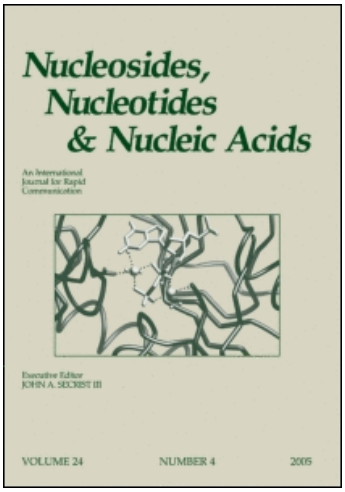

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Synthesis of Novel mRNA 5′ Cap-Analogues: Dinucleoside P^1, P^3-Tri-, P^1, P^4-Tetra-, and P^1, P^5-Pentaphosphates

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ABSTRACT

A series of new mRNA anti reverse cap analogues (ARCA) was designed to obtain a tool for studying the mechanism of protein translation. Dinucleoside P^1, P^3-tri-, P^1, P^4-tetra- and P^1, P^5-pentaphosphates, linked by a 5′-to-5′ phosphate bridge and composed of modified 7-methylguanosine and guanosine, have been synthesized. The hydroxyl group (2′OH or 3′OH) in 7-metylguanosine moiety was replaced by $-OCH_3$ or -H in order to obtain the cap analogues capable to be correctly incorporated into synthetic mRNA transcripts. Tri-, tetra-, and pentaphosphates were prepared by $ZnCl_2$ catalyzed condensation in DMF of derivatives of the 7-methylguanosine diphosphates with the guanosine mono-, di- and triphosphate P-imidazolides, respectively. The structures of the novel compounds were established by means of ^1H and ^{31}P NMR spectra.

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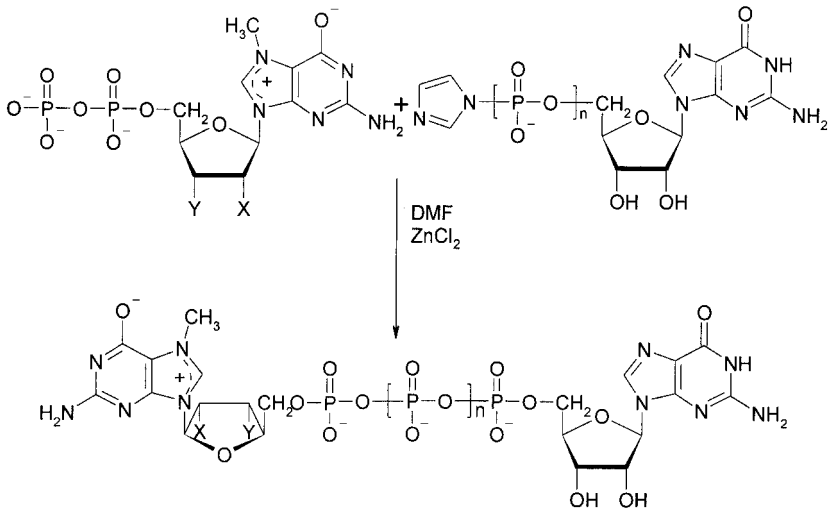
INTRODUCTION

The 5′-cap structure is present in almost all eukaryotic mRNAs. Among its several regulatory functions, cap plays an essential role in facilitating recruitment of the ribosome to the starting codon during the translation initiation.[1] This process occurs by interaction of 5′-cap with a cap binding protein, eukaryotic initiation factor 4E (eIF4E).[2] Synthetic analogues of the mRNA cap proved to be very useful in studying eIF4E function in cap-dependent translation.[3] The dinucleoside triphosphates, with their most representative member m^7 GpppG, are widely used in in vitro synthesis of 5′ capped mRNAs and U snRNAs by bacteriophage RNA polymerases.[4] Such synthetic transcripts are widely applied in search for mechanisms of protein translation, RNA transport, and pre-mRNA splicing.[5] However, it was found that one-third to one-half of the cap is incorporated in the unwanted, reverse orientation, with the m^7 Guo moiety linked by a 3′-5′ phosphodiester bond to the first nucleoside of the RNA chain.[6] Recently we synthesized two novel analogues in which 3′OH group in m^7 Guo was replaced by either 3′OCH_3 or 3′deoxy.[7] These modifications made the anti-reversed cap analogues (ARCAs) incapable of being incorporated in the reverse orientation. ARCAs retained all their inhibitory activity as compared with unmodified m^7 GpppG. Moreover, the translation efficiency of



Abbreviation	X	Y	n
$m^7$3'OMeGp$_3$G	OH	OMe	1
$m^7$3'dGp$_3$G	OH	H	1
$m^7$2'dGp$_3$G	H	OH	1
$m^7$2'OMeGp$_3$G	OMe	OH	1
$m^7$3'OMeGp$_4$G	OH	OMe	2
$m^7$2'OMeGp$_4$G	OMe	OH	2
$m^7$2'dGp$_4$G	H	OH	2
$m^7$3'OMeGp$_5$G	OH	OMe	3

Scheme 1. Synthesis of dinucleoside P^1, P^3-tri-, P^1, P^4-tetra- and P^1, P^5-pentaphosphates in coupling reaction catalyzed by $ZnCl_2$.

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ARCA-capped transcripts was 2-fold higher than for the m^7 GpppG-capped transcripts,[7] what renders the ARCA containing RNA transcripts very promising in many areas of gene expression research. We present here the synthesis of a new series of dinucleoside tri-, tetra- and pentaphosphates with modifications at 2′ and 3′ positions of 7-methylguanosine.

RESULTS

3′-Deoxyguanosine (purchased from Sigma-Aldrich), 2′-O- and 3′-O-methylguanosine obtained as described earlier[8] and 2′-deoxyguanosine 5′-monophosphate (from Calbiochem) were the starting compounds for the synthesis of ribose-modified 7-methylguanosine 5′-diphosphate, the key intermediate in preparation of cap analogues. 3′-O-Methylguanosine-, 2′-O-methylguanosine- and 3′-deoxyguanosine 5′-monophosphates were prepared according to the standard Yoshikawa's phosphorylation.[9] Monophosphates were converted into diphosphates in a two-step procedure that comprised preparation of nucleoside 5′-monophosphate imidazolides[10] and their reaction with triethylammonium phosphate.[7] Diphosphates of the modified guanosine nucleosides were methylated with methyl iodide in DMSO.[11] The crucial step in the synthesis of the dinucleotide 5′, 5′-tri-, tetra- and pentaphosphates was coupling of ribose-modified 7-methylguanosine 5′-diphosphates with the corresponding imidazolide of guanosine 5′-phosphates.[7] The coupling reaction was carried out in dimethylformamide, in the presence of $ZnCl_2$ as a catalyst.[12] These reaction conditions improved significantly the coupling efficiency and allowed to avoid decomposition of the unstable imidazolides. Imidazolides of the guanosine 5′-mono-, di- and triphosphates were prepared by condensation of GMP, GDP and GTP, respectively, with imidazole, using 2,2′-dithiodipyridine and triphenylphosphine as a condensing agent.[10]

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