



**GLEN RESEARCH**

22825 DAVIS DRIVE  
STERLING, VIRGINIA  
20164

PHONE

703-437-6191

800-327-GLEN

FAX

703-435-9774

INTERNET

WWW.GLENRES.COM

## DEPROTECTION OF OLIGORIBONUCLEOTIDES CONTAINING 4-THIO-U

4-Thio-U TOM phosphoramidite allows the efficient incorporation of a pyrimidine thiocarbonyl into an oligoribonucleotide. The introduction of cyanoethyl protection for sulfur prevents oxidation of sulfur by  $I_2^1$  and provides a convenient synthesis route for a series of thiocarbonyl purine and pyrimidine phosphoramidites. Unfortunately, S-cyanoethyl is also a good leaving group and the C4 carbon of pyrimidines is susceptible to nucleophilic attack with displacement of sulfur by the attacking nucleophile. Hydrolysis of S-cyanoethyl and conversion to a thiocarbonyl by treatment with DBU prior to base deprotection<sup>2</sup> significantly reduced sulfur loss during base deprotection as does including sodium hydrosulfide (NaSH) to act as a competing nucleophile in the deprotection solution.<sup>3</sup> Additionally, we have found that base deprotection using a more hindered nucleophile such as *tert*-butylamine further reduces sulfur loss. A deprotection mix of *tert*-butylamine:MeOH:H<sub>2</sub>O (1:1:2) has been used successfully to deprotect oligos containing the base labile fluorophore TAMRA.<sup>4</sup>

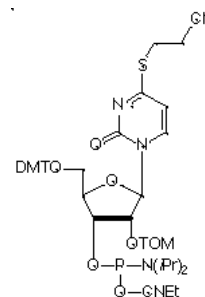
### Materials

DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene), Aldrich # 19,900 or equivalent.  
Anhydrous acetonitrile.  
*Tert*-Butylamine, Aldrich # 39,143-3 or equivalent.  
Methanol, HPLC grade or equivalent.  
Sodium Hydrosulfide hydrate (NaSH.xH<sub>2</sub>O), Aldrich # 16,152-7 or equivalent.  
Triethylamine trihydrofluoride (TEA.3HF), Aldrich # 34,464-8 or equivalent.

### Methods

- Synthesis:**  
Synthesize oligoribonucleotide using standard TOM RNA chemistry (6 min. coupling time).
- Cyanoethyl Deprotection:**  
Remove S-cyanoethyl protection by treatment of the CPG with 2 ml of 1.0 M DBU in anhydrous acetonitrile for 2 hours at RT. This can be done in the column using 2 disposable polypropylene syringes.

Following DBU treatment wash CPG thoroughly with acetonitrile to completely remove DBU and dry the support. (Residual DBU in the deprotection solution can cause loss of sulfur during the base deprotection step.)



4-Thio-U-TOM-CE  
Phosphoramidite

- Base Deprotection:**  
Transfer CPG to a cleavage vial, add 1 ml of *tert*-butylamine:MeOH:H<sub>2</sub>O (1:1:2) containing 50 mM NaSH and deprotect for 3 hours at 55 °C. Cool vial and collect supernatant by filtration and desalt on NAP-10 column to remove NaSH. Dry desalted oligoribonucleotide in vacuum concentrator.
- Silyl Deprotection:**  
Remove 2'-hydroxyl protecting groups using TEA.3HF, as described in RNA Technical Bulletin. *tert*-Butylammonium fluoride in tetrahydrofuran (TBAF) can also be used, however, it has been shown to degrade 6-thio-G.<sup>5</sup>

### References

- Coleman, RS., and Siedlecki, J.M., *Tetrahedron Lett.*, 1991, **32**, 3033-3034.
- Coleman, RS., and Siedlecki, J.M., *Journal of the American Chemical Society*, 1992, **114**, 9229-9230.
- Coleman, RS., and Kesicki, E.A., *Journal of the American Chemical Society*, 1994, **116**, 11636-11642.
- Mullah, B., and Andrus, A., *Tetrahedron Lett.*, 1997, **38**, 5751-5754.
- Adams, C.J., Murray, J.B., Farrow, M.A., Arnold, J.R.P., and Stockley, P.G., *Tetrahedron Lett.*, 1995, **36**, 5421-5424.