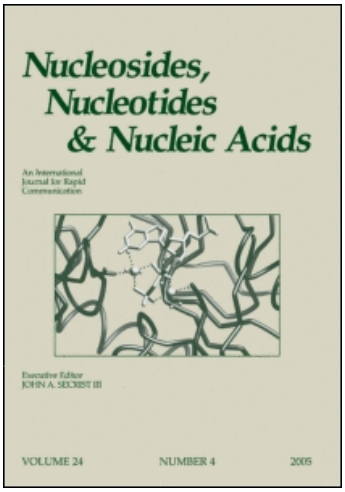

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SYNTHESIS OF ^{3}H AND ^{13}C LABELED mRNA CAP DINUCLEOTIDES—USEFUL TOOLS FOR NMR, BIOCHEMICAL, AND BIOLOGICAL STUDIES

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□ *For deeper understanding the roles of the mRNA cap structure in cellular processes isotopically labeled dinucleotide cap analogues have been synthesized as tools for NMR and in vivo studies. Tritium or carbon C-13 labeled methyl iodide was used as a source of the isotope material. In order to minimize the number of steps during the radioisotopic synthesis the methylation with tritium labeled methyl iodide was performed with Gp_3G as a substrate. The C-13 isotope was introduced into the cap dinucleotide by methylation of GDP with C-13 Methyl iodide, followed by coupling the product with guanosine 5′-phosphorimidazolide in DMF with zinc chloride as a catalyst.*

Keywords 5′ mRNA Cap; tritium; carbon 13; isotope labeling

RESULTS AND DISCUSSION

The cap structure at the 5′-end of eukaryotic mRNA plays a pivotal role in several cellular processes,[1] including RNA splicing, nucleocytoplasmic transport, efficient translation, and protection against degradation by cellular 5′-exonucleases. All these processes are extensively studied in vitro using synthetic cap analogues. We have recently extended our study to in vivo in order to better understanding of these processes occurring inside the cells.[2] A major disadvantage of the in vivo experiments is a difficulty with detection of the cap analogues inside the cell. Thus, we can neither verify that such an analogue remains intact over the course of the measurement, nor we know its intracellular concentration. We have addressed this problem by synthesizing isotope-labeled cap analogues. We have developed simple and efficient methods of the synthesis of m^7GpppG labeled in N7-methyl group of 7-methylguanosine moiety either with

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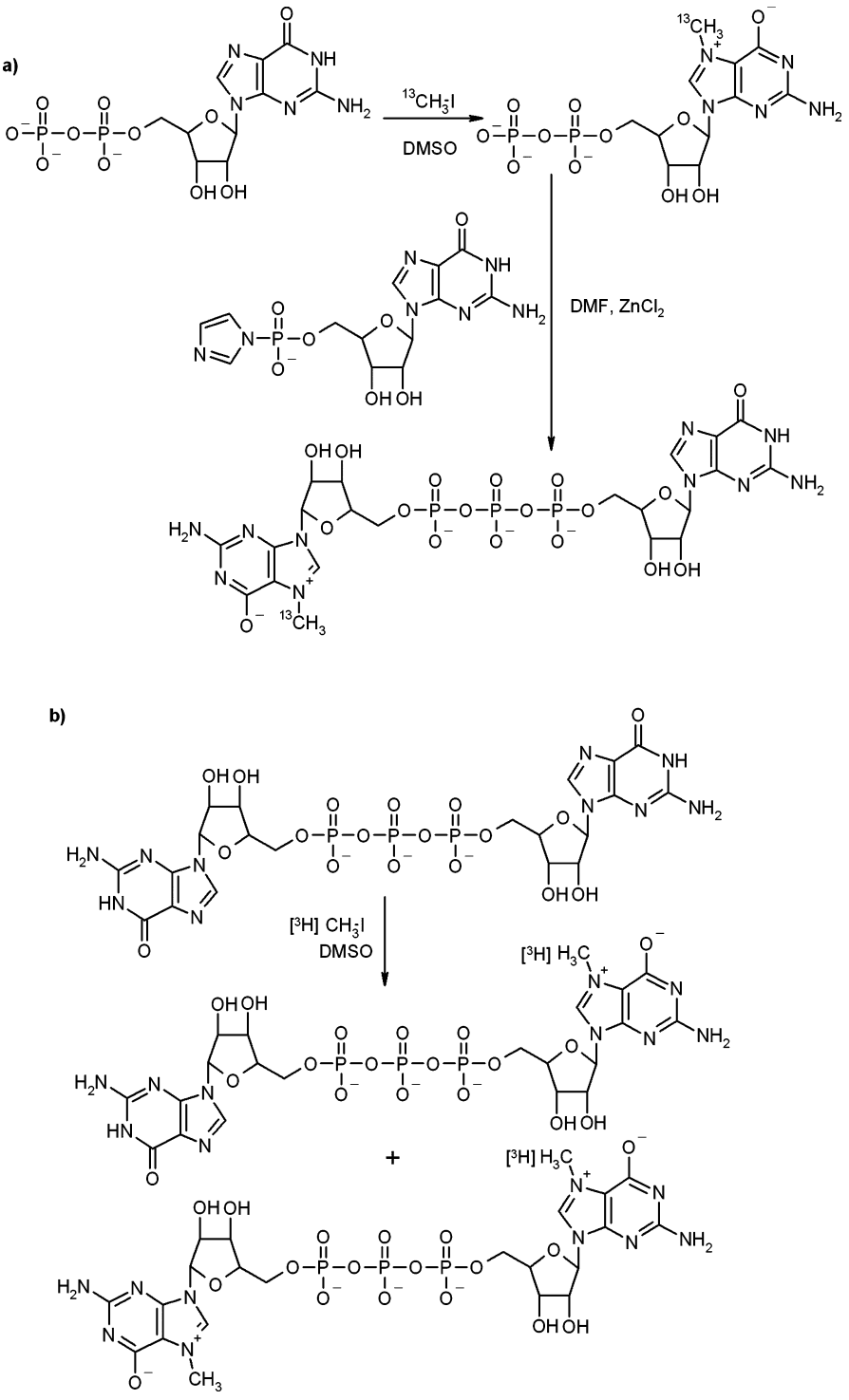

