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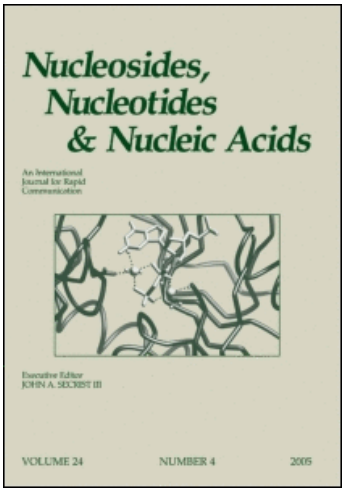

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SYNTHESIS, ANTI-HIV ACTIVITY, AND BIOLOGICAL PROPERTIES OF 2',3'-DIDEHYDRO-2',3'-DIDEOXYTHYMIDINE (D4T)

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Abstract. D4T is a thymidine analogue with an in vitro potency against HIV comparable to that of AZT but is less toxic to a variety of cell lines including human hemopoietic progenitor cells.

The majority of commercial and experimental drugs for the treatment of viral infections have been nucleoside analogues. For AIDS, the drug AZT (zidovudine) is the only agent approved to date.[1] The toxicities seen in AIDS patients treated with AZT include anemia which can require transfusions and neutropenia that often forces discontinuation of treatment.[2] The goal for the development of new compounds for the treatment of AIDS is to identify those substances which could have a superior therapeutic index compared to AZT.

D4T is a thymidine derivative which is similar in structure to AZT and has been reported to have a comparable potency against HIV.[3-6] Large scale synthesis of D4T has been carried out to allow for the detailed biological evaluation of this promising anti-HIV substance.

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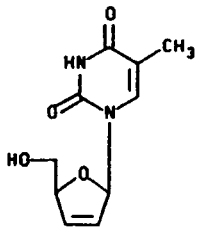
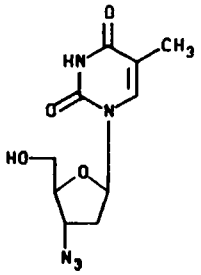


D4T



AZT

RESULTS AND DISCUSSION

A synthetic procedure for the preparation of D4T that was described by Horwitz et al.[7] was modified so that larger quantities could be prepared. Starting from thymidine the formation of dimesylate 1 was carried out as described to give the product in 98 % yield by a straightforward isolation procedure. Oxetane 2 was produced by the treatment of 1 with aqueous NaOH. Acidification with HCl afforded the pure product which was obtained in 74 % yield after recrystallization from ethanol.

The final reaction (2 to D4T) was the scale limiting step of the Horwitz procedure because of the instability of D4T under the conditions of evaporation of DMSO at elevated temperatures. D4T is heat and base sensitive and decomposes by a mechanism presumably involving deprotonation of the 4'-H to give a furan derivative and thymine. The final step of the base catalyzed elimination of the oxetane must be carried out quickly to avoid decomposition of the product. DMSO was an ideal solvent because the reaction remained homogeneous allowing for rapid conversion. To avoid the evaporation procedure, the potassium salt of D4T was simply precipitated from the reaction mixture by addition of toluene, collected by filtration, and redissolved in water. D4T was crystallized by neutralization to pH 7 with HCl. After redissolution of the solid in hot acetone, filtration, and evaporation, D4T was isolated in 57 % yield.

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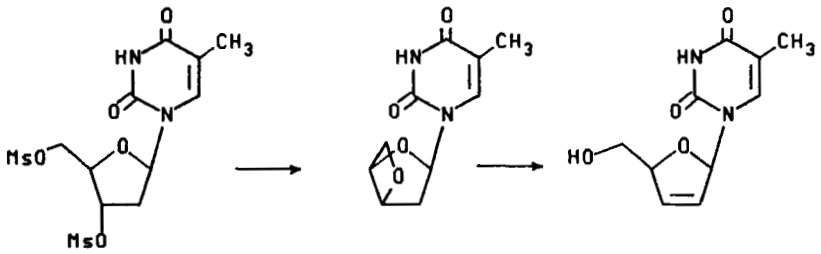

1 2 D4T

The anti-HIV and cytotoxic activities of D4T were compared to AZT. The two substances showed comparable potency against HIV 1 (LAV strain) as indicated by the reduction of p24 antigen in HIV infected CEM cells. D4T was only slightly less toxic than AZT to the CEM cells. However, a major difference was seen when the toxicities of D4T and AZT were assayed in human hemopoietic progenitor cells. This assay is thought to provide some predictability of the potential of a nucleoside analogue to induce neutropenia and anemia in the clinic[8]. D4T was 100 fold less toxic to granulocyte-monocyte precursors (CFU-GM) and slightly less toxic to erythroid precursors (BFU-E) (Table 1).

Because of the lack of a predictive animal model for AIDS, biochemical studies can provide important indications for possible clinical efficacy. The mechanism of action of nucleoside analogues against HIV involves enzymatic phosphorylation of the analogue to a triphosphate species which then inhibits the virus enzyme reverse

TABLE 1. ANTI-HIV ACTIVITY AND CELLULAR TOXICITIES OF D4T AND AZT

	ID_{50}, uM			
	HIV	CEM	CFU-GM	BFU-E
D4T	0.15	90	100	10
AZT	0.10	29	1	6.7

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transcriptase (RT) and thus virus replication. Inhibition of RT by the triphosphates of D4T and AZT was assayed using poly(rA):oligo(dT) as the RNA template/primer pair. The results demonstrated that the triphosphates of D4T and AZT are potent inhibitors of the enzyme with K_i's of 0.032 and 0.007 uM, respectively. These affinities are high relative to the K_m of 5 uM for thymidine triphosphate.

A study of the phosphorylation of ^{3}H-D4T in CEM cells showed that, unlike AZT,[9] the monophosphate of D4T does not accumulate. Instead, the monophosphate is efficiently metabolized to D4T triphosphate. After removal of D4T from the medium, the concentration of the triphosphate form of D4T diminished with a long half-life of 190 minutes. The persistence of the triphosphate may justify dosing only twice a day in the clinic.

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