Letter

Pyridoxal-Catalyzed Racemization of #-Aminoketones Enables the Stereodivergent Synthesis of 1,2-Amino Alcohols Using Ketoreductases

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Pyridoxal-Catalyzed Racemization of α-Aminoketones Enables the Stereodivergent Synthesis of 1,2-Amino Alcohols Using Ketoreductases

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ABSTRACT: Differentially substituted 1,2-amino alcohols are a prevalent motif in a variety of pharmaceutical and agrochemical molecules. Dynamic kinetic resolutions (DKRs) that involve the asymmetric reduction of α-amino ketones are an attractive strategy for preparing this motif, however, methods for racemizing the stereogenic α-carbon under mild conditions are underdeveloped. Here we report a chemoenzymatic DKR involving ketoreductases (KREDs), where pyridoxal-5-phosphate (PLP) is used to catalyze racemization of the starting racemic α-aminoketone. This strategy enables access to a variety of 1,2-amino alcohols with high levels of diastereo- and enantioselectivity. Using commercial available KREDs, all four possible stereoisomers can be accessed, highlighting a benefit to this approach.

Keywords: biocatalysis, dynamic kinetic resolution, racemization, pyridoxal, ketoreductase

Vicinal amino alcohols are ubiquitous in natural products and pharmaceutically important molecules.[1] The prevalence of this motif has spurred the development of catalytic methods for preparing 1,2-amino alcohols with high levels of stereoselectivity. Among these methods, the aminohydroxylation of alkenes and nucleophilic opening of epoxides and aziridines catalyzed by enzymes and small molecule catalysts are the most well developed. [2,3,4] While these methods are highly effective, they involve stereospecific reaction mechanisms, limiting their use to the synthesis of specific stereoisomers. Alternatively, asymmetric reductive amination. The asymmetric reduction of *α*-aminoketones is an attractive alternative to these approaches because it is not mechanistically restricted to providing certain stereochemical outcomes.[5] When coupled to a mechanism for *α*-aminoketone racemization,[6] stereoselective reduction catalysts can, in theory, be employed in to access all possible stereochemical outcomes from racemic starting materials.[7,8] To achieve this goal, however, catalysts scaffolds need to be identified that are capable of furnishing all possible stereoisomers while also tolerating under the basic conditions required for racemization.

Ketoreductases (KREDs) are ideal catalysts for stereoselective carbonyl reductions because of their substrate promiscuity and ability to be tuned, using directed evolution, to provide desired stereochemical outcomes.[9] Thanks to these features, collections of structurally diverse KREDs have been compiled for use in chemical synthesis.[10] These panels have been applied to a variety of synthetic scenarios,[11] including in dynamic kinetic resolutions. KREDs generally operate in a relatively narrow pH window (pH 6-8), consequently substrates for DKRs require acidic α-protons site with pKa's between 7-12 (Figure 1a).[12] As most α-aminoketones have less acidic α-protons, only substrates containing addition electron withdrawing groups can be racemized under these conditions.[13]

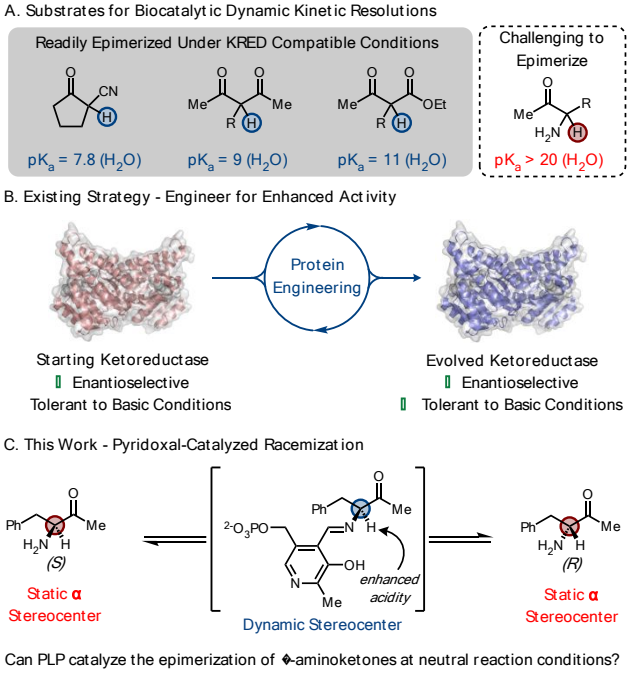


Figure 1. Biocatalytic Dynamic Kinetic Resolutions and Pyridoxal-5-Phosphate Catalysis (as a strategy for labializing the *α*-amino proton). A. Substrates for Biocatalytic Dynamic Kinetic Resolutions. B. Existing Protein Engineering Strategy. C. This Work using Pyridoxal-Catalyzed Racemization.

