Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Asymmetric synthesis of chiral amine from cyclic imine by bacterial whole-cell catalyst of enantioselective imine reductase

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Chemicals

2-Methyl-1-pyrroline (2-MPN) and racemic, *R*- and *S*-2-methylpyrrolidine (2-MP) were purchased from Sigma-Aldrich (Tokyo, Japan). 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) and phenyl isothiocyanate (PITC) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Screening of Imine-reducing Strains

After cultivation of each microorganismin a nutrient medium (4 ml) containing 0.1 % (v/v) 2-methyl-1-pyrroline (2-MPN), the cells harvested by centrifugation were washed thrice with 0.85% (w/v) NaCl. Conversion of 10 mM 2-MPN was performed using whole cells from 2 ml of the culture broth in 100 mM phosphate buffer (pH 7.0) at 25ºC for 24 h with reciprocal shaking (115 strokes/min). The formation of 2-MP from 2-MPN was initially checked on TLC (silica gel 60 F254 plate, Merck) using developing solvent (*n*-butanol:acetic acid: water=2:1:1) and ninhydrin reagent. Then both concentration and optical purity of 2-MP formed were determined by HPLC after 2-MP was derivatized with GITC.
The following genera and soil isolates (unidentified) were used for screening: **bacterium**; *Achromobacter, Acinetobacter, Aeromonas, Agrobacterium, Alcaligenes, Arthrobacter, Aureobacterium, Bacillus, Brevibacterium, Brevundimonas, Cellulomonas, Citrobacter, Comamonas, Corynebacterium, Enterobacter, Erwinia, Escherichia, Flavobacterium, Gordona, Hafnia, Hydrogenophaga, Iodobacter, Micrococcus, Moraxella, Pantoea, Paracoccus, Pimelobacter, Pseudomonas, Ralstonia, Rhizobium, Rhodobacter, Rhodococcus, Rhodospirillum, Salmonella, Serratia, Xanthobacter*, *Actinomyces, Actinomadura, Nocardia* and *Streptomyces*; **fungus**; *Agaricales, Alternaria, Amylomyces, Amylomyces, Armillariella, Arthrinium, Aspergillus, Beauveria, Byssochlamys, Calonectria, Calcarisporium, Caldariomyces, Chaetomium, Chlamydoabsidia, Chloridium, Cladobotryum, Cladosporium, Clonostachys, Coelomycetes, Colletotrichum, Coniothyrium, Curvularia, Dactylaria, Dicranophora, Dothiora, Emericella, Eupenicillium, Eurotium, Eurotium, Exophiala, Fusarium, Ganoderma, Gibberella, Gliocladium, Grifola, Halteromyces, Hormonema, Hypocrea, Mariannaea, Memnoniella, Micronectriella, Monocillium, Mortierella, Mucor, Mucorales, Mycotypha, Myrothecium, Nectria, Oidiodendron, Paecilomyces, Penicillium, Periconia, Phaeococcomyces, Phoma*, *Phycomyces, Phytophthora, Pilaira, Pilobolus, Plectosphaerella, Pringsheimia, Protomycocladus, Radiomyces, Rhizopus, Sesquicillium, Stachybotrys, Sydowia, Syncephalastrum, Syncephalis, Syzygites, Thamnostylum, Torula, Trichoderma, Trichophyton, Umbelopsis, Verticillium, Volutella* and *Zygorhynchus*; **yeast**; *American yeast, Awamori yeast, Baker's yeast, Candida, Citeromyces, Cryptococcus, Debaryomyces, Hansenula, Hanseniaspora, Kloeckera, Pichia, Rhodotorula, Saccharomyces, Saccamycodes, Satumispora, Torulopsis and Williopsis*.

