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Biotransformations of γ-Methyl-β-Ketosulfones: Stereoselectivity of 3-Methyl-1-(Phenylsulfonyl)Hexan-2-One Reductions by Various Yeasts.

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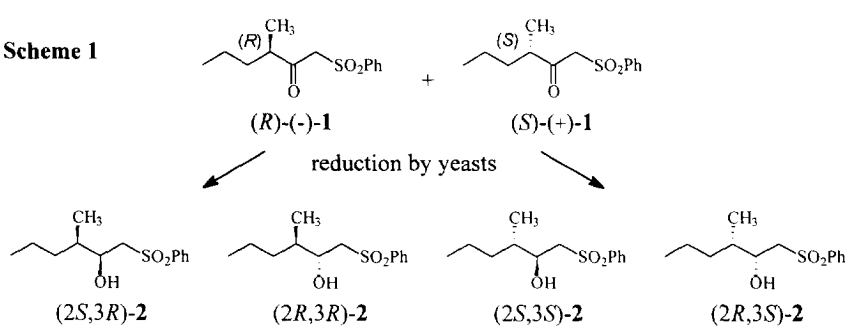
Abstract: The stereoselectivity of the reduction of *rac*-3-methyl-1-(phenylsulfonyl)hexan-2-one (**1**) to 3-methyl-1-(phenylsulfonyl)hexan-2-ol (**2**) diastereomers by more than 20 yeasts was studied. Reduction of carbonyl group in **1** proceeds with a high *Re*-face enantioselectivity: *Candida guillermondii* (98.9% *e.e.*), *C. zeylanoides* (>99.9%), and *Kloeckera apiculata* (99.6%), respectively and the (*R*)-**1** enantiomer usually reacted faster. The enantioselectivity was determined by GC on chiral cyclodextrine phases and absolute configurations of products were assigned by NMR spectroscopy and a chemical correlation.

Introduction

Enzymatic enantioselective reductions of β-ketophenylsulfones have attracted considerable attention from synthetic chemists in the past decade.[1,2,3] The chiral nonracemic β-hydroxyphenylsulfones formed are useful building blocks in natural product synthesis, since: 1) reductions of the keto group proceed with a very high stereoselectivity and 2) phenylsulfonyl group allows further functionalizations[4] at the α-carbon atom; moreover, this group can be easily removed from the skeleton without any racemization.[5] Readily available baker's yeast or more defined *Saccharomyces cerevisiae* cultures have been the most extensively studied microorganisms in this respect. As the 1-hydroxy-2-methylalkyl motifs are widespread among natural products,[6] the ketosulfone **1** seems to be an ideal starting material for their synthesis (Scheme 1). However, in the absence of information about the influence of methyl-branching on both conversions and stereoselectivity in enzymatic reductions of γ-methyl-β-ketosulfones, optimizing the bioreductions can only be achieved empirically.

Scheme 1

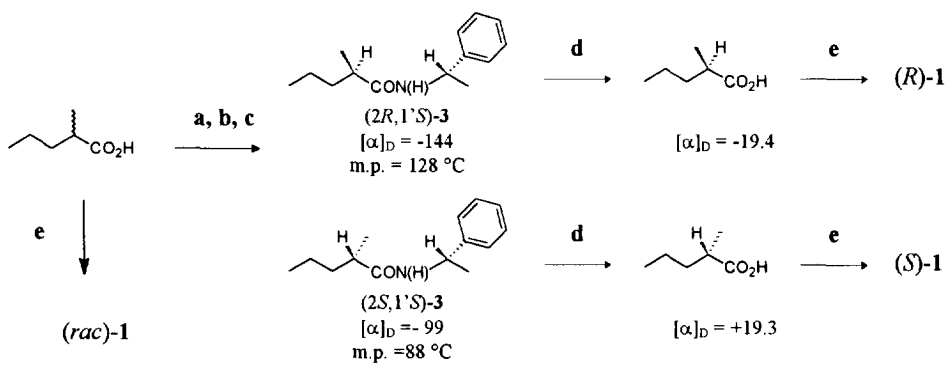


Here we report on the activity and stereoselectivity of a variety of yeasts in the reductive biotransformation of *rac*-3-methyl-1-(phenylsulfonyl)-hexan-2-one **1** to the 3-methyl-1-(phenylsulfonyl)-hexan-2-ol **2** diastereomers. It has been proposed that all four enantiomers of **2** could be employed in the synthesis of compounds of agricultural importance, e.g. pheromones.

