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A Biocatalytic Route to Highly Enantioenriched β -Hydroxydioxinones

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Abstract

A novel biocatalytic system to access a wide variety of β -hydroxydioxinones from β -ketodioxinones employing commercial engineered ketoreductases has been developed. This practical system provides a remarkably straightforward solution to limitations in accessing certain chemical scaffolds common in β -hydroxydioxinones that are of great interest due to their diversification capabilities. A few highlights of this system are that it is high yielding, highly enantioselective, and chromatography-free. We have demonstrated both a wide substrate scope and a high degree of scalability.

Keywords

asymmetric synthesis; enzyme catalysis; hydroxydioxinones; ketoreductases

The synthesis of chiral β -hydroxydioxinones has attracted significant interest because of their use as building blocks to access functionally diverse products, such as δ -hydroxy- β -keto esters and the resulting *syn*- and *anti*- β,δ -diol esters.^[1] These motifs are commonly found in polyketide natural products, which are known for their potent biological activity and structural diversity. The complexity of these targets typically necessitates the construction of these polyol subunits with high selectivity and efficiency to mirror what is achieved in nature by polyketide synthetases.^[2] δ -Hydroxy- β -keto ester scaffolds are also common in a variety of commercially available statins and have been shown to be crucial for the observed biological function of these drugs.^[3] Additionally, β -hydroxydioxinones are also commonly used to access substituted tetrahydropyranones^[4] and δ -lactones^[5] and their corresponding dihydropyranones,^[6] all of which are also seen in a variety of biologically active natural products.^[7] These efforts have been of significant interest to our research group for the synthesis of a variety of natural products such as exiguolide,^[8] neopeltolide,^[9] and okilactomycin,^[10] as well as the total syntheses of many other naturally occurring compounds.^[11]

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A conventional approach (Figure 1) to β -hydroxydioxinones (**2**) involves the Lewis acid-catalysed addition of dioxinone-derived silyl dienolates (**1**) to aldehydes *via* a vinylogous Mukaiyama aldol reaction.^[12] A variety of chiral catalysts such as Ti(IV)-BINOL complexes,^[13] Ti(IV)-Schiff base complexes,^[14] Si(IV)-phosphoramidite complexes,^[15] and TADDOL catalysts^[16] have proven to be competent for this general transformation.^[12b,13–17] Even with the advances in this Mukaiyama aldol platform, there are still significant substrate limitations (see below) and the reactions typically require anhydrous conditions. Notably, a recent report by Willis using transfer hydrogenation with a chiral Ru catalyst provides an alternative non-aldol approach to β -hydroxydioxinone targets.^[18] While these approaches have been utilized in total synthesis efforts,^[19] **these methods are typically limited by the need for an sp^2 center adjacent to the newly formed stereogenic center for high levels of selectivity, i.e., unsaturated aldehyde substrates** (Figure 2). Based on our extensive experience with the creation and use of β -hydroxydioxinones, this pervasive restriction of substrate scope for the established strategies discussed above has implications in the application of dioxinone-derived aldol reactions in target synthesis.

For substrates outside of the α,β -unsaturated/aryl aldehyde limitation, Evans reported the use of benzyloxyacetaldehyde with a copper-bisoxazoline catalyst, but the reaction is restricted to this singular chelating aldehyde.^[17c] Rawal demonstrated that glyoxalate electrophiles could benefit similarly to the aforementioned chelation system using TADDOL hydrogen-bond donors to allow for high selectivity.^[16] Notably, in their recent total synthesis of lymbouilloside, Cossy disclosed that using vinylogous Mukaiyama conditions did not provide their desired β -hydroxydioxinone adduct in a stereoselective manner, requiring them to use preparative supercritical fluid chromatography to separate the enantiomers.^[20] These examples, combined with previous methods that are only practically selective for sp^2 -type substrates highlight situations where there is limited enantio- and diastereocontrol, force the need for step-uneconomical processes.^[21] Taken collectively, these widespread limitations compelled us to investigate a new, complementary approach to the enantioselective construction of β -hydroxydioxinones with broad substrate scope and functional group tolerability.

