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Homochiral (*R*)- and (*S*)-1-heteroaryl- and 1-aryl-2-propanols via microbial redox

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Abstract

Preparation of various heteroaryl propanols **2a–g** and of the corresponding propanones **3a–g** as starting materials for microbial redox is described. The kinetic resolution of the racemic propanols **2a–g** is obtained via oxidation with *Pseudomonas paucimobilis* and *Bacillus stearothermophilus* [(*R*)-alcohols, ee 74–100%]. Similar results are achieved with 3-(2-hydroxypropyl)trifluoromethylbenzene **7** (44%, ee 100% of the (*R*)-alcohol **6**). The reduction of the propanones **3a–d** and **3g** with baker's yeast and other fungi gives the (*S*)-alcohols (ee 100%). The pure (*S*)-alcohols are also obtained by reduction of 1-[3-(trifluoromethyl)phenyl]-2-propanone **7**. 1-[(4,4-Dimethyl)-2-(Δ^2)oxazolinyl]-2-propanone **3e** and 1[2-(Δ^2)-thiazolinyl)-2-propanone **3f** are not reduced. The heterocyclic rings of (*S*)-5-(2-hydroxypropyl)-3-methylisoxazole **2d** and of (*S*)-2-(2-hydroxypropyl)-4-methylthiazole **2g** are deblocked to the homochiral enamino ketone **8** (78%) and to the protected β-hydroxy aldehyde **9** (73%), respectively. The (*R*)-3-(2-hydroxypropyl)trifluoromethylbenzene **6** is transfomed into the homochiral precursor of (*S*)-fenfluramine **10** (overall yield 65%).

1. Introduction

Enantiomerically pure secondary alcohols are useful chiral auxiliaries in organic chemistry both for analytical and synthetic applications.[1] In particular chiral heteroaryl secondary alcohols, synthesized with the aim of unmasking a hidden functionality after the desired transformations,[2] have been mainly obtained from the corresponding ketones by catalytic asymmetric hydrogenation,[3] by treatment with a hydride reagent modified with a chiral auxiliary,[3] or by microbial reductions with baker's yeast[4] or other microrganisms.[5,6]

Compared with the reduction of ketones, however, there are few reports of microbial oxidations of alcohols[7] because in general their oxidations are thermodynamically unfavoured and inhibited by

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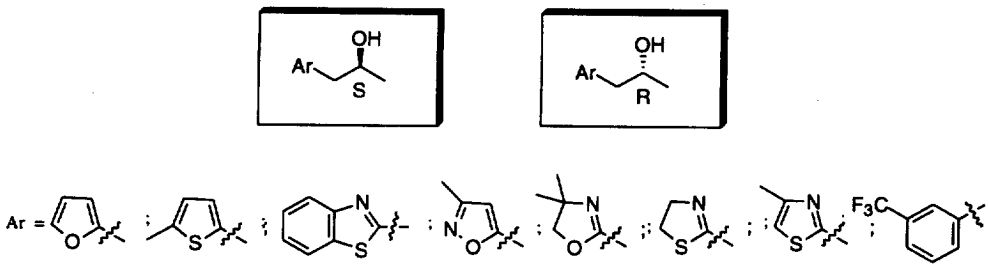


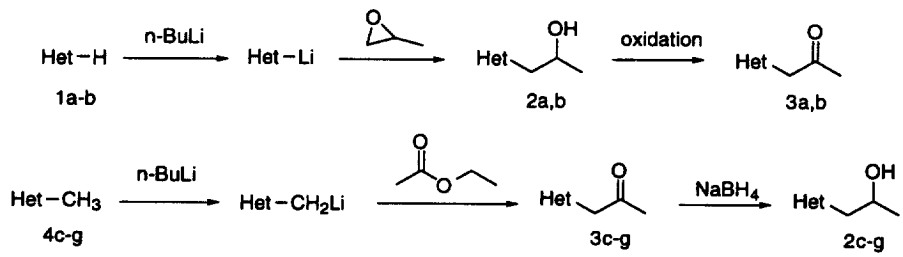
Fig. 1.

the products formed.[8] Recently we reported the kinetic resolution of racemic 1-heteroaryl- and 1-arylethanols via oxidation with whole *Bacillus stearothermophilus*[9] cells and baker's yeast.[10]

In this paper we describe the synthesis of homochiral (*R*)- and/or (*S*)-1-heteroaryl- and 1-aryl-2-propanols (Fig. 1) by kinetic resolution of the racemic alcohols via oxidation with *Pseudomonas paucimobilis* and *Bacillus stearothermophilus* or by reduction of the corresponding ketones with baker's yeast and other fungi (yeasts and moulds).

2. Results and discussion

The preparation of the starting materials (racemic alcohols or ketones) are achieved by lithiation of the appropriate heterocycle followed by quenching with the appropriate electrophile (Scheme 1).



a; Het = 2-furanyl
b; Het = 5-methyl-2-thiophenyl
c; Het = 2-benzothiazolyl
d; Het = 3-methyl-5-isoxazolyl
e; Het = 4,4-dimethyl-2-(Δ^2)-oxazolinyl
f; Het = 2-(Δ^2)-thiazolinyl
g; Het = 4-methyl-2-thiazolyl

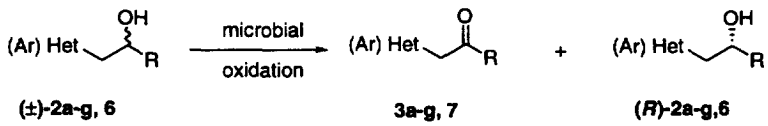
Scheme 1.