SCHEME 1 Syntheses of m^7Gp_3G labeled at N7-methyl group with: a) ^{13}C and b) ^3H.

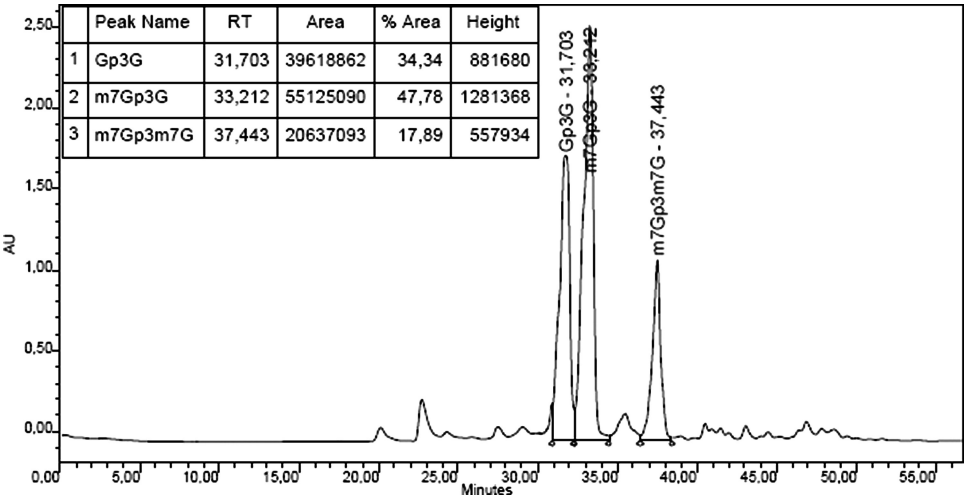

FIGURE 1 400 MHz ^{1}H NMR and ESI-MS spectra of [7-^{13}C] methylguanosine(5′)triphospho (5′)-guanosine (m^7Gp_3G).

carbon ^{13}C or tritium (^{3}H), for NMR and biochemical studies, respectively. As a source of isotopic material appropriately labeled methyl iodide was used in both cases. The synthesis of ^{13}C labeled cap analogue was as follows (Scheme 1a): Guanosine 5′-diphosphate was methylated with [^{13}C] methyl iodide gave [7-^{13}C]-methylguanosine 5′-diphosphate, and the product was coupled with guanosine 5′ phosphorimidazolide in DMF containing zinc chloride.[3,4] According to MS and NMR studies (Figure 1) the enrichment of ^{13}C isotope in 7-methyl group of the final product [7-^{13}C]-methylguanosine(5′)triphospho(5′)-guanosine, was over 99%.

