Electronic Supplementary Information

Enoyl acyl carrier protein reductase (FabI) catalyzed asymmetric reduction of C-C double bond of α, β-unsaturated ketones: preparation of (*R*)-2-alkyl-cyclopentanones

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1. Chemicals, plasmids and strains

2-Pentyl-cyclopentanone **2c** (>95%) was purchased from Sigma-Aldrich. 2-Heptyl-cyclopentanone **2a** (>99%), 2-hexylcyclopentanone **2b** (>96%) and 2-butyl-cyclopentanone **2d** (>95%) were purchased from TCI (Japan). Ethyl acetate (HPLC grade), silica gel 60 (200-300 mesh), and TLC Silica gel 60 plates were bought from Merck. *n*-Dodecane (>99%) were obtained from Acros Organics. *n*-Hexane (HPLC grade) and isopropanol (HPLC grade) were purchased from Tedia. 2-hetpylidene-cyclopentanone **1a**, 2-hexylidene-cyclopentanone **1b**, 2-pentylidene-cyclopentanone **1c** and 2-butylidene-cyclopentanon **1d** were prepared as reported before. [S1]

The restriction enzyme BamHI, HindIII, NdeI and XhoI were purchased from NEB. pRSF-Duet, pCDF-Duet, pET-28a plasmids and *E. coli* BL21 (DE3) strain were bought from Novagen.

2. Analytic methods

The concentrations of 2-alkylidnenecyclopentanones **1a-d** and 2-alkyl-cyclopentanones **2a-d** were determined with an Agilent 7890A gas chromatography on a HP-5 capillary column (30 m×322 μm×0.25 μm). Helium was used as the carrier gas at the flow rate of 2 mL/min. The chemical components in sample were detected with a FID detector. Inlet and detector temperature was 220 °C and 270 °C, respectively. The oven temperature started at 120 °C, increased to 200 °C at the rate of 20 °C/min, increased to 250 °C within 1 min and hold at this temperature for 2 min. Retention times were listed in the Table S1. 2 mM of *n*-dodecane was used as the internal standard, with a retention time of 2.9 min. By using the internal standard, calibration curves of **1a-d** and **2a-d** at different concentration were established. By comparing

the ratio of peak areas of the target compound and the internal standard in GC chromatogram the concentrations of the substrate and product in the samples were calculated.

Table S1. Retention time of 2-alkylidene-cyclopentanoones **1a-d** and 2-alkyl-cyclopentanones **2a-d** in the GC chromatograms

Compound	Retention time (min)	Compound	Retention time (min)
1a	4.2	**2a**	4.6
1b	3.7	**2b**	4.1
1c	3.2	**2c**	3.7
1d	2.7	**2d**	3.1

The *ee* values of **2a-d** were analyzed with a Shimadzu HPLC system (LC-20AD) equipped with Daicel Chiralpak IA-3 column (250 mm×4.6 mm). The product **2a-d** were detected by UV at 300nm. The mobile phase was a mixture of *n*-hexane and isopropanol (97:3) at the flow rate of 0.5 mL/min. The oven temperature was 25°C. Retention times were as listed in the Table S2. Enantiomeric excess (*ee*) were calculated from the peak areas of the two enantiomers.

Table S2. Retention time of the enantiomers of 2-alkyl-cyclopentanones **2a-d** in the chiral HPLC chromatograms

Compound	Retention time (min)	Compound	Retention time (min)
(*R*)-**1a**	8.6	(*S*)-**1a**	9.0
(*R*)-**1b**	8.7	(*S*)-**1b**	9.2
(*R*)-**1c**	9.0	(*S*)-**1c**	9.5
(*R*)-**1d**	9.2	(*S*)-**1d**	9.8

3. Purification of the ene-reductase from *Acinetobacter* sp. RS1

The general procedure of the purification of ene-reductase from *Acinetobacter* sp. RS1 was shown in Figure S1.

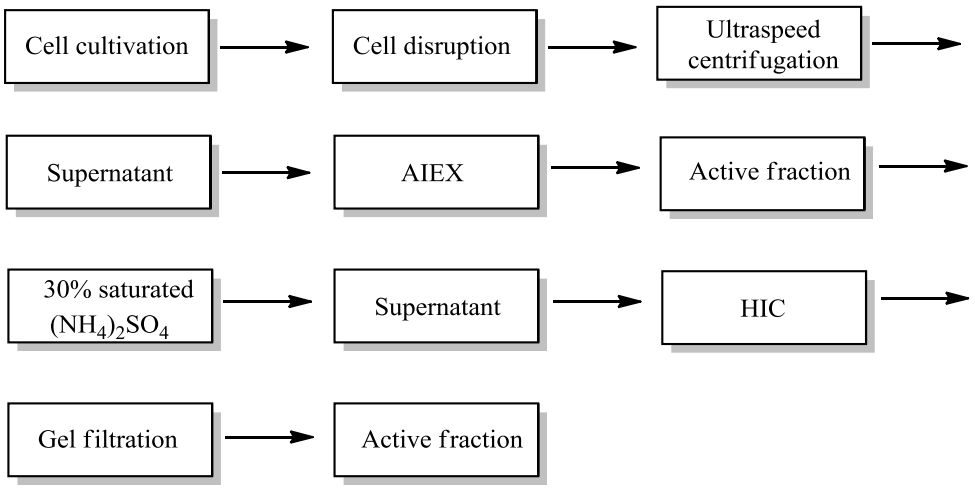

Figure S1. General procedures of the purification of the ene-reductase of *Acinetobacter* sp. RS1.

Acinetobacter sp. RS1 was cultivated with TB medium at 30 °C and 250 rpm for 12 h.[S1] Cells were harvested by centrifugation (3,000 g, 10 min) and resuspended in 250 mL Tris buffer (100 mM, pH 8.0). The harvested cells were disrupted with APV-1000 homogenizer. Cell debris was then removed by centrifugation at 15,000 g for 60 min and the supernatant was filtered through a PVDF syringe filter (pore size: 0.45μm) to give 250 mL of cell-free extract (CFE) with 60 mg/mL of protein and a specific activity for the reduction of **1b** of around 0.3U/g. The CFE was subjected to anion exchange chromatography (AIEXC), hydrophobic interaction chromatography (HIC), and gel filtration chromatography. The AIEXC was carried out by using a Sepharose Q XL 16/20 column with aqueous NaCl solution as eluent in a gradient from 0 to 0.6M NaCl in 15 column volumes. The HIC purification was accomplished by using a butyl HP column with aqueous ammonium sulfate solution as eluent in a gradient from 1.3 M to 0 M in 20

column volumes. The gel filtration chromatography was done with Superdex 10/30 GL column using Tris buffer (50 mM, pH 7.5) as eluent.

