EurJOC
European Journal of Organic Chemistry

Full Papers
doi.org/10.1002/ejoc.202100174

Chemistry Europe
European Chemical Societies Publishing

Special Collection

Biocatalytic Approaches to the Enantiomers of Wieland–Miescher Ketone and its Derivatives

Susanna Bertuletti,[a, b] Ikram Bayout,[a, c] Ivan Bassanini,[a] Erica E. Ferrandi,[a] Nassima Bouzemi,[c] Daniela Monti,[a] and Sergio Riva*[a]

This manuscript is dedicated to Prof. Franco Cozzi on the occasion of his 70th birthday.

Biocatalytic approaches have been investigated in order to isolate the enantiomers of Wieland–Miescher ketone (**1**) and of its alcoholic derivatives (*cis*-**2** and *trans*-**3**). Specifically, two enzymes from our in-house metagenomic collection of oxidoreductases, IS2-SDR and Dm7α-HSDH, catalyzed the kinetic resolution of the starting racemic ketone **1** or its complete conversion into two diastereomeric products, respectively. Moreover, the kinetic resolution of the racemic *cis*-alcohol (**2**) was very efficiently obtained (E≅2.000) by lipase PS catalyzed acetylation in dry acetone. All the products were isolated with ee ≥ 95 %. Simple chemical elaborations of some of them allowed to isolate the missing enantiomers.

Introduction

The so-called Wieland-Miescher ketone (**1**, Scheme 1) is an important intermediate widely used in the total synthesis of complex natural products, predominantly with a terpenoid structure.[1–3] It can be easily prepared in its racemic form from 2-methylcyclohexane-1,3-dione and methyl vinyl ketone *via* the Robinson annulation, a tandem process proposed in 1935 including a Michael addition and an intramolecular aldol condensation.[4]

The enantiomerically enriched form of **1** was firstly described in 1971, when two different groups of industrial chemists independently proposed an asymmetric version of the Robinson annulation catalyzed by proline.[5–7] Since then, the enantioselective synthesis of one of the enantiomers of **1** ((8a*S*)-**1a** and (8a*R*)-**1b**) has become a model target to evaluate the performances of new chiral organocatalyst,[8–10] as it has been recently carefully reviewed.[11,12]

The unselective reduction of the C_1 carbonyl moiety of **1** should theoretically afford a complex mixture of stereoisomeric alcohols (5-hydroxy-4a-methyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one) which exist as two couples of *cis*- and *trans*-enantiomers (compounds (4a*S*, 5*S*)-**2a**, (4a*R*, 5*R*)-**2b** and (4a*S*, 5*R*)-**3a**, (4a*R*, 5*S*)-**3b** in Scheme 1). These chiral derivatives, when obtained as enantiomerically enriched species, are known to be important building blocks for the synthesis of natural, bioactive products or compounds of pharmaceutical interest.[13–17] The presence of an enone group, in fact, easily allows to further manipulate their molecular skeleton and to transform them into structurally complex compounds.

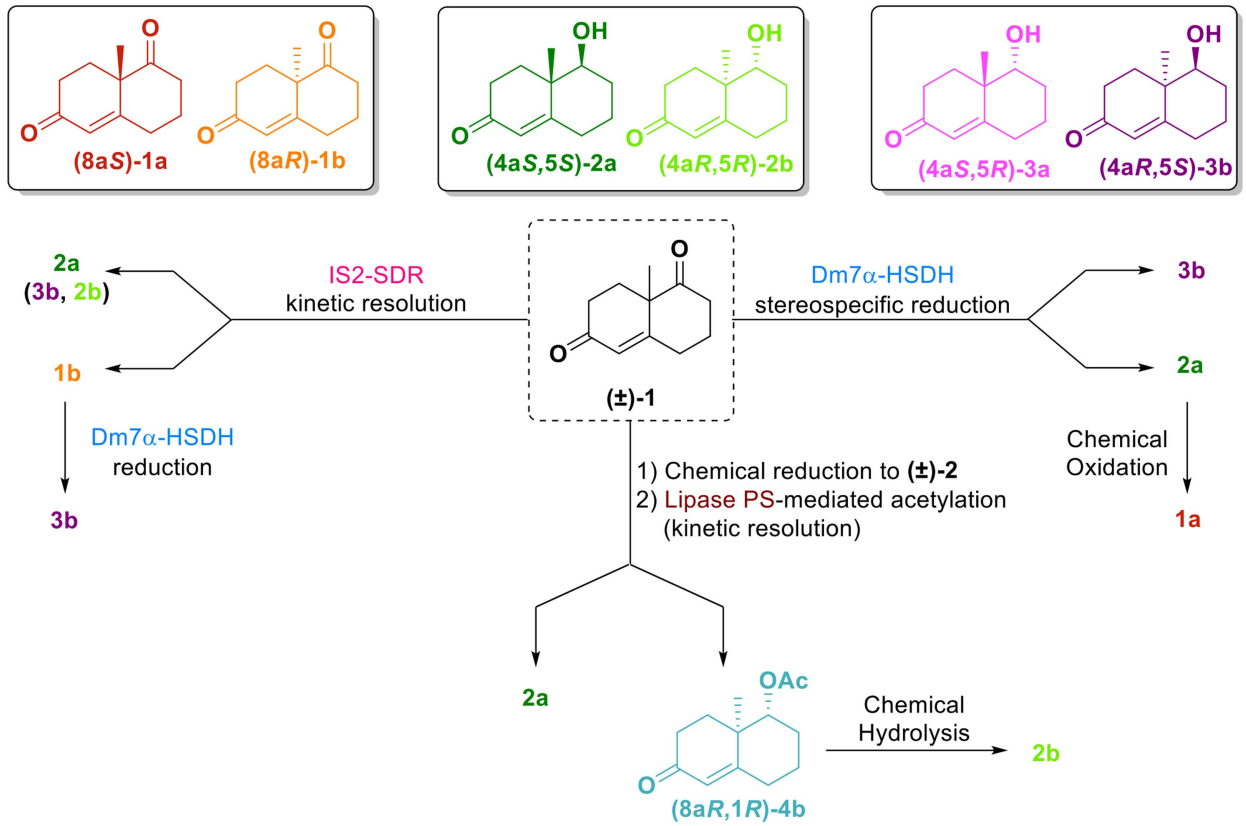
From a chemical point of view, the reduction of racemic **1** with common hydride-based reducing agents (e.g., $NaBH_4$), due to the steric hindrance determined by the adjacent C_{8a} methyl group, usually gives access only to a racemic mixture of the *cis*-alcohols (**2a** and **2b**).[18] The two *trans*-alcohols **3a** and **3b** appear to be accessible only from the *cis*-isomers *via* low-efficient protocols of stereoinversion.[19]

