Supporting Information

One-Pot Cascade Synthesis of Mono- and Disubstituted Piperidines and Pyrrolidines using Carboxylic Acid Reductase (CAR), ω-Transaminase (ω-TA) and Imine Reductase (IRED) Biocatalysts

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1 Contents

2 Materials and Equipment

2.1 Chemicals and Equipment

Commercial reagents and anhydrous solvents were purchased from Sigma Aldrich, Alfa Aesar or Fluorochem and used without further purification. Specifically substrates **1a**, **1b**, and **14** were purchased from Sigma Aldrich and **1d** and **1e** were purchased from Fluorochem. Amine standards **2a** was purchased from Sigma Aldrich, **2b** was purchased from TCI Chemicals and **2c-2e** prepared as previously reported.[1,2] Column chromatography was performed with Fluka silica gel, 220-440 mesh and TLC carried out on Polygram SIL G/UV254. NMR spectra were recorded using a Bruker Avance 400 spectrometer with chemical shifts reported in ppm relative to residual protic solvent signals ($CHCl_3$ in $CDCl_3$, ^1H = 7.27; $CDCl_3$, ^{13}C = 77.0; CHD_2OD in CD_3OD, ^1H = 3.31; CD_3OD, ^{13}C = 49.0; CHD_2SOCD_3 in $(CD_3)_2SO$, ^1H = 2.50; $(CD_3)_2SO$, ^{13}C = 39.52).[3] The coupling constants (*J*) are quoted in Hz to the nearest 0.1 Hz. Signal multiplicities are assigned as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), sextet (sxt), multiplet (m), broad (br) or a combination of these. Low resolution mass spectrometry (MS) was performed on a Micromass Platform II instrument or on a HP-6890 GC connected to a HP5973 MS detector. High resolution mass spectrometry (HRMS) was performed on a Waters LCT-TOF instrument or an Agilent 1200 series LC system, coupled to an Agilent 6520 QTOF mass spectrometer, ESI positive mode with 50 % MeCN 0.1% formic acid eluent. Optical rotation measurements were taken on a Bellingham + Stanley ADP400+ digital polarimeter with the temperature, solvent and concentration stated. Microwave reactions were carried out in a CEM Discoverer microwave reactor using sealed reaction vessels. Maximum power output was set to 200 W and maximum pressure was set to 247 psi. All microwave reactions were conducted under stirring.

2.2 HPLC, GC and GCMS Analysis

Chiral normal phase HPLC was performed on an Agilent system equipped with a G1379A degasser, G1312A binary pump, a G1367A well plate autosampler unit, a G1316A temperature controlled column compartment and a G1315C diode array detector. CHIRALPAK®IB, CHIRALPAK®IC and CHIRALCEL OD-H analytical columns were purchased from Daicel (Osaka, Japan). All columns possess dimensions of 250 mm length, 4.6 mm diameter, 5 μm particle size columns were used. An injection volume of 10 μL was used and chromatograms were monitored at 265 nm.

GC analysis was performed on an Agilent 6850 GC with a flame ionization detector (FID) on a 25 m CP-Chirasil-DEX CB column with 0.25 mm inner diameter and 0.25 μm film thickness (Agilent, Santa Clara, CA, USA). Where stated, samples were derivatized using acetic anhydride with an excess of triethylamine at room temperature prior to GC-FID analysis.

GCMS analysis was performed on a HP-6890 Series GC coupled to a HP5973 MS detector, EI positive mode.

Analysis and *ee* determination of amines **2a-2e** based on previously reported HPLC or GC-FID analysis on a chiral stationary phase.[1,2]

When diketone substrates **11a-11d** and **14** were extracted under basic conditions, intramolecular aldol condensation products were observed. The resulting α,β-unsaturated ketone products were confirmed by GCMS analysis.

3 General Procedures for Expression of CAR Gene and Preparation of Whole-Cell Biocatalyst.

ATA-113, ATA-117, glucose dehydrogenase CDX-901 (GDH) and lactate dehydrogenase LDH-103 (LDH) biocatalysts were purchased as powdered lysates from Codexis. The (*R*)-IRED and (*S*)-IRED whole-cell biocatalysts were prepared as previously reported.[1,2]

Transformation of chemically competent cells was performed as per manufacturer's instructions (NEB).