Efforts to overcome the pKa limitation have primarily focused on engineering KREDs to function under more basic conditions (Figure 1b). In their synthesis of Vibegron, Merck & Co. partnered with Codexis to demonstrate that a KRED could be evolved to operate at pH =10.0, enabling racemization of Boc-protected *α*-aminoarylketones.[14] We imagined that the need for protein engineering could be overcome by developing a strategy for racemizing *α*-

aminoketones at neutral pH. This would enable commercially available kits of structurally diverse KREDs to be rapidly deployed in DKRs to prepare 1,2-amino alcohols, thereby eliminating the need to engineer a base-tolerant KREDs.[15]

Amine activation via aldehyde catalysis is widely used in nature to facilitate amine metabolism. Nature's aldehyde of choice is pyridoxal-5-phosphate (PLP), a cofactor found in a host of enzyme classes,[16] including transaminase, tryptophan synthase, threonine aldolase, tyrosine decarboxylase, and alanine racemase.[17] In these enzymes, PLP forms a Schiff base with the amine enabling stabilization of negative charge generated at the α-position of the amine (Figure 1c).[18] It is calculated that PLP can increase the acidity of the α-position of glycine by 18 orders of magnitude.[19] Inspired by this profound effect, we questioned whether PLP could catalyze the racemization of α-aminoketones in the absence of protein at neutral pH. Previous reports demonstrate that PLP can mediate the racemization of α-aminoesters in 95% organic solvent.[20] In the presence of solvent stable hydrolases, a dynamic kinetic resolution is achieved to prepare enantioenriched amino acids which precipitated from solution to prevent further racemization.[21]We hypothesized that PLP should also be able catalyze the racemization of α-aminoketones under aqueous conditions. When run in the presence of KREDs, an enantio- and diastereoselective synthesis of 1,2-amino alcohols would be achieved, with the decreased acidity of the α-amino proton in the product precluding product epimerization.

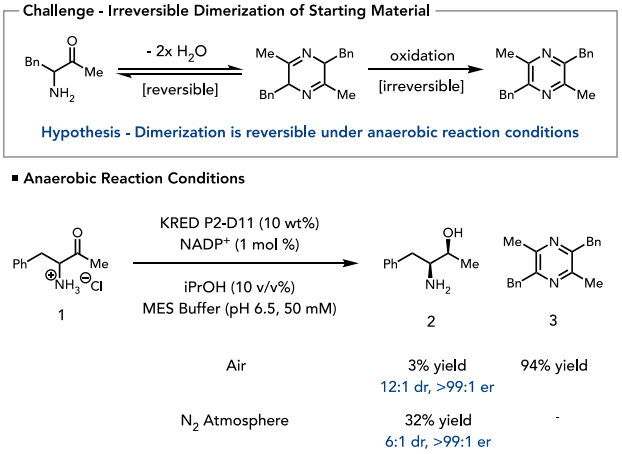


Figure 2. Pyrazine Formation and Dynamic Kinetic Resolution Development

At the outset, we recognized that a challenge inherent to the proposed reactivity was dimerization of the α-aminoketone to afford a dihydropyrazine, which upon oxidation would provide the pyrazine (Figure 2). We hypothesized that under aerobic reaction conditions, formation of the dihydropyrazine is reversible, while the oxidation to form the pyrazine is irreversible. Based on this understanding, we reasoned that anaerobic reaction conditions should diminish formation of the undesired pyrazine side product.[22] To test this hypothesis, we explored the reduction of phenylalanine-derived methyl ketone **1** with a panel of KREDs purchased from Codexis® under aerobic conditions and observed pyrazine **3** as the major product, with < 5% formation of the desired 1,2-amino alcohol **2** (Figure 2). When the same reaction was tested under anaerobic conditions, KRED P2-D11 provided the desired 1,2-amino alcohol **2** in 32% yield with 6:1 dr and >99:1 er, with the remaining mass balance being unreacted starting material (Figure 2).

