An Efficient Synthesis of the Bicyclic Darunavir Side Chain Using Chemoenzymatic Catalysis

Paul S. Riehl[+], *Jesmine Lim*[‡], *James D. Finnigan*[‡], *Simon J. Charnock*[‡], *Todd K. Hyster*[+]*

[+]Cornell University, Department of Chemistry and Chemical Biology, Ithaca NY 14853
[‡] Prozomix, Building 4, West End Ind. Estate, Haltwhistle, UK

Dynamic Kinetic Resolution, Ketoreductase, Biocatalysis, Antiviral

ABSTRACT

Herein, we describe a chemoenzymatic synthesis of the bicyclic fragment of Darunavir. A ketoreductase was identified using metagenomic mining to catalyze a highly enantio- and diastereoselective dynamic kinetic resolution of a β-ketolactone. Subsequent lactone reduction with diisobutylaluminium hydride and phase transfer cyclization affords the bicyclic acetal fragment in 39% yield over four steps.

MAIN TEXT

Darunavir is a protease inhibitor used to treat HIV-AIDS. Given its effectiveness against multi-drug resistant variants and low toxicity, it is an ideal treatment for individuals in the developing world.[1] However, it is currently too expensive to be widely administered to populations with

limited resources. Consequently, there is considerable interest in developing an efficient and cost-effective synthesis that would enable the broader distribution of this medicine. Recent reports suggest that the costliest component of this molecule is the bicyclic acetal side chain (**4**) of the molecule.[2] It contains three contagious stereocenters across only six Csp3-hydridized carbons, making it a challenging molecule to prepare.

The bicyclic acetal fragment has been synthesized using numerous strategies (Figure 1). Enzymatic resolution of the racemic alcohol is commonly employed but limits the overall yield.[3] A catalytic asymmetric synthesis offers the potential for higher yields, but many examples to date have struggled to provide high selectivity in a short synthetic route. For instance, the asymmetric addition of dihydrofuran to glycolaldehyde using a chiral Lewis acid prepares the bicyclic acetal in a single step with high diastereoselectivity but poor enantioselectivity.[4] In contrast, a Mukiyama-Evans aldol using similar starting materials provides high enantioselectivity but provides the wrong alcohol epimer necessitating an oxidation/reduction sequence to invert that stereocenter.[5] Organocatalytic aldol reaction between aliphatic aldehydes and ethyl glyoxalate provides high diastereo- and enantioselectivity but requires additional protection and deprotection steps to afford the desired alcohol.[6] Inspired by these approaches, we sought to develop a chemoenzymatic synthesis that would provide high levels of selectivity while also limiting the number of chemical manipulations required to prepare the core.[7]

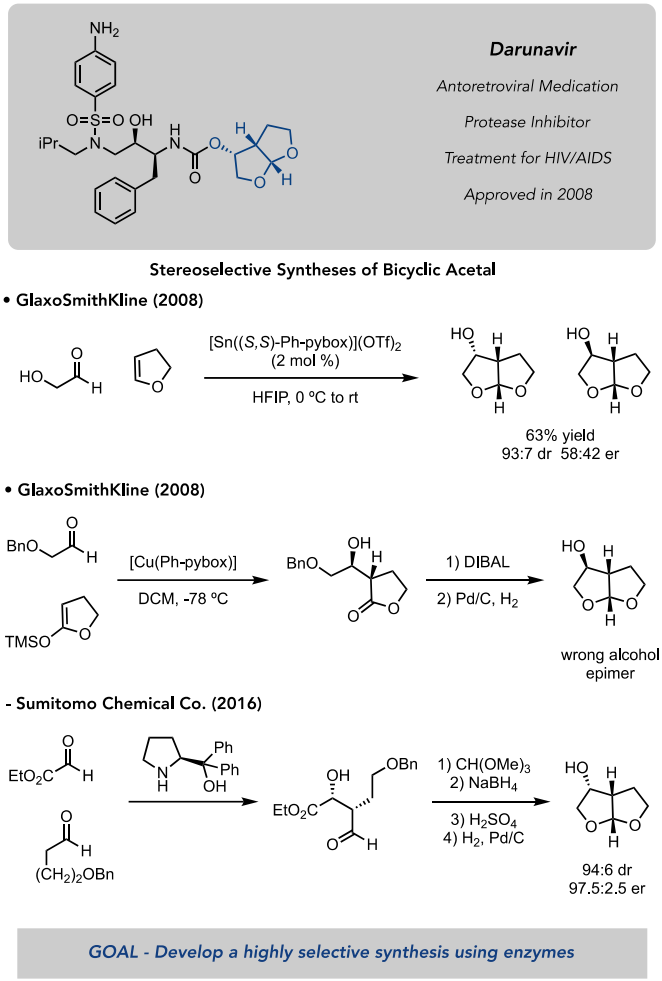


FIGURE 1. Darunavir is a protease inhibitor used for the treatment of HIV/AIDS. The bicyclic acetal (blue) is the most cost-intensive fragment to prepare. Existing catalytic approaches prepare this core with low enantioselectivity, favor the wrong diastereomer, or involve multiple protection and deprotection steps.

