Dynamic kinetic resolution of *tert*-butyl 4-methyl-3,5-dioxohexanoate through enzymatic reduction

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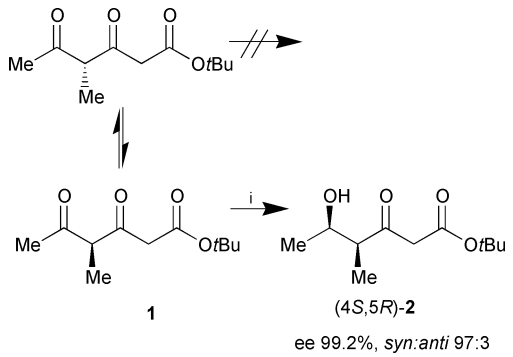
An entirely new method for the dynamic kinetic resolution of a racemic, 2-methyl substituted, unsymmetrical 1,3-diketone *via* enzymatic reduction to give an enantiomerically pure compound is introduced.

The dynamic kinetic resolution of α-substituted β-keto esters by chemical[1] or biocatalytical[2] reduction is particularly useful due to the simultaneous introduction of two stereogenic centres into the molecule in combination with a theoretical maximum yield of 100%. Although this method has proven broad applicability in stereoselective synthesis, the corresponding dynamic kinetic resolution of 2-substituted 1,3-diketones is rarely found in the literature.[3] Our aim is directed toward extending dynamic kinetic resolution to enantio- and regioselective reduction of alkyl-substituted 3,5-dioxoesters, which would enable the introduction of up to four stereogenic centers by two consecutive reduction steps.

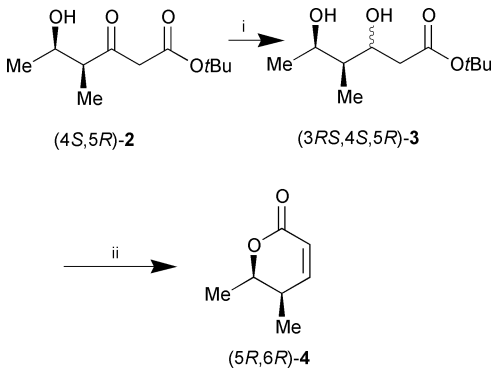
The attempted enantioselective ketone reduction of 3,5-dioxohexanoate esters by chemical methods[4] or biotransformation[5] usually results in complex mixtures of several stereo- and regioisomeric products with one or both keto groups reduced. We figured out that this difficult transformation can be accomplished by using isolated enzymes to afford optically pure 5-hydroxy-3-oxohexanoates in high yield.[6] Herein we wish to report in preliminary form on the first enantio- and regioselective enzymatic reduction of 4-alkyl-3,5-dioxohexanoates resulting in formation of one out of a total of 8 monoreduction and 8 bisreduction products.

tert-Butyl 4-methyl-3,5-dioxohexanoate (**1**) was prepared by acylation of the bisenolate of *tert*-butyl 3-oxovalerate with commercially available Weinreb acetamide.[7] For the enzymatic reduction recombinant alcohol dehydrogenase from *Lactobacillus brevis* (recL*B*ADH) was chosen, which has been cloned and overexpressed in *E. coli*.[8] recL*B*ADH exhibits a broad substrate range and considerable stability even towards highly reactive compounds like 6-chloro-3,5-dioxohexanoates.[6,8] Cofactor (NADPH) regeneration succeeds *via* a coupled-substrate process. Propan-2-ol (200 mM) was applied in excess to the reaction mixture as an auxiliary substrate in order to shift the equilibrium of the reaction towards the desired direction (Scheme 1).[9]

NMR data of the major product (4*S*,5*R*)-**2** which was obtained in 66% isolated yield, clearly proved the regioselective monoreduction of the keto group at C-5. Additionally, from GC-MS data of the crude product after derivatisation with $(F_3CCO)_2O$, pyridine, no evidence could be found for the reduction of the keto group at C-3. In order to verify the proposed absolute configuration and to enable precise determination of the enantiomeric excess, (4*S*,5*R*)-**2** was transformed through sodium borohydride reduction into lactone **4** *via* diol (3*RS*,4*S*,5*R*)-**3**. Lactonisation and dehydration gave the unsaturated lactone (5*R*,6*R*)-**4** which is known in racemic form[10] (Scheme 2).



Scheme 1 *Reagents and conditions*: i, **1** (20 mM), propan-2-ol (200 mM), $NADP^+$ (1 mM), pH 6.5, recL*B*ADH (360 U), 23 h, rt (66%).



Scheme 2 *Reagents and conditions*: i, $NaBH_4$, EtOH, 0 °C; ii, cat. TsOH, toluene, reflux, 2 h (60% over two steps).

