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CHEMICAL PHOSPHORYLATION REAGENT II

INTRODUCTION

Chemical Phosphorylation Reagent II (CPR II) has become[1] a very popular chemical phosphorylation reagent (CPR) for phosphorylating oligos at the 5' terminus. While CPR II is most commonly used DMT-on to allow simple cartridge purification of the oligos produced, it can also be used DMT-off if the 5'-phosphorylated oligos can be used without purification in the same way as our original CPR.[2] One minor drawback in the usage of these two CPRs is the fact that they are both viscous oils. We offer these products prepackaged in serum vials but it is sometimes useful in high throughput situations to be able to weigh powder into a bottle in the exact quantity needed for the synthesis session. The answer is Solid CPR II.

Solid CPR II is the dimethylamide analogue of CPR II so it is more stable than CPR II to the conditions of oligonucleotide synthesis. It can also be used at the 3' terminus in situations where CPR is too labile for the synthesis cycles and any special manipulations during the synthesis. For example, this amide structure was used[3] for the synthesis of a long oligo in which a silyl protecting group had to be removed with a fluoride reagent which proved to be too basic for our standard 3'-phosphate support.

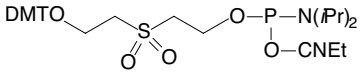
A more significant issue is that our original support for 3'-phosphorylation, 3'-phosphate CPG, does not allow users to carry out ß-elimination of cyanoethyl protecting groups prior to deprotection with, for example, diethylamine since the linker itself is labile to ß-elimination. 3'-CPR II CPG is compatible with regular oligonucleotide cleavage and deprotection while being stable to pretreatment of the support for ß-elimination.

The use of these reagents offers an alternative to enzymatic techniques for oligonucleotide phosphorylation. They also offer the advantages of allowing determination of phosphorylation efficiency or compatibility with DMT-ON purification techniques.

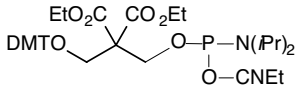
References:

1. A. Guzaev, H. Salo, A. Azhayev, and H. Lonnberg, *Tetrahedron*, 1995, **51**, 9375-9384.
2. T. Horn, and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.
3. P.J. Brooks, *et al.*, *J Biol Chem*, 2000, **275**, 22355-62.

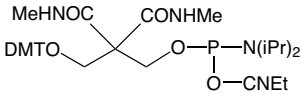
FIGURE 1: CPR STRUCTURES



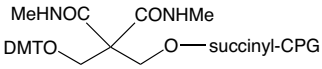
10-1900: Chemical Phosphorylation Reagent (CPR)



10-1901: Chemical Phosphorylation Reagent II (CPR II)



10-1902: Solid CPR II



20-2903: 3'-CPR II CPG

KEY POINTS

- UPDATE 7/13: Recent work has shown that it is no longer necessary to omit the capping step in the last cycle with our current high purity CPR II.
- CPR II is not sufficiently stable to regular synthesis cycles and should not be used for 3'-phosphorylation.

USE OF CPR II

Diluent: Anhydrous Acetonitrile
Add fresh diluent to the product vial to the recommended concentration and swirl the vial occasionally over several minutes until the product is completely dissolved. (Some oils may require between 5 and 10 minutes.) Use care to maintain anhydrous conditions. In case of transfer to an alternate vial type, ensure recipient vial has been pre-dried. For more information, see: http://www.glenresearch.com/Technical/TB_ABITransfer.pdf.

Coupling: 6 minute coupling time. It is no longer necessary omit the capping step a**fter the addition of this reagent!**

Deprotection: Deprotect as required by nucleobases.

Storage: Refrigerated storage, maximum of 2-8 °C, dry

Stability in Solution: 2-3 days

USE OF SOLID CPR II

Diluent: Anhydrous Acetonitrile

Coupling: No changes needed from standard method recommended by synthesizer manufacturer.

Deprotection: If the final DMT is removed during synthesis, deprotection under standard conditions will eliminate the Solid CPR II to yield a 5' Phosphate. If the DMT is retained, see the Purification section below.

Storage: Refrigerated storage, maximum of 2-8 °C, dry

Stability in Solution: 2-3 days

USE OF 3'-CPR II CPG

Coupling: This support should be used in a manner identical to normal protected nucleoside supports since it contains the DMT group.

Deprotection: Cleavage of the oligonucleotide from this support requires 2 hours at room temperature with ammonium hydroxide. Complete the deprotection using the protocol required by the nucleobases.

