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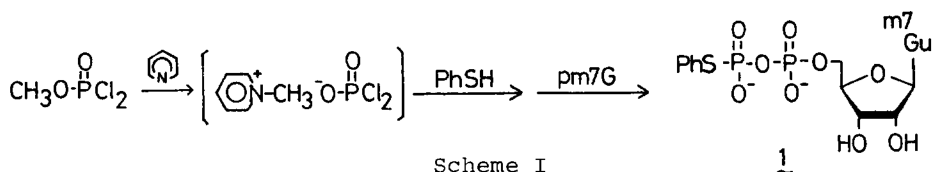
AN EFFECTIVE METHOD FOR THE SYNTHESIS OF THE CAP STRUCTURE OF EUKARYOTIC MESSENGER RIBONUCLEIC ACIDS

Takashi Kamimura, Yumi Osaki, Mitsuo Sekine, and Tsujiaki Hata
Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku Yokohama 227, Japan

Summary: A new promising "capping agent" (9) was synthesized by use of a lipophilic amino protecting group and an activatable pyrophosphate protecting group. This agent reacted homogeneously with 5'-nucleotides in the presence of $AgNO_3$ to afford the cap structures (m7G$^{5'}$pppNu).

The "cap" structure (m7G$^{5'}$pppNupNu···) is well recognized as the common 5'-terminal structure of eukaryotic mRNAs. Biological function of the 5'-terminal 7-methylguanosine triphosphate bridge has extensively been studied in a number of laboratories.[1)] In order to satisfy the requirement of biologists, a convenient and highly reproducible method for the preparation of a "capping agent" in a moderate scale should be explored. Although, several years ago, we reported a capping agent (1), which joined m7G$^{5'}$pp to pNu, by one-pot reaction as shown in Scheme I, the following problems have still remained:



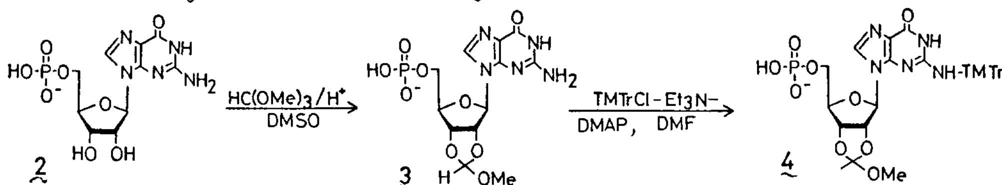
Scheme I

1) The in situ introduction of the PhS-P(O)(O⁻)-O-P(O)(O⁻)- function into 5'-7-methylguanylic acid (pm7G) gives a complicated mixture that causes difficult isolation of the capping agent 1. 2) The reproducibility of the capping reaction is invariably poor since the reaction depends mainly on how to make the anhydrous conditions under which pm7G is extremely insoluble and somewhat unstable. 3) At the purification stage, 1 can be separated only by ion-exchange chromatography using protic solvents. Through the time-consuming operation, 1 has often been decomposed at the alkali-labile 7-methylguanine residue and at the P-S bond.

Therefore, we have tackled to find a more synthetically useful capping agent. In this paper, we wish to report an effective capping agent for the synthesis of m7G$^{5'}$pppNu.

In order to monitor the reactions and reduce laborious efforts of purification, the 2-amino and 2',3'-cis-diol functions of 5'-guanylic acid (2) were protected with the 4,4',4"-trimethoxytrityl (TMTr) and methoxymethylene

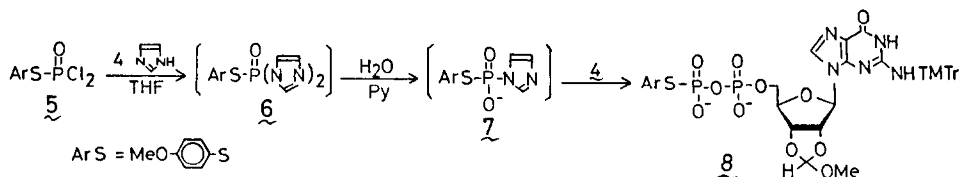
(MM) groups, respectively. The former was chosen because the stability of the N^2-TMTr group was extremely enhanced after the N^7-methylation.[2a] The acid-labile protecting groups were chosen because of the great instability of the 7-methylguanine residue in basic and even neutral media.[3] The protected 5'-guanylic acid (4) was obtained via 3 as shown in Scheme II.



