A New Silyl Ether-Type Linker Useful for the Automated Synthesis of Oligonucleotides Having Base-Labile Protective Groups

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A new silyl-type linker for the automated solid-phase synthesis of oligodeoxynucleotides was developed. A hydrosilane-type reagent for introduction of the silyl linker was synthesized in an overall yield of 50% from 1,4-dibromobenzene. By using this linker, an oligonucleotide having base-labile protecting groups could be isolated from the controlled pore glass under neutral conditions.

Over the past several years, a number of approaches for gene therapy have been explored by use of oligonucleotide analogues as drugs.[1] In particular, phosphorothioate (PS) oligonucleotides have been well studied as the first-generation antisense molecules both *in vitro* and *in vivo*, and their usefulness has been demonstrated by many examples of successful inhibition of expression of the target genes.[2] However, side effects were observed in toxicity studies in mice, rats and monkeys.[3] Therefore, various oligonucleotides as the second-generation antisense oligonucleotides were required to overcome these problems. Furthermore, synthetic oligonucleotides were widely used in the field of the analysis of single nucleotide polymorphism (SNP).[4] To develop the antisense therapy and DNA diagnosis, large quantities of oligonucleotides with various functional groups involving base-labile ones should be supplied rapidly and inexpensively by the method of automated oligonucleotides synthesis.[5]

In the current automated solid-phase synthesis of DNA, oligonucleotides bound to solid supports *via* a succinyl linker are liberated by treatment with aqueous ammonia.[6] However, DNA oligomers having base-labile functional derivatives could not be synthesized by the standard method. An approach to improve the oligonucleotide synthesis involves the use of an oxalyl linker, which was reported by Letsinger in 1991.[7] By using this linker, oligonucleotides could be released from the solid support by treatment with propylamine under anhydrous conditions, and oligodeoxynucleotides having *N*-benzoylated deoxycytidines were synthesized. Fraser *et al.* reported a diisopropylsilanediyl linker[8] (**1a**) which allowed the release of oligodeoxynucleotides from solid supports under neutral conditions. They also synthesized oligodeoxynucleotides having *N*-benzoylated deoxycytidines. However, partial cleavage of the silanediyl group of the linker was observed during the detritylation step under acidic conditions.[9] Recently, Lipshutz *et al.* reported the silyl linker (**1b**) for the solid-phase peptide synthesis using *para*-bromopolystyrene support (1% crosslinked, 200-400 mesh, 2.6 μmol/g).[10] Therefore, we designed a silyl-type linker (**1c**) bound to aminopropyl controlled pore glass (AP-CPG) which was perfectly compatible with oligonucleotide synthesizers, as shown in Figure 1.

Scheme 1 shows a simple and efficient synthetic route to the T-loaded AP-CPG support bridged by the linker **1c**. Triethylammonium 4-(diisopropylsilyl)benzoate (**4**) was synthesized from 1,4-dibromobenzene (**2**) by using stepwise halogen-metal exchange

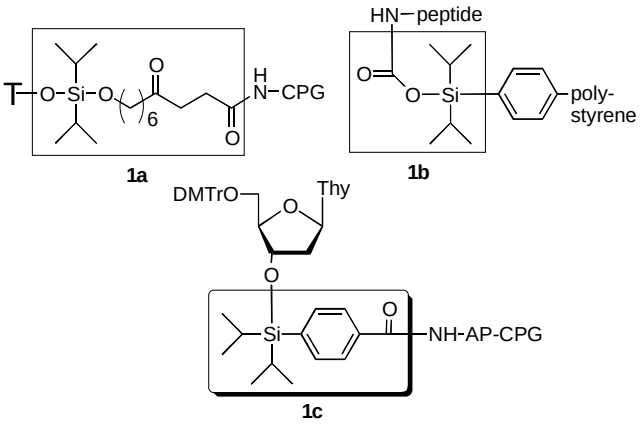
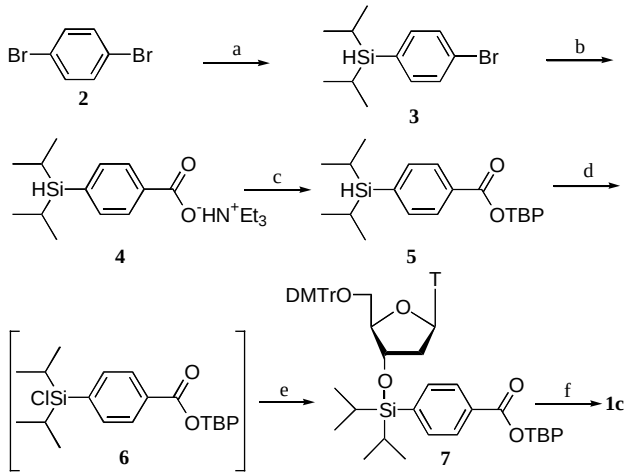


Figure 1.