Alternative "bio"-based strategies have been investigated to build a convenient entry to the enantiomers of **1** and/or to isolate compounds **2a**–**b** and **3a**–**b** as stereo-enriched species. On this respect, it deserves to be mentioned the outstanding results obtained by Lerner, Danishefsky and coworkers, who developed a catalytic antibody able to catalyze the enantioselective Robinson annulation.[20] The reaction was performed at a 110 mg scale and the *S*-enantiomer **1a** could be isolated in 94 % yield and 96 % optical purity.

More recently a biocatalyzed approach to the synthesis of **1**, *via* the Robinson annulation, has been proposed, exploiting the catalytic promiscuity[21] of a commercial lipase from porcine pancreas.[22] However, a more detailed investigation of the protein components of that crude preparation demonstrated that non-lipase contaminant enzymes, and specifically an α-amylase, were apparently responsible for the observed transformation. In any case, even under optimized conditions, the results both in terms of yield and enantiomeric excess of **1** were significantly lower when compared with a control organocatalyzed (with *L*-proline) reaction.[23]

[a] S. Bertuletti, I. Bayout, Dr. I. Bassanini, Dr. E. E. Ferrandi, Dr. D. Monti, Dr. S. Riva
Istituto di Scienze e Tecnologie Chimiche "Giulio Natta"
Consiglio Nazionale delle Ricerche
Via Mario Bianco 9, Milano, 20131, Italy
E-mail: sergio.riva@scitec.cnr.it
http://www.scitec.cnr.it/personale/bianco-ita/sergio-riva
[b] S. Bertuletti
Pharmaceutical Sciences Department
University of Milan
Via Mangiagalli 25, Milano, 20133, Italy
[c] I. Bayout, Dr. N. Bouzemi
Ecocompatible Asymmetric Catalysis Laboratory (LCAE). Badji Mokhtar Annaba-University,
BP12, Annaba 23000, Algeria

Special Collection Part of the "Franco Cozzi's 70th Birthday" Special Collection.Supporting information for this article is available on the WWW under https://doi.org/10.1002/ejoc.202100174



Scheme 1. (Chemo)enzymatic manipulation of racemic Wieland–Miescher ketone (±**1**) and of its alcoholic derivatives (±**2**).

A partial kinetic resolution of racemic **1** was obtained by submitting this compound to the action of a cyclohexanone monooxygenase from *Acinetobacter calcoaceticus*, but the preferred enantiomer was irreversibly oxidized to the corresponding lactone.[18] Nevertheless, a biocatalyzed synthetic strategy, exploiting the intrinsic enantioselectivity of enzymes, might offer a simple and efficient solution to the separation of the two enantiomers of the easily obtainable racemic **1**. To our surprise, quite few reports can be found in the literature on this topic so far, most of them describing biotransformations with microbial whole cells. Stereoselective reduction of racemic **1** was firstly described by Prelog using whole cells of *Curvularia falcata*.[24–27] Later on, Sugai described the results obtained with yeast strains of *Torulaspora delbrueckii* and *Candida melibiosica*,[28,29] and Kodama with baker's yeast.[30] More recently Janeczko described the reductive performances of *Didymosphaeria igniaria* and *Coryneum betulinium* strains.[31]

Apparently, no examples of the stereoselective reduction of **1** catalyzed by isolated enzymes have been reported to date. Therefore, we considered racemic **1** an interesting substrate to evaluate the performance of our in-house collection of dehydrogenases, including new metagenomic and extremophilic enzymes.[32]

Moreover, complementary biocatalyzed strategies, i.e., the well-known kinetic resolution of racemic alcohols catalyzed by hydrolases, deserve to be explored to build alternative entries to Wieland-Miescher ketone derivatives as enantiomerically enriched species. On this respect, Shimizu *et al.* were able to obtain the stereoisomeric alcohol derivatives of **1** by conducting an enzymatic asymmetric hydrolysis (exploiting a library of hydrolases of different origins)[33] of the acetylated racemates of alcohols **2** and **3** obtained from **1** according to the above mentioned protocols.[18,19] Best results were obtained on the *cis* racemate **2** using a β-amilase from wheat. On the other hand, to our surprise again, we could not find examples related to the use of lipases in organic solvents[34] to catalyze the kinetic resolution, by esterification, of racemic **2** and **3**.

In the following we report the results that we have obtained investigating the performances of new oxidoreductases and a well-known lipase, together with a critical comparison with the literature data.

Results and Discussion

One of the most interesting properties of enzymes, from a synthetic point of view, is their substrate promiscuity, that is their ability to transform compounds with chemical structures significantly different from those of their natural substrates, still maintaining high levels of stereoselectivity. On this respect, we recently reported on the performances of a collection of new hydroxysteroid dehydrogenases (HSDHs), identified either by genome mining or by metagenomics approaches, in the reduction of a panel of substrates including, in addition to the

"natural" hydroxysteroids, α-ketoesters and selected ketones partially resembling the structural features of steroids.[32]

The results obtained in the reduction of racemic **1** *via* the preliminary screening of our panel of dehydrogenases is summarized in Table 1. Among the 15 (mostly new) enzymes tested, including 13 HSDHs (7α-HSDHs, 7β-HSDHs, and 12α-HSDHs) and two short-chain dehydrogenases/reductases (SDRs), only four accepted **1** as a substrate, two of them with significantly higher conversion values. The reactions with these two enzymes, IS2-SDR (a SDR identified from a metagenome obtained from Icelandic hot spring sediments) and Dm7α-HSDH (a 7α-hydroxysteroid dehydrogenase identified in the genome of the extremophilic bacterium *Deinococcus marmoris* strain PAMC 26562, a radiation-resistant and psychro- and draught-tolerant bacterium isolated from an Antarctic rock sample), were analyzed in more details as their chiral phase HPLC chromatograms showed two different reaction outcomes. As reported in Figure 1, while with Dm7α-HSDH a complete conversion of **1** into two products was obtained, IS2-SDR catalyzed a kinetic resolution of the starting racemic ketone.

