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Stereoselective Synthesis of Three Isomers of *tert*-Butyl 5-Hydroxy-4-methyl-3-oxohexanoate through Alcohol Dehydrogenase-Catalyzed Dynamic Kinetic Resolution

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Abstract: Regioselective reduction of the 5-keto group of *tert*-butyl 4-methyl-3,5-dioxohexanoate (**1**) leads to a stereodiad of *tert*-butyl 5-hydroxy-4-methyl-3-oxohexanoate (**2**). Alcohol dehydrogenases from *Lactobacillus brevis* (LBADH), *Rhodococcus* sp. (RS 1-ADH) and *Saccharomyces cerevisiae* (YGL157w) reduce **1** under dynamic kinetic resolution conditions, thereby establishing two chiral carbons with a single reduction step. While it had been shown previously that LBADH reduction of **1** stereoselectively leads to *syn*-(4*S*,5*R*)-**2**, alcohol dehydrogenase-mediated dynamic kinetic resolution now allows easy access to *syn*-(4*R*,5*S*)-**2** (RS 1-ADH; 97.6% *ee*, *syn*:*anti* = 92:8, 66% conversion, 37% isolated yield) and *anti*-(4*S*,5*S*)-**2** (YGL157w; 90% *ee*, *anti*:*syn* = 93:7, 64% conversion, 42% isolated yield), as well. Thus three out of four possible stereoisomers were formed selectively upon reduction of **1**.

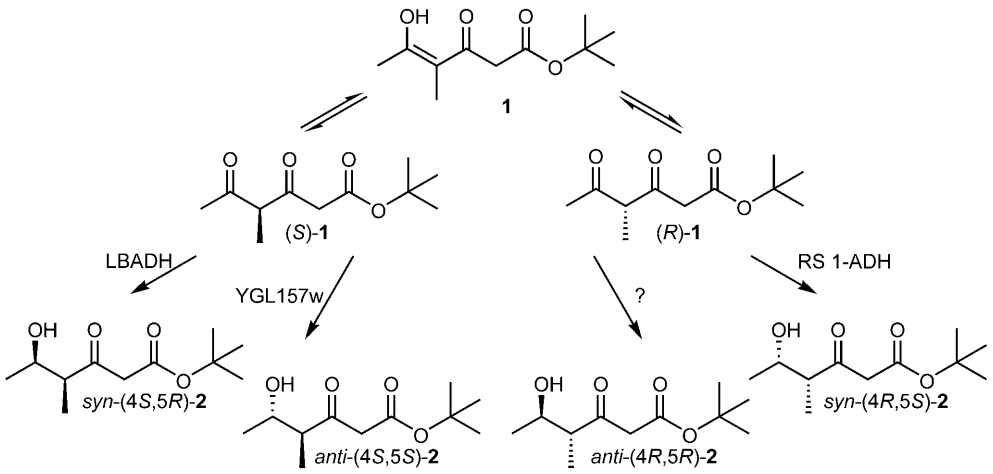
Keywords: alcohol dehydrogenase; enzyme catalysis; polyketide analogues; stereoselectivity

Introduction

Polyketides like macrolide antibiotics feature alternating motifs of keto or hydroxy groups and alkyl side chains.[1] Their arrangement and stereochemistry is well-defined and crucial for the active conformation of polyketidic drugs.[2] Since 5-hydroxy-3-oxohexanoates can serve as building blocks for the synthesis of polyketide analogues[3] or polyketide-derived natural products[4] they serve as important model compounds for polyketide chemistry. Methylated 5-hydroxy-3-oxohexanoates represent likewise prototypes for polyketidic structures formed of propionate subunits.[2]

Stereoselective synthesis of a methylated hydroxy keto ester through dynamic kinetic resolution had been introduced for reduction of **1**. The asymmetric carbon C-4 of formally chiral diketo esters (*R*)-**1** and (*S*)-**1** is subject to racemization through a tautomeric equilibrium with the corresponding hydroxy-ene species (Scheme 1). Using alcohol dehydrogenase from *Lactobacillus brevis* (LBADH), which prefers (*S*)-**1** as a substrate, *syn*-(4*S*,5*R*)-*tert*-butyl 5-hydroxy-4-methyl-3-oxohexanoate [*syn*-(4*S*,5*R*)-**2**] had been obtained with good yield and high stereoselectivity (99.2% *ee*, *syn*:*anti* = 97:3) for enzymatic reduction of the 5-keto group of **1**.[5]

