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# Enzymatic hydrogen transfer reduction of α-chloro aromatic ketones catalyzed by a hyperthermophilic alcohol dehydrogenase

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ABSTRACT

An alcohol dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus* (PFADH) effectively catalyzed the reductions of various substituted α-chloroacetophenones to furnish the corresponding (*R*)-configurated α-chlorohydrins with excellent enantiomeric purity. The co-factor NADH could be recycled with D-glucose dehydrogenase/D-glucose system or in a coupled substrate approach using *iso*-propanol as the hydrogen donor. The hydrogen transfer mode should be more cost-effective. Thus, the PFADH-catalyzed hydrogen transfer reductions of some substrates were carried out on the preparative scale, demonstrating that this enzyme would be a valuable biocatalyst for the preparation of chiral chlorohydrins of pharmaceutical interest.



## 1. Introduction

Optically active chlorohydrins are versatile intermediates for the syntheses of biologically active compounds of pharmaceutical and agricultural interest. For example, they have been widely used in the preparation of a large group of β-adrenergic receptor agonists [1,2], 2-aryl-substituted morpholine derivatives, which have a τ protein kinase 1 inhibitory activity and are useful as a therapeutic drug for Alzheimer's disease [3], and substituted pyrrolidines as molanocortin receptor agonists [4]. (*R*)- or (*S*)-2-chloro-1-(2′,4′-dichlorophenyl) ethanol has been used to construct optically active imazalil as a fungicide [5]. A straightforward approach to the synthesis of optically active chlorohydrins is the asymmetric reduction of α-chloroketones, which can be achieved by traditional chemical [2,6–9] or biocatalytic [10–16] methods. Biocatalytic reduction processes are usually carried out under neutral reaction conditions, which would greatly benefit the reduction of highly base-labile α-chloroketones [17–19]. Although biocatalysts possess such an advantage, most studies on the biocatalytic reduction of α-chloroketones have been limited to 2-chloroacetophenone itself, and only a few reports have examined the bioreduction of substituted α-chloroacetophenones [12,20–22]. The scarcity of such studies and the prevalence of chiral chlorohydrins as key building blocks for the preparation of a variety of pharmacologically active compounds have stimulated our interest in developing enzymatic processes for the synthesis of optically active chlorohydrins [23]. Recently, we have assessed the substrate specificity and enantioselectivity of an alcohol dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus* (PFADH) toward a variety of ketones including aryl ketones, α- and β-ketoesters [24,25]. It has been found that the hyperthermophilic alcohol dehydrogenase effectively catalyzed the reduction of α-chloroacetophenone to (*R*)-2-chloro-1-phenylethanol in essentially optically pure form. This alcohol dehydrogenase also showed high tolerance of organic solvents such as dimethyl sulfoxide, *iso*-propanol, methyl *tert*-butyl ether and hexane, a particularly important and useful feature for the reduction of ketones with low solubility in aqueous buffers [24]. Since α-chloro aromatic ketones are quite hydrophobic, the enzymatic reductions of a series of α-chloroacetophenone derivatives were investigated first in aqueous buffer with this alcohol dehydrogenase, and then in organic/buffer systems. Interestingly, it has been found that PFADH also catalyzed the reduction in a substrate-coupled mode using *iso*-propanol as hydrogen donor, which was applied in the synthesis of some optically active α-chlorohydrins of pharmaceutical importance to assess its applicability.

