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Stereoselective synthesis of aryl γ,δ-unsaturated β-hydroxyesters by ketoreductases

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ABSTRACT

The biocatalytic reduction of aryl γ,δ-unsaturated-β-ketoesters was evaluated utilizing 24 different commercially available ketoreductases. In all cases, both (*R*) and (*S*)-enantiomers of γ,δ-unsaturated β-hydroxyesters were synthesized by one or more ketoreductases in excellent optical purity and yields.

1. Introduction

Enantiomerically pure γ,δ-unsaturated-β-hydroxyesters and their derivatives are important chiral building blocks for synthesis of many biologically active compounds, pharmaceutical products and their intermediates, such as (−)-CP_2-Disorazole C_1 [1], turnagainolides A and B [2], epothilone [3] and Seimatopolide A [4]. Many traditional methods have been addressed and provide pathways to make β-hydroxyesters asymmetrically, for example, (1) enantioselective Mukaiyama aldol addition between aldehyde and methyl (ethyl) acetate *O*-silyl enolates with a chiral Ti(IV) complexes as catalyst [5–7], (2) enantioselective ruthenium-catalyzed hydrogenation of saturated α-ketoesters [8], β-ketoesters [9–18] and α,β-unsaturated carbonyls [19,20], and (3) chiral oxazaborolidine-catalyzed reduction of prochiral ketones, cyclic and acyclic α, and β-enones [21–25].

All of these methods, while potentially providing high enantioselectivity, have disadvantages. The Mukaiyama aldol methods often require an expensive chiral ligand (e.g. BINAP) and multiple steps to make the catalyst [7]. While ruthenium-catalyzed hydrogenation has been shown to selectively reduce the carbonyl of β-ketoesters chemoselectively in the presence of a nonconjugated carbon–carbon double bond [10,26], we and others [27] have found that partial hydrogenation of the double bond of conjugated γ,δ-unsaturated-β-ketoesters often occurs, and is difficult to control. Recently, Ma et al. published a Ru-catalyzed hydrogenation of γ-halo-γ,δ-unsaturated-β-ketoesters in up to 97% ee [27], where halogen at the γ-position is needed to protect the γ,δ-carbon–carbon double bond with further organometallic transformation required to remove the vinyl halide moiety. The enantioselectivity of chiral oxazaborolidines catalysts are sensitive to moisture and temperature and must be conducted under strictly anhydrous conditions and typically at low temperature (−20 °C and below). Also, the use of BH_3·THF (or BH_3·Me_2S) is often incompatible with carbon–carbon double bonds. Although these chemical methods using chiral auxiliaries are frequently used, other limitations remain in many cases; the incompatibility of catalytic conditions with a number of functionalities [28–30] and the catalytic behavior (enantiomeric purity and productivity) being sensitive to even slight modifications in the substances due to the change of steric and electronic properties.

With the advantages of high enantioselectivity, broader substrate acceptance, mild and environmental friendly reaction conditions, tolerance of organic solvents and easy separation, biocatalysts using whole cells and isolated enzymes have received increasing interest toward the production of optically pure β-hydroxyesters and their derivatives [31–36]. For instances, the asymmetric reduction of α- and/or β-ketoesters was evaluated by means of whole cells of *Candida parapsilosis* ATCC 7330 (on the reduction alkyl 2-oxo-4-arylbutanoates) [37], the recombinant *Escherichia coli* (for the synthesis of 3-hydroxybutyrate) [38], *Chlorella strains* (toward the reduction of α-ketoesters) [39], yeast (reduction of α- and β-ketoesters) [34,40], carbonyl reductase from *Candila magnolia* [33], nicotinamide-dependent ketoreductases

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(reduction of β-ketoesters with alkyl substituents at α- or β-position) [41,42], and NADPH-dependent ketoreductases (toward the reduction of α-alkyl-β-ketoesters) [43]. What is more, *Candida parapsilosis* ATCC 7330-induced deracemisation of unsaturated aryl β-hydroxyesters to a single enantiomer has also been reported [44,45].

