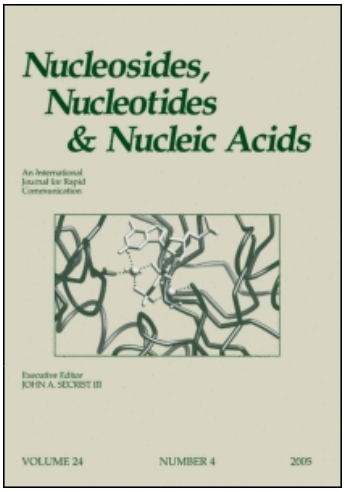

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IMPROVED DEPROTECTION PROCEDURE FOR DPSE PROTECTED PHOSPHOROTHIOATES

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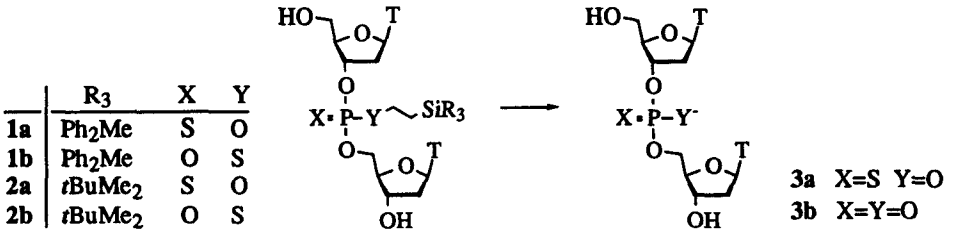
ABSTRACT. Deprotection of 2-(diphenylmethylsilyl)ethyl (DPSE) protected (*O,O,O*)- and (*O,O,S*)-trialkyl phosphorothioates with tetrabutylammonium fluoride affords the corresponding phosphorothioate. Formation of phosphodiester linkages is not observed.

The efficacy of phosphorothioate oligonucleotides as antisense therapeutic agents has been demonstrated.[1] The currently preferred method for phosphorothioate synthesis includes coupling of activated β-cyanoethyl 3'-phosphoramidites to the free 5'-hydroxyl group of the growing chain which is attached to a solid support followed by stepwise sulfurization. Selective deprotection of the internucleotide linkage occurs through *β-elimination.*[2] Recently, we and others have demonstrated that the 2-(diphenylmethylsilyl)ethyl (DPSE) group is a suitable protecting group for the synthesis of DNA and phosphorothioate oligonucleotides.[3] In this case, deprotection occurs through a *β-fragmentation* mechanism.[4] The properties of various β-(trialkylsilyl)ethyl protecting groups in the context of phosphorothioate synthesis have also been reported.[5]



Using the previously described protocol[3a] for the synthesis and deprotection of DPSE-protected phosphorothioate oligomers a slightly increased phosphodiester content in the final oligonucleotide product was observed. Here, we report a modified deprotection pro-

Table 1. Half-life times and deprotection products of (*O,O,O*)- and (*O,O,S*)-trialkyl phosphorothioates.[1]

reagent	educt	$t_{1/2}$ [h]	3a [%]	3b [%]
NH_4OH (30%)/D_2O/ EtOH 63:7:30, *v/v/v*, r.t.	**1a**	88	96	4
	1b	9	3	92
	2a	4	61	38
	2b	13	0	96
NH_4OH (30%), 60 °C	**1a**	0.3	97	3
	1b	<0.1	7	90
	2a	<0.1	64	33
	2b	<0.1	00	>99
aqu. CH_3NH_2 (40%), r.t.	**1a**	2	>99	0
	1b	0.5	8	92
	2a	1.7	63	37
	2b	0.7	0	>99
$nBu_4N^+F^-$ (1M in THF), r.t.	**1a**	<0.01	>99	0
	1b	<0.01	>99	0
	2a	<0.01	>99	0
	2b	<0.01	17	n.d.[2]

[1] by ^{31}P NMR [2] not determined, multiple reaction products

cedure, that affords phosphorothioate oligonucleotides with high phosphorothioate content. 2-Trialkylsilylethyl groups of (*O,O,O*)-trialkyl phosphorothioates migrate rapidly from oxygen to sulfur ($t_{1/2}$ = 0.9 - 5.8 h) to form the thermodynamically more stable (*O,O,S*)-trialkyl phosphorothioate isomer. In contrast, the DPSE group migrates only very slowly under the same conditions ($t_{1/2}$ = 385 h).[5] Since acid treatment for removal of the 4,4'-dimethoxytrityl protecting group is one of the steps in the oligomerization cycle used for the synthesis of phosphorothioate oligomers various amounts of (*O,O,S*)-β-silylethyl protected phosphorothioates may be present at the end of the synthesis. Therefore it was important to find deprotection conditions, that would allow for selective conversion of *both*, (*O,O,O*)- and (*O,O,S*)-trialkylphosphorothiates to the desired phosphorothioate diester without formation of undesired phosphodiester linkages.

