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Enantioselective stereoinversion of *sec*-alkyl sulfates by an alkylsulfatase from *Rhodococcus ruber* DSM 44541

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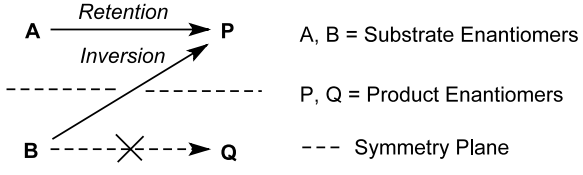
Abstract—Enantioselective biohydrolysis of *sec*-alkyl sulfate esters using a bacterial alkylsulfatase from *Rhodococcus ruber* DSM 44541 proceeded in a stereoselective fashion though *inversion* of configuration. Thus, from racemic substrates, the corresponding (*R*)-enantiomers were hydrolyzed selectively to furnish the corresponding *sec*-alcohol and non-reacted sulfate ester, both of (*S*)-configuration, which represents a *homochiral* product mixture. The enantioselectivities were found to depend on the substrate structure and were optimal for *sec*-sulfate esters in the ω-1 position (up to $E=21$). Since the enzyme was inactive on *prim*-sulfate esters, it can be classified as a *sec*-alkylsulfatase [EC 3.1.6.X].

1. Introduction

Driven by the demand to improve the economic balance of chemical processes, strategies are currently being sought which avoid the formation of unwanted stereoisomers derived from kinetic resolution of racemates.[1,2] A major topic among these so-called 'deracemization processes' is the development of enantioconvergent transformations (Scheme 1). The key principle of the latter is the enantioselective transformation of each individual substrate enantiomer (**A**, **B**) via individual *opposite* stereochemical pathways. Thus, for instance, whereas **A** is converted into **P** with *retention* of configuration, the reaction arrow from **B**→**P** crosses the plane of symmetry, and, as a consequence, *inversion* of configuration is achieved. As a consequence, the flow of both enantiomeric starting materials (**A**+**B**) converges at a single stereochemical product (**P**) and the occurrence of the unwanted stereoisomer **Q** is entirely avoided. Such processes, which show the advantage of a 100% theoretical yield of a single enantiomeric product from racemic starting material have been aptly denoted as 'enantioconvergent'.[3–5]



Scheme 1. Principle of enantioconvergent transformations.

From the plethora of enantioselective transformations available to date, the vast majority proceed with *retention* of configuration and thus can serve as a repertoire for transformations from **A**→**P**. In contrast, the crucial prerequisite for *inverting* processes (**B**→**P**) is a severe limitation, as the number of stereoselective reactions to chose from is very limited. In order to circumvent this bottleneck, we initiated a search for suitable biocatalysts, which are able to act through different stereochemical pathways - sulfatases.[6]

Sulfatases [EC 3.1.6.X] are a heterogenic group of enzymes, that catalyze the hydrolytic cleavage of the sulfate ester bond by liberating inorganic sulfate and the corresponding alcohol.[7] In terms of possible chiral recognition of racemic substrates, alkylsulfatases are prime candidates,[8] since they bear the potential for the kinetic resolution of *rac*-*sec*-alkyl sulfate esters or for the desymmetrization of bifunctional *meso*- or prochiral substrate analogues. Depending on the nature of the enzyme and its catalytic mechanism, enzymatic hydrolysis of *sec*-alkyl sulfates may proceed through *retention* or *inversion* of configuration at the stereogenic carbon atom; the latter is caused by the cleavage of the C–O bond in favor of the S–O bond and vice versa (Scheme 2).[9–11]

A small number of alkylsulfatases have been biochemically characterized,[8] but to date, these enzymes have

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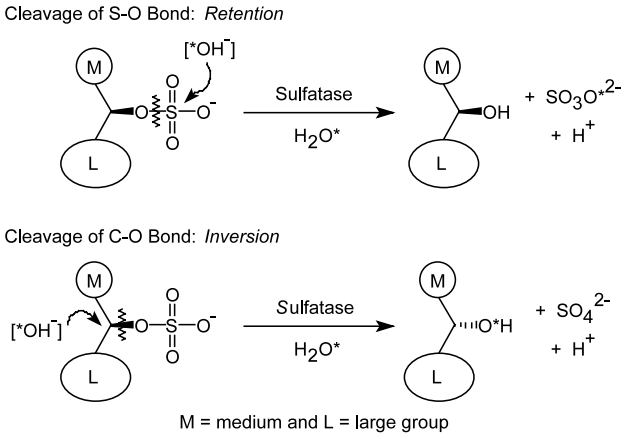