In this paper, we describe the synthesis of a series of aryl γ,δ-unsaturated-β-hydroxyesters from aryl γ,δ-unsaturated-β-ketoesters via the action of 24 isolated NAD(P)H dependent ketoreductases from Codexis. Either enantiomer of γ,δ-unsaturated β-hydroxyesters can be synthesized with high enantioselectivity (>99% ee) and chemoselectivity (no olefin reduction) in good to excellent yield in one step by one or more enzymes. Additionally, conversions and purifications were achieved economically, safely and readily under the enzymatic reaction condition used.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma–Aldrich Chemical Company unless otherwise noted. Spectroscopy grade chloroform was used for all optical rotation measurements. Cyclodextrins used for capillary electrophoresis, heptakis (2,3-di-O-methyl-6-O-sulfobutyl) cyclomaltoheptaose, sodium salt (NaSBDM-β-CD) and heptakis (2,3-di-O-ethyl-6-O-sulfopropyl) cyclomaltoheptaose potassium salt (KSPDE-β-CD), were synthesized according to procedures reported in the literature [46]. Codex® KRED screening kit was purchased from Codexis, Inc. Both reconstituted KRED Recycle Mix N (containing 250 mM potassium phosphate, 2 mM magnesium sulfate, 1.1 mM NADP+, 1.1 mM NAD+, 80 mM D-glucose, 10 U/mL glucose dehydrogenase, pH 7.0) and reconstituted KRED Recycle Mix P (containing 125 mM potassium phosphate, 1.25 mM magnesium sulfate, 1.0 mM NADP+, pH 7.0) are available from Codexis, Inc. Aluminum coated silica gel WF_{254s} plates were used to monitor reactions products and flash chromatography eluents. Column chromatography was performed with silica gel SiliaFlash®P60 (40–60 μm, 230–400 mesh).

2.2. Instrument

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with a Varian 300 MHz instrument. Chemical shifts are reported in ppm relative to TMS as internal standard. HPLC was performed on Agilent 1100 series with isocratic pump and UV-visible detector. A Phenomenex® Lux 3 μ cellulose-1 column (50 mm × 4.60 mm) was used for the chiral separation at 23 °C. The mobile phase consisted of hexanes and isopropanol in the ratio of 90:10, and flow rate of 0.5 mL/min. Optical rotations were measured on Krüss P3000 polarimeter operating at the sodium D line 589 nm and reported as follows: $[\alpha]_{589}^{23}$, concentration (g/100 mL), and solvent. Capillary electrophoresis was performed with an Agilent 3D Capillary Electrophoresis System using bare fused silica capillary (purchased from Polymicro Technologies, L.L.C.) (50 μm i.d., 32.5 cm total length, 24.0 cm to detector) under reverse polarity (−10 or −15 kV), and detection was by UV absorbance at 254 nm. Prior to first use, the capillary was primed for 2 min with 1 M NaOH solution and then for 2 min with 0.1 M NaOH solution. For each use, the capillary was preconditioned for 1 min using background electrolyte (BGE). BGE was either 5.0 mM NaSBDM-β-CD or 5.0 mM KSPDE-β-CD as chiral selector in 25 mM tris buffer, pH 2.5. Samples (diluted 10 fold into 90:10 v/v deionized water/acetone solvent) were injected into the capillary under 50.0 mbar pressures for 3 s. At the end of each run, the capillary was post-conditioned with 0.1 M NaOH solution for 1 min and then with deionized water for 1 min.

2.3. Synthesis of γ,δ-unsaturated β-keto ethyl esters **2A–2F**

(E)-Ethyl 4-(diethoxyphosphinyl)-3-oxobutanoate (2.234 g, 6.6 mmol), prepared according to the literature [47], in 20 mL anhydrous THF was reacted with 2.0 equiv. of n-BuLi (6.0 mL, 13.2 mmol, 2.2 M in hexanes) at 0 °C. After gas formation ceased, strirring was continued for 1 h at room temperature. One equivalent of aldehyde (6.0 mmol) was added over 10 min, and the reaction mixture was stirred at room temperature for another 2.5 h. After completion, the reaction was quenched by adding 15 mL of saturated NH_4Cl. The reaction mixture was concentrated under vacuum at 50–60 °C. The residue was extracted by CH_2Cl_2 (3× 15 mL), the combined organic extracts were washed with saturated NaCl (2× 15 mL), and then dried with anhydrous Na_2SO_4. The organic solvent was removed under vacuum at 30 °C. Product was isolated by chromatography on silica gel using 10:1 CH_2Cl_2/EtOAc and verified by ^1H and ^{13}C NMR spectroscopy.

