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Biocatalytic oxidative kinetic resolution of *sec*-alcohols: stereocontrol through substrate-modification

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

Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010Graz, Austria

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Abstract—Whole lyophilised cells of *Rhodococcus ruber* DSM 44541 were employed for the oxidative kinetic resolution of *sec*-alcohols using acetone as hydrogen acceptor. The enantioselectivity of this process could be controlled effectively by introducing C–C multiple bonds into substrates, which were inefficiently recognised, in particular short-chain (ω-1)-alcohols and (ω-2)-analogs. Thus, the enantioselectivities of *rac*-2-pentanol ($E=16.8$) and *rac*-3-octanol ($E=13.3$) were significantly improved by introducing a C=C bond adjacent to the alcohol moiety to give racemic (*E*)-pent-3-en-2-ol and 4-(*E*)-octen-3-ol, which were resolved with excellent selectivities ($E>100$ and 50, respectively). In addition, it was found that high stereodifferentiation between the *E*- and *Z*-configured double bonds occurred, as the corresponding (*Z*)-isomers were not converted. Similar selectivity-enhancing effects were observed with acetylenic analogs.

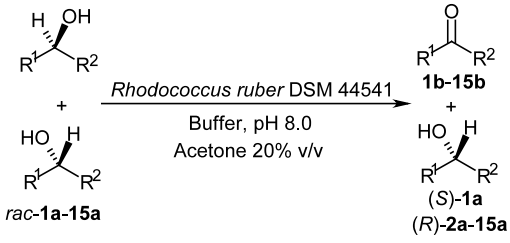
1. Introduction

Oxidative kinetic resolution of *sec*-alcohols via asymmetric hydrogen transfer employing (transition) metals and chiral organic ligands allows the synthesis of non-racemic *sec*-alcohols.[1–4] Although satisfactory enantioselectivities were achieved, it is still difficult to shift the reaction towards completion and to suppress racemisation.[2]

Biocatalytic methods for asymmetric hydrogen transfer are based on alcohol dehydrogenases and show several advantages over chemical methods, such as their stability towards molecular oxygen, their intrinsic asymmetry and the absence of side reactions (in particular the aldol condensation). In addition, they operate under mild reaction conditions and prefer an aqueous medium. However, their general application has been impeded by (i) their requirement for cofactor-recycling, which increases the complexity of systems,[5,6] (ii) their instability towards elevated concentrations of substrates and co-substrates (such as acetone or 2-propanol), and (iii) inhibition phenomena.[7] The instability of most enzymes towards elevated concentrations of acetone has prevented their use as effective hydrogen acceptors for dehydrogenase-catalysed oxidation reactions. As a consequence, biochemical oxidations employing *sec*-alcohol dehydrogenases were restricted to the use of fermenting cells,[8–10] the use of hydrogen acceptors of low volatility such as cyclohexanone,[11] or the use of membrane reactors.[12] In order to broaden the applicability of our 'green' oxidation method towards substrates which were transformed with low reaction rates or insufficient stereoselectivites, we investigated the possibility of selectivity-enhancement by rational substrate modification.[13] This technique has been successfully applied to lipases[14–16] and epoxide hydrolases.[17]

2. Results and discussion

Recently, we have shown that whole cells of *Rhodococcus ruber* DSM 44541 represent an efficient biocatalyst for the oxidative kinetic resolution of racemic secondary alcohols due to their high stability in the presence of elevated concentrations of the hydrogen acceptor, acetone (Scheme 1).[18] Initial studies on the



Scheme 1. Biocatalytic oxidative kinetic resolution of racemic alcohols.

