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Stereoselective Reduction of α-Fluoro-β-ketoesters by NADH and NADPH-dependent Ketoreductases

Thomas K. Green*, Anil Damarancha, Matthew Vanagel, Brandon Showalter, Sandra Kolberg, and Alexander Thompson

Abstract: Racemic α-Fluoro-β-ketoesters were stereoselectively transformed to α-fluoro-β-hydroxyesters through dynamic reductive kinetic resolution (DYRKR) using commercially-available NAD(P)H-dependent ketoreductases. Aromatic, alkenyl and alkyl substrates were all reduced in high optical purities and yields. For most substrates, either *anti* or *syn* diastereomers could be produced with high enantiomeric excess, depending on the enzyme employed. The enzyme reactions with ethyl α-fluoroacetoacetate were conveniently monitored in real time by *in situ* ^{19}F NMR spectroscopy. These commercially-available enzymes provide convenient access to stereoisomers of α-fluoro-β-hydroxyesters from easily accessible racemic substrates.

Introduction

The introduction of the C-F bond into a compound can dramatically alter its physical and chemical properties[1] including its solubility, pK_a, and molecular conformation. Fluorine can also have a dramatic effect on the bioactive properties of pharmaceutical drugs, including potency, membrane permeability, enzyme binding, and metabolic stability.[2–7] Indeed, some of the best performing drugs on the market contain one or more fluorine atoms incorporated into their structure.[1,8] Due to the high, and often beneficial, impact of fluorine incorporation into drugs, the development of new methodologies for the synthesis of fluorine-containing compounds remains an active area of research. Given that most drugs possess one or more chiral centers, it comes as no surprise that recent efforts have focused on the stereoselective incorporation of fluorine. This paper focuses on the stereoselective synthesis of α-fluoro-β-hydroxyesters, with two chiral centers, using a set of commercially-available ketoreductases (KRED) from Codexis, Inc.

A number of chemical methods have been developed for the stereoselective synthesis of α-fluoro-β-hydroxyesters including radical reduction of α-bromo-α-fluoro-β-hydroxyesters,[9] using either trimethyl aluminum (*syn*) or tris(trimethylsilyl)silane (*anti*) as reductant. Using similar conditions, the α-allyl-α-fluoro-β-hydroxyesters could be produced with high diastereomeric excess.[10] β-Fluoro-α-hydroxyesters have been synthesized by enantioselective Pd-catalyzed β-fluorination of the α-ketoester followed by diastereoselective chemical reduction of the keto function.[11] *Anti*-α-fluoro-β-hydroxy ketones were synthesized with high stereoselectivity by L-prolinol-catalyzed aldol condensation.[12] Chiral fluorinated hydroxyketones, derived from p- and m-trifluoroacetyl substituted acetophenones, have also been synthesized in high yield and enantiomeric excess (>98%) using commercially available KREDs.[13] Racemic ethyl-α-fluoroacetoacetate was hydrogenated over a Cinchona-modified Pt catalyst to favor (2*S*,2*R*)-α-fluoro-β-hydroxyester in 82% enantiomeric excess and a 99/1 *anti*/*syn* ratio.[14] The aldolase-catalyzed reaction of fluoro pyruvate with aromatic and heteroaromatic aldehydes leads to *syn* *α*-fluoro *β*-hydroxy carboxylic acids with high diastereo- and enantioselectivity.[15]

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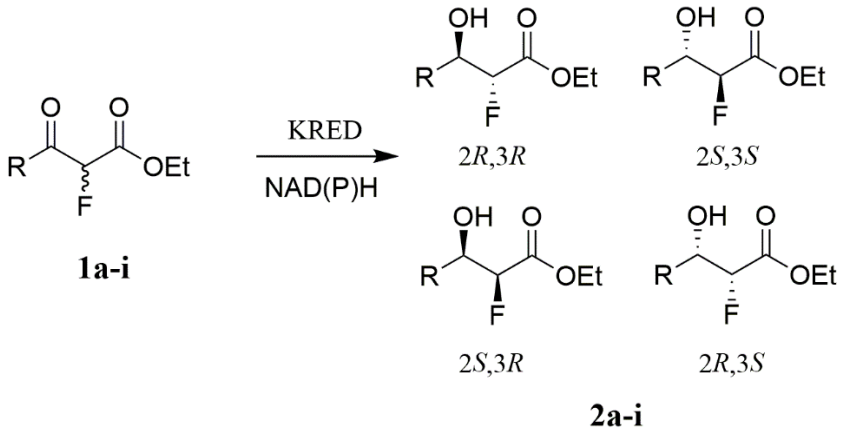
There are numerous reports of KRED-catalyzed enantioselective reductions of β-ketoesters to β-hydroxyesters possessing one chiral center.[16–21] KREDs have also been employed in the synthesis of α-alkylated- and α-chloro-β-hydroxyesters possessing two chiral centers (four stereoisomers).[22,23] These products form by reduction of the prochiral ketone with epimierzation [24] at the α-carbon, termed dynamic reductive kinetic resolution (DYRKR)[25], to often yield a single stereoisomer.[26] The stereoselective reduction of the ethyl α-fluoroacetoacetate on a large scale using a dehydrogenase has been accomplished, with 85% diastereomeric and >98% enantiomeric excess.[27] In this paper, we sought to broaden the scope of α-fluorinated-β-ketoesters as substrates that might be candidates for DYRKR. We employ 24 isolated NAD(P)H dependent ketoreductases from Codexis to reduce alkyl-, alkenyl- and aryl-substituted α-fluorinated-β-ketoesters. In many instances, a single stereoisomer of four possible α-fluorinated-β-hydroxyesters can be synthesized in high yield and high diastereo- and enantioselectivity.