Lithiation of the 2-H heterocycles **1a,b** and quenching with propylene oxide[11] afford the racemic alcohols **2a,b** in good yield (86–90%). The corresponding ketones **3a,b** are obtained by oxidation with pyridinium chlorochromate (PCC) (**3a**, 88%) or Jones' reagent (**3b**, 95%).

On the other hand, metallation of the 2-methyl heterocycles **4c–g** and treatment with ethylacetate produce the ketones **3c–g** (82–95%). All the ketones, including the commercially available 1-[3-(trifluoromethyl)-phenyl]2-propanone **7**, are quantitatively reduced to the corresponding racemic alcohols with $NaBH_4$.

On the basis of results obtained in our laboratory in the kinetic resolution of 1-heteroaryl- and 1-arylethanols,[9,10] the racemic 1-heteroaryl-2-propanols **2a–g** and 3-(2-hydroxylpropyl)-

trifluoromethylbenzene **6** are oxidized with *Bacillus sterarothermophilus* ATCC 2027 and with *Pseudomonas paucimobils*[11] (Scheme 2). The results are summarized in Table 1.



(±)-2a-g, 6 **3a-g, 7** **(*R*)-2a-g,6**

Scheme 2.

The racemic alcohols were added to a growing culture of *Bacillus sterothermophilus* and *Pseudomonas paucimobilis* as a concentrated solution in DMF and the incubation was carried out for 48 h at 39°C and at 29°C, respectively. All the reactions have been carried out on an analytical scale in various culture media and monitored by GLC. The best results have been confirmed on a preparative scale.

The oxidation of the racemic alcohols **2a**, **2f** and **2g** with *Bacillus stearothermophilus* gives the corresponding ketones **3a**, **3f** and **3g** (52–60%) and the homochiral (*R*)-alcohols (40–48% yield, ee 95–100%). The same substrates are also resolved by oxidation with *Pseudomonas paucimobilis* on an analytical scale and the results are confirmed on a preparative scale (40–55% yield, ee 74–100%). The oxidation with *Pseud. pauc.* is also very efficient for the racemic alcohols **2b–e** and **6** (40–47% yield, ee 90–100% of the recovered (*R*)-alcohols), while *Bac. sterarotherm.*, that affords ketone **3b** in good yield (51%) and leaves the (*R*)-alcohol **2b** (49%) with good enantiomeric excess (72%), produces ketones **3c–e** in low yields (10–25%) and do not oxidize alcohol **6**. On the other hand the (*S*)-alcohols are obtained in good yields and excellent enantiomeric excesses by microbial reduction (Scheme 3). The results are summarized in Table 2.

The ketones were added to a growing culture of various microrganisms or to a suspension of baker's yeast as a concentrated solution in DMF and the incubation was carried out for 48 h at 29°C. All the reactions have been carried out on an analytical scale in the appropriate culture medium and monitored by GLC. The reductions with baker's yeast have been repeated on a preparative scale.

The ketones **3a–d**, **3g**, and **7** are enantioselectively reduced with baker's yeast to give the corresponding (*S*)-alcohols (80–92%, ee 100%). Also other yeasts and moulds are checked to obtain the enatiomerically pure alcohols. *Yarrowia lipolytica* YL10 is very efficient in the reduction of the ketones **3g** and **7** (92–95%, ee 100% of the (*S*)-alcohols) and *Mucor spirescens* gives almost quantitative yields of the (*S*)-alcohols **2a** (ee 94%). On the other hand *Trichoderma* affords the pure (*S*)-alcohols **2b** and **2g** (89–96% yields) while the alcohols **2a**, **2c** and **2d** (89–98% yields) are obtained with poor enantiomeric excesses (46–52%).

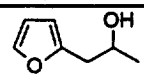
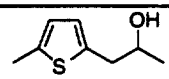
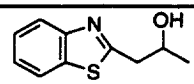
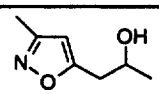
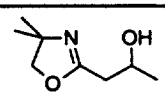
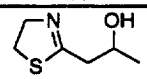
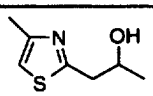
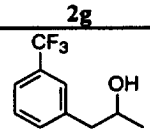
Because one of the most important characteristics of these heteroaryl rings is the possibility of unmasking various functions after obtaining the chiral side-chain, the isoxazolic ring of the (*S*)-alcohol **2d** was reductively cleaved[12] to the enantiomerically pure (*S*)-enamino ketone **8** using hydrogen with PtO_2 and Ni/Raney to give 78% yield and 100% ee (Scheme 4).

On the other hand the equivalence of the thiazole ring to a formyl group[13] permits the preparation of chiral β-hydroxy aldehyde **9** (73%, ee 100%) from the (*S*)-2-(2-hydroxypropyl)-4-methylthiazole **2g** (Scheme 5).

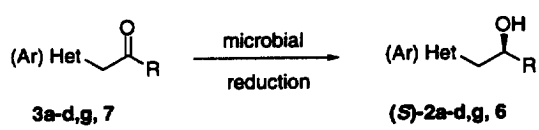
A very interesting application is the synthesis of (*S*)-fenfluramine,[14] an anorectic agent, starting from the (*R*)-alcohol **6** obtained from kinetic resolution of the racemic mixture with *Pseudomonas paucimobilis* (Scheme 6).

