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INSERTION OF THE FRAGILE 2'-DEOXYRIBOSYLUREA RESIDUE INTO OLIGODEOXYNUCLEOTIDES

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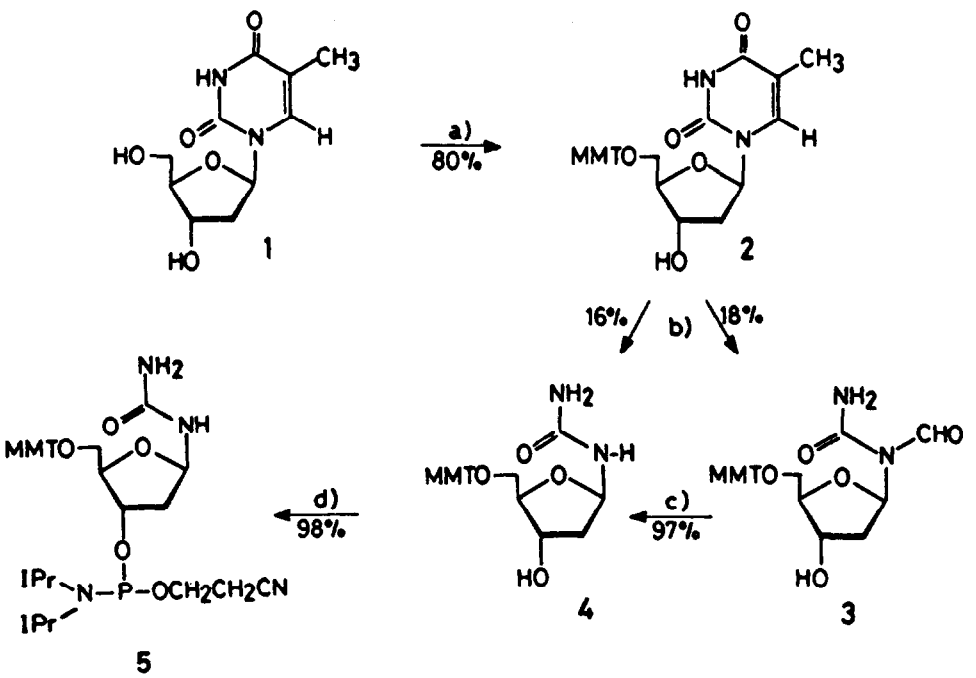
SUMMARY: Deoxyribosylurea is an alkali sensitive DNA defect induced by the action of ionizing radiation. Oligonucleotides bearing this residue were synthesized using 5'-O-(4-methoxytrityl)-2'-deoxyribosylurea-3'-O-(2-cyanoethyl-N,N'-diisopropyl) phosphoramidite and PAC protected deoxynucleoside phosphoramidites.

Urea is a fragmentation product of thymidine and 2'-deoxycytidine radiolysis. When DNA is submitted to the action of ionizing radiation urea is retained on the polymer chain backbone (1-3). This radiation induced DNA lesion is recognized and excised by specific repair enzymes (4-6). *Escherichia coli* exonuclease III, a complex enzyme that contains 3'-exonuclease and phosphatase activities as well as apurinic endonucleolytic activity, recognizes the DNA-urea residue but not the DNA-thymine glycol residue. No urea is released, supporting the idea that exonuclease behaves as an endonuclease nicking on the 5'- site of the deoxyribosylurea residue. *Escherichia coli* endonuclease VIII incises DNA containing an urea residue and may substitute for endonuclease III which has a N-glycosylase activity in the initiation of the repair of this lesion (7). Urea seems to block the copy of DNA by *Escherichia coli* DNA polymerase *in vitro* (8). These biological experiments have been performed with OsO_4-oxidized DNA and subsequent alkali treatment. In these conditions, the formation of different oxidative products cannot be definitively ruled out and there is a need for a DNA substract bearing only one well defined defect. Therefore, to investigate mutagenic and repair properties of biological systems, an urea nucleotide has been selectively incorporated into chemically synthesized DNA fragments.

The method (9) used to prepare the protected deoxyribosylurea phosphoramidite, which is required for the oligonucleotide synthesis, is outlined below. Protection of the 5'-hydroxyl function of thymidine **1** using monomethoxytritylchloride gave **2** in 80 % yield. Permanganate oxidation of 5'-O-methoxytritylthymidine **2** in acetone-pyridine solution, at pH 8, at room temperature for 2 h, was followed by lead tetraacetate treatment to obtain final products **3** and **4**. Quantitative ammonolysis of **3** with 28 % NH_4OH at room temperature for 10 minutes provided the ureido compound **4**.

Chromatography on silica gel column yielded 33 % of methoxytrityl-deoxyribosylurea **4**. Phosphitylation of **4** to give the phosphoramidite **5** was carried out with 0.9 eq. of cyanoethyl-tetraisopropyl-phosphorodiamidite and diisopropylammonium tetrazolide in anhydrous CH_2Cl_2, at 25°C, for 30 min. (4 diastereoisomers, 98 %). The structure of the pure isolated products was confirmed by NMR and mass spectrometry (10).

