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2-DIPHENYLMETHYLSILYLETHYL GROUP AS A NEW PROTECTING GROUP OF INTERNUCLEOTIDIC PHOSPHATES IN OLIGONUCLEOTIDE SYNTHESIS

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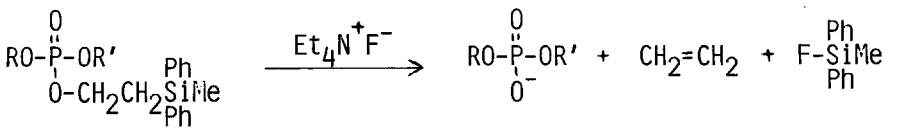
Abstract: *Internucleotidic phosphates were protected by 2-diphenylmethylsilylethyl group which was selectively removed by treatment with tetrabutylammonium fluoride.*

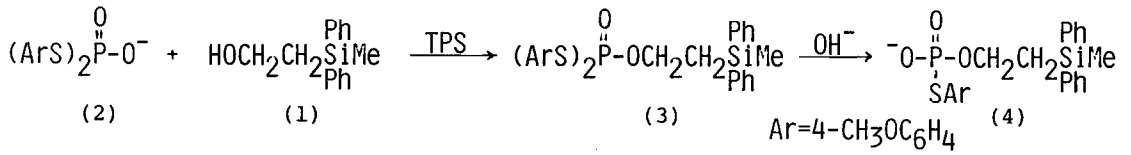
There have been several successful reports on the synthesis of oligonucleotides *via* phosphotriester approach,[1] however, there still remain crucial problems of the removal of protecting groups of internucleotidic phosphates.[2] The problem may be solved to find more suitable protecting group which can be removed by β-elimination mechanism from the internucleotidic phosphates. Up to date, there have been a few phosphate protecting groups which satisfy the above requirement. For example, 2-cyanoethyl,[3] 2,2,2-trichloroethyl,[4] and 2-p-nitrophenylethyl[5] groups were proposed and examined in several laboratories.

On the other hand, Gerlach[6] and Carpino[7] reported that 2-trimethylsilylethyl group could be used for protection of carboxylic acids and the ester linkage could be easily cleaved by treatment with tetrabutylammonium fluoride (TBAF). 2-Trimethylsilylpropen-2-yl group was proposed by Chan[8] and applied to simple phosphates. We have tried to apply these protecting groups containing silicon atom to the synthesis of oligonucleotides. However, they were found to be too labile under any conditions for the condensation reactions. In order to overcome the instability of such a type of protecting groups containing silicon atom, several 2-tri-alkyl(or aryl)silylethyl groups were examined. Finally, we could find 2-diphenylmethylsilylethyl group (se) which could be introduced easily and removed selectively by treatment with TBAF from the phosphotriesters along with the formation of ethylene and di-phenylmethylsilyl fluoride.



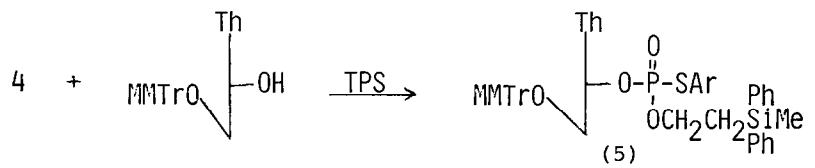
A new phosphorylating agent, 2-diphenylmethylsilylethyl S-p-methoxyphenyl