We envisioned a rapid synthetic strategy relying on a biocatalytic dynamic kinetic resolution (DKR). The key β-ketolactone **1** can be prepared from commercially available starting materials in a single step. Reduction of the ketone using a ketoreductase (KRED) would afford

chlorohydrin **2**, which upon lactone reaction and spontaneous cyclization would afford the desired bicyclic acetal **4** in three synthetic steps (Figure 2a). This approach is attractive because the biocatalytic DKR would set two of the three stereocenters in bicyclic acetal **4** with the thermochemistry of the [3.3.0] bicycle parlaying the stereochemical information into the third stereocenter (Figure 2b).[8]

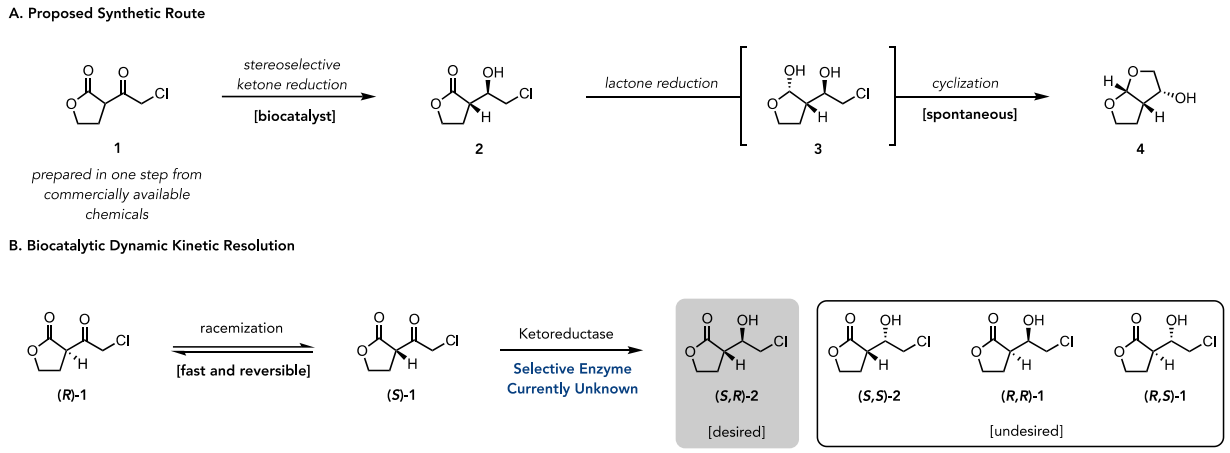


FIGURE 2. A. The proposed ketoreductase-based synthetic approach to the bicyclic acetal of Darunavir. B. Dynamic kinetic resolution is a valuable strategy to access a single stereoisomer of product in high yields.

We began by preparing the chlorinated β-keto ester **1** from abundant, low-cost γ-butyrolactone and ethyl chloroacetate. (Figure 3). Initial reaction conditions (0.45 M, 1hr) afforded the product in up to 89% yield contaminated with inseparable γ-hydroxybutyric acid. Increasing the reaction concentration to 0.65 M and extending the reaction time to 3 hours suppressed the formation of this byproduct and readily delivered large quantities of product in excellent yield of 98% as a solid.

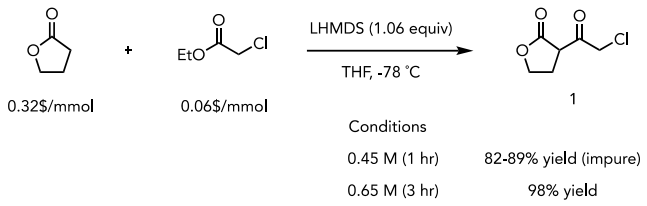
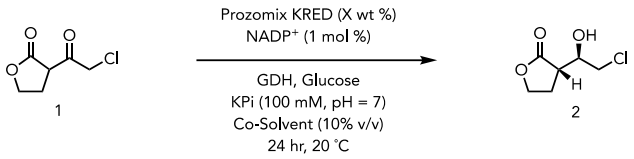


FIGURE 3. Acylation to form β-keto ester **1** from low-cost starting materials (Prices calculated based on 20 kg for butyrolactone and 2.5L for ethyl chloroacetate from Sigma-Aldrich at >99% purity). Conditions: γ-butyrolactone (94 mmol), LiHMDS (1M in THF, 103 mmol), ethyl chloroacetate (100 mmol) in THF (0.45-0.65M), -78 °C, 1-3 hr.

