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Hydrolysis of Oligonucleotides containing 8-Substituted Purine Nucleosides. A New Route for Preparing Abasic Oligodeoxynucleotides.

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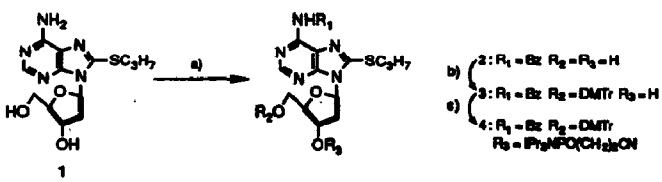
Abstract : 2'-Deoxyadenosine substituted at C-8 by a propylthio group was introduced into oligodeoxyribonucleotides by solid phase synthesis. Oxidation by potassium persulfate (oxone) occurred selectively on the sulfur containing nucleoside causing a weakening of the glycosidic bond. Subsequent hydrolytic treatment led to selective removal of the modified base and generation of an abasic site. This constitutes a novel and convenient route for the chemical synthesis of oligodeoxyribonucleotides containing an abasic site at a preselected position in the sequence.

Abasic sites in DNA result from cleavage of the N-glycosidic bond and removal of the base. Depurination is one of the most frequent forms of chemical damage to DNA. Hydrolysis of the glycosidic bond is accelerated by chemical alteration of bases with alkylating agents[1] or through ionizing radiation.[2] In fact, abasic sites are common intermediates in the biological repair of most base damage in DNA, being produced by specific glycosylases that remove the abnormal or modified bases.[3]

In connection with ongoing programs aimed at devising molecules that recognize this lesion[4], oligonucleotides containing an abasic site at predetermined positions in the sequence were required. A few chemical methods have appeared in the literature concerning the synthesis of abasic oligonucleotides that make use of a photolabile nitrobenzyl group[5] or an acid labile *tert*-butyldimethylsilyl group[6] to protect the anomeric hydroxyl function of the 2-deoxy-D-ribofuranose building block used in automated synthesis. A synthesis has been reported in which 2-pyrimidinone-2'-deoxynucleosides were incorporated in oligodeoxynucleotides.[7] The acidic glycosidic bond cleavage of the modified nucleoside then led to the abasic site.

We report a new route for the preparation of oligodeoxyribonucleotides containing an abasic site at any preselected position in the sequence. The method involves three steps: (1) synthesis of a stable oligonucleotidic precursor containing the modified nucleoside 8-propylthio-2'-deoxyadenosine **1**, (2) mild oxidative treatment of the resulting oligo for selective oxidation at sulfur of the modified nucleoside, (3) hydrolytic removal of the oxidized base. This strategy is based on recent observations we made concerning the hydrolysis of modified nucleosides.[8] We have shown that oxidation of a sulfide located at the C-8 position of a deoxypurine nucleoside to a sulfone greatly increases the rate of cleavage of the glycosidic bond. The acceleration is close to four orders of magnitude in the deoxyguanosine series. The increase could not be determined for deoxyadenosine derivatives,

SCHEME



1

a) PhCOCl, pyr. then NaOH b) DMTrCl, pyr. c) $(iPr)_2N$-P-(Cl)-O$(CH_2)_2$CN

however, as cleavage of the glycosidic bond occurred during oxidation (8-propylsulfonyladenine resulted from oxidation of 8-propylthio-2'-deoxyadenosine). We describe the synthesis of two abasic oligonucleotides: the trimer d(GXA) 7, which contains only purine bases, and the heptamer d(AGCXGAT) 8, which includes the four natural bases.

(1) Preparation of the building block 4

Preparation of the necessary phosphoramidite building block 4 was accomplished by a straigthforward route (Scheme). The propylthio substituted nucleoside 1 was obtained in three steps starting from 2'-deoxyadenosine, namely bromination at C-8, followed by hydrogen sulfide treatment and alkylation of the resulting thiol.[9] The N-6 amino function in 1 was protected by a benzoyl group introduced by nonselective benzoylation of the amino- and hydroxyl functions, followed by controlled basic hydrolysis of the tetrabenzoylated intermediate. Protection at 5'-OH was achieved by dimethoxytritylation and introduction of the phosphite at 3'-OH was accomplished by conventional treatment with 2-cyanoethyl-N,N-diisopropyl chlorophosphoamidite in dry CH_2Cl_2 in the presence of N,N-diisopropylethylamine.[10] The resulting building block 4 was obtained as a white solid in an overall yield of 30 %.

