Solid Phase Synthesis of DNA- and RNA 5’-Triphosphates Using *cyclo*Sal-Phosphoramidites

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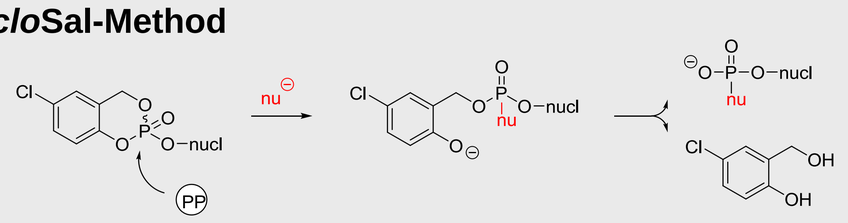
Introduction

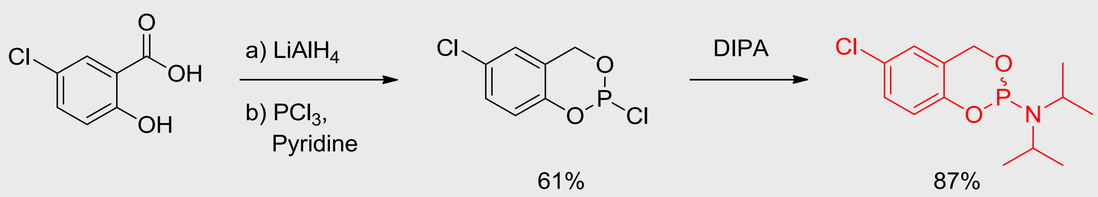
2’-Deoxyribo- and ribonucleoside 5’-triphosphates are the building blocks for enzymatic synthesis of DNA and RNA *in vivo* and *in vitro*.[1] While DNA 5’-triphosphates are mostly used in biotechnology industry to obtain synthetic genes, RNA 5’-triphosphates have a broader spectrum of applications. For example, RNA 5’-triphosphates are used for the induction of antiviral immunity and for the ligation of RNA fragments.[2] Nucleoside 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 *cyclo*Sal-Method



Acceptor-substituted *cyclo*Sal-phosphate triesters can be used as activated phosphate donors in synthesis.[3] The attack of different nucleophiles at the activated phosphorus atom of a *cyclo*Sal-nucleotide provides access to a variety of phosphorylated biomolecules. Using pyrophosphate as nucleophile, the corresponding triphosphates were obtained.[4] We transferred the *cyclo*Sal-strategy in order to obtain DNA- and RNA oligonucleotide 5’-triphosphates.

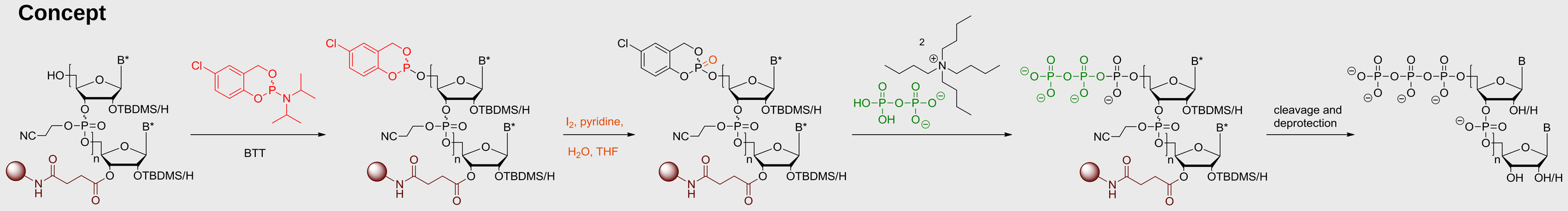
Synthesis of 5-Chlorosaligenyl-*N*,*N*-di*iso*propylphosphoramidite



The synthesis of 5-chloro-saligenyl-*N*,*N*-di*iso*propylphosphoramidite was achieved in 3 steps in an overall yield of 53%. First, 5-chlorosalicylic acid was reduced with $LiAlH_4$ to give 5-chlorosaligenol which was then reacted with PCl_3 in the presence of pyridine to 5-chloro-*cyclo*Saligenylchlorophosphite. Next, this compound was reacted with $(iPr)_2NH$ and the formed 5-chloro-saligenyl-*N*,*N*-di*iso*propylphosphoramidite was obtained after purification as a colourless solid suitable to be used in a DNA/RNA synthesizer.

