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Highly stereoselective biocatalytic reduction of alpha-halo ketones

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ABSTRACT

The use of recombinant carbonyl reductase biocatalysts for the reduction of alpha-halo ketone intermediates to their corresponding alpha-halo alcohols has been investigated. The alpha-halo alcohol is obtained in good yield from the corresponding ketone in a stereoselective manner.

1. Introduction

The application of biocatalysts for the synthesis of important enantiomerically pure compounds by the fine chemical and pharmaceutical industries has increased greatly over the last decade.[1] The asymmetric reduction of prochiral ketones using carbonyl reductase (CRED) biocatalysts is becoming a straightforward approach for the synthesis of chiral alcohols.[2–5] These biocatalysts have received much attention from both academic and industrial groups and many recombinant CREDs have been reported.[6–12] CRED biocatalysts have now received acceptance in the chemist's toolbox due to their high specificity and enantioselectivity under physiological conditions. For these systems to be used at scale, CREDs require cofactor recycling, and several methods have been employed to multi-kilogram scale including glucose dehydrogenase (GDH)[13–15] and isopropyl alcohol (IPA), Figure 1.[16–18] The use of IPA as the cofactor recycle system has the added advantage that pH control is not required and it can also be used as a co-solvent.

Due to their versatility, CREDs must now be considered as an accepted tool for organic synthesis in the preparation of chiral alcohol intermediates. For instance, ketones of type **1** can be reduced stereoselectively and used in the preparation of a number of pharmaceutically active compounds such as amprenavir **2**,[19] fosamprenavir **3**[20] and atazanavir **4**.[21]

There are numerous examples in the patent and non-patent literature of disclosures relating to the stereoselective reduction of alpha-chloroketones to the corresponding alpha-chloroalcohols. Conventional methods involve the use of inorganic reducing agents to reduce the ketone functionality.[22] In reactions where stereochemical control is required, asymmetric catalysts and/or asymmetric reducing agents may be used. For example, the reaction may be carried out as a transfer hydrogenation using a chiral metal catalyst.[23]

More recently, the chemical industry has turned to enzymatic and fermentation processes in order to obtain enantiomerically or diastereomerically enriched products from reductions of this type.[24] The major advantage of using whole living cells relative to isolated biocatalysts is that the cells can regenerate their own cofactors. In addition, whole cells are often easy to produce, and are relatively inexpensive. The drive towards increasing purity and yield, together with the need to use environmentally less damaging materials, has also encouraged the use of whole living cells. However, one disadvantage of using whole cells is that the volume efficiency of the reaction may be low. The reactions also frequently produce low yields. There has been a significant drive towards the use of recombinant biocatalysts as cell-free extracts to increase volume efficiency and make these biotransformations commercially viable.

Herein we report the use of an isolated recombinant biocatalyst for the reduction of alpha-halo ketones **1** (Scheme 1).

2. Results and discussion

2.1. Carbonyl reductase library screen

A library of commercially available CREDs was screened for the reduction of ketones (*S*)-**1a** and (*S*)-**1b**. A selection of the screening results is shown in Tables 1 and 2, respectively.

These results show that changing the substrate-protecting group can significantly alter the diastereomeric excess (de) of the product alcohol. This is most clearly observed with the results for CRED 119, for which the % de notably varies from 80% (*S*,*S*) to 100% (*S*,*R*).

For both substrates, there are a number of CREDs that afford alcohols with 100% de. This high selectivity clearly illustrates the benefits of biocatalysis for the synthesis of chiral compounds. For comparative purposes, Table 3 shows the results of conventional hydrogenation reactions such as a transfer hydrogenation performed using known catalysts[23] and a sodium borohydride reduction.[22] It should be noted that from a panel of CRED biocatalysts, all

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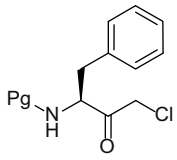
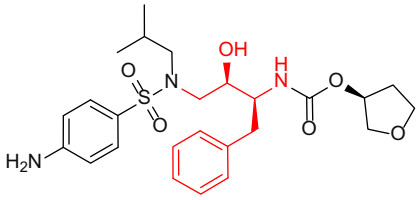


1a, Pg = Boc
1b, Pg = Cbz



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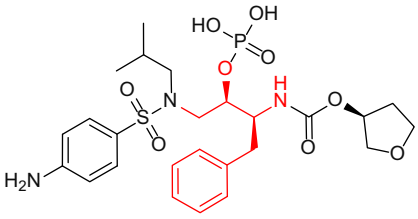

