*Supplementary information*

# Improvement of (*S*)-Selective Imine Reductase GF3546 for Synthesis of Chiral Cyclic Amines

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## Materials and methods

### S.1 General information

Commercially available reagents were used without purification and purchased from Tokyo Chemical Industry Co. Ltd., FUJIFILM Wako Pure Chemical Corporation and Sigma-Aldrich unless stated otherwise. The following products from each supplier were used: polypeptone (Nippon Seiyaku, Tokyo, Japan), meat extract (Kyokuto Seiyaku, Tokyo, Japan), and yeast extract (Oriental Yeast, Tokyo, Japan). Thin-layer chromatography (TLC) was performed on TLC silica gel $60F_{254}$ (Merck KGaA, Darmstadt, Germany). Column chromatography was performed on Wakosil® 60 (FUJIFILM Wako Pure Chemical Corporation, spherical, 64–210 µm). HPLC analyses were performed using LC-20AD pump, SPD-10A detector (Shimadzu, Kyoto, Japan), Atlantis dC18 5µm 4.6 × 150 mm column (Waters, Massachusetts, USA), and CHIRALPAK AD-RH 5µm 4.6 × 250 mm column (Daicel, Osaka, Japan). Conversion rate and optical purity of amines were determined by HPLC after derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) or methyl isothiocyanate (MITC) at 40°C for 1 h. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a JEOL ECA600 spectrometer (600 MHz for $^{1}H$ and 150 MHz for $^{13}C$) in $CDCl_3$ or $CD_3OD$ using tetramethylsilane as an internal standard (δ = 0 ppm). Polymerase chain reaction (PCR), restriction enzyme digestion, and DNA ligation were performed using TaKaRa PCR Thermal Cycler Dice® mini (Takara Bio, Shiga, Japan).

### S.2 Synthesis of cyclic nitrogen-containing compounds

*N*-Boc-2-pyrrolidone

A solution of 2-pyrrolidone (5.43 g, 63.82 mmol) and *N*,*N*-(dimethylamino)pyridine (17.3 mg) in CPME (10 mL) was added to di-*tert*-butyl dicarbonate (13.91 g, 63.75 mmol) at room temperature, and the mixture was stirred at 25–30°C overnight. The reaction mixture was diluted with 200 mL ethyl acetate, washed with 5% (w/v) $NaH_2PO_4$, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, *n*-hexane:ethyl acetate = 3:2 and 1:1) to yield *N*-Boc-2-pyrrolidone (11.07 g, 94% yield) as a colorless oil. $^{1}H$ NMR (600 MHz, $CDCl_3$): δ (ppm) 1.53 (s, 9H), 2.00 (quin, *J* = 7.7 Hz, 2H), 2.52 (t, *J* = 8.1 Hz, 2H), 3.75 (t, *J* = 14.4 Hz, 2H); $^{13}C$ NMR (150 MHz, $CDCl_3$): δ (ppm) 17.35, 28.0, 32.91, 46.43, 82.72, 150.18, 174.30.

2-Phenyl-1-pyrroline

Phenyl magnesium bromide (1 M solution in THF, 43.6 mL) was added dropwise to a solution of *N*-Boc-2-pyrrolidone (6.05 g, 32.69 mmol) in CPME at −78°C under nitrogen atmosphere, and the mixture was stirred overnight. The reaction was quenched by adding 2 M HCl (22 mL) and 10% (w/v) $NaH_2PO_4$ (20 mL). The mixture was extracted with ethyl acetate (3 x 100 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give white solid (8.47 g). This crude product was dissolved in THF (5 mL) and CPME (30 mL). 4 M HCl in CPME (40 mL) was added to the solution and stirred at room temperature overnight. The resulting precipitate was filtered and washed with ethyl acetate. The white solid was dissolved in $H_2O$ (20 mL) and MTBE (20 mL), and 20% (w/v) NaOH (40 mL) was then added dropwise. The mixture was extracted with MTBE (3 x 70 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give 2-phenyl-1-pyrroline (4.55

g, 97% yield) as a red-brown oil. $^{1}$H NMR (600 MHz, $CDCl_3$): δ (ppm) 2.02 (quin, *J* = 7.8 Hz, 2H), 2.91 (tt, *J* = 1.8, 8.4 Hz, 2H), 3.84 (s, 3H), 4.03 (tt, *J* = 1.8, 7.2 Hz, 2H), 6.92 (dt, *J* = 3.0, 8.4 Hz, 2H), 7.79 (dt, *J* = 2.4, 9.0 Hz, 2H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ (ppm) 22.69, 34.80, 55.27, 61.29, 113.66, 127.45, 129.12, 161.23, 172.55.

2-(4-Methoxyphenyl)-1-pyrroline

To a stirred suspension of Mg (0.51 g, 20.95 mmol) and small piece of iodine in dry THF (10 mL) under nitrogen atmosphere, 4-bromoanisole (3.74 g, 20.00 mmol) in dry THF (7 mL) was added dropwise at room temperature and stirred for 1 h. After the mixture was cooled to −78°C, *N*-Boc-2-pyrrolidone (3.50 g, 18.90 mmol) in CPME (15 mL) was added dropwise and stirred overnight. The reaction was quenched by adding 2 M HCl (10 mL) and 10% (w/v) $NaH_2PO_4$ (20 mL). The mixture was extracted with ethyl acetate (3 x 70 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give off-white solid (5.11 g). This crude product was dissolved in THF (5 mL) and CPME (5 mL). 4 M HCl in CPME (20 mL) was added to the solution and stirred at room temperature overnight. The resulting precipitate was filtered and washed with ethyl acetate. The white solid was dissolved in $H_2O$ (20 mL) and MTBE (20 mL), and 20% (w/v) NaOH (40 mL) was then added dropwise. The mixture was extracted with MTBE (3 x 70 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give 2-(4-methoxyphenyl)-1-pyrroline (1.51 g, 46% yield) as a pale-orange solid. $^{1}$H NMR (600 MHz, $CDCl_3$): δ (ppm) 2.02 (quin, *J* = 7.8 Hz, 2H), 2.91 (tt, *J* = 1.8, 8.4 Hz, 2H), 3.84 (s, 3H), 4.03 (tt, *J* = 1.8, 7.2 Hz, 2H), 6.92 (dt, *J* = 3.0, 8.4 Hz, 2H), 7.79 (dt, *J* = 2.4, 9.0 Hz, 2H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ (ppm) 22.69, 34.80, 55.27, 61.29, 113.66, 127.45, 129.12, 161.23, 172.55.

*rac*-2-(4-Methoxyphenyl)pyrrolidine

2-(4-methoxyphenyl)-1-pyrroline (179 mg, 1.02 mmol) and $NaBH_4$ (38.8 mg, 1.03 mmol) were dissolved in *i*PrOH (5 mL). Methanol (2 mL) was added and the mixture was stirred at room temperature overnight. The reaction was quenched by adding 2 M HCl (2 mL) and the mixture was stirred for 30 min. 2 M NaOH (5 mL) was then added and the mixture was extracted with MTBE (3 x 20 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (methanol-washed silica gel, methanol) to yield *rac*-2-(4-methoxyphenyl)pyrrolidine (135 mg, 74% yield) as a pale-yellow oil. $^{1}$H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.66 (dq, *J* = 8.6, 12.6 Hz, 1H), 1.80–1.95 (m, 2H), 2.14 (dddd, *J* = 4.2, 7.2, 7.8, 12.6 Hz, 1H), 2.73 (bs, 1H), 2.98 (ddd, *J* = 7.2, 8.4, 10.2 Hz, 1H), 3.18 (ddd, *J* = 5.4, 7.8, 10.2 Hz, 1H), 3.79 (s, 3H), 4.05 (t, *J* = 7.8, 1H), 6.85 (dt, *J* = 3.0, 8.4 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ (ppm) 25.40, 34.03, 46.68, 55.22, 62.04, 113.70, 127.65, 136.13, 158.51.

1-Methyl-3,4-dihydroisoquinoline

Acetyl chloride (7.8 mL, 109 mmol) was added to a solution of 2-phenylethylamine (12.16 g, 99.55 mmol) and triethylamine (10.24 g, 101.2 mmol) in acetonitrile (100 mL) at 0°C with stirring. The reaction mixture was stirred at room temperature overnight. CPME (20 mL) was added and the mixture was filtered. The

supernatant was concentrated under reduced pressure. The residue was diluted with 200 mL ethyl acetate, washed with 0.2 M HCl and 5% (w/v) $NaHCO_3$, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The resulting yellow solid (15.71 g, 97% yield) was used without further purification. The afforded *N*-acetyl-2-phenylethylamine (1.42 g, 8.716 mmol) was added to polyphosphoric acid (21.4 g) and $P_2O_5$ (2.2 g). The mixture was heated to 110°C for 15 min then 180°C for 4 h. 11% (w/v) NaOH (200 mL) was added to the reaction mixture cooled with ice water. The mixture was extracted with ethyl acetate (3 x 100 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, ethyl acetate) to yield 1-methyl-3,4-dihydroisoquinoline (0.837 g, 66% yield) as a yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) 2.39 (t, *J* = 1.5 Hz, 3H), 2.71 (t, *J* = 7.5 Hz, 2H), 3.66 (tq, *J* = 1.6, 7.4 Hz, 2H), 7.18 (dd, *J* = 0.9, 7.5 Hz, 1H), 7.30 (tt, *J* = 0.7, 7.6 Hz, 1H), 7.35 (td, *J* = 1.2, 14.4 Hz, 1H), 7.48 (dd, *J* = 0.9, 7.5 Hz, 1H); $^{13}$C NMR (150 MHz, $CDCl_3$): δ (ppm) 23.23, 25.99, 46.86, 125.26, 126.83, 127.37, 129.52, 130.52, 137.36, 164.28.

**S.3 Cloning and overexpression**

**Plasmid construction**

pET-21a(+)/SIR46

The *SIR46* gene on pT7Blue vector was amplified by PCR with *TaKaRa Ex Taq*® (Takara Bio) using two primers containing *Nde* I or *EcoR* I restriction site. The amplified DNA fragment and pET-21a(+) vector (Merck KGaA) were digested with *Nde* I and *EcoR* I, and ligated using DNA Ligation Kit (Takara Bio). The resulting plasmid was transformed into *E. coli* JM109 and isolated from the recombinant cells.

pACYDuet-1/BsGDH and pACYCDUet-1/BsGDH-SIR46

The genome DNA of *Bacillus subtilis* NBRC3026 was prepared using ISOPLANT II (NIPPON GENE, Tokyo, Japan). The *BsGDH* gene on the genome DNA was amplified by PCR with *TaKaRa Ex Taq*® (Takara Bio) using two primers containing *Nde* I or *Xho* I restriction site. The amplified DNA fragment and pT7Blue T-vector (Merck KGaA) were ligated using DNA Ligation Kit (Takara Bio). The resulting plasmid was transformed into *E. coli* JM109. The obtained plasmid was digested with *Nde* I and *Xho* I, and subcloned into MCS2 of pACYCDuet-1 vector (Merck KGaA) using DNA Ligation Kit (Takara Bio). The resulting plasmid was transformed into *E. coli* JM109 and isolated from the recombinant cells. The *SIR46* gene was further subcloned into MCS1 of the obtained pACYCDuet-1/BsGDH(MCS2). pACYCDuet-1/BsGDH(MCS1) and pACYCDuet-1/BsGDH(MCS1)-SIR46(MCS2) were also prepared in the same way as shown above, using *Nco* I and *EcoR* I restriction site for subcloning into MCS1 of pACYCDuet-1 vector.

pTipRT2/SIR46 and pTipQC1/BsGDH

The *SIR46* or *BsGDH* gene on pT7Blue vector were amplified by PCR with PrimeSTAR® Max DNA Polymerase (Takara Bio) using two primers containing *Nde* I or *Xho* I restriction site. Additionally, a *BsGDH* gene was commercially synthesized with codon optimization for efficient expression in *Rhodococcus* (Eurofins Genomics, Tokyo, Japan) and used in the same way as shown here. The amplified DNA fragment and pTip vectors (Hokkaido System Science, Hokkaido, Japan) were digested with *Nde* I and *Xho* I, and ligated using

DNA Ligation Kit (Takara Bio). The resulting plasmid was transformed into *E. coli* JM109 and isolated from the recombinant cells.

**Expression**

Co-expression of SIR46 and BsGDH genes in *E. coli* BL21 (DE3)

*E. coli* BL21 (DE3) was transformed with pET-21a(+)/SIR46 according to SEM transformation [1]. The resulting transformant was further used for transformation with pACYDuet-1/BsGDH. The obtained recombinant cells were cultivated at 120 rpm and 28°C in LB medium supplemented with 50 μg $mL^{-1}$ ampicillin and 20 μg $mL^{-1}$ chloramphenicol. When the optical density at 610 nm ($OD_{610}$) reached to 0.6–1.0, 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added for induction of protein expression and the culture was incubated at 28°C and 120 rpm for 5 h. The cells were harvested by centrifugation, washed with 0.85 (w/v) NaCl, and resuspended in 50 mM potassium phosphate buffer (pH 7.0). To prepare cell-free extract, 0.1 mM dithiothreitol was added and disrupted by ultrasonication at 100 W for 5 min at 0°C using a cell disrupter (19 kHz, Insonator 201 M; Kubota, Japan). The cell debris was removed by centrifugation at 4°C and 12,000 rpm for 30 min and the supernatant solution was used as cell-free extract. The amount of the protein extracted from the cells was approximately 0.5 g per liter of culture medium.

Expression of SIR46 gene in *R. erythropolis* L88

*R. erythropolis* L88 (Hokkaido System Science) was transformed with pTipRT2/SIR46 by electroporation using Eporator® (Eppendorf, Hamburg, Germany). The transformant was cultivated with 10 μg $mL^{-1}$ tetracycline at 120 rpm and 28°C in 5 mL of nutrient medium A containing 10 g $L^{-1}$ tryptone, 5 g $L^{-1}$ yeast extract, 4 g $L^{-1}$ $Na_2HPO_4$, 1 g $L^{-1}$ $KH_2PO_4$, 1 g $L^{-1}$ NaCl, 0.2 g $L^{-1}$ $MgSO_4 \cdot 7H_2O$, and 10 mg $L^{-1}$ $CaCl_2 \cdot 7H_2O$ in tap water. The preculture was inoculated in 90 mL of the nutrient medium with 0.2 μg $mL^{-1}$ thiostrepton and incubated at 20°C and 120 rpm for 24 h. The cells were harvested by centrifugation and washed twice with 50 mM potassium phosphate buffer (pH 7.0). To prepare cell-free extract, the cells were resuspended in 50 mM potassium phosphate buffer (pH 7.0) with 1.0 mg $mL^{-1}$ lysozyme and 0.1 mM dithiothreitol, and incubated at 4°C overnight. The treated cells were disrupted by ultrasonication at 100 W for 20 min at 0°C using a cell disrupter (19 kHz, Insonator 201 M; Kubota, Japan). The cell debris was removed by centrifugation at 4°C and 12,000 rpm for 30 min and the supernatant solution was used as cell-free extract. The amount of the protein extracted from the cells was 0.5–0.6 g per liter of culture medium.

Co-expression of SIR46 and BsGDH genes in *R. erythropolis* L88

The recombinant *R. erythropolis* L88_pTipRT2/SIR46 was cultivated at 120 rpm and 28°C in 5 mL of nutrient medium A supplemented with 10 μg $mL^{-1}$ tetracycline until $OD_{610}$ reached to 1.0. The cells were harvested by centrifugation, washed with water, and resuspended in 10% (v/v) glycerol supplemented with 1% (v/v) trehalose. The cell suspension was used for transformation of *R. erythropolis* L88_pTipRT2/SIR46 with pTipQC1/BsGDH. The resulting transformant was cultivated with 10 μg $mL^{-1}$ tetracycline at 120 rpm and 28°C in 5 mL of nutrient medium A supplemented with 10 μg $mL^{-1}$ tetracycline and 30 μg $mL^{-1}$ chloramphenicol. Induction of protein expression in the recombinant cells was performed in the same way

as single expression of *SIR46* in *R. erythropolis* L88. The amount of the protein extracted from the cells was approximately 0.5 g per liter of culture medium.

**S.4 Variant construction**

SIR46 variants were generated by amplifying full-length plasmid pTipRT2-SIR46 by PCR according to the instructions of PrimeSTAR® Mutagenesis Basal Kit (Takara-Bio). The PCR products were digested with Dpn I and purified by gel electrophoresis. The resulting DNAs were used to transform *E coli* JM109 for plasmid preparation. Introduced mutations were verified by dye-terminator sequencing.

**S.5 Biotransformation**

Biotransformations were performed at 30°C with shaking (120 rpm) in potassium phosphate buffer (pH 7.0) containing imine substrate, glucose and whole cells or cell-free extract. Samples were collected multiple times and analyzed by HPLC after amine derivatization with GITC.

