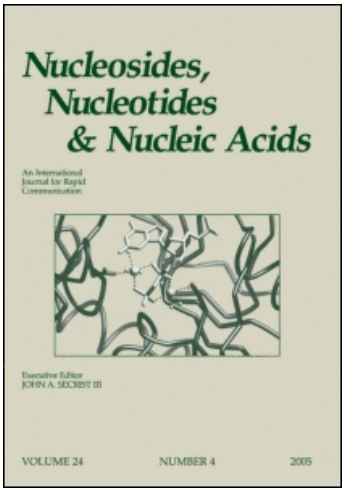

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An Industrial Process for Selective Synthesis of 7-methyl Guanosine 5'-Diphosphate: Versatile Synthon for Synthesis of mRNA Cap Analogues

Anilkumar R. Kore[a]; Gaurang Parmar[a]
[a] Bioorganic Chemistry Division, Ambion, Inc., Austin, Texas, USA

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AN INDUSTRIAL PROCESS FOR SELECTIVE SYNTHESIS OF 7-METHYL GUANOSINE 5′-DIPHOSPHATE: VERSATILE SYNTHON FOR SYNTHESIS OF mRNA CAP ANALOGUES

Anilkumar R. Kore and Gaurang Parmar □ *Ambion, Inc., Bioorganic Chemistry Division, Austin, Texas, USA*

□ *We report an industrial scale facile synthesis of 7-methyl guanosine 5′-diphosphate, which plays an important role in synthesis of various mRNA cap analogs. An efficient and selective methylation at position 7 of guanosine 5′-diphosphate was achieved by dissolving guanosine 5′-diphosphate in water and drops wise addition of dimethyl sulfate over a period of 1 h at room temperature. The reaction was completed within 2 h and resulted in more than a 96% yield. The desired product, 7-methyl GDP was purified by using BPG column on AKTA Purifier 100. Certainly, this method has advantages over the known methylation method, in terms of yield, economy, safety, and environmental concerns.*

Keywords Guanosine 5′Diphosphate; GDP; 7-Methyl Guanosine 5′-Diphosphate; 7mGDP; Cap Analog

INTRODUCTION

Alkylation of nucleic acids and their components has been the subject of many chemical and biological studies. A substantial number of N-methylpurines have been isolated from various biological sources and identified in recent years.[1] The most interesting aspects of the problem have arisen from the discovery of various kinds of methylated ribonucleosides from RNA,[2] and from studies of mutagenic and carcinogenic effects that were observed in living systems upon administration of alkylating agents.[3]

In the course of our continued efforts, to establish an industrial-scale synthesis of various cap analogs, the main challenging synthon was methylation of guanosine 5′ diphosphate at position 7. Our attention has been primarily on selective N 7 methylation of GDP, and there are very few reports available on selective methylation of GDP. Recently, methylation of guanosine

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Address correspondence to Anilkumar Kore, Ambion, Inc., Bioorganic Chemistry Division, 2130 Woodward St., Austin, TX, 78744. E-mail: akore@ambion.com

5′-diphosphate has been reported[4] at position 7, as a one of the valuable intermediate in the synthesis of various cap analogs. In this report, a mixture of dimethylformamide and dimethylsulfoxide to dissolve GDP has been used, along with methyl iodide as an alkylating reagent and reported yield was 28%. We tried the methylation under similar conditions, but the GDP itself never dissolved into mixture of DMF and DMSO. This could be due to the fact that GDP might require into certain form of different counter ions, which is not clear in their experiment. In another report[5] for the synthesis of chain terminating dinucleotide mRNA cap analog, they have carried out methylation of guanosine 5′ monophosphate at position 7 by dissolving pyridinium salt of GMP into 1-methylpyrrolidone (MPD) using methyl iodide as a alkylating agent.

In this communication, we wish to report an industrial-scale selective synthesis of 7-methyl guanosine 5′-diphosphate, which has a considerable value in a variety of applications. 7mGDP plays an important role in synthesis of various mRNA cap analogs. Eukaryotic messenger RNA contains the cap structure, 7mGpppN (where N is any nucleotide), in which 7-methylguanosine is linked to the 5′ end of RNA via a 5′-5′ triphosphate bridge.[6,7] Cap plays critical roles in many aspects of messenger RNA metabolism, including processing, nuclear transport, translation, and the protection of mRNA from untimely degradation.[8,9]

