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Chemo- and Enantioselective Routes to Chiral Fluorinated Hydroxyketones Using Ketoreductases

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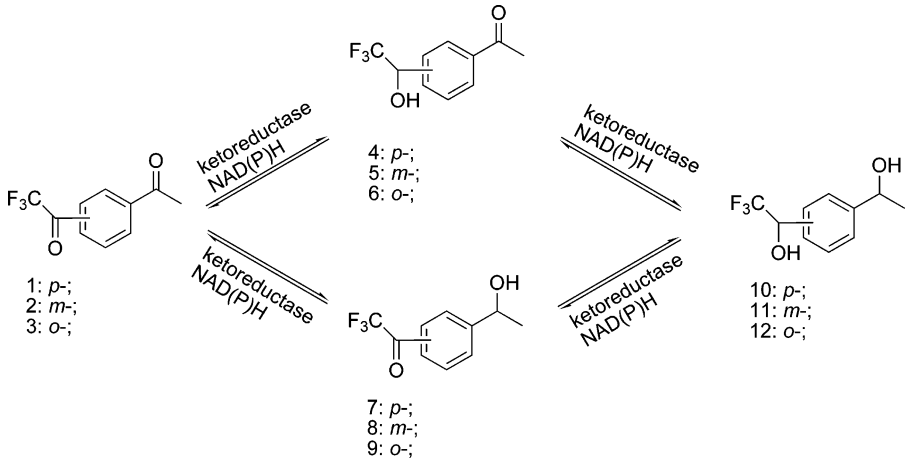
ABSTRACT



Chiral fluorinated hydroxyketones were synthesized with excellent ee (>98%) and yield by a chemo- and stereoselective reduction of prochiral methyl/trifluoromethyl diketones using commercially available ketoreductase enzymes. By using *p*- and *m*-trifluoroacetyl substituted acetophenones, we demonstrate that ketoreductases can selectively differentiate between methyl and trifluoromethyl ketones within the same molecule. As a result, useful catalysts were identified that eliminated the need for costly and time-consuming protection/deprotection of the ketone moiety, enabling a more convergent synthesis of hydroxyketones. Further, a route to chiral methyl hydroxyketones is provided where an enzyme selectively reduces the unactivated ketone.

Over the past decade, there has been a dramatic increase in the demand for fluorinated intermediates[1] as chemists routinely incorporate fluorine into molecules in order to modify the electronic properties, binding affinities, and bioavailabilities of compounds.[2] As Swinson indicates, fluorines are prevalent in antidepressant, antibacterial, antiinflammatory, and cholesterol-lowering drugs. Therefore, the ability to produce optically pure halogenated intermediates is of great value to the pharmaceutical industry in order to develop robust, cost-efficient syntheses of complex molecules. One potentially useful intermediate for synthesis that is not readily available is a fluorinated chiral hydroxyketone. An elegant and efficient method to access these compounds is utilizing a chemo- and stereoselective reduction of a prochiral diketone. This route will allow access to a chiral hydroxyketone without the need for protecting groups and additional steps simply by using a commercially available catalyst.

In this paper, we describe the highly chemo- and enantioselective preparation of chiral fluorinated hydroxyketones employing reduction of a *p*- and *m*-diketone, **1** and **2** respectively, catalyzed by commercially available ketore-

(1) Thayer, A. M. *Chem. Eng. News* **2006**, *84*, 15−24.
(2) Swinson, J. *PharmaChem* **2007**, *6*, 4, 38−41; Swinson, J. *PharmaChem* **2005**, *4*, 6, 26−30.

