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Stereoselective microbial reduction of racemic acetyl(t-butyl)methylphenylsilane by *Trigonopsis variabilis* (DSM 70714) and *Corynebacterium dioxydans* (ATCC 21766) *

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Abstract

(Si*R*,C*R*)- and (Si*S*,C*R*)-t-butyl(1-hydroxyethyl)methylphenylsilane [(Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3**] have been prepared by (*R*)-selective microbial reduction of racemic acetyl(t-butyl)methylphenylsilane (*rac*-**1**) using resting free cells of the yeast *Trigonopsis variabilis* (DSM 70714) or the bacterium *Corynebacterium dioxydans* (ATCC 21766). The biotransformations were carried out on a 10 g scale. After separation by column chromatography on silica gel, the optically active diastereomers (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** produced by *T. variabilis* were obtained in good yields [74% ((Si*R*,C*R*)-**2**), 78% ((Si*S*,C*R*)-**3**)]. The products obtained from the reduction with *C. dioxydans* were isolated in significantly lower yields [20% ((Si*R*,C*R*)-**2**), 20% ((Si*S*,C*R*)-**3**)]; reaction conditions not optimized). Both bioconversions gave products with high enantiomeric purities [*T. variabilis*: 97% ee ((Si*R*,C*R*)-**2**), 96% ee ((Si*S*,C*R*)-**3**); *C. dioxydans*: ⩾ 99% ee ((Si*R*,C*R*)-**2**), ⩾ 99% ee ((Si*S*,C*R*)-**3**)].

To throw light on the stereochemical aspects of these biotransformations, an X-ray diffraction study was carried out on the 3,5-dinitrobenzoate of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3**. In addition, ^{1}H NMR spectroscopic stereochemical correlation studies were performed with the (*S*)-MTPA esters derived from (Si*R*,C*R*)-**2**, (Si*S*,C*R*)-**3**, *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** [*rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** were obtained by reduction of *rac*-**1** with $LiAlH_4$ in diethyl ether, followed by chromatographic separation of the diastereomers on silica gel]. These stereochemical studies allowed assignment of the absolute configurations and enantiomeric purities of the biotransformation products.

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* Dedicated to Professor Dr. E. Hengge on the occasion of his 60th birthday

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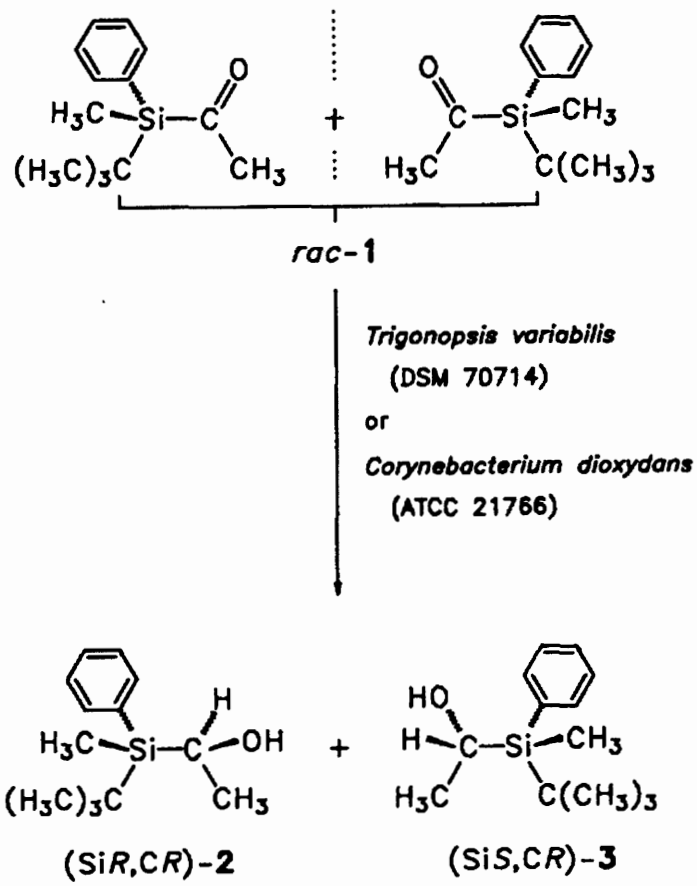
Introduction

In screening experiments [1] it was shown that the achiral model compound acetyldimethylphenylsilane is reduced enantioselectively by resting free cells of the yeast *Trigonopsis variabilis* (DSM 70714) and the bacterium *Corynebacterium dioxydans* (ATCC 21766) to give optically active (*R*)-(1-hydroxyethyl)dimethylphenylsilane [$PhMe_2SiC(O)Me \rightarrow$ (*R*)-$PhMe_2SiCH(OH)Me$]. In continuation of our studies on the biocatalyzed synthesis of optically active silanes (for reviews see refs. 2–4; for original papers see refs. 5–7), we have investigated analogous biotransformations of the chiral substrate *rac*-acetyl(t-butyl)methylphenylsilane (*rac*-**1**) into the diastereomeric reduction products (Si*R*,C*R*)- and (Si*S*,C*R*)-t-butyl(1-hydroxyethyl)methylphenylsilane [(Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3**] (Scheme 1). Here we report on methods for the synthesis of (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** by (*R*)-selective reduction of *rac*-**1** using resting free cells of *T. variabilis* or *C. dioxydans*. Both bioconversions were carried out on a 10 g scale. After chromatographic separation on silica gel the resulting optically active biotransformation products were isolated with high enantiomeric purities.

Preliminary results concerning the microbial reduction of *rac*-**1** with *T. variabilis* have already been published elsewhere [2–4,8]. The synthesis of the substrate *rac*-**1** was recently described [9].



