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Lewis acid deprotection of silyl-protected oligonucleotides and base-sensitive oligonucleotide analogues

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Abstract—Oligonucleotides protected with *N*-(trimethylsilylethoxycarbonyl) (Teoc) and *P*-(trimethylsilylethanol) (Tse) groups were synthesized and deprotected by a single $ZnBr_2$ treatment. Teoc group stabilized dA against depurination. This strategy was applied to the synthesis of base-sensitive oligonucleotide prodrugs bearing *S*-acetyl-2-thioethyl (Sate) phosphotriesters.

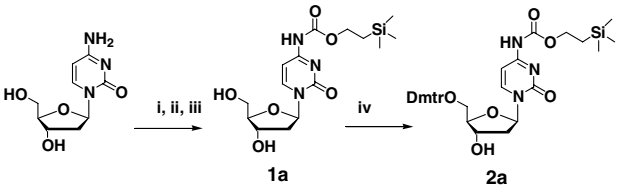
Prodrugs of oligonucleotides (prooligos) are designed to circumvent the main limitations of the oligonucleotides as therapeutics: that is degradation by nucleases and poor uptake. Thus we have shown that prooligos are nuclease resistant, and rapidly and highly taken up by cells.[1] Since prooligos are constituted of phosphate protected with base-sensitive *S*-acetyl-2-thioethyl (Sate) groups, it is compulsory to use protecting groups that will be removed without ammonia treatment. For that purpose, our group develop several strategies.[2–4]

Herein we present the synthesis of base-sensitive prooligos using silyl protecting groups either on the nucleobases or on the phosphate.[5]

We chose to use the trimethylsilylethoxycarbonyl (Teoc) group that could be rapidly introduced on the nucleobases starting either from its *p*-nitrophenol derivative[6] for C or from its chloride derivative[7] for A and G. Teoc group was described for amino protection[8] in peptide synthesis and for hydroxyl protection[9] in oligonucleotide synthesis, but never as protection of nucleobases. This protecting group could be removed under several conditions[10] and specially under a mild treatment with Lewis acid like $ZnCl_2$ or $ZnBr_2$.[9,11] Furthermore, since prooligos should exhibit some charges to be soluble in aqueous media, they were introduced through trimethylsilylethyl (Tse) phosphotriester as already reported.[4,12] Thus after deprotection they will yield phosphodiester linkages. The Tse groups will be removed in the same manner than the Teoc ones.

Firstly we synthesized the three nucleosides corresponding to dC, dA and dG with the Teoc protecting group. The dC was efficiently protected using a transient 3′ and 5′-OH protection with trimethylsilyl group and then with 4-nitrophenyl-2-(trimethylsilyl) ethylcarbonate in presence of DMAP as catalyst. After 16 h, a 20 min treatment with ammonia yielded the expected N^4-Teoc-dC **1a** (80%), (Scheme 1). Finally it was 5′-dimethoxytritylated to yield **2a** (85%).

Since the amino function of dA and dG is not enough nucleophilic the 2-(trimethylsilyl)ethyl-carbonochloridate (TeocCl) was used instead of the 4-nitrophenyl derivative. This reagent was fleshly prepared starting from 2-(trimethylsilyl)ethanol and phosgene in toluene in the presence of powdered anhydrous potassium carbonate.[7] Introduction of Teoc group was performed



Scheme 1. Reagents: (i) TMSCl, dry pyridine; (ii) 4-nitrophenyl-2-(trimethylsilyl) ethylcarbonate, DMAP; (iii) NH_4OH; (iv) DmtrCl dry pyridine.

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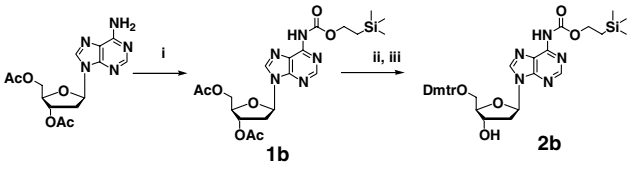


Scheme 2. Reagents: (i) TeocCl, *N*-methyl imidazolo CH_2Cl_2; (ii) 0.2 N NaOH, THF–MeOH–H_2O; (iii) DmtrCl dry pyridine.

starting from 3′,5′-di-*O*-acetyl dA and dG.[13] On the one hand (Scheme 2), the *N*-6 of adenine was protected by reaction with TeocCl in presence of *N*-methyl imidazole in dichloromethane for 16 h (95%). Then a 10-min treatment with 0.2 N NaOH in THF–MeOH–H_2O (25:15:10, v/v/v) led to the expected N^6-Teoc-dA. After work up, the crude was directly dimethoxytritylated to give **2b** (80%).