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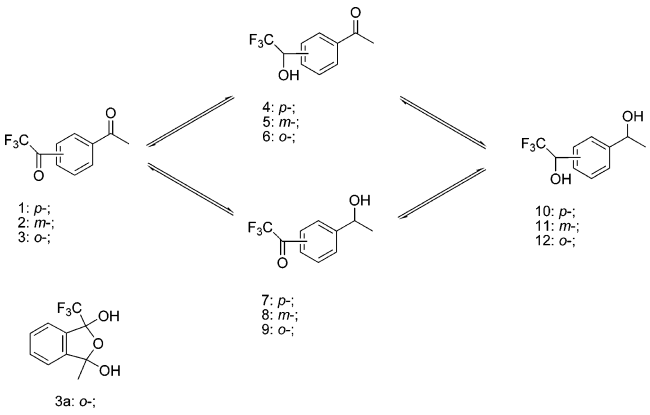
Scheme 1. Chemoselective Reductions of Methyl/Trifluoromethyl Diketones To Produce Hydroxyketones[a]



[a] **3a**: The *o*-diketone was isolated as a cyclized dihydrate and was inert in all reactions.

ductase enzymes (Scheme 1). This new method of producing optically pure hydroxyketones eliminates the need to protect prochiral diketones and allows a more efficient and convergent synthesis. Importantly, we demonstrate a route to (*S*)-methyl hydroxyketones (**7**, **8**) which are difficult compounds to synthesize due to the relative ease of a chemical reduction of a trifluoromethyl ketone located within five bonds of the methyl carbonyl.

The chemoselective reduction (Scheme 1) of these compounds is reported in the literature,[3] but this reaction has limited application in the synthesis of pharmaceutical intermediates as it produces racemic hydroxyketones. Synthesizing chiral trifluoromethyl hydroxyketones by a chemoselective asymmetric hydrogenation of fluorinated β-diketones was demonstrated using Pt/Al_2O_3 and chiral modifiers.[4] However, this metal-catalyzed transformation is limited to reduction of the activated ketone in the β-diketones examined.

The aim of our research was to demonstrate that keto reductases can chemo- and stereoselectively differentiate between methyl and trifluoromethyl ketones within the same molecule by using *p*- and *m*-trifluoroacetyl-substituted acetophenones. Enzymes are an excellent choice as catalysts to mediate this chemoselective diketone reduction as the elaborate chiral environment should be able to differentiate between the ketones due to the steric and electronic differences of the carbonyls. However, prior to the results presented here, there are no examples in the literature of an enzymatic chemoselective reduction of methyl/trifluoromethyl diketones, although there are a number of examples of enzymes catalyzing the regio- and enantioselective reductions of substrates such as α-diketones[5−7] and β-diketones.[7−9] Additionally, these biocatalysts have been shown to have a broad substrate range including substituted acetophenones,[10−13] as well as 2,2,2-trifluoroacetophenone.[14−16] Finally, the catalysts that we screened are synthetically useful as they are commercially available[17] as isolated enzymes and have been used to deliver multikilograms of optically pure chiral alcohol.[13]

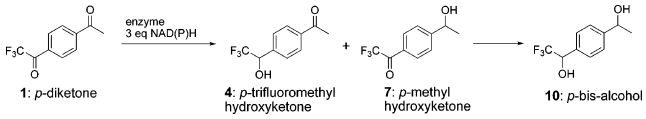
The methyl/trifluoromethyl diketones **1**, **2**, and **3** were synthesized by modifying the literature procedure.[3] Ketals were formed from the corresponding bromo-acetophenones using ethylene glycol and were catalyzed by *p*-toluene sulfonic acid. This step was followed by a metal/halogen exchange using *n*-hexyl lithium and quench with methyl trifluoroacetate. Deprotection with 1 N HCl and crystallization afforded the trifluoroacetyl-substituted acetophenones **1**, **2**, and **3** as solids with 60−80% isolated yield. The three diketones **1**, **2**, and **3** were screened against a library consisting of 72 commercially available enzymes in a 96-well format.[17] The *o*-diketone was isolated as a cyclized dihydrate **3**[a] (Scheme 1) and found to be inert in all chemical and enzymatic reactions. The reactions were monitored by an achiral GC assay to determine the product ratios and conversions. The ee value of hydroxyketones was determined by chiral SFC for **4** and **5** and by chiral GC for **7** and **8**.

