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Use of 2-(*tert*-Butyldiphenylsilyloxymethyl) Benzoyl as *N*-Protecting Group for the Synthesis of Prooligonucleotides

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Abstract—A short TCCT Me-SATE prooligonucleotide was successfully synthesized using 2-(*tert*-butyldiphenyloxymethyl) benzoyl protecting group, after its removal by means of trimethylsilyl chloride and water.

Antisense oligonucleotides (oligos) are designed to interact with mRNA targets to downregulate protein expression. However, these polyanionic compounds pass through the hydrophobic lipid cellular membrane with only poor efficiency. To overcome that limitation we applied a prodrug strategy to the oligos with the synthesis of prooligonucleotides (prooligos).[1] We have shown that polythymidine prooligos are rapidly and efficiently taken up by HeLa cells.[2] Now, to evaluate the efficiency of the prooligonucleotide approach to inhibit gene expression it is necessary to synthesize prooligos bearing the three other nucleobases (i.e., A, C, and G). Since prooligos are highly sensitive to ammonia treatment, we cannot use the standard acyl protecting groups (i.e., benzoyl and isobutyryl) for the protection of the exocyclic amine of the nucleosides. Several protecting groups were evaluated for this purpose in our laboratory.[3] Using photocleavable 2,2′-bis(2-nitrophenyl) ethoxycarbonyl protection, we were able to synthesize the first prooligonucleotides constituted with the four nucleobases.[4] However, a photolabile protecting group presents some drawbacks as we must photolyze at low concentration to avoid any saturation because of the absorption of the nucleobases. Hence the longer the oligo, the harder the photolysis.

We present here the use of 2-(*tert*-butyldiphenylsilyloxymethyl) benzoyl (SiOMB) as a protecting group for the synthesis of prooligonucleotides. This acyl protecting group was introduced previously for the synthesis of RNAs which are sensitive to long ammonia treatment.[5] The SiOMB group was removed by treatment with TBAF which leads to the hydrolysis of the silyl ether and then the free hydroxyl via an intramolecular nucleophilic attack yields the deprotected nucleobase and the phthalide. Such a treatment could not be used with prooligos but silyl groups could be removed under a wide type of mild conditions.

To evaluate the interest of this protection for the synthesis of prooligos, we first introduced the SiOMB on deoxycytidine, converted it as a phosphoramidite building block, and then we synthesized a short Me-SATE TCCT prooligo on a photolabile solid support.[6] The deprotection was performed by means of a solution of trimethylsilyl chloride (TMSCl)/water in acetonitrile.

The synthesis of the Me-SATE TCCT prooligo requires the preparation of Me-SATE phosphoramidite building blocks of thymidine[7] and deoxycytidine. This latter was synthesized as follows. 4-*N*-[2-(*tert*-Butyldiphenylsilyloxymethyl)]benzoyl deoxycytidine was synthesized according to the transient di-*O*-3′,5′-trimethylsilyl protection (Fig. 1).[8] The deoxycytidine hydrochloride, dissolved in dry pyridine, was treated with 1 equiv of triethylamine to recover the free amine. The 3′- and 5′-hydroxyls were transitorily protected with trimethylsilyl groups and the *N*-4 amine was treated with the 2-(*tert*-butyldiphenylsilyloxymethyl) benzoyl chloride (1.1 equiv)[9] to yield the fully protected deoxycytidine **1**. The

