Total Synthesis of (±)-Epibatidine Using a Biocatalytic Approach

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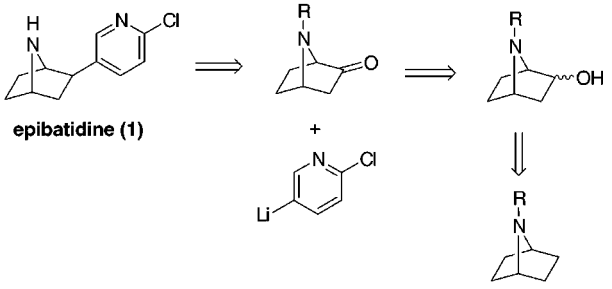
Epibatidine (**1**) is a natural product that was isolated from the skin of the Ecuadorian poison frog *Epipedobates tricolor* in trace amounts (less than 1 mg from 700 frogs).[1] This alkaloid has attracted a lot of attention because it showed remarkable analgesic activity (200−500 times more potent than morphine) and displayed a very distinct mode of action.[2] Interestingly, both optical isomers displayed similar activity.[3] Since its structural elucidation by Daly in 1992, a surprisingly large number of syntheses have appeared in the literature.[4] However, no total synthesis using a biocatalytic approach has yet been described.[5]



Microbial oxidation of unfunctionalized carbons can be a powerful tool for providing hydroxylated molecules that otherwise might not be easily accessible. We envisioned that an appropriately N-substituted 7-azanorbornane derivative would be a good substrate for oxidation of an unfunctionalized carbon and that the metabolite would make a valuable intermediate for a total synthesis of epibatidine. Oxidation of N-substituted 7-azanorbornanes with the fungus *Beauveria bassiana* has been studied independently by Johnson's group and ourselves.[5] We recently reported that easily prepared 7-azanorbornanes carrying an appropriate N-substituent are microbially oxidized stereoselectively on an unfunctionalized methylene carbon.[5,6] In this note, we communicate our total synthesis of epibatidine using a selected metabolite generated from this biotransformation.

Results and Discussion

We selected the *N*-benzoyl group as the anchoring/directing group in the microbial oxidation of 7-azanorbornane because of the reproducibility of the biotransformation experiments, good yield, and minimal production of side products.[5b] The desired *N*-benzoyl-7-azanorbornane (**2a**) was easily prepared in three steps from commercially available *trans*-4-aminocyclohexanol hydrochloride (Scheme 1). Microbial hydroxylation of substrate **2a** utilizing *B. bassiana* furnished stereoselectively 2-*endo*-hydroxy-7-azanorbornane (**3a**) in 56% yield and 22% ee.[5b] Optical rotation experiments showed that the slightly favored enantiomer generated in the microbial transformation was (−)-(*1R*). NMR spectra of substrate **2a** and metabolite **3a** showed the presence of a mixture of rotamers. Thus, we reduced the benzamides **2a** and **3a** to their corresponding benzylic amines with lithium aluminum hydride to facilitate their structural assignment. ^{1}H- and ^{13}C NMR spectra of the benzylic amine **3b** were identical to those reported by Fletcher.[7] We have also studied the effect of several phosphorus-containing N-substituents on the microbial hydroxylation of 7-azanorbornanes.[6] We and Johnson's group have found that *B. bassiana* is able to accept a variety of functionalities on the heteroatom of the substrate.[5,6] We found that good reproducible yields are obtained when the N-substituent is a benzoyl group and therefore selected the corresponding metabolite as the intermediate to complete a synthesis of epibatidine. We might attribute the low enantioselectivity of the microbial oxidation to the hindered rotation of the *N*-benzoyl group, which was improved when utilizing phosphorus-containing N-substituents albeit in slightly lower isolated yields.[6]

Thus, oxidation of microbially hydroxylated 7-azanorbornane **3a** with TPAP−NMO gave ketone **4** (Scheme 2). Transmetalation of 2-chloro-5-iodopyridine[8] with *n*-butyllithium and addition to ketone **4** at − 78 °C yielded exclusively *endo*-alcohol **5**. Deoxygenation of tertiary alcohol **5** was carried out successfully in two steps following a modification of the Dolan−MacMillan protocol.[9] Alcohol **5** was esterified to the mixed anhydride **6** in quantitative yield. Methyl oxalate **6** was treated with tributyltin hydride to furnish exclusively *endo*-chloropyridyl isomer **7a**. Equilibration to a mixture of separable

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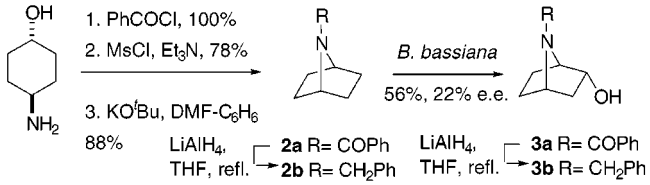
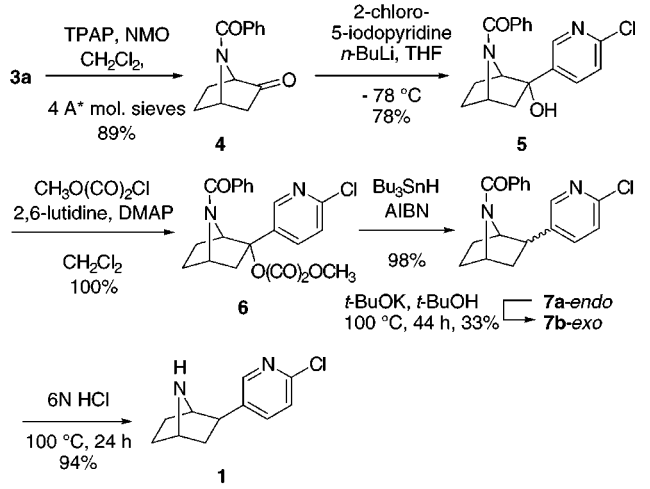
Scheme 1. Preparation of Substrate and Microbial Transformation



Scheme 2. Synthesis of Epibatidine from Hydroxylated Metabolite



endo- and *exo*-isomers was possible when the *endo*-isomer **7a** was subjected to 6 equiv of potassium *tert*-butoxide in *tert*-butyl alcohol at 100 °C in a sealed tube.[9a] Removal of the *N*-benzoyl group was achieved using acid hydrolysis to afford epibatidine (**1**).[10]

In summary, we have synthesized epibatidine in 10 steps from commercially available *trans*-4-aminocyclohexanol. The three key steps involve an intramolecular nucleophilic displacement of a *trans*-4-aminocyclohexanol derivative, microbial oxidation of an unfunctionalized carbon, and coupling of the chloropyridyl ring to a 7-azanorbornanone. We have shown how biocatalysis can be a useful tool to provide access to molecules that otherwise may require lengthier synthetic sequences. This work demonstrates how preparative biotransformations can be successfully blended with modern synthetic organic chemistry to prepare a molecule of great biological interest.

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Supporting Information Available: Experimental procedures for the preparation of compounds **2a**−**7b** and ^{1}H and ^{13}C NMR spectra for compounds **1**−**7b**. This material is available free of charge from the Internet at http://pubs.acs.org.

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