Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 2709–2713

TETRAHEDRON: *ASYMMETRY*

Enantioselective hydrolyses with *Yarrowia lipolytica*: a versatile strain for esters, enol esters, epoxides, and lactones

Giancarlo Fantin, Marco Fogagnolo, Alessandra Guerrini, Alessandro Medici,* Paola Pedrini and Silvia Fontana

Dipartimento di Chimica, Università di Ferrara, Via L. Borsari 46, I-44100 Ferrara, Italy

Received 18 September 2001; accepted 19 October 2001

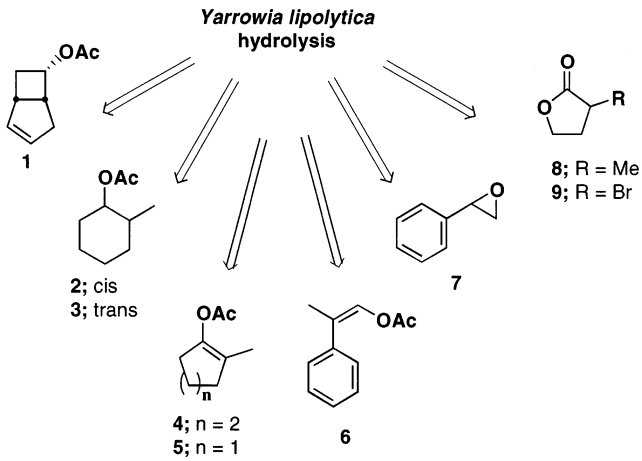
Abstract—Racemic secondary esters **1**–**3**, γ-lactones **8**–**9**, and styrene oxide **7** are kinetically resolved via hydrolysis with *Yarrowia lipolytica* YL2 strain. The enantioselective hydrolysis of prochiral enol esters **4**–**6** to the corresponding homochiral carbonyl compounds **13**–**15** is also described. Subsequent reduction of the ketone **13** and of the aldehyde **15** can be avoided using lyophilised cells. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Among biocatalyzed reactions the hydrolytic transformations involving amide and ester bonds are most easy to perform and are widely used to obtain homochiral secondary alcohols by proteases, esterases and most of all lipases.[1] Other types of applications of hydrolytic enzymes involving the cleavage of phosphate esters, nitriles and epoxides are generally more complicated.[2] Whole microbial cells have also been used to catalyse some esterolytic reactions: interesting examples can be found for both bacteria and fungi, i.e. *Bacillus subtilis*,[3] *Brevibacterium ammoniagenes*,[4] *Bacillus coagulans*,[5] *Pichia miso*,[6] *Rhizopus nigricans*,[7] and *Rhizopus oryzae*.[8] On the other hand, the examples of microbial hydrolysis of epoxides[9–12] and nitriles[13–15] are not so common, while only a few papers describe the hydrolytic conversions by baker's yeast,[16–18] although the use of this microorganism is very simple and obviates the need for sterile fermentation equipment. Herein, we describe the very efficient microbial hydrolysis of compounds **1**–**9** with *Yarrowia lipolytica* strains (Scheme 1). These microorganisms are yeasts, distributed over a wide range of food systems, that use oils and fats as the sole carbon source and previously have been efficiently employed in the enantioselective reduction of prochiral ketones[19] and in the kinetic resolution of racemic secondary alcohols via oxidation.[20]

2. Results and discussion

Seventeen strains of *Y. lipolytica*[19] have been employed in hydrolytic screening of compounds **1**–**9** and the results have been monitored by GLC. These preliminary results have focused our attention on *Y. lipolytica* YL2, that had been the strain of choice in the oxidation of secondary alcohols[20] and that, also in this case, show higher efficiency in the hydrolysis of all substrates. Most of the hydrolyses with *Y. lipolytica* YL2 are repeated on preparative scale and the best results, both on analytical and preparative scale, are summarised in Table 1.

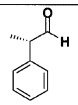


Scheme 1.