Derivatization of 2-MP with GITC

The derivatization of 2-methylpyrrolidine (2-MP) was carried out at 40ºC for 1h using each sample (50 μL), 0.8% (w/v) GITC (100 μL) and 0.2% (w/v) triethylamine (50 μL) in acetonitrile. The derivative was analyzed at 254 nm at a flow rate of 1.0 ml/min using HPLC with a Wakosil-II 5C18 RS (4.6 mm×150 m; Wako) and 10 mM KH_2PO_4/H_3PO_4 (pH 2.5)/methanol (55:45 v/v). *S*- and *R*-2-MP derivatives were detected at 24.9 min and 26.3 min, respectively. 2-MP concentration was determined using the calibration curves of authentic *S*- and *R*-2-MP derivatives.

Synthesis of PITC-derivatized 2-MP

PITC-derivatized 2-MP was synthesized from 2-MP at 40ºC using PITC and triethylamine in acetonitrile for NMR and MS analyses. After extraction of reaction product with ethyl acetate, the crude product was purified by silica gel chromatography with the following solvent (*n*-hexane : ethyl acetate=8:1, 4:1, 2:1 and 1:1). The PITC-derivative (54 mg, 0.245 mmol) was obtained from 0.39 mmol *R*-2-MP (>99%e.e.) formed by whole cells of GF3587 with 63% isolated yield.
PITC-derivatized 2-MP, 1-(2-methylpyrrolidine)thiocarboxanilide : δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.31 (3 H, d, *J* 6.3 CHCH_3), 1.65-2.13 (4 H, m, CHCH_2CH_2N), 3.53 (1 H, br s, CH_aH$_b$N), 3.63 (1 H, br s, CH$_a$$H_b$N), 4.51 (1 H, br s, NC$HCH_3$), 7.06 (1 H, s, N$H$Ph), 7.15-7.35 (5 H, m, Ph); δ_C(125 MHz; $CDCl_3$; Me_4Si) 18.8, 23.2, 32.2, 49.6, 56.8, 125.4, 125.6, 128.3, 139.3 and 177.8; *m*/*z* (EI) 220 (M^+; 100%), 187 (65) and 128 (89). All spectral data were coincident with those of PITC- derived authentic racemic 2-methylpyrrolidine.

Cultivation of 2-MPN-reducing *Streptomyces* Strains

Streptomyces sp. GF3587 was cultivated at 28ºC for 24 h with reciprocal shaking (115 strokes/min) in a 500-ml shaking flask containing 40 ml of the nutrient medium containing 8 g/L glucose, 20 g/L malt extract (Difco, USA), 4 g/L NZ amine (Wako Pure Chemical Industries, Osaka, Japan) and 5 mg/L $FeSO_4 \cdot 7H_2O$ at pH 7.3. Dry cell weight was determined to be 0.51 mg per ml of culture broth per OD (optical density) of 1.0 at 610 nm.

Streptomyces sp. GF3546 was cultivated at 28ºC for 72 h with reciprocal shaking (115 strokes/min) in a 500-ml shaking flask containing 40 ml of the nutrient medium containing 20 g/L glucose, 30 g/L meat extract (Kyokuto Pharmaceutical Industrial, Takahagi, Ibaraki, Japan), 20 g/L yeast extract (Oriental Yeast, Osaka, Japan), 10 g/L NZ amine and trace elements solution supplemented with 1 mg/L $FeSO_4 \cdot 7H_2O$, 1 mg/L $MnCl_2 \cdot 4H_2O$ and 1 mg/L $ZnSO_4 \cdot 7H_2O$ at pH 7.3. Dry cell weight was determined to be 9.1 mg per ml of culture broth.

