Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

A carbonyl reductase from *Candida parapsilosis* ATCC 7330: Substrate selectivity and enantiospecificity

Sneha Sudhakara[a] and Anju Chadha*[a,b]

[a]*Laboratory of Bioorganic Chemistry, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600 036, India.*
E mail: snehasudhakara@gmail.com

[b]*National Center for Catalysis Research, Indian Institute of Technology Madras, Chennai 600 036, India.*
Email: anjuc@iitm.ac.in; Fax: +91-044-22574102; Tel: +91-044-22574106

I. Information about compound identity

As per the requirement, following are the details of compounds used as substrates as mentioned in Table 1 of the main article. Substrates 1-9 given below were procured.

Entry	Substrate	Company
	α-Ketoesters	
1	Ethyl-2-oxo-4-phenylbutanoate	Alfa Aesar, Great Britain
2	Ethyl-2-oxo-4-phenylacetate	Alfa Aesar, Great Britain
	Ketones	
3	2-Hydroxyacetophenone	TCI Chemicals, Tokyo, Japan
4	2-Chloroacetophenone	Avra Synthesis Pvt. Ltd., Hyderabad, India
5	Acetophenone	Fisher Scientific, Mumbai, India
6	*p*-bromoacetophenone	Sisco Research Laboratories Pvt. Ltd., Mumbai, India
7	*p*-methylacetophenone	Sisco Research Laboratories Pvt. Ltd., Mumbai, India
	Aldehydes	
8	Benzaldehyde	Fisher Scientific, Mumbai, India
9	Anisaldehyde	SD Fine-Chem Limited, Mumbai, India

Substrates **1-4** mentioned below were subjected to enzymatic asymmetric reduction. The corresponding racemic alcohols **6-8** were prepared by reported methods and were characterized by ^{1}H NMR and ^{13}C NMR that matched with the references B-D.

1-phenyl-1,2-ethanediol (**5**) was procured [Reference A].

These racemic alcohols served as standards in HPLC analysis.

Entry	Substrate	Entry	Product
1	2-Hydroxyacetophenone	**5**	1-Phenyl-1,2-ethanediol
2	2-Chloroacetophenone	**6**	2-Chloro-1-phenylethan-1-ol
3	Ethyl-2-oxo-4-phenylbutanoate	**7**	Ethyl-2-hydroxy-4-phenylbutanoate
4	Ethyl-2-oxo-2-phenylacetate	**8**	Ethyl-2-hydroxy-2-phenylacetate

References

A. Procured from Sigma-Aldrich, Missouri, USA

B. C. Rodríguez, W. Borzęcka, J. H. Sattler, W. Kroutil, I. Lavandera, and V. Gotor, *Org. Biomol. Chem.*, 2014, **12** (4), 673-681.

C. Z. M. Ou and R. W. Li, *Adv. Mat. Res.*, 2012, **560**, 333-337

D. C. Rodríguez, W. Borzęcka, J. H. Sattler, W. Kroutil, I. Lavandera, and V. Gotor, *Org. Biomol. Chem.*, 2014, **12** (4), 673-681.

II. SDS PAGE analysis of *S*RED

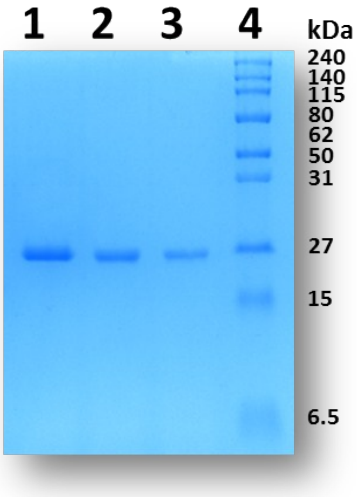

Fig. 1 SDS PAGE followed by Coomassie Brilliant Blue R250 staining of purified *S*RED; Lane 1, 6 µg of *S*RED; Lane 2, 4 µg of *S*RED; Lane 3, 2 µg of *S*RED; Lane 4, protein ladder.

III. Cofactor selectivity of *S*RED

Cofactor selectivity of this enzyme was determined by reducing the substrates **1-4** referred below in presence of NAD(P)H. After the reduction of substrates **1-4**, the extracted reaction mixture was analyzed by GC-MS (Shimadzu GCMS-QP 2010 Ultra).

