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DEPROTECTION OF OLIGORIBONUCLEOTIDES CONTAINING 4-THIO-U

4-Thio-U TOM phosphoramidite (10-3052) 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. Elimination of S-cyanoethyl and conversion to a thiocarbonyl by treatment with DBU prior to base deprotection[2] significantly reduced sulfur loss during base deprotection, as does including sodium hydrosulfide (NaSH) to act as a competing nucleophile in the deprotection solution.[3] Additionally, we have found that base deprotection using a more hindered nucleophile such as *tert*-butylamine further reduces sulfur loss. *Tert*-butylamine has been used successfully to deprotect oligos containing the base labile fluorophore TAMRA.[4]

Materials

DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene), Sigma-Aldrich # 19,900 or equivalent.
Anhydrous acetonitrile.
Tert-Butylamine, Sigma-Aldrich # 391433 or equivalent.
Sodium Hydrosulfide hydrate ($NaSH.xH_2O$), Sigma-Aldrich # 161527 or equivalent.
Triethylamine trihydrofluoride (TEA.3HF), Sigma-Aldrich # 344648 or equivalent.

Methods

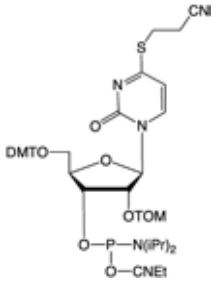
1. *Synthesis:*

Synthesize oligoribonucleotide using standard TOM RNA chemistry (6 min. coupling time).

2. *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

3. *Base Deprotection:*

Transfer CPG to a cleavage vial, add 1 ml of *tert*-butylamine:H_2O (1:3) containing 50 mM NaSH and deprotect for 4 hours at 60 ˚C.
Cool vial and collect supernatant by filtration and desalt on Glen Gel-Pak™ 1.0 or NAP-10 column to remove NaSH.
Dry desalted oligoribonucleotide in vacuum concentrator.

4. *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.[5]

References:

(1) Coleman, RS., and Siedlecki, J.M., *Tetrahedron Lett.*, 1991, **32**, 3033-3034.
(2) Coleman, RS., and Siedlecki, J.M., *J. Amer. Chem. Soc.*, 1992, **114**, 9229-9230.
(3) Coleman, RS., and Kesicki, E.A., *J. Amer. Chem. Soc.*, 1994, **116**, 11636-11642.
(4) Mullah, B., and Andrus, A., *Tetrahedron Lett.*, 1997, **38**, 5751-5754.
(5) Adams, C.J., Murray, J.B., Farrow, M.A., Arnold, J.R.P., and Stockley, P.G., *Tetrahedron Lett.*, 1995, **36**, 5421-5424.