

## Supporting Information

Identification of key residues in *Debaryomyces hansenii* carbonyl reductase for highly productive preparation of (*S*)-aryl halohydrins

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## 1. General remarks

Prochiral ketones were purchased from commercial source. Molecular cloning and expression of genes were carried out according to molecular cloning manual. Recombinant proteins were purified as our previously described.<sup>1</sup>

## 2. Molecular modeling

Protein modeling was implemented with EasyModeller 4.0 using protein 1VL8, 3CTM, 3GDF as templates and verified with SAVES (The Structure Analysis and Verification Server version 4).<sup>2</sup> Tunnels in *DhCR* were identified using CAVER 3.0, with coordinates of the catalytic tyrosine as starting points.<sup>3</sup> All docking calculations were accomplished with AutoDock Vina 1.1, with the docking algorithm that took account of ligand flexibility but kept the protein rigid.<sup>4</sup> Docking runs were carried out using the standard parameters of the program for interactive growing and subsequent scoring. Stereo views were constructed using Pymol.<sup>5</sup> Consensus analysis was performed with WebLogo 3.4, using the following 20 carbonyl reductases.<sup>6</sup> Uniprot accession no.: Q6BQ25 (This work, *Debaryomyces hansenii*), O42703 ((*S*)-specific carbonyl reductase, *Candida parasilosis*), Q9C4B3 (Carbonyl reductase S1, *Candida magnoliae*), P87219 (Sorbitol reductase SOU1, *Candida albicans*), P87218 (Sorbitol reductase homolog SOU2, *Candida albicans*), C5DIK0 (*KtCR*, *Kluyveromyces thermotolerans*), A3GF07 (PsCRI, *Pichia stipitis*), A3GF05 (PsCRII, *Pichia stipitis*), Q75WS0 (KaCR1, *Kluyveromyces aestuarii*), A5DQB5 (PgCR, *Pichia guilliermondii*), D5G306 (SCRI, *C. parasilosis*), D5G304 (SCRII, *C. parapsilosis*), D5G305 (SCRIII, *C. parapsilosis*), Q6CEE9 (Mannitol dehydrogenase, *Candida lipolytica*), C5DNV9 (Putative carbonyl reductase, *Zygosaccharomyces rouxii*), Q5AUB7 (Putative SDR, *Aspergillus nidulans*), B8NF06 (Short-chain dehydrogenase, *Aspergillus flavus*), Q0CIH3 (L-xylulose reductase, *Aspergillus terreus*), A5AB79 (Short-chain dehydrogenase, *Aspergillus niger*), F9FI58 (SDR member, *Fusarium oxysporum*).

## 3. Construction of CASTing libraries

Through overlap PCR, the gene products containing mutation sites were obtained using primers as listed in **Table S1** with NDT degeneracy.<sup>7</sup> After digested with *EcoRI*

and *XhoI*, the resultant products were ligated with pET28a (linearized with *EcoRI* and *XhoI*) and transformed into *E. coli* BL21. Then the mono-colonies were picked and inoculated into 96 deep well plates containing 300  $\mu\text{L}$  LB medium per well, and cultivated for 12 h at 37 °C and 200 rpm. Fifty microliter cultures were transferred into a new 96 deep well plates containing 550 mL LB medium and incubated for 2 h at 37 °C and 200 rpm. About 0.5 mM IPTG (final concentration) was added and induced at 25 °C and 200 rpm further for 12 h. The cultures were centrifuged at 3,200 rpm for 10 min to get the variants library in the bottom of plates. The plates were frozen at  $-80$  °C for at least 2 h. After melted at room temperature, 200  $\mu\text{L}$  disruption buffer (pH 7.5, 10 mM Tris-HCl; 750  $\text{mg}\cdot\text{L}^{-1}$  lysozyme; and 10  $\text{mg}\cdot\text{L}^{-1}$  DNA nuclease) was added into each well and mixed for 5 min, further incubated at 37 °C for 1 h. After centrifuged at 3,200 rpm for 20 min, the resultant supernatants (crude extracts) were used for screening of variant's library.

Eight variants with improved  $\alpha$ -chloroacetophenone reduction activity were chosen and mixed as the templates for DNA shuffling. The DNA shuffling was performed as described by Stemmer and Zhang et al. Reassembly of the full-length was achieved using primers listed in [Table S1](#).<sup>8-9</sup> The resultant genes were ligated into pET28a between *EcoRI* and *XhoI* sites, followed by cultivation and induction as above to get the crude extracts of combinatorial library.

#### **4. Activity determination and high-throughput screening**

High-throughput activity screening was performed spectrophotometrically at 340 nm by determining the decrease of NADPH. The reaction mixture consisted of 170  $\mu\text{L}$  PBS (pH 6.5, 100 mM), 10  $\mu\text{L}$   $\alpha$ -chloroacetophenone (0.4 M, dissolved in ethanol), 10  $\mu\text{L}$  NADPH stock solution (10 mM, dissolved in ddH<sub>2</sub>O) and 10  $\mu\text{L}$  of the crude enzyme solution (diluted to an appropriate concentration). Control experiments were performed with 180  $\mu\text{L}$  PBS (pH 6.5, 100 mM), 10  $\mu\text{L}$   $\alpha$ -chloroacetophenone (0.4 M, dissolved in ethanol), 10  $\mu\text{L}$  NADPH stock solution (10 mM, dissolved in ddH<sub>2</sub>O). After incubation at 30 °C for 1 min, the changes of absorbance at 340 nm ( $A_{340}$ ) during 5 min were monitored. One unit of the reductase

was defined as the amount of enzyme required for the oxidation of 1  $\mu\text{mol}$  NADPH per minute under above mentioned assay condition.

## 5. Enzyme characterization

The pH and temperature profiles of *DhCR* variants were determined as described previously.<sup>1</sup> Kinetic constants were assayed and calculated according to the Lineweaver Burk plot of reciprocal curves. Substrate specificities were tested with 2 mM different prochiral ketones.

## 6. Asymmetric reduction of $\alpha$ -chloroacetophenone

Reactions were carried out with 100  $\text{g}\cdot\text{L}^{-1}$   $\alpha$ -chloroacetophenone in a system with 50 mL toluene, an appropriate amount of whole cells (30  $\text{g}\cdot\text{L}^{-1}$  *E. coli* BL21(DE3)/pET28-*bmgdh-dhcr* or 7.0  $\text{g}\cdot\text{L}^{-1}$  *E. coli* BL21(DE3)/pET28-*bmgdh-dhcr*<sub>v9</sub>) and 200  $\text{g}\cdot\text{L}^{-1}$  glucose in 50 mL KPB buffer (pH 6.5, 100 mM). The reaction was maintained at pH 6.5 by titration of 1.0 M  $\text{Na}_2\text{CO}_3$ . Twenty microliters of reaction mixture were removed periodically and mixed with 480  $\mu\text{L}$  of KPB buffer and 500  $\mu\text{L}$  of ethyl acetate containing dodecane as an internal standard. After centrifuge at  $8,000 \times g$  for 5 min, the organic phase was transferred and dried over anhydrous  $\text{Na}_2\text{SO}_4$ , then analyzed with GC equipped with CP7502 column. The temperatures of injector and detector were set at 280  $^\circ\text{C}$ , while the column temperature was 140  $^\circ\text{C}$ . The retention times of  $\alpha$ -chloroacetophenone, (*S*)- $\alpha$ -chloroacetophenol and (*R*)- $\alpha$ -chloroacetophenol were 5.73, 10.71 and 10.86 min, respectively. The amount of biocatalyst in the whole-cell reactions was calculated by divided the total activity of whole cells with specific activity of purified enzymes. Space-time yield was defined as the production of (*S*)- $\alpha$ -chloroacetophenol per liter of reaction mixture per day. Total turnover number (TTN) per cofactor of reductase was the number of (*S*)- $\alpha$ -chloroacetophenol per  $\text{NADP}^+$  in the asymmetric reaction.

## 7. Preparation of cross-linked *E. coli* BL21/pET28-*bmgdh-dhcr*<sub>v9</sub> cells

Recombinant whole cells of *E. coli* BL21 harboring pET28-*bmgdh-dhcr*<sub>v9</sub> were cultivated and induced at 25  $^\circ\text{C}$  for 12 h. Then 1% polyethyleneimine (PEI) and 2% glutaraldehyde (GA) were added and the cell suspension was further shaken for 30

min. The cross-linked whole cells were harvested through centrifuge at  $4000 \times g$  for 10 min and washed twice with 0.85% physiological saline.

## **8. Evaluation of the cross-linked *E. coli* BL21/pET28-*bmgdh-dhcr*<sub>V9</sub>**

The stability of free and cross-linked *E. coli* BL21 whole cells harboring pET28-*bmgdh-dhcr*<sub>V9</sub> were investigated by incubating 20 mg·mL<sup>-1</sup> whole cells at 30 °C and 900 rpm. An appropriate amount of sample was removed periodically and the activity was assayed in 500 μL KPB (pH 6.5, 100 mM) at a constant concentration of 1.0 mg·mL<sup>-1</sup> cell, supplemented with 20 mM α-chloroacetophenone and 30 mM glucose. After reaction at 30 °C and 900 rpm for a certain period of time (<10% conversion), the product was extracted by an equal volume of ethyl acetate with dodecane as the internal standard. The organic phase was isolated and determined employing above mentioned method. Each reaction was carried out in triplicates.

The reusability of cross-linked *E. coli* BL21(DE3) cells harboring pET28-*bmgdh-dhcr*<sub>V9</sub> was performed in three-neck flasks (250-mL). Cross linked whole cells (10 g) were added with 1.5 equiv. glucose (*ca.* 9.74 g) in 50 mL KPB (pH 6.5, 100 mM). Then 5.0 g of α-chloroacetophenone dissolved in 50 mL toluene was added to start the reaction with mechanical agitation at 30 °C and 120 rpm, titrated with 0.5 M Na<sub>2</sub>CO<sub>3</sub> to maintain the pH around 6.5. After about 4 h, the reaction was completed, and terminated by separating the two phases. Another 1.0 equiv. glucose and 5.0 g α-chloroacetophenone in 50 mL toluene were added to start the reaction. Reactions were repeated until the conversion rate was lower than 80% within 4 h.

## **9. Settings of grid box dimensions and centers in flexible docking**

All docking calculations were accomplished with AutoDock Vina 1.1. A docking algorithm that took account of ligand flexibility but kept the protein rigid was employed. Docking runs were carried out using the standard parameters of the program for interactive growing and subsequent scoring, except for the parameters for setting grid box dimensions and center as follows.