On the other hand, the protection of the *O*-6 position of dG was necessary to obtain a good yield.[14] Without, the di-*O*-3′,5′-acetyl N^2-Teoc-dG was obtained with only 15% yield. Thus, the *O*-6 of guanine was protected with Tse group in two steps. First (Scheme 3) 2,4,6-triisopropylbenzenesulfonyl chloride in presence of DMAP and TEA reacted on the *O*-6, then the trimethylsilylethanol in presence of DABCO displaced this leaving group to yield the O^6-Tse-dG (60%). The Teoc group was finally introduced on *N*-2 by treatment with TeocCl in presence of *tert*-butyl magnesium chloride in THF for 16 h (70%). Acetyl groups were removed by means of a solution of 0.2 N NaOH in THF–MeOH–H_2O (25:15:10, v/v/v) for 10 min. After work up, the crude was directly dimethoxytritylated to give **2c** (75%).

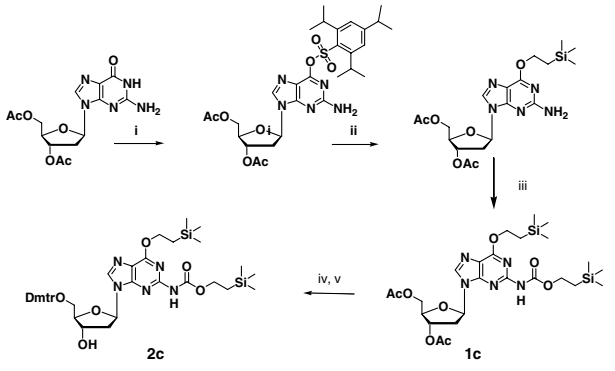
In order, to find the best treatment for the removal of Teoc group we tried several conditions at the nucleoside level (N^4-Teoc-dC **1a**, 3′,5′-di-*O*-Ac-N^6-Teoc-dA **1b** and 3′,5′-di-*O*-Ac-O^6-Tse-N^2-Teoc-dG **1c**). Treatment with fluorine reagents (Et_3N–3HF, TBAF, TBAF–AcOH and HF–pyridine) led to no or incomplete deprotection, while treatment with acid Lewis like $ZnBr_2$ and $ZnCl_2$ gave rapid deprotection of the Teoc group. The full deprotection was faster with $ZnBr_2$ (30–60 min) than with $ZnCl_2$ (about 2 h). The order of silyl removal was dC > dA > dG. It is noteworthy that Tse group on *O*-6 of dG was removed by the same way. With $BiCl_3$ we observed some depurination. As a DNA synthesis cycle involves an acidic treatment, we studied the stability of 3′,5′-di-*O*-Ac-N^6-Teoc-dA **1b** and N^6-Bz-dA in acidic conditions. While N^6-Bz-dA was depurinated in 80% acetic acid at 20 °C within 1 h, **1b** was fully stable up to 2 h. On the contrary **1b** was degraded in a 5% TFA CH_2Cl_2 solution, but fortunately stable in 3% TCA or 2.5% DCA in CH_2Cl_2. Hence we could use the standard detritylation solution on the synthesizer.

As Teoc and Tse groups were rapidly removed by $ZnBr_2$ treatment and were stable under DCA or TCA treatment, they are fully compatible to be used for the synthesis of base-sensitive oligonucleotides like prooligos.

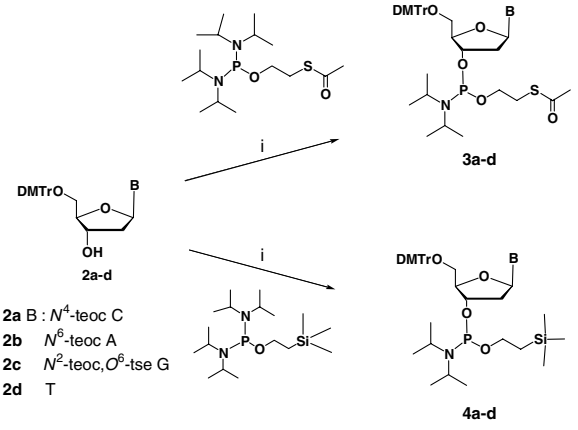
To be soluble in aqueous media the prooligos should have some charges. For that purpose, we have shown that Tse group on the phosphate could be removed by a Et_3N–3HF treatment without the hydrolysis of the Sate groups.[4] Thus the 5′-*O*-Dmtr-*N*-protected nucleosides (**2a**–**d**) were converted into Sate (**3a**–**d**) and Tse (**4a**–**d**) phosphoramidite derivatives (Scheme 4) in presence of diisopropyl ammonium tetrazolide as catalyst in CH_2Cl_2 using Sate bis-*N*,*N*′-diisopropyl phosphine[15] and Tse bis-*N*,*N*′-diisopropyl phosphine,[12] respectively. **3a**, (80%); **3b** (70%); **3c** (83%); **3d** (75%), **4a** (90%); **4b** (80%); **4c** (85%); **4d** (90%).

