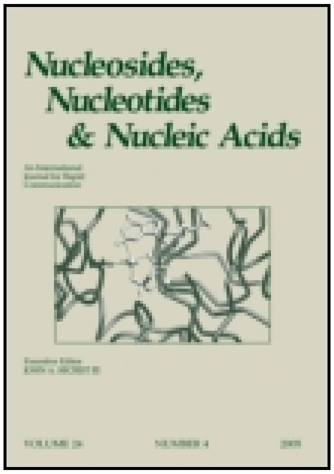

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

SILYL PROTECTING GROUPS FOR OLIGONUCLEOTIDE SYNTHESIS REMOVED BY A $ZnBr_2$ TREATMENT

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Published online: 15 Nov 2011.

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):1009–1013, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060342

SILYL PROTECTING GROUPS FOR OLIGONUCLEOTIDE SYNTHESIS REMOVED BY A $ZnBr_2$ TREATMENT

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▫ *An oligonucleotide protected with N-(trimethylsilyloxycarbonyl) (Teoc) and P-(trimethylsilylethanol) (Tse) groups was synthesized and deprotected by a single $ZnBr_2$ treatment. Finally it was released from the solid support by cleavage of a disulfide linkage with TCEP. The oligonucleotide was obtained without any basic treatment.*

INTRODUCTION

Protecting groups for the amino functions of nucleobase have been extensively developed. Protecting groups able to be removed under mild or neutral conditions are of great interest for the synthesis of base-sensitive oligonucleotides.

Here we present a strategy to synthesize oligonucleotides without any basic treatment by using the trimethylsilylethoxycarbonyl (Teoc) group for the protection of nucleobases and of the trimethylsilylethyl (Tse) group[1,2] for the protection of the internucleotidic linkages.

RESULTS AND DISCUSSION

Deoxycytidine was efficiently protected using a transient 3′ and 5′-OH protection with the trimethylsilyl group and then with 4-nitrophenyl-2-(trimethylsilyl) ethylcarbonate[3] in presence of DMAP as catalyst. After 16 h, a 20 min treatment with ammonia yielded the expected N^4-Teoc-dC **3** (80%), (Scheme 1). Finally, it was 5′-dimethoxytritylated **10a** (85%).

For the protection of deoxyadenosine and deoxyguanosine, the more reactive 2-(trimethylsilyl)ethyl-carbonochloridate (TeocCl)[4] must be used. Introduction of

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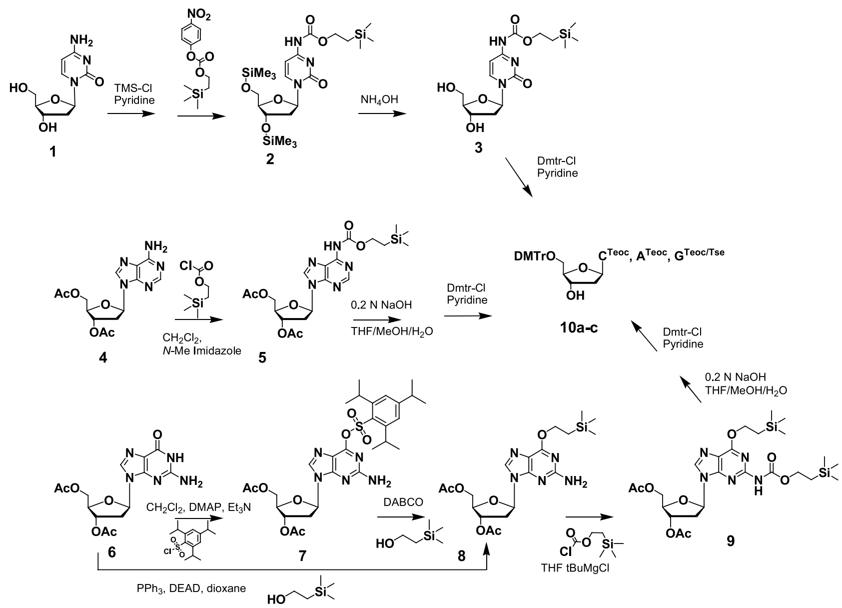
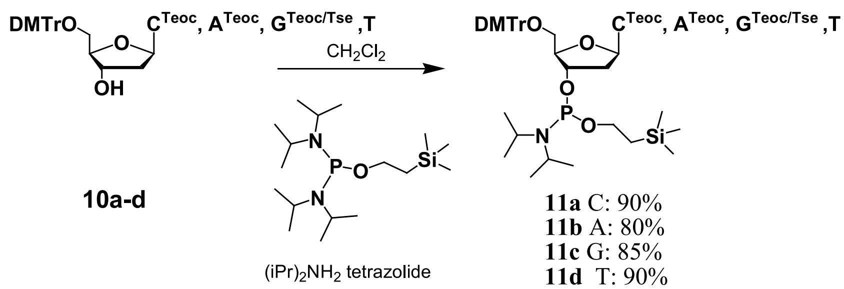