* Corresponding author. E-mail: mda@dns.unife.it

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00463-3

Table 1. Hydrolysis of compounds **1–9** with *Y. lipolytica* YL2

Substrate	Time (h)	Hydrolysis product	Starting materials	Reduction product
 rac-**1**	1[a] 2[b]	 (*1S,5R,6S*)-**10** 50% (ee 60%) 79% (ee 26%)	 (*1R,5S,6R*)-**1** 50% (ee 67%) 21% (ee >95%)	
 rac-**2**	18[a] 24[b]	 (*1S,2R*)-**11** 56 % (ee 60 %) 95% (ee 10%)	 (*1R, 2S*)-**2** 44 % (ee 95 %) 5% (ee 100%)	
 rac–**3**	2[a] 4[b]	 (*1S,2S*)-**12** 56% (ee 39%) 90% (ee 19%)	 (*1R,2R*)-**3** 44% (ee 42%) 10% (ee 100%)	
 **4**	2[a] 2[b,c] 2[b,d]	 (*S*)-**13** 78% (ee > 95%) 71% (ee 100%) 91 % (ee 53%)		 (*1S,2R*)-**11** 22% (ee 100%) 28% (ee 100%) --
 **5**	30 min[b]	 (*R*)-**14** 100% (ee 22%)		
 **6**	20 min[b] 30 min [a,e]	 (*S*)-**15** 6% (ee 28%) 76% (ee 57%)[f]		 **16** 92% (ee 0 %)
 rac-**7**	8[b,c] 24[b,c]	Not detected	 (*R*) – **7** 57% (ee 20%) 22% (ee 52%)	
 rac-**8**	24[a] 48[b]	Not detected	 (*S*)-**8** 65% (ee 40%) 48% (ee 72%)	
 rac-**9**	2[b] 4[b]	Not detected	 (*S*)-**9** 56% (ee 12%) 36% (ee 22%)	

[a] Reaction on preparative scale.

[b] The yield was determined by chromatography using 4-methylcyclohexanone as internal standard.

[c] Hydrolysis carried out in phosphate buffer pH 7.

[d] Reaction carried out with lyophilised cells in phosphate buffer pH 6.

[e] Reaction carried out with lyophilised cells in phosphate buffer pH 7.

[f] The enantiomeric excess is determined reducing the aldehyde with $NaBH_4$ to the *S*-alcohol **16**.

The hydrolysis of the *endo*-bicyclo[3.2.0]hept-2-en-6-ol acetate **1**, after 1 h incubation with growing cells of *Y. lipolytica* YL2, produces the (1*S*,5*R*,6*S*)-*endo*-alcohol **10** in 50% yield (ee 60%) and leaves the (1*R*,5*S*,6*R*)-*endo*-acetate **1** (50%, ee 67%). The same acetate is recovered enantiomerically pure (21%) after 2 h incubation and the hydrolysis is complete after 4 h. The screening reveals, however, two strains, i.e. *Y. lipolytica* YL4 and YL10, that give the opposite enantiomer (1*R*,5*S*,6*R*)-*endo*-alcohol **10** in 32% yield (ee 100%, 6 h incubation) and in 46% yield (ee 66%, 4 h incubation), respectively. Obviously in both cases the (1*S*,5*R*,6*S*)-*endo*-acetate **1** is recovered in 68% yield (ee 47%) and in 45% yield (ee 34%), respectively.

Y. lipolytica YL2 is also efficient in the hydrolysis of racemic *cis*- and *trans*-2-methylcyclohexanol acetates **2** and **3**: the rate is different but both racemic acetates afford the 1*S*-alcohols **11** and **12** leaving the 1*R*-acetates unreacted. In particular the (1*R*,2*S*)-*cis* acetate **2** is resolved after 18 h incubation (yield 44%, ee 95%) producing the (1*S*,2*R*)-alcohol **11** (56%, ee 60%), while the (1*R*,2*R*)-t*rans*-acetate **3** is obtained in 44% yield only after 2 h but with a lower enantiomeric excess (42%): in this case the (1*S*,2*S*)-alcohol **12** (56%, ee 39%) is produced.