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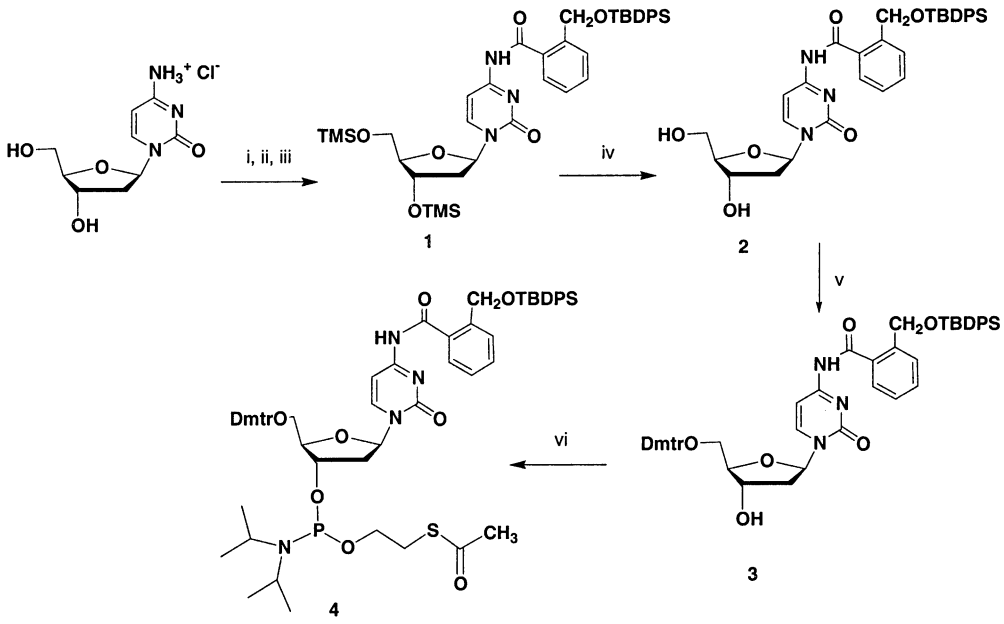


Figure 1. Synthesis of deoxycytidine Me-SATE phosphoramidite bearing the SiOMB protecting group. (i) Et_3N (1 equiv), dry pyridine; (ii) trimethylsilyl chloride (10 equiv); (iii) SiOMBCl (2.2 equiv); (iv) MeOH, H_2O; (v) DmtrCl (1.2 equiv), dry pyridine; (vi) *N*,*N*,*N*′*N*′-tetraisopropyl-*S*-acetyl-2-thioethyl phosphorodiamidite (1.2 equiv), diisopropylammonium tetrazolide (0.5 equiv), dry methylene chloride.

trimethylsilyl groups were removed by treatment with methanol/water to give **2** (overall yield 42%). The *N*-4-SiOMB deoxycytidine was 5′-dimethoxytritylated and then converted into the phosphoramidite derivative by reaction with *N*,*N*,*N*′*N*′-tetraisopropyl-*S*-acetyl-2-thioethyl phosphorodiamidite activated with diisopropylammonium tetrazolide (0.5 equiv) in freshly distilled methylene chloride (**4**: 50%, ^{31}P NMR 148.9 and 149.2 ppm).

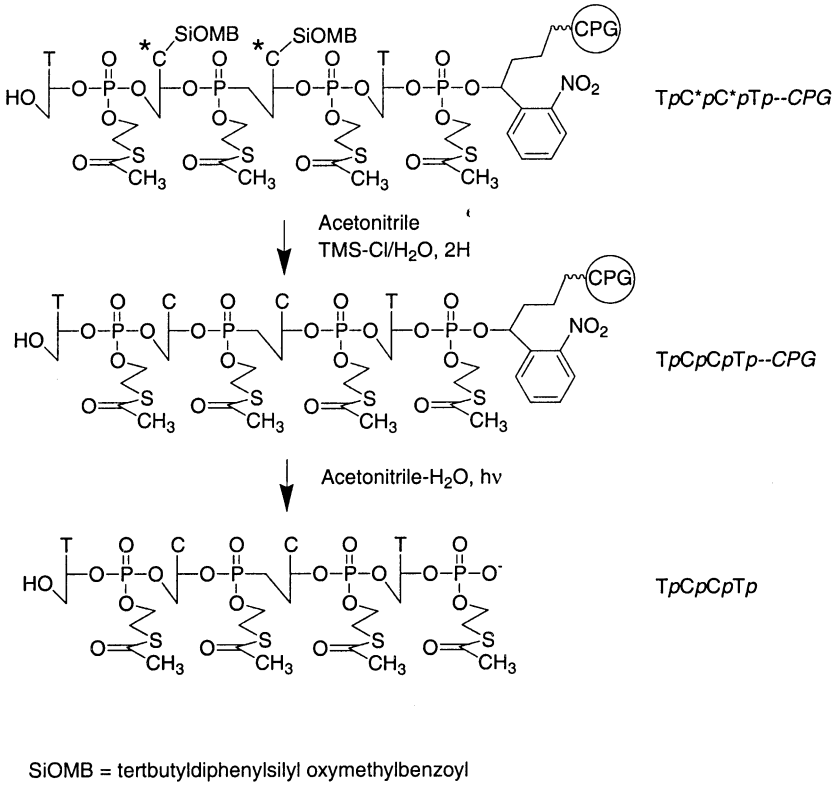
The TCCT Me-SATE prooligo was synthesized on a photolabile solid support according to the already published procedure[7] (average coupling 97% calculated from the trityl assay).