The reactions were scaled-up in order to isolate and characterize the products, using the ancillary enzyme glucose dehydrogenase (GDH) to regenerate *in situ* the needed reduced cofactor (NADH for Dm7α-HSDH and NADPH for IS2-SDR). As summarized in the left upper part of Scheme 1, the kinetic resolution of **1** in the reduction catalyzed by IS2-SDR allowed

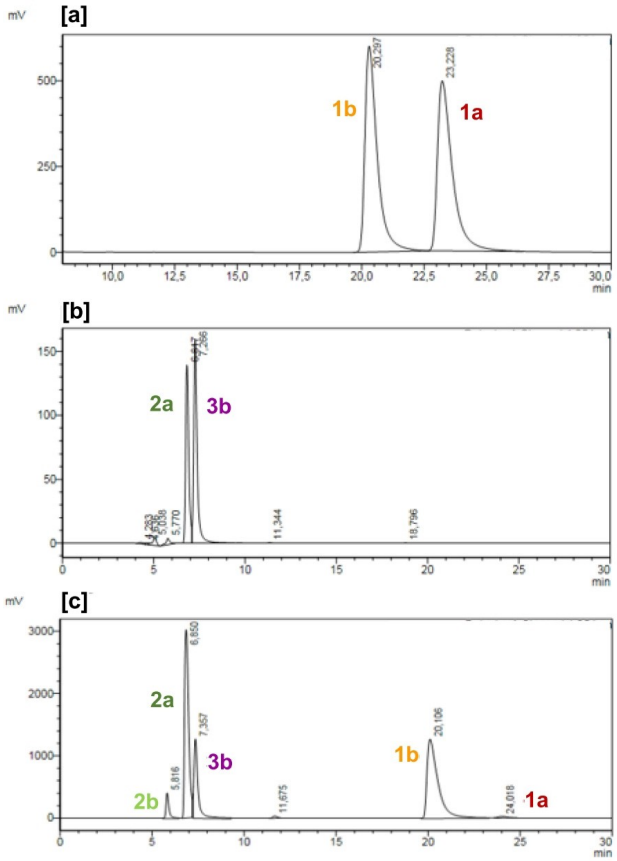


Figure 1. HPLC chromatograms of [a] racemic **1**; [b] Dm7α-HSDH stereoselective biocatalyzed reduction of racemic **1**; [c] IS2-SDR biocatalyzed kinetic resolution of racemic **1**.

Table 1. Screening of dehydrogenases for the reduction of racemic **1**.[a]

Entry	Enzyme[b]	Source[c]	Cofactor	Conversion[d]
1	Ca7α-HSDH	*Clostridium absonum*	NADP (H)	<1%
2	Dm7α-HSDH	*Deinococcus marmoris*	NAD(H)	>99%
3	Ec7α-HSDH	*Escherichia coli*	NAD(H)	6%
4	Hh7α-HSDH	*Halomonas halodenitrificans*	NAD(H)	<1%
5	NGI7α-HSDH	Norwegian metagenome	NAD(H)	<1%
6	Bsp7β-HSDH	*Bacillus* sp.	NAD(H)	<1%
7	Ca7β-HSDH	*Clostridium absonum*	NADP (H)	<1%
8	Cae7β-HSDH	*Collinsella aerofaciens*	NADP (H)	<1%
9	Hh7β-HSDH	*Halomonas halodenitrificans*	NAD(H)	<1%
10	Rs7β-HSDH	*Rhodobacter sphaeroides*	NAD(H)	<1%
11	Sc7β-HSDH	*Stanieria cyanosphaera*	NAD(H)	<1%
12	Csp12α-HSDH	*Clostridium* sp.	NADP (H)	<1%
13	Ls12α-HSDH	*Lysinibacillus sphaericus*	NAD(H)	<1%
14	IS2-SDR	Icelandic metagenome	NADP (H)	66%
15	NGI7-SDR	Norwegian metagenome	NAD(H)	12%

[a] Reaction conditions: 10 mM substrate; 50 mM phosphate buffer pH 7; 5% v/v DMSO; 50 mM glucose; 0.4 mM cofactor; 4 U mL^{-1} DH; 1 U mL^{-1} GDH; 0.4 M NaCl (only for entries 4 and 9); 25 °C, 100 rpm. [b] The library is composed both of HSDHs (entries 1–13) and of short chain reductases (SDRs, entries 14 and 15). [c] The recombinant enzymes were either from defined chemical sources or coming from metagenomic samples, as detailed in ref [32]. [d] Conversions were evaluated after 48 h by GC/MS and, for the entries 2, 3, 14, and 15, also by chiral phase HPLC analyses.

the isolation of the enantiomer **1b** with a 96.5% e.e ($[\alpha]_D^{22}$ –88.7°, c 1.0 in $CHCl_3$), in 32% yield. This result compares well, both in terms of selectivity and reaction outcome, with those obtained by Sugai with whole cells of *T. delbrueckii*,[28] by Kodama with baker's yeast,[30] and by Janeczko with *D. igniaria*.[31]

Later on this reaction was investigated in more details by submitting the two separate enantiomers to the catalytic action of IS2-SDR. It was confirmed that the enantiomer **1a** (8a*S* configuration) was the preferred one, the relative ratio of the reaction rates of its reduction *vs* that of **1b** being approximately 3 to 1. Moreover, the IS2-SDR-catalyzed reduction of **1a** was stereoselective and the *cis* alcoholic enantiomer **2a** was the only product. On the contrary, the slow reduction of **1b** gave predominantly the *trans* alcohol **3b**, accompanied by the *cis* diastereoisomer **2b** (relative ratio **3b** vs **2b**:4:1).