Mitsunobu reaction[15] with phthalimide affords the compound **10** with inverted configuration. Treatment with hydrazine and successively with acetic anhydride and pyridine gives the N-acetyl derivative

Table 1
Kinetic resolution via oxidation of the racemic alcohols **2a–g** and **6** with *Bacillus stearothermophilus* and *Pseudomonas paucimobilis*

R/S Alcohol	Microrganism (culture medium)[a]	Products (yield %)[b]	ee % (abs. conf.)
 **2a**	*Bacillus stearothermophilus* (A)[c]	**3a** (52)	
		2a (48)	**100** (R)
	Pseudomonas paucimobilis (C)[d]	**3a** (43)	
		2a (40)	**100** (R)
 **2b**	*Bacillus stearothermophilus* (A)	**3b** (51)	
		2b (49)	**72** (R)
	Pseudomonas paucimobilis (B)[d]	**3b** (42)	
		2b (40)	**100** (R)
 **2c**	*Bacillus stearothermophilus* (C)[c]	**3c** (25)	
		2c (75)	**24** (R)
	Pseudomonas paucimobilis (B)[d]	**3c** (50)	
		2c (43)	**100** (R)
 **2d**	*Bacillus stearothermophilus* (A)[c]	**3d** (10)	
		2d (90)	**16** (R)
	Pseudomonas paucimobilis (B)[d]	**3d** (44)	
		2d (40)	**90** (R)
 **2e**	*Bacillus stearothermophilus* (C)[c]	**3e** (10)	
		2e (90)	**5** (R)
	Pseudomonas paucimobilis (B)[c]	**3e** (39)	
		2e (47)	**95** (R)
 **2f**	*Bacillus stearothermophilus* (A)[c]	**3f** (60)	
		2f (40)	**95** (R)
	Pseudomonas paucimobilis (C)[c]	**3f** (45)	
		2f (55)	**74** (R)
 **2g**	*Bacillus stearothermophilus* (A)[c]	**3g** (52)	
		2g (48)	**98** (R)
	Pseudomonas paucimobilis (C)[c]	**3g** (55)	
		2g (45)	**100** (R)
 **6**	*Bacillus stearothermophilus* (A,C)[c]	**7** (0)	
		6 (100)	–
	Pseudomonas paucimobilis (B)[d]	**7** (43)	
		6 (44)	**100** (R)

[a] For culture medium see experimental. [b] Time of oxidation: 48 h. [c] Reactions on analytical scale: yields are determined by GLC [d] Reactions on preparative scale: yields are calculated on isolated products (chromatography).



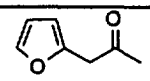
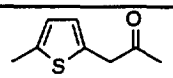
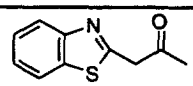
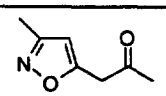
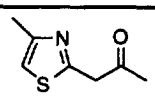
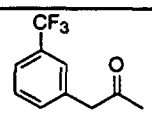
3a-d,g, 7 **(*S*)-2a-d,g, 6**

Scheme 3.

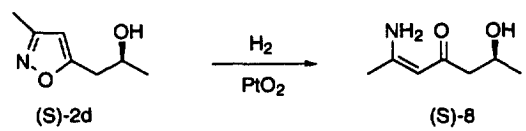
11 (overall yield 65%, ee 100%). Reduction with $LiAlH_4$ as reported in a previous work[14] affords the (*S*)-fenfluramine.

In conclusion 1-heteroaryl- and 1-aryl-2-propanols are obtained in the pure (*R*)-form by kinetic resolution of the corresponding racemates via oxidation with *Pseudomonas paucimobilis* while it is

Table 2
Microbial reduction of the ketones **3a–d**, **3g**, and **7**

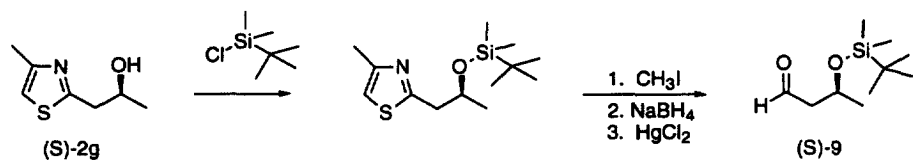
Ketone	Microrganism (culture medium)[a]	Product (yield %)[b]	ee % (abs. conf.)
 **3a**	Baker's yeast (D)[c]	**2a** (81)	**100** (S)
	Mucor spirescens (E)[d]	**2a** (96)	**94** (S)
	Trichoderma sp. (E)[d]	**2a** (89)	**46** (S)
 **3b**	Baker's yeast (D)[c]	**2b** (84)	**100** (S)
	Saccharromyces cerevisiae SC14 (E)[d]	**2b** (78)	**100** (S)
	Trichoderma sp. (E)[d]	**2b** (89)	**100** (S)
 **3c**	Baker's yeast (D)[c]	**2c** (80)	**100** (S)
	Saccharromyces cerevisiae SC14 (E)[d]	**2c** (30)	**100** (S)
	Trichoderma sp. (E)[d]	**2c** (97)	**52** (S)
 **3d**	Baker's yeast (D)[c]	**2d** (77)	**100** (S)
	Saccharromyces cerevisiae SC14 (E)	**2d** (30)	**100** (S)
	Trichoderma sp. (E)	**2d** (98)	**52** (S)
 **3g**	Baker's yeast (D)[c]	**2g** (82)	**100** (S)
	Yarrowia lipolytica YL10 (E)[d]	**2g** (95)	**100** (S)
	Trichoderma sp. (E)[d]	**2g** (96)	**100** (S)
 **7**	Baker's yeast (D)[c]	**6** (86)	**100** (S)
	Yarrowia lipolytica YL10 (E)[d]	**6** (92)	**100** (S)

[a] For culture medium see experimental. [b] Time of oxidation: 48 h. [c] Reactions on preparative scale: yields are calculated on isolated products (chromatography). [d] Reactions on analytical scale: yields are determined by GLC.



