ACS Catalysis

Research Article

pubs.acs.org/acscatalysis

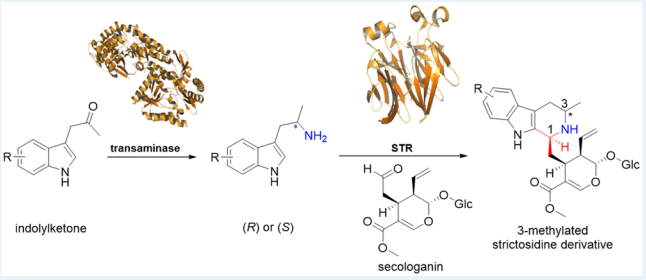
Stereoselective Cascade to C3-Methylated Strictosidine Derivatives Employing Transaminases and Strictosidine Synthases

Eva-Maria Fischereder, Desiree Pressnitz, and Wolfgang Kroutil*

Institute of Chemistry, Organic and Bioorganic Chemistry, University of Graz, NAWI Graz, Heinrichstrasse 28, 8010 Graz, Austria

Supporting Information

ABSTRACT: (*S*)-Strictosidine represents the first key intermediate in the biosynthesis of several pharmaceutically relevant monoterpenoid indole alkaloids. Optically pure C3-methyl-substituted strictosidine derivatives were prepared by setting up the two stereogenic centers at the β-carboline core via two enzymatic steps catalyzed by the enzymes transaminase and strictosidine synthase in a one-pot cascade fashion. The two enzymatic steps were performed simultaneously as well as in a stepwise fashion. The amination of the prochiral ketones led to optically pure amines with up to >98% enantiomeric excess. Depending on the enzyme used, the (*S*)- and (*R*)-enantiomers were prepared in most cases. Selected amines were then condensed with secologanin in a Pictet−Spengler reaction catalyzed by strictosidine synthase leading to diastereomerically pure products (>98% diastereomeric excess).

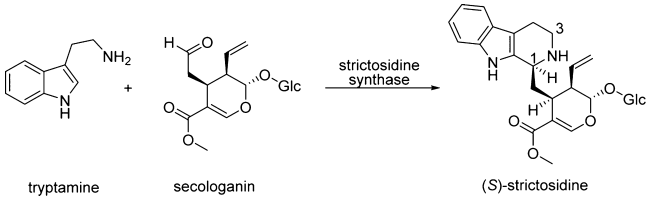


KEYWORDS: *indole alkaloids, strictosidine synthase, transaminases, biocatalytic cascade, strictosidine derivatives*

INTRODUCTION

Indole alkaloids make up one of the largest structurally diverse groups in the alkaloid superfamily (~2000 members).[1,2] Several ambassadors of this class are well-known for their pharmaceutical activity, including treatments for cancer (vinblastine), antiarrhythmic heart disorders (ajamaline), hypertension, and schizophrenia (reserpine).[3−5] In the plant's biomachinery, these highly complex structures are synthesized through a conserved sequence of various different enzymes.[1] Strictosidine synthase (STR) is considered as the gate enzyme in the alkaloid pathways, condensing tryptamine and secologanin to form (*S*)-strictosidine via a Pictet−Spengler reaction (Scheme 1).[6−8]

Scheme 1. Natural Reaction Catalyzed by Strictosidine Synthase



The chemical Pictet−Spengler reaction is broadly applied in organic chemistry for the synthesis of tetrahydroisoquinolines.[9] The first mention in the literature of strictosidine synthase goes back to 1975, employing cell cultures of *Catharanthus roseus* for the biosynthesis of the alkaloid ajmalicine.[10] Since then, strictosidine synthases have been biochemically characterized and their substrate scope has been investigated.[5,8,11−14] They are described in general as being restricted to close derivatives of secologanin as the aldehyde substrate, whereas various substituted tryptamine derivatives are accepted by the enzymes.[8,14−16]

Because (*S*)-strictosidine is a central building block in nature leading to many bioactive compounds, we aimed to investigate the formation of (*S*)-strictosidine derivatives **3** possessing an additional stereogenic center at C3 of the tetrahydro-β-carboline core via a biocatalytic cascade in which one stereogenic center is established in each step.[17−22]

RESULTS AND DISCUSSION

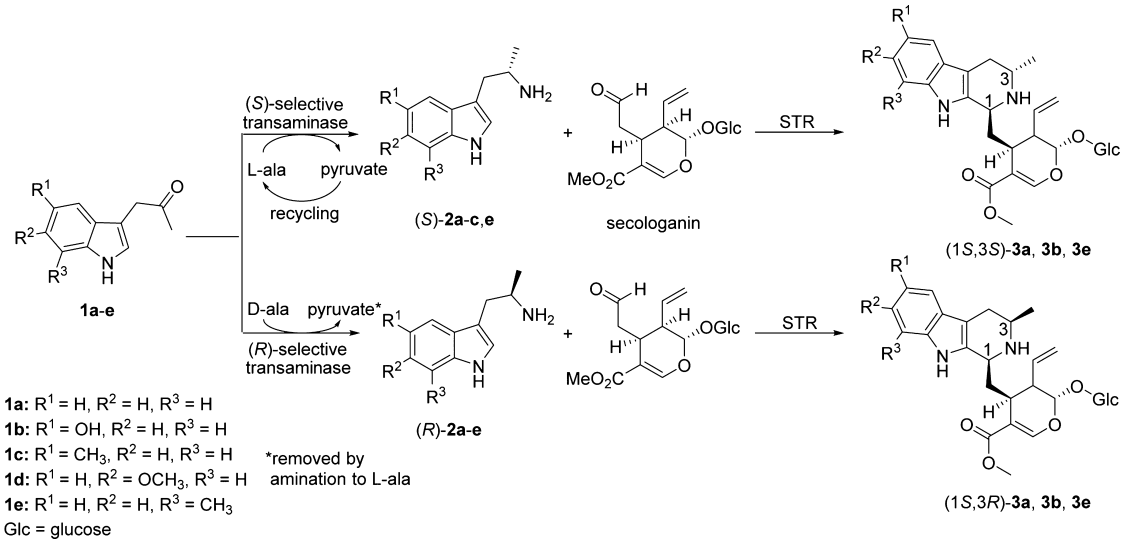
A two-step cascade was envisioned for the biocatalytic preparation of optically pure C3-methyl-substituted strictosidine derivatives **3**: a stereoselective amination of prochiral ketones **1** by ω-transaminases[23−38] leading to α-methyltryptamine (AMT) **2** in a first step is followed by the second step, a Pictet−Spengler condensation of amines **2** with secologanin catalyzed by strictosidine synthases (Scheme 2). By using stereocomplementary ω-transaminases, both enantiomers of **2** should be accessible, allowing the preparation of both C3 epimers. In this study, lyophilized *Escherichia coli* cells containing the recombinant (*S*)- or (*R*)-selective TAs or the recombinant strictosidine synthase were used, because the *E. coli* host did not show any transaminase or strictosidine synthase background activity. This procedure simplifies catalyst preparation, ensuring that reactions can be scaled up

Received: August 19, 2015
Revised: November 10, 2015

 © XXXX American Chemical Society

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

Scheme 2. Biocatalytic Cascade to C3-Methyl-Substituted Optically Pure Strictosidine Derivatives



economically. Activities of the freeze-dried catalyst preparations are given in the Supporting Information (Tables S5 and S6).