* Corresponding author. Tel.: +43-316-380-5350; fax: +43-316-380-9840; e-mail: wolfgang.kroutil@uni-graz.at

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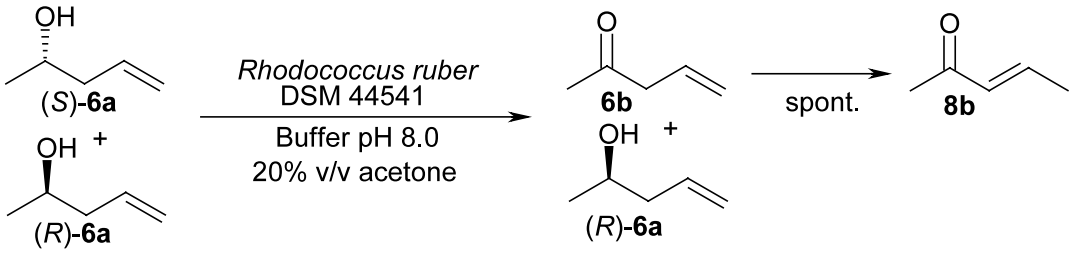
Table 1. Microbial oxidation of short-chain alcohols

Substrate	Time (h)	Conv. (%)	E.e. (%)	*E*
rac-2-Butanol *rac*-**1a**	13	85.7	45.3 (*S*)	1.6
rac-3-Butyn-2-ol *rac*-**2a**	38	n.c.	–	–
rac-2-Pentanol *rac*-**3a**	38	57.1	92 (*R*)	16.8
rac-4-Pentyn-2-ol *rac*-**4a**	38	<1.0	1.5	n.d.
rac-3-Pentyn-2-ol *rac*-**5a**	38	36.8	64 (*R*)	>100
rac-4-Penten-2-ol *rac*-**6a**	38	48.4[a]	58 (*R*)	7.6
rac-(*Z*)-3-Penten-2-ol *rac*-**7a**	38	5.0	2.3	n.d.
rac-(*E*)-3-Penten-2-ol *rac*-**8a**	38	53.0	>99 (*R*)	>100

[a] Isomerisation of 4-penten-2-one to 3-penten-2-one; n.c. = no conversion; n.d. = not determined due to exceedingly low conversion.



Scheme 2. Spontaneous isomerisation of 4-penten-2-one after bio-oxidation of *rac*-4-penten-2-ol.

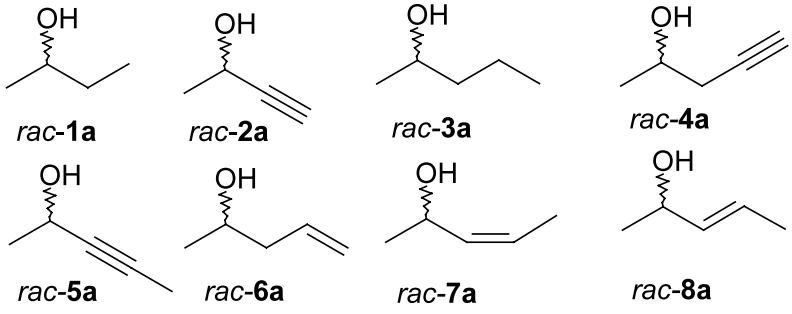


Chart 1.

substrate-selectivity pattern of this enzyme system showed that excellent enantioselectivities were obtained with (ω-1)-alkanols of medium chain length, typically C-8 to C-10, but two severe limitations exist: (i) short-chain alkanols and sterically less accessible (ω-2)-alkanols were transformed either with insufficient enantioselectivities or at exceedingly low reaction rates, respectively.

For instance, the enantioselectivities for short-chain (ω-1)-alkanols, such as *rac*-2-butanol (*rac*-**1a**, $E=1.6$) and *rac*-2-pentanol (*rac*-**3a**, $E=16.8$) were rather low (Table 1). In order to circumvent this limitation we attempted to improve the selectivity by substrate modification by introducing carbon–carbon multiple bonds. When terminal olefinic groups were employed, the results were disappointing: *rac*-3-butyn-2-ol (*rac*-**2a**)—a building block for 5-lipoxygenase inhibitors[19]—and *rac*-4-pentyn-2-ol (*rac*-**4a**) were not accepted.