The use of evolved ketoreductase^[22] and p450^[23] enzymes in synthesis continues to grow rapidly since these complementary catalyst systems (reduction *vs.* oxidation, respectively) can produce chiral alcohols with high efficiency and superb selectivity.^[24] Specifically, ketoreductases have been utilized in the production of a variety of statins,^[25] β -lactam antibiotics,^[26] anti-inflammatory medicines,^[27] cancer treatments^[28] and other various pharmaceutical building blocks.^[29] Whole cell systems for biocatalytic reductions have been employed, but these processes are traditionally plagued by low yields and low selectivity. These detracting characteristics are primarily due to the presence of multiple ketoreductases catalysing the reaction as well as producing undesired impurities.^[30] Isolated enzymes are ideal because they can be evaluated through operationally straightforward, mild reaction conditions with minimal components necessary for optimization. These attributes make them amenable for rapid reaction development, a component that is especially attractive in the pharmaceutical industry. Ketoreductases in nature require NADH or NADPH as a

cofactor for function alongside a dehydrogenase (formate or glucose) for cofactor regeneration (Figure 3).^[31]

While these tandem systems have been used extensively,^[31] *they are potentially problematic when the optimal conditions for the ketoreductase and the dehydrogenase are not the same.* This additional variable can necessitate further rounds of enzyme evolution to obtain an optimal match between the two enzymes. Furthermore, due to the use of glucose in these systems to turnover glucose dehydrogenase, the resulting gluconolactone is spontaneously hydrolyzed to gluconic acid, requiring additional process controls to maintain the pH of the reaction system in the optimal range. Engineered ketoreductases capable of using isopropyl alcohol (IPA) to regenerate the cofactor overcome (i) the tandem optimization issue and (ii) the process parameters for pH control. Typically, the only additional variable beyond which specific engineered ketoreductase to use that needs to be considered is its tolerance to organic solvents. This advantage provides unique platforms that grant access to substrates that are highly insoluble under aqueous conditions because of the use of organic solvents. Surprisingly, the application of ketoreductases to the synthesis of highly enantioenriched β -hydroxydioxinones has not been reported to the best of our knowledge.

With this knowledge in hand, we initiated our studies with β -ketodioxinone **3a** by surveying a panel of isolated enzymes from Codexis Inc. (Table 1) following the prescribed screening protocol (see the Supporting Information for details). We selected **3a** since it represented the least biased/most challenging Csp^3 substrate with a simple methyl group. In this initial screen, both KRED-P01-H08 and KRED-P01-C01, two enzymes that utilized NADPH as the cofactor and IPA as the reductant, gave β -hydroxydioxinone **4a** in >75% yield and >99:1 *er* (Figure 4). Notably, all of the enzymes that utilized NADPH as the cofactor provided the (*R*)-enantiomer (entry 1–19), where the enzymes that utilized NADH provided the (*S*)-enantiomer (entry 20–24). After these initial results, we sought to improve this process by lowering the catalyst loading while maintaining greater than 98% conversion, 98% chemical purity, and 99:1 *er* (Figure 4).^[32]

Initial attempts to optimize the process using KRED-P01-H08 were undertaken because it showed the highest selectivity among the enzymes screened.^[27] However, after considerable effort, the process still required unreasonably high catalyst loading (10 wt%) which would not be viable for larger scale reactions (> 20 g) that were of interest in our laboratory. We then turned to KRED-P01-C01, and were pleased to find that β -hydroxydioxinone (**4**) could be obtained in 68% yield and 99:1 *er* simply by lowering the catalyst loading to 5 wt% from the recommended 100 wt% loading in the screening protocol (Table 2). Evaluating pH outside of the standard screening conditions (pH 7) proved to be deleterious to the conversion (entries 2–6), suggesting that the ketoreductase was unstable outside of a neutral pH. We next investigated the use of different co-solvents (@ 10%) with water/IPA (0.1M phosphate buffer with 10% IPA) to find that ethereal solvents such as THF, 2-MeTHF, CPME, MTBE increased yield of this process (entries 7, 11–13). More hydrophobic solvents such as hexanes and toluene showed slightly decreased yields (entries 8 and 10). Further investigations with different co-solvent percentages showed that organic solvent ratios totaling greater than 20% (e.g., 10% IPA, 10% CPME) reduced the overall yield of product (entries 15–17). The reductions in enzyme catalyst loading to 2 wt% or 1 wt% were not