Results

Synthesis

Racemic ketosulfone **1** was prepared from 2-methylpentanoyl chloride upon treatment with phenylsulfonylmethylene α-carbodianion.[7] The enantiomerically enriched (3*R*)- and (3*S*)-**1** were prepared in the same way, the optically active chlorides being available from resolved acids. Corresponding (*R*)- and (*S*)-2-methylpentanoic acids were obtained by resolution of the racemic 2-methylpentanoic acid *via* the corresponding phenethylamides separable both by chromatography on silica gel and fractional crystallization.[8] The acids were liberated from the diastereomerically pure amides (>99.9%) either by an acidic hydrolysis,[9] or by using the Sonnet procedure.[10] The enantiomeric purity of the acids and ketosulfones was determined by gas chromatography on chiral cyclodextrine phases. It is to be noted that while the acidic hydrolysis[9] of the amides proceeded with a notable racemization, the Sonnet procedure[10] produced enatiomerically pure acids. Because of the mentioned racemization the prepared ketosulfone enantiomers have 50% enantiomeric purity (*e.p.*) for the (3*S*)- and 80% for the (3*R*)-**1**, respectively.



Scheme 2 Resolution of 2-methylpentanoic acid and synthesis of the ketosulfone **1**
a) $SOCl_2$, b) (*S*)-phenethyl amine, c) MPLC (hexane/ethylacetate) and crystallization in petroleum ether/ether/ethanol, d) LDA, oxirane, $HClO_4$[10], e) Cl_2CHOCH_3, $Li_2^+[CH_2SO_2Ph]^{2-}$

Microbial reductions

The yeast biomass was produced on a malt extract liquid medium and subsequently separated by centrifugation. All bioreductions were performed in a glucose solution. The substrate **1** was introduced as a dimethylsufoxide (DMSO) solution to increase the solubility and mass transport (DMSO permeabilizes cell membrane). With

fermenting yeast we performed both aerobic and anaerobic experiments to determine the influence of the aerobic and the anaerobic metabolism on the reduction yields and stereoselectivity. No significant effects on the stereoselectivity were observed while the conversions were higher under aerobic conditions.

Table 1. Results of reductions of ketosulfone **1** by selected yeasts[a]

Yeast	% of **1** converted/ mg of yeast[d]	Compound [%][b]					Class
			Absolute configurations[c]				
		1	1. peak (2*R*,3*R*)-**2**	2. peak (2*S*,3*S*)-**2**	3. peak (2*R*,3*S*)-**2**	4. peak (2*S*,3*R*)-**2**	
Candida guillermondii	15.9	46	0	10	0	43	A
Candida pseudotropicalis	5.3	83	1	11	0	4	A[e]
Candida zeylanoides	25.9	4	0	50	0	45	A
Cryptococcus spec. WS	27.7	56	1	3	0	40	A[f]
Hansenula anomala	10.6	4	1	42	0	53	A
Kloeckera apiculata	14.0	34	0	10	0	55	A[g]
Nematospora coryli	7.4	62	0	7	0	31	A
Schizosaccharomyces pombe.	6.7	73	1	7	0	19	A[h]
Candida curvata	1.7	89	2	0	1(sh)	9	B
Zygofabospora krasilnikovi	3.2	84	7	2	1	6	B
Cryptococcus laurentii	1.4	99	0	0	0	1	C
Endomyces capsularis	0.2	98	0	1	0	1	C
Kloeckera apiculata	1.6	93	2	2	1	4	C
Pichia fermentans	0.5	98	0	0	0	1	C
Rhodotorula glutinis	5.6	74	5	1	6	15	C
Saccharomyces chevalieri	9.7	62	6	3	3 (sh)	26	C
Saccharomyces uvarum	12.0	52	3	7	3 (sh)	35	C
Saccharomyces cerevisiae	5.5	72	9	3	4	13	C
Sporobolomyces salmonicolor	5.9	90	1	2	5	1	C
Trichosporon margaritiferum	0.6	95	0	1	0	3	C

[a] 50 mg of **1** in 50 ml of 2% sucrose solution for 48h, [b] determined by GC on Cyclodex B; elution order is as presented in the Table; r_t= 38.95/39.42/39.99/40.30 [min], [c] from ^{1}H NMR and chemical correlation, [d] % of **1** per mg of yeast dry wt./ml/48h, [e] reversed diastereoselectivity, [f] *e.e.*= 94.3 %, [g] *e.e.*= 99.6 %, [h] *e.e.*= 89.7% (all determined on Lipodex E).