## 2. Experimental

### 2.1. General methods

The chiral HPLC analysis was performed on an Agilent 1100 series high-performance liquid chromatography system with (S,S)-

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**Table 1**
Reduction of the substituted α-chloroacetophenones catalyzed by PFADH in aqueous buffer[a]

| X | Yield (%) | ee (%) |
|---|---|---|
| 4′-H (**1**) | 55 | 99[b,c] |
| 4′-F (**2**) | 95 | 96[b,d] |
| 4′-Cl (**3**) | 17 | 98[c] |
| 4′-$NO_2$ (**4**) | 95 | 98[c] |
| 4′-$CH_3CONH$ (**5**) | 97 | 99[e] |
| 4′-$CH_3SO_2NH$ (**6**) | 20 | 98[c] |
| 2′,4′-$(F)_2$ (**7**) | 100 | 98[b,d] |
| 3′-Cl (**8**) | 100 | 98[c] |
| 2′,4′-$(Cl)_2$ (**9**) | 100 | 98[d] |
| 3′,4′-$(Cl)_2$ (**10**) | 45 | 98[c] |
| 3′-$NO_2$-4′-$CH_3CONH$ (**11**) | 37 | 98[d] |

[a] The reactions were carried out in potassium phosphate buffer and NADH was regenerated with D-glucose dehydrogenase and D-glucose system. The yields and ee values were measured by chiral HPLC analysis, except indicated otherwise.
[b] Chiral GC analysis.
[c] The absolute configuration was assigned by comparing with the standard samples [23].
[d] The absolute configuration was assigned by the sign of specific rotations.
[e] The absolute configuration was assigned by analogy with other chlorohydrins.

Whelk-O 1 column (25 cm × 4.6 mm, Regis Technologies Inc.). The chiral GC analysis was performed on a Hewlett Packard 5890 series II plus gas chromatograph equipped with autosampler, EPC, split/splitless injector, FID detector and CP-Chirasil-Dex CB chiral capillary column (25 m × 0.25 mm). All the ketone substrates were obtained from Aldrich and used as received. The racemic alcohol standard samples were prepared via the reduction with sodium borohydride at 0 °C. The alcohol dehydrogenase from hyperthermophilic archaeon *P. furiosus* (GenBank accession number: AE010289.1, PFADH) and glucose dehydrogenase from *Bacillus subtilis* (GenBank accession number: M12276.1) were prepared as previously reported [24].

### 2.2. Enzymatic reduction of α-chloroketones with D-glucose dehydrogenase/D-glucose as the NADH recycling system

The general procedure was as follows: D-glucose (4.0 mg), D-glucose dehydrogenase (0.5 mg), NADH (0.5 mg), alcohol dehydrogenase (1.0 mg) and ketone solution in DMSO (50 μl, 0.25 M) were mixed in a potassium phosphate buffer (1.0 ml, 100 mM, pH 7.0; Table 1) or a potassium phosphate buffer (100 mM, pH 7.0) containing *iso*-propanol (85/15 v/v, total 1.0 ml; Table 2). The mixture was shaken overnight at 37 °C. The mixture was extracted with methyl *tert*-butyl ether (1.0 ml). The organic extract was dried over anhydrous sodium sulfate and was subjected to chiral HPLC or GC analysis to determine the yield and enantiomeric excess. The absolute configurations of product alcohols were identified as described in the footnotes of Table 1.

**Table 2**
Reduction of the substituted α-chloroacetophenones catalyzed by PFADH in the buffer with *iso*-propanol[a]

| X | Yield (%) | ee (%) |
|---|---|---|
| 4′-H (**1**) | 99 | 99[b] |
| 4′-F (**2**) | 97 | 99[b] |
| 4′-Cl (**3**) | 75 | 99 |
| 4′-$NO_2$ (**4**) | 96 | 98 |
| 4′-$CH_3CONH$ (**5**) | 97 | 99 |
| 4′-$CH_3SO_2NH$ (**6**) | 41 | 98 |
| 2′,4′-$(F)_2$ (**7**) | 100 | 99[b] |
| 3′-Cl (**8**) | 75 | 99 |
| 2′,4′-$(Cl)_2$ (**9**) | 90 | 99 |
| 3′,4′-$(Cl)_2$ (**10**) | 100 | 99 |
| 3′-$NO_2$-4′-$CH_3CONH$ (**11**) | 87 | 98 |

[a] The reactions were carried out in potassium phosphate buffer containing 15% (v/v) *iso*-propanol and NADH regeneration system of D-glucose dehydrogenase and D-glucose. The yields and ee values were measured by chiral HPLC analysis, except indicated otherwise.
[b] Chiral GC analysis.

