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2-(Trimethylsilyl)ethyl as a Phosphate Protecting Group in Oligonucleotide Synthesis

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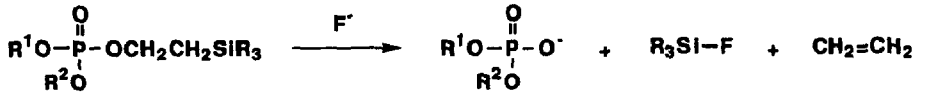
Abstract: The 2-(trimethylsilyl)ethyl (TSE) group was found to be effective as a protecting group for the internucleotidic phosphate in oligonucleotide synthesis. Phosphoramidite building blocks having the TSE group were prepared in good yields. In the case of deoxyguanosine, a 2-*N*-unprotected phosphoramidite building block was synthesized. These compounds were applied to the solid-phase synthesis of oligodeoxyribonucleotides. A side reaction associated with the unprotected guanine moiety was elucidated by means of ^{31}P NMR.

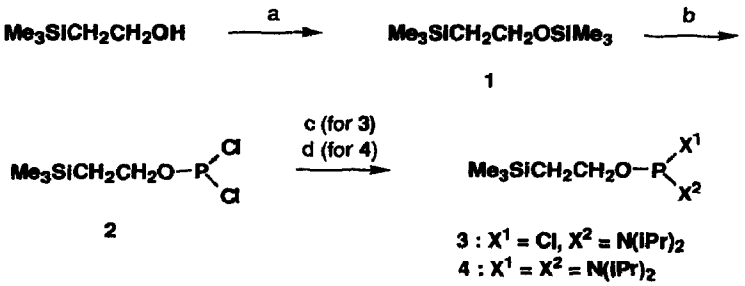
In the chemical synthesis of natural products having phosphoric acid esters, the selection of the phosphate protecting group is of great importance. A number of protecting groups have been developed for mono- and di-alkyl phosphates. Up to date, a few silicon-based phosphate protecting groups have been proposed. For instance, 2-(trimethylsilyl)propen-2-yl,[1] 2-(triphenylsilyl)ethyl,[2] and 2-(trimethylsilyl)ethyl[2,3,4] groups were explored for the phosphomonoester protection. These groups involve "trialkylsilylethyl" backbone structures, which can be deprotected by fluoride ion via β-elimination mechanism.



The only example of silicon-based protecting group for the internucleotidic phosphodiester was the 2-(diphenylmethylsilyl)ethyl group which was applied to the phosphotriester approach.[5] The 2-(trimethylsilyl)ethyl (TSE) group was also examined for the internucleotidic phosphodiester protection in the same literature. However, this group was found to be too labile during phosphotriester coupling reactions. This instability of the TSE group bound to the phosphate is attributed to the electron-withdrawing character of a phosphorus(V) intermediate produced by a strong condensing reagent. In contrast to this fact, the trialkylsilylethyl groups were proved to be stable under coupling conditions via phosphorus(III) intermediates.[2,3,4] In this paper, we wish to describe a novel strategy for DNA synthesis by the use of TSE for the internucleotidic phosphate protection based on the phosphoramidite chemistry.

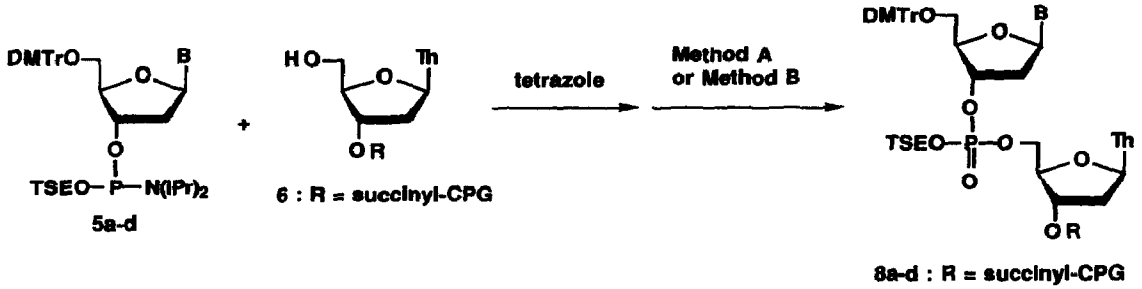
Preparation of phosphitylating reagents having the TSE group was examined. 2-(Trimethylsilyl)ethanol was silylated with hexamethyldisilazane in the presence of a catalytic amount of trimethylsilyl chloride at 120 °C for 6 h to afford 1-trimethylsilyl-2-trimethylsilyloxyethane **1** (bp 56 °C / 19 mmHg) in 98% yield. It was treated