(S)-2d (S)-8

Scheme 4.



(S)-2g (S)-9

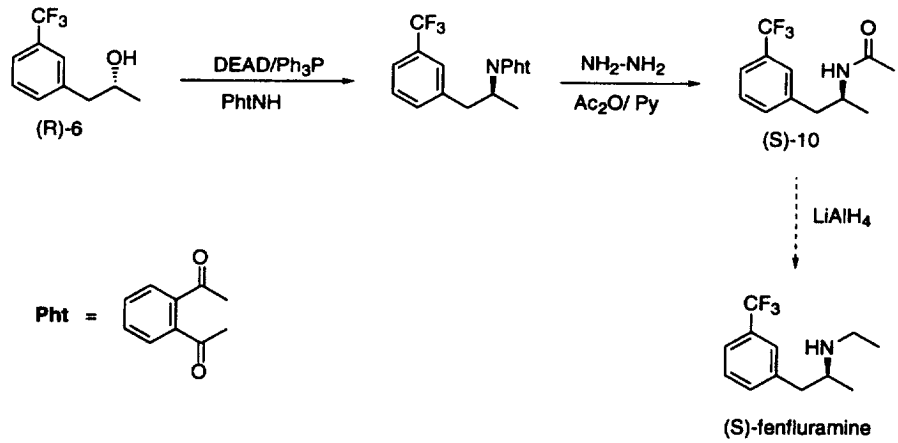
Scheme 5.

possible to achieve the (*S*)-enantiomers by reduction with baker's yeast and other yeasts and moulds. Some applications of these homochiral compounds confirm their synthetic utility.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra (in $CDCl_3$) were obtained with a Varian Gemini 300 spectrometer. Chemical shifts were given in parts per million from Me_4Si as an internal standard. IR spectra were recorded on a Perkin–Elmer Model 297 grating spectrometer. Gas chromatographic analyses were perfomed on a



Scheme 6.

Carlo Erba HRGC 5160 Mega series chromatograph. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter.

Furan **1a**, 2-methylthiophene **1b**, 2-methylbenzothiazole **4c**, 3,5-dimethylisoxazole **4d**, 2,4,4-trimethyl-2-oxazoline **4e**, 2-methyl-2-thiazoline **4f**, 2,4-dimethylthiazole **4g** and 1-[3-(trifluoromethyl)phenyl]-2-propanone **7** are commercially available. Metallation reactions have been carried out with dried THF ($LiAlH_4$) and under a nitrogen atmosphere.

Yarrowia lypolitica YL10, *Saccharomyces cerevisiae* SC4 and SC14, *Mucor* sp., *Trichoderma* sp., and *Pseudomonas paucimobilis*, except *Bacillus stearothermophilus* ATCC 2027, belong to the Dipartimento di Chimica collection.

3.2. Enantiomer separation

Enantiomer separation was obtained on a Megadex 1 column (25 m×0.32 mm) containing permethylated β-cyclodextrin in OV 1701 from Mega s.n.c.: carrier gas helium (0.8 atm). For compound **2a** temp. 70–200°C (2°C/min), retention time in min: *S*-**2a**, 9.92; *R*-**2a**, 10.33. For compound **2b** temp. 115–200°C (2°C/min), retention time in min: *S*-**2a**, 9.04; *R*-**2b**, 9.36. For compound **2c** temp. 150°C (22 min) and 150–200°C (20°C/min), retention time in min: *S*-**2c**, 24.45; *R*-**2c**, 24.56. For compound **2d** temp. 130–200°C (1°C/min), retention time in min: *S*-**2d**, 7.08; *R*-**2d**, 7.34. For compound **2e** temp. 90–200°C (1.5°C/min), retention time in min: *S*-**2e**, 7.21; *R*-**2e**, 7.37. For compound **2f** temp. 95–200°C (2°C/min), retention time in min: *S*-**2f**, 12.95; *R*-**2f**, 13.12. For compound **2g** temp. 100–200°C (2°C/min), retention time in min: *S*-**2g**, 10.30; *R*-**2g**, 10.62. For compounds **6** and **7** temp. 120–200°C (3°C/min), retention time in min: **7**, 7.61; *S*-**6** (as acetyl derivative), 8.82; *R*-**6** (as acetyl derivative), 9.38. The absolute configurations are assigned on the basis of the reductions with baker's yeast that afforded the (*S*)-enantiomers on the basis of the Prelog rule.

3.3. Synthesis of 2-(2-hydroxypropyl)furan 2a and 2-(2-hydroxypropyl)-5-methylthiophene 2b. General procedure

To a stirred and cooled (0°C) solution of the proper heterocycle **1** (50 mmol) in dry THF (50 ml) a solution of *n*-butyllithium 1.6 M in hexane (35 ml, 55 mmol) was added. After 2 h stirring at the same temperature, a solution of propylene oxide (4.3 g, 75 mmol) in THF (30 ml) was added dropwise and the temperature was maintained at 0°C for a further 1 h. The reaction mixture was allowed to warm to room

temperature, treated with a saturated aqueous solution of $NaHCO_3$ (50 ml) and extracted with diethyl ether (2×50 ml). The organic layer was washed with a saturated aqueous solution of NaCl (2×50 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum.