The stereospecific reduction of **1** was obtained in the reaction catalyzed by Dm7α-HSDH, which gave a 1:1 mixture of two diastereoisomers, whose preparative separation was quite troublesome and could be achieved by using a Biotage® SP1 system for flash chromatography. The two products proved to be the *cis*-alcohol **2a** derived from the enantiomer **1a** and the *trans*-alcohol **3b** derived from the enantiomer **1b** (Scheme 1, right upper part). Pure **3b** (>99% ee, $[\alpha]_D^{22}$ –111.6°, c 1.0 in

$CHCl_3$) was also obtained by Dm7α-HSDH-catalyzed reduction of the previously isolated enantiomer **1b** (Scheme 1, left upper part). In terms of stereoselectivity, the production of the *trans*-alcohol **3b** from the ketone enantiomer **1b** is in agreement with data reported by Sugai with whole cells of *C. melibiosica* (lower ee values were obtained)[28] and by Prelog with *C. falcata*.[24] A similar outcome but with much lower selectivity was observed by Janeczko in the biotransformations catalyzed by strains of *D. igniaria* and of *C. betulinium* whole cells.[31]

It is worthy to be reminded that the *trans*-alcohol **3** is not obtained using the classical chemical reductive reagents and only the *cis*-alcohol **2** could be isolated.[18,35] Moreover, once again it was possible to observe a significant selectivity of a HSDH, in this case devoted to the regio- and stereoselective modification of oxygenated substituent at the position C_7 of the steroid skeleton, with compounds not structurally related to a steroid. For additional examples of the substrate promiscuity of HSDHs see ref 33 and 36–41.

The easy availability of the racemic mixture of the *cis*-alcohol **2a** and **2b** prompted us also to evaluate an alternative biocatalyzed approach, exploiting the well-known enantioselectivity of hydrolases in the acylation of secondary alcohols in organic solvents.[34] As previously stated, to our surprise, apparently this substrate has never been considered in previous investigations, despite the fact that it is clearly a suitable target to be acylated by lipases, as shown by Franssen and coworkers with a structurally not so different compound.[42]

Compound **2** was dissolved in dry acetone containing a large excess (10% v/v) of the acylating agent vinyl acetate and different lipases were added. The reactions were shaken at 45 °C and monitored by TLC and chiral phase HPLC. As shown in Figure 2 we were pleased to find that the so-called lipase PS, adsorbed on celite, showed a really significant enantioselectivity. Actually, the value of enantiomeric ratio "E", that could be calculated from the values of degree of conversion and ee of the residual substrate according to the well-known formula proposed by Sih years ago,[43] was very high: approximately 2.000. This value makes this biocatalyzed acetylation really close to an ideal kinetic resolution process that spontaneously stops after the preferred enantiomer is consumed. And this was indeed the case, and the two pure *cis* enantiomers, the alcohol **2a** and the acetate **4b**, could be isolated and characterized.

The same protocol was applied to the diastereomeric mixture of alcohols **2a** and **3b** obtained by the reaction catalyzed by Dm7α-HSDH, but no conversion was observed with any of the lipases tested, leaving the above-described chromatographic protocol as the only way to separate these two compounds. The hydrolysis of the ester **4b** furnished the pure missing enantiomer of the alcohol, that is **2b**, while oxidation of the alcohol **2a** gave an easy and efficient entry to ketone **1a**.

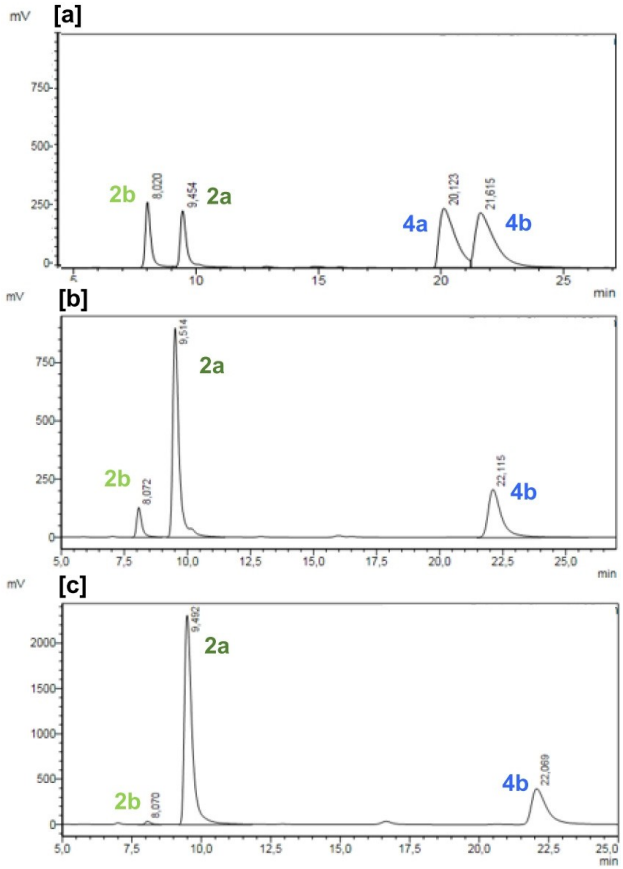


Figure 2. HPLC chromatograms of [a] racemic **2** + racemic **4**; [b] Lipase PS-biocatalyzed kinetic resolution of racemic **2** after 72 h; [c] After 120 h.

Conclusion

Wieland–Miescher ketone (**1**) has been chosen as a model synthon to evaluate the selectivity of a panel of dehydrogenases, including enzymes with different substrate scope as well as biological origin. The obtained results further exemplify the substrate promiscuity of this group of enzymes. Moreover, a more classical approach based on the kinetic resolution of a racemic mixture of the *cis* alcohol (**2**) catalyzed by lipase PS in acetone proved to be particularly efficient, very close to an ideal process in terms of enantioselectivity of the biocatalyst.

The dehydrogenase-catalyzed reactions were performed on a 50–100 mg scale, whereas the lipase-catalyzed kinetic resolution was obtained starting from 1 g of the substrate. All the reactions can be easily scaled-up as the DHs are available as recombinant overexpressed proteins, while lipase PS is a cheap commercially available enzyme. Specifically, the lipase-catalyzed reaction has not been optimized in terms of substrate concentration and substrate/biocatalyst ratio, therefore further improvements of productivity and solvent consumption are likely to be easily achieved at will.

The *trans* enantiomer **3a** is presently the only isomer that could not be produced by an enzyme-catalyzed reaction and this target will be the object of further future investigations.