Center\_x=52.811

Center\_y=13.448

Center\_z=-2.505

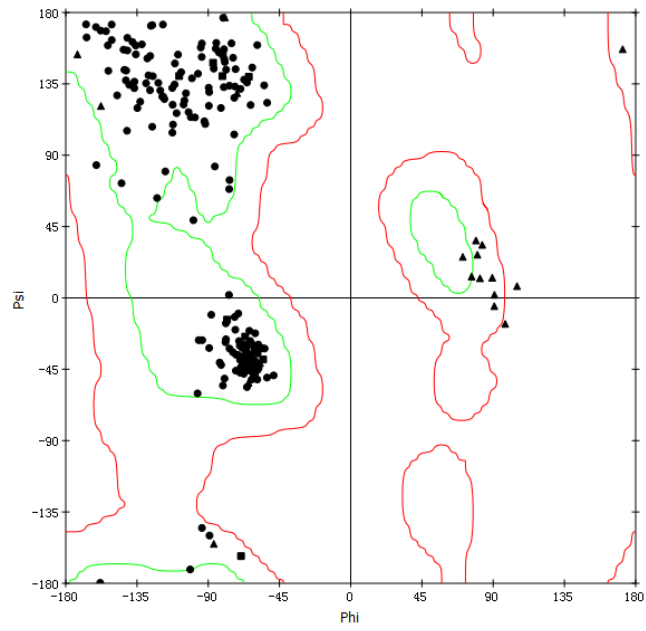
Size\_x=20

Size\_y=20

Size\_z=22

**Table S1** Primers used in the construction of mutants library

<b>Primer</b>	<b>Sequence (5'→3')</b>	<b>Length</b>
<i>Dh</i> CR-F	CCG <u>GAA</u> TTTCATGTCACACCCCAACATCAGTTATC	33
<i>Dh</i> CR-R	CCG <u>CTC</u> GAGCTATGGGGCGGAGTAACCACCATC	33
R1-F	GGTGTGNDTNDTACCGAGGGCAGAAGTATT	30
R1-R	GGTAHNAHNCACACCAGCATTAGCGACAAA	30
R2-F	ATGNDTGGCCACATTGTGNDTGTTCACAAAATGC	34
R2-R	AACAHNCACAATGTGGCCAHNCAATTGATGCGCAT	34
R3-F	AACACCNDTNDTCCAGGTTACATTGCTACA	30
R3-R	ACCTGGAHNAHNGGTGTAACTCTAGCGAA	30
R4-F	TCTCCANDTNDTATTGCTACAGAGATTTCC	30
R4-R	AGCAATAHNAHNTGGAGAAATGGTGTTAAC	30
R5-F	ACAGAGNDTNDTGATTTTGTTCCTGCCGAA	30
R5-R	AAAATCAHNAHNCTCTGTAGCAATGTAACC	30
<i>Kt</i> CR-N181S-F	GTGGTCTCGGTGCCGCAGCTG	21
<i>Kt</i> CR-N181S-R	CGGCACCGAGACCACGTGCC	21



**Figure S1** Ramachandran plot of *DhCR* homology structure. Green: Core zone, Red: Maximum allow zone, Black dots: residues.



PROVE  
DhCR-NADP.pdb  
Analysis of entire structure

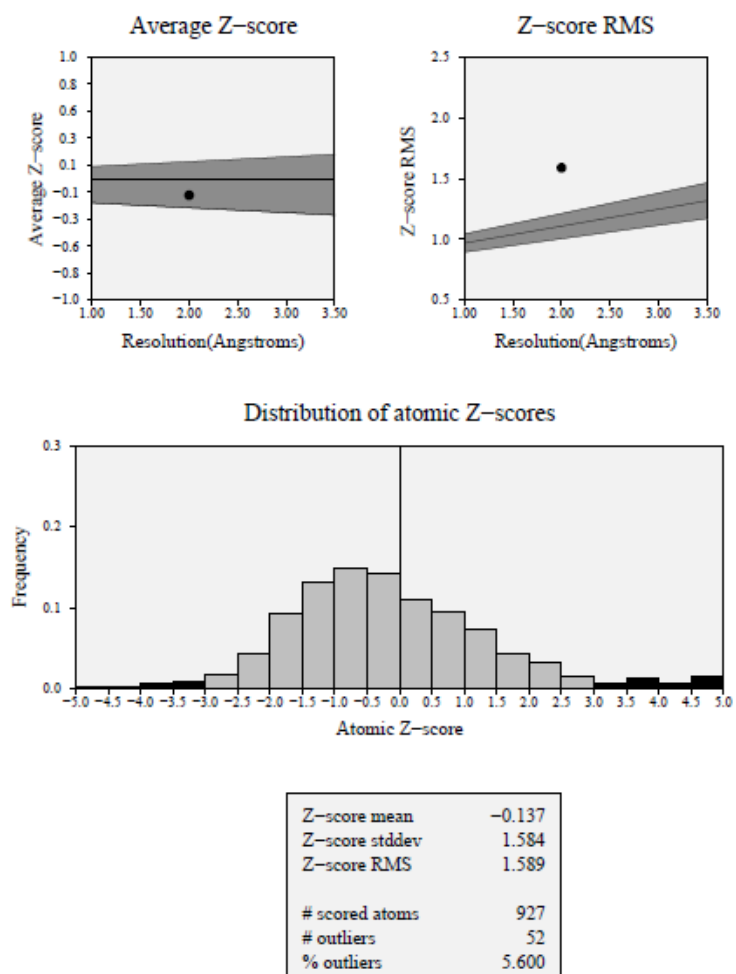
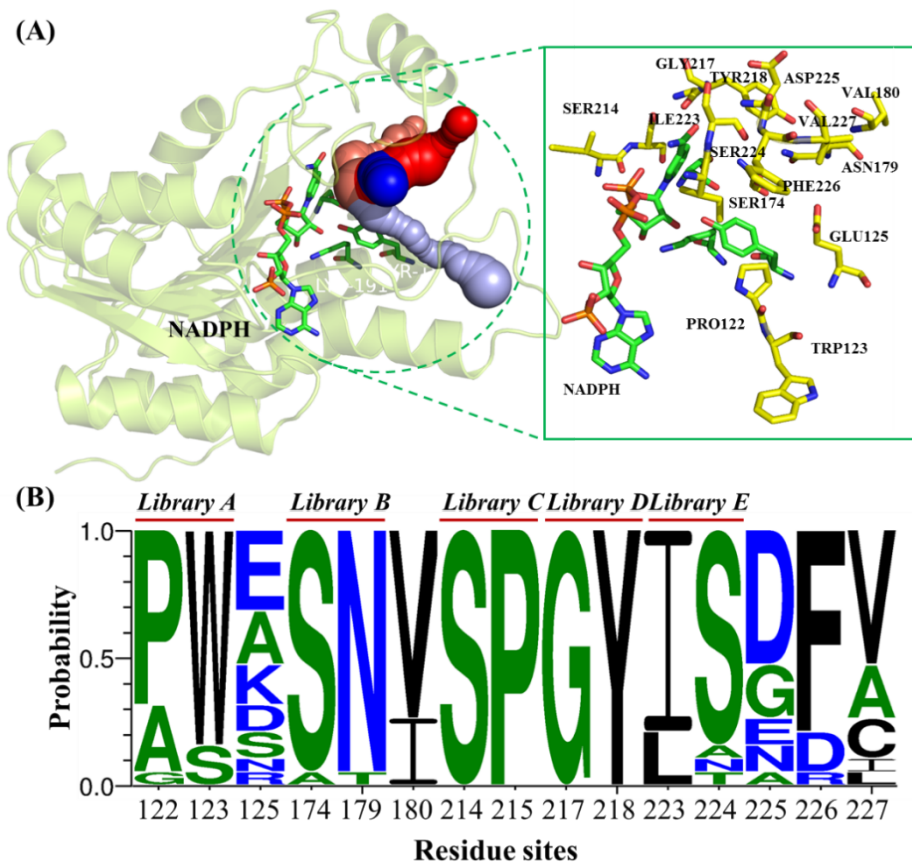
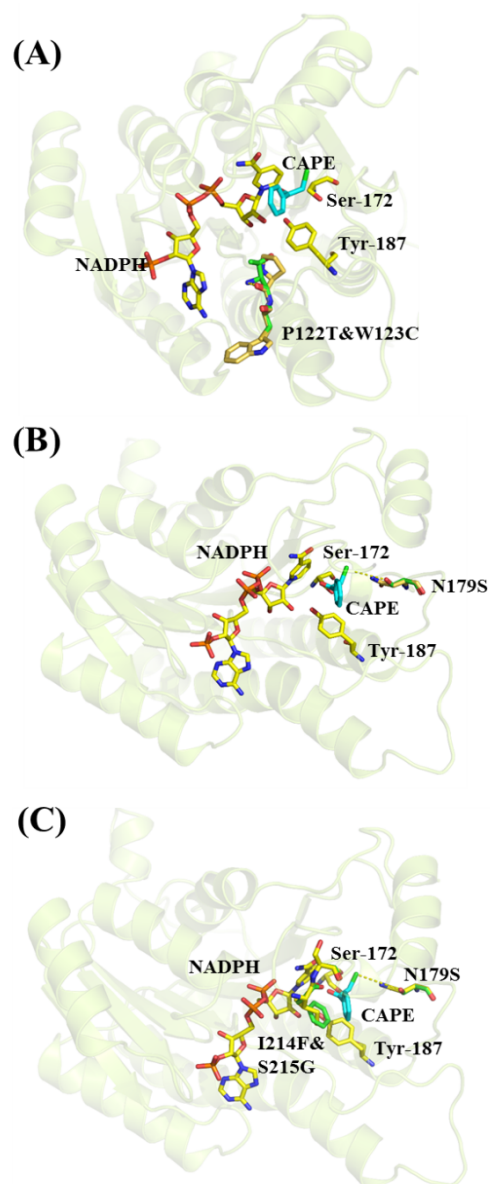


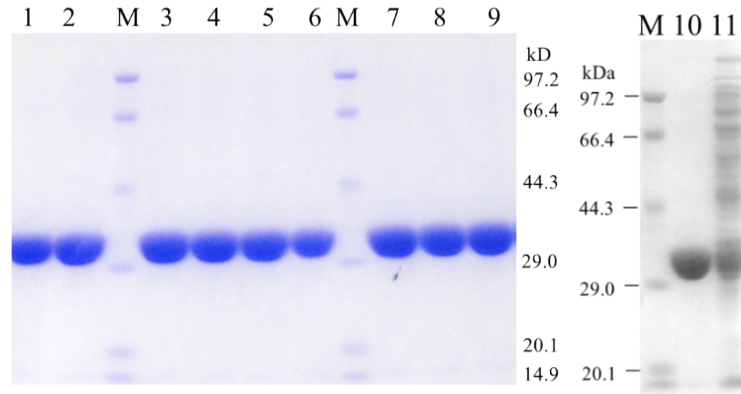
Figure S2 PROVE result of the *DhCR* homology model.