Scheme II

5'-Guanylic acid 2 was treated with trimethyl orthoformate (20 equiv.) and trifluoroacetic acid (60 equiv.) in dry dimethyl sulfoxide at room temperature for 12 h. The excess formate and trifluoroacetic acid were evaporated in vacuo and the residue wsa poured into ether to precipitate the methoxymethylene derivative 3. Compound 3 was further treated with 4,4',4"-trimethoxytrityl chloride (4 equiv.) and triethylamine (4 equiv.), in the presence of 4-dimethylaminopyridine (DMAP, 0.04 equiv.) in dry DMF at room temperature for 4 h. The reaction mixture was poured into ether and the gummy residue was suspended in 1 M triethylammonium bicarbonate buffer (pH 7.0). The desired fully protected derivative 4 could be selectively extracted with methylene chloride—n-BuOH (3:1 v/v) owing to high lipophilicity of the TMTr group. The extracts were combined and the solvent was exaporated in vacuo. The residue was poured into ether to give 4 as an analytical pure triethylammonium salt[4] in 81% yield from 2.

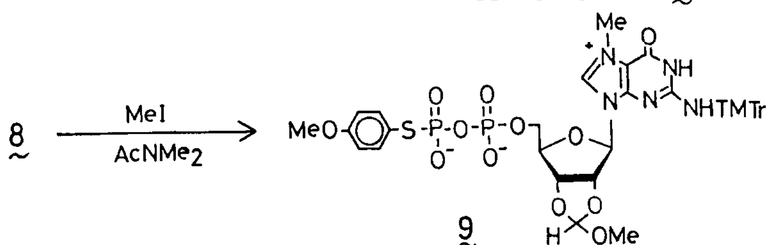
Next, the synthesis of "capping agent" (9) has been investigated. A phosphorylating agent (7) was synthesized as shown in Scheme III. According to Cramer's method,[5] the phosphorodiimidazolide (6) initially obtained from S-phenyl phosphorodichloridothioate (5) was converted to 6 by partial hydrolysis with 1 equiv. of water in pyridine. Without isolation of 7, the protected 5'-guanylic acid was allowed to react with 8 in dry pyridine at room temperature for 1 h to give the desired diphosphate (8) in 83% yield.



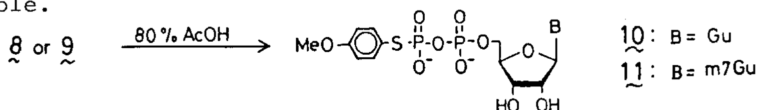
Scheme III

All the reactions shown in Scheme III were monitored by tlc and any byproducts were not detected. Moreover, 7 could be purified readily by silica gel colum chromatography (Merck Kieselgel 60 Silanisielt) developed with solvent system

CH_3CN-conc. NH_4OH-H_2O (18:1:1 v/v/v). The employment of this system reduced the total time required for purification. The introduction of the electron-donating 4-methoxy group to the phenylthio group stabilized the P-S bond and no decomposition was observed during the purification. Finally, the N^7-methylation of 8 was carried out at room temperature by treatment with an excess amount of methyl iodide (30 equiv.) in dimethylacetamide. The reaction was completed after 9 h to afford the new "capping agent" (9) quantitatively.



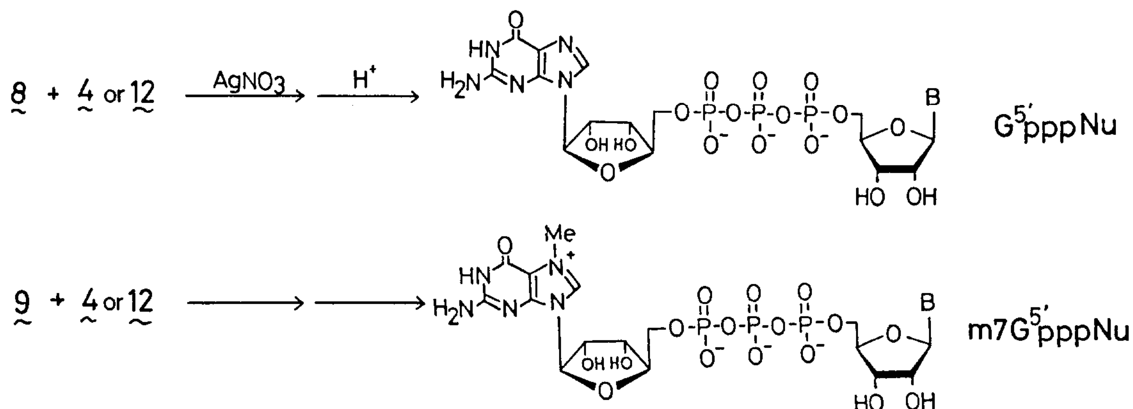
After acid treatment of 8 and 9, the deblocked compounds (10, 11) were isolated by preparative paper electrophoresis. The structures of 9 and 10 were determined by UV and fluorescence spectra, paper electrophoresis, enzymatic assay[6), silver nitrate treatment,[6) and HPLC.[7) The results are shown in the following Table.