With an efficient synthesis of **1** in hand, we turned our attention to developing an enzymatic DKR. This substrate is ideally suited for a DKR because of the acidic α-proton.[9] Previous reports demonstrated that KREDs can participate in related DKRs; however, examples on corresponding substrates provided high yields and enantioselectivity but modest diastereoselectivity.[10] To identify a superior variant, we tested a collection of 384 wild-type KREDs assembled by Prozomix using the established colorimetric assay (KREDy-to-go™) to quickly identify promising catalysts.[11] Prozomix KRED300 proved to be the most selective, affording halohydrin **2**. The initial reaction conditions involved high enzyme loading (200 wt%) and dilute reaction conditions (24 g/L substrate **1**) and provided the product in 82% yield as a single stereoisomer (>99:1 er and >19:1 dr; Table 1, entry 1). This enzyme affords product in 85 % yield when challenged with decreased enzyme loading and increased substrate concentration (50 wt% enzyme lysate and 100 g/L substrate; Table 1, entry 2).



Entry	1 (g/L)	KRED (wt %)	Co-Solvent	Yield (%)	e.r.	d.r
1	24	KRED300 (200)	DMSO	82	>99:1	>19:1
2	100	KRED300 (50)	DMSO	85	>99:1	>19:1
3	100	KRED300 (25)	DMSO	48	>99:1	>19:1
4	50	KRED300-H2 (10)	DMSO	95	>99:1	>19:1
5	50	KRED300-H2 (10)	MeCN	99	>99:1	>19:1

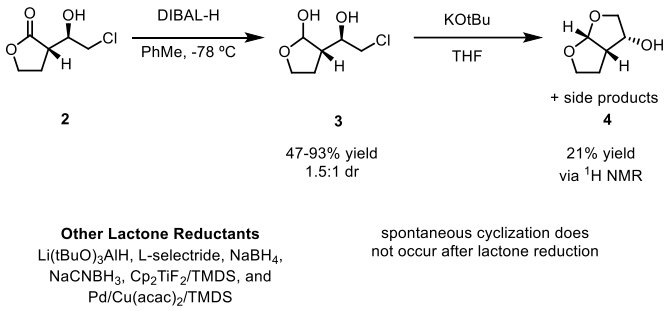
Table 1. Initial evaluations of Prozomix KRED300 for reduction of **1** showed high levels of stereoselectivity. Conditions: **1** (0.15-6.2 mmol), 100 mM Kpi (pH = 7, 10% v/v cosolvent, 24-100 g/L substrate **1**), KRED (10-200 wt%) glucose (6 equivalents), $NADP^+$ (1 mol%), GDH-105 (10-25 wt%), room temperature, 24 hr. Yields determined by isolation (entries 1-3) or HPLC (entries 4-5). e.r. measured by chiral GC and d.r. measured by NMR.

Having identified a starting point, we evaluated the Prozomix metagenome library for related homologs, using the identified lead enzyme (KRED300) as the starting point.[12] A total of 15,457,686 predicted open reading frames from 11 environmental samples taken from within the United Kingdom (terrestrial and marine) were searched, revealing 21,758 genes, with the closest related gene at 82.72% ID. A total of 22 targets were selected from the 21,758 potential genes, where the closest homologs were selected while removing any targets above 95% ID to other genes within the dataset. Evaluation of a suite of homologous KREDs with 50 wt% revealed multiple improved homologs with yields of more than 80% and high enantioselectivity (Supplemental Table 2). The best homolog was KRED300-H2, which afforded product in >99% yield as a single stereoisomer and maintained excellent yield of 95% when lowering the loading to 10 wt% (Table 1, entry 4).