Results and Discussion

The enzymatic reduction α-fluoro-β-ketoesters is shown in Scheme 1. Ethyl α-fluoroacetoacetate **1a** was obtained commercially as a racemic mixture. Substrates **1b** – **1i** were synthesized by Reformatsky reaction of the corresponding aldehyde with ethyl bromofluoroacetate.[28,29] followed by oxidation with Dess-Martin periodinane to the corresponding ketone (Supporting Information).[30] The Reformatsky product was a mixture of all four stereoisomers shown in Scheme 1, which was used as a standard for stereochemical analysis by NMR spectroscopy.



1a-i **2a-i**

R: **a**, methyl; **b**, pentyl; **c**, heptyl; **d**, 1-heptenyl; **e**, 1-undecenyl;
f, phenyl; **g**, p-methoxyphenyl; **h**, p-trifluoromethylphenyl; **i**, styrenyl

Scheme 1. Enzymatic reduction of α-fluoro-β-ketoesters.

Initially, the substrate (+/-)-ethyl-α-fluoroacetoacetate **1a** (R=CH_3) was subjected to KRED-catalyzed reduction for 24 hours at 37 °C using a set of 24 commercially-available ketoreductases obtained from Codexis, Inc. using conditions previously employed (Supporting Information).[16] Typically, 25, 50, 100 or 200 μmol of substrate was reacted in water/methanol with either isopropyl alcohol or glucose/glucose dehydrogenase for co-factor recycling. All KREDs utilize NADPH except for two enzymes, which utilize NADH. Crude products were analyzed by quantitative ^{19}F NMR spectroscopy to assess diastereomeric ratio (*anti*:*syn* ratio) and percent conversion, which were typically 86-97% isolated yields. Downfield ^{19}F NMR signals correspond to the *anti* configuration (2*S*,3*S* and 2*R*,3*R*) according to the literature.[28,31] The *anti* and *syn* configuration exhibit characteristic three-bond ^{19}F-^{1}H couplings of 17.8 and 22.1 Hz, respectively, which aids in identification. The *anti* and *syn* signals in the ^{1}H NMR spectra were identified by the three-bond ^{1}H-^{1}H coupling of 4.0 Hz and 3.3 Hz, respectively.[31]

KRED products were subjected directly without purification to *in-NMR-tube* Mosher ester derivatization[32,33] and analyzed by ^{19}F NMR spectroscopy to establish carbinol stereochemistry (C-3) and relative amounts of each stereoisomer (Supporting Information). The percent *anti* or *syn* diastereomer determined from separate integration of the ^{19}F resonances of the KRED and MPTA products agreed to within a standard deviation of ±1.1% (23 product analyses), indicative of complete and nonselective MPTA derivatization. The *anti*/*syn* ratios in Tables 1 and 2 were determined by integration of the MPTA ester ^{19}F resonances.

Preliminary screening results with all 24 enzymes with **1a** as substrate revealed that conversions were typically >99%, with no substrate detected by NMR spectroscopy after product work-up. Most KREDs show *anti* selectivity. Seven KREDs resulted in at least 90% *anti* configuration in high yield, as shown in Table I. Five KREDs produced the 2*S*,3*S* enantiomer as the predominant *anti* configuration (≥ 88%). Two KREDs produced the 2*R*,3*R* enantiomer as the predominant stereoisomer (88%). Six enzymes show *syn* selectivity, although less impressively at 53-73% relative to *anti* (Supporting Information).

Table 1. Enzyme-Catalyzed Stereoselective Reduction of (+/-)-Ethyl-α-fluoroacetoacetate (**1a**).[a],[b]

Entry	KRED	Anti/Syn[c]	2*R*,3*R*	2*S*,3*S*	2*S*,3*R*	2*R*,3*S*
1	119	88/12	nd[d]	88	nd	12
2	130	85/15	1	84	1	14
3	NADH-110	97/3	56	41	3	nd
4	P1-A04	83/17	83	nd	17	nd
5	P1-H10	97/3	88	9	3	nd
6	P2-C11	94/6	32	62	4	2
7	P2-G03	98/2	6	92	1	1
8	P2-H07	88/12	88	nd	12	nd
9	P3-B03	95/5	nd	95	nd	5
10	P3-G09	92/8	nd	92	nd	8
11	P3-H12	94/6	1	93	nd	6

[a] Conditions: Entries 1-3; *β*-ketoester (50 μmol), methanol (100 μL), KRED Mix N (114.8 mg in 2.0 mL H_2O), ketoreductase (2 mg), 37 ± 1 °C, 24 h: Entries 4-11; Same conditions except 2-propanol (800 μL) added and KRED Mix P (58.2 mg).