During the purification process, the protein fractions after each column were tested for the reduction of 2-hexylidene-cyclopentanone **1b**. The reaction was conducted in 50mM Tris buffer with 0.1 mL protein fraction, 5 mM substrate and 10 mM NADH at 30 °C and 250 rpm for 30 min and the product formation was analyzed by GC. The result of the purification was summarized in Table S3. The specific activity of the purified active fraction after 3 columns reached 110 U/g protein, giving the purification factor of 370.

Table S3. Summarized results of the 3-column chromatography purification of the functional ene-reductase from *Acinetobacter* sp. RS1.

Active fraction	Spec. activity (U/g protein) [a]	Conc. (mg protein/mL)	Vol. (mL)	Total activity (U)	Recov. (%)	Purification factor
CFE	0.3	60	250	4050	-	-
AIEXC	3.1	2.9	100	899	22.5	11.5
HIC	11.7	1	50	585	14.4	73
Gel filtration	110	0.1	5	55	1.3	370

[a] The activity was measured with the substrate **1b** in 50mM Tris buffer, 30ºC, 250rpm in 30 min.

However, the purified ene-reductase only accounted for 60% of the total protein content (Fig. S2). Thus the purity and total protein amount of the protein sample could not enough for protein sequencing. The active fraction of gel filtration chromatography was analyzed with MALDI-TOF to give the molecular weight of the major protein as 27.5kDa.

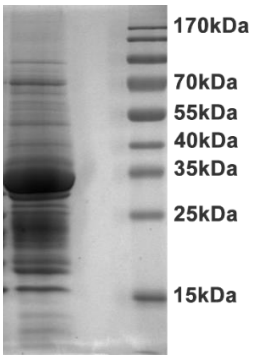

Figure S2. SDS-PAGE of the active fraction after gel filtration chromatography. Left: the purified ene-reductase; right: protein marker.

4. Cloning of *A*-FabI reductase from *Acinetobacter* sp. RS1 and construction of *E. coli* (*A*-FabI)

The genome of *Acinetobacter* sp. RS1 was sequenced by Beijing Genomics Institute (BGI). Based on the genome information, 7 C-C and C-N double bond reductases were predicted (Table S4). Out of those reductases, the *Acinetobacter* enoyl-ACP reductase (*A*-FabI) showed similar molecular weight (28.5kDa) to that of the partially purified ene-reductase from *Acinetobacter* sp. RS1. *A*-FabI was thus chosen for cloning and activity test.

Table S4. Predicted C-C and C-N double bond reductases in *Acinetobacter* sp. RS1

Gene Annotation	Size (kDa)	Nature substrate	Coenzyme or cofactor
Acyl coenzyme A dehydrogenase	65.1	Acyl-CoA	FAD(H)
Enoyl-ACP reductase (*A*-FabI)	28.5	Enoyl-ACP	NAD(P)H
Predicted acyl-CoA reductase	41.8	Acyl-CoA	-
Isovaleryl CoA dehydrogenase	43.2	Isovaleryl-CoA	-
Pyrroline-5-carboxylate reductase	30.1	L-proline	NAD(P)H
Predicted OYE	41.1	N-ethylmaleimide	NAD(P)H
2,4-dienoyl-CoA reductase	73.5	2,4-dienoyl-CoA	NADH

A-FabI was chosen for cloning and activity test. *A*-FabI gene was amplified with PCR using the primers as shown below.

A-FabI-S (NdeI): TTTCATATGGCACAAGGACTATTGGCAGG
A-FabI-A (XhoI): GGGCTCGAGCTATTCGCTGTCCATCATTGAC

The PCR product was purified and treated with NdeI and XhoI. The digested PCR products were purified and ligated to predigested pET-28a plasmid using the same restriction enzymes. A His-tag was fused to the 5'-terminal of the *A*-FabI gene. The recombinant plasmid was transferred to *E. coli* (BL21, DE3) competent cells and spread on LB agar plate containing 50 μg/mL kanamycin. The plate was cultivated at 37 ºC for 12 h. The clones were picked up and grown in LB medium at 37 ºC for 12 h. Plasmid was extracted from the cell culture for gene sequencing, and the cloned *A*-FabI gene was confirmed. The obtained recombinant is named as *E. coli* (*A*-FabI).

5. Purification of *A*-FabI from *E. coli* (*A*-FabI)

E. coli (*A*-FabI) recombinant was inoculated to 200 mL TB medium. The cell cultivation was conducted at 37 ºC and 250 rpm, and monitored by OD measurement at 600nm. After 2 h (OD_{600} of 0.6), IPTG was added to 0.1 mM to induce the expression of *A*-FabI. Afterwards the cells were grown for 12 h and then harvested by centrifugation. The cell pellets were resuspended in 50mM of phosphate buffer (pH 7.5) and disrupted using APV-1000 homogenizer. The cell-free extract (CFE) was obtained by centrifugation of the lysate at 15,000 g and 4 ºC for 10 min. The CFE was filtered through 0.2 μm PTFE membrane and subsequently loaded to HisPrep FF 16/10 affinity column (column volume, CV=20 mL). The non-his-tagged proteins were removed by flushing the column with 3 CVs of phosphate buffer (pH 7.0) containing 30 mM imidazole. Final elution was accomplished with 5 CVs of 300 mM imidazole and resulted in 30mL of protein

solution (2 mg protein/mL). The *A*-FabI accounted for more than 95% of the whole protein in the solution based on the SDS-PAGE (Fig. S3).

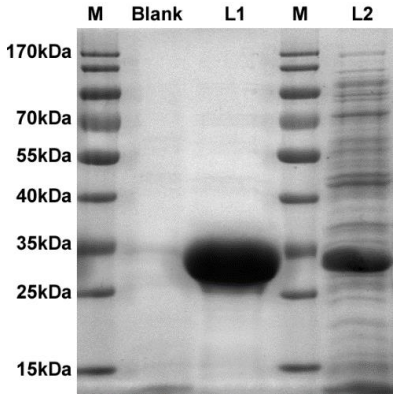

Figure S3. SDS-PAGE of purified *A*-FabI protein. L1: purified A-FabI; L2: cell-free extract of *E. coli* (*A*-FabI). M: marker.

