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THIOL-MODIFIER S-S PHOSPHORAMIDITE AND SUPPORTS

INTRODUCTION

Thiol-modification of oligonucleotides is important for:

- Labelling with thiol-specific tages like iodoacetamides and maleimides,
- Conjugation of enzymes, especially horseradish peroxidase, and
- Attachment of oligonucleotides to gold surfaces.

Thiol-Modifiers for the production of sulfhydryl groups at the 5'-terminus have been readily available commercially, including from Glen Research. These products are based[1,2,3] on blocking the thiol group during synthesis with a trityl protecting group. Although this procedure has been successfully used, several problems exist including a low level of oxidative detritylation during oligonucleotide synthesis and the use of silver nitrate is cumbersome during final deblocking. For these reasons, we have introduced disulfide versions which require a simple reductive cleavage with DTT or TECP to generate the thiol group. 3'-Thiol-modified oligonucleotides are especially interesting in cases where a different label is desired for the 5'-terminus.

USE OF THIOL-MODIFIER C6 S-S

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

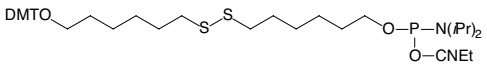
Coupling: Standard coupling time.

Deprotection: After normal deprotection, the disulfide can be cleaved at room temperature in 30 minutes with 100 mM DTT pH 8.3 - 8.5 in the buffer of your choice.

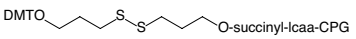
Storage: Freezer storage, -10 to -30°C, dry

Stability in Solution: 2-3 days

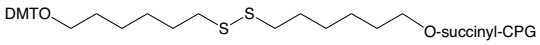
FIGURE 1: STRUCTURE



10-1936: Thiol-Modifier C6 S-S Phosphoramidite



20-2933: 3'-Thiol-Modifier C3 S-S CPG



20-2936: 3'-Thiol-Modifier C6 S-S CPG

KEY POINTS

- If the phosphoramidite is added only at the 5'-terminus, carry out the oxidation step of the final cycle with 0.02M Iodine solution to avoid oxidative cleavage of the disulfide linkage. If used at the 3'-terminus, all oxidation steps should use 0.02M Iodine solution.

- Thiol-Modifier C6 S-S Phosphoramidite can be used to add a thiol group to either the 3'- or 5'-terminus.

THIOL GROUP AT THE 5'-TERMINUS

DMT-off Synthesis

Add the Thiol-Modifier C6 S-S Phosphoramidite at the 5'-terminus of the oligonucleotide in the automated DMT-off synthesis mode. The DMT release from the last cycle can be used to determine coupling efficiency. Carry out deprotection in the normal manner. This procedure removes the base protecting groups. Cleave the disulfide linkage using 100 mM DTT, pH 8.3 - 8.5, at room temperature for 30 minutes.[5] Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures.

DMT-ON Synthesis

Add the Thiol-Modifier C6 S-S Phosphoramidite at the 5'-terminus of the oligonucleotide in the automated DMT-ON synthesis mode. Carry out deprotection in the normal manner. Purify the trityl containing oligonucleotide on a reverse phase cartridge omitting the 2% TFA step. Evaporate the product solution to dryness. Cleave the disulfide linkage using 100 mM DTT, pH 8.3 - 8.5, at room temperature for 30 minutes.[5] Desalt the oligonucleotide. If using a reverse phase

cartridge, be sure to include a 10 mL rinse of 5% acetonitrile (ACN) in 0.1 M TEAA. This will remove any residual DTT that is bound to the cartridge without any loss of oligo. Elute the oligo from the cartridge as usual with ACN in water. (The DMT containing thiol will remain attached to the cartridge.)

