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CHIRAL, PIPERIDINE-BASED ANALOGUES OF AF64A AND ACETYLCHOLINE

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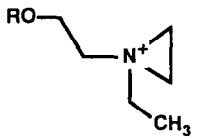
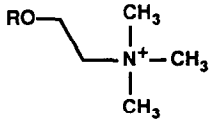
Abstract. Chiral analogues of acetylcholine and AF64A were prepared from *l*-glutamic acid via the central intermediate, (S)-3-acetoxypiperidine.

Ethylcholine mustard aziridinium ion (**1**; AF64A, MEChMAz) is a selective, irreversible inhibitor of high-affinity choline transport (HAChT),[1] choline acetyltransferase,[2] and choline dehydrogenase.[3] The *in vivo* administration of AF64A causes persistent central cholinergic hypofunction and can produce reductions in presynaptic cholinergic markers like acetylcholine (**2a**; ACh). As a cholinergic neuronspecific neurotoxicant, AF64A has been proposed to simulate the condition believed to exist in Alzheimer's disease.[4] Since AF64A is an analogue of choline (**2**) and contains a highly reactive aziridinium ion, this agent's mechanism of action proceeds through formation of a covalent bond with a nucleophilic moiety in the active site of the target enzyme.[5]



1 (R=H): AF64A
1a (R=Ac): Ac-AF64A

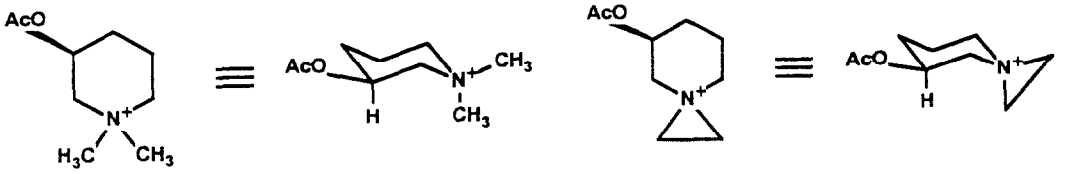


2 (R=H): choline
2a (R=Ac): acetylcholine

Two important features of **2/2a** render them insufficient probes of biomolecules; they bear no center of asymmetry and are capable of rapid conformational interconversion. To evaluate these aspects prior researchers investigated the required pharmacophoric conformation of ACh for the muscarinic receptor. For example, the anti conformation of *trans*-decalin stereoisomers exhibited the greatest receptor activity.[6] *Trans*- and *cis*-1-acetoxy-2-trimethylammonio-cyclopropanes were prepared and tested as cholinomimetics. The (+)-trans isomer was found to be equal to choline in activity whereas the (-)-trans isomer and racemic cis-isomer were much less active.[7] The *erythro*-isomer of 2,3-dimethylacetylcholine was approximately 400-times more potent than the *threo* isomer when compared to ACh potency.[8] The modest activity for the threo isomer was anticipated based upon the decalin study [6] but the erythro activity (14-fold of ACh) is surprising. These studies not only suggest that ACh binds in the anti-conformation but that the role of stereochemistry strongly affects the binding phenomena.

We sought to more precisely inspect the configurational requirements of cholinomimetics

