Supplementary Information

A highly efficient designer cell for enantioselective reduction of ketones

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1. Demonstration of the expression of *surf-crs* on the surface of designer cell

The recombinant cells grown in 100 mL culture media were isolated by centrifugation and washed with 50 mM phosphate buffer (pH- 7.0). The cells were then suspended in 5 mL lysis buffer (50 mM NaH_2PO_4, 150 mM NaCl, 1mg mL^{-1} Lysozyme, pH 8.0) for 30 min at 4 °C. The cell suspension was sonicated with 30 sec pulse on and 30 sec pulse off at 4 °C for 20 min. The cell debris was removed by centrifugation at 14,000 rpm for 30 min. The supernatant (cell-free extract) was then subjected to ultra-centrifugation at 1,00,000*g* for 2 h at 4 °C for separation of membrane fraction and soluble fraction. The sediment containing the membrane fraction was washed with the same buffer and re-suspended in membrane solublization buffer (25 mM Tris HCl, 20% Glycerol and 2% Triton X100, pH 7.5). All three fractions, cell-free extract, membrane fraction and soluble protein fraction were assayed for their activity using ethyl 4-chloro-3-oxobutyrate (ECOB) (**1a**) as substrate. In brief, the reaction mixture (1 mL) in 50 mM phosphate buffer pH 7.0, containing 0.2 mM NADPH, 2.0 mM **1a** and 1-50 µl of the sample was incubated at 30 ^{o}C and the total activity was determined.[1] The results have been summarized in Table 1S. As expected, most of the activity was recovered from membrane fraction. Significantly, membrane fraction of the *E. coli* BL21 (DE3) + *pET23(a)* (negative control) was devoid of any activity.

Table 1S: Carbonyl reductase (crs) activity of various fractions obtained from *E. coli* BL21(DE3) + *pET23(a)* and *E. coli* BL21 + *pET 23(a)-omp-crs*.

Entry	**Fractions of *E. coli***	***E. coli* BL21(DE3) + *pET 23(a)*, (negative control) Total activity (nmol/min)**	***E. coli* BL21(DE3) + *pET 23(a)-omp-crs*, Total activity (nmol/min)**
1	Cell-free extract	376	1673
2	Soluble fraction	76	350
3	Membrane fraction	0.0	1192

The presence of crs on the surface of *E. coli* BL21(DE3) + *pET 23(a)-omp-crs* was further confirmed by EM immunogold labeling studies carried out with ultrathin sections of expressed *E. coli* BL21(DE3) + *pET 23(a)-omp-crs* cells as described in detail in Experimental Section. Briefly, *anti-crs* polyclonal antibody was raised against the purified crs in rabbit and was assayed for their specificity by Western blotting. The purified crs was run on SDS-PAGE under the reducing condition and after electro-blotting on to nitrocellulose

membrane was probed with rabbit anti-*crs* polyclonal antibody which was further probed with alkaline phosphatase conjugated goat *anti-rabbit IgG* (whole molecule) secondary antibody. The blot was then developed by dipping in the substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP, 0.15 mg/mL), nitro blue tetrazolium (NBT, 0.30 mg/mL), tris-HCl (100 mM) and $MgCl_2$ (5 mM), pH 9.5 for 10 min.[1, 2] The polyclonal antibody that specifically labeled pure crs (Lane 2, Figure 1S) was also able to specifically label omp-crs (Lane 4, Figure 1S).

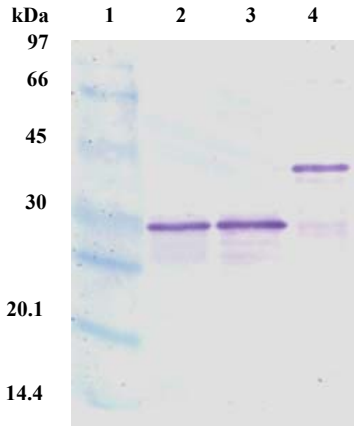


Figure 1S. **Specificity of *anti-crs* Ab against *crs* by Western blotting.** Lane 1: Molecular weight marker, Lane 2- Purified crs, Lane 3- Whole cell proteome of *E. coli* BL21(DE3) + *pET23(a)-crs*, Lane 4- Whole cell proteome of *E. coli* BL21(DE3) + *pET23(a)-omp-crs*.

After several dehydration steps, cells of recombinant *E. coli* BL21(DE3) + *pET 23(a)* (negative control) and *E. coli BL21(DE3) + pET 23(a)-omp-crs* were embedded in LR white resin, which was then dehydrated in several steps using 0.2% glutaraldehyde as fixative. Thin sections cut using an ultramicrotome were incubated with rabbit anti-*crs* polyclonal antibody followed by nanogold-labeled goat *anti-rabbit IgG* (whole molecule) secondary antibody and visualized under the transmission electron microscope. Different fields were observed and the gold particles were found to be exclusively present on the surface of the cells (Figure 2S b-c). No labeling occurred with cells used as the negative control (Figure 2S a).

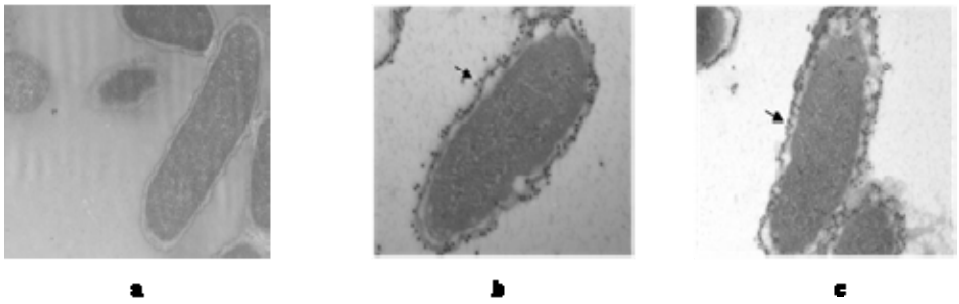


Figure 2S. **Transmission electron micrograph of 'Designer whole-cell biocatalyst'**. 'Designer whole-cell biocatalyst' was probed with rabbit anti-*crs* polyclonal antibody followed by nanogold labeled goat *anti-rabbit IgG* (whole molecule) secondary antibody. Arrowheads denote gold particles. (a) *E. coli* BL21(DE3) + *pET 23(a) (negative control)*, (b) & (c) *E. coli* BL21(DE3) + *pET 23(a)-omp-crs*.

2. Relative expression levels of cytoplasmic and surface expressed crs in recombinant *E. coli*

Fresh culture of recombinant *E. coli* BL21(DE3) + *pET 23(a)* (negative control), *E. coli BL21(DE3) + pET 23(a)-crs* and *E. coli BL21(DE3) + pET 23(a)-omp-crs* were grown and the whole cell proteome was run on 12.5% SDS-PAGE under reducing condition (Figure 3S). To the naked eye, the intracellular expression of crs (Lane 1, Figure 3S) appeared to be much higher than the surface expressed *crs* **(**Lane 9, Figure 3S). There were no expression in induced (Lane 3 & 7, Figure 3S) and uninduced (Lane 4 & 6, Figure 3S) *E. coli* BL21(DE3) + *pET 23(a)* (negative control). For quantitative determination, the proteome obtained from various concentrations of cells was run on 12.5% SDS-PAGE under reducing conditions. After electro blotting on to nitrocellulose membrane, it was probed with rabbit *anti-crs* polyclonal antibody, which was further probed with alkaline phosphatase conjugated goat-*anti rabbit IgG* antibody. Probing with antibody was followed by developing the blot by dipping in the substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP, 0.15 mg mL^{-1}), nitro blue tetrazolium (NBT, 0.30 mg mL^{-1}), Tris HCl (100 mM) and $MgCl_2$ (5 mM), pH 9.5 for 10 min (Figure 4S).[2] The expression of the crs was determined by analyzing the band intensity by software Scion Image of the corresponding clone. The intensity was plotted against amount of the cells taken and the slope (dy/dx) for the intracellular expression (Figure 5S a) and surface expression (Figure 5S b) compared. The expression of crs on the surface of the cells as omp-crs fusion protein was found to be 17.9-fold less as compared to crs expressed in the cytoplasm.

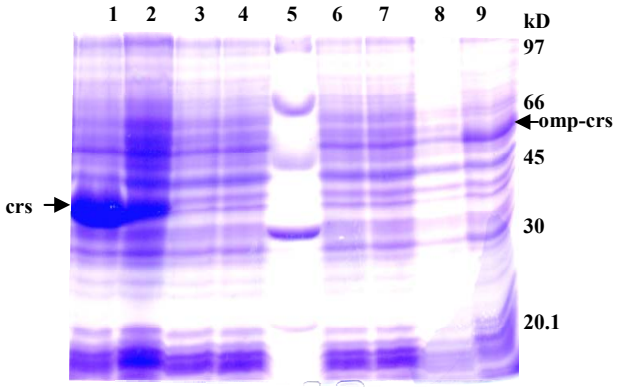


Figure 3S

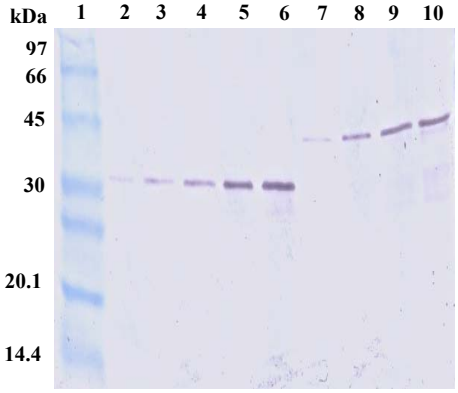
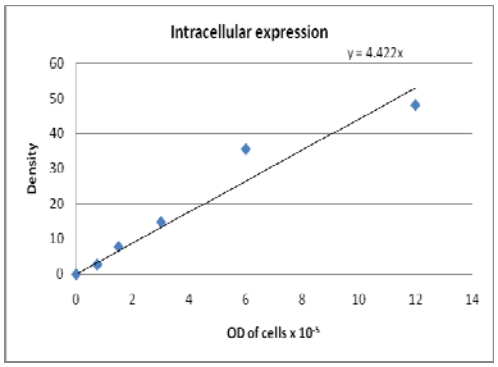


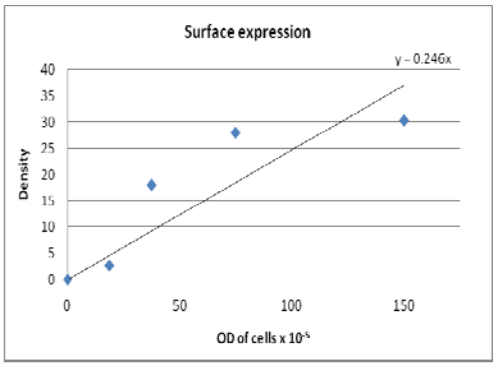
Figure 4S

Figure 3S. **Expression level of crs.** Lane 1- Induced *E.coli* BL21(DE3) + *pET 23(a) –crs*, Lane 2- Uninduced *E.coli* BL21(DE3) + *pET 23(a) -crs*, Lane 3 & 7- Induced *E.coli* BL21(DE3) + *pET 23(a)*, Lane 4 & 6- Uninduced *E.coli* BL21(DE3) + *pET 23(a)*, Lane 5- Molecular weight marker, Lane 8- Uninduced *E.coli* BL21 + *pET 23(a) –omp-crs*, Lane 9- Induced *E.coli* BL21 + *pET 23(a)–omp-crs*.

Figure 4S. **Quantitative expression level of crs.** Lane 1-Molecular weight marker, Lane 2 to 6- cell free extract from 0.75, 1.5, 3, 6 and 12 x 10^{-5} OD cells of *E. coli* BL21(DE3) + *pET23(a)-crs*, Lane 7 to 10- cell free extract from 18.75, 37.5, 75 and 150 x 10^{-5} OD cells of *E. coli* BL21(DE3) + *pET23(a)-omp-crs*.



a



b

Figure 5S. **Quantification of crs expression level.** Intensity of the bands in Figure 16 was determined by Scion Image and plotted against optical density of the cell. (a) *E. coli* BL21(DE3) + *pET23(a)-crs* and (b) *E. coli* BL21(DE3) + *pET23(a)-omp-crs*.

3. SDS-PAGE showing expression of surf-crs, surf-gdh, cyto-crs and cyto-gdh by various designer cells

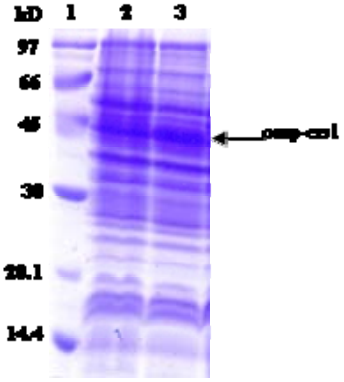


Figure 6S

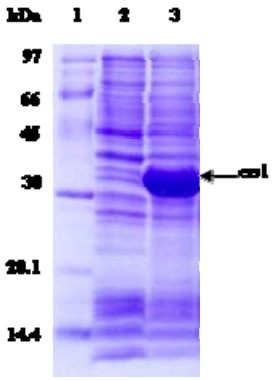


Figure 7S

Figure 6S. **Expression of *surf-crs* fusion protein.** Lane 1- Molecular weight marker, Lane 2- Induced *E. coli* BL21(DE3) + *pET 23(a)*, Lane 3- Induced *E. coli* BL21 + *pET 23(a)-omp-crs*. Arrow is representing the expression of 44.45 kD omp-crs fusion protein.

Figure 7S. **Expression of cyto-*crs*.** Lane 1- Molecular weight marker, Lane 2- Induced *E.coli* BL21(DE3) + *pET 23(a) -crs* and Lane 3- Induced *E.coli* BL21(DE3) + *pET 23(a)-crs*.

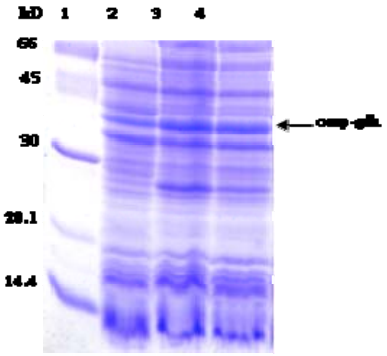


Figure 8S

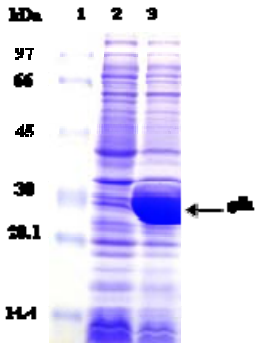


Figure 9S

Figure 8S. **Expression of *surf-gdh* fusion protein.** Lane 1- Molecular weight marker, Lane 2- Induced *E. coli* BL21(DE3) + *pET 29(a)*, lane 3 & 4- Induced *E. coli* BL21 + pET 29(a)-omp-gdh. Arrow is representing the expression of 42.2 kD omp-gdh fusion protein.

Figure 9S. **Expression of *cyto-gdh*.** Lane 1- Molecular weight marker, Lane 2- Induced *E. coli* BL21(DE3) + *pET 29(a)* and Lane3- Induced *E. coli* BL21(DE3) + *pET 29(a)-gdh*.