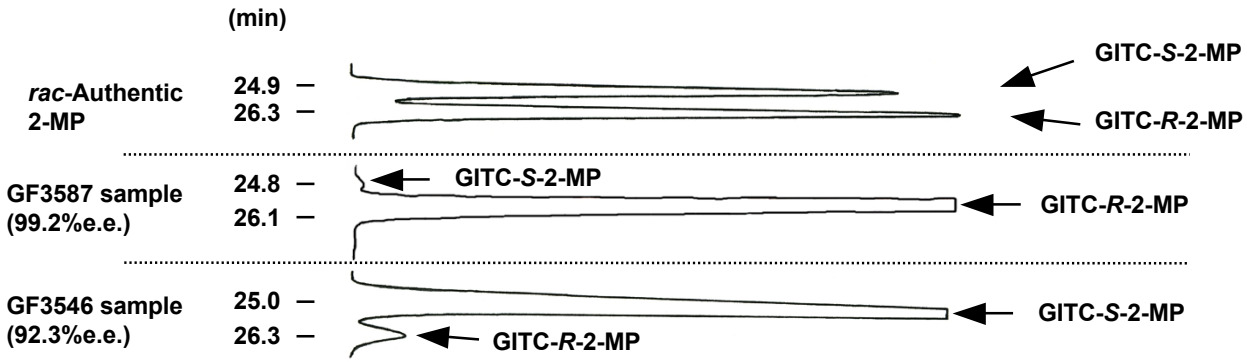
Production of *R*-and *S*-2-MP with whole-cell catalysts

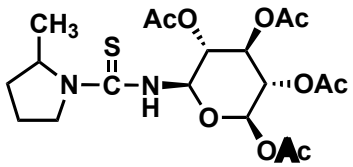
After cultivation, the cells of GF3587 were harvested by centrifugation at 12,000 *g* for 20 min, GF3546 cells were collected by filtration. Each of the cells suspended with an appropriate volume of 0.85% NaCl was used as a whole-cell catalyst (GF3587; 455 mg dry cell weight, GF3546; 83 mg dry cell weight). The reaction was carried out at 25ºC in the presence of glucose (2% or 4% w/v) with monitoring 2-MP formation by HPLC.

Syntheses of *R*-and *S*-2-MP hydrochlorides

R- and *S*-2-methylpyrrolidine hydrochloride were synthesized from reaction solution via both protection of amine and deprotection of *N*-Boc group.
R-2-methylpyrrolidine hydrochloride: δ_H(600 MHz; D_2O; DDS) 1.39 (3 H, d, *J* 6.2), 1.62-1.69 (1 H, m), 1.96-2.13 (2H, m), 2.17-2.26 (1H, m), 3.26-3.38 (2H, m), 3.66-3.74 (1H, m); δ_C(150 MHz; D_2O; DDS) 16.8, 23.2, 31.3, 45.1, 56.6. The spectra of *S*-2-methylpyrrolidine hydrochloride were coincident with those of *R*-2-MP hydrochloride: All spectral data were coincident with those of authentic racemic 2-methylpyrrolidine hydrochloride.

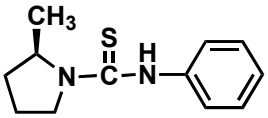
HPLC Chromatograms of GITC-derivatized 2-Methylpyrrolidine





2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate-2-methylpyrrolidine (GITC-2-MP)

^{1}H NMR (500 MHz, $CDCl_3$)



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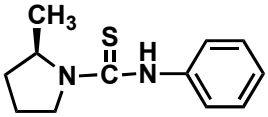

