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Synthesis and Deprotection of β-Silylethyl Protected *O,O,O*- and *O,O,S*-Trialkylphosphorothioates

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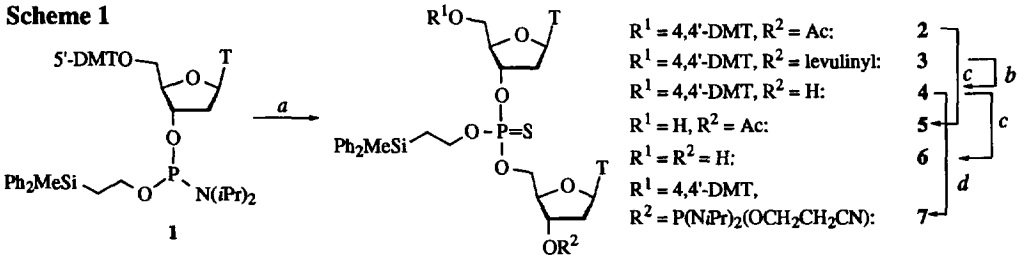
Abstract: Functionalized 2-(diphenylmethylsilyl)ethyl protected thymidyl-thymidine phosphorothioate dimers are easily accessible and stable under conditions used in oligophosphorothioate synthesis. Deprotection with ammonium hydroxide occurs through β-fragmentation. Methylamine and tetrabutylammonium fluoride rapidly and selectively remove the DPSE protecting group of *O,O,O*- and *O,O,S*-trialkylphosphorothioates.

The potential of modified oligonucleotides as antisense therapeutic agents has been demonstrated.[1] Among the DNA modifications reported to date, phosphorothioates are the first class of compounds to undergo clinical trials in humans.[1b] Very recently, we have successfully used the 2-(diphenylmethylsilyl) ethyl (DPSE) group for phosphate protection in DNA and phosphorothioate synthesis *via* the amidite approach.[2] Deprotection of the internucleotide linkage with methylamine or tetrabutylammonium fluoride (TBAF) leads exclusively to the corresponding dialkylphosphorothioate.[3]

Here, we describe the synthesis of DPSE-protected trialkylphosphorothioate thymidyl-thymidine dinucleosides with different 3' and 5' protecting group motifs. Phosphoramidite **7** was synthesized as a dimeric block synthon for phosphorothioate synthesis. Different mechanisms of deprotection of the internucleotide linkage of *O,O,O*- and *O,O,S* -trialkylphosphorothioates are discussed.

Scheme 1



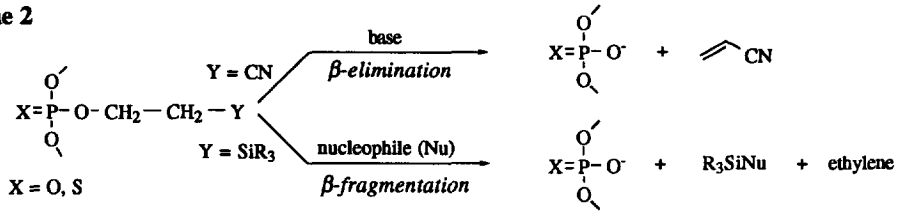
a: 1*H*-tetrazole (4 eq)/3'-*O*-Lev-T or 3'-*O*-Ac-T/Beaucage reagent (5 eq), dry CH_3CN; *b*: hydrazine, acetic acid/pyridine 2:3 (*v/v*); *c*: 2% dichloroacetic acid/CH_2Cl_2; *d*: $(NCCH_2CH_2O)P(NiPr_2)_2$, 1*H* tetrazole; T = N^1-thyminyl.

The synthesis of DPSE protected dimers **2-6** was performed on a multi-gram scale in solution (Scheme 1). Phosphoramidite **1**[2] was coupled with 3'-*O*-acetylthymidine or 3'-*O*-levulinylthymidine[4] in the presence of 1*H*-tetrazole (4 eq) in anhydrous acetonitrile, followed by oxidation of the trialkylphosphite intermediate with 3*H*-1,2-benzodithiol-3-one-1,1-dioxide (Beaucage reagent)[5] (5 eq) to afford the fully protected dimers **2** and **3**, respectively, as mixtures of R_p/S_p diastereomers in high yield (88 %). Removal of the 3'-acetate group of **2** with 30% NH_4OH/H_2O/ethanol (5:1:4, *v/v/v*) at r. t. was complete in 4 h. HPLC

indicated ca. 4% deprotection of the phosphorothioate moiety. In the case of **3**, treatment with hydrazine monohydrate in pyridine/acetic acid (3:2, *v/v*)[4] resulted in selective deprotection of the 3' terminus within minutes without affecting the phosphorus protecting group,[6] furnishing **4** in excellent yield (coupling, sulfurization, deprotection, 81%). Deprotection of the 5' terminus of **2** and **4** with 2% dichloroacetic acid in CH_2Cl_2 for 5 min provided **5** and **6**, respectively, in essentially quantitative yield (95%) indicating that the DPSE protecting group is also stable under conditions required for 5'-DMT deprotection. Phosphitylation of **4** with *O*-(2-cyanoethyl)-*N,N,N',N'*-tetraisopropylphosphordiamidite according to standard procedures followed by aqueous work-up and flash chromatography on silica provided **7** in 85% yield.[7]

