Custom 5’-triphosphate oligonucleotides for diagnostics and gene therapeutics

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Introduction

5’-Triphosphate (5’-PPP) oligonucleotides play versatile roles in biomedicine and biological processes, particularly in RNA biology. The incorporation of 5’-PPP into RNA or DNA oligos enables:

(1) Stimulation of antiviral responses for immunotherapy and vaccine adjuvants.
(2) Enhanced stability, specificity, and efficacy of guide RNA in gene editing.
(3) Inhibition of DNA-binding proteins.

5’-triphosphorylation is historically done using enzymatic methods, but the application is limited to natural nucleotides, length, and sequence design. A more favorable approach is chemical triphosphorylation, which utilizes solid-phase phosphoramidite synthesis (**Figure 1**). Chemical triphosphorylation allows ease of access to 5’-PPP oligonucleotides of arbitrary design and non-natural modification. 5’-Monophosphate and 5’-diphosphate are common impurities formed during synthesis, particularly when using the Ludwig and Eckstein strategy. Here, we show a reliable synthesis method capable of delivering more than 90% pure 5’-triphosphate oligonucleotides. This method is compatible with most length and modification up to a few hundred of grams scale, free from monophosphate or diphosphate contaminants.

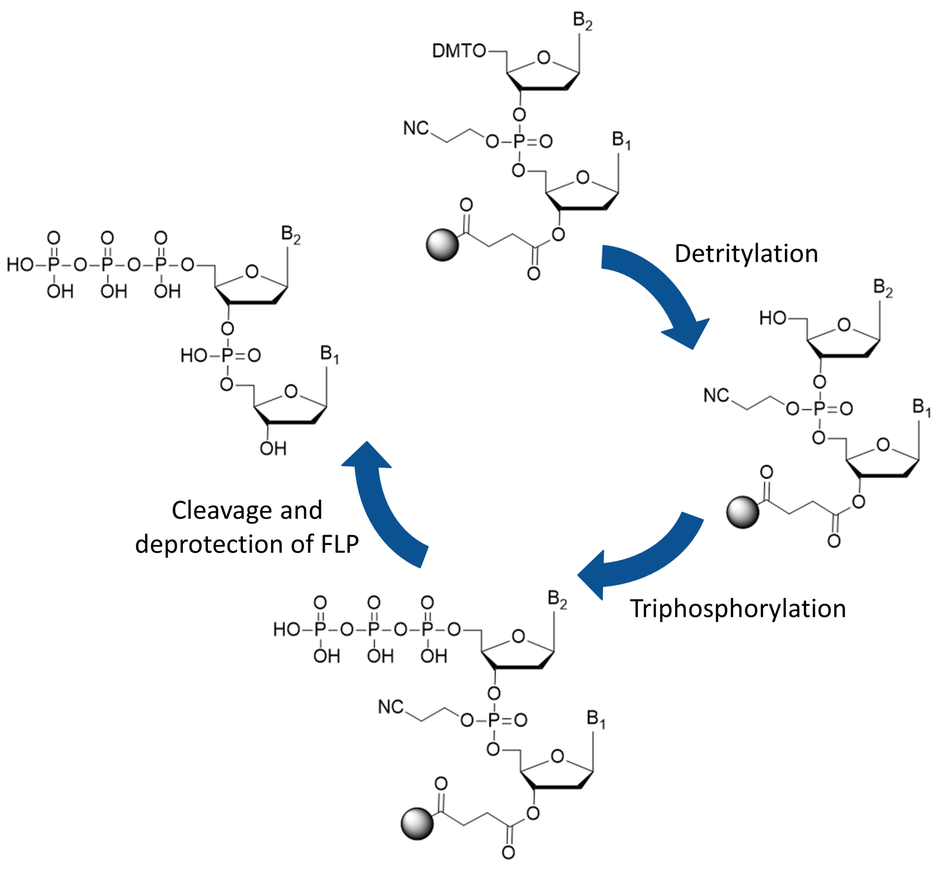

Figure 1. Triphosphorylation of oligonucleotide

Results

Using our in-house synthesis method, 5’-triphosphate oligonucleotide could be synthesized in 2 hours with 69.6% yield (**Figure 2, panel A**). The crude oligo contained 3-5% of common contaminants such as 5’-monophosphate and 5’-diphosphate, as expected. Purification afforded the pure 5’-PPP oligo, free from impurities (**Figure 2, panel B**).

