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Overcoming Mass Transfer Limitations in hCAII-Catalyzed Bioreduction of Bulky Ketones via Pulsed Phenylsilane Addition

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Human carbonic anhydrase II (hCAII) facilitates the abiotic, enantioselective reduction of ketones into chiral alcohols, utilizing phenylsilane as a hydride donor. While the conventional protocol—employing three equivalents of phenylsilane in a 30% (v/v%) DMSO:water mixture—yields excellent performance for soluble model substrates, it is largely ineffective for the bulky, poorly water-soluble ketones typically required for fine chemical synthesis. We have identified a fundamental kinetic bottleneck: the slow mass transfer of hydrophobic substrates into the hCAII active site occurs at a rate that cannot compete with the inherent instability of phenylsilane, which is prone to degradation and volatilization. At low concentrations, this kinetic mismatch is intensified, resulting in poor conversions even when excess reagents are provided.

Our methodology—utilizing pulsed phenylsilane addition—addresses this by maintaining a consistently high concentration of the hydride donor throughout the prolonged substrate ingress phase. When applied within NMP:water or DMSO:water solvent systems, this strategy yielded significantly higher conversions for 2-acetylnaphthalene compared to traditional single-dose methods. Observations using 4-chloroacetophenone as a model substrate provided a critical breakthrough: the success of the reaction is governed by the absolute concentration of phenylsilane rather than the stoichiometric ratio. Low concentrations proved unsuccessful regardless of excess equivalents, whereas high, sustained concentrations achieved success using only two total equivalents.

Detailed extraction analysis further revealed that the precipitation of substrates and products during the workup phase often skews apparent conversion data, reinforcing the conclusion that mass transfer is the primary rate-limiting factor. By decoupling substrate solvation from hydride availability, pulsed delivery allows for industrially viable concentrations (>10 mM) even with challenging, poorly soluble ketones. Chiral chromatography confirms that these gains in efficiency do not compromise high enantioselectivity. Ultimately, this concentration-driven approach bypasses the constraints of fixed-equivalent protocols, establishing hCAII as a robust and practical biocatalyst for the synthesis of chiral alcohols from demanding substrates.

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