Biocatalytic Reduction of 2-Monosubstituted 3-Thiazolines Using Imine Reductases

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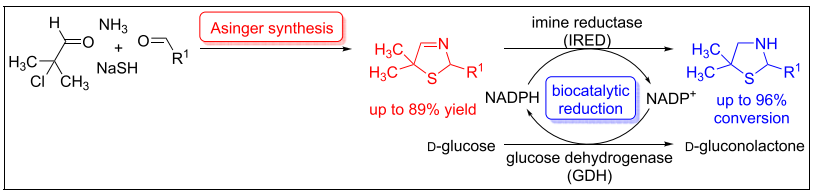

The application of a straightforward biocatalytic technology for the reduction of racemic 2-monosubstituted 3-thiazolines, which are easily prepared *via* Asinger-multicomponent reaction, is reported. The biocatalytic reduction yields racemic 2-monosubstituted 3-thiazolidines, which are difficult to be prepared by means of classic chemical routes, in moderate to high yields. Moreover, our study clarifies the stereochemical reaction course of the biocatalytic reduction. Furthermore, the efficiency of this biocatalytic technology is demonstrated in an experiment at an elevated substrate concentration of 60 mM leading to 96% conversion.

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INTRODUCTION

The 3-thiazolidine scaffold represents an important heterocyclic structural motif in both medicinal chemistry and agrochemistry [1–5]. Furthermore, this heterocycle is widely present in natural products and is a key structure in penicillins as presumably most prominent example. For their preparation, a range of methods are conceivable. As 3-thiazolines are readily accessible *via* Asinger-synthesis starting from cheap commodity chemicals [6–9], their reduction appears as an attractive retrosynthetic route (Scheme 1). For decades, however, the realization of such an approach remained a challenge because numerous established methods for C=N double bond reduction failed due to, for example, undesired side-reactions and catalyst deactivation by sulfur [9–12]. Very recently, we could demonstrate that these hurdles could be overcome when applying enzyme catalysis. In detail, 3-thiazolines have been efficiently converted into the corresponding 3-thiazolidines when utilizing imine reductases (IREDs) as biocatalysts and glucose as reducing agent in combination with a glucose dehydrogenase (GDH) for *in situ* regeneration of the cofactor NADPH [12]. Stereochemical aspects have been studied as well and when starting from prochiral 3-thiazolines as substrates, high enantioselectivities are obtained [12].

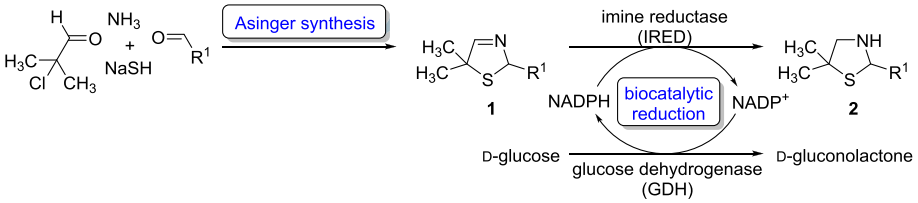
A remaining open question, however, is related to the reduction of 2-monosubstituted 3-thiazolines of type **1** (Scheme 1). As such molecules are racemic when being prepared *via* Asinger-synthesis, in the presence of enzymes as chiral catalysts both enantiomeric recognition and non-selective conversions are conceivable options. Notably, both options can be synthetically useful for various purposes. In the following, we report an approach towards a straightforward synthesis of 2-monosubstituted 3-thiazolidines. Moreover, our study gives an insight into the clarification of the stereochemical research issue towards recognition of the absolute configuration of the stereogenic center in 2-position.

RESULTS AND DISCUSSION

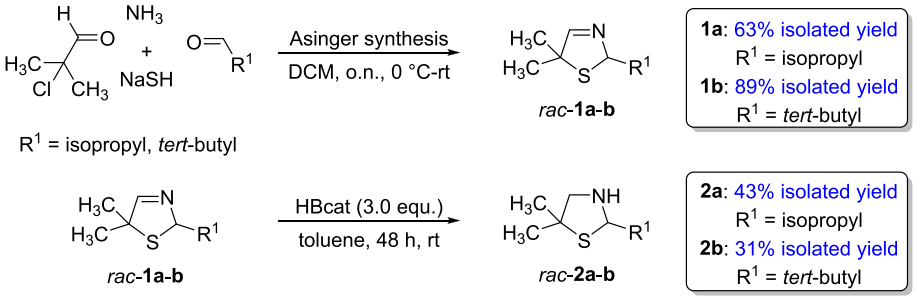
In the first step, two 2-monosubstituted 3-thiazolines serving as model substrates for this class of compounds were prepared through modified Asinger-synthesis starting from preformed 2-chloroisobutyraldehyde, sodium hydrosulfide, ammonia, and either isobutyraldehyde or pivaldehyde. Following a literature-known procedure [8,11,13], the 2-monosubstituted 3-thiazolines *rac*-**1a** and *rac*-**1b** bearing either an isopropyl or a *tert*-butyl substituent were obtained in yields of 63% and 89%, respectively (Scheme 2). In the next step, the racemic 3-thiazolidines (*rac*-**2a–b)** were prepared by chemical reduction using catecholborane (HBcat) as reference compounds for analytical purpose (Scheme 2). In accordance with previous work [11,12], low yields were obtained for *rac*-**2a–b** (43% and 31%), thus underlining the difficulty for the selective reduction of 3-thiazolines **1** with established reducing agents such as metal hydrides.