The carboxylic acid reductase (CAR) biocatalysts were prepared as follows: chemically competent *E. coli* BL21 (DE3) cells were co-transformed with pET21 plasmid vectors containing either *Mycobacterium marinum* CAR or *Nocardia iowensis* CAR codon-optimized (for *E. coli*) genes and pCDF vector containing the phosphopantetheinyl transferase (Sfp) gene from a *Bacillus subtilis* strain. Transformants were selected by growth on LB agar plates containing appropriate selective agents (100 µg/mL ampicillin and 50 µg/mL spectinomycin). Single colonies were picked to initiate 10 mL overnight cultures of LB-Broth Miller containing 100 µg/mL ampicillin and 50 µg/mL spectinomycin in 50 mL Falcon tubes. These overnight cultures were grown for 16 h at 37°C, and shaking at 250 rpm, before being diluted 1:100 into 500 mL of auto-induction medium (according to Studier),[4] contained in a 2 L baffled conical flask. These expression cultures were incubated at 20°C and 250 rpm for 48 h. Cells were harvested by centrifugation at 4000 rpm and 4°C. Cells were washed with 100 mM pH 7.0 NaPi buffer and again harvested at 4000 rpm at 4°C and stored on ice until required.

3.1 CAR Gene Sequences

3.1.1 *Mycobacterium marinum* CAR (MCAR)

ATGAGCCCGATTACCCGTGAAGAACGTCTGGAACGTCGTATTCAGGATCTGTATGCGAACGATCCG
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CCCTGGCACGTCTGGGTGCTGTCGCTGTCCCGCTGCAGACCTCTGCTGCGATTACCCAGCTGCAGC

CGATTGTGGCGGAAACCCAGCCGACCATGATTGCGGCGAGCGTGGATGCCCTGGCCGATGCGACC

GAACTGGCACTGAGTGGTCAAACGGCTACGCGTGTGCTGGTGTTTGATCATCATCGTCAGGTGGAT

GCGCATCGTGCGGCGGTTGAAAGCGCGCGTGAACGTCTGGCCGGTAGCGCGGTGGTTGAAACCCT

GGCCGAAGCGATTGCGCGTGGTGATGTGCCGCGTGGTGCGAGCGCGGGTAGCGCACCGGGCACCG

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3.1.2 *Nocardia Iowensis* CAR (NCAR)

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3.1.3 *Bacillus subtilis* Sfp

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GATCCCGGCTACAAAATGGCTGTATGCGCCGCACACCCTGATTTCCCTGAGGATATCACAATGGTC

TCGTACGAAGAGCTTTTATAA

4 General Biotransformation Procedures

Analytical-scale reactions were carried out in 2 mL Eppendorf tubes with a total reaction volume of 500 μL. Preparative-scale reactions were carried out in non-baffled conical flasks with a total reaction volume to suit the amount of substrate to be converted.

4.1 CAR-TA-IRED Cascade General Procedure

Each biotransformation contained 5 mM substrate, 75 mg/mL CAR wet whole cells, 50 mg/mL IRED wet whole cells, 2.5 mg/mL ATA-113, 1 mg/mL GDH (CDX-901), 0.5 mg/mL LDH (LDH-103), 250 mM racemic DL-alanine, 100 mM glucose, 1.5 mM, NAD^+ and 1 mM PLP in 500 mM pH 7.0 sodium phosphate buffer and 1% v/v DMSO (from addition of substrate as a solution in DMSO). The reaction mixture was incubated at 30°C, 250 rpm for 24 h. Reactions were then basified to pH 12.0 with 10 M sodium hydroxide and extracted into methyl-tert-butyl ether or CH_2Cl_2 (1 mL for analytical-scale reactions) followed by analysis by normal phase HPLC, GC-FID or GC-MS. The aqueous layer was acidified to pH 1.0 with conc. HCl and extracted with methyl-tert-butyl ether or CH_2Cl_2 (1 mL for analytical-scale reactions) followed by analysis on HPLC to assay for keto acid consumption. Conversions based on HPLC, GC-FID and/or GC-MS analysis of imine and amine (and keto alcohol if present) peaks when consumption of keto acid was complete.

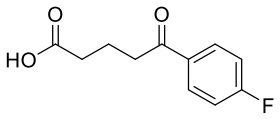
4.2 TA-IRED Cascade General Procedures

Starting from keto aldehyde: The CAR-TA-IRED Cascade General Procedure (4.1) was followed using 100 mM pH 7.0 sodium phosphate buffer, 45 mg/mL IRED wet whole cells and without the addition of CAR.