Scheme 1. a) BuLi (1 equiv), THF, −78 °C, 5 min. then diisopropylchlorosilane (1 equiv), 15 min. 86%. b) BuLi(1 equiv), THF, −78 °C, 5 min. then excess dryice. 69% c) 2,4,6-tribromophenol(TBPOH)(2 equiv), BOPCl (4 equiv), pyridine, rt, 30 min. quant. d) 1,3-dichloro-5,5-dimethylhydantoine (2 equiv), CH_2Cl_2, rt, 1 h. e) DMTrT (2 equiv), imidazole(10 equiv), CH_2Cl_2, rt, 1 h. 84%. f) aminopropyl-CPG, CH_2Cl_2, rt, 36 h.

reactions[11] and purified by silica-gel column chromatography containing 1% triethylamine. The tribromophenyl (TBP) ester **5**[12] was obtained by condensation of **2** with tribromophenol in the presence of BOPCl. Compound **5** was converted to a silyl chloride derivative **6** by treatment with 1,3-dichloro-5,5-dimethylhydantoine.[13] The *in situ* generated silyl chloride was allowed to react with 5'-*O*-DMTr-thymidine in the presence of imidazole to give the anchor nucleoside **7** in an overall yield of 50% from commercially available 1,4-dibromobenzene (**2**). When the pentafluorophenyl ester was employed, it partially decomposed during the 3'-*O*-silylation. Derivatization of aminopropyl-con-

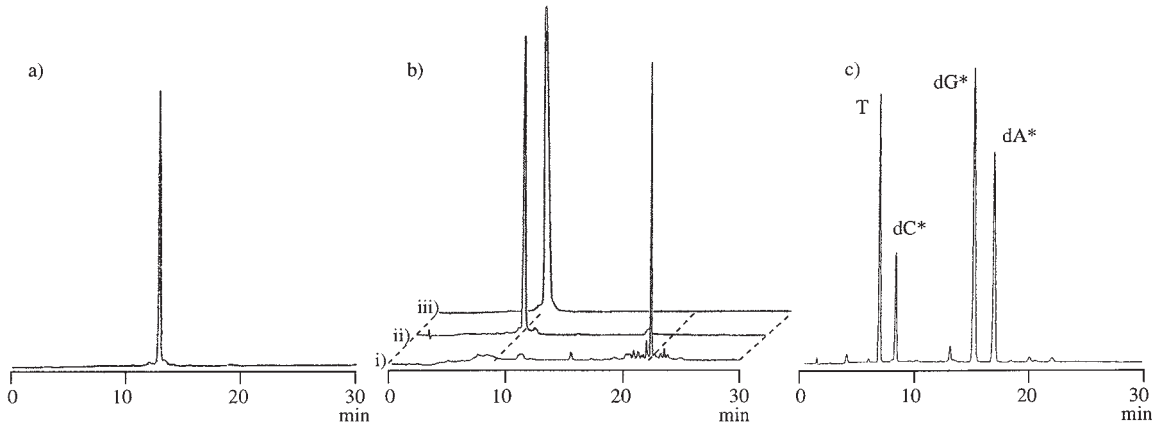


Figure 2. a) Anion exchange HPLC profiles of TTTTTTTT synthesized from the anchor nucleoside **1**. b) Reversed-phase HPLCprofiles of i) d(C*A*astG*T)$_3$ synthesized from the anchor nucleoside **1** ii) d(C*A*G*T)$_3$ after NH_3 treatments iii) d(CAGT)$_3$ synthesized from the standard succinate linker. c) Reversed-phase HPLC profiles of the mixture of T, dC*, dG* and dA* which was obtained after treatment of d(C*A*G*T)$_3$ with SVPD and AP.

trolled pore glass (AP-CPG, 89.2 μmol/g) was performed with **7** at room temperature for 36 h. After capping of the unreacted amino functions with Ac_2O in the presence of DMAP, the DMTrT-loaded resin (**1**: 20.8 μmol/g) was obtained. When this resin was treated with 1 M TBAF-AcOH in THF for 1 h, 90% of the nucleoside was released from the solid support, as evidenced by the DMTr assay.

To demonstrate the practical value of the silyl linker in the automated DNA synthesis, TTTTTTTT was prepared from **1c** in the phosphoramidite approach[14] on an Applied Biosystems 392 DNA/RNA synthesizer. As the result, the average coupling yield was >98% which was measured by the DMTr cation assay. Treatment of 10% DBU/CH_3CN for 30 s for deprotection of the cyanoethyl group was required before the cleavage of the oligonucleotide using 1 M TBAF-AcOH in THF for 1 h. After SepPak chromatography purification,[15] TTTTTTTT was obtained in an overall yield of 73% from the T-loaded resin having the linker **1c**. The anion exchange HPLC profile of the final product is shown in Figure 2(a). Furthermore, an oligodeoxynucleotide 12mer including *N*-acetyldeoxycytidine dC*,[16] *N*-benzoyldeoxyadenosine dA* and *N*-isobutyryldeoxyguanosine dG* whose acyl groups are known to be easily eliminated under basic conditions were similarly synthesized from the T-loaded resin with the average coupling yield of >98%. The reversed-phase HPLC profile of d(C*A*G*T)$_3$ thus obtained is shown in Figure 2b-(i). Figure 2b-(ii) shows the HPLC profiles of the reaction mixtures obtained by treatment of d(C*A*G*T)$_3$ with NH_3aq for 12 h at 60 °C. It was shown that the *N*-acylated DNA oligomer could be converted to the natural-type DNA oligomer under basic conditions. The structures of these purified products were further confirmed by MALDI-TOF mass. The *N*-acylated oligonucleotide d(C*A*G*T)$_3$ was treated successively with snake venom phosphodiesterase (SVPD) and calf intestinal alkaline phosphatase (AP) to give T, dC*, dG* and dA* in the expected ratio, as shown in Figure 2c. From these results, it was found that d(C*A*G*T)$_3$ was synthesized without elimination of the acyl group through the DNA synthesis.

In conclusion, we have demonstrated the utility of the silyl linker for DNA solid-phase synthesis. The linker was fully matched to the present automated DNA synthesizer, and could be used for the synthesis of oligonucleotides having various base-labile functional groups. Further studies are now under way.

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This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 75th birthday

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