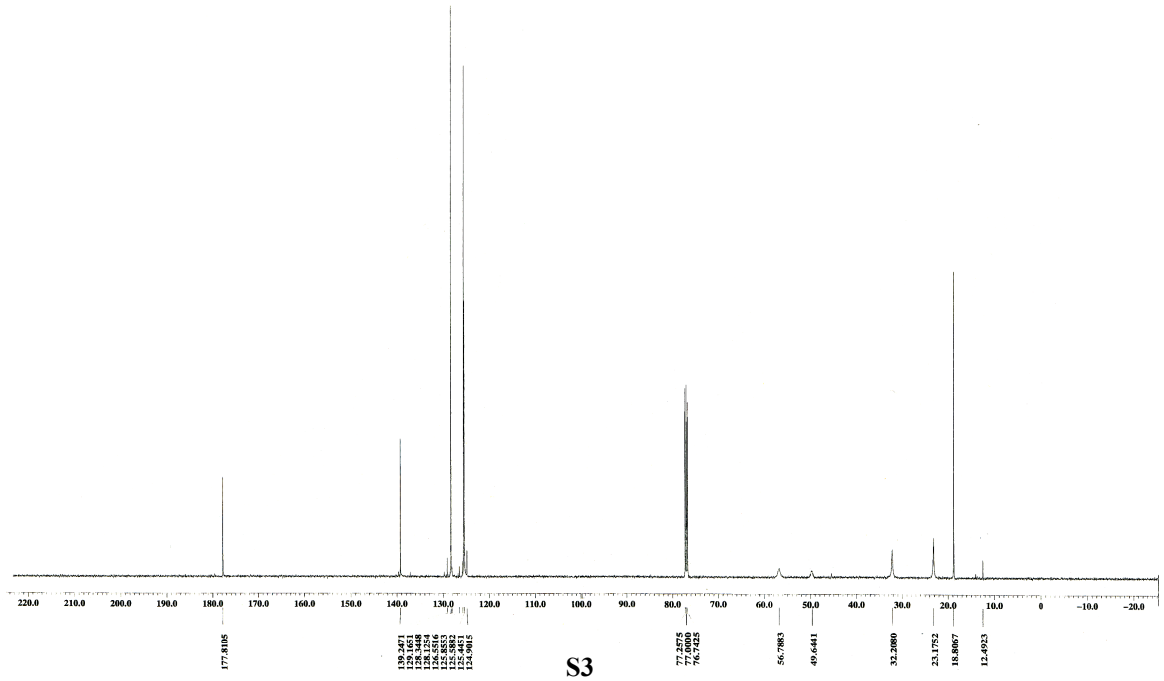

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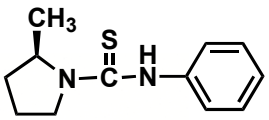
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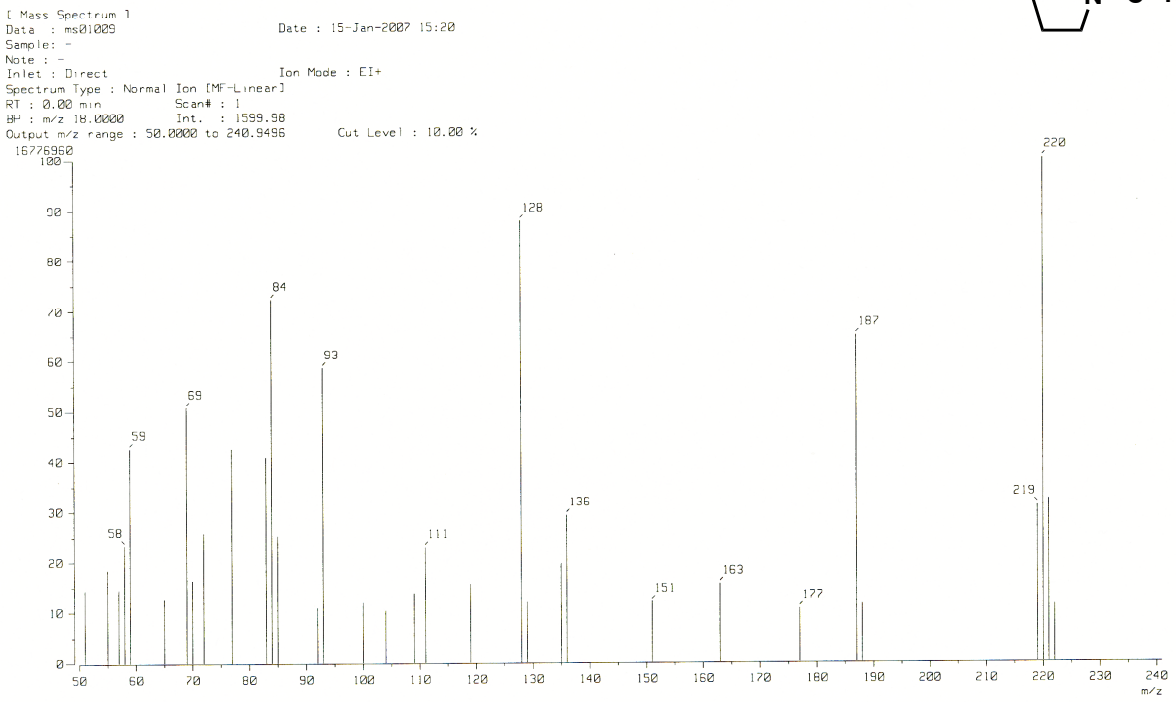
N
C
N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150