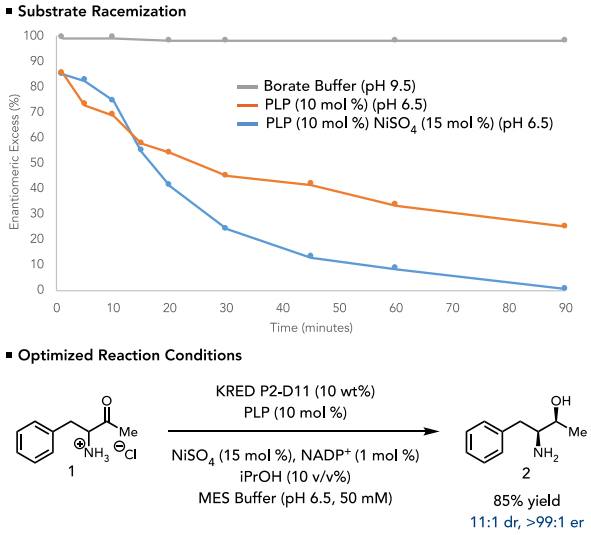
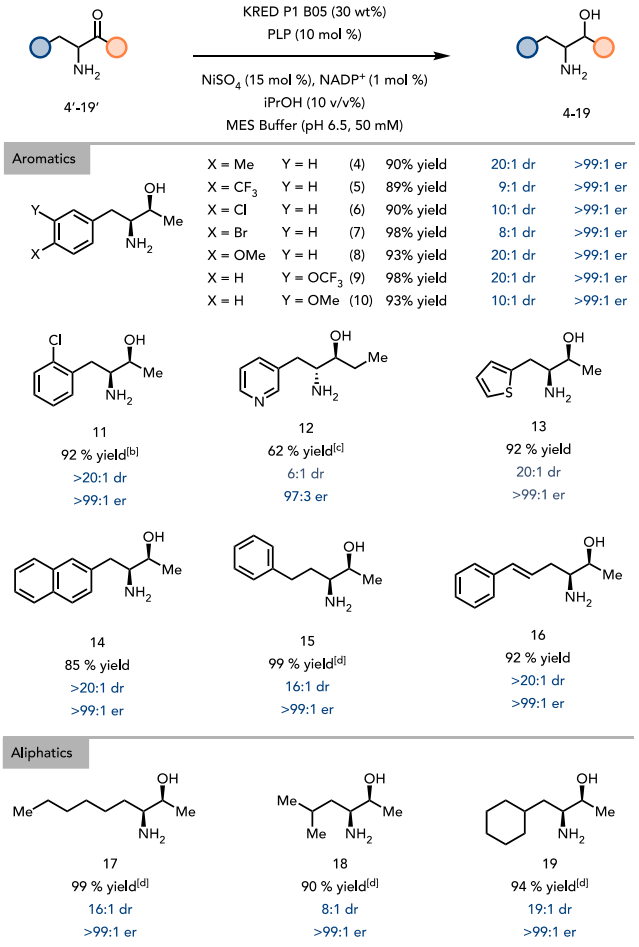


Figure 3. PLP-Catalyzed Substrate Racemization

With a promising enzyme in hand, we shifted our attention to developing conditions to racemize the α-aminoketone **1**. In preliminary experiments, we found that the starting material does not racemize in buffer at pH's ranging from 6.5-9.5 (Figure 3). Upon addition of PLP (10 mol %) to 2-(*N*-morpholino)ethanesulfonic acid buffer (MES) at pH 6.5, racemization of the starting material from 96% ee to 20% ee occurred within two hours (Figure 3). We recognized that enhanced rates of racemization could be necessary for more kinetically active KREDs. Snell and coworkers previously demonstrated that metal salts can enhance the rate of pyridoxal catalyzed α-aminoester racemization by forming stable metal chelates.[23] After testing a small collection of metal salt additives, we found that addition of catalytic $NiSO_4$ (15 mol %) enabled complete racemization within 90 minutes. With ideal racemization conditions in hand, we explored the impact these conditions have on the stereochemical integrity of amino alcohol **2**. We found that the product did not decompose or epimerize under the reaction conditions, presumably due to the insufficient acidity of the α-amino proton (Scheme S1). Having identified optimal conditions for substrate racemization, we tested the racemization conditions in the presence of ketoreductase P2-D11 and

achieved an effective dynamic kinetic resolution of **1** to afford 1,2-aminoalcohol **2** in 85% yield as an 11:1 dr and 99:1 er (Figure 3).

Table 1. Substrate Scope



[a] Reaction performed with α-aminoketone salt (0.03 mmol, 1 equiv), $NiSO_4$ (15 mol%), pyrodoxial 5'-phosphate (10 mol%), $NADP^+$ or NAD^+ (1 mol%), degassed isopropyl alcohol (10 v/v%) or GDH 105 with Glucose (6 equiv), ketoreducatse lysate (30~40 wt%) in degassed MES buffer (1.5 mL, 50 mM MES and 1.25 mM $MgCl_2$) under anaerobic enviroment at room temperature for 48 hr unless otherwise indicated. [b] Performed for 24 hr. [c] KRED P2 C02 (10 wt%). [d] KRED P2 D11 (10 wt%).

The scope and limitations of the transformation were tested on a variety of substrates (Table 1). Codexis® KRED P1-B05 proved most effective for phenylalanine-derived methyl ketones, with various functional groups appended to the aromatic ring being well-tolerated in this reaction. Electron donating groups provided slightly higher levels of diastereoselectivity by comparison to electron withdrawing substituents (Table 1, **4**-**10**). Differences in diastereoselectivity are due to either a change in the rate of racemization or variations in the rate and selectivity of ketone reduction. Substrates with *ortho*- substituents are also compatible with the reaction conditions. Beyond simple aromatics, heteroaromatics, such as pyridine and thiophene, are well-tolerated, affording product with good yields and selectivities. More sterically demanding naphthyl substituted ketones are also effective substrates for this reaction.