GC:

- Supelco OmegawaxTM 320 capillary column (30 m X 0.32 mm X 0.25 µm film thickness)
- Injector / ion source temperature of 250 ^{o}C / 250 ^{o}C
- Temperature profile: 50 ^{o}C for 2 min, increased to 210 ^{o}C at the rate of 4 ^{o}C min^{-1} and then held for 6 min

MS (EI):

- Ion source temperature: 250 ^{o}C
- Interface temperature: 200 ^{o}C
- Voltage: 70 eV
- Scan mode: *m/z* 45.00 – 900.00

Entry	Substrate	**Entry**	Product
1	2-Hydroxyacetophenone	**5**	1-Phenyl-1,2-ethanediol
2	2-Chloroacetophenone	**6**	2-Chloro-1-phenylethan-1-ol
3	Ethyl-2-oxo-4-phenylbutanoate	**7**	Ethyl-2-hydroxy-4-phenylbutanoate
4	Ethyl-2-oxo-2-phenylacetate	**8**	Ethyl-2-hydroxy-2-phenylacetate

Following are the GC chromatograms for the reduction of substrates **1-4** with NADPH and NADH, and the mass spectra of the substrates and products.

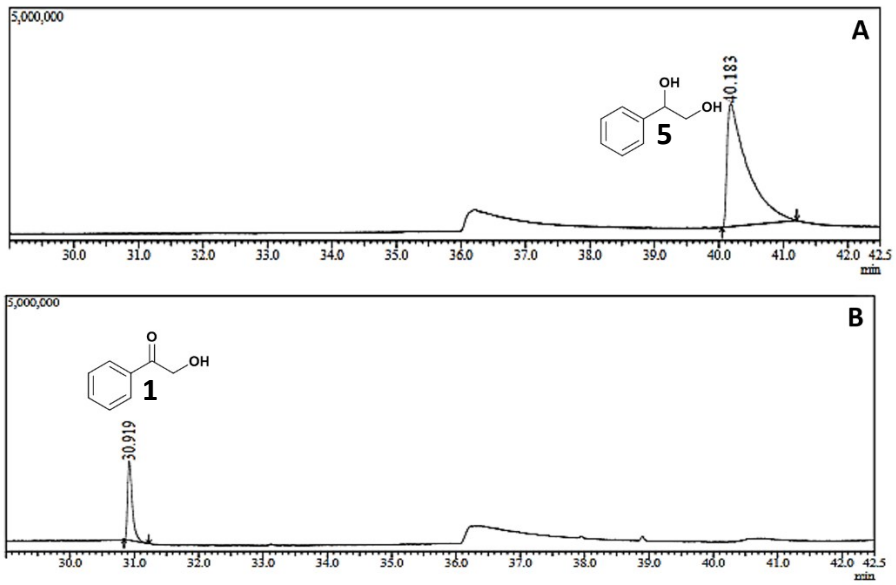

Fig. 2a GC chromatogram for reduction of 2-hydroxyacetophenone (**1**) [RT 30.92 min] to 1-phenyl-1,2-ethanediol (**5**) [RT 40.18 min]; A: with NADPH (conversion 100%); B: with NADH (no conversion).

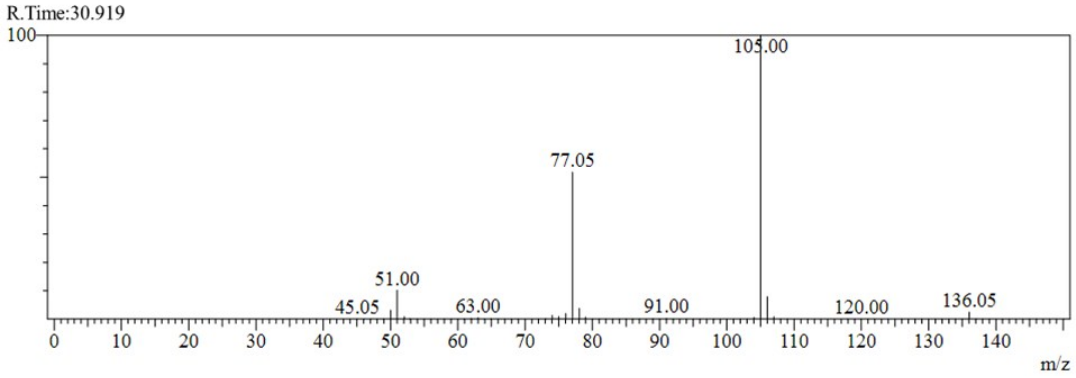

Fig. 2b Mass spectrum of 2-hydroxyacetophenone (**1**) [*m/z* 136.05 (M^+, 2.44 %), 105.00 (100.00), 77.05 (51.83), 51.00 (10.28)]