In contrast to terminal triple bonds, a substrate possessing a terminal C–C double bond located away from the alcohol moiety (*rac*-4-penten-2-ol, *rac*-**6a**) was well accepted by the enzyme, but the enantioselectivity deteriorated even further ($E=7.6$) in comparison to the saturated substrate *rac*-**3a** ($E=16.8$). Furthermore, during kinetic resolution of *rac*-**6a** the corresponding ketone obtained (**6b**) spontaneously isomerised to furnish the conjugated α,β-unsaturated analogue 3-penten-2-one (**8b**) (Scheme 2). The spontaneous (non-enzymatic) nature of this isomerisation was proven in separate experiments by shaking ketone **6b** in buffer in the absence of cells.

In contrast, the strategy turned out to be very successful when the C–C multiple bond was moved towards the interior of the side chain adjacent to the alcohol moiety. Thus, *rac*-3-pentyn-2-ol (*rac*-**5a**) was resolved with excellent enantioselectivity (E >100) on preparative scale at a (non-optimised) substrate concentration of 32 g/L (Chart 1).For internal olefinic substrate analogues *rac*-**7a** and *rac*-**8a**, remarkable stereodiscrimination between the *E*- and *Z*-configured alkene moieties was observed: Whereas *rac*-(*Z*)-4-penten-2-ol (*rac*-**7a**) was not oxidised, the corresponding (*E*)-analogue (*rac*-

Table 2. Microbial oxidation of C-8 straight chain alcohols.

Substrate	Time (h)	Conv. (%)	E.e. (%)	*E*
rac-3-Octanol *rac*-**9a**	20	21.1	24.4 (*R*)	13.3
rac-1-Octen-3-ol *rac*-**10a**	20	2.7	n.d.	n.d.
rac-1-Octyn-3-ol *rac*-**11a**	20	n.c.	–	–
rac-4-(*Z*)-Octen-3-ol *rac*-**12a**	20	n.c.	–	–
rac-4-(*E*)-Octen-3-ol *rac*-**13a**	20	42.1	67.0 (*R*)	49.2
rac-5-(*E*)-Octen-3-ol *rac*-**14a**	20	22.6	19.8 (*R*)	6.4
rac-4-Octyn-3-ol *rac*-**15a**	22	41.2	84.0 (*R*)	>100

n.c. = no conversion; n.d. = not determined due to exceedingly low conversion.

8a) was resolved with excellent enantioselectivity (*E* >100) giving access to enantiopure (*R*)-(*E*)-3-penten-2-ol at a rate comparable to that seen for the saturated substrate, *rac*-2-pentanol (*rac*-**3a**).

Encouraged by these results, we chose to apply this approach for the oxidative kinetic resolution of (ω-2)-alcohols, such as *rac*-3-octanol (*rac*-**9a**), which were impeded by reduced reaction rates and insufficient enantioselectivities, presumably due to steric hindrance. The enantioselectivity for *rac*-**9a** (*E* = 13.3, Table 2) is similar to that of *rac*-2-pentanol *rac*-**3a** (*E* = 16.8, Table 1). We were pleased to see that the results from short-chain substrates could be generalised for long-chain analogues: Both of the unsaturated derivatives *rac*-1-octen-3-ol (*rac*-**10a**) and *rac*-1-octyn-3-ol (*rac*-**11a**) possessing a terminal double or triple bond, respectively, adjacent to the alcohol moiety were not transformed (Table 2, Chart 2).

