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# Biocatalytic Asymmetric Hydrogen Transfer Employing *Rhodococcus ruber* DSM 44541

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Nonracemic *sec*-alcohols of opposite absolute configuration were obtained either by asymmetric reduction of the corresponding ketone using 2-propanol as hydrogen donor or by enantioselective oxidation through kinetic resolution of the *rac*-alcohol using acetone as hydrogen acceptor employing whole lyophilized cells of *Rhodococcus ruber* DSM 44541. The microbial oxidation/reduction system exhibits not only excellent stereo- and enantioselectivity but also a broad substrate spectrum. Due to the exceptional tolerance of the biocatalyst toward elevated concentrations of organic materials (solvents, substrates and cosubstrates), the process is highly efficient. The simple preparation of the biocatalyst and its ease of handling turns this system into a versatile tool for organic synthesis.

## Introduction

Catalytic asymmetric hydrogen transfer based on (transition) metals and chiral organic ligands constitutes a general approach to synthesize nonracemic *sec*-alcohols by asymmetric reduction[1] of the corresponding ketone or by kinetic resolution of *rac*-*sec*-alcohols via enantioselective oxidation.[2] Recently, the use of asymmetric hydrogen transfer catalysts compatible with aqueous solution—a tremendous opportunity for the practice of "green chemistry"—has garnered considerable interest and resulted in the publication of the first water-compatible or -soluble catalyst analogues.[1c,e,f,i]

All biocatalytic methods for the asymmetric hydrogen transfer are based on alcohol dehydrogenases requiring nicotinamide-cofactors. They show several advantages over the chemical methods, such as their intrinsic asymmetry, the absence of side reactions (in particular the aldol condensation), the essentially mild reaction conditions needed, and the preference for an aqueous medium. However, their large-scale application has been impeded by (i) their requirement for cofactor-recycling,[3] (ii) their instability toward elevated concentrations of organic materials (such as substrates and acetone or 2-propanol used as cosubstrates), and (iii) inhibition phenomena.[4] As a consequence, biochemical reductions/oxidations employing *sec*-alcohol dehydrogenases on a large scale are restricted to the use of fermenting cells[5] and are impeded by low (co)substrate concentration(s).[6]

We have recently presented a highly enantioselective *sec*-alcohol dehydrogenase[7] from *Rhodococcus ruber* DSM 44541 that is exceptionally stable toward organic sol-

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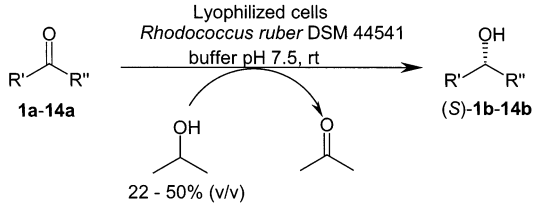


**FIGURE 1.** Asymmetric reduction of ketones employing lyophilized whole cells.

**TABLE 1. Biocatalytic Asymmetric Reduction of Ketones Employing *R. ruber* DSM 44541 (see Figure 1)**

| substr | R′ | R″ | activity ($\mu$mol $h^{-1}$ $g^{-1}$) | product | % conv[a] | % ee |
|---|---|---|---|---|---|---|
| **1a** | $CH_3$ | $n$-$C_8H_{17}$ | 69 | (*S*)-**1b** | 94 | >99 |
| **2a** | $CH_3$ | $n$-$C_7H_{15}$ | 84 | (*S*)-**2b** | 94 | >99 |
| **3a** | $CH_3$ | $n$-$C_6H_{13}$ | 97 | (*S*)-**3b** | 92 | >99 |
| **4a** | $CH_3$ | $n$-$C_5H_{11}$ | 93 | (*S*)-**4b** | 92 | >99 |
| **5a** | $CH_3$ | $(CH_3)_2C{=}CH(CH_2)_2$ | 95 | (*S*)-**5b** | 93 | >99 |
| **6a** | $C_2H_5$ | $n$-$C_5H_{11}$ | 74 | (*S*)-**6b** | 79 | 97 |
| **7a** | $CH_3$ | Ph | 103 | (*S*)-**7b** | 81 | >99 |
| **8a** | $CH_3$ | $p$-$MeOC_6H_4$ | nd | (*S*)-**8b** | 61 | >99 |
| **9a** | $CH_3$ | $c$-$C_6H_{11}$ | 90 | (*S*)-**9b** | 87 | >99 |
| **10a** | $CH_3$ | 2-naphthyl | 64 | (*S*)-**10b** | 82 | >99 |
| **11a** | $CH_3$ | (*E*)-Ph-HC=CH | nd[b] | (*S*)-**11b** | 52 | >99 |

