

# Overcoming Equilibrium Issues with Carbonyl Reductase Enzymes

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**ABSTRACT:** We report herein the screening, optimisation and scale up to 100 g of a bioreduction process that employs an in situ product removal (ISPR) technique to overcome the inherent equilibrium problem associated with the coupled-substrate approach to biocatalytic carbonyl reduction. This technique allowed the valuable chiral alcohol, (S)-2-bromo-2-cyclohexen-1-ol, to be isolated in 88% yield and 99.8% ee without the need for further purification, validating the general applicability of this experimental setup.

## INTRODUCTION

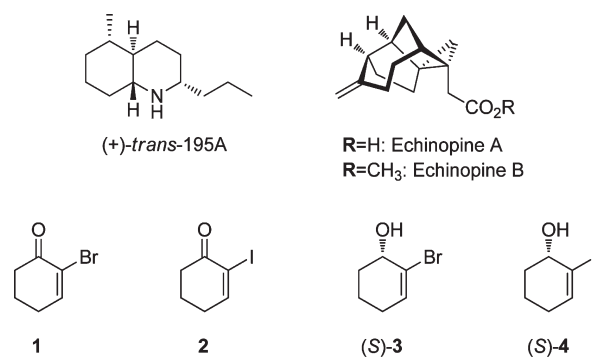
$\alpha$ -Halogenated,  $\alpha,\beta$ -unsaturated compounds represent a useful class of intermediates in organic synthesis. Their versatility extends from their ability to serve as  $\alpha$ -ketovinyl anion equivalents as well as enabling access to  $\alpha$ -substituted enones.  $\alpha$ -Bromocyclohex-2-enone (**1**) is frequently encountered as the starting point in a number of natural product syntheses including the total synthesis of the sesquiterpenes echinopines A and B<sup>1</sup> and (+)-*trans*-195A,<sup>2</sup> the name assigned to a decahydroquinoline alkaloid isolated from the skin of dendrobatid frogs, as shown in Figure 1. Nicolaou and co-workers generated chiral alcohol (S)-**3** in 90% yield and  $\geq 95\%$  ee,<sup>2</sup> while Blechert and co-workers used a modified CBS reagent to afford (S)-**3** in 95% yield and 99% ee.<sup>3</sup> Unfortunately, both procedures necessitated subsequent purification by chromatographic means.

Almac recently had a requirement to synthesise kilogram quantities of  $\alpha$ -halo,  $\alpha,\beta$ -unsaturated chiral alcohols (S)-**3** or (S)-**4** shown in Figure 1. These were required as a key intermediate for the synthesis of a novel therapeutic agent currently under development.

The interest of the synthetic organic chemistry community in biocatalysis has exploded over the past decade.<sup>3</sup> This is true, in particular, for carbonyl reductase (CRED) enzymes, which have now become the method of choice for the asymmetric reduction of prochiral ketones.<sup>4</sup> These enzymes have received much attention by both academic and industrial groups and many cloned reductases have been reported.<sup>5</sup> In order for this approach to be considered economically viable at scale, CRED enzymes require cofactor recycling, and several methods have been employed to date, most notably glucose dehydrogenase (GDH)<sup>6</sup> and isopropyl alcohol (IPA), (Scheme 1).<sup>7</sup> The use of IPA as the cofactor recycle system is generally preferred not only for economic reasons (using one enzyme as opposed to two), but also because it has the added advantage that pH control is not required; a most desirable attribute during the optimisation phase of process development. Most crucially, in this case bioreduction would offer access to the required chiral alcohols (S)-**3** and/or (S)-**4** without the need for chromatographic purification ensuring ease of scalability.

## RESULTS AND DISCUSSION

**Screening.** The desired ketone substrates were synthesised according to known literature precedents from the cheap,



**Figure 1.** Recent natural product synthesis targets, (+)-*trans*-195A and echinopines A and B, which require (S)-**3** or (S)-**4** as intermediates derived from ketones **1** or **2**, respectively.

commercially available, 2-cyclohexen-1-one (**5**) as shown in Scheme 2. Compounds **1** and **2** were obtained in 36% and 74% yield respectively. These reactions were not optimised in any way.