For internal C–C multiple bonds, however, analogous results were obtained: Thus, *rac*-4-octyn-3-ol (*rac*-**15a**) was resolved with excellent enantioselectivity (*E* >100) and high stereorecognition was observed for the olefinic analogues. As expected, the *E*-isomer *rac*-(*E*)-4-octen-3-ol (*rac*-**13a**, *E* = 49) was resolved with markedly improved enantioselectivity in comparison to the saturated substrate *rac*-**9a** (*E* = 13.3), whereas the (*Z*)-isomer (*rac*-**12a**) was a non-substrate. Interestingly, the selectivity-enhancing effect of an (*E*)-configured olefinic bond entirely vanished when the C=C bond was not adjacent to the alcohol moiety: Substrate *rac*-**14a** showed an *E*-value of only 6.4. In this case, no spontaneous isomerisation of the ketone to furnish the conjugated analogue **13b** (as was proven for 4-penten-2-one) was observed.

The stereopreference of the biooxidation was shown to follow Prelog's rule,[20] i.e. (*S*)-enantiomers were preferentially transformed from the racemate, under the precondition that the 'small' substituent of the Prelog model has CIP priority over the 'large' one. The only exception being *rac*-2-butanol (*rac*-**1a**), where—with very low enantioselectivity (*E* = 1.6)—a slight preference for the (*R*)-enantiomer was observed.

3. Conclusion

The oxidative kinetic resolution of *rac*-*sec*-alcohols employing the hydrogen-transfer system of *R. ruber* DSM 44541 was successfully extended to incorporate (ω-2)- and short-chain (ω-1)-alkanols via rational substrate modification. Thus, significant selectivity-enhancement was achieved by introducing an internal acetylenic or (*E*)-configured carbon–carbon bond into the side chain adjacent to the alcohol moiety. In contrast, (*Z*)-analogues and substrates possessing terminal olefinic or acetylenic moieties were unreactive. Thus, *rac*-4-(*E*)-octen-3-ol (*rac*-**13a**) is a suitable substitute to produce enantiopure (*R*)-3-octanol and derivatives of 3-octanol bearing a multiple carbon–carbon bond at C-4. Similarly, *rac*-3-pentyn-2-ol (*rac*-**5a**) or *rac*-(*E*)-3-penten-2-ol (*rac*-**8a**) are the substrates of choice to afford enantiopure (*R*)-(*Z*)-3-penten-2-ol (via hydrogenation with Lindlar's catalyst) or (*R*)-2-pentanol (using H_2, Pd/C).

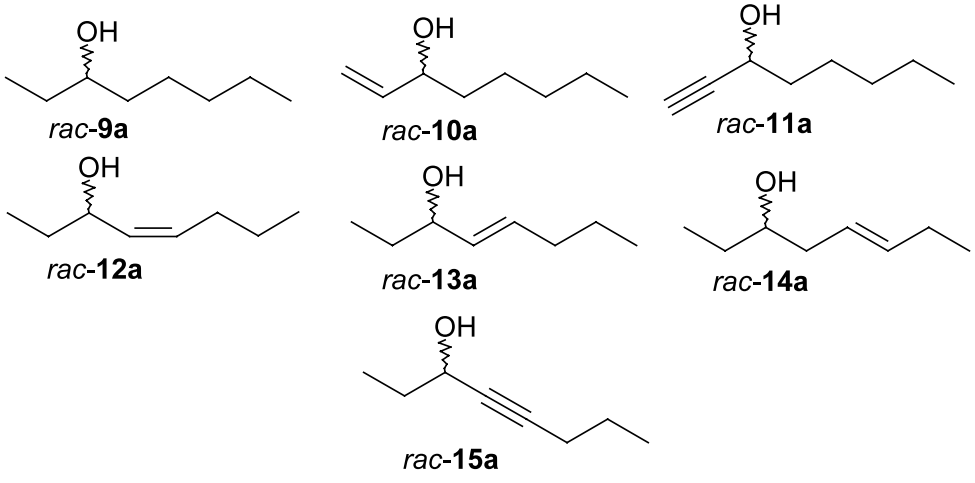


Chart 2.