The enantiomer *syn*-(4*R*,5*S*)-**2** had previously been obtained from Claisen condensation of *tert*-butyl acetate and *syn*-(2*R*,3*S*)-ethyl 2-methyl-3-hydroxybutanoate from baker's yeast whole cell reduction of ethyl 2-methyl-3-oxobutanoate.[5,6] For the stereoselective reduction of ethyl 2-methyl-3-oxobutanoate several enzymatic reactions had been described, leading to different stereoisomers of ethyl 2-methyl-3-hydroxybutanoate.[7] Thus, various stereoisomers of **2** should be accessible through Claisen condensation of stereoselectively obtained isomers of ethyl 2-methyl-3-oxobutanoate and *tert*-butyl acetate. From this approach, however, no information about the regioselectivity of the crucial reduction step can be drawn while stereoselective access to 5-hydroxy-4-methyl-3-oxohexanoates through dynamic kinetic resolution of **1** requires also highly regioselective reduction of the 5-keto group. This requirement is fulfilled for the two enzymes which have been used in this study. Reduction of **1** catalyzed by alcohol dehydrogenase 1 from *Rhodococcus* sp. (RS 1-ADH) selectively led to *syn*-



Scheme 1. Regioselective reduction of the 5-keto group of racemic **1** leads to a diastereomeric stereodiad. Since **1** racemizes rapidly through a tautomeric equilibrium, stereoselective and substrate-specific alcohol dehydrogenases allow dynamic kinetic resolution leading to enzyme-specific isomers of the stereodiad.

(4*R*,5*S*)-**2**, the enantiomer of the LBADH reduction product. The diastereomeric reduction product *anti*-(4*S*,5*S*)-*tert*-butyl 5-hydroxy-4-methyl-3-oxohexanoate was obtained selectively using a reductase from *Saccharomyces cerevisiae* (baker's yeast) encoded by the gene YGL157w.

Thus, three out of four possible isomers can be selectively accessed through dynamic kinetic resolution of the same substrate **1** (Scheme 1), with all enzymatic reactions being performed at neutral pH and ambient temperature and allowing for economical regeneration of cofactors.

Results and Discussion

Compound **1** was obtained through acylation of *tert*-butyl 3-oxovalerate using Weinreb acetamide as acylating agent.[5,8] LBADH-catalyzed reduction of **1** was reproduced as described in the literature.[5] The resulting *syn*-(4*S*,5*R*)-**2** was used as a reference for the assignment of relative and absolute configurations of the reduction products for new enzymatic reductions of **1**.

Using different variants of β-keto esters as substrates it could be shown that LBADH selectively leads to (*R*)-3-hydroxy esters[5,9] which suggests selective hydride transfer to the *si*-face of the carbonyl moiety (anti-Prelog-type stereospecificity).[10] For the production of hydroxy esters by baker's yeast whole cell reductions a Prelog-type stereospecificity is usually observed.[10] Whole cell reduction of the methylated β-keto ester ethyl 2-methyl-3-oxobutanoate predominantly leads to *syn*-(2*R*,3*S*)-ethyl 2-methyl-3-hydroxybutanoate.[11] Actually a systematic screening of carbonyl reductases from baker's yeast in most cases resulted in Prelog-type reduction of β-keto esters.[12] Similar to most reductases from baker's yeast, alcohol dehydrogenases from different *Rhodococcus* species also typically exhibit Prelog-type stereospecificity, as observed for substrate screenings with whole cell preparations of *Rhodococcus ruber*[13] and for isolated enzymes from *Rhodococcus ruber*[14] and *Rhodococcus erythropolis*.[15]

In order to obtain *syn*-(4*R*,5*S*)-**2** through reduction of **1** we used commercially available NADH-dependent alcohol dehydrogenase 1 from *Rhodococcus* sp. (RS 1-ADH, Julich Chiral Solutions GmbH, Jülich, Germany). For cofactor regeneration a coupled substrate process with 2-propanol was chosen.[16] RS 1-ADH in the presence of catalytic amounts of NAD^+ and a ten-fold excess of 2-propanol led to 66% (NMR) conversion of **1**. From NMR spectroscopy it was obvious that only the keto group at C-5 was reduced resulting in the formation of **2** as observed for LBADH reductions.[5] For the determination of the relative stereochemistry ^{1}H NMR spectroscopy was sufficient since signals for *syn* and *anti* reduction products could easily be distinguished (see Supporting Information, Figure S1). Assignments of significant *syn* and *anti* signals in the ^{1}H NMR spectrum had previously been described, e.g., for α-hydroxy-β-carbonyl compounds and for 5,6-dimethyldihydropyran-2,4-diones.[17] To obtain a diastereomeric mixture *syn*/*anti*-*rac*-**2** was synthesized non-stereoselectively by aldol addition of *tert*-butyl 3-oxovalerate and acetaldehyde. Signals for *syn*-**2** were assigned by using data of *syn*-(4*S*,5*R*)-**2** from LBADH reduction as a reference.[5] The corresponding signals of the RS 1-ADH reduction product appeared with identical chemical shifts and signal splitting as described for the product from LBADH-catalyzed reduction (see Supporting Information). Therefore, the relative configuration of the RS 1-ADH main reduction product was assigned to

syn-**2** with a diastereomeric ratio $dr_{syn:anti}$ of 90.5:9.5 (^{1}H NMR spectrum of raw product, not shown).