With the starting materials and reference compounds in hand, analytical methods for determining the conversion

Scheme 1. Conceptual approaches towards 3-thiazolidines **2** by combination of Asinger-synthesis and enzymatic C=N reduction using imine reductases. [Color figure can be viewed at wileyonlinelibrary.com]



Scheme 2. Synthesis of 2-monosubstituted 3-thiazolines **1** and their corresponding 3-thiazolidine derivatives **2** *via* Asinger-synthesis and subsequent reduction. [Color figure can be viewed at wileyonlinelibrary.com]

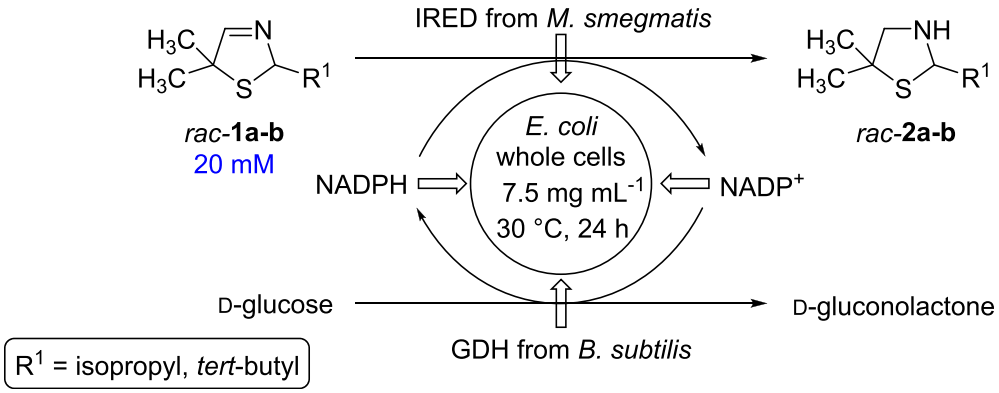


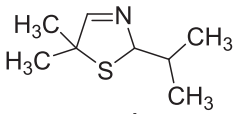
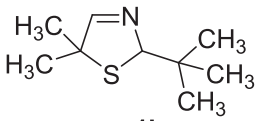
(by achiral GC) and enantioselectivity (by chiral SFC-HPLC) were established (see Experimental section). Attracted by the recent successful utilization of the IRED from *Mycobacterium smegmatis* [14] for the reduction of a range of prochiral 3-thiazolines [12], we next conducted synthetic experiments at substrate concentrations of 20 mM *rac*-**1a** and *rac*-**1b** in order to get an insight into the suitability of this IRED to form the desired products. Towards this end, we used a tailor-made recombinant whole-cell biocatalyst [12], which contains the IRED from *M. smegmatis* and the GDH from *Bacillus subtilis* for *in situ* recycling of the cofactor NADPH (utilized in catalytic amount) using D-glucose as a co-substrate (Table 1). We were pleased to find that the racemic 3-thiazolines *rac*-**1a** and *rac*-**1b** were converted to the corresponding 3-thiazolidines **2** in the presence of the whole-cell catalyst (7.5 mg/mL lyophylized whole-cells) after 24 h of reaction time at 30°C. Even under non-optimized reaction conditions *rac*-**1a** was converted with a good conversion of 77%, whereas conversion of *rac*-**1b** was lower with 21% under the same reaction conditions (Table 1). We assume that the lower conversion towards reduction of *rac*-**1b** is due to the more sterically demanding *tert*-butyl substituent at R^1 compared with the isopropyl substituent.