The protected phosphoramidite **5** and the new commercially available (Pharmacia) PAC phosphoramidites (11, 12) (phenoxyacetyl for adenine and guanine, and isobutyryl for cytosine as protective



MMT = monomethoxytrityl, Ipr = isopropyl. **a)** 38 mmol thymidine, 16.4 mmol MMT-Cl, pyridine 25°C, 2 hr. **b)** 8 mmol MMT-Thymidine, 16.4 mmol $KMnO_4$, Pyridine/acetone, pH = 8, 1 hr at 20°C, 1 M aq. $NaHSO_3$, filter off and evaporate then add 8.4 mmol anh. $Pb(OAc)_4$, dry pyridine, 2 hr at 20°C. **c)** ammonolysis at r.t. 28 % NH_4OH/pyridine (4v/1v), 10 min. **d)** 2 mmol MMT-deoxyribosylurea, 1.8 mmol CNEt-tetraisopropyl phosphoro-diamidite, 1.2 mmol diisopropylammonium tetrazolide, anh. CH_2Cl_2, 30 min. at 25°C.

groups) were used for the synthesis of the modified oligodeoxynucleotides on silica gel support. The usual isobutyryl deoxyguanosine and benzoyl deoxyadenosine phophoramidites (13) are not convenient, because deprotection of the base amino groups needs too drastic alkaline conditions. A set of seven DNA fragments (14) ranging from 5 to 47 nucleotides units was prepared for biological purposes on a DNA synthesizer using the cyanoethyl-diisopropylphosphoramidite method at a flow rate of 2.3 ml.min.$^{-1}$ for optimal coupling efficiency.

During the detritylating step of the synthesis cycle monomethoxytrityl and dimethoxytrityl protecting groups were eliminated in the same mild acid conditions (3 % trichloroacetic acid in dichloromethane for 120 sec) without any change in the coupling yield (15). Crude oligomers were obtained in a high yield (77 % and 81 % for the 47 mer oligonucleotides). Deprotection was performed at 20°C in 28 % ammonia for 5 hr and then the crude products were purified by HPLC or preparative polyacrylamide gel electrophoresis. The overall yields of oligomers **9** and **10** isolated from HPLC on anion exchange column and dialysis were 46 % and 49 % respectively.

The purity of the isolated material was confirmed by ^{32} P-labeling with polynucleotide kinase and gamma ^{32}P-ATP followed by gel electrophoresis. The stability and the authenticity of the ureido residue (Ur) was confirmed on a short model sequence dCG (Ur)AT by fast atom bombardment mass spectrometry (16). To check the stability for the longer oligonucleotides the same sequence was submitted to 47 cycles of acidic detritylation, CH_3CN washing, phenoxyacetic anhydride capping and 0.1M I_2-oxidation steps and to the final ammonia deprotection. Molecular weight measurement of oligonucleotide confirmed the presence of the urea residue.

Most of the radiation induced DNA base defects are alkali labile. The use of PAC phosphoramidites allows milder deprotection conditions with ammonia and opens new possibilities for the preparation of modified oligonucleotide bearing a specific base oxidative damage in a well defined position in DNA sequence. These modified oligonucleotides should be of great interest for the study of the biological effects of this unique lesion.

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References and footnotes

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9. Attempts to synthesize protected deoxyribosylurea phosphoramidite by direct oxidation of thymidine and dimethoxytrityl protection followed by phosphitylation were unsuccessful. We observed extensive decomposition of the dimethoxytritylated compounds during isolation by silica gel chromatography and purification by high performance liquid chromatography.
10. **Product 4 : ^{1}H-NMR** (200 MHz, CD_3OD, 2 isomers, in ppm relative to TMS) : 1.78 (m, 0.6H, H2", isomer 1), 1.93 (m, 0.4, H-2', isomer 2), 2.08 (m, 0.4H, H-2", isomer 2), 2.37 (m, 0.6H, H-2', isomer 1), 3.11 (m, 2H, H-5'

and H-5", isomers 1 and 2) 3.75 (s, 1H, OCH_3 (MMT)), 3.92 (m, 0.4H, H-4', isomer 2), 4.10 (m, 0.6H, H-4', isomer 1), 4.25 (m, 1H, H-3', isomers 1 and 2), 5.75 (m, 1H, H-1', isomers 1 + 2), 6.75-7.5 (m, 14H, H-Ar) **HR FAB-MS** (neg.ions, PEG 200 matrix) : 447.1941 $(M-H)^-$, $C_{26}H_{28}O_5N_2$, calc. 447.1920 ; **FAB-MS** (neg. ions, glycerol matrix) : 447 (100, $(M-H)^-$), 175 (83, $(M-H-MMT)^-$). **Product 5** : ^{31}**P-NMR** (101.2 MHz, CD_3CN, in ppm relative to 85 % H_3PO_4, 2 diastereoisomers) : 149.7 and 149.4 ; **FAB-MS** (pos. ions, PEG 200 matrix, NaI), 671 $(M+Na)^+$, 649 $(M+H)^+$, 377 $(MH-MMT)^+$; **HR FAB-MS** (neg. ions, PEG 200 matrix) 594.2716 $(M-CNEt)^-$, $C_{32}H_{41}N_3O_6P$, calc. 594.2733.

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14. Sequence of chemically synthesized oligodeoxynucleotides with 2' - deoxyribosylurea residue (Ur) :
    - **6** d (ACGACTTAGCGAGGUrT)
    - **7** d (ACGACTTAGCGAGUrTT)
    - **8** d (ACGACTTAGCGAGUrGTT)
    - **9** d (GTGGTATGGCACTTCGGAACGTGUrTGCGGCTAACCTCGCTAAGTCGT)
    - **10** d (GTGGTATGGCACTTCGGAACGTCUrTGCGGCTAACCTCGCTAAGTCGT)
    - **11** d (CCATGUrTCGC)
    - **12** d (CGUrAT)
15. Relative to the amount of released trityl cation.
16. Oligonucleotide dCG(Ur)AT : **FAB-MS** (neg. ions,GLYCEROL matrix) : 1410.1 $(M-H)^-$, calc. 1410.3.

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