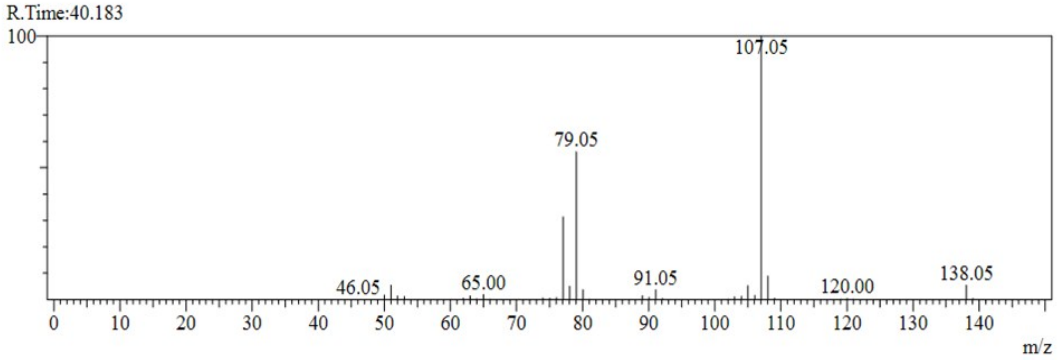

Fig. 2c Mass spectrum of 1-phenyl-1,2-ethanediol (**5**) [*m/z* 138.05 (M^+, 5.33 %), 107.05 (100.00), 79.05 (57.38), 77.05 (32.00)]

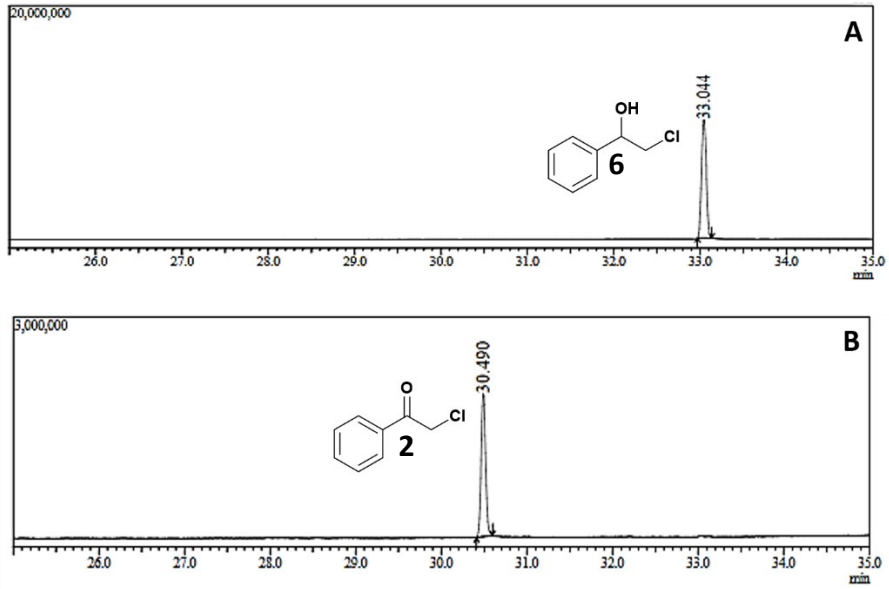

Fig. 3a GC chromatogram for reduction of 2-chloroacetophenone (**2**) [RT 30.49 min] to 2-chloro-1-phenylethan-1-ol (**6**) [RT 33.04 min]; A: with NADPH (conversion 100%); B: with NADH (no conversion).

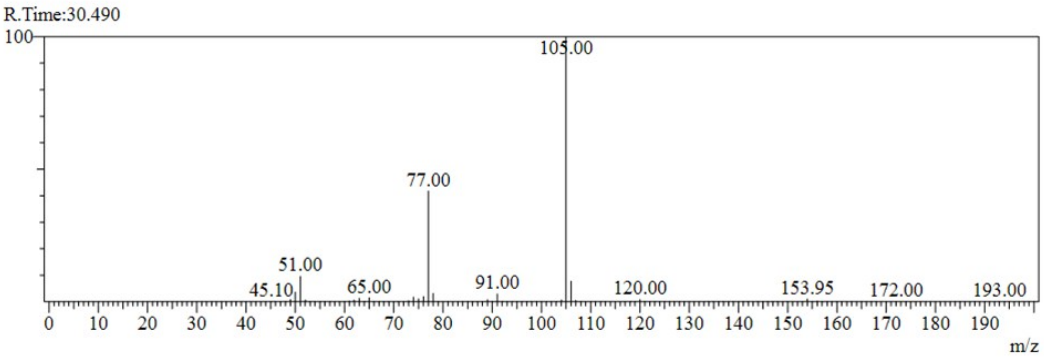

Fig. 3b Mass spectrum of 2-chloroacetophenone (**2**) [*m/z* 153.95 (M^+, 1.03 %), 105.00 (100.00), 77.00 (41.80), 51.00 (9.60)]