phosphorothioate (4) was prepared as follows: 2-Diphenylmethylsilylethanol (1) (53.6 g, 73%), bp. 153-155°(0.5 mmHg), was obtained by a modification of the procedure of Gerlach[6] from the reaction of diphenylmethylsilyl acetate[9] (85.0 g, 0.30 mol) with $LiAlH_4$ (21.4 g, 0.56 mol) in dry ether (600 ml) under reflux for 3 h. When 1 (2.86 g, 11.8 mmol) was treated with S, S'-di-p-methoxyphenyl phosphorodithioate (2)[10] (5.30 g, 12 mmol) in the presence of TPS (4.38 g, 14.5 mmol) in dry pyridine (30 ml), the desired phosphotriester (3) was obtained and separated by silica gel column chromatography with ether-CH_2Cl_2 [1:9(v/v)]. After removal of the solvent, the residue was treated with alkaline solution [0.2 M NaOH/dioxane, 1:1(v/v)] for 30 min at room temperature in order to remove selectively one of the two arylthio groups of the triester.[10b] The desired phosphorylating agent (4) was isolated as a cyclohexylammonium salt.



Compound 4 was applied to the synthesis of oligothymidylates via phosphotriester approach.

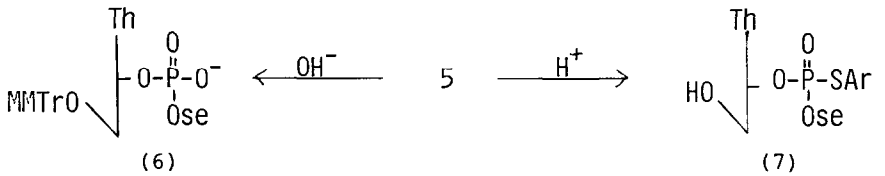
For example, the condensation reaction of 5'-O-monomethoxytritylthymidine (1.55 g, 3.0 mmol) with 4 (2.12 g, 3.9 mmol) in the presence of TPS (2.60 g, 8.6 mmol) in dry pyridine (5 ml) gave 2-diphenylmethylsilylethyl S-p-methoxyphenyl 5'-O-monomethoxytritylthymidine 3'-phosphorothioate (5)(2.60 g, 92%) [Rf: 0.52, solvent A, CH_2Cl_2/MeOH, 9:1 (v/v)] which was separated by silica gel column chromatography by using CH_2Cl_2 containing a small amount of methanol (0–3%).



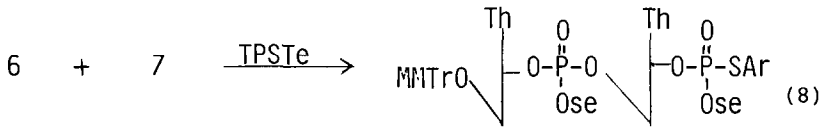
The arylthio group was selectively removed from 5 by treatment with alkaline solution [0.2 M NaOH/dioxane, 1:1(v/v)] for 1 h at room temperature to give 2-diphenylmethylsilylethyl 5'-O-monomethoxytritylthymidine 3'-phosphate (6) in almost quantitative yield.

On the other hand, monomethoxytrityl group could be selectively removed from 5 by treatment with 2% trifluoroacetic acid in CH_2Cl_2 at 0°, giving the thymidine 3'-phosphotriester derivative (7) in 88% yield (Rf 0.45: solvent A). The condensation reaction of pyridinium salt of 6 (110 mg, 0.13 mmol) with 7 (69 mg, 0.1 mmol) in the presence of 2,4,6-triisopropylbenzenesulfonyltetrazole (TPSTe) (0.4 mmol) formed by the reaction of TPS (121 mg, 0.4 mmol) with tetrazole (28 mg, 0.4 mmol) in the presence of triethylamine (56 ul, 0.4 mmol) in situ, was carried out in dry pyridine (0.5 ml) at room temperature for 3 h and gave the fully

protected dithymidylate, MMTrTp(se)Tp(se)(SAr), (8) in 72% (108 mg) yield (Rf. 0.42, solvent A). In the above reaction other condesing agents, e.g., TPS and mesitylenesulfonyltriazole were found to be ineffective.