Determination of enantiomeric purity of the alcohol **2** was rather complicated. The hydroxyl group in the hydroxysulfone **2** was found to be unexpectedly unreactive, and the Mosher's esters could not be prepared. Also, the use of a chiral HPLC column (β-CDX-silica bonded) did not result in any separations. Poor resolution of enantiomers can be attributed to complex equilibrium observed[11] for species with free and intramoleculary bounded OH group to SO_2 oxygens. Nevertheless, resolution of all four diastereomers was obtained on a chiral GC column (Cyclodex B) using hydrogen as a carrier gas. Another GC chiral column tested (Lipodex E) was less effective, however it proved to be useful in particular cases. Because of the no-baseline separations, the accuracy of the determinations was affected by an error (±5 rel %). Coinjections of the samples with a racemic mixture containing all stereoisomers of **2** were performed for each experiment.

Table 2 Reduction of enantiomers of ketosulfone **1** by selected yeasts[a]

Yeast substrate/time [h]	% of **1** converted[b]	Compound [%]					*e.e.*[c] [%]
		Absolute configurations					
		1	(2*R*,3*R*)-**2**	(2*S*,3*S*)-**2**	(2*R*,3*S*)-**2**	(2*S*,3*R*)-**2**	
Candida guillermondii							
1/24	12.9	70	0	4	0	25	
1/48	17.9	52	0	10	0	38	
1/52	nd	25	0	24	0	51	98.4
(*S*)-**1**/48	5.8	60	0	13	0	27	
(*R*)-**1**/48	8.8	40	0	4	0	55	98.9
(*R*)-**1**/58	18.0	15	0	7	0	78	
Candida zeylanoides							
(*S*)-**1**/48	13.0	0	0	71	0	29	>99.9
(*R*)-**1**/48	11.0	8	0	18	0	74	>99.9
Zygofabospora krasilnikovi							
(*S*)-**1**/52	0.1	99	1	0	0	0	
(*R*)-**1**/52	2.1	85	6	0	0	8	

[a] see Table 1 [b] % of **1** per mg of yeast dry wt/ml/time [h] ,[c] enantioselectivity of C(2) carbonyl reduction determined using Lipodex E capillary column, for details see Exp., nd= not determined

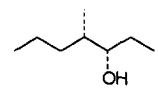
The absolute configuration of **2** was determined by a combination of ^{1}H NMR, chromatography on chiral GC phases, and a chemical correlation. A sample of **2** obtained by sodium borohydride reduction of **1** gave four peaks on a Cyclodex B chiral column with the same abundance. However, when the enantiomerically enriched (*R*)-**1** was subjected to the sodium borohydride reduction, the GC analysis showed that the reaction mixture consists of the 1st and the 4th peak, while the reduction of the (*S*)-**1** provided a mixture of alcohols with 2nd and 3rd peaks prevailing. Determination of a relative stereochemistry of $C_{(3)}$ methyl and $C_{(2)}$ hydroxyl groups in diastereomers of the alcohol **2** (obtained from (*S*)-**1** and (*R*)-**1** enriched precursors) is based on a comparison of their ^{1}H and ^{13}C NMR chemical shifts with the corresponding data of models **4** and **5** published in the literature.[6,12] The relative orientation of substituents at $C_{(2)}$ and $C_{(3)}$ consistently affects chemical shifts of CH-O proton and carbon atoms in model compounds. The differences in published shifts ($\Delta\delta$) were used for the determination of *syn* or *anti* orientations of hydroxyl and methyl group in **2** (Table 3).

Thus, the measured chemical shifts (^{1}H and ^{13}C) of the major isomer in the mixture obtained from the bioreduction of enantiomerically enriched (*R*)-**1** by *C. guillermondii* (85% yield, 2nd and 4th peak: 1/10, Cyclodex B) correspond to *syn* (threo) relationship of the methyl and the hydroxyl group. As the configuration at C(3) in the starting material was fixed as the *R* configuration, the absolute configuration of the enantiomer eluted as the 4th peak is to be (2*S*,3*R*)-**2**. Another bioreduction of (*S*)-**1** with *C. zeylanoides* provided a mixture of alcohols, where the 2nd peak (Cyclodex B) predominated. NMR analyses indicated that the hydroxyl and methyl groups have *anti* (erythro) orientation and the absolute configuration of the major diastereomer is

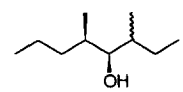
(2*S*,3*S*)-**2**. The values of measured coupling constants for *syn*- (3.9 Hz) and *anti*-**2** (4.9 Hz) were not useful for the relative geometry assignment.

