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# Enantioselective Reduction of 4-Fluoroacetophenone at High Substrate Concentration using a Tailor-Made Recombinant Whole-Cell Catalyst

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Dedicated to Professor Dr. Masakatsu Shibasaki on the occasion of his 60th birthday.

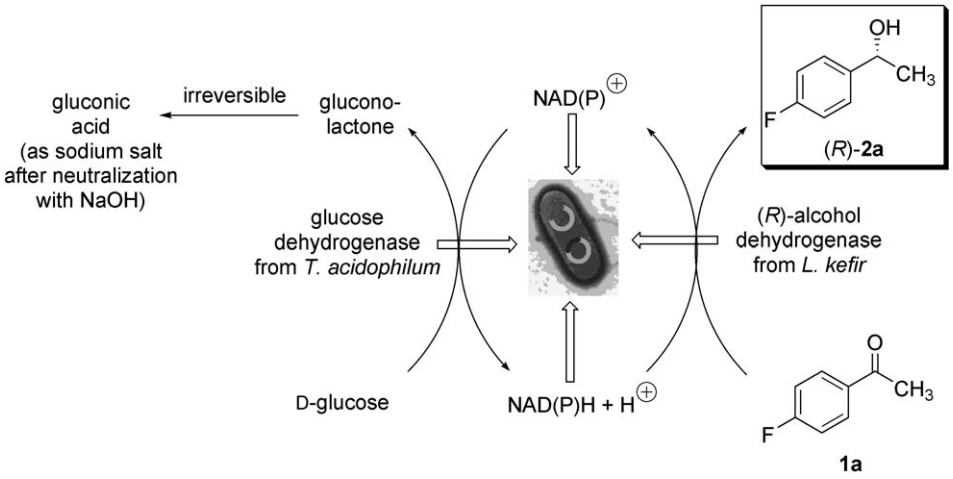
**Abstract:** A practical and highly efficient biocatalytic synthesis of optically active (*R*)-4-fluorophenylethan-1-ol has been developed based on reduction of the corresponding 4-fluoroacetophenone in the presence of a tailor-made recombinant whole-cell biocatalyst, containing an alcohol dehydrogenase and a glucose dehydrogenase. The reaction proceeds in a pure aqueous solvent media at a substrate concentration of *ca.* 0.5 M, and gives the desired product with high conversion (>95%), good yield (87%) and with an excellent enantioselectivity of >99% *ee*. In addition, activity tests further showed that also the analogous 2- and 3-fluoroacetophenones are promising substrates.

**Keywords:** alcohols; asymmetric catalysis; biotransformations; enzyme catalysis; enzymes; reduction

## Introduction

Optically active, fluorinated compounds play a key role in medicinal chemistry.[1] Among the favoured synthetic approaches for the corresponding fluorinated intermediates are catalytic enantioselective processes. A popular access towards enantiomerically pure fluoro-substituted secondary alcohols is an enantioselective reduction of the corresponding ketones. Several procedures for this type of reactions have already been applied successfully using both metal catalysts[2] as well as biocatalysts.[3] With respect to technical process development, an impressive synthesis of (*S*)-4-fluorophenylethan-1-ol on a kilogram scale by means of an enantioselective ketone hydrogenation in the presence of a chiral ruthenium-phosphane complex as a catalyst has been recently reported by Ramsden and co-workers from Chirotech Technology Limited.[2b] The desired product (*S*)-4-fluorophenylethan-1-ol, (*S*)-**2a**, was obtained in 94% yield and with 98.3% *ee*. Interestingly, however, direct use of some commercial samples of 4-fluoroacetophenone, **1a**, as a substrate gave significantly less satisfactory results, thus requiring distillation of the substrate as an initial step. Besides this metal-catalyzed approach, efficient and industrially feasible biocatalytic reduction methodologies would represent an attractive alternative asymmetric route to fluorinated phenylethan-1-ols due to the advantages "white biotechnology" offers for industrial production with respect to, e.g., process efficiency and economy as well as environmental aspects. In continuation of our study on the synthetic potential of recombinant whole-cell catalysts for technically feasible redox processes,[4] in the following we report the application of this whole-cell redox technology platform for an (alternative) biocatalytic enantioselective synthesis of fluorinated phenylethan-1-ols. This type of reaction is exemplified for the synthesis of the analogue (*R*)-enantiomer of 4-fluorophenylethan-1-ol, (*R*)-**2a**, by means of a reduction of 4-fluoroacetophenone, **1a**, as a model substrate in the presence of a tailor-made recombinant whole-cell catalyst. It is also demonstrated that the desired reduction of 4-fluoroacetophenone, **1a**, proceeds with high conversion and excellent enantioselectivity at high substrate concentrations, even when using a commercial sample of substrate directly without further purification (Scheme 1).