First, we used ^{31}P NMR spectroscopy to analyze the deprotection products of (*O,O,O*)- and (*O,O,S*)-β-silylethyl protected dimers **1** and **2** obtained under different deprotection conditions. An inverse proportionality between NH_4OH concentration used for deprotection and phosphodiester content was observed. In 30% NH_4OH mainly phosphorothioate **3a** (96-97%) but also small amounts of **3b** (3-4%) were obtained.[5] Deprotection of **1a** in methylamine/water is ca. 20 times faster and in 40% aqueous methylamine phosphodiester formation is completely suppressed. Deprotection with even more nucleophilic reagents like $Bu_4N^+F^-$ (TBAF) in THF under anhydrous conditions is complete within minutes with no **3b** being detected. Deprotection of **2a** with aqueous amine bases is faster

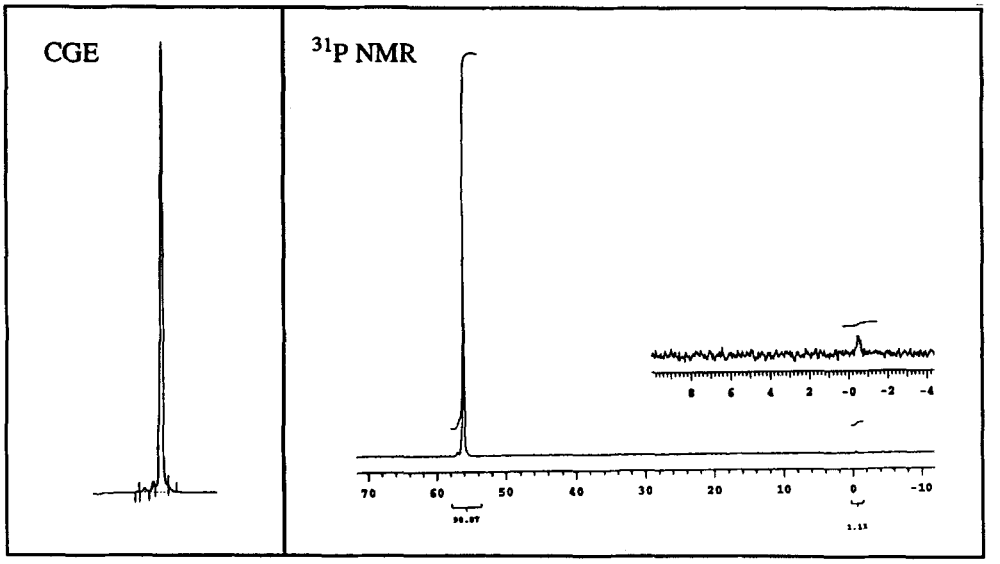


Figure 1. (left) CGE trace of T_{19} after HPLC purification. (right) ^{31}P NMR (161.9 MHz, D_2O) spectrum of T_{19}.

compared to **1a**, however only little selectivity is obtained (**3a/3b** = 61:38). (*O,O,S*)-Trialkyl phosphorothioates **1b** and **2b** afford under aqueous base conditions mainly phosphodiester **3b**, in case of **2b**, with very high selectivity. Deprotection with TBAF is very rapid, and most importantly, in case of DPSE protection *both*, **1a** and **1b** form only the desired phosphorothioate **3a**. TBAF treatment of **2b** gave multiple reaction products. Treatment of dimer DMTr$^{5'}$*O*-dA^{Bz}-P(S)(*O*-DPSE)-dC^{Bz}-$^{3'}$*O*Lev and trimer DMTr$^{5'}$*O*-dG^{ibu}-P(S)(*O*-DPSE)-T-P(S)(*O*-DPSE)-T-$^{3'}$*O*Lev with TBAF/THF gave the corresponding phosphorothioate diesters in less than 2 min with no phosphodiester linkages being detected.

Based on these results we developed a deprotection procedure for oligonucleotides. A DPSE protected T_{19} phosphorothioate bound to CPG was first treated with NH_4OH for 90 min to effect cleavage from the support and was subsequently treated with TBAF in THF (see *Experimental*). The CGE trace of the DMTr-on fraction from HPLC and the ^{31}P NMR spectrum of the crude oligomer product are shown in Fig. 1. The high phosphorothioate content (99%) compares well with what we typically see using β-cyanoethyl protected phosphoramidites.

In summary, 2-(diphenylmethylsilyl)ethyl (DPSE) protected amidites are useful synthons for the synthesis of phosphorothioate oligonucleotides. Treatment of DPSE-protected (*O,O,O*)- and (*O,O,S*)-trialkyl phosphorothioates with TBAF/THF leads to dialkyl phosphorothioates, with no phosphodiester linkages being detected.

Experimental: Oligonucleotide synthesis, deprotection and purification:
A T_{19} phosphorothioate oligonucleotide was assembled on 1 μmol scale on CPG support on an ABI 394 DNA/RNA synthesizer. DPSE protected phosphoramidite[3c] (0.1 M in CH_3CN, coupling time 900 s), 3*H*-1,2-benzodithiol-3-one-1,1-dioxide[6] (0.2 M in CH_3CN, 900 s) and standard ABI reagents were used. The oligonucleotide was synthesized DMTr-on to help separation of DMT-on full length oligonucleotide from capped failure sequences by HPLC. Deprotection: The CPG-bound oligomer was treated with NH_4OH (30%, 1 ml) for 90 min at r. t. and concentrated in vacuo. The dry powder was treated with tetrabutylammonium fluoride (1 ml, 1 M in THF) for 24 h at r. t. Water (1 ml) was added and the mixture was filtered (0.45 micron). The solid was rinsed with ethanol/water (1:1, 3 x 0.5 ml). The combined solutions were concentrated. Water (8 ml) was added and the solution was loaded on a SEP-PAK (Waters). Water (8 ml) was passed through the SEP-PAK, followed by CH_3CN/water (60:40, *v/v*) to elute the oligomer. The solution was concentrated in vacuo and subsequent HPLC-purification[7] afforded the DMTr-on fraction which was detritylated by treatment with acetic acid.

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7. Reversed phase C_{18} column (Waters Nova Pak) 3.9 x 300 mm, flow rate: 0.7 ml min^{-1}, CH_3CN (A)/triethylammonium acetate (0.125 M) gradient: 0-10 min: 10 to 35% A, 10 to 40 min: 35 to 50% A. t_R(DMT-T_{19}) 22-24 min.

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