To distinguish the *syn*-hydroxy keto esters from LBADH- and RS 1-ADH-catalyzed reductions, respectively, we used vibrational circular dichroism (VCD; Figure 1 **a**). VCD signals of *syn*-**2**, however, are rather weak. Nevertheless, prominent difference signals at 1731 (−)/1697 (+) cm^{-1}, 1412 (+) cm^{-1} and 1394 (+)/1381 (−) cm^{-1} for *syn*-**2** from the RS 1-ADH-catalyzed reduction of **1** appear with opposite sign in the VCD difference spectrum of *syn*-(4*S*,5*R*)-**2** from the LBADH-catalyzed reduction. Therefore, from VCD and ^{1}H NMR data, the product from the RS 1-ADH-catalyzed reduction could be unequivocally identified as *syn*-(4*R*,5*S*)-**2**, the enantiomer of the product from the LBADH-catalyzed reduction of **1**.

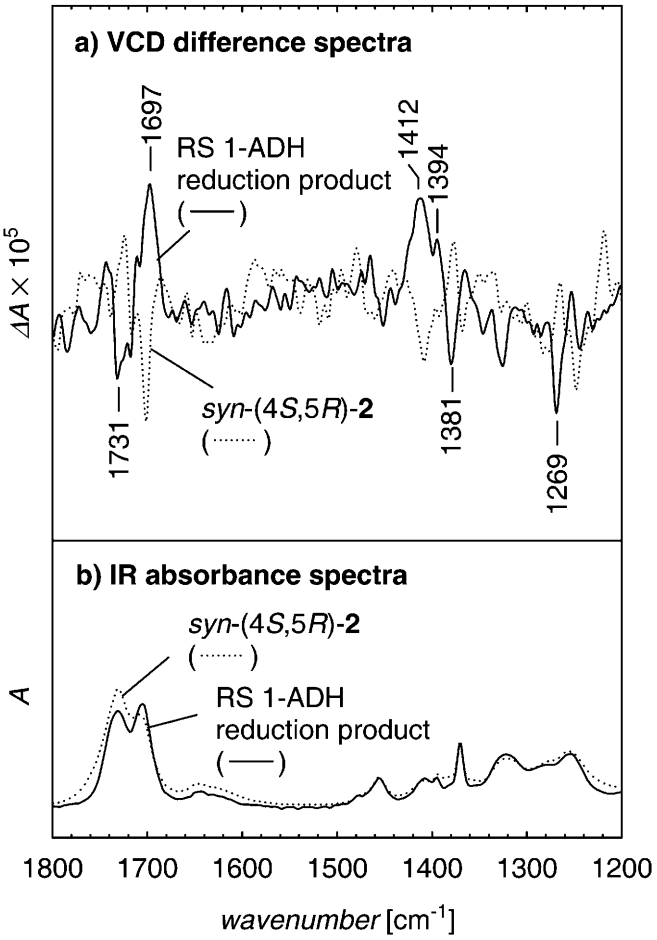


Figure 1. Vibrational spectra of *syn*-**2** from RS 1-ADH- and LBADH-catalyzed reductions of **1.** **a**) Vibrational circular dichroism (VCD) difference spectra corrected for $CDCl_3$ background in the spectral range between 1800 and 1200 cm^{-1}. Most prominent difference signals for the RS 1-ADH reduction product (black line) emerging at 1731 (−)/1697 (+) cm^{-1}, 1412 (+) cm^{-1}, 1394 (+)/1381 (−) cm^{-1} and 1269 (−) cm^{-1} appear with opposite sign compared to the VCD difference spectrum of *syn*-(4*S*,5*R*)-**2** (dotted line). **b**) IR absorbance (corrected for $CDCl_3$ background) of both samples in the same spectral range.