After this proof of concept for the usefulness of IREDs to synthesize 2-substituted 3-thiazolidines **2** through reduction of the corresponding 3-thiazolines, we wanted to get an insight into the stereoselectivity of this biocatalytic reduction. In principle, both an enantioselective and a non-enantioselective reaction course are of preparative interest because in the first case, a racemic resolution is conceivable (albeit at a maximum yield of 50% for each enantiomer), whereas the latter case enables an elegant route with (theoretically) full conversion to racemic 3-thiazolidines **2** (which are difficult to form by means of other routes). Utilizing chiral supercritical fluid chromatography-high performance liquid chromatography (SFC-HPLC), we analyzed products **2a** and **2b** after derivatization and found that both enantiomers are present to the same extent and the enantiomeric excess is therefore 0% *ee* (Table 1). However, it should be added that obtaining a racemate does not necessarily mean a non-enantioselective reduction as the stereogenic center is an acetal-type carbon which might be prone to racemization in particular in case of the saturated 3-thiazolidines (e.g., due to reversible ring cleavage and formation). Although taking into account the neutral reaction conditions such an effect is unlikely, we wanted to clarify this issue. Therefore, the enantiomeric excess of the remaining starting material had to be determined. Because it was not possible to separate the enantiomers of *rac*-**1a** and *rac*-**1b** by means of chiral GC, we performed a biotransformation of *rac*-**1b** at an elevated substrate concentration of 60 mM, using 5 mg/mL of IRED cell crude extract from *M. smegmatis* and stopped the biotransformation at a low conversion of 6%. After separation of the remaining starting material and product by flash column chromatography, the remaining starting material was used as starting material in a non-

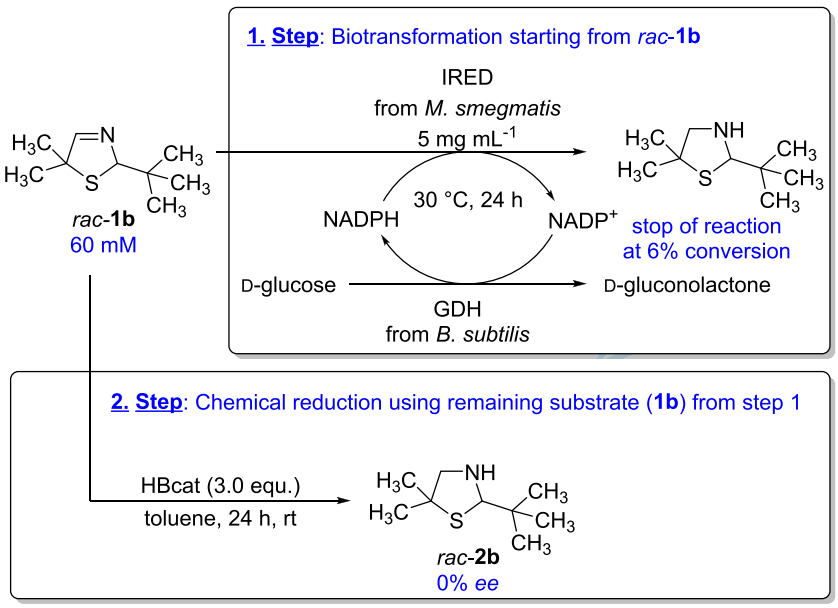
Table 1

Initial biotransformation of 2-monosubstituted 3-thiazolines *rac*-**1a**–**b**.

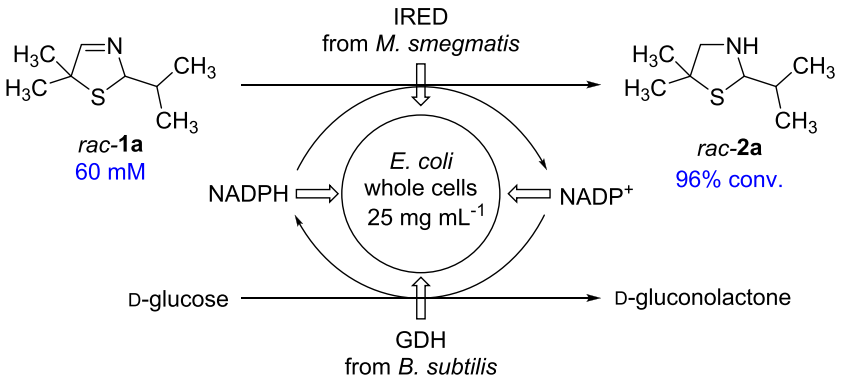


Entry	Substrate	Conversion [%]	*ee* of product [%]
1	 *rac*-**1a**	77	0
2	 *rac*-**1b**	21	0

Scheme 3. Biotransformations and subsequent chemical reductions of remaining starting material **1b** for clarification of the stereochemical reaction course. [Color figure can be viewed at wileyonlinelibrary.com]



Scheme 4. Process development and optimized biotransformation for the racemic 3-thiazoline *rac*-**1a**. [Color figure can be viewed at wileyonlinelibrary.com]



enantioselective chemical reduction using catecholborane. The corresponding 3-thiazolidine **2b** was then analyzed by means of chiral SFC-HPLC, after derivatization with phenylisocyanate (Scheme 3). As a result, we found the same ratio of both enantiomers of **2b**, corresponding to an enantiomeric excess of 0% *ee*. This result indicates that the IRED from *M. smegmatis* catalyzes the reduction of *rac*-**2b** in a non-enantioselective fashion regarding the chiral center at C2, allowing a (theoretically) full conversion towards the racemic 3-thiazolidine due to the recognition of both enantiomers of the substrate *rac*-**1b**. Thus, we assume that this stereochemical behavior is general also when using other 2-substituted 3-thiazolines in the presence of the IRED from *M. smegmatis*.