[a] 22% v/v 2-propanol, 22 h reaction time. [b] nd, not determined.

vents. The enzyme's activity remains high at concentrations of up to 20% (v/v) acetone and 50% (v/v) 2-propanol, allowing one to perform asymmetric hydrogen transfer in aqueous systems at neutral pH. For preparative-scale applications, whole lyophilized or resting cells can be used as a readily available alternative.

## Results and Discussion

**Reduction.** Hydrogen-transfer reactions are generally reversible and preparative-scale reactions are usually driven into the desired reduction or oxidation direction by employing the corresponding cosubstrate as hydrogen acceptor/donor in excess to shift the system out of equilibrium.

Metal-catalyzed hydrogen-transfer reductions described so far are often limited by the reversibility of the reaction due to the low cosubstrate concentration used (0.1 M 2-propanol[2a]). In contrast, whole lyophilized cells of *R. ruber* DSM 44541 perform the asymmetric hydrogen transfer reaction at 2-propanol concentrations up to 50% v/v ≈7.9 M[7], thus avoiding the problem of reversibility due to the excess of cosubstrate (Figure 1).

*n*-Alkan-2-ones **1a**−**4a** were reduced to the corresponding (*S*)-alcohols **1b**−**4b** with excellent stereoselectivity (Table 1) using 22% (v/v) 2-propanol as cosubstrate. Branching and the presence of a C=C double bond in the alkyl chain at the $\omega$-position of the alkyl chain (substrate **5a**) does not influence the ee of the product nor the activity. A slight decrease in stereoselectivity was observed when the carbonyl moiety was moved to the more hindered 3-position (substrate **6a**). Acetophenone **7a** and even *p*-methoxyacetophenone **8a**, which can be reduced only with difficulties using metal-catalyzed hydrogen-

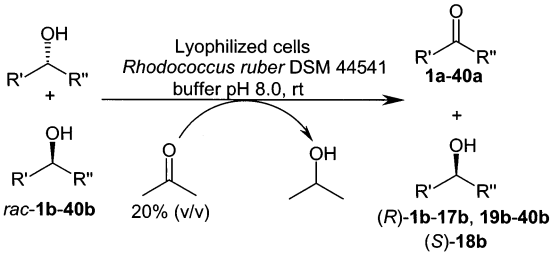


**FIGURE 2.** Biocatalytic kinetic resolution of *sec*-alcohols by enantioselective oxidation.

**CHART 1**

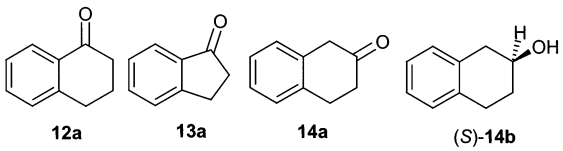


**TABLE 2. Biocatalytic Reduction of Cyclic Ketones (see Figure 1 and Chart 1)**

| substr | product | % conversion[b] | % ee |
|---|---|---|---|
| **12a** | **12b**[a] | <0.2 | nd[c] |
| **13a** | **13b**[a] | 0.2 | 7.8 |
| **14a** | (*S*)-**14b** | 19.8 | 83.2 |

[a] Absolute configuration was not determined due to low conversion. [b] 22 h reaction time. [c] nd, not determined due to low conversion.

transfer protocols,[2a] can be transformed to their corresponding (*S*)-alcohols with exceptional prochiral recognition. Enantiopure 4-phenyl-but-3-en-2-ol **11b** can be obtained either by kinetic resolution of the *rac*-alcohol employing Sharpless epoxidation[8] or via asymmetric hydrogenation of ketone **11a** in 97% ee;[9] however, metal-catalyzed hydrogen-transfer protocols failed due to insufficient chemodifferentiation between the C=C and C=O double bond. Based on the intrinsic excellent chemoselectivity of alcohol dehydrogenases, the (*S*)-alcohol **11b** was obtained from ketone **11a** employing whole cells of *R. ruber* DSM 44541 in >99% ee.