An oligo phosphodiester GCATTAGCATpOCH$_3$ was synthesized from the Tse phosphoramidites to evaluate their efficacy. They were used at a standard 0.1 M concentration in dry acetonitrile with a 120 s coupling step. Since acetic anhydride usually used for capping step could also react on the exocyclic amino function specially of adenine we capped with di-*tert*-butyl diethyl phosphoramidite (0.05 M in CH_3CN) for 10 s. Oxidation was performed with a 0.067% 2-butanoneperoxyde solution in CH_2Cl_2 for 60 s[16] and detritylation with standard 3% TCA solution for 60 s.

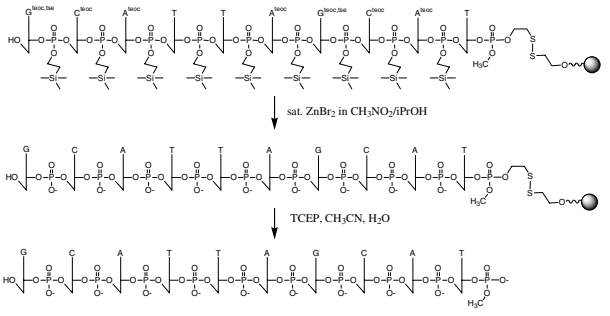
In order, to be released from the solid support without ammonia treatment we used a solid support with a disul-



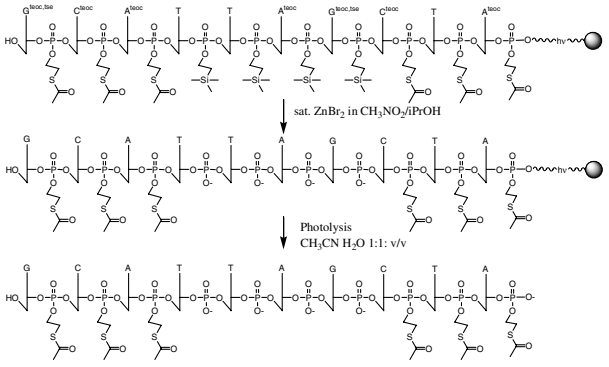
Scheme 3. Reagents: (i) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_2Cl_2; (ii) TseOH, DABCO; (iii) TeocCl, *t*-Bu–MgCl, THF; (iv) 0.2 N NaOH, THF–MeOH–H_2O; (v) DmtrCl dry pyridine.



Scheme 4. Synthesis of Sate and Tse phosphoramidites derivatives. Reagents: (i) diisopropyl ammonium tetrazolide, CH_2Cl_2.



Scheme 5. Deprotection of oligo GCATTAGCATpOCH$_3$ by $ZnBr_2$ treatment and release from solid support by TCEP treatment.



Scheme 6. Deprotection of oligo GCATTAGCTAT by $ZnBr_2$ treatment and release from solid support by photolysis.

fide linkage[17] that will be cleaved by tris-2-carboxyethylphosphine (TCEP).

After elongation, the CPG-supported oligo was treated overnight with a saturated solution of $ZnBr_2$ in nitromethane–isopropanol (1:1, v/v) (Scheme 5). Then the beads were thoroughly washed with water and with a 0.1 M EDTA solution to scavenge the Zn^{2+} cations. Finally, the oligo was cleaved from the solid support by treatment with TCEP in triethylammonium acetate buffer pH 7 with 80% acetonitrile for 2 h. This treatment led to a 3′-phosphotriester with a thio-ethyl group that eliminated spontaneously to a 3′-phosphodiester after elimination of episulfide. As that elimination is very slow on diester due to the negative charge, we started the synthesis with methoxyphosphoramidite[18] to obtain after $ZnBr_2$ treatment a 3′-methyl triester linkage and then the expected 3′-methyl phosphodiesters as confirmed by MALDI-TOF MS (negative mode *m/z* for $C_{99}H_{126}N_{37}O_{61}P_{10}$ calcd 3120.07, found 3121.90). The crude HPLC showed a broad peak (Fig. 1 left) with peaks at higher retention times corresponding to the short mers 5′-di-*tert*-butylphosphotriester as determined by MALDI-TOF MS (Fig. 1 middle). After purification the pure decamer was obtained (Fig. 1 right).

A first oligo was synthesized using silyl protecting groups on the nucleobase (Teoc + Tse for G) and on the phosphorous (Tse). By a simple treatment with Lewis acid like $ZnBr_2$ all the protecting groups were efficiently removed, without any ammonia treatment. This strategy opens the way to the synthesis of base-sensitive prooligos.