Scheme 1

Results and discussion

(a) Biotransformation with T. variabilis

In preliminary studies the acetylsilane *rac*-**1** was reduced by growing cells of *T. variabilis* on a 500 mg scale to yield the corresponding (1-hydroxyethyl)silanes (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** with satisfactory yields (≈ 50%) and high enantiomeric purities (> 90% ee) [8]. After optimization of the reaction conditions with resting free cells on an analytical scale (data not given), we have performed this biotransformation in a 30 l bioreactor on a 10 g scale (cell wet mass 1500 g, 1280 g glucose, 0.1 *M* Sörensen buffer, pH 6.8, 37 °C, substrate concentration 0.35 g/l, 1500 rpm). After an incubation period of 68 min (turnover 99% as monitored by GLC), the biotransformation products (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** were isolated by extraction with ethyl acetate and separated by column chromatography on silica gel, followed by Kugelrohr distillation. The optically active silanes (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** were obtained in good yields [74% ((Si*R*,C*R*)-**2**), related to (*R*)-**1**; 78% ((Si*S*,C*R*)-**3**), related to (*S*)-**1**] and with high enantiomeric purities [97% ee ((Si*R*,C*R*)-**2**), 96% ee ((Si*S*,C*R*)-**3**)]. Both compounds were isolated as colourless liquids; however, (Si*S*,C*R*)-**3** crystallized upon cooling of a solution in n-pentane to −20 °C, resulting in an increase of enantiomeric purity [≥ 99% ee, crystallized (Si*S*,C*R*)-**3**].

In conclusion, the preparative procedure described here represents an efficient method for the synthesis of (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3**.

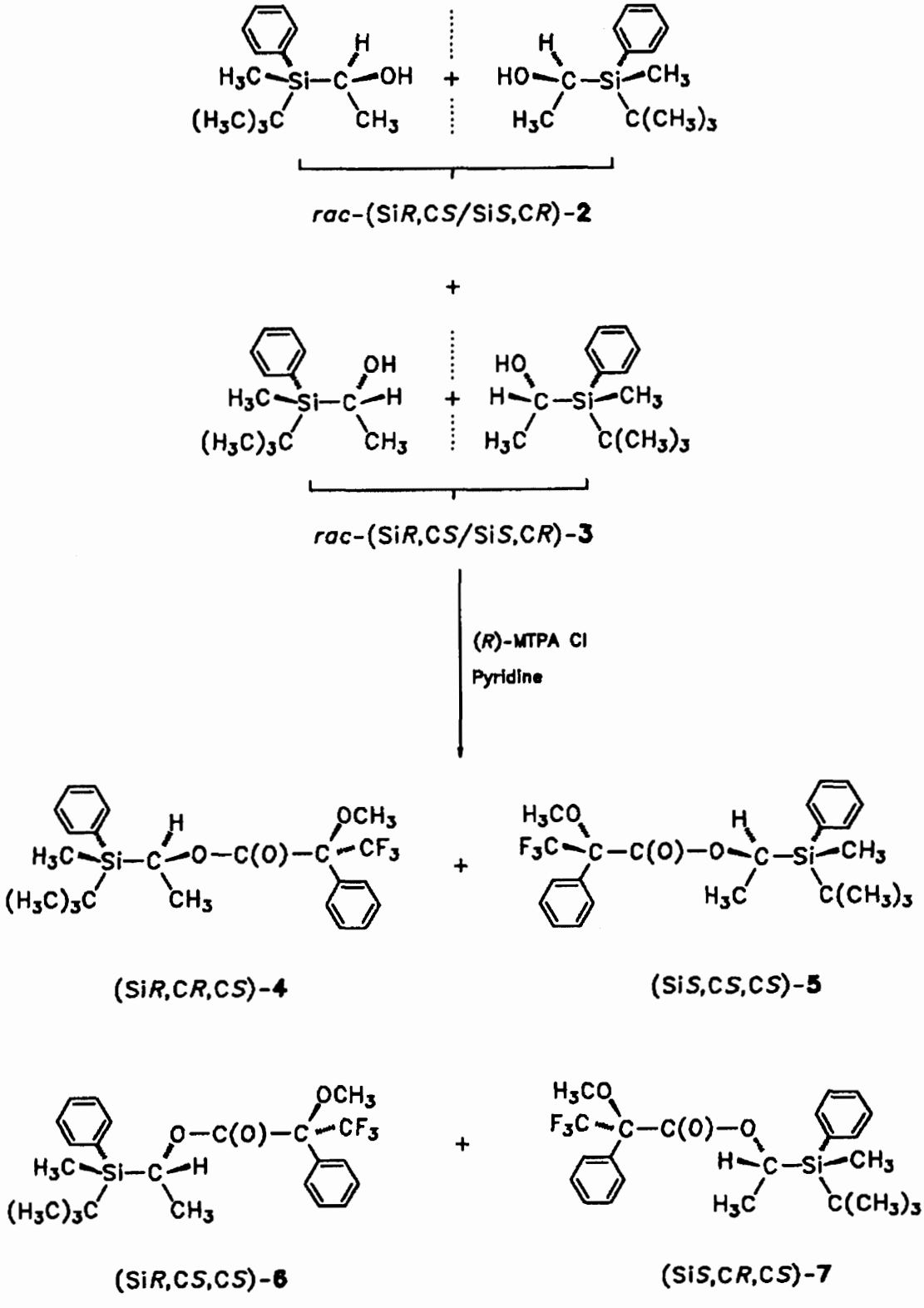
(b) Biotransformation with C. dioxydans

In preliminary experiments the acetylsilane *rac*-**1** was also found to be reduced (*R*)-selectively to the corresponding (1-hydroxyethyl)silanes (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** when using resting free cells of *C. dioxydans*. This biotransformation (reaction conditions not optimized) was also performed in a 30 l bioreactor on a 10 g scale (cell wet mass 1200 g, 600 g glucose, 0.16 *M* Tris buffer, pH 6.8, 27 °C, substrate concentration 0.35 g/l, 1500 rpm). After an incubation period of 64 h (turnover 81% as monitored by GLC), the biotransformation products (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** were isolated by extraction of the culture broth with ethyl acetate and extraction of the broken-up cell mass with dichloromethane/methanol, followed by column chromatography (silica gel) and Kugelrohr distillation of the diastereomers. The (1-hydroxyethyl)silanes (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** were obtained in acceptable yields [20% ((Si*R*,C*R*)-**2**), related to (*R*)-**1**; 20% ((Si*S*,C*R*)-**3**), related to (*S*)-**1**] and high enantiomeric purities [≥ 99% ee ((Si*R*,C*R*)-**2**), ≥ 99% ee ((Si*S*,C*R*)-**3**)]. t-Butylmethylphenylsilanol [MePh(t-Bu)SiOH, see ref. 9] was formed as a by-product in the course of this bioconversion (not observed in the case of *T. variabilis*), but could easily be separated when purifying (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** by column chromatography. By analogy to the method described above, both biotransformation products were obtained as colourless liquids. Upon cooling to −20 °C, (Si*S*,C*R*)-**3** crystallized whereas (Si*R*,C*R*)-**2** remained liquid.