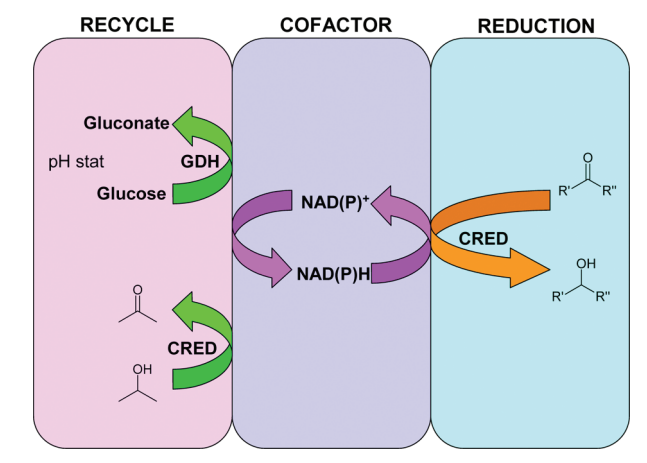
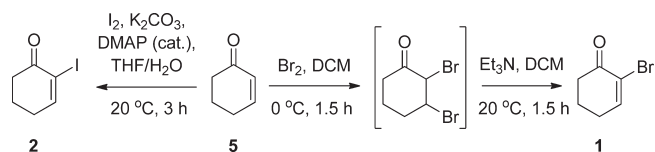
Almac's CESK-5000 carbonyl reductase enzyme kit<sup>8</sup> was screened for the reduction of ketones **1** and **2**. All of the reactions were performed utilising the coupled-enzyme approach, employing a glucose/GDH system, to regenerate the required cofactor. However, for enzymes that exhibit tolerance towards IPA, a second reaction was performed in tandem utilising the coupled-substrate approach. Racemic standards were prepared by carrying out NaBH<sub>4</sub>-mediated reductions of ketones **1** and **2**. Absolute stereochemistry was assigned by comparison of  $[\alpha]_D$  measurements with known literature values. Selections of the screening results for ketones **1** and **2** are shown in Tables 1 and 2, respectively.

As can be seen from Table 1, entry 6 shows that CRED A-601 exhibited high conversion and high enantioselectivity generating the required (S)-isomer quantitatively in 98.8% ee, albeit in the glucose/GDH-coupled system.

As can be seen from Table 2 entry 4, (S)-**4** was also formed in high ee, albeit in lower yield than (S)-**3** under screening conditions. For this reason, the decision was made to move forward with  $\alpha$ -bromoketone **1** as the preferred CRED substrate.

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Scheme 1. Cofactor recycle systems used in CRED asymmetric reductions of prochiral ketones<sup>5</sup>Scheme 2. Synthesis of ketones **1** and **2**, precursors to chiral alcohols **3** and **4**, respectively

**Process Development.** As can be seen from Tables 1 and 2, the lead enzyme/substrate combination during the screening phase of this process was found to be A-601 acting on ketone **1** using the glucose/GDH recycle system (99.9% conversion, 98.8% ee). The use of IPA for cofactor regeneration resulted in a marginally poorer result (95.7% conversion, 96.8% ee). The main advantage of IPA over glucose/GDH for cofactor recycling is cost. In addition, the absence of a requirement for pH control, the potential to use IPA as both cofactor and also organic cosolvent, and the added simplicity of optimising a one-enzyme versus a two-enzyme system encouraged us to proceed with the IPA dependent system.

Brief optimisation studies were carried out to assess the effect of a number of variables, namely, temperature, pH, % IPA loading (v/v), % substrate loading (w/v), and cosolvent. The thermostability of the enzyme was first assessed, as once this has been set, all further optimisation could be carried out using an isothermal parallel reactor. These reactions were carried out under the screening conditions employed during the enzyme selection phase of development.

As can be seen from Figure 2, this process benefits from an increase in temperature up to 30 °C. Beyond this point, the rate of conversion decreases rapidly with increasing temperature. This is undoubtedly due to the denaturation of the carbonyl reductase enzyme. All subsequent reactions were carried out at 30 °C.

The effect of pH on reaction conversion was also assessed by carrying out five parallel reactions covering the pH range of 6–8. The results are depicted in Figure 3.