After having characterized the enzymatic reaction in terms of activity and conversion as well as enantioselectivity, we were further interested to extend this reaction towards an efficient synthetic transformation. Thus, an initial biotransformation experiment at an elevated substrate concentration of 60 mM *rac*-**1a** was carried out (Scheme 4). As a biocatalyst, again the tailor-made recombinant whole cell biocatalyst [12] containing both required enzymes (IRED and GDH) in overexpressed form was utilized. After 65 h of reaction time at 30°C, we were pleased to find that the reduction of *rac*-**1a** proceeds smoothly, leading to the formation of the desired 3-thiazolidine *rac*-**2a** with an excellent conversion of 96% (Scheme 4).

CONCLUSION

The successful application of a biocatalytic reduction technology using IREDs towards the reduction of racemic 2-monosubstituted 3-thiazolines is reported, yielding the corresponding 3-thiazolidines in moderate to high conversions. Furthermore, an insight into the stereochemical reaction course is provided revealing the absence of an enantiopreference of the biocatalyst for one of the enantiomers of the 3-thiazoline substrates of type **1** and formation of the desired 3-thiazolidine products as a racemate. In addition, the efficiency of this type of biotransformation has been demonstrated by means of an experiment at a 10 mL scale and at a substrate concentration of 60 mM, leading to the formation of the desired product with 96% conversion.

EXPERIMENTAL

General information. The commercially available reagents (Acros, Alfa Aesar, Merck, VWR, Acros, Fisher Scientific, TCI) were used without further purification. Solvents were used in high-grade purity or purified prior to use. NMR spectra were recorded on Bruker Advance III 500 or Bruker Advance III 500HD at a frequency of 500 MHz (^{1}H) or 126 MHz (^{13}C). The chemical shift δ is given in ppm and referenced to the corresponding solvent signal ($CDCl_3$). Coupling constants (*J*) are given in Hz. Nano-ESI mass spectra were recorded using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a standard nano-ESI source. Samples were introduced by static nano-ESI using in-house pulled glass emitters. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a Bruker nitrogen generator NGM 11. Helium served as cooling gas for the ion trap and collision gas for MS^n experiments. HRMS-ESI mass spectra are recorded using an Agilent 6220 time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) in extended dynamic range mode equipped with a Dual-ESI source, operating with a spray voltage of 2.5 kV. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a nitrogen generator NGM 11. Samples are introduced with a 1200 HPLC system consisting of an autosampler, degasser, binary pump, column oven, and diode array detector (Agilent Technologies, Santa Clara, CA, USA) using a C18 Hypersil Gold column (length: 50 mm, diameter: 2.1 mm, particle size:

1.9 μm) with a short gradient (in 4 min from 0% B to 98% B, back to 0% B in 0.2 min, total run time 7.5 min) at a flow rate of 250 μL/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9% water, 5% acetonitrile and 0.1% formic acid, solvent B of 5% water, 94.9% acetonitrile and 0.1% formic acid. The mass axis was externally calibrated with ESI-L Tuning Mix (Agilent Technologies, Santa Clara, CA, USA) as calibration standard. EI mass spectra were recorded using an Autospec X magnetic sector mass spectrometer with EBE geometry (Vacuum Generators, Manchester, UK) equipped with a standard EI source. Samples were introduced by push rod in aluminum crucibles if not otherwise noted. Ions were accelerated by 8 kV in EI mode. Flash column chromatography was performed using Biotage "Isolera One" flash chromatography system with cyclohexane/ethyl acetate mixtures.

Recombinant expression of imine reductases. The IREDs from *M. smegmatis* [14] is recombinantly expressed in *Escherichia coli* (*E. coli*) BL21(DE3), according to a literature-known procedure [12]. A preculture (10 mL LB medium, containing 100 μg/mL of carbenicillin) of *E. coli* BL21(DE3) carrying the recombinant plasmid is cultivated overnight at 37°C. The main culture (300 mL TB medium, containing 100 μg/mL of carbenicillin) is inoculated with the preculture to a final concentration of 1%. The production of recombinant protein is induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) (final concentration 0.5 mM) at an OD_{600} between 0.4 and 0.6. Cultures are shaken at 25°C for 20 h and 140 rpm and harvested by centrifugation at 4000 *g* and 4°C for 30 min. Cell pellets are stored at −20°C. Cell disruption is performed after resuspension of the cells (3 mL 50 mM KP_i pH 7 per gram wet cell weight) by sonification (Bandelin Sonopuls HD 2070) with 3 × 120 s burst (5 cycles, 20% energy) and 120 s intervals and both steps being carried out on ice. After centrifugation at 21,000 *g* for 30 min at 4°C, the crude extract is obtained as supernatant. The protein concentration is determined *via* the Bradford assay against BSA as a concentration standard.