**S.6 Enzyme assay**

Imine reductase activity was determined by monitoring the decrease of NADPH with UV1600PC (Shimadzu, Tokyo, Japan) at 340 nm ($\varepsilon$ = 6.22 $mM^{-1}$ $cm^{-1}$) at 30°C. The reaction mixture (1 mL) generally contained 100 mM potassium phosphate buffer (pH 7.0), 10 mM 2-methyl-1-pyrroline, 0.15 mM NADPH and 20-100 µL cell-free extract. The reaction was started by adding cell-free extract to the mixture. One unit (U) of imine reductase activity was defined as the amount of protein that oxidized 1 µmol of NADPH at 30°C per minute. Glucose dehydrogenase activity was determined by monitoring the increase of NADPH. The reaction mixture (1 mL) generally contained 100 mM potassium phosphate buffer (pH 7.0), 10 mM glucose, 0.15 mM $NADP^+$ and 0.4 µL cell-free extract. One unit (U) of glucose dehydrogenase activity was defined as the amount of protein that reduced 1 µmol of $NADP^+$ at 30°C per minute. To examine the activity of NADPH regeneration in the cell-free extract of *Rhodococcus* expressing wild-type SIR46 gene, 0.5 mM ATP and 1 mM $MgCl_2$ were further added if necessary. The NADPH-regenerating activity with glucose 6-phosphate instead of glucose was also examined.

**S.7 Effect of temperature, pH and organic solvent on SIR46 activity**

To determine the thermal stability of SIR46, the cell-free extract containing SIR46 variant was pre-incubated at temperatures from 40 to 60°C for 30 min and cooled on ice before imine reductase activity was determined. Effect of temperature was examined in a 100 mM potassium phosphate buffer (pH 7.0) containing 10 mM 2-methyl-1-pyrroline, 0.15 mM NADPH and 20 or 50 µL cell-free extract at temperatures from 35 to 60°C. Effect of pH was examined at 30°C using various 100 mM buffers with different pH values including potassium phosphate buffer (KPB) (pH 6.0–8.0), Tris-HCl (pH 7.5–9.5) and HEPES (pH 7.0–8.0), containing 10 mM 2-methyl-1-pyrroline, 0.15 mM NADPH and 50 µL cell-free extract. Effect of organic solvent was examined at 30°C in 100 mM potassium phosphate buffer containing 10 mM 2-methyl-1-pyrroline, 0–20% (v/v) organic solvent, 0.15 mM NADPH and 50 µL cell-free extract.

**S.8 Protein analysis**

Protein concentration was determined by the Bradford method [2], using a protein assay kit (Bio-Rad Laboratories, Inc., California, USA) and bovine serum albumin as a standard. In column chromatography, absorbance at 280 nm was measured to detect eluted protein. SDS-PAGE was performed on 12% (w/v) polyacrylamide gel with Tris-glycine buffer system according to Laemmli [3]. Proteins were stained with Coomassie Brilliant Blue R250 and gels destained in ethanol/acetate/$H_2O$ (3:1:6, v/v/v).

**S.9 DNA and amino acid sequences**

(*S*)-Selective imine reductase from *Streptomyces* sp. GF3546 (SIR46)

Wild-type SIR46 DNA sequence

ATGAGCAAGCAGTCGGTAACGGTCATCGGTCTGGGCCCGATGGGCCAGGCGATGGTGAACACCTTCCTGGACAACGG
CCACGAGGTGACCGTGTGGAACCGGACCGCCAGCAAGGCCGAGGCGCTCGTGGCCCGGGGTGCCGTACTGGCTCCC
ACCGTCGAGGACGCGCTCAGCGCCAATGAGCTGATCGTGCTCAGCCTGACGGACTACGACGCGGTGTACGCCATTCTT
GAGCCGGTGACGGGTTCCCTGTCCGGCAAGGTCATCGCCAACCTCAGCTCCGACACCCCGGACAAGGCCCGCGAGG
CGGCCAAGTGGGCGGCCAAGCACGGTGCGAAGCACCTCACCGGTGGTGTGCAGGTGCCGCCGCCGCTGATCGGCAA
GCCCGAGTCCTCCACCTACTACAGCGGTCCCAAGGATGTCTTCGACGCCCATGAGGACACCCTGAAGGTCCTCACCAA
CGCGGACTACCGCGGCGAGGACGCCGGCCTCGCGGCCATGTACTACCAGGCCCAGATGACCATCTTCTGGACCACGAT
GCTGAGCTACTACCAGACCCTCGCGCTGGGCCAGGCCAACGGTGTCTCGGCGAAGGAACTGCTGCCCTACGCCACGA
TGATGACGTCGATGATGCCGCACTTCCTGGAGCTGTACGCCCAGCACGTGGACTCCGCGGACTACCCGGGCGACGTG
GACCGGCTCGCGATGGGGGCGGCCAGTGTCGACCACGTCCTGCACACGCACCAGGACGCGGGCGTCAGCACCGTGC
TGCCGGCCGCCGTCGCCGAGATCTTCAAGGCGGGCATGGAGAAGGGCTTCGCCGAGAACAGCTTCTCCAGCCTCATC
GAGGTGCTCAAGAAGCCGGCGGTCTGA

Wild-type SIR46 amino acid sequence

MSKQSVTVIGLGPMGQAMVNTFLDNGHEVTVWNRTASKAEALVARGAVLAPTVEDALSANELIVLSLTDYDAVYAILEPV
TGSLSGKVIANLSSDTPDKAREAAKWAAKHGAKHLTGGVQVPPPLIGKPESSTYYSGPKDVFDAHEDTLKVLTNADYRGED
AGLAAMYYQAQMTIFWTTMLSYYQTLALGQANGVSAKELLPYATMMTSMMPHFLELYAQHVDSADYPGDVDRLAMG
AASVDHVLHTHQDAGVSTVLPAAVAEIFKAGMEKGFAENSFSSLIEVLKKPAV*

SIR46 M9 variant DNA sequence

ATGAGCAAGCAGTCGGTAACGGTCATCGGTCTGGGCCCGATGGGCCAGGCGATGGTGAACACCTTCCTGGACAACGG
CCACGAGGTGACCGTGTGGAACCGGACCGCCAGCAAGGCCGAGCCGCTCGTGGCCCGGGGTGCCACGCTGGCTCCC
ACCGTCGAGGACGCGCTCAGCGCCAATGAGCTGATCGTGCTCAGCCTGACGGACTACGACGCGGTGTACGCCATTCTT
GAGCCGGTGACGGGTTCCCTGTCCGGCAAGACCATCGTCAACCTCAGCTCCGACACCCCGGACAAGGCCCGCGAGAT
GGCCAAGTGGGCGGCCAAGCACGGTGCGAAGCACCTCACCGGTGGTGTGCAGGTGCCGCCGCCGCTGATCGGCAAG
CCCGAGTCCTCCACCTACTACAGCGGTCCCAAGGATGTCTTCGACGCCCATGAGGACACCCTGAAGGTCCTCACCAAC
GCGGACTACCTCGGCGAGGACCCCGGCCTCGCGGCCATGTACTACCAGGCCCAGATGACCATCTTCTGGACCACGATG

CTGAGCTACTACCAGACCCTCGCGCTGGGCCAGGCCAACGGTGTCTCGGCGAAGGAACTGCTGCCCTACGCCACGAT
GACCACGCGCATGATGCCGCACTTCCTGGAGCTGTACGCCCAGCACGTGGACTCCGCGGACTACCCGGGCGACGTGG
ACCGGCTCGCGATGGGGGCGGCCAGTGTCGACCACGTCCTGCACACGCACCAGGACGCGGGCGTCAGCACCGTGCT
GCCGGCCGCCGTCGCCGAGATCTTCAAGGCGGGCATGGAGAAGGGCTTCGCCGAGAACAGCTTCTCCAGCCTCATCG
AGGTGCTCAAGAAGCCGGCGGTCTGA

SIR46 M9 variant amino acid sequence

MSKQSVTVIGLGPMGQAMVNTFLDNGHEVTVWNRTASKAEPLVARGATLAPTVEDALSANELIVLSLTDYDAVYAILEPV
TGSLSGKTIVNLSSDTPDKAREMAKWAAKHGAKHLTGGVQVPPPLIGKPESSTYYSGPKDVFDAHEDTLKVLTNADYLGED
PGLAAMYYQAQMTIFWTTMLSYYQTLALGQANGVSAKELLPYATMTTRMMPHFLELYAQHVDSADYPGDVDRLAMGA
ASVDHVLHTHQDAGVSTVLPAAVAEIFKAGMEKGFAENSFSSLIEVLKKPAV*

Glucose dehydrogenase from *Bacillus subtilis* NBRC3026 (BsGDH)

BsGDH DNA sequence

ATGGGCCACCATCACCATCACCATATGTATCCGGATTTAAAAGGAAAAGTCGTCGCTATTACAGGAGCTGCTTCAGGGC
TCGGAAAGGCGATGGCCATTCGCTTCGGCAAGGAGCAGGCAAAAGTGGTTATCAACTATTATAGTAATAAACAAGATC
CGAACGAGGTAAAAGAAGAGGTCATCAAGGCGGGCGGTGAAGCTGTTGTCGTCCAAGGAGATGTCACGAAAGAGG
AAGATGTAAAAAATATCGTGCAAACGGCAATTAAAGAGTTCGGCACACTCGATATTATGATTAATAATGCCGGTCTTGAA
AATCCTGTACCATCTCACGAAATGCCGCTCAAGGATTGGGATAAAGTCATCGGCACGAACTTAACGGGTGCCTTTTTAG
GAAGCCGTGAAGCGATTAAATATTTCGTAGAAAACGATATCAAGGGAAATGTCATTAACATGTCCAGTGTGCACGAAGT
GATTCCTTGGCCGTTATTTGTCCACTATGCGGCAAGTAAAGGCGGGATAAAGCTGATGACAGAAACATTAGCGTTGGA
ATACGCGCCGAAGGGCATTCGCGTCAATAATATTGGGCCAGGTGCGATCAACACGCCAATCAATGCTGAAAAATTCGCT
GACCCTAAACAGAAAGCTGATGTAGAAAGCATGATTCCAATGGGATATATCGGCGAACCGGAGGAGATCGCCGCAGTA
GCAGCCTGGCTTGCTTCGAAGGAAGCCAGCTACGTCACAGGCATCACGTTATTCGCGGACGGCGGTATGACACAATAT
CCTTCATTCCAGGCAGGCCGCGGTTGA

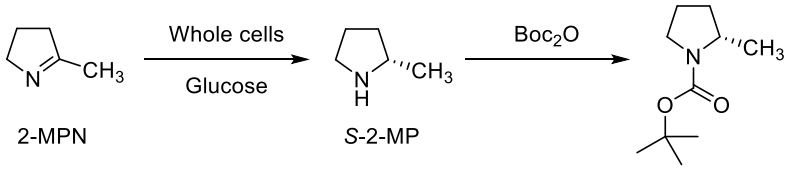
Codon-optimized BsGDH DNA sequence

ATGGGCCACCATCACCATCACCATATGTACCCCGACCTCAAGGGGAAGGTGGTCGCCATCACCGGCGCGGCCTCCGGC
CTGGGTAAGGCCATGGCGATCCGCTTCGGCAAGGAGCAGGCGAAGGTCGTCATCAACTACTACAGCAACAAGCAGGA
CCCGAACGAGGTCAAGGAGGAAGTCATCAAGGCCGGCGGCGAAGCCGTCGTCGTGCAGGGCGACGTCACGAAGGA
GGAGGACGTCAAGAACATCGTCCAGACCGCCATCAAGGAGTTCGGCACGCTCGACATCATGATCAACAACGCCGGCC
TGGAGAACCCCGTCCCGTCGCACGAGATGCCGCTGAAGGACTGGGACAAGGTGATCGGCACCAACCTGACCGGCGC
CTTCCTCGGCAGCCGGGAGGCCATCAAGTACTTCGTGGAGAACGACATCAAGGGCAACGTGATCAACATGTCCTCCGT
GCACGAGGTCATCCCGTGGCCGCTGTTCGTCCACTACGCCGCCTCCAAGGGCGGGATCAAGCTGATGACCGAAACCCT
GGCGCTGGAGTACGCCCCGAAGGGCATCCGCGTGAACAACATCGGTCCCGGCGCCATCAACACGCCCATCAACGCCG
AGAAGTTCGCGGACCCGAAGCAGAAGGCCGACGTCGAGTCGATGATCCCCATGGGCTACATCGGGGAACCGGAGGA
GATCGCCGCGGTGGCGGCGTGGCTGGCCTCGAAGGAGGCGTCGTACGTCACCGGCATCACGCTCTTCGCCGACGGC
GGCATGACCCAGTACCCGTCCTTCCAGGCGGGTCGCGGGTGA

BsGDH amino acid sequence

MGHHHHHHMYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYSNKQDPNEVKEEVIKAGGEAVVVQGDVTKEE
DVKNIVQTAIKEFGTLDIMINNAGLENPVPSHEMPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIP
WPLFVHYAASKGGIKLMTETLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAWLAS
KEASYVTGITLFADGGMTQYPSFQAGRG*

**S.10 Preparative-scale biotransformation of 2-MPN**




(*S*)-*N*-Boc-2-methylpyrrolidine

Cell suspension of *R. erythropolis* L88 expressing SIR46 M9 and BsGDH genes in 50 mM potassium phosphate buffer (5.0 mL, $OD_{610}$ = 50, pH 7.0) was added to a solution of 2-MPN (0.25 g, 3.00 mmol) and glucose (0.54 g, 3.60 mmol) in potassium phosphate buffer (5.0 mL, 200 mM, pH 7.75). The mixture (10 mL) was incubated at 30°C and 70 rpm for 7 days in 50 mL falcon tube. The reaction mixture was centrifugated to remove the cells. NaOH (0.51 g, 12.6 mmol) in 10 mL $H_2O$ and di-*tert*-butyl dicarbonate (1.07 g, 4.92 mmol) in 10 mL MTBE was added to the supernatant, and the mixture was stirred at room temperature overnight. After MTBE (40 mL) was added to the reaction mixture, organic phase was washed with $H_2O$ (20 mL) and 5% (w/v) $NaH_2PO_4$ (2 x 25mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, *n*-hexane:ethyl acetate = 20:1) to yield (*S*)-*N*-Boc-2-methylpyrrolidine (0.33 g, 1.79 mmol, 60% yield, 92.4% *ee*) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.15 (bs, 3H), 1.46 (s, 9H), 1.51–1.56 (m, 1H), 1.75–1.81 (m, 1H), 1.85–1.90 (m, 1H), 1.98 (bs, 1H), 3.36 (bs, 2H), 3.87 (bd, *J* = 50.4 Hz, 1H); $^{13}$C NMR (150 MHz, $CDCl_3$): δ (ppm) 20.11, 20.75, 22.93, 23.60, 28.54, 32.52, 33.28, 46.09, 46.41, 52.74, 78.76, 154.58.

**Figures and tables**

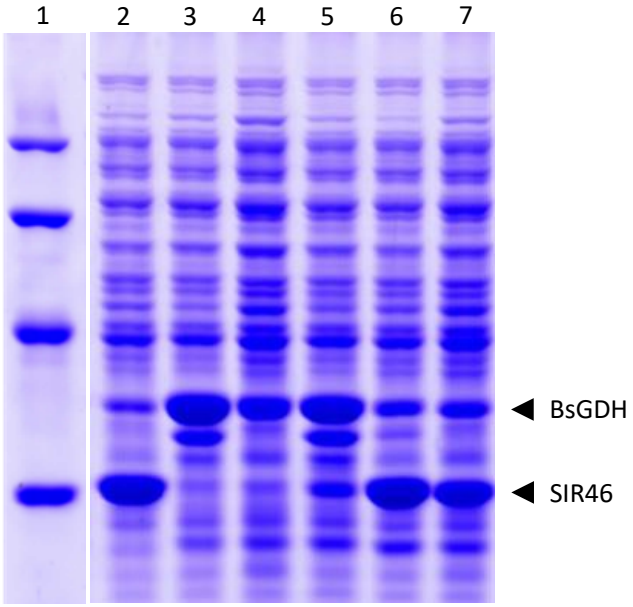



**Figure S1.** SDS-PAGE analysis of wild-type SIR46 and BsGDH expressed in *E. coli* BL21. Lane 1: molecular weight markers 97 kDa, 66 kDa, 45 kDa, 30 kDa. Lanes 2: cell-free extract of *E. coli* BL21 (pET-21a(+)/SIR46) expressing wild-type SIR46 (CFE pET/SIR46). Lanes 3: CFE pACYCDuet/BsGDH(MCS2). Lanes 4: CFE pACYCDuet/BsGDH(MCS1). Lanes 5: CFE pACYCDuet/SIR46(MCS1)-BsGDH(MCS2). Lane 6: CFE pET-21a(+)/SIR46 and pACYCDuet/BsGDH(MCS2). Lane 7: CFE pACYCDuet/BsGDH(MCS1)-SIR46(MCS2).