### 2.3. Enzymatic reduction of α-chloroketones in the coupled substrate mode with iso-propanol as hydrogen donor

The general procedure was as follows: NADH (0.5 mg), alcohol dehydrogenase (1.0 mg) and ketone solution in DMSO (50 μl, 0.25 M) were mixed in a potassium phosphate buffer (0.85 ml, 100 mM, pH 7.0) and *iso*-propanol (0.15 ml) was added. The reaction mixture was shaken overnight at 37 °C. The mixture was extracted with methyl *tert*-butyl ether (1.0 ml). The organic extract was dried over anhydrous sodium sulfate and was subjected to chiral HPLC or GC analysis to determine the yield and enantiomeric excess. The absolute configurations of product alcohols were identified as described in the footnotes of Table 1.

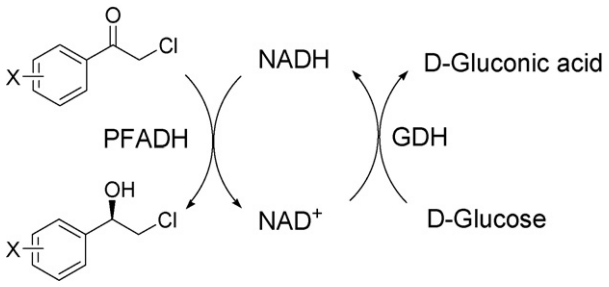
### 2.4. Preparative scale reduction of α-chloroketones in the coupled substrate mode with iso-propanol as hydrogen donor

The general procedure was as follows (using 2-chloro-4′-fluoroacetophenone as an example): NADH (5 mg), alcohol dehydrogenase (10 mg) and ketone (200 mg, 1.16 mmol) were mixed in a potassium phosphate buffer (85 ml, 100 mM, pH 7.0) and *iso*-propanol (15 ml) was added. The reaction mixture was shaken at 37 °C until conversion reached >95% (about 24–36 h). The mixture was extracted with methyl *tert*-butyl ether. The organic extract was dried over anhydrous sodium sulfate and removal of solvent gave the product chlorohydrins, which were purified by column chromatography on silica gel with ethyl acetate/hexane as eluent. The yields, ee values and specific rotations are summarized in Table 4. (*R*)-2-Chloro-1-(4′-fluorophenyl) ethanol (**2a**) [26], $^1$H NMR $\delta$ (ppm, $CDCl_3$) 2.85 (s, 1H), 3.60 (t, $J$ = 8.1 Hz, 1H), 3.75 (d, $J$ = 8.1 Hz, 1H), 4.95 (d, $J$ = 8.1 Hz, 1H), 7.10 (m, 2H), 7.40 (m, 2H); $^{13}$C NMR $\delta$ (ppm, $CDCl_3$) 51.1, 73.8, 116.0 (d, $^2J_{C-F}$ = 21 Hz), 128.2(d, $^3J_{C-F}$ = 8 Hz), 129.2, 136.1(d, $^4J_{C-F}$ = 3 Hz), 163.1 (d, $^1J_{C-F}$ = 245 Hz). (*R*)-2-Chloro-1-(2′,4′-difluorophenyl) ethanol (**7a**) [4], $^1$H NMR $\delta$ (ppm, $CDCl_3$) 2.87 (s, 1H), 3.65 (m, 1H), 3.81 (m, 1H), 5.18 (m, 1H), 6.82 (m, 1H), 6.92 (m, 1H), 7.53 (m, 1H); $^{13}$C NMR $\delta$ (ppm, $CDCl_3$) 49.5, 67.8, 103.8 (t, $^2J_{C-F}$ = 25 Hz), 111.6 (dd, $^2J_{C-F}$ = 21 Hz, $^3J_{C-F}$ = 4 Hz), 122.9 (t, $^3J_{C-F}$ = 4 Hz), 128.6 (dd, $^2J_{C-F}$ = 10 Hz, $^4J_{C-F}$ = 5 Hz), 159.7 (dd, $^1J_{C-F}$ = 247 Hz, $^3J_{C-F}$ = 12 Hz), 162.8 (dd, $^1J_{C-F}$ = 248 Hz, $^3J_{C-F}$ = 12 Hz). (*R*)-2-Chloro-1-(2′,4′-dichlorophenyl) ethanol (**9a**) [27], $^1$H NMR $\delta$ (ppm, $CDCl_3$) 3.01 (s, 1H), 3.51 (dd, $J$ = 15.3, 8.4 Hz, 1H), 3.85 (dd, $J$ = 11.3, 2.8 Hz, 1H), 5.25 (d, $J$ = 8.1 Hz, 1H), 7.29 (m, 2H), 7.56 (d, $J$ = 8.1 Hz, 1H); $^{13}$C NMR $\delta$ (ppm, $CDCl_3$) 49.0, 70.2, 127.5, 128.5, 129.2, 132.4, 134.5, 135.9. (*R*)-2-Chloro-1-(3′-nitro-4′-acetamidophenyl) ethanol (**11a**) [28], $^1$H NMR $\delta$ (ppm, acetone-$d_6$) 2.20 (s, 3H), 3.72 (m, 1H), 3.80 (m, 1H), 5.02 (s, 1H), 5.15 (s, 1H), 7.75 (m, 1H), 8.18 (s, 1H), 8.38 (m, 1H), 9.95 (s, 1H); $^{13}$C NMR $\delta$ (ppm, $CDCl_3$) 24.1, 49.8, 71.9, 123.0, 123.2, 133.0, 133.3, 138.1, 138.5, 168.8.