RESULTS AND DISCUSSION

In our search for a short and efficient convergent synthesis toward selective methylation at position 7 of guanosine 5′ diphosphate, here we describe an efficient method for the large-scale synthesis of 7-methyl guanosine 5′-diphosphate. In a typical experimental procedure 10 g (20.5 mmol) of guanosine 5′-diphosphate, either in free acid or sodium as a counter ion form, was dissolved in 200 mL of water and pH was adjusted to 4.0 with glacial acetic acid. Over a period of 1 h dimethyl sulfate 20 mL (119.04 mmol) was added with constant stirring at room temperature and the reaction was allowed to continue for an additional 1 h (Figure 1). As the reaction proceeds, the pH of the reaction tends to go more acidic below 3, but it was kept constant between 3.8 to 4.0 by drop-wise addition of 10 mM NaOH. During the course of the reaction, it was monitored by analytical HPLC for its progress and it was revealed that the methylation was completed 98% within 2 h. In order to remove the excess dimethyl sulfate from the reaction mixture, 200 mL of chloroform was added and the reaction mixture was extracted in a separatory funnel. The extraction was repeated three times (3 × 200 mL of chloroform).

The resulting aqueous layer was further evaporated on a rotary evaporator to get rid of any chloroform traces, and the aqueous layer was further diluted up to a 1.5 L with water and loaded on anion exchange resin, i.e.,

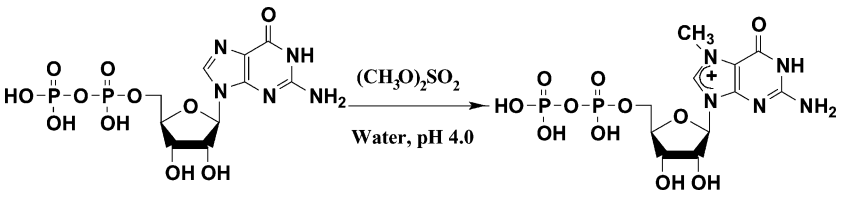

FIGURE 1 Selective methylation of guanosine 5′-diphosphate (GDP) at the N-7 position.

DEAE Sepharosa fast flow packed in a BPG 100 column. (Amersham GE, Piscataway, NJ, USA). Specification of BPG (biological process glass) 100/500 column, where 100 is a column diameter (mm) and 500 is a column height in mm (50 cm), was packed with DEAE Sepharosa fast flow resin up to the bed volume 400 mm. The desired compound was eluted by using four bed volumes of gradient from 0 to 80% of 1 M TEAB buffer pH 7.5. (triethylammonium bicarbonate) at a flow rate of 100 ml/min, by using AKTA purifier 100 FPLC (Amersham GE). At 45% of TEAB buffer, the desired product (7-methylguanosine 5′-diphosphate) was eluted a large broad peak, which showed a strong ultraviolet absorbance at 254 nm. Considering the stability and possibility of imidazole ring opening in alkaline solution,[10] the purification conditions were developed and well suited for the purification of positively charged imidazole ring in guanosine 5′-diphosphate. The latter fractions, about 3 L, were combined and subjected to evaporation under reduced pressure by using a Rotary Evaporator LR 20 (Heidolph Electro GmbH & Co., Kelheim, Germany). The excess TEA salt was removed by co-evaporating with methanol 3 × 600 mL. The resulting residue was placed in a centrifuge tube, and sodium perchlorate 8.9 g dissolved in acetone 1.1 L were added. After cooling for 2 h in a refrigerator, the mixture was centrifuged and the supernatant was discarded. The precipitate was ground with new portion of acetone, cooled, and centrifuged again. The process was repeated once more, and the precipitate was dried in a vacuum desiccator over P_2O_5. The 7 methylguanosine 5′-diphosphate thus obtained was an amorphous white powder, 8.7 g in weight and overall after purification gave 87% yield, and the purity was 98% by analytical HPLC. The spectroscopic analysis data from ^{1}H NMR (D_20, 400 MHz) and ^{31}P NMR (D_2O, 161 MHz) obtained from Spectral Data Services, Inc., was in well agreement with the previously published data.[11]

We were profoundly interested in developing an efficient synthetic route for the selective methylation of guanosine 5′-diphosphate because of its versatility and considerable importance in synthesis of various cap analogs on an industrial scale.

CONCLUSION

An efficient industrial process for the selective methylation of guanosine 5′-diphosphate at position N 7 has been accomplished in high purity and

yield by employing simple reaction conditions and inexpensive reagents. Purification of 7m GDP has been achieved by using anion exchange resin without destabilizing the N 7 substituted, positively charged imidazole ring in guanosine 5′-diphosphate. An improved process has been successfully implemented in pilot plant with reproducible results.

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