Construction and preparation of whole cell catalyst. The whole cell catalyst is constructed and prepared according to reference [12].

2-Chloro-2-methylpropanal (according to [12]). Sulfuryl chloride (41.0 mL, 0.50 mol) is added under cooling at a vacuum of 900 mbar to isobutanal (46.0 mL, 0.50 mol), keeping the temperature stable at 40°C. The reaction mixture is stirred afterwards for 2 h at 45°C and 900 mbar. 2-Chloro-2-methylpropanal (44.4 g, 0.42 mol, 83%) is obtained as colorless liquid by fractional distillation (boiling point 90°C). **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 9.43 (s, 1H, C1-**H**), 1.64 (s, 6H, C2(C**H$_3$**)$_2$). **^{13}C NMR** (126 MHz, $CHCl_3$): δ [ppm] = 195.32 (**C**1), 69.64 (**C**2(CH$_3$)$_2$), 26.20 (C2(**C**H$_3$)$_2$). **MS (EI)** *m/z* calculated for C_4H_7ClO $[M]^+$: 106.02, found: 106.0. The analytical data correspond with literature data [12].

General procedure 1 for the synthesis of 3-thiazolines (according to references [11,13]). Sodium hydrosulfide monohydrate (0.10 mol), ammonia-solution (13.3 M, 22.5 mL), and aldehyde (0.10 mol) are mixed and cooled to 0°C. α-Chlorinated aldehyde (0.10 mol) is dissolved in dichloromethane (100 mL) and added to the yellow mixture, keeping the temperature under 10°C. The reaction mixture is stirred overnight at room temperature, phases are separated, and the aqueous phase is extracted with dichloromethane (3 × 30 mL). The combined organic phases are dried over magnesium sulfate; the solvent is evaporated *in vacuo*; and the crude product is purified by flash column chromatography.

***rac*-2-Isopropyl-5,5-dimethyl-3-thiazoline (*rac*-1a).** *rac*-**1a** is prepared according to general procedure **1** using sodium hydrosulfide monohydrate (14.8 g, 200 mmol), ammonia-solution (13.3 M, 30 mL), and isobutanal (18.2 mL, 200 mmol), yielding *rac*-**1a** (19.8 g, 126 mmol, 63%) as colorless oil after flash column chromatography (5–28% ethyl acetate in cyclohexane). **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 0.93 (d, *J* = 6.7 Hz, 3H, C2-CH(C**H$_3$**)$_2$), 1.02 (d, *J* = 6.8 Hz, 3H, C2-CH(C**H$_3$**)$_2$), 1.52 (s, 3H, C5-(C**H$_3$**)$_2$), 1.53 (s, 3H, C5-(C**H$_3$**)$_2$), 2.13–2.09 (m, 1H, C$_2$-C**H**(CH$_3$)$_2$), 5.62–5.61 (m, 1H, C2-**H**), 7.03 (d, *J* = 2.6 Hz 1H, C4-**H**). **^{13}C NMR** (126 MHz, $CDCl_3$): δ [ppm] = 18.3 (C2-CH(**C**H$_3$)$_2$), 19.9 (C2-CH(**C**H$_3$)$_2$), 29.3 (C5-(**C**H$_3$)$_2$), 29.7 (C5-(**C**H$_3$)$_2$), 34.8 (C2-**C**H), 63.5 (**C**5), 90.8 (**C**2), 168.4 (**C**4). **HRMS (ESI)** *m/z* calculated for $C_8H_{16}NS$ $[M + H]^+$: 158.0998, found: 158.0994. **IR** (neat) [cm^{-1}]: 2958, 2924, 2870, 1650, 1362.