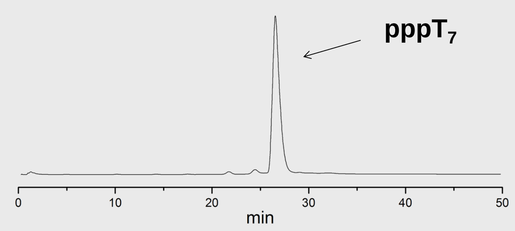
The *cyclo*Sal-phosphoramidite showed comparable stability as normal nucleoside phosphoramidites and it can be stored in the freezer for months without degradation.

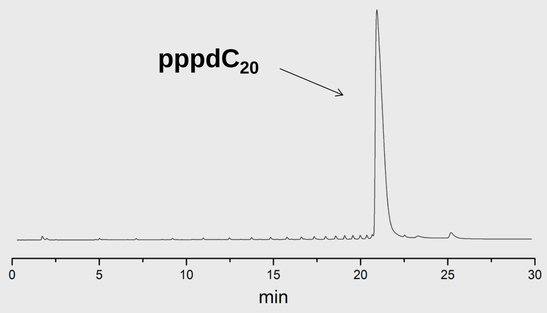
Concept



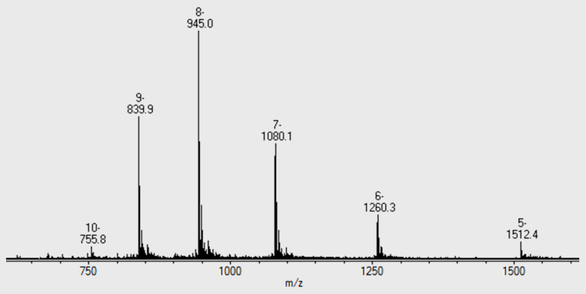
Synthesis of DNA 5’-Triphosphates

crude IEX HPLC chromatograms



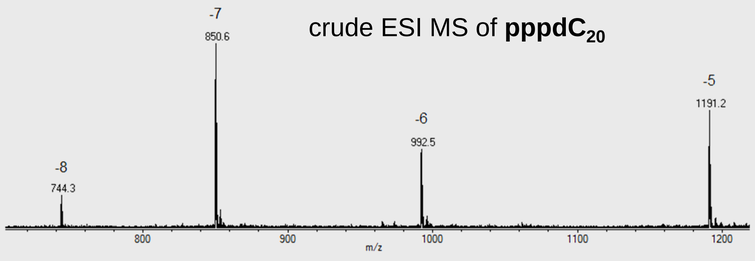


ESI MS of crude ppp[d(GCG AGC TAT ATC ATC TCT GAA CTG)]