To investigate a broad substrate scope in addition to commercially available 3-indoleacetone **1a**, four ketone derivatives (**1b–e**) bearing electron-donating as well as electron-withdrawing groups at various positions on the indole core were synthesized. For their synthesis, a two-step procedure involving transformation of the corresponding indole derivatives via a Michael addition and Nef reaction was adapted from the literature.[39] The so obtained prochiral ketones **1a–e** were subsequently tested in the asymmetric amination employing various (*S*)- and (*R*)-selective transaminases using L- and D-alanine, respectively, as the amine donors; L-Ala was required for (*S*)-selective transaminases, while D-Ala was needed for the (*R*)-selective enzymes.

The coproduct pyruvate was recycled/removed to L-alanine by using an alanine dehydrogenase and a formate dehydrogenase for NADH recycling. Thereby, the (*R*)-selective transaminase from *Arthrobacter* sp. (ArR-TA)[40] turned out to be the most efficient enzyme for synthesizing the corresponding (*R*)-enantiomers of all α-methyltryptamine derivatives (**2a–e**) (Table 1). On the other hand, the stereocomplementary (*S*)-selective transaminase from *Silibacter pomeroyi* (SP-TA)[41] gave the best conversion and enantiomeric excess (ee) values for (*S*)-amines (*S*)-**2a**, (*S*)-**2b**, and (*S*)-**2e**, while the transaminase from *Bacillus megaterium* (BM-TA)[42] was best suited for the preparation of (*S*)-**2c**.

In addition to the TAs mentioned above, TAs from other sources such as *Aspergillus terreus*,[43] *Pseudomonas fluorescence*,[44] or *Chromobacterium violaceum*[45] were also able to aminate the ketones **1** with reasonable conversions as well as ee (see the Supporting Information). Interestingly, only in the case of ketone **1d**, which bears a methoxy group at position 6 of the indole core, was no suitable (*S*)-selective transaminase found, although the (*R*)-enantiomer was obtained with ArR-TA. In all other cases, both enantiomers of the α-methyltryptamines **2** were isolated. α-Methyltryptamines are potent psychoactive drugs because they inhibit the re-uptake of the neurotransmitters serotonin, dopamine, and norepinephrine.[46−48] Several chemical procedures for the synthesis of these amines have been published,[39,49−51] but to the best of our knowledge, this is the first example on transaminase catalysis leading to these compounds.

Table 1. Asymmetric Reductive Amination of Ketones 1a–e to Amine 2

entry	substrate	enzyme	conversion (%)[a]	yield [mg (%)][b]	ee (%)[c]
1	**1a**	SP-TA	98	49 (57)	>98 (*S*)
2	**1a**	ArR-TA	98	44 (51)	>98 (*R*)
3	**1b**	SP-TA	98	18 (48)	97 (*S*)
4	**1b**	ArR-TA	98	22 (58)	>98 (*R*)
5	**1c**	BM-TA	98	22 (30)	>98 (*S*)
6	**1c**	ArR-TA	98	23 (31)	>98 (*R*)
7	**1d**	ArR-TA	98	16 (20)	>98 (*R*)
8	**1e**	SP-TA	>99	33 (30)	>98 (*S*)
9	**1e**	ArR-TA	>99	34 (31)	>98 (*R*)

[a]Reaction conditions: substrates **1a–e** (50 mM), lyophilized *E. coli* cells containing the overexpressed (*S*)/(*R*)-selective TAs (20 mg), D- or L-alanine (250 mM), ammonium formate (150 mM), potassium phosphate buffer (100 mM, pH 7), PLP (1 mM), NAD^+ (1 mM), FDH (11 units), L-AlaDH (1.4 units), DMSO [10% (v/v)], 48 h, 30 °C, reaction volume of 1 mL. Conversion was determined by GC-FID. [b]Isolated yield after two purification steps. [c]Determined by high-performance liquid chromatography on a chiral phase after acetylation of the amines to the corresponding acetamide derivatives.

In the next step, the α-methyltryptamines **2** were transformed with secologanin employing strictosidine synthases (STRs) to investigate whether amines **2** are suitable substrates for these enzymes. Five different STRs from various plant sources were applied for this purpose: a STR from *Rauvolfia serpentina* (RsSTR)[52] and its V208A variant (RvSTR)[53] and STRs from *C. roseus* (CrSTR),[54] *Ophiorriza pumila* (OpSTR),[55] and *Ophiorriza japonica* (OjSTR).[56] The STRs from *C. roseus* and *R. serpentina* origin from the Apocynaceae family and have sequences that are 81% identical. The STRs from *O. pumila* and *O. japonica* belong to the Rubiaceae family and have sequences that are 60% identical when compared to the sequence of STR from *R. serpentina*.

With the library of STRs in hand, a cascade consisting of the simultaneous amination of ketones **1a–e** as well as the Pictet–Spengler reaction was designed. In the Pictet–Spengler reaction, the enantiomerically pure α-methyltryptamines **2a–e**

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

of the first step were reacted with secologanin to give final product **3**. Only the most suitable TAs were employed for the amination step. Because the two steps were performed simultaneously, both enzymes, the ω-TA and the STR, as well as both substrates (ketone **1a**−**e** and secologanin) were added at the beginning of the reaction; this requires that both reaction steps be compatible to each other. Of the library of five STRs, only OpSTR, RsSTR, and its variant RvSTR led to formation of product **3**. OpSTR proved to be the most active STR leading to the highest conversions (Table 2). Compared to OpSTR, the two other active enzymes (RsSTR and RvSTR) displayed ∼3-fold lower activity for the amines transformed (see the Supporting Information) whereby amines **2a**, **2b**, and **2e** turned out to be suitable substrates for certain STRs, while **2c** and **2d** were not transformed. The diastereomeric excess (de) of the obtained products **3** proved to be excellent in all cases (>98% de). Blank reactions showed that in the absence of catalyst (no cells or cells void of TA and STR) no reaction occurred.

Table 2. Simultaneous Amination and Pictet−Spengler Reaction

entry	substrate	TA	selectivity of TA	product	**3** (%)[a]	de of **3** (%)[b]
1	**1a**	SP	*S*	(1*S*,3*S*)-**3a**	96 ± 1.6	>98
2	**1a**	ArR	*R*	(1*S*,3*R*)-**3a**	97 ± 1.1	>98
3	**1b**	SP	*S*	(1*S*,3*S*)-**3b**	31 ± 0.3	>98
4	**1b**	ArR	*R*	(1*S*,3*R*)-**3b**	74 ± 0.4	>98
5	**1e**	SP	*S*	(1*S*,3*S*)-**3e**	64 ± 0.1	>98
6	**1e**	ArR	*R*	(1*S*,3*R*)-**3e**	86 ± 0.3	>98

[a]Percentage of **3** formed determined by high-performance liquid chromatography−ultraviolet on an achiral phase. Reaction conditions: lyophilized *E. coli* cells containing the overexpressed (*S*)/(*R*)-selective TAs (10 mg)/STR from *O. pumila* (90 milliunits), D- or L-alanine (250 mM), ammonium formate (150 mM), potassium phosphate buffer (100 mM, pH 7), PLP (1 mM), NAD^+ (1 mM), FDH (11 units), L-AlaDH (1.4 units), DMSO [5% (v/v)], ketones **1a**−**e** (2 mM), secologanin (4 mM), 24 h, 30 °C, reaction volume of 1 mL. All experiments were performed in triplicate. [b]Determined by high-performance liquid chromatography−ultraviolet on an achiral phase.