[b] Conversion to product >99%, except for P3-B03 (95%). Conversion determined from integration of ^{19}F resonances of products upon work-up. *In situ* ^{19}F NMR analysis of P1-H10, P2-C11, and P3-G09 reactions revealed formation of 8-20% of acid from ester hydrolysis, which was not recovered upon work-up.

[c] Percentages of stereoisomers determined by integration of the ^{19}F NMR resonances of the corresponding MPTA esters.

[d] Not detected.

The reactions of **1a** were also screened by *in situ* ^{19}F NMR spectroscopy for some enzymes to establish conversion products and rates. The reactions were carried out in both H_2O and D_2O. In each case, the substrate was mixed with co-factor recycling mix supplied by Codexis dissolved in H_2O or D_2O, methanol and/or isopropyl alcohol. Under these conditions, the ketoester **1a** was in equilibrium with the hydrate (~40%), as shown in Figure 1. Then enzyme was added, and the reaction monitored at 37°C by ^{19}F NMR spectroscopy. In D_2O, prior to adding enzyme, deuterium exchange takes place rapidly at the α-carbon (C-2), with exchange up to 98% deuterium incorporation in less than one hour. For all reactants and products, the ^{19}F chemical shifts of the α-deuterated form appeared upfield of the α-protiated form, as shown in Figure 1, with a change in multiplicity consistent with two-bond ^{19}F-D coupling. KRED DYRKR reactions in D_2O may offer convenient access to diastero- and enantiomerically pure [2-*d*]-2-fluoro-3-hydroxyacids and related derivatives. For example, stereochemically pure [2-*d*]-2-methyl-3-hydroxyacids have proved valuable in mechanistic studies of epimerization and reduction by ketoreductases,[24] but a lengthy six-step process was required for their synthesis.

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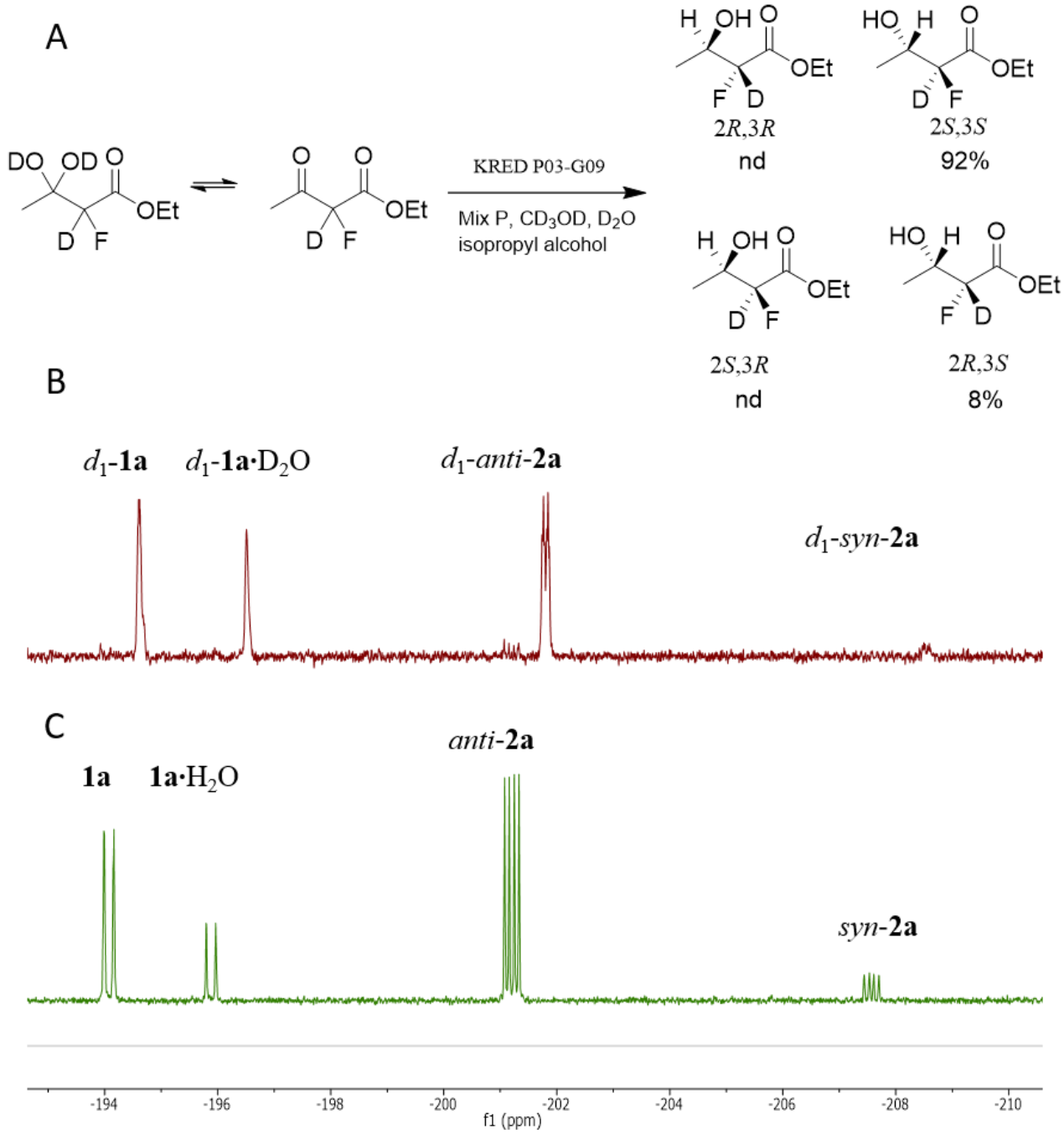