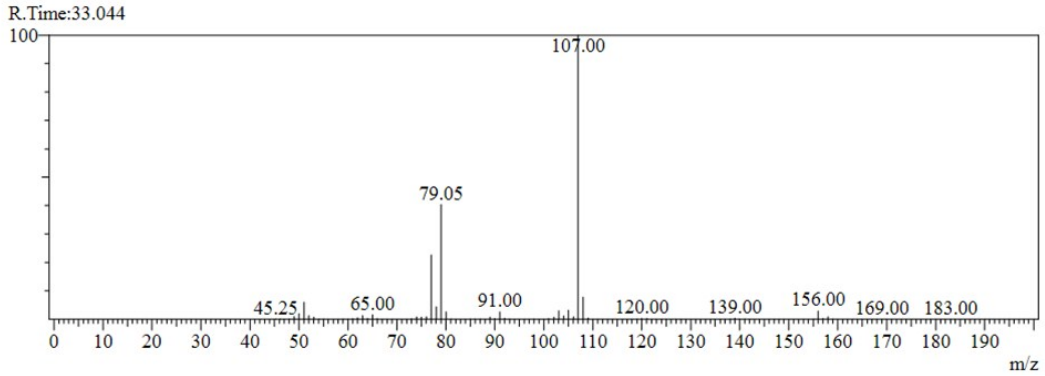

Fig. 3c Mass spectrum of 2-chloro-1-phenylethan-1-ol (**6**) [*m/z* 156.00 (M^+, 2.94 %), 107.00 (100.00), 79.05 (40.39), 77.00 (22.65)]

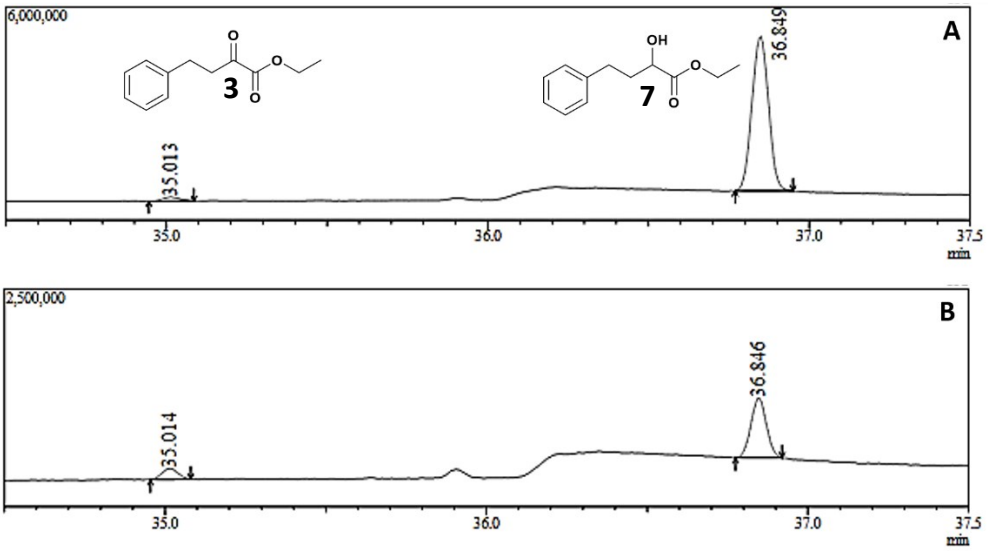

Fig. 4a GC chromatogram for reduction of ethyl-2-oxo-4-phenylbutanoate (**3**) [RT 35.01 min] to ethyl-2-hydroxy-4-phenylbutanoate (**7**) [RT 36.85 min]; A: with NADPH (conversion 98%); B: with NADH (conversion 84%).