To investigate whether the STRs control the diastereoselectivity in this Pictet−Spengler reaction or whether the diastereoselectivity is intrinsic for this reaction, the isomeric composition of the chemical Pictet−Spengler reaction of optically pure (*R*)-**2a** and (*S*)-**2a** as well as of the racemic amine *rac*-**2a** with secologanin was compared to that after the enzymatic transformation of (*S*)-**2a** with secologanin (Figure 1). While the enzymatic transformation of (*S*)-**2a** led to the optically pure diastereomer (1*S*,3*S*)-**3a** (track C, >98% de), the chemical reaction of (*S*)-**2a** led to the predominant formation of the opposite diastereomer (1*R*,3*S*)-**3a** (track A) in 48% de. This clearly shows that STR controls the diastereoselectivity. Another clear indication of the enzyme-controlled diastereoselectivity is the fact that both enantiomers of the amines led to an (*S*)-configured center at C1 of the indole in a manner independent of the absolute configuration at C3 as shown for (*S*)-**2a** giving (1*S*,3*S*)-**3a** and (*R*)-amine **2b** giving (1*S*,3*R*)-**3b**.

The results listed in Table 2 indicate that OpSTR may possess a certain enantioselectivity for the transformation of one enantiomer of **2**, especially in the case of **2b** and **2e**. For instance, the (*R*)-enantiomer (*R*)-**2b** and **2e** led to higher conversions of products **3** in both cases. To test whether this is also the case for amine **2a**, the conversion of optically pure (*R*)-**2a** and (*S*)-**2a** as well as the racemate *rac*-**2a** was followed during the first 1.5 h of reaction (Figure 2). Employing optically pure amines as substrates showed that (*S*)-**2a** was transformed faster than (*R*)-**2a**; in contrast, in the case using the racemic mixture as a substrate, the (*R*)-**2a** enantiomer was transformed faster. The estimated enantioselectivity *E* was low in both cases, namely, ∼3 in the case of the optically pure amines with an enantiopreference for the (*S*)-enantiomer and 5−7 using the racemic substrate with an enantiopreference for the (*R*)-enantiomer; the low enantioselectivity allowed the complete transformation of both enantiomers within 24 h. Obviously, OpSTR shows only a low level of recognition for a

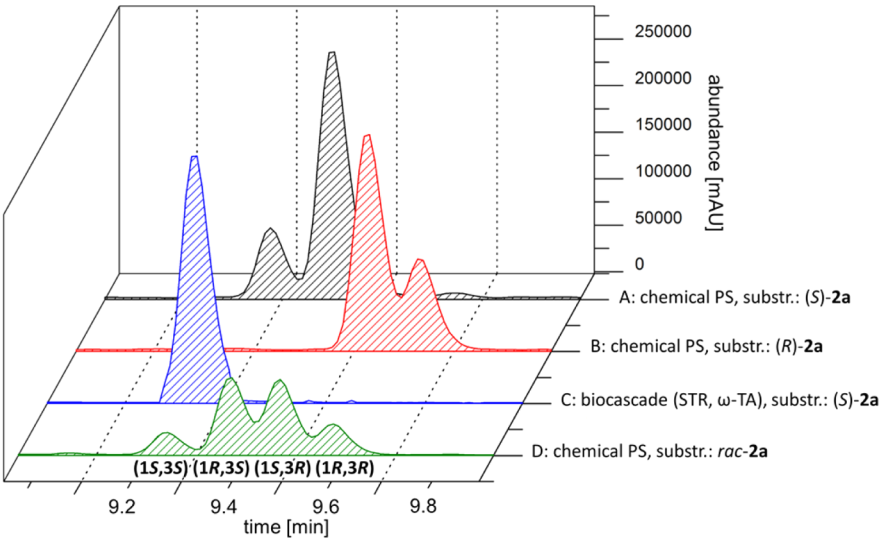


Figure 1. Isomeric composition of derivative **3a** obtained by chemical Pictet−Spengler (PS) reaction (A, B, and D) and from biotransformation (C): (A) substrate (*S*)-**2a**, (B) substrate (*R*)-**2a**, (D) substrate *rac*-**2a**, and (C) substrate (*S*)-**2a** (formed by TA from *S. pomeroyi* in the cascade) and transformed with OpSTR. Reaction conditions for chemical transformation: (*S*)/(*R*)/*rac*-**2a** (2 mM), DMSO [5% (v/v)], maleic acid buffer (10 mM, pH 2.8, 475 μL), secologanin (4 mM), 24 h at 60 °C.

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

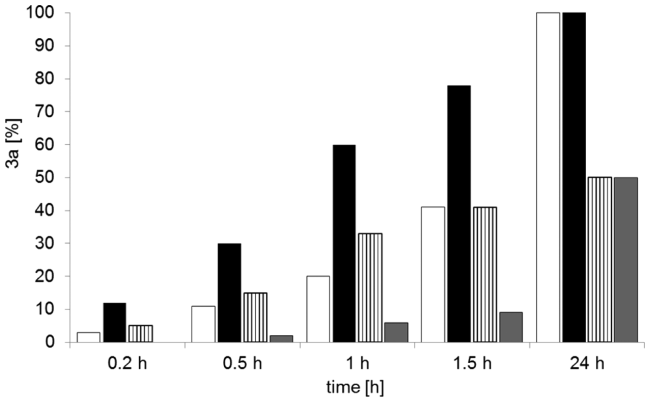


Figure 2. Formation of strictosidine derivative **3a** over time: white bars, substrate (*R*)-**2a**; black bar, substrate (*S*)-**2a**; white bar with lines, transformation of (*R*)-**2a** of *rac*-**2a**; gray bar, transformation of (*S*)-**2a** of *rac*-**2a**. Reaction conditions: lyophilized *E. coli* cells containing overexpressed STR from *O. pumila* (90 milliunits), PIPES buffer (100 mM, pH 6.8), DMSO [5% (v/v)], (*S*)/(*R*)/*rac*-**2a** (1 mM), secologanin (2 mM), 30 °C, reaction volume of 0.5 mL.

remote stereogenic center,[57] which makes this enzyme applicable for the transformation of both enantiomers of **2a**.

As an alternative to the simultaneously performed biotransformation, a stepwise cascade was tested, thus performing the sequence in one pot, whereby the second step is started ideally after the first is finished. In this setup, the TA-catalyzed amination was run for 24 h, followed by the removal of the enzyme and subsequent addition of the STR and secologanin. The Pictet−Spengler condensation was then allowed to proceed for an additional 24 h. Via comparison of the simultaneous and stepwise cascade (Tables 2 and 3), the

Table 3. Stepwise Cascade of Amination and Pictet−Spengler Reaction

entry	product	**3** (%)[a]	isolated yield (%)	de of **3** (%)[b]
1	**3a**	>99 ± 0.0	70	>98 (1*S*,3*S*)
2	**3a**	>99 ± 0.0	not determined	>98 (1*S*,3*R*)
3	**3b**	56 ± 0.1	not determined	>98 (1*S*,3*S*)
4	**3b**	89 ± 0.8	75	>98 (1*S*,3*R*)
5	**3e**	98 ± 0.3	not determined	>98 (1*S*,3*S*)
6	**3e**	>99 ± 0.0	80	>98 (1*S*,3*R*)

[a]Percentage of **3** formed. Reaction conditions: lyophilized *E. coli* cells containing the (*S*)/(*R*)-selective TAs (10 mg), D- or L-alanine (250 mM), ammonium formate (150 mM), potassium phosphate buffer (100 mM, pH 7), PLP (1 mM), NAD^+ (1 mM), FDH (11 units), L-AlaDH (1.4 units), DMSO [5% (v/v)], ketone (**1a**, **1b**, or **1e**; 2 mM), 24 h, 30 °C. Removal of TAs by centrifugation (20 min at 13000 rpm). Addition of lyophilized *E. coli* cells containing overexpressed STR from *O. pumila* (90 milliunits) and secologanin (4 mM), 24 h, 30 °C. Experiments were performed in triplicate. [b]Determined by high-performance liquid chromatography−ultraviolet on an achiral phase.

stepwise approach led to higher conversions employing OpSTR (Table 3) as well as for RvSTR and RsSTR (see the Supporting Information). One possible explanation might be that the aldehyde secologanin required for the second step can also serve as a substrate for the amination by the transaminase in the first step, leading thereby to an unwanted side reaction.

