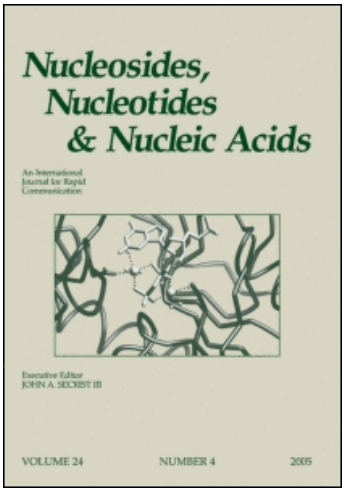

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A Novel Approach to Solid Phase Chemical Synthesis of Oligonucleotide mRNA Cap Analogs

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A NOVEL APPROACH TO SOLID PHASE CHEMICAL SYNTHESIS OF OLIGONUCLEOTIDE mRNA CAP ANALOGS

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▫ *A novel approach for the synthesis of 5′-capped 2′-O-methyloligoribonucleotides on a disulfide-tethered solid support is described. The key step of the synthesis is $ZnCl_2$ promoted coupling of m^7GDP imidazolide to a fully deprotected oligonucleotide 5′-phosphate on-support. By this methodology $m^7G^{5'}pppm^{2'}Apm^{2'}Upm^{2'}Ap$ has been prepared.*

Keywords Oligonucleotide Synthesis, mRNA Cap Analogs, Disulfide Linker, Solid Support

INTRODUCTION

So far oligonucleotides bearing a 5′-terminal 7-methylguanosine 5′-triphosphate *cap* have been prepared in solution-phase,[1–5] since the presence of highly base-labile 7-methylguanine residue excludes conventional approaches of solid-supported oligonucleotide synthesis. In fact, the only example of a successful synthesis of 5′-capped oligonucleotides on a solid support is offered by preparation of a N^2,N^2,7-trimethylguanosine capped 2′-*O*-methyltrinucleotide, $m_3^{2,2,7}G^{5'}pppm^{2'}Apm^{2'}UpA$, by Kadokura et al.[6] The trinucleotide was assembled on a phosphoramidate linker and converted to its 5′-diphosphate by two successive phosphorylations. The base moiety protections were then removed and the cap

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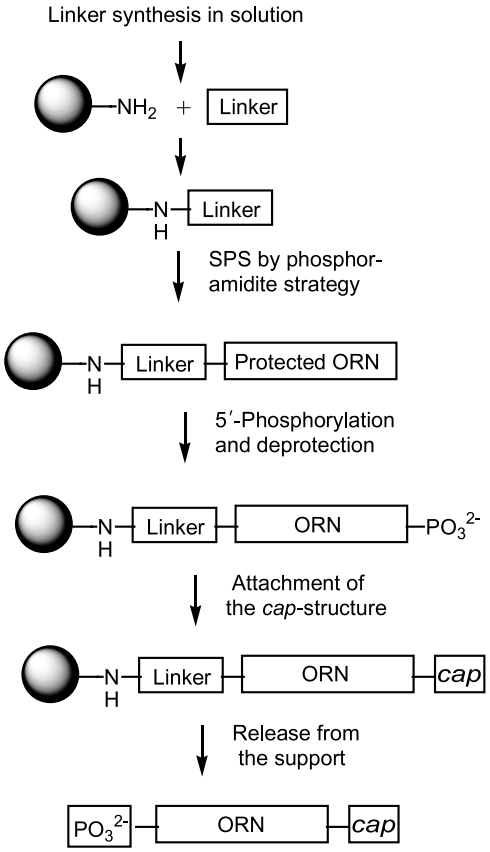
nucleoside was introduced as a 2′,3′-phenylboronylated 5′-phosphoroimidazolide. Finally, the conjugate was released with acetic acid, the 2′-*O*-TBDMS protection of the 3′-terminal nucleoside was removed with hydrogen chloride, and the 3′-terminal phosphate with alkaline phosphatase. We now report on an alternative approach exploiting a disulfide linker and unprotected 7-methylguanosine 5′-diphosphoroimidazolide as a capping agent.

