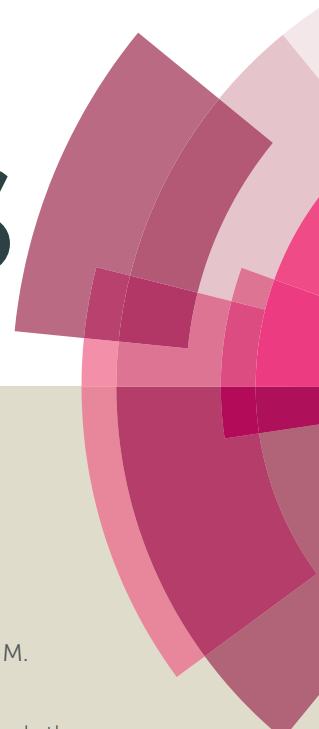


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## ARTICLE

# Enzyme-catalysed regio- and enantioselective preparative scale synthesis of (S)-2-hydroxy alkanones

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$\alpha$ -Hydroxy alkanones were synthesised with high enantiomeric purity by stereoselective enzyme-catalysed diketone reduction. Both diketone reduction and cofactor regeneration were accomplished with purified carbonyl reductase from *Candida parapsilosis* (CPCR2). Reaction products were isolated by column chromatography and analysed by chiral GC measurements,  $^1\text{H}$ -NMR spectroscopy and determination of optical rotations. Preparative-scale biotransformations yielded 350 - 600 mg of pure aliphatic  $\alpha$ -hydroxy ketones including the difficult to obtain (S)-2-hydroxypentane-3-one. For all products good enantiomeric excesses in the range of 89 – 93 % were achieved.

## Introduction

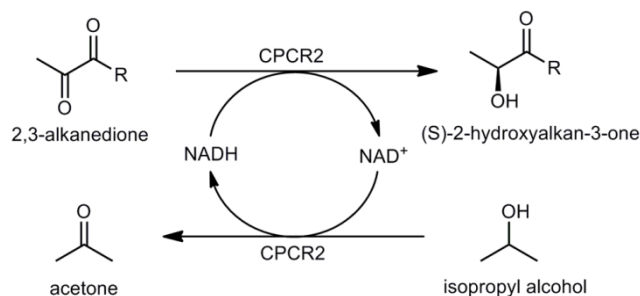
Chiral  $\alpha$ -hydroxy ketones are important and valuable building blocks for the synthesis of several different pharmaceuticals such as anti-depressants and fungicides.<sup>[1]</sup> They contain a chiral centre at the carbon atom of the hydroxy function, and in case of unequal substituents at the carbonyl and the hydroxy moiety not only enantiomers, but also structural isomers occur. Thus, synthesis of this type of compounds requires regio- as well as stereoselective approaches.

Presently, various organochemical methods for the synthesis of  $\alpha$ -hydroxy ketones are available. Examples are ranging from peroxy acid catalysed oxidations<sup>[2]</sup> to metal catalysed diketone reductions applying zinc<sup>[3]</sup> or titan iodide.<sup>[4]</sup> Some catalysts, such as transition metal complexes, exhibit a notable regio- and stereoselectivity, mostly, however, restricted to phenyl-substituted hydroxy ketones.<sup>[5,6,7,8,9,10,11]</sup> Linear aliphatic  $\alpha$ -hydroxy ketones comprise particularly challenging targets due to the structural similarity of the substituents.

In recent years, different biocatalytic approaches for the production of  $\alpha$ -hydroxy ketones have been introduced as alternatives to chemical methods, including the use of isolated enzymes such as lipases, lyases, and oxidoreductases.<sup>[1,12,13]</sup> Lyases have been successfully applied in the synthesis of symmetric  $\alpha$ -hydroxy ketones such as acetoin<sup>[14]</sup> and propionin.<sup>[15,16,17]</sup> Utilizing a lyase and decarboxylase in cascade, some non-symmetric compounds could also be synthesized.<sup>[18]</sup> With lipases, kinetic resolutions of esters were conducted yielding enantiopure aliphatic  $\alpha$ -hydroxy ketones especially for larger substituents.<sup>[19]</sup> The application of alcohol dehydrogenases for the synthesis of aliphatic  $\alpha$ -hydroxy ketones by means of diol oxidation<sup>[20,21]</sup> or diketone reduction<sup>[21,22,23]</sup>, was demonstrated.