Figure 1. *In situ* monitoring of **1a** with KRED P03-G09 by ^{19}F NMR spectroscopy at 37°C; (A) Overview of reaction; (B) KRED P3-09 in D_2O; (C) P2-09 in H_2O. Reaction times were <30 min. Final product distributions were 2*S*,3*S* (92%) and 2*R*,3*S* (8%). Protiated forms of reactants appear at ~0.6 ppm higher frequency (left) of the deuterated forms. Stereochemistry was determined by ^{19}F NMR Mosher ester analysis.

For some enzymes, the conversion rate of **1a** was monitored in H_2O by ^{19}F NMR spectroscopy. Using P1-H10, P2-C11, and P3-G09, conversion to **2a** was complete in 1-2 hours. For P1-H10 and

P3-G09, disappearance of **1a** was linear up to ~80-90% at 17.2 mM substrate/mg KRED, but conversion using P2-C11 was linear only to ~20-30% at this concentration, as shown in Figure 2. Reactions were also monitored at higher concentrations of **1a** for P2-C11 (68.8 and 138 mM) using 1 mg of enzyme (Figure 3). Initial rates of disappearance of **1a** increased with substrate concentration, as shown in Figure 3, indicating that substrate concentrations were below saturation levels for P2-C11. The *in situ* ^{19}F NMR technique offers potential of establishing Michaelis-Menten constants for these reactions. KRED NADH-110 quantitatively converts **1a** to *anti* **2a (***anti:syn* 97:3, Table I) in *less than 10 min*, which was too rapid to follow by *in situ* ^{19}F NMR spectroscopy.

For the *in situ* reactions, we observed that 8 -20% of substrate is hydrolyzed to carboxylic acid as identified by the^{19}F doublet at 184 ppm (J=51.3 Hz).[34] Hydrolysis of the ester competes with ketoreduction by the enzyme and will lower yield. Hydrolysis occurs both in the absence and presence of enzyme; substrate **1a** was quantitative hydrolyzed within 5 h in the absence of enzyme using the Mix P system (Supporting Information). More rapid reduction (e.g. NADH-110) minimizes hydrolysis product. The carboxylic acid is, however, not recovered upon workup, presumably due to its high water solubility.

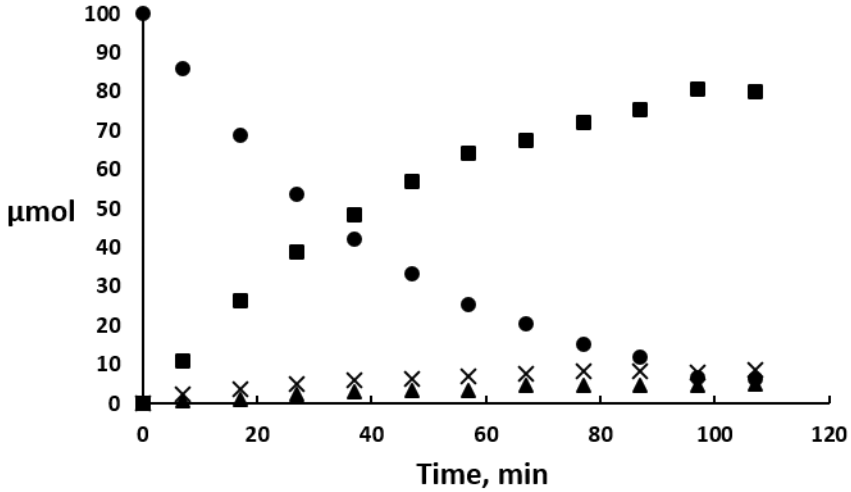

Figure 2. Conversion of **1a** with KRED P2-C11 monitored by *in situ* ^{19}F NMR spectroscopy at 37 ºC. Conditions: 100 µmol in 400 µL isopropanol and 50 µL methanol, KRED Mix P (29.1 mg in 1.0 mL H_2O) followed by 1.0 mg ketoreductase. Substrate concentration was 68.8 mM. ● **1a, 1a-H_2O;** ■ anti **2a**; ▲ syn **2a**; × acid of **1a**.