detrimental to the yield, further demonstrating the robustness of this process (entries 18 and 19).

For comparison, this optimized biocatalytic reduction was benchmarked against traditional routes to β -hydroxydioxinone **4a** from β -ketodioxinone **3a** including chiral hydride sources (i.e., CBS reductions),^[33] transfer hydrogenation and baker3s yeast biocatalysis (Figure 4). The collected data indicate that this new ketoreductase protocol provides the desired alcohol **4a** in superior yield and selectivity relative to traditional routes (see the Supporting Information for details of the experimental conditions).

With these optimized conditions in hand, the scope for the asymmetric reduction was explored (Table 3). Overall, the products were obtained in excellent yield (>90%) and enantioselectivity (>95:5). *In all cases, the products were >95% pure after aqueous work-up, thereby typically obviating the need for chromatography.* Aryl substrates that were electron-neutral, as well as substrates bearing electron-rich and electron-poor substituents were well tolerated (**4b–4m**). Substrates with carbon centers adjacent to the newly formed stereocenter changed from sp^2 to sp^3 (i.e., “saturated” substrates) provided exquisite selectivity and yield (**4n, 4a, 4s**). These products correspond to the reaction of saturated aldehydes and **1** under Mukaiyama aldol conditions *but, as noted above, the typical selectivities for these reactions are <90:10 er*. Additionally, sterically encumbered groups adjacent to the ketone were well tolerated, providing the resulting alcohol in excellent yield and enantioselectivity (**4o**). Various functional groups such as TMS-protected alkynes, Cbz-protected amines, benzyl-protected alcohols, alkyl-protected esters and terminal alkenes performed well in this reaction, further highlighting the tolerance of functional diversity of this methodology (**4p, 4q, 4r, 4t, 4u**). *Notably, these substrates have not been accessible in a stereoselective manner using previous methodologies,* allowing for access to more complex scaffolds from these chiral β -hydroxydioxinones.

To further investigate the synthetic utility of this process, larger scale-up reactions were pursued. Lowering the enzyme loading to 0.5 wt% (at a concentration of 100 gL⁻¹) predictably lengthened the overall reaction time (from 24 h to 72 h) and **20 g of β -hydroxydioxinone 4a in 99% yield with >99:1 er were isolated after a simple extraction (>99% pure as determined by HPLC)**. Additionally, we wanted to determine if we could access the opposite enantiomer of β -hydroxydioxinone **4**. We were pleased to find that *without optimization*, we were able to access β -hydroxydioxinone *ent-4a* in 97% yield with 89:11 *er* (Figure 5).

The resulting β -hydroxydioxinones can be converted into a variety of motifs relevant to several families of natural products using previously established chemical routes. Of particular note, dioxinones have extensive precedents in photocycloadditions to access fused cyclic structures common in diterpene natural products **5**,^[34] thermal opening to provide macrocycles of varying sizes **6**,^[35] and Lewis acid/Bronsted acid-catalysed reactions to form substituted tetrahydropyranones **7**^[4,36] (Figure 6).

In conclusion, we have described herein a biocatalytic method for the enantioselective reduction of β -ketodioxinones with sp^3 carbons adjacent to the stereogenic center, as well as

demonstrating access to value-added molecules previously inaccessible in a stereoselective manner. This approach is an attractive, complementary approach to previously established aldol-focused methods because of its much broader substrate scope and operational simplicity. This methodology allows for mild conditions and does not require the use of high temperature, pressure, or oxygen-free environments. Investigations in our laboratory towards diastereoselective reductions using ketoreductases as well as enzyme immobilization are currently ongoing.

