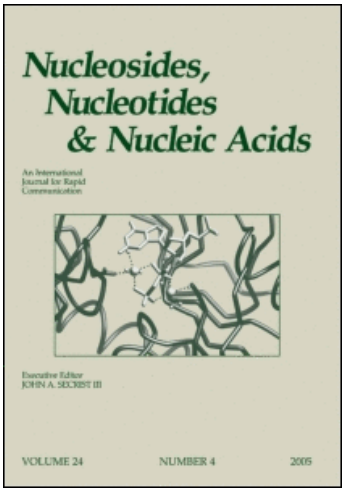

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Synthesis and Photochemical Conversion of Oligonucleotides Containing 2-Chloro-2'-Deoxyadenosine

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SYNTHESIS AND PHOTOCHEMICAL CONVERSION OF OLIGONUCLEOTIDES CONTAINING 2-CHLORO-2'-DEOXYADENOSINE

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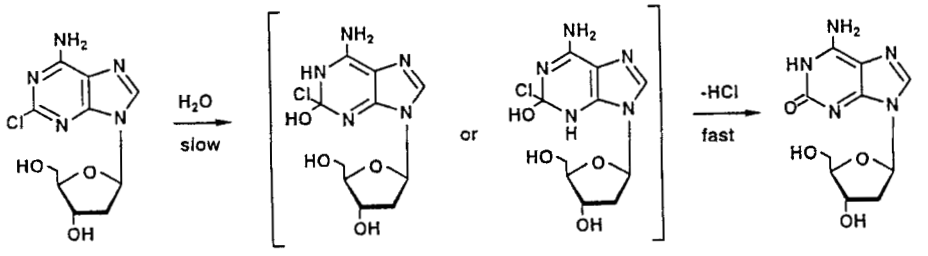
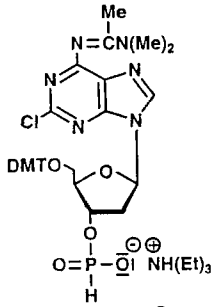
Abstract. The rate and velocity of the photoconversion of 2-chloro-2'-deoxyadenosine into 2'-deoxyisoguanosine within oligonucleotides was found to be sequence-specific and depends on the nearest neighbor.

2-Chloro-2'-deoxyadenosine (Cladrabine, Cl^2A_d, **1**) is an adenosine deaminase (ADA)-resistant analogue of deoxyadenosine currently undergoing clinical trials [1]. A major contribution to the cytotoxicity of Cl^2A_d probably causes the termination of DNA synthesis after its incorporation into DNA by human polymerases α and ß [2]. Another phenomenon which may cause cytotoxic events of Cl^2A_d is its UV sensitivity. Upon irradiation at 254 nm it is converted into 2'-deoxyisoguanosine (iG_d, **2**) [3] which shows an altered base pairing pattern. The photoreaction of Cl^2A_d, performed in H_2O as well as in D_2O suggests an addition-elimination mechanism according to the scheme shown below.



1 **2**

The photoconversion of oligonucleotides containing Cl^2A_d has now been investigated. For this purpose the dodecamers **5-9** were prepared by solid-phase synthesis using the phosphonate **3** or the phosphoramidite **4**.



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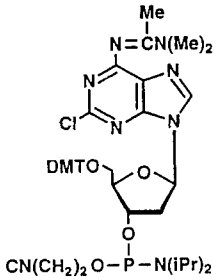

4

5'-d(Cl^2A-T)$_6$ **5**

5'-d(Cl^2A-G)$_6$ **6**

5'-d(Cl^2A-C)$_6$ **7**

5'-d(Cl^2A-A)$_6$ **8**

5'-d(Cl^2A_{11}-A) **9**

The reaction rate and the completeness of the Cl^2A_d photoconversion into iG_d within the oligonucleotides **5-9** were studied by reversed phase HPLC upon hydrolysis with snake venom phosphodiesterase followed by alkaline phosphatase after 10 and 50 min of UV irradiation as well as UV spectroscopy (appearance of a new absorption at 300 nm).