Figure 3 shows that the rate of conversion of **1** to (*S*)-**3** decreases rapidly once the buffer pH becomes even slightly alkaline. It is worth noting also that although the rate of reaction remained high in slightly acidic media (pH = 6.5/6), the presence of small quantities (<10% total GC area) of unidentified side products

Table 1. Selection of results from the CRED library screen of ketone **1**

entry	enzyme	cofactor	cofactor recycle	% conversion <sup>a</sup>	% ee <sup>b</sup>
1	A101	NADP <sup>+</sup>	GDH	85.3	98.0 ( <i>S</i> )
2	A201	NADP <sup>+</sup>	IPA	6.0	94.7 ( <i>S</i> )
3	A301	NADP <sup>+</sup>	GDH	1.4	63.2 ( <i>S</i> )
4	A401	NADP <sup>+</sup>	GDH	83.4	40.7 ( <i>R</i> )
5	A501	NADP <sup>+</sup>	GDH	4.1	92.1 ( <i>S</i> )
6	A601	NADP <sup>+</sup>	GDH	99.9	98.8 ( <i>S</i> )
7	A131	NAD <sup>+</sup>	IPA	97.8	18.8 ( <i>S</i> )
8	A161	NADP <sup>+</sup>	GDH	82.8	55.4 ( <i>R</i> )
9	A171	NAD <sup>+</sup>	GDH	77.9	90.6 ( <i>S</i> )
10	A231	NADP <sup>+</sup>	GDH	100.0	34.7 ( <i>R</i> )
11	A411	NAD <sup>+</sup>	GDH	5.6	91.7 ( <i>S</i> )
12	A441	NAD <sup>+</sup>	GDH	59.9	97.0 ( <i>R</i> )
13	A451	NAD <sup>+</sup>	IPA	30.8	72.6 ( <i>S</i> )
14	A461	NAD <sup>+</sup>	GDH	28.1	97.2 ( <i>R</i> )
15	A481	NAD <sup>+</sup>	GDH	1.5	68.2 ( <i>S</i> )
16	A511	NAD <sup>+</sup>	GDH	19.4	87.8 ( <i>R</i> )
17	N701	NADP <sup>+</sup>	GDH	8.9	86.4 ( <i>S</i> )

<sup>a</sup> Conversion determined by GC analysis on a Supelco Beta Dex 225 (30 m × 0.25 mm × 0.25 μm). <sup>b</sup> Ee determined by GC analysis on a Supelco Beta Dex 225 (30 m × 0.25 mm × 0.25 μm).

Table 2. Selection of results from the CRED library screen of ketone **2**

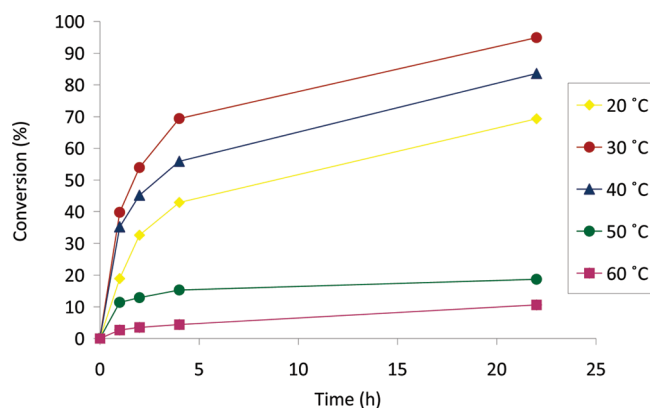
entry	enzyme	cofactor	cofactor recycle	conversion (%) <sup>a</sup>	ee (%) <sup>b</sup>
1	A101	NADP <sup>+</sup>	GDH	45.2	96.5 ( <i>S</i> )
2	A201	NADP <sup>+</sup>	GDH	33.4	67.5 ( <i>S</i> )
3	A401	NADP <sup>+</sup>	GDH	2.7	64.7 ( <i>R</i> )
4	A501	NADP <sup>+</sup>	GDH	3.7	97.3 ( <i>S</i> )
5	A601	NADP <sup>+</sup>	GDH	53.7	95.2 ( <i>S</i> )
6	A131	NAD <sup>+</sup>	GDH	93.4	22.6 ( <i>S</i> )
7	A151	NAD <sup>+</sup>	GDH	4.6	84.6 ( <i>S</i> )
8	A161	NADP <sup>+</sup>	GDH	80.2	23.0 ( <i>R</i> )
9	A171	NAD <sup>+</sup>	GDH	8.3	92.0 ( <i>S</i> )
10	A231	NADP <sup>+</sup>	GDH	94.8	90.2 ( <i>R</i> )
11	A441	NAD <sup>+</sup>	GDH	47.0	98.1 ( <i>R</i> )
12	A451	NAD <sup>+</sup>	GDH	8.6	73.8 ( <i>S</i> )

<sup>a</sup> Conversion determined by GC analysis on a Supelco Beta Dex 225 (30 m × 0.25 mm × 0.25 μm). <sup>b</sup> Ee determined by GC analysis on a Supelco Beta Dex 225 (30 m × 0.25 mm × 0.25 μm).

