These results demonstrate that both aldol enantiomers could be accessed through aldol or retro-aldol reactions using the same antibody 84G3. In order to assign the absolute configurations, the enantiopure products were synthesized by independent chemical asymmetric synthesis for compounds **9, 11**, and **13**.[9] The absolute configuration of compound **7** was assigned by analogy with compound **9**.

In conclusion, we described here the first aldolase antibody, ab84G3, capable of rerouting the regioselectivity of a series of cross aldol reactions which led to the formation of the otherwise disfavored products. This new reactivity highlights the scope of the reactive immunization strategy developed by the groups of Lerner and Barbas for catalyst design. This work further increases the repertoire and efficiency of antibody-catalysed aldol reactions. Further studies on the reactivity of ab84G3 are currently in progress.

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Biocatalytic Asymmetric Hydrogen Transfer**

Wolfgang Stampfer, Birgit Kosjek, Christian Moitzi, Wolfgang Kroutil,* and Kurt Faber

Driven by the increased public awareness on hazards derived from chemical production, environmentally benign oxidation methods have gained increasing importance.[1] In this context, the reduction of ketones at the expense of a sacrificial secondary alcohol and the corresponding reverse reaction (known since the 1920s as the Meerwein–Ponndorf–Verley reduction (MPVRed) and Oppenauer-oxidation (OOx), respectively, constitute typical "green" redox reactions.[2] As a consequence, they have been re-investigated recently[3] to replace the previously employed metal alkoxides with catalysts showing improved efficiency,[3c] better recoverability,[3g] avoiding aldol condensation as side reaction,[3f] and water-soluble analogues.[3b] Asymmetric variants have been pursued by using enantioselective hydride-transfer methods based on chiral transition metal complexes[3e] or chiral hydride sources.[3a]

All biocatalytic methods for the asymmetric hydrogen transfer are based on alcohol dehydrogenases requiring nicotinamide cofactors. They have several advantages over the chemical methods, such as 1) their intrinsic asymmetry, 2) absence of side reactions, such as aldol condensation, and 3) they operate under essentially mild reaction conditions. However, their large-scale application has been impeded by the requirement for cofactor-recycling.[4] Since the sacrificial secondary alcohol used as cosubstrate (for MPVRed) or the carbonyl compound (for OOx) has to be employed in excess to drive the reaction from equilibrium towards completion, cosubstrate inhibition is common in such a "coupled-substrate" approach based on the use of a single enzyme.[5] Although this drawback has been surmounted to some extent by using a second dehydrogenase, which is highly specific for the sacrificial cosubstrate,[6] these so-called "coupled-enzyme" methods are rather complex and require the handling of isolated enzymes and cofactor(s). As a consequence, biochemical MPVReds and OOxs on a large scale are limited by the use of fermenting cells[7] and/or low (co)substrate concentration(s).[8]

We have recently isolated a highly enantioselective secondary-alcohol dehydrogenase[9] from *Rhodococcus ruber* DSM 44541, which is exceptionally stable towards organic solvents. The activity of the enzyme remains high at concentrations of up to 20% (v/v) acetone and 50% (v/v) 2-propanol. This activity enables the use of the enzyme for MVRed and OOx in the "coupled-substrate" approach. For preparative-

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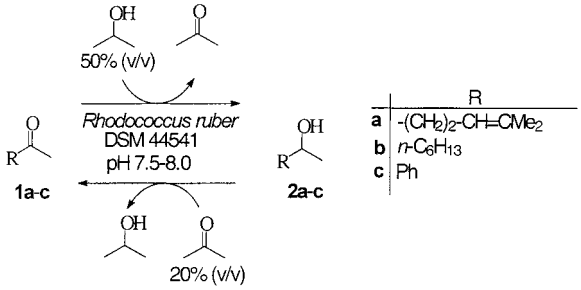
scale applications, whole lyophilized or resting cells can be used as a readily available alternative.