2-(2-Hydroxypropyl)furan **2a** was purified by distillation (5.67 g, 90%): bp 42°C (0.3 mmHg) [lit.[16] bp 47–50°C (0.3 mmHg)]; IR (film) 3500, 1600, 1500, 1150 cm^{-1}; ^1H NMR δ 1.20 (d, 3H, *J*=7 Hz), 1.9 (br, 1H), 2.74 (dd, 1H, *J*=6.9 and 13.8 Hz), 2.82 (dd, 1H, *J*=4.2 and 13.8 Hz), 4.20 (m, 1H), 6.10 (d, 1H, *J*=3.4 Hz), 6.30 (m, 1H), 7.34 (d, 1H, *J*=1.3 Hz).

2-(2-Hydroxypropyl)-5-methylthiophene **2b** was chromatographed (silica gel, cyclohexane:diethyl ether=6:4) (6.7 g, 86%): oil; IR (film) 3380, 1580, 1540, 1450 cm^{-1}; ^1H NMR δ 1.25 (d, 3H, *J*=7 Hz), 1.9 (br, 1H), 2.44 (s, 3H), 2.80 (dd, 1H, *J*=8.2 and 13.8 Hz), 2.92 (dd, 1H, *J*=4.1 and 13.8 Hz), 4.96 (m, 1H), 6.62 (m, 2H). Anal. calcd for $C_8H_{12}OS$: C, 61.50; H, 7.74. Found: C, 61.58; H, 7.68.

3.4. Synthesis of 1-heteroaryl-2-propanones 3c–g. General procedure

To a stirred and cooled (−78°C) solution of the proper heterocycle **4** (40 mmol) in THF (60 ml) a solution of *n*-butyllithium 1.6 M in hexane (28 ml, 44 mmol) was added. After 2 h stirring at the same temperature, a solution of ethyl acetate (7 ml, 75 mmol) in THF (40 ml) was added dropwise and the temperature was maintained at −78°C for a further 1 h. The reaction mixture was allowed to warm to room temperature, treated with a saturated aqueous solution of $NaHCO_3$ (50 ml) and extracted with diethyl ether (2×50 ml). The organic layer was washed with a saturated aqueous solution of NaCl (2×50 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum.

1-(2-Benzothiazolyl)-2-propanone **3c** was purified by crystallization from diethyl ether–hexane (7.02 g, 92%): mp 118–120°C [lit.[17] mp 112°C]; IR (KBr) 3070, 1600, 1580, 1520 cm^{-1}; ^1H NMR δ 2.1 (s, 0.6H, enolic CH_3), 2.34 (s, 2.4H, ketonic CH_3), 4.25 (s, 1.6H, ketonic CH_2), 5.63 (s, 0.2H, enolic =CH), 7.3–8.05 (m, 4H). From NMR spectrum the keto:enolic ratio is 4:1.

1-[(3-Methyl)-5-isoxazolyl]-2-propanone **3d**[18] was chromatographed (silica gel, diethyl ether:cyclohexane=8:2) (4.56 g, 82%): bp 85°C (2 mmHg); IR (film) 1720, 1610, 1580, 1420 cm^{-1}; ^1H NMR δ 2.22 (s, 3H), 2.27 (s, 3H), 3.82 (s, 2H), 6.05 (s, 1H).

1-[(4,4-Dimethyl)-2-(Δ^2)-oxazolinyl]-2-propanone **3e**[19] was chromatographed (silica gel, diethyl ether:cyclo-hexane=7:3) (5.4 g, 87%): mp 116–119°C [lit.[19] mp 125–127°C]; IR (KBr) 3260, 1630, 1550 cm^{-1}; ^1H NMR (enamine form) δ 1.40 (s, 6H), 2.02 (s, 3H), 4.07 (s, 2H), 4.87 (s, 1H), 9.2 (br, 1H).

1-[2-(Δ^2)-Thiazolinyl]-2-propanone **3f** was chromatographed (silica gel, ethyl acetate:cyclohexane=8:2) (5.4 g, 95%): oil; IR 3050, 1635, 1565, 1500 cm^{-1}; ^1H NMR (enolic form) δ 2.02 (s, 3H), 3.20 (t, 2H, *J*=6.6 Hz), 3.85 (t, 2H, *J*=6.6 Hz), 5.30 (s, 1H), 10.2 (br, 1H). Anal. calcd for C_6H_9NOS: C, 50.32; H, 6.33; N, 9.78. Found: C, 50.40; H, 6.26; N, 9.83.

1-[(4-Methyl)-2-thiazolyl]-2-propanone **3g** was distilled (5.1 g, 82%): bp 130–131°C (20 mmHg); IR (film) 1710, 1630, 1605, 1520 cm^{-1}; ^1H NMR δ 2.27 (s, 3H), 2.42 (s, 3H), 4.1 (s, 2H), 6.85 (s, 1H). Anal. calcd for C_7H_9NOS: C, 54.37; H, 5.82; N, 8.99. Found: C, 54.44; H, 5.80; N, 8.94.