In these enzymatic processes enantiomeric excesses between 90 and 99 % were achieved. If determined, isolated yields ranged between 23 and 70 %. However, especially the diketone reduction has not been sufficiently exploited for efficient preparation of  $\alpha$ -hydroxy ketones so far.<sup>[1]</sup>

Here, we demonstrate the application of a carbonyl reductase from *Candida parapsilosis* CPCR2<sup>[24,25,26,27]</sup> for the highly selective asymmetric reduction of prochiral linear aliphatic diketones to a set of corresponding (S)-configured  $\alpha$ -hydroxy ketones in preparative scale. At the same time, the required cofactor nicotinamide adenine dinucleotide (NADH) was efficiently regenerated by oxidation of isopropyl alcohol with CPCR2 (Figure 1). The reaction products were purified by silica gel chromatography and analysed via gas chromatography,  $^1\text{H}$ -NMR spectroscopy and determination of optical rotation. The absolute configuration was determined via  $^1\text{H}$ -NMR analysis of Mosher's esters obtained from the reaction products.



**Figure 1.** Reduction of 2,3-alkanedione substrates to the corresponding (S)-2-hydroxy alkan-(3)-ones catalysed by CPR2. Cofactor regeneration is achieved by oxidation of the co-substrate isopropyl alcohol to acetone, also catalysed by CPR2.

## Results and Discussion

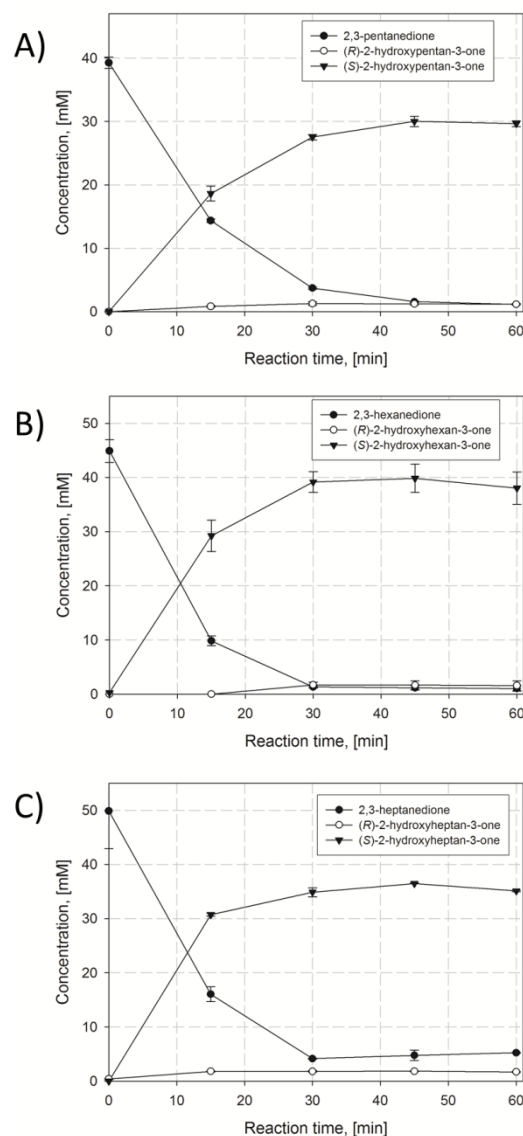
For  $\alpha$ -diketone substrates with varying structural determinants and therefore varying potential for reduction by CPR2, the specific catalytic activity of the enzyme was investigated. A selection of results is shown in Table 1. It was found that cyclic diketones were not accepted at all, and substrates with substituents larger than a methyl group next to both carbonyl functions were converted with only low reaction rates. Aliphatic diketones comprising one methyl group next to the carbonyl functions, on the other hand, were converted with reaction rates up to  $10 \text{ U} \cdot \text{mg}^{-1}$ , which is quite good. Accordingly, 2,3-alkanedione substrates were investigated for biotransformation in preparative scale.