Starting from diketone: Each biotransformation contained 5 mM substrate, 2.5 mg/mL ATA-113 or ATA-117, 1 mg/mL GDH (CDX-901), 0.5 mg/mL LDH (LDH-103), 250 mM L-alanine (with ATA-113) or D-alanine (with ATA-117), 100 mM glucose, 1.5 mM, NAD^+ and 1 mM PLP in 100 mM pH 7.0 sodium phosphate buffer and 1% v/v DMSO (from addition of substrate as a solution in DMSO). The reaction mixture was incubated at 30°C, 250 rpm for 24 h to facilitate full conversion of starting diketone by the transaminase before addition of 200 mg/mL IRED wet whole cells and a further 24 h incubation time. Reactions were then basified to pH 12.0 with 10 M sodium hydroxide and extracted into ethyl acetate or diethyl ether (1 mL for analytical-scale reactions) followed by analysis by GC-FID or GC-MS. Conversions based on GC-FID analysis of keto acid, imine and amine peaks.

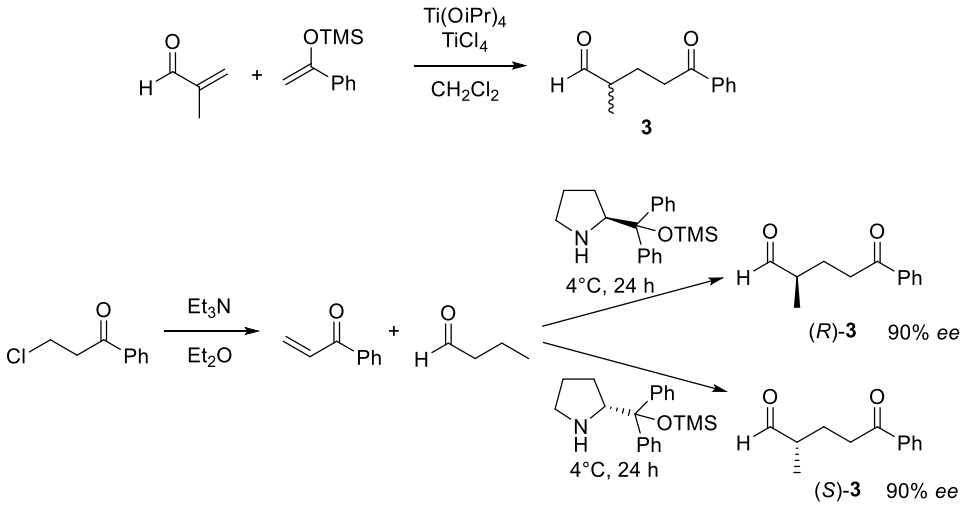
5 Keto Acid, Keto Aldehyde and Diketone Substrate Synthesis

4-(*p*-Fluorobenzoyl)butyric acid



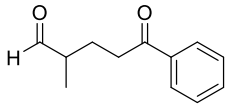
To a suspension of anhydrous aluminium chloride (18.77 g, 140.8 mmol) in CH_2Cl_2 (50 mL) at 0°C was added a solution of glutaric anhydride (7.67 g corrected for 95 % purity, 64 mmol) in CH_2Cl_2. The mixture was stirred vigorously at 0°C for 30 mins. A solution of *p*-fluorobenzene (6.15 g, 64.0 mmol) in CH_2Cl_2 (12 mL) was added drop-wise and stirred for 3 h. The reaction was quenched by pouring into a mixture of ice-water (1:1, 100 g) with vigorous stirring. The precipitate was collected by filtration, and washed with cold water until the washings were neutral. The precipitate was dissolved in NaOH solution (2 M, excess) and washed once with CH_2Cl_2. The product was precipitated by acidification with conc. HCl, collected by filtration and air dried. The product was recrystallized from ethanol-water to give the title compound as white leaflets, (8.03 g, 38 mmol, 60%). **^{1}H NMR** δ_H (400 MHz, $(CD_3)_2SO$) 12.01 (s, 1H), 8.07-7.99 (m, 2H), 7.37-7.28 (m, 2H), 3.04 (t, *J* = 7.0 , 2H), 2.30 (t, *J* = 7.5 , 2H), 1.82 (quint, *J* = 7.5, 2H); **^{13}C NMR** δ_C (100 MHz, $(CD_3)_2SO$) 198.2, 174.3, 165.0 (d, *J* = 251.5), 133.4 (d, *J* = 3.0), 130.8 (d, *J* = 9.5), 115.7 (d, *J* = 22.0), 37.1, 32.8, 19.2. NMR data consistent with literature values.[5]