***rac*-2-(*tert*-Butyl)-5,5-dimethyl-3-thiazoline (*rac*-1b).** *rac*-**1b** is prepared according to general procedure **1** using sodium hydrosulfide monohydrate (11.1 g, 0.10 mol), ammonia-solution (13.3 M, 22.5 mL), and 2,2-dimethylpropanal (16.3 mL, 100 mmol), yielding *rac*-**1b** (15.3 g, 89.0 mmol, 89%) as colorless oil after flash column chromatography (5–40% ethyl acetate in cyclohexane). **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 0.99 (s, 9H, C2-C(C**H$_3$**)$_3$), 1.52 (s, 3H, C5-(C**H$_3$**)$_2$), 1.53 (s, 3H, C5-(C**H$_3$**)$_2$), 5.56 (d, *J* = 2.7 Hz, 1H, C2-**H**), 7.03 (d, *J* = 2.7 Hz, 1H, C4-**H**). **^{13}C NMR** (126 MHz, $CDCl_3$): δ [ppm] = 26.7 (C2-C (**C**H$_3$)$_3$), 29.0 (C5-(**C**H$_3$)$_2$), 29.7 (C5-(**C**H$_3$)$_2$), 36.1 (C2-**C**(CH$_3$)$_3$), 63.5 (**C**5), 95.2 (**C**2), 168.5 (**C**4). **MS (ESI)** *m/z* calculated for $C_9H_{18}NS$ $[M + H]^+$: 171.11, found: 172.00. The analytical data correspond with literature data [15].

General procedure 2 for the reduction of 3-thiazolines using catecholborane (according to [11]). 3-Thiazoline (15.0 mmol) is dissolved in toluene (60 mL), and

catecholborane (45.0 mmol), dissolved in toluene (15 mL), is added slowly under argon atmosphere at room temperature. The reaction mixture is stirred at room temperature for 48 h; distilled water (dH_2O) (60 mL) is carefully added; the phases are separated; and the organic phase is extracted with sodium hydroxide solution (2 M, 3×). The combined organic phases are washed with brine, dried over magnesium sulfate, and the solvent is evaporated *in vacuo*. The crude product is purified *via* column chromatography.

***rac*-2-Isopropyl-5,5-dimethyl-3-thiazolidine (*rac*-2a).** *rac*-**2a** is prepared according to general procedure **2** using *rac*-**1a** (2.00 g, 12.7 mmol). *rac*-**2a** (0.94 g, 5.43 mmol, 43%) is obtained as colorless oil after purification *via* flash column chromatography (5–40% ethyl acetate in cyclohexane). **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 1.00 (d, *J* = 6.7 Hz, 3H, C2-CH(CH_3)$_2$), 1.05 (d, *J* = 6.7 Hz, 3H, C2-CH(CH_3)$_2$), 1.36 (s, 3H, C5-(CH_3)$_2$), 1.48 (s, 3H, C5-(CH_3)$_2$), 1.86–1.93 (m, 1H, C2-C**H**(CH_3)$_2$), 2.77 (d, *J* = 12.3 Hz, 1H, C4-**H**$_2$), 3.02 (d, *J* = 12.3 Hz, 1H, C4-**H**$_2$), 4.51 (d, *J* = 7.2 Hz, 1H, C2-**H**). **^{13}C NMR** (126 MHz, $CHCl_3$): δ [ppm] = 20.8 (C2-CH(**C**H_3)$_2$), 21.2 (C2-CH(**C**H_3)$_2$), 29.9 (C5-(**C**H_3)$_2$), 32.5 (C5-(**C**H_3)$_2$), 35.1 (C2-**C**H (CH_3)$_2$), 57.1 (**C**5), 66.6 (**C**4), 80.9 (**C**2). **HRMS (ESI)** *m/z* calculated for $C_8H_{18}NS$ [M + H]$^+$: 160.1155, found: 160.1157. **IR** (neat) [cm^{-1}]: 2954, 2921, 2863, 1453, 1364.

***rac*-2-(*tert*-Butyl)-5,5-dimethyl-3-thiazolidine (*rac*-2b).** *rac*-**2b** is prepared according to general procedure **2** using *rac*-**1b** (2.51 g, 15.0 mmol), yielding *rac*-**2b** (0.80 g, 4.62 mmol, 31%) as colorless oil after purification *via* flash column chromatography (5–40% ethyl acetate in cyclohexane). **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 1.02 (s, 9H, (C2-C(**C**H_3)$_3$), 1.32 (s, 3H, C5-(**C**H_3)$_2$), 1.48 (s, 3H, C5-(**C**H_3)$_2$), 2.77 (d, *J* = 12.2 Hz, 1H, C4-**H**$_2$), 3.03 (d, *J* = 12.2 Hz, 1H, C4-**H**$_2$), 4.63 (s, 1H, C2-**H**).**^{13}C NMR** (126 MHz, $CHCl_3$): δ [ppm] = 27.2 (C2-C(**C**H_3)$_3$), 29.2 (C5-(**C**H_3)$_2$), 31.7 (C5-(**C**H_3)$_2$), 34.5 (C2-**C**(CH_3)$_3$), 56.1 (**C**5), 66.4 (**C**4), 84.3 (**C**2). **HRMS (ESI)** *m/z* calculated for $C_9H_{20}NS$ [M + H]$^+$: 174.1311, found: 174.1306. **IR** (neat) [cm^{-1}]: 2952, 2924, 2863, 1454, 1363.