Notes:

1. A Glen-Pak™ procedure for DMT-ON purification of 5'-Thiol-Modifier C6 S-S oligos and subsequent reduction and desalting of 5'-Thiol-Modified Oligonucleotides is described in the User Guide on our web site: http://www.glenresearch.com/Technical/GlenPak_UserGuide.pdf

THIOL GROUP AT THE 3'-TERMINUS

Using Disulfide Phosphoramidite

Add the Thiol-Modifier C6 S-S Phosphoramidite to any nucleoside support and then synthesize the desired oligonucleotide. Carry out deprotection in the normal manner. This procedure removes the base protecting groups. Cleave the disulfide linkage using 100 mM DTT, pH 8.3 - 8.5, at room temperature for 30 minutes.[5] Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures. Just prior to conjugation, cleave the disulfide with 100mM DTT/ pH 8.3 - 8.5/RT/30min. Desalt on a Glen Gel-Pak™ (or equivalent) desalting column equilibrated with conjugation buffer. Continue to the conjugation reaction.

Using Disulfide Support

3'-Thiol-Modifier C3 S-S CPG or 3'-Thiol-Modifier C6 S-S CPG is designed to introduce a thiol group to the 3'-terminus of a target oligonucleotide[4]. Carry out deprotection in the normal manner. This procedure removes the base protecting groups. Cleave the disulfide linkage using 100 mM DTT, pH 8.3 - 8.5, at room temperature for 30 minutes.[5] Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures. Just prior to conjugation, cleave the disulfide with 100mM DTT/ pH 8.3 - 8.5/RT/30minutes. Desalt on a Glen Gel-Pak™ (or equivalent) desalting column equilibrated with conjugation buffer. Continue to the conjugation reaction.

References:

1. B.A. Connolly and R. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. B.A. Connolly, *Nucleic Acids Res.*, 1987, **15**, 3131-3139.
3. N.D. Sinha and R.M. Cook, *Nucleic Acids Res.*, 1988, **16**, 2659.
4. A. Kumar, S. Advani, H. Dawar, and G.P. Talwar, *Nucleic Acids Res.*, 1991, **19**, 4561.
5. Gregg Morin, Geron Corporation, Personal Communication.

GENERAL PROCEDURE FOR LABELLING OF THIOL-MODIFIED OLIGONUCLEOTIDES

Step 1: Oligo Sulfhydryl activation (Oligo-disulfide)

Dissolve oligo disulfide (15-25 A_{260} units) in 0.25mL 100mM DTT, pH 8.3 - 8.5. Incubate at room temperature for 30 minutes.

Step 2: Removal of DTT and other reaction byproducts and change to conjugation buffer

Load entire sample on a Glen Gel-Pak™ 1.0 (or equivalent) desalting column equilibrated with 50mM sodium phosphate, pH 6.0. Allow to drip through. Add 0.75mL of 50mM sodium phosphate, pH 6.0 and allow to drip through. Elute with 1mL sodium phosphate pH 6.0. Collect for conjugation.

Step 3: Conjugation

Dissolve thiol reactive ligand in sodium phosphate, pH 6.0 (≈ 20mM final concentration). *If thiol reactive ligand is not soluble in water dissolve in appropriate solvent (DMF or DMSO) at the same concentration.* Add 0.2mL thiol reactive ligand to activated, desalted thiol modified oligo from step 2. Vortex and incubate at RT for 2-4 hours or overnight at 4°C.

Step 4: Purification

Purify oligo conjugate by PAGE, RP HPLC or ion exchange HPLC.

Notes:

1. Thiol-modified oligonucleotides should be kept either under an inert atmosphere or in a solution containing DTT (10mM) to avoid oxidative disulfide formation. The DTT can be extracted from the solution using ethyl acetate or removed by desalting prior to conjugation.
2. When desalting an oligonucleotide from a solution containing DTT on a reverse phase cartridge, it is necessary to include a 10 mL rinse of 5% acetonitrile (ACN) in 0.1 M TEAA. This will remove any residual DTT that is bound to the cartridge without any loss of oligo. Elute the oligo from the cartridge as usual with ACN in water.