However, in contrast to metal-catalyzed hydrogen-transfer methods, 1-tetralone **12a** and 1-indanone **13a** (Chart 1) could not be reduced by employing the microbial method. Nevertheless, 2-tetralone **14a** was reduced with reduced activity and moderate stereopreference; thus, alcohol (*S*)-**14b** was obtained in 83% ee.

The stereopreference of the dehydrogenase(s) involved follows Prelog's rule;[10] thus, the attack of the (formal) hydride preferentially occurs from the Re-side of the ketone by assuming that the smaller group possesses the lower CIP-priority.

**Oxidation.** The microbial alcohol dehydrogenase system not only tolerates 2-propanol but also acetone in concentrations of up to 20% (v/v) to effect the reverse

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**TABLE 3. Biocatalytic Kinetic Resolution by Oxidation of *rac*-Alkanols Employing Lyophilized Cells of *R. ruber* DSM 44541 (see Figure 2)**

| substr. | R′ | R″ | activity ($\mu$mol h$^{-1}$ g$^{-1}$) | time (h) | % conv | alcohol | % ee | *E* value |
|---|---|---|---|---|---|---|---|---|
| **15b** | $CH_3$ | *n*-$C_{10}H_{21}$ | 140 | 24 | 8.3 | **15b**[a] | 10.2 | nd[b] |
| **1b** | $CH_3$ | *n*-$C_8H_{17}$ | 420 | 24 | 55 | (*R*)-**1b** | 96.5 | 34 |
| **2b** | $CH_3$ | *n*-$C_7H_{15}$ | 840 | 24 | 56 | **2b**[a] | 99.0 | 43 |
| **3b** | $CH_3$ | *n*-$C_6H_{13}$ | 1260 | 24 | 50 | (*R*)-**3b** | >99 | >100 |
| **4b** | $CH_3$ | *n*-$C_5H_{11}$ | 1400 | 24 | 52 | (*R*)-**4b** | >99 | >100 |
| **16b** | $CH_3$ | *n*-$C_4H_9$ | 1120 | 24 | 49 | (*R*)-**16b** | >99 | >100 |
| **17b** | $CH_3$ | *n*-$C_3H_7$ | 560 | 24 | 56 | (*R*)-**17b** | 97.5 | 33 |
| **18b** | $CH_3$ | $C_2H_5$ | 1540 | 24 | 96 | (*S*)-**18b** | 34 | 1.3 |
| **5b** | $CH_3$ | $(CH_3)_2C{=}CH(CH_2)_2$ | 1550 | 24 | 50 | (*R*)-**5b** | >99 | >100 |
| **6b** | $C_2H_5$ | *n*-$C_5H_{11}$ | 5 | 22 | 49.5 | (*R*)-**6a** | 33.0 | 2.7 |
| **19b** | $CH(CH_3)_2$ | *n*-$C_5H_{11}$ | nd | 24 | 0.7 | **19b**[a] | nd | nd |
| **20b** | *n*-$C_3H_7$ | *n*-$C_4H_9$ | nd | 24 | 36.9 | **20b**[a] | 0 | ~1 |

[a] Absolute configuration of remaining alcohol not determined due to low conversion;. [b] nd, not determined due to low conversion.

reaction, namely, the kinetic oxidative resolution of *rac-sec*-alcohols via biocatalytic hydrogen transfer (Figure 2). The biocatalyst not only displays excellent stereoselectivity for most substrates investigated but is also able to differentiate between primary and secondary alcohols. Only *sec*-alcohols are oxidized and primary alcohols (e.g. 1-octanol, 2-phenylethanol) remain essentially untouched. Since the catalyst always acts on the same enantiomer—the ketone is reduced to the (*S*)-alcohol—the (*S*)-enantiomer is oxidized from a racemate in the oxidation mode, by leaving the (*R*)-enantiomer untouched. By these means, both enantiomers are accessible in nonracemic form using the same catalyst.[2b]