In conclusion, this bioconversion approach also represents a suitable method for the preparation of (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3**. The processing was more difficult and the yield was lower (reaction conditions not optimized) as compared to the conversion with *T. variabilis*, but the enantiomeric purity of the biotransformation products was somewhat higher.

(c) Absolute configurations and enantiomeric purities

The absolute configurations and enantiomeric purities of the biotransformation products (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3** were determined, after derivatization with



Scheme 2

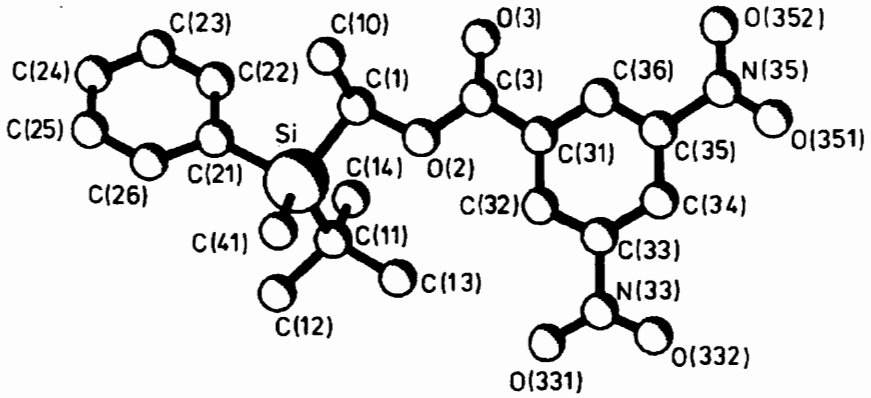


Fig. 1. Molecular structure of the enantiomer (Si*R*,C*S*)-**8** as observed in the crystal lattice of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8**.

(*R*)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride [(*R*)-MTPA Cl], by ^{1}H NMR spectroscopic studies of the corresponding MTPA esters by the general method described in ref. 10. For comparison, a mixture of the racemates (Si*R*,C*R*/

Table 1

Positional parameters and equivalent temperature factors of the non-hydrogen atoms of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8**

	x	*y*	*z*	U_{eq}
Si	0.7895(1)	0.0203(1)	0.1461(1)	0.050(0)
C(1)	0.6552(3)	0.0681(3)	0.1008(2)	0.047(1)
O(2)	0.6328(2)	0.1970(2)	0.1343(1)	0.050(1)
C(3)	0.5602(3)	0.2675(3)	0.0918(2)	0.048(1)
O(3)	0.5121(2)	0.2327(2)	0.0304(1)	0.065(1)
C(31)	0.5478(3)	0.3964(3)	0.1292(2)	0.041(1)
C(32)	0.6157(3)	0.4457(3)	0.1906(2)	0.045(1)
C(33)	0.5995(3)	0.5672(3)	0.2195(2)	0.045(1)
N(33)	0.6703(3)	0.6191(3)	0.2868(2)	0.062(1)
O(331)	0.7424(2)	0.5518(3)	0.3115(2)	0.090(1)
O(332)	0.6515(2)	0.7262(3)	0.3123(2)	0.091(1)
C(34)	0.5184(3)	0.6428(3)	0.1906(2)	0.048(1)
C(35)	0.4518(3)	0.5925(3)	0.1294(2)	0.045(1)
N(35)	0.3623(3)	0.6715(3)	0.0983(2)	0.061(1)
O(351)	0.3509(2)	0.7763(3)	0.1287(2)	0.081(1)
O(352)	0.3071(2)	0.6250(3)	0.0421(2)	0.083(1)
C(36)	0.4659(3)	0.4724(3)	0.0986(2)	0.046(1)
C(10)	0.5714(3)	−0.0257(4)	0.1211(2)	0.067(1)
C(11)	0.8937(3)	0.1331(4)	0.1162(2)	0.056(1)
C(12)	0.9992(4)	0.0785(6)	0.1495(4)	0.081(2)
C(13)	0.8833(6)	0.2679(6)	0.1516(6)	0.099(2)
C(14)	0.8942(5)	0.1422(6)	0.0230(3)	0.079(2)
C(21)	0.8101(3)	−0.1452(3)	0.1051(2)	0.050(1)
C(22)	0.7844(3)	−0.1785(4)	0.0243(3)	0.062(1)
C(23)	0.7998(4)	−0.2992(4)	−0.0060(3)	0.070(1)
C(24)	0.8445(4)	−0.3920(5)	0.0438(3)	0.086(2)
C(25)	0.8693(5)	−0.3653(5)	0.1246(3)	0.098(2)
C(26)	0.8541(4)	−0.2426(4)	0.1538(3)	0.077(1)
C(41)	0.7867(6)	0.0161(7)	0.2595(3)	0.079(2)