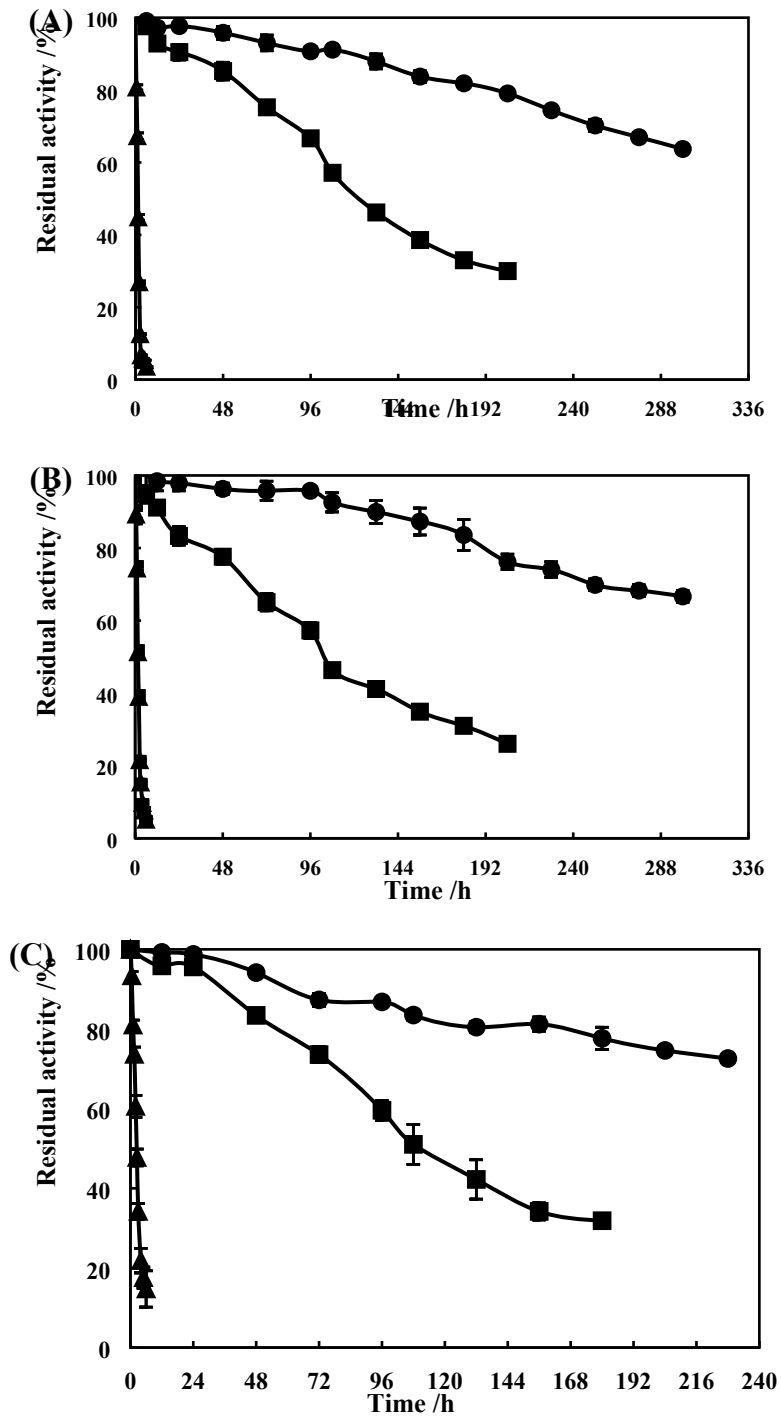
**Figure S3** Structural model of *DhCR* and consensus residues around the substrate tunnels. (A) Tunnels in the *DhCR* structure around the catalytic tyrosine (created by Pymol with CAVER 3.0 plugin). (B) Consensus analysis of the residues around the tunnels among twenty similar carbonyl reductases (created with WebLogo 3.4). NADPH and catalytic triads are shown in green, residues around the substrate tunnels are depicted in yellow.



**Figure S4** Close-ups of the active sites of the *DhCR* and variants. (A) *DhCR* and *DhCR*<sub>V1</sub>; (B) *DhCR* and *DhCR*<sub>V3</sub>; (C) *DhCR* and *DhCR*<sub>V9</sub>. Catalytic residues and NADPH are depicted in yellow.  $\alpha$ -Acetophenone molecules are in blue, while mutant residues are in green.

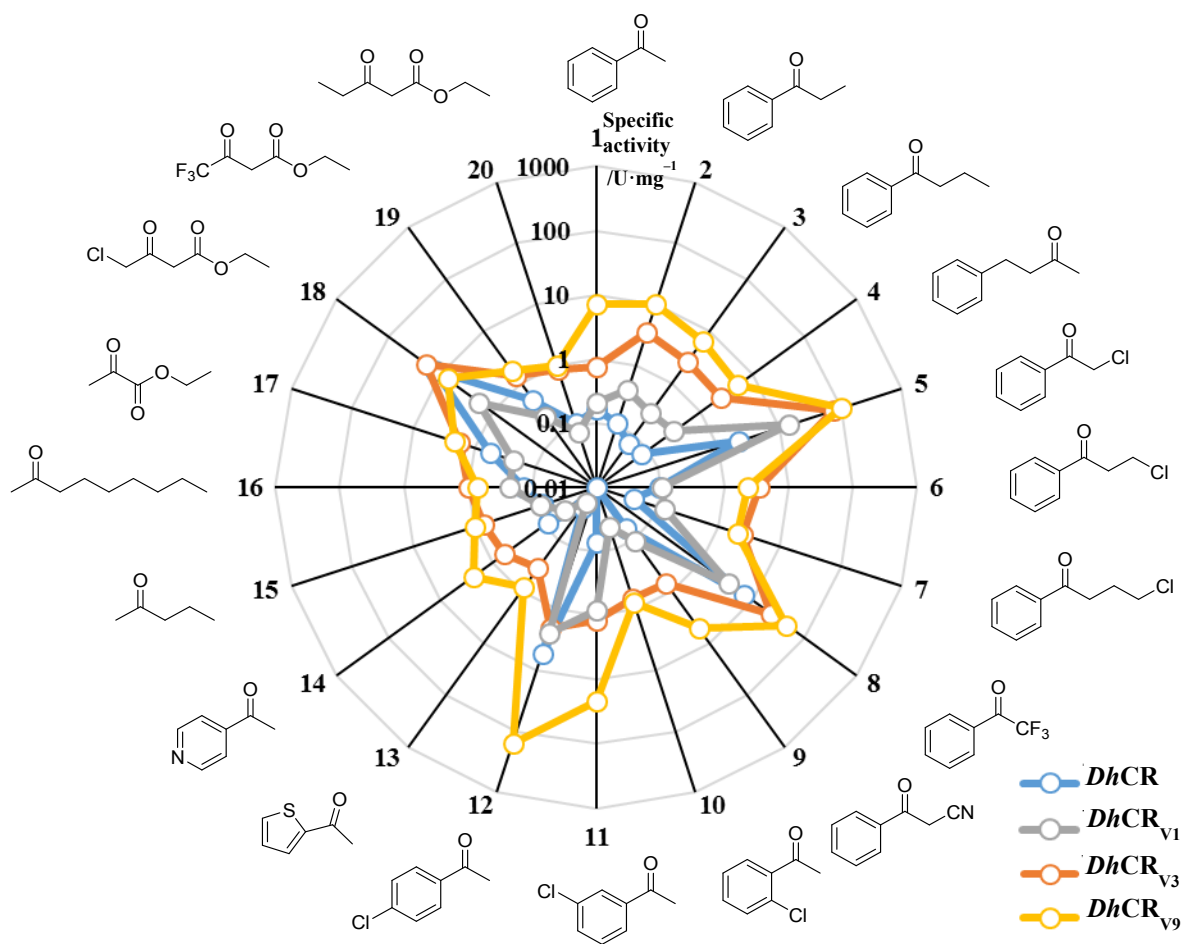


**Figure S5** SDS-PAGE analysis of the purified *DhCR* and variants. Lanes 1–9, *DhCR* variants from V1 to V9; Lane 10, purified *DhCR*; Lane 11: crude extract of *DhCR*; Lane M, low molecular protein markers.

