Tetrahedron Letters, Vol. 34, No. 50, pp. 8101-8102, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

The Synthesis of 5-Hydroxy-5-methylhydantoin Nucleoside and its Insertion into Oligodeoxyribonucleotides

André Guy*, Josselin Dubet and Robert Téoule

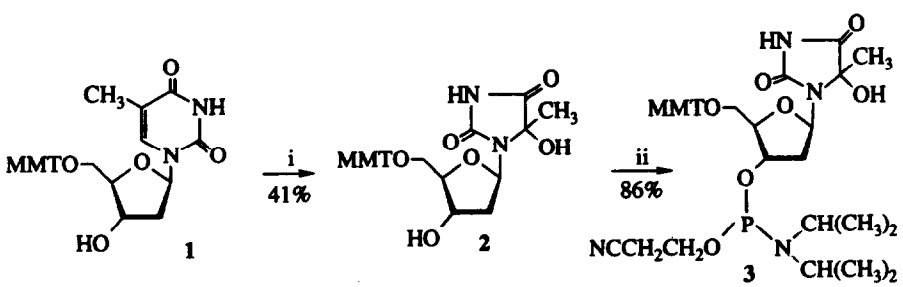
CEA, Département de Recherche Fondamentale sur la Matière Condensée, SESAM, 85X, 38041 Grenoble Cedex, France.

Abstract : 5-hydroxy-5-methylhydantoin is a nucleobase lesion induced by the action of ionizing radiation on thymine residue in DNA. The protected nucleoside cyanoethyldiisopropylphosphoramidite has been synthesized and used to prepare chemical DNA fragments containing 5-hydroxy-5-methylhydantoin moieties.

Base moieties in nucleic acids are the major targets of free radicals produced by ionizing radiation leading to DNA lesions wich are involved in mutation and in carcinogenesis induction [1]. One of the major problem encountered in the study of DNA base damage is to determine the nature of lesions and to localize them along the irradiated DNA chain. To establish which lesion is responsible of the particular biological effect, oligonucleotides bearing only one DNA damage in a well defined position of a particular sequence are of great interest.

We have focused our attention on 5-hydroxy-5-methylhydantoin (HMH) a specific thymine modification induced by the action of ionizing radiation on DNA [2,3]. We report herein the synthesis of phosphoramidite synthon 3 suitable for oligodeoxyribonucleotide assembly using fast deprotection chemistry.

Previous studies suggested that methoxytrityl thymidine could be a convenient substrate for Mn(VII) oxidation and that the introduction of 4-methoxytrityl (MMT) protecting group at the first step of the synthesis could be a convenient marker for following reactions and purifications. Based on this approach we have developed a route starting from 1 to synthesize the protected HMH nucleoside 2 and its phosphoramidite 3 (see scheme).



i. $KMnO_4$, $Pb(OAc)_4$; ii. $NCCH_2CH_2OP(N(iPr)_2)_2$

The typical procedure for Mn (VII) oxidation and Pb $(OAc)_4$ treatment is as follows. To a solution of 1 (10 g, 19.4 mmol) in pyridine (25 ml) and 4M $NaHPO_4$ (50 ml) fine needles of $KMnO_4$ (4 g, 25 mmol) were added under stirring (4 h, pH 5.2). Aqueous $NaHSO_3$ (1 M, 50 ml) was added, salts were filtered off and filtrate evaporated. The residue obtained was dissolved in dry pyridine and anhydrous powdered $Pb(OAc)_4$ (9g, 20 mmol) was added. After stirring (2 h) and TLC monitoring, pyridine was removed in *vacuo*. The oily residue was dissolved in CH_2Cl_2 (2 x 200 ml) and washed with saturated $NaHCO_3$ solution, water and dried (Na_2SO_4). The residue obtained after removal of solvent was purified by flash chromatography on silica gel column to give 2. Purification by HPLC on silica gel column (260 g, 4 x 30 cm) using a linear gradient of 0-5 % iPrOH in $CHCl_3$ gave a 41 % yield of pure compound 2.

Selective 3'-O-phosphitylation of 2 to give the corresponding phosphoramidite 3 was carried out with 1.1 eq of 2-cyanoethyl-tetraisopropylphosphorodiamidite, 0.5 eq of diisopropylammonium tetrazolate in anhydrous CH_2Cl_2 at room temperature for 2 h (precipitation in hexane at -70°C gave 86 % of 3). The structure of the pure isolated products was confirmed by NMR and mass spectrometry [4].

The efficiency of this protected 3'-(cyanoethyl-N-diisopropyl) phosphoramidite 3 has been evaluated for the synthesis of modified oligodeoxyribonucleotides on suitable alkali labile protected nucleoside bound supports. To minimize HMH base modification we used the phosphoramidites set with easily removable amino protecting groups (phenoxyacetyl for adenine and guanine, and isobutyryl for cytosine) which can be eliminated with 28 % NH_4OH within 5 h at room temperature [5,6]. DNA fragments ranging from 5 to 47 nucleotides were prepared using N-methylimidazole and phenoxyacetic anhydride capping solution [7].

These modified oligonucleotides sequences bearing a site specific oxidative lesion are under investigation to know whether this lesion blocks DNA polymerase synthesis and to determine whether or not this defect is processed by DNA repair enzymes.

REFERENCES and NOTES

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4. **Product 2 :** TLC ; SiO_2, $CHCl_3$/iPrOH : 90/10, Rf = 0.75. ^{1}H-NMR (400 MHz, CD_6 $COCD_6$) 1.58 (s, 1H, CH_3 base), 2.83-2.98 (m, 2H, $H_{2'}$ & $H_{2''}$), 3.37-3.42 (m, 2H, $H_{5'}$ & $H_{5''}$), 3.90 (s, 3H, CH_3O trityl), 4.03 (m, 1H, H_4), 4.65 (m, 1H, H_3), 6.0 (t, 1H, H_1), 7.0-7.60 (H arom.). FAB-MS (glycerol matrix, relative intensity, neg. ions, m/z) 517 $(M-H)^-$.
   Product 3 : TLC ; SiO_2, $CHCl_3$/iPrOH/TEA : 89/10/1, Rf = 0.65. ^{31}P-NMR (101 MHz, CD_6COCD_6, δ in ppm relative to 85 % H_3PO_4, 4 diastereomers) : 149.9, 149.6, 149.4, 149.1. FAB-MS (neg. ions, PEG matrix, m/z) : 717 (30, $[M-H]^-$), 445 (10, $[M-H-MMT]^-$).
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(Received in France 16 September 1993; accepted 7 October 1993)