Reduction mode: Whole lyophilized cells of *Rhodococcus ruber* DSM 44541[10, 11] grown on a standard medium (glucose, peptone, yeast extract) without enzyme induction catalyzed the reduction of **1a** in aqueous buffer at pH 7.5 with excellent enantioselectivity to furnish (*S*)-sulcatol (**2a**; Scheme 1) with



Scheme 1. Biocatalytic oxidation/reduction of secondary alcohols/ketones employing lyophilized cells in buffer and cosubstrate [20% (v/v) acetone/ 50% (v/v) 2-propanol].

an *ee* value of >99%, while the reaction was driven towards completion by addition of 2-propanol. When the cosubstrate concentration was gradually increased,[12] the reaction rate reached a maximum at 50% (v/v) of 2-propanol (Figure 1).[13] To explore the limits of the productivity of the system, the substrate concentration was varied by keeping the concentration of 2-propanol at a constant level of 50% (v/v). A broad optimum for the substrate concentration was found within a range of 0.5 to 1.0 mol L^{-1}, which corresponds to 76 g L^{-1} to 126 g L^{-1} for this substrate (Figure 1).[14, 15]

The flexibility of this system was demonstrated by the reduction of substrates containing an aromatic moiety or α,β-unsaturated ketones (Table 1) to furnish the corresponding alcohol with excellent *ee* values.

A preparative-scale reaction was repeated at high substrate and cosubstrate concentration. Without further optimization,

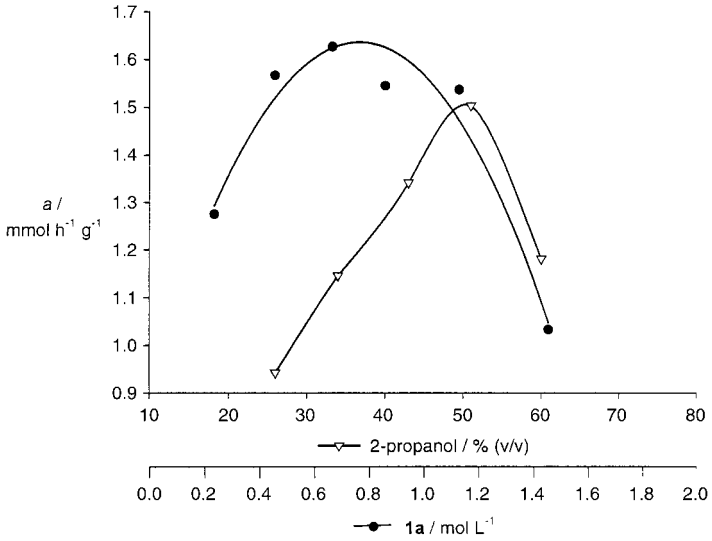


Figure 1. Activity *a* of whole cells of *Rhodococcus ruber* DSM 44541 for the reduction of **1a** in phosphate buffer (pH 7.5) at different concentrations of 2-propanol (▽) and at different substrate concentration **1a** [●, 50% (v/v) 2-propanol].

Tabelle 1. Results of the biocatalytic reduction of various ketones employing lyophilized whole cells of *Rhodococcus ruber* DSM 44541 with 50% (v/v) 2-propanol in buffer.

Substrate	*t* [h]	*ee* (Product) [%]	Conversion [%]
1a	22	>99 (*S*)	70
1b	22	>99 (*S*)	91
1c	22	>99 (*S*)	81
3-octanone	22	97 (*S*)	79
2-decanone	22	>99 (*S*)	65
1-cyclohexyl ethanone	22	>99 (*S*)	92
1-(naphth-2-yl) ethanone	22	>99 (*S*)	82
oct-3-en-2-one	96	>99 (*S*)	67

1a (1.0 g, 125 g L^{-1}, 0.99 mol L^{-1}) was reduced at 24 °C within 24 h employing lyophilized whole cells of *Rhodococcus ruber* DSM 44541 (0.3 g) in phosphate buffer (pH 7.5, 50 mM) containing 50% 2-propanol to give (*S*)-sulcatol **2a** in 67% yield and >99% *ee*.[16, 17]

Oxidation mode: Encouraged by these results, we examined the applicability of the system for the reverse reaction, that is, the oxidation of *rac* secondary alcohols at the expense of acetone as sacrificial cosubstrate.[18] Indeed, whole cells of *Rhodococcus ruber* DSM 44541 exclusively oxidized the *S* enantiomer from racemic substrates *rac*-**2a**, *rac*-**2b**, and *rac*-**2c** (Table 2). The relative rate of oxidation for (*S*)-**2b** was about 55 times higher than that of (*S*)-**2c**. Optimization of the system showed that the oxidation rate for *rac*-**2c** measured at different acetone concentrations reached a maximum at 5% (v/v) of acetone (Figure 2). However, the reaction ceased before the maximum obtainable conversion of 50% (for a kinetic resolution) was reached. Complete reaction (conversion = 50%) occurred when the acetone concentration was increased to 20% (v/v), however, a reduction of reaction rate was observed, and preliminary results indicated that enzyme inhibition occurred. A compromise between a maximum reaction rate and minimum enzyme inhibition was found, the best results were obtained with an acetone concentration of 5% (v/v) at start followed by continuous addition throughout the reaction reaching a final acetone concentration of 20% (v/v).[19]

Tabelle 2. Results of the biocatalytic kinetic resolution by oxidation of secondary alcohols employing lyophilized whole cells of *Rhodococcus ruber* DSM 44541.