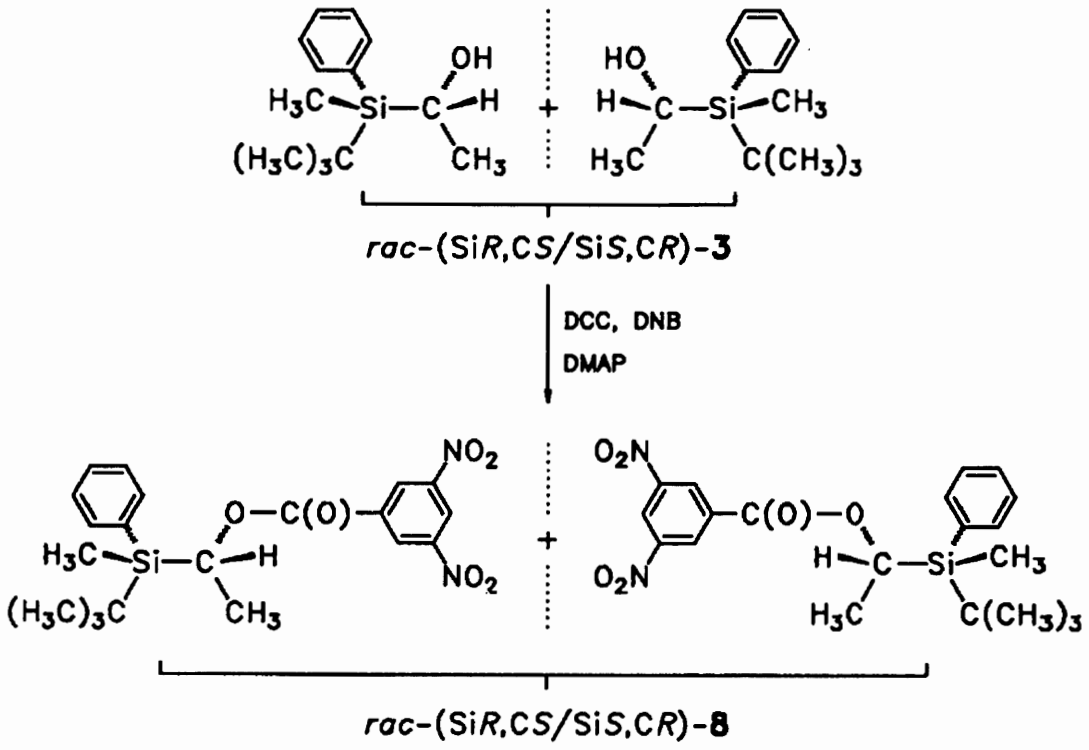
Si*S*,C*S*)-**2** and (Si*R*,C*S*/Si*S*,C*R*)-**3** (available by reduction of *rac*-**1** with $LiAlH_4$ in diethyl ether) was transformed into the corresponding MTPA esters (Si*R*,C*R*,C*S*)-**4**, (Si*S*,C*S*,C*S*)-**5**, (Si*R*,C*S*,C*S*)-**6** and (Si*S*,C*R*,C*S*)-**7** as outlined in Scheme 2.

Before these studies were performed, the relative configurations of *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** were determined by a single crystal X-ray structural analysis of the 3,5-dinitrobenzoate *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8** (Fig. 1, Tables 1 and 2). This compound was prepared by esterification of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** with 3,5-dinitrobenzoic acid by a method described in ref. 12 (Scheme 3).

Table 2

Molecular geometry of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8**

Bond lengths [pm]			
C(1)–Si	190.3(3)	C(11)–Si	187.5(4)
C(21)–Si	186.8(4)	C(41)–Si	186.1(5)
O(2)–C(1)	148.1(4)	C(10)–C(1)	151.1(5)
C(3)–O(2)	134.3(4)	O(3)–C(3)	119.9(4)
C(31)–C(3)	148.4(5)	C(32)–C(31)	138.4(4)
C(36)–C(31)	138.4(5)	C(33)–C(32)	136.7(5)
N(33)–C(33)	148.2(5)	C(34)–C(33)	136.6(5)
O(331)–N(33)	121.0(5)	O(332)–N(33)	121.7(5)
C(35)–C(34)	137.5(5)	N(35)–C(35)	147.7(5)
C(36)–C(35)	136.0(5)	O(351)–N(35)	120.9(4)
O(352)–N(35)	122.2(4)	C(12)–C(11)	153.8(6)
C(13)–C(11)	152.2(8)	C(14)–C(11)	153.1(7)
C(22)–C(21)	138.5(5)	C(26)–C(21)	138.3(6)
C(23)–C(22)	136.6(6)	C(24)–C(23)	136.3(7)
C(25)–C(24)	136.7(8)	C(26)–C(25)	137.8(7)
Bond angles [°]			
C(11)–Si–C(1)	112.8(2)	C(21)–Si–C(1)	104.4(2)
C(21)–Si–C(11)	110.8(2)	C(41)–Si–C(1)	108.2(2)
C(41)–Si–C(11)	109.9(3)	C(41)–Si–C(21)	110.5(2)
O(2)–C(1)–Si	106.7(2)	C(10)–C(1)–Si	113.3(2)
C(10)–C(1)–O(2)	109.6(3)	C(3)–O(2)–C(1)	116.5(2)
O(3)–C(3)–O(2)	124.9(3)	C(31)–C(3)–O(2)	111.6(3)
C(31)–C(3)–O(3)	123.5(3)	C(32)–C(31)–C(3)	123.4(3)
C(36)–C(31)–C(3)	117.8(3)	C(36)–C(31)–C(32)	118.7(3)
C(33)–C(32)–C(31)	119.1(3)	N(33)–C(33)–C(32)	119.3(3)
C(34)–C(33)–C(32)	122.8(3)	C(34)–C(33)–N(33)	117.9(3)
O(331)–N(33)–C(33)	117.3(3)	O(332)–N(33)–C(33)	117.3(3)
O(332)–N(33)–O(331)	125.4(3)	C(35)–C(34)–C(33)	117.3(3)
N(35)–C(35)–C(34)	118.3(3)	C(36)–C(35)–C(34)	121.6(3)
C(36)–C(35)–N(35)	120.2(3)	O(351)–N(35)–C(35)	118.2(3)
O(352)–N(35)–C(35)	116.1(3)	O(352)–N(35)–O(351)	125.6(3)
C(35)–C(36)–C(31)	120.4(3)	C(12)–C(11)–Si	108.2(3)
C(13)–C(11)–Si	112.8(4)	C(13)–C(11)–C(12)	107.7(4)
C(14)–C(11)–Si	110.7(3)	C(14)–C(11)–C(12)	107.9(4)
C(14)–C(11)–C(13)	109.2(5)	C(22)–C(21)–Si	122.8(3)
C(26)–C(21)–Si	121.8(3)	C(26)–C(21)–C(22)	115.4(4)
C(23)–C(22)–C(21)	123.0(4)	C(24)–C(23)–C(22)	119.7(5)
C(25)–C(24)–C(23)	119.7(5)	C(26)–C(25)–C(24)	119.6(5)
C(25)–C(26)–C(21)	122.4(4)		



