484d Solid-Phase Combinatorial Biocatalysis of the Natural Product Bergenin

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The natural flavonoid bergenin has been employed as a model substrate to evaluate the feasibility of solid phase combinatorial biocatalysis as a complement to combinatorial chemistry and rational drug design methods for the optimization of pharmaceutical leads. Substrate immobilization efforts thus far have focused on optimizing the choices of both the support and immobilization chemistry. After performing a series of preliminary immobilization chemistries, 2 types of supports - controlled-pore glass (CPG) and monomethoxy-polyethelene glycol (MPEG) polymer – and 2 reaction chemistries – primary hydroxyl coupling to an activated-carboxylic acid support (R-COOH) and vinyl ester coupling to a primary amine support (R-NH2) – were chosen. Removal of the immobilized substrate has been accomplished both chemically and with the use of enzyme-cleavable linkers. In addition, preliminary reaction kinetic measurements have been compared for the halogention of bergenin in both in the solid-phase as well as in solution. A similar comparison for bergenin acylation is currently underway. In parallel to immobilizing bergenin onto various supports, a series of enzymes have been solubilized for use in solid-phase organic reactions, as enzymes are typically insoluble in such systems. In particular a new method for solubilizing enzymes, termed “direct solubilization,” has been developed. This method differs from the previously employed extraction method in that a single phase is used for the solubilization instead of a bi-phasic extraction. Solubilization efficiencies for this method are far greater than those for the extraction method for several enzymes tested. More important, however, is the dramatic increase in reaction conversions observed for the directly solubilized enzyme compared to the extracted enzyme, especially in non-polar and highly polar solvents for subtilisin carlsberg. Current and future work in this area will focus on structurally characterizing directly-solubilized enzymes, in hopes of elucidating the mechanisms for the pronounced increases observed in both solubilization efficiency and resulting enzyme activity.