Substrate	Relative activity[a]	Enantioselectivity *E*[b]	Conversion [%]	*ee* [%]
rac-**2a**	1000	>100	49.0	97.2(*R*)
rac-**2b**	813	>100	49.4	97.8(*R*)
rac-(*E*)-3-octen-2-ol	374	>100	46.7	98.5(*R*)
rac-4-phenyl-2-butanol	338	>100	49.7	95.7(*R*)
cyclopentanol	178	–	98.2	–
rac-1-(naphth-2-yl)ethanol	62	>100	49.3	98.2(*R*)
rac-**2c**	15	>100	44.4	77.8(*R*)
rac-3-octanol	3	2.7	49.5	33.0(*R*)
rac-4-octanol	2.6	~1	36.9	0

[a] The relative activity for **1a** was arbitrarily set to 1000, which corresponds to 1.55 mmol transformed substrate $h^{-1}g^{-1}$ lyophilized cells. [b] Enantioselectivity was calculated from *ee* values and conversion: $E = \{\ln[(1-c)(1-ee)]\}/\ln[(1-c)(1+ee)]$. All experiments were performed employing *Rhodococcus ruber* DSM 44541 with 20% (v/v) acetone in phosphate buffer at 24 °C and at pH 8.0.

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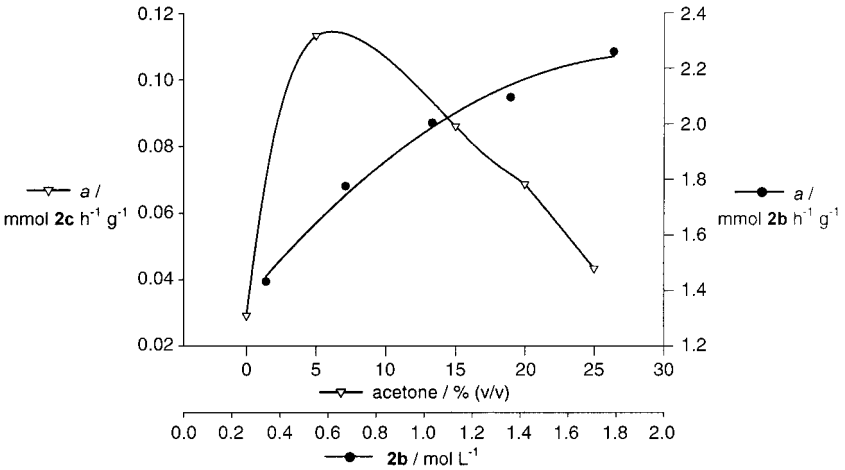

Figure 2. Activity *a* of whole cells of *Rhodococcus ruber* DSM 44541 for the oxidation of *rac*-**2c** in phosphate buffer (pH 8.0) at different concentrations of acetone (▽) and for the oxidation of *rac*-**2b** at different substrate concentration (●) at 20% (v/v) acetone.

Surprisingly, no maximum was reached when we tried to optimize the substrate concentration for *rac*-**2b** at a fixed acetone concentration of 20% (v/v) (Figure 2); the reaction rate increased continuously with increasing substrate concentration of up to 1.8 mol L^{-1}, which corresponds to 237 g L^{-1} for *rac*-2-octanol **2b**. Beyond this value, no reliable data could be obtained as the mixture turned very viscous.

These results were confirmed on a preparative scale by kinetic resolution of *rac*-2-octanol (1.0 g, concentration 111 g L^{-1}, 0.84 mol L^{-1}) by oxidation using 0.6 g of lyophilized *Rhodococcus* cells and a final acetone concentration of 20% (v/v) in buffer at 24 °C. Within 24 h, a gas chromatography (GC) conversion of 48.8% was reached and unreacted (*R*)-2-octanol was isolated in 48% yield and 96% *ee* together with 2-octanone (37% yield).[20] The enantioselectivity of the reaction was calculated as $E > 100$ (for a definition of *E* see Table 2).