with phosphorus trichloride under reflux for 1 h to give dichloro[2-(trimethylsilyl)ethoxy]phosphine (2)[6] in 72% yield. The resulting dichlorophosphine 2 was treated with diisopropylamine in Et_2O to afford the corresponding chloro(*N*,*N*-diisopropylamino)phosphine (3),[7] and bis(*N*,*N*-diisopropylamino)phosphine (4)[8] in 76% and 66% yields, respectively. These phosphitylating reagents had lower boiling points and were easier to handle than the corresponding 2-cyanoethoxy derivatives.



Reagents: (a) $Me_3SiNHSiMe_3$ (0.55 equiv), Me_3SiCl (cat); (b) PCl_3 (3 equiv); (c) iPr_2NH (2 equiv) / Et_2O; (d) iPr_2NH (5 equiv) / Et_2O.

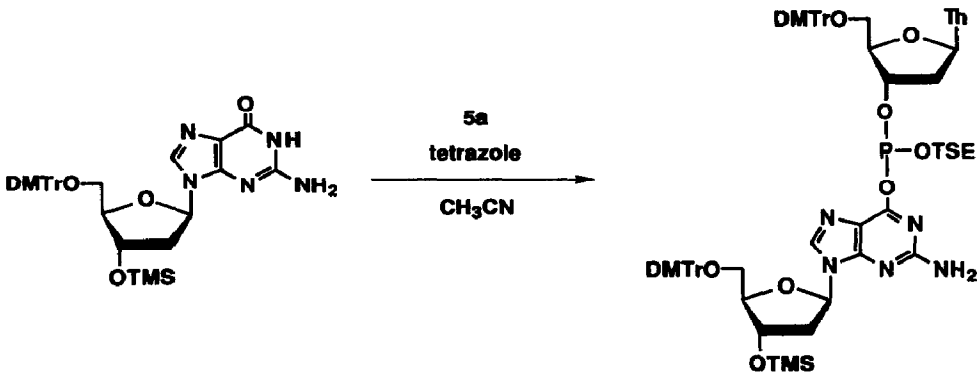
The phosphitylating reagent 3 or 4 was allowed to react with 5'-*O*-dimethoxytritylthymidine, 5'-*O*-dimethoxytrityl-6-*N*-benzoyldeoxyadenosine, and 5'-*O*-dimethoxytrityl-4-*N*-benzoyldeoxycytidine in the usual manner[9,10] to afford phosphoramidites building blocks **5a-c** in 80-86% yields. Recently, Letsinger has reported that the phosphoramidite approach by use of phosphoramidite units having unprotected bases,[11,12] where the free amino groups of deoxyadenosine and deoxycytidine were considerably phosphitylated with activated phophoramidite reagents but the amino group of deoxyguanosine was relatively resistant. On the basis of this fact, we also tried to prepare a phosphoramidite unit of deoxyguanosine without 2-*N*-acyl protection. 5'-*O*-Dimethoxytritylideoxyguanosine was phosphitylated with 3 in the presence of diisopropylammonium tetrazolide in CH_2Cl_2 to afford the desired phosphoramidite unit **5d** in 92% yield. The phosphoramidite building blocks having the TSE group were highly lipophilic and allowed easy purification by silica gel column chromatography.



a : B = Th; b : B = Ad^{bz}; c : B = Cy^{bz} ; d : B = Gu

Metod A: (1) I_2 oxidation, (2) capping; Method B: (1) capping, (2) I_2 oxidation.