## 3. Results and discussion

The alcohol dehydrogenase from the hyperthermophilic archaeon *P. furiosus* was produced as previously described [24]. The reductions of various substituted α-chloroacetophenones were first examined in aqueous phosphate buffer using glucose dehydrogenase/D-glucose as NADH regeneration system (Scheme 1). The results are summarized in Table 1.

It can be seen from Table 1 that PFADH catalyzed the enantioselective reduction of various substituted α-chloroacetophenones to




**Scheme 1.** Reduction of ketones with NADH regeneration system of D-glucose dehydrogenase and D-glucose.

give the corresponding (*R*)-configurated chlorohydrins with excellent enantiomeric excess values (>96%). Similar to our previous observation for the reduction of substituted acetophenones with PFADH as a biocatalyst [24], the substituents did not exert significant effects on the enantioselectivity. However, the substituents at the phenyl ring greatly affected the yield of the product, although no clear trend was observed. The reason might be that the enzyme activity was affected by both electronic and steric factors as well as the solubility of the substrates in the aqueous buffer.

It has been found that PFADH showed high tolerance of organic solvents such as dimethyl sulfoxide, *iso*-propanol, methyl *tert*-butyl ether and hexane [24]. Therefore, the reduction of the substrate (**11**) was evaluated in the mixtures of potassium phosphate buffer with these organic solvents in an effort to increase the product yield. The results are presented in Fig. 1. The data showed that addition of dimethyl sulfoxide, methyl *tert*-butyl ether or hexane into the reaction buffer did not significantly change the yield. Fortunately, the alcohol yield was greatly improved with the addition of *iso*-propanol. As shown in Fig. 1, the yield reached 87% and 86% in 15% and 30% of *iso*-propanol, respectively.