Table 3 Chemical shifts of CH-O proton/carbon and their diferences for *syn* and *anti* isomers of **2** and model compounds **4** and **5** (in ppm).

	^{1}H NMR		^{13}C NMR		
Compound	**2**	**4**[6]	**2**	**4**[6]	**5**[12]
syn	4.10	3.30	59.37	76.71	77.8
anti	4.05	3.20	60.42	77.49	78.2
Δδ	-0.05	-0.10	+1.05	+0.78	+0.4

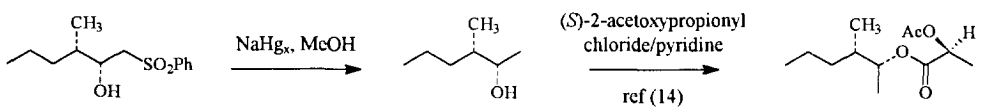


4



5

Combining the results of NMR spectrometry with the elution orders of alcohols obtained from the borohydride reductions the absolute configurations of unassigned peaks can be directly established (Table 1). The final proof of our GC and NMR-based assignments was obtained from the chemical correlation shown in Scheme 3. Two diastereomers of hydroxysulfone **2** (peaks 2 and 4, Cyclodex B) from *C. guillermondii* reduction of *rac*-**1** were converted to 3-methylhexan-2-ol **6** by $NaHg_x$ reduction[13] and the alcohol was esterified using (*S*)-2-acetoxypropionyl chloride/pyridine.[14] A standard mixture of four diasteromeric esters **7** (see Exp.) and the esters from the correlation were measured on DB-WAX (Carbowax equivalent) capillary GC column and the elution order was compared with published data.[14] The elution orders shown in Table 4 demonstrated that the major enantiomer of **7** in the correlated sample has absolute configuration (2*R*,3*R*) and the minor one has (2*R*,3*S*) configuration. These findings are consistent with the (2*S*,3*R*)-**2** and (2*S*,3*S*)-**2** absolute configurations, respectively (note that the "CIP" nomenclature order of substituents on $C_{(2)}$ in **2** has changed) and thus corroborating our previous assignment.



Scheme 3 Chemical correlation **6** **7**

Discussion

Tested yeasts can be divided into three classes according to the observed stereoselectivity. Class **A** is formed by yeasts that reduce keto group only from one prochiral face (*Re*), the (3*R*)-**1** being reduced in most cases faster and with substantial diasteroselectivity (*syn*/*anti*-ratio: 0.4-13.3). In those cases where the carbonyl reduction was fast (within 48 h) we obtained mixtures of diastereomers which reflected enantiomeric

composition of the substrate (e.g. *C. zeylanoides* and *Hansenula anomala*). When the rate was slower (*C. guillermondi* and *Cryptococus sp. WS*), the *syn* isomer [(2*S*,3*R*)-**2**] was formed in 72% and 86% *d.e.*, respectively (see Tables 1 and 2). The *anti*-isomer (2*S*,3*S*) was formed in 47% *d.e.* by *Candida pseudotropicalis*, and also *e.e.* was lower than in the former examples. In particular cases, the results of GC analyses on Cyclodex B were confirmed on a Lipodex E phase. The γ-cyclodextrine chiral GC phase base-line-separated diastereomeric pairs (2*R*,3*R*)/(2*R*,3*S*) and (2*S*,3*S*)/(2*S*,3*R*). this allowed us to obtain more accurate values for enantioselectivity of $C_{(2)}$ carbonyl reductions. Using this phase the *e.e.* of the reduction of **1** was found to be more than 99.9% for *C. zeylanoides*.

Yeast *Zygofabospora krasilnikovi* reduces preferentially the 3*R* enantiomer of **1** in a nonstereospecific fashion and falls into the second class (**B**). The other microbial systems which were not active enough or yielded a complex mixtures of diastereomers and they were not useful for us, were combined in class **C**.

