. Up to date, we have reported several methods for the synthesis of capped RNAs[2]. In these methods, the protecting groups were required and introduced to guanosine and 7-methylguanosine on the base and sugar moieties for masking of the functional groups and also increasing the solubility of guanosine derivatives in organic solvents. In this paper, a simple method for large scale synthesis of the cap structure without using any protecting groups was described.



$m^7G^{5'}pppG$

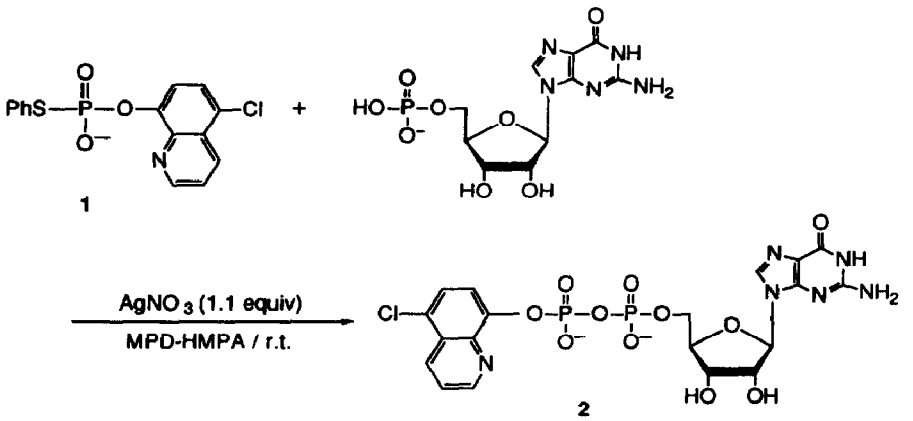
Unprotected guanosine 5'-phosphate(pG) and 7-methylguanosine 5'-phosphate(pm^{7}G) are poorly soluble in aprotic organic solvents so that reproducible results were not expected. To overcome the solubility problem, we have examined a lot of polar organic solvents. Finally we found both of pG and pm^{7}G were solved in a mixture of 1-methylpyrrolidone(MPD)-HMPA(3:1, v/v). In the above mixed solvent, *O*-8-(5-chloroquinolyl)-*S*-phenyl phosphorothioate **1** was proposed as a bifunctional phosphorylating reagent [3]. The phenylthio and 5-chloro-8-quinolyl[4] groups were chosen as activatable groups. Since the phenylthio group could be activated with silver nitrate and the 5-chloro-8-quinolyl group was activated with copper(II) chloride, unsymmetrical triphosphate, m^{7}G$^{5'}$pppG by using **1** with pG and pm^{7}G should be selectively obtained step by step in one flask reaction.

First of all, bifunctional phosphorylating reagent **1** was synthesized as shown in Scheme 1: When a mixture of one equiv of 5-chloro-8-quinolyl phosphate[5] and 5 equiv of diphenyl disulfide in dry pyridine was treated with 5 equiv of tributylphosphine at room temperature for 2 h[6], a new type of bifunctional phosphorylating reagent **1** was obtained. Compound **1** was extracted with chloroform and the solvent was removed. Residual oil was dissolved in dichloromethane containing 1.5 equiv of cyclohexylamine. After addition of acetonitrile, the solution was concentrated and cyclohexylammonium salt of **1** was precipitated as white powder in 84% yield[7]. It was stable enough in refrigerator.