| Substrate             | Spec. activity<br>[ $\text{U} \cdot \text{mg}^{-1}$ ] |
|-----------------------|-------------------------------------------------------|
| 2,3-Pentanedione (1a) | $10.0 \pm 0.3$                                        |
| 2,3-Hexanedione (2a)  | $8.9 \pm 0.2$                                         |
| 2,3-Heptanedione (3a) | $9.1 \pm 0.1$                                         |
| 3,4-Hexanedione       | $1.7 \pm 0.1$                                         |
| 1,2-Cyclohexane-dione | $0.0 \pm 0.0$                                         |

**Table 1.** Specific catalytic activity of CPR2 in the reduction of diketones.

Preparative-scale biotransformations were successfully conducted for 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione (entries 1a, 2a and 3a, respectively, in Table 1). Conversions between 88 and 97 % were observed within only 30 minutes (Figure 2). The yields after 1 h of reaction time ranged between 70 and 87%. Next to the formation of the main (S)-product very small amounts of the (R)-product were detected, leading to decent enantiomeric excesses (*ee*-values) (Table 2). Taking both chiral products into account the mass balance of the reactions is imbalanced by 7-19 % on the product side. This divergence between the measured decrease of substrate and increase of product may be explained by the substrate evaporation and adsorption to the reaction vessel, and a light-induced side-reaction of diketones as described by Maekinen and co-workers.<sup>[28]</sup>

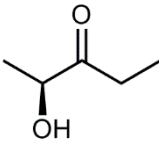
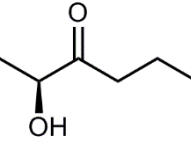
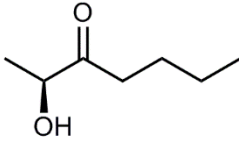
After completion of the reaction after one hour, the reactants were retrieved by a four-fold extraction with equal volumes of ethyl acetate. Purification of the reaction products by column chromatography yielded highly pure fractions of the 2-hydroxy-alkan-3-ones. After solvent removal  $^1\text{H-NMR}$  analysis was conducted showing no major impurities in the reaction products (supporting material, Figures S4-6). Hence, for the investigated group of substrates, the reaction is chemoselective regarding the reduction of diketones to hydroxy ketones, and regioselective regarding the carbonyl function on C2-position. In addition, the reaction is stereoselective, yielding *ee*-values between 89 and 95% (Table 2). A significant effect of the reaction parameters temperature, pH-value und cosubstrate concentration was not observed (Figure S7).



**Figure 2.** Progress of 2,3-alkanedione reduction and corresponding product formation in preparative scale biotransformations. A) 2,3-pentanedione, B) 2,3-hexanedione, C) 2,3-heptanedione.

Performance of cofactor regeneration by using isopropyl alcohol as co-substrate effectively supplied the reaction with reduced cofactor. Turnover numbers (TONs) of NADH were 300-450 (Table 3), which is a reasonable range. Improvement would certainly be possible by decreasing NADH concentration, but most probably at the cost of reaction rates. The TON of the enzyme was in the order of 105, which is excellent and a good measure for the efficiency of the catalyst.<sup>[29]</sup> The procedure yielded 360–600 mg of the pure (S)- $\alpha$ -hydroxy ketones, corresponding to isolated yields between 36–46%. On the one hand, these low values may be explained by the factors already discussed for the reaction yield. On the other hand the purification of the products by silica gel chromatography was not optimized and specifically difficult for the short hydrophilic substrates. Nevertheless, high space time yields (STY) between 43 and  $72 \text{ g} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$  were achieved (Table 3), mainly due to the fast conversion of substrates.