Experimental Section

General Information

All reactions were carried out under a nitrogen atmosphere in oven-dried glassware with magnetic stirring. All organic solvents were purified by passage through a bed of activated alumina.^[37] Reagents were purified prior to use unless other-wise stated following the guidelines of Chai and Armarego.^[38] Purification of reaction products was carried out by flash chromatography using EM Reagent or Silicycle silica gel 60 (230–400 mesh). Analytical thin layer chromatography was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and ceric ammonium nitrate stain, potassium permanganate stain or ninhydrin stain followed by heating. ¹H NMR spectra were recorded on a Bruker Avance 500 MHz w/direct cryoprobe (500 MHz) spectrometer and are reported in ppm using solvent as an internal standard (CDCl₃ at 7.26 ppm). Data are reported as [ap=apparent, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad; coupling constant(s) in Hz, integration]. Proton-decoupled ¹³C NMR spectra were recorded on a Bruker Avance 500 MHz w/direct cryoprobe (125 MHz) spectrometer and are reported in ppm using solvent as an internal standard (CDCl₃ at 77.16 ppm). Mass spectra data were obtained on a Waters Acquity-H UPLC-MS with a single quadrupole ESI Spectrometer or on a Gas Chromatography Mass Spectrometer (Agilent 7890A/5975C GCMS System).

CCDC 1516986 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

General Procedure for the Preparation of β -Hydroxydioxinones 4a–4u

50 mg of the KRED Recycle Mix P (contains NADPH) were added to a vial. 0.8 mL of 0.1M phosphate buffer (pH 7.0) were added to the KRED Recycle Mix P, and stirred until it was homogeneous, whereupon 1 mg of KRED-P01-C01 was then added. In a separate vial, 100 mg of β -ketodioxinone substrate, 0.1 mL isopropyl alcohol (IPA) and 0.1 mL cyclopentylmethyl ether (CPME) were added. Once the β -ketodioxinone substrate had completely dissolved, this solution was added to the solution containing KRED-P01-C01. The reaction mixture was stirred for 24 hours at 30°C. Upon reaction completion, a small amount of solid NaCl was added to the reaction mixture. The solution was then filtered, extracted with ethyl acetate (30 $\times$ 15 mL), dried with MgSO₄, filtered, and then concentrated under vacuum to obtain the >99% pure β -hydroxydioxinone.

Procedure for the Large-Scale Preparation of 4a

160 mL of 0.1M phosphate buffer (pH 7.0) were added to a 500-mL, 3-neck, round-bottom flask equipped with an overhead stirrer, a nitrogen inlet, and a septum. 100 mg of KRED-P01-C01 were then added followed by 50 mg of NADPH. In a separate 100-mL round-bottom flask, 20 g of β -ketodioxinone substrate **3a**, 20 mL isopropyl alcohol (IPA) and 20 mL cyclopentyl methyl ether (CPME) were added. Once the β -ketodioxinone substrate had completely dissolved (required slight heating and stirring), this solution was added to the solution containing KRED-P01-C01. The reaction mixture was stirred for 72 hours at 30°C. Upon reaction completion, solid NaCl was added to the reaction mixture. The solution was then filtered, extracted with ethyl acetate (50150 mL), dried with MgSO₄, filtered, and then concentrated under vacuum to obtain the >99% pure β -hydroxydioxinone **4a**.