Selective deprotection of the phosphorothioate internucleotide linkage is of crucial importance for an efficient oligonucleotide synthesis. The most widely used protecting group in amidite chemistry, the β-cyanoethyl group, is selectively removed with NH_4OH through *β-elimination*. In case of the DPSE group, selective deprotection upon treatment with nucleophiles also occurs, the electronic properties of the silyl group at the β-carbon favor a *β-fragmentation* as readily demonstrated by product analysis, eliminating acrylonitrile as a potential product contaminant.

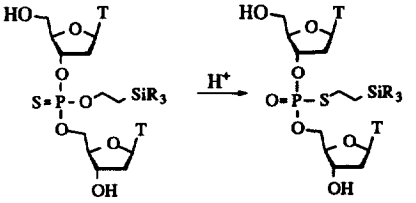
Scheme 2



Treatment of **5** with 30% NH_4OH/D_2O/ethanol (63:7:30, *v/v/v*) furnished phosphorothioate **8a** (^{31}P δ = 56.1, 56.2 ppm, 96%) and also dialkylphosphate **8b** (0.1 ppm, 3-4%). The kinetic analysis of the P deprotection at r. t. gave a pseudo-first-order rate constant of 2.2×10^{-5} s^{-1}; the rate equivalent to a half-life time of 88 h. Considering several mechanisms for DPSE deprotection such as (*a*) nucleophilic attack on the phosphorus or at the α-carbon, (*b*) β-elimination, or (*c*) nucleophilic attack at the silicon and subsequent β-fragmentation, one would expect corresponding formation of 2-(diphenylmethylsilyl)ethanol (**9**), diphenylmethylvinylsilane (**10**) or diphenylmethylsilanol (**11**) and ethylene, respectively. To determine the fate of the DPSE group on removal, we compared HPLC retention times of the reaction products with authentic samples of potential reaction products **9**, **10** and **11**.[8] A solution of **5** in 30% NH_4OH/EtOH (8:2, *v/v*) was kept at 62 °C in a sealed vial for 20 h. In addition to the fast eluting phosphorothioate **8a** an additional peak has been detected in the HPL-chromatogram. Co-injection of the reaction mixture and **9**, **10** (20 mol-%) and **11** (50 mol-%) showed that **9** and **10** were not present in the reaction mixture and that silanol **11** co-eluted with the new peak. A time course experiment showed that the peak area of **11** increased in proportion to the amount of **8** formed. The almost quantitative formation of **11** supports our hypothesis that deprotection of DPSE-protected *O,O,O*-trialkylphosphorothioates proceeds exclusively *via* a β-fragmentation mechanism. Comparing the ^1H NMR spectra of a solution of **6** in perdeuteroammonium deuteroxide before and after heating at 62 °C for 4 h showed the disappearance of a triplet at δ = 2.04 (J = 7.5 Hz, Si-CH_2) as expected when ethylene is formed as a volatile reaction product.

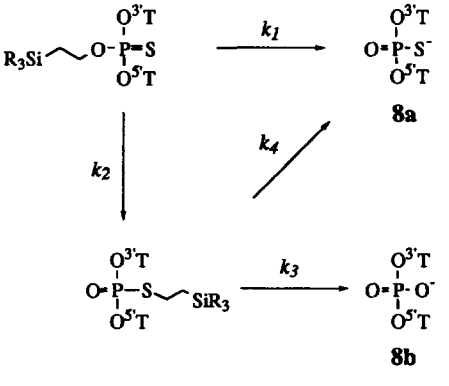
To provide further evidence that the dialkylphosphate formation during deprotection of **5** with NH_4OH has its origin in a thiono-thiolo rearrangement[3] we synthesized the presumed intermediate **12**. (Scheme 3) Heating a solution of **5** in 1% dichloroacetic acid in chloroform at reflux (3d) afforded **12**.[9] For comparison, rearrangement of **13** to **14** is complete within 36 h at r. t.. The stability and the deprotection products of trialkylsilyl and diarylalkyl substituted silylethyl protected *O,O,O*-and *O,O,S*-trialkylphosphorothioates under different deprotection conditions are compared in **Table 1**. All compounds are readily deprotected with NH_4OH, aqueous methylamine or TBAF/THF. The major products are phosphorothioate **8a** through β-fragmentation (k_1, k_4) and dialkylphosphate **8b** through hydrolysis at the phosphorus center (k_3) (Scheme 4). For *O,O,O*-trialkylphosphorothioates the ratio of **8a/8b** is determined by the ratio of k_1/k_2, k_2 is the rate constant for the competing thiono-thiolo rearrangement. In case of **5**, deprotection with NH_4OH is rather slow, leading to about 96% **8a**. Under the conditions, rearrangement of **5** to **12** can take place, which leads to formation of **8b**. Methylamine and TBAF increase the deprotection rate k_1 significantly, therefore the formation of **8b** is suppressed. The reactivities of NH_4OH and $MeNH_2$ toward **13**, which rearranges to the *O,O,S*-trialkyl isomer significantly faster than **5**, are very similar and consequently low **8a/8b** ratios are obtained. With TBAF, however, deprotection is very rapid, only **8a** is obtained. Deprotection of *O,O,S*-trialkylphosphorothioates with NH_4OH and $MeNH_2$ yields about 92% **8b** and 3-8% **8a** in case of **12**. TBAF completely shifts the mechanism of deprotection of **12** to a β-fragmentation as **8a** is the only product observed. Treatment of **14** with amine bases furnishes **8b** as the major product, with no **8a** being detected. TBAF treatment of **14** gave multiple reaction products.