2.4. Synthesis of racemic γ,δ-unsaturated β-hydroxyesters **3A–3F**

In a round bottom flask β-ketoester (1 mmol) was dissolved in 5 mL ethanol. In portion, cautiously and intermittently, 0.4 equiv. of $NaBH_4$ (15 mg, 0.4 mmol) was added and the mixture was stirred for 30 min at room temperature. The reaction mixture was concentrated under vacuum at 60 °C. The product was isolated by chromatography on silica gel using 10:1 CH_2Cl_2/EtOAc and verified by ^1H and ^{13}C NMR spectroscopy.

2.5. Stereoselectivity of enzymatic formation of β-hydroxyesters **3A–3F** using NADH system (enzyme 1–5)

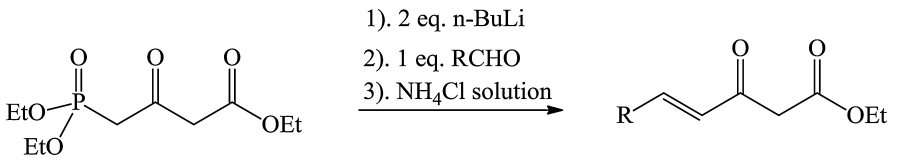
Into a solution of β-ketoester (25 μmol) in 50 μL methanol, was added KRED Mix N (57.4 mg in 1.0 mL deionized H_2O) and 1.0 mg ketoreductase. The mixture was shaken at 32 ± 1 °C. After 24 h, the reaction was extracted by EtOAc (2× 1 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and was subjected to chiral HPLC or CE analysis, and ^1H NMR spectroscopy.

2.6. Stereoselectivity of enzymatic formation of β-hydroxyesters **3A–3F** using NADPH system (enzyme 6–24)

Into a solution of β-ketoester (25 μmol) in 400 μL isopropanol and 50 μL methanol, was added KRED Mix P (29.1 mg in 1.0 mL deionized H_2O) and 1.0 mg ketoreductase. The mixture was stirred at 32 ± 1 °C. After 24 h, the reaction was extracted by EtOAc (2× 1 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and was subjected to chiral HPLC or CE analysis, and ^1H NMR spectroscopy. For β-ketoesters **2A–2E** except **2D**, the reactions were scaled up by a factor of 20 or 100 using enzymes that yielded high conversions and ee, and all products were isolated by chromatography on silica gel using 10:1 CH_2Cl_2/EtOAc and verified by ^1H and ^{13}C NMR spectroscopy.

2.7. Synthesis of MTPA ester of γ,δ-unsaturated β-hydroxyesters [48]

Into a solution of 64 μmol β-hydroxyester (racemic or made by enzyme 8 KRED-P1-B05 or enzyme **23** KRED-P3-G09) and dry pyridine (16 μL, 200 μmol, 3.1 equiv.) in 1.0 mL anhydrous CH_2Cl_2, was added the *R*-(−)-MTPA-Cl (23 μL, 120 μmol, 1.9 equiv.). The reaction mixture was stirred at ambient temperature till the reaction was completed as monitored by TLC plate (eluent: 10:1 = CH_2Cl_2:EtOAc) (usually 3 h). After completion, the reaction was quenched by 2 mL H_2O, extracted by EtOAc (2× 5 mL). The combined organic layer were dried over anhydrous Na_2SO_4 and removed under vacuo. The product MTPA ester of γ,δ-unsaturated



1

R

2A phenyl
2B 4-Me-phenyl
2C 4-Cl-phenyl
2D 4-NO_2-phenyl
2E 4-*n*Bu-phenyl
2F benzyl

Scheme 1. Synthesis of γ,δ-unsaturated-β-ketoesters from appropriate aldehydes and 4-(diethoxyphosphinyl)-3-oxobutanoate in the presence of two equivalents of n-BuLi.