SCHEME 1 Synthesis of 5′-*O*-dimethoxytrityl-*N*-Teoc nucleosides.

the Teoc group was performed starting from 3′,5′-di-*O*-acetyl dA and dG.[5] The *N*-6 of adenine was protected by reaction with TeocCl in presence of *N*-methyl imidazole in dichloromethane for 16 h (95%) (Scheme 1). 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 product was directly dimethoxytritylated **10b** (80%). The *O*-6 of guanine was protected with Tse group in two steps. First 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 **8** (60%). Alternatively it was synthesized by a Mitsonobu reaction using tri phenylphosphine, diethyl azodicarboxylate and trimethylsilylethanol in dioxane with 48% yield. The Teoc group was 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 a solution of 0.2 N NaOH in THF/MeOH/H_2O for 10 min. After work up, the crude product was directly dimethoxytritylated **10c** (75%).

The conditions of removal of Teoc group were studied at the nucleoside level. Treatment with various fluoride reagents (HF.pyridine, $Et_3N.3HF$, TBAF-AcOH, and TBAF), in THF, led to no or incomplete deprotection. In contrast, treatment with $ZnBr_2$ and $ZnCl_2$ gave rapid deprotection. The full deprotection was faster with $ZnBr_2$ (30 to 60 min) than with $ZnCl_2$ (about 2 h). The order of silyl removal was dC > dA > dG. Note that Tse group on *O*-6 of dG was removed by the same way. As a DNA synthesis cycle involves an acidic treatment, we studied the stability of N^6-Bz-dA and 3′,5′-di-*O*-Ac-N^6-Teoc-dA in acidic conditions. While the former

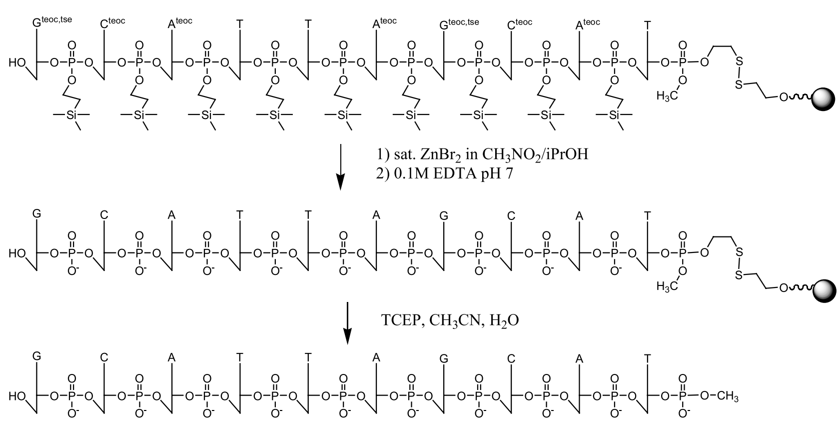


SCHEME 2 Synthesis of Tse phosphoramidites derivatives.

was fully depurinated in 80% acetic acid at 20°C within 1 h the latter was fully stable up to 2 h. Furthermore 3′,5′-di-*O*-Ac-N^6-Teoc-dA was found stable in 3% TCA or 2.5% DCA in CH_2Cl_2. Hence, the standard detritylation solution could be used on synthesizer.