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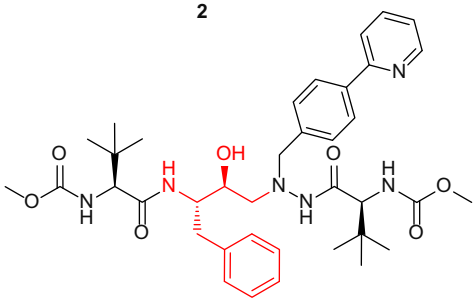

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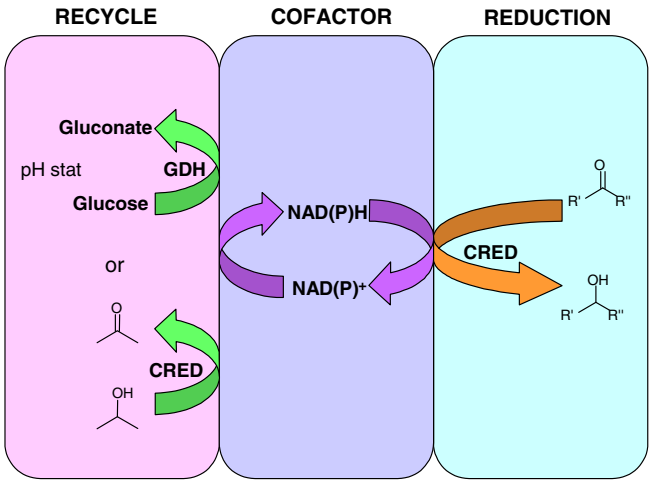


Figure 1. Cofactor recycle systems used in CRED asymmetric bioreductions of prochiral ketones.

four potential products (*S*,*R*)-**5a**, (*S*,*S*)-**5a**, (*S*,*R*)-**5b** and (*S*,*S*)-**5b** are attainable with 100% de.

2.2. Process development

To demonstrate the effectiveness of a CRED for the synthesis of a diastereoselective alcohol, process development experiments were carried out on substrate (*S*)-**1a**, using NADH-dependent CRED C1E. We discovered that this CRED is able to utilise isopropanol (IPA) for cofactor regeneration, precluding the requirement for GDH (Scheme 1) and therefore the need for pH control.

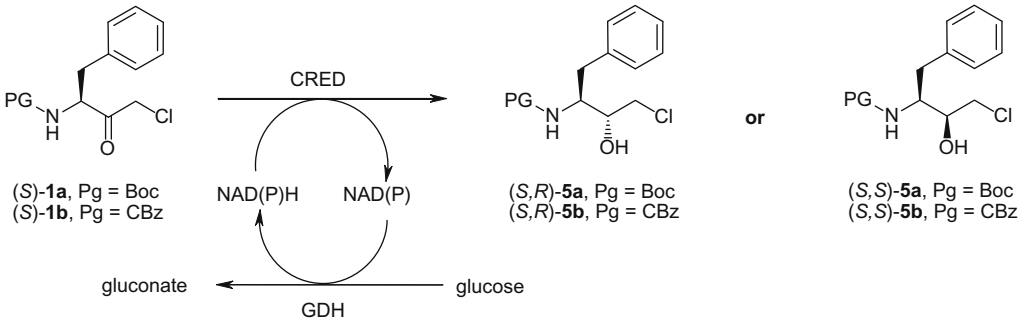
An initial experiment was carried out to establish the optimum % IPA (v/v) in the reaction mixture. A substrate loading of 10 g/L was used. Samples were taken at time points and % conversions were determined by HPLC analysis (Fig. 2). The optimum IPA concentration for this reduction was found to be 30% v/v.

Screening reactions were then carried out to assess the effect of a range of solvents, at approximately 30% (v/v), on enzyme activity.

Table 1
Carbonyl reductase screening data for the reduction of compound (*S*)-**1a**

Enzyme catalyst	Conversion[a] (%)	de[b] (%)	Cofactor dependency
CRED 111	28.2	76 (*S*,*S*)	NADPH
CRED 112	26.1	100 (*S*,*S*)	NADPH
CRED 113	18.4	100 (*S*,*S*)	NADPH
CRED 119	10.8	80 (*S*,*S*)	NADPH
CRED 121	18.6	84 (*S*,*S*)	NADPH
CRED 125	48.2	48 (*S*,*S*)	NADPH
CRED 128	18.4	86 (*S*,*S*)	NADPH
CRED 130	24.0	67 (*S*,*S*)	NADPH
CRED C1E	36.2	100 (*S*,*R*)	NADH
CRED C1A	58.3	100 (*S*,*S*)	NADH

[a] Determined after 16 h reaction.
[b] Determined by HPLC using a Phenomenex Sphereclone 5μ silica column (4.6 × 150 mm) with 95:5 hexane/IPA as eluent.