Using the eight phosphoramidites derivative **3a–d** and **4a–d** a prooligo exhibiting the four nucleobases and bearing on each side three phosphotriester SATE linkages ($Gp_{Sate}Cp_{Sate}Ap_{Sate}TTAGCp_{Sate}Tp_{Sate}Ap_{Sate}$) was synthesized on a solid support with a photolabile linker.[19]

After elongation according to the same cycle than for the previous oligo, the prooligo was deprotected (Scheme 6) by treatment with $ZnBr_2$ in nitromethane isopropanol (7 h). The deprotection was monitored by MALDI-TOF MS[20] thanks to the photolabile linker. We observed that this treatment also led to few hydrolysis of one Sate group.

Then a 20 min photolysis released the prooligo in solution. Nevertheless, one Sate group was partially removed as showed by HPLC (Fig. 2) peaks 13–14.5 min and MALDI-TOF MS (M − 102 Da). The prooligo with the six Sate groups was eluted as a massif between 14.5 and 17 min owing to the 2^6 diastereoisomers. Each main peak was characterized by MALDI-TOF MS and exhibited the same mass corresponding to the expected prooligo with six Sate (Negative mode *m/z* for $C_{122}H_{160}N_{37}O_{67}P_{10}S_6$ calcd 3718.97, found 3722.10).

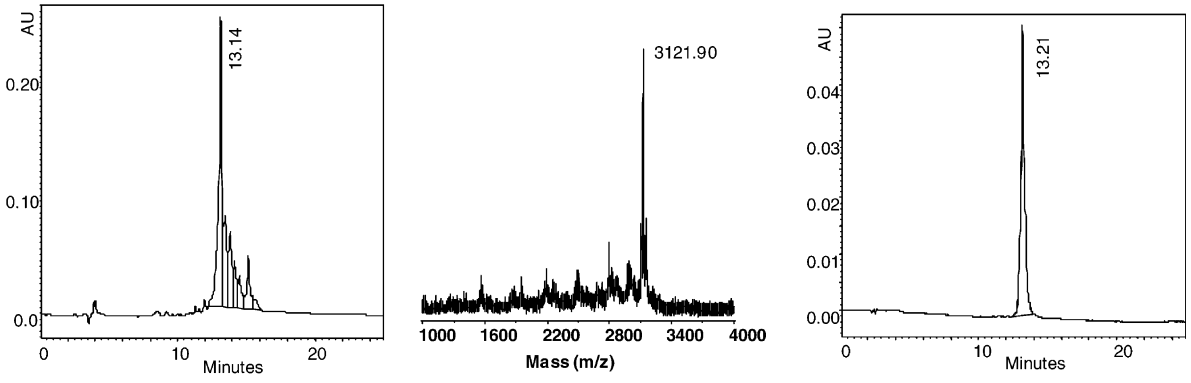


Figure 1. C_{18} reverse phase HPLC profile of GCATTAGCATpoCH$_3$ crude (left) and purified (right). MALDI-TOF MS of crude (middle).

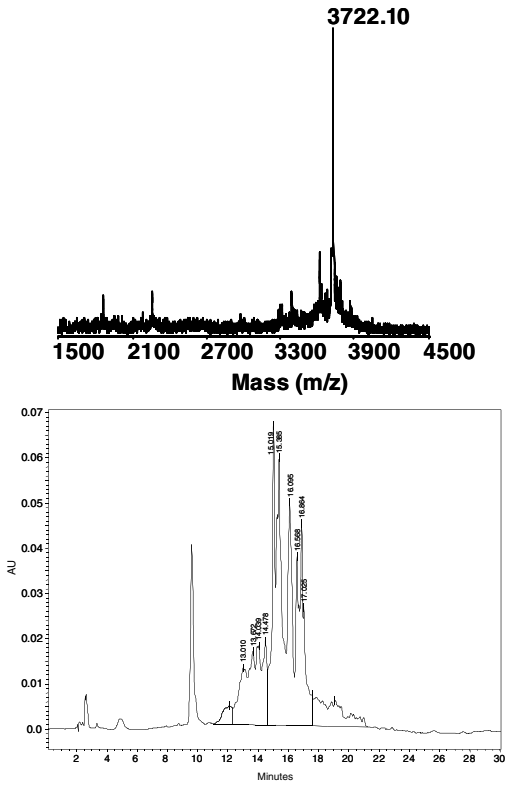


Figure 2. MALDI-TOF MS and C_{18} reverse phase HPLC of the crude prooligo $Gp_{Sate}Cp_{Sate}Ap_{Sate}TTAGCp_{Sate}Tp_{Sate}Ap_{Sate}$.

In conclusion a strategy using silyl protecting groups on the nucleobases and on the phophate was successfully applied to the synthesis of regular and base-sensitive oligonucleotide. The Teoc and Tse silyl groups were efficiently removed by treatment with $ZnBr_2$. It is noteworthy that the Teoc is poorly removed by fluoride reagents. Furthermore Teoc group stabilizes dA against depurination.

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