Compd.	UV (nm) max	UV (nm) min	Fluorescence (nm) ex	Fluorescence (nm) em	E.P. (pH 3.5) mobility relative to pG	HPLC (min)
10	270 (sh) 244	227	-	-	1.55	6.4
11	278 (sh) 244	228	279	393	0.89	2.2

After evaporation of the excess methyl iodide and dimethylacetamide, the TMTr group was partially (20%) released but the reaction mixture could be used without difficulty in the next capping reaction. The reaction of 8 or 9 with 2 equiv. of 4 or tributylammonium 5'-adenylic acid (12) was performed by activation of the ArS group with silver nitrate (30 equiv.)[a) as shown in Scheme IV. The homogeneous reaction was kept at room temperature for 2 h and then the excess silver ion was removed as Ag_2S by bubbling with hydrogen sulfide gas followed by centrifugation. The supernatant was evaporated and coevaporated with toluene. The residue was further treated with 80% acetic acid at room temperature for 16 h and the resulting mixture was monitored by HPLC and separated by preparative paper electrophoresis. The resulting 7-methylguanosine derivatives were stable during purification and could be easily detected as major bands with fluorescence which was a specific indicator. The desired

triphosphate derivatives were eluted with water and the yields were estimated by UV spectrophotometry. Their physical properties were identical to those previously reported.[2a,b] Thus, $G^{5'}$pppG and $G^{5'}$pppA were obtaind in 72 and 50% yields, respectively. The natural cap structures, m7$G^{5'}$pppG and m7$G^{5'}$pppA were obtained in 45 and 57% yields, respectively, with high reproducibility.



Scheme IV

In conclusion, the present method can provide several hundred miligrams of 8 which is the stable precursor of "capping agent" 9. By preparative paper electrophoresis, sufficient amounts of m7$G^{5'}$pppNu could be obtained for biological studies. The capping reaction with oligoribonucleotides to know the biological meaning of the cap structure is now in progress.

References and Notes

1) a) Y. Furuichi and K. Miura, Nature, 253, 374 (1975); b) A. J. Shatkin, Cell, 9, 645 (1976); c) K. Miura, Adv. Biophysics, 14, 205 (1981).
2) a) T. Hata, I. Nakagawa, K. Shimotohno, and K. Miura, Chem. Lett., 987 (1976); b) I. Nakagawa, S. Konya, S. Ohtani, and T. Hata, Synthesis, 556 (1980); c) T. Hata, N. Gokita, N. Sakairi, K. Yamaguchi, M. Sekine, and Y. Ishido, Bull. Chem. Soc. Jpn., 55, 2949 (1982); d) T. Kamimura, M. Sekine, and T. Hata, Chem. Lett., 951 (1983); e) M. Sekine, T. Kamimura, and T. Hata, submitted for publication to J. Org. Chem.
3) J. A. Haines, C. B. Reese, and L. Todd, J. Chem. Soc., 5281 (1962).
4) ^{1}H-nmr (d_6-DMSO): δ 1.15(t, 9, J=8Hz, CH_3CH_2-N), 3.02(m, 6, CH_3CH_2-N), 3.20, 3.27(s, 3, $C\underline{H}_3OCH_2$), 3.78(s, 9, CH_3O- of TMTr group), 4.20-4.40(m, 3,4'-H and 5'-H), 4.70-4.90(m, 2, 2'-H and 3'-H), 5.65(d, 1, J=4Hz, 1'-H), 6.03 (s, 1, $CH_3OC\underline{H}_2$), 6.96(d, 6, J=9Hz, 2-H and 6-H of TMTr group), 7.23(d, 6, 3-H and 5-H of TMTr group), 7.80(br s, 1, 2-NH), 7.87(s, 1, 8-H). Anal. Calcd for $C_{40}H_{51}N_6O_{12}P \cdot H_2O$: C, 56.07; H, 6.23; N, 9.81. Found: C, 56.15; H, 5.90; N, 9.73.
5) F. Cramer, H. Schaller, and H. A. Staab, Chem. Ber., 94, 1612 (1961). H. Schaller, H. A. Staab, and F. Cramer, ibid. 94, 1621 (1961).
6) Enzymatic and chemical treatments of 10 or 11 with venom phosphodiesterase and with I_2 resulted in pG or pm7G and ppG or ppm7G, respectively.
7) The purity of the samples was analyzed by using HPLC under the following conditions; colum: Permaphase AAX (2 mmϕ x 1 m); Buffer: a linear gradient of 0.005 M KH_2PO_4 (pH 4.1) to 0.05 M KH_2PO_4-0.5 M KCl (pH 4.5) for 32 min.

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