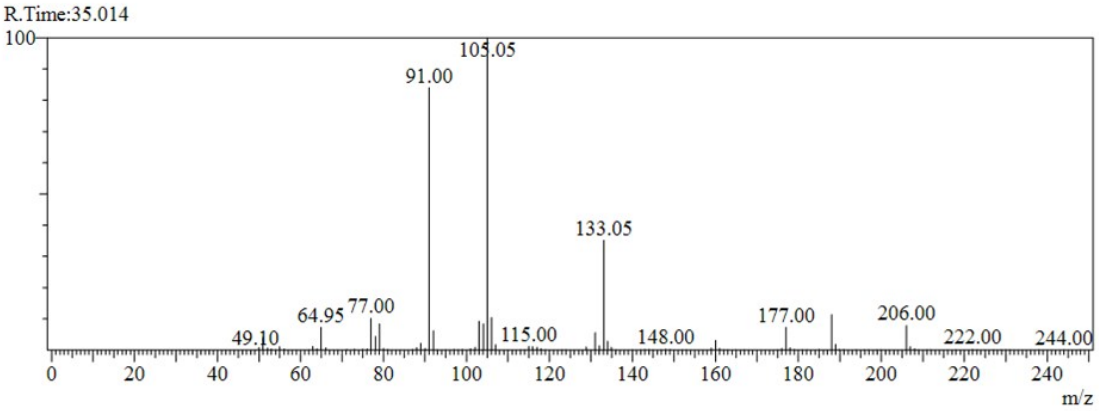

Fig. 4b Mass spectrum of ethyl-2-oxo-4-phenylbutanoate (**3**) [*m/z* 206.00 (M^+, 7.89 %), 188.00 (11.39), 133.05 (35.18), 105.05 (100.00), 91.00 (84.08), 77.00 (10.17)]

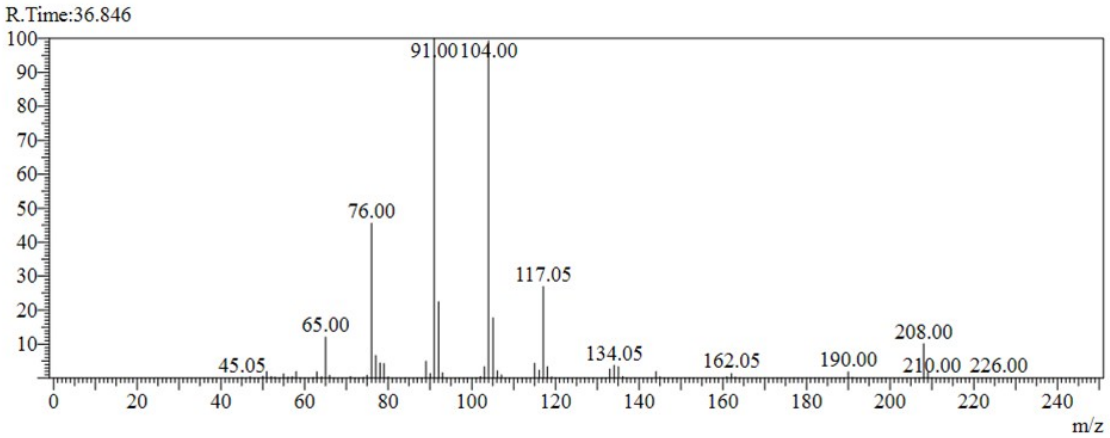

Fig. 4c Mass spectrum of ethyl-2-hydroxy-4-phenylbutanoate (**7**) [*m/z* 208.00 (M^+, 10.13 %), 117.05 (26.98), 104.00 (99.33), 91.00 (100.00), 76.00 (45.63), 65.00 (12.11)]

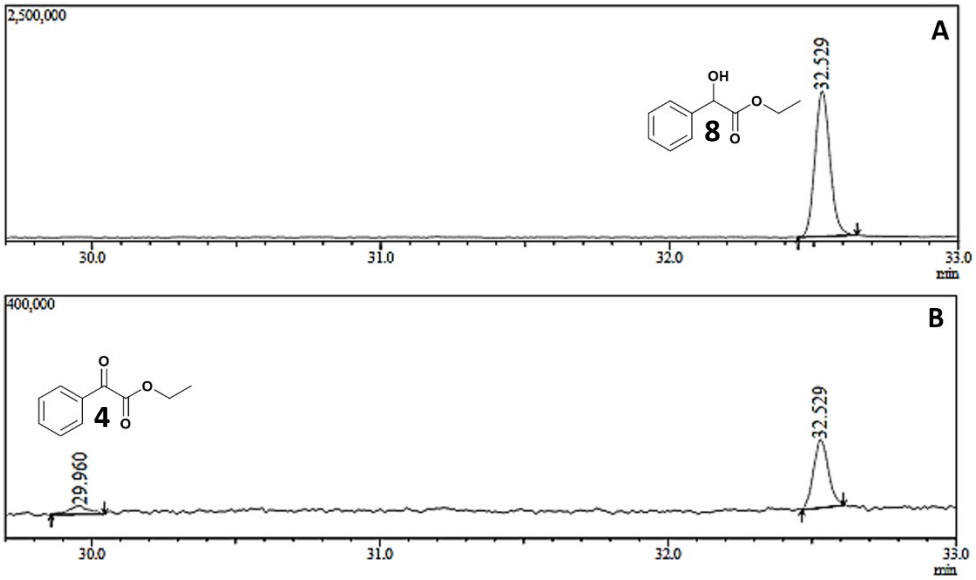

Fig. 5a GC chromatogram for reduction of ethyl-2-oxo-2-phenylacetate (**4**) [RT 29.96 min] to ethyl-2-hydroxy-2-phenylacetate (**8**) [RT 32.53 min]; A: with NADPH (conversion 100%); B: with of NADH (conversion 86%).