For selected enzymes, the final strictosidine derivatives were isolated (Table 3, entries 1, 4, and 6). The absolute configuration at C1 formed during the biocatalytic Pictet−Spengler reaction was proven for (1*S*,3*S*)-**3a**, (1*S*,3*R*)-**3b**, and (1*S*,3*R*)-**3e** by NOESY experiments exploiting the already known fixed stereochemistry at the second stereogenic center originating from the chiral amine. Thus, it was shown that the products were (*S*)-configured at C1 (see the Supporting Information).

CONCLUSION

Novel C3-methyl-substituted strictosidine derivatives were prepared via a two-step biocatalytic cascade whereby in each step one new stereogenic center was established. The first step of the reaction sequence was the reductive amination of prochiral indolyl ketones via transaminases to give optically pure α-methyltryptamines. In the second step, the optically pure α-methyltryptamines were condensed with segologanin catalyzed by strictosidine synthase to yield the final products. The reaction conditions of TAs and STRs were compatible, allowing the simultaneous performance of the two biocatalytic reactions; however, higher conversions were achieved by running the two reactions in a stepwise fashion. By using an appropriate ω-TA, the amines were obtained with up to >98% ee. Three amines were accepted by the strictosidine synthases from *O. pumila* as well as *R. serpentina*. The final products were obtained with diastereoselectivities of >98%.

EXPERIMENTAL SECTION

General Remarks and Analytical Techniques. All chemicals and solvents used in this study were purchased in the highest available purity and were used without further purification unless stated otherwise. Preparative chromatographic separations were performed by column chromatography or thin layer chromatography (TLC) on Merck silica gel 60 (0.063−0.200 μm). Optical rotations at the sodium D-line were measured at 20 °C on a PerkinElmer model 341 polarimeter. Gas chromatography (GC) and GC−mass spectrometry (MS) data were recorded with an Agilent 7890A GC system. The GC−MS system was equipped with an Agilent 5975C mass-selective detector and an HP-5 MS column [30 m × 0.25 μm; helium as the carrier gas (flow rate of 0.55 mL min^{-1})]. ^1H and ^{13}C NMR spectra were recorded at 20 °C on a 300 Bruker NMR unit; chemical shifts are given in parts per million related to Me_4Si or related to the resonance of the solvent. High-performance liquid chromatography (HPLC) measurements were recorded with a Shimadzu system equipped with a UV detector. For measurements, the system was equipped either with a Luna C18 column (250 mm × 4.6 mm) or with a chiracel OJ column (250 mm × 4.6 mm). For preparative HPLC purification, a Shimadzu system equipped with a UV detector and a semiprep VP Nucleodur C18 column (5 μm, 250 mm × 16 mm) was used. In the case of liquid chromatography (LC)−MS measurements, an Agilent 1200 Infinity system was equipped with a Zorbax SB-C18 column (2.1 mm × 50 mm). Formate dehydrogenase (2.1 units mg^{-1}) was purchased from Evocatal (Evo-1.1.230). L-Alanine dehydrogenase from *Bacillus subtilis* was prepared and purified as described previously.[58] All TAs were overexpressed in *E. coli* BL21(DE3) and used as lyophilized cell preparations as reported recently.[40−45] The *E. coli* codon-optimized gene sequences from *R. serpentina* (RsSTR) (GenBank entry Y00756.1), the variant of *Rauvolfia* (RvSTR) (GenBank entry CAA44208.1), *C. roseus* (GenBank entry CAA43936.1), *O. pumila* (sequence taken from ref 55), and *O. japonica*

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

(GenBank entry ACF21007.1) were ordered from Life Technologies (Darmstadt, Germany). Oligonucleotide primers were obtained from Eurofines MWG Operon (Ebersberg, Germany). Expression vector pET-28a(+) was purchased from Novagen (Darmstadt, Germany). *E. coli* strain BL21(DE3), C43(DE3), and chemically competent *E. coli* Top10 cells were obtained from Invitrogen (Karlsruhe, Germany). QIAquick Gel Extraction Kits and Miniprep Kits from Qiagen (Hilden, Germany) were used for DNA isolation and purification. Restriction enzymes *Bam*HI and *Sac*I as well as Phusion-DNA polymerase and T4 DNA ligase were obtained from New England Biolabs (Frankfurt/Main, Germany). *Taq*-DNA polymerase was obtained from Peqlab (Polling, Austria).

Construction of STR Expression Vectors. The codon-optimized strictosidine synthase genes were amplified via polymerase chain reaction (PCR) to introduce *Bam*HI and *Sac*I restriction sites into RsSTR, RvSTR, CrSTR, and OjSTR. In the case of OpSTR, *E. coli* codon-optimized sequences comprising *Nco*I and *Xho*I were ordered and used without further modifications (for enzyme/primer sequences, see the Supporting Information). All PCR fragments were purified and digested with the corresponding restriction enzymes for subcloning into pET-28a(+). Following confirmation of the correct DNA sequence, plasmids were transformed into *E. coli* C43(DE3).

Heterologous Overexpression of STRs. *E. coli* C43(DE3) host cells containing pET28a(+)/STRs expression plasmids were cultured in LB medium supplemented with kanamycin (30 μg/mL) overnight. Aliquots were used to inoculate fresh culture media, and cells were incubated at 37 °C until the cell density reached an OD_{600} of 0.6−0.8. Protein expression was started by addition of isopropyl β-D-1-thiogalactopyranoside (final concentration of 0.6 mM), and incubation at 20 °C was continued for 24 h. Cells were harvested by centrifugation, and pellets were washed once with buffer [50 mM PIPES (pH 6.8)]. Finally, cells were resuspended in 15 mL of PIPES buffer and lyophilized. Freeze-dried cell pellets were stored at 4 °C until further use.

General Procedure for the Synthesis of Indolylpropanamines 2a−e (analytical scale). Lyophilized cells of *E. coli* BL21(DE3) containing the corresponding overexpressed transaminases (20 mg) were rehydrated in potassium phosphate buffer (0.9 mL, 100 mM, pH 7) containing PLP (0.2 mg, 1 μmol, final concentration of 1 mM), ammonium formate (9.4 mg, 150 μmol, final concentration of 150 mM), L- or D-alanine (22 mg, 250 μmol, final concentration of 250 mM), formate dehydrogenase (11 units), and NAD^+ (0.6 mg, 1 μmol, final concentratioon of 1 mM) for 30 min at 30 °C and 120 rpm on an orbital shaker placed on its side. Then ketones **1a−e** (final concentration of 50 mM) dissolved in DMSO [100 μL, 10% (v/v)] and L-alanine dehydrogenase (1.4 units) were added. The mixture was shaken at 30 °C and 120 rpm for 48 h on an orbital shaker placed on its side.

*Workup for **2a** and **2c−e**.* The reaction was terminated by addition of a saturated $NaHCO_3$ solution (100 μL) and the mixture extracted with ethyl acetate (2 × 500 μL). The combined organic phases were dried over Na_2SO_4, and the conversion was measured by GC-FID.

*Workup for **2b**.* The reaction was terminated by addition of MeOH (500 μL). Then the reaction mixture was centrifuged (30 min at 13000 rpm), filtered (22 μm filter), and directly subjected to HPLC−UV analysis.

