Bacterial steroid modifying/degrading enzymes are attractive as alternatives to traditional chemical reagents for catalyzing steroid drug synthesis. From gene knockout studies in Proteobacteria, two enzymes, Sal (Steroid aldolase) and Sad (Steroid aldehyde dehydrogenase) were shown to be involved in the degradation of the 5-carbon side-chain of cholic acid. Sal is homologous to Ltp2, a previously characterized aldolase involved in degradation of 3-carbon side-chains of steroids. Ltp2 associates with a hydratase, which catalyzes the preceding reaction in the pathway, through an interaction with a DUF35 domain of the hydratase (DUF stands for domain of unknown function). Interestingly, sal is upstream of a gene, shy (Steroid hydratase), that also encodes a hydratase containing a DUF35 domain. I hypothesize that Sal associates with the DUF35 domain of Shy. However, the catalytic residues identified in Ltp2 are not conserved in Sal. By site-specific mutagenesis and 3D-structure determination of Sal, residues involved in catalyzing the retro-aldol reaction will be identified. Similar structure-function studies of Sad will be performed to determine how this unique aldehyde dehydrogenase binds to bulky steroid substrates for catalysis. The results from this work will facilitate future application of the enzymes as biocatalysts to produce novel steroids with variable side chain length or structure that may have useful pharmacological properties.