The HPLC profiles (Fig. 1) indicate that after 10 min of irradiation (254 nm, H_I = 842 kW/m^2·h) 5'-d(Cl^2A-A)$_6$ (**8**) was almost completely converted to the iG_d-containing product (Fig. 1B and 1C). However, a significant amount of non-digestible high-molecular photoproducts was formed (Fig. 1D) indicating probably the formation of covalent bonds between the nucleotide moieties.

In contrast to this almost no formation of non-cleavable photoproducts was observed for 5'-d(Cl^2A-G)$_6$ (**6**) and photoconversion was complete after the same time of irradiation (10 min) under formation of 5-d(iG-G)$_6$ [4].

In the case of the dC-containing oligomer **7** after 10 min of irradiation a significant amount of unreacted Cl^2A_d was found (Fig. 2B). Further irradiation (50 min) leads to a complete conversion of remaining Cl^2A_d (Fig. 2C); comparably small amounts of non-digested photoproducts were formed (Fig. 2D). Under the same irradiation conditions the oligonucleotide 5'-d(Cl^2A-T)$_6$ (**5**) shows a similar behavior as oligomer **7**. The reason is that pyrimidine nucleosides readily undergo side photoreactions (photohydration, photodimerization, homolytic addition of hydroxyl radicals, etc.) under the influence of UV light. The photoreactivity of stable dA is significantly enhanced when it is incorporated into oligonucleotides [5].

These results were confirmed by UV spectra of the irradiated oligonucleotide mixtures run at different time intervals. It can be seen (Fig. 3) that after 10 min of UV irradiation the photoconversion of only the purine-containing oligonucleotides **6** and **8** was terminated (Fig. 3A) but that a

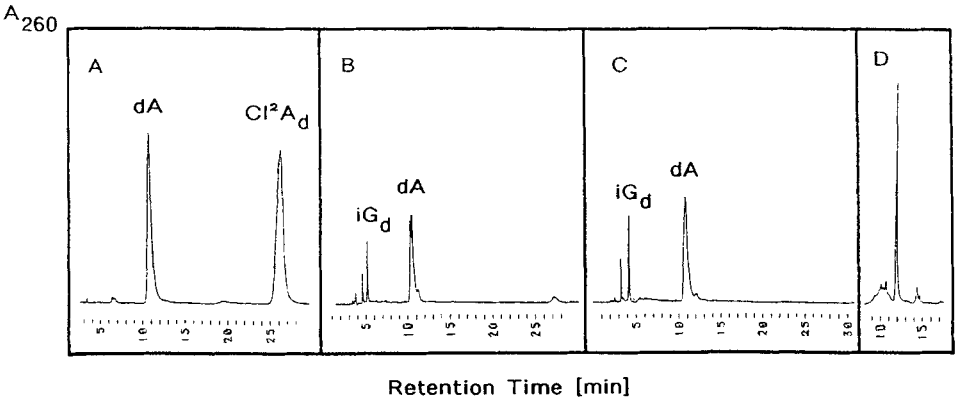

Fig. 1. HPLC Profile of 5'-d(Cl^2A-A)$_6$; A) enzymatic digest of the unirradiated oligonucleotide; B) enzymatic digest after 10 min of irradiation; C) enzymatic digest after 50 min of irradiation; D) non-digested photoproducts.