6. Cloning of *E*-FabI reductase from *E. coli* and construction of *E. coli* (*E*-FabI)

The protein sequence of *A*-FabI enzyme was used to identify homologous reductase with blast search within the released *E. coli*'s genome information (GeneBank: U00096). *Escherichia coli* FabI reductase (*E*-FabI) was founded to share 60% of sequence identify with *A*-FabI (Fig. S4).

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A-FabI  GLLAGKRFLIAGIASKLSIAFGIAQALHREGAELAFTYPNDKLKKRVDDFAEQFGSTLVF
        G L+GKR L+ G+ASKLSIA+GIAQA+HREGAELAFTY NDKLK RV++FA Q GS +V
E-FabI  GFLSGKRILVTGVASKLSIAYGIAQAMHREGAELAFTYQNDKLKGRVEEFAAQLGSDIVL

A-FabI  PCDVAVDAEIDHAFAELAKHWDGLDGVVHSIGFAPAHTLDGDFTDVTDREGFKIAHDISA
         CDVA DA ID  FAEL K W   DG VHSIGFAP   LDGD+ +   REGFKIAHDIS+
E-FabI  QCDVAEDASIDTMFAELGKVWPKFDGFVHSIGFAPGDQLDGDYVNAVTREGFKIAHDISS

A-FabI  YSFVAMARAAKPLLLARQGCLLTLTYQGSESVMPNYNVMGMAKASLEAGVRYLASSLGAE
        YSFVAMA+A +  +L     LLTL+Y G+E  +PNYNVMG+AKASLEA VRY+A+++G E
E-FabI  YSFVAMAKACRS-MLNPGSALLTLSYLGAERAIPNYNVMGLAKASLEANVRYMANAMGPE

A-FabI  GIRVNAISAGPIRTLAASGIKSFRKMLDANEKIAPLKRNVTIEDVGNAALFLCSPWANGI
        G+RVNAISAGPIRTLAASGIK FRKML   E + P++R VTIEDVGN+A FLCS  + GI
E-FabI  GVRVNAISAGPIRTLAASGIKDFRKMLAHCEAVTPIRRTVTIEDVGNSAAFLCSDLSAGI

A-FabI  TGEILYVDAGFNTVGMSQ
        +GE+++VD GF+   M++
E-FabI  SGEVVHVDGGFSIAAMNE
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Figure S4. Sequence alignment of *A*-FabI and *E*-FabI reductase. The alphabet between the 2 protein sequences indicates identical amino acids. '+' denotes the residue of similar property and the blank indicates a mismatch.

To clone *E*-FabI, the gene was amplified with the following primers:

E-FabI-S (NdeI): TTTCATATGGGTTTTCTTTCCGGTAAGCGCA
E-FabI-A (XhoI): TTTCTCGAGTTATTTCAGTTCGAGTTCGTTC

The PCR product was purified, digested and ligated to the predigested pET-28a plasmid. A His-tag was introduced to the 5' end of the gene to facilitate the follow-up purification of the enzyme. The recombinant plasmid was transferred to *E. coli* (BL21, DE3) competent cells and cultivated at 37 ºC for 12 h on a LB agar plate supplemented with kanamycin (50 μg/mL). The obtained colonies were cultivated in LB medium at 37 ºC for 12h. The plasmid was then extracted from the cell culture and submitted to gene sequencing to confirm the success of the cloning. The obtained *E. coli* recombinant strain over-expressing *E*-FabI is named as *E. coli* (*E*-FabI).

7. Purification of *E*-FabI from *E. coli* (*E*-FabI)

The cells of *E. coli* (E-FabI) were grown by using the same procedure as described for *E. coli* (*A*-FabI). The cells were harvested and disrupted. The cell-lysate was centrifuged at 15,000 g for 10 min to give CFE. His-tagged *E*-FabI protein in the CFE was purified by affinity chromatography using the same protocol as described for the purification of *A*-FabI, giving L1 fraction with 4 mg protein/mL. *E*-FabI protein accounted for 70% based on the SDS-PAGE (Fig. S5).

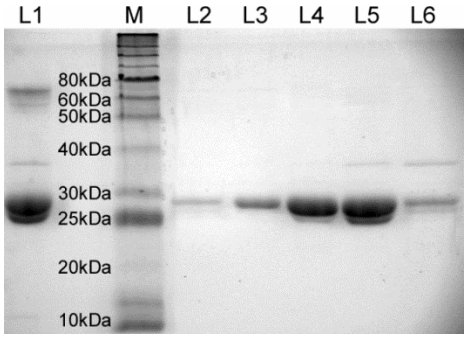

Figure S5. SDS-PAGE of purified *E*-FabI protein. L1: elution of affinity chromatography; L2-L6: five fractions by gel filtration chromatography.

To further improve the purity of *E*-FabI, 2 mL L1 fraction (Fig. S5) was loaded to a Superdex 10/300 GL column. The elution was performed with 1.5 CVs of 20 mM phosphate buffer containing 100 mM NaCl. Fractions were collected in 1mL volume and totally 5 fractions were collected. As shown in the Fig. S5, highly pure *E*-FabI (95% of purity, 2.3 mg protein/mL) was obtained from L4 fraction of the gel filtration purification.

8. Activity assay of the purified *A*-FabI and *E*-FabI for the reduction of 2-hexylidene-cyclopentnone 1b, respectively

The purified FabI proteins were tested for the reduction of the substrate **1b** (Table S5). The catalysis was conducted in 1 mL Tris buffer containing 1 mg/mL of FabI enzyme, 6 mM **1b** and 10 mM NADPH. The reaction mixture was incubated at 30 ºC and 250 rpm for 30 min, followed by GC analysis. The results are summarized in Table S5.

Table S5. Activity of the purified protein FabIs and partially purified reductase from *Acinetobacter* sp. RS1

Protein	Conc. (mg protein/mL)	Substrate	Cofactor	Time (min)	Prod.	Spec. Act. (U/g protein)	*ee* (%)
Purified *A*-FabI [a]	2	**1b**	NADPH	30	(*R*)-**2b**	300	88
Purified *E*-FabI [a]	2.3	**1b**	NADPH	30	(*R*)-**2b**	700	69
Purified reductase from RS1 strain [b]	0.1	**1b**	NADPH	30	(*R*)-**2b**	110	83

a. Reaction was performed with 1 mL Tris buffer containing 1 mg/mL enzyme, 6 mM **1b** and 10 mM NADPH at 30 ºC and 250 rpm for 30 min.

b. Reaction was performed with 1 mL Tris buffer containing 0.1 mg/mL enzyme, 6 mM **1b** and 10 mM NADPH at 30 ºC and 250 rpm for 30 min.