The majority of the ketoreductase library was active toward the *p*-diketone **1** and produced the trifluoromethyl hydroxyketone **4** in excess. The enzymes listed in Table 1 showed the highest chemoselectivity toward the *p*-diketone. In fact, 49 of the enzymes had a mixture of trifluoromethyl hydroxyketone **4** and bis-alcohol **10** when sampled.

The results of screening the *p*-diketone **1** toward the library demonstrates that by using isolated enzymes one can chemoselectively access both enantiomers of the trifluoromethyl hydroxyketone **4** in high enantiomeric excess. In fact, there

(3) Sasaki, S.; Yamauchi, T.; Kubo, H.; Kanai, M.; Ishii, A.; Higashiyama, K. *Tetrahedron Lett.* **2005**, *46*, 1497−1500.
(4) Hess, R.; Diezi, S.; Mallat, T.; Baiker. A. *Tetrahedron: Asymmetry* **2004**, *15*, 251−257.
(5) Boutante, P.; Mousset, G.; Veschambre, H. *New J. Chem*. **1998**, 247−251.
(6) Mahmoodi, N. O.; Mohammadi, H. G. *Monatsh. Chem*. **2003**, *134*, 1283−1288.
(7) Edegger, K.; Stampfer, W.; Seisser, B; Faber, K; Mayer, S. F.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Eur. J. Org. Chem.* **2006**, 1904−1909.
(8) Muller, M.; Wolberg, M.; Shubert, T.; Hummel, W. *Adv. Biochem. Eng. Biotechnol*. **2005**, *92*, 261−287.
(9) Wolberg, M.; Hummel, W.; Wandrey, C.; Muller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 23, 4306−4308.
(10) Zhu, D.; Rios, B. D.; Rozzell, J. D.; Hua, L. *Tetrahedron: Asymmetry* **2005**, *16*, 1541−1546.
(11) Groger, H.; Hummel, W.; Rollman, C.; Chamouleau, F.; Husken, H.; Werner, H.; Wunderlich, C.; Abokitse, K.; Drauz, K.; Buchholz, S. *Tetrahedron* **2004**, *60*, 633−640.
(12) Rosen, T. C.; Feldmann, R.; Dunkelmann, P.; Daubmann, T. *Tetrahedron Lett.* **2006**, *47*, 4803−4806.
(13) Pollard, D.; Truppo, M.; Pollard, J.; Chen, C.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554−559.
(14) Yamazaki, Y.; Kobayashi, H. *Tetrahedron: Asymmetry* **1993**, *6*, 1287−1294.
(15) Bradshaw, C. W.; Hummel, W.; Wong, C. H. *J. Org. Chem*. **1992**, *57*, 1532−1536.
(16) Caron, D.; Coughlan, A. P.; Simard, M.; Bernier, J.; Piche, Y.; Chenevert, R. *Biotechnol. Lett.* **2005**, *27*, 713−716.
(17) Isolated enzymes were used as lyophilized solids or liquid preparations. KRED-XXX enzymes, alcohol dehydrogenase from *Rhodococcus erythropolis* (ADH-RE), alcohol dehydrogenase from *Candida parapsilosis* (ADH-CP), glucose dehydrogenase-103 (GDH-103), NAD^+, NADH, and NADPH were purchased from Biocatalytics, Inc. (Pasedena, Ca, U.S.A.). ADH CDX010, ADH CDX013, alcohol dehydrogenase from *Lactobacillus brevis* (ADH-LB), and alcohol dehydrogenase from *Thermoanaerobacter sp*. (ADH T) were purchased from Julich Chiral Solutions, Inc (Julich, Germany). All chemicals used were certified as reagent grade and purchased from Sigma-Aldrich and Fisher Scientific. Screening conditions are listed in Tables 1 and 2 under footnote "a".

