The efficiency of incorporation of dTTP opposite 2ClA is 25 - 50-fold lower than opposite A and extension of an 2ClA·T pair is 3-fold lower than of an A·T pair. This indicates that the presence of 2ClA in DNA slows replication, but does not lead to base-substitution mutations. From the analysis of the parameters of dTTP incorporation we conclude that formation of a 2ClA·T pair is thermodynamically, but not kinetically controlled. The diffrence in binding energy (DDG) between 2ClA·T and A·T pairs in the environment of the polymerase active site is 2 kcal/mol.

2'-Deoxyisoguanosine (diG, 2-oxo-2'deoxyadenosine) is formed in DNA in a reaction of 2'-deoxyadenosine with reactive oxygen species. In order to study its miscoding properties, 25-mer deoxyoligonucleotide templates with diG located site-specifically, were prepared.

**2.2. Repair of cyclic adducts.**

The combined action of glycosylases and abasic site-specific endonucleases on damaged bases in DNA results in single strand breaks. In plasmid DNA, as a consequence, the covalently closed circular (*ccc*) form is converted to the open circular (*oc*) form, and this can be quantitated by agarose gel electrophoresis. We studied DNA lesions sensitive to *E. coli* AlkA and cloned human ANPG-40 glycosylases which are known to excise alkylated bases and etheno adducts. To our surprise pBR322 and pAlk10 plasmids not pretreated with mutagens were cleaved by both glycosylases in the presence of enzymes possessing endonucleolityc activity (Nth, Fpg, ExoIII), which indicates that plasmids contain unknown, endogenously formed adducts. Plasmid pretreated with chloroacetaldehyde, a mutagen forming etheno adducts, exhibited enhanced sensivity as expected. Adducts formed by acrolein and croton aldehyde are excised by AlkA, but not by ANPG-40, whereas malondialdehyde adducts are not excise by either glycosylase. Bulky *p*-benzochinone adducts are not excised by AlkA, however, plasmid pretreated with this mutagen is incised by endonucleases, possibly without prior generation of an abasic site. These examples show that examination of conformational changes of plasmid DNA can be exploited to study the specificity of N-alkylpurine-DNA glycosylases.

**Publications: 3121, 1009/A, 1011/A**


## 3. PYRIMIDINE NUCLEOSIDE ANALOGUES, POTENTIAL CHEMOTHERAPEUTIC AGENTS, AND SUBSTRATES/INHIBITORS IN VARIOUS ENZYME SYSTEMS

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Pyrimidine nucleoside analogues are an important class of compounds with antimetabolic (antitumor, antiparasitic and antiviral) properties. The synthesis of thiated nucleoside and nucleotide analogues, determination of structures, conformation and dissociation constants, their potential chemotherapeutic activities, and their

substrate/inhibitor properties in various enzyme systems, with emphasis on enzymes related to chemotherapeutic activities, were investigated.

In the series of thionated inhibitors of thymidylate synthase (TS), potential antitumor agents, regioselective syntheses were elaborated for 2- and 4-thio, and 2,4-dithio derivatives of 2'-deoxyuridine (dUrd), 5-fluoro-2'-deoxyuridine (FdUrd), and several other 5-fluoro, 5-bromo- and 5-trifluoromethyl congeners, and the 2-thio derivatives of FdUrd and its ∝-anomer, which proved to be selective agents with high cytotoxicities correlated with the inhibitory activities *vs* TS of their corresponding 5'-monophosphates.

Regioselective syntheses were also elaborated for 2'-deoxycytidine and 5-fluoro-2'-deoxycytidine derivatives. Solution conformations of these nucleosides were deduced from high- resolution (500 MHz) $^1$H NMR spectra. Substrate/inhibitor properties of 2-thio-2'-deoxycytidine ($S^2$dCyd) and 5-fluoro-2-thio-2'-deoxycytidine ($S^2$FdCyd) with respect to human leukemic spleen deoxycytidine kinase have been examined. Both are substrates, and also good inhibitors, of phosphorylation of 2'-deoxycytidine and 2'-deoxyadenosine. Particular attention was directed to the specificity of the NTP phosphate donor for several nucleoside kinases, and procedures have been developed for distinguishing between ATP and other NTP donors, a problem of importance in chemotherapy with nucleoside analogues.

Biological properties of the newly synthesized thiated pyrimidine 2',3'-dideoxy-3'-fluoronucleosides, $S^2$,3'-FddUrd and $S^2$,3'-FddThd, were also investigated. Thiated 3'-fluoronucleosides were moderate substrates for thymidine phosphorylase and were quite inactive *vs* uridine phosphorylase. $S^2$,3'-FddUrd proved to be a moderate, and $S^2$,3'-FddThd a potent and selective, inhibitor of the replication of HIV-1 in CEM4 cells, comparable to the activity of the known antiretroviral agent AZT.

5-Fluorouridine and 5-fluorocytidine are potent antitumor agents, but too toxic to be used as drugs. To decrease this toxicity, a new class of branched-chain sugar nucleosides, pyrimidine and purine hamamelose nucleosides, was synthesized, with uracil and 5-fluorouracil derivatives being the most potent antileukemic agents, activities being comparable to that of 5-fluorouridine.

**Publications: 3098, 3112,+3113, 3114, 3118, 3137, 3138, 3139, 3142, 3150, 3175, 3182, 3191, 3225, +3226, 3236, 59/N**

## 4. MECHANISMS OF PUVA (Psoralen + UVA) PHOTOCHEMOTHERAPY

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Our studies are aimed at understanding the action of PUVA phototherapy on the lym-phocytes membrane. The investigations are carried out on model lecithins having in the second position oleic/linoleic acid as the majority of lecithins have. Previous investigations led to a discovery of a new class of photoadducts which accompany the photolytic breakdown of lecithin. The new photoadducts were obtained