4. Analytical Methods and Experimental procedures

4.1. General Notes

Polymerase chain reaction was performed by Eppendorf thermocycler. All solvents used for flash chromatography and synthesis were purified before use. Thin layer chromatography (TLC) was used for monitoring the reaction and for comparison with authentic samples. Aluminium sheets pre-coated with silica-gel 60 F_{254} of Merck, Germany (Product no. 105554) were used for TLC. Separated compounds were visualized by exposure to iodine vapors or by staining with 1% $KMNO_4$ and 2% sodium bicarbonate aqueous solution followed by heating with heat gun (250 ^oC) for few seconds. Flash chromatography was performed on silica gel (200-400 mesh). NMRs were run on Jeol ECX 300 NMR spectrometer using $CDCl_3$ as solvent. Chemical shifts are reported as downfield from TMS used as internal standard. Values of coupling constants *J* are reported in Hz. HPLC was done using Dionex Summit HPLC system equipped with high pressure gradient dual pump, manual injector, variable temperature column compartment and PDA detector. Analysis was done using chromeleon® version 6.50 software. Optical rotation was recorded on the Polarimeter (Autopol IV, Rudolph Research, USA). All evaporation of solvents was done at 40 ^oC under reduced pressure on BÜCHI Rotavapor-R114, Switzerland. Protein purification was performed on FPLC (Amersham Biosciences AKTA prime). Spectrophotometric analysis was done on Perkin Elmer, Lambda 25 UV/VIS spectrometer.

4.2 Enzymes and chemicals

Recombinant DNA procedures were carried out using standard procedures.[3] Enzymes required for molecular biology were purchased from *New England BioLabs* (United Kingdom) and used as recommended. QIAprep Spin Miniprep Kit for plasmid isolation, QIAquick Gel Extraction Kit for extraction of DNA from the agarose gel and Ni-NTA Agarose for purification of 6xHis-tagged proteins was purchased from *Qiagen* and used according to the manufacturer protocol. Antibiotic penicillin and kanamycin was purchased from Sigma-Aldrich. PD-10 column used for desalting of protein was from GE Healthcare.

NADPH, ethyl 4-chloro-3-oxobutanoate (**1a**), ethyl 3-oxobutanoate (**1c**) and 1-(4-nitrophenyl)ethanone (**1n**) were purchased from Sigma-Aldrich. Acetophenone (**1g) and** 1-(4-chlorophenyl)ethanone (**1h**) were from Lanchater, UK. NADP, monosodium salt was from

SISCO, India. All other ketones (**1b**, **1d**, **1f**, **1i-1m**) used in this study were from Acros Organics, USA. Solvents including HPLC solvents and all other chemicals were from Merck Specialties Pvt Ltd, India. Racemic alcohols were prepared by borhydride reduction of corresponding ketone.

4.3. Enzyme assays

Assay for carbonyl reductase (crs) activity: Whole-cell biocatalyst (10^6-10^7 cells) was added to 1 mL of reaction mixture consisting of 50 mM phosphate buffer, pH 6.5, 2 mM **1a**, 0.2 mM NADPH and the reaction monitored spectrophotometrically at 340 nm (molar absorption coefficient of 6800 $M^{-1}cm^{-1}$) for the oxidation of NADPH.[1]

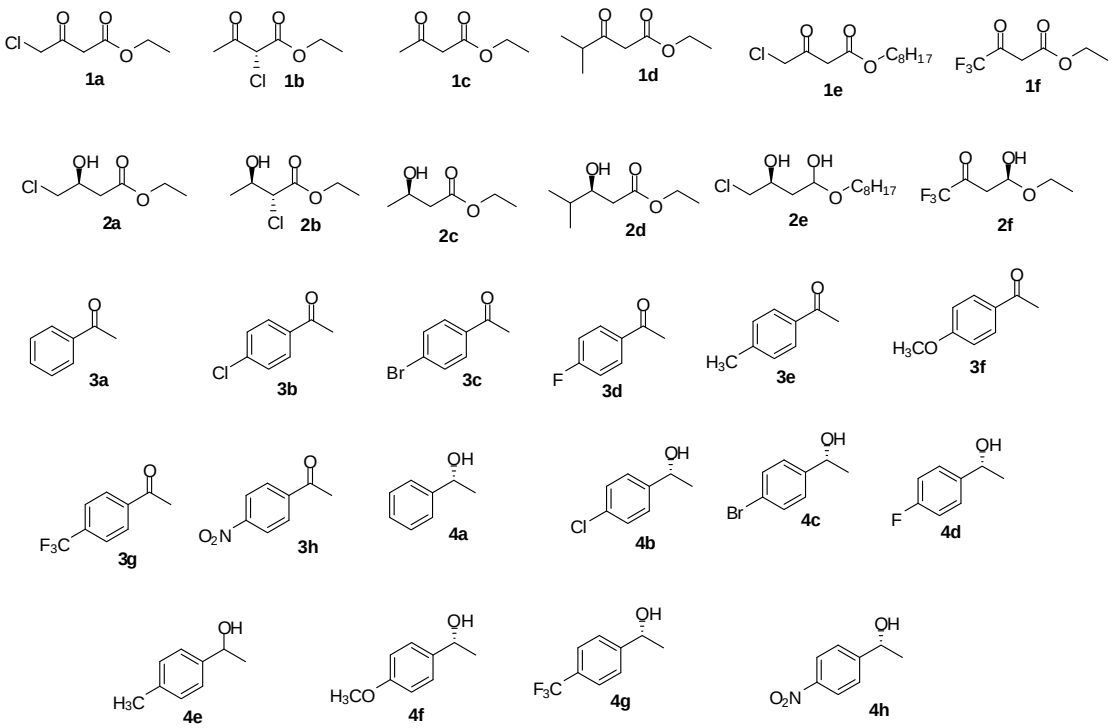
Assay for glucose dehydrogenase (gdh) activity: Whole-cell biocatalyst (10^6-10^7 cells) was added to 1 mL of reaction mixture consisting of 100 mM tris-HCl buffer, pH 8.0, 10 mM glucose, 0.5 mM $NADP^+$ and the reaction monitored spectrophotometrically at 340 nm (molar absorption coefficient of 6800 $M^{-1}cm^{-1}$) for the reduction of $NADP^+$.[4]

4.4 Experimental procedures

4.4.1. General procedure for the preparation of biocatalyst: Chemically competent cells of *E. coli* BL21(DE3) or *E. coli* C41(DE3)[7, 8] were transformed with the plasmid pETDuet1-GJSCG or pETDuet1-GJCCG (Figure 1) as described previously.[3] The plasmid pETDuet1-GJSCG encoded for an artificial surf-crs and artificial surf-gdh, which coexpress crs and gdh, respectively on the surface of the cell. The plasmid pETDuet1-GJSCG encoded for cyto-crs and cyto-gdh, which coexpress crs and gdh, respectively in the cytoplasm of cell. Active cells were prepared by incubation of a single colony *of E. coli* in 5 mL LB medium containing antibiotics supplement (100 μgL^{-1} ampicillin) with shaking (200 rpm) at 37 ^oC for 12 h. The culture was diluted 1:100 with fresh LB medium containing antibiotic supplement and incubated with shaking (200 rpm) at 37 ^oC. When the OD at 600 nm reached to 0.4-0.6, the culture was induced with 0.2 mM IPTG and culture incubated further at 37 ^oC for 16 h at 20 ^oC. The cells were harvested by centrifugation washed with 50 mM phosphate buffer (pH 6.5) and stored at 4 °C.

4.4.2. Relative activity of surf-crs-gdh and cyto-crs-gdh for ketones (1a-f and 3a-h): The assays were done in 96-well ELISA plate. The reaction mixture consisting of 0.2 mM nicotinamide adenine dinucleotide phosphate, reduced (NADPH), 2.0 mM substrate, 50µg/ml *Escherichia coli* cells (for calculation of cell mass, OD_{600} = 1 of cell suspension was taken as equivalent to 0.25 mg/ml dry cell weight) in 50 mM phosphate buffer, pH 7.0 was incubated at 30 °C for about 30 min to 12 hr depending upon the consumption of the substrate. The consumption of nicotinamide adenine dinucleotide phosphate, reduced (NADPH) was monitored by decrease in the absorbance at λ_{340}.

4.4.3. Preparative scale biocatalytic reduction of ketones 1a-f to chiral alcohols 2a-f and ketones 3a-h to alcohols 4a-h:



4.4.3.1. **Biocatalytic reduction of Ethyl 4-chloro-3-oxobutyrate (1a):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (5.4 g, 30 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **1a** (3.29 g, 20 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 9.5 h when TLC of reaction showed absence of

starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a pale yellow residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2a** (3.18 g; Yield 95.5%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.28 (3H, t, *J* = 7.2 Hz); 2.63 (3H, m); 3.61 (2H, dd, *J* = 7.2, 5.4 Hz); 4.18 (2H, q, *J* = 7.2 Hz); 4.23 (1H, m). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.20, 38.58, 48.22, 61.11, 68.03, 171.89. Ee >99%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 14.3. The absolute configuration was assigned as (*S*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = -22.1 (c = 8.72, $CHCl_3$) [lit.[5] (*R*) $[\alpha]_D^{25}$= +20.1 (c = 8.24, $CHCl_3$) 96% ee].

4.4.3.2. **Biocatalytic reduction of Ethyl 2-chloro-3-oxobutyrate (1b):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (5.4 g, 30 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **1b** (3.29 g, 20 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 10 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2b** (3.02 g; Yield 90.7%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.34 (m, 6H); 4.25 (m, 4H). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.09, 19.25, 61.00, 62.42, 69.08, 168.74. De 98% *anti*, determined by GLC (Factorfour^{TM} Varian, 30m x 0.25mm, 140 ^{o}C, N_2 1 kg min^{-1}, Detection FID), retention time 8.90; Ee >98%, determined by GLC (betaDex^{TM} Supelco, 30m x 0.25mm, 140 ^{o}C, N_2 1 kg min^{-1}, Detection FID), retention time 13.1; tentatively assigned (*2R*, *3R*) configuration based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = -3.8 (c = 1.13, $CHCl_3$). [lit.[6] (2*S*,3*S*)$[\alpha]_D^{25}$ = +4.0 (c = 1.1, $CHCl_3$), 97% de, 99% ee).

4.4.3.3. **Biocatalytic reduction of Ethyl 3-oxobutyrate (1c):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (5.4 g, 30 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **1c** (2.60 g, 20 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 27 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2c** (2.35g; Yield 89%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.21 (3H, d, *J* = 6.5 Hz); 1.26 (3H, t, *J* = 6.8 Hz); 2.46 (2H, m); 3.00 (1H, bs); 4.16 (3H, m). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.27, 22.48, 42.80, 60.77, 64.34. Ee 95%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 9.4 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = -44.2 (c = 2.03, $CHCl_3$) [lit.[7] (*S*) $[\alpha]_D^{25}$ = +32.8 (c = 3.0, $CHCl_3$) 99% ee].

4.4.3.4. **Biocatalytic reduction of Ethyl 4-methyl-3-oxopentanoate (1d):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (5.4 g, 30 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **1d** (3.15g, 19.9 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 16 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2d** (2.93 g; Yield 91.9%). **^{1}H NMR**

(300 MHz, $CDCl_3$): 0.90 and 0.93 (each 3H, each t, *J* = 6.8 Hz); 1.26 (3H, t, *J* = 7.2 Hz); 1.69 (1H, m); 2.38 (1H, dd, *J* = 9.6, 16.5 Hz); 2.52 (1H, dd, *J* = 2.8, 16.5 Hz); 2.94 (1H, bs); 3.75 (1H, m,); 4.14 (2H, q, *J* = 7.2 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.25, 17.83, 18.42, 33.24, 38.54, 60.79, 72.82, 173.62. Ee >99%, determined by HPLC (Chiralcel OD-H, λ_{217}, hexane:isopropanol 95:5, 1 ml/min). Retention time 5.3 min. Absolute configuration was assigned as (*S*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = -40.8 (c = 2.56, $CHCl_3$) [lit.[8] (*R*) $[\alpha]_D^{20}$= +34.3 (c = 0.078, $CHCl_3$) 83% ee].

4.4.3.5. **Biocatalytic reduction of Octyl 4-chloro-3-oxobutanoate (1e):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (5.4 g, 30 mmol). The contents were mixed by gentle stirring at 30 ^oC and a solution of **1e** (4.95g , 19.9 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^oC and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^oC, the stirring was continued for another 29 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2e** (4.39 g; Yield 88%). **^{1}H NMR** (300 MHz, $CDCl_3$): 0.86 (3H, t, *J* = 6.9 Hz, H_2 CH_3); 1.27 (10H, m, 5 x CH_2); 1.61 (2H, m); 2.62 (2H, m); 3.60 (2H, m); 4.11 (2H, t, *J* = 6.8 Hz); 4.14 (1H, m, H3). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.16, 22.71, 25.94, 28.59, 29.24, 31.84, 38.50, 48.19, 65.35, 68.06, 172.02. Ee >99%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 7.6 min. Absolute configuration was assigned as (*S*) based on comparison of optical rotation with literature $[\alpha]_D^{25}$ = -15.9 (c = 4.60, $CHCl_3$) [lit.[9] (*R*) $[\alpha]_D^{23}$= +15.1 (c = 4.66, $CHCl_3$) 97% ee].

4.4.3.6. **Biocatalytic reduction of Ethyl 4,4,4-trifluoro-3-oxobutyrate (1f):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (2.7 g, 15 mmol).

The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **1f** (1.84 g , 10 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 19 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **2f** (1.59 g; Yield 85.5%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.29 (3H, t, *J* = 7.2 Hz); 2.70 (2H, m); 4.21 (2H, q, J = 7.2 Hz); 4.43 (1H, m). **^{13}C NMR** (75 MHz, $CDCl_3$): 14.06, 34.87, 61.68, 67.21 (q, J = 32 Hz), 124.51 (q, J = 289 Hz), 170.94. Ee and absolute configuration (*S*) was determined based on comparison of optical rotation with literature $[\alpha]_D^{25}$ = -20.3 (c = 1.87, $CHCl_3$) [lit.[10] $[\alpha]_D^{23}$= -12.1 (c = 1, $CHCl_3$) 64.5% ee].

4.4.3.7. **Biocatalytic reduction of Acetophenone (3a):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (2.7 g, 15 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **3a** (1.20 g , 10 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 21 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4a** (1.09 g; Yield 89.2%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.49 (3H, d, *J* = 6.5 Hz); 2.10 (1H, bs); 4.87 (1H, q, *J* = 6.5 Hz); 7.35 (5H, m). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.25, 70.53, 125.47, 127.58, 128.60, 145.88. Ee 99%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 9.2 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with

literature. $[\alpha]_D^{25}$ = +54.8 (c = 2.74, $CHCl_3$) [lit.[11] (*S*)] $[\alpha]_D^{25}$= -49.5 (c = 0.05, CH_2Cl_2) 97% ee].

4.4.3.8. **Biocatalytic reduction of 4-Chloroacetophenone (3b):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (2.7 g, 15 mmol).. The contents were mixed by gentle stirring at 30 ^oC and a solution of **3b** (1.55 g, 10 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^oC and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^oC, the stirring was continued for another 23 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4b** (1.44 g; Yield 91.9%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.47 (3H, d, *J* = 6.5 Hz); 2.1 (1H, bs, OH); 4.87 (1H, q, *J* = 6.5 Hz); 7.31 (4H, s). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.37, 69.85, 126.88, 128.69, 133.16, 144.33. Ee 99%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 10.5 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +49.2 (c = 1.83, ether) [lit.[11] (*S*)][$\alpha]_D^{25}$= -47.4 (c = 0.06, ether) 94% ee].