**Table 2.** Conversion, reaction yield, enantiomeric excess and isolated yield of 2,3-alkanedione reduction with CPCR2 on a preparative scale. a) Substrate consumption after 1 h, measured via GC; b) Product concentration after 1 h, measured via GC.

| Entry | Product                                                                           | Conversion <sup>a)</sup><br>[%] | Reaction<br>Yield <sup>b)</sup> [%] | ee [%]     | Yield<br>[mg] | Isolated<br>Yield [%] |
|-------|-----------------------------------------------------------------------------------|---------------------------------|-------------------------------------|------------|---------------|-----------------------|
| (1b)  |  | 97.1 ± 0.2                      | 75.5 ± 1.2                          | 94.8 ± 1.2 | 362           | 36.2                  |
| (2b)  |  | 97.7 ± 0.1                      | 87.0 ± 0.5                          | 93.5 ± 1.3 | 529           | 46.3                  |
| (3b)  |  | 89.5 ± 0.1                      | 70.4 ± 0.3                          | 88.7 ± 0.8 | 601           | 46.9                  |

For determination of the absolute configuration of the main products, Mosher's esters of the reaction products were synthesised and analysed via <sup>1</sup>H-NMR<sup>[30]</sup>. This method is based on an anisotropic effect of a phenyl group in the derivatization agent MTPA on the chemical shift of the protons in the substituents of a target hydroxyl compound. Both, the (*R*)- and (*S*)-MTPA esters of each reaction product were synthesized and <sup>1</sup>H-NMR spectra were recorded. The differences between the chemical shifts for protons, in the same position in both esters, are described as Δδ values. For all reaction products, these Δδ values could only be determined for the protons on C1-position, ranging between -0.05 and -0.06 ppm (Table 4). Considering the spatial position of the MTPA-phenyl group, the position of the small substituent relative to the large substituent could be determined, yielding (*S*)-configuration at the hydroxy group.<sup>[30]</sup> Results of the analysis are illustrated in the supporting information (Figures S8, S9 and S10).

The optical rotation of the reaction products was measured applying the conditions described by Martre and coworkers ([α]<sub>D</sub><sup>25</sup> (c=0.9, chloroform)<sup>[11]</sup>). With +43.8° the optical rotation of (*S*)-2-hydroxy-pentane-(3)-one is in good agreement with +48°, determined in the reference study<sup>[11]</sup> (Table 4). With +52.0° for (*S*)-2-hydroxy-hexane-(3)-one and +59.2° (*S*)-2-hydroxy-heptane-(3)-one, the reaction products exhibited increasing optical rotations with increasing size of the large substituent. For (*S*)-2-hydroxy-hexane-(3)-one the measurement was repeated under the conditions applied by Bortolini and coworkers ([α]<sub>D</sub><sup>20</sup> (c=2.5, chloroform)<sup>[23]</sup>). The measured value of +55.2° is in good agreement with the reference (57°<sup>[23]</sup>). In case of (*S*)-2-hydroxy-heptane-(3)-one, no reference value was detected. Thus, where available, the measured optical rotations were comparable to literature, with a tendency to lower values. This may be explained by enantiomeric impurities resulting from the biotransformation.

| Product | TON NADH | TON CPCR2             | STY [gL <sup>-1</sup><br>d <sup>-1</sup> ] |
|---------|----------|-----------------------|--------------------------------------------|
| 1b      | 297      | 2.8 × 10 <sup>5</sup> | 43                                         |
| 2b      | 447      | 4.2 × 10 <sup>5</sup> | 64                                         |
| 3b      | 351      | 3.3 × 10 <sup>5</sup> | 72                                         |

**Table 3.** Process relevant data from preparative scale biotransformations of diketones to the corresponding chiral α-hydroxy ketones. Turnover numbers (TON) for biocatalyst and cofactor, and space time yield (STY) of the reaction.