With a superior enzyme in hand, we optimized the reaction parameters. In testing organic cosolvents, we found acetonitrile and dimethylformamide (Supplemental Table 3) to provide the highest yields. Next, we tested whether isopropanol could function as a terminal reductant as this would eliminate the need for glucose dehydrogenase and glucose. Unfortunately, removing glucose dehydrogenase and glucose and running the reaction with *i*PrOH (10 v/v%) failed to provide the product, suggesting that KRED300-H2 cannot oxidize *i*PrOH (Supplemental Scheme 1). Reaction performance decreased as the temperature was increased from 20 ºC to 40 ºC (Supplemental Table 4). Finally, we explored the reaction's substrate loading and found that 50 g/L substrate provides the highest yields with no observed side product formation. The final optimized reaction conditions used 10 wt% KRED with 50 g/L substrate concentration affording product in quantitative yield with >99:1 e.r. and >19:1 d.r. This reaction can be run on a preparative scale with no loss in yield or selectivity (Table 1, Entry 5).

Next, we explored the reduction of lactone **2** to the lactol and subsequent cyclization to provide the bicyclic acetal **4**. We began by testing a series of reductants known to reduce lactones to lactols, such as Li(*t*BuO)$_3$AlH, L-selectride, $NaBH_4$, and $NaCNBH_3$. While all these reagents could reduce the lactone, they afforded complicated mixtures of over-reduced products. Alternatively, Cp_2TiF_2/TMDS[13], and Pd/Cu(acac)$_2$/TMDS[14] failed to reduce the lactone and returned only starting material. Ultimately, diisobutylaluminum hydride (DIBAL) proved to be most effective, providing the lactol **3** in variable yields (47–93 %) as a 1:1.5 mixture of lactol epimers with negligible formation of over reduced side products (Scheme 1). Unfortunately, the lactol did not spontaneously cyclize to form the bicyclic acetal **4**. Attempts to induce cyclization by running the reaction at higher temperatures or heating the reaction after the reduction led to decomposed material (Supplemental Information). Gratifyingly, when the crude lactol **3** was

isolated and subjected to KO*t*Bu in THF, the desired acetal **4** was formed in 21% yield; however, the remaining mass balance was decomposed products (Scheme 1). We hypothesize that the challenge with this reaction is the competing collapse of the halohydrin to the corresponding epoxide; poor orbital overlap in the desired cyclization renders it a less facile reaction.



SCHEME 1. DIBAL Reduction and Base Induced Cyclization.

Challenged by the proposed synthesis, we considered alternative routes that would utilize hydrohydrin **2** (Figure 4). A recently reported synthesis of the bicyclic acetal demonstrated that the corresponding diol **5** would readily cyclize under acid conditions.[15] Inspired by this report, we sought to convert the halohydrin **2** to the corresponding diol, which upon lactone reduction and acidic workup affords the desired acetal **4**. Attempting to form the epoxide under basic conditions quickly afforded enone **6**, presumably formed via an E1cB mechanism. Given the sensitivity of the halohydrin, we considered an enzymatic conversion using halohydrin dehalogenases (HHDHs) and nitrite, formate, and acetate as oxygen nucleophiles.[16] We tested a small collection of Prozomix HHDHs and found that while the epoxide could be detected by GC analysis, none of these enzymes were reactive towards the tested oxygen nucleophiles. We next investigated displacement of the chloride with sodium acetate using a phase transfer catalyst; however, this method proved ineffective.

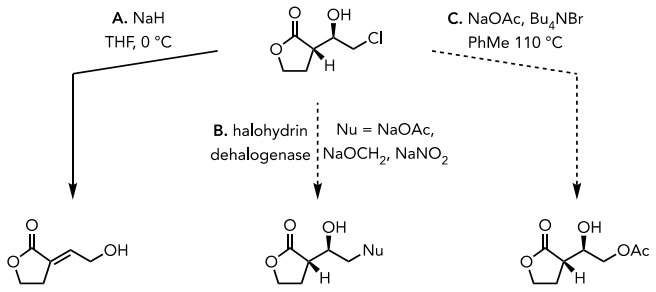
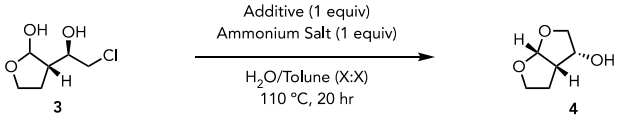


FIGURE 4. Alternative, unsuccessful cyclization approaches. A. Epoxide formation rapidly leads to allylic alcohol via E1cB elimination. B and C. Neither HHDH enzymes nor *S_N2* conditions give diol adducts.