4. Experimental

The following chemicals were purchased from Aldrich: 2-butanone **1b** (36,047), *rac*-2-pentanol **3a** (P801-6), 2-pentanone **3b** (P810-6), *rac*-4-pentyn-2-ol **4a** (26,899-2), *rac*-4-penten-2-ol **6a** (P880-7), *rac*-(*E*)-3-penten-2-ol **8a** (11,128-7) and *rac*-1-octen-3-ol **10a** (O-528-4). *rac*-3-Butyn-2-ol **2a** (6239), 1-octen-3-one **10b** (6312) and *rac*-1-octyn-3-ol **11a** (8487) were purchased from Lancaster, *rac*-2-butanol **1a** (822263) and 3-octanone **9b** (821860) from Merck and *rac*-3-pentyn-2-ol **5a** (77056) and *rac*-3-octanol **9a** (74870) from Fluka.

4.1. General procedure for the microbial oxidation of *rac-sec*-alcohols

Lyophilised cells (40 mg) were rehydrated in phosphate buffer (50 mM, pH 8.0, 0.50 mL) in 1.5 Eppendorf tubes at 30°C before alcohol (20 mg, 15 mg for C-4 and C-5 alcohols) and acetone (125 μL) was added (V_{tot} = 0.625 mL). The vials were shaken at 30°C at 130 rpm for the indicated time. The reaction was stopped by extraction with EtOAc (1 mL). After centrifugation, the organic phase was separated, dried (Na_2SO_4) and analysed by GC. Products were identified by NMR and/or co-injection with independently obtained reference samples on GC.

4.2. Synthesis of substrates (*rac*-alcohols)

4.2.1. *rac*-(*Z*)-3-Penten-2-ol, *rac*-7a. To a solution of *rac*-3-pentyn-2-ol *rac*-**5a** (3.13 g, 37.2 mmol) in abs. EtOH (15 mL) and quinoline (5 mL), Pd (0.35 g, 5% on $CaCO_3$) was added and the resulting mixture was vigorously stirred under H_2 for 3 days. The *Z*:*E* ratio dropped from 55 (1 d) to 20 (3 d). After completion, the solids were removed by filtration through a plug of Hyflo Super Cel and crude (*Z*)-3-penten-2-ol was obtained from the filtrate by distillation to give 2.1 g *rac*-**7a** (65%, *Z*:*E* = 10). The product was identified by comparison of NMR data with literature values.[21]

4.2.2. *rac*-(*Z*)-4-Octen-3-ol, *rac*-12a. Compound **12a** was synthesised from *rac*-4-octyn-3-ol *rac*-**15a** (1.40 g, 11 mmol) as described for *rac*-**7a**. Flash chromatography (petroleum ether/EtOAc) afforded (*Z*)-4-octen-3-ol *rac*-**12a** [1.23 g (86%), *Z*:*E* ~20]. The product was identified by comparison of NMR data with literature values.[22]

4.2.3. *rac*-(*E*)-4-Octen-3-ol, *rac*-13a. *rac*-4-Octyn-3-ol *rac*-**15a** (0.97 g, 7.8 mmol) was dissolved in dry Et_2O (15 mL) and $LiAlH_4$ (0.45 g, 11.5 mmol, 97%) was added in small portions. The mixture was refluxed until completion (24 h), then the reaction was quenched with 2N HCl. The organic phase was extracted with water and aq. $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (petroleum ether/EtOAc) afforded *E*-alcohol *rac*-**13a** (0.61 g, 61%) as a colourless liquid. The product was identified by comparison of NMR data with literature values.[23]

