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## SOLID CHEMICAL PHOSPHORYLATION REAGENT II

### Introduction

CPR II (10-1901-XX) (1) has become<sup>1</sup> 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 (10-1900-XX) (2).<sup>2</sup> 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 simple – Solid CPR II (10-1902-XX) (3).

Solid CPR II is the dimethylamide analogue of CPR II so it is more stable than CPR II to the conditions of oligonucleotide synthesis. Consequently, it can be used at the 5' terminus without the need to miss out the capping step in the last cycle, which is the case for CPR II. 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<sup>3</sup> 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.

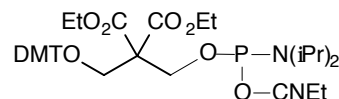
### Synthesis

No changes are needed from the standard method recommended by the synthesizer manufacturer. The final DMT may be retained for purification (DMT On) or removed to determine coupling efficiency (DMT Off).

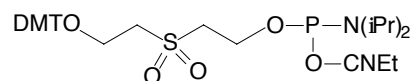
### DMT-Off

Deprotection under standard conditions will eliminate the Solid CPR II to yield a 5' Phosphate.

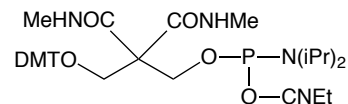
**FIGURE 1: STRUCTURE**



(1) Chemical Phosphorylation Reagent II



(2) Chemical Phosphorylation Reagent



(3) Solid Chemical Phosphorylation Reagent II

### DMT-On

After purification by Glen-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.

### 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.

Glen-Pak is a trademark of Glen Research Corporation. CPR II and Solid CPR II are covered by patents - US5,959,090 and EP0186368.