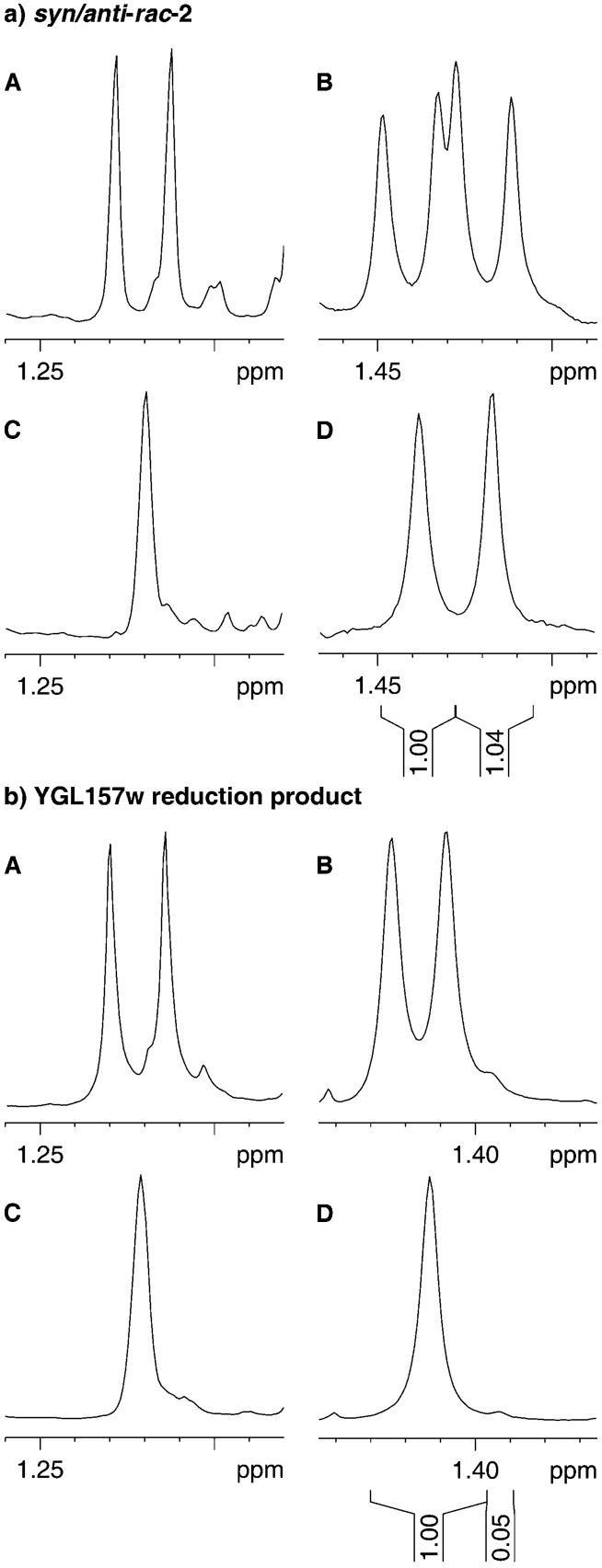
The enantiomeric composition of the products from the RS 1-ADH reduction was determined using chiral stationary phase (CSP) HPLC after derivatization of reduction product **2** (see Supporting Information, Figure S2).[5]

Now having selective access to both *syn* enantiomers of the stereodiad of **2**, we focused on an enzyme capable of selectively forming one of the desired *anti* products through dynamic kinetic resolution of **1**. An NADPH-dependent alcohol dehydrogenase from baker's yeast encoded by the gene YGL157w had been described to reduce the β-keto ester ethyl 2-methyl-3-oxobutanoate leading to *anti*-(2*S*,3*S*)-ethyl 3-hydroxy-2-methylbutanoate.[12] This enzyme also had previously been used for the Prelog-type reduction of the β,δ-diketo ester *tert*-butyl 6-chloro-3,5-dioxohexanoate in order to obtain (*R*)-*tert*-butyl 6-chloro-5-hydroxy-3-oxohexanoate,[10,18] a molecule that, like the (*S*)-enantiomer, can serve as a building block for natural products.[19]

We sub-cloned this gene from baker's yeast cDNA (Biocat, Heidelberg, Germany) into an expression vector and expressed the enzyme in *E. coli* BL21-(DE3) cells. Lysate containing recombinant YGL157w reduced **1** in the presence of excess NADPH. A coupled substrate cofactor regeneration process with 2-propanol, as it had been successfully applied for the RS 1-ADH reduction and the LBADH reduction, failed here. The enzyme evidently seems to be intolerant towards 2-propanol, which was indicated by precipitation of enzyme in a solution containing 2-propanol after reacting for 20 h. In a control experiment without 2-propanol no opaqueness was observed.