Scheme 2. Stereochemical course of enzymatic sulfate ester hydrolysis.

not been applied to preparative biotransformations.[12] In this study, we describe the use of an enantio- and stereoselective *sec*-alkylsulfatase from bacterial origin for the transformation of *rac*-*sec*-alkyl sulfates into homochiral products, i.e. the corresponding (*S*)-*sec*-alcohols and unreacted (*S*)-*sec*-sulfate ester.[13]

2. Results and discussion

2.1. Screening for alkylsulfatase activity

The existence of simple alkylsulfatases was first indicated through the chance observation of a bacterial contamination in a commercial shampoo preparation. During a more detailed study, a range of bacterial strains possessing alkylsulfatase activity were isolated from sewage sludge. The latter were identified on the basis of their ability to accept alkyl sulfates as the sole carbon source.[14] To date, the most detailed studies on alkylsulfatases were performed on Gram-negative bacteria, such as *Pseudomonas* C12B (NCIMB 11753 = ATCC 43648) and *Comanomas terrigena* (NCIMB 8193).[8] It was shown that—under appropriate culture conditions—*Pseudomonas* C12B produced up to five different alkylsulfatases, i.e. three *sec*- and two *prim*-alkylsulfatases.[15–17] In contrast, *Comamonas terrigena* produced only two *sec*-alkylsulfatases, irrespective of the culture conditions employed.[18,19] To date, alkylsulfatases have been mostly found in Gram-negative bacteria, the only exceptions are *Bacillus cereus*[20] and a coryneform sp. B1a.[21]

Our screening for alkylsulfatase activity was focused on *Actinomyces* spp. bearing in mind that they are exceptionally rich in their secondary metabolism. In addition, several fungal strains known for their flexible biotransformation activity were checked as well. All strains were grown on a standard medium in the absence of sulfate esters as inducers. Thus, lyophilized whole (resting) cells were tested for their ability to hydrolyze *rac*-2-octyl sulfate[22] as test substrate (Scheme 3). Cells of *Pseudomonas* C12B served as a biological standard to prove the validity of the experimental setup. The results (depicted in Table 1) were encouraging: Whereas the positive activity of *Pseudomonas* C12B proved that the experimental conditions were correct, the most active strains were found within *Actinomycetes* strains, in particular *Rhodococcus* spp. Besides the (expected) formation of 2-octanol **1b** and inorganic sulfate, several strains produced a certain amount of 2-octanone **1c** as side product due to biooxidation of the 2-octanol formed.[23] In contrast, *Streptomyces*, *Mycobacterium*, *Bacillus*, *Corynebacterium* and *Mycoplana* spp. showed no sulfatase activity, as well as all of the fungi tested.

2.2. Substrate tolerance, enantioselectivity and stereochemical pathway of *Rhodococcus* sulfatase RS2 from *Rhodococcus ruber* DSM 44541

The alkylsulfatase from the most promising strain showing good activity and selectivity—*R. ruber* DSM 44541—was investigated in more detail.

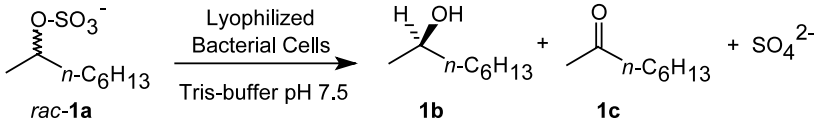
(i) The *sec*-alkylsulfatase activity was constitutive with respect to enzyme induction and was present in resting cells when grown on a complex medium in the absence of any sulfate ester which might serve as an inducer.