Kinetic resolution of *rac*-*n*-2-alkanols of varying chain length (C3−C10, substrate **1b**−**4b** and **15b**−**17b**) revealed that the oxidative activity peaks at a maximum of C7, as (*S*)-2-heptanol (*S*)-**4b** was oxidized fastest (Table 3). The activity for 2-dodecanol **15b** was only one tenth of the rate for 2-heptanol **4b**. For 2-alkanols exceeding 10 carbon atoms (C10, C12) but also for shorter 2-alkanols (C5), both the activity and also the enantioselectivity was reduced. Thus, for 2-pentanol **17b** the enantioselectivity decreased to $E = 33$. However, upon reducing the length of the carbon chain from C5 to C4 (substrate **18b**), an unexpected phenomenon was observed: The activity almost tripled but the enantioselectivity was very low ($E = 1.3$), going in hand with *opposite* "anti-Prelog" stereopreference. Thus, the (*R*)-enantiomer was preferentially oxidized instead of the (*S*)-enantiomer. By comparing the data for the oxidation with that of the reduction, it becomes clear that stereoselectivities match for 6-methyl-hept-5-en-2ol **5b**/6-methyl-hept-5-en-2one **5a** but mismatch for 3-octanol **6b**/3-octanone **6a**. Although the enantioselectivity for the oxidation of *rac*-3-octanol **6b** was low ($E \sim 5$, Table 3)—the (*S*)-enantiomer was oxidized only 5 times faster than (*R*)-**6b**—the corresponding reduction of ketone **6a** furnished alcohol (*S*)-**6b** in 97% ee at 79% conversion (Table 1). The same is true for 2-decanol **1b**/2-decanone **1a**. Nevertheless, more sterically hindered alcohols, such as **6b** and **19**−**20b**, were transformed much more slowly.

Sterically encumbered cyclic ketones α-tetralone **12a** and α-indanone **13a** could not be reduced by this method (see Table 2), but their corresponding *rac*-alcohols (α-tetralol *rac*-**12b**) and α-indanol (*rac*-**13b**) (Chart 2) were resolved through oxidation, albeit at low rates and insufficient enantioselectivities (Table 4). In contrast,

**CHART 2**

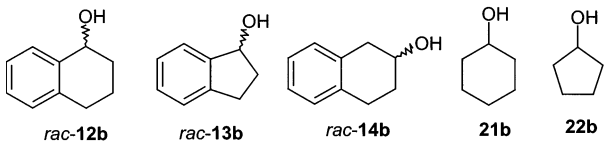


**TABLE 4. Biocatalytic Oxidation (for Kinetic Resolution) of Cyclic Alkanols (see Figure 2 and Chart 2)**

| substrate | time (h) | % conv[b] | alcohol | % ee | *E* value |
|---|---|---|---|---|---|
| *rac*-**12b** | 24 | 4.9 | (*S*)-**12b** | 4.0 | 8.3 |
| *rac*-**13b** | 24 | 11.3 | **13b**[a] | 7.8 | 4.5 |
| rac-**14b** | 24 | 0.2 | **14b**[a] | 0.3 | nd[c] |
| **21b** | 24 | 8.7 | | na[c] | na |
| **22b** | 24 | 98.0 | | na | na |

[a] Absolute configuration of remaining alcohol not determined due to low conversion. [b] 24 h reaction time. [c] na, not applicable; nd, not determined due to low conversion.

although β-tetralol *rac*-**14b** was not oxidized, the corresponding ketone **14a** was reduced with moderate stereopreference (ee = 83%, Table 2). Interestingly, cyclohexanol **21b** was oxidized at rather low rate (8.7% conversion within 24 h), while cyclopentanol **22b** was an excellent substrate (98% conversion within 24h).

Racemic 1-phenyl ethanol *rac*-**7b** was resolved by kinetic oxidative resolution with excellent enantioselectivity ($E > 100$) (Table 5). However its *p*-amino **23b** derivative was not accepted; similarly, *p*-hydroxy-1-phenylethanol **24b** exhibited moderate selectivity ($E \sim 7$). All other 1-phenylethanol derivatives possessing a substituent in the para-position, such as *p*-MeO **8b**, *p*-F **25b**, *p*-Cl **26b**, *p*-Br **27b**, and *p*-methyl **28b**, gave excellent results in kinetic resolution. Steric hindrance may be the reason that ortho-substituted 1-phenylethanol derivatives, e.g., **30b** (*o*-Me-), **32b** (*o*-Cl), or **34b** (*o*-methoxy-1-phenylethanol), were not accepted. However, no difference in enantioselectivity was observed for the para- and meta-position for a Me (**29b**), Cl (**31b**), or methoxy (**33b**) derivative, and relative activities of meta-derivatives were even higher than that of the para-analogues. Interestingly, whereas the saturated system 1-cyclohexylethanol **9b** was resolved with low enantioselectivity, the corresponding reduction of 1-cyclohexylethanone **9a** proceeded in excellent stereoselectivity (ee >99%) and yield (Table 1). Expanding the aromatic