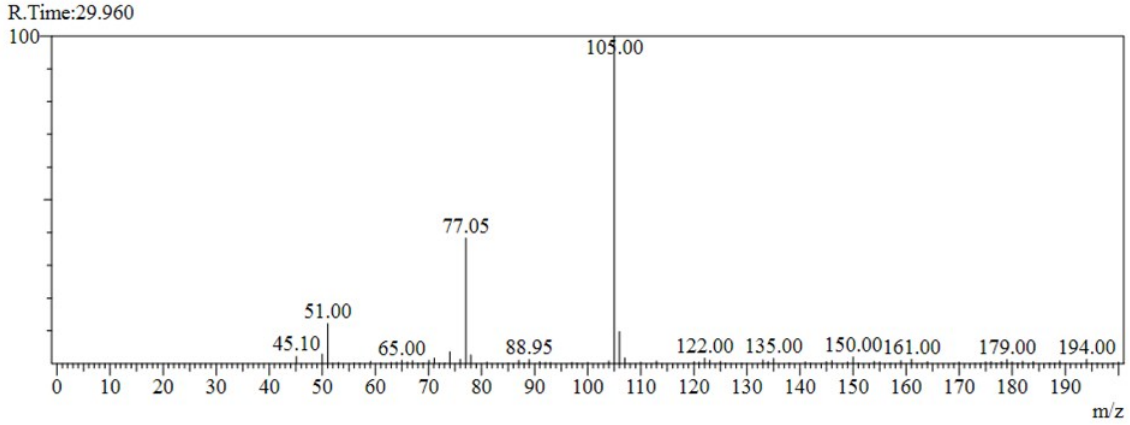

Fig. 5b Mass spectrum of ethyl-2-oxo-2-phenylacetate (**4**) [*m/z* 179.00 (M^{+1}, 1.21 %), 105.00 (100.00), 77.05 (38.37), 51.00 (12.20)]

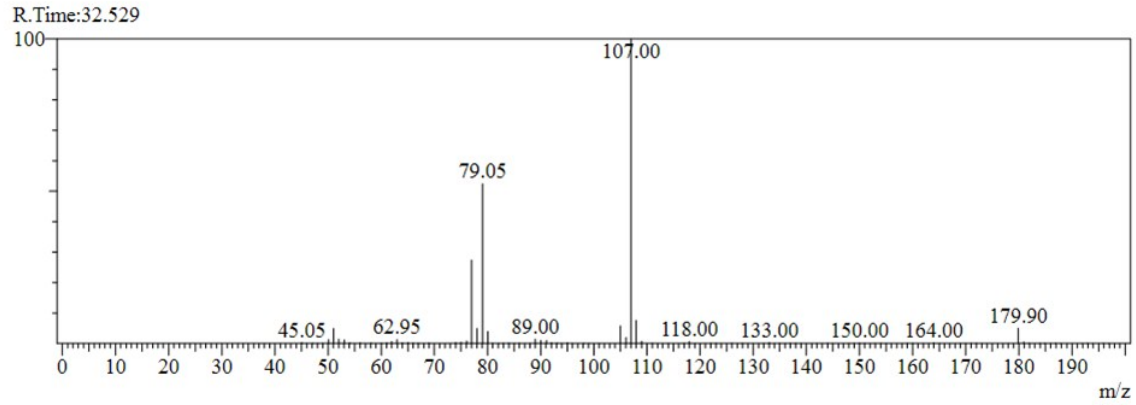

Fig. 5c Mass spectrum of ethyl-2-hydroxy-2-phenylacetate (**8**) [*m/z* 179.90 (M^{+}, 5.01 %), 107.00 (100.00), 79.05 (52.43), 77 (27.41)]

IV. Enantiospecificity of *S*RED

Ketone (**1,2**) and α-ketoester (**3,4**) substrates were reduced by *S*RED in presence of NADPH. The enantiomeric excess of the reaction mixture after extraction was calculated by analyzing the samples by HPLC (Jasco PU 1580 with MD 1515 detector). The corresponding (*RS*) - alcohols (**5-8 a,b**) were used as standards.

