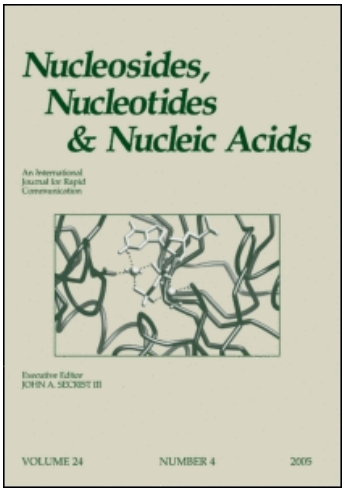

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SULFURIZATION EFFICIENCY IN THE SOLUTION PHASE SYNTHESIS OF DEOXYRIBONUCLEOSIDE PHOSPHOROTHIOATES - COMPARISON OF SULFUR TRIETHYLAMINE WITH VARIOUS SULFURIZING AGENTS[1]

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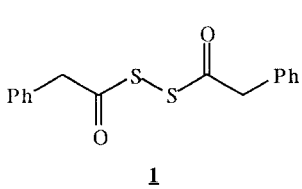
Abstract: Efficient solution-phase synthesis of nucleoside phosphorothioates utilizing phosphoramidite approach is described. Elemental sulfur in combination with triethylamine is the prefered choice for sulfurization of phosphite triester to phosphorothioates.

The antisense therapeutic principle has undergone revolutionary developments during the last few years.[2-10] This strategy is proving to be extremely promising and has thus raised the key question issue of manufacturing large quantities of oligonucleotide analogs. The phosphorothioates are the first class of compounds to reach the clinic. In order to develop cost-effective synthetic processes, issues related to efficient synthesis, scaleability and product purification are being investigated with renewed attention. One of the avenues to be explored for reducing the cost of antisense drugs is solution phase synthesis.[11-13] Cost of reagents, reproduceibility, and scaleability are important practical considerations. Even though much work has been done using the phosphotriester approach, there is no report on large scale solution phase synthesis utilizing phosphoramidite chemistry. Recently, Bonora and co-workers have reported the small scale liquid phase (HELP) synthesis of oligonucleotides using polyethylene glycol (PEG) as soluble support.[14-16] Since phosphoramidite chemistry has several advantages over the phosphotriester approach, there is an urgent need to further investigate and extent this route to phosphorothioates.

Recently, we reported a novel protecting group for internucleotidic phosphates, viz. 2-diphenylmethylsilylethyl (DPSE), and utilized it in the solid supported synthesis of oligonucleotides.[17-21] This protecting group is stable under acidic conditions and can be removed by a β-fragmentation mechanism under mild conditions using aqueous ammonium hydroxide. During synthesis, we observed that the DPSE group affords a highly lyphophilic character to the oligomers. Encouraged by this observation, we became interested in utilizing this group in the solution phase synthesis of deoxyribonucleotide phosphorothioates. We also have recently reported the investigation of sulfurization in the solid supported synthesis of phosphorothioates.[22] However, since the kinetics and results of sulfurization could be different on solid support and in solution, we were interested in further investigating the efficiencies of various sulfurizing reagents in solution. We report here for the first time an investigation of the solution-phase sulfurization of fully protected dinucleoside phosphites, using DPSE as the phosphorus protecting group via phosphoramidite approach.

In addition to elemental sulfur, four organosulfur reagents[23-27] were investigated. Phenylacetyl disulfide (**1**), tetraethylthiuram disulfide (TETD) (**2**), 3H-1,2-benzodithiol-3-one 1,1-dioxide (**3**), and bis(*O*,*O*-diisopropoxyphosphinothioyl)disulfide (**4**). Table **1** shows the sulfurization conditions for various reagents and Tables **2** and **3** show the results of sulfurization in the synthesis of thymidine dimer and hetero-dimers respectively.



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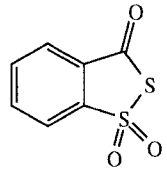

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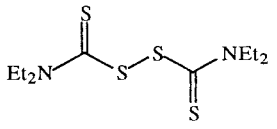

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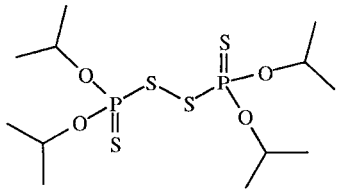