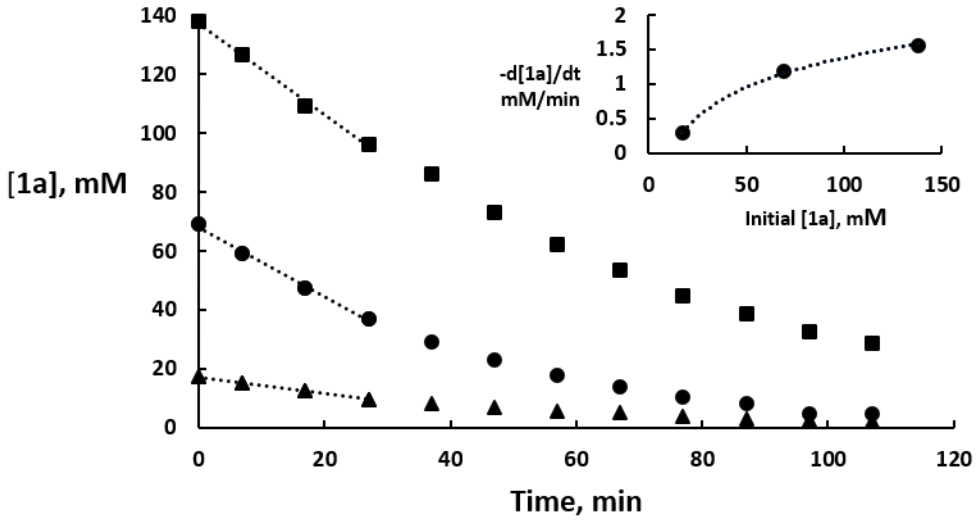

Figure 3. Disappearance of **1a** as a function of substrate concentration using KRED P2-C11. Conditions: See Figure 2. Concentrations: ■ 200 µmol, 138 mM; ● 100 µmol**,** 68.8 mM**;** ▲ 25 µmol, 17.2 mM. Initial slopes from 0-27 min: ■ -1.56; ● -1.18; ▲ -0.284 mM/min. Insert plot is rate of disappearance of **1a** (slope) against initial [**1a**].

Initial KRED reductions of substrates with larger R groups were carried out and analyzed in a similar manner using KREDs NADH-110 and 130, since these KRED resulted in rapid conversions with either high *anti* or *syn* selectivity. *In situ* ^{19}F NMR studies also indicated that these substrates were more resistant to ester hydrolysis than **1a**. For reactions, conversions were >99% by ^{19}F NMR spectroscopy. For all substrates, the ^{19}F NMR resonances of the *anti* diastereomers were more downfield. Additionally, the *anti* three-bond ^{1}H-^{19}F coupling constants were smaller (14-20 Hz) than the corresponding *syn* coupling constants (22-24 Hz), consistent with the literature. [28,31]

The results in Table 2 indicate that NADH-110 selects for *anti* configuration for all substrates, with 2*S*,3*S* isomer strongly favored in high enantiomeric ratio, especially for the aromatic substrates. KRED 130 showed *syn* selectivity for all substrates except **1a**, favoring the formation of the 2*S*,3*R* configuration in all cases. For the major diastereomer, high enantiomeric ratio was achieved especially for the aromatic substrates (**1f-1h**). Substrate **1g** was scaled (200 mg) using the same reactions conditions to obtain the same result in 77% yield. Substrate **1g** was also examined by *in situ* ^{19}F NMR with both KRED NADH-110 and 130. Using NADH-110, **1g** undergoes rapid, quantitative reduction to >99% 2*S*,3*S*-**2g** in *less than 10 min*. Using KRED 130, **1g** is reduced quantitatively to 90% 2*S*,3*R* in less than 30 min. In contrast, certain KREDs resulted in poor yield, for example, **1g** and P1-H10 gave only about ~10% **2g** (^{19}F NMR yields). Our studies also reveal that certain Codexis enzymes favor the *syn* 2*R*,3*S* isomer (P2-C02 and P1-B02, Table 2), demonstrating that 3 of 4 possible stereoisomers can be synthesized with high diastereomeric and enantiomeric ratio.