Table 1. Alkylsulfatase activities of Gram-positive whole bacterial cells

Strain	Relative activity[a]
Rhodococcus sp. NCIMB 11216	±
Rhodococcus erythropolis DSM 312[b]	+
R. ruber DSM 44539	++
R. ruber DSM 44540	++
R. ruber DSM 44541	++
R. ruber DSM 43338	+++
R. equi IFO 3730	+++
Rhodococcus sp. R312 (CBS 717.73)	+++
Pseudomonas C12B (NCIMB 11753)	+++

[a] Relative activity denoted within a range of '±' (low) to '+++' (excellent).

[b] Formerly classified as *Arthrobacter* sp. The following strains showed no activity: *Streptomyces lavendulae* ATCC 55209, *Bacillus megaterium* DSM 32, *Mycobacterium paraffinicum* NCIMB 10420, *Mycoplana rubra* R14 (FCC009), *Corynebacterium glutamicum* ATCC 13032, *Beauveria bassiana* ATCC 7159, *Cunninghamella blakesleeana* DSM 1806, *Helminthosporium* sp. NRRL 4671, *Mortierella alpina* ATCC 8979, *Mucor plumbeus* CBS 110.16, *Syncephalastrum racemosum* ATCC 18192.



Scheme 3. Screening for alkylsulfatase activity.

(ii) In whole cells, the activity could be preserved upon lyophilization in Tris–HCl buffer (pH 7.5, 10 mM) and could be maintained for several months in the absence of any stabilizer when stored at +4°C. This fact is particularly important in view of preparative-scale biotransformations.

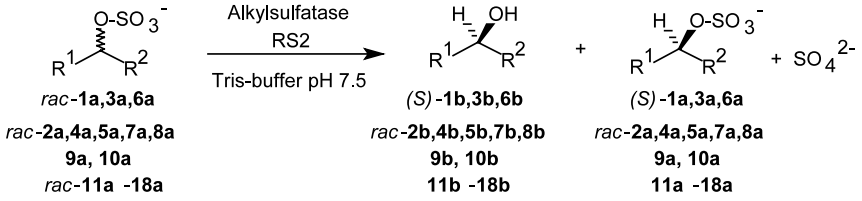
For all stereochemical studies, such as substrate-tolerance and enantioselectivities, as well as the stereochemical course of sulfate ester hydrolysis, the undesired oxidation observed when whole cells were used, had to be suppressed. This was accomplished by protein purification.[24] For all stereochemical investigations described below, a semi-purified alkylsulfatase preparation termed RS2—devoid of oxidation activity—was used.[25]

(iii) The stereochemical pathway of sulfate ester hydrolysis was found to proceed with *inversion* of the configuration of the stereogenic center. Thus, when enantiopure (*R*)-2- or (*R*)-3-octyl sulfate was used as substrate, (*S*)-(+)-2- and (*S*)-(+)-3-octanol, respectively, were obtained without racemization in >98% e.e.

(iv) In order to evaluate the biocatalytic activity for preparative biotransformations, the substrate tolerance and the respective enantioselectivities were investigated. For this purpose, a series of *rac-sec*-alkyl sulfate esters were synthesized and subjected to biohydrolysis (Scheme 4, Table 2). The following structural parameters of the substrate were investigated: (i) Overall chain length of ω-1 sulfates (*rac*-**1a**–**5a**), (ii) relative position of the sulfate group within the chain (*rac*-**6a**–**8a**, **9a**, **10a**), (iii) tolerance of branched (*rac*-**12a**) and cyclic substrates (*rac*-**15a**–**17a**), as well as (iv) functional groups (*rac*-**11a**, **13a**, **14a**), see Scheme 5. Compound *rac*-**18a** proved to be unsuitable as substrate due to its strong tendency towards spontaneous decomposition. This fact can be attributed to the presence of an activated sulfate group in the benzylic position.

The data shown in Table 2 reveal several trends:

Enzyme RS2 showed absolute regioselectivity for *rac-sec*-alkyl sulfates, which were readily accepted as substrates. In contrast, *prim*-sulfate esters **9a**,**10a** were not converted at all. Thus, the enzyme can be biochemically

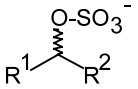


Scheme 4. Enantioselective biohydrolysis of sulfate esters using semipurified alkylsulfatase RS2.

