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A Facile Stereoselective Biocatalytic Route to the Precursor of Woody Acetate

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ABSTRACT: Carbonyl reductase (CRED) technology has been shown to be an important tool for the rapid and efficient preparation of *cis*- and *trans*-4-*tert*-butylcyclohexanol, the precursors to the high volume fragrance ingredients known as Woody Acetate.

INTRODUCTION

Both *cis* and *trans* forms of 4-*tert*-butylcyclohexyl acetate, **1** and **2**, respectively (Figure 1), are widely used as a perfume for cosmetics including soaps. The fragrance of its *cis*-isomer is more favorable than that of its *trans*-isomer, having a creamy, woody, and sweet odor.[1] *cis*-4-*tert*-Butylcyclohexyl acetate **1** is obtained by acetylation of the corresponding alcohol **3** (Scheme 1).

Current methods for Woody Acetate production include the hydrogenation of 4-*tert*-butylphenol in the presence of a catalyst supported on a carrier under a superatmospheric pressure of hydrogen and at an elevated temperature. Sumitomo patented a hydrogenation process based on rhodium on carbon with HCl or sulfuric acid[2] that yields isomer ratios of approximately 80:20 to 90:10 *cis*/*trans*. More recently Abdur-Rashid et al. have submitted a patent application[3] for the stereoselective reduction of 4-*tert*-butylcyclohexanone with ruthenium-aminophosphine complexes in the presence of hydrogen gas and base. Ratios of 96:4 *cis*/*trans* have been achieved. Whilst the *cis*/*trans* ratios obtained are acceptable, these processes suffer from a number of drawbacks, namely, the use of expensive metal catalysts and/or corrosive reagents. With these facts in mind, the investigation of a biocatalytic route to generate *cis*-4-*tert*-butylcyclohexyl acetate becomes increasingly attractive.

The application of biocatalysts to the synthesis of important enantiomerically pure compounds by the fine chemical and pharmaceutical industries has increased exponentially over the past decade.[4] The asymmetric reduction of prochiral ketones using carbonyl reductase (CRED) biocatalysts is becoming a straightforward approach for the synthesis of chiral alcohols.[5] CRED enzymes have received much attention by both academic and industrial groups, and many cloned reductases have been reported.[6] These biocatalysts have now received acceptance in the chemist's tool-box due to their high specificity and enantioselectivity under physiological conditions. For these systems to be used at scale, CRED enzymes require cofactor recycling, and several methods have been employed at multikilogram scale including glucose dehydrogenase (GDH)[7] and isopropyl alcohol (IPA) (Scheme 2).[8] The use of IPA as the cofactor recycle system has the added advantage that pH control is not required and can also be used as cosolvent.

Due to their versatility, CRED enzymes must now be considered as an accepted tool in organic synthesis for the preparation of chiral alcohol intermediates.

RESULTS AND DISCUSSION

Almac's CESK 5000 carbonyl reductase enzyme kit was screened for the reduction of 4-*tert*-butylcyclohexanone. A selection of the screening results is shown in Table 1.

The screening results show that both the *cis*- and *trans*-isomers are available in high yield and diastereomeric excess. For example, entry 13 shows that enzyme A161 gave 100% de for the *cis*-isomer with 100% conversion after 24 h, while entry 28 shows that the *trans*-isomer was obtained in 100% de with 95% conversion after 24 h, screening with enzyme N151.

Process Development. To demonstrate the effectiveness of a carbonyl reductase for the synthesis of **1**, process development experiments were carried out on 4-*tert*-butylcyclohexanone, using NADH-dependent CRED A161. Gratifyingly, this enzyme was found to utilize isopropanol (IPA) for cofactor regeneration, precluding the requirement for GDH (Scheme 2) and therefore the need for pH control. The key driver for the production of Woody Acetate is cost, and therefore the application of a one enzyme system is desirable to minimize the cost contribution of the catalyst.

Screening reactions were carried out to assess the effect of a number of variables, namely, cosolvent, pH, temperature, % IPA loading (v/v), % cell paste loading (w/w) and % substrate loading (w/v). The cosolvent screen was carried out with a substrate loading of 10 g/L using 1.5 vol (1.8 mL) of cosolvent. All cosolvents, with the exception of acetonitrile, had negligible impact on the enzymatic activity with conversions of 100% achieved after 4 h as shown in Figure 2. As such, MtBE was employed as cosolvent in all future reactions to solubilise the substrate, 4-*tert*-butylcyclohexanone, for economical reasons and also because it was known to possess both a low boiling point and low water miscibility, favorable attributes for reaction workup.

