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## 5'-THIOL-MODIFIER C6

### INTRODUCTION

The 5'-Thiol-Modifier C6 is used to produce a thiol group at the 5'-terminus of a synthetic oligonucleotide<sup>1</sup>. The thiol group can be used to attach a variety of products to the oligonucleotide, including fluorescent tags<sup>1,3</sup>, biotin<sup>2</sup>, and alkaline phosphatase<sup>4</sup>.

### USE OF 5'-THIOL-MODIFIER C6

*Diluent:* Anhydrous Acetonitrile

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

*Coupling:* Standard coupling time. Use 0.02 M Iodine for Oxidation.

*Deprotection:* The Trityl group protecting the sulfur must be removed with silver nitrate.

*Storage:* Freezer storage, -10 to -30°C, dry

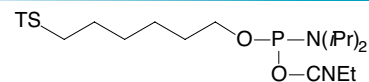
*Stability in Solution:* 2-3 days

### DEPROTECTION AND PURIFICATION

The trityl group used to protect the thiol is not acid labile and therefore can not be removed on a DNA synthesizer using the normal acid deprotection. Cleavage of the oligonucleotide from the support and removal of the base protecting groups are carried out in the normal manner. If purification is desired, it should be done before removing the trityl group. The presence of the trityl group allows standard trityl-on reverse phase (RP) purification techniques to be used.

Final deblocking of the oligonucleotide involves cleavage of the trityl-sulfur bond. This is accomplished by oxidation with silver nitrate with the excess silver nitrate being precipitated with dithiothreitol (DTT). Excess DTT can be removed by extraction with ethyl acetate, by desalting or by ethanol precipitation.

FIGURE 1: STRUCTURE



10-1926: 5'-Thiol-Modifier C6

### KEY POINT

- The oxidation of the final cycle of synthesis must use 0.02M iodine solution to minimize oxidative cleavage of the trityl-S-linkage.

### PROCEDURE

1. Deprotect with ammonium hydroxide in the normal manner.
2. Purify the trityl containing oligonucleotide by HPLC or Poly-Pak cartridge.
3. Evaporate the product solution to dryness.
4. Suspend the product in 0.1M triethylammonium acetate (TEAA), pH6.5 at a concentration of approximately 100 A260 units/mL.
5. Add 0.15 volumes of 1M aqueous silver nitrate solution, mix thoroughly, and leave to react at room temperature for 30 minutes.
6. Add 0.20 volumes of 1M aqueous DTT solution, mix thoroughly, and leave at room temperature for 5 minutes.
7. Centrifuge the suspension to remove the silver DTT complex. Remove the supernatant. Wash the precipitate with 1 volume of 0.1M TEAA. Centrifuge and combine the supernatant with the first volume. (Alternatively, vortex the suspension and apply to a desalting column equilibrated with conjugation buffer.)
8. Remove the excess DTT from the supernatant by desalting on either an RP cartridge or NAP-25 column and proceed directly to the conjugation reaction. (If not used immediately, the free thiol oligonucleotide must be stored under an inert atmosphere to avoid oxidative dimerization to the disulfide.)

### References

1. B.A. Connolly and P. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. B.S. Sproat, B.S. Beijer, P. Rider, and P. Neuner, *Nucleic Acids Res.*, 1987, **15**, 4837.
3. R. Zuckerman, D. Corey, and P. Shultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
4. P. Li, et al., *Nucleic Acids Res.*, 1987, **15**, 5275.

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