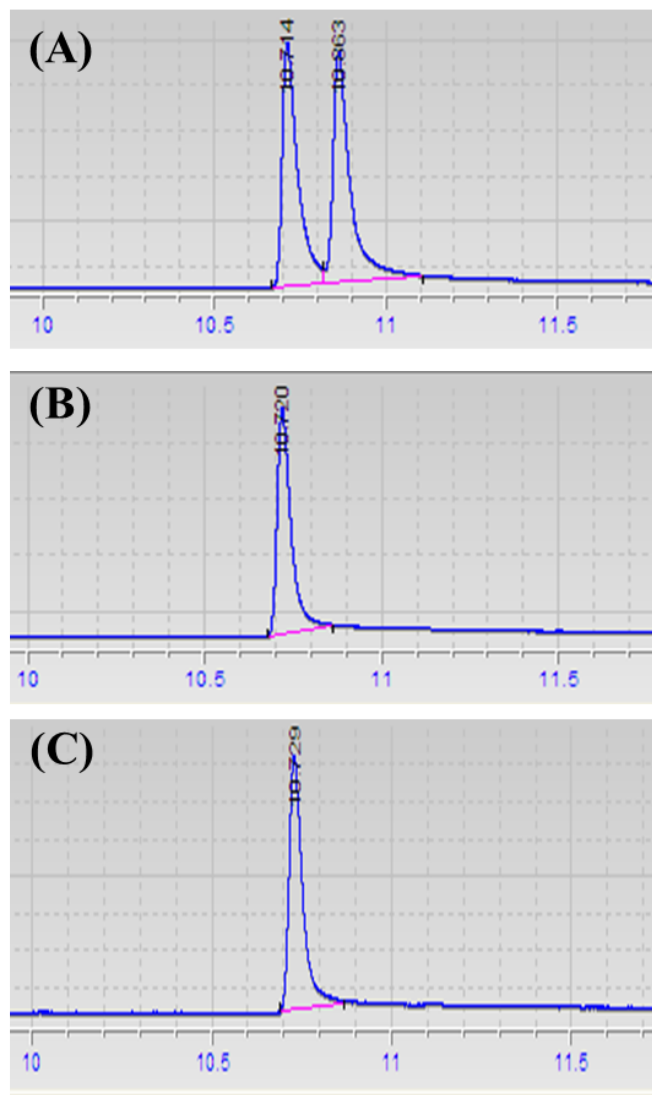


**Figure S6** Thermostability of *DhCR<sub>v3</sub>*, *DhCR<sub>v9</sub>* and *DhCR* at different temperatures.

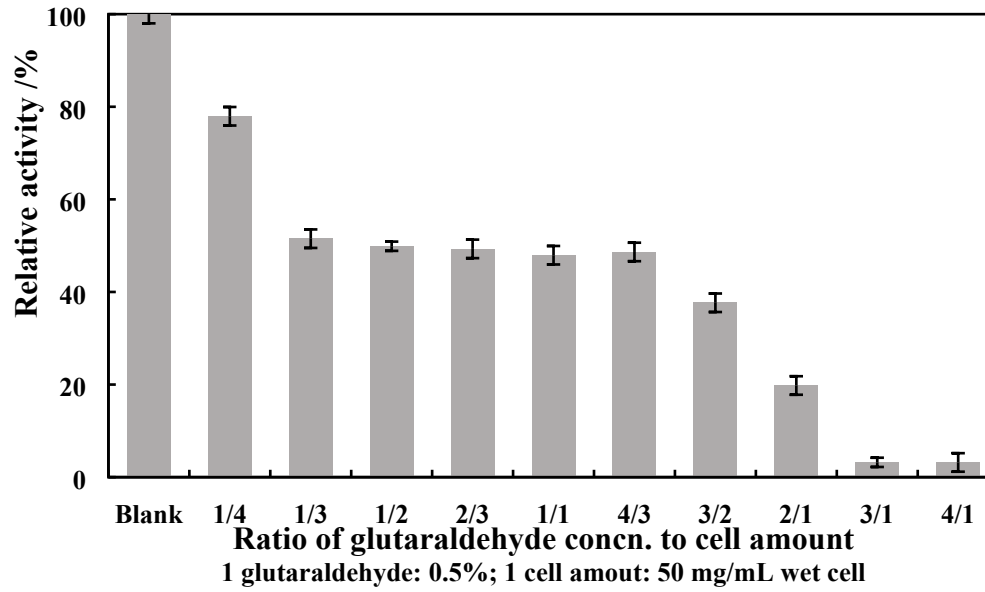
(A) *DhCR<sub>v3</sub>*; (B) *DhCR<sub>v9</sub>*; (C) *DhCR*. (●) 30°C; (■) 40°C; (▲) 50°C.



**Figure S7** Substrate profiles of *DhCR* variants. Specific activities are shown in logarithmic form in the radar graph.

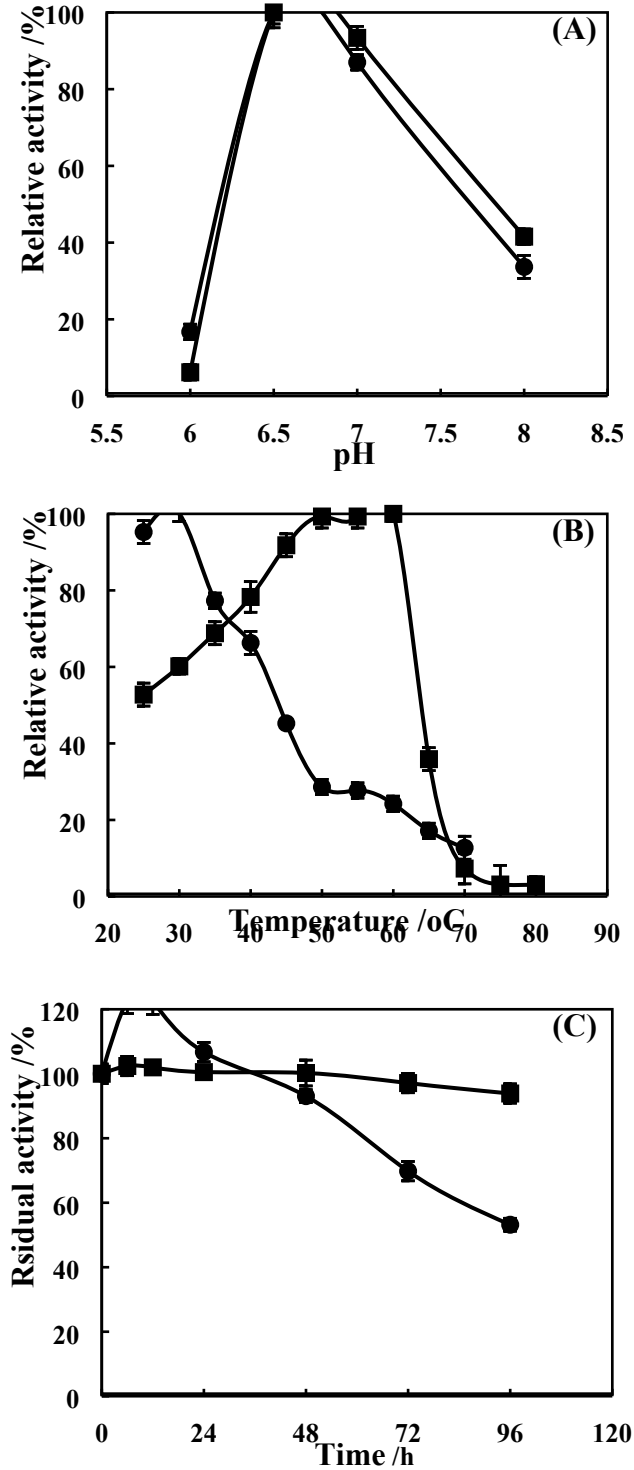


**Figure S8** Enantioselectivity of the DhCR variants. (A) *rac.*  $\alpha$ -chlorophenylethanol; (B) (*S*)- $\alpha$ -chlorophenylethanol produced by DhCR; (C) (*S*)- $\alpha$ -chlorophenylethanol produced by DhCR<sub>V9</sub>.



**Figure S9** Effect of the ratio of glutaraldehyde and cell amounts on the activity recovery of cross-linked recombinant *E. coli* BL21 cells harboring pET28-*bmgdh-dhcr*<sub>19</sub>. 1 of glutaraldehyde: 5 mM (0.5% in v/v). 1 of cell amount: 50 mg/mL.



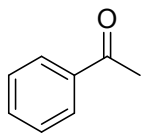


**Figure S10** Optimum pH (A), temperature (B) and thermostability (C) of free and cross-linked recombinant *E. coli* BL21 cells harboring pET28-*bmgdh-dhcr<sub>v9</sub>*. (●): *freeDhCR<sub>v9</sub>* cells; (■): *clDhCR<sub>v9</sub>*. The stability of free and cross-linked *E. coli* BL21 whole cells harboring pET28-*bmgdh-dhcr<sub>v9</sub>* were investigated by incubating 20 mg·mL<sup>-1</sup> whole cells at 30°C and 900 rpm.

## 10. Determination the stereoselectivities of *DhCR* and *DhCR<sub>V9</sub>*

Reaction mixtures (300  $\mu$ L) containing 5 mM substrate **1–20**, appropriate purified enzyme solutions (0.1 mg/mL, 50  $\mu$ L *DhCR* or 5  $\mu$ L *DhCR<sub>V9</sub>*), 5 mM NADPH in KPB buffer (pH 6.5 100 mM), were incubated at 30°C, 180 rpm for 2 h. Afterwards, the reaction mixtures were extracted with 500  $\mu$ L ethyl acetate. The organic phases were transferred to new EP tubes and dehydrated with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Racemic alcohols or products prepared by unselective reductases were regarded as standards. Every samples were analyzed as following:

## Substrate 1



### HPLC Analysis

Column: Chiralcel OD-H column (4.6 mm × 250 mm)

Column temperature: 30°C

Detector wavelength: 228 nm

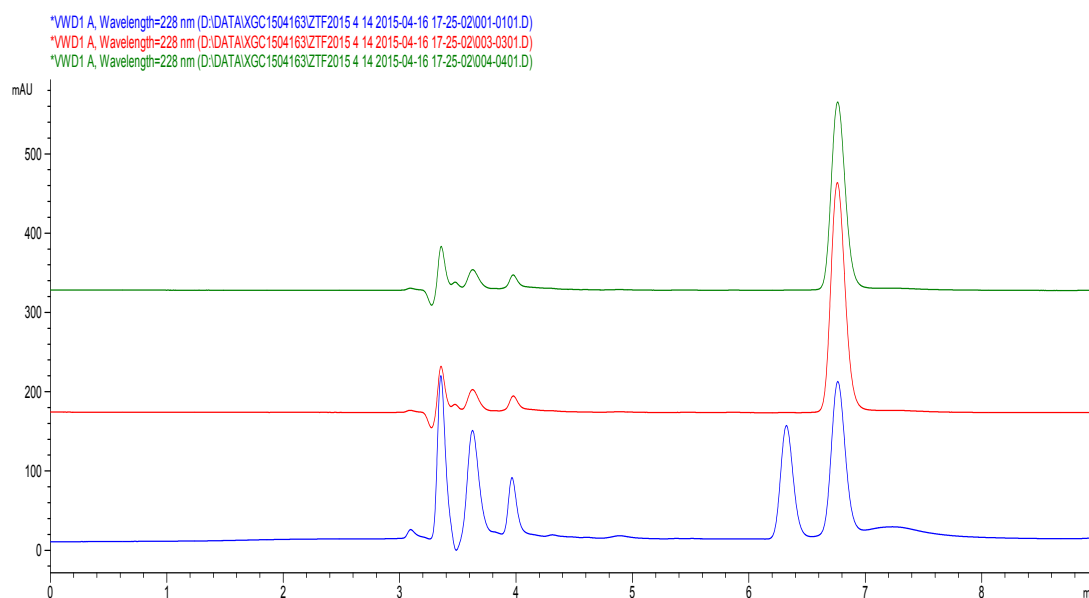
Mobile phase: n-Hexane/isopropanol (95/5)

Flow rate: 1 mL/min

Injection volume: 10 µL

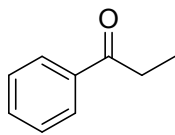
Retention times: (*R*)-alcohol (6.764 min), (*S*)-alcohol (6.322 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [6.764 min]	Peak area of ( <i>S</i> )-alcohol [6.322 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	2253.6	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	2690.5	0	>99