Procedure for the Preparation of β -Hydroxydioxinone *ent*-4a

50 mg of the KRED Recycle Mix N (contains NADH) were added to a vial. 1 mL of 0.1M phosphate buffer (pH 7.0) was added to the KRED Recycle Mix N, and stirred until it was homogeneous, whereupon 1 mg of KRED-NADH 101 was then added. 100 mg of β -ketodioxinone substrate were then added and the reaction mixture was stirred for 24 hours at 30°C. Upon reaction completion, a small amount of solid NaCl was added to the reaction mixture. The solution was then filtered, extracted with ethyl acetate (30 $\times$ 15 mL), dried with MgSO₄, filtered, and then concentrated under vacuum to obtain the >99% pure β -hydroxydioxinone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Krüger J, Carreira EM. Tetrahedron Lett. 1998; 39:7013.
2. Staunton J, Weissman KJ. Nat Prod Reports. 2001; 18:380.
3. a) Wu Y, Xiong F-J, Chen F-E. Tetrahedron. 2015; 71:8487. b) Oliveira EF, Santos-Martins D, Ribeiro AM, NF, Brás AM, Cerqueira NS, Sousa SF, Ramos MJ, Fernandes PA. Expert Opinion on Therapeutic Patents. 2016:1.
4. Morris WJ, Custar DW, Scheidt KA. Org Lett. 2005; 7:1113. [PubMed: 15760152]
5. a) Gleitz J, Friese J, Beile A, Ameri A, Peters T. Eur J Pharmacol. 1996; 315:89. [PubMed: 8960869] b) Reddy PP, Yen KF, Uang BJ. J Org Chem. 2002; 67:1034. [PubMed: 11856060] c) Stokker GE, Hoffman WF, Alberts AW, Cragoe EJ, Deana AA, Gilfillan JL, Huff JW, Novello FC, Prugh JD. J Med Chem. 1985; 28:347. [PubMed: 3973903]
6. a) Goel A, Ram VJ. Tetrahedron. 2009; 65:7865. b) Aytemir MD, Çali Ü, Özalp M. Arch Pharm. 2004; 337:281.
7. Li W, Li X-B, Li L, Li R-J, Lou H-X. Phytochemistry Lett. 2015; 12:22.
8. Crane EA, Zabawa TP, Farmer RL, Scheidt KA. Angew Chem. 2011; 123:9278. Angew Chem Int Ed. 2011; 50:9112.