Summarising, the asymmetric reduction of three different 2,3-alkanediones with CPCR2 yielded the corresponding (*S*)-2-hydroxy-alkane-3-ones with good enantiomeric excesses. Due to a considerably small substrate binding pocket<sup>[25]</sup>, the substrate specificity of CPCR2 restricts its application to compounds with a carbonyl group on C2-position, which on the other hand allows a highly regioselective reduction of asymmetric diketones. In addition, no consecutive reduction of the hydroxy ketone to the corresponding diol was observed as described in previous studies with ADHs.<sup>[20,21]</sup> Here again the limitation of the substrate scope of the enzyme is turned into an advantage since the reaction selectively stops at the hydroxy ketone level. Thus, low molecular weight α-hydroxy ketones, containing the hydroxy function on C2-position, may be synthesised chemo-, regio- and stereoselectively utilising CPCR2.

In contrast, the application of lipases for the synthesis of aliphatic hydroxy ketones shows improving enantiomeric excesses with an increasing size of both substituents.<sup>[19]</sup> This is also true for lyases, where symmetric α-hydroxy ketones with C5-substituents were synthesized with the highest enantiomeric excesses.<sup>[17]</sup> Thus, the comparably small substrate scope of the asymmetric diketone reduction with CPCR2 is compensated by the fact that the products obtained in this study are difficult to synthesize applying other enzymatic approaches.



| Product | Hydroxy ketone | (R)-Ester | (S)-Ester | $\Delta\delta^{\text{SR}}$ | Optical rotation                                                  |
|---------|----------------|-----------|-----------|----------------------------|-------------------------------------------------------------------|
| 1b      | 1.34           | 1.43      | 1.49      | -0.06                      | $+43.8^\circ \pm 1.3^{\text{a}}$                                  |
| 2b      | 1.34           | 1.43      | 1.48      | -0.05                      | $+52.0^\circ \pm 1.3^{\text{a}} / +55.2^\circ \pm 2.5^{\text{b}}$ |
| 3b      | 1.34           | 1.43      | 1.49      | -0.05                      | $+59.2^\circ \pm 1.9^{\text{a}}$                                  |

**Table 4.** Chemical shifts and  $\Delta\delta^{\text{SR}}$  values (in ppm) for hydrogen atoms at C1-position of hydroxy ketones and corresponding Mosher's esters. a) measured at  $[\alpha]_{\text{D}}^{25}$  (c=0.9, chloroform) b) measured at  $[\alpha]_{\text{D}}^{20}$  (c=2.5, chloroform)

## Experimental section

If not stated otherwise all chemicals were purchased from Sigma-Aldrich (St. Louis, USA). All reactions were performed in aqueous solution.

### Enzyme preparation and characterization

CPCR2 was prepared according to a modified protocol of Jakoblinnert et al.<sup>[27]</sup> Fermentation was performed in 1 L scale in LB medium with ampicillin ( $100 \mu\text{g}\cdot\text{mL}^{-1}$ ) in a stirred tank reactor (Biostat B Plus, Sartorius, Germany). The medium was inoculated from a preculture of *E.coli* BL21 (DE3) containing the plasmid pET22b(+):CPCR2 to a final OD<sub>600</sub> of 0.1. Temperature ( $37^\circ\text{C}$ ), pH (7.0) and dissolved oxygen (50%) were controlled automatically. After reaching an OD<sub>600</sub> of 0.8, the temperature was reduced to  $20^\circ\text{C}$  and protein expression was induced with IPTG (Isopropyl  $\beta$ -D-1-thiogalactopyranoside) at a final concentration of  $0.1 \text{ mmol}\cdot\text{L}^{-1}$ . After 4 hours incubation, cells were harvested by centrifugation (10 min,  $5000 \text{ g}$ ,  $4^\circ\text{C}$ ).