4.4.3.9. **Biocatalytic reduction of 4-Bromoacetophenone (3c):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), β-NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (4.32 g, 24 mmol). The contents were mixed by gentle stirring at 30 ^oC and a solution of **3c** (3.18 g, 16 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^oC and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^oC, the stirring was continued for another 23h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation

and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4c** (2.85 g; Yield 88.6%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.47 (3H, d, *J* = 6.5 Hz); 2.08 (1H, bs); 4.87 (1H, q, *J* = 6.5 Hz); 7.25 and 7.47 (each 2H, each d, *J* = 8.7 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.34, 69.87, 121.25, 127.25, 131.64, 144.86. Ee 97%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 11.4 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +38.3 (c = 1.55, $CHCl_3$) [lit.[11] (*S*)]$[\alpha]_D^{25}$= -37.5 (c = 0.07, $CHCl_3$) 96% ee].

4.4.3.9. **Biocatalytic reduction of 4-Fluoroacetophenone (3d):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (4.32 g, 24 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **3d** (2.21 g, 16 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 19 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4d** (2.01 g; Yield 89.7%). **^{1}H NMR** (300 MHz, $CDCl_3$): 1.49 (3H, d, *J* = 6.5 Hz); 2.03 (1H, bs); 4.89 (1H, q, *J* = 6.5 Hz); 7.01 and 7.03 (each 2H, each d, *J* = 8.6 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): 69.80, 115.26 (d, J = 24Hz), 127.14 (d, J = 7.9 Hz), 141.64, 162.17 (d, J = 243 Hz). Ee 97% determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min). Retention time 8.8 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +48.8 (c = 1.4, $CHCl_3$) [lit.[11] (*S*)]$[\alpha]_D^{25}$= -47.4 (c = 0.06, $CHCl_3$) 97% ee].[9, 10]

4.4.3.10. **Biocatalytic reduction of 4-Methylacetophenone (3e):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100

mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (2.7 g, 15 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **3e** (1.35 g , 10.1 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 20 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4e** (1.17 g; Yield 85%).**^{1}H NMR** (300 MHz, $CDCl_3$): 1.48 (3H, d, *J* = 6.5 Hz); 2.01 (1H, bs); 2.38 (1H, s); 4.87 (1H, q, *J* = 6.5 Hz); 7.15 and 7.26 (each 2H, each d, *J* = 7.9 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): 21.19, 25.18, 70.37, 125.44, 129.26, 137.27, 142.95. Ee 99%, determined by HPLC (Chiralcel OB-H, λ_{217}, hexane:isopropanol 95:5, 1 ml/min). Retention time 12.9 min. Absolute configuration was assigned as (*R*) based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +52.1 (c = 1.98, $CHCl_3$) [lit.[11] (*S*)] $[\alpha]_D^{25}$= -51.1 (c = 1, $CHCl_3$) 96% ee].

4.4.3.11. **Biocatalytic reduction of 4-Methoxyacetophenone (3f):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (1.0 g, wet biomass), NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (2.7 g, 15 mmol). The contents were mixed by gentle stirring at 30 ^{o}C and a solution of **3f** (1.5 g, 10 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^{o}C and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^{o}C, the stirring was continued for another 29 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4f** (1.32 g; Yield 86.7%).**^{1}H NMR**(300 MHz, $CDCl_3$): 1.46 (3H, d, *J* = 6.5 Hz); 2.08 (1H, bs); 3.80 (3H, s); 4.84 (1H, q, *J* = 6.5 Hz); 6.86

and 7.28 (each 2H, each d, J = 8.2). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.11, 55.39, 70.08, 113.94, 126.76, 138.09, 159.07. Ee and absolute configuration (*R*) was determined based on comparison of optical rotation with literature. $[\alpha]_D^{22}$= +51.4 (c 1.72, $CHCl_3$) [lit.[12] (*S*) $[\alpha]_D^{25}$= -51.9 (c 0.72, $CHCl_3$) 99% ee).

4.4.3.9. **Biocatalytic reduction of 4-trifluoromethylacetophenone (3g):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (4.32 g, 24 mmol). The contents were mixed by gentle stirring at 30 ^oC and a solution of **3g** (3.0 g, 16 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^oC and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^oC, the stirring was continued for another 14 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4g** (2.86 g; Yield 94%).**^{1}H NMR** (300 MHz, $CDCl_3$): 1.5 (3H, d, J = 6.51 Hz); 2.33 (1H, bs); 4.98 (1H, q, J = 6.5 Hz); 7.48 and 7.60 (each 2H, each d, J = 8.2 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.49, 69.92, 125.51, 125.56, 125.73, 149.76. Ee and absolute configuration (*R*) was determined based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +27.2 (c 2.08, MeOH) [lit.[12] (*S*) $[\alpha]_D^{22}$= -28.1 (c 1.13%, MeOH) 99% ee].

4.4.3.9. **Biocatalytic reduction of 4-Nitroacetophenone (3h):** An auto titrator apparatus equipped with a magnetic stirrer was charged with aq. phosphate buffer solution (20 mL, 100 mM; pH 6.5) followed by sequential addition of recombinant *E. coli* (0.4 g, wet biomass), NADP, mono sodium salt (7.65 mg, 10 nmol) and D-glucose (4.32 g, 24 mmol). The contents were mixed by gentle stirring at 30 ^oC and a solution of **3h** (2.64 g, 16 mmol) in di-n-butyl ether (7 mL) added drop wise over a period of 1 h maintaining the temperature at 30 ^oC and pH at 6.5 with 5N NaOH. While maintaining the pH at 6.5 and temperature at 30 ^oC, the stirring was continued for another 11 h when TLC of reaction showed absence of starting material. For TLC, an aliquot of 0.1 mL was withdrawn in an eppendorf tube and thoroughly

mixed with equal volume of ethyl acetate. The organic phase was separated by centrifugation and subjected to analysis by TLC. After completion of reaction, the product was extracted in ethyl acetate (3x25mL), washed once with brine (10 mL), dried over anhyd. sodium sulfate and solvents removed to leave a pale yellow residue, which was further purified by flash chromatography over silica gel (200-400 mesh) to obtain **4h** (2.53 g; Yield 94.6%).**^{1}H NMR** (300 MHz, $CDCl_3$): 1.52 (3H, t, *J* = 6.5 Hz, CH_3); 2.50 (1H, bs, OH); 5.02 (1H, q, *J* = 6.5 Hz, CH); 7.54 and 8.12 (each 2H, each d, *J* = 8.9 Hz, aryl). **^{13}C NMR** (75 MHz, $CDCl_3$): 25.55, 69.55, 123.82, 126.22, 147.21, 153.26. Ee and absolute configuration (*R*) was determined based on comparison of optical rotation with literature. $[\alpha]_D^{25}$ = +31.4 (c = 3.99, $CHCl_3$) [lit.[7] (*S*)] $[\alpha]_D^{25}$= -30.5 (c = 4.0, $CHCl_3$) 96% ee].

4.5. List of primers.

Entry	Oligomer	Sequence of the primer (5'→3')
1.	crsF	ATTATCCATATGGCTAAGAACTTCTCCAACG
2.	crsR	ATCTTTCTCGAGGGGAAGCGTGTAGCCACC
3.	ogF	TTGTTGTTCATATGAAAGCTACTAAACTGGTACTG
4.	ogR	TTGTTGTTCTCGAGTTATCCGCGTCCTGCTTGG
5.	oep-ogR	CTTTATACATGCCTGGGATGCCGTTGTC
6.	oep-ogF	CATCCCAGGCATGTATAAAGATTTAGAAGGG
7.	gdhF	TTGTTATTCATATGTATAAAGATTTAGAAGGGAAAG
8.	gdhR	TTGTTGTTCTCGAGTCCGCGTCCTGCTTGGAAT
9.	oc1F	TATCGCATTCCATGGGCAAAGCTACTAAACTGGTAC
10.	oc1R	GTTATGTTCAAGCTTTTACGGCAGGGTATAACC
11.	crsDF	ATTATCCCATGGGCGCTAAGAACTTCTCCAACG
12.	crsDR	ATCTTTAAGCTTGGGAAGCGTGTAGCCACC

4.6. Construction of plasmids

Construction of the 4.9 kb *pET 23(a)-omp-crs* (pET23(a)-GJSC) expression plasmid

The custom synthesized complete coding sequence for *omp-crs* (Sequence No. 7) in pUC 19 were double digested by *Nde1* and *EcoR1* and product was separated by agarose gel electrophoresis and 1.3 Kb *omp-crs* was purified from the gel with *Qiaquick kit* (Qiagen).

The 1.3 kb *omp-crs* gene was cloned downstream of the *lac* promoter of *Nde1–EcoR1* treated *pET 23(a)*, which was previously dephosphorylated by calf intestinal alkaline phosphatase. The 4.9 Kb plasmid *pET 23 (a)-omp-crs* was used to transformed *E. coli* BL21(DE3) or *E. coli* C41(DE3).

Construction of the 4.4 kb *pET 23(a)-crs* (pET23(a)-GJCC) expression plasmid

The *crs* gene was amplified from the custom synthesized *omp-crs* in pUC 19 plasmid as a template by polymerase chain reaction (PCR) with the primers crsF and crsR. The PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles of 95 °C for 60 s, 58 °C for 60 s and 72 °C for 60 s as well as a final extension step of 72 °C for 5 min. The PCR product (Sequence No. 8) was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pET 23(a)* through *Nde1*and *Xho1* site and the resultant 4.4 Kb *pET 23(a)-crs* plasmid was transformed into *E. coli* BL21(DE3) expressing *crs* with C-terminal His6 inside the cytoplasm.

Construction of the 6.5 kb *pET 29(a)-omp-gdh* (pET29(a)-GJSG) expression plasmid

The *omp-gdh* gene (Sequence No. 9) was constructed by applying the overlapping extension PCR strategy. Briefly, *omp* gene was amplified from the plasmid *pET 23 (a)-omp-crs* by polymerase chain reaction (PCR) with the primers ogF and oep-ogR and *gdh* gene was amplified from the plasmid *pET 29 (a)-gdh* by polymerase chain reaction (PCR) with the primers oep-ogF and ogR. Eqavimolar concentration of *omp* and *gdh* were used as a template for polymerase chain reaction (PCR) with the primers ogF and ogR for the fusion of *omp* with *gdh* The PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles of 95 °C for 60 s, 58 °C for 60 s and 72 °C for 90 s as well as a final extension step of 72 °C for 5 min. The 1.23 Kb PCR product was cloned into the pUC19 to get 3.9 Kb *pUC19-omp-gdh* plasmid. The 1.23 Kb *omp-gdh* fragment was amplified by using ogF and ogR primer and the resultant PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pET 29(a)* through *Nde1*and *Xho1* site and get the resultant 6.5 Kb *pET 29(a)-omp-gdh* plasmid was transformed into *E. coli* BL21(DE3) or *E. coli* C41(DE3).

Construction of the 6.1 kb *pET 29(a)-gdh* (pET29(a)-GJCC) expression plasmid

The *gdh* gene (Sequence No. 10) was amplified from the genomic DNA of the *Bacillus megaterium* as a template by polymerase chain reaction (PCR) with the primers gdhF and gdhR. The PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles

of 95 °C for 60 s, 52 °C for 60 s and 72 °C for 60 s as well as a final extension step of 72 °C for 5 min. The PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pET 29(a)* through *Nde1*and *Xho1* site and the resultant 6.1 kb *pET 29(a)-gdh* plasmid was transformed into *E. coli* BL21(DE3).

Construction of the 7.8 kb *pETDuet1-omp-crs;omp-gdh* (pETDuet1-GJSCG) expression plasmid

The *omp-crs* gene (Sequence No. 7) was amplified from the custom synthesized *omp-crs* in pUC 19 plasmid as a template by polymerase chain reaction (PCR) with the primers ocF and ocR. The PCR conditions were initial denaturing at 95 °C for 5 min followed by 30 cycles of 95 °C for 60 s, 58 °C for 60 s and 72 °C for 90 s as well as a final extension step of 72 °C for 5 min. The PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pETDuet1* through *Nco1* and *Hind3* site and got the resultant 6.65 kb *pETDuet1-omp-crs* plasmid. The further extension of the *omp-gdh* gene in 6.65 kb *pETDuet1-omp-crs* plasmid was done by amplification of the 1.2 kb omp-gdh gene (Sequence No. 9) from the *pET 29(a)-omp-gdh* plasmid as a template by polymerase chain reaction (PCR) with the primers ogF and ogR. The PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles of 95 °C for 60 s, 54 °C for 60 s and 72 °C for 90 s as well as a final extension step of 72 °C for 5 min. The PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pETDuet1-omp-crs* plasmid through *Nde1* and *Xho1* site and the resultant 7.8 kb *pETDuet1-omp-crs;omp-gdh* plasmid was transformed into the *E. coli* BL21(DE3) or *E. coli* C41(DE3).

Construction of the 7.0 kb *pETDuet1-crs; gdh* (pETDuet1-GJCCG) expression plasmid

The *crs* gene (Sequence No. 8) was amplified from the custom synthesized *omp-crs* in pUC 19 plasmid as a template by polymerase chain reaction (PCR) with the primers crsDF and crsDR. The PCR conditions were initial denaturing at 95°C for 5 min followed by 30 cycles of 95 °C for 60 s, 58 °C for 60 s and 72 °C for 60 s as well as a final extension step of 72 °C for 5 min. The PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pETDuet1* through *Nco1* and *Hind3* site and got the resultant 6.2 kb *pETDuet1-omp-crs* plasmid. The further extension of the *gdh* gene in 6.2 kb *pETDuet1-crs* plasmid was done by amplification of the 0.75 kb gdh gene (Sequence No. 10) from the *pET 29(a)-omp-gdh* plasmid as a template by polymerase chain reaction (PCR) with the primers ogF and ogR. The PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles of

95 °C for 60 s, 54 °C for 60 s and 72 °C for 90 s as well as a final extension step of 72 °C for 5 min. The PCR product was recovered by a QIAquick Gel extraction kit (Qiagene) and cloned into *pETDuet1-crs* plasmid through *Nde1* and *Xho1* site and the resultant 7.0 kb *pETDuet1-crs;gdh* plasmid was transformed into the *E. coli* BL21(DE3) or *E. coli* C41(DE3).

4.7. Expression of the recombinant protein in *E. coli* strain

Fresh culture of recombinant *E. coli* strain harboring plasmid was grown in 20 mL LB media containing either ampicillin (100 µg/mL) or kanamycin (50 µg/mL) or both at 37 °C. After 6 h 5 mL of the culture was inoculated in 500 mL fresh LB media containing antibiotic depending upon the plasmid and grown at 37 °C under 200 rpm shaking condition. When the OD at 600 nm reached 0.4-0.6, the culture was induced with final concentration of 0.2 mM IPTG and the culture incubated further for 16 h at 20 °C under 200 rpm shaking condition. The cells were isolated by centrifugation, washed twice with 50 mM phospahate buffer, pH 6.5 and used for future experiments. Expression of the protein were checked by SDS-PAGE, which was carried out according to protocol of LaemmLi with some modifications.[13]

4.8. Demonstration of cell surface display of crs and gdh

Induced cells from 100 mL culture were centrifuged and washed with 50 mM phosphate buffer, and then suspended in 5 mL lysis buffer (50 mM NaH_2PO_4, 150 mM NaCl, 1 mg/mL Lysozyme, pH-8.0), at 4 °C for 30 min, and then sonicated for 20 min with 30 sec pulse on and 30 sec pulse off. The cell debris was removed by centrifugation at 14000 rpm for 30 min. The membrane was separated at 100000g for 2 h and washed with the same buffer and then suspended in 1 mL membrane solublization buffer (25 mM Tris HCl, 20% Glycerol and 2% Triton X100, pH-7.5) and the activity for *gdh* and *crs* was determined by as described below.

