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Enzymatic Synthesis of 3'-Azido-3'-Deoxy-5'-O-(1,2-Dihexadecanoyl-3-sn-Phosphatidyl)thymidine Involving Commercially Available Phospholipase D Type VII.

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In 1987 Shuto and co-workers[1] described the facile synthesis of 5'-phosphatidylnucleosides. The authors demonstrated that phospholipase D from Streptomyces sp. AA 586 effectively catalyzes the transfer of the phosphatidyl residue from phosphatidylcholine to the 5'-hydroxyl group of a nucleoside. In 1990 Hostetler and co-workers[2] described the chemical synthesis of phospholipid analogs of 3'-azido-3'-deoxythymidine and other antiviral nucleosides. In order to explore the utility of liponucleotide prodrugs, we elected to explore the enzymatic synthesis of these compounds. Several commercially available Phospholipase D enzymes were tested as potential catalysts. The results of these studies will be presented.

1) Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Matsuda, A; Ueda, T. *Tetrahedron Lett.* **1987**, 28, 199.
2) Hostetler, K. Y.; Stuhmiller, L. M.; Lenting, H. B. M.; van den Bosch, H.; Richman, D. D. *J. Biol. Chem.* **1990**, 265, 6112.

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5-Halo-6-alkoxy(or azido)-5,6-dihydro-3'-azido-3'-deoxythymidine Diastereomers as Potential Prodrugs of 3'-Azido-3'-deoxythymidine (AZT). E. E. Knaus*[1], L. I. Wiebe[1], L. Wang[1], R. Kumar[1], K. W. Morin[1], K. G. Todd[2] and G. B. Baker[2]. [1]Faculty of Pharmacy and [2]Department of Psychiatry, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

The title 5,6-dihydro analogs of AZT were designed to enhance the duration of action, lipophilicity and cephalic delivery to the CNS. These 5,6-dihydro AZT analogs (5R,6R; 5S,6S; 5S,6R; 5R,6S), which were synthesized by the regiospecific addition of XR (X = Br, Cl, I; R = alkoxy, azido) to the 5,6-olefinic bond of AZT, are more lipophilic (P = 3.3-18.8 range) than AZT (P = 1.29). Regeneration of the 5,6-olefinic bond to give AZT, upon incubation with glutathione, mouse blood or liver homogenate, was dependent upon the nature of the 5-halo substituent (I > Br >> Cl). The 5-X-6-OMe-5,6-dihydro AZT diastereomers (5R,6R; 5S,6S) had a higher affinity (K_i = 0.2-0.5 mM) for the NBMPR nucleoside erythrocyte transporter relative to AZT (K_i=1.3 mM). The amounts of radioactivity in mouse brain after iv injection of [2-^{14}C]-labelled (5R,6R)- or (5S,6S)-5-Br-6-OMe, or (5R,6R)-5-Br-6-OEt, 5,6-dihydro analogs of AZT were 2-4 fold higher than for AZT ($P < 0.05$). The radioactivity remaining in blood after dosing with these 5-Br-6-alkoxy-5,6-dihydro analogs of AZT was up to 20-fold higher than after injection of [2-^{14}C]-AZT at longer time intervals post-injection. Radioactivity present in bone following injection of [2-^{14}C]-AZT, or these 5-bromo-6-alkoxy-5,6-dihydro analogs of AZT were similar. Subcellular and regional distributions of [2-^{14}C]-labelled AZT, (5R,6R)-5-Br-6-OMe-5,6-dihydro-AZT or (5R,6R)-5-Br-6-ethoxy-5,6-dihydro-AZT in mouse brain did not show preferential concentration in any particular subcellular fraction, nor a marked preferential localization for either AZT or these 5,6-dihydro prodrugs of AZT. *In vitro* anti-HIV structure-activity correlations (CEM cells) will be described.