Experimental Section

General

Wieland-Miescher ketone (8a-methyl-3,4,8,8a-tetrahydronaphthalene-1,6(2H,7H)-dione, purity 98.5%) was purchased from Fluorochem (Hadfield, UK). Amano lipase PS (adsorbed by us on celite)[44] was from Merck (Darmstadt, Germany). All reagents and solvents were purchased from Merck and used without further purification, unless otherwise stated.

Reactions were monitored *via* TLC (thin-layer chromatography) on pre-coated glass plates silica gel 60 with fluorescent indicator UV_{254} and treated with an oxidizing solution [4-hydroxybenzaldehyde (6.3 g), H_2SO_4 (50% v/v in H_2O, 40 mL), MeOH (400 mL)].

Abbreviations

HSDH: Hydroxysteroid Dehydrogenase; U: enzymatic unit; SDR: Short Chain Dehydrogenase/Reductase; BmGDH: Glucose Dehydrogenase from *Bacilllus megaterium*; IPTG: Isopropyl β-D-1-thiogalactopyranoside.

Enzyme Preparation

HSDHs/SDRs expression and purification

Expression and purification of HSDHs, SDRs, and BmGDH were carried out as previously described.[32]

Expression conditions of Dm7α-HSDH were optimized by expression trials carried out as follows: *E. coli* BL21(DE3) harboring the expression plasmid pETiteDm7aHSDH was inoculated overnight in 50 mL LB medium supplemented with 30 $\mu g\,mL^{-1}$ kanamycin (LB_{kan30}) and grown at 37 °C, 220 rpm. The overnight culture was subsequently inoculated in 1 L LB_{kan30} medium and incubated at 37 °C, 220 rpm. Protein expression was then induced with 1 mM IPTG at an OD_{600} of 0.5–1. The culture was transferred to 30, 25 or 17 °C with shaking at 220 rpm and grown for 24, 48 or 72 h, respectively. The cells were harvested by centrifugation at 6000 rpm for 10 min at 4 °C and resuspended in 20 mL lysis buffer (20 mM potassium phosphate (KP) buffer, pH 7.0, 500 mM NaCl, 20 mM imidazole). After disruption by sonication at 4 °C, the cell extract was centrifuged at 12000 rpm at 4 °C for 30 min and clear lysates were assessed for the presence of soluble protein by SDS-PAGE (12% T, 2.6% C). Protein purification was performed as previously described.[32] Cell incubation at 17 °C for 72 h after induction resulted in the best expression yields (i.e., 108 mg of pure Dm7α-HSDH (11.575 U) were obtained after protein purification).

Activity assays

Dehydrogenase activities of HSDHs and BmGDH were determined spectrophotometrically by measuring the reduction of $NAD(P)^+$ at 340 nm (ε: 6.22 $mM^{-1}cm^{-1}$), while the activity of IS2-SDR was measured by following the oxidation of NADPH at the same wavelength. Assays were carried out in polyethylene cuvettes at room temperature by adding the opportune purified dehydrogenase (1–20 μL) to the following assay mixtures (1 mL final volume):

HSDH assay: 2.5 mM substrate (cholic acid for 7α- and 12α-HSDHs, ursodeoxycholic acid for 7β-HSDHs); 50 mM potassium phosphate buffer, pH 9.0; 0.20 mM $NAD(P)^+$.

IS2-SDR assay: 10 mM methyl benzoylformate; 50 mM potassium phosphate buffer, pH 8.0; 0.20 mM NADPH.

BmGDH assay: 50 mM glucose; 50 mM potassium phosphate buffer, pH 7.0; 0.20 mM $NAD(P)^+$.

One unit (U) is defined as the enzyme activity that reduces/oxidizes 1 μmol of NAD(P)(H) per min under the assay conditions described above.

Analytical methods

At scheduled times, reaction samples (50 μL) were extracted with AcOEt and dried over Na_2SO_4 to afford 10 mM samples, submitted to GC-MS analysis, or evaporated, resuspended in *i*-PrOH to a 10 mM final concentration and analyzed by chiral HPLC.

GC-MS analyses were performed using an Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm) on a Finnigan TRACE DSQ GC/MS instrument (ThermoQuest, San Jose, CA). Elution conditions: 60 °C, 1 min; + 10 °C min^{-1} until 300 °C; hold 1 min; flow rate: 1.0 mL min^{-1}; inlet temperature: 250 °C; ion source temperature: 250 °C; MS transfer line temperature: 250 °C. Retention times: (**1a**,**1b**): 13.7 min; (**2a**,**2b**): 14.6 min; (**3a**,**3b**): 14.8 min; (**4a**,**4b**): 15.4 min.

Chiral phase HPLC analyses were performed on a Shimadzu LC-20AD high performance liquid chromatography system equipped with a Shimadzu SPD-20 A UV detector and a Phenomenex Lux 3u Cellulose-2 chiral column (250 mm × 4.6 mm). HPLC conditions: injection volume 10 μL; mobile phase: *i*-PrOH: petroleum ether = 1:1; flow rate: 0.7 mL min^{-1}; detection λ: 254 nm; temperature: 33 °C. Retention times: (**1a**): 22.4 min; (**1b**): 19.1 min; (**2a**): 6.9 min; (**2b**): 5.9 min; (**3b**): 7.3 min; (**4a**): 16.1 min; (**4b**): 15.8 min. Prior to performing HPLC analyses, the molar extinction coefficients of **1**, **2** and **4** were determined, to be able to assess conversions while considering the different molar absorbivity of the molecules under analysis.

The nature of the obtained compounds, which have been previously characterized in the literature, was confirmed by means of ^{1}H-NMR, mass spectrometry and optical rotation measurements.

The NMR spectra were acquired in $CDCl_3$ or in DMSO-d_6 at rt on a Bruker AV 400 MHz spectrometer with a z gradient at 400 MHz for ^{1}H-NMR analysis and 101 MHz for ^{13}C-NMR.

ESI-MS spectra were recorded on a Bruker Esquire 3000 PLUS instrument (ESI Ion Trap LC/MSn System), equipped with an ESI source and a quadrupole ion trap detector (QIT). The samples were dissolved in methanol to 1–2 g L^{-1} and then directly syringed in the ESI-MS at 4 μL min^{-1} rate. The analyses were performed in positive mode. The acquisition parameters were optimized as such: 4.5 kV needle voltage, 10 L h^{-1} N_2 flow rate, 40 V cone voltage, trap drive set to 46, 115.8 V capillary exit, 13000 (m/z) s^{-1} scan resolution over the 35–900 m/z mass/charge range, source temperature 250 °C.