9. Cofactor dependency of *A*-FabI and *E*-FabI-catalyzed reductions, respectively

The cofactor dependency of the FabI enzymes for the reduction of 2-alkylidene-cyclopentanones **1a-c** was tested in the presence of NADH or NADPH. 1 mL of Tris buffer (50

mM, pH 7.5) containing *E*-FabI (0.3 mg protein/mL) or *A*-FabI (1 mg protein/mL), 5-10 mM substrate and excess amount of the cofactor was shaken at 30°C and 250 rpm for 10min. The product formation was analyzed by GC. As shown in Table S6, both *E*-FabI and *A*-FabI accepted NADH and NADPH for the reduction of **1a-c**.

Table S6. Cofactor dependency of FabI in the reduction of 2-alkylidene-cyclopentanones **1a-c**

FabI [a]	Enzyme Conc. (mg/mL)	Substrate	Cofactor	Time (min)	Conv. (%)
E-FabI	0.3	**1a** (5 mM)	NADH (10 mM)	10	73
E-FabI	0.3	**1a** (5 mM)	NADPH (10 mM)	10	75
E-FabI	0.3	**1b** (6 mM)	NADH (10 mM)	10	88
E-FabI	0.3	**1b** (6 mM)	NADPH (10 mM)	10	90
E-FabI	0.3	**1c** (7 mM)	NADH (10 mM)	10	36
E-FabI	0.3	**1c** (7 mM)	NADPH (10 mM)	10	35
A-FabI	1	**1a** (10 mM)	NADH (15 mM)	60	63
A-FabI	1	**1a** (10 mM)	NADPH (15 mM)	60	82
A-FabI	1	**1b** (10 mM)	NADH (15 mM)	60	61
A-FabI	1	**1b** (10 mM)	NADPH (15 mM)	60	67

[a] The reaction was conducted in 1 mL Tris buffer (50 mM, pH 7.5) at 30 °C and 250 rpm for 10 min.

10. Bioreduction of 1a-d with *A*-FabI and *E*-FabI, respectively

The purified *A*-FabI and *E*-FabI were further tested for the bioreduction of 2-alkylidene-cyclopentanones **1a-d** at different substrate concentrations (10-50 mM). The catalysis was conducted in 3 mL Tris buffer (5 0mM, pH7.5) containing 3 mg purified FabI as well as substrate and NADH at a molar ratio of 1:1.5. The reaction mixture was incubated at 30 ºC and 300 rpm for 1h, and the product formation was analyzed by GC. The results are summarized in Table S7.

Table S7. Reduction of **1a-d** with the purified *A*-FabI and *E*-FabI.

E-FabI [a]							
Substrate	Conv. (%)	Substrate	Conv. (%)	Substrate	Conv. (%)	Substrate	Conv. (%)
1a (10mM)	100	**1b** (10mM)	100	**1c** (7mM)	100	**1d** (10mM)	100
1a (20mM)	100	**1b** (20mM)	100	**1c** (15mM)	100	**1d** (20mM)	45
1a (30mM)	100	**1b** (30mM)	100	**1c** (30mM)	53	**1d** (30mM)	39
1a (40mM)	100	**1b** (40mM)	100	**1c** (40mM)	44	**1d** (40mM)	9
1a (50mM)	72	**1b** (50mM)	81	**1c** (80mM)	15	**1d** (50mM)	3
1a (75mM)	53	**1b** (75mM)	41				
1a (100mM)	39	**1b** (100mM)	33				
A-FabI [a]							
1a (10mM)	63	**1b** (10mM)	61	**1c** (10mM)	100	**1d** (5mM)	81
1a (20mM)	31	**1b** (20mM)	45	**1c** (20mM)	75	**1d** (10mM)	37
1a (30mM)	28	**1b** (30mM)	31				

[a] Reaction was performed in 3 mL Tris buffer (50mM, pH7.5) containing 3mg purified FabI as well as substrate and NADH in a molar ratio of 1:1.5 at 30 ºC and 300 rpm for 1h.

11. Determination of the kinetics of *E*-FabI towards the reduction of 1a-c

The kinetics of *E*-FabI was determined and the kinetics data were obtained by using Lineweaver-Burk plot. The assay was performed at room temperature with 3 mL 30 mM PIPES containing 150 mM NaCl, 0.5 mM NADH, 7 mg protein and different amount of the substrate. The initial catalytic rate was determined by on-line monitoring the consumption of NADH at UV of 339 nm during the reactions. The determined kinetics data are summarized in Table S8.

Table S8. Kinetics data of *E*-FabI towards the reduction of **1a-c**

Sub.	Kinetics			
	K_m (μM)	k_{cat} (s^{-1})	V_{max} (U mg^{-1})	K_{cat}/K_m ($s^{-1}M^{-1}$)
1a	49	2.2	4.3	4.5×10^4
1b	31	2.0	4.0	6.5×10^4
1c	46	1.2	2.4	2.6×10^4

12. Construction of *E. coli* (*E*-FabI-GDH) coexpressing *E*-FabI and GDH

The GDH gene from *B. subtilis* was amplified using the following primers.

GDH-S (BamHI/NdeI):GGG(GGATCC/CATATG)ATGTATCCGGATTTAAAAGGAA

GDH-A (XhoI):AAACTCGAGTTAACCGCGGCCTGCCTG

To clone the gene into different plasmids, the restriction site of BamHI or NdeI was introduced into the sense primer; the restriction site of XhoI was engineered into the anti-sense primer. The PCR product was purified and digested with BamHI-XhoI or NdeI-XhoI. The digested PCR products were purified and ligated to the cloning plasmid (pRSF-Duet, pCDF-Duet or pET-28a) and transferred to *E. coli* (BL21, DE3) competent cells to give the *E. coli* (GDH) recombinants (recombinant 1, 2 and 7).

The recombinant 5 was obtained by transferring pCDF-Duet-GDH recombinant into the constructed *E. coli* (*E*-FabI) competent cell.

For the construction of recombinant 4 and 6, the *E*-FabI gene was amplified with the following primers.