Determination of Optical Purity. The enantiomeric excess of amines **2a−e** was analyzed by HPLC−UV on a chiral phase after derivatization to the corresponding acetoamides. For amines **2a** and **2c−e**, the corresponding amides were prepared by addition of 4-(dimethylamino)-pyridine (5 mg) in acetic anhydride (100 μL). After the samples had been washed with water and dried (Na_2SO_4), the ee values were measured. In case of amine **2b**, the acetoamide was prepared directly from the biotransformation samples via the addition of triethylamine (100 μL) and ESOF [ethyl-(succimidooxy)formate, 50 mg] predissolved in acetonitrile (100 μL). The mixture was shaken at 45 °C overnight. Then the samples were extracted with methyl *tert*-butyl ether (2 × 500 μL). The combined organic phases were dried (Na_2SO_4), and the ee values were measured via HPLC−UV.

General Procedure for the Synthesis of Indolylpropanamines 2a−e (preparative scale). For the preparative biotransformations, multiple analytical scale experiments were conducted: **1a** (10 experiments; 87 mg of **1a** in total), **1b** (4 experiments; 38 mg of **1b** in total), **1c** (8 experiments; 74 mg of **1c** in total), **1d** (8 experiments; 81 mg of **1d** in total), and **1e** (12 experiments; 112 mg of **1e** in total). Reactions were terminated through addition of MeOH (500 μL). Then the samples were centrifuged (13000 rpm for 10 min), and the supernatant was filtered (22 μm filter). Afterward, water was removed under reduced pressure. The remaining yellowish highly viscous liquids were taken up in the column chromatography eluent (80/19/1 CH_2Cl_2/MeOH/NH_4OH) and purified via flash chromatography (silica; 80/19/1 CH_2Cl_2/MeOH/NH_4OH). The corresponding product fractions were combined and filtered through Celite, and the solvent was removed under reduced pressure. The remaining products were resuspended in MeOH (700 μL) and subjected to preperative TLC (500 μm silica coating; 98/2 MeOH/25% aqueous NH_3 solution). The product band was scraped off the plate and incubated with MeOH (70 mL) for 1.5 h. Afterward, the suspension was filtered and the solvent evaporated under reduced pressure.

(*R*)/(*S*)-1-(Indol-3-yl)-2-aminopropane 2a. *(R)-**2a**.* 44 mg of a yellowish solid (51%); $[\alpha]_D^{20}$ = −40 (*c* = 0.5, $CHCl_3$) [lit.[39] $[\alpha]_D^{20}$ = −35 (*c* = 0.5, MeOH)]; TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.33; mp 103−105 °C (lit.[39] 127−127 °C).

*(S)-**2a**.* 49 mg of a yellowish solid (57%); $[\alpha]_D^{20}$ = +34 (*c* = 0.5, MeOH) [lit.[39] $[\alpha]_D^{20}$ = +34 (*c* = 0.5, MeOH)]; TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.33; mp 103−105 °C (lit.[39] 129−130 °C).

^{1}H NMR data are in accordance with the literature.[39]

^{13}C NMR (CD_3OD, 75 MHz) δ 20.82, 34.08, 110.9, 111.3, 117.9, 118.2, 120.9, 122.7, 127.4, 136.8; MS (EI, 70 eV) *m*/*z* 174 (M, 4.3), 131 (100), 103 (8.6), 77 (11), 44 (37).

(*R*)/(*S*)-1-(5-Hydroxy-indol-3-yl)-2-aminopropane 2b. *(R)-**2b**.* 22 mg of a brownish solid (58%); $[\alpha]_D^{20}$ = −34 (*c* = 0.5, MeOH) [lit.[39] $[\alpha]_D^{20}$ = −37 (*c* = 0.5, MeOH)]; TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.26; mp 45−48 °C.

*(S)-**2b**.* 18 mg of a brownish solid (48%); $[\alpha]_D^{20}$ = +35 (*c* = 0.5 MeOH) [lit.[39] $[\alpha]_D^{20}$ = +35 (*c* = 0.5, MeOH)]; TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.26; mp 45−48 °C.

^{1}H NMR data are in accordance with the literature.[39]

^{13}C NMR (CD_3OD, 75 MHz) δ 18.29, 31.14, 83.98, 102.2, 108.4, 111.7, 111.8, 124.3, 128.0, 131.4, 150.9; MS (EI, 70 eV) *m*/*z* 190 (M, 5.2), 147 (100), 117 (8.6), 91 (6.9), 44 (39).

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

(*R*)/(*S*)-1-(5-Methyl-indol-3-yl)-2-aminopropane 2c. *(R)-**2c***. 23 mg of an off-white solid (31%); $[\alpha]_D^{20}$ = −33 (*c* = 1.1, MeOH); TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.27; mp 131−135 °C.

*(S)-**2c***. 22 mg of an off-white solid (30%); $[\alpha]_D^{20}$ = +34 (*c* = 1.0, MeOH); TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.27; mp 131−135 °C.

^{1}H NMR (CD_3OD, 300 MHz) δ 1.01 (3H, d, *J* = 6.0 Hz, CH_3), 2.30 (3H, s, CH_3-aryl), 2.55 (1H, dd, J_1 = 6.0 Hz, J_2 = 15 Hz, CH_2), 2.68 (1H, dd, J_1 = 6.0 Hz, J_2 = 15 Hz, CH_2), 3.07 (1H, q, *J* = 6 Hz, CH), 6.82 (1H, d, *J* = 9.0 Hz, aryl), 6.90 (1H, s, C*H*-NH), 7.11 (1H, d, *J* = 6 Hz, aryl), 7.22 (1H, s, aryl).

^{13}C NMR (CD_3OD, 75 MHz) δ 20.27, 21.51, 34.84, 47.09, 110.5, 111.3, 117.6, 122.5, 122.7, 127.2, 127.7, 136.1; MS (EI, 70 eV) *m*/*z* 188 (M, 4.3), 145 (100), 115 (13), 44 (45).

(*R*)-1-(6-Methoxy-indol-3-yl)-2-aminopropane 2d. *(R)-**2d***. 15 mg of an off-white solid (20%); $[\alpha]_D^{20}$ = −30 (*c* = 0.5, MeOH) [lit.[39] $[\alpha]_D^{20}$ = −27 (*c* = 0.5, MeOH)]; TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.23; mp 110−112 °C (lit.[39] 110−112 °C).

^{1}H NMR data are in accordance with the literature.[39]

^{13}C NMR (CD_3OD, 75 MHz) δ 21.19, 34.56, 47.18, 54.56, 94.07, 108.5, 111.6, 118.5, 121.4, 121.9, 137.4, 156.1; MS (EI, 70 eV) *m*/*z* 204 (M, 11), 161 (100), 146 (27), 117 (13), 44 (33).

(*R*)/(*S*)-1-(7-Methyl-indol-3-yl)-2-aminopropane 2e. *(R)-**2e***. 34 mg of an off-white solid (31%); $[\alpha]_D^{20}$ = −32 (*c* = 1.2, MeOH); TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.27; mp 115−118 °C (lit.[59] 106−107 °C).

*(S)-**2e***. 33 mg of an off-white solid (30%); $[\alpha]_D^{20}$ = +28 (*c* = 1.0, MeOH); TLC (80/19/1 CH_2Cl_2/MeOH/NH_4OH) R_f = 0.27; mp 115−118 °C (lit.[59] 106−107 °C).