4.9. Immuno-localization of carbonyl reductase in *E. coli* BL21(DE3)

4.9.1. anti-*crs* polyclonal antibody

350 µg of purified crs in 400 µL of buffer was emulsified with equal amount of adjuvants and injected to rabbits. First injection was given with Freund's complete adjuvant, while 4 booster doses of 350 µg of purified crs in 400 µL of buffer, emulsified with equal amounts of Freund's incomplete adjuvant, given continuously at the interval of 21 days. Samples of serum were withdrawn on fifth day after each booster to check antibody titer. Sample collected after 5 days of the 4^{th} booster was used for the further experiment. Pre-immune sera was collected as a control before the first immunization to check the cross-reactivity if any.

4.9.2. Western blotting

The antibodies raised in rabbit were tested for their specificity against the enzyme by western blotting. 12.5% SDS-PAGE was run and the protein was transferred to PVDF membrane by applying a current of 100 mA for 1h. The membrane was temporarily stained with Ponceau S stain to check the transfer. It was kept for blocking with 10% skimmed milk in phosphate buffer saline for 2h at RT. This was then washed thrice with 0.05% Tween 20 in 1X PBS for 5 min and once with PBS alone for 10 min. The strips were cut for each lane to be incubated with different dilution of the antibody. Different dilutions of primary antibodies were made in 0.1% skimmed milk in 1X PBS. The antibodies from pre-immunized sera were used as control in this case to check the cross-reactivity of protein. The antibody dilution 1/1,00,000 gave a good signal. After incubating with primary antibody for 2 h at room temperature, this was washed thrice with 0.05% Tween 20 in 1X PBS for 5 min and once with PBS alone for 10 min. It was next incubated with alkaline phosphatase conjugated goat anti-rabbit antibody for 1 h. Washing was done after each incubation as previously described. The strips were then dipped in the substrate solution contains 5-bromo-4-chloro-3-indolyl phosphate (BCIP, 0.15 mg/mL), nitro blue tetrazolium (NBT, 0.30 mg/mL), Tris HCl (100 mM) and $MgCl_2$ (5 mM), pH 9.5 for 10 min.[1, 2] The blot was analyzed by Scion Image software for comparative study of the protein.

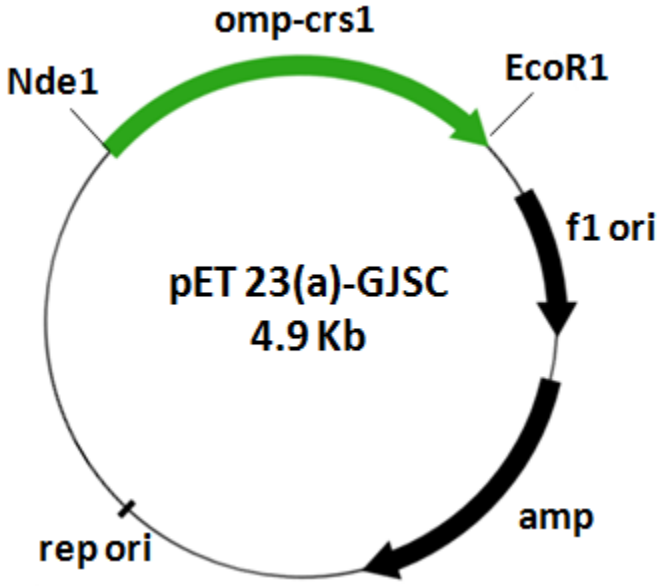
4.9.3. Transmission electron microscopy

Cells were harvested by centrifugation at 5,000 x g at 4 °C and washed 4 times with Dulbecco's phosphate-buffered saline (PBS), re-suspended and kept at 4 °C in 0.5% paraformaldehyde and 0.5% glutaraldehyde for 30 min. These were washed with PBS and a suspension was made in 2% agarose solution. The agarose blocks were cut into small pieces and were dehydrated with graded series of ethanol and embedded in LR White resin (polymerization at 60 ^{o}C for 48-74 h). Ultrathin sections cut with a Reichert Ultracut Ultramicrotome (Leica Reichart Jung, Austria) were picked up on 200-mesh nickel grids. Nonspecific sites were blocked with 0.1M PBS with 3% fish gelatin and 0.25% Tween 20 for 2 h at room temp (blocking buffer). The grids carrying the ultrathin Sections were then washed in 0.05% Tween 20 in PBS (washing buffer) and incubated overnight with rabbit anti-*crs* polyclonal antibody (diluted 1:2000 in 1:10 diluted blocking buffer) at 4 °C. The grids were washed in washing buffer and incubated for 2 h at room temp with goat anti-rabbit antibody conjugated to 10 nm gold spheres (diluted 1:200 in 1:100 diluted blocking

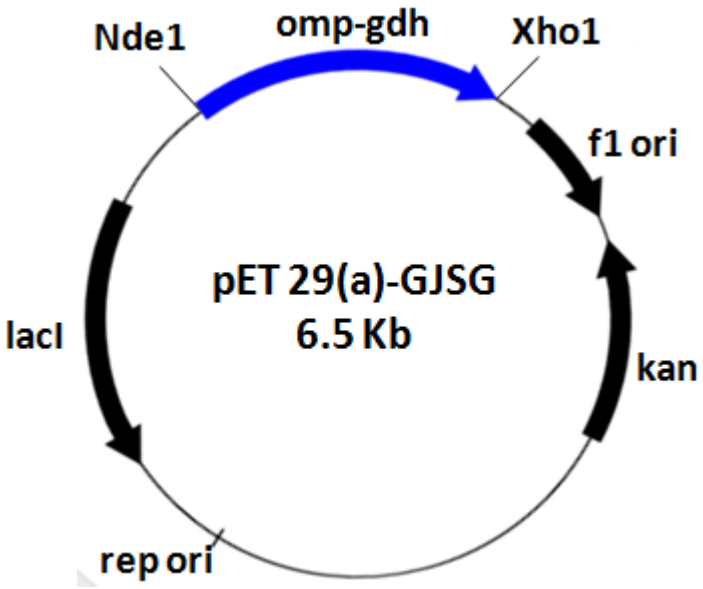
buffer). This was followed by washing the grids in washing buffer and subsequently in 0.1M phosphate buffer. The sections were then fixed with 1% glutaraldehyde in phosphate buffer for 15 min, and then washed in milliQ water; these were then stained in 2% aqueous uranyl acetate for 30 min at room temperature in dark followed by final washing with milliQ water. The grids thus prepared were examined in a Philips CM-10 transmission electron microscope (TEM, operating voltage 60-80kV), and random fields were photographed. The prints of the micrographs were then made at the desired magnification for further analysis. Controls included the labeling of each set of samples with pre-immune serum (i.e. normal rabbit serum) instead of anti-*crs* serum and cells which does not expressing *crs*, treated with rabbit anti-*crs* polyclonal antibody.

5. Plasmid maps

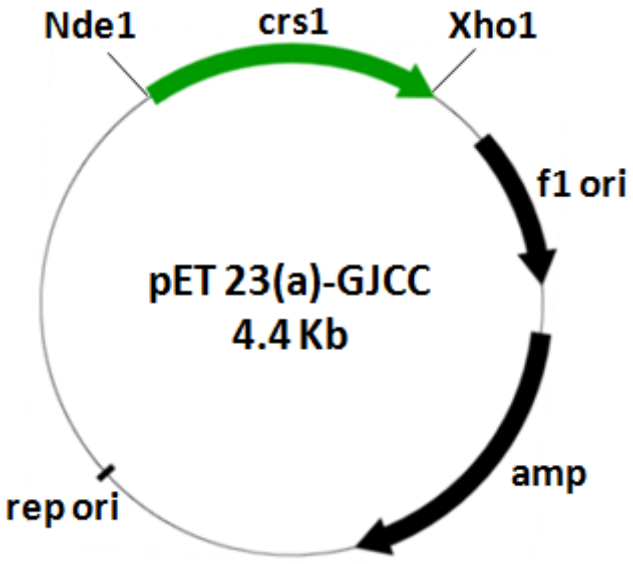
1- Pet23(a)-GJSC



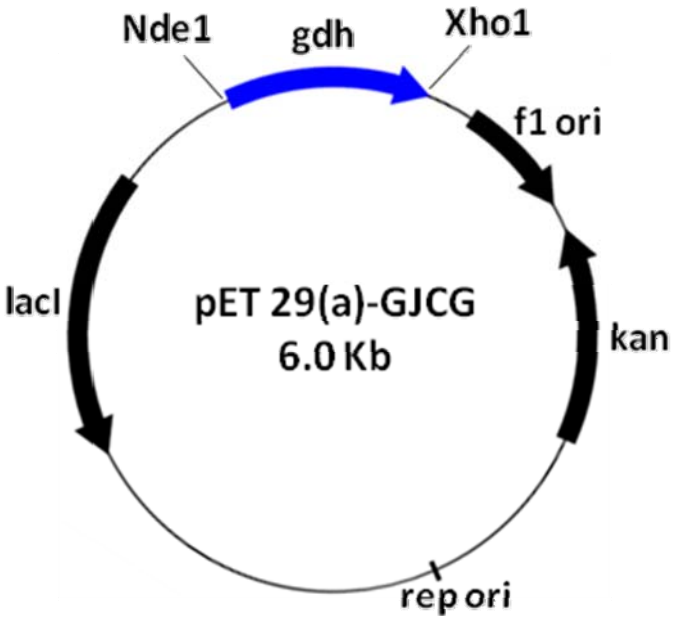
2- Pet29(a)-GJSG



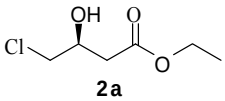
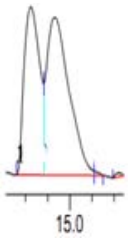
3- Pet23(a)-GJCC

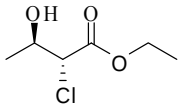
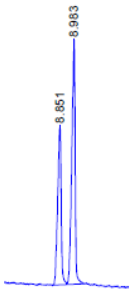
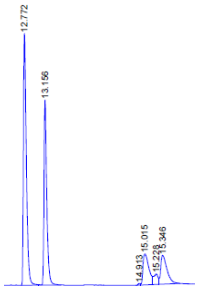
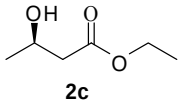
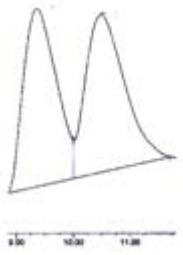
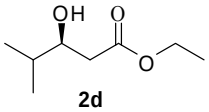
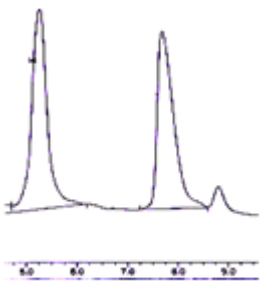
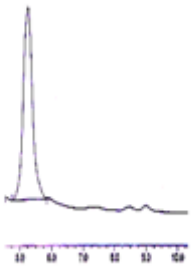
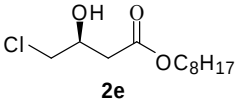
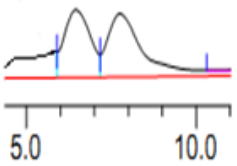
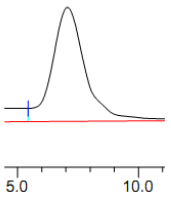


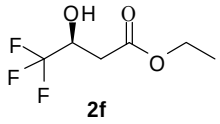
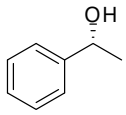
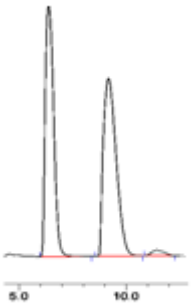
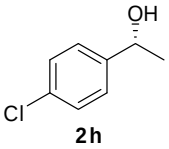
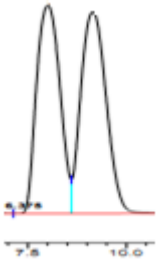
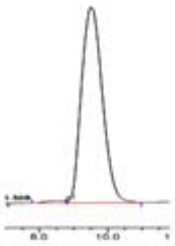
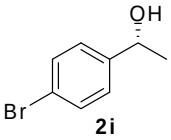
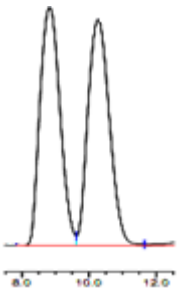
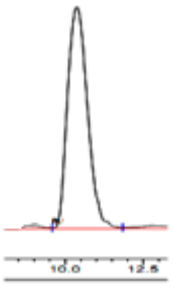
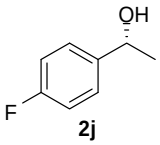
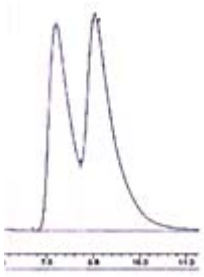
4- Pet23(a)-GJCG

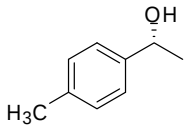
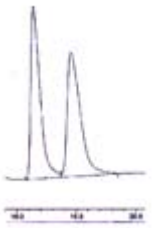
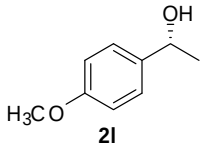
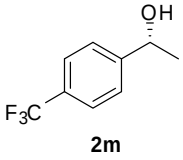
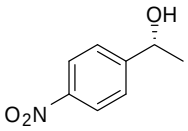


6. HPLC/GLC traces

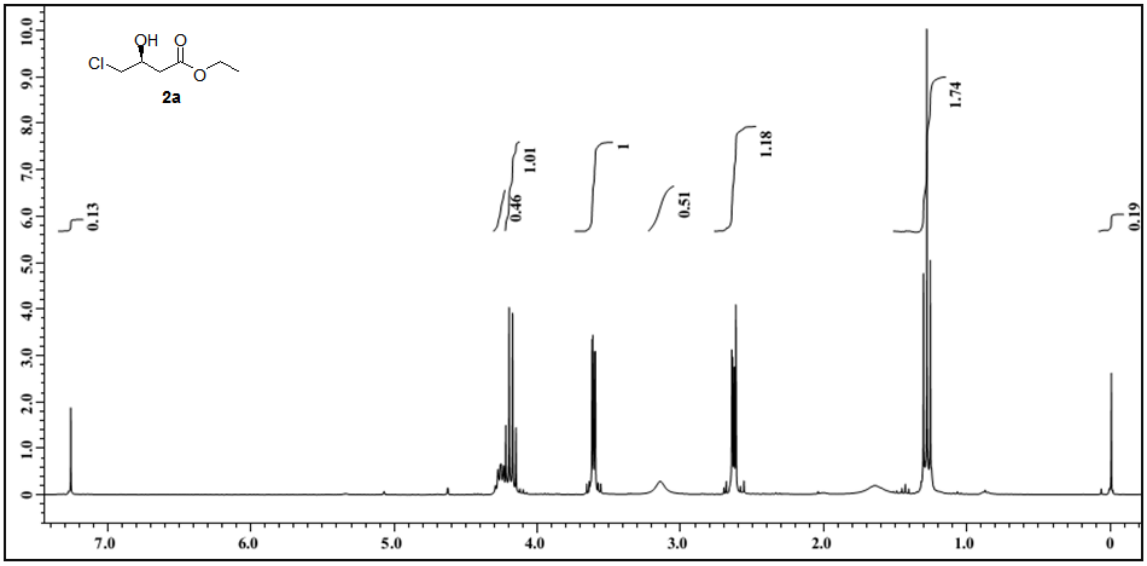
Entry	Product	HPLC/GLC conditions (column, detection wavelength, mobile phase, flow rate) Injection: manual	*rac*-alcohol	Biocatalyzed alcohol
1	 **2a**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		