Table 2. Enzyme-Catalyzed Stereoselective Reduction of Racemic α-fluoro-β-ketoesters.[a],[b]

NADH-110	**Anti/Syn** [c]	**2*R*,3*R***	**2*S*,3*S***	**2*S*,3*R***	**2*R*,3*S***
1a	97/3	56	41	3	nd
1b	99/1	4	96	0	nd
1c	86/14	nd	86	3	11
1d	78/22	1	77	nd	22
1e	93/7	nd	93	nd	7
1f	99/1	nd	>99	nd	nd
1g	99/1	nd	>99	nd	nd
1h	99/1	nd	>99	nd	nd
1i	99/1	nd	>99	nd	nd
130	**Anti/Syn**	**2*R*,3*R***	**2*S*,3*S***	**2*S*,3*R***	**2*R*,3*S***
1a	85/15	1	84	1	14
1c	17/83	3	14	83	nd
1d	13/87	6	7	84	3
1e	14/86	5	9	79	7
1f	8/92	8	nd	92	nd
1g	10/90	10	nd	90	nd
1h	11/89	11	nd	89	nd
1i	23	77	16	77	nd
P2-C02	**Anti/Syn**	**2*R*,3*R***	**2*S*,3*S***	**2*S*,3*R***	**2*R*,3*S***
1d	17/83	4	13	2	81
1e	12/88	2	10	7	81
P1-B02	**Anti/Syn**	**2*R*,3*R***	**2*S*,3*S***	**2*S*,3*R***	**2*R*,3*S***
1d	9/91	5	4	5	86
1g	1/99	nd	nd	18	82

[a] Conditions: NADH-110 and 130; *β*-ketoester (50 μmol), methanol (100 μL), KRED Mix N (114.8 mg in 2.0 mL H_2O), ketoreductase (2 mg), 37 ± 1 °C, 24 h: P2-C02 and P1-B02; Same conditions except 2-propanol (800 μL) added and KRED Mix P (58.2 mg).
[b] Conversion to product >99%. Conversion determined from integration of ^{19}F resonances of products upon work-up.
[c] Percentages of stereoisomers determined by integration of the ^{19}F NMR resonances of the corresponding MPTA esters.

Conclusions

Of the substrates employed in this study, except for **1a**, we conclude that KRED NADH-110 selects for the 2*S*,3*S* configuration with high enantiomeric and diastereomeric ratio. For at least two substrates, **1a** and **1g**, reduction is rapid and quantitative, with reaction complete in less than 10 min. KRED 130 can be used to select for the 2*S*,3*R* configuration, and KREDs P1-B02 and P2-C02 can be used to select for the 2*R*,2*S* configuration. The presence of the ^{19}F nucleus allows for convenient *in situ* ^{19}F NMR monitoring of the enzyme reaction, as well as convenient analysis of Mosher ester products for stereochemical assignments and quantification. These α-fluoro-β-hydroxyesters may serve as useful intermediates in the synthesis of 2-fluorinated D-erythro sphingosine analogues by α-amination reactions.[35] Such intermediates may also prove useful toward synthesis of α-fluoro-α-amino acids.[36,37]

Experimental Section

Synthesis of racemic 2-fluoro-3-hydroxyesters: Racemic **2b-2i** were synthesized by Reformatsky reactions using zinc dust, ethyl bromofluoroacetate and the corresponding aldehyde as described in the literature.[28,29] See Supporting Information for experimental procedure, yield data and NMR characterization, including ^{1}H, ^{13}C, and ^{19}F NMR spectra.

Synthesis of 2-fluoro-3-ketoesters: Ketoesters **1b** and **1c** were synthesized by alkylation of ethyl-2-fluoro-acetoacetate **1a** according to the literature.[38] Ketoesters **1d-1i** were synthesized by Dess-Martin periodinane oxidation of racemic **2d-2i** according to the literature.[30] See Supporting Information for experimental procedure, yield data and NMR characterization, including ^{1}H, ^{13}C, and ^{19}F NMR spectra

KRED Screening Reactions: A procedure similar to previously described [16] was used. NADH system (Entries 1-3, Table I): Into a solution of *β*-ketoester (50 μmol) in 100 μL methanol, was added KRED Mix N (114.8 mg in 2.0 mL deionized H_2O) and 2.0 mg ketoreductase. The mixture was stirred at 37 ± 1 °C. After 24 h, the reaction was extracted by EtOAc (2 x 1 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and was subjected to ^{1}H and ^{19}F NMR spectroscopy.

NADPH system (Entries 4-11, Table I): Into a solution of *β*-ketoester (50 μmol) in 800 μL isopropanol and 100 μL methanol, was added KRED Mix P (58.2 mg in 2.0 mL deionized H_2O) and 2.0 mg ketoreductase. The mixture was stirred at 37 ± 1 °C. After 24 h, the reaction was extracted by EtOAc (2 x 1 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and was subjected to ^{1}H and ^{19}F NMR spectroscopy.

^{1}H and ^{19}F NMR spectra for crude KRED products were obtained in $CDCl_3$ at 300 MHz. ^{1}H NMR data for H-2 and ^{19}F NMR data were both diagnostic for *anti* and *syn* configuration. **2a:** Ethyl-2-fluoro-3-hydroxybutanoate (**2a**).[14] ^{1}H NMR (300 MHz, $CDCl_3$) *anti* δ 4.83 (dd, J = 48.5, 4.0 Hz, 1H, H-2), *syn* δ 4.75 (dd, J = 48.3, 3.3 Hz, 1H, H-2); ^{19}F NMR (282 MHz, $CDCl_3$) *anti* δ -201.05 (dd, J = 48.5, 17.8 Hz), *syn* δ -206.6 (dd, J = 48.3, 22.1 Hz).