^{1}H NMR (CD_3OD, 300 MHz) δ 1.11 (3H, d, *J* = 6.0 Hz, CH_3), 2.46 (3H, s, CH_3-aryl), 2.68 (1H, dd, J_1 = 6.0 Hz, J_2 = 15 Hz, CH_2), 2.80 (1H, dd, J_1 = 6.0 Hz, J_2 = 15 Hz, CH_2), 3.18 (1H, q, *J* = 6 Hz, CH), 6.86−6.93 (2H, m, aryl), 7.05 (1H, s, C*H*-NH), 7.36 (1H, d, *J* = 6 Hz, aryl).

^{13}C NMR (CD_3OD, 75 MHz) δ 15.51, 21.39, 34.83, 47.16, 112.1, 115.8, 118.5, 120.2, 121.4, 122.5, 127.1, 136.2; MS (EI, 70 eV) *m*/*z* 188 (M, 4.3), 145 (100), 115 (13), 44 (45).

The absolute configuration for **2a**, **2b**, and **2d** was obtained by comparison of the optical rotation with literature values. The absolute configuration for **2c** and **2e** was assumed on the basis of three matching indicators: (i) comparison of the sign of the optical rotation with those of related compounds (*R*)/(*S*)-**2a**, (*R*)/(*S*)-**2b**, and (*R*)/(*S*)-**2d**, (ii) comparison of elution order with compounds **2a**, **2c**, and **2e**, and (iii) preference of the enzyme.

General Procedure for the Synthesis of Strictosidine Derivatives 3a, 3b, and 3e [one-pot, one-step cascade (analytical scale)]. Lyophilized *E. coli* BL21(DE3)/C43(DE3) cells containing the corresponding overexpressed transaminase (10 mg) and strictosidine synthase (OpSTR, 90 milliunits; RsSTR, 90 milliunits; RvSTR, 12 milliunits; CrSTR, 90 milliunits; OjSTR, 20 mg) were rehydrated in potassium phosphate buffer (0.9 mL, 100 mM, pH 7) containing PLP (0.2 mg, 1 μmol, final concentration of 1 mM), ammonium formate (9.4 mg, 150 μmol, final concentration of 150 mM), L- or D-alanine (22 mg, 250 μmol, final concentration of 250 mM), formate dehydrogenase (11 units), and NAD^+ (0.6 mg, 1 μmol, final concentration of 1 mM) for 30 min at 30 °C and 120 rpm on an orbital shaker placed on its side. Then ketones **1a**−**e** (final concentration of 2 mM) dissolved in DMSO (50 μL), secologanin (1.5 mg, 4 μmol, final concentration of 4 mM) dissolved in KP_i buffer (50 μL), and L-alanine dehydrogenase (1.4 units) were added. The mixture was shaken at 30 °C and 800 rpm for 24 h on an orbital shaker placed on its side. The reaction was terminated by addition of MeOH (100 μL) followed by centrifuged (25 min at 13000 rpm) and filtration (22 μm filter) of the supernatant, which was directly subjected to HPLC−UV analysis.

General Procedure for the Synthesis of Strictosidine Derivatives 3a, 3b, and 3e [one-pot, two-step cascade (analytical scale)]. Lyophilized *E. coli* BL21(DE3) cells containing the corresponding overexpressed transaminase (10 mg) were rehydrated in potassium phosphate buffer (0.9 mL, 100 mM, pH 7) containing PLP (0.2 mg, 1 μmol, final concentration of 1 mM), ammonium formate (9.4 mg, 150 μmol, final concentration of 150 mM), L- or D-alanine (22 mg, 250 μmol, final concentration of 250 mM), formate dehydrogenase (11 units), and NAD^+ (0.6 mg, 1 μmol, final concentration of 1 mM) for 30 min at 30 °C and 120 rpm on an orbital shaker placed on its side. Then ketones **1a**−**e** (final concentration of 2 mM) dissolved in DMSO (50 μL) and L-alanine dehydrogenase (1.4 units) were added. The mixture was shaken at 30 °C and 800 rpm for 24 h on an orbital shaker placed on its side. Afterward, the transaminases were removed from the biotransformation mixture by centrifugation (25 min at 13000 rpm). The supernatant was applied to lyophilized *E. coli* C43(DE3) cells containing the corresponding overexpressed strictosidine synthase (OpSTR, 90 milliunits; RsSTR, 90 milliunits; RvSTR, 12 milliunits). Afterward, secologanin (1.5 mg, 4 μmol, final concentration of 4 mM) dissolved in KP_i buffer (50 μL) was added, and the biotransformation mixture was shaken for an additional 24 h at 800 rpm. The reaction was terminated by addition of MeOH (100 μL) followed by centrifugation (25 min at 13000 rpm) and filtration (22 μm filter) of the supernatant, which was directly subjected to HPLC−UV analysis.

General Procedure for the Synthesis of Strictosidine Derivatives 3a, 3b, and 3e [one-pot, two-step cascade (preparative scale)]. For the preparative biotransformations, multiple analytical scale experiments were conducted. All experiments in the cascade were performed with the strictosidine synthase from *O. pumila*. Reactions were terminated by addition of MeOH (500 μL). Then the samples were centrifuged (13000 rpm for 10 min), and the supernatant was filtered (22 μm filter). Afterward, water was removed under reduced pressure. The remaining yellowish highly viscous liquids were subjected to purification via column chromatography and/or preparative HPLC.

*(1S,3S)-3-Methylstrictosidine **3a***. First, **3a** was purified via flash chromatography [silica, 80/20 CH_2Cl_2/MeOH, R_f (80/20 CH_2Cl_2/MeOH) = 0.25], which yielded **3a** (22 mg, 110%) as an off-white solid. For further purification, the solid was dissolved in MeOH (2 mL) and subjected to preparative HPLC [reversed phase column; gradient from 90/10 to 20/80 buffer (NH_4COOH, 30 mM, pH 2.8)/acetonitrile over 24 min]. Pure (1*S*,3*S*)-**3a** (15 mg, 70%) was isolated as an off-white solid and characterized by NMR (>98% de): ^{1}H NMR (CD_3OD, 300 MHz) δ 1.49 (3H, d, *J* = 7 Hz, H-3″), 2.20−2.37 (2H, m, H-14), 2.27−2.89 (2H, m, H-4, H-20), 3.05−3.13 (1H, m, H-15), 3.19−3.26 (3H, m, H-4, GlcH-2′, GlcH-4′), 3.34−3.40 (2H, m, GlcH-5′, GlcH-3′) 3.61 (1H, dd, J_1 = 3 Hz, J_2 = 12 Hz, GlcH-6′), 3.78 (3H, s, H-22′), 3.96 (1H, dd, J_1 = 3 Hz, J_2 = 12 Hz, GlcH-6′), 4.07 (1H, q, *J* = 6 Hz, H-3), 4.66 (1H, dd, J_1 = 3 Hz,

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

J_2 = 9 Hz, H-1), 4.79 (1H, d, J = 9 Hz, GlcH-1′), 5.28 (1H, tt, J_1 = 3 Hz, J_2 = 9 Hz, H-18*cis*), 5.37 (1H, tt, J_1 = 3 Hz, J_2 = 18 Hz, H-18*trans*), 5.80−5.92 (2H, m, H-19, H-21), 7.04 (ddt, J_1 = 1 Hz, J_2 = 6 Hz, H-8), 7.13 (ddt, J_1 = 1 Hz, J_2 = 6 Hz, H-9), 7.32 (1H, d, J = 6 Hz, H-7), 7.46 (1H, d, J = 9 Hz, H-10), 7.79 (1H, s, H-17); ^{13}C NMR (CD_3OD, 75 MHz) δ 15.49, 25.13, 30.95, 33.74, 51.07, 61.54, 70.31, 73.25, 76.57, 77.35, 95.97, 99.01, 104.6, 107.7, 110.8, 117.6, 118.4, 119.1, 122.1, 126.3, 127.9, 134.0, 137.0, 155.2, 169.6.