9. a) Custar DW, Zabawa TP, Scheidt KA. *J Am Chem Soc.* 2008; 130:804. [PubMed: 18161979] b) Custar DW, Zabawa TP, Hines J, Crews CM, Scheidt KA. *J Am Chem Soc.* 2009; 131:12406. [PubMed: 19663512]
10. Tenenbaum JM, Morris WJ, Custar DW, Scheidt KA. *Angew Chem.* 2011; 123:6014; *Angew Chem Int Ed.* 2011; 50:5892.
11. a) Nicolaou KC, Guduru R, Sun YP, Banerji B, Chen DYK. *Angew Chem.* 2007; 119:6000. *Angew Chem Int Ed.* 2007; 46:5896. b) Kan SBJ, Ng KKH, Paterson I. *Angew Chem.* 2013; 125:9267. *Angew Chem Int Ed.* 2013; 52:9097. c) Cookson R, Barrett TN, Barrett AGM. *Acc Chem Res.* 2015; 48:628. [PubMed: 25689674]
12. a) Casiraghi G, Battistini L, Curti C, Rassu G, Zanardi F. *Chem Rev.* 2011; 111:3076. [PubMed: 21434642] b) Casiraghi G, Zanardi F, Appendino G, Rassu G. *Chem Rev.* 2000; 100:1929. [PubMed: 11749280]
13. De Rosa M, Acocella MR, Villano R, Soriente A, Scettri A. *Tetrahedron: Asymmetry.* 2003; 14:2499.
14. Singer RA, Carreira EM. *J Am Chem Soc.* 1995; 117:12360.
15. Denmark SE, Wynn T, Beutner GL. *J Am Chem Soc.* 2002; 124:13405. [PubMed: 12418891]
16. Gondi VB, Gravel M, Rawal VH. *Org Lett.* 2005; 7:5657. [PubMed: 16321015]
17. a) Krüger J, Carreira EM. *J Am Chem Soc.* 1998; 120:837. b) Denmark SE, Heemstra JR, Beutner GL. *Angew Chem.* 2005; 117:4760. *Angew Chem Int Ed.* 2005; 44:4682. c) Evans DA, Kozłowski MC, Murry JA, Burgey CS, Campos KR, Connell BT, Staples RJ. *J Am Chem Soc.* 1999; 121:669.
18. Fang Z, Clarkson GJ, Wills M. *Tetrahedron Lett.* 2013; 54:6834.
19. a) Kim Y, Singer RA, Carreira EM. *Angew Chem.* 1998; 110:1321. *Angew Chem Int Ed Engl.* 1998; 37:1261. b) Evans DA, Hu E, Burch JD, Jaeschke G. *J Am Chem Soc.* 2002; 124:5654. [PubMed: 12010035] c) Paterson I, Davies RDM, Marquez R. *Angew Chem.* 2001; 113:623. *Angew Chem Int Ed.* 2001; 40:603. d) Denmark SE, Fujimori S. *J Am Chem Soc.* 2005; 127:8971. [PubMed: 15969572] e) Dalby SM, Goodwin-Tindall J, Paterson I. *Angew Chem.* 2013; 125:6645. *Angew Chem Int Ed.* 2013; 52:6517.
20. Kolleth A, Gebauer J, ElMarrouni A, Lebeuf R, Prévost C, Brohan E, Arseniyadis S, Cossy J. *Frontiers in Chemistry.* 2016; 4. [PubMed: 26904536]
21. a) Snider BB, Song F. *Org Lett.* 2001; 3:1817. [PubMed: 11405719] b) Munakata R, Katakai H, Ueki T, Kurosaka J, Takao K-i, Tadano K-i. *J Am Chem Soc.* 2003; 125:14722. [PubMed: 14640644]
22. a) Huisman GW, Liang J, Krebber A. *Curr Opin Chem Biol.* 2010; 14:122. [PubMed: 20071211] b) Bezborodov AM, Zagustina NA. *Applied Biochemistry and Microbiology.* 2016; 52:237.
23. a) Fasan R. *ACS Catal.* 2012; 2:647. b) Renata H, Wang ZJ, Arnold FH. *Angew Chem.* 2015; 127:3408. *Angew Chem Int Ed.* 2015; 54:3351.
24. Gröger, H., Hummel, W., Borchert, S., Krauß, M. *Enzyme Catalysis in Organic Synthesis.* Wiley-VCH; Weinheim: 2012. p. 1035
25. a) Tartaglia S, Fogal S, Motterle R, Ferrari C, Pontini M, Aureli R, Lucchi OD. *Eur J Org Chem.* 2016:3162. b) Ma SK, Gruber J, Davis C, Newman L, Gray D, Wang A, Grate J, Huisman GW, Sheldon RA. *Green Chem.* 2010; 12:81.
26. Liang J, Mundorff E, Voladri R, Jenne S, Gilson L, Conway A, Krebber A, Wong J, Huisman G, Truesdell S, Lalonde J. *Org Process Res Dev.* 2010; 14:188.
27. Liang J, Lalonde J, Borup B, Mitchell V, Mundorff E, Trinh N, Kochrekar DA, Nair Cherat R, Pai GG. *Org Process Res Dev.* 2010; 14:193.
28. Ju X, Tang Y, Liang X, Hou M, Wan Z, Tao J. *Org Process Res Dev.* 2014; 18:827.
29. a) Patel RN. *ACS Catal.* 2011; 1:1056. b) Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, Robins K. *Nature.* 2012; 485:185. [PubMed: 22575958] c) Kuethe JT, Janey JM, Truppo M, Arredondo J, Li T, Yong K, He S. *Tetrahedron.* 2014; 70:4563.
30. Pollard DJ, Telari K, Lane J, Humphrey G, McWilliams C, Nidositko S, Salmon P, Moore J. *Biotechnol Bioeng.* 2006; 93:674. [PubMed: 16395718]
31. Matsuda T, Yamanaka R, Nakamura K. *Tetrahedron: Asymmetry.* 2009; 20:513.
32. Pollard DJ, Woodley JM. *Trends Biotechnol.* 2007; 25:66. [PubMed: 17184862]