For the reduction of ethyl 2-methyl-3-oxobutanoate by YGL157w a different NADPH regeneration process was described using a three-fold excess of glucose 6-phosphate in the presence of glucose 6-phosphate dehydrogenase.[12] Since excessive use of glucose 6-phosphate might be non-economical for large-scale *in vitro* preparations we used glucose dehydrogenase from *Bacillus subtilis* subsp. *subtilis*[20] (Evocatal GmbH, Düsseldorf, Germany), instead. Using a five-fold excess of β-D-glucose, which is oxidized to D-glucono-1,5-lactone, resulted in a 64% conversion of **1** (NMR). The main reduction product could be easily identified as *anti*-**2** by ^{1}H NMR (see Supporting Information, Figure S1) with a diastereomeric ratio $dr_{anti:syn}$ of 93.4:6.6 (^{1}H NMR spectrum of raw product, not shown).

Only Prelog-type stereospecificity had been reported for the YGL157w-catalyzed reduction of keto esters in previous studies, where *anti*-(2*S*,3*S*)-ethyl 3-hydroxy-2-methylbutanoate[12] and (*R*)-*tert*-butyl 6-chloro-5-hydroxy-3-oxohexanoate[18] had been formed



upon YGL157w-catalyzed reductions of the respective keto esters. Consideration of these findings would lead to a 4*S*,5*S* stereochemistry of the product from the YGL157w-catalyzed reduction of **1**. This is in agreement with deductive assignment of the absolute configuration using the peaks of diastereomeric by-products in CSP-HPLC (see Supporting Information, Figure S2) and allows for the assignment of the YGL157w-catalyzed reduction product as *anti*-(4*S*,5*S*)-**2**.

Since the *anti*-**4** enantiomers could not be separated on CSP-HPLC (see Supporting Information, Figure S2), we employed ^{1}H NMR spectroscopy with 0.4 equivalents of a chiral lanthanide shift reagent (LSR). Europium(III) tris[3-(trifluoromethylhydroxymethylene)-D-camphorate] (Acros Organics, Geel, Belgium) was added for discrimination of the enantiomers and for calculation of *ee* values of *anti*-(4*S*,5*S*)-**2**.[21] This was conveniently achieved by observing the shift of the doublet corresponding to H-6 from the terminal C-6 methyl group (assigned by ^{1}H total correlation spectroscopy, see Supporting Information, Figure S2). Representing three protons, this signal is strong enough for reliable quantitative evaluations and shows high sensitivity towards chiral LSR without the interference of other signals.

The effect of the chiral LSR on the H-6 doublet of *syn*/*anti-rac*-**2** is shown in Figure 2 **a**. Formation of a diastereomeric complex with chiral LSR induces different chemical shifts for *anti*-(4*S*,5*S*)-**2** and for *anti*-(4*R*,5*R*)-**2** resulting in two doublets at 1.44 and 1.42 ppm, respectively (Figure 2 **a**, **B**). To facilitate quantitative evaluation we used proton decoupling, rendering two well separated singlets (Figure 2**a**, **D**). Integration of these singlets delivers for *syn*/*anti-rac*-**2** the expected statistical enantiomeric ratio of 1.00:1.04 [*anti*-(4*S*,5*S*)-**2** *vs. anti*-(4*R*,5*R*)-**2**].

Figure 2. Discrimination of the enantiomers of *anti*-**2** by ^{1}H NMR spectroscopy using chiral LSR. The doublet at 1.22 ppm representing H-6 (terminal C-6 methyl group of **2**, see Supporting Information, Figure S1, Figure S3) was used as a probe signal to quantify the enantiomeric composition of *anti*-**2** species. **a**) H-6 signals taken from the corresponding ^{1}H NMR spectra of *syn*/*anti-rac*-**2**: doublet without chiral LSR (**A**); two doublets representing both enantiomers of *anti*-**2** in presence of chiral LSR in a molar ratio of 0.4 (**B**); singlet by suppression of proton coupling to H-5 without chiral LSR (**C**); two singlets with a 1:1 ratio of signal integrals in presence of chiral LSR and proton decoupling (**D**). **b**) C-6 methyl group signals of the YGL157w reduction product: doublet without chiral LSR (**A**); single doublet representing *anti*-(4*S*,5*S*)-**2** in presence of chiral LSR with a molar ratio of 0.4 (**B**); singlet without chiral LSR and proton decoupling (**C**); two singlets with 1:0.05 ratio of signal integrals in presence of chiral LSR and proton decoupling allowing calculation of 90% *ee* for *anti*-(4*S*,5*S*)-**2** after YGL157w-catalyzed reduction of **1** (**D**).