Selected yeasts (class **A**) reduce the prochiral keto group *Re*-face. The enantioselectivity of the reduction is very high (>99.9% *e.e.*) and the diastereoselectivity is up to 86% of *syn*-isomer (*Cryptococus sp.*, at 44 % conversion). Note that similar preference for *syn* isomers was observed[15] by Kozikowski et al in reductions of α-methyl-β-ketosulfones by baker's yeast. The most frequently used yeast in microbial reductions[3], *S. cerevisiae*, did not show any reasonable selectivity. Also *Geotrichum candidum*, which usually reduces the keto groups *Si*-face[5,16] yielded only very low conversions (<1%) in our case.

We can conclude from our screening that *C. zeylanoides* or *C. guillermondii* are able to produce two diastereomers of alcohol **2** each in 99-99.9 % *e.p.* These diastereoisomeric alcohols can be further purified using HPLC technique on a silica gel column. Although a better method of purification would be crystallization, we were not able to prepare suitable crystalline derivatives of the alcohol **2**. The enantiomerically enriched sample of **1** can be obtained by the chemical resolution of 2-methylalkanoic acids -*vide infra* - or employing much faster reductions of (*R*)-**1** by microorganisms from the class **B**. The diastereomeric alcohols formed can be reoxidized to (*R*)-**1** and the remaining ketone from the reduction could supply the opposite enantiomer. We are currently examining other microorganisms for their potential in reducing the keto group in **1** from *Si*-face, as well as a possibility to invert the configurations on $C_{(2)}$ chiral center using the Mitsunobu esterification.[17]

Experimental Section

Instrumentation

GC analyses were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a split/splitless injector and an FID detector. Following capillary GC columns and conditions were used: DB-5 (J&W Scientific) 30m x 0.25 mm (i.d.) film thickness 0.25 mm, carrier gas: He, temperature program: 60 °C for 4 min. rised 10 °C/min to 280 °C, and held for 5 min; Cyclodex B (permethylated β-cyclodextrin, J&W) 30m x 0.25 mm (i.d.), film thickness 0.25 μm, H_2 was used as a carrier gas, isothermaly at 170 °C; and Lipodex E

(dipentyl butyryl γ-cyclodextrin, Macherey-Nagel) 20 m x 0.25 mm (i.d.), carrier gas H_2, at 150 °C (for **1**) and 160 °C (**2**), and DB-WAX (J&W) 30 m x 0.25 mm (i.d.) film thickness 0.25 μm, He was used as a carrier gas (18.7 cm/s) at temperature program: 45 °C for 2 min, rised to 110 °C at rate 70 °C/min and than by 5 °C/min to 200 °C, and held for 5 min at this temperature. HPLC analyses were performed on an HP 1090 chromatograph on two serial silica gel columns 2 x 150 x 3.1 mm (i.d.) (Tessek, Prague) eluated with hexane/2-propanol (99/1) mixture at 1 ml/min. MPLC preparative chromatography was run on Büchi apparatus on silica gel columns using hexane/ethylacetate mixtures as a mobile phase. Optical rotation measurements were taken on a Perkin-Elmer 241 polarimeter, NMR spectra were determined in C^2HCl_3 solutions on a Varian UNITY-500, operating at 499.5 MHz for ^1H and at 128 MHz for ^{13}C NMR spectra, respectively. Absorptions are expressed in δ (ppm) scale relative to TMS for ^1H and relative to C^2HCl_3 signal (77.00 ppm) for ^{13}C NMR, respectively. The IR spectra were recorded on a Bruker IFS 88 FT-IR spectrometer in KBr pellets or in CCl_4 solutions, and electron impact (70 eV) mass spectra were obtained on ZAB-EQ (VG, England) instrument.

Chemical synthesis

Resolution of 2-methylpentanoic acid [8]

Diastereomeric amides (2*R*,1'*S*)-**3** and (2*S*,1'*S*)-**3** were prepared from *rac*-2-methylpentanoyl chloride and (*S*)-phenethyl amine and separated into individual diastereoisomers by MPLC on silica gel, and finally purified by fractional crystallization from hexane/ether/ethanol mixture to the final purity >99.9% (by GC on DB-5).