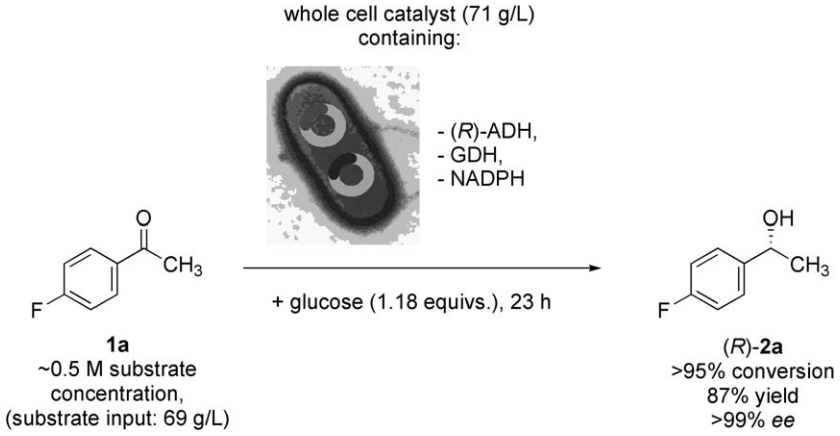

**Scheme 1.**

## Results and Discussion

For the (*R*)-enantioselective reduction of the fluorinated acetophenone **1a**, we envisioned that our recently developed (*R*)-enantioselective whole-cell biocatalyst[4c] might be suitable. This recombinant *E. coli* whole-cell biocatalyst, which turned out to be a highly efficient catalyst for the reduction of a broad range of ketones, contains an (*R*)-enantioselective alcohol dehydrogenase from *Lactobacillus kefir* (LK-ADH) and a glucose dehydrogenase from *Thermoplasma acidophilum* in overexpressed form.[4c] In ref.[4c] we also described a preparative example for a 10-L scale-up using this whole-cell biocatalyst. Furthermore, this tailor-made recombinant *E. coli* whole-cell biocatalyst can be produced by means of high cell density fermentation when used for industrial scale processes.

The biotransformation concept for the desired reduction of 4-fluoroacetophenone, **1a**, with this whole-cell biocatalyst at a substrate concentration of $>50$ g/L of **1a** is graphically shown in Scheme 1. A key step therein is the LK-ADH-catalyzed reduction of the ketone, **1a**, in aqueous buffer under consumption of the cofactor NADPH as a reducing agent and formation of the oxidized form, $NADP^+$. Subsequent *in situ* reduction of this oxidized form under formation of the required reduced form, NADPH, is catalyzed by the glucose dehydrogenase by means of an oxidative transformation of D-glucose into D-gluconolactone.[5] Opening of D-gluconolactone under formation of D-gluconic acid and neutralization to the corresponding sodium salt thereof makes the whole process irreversible, thus shifting the equilibrium towards the direction of the desired product. Since not only both enzymes but also the required cofactor are available in the whole-cell biocatalyst, an external amount of NADPH – as needed when using isolated enzymes – is not added in this whole-cell-catalyzed reduction of 4-fluoroacetophenone, **1a**. Due to the high price of NADPH, the avoidance of addition of cofactor – even at catalytic amounts – has a positive economic impact.

Based on this reduction concept, a reaction has been carried out on preparative scale at a substrate concentration of *ca.* 0.5 M of 4-fluoroacetophenone **1a** (Scheme 2; for preparative details, see Experimental




**Scheme 2.**

Section). The co-substrate D-glucose was added at 1.18 equivalents, and the biocatalyst input of the *E. coli* DSM14459 catalyst, bearing the ADH from *L. kefir* and GDH from *T. acidophilum*, was 71 g/L of wet biomass. An external amount of the cofactor NADPH was not added. After a reaction time of 23 h, the desired product (*R*)-4-fluorophenylethan-1-ol, (*R*)-**2a**, was formed with a conversion of >95%. The substrate does not need to be purified prior to the reaction and was used directly as commercially available sample. Subsequent downstream processing steps have been done according to a previously reported protocol,[4c] consisting of the standard operations filtration (after decreasing the pH and addition of a filter aid material), extraction with methyl *tert*-butyl ether (MTBE) and evaporation of this solvent. The desired (*R*)-alcohol (*R*)-**2a** was obtained as a crude product in 92% yield with a purity of >95% (main impurity: MTBE solvent) and a high enantiomeric excess of >99%. The crude product was further purified by means of a short-path distillation under vacuum, leading to the purified isolated product (*R*)-**2a** in a yield of 87% and with >99% *ee*.

In addition, in subsequent preliminary activity tests other fluorinated acetophenones have also been shown to be accepted as a substrate by the alcohol dehydrogenase from *L. kefir* (Scheme 3). These activity tests have been carried out by means of standardized photometer assays in analogy to a protocol reported earlier,[6] and are based on the measurement of consumption of the reduced cofactor NADPH in the presence of the substrate and isolated enzyme. When using the *m*-substituted analogue, 3-fluoroacetophenone, **1b**, and the ADH from *L. kefir* an activity of 92% compared to acetophenone (which is known to be a good substrate with a specific activity of 99.1 U/mg of purified ADH from *L. kefir*; see ref.[7]) has been achieved, thus being in a similar range. Also 2-fluoroacetophenone, **1c**, is accepted as a substrate. However, the resulting activity was somewhat lower with 67%, thus indicating a negative impact of the *o*-substituent on the enzyme's activity (which is in accordance to previous observations with other types of *o*-substituted acetophenones and alcohol dehydrogenases, see for example ref.[6]).