RESULTS AND DISCUSSION

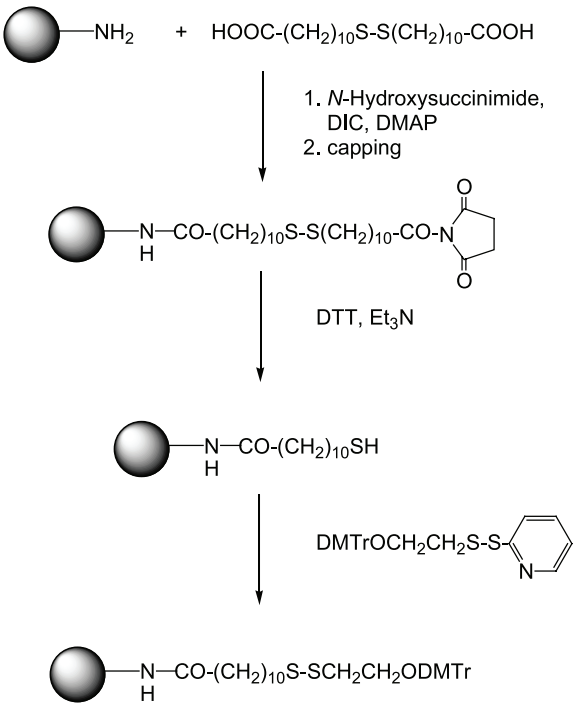
The general course of the synthesis of $m^7G^{5'}pppm^{2'}Apm^{2'}Upm^{2'}Ap$ is outlined in Scheme 1. The disulfide linker allows the chain assembly by normal phosphoramidite chemistry withstands removal of base protections by ammonolysis and may be reductively cleaved under mild conditions.[7] The 2-mercaptoethyl group that remains bonded to the 3′-terminal phosphate upon cleavage of the disulfide bond is released as episulfide at slightly basic pH.



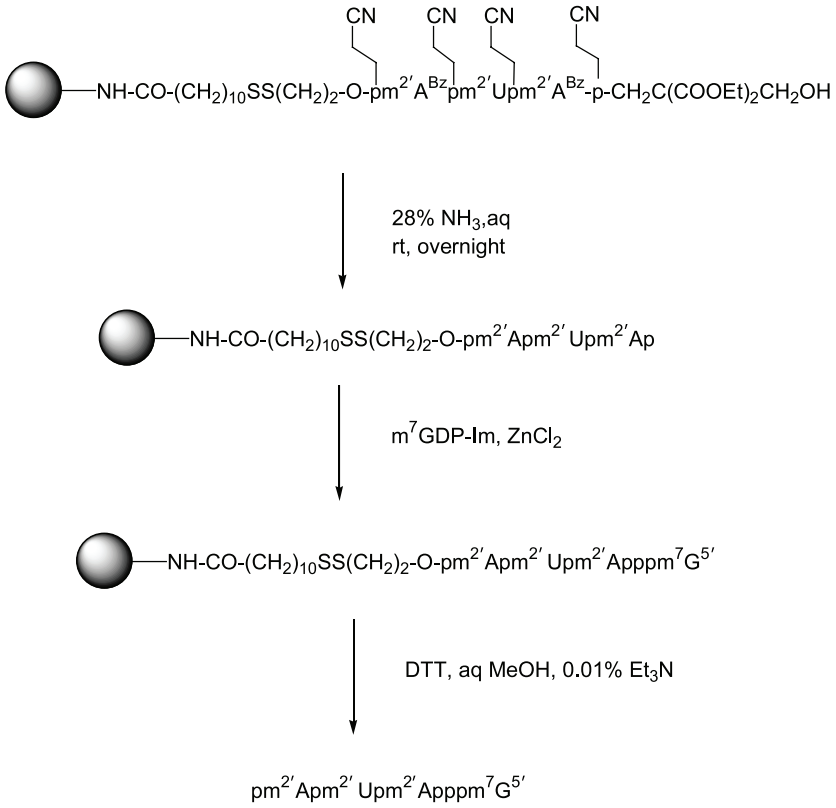
SCHEME 1 Strategy for the synthesis of cap oligonucleotides on solid support.