The removal of the SiOMB groups was performed directly on solid support by treatment with a solution of trimethylsilyl chloride/water/acetonitrile (5 μL/2.5 μL/50 μL).[10] This treatment leads to the in situ generation of a small amount of HCl that hydrolyzes the *tert*-butyldiphenylsilyl group and then the free hydroxyl attacks the carbonyl to give the unprotected nucleobase. The deprotection was directly monitored on the support by MALDI-TOF mass spectrometry.[11] Thus, after 30, 90 and 120 min, few beads were withdrawn, mixed with matrix (2,4,6-trihydroxyacetophenone, THAP), spotted on the target to cocrystallize and then analyzed. Spectra showed that after 30 min only a small amount of prooligo still bearing one SiOMB group was present (Fig. 2A). After 90 min, the prooligo was fully deprotected to afford the Me-SATE T*p*C*p*C*p*T*p* prooligo still bearing the Me-SATE groups on the phosphates (Fig. 2B). A small peak corresponding to a loss of one Me-SATE group (1510.20 Da) was also detected but extended reaction time did not give much more release of Me-SATE groups (Fig. 2C). We always observe a small amount of prooligo having lost one Me-SATE and we believe that phenomenon mainly occurred during the synthesis and not during the treatment with TMSCl/water.

Finally, the prooligo was released from the solid support by UV irradiation.[7] The crude mixture was analyzed by HPLC on a C_{18} reverse-phase column (Fig. 3). The HPLC profile showed two main peaks centered at 32.4 and 39.4 min corresponding to the T*p*C*p*C*p*T*p* with three Me-SATE and four Me-SATE groups, respectively.

Since the phosphate group is chiral diastereoisomers are visible as multiple peaks for each prooligo. To verify that point, we withdrew one drop of solution from each peak (32.4, 38.6, 39.4 and 40.2 min) during the HPLC purification and analyzed by MS MALDI-TOF. The first peak had a mass of 1510.26 corresponding to that of TCCT prooligo bearing 3 Me-SATE groups (calcd 1510.25, negative mode). The three other peaks displayed the same mass spectrum (1612.79, 1612.63 and 1612.53, respectively) corresponding to the fully Me-SATE prooligo. Finally, the pure prooligo was characterized by MS MALDI-TOF (MS calcd 1612.40, found 1612.64, negative ion).

Using SiOMB protecting group, we were able to synthesize a short prooligo constituted with deoxycytidine. This protection will soon be applied to the two other nucleobases (i.e., adenine and guanine). Thus, we believe that using *tert*-butyldiphenyl-silyloxymethyl benzoyl group we could synthesize prooligos, constituted with the four nucleobases, targeted against specific genes.