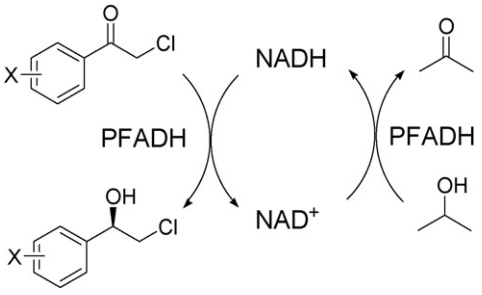
Since doubling the percentage of *iso*-propanol did not change the yield and enantioselectivity, the reductions of other substituted α-chloroacetophenones were carried out in the mixture of potassium phosphate buffer with 15% (v/v) *iso*-propanol. The yield and enantiomeric excess are presented in Table 2. It can be seen from the results that the yield was improved in the cases where X is 4′-H (**1**), 4′-Cl (**3**), 4′-$CH_3SO_2NH$ (**6**), 3′,4′-$(Cl)_2$ (**10**) and 3′-$NO_2$-4′-$CH_3CONH$ (**11**), but when X is 3′-Cl (**8**) and 2′,4′-$(Cl)_2$ (**9**), the yield decreased. For the other substrates, addition of *iso*-propanol into the reaction buffer did not affect the yield. It is interesting to note that the enantioselectivity increased for the reduction of 2-chloro-4′-fluoroacetophenone when it was carried out in the mixture of potassium phosphate buffer and *iso*-propanol.

**Table 3**
Hydrogen transfer reduction of the substituted α-chloroacetophenones catalyzed by PFADH[a]

| X | Yield (%) | ee (%) |
|---|---|---|
| 4′-H (**1**) | 96 | 97[b] |
| 4′-F (**2**) | 99 | 98[b] |
| 4′-F (**2**)[c] | 28 | 98[b] |
| 4′-F (**2**)[d] | 10 | 98[b] |
| 4′-Cl (**3**) | 55 | 98 |
| 4′-$NO_2$ (**4**) | 45 | 98 |
| 4′-$CH_3CONH$ (**5**) | 72 | 98 |
| 4′-$CH_3SO_2NH$ (**6**) | 10 | 98 |
| 2′,4′-$(F)_2$ (**7**) | 99 | 99[b] |
| 3′-Cl (**8**) | 94 | 99 |
| 2′,4′-$(Cl)_2$ (**9**) | 96 | 99 |
| 3′,4′-$(Cl)_2$ (**10**) | 42 | 99 |
| 3′-$NO_2$-4′-$CH_3CONH$ (**11**) | 98 | 98 |

[a] The reactions were carried out in potassium phosphate buffer containing 15% (v/v) *iso*-propanol and NADH was regenerated with *iso*-propanol as hydrogen donor, except stated otherwise. The yields and ee values were measured by chiral HPLC analysis, except indicated otherwise.
[b] Chiral GC analysis.
[c] Co-solvent *iso*-propanol was replaced with 4-methyl-2-pentanol.
[d] Co-solvent *iso*-propanol was replaced with 2-octanol.

It is known that the co-factor NADH or NADPH could be regenerated via simple hydrogen transfer mode using *iso*-propanol as the hydrogen donor [12,13,29–31]. We thus reasoned that the reduction of the substituted α-chloroacetophenones catalyzed by PFADH might also proceed via a simple hydrogen transfer mode, in which the co-factor NADH is regenerated with *iso*-propanol as the hydrogen donor. In order to test this hypothesis, the reductions were performed in the mixture of potassium phosphate buffer and *iso*-propanol (85/15, v/v) without D-glucose dehydrogenase and D-glucose. The yields and ee values are presented in Table 3.