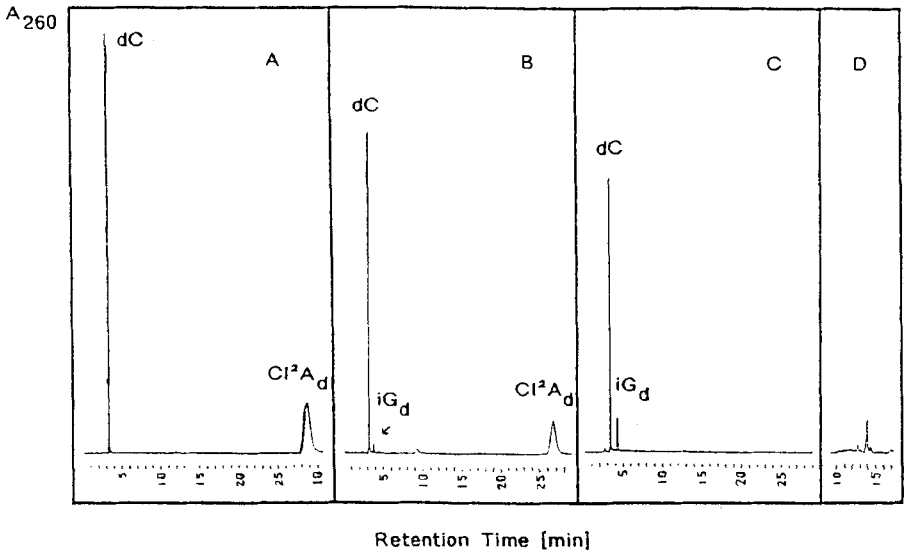

Fig. 2. HPLC Profile of 5'-d(Cl^2A-C)$_6$; A) enzymatic digest of the unirradiated oligonucleotide; B) enzymatic digest after 10 min of irradiation; C) enzymatic digest after 50 min of irradiation; D) non-digested photoproducts.

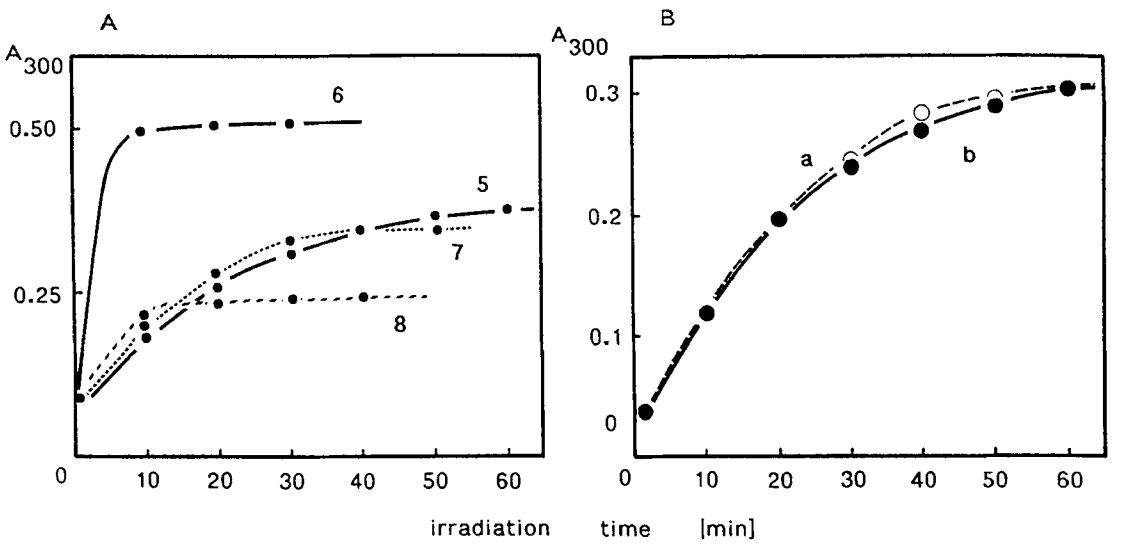

Fig. 3. Normalized profiles of UV absorbance as function of irradiation time; A) oligomers **5-8**; B) Cl^2A_d + dG (a) and Cl^2A_d + dT (b).

complete conversion of the pyrimidine-containing oligonucleotides **5** and **7** as well as of the corresponding nucleoside mixtures (Fig. 3B) needs about 50 min.

Irradiation of the oligomer 5'-d(Cl^2A_{11}-A) (**9**) gives an almost complete formation of enzymatically non-cleavable photoproducts.

These findings indicate that the photoconversion of Cl^2A_d into iG_d within an oligonucleotide chain is sequence-specific; an almost clean reaction is only possible in the presence of dG as nearest neighbor.

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