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Synthesis of 1-(2-Indolyl)pyridinium Salts: A Prodrug Approach to Acetylcholinesterase Inhibition

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Abstract: 1-(2-Indolyl)pyridinium bromide 2 has been synthesized as a potential acetylcholinesterase inhibitor suitable for administration in the form of a dihydropyridine prodrug. The synthesis of salt 3 is also described.

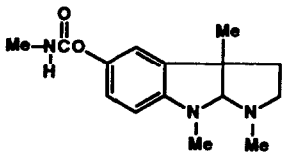
Although the cause of Alzheimer's Disease is unknown, much evidence indicates that dysfunction in transmission of the neurotransmitter acetylcholine (AcCh) contributes to the disease pathology and dementia.[1-3] This suggests that improvements in cholinergic function might represent a rational treatment approach. One way in which this might be accomplished is through inhibition of the enzyme acetylcholinesterase (AcChE), the enzyme responsible for AcCh hydrolysis and inactivation.[3]

The most well studied AcChE inhibitor is physostigmine (Phy, 1). Memory improvement has been documented in a number of clinical studies with Phy;[4] however, it can safely be said that improvements are subtle with little improvement in quality of patient life.[3] Phy is also plagued by a number of problems including high toxicity, adverse side effects and a narrow therapeutic window.[5,6] These problems may be related to a lack of specific delivery of the drug to the brain. Phy is a tertiary amine with a pK_a of 7.9,[7] meaning that at physiological pH, Phy exists as approximately a 3:1 ratio of charged to uncharged drug. Thus, although there is sufficient uncharged material available for penetration of the blood brain barrier (BBB), following administration, a good deal of the drug, as the charged form, remains in the periphery, leading to peripheral adverse effects. Further complicating the issue is the fact that the form of Phy responsible for enzyme inhibition is the charged form.[8,9] Therefore, the use of Phy presents a basic dilemma in that the form of drug needed for optimum and specific BBB penetration is not the same as the form required for biological action. A final problem is the drug's short half-life.[6] Phy rapidly undergoes metabolism in peripheral tissues,[3] presumably via decarbamylation or N(1) demethylation processes.[10]

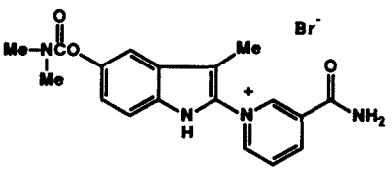
We felt it might be possible to design a new AcChE inhibitor that would circumvent the problems associated with Phy use by applying key concepts from the Chemical Delivery System designed by Bodor.[11] In this system, a drug which penetrates the BBB poorly is chemically coupled to a

dihydropyridine carrier. Administration of this drug-carrier molecule leads to significant crossing of the BBB due to the lipophilic nature of the dihydropyridine ring. *In vivo* oxidation provides the pyridinium salt in both the brain and periphery. Peripheral salt is excreted due to its ionic hydrophilic nature; however, the charge of the pyridinium molecule leads to a "lock-in" effect in the brain, preventing it from exiting into the periphery. Slow cleavage of the drug-carrier molecule leads to a sustained release of the drug in the brain. Bodor has successfully utilized this strategy in the specific brain delivery of many agents including phenethylamine,[11a] dopamine,[11b] testosterone,[11c] and estradiol.[11d]

An application of the overall Bodor strategy could be utilized in the design of Phy analogs such as 2 which incorporate a pyridinium moiety directly into the inhibitor structure. The location of the charged nitrogen and the carbamate would mimic that of Phy and thus serve to enhance binding to the enzyme. In this way, the drug could be administered in the form of a dihydropyridine prodrug, which would be stabilized by the presence of the electron withdrawing group on the pyridine ring.[12] Administration of this moiety may be expected to lead to extensive BBB penetration. *In vivo* oxidation would then lead to generation of the charged pyridinium species, ideally suited for enzyme inhibition. This method might allow for selective delivery of the AcChE inhibitor to the brain since charged inhibitor formed in the periphery would be readily excreted while charged inhibitor formed in the brain would not be expected to easily leave the central nervous system. This approach differs fundamentally from the Bodor Chemical Delivery System which utilizes the pyridinium ring only for delivery purposes. Additionally, one would expect structures such as 2 to be inherently more stable than that of Phy. It has been shown that N,N-dimethylcarbamates are more stable than N-methyl carbamates (such as in Phy) under physiological conditions.[13] Moreover, decarbamylation metabolism should be slower due to this modification and N(1) demethylation is no longer an issue due to the pyridine ring introduction in the target molecule. Herein, we describe the synthesis of proposed Ache inhibitor 2, as well as the synthesis of pyridinium salt 3. We also document the preparation of indolyldihydropyridine 4.