Optical rotations were measured on a Jasco P-2000 polarimeter. The specific rotation was calculated as the $[\alpha]\lambda T = \alpha 100/cd$, where α represents the recorded optical rotation, c the analyte concentration (mg mL^{-1}), d the cuvette length (dm). As such, the specific rotation is expressed as 10^{-1} deg cm^{-2} g^{-1}. λ is reported in nm and T in °C. λ corresponds to sodium D line (589 nm), thus the optical rotation is referred to as $[\alpha]_D$. T, c and the solvent were chosen according to references reported in literature (see below).

Determination of the molar absorbivity coefficients of compounds 1, 2 and 4

The molar extinction coefficients of ketone **1**, *cis*-alcohol **2** and *cis*-acetate **4** were determined at 254 nm. For each compound, a 10 mM solution in CH_3CN was prepared and it was used as a mother solution to test the absorption of each molecule in 1 mL quartz cuvettes in the concentration range of 0.1-25 μM in CH_3CN (where linearity of data was observed). ε_{254} (**1**): 6508 $M^{-1}cm^{-1}$; ε_{254} (**2**): 5889 $M^{-1}cm^{-1}$; ε_{254} (**4**): 2620 $M^{-1}cm^{-1}$ (for more details see Paragraph 1, Supporting information).

Characterization of commercially available racemic 1

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (d, J = 1.8 Hz, 1H), 2.84–2.65 (m, 2H), 2.61–2.39 (m, 4H), 2.30–2.04 (m, 3H), 1.72 (qt, J = 13.3, 4.4 Hz, 1H), 1.46 (s, 3H). ESI-MS: $[M+Na]^+$: 201.0.

Synthetic chemistry

Preparation of standard racemate 2, (±)-4 a,5-cis-(5-hydroxy-4 a-methyl-4,4 a,5,6,7,8-hexahydronaphthalen-2(3H)-one)[18]

The reduction of substrate **1** was performed following a standard protocol with $NaBH_4$. To a stirred solution of 0.4 M substrate (1 eq, 100 mg) in EtOH (5 mL) at 0 °C, an ethanolic suspension (0.4 M) of $NaBH_4$ (1 eq, 13 mg) was added dropwise. After 4 hours, the reaction was quenched with a saturated aqueous solution of NH_4Cl, then it was extracted with AcOEt (3x). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield a crude extract. A flash chromatography purification afforded the desired products (**2 a** + **2 b**) in up to 90% yield. The racemic product **2** was characterized by chiral HPLC (see analytical methods) and ^{1}H-NMR analyses. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.78 (d, J = 1.8 Hz, 1H), 3.42 (dd, J = 11.6, 4.3 Hz, 1H), 2.50–2.28 (m, 3H), 2.25–2.13 (m, 2H), 1.94–1.78 (m, 3H), 1.76–1.64 (ddd, J = 16.9, 13.3, 4.2 Hz, 1H), 1.48–1.33 (qt, J = 13.2, 4.0 Hz, 1H), 1.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.66, 199.56, 168.43, 125.50, 78.28, 41.63, 34.27, 33.72, 32.03, 30.31, 23.20, 15.27. ESI-MS: $[M+Na]^+$: 203.0.

Preparation of standard racemate 4, (±)-8 a,1-cis-(-8 a-methyl-6-oxo-1,2,3,4,6,7,8,8 a-octahydronaphthalen-1-yl acetate)[46]

The acetylation of racemate substrate **2** was performed according to a standard acetylation protocol with acetyl chloride. To a stirred solution of 0.1 M substrate (1 eq, 50 mg) in CH_2Cl_2 (2.8 mL), pyridine (1.2 eq, 27 μL) and acetyl chloride (1.2 eq, 24 μL) were added. The reaction was stirred overnight at room temperature. Then, CH_2Cl_2 was evaporated and the crude extract was resuspended in saturated aqueous $NaHCO_3$, then extracted (3×) with AcOEt. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to give the desired products (**4 a** + **4 b**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (d, J = 1.8 Hz, 1H), 4.65 (dd, J = 11.8, 4.2 Hz, 1H), 2.44–2.30 (m, 3H), 2.30–2.21 (m, 1H), 2.08 (s, J, 3H), 1.98–1.66 (m, 5H), 1.55–1.41 (m, 1H), 1.27 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.82, 170.32, 166.67, 125.80, 79.17, 40.39, 33.98, 33.47, 31.76, 26.87, 22.95, 21.07, 16.64. ESI-MS: $[M+H]^+$: 223.1. ESI-MS: $[M+Na]^+$: 245.0.

Enzymatic reduction of racemic ketone 1 with HSDHs/SDRs

General protocol for the enzymatic reduction: the reactions catalyzed by HSDHs were coupled with a glucose/GDH system to regenerate NAD(P)H. For the initial activity screening of the whole library of enzymes, the general reaction protocol was as follows: 50 mM glucose; 0.2 U mL^{-1} GDH; 0.4 mM $NAD(P)^+$; 3.4 U mL^{-1} HSDH; 10 mM substrate; 5% v/v DMSO; 50 mM potassium phosphate buffer, pH 7.0 (total volume: 1 mL). Reactions catalyzed by Hh7α-HSDH and Hh7β-HSDH were performed both in the presence and in the absence of 0.4 M NaCl. The mixtures were shaken at 25 °C and 100 rpm for 24 to 48 h and monitored over time *via* TLC (eluent CH_2Cl_2 : AcOEt = 9 : 1). Reaction conversions and enantiomeric excesses were evaluated by GC-MS and chiral HPLC analyses. The isolation of pure products was obtained *via* extraction with AcOEt of the reaction mixtures and subsequent flash chromatography on silica gel 60 (70–320 mesh, Merck, eluent CH_2Cl_2/AcOEt mixtures). The absolute configurations of the residual substrate **1 b**, if any, and of the products **2 a** and **3 b** were assigned via optical rotation measurements and subsequent confrontation with values reported in literature.[24–27]

Enzymatic reduction of 1 catalyzed by IS2-SDR

Following the aforementioned general protocol on a 100 mg scale (10 mM substrate, 56 mL reaction volume) and stopping the reaction at 58% conversion, the following products were isolated after flash chromatography (eluent: CH_2Cl_2 : AcOEt = 9 : 1). **1 b**: ee: 96.7%; $[\alpha]_D^{22}$: −97.0° (c: 1.0 in $CHCl_3$);[47] isolated yield: 33 mg (32%); **2 a**: ee: 90.7%; isolated yield: 30 mg, (29%). NMR and ms spectra are in accordance with the proposed structures (see Supplementary).