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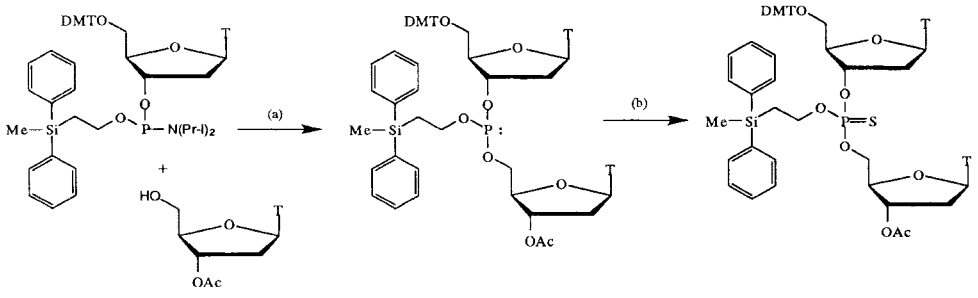

(a) 1H-Tetrazole, rt. (b) Sulfurizing reagent, rt.

Table 1. Sulfurization using various reagents.

Reagent	Solvent	Concentration	Equivalents
(1)	Dichloroethane/Collidine, 4/1, v/v	0.17 M	5
(2)	CH_3CN	0.5 M	30
(3)	CH_3CN	0.2 M	5
(4)	Pyridine	0.2 M	5
Sulfur/$(C_2H_5)_3N$	CH_2Cl_2	-	40

Table 2. Analysis and yields of the synthesized homo-thymidine dimers.[28,29]

Reagent	P=S / P=O	Isolated Yield (%)
(1)	100 / 0	68
(2)	76 / 24	81
(3)	> 99.5 / < 0.5	90
(4)	100 / 0	47
Sulfur/$(C_2H_5)_3N$	99.8 / 0.2	95

Table 3. Analysis and yields of the synthesized dimers.[28]

Dimers	Isolated Yield (%)
5' - DMT -TpsT - OAc	95
5' - DMT -d(GpsT) - OAc	86
5' - DMT -d(CpsT) - OAc	94
5' - DMT -d(ApsT)- OAc	85

5'-*O*-(4,4'-dimethoxytrityl)thymidine-3'-*O*-(2-diphenylmethylsilyl-ethyl *N*,*N*-diisopropyl phosphoramidite) was prepared according to the published procedure.[17] All other sulfurizing reagents were used as received. Elemental sulfur is inexpensive[30] and therefore important as a sulfurizing agent for large scale phosphorothioate oligomer synthesis. Elemental sulfur has been difficult to use in solid-supported synthesis due to its poor solubility in the typical reaction solvents and the resulting tendency to clog the apparatus. Carbon disulfide, normally the solvent of choice for elemental sulfur, is dangerous in large scale use.

In the present solution phase work, these problems were circumvented by adding 4 equivalents of triethyamine to elemental sulfur in dichloromethane, then using the resulting solution as sulfurization reagent. Sulfur triethylamine compares favorably with organosulfur reagents **1** - **4** in efficiency, and sharply reduces cost of phosphorothioate synthesis. No organic by-products are formed in the

sulfurization reaction, in contrast with reagents **1 - 4**, which makes product purification easier. Thus, in comparison to other reagents, sulfur triethylamine can be a preferred reagent for the solution phase synthesis of oligonucleotide phosphorothioates.

Typical Experimental Procedure: To a stirred solution of 3'-O-acetylthymidine (0.142 g; 0.5 mmole) and 1H-tetrazole (0.035 g; 0.5 mmole) in anhydrous acetonitrile (5 ml) at room temperature under argon was added a solution of 5'-*O*-(4,4'-dimethoxytrityl)thymidine-3'-*O*-(2-diphenylmethylsilylethyl *N,N*-diisopropyl phosphoramidite) (0.550 g; 0.6 mmole) in acetonitrile (5 ml). After stirring for 3 h, the sulfurizing reagent in appropriate solvent was added all at once. After 5 h, the reaction mixture was filtered and concentrated. The crude product was purified by flash chromatography using silica gel and ethylacetate/hexane as eluents. ^{31}P NMR ($CDCl_3$): 66.4, 66.6.

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28. No attempts were made to optimize the yields.
29. The P=S / P=O ratio were determined based on ^{31}P NMR with a signal to noise ratio value of 500.
30. At current rate the cost of various reagents are as follows: Sulfur : $ 0.01/g ($ 0.0007 / mmole), (**1**) : $ 1.80/g ($ 0.54 / mmole); TETD : $ 0.02/g ($ 0.006 / mmole); (**3**) : $ 10/g ($ 2.00 / mmole); (**4**) : $ 4/g ($ 1.70 / mmole).

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