Table 2. Relative activities and enantioselectivities of enzymatic sulfate ester hydrolysis

Substrate	R^1	R^2	Relative activity (%)[a]	Conversion (%)	E.e.$_p$ (%)	Enantioselectivity *E*
rac-**1a**	*n*-C_6H_{13}	CH_3	71	46	82	21
rac-**2a**	*n*-C_5H_{11}	CH_3	6	4	n.d.	n.d.
rac-**3a**	*n*-C_7H_{15}	CH_3	35	23	24	1.7
rac-**4a**	*n*-C_8H_{17}	CH_3	7	4	n.d.	n.d.
rac-**5a**	*n*-$C_{10}H_{21}$	CH_3	<1	<1	~0	n.d.
rac-**6a**	*n*-C_5H_{11}	C_2H_5	100	65	40	3
rac-**7a**	*n*-C_4H_9	*n*-C_3H_7	100	68	~0	~1
rac-**8a**	*n*-C_6H_{13}	*n*-C_3H_7	8	5	n.d.	n.d.
9a	*n*-C_7H_{15}	H	<1	<1	n.a.	n.a.
10a	*n*-$C_{11}H_{23}$	H	<1	<1	n.a.	n.a.
rac-**11a**	*n*-C_5H_{11}	$CH{=}CH_2$	74	48	<5	~1

[a] In relation to *rac*-**6a**,**7a** set as standard (100%); n.d. = not determined due to low conversion; n.a. = not applicable.



rac-**12a -18a**

Substrate	R^1	R^2
rac-**12a**	*n*-C_5H_{11}	$CH(CH_3)_2$
rac-**13a**	$(CH_2)_2CH{=}C(CH_3)_2$	CH_3
rac-**14a**	$(CH_2)_6$-Br	CH_3
rac-**15a**	*c*-C_6H_{11}	CH_3
rac-**16a**	$(CH_2)_2Ph$	CH_3
rac-**17a**	CH_2Ph	CH_3
rac-**18a**	Ph	CH_3

Scheme 5. Non-substrates for alkylsulfatase RS2.

denoted as a *sec*-alkylsulfatase [EC 3.1.6.X]. The relative reaction rates of *sec*-sulfates were shown to depend significantly on the overall chain length of the substrate: whereas medium-chain substrates of a total carbon number ranging from C_7 to C_9 were hydrolyzed with good rates, significantly reduced reaction rates were found for long-chain analogues (C_{10}–C_{12}).

The enantioselectivity - expressed as the enantiomeric ratio (*E* value[26]) - was optimal for substrate *rac*-**1a** ($E=21$) where the relative size of substituents R^1 and R^2 differs significantly. This fact has been shown to dominate the chiral recognition process in many biocatalyzed transformations.[27] In contrast, reduced enantioselectivities were observed when the functional group was gradually moved towards the center of the molecule, with R^1 and R^2 becoming similar in size to give near-symmetrical compounds *rac*-**6a** and *rac*-**7a**. In several of these cases, poor enantioselectivities could be dramatically improved by addition of so-called 'enhancers', such as metal ions, carbohydrates and detergents—i.e. components which are known to influence the chiral recognition process of enzymes.[28,29]

In general, the stereopreference was shown to be (*R*), i.e. (*R*)-configured substrate enantiomers were transformed with *inversion* of configuration to give the corresponding (*S*)-alcohols, while (*S*)-sulfate esters remained unreacted. As a consequence, a *homochiral* mixture of unreacted substrate and inverted product was formed from the racemic starting material.

The limitations of the substrate tolerance became clear with further structural variations: Branched substrates (*rac*-**12a**,**13a**) or derivatives bearing cyclic structural elements (*rac*-**15a**–**17a**), as well as derivatives bearing lipophilic functional groups, such as halogen moieties (*rac*-**14a**) were not converted at all. Although the unsaturated substrate *rac*-**11a** was well accepted, the enantioselectivity was poor.

In conclusion, an enantio- and stereoselective *sec*-alkylsulfatase acting with *inversion* of configuration was found in *R. ruber* DSM 44541, which allows the biocatalytic transformation of *rac*-*sec*-alkyl sulfates into homochiral (*S*)-configured products. This constitutes an important step en route to the deracemization of *sec*-alcohols via the stereo- and enantioselective biohydrolysis of their corresponding sulfate esters.