**Table S1.** Bioconversion of 2-MPN by whole cells of *E. coli* BL21 (DE3) expressing wild-type SIR46 and BsGDH genes.[1]

| Plasmid vector | Whole cells[2] | 2-MPN (mM) | Glucose (mM) | Product (mM) |
|---|---|---|---|---|
| pACYCDuet/SIR46(MCS1)-BsGDH(MCS2) | 10-fold | 10 | 50 | 2.8 (with NADPH)<br>1.9 (no coenzyme) |
| pACYCDuet/BsGDH(MCS1)-SIR46(MCS2) | 10-fold | 10 | 50 | 4.8 (with NADPH)<br>1.7 (no coenzyme) |
| pET-21a(+)/SIR46 and pACYCDuet/BsGDH(MCS2) | 5-fold | 10 | 50 | 9.4 (with NADPH)<br>9.9 (with $NADP^+$)<br>3.3 (no coenzyme) |
| | 10-fold | 30 | 100 | 27.1 (with $NADP^+$) |

[1] Reactions were performed at 30°C for 24 h in 100 mM potassium phosphate buffer (pH 7.0) containing 2-MPN, glucose, 0 or 0.2 mM NADP(H), and whole cells expressing wild-type SIR46 and BsGDH genes.

[2] One-fold whole cell is the turbidity of culture broth when harvested.

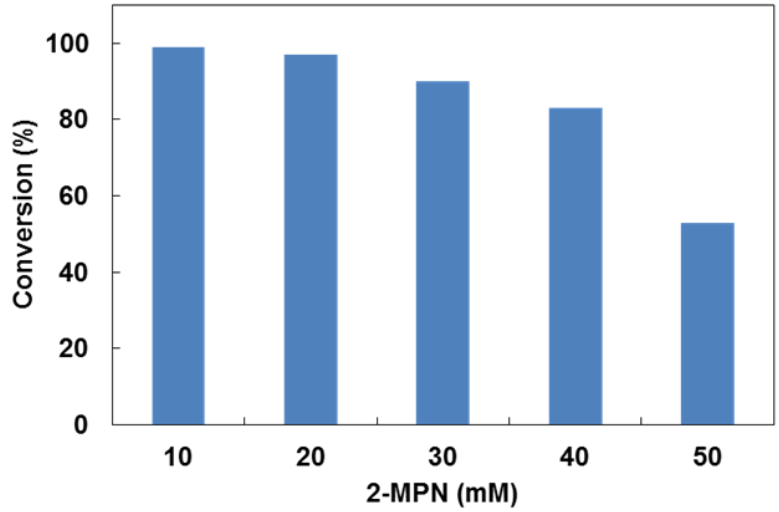



**Figure S2.** Effect of 2-MPN concentration on conversion of 2-MPN by wild-type SIR46. Reactions were performed at 30°C for 24 h in 100 mM potassium phosphate buffer (pH 7.0) containing 10–50 mM 2-MPN, 100 mM glucose, 0.2 mM $NADP^+$, and 10-fold whole cells of *E. coli* BL21 (pET-21a(+)/SIR46WT and pACYCDuet/BsGDH (MCS2)).

