# EFFICIENT CHEMICAL SYNTHESIS OF OLIGODEOXYNUCLEOTIDES CONTAINING A TRUE ABASIC SITE

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<u>ABSTRACT</u>. The photolabile *o*-nitrobenzyl group has been used to protect the anomeric hydroxyl function of a 2deoxy-D-ribofuranose building block which was incorporated into DNA oligomers using the standard phosphoramidite method for automated DNA synthesis. The resulting stable *o*-nitrobenzyl protected oligomers have been purified by HPLC and then quantitatively converted upon long-wavenlength UV irradiation into the corresponding abasic DNA sequences (up to 30- mer).

The abasic site is one of the most common chemical lesions in DNA. Abasic sites (apurinic or apyrimidinic) arise from hydrolytic cleavage of the glycosidic bond between a nucleobase (purine or pyrimidine) and the corresponding deoxyribose moiety and are considered to be common intermediates in mutagenesis<sup>1</sup>. High lability of abasic DNA strands towards base<sup>2,3</sup> represents a hindrance to the synthesis of short oligodeoxynucleotides containing an abasic site at preselected positions. Three years ago, we reported the first chemical synthesis of a short apurinic oligodeoxynucleotide<sup>4</sup>. However, the method we used, based on the selective acid hydrolysis of a deoxyadenosine glycosidic bond, is limited to the synthesis of oligodeoxypyrimidines containing apurinic sites.

More recently, mild acid hydrolysis of oligodeoxynucleotides containing either a 2-pyrimidinone deoxynucleoside<sup>5</sup> or a 1-O-(*tert*-butyldimethylsilyl)-2-deoxy-D-ribofuranose<sup>6</sup> was shown to generate abasic sites in sequences including purines. Surprisingly, although 2-pyrimidinone deoxynucleosides and 1-O-(*tert*-butyldimethylsilyl)-2-deoxyribofuranose are both acid labile, the corresponding phosphoramidite derivatives have been used, without any detected side reaction, as building blocks in automated synthesis using cycles which, in both cases included a 1.5 min treatment with 2% dichloroacetic or 3% trichloroacetic acid in methylene chloride. This last point is of importance, since unwanted premature unprotection of the deoxyribose residue during the assembling may generate undesired oligomers branched at the released anomeric hydroxyl function.

Alternatively, we report here the use of the photolabile *o*-nitrobenzyl group for the protection of the anomeric hydroxyl function of deoxyribofuranose during the solid support synthesis of abasic oligodeoxynucleotides by the phosphoramidite method. This protecting group has successfully been used for the 2'-OH protection in oligoribonucleotide synthesis<sup>7-9</sup>. In particular, it has been shown to be completely resistant to various conditions which are used in DNA synthesis, including acid treatments, and then quantitatively removed upon long-wavenlength UV irradiation in conditions which are safe for the oligomer integrity.

A regular Koenigs-Knorr type glycosidation procedure using 3,5-di-O-toluyl 2-deoxyribofuranosyl chloride and o-nitrobenzyl alcohol in the presence of silver trifluoromethanesulfonate and subsequent alkaline treatment afforded a mixture (73% overall yield) of anomeric o-nitrobenzyl 2-deoxy-D-ribofuranosides 1a and  $1b^{10}$  in nearly equal amounts (scheme). The two anomeric compounds have been separated by silica-gel column chromatography and their structures ascertained by NOE difference spectroscopy. The  $\beta$ -configuration was assigned to low-Rf isomer 1b in which a trans relationship was evidenced between H-3 and H-1 of the deoxyribose ring through comparison of NOEs induced by irradiating vicinal H-2 and H-2<sup>11</sup>. The result is in agreement with the large absorption band width (0.61 ppm) of the two H-2 and H-2' observed for high Rf isomer 1a as compared to 1b (0.21 ppm)<sup>12</sup>. In order to ascertain the stability of the *o*-nitrobenzyl protecting group under acidic conditions, glycosides 1a and 1b were treated with 3% trichloroacetic acid in methylene chloride at room temperature. TLC analysis indicated that no hydrolysis was detectable after 30 minutes, which represents the cumulative effect of detritylation steps from 20 cycles on a DNA synthesizer.