Scheme 3

The silane *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** was obtained by reduction of *rac*-**1** with $LiAlH_4$, followed by chromatographic separation from *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** [*rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** was eluted with diethyl ether/n-hexane (1/1.8, v/v) as the second product; see Experimental section].

The bond distances and angles (Table 2) of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8** are within the normally observed range and so do not need further discussions. The arrangement of the NO_2 groups and the CC(O) moiety relative to the phenyl ring is nearly coplanar (maximum deviation of the O–N–C–C torsional angles from 0° or 180°: 3.5°; maximum deviation of the O–C–C–C torsional angles from 0° or 180°: 12.4°). In addition, the conformation can be described by the following torsional angles: C(1)–O(2)–C(3)–O(3) 1.2°, Si–C(1)–O(2)–C(3) 158.7°, C(11)–Si–C(1)–C(10) 177.3°.

Experimental

(a) Chemical syntheses

All reactions were performed in dried solvents under dry nitrogen unless otherwise indicated. Melting points were determined with a Kofler apparatus (Reichert). ^1H and ^{13}C NMR spectra were recorded either on a Bruker AM-400 spectrometer or a Bruker WM-400 spectrometer at 400.1 MHz (^1H) and 100.6 MHz (^{13}C), respectively. Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^1H, δ 7.25) or $CDCl_3$ (^{13}C, δ 77.05). Assignment of the ^{13}C data was supported by DEPT experiments. The results of these experiments are included in the assignments. Mass spectra were obtained with a Finnigan MAT 8430 mass spectrometer (EI MS, 70 eV); the *m/z* values given refer to the isotopes ^1H, ^{12}C, ^{14}N, ^{16}O and ^{29}Si.

rac-*Acetyl(t-butyl)methylphenylsilane* (rac-*1*)

This compound was synthesized according to ref. 9.

rac-*(SiR,CR / SiS,CS)-t-Butyl(1-hydroxyethyl)methylphenylsilane ((SiR,CR / SiS,CS)-2) and* rac-*(SiR,CS / SiS,CR)-t-butyl(1-hydroxethyl)methylphenylsilane ((SiR,CS/SiS,CR)-3))*

A solution of *rac*-**1** (0.82 g, 3.72 mmol) in diethyl ether (15 ml) was added dropwise at 0 °C (ice cooling) during 10 min to a stirred suspension of $LiAlH_4$ (0.04 g, 1.05 mmol) in diethyl ether (20 ml). After 2 h stirring at room temperature the mixture was added carefully (ice cooling) to 6 *N* hydrochloric acid (10 ml). The ethereal phase was separated and the aqueous layer extracted twice with 25 ml portions of diethyl ether. After neutralization of the combined organic extracts with saturated aqueous $NaHCO_3$ solution and washing with water, the organic phase was dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by Kugelrohr distillation (oven temperature max. 150 °C, 0.3 Torr) to yield 0.77 g (93%) of a colourless viscous liquid consisting of 58% *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and 42% *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3**. The diastereomers were separated by column chromatography on silica gel [Lobar® pre-packed column, size B (310–25), Lichroprep Si60 (40–63 μm), Merck 10401; elution with diethyl ether/n-hexane (1/1.8, v/v); R_f((Si*R*,C*R*/Si*S*,C*S*)-**2**) > R_f((Si*R*,C*S*/Si*S*,C*R*)-**3**) as monitored on TLC plates (silica gel 60, Merck 5554; diethyl ether/n-hexane (1/1.8, v/v), UV detection)] and finally purified by Kugelrohr distillation (oven temperature max. 150 °C, 0.1 Torr).

(SiR,CR/SiS,CS)-2. ^{1}H NMR ($CDCl_3$): δ 0.28 (s, 3H; $SiCH_3$), 0.97 (s, 9H; $C(CH_3)_3$), 1.37 (d, ^3J 7.4 Hz, 3H; $SiCHCH_3$), 4.02 (q, ^3J 7.4 Hz, 1H; $SiCHCH_3$), 7.3–7.4 and 7.6–7.7 (m, 5H; SiC_6H_5), OH not localized. ^{13}C NMR ($CDCl_3$): δ −10.1 ($SiCH_3$), 17.4 ($SiC(CH_3)_3$), 20.6 ($SiCHCH_3$), 27.4 ($C(CH_3)_3$), 59.3 ($SiCHCH_3$), 127.8 (C_m, SiC_6H_5), 129.3 (C_p, SiC_6H_5), 134.5 (C_i, SiC_6H_5), 135.3 (C_o, SiC_6H_5). MS: *m/z* 222 (6%, M^+), 137 (100%). Anal. Found: C, 70.3; H, 10.0. $C_{13}H_{22}OSi$ (222.4) calc: C, 70.21; H, 9.97%.