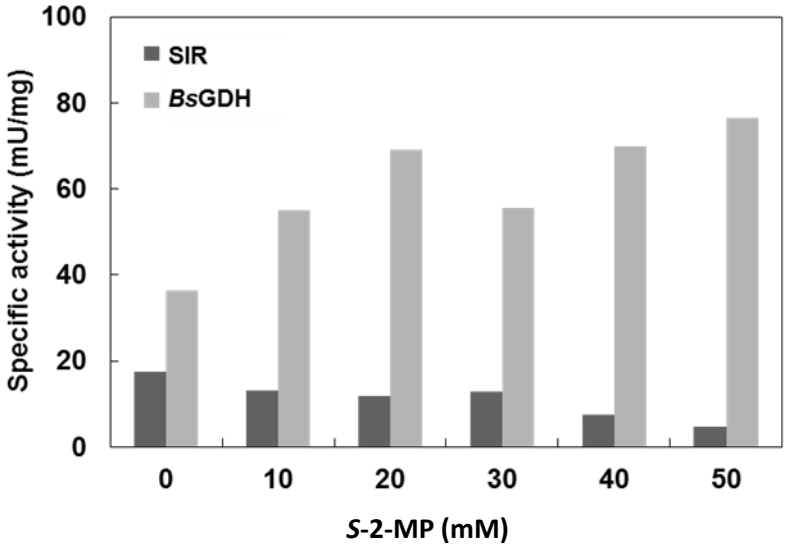



**Figure S3.** Effect of *S*-2-MP concentration on wild-type SIR46 and BsGDH activities.

**Table S2.** NADPH-generating activity in the cell-free extract of *Rhodococcus* expressing wild-type SIR46 gene

| Coenzyme | Substrate | Specific activity (mU $mg^{-1}$) |
|---|---|---|
| $NAD^+$ | Glucose | 3.3 |
| $NAD^+$ | Glucose 6-phosphate | 1.3 |
| $NADP^+$ | Glucose | 5.6 |
| $NADP^+$ | Glucose 6-phosphate | 31.9 |
| $NADP^+$, ATP | Glucose | 5.6 |

```
SIR46            ------------------MSKQSVTVIGLGPMGQAMVNTFLDNGHEVTVWNRTASKAEAL    42
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WP_206300583.1   ------------------MSKQSVTVIGLGPMGQAMVNTFLENGHEVTVWNRTASKAEAL    42
WP_189220594.1   ------------------MSKKSLTLIGLGPMGQAMVNTYLDNGYDVTVWNRTASKAEPL    42
WP_037679270.1   ------------------MSKQSVTVIGLGPMGQAMVGAFIDNGHEVTVWNRTASKAEAL    42
WP_086884371.1   ------------------MSKQPVTVIGLGPMGQAMVSTFLEQGHAVTVWNRTPAKAEAL    42
WP_229819828.1   ------------------MSKQAVTVIGLGPMGQAMVTAFLENGHPVTVWNRTASKADEL    42
WP_229356653.1   -------------MRGEPVSKQAVTVIGLGPMGQAMVAAFLENGHPVTVWNRTASKAAEL    47
WP_127489187.1   MNSSNLKEDSSVGNASAATNRKSVTVMGLGPMGQAMAGVFLECGYAVTVWNRTASKADEL    60
WP_144878318.1   -MNQNDQRSKHEATSAGERNRPSVTIIGLGPMGQAMAAVFLDRGYEVTVWNRTAGKSDAL    59
WP_043615602.1   ------------------MSGKQSVTVIGLGPMGQAMVQAFLDKGHPVTVWNRTPSRADAL    43
WP_030554141.1   ----------------MSSTKQSVTVIGLGPMGQAMAGAFLDRGYDVTVWNRTASRADAL    44
WP_129772310.1   ------MPGTSATPVTPTASATPVTVIGLGPMGRAMVGAFLDGGHQVTVWNRTASRADEL    54
WP_073792319.1   ------------------MDKQAVTLIGLGPMGQAMGAAFLDRGHALTVWNRSPGKADGL    42
WP_063484289.1   ------------------MSKHEVTVIGLGPMGRAMASTFLGKGYRVTVWNRTAGKADEL    42
RIR87            ----------------MGDNRTPVTVIGLGLMGQALAAAFLEAGHTTTVWNRSAGKAEQL    44
WP_164356008.1   ----------------MGDNRTPVTVIGLGLMGQALAAAFLEAGHTTTVWNRSAGKAEQL    44
WP_192770552.1   -------------MRNHDAGHTPVTVIGLGLMGQALAGAFLRAGHPTTVWNRTAAKAEDL    47
WP_189106074.1   -----------------MNATTPVTVLGLGLMGRALAEKFLREGHATTVWNRTAARAEPL    43
WP_132386371.1   ----------------MGDGRVPVTVIGLGMMGRALARAFLSGEHPTTVWNRTAGRAEEL    44
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WP_206300583.1   VAKGATLAPTVEDALGANELIVLSLTDYDAVNAILEPVAASLSGKVIANLSSDTPDKTRE    102
WP_189220594.1   VARGAVLAPTVEEALAANELIVLSLTDYDAVYAILEPVADHLSGKVIANLTSDTPEKARE    102
WP_037679270.1   VAKGAVLAPTLRDALAANELVVLSLTDYDAVYAVLKPVADSLSGKVIANLSSDTPERARE    102
WP_086884371.1   VAKGAVLASTVSEALAANELIVLSLTDYDAVYAVLEQAPDALSGKVIANLSSDTPERARE    102
WP_229819828.1   VAKGAVLAPSIEDALKANELVVLSLTDYDAMYAILEPATAALDGRVLINLSSDTPEKARA    102
WP_229356653.1   VAKGAVLAPSVEDALRANELVVLSLTDYDAMYAILEPATAALDGRVLINLSSDTPEKARA    107
WP_127489187.1   VAKGATRASTVNEALAANELVILSLTDYDVMYAILEPASMNLSGKVLVNLSSDTPEKVRK    120
WP_144878318.1   AAKGAIKASSVDEAIAANDLIILSLTDYDAMYAILEKAARDLSGKVFVNLSSDTPEKARE    119
WP_043615602.1   VARGAVLAGSVEEALAANELVILSLTDYDAMYTVLEPAAHALSGRVLVNLSSDTPEKTRA    103
WP_030554141.1   VTRGAVLAPNVKEALAAHELVILSLTDYDAMYAVLEPAEAALTGRTLVNLSSDTPEKARD    104
WP_129772310.1   VARGAVRAATVDEALAANELVILSLTDYDAMYALLGPATGALAGRVLVNLSSDTPKRAHE    114
WP_073792319.1   VARGAVLADSVEAALRANDLVVLSLTDYDATYGVLGPAQDALAGRVVVNLSSDTPDRARE    102
WP_063484289.1   VVAGAHRAATVRDALTASELVILSLTDHHAMYAVLESATDALSGRTLVNLGSDTPESARR    102
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WP_164356008.1   VSQGAVQAATPADAVAASELVVVCLSTYDNMHDVIGSLGESLRGKVIVNLTSGSSDQGRE    104
WP_192770552.1   VAQGATLAGSVGDAVAASPLVVVCVSNYEAMHELLDPLGGVLDGRVLVNLTSGTSRVARE    107
WP_189106074.1   VAEGAELAGSVADAVAASPLVVVCVLDYDAVHALLDPVGDALDGRVLVNLTSGTSEGARE    103
WP_132386371.1   VAAGATSTDNVAAAVSASPLVVVCVRDYQAAYEILGPVSDTLAGRVLVNLTSGTSAQARA    104
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WP_206300583.1   AAKWATKHGAKHLTGGVQVPPPLIGKPGSSTYYSGPRDVFEAHEETLKVLTD-ADYRGED    161
WP_189220594.1   AFKWAAKHGAQHITGGVQVPPPLIGKPESSTYYSGPKDAFDTHEEALKVLTN-ADYRGED    161