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190
200
210
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230
240
m/z
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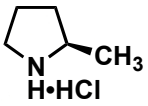
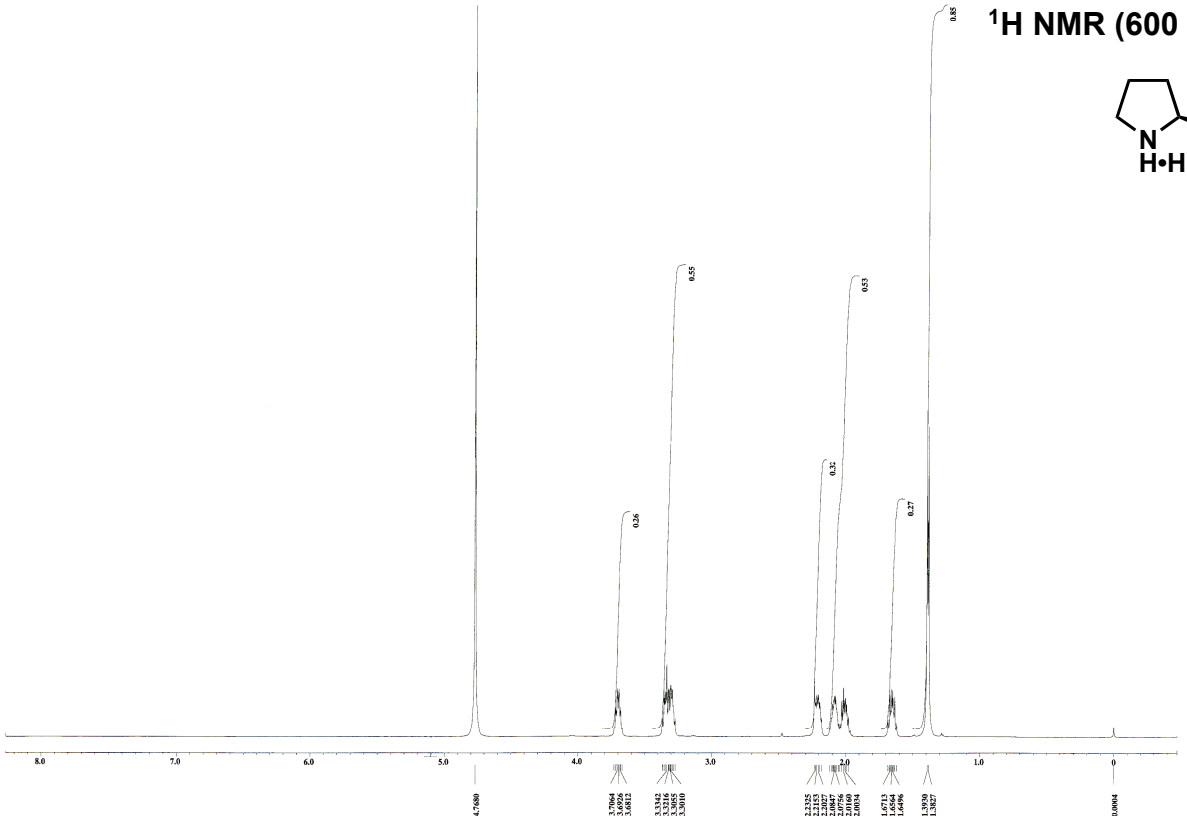
^{1}H NMR (600 MHz, D_2O)





^{13}C NMR (150 MHz, D_2O)