E-FabI-S (BamHI): TTTGGATCC GGTTTTCTTTCCGGTAAGCGCA
E-FabI-A (XhoI): TTTCTCGAGTTATTTCAGTTCGAGTTCGTTC

The gene was cloned to pRSF-Duet and pCDF-Duet plasmid, respectively using the same procedures as described in the section 6. The pRSF-*E*-FabI plasmid was extracted from the cell culture of the recombinant 4, digested with NdeI and XhoI, ligated with the digested GDH gene and transformed into the E. coli BL21 (DE3) competent cell to give recombinant 8.

To assess the catalytic activity of different recombinant, each constructed strain was cultivated in 50 mL TB medium at 37 °C for 12 h. IPTG (0.1 mM) was added to induce the protein expression. The cells were subsequently harvested and the cell pellet was suspended in Tris buffer (50 mM,

pH7.5) to a density of 10 g cdw/L for the reduction of **1a**. The catalysis was conducted with 1mL of cell suspension containing 20 mg/mL glucose and 40 mM **1a**, and the conversion was determined by GC analysis.

As shown in Table S9, the recombinant 8 co-expressing both FabI and GDH showed highest conversion. The recombinant was hence chosen and hereafter referred as *E. coli* (*E*-FabI-GDH).

Table S9. Construction of recombinant *E*-FabI and GDH and bioreduction of **1a** with the resting cell of the recombinants

Recomb.	*E*-FabI [a]	GDH [a]	Expression	His-tag	Conv. of **1a** (40mM)[b]
1	-	pRSF-GDH(BX) [a]	+	+	29
2	-	pCDF-GDH(BX)	+	+	34
3	pET-28-*E*-FabI(NX)	-	+	+	49
4	pRSF- *E*-FabI (BH)	-	+	-	55
5	pET-28- *E*-FabI (NX)	pCDF-GDH(BX)	+	+	82
6	pCDF- *E*-FabI (BH)	-	+	-	60
7	-	pRSF-GDH(NX)	+	+	N.D.
8	pRSF- *E*-FabI (BH)-GDH(NX)	-	+	+	100

a. The bracketed letters denote the restriction enzymes used for cloning. B, *BamH*I; H, *Hind*III, N, *Nde*I; X, *Xho*I. N.D., not determined.

b. The catalysis was conducted with 1 mL Tris buffer containing 10 g cdw/L of cells, 20 mg/mL of glucose and 40 mM **1a** at 30 °C and 250 rpm for 3 h.

The SDS-PAGE of the CFE of the constructed recombinants was shown in Fig. S6. Both recombinants 5 and 8 coexpressed *E*-FabI and GDH.

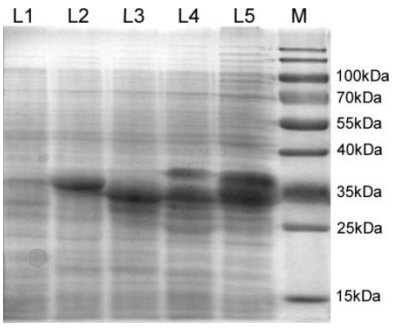

Figure S6. SDS-PAGE of constructed *E*-FabI and GDH recombinants. GDH (33 kDa) is slightly larger than *E*-FabI (30 kDa). L1, *E. coli* BL21 (DE3); L2, recombinant 2; L3, recombinant 3; L4, recombinant 5; L5, recombinant 8.

13. Growth curve and specific activity of *E. coli* (*E*-FabI-GDH)

The cells of *E. coli* (*E*-FabI-GDH) were inoculated into 100 mL TB medium in a 500 mL shaking flask containing kanamycin (50 μg/mL) and cultivated at 37 °C and 250 rpm for 24 h. IPTG (0.1 mM) was added at the 5 h to induce the expression of FabI and GDH. Afterwards 1 mL culture was withdrawn every hour for the OD measurement at 600nm. From early exponential phase to stationary phase, cells were also taken for the activity test through whole cell biotransformation. Biotransformation was performed at cell density of 10g cdw/L in 50 mM of Tris buffer (pH 7.5) containing 6 mM of **1a-b** and 20 mg/mL of glucose at 30 °C and 250 rpm for 1 h. The results were analyzed by using GC, and the catalytic activity was calculated for the first 30min of bioreduction. The growth curve and specific activity of the *E. coli* (*E*-FabI-GDH) were shown in Fig. S7.

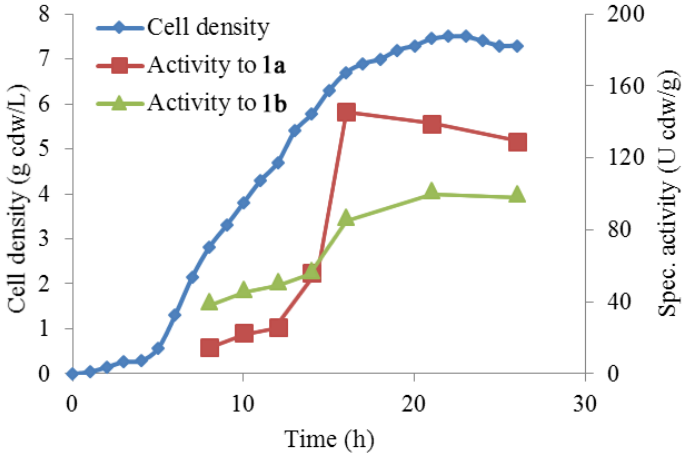

Figure S7. Time course of cell growth and curve of *E. coli* (*E*-FabI-GDH) in TB medium and the succession of the specific activities towards the **1a-b**.

14. One-pot sequential cascade biotransformation of 1a-d with the resting cells of *E. coli* (*E*-FabI-GDH) and *E. coli* (CHMO-GDH)

The one-pot cascade biotransformation of **1a-d** was started with 5mL Tris buffer (50mM, pH7.5) containing 20g cdw/L of *E. coli* (*E*-FabI-GDH), 20 mg/mL of glucose and 40mM **1a-c** or 30mM **1d** at 30°C and 250rpm for 1 h (for **1a-c**) or 3 h (for **1d)**. During the biotransformation, 150 μL reaction mixture was taken and the concentration of the substrates **1a-d** and products **2a-d** was determined by GC analysis. After finishing the reduction, the pH of the reaction mixture was adjusted to 7.5 and 1-5 mL of Tris buffer containing 8 g cdw/L of *E. coli* (CHMO-GDH) cells and 20 mg/mL of glucose were added to the reaction mixture. The oxidation was conducted at 30°C and 250rpm for 1 h, and 200 μL reaction mixture was taken every 15min for the determination of the concentration and *ee* of **2a-d** by GC and Chiral GC analysis. The time courses of the one-pot cascade biotransformation of **1a-d** were shown in Fig. S8.