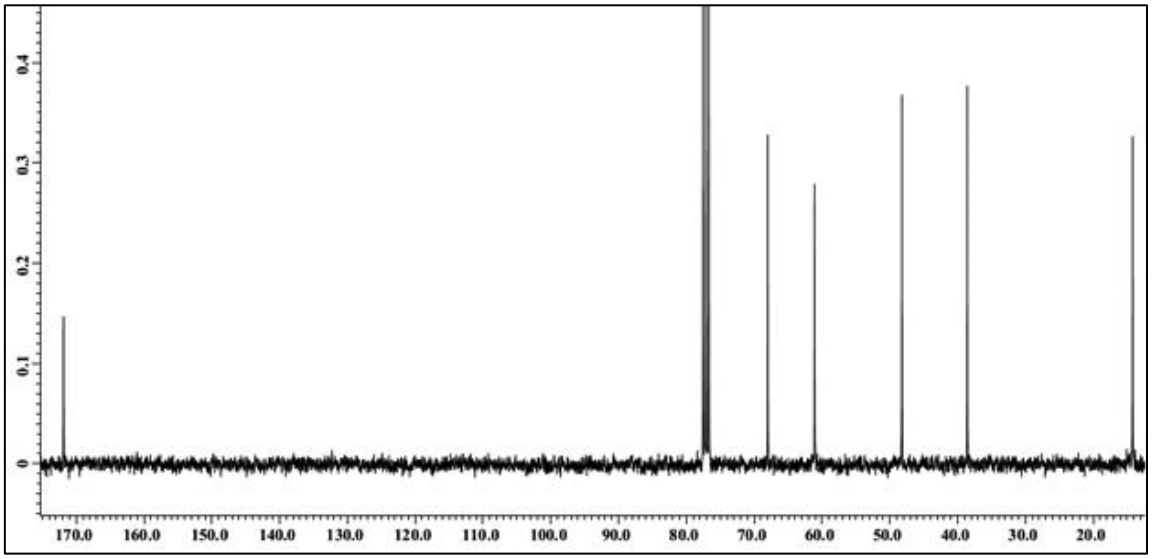
2	 **2b**	**GLC for d.e.** determination Factorfour™ (Varian, 30m x 0.25mm, 140 °C, N_2 1 kg min^{-1}, detection FID)		
		GLC for e.e. betaDex™ (Supelco, 30m x 0.25mm, 140 °C, N_2 1 kg min^{-1}, detection FID)		
3	 **2c**	OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		
4	 **2d**	Chiralcel OD-H, λ_{217}, hexane:isopropanol 95:5, 1 ml/min		
5	 **2e**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		

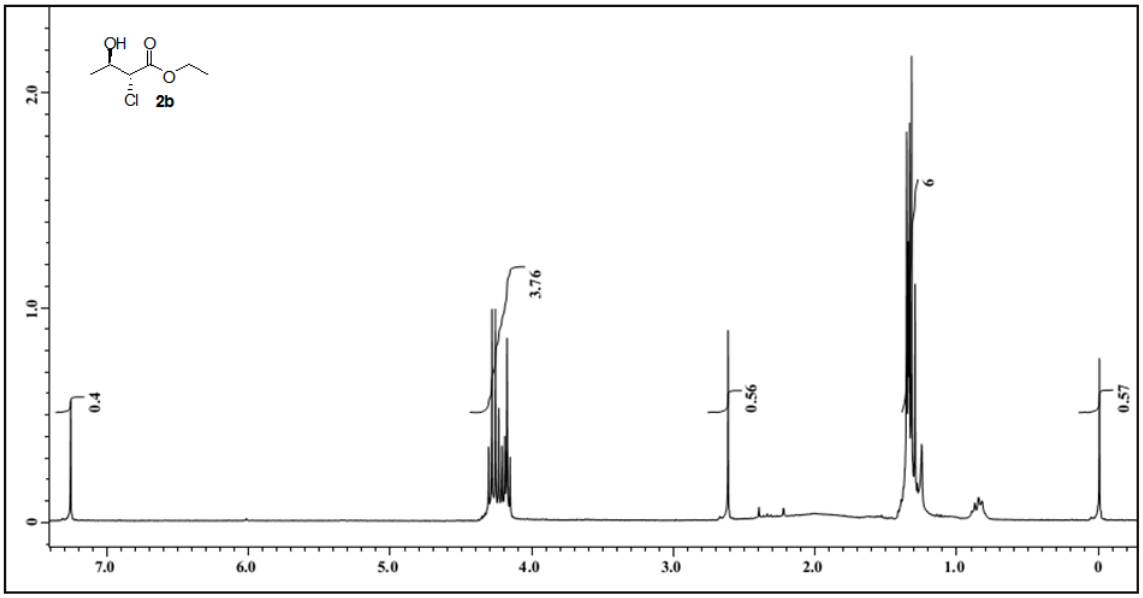
6	 **2f**	OB-H, OD-H and OJ column	Failed to resolve	$[\alpha]_D^{25}$ = -20.3 (c = 1.87, $CHCl_3$) [lit.[10] $[\alpha]_D^{23}$= -12.1 (c = 1, $CHCl_3$) 64.5% ee]
7	 **2g**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min.		
8	 **2h**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		
9	 **2i**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		
10	 **2j**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 96:4, 1 ml/min		

12	 **2k**	Chiralcel OB-H, λ_{217}, hexane:isopropanol 95:5, 1 ml/min		
13	 **2l**	OB-H, OD-H and OJ column	Failed to resolve	$[\alpha]_D^{22}$= +51.4 (c 1.72, $CHCl_3$) [lit.[12] (*S*)$[\alpha]_D^{25}$= -51.9 (c 0.72, $CHCl_3$) 99% ee)
11	 **2m**	OB-H, OD-H and OJ column	Failed to resolve	$[\alpha]_D^{25}$ = +27.2 (c 2.08, MeOH) [lit.[12] (*S*) $[\alpha]_D^{22}$= -28.1 (c 1.13%, MeOH) 99% ee]
14	 **2n**	OB-H, OD-H and OJ column	Failed to resolve	$[\alpha]_D^{25}$ = +31.4 (c = 3.99, $CHCl_3$) [lit.[7] (*S*)] $[\alpha]_D^{25}$= -30.5 (c = 4.0, $CHCl_3$) 96% ee]

7. **NMR SPECTRA**






OH
O
O
Cl
2b
2.0
1.0
0
0.4
3.76
0.56
6
0.57
7.0
6.0
5.0
4.0
3.0
2.0
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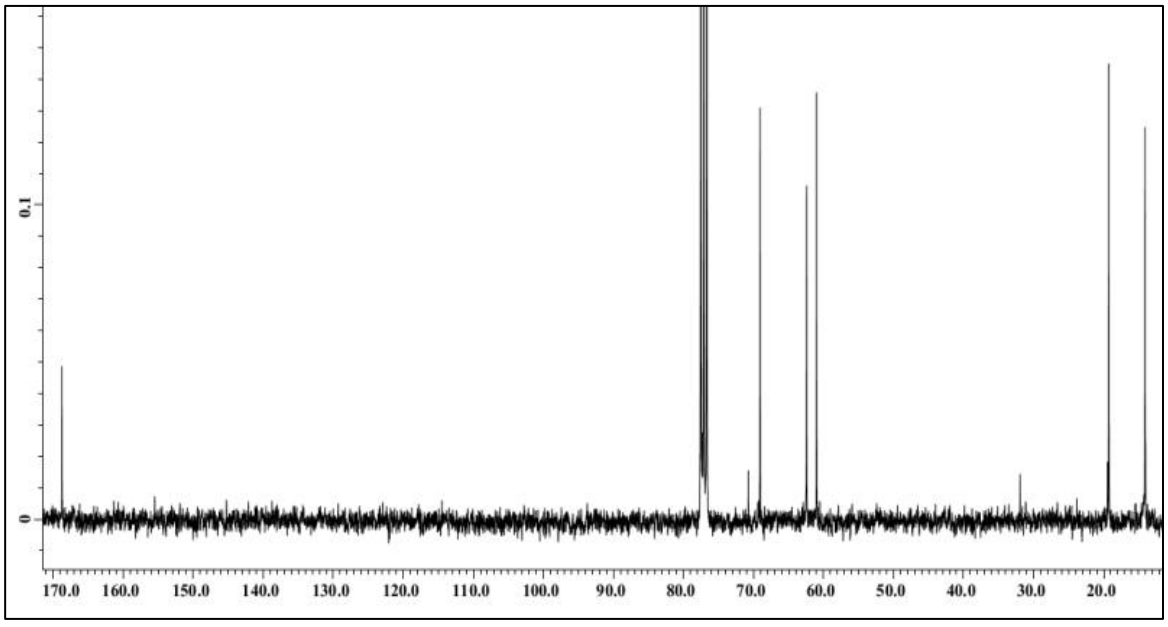
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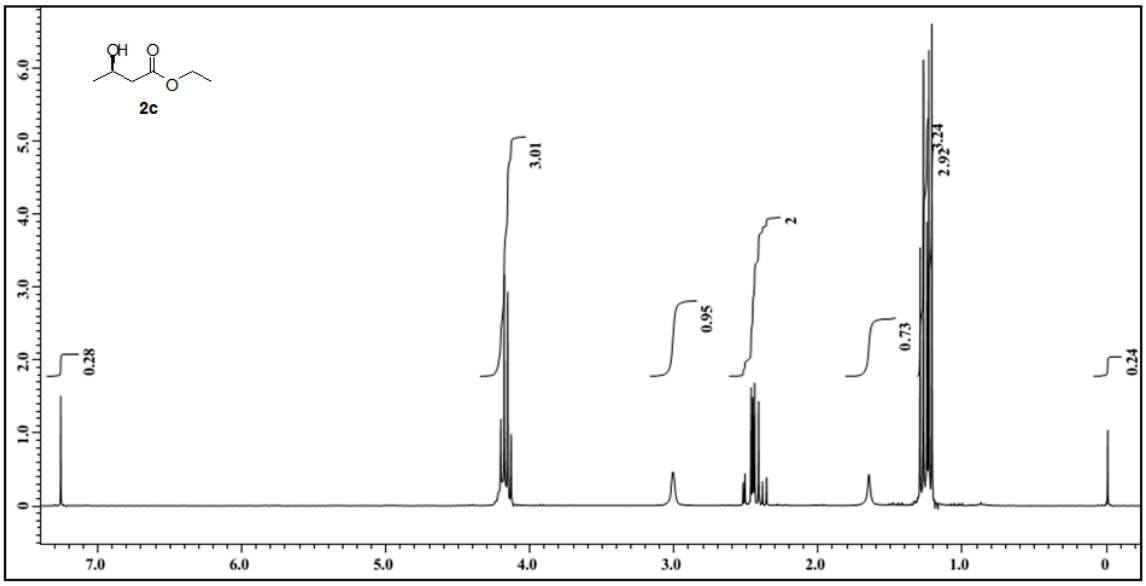
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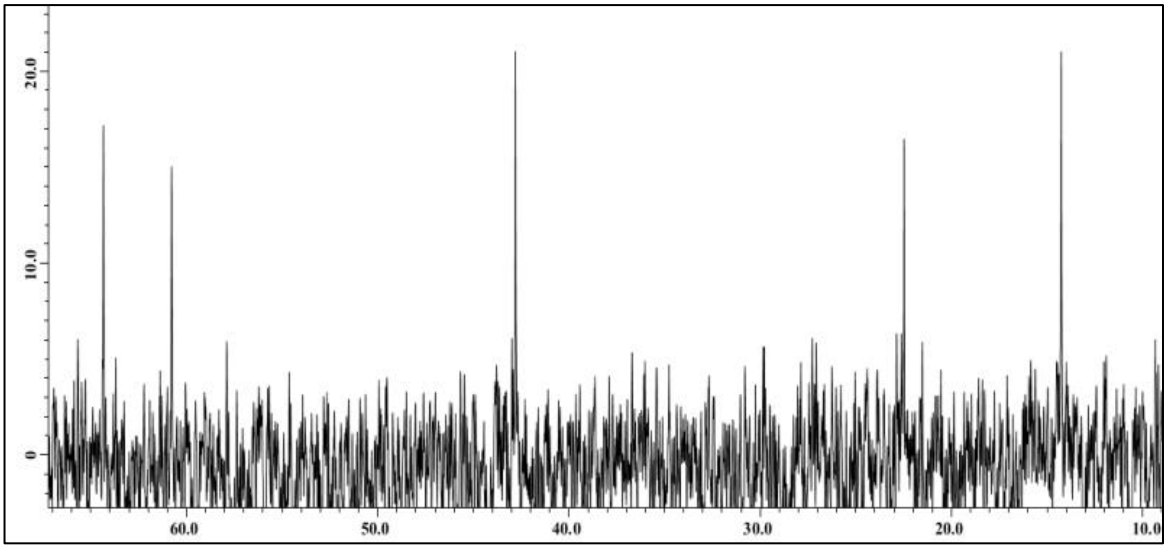
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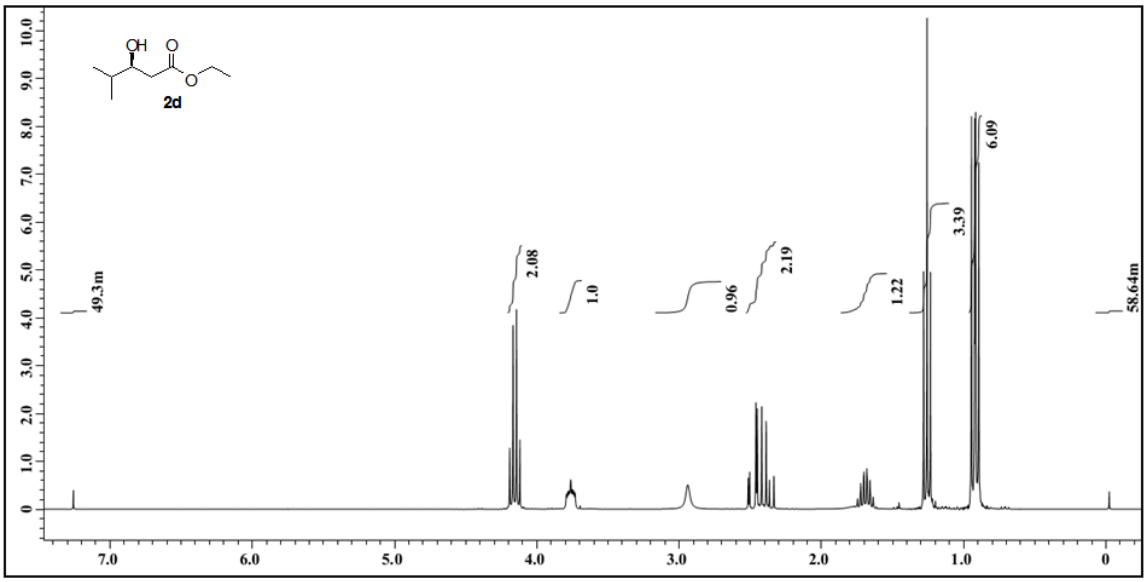
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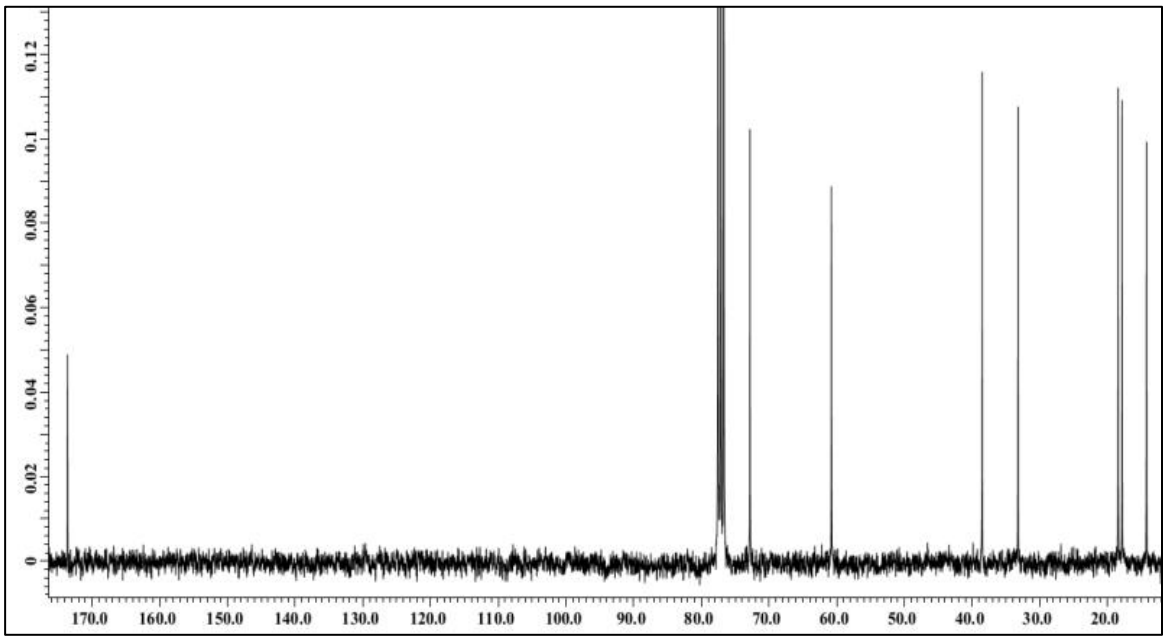