3.5. Oxidation of alcohol 2a

To a stirred solution of 2-(2-hydroxypropyl)furan **2a** (0.5 g, 4 mmol) in dichloromethane (30 ml) pyridinium chlorochromate (2 g, 9.3 mmol) and Al_2O_3 (1 g) were added. After 24 h stirring the reaction mixture was filtered on silica gel and treated with a saturated aqueous solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with dichloromethane (20 ml). After treatment with anhydrous Na_2SO_4 the solvent was removed in vacuo and the residue chromatographed (silica

gel, cyclohexane:diethyl ether=6:4) to give the pure 1-(2-furanyl)-2-propanone **3a**[20] (0.44 g, 88%): bp 62–64°C (8 mmHg); IR (film) 1720, 1600, 1580, 1350 cm^{-1}; ^1H NMR δ 2.18 (s, 3H), 3.70 (s, 2H), 6.20 (d, 1H, *J*=3.2 Hz), 6.35 (m, 1H), 7.38 (d, 1H, *J*=1.1 Hz).

3.6. *Oxidation of alcohol* **2b**

To a stirred and cooled (0°C) solution of 2-(2-hydroxypropyl)-5-methylthiophene **2b** (0.5 g, 3.2 mmol) in freshly distilled acetone (10 ml) Jones' reagent[21] (prepared dissolving 10.3 g of CrO_3 and 8.7 ml of 98% H_2SO_4 in 30 ml of H_2O) was added dropwise. The oxidation is complete when the reaction colour does not change from yellow to green by adding the Jones' reagent. Isopropanol (0.5 ml) was added and the reaction mixture was filtered through Celite washing the chromium salts with acetone several times. The solvent was removed in vacuo and the crude reaction mixture was chromatographed (silica gel, cyclohexane:diethyl ether=6:4) to give 0.47 g (95%) of 1-[(5-methyl)-2-thiophenyl]-2-propanone **3b**: oil; IR (film) 3380, 1640, 1580, 1450 cm^{-1}; ^1H NMR δ 2.22 (s, 3H), 2.43 (s, 3H), 3.80 (s, 2H), 6.60 (d, 1H, *J*=3.2 Hz), 6.65 (d, 1H, *J*=3.2 Hz). Anal. calcd for $C_8H_{10}OS$: C, 62.30; H, 6.54. Found: C, 62.35; H, 6.51.

3.7. *Reduction with* $NaBH_4$ *of the ketones* **3c–g**, *and* **7**

To a stirred and cooled (0°C) solution of the appropriate ketone (5 mmol) in diethyl ether (5 ml) and methanol (5 ml) $NaBH_4$ (0.3 g, 10 mmol) was added portionwise. After 30 min of stirring the reaction mixture was concentrated, treated with a saturated aqueous solution of NH_4Cl (10 ml) and extracted with diethyl ether (or dichloromethane) (20 ml). The organic layer was dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent under vacuum afforded the pure racemic alcohols **2c–g**, **6g** and **8** in quantitative yield.

2-(2-Hydroxypropyl)benzothiazole **2c** showed the following: mp 94–97°C; IR (Nujol) 3450, 1675 cm^{-1}; ^1H NMR δ 1.36 (d, 3H, *J*=7 Hz), 3.13 (dd, 1H, *J*=8.3 and 15.2 Hz), 3.26 (dd, 1H, *J*=3.4 and 15.2 Hz), 3.85 (br, 1H), 4.2 (m, 1H), 7.38 (t, 1H, *J*=6.3 Hz), 7.49 (t, 1H, *J*=6.3 Hz), 7.85 (d, 1H, *J*=6.9 Hz), 7.98 (d, 1H, *J*=6.9 Hz). Anal. calcd for $C_{10}H_{11}NOS$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.21; H, 5.70; N, 7.30.

5-(2-Hydroxypropyl)-3-methylisoxazole **2d**: oil; IR (film) 3400, 1610, 1605, 1420 cm^{-1}; ^1H NMR δ 1.22 (d, 3H, *J*=6.9 Hz), 2.22 (d, 3H, *J*=0.7 Hz), 2.7 (br, 1H), 2.82 (m, 2H), 4.13 (m, 1H), 5.9 (s, 1H). Anal. calcd for $C_7H_{11}NO_2$; C, 59.56; H, 7.85; N, 9.92. Found: C, 59.50; H, 7.82; N, 9.87.

4,4-Dimethyl-2-(2-hydroxypropyl)-Δ^2-oxazoline **2e**: oil; IR (film) 3350, 2960, 1655 cm^{-1}; ^1H NMR δ 1.22 (d, 3H, *J*=7 Hz), 1.25 (s, 6H), 2.25 (dd, 1H, *J*=7.7 and 14.7 Hz), 2.35 (dd, 1H, *J*=2.8 and 14.7 Hz) 3.88 (s, 2H), 4.10 (m, 1H), 4.3 (br, 1H). Anal. calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.16; H, 9.55; N, 8.88.

2-(2-Hydroxypropyl)-Δ^2-thiazoline **2f**: oil; IR (film) 3350, 2980, 1620 cm^{-1}; ^1H NMR δ 1.21 (d, 3H, *J*=6.8 Hz), 2.47 (dd, 1H, *J*=9 and 16.5 Hz), 2.58 (dd, 1H, *J*=1.3 and 16.5 Hz), 3.26 (pseudot, 2H, *J*=7.6 Hz), 4.18 (br, 1H), 4.21 (m, 4H). Anal calcd for $C_6H_{11}NOS$: C, 49.62; H, 7.64; N, 9.65. Found: C, 49.55; H, 7.71; N, 9.60.