(2*R*,1'*S*)-**3**: m.p. 128 °C, $[\alpha]_D$= -144° (c=1.0, ethanol), **^{1}H NMR** (500 MHz, C^2HCl_3, δ) 7.36-7.24 (5H, m, Ph), 5.60 (1H, bd, *J*= 6.6 Hz, NH), 5.15 (1H, m, benzyl H), 2.17 (1H, ddq, *J*= 6.1, 3x 6.8, 8.1 Hz, HC-C=O), 1.62 (1H, dddd, *J*= 6.1, 8.1, 9.0, 13.2 Hz, $CHCH_a$), 1.49 (3H, d, *J*= 6.9 Hz, CH_3CHPh), 1.34 (1H, dddd, *J*= 6.1, 6.6, 9.0, 13.2, $CHCH_b$), 1.30-1.22 (2H, m, CH_2), 1.14 (1H, d, *J*= 6.8 Hz, $C\underline{H}_3CH$), 0.97 (3H, t, *J*=7.2, $C\underline{H}_3CH_2$); **IR** (KBr, cm^{-1}) 3298 vs, ν(NH), 1639 vs, amid I, 1547 vs, amid II; **MS** [EI, 70 eV, *m/z*(rel. intens.)] 219(50, M^+), 177(50), 120(25), 105(100), 77(15), 71(20), 43(25). (2*S*,1'*S*)-**3**: m.p. 88 °C, $[\alpha]_D$= -99° (c=1.0, ethanol), **^{1}H NMR** (500 MHz, C^2HCl_3, δ) 7.37-7.24 (5H, m, Ph), 5.59 (1H, bd, *J*= 6.8 Hz, NH), 5.15 (1H, m, benzyl H), 2.16 (1H, ddq, *J*= 5.8, 3x 6.8, 8.7 Hz, HC-C=O), 1.65 (1H, m, $CHCH_a$), 1.50 (3H, d, *J*= 6.8 Hz, CH_3CHPh), 1.40-1.28 (1H, m, $CHCH_b$), 1.40-1.28 (2H, m, CH_2), 1.12 (1H, d, *J*= 6.8 Hz, $C\underline{H}_3CH$), 0.91 (3H, t, *J*=7.2, $C\underline{H}_3CH_2$); **IR** (KBr, cm^{-1}) 3281 vs, ν(NH), 1634 vs, amid I, 1540 vs, amid II; **MS** [EI, 70 eV, *m/z*(rel. intens.)] 219(50, M^+), 177(50), 120(25), 105(100), 77(15), 71(20), 43(25).

The corresponding opposite diastereomeric pair, (2*R*,1'*R*)-**3** and (2*S*,1'*R*)-**3**, was prepared using (*R*)-phenethyl amine: (2*R*,1'*R*)-**3** $[\alpha]_D$= 141 (c=1.0, ethanol) and (2*S*,1'*R*)-**3** $[\alpha]_D$= 95 (c=1.0, ethanol).

(*R*)- and (*S*)-2-Methylpentanoic acids

The amides (2*R*,1'*S*)-**3** and (2*S*,1'*S*)-**3** were converted to enantiopure acids according to the Sonnet procedure.[10] (*R*)-2-Methylpentanoic acid: : 98.1% *e.p.* by GC on Cyclodex B, b.p. 110-115 °C/15 torr, $[\alpha]_D$=

19.4 (c= 3.0 , ether); (*S*)-2-Methylpentanoic acid: 97.1% *e.p.* by GC on Cyclodex B, b.p. 110 °C/14 torr, $[\alpha]_D$= +19.3 (c= 3.5 , ether). Engel[18] value for (*R*) acid: $[\alpha]_D$= -20.1 (c= 5.5 , ether).