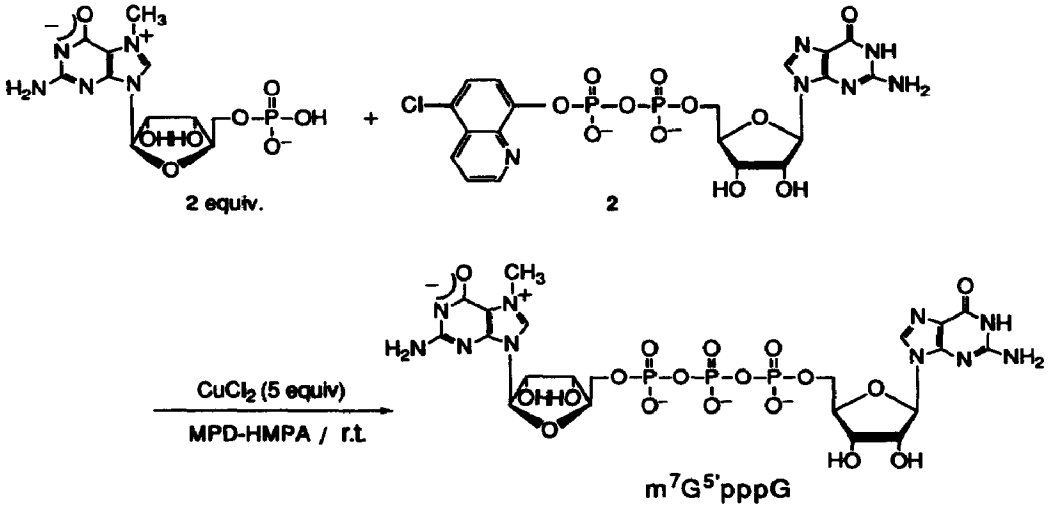
Scheme 1

Next, compound **1** was successfully applied to the synthesis of the cap structure, m^{7}G$^{5'}$pppG: A mixture of **1** (0.41 mmol) and pG (0.41 mmol) was treated with silver nitrate (0.45 mmol, 1.1 equiv) in MPD-HMPA (3:1, v/v, 26ml) at room temperature for 5min. The phenylthio group was activated with a solution of silver



nitrate in pyridine and AgSPh was precipitated. According to monitoring ^{31}P NMR[8] and anion exchange HPLC[9] analysis, the diphosphate **2** was formed quantitatively.

Without isolating **2**, the resulting mixture was treated with pm^{7}G (0.82 mmol, 2 equiv)[10] and anhydrous copper(II) chloride (276 mg, 5 equiv) in MPD at room temperature for 24 h. m^{7}G$^{5'}$pppG was obtained in 55% yield (193 mg) by use of a DEAE Sephadex A-25 column chromatography[11]. Although 5-chloro-8-quinolyl group could also be activated at a relatively high temperature(60 ℃) for 1 h, the yield of the main product, m^{7}G$^{5'}$pppG was decreased. On the other hand, when **1** was treated with copper(II) chloride before treatment with silver nitrate, P^1-S-Phenyl P^2-guanosine-5'-pyrophosphorothioate (PhSppG) and **2** were formed owing to activating both 5-chloro-8-quinolyl group and phenylthio group. Therefore, the order of addition of metal salts is important for the selective synthesis of m^{7}G$^{5'}$pppG.



In a similar manner, when adenosine 5'-phosphate(pA) was employed in place of pG, m^{7}G$^{5'}$pppA was obtained in 53% yield[12] based on **1** after the purification procedure.

In conclusion, it is noted that the procedure described here is very useful for the preparation of nucleoside triphosphate derivatives which have occasionally crucial problems during the synthesis and several hundred miligrams of the cap structure can be obtained. In addition, a mixed solvent of MPD and HMPA is remarkably effective for the preparation of the cap structure. The new bifunctional phosphorylating reagent **1** would be widely applicable to the synthesis of other polyphosphate derivatives. Further investigation is now in progress.

Acknowledgement: This work was supported by the Grant-in-Aid for Scientific Research on Priority Areas No. 04226105 from the Ministry of Education, Science and Culture, Japan.

References and Notes

1. Green, M. R.; Maniatis, T.; Melton, D. A. *Cell* **1983**, 32, 681-694. Konarska, M. M.; Padgett, R. A.; Sharp, P. A. *Cell* **1984**, 38, 731-736. Pelletier, J.; Sonnenberg, N. *Cell* **1985**, 40, 515-526. Nielsen, D. A.; Shapiro, D. J. *Nucleic Acids Res.* **1986**, 14, 5936.