Reduction of ketone substrates (**1,2**) were analyzed by

- Column: Chiralcel OB-H (Daicel, 0.46 cm X 25 cm)
- Mobile phase: Hexane:Isopropanol = 90:10
- Flowrate: 1 ml min^{-1}

Reduction of α-ketoester substrates (**3,4**) were analyzed by

- Column: Chiralcel OD-H (Daicel, 0.46 cm X 25 cm)
- Mobile phase: Hexane:Isopropanol = 98:02
- Flowrate: 1 ml min^{-1}

Entry	Substrate	**Entry**	Product
1	2-Hydroxyacetophenone	**5 a,b**	(*RS*)-1-Phenyl-1,2-ethanediol
2	2-Chloroacetophenone	**6 a,b**	(*RS*)-2-Chloro-1-phenylethan-1-ol
3	Ethyl-2-oxo-4-phenylbutanoate	**7 a,b**	(*RS*)-Ethyl-2-hydroxy-4-phenylbutanoate
4	Ethyl-2-oxo-2-phenylacetate	**8 a,b**	(*RS*)-Ethyl-2-hydroxy-2-phenylacetate

Following are the HPLC chromatograms for the reduction of substrates **1-4** with NADPH.

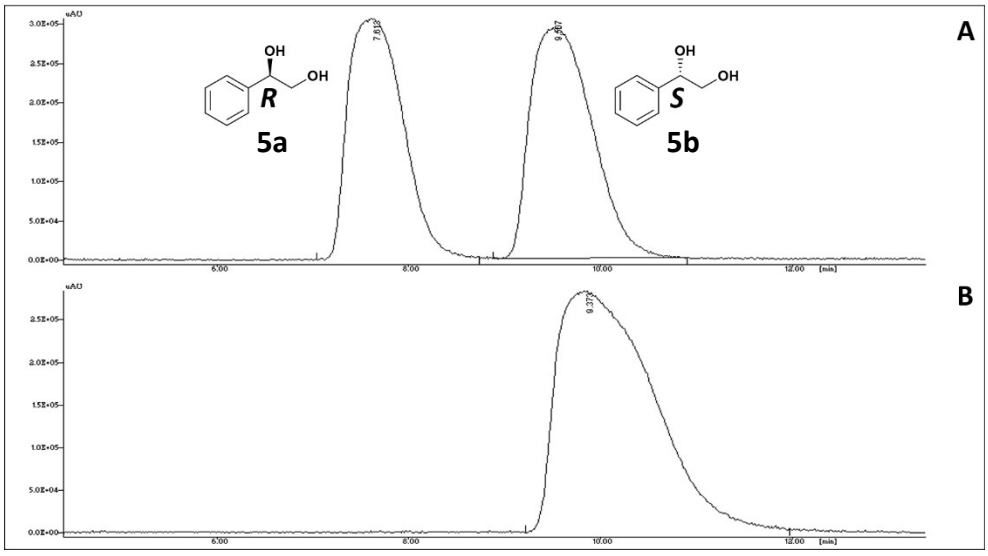

Fig. 6 HPLC chromatogram for reduction of 2-hydroxyacetophenone (**1**) to (*S*)-1-phenyl-1,2-ethanediol (**5b**); A: racemic mixture of 1-phenyl-1,2-ethanediol (RT of *R*- 7.6 min and *S*- 9.5 min); B: reaction mixture (ee > 99% of *S*).

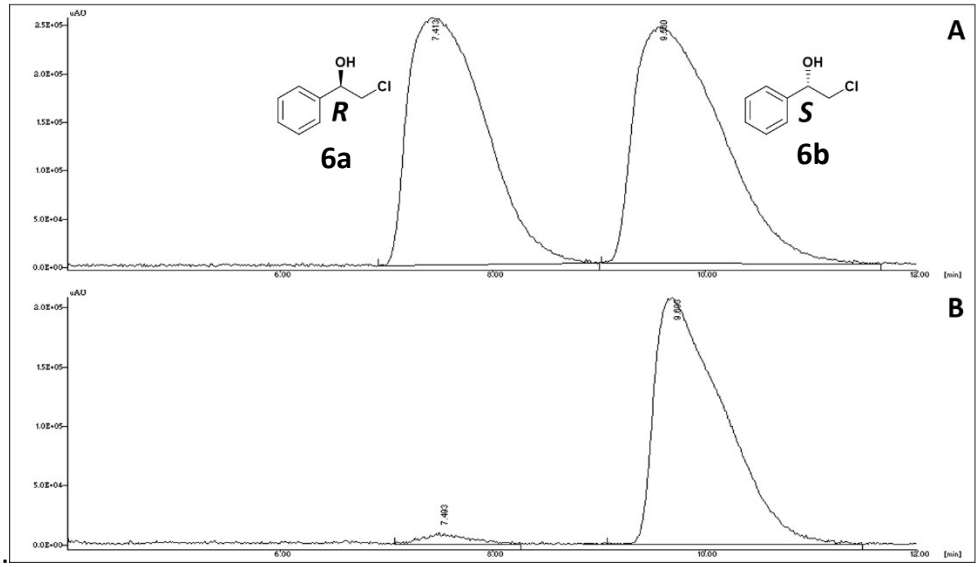

Fig. 7 HPLC chromatogram for reduction of 2-chloroacetophenone (**2**) to (*S*)-2-chloro-1-phenylethan-1-ol (**6b**); A: racemic mixture of 2-chloro-1-phenylethan-1-ol (RT of *R*- 7.4 min and *S*- 9.6 min); B: reaction mixture (ee 99% of *S*).