(*S*)-**1a**, Pg = Boc
(*S*)-**1b**, Pg = CBz

(*S*,*R*)-**5a**, Pg = Boc
(*S*,*R*)-**5b**, Pg = CBz

(*S*,*S*)-**5a**, Pg = Boc
(*S*,*S*)-**5b**, Pg = CBz

Scheme 1. Enzymatic asymmetric reduction of ketone **1** with carbonyl reductase, including NAD(P)H cofactor recycling system using glucose dehydrogenase (GDH).

Table 2
Carbonyl reductase screening data for the reduction of compound (*S*)-**1b**

Enzyme catalyst	Conversion[a] (%)	de[b] (%)	Cofactor dependency
CRED 111	41.6	56 (*S*,*S*)	NADPH
CRED 112	43.5	100 (*S*,*S*)	NADPH
CRED 113	49.3	100 (*S*,*S*)	NADPH
CRED 119	40.1	100 (*S*,*R*)	NADPH
CRED 121	38.9	27 (*S*,*S*)	NADPH
CRED 125	25.8	73 (*S*,*S*)	NADPH
CRED 128	34.1	100 (*S*,*S*)	NADPH
CRED 130	13.8	48 (*S*,*R*)	NADPH

[a] Determined after 16 h reaction.
[b] Determined by HPLC using a Phenomenex Luna 5μ silica column (4.6 × 150 mm) with 98:2 hexane/IPA as eluent.

Table 3
Chemical reduction of ketones (*S*)-**1a** and (*S*)-**1b**

Entry	Reducing system	de of **5a** (%)	de of **5b** (%)
1	$NaBH_4$	50 (*S*,*S*)	50 (*S*,*S*)
2	CATHy™	80 (*S*,*R*)	80 (*S*,*R*)
3	(*S*,*S* TSDPEN)Ru(Mes)	96 (*S*,*S*)	40 (*S*,*S*)
4	(*R*,*R* TSDPEN)Ru(Mes)	70 (*S*,*R*)	72 (*S*,*R*)

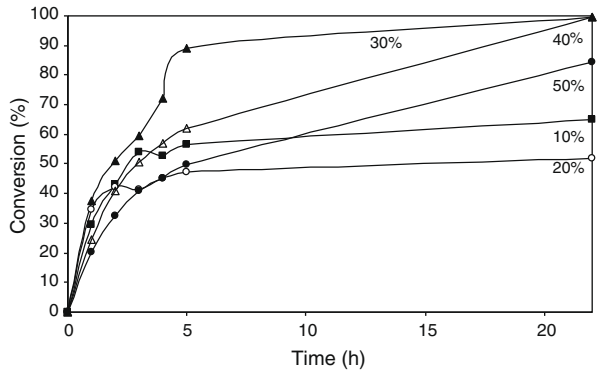


Figure 2. Effect of % IPA (v/v) on the reduction of (*S*)-**1a** by CRED C1E.

An IPA concentration of 30% (v/v), and a substrate loading of 10 g/L were used (Fig. 3). All the co-solvents investigated resulted in an overall reduction of activity with the exception of heptane, which exhibited a stimulatory effect. This is likely due to the improved solubility of the substrate in the presence of heptane and the lack of a significant concurrent inhibitory effect due to the solvent.

These optimised conditions were then used to assess the effect of substrate concentration on the activity of CRED C1E. Reactions were run using substrate concentrations varying from 50 g/L[1] to 200 g/L. The insolubility of substrate (*S*)-**1a** meant that accurate sampling from the heterogeneous reaction mixture at various time points was difficult. Therefore each reaction mixture was completely extracted with organic solvent after 21 h to determine the conversion, and consequently the concentration of alcohol product formed (g/L) (Fig. 4). A substrate concentration of 150 g/L provided 62 g/L of product.

To investigate the effect of pH on the activity of CRED C1E, four reactions were carried out at different pH values, each containing 30% IPA (v/v), 30% heptane (v/v) and substrate (*S*)-**1a** (150 g/L). The pH of each reaction was modified by the addition of either 1 M NaOH or 1 M HCl solutions. Extraction of each reaction mixture after 21 h and analysis by HPLC revealed that CRED C1E operates best at pH 7 (Fig. 5). However, it seems to be able to tolerate from pH 6.5 to 7.5 without major impact on the activity. This pH control is easily attainable on a pilot plant scale, and we have controlled to within a range of <0.1. It should be noted that in all reactions, the % de of the alcohol product was consistently 100% (*S*,*R*).