Storage: Controlled Room Temperature (or lower), dry

5'-PHOSPHORYLATION

The DMT group may be removed on the synthesizer by the standard deblocking method to determine coupling efficiency, if desired. Standard deprotection is used to cleave the modified oligonucleotide from the support and to remove all other protecting groups as well as the CPR linkers to generate the 5'-phosphate.

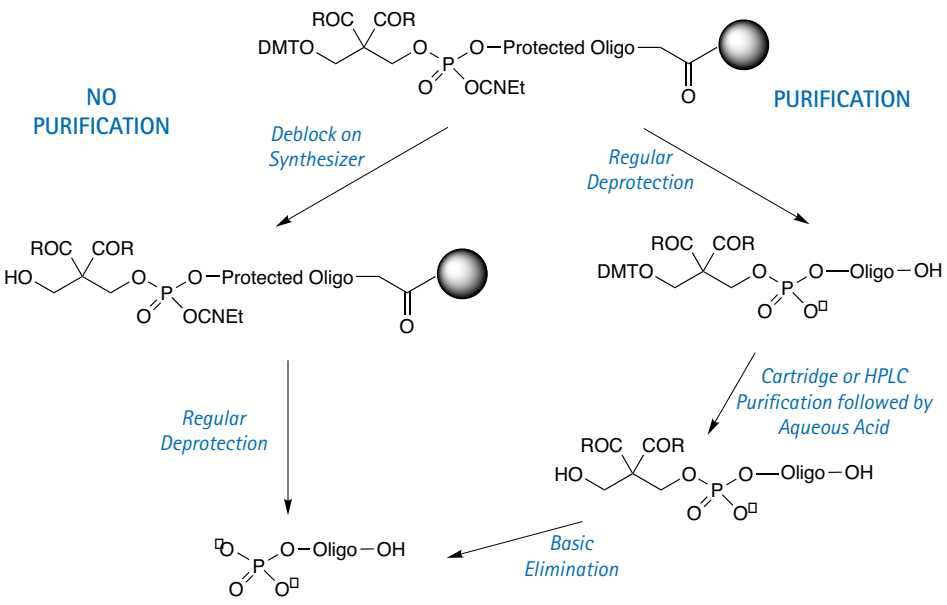
However, the most common use is to carry out the synthesis DMT-on and use the DMT group for purification by cartridge or HPLC. Details are provided in the Purification section below.

3'-PHOSPHORYLATION

Using 3'-CPR II CPG

A simple approach to 3'-phosphorylation is to use 3'-CPR II CPG. In this case, the 3'-nucleotide derives from the first nucleoside phosphoramidite addition.

FIGURE 2: OPTIONS FOR 5'-PHOSPHORYLATION



PURIFICATION

DMT-ON - CPR II

Carry out standard purification on Glen-Pak™ or Poly-Pak™ cartridges and elute the purified oligonucleotide as normal. The 5'-phosphate still has the CPR II side chain attached and it must be removed under basic conditions. Add an equal volume of concentrated ammonium hydroxide to the eluent. For HPLC purification, remove the DMT using acetic acid:water (20:80) for 1 hour at room temperature. Dry the oligonucleotide down and add ammonium hydroxide. Leave at room temperature for 15 minutes to achieve complete elimination of the side chain to the 5'-phosphate.

DMT-ON - Solid CPR II

After purification by Glen-Pak™, Poly-Pak™ or RP HPLC methods, remove the DMT according to standard methods either on the cartridge or in solution (e.g., 20% acetic acid for 1 hour at room temperature). Dry the oligonucleotide down and use one of the following deprotection conditions to complete the elimination of the Solid CPR II to yield the 5'-phosphate.

1) 30% Ammonium hydroxide for 2 hours at 55 °C
2) AMA (30% Ammonium hydroxide/40%Methylamine 1:1 v/v) for 10 minutes at 65 °C
3) 0.1 M NaOH at room temperature for 5 minutes. Note the 0.1 M NaOH has to be neutralized or desalted prior to drying down. For neutralization, add 7 µL of glacial acetic acid per mL of 0.1 M NaOH.

3'-Phosphates

For 3'-phosphorylated oligonucleotides, the final DMT group may be removed on the synthesizer or it may be retained to aid in purification. If the DMT group is retained, it may be removed on a purification cartridge or, following purification, by treating the oligonucleotide with acetic acid:water (20:80) at room temperature for 1 hour.