WP_037679270.1   AAKWAAEHGAQHLTGGVQVPPPMIGTSDASAYYSGPEDVFNAHRSTLAVLTN-TDFRGED    161
WP_086884371.1   AAKWAERHGAVHITGGVQVPPPMIGKPESSTYYSGPKDAFDKYESTLKVLTG-ADFRGED    161
WP_229819828.1   AFEWAAGHGAKHVTGGVQVPPPLIGKEGSSTYYSGPRDVFDTYKETLEVLTA-ADYRGED    161
WP_229356653.1   AFAWAAGHGARHLTGGVQVPPPMIGKEGSSTYYSGPRDVFDTYRETLAVLTG-TDYRGED    166
WP_127489187.1   AAKWLAGRGARHVTGGVQVPPSGIGKSESSTYYSGPREVFEAHRETLEVLTG-TDYRGED    179
WP_144878318.1   AAKWLEARGARHLTGGVQVPPSGIGKAESSTYYSGTREVFDAHKKTLEVLTG-ADYRGED    178
WP_043615602.1   GAKWVAGHGGVQLSGGVTVPPSGIGKPESSTFYSGPRGPFETHRPTLEALTG-TDYRGED    162
WP_030554141.1   AARWAAEQGAVQLTGGVTVPPSGIGQAESSTFYSGPRDVFDRHRPALEVITGRTDHRGED    164
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WP_073792319.1   AAAWIAERGATHLTGGVRVPPSGIGQPGTSTFYSGPREVFERHKETLETLTG-ADYLGED    161
WP_063484289.1   AARWASEHGARYLTGGVQASPPGIGQPGFSTFYSGPQEVFDAHQEALEALTG-ADYRGED    161
RIR87            TAAWAEKQGVEYLDGAIMITPPGIGTETAVLFYAGTQSVFEKYEPALKLLGGGTTYLGTD    164
WP_164356008.1   TAAWAEKQGVEYLDGAIMITPPGIGTETAVLFYAGTQSVFEKYEPALKLLGGGTTYLGTD    164
WP_192770552.1   TAQRAAPLGGTYLDGAIMAIPQAIGTADAVILYSGPQAAFDSYESTLRSLG-AGTYLGAD    166
WP_189106074.1   MAEWAAGHGATYLDGAILSDPDGVGTADAVILYSGPRAAFDAHEPVLKLLGGATTHLGED    163
WP_132386371.1   MAGWAAERGAGYLDGAIMMTPPGIGTAEALILYSGPSETFAAHRPALTALGGGTTHLGAD    164
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WP_206300583.1    AGLAAMYYQAQMTIFWTTMLSYYQMLALGKANGVSAKELLPYATMMTSMMPHFLELYAQH    221
WP_189220594.1    AGLAAMYYQAQMTIFWTSMLSFYQALALAQANGVAAKDMVPYAEMQFAMMPHFLGLYAQH    221
WP_037679270.1    PGLAAMYYQAQMTIFWTTMLSYYQTLALGKANGVSAKELLPYASMMTTMMPHFLELYAQH    221
WP_086884371.1    PGLAAMYYQAQMTIFWTTMLSFYQMVALGQANGVSAKELLPYATMMTQMMPHFLELYAGH    221
WP_229819828.1    PGLAAMYYQAQMIIFWTSMLSFYQALALGKANGVSAKELLPYASNMMTMMPHFLELYTQH    221
WP_229356653.1    PGLAAMYYQAQMIIFWTSMLSYYQALALAKANGVSAGELLPYASGMMTMMPHFLELYARH    226
WP_127489187.1    PGLAMLYYQIQMDIFWTSMLSYLHALAVATANGITAEQFLPYASATLSSLPKFVEFYTPR    239
WP_144878318.1    PGLAALYYQIQMDMFWTSMLSYLHALSLAGANGLTAEQIRPYAIETMKSLPMFIEFYTPR    238
WP_043615602.1    PGLAALFYQIGMVMFWTSMLSYWQAVALADANGLTAADILPHATETMATMPNFLSFYAAR    222
WP_030554141.1    PGLAALMYQIGMVMFWTSMLSYWQAIALADANGLTAADILPHAVETANSLPGFFSFYAER    224
WP_129772310.1    PALAPLYYQLQMDIFWTAMTSSLHALAVARAHGISAGDFLPYAVPTLASLPDFLAFYAPR    233
WP_073792319.1    PGRAALFYQLQMTVFWTTMLSWLQAVALAGAHGVTAEELVPYVKDTVDIG-QFLDFYSAR    220
WP_063484289.1    PGLAALYYQVGMDLFWTTVLGWLHALALADAHGVPAEEILPSASSVLSGMPEFMAFYTQR    221
RIR87             HGMPALYDVSLLGLMWGTLNSFLHGVAVVETAGVGAQQFLPWAHMWLEAIKMFTADYAAQ    224
WP_164356008.1    HGMPALYDVSLLGLMWGTLNSFLHGVAVVETAGVGAQQFLPWAHMWLEAIKMFTADYAAQ    224
WP_192770552.1    HGLSSLYDVALLGVMWSVLNGFLQGAALAGTAGVDATTFAPIANTVIRTVTDWVPGYARQ    226
WP_189106074.1    HGLASLHDVAVLGLMWGVLNSFLQGAAVLGAAGVTASAFAPLATTSIKMVADWVNGYAEQ    223
WP_132386371.1    VGLASVYDVALLGIMWSTFNGFMHAAALVGSENVPATAFLPLARQWLTGVASFLTPYAEQ    224
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WP_206300583.1    VDSAT--YPGDVDRLAMGAASIDHVLHTHKDAGVSTVLPAAVAEIFHAGMDKGFAENSFS    279
WP_189220594.1    VDSAS--YPGDVDRLAMGAASIDHVTHTHADAGVNTTLMEAVSKIFHAGMDKGFAENSFS    279
WP_037679270.1    VDSGE--YPGAVDRLAMGAASADHVLHTHKDAGVDTTLPAAVAEIFHKGMERGFAENSFS    279
WP_086884371.1    VDSGE--YPGDVDRLAMGAASVDHILHTHKDAGISTALPAVVAEIFHKGMDAGFADNSFS    279
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WP_229356653.1    VDDAE--YPGDVDRLAMGAASVDHILHTSEDAGVDTTLLKAVQDVFRRGMDRGFEDNSFS    284
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WP_144878318.1    IDAGE--HPGDVDRLGMGVASVDHIVHTSKDAGIDASLPAAVLEVFKRGVANGQAGNSFT    296
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WP_030554141.1    IDAGR--HIGDVDRLAMGMASIEHVLHTNADAGVDTTLPAAVVELARRGMDAGHAADSFS    282
WP_129772310.1    IDRGE--YPGDVDRLAMGAASVDHVVHTTGDAGVDTTLPAAVLGLFRRGVTAGHAADSFT    291
WP_073792319.1    VDRGE--HPGDVERLTMGVASIEHVVHTARDSGVDSTLPSAVHDIFRRGVAAGRGADSFT    278
WP_063484289.1    IDAGE--YPGDIDRLAMGVASVDHVLRTARDAGVDTSLPTAVREIFRRGTAAGHADDSVT    279