## Substrate 2



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

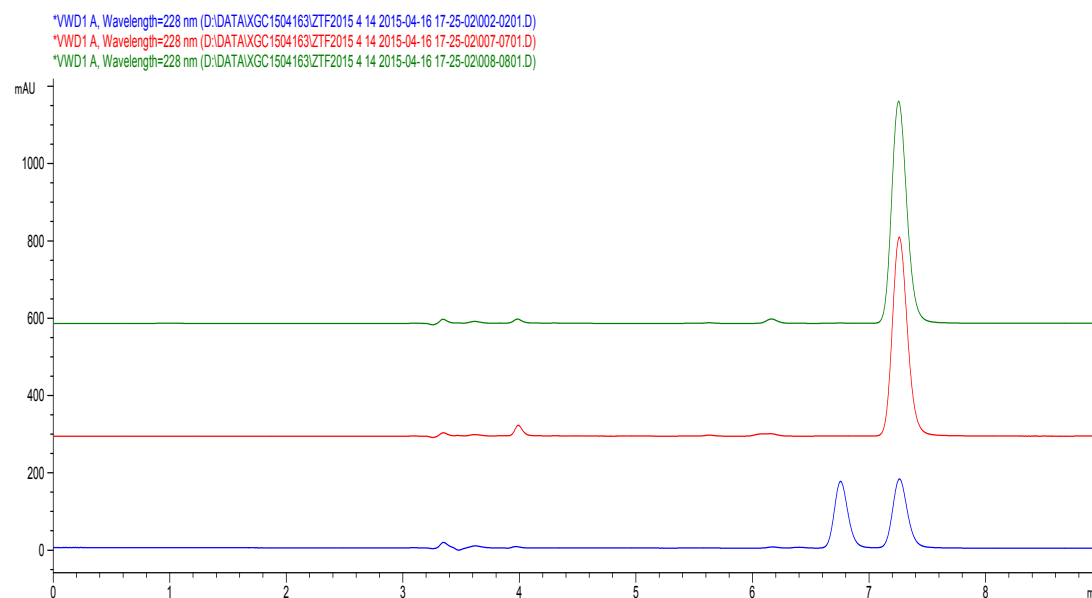
**Mobile phase:** n-Hexane/isopropanol (90/10)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

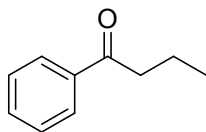
**Retention times:** (*R*)-alcohol (7.261 min), (*S*)-alcohol (6.756 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [7.261 min]	Peak area of ( <i>S</i> )-alcohol [6.756 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	4760.8	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	5327.7	0	>99

## Substrate 3



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

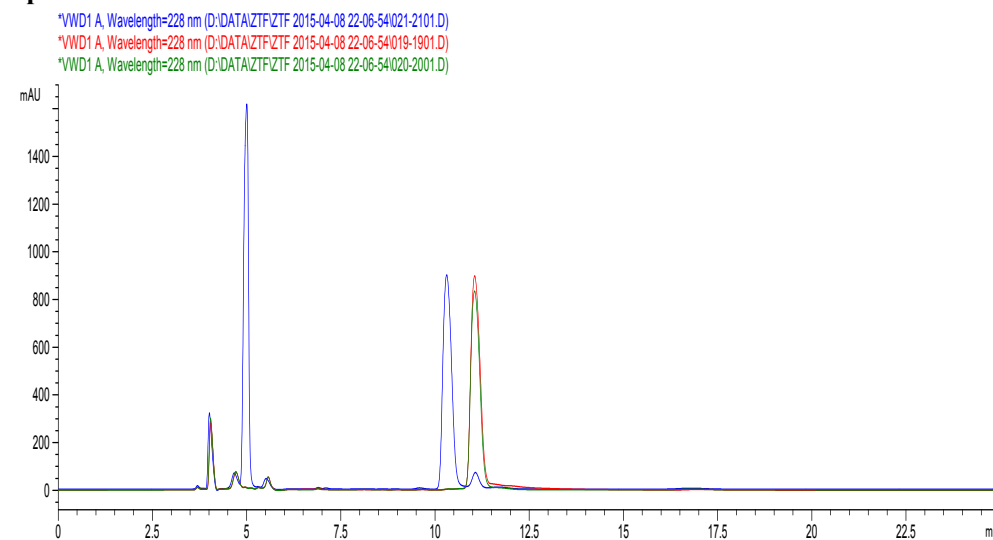
**Mobile phase:** n-Hexane/isopropanol (93/7)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

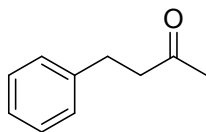
**Retention times:** (*R*)-alcohol (11.073 min), (*S*)-alcohol (10.310 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [11.073 min]	Peak area of ( <i>S</i> )-alcohol [10.310 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	14429.3	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	17687.1	0	>99

## Substrate 4



### HPLC Analysis

Column: Chiralcel OD-H column (4.6 mm × 250 mm)

Column temperature: 30°C

Detector wavelength: 228 nm

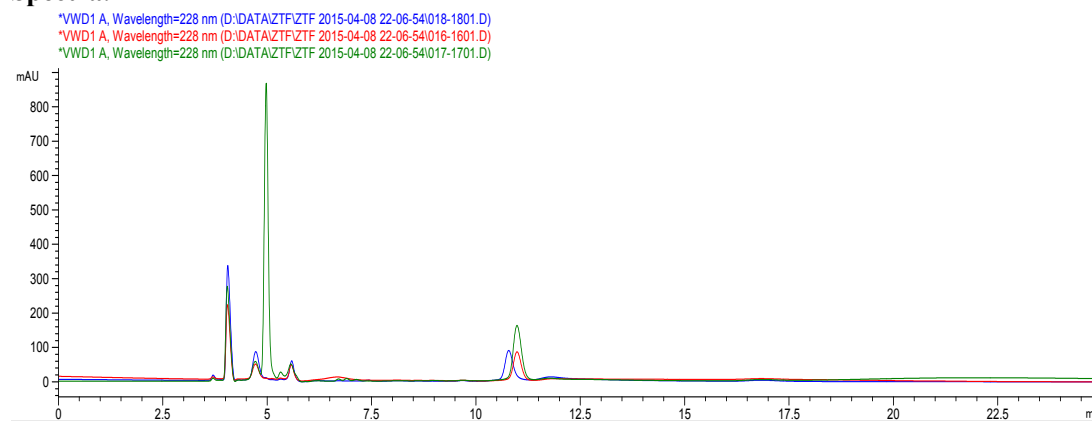
Mobile phase: n-Hexane/isopropanol (93/7)

Flow rate: 1 mL/min

Injection volume: 10 µL

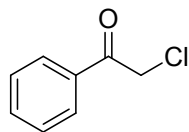
Retention times: (*R*)-alcohol (10.986 min), (*S*)-alcohol (10.788 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [10.986 min]	Peak area of ( <i>S</i> )-alcohol [10.788 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	1154.7	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	1492.4	0	>99

## Substrate 5



### HPLC Analysis

Column: Chiralcel OD-H column (4.6 mm × 250 mm)

Column temperature: 30°C

Detector wavelength: 228 nm

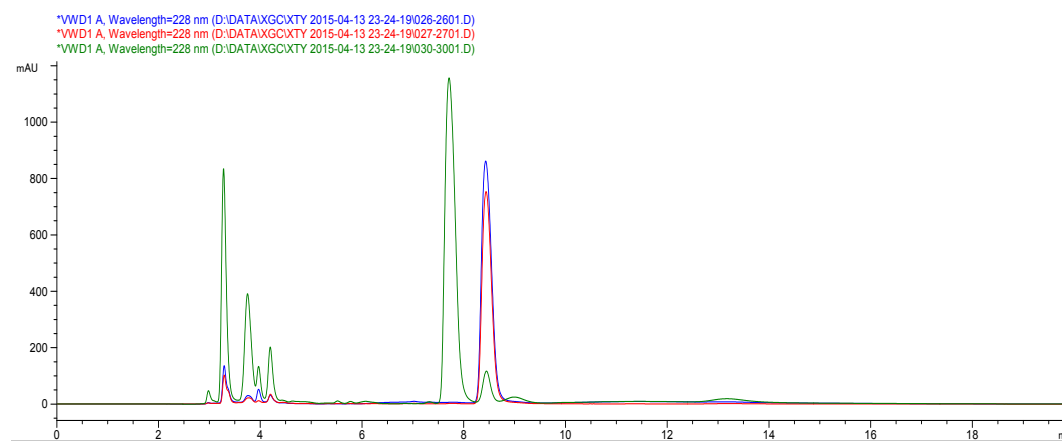
Mobile phase: n-Hexane/isopropanol (95/5)

Flow rate: 1 mL/min

Injection volume: 10 µL

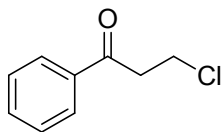
Retention times: (*R*)-alcohol (7.712 min), (*S*)-alcohol (8.446 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [7.712 min]	Peak area of ( <i>S</i> )-alcohol [8.446 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	0	16489.5	>99
<i>DhCR</i> <sub>V9</sub> (—)	0	17152.7	>99

## Substrate 6



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

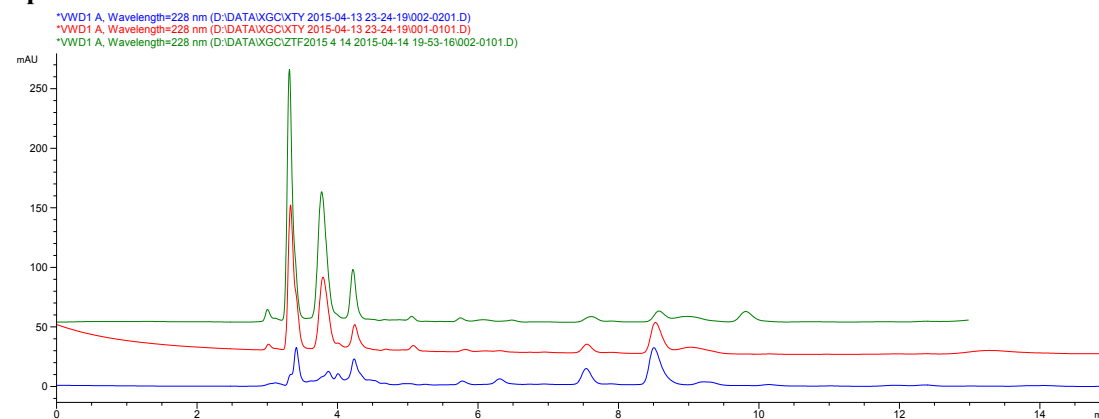
**Mobile phase:** n-Hexane/isopropanol (95/15)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**Retention times:** (*R*)-alcohol (8.509 min), (*S*)-alcohol (9.858 min)

### Spectra:

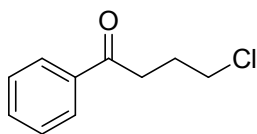


	Peak area of ( <i>R</i> )-alcohol [8.509 min]	Peak area of ( <i>S</i> )-alcohol [9.858 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	315.3	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	460.1	0	>99

Note: 3-chloro-1-phenylpropanol was not stable and may be dehalogenated spontaneously.