(2) Synthesis of oligonucleotides 5 and 6 containing 8-propylthio-2'-deoxyadenosine

As a prerequisite to the synthesis of the oligonucleotides, we studied the stability of the modified adenine moiety to be introduced. The behaviour of 8-propylthio-2'-deoxyadenosine 1 was examined in all conditions of the solid phase synthesis (especially in the phosphite oxidation step). No decomposition of the thioether group was observed. Consequently phosphoramidite 4, together with the phosphoramidites of the four naturally occurring nucleosides, was used in standard automated solid phase synthesis to prepare the trimer d($GX^{SPr}A$) 5 and the heptamer d($AGCX^{SPr}GAT$) 6. In the two cases, all coupling yields, including that with the modified purine synthon 4, were higher than 98% (as indicated by release of the trityl cation). In particular, no side products resulting from oxidation at sulfur could be detected. After applying standard procedures for the cleavage from the support and deprotection, the oligonucleotides 5 and 6 were purified by reverse-phase HPLC (Figure 1A). The base composition was ascertained by enzymatic hydrolysis using nuclease P1 and bacterial alkaline phosphatase. In particular, no peak corresponding to oxidation of the sulfur could be detected. The base composition was further confirmed by acidic hydrolysis, which released the corresponding bases. The MALDI mass spectrum of the trimer 5 (Figure 2A) clearly showed the molecular ion M^+ at 965, which confirmed that the propylthio nucleoside was included in the sequence.

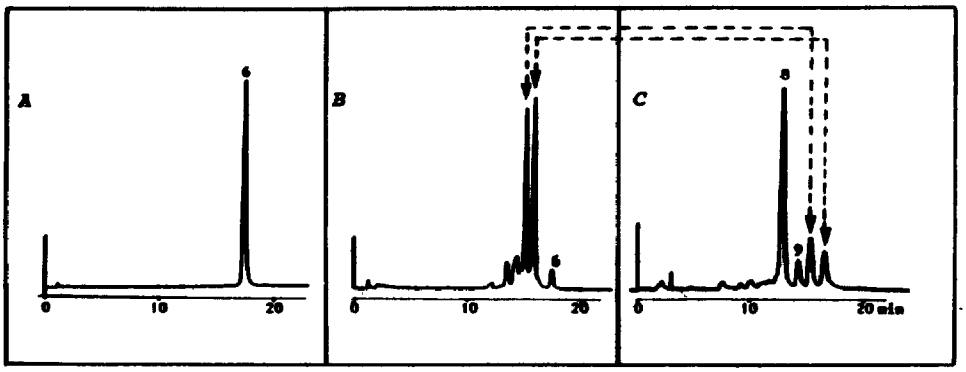


Figure 1: HPLC profiles: (A) heptamer d($AGCX^{SPr}GAT$) 6, X^{SPr} = 8-propylthio-2'-deoxyadenosine; (B) oxidation of 6: crude reaction mixture; (C) hydrolysis step giving the abasic oligodeoxynucleotide 8: the chromatogram corresponds to the reaction mixture before completion of the hydrolysis; the two components of the starting material can be seen. The solvent gradients have been varied in (B) and (C) for better separation of the components. Peak 9 corresponds to 8-propylsulfonyladenine released in the reaction. HPLC conditions: analytical μBondapack C_{18} column, flow rate 2.5 mL/min., mobile phases: I H_2O-NaH_2PO_4 20 mM, pH 5.5; II MeOH, linear gradiant 0 to 35% II in 20 min. in case of (A) and (B). Non linear gradiant in case of (C).

(3) Synthesis of oligonucleotides 7 and 8 containing an abasic site

Selective oxidation of the propylthio adenine residues in oligonucleotides **5** and **6** was achieved by treatment under the carefully controlled oxidation conditions defined at the nucleoside level.[8] Reaction of **5** and **6** with an aqueous solution of "oxone"[11-12] ($2KHSO_5$, $KHSO_4$, K_2SO_4) at rt for 1 h gave the oxidized oligonucleotides, which were isolated. The oxidized oligos (a mixture of two products, see below[14]) were treated without further purification under hydrolytic conditions (heating at 65°C at pH 3, formate buffer, 30 min) for selective removal of the oxidized adenine derivative to give the abasic oligonucleotides **7** and **8**. The HPLC profile of the crude hydrolysis mixture (Figure1C) showed that the abasic heptamer **8** was formed as a very major product from the mixture of oxidized oligonucleotides (Figure 1B). The abasic oligonucleotides **7** and **8** were analyzed for nucleoside composition by digestion with nuclease P1 and alkaline phosphatase followed by HPLC analysis, which indicated correct base composition in both cases. No peak corresponding to a modified deoxyadenosine derivative could be detected. The presence of one abasic site in **7** and **8** was indicated by their cleavage under alkaline conditions. Moreover, the appearance of a new HPLC peak after reaction of the oligo with methoxyamine (formation of the Schiff base adduct with the abasic site aldehydic function) also confirmed the presence of the abasic site.[15] Finally, the presence of the peak at 775, corresponding to the calculated molecular ion (MW = 775) in the MALDI mass spectrum of the trimer **7** (figure 2B), confirmed the existence of the abasic site in the structure.