**TABLE 5. Biocatalytic Kinetic Resolution of *rac*-1-Phenylethanol Derivatives by Oxidation Employing *R. ruber* DSM 44541 (see Figure 2)**

| substr | R′ | R″ | activity ($\mu$mol $h^{-1}$ $g^{-1}$) | % conv[b] | alcohol | % ee | *E* value |
|---|---|---|---|---|---|---|---|
| **7b** | Ph | $CH_3$ | 133 | 44.4 | (*R*)-**7b** | 77.8 | >100 |
| **23b** | *p*-$NH_2$-$C_6H_4$ | $CH_3$ | <1 | nc[c] | **23b**[a] | | |
| **24b** | *p*-$OHC_6H_4$ | $CH_3$ | nd[c] | 36 | (*R*)-**24b** | 37 | 6.6 |
| **8b** | *p*-$MeOC_6H_4$ | $CH_3$ | 456 | 49.8 | (*R*)-**8b** | >99 | >100 |
| **25b** | *p*-$FC_6H_4$ | $CH_3$ | 241 | 43.5 | (*R*)-**25b** | 73.5 | 96 |
| **26b** | *p*-$ClC_6H_4$ | $CH_3$ | 257 | 41.3 | (*R*)-**26b** | 78.2 | >100 |
| **27b** | *p*-$BrC_6H_4$ | $CH_3$ | 208 | 43.8 | (*R*)-**27b** | 82.5 | >100 |
| **28b** | *p*-Me-$C_6H_4$ | $CH_3$ | 432 | 47.6 | (*R*)-**28b** | 97.3 | >100 |
| **29b** | *m*-Me-$C_6H_4$ | $CH_3$ | 573 | 50.1 | (*R*)-**29b** | >99 | >100 |
| **30b** | *o*-Me-$C_6H_4$ | $CH_3$ | <1 | nc | **30b**[a] | | |
| **31b** | *m*-$ClC_6H_4$ | $CH_3$ | 299 | 42.7 | (*R*)-**31b** | 76.1 | >100 |
| **32b** | *o*-$ClC_6H_4$ | $CH_3$ | <1 | nc | **32b**[a] | | |
| **33b** | *m*-$MeOC_6H_4$ | $CH_3$ | nd | 45.4 | (*R*)-**33b** | 85.7 | >100 |
| **34b** | *o*-$MeOC_6H_4$ | $CH_3$ | <1 | nc | **34b**[a] | | |
| **9b** | cyclohexyl | $CH_3$ | nd | 32.0 | (*R*)-**9b** | 26 | 31 |
| **10b** | 2-naphthyl | $CH_3$ | nd | 48.9 | (*R*)-**10b** | >99 | >100 |

[a] Absolute configuration of remaining alcohol not determined due to low conversion. [b] 24 h reaction time. [c] nc, no conversion; nd, not determined due to low conversion.

**TABLE 6. Results of the Biocatalytic Kinetic Resolution by Oxidation of *rac*-1-Phenylethanol Derivatives Employing Lyophilized Whole Cells of *R. ruber* DSM 44541 (see Figure 2)**

| substr | R′ | R″ | % conv[b] | alcohol | % ee | *E* value |
|---|---|---|---|---|---|---|
| **35b** | Ph | $C_2H_5$ | 5.4 | (*R*)-**35b** | 2.0 | nd[c] |
| **36b** | Ph | $C_3H_7$ | 3.2 | **36b**[a] | nd | nd |
| **37b** | Ph | $C_4H_9$ | 7.4 | (*R*)-**37b** | 5.6 | nd |
| **38b** | Ph | $C_5H_{11}$ | 6.4 | **38b**[a] | nd | nd |
| **39b** | Ph-$CH_2$ | $CH_3$ | 22.8 | (*R*)-**39b** | 34.0 | >100 |
| **40b** | Ph-$(CH_2)_2$ | $CH_3$ | 49.5 | (*R*)-**40b** | 96.1 | >100 |

[a] Absolute configuration of remaining alcohol not determined due to low conversion. [b] 24 h reaction time. [c] nd, not determined due to low conversion.

system from a phenyl to a naphthyl moiety (substrate **10b**) resulted in an excellent kinetic resolution.