## Substrate 7



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

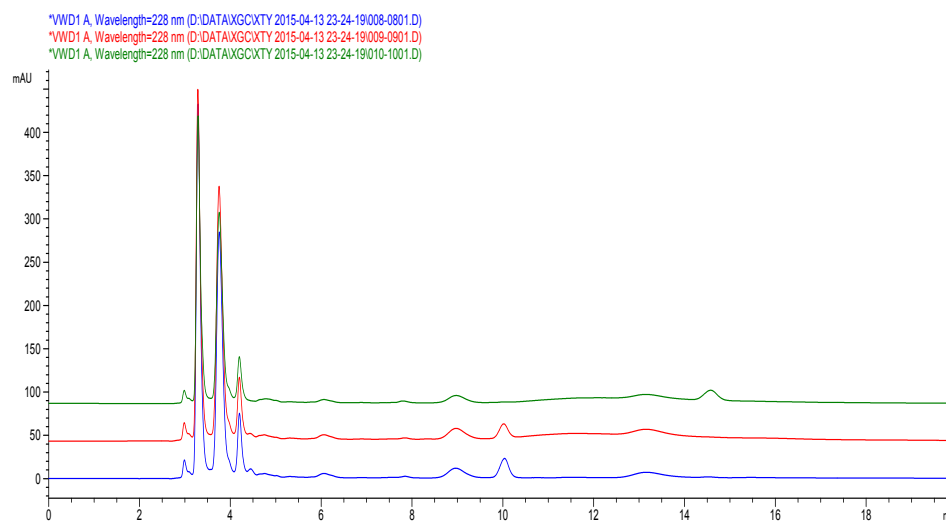
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**Retention times:** (*R*)-alcohol (10.089 min), (*S*)-alcohol (14.576 min)

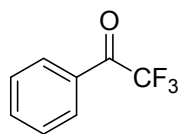
**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [10.089 min]	Peak area of ( <i>S</i> )-alcohol [14.576 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	295	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	349.4	0	>99

Note: 4-chloro-1-phenylbutanol was not stable and may be dehalogenated spontaneously.

## Substrate 8



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

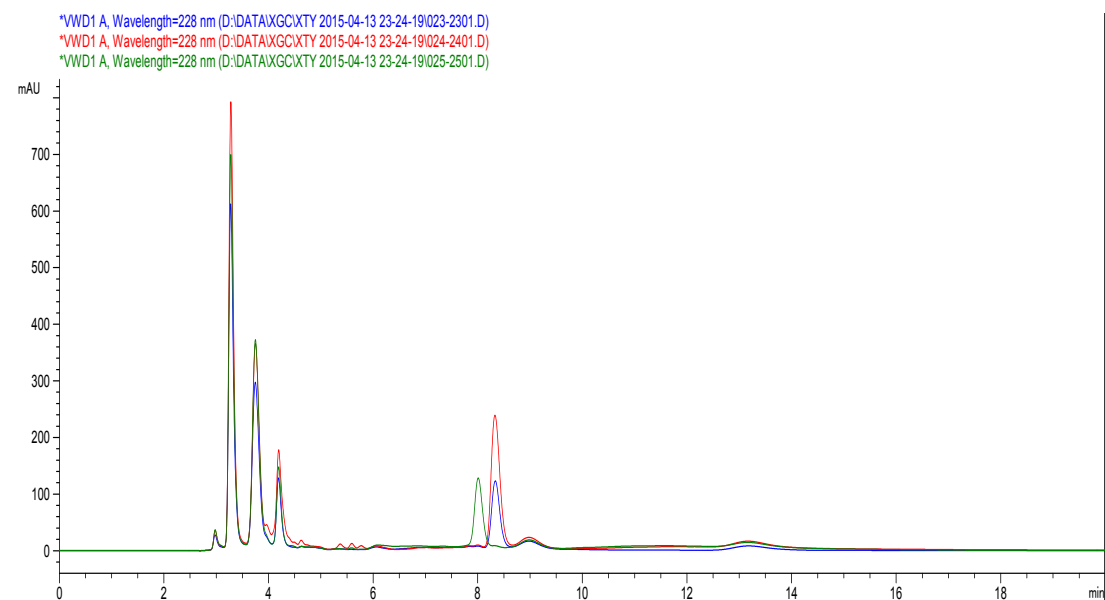
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

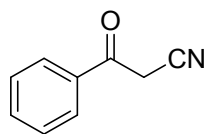
**Retention times:** (*R*)-alcohol (7.998 min), (*S*)-alcohol (8.378 min)

**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [7.998 min]	Peak area of ( <i>S</i> )-alcohol [8.378 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	0	2225.7	>99
<i>DhCR</i> <sub>V9</sub> (—)	0	2894.5	>99

## Substrate 9



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

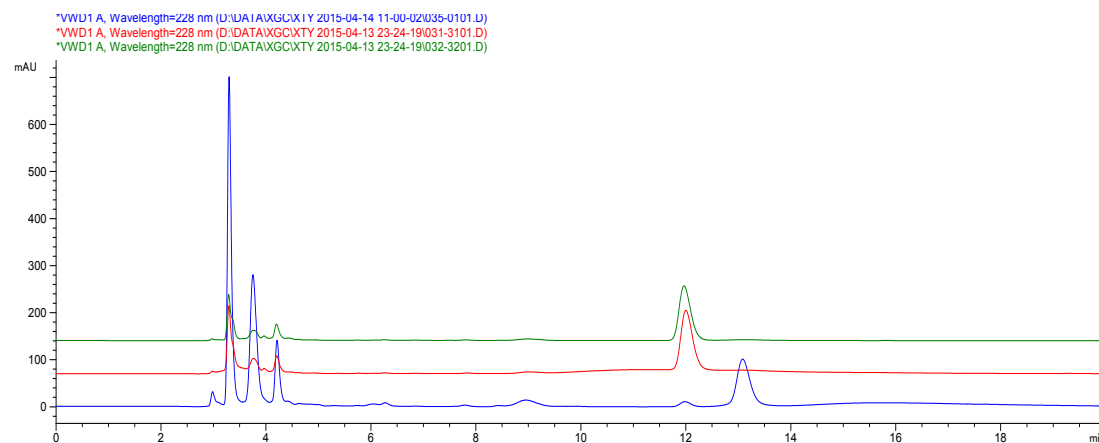
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

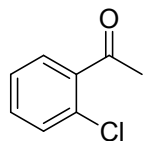
**Retention times:** (*R*)-alcohol (11.985 min), (*S*)-alcohol (13.084 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [11.985 min]	Peak area of ( <i>S</i> )-alcohol [13.084 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	1830.3	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	1898.5	0	>99

## Substrate 10



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

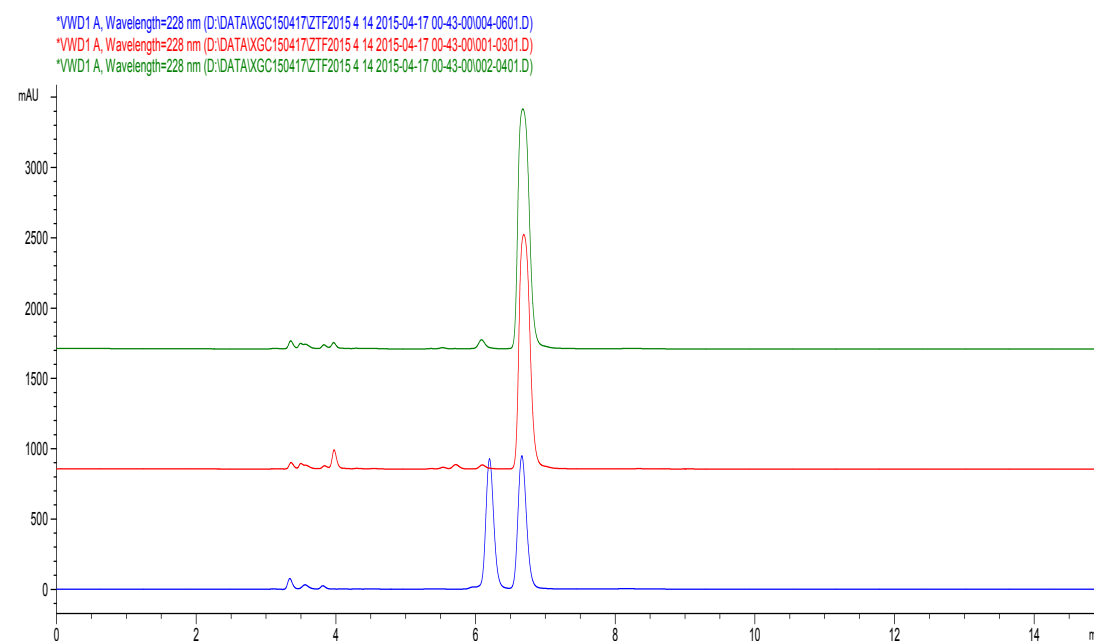
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

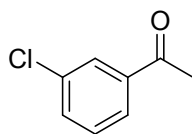
**Retention times:** (*R*)-alcohol (6.663 min), (*S*)-alcohol (6.200 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [6.663 min]	Peak area of ( <i>S</i> )-alcohol [6.200 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	18022.6	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	19197	0	>99