β-hydroxyesters was isolated by chromatography on silica gel using 10:1 CH_2Cl_2/EtOAc. (*S*)-MTPA ester spectra are included in Supporting Information.

3. Results and discussion

3.1. Preparation of γ,δ-unsaturated β-keto ethyl esters **2A–2F**

By optimizing previously reported procedures [49–53], synthesis of γ,δ-unsaturated β-keto ethyl esters **2A–F**, shown in Scheme 1, was accomplished via the condensation of appropriate aldehydes with pretreated 4-(diethoxyphosphinyl)-3-oxobutanoate [47] by two equivalents of n-BuLi at room temperature. No *Z*-olefins were detected by ^{1}H NMR (*E*-olefins have a coupling constant with a value of 15 Hz in ^{1}H NMR spectrometry). The condensation afforded yields of 75–90% for **2A–E**, but the ketoester **2F** was formed in only 15% yield when phenylacetaldehyde **1F** was used under the same reaction condition. A significant by-product was obtained because α-hydrogen of phenylacetaldehyde is activated by the phenyl and formyl groups and easily deprotonated under basic conditions, followed by intermolecular aldol condensation.

3.2. Stereoselectivity of enzymatic formation of β-hydroxyesters **3A–3F**

The five *para*-substituted phenyl (**2A–E**) and one benzyl (**2F**) γ,δ-unsaturated β-ketoesters were selected as substrates to evaluate the stereoselectivity of twenty-four isolated ketoreductases listed in Table 1 from the Codex® ketoreductase screening kit which includes five wild type KREDs 1–5 and nineteen engineered KREDs 6–24. All KREDs reactions used NADPH recycle system except enzyme 4 (KRED-NADH-101) and 5 (KRED-NADH-110) which used NADH instead of NADPH, as shown in Scheme 2. For engineered KREDs 6–24 which have high tolerance to high concentration

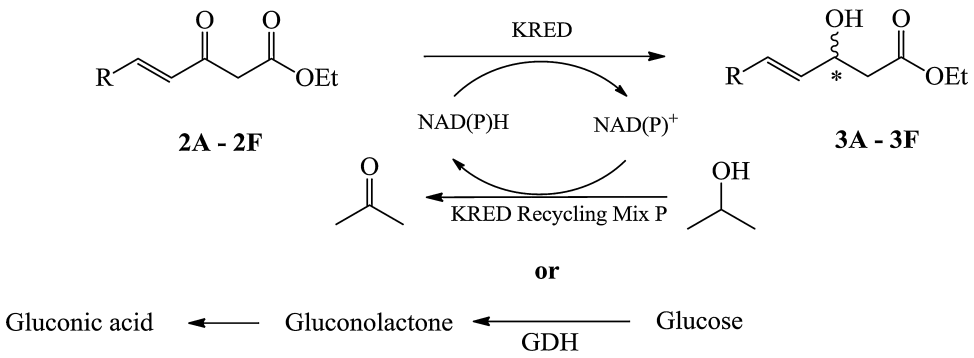
Table 1
Stereocontrolled reduction of γ,δ-unsaturated β-ketoesters to γ,δ-unsaturated β-hydroxyesters by 24 Codex® ketoreductases.