(1S,3R)-3-Methyl-8-hydroxystrictosidine ***3b***. **3b** was taken up in MeOH (2 mL) and subjected to preparative HPLC [reversed phase column; gradient from 90/10 to 50/50 buffer (NH_4COOH, 30 mM, pH 2.8)/acetonitrile over 16 min]. Product fractions were collected and freeze-dried. The residual white solid was again subjected to preparative HPLC (reversed phase column; gradient from 90/10 to 40/60 water/acetonitrile over 16 min). Analytically pure (1*S*,3*R*)-**3c**·$^1/_2HCO_2H$ (16 mg, 75%) was isolated as a white solid and characterized by NMR (>98% de): ^{1}H NMR (CD_3OD, 300 MHz) δ 1.61 (3H, d, J = 6 Hz, H-3″), 2.12 (1H, ddt, J_1 = 3 Hz, J_2 = 13 Hz, H-14), 2.38 (1H, ddt, J_1 = 3 Hz, J_2 = 13 Hz, H-14), 2.69−2.78 (2H, m, H-4, H-20), 3.01 (1H, dd, J_1 = 3 Hz, J_2 = 14 Hz, H-4), 3.10−3.17 (1H, m, H-15), 3.21−3.27 (2H, m, GlcH-2′, GlcH-4′), 3.37−3.45 (2H, m, GlcH-5′, GlcH-3′), 3.53−3.61 (1H, m, H3), 3.67 (1H, dd, J_1 = 6 Hz, J_2 = 12 Hz, GlcH-6′), 3.81 (3H, s, H-22′), 4.01 (1H, dd, J_1 = 3 Hz, J_2 = 12 Hz, GlcH-6′), 4.55 (1H, d, J = 12 Hz, H-1), 4.81 (1H, d, J = 9 Hz, GlcH-1′), 5.26 (1H, tt, J_1 = 1 Hz, J_2 = 10 Hz, H-18*cis*), 5.35 (1H, tt, J_1 = 1 Hz, J_2 = 18 Hz, H-18*trans*), 5.79−5.87 (1H, m, H-19), 5.90 (1H, d, J = 9 Hz, H-21), 6.68 (1H, dd, J_1 = 2 Hz, J_2 = 9 Hz, H-9), 6.80 (1H, d, J = 2 Hz, H-7), 7.12 (1H, d, J = 9 Hz, H-10), 7.82 (1H, s, H-17), 8.52 (0.5 H, s, HCO_2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 18.47, 26.86, 30.91, 33.34, 44.03, 52.17, 52.54, 61.19, 70.35, 73.25, 76.59, 77.45, 95.93, 99.01, 101.9, 105.7, 107.4, 111.2, 111.6, 118.3, 126.5, 126.5, 126.8, 131.7, 133.9, 138.9, 150.4, 155.78, 168.6, 170.3.

(1S,3R)-3,10-Methylstrictosidine ***3e***. **3e** was taken up in MeOH (2 mL) and subjected to preparative HPLC (reversed phase column; gradient from 80/20 to 10/90 water with 0.1% TFA/acetonitrile over 20 min). Analytically pure (1*S*,3*R*)-**3e** (12 mg, 80%) was isolated as an off-white solid and characterized by NMR (>98% de): ^{1}H NMR (CD_3OD, 300 MHz) δ 1.65 (3H, d, J = 6.0 Hz, H-3″), 2.11−2.21 (1H, m, H-14), 2.46 (1H, s, H-10′), 2.26−2.89 (3H, m, H-14, H-20, H-4), 3.10−3.23 (4H, m, H-4, H-15, GlcH-1′, GlcH-4′), 3.45−3.38 (2H, m, GlcH-3′, GlcH-4′), 3.59−3.72 (2H, m, GlcH-6′, H-3), 3.82 (3H, s, H-22′), 4.03 (1H, dd, J_1 = 3 Hz, J_2 = 12 Hz, GlcH-6′), 4.68 (1H, d, J = 12 Hz, H-1), 4.83 (1H, d, J = 9 Hz, GlcH-1′), 5.26 (1H, tt, J_1 = 3 Hz, J_2 = 9 Hz, H-18*cis*), 5.35 (1H, tt, J_1 = 3 Hz, J_2 = 18 Hz, H-18*trans*), 5.86 (1H, ddt, J_1 = 7 Hz, J_2 = 10 Hz, J_3 = 17 Hz, H-19), 6.01 (1H, d, J = 9 Hz, H-21), 6.91−6.98 (2H, m, H-8, H-7), 7.28 (1H, dd, J_1 = 3 Hz, J_2 = 6 Hz, H-9), 7.86 (1H, s, H-17); ^{13}C NMR (CD_3OD, 75 MHz) δ 15.53, 18.12, 26.50, 31.28, 32.83, 43.87, 51.47, 52.41, 52.71, 61.89, 70.50, 73.21, 76.56, 77.64, 95.88, 99.02, 106.7, 107.0, 115.3, 118.2, 119.5, 120.6, 122.7, 125.6, 128.1, 133.9, 136.4, 156.3, 170.8.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b01839.

Synthetic procedures, HPLC−UV, GC-FID, and NMR data, and enzyme/primer sequences and additional figures and tables (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wolfgang.kroutil@uni-graz.at. Telephone: +43 (0)316 380−5350.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research leading to these results was funded by the European Union's 7th framework program FP7/2007−2013 under Grant 289646 (KyroBio). COST Action CM1303 "Systems Biocatalysis" is acknowledged. Klaus Zangger (University of Graz, Graz, Austria) and his group are acknowledged for recording the NMR spectra.

■ REFERENCES

(1) Facchini, P. J. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2001**, *52*, 29−66.
(2) Saxton, E. J. *Nat. Prod. Rep.* **1997**, *14*, 559−590.
(3) van Der Heijden, R.; Jacobs, D. I.; Snoeijer, W.; Hallard, D.; Verpoorte, R. *Curr. Med. Chem.* **2004**, *11*, 607−628.
(4) Jonson, I. S.; Wright, H. F.; Svoboda, G. H. *J. Lab. Clin. Med.* **1959**, *54*, 173−178.
(5) Ma, X.; Panjikar, S.; Koepke, J.; Loris, E.; Stöckigt, J. *Plant Cell* **2006**, *18*, 907−920.
(6) Stöckigt, J.; Zenk, M. H. *J. Chem. Soc., Chem. Commun.* **1977**, *18*, 646−648.
(7) Treimer, J. F.; Zenk, M. H. *Eur. J. Biochem.* **1979**, *101*, 225−233.
(8) Stöckigt, J.; Antonchick, A. P.; Wu, F.; Waldmann, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 8538−8564.
(9) Pictet, A.; Spengler, T. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 2030−2036.
(10) Scott, A. I.; Lee, S. L. *J. Am. Chem. Soc.* **1975**, *97*, 6906−6908.
(11) Mizukami, H.; Nordlöv, H.; Lee, S. L.; Scott, A. I. *Biochemistry* **1979**, *18*, 3760−3763.
(12) Hampp, N.; Zenk, M. H. *Phytochemistry* **1988**, *27*, 3811−3815.
(13) Kutchan, T. M.; Dittrich, H.; Bracher, D.; Zenk, M. H. *Tetrahedron* **1991**, *47*, 5945−5954.
(14) Yang, L.; Zou, H.; Zhu, H.; Ruppert, M.; Gong, J.; Stöckigt, J. *Chem. Biodiversity* **2010**, *7*, 860−870.
(15) McCoy, E.; Galan, M. C.; O'Connor, S. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2475−2478.
(16) Stöckigt, J.; Barleben, L.; Panjikar, S.; Loris, E. A. *Plant Physiol. Biochem.* **2008**, *46*, 340−355.
(17) O'Reilly, E.; Turner, N. J. *Perspectives in Science* **2015**, *4*, 55−61.
(18) Muschiol, J.; Peters, C.; Oberleitner, N.; Mihovilovic, M. D.; Bornscheuer, U. T.; Rudroff, F. *Chem. Commun.* **2015**, *51*, 5798−5811.
(19) Köhler, V.; Turner, N. J. *Chem. Commun.* **2015**, *51*, 450−464.
(20) Simon, R. C.; Richter, N.; Busto, E.; Kroutil, W. *ACS Catal.* **2014**, *4*, 129−143.
(21) Schrittwieser, J. H.; Sattler, J.; Resch, V.; Mutti, F. G.; Kroutil, W. *Curr. Opin. Chem. Biol.* **2011**, *15*, 249−256.
(22) Ricca, E.; Brucher, B.; Schrittwieser, J. W. *Adv. Synth. Catal.* **2011**, *353*, 2239−2262.
(23) Tufvesson, P.; Lima-Ramos, J.; Jensen, J. S.; Al-Haque, N.; Neto, W.; Woodley, J. M. *Biotechnol. Bioeng.* **2011**, *108*, 1479−1493.
(24) Seo, J.-H.; Kyung, D.; Joo, K.; Lee, J.; Kim, B.-G. *Biotechnol. Bioeng.* **2011**, *108*, 253−263.
(25) Koszelewski, D.; Lavandera, I.; Clay, D.; Guebitz, G. M.; Rozzell, D.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 9337−9340.
(26) Truppo, M. D.; Rozzell, J. D.; Turner, N. J. *Org. Process Res. Dev.* **2010**, *14*, 234−237.