KRED Scale-up reaction (100x): Ethyl (2*S*,3*S*)-2-fluoro-3-(4-methoxyphenyl)propanoate **(2g)**:[39] The reaction was scaled by a factor of 100 compared to the screening reactions. Into a solution of **1g** (200 mg, 0.823 mmol) in 1.67 mL methanol, was added KRED Mix N (1.91 g in

33.3 mL deionized H_2O) and 33.3 mg ketoreductase. The mixture was stirred at 37 ± 1 °C. After 24 h, the reaction was extracted by EtOAc (2 x 10 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and was subjected to ^1H, ^{13}C, and ^{19}F NMR spectroscopy; 156 mg, 77%, >99% 2*S*,2*S*. ^1H NMR (300 MHz, $CDCl_3$);[39] *anti* δ 7.29 (m, 2H), 6.86 (m, 2H), 5.03 (dd, J = 16.4, 4.9 Hz, 1H, H-3), 5.01 (dd, *J* = 49.5, 4.9 Hz, 1H, H-2), 4.17 (q, J = 7.1 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9 (d, *J* = 23.1 Hz), 159.7, 129.8 (d, *J* = 2.6 Hz), , 128.2 (d, *J* = 1.4 Hz), 113.8, 91.1 (*J* = 190.5 Hz), 73.2 (d, *J* = 21.9 Hz), 61.8, 55.2, 14.0; ^{19}F NMR (282 MHz, $CDCl_3$) *anti* δ -198.4 (dd, *J* = 49.8, 14.9 Hz).

Mosher ester synthesis and analysis. An *in-tube* derivatization similar to that previously described was used to synthesize MPTA (α-methoxy-α-trifluoromethylphenylacetic acid) esters of the product alcohols.[33] The NMR tube contained approximately 50 μmol of KRED-reduced product in 0.6 mL of $CDCl_3$. Half of the NMR tube volume (0.3 mL) was mixed with a $CDCl_3$ solution (0.3 mL) containing either *R*-MPTA or *S*-MPTA (0.0176 g, 75 μmol, 3 eq), N,N'-dicyclohexylcarbodiimide (DCC) (0.0154 g, 75 μmol, 3 eq), and 4-dimethylaminopyridine (DMAP) (0.00915 g, 75 μmol, 3 eq). A small quantify of 3 Å molecular sieves was added (0.5 cm in height). The NMR tube was then allowed to react overnight at 37°C. A solid precipitate sometimes formed on top, and usually redissolved upon mixing. The NMR tube contents was analyzed directly by ^{19}F NMR spectroscopy to assess stereoisomeric ratios. A minimum of 2000 transients was collected to minimize S/N ratio. A typical spectrum yielded the following chemical shift and coupling information. ^{19}F NMR spectrum of S-MPTA esters of **2d** (R = heptenyl). ^{19}F NMR (282 MHz, $CDCl_3$) 2*S*,3*S*, δ -199.0 (dd, *J* = 48.6, 21.2 Hz); 2*R*,3*R*, -201.1 (dd, *J* = 48.8, 21.9 Hz); 2*R*,3*S* -201.4 (dd, *J* = 47.4, 22.0 Hz); 2*S*,3*R*, -203.3 (dd, *J* = 47.2, 23.3 Hz).

Monitoring reaction by *in situ* ^{19}F NMR Spectroscopy. Substrate **1a** (25, 50, 100 or 200 μmol) was mixed with 400 μL isopropanol and 50 μL methanol, KRED Mix P (29.1 mg in 1.0 mL H_2O) followed by 1.0 mg KRED P1-H10, P2-C11, or P3-GO9. A capillary insert containing D_2O was placed in the NMR tube for locking and shimming purposes. A sequence of spectra was obtained in an arrayed experiment using the available software, with 64 transients per spectrum, collected every 10 minutes for 120 minutes. Spectra were processed, phased and baseline corrected prior to integration. Absolute areas of each integral were summed and used to calculate relative amounts of reactant and products.

Keywords: enantioselectivity, enzymes, ketoreductases, α-fluoro-β-hydroxyesters, α-fluoro-β-ketoesters

REFERENCES

[1] D. O'Hagan, *Chem. Soc. Rev.* **2008**, *37*, 308–319.

[2] N. A. Meanwell, *J. Med. Chem.* **2018**, *61*, 5822–5880.

[3] S. Swallow, *Prog. Med. Chem.* **2015**, *54*, 65–133.

[4] E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, *J. Med. Chem.* **2015**, *58*, 8315–8359.

[5] W. K. Hagmann, *J. Med. Chem.* **2008**, *51*, 4359–4369.