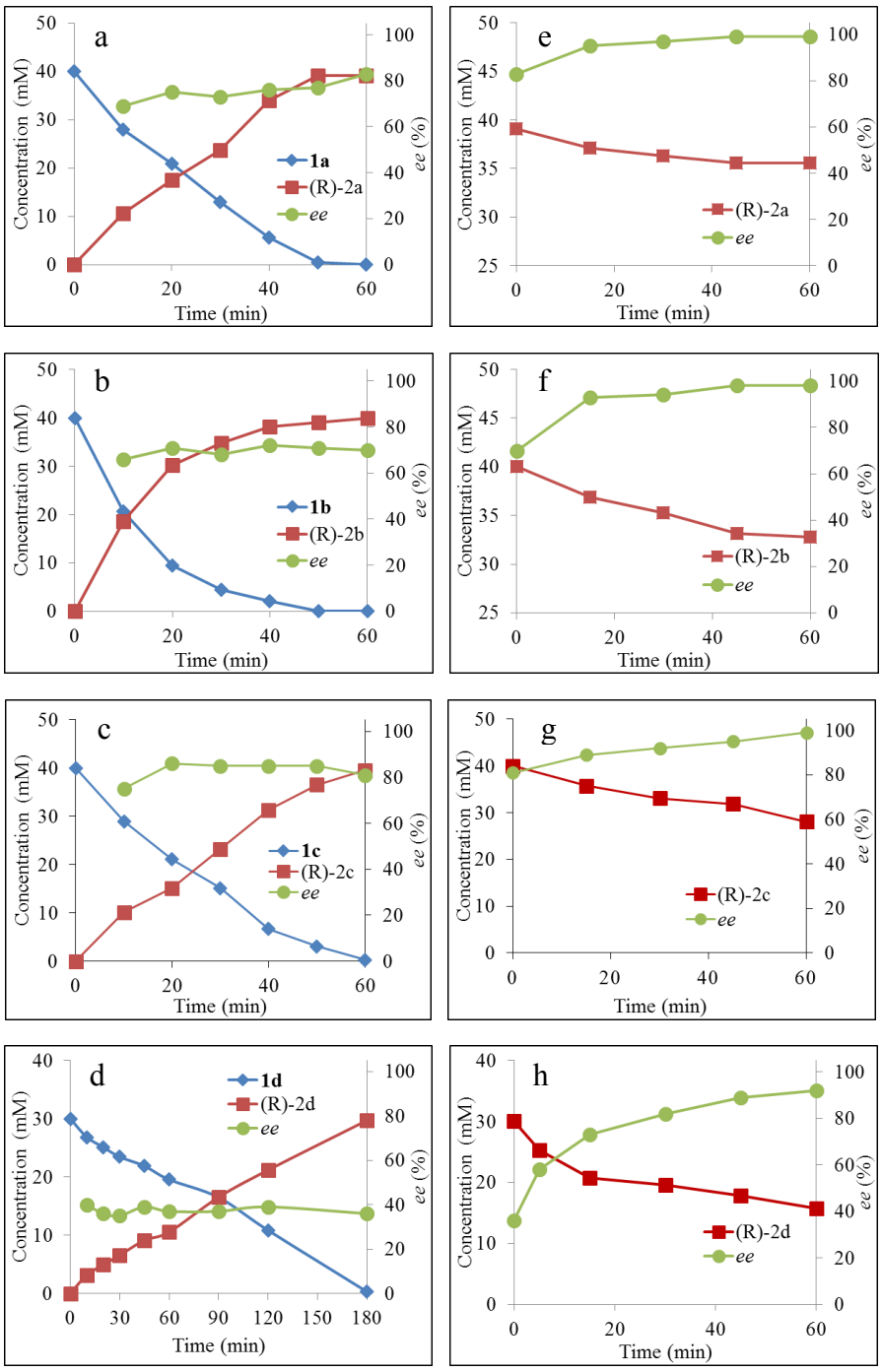

Figure S8. Time courses of the one-pot cascade biotransformation of **1a-d** for the preparation of (*R*)-**2a-d**. (a-d) Bioreduction of **1a-d** to (*R*)-**2a-d** with *E. coli* (*E*-FabI-GDH). (e-h) *S*-enantioselective Baeyer-Villiger oxidation of **2a-d** with *E. coli* (CHMO-GDH).

15. Chiral HPLC chromatograms of (*R*)-2a-d produced from the cascade biotransformation of 1a-d

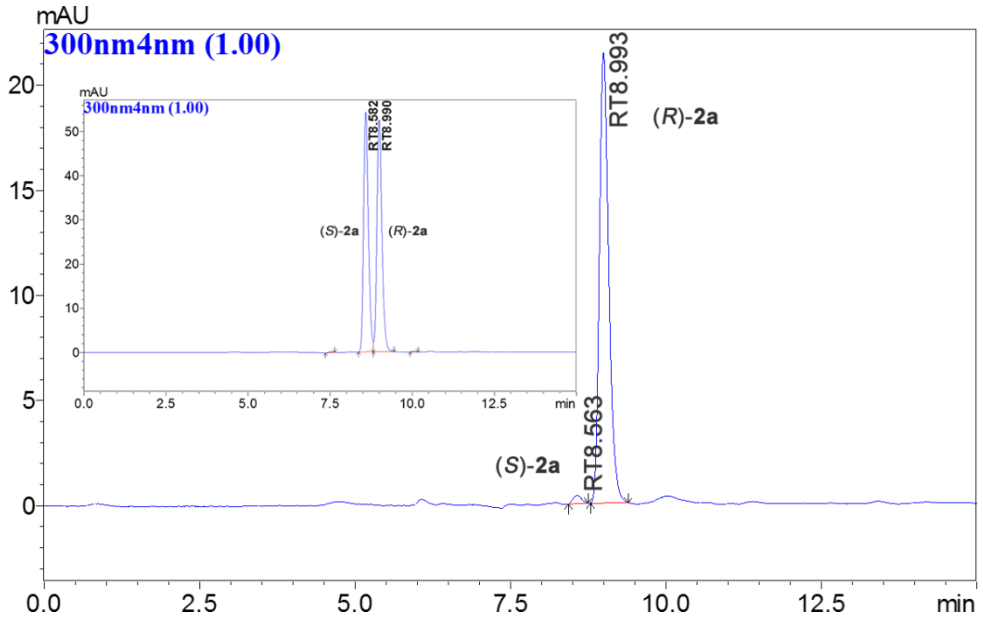

Figure S9. Chiral HPLC chromatogram of the produced (*R*)-**2a** from the cascade biotransformation of **1a** with *E. coli* (*E*-FabI-GDH) and *E. coli* (CHMO-GDH).