The linker was synthesized as depicted in Scheme 2. Accordingly, 11-mercaptoundecanoic acid, prepared as described earlier,[7] was allowed to dimerize to a disulfide and this was then immodized by diisopropylcarbodiimide (DIC)/*N*-hydroxysuccinimide activation to an amino-functionalized support (Tentagel, 260 μmol/g) as a disulfide dimer. The disulfide bond was reductively cleaved with DTT and 2-pyridylsulfide activated 2-(4,4′-dimethoxytrityloxy)-etanethiol was attached. According to dimethoxytrityl (DMTr) cation assay, the loading was 150 μmol/g). After removal of the DMTr group, the trinucleotide sequence was assembled by normal phosphoramidite chemistry and the 5′-hydroxy function was phosphitylated with 3-DMTrO-2,2-bis(ethoxycarbonyl)propyl 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite and oxidized to a phosphate triester (Scheme 3).[8] The base and phosphate protections were removed by standard DMTr-off ammonolysis. The support-anchored oligonucleotide 5′-phosphate was then treated with 7-methylguanosine 5′-diphosphate imidazolide[2] in DMF in the presence of 8-fold excess of $ZnCl_2$. The disulfide bond was finally cleaved with DTT and the 2-mercaptoethyl group was allowed to eliminate in aq methanol containing 0.01% triethylamine. On the basis of HPLC and MALDI-MS (Figure 1) analysis, the yield of the capping step was 50%.

The method described in all likelihood also allows synthesis of longer oligonucleotide sequences. If desired, the 3′-terminal phosphate group may be



SCHEME 2 Preaparition of the resin bridged with disulfide linker.



SCHEME 3 Synthesis of 7-methyl guanosine capped tetranucleotide.

removed by alkaline phosphatase, as shown previously.[6] Non-methylated ribonucleosides may in principle be inserted as 2′-*O*-TBDMS derivatives, since it has been shown that the cap-structure withstands acidolytic removal of this group.[6]

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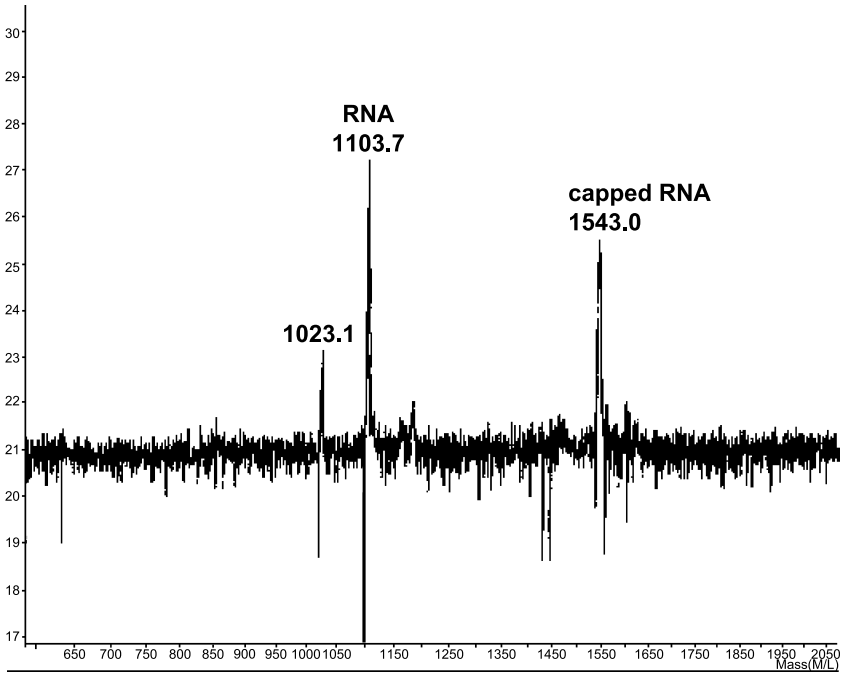

FIGURE 1 MALDI mass spectrum (negative mode) of the reaction mixture (final product release from the solid support into the solution).

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