RIR87             IDAGDGKFPANDATLETHLAALKHLVHESEALGIDAELPKYSEALMERVISQGHAKNSYA    284
WP_164356008.1    IDAGDGKFPANDATLETHLAALKHLVHESEALGIDAELPKYSEALMERVINQGHAKNSYA    284
WP_192770552.1    IDEGA--YPADDSTIDTHLGAMAHLVHESEFLGVNADLPRHIKALTDQAVTDGHGGSGYA    284
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WP_132386371.1    IDTGD--YPASDATLETHLSPVEHLIEESRARGIDATAAEYTKRLVEEAVADGHALDSYA    282
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WP_206300583.1    SLIEVLKKPSA----------------    290
WP_189220594.1    SLIEILKKQAG----------------    290
WP_037679270.1    SLIEVLKKQG-----------------    289
WP_086884371.1    SLIEVLKKPSA----------------    290
WP_229819828.1    SLIEVLKKPSA----------------    290
WP_229356653.1    SLIEVLKKPSA----------------    295
WP_127489187.1    SLIEIFKNSIRP---------------    309
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WP_073792319.1    SLLEVLRKPAA----------------    289
WP_063484289.1    SLIEVLKKPAARSLPLSGGHADPGSA    305
RIR87             AVLKAFRKPSE----------------    295
WP_164356008.1    AVLKAFRKPSE----------------    295
WP_192770552.1    AMIEQFRRPSEARG-------------    298
WP_189106074.1    AMIEIFRKPSATRE-------------    295
WP_132386371.1    RIVEHFAPARRA---------------    294
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```
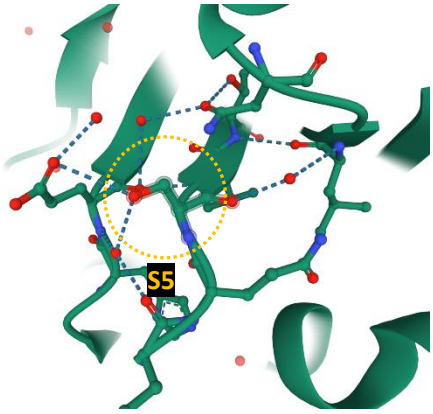
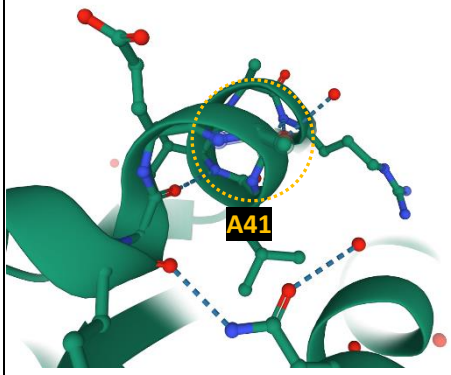
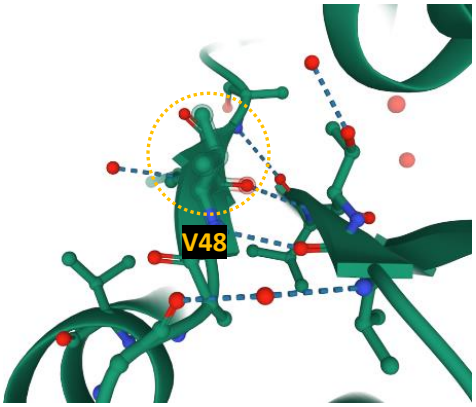
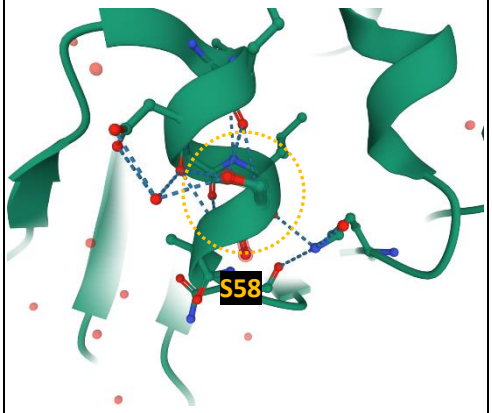
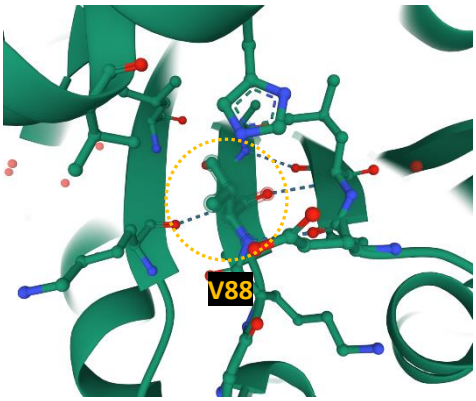
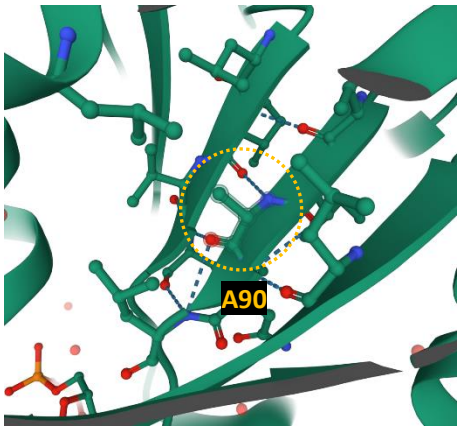
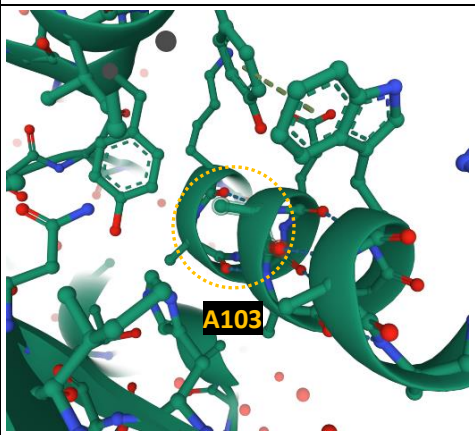
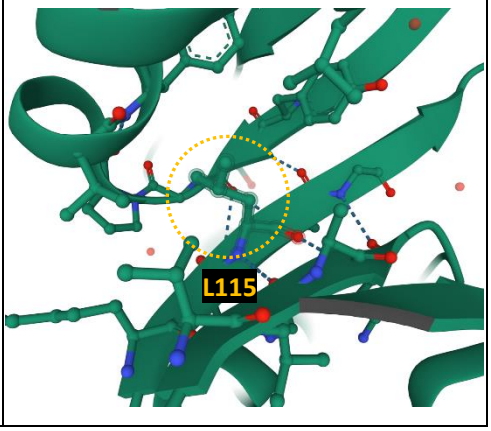
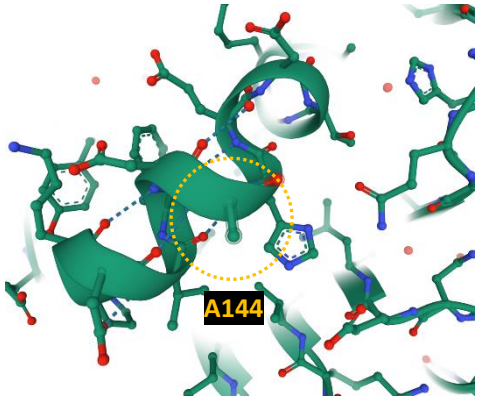
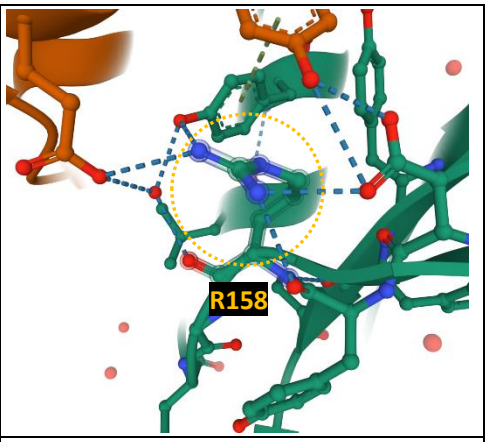
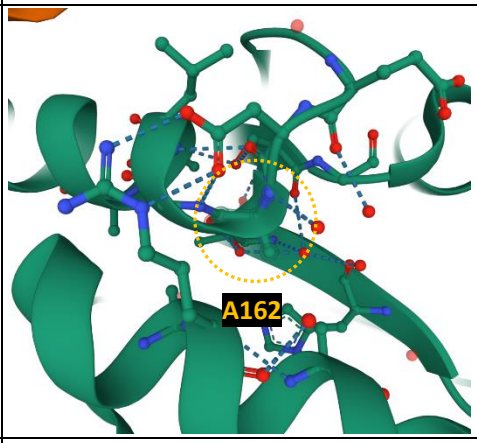
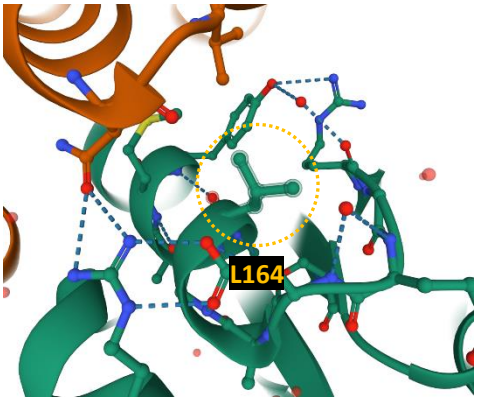
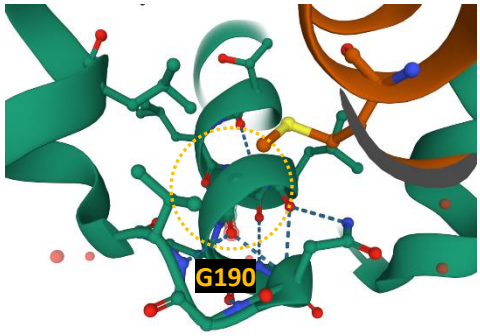
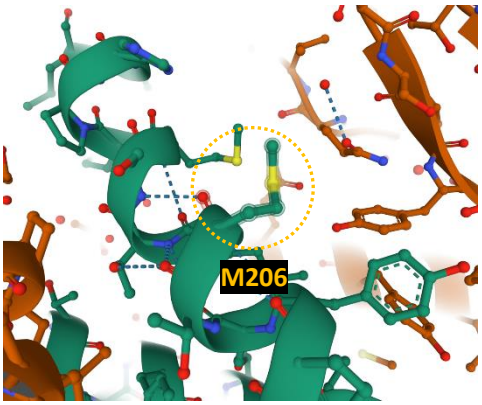
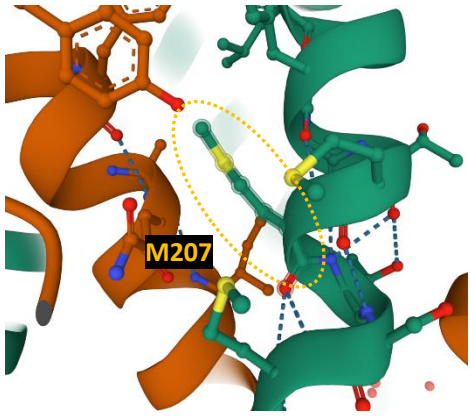
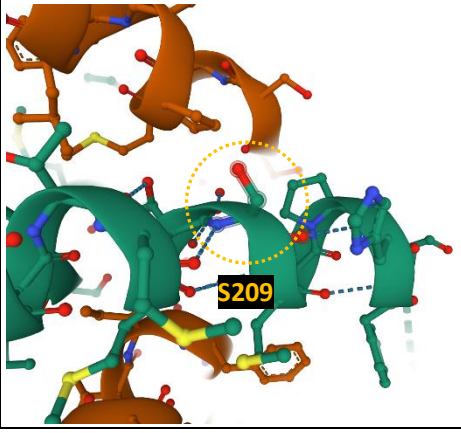

**Figure S4.** Multiple sequence alignments with SIR46. Alignments performed with clustal W using protein sequences obtained by BLAST searches with protein sequences of SIR46 and RIR87. The mutation candidates for improvement of SIR46 were shown in blue.

**Table S3.** Target residues in SIR46 (pdb 4oqy) (browsed by RCSB PDB web site)

| Residue | Structual position | Residue | Structual position |
|---|---|---|---|
| S5 |  | A41 |  |
| V48 |  | S58 |  |
| V88 |  | A90 |  |
| A103 |  | L115 |  |

| | | | |
|---|---|---|---|
| A144 |  | R158 |  |
| A162 |  | L164 |  |
| G190 |  | M206 |  |
| M207 |  | S209 |  |

| | | | |
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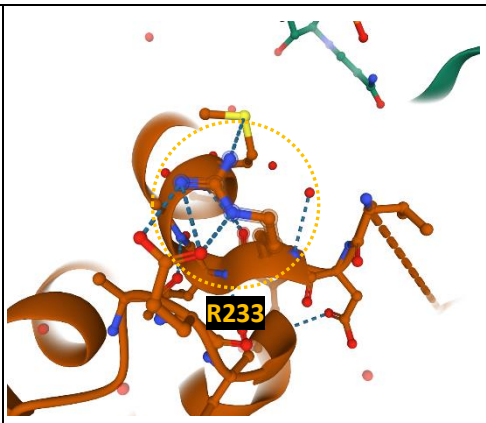
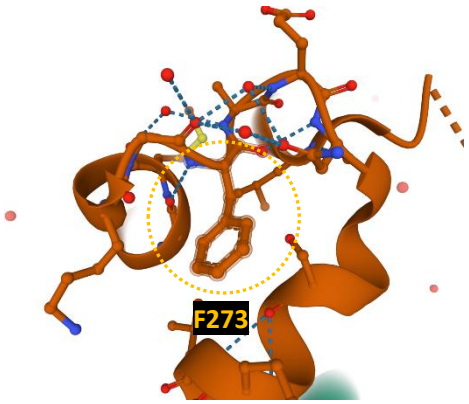
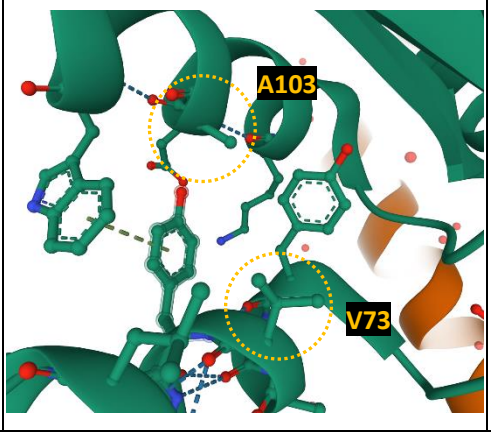
| R233 |  | F273 |  |
| V73 and A103 |  | | |

**Table S4.** Specific activity and thermostability of SIR46 variants

| Variant | Specific activity (mU mg$^{-1}$) | Residual activity[1] (%) | | | |
|---|---|---|---|---|---|
| | | 40°C | 45°C | 50°C | 55°C |
| Wild type (WT) | 12.6 | 66 | 0 | n.d. | n.d. |
| WT + His6 | 8.8 | 58 | 0 | n.d. | n.d. |
| R158L | 23.4 | 91 | 6 | n.d. | n.d. |
| V48T | 16.2 | 57 | 0 | n.d. | n.d. |
| A90V | 4.6 | 78 | 0 | n.d. | n.d. |
| A103M | 21.2 | 85 | 15 | n.d. | n.d. |
| S209R | 20.4 | 88 | 27 | n.d. | n.d. |
| S5P | 13.8 | 60 | 0 | n.d. | n.d. |
| A41P | 13.4 | 67 | 0 | n.d. | n.d. |
| L164R | 13.1 | 0 | n.d. | n.d. | n.d. |
| M206E | 2.4 | 0 | n.d. | n.d. | n.d. |
| R233T | 9.0 | 93 | 34 | n.d. | n.d. |
| S58R/R158L | 19.7 | 96 | 16 | n.d. | n.d. |
| R158L/G190A | 7.4 | 69 | 0 | n.d. | n.d. |
| R158L/F273H | 9.5 | 10 | 0 | n.d. | n.d. |
| M7 | 35.7 | n.d. | n.d. | 100 | 48 |
| M7 + His6 | 33.4 | n.d. | n.d. | 97 | 33 |
| M7 + V88T | 31.6 | n.d. | n.d. | 104 | 58 |
| M7 + L115V | 35.1 | n.d. | n.d. | 87 | 0 |
| M7 + A144R | 31.8 | n.d. | n.d. | 105 | 55 |
| M7 + M207T (M8) | 36.6 | n.d. | n.d. | 94 | 63 |