5.1 Preparation of 2-Methyl-5-oxo-5-phenylpentanal, 3



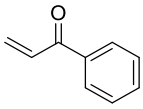
Scheme S1: Route to (±)-**3**, (*R*)-**3** and (*S*)-**3**

2-Methyl-5-oxo-5-phenylpentanal, (±)-3



Title compound prepared by a method based on work by Narasaka *et al*..[6] To a solution of $TiCl_4$ (189 mg, 1.00 mmol) in CH_2Cl_2 (4 mL) at -78°C under nitrogen was added $Ti(O^iPr)_4$ (284 mg, 1.00 mmol). To this mixture was added a solution of 1-phenyl-1-trimethylsiloxyethylene (192 mg, 1.00 mmol) in CH_2Cl_2 (3 mL) and a solution of methacrolein (70 mg, 1.00 mmol). The reaction was stirred for 30 mins then quenched with K_2CO_3 (aq) solution (0.5 M, 12 mL). The mixture was allowed to warm to room temperature and filtered to remove solid inorganic material. The product was extracted into CH_2Cl_2 (3 x 10 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was subjected to column chromatography (silica, 9:1 cyclohexane:EtOAc, R_f = 0.24) to afford **(±)-3** as a colorless oil (102 mg, 0.53 mmol, 53%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 9.68 (d, *J* = 1.8, 1H), 7.97 (m, 2H), 7.58 (m, 1H), 7.48 (m, 2H), 3.12-3.98 (m, 2H), 2.55-2.46 (m, 1H), 2.21-2.12 (m, 1H), 1.87 (ddt, *J* = 14.3, 8.1, 6.3, 1H), 1.19 (d, *J* = 7.2, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 204.5, 199.3, 136.7, 133.2, 128.6, 128.0, 45.6, 35.5, 24.6, 13.6; **IR** ν_{max}/cm^{-1} 2969 (C-H), 2932 (C-H), 1721 (C=O), 1682 (C=O), 1580 (C=C); **MS** *m*/*z* 213 $[M+Na]^+$; **HRMS** calcd. for $C_{12}H_{14}O_2Na$ 213.0891$[M+Na]^+$, found 213.0882. Data consistent with literature values.[7]

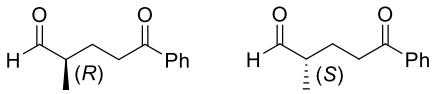
1-Phenylprop-2-en-1-one



To a solution of 3-chloropropiophenone (1.00 g, 5.93 mmol) in diethyl ether (15 mL) was added triethylamine (2.00 mL, 14.35 mmol) and the reaction stirred at room temperature for 18 h. The reaction was filtered to remove the white precipitate by-product formed and the solvent was removed under reduced pressure. The residue was subjected to column chromatography (silica, 95:5 cyclohexane:EtOAc, R_f = 0.46 with 9:1 cyclohexane:EtOAc) to afford the title compound as a colorless oil (687 mg, 5.20 mmol, 88%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.98-7.93 (m, 2H), 7.62-7.56 (m, 1H), 7.53-7.46 (m, 2H), 7.17 (dd, *J* = 17.2, 10.5, 1H), 6.45(dd, *J* = 17.2, 1.6, 1H), 5.95 (dd, *J* = 10.5, 1.6, 1H); **^{13}C NMR** δ_C (100 MHz

$CDCl_3$) 191.1, 137.3, 133.0, 132.4, 130.2, 128.7, 128.6; **IR** ν_{max}/cm^{-1} 3061 (C-H), 1670 (C=O); **MS** *m*/*z* 133 $[M+H]^+$, 303 $[2M+Na]^+$. NMR data consistent with literature values.[8]

(*R*)- and (*S*)-2-Methyl-5-oxo-5-phenylpentanal, (*R*)-3 and (*S*)-3



Title compound prepared by a method based on work by Gellman *et al*.[9] and previously reported by Feuillastre *et al*..[7] To a mixture of neat propionaldehyde (1 equiv.) and 1-phenylprop-2-en-1-one (1.5 equiv.) at 0°C was added (*R*)- or (*S*)-α,α-diphenyl-2-pyrrolidinemethanol trimethylsilyl ether (0.05 equiv.) and ethyl 3,4-dihydroxybenzoate (0.2 equiv.). The reaction was stirred at 4°C for 24 h. The crude residue was immediately subjected to column chromatography (silica, 9:1 cyclohexane:EtOAc, R_f = 0.22) to afford either (*R*)- or (*S*)-**3**.