Table 1. Enzyme-Catalyzed Reduction of *p*-Diketone **1**



entry	enzyme	**4** yield (%)[b]	**7** yield (%)[b]	**10** yield (%)[b]	ee (%)[c,d]
1	KRED-112	100	0	0	>99 (*S*)
2	KRED-129	100	0	0	>99 (*R*)
3	KRED-131	100	0	0	>99 (*R*)
4	KRED-A1i	100	0	0	>99 (*S*)
5	KRED-A1n	100	0	0	>99 (*R*)
6	KRED-A1x	100	0	0	>99 (*R*)
7	CDX-013	92	0	8	>99 (*S*)
8	KRED-111	92	0	8	97 (*S*)
9	KRED-B1d	90	0	10	>99 (*S*)
10	KRED-B1e	88	0	12	>99 (*S*)
11	KRED-113	86	0	14	>99 (*S*)
12	CDX-010	71	9	20	>99 (*R*)
13	GDH-103	72	0	28	13 (*R*)[e]
14	ADH-RE	47	10	43	>99 (*R*)[f]
15	ADH-T	27	0	73	>99 (*R*)
16	ADH-CP	0	100	0	98 (*S*)
17	KRED-A1p	0	87	13	98 (*S*)

[a] Reaction conditions: 0.4 g/L enzyme, 2.5 g/L diketone, 3 equiv of NAD(P)H, 5% v/v DMF, in 100 mM potassium phosphate buffer (pH 7) at 30 °C. [b] Analyzed using achiral GC assay. [c] Analyzed using chiral SFC for **4** and chiral GC assays for **7**. [d] Absolute configuration for **4** and **7** was determined by using the modified Mosher method[21] and chiral hydroxyketones produced from the catalysts listed in entries 1 and 16. [e] Entry 13 is listed although it shows moderate chemoselectivity and low enantioselectivity due to relevance of GDH-103 as a cofactor recycling enzyme and its activity toward **1**. [f] ee and absolute configuration is listed for trifluoromethyl hydroxyketone **4**.

are a number of enzymes whereby one can choose to produce either of the enantiomers with high yield. With perfect chemoselectivity, KRED-112 or KRED-A1i (Table 1, entries 1 and 4) can be used to synthesize the (*S*)-trifluoromethyl hydroxyketone **4**, while KRED-129, KRED-131, KRED-A1n, and KRED-A1x (Table 1, entries 2, 3, 5, and 6) produce the (*R*)-enantiomer **4**.

To confirm the results of the screen and isolate the chiral hydroxyketones, reactions were run at the 100-mg scale. These experiments indicate that **4** is synthesized by two routes. One route, demonstrated by KRED-112 (Table 1, entry 1), shows a perfectly chemoselective reduction of the trifluoromethyl carbonyl, producing the (*S*)-enantiomer of **4** cleanly with less than 1% bis-alcohol **10** and no production of the other regioisomer **7**. The second route to **4** is through a mechanism of chemoselective oxidation with the bis-alcohol **10** acting as an internal cofactor recycling reagent. This route (Figure 1) is demonstrated using alcohol dehydrogenase from *Rhodococcus erythropolis* (ADH-RE, Table 1, entry 14) which rapidly reduces methyl carbonyl of **1** to produce **7**. Gradually, compounds **4** and **10** begin to accumulate in the system. At 16 h, additional NADH was charged to drive the reaction forward and results in an increase of **7** and **10** with a decrease of **4**. At the end of the reaction, the sole products are **4** and **10** in a 1:1 mixture. Considering the instability of NADH under the reaction

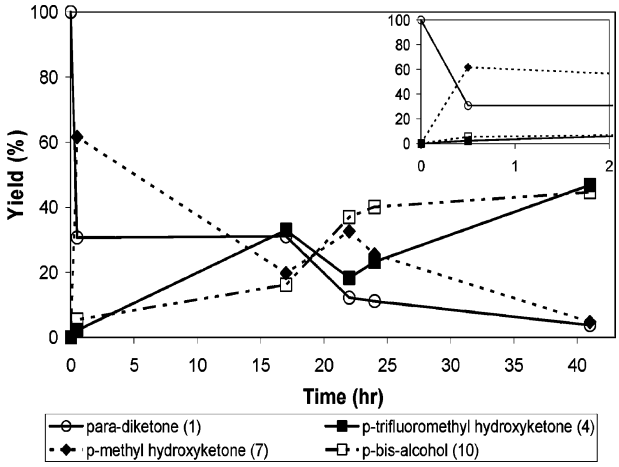


Figure 1. ADH-RE-catalyzed chemoselective oxidation of *p*-bis alcohol **10**. At 16 h timepoint, 1 equiv of NADH was added to drive the reaction to completion due to instability of the cofactor under the reaction conditions.[18]