With strategies to modify the halohydrin **2** being unsuccessful, we questioned whether solvolysis of the lactol halohydrin **3** to the corresponding diol would prove more successful. The crude lactol was isolated after DIBAL reduction and suspended in water at 80 ºC, and we observed measurable quantities of the bicyclic acetal **4** (Table 2, entry 1). We hypothesized the poor solubility of the lactol led to lower yields, prompting us to test a series of organic cosolvents. Ultimately, a 1:1 mixture of toluene and H_2O proved most effective, affording the acetal product **4** in 26% yield (Table 2, entry 2). We tested a series of additives and found that the addition of sodium acetate improved the yield to 45%, presumably by nucleophilic displacement of the chloride and subsequent hydrolysis of the resultant acetate facilitates the formation of triol **6** (Table 2, entry 3). The addition of a phase transfer reagent Bu_4NBr in combination with sodium acetate improved the yield significantly to 72% (Table 2, entry 4). We attribute this improvement in yield to both the ability of the Bu_4NBr to activate the chloride for *S_N2* substitution via a Finkelstein reaction and its phase transfer properties improving the solubility of the acetate nucleophile in the organic layer of the biphasic mixture. Finally, increasing the ratio of water to toluene to 5:1 further improved the yield to 84% (Table 2, entry 5).

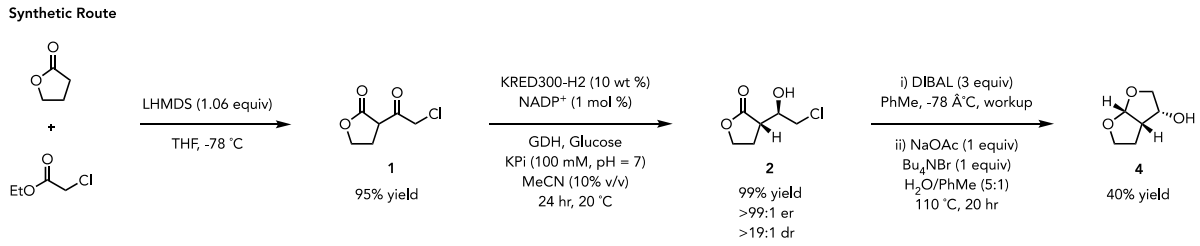
TABLE 2. Cyclization via Solvolysis



Entry	Additive	Ammonium Salt	H_2O/Tolune (X:X)	Yield (%)
1	-	-	1:0	7
2	-	-	1:1	26
3	NaOAc	-	1:1	45
4	NaOAc	Bu_4NBr	1:1	72
5	NaOAc	Bu_4NBr	5:1	84

Conditions: **3** (0.06 mmol), NaOAc (0 or 0.06 mmol), Bu_4NBr (0 or 0.06 mmol), H_2O/toluene (0.05 M), 110 °C, 20 hours.

Having established effective conditions for each of two steps in the reduction-cyclization cascade, we carried out a preparative scale reduction and cyclization (Scheme 2) under these best conditions. The crude yield of the DIBAL reduction step was 93%, and the overall yield of the bicyclic acetal **4** after purification was 40% as a single enantiomer. This demonstrates a highly enantioselective, enzyme-dependent strategy to prepare the bicyclic acetal fragment of Darunavir in just 4 synthetic steps from two low-cost commodity chemicals.



SCHEME 2. Overall Synthetic Route to **4**

Overall, this sequence rapidly and selectively delivers bicyclic acetal **4** in 39% yield and greater than 19:1 d.r. and 99:1 e.r. The initial acylation step combines two low-cost commodity chemicals and is easily scaled up to deliver β-keto ester **2** in 98% yield. Enzymatic reduction using KRED300-H2 on gram-scale provides 99% yield and over 99:1 e.r. (>19:1 d.r.). Finally, DIBAL reduction and subsequent cyclization afford the acetal **4** in 40% yield over two steps. This synthetic route demonstrates that enzymes, such as ketoreductases, can be used to set multiple stereocenters with high levels of activity and selectivity without requiring extensive rounds of protein engineering. We hope that this report can inspire more chemoenzymatic syntheses of drugs to impact global health.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge at

Content of the supplementary materials includes (1) general information (2) screening data, (3) supporting experiments, and (4) ^{1}H and ^{13}C NMR for isolated intermediates.

AUTHOR INFORMATION

Corresponding Author

* Todd K. Hyster – Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, United States; E-mail - thyster@cornell.edu

Funding Sources

The Bill & Melinda Gates Foundation funded this work.

ACKNOWLEDGMENT

We would like to thank Dr. John Dillon, Dr. Trevor Laird and Dr. Silpa Sundaram for their helpful comments and advice during this project. We gratefully acknowledge Prof. Geoff Coates and his research group for allowing us use of their chiral GC system. We thank Christina Kraml and Lotus Separations, LLC for preparative separation of stereoisomers of **2**.

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