	KRED	**3A**		**3B**		**3C**		**3D**		**3E**		**3F**		**3F'**	
		e.e.[a,b] %	Conv.[c] (%)	e.e.[a,b] %	Conv.[c] (%)	e.e.[b] %	Conv.[c] (%)	e.e.[a,b] %	Conv.[c] (%)	e.e.[a] %	Conv.[c] (%)	e.e.[a] %	Conv.[c] (%)	e.e.[a] %	Conv.[c] (%)
1	101	>99	92	>99	67	>99	77	>99	62	>99	49	91	>99	–	–
2	119	>99	94	>99	96	94	95	>99	24	>99	87	96	>99	–	–
3	130	–>99	84	–>99	84	–93	85	–>99	6	–>99	74	–85	81	–	–
4	NADH-101	–>99	w	–>99	w	–	–	<3	<3	–	–	–>99	50	–	–
5	NADH-110	>99	94	>99	75	>99	66	>99	13	>99	8	>99	>99	–	–
6	P1-A04	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	P1-B02	84	88	90	90	>99	>99	>99	86	54	87	98	97	8	3
8	P1-B05	>99	>99	>99	79	>99	>99	>99	92	>99	>99	82	89	90	11
9	P1-B10	53	28	84	13	68	40	72	<3	–	–	52	<3	–68	<3
10	P1-B12	49	31	45	28	50	<3	>99	<3	–	–	38	<3	–76	<3
11	P1-C01	90	<3	–	–	–	–	–	–	–	–	–	–	–	–
12	P1-H08	98	<3	95	8	40	<3	54	<3	–	–	–	–	–	–
13	P1-H10	–	–	–	–	–	–	–	–	–	–	48	<3	14	<3
14	P2-B02	>99	>99	>99	>99	>99	>99	96	96	>99	>99	>99	97	95	3
15	P2-C02	>99	93	>99	>99	>99	>99	25	90	>99	>99	>99	98	>99	2
16	P2-C11	>99	96	>99	93	>99	>99	>99	96	>99	98	>99	94	>99	6
17	P2-D03	94	<3	–	–	80	<3	–	–	–	–	–	–	–	–
18	P2-D11	96	33	>99	10	92	<3	–	–	–	–	–	–	–	–
19	P2-D12	95	<3	96	<3	82	<3	–	–	–	–	–	–	–46	<3
20	P2-G03	>99	94	>99	97	>99	>99	>99	81	>99	>99	>99	>99	–	–
21	P2-H07	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22	P3-B03	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	P3-G09	–>99	15	–>99	38	–>99	>99	–>99	73	–>99	40	–>99	43	–>99	9
24	P3-H12	–>99	8	–>99	<3	–>99	20	–>99	43	–>99	15	–90	62	21	31

[a] The percent ee was measured by chiral HPLC. The positive ee value indicates that (*R*)-enantiomer is the major product, while negative ee value indicates that (*S*)-enantiomer is the major product. In each case, the racemic mixture was prepared for calibration. Absolute configuration of stereogenic C-3 was identified by ^{1}H NMR analysis of corresponding MTPA ester [48].

[b] The percent ee was determined by CE using charged β-CD as a chiral selector.

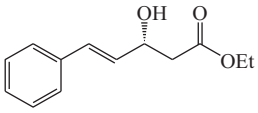
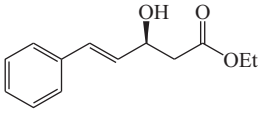
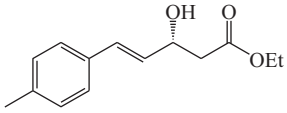
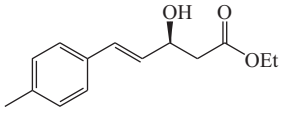
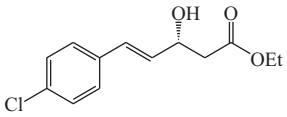
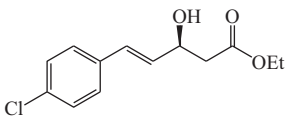
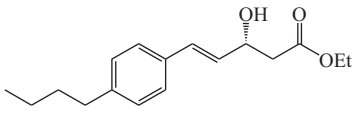
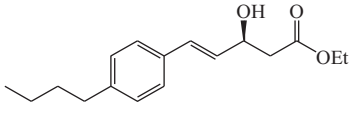
[c] The conversion was obtained by ^{1}H NMR integration using *d*-chloroform as a solvent. When <3% conversion is reported, the presence of byproducts made the measurement uncertain, although a small amount of product is detected. If no value is given, the product was not detected by either HPLC, CE, ^{1}H NMR spectroscopy or TLC.



