Solid Phase Synthesis of DNA- and RNA 5'-Triphosphates Using cycloSal-Phosphoramidites

Ivo Sarac and Chris Meier

Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Introduction
2'-Deoxyribonucleoside 5'-triphosphates are the building blocks for enzymatic synthesis of DNA and RNA in vitro. While DNA 5'-triphosphates are mostly used in biotechnology to obtain synthetic genes, RNA 5'-triphosphates have a broader spectrum of applications. For example, RNA 5'-triphosphates are used for the induction of arterial immunity and for the ligation of RNA fragments.2 Nucleosides as well as oligonucleotide 5'-triphosphates are not only very important compounds in biological systems but have also therapeutic applications. Although, there have been a number of different approaches reported, a general and reliable access to these important classes of compounds is still a challenge. Therefore, we attempted to develop a broadly applicable route to both nucleoside 5'-triphosphates and oligonucleotide 5'-triphosphates.

The cycloSal-Method
Acceptor-substituted cycloSal-phosphate triesters can be used as activated phosphate donors in synthesis.7 8 The attack of different nucleophiles at the activated phosphorus atom of a cycloSal-nucleotide provides access to a variety of phosphorylated biomolecules. Using phosphorylate as nucleophile, the corresponding triphosphates were obtained.8 9 We transferred the cycloSal-strategy in order to obtain DNA- and RNA-oligonucleotide 5'-triphosphates.

Synthesis of 5-Chlorosaligenyl-N,N-diisopropylphosphoramidite

The synthesis of 5-chlorosaligenyl-N,N-diisopropylphosphoramidite was achieved in 3 steps in an overall yield of 53%. First, 5-chlorosaligenyl acid was reduced with LiAlH4 to give 5-chlorosaligenol which was then reacted with PCl3 in the presence of pyridine to 5-chloro-cycloSaligenylchlorophosphate. Next, this compound was reacted with (Pr)2NH and the formed 5-chloro-saligenyl-N,N-diisopropylphosphoramidite was obtained after purification as a colourless solid suitable to be used in a DNA/RNA synthesizer.

The cycloSal-phosphoramidite showed comparable stability as normal nucleoside phosphoramidites and it can be stored in the freezer for months without degradation.

5'-Phosphate Synthesis

The cycloSal-phosphoramidite can be used also for the preparation of 5'-monophosphorylated oligonucleotides. The crude IEX HPLC chromatogram and ESI MS result of a 20mer oligonucleotide is shown below that was made by coupling the cycloSal-phosphoramidite, standard oxidation and deprotection with NH4OH for 2 hours at room temperature to form the 5'-monophosphate.

\[ p(d(ACT GTG CAA TCG CAG TAC GT)) \]

Conclusion
• DNA/RNA 5'-triphosphates were synthesized using a cycloSal-phosphoramidite approach
• The synthesis was carried out fully automated on a standard oligonucleotide synthesizer
• The coupling reaction of the cycloSal-phosphoramidite is quantitative
• Standard coupling reagent and iodine oxidation is used
• This is a convenient and reliable way to synthesize 5'-triphosphorylated DNA- and RNA oligonucleotides

References

Acknowledgement
We are grateful for financial support by the University of Hamburg. The research was funded by the EU project SILVER "Small-molecule Inhibitor Leads Versus Emerging and neglected RNA viruses".