As a standard a racemic 1:1 mixture of *syn*- and *anti*-lactone *rac*-**4** was synthesised from keto ester **5** by sodium borohydride reduction, subsequent chain elongation, and, finally, lactone formation as described above (Scheme 3). The four stereoisomers of *syn/anti-rac*-**4**, which were formed in equal amounts, can be separated by HPLC on chiral stationary phase (Daicel Chiracel OB).

An authentic sample of the enantiomeric *syn*-lactone (5*S*,6*S*)-**4** was synthesised by the same sequence starting from bakers' yeast reduction of **5** *via* the known[11] ethyl (2*R*,3*S*)-2-methyl-3-hydroxybutyrate (2*R*,3*S*)-**6** (Scheme 4).

The spectroscopic data (^{1}H-NMR, ^{13}C-NMR, MS) of (5*S*,6*S*)-**4** and of (5*R*,6*R*)-**4**, produced *via* enzymatic (recL*B*ADH) reduction of **1**, are identical. Comparison of the CSP-HPLC data of both lactones revealed the (4*S*,5*R*)-absolute configuration for the product **2** of the recL*B*ADH reduction. This product is formed in almost enantiomerically pure form (99.2% ee, HPLC data); the diastereomeric ratio of *syn*:*anti* 97:3 is likewise very high (NMR and HPLC data).

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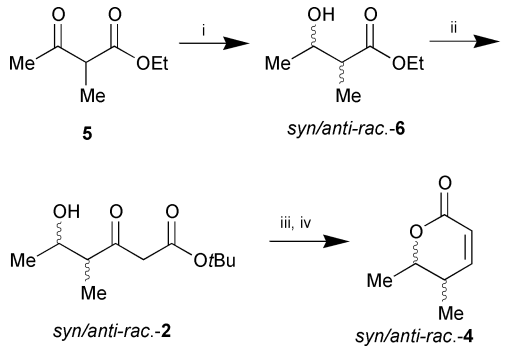
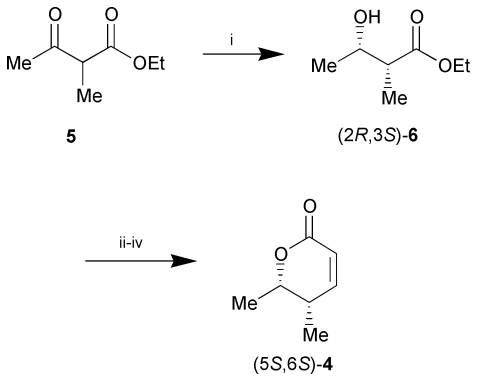


Scheme 3 *Reagents and conditions*: i, $NaBH_4$, EtOH, 0 °C (83%); ii, CH_2=C(OLi)O*t*Bu, THF, −30 °C (53%); iii, $NaBH_4$, EtOH, 0 °C; iv, cat. TsOH, toluene, reflux, 2 h (60% over two steps).



Scheme 4 *Reagents and conditions*: i, bakers' yeast, 10% aq. EtOH, (50%); ii, CH_2=C(OLi)O*t*Bu, THF, −30 °C; iii, $NaBH_4$, EtOH, 0 °C; iv, cat. TsOH, toluene, reflux, 2 h (53% over three steps).

In summary, we have shown the regio- and enantioselective reduction of *tert*-butyl 4-methyl-3,5-dioxohexanoate *via* dynamic kinetic resolution to give an almost enantiomerically and diastereomerically pure compound introducing two stereogenic centers can be done efficiently by enzyme-catalysed reduction. This method represents a novel entry into the chemistry of polypropionates based on a biomimetic approach *via* polyketides. This method should be extendable towards dynamic kinetic resolution of other 2-alkyl-substituted unsymmetrical 1,3-diketones.

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9 Enzymatic transformation: A solution of recLBADH was prepared by mechanically disrupting wet cells of recombinant *E. coli* strain recADH-HB101+.[8] One unit (U) enzyme activity is defined as the amount of recLBADH that catalyses the oxidation of 1 μmol NADPH per minute when incubated with acetophenone (10 mM) and NADPH (0.25 mM) at 25 °C and pH 6.5 (100 mM phosphate buffer, 1 mM $MgCl_2$). In a round bottom flask, a solution of diketo ester **1** (0.53 g, 2.5 mmol) in propan-2-ol (1.9 mL, 25 mmol) was added to 120 mL phosphate buffer (100 mM, pH 6.5) containing 1 mM $MgSO_4$, and the mixture was ultrasonicated for 1 minute. The reaction was started by addition of $NADP^+$ (105 mg, 120 μmol; FLUKA Nr. 93210, 90%) and recLBADH (360 U). After slowly stirring for 23 h at ambient temperature, 20 g NaCl were added and the solution was extracted with ethyl acetate three times. The combined organic phases were dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (silica, ethyl acetate isohexane 40/60 (v/v)), yielding 0.35 g (66%) hydroxyketo ester (4*S*,5*R*)-**2** as a colourless oil.
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