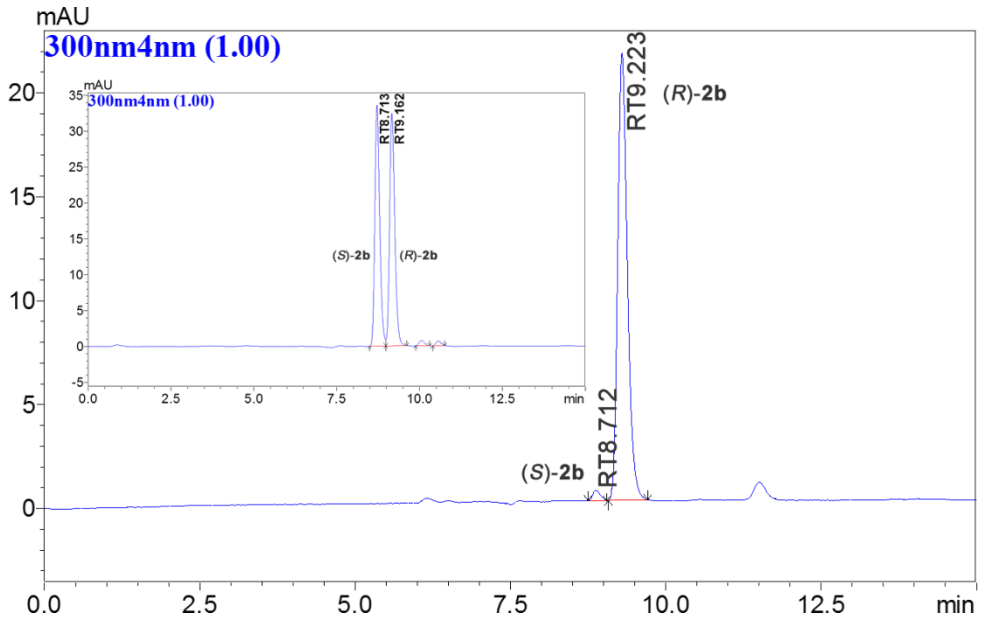

Figure S10. Chiral HPLC chromatogram of the produced (*R*)-**2b** from the cascade biotransformation of **1b** with *E. coli* (*E*-FabI-GDH) and *E. coli* (CHMO-GDH).

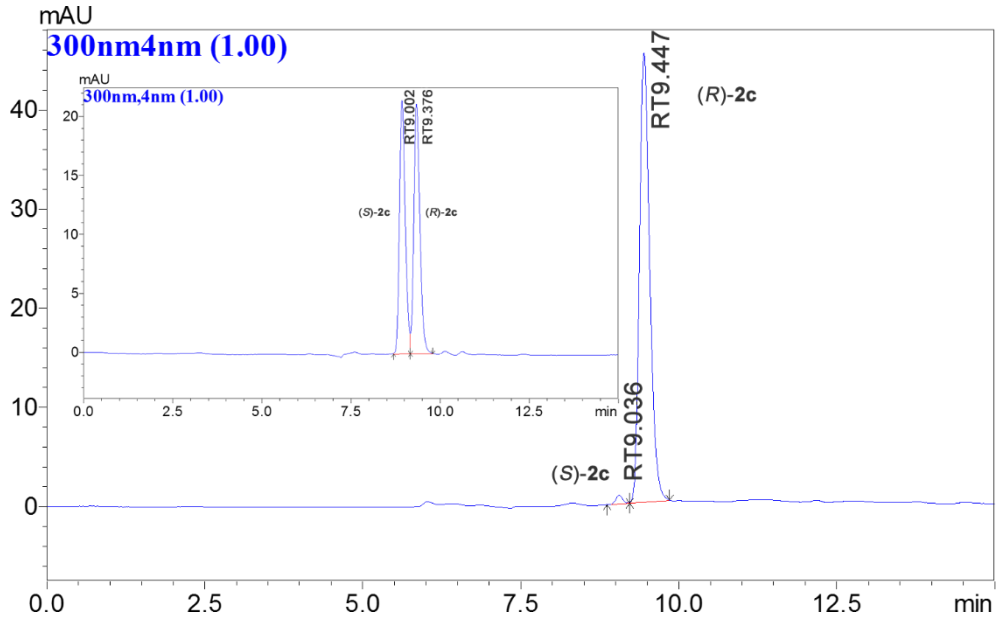

Figure S11. Chiral HPLC chromatogram of the produced (*R*)-**2c** from the cascade biotransformation of **1c** with *E. coli* (*E*-FabI-GDH) and *E. coli* (CHMO-GDH).