For cell lysis, the cell pellet was resuspended in 30 mL triethanolamin (TEA) buffer ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH = 8.0) adding  $50 \mu\text{g}\cdot\text{mL}^{-1}$  DNaseI (Applichem GmbH, Germany),  $500 \mu\text{g}\cdot\text{mL}^{-1}$  lysozyme and  $1 \text{ mmol}\cdot\text{L}^{-1}$  phenylmethylsulfonyl fluoride (PMSF). The lysate was cleared by centrifugation (20 min,  $16000 \text{ g}$ ,  $4^\circ\text{C}$ ). CPCR2 was purified by StrepTag affinity chromatography applying a fast protein liquid chromatography system (Äkta 920, General Electrics, USA) equipped with a 5 mL StrepTrap® column (General Electrics, USA) according to modified manufacturer's instructions. As washing buffer, a TEA buffer ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH = 8.5) with  $500 \text{ mmol}\cdot\text{L}^{-1}$  sodium chloride, 10% (v/v) glycerol, and  $1 \text{ mmol}\cdot\text{L}^{-1}$  ethylene diamine tetra acetic acid (EDTA) was applied. For elution a TEA buffer ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH = 8.5) with  $500 \text{ mmol}\cdot\text{L}^{-1}$  NaCl, 10% (v/v) glycerol, and  $2.5 \text{ mmol}\cdot\text{L}^{-1}$  desthiobiotin was used.

CPCR2 concentration for determination of specific activities was quantified via BCA-assay (Pierce, USA) according to the manufacturer's instructions. Enzyme activity assays were performed at  $25^\circ\text{C}$  with  $6.2 \mu\text{g}\cdot\text{mL}^{-1}$  CPCR2 in 1 mL TEA buffer ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH 7.3) applying  $3 \text{ mmol}\cdot\text{L}^{-1}$  substrate. Reactions were started by addition of nicotinamide adenine dinucleotide (NADH) to a final concentration of  $250 \mu\text{mol}\cdot\text{L}^{-1}$ . For all substrates the initial reaction rate was measured photometrically at 340 nm for 2 minutes. Measurements were performed in triplicates.

For determination of stereoselectivity, activity assays were performed on  $500 \mu\text{L}$ -scale. The reaction mixture, containing  $25 \text{ mmol}\cdot\text{L}^{-1}$  of substrate (1(a): 480 ng, 2(a): 533 ng, 3(a): 590 ng),  $250 \mu\text{mol}\cdot\text{L}^{-1}$  NADH, 5% (v/v) isopropyl alcohol and  $4 \text{ mmol}\cdot\text{L}^{-1}$  dithiothreitol (DDT) in TEA buffer ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH 7.3), was prepared and preincubated at  $25^\circ\text{C}$  for 5 min. Conversion was started by addition of  $1 \mu\text{g}\cdot\text{mL}^{-1}$  CPCR2.

Reactions were performed at room temperature in a shaker (Vibramax 110, Heidolph, Germany) at 1000 rpm for 30 min.  $125 \mu\text{L}$  samples were withdrawn every 10 min and extracted with  $125 \mu\text{L}$  ethyl acetate. Product concentrations were determined via chiral GC.