<u>Scheme</u>. (i) silver trifluoromethanesulfonate/2,4,6-trimethylpyridine in  $CH_2Cl_2$  at -30°C. (ii) 0.05 M NaOH in 5% aqueous MeOH. (iii) 4,4'-dimethoxytrityl chloride in pyridine. (iv) bis(diisopropylamino)(2-cyanoethoxy)phosphane/diisopropylammonium tetrazolide in  $CH_2Cl_2$ .

Anomers 1a and 1b were separately reacted with 4,4'-dimethoxytrityl chloride and then with bis (diisopropylamino) (2-cyanoethoxy)phosphane in presence of diisopropylammonium tetrazolide<sup>13</sup> as the activator. Both anomeric phosphoramidite building blocks 2a and 2b were obtained as colorless and amorphous powder after silica-gel chromatography and lyophilization from benzene. Their purity was checked by <sup>31</sup>P-NMR<sup>14</sup>. The phosphoramidite 2b was thus used like each of the four natural deoxynucleoside phosphoramidites for the automated solid-phase synthesis of oligodeoxynucleotides.

o-Nitrobenzyldeoxyribofuranose containing oligonucleotides  $^{15}$  3 and 4 (Fig. 1) were assembled on a DNA synthesizer, using the lµmole standard cycle. The coupling efficiency for the abasic building block 1b was better than 98% as judged by photometric trityl assay. After applying standard procedure for the cleavage from the support and deprotection, the nitrobenzyl protected oligomers 3 and 4 were purified by reverse-phase HPLC.

## d(CTTXTCC)

#### 3 (X= o-nitrobenzyl 2-deoxy- $\beta$ -D-ribofuranoside); 5 (X= 2-deoxy-D-ribofuranose).

## d(AACGTGAGTGCCGTGXGTGCCGTGAGTGCA)

4 (X= o-nitrobenzyl 2-deoxy- $\beta$ -D-ribofuranoside); 6 (X= 2-deoxy-D-ribofuranose).

Figure 1. Synthesized abasic oligodeoxynucleotides

Efficient removal of the *o*-nitrobenzyl protecting group was achieved by irradiating solutions of the purified oligomers in 0.2 M ammonium formate  $(pH 4)^{16}$  at 20°C through a 2 mm pyrex filter with a high pressure mercury lamp. After 10 min. irradiation, HPLC analysis (Fig. 2) indicated quantitative conversion of the starting heptamer 3 ( $R_T$  18.26 min) into a faster eluting compound 5 ( $R_T$  12.35 min). Structure of this latter compound was ascertained after HPLC analysis of the products obtained upon alkaline degradation and subsequent treatment with alkaline phosphatase. As expected, compound 5 was degradated and two peaks ( $R_T$  9.29 and 10.55 min) corresponding respectively to those of authentic samples of d(TCC) and d(CTT) were observed, whereas *o*-nitrobenzyl protected heptamer 3 was kept unchanged upon the same treatment. Similarly, photolysis of *o*-nitrobenzyl protected 30- mer 4 afforded the corresponding abasic 30-mer 6 which upon alkaline degradation and phosphatase treatment was converted into two distinct oligomers of shorter size as monitored by denaturing polyacrylamid gel electrophoresis (data not shown).



Figure 2. Reverse-phase HPLC analysis of crude heptamer 3 (---); crude abasic heptamer 5 obtained upon photolysis of 3 (---) and products resulting from alkaline and phosphatase treatments of crude 5(--).

In conclusion, these results demonstrate the usefulness of the photolabile *o*-nitrobenzyl group for the protection of anomeric hydroxyl functions of deoxyribofuranose with regard to the specific introduction of abasic sites into synthetic oligodeoxynucleotides up to 30 bases in length without any restriction concerning the base

sequence. The protected deoxyribofuranose 3'-phosphoramidite can be easily obtained in gram amounts and then be used on a DNA synthesizer as any usual deoxynucleoside phosphoramidite and without any modifications of standard cycles. *o*-Nitrobenzyl-protected abasic oligonucleotides are stable and can be purified as well as normal oligomers. Moreover, the synthesis of abasic oligonucleotides, when compared to that of normal oligomers, requires only one extra step (photolysis) which is fast and quantitative.

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### **REFERENCES AND NOTES.**

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