(SiR,CS/SiS,CR)-3. ^{1}H NMR ($CDCl_3$): δ 0.34 (s, 3H; $SiCH_3$), 0.99 (s, 9H; $C(CH_3)_3$), 1.28 (d, ^3J 7.5 Hz, 3H; $SiCHCH_3$), 4.09 (q, ^3J 7.5 Hz, 1H; $SiCHCH_3$), 7.3–7.5 (m, 5H; SiC_6H_5), OH not localized. ^{13}C NMR ($CDCl_3$): δ −11.0 ($SiCH_3$), 17.6 ($C(CH_3)_3$), 21.2 ($SiCHCH_3$), 27.5 ($C(CH_3)_3$), 60.0 ($SiCHCh_3$), 127.6 (C_m, SiC_6H_5), 129.1 (C_p, SiC_6H_5), 134.9 (C_o, SiC_6H_5), 135.7 (C_i, SiC_6H_5). MS: *m/z* 165 (68%, $M^+ - C(CH_3)_3$), 137 (100%). Anal. Found: C, 70.3; H, 10.0. $C_{13}H_{22}OSi$ (222.4) calc: C, 70.21; H, 9.97%.

Transformation of a mixture of rac-*(SiR,CR/SiS,CS)-2 and* rac-*(SiR,CS/SiS,CR)-3 into the corresponding MTPA esters (SiR,CR,CS)-4, (SiS,CS,CS)-5, (SiR,CS,CS)-6 and (SiS,CR,CS)-7*

(*R*)-α-Methoxy-α-trifluoromethyl-phenylacetyl chloride [(*R*)-MTPA Cl; prepared from (*S*)-(−)-α-methoxy-α-trifluoromethyl-phenylacetic acid (Fluka 65364) as described in ref. 11] (52 μl) was added at room temperature to a stirred mixture of tetrachloromethane/pyridine (1/1, v/v; 1.2 ml) and *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2**/*rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** (0.2 mmol). After 3 h stirring at room temperature [complete conversion as monitored by TLC; silica gel plates (Merck 5735), n-hexane/diethyl ether (2/1, v/v), UV detection], 3-dimethylamino-1-propylamine (48 μl) was

added and the mixture was stirred for 10 min. After addition of diethyl ether (20 ml) and of 2% hydrochloric acid (20 ml), the organic layer was separated and shaken first with saturated aqueous Na_2CO_3 solution (20 ml) and then with saturated aqueous NaCl solution (20 ml). The organic phase was dried over $MgSO_4$, the solvent removed under reduced pressure, and the residue dissolved in $CDCl_3$ (0.5 ml). To remove traces of diethyl ether the solvent was again evaporated and the residue redissolved in $CDCl_3$. The samples obtained by this procedure were directly used for the NMR spectroscopic studies. The assignment of the absolute configuration at the carbon atoms of the $SiC^*H(OR)CH_3$ moieties of the MTPA esters [R = $C(O)C(OCH_3)(CF_3)C_6H_5$] was performed by the correlation method described in ref. 10.

*(Si*R,*C*R,*CS)-4.* ^{1}H NMR ($CDCl_3$): δ 0.33 (s, 3H; $SiCH_3$), 0.91 (s, 9H; $C(CH_3)_3$), 1.40 (d, ^3J 7.4 Hz, 3H; $SiCHCH_3$), 3.29 ('q', ^5J(H–F) 0.98 Hz, 3H; OCH_3), 5.51 (q, ^3J 7.4 Hz, 1H; $SiCHCH_3$), 7.2–7.7 (m, 10H; SiC_6H_5, CC_6H_5).

*(Si*S,*CS*,*CS)-5.* ^{1}H NMR ($CDCl_3$): δ 0.29 (s, 3H; $SiCH_3$), 0.78 (s, 9H; $C(CH_3)_3$), 1.44 (d, ^3J 7.4 Hz, 3H; $SiCHCH_3$), 3.42 ('q', ^5J(H–F) 1.03 Hz, 3H; OCH_3), 5.44 (q, ^3J 7.4 Hz, 1H; $SiCHCH_3$), 7.2–7.7 (m, 10H; SiC_6H_5, CC_6H_5).

*(Si*R,*CS*,*CS)-6.* ^{1}H NMR ($CDCl_3$): δ 0.32 (s, 3H; $SiCH_3$), 0.76 (s, 9H; $C(CH_3)_3$), 1.33 (d, ^3J 7.4 Hz, 3H; $SiCHCH_3$), 3.62 ('q', ^5J(H–F) 1.03 Hz, 3H; OCH_3), 5.42 (q, ^3J 7.4 Hz, 1H; $SiCHCH_3$), 7.2–7.7 (m, 10H; SiC_6H_5, CC_6H_5).

*(Si*S,*C*R,*CS)-7.* ^{1}H NMR ($CDCl_3$): δ 0.41 (s, 3H; $SiCH_3$), 0.86 (s, 9H; $C(CH_3)_3$), 1.28 (d, ^3J 7.4 Hz, 3H; $SiCHCH_3$), 3.45 ('q', ^5J(H–F) 0.66 Hz, 3H; OCH_3), 5.44 (q, ^3J 7.4 Hz, 1H; $SiCHCH_3$), 7.2–7.6 (m, 10H; SiC_6H_5, CC_6H_5).

rac-*(Si*R,*CS*/*Si*S,*C*R)-*t*-Butylmethyl-[1-(3.5-dinitrobenzoyloxy)ethyl]phenylsilane *((Si*R,*CS*/*Si*S,*C*R)-*8)*