On the other hand, 1-acetoxy-2-methylcyclohexene **4** is enantioselectively hydrolysed by *Y. lipolytica* YL2 producing, after 2 h incubation, (*S*)-2-methylcyclohexanone **13** (78% yield, ee >95%) together with the pure reduction product (1*S*,2*R*)-*cis*-2-methylcyclohexanol **11** (22%). Under these conditions (Sabouraud medium) the reduction cannot be avoided because the ratio between the hydrolysis and reduction product is also the same after 10 min incubation and keeps uniform during the biotranformation. In order to obtain only the (*S*)-ketone, *Y. lipolytica* YL2 cells were harvested by centrifugation and suspended in phosphate buffer at pH 6, 7 and 8. The incubation at pH 7 gave the best result but similar (see Table 1) to that obtained in Sabouraud, while in the other cases only lower yields were obtained. The *cis*-alcohol **11** was not produced carrying out the hydrolysis only with lyophilised cells of *Y. lipolytica* YL2 suspended in phosphate buffer at pH 6: the enantiomeric excess of the (*S*)-2-methylcyclohexanone **13** (91%, ee 53%) was lower, while at pH 7 it increased (ee 64% of *S*-**13**) but again the *cis*-alcohol **11** (15%, ee 100%) was obtained. No reduction product was detected in the *Y. lipolytica* hydrolysis of 1-acetoxy-2-methylcyclopentene **5**: the reaction is very fast and affords, after 30 min incubation, only the (*R*)-ketone **14** with low enantiomeric excess (100% yield, ee 22%). The ee does not change during the biotranformation.

However, the hydrolysis of 2-phenyl-2-propen-1-ol acetate **6** to the corresponding aldehyde **15** (54%) followed by reduction to 2-phenyl-1-propanol **16** (31%) was practically immediate (30 s). In fact, after 20 min incubation only 6% of (*S*)-aldehyde **15** was detected (ee 28%) together with the racemic alcohol **16** (92%). Also in this case, the reduction was inhibited using lyophilised cells of *Y. lipolytica* YL2 in phosphate buffer at pH 7. After 30 min incubation the (*S*)-aldehyde **15** was obtained (76% yield, ee 57%) and no reduction product was detected even after 1 h (100% yield, ee 34%). In order to avoid the easy racemization, the absolute configuration of the aldehyde was determined by reduction to the corresponding (*S*)-alcohol **16**.

Styrene oxide **7** was totally hydrolysed after 2 h incubation with growing cells of *Y. lipolytica* YL2 in Sabouraud. On the contrary, the reaction carried out with the cells, suspended in phosphate buffer at pH 7, leaves, after 8 h, the (*R*)-styrene oxide **7** (57% yield, ee 20%). Obviously when only the 22% of the starting product remains (24 h) the enantiomeric excess of the (*R*)-isomer increases (52%).

The hydrolysis, moreover, of the α-methyl-γ-butyrolactone **8** with *Y. lipolytica* YL2 kinetically resolves (48 h incubation) the (*S*)-enantiomer (48% yield, ee 72%). On the other hand, the hydrolysis of the α-bromo derivative **9** is faster but less selective. After 2 h incubation the (*S*)-enantiomer **9** was recovered in 56% yield but with low enantiomeric excess (12%) and with 36% yield (ee 22%) after 4 h.

3. Conclusion

In this screening the yeast strain *Y. lipolytica* YL2, already amply utilized in reduction of prochiral carbonyls and in oxidation of racemic secondary alcohols, has showed a great versatility, efficiency and aptitude for hydrolysing various substrates. The possibility of utilizing lyophilised cells with only minor lack of activity will be studied in order to use this strain in organic solvents both in hydrolysis and in esterification. *Y. lipolytica* YL2 appears as a candidate to become a valid choice towards hydrolytic enzymes.

4. Experimental

The acetyl derivatives of the commercially available *endo*-bicyclo[3.2.0]hept-2-en-6-ol, *cis*-2-methyl-cyclohexanol, and *trans*-2-methylcyclohexanol were prepared with acetic anhydride and pyridine. 1-Acetoxy-2-methylcyclohexene and 1-acetoxy-2-methylcyclopentene were prepared by treatment of the corresponding ketone with perchloric acid and acetic anhydride.[21] 2-Phenyl-2-propen-1-ol acetate was prepared from the corresponding aldehyde with acetic anhydride, potassium carbonate and sodium acetate.[22] Styrene oxide, α-methyl- and α-bromo-γ-butyrolactone, and 4-methylcyclohexanone are commercially available. The fermentation was obtained in a Bioindustrie Mantovane bioreactor Model BM 3000 CLP. Lyophilization was achieved with an Edwards E-C Modulyo lyophilizator.