1



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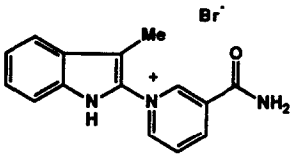

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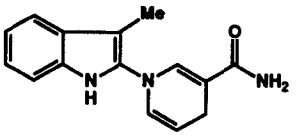
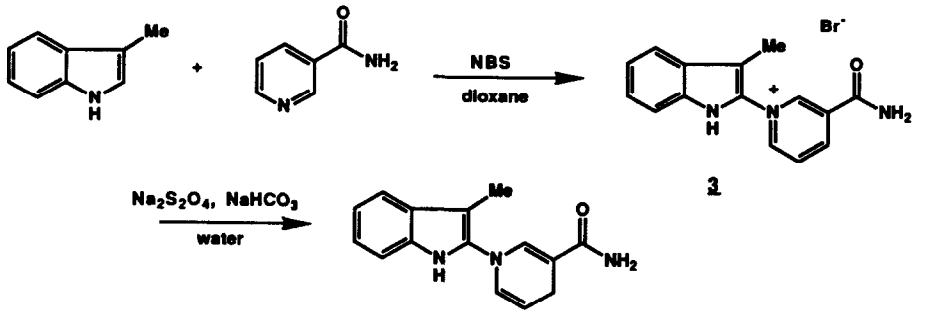


4

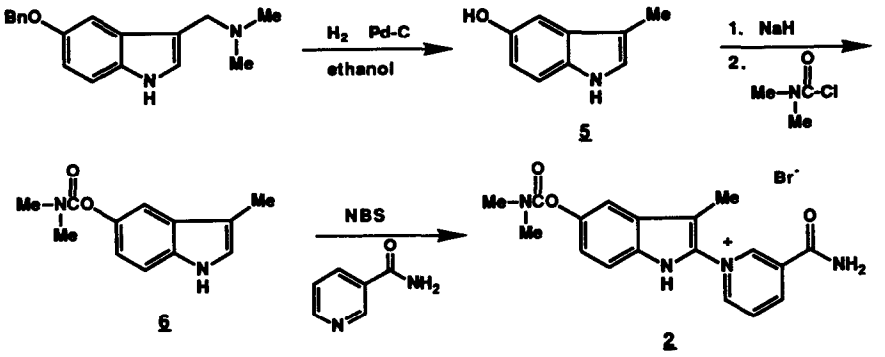
Hino[14] has demonstrated that treatment of 3-methylindole with N-bromosuccinimide (NBS) in pyridine-dioxane led to pyridine addition at the two position of the indole ring. Thus we chose to initially investigate the feasibility of a similar nicotinamide addition process. Treatment of 3-methylindole with NBS (1 equivalent) and nicotinamide (1.35 equivalents) in dioxane (24 hr., r.t.) led to formation of indolylpyridinium salt 3[15] in 51% recrystallized yield (Scheme 1). Bromination at C-3, followed by nicotinamide attack on the resulting iminium ion with subsequent loss of HBr, is the proposed mechanistic sequence. Reduction with sodium dithionite and sodium bicarbonate in water[11c] afforded dihydropyridine 4 in 57% yield,[15,16] confirming the potential for prodrug synthesis.

SCHEME 1



The desired Phy pyridinium analog 2 was synthesized in three steps as indicated in Scheme 2. Catalytic hydrogenolysis of commercially-available 5-benzyloxygramine (H_2, 10% Pd-C, ethanol, 48 hr., r.t., atmospheric pressure) afforded 5-hydroxy-3-methylindole 5 in 76% yield. Silica gel flash chromatography of the filtered and concentrated reaction mixture resulted in a significantly higher yield than that previously reported for this reaction.[17] Phenolic deprotonation with one equivalent of sodium hydride (acetonitrile, 20 minutes, 0°C), followed by treatment with dimethylcarbamyl chloride (3 hr., 0°C) afforded carbamate 6 in 78% yield following silica gel flash chromatography (2:1, hexane:EtOAc). Finally, exposure of 6 to the conditions developed in the synthesis of 3, followed by recrystallization from ethanol, afforded yellow 2 in 60% yield.[15]

SCHEME 2



Indolylpyridinium salt 2 was examined for AcChE inhibition activity[18] using commercial electric eel enzyme. In this system, 2 was found to have an IC_{50} value of 5×10^{-5} M. As a control, Phy was found to demonstrate an IC_{50} value of 1×10^{-9} M. This low degree of potency relative to Phy would preclude further prodrug development of this compound. The design, synthesis and evaluation of a more potent pyridinium-based Ache inhibitor will be the subject of future reports.

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