A procedure analogous to that described in ref. 12 was used. A solution of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** (100 mg, 0.45 mmol) in dichloromethane (5 ml) was added during 10 min at room temperature to a stirred solution of 3,5-dinitrobenzoic acid (143 mg, 0.674 mmol), *N*,*N*′-dicyclohexylcarbodiimide (110 mg, 0.533 mmol) and 4-(dimethylamino)pyridine (4.5 mg, 36.8 μmol) in dichloromethane (10 ml). After 48 h stirring at room temperature [complete conversion of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** as monitored by TLC] the precipitate was filtered off and the filtrate was concentrated in vacuo. The residual oil was purified by column chromatography on silica gel (silica gel 60, 0.063–0.200 mm, Merck 7734; elution with dichloromethane) to yield a solid product. Recrystallization from n-hexane/dichloromethane (10/1, v/v) gave 174 mg (yield 93%) of the pure crystalline product, mp. 107–108°C. ^{1}H NMR ($CDCl_3$): δ 0.53 (s, 3H; $SiCH_3$), 0.97 (s, 9H; $C(CH_3)_3$), 1.40 (d, ^3J 7.5 Hz, 3H; $SiCHCH_3$), 5.68 (q, ^3J 7.5 Hz, 1H; $SiCHCH_3$), 7.4–7.6 (m, 5H; SiC_6H_5), 9.15 (d, ^4J 2.5 Hz, 2H; C(2)-H and C(6)-H, $C_6H_3(NO_2)_2$), 9.23 (t, ^4J 2.5 Hz, 1H; C(4)-H, $C_6H_3(NO_2)_2$). ^{13}C NMR ($CDCl_3$): δ −10.3 ($SiCH_3$), 17.4 ($SiCHCH_3$), 17.5 ($C(CH_3)_3$), 27.3 ($C(CH_3)_3$), 66.0 ($SiCHCH_3$), 122.2, 128.0, 129.3, 129.7, 133.6, 134.6 and 134.8 (aromatic *C*), 148.8 (C(3) and C(5), $C_6H_3(NO_2)_2$), 162.7 (CO). MS: *m*/*z* 177 (35%, $M^+ - CH(CH_3)OC(O)C_6H_3(NO_2)_2$), 359 (100%, $M^+ - C(CH_3)_3$). Anal. Found: C, 57.7; H, 5.9. $C_{20}H_{24}N_2O_6Si$ (416.5) calc: C, 57.68; H, 5.81%.

(b) X-Ray diffraction study

Crystals of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8**, obtained by crystallization from n-hexane/trichloromethane (10/1, v/v), have monoclinic symmetry, space group $P2_1/c$. The

unit cell [$a = 1292.43(12)$, $b = 1036.62(9)$, $c = 1637.77(17)$ pm, $\beta = 94.17(24)°$] contains four molecules yielding a calculated density of 1.26 g cm^{-3}. The data were collected at room temperature on a Syntex diffractometer (type $P2_1$) using monochromatized Mo-K_α radiation ($\lambda = 71.07$ pm).

Intensities were measured in the θ–2θ mode with a scan rate between 2.93 and 29.30°/min depending on the intensities of the reflections. The data were corrected for Lorentz and polarization effects, but not for absorption effects ($\mu = 0.105$ mm^{-1}). The structure was solved by a combination of direct methods and difference Fourier syntheses. Hydrogen atoms were localized from difference Fourier maps and refined isotropically. The refinement using 2266 out of 3814 measured independent reflections ($F \geqslant 3.0\sigma(F)$, $3° \leqslant 2\theta \leqslant 50°$) converged at $R = 0.065$. A final difference Fourier map displayed no electron density higher than 0.35×10^{-6} e/pm^3. The program SHELX-76 [13] and our own programs were used and complex atom scattering factors [14] were employed. The positional parameters and equivalent temperature factors of the non-hydrogen atoms of *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**8** are listed in Table 1. Bond lengths and angles are given in Table 2 and the atomic numbering scheme is shown in Fig. 1. Lists of thermal parameters and observed and calculated structure factors are available from the authors.

According to these studies, racemic **8** consists of a 1 : 1 mixture of the respective (*R*/*S*)- and (*S*/*R*)-enantiomers.

(c) Biotransformations

Microorganisms

The microorganisms *Trigonopsis variabilis* (DSM 70714) and *Corynebacterium dioxydans* (ATCC 21766) were obtained from public type culture collections (DSM, ATCC).

Cultivation of the microorganisms

T. variabilis was cultivated under aerobic conditions at pH 6.8 and 27 °C in a medium containing 0.5% yeast extract, 2% malt extract, 1% peptone and 2.5% glucose. The cultivation was performed either in shake flasks (500 ml) containing 100 ml of the medium or in a 20 l bioreactor as described previously (see ref. 5).

C. dioxydans was cultivated under aerobic conditions at pH 7.5 and 27 °C in a medium containing 1% yeast extract, 1% bacto peptone, 0.3% NaCl, 0.01% $MgCl_2$ and 1% glucose. The cultivation was performed either in shake flasks (2000 ml) containing 500 ml of the medium or in a 50 l bioreactor (Braun–Melsungen AG, Melsungen, FRG) equipped with flat blades. Physiological activity was monitored using a pH electrode, a pO_2 electrode as well as oxygen and carbon dioxide analyzers (Maihak, Hamburg, FRG). During the cultivations a constant pH value (pH 7.5) was maintained by titration with 10% aqueous NaOH solution or 12.5% H_3PO_4 solution. The time course of the cultivations was monitored by measuring the optical density at 546 nm (OD_{546}), the cell dry mass and the glucose concentration. The cells produced in the bioreactor were harvested after the glucose had been consumed.

Harvesting of the cell wet mass

Cells from shake flask cultivations were harvested by centrifugation for 30 min at 4600 rpm and 10 °C in a Cryofuge M 6000 (Heraeus-Christ, Osterode, FRG). The

cells produced in the bioreactor were harvested by centrifugation at 20000 rpm (continuous centrifugation) with a Padberg centrifuge (type 41, Carl Padberg GmbH, Lahr, Germany).

Monitoring of the bioconversions

After starting the biotransformation, samples of 1 ml of the culture broth were taken in regular intervals and subsequently extracted with 1 ml of n-hexane or dichloromethane. 1 µl of this extract was analyzed by GLC using a Chrompack gas chromatograph [model 436, Chrompack, Frankfurt am Main, FRG; capillary column Cp-Sil 5 CB, 10 m; 110 °C isotherm, carrier gas hydrogen; retention time 0.9 min (**1**) and 1.24 min (**2**/**3**)].