2-(2-Hydroxypropyl)-4-methylthiazole **2g**: oil; IR (film) 3400, 1610, 1520 cm^{-1}; ^1H NMR δ 11.3 (d, 3H, *J*=6.9 Hz), 2.42 (s, 3H), 2.96 (dd, 1H, *J*=7.6 and 14.5 Hz), 3.10 (dd, 1H, *J*=2.8 and 14.5 Hz), 3.70 (br, 1H), 4.22 (m, 1H), 6.72 (s, 1H). Anal. calcd for $C_7H_{10}NOS$: C, 53.82; H, 6.45, N, 8.97. Found: C, 53.90; H, 6.48; N, 9.02.

3-(2-Hydroxypropyl)trifluoromethylbenzene **6**:[14] oil; IR (film) 3380, 1580, 1490 cm^{-1}; ^1H NMR δ 1.22 (d, 3H, *J*=7 Hz), 1.71 (br, 1H), 2.75 (dd, 1H, *J*=7.7 and 14.5 Hz), 2.84 (dd, 1H, *J*=4.8 and 14.5 Hz), 4.05 (m, 1H), 7.45 (m, 4H).

3.8. Culture media

Culture medium **A** for *Bacillus sterothermophilus*: bactotryptone (20 g), yeast extract (10 g), sucrose (20 g), K_2SO_4 (2.6 g) and Na_2HPO_4 (6.4 g) in H_2O (1 l).

Culture medium **B** for *Pseudomonas paucimobilis*: bactotryptone (10 g), yeast extract (5 g), glucose (2 g), and K_2HPO_4 (0.4 g) in H_2O (1 l).

Culture medium **C**: bactotryptone (20 g), yeast extract (10 g) and sodium ossalate (3 g) in H_2O (1 l).

Culture medium **D** for moulds and yeasts: glucose (50 g), $(NH_4)_2PO_4$ (5 g), K_2HPO_4 (2 g), $CaCl_2$ (0.25 g), $MgSO_4 \cdot 7H_2O$ (0.25 g), inositol (25 mg), H_3BO_3 (1 mg), $ZnSO_4$ (1 mg), $MnCl_2$ (1 mg), $FeCl_2$ (0.5 mg), $CuSO_4$ (0.1 mg), KI (0.1 mg), tiamine (0.3 mg), biotine (0.025 mg, calcium pantothenate (0.3 mg), pyridoxine (0.3 mg), and nicotinic acid (0.3 mg) in H_2O (1 l).

Culture medium **E** for baker's yeast: glucose (31 g) in H_2O (1 l).

3.9. Screening of microbial oxidation of the racemic alcohols 2a–g, and 6. General procedure

A sterilized nutrient broth (**A** or **C** for *Bac. stearotherm.* and **B** or **C** for *Pseud. pauc.*) (10 ml) was inoculated with a loopful of the proper microrganism. The mixture was incubated on a reciprocatory shaker for 48 h at 39°C for *Bac. stearotherm* and at 29°C for *Pseud. pauc.*, respectively. To the resulting suspension of grown cells the appropriate racemic alcohols (10 mg) in DMF (0.1 ml) were added. After 48 h of further incubation, the reaction mixture was extracted with diethyl ether and dried over anhydrous Na_2SO_4. After filtration the solvent was evaporated and the crude reaction mixture analyzed by GLC. The best results are reported in Table 1.

3.10. Oxidation with Pseudomonas paucimobilis on a preparative scale. General procedure

The reactions were repeated on a preparative scale inoculating with a loopful of *Pseudomonas paucimobilis* 400 ml of the proper nutrient broth (see Table 1) and adding, after 48 h of incubation, the racemic alcohols (0.5 g) in DMF (1 ml). After 48 h under vigorous stirring at 29°C, the reaction mixtures were extracted with diethyl ether (200 ml) with a continous liquid–liquid extractor, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, diethyl ether:cyclohexane=6:4) to give the ketones and the unreacted alcohols. The enantiomeric excesses of the recovered alcohols are determined by GLC (see Table 1).

3.11. Screening of microbial reduction of ketones 3a–g and 7. General procedure

A sterilized culture medium **D** (10 ml) was inoculated with a spore suspension (yeast or mould) and grown for 48 h at 29°C. Each substrate is tested for *Yarrowia lypolitica* YL10, *Saccharomyces cerevisiae* SC4 and SC14, *Mucor* sp. and *Trichoderma* sp. To the resulting suspension of grown cells the appropriate ketones (10 mg) in DMF (0.1 ml) were added. After 48 h of further incubation, the reaction mixture was extracted with diethyl ether and dried over anhydrous Na_2SO_4. After filtration the solvent was evaporated and the crude reaction mixture analyzed by GLC.

The reaction with baker's yeast was carried out as above starting from 2 g of yeast in 10 ml of unsterilized culture medium **E**. The best results are reported in Table 2.

3.12. Reductions with baker's yeast on a preparative scale. General procedure

To a vigorously stirred culture medium **E** (400 ml) baker's yeast (80 g) was added and stirring continued for 1 h at 29°C. To the stirred suspension, the appropriate ketone (0.5 g) in DMF (1 ml) was added and incubation was continued for a further 48 h at the same temperature. The reaction mixture was extracted with diethyl ether (200 ml) with a continuous liquid–liquid extractor, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, diethyl ether:cyclohexane=6:4) to give the (*S*)-alcohols. The enantiomeric excesses are determined by GLC (see Table 2).