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30

(27) Hailes, H. C.; Dalby, P. A.; Lye, G. J.; Baganz, F.; Micheletti, M.; Szita, N.; Ward, J. M. *Curr. Org. Chem.* **2010**, *14*, 1883−1893.
(28) Park, E.; Kim, M.; Shin, J.-S. *Adv. Synth. Catal.* **2010**, *352*, 3391−3398.
(29) Cassimjee, K. E.; Branneby, C.; Abedi, V.; Wells, A.; Berglund, P. *Chem. Commun.* **2010**, *46*, 5569−5571.
(30) Höhne, M.; Bornscheuer, U. T. *ChemCatChem* **2009**, *1*, 42−51.
(31) Simon, R. C.; Richter, N.; Busto, E.; Kroutil, W. *ACS Catal.* **2014**, *4*, 129−143.
(32) Sehl, T.; Hailes, H. C.; Ward, J.; Wardenga, R.; von Lieres, E.; Offermann, H.; Westphal, R.; Pohl, M.; Rother, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 6772−6775.
(33) Siirola, E.; Frank, A.; Grogan, G.; Kroutil, W. *Adv. Synth. Catal.* **2013**, *355*, 1677−1691.
(34) Ghislieri, D.; Turner, N. J. *Top. Catal.* **2014**, *57*, 284−300.
(35) Kohls, H.; Steffen-Munsberg, F.; Höhne, M. *Curr. Opin. Chem. Biol.* **2014**, *19*, 180−192.
(36) Schmidt, N. G.; Simon, R. C.; Kroutil, W. *Adv. Synth. Catal.* **2015**, *357*, 1815−1821.
(37) Holzer, A. K.; Hiebler, K.; Mutti, F. G.; Simon, R. C.; Lauterbach, L.; Lenz, O.; Kroutil, W. *Org. Lett.* **2015**, *17*, 2431−2433.
(38) Richter, N.; Farnberger, J. E.; Pressnitz, D.; Lechner, H.; Zepeck, F.; Kroutil, W. *Green Chem.* **2015**, *17*, 2952−2958.
(39) Nichols, D. E.; Lloyd, D. H.; Johnson, M. P.; Hoffman, A. J. *J. Med. Chem.* **1988**, *31*, 1406−1412.
(40) Yamada, Y.; Iwasaki, A.; Kizaki, N. (K. Corporation). European Patent EP 0987332A1, 2000.
(41) Hanson, R. L.; Davis, B. L.; Chen, Y.; Goldberg, S. L.; Parker, W. L.; Tully, T. P.; Montana, M. A.; Patel, R. N. *Adv. Synth. Catal.* **2008**, *350*, 1367−1375.
(42) Steffen-Munsberg, F.; Vickers, C.; Thontowi, A.; Schätzle, S.; Meinhardt, T.; Svedendahl Humble, M.; Land, H.; Berglund, P.; Bornscheuer, U. T.; Höhne, M. *ChemCatChem* **2013**, *5*, 154−157.
(43) Höhne, M.; Schätzle, S.; Jochens, H.; Robins, K.; Bornscheuer, U. T. *Nat. Chem. Biol.* **2010**, *6*, 807−813.
(44) Kawano, S.; Ito, N.; Yasohara, Y. WO 2007/139055, 2007.
(45) Kaulmann, U.; Smithies, K.; Smith, M. E. B.; Hailes, H. C.; Ward, J. M. *Enzyme Microb. Technol.* **2007**, *41*, 628−637.
(46) Nagai, F.; Nonaka, R.; Satoh Hisashi Kamimura, K. *Eur. J. Pharmacol.* **2007**, *559*, 132−137.
(47) Glennon, R. A. *J. Med. Chem.* **1987**, *30*, 1−12.
(48) Arunotayanun, W.; Dalley, J. W.; Huang, X. P.; Setola, V.; Treble, R.; Iversen, L.; Roth, B. L.; Gibbons, S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3411−3415.
(49) Rodriguez-Mata, M.; Gotor-Fernandez, V.; Gonzalez-Sabin, J.; Rebolledo, F.; Gotor, V. *Org. Biomol. Chem.* **2011**, *9*, 2274−2278.
(50) Pietra, S.; Tacconi, G. *Farmaco. Sci.* **1958**, *13*, 893−910.
(51) Pradhan, P. K.; Dey, S.; Jaisankar, P.; Giri, V. S. *Synth. Commun.* **2005**, *35*, 913−922.
(52) Bracher, D.; Kutchan, T. M. *Arch. Biochem. Biophys.* **1992**, *294*, 717−723.
(53) Loris, E. A.; Panjikar, S.; Ruppert, M.; Barleben, L.; Unger, M.; Schübel, H.; Stöckigt, J. *Chem. Biol.* **2007**, *14*, 979−985.
(54) Pasquali, G.; Goddijn, O. J.; de Waal, A.; Verpoorte, R.; Schilperoort, R. A.; Hoge, J. H.; Memelink, J. *Plant Mol. Biol.* **1992**, *18*, 1121−1131.
(55) Bernhardt, P.; Usera, A. R.; O'Connor, S. E. *Tetrahedron Lett.* **2010**, *51*, 4400−4402.
(56) Lu, Y.; Wang, H.; Wang, W.; Qian, Z.; Li, L.; Wang, J.; Zhou, G.; Kai, G. *Mol. Biol. Rep.* **2009**, *36*, 1845−1852.
(57) Alfaro Blasco, M. A.; Gröger, H. *Bioorg. Med. Chem.* **2014**, *22*, 5539−5546.
(58) Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Sattler, J. H.; Kroutil, W. *Adv. Synth. Catal.* **2011**, *353*, 3227−3233.
(59) Franklin, C. S.; White, A. C. *J. Chem. Soc.* **1963**, 1335−1336.

DOI: 10.1021/acscatal.5b01839
ACS Catal. 2016, 6, 23−30