3. Experimental

3.1. General remarks

NMR spectra were recorded in MeOD using a Bruker AMX 360 at 360 (1H) and 90 (13C) MHz or a Bruker DMX Avance 500 at 500 (^{1}H) and 125 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) with $CHCl_3$ as internal standard [δ 7.23 (^{1}H) and 76.90 (^{13}C)], coupling constants (*J*) are given in Hz. GC analyses were carried out on a Varian 3800 gas chromatograph and on a HP 6890 gas chromatograph, both equipped with FID and either a HP 1301 (30 m×0.25 mm×0.25 μm film, N_2) or a HP-1 (30 m×0.53 mm×0.5 μm, N_2) capillary column. Enantiomeric purities were analyzed on columns Chrompack CP7500 (25 m×0.25 mm×25 μm; column A) and Chrompack Chirasil-Dex CB/G-PN, (30 m×0.32 mm, column B) using H_2 as carrier gas.

Alcohols were purchased from Aldrich (**1b**–**4b**, **8b**, **11b**, **13b**, **17**–**18b**), Fluka (**6**–**7b**, **9b**), Lancaster (**12b**, **16b**) or Merck (**5a**). 1-Bromooctan-7-ol (**14b**) was synthesized according to Yadav et al.[30] from *rac*-octane-2,8-diol, which was obtained by reduction of 7-oxooctanoic acid.[31] 1-Cyclohexylethanol (**15b**) was obtained through reduction of 1-cyclohexylethanone (Fluka) with $NaBH_4$ (MeOH, rt). Sodium dodecyl sulfate (**10a**) was used as received (Sigma). TLC analyses were run on silica gel Merck (F_{254}) and compounds were visualized by spraying with vanillin/H_2SO_4 conc. and heat treatment. For the biotransformations, lyophilized cells and partially purified enzyme RS2 were used, respectively. All strains were obtained from the respective culture collections, FCC stands for 'Fab-Crew-Collection'.

3.2. Synthesis of substrates

3.2.1. General procedure. Sodium alkyl sulfates **1a**–**17a** were prepared by sulfatation of the corresponding alcohols **1b**–**17b** using the triethylamine–SO_3 complex following a known procedure[32] with the following modifications. Sodium hydride (0.132 g, 5.52 mmol, 60% dispersion in mineral oil, washed with petroleum ether prior to use) was suspended in 2 ml dioxane under an atmosphere of argon. To this suspension, 2.48 mmol of *sec*-alcohol was slowly added dropwise through a septum using a syringe. The mixture was stirred at rt for 1 h. Sulfur trioxide triethylamine complex (0.5 g, 2.76 mmol) was dissolved in 4 ml of anhydrous dioxane with external warming by using a heatgun. This solution was subsequently added dropwise to the sodium alcoholate. After stirring overnight at rt, the reaction was quenched by addition of a few ml of dist. water. The solution was concentrated almost to dryness by rotary evaporation. The residue was diluted with ca. 15 ml of dest. water and extracted 5× with 10 ml of ethyl acetate. Water was removed by lyophilization, the remaining powder was dissolved in 30 ml methanol. After filtration, the organic solvent was evaporated under reduced pressure.

3.2.2. NMR data and yields for alkyl sulfates 1a–17a. ***rac*-2-Octyl sulfate**, **1a**: ^{1}H NMR (500 MHz, CD_3OD): 0.95 (3H, t, $J=6.9$ Hz), 1.34–1.69 (10H, m), 1.94 (3H, s), 4.45 (1H, m); ^{13}C NMR (CD_3OD): 11.4, 18.1, 20.7, 23.3, 27.3, 30.0, 35.1, 74.3 (C-2); 90% yield.

***rac*-2-Heptyl sulfate**, **2a**: ^{1}H NMR (500 MHz, CD_3OD): 0.96 (3H, t, $J=7$ Hz), 1.34–1.79 (8H, m), 1.94 (3H, s), 4.45 (1H, m); ^{13}C NMR: (CD_3OD) 13.7, 20.3, 22.9, 25.3, 32.2, 37.3, 76.6 (C-2); 85% yield.