3.13. Synthesis of (S)-2-amino-4-oxo-2-hepten-6-ol 8

A solution of the (*S*)-alcohol **2d** (0.3 g, 2 mmol) in methanol (20 ml) was reduced at atmospheric pressure over a PtO_2 catalyst (0.04 g) pre-reduced by adding a small amount of Raney nickel in methanol (4 ml). After hydrogenation was complete (4 h), the mixture was filtered through Celite and concentrated under reduced pressure. Chromatography of the residue (silica, diethyl ether) gives the enamino-ketone **8** (0.11 g, 78%): mp 82–85°C; $[\alpha]_D^{20}$=26.0 (*c* 6.0, $CHCl_3$); IR (Nujol) 3300, 1590, 1525, 1300 cm^{-1}; ^1H NMR δ 1.18 (d, 3H, *J*=7 Hz), 1.93 (s, 3H), 2.33 (dd, 1H, *J*=9.1 and 15.5 Hz), 2.46 (dd, 1H, *J*=3 and 15.5 Hz), 4.15 (m, 1H), 4.98 (s, 1H), 5.3 (br, 1H), 9.7 (br, 2H).

3.14. Synthesis of (S)-3-dimethyl-t-butylsilyloxy-butanal 9

To a stirred solution of the alcohol *S*-**2g** (0.4 g, 3 mmol) and imidazole (0.4 g, 6 mmol) in DMF (20 ml) dimethyl-*t*-butylsilyl chloride (0.8 g, 6 mmol) was added. After 12 h stirring the solvent was removed in vacuo, the residue was treated with a saturated aqueous solution of $NaHCO_3$ (20 ml) and extracted with diethyl ether (20 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The crude O-dimethyl-*t*-butylsilyl derivative was diluted with acetonitrile (10 ml) and after adding methyl iodide (2 ml, 30 mmol) refluxed for 12 h. The reaction mixture was allowed to cool to room temperature and the solvent was removed in vacuo. The crude N-methyl derivative was dissolved in methanol (20 ml) and $NaBH_4$ (0.12 g, 3 mmol) was added portionwise at 0°C. After 30 min stirring, the solvent was evaporated and the mixture treated with a saturated solution of $NaHCO_3$ (20 ml) and diethyl ether (30 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was dissolved in a mixture of acetonitrile:water (4:1, 5 ml) and $HgCl_2$ (0.81 g, 3 mmol) was added slowly with stirring. A white precipitate was formed. After 15 min the reaction was concentrated in vacuo, diethyl ether (15 ml) was added and the mixture was filtered over Celite. The filtrate was treated with saturated NaCl (10 ml), the organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. Chromatography of the residue (silica, diethyl ether:cyclohexane=3:7) afforded the protected β-hydroxy aldehyde **9** (0.4 g, 73%): oil; $[\alpha]_D^{20}$=12.1 (*c* 4.6, $CHCl_3$); IR (film) 2710, 1725, 1460 cm^{-1}; ^1H NMR δ 0.04 (s, 3H), 0.06 (s, 3H), 0.87 (s, 9H), 1.23 (d, 3H, *J*=6.4 Hz), 2.43 (ddd, 1H, *J*=2, 5 and 16 Hz), 2.56 (ddd, 1H, *J*=2.8, 7 and 16 Hz), 4.34 (m, 1H), 9.80 (m, 1H).

3.15. Synthesis of (S)-3-(N-acetyl-2-aminopropyl)trifluoromethylbenzene **10**

To a stirred solution of the (*R*)-alcohol 6 (0.4 g, 2 mmol), phthalimide (0.29 g, 2 mmol and triphenylphosphine (0.52 g, 2 mmol) in THF (10 ml) diethylazodicarboxylate (0.35 g, 2 mmol) was added dropwise at room temperature. After 12 h stirring the solvent was evaporated in vacuo, the residue was treated with diethyl ether (30 ml) and the mixture was filtered. The solvent was removed under vacuum and the residue was chromatographed (silica, cyclohexane:diethyl ether=7:3) to give the N-phthalimide derivative (0.52 g, 79%) that was used without further purification in the next step. The ^{1}H NMR spectrum of the intermediate was the following: δ 1.55 (d, 3H, *J*=7 Hz), 3.15 (dd, 1H, *J*=7 and 14 Hz), 3.40 (dd, 1H, *J*=9.5 and 14 Hz), 4.64 (m, 1H), 7.40 (m, 4H), 7.67 (m, 2H), 7.76 (m, 2H).

A solution of the intermediate (0.5 g, 1.5 mmol) and hydrazine hydrate (200 mg) in ethanol (5 ml) was refluxed for 2 h. The reaction mixture was treated with 10% HCl and filtered. The filtrate was extracted with diethyl ether (20 ml). The aqueous layer was basified with 1 N NaOH and extracted with diethyl ether (2×20 ml). The organic layers were combined, dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was treated wth acetic anhydride (0.3 ml) and pyridine (1 ml). After 1 h the reaction mixture was diluted with diethyl ether (10 ml) and treated with 5% HCl. The organic layer was washed with H_2O (5 ml) and then with a saturated solution of $NaHCO_3$ (5 ml) and dried over anhydrous Na_2SO_4. The removal of the solvent gave the N-acetyl derivative **10** (0.32 g, overall yield 68%) with 100% enantiomeric excess determined by GLC on a chiral column.

The N-acetyl derivative showed the following: oil; $[\alpha]_D^{20}=-19.1$ (*c* 1.5, $CHCl_3$); IR (film) 3290, 1730, 1650, 1550 cm^{-1}; ^{1}H NMR δ 1.1 (d, 3H, *J*=6.8 Hz), 1.92 (s, 3H), 2.71 (dd, 1H, *J*=6.8 and 13 Hz), 2.9 (dd, 1H, *J*=7.5 and 13.6 Hz), 4.20 (m, 1H), 7.38 (m, 4H).

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