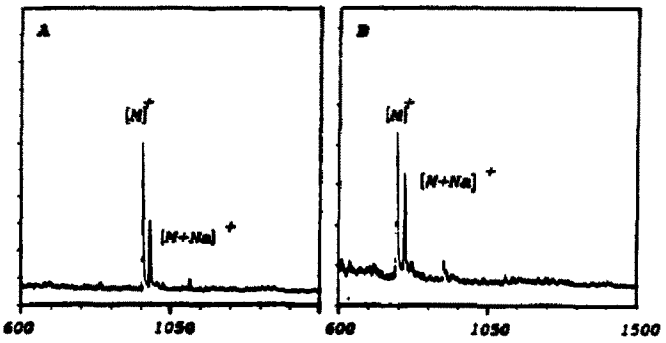


Figure 2: MALDI/MS spectra of (A) trimer d(GX^{3Pr}A) **5** (M^+ = 965) and (B) d(GXA) **7** (M^+ = 775) (X = 2-deoxy-D-ribofuranose). Matrix: 2,4,6-trihydroxyacetophenone.

In conclusion, the strategy based on the oxidation-triggered selective hydrolysis of a propylthiodeoxyadenosine moiety in an oligo affords an efficient synthetic method for preparing DNA fragments containing abasic sites at preselected positions. The necessary 8-propylthio-2'-deoxyadenosine building block can readily be obtained in gram quantities, stores well, and importantly exhibits properties and behavior quite close to those of the natural nucleosides in the automated phosphoramidite synthesis.

Acknowledgments

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9. Data for **1**: mp 105-106°C. ^{1}H-NMR (DMSO d_6): δppm 8.00 (1H, s, Ade-C$_2$H), 7.05 (2H, s, Ade-NH$_2$), 6.23 (1H, t, J = 6.2 Hz, C$_1$·H), 5.30 (1H, q, J = 4.1 Hz, C$_5$·OH), 5.20 (1H, d, J = 4.0 Hz, C$_3$·OH), 4.44 (1H, m, C$_3$·H), 3.85 (1H, m, C$_4$·H), 3.65 (1H, m, C$_5$·H), 3.30 (1H, m, C$_5$·H), 3.25 (2H, m, SCH$_2$), 3.12 (1H, m, C$_2$·H), 2.12 (1H, m, C$_2$·H), 1.72 (2H, m, SCH$_2$CH$_2$), 1.00 (3H, t, J = 7.3 Hz, CH$_3$). ^{13}C-NMR (DMSO d_6): δppm 154.3 (Ade-C$_6$), 151.1 (Ade-C$_2$), 150.4 (Ade-C$_4$), 148.1 (Ade-C$_8$), 119.5 (Ade-C$_5$), 88.1 (C$_4$·), 84.7 (C$_1$·), 71.2 (C$_3$·), 62.1 (C$_5$·), 37.3 (C$_2$·), 34.0 (S-CH$_2$), 22.0 (S-CH$_2$-CH$_2$), 12.8 (CH$_3$). MS (FAB(+)): m/z (relative intensity) = 326 (100, [M+H]$^+$), 210 (71, [M+H-dRib]$^+$), 136 (13, [M+H-dRib-SC$_3$H$_7$]$^+$). Anal. Calcd for $C_{13}H_{19}N_5O_3S$: C 47.99, H 5.89, N 21.52, S 9.85. Found C 47.77, H 5.84, N 21.18, S 9.92.
10. Data for **4**: ^{31}P-NMR ($CDCl_3$): δppm 147.1 (s), 146.7 (s). MS (FAB(-)): *m/z* (relative intensity) = 930 (20, [M-H]$^-$), 732 (5, [M - loss of phosphoramidite]$^-$), 312 (20, [heterocyclic base]$^-$), 303 (100, [trityl]$^-$).
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12. Kettani *et al*[13] have reported adenosine monophosphate oxidation at the N-1 position by oxone. Under the conditions we use the concentration of the oxidizing agent is much lower ($KHSO_5$ 2.7×10^{-3}M; oligonucleotide 5×10^{-4}M) and the four natural deoxyribonucleosides remain totally unchanged after a six-hour treatment, as determined by HPLC analysis.
13. Kettani, A.E.C.; Bernadou, J.; Meunier, B. *J. Org. Chem.* **1989**, *54*, 3213-3215.
14. The oxidation conditions must be strictly controlled. Longer reaction times than indicated led to complex mixtures. The heptamer and the trimer behaved similarly. Oxidation gave a mixture of two oligonucleotides. One probably contains 8-propylsulfonyldeoxyadenosine, as the peak **9** corresponding to the base released in the hydrolytic treatment has the characteristics (retention time and UV absorption ratio at 260/280 nm) of synthetic 8-propylsulfonyladenine. The second oligonucleotide could contain the base oxidized to the intermediate sulfoxide stage. In the case of the trimer **5**, we separated the two oxidized oligomers and treated them separately under hydrolytic conditions; both were converted to the same abasic trimer **7**.
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