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 chemical1 or biocatalytical2 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-dioxohexanoic esters by chemical methods4 or biotransformation5 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 bireduction products.

tert-Butyl 4-methyl-3,5-dioxohexanoate (1) was prepared by acylation of the bisenolate of tert-butyl-3-oxoalrate with commercially available Weinreb aceticide.7 For the enzymatic reduction recombinant alcohol dehydrogenase from Lactobacillus brevis (recLADH) was chosen, which has been cloned and overexpressed in E. coli.8 recLADH exhibits a broad substrate range and considerable stability even towards highly reactive compounds like 6-chloro-3,5-dioxohexanoates.8,9 Co-factor (NADPH) regeneration succeeds via a coupled-substrate process. Propan-2-ol (200 mM) was applied in excess to the substrate range and considerable stability even towards highly reactive compounds like 6-chloro-3,5-dioxohexanoates.8,9 Co-factor (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 (4S,5R)-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 (F3CCO)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, (4S,5R)-2 was transformed through sodium borohydride reduction into lactone 4 via diol (3RS,4S,5R)-3. Lactonisation and dehydrogenation gave the unsaturated lactone (5R,6R)-4 which is known in racemic form10 (Scheme 2).

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 Chiralcel OB).

An authentic sample of the enantiomeric syn-lactone (5S,6S)-4 was synthesised by the same sequence starting from bakers’ yeast reduction of 5 via the known11 ethyl (2R,3S)-2-methyl-3-hydroxybutyrate (2R,3S)-6 (Scheme 4).

The spectroscopic data (1H-NMR, 13C-NMR, MS) of (5S,6S)-4 and of (5R,6R)-4, produced via enzymatic (recLADH) reduction of 1, are identical. Comparison of the C5P-HPLC data of both lactones revealed the (4S,5R)-absolute configuration for the product 2 of the recLADH 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).
Reagents and conditions: i, NaBH₄, EtOH, 0 °C (83%); ii, CH₃=CH(O)LiOrBu, THF, −30 °C (53%); iii, NaBH₄, EtOH, 0 °C; iv, cat. TsOH, toluene, reflux, 2 h (66% over two 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 1,3-diketones.

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Notes and references
9 Enzymatic transformation: A solution of recLBADH was prepared by mechanically disrupting wet cells of recombinant E. coli strain recADH-HB101+. 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 MgCl₂). 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 2.5) during ultrasonication 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 MgSO₄ 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 as a colourless oil.