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USE OF 2-DIPHENYLMETHYLSILYLETHYL (DPSE) PROTECTING GROUP IN OLIGONUCLEOTIDE SYNTHESIS VIA PHOSPHORAMIDITE APPROACH

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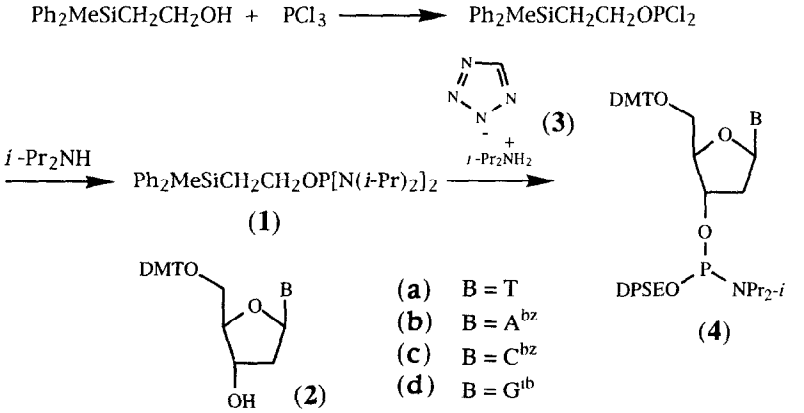
Abstract: 2-Diphenylmethylsilylethyl (DPSE) is a new protecting group for the internucleotidic bonds in the synthesis of deoxyribooligonucleotides by the phosphoramidite approach. This group is stable to acidic conditions and can be removed under mild conditions using aqueous ammonium hydroxide.

There have been several successful reports on the synthesis of oligonucleotides via phosphoramidite approach.[1,2] However there still remains some crucial problems of the removal of protecting groups of internucleotidic phosphates. The problems may be solved by finding more suitable protecting groups which can be removed under mild conditions. Till today few phosphate protecting groups satisfy these requirements. For example, 2-cyanoethyl,[3] 2,2,2-trichloro-1,1-dimethylethyl,[4,5] *p*-nitrophenylethyl,[6-8] and allyl[9,10] groups were reported by several laboratories.

We now wish to report that a) the easily accessible reagent bis[*N*,*N*-diisopropylamino]-2-diphenylmethylsilylethoxyphosphine(1) has excellent phosphitylating properties and b) the diphenyl methylsilylethyl (DPSE) group for protecting P-O can be removed under mild conditions using aqueous ammonium hydroxide.

First we examined the preparation of bis[*N*,*N*-diisopropyl amino]-2-trimethylsilylethoxyphosphine. This compound which was obtained as a colorless viscous liquid (^{31}P NMR δ 124), however was found to be too labile under any conditions for the subsequent condensation reactions. Next we turned our attention to the more stable diphenylmethylsilylethyl group. The favourable properties of diphenylmethylsilylethyl as a protecting group has been amply demonstrated by Honda and Hata in the synthesis[11] of oligo nucleotides using the phosphotriester approach. In order to find out

if these properties could be extended to the phosphoroamidite approach we set out to synthesize the phosphitylating reagent (**1**). To a 1.35 molar excess of phosphorus trichloride in anhydrous ether was added dropwise, during 30 min at 0^0C, 2-diphenylmethyl silylethanol[12] in ether. After 3 h at room temperature , the solution was concentrated[13] to remove excess phosphorus trichloride and redissolved in anhydrous ether. To this solution at 0^0C, was added a solution of diisopropylamine in ether. After stirring at room temperature overnight, the reaction mixture was filtered and concentrated[13] to afford the phosphitylating agent in almost quantitative yield. The crude phosphine was treated with nucleoside (**2a**) in the presence of (**3**) in acetonitrile. After 3 h, at room temperature, the usual aqueous work-up followed by silica gel flash chromatography gave (**4a**) in 77% yield. The ^{31}P NMR of (**4a**) showed two characteristic signals corresponding to a diastereomeric mixture and no 3'-3' dinucleoside phosphite could be detected. The phosphoramidites (**4b-d**) were prepared by a similar procedure in 68-74% yields.



The applicability of phosphoramidites (**4**) were demonstrated by the synthesis of four homo-dimers d(TpsT), d(CpsC), d(ApsA) and d(GpsG) (yields > 99%) on solid support. Sulfurization was effected using Beaucage reagent.[14,15] The compounds were identified by comparison with the same homo-dimers independently synthesized using cyanoethoxy protection on the phosphate backbone.

Table 1. Yields and ^{31}P NMR data of phoroamidites (**4a-d**)

Compound	Yield (%)	^{31}P NMR Chemical Shift (ppm)
4a	77	146.09, 146.32
4b	68	146.09, 146.48
4c	74	146.28, 146.68
4d	65	145.48, 146.18

The chemical shifts are given in ppm relative to the external standard of 85% H_3PO_4.

To check the stability of DPSE group in presence of aqueous ammonium hydroxide, the DPSE protected phosphorothioate homo-dimers d(TpsT), d(CpsC), d(ApsA) and d(GpsG) after cleavage from solid support were analysed by ^{31}P NMR at 55^{0}C. Complete deprotection takes place within 10 min. as shown by the shift in values from *ca.* 67 to 56 ppm. This indicates that the removal of the DPSE group is fast and a selective process as the cleavage of the internucleotide bond was not observed.

Synthesis of homo-thymidine heptamer: The applicability of DPSE protection was extended to the synthesis of a homo-thymidine phosphorothioate heptamer on a solid support. The following elongation cycle was utilised.

Steps	Solvents and reagents	Time (min)
1	CH_3CN	0.5
2	CH_2Cl_2	0.5
3	3% dichloroacetic acid in dichloromethane	2
4	CH_3CN	3
5	(**4a**) (0.2 M solution) in CH_3CN + Tetrazole (0.4 M solution) in CH_3CN	5
6	CH_3CN	0.5
7	Beaucage reagent (0.5 M solution) in CH_3CN	3
8	Beaucage reagent (0.5 M solution) in CH_3CN	3
9	CH_3CN	0.5
10	Cap A + Cap B	1

The overall coupling efficiency was found to be 99% as determined by the usual spectrophotometric quantitation of released *p,p'*-

dimethoxytriphenylmethyl cation. After the synthesis of heptamer, the CPG was treated first with NH_4OH at room temperature for 1 h and then at 55^0C for 1 h to afford the deprotected oligonucleotide.

In summary, the DPSE is a suitable protecting group for internucleotidic phosphate protecting group. Compared with other common protecting groups, as for example the cyanoethoxy group which is removed via a β-elimination mechanism, it is to be noted that the DPSE group can be easily removed by ammonium hydroxide via a β-fragmentation mechanism. Further applications of the DPSE group in the oligonucleotide synthesis are in progress.

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