conditions[18] and the initial reduction rates of the carbonyls, our hypothesis is that the oxidized cofactor (NAD^+) accumulates within the system and the enzyme chemoselectively oxidizes the methyl alcohol of **10** to yield highly enantiopure **4**. This oxidation was observed as a result of our screening conditions employing excess cofactor. In contrast, addition of an enzyme that recycles cofactor to the reaction to regenerate NADH yields bis-alcohol **10** as the sole product.

A similar regioselective oxidation of diols was presented by Edegger, et al.[7] as a route to provide enantiopure hydroxyketones. In order to prove that we were observing a chemoselective oxidation, we ran reactions using ADH-RE and charged racemic bis-alcohol (**10**) and excess NAD^+, which yielded 21% of **4** and no methyl hydroxyketone **7**. Therefore, the chemoselective oxidation of similar bis-alcohols using enzymes such as ADH-RE may allow a route to produce chiral hydroxyketones when combined with a system to recycle the oxidized cofactor.[19]

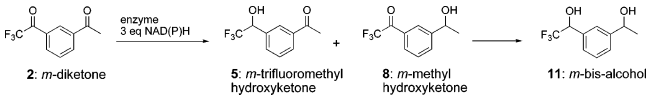
Identifying catalysts that chemoselectively reduce the methyl ketone proved to be more difficult. Yet as shown in Table 1 (entries 16 and 17), the (*S*)-enantiomer of the methyl hydroxyketone **7** can be synthesized. Two enzymes, the alcohol dehydrogenase from *Candida parapsilosis* (ADH-CP) and KRED-A1p demonstrated chemoselectivity toward the reduction of the methyl carbonyl producing the methyl hydroxyketone **7** with low levels of bis-alcohol **10** and none of the other regioisomer **4**. This result was confirmed at larger scale with a 94% yield, 98% ee, and less than 1% of **10**. The ability of the enzyme to successfully mediate this chemoselective reduction may be due to a combination of factors including a pseudoprotection of the trifluoromethyl carbonyl as a stable hydrate under aqueous conditions (observed by ^1H NMR), an electronic/hydrophobic effect of the fluorines within the active site, and a steric difference in

(18) Wu, J. T.; Wu, L. H.; Knight, J. A. *Clin. Chem.* **1986**, *3*, 314−319.
(19) Hummel, W.; Riebel, B. *Biotechnol. Lett.* **2003**, *25*, 51−54.

the relative size of a trifluoromethyl group as compared to the methyl analogue.[20]

The *m*-diketone **2** results indicate that both enantiomers of the *m*-trifluoromethyl hydroxyketone **5** can be synthesized with high ee and good chemoselectivity. The enzymes listed in Table 2 show the highest chemoselectivity toward the *m*-diketone under the screening conditions. Sixty-eight of the catalysts (94%) had a mixture of *m*-trifluoromethyl hydroxyketone **5** and bis-alcohol **11**. Overall, the chemoselectivity of the catalysts under the conditions employed was lower for the *m*-diketone **2** as compared to the *p*-diketone **1**. Again, the (*S*)-enantiomer of the *m*-trifluoromethyl hydroxyketone **5** can be synthesized in high ee (>99%) by a number of catalysts, such as KRED-A1i and KRED-112 (Table 2, entries 1 and 6) while the (*R*)-enantiomer was achieved using ADH-RE (Table 2, entry 14). A time course analysis of the reactions using 100 mg of **2** and the catalysts, KRED-112 (Table 2, entry 6) and KRED-A1y (Table 2, entry 2) revealed excellent chemoselectivity to **5** with <1% bis-alcohol **11** when harvested at complete consumption of the diketone **2**.