(*R*)-**3**; Propionaldehyde (116 mg, 2.00 mmol) as limiting reagent with (*S*)-α,α-diphenyl-2-pyrrolidinemethanol trimethylsilyl ether catalyst afforded (*R*)-**3** as a colorless oil (226 mg, 1.19 mmol, 60 %) after column chromatography. NMR data was identical to the racemic compound.

(*S*)-**3**; Propiophenone (65 mg, 1.12 mmol) as limiting reagent with (*R*)-α,α-diphenyl-2-pyrrolidinemethanol trimethylsilyl ether catalyst afforded (*S*)-**3** as a colorless oil (145 mg, 0.76 mmol, 68 %) after column chromatography. NMR data was identical to the racemic compound.

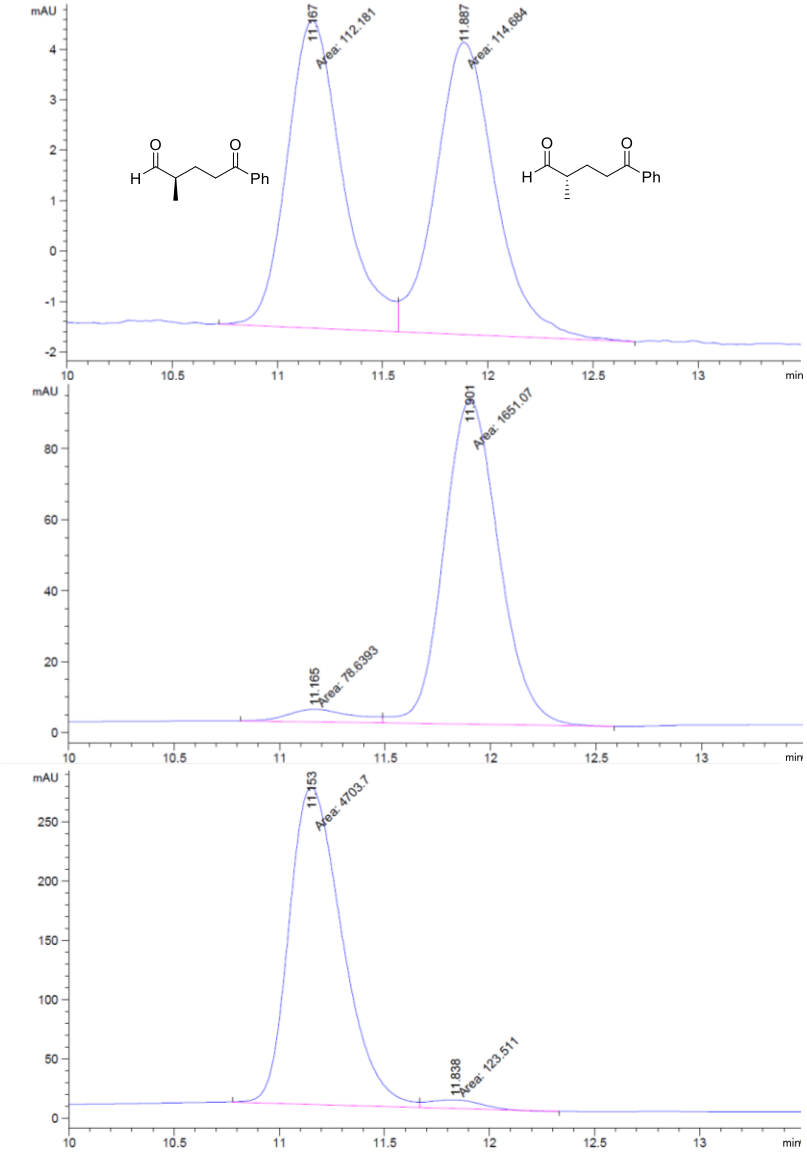
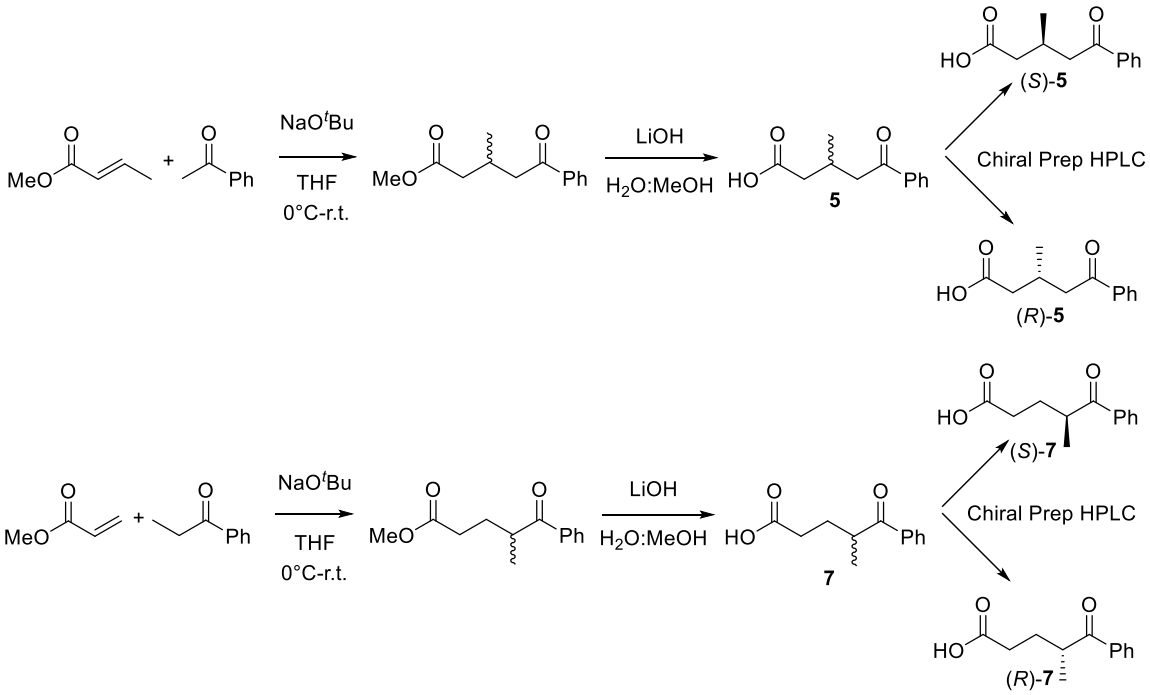


