Studies Directed Toward the Total Synthesis of Kobusine

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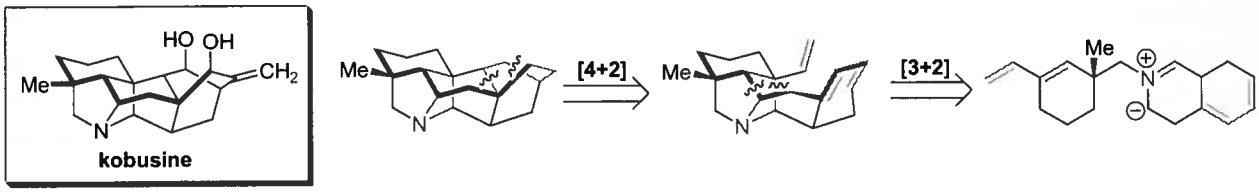
The genera of *Aconitum* (commonly known as Monkshood) and *Delphinium* have long been recognized as a rich source of alkaloid natural products. Among these, kobusine and related hetisine alkaloids are particularly interesting due to their biological activity and their highly complex carbon skeleton, thus making them attractive synthetic targets. Our synthetic strategy utilizes an early stage intramolecular dipolar [3+2] cycloaddition followed by a late stage [4+2] cycloaddition as the key steps of the synthesis. Current work is focusing on examination of the proposed dipolar [3+2] cycloaddition.



Evolutionary Potential of $(\beta/\alpha)_8$-Barrels: Enhancing the Functional Promiscuity of the Enolase Superfamily

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The enolase superfamily is a mechanistically diverse group of homologous enzymes that share a catalytically responsible $(\beta/\alpha)_8$-barrel, a common enolate anion intermediate, and an N-terminal capping domain responsible for substrate binding. The superfamily is further characterized by superimposable three-dimensional structures and three subgroups that have conserved catalytic residues, but low overall sequence identity. Two muconate lactonizing subgroup members from *Ecsherichia coli,* L-Ala-D/L-Glu Epimerase (AEE) and *ortho*-succinylbenzoate synthase (OSBS), have identical catalytic residues. The AEE scaffold, with a single D297G mutation, can complement an anaerobic auxotroph that has had its OSBS removed while exhibiting decreased AEE activity. Although D297G has measurable OSBS activity, it remained almost 10^6 fold slower than natural OSBSs. In order to better understand how new functions evolve, D297G was used as the template for error-prone PCR. Anaerobic selection was used, and a 2nd mutant (D297G/I19F) was identified that further enhanced the OSBS activity, but with a further loss of AEE activity. The I19F mutation is located in the N-terminal capping domain. Randomization of I19 identified multiple additional amino acids that demonstrate increased OSBS activity in comparison to D297G alone. These mutants resulted in a further drop in the original AEE activity due to the removal of the Glu297, which is responsible for stabilizing the dipeptides within the active site. Randomization of alternative positions within the active site from the N-terminal domain have identified other mutations that enhance the OSBS activity of AEE D297G, including R21Y, R21S and Y48F. Future investigations will include generating additional error-prone libraries using AEE D297G/I19X mutants and the directed randomization of multiple sites at once.