The TSE-phosphoramidite (**5a**, **5b**, **5c**, or **5d**, 0.1 M in CH_3CN, 20 μmol) was condensed with thymidine 3'-*O*-succinate bound to controlled pore glass (CPG) **6** (1 μmol) in the presence of 1*H*-tetrazole (1.0 M in CH_3CN, 200 μmol) for 5 min. After oxidation with 0.1M I_2 in aqueous pyridine for 1 min and the successive capping reaction using Ac_2O-0.1M DMAP in pyridine for 2 min (Method A), the yields of the dimers **8a-d** were estimated by DMTr assay. By use of the thymidine, deoxyadenosine, and deoxycytidine units, the coupling yields were higher than 99%. In the case of deoxyguanosine unit, the coupling yield was 103%. This result indicated that some side reactions occurred on the unprotected guanine moiety to some extent. In order to elucidate the side reaction of the guanine moiety with activated phosphoramidite reagent, a model reaction of 3'-*O*-trimethylsilyl-5'-*O*-dimethoxytrityldeoxyguanosine[13] with **5a** was examined. ^{31}P NMR analysis of the model reaction in CH_3CN-CD_3CN (3:2, v/v) showed resonance peaks of products at 134.1 and 134.4 ppm. Ogilvie reported that similar chemical shifts (133.8 and 134.0 ppm) which were assigned to the diastereomeric O^6-phosphitylated 2-*N*-isobutyryl deoxyguanosine derivatives.[14] In comparison with this fact, we concluded that phosphitylation took place at the O^6-position of unprotected guanine. The O^6-phosphitylated guanine derivatives were found to be converted to the unmodified guanine derivative immediately by treatment with the capping solution as described by Ogilvie.[14,15] When the oxidation and capping steps were reversed in the solid-phase synthesis (Method B), the coupling yield of the deoxyguanosine unit was reduced to 99%. This fact suggested that the O^6-phosphitylated guanine base moiety was recovered by treatment with the capping solution.



Next, solid-phase synthesis of a hexadeoxyribonucleotide (dTGACTT) was examined. Thymidine 3'-*O*-oxalate bound to CPG 7[16] (1 μmol) was used as an anchor nucleoside and the fully protected hexamer was synthesized according to the conventional chain elongation procedure with 99% average coupling yield. The fully protected hexamer bound to CPG was treated with concd NH_3 at room temperature for 10 min to cleave the oxalyl linkage. The internucleotidic TSE groups were deprotected by treatment with 1.0 M Bu_4NF in THF for 5.5 h. The crude mixture was treated successively with concd NH_3-pyridine (9:1, v/v) at room temperature for 24 h and with 80% acetic acid for 15 min. The crude product was purified by C_{18} reversed phase HPLC (Fig. 1) and 25 A_{260} units of pure dTGACTT was obtained in 53% yield. The hexamer was completely digested with snake venom phosphodiesterase and dephosphorylated with calf intestinal alkaline phosphatase to give dT, dC, dA, and dG in the reasonable ratio (Fig. 2). It was noteworthy that the modified deoxyguanosine derivative was not detected by the HPLC analysis.

In conclusion, the 2-(trimethylsilyl)ethyl (TSE) group has proved to be potentially useful as a new protecting group for the internucleotidic phosphate in oligonucleotide synthesis.

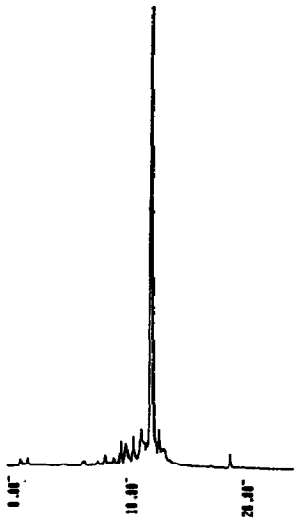


Fig. 1 Reversed phase HPLC profile of crude dTGACTT.

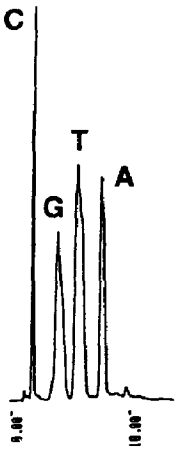


Fig. 2 Reversed phase HPLC profile after treatment with snake venom phosphodiesterase and calf intestinal alkaline phosphatase.

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References and Notes

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6. bp 38-39 °C / 0.9 mmHg, ^{31}P NMR ($CDCl_3$) δ 161.3.
7. bp 100-101 °C / 1.4 mmHg, ^{31}P NMR ($CDCl_3$) δ 181.8.
8. bp 103-105 °C / 0.6 mmHg, ^{31}P NMR ($CDCl_3$) δ 123.6.
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