### Preparative Biotransformation

Preparative biotransformations were conducted in 200 mL-scale in 500 mL shake-flasks mixed with a magnetic stir bar (80 rpm) at room temperature. The reaction mixture contained: CPCR2  $8.5 \mu\text{g}\cdot\text{mL}^{-1}$ , substrate  $50 \text{ mmol}\cdot\text{L}^{-1}$  (1(a): 961 mg, 2(a): 1066 mg, 3(a): 1179 mg), NADH  $100 \mu\text{mol}\cdot\text{L}^{-1}$ , isopropyl alcohol 5 % (v/v), TEA ( $100 \text{ mmol}\cdot\text{L}^{-1}$ , pH 7.3), dithiothreitol (DDT)  $4 \text{ mmol}\cdot\text{L}^{-1}$ . Like for activity assays isopropyl alcohol acts as cosubstrate for cofactor regeneration. 5 % (v/v) isopropyl alcohol corresponds to a concentration of  $650 \text{ mmol}\cdot\text{L}^{-1}$  yielding a 13-fold excess of cosubstrate over the substrate. All components except for CPCR2 were premixed under the given conditions until the substrate was dissolved completely. Then the reaction was initiated by the addition of CPCR2. The biotransformations were stirred for one hour and samples were withdrawn at definite time intervals (15 min) and extracted with one sample volume of ethyl acetate. Conversion of substrates and synthesis of products was assessed by GC.

### Product purification and analytics

Reaction products were isolated by four consecutive extractions with 200 mL of ethyl acetate. Purification was performed via column chromatography using silica gel 60 (Sigma Aldrich, Germany) as solid phase and a solvent mixture of n-hexane and ethyl acetate at a ratio of 4:1 (compounds 2,3) and 1:1 (compound 1), respectively. Purification was monitored via GC and thin layer chromatography (TLC) in combination with cerium sulfate staining.

GC analysis of the reaction products was performed on a Shimadzu GC2010 gas chromatograph with flame ionization detector (FID) and an AOC20i Autosampler. For separation of all chiral compounds Hydrodex  $\gamma$  DIMOM (Macherey-Nagel, Germany) was applied as solid phase using different temperature gradients for each compound. The carrier gas N<sub>2</sub> was applied with a pressure of 70 kPa.  $2 \mu\text{L}$  of the extracted samples were injected to the column without split flow. Exemplary chromatograms are provided in the supporting information (Figures S1-S3).

2,3-Pentanedione: A temperature gradient from  $95$ – $115^\circ\text{C}$  was applied with a ramp of  $2^\circ\text{C}\cdot\text{min}^{-1}$ . Retention times were determined at 3.3 min for 2,3-pentanedione 1(a), 5.6 min for (R)-2-hydroxy-pentan-(3)-one and 7.8 min for (S)-2-hydroxy-pentan-(3)-one 1(b) (supporting information, Figure S1).

2,3-Hexanedione: A temperature gradient from  $98$ – $122^\circ\text{C}$  was applied with a ramp of  $2^\circ\text{C}\cdot\text{min}^{-1}$ . Retention times were determined at 3.8 min for 2,3-hexanedione 2(a), 8.6 min for (R)-2-hydroxy-hexan-(3)-one and 9.2 min for (S)-2-hydroxy-hexan-(3)-one 2(b) (supporting information, Figure S2).

2,3-Heptanedione: A temperature gradient from  $105$ – $125^\circ\text{C}$  was applied with a ramp of  $2^\circ\text{C}\cdot\text{min}^{-1}$ . Retention times were determined at 4.4 min for 2,3-heptanedione 2(a), 9.1 min for (R)-2-hydroxy-heptan-(3)-one and 9.4 min for (S)-2-hydroxy-heptan-(3)-one 2(b) (supporting information, Figure S3).

Reaction products were identified via  $^1\text{H-NMR}$  spectroscopy ( $500 \text{ MHz}$ , Bruker DRX-500) using chloroform- $d$  ( $^1\text{H}$ :  $\delta$  7.26).  $^1\text{H-NMR}$  spectra are provided in the supporting information (Figures S4-S6).