Then, the 5′-*O*-Dmtr-*N*-protected nucleosides **10a–d** were converted into Tse phosphoramidite derivatives (Scheme 2) in the presence of diisopropyl ammonium tetrazolide as catalyst in CH_2Cl_2 using Tse bis-*N,N′*-diisopropyl phosphine:[1] 90% for dC **11a**; 80% for dA **11b**; 85% for dG **11c**, and 90% for dT **11d**.

An oligonucleotide GCATTAGCATpOCH_3 was synthesized from the Tse phosphoramidites on a solid support with a disulfide linkage. The phosphoramidite building blocks were used at a standard 0.1 M concentration in dry acetonitrile with a 120-s coupling step. Since acetic anhydride usually used for the capping step could also react on the exocyclic amino function especially of adenine, we capped with di-*tert*-butyl diethyl phosphoramidite (0.05M in CH_3CN) for 10 s. Oxidation was performed with a 0.067% 2-butanoneperoxide solution in CH_2Cl_2 for 60 s[6] 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 disulfide linkage[7] that can be cleaved by tris-2-carboxyethylphosphine (TCEP).



SCHEME 3 Deprotection of oligo GCATTAGCATpOCH_3 by $ZnBr_2$ and release from solid support by TCEP.

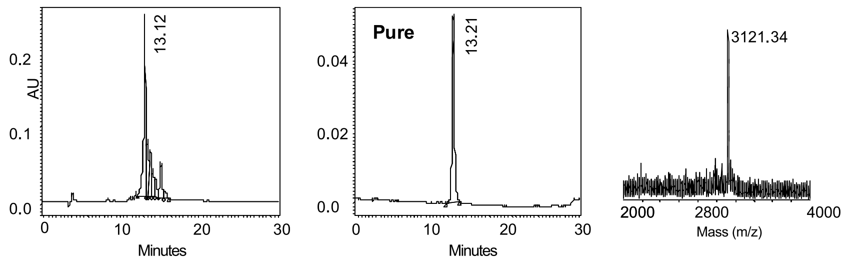


FIGURE 1 HPLC profiles of the crude and pure oligonucleotide and MALDI-TOF MS.

After elongation, the CPG-supported oligonucleotide was treated overnight with a saturated solution of $ZnBr_2$ in nitromethane/isopropanol (1:1, v/v) (Scheme 3). Then the beads were thoroughly washed with water and with a 0.1M 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 rearranged spontaneously to a 3′-phosphodiester after elimination of episulfide. As that elimination is very slow with a diester due to the negative charge, we started the synthesis using a methoxyphosphoramidite to obtain after $ZnBr_2$ treatment a 3′-methyl triester linkage that rearranged the expected 3′-methyl phosphodiesters. The crude HPLC showed a peak (Figure 1, left) with other peaks at higher retention times corresponding to the short of mers 5′-di-*tert*-butylphosphotriester as determined by MALDI-TOF MS. After purification, the pure decamer was obtained (Figure 1 middle) and characterized by MALDI-TOF MS (negative mode m/z for $C_{99}H_{126}N_{37}O_{61}P_{10}$ calc. 3120.07, found 3121.34) (Figure 1, right).

CONCLUSION

Using silyl protecting groups on the nucleobases (Teoc) and on the phosphate (Tse), we synthesized an oligonucleotide and deprotected it by a single treatment with a $ZnBr_2$ solution. This strategy could be applied to the synthesis of base-sensitive oligonucleotides.

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