Table 2. Enzyme-Catalyzed Reduction of *m*-Diketone **2**



entry	enzyme	**5** yield (%)[b]	**8** yield (%)[b]	**11** yield (%)[b]	ee (%)[c,d]
1	KRED-A1i	89	0	11	>99 (*S*)
2	KRED-A1y	88	0	12	76 (*R*)
3	KRED-B1e	88	0	12	>99 (*S*)
4	KRED-124	87	0	13	>99 (*S*)
5	KRED-B1d	87	0	13	>99 (*S*)
6	KRED-112	86	0	14	>99 (*S*)
7	KRED-113	86	0	14	>99 (*S*)
8	KRED-115	86	0	14	96 (*S*)
9	KRED-129	86	0	14	63 (*R*)
10	GDH-103	85	0	15	15 (*S*)[e]
11	KRED-A1q	84	0	16	>99 (*S*)
12	KRED-126	83	0	17	98 (*S*)
13	ADH-LB	83	0	17	>99 (*S*)
14	ADH-RE	82	0	18	>99 (*R*)
15	CDX-013	78	0	22	>99 (*S*)
16	KRED-130	76	0	24	42 (*R*)
17	ADH-CP	4	76	20	90 (*S*)[f]

[a] Reaction conditions: 0.4 g/L enzyme, 2.5 g/L diketone, 3 equiv of NAD(P)H, 5% v/v DMF, in 100 mM potassium phosphate buffer (pH 7) at 30 °C. [b] Analyzed using achiral GC assay. [c] Analyzed using chiral SFC for **5** and chiral GC assays for **8**. [d] Absolute configuration for **5** and **8** was determined by using the modified Mosher method[21] and chiral hydroxyketones produced from the catalysts listed in entries 6 and 17. [e] Entry 13 is listed although it shows low enantioselectivity due to relevance of GDH-103 as a cofactor recycling enzyme and its activity toward **2**. [f] Absolute configuration is listed for methyl hydroxyketone **8**.

ADH-CP (Table 2, entry 17) produces the (*S*)-enantiomer of the *m*-methyl hydroxyketone **8** in moderate ee and chemoselectivity. A switch from the *p*-diketone to the *m*-diketone showed reduction of stereoselectivity and chemoselectivity for this catalyst.

In fact, there were a number of enzymes that showed differences in chemo- and stereoselectivity against the *p*- and *m*-diketones **1** and **2** under the same conditions. The substrate-dependent variations in enzyme selectivity are likely due to the role that steric and electronic effects play within the active site of these catalysts. Therefore, there is value in screening a moderate sampling of distinct enzymes[22] to ensure that one identifies the best catalyst for a particular substrate.

The use of readily available commercial ketoreductases has been demonstrated to be very effective for reducing the *p*- and *m*-diketones **1** and **2** with high ee and minimal formation of side products. We have successfully demonstrated the direct access to both enantiomers of the trifluoromethyl hydroxyketones **4** and **5**. Additionally, we have provided a route to the (*S*)-enantiomers of methyl hydroxyketones **7** and **8**.

Note Added after ASAP Publication. Compound **1** was incorrect in Table 1 in the version published ASAP October 31, 2007; the corrected version was published ASAP November 2, 2007.

Supporting Information Available: Experimental procedures for synthesizing the substrates and products as well as analytical methods and spectroscopic data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Timperley, C. M.; White, W. E. *J. Fluorine Chem.* **2003**, *123*, 65−70.
(21) Xiao, L.; Yamazaki, T.; Kitazume, T.; Yonezawa, T.; Sakamoto, Y.; Nogawa, K. *J. Fluorine Chem.* **1997**, *84*, 19−23.
(22) Homann, M. J.; Vail, R. B.; Previte, E.; Tamarez, M.; Morgan, B.; Dodds, D. R.; Zaks, A. *Tetrahedron.* **2004**, *60*, 789−797.