Achiral gas chromatography analysis. Gas chromatography analysis (determination of the conversions for the biotransformations of 3-thiazolines [*rac*-**1a**–**b**] to the corresponding 3-thiazolidines [*rac*-**2a**–**b**]) is carried out using the gas chromatograph system GC-2010 Plus (Shimadzu, Kyoto, Japan) equipped with ZB-5MSi column (Phenomenex, 30 m × 0.25 mm × 0.25 µm; N_2; linear velocity 46.9 cm/s split mode 1:10; total flow 28.8 mL/min; purge flow 3.0 mL/min; column flow 2.34 mL/min; pressure 140.4 kPa) and coupled to an AOC-20i/s auto injector/auto sampler. Temperature program: 40–200°C, 10°C/min. t_R (*rac*-**1a**): 5.5 min; t_R (*rac*-**2a**): 6.1 min; t_R (*rac*-**1b**): 6.0 min; t_R (*rac*-**2b**): 6.6 min.

General procedure 3 for derivatization of 3-thiazolidines (according to [11,12]). 3-Thiazolidine (1.25 mmol) is dissolved in diethylether (10 mL), phenylisocyanate (1.30 mmol), and cyclohexane (5 mL) are added, and the reaction mixture is stirred overnight while the solvent is evaporated in the meantime. The white solid is dried *in vacuo*.

***rac*-2-Isopropyl-5,5-dimethyl-*N*-phenylthiazolidine-3-carboxamide (*rac*-3a).** Preparation according to general procedure **3** using *rac*-**2a** (50.0 mg, 0.29 mmol), yielding *rac*-2-isopropyl-5,5-dimethyl-*N*-phenylthiazolidine-3-carboxamide (80.0 mg, 0.29 mmol, 99%) as colorless solid. **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 0.98 (d, *J* = 4.4 Hz, 3H, C2-C(C**H**$_3$)$_2$), 0.99 (d, *J* = 4.4 Hz, 3H, C2-C(C**H**$_3$)$_2$), 1.41 (s, 3H, C5-(C**H**$_3$)$_2$), 1.43 (s, 3H, C5-(C**H**$_3$)$_2$), 2.19–2.26 (m, C2-C**H**(CH_3)$_2$), 3.26 (d, *J* = 11.2 Hz, 1H, C4-**H**$_2$), 4.04 (d, *J* = 11.2 Hz, 1H, C4-**H**$_2$), 5.30 (d, *J* = 6.6 Hz, 1H, C2-**H**), 7.04–7.07 (m, 1H, Ar–**H**), 7.28–7.30 (m, 2H, Ar–**H**), 7.35–7.37 (m, 2H, Ar–**H**). **^{13}C NMR** (126 MHz, $CHCl_3$): δ [ppm] = 17.6 (C2-CH(**C**H_3)$_2$), 20.1 (C2-CH(**C**H_3)$_2$), 26.5 (C5-(**C**H_3)$_2$), 27.2 (C5-(**C**H_3)$_2$), 34.7 (C2-**C**H(CH_3)$_2$), 52.4 (**C**5), 62.1 (**C**4), 70.5 (**C**2), 120.2 (Ar–**C**), 123.7 (Ar–**C**), 129.3 (Ar–**C**), 138.9 (Ar–**C**), 154.6 (**C**=O). **HRMS (ESI)** *m/z* calculated for $C_{15}H_{23}N_2OS$ [M + H]$^+$: 279.1526, found: 279.1521. **IR** (neat) [cm^{-1}]: 2958, 2917, 1640, 1442, 1376. T_m: 164°C.

***rac*-2-(*tert*-Butyl)-5,5-dimethyl-*N*-phenylthiazolidine-3-carboxamide (*rac*-3b).** Preparation according to general procedure **3** using *rac*-**2a** (104 mg, 0.58 mmol), yielding *rac*-2-(*tert*-butyl)-5,5-dimethyl-*N*-phenylthiazolidine-3-carboxamide (169 mg, 0.578 mmol, 98%) as colorless solid. **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 1.02 (s, 9H, C2-C(C**H**$_3$)$_3$), 1.37 (s, 3H, C5-(C**H**$_3$)$_2$), 1.47 (s, 3H, C5-(C**H**$_3$)$_2$), 3.24 (d, *J* = 11.7 Hz, 1H, C4-**H**$_2$), 4.02 (d, *J* = 11.7 Hz, 1H, C4-**H**$_2$), 5.48 (s, 1H, C2-**H**), 7.05–7.07 (m, 1H, Ar–**H**), 7.28–7.35 (m, 4H, Ar–**H**). **^{13}C NMR** (126 MHz, $CHCl_3$): δ [ppm] = 26.1 (C5-(**C**H_3)$_2$), 26.8 (C2-C(**C**H_3)$_3$), 31.1 (C5-(**C**H_3)$_2$), 38.8 (C2-C(**C**H_3)$_3$), 53.2 (**C**5), 63.5 (**C**4), 73.7 (**C**2), 120.3 (Ar–**C**), 123.8 (Ar–**C**), 129.4 (Ar–**C**), 139.0 (Ar–**C**), 156.4 (**C**=O). **HRMS (EI)** *m/z* calculated for $C_{16}H_{25}N_2OS$ [M + H]$^+$: 293.1682, found: 293.1676. **IR** (neat) [cm^{-1}]: 3307, 2968, 2949, 1750, 1632, 1539, 1520. T_m: 162°C.