3-Methyl-1-(phenylsulfonyl)hexan-2-one *rac*-**1**

A solution of phenylmethylsulfone (20 mmol, 3.12g) in dry THF (80 ml) was treated with *n*-butyl lithium hexane solution (2.2 M, 19.5 ml) at -50 to -35 °C for 15 min. The yellow slurry was treated with 2-methylpentanoyl chloride (22.3 mmol, 3.0 g) at the same temperature for 20 min. Than the solution was warmed to room temperature and it was poured into a cold ammonium chloride solution. Usual workup, flash chromatography and recrystalization from hexane/ether mixtures provided ketosulfone **1** (3.86 g, 76 % of th. yield) as white crystals (m.p. = 44-44.5 °C). **^{1}H NMR** (200MHz, C^2HCl_3, δ) 7.62-7.93 (5H, m, Ph), 4.26 (1H, d, *J*=13.7, $COCH_2SO_2Ph$), 4.18 (1H, d, *J*=13.7, $COCH_2SO_2Ph$), 2.83 (1H, m, *J*=6.9, $C\underline{H}CH_3$), 1.17-1.35 (4H, m, CH_2), 1.07 (3H, d, *J*=6.9, $C\underline{H}_3CH$), 0.89 (3H, d, *J*=7.1, $C\underline{H}_3CH_2$); **IR** (KBr, cm^{-1}) 3067 m, 20a(Ph), 1716 ν(C=O) vs, 1449 m, 18b(Ph), 1379 m, σ(CH_3), 1323 s, $\nu_A(SO_2)$, 1156 s, $\nu_S(SO_2)$, **MS** [EI, 70eV, *m/z*(rel. intens.)] 254(2, M^+), 212(100, M^+-C_3H_6), 156(19), 143(40), 141(50, $PhSO_2$), 77(67, Ph), 71(C_5H_{11}), 43(60).

(*R*)-3-Methyl-1-(phenylsulfonyl)hexan-2-one (*R*)- **1**

Acidic hydrolysis of amides [9]

A mixture of (2*R*,1'*S*)-**3** (4.6g, 21 mmol), 6N HCl (60 ml) and fenol (3g) was heated at reflux for 30 h. Subsequent extraction with ethylacetate (10 x 50 ml), evaporation of solvents at reduced pressure, and distillation afforded the acid (2.20 g, 90 % yield) with 88.9% *e.p* (GC on Cyclodex B).

Acylation

A mixture of 2-methylpentanoic acid (2.0 g; 17.2 mmol) and $CHCl_2OCH_3$ (11.9 g; 0.103 mol) was stirred at 50°C for 40 min. The product was concentrated *in vacuo* (2.7 kPa) yielding the crude chloride (2.3 g; 17.2 mmol). This material was added dropwise to a stirred and cooled (-30°C) solution of methylphenylsulfonyl dianion, prepared from methylphenyl sulfone (3.12 g; 20 mmol) and *n*-butyllithium (25 ml of 1.6M solution; 40 mmol), according to the above procedure. Purification by prep. MPLC and crystallization from hexane/ether mixtures gave 1.19 g (27 %) of the ketosulfone (*R*)-**1**. m.p. = 59-61.5 °C; $[\alpha]_D$= -25.6 (c= 0.4 , $CHCl_3$).

(*S*)-3-Methyl-1-(phenylsulfonyl)hexan-2-one (*S*)- **1**

Starting from (2*S*,1'*S*)-**3** (2.50g, 11.3mmol) of the ketosulfone (*S*)-(**1**) (1.36 g, 53 %) was prepared following the above procedure; m.p. = 48-49.5 °C; $[\alpha]_D$= +14.9 (c= 0.4 , $CHCl_3$).

Yeast cultivations and biotransformation

Yeasts from the Collections of the Institute of Microbiology were cultivated on a malt extract liquid medium (80 g/l, 50 ml) in 250 ml connical flasks at 28°C on the reciprocal shaker for 35 h. The biomass was separated by centrifugation, resuspended in a sterile 2% (w/v) solution of glucose (50 ml) and supplemented with the

substrate **1** (50 mg) dissolved in dimethylsulfoxide (0.5 ml). Biotransformations proceeded on a reciprocal shaker at 28°C for 48 h, unless stated otherwise. The whole cultures were frozen and filtered after thawing with addition of Cellite. The filtrate was passed through SM-2 prepacked column (Biorad) equilibrated with water and the column was successively washed with water and acetone. The solvent was evaporated and products were taken up by ethylacetate. The conversions and the distribution of diastereomers were determined by GC and HLPC. The samples of ketone **1** and alcohol diastereomers for spectral measurements were additionlly purified using silica gel column chromatography.