On the one hand, ω-phenylalkan-2-ols and allylic alcohols, as well as cyclopentanol are very good substrates (Table 2), while secondary alkanols having a more remote hydroxy moiety in the (ω-2) or (ω-3) position are oxidized with lower activity.

To our knowledge, this is the first report of a preparative-scale biocatalytic oxidation of secondary alcohols using acetone as a cosubstrate, which is presumably because the oxidation of alcohols is thermodynamically unfavored and cosubstrate[21] or product[22] inhibition are common phenomena for this type of reaction. In addition, acetone is known to deactivate enzymes.

The biocatalytic redox-system described here is a simple and flexible method, at high substrate concentration, for the Meerwein–Ponndorf–Verley reduction of ketones and its reverse counterpart, the Oppenauer oxidation of secondary alcohols by a simple switch of cosubstrate. The method is highly regio- and enantioselective, and it represents essentially "clean" chemistry.

Experimental Section

Optimum of cosubstrate concentration: Cells of *Rhodococcus ruber* DSM 44541 (40 mg) were rehydrated in phosphate buffer (400 μL, 50 mM, pH 7.5 for reduction; pH 8.0 for oxidation) for 30 min at 30 °C. 2-Propanol or acetone, respectively, was added at a certain concentration. The substrate concentration was constant for all experiments (**1a**, 126 g L^{-1}; *rac*-**2c**, 18 g L^{-1}). Samples were shaken in Eppendorf vials at 130 rpm and 24 °C on a rotary shaker for 2.3 (**1a**) or 4 h (*rac*-**2c**), respectively. The reactions were quenched by addition of ethyl acetate (0.6 mL) followed by centrifugation.

The optimum for the substrate concentration for the reduction [oxidation] was determined by rehydrating whole lyophilized cells of *Rhodococcus ruber* DSM 44541 (20 mg) [30 mg] in phosphate buffer (650 μL, 50 mM, pH 7.5/reduction, pH 8.0/oxidation) for 30 min at 30 °C. 2-Propanol [acetone] was added at a concentration of 50% (v/v) [20% (v/v)]. Substrate **1a** [*rac*-**2b**] was added and the reaction shaken at 24 °C at 130 rpm for 3 h.

The enantiomeric excess was determined on a Chrompack Chirasil Dex column (25 m × 0.32 mm × 0.25 μm, H_2): **2a**: 80 °C isotherm, 3.4 min (*S*), 3.7 min (*R*); **2c**: 100 °C/5 min – 12 °C/min – 160 °C/0 min, 6.4 min (*R*), 7.6 min (*S*); a G-PN column (30 m × 0.32 mm, NG 9908-08, H_2) was used for the determination of **2b** as its acetate, 9.8 min (*S*), 10.2 min (*R*). The absolute configuration of (*S*)-**1a** and (*S*)-(*E*)-3-octen-2-ol was proven by comparison of the optical rotation with the literature,[23] and that of (*R*)-**2b** and (*R*)-**2c** by co-injection with commercially available nonracemic samples on chiral GC.

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concentrations see: e) C. Heiss, R. S. Phillips, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2821 – 2825 [15 % (v/v) cosubstrate 2-propanol, 0.12 mol L^{-1} substrate]; f) A. Matsuyama, H. Yamamoto, N. Kawada, Y. Kobayashi, *J. Mol. Catal. B* **2001**, *11*, 513 – 521 (0.3 mol L^{-1} substrate, no details on the NAD^+/NADH recycling system); g) H. D. Simpson, D. A. Cowan, *Protein Pept. Lett.* **1997**, *4*, 25 – 31 [11 % (v/v) cosubstrate 2-propanol, 0.44 mol L^{-1} substrate].

[9] Enzyme purification showed that at least four NAD^+/NADH-dependent active fractions are present in the cells, of which only one had a stability towards acetone and 2-propanol comparable to that of the whole cells. The other NAD^+/NADH depending dehydrogenases were either unstable or could not use 2-propanol/acetone as cosubstrate. However, all dehydrogenases displayed the same stereopreference.

[10] Lyophilized cells can be stored at +4 °C for several months without noticeable loss of activity.

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[12] All optimization experiments were performed at 30 °C.

[13] Whole lyophilized cells lost 50 % of their activity in 30 % 2-propanol after 35 h, in 50 % 2-propanol already after 3 h.

[14] Since the solubility of most substrates in aqueous buffer is limited, apparent substrate concentrations are used throughout this study which correspond to mol substrate/total volume of the system.