***rac*-2-Nonyl sulfate**, **3a**: ^{1}H NMR (360 MHz, CD_3OD): 0.87 (3H, t, $J=6.8$ Hz), 1.27–1.65 (12H, m), 1.88 (3H,

s), 4.40 (1H, m); ^{13}C NMR (CD_3OD): 13.6, 20.3, 23.0, 25.6, 29.6, 29.9, 32.2, 37.4, 76.6 (C-2); 78% yield.

***rac*-2-Decyl sulfate**, **4a**: ^{1}H NMR (360 MHz, CD_3OD): 0.86 (3H, t, $J=6.8$ Hz), 1.25–1.47 (14H, m), 1.85 (3H, s), 4.38 (1H, m); ^{13}C NMR (CD_3OD): 13.7, 20.4, 23.0, 23.5, 25.6, 29.7, 30.0, 30.0, 32.3, 37.4, 76.6 (C-2); 70% yield.

***rac*-2-Dodecyl sulfate**, **5a**: ^{1}H NMR (500 MHz, CD_3OD): 0.87 (3H, t, $J=6.8$ Hz), 1.26–1.60 (18H, m), 1.87 (3H, s), 4.40 (1H, m); ^{13}C NMR: (CD_3OD) 11.6, 18.2, 20.9, 23.5, 27.6, 27.9 (4×C), 30.2, 35.3, 74.4 (C-2); 60% yield.

***rac*-3-Octyl sulfate**, **6a**: ^{1}H NMR (360 MHz, CD_3OD): 0.83–0.92 (6H, m), 1.28–1.40 (6H, m), 1.52–1.68 (4H, m), 4.25 (1H, m); ^{13}C NMR (CD_3OD): 9.0, 13.8, 22.9, 25.0, 27.1, 32.3, 33.8, 81.6 (C-3); 87% yield.

***rac*-4-Octyl sulfate**, **7a**: ^{1}H NMR (500 MHz, CD_3OD): 0.85–1.0 (6H, m), 1.25–1.5 (6H, m), 1.55–1.7 (4H, m), 4.3 (1H, m); ^{13}C NMR (CD_3OD): 13.6, 13.7, 18.4, 23.0, 27.4, 34.2, 36.7, 80.0 (C-4); 78% yield.

***rac*-4-Decyl sulfate**, **8a**: ^{1}H NMR (360 MHz, CD_3OD): 1.00–1.08 (6H, m), 1.43–1.58 (10H, m), 1.70–1.78 (4H, m), 4.47 (1H, m); ^{13}C NMR (CD_3OD): 11.2, 11.2, 16.0, 20.4, 22.8, 27.2, 29.7, 32.2, 34.4, 77.7 (C-2); 70% yield.

***rac*-1-Octyl sulfate**, **9a**: ^{1}H NMR (360 MHz, CD_3OD): 0.85 (3H, t, $J=7.0$ Hz), 1.26–1.35 (8H, m), 1.58–1.63 (2H, m), 1.85 (2H, m), 3.95 (2H, t, $J=6.6$ Hz); ^{13}C NMR (CD_3OD): 12.6 (C-8), 21.9, 25.1, 28.6 (3×C), 31.2, 67.4 (C-1); 96% yield.

***rac*-1-Octen-3-yl sulfate**, **11a**: ^{1}H NMR (360 MHz, CD_3OD): 1.04 (3H, t, $J=6.8$ Hz), 1.46–1.55 (6H, m), 1.83–1.87 (2H, m), 4.87 (1H, m), 5.27 (1H, d, $J=10.6$ Hz), 5.42 (1H, d, $J=17.1$ Hz), 6.0 (1H, m); ^{13}C NMR (CD_3OD): 11.1, 20.3, 22.3, 29.6, 33.0, 77.9 (C-3), 113.2 (C-1), 135,8 (C-2); 60% yield.

***rac*-2-Methyl-3-octyl sulfate**, **12a**: ^{1}H NMR (360 MHz, CD_3OD): 1.06 (9H, m), 1.45 (6H, m), 1.74 (2H, m), 2.2 (1H, m), 4.29 (1H, m); ^{13}C NMR (CD_3OD): 11.1, 14.9, 20.3, 20.9, 22.7, 28.2, 28.7, 29.8, 82.3 (C-3); 57% yield.