4.2.4. *rac*-(*E*)-5-Octen-3-ol, *rac*-14a. Compound **14a** was synthesised from *rac*-5-octyn-3-ol *rac*-**15a** (0.50 g, 4.0 mmol) described for *rac*-**13a**. Flash chromatography (petroleum ether/EtOAc) afforded (*E*)-alcohol *rac*-**14a** (0.42 g, 83%) as a slightly yellow liquid. ^{1}H NMR (360 MHz, $CDCl_3$): δ 5.56 (H, dt, J = 15.3, 6.2 Hz), 5.40 (H, dt, J = 15.2, 1.5 Hz), 3.48 (H, m), 2.15–2.25 (H, m), 2.02 (3H, hex, J = 6.8 Hz), 1.77 (OH, bs), 1.40–1.52 (2H, m), 0.96 (3H, t, J = 7.4 Hz), 0.92 (3H, t, J = 7.5 Hz); ^{13}C NMR (90 MHz, $CDCl_3$): δ 136.1, 124.9, 72.3, 40.2, 29.4, 25.7, 13.8, 10.0.

4.2.5. *rac*-4-Octyn-3-ol, *rac*-15a. Commercial 1-pentyne (8.0 g, 40.3 mmol) was dissolved in anhydrous THF (120 mL) and cooled to −85°C under argon. *t*-BuLi (26 mL, 1.7 M in *n*-hexane, 1.1 equiv.) was added slowly, while the temperature was maintained below −70°C. After 5 min at −80°C the solution was allowed to warm to −15°C within 1.5 h. Propanal (2.34 g, 40.3 mmol, freshly distilled, in 10 mL THF) was added slowly at −80°C while the temperature was maintained below −65°C. After 10 min at −80°C the solution was allowed to warm slowly to room temperature. After 18 h the reaction was quenched by acidification (dil. aq. HCl) and the two-phase system was extracted with Et_2O. The organic phase was washed once with saturated $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. Flash chromatography (EtOAc/petroleum ether) afforded *rac*-4-octyn-3-ol *rac*-**15a** (8.2 g, 84%) as a yellow liquid. ^{1}H NMR (360 MHz, $CDCl_3$): δ 4.29 (H, m), 2.20 (2H, dt, J = 7.05, 1.93 Hz), 1.80 (OH, bs), 1.66–1.72 (2H, m), 1.54 (2H, hex, J = 7.2 Hz), 1.01 (3H, t, J = 7.0 Hz), 0.99 (3H, t, J = 7.4 Hz); ^{13}C NMR (90 MHz, $CDCl_3$): δ 85.3, 81.4, 63.9, 31.2, 22.2, 20.7, 13.4, 9.5.

4.2.6. (*R*)-4-Octyn-3-ol, (*R*)-15a. $[\alpha]_D^{21}$ +8.4 (c 0.79, Et_2O, 72% e.e.), lit.[24] $[\alpha]_D^{21}$ −33.5 (c 0.40 Et_2O) 95.5% e.e. (*S*)-enantiomer.

4.3. Synthesis of reference compounds (ketones)

4.3.1. 3-Pentyn-2-one, 5b. A solution of *rac*-3-pentyn-2-ol *rac*-**5a** (0.52 g, 6.15 mmol) in CH_2Cl_2 (8 mL) was titrated with a solution of 8N Jones' reagent (8 h) at room temperature using a water bath. After complete consumption of the alcohol the organic phase was extracted with water, dried (Na_2SO_4) and concentrated. Flash chromatography (*n*-pentane/CH_2Cl_2) afforded pure 3-pentyn-2-one (240 mg, 47%). The product was identified by comparison of NMR data with literature values.[25]

4.3.2. 4-Penten-2-one, 6b. A suspension of *rac*-4-penten-2-ol *rac*-**6a** (0.42 g, 5.4 mmol, in 10 mL CH_2Cl_2) and PDC (3.8 g, 10 mmol) was vigorously stirred at room temperature for 8 days. The solids were centrifuged (13000 rpm). After addition of Et_2O (10 mL) the solution was extracted with 5% aq. HCl and after neutralisation (extraction with aq. $NaHCO_3$ solution and water) it was dried (Na_2SO_4). Flash chromatography (*n*-pentane/CH_2Cl_2) afforded pure 4-penten-2-one **6b** (20 mg, 5%). ^{1}H NMR (360 MHz, $CDCl_3$): δ 5.82–6.04

(H, m), 5.17 (H, d, $J=11.0$ Hz), 5.11 (H, d, $J=20.3$ Hz), 3.16 (2H, d, $J=7.1$), 2.12 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$): δ 206.8, 130.5, 118.9, 48.6, 29.5.