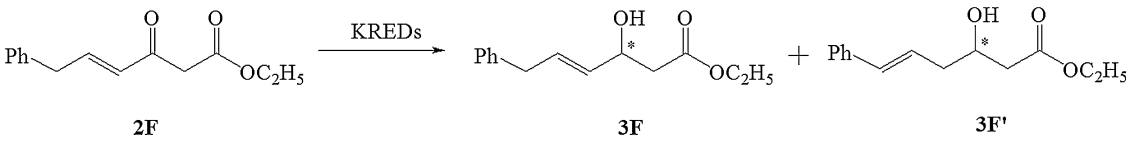
Scheme 2. Enzymatic reduction of γ,δ-unsaturated β-ketoesters with NAD(P)H-dependent Codex® ketoreductases.

of isopropanol (IPA), IPA was used to assist in dissolving poor water soluble substances and served to recycle NADPH cofactor from NADP+, and the reconstituted KRED recycle Mix P serviced as the reaction medium. However, for wild type KREDs 1–5, the reconstituted KRED recycle Mix N containing D-glucose/glucose dehydrogenase (GDH) and NAD(P)+ was used to provide a cofactor recycle system. In each case, methanol was used as co-solvent to enhance the solubility of β-ketoesters, but the amount of co-solvent was no more than 5% of the total volume of reaction solution as recommended in the protocol provided by Codexis.

Table 2
Isolated yields of γ,δ-unsaturated-β-hydroxyesters 3 using ketoreductases which provide both good yield and high enantioselectivity.

#	Substance	KRED	Isolated yield (%)	ee[a] (%)	$[\alpha]_{589}^{23}$
3A		P2-B02	90	>99	+14° (c 0.57, $CHCl_3$) lit. +13.6° [5]
		130	70	–>99	−16° (c 0.34, $CHCl_3$) lit. −2.6° [44], −6.8° [45]
3B		P1-B05	79	>99	+20° (c 0.10, $CHCl_3$)
		130	75	–>99	−15° (c 0.33, $CHCl_3$)
3C		P2-C11	85	>99	+15° (c 0.13, $CHCl_3$)
		P3-G09	81	–>99	−16° (c 0.16, $CHCl_3$)
3E		P1-B05	77	>99	+20° (c 0.10, $CHCl_3$)
		130	64	–>99	−11° (c 0.55, $CHCl_3$)

[a] The percent ee was measured by chiral HPLC.



2F 3F 3F'

Scheme 3. Enzymatic reduction of **2F** to **3F** and rearranged product **3F'**.

In the protocol provided by Codex®, 1–4 mg of KREDs was recommended to reduce 1 mmol of substance. Different from that in the protocol, in our experiment 40 mg/mmol ratio of KREDs relative to unsaturated β-ketoesters was used to perform all the microscale reactions (using 25 μmol of unsaturated β-ketoesters) shown in Table 1. All reactions were carried at 30–32 °C. Approximate yields for the microscale reactions were determined by integration of the vinyl hydrogen regions of the ^{1}H NMR spectra of the crude mixtures, which also served to establish whether substantial byproducts formed and whether the double bond was reduced. There was no evidence of double bond reduction in any reaction. When conversion of β-ketoesters was greater than 3%, only the presence of unreacted β-ketoesters and product β-hydroxyester was observed in the spectrum (See example spectra in Fig. 43s).

To determine the absolute configuration of β-hydroxyesters, the reductions of β-ketoesters with enzyme **8** and **23**, which provide >99% ee of R and S isomers, respectively, were scaled by a factor of 10 (0.25 mmol) by using the same relative ratio of enzyme to β-ketoesters as the microscale reaction. The Mosher ester method was then used to determine the absolute configuration of each enantiopure β-hydroxyester from enzyme **8** or **23** by comparison with the racemic mixture (Fig. 38s–42s) [48]. Optical rotations of both enantiomers of aryl γ,δ-unsaturated β-hydroxyesters **3** except **3D** were also measured (Table 2). Comparing the signs of measured values of 3A with those of known literature values, the absolute configurations of β-hydroxyesters were further confirmed [5,44]. The percent enantiomeric excess (% ee) was determined by either chiral HPLC or chiral capillary electrophoresis using a charged cyclodextrin as chiral selector (Fig. 44s–48s and Fig. 49s).

The data reported in Table 1 present the results of the reduction of γ,δ-unsaturated β-ketoesters under the action of 24 KREDs **1–24**. With exceptions of eight enzymes (P1-A04, P1-C01, P1-H08, P1-H10, P2-D03, P2-D12, P2-H07 and P2-D11) which have no (or very weak) effects on the conversion of β-ketoesters and two enzymes (P1-B10 and P1-B12) which provide low to medium enantiomeric excess of the desired β-hydroxyesters, most enzymes showed activity and excellent stereoselectivity for the reduction of β-ketoesters **2A–2F** toward β-hydroxyesters **3A–3F**. We do not discern any trends, either in terms of ee or conversion, as the substituent on the aromatic rings changes from weakly electron-donating (**2B** and **2E**, alkyl) to strongly electron-withdrawing (**2D**, nitro).