***rac*-6-Methyl-5-hepten-2-yl sulfate**, **13a**: ^{1}H NMR (360 MHz, CD_3OD): 1.27 (3H, d, $J=6.3$ Hz), 1.57 (3H, s), 1.62 (3H, s), 1.85–2.05 (4H, m), 4.40 (1H, m), 5.08 (1H, m); ^{13}C NMR (CD_3OD): 16.1, 19.3, 23.2, 24.2, 36.5, 75.4 (C-2), 123.4 (C-5), 130.8 (C-6); 80% yield.

***rac*-1-Bromo-7-octyl sulfate**, **14a**: ^{1}H NMR (360 MHz, CD_3OD): 1.27 (3H, d, $J=6.3$ Hz), 1.33–1.86 (10H, s), 3.32 (2H, m, obscured by solvent peak), 4.41 (1H, m); ^{13}C NMR (CD_3OD): 20.4, 25.4, 28.4, 29.0, 33.2, 33.7, 37.2, 76.4 (C-2); 80% yield.

***rac*-1-Cyclohexylethyl sulfate**, **15a**: ^{1}H NMR (500 MHz, CD_3OD): 1.33 (3H, d, $J=6.39$ Hz), 1.56–1.91 (11H, m), 2.71–2.79 (2H, m) 4.29 (1H, m); ^{13}C NMR (CD_3OD): 17.0, 26.5 (2×C), 26.9, 28.5, 28.9, 44.0, 80.3 (C-1); 95% yield.

***rac*-4-Phenyl-2-butyl sulfate**, **16a**: ^{1}H NMR (500 MHz, CD_3OD): 1.37 (3H, d, $J=6.27$ Hz), 1.85–1.93 (2H, m), 2.71–2.79 (2H, m) 4.52 (1H, m), 7.15–7.26 (5H, m); ^{13}C NMR (CD_3OD): 20.4, 31.8, 39.3, 76.2 (C-2), 126.0, 128.5 (2×C), 128.6 (2×C), 142.6; 80% yield.

***rac*-1-Phenyl-2-propyl sulfate**, **17a**: ^{1}H NMR (500 MHz, CD_3OD): 1.25 (3H, d, $J=6.15$ Hz), 1.95 (2H, m), 4.7 (1H, m), 7.22–7.30 (5H, m); ^{13}C NMR (CD_3OD): 19.3, 43.2, 76.8, 126.6, 128.5 (2×C), 130.0 (2×C), 138.2; 90% yield.

3.3. Biocatalytic procedures

3.3.1. Growth of strains. All bacteria strains were grown under aerobic conditions in baffled Erlenmeyer flasks at 30°C and 130 rpm using the following medium: 10 g/l glucose, 10 g/l peptone, 10 g/l yeast extract, 2 g/l NaCl, 1.5 g $MgSO_4 \cdot 7\ H_2O$, 1.3 g/l NaH_2PO_4 and K_2HPO_4. Cell growth was monitored by measurement of the optical density via the absorption at 546 nm.

3.3.2. Screening for biocatalytic activity. Lyophilised cells (50 mg) were rehydrated in Tris-buffer (pH 7.5, 0.1 M, 600 μl) and shaken for 1 h at 24°C. To the rehydrated cells, 200 μl of a 88 mM *rac*-2-octyl sulfate (*rac*-**1a**) stock solution (20.5 mg/ml, 0.1 M Tris, pH 7.5 buffer) was added and the vials were shaken for 5 days. Finally, 400 μl of the suspension were extracted with 400 μl of ethyl acetate. The reaction was monitored by TLC and GC. For GC-analysis, 200 μl of the organic phase was mixed with a stock solution of 5 μl (25 μl) menthol in ethanol [*c* 15.4 mg/ml (77 mg/ml)], which served as an internal standard. For each substrate, any spontaneous non-enzymatic hydrolysis was checked by blank-experiments in the absence of enzyme. With the exception of *rac*-**18a**, which was prone to decomposition, the degree of spontaneous hydrolysis was negligible.