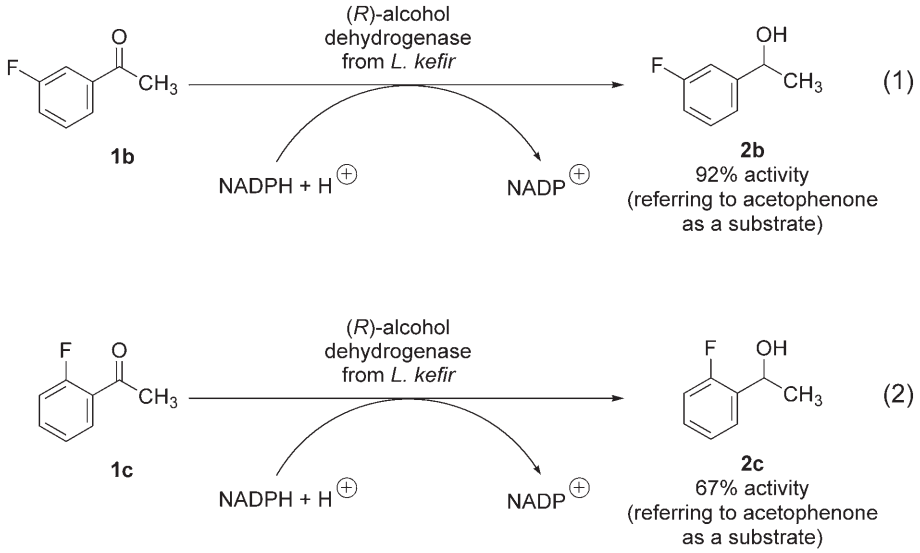
## Conclusions

In conclusion, a practical biocatalytic synthesis of optically active (*R*)-4-fluorophenylethan-1-ol, (*R*)-**2a**, has been developed. The synthesis is based on a reduction of the corresponding 4-fluoroacetophenone, **1a**, in the presence of a tailor-made whole-cell biocatalyst, containing an alcohol dehydrogenase and a glucose dehydrogenase. The reaction proceeds in a pure aqueous solvent media at a substrate concentration of *ca.* 0.5 M, and gives the desired product (*R*)-**2a** with high conversion (>95%), good yield (87%) and with an excellent enantioselectivity of >99% *ee*. In addition, activity tests further showed that also the analogous 2- and 3-fluoroacetophenones are promising substrates. With respect to these substrates **1b** and **1c**, the next steps will be reduction experiments on preparative scale and the investigation of enantioselectivity and volumetric productivity.

## Experimental Section

### Construction of the Whole-Cell Biocatalyst Bearing an (*R*)-Alcohol Dehydrogenase from *Lactobacillus kefir* and a Glucose Dehydrogenase from *Thermoplasma acidophilum*

The preparation of active cells *E. coli* DSM14459, bearing an (*R*)-alcohol dehydrogenase from *Lactobacillus kefir* and




**Scheme 3.**

a glucose dehydrogenase from *Thermoplasma acidophilum*, has been described in ref.[4c] The corresponding cell pellets were – after storage at −20 °C – used in the reduction of 4-fluoroacetophenone, **1a**, as described in the procedure below.

### Procedure for the Biocatalytic Synthesis of (*R*)-4-Fluorophenylethan-1-ol *via* Reduction of 4-Fluoroacetophenone at a 0.5 M Substrate Concentration

In a Titrino-reaction apparatus, 3.55 g of the whole cell catalyst *E.coli* DSM14459, containing an (*R*)-alcohol dehydrogenase from *L. kefir* as well as a glucose dehydrogenase from *Thermosplasma acidophilum*, leading to a cell concentration of 71 g of wet biomass per L reaction volume, 29.3 mmol of D-glucose (1.18 equivalents referring to the molar amount of ketone **1a**) and 24.8 mmol of 4-fluoroacetophenone, **1a** (directly used as commercially available sample, which was purchased from Merck KgaA, Germany), are added to 20 mL of an aqueous phosphate buffer solution (0.2 M; adjusted to pH 7.0). Then, water is added until a volume of 50 mL is reached. The reaction mixture is stirred at room temperature, and the pH is kept constantly at ~6.5 by dosage of aqueous sodium hydroxide (5 M NaOH). After a reaction time of 23 h, a conversion of >95% has been achieved (according to HPLC and NMR spectroscopy). The work-up was carried out by decreasing the pH to 2 to 3 with concentrated hydrochloric acid and addition of 5.2 g of the filter aid material Celite Hyflo Supercel to the reaction mixture, and subsequent filtration. The filter cake was washed 3 times with 50 mL of MTBE, and the aqueous phase was extracted with the three obtained organic MTBE fractions. After drying over magnesium sulfate the collected organic phases were evaporated. Subsequently, the resulting crude product was distilled in vacuum (3.5 mbar) by means of a short-path distillation furnishing the purified (*R*)-4-flurophenylethan-1-ol, (*R*)-**2a**; yield: 87%, >99% *ee*.

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