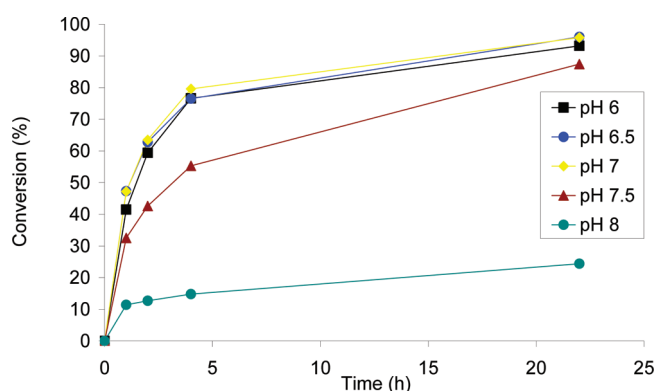
was observed in these cases, suggesting that the vinyl bromide moiety is inherently unstable under acidic conditions. In any case, the optimum level was found to be pH 7, and all subsequent reactions were carried out under this condition.

The effect of IPA loading on the process was explored with another parallel set of experiments, the results of which are outlined in Figure 4.

Figure 4 shows that the optimum IPA loading (v/v with respect to buffer) was 20%. The unusual trend observed here at lower IPA loadings can be explained by the fact that a 1% IPA loading is approximately equal to 0.75 mol equivalents with respect to the substrate and is therefore depleted before reaction



**Figure 2.** Thermostability assessment for A-601 in the conversion of ketone **1** to alcohol (S)-**3**.



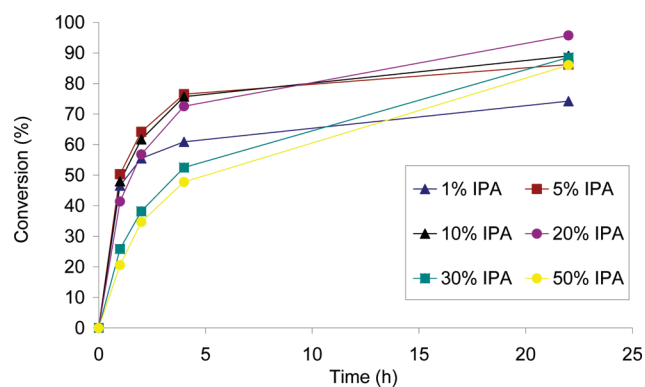
**Figure 3.** Effect of buffer pH in the A-601-mediated conversion of ketone **1** to alcohol (S)-**3**.

completion. It is also evident that higher IPA loadings (30% and 50%) produce a detrimental effect on reaction rate.

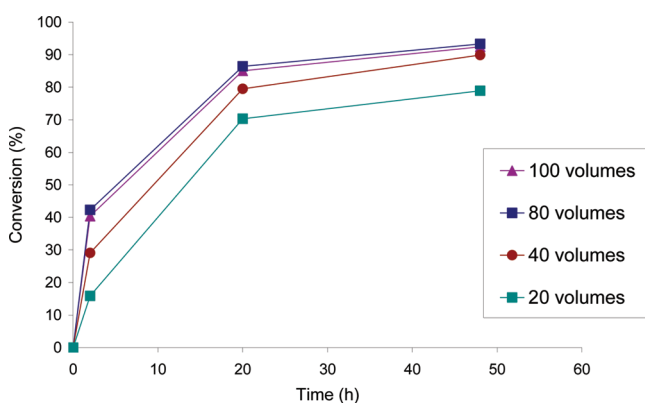
The final parameter that was assessed in this fashion was substrate loading (v/v with respect to buffer). In an effort to move towards a more practically useful, economical process, the cell-free extract (CFE) form of the enzyme that was used up until this point was replaced with crude cell pellet. The results are shown in Figure 5.