Chiral supercritical fluid chromatography-high performance liquid chromatography analysis. Chiral SFC-HPLC analysis (for determination of the enantiomeric excess of *rac*-**2a** and *rac*-**2b** after derivatization with phenylisocyanate to *rac*-**3a**/*rac*-**3b**) is performed using the LC2000 SFC-HPLC system from Jasco (Easton, USA) with the HPLC column Chiralpak

IC from Daicel (Tokyo, Japan) (supercritical CO_2:EtOH (Et_2NH) = 90:10 (0.01), 1 mL/min, 20°C, 10 MPa, 210 nm). Enantiomeric excess is determined based on area% of the enantiomers. t_R (*rac*-**3a**): 9.5 min, 12.9 min; t_R (*rac*-**3b**): 8.6 min, 10.2 min.

General procedure for biotransformations of 2-monosubstituted 3-thiazolines. Biotransformations of 2-monosubstituted 3-thiazolines *rac*-**1a**–**b** are performed on a 10 mL scale at 30°C and 1000 rpm in 100 mM KP_i buffer pH 7, containing 2% of methanol as co-solvent, 40 mM D-glucose, 20 mM *rac*-**1a**–**b**, 7.5 mg/mL of lyophilized whole cell catalyst (which is prepared from a 14 mg/mL of cell suspension in 200 mM KP_i buffer pH 7) and 0.1 mM $NADP^+$. After 24 h, the reaction is stopped by adding 1 mL of 32% NaOH solution and 10 mL of dichloromethane. Phase separation is promoted by centrifugation. The conversions are determined by analyzing the organic phase by achiral GC, and the enantiomeric excess is determined by means of chiral SFC-HPLC, after derivatization of the samples with phenylisocyanate according to general procedure 3.

Biotransformation of *rac*-1b and chemical reduction for clarification of the stereochemical reaction course. The biotransformation for the clarification of the sterochemical reaction course (10 mL) is performed from a 60 mM concentration of *rac*-**1b**, 5.7 mg/mL of IRED crude extract from *M. smegmatis*, 280 U of GDH, 120 mM of D-glucose, 0.1 mM of $NADP^+$, and 2% of methanol as co-solvent in distilled water. The reaction mixture is stirred at 30°C for 23.5 h. The reaction is stopped by adding 1 mL of 32% NaOH solution and 10 mL of dichloromethane. Phase separation is promoted by centrifugation. The organic phase is dried over magnesium sulfate. The conversions are determined by analyzing the organic phase by achiral GC. The solvent is evaporated *in vacuo*, product and remaining starting material *rac*-**1b** are separated by flash column chromatography (5–40% ethyl acetate in cyclohexane). The isolated, remaining *rac*-**1b** is reduced using catecholborane *via* general procedure **2** (24 h reaction time in this case). The corresponding 3-thiazolidine *rac*-**2b** is purified *via* preparative layer chromatography and derivatized according to general procedure 3. The corresponding colorless solid is dissolved in dichloromethane and analyzed by means of chiral SFC-HPLC.

Semi-preparative scale biotransformation of *rac*-1a. The semi-preparative scale biotransformation (10 mL) is performed from a 60 mM concentration of *rac*-**1a**, 25 mg/mL of lyophilized whole cell catalyst (which is prepared from a 50 mg/mL of cell suspension in 200 mM KPi buffer pH 7), 140 mM of D-glucose, 0.1 mM of $NADP^+$, and 2% of methanol as co-solvent in distilled water. The reaction mixture is stirred at 30°C for 65 h. The reaction is stopped by adding 1 mL of 32% NaOH solution and 20 mL of dichloromethane. Phase separation is promoted by centrifugation. The organic phase is dried over magnesium sulfate. The conversions are determined by analyzing the organic phase by achiral GC, and the enantiomeric excess is determined by means of chiral SFC-HPLC, after derivatization of the samples with phenylisocyanate.

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