Deconv. mass: 7564.9 Da, calculated 7564.2 Da

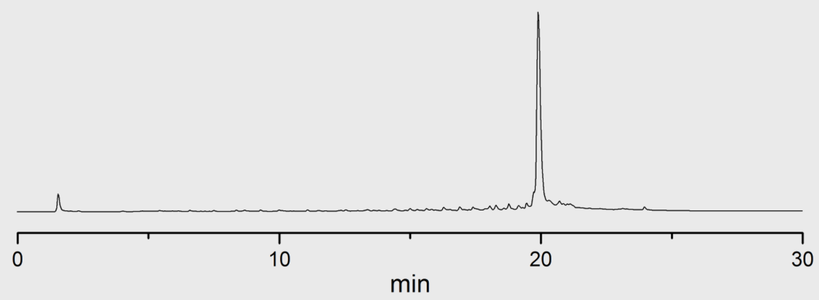
crude ESI MS of **pppdC20**

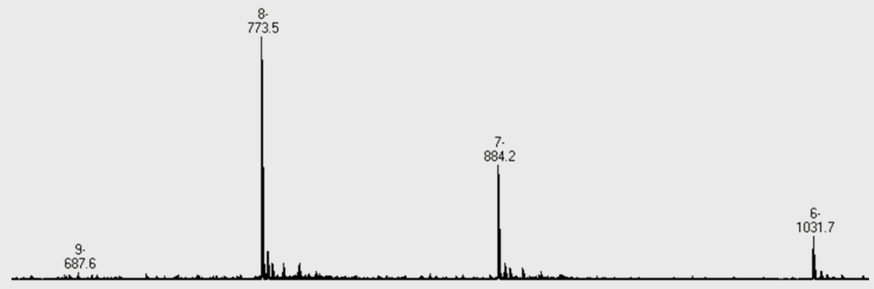


5’-Phosphate Synthesis

The *cyclo*Sal-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 *cyclo*Sal-phosphoramidite, standard oxidation and deprotection with NH_4OH for 2 hours at room temperature to form the 5’-monophosphate.

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

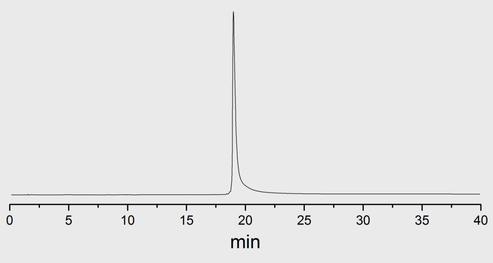




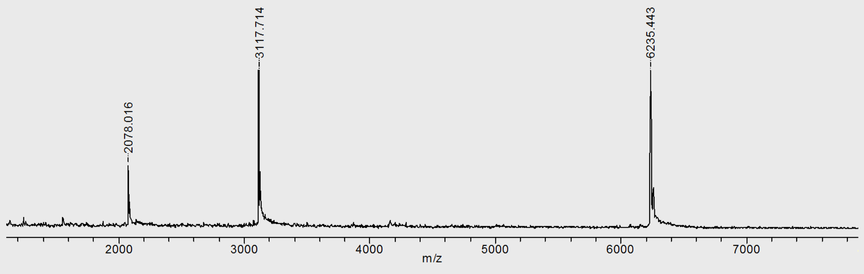
IEX HPLC chromatogram of crude p[d(ACT GTG CAA TCG CAG TAC GT)] was performed on a Dionex DNAPac PA200 (4 x 250mm) column from Thermo Fisher Scientific.

Synthesis of RNA 5’-Triphosphates

purified pppACU GUU UCA ACG UAC UGU G



MALDI MS of purified pppACU GUU UCA ACG UAC UGU G



Conclusion

- DNA/RNA 5’-triphosphates were synthesized using a *cyclo*Sal-phosphoramidite approach
- The synthesis was carried out fully automated on a standard oligonucleotide synthesizer
- The coupling reaction of the *cyclo*Sal-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

[1] Burgess, K., Cook, *D. Chem Rev.* **2000**, *100*, 2047-2059. **[2]** Zlatev, I., Lavergne, T., Debart, F., Vasseur, J-J., Manoharan, M., Morvan, F. *Org. Lett.* **2010**, *12*, 2190-2193. **[3]** Warnecke, S., Meier, C. *Nucleic Acids Symp.* **2008**, *52*, 583-584. **[4]** Tonn, V. C., Meier, C. *Chem. Eur. J.* **2011**, *17*, 9832-9842.

Acknowledgement

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