Substitution of the $CH_3$ moiety in 1-phenylethanol by a longer alkyl group resulted in a decrease of activity (see substrates **35b**−**38b**, Table 6). In contrast, by keeping the $CH_3$ moiety and moving the phenyl moiety further away from the alcohol group through $-CH_2-$ spacer groups (substrates **39b** and **40b**), the activity did not decrease and no negative influence on the enantioselectivity was observed ($E$ > 100). Substrate **40b** is of practical interest, since it constitutes a precursor for the synthesis of antihypertensive agents, such as bufeniode or labetalol,[11] and spasmolytics or antileptics, such as emepronium bromide.[12] Both enantiomers were obtained either by oxidative kinetic resolution of *rac*-**40b** to give (*R*)-**40b** (see Table 6) or reduction of ketone **40a** to give (*S*)-**40b**, with >99% ee at 98% conversion within 4.5 h at 50% v/v 2-propanol.

## Conclusions

We have shown that the whole-cell microbial oxidation/reduction system of *R. ruber* DSM 44541 represents a versatile tool for organic synthesis for the following reasons: (1) A broad spectrum of ketones can be reduced in an asymmetric fashion giving (*S*)-configured alcohols in high yields and excellent ee using 2-propanol as a hydrogen donor at 22% v/v. (2) Likewise, the system can tolerate acetone as a hydrogen acceptor for oxidation reactions at a concentration of 20% v/v, and a large variety of *rac*-alcohols can be resolved by kinetic resolution to furnish nonreacted (*R*)-alcohols and the corresponding ketone, which may be recycled. (5) High substrate concentrations make these systems applicable to large-scale applications.[13]

## Experimental Section

Cells were cultivated and prepared according to literature methods.[14]

The following substrates and reference compounds were commercially available:

**1a**−**40a**, **1b**−**7b, 12b**, **13b**, **15b**−**23b**, **28b**−**31b**, **35b**, **37b**, **39b**, and **40b**.

The following *rac*-alcohols were synthesized by reduction ($NaBH_4$, EtOH) of the corresponding ketone as previously reported, and products were identified by comparison of NMR data:

**8b**,[15] **9b**,[16] **10b**,[16] **11b**,[16] **14b**,[17] **24b**,[18] **25b**,[15] **26b**,[15] **27b**,[15] **32b**,[15] **33b**,[15] **34b**,[15] **36b**,[19] and **38b**.[19]

**Microbial Transformations.** In a typical oxidation procedure, lyophilized cells (40 mg) were rehydrated in sodium phosphate buffer (0.50 mL, pH 8.0, 50 mM) for 0.5 h before substrate (200 $\mu$mol) and acetone (125 $\mu$L) were added, and the reaction mixture was shaken for 24 h at 130 rpm (24 °C).

In a typical reduction procedure, lyophilized cells (40 mg) were rehydrated in sodium phosphate buffer (0.50 mL, pH 7.5, 50 mM) for 0.5 h before substrate (100 $\mu$mol) and 2-propanol (140 $\mu$L) were added, and the reaction mixture was shaken for 22 h at 130 rpm (24 °C).

Biotransformations were stopped by addition of ethyl acetate (0.7 mL), mixing, and centrifugation (5 min, 13 000 rpm). The organic phase was dried ($Na_2SO_4$) and analyzed by GC on a chiral stationary phase (see below).

**Determination of Absolute Configuration.** The absolute configuration of the followings compounds was elucidated by coinjection on GC using a chiral stationary phase and commercially available reference samples: (*S*)-**3b**, (*S*)-**4b**, (*S*)-**7b**, (*S*)-**10b**, (*S*)-**12b**, (*R*)-**16b**, (*R*)-**17b**, (*S*)-**18b**, and (*R*)-**39b**. The absolute configuration of (*S*)-**8b**, (*R*)-**35b**, and (*R*)-**37b** was proven by comparison of GC data with that of the literature.[20] The absolute configuration of (*S*)-**11b** was determined by hydrogenation of the C=C double bond (5% Pd/C, EtOH, $H_2$ 1 bar) and comparison of the resulting product **40b** with (*S*)-**40b** by coinjection on GC using a chiral stationary phase. The absolute configuration of the remaining compounds was determined by comparison of optical rotation values with literature data (see the Supporting Information).

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**Supporting Information Available:** Achiral and chiral GC data as well as the $[\alpha]^{20}_{D}$ values. This material is available free of charge via the Internet at http://pubs.acs.org.

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