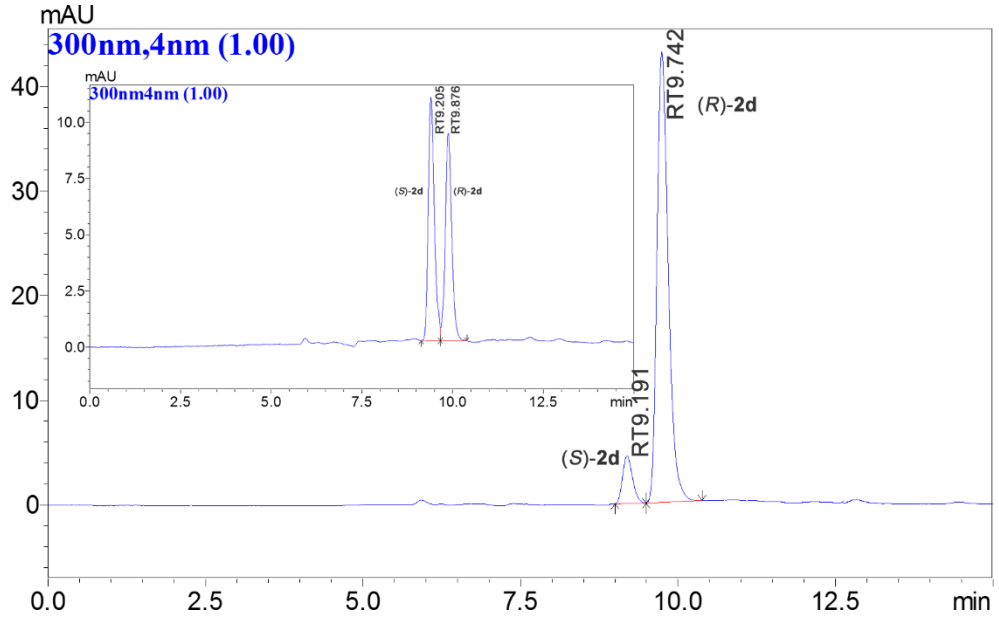

Figure S12. Chiral HPLC chromatogram of the produced (*R*)-**2d** from the cascade biotransformation of **1d** with *E. coli* (*E*-FabI-GDH) and *E. coli* (CHMO-GDH).

16. Molecular docking of 1a-c in *A*-FabI and *E*-FabI

Docking of substrate **1a-c** in A-Fab I or *E*-FabI was accomplished with Autodock Vina software. The structure of *E*-FabI was obtained from PDB database (1MFP, 2.33Å). Water and other non-protein components were removed from the X-ray structure except the co-crystalized NAD^+ molecule. The structure of *A*-FabI was generated by homology modeling using *E*-FabI structure (Swiss-Model, swissmodel.expasy.org). Approximately 20 docking poses were generated for each docking prediction. The most possible catalytic active pose was then screened out with the following geometric criteria 1) the distance for the hydride transferring from the cofactor to the C_β carbon is smaller than 5 Å and 2) hydrogen bond (H-bond) is formed between substrate and the catalytic tyrosine (*E*-FabI, Tyr146 and Tyr156; A-FabI, Tyr149 and Tyr159).

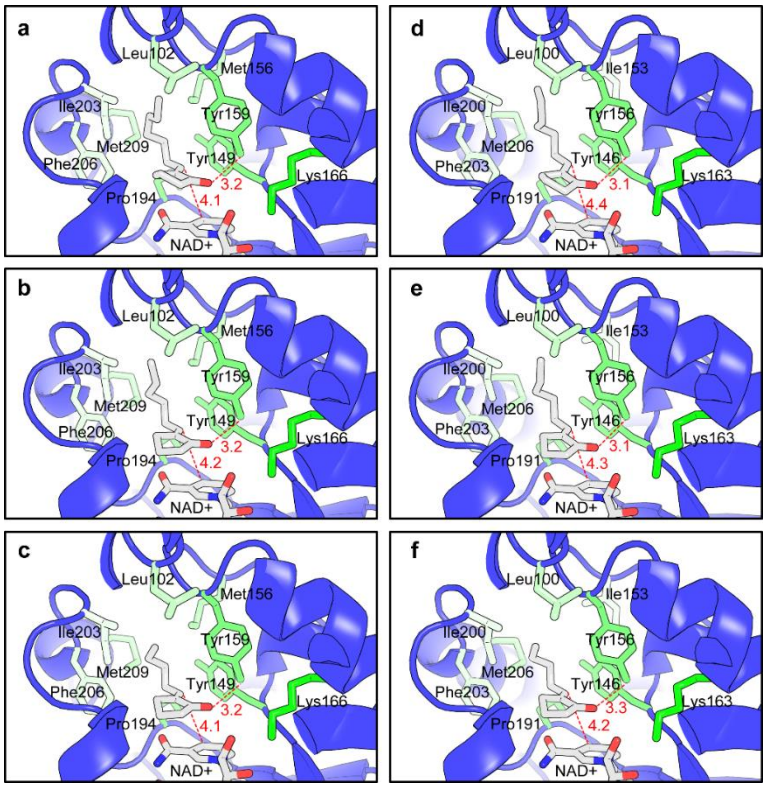

Figure S13. Predicted active docking pose of 2-alkylidene-cyclopentanones **1a-c** in *A*-FabI (a-c) and *E*-FabI (d-f).

Similar active binding pose was predicted with **1a-c** in both FabI enzymes (Fig. S13). The substrates form a 'U-shape pose' with C-C double bond in parallel to the nicotinamide ring of the cofactor. The side chain of the substrate is buried into a hydrophobic cluster consisting of hydrophobic residues from the adjacent loop structures. The distance for hydride transfer is between 4.1 Å ~4.4 Å. H-bond is formed between the carbonyl group of the substrate and Tyr156; while Tyr146 falls aside of the carbonyl group leading to less potential for an H-bond. In all of the docking case, the active pose shown in Fig. S13 was the one with lowest docking energy.

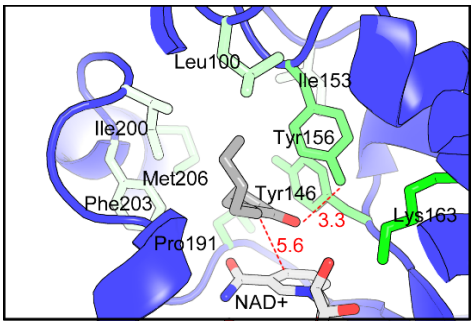

Figure S14. Predicted docking pose of 2-pentyl-cyclopentanones **1c** in *E*-FabI that would lead to the formation of (*S*)-product.

No other alternative active pose was predicted in all docking cases. However, a substrate binding pose obtained with **1c** in *E*-FabI would lead to the formation of (*S*)-product in the bioreduction (Fig. S14). In this pose, the substrate flips over at the axis of the carbonyl group so the side chain is orientating outwards the binding pocket, similar to the recorded 'flipped binding' in PETN reductase.[S2] A H-bond is formed between the carbonyl group of the substrate and the catalytic Tyr156. Following the *cis*-addition mechanism, this pose would give (*S*)-product. However the distance for the hydride transferring from the cofactor to substrate is 5.6 Å, indicating a non-active substrate pose.

References

[S1] J. Liu and Z. Li, *ACS Catal.*, 2013, **3**, 908-911.

[S2] H. S. Toogood, J. M. Gardiner and N. S. Scrutton, *ChemCatChem*, 2010, **2**, 892-914.