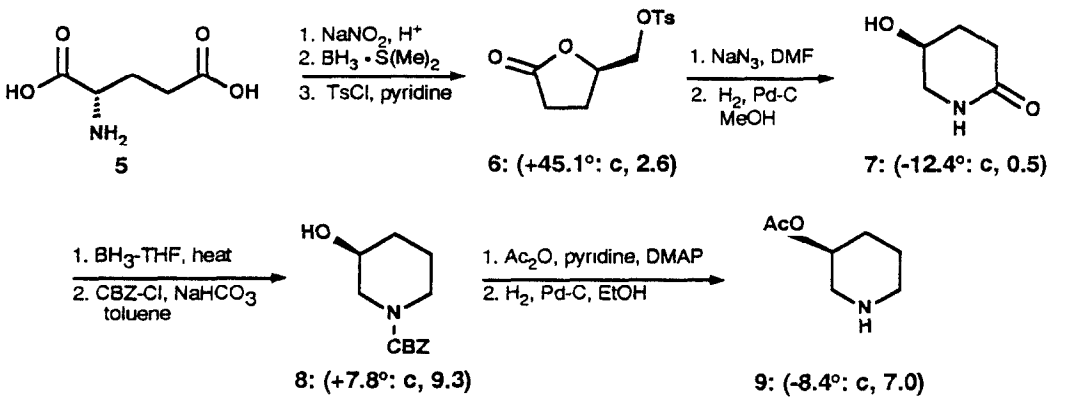
without dramatically altering the steric component. Unfortunately, the alkylating probes **1**/**1a** also suffer from an inability to discriminate active or receptor site topologies. This goal could possibly be achieved by combining the aforementioned informative leads[6-8] and the known utility of **1**/**1a** in the construction of chiral, piperidine-based analogues. Compounds **3** and **4** bear the requisite quaternary ethanolamine linkage in an anti-conformation and, by design, have the (S)-configuration installed. Therefore, [acetyl]choline analog (**3**) and AF64A analog (**4**) were chosen as our primary synthetic targets.



3 **4**

The sequence to chiral piperidone (**7**) (envisioned as a precursor to **3** and **4**) followed a combination of previously reported procedures[9-14] and newer modifications that are outlined in Scheme 1. Diazotization of *l*-glutamic acid (**5**)[9-11] affords (S)-butyrolactone carboxylic acid that is converted into (S)-(+)-tosyloxymethyl-γ-butyrolactone (**6**) following borane-dimethylsulfide reduction[11] and tosylation[15] in 37% overall yield. Displacement of the tosyl moiety with N_3^- and catalytic hydrogenation over 10% Pd resulted in rearrangement to (S)-5-hydroxy-piperidin-2-one (**7**) in 80% yield for the two steps.[14]

Scheme I



5 **6: (+45.1°: c, 2.6)** **7: (-12.4°: c, 0.5)**

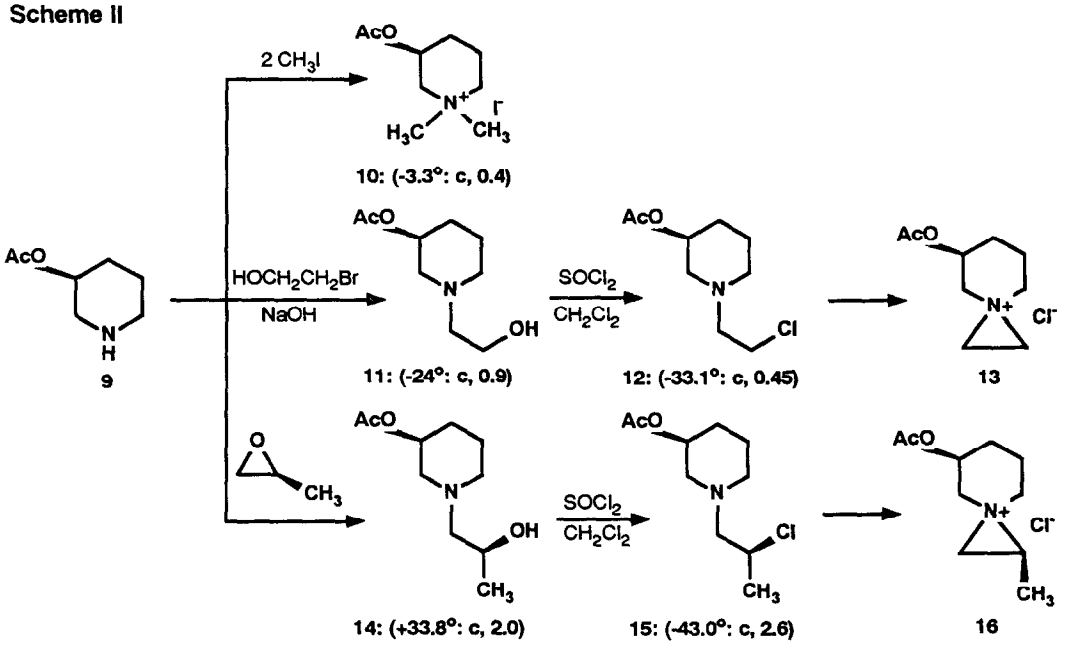
8: (+7.8°: c, 9.3) **9: (-8.4°: c, 7.0)**