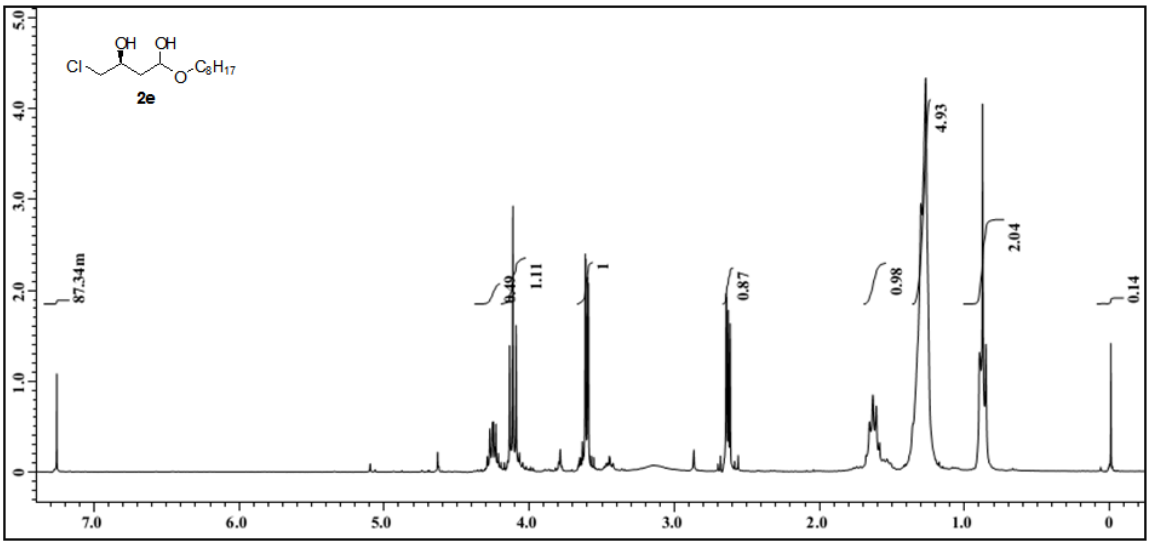
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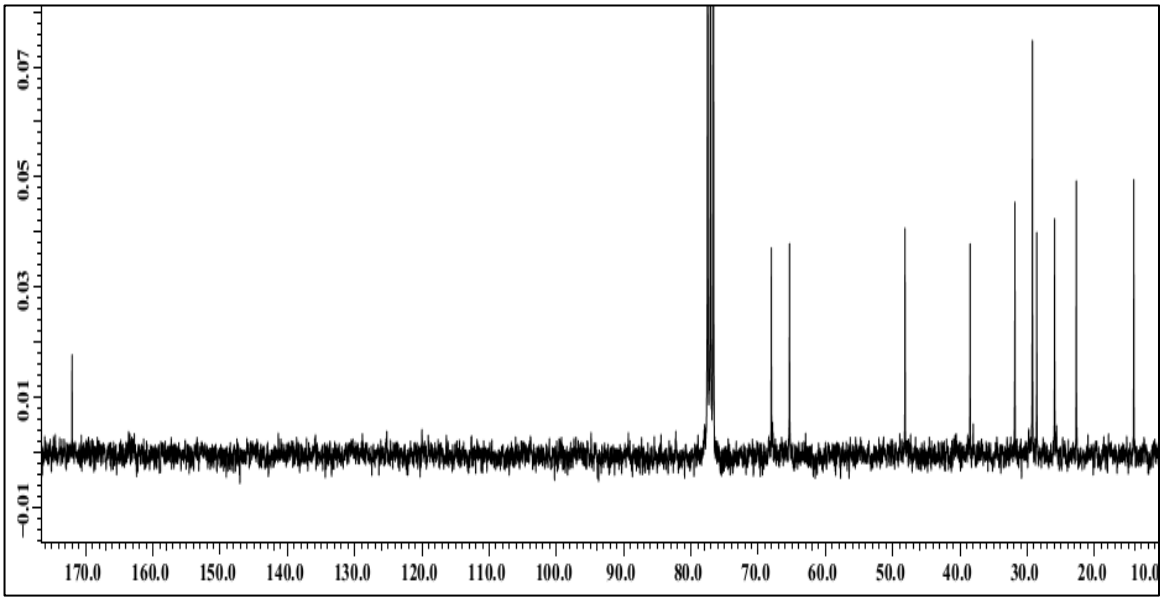
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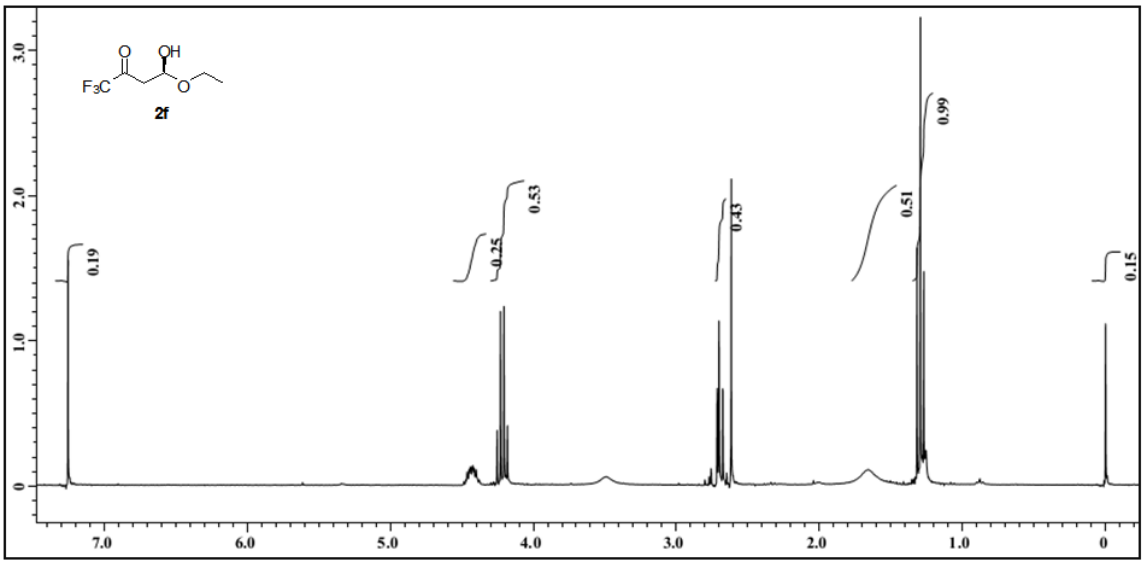
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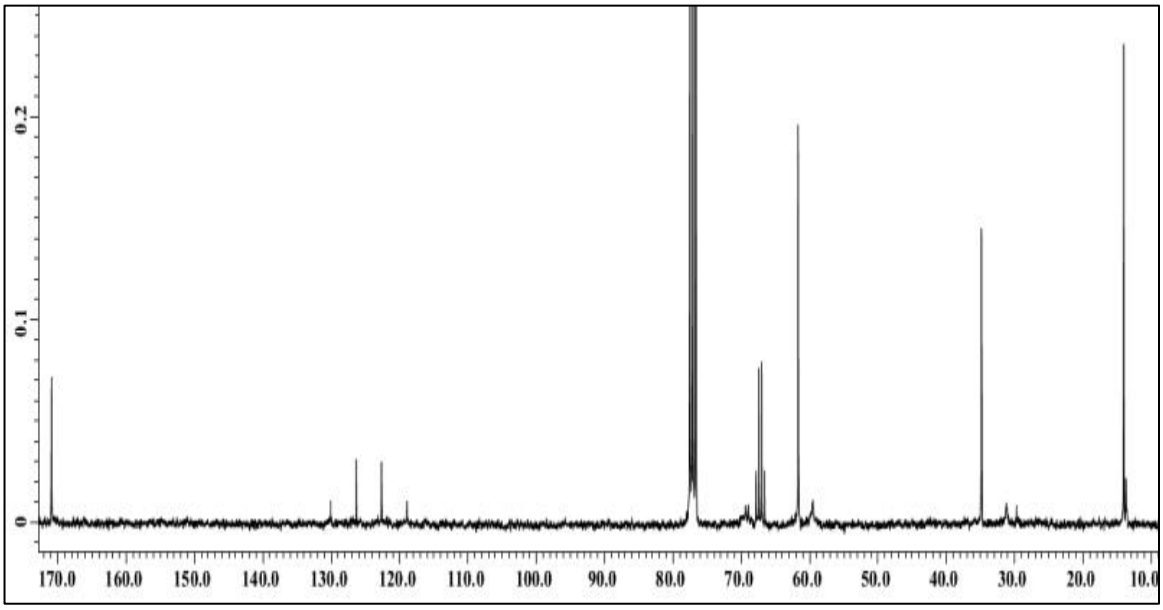
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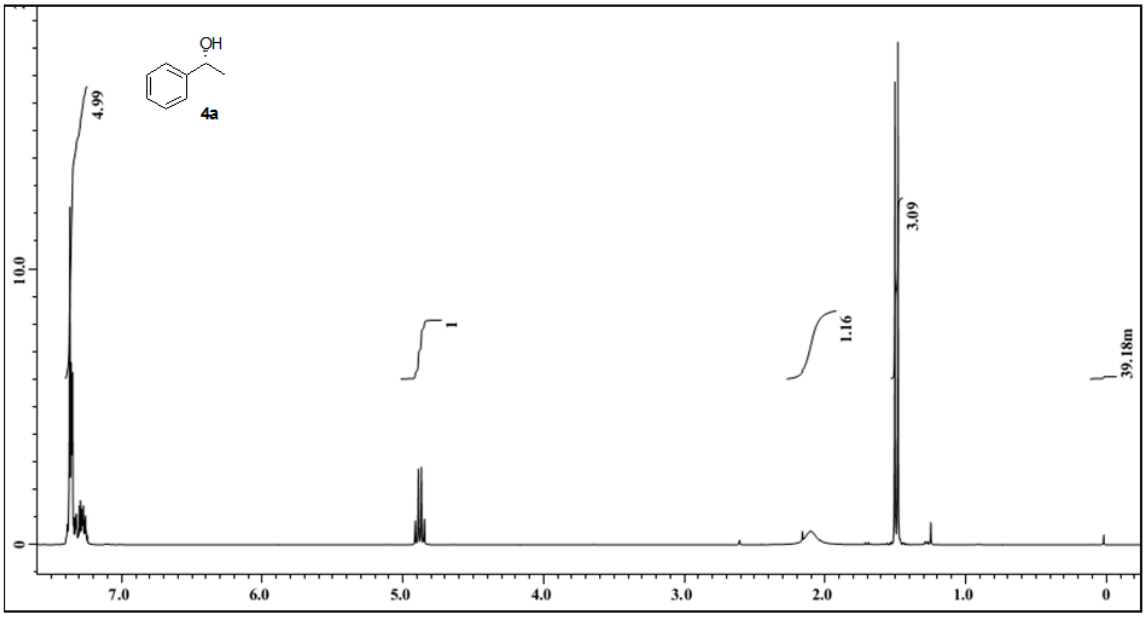
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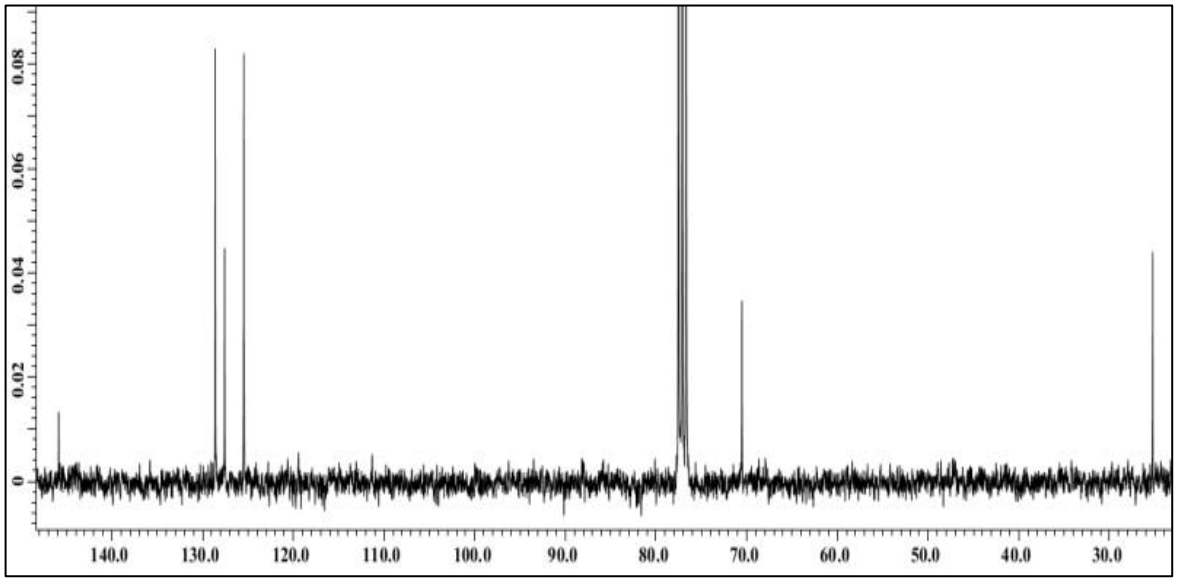
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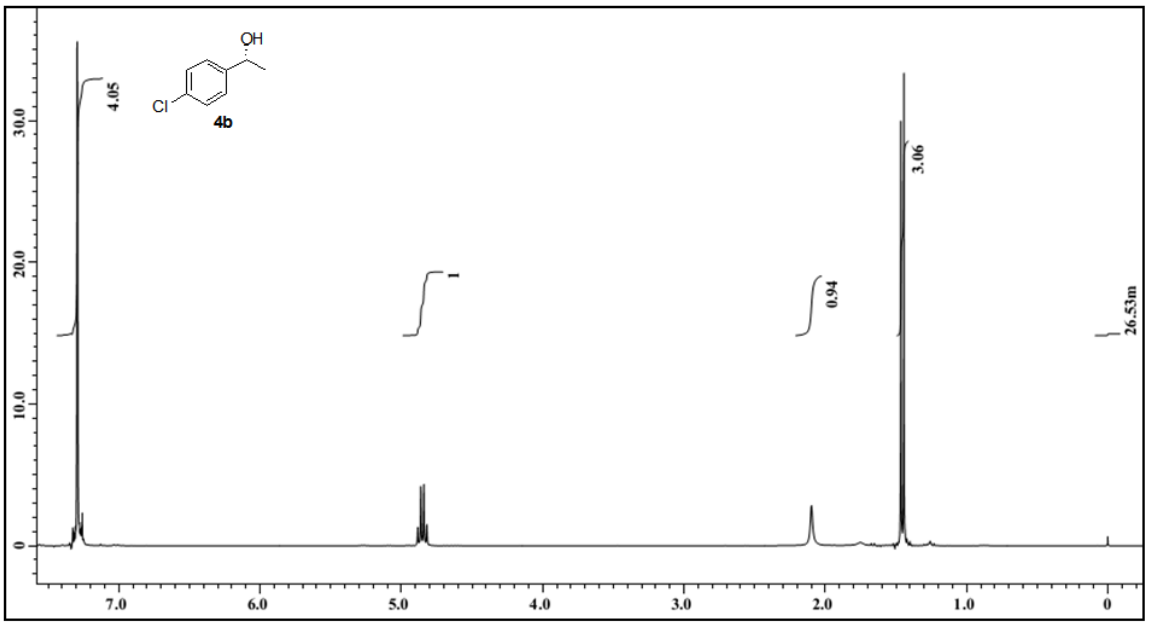