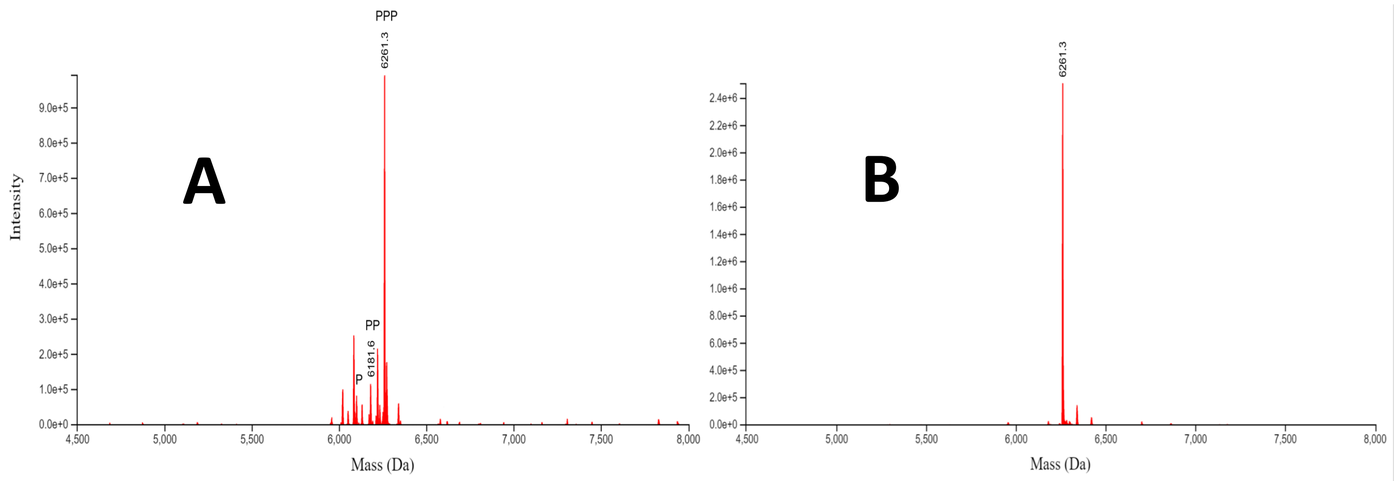

Figure 2. ESI-MS chromatograms of 5’-PPP oligo before (**A**) and after purification (**B**)

Quality Assurance

Quantification of the 5’-monophosphate and 5’-diphosphate impurities is difficult by mass spectrometry due to ionization or peak overlap with the FLP. Therefore, it is crucial the purity of triphosphate nucleotides is conjecturally accessed by analytical HPLC to ensure the highest quality, especially in therapeutics. Analysis by RP-HPLC showed the 5’-PPP oligo had a 94% purity (**Figure 3**).

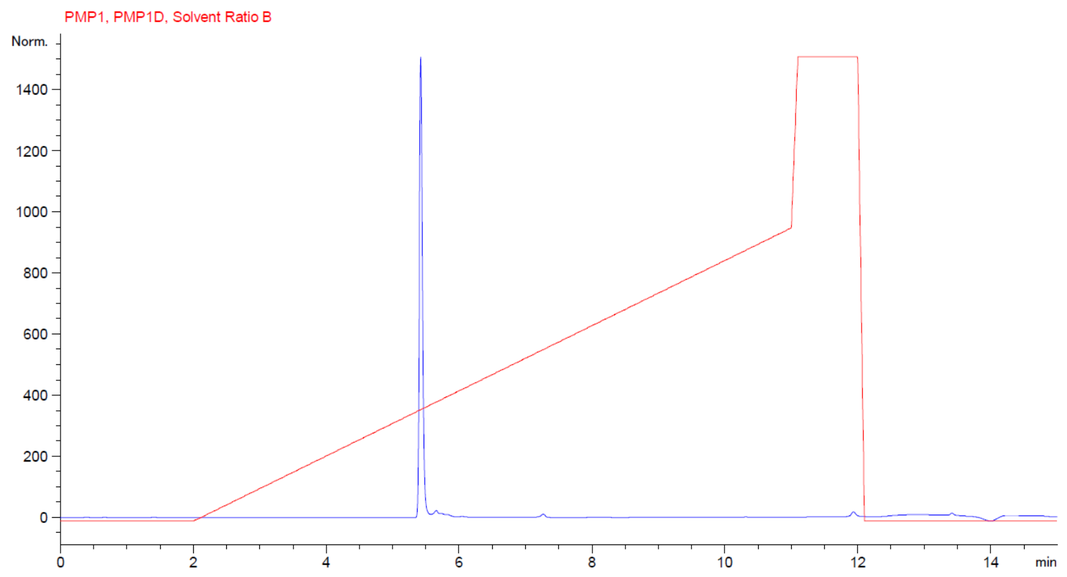

Figure 3. HPLC chromatogram of 5’-PPP oligo

Conclusion

- Synthesis method delivered triphosphate oligonucleotides in 2 hours.
- Quality of 5’-PPP oligo exceeded 90% purity according to RP-HPLC.
- Method is compatible with more challenging oligonucleotides such as highly modified DNA/RNA or shortmer, like 5-mer in various scales (not shown here).

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