4.1. Enantiomer separation

Gas chromatographic analyses were performed on a Carlo Erba GC 6000 Vega series 2. Enantiomer separa-

tion for compound **1** was achieved on Megadex 5 column (25 m×0.25 mm) containing *n*-pentyl dimethyl β-cyclodextrin in OV 1701 from Mega s.n.c.: carrier gas: helium 82 kPascal. Enantiomer separation for compounds **2**–**9** was achieved on a Megadex DETTBSβ column (25 m×0.25 mm) containing diethyl-*tert*-butylsilyl β-cyclodextrin in OV 1701 from Mega s.n.c.: carrier gas: helium 100 kPascal. The yield of the reaction on analytical scale was determined by GC using 4-methylcyclohexanone as internal standard.

For the hydrolysis of **1**, temperature 90–200°C (1°C/min), retention time (min): (1*R*,5*S*,6*R*)-**10**, 19.04; (1*S*,5*R*,6*S*)-**10**, 19.29; (1*S*,5*R*,6*S*)-**1**, 20.50; (1*R*,5*S*,6*R*)-**1**, 22.30. For the hydrolysis of **2**, temperature 70–200°C (1.5°C/min), retention time (min): **11**, 10.31; (1*R*,2*S*)-**2**, 10.77; (1*S*,2*R*)-**2**, 12.01. The enantiomeric excess of the *cis*-alcohol **11** was calculated after separation and acetylation with acetic anhydride and pyridine. For the hydrolysis of **3**, temperature 70–200°C (1.5°C/min), retention time (min): (1*S*,2*S*)-**12**, 9.60; (1*R*,2*R*)-**12**, 9.78; (1*R*,2*R*)-**3**, 10.61; (1*S*,2*S*)-**3**, 10.78. For the hydrolysis of **4**, temperature 70–200°C (1.5°C/min), retention time (min): (*S*)-**13**, 8.27; (*R*)-**13**, 8.53; **11**, 10.48; **4**, 13.99. For the hydrolysis of **5**, temperature 80–200°C (1°C/min), retention time (min): (*S*)-**14**, 3.93; (*R*)-**14**, 4.17; **5**, 6.45. For the hydrolysis of **6**, temperature 100–200°C (5°C/min), retention time (min): (*S*)-**15**, 5.10; (*R*)-**15**, 5.23; **16**, 7.18; **6**, 9.28. For the hydrolysis of **7**, temperature 100°C, retention time (min): (*R*)-**7**, 5.96; (*S*)-**7**, 6.48. For the hydrolysis of **8**, temperature 100°C, retention time (min): (*R*)-**8**, 4.64; (*S*)-**8**, 5.36. For the hydrolysis of **9**, temperature 90–200°C (5°C/min), retention time (min): (*R*)-**9**, 9.07; (*S*)-**9**, 9.43.

The absolute configurations of the compounds were determined comparing the sign of their specific rotation with those of the literature: for (1*S*,5*R*,6*S*)-**1**[23] $[\alpha]_D = -36.3$ (*c* 2.27, $CHCl_3$); for (1*R*,5*S*,6*R*)-**10**[24] $[\alpha]_D = -68$ (*c* 1.1, $CHCl_3$); for (1*R*,2*S*)-**2**[25] $[\alpha]_D = -37.4$ (*c* 1.50, $CHCl_3$); for (1*S*,2*R*)-**11**[26] $[\alpha]_D = 18$ (*c* 1.0, MeOH); for (1*S*,2*S*)-**3**[27] $[\alpha]_D = 69.9$ (*c* 0.64, EtOH); for (1*R*,2*R*)-**12**[28] $[\alpha]_D = -38.2$ (*c* 9.6, EtOH); for (*R*)-**13**[26] $[\alpha]_D = 14$ (*c* 0.23, MeOH); for (*S*)-**16**[28] $\alpha_D = -17$ (neat); for (*S*)-**7**[29] $\alpha_D = -33$ (neat); for (*R*)-**8**[30] $[\alpha]_D = 21.2$ (*c* 8.6, EtOH). The absolute configurations of compounds **9**[31] and **14**[32] were assigned comparing the signs of their specific rotation with those of the literature: (*R*)-(−)-**9** and (*R*)-(−)-**14**.