As is clearly evident from this data, more attractive volume efficiency results in poor conversion, even after 48 h. The increase in substrate concentration markedly amplifies an underlying problem associated with the coupled-substrate approach to cofactor recycling. The obvious drawback of employing the IPA cofactor regeneration approach is the competition that occurs between all four components of the system; substrate, product, co-substrate, and coproduct. The thermodynamic equilibrium that is established determines the maximum conversion to product that can occur and is independent of the kinetics or catalyst used. It has been demonstrated that removal of acetone from the equilibrium can drive the desired reaction to completion.<sup>9–11</sup> This is a form of ISPR, a concept first pioneered in the area of biotechnology in the 1960s.<sup>12</sup>

A series of experiments were conducted in an effort to monitor the effect of reducing the atmospheric pressure in the reaction vessel. Temperature was maintained constant throughout. A representative sample of the data obtained is shown in Figure 6.



**Figure 4.** Effect of IPA loading on the conversion of ketone **1** to alcohol (S)-**3**.



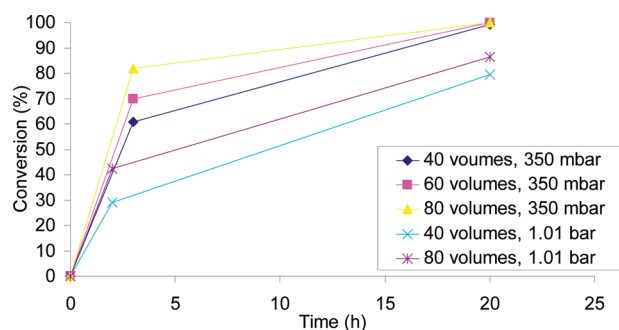
**Figure 5.** Effect of concentration on the A-601 reduction of ketone **1** to afford alcohol (S)-**3**.

Gratifyingly, the reaction could be driven to completion by reducing the pressure to 350 mbar. Unfortunately, it was found to be necessary to maintain the level of IPA in the reaction as this was concomitantly removed with the acetone. This required regular in situ sampling of the reaction by <sup>1</sup>H NMR spectroscopy and addition of the appropriate quantity of IPA to maintain the optimum 20% v/v level. An alternative solution to the problem is illustrated in Figure 7.

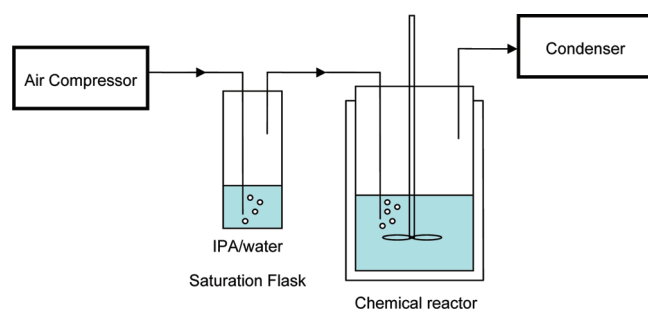
In the experimental setup shown in Figure 7, the saturation flask contains the desired IPA/H<sub>2</sub>O ratio (20% v/v). This allows the level of IPA in the chemical reactor to remain relatively constant throughout the course of the reaction.

Up until this point, all optimization experiments were conducted with DMSO as the organic cosolvent. It was deemed prudent to investigate while another non-volatile solvent might confer a positive effect on the reaction rate, thereby allowing further reduction in key cost contributors such as enzyme loading and/or NADP<sup>+</sup> loading prior to scale-up.<sup>13</sup> Of the cosolvents tested, only DMF achieved comparable results. It was decided to proceed with DMSO as cosolvent.

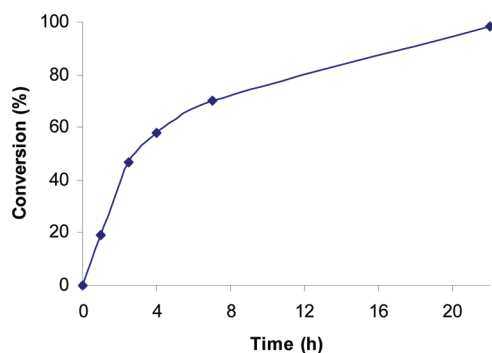
**Scale-Up.** Following a trial reaction on a 10-g scale, the reaction was performed on 100 g of **1**. The reaction profile is shown in Figure 8. There is no negative impact on reaction rate observed when operating at this scale. It is envisaged that when required, this reaction will scale to kilogram quantities without the need for further optimisation.



**Figure 6.** Effect of reduced pressure at a range of substrate loadings on the reduction of ketone **1** to afford alcohol (S)-**3**.