[15] The reason for the decrease of activity at high substrate concentration may be inhibition, deactivation, or limitations of the substrate transport (diffusion, transport into the cell). However, the reason was not clarified for whole cells because of the complexity of the system, but will be elucidated for the isolated enzyme.

[16] This corresponds to a productivity of 26 mmol product $h^{-1}L^{-1}$ employing 0.3 g whole lyophilized cells, or 2.3 g product g^{-1} cells. Literature values for g product g^{-1} whole cells for reductions are at 0.006 g g^{-1}[7d] or 0.02 g g^{-1}.[8c]

[17] The moderate isolated yield results from the limited half-life of whole cells at 50 % (v/v) 2-propanol and may be increased by either using more cells or by starting at a lower concentration of 2-propanol [e.g. 30 % (v/v)] and by continuously increasing it.

[18] The use of different aldehydes/ketones such as, octanal, propanal, isobutyraldehyde, cyclohexanone, cyclobutanone, cyclopentanone, octan-2-one, chloroacetone, and others for cofactor recycling has been reported, although no detailed data are available: see ref. [8a]; J. Grunwald, B. Wirz, M. P. Scollar, A. M. Klibanov, *J. Am. Chem. Soc.* **1986**, *108*, 6732 – 6734; K. Nakamura, Y. Inoue, A. Ohno, *Tetrahedron Lett.* **1994**, *35*, 4375 – 4376; A. M. Snijder-Lambers, E. N. Vulfson, H. J. Doddema, *Recl. Trav. Chim. Pays-Bas* **1991**, *100*, 226 – 230; C. Gorrebeeck, M. Spanoghe, D. Lanens, G. L. Lemière, R. A. Dommisse, J. A. Lepoivre, F. C. Alderweireldt, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 231 – 235; H. Weenen, R. L. G. M. Boog, W. Apeldoorn, *Proceedings of the international conference of Bioflavour 95* (Dijon, France), **1995**, pp. 375 – 380.

[19] Whole lyophilized cells lost 50 % of their activity in 20 % acetone after 40 h.

[20] This corresponds to a productivity of 18 mmol product $h^{-1}L^{-1}$ employing 0.6 g whole lyophilized cells or 0.8 g product g^{-1} cells. A literature value for g product g^{-1} cells of a comparable reaction is 0.01 g product g^{-1} cells.[8a]

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Compartmentalization of a Gadolinium Complex in the Apoferritin Cavity: A Route To Obtain High Relaxivity Contrast Agents for Magnetic Resonance Imaging**

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The search for high relaxivities continues to be a central item in the development of paramagnetic contrast agents (CA) for magnetic resonance imaging (MRI).[1–3] This class of diagnostic agents is mainly represented by highly stable Gd^{III} chelates since the Gd^{III} ion, with its seven unpaired electrons, provides both a very high magnetic moment and a long electronic relaxation time. The observed relaxivity r_1 for the free Gd^{III} chelates may be considered as the sum of three contributions arising from the water molecule(s) directly coordinated to the paramagnetic ion (inner-sphere term),[1, 4] from water molecules hydrogen bonded at the surface of the complex (second coordination sphere term),[5] and from water molecules diffusing in the proximity of the complex (outer-sphere term).[1, 4] By using the theory of paramagnetic relaxation, the design of improved systems has been pursued through the optimization of the determinants of each contribution to the overall relaxivity. However, it was found early on that the observed relaxivity could only be explained if further contributions are operative when interaction with the surface of the protein occurs. For example, the binding of GdDOTP (H_8DOTP = 1,4,7,10-tetrakis(methylenephosphonic acid)-1,4,7,10-tetraazacyclododecane), which does not contain any inner-sphere water molecules, to human serum albumin (HSA) causes a relaxation enhancement of approximately five times.[1, 6] It was straightforward to assign such additional contributions to water molecules and exchangeable protons on the surface of the protein in the proximity of the binding site(s) of the paramagnetic complex. Clearly the microenvironment of the paramagnetic chelate is highly relevant to the determination of the relaxation-enhancing capability of a given Gd^{III} chelate. We deemed it of interest to explore new routes for the attainment of high relaxivities by exploiting such "protein surface" effects. The aim was to design systems containing a large proteic surface for interaction with the paramagnetic complex that would affect a large number of hydration water molecules and mobile protons, which, in turn, would exchange with the bulk solvent and act as amplifiers of the presence of the CA. One way to deal with a large surface is to design a spherical compartment in which the CA is trapped while the water molecules are free

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