Next, we tested a series of substrates lacking aromatic substituents. While a different KRED was required for high levels of selectivity, *n*-hexyl glycine and derived *c*-hexylalanine derived α-aminoketone afforded product in high yields, diastereoselectivities and enantioselectivities. (**17, 19**). Leucine-derived α-aminoketone is also an effective substrate for this reaction, although product is formed with slightly diminished diastereoselectivity **18** (90% yield, 8:1 dr, >99:1 er).

An attractive feature of this approach is the possibility to access all four possible stereoisomeric products simply by exchanging the KRED involved in the kinetic resolution. We found that by screening the collection of the 24 ketoreductases in the Codexis® kit, enzymes can be identified that provide all possible stereoisomers in high yield and enantioselectivity and with good to excellent levels of diastereoselectivity (Figure 4). The general selectivity trends with these enzymes are observed with structurally related substrates (Figure S1). These examples demonstrate the possibility of novel racemization mechanisms to be paired with curated collections of enzymes to provide rapid access to the desired 1,2-amino alcohol stereoisomer.

As a demonstration of the versatility of this method, we sought to utilize this reaction to prepare amino alcohols found in medicinally important molecules. We recognized that amino alcohol motif found in HIV antiviral drugs Darunavir[24] and Atazanavir[25] could be accessed using this approach via conversion of a chloroaminoketone into an amino alcohol. After screening the commercial collection of KREDs, we found that KRED P1-B10 provided product in excellent yield, enantioselectivity and diastereoselectivity.

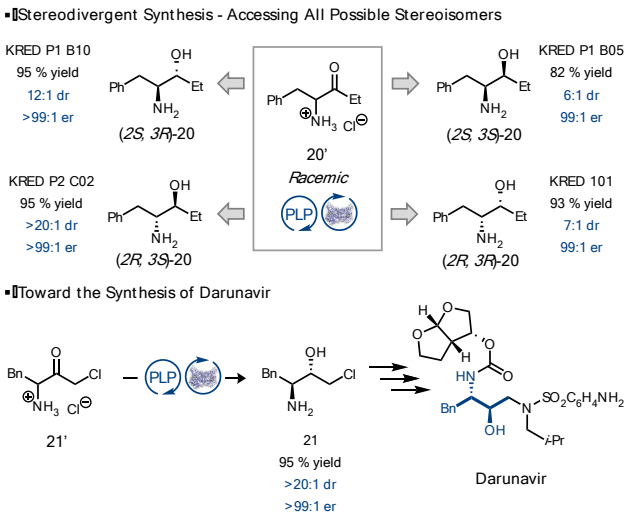


Figure 4. Stereodivergent Enzymes and Synthetic Applications

In conclusion, by merging nonenzymatic PLP racemization and diastereoselective reduction using ketoreductases, we developed a dynamic kinetic resolution

process for the preparation of chiral aminoalcohols from α-aminoketone starting materials. Utilizing commercially available KREDs, all four possible stereoisomers can be accessed using the same protocol, highlight the advantage of this approach by comparison to existing amino alcohols syntheses. As this racemization strategy operates at neutral conditions, it should be compatible with many evolved and naturally occurring KREDs, enabling it to be applied to the synthesis of a range of structurally diverse α-aminoalcohols.[26]

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org

General experimental information, experimental procedure with chemical structures and characterization data, experiments, experimental procedure for the synthetic applications with chemical structures and characterization data, and NMR spectra (PDF)

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Notes

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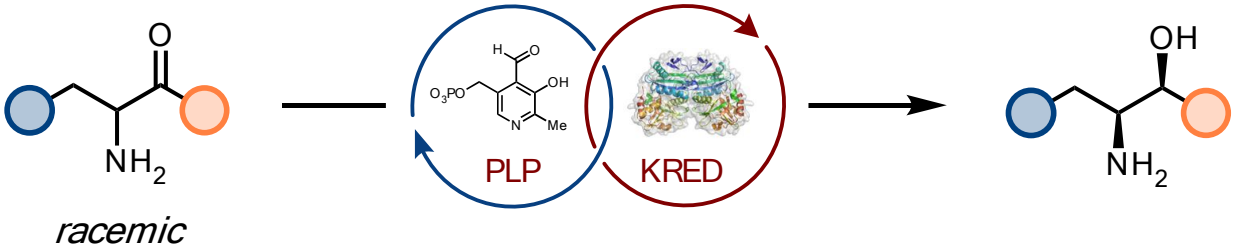

Access to All Possible Stereoisomers