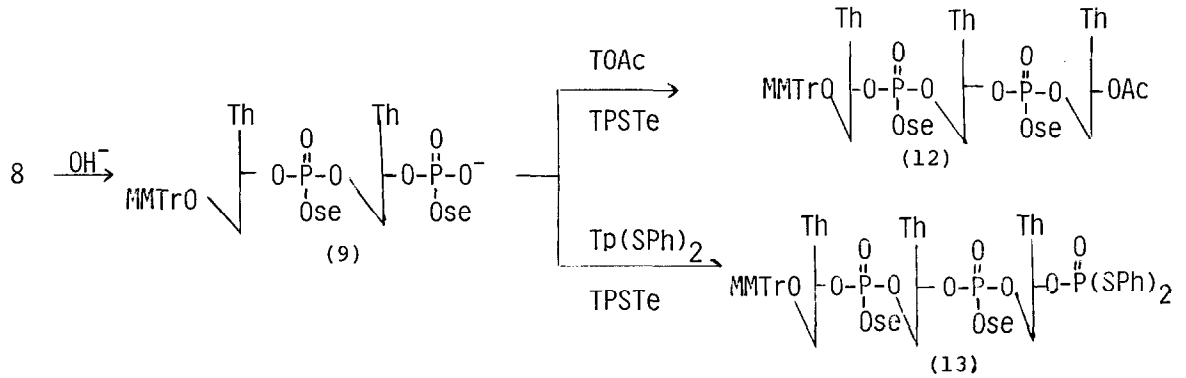
(6) (7)



(8)

Similary, the fully protected dinucleoside monophosphates, MMTrTp(se)TOAc (10) and MMTrA^{bz}p(se)TOAc (11) were synthesized in 60% and 72% yield, respectively. In both cases, the diastereoisomers were separated on tlc (see Table 1).

Further, the fully protected trinucleotides e.g., MMTrTp(se)Tp(se)TOAc (12) and MMTrTp(se)Tp(se)Tp(SPh)$_2$ (13) were synthesized in 55% and 47% yields respectively. The reaction conditions and the results are summarized in Table 1.



Deprotection of 11 was performed as follows: Compound 11 was treated with 0.05 M TBAF (2 equiv.) in (THF/pyridine/water-8:1:1) at room temperature for 24 h, followed by treatment of methanolic ammonia and 80% acetic acid in the usual workup. Dinucleoside phosphate, ApT, was obtained by paper chromatography (Whatman 3MM) in almost quantitative yield. The structure of ApT was confirmed by the enzymatic degradation with snake venom phosphodiesterase, giving a reasonable ratio of Ap:T (1.13:1:0).

Deprotection of both 10 and 12 was performed in the same manner, to afford TpT and TpTpT in almost quantitatively.

Table 1 Synthesis of the Fully Protected Di- and Tri-nucleotides

Phosphate component (mmol)	hydroxyl component (mmol)	condensing agent (mmol)	pyridine (ml)	time (h)	product (yield)	Rf value (solvent A*)
MMTrTp(se) (0.26)	TOAc (0.20)	MSTe*** (0.82)	1.5	3.5	10 (72%)	0.68 and 0.58**
MMTrA^{bz}p(se) (0.41)	TOAc (0.33)	TPSTe (1.22)	2.0	5	11 (60%)	0.49 and 0.40**
MMTrTp(se)Tp(se) (0.07)	TOAc (0.09)	TPSTe (0.21+0.14)	1.0	40	12 (55%)	0.30
MMTrTp(se)Tp(se) (0.085)	Tp(SPh)$_2$ (0.08)	TPSTe (0.424)	2.0	37	13 (47%)	0.37

* Solvent A : CH_2Cl_2/MeOH, 9:1 (v/v)
** diastereoisomers; *** MSTe refers to mesitylenesulfonyltetrazole.

In these cases, the cleavage of internucleotide bond didn't take place during the deprotection since any other spots were not detected on paper chromatogram.

Compared with other protecting groups, it is noted that the se group can be easily removed by an attack of fluoride ion from TBAF not on phosphorus atom but on silicon atom via β-elimination mechanism, so that reactive intermediate, the phosphorofluoridate does not produced during the deprotection process.

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