Both (*R*) and (*S*) enantiomers of each β-hydroxyester were produced impressively in optically pure form (>99% ee) by a number of KREDs. (*S*)-enantiomers β-hydroxyesters were made from four enzymes (130, NADH-101, P3-G09 and P3-H12) while (*R*)-enantiomers can be accessed using nine enzymes (101, 119, P1-B02, P1-B05, P2-B02, P2-C02, P2-C11, P2-D11 and P2-G03). For each β-ketoesters, at least one enzyme could be used to catalyze the formation of the corresponding (*S*)-enantiomer and several enzymes could be used to produce the corresponding (*R*)-enantiomer, both with excellent stereoselectivity and good conversion. For example, in the formation of (*S*)-**3A**, three out of four enzymes (NADH-101, P3-G09 and P3-H12) had low conversion with over 99% enantiomeric excess. However, enzyme **3** (**130**) could catalyze formation of (*S*)-**3A** with both excellent stereoselectivity (>99%) and conversion (84%). Seven out of nine enzymes catalyzed the formation of (*R*)-**3A** with over 99% ee and 83–99% conversion. Impressively, the ^{1}H NMR spectra of several crude products (e.g. those from enzyme 8) showed no impurities, and were essentially identical to the spectrum of the "purified" product.

For β-ketoesters **2A–E** except **2D**, the reactions were scaled up by a factor of 20 or 100 using chosen ketoreductases, as shown in Table 2. The product γ,δ-unsaturated β-hydroxyesters 3 were isolated by chromatography on silica gel using 10:1 CH_2Cl_2/EtOAc. Isolated yields were slightly lower compared to NMR yields in Table 1. The same optical purity was obtained in all scaled reactions, compared to the one obtained from crude product as shown in Table 1.

In the case of β-ketoester **2F**, 1,3-hydrogen rearrangement occurred to a small extent when some ketoreductases were used, which produced not only ethyl (4E)-3-hydroxy-6-phenylhex-4-enoate **3F**, but also ethyl (5E)-3-hydroxy-6-phenylhex-5-enoate **3F'**, as shown in Scheme 3. In the rearrangement process, the hydrogen was transferred without formation of the Z-isomer. Evidence of 1,3-hydrogen rearrangement comes from the HPLC ultraviolet absorption spectra of **3F** and **3F'** which show different absorption maxima (λ_{max}) for **3F** and **3F'**. Indeed, a λ_{max} at 250 nm is observed in the UV spectrum of **3F'** while a λ_{max} of 206 nm is the spectrum of **3F** (Fig. 34s and 35s). Further conclusive evidence for allylic rearrangement was obtained by examination of the ^{1}H NMR spectra of the crude products (Fig. 33s).

The pathway for the formation of **3F'** is not clear. Double bond migration of **2F** may occur prior to enzymatic reduction to **3F'**. Alternatively, 2F may be enzymatically reduced to **3F**, followed by isomerization to **3F'**. Current literature is lacking on the mechanistic aspects of this process and further studies will be required. Nevertheless, product (*R*)-**3F** can be obtained in pure form with high ee and conversion (Enzymes 5 and 20) but further optimization is required for obtaining pure (*S*)-**3F** with high yield and conversion.

4. Conclusion

We have developed an efficient enzymatic method utilizing isolated NAD(P)H dependent ketoreductases for the direct and stereoselective synthesis of either enantiomer of aryl γ,δ-unsaturated β-hydroxyesters in excellent optical purity (>99%) with good to excellent conversion for at least one ketoreductase. The scaled reaction with chosen enzyme shows that the isolated yields are very close to the conversion monitored by NMR spectroscopy and with same optical purity (>99%). These results suggest that Codexis ketoreductases offer potential for large scale synthesis of either enantiomer of aryl γ,δ-unsaturated-β-hydroxyesters.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.09.003.

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