2.3. Scale-up

A bioreduction of (*S*)-**1a** by CRED C1E was carried out on a 10 g scale to demonstrate the viability of this process (reaction conditions described in Section 4.4). The reaction reached >98.5% conversion over a 21 h period at 50 g/L (Fig. 6).

3. Conclusion

This work successfully demonstrates the utility of a recombinant CRED biocatalyst for the stereoselective synthesis of an al-

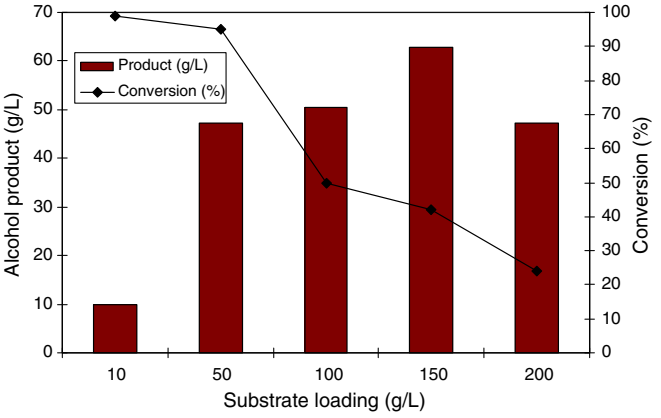


Figure 4. Effect of different substrate concentrations on the reduction of (*S*)-**1a** by CRED C1E.

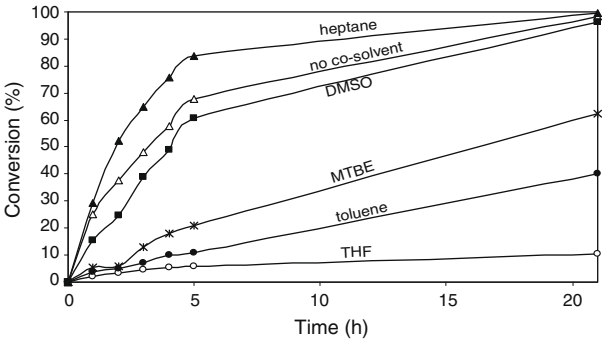


Figure 3. Effect of co-solvents on the reduction of (*S*)-**1a** by CRED C1E.

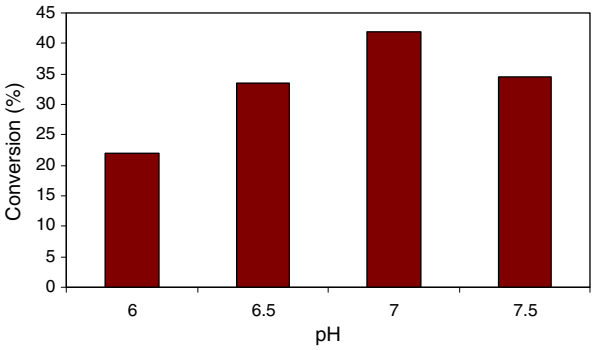


Figure 5. Effect of pH on the reduction of (*S*)-**1a** by CRED C1E.

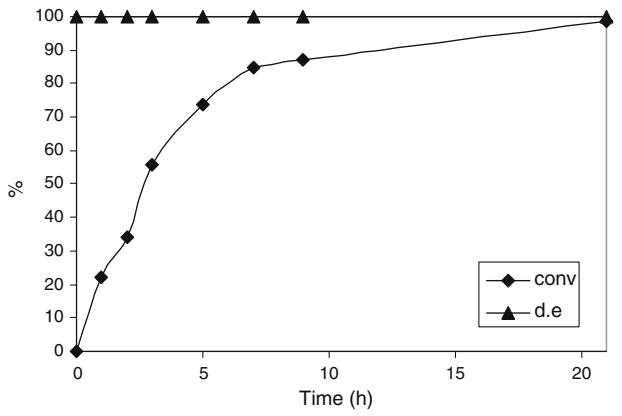


Figure 6. Scaled-up reduction of **1a** by CRED C1E.

pha-halo alcohol yielding both diastereoisomers by enzymatic reduction of the corresponding ketone precursor. IPA was shown to be a suitable means of cofactor regeneration without the need for pH control.