## Substrate 11



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

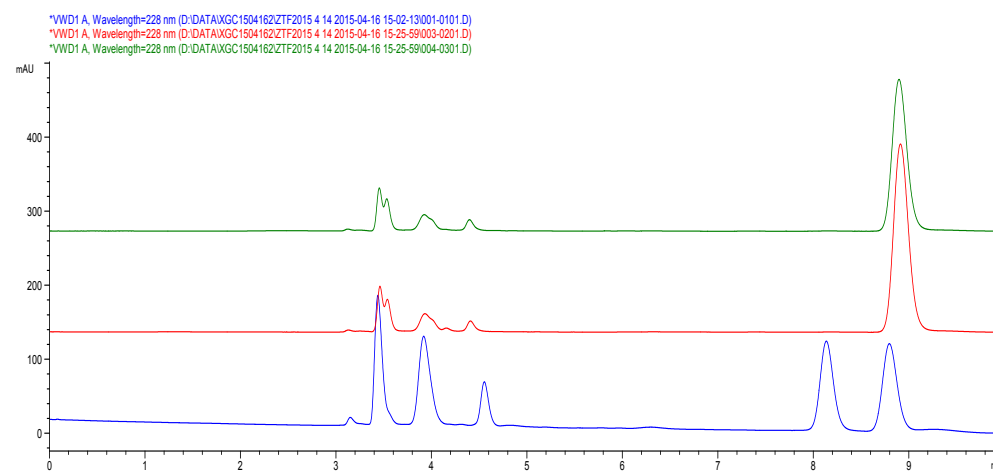
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

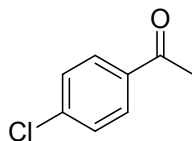
**Retention times:** (*R*)-alcohol (8.899 min), (*S*)-alcohol (8.178 min)

**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [8.899 min]	Peak area of ( <i>S</i> )-alcohol [8.178 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	2267.5	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	2837.8	0	>99

## Substrate 12



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

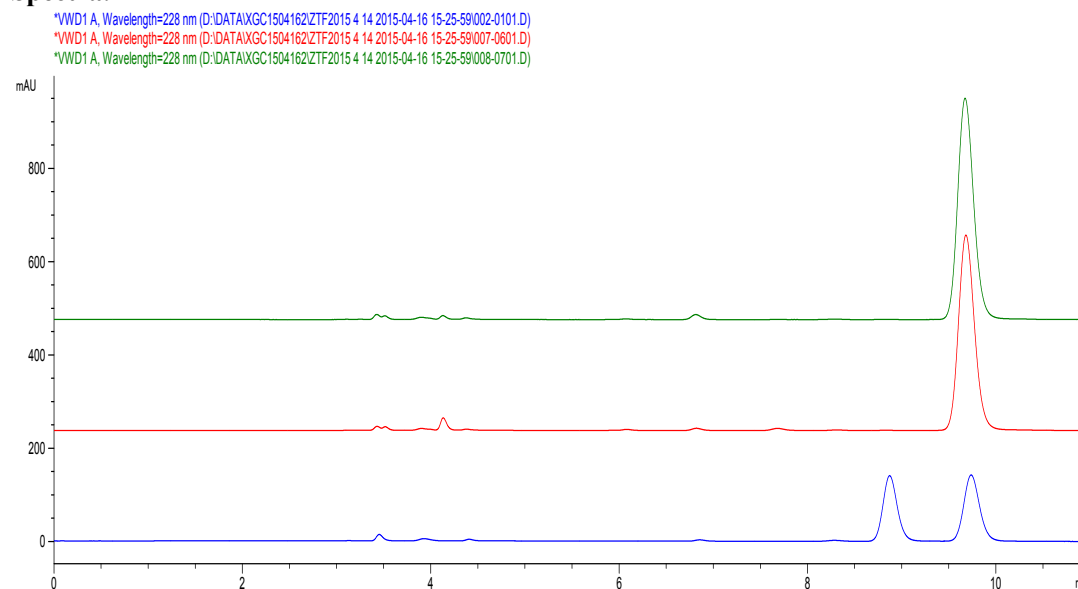
**Mobile phase:** n-Hexane/isopropanol (95/5)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

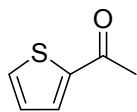
**Retention times:** (*R*)-alcohol (9.683 min), (*S*)-alcohol (8.878 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [9.683 min]	Peak area of ( <i>S</i> )-alcohol [8.878 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	4998.8	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	5651	0	>99

## Substrate 13



### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

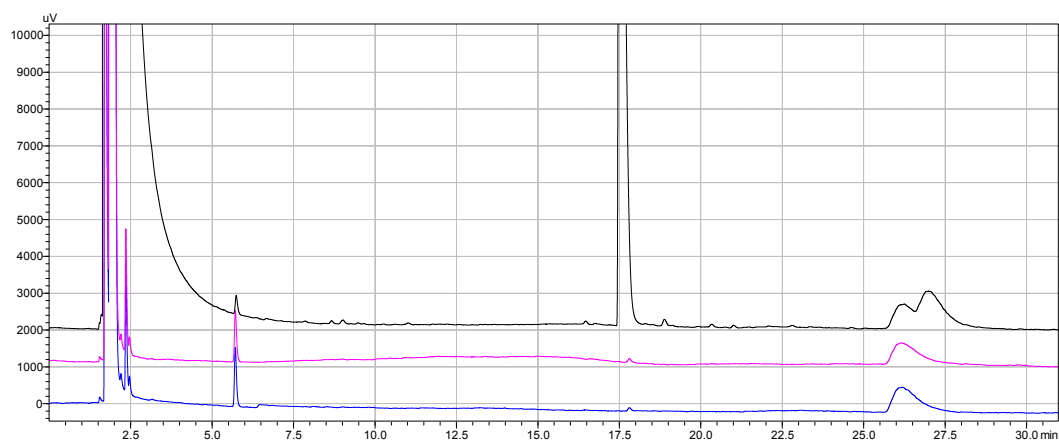
**Column temperature:** 80°C kept for 10 min, 5°C/min increased to 180°C, then kept for 2 min

**Injection volume:** 0.5 µL

**Split ratio:** 50

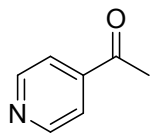
**Retention times:** ketone (17.815 min), (*R*)-alcohol (26.251 min), (*S*)-alcohol (26.991 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [26.251 min]	Peak area of ( <i>S</i> )-alcohol [26.991 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	37377	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	34825	0	>99

## Substrate 14



### HPLC Analysis

**Column:** Chiralcel OD-H column (4.6 mm × 250 mm)

**Column temperature:** 30°C

**Detector wavelength:** 228 nm

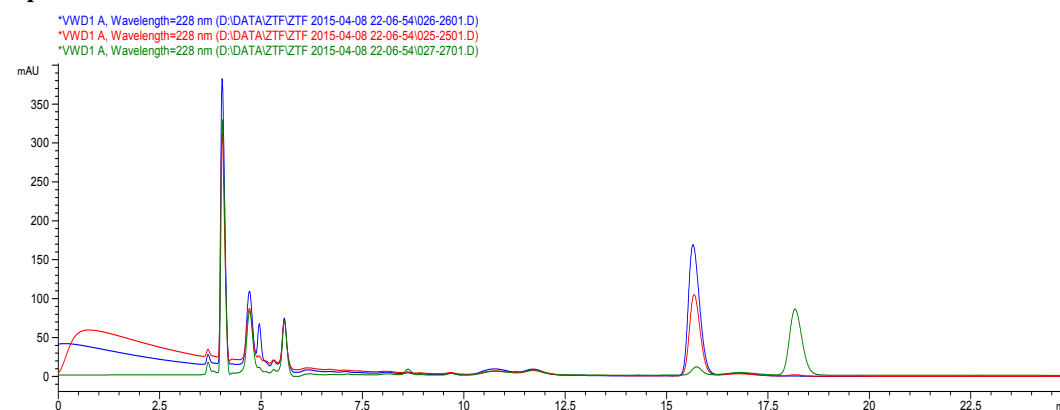
**Mobile phase:** n-Hexane/isopropanol (93/7)

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**Retention times:** (*R*)-alcohol (15.733 min), (*S*)-alcohol (18.167 min)

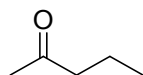
### Spectra:



	Peak area of ( <i>R</i> )-alcohol [15.733 min]	Peak area of ( <i>S</i> )-alcohol [18.167 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	1980.6	10.1	99.0
<i>DhCR</i> <sub>V9</sub> (—)	3303.4	15.8	99.0



## Substrate 15



### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

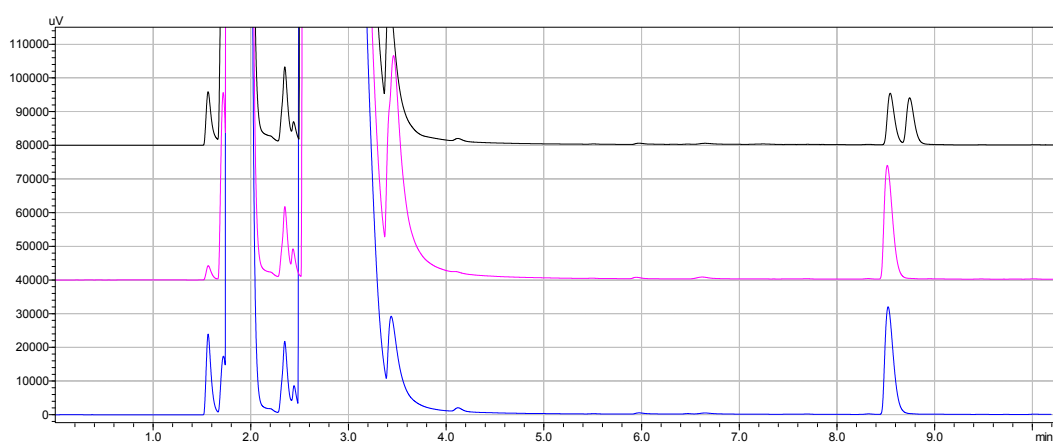
**Column temperature:** 60°C kept for 4 min, 10°C/min increased to 120°C, then kept for 2 min

**Injection volume:** 0.5 µL

**Split ratio:** 50

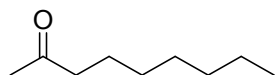
**Retention times:** ketone (6.678 min), (*R*)-alcohol (8.545 min), (*S*)-alcohol (8.744 min)

**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [8.545 min]	Peak area of ( <i>S</i> )-alcohol [8.744 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	33779	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	31901	0	>99

## Substrate 16



Products were ethylated first with appropriate amount of acetic anhydride and pyridine in boiling water for 1 h.

### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

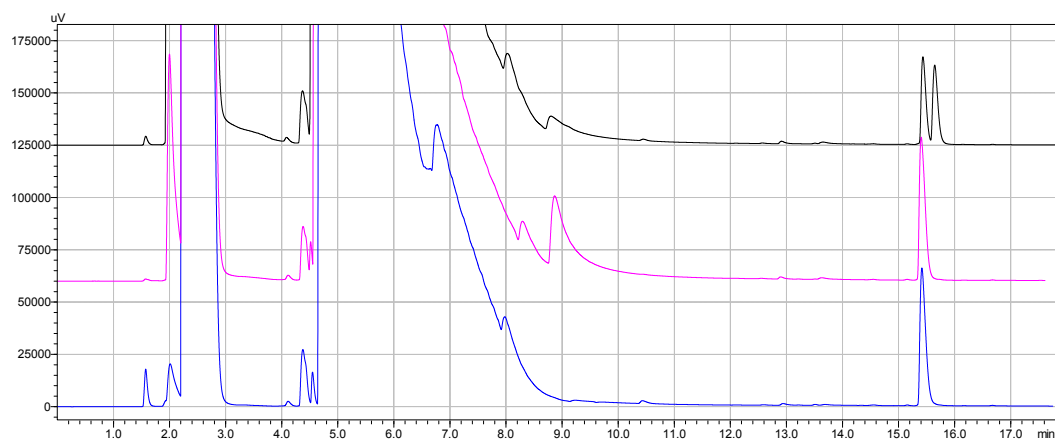
**Column temperature:** 60°C kept for 2 min, 5°C/min increased to 150°C, then kept for 2 min

**Injection volume:** 0.5 µL

**Split ratio:** 50

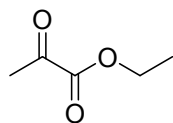
**Retention times:** ketone (8.978 min), (*R*)-alcohol (15.434 min), (*S*)-alcohol (15.645 min)

**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [8.545 min]	Peak area of ( <i>S</i> )-alcohol [8.744 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	498874	0	>99
<i>DhCR</i> <sub>v9</sub> (—)	471353	0	>99

## Substrate 17



### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

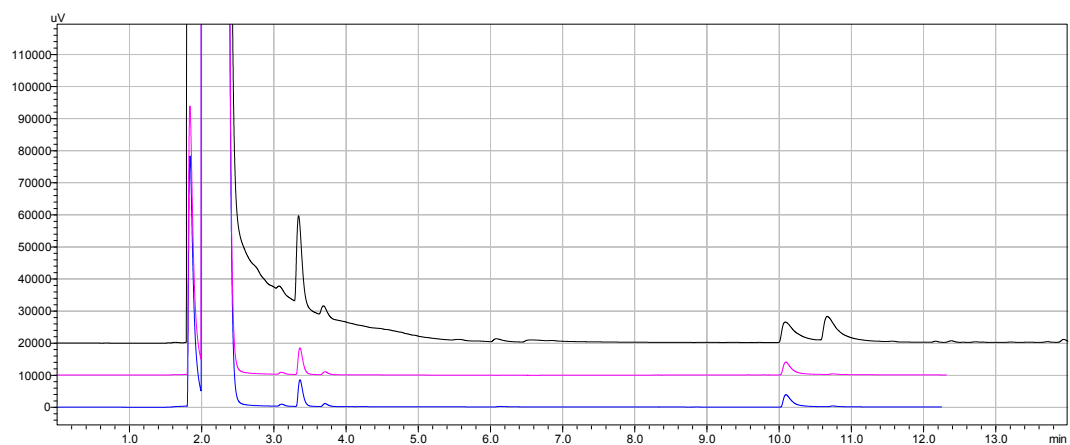
**Column temperature:** 60°C kept for 4 min, 10°C/min increased to 120°C, then kept for 2 min

**Injection volume:** 0.5 µL

**Split ratio:** 50

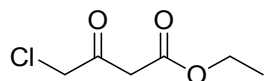
**Retention times:** ketone (6.178 min), (*R*)-alcohol (10.085 min), (*S*)-alcohol (10.666 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [10.085 min]	Peak area of ( <i>S</i> )-alcohol [10.666 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	33754	156	99.0
<i>DhCR</i> <sub>v9</sub> (—)	34929	163	99.1

## Substrate 18



Products were ethylated first with appropriate amount of acetic anhydride and pyridine in boiling water for 1 h.

### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

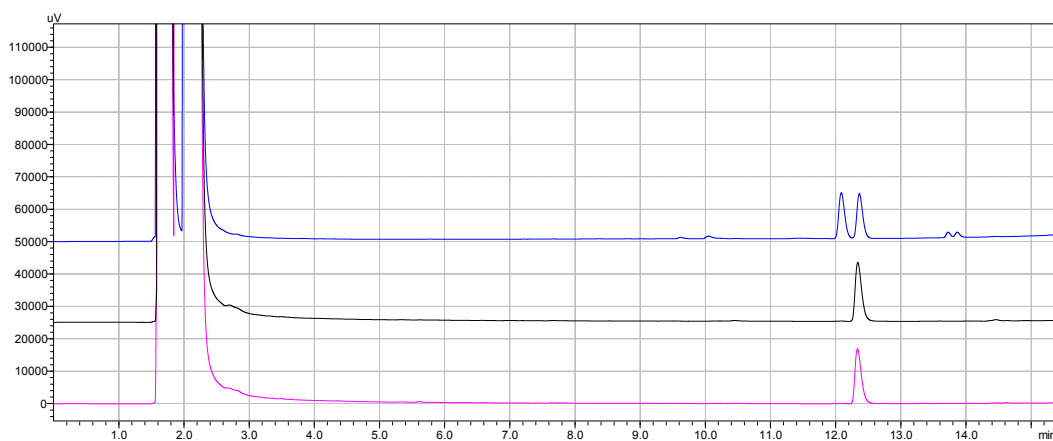
**Column temperature:** 110°C kept for 2 min, 2°C/min increased to 126°C, kept for 5 min, and then 2°C/min increased to 160°C

**Injection volume:** 0.5 µL

**Split ratio:** 50

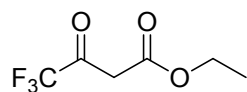
**Retention times:** ketone (9.058 min), (*R*)-alcohol (12.053 min), (*S*)-alcohol (12.356 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [12.053 min]	Peak area of ( <i>S</i> )-alcohol [12.356 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	0	102130	>99
<i>DhCR</i> <sub>V9</sub> (—)	0	116092	>99

## Substrate 19



Products were ethylated first with appropriate amount of acetic anhydride and pyridine in boiling water for 1 h.

### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

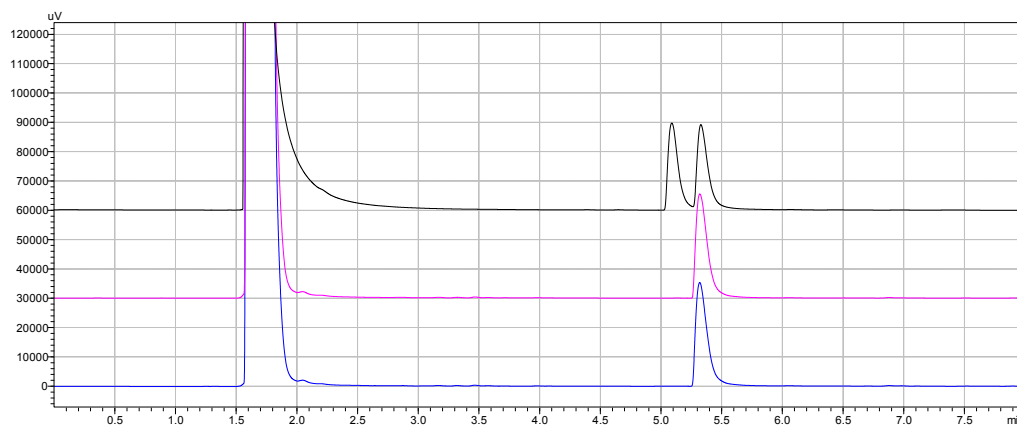
**Column temperature:** 130°C

**Injection volume:** 1.0 µL

**Split ratio:** 50

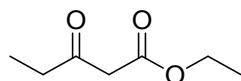
**Retention times:** ketone (2.058 min), (*R*)-alcohol (5.089 min), (*S*)-alcohol (5.329 min)

**Spectra:**



	Peak area of ( <i>R</i> )-alcohol [5.089 min]	Peak area of ( <i>S</i> )-alcohol [5.329 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	0	241297	>99
<i>DhCR</i> <sub>v9</sub> (—)	0	234445	>99

## Substrate 20



Products were ethylated first with appropriate amount of acetic anhydride and pyridine in boiling water for 1 h.

### GC Analysis

**Column:** Chirasil-DEX CB CP7502 column (25 m × 0.25 mm)

**Injector temperature:** 280°C

**Detector temperature:** 280°C

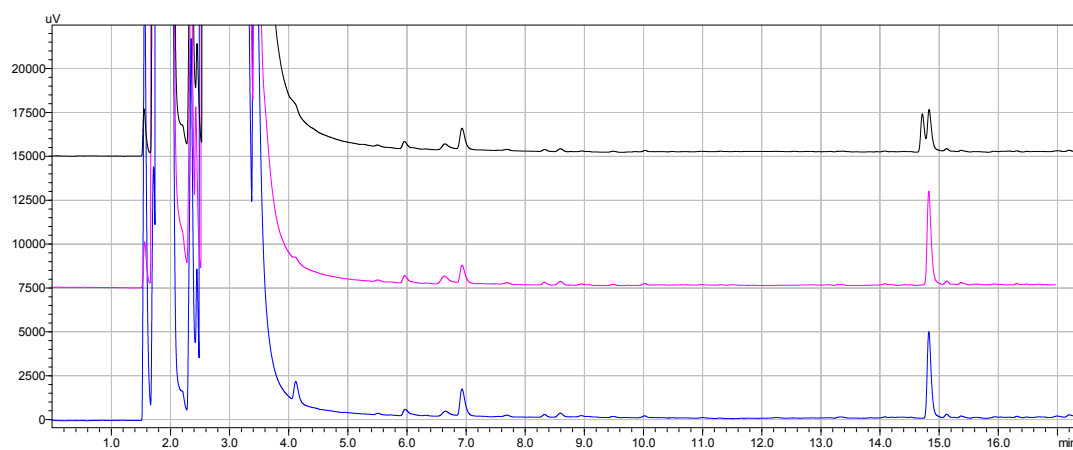
**Column temperature:** 110°C kept for 2 min, 2°C/min increased to 126°C, kept for 5 min, and then 2°C/min increased to 160°C

**Injection volume:** 1.0 µL

**Split ratio:** 50

**Retention times:** ketone (6.918 min), (*R*)-alcohol (14.716 min), (*S*)-alcohol (14.830 min)

### Spectra:



	Peak area of ( <i>R</i> )-alcohol [14.716 min]	Peak area of ( <i>S</i> )-alcohol [14.830 min]	<i>ee</i> [%]
<i>DhCR</i> (—)	203834	0	>99
<i>DhCR</i> <sub>V9</sub> (—)	193265	0	>99

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