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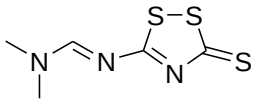
Guidelines for the Use of DDTT as a Sulfur Transfer Reagent in Synthesis of Oligonucleotide Phosphorothioates

Version 1.4

Introduction

DDTT is a novel sulfur transfer reagent designed, manufactured and marketed by AM Chemicals[1], for efficient oligonucleotide synthesis. Please feel free to contact us for additional information, comments, or suggestions.



Properties of DDTT. 3-((Dimethyl aminomethylene)amino)-3*H*-1,2,4-dithiazole-5-thione (DDTT) is a bright-yellow crystalline compound with molecular mass of 205.3. The fact that DDTT does not contain any oxygen results in a very low percentage of oxidation in the process of oligonucleotide synthesis (less than 2% for mixed-base 20-mer oligonucleotide phosphorothioates). DDTT is a proprietary agent developed and marketed by our company.[1]

The Use of DDTT in DNA Synthesis. Regardless of the sulfurization agent used, the presence of water in the reaction mixtures increases the degree of oxidation and leads to higher PO/PS ratio. *The use of anhydrous solvents for the preparation of the solutions of DDTT is strongly recommended.* In our experience, the sulfurization step in the synthesis of 2'-deoxyoligonucleotide phosphorothioates on a small scale requires the exposure of the solid phase to 5 to 6 molar excess DDTT (contact times of 30 s, 1, 2, and 2.5 min for 0.1 M, 0.05 M, 0.03 M, and 0.02 M solutions, respectively). Under these conditions, the ESMS and the RP HPLC profiles of the products were indistinguishable from those obtained using EDIT as a sulfur transfer reagent.

The Use of DDTT in RNA Synthesis. *The use of anhydrous solvents for the preparation of the solutions of DDTT is strongly recommended.* To synthesize full-length RNA phosphorothioates, 0.05 M solution of DDTT is recommended, with a contact time of 4 min. Under these conditions, the yield of the full-PS random 20-mer RNA greater than 90% may be expected. RNA sequences containing stretches of purine nucleoside residues are more difficult to sulfurize irrespective of the reagent used. To obtain high degree of sulfurization with those oligonucleotides, 0.1 M solution of DDTT and/or extended contact time may be required.

Table 1. Solubility of DDTT in Organic Solvents.

Concentration of DDTT, M	Solvents and their ratio (v/v)	
	Py - MeCN	Py - THF
0.1	100:0	40:60
0.06	50:50	-
0.05	40:60	20:80
0.03	30:70	-
0.02	20:80	0:100

Solubility and Stability of DDTT. The solubility of DDTT in mixtures of anhydrous Py and MeCN or THF is relatively limited and increases with the increasing concentration of Py. Table 1 lists the various compositions of co-solvents.

Under these conditions, DDTT forms stable solutions that do not display any loss of functional activity or precipitation of the reagent for a period of over 6 months. To prepare solutions of the desired concentration, we recommend first dissolving DDTT in the calculated amount of pyridine, which may require a mild heat, followed by diluting the obtained solution with the required volume of MeCN or THF.

[1] **US Patents 7,723,528** and **8,552,175**