| M8 + V88T (M9) | 32.9 | n.d. | n.d. | 102 | 70 |
| M9 + A144R (M10) | 32.6 | n.d. | n.d. | 98 | 71 |
| M10 + L115V | 30.6 | n.d. | n.d. | 97 | 54 |

[1] Residual activity after incubation at 40°C–55°C for 30 min.

**Table S5.** Effect of various organic solvents on SIR46 M9 or M10 activity for selection of the best variant.

| Organic solvent | SIR46 M9 | SIR46 M10 |
|---|---|---|
| None | 100% (35.0 mU $mg^{-1}$) | 100% (35.0 mU $mg^{-1}$) |
| 5% MeOH | 89 | 85 |
| 10% MeOH | 76 | 70 |
| 15% MeOH | 65 | 59 |
| 20% MeOH | 50 | 45 |
| 5% DMSO | 77 | 75 |
| 10% DMSO | 59 | 54 |
| 15% DMSO | 43 | 41 |
| 20% DMSO | 30 | 26 |
| 5% EtOH | 60 | 51 |
| 10% EtOH | 35 | 32 |
| 5% MeCN | 41 | 40 |
| 5% AcOEt | 4 | 6 |

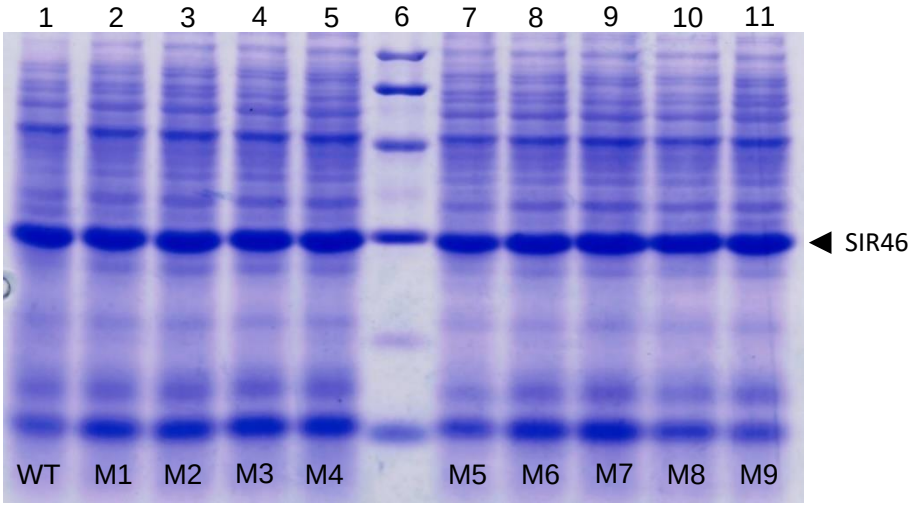



**Figure S5.** SDS-PAGE analysis of SIR46 variants expressed in *R. erythropolis* L88. Lanes 1–5, 7–11: cell-free extract of *R. erythropolis* L88 expressing SIR46 variants gene. Lane 6: molecular weight markers 97 kDa, 66 kDa, 45 kDa, 30 kDa, 20.1 kDa, 14.4 kDa.

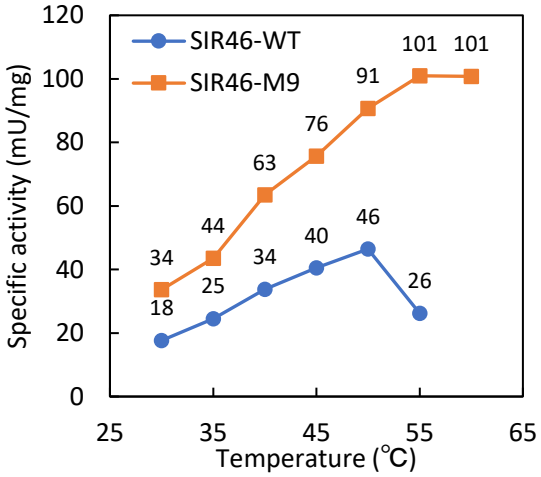



**Figure S6.** Effect of temperature on SIR46 activity.

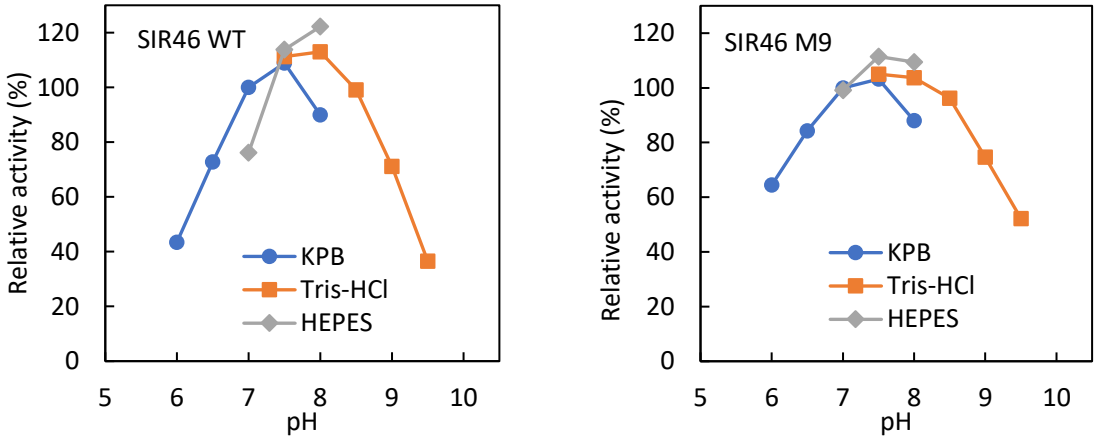



**Figure S7.** Effect of pH on SIR46 activity. 100% = 18.0 mU $mg^{-1}$ (WT), 33.7 mU $mg^{-1}$ (M9) in 100 mM KPB (pH 7.0).

**Table S6.** Residual activity of wild-type SIR46 and the variant M9 in cell-free extract on several conditions.

| Condition | Residual activity (%) | |
|---|---|---|
| | Wild type | M9 |
| None | 100 (17.6 mU $mg^{-1}$) | 100 (32.7 mU $mg^{-1}$) |
| Storage at 4°C for 15 days | 65 | 107 |
| Storage at 4°C for 35 days | 34 | 110 |
| Storage at 4°C for 53 days | 15 | 106 |
| Incubation at 35°C for 24 h | 4 | 102 |

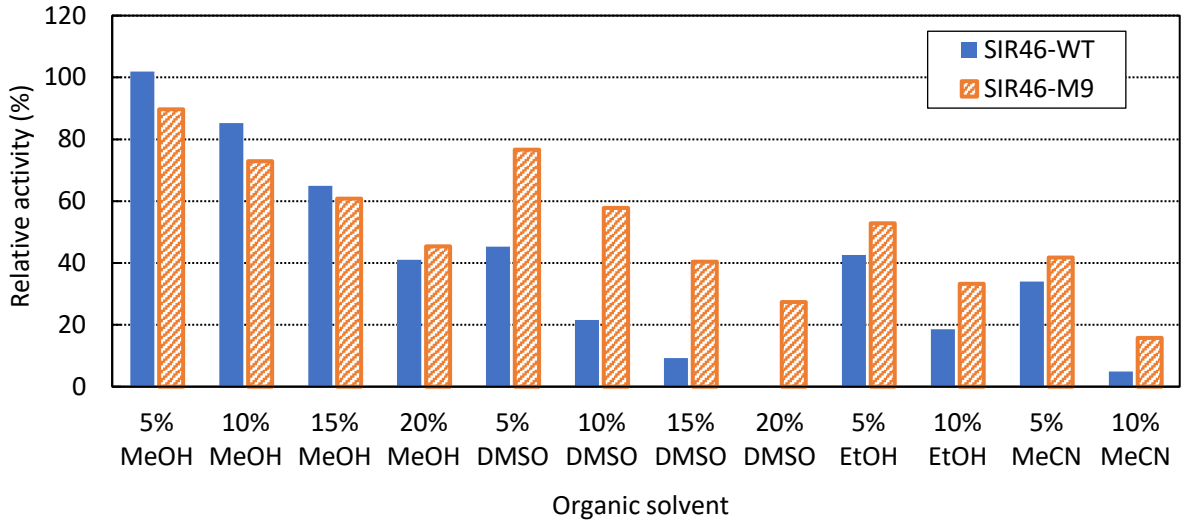



**Figure S8.** Effect of various organic solvents on SIR46 activity. None = 100% = 17.6 mU $mg^{-1}$ (WT), 33.6 mU $mg^{-1}$ (M9)

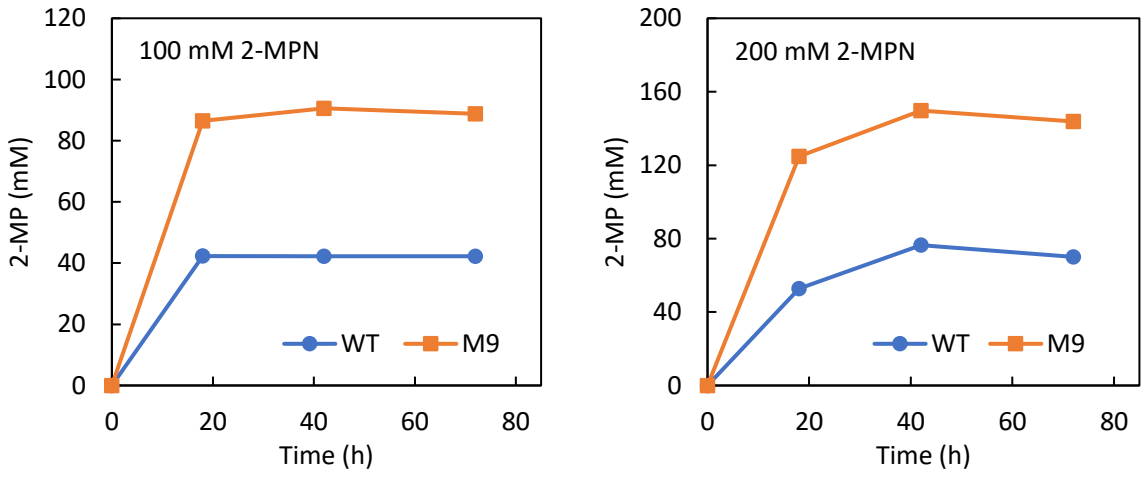



**Figure S9.** Evaluation of imine reductase activity of SIR46 WT or M9 in the presence of high concentration of 2-MPN. Reactions were performed at 30°C in potassium phosphate buffer (pH 7.5) containing 100 or 200 mM 2-MPN, glucose (1.25 eq.), 0.2 mM $NADP^+$, and 3.0 mg $mL^{-1}$ cell-free extract of the recombinant cells overexpressing SIR46 and BsGDH genes.

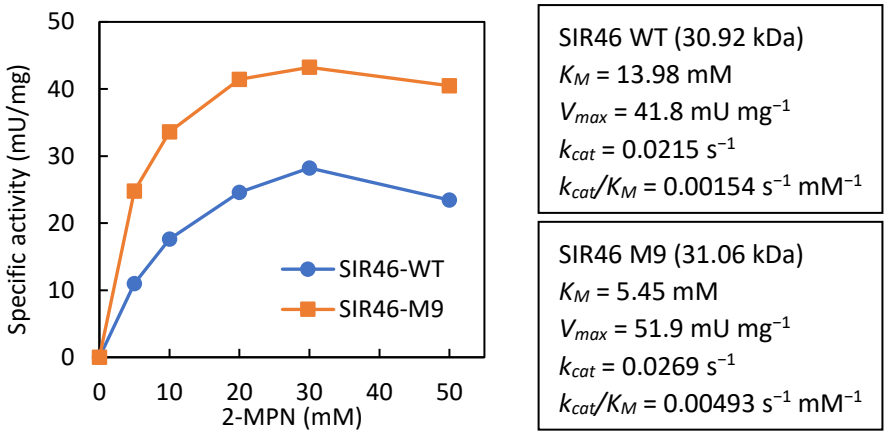



**Figure S10.** Determination of kinetic constants for 2-MPN reduction by SIR46. Kinetic constants were calculated using the values of specific activity at 5–30 mM 2-MPN.

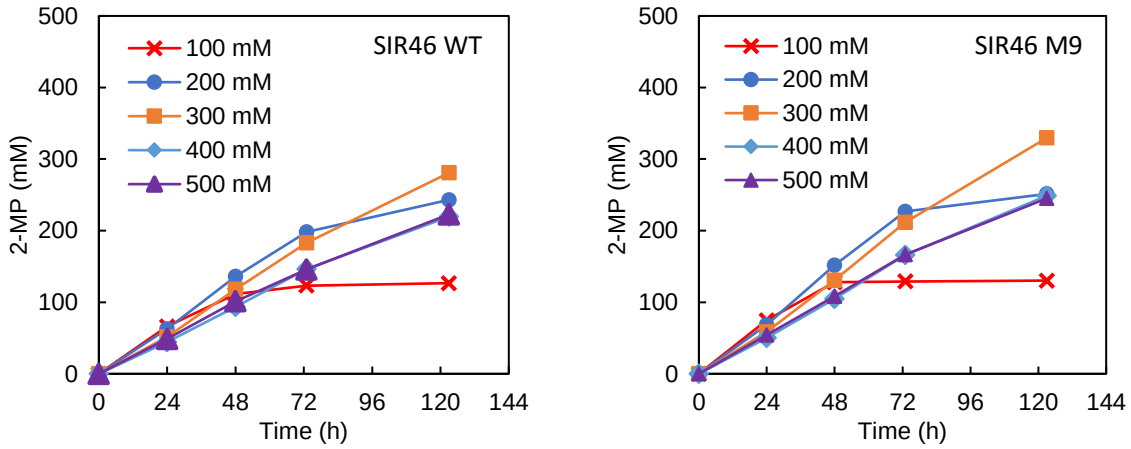



**Figure S11.** Bioconversion of high concentration of 2-MPN by SIR46 WT or M9. The reactions were performed at 30°C in potassium phosphate buffer (pH 7.5) containing 100–500 mM 2-MPN, glucose (1.2 eq.), and whole cells expressing SIR46 WT or M9 gene ($OD_{610}$ = 25).

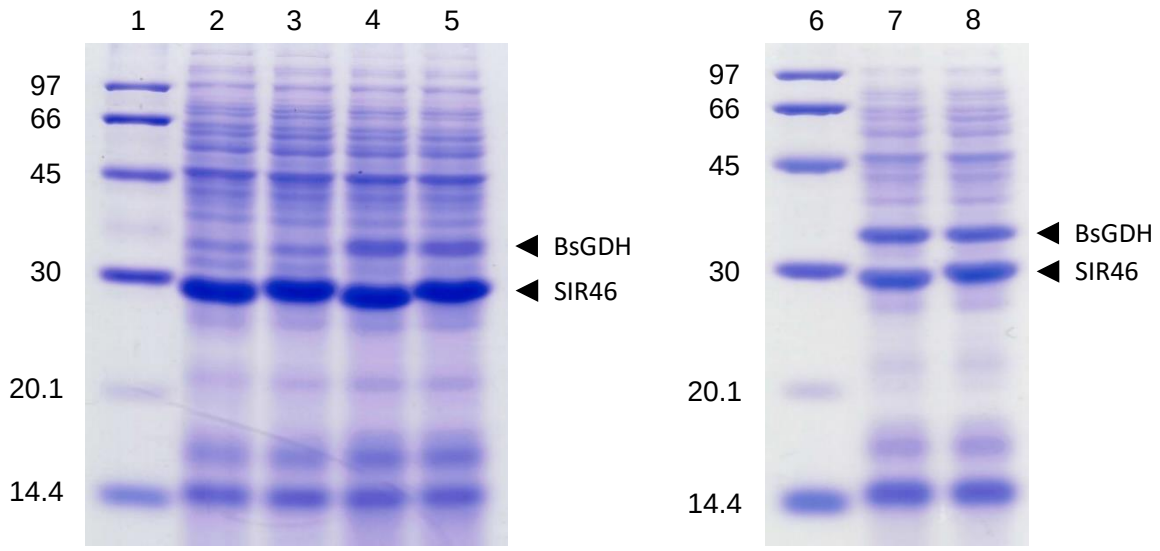



**Figure S12.** SDS-PAGE analysis of SIR46 M9 and BsGDH expressed in *R. erythropolis* L88. Lane 1, 6: molecular weight markers 97 kDa, 66 kDa, 45 kDa, 30 kDa, 20.1 kDa, 14.4 kDa. Lanes 2: cell-free extract of *R. erythropolis* L88 / wild-type SIR46 (CFE SIR46WT). Lanes 3: CFE SIR46WT. Lanes 4: CFE SIR46 WT and BsGDH. Lanes 5: CFE SIR46 M9 and BsGDH. Lane 7: CFE SIR46 WT and BsGDH (codon-optimized gene). Lane 8: CFE SIR46 M9 and BsGDH (codon-optimized gene).

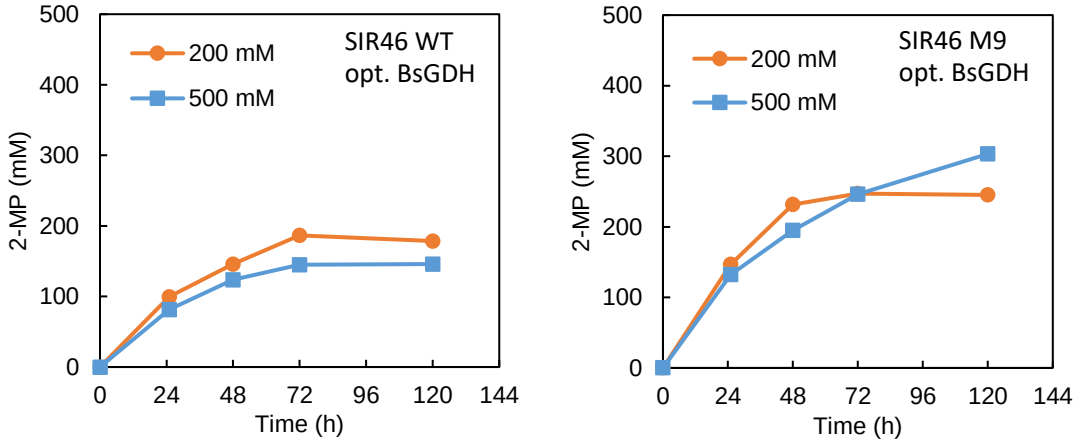



**Figure S13.** Bioconversion of high concentration of 2-MPN by SIR46 WT at 35°C. Reactions were performed at 35°C in potassium phosphate buffer (pH 7.5) containing 200 or 500 mM 2-MPN, glucose (1.2 eq.), and whole cells expressing SIR46 WT and codon-optimized BsGDH gene ($OD_{610}$ = 25).

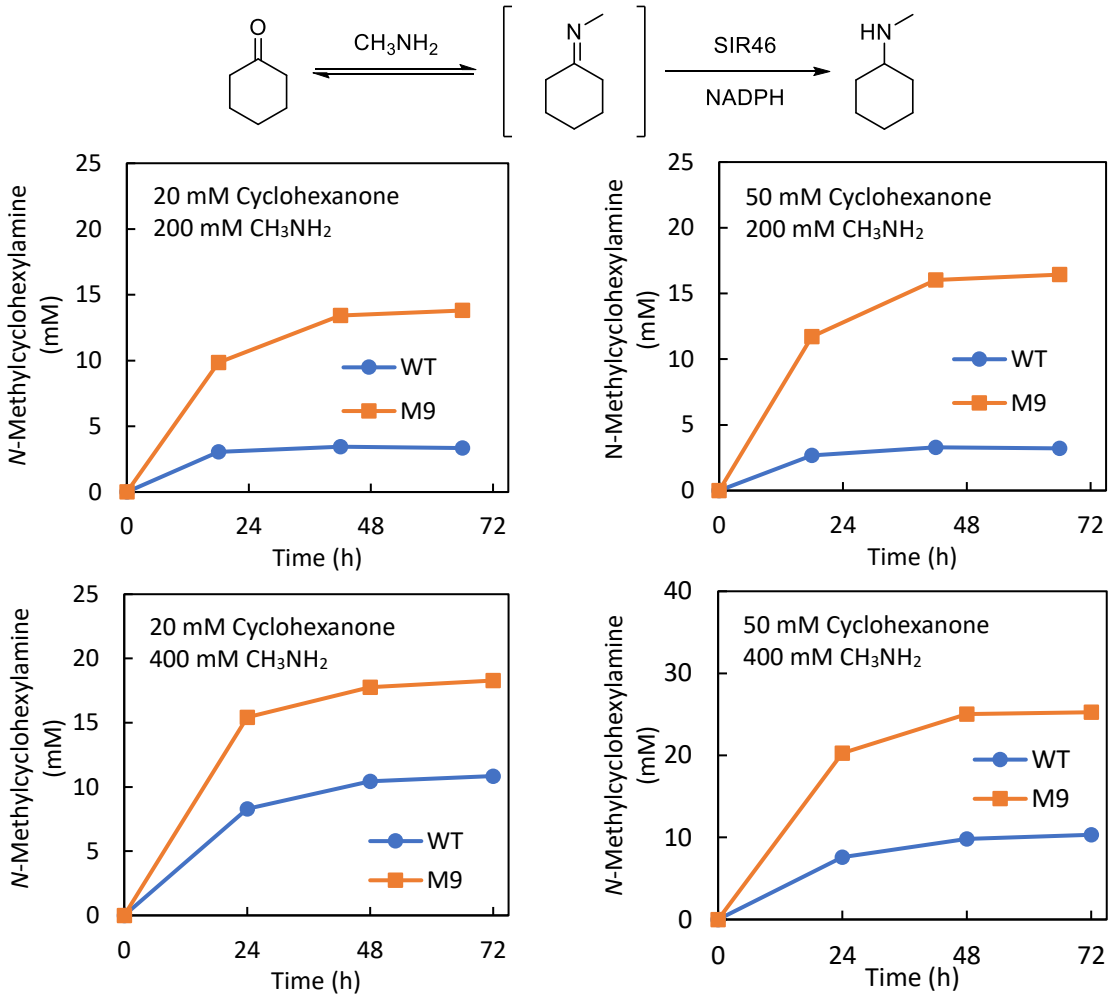



