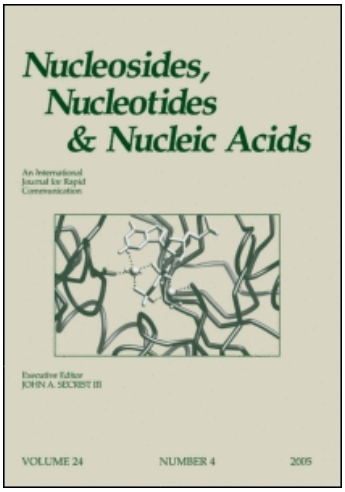

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Synthesis of Oligodeoxynucleotides Bearing a Radiation Induced DNA Damage: Deoxyribosylurea and Deoxyribosylformamide

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SYNTHESIS OF OLIGODEOXYNUCLEOTIDES BEARING A RADIATION INDUCED DNA DAMAGE: DEOXYRIBOSYLUREA AND DEOXYRIBOSYLFORMAMIDE

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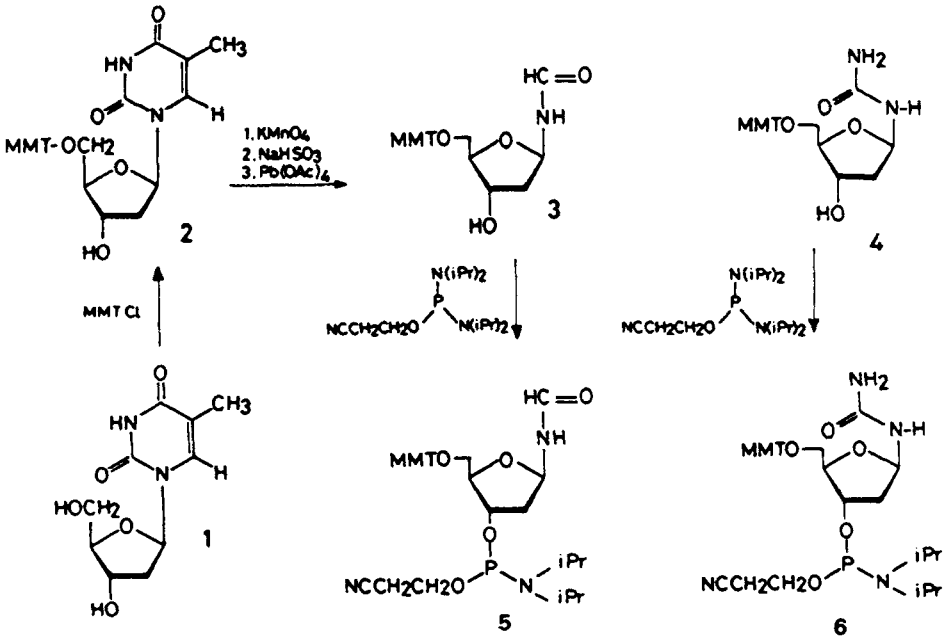
ABSTRACT : Formamido and ureido residues were introduced in DNA fragments using PAC cyanoethylphosphoramidite methodology. Mild alkaline conditions were used for the deprotection of the oligonucleotides and the integrity of these fragile defects was confirmed by FAB Mass spectrometry.

Deoxyribosylurea and deoxyribosylformamide are two major DNA modifications induced by the action of ionizing radiation[1]. In order to investigate the mutagenic effects and the repair of these lesions, oligodeoxynucleotides bearing only one DNA damage in a well defined position are of great interest. That is why urea nucleotide



and formamide nucleotide have been selectively introduced into chemically synthesized DNA fragments.

These modified nucleosides are produced in low yield by gamma irradiation of aqueous thymidine solution, therefore irradiation cannot conveniently be used to prepare them. To synthesize the protected 2'-deoxyribosylformamido phosphoramidite **5** and 2'-deoxyribosylurea phosphoramidite **6** wich are needed in the phosphoramidite synthetic methodology we used a 3-step reaction scheme.

Using the monomethoxytrityl group to protect the 5'-hydroxyl function of thymidine **1** we obtained **2** in 80 % yield. Direct permanganate oxidation of **2** in acetone-pyridine solution (pH 8) for two hours at room temperature was followed by lead tetraacetate treatment to give compounds **3** and **4**. Chromatography on silica gel column yielded 32 and 30 % respectively of monomethoxytrityl-deoxyribosylformamide **3** and monomethoxytrityl-deoxyribosylurea **4**. Compounds **3** and **4** were phosphitylated by the action of cyanoethyl-tetraisopropyl-diamino phosphine according to a procedure described by Barone [2] to give protected phosphoramidites **5** and **6** in high yield (mass spectrometry and ^{31}P-NMR analysis confirmed the structure).

The protected compounds **5** or **6** and the cyanoethyldiisopropyl phosphoramidites with labile base protection [3] were used for the synthesis of oligodeoxynucleotides on derivatized silica gel support. A set of DNA fragments ranging from 5 to 47 nucleotide units and containing the ureido or the formamido residue were prepared for biological purposes. The oligodeoxynucleotides were then deprotected according to a procedure previously described [4].

To evaluate the presence of these defects in the final synthetic DNA fragments we prepared a model sequence CGMAT (M is ureido or formamido residue) and we confirmed their stability and their authenticity by fast atom bombardment mass spectrometry. To check the stability for the longer oligonucleotides the same sequence was submitted to repetitive 42 cycles of acidic detritylation, CH_3CN washing, capping and oxidating steps. Molecular weight measurement of the oligonucleotides identified unambigously ureido and formamido nucleosides.

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