4.3.3. (*E*)-3-Penten-2-one, 8b. *rac*-4-Penten-2-ol *rac*-**6a** was oxidised as described for the synthesis of **13b**. The product was identified by comparison of NMR data with literature values.[26]

4.3.4. (*Z*)-4-Octen-3-one, 12b. Compound **12b** was synthesised as described for **6b**. Flash chromatography (*n*-pentane/CH_2Cl_2) afforded (*Z*)-4-octen-3-one **12b** (54 mg, 39%, $Z:E=6$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 6.10 (2H, m), 2.58 (2H, q, $J=7.5$ Hz); 2.48 (2H, q, $J=7.3$ Hz), 1.45 (2H, hex, $J=7.5$ Hz), 1.07 (3H, t, $J=7.3$ Hz), 0.93 (3H, t, $J=7.4$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$): δ 202.1, 148.2, 126.5, 37.3, 31.4, 22.4, 13.3, 7.8.

4.3.5. (*E*)-4-Octen-3-one, 13b. *rac*-(*E*)-4-Octen-3-ol *rac*-**13a** was oxidised with whole cells of *R. ruber* DSM 44541 (200 mg alcohol, 800 mg cells, 2.5 mL acetone, 10 mL phosphate buffer pH 8.0, 22 h, 30°C, 130 rpm). After extraction with ethyl acetate, purification of the crude product by flash chromatography afforded ketone **13b** (75 mg) in 38% yield. ^{1}H NMR (360 MHz, $CDCl_3$): δ 6.83 (H, dt, $J=15.9$, 6.9 Hz), 6.10 (H, dt, $J=15.9$, 1.5 Hz), 2.56 (2H, q, $J=7.8$ Hz), 2.19 (2H, dq, $J=7.5$, 1.4 Hz), 1.50 (2H, hex, $J=7.4$ Hz), 1.10 (3H, t, $J=7.3$ Hz), 0.94 (3H, t, $J=7.4$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$): δ 201.2, 146.9, 130.3, 34.7, 34.4, 21.4, 13.7, 8.2.

4.3.6. 4-Octyn-3-one, 15b. *rac*-4-Octyn-3-ol *rac*-**15a** was oxidised as described for **13b**. Yield: 81 mg, 41%. ^{1}H NMR (360 MHz, $CDCl_3$): δ 2.55 (2H, q, $J=7.4$ Hz), 2.34 (2H, t, $J=7.0$ Hz), 1.61 (2H, q, $J=7.2$ Hz), 1.13 (3H, t, $J=7.4$ Hz), 1.01 (3H, t, $J=7.4$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$): δ 188.9, 94.1, 80.9, 38.8, 21.3, 20.9, 13.5, 8.1.

4.4. Verification of spontaneous isomerisation of 4-penten-2-one, 6b

4-Penten-2-one **6b** (10 μL) was shaken in phosphate buffer (0.50 mL, pH 8.0, 50 mM) at 24°C (130 rpm) in 1.5 mL vials with and without lyophilised cells (30 mg). In both cases isomerisation occurred as shown by GC after work up as described for the microbial oxidation.