**Figure S14.** Reductive amination activity of SIR46 WT or M9. Reactions were performed at 30°C in potassium phosphate buffer (pH 8.0) containing 200 or 400 mM methylamine hydrochloride, 100 mM glucose, 0.2 mM $NADP^+$, 20 or 50 mM cyclohexanone, and 1.5 mg $mL^{-1}$ cell-free extract of the recombinant cells overexpressing SIR46 and BsGDH genes.

**Table S7.** Specific activities of SIR46 and BsGDH in the cell-free extract of the recombinant cell.

| Coexpression system | Substrate | Coenzyme | Specific activity (mU $mg^{-1}$) |
|---|---|---|---|
| *E. coli* BL21 (DE3) pACYCDuet/SIR46(MCS1)-BsGDH(MCS2) | 2-MPN | NADPH | 2.3 |
| | Glucose | $NADP^+$ | 628.3 |
| *E. coli* BL21 (DE3) pACYCDuet/BsGDH(MCS1)-SIR46(MCS2) | 2-MPN | NADPH | 10.0 |
| | Glucose | $NADP^+$ | 1.4 |
| *E. coli* BL21 (DE3) pET-21a(+)/SIR46 and pACYCDuet/BsGDH(MCS2) | 2-MPN | NADPH | 22.7 |
| | Glucose | $NADP^+$ | 133.1 |
| *R. erythropolis* L88 pTipRT2/SIR46 WT and pTipQC1/BsGDH | 2-MPN | NADPH | 14.4 |
| | Glucose | $NADP^+$ | 5.2 |
| *R. erythropolis* L88 pTipRT2/SIR46 M9 and pTipQC1/BsGDH | 2-MPN | NADPH | 33.2 |
| | Glucose | $NADP^+$ | 4.5 |
| *R. erythropolis* L88 pTipRT2/SIR46 WT and pTipQC1/opt. BsGDH [1] | 2-MPN | NADPH | 11.1 |
| | Glucose | $NADP^+$ | 6841 |
| | Glucose | $NAD^+$ | 7314 |
| *R. erythropolis* L88 pTipRT2/SIR46 WT and pTipQC1/opt. BsGDH [1] | 2-MPN | NADPH | 25.1 |
| | Glucose | $NADP^+$ | 6110 |
| | Glucose | $NAD^+$ | 6083 |

[1] Codon-optimized BsGDH gene was expressed in the recombinant cell.

**Table S8.** HPLC analysis conditions for detection of cyclic amines.

| Cyclic amine[1] | Column | Solvent | Retention time[2] (min) |
|---|---|---|---|
| 2-Methylpyrrolidine (2-MP)[3] | Atlantis dC18 5μm 4.6×150 mm and YMC-Triart C18 5μm 4.6×150 mm | Sodium phosphate buffer (10 mM, pH 3.0) : MeOH = 11 : 10 | 32.8 (*S*)<br>34.6 (*R*) |
| 2-Phenylpyrrolidine (2-PP) | Atlantis dC18 5μm 4.6×150 mm | Sodium phosphate buffer (10 mM, pH 3.0) : MeOH = 4 : 5 | 14.9 (S)<br>15.7 (*R*) |
| 2-(4-Methoxyphenyl) pyrrolidine (2-MeOPP) | Atlantis dC18 5μm 4.6×150 mm | Sodium phosphate buffer (10 mM, pH 3.0) : MeOH = 4 : 5 | 16.4 (*S*)<br>16.9 (*R*) |
| 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1-MTIQ) | CHIRALPAK AD-RH 5μm 4.6×150 mm | sodium phosphate buffer (10 mM, pH 3.0) : MeCN = 3 : 2 | 12.8 (*R*)<br>13.7 (*S*) |
| *N*-Methyl-cyclohexylamine | Atlantis dC18 5μm 4.6×150 mm | sodium phosphate buffer (10 mM, pH 3.0) : MeOH = 4 : 5 | 18.0 |

[1] Cyclic amines except for 1-MTIQ were derivatized with GITC to determine the yield and optical purity. 1-MTIQ was derivatized with MITC.

[2] Absolute configurations of chiral cyclic amines were determined by using commercially available chiral reagents or the reference.

[3] HPLC analysis of 2-MP was performed with two columns connected in series (Atlantis column in front, YMC column in back).

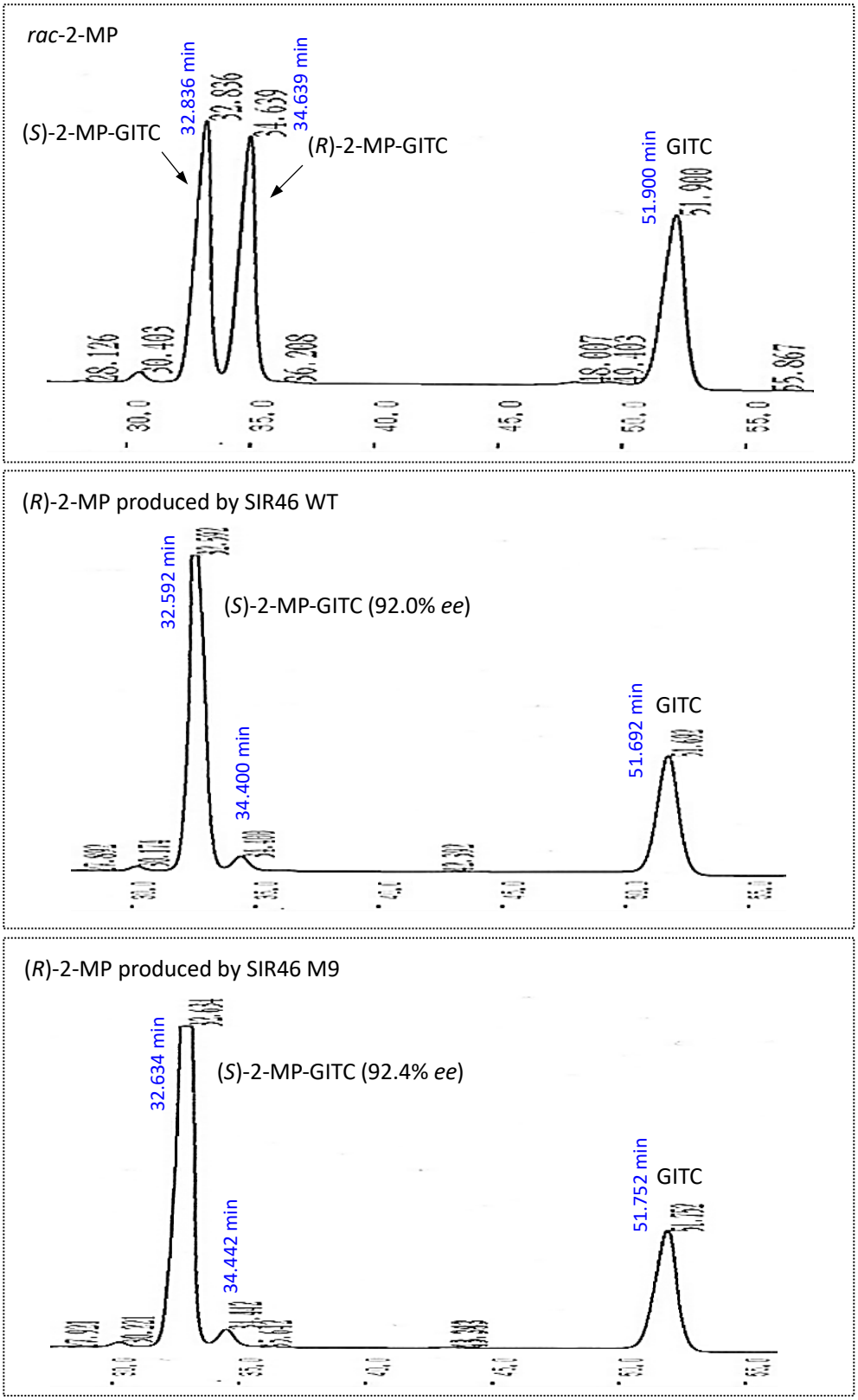



**Figure S15.** HPLC chromatograms of 2-MP-GITC.

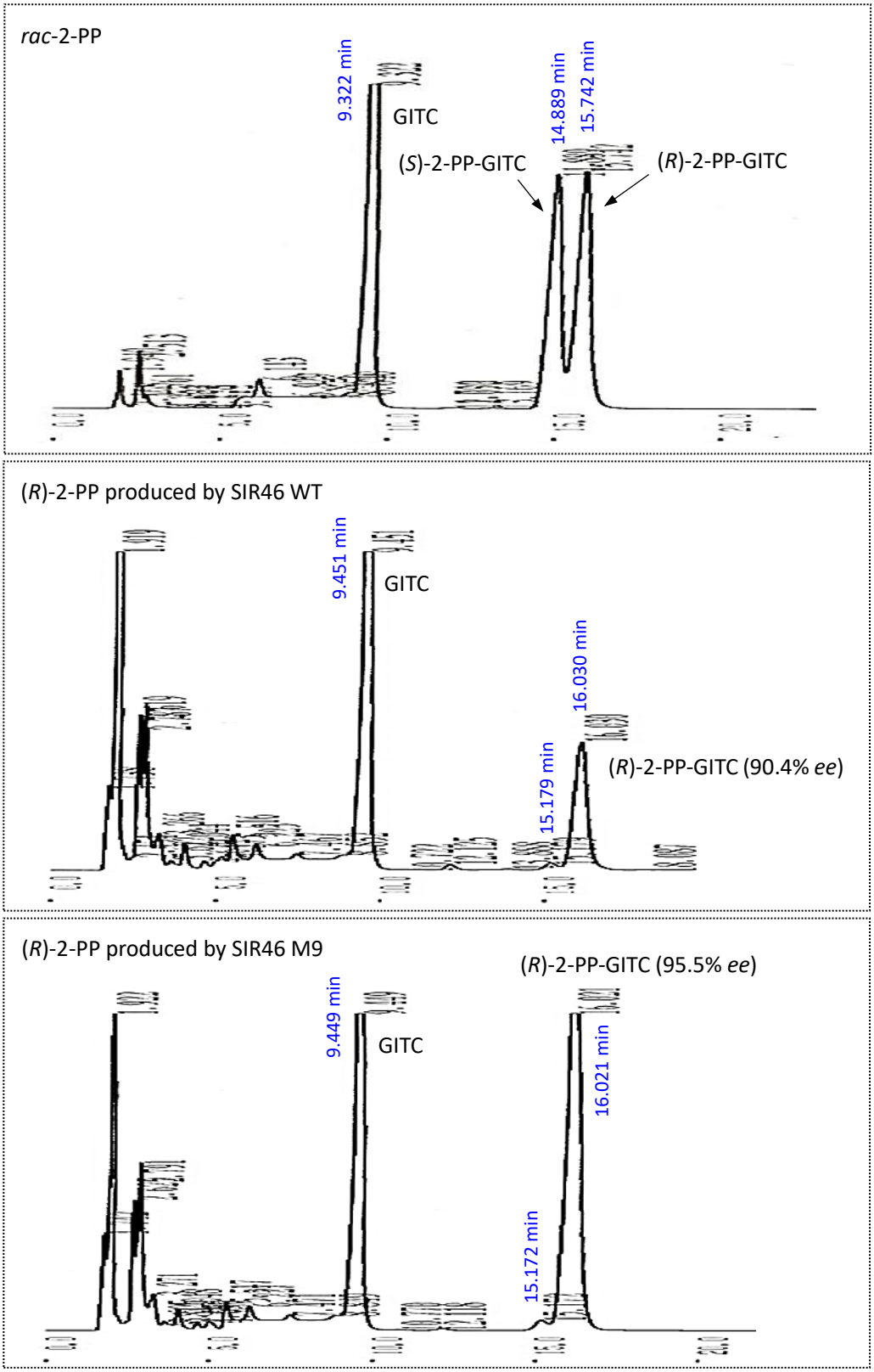



**Figure S16.** HPLC chromatograms of 2-PP-GITC.

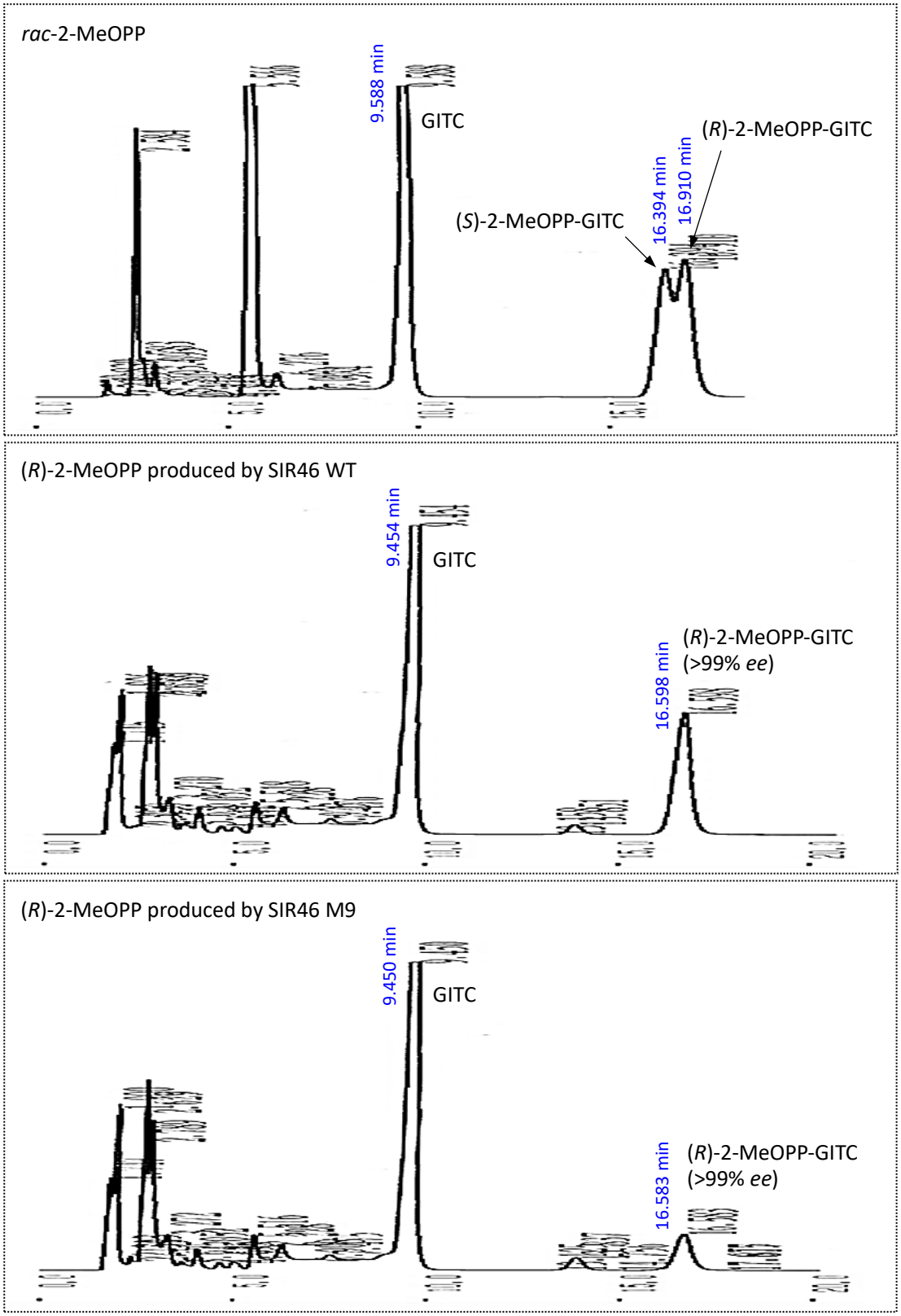



**Figure S17.** HPLC chromatograms of 2-MeOPP-GITC.

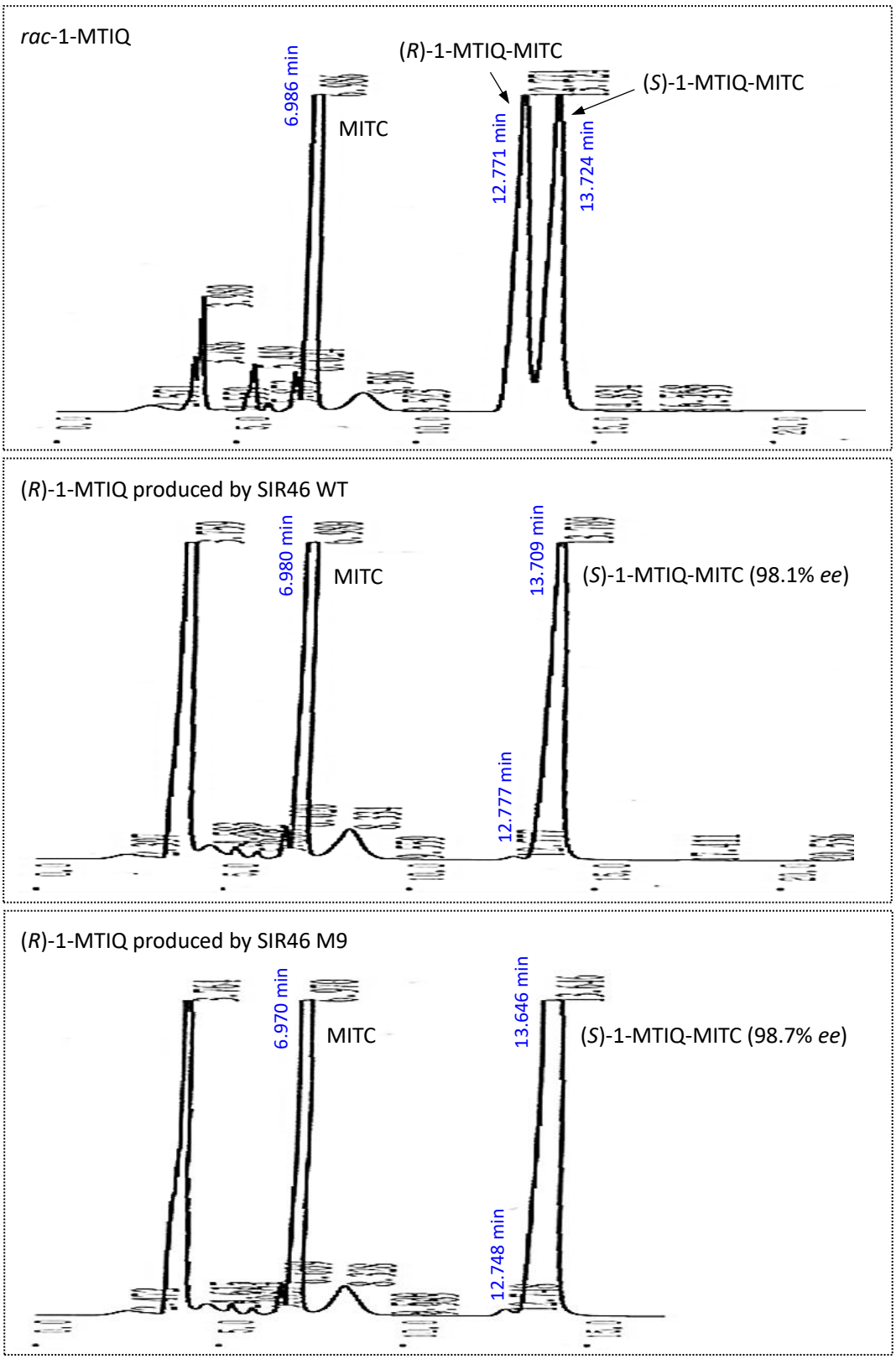
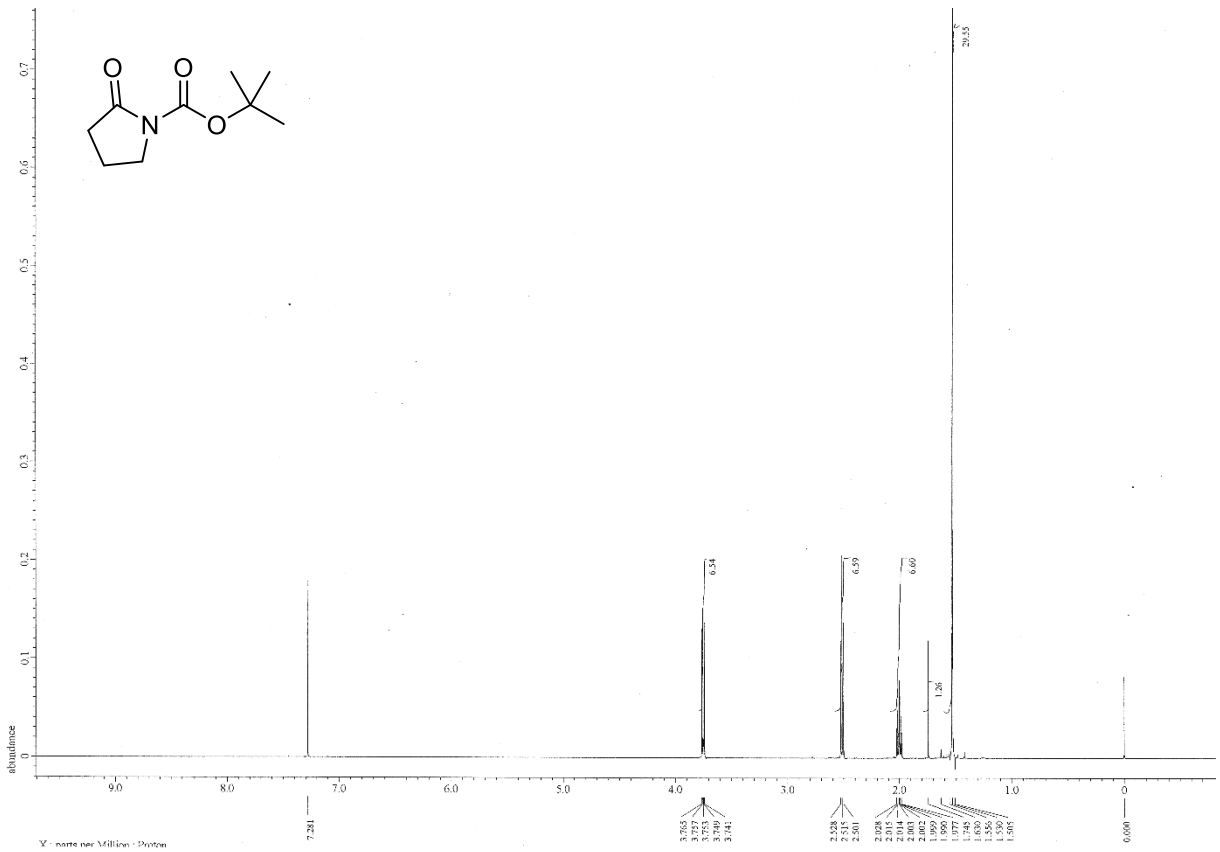



**Figure S18.** HPLC chromatograms of 1-MTIQ-MITC.



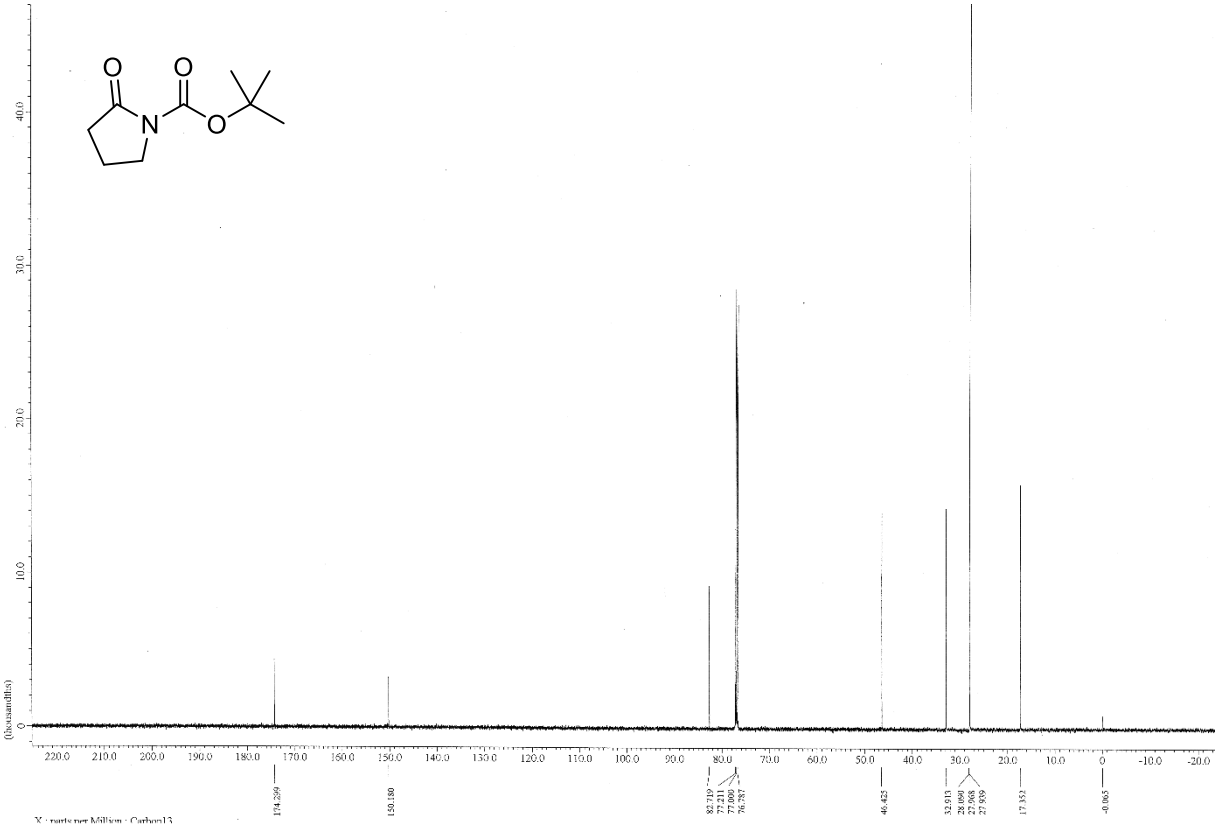




**Figure S19.** $^{1}$H NMR and $^{13}$C NMR spectra of *N*-Boc-2-pyrrolidone

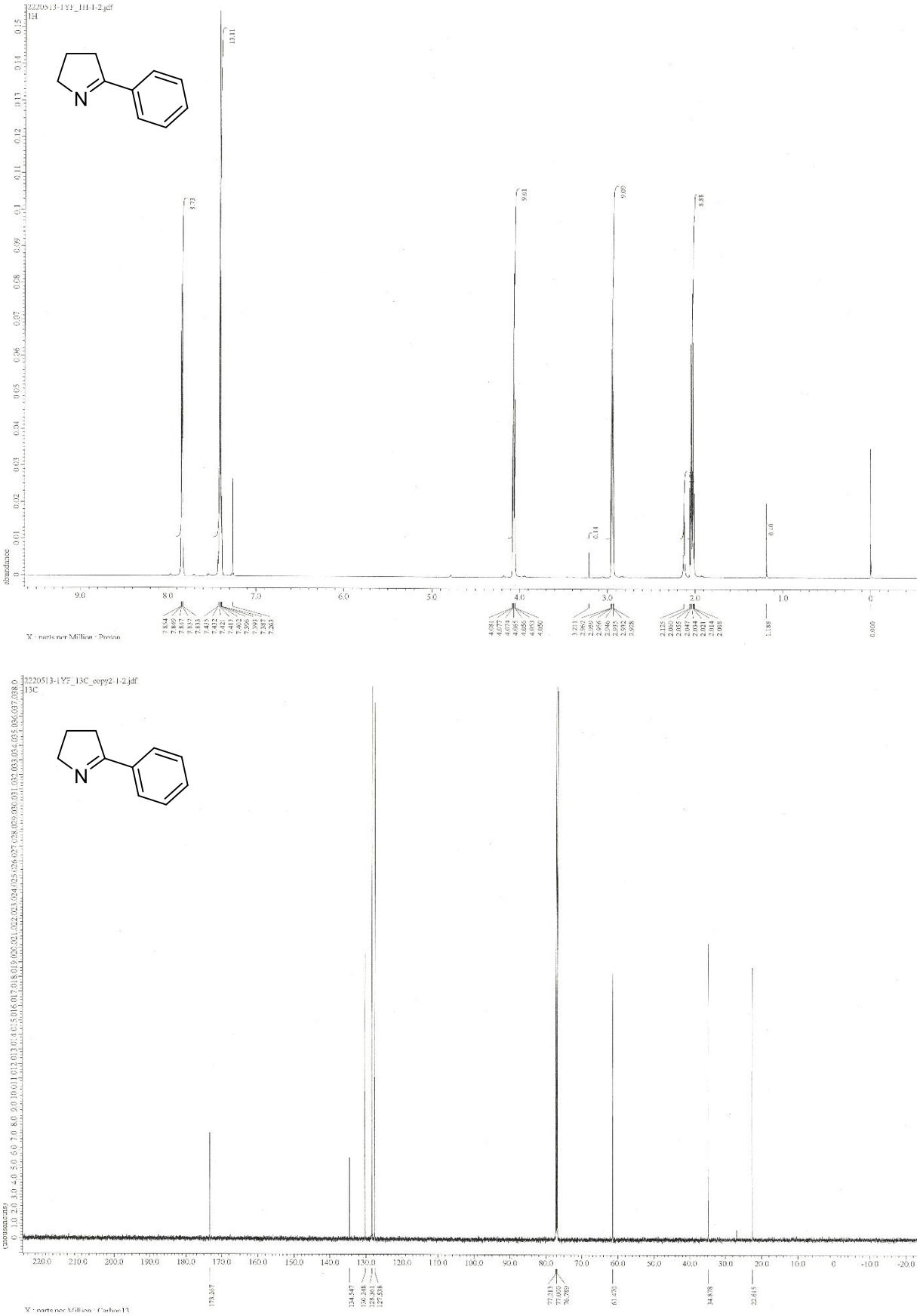
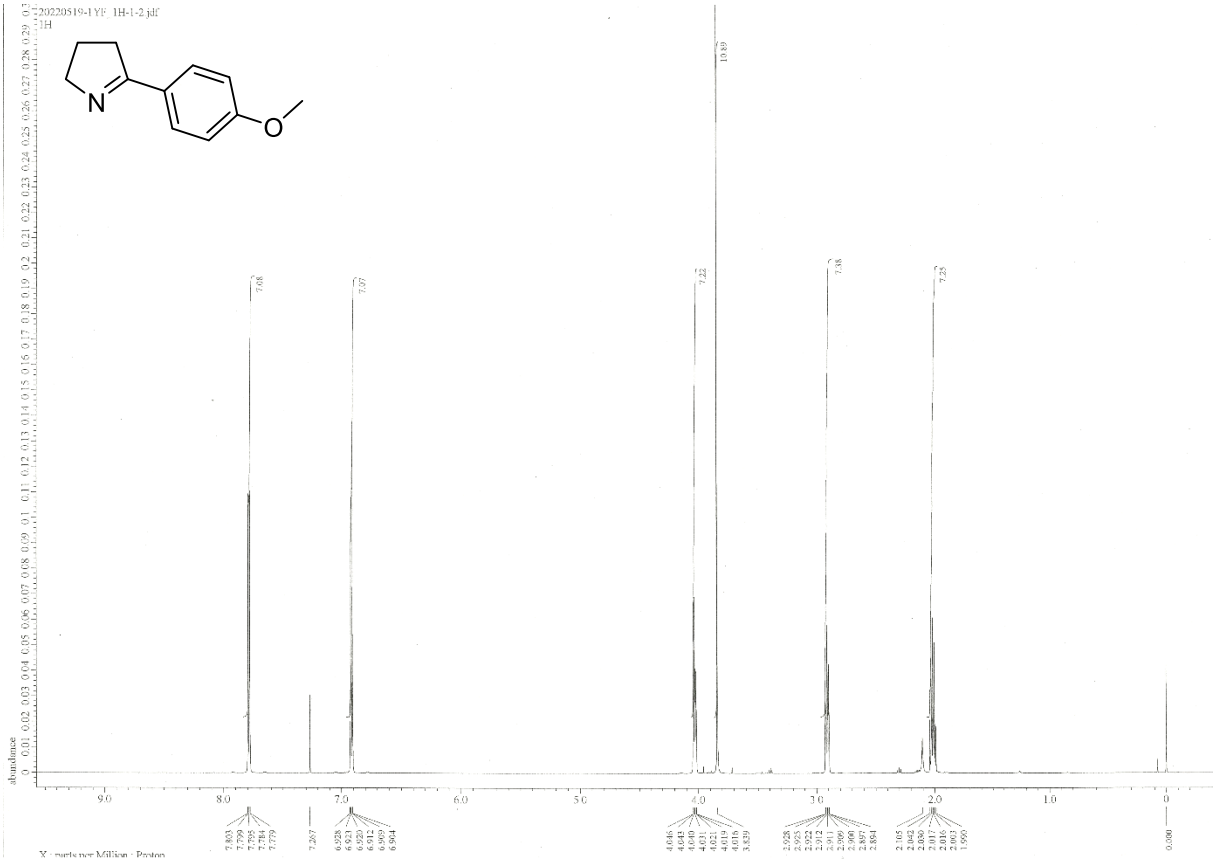


**Figure S20.** $^{1}H$ NMR and $^{13}C$ NMR spectra of 2-phenyl-1-pyrroline



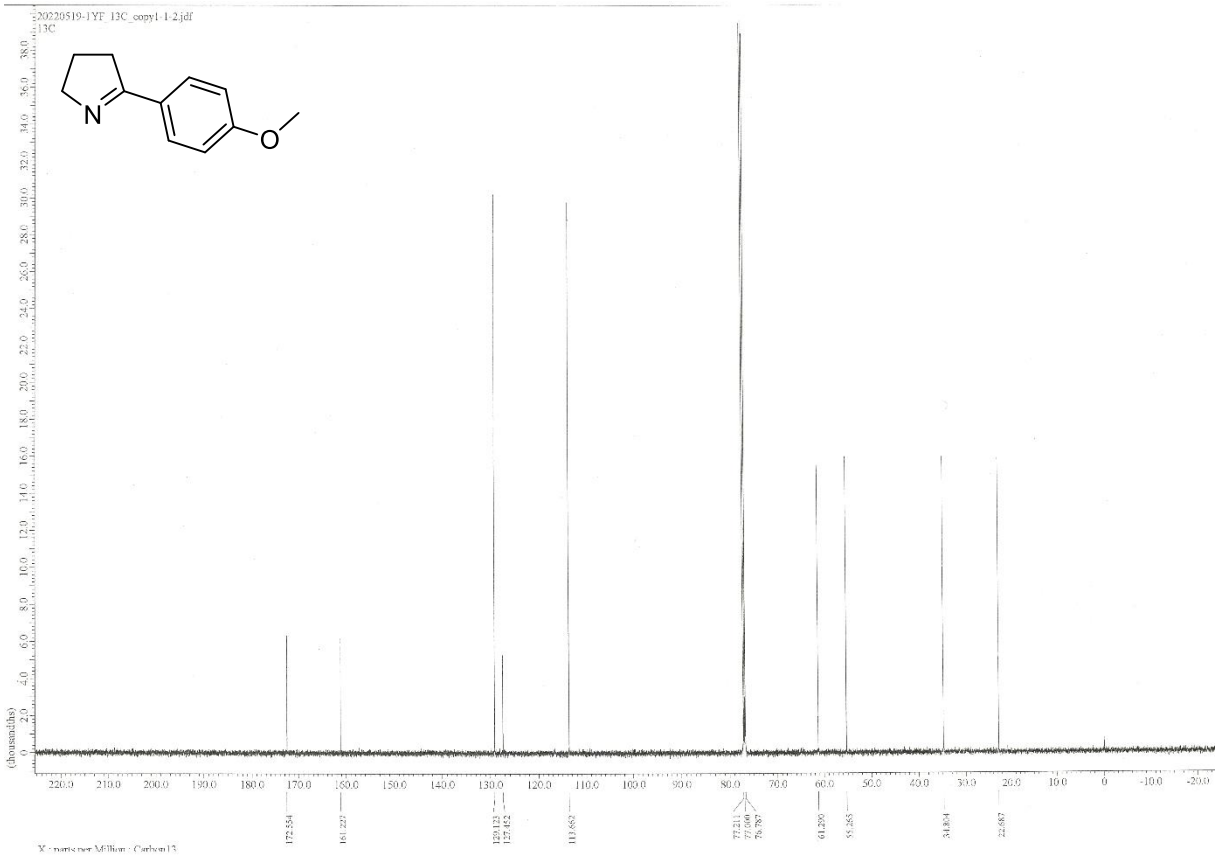




**Figure S21.** $^{1}$H NMR and $^{13}$C NMR spectra of 2-(4-methoxyphenyl)-1-pyrroline


**Figure S22.** $^{1}$H NMR and $^{13}$C NMR spectra of *rac*-2-(4-methoxyphenyl)pyrrolidine

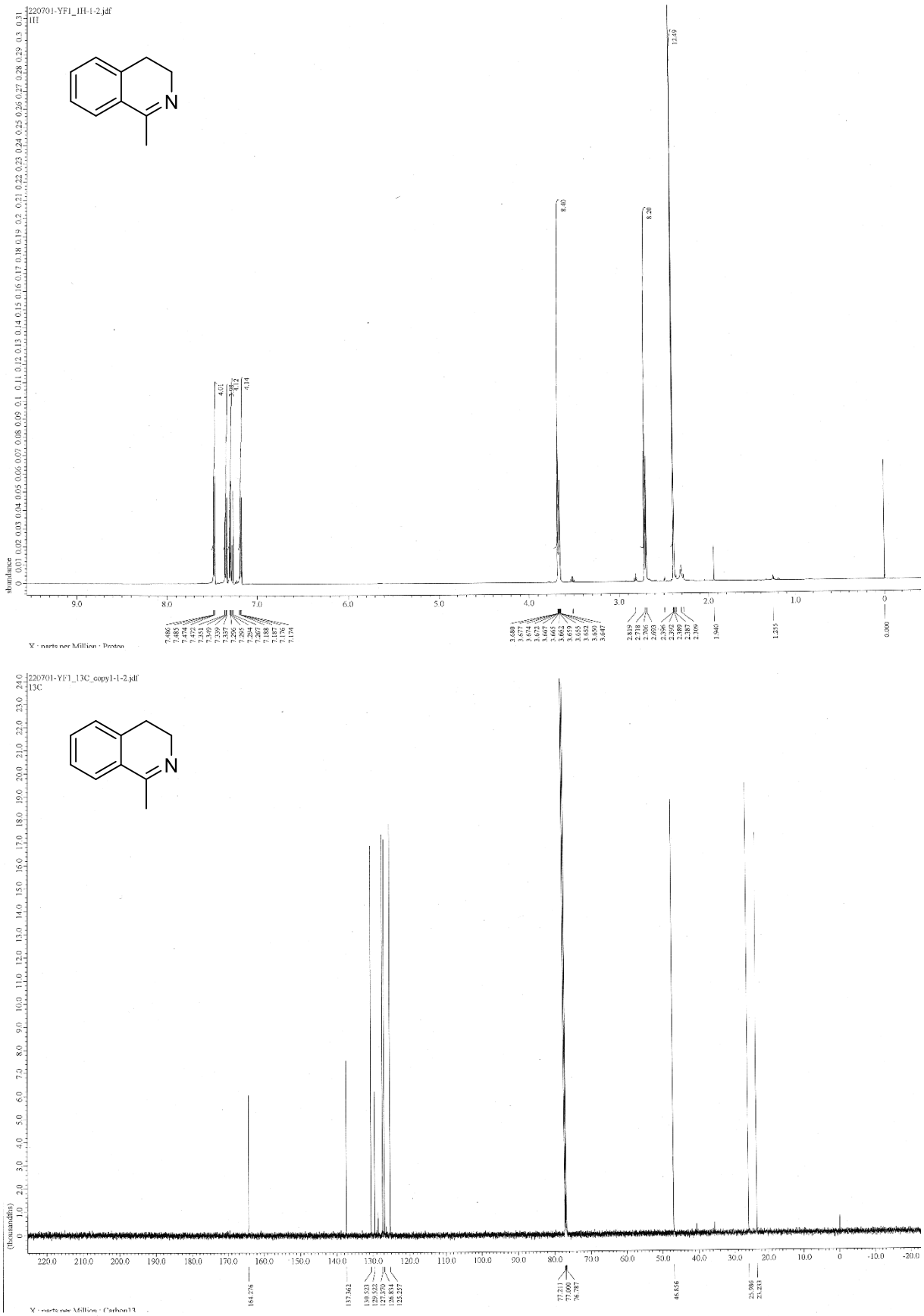



**Figure S23.** $^{1}$H NMR and $^{13}$C NMR spectra of 1-methyl-3,4-dihydroisoquinoline

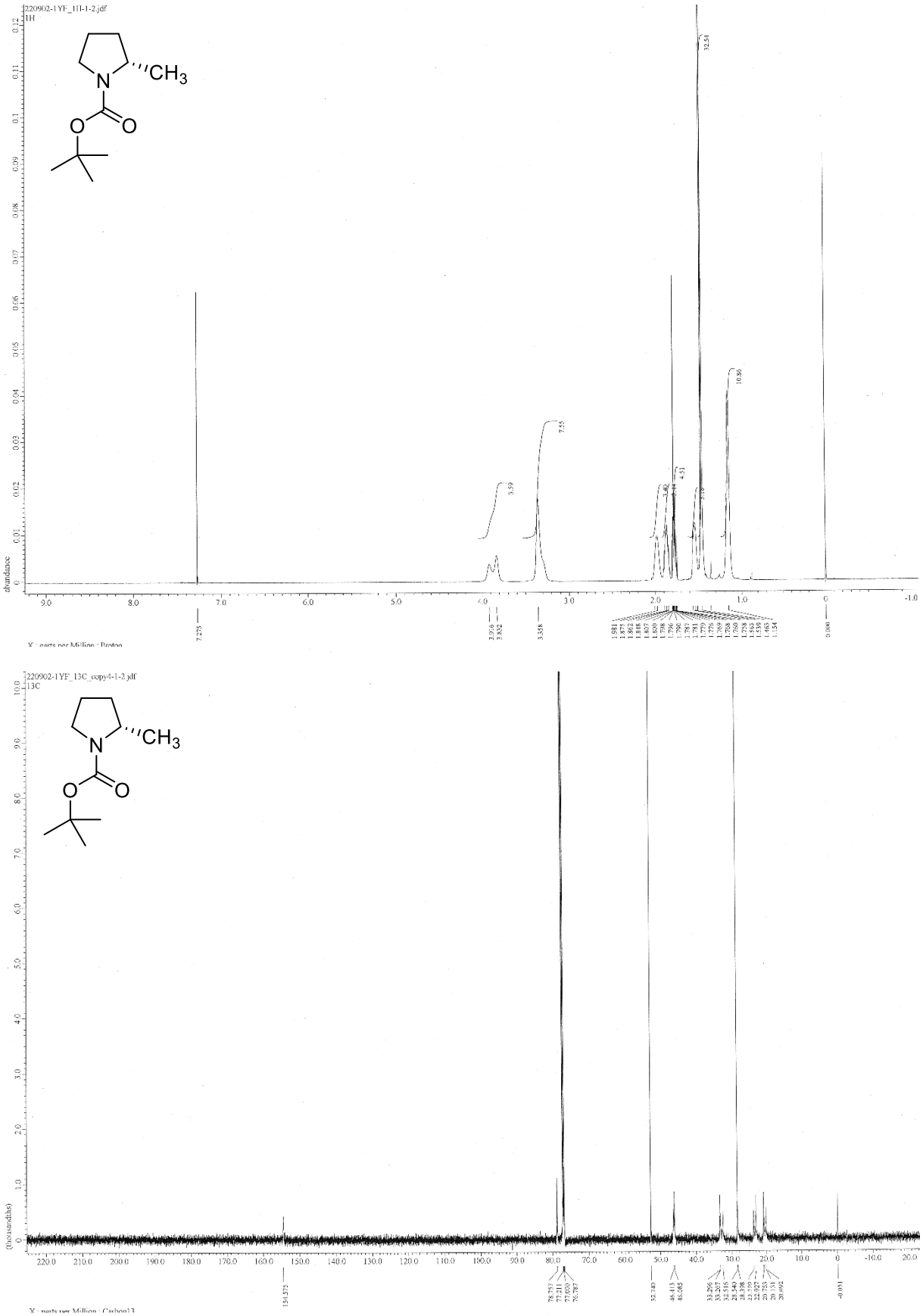



**Figure S24.** $^{1}$H NMR and $^{13}$C NMR spectra of (*S*)-*N*-Boc-2-methylpyrrolidine

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