*Preparation of (Si*R,C*R)-2 and (Si*S,C*R)-3 by reduction with resting cells of* T. variabilis

The bioconversion was performed in a 30 l bioreactor (intensor system; surface aeration; model B20, Giovanola Freres, Switzerland). The substrate *rac*-**1** (10 g, 45.4 mmol) was added at 37 °C to a stirred suspension (1500 rpm) of freshly harvested cells of *T. variabilis* (1500 g cell wet mass) in 0.1 *M* Sörensen buffer (pH 6.8, 30 l) containing glucose (1280 g). After an incubation period of 68 min (turnover 99% as monitored by GLC), the culture broth was extracted once with ethyl acetate (60 l) using a roll-round pump (model B4-DA 6-300, K. Lutz, FRG; 6900 rpm, 6 atm). The extract was concentrated in vacuo (rotary evaporator) and then dried over Na_2SO_4. After evaporation of the solvent (rotary evaporator) the crude product was purified by Kugelrohr distillation (oven temperature max. 175 °C, 0.1 Torr) to yield 8.5 g (84%, related to *rac*-**1**) of a mixture consisting of (Si*R*,C*R*)-**2** and (Si*S*,C*R*)-**3**. These diastereomers were separated by column chromatography on silica gel [Lobar® pre-packed column, size B (310–25), Lichroprep Si60 (40–63 µm), Merck 10401; elution with diethyl ether/n-hexane (1/1.8, v/v)] to give 3.72 g (Si*R*,C*R*)-**2** [74%, related to (*R*)-**1**] and 3.95 g (Si*S*,C*R*)-**3** [78%, related to (*S*)-**1**]. Both compounds were obtained as viscous colourless liquids [(Si*S*,C*R*)-**3** crystallized from a solution in n-pentane upon cooling to −20 °C; mp < 20 °C]. The analytical properties (elemental analysis, spectroscopic data) of the biotransformation products were identical with those obtained from the chemically prepared racemates *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** (see above).

The enantiomeric purities of the biotransformation products were determined by ^{1}H NMR spectroscopic investigation of the respective MTPA esters as described above ((Si*R*,C*R*)-**2**: 97% ee; (Si*S*,C*R*)-**3**: 96% ee).

*Preparation of (Si*R,C*R)-2 and (Si*S,CR)-3 by reduction with resting cells of* C. dioxydans

The bioconversion was performed in a 30 l bioreactor (intensor system; surface areation model B20, Giovanola Freres, Switzerland). The substrate *rac*-**1** (10.6 g, 48.1 mmol) was added at 27 °C to a stirred suspension (1500 rpm) of freshly harvested cells of *C. dioxydans* (1200 g cell wet mass) in 0.16 *M* Tris buffer (pH 6.80, 30 l) containing glucose (600 g). After an incubation period of 64 h (turnover 81% as monitored by GLC), the cell mass was separated by centrifugation of the culture broth (Padberg centrifuge type 41, Carl Padberg GmbH, Lahr, Germany; 20 °C, 20000 rpm, continuous centrifugation) and the cells and the supernatant liquid were then worked up separately as described below.

Cell mass. The cell mass was broken up by stirring (500 rpm) with methanol/dichloromethane (1/2, v/v; 5 l). After centrifugation (Cryofuge M 6000, Heraeus–Christ, Osterode, FRG; 10 °C, 4600 rpm), the supernatant liquid was transferred into a separation funnel, the organic layer was separated and the aqueous layer extracted twice with dichloromethane. The combined organic extracts were concentrated to 100 ml under reduced pressure and dried over Na_2SO_4. After evaporation of the solvent in vacuo the orange-coloured, viscous residue was filtered over silica gel (silica gel 60, 0.063–0.200 mm, Merck 7734) to remove residual cell particles. Further purification by column chromatography on silica gel [silica gel 60, Merck 7734; elution with diethyl ether/n-hexane (1/1.8, v/v)] and subsequent Kugelrohr distillation (oven temperature max. 175 °C, 0.1 Torr) yielded 4.07 g of the product mixture consisting of (Si*R*,C*R*)-**2**, (Si*S*,C*R*)-**3** and traces of t-butylmethylphenylsilanol.

Supernatant. The supernatant was extracted once with ethyl acetate (60 l) using a roll-round pump (model B4-DA 6-300, K. Lutz, FRG; 6900 rpm, 6 atm). The extract was concentrated in vacuo (rotary evaporator) to 500 ml and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure the residue (8 g) was purified by column chromatography on silica gel (conditions as described above) and subsequent Kugelrohr distillation to yield 0.53 g of a mixture consisting of (Si*R*,C*R*)-**2**, (Si*S*,C*R*)-**3** and traces of t-butylmethylphenylsilanol.

The combined product (4.6 g; obtained by processing of the cell mass and the supernatant liquid) consisted of 46 mol-% (Si*R*,C*R*)-**2**, 48 mol-% (Si*S*,C*R*)-**3** and 6 mol-% t-butylmethylphenylsilanol. Column chromatography on silica gel [Lobar® pre-packed column, size B (310–25), Lichroprep Si60 (40–63 μm), Merck 10401; elution with diethyl ether/n-hexane (1/1.8, v/v)] and subsequent Kugelrohr distillation (oven temperature max. 150 °C, 0.3 Torr) yielded 1.06 g of (Si*R*,C*R*)-**2** [20%, related to (*R*)-**1**] and 1.04 g (Si*S*,C*R*)-**3** [20%, related to (*S*)-**1**]. Both compounds were obtained as viscous colourless liquids [(Si*S*,C*R*)-**3** crystallized upon cooling of a solution in n-pentane to −20 °C; mp < 20 °C]. The analytical properties (elemental analysis and spectroscopic data) of the biotransformation products were identical with those obtained from the chemically prepared racemates *rac*-(Si*R*,C*R*/Si*S*,C*S*)-**2** and *rac*-(Si*R*,C*S*/Si*S*,C*R*)-**3** (see above).

The enantiomeric purities of the biotransformation products were determined by ^{1}H NMR spectroscopic investigations of the respective MTPA esters as described above [(Si*R*,C*R*)-**2**: ⩾ 99% ee; (Si*S*,C*R*)-**3**: ⩾ 99% ee].

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