4.5. Determination of absolute configuration

The absolute configuration of (*S*)-2-butanol (*S*)-**1a** and (*R*)-2-pentanol (*R*)-**3a** was proven by co-injection with commercially available nonracemic reference samples on GC using a chiral stationary phase. The absolute configuration of (*R*)-3-octanol (*R*)-**9a** was proven by comparison of the optical rotation with literature data. (*R*)-3-Octanol (*R*)-**9a**: $[\alpha]_D^{22}$ −9.15 (c 4.33, $CHCl_3$, 97% e.e.), lit.[27] $[\alpha]_D^{21}$ +8.8 (c 0.49 $CHCl_3$) for the (*S*)-enantiomer.

The assignment of the absolute configuration of all other nonracemic alcohols was performed via catalytic reduction (H_2, 1 atm, 5% Pd/C, MeOH, room temperature, 0.5 h) of the corresponding unsaturated acetate ester and co-injection with the acetate esters of racemic and nonracemic 2-pentanol **3a** and 3-octanol **9a**, respectively.

4.6. General procedure for the acetylation of alcohols for GC analysis

A sample of alcohol was dried (Na_2SO_4) and a proper volume (0.1–0.3 mL) of acetic anhydride and a catalytic amount of DMAP was added. After 30 min at room temperature, water (1.0 mL) and pentane (about 0.5 mL) was added. After shaking the vial (2.0 mL) for 10-30 s, the aq. phase was removed and replaced by 1.0 to 1.5 mL of water. The organic phase was extracted three times following this procedure, dried again and analysed by GC.

4.7. GC methods

4.7.1. Non-chiral methods. Column J&W Scientific Agilent Technologies HP-1 (30 m×0.25 mm×0.25 μm, 1.0 bar N_2) was used with the following temperature program: 40°C/5 min–0°C/min–60°C/1 min; retention times: **1a** 2.6 min; **1b** 2.5 min; **2a** 2.5 min; **3a** 3.7 min; **3b** 3.5 min; **4a** 3.5 min; **5a** 5.3 min; **5b** 5.0 min; **6a** 3.4 min; **6b** 3.3 min; **7a** 4.0 min; **8a** 3.9 min; **8b** 4.5 min.

4.7.2. Temperature program. 70°C/7 min–10°C/min–110°C/0 min–30°C/min–250°C/0 min; retention times: **9a** 8.8 min; **9b** 8.3 min; **10a** 8.3 min; **11a** 8.6 min; **12a** 8.3 min; **12b** 9.4 min; **13a** 8.5 min; **13b** 9.5 min; **14a** 8.6 min; **14b** 8.8 min; **15a** 9.5 min; **15b** 9.6 min. For data from chiral analysis, see Table 3.

Table 3. Chiral GC analysis

Substrate	Retention times (min)		Method	Column
2-Butanol **1a**[a]	2.3 (*S*)	2.5 (*R*)	32°C isotherm	A
2-Pentanol **3a**[a]	4.8 (*S*)	8.7 (*R*)	50°C isotherm	B
3-Pentyn-2-ol **5a**	7.1 (*S*)	8.8 (*R*)	50°C isotherm	B
4-Penten-2-ol **6a**[a]	5.1 (*S*)	8.1 (*R*)	50°C isotherm	B
(*E*)-3-Penten-2-ol **8a**[a]	4.4 (*S*)	7.3 (*R*)	50°C isotherm	B
3-Octanol **9a**[a]	11.8 (*S*)	12.4 (*R*)	Method A	B
(*E*)-4-Octen-3-ol **13a**[a]	11.7 (*S*)	11.2 (*R*)	Method A	B
(*E*)-5-Octen-3-ol **14a**[a]	11.9 (*S*)	12.3 (*R*)	Method A	B
4-Octyn-3-ol **15a**[a]	12.2 (*S*)	12.6 (*R*)	Method A	B

[a] Alcohol analysed as the corresponding acetate ester; Method A: 50°C/8 min–10°C/min–150°C/2 min; Column A: Astec Chiraldex G-PN (30 m×0.32 mm, 1.0 bar H_2); Column B: Chrompack Chirasil Dex (25 m×0.32 mm×0.25 μm, H_2).

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