**Scheme 2.** Hydrogen transfer reduction of ketones catalyzed by PFADH.

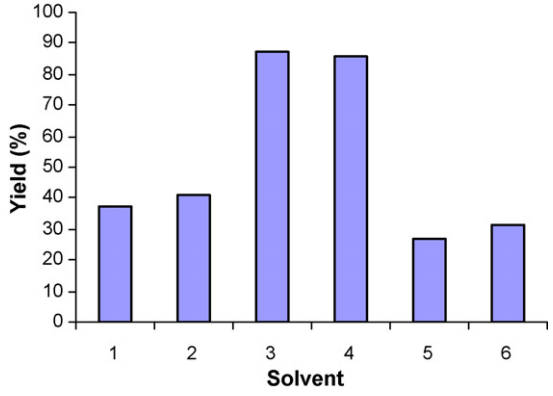



**Fig. 1.** Reduction of the substrate (**11**) in the mixtures of potassium phosphate buffer with different organic solvents. (1) Potassium phosphate buffer (no organic solvent); (2) 15% (v/v) dimethyl sulfoxide; (3) 15% (v/v) *iso*-propanol; (4) 30% (v/v) *iso*-propanol; (5) 30% (v/v) methyl *tert*-butyl ether; (6) 30% (v/v) hexane.

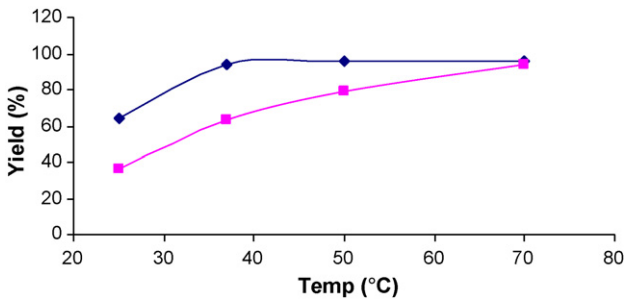
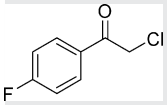
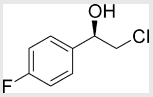
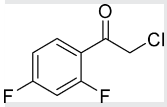
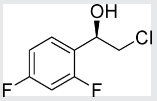
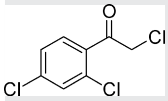
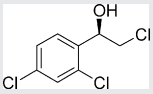
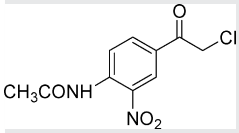
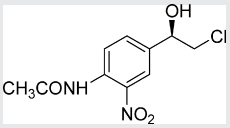



**Fig. 2.** Temperature impact on the enzyme activity. Reaction conditions: NADH (1.0 mg), alcohol dehydrogenase (1.0 mg) and α-chloroacetophenone (12.5 μmol) were mixed in a potassium phosphate buffer (0.85 ml, 100 mM, pH 7.0) and *iso*-propanol (0.15 ml). Reaction time: 2 h (square) and 4 h (diamond).

**Table 4**
Preparation of chiral chlorohydrins via PFADH-catalyzed hydrogen transfer reduction[a]

| Ketones | Product | Yield (%) | ee (%) | $[\alpha]_D^{22}$[d] |
|---|---|---|---|---|
|  |  | 87 | 98[b] | −32.4 |
| **2** | **2a** | 89[e] | 98[b,e] | |
|  |  | 83 | 99[b] | −37.2 |
| **7** | **7a** | 92[e] | 99[b,e] | |
|  |  | 92 | 99[c] | −52.8 |
| **9** | **9a** | | | |
|  |  | 85 | 98[c] | −9.4 |
| **11** | **11a** | | | |

[a] Reaction as described in Section 2.4, except when stated otherwise.
[b] The ee value was measured by chiral GC analysis.
[c] The ee value was measured by chiral HPLC analysis.
[d] The specific rotation was measured in methanol ($c$ = 1.0).
[e] The reaction was carried at gram scale: NADH (25 mg), alcohol dehydrogenase (50 mg) and ketone (2.0 g) were mixed in a potassium phosphate buffer (85 ml, 100 mM, pH 7.0) and *iso*-propanol (15 ml), and shaken at 37 °C for 24 h.

Fortunately, PFADH catalyzed the hydrogen transfer reduction of the substituted α-chloroacetophenones (Scheme 2), although the yield decreased in some cases. When compared with the data in Table 2, no significant change was observed for the enantioselectivity. Therefore, under the reaction conditions of Table 2, these two NADH regeneration modes should be concomitant. In the reduction of 2-chloro-4′-difluoroacetophenone, when the co-solvent *iso*-propanol was replaced with 4-methyl-2-pentanol or 2-octanol, the yields were much lower (Table 3).