Since the exact ratio of analyte *vs.* LSR is difficult to reproduce, chemical shifts in the corresponding experiment for the YGL157w reduction product (Figure 2**b**, **B**) deviated slightly from the experiment with *syn*/*anti*-*rac*-**2** (Figure 2**a**, **B**). In Figure 2**b**, **B** only one doublet at 1.42 ppm representing *anti*-(4*S*,5*S*)-**2** can be observed. The respective doublet for *anti*-(4*R*,5*R*)-**2** appears as a shoulder at 1.40 ppm. Proton decoupling delivered two separated singlets with an area ratio of 1.00:0.05 (Figure 2**b**, **D**) leading to an *ee* value of 90% for the synthesis of *anti*-(4*S*,5*S*)-**2** by YGL157w reduction of **1**.

Conclusions

Using easily accessible alcohol dehydrogenases LBADH, RS 1-ADH and YGL157w three out of four stereoisomers of **2** could be obtained through dynamic kinetic resolution of **1**, which is the same starting material for all reactions (Scheme 1). An overall conversion of $>50\%$ proved the concept of dynamic kinetic resolution for RS 1-ADH (66%) and YFL157w (64%) catalyzed reductions. Using the 6-carbon β,δ-diketo ester **1** as a substrate and taking advantage of the excellent regioselectivity of the tested enzymes, this study supplements previous data on alcohol dehydrogenase-catalyzed dynamic kinetic resolution of alkylated β-keto esters and 1,3-diketones.[7,22] The theoretical yield of dynamic kinetic resolution is 100%, which suggests that the presented methods have an even higher potential than that shown here. Therefore, improvement of biocatalysis conditions should be subject to future studies, in particular since moderate resolution of **1** and **2** leads to reduced yield after chromatography (37%; 42%).

Nevertheless, the described enzymatic reductions of **1** catalyzed by LBADH,[5] RS 1-ADH and YGL157w allow easy, highly stereoselective and cost-effective access to polypropionate-like building blocks, since they work at neutral pH and ambient temperature with efficient regeneration of cofactors.

syn-(4*S*,5*R*)-**2**, *syn*-(4*R*,5*S*)-**2** and *anti*-(4*S*,5*S*)-**2** could be obtained using biocatalysis by recombinant alcohol dehydrogenases which are not connected to polyketide synthesis in their host organisms. Stereoselective synthesis of *anti*-(4*R*,5*R*)-**2** through enzyme-catalyzed reduction of **1** might be challenging, however, since the desired (*R*,*R*)-*anti*-stereochemistry is rarely found in natural metabolites. A solution to this problem might be TylKR$_1$, a ketoreductase domain from the tylosin polyketide synthase complex, which had been shown to deliver selectively *anti*-(2*R*,3*R*)-3-hydroxy-2-methylpentanoic acid *N*-acetylcysteamine thioester upon reduction of the racemic dipropionate 2-methyl-3-oxopentanoic acid *N*-acetylcysteamine thioester.[23] Furthermore, stereoselective anti-Prelog-type reductions of 4-carbon β-keto esters had also been performed by non-PKS derived enzymes from yeast organisms.[24] Future studies will have to deal with the question if such enzymes are applicable for stereoselective synthesis of *anti*-(4*R*,5*R*)-**2**, the missing isomer of the stereodiad.

Experimental Section

Molecular Cloning of Baker's Yeast Reductase Gene YGL157w in *E. coli*

Molecular biology was carried out using standard procedures.[25] For all DNA procedures enzymes from commercial suppliers were used as recommended in the standard kit protocols. The YGL157w gene was amplified by PCR from a baker's yeast cDNA clone purchased from Biocat, Heidelberg, Germany. Primers used for PCR were synthesized by MWG, Ebersberg, Germany. The synthetic oligonucleotides carried a stop codon on the 3′-terminus and restriction sites for *NdeI* and *XhoI* (New England Biolabs GmbH, Frankfurt, Germany). The PCR amplified YGL157w was inserted into a pET19b vector (Novagen, Darmstadt, Germany). The resulting vector was transformed in TG1 competent *E. coli* cells for amplification.