Enzymatic reductions of 1 and 1 b catalyzed by Dm7α-HSDH

Following the general protocol on a 50 mg scale (10 mM substrate, 28 mL reaction volume) and bringing the reaction to full conversion from racemic **1**, the following products were isolated *via* flash chromatography on a Biotage SP1 working with 50 mg of crude mixture and a 10 g silica gel Biotage® cartridge in a gradient of AcOEt in DCM (10 CV 5% AcOEt; 5 CV from 5% to 15% AcOEt, 10 CV 15% AcOEt).

2 a: 20 mg, ee: 95.3%, isolated yield: 40.0%. NMR and ms spectra in accordance with the proposed structure (see Supplementary).

3 b: 25 mg, ee: >99%, isolated yield: 50.0%.^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (br s, 1H), 3.65 (br s, 1H), 2.74–2.35 (m, 4H), 2.35–2.23 (m, 1H), 2.14–1.98 (m, 1H), 1.96–1.66 (m, 3H), 1.57–1.46 (m, 1H), 1.24 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.41, 167.68, 127.03, 75.33, 40.93, 34.02, 31.73, 30.80, 28.71, 21.83, 19.83; ee: >99%; $[\alpha]_D^{22}$: −111.6° (c: 1.0 in $CHCl_3$);[28] ESI-MS: $[M+Na]^+$: 203.0.[19]

Using the enantiomer **1 b** as a substrate (30 mg scale), the *trans*-alcohol **3 b** was obtained as sole product following, again, the general protocol. **3 b**: yield: 91%; ee: >99%.

Enzymatic acetylation of substrate 2 b catalyzed by Amano lipase PS

Racemic substrate **2** (1040 mg, 1 eq) was dissolved in dry acetone (80 mM substrate, 70 mL reaction volume). Then, excess vinyl acetate (10% v/v, 14 eq) and Amano lipase PS on celite (1000 mg, 1% wt, catalytic amount) were added. The reaction was shaken at 45 °C, 200 rpm for six days. The enzyme was filtered away and the solvent was evaporated to afford a crude mixture of **2 a** and **4 b**. The two products were separated *via* flash chromatography (CH_2Cl_2: AcOEt gradient from 9 : 1 to 7 : 3) to give the subsequent products: **2 a**: 660 mg, 50% isolated yield, ee: 97.3%; $[\alpha]_D^{22}$: +173.4° (c: 1.0 in $CHCl_3$).[33] **4 b**: 407 mg, 39% isolated yield, ee: >99%; $[\alpha]_D^{22}$: −69.5° (c: 1.0 in $CHCl_3$).[33]

Chemical hydrolysis of 4b to 2b

Substrate **4b** (100 mg, 0.450 mmol, 1 eq) was dissolved in EtOH (0.5 M). Then, a 1 M aqueous solution of NaOH was added dropwise (0.675 mL, 0.675 mmol, 1.5 eq). The reaction was stirred at room temperature for 2 h, then quenched with a saturated aqueous solution of $NaHCO_3$ and the aqueous phase thus obtained was extracted with AcOEt (3x). The organic phase was then dried over Na_2SO_4 and evaporated *in vacuo* to give the desired product **2b** (0.377 mmol, 90.0% yield). $[\alpha]_D^{22}$: $-180.5°$ (c: 1.0 in $CHCl_3$).[28] ee (HPLC): 98.5%.NMR and ms spectra are in accordance with the proposed structures (see Supplementary).

Chemical re-oxidation of 2a to 1a

2-Iodoxybenzoic acid (IBX,[45] 0.289 mmol) was dissolved 0.4 M in DMSO. Then, a 0.3 M solution of **2a** (0.172 mmol) in DMSO was added. The reaction was stirred for 17 h at room temperature, then quenched with an aqueous saturated solution of $NaHCO_3$. The phase thus obtained was extracted with AcOEt (3x). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to afford a yellow oil. (0.168 mmol, 97.9% yield). $[\alpha]_D^{22}$: $+102.9°$ (c: 1.0 in $CHCl_3$);[48] ee (HPLC): 96.2%. NMR and ms spectra in accordance with the proposed structure (see Supplementary)

Acknowledgements

The technical contributions by M.Sc. Marta Vanoni and M.Sc. Eleonora Celata are gratefully acknowledged.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Biocatalysis · Dehydrogenases · Enzymes · Lipases · Wieland-Miescher ketone