(S)-2-Hydroxy-pentan-3-one (1b):  $[\alpha]_D^{25} = +43.8^\circ \pm 1.3$  (c=0.9, chloroform) Yellowish oil, ( $^1\text{H-NMR}$ , 500 MHz)  $\delta$  (ppm): 4.19 (1H, q, j = 7.08, CH-OH), 3.40 (1H, bs, OH), 2.45 (2H, m, CH<sub>2</sub>), 1.32 (3H, d, j = 7.07, CH<sub>3</sub>), 1.05 (3H, t, j = 7.32, CH<sub>3</sub>), (supporting information, Figure S4)

Reference:  $[\alpha]_D^{25} = +48^\circ$  (c=0.9, chloroform)<sup>[11]</sup>

(S)-2-Hydroxy-hexan-3-one (2b):  $[\alpha]_D^{25} = +52.0^\circ \pm 1.3$  (c=0.9, chloroform),  $[\alpha]_D^{20} = +55.2^\circ \pm 2.5$  (c=2.5, chloroform) Yellowish oil, ( $^1\text{H-NMR}$ , 500 MHz)  $\delta$  (ppm): 4.23 (1H, q, j = 7.08, CH-OH), 3.52 (1H, bs, OH), 2.45 (2H, m, CH<sub>2</sub>), 1.66 (2H, m, CH<sub>2</sub>), 1.38 (3H, d, j = 7.08, CH<sub>3</sub>), 0.93 (3H, t, j = 7.31, CH<sub>3</sub>), (supporting information, Figure S5)

Reference:  $[\alpha]_D^{20} = +57^\circ$  (c=2.5, chloroform)<sup>[23]</sup>

(S)-2-Hydroxy-heptan-3-one (3b):  $[\alpha]_D^{25} = +59.2^\circ \pm 1.9$  (c=0.9, chloroform) Yellow oil, ( $^1\text{H-NMR}$ , 500 MHz)  $\delta$  (ppm): 4.22 (1H, q, j = 7.09, CH-OH), 3.56 (1H, bs, OH), 2.45 (2H, m, CH<sub>2</sub>), 1.60 (2H, m, CH<sub>2</sub>), 1.35 (3H, d, j = 7.14, CH<sub>3</sub>), 1.31 (2H, m, CH<sub>2</sub>), 0.89 (3H, t, j = 7.35, CH<sub>3</sub>), (supporting information, Figure S6)

### Absolut configuration of $\alpha$ -hydroxy ketones.

The absolute configuration of reaction products was determined via  $^1\text{H-NMR}$  analysis of Mosher's esters of the products according to the protocol of Hoyer and coworkers (Procedure: 1.b).<sup>[30]</sup> In this method, chiral alcohols are esterified with both enantiomers of a phenyl substituted chiral acid. Electrostatic shielding effects lead to a shift in the  $^1\text{H-NMR}$ -spectrum, which can be correlated with the absolute configuration of the analysed alcohol. The reaction was performed in dichloromethane-*d* ( $^1\text{H}$ :  $\delta$  5.32) on 50  $\mu\text{g}$ -scale. The synthesis of (S)-Mosher's esters of hydroxy ketones was performed by esterification with the (R)-MTPA-Cl ((R)-(-)- $\alpha$ -methoxy- $\alpha$ -(trifluoro-methyl)phenylacetyl chloride, Sigma-Aldrich, Germany), while esterification with the (S)-MTPA-Cl gives rise to (R)-Mosher's esters. The absolute configuration was then determined by comparison of the  $^1\text{H-NMR}$  spectra and the chemical shifts of the two diastereomeric esters, and subsequent determination of  $\Delta\delta^{\text{SR}}$ .

### Conclusions

The asymmetric reduction of prochiral diketones with the alcohol dehydrogenase CPCR2 proves to be a fast and efficient method for the synthesis of (S)-2-hydroxy alkanones and a good addition to the existing biocatalytic approaches. While lyases are good catalysts for the synthesis of symmetric hydroxy ketones, lipases are suitable for higher molecular weight compounds. Substrate scope and selectivity make the CPCR2 a valuable biocatalyst for the synthesis of asymmetric low molecular weight hydroxy ketones as described in this study, significantly improving the accessibility of this class of chiral compounds by biochemical synthesis.

### Acknowledgements

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### Notes and references

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