A screen 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 various time points, and % conversions were determined by GC analysis. IPA (30%, v/v) was established as the optimum concentration for this reduction (Figure 3).

Temperature optimization studies were carried out between 20 and 60 °C as shown in Figure 4, and as expected, the reaction showed a temperature profile with maximum activity being achieved between 40 and 50 °C. Above this temperature the

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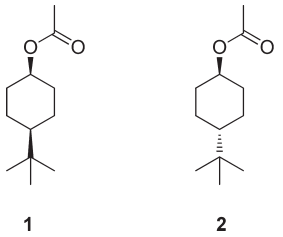
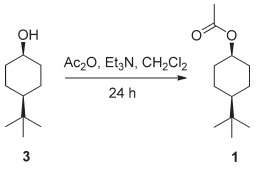
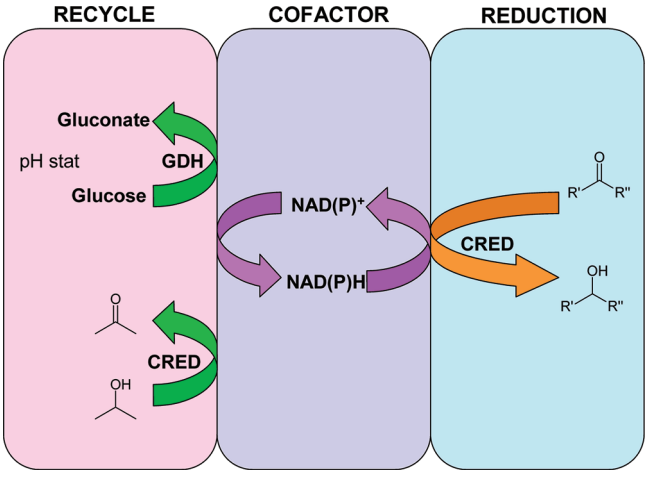


Figure 1. *cis* and *trans* forms of Woody Acetate.

Scheme 1. Woody Acetate Is Synthesised by Acylation of the Corresponding Alcohol 3



Scheme 2. Cofactor Recycle Systems Used in CRED Asymmetric Reductions of Prochiral Ketones



enzyme activity drops off significantly, probably due to protein denaturation.

The effect of pH on the activity of CRED A161 was also investigated. The results are shown in Figure 5. The pH profiles showed minimal change in the rates of conversion over a given time period, demonstrating the enzymes pH stability over the pH range 6.5−8.0.

With increasing pressures to lower cost, the enzyme cell paste loading was also investigated as shown in Figure 6. The reduction is complete within 24 h with 10% (w/w) cell paste loading. Cell paste was chosen as the formulation of the enzyme for subsequent reactions as this is the most economical way to access the catalyst directly from fermentation.

These optimized conditions were then used to assess the effect of substrate concentration on the activity of CRED A161. Reactions were run using substrate concentrations varying from 10 to 500 g/L. Samples were taken from each reaction after 24 h and analyzed by GC to determine conversion and, consequently, the concentration of alcohol product formed (g/L) (Figure 7). A substrate concentration of 500 g/L provided 370 g/L of product after a 24 h period.

Table 1. Selection of Results from the Carbonyl Reductase Library Screen of 4-*tert*-Butylcyclohexanone

entry	enzyme	conversion (%)[a]	de (%)[b]	*cis*/*trans*
1	A101	4	10.9	*cis*
2	A201	88	6.6	*trans*
3	A401	100	3.5	*trans*
4	A601	68	54.3	*cis*
5	A701	23	13.1	*trans*
6	A801	8	50.5	*cis*
7	A901	27	73.4	*cis*
8	A111	9	58.7	*cis*
9	A121	23	70.5	*cis*
10	A131	98	100	*cis*
11	A141	12	60.8	*cis*
12	A151	69	100	*cis*
13	A161	100	100	*cis*
14	A171	70	81.8	*trans*
15	A231	46	100	*cis*
16	A281	68	21.8	*cis*
17	A291	37	67.0	*cis*
18	A311	61	90.4	*cis*
19	A321	27	100	*cis*
20	A341	31	72.2	*cis*
21	A371	72	19.2	*cis*
22	A411	37	100	*cis*
23	A431	35	100	*cis*
24	A451	91	100	*cis*
25	A471	75	99.8	*trans*
26	N121	8	90.1	*trans*
27	N131	68	99.1	*trans*
28	N151	95	100	*trans*

[a] Conversion determined by GC analysis. [b] de determined by GC analysis on a Supelco Beta Dex 225 (30 M × 0.25 mm × 0.25 μm).