[1] P. Wieland, K. Miescher, *Helv. Chim. Acta* **1950**, *33*, 2215–2228.
[2] Z.-Q. Liu, *Curr. Org. Synth.* **2019**, *16*, 328–341.
[3] H. Hagiwara, *Nat. Prod. Commun.* **2020**, *15*, 1934578X2092534.
[4] Z.-Q. Liu, *Curr. Org. Chem.* **2018**, *22*, 1347–1372.
[5] U. Eder, G. Sauer, R. Wiechert, *Asymmetrische Synthese Polycyclischer Organischer Verbindungen* **1971**, DE 2102623.
[6] U. Eder, G. Sauer, R. Wiechert, *Asymmetrische Synthese Polycyclischer Organischer Verbindungen* **1971**, DE 2014757.
[7] U. Eder, G. Sauer, R. Wiechert, *Angew. Chem. Int. Ed. Engl.* **1971**, *10*, 496–497.
[8] S. Canellas, C. Ayats, A. H. Henseler, M. A. Pericàs, *ACS Catal.* **2017**, *7*, 1383–1391.
[9] V. Srivastava, *J. Chem. Sci.* **2013**, *125*, 1523–1527.
[10] D. B. Ramachary, M. Kishor, *J. Org. Chem.* **2007**, *72*, 5056–5068.
[11] X. Yang, J. Wang, P. Li, *Org. Biomol. Chem.* **2014**, *12*, 2499–2513.
[12] B. Bradshaw, J. Bonjoch, *Synlett* **2012**, *23*, 337–356.
[13] M. Scheck, M. A. Koch, H. Waldmann, *Tetrahedron* **2008**, *64*, 4792–4802.
[14] Y. S. Lu, X. S. Peng, *Org. Lett.* **2011**, *13*, 2940–2943.
[15] K. Ma, C. Zhang, M. Liu, Y. Chu, L. Zhou, C. Hu, D. Ye, *Tetrahedron Lett.* **2010**, *51*, 1870–1872.
[16] S. K. Yeo, N. Hatae, M. Seki, K. Kanematsu, *Tetrahedron* **1995**, *51*, 3499–3506.
[17] X. S. Peng, H. N. C. Wong, *Chem. Asian J.* **2006**, *1*, 111–120.
[18] a) G. Ottolina, G. De Gonzalo, G. Carrea, B. Danieli, *Adv. Synth. Catal.* **2005**, *347*, 1035–1040; b) S. K. Yeo, N. Hatae, M. Seki, K. Kanematsu, *Tetrahedron* **1995**, *51*, 3499–3506.
[19] T. Shimizu, S. Hiranuma, T. Nakata, *Tetrahedron Lett.* **1996**, *37*, 6145–6148.
[20] G. Zhong, T. Hoffmann, R. A. Lerner, S. Danishefsky, C. F. Barbas, *J. Am. Chem. Soc.* **1997**, *119*, 8131–8132.
[21] Y. Miao, M. Rahimi, E. M. Geertsema, G. J. Poelarends, *Curr. Opin. Chem. Biol.* **2015**, *25*, 115–123.
[22] Y. F. Lai, P. F. Zhang, *Res. Chem. Intermed.* **2015**, *41*, 4077–4082.
[23] M. P. Patel, N. T. Green, J. K. Burch, K. A. Kew, R. M. Hughes, *Catalysts* **2020**, *10*, 1–13.
[24] V. Prelog, W. Acklin, *Helv. Chim. Acta* **1956**, *39*, 748–757.
[25] W. Acklin, V. Prelog, A. P. Prieto, *Helv. Chim. Acta* **1958**, *41*, 1416–1424.
[26] W. Acklin, D. Dütting, V. Prelog, *Helv. Chim. Acta* **1958**, *41*, 1424–1427.
[27] W. Acklin, V. Prelog, D. Zäch, *Helv. Chim. Acta* **1958**, *41*, 1428–1437.
[28] K. I. Fuhshuku, N. Funa, T. Akeboshi, H. Ohta, H. Hosomi, S. Ohba, T. Sugai, *J. Org. Chem.* **2000**, *65*, 129–135.
[29] K. I. Fuhshuku, M. Tomita, T. Sugai, *Adv. Synth. Catal.* **2003**, *345*, 766–774.
[30] H. Hioki, T. Hashimoto, M. Kodama, *Tetrahedron: Asymmetry* **2000**, *11*, 829–834.
[31] T. Janeczko, J. Dmochowska-Gładysz, E. Kostrzewa-Susłow, *World J. Microbiol. Biotechnol.* **2010**, *26*, 2047–2051.
[32] S. Bertuletti, E. E. Ferrandi, S. Marzorati, M. Vanoni, S. Riva, D. Monti, *Adv. Synth. Catal.* **2020**, *362*, 2474–2485.
[33] N. Shimizu, H. Akita, T. Kawamata, *Tetrahedron: Asymmetry* **2002**, *13*, 2123–2131.
[34] G. Carrea, S. Riva, *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2226–2254.
[35] S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Snyder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, C. A. Alaimo, et al., *J. Am. Chem. Soc.* **1996**, *118*, 2843–2859.
[36] S. Butt, H. G. Davies, M. J. Dawson, G. C. Lawrence, J. Leaver, S. M. Roberts, M. K. Turner, B. J. Wakefield, W. F. Wall, J. A. Winders, *J. Chem. Soc. Perkin Trans. 1* **1987**, *0*, 903–907.
[37] U. C. T. Oppermann, E. Maser, *Eur. J. Biochem.* **1996**, *241*, 744–749.
[38] E. Maser, G. Bannenberg, *Biochem. Pharmacol.* **1994**, *47*, 1805–1812.
[39] M. De Amici, C. De Micheli, G. Molteni, D. Pitrè, G. Carrea, S. Riva, S. Spezia, L. Zetta, *J. Org. Chem.* **1991**, *56*, 67–72.
[40] D. Zhu, J. E. Stearns, M. Ramirez, L. Hua, *Tetrahedron* **2006**, *62*, 4535–4539.
[41] M. Zorko, H. E. Gottlieb, M. Žakelj-Mavric, *Steroids* **2000**, *65*, 46–53.
[42] M. C. R. Franssen, H. Jongejan, H. Kooijman, A. L. Spek, R. P. L. Bell, J. B. P. A. Wijnberg, A. De Groot, *Tetrahedron: Asymmetry* **1999**, *10*, 2729–2738.
[43] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
[44] M. M. Cruz Silva, M. L. Sá E Melo, M. Parolin, D. Tessaro, S. Riva, B. Danieli, *Tetrahedron: Asymmetry* **2004**, *15*, 21–27.
[45] M. Frigerio, M. Santagostino, *Tetrahedron Lett.* **1994**, *35*, 8019–8022.
[46] N. Bensel, H. Marschall, P. Weyerstahl, R. Zeisberg, *Liebigs Ann. Chem.* **1982**, 10, 1781–807.
[47] K. Mori, T. Suzuki, T. Noji, M. Yamanaka, T. Akiyama, *Angew. Chem. Int. Ed.* **2009**, *48*, 9652–9654.
[48] A. B. Smith, K. Laszlo, A. H. Davulcu, Y. S. Cho, *Org. Process Res. Dev.* **2007**, *11*, 19–24.

Manuscript received: February 11, 2021
Revised manuscript received: March 15, 2021
Accepted manuscript online: March 16, 2021

FULL PAPERS