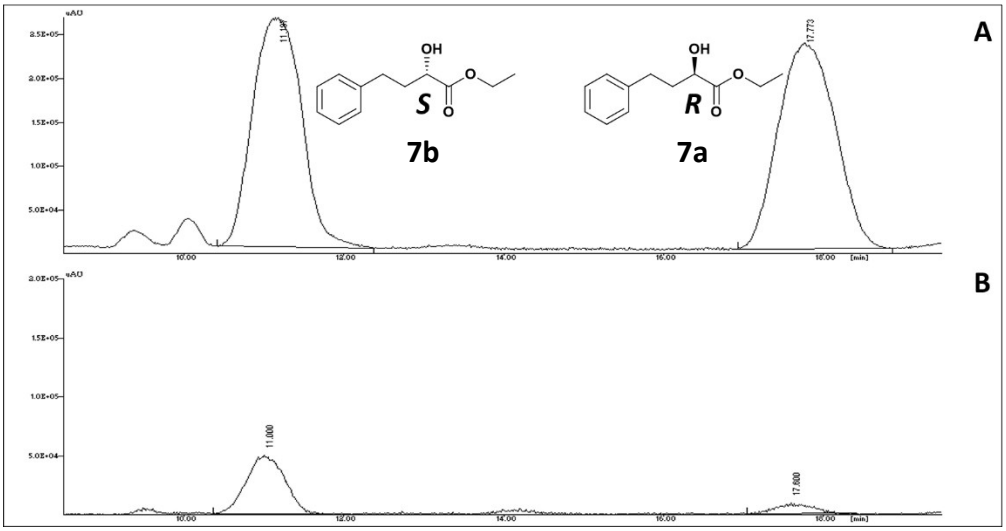

Fig. 8 HPLC chromatogram for reduction of ethyl-2-oxo-4-phenylbutanoate (**3**) to (*S*)-ethyl-2-hydroxy-4-phenylbutanoate (**7b**); A: racemic mixture of ethyl-2-hydroxy-4-phenylbutanoate (RT of *S*- 11.2 min and *R*- 17.7 min); B: reaction mixture (ee 70% of *S*).

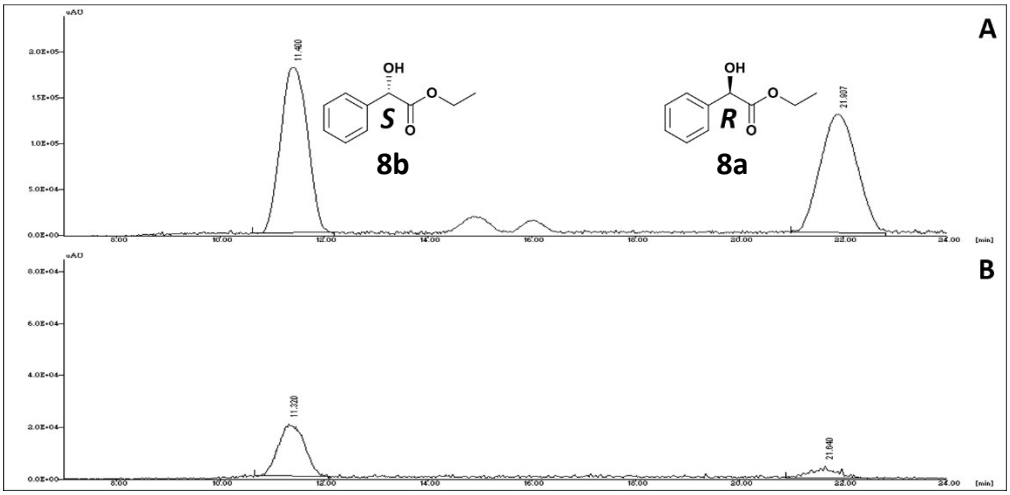

Fig. 9 HPLC chromatogram for reduction of ethyl-2-oxo-2-phenylacetate (**4**) to (*S*)-ethyl-2-hydroxy-4-phenylacetate (**8b**); A: racemic mixture of ethyl-2-hydroxy-2-phenylacetate (RT of *S*- 11.4 min and *R*- 21.9 min); B: reaction mixture (ee 70% of *S*).