Overexpression of YGL157w in *E. coli* BL21(DE3) cells

E. coli BL21(DE3) cells transformed with the YGL167w-containing expression vector were grown in 800 mL Lennox LB-broth (10% tryptone, 0.5% yeast extract, 0.5% NaCl; Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 100 mg/L of ampicillin. The culture was shaken at 37 °C (120 rpm) until an optical density (600 nm) of 0.5–0.8 was reached. Then expression was started by adding IPTG (final concentration 400 μM) and the culture was shaken for additional 6 h at 37 °C (120 rpm). Cells were finally collected by centrifugation and washed with water. 3 g of cell pellet were resuspended in 40 mL KPi 50 mM, pH 7.0 containing 10 mM of PMSF protease inhibitor. For cell lysis 40 mg of lysozyme were added and incubated on ice for 1 h before ultrasonic cell disruption using 40 s pulse cycles of a Branson Sonifier 250 (Branson Ultrasonics, Danbury, CT, USA). Cell debris was removed by centrifugation at 7800 × *g* for 30 min at 4 °C.

tert-Butyl 3-Oxopentanoate

Diisopropylamine (14 mL, $\rho = 0.754$ g/mL, 90 mmol) was suspended in 250 mL of THF. *n*-Butyllithium (57 mL, 1.6 M in *n*-hexane, 90 mmol)) was added at 0 °C to create LDA. After cooling to −75 °C *tert*-butyl acetate (11 mL, $\rho = 0.866$, 82.5 mmol) was added and stirred for 30 min before the reaction vessel was transferred into a cooling bath where a temperature of −40 °C was established by a cryostat. Then ethyl propionate (2.5 mL, $\rho = 0.885$, 25 mmol) was added and stirred for 2 h. Afterwards it was quenched with 12.5 mL of a 1:4 (v/v) mixture of glacial acetic acid in water at −40 °C. After warming up to room temperarture the reaction mixture was extracted twice with methyl *tert*-butyl

ether. The combined organic phases were washed with brine and dried over $MgSO_4$. Vacuum distillation at 1 mbar and 40 °C afforded a pale yellow oil; yield: 2.0 g (47%). Analytical data were consistent with literature values.[26]

tert-Butyl 4-Methyl-3,5-dioxohexanoate (1)

Ester **1** was synthesized by acylation of *tert*-butyl 3-oxopentanoate using Weinreb amide,[8] *N*-methoxy-*N*-methylacetamide, in accordance with the literature method.[5]

tert-Butyl 5-Hydroxy-4-methyl-3-oxohexanoate (*syn*/*anti*-*rac*-2)

NaH (416 mg, 60% in petroleum, 10.4 mmol) was suspended in THF (40 mL) and chilled on ice. *tert*-Butyl 3-oxopentanoate (1.2 mL, 8 mmol) was added dropwise, stirred for 10 min and cooled to −50 °C. *n*-Butyllithium (6.5 mL, 1.6 M, 10.4 mmol) was added dropwise at −45 °C and stirred for another 20 min. Chilled acetaldehyde (350 mg, 8 mmol) was added at −20 °C, warmed to room temperature and stirred for 30 min. Then it was quenched in 60 mL of a 1:1 (v/v) mixture of saturated NH_4Cl solution and ethyl acetate. After phase separation the aqueous phase was extracted twice with ethyl acetate (50 mL). The pooled organic phases were washed twice with brine and dried over $MgSO_4$. Flash column chromatography on silica (mobile phase: cyclohexane/ethyl acetate 2:1, $R_f = 0.33$) afforded *syn*/*anti*-*rac*-**2** as a yellow oil; yield: 741 mg (43%). For ^{1}H NMR and ^{13}C NMR data, see Supporting Information.

Enzymatic Reductions

Enzyme activity was measured before performance of enzymatic reactions and was calculated as enzyme units (U, that is, μmol/min) from linear regression of the decay of NAD(P)H absorbance (340 nm; $\varepsilon = 6{,}220\ M^{-1} \times cm^{-1}$) in a buffer solution containing the enzyme and a respective standard substrate. LBADH was tested in a 11 mM solution of acetophenone in 50 mM KPi (pH 6.5) containing 1 mM $MgCl_2$ and 190 μM of NADPH. RS 1-ADH was assayed in a 1.5 mM solution of *p*-chloroacetophenone in 50 mM KPi (pH 6.5) containing 200 μM of NADH. YGL157w activity was measured in a 11 mM solution of ethyl acetoacetate in 50 mM KPi (pH 7.0) containing 190 mM of NADPH.