3.3.3. Determination of conversion. The degree of conversion and relative enzyme activities were measured using 15 mM substrate in Tris buffer (0.1 M, pH 7.5). Enzyme solution [400 μl, partially purified enzyme solution after chromatography on a Q6 column or lyophilized enzyme powder, after chromatography on Phenyl Sepharose, resuspended in Tris buffer (0.1 M, pH 7.5)] was mixed with 400 μl of a 30 mM substrate solution. This mixture was shaken at 24°C at 130 rpm for 6 or 12 h, depending on the range of relative activity. An aliquot of 200 μl from the reaction mixture was extracted with 200 μl of ethyl acetate. After vigorous vortexing (30 s) and centrifugation (13.000 rpm, 5 min), 100 μl of the organic phase was mixed with a stock solution of 5 μl (25 μl) menthol in ethanol [*c* 15.4 mg/ml (77 mg/ml)], which served as an internal standard. The conversion was calculated from calibration curves for *rac*-2-, 3- and 4-octyl sulfate.

Table 3. GC retention times of enantiomeric trifluoroacetate or acetate ester derivatives of alcohols **1b**, **3b**, **6b**, **7b**, **11b** on GC using a chiral stationary phase

Compound	Column	Conditions	Retention time/min (configuration)
1b (-$COCF_3$)	A	14.5 psi H_2, 65°C (iso)	7.66 (*S*), 8.00 (*R*)
1b (-$COCF_3$)	B	10.0 psi H_2, 40°C (iso)	16.63 (*R*), 17.60 (*S*)
3b (-$COCH_3$)	B	10.0 psi H_2, 40°C (iso)	13.50, 13.70
6b (-$COCF_3$)	A	14.5 psi H_2, 60°C (iso)	7.65 (*S*), 8.08 (*R*)
7b (-$COCF_3$)	A	14.5 psi H_2, 55°C (iso)	7.84, 8.11
11b (-$COCH_3$)	B	10.0 psi H_2, 40°C (iso)	9.20, 9.85

3.3.4. Determination of e.e.$_p$. For the determination of the enantiomeric excess of alcohols formed during biohydrolysis, the latter had to be derivatized with trifluoroacetic anhydride (TFA) in order to achieve enantioseparation on a chiral GC-column. Thus, 400 μl samples from the enzyme reaction mixture were extracted with CH_2Cl_2 (500 μl) and dried over anhydrous sodium sulfate. After addition of TFA (40 μl), the capped vials were heated at 60°C for 20 min. After cooling to room temperature, the sample was extracted twice with 5% aqueous sodium bicarbonate solution (0.5 ml). After final drying of the organic phase over anhydrous sodium sulfate, the samples were subjected to GC analysis. Alternatively, alcohols were analyzed as their corresponding acetates (after derivatization with acetic anhydride). Thus, a 400 μl sample of the enzyme reaction mixture was extracted with ethyl acetate (400 μl) and dried over anhydrous sodium sulfate. After addition of acetic anhydride (80 μl) and cat. *p*-dimethylaminopyridine, the capped Eppendorff vials were shaken at room temperature overnight. Finally, the samples were extracted twice with water (0.5 ml). After drying the organic phase over anhydrous sodium sulfate, the samples were subjected to GC analysis. It should be noted that the relative elution order of trifluoroacetate versus acetate esters was reversed. For retention times see Table 3.

3.3.5. Elucidation of absolute configuration. The determination of the absolute configuration was performed by allocation of the peaks on GC using a chiral stationary phase via coinjection with an independent reference sample of the (*R*)- or (*S*)-alcohol, respectively. Retention times are listed in Table 3.

3.3.6. Elucidation of the stereochemical course of biohydrolysis. In order to determine the stereochemical pathway of enzymatic sulfate ester bond hydrolysis (i.e. S–O versus C–O bond cleavage), enantiomerically pure (*R*)-2- or (*R*)-3-octyl sulfate was used as a substrate, which was prepared from (*R*)-(−)-2-octanol (Fluka, e.e. 99%) and (*R*)-(−)-3-octanol, respectively, as described for *rac*-**1a**. GC-Analysis on a chiral stationary phase revealed that (*S*)-(+)-2- and (*S*)-(+)-3-octanol, respectively, was the only product from the enzymatic hydrolysis.

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