33. Corey EJ, Helal CJ. *Angew Chem.* 1998; #110:2092. *Angew Chem Int Ed Engl.* 1998; 37:1986.
34. a) Winkler JD, Henegar KE, Williard PG. *J Am Chem Soc.* 1987; 109:2850. b) Winkler JD, Muller CL, Hey JP, Ogilvie RJ, Haddad N, Squattrito PJ, Williard PG. *Tetrahedron Lett.* 1989; 30:5211. c) Winkler JD, Shao B. *Tetrahedron Lett.* 1993; 34:3355. d) Winkler JD, Doherty EM. *Tetrahedron Lett.* 1998; 39:2253. e) Winkler JD, McLaughlin EC. *Org Lett.* 2005; 7:227. [PubMed: 15646964]
35. a) Boeckman RK, Pruitt JR. *J Am Chem Soc.* 1989; 111:8286. b) Boeckman RK Jr, Barta TE, Nelson SG. *Tetrahedron Lett.* 1991; 32:4091. c) Boeckman RK, Shao P, Wroblewski ST, Boehmler DJ, Heintzelman GR, Barbosa AJ. *J Am Chem Soc.* 2006; 128:10572. [PubMed: 16895426] d) Reber KP, Tilley SD, Sorensen EJ. *Chem Soc Rev.* 2009; 38:3022. [PubMed: 19847338] e) Tilley SD, Reber KP, Sorensen EJ. *Org Lett.* 2009; 11:701. [PubMed: 19119873] f) Hoye TR, Danielson ME, May AE, Zhao H. *Angew Chem.* 2008; 120:9889. *Angew Chem Int Ed.* 2008; 47:9743. g) Hoye TR, Danielson ME, May AE, Zhao H. *J Org Chem.* 2010; 75:7052. [PubMed: 20932014] h) Zhao YM, Maimone TJ. *Angew Chem.* 2015; 127:1239. *Angew Chem Int Ed.* 2015; 54:1223.
36. a) Wang J, Crane EA, Scheidt KA. *Org Lett.* 2011; 13:3086. [PubMed: 21591768] b) Cui J, Chai DI, Miller C, Hao J, Thomas C, Wang J, Scheidt KA, Kozmin SA. *J Org Chem.* 2012; 77:7435. [PubMed: 22860634]
37. Pangborn AB, Giardello MA, Grubbs RH, Rosen RK, Timmers FJ. *Organometallics.* 1996; 15:1518.
38. Armarego, WLF., Chai, CLL. *Purification of Laboratory Chemicals.* 6. Armarego, WLF., Chai, CLL., editors. Butterworth-Heinemann; Oxford: 2009. p. 88

Figure 1.
Biologically active scaffolds accessed through β-hydroxydioxinones.

Figure 2.
Routes to access chiral β -hydroxydioxinones.

Figure 3.
Catalytic cycle of ketoreductase enzymes.

Figure 4.
Results from conventional routes to β-hydroxydioxinones.

Figure 5.
Large-scale reduction of β -ketodioxinone and access to both enantiomers of β -hydroxydioxinone **4a**.

Figure 6.
Diverse scaffolds accessible with β -hydroxydioxinones.

Table 1

Initial enzyme screening results and process target.

[a]

[b]

[b]

Entry	Catalyst [100 wt%]	Cofactor	Conversion [%]	<i>er</i>
1	P1-A04	NADPH	51	55:45
2	P1-A12	NADPH	10	60:40
3	P1-B01	NADPH	45	71:29
4	P1-B05	NADPH	21	52:48
5	P1-B10	NADPH	08	72:28
6	P1-B12	NADPH	21	83:17
7	P1-C01	NADPH	99	99:1
8	P1-H08	NADPH	92	99:1
9	P2-B02	NADPH	17	67:33
10	P2-C02	NADPH	79	51:49
11	P2-C11	NADPH	24	51:49
12	P2-D03	NADPH	63	58:42
13	P2-D11	NADPH	48	63:37
14	P2-D12	NADPH	67	68:32
15	P2-G03	NADPH	89	72:28
16	P2-H07	NADPH	23	65:35
17	P3-B03	NADPH	41	70:30
18	P3-G09	NADPH	20	64:36
19	P3-H12	NADPH	28	51:49
20	101	NADH	55	37:63
21	119	NADH	63	28:72
22	130	NADH	68	35:75
23	NADH-101	NADH	97	11:89
24	NADH-110	NADH	71	39:61