4. Experimental

4.1. Materials

Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purifications. CREDs were purchased from Biocatalytics Inc. Information on the source of each CRED is not provided. Compounds (*S*)-**1a** and (*S*)-**1b** were prepared according to literature procedures.[25] Standards were prepared via sodium borohydride reduction of the ketone.[23] Conversion and diastereomeric excess were determined on a Shimadzu CDD 10A VP HPLC detector using a Phenomenex Sphereclone 5μ silica column (4.6 × 150 mm) with a solvent eluent of 95:5 hexane/IPA and UV detection (λ = 254 nm) for reactions with (*S*)-**1a**, and a Phenomenex Luna 5μ silica column (4.6 × 150 mm) with a solvent eluent of 98:2 hexane/IPA and UV detection (λ = 254 nm) for reactions with (*S*)-**1b**.

4.2. Screening conditions for the enzymatic reduction of (*S*)-1a and (*S*)-1b

A solution of chloroketone (*S*)-**1a** or (*S*)-**1b** (5 mg) in dimethylsulfoxide (200 μL) was added to a mixture of 0.1 M KH_2PO_4 buffer at pH 7 (1 mL) and NADPH (2 mg). Lyophilised CRED biocatalyst (2 mg) and GDH (2 mg) were added; the vial was sealed and shaken overnight at room temperature. Ethyl acetate (0.5 mL) was added to the vial and the organic layer was separated, filtered through cotton wool and dried ($MgSO_4$). The ethyl acetate was evaporated in an oven at 60 °C and the residue was redissolved in the appropriate HPLC solvent. Samples were analysed by HPLC as described in Section 4.1. Retention times for the reduction of (*S*)-**1a**: 3.3 min (*S,R*), 3.6 min (*S,S*). Retention times for the reduction of (*S*)-**1b**: 11.1 min (*S,R*), 17.0 min (*S,S*).

4.3. Experimental for asymmetric transfer hydrogenations

IPA as a hydrogen source: Potassium hydroxide (4 mg) and Ru(mesitylene)(TSDPEN) catalyst (4 mg) were added to a solution of chloroketone (200 mg) in IPA under nitrogen atmosphere, then heated at 60 °C for 60 h. The reaction was diluted with water (10 mL), extracted into ethyl acetate (2 × 20 mL), dried (Na_2SO_4) and concentrated. NMR values were consistent with those mentioned in the literature.[23] Samples were analysed by HPLC (Phenomenex Sphereclone 5μ silica), eluent 95:5 hexane/IPA.

Triethylammonium formate (TEAF) as a hydrogen source: Freshly distilled TEAF (0.4 mL) was added to a solution of chloroketone (200 mg) in degassed ethyl acetate (2 mL) followed by the addition of Ru(mesitylene)(TSDPEN) catalyst (4 mg), and stirred at 30 °C for 60 h. The reaction was diluted with water (10 mL), extracted into ethyl acetate (2 × 20 mL), dried (Na_2SO_4) and concentrated. Samples were analysed by HPLC (Phenomenex Sphereclone 5μ silica), eluent 95:5 hexane/IPA.

4.4. Enzymatic reduction of ketone (*S*)-1a using IPA for cofactor regeneration

Compound (*S*)-**1a** (10 g, 33.58 mmol) was added to 0.1 M KH_2PO_4 buffer (pH 7, 120 mL) followed by DMSO (6 mL), IPA (37 mL) and heptane (37 mL). Next, NAD (100 mg) was added, followed by CRED C1E (200 mg), and the reaction was stirred at 30 °C. Samples were taken at time points and run on HPLC to monitor the reaction progress. Conversion and diastereomeric excess were determined on a Shimadzu CDD 10A VP HPLC detector using a Phenomenex Sphereclone 5μ silica column (4.6 × 150 mm) with a solvent eluent of 95:5 hexane/IPA with UV detection (λ = 254 nm). After 21 h, the reaction reached 98.5% completion. The mixture was extracted with EtOAc (4 × 40 mL), dried over $MgSO_4$ and evaporated to dryness, yielding compound (*S,R*)-**5a** (9.36 g, 31.22 mmol, 93%) as a white solid with 100% de and 98.2% purity by wt/wt NMR. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.91 (dd, *J* = 8.2, 13.5 Hz, 1H), 3.00 (dd, *J* = 7.2, 13.5 Hz, 1H), 3.55 (m, 2H), 3.74 (m, 1H), 3.84 (m, 1H), 4.90 (br d, *J* = 8.6 Hz, 1H), 7.15–7.35 (m, 5H); $[\alpha]_D^{20} = -40$ (*c* 0.1, $CHCl_3$); mp 83–84 °C ($CHCl_3$).

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