[6] S. Swallow, *Prog. Med. Chem.* **2015**, *54*, 65–133.

[7] B. E. Smart, *J. Fluor. Chem.* **2001**, *109*, 3–11.

[8] J. Wang, M. Sánchez-Roselló, J. L. Aceña, C. del Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok, H. Liu, *Chem. Rev.* **2014**, *114*, 2432–2506.

[9] K. Mima, T. Ishihara, S. Kuwahata, T. Konno, H. Yamanaka, *Tetrahedron* **2002**, *58*, 2369–2376.

[10] T. Ishihara, K. Mima, T. Konno, H. Yamanaka, *Tetrahedron Lett.* **2002**, *43*, 3493–3497.

[11] S. Suzuki, Y. Kitamura, S. Lectard, Y. Hamashima, M. Sodeoka, *Angew. Chemie - Int. Ed.* **2012**, *51*, 4581–4585.

[12] G. Zhong, J. Fan, C. F. Barbas, *Tetrahedron Lett.* **2004**, *45*, 5681–5684.

[13] B. T. Grau, P. N. Devine, L. N. DiMichele, B. Kosjek, *Org. Lett.* **2007**, *9*, 4951–4954.

[14] K. Szori, G. Szöllosi, M. Bartók, *Adv. Synth. Catal.* **2006**, *348*, 515–522.

[15] J. K. Howard, M. Müller, A. Berry, A. Nelson, *Angew. Chemie - Int. Ed.* **2016**, *55*, 6767–6770.

[16] Z. Dai, K. Guillemette, T. K. Green, *J. Mol. Catal. B Enzym.* **2013**, *97*, 264–269.

[17] T. Matsuda, R. Yamanaka, K. Nakamura, *Tetrahedron Asymmetry* **2009**, *20*, 513–557.

[18] D. Zhu, C. Mukherjee, J. D. Rozzell, S. Kambourakis, L. Hua, *Tetrahedron* **2006**, *62*, 901–905.

[19] N. J. Turner, *Nat. Chem. Biol.* **2009**, *5*, 567–573.

[20] I. A. Kaluzna, T. Matsuda, A. K. Sewell, J. D. Stewart, *J. Am. Chem. Soc.* **2007**, *126*, 12827–12832.

[21] G. W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol.* **2010**, *14*, 122–129.

[22] D. Kalaitzakis, J. David Rozzell, S. Kambourakis, I. Smonou, *Org. Lett.* **2005**, *7*, 4799–4801.

[23] G. A. Applegate, R. W. Cheloha, D. L. Nelson, D. B. Berkowitz, *Chem. Commun.* **2011**, *47*, 2420–2422.

[24] A. Garg, C. Khosla, D. E. Cane, *J. Am. Chem. Soc.* **2013**, *135*, 16324–16327.

[25] G. A. Applegate, D. B. Berkowitz, *Adv. Synth. Catal.* **2015**, *357*, 1619–1632.

[26] J. Mangas-Sánchez, E. Busto, V. Gotor, V. Gotor-Fernández, *Org. Lett.* **2013**, *15*, 3872–3875.

[27] D. Dascier, S. Kambourakis, L. Hua, J. David Rozzell, J. D. Stewart, *Org. Process Res. Dev.* **2014**, *18*, 793–800.

[28] R. J. Linderman, D. M. Graves, *J. Org. Chem.* **1989**, *54*, 661–668.

[29] A. R. Gholap, A. P. Chavan, *J. Chem. Res.* **2009**, *2003*, 374–376.

[30] S. D. Meyer, S. L. Schreiber, *J. Org. Chem.* **1994**, *59*, 7549–7552.

[31] R. Ocampo, J. R. Dolbier, K. A. Abboud, F. Zuluaga, *J. Org. Chem.* **2002**, *67*, 72–78.

[32] T. R. Hoye, C. S. Jeffrey, F. Shao, *Nat. Protoc.* **2007**, *2*, 2451–2458.

[33] J. Gao, S. Rajan, B. Wang, *Magn. Reson. Chem.* **2017**, *55*, 269–273.

[34] B. W. Thuronyi, T. M. Privalsky, M. C. Y. Chang, *Angew. Chemie - Int. Ed.* **2017**, *56*, 13637–13640.

[35] Z. Dai, T. K. Green, *J. Org. Chem.* **2014**, *79*, 7778–7784.

[36] D. P. Huber, K. Stanek, A. Togni, *Tetrahedron Asymmetry* **2006**, *17*, 658–664.

[37] A. Tarui, K. Sato, M. Omote, I. Kumadaki, A. Ando, *Adv. Synth. Catal.* **2010**, *352*, 2733–2744.

[38] M. F. Ibad, O. U. R. Abid, M. Adeel, M. Nawaz, V. Wolf, A. Villinger, P. Langer, *J. Org. Chem.* **2010**, *75*, 8315–8318.

[39] X. T. Huang, Q. Y. Chen, *J. Org. Chem.* **2002**, *67*, 3231–3234.