**Figure 7.** IPA/H<sub>2</sub>O-saturated air sparge.



**Figure 8.** Reaction profile of the bioreduction of ketone **1** to alcohol (S)-**3** at 100-g scale.

## CONCLUSION

The synthesis, screening, and process development work described herein has resulted in an efficient, scalable, environmentally friendly route to the required enantioenriched alcohol, (S)-**3**. The use of IPA/H<sub>2</sub>O-saturated air sparging has effectively overcome the inherent unfavorable equilibrium of the system of interest.

## EXPERIMENTAL SECTION

**General.** <sup>1</sup>H NMR spectra were recorded at 300 MHz on a Bruker AV-300 spectrometer. <sup>13</sup>C NMR spectra were recorded at 100 MHz. All chemical shifts are relative to internal TMS. The term ‘volumes’ is used throughout this paper to describe the quantity of solvent used in a given experiment with respect to

mass input of substrate. For example, 100 g of substrate in 1 L equates to 10 volumes.

**Chemicals and Enzymes.** Chemicals were purchased from Sigma Aldrich. CESK-5000 enzyme screening kit and subsequent gram quantities of CRED enzyme A601 were supplied by Almac.

**Analytical Methods.** Conversion and enantiomeric excess measurements were determined by GC analysis using a Perkin Elmer AutoSystem XLGC equipped with Supelco  $\beta$ -DEX 225 column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). Hydrogen was used as a carrier gas. Method: 80 °C hold for 3 min, 5 °C min<sup>-1</sup> until 180 °C, hold for 2 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min<sup>-1</sup>. Retention times for the reduction of (S)-**3** and (R)-**3** from 2-bromo-cyclohexen-1-one (**1**) were 19.6, 21.3, and 31.4 min, respectively. Retention times for the reduction of (S)-**4** and (R)-**4** from 2-iodo-cyclohexen-1-one (**2**) were 18.6, 19.1, and 23.6 min, respectively. Assignment of absolute stereochemistry for each enantiomer was achieved by carrying out the required selective enzymatic reductions at 500 mg scale and comparison of  $[\alpha]_D$  measurement with known literature values.<sup>3,14</sup>

**2-Bromocyclohex-2-enone (1).** A solution of Br<sub>2</sub> (108 mL, 2.11 mol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 L) was added dropwise to a solution of  $\alpha$ -cyclohexen-1-one (**5**) (200 mL, 2.07 mol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 L) at 0 °C over 45 min. The solution was stirred for 1.5 h before dropwise addition of triethylamine (480 mL, 3.44 mol). The reaction mixture was allowed to warm to room temperature and stirred for a further 1.5 h before quenching with 2 M HCl (250 mL). The layers were separated, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  250 mL). The combined organic phases were washed with aqueous NaOH (pH 12.5) to remove phenolic impurities and subsequently washed with brine (300 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Recrystallisation using EtOAc/hexane (1:4 v/v) afforded the product as an off-white solid (130.5 g, 36%). Mp 40–42 °C, lit. 38–40 °C<sup>15</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.09 (2H, m, CH<sub>2</sub>), 2.42–2.52 (2H, m, CH<sub>2</sub>), 2.65 (2H, t,  $J$  = 6.4, CH<sub>2</sub>), 7.44 (1H, t,  $J$  = 4.4, CH); <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 22.7, 28.4, 38.4, 123.9, 151.2, 191.3, lit.<sup>16</sup>

**Racemic 2-Bromo-2-cyclohexen-1-ol (3).** Cerium(III) chloride heptahydrate (2.13 g, 5.71 mmol) was added to a solution of 2-bromocyclohex-2-enone (**1**) (1.00 g, 5.78 mmol) in methanol (15 mL) at 0 °C. The reaction mixture was stirred for 30 min before slow addition of NaBH<sub>4</sub> (0.26 g, 6.88 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h before quenching with ice water (10 mL). The reaction mixture was extracted with diethyl ether (3  $\times$  40 mL), and the combined organic phases were washed with brine (30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo to afford the product as a pale-yellow crystalline solid (0.96 g, 95%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.62–1.77 (2H, m), 1.83–1.98 (2H, m), 2.03–2.13 (2H, m), 2.40 (1H, d,  $J$  = 4.2), 4.22 (1H, t,  $J$  = 4.3), 6.21 (1H, t,  $J$  = 4.2). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 17.6, 27.8, 32.0, 69.9, 125.8, 132.6, lit.<sup>2</sup> HRMS (EI): calcd for C<sub>6</sub>H<sub>9</sub>BrO [M]<sup>+</sup> 175.9837, found 175.9845.