Scale-Up. To test the scalability of the CRED reduction, the reaction was performed on 500 g of 4-*tert*-butylcyclohexanone. The reaction profile is shown in Figure 8. There is no apparent impact on reaction rate when moving from single gram quantities to 500 g scale.

CONCLUSION

This work has resulted in a cost-effective, easily scalable, volume efficient process for the generation of pure *cis*-*tert*-butylcyclohexanol, **1**, a valuable intermediate in the synthesis of Woody Acetate. The mild reaction conditions, ease of workup, and relatively benign waste streams make this a more attractive process than asymmetric catalysis reactions involving high temperatures/pressures, corrosive reagents, and expensive metal catalysts.

EXPERIMENTAL SECTION

Chemicals and Enzymes. Chemicals were purchased from Alfa Aesar. CESK 5000 enzyme screening kit and subsequent gram quantities of CRED enzyme A161 were supplied by Almac.

Analytical Methods. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer; shifts are relative to internal TMS.

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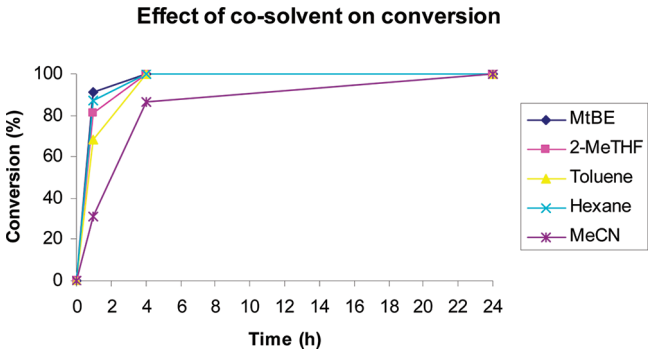

Figure 2. Cosolvent screen for the CRED reduction of 4-*tert*-butylcyclohexanone.

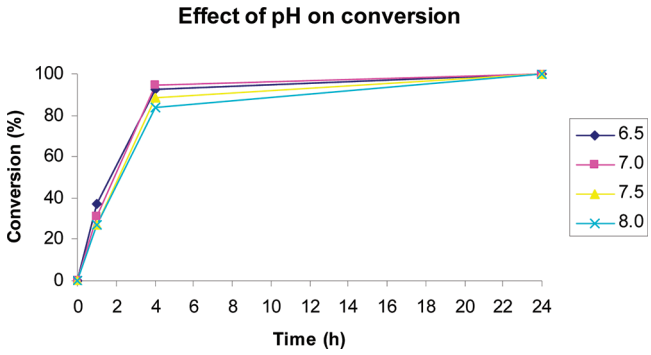

Figure 5. Effect of pH on the CRED reduction of 4-*tert*-butylcyclohexanone.

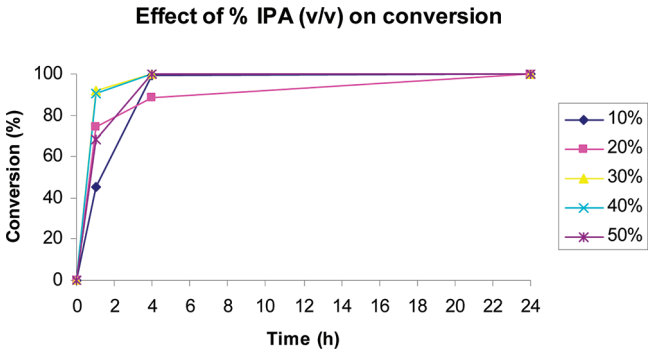

Figure 3. Effect of IPA concentration (v/v) on the CRED reduction of 4-*tert*-butylcyclohexanone.

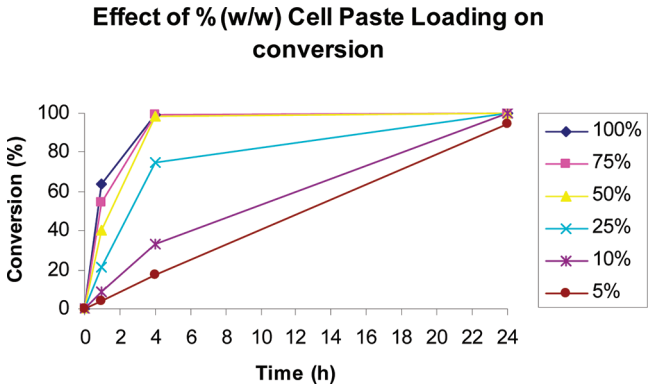

Figure 6. Effect of % (w/w) cell paste loading on the CRED reduction of 4-*tert*-butylcyclohexanone.