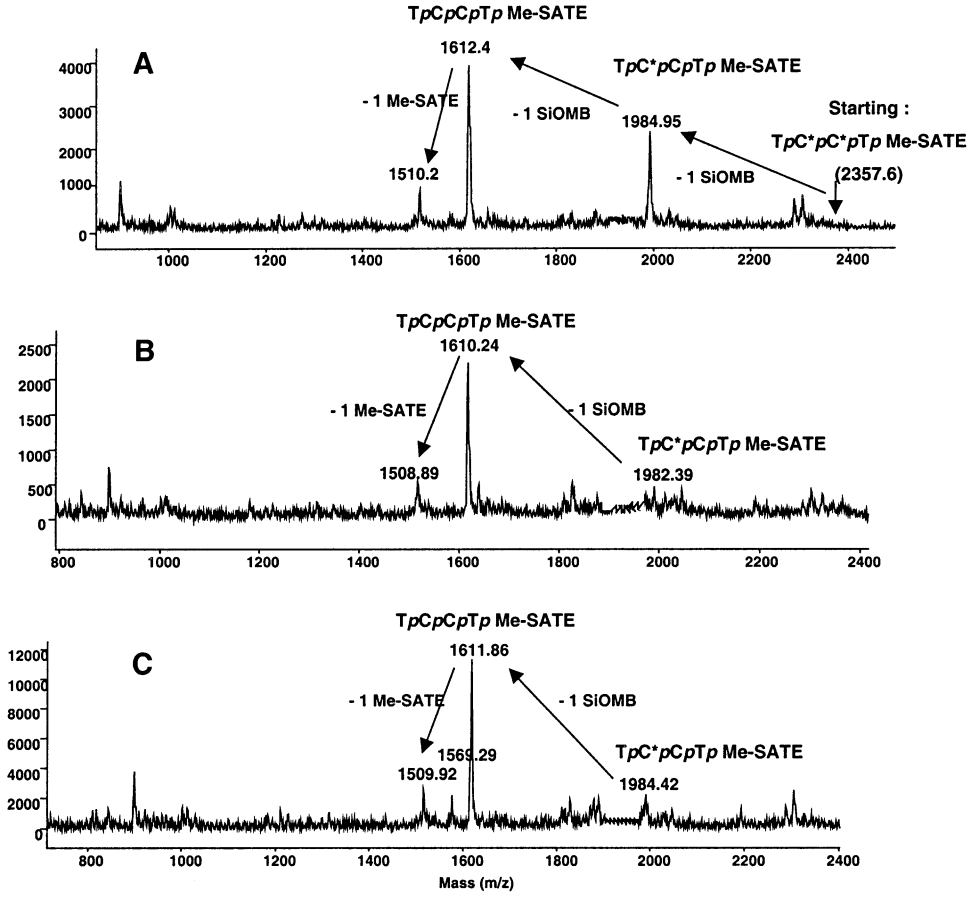


Figure 2. (Top) structure of Me-SATE TCCT prooligo; (bottom) monitoring of the deprotection by MALDI-TOF analysis directly on the solid support bearing a photolabile linker. Treatment with $TMSCl/H_2O$/acetonitrile: (A) 30 min; (B) 90 min; (C) 120 min.

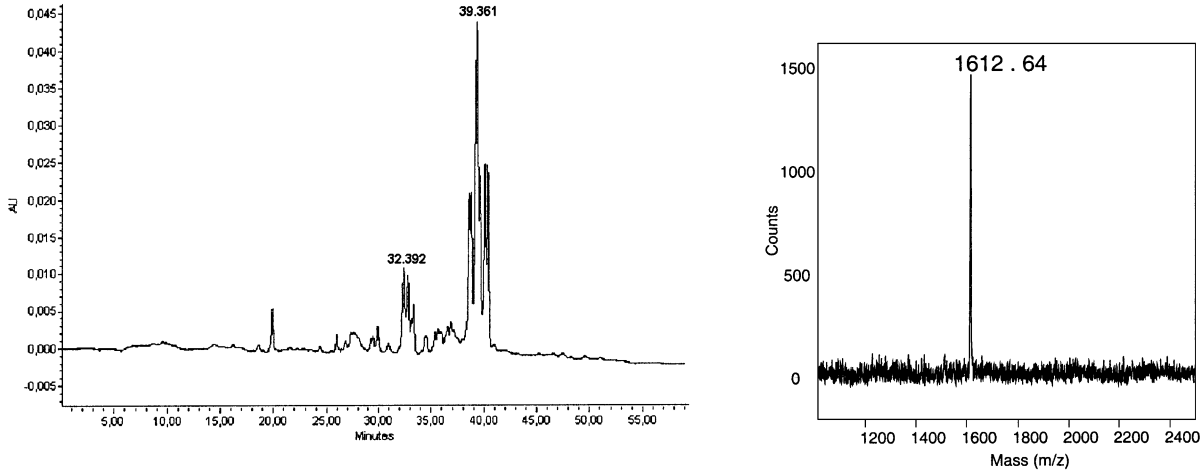


Figure 3. (Left) crude HPLC profile of the Me-SATE TCCT prooligo after deprotection; (right) MALDI-TOF spectra of Me-SATE TCCT prooligo (negative mode) after purification by HPLC.

Acknowledgements

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