Scheme 3



5 R_3 = Ph_2Me **12** R_3 = Ph_2Me
13 R_3 = tBuMe$_2$ **14** R_3 = tBuMe$_2$

Scheme 4



8a **8b**

Table 1. Half-life times and deprotection products of *O,O,O*- and *O,O,S*-trialkylphosphorothioates.

reagent	educt	$t_{1/2}$ [h]	**8a** [%]	**8b** [%]
NH_4OH (30%)/D_2O/ EtOH 63:7:30, *v/w/v*, r.t.	**5**	88	96	4
	12	9	3	92
	13	4	61	38
	14	13	0	96
NH_4OH (30%), 60 °C	**5**	0.3	97	3
	12	<0.1	7	90
	13	<0.1	64	33
	14	<0.1	00	>99
aqu. CH_3NH_2 (40%), r.t.	**5**	2	>99	0
	12	0.5	8	92
	13	1.7	63	37
	14	0.7	0	>99
nBu$_4$N$^+$F$^-$ (1M in THF), r.t.	**5**	<0.01	>99	0
	12	<0.01	>99	0
	13	<0.01	>99	0
	14	<0.01	17	n.d.

In summary, we have shown the preparation of useful DPSE protected phosphorothioate dimers. We provided experimental evidence that deprotection of 2-(diphenylmethylsilyl)ethyl-protected (*O,O,O*)-trialkyl phosphorothioates with NH_4OH proceeds through β-fragmentation. Deprotection of β-silylethyl *O,O,O*- and *O,O,S*-trialkylphosphorothioates furnishes dialkylphosphorothioate or dialkylphosphate depending on the reaction conditions.

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References and Notes

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6. A solution of **3** in 2.5% hydrazine in pyridine/actetic acid (3:2) was kept at room temperature for 24 h. ^{31}P NMR showed that the phosphorus protecting group is completely stable under these conditions.
7. Dimer amidite **7**: ^{31}P NMR (81 MHz, $CDCl_3$): 67.67, 67.3, 67.84, 149.5, 149.7, 150.0 ppm.
8. The HPLC system used consisted of a 600E System Controller, a 996 Photodiode Array Detector and a 717 Autosampler from Waters. We used a reversed phase C_{18} column (Waters Nova Pak) 3.9 x 300 mm, flow rate: 1 ml min^{-1}, acetonitrile (A)/water gradient: 0-5 min: 5% A, 5-10 min: 5 to 45% A, 10-40 min: 45 to 65% A, 40-45 min: 65 to 90% A, 45-55 min: 90% A.
   9 (Fluka), t_R = 31.3 min. Chlorodiphenylmetylsilane (Aldrich) was reacted with vinyl magnesium bromide in THF. Aqueous work-up, flash chromatography on silica and Kugelrohr-distillation afforded diphenylmethylvinylsilane **10**, t_R = 52.1 min. **11** was obtained by hydrolysis of chlordiphenylmethylsilane with aqueous lithium hydroxide, t_R = 29.5 min.
9. *O,O,S*-Trialkylphosphorothioate **12**, *experimental procedure*: A solution of **5** (500 mg, 0.64 mmol) in $CHCl_3$ (100 ml) containing dichloroacetic acid (1 ml) was heated at reflux for 3d. The solution was extracted twice with $NaHCO_3$ (0.5 M, 25 ml), dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography on silica (20 x 2 cm, gradient-elution: 1-8% methanol in ethyl acetate) furnished **12** (280 mg, 56%) and starting material. ^{31}P NMR (81 MHz, $CDCl_3$): δ 34.4, 34.5 ppm.

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