In the case of radioisotopically labeled mRNA cap analogues, in order to minimize the number of steps with the radioactive material an alternative route of synthesis was used (Scheme 1b) Gp_3G was methylated with equimolar quantity of [^{3}H]-methyl iodide. Similar approach was applied previously for the synthesis of unlabeled mRNA cap analogues, where 3-fold molar excess of "cold" methyl iodide was used.[5,6] After 6 hours the reaction was quenched at 50% yield of the desired product ([7-^{3}H]-m^7Gp_3G), 20% of the double methylated by-product ([7-^{3}H]-$m^7Gp_3m^7G$), and 25% of unreacted Gp_3G, as shown by HPLC (Figure 2). Both radiolabeled products were purified using preparative RP HPLC, and their specific activity after lyophilization was determined using a scintillation counter and liquid scintillators for the aqueous samples (8.4 MBq/mg).

In summary, we have synthesized dinucleotide cap analogues specifically labeled in N7-methyl group of 7-methylguanosine moiety with C-13 and tritium. The C-13 labeled analog shows high enrichment of C-13 isotope in N7-methyl position (over 99%). Tritium labeled cap analogue was synthesised according to procedure in which only one step involved use of

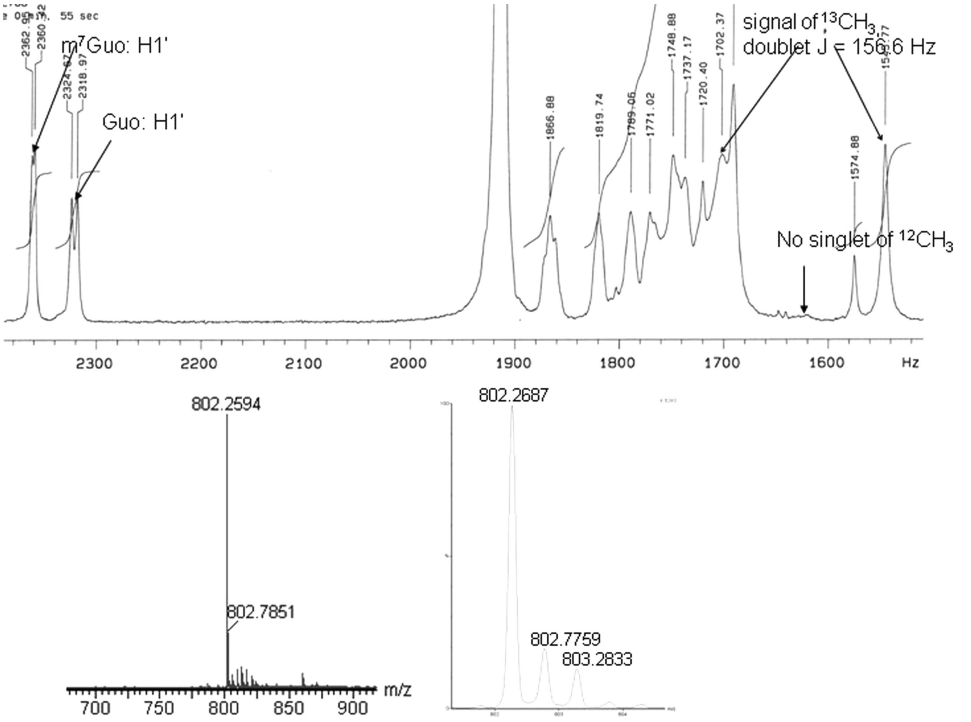

FIGURE 2 HPLC separation of the reaction mixture on RP column, Waters Nova-Pak C18, 6 mm 19 × 300 mm, with a linear gradient of B, 0% to 100% in A, for 45 minutes; A, 0.05 M CH_3COONH_4, pH = 5.9; B, 1:1 (v/v) mixture of buffer A and methanol.

radioisotopes. The product was obtained with moderate yield and relatively high specific activity (7.17 GBq/mmol). Both dinucleotides appeared to be useful tools for studying the processes in which mRNA cap is involved. Their use in NMR and in vivo studies is under investigation and will be reported elsewhere.

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