4.2. Microorganisms

Y. lipolytica strains[19] were isolated from various habitats and belong to DPVA (Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy). Seventeen strains were tested in the oxidation of racemic alcohols: YL1 (Y2), YL2 (Y9), YL4 (Y21), YL5 (PO5), YL6 (Y5), YL7 (RO3), YL8 (1A), YL9 (PO19), YL10 (Y10), YL12 (RO13), YL13 (RO18), YL14 (RO21), YL15 (16B), YL16 (27D), YL17 (PO6), YL18 (PO17), YL19 (PO23).[33]

4.3. Screening of hydrolysis with *Y. lipolytica* strains of compounds 1–9 on analytical scale. General procedure

The sterilized (120°C for 20 min) culture medium (Sabouraud, 10 mL), containing glucose (40 g/L) and peptone (10 g/L), was inoculated with a loopful of the selected *Y. lipolytica* and grown for 48 h at 28°C. To the culture was added the selected substrate solution (100 μL, 10 mg) (the solution was prepared dissolving 0.1 g of the selected substrate in 1 mL of DMF). Aliquots were withdrawn at 2, 4, 6, 24 h, extracted with diethyl ether, dried over anhydrous Na_2SO_4 and monitored by GLC on chiral column using 4-methylcyclohexanone as internal standard. In Table 1 are reported the results obtained with *Y. lipolytica* YL2 that is the more efficient strain in the hydrolysis of all screened substrates.

Some biotransformations were carried out suspending the cells harvested by centrifugation in phosphate buffer (10 mL) (see Table 1).

4.4. Hydrolysis of compounds 1–4, 6, 8 with *Y. lipolytica* YL2 on preparative scale. General procedure

The reaction was carried out as above starting from 200 mL of the culture medium and 0.2 g of the substrate in 2 mL of DMF (see Table 1). After the appropriate time (monitored by GLC) the reaction mixture was extracted with diethyl ether (200 mL) by a continuous liquid extractor and dried over anhydrous Na_2SO_4. The crude reaction products and the enantiomeric excesses were analysed by GLC. Chromatography of the crude reaction mixture (silica gel, cyclohexane/diethyl ether, 80:20) gave the purified products (see Table 1).

4.5. Lyophilisation of *Y. lipolytica* cells

The sterilized (120°C for 20 min) culture medium (10 mL), containing glucose (40 g/L) and peptone (10 g/L), was inoculated with a loopful of *Y. lipolytica* YL2 and grown for 24 h at 28°C. This culture (1 mL) was inoculated in 50 mL of the same broth (freshly sterilized) and grown for a further 24 h at 28°C. The last culture (50 ml) was added to the same culture medium (2 L) in a bioreactor and the grown is continued for further 48 h at 28°C monitoring the following parameters: pH range 2–9; O_2 pressure, 30 mmHg; gas flow, 1.0 Nl/min; stirring 300–900 rpm. The cells (43 g) were harvested by centrifugation (5 min, 9000 rpm) and saccharose 20% (140 mL) was added. The suspension was frozen and lyophilised to obtain 37 g of lyophilised cells.

4.6. Hydrolysis of 1-acetoxy-2-methylcyclohexene 4 with lyophilised cells of *Y. lipolytica* YL2

Y. lipolytica lyophilised cells (1 g) in phosphate buffer pH 7 (10 mL) were stirred for about 10 min and then the enol acetate solution (100 μL, 10 mg) (the solution was prepared dissolving 0.1 g of the substrate in 1 mL of DMF) was added. After 2 h incubation at 28°C, the suspension was extracted with diethyl ether and dried

over anhydrous Na_2SO_4. GLC analysis on chiral column using 4-methylcyclohexanone as internal standard showed the presence of (*S*)-methylcyclohexanone **13** in 85% yield (ee 64%), together with the pure (1*S*,2*R*)-*cis* methylcyclohexanol **11** (15% yield). The reaction was repeated in phosphate buffer at pH 6 and after 2 h only the (*S*)-ketone **13** was obtained (91% yield, ee 53%).

4.7. Hydrolysis of 2-phenyl-2-propen-1-ol acetate 6 with lyophilised cells of *Y. lipolytica* YL2

Y. lipolytica lyophilised cells (10 g) in phosphate buffer pH 7 (100 mL) were stirred for about 10 min and then the enol acetate **6** (0.1 g) in 1 mL of DMF was added. After 30 min and usual work up, GLC analysis on chiral column using 4-methylcyclohexanone as internal standard showed the presence of the (*S*)-aldehyde **15** in 76% yield (ee 57%). After 1 h the hydrolysis was complete (100% yield, ee 34%) with no reduction product.