**2-Iodo-2-cyclohexen-1-one (2).** To a solution of 2-cyclohexen-1-one (**5**) (1.58 g, 16.4 mmol) in THF/H<sub>2</sub>O (80 mL, 1:1 v/v) was added K<sub>2</sub>CO<sub>3</sub> (2.73 g, 19.7 mmol), I<sub>2</sub> (6.26 g, 24.7 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.4 g, 3.3 mmol). The reaction mixture was stirred for 3 h at room temperature and then diluted with EtOAc (80 mL). The organic phase was washed with saturated Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (60 mL), 0.1 M HCl (60 mL) and brine (60 mL) before it was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and



concentrated in vacuo to afford the product as a low-melting solid, which was subsequently recrystallised from hexane/EtOAc (4:1 v/v) (2.70 g, 74%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.98–2.07 (2H, m), 2.37–2.42 (2H, m), 2.60 (2H, t,  $J$  = 6.7), 7.72 (1H, t,  $J$  = 4.4);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 23.3, 30.3, 37.7, 104.3, 159.9, 192.6, lit.<sup>17</sup>

**Racemic 2-Iodo-2-cyclohexen-1-ol (4).** 2-Iodo-2-cyclohexen-1-one (2) was reduced as for 3 to yield the title compound as a colourless crystalline solid (1 g, 90%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.59–2.15 (6H, m), 2.28 (1H, s), 4.20 (1H, s), 7.72 (1H, t,  $J$  = 4.4);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 17.7, 29.4, 32.0, 72.1, 103.6, 141.0, lit.<sup>18</sup>

**Screening Conditions for the Enzymatic Reduction of 2-Bromocyclohex-2-enone (1).** A solution of 2-bromocyclohex-2-enone (1) (20 mg) in DMSO (50  $\mu\text{L}$ ) was added to a solution of  $\text{NADP}^+$  or  $\text{NAD}^+$  (2 mg), depending on cofactor preference, in pH 7  $\text{KH}_2\text{PO}_4$  buffer (0.1 M, 1.5 mL). Lyophilized CRED biocatalyst (2 mg), glucose (50 mg), and GDH (3 mg) were added. The vial was sealed and shaken overnight at 30 °C. MtBE (1.5 mL) was added to the vial, and the organic layer was separated, filtered through a cotton wool plug containing anhydrous  $\text{MgSO}_4$ , and analysed by GC.

**(5)-2-Bromo-2-cyclohexen-1-ol ((5)-3).** CRED A601 cell paste (20 g) was resuspended in a solution of pH 7  $\text{KH}_2\text{PO}_4$  buffer (3.9 L, 0.1 M) containing  $\text{NADP}^+$  (1.0 g, 1.3 mmol) and shaken at 20 °C for 30 min. The suspension was transferred to a 5-L reactor to which was added a solution of 2-bromocyclohex-2-enone (1) (100 g, 0.57 mol) dissolved in IPA (800 mL) and DMSO (200 mL). In addition, polypropylene glycol (1.5 mL) was added to prevent excessive foaming. The reaction mixture was stirred mechanically using an overhead stirrer and incubated at 35 °C for 24 h. The depletion of IPA and water was counterbalanced by bubbling a pressurised airflow saturated with 2-propanol and water (1:4 v/v) through the reaction mixture. Upon completion, the resulting mixture was extracted with MtBE (3  $\times$  5 L) and washed with water (2  $\times$  2.5 L) and brine (2  $\times$  2.5 L) and dried over  $\text{MgSO}_4$ . Solvent was removed under reduced pressure to afford the title compound as a colourless oil (89 g, 88%) with an enantiomeric excess of 99.8% as determined by GC analysis.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.62–1.77 (2H, m), 1.83–1.98 (2H, m), 2.03–2.13 (2H, m), 2.40 (1H, d,  $J$  = 4.2), 4.22 (1H, t,  $J$  = 4.3), 6.21 (1H, t,  $J$  = 4.2).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 17.6, 27.8, 32.0, 69.9, 125.8, 132.6, lit.<sup>1</sup>  $[\alpha]_{\text{D}}^{20}$  = –80.3, (c 1.77,  $\text{CHCl}_3$ ); lit.<sup>2</sup>  $[\alpha]_{\text{D}}^{20}$  = –77 (c 1.74,  $\text{CHCl}_3$ ).