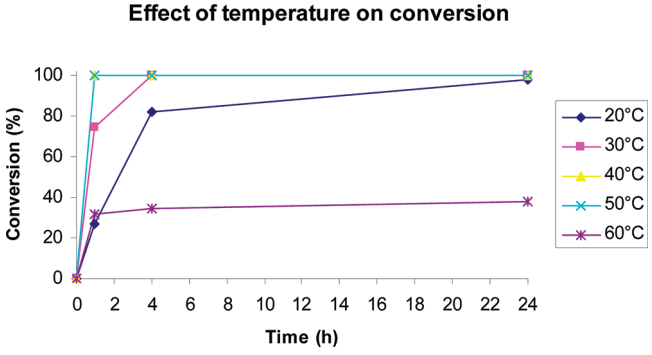

Figure 4. Temperature profiles for the CRED reduction of 4-*tert*-butylcyclohexanone.

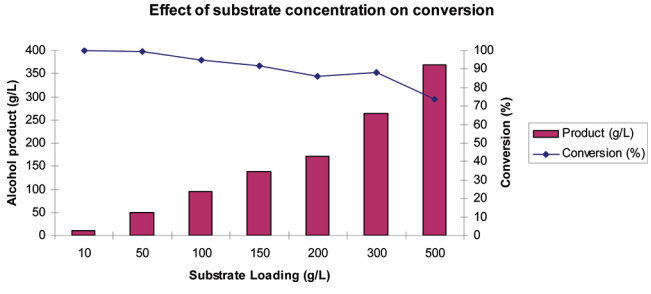

Figure 7. Effect of substrate concentration on the CRED reduction of 4-*tert*-butylcyclohexanone.

The diastereomeric excesses were measured by chiral stationary phase GC on a Supelco Beta Dex 225 column (30 m × 0.25 mm × 0.25 μm). Typical retention times were 8.0 min for *cis*-4-*tert*-butylcyclohexanol, 8.4 min for *trans*-4-*tert*-butylcyclohexanol, and 10.5 min for 4-*tert*-butylcyclohexanone.

Screening Conditions for the Enzymatic Reduction of 4-*tert*-Butylcyclohexanone. A solution of 4-*tert*-butylcyclohexanone (20 mg) in DMSO (50 μL) was added to a vial containing 0.1 M KH_2PO_4 buffer, pH 7 (2 mL), NAD (1 mg), GDH (2 mg), glucose (75 mg), and lyophilized CRED enzyme (5−10 mg). The vial was sealed and shaken for 16 h at 25 °C. MtBE (2 mL) was added to the vial, and the organic layer was separated, dried over anhydrous sodium sulphate, and filtered through a cotton wool plug. The samples were analyzed directly by GC as described in Analytical Methods.

Process Development Reaction Conditions. Process development experiments were carried out using a Radley's Tornado parallel reactor at 400 rpm. All reactions were scaled up/down as required to run at a standardized 120 mL total reaction volume in order to eliminate extraneous environmental factors. Biphasic samples (1 mL) were withdrawn from the reaction media while stirring was maintained. MtBE (2 mL) was added, and the sample was shaken vigorously. The organic layer was separated, dried over anhydrous sodium sulphate, and filtered through cotton wool. The samples were analyzed directly by GC as described in Analytical Methods.

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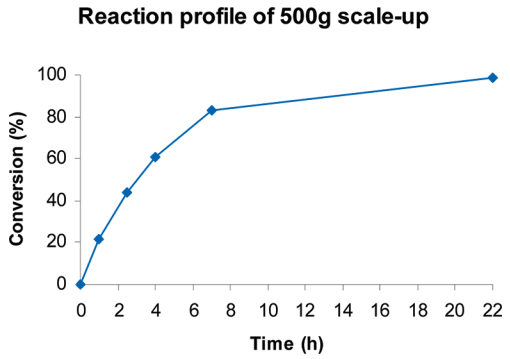

Figure 8. Reaction profile at 500 g scale.