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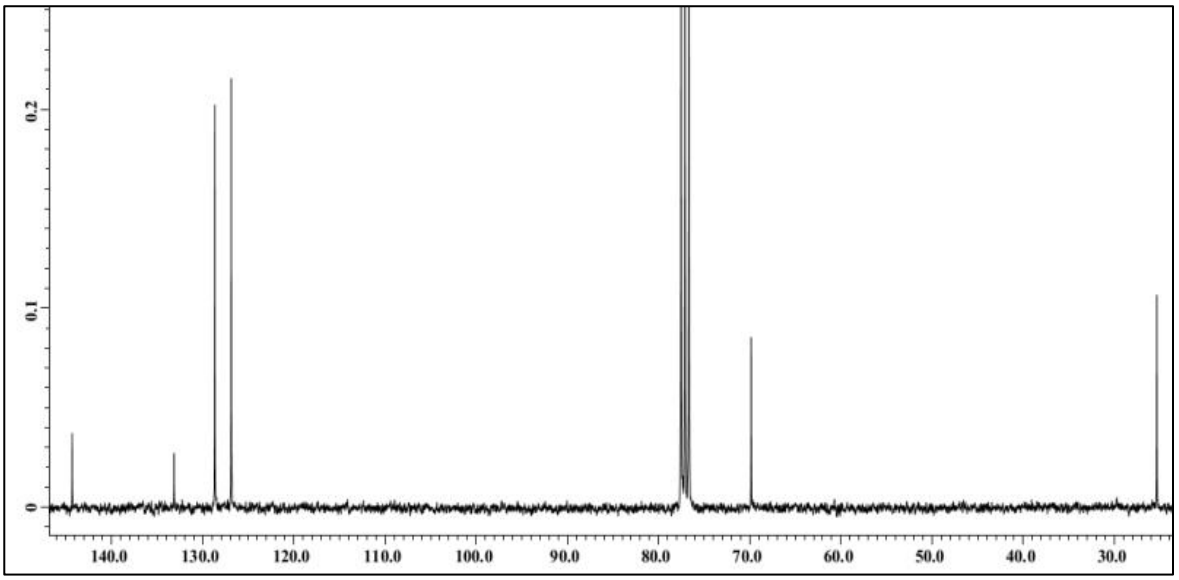
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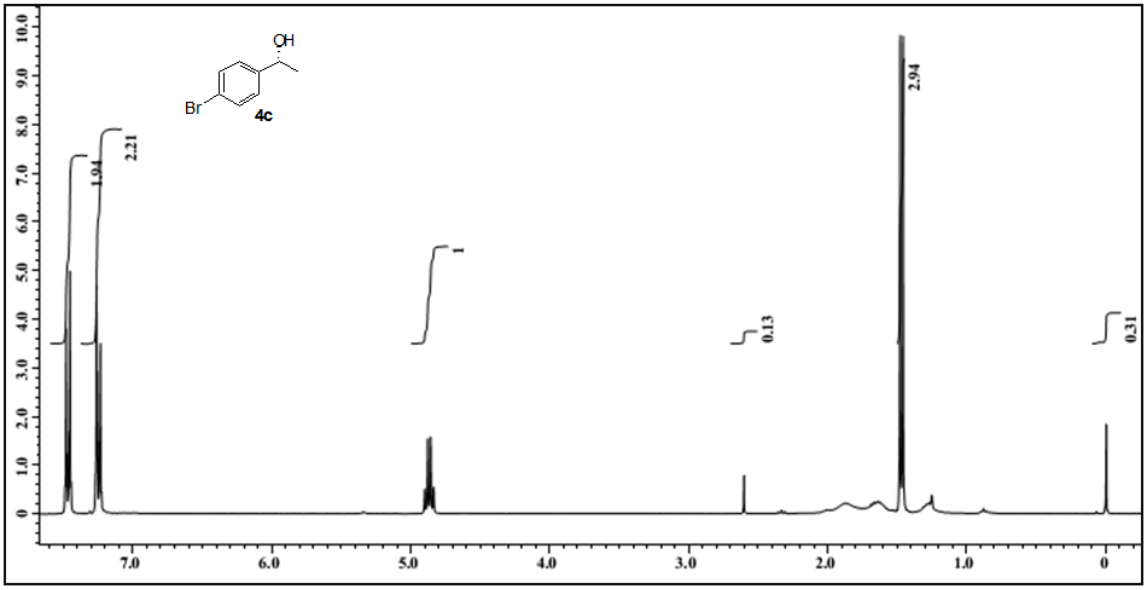
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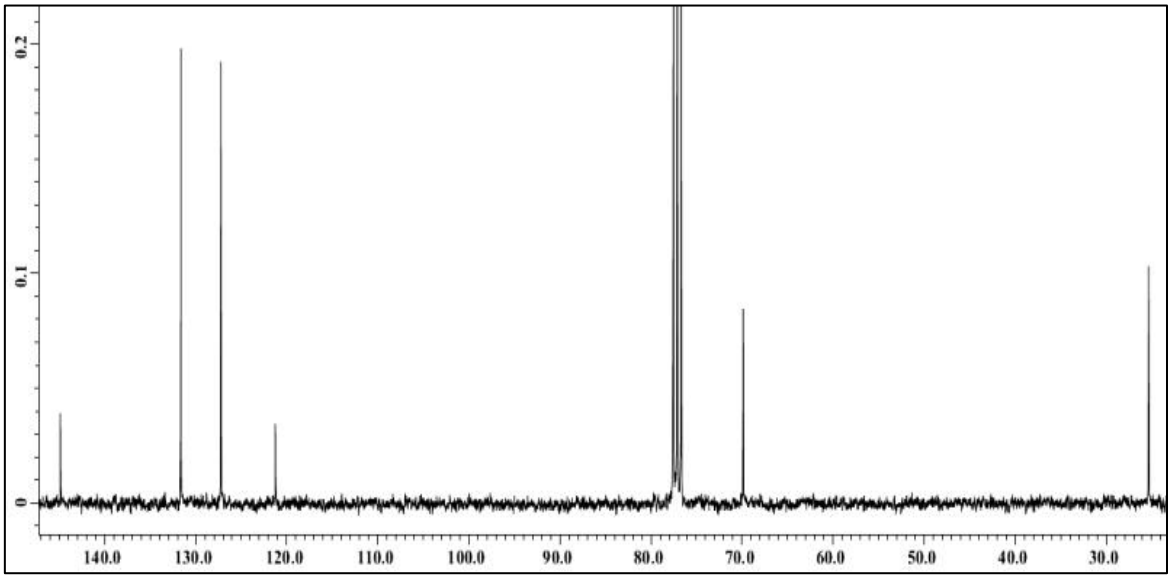
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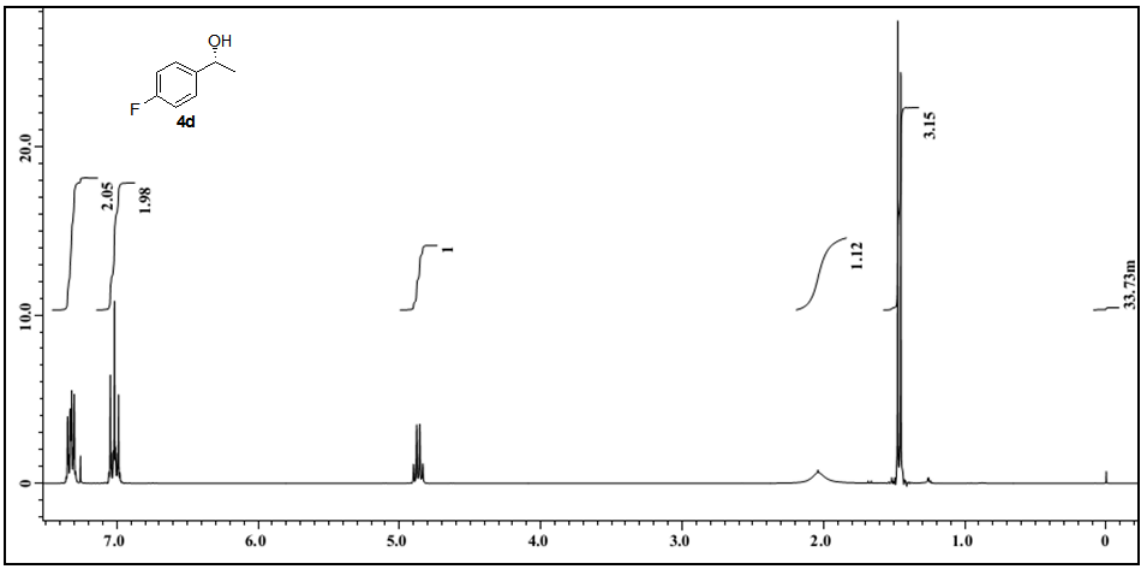
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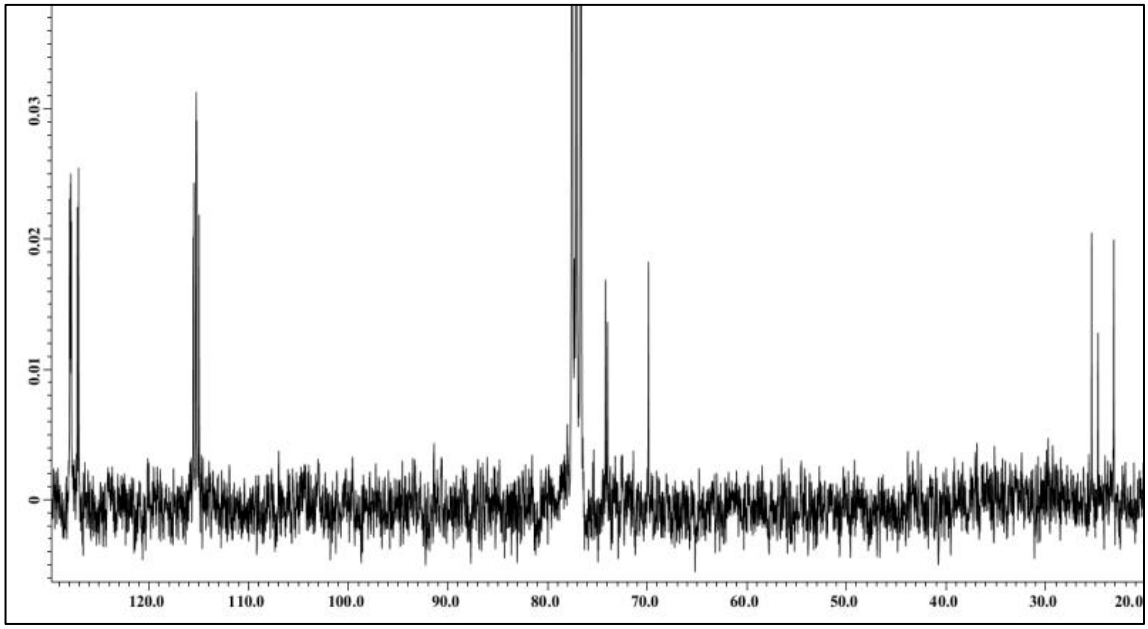
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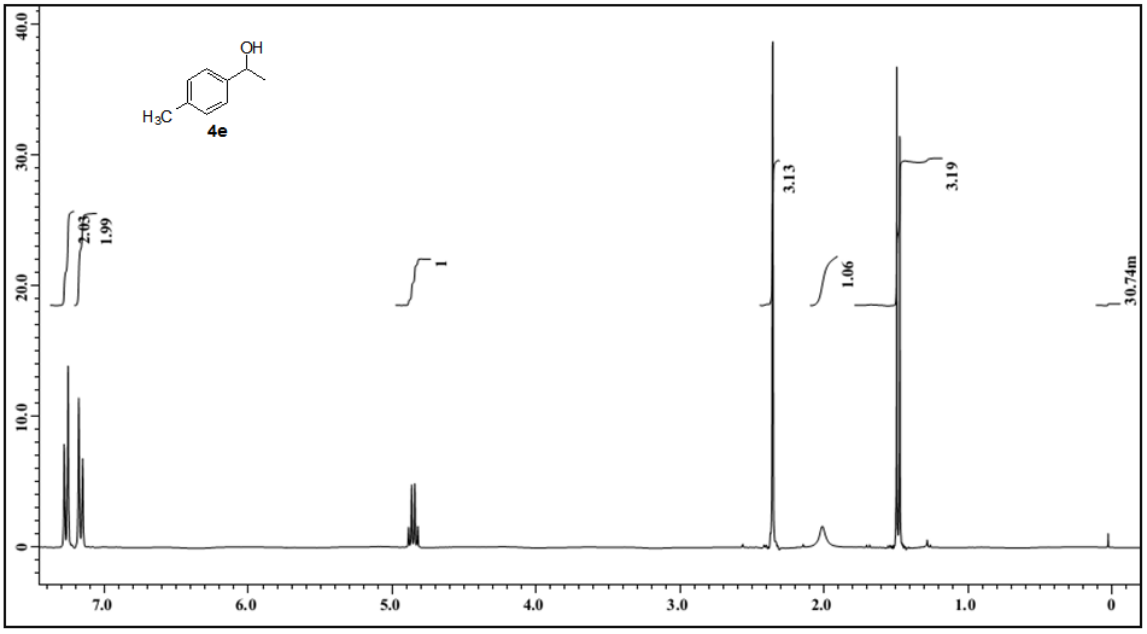
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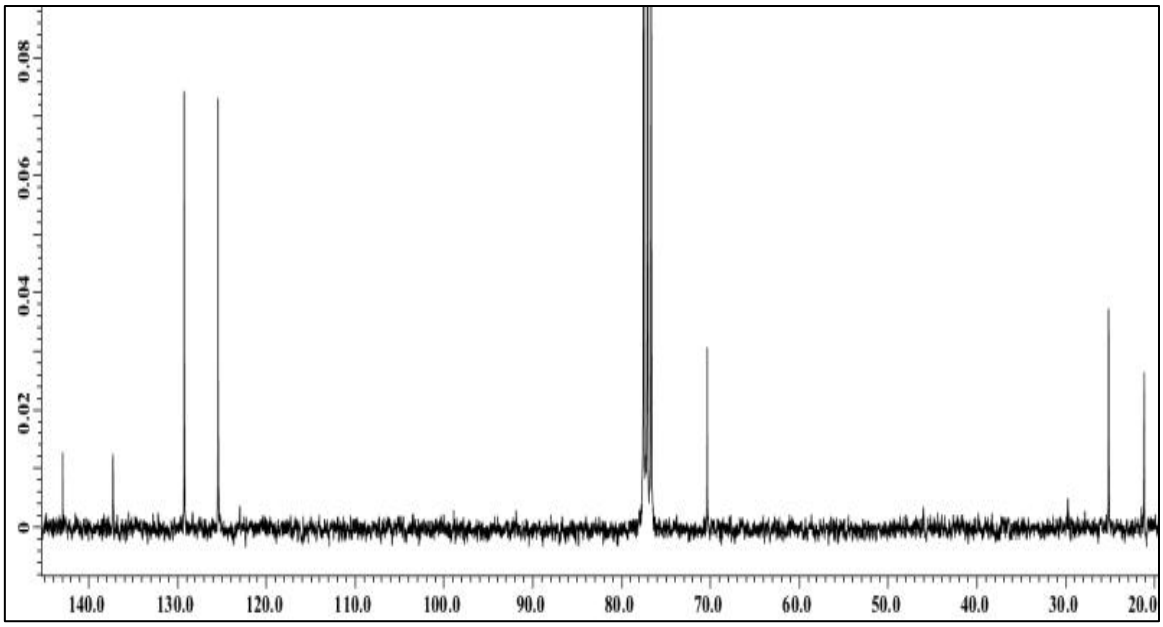