Rac-**1** (500 mg, 1.97 mmol) was dispensed into 10 conical flasks with *Candida guillermondii* cultures and biotransformed according to the procedure described above. It furnished two diastereomeric alcohols accompanied by nonreduced ketosulfone. The mixture was separated by column chromatography on silica gel providing **1** partially enriched in (*S*) enantiomer {35 mg, 7 %; $[\alpha]_D$= +14.9 (c= 0.4 , $CHCl_3$)} and 68:32 mixture (135 mg, 27 %) of (2*S*,3*R*)-**2** and (2*S*,3*S*)-**2**. **^{1}H NMR** (500 MHz, C^2HCl_3, δ) (*syn* isomer) 7.59-7.97 (5H, m, Ph), 4.10 (1H, ddd, *J*=1.5, 3.9, 9.8, C<u>H</u>OH), 3.24 (1H, dd, *J*=9.8, 14.1, CH_2SO_2Ph), 3.14 (1H, dd, *J*=1.5, 14.1, CH_2SO_2Ph), 1.54 (1H, m, J=3.9, 4.7, 3 x 6.8, 8.8, C<u>H</u>CH_3), 1.03-1.47 (4H, m, CH_2), 0.87 (3H, t, J=7.2, C<u>H</u>$_3CH_2$), 0.84 (3H, d, J=6.8, C<u>H</u>$_3$CH); (*anti* isomer) 7.59-7.97 (5H, m, Ph), 4.05 (1H, ddd, *J*=2.4, 4.9, 8.8, C<u>H</u>OH), 3.20 (1H, dd, *J*=8.8, 14.1, CH_2SO_2Ph), 3.16 (1H, dd, *J*=2.4, 14.1, CH_2SO_2Ph), 1.67 (1H, m, J=4.0, 4.9, 3 x 6.9, 9.0, C<u>H</u>CH_3), 1.03-1.47 (4H, m, CH_2), 0.86 (3H, t, J=6.9, C<u>H</u>$_3$CH), 0.84 (3H, t, J=7.1, C<u>H</u>$_3CH_2$); **^{13}C NMR** (125.7 MHz, C^2HCl_3, δ) (*syn* isomer) 134.0 (*p*-C_6H_5), 132.4 (*i*-C_6H_5), 129.4 (*m*-C_6H_5), 127.9 (*o*-C_6H_5), 68.8 (C-2), 60.4 (C-1), 38.1 (C-3), 34.5 (C-4), 20.2 (C-5), 14.1 (C-7), 13.8 (C-6); (*anti* isomer) 134.0 (*p*- C_6H_5), 132.4 (*i*- C_6H_5), 130.9 (*m*- C_6H_5), 128.8 (*o*- C_6H_5), 69.4 (C-2), 59.4 (C-1), 37.9 (C-3), 34.0 (C-4), 20.0 (C-5), 14.5 (C-7), 14.0 (C-6); **IR** (CCl_4, cm^{-1}) 3540m, ν(OH), 3087 m, 20a(Ph), 1448 m, 18b(Ph), 1383 m, σ(CH_3), 1308 s, $\nu_A(SO_2)$, 1149 s, $\nu_S(SO_2)$; **MS** [EI, 70eV, *m/z*(rel. intens.)] 256(2, M^+), 185(100, M^+-C_5H_{11}), 141(44, $PhSO_2$), 77(67, Ph), 43(24).

Chemical correlation

A methanolic solution (300 μl) of hydroxysulfone **2** from yeast reduction (Table 2, *Candida guillermondii*/**1**/52) (29 mg, 0.11 mmol) was vigorously vortexed with sodium dihydrogenphosphate (29 mg) and with $NaHg_x$ (2.5% Na, 500 mg) at room temperature for 4 h. The reaction mixture was dilluted with cold water (1 ml) and after mercury separation, the extractive workup with dichlormethane (4x 250 μl) furnished a mixture of diastereoisomers of **6** which was subsequently esterified with (*S*)-2-acetoxypropionyl chloride/pyridine according to the published procedure.[14] Standard of the four diastereomeric esters **7** was formed in the same way[15] from *rac*-**6**, which was prepared from *rac*-2-methylpentanal and methylmagnesium bromide. Retention times of prepared esters measured on DB-WAX capillary column are presented in Table 4.

Table 4 Comparison of measured and published[14] retention times of diastereomeric ester **7**

Ret. time [min] on DB-WAX (abundance in %)		Retention times [min] (absolute configurations of **7**)[14]
Standard of *rac*-**7**	Mixture from the correlation	
15.16 (32)	15.16 (67)	50.6 (**2*R*,3*R***)
15.33 (22)	15.33 (33)	53.4 (**2*R*,3*S***)
15.60 (25)	--	55.6 (2*S*,3*S*)
15.75 (21)	--	57.3 (2*S*,3*R*)

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