Since PFADH showed high resistance to thermal inactivation [24,25], the temperature impacts on the enzyme activity and enantioselectivity in the hydrogen transfer mode were investigated using α-chloroacetophenone as the substrate and the results were presented in Fig. 2. It can be seen that the enzyme activity enhanced as the temperature increased. The enantioselectivity slightly decreased at higher reaction temperatures; the ee values of 2-chloro-1-phenylethanol at 4 h were >99, 99, 98 and 96 at 25, 37, 50 and 70 °C, respectively. It was also observed that the product ee value slightly decreased as the reaction time increased. Therefore, considering the activity and enantioselectivity, the optimal reaction temperature should be around 37 °C.

In the hydrogen transfer mode, *iso*-propanol might act as the hydrogen donor and also increase the solubility of hydrophobic ketone substrates. From the practical point of view, the hydrogen transfer reduction with *iso*-propanol as hydrogen donor is more cost-effective, because the requirement of D-glucose dehydrogenase and D-glucose is eliminated. To demonstrate the applicability of PFADH-catalyzed hydrogen transfer reduction in the synthesis of optically active chlorohydrins, the reductions of 2-chloro-4′-fluoroacetophenone (**2**), 2-chloro-2′,4′-difluoroacetophenone (**7**), 2,2′,4′-trichloroacetophenone (**9**), (4′-chloroacetyl-2′-nitrophenyl) acetilidine (**11**) were carried on the preparative scale. The corresponding chiral chlorohydrins (**2a**, **7a**, **9a** and **11a**) were isolated and characterized by $^1$H and $^{13}$C NMR analysis. The isolated yields, ee values and specific rotations were listed in Table 4. These pharmaceutically important chlorohydrins were obtained via PFADH-catalyzed hydrogen transfer reduction in high yield and excellent enantiomeric purity. Ketones 2 and 7 were also tested at substrate concentration of about 100 mM and the reduction proceeded smoothly to afford the corresponding products in excellent yields.

## 4. Conclusion

The alcohol dehydrogenase from the hyperthermophilic archaeon *P. furiosus* effectively catalyzes the reductions of various substituted α-chloroacetophenones to form the (*R*)-enantiomer of the corresponding chlorohydrins with excellent ennatiomeric purity. The co-factor NADH can be recycled by the D-glucose dehydrogenase and D-glucose regeneration system or via the simple hydrogen transfer mode using *iso*-propanol as the hydrogen donor. The applicability of PFADH-catalyzed hydrogen transfer reduction in the synthesis of optically active chlorohydrins has been demonstrated by carrying out several reductions on the preparative scale. Thus PFADH is a valuable biocatalyst for the preparation of chiral chlorohydrins of pharmaceutical interest.

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## References

[1] C.P. Kordik, A.B. Reitz, J. Med. Chem. 42 (1999) 181–201.
[2] E.J. Corey, C.J. Helal, Angew. Chem. Int. Ed. 37 (1998) 1986–2012.
[3] M. Okuyama, F. Uehara, H. Iwamura, K. Watanabe, WO 2007011065 to Mitsubishi Pharma Corporation, Japan; Sanofi-Aventis, 2007.
[4] J.Y.L. Chung, R. Cvetovich, J. Amato, J.C. McWilliams, R. Reamer, L. DiMichele, J. Org. Chem. 70 (2005) 3592–3601.