2. Hata, T.; Furusawa, K.; Sekine, M. *J. Chem. Soc., Chem. Commun.* **1975**, 196-197. Hata, T.; Nakagawa, I.; Shimotohno, K.; Miura, K. *Chem. Lett.* **1976**, 987-990. Furusawa, K.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. I* **1976**, 1711-1716. Nakagawa, I.; Konya, S.; Ohtani, S.; Hata, T. *Synthesis* **1980**, 556-557. Yamaguchi, K.; Nakagawa, I.; Sekine, M.; Hata, T.; Shimotohno, K.; Hiruta, H.; Miura, K. *Nucleic Acids Res.* **1984**, 12, 2939-2954. Kamimura, T.; Osaki, Y.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1984**, 25, 2683-2686. Sekine, M.; Nishiyama, S.; Kamimura, T.; Osaki, Y.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, 58, 850-860. Iwase, R.; Sekine, M.; Hata, T.; Miura, K. *Tetrahedron Lett.* **1988**, 29, 2969-2972. Sekine, M.; Iwase, R.; Hata, T.; Miura, K. *J. Chem. Soc., Perkin Trans. I* **1989**, 969-978. Iwase, R.; Maeda, M.; Fujiwara, T.; Sekine, M.; Hata, T.; Miura, K. *Nucleic Acids Res.* **1992**, 20, 1643-1648.

3. van der Marel, G. A.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Tetrahedron Lett.* **1981**, 22, 3887-3890.

4. Takaku, H.; Konishi, T.; Hata, T. *Chem. Lett.* **1977**, 655-658.

5. Takaku, H.; Yamaguchi, R.; Nomoto, T.; Hata, T. *Tetrahedron Lett.* **1979**, 20, 3387-3860.

6. Compound 1 was prepared by a modification of the procedure for *S*-phenyl nucleoside phosphorothioates synthesis: Hata, T.; Sekine, M. *Chem. Lett.* **1974**, 837-838.

7. Data of 1: ^{1}H NMR ($CDCl_3$, 270.05 MHz) δ 8.61 (bs, 3H), 8.40 (dd, J=1.3, 4.3 Hz, 1H), 8.23 (dd, J=1.3, 8.6 Hz, 1H), 7.72 (d, J=8.6 Hz, 1H), 7.41 (dd, J=1.3, 6.8 Hz, 2H), 7.36 (d, J=8.6 Hz,1H), 7.05-7.22 (m, 4H), 2.63 (bs, 1H), 0.92-1.85 (m, 10H). ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 148.9, 147.4, 140.0, 134.9, 133.2, 130.4, 128.5, 127.7, 126.7, 126.4, 124.2, 121.7, 117.5, 50.1, 31.6, 25.1, 24.7. ^{31}P NMR ($CDCl_3$, 109.25 MHz) δ 11.98.

8. ^{31}P NMR data of 2 : ^{31}P NMR (pyridine-d_5, 109.25 MHz) δ -11.17 (d, J=18.6 Hz), -16.05 (d, J=19.7 Hz), ^{31}P-chemical shifts are given relative to 85% H_3PO_4 as external standard.

9. Compound 2 and m^7G^5pppG were analyzed by using HPLC under the following conditions; column: Whatman Partisil 10 SAX (25 cm); flow: 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.

10. pm^7G was prepared from pG and 4 equiv of CH_3I in MPD for 8h. After CH_3I was removed under reduced pressure, a small amount of dry pyridine was added. Since pm^7G was formed quantitatively by this procedure, it was used without further purification.

11. Data of m^7G^5pppG: ^{1}H 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 are given relative to 85% H_3PO_4 as external standard.

12. Data of m^7G^5pppA: ^{1}H NMR (D_2O, 270.05 MHz) δ 8.42 (s, 1H), 8.19 (s, 1H), 6.01 (d, 5.9 Hz, 1H), 5.87 (d, 3.6 Hz, 1H), 4.66 (t, 5.6 Hz, 1H), 4.22-4.54 (m, 9H), 4.00 (m, 3H). ^{31}P NMR (D_2O, 109.25 MHz) δ -10.88 (d, 17.0 Hz, P_α and P_γ), -22.46 (t, 19.4 Hz, P_β), ^{31}P-chemical shifts are given relative to 85% H_3PO_4 as external standard.

(*Received in Japan* 25 *October* 1993)