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## REFERENCES

- (1) Nicolaou, K. C.; Ding, H.; Richard, J.-A.; Chen, D. Y.-K. *J. Am. Chem. Soc.* **2010**, 132, 3815.
- (2) Holub, N.; Neidhöfer, J.; Blechert, S. *Org. Lett.* **2005**, 7, 1227.
- (3) Moody, T. S.; Taylor, S. *Speciality Chemicals*; Quartz Business Media: Redhill, Surrey, U.K., 2009; p51.

- (4) (a) Brown, G.; Mangan, D.; Miskelly, I.; Moody, T. S. *Org. Process Res. Dev.* **2011**, 5, 1036. (b) Matsuda, T.; Yamanaka, R.; Nakamura, K. *Tetrahedron: Asymmetry* **2009**, 20, 513. (c) Borges, K. B.; de Souza Borges, W.; Durán-Patrón, R.; Pupo, M. T.; Bonato, P. S.; Collado, I. G. *Tetrahedron: Asymmetry* **2009**, 20, 385. (d) Kaluzna, I. A.; Rozzell, J. D.; Kambourakis, S. *Tetrahedron: Asymmetry* **2005**, 16, 3682. (e) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, 14, 2659.
- (5) (a) Yang, Y.; Zhu, D.; Piegat, T. J.; Hua, L. *Tetrahedron: Asymmetry* **2007**, 18, 1799. (b) Padha, S. K.; Kaluzna, I. A.; Buisson, D.; Azerad, R.; Stewart, J. D. *Tetrahedron: Asymmetry* **2007**, 18, 2133. (c) Panizza, P.; Onetto, S.; Rodríguez, S. *Biocatal. Biotransform.* **2007**, 25, 414. (d) Weckbecker, A.; Hummel, W. *Biocatal. Biotransform.* **2006**, 24, 380. (e) Hanson, R. L.; Goldberg, S.; Goswami, A.; Tully, T. P.; Patel, R. N. *Adv. Synth. Catal.* **2005**, 347, 1073. (f) Engelking, H.; Pfaller, R.; Wich, G.; Weuster-Botz, D. *Tetrahedron: Asymmetry* **2004**, 15, 3591.
- (6) (a) Kosjek, B.; Nti-Gyabaah, J.; Telari, K.; Dunne, L.; Moore, J. C. *Org. Process Res. Dev.* **2008**, 12, 584. (b) Zhu, D.; Yang, Y.; Hua, L. *J. Org. Chem.* **2006**, 71, 4202.
- (7) (a) Lavandera, I.; Höller, B.; Kern, A.; Ellmer, U.; Glieder, A.; de Wildeman, S.; Kroutil, W. *Tetrahedron: Asymmetry* **2008**, 19, 1954. (b) Matsuda, T.; Yamagishi, Y.; Koguchi, S.; Iwai, N.; Kitazume, T. *Tetrahedron Lett.* **2006**, 47, 4619. (c) Amidjojo, M.; Weuster-Botz, D. *Tetrahedron: Asymmetry* **2005**, 16, 899.
- (8) See: [www.almacgroup.com/biocatalysis](http://www.almacgroup.com/biocatalysis).
- (9) Stillger, T.; Bönits, M.; Filho, M. V.; Liese, A. *Chem. Ing. Tech.* **2002**, 74, 1035.
- (10) Goldberg, K.; Edegger, K.; Kroutil, W.; Liese, A. *Biotechnol. Bioeng.* **2006**, 95, 192.
- (11) Dausmann, T.; Rosen, T. C.; Dünkemann, P. *Eng. Life Sci.* **2006**, 6, 125.
- (12) Stark, D.; von Stockar, U. *Process Integration in Biochemical Engineering*; Springer-Verlag: Berlin, 2003.
- (13) Andrade, L. H.; Piovan, L.; Pasquini, M. D. *Tetrahedron: Asymmetry* **2009**, 20, 1521.
- (14) Kim, J.; Bruning, J.; Park, K. E.; Lee, D. J.; Singaram, B. *Org. Lett.* **2009**, 11, 4358.
- (15) Sonnerberg, J. J. *Org. Chem.* **1962**, 27, 748.
- (16) Kangying, L.; Alexandre, A. *Angew. Chem., Int. Ed.* **2006**, 45, 7600.
- (17) Krafft, M. E.; Cran, J. W. *Synlett* **2005**, 8, 1263.
- (18) Sha, C.-K.; Huang, S.-J.; Zhan, Z.-P. *J. Org. Chem.* **2002**, 67, 831.