Large Scale Bioreduction of 4-*tert*-Butylcyclohexanone. To a stirred solution (~350 rpm) of 0.1 M KH_2PO_4 buffer, (pH 7, 700 mL) and IPA (300 mL, 235 g, 3.92 mol) were added enzyme A161 cell paste (50 g) and NAD (1 g, 1.5 mmol). 4-*tert*-Butylcyclohexanone (500 g, 3.244 mol) was dissolved in the minimum volume of MtBE (750 mL) and charged to the reactor. The biphasic mixture was heated to 45 °C. Samples were removed as described above for analysis at various time points until reaction completion was observed after 22 h. Celite (25 g) was added to the reaction mixture. Agitation was continued for a further 2 min before filtering through a small pad of Celite. The lower aqueous layer was separated and extracted with MtBE (2 × 300 mL). All organic extracts were combined and washed with brine (300 mL), dried over anhydrous sodium sulphate, and concentrated under vacuum to yield 462 g of **3** as a white crystalline solid (2.959 mol, 91%); ≥98% w/w purity established by ^{1}H NMR assay. ^{1}H NMR ($CDCl_3$, 400.13 MHz) 0.86 (9H, s), 1.30−1.85 (9H, m), 4.03 (1H, br t, *J* 2.5); ^{13}C NMR ($CDCl_3$, 100.62 MHz) 20.86, 27.45, 32.53, 33.34, 47.98, 65.89, lit.[9]; HRMS (EI+) calcd for $C_{10}H_{20}O$ 156.1514, found 156.1514.

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REFERENCES

(1) Arctander, S. *Perfume and Flavor Chemicals*; Steffen Arctander: Montclair, NJ, 1969; Monograph no. 441.

(2) Sekiguchi, M.; Tanaka, S. Processes for preparing 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate. U.S. Patent 1999/5977402, 1999.

(3) Abdur-Rashid, K.; Chen, X.; Guo, R.; Jia, W. Method for the preparation of *cis*-4-*tert*-butylcyclohexanol. U.S. Patent 2010/0204524, A1, 2010.

(4) Moody, T. S.; Taylor, S. *Spec. Chem.* **2009**, No. January/February Issue, 51.

(5) (a) Matsuda, T.; Yamanaka, R; Nakamura, K. *Tetrahedron: Asymmetry* **2009**, *20*, 513. (b) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659. (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.

(6) (a) Weckbecker, A.; Hummel, W. *Biocatal. Biotransform.* **2006**, *24*, 380. (b) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M; Shimizu, S. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 1430. (c) Engelking, H.; Pfaller, R.; Wich, G.; Weuster-Botz, D. *Tetrahedron: Asymmetry* **2004**, *15*, 3591. (d) Hanson, R. L.; Goldberg, S.; Goswami, A.; Tully, T. P.; Patel, R. N. *Adv. Synth. Catal.* **2005**, *347*, 1073. (e) Yang, Y.; Zhu, D.; Piegat, T. J.; Hua, L. *Tetrahedron: Asymmetry* **2007**, *18*, 1799. (f) Padha, S. K.; Kaluzna, I. A.; Buisson, D.; Azerad, R.; Stewart, J. D. *Tetrahedron: Asymmetry* **2007**, *18*, 2133. (g) Panizza, P.; Onetto, S.; Rodríguez, S. *Biocatal. Biotransform.* **2007**, *25*, 414.

(7) (a) Zhu, D.; Yang, Y.; Hua, L. *J. Org. Chem.* **2006**, *71*, 4202. (b) Kosjek, B.; Nti-Gyabaah, J.; Telari, K.; Dunne, L.; Moore, J. C. *Org. Process Res. Dev.* **2008**, *12*, 584. (c) Kataoka, M.; Yamamoto, K.; Kawabata, H.; Wada, M.; Kita, K.; Yanase, H.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 486.

(8) (a) Matsuda, T.; Yamagishi, Y.; Koguchi, S.; Iwai, N.; Kitazume, T. *Tetrahedron Lett.* **2006**, *47*, 4619. (b) Amidjojo, M.; Weuster-Botz, D. *Tetrahedron: Asymmetry* **2005**, *16*, 899. (c) Lavandera, I.; Höller, B.; Kern, A.; Ellmer, U.; Glieder, A.; de Wildeman, S.; Kroutil, W. *Tetrahedron: Asymmetry* **2008**, *19*, 1954.

(9) Spiniello, M.; White, J. M. *Org. Biomol. Chem.* **2003**, *1*, 3094.