[c]

	hit criteria in initial screen	process target
substrate loading	1 g/L	100 g/L
KRED loading	1 g/L/[d,e]	1 g/L/[d,e]
conversion	75%	98%
reaction time	24 h	24 h
chemical purity (HPLC)	90%	98%

[a]

[b]

[b]

Entry	Catalyst [100 wt%]	Cofactor	Conversion [%]	<i>er</i>
optical purity of the product			95:5 <i>er</i>	99:1 <i>er</i>

[a] Desired biocatalytic reduction.

[b] Enantioselectivity and conversion of ketoreductases in Codexis KRED Screening Kit.

[c] Hit criteria in initial screen and final KRED process target.

[d] Lyophilized cell lysate. Approximately 20–30 wt% of the lyophilized powder is the catalyst of interest. All references to “gL⁻¹” used herein will be based on this preparation.

[e] On a *molar* basis, the catalyst loading is on the order of 10⁻⁴ to 10⁻³ mol%.

Table 2

Optimization of the reaction conditions.^[a]

Entry	Catalyst [wt%]	pH	Co-solvent (%)	Yield [%] ^[b]	ee [%] ^[c]
1	5	7	N/A	68	99:1
2	5	6	N/A	52	N.D.
3	5	5	N/A	42	N.D.
4	5	4	N/A	21	N.D.
5	5	8	N/A	47	N.D.
6	5	9	N/A	19	99:1
7	5	7	THF (10%) ^[d]	73	N.D.
8	5	7	toluene (10%) ^[d]	45	N.D.
9	5	7	EtOAc (10%) ^[d]	51	99:1
10	5	7	hexanes (10%) ^[d]	32	99:1
11	5	7	2-MeTHF (10%) ^[d]	74	99:1
12	5	7	CPME (10%) ^[d]	97	99:1
13	5	7	MTBE (10%) ^[d]	89	99:1
14	5	7	CPME (5%) ^[e]	91	99:1
15	5	7	CPME (15%) ^[f]	94	99:1
16	5	7	CPME (20%) ^[g]	85	99:1
17	5	7	CPME (25%) ^[h]	71	99:1
18	2	7	CPME (10%) ^[d]	96	99:1
19	1	7	CPME (10%) ^[d]	98	99:1

^[a] Conditions: **3a** (0.05 mmol), KRED-P01-C01 (0.5 mg), NADPH (0.1 mg) 1:9 IPA:phosphate buffer (0.1M) at 30°C for 24 h.^[b] Determined by HPLC with naphthalene as internal standard.

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[c] Determined by HPLC analysis.

[d] 1:1:8 IPA:co-solvent:phosphate buffer (0.1M).

[e] 1:0.5:8.5 IPA:co-solvent:phosphate buffer (0.1M).

[f] 1:1.5:7.5 IPA:co-solvent:phosphate buffer (0.1M).

[g] 1:2:7 IPA:co-solvent:phosphate buffer (0.1M).

[h] 1:2.5:6.5 IPA:co-solvent:phosphate buffer (0.1M). THF= tetrahydrofuran, EtOAc=ethyl acetate, CPME=cyclopentyl methyl ether, MTBE=methyl *tert*-butyl ether.

Table 3

Substrate scope.^[a]

^[a] See the Supporting Information for details. Yields are of isolated product after extraction. Enantiomeric ratio was determined by HPLC analysis on a chiral stationary phase.

^[b] Absolute configuration was determined by X-ray crystallography of **4b** and all other analogues were assigned by analogy.