Prior reports[16] indicated the use of $LiAlH_4$ for the reduction of the lactam to form (S)-3-hydroxy piperidine. However, we were able to achieve only 30-40% yields by this process. Owing to the volatile nature and water solubility of the piperidinol, much material was lost in the purification. We examined alternative reducing agents and found that borane-THF complex gave the desired product in 60-70% yield, which provided the product in high enough purity to

proceed directly to the next step. Yet, direct acetylation using acetic anhydride in acetic acid did not furnish 3-acetoxy piperidine, rather, acetylation at nitrogen occurred. Therefore, immediately following reduction of **7** by BH_3-THF, the crude piperidinol was converted to the CBZ-derivative (**8**) in 75% yield.[17] Dimethylaminopyridine (DMAP)-promoted acetylation of **8**, followed by hydrogenolysis gave (S)-3-acetoxypiperidine (**9**) in 74% yield for the two steps.

From key intermediate **9**, the synthesis of the target analogues was next undertaken. Reaction of **9** with 2 equiv. of methyl iodide in dry methanol deposited (S)-3-acetoxy-N,N-dimethylpiperidinium iodide (**10**) as needles in quantitative conversion (Scheme II).

Scheme II



Preparation of the spirocyclic aziridinium AF64A analog (**13**) was not readily accomplished. Several attempts were made to install a N-2-substituted ethane moiety that would serve as a cyclization precursor. Included in this investigation were reaction with ethylene oxide, ethylene carbonate, 1-bromo-2-chloroethane, 1-bromo-2-chloroethane with catalytic NaI, 1,2-dibromoethane, and bromoethanol. Reaction of **9** with one equivalent of dihalide reagent led only to small amounts of product, the majority of reaction expectedly leading to dialkylation and dimerization. Based on these failures, conversion to the ethanolamine precursor (**11**) was pursued. Ethylene carbonate failed to react with **9** even after forcing conditions. Finally, mono-alkylation with ethylene oxide (formed *in situ* from bromoethanol and NaOH) at low temperature gave **11** in 38% yield although dialkylation was a competing reaction. (S)-3-Acetoxy-N-(2-hydroxyethyl)piperidine (**11**) was reacted with neat thionyl chloride to afford the β-chloroethylamine hydrochloride salt, which was free-based to give derivative **12** in 70% yield

following flash chromatography. Upon storage, compound **12** cyclized to form the aziridinium (**13**) and dimer in equal quantities as determined by NMR integration. Treatment of **12** with $AgClO_4$[18] to induce aziridinium formation was inconclusive by ^{1}H-NMR. Since alignment of AF64A in the receptor site is expected to be configurationally biased prior to reaction with a nucleophile, it is expected that this Sn_2 reaction would be influenced by substituents on the aziridinium ring. To study this feature, we also sought to prepare an analog that bears substituents of known configuration at both acetoxy and reactive aziridinium centers. In this instance, a nucleophilic moiety on the biomolecule would be expected to attack the methylene center opposite to the juxtaposed substituent. Thus, compound **9** was reacted with (S)-propylene oxide to afford (S)-3-acetoxy-N-[(S)-2-hydroxypropyl]piperidine (**14**) in 48% yield. Reaction with thionyl chloride gave **15** with retention of configuration in 70% yield following crystallization. The identical sequence with (R)-propylene oxide also was conducted.

Racemic materials also have been synthesized. We currently are preparing the 3-(R)-isomers of compounds **10**, **13** and **16** and are investigating the interaction of these novel analogues with biomolecules.

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