[5] R.A. Nelson, N.W. Thomas, G.W. Matcham, S.L. Lin, M. Zhang, C. Lewis, WO 2000038521 to Celgene Corporation, USA, 2000.
[6] T. Ohkuma, K. Tsutsumi, N. Utsumi, N. Arai, R. Noyori, K. Murata, Org. Lett. 9 (2007) 255–257.
[7] D.S. Matharu, D.J. Morris, A.M. Kawamoto, G.J. Clarkson, M. Wills, Org. Lett. 7 (2005) 5489–5491.
[8] D.J. Morris, M. Wills, Pharma. Chem. 5 (2006) 8–11.
[9] D.J. Morris, A.M. Hayes, M. Wills, J. Org. Chem. 71 (2006) 7035–7044.
[10] W. Yang, J.-H. Xu, Y. Xie, Y. Xu, G. Zhao, G.-Q. Lin, Tetrahedron: Asymmetry 17 (2006) 1769–1774.
[11] T. Ema, H. Yagasaki, N. Okita, M. Takeda, T. Sakai, Tetrahedron 62 (2006) 6143–6149.
[12] T.M. Poessl, B. Kosjek, U. Ellmer, C.C. Gruber, K. Edegger, K. Faber, P. Hildebrandt, U.T. Bornscheuer, W. Kroutil, Adv. Synth. Catal. 347 (2005) 1827–1834.
[13] K. Inoue, Y. Makino, N. Itoh, Tetrahedron: Asymmetry 16 (2005) 2539–2549.
[14] T. Ema, H. Moriya, T. Kofukuda, T. Ishida, K. Maehara, M. Utaka, T. Sakai, J. Org. Chem. 66 (2001) 8682–8684.
[15] N. Itoh, N. Mizuguchi, M. Mabuchi, J. Mol. Catal. B: Enzymatic 6 (1999) 41–50.
[16] Z.-L. Wei, Z.-Y. Li, G.-Q. Lin, Tetrahedron 54 (1998) 13059–13072.
[17] *R*-Chloroacetophenone undergoes a Darzens-like condensation in a basic alcoholic solvent.
[18] H.H. Wasserman, N.E. Aubrey, H.E. Zimmerman, J. Am. Chem. Soc. 75 (1953) 96–98.
[19] C.L. Stevens, R.J. Church, V.J. Traynelis, J. Org. Chem. 19 (1954) 522–532.
[20] H. Hamada, T. Miura, H. Kumobayashi, T. Matsuda, T. Harada, K. Nakamura, Biotechnol. Lett. 23 (2001) 1603–1606.
[21] C. Barbieri, E. Caruso, P. D'Arrigo, G.P. Fantoni, S. Servi, Tetrahedron: Asymmetry 10 (1999) 3931–3937.
[22] N. Itoh, M. Matsuda, M. Mabuchi, T. Dairi, J. Wang, Eur. J. Biochem. 269 (2002) 2394–2402.
[23] D. Zhu, C. Mukherjee, L. Hua, Tetrahedron: Asymmetry 16 (2005) 3275–3278.
[24] D. Zhu, H.T. Malik, L. Hua, Tetrahedron: Asymmetry 17 (2006) 3010–3014.
[25] R. Machielsen, A.R. Uria, S.W.M. Kengen, J. van der Oost, Appl. Environ. Microbiol. 72 (2006) 233–238.
[26] T.R. Nieduzak, A.L. Margolin, Tetrahedron: Asymmetry 2 (1991) 113–122.
[27] M. Srebnik, P.V. Ramachandran, H.C. Brown, J. Org. Chem. 53 (1988) 2916–2920.
[28] A. Mukherjee, S. Kumar, M. Seth, A.P. Bhaduri, Indian J. Chem., Sect. B 28B (1989) 391–396.
[29] K. Edegger, W. Stampfer, B. Seisser, K. Faber, S.F. Mayer, R. Oehrlein, A. Hafner, W. Kroutil, Eur. J. Org. Chem. (2006) 1904–1909.
[30] W. Stampfer, B. Kosjek, K. Faber, W. Kroutil, J. Org. Chem. 68 (2003) 402–406.
[31] W. Stampfer, B. Kosjek, C. Moitzi, W. Kroutil, K. Faber, Angew. Chem. Int. Ed. 41 (2002) 1014–1017.