The absolute configuration of the aldehyde **15** was determined by reduction to the corresponding alcohol **16**. The suspension obtained after 1 h incubation was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude reaction mixture was dissolved in methanol (5 mL) and $NaBH_4$ (1.5 equiv.) was added at 0°C. Usual work and chromatography (silica, petroleum ether/diethyl ether, 8:2) afforded the pure alcohol **16** in quantitative yield.

References

1. Schmidt, R. D.; Verger, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1609.
2. Faber, K. *Biotranformations in Organic Chemistry*; Springer-Verlag: Berlin, Heidelberg, 1992; p. 23.
3. Sugai, T.; Kuwahara, S.; Hishino, C.; Matsuo, N.; Mori, K. *Agric. Biol. Chem.* **1982**, *46*, 2579.
4. Oritani, T.; Yamashita, K. *Agric. Biol. Chem.* **1980**, *44*, 2407.
5. Ohta, H.; Miyamae, Y.; Kimura, Y. *Chem. Lett.* **1989**, 379.
6. Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 9614.
7. Ziffer, H.; Kawai, K.; Kasai, K.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017.
8. Demir, A. S.; Hamamci, H.; Tanyeli, C.; Akhmedov, I. M.; Doganel, F. *Tetrahedron: Asymmetry* **1998**, *9*, 1673.
9. Weijers, C. A. G. M. *Tetrahedron: Asymmetry* **1997**, *8*, 639.
10. Weijers, C. A. G. M.; Botes, A. L.; van Dyk, M. S.; de Bont, J. A. M. *Tetrahedron: Asymmetry* **1998**, *9*, 467.
11. Goswami, A.; Totleben, M. J.; Singh, A. K.; Patel, R. N. *Tetrahedron: Asymmetry* **1999**, *10*, 3167.
12. Botes, A. L.; Weijers, C. A. G. M.; Botes, P. J.; van Dyk, M. S. *Tetrahedron: Asymmetry* **1999**, *10*, 3327.
13. Crosby, J.; Moilliet, J.; Parratt, J. S.; Turner, N. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1679.
14. Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099.
15. Matoishi, K.; Sano, A.; Imai, N.; Yamazaki, T.; Yokoyama, M.; Sugai, T.; Ohta, H. *Tetrahedron: Asymmetry* **1998**, *9*, 1097.
16. Servi, S. *Synthesis* **1990**, 1.
17. Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49.
18. Fouché, G.; Horak, R. M.; Meth-Cohn, O. *J. Chem. Soc., Chem. Commun.* **1993**, 119.
19. Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P.; Gardini, F.; Lanciotti, R. *Tetrahedron* **1996**, *52*, 3547.
20. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Fontana, S. *Tetrahedron: Asymmetry* **2000**, *11*, 2367.
21. Gall, M.; House, H. *Org. Synth. Vol. 1* **1988**, 121.
22. Barbier, P.; Benezra, C. *J. Org. Chem.* **1983**, *48*, 2705.
23. Klempier, N.; Geymayer, P.; Stadler, P.; Faber, K.; Griengl, H. *Tetrahedron: Asymmetry* **1990**, *1*, 111.
24. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Rosini, G. *Tetrahedron: Asymmetry* **1994**, *5*, 1635.
25. Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385.
26. Matsumoto, K.; Kawabata, Y.; Takahashi, J.; Fujita, Y.; Hatanaka, M. *Chem. Lett.* **1998**, 283.
27. Hirata, T.; Izumi, S.; Akita, K.; Yoshida, H.; Gotoh, S. *Tetrahedron: Asymmetry* **1993**, *4*, 1465.
28. The (*S*)-(−)-2-phenylpropanol **16** is commercially available from Aldrich.
29. The (*S*)-(−)-styrene oxide **7** is commercially available from Aldrich.
30. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
31. Francotte, E.; Lohmann, D. *Helv. Chim. Acta* **1987**, *70*, 1569.
32. Tanaka, K.; Honke, S.; Urbanczyk-Lipokowska, Z.; Toda, F. *Eur. J. Org. Chem.* **2000**, 3171.
33. In parenthesis the identification label from Ref. 19.