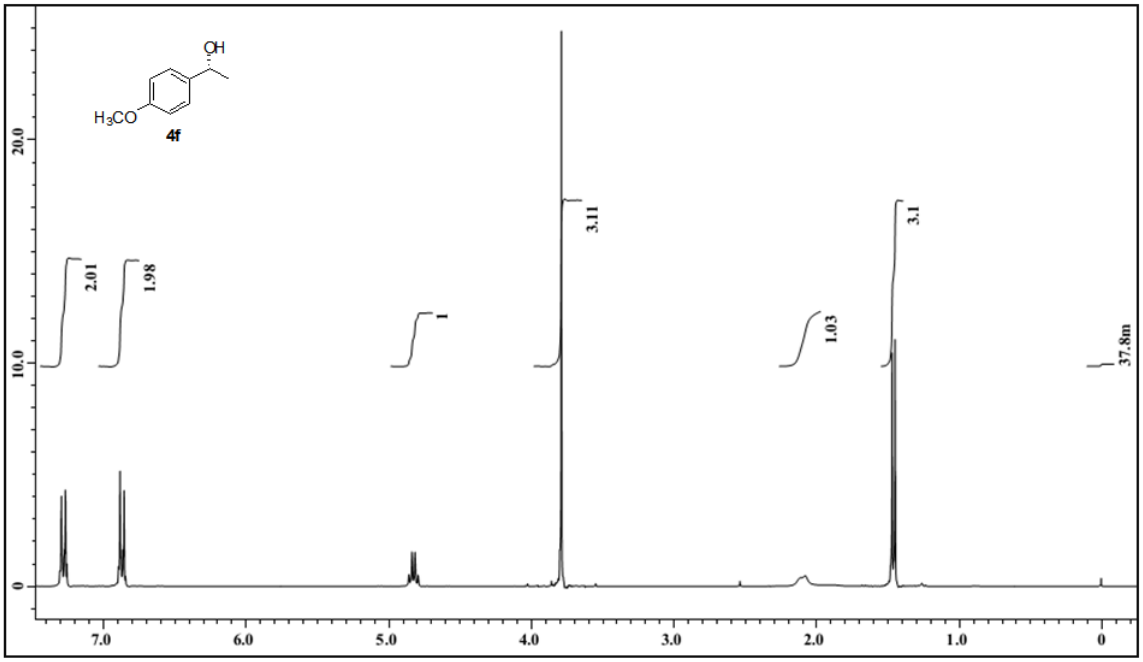
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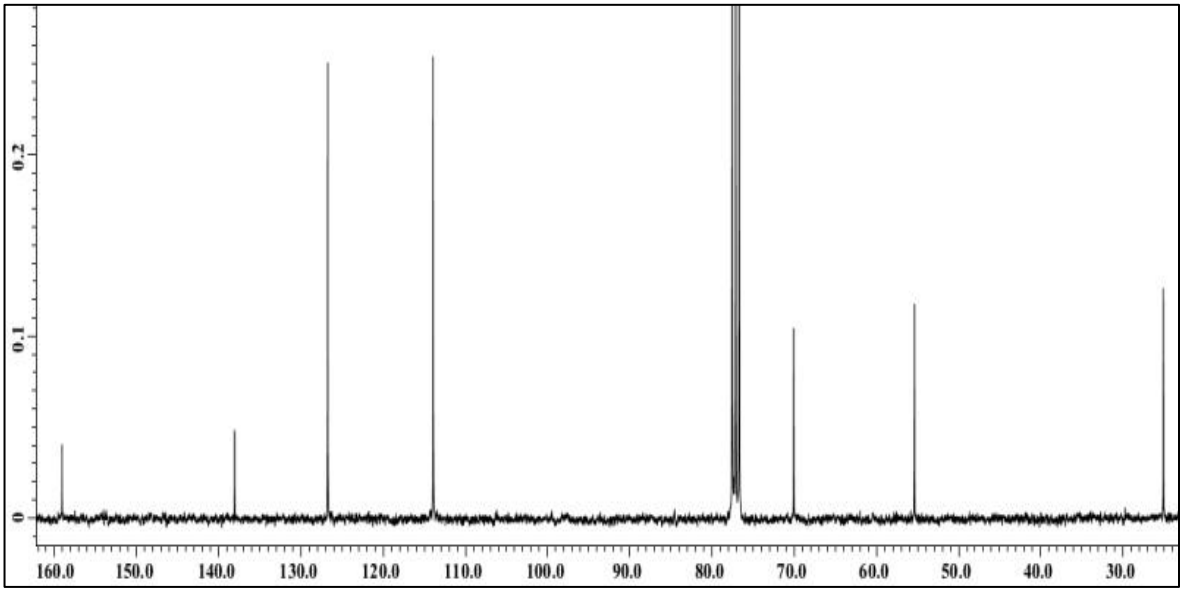
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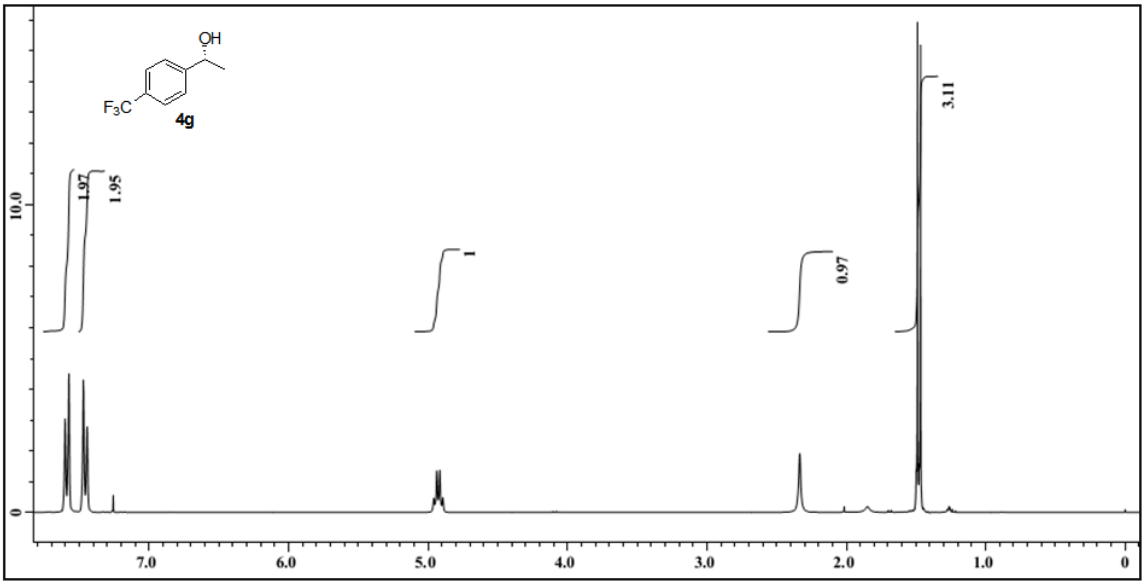
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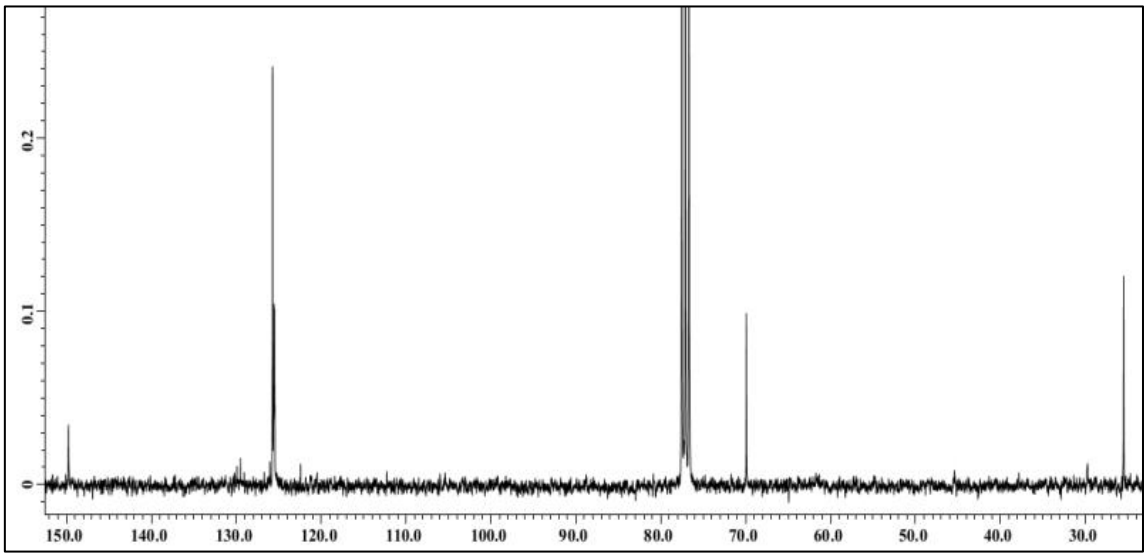
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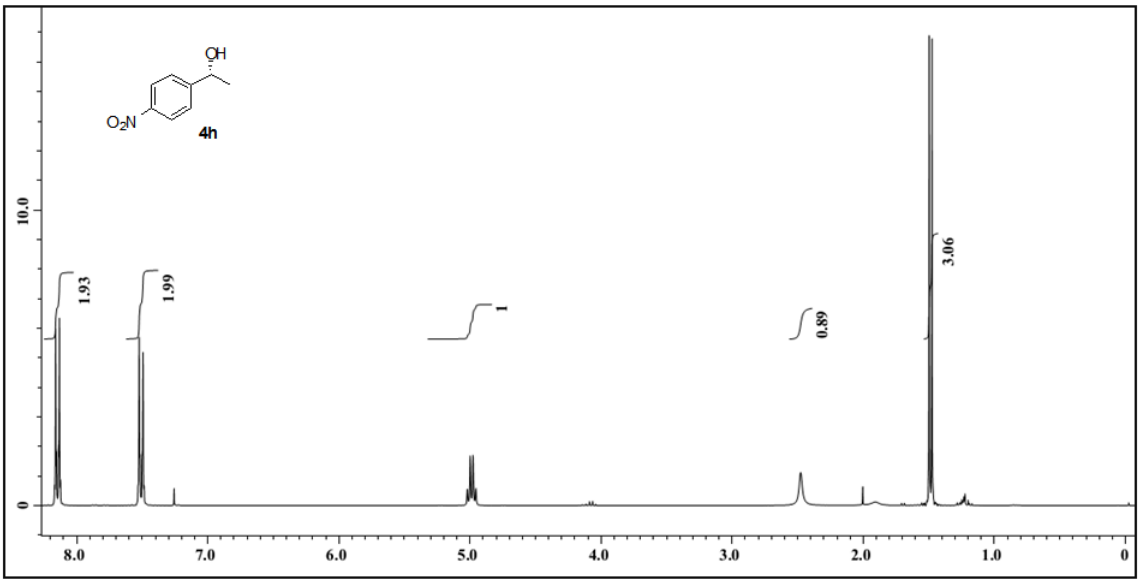
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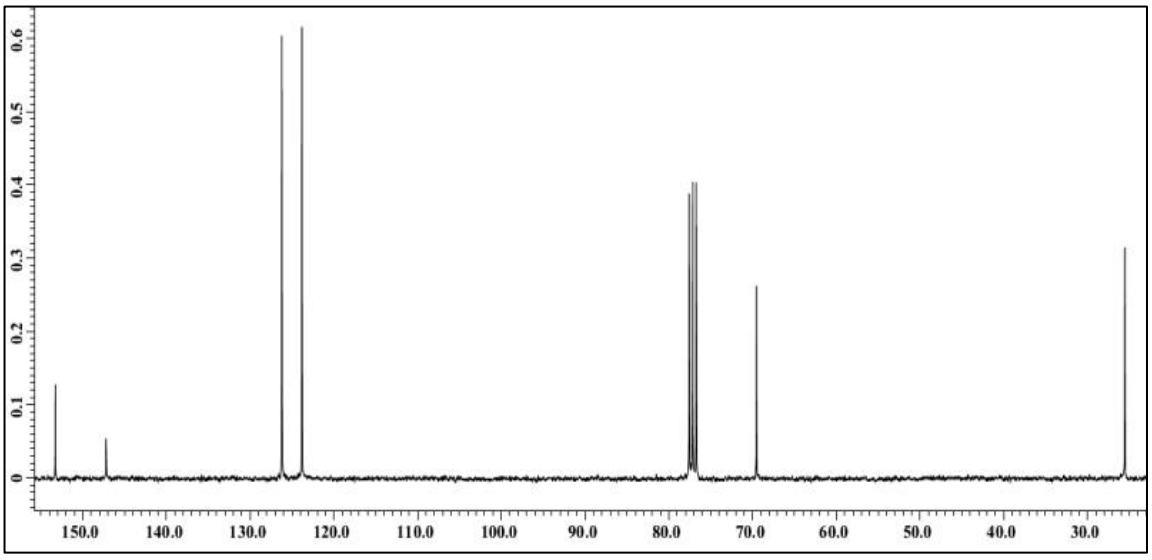
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8. Gene sequence listing

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Plasmid pETDuet1-GJCCG coexpresses carbonyl reductase and glucose dehydrogenase in the cytoplasm of *E. coli* strain

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Plasmid pETDuet1-GJSCG coexpresses carbonyl reductase and glucose dehydrogenase on the surface of *E. coli* strain

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Plasmid pET23(a)-GJSC expresses carbonyl reductase on the surface of *E. coli* strain

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Plasmid pET29(a)-GJSG expresses glucose dehydrogenase on the surface of *E. coli* strain

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<213> pET23(a)-crs (**pET23(a)-GJCC**)

Plasmid pET23(a)-GJCC expresses carbonyl reductase from *Candida magnoliae* in the cytoplasm of *E. coli* strain

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Plasmid pET29(a)-GJCG expresses glucose dehydrogenase in the cytoplasm of *E. coli* strain

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