***syn*-(4*S*,5*R*)-*tert*-Butyl 5-hydroxy-4-methyl-3-oxohexanoate [*syn*-(4*S*,5*R*)-2]:** Prepared by LBADH-catalyzed reduction of **1** as described in the literature.[5]

***syn*-(4*R*,5*S*)-*tert*-Butyl 5-hydroxy-4-methyl-3-oxohexanoate [*syn*-(4*R*,5*S*)-2]:** 107 mg (500 μmol) of **1** were dissolved in 385 μL (5 mmol) of 2-propanol and dispersed in 100 mM KPi buffer (pH 6.5). 2 mL (25 mM, 37.5 μmol) of NAD^+ solution were added for a final concentration of 1 mM. The reaction was started by addition of 25 U of RS 1-ADH (70 U/mL) for a final enzyme activity of 1 U/mL and stirred for 24 h at room temperature using a fish-clip to avoid enzyme degradation by shearing. The reaction was stopped by adding 4 g of NaCl. After extracting three times with ethyl acetate (30 mL) it was filtered through Celite 535 coarse and dried over $MgSO_4$. Purification by flash column chromatography on silica (mobile phase: cyclohexane/ethyl acetate 2:1, $R_f = 0.33$) afforded *syn*-(4*R*,5*S*)-**2** as a pale yellow oil; yield: 40 mg (37%). For ^{1}H NMR and ^{13}C NMR data see Supporting Information.

***anti*-(4*S*,5*S*)-*tert*-Butyl 5-hydroxy-4-methyl-3-oxohexanoate [*anti*-(4*S*,5*S*)-2]:** 174 mg (800 μmol) of **1** were dispersed in 50 mM KPi buffer (pH 7.0). 3.2 mL (12.5 mM, 40 μmol) of $NADP^+$ solution and 720 mg (4 mmol) of β-D-glucose were added for a final concentration of 1 mM $NADP^+$ and 100 mM glucose. YGL157w-containing lysate (20 mL, 1.7 U/mL, 40 U) was added establishing a final enzyme activity of 1 U/mL. The reaction was started by addition of GDH (80 μL, 1.5 kU/mL, 120 U) for a final enzyme activity of 3 U/mL. The total volume was adjusted to 35 mL. The reaction mixture was stirred at room temperature using a fish-clip to avoid shearing. The reaction was stopped after 24 h by adding 6 g of NaCl. After extracting three times with ethyl acetate (50 mL) it was filtered through Celite 535 coarse and dried over $MgSO_4$. Flash column chromatography on silica (mobile phase: cyclohexane/ethyl acetate 2:1, $R_f = 0.33$) afforded *anti*-(4*S*,5*S*)-**2** as a pale yellow oil; yield: 73 mg (42%). For ^{1}H NMR and ^{13}C NMR data see Supporting Information.

General Procedure for Preparation of α,β-Unsaturated Lactone Derivatives for Chiral Solid Phase (CSP) HPLC

Enzymatic reduction products **2** and *syn*/*anti*-*rac*-**2** from the non-stereoselective aldol addition were derivatized prior CSP-HPLC[5] to achieve better chromatographic performance and higher sensitivity of UV detection. To avoid detection of artificial enantiomeric impurities through $NaBH_4$ reduction of **1**, only purified reduction products were used for the derivatization reactions. All reaction steps were performed in 1 mL glass vials. 3 μL of *syn*/*anti*-*rac*-**2**, *syn*-(4*S*,5*R*)-**2**, *syn*-(4*S*,5*R*)-**2** or *anti*-(4*S*,5*S*)-**2** were dissolved in 250 μL of toluene. A small amount of $NaBH_4$ was added and shaken for 1 h at room temperature and 300 rpm (Eppendorf Thermomixer comfort). Then 60 μL of acetic acid were added, the mixture was washed with 250 μL of brine and extracted with 250 μL of toluene. A small amount of *p*-toluenesulfonic acid was added to the combined organic phases and the vial was sealed. After heating for 3 h at 118 °C it was washed with 300 μL of $NaHCO_3$ 5% solution containing a small amount of NaCl. After removing the solvent under vacuum the resultant *syn*/*anti*-*rac*-**4**, *syn*-(5*R*,6*R*)-**4**, *syn*-(5*S*,6*S*)-**4** or *anti*-(5*R*,6*S*)-**4** could be used for CSP-HPLC analysis.

^{1}H NMR Shift Experiments using Chiral LSR

For shift experiments we mixed 635 μL of 80 mM solutions of *syn*/*anti*-*rac*-**2**, *syn*-(4*S*,5*R*)-**2**, *syn*-(4*R*,5*S*)-**2**, and *anti*-(4*S*,5*S*)-**2** in $CDCl_3$ with different aliquots of a 250 mM solution of europium(III) tris[3-(trifluoromethylhydroxymethylene)-D-camphorate] (Acros Organics, Geel, Belgium) in $CDCl_3$ to test for the optimal sample/LSR ratio. Best separation of enantiomers was achieved by adding 76 μL of reagent solution corresponding to a molar ratio of 0.4.

Supporting Information

General methods and spectral data are given in the Supporting Information.

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