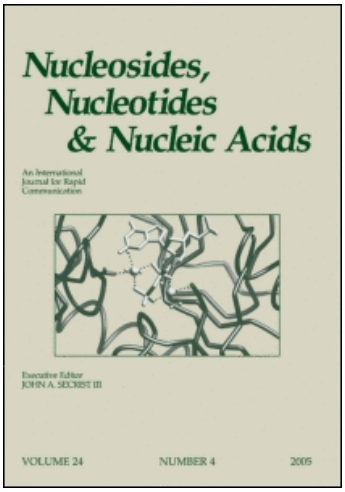

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Chemical Synthesis of Oligonucleotides Containing The (6-4) Photoproduct at the Thymine-Cytosine Site and Its Repair By (6-4) Photolyase

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CHEMICAL SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING THE (6–4) PHOTOPRODUCT AT THE THYMINE–CYTOSINE SITE AND ITS REPAIR BY (6–4) PHOTOLYASE

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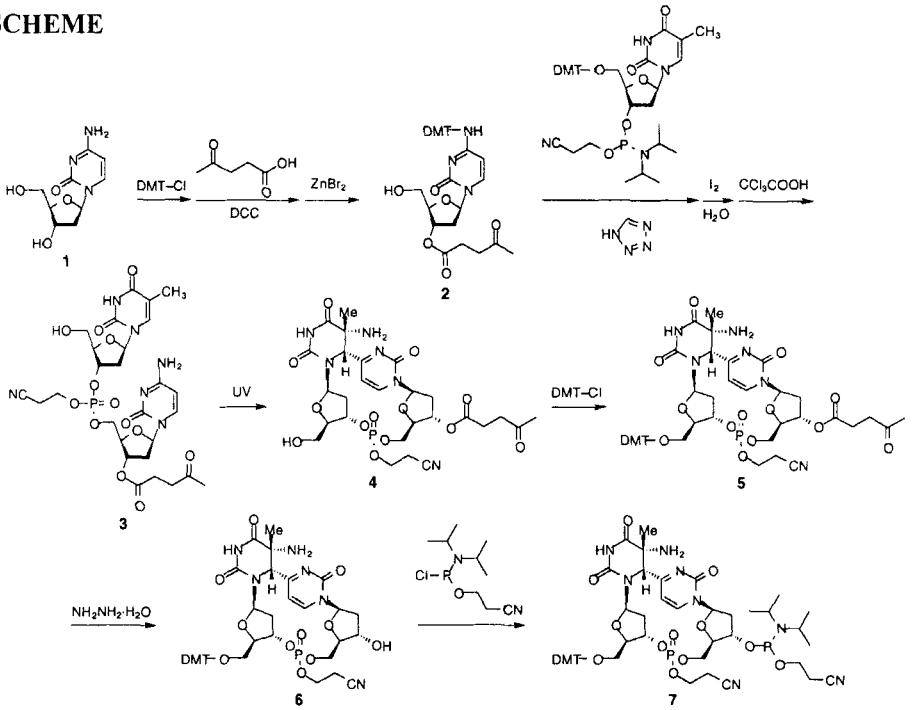
ABSTRACT: A phosphoramidite building block of the T(6–4)C photoproduct was synthesized. One of the differences from T(6–4)T was formation of cytosine hydrates by UV irradiation, and the other was acylation of the amino function with the capping reagent. The capping step was omitted to improve the yield of the desired oligonucleotides. Characterization of the (6–4) photolyase using one of the oligonucleotides revealed that this enzyme restores the pyrimidines in T(6–4)C to their original structures.

Ultraviolet light, especially UV-C radiation with wavelengths around the absorption maximum of nucleic acids, causes various damages in DNA. At dipyrimidine sites, two major types of photolesions, i.e. cyclobutane pyrimidine dimers and pyrimidine(6–4)pyrimidone photoproducts, are produced. Previously, we synthesized a phosphoramidite building block of the (6–4) photoproduct of thymidylyl(3'–5')thymidine (T(6–4)T) and incorporated it into oligonucleotides,[1] which have been applied to molecular biology of mutation[2,3] and repair[4,5]. However, it has been reported that the (6–4) photoproduct is formed most efficiently at the TC sequence. Therefore we studied the synthesis of oligonucleotides containing the (6–4) photoproduct between thymine and cytosine (T(6–4)C) using a dimer building block, and one of the oligonucleotides was applied to characterization of *Xenopus* (6–4) photolyase.

UV irradiation of thymidylyl(3'–5')deoxycytidine bearing protecting groups at the phosphate and the 3'-hydroxyl function (**3**) gave hydrates of cytosine as main products, and formation of the (6–4) photoproduct (**4**) reached a plateau faster than that from the thymidylyl(3'–5')thymidine derivative. For preparation, a 2 mM aqueous solution of **3** was irradiated at a total UV dose of 40 J/cm^2, and the two diastereomers of **4** were separated by chromatography on alkylated silica gel. The major isomer was purified further, and its yield from **3** was 4%. The structure and the stereochemistry of the photoproduct were confirmed by NMR spectroscopy. As shown in SCHEME, a phosphoramidite building block (**7**) was synthesized in three steps from **4**.

SCHEME



When compound **7** was used for the usual oligonucleotide assembly on a solid support, it was found that acylation of the amino group of the 5' pyrimidine component occurred at the capping step. Oligonucleotides were synthesized successfully by omitting the capping steps after coupling of the (6–4) photoproduct unit. A 12-mer, d(CAT(6–4)CAGCACGAC), which was prepared previously by UV irradiation of the undamaged

oligonucleotide,[2] a 14-mer, d(AAAAAAAAT(6–4)CAAAA), and five 30-mers with defined sequences were synthesized on a 0.2 μmol scale by this method.

The 14-mer was treated with *Xenopus* (6–4) photolyase, and HPLC analyses of the photoreactivation product and its nucleoside composition revealed that this (6–4) photoproduct was repaired to its original pyrimidine components by this enzyme.

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