Figure S1: HPLC analysis of **3**. (Daicel CHIRALPAK®IC 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/diethylamine = 80/20/0.1, 1 mL/min, 265 nm). a) (±)- **3**, b) (*R*)-**3** prepared using (*S*)-α,α-diphenyl-2-pyrrolidinemethanol trimethylsilyl ether catalyst, c) (*S*)-**3** prepared using (*S*)-α,α-diphenyl-2-pyrrolidinemethanol trimethylsilyl ether catalyst.

5.2 Preparation of Chiral Keto Acids, 5 and 7.

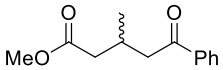


Scheme S2: Synthesis of keto acids **5** and **7**

5.2.1 General Method 1: Michael Addition to form Chiral Keto Esters

Method based on a procedure by Pan *et al*..[10] To a stirred mixture of sodium *tert*-butoxide (1.1 equiv.) in anhydrous THF (5 mL/mm of limiting reagent) under nitrogen at 0°C was added ketone (1 equiv.) and α,β-unsaturated ester (1.1 equiv.). The reaction was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ and EtOAc were added. The organic layer was separated and the aqueous layer extracted with EtOAc (3 x). The organic layers were combined, washed with brine, dried over $MgSO_4$ the solvent removed under reduced pressure. The crude product was subjected to column chromatography to afford the keto ester.

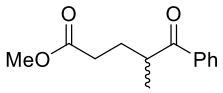
Methyl 3-methyl-5-oxo-5-phenylpentanoate



Following General Method 1 with acetophenone (1.16 mL, 10.0 mmol) and methyl crotonate (1.16 mL, 11.0 mmol) and subjecting the crude product to column chromatography (silica, 9:1 cyclohexane:EtOAc, R_f = 0.20), the title compound was afforded as a colorless oil (951

mg, 4.32 mmol, 43%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.00-7.95 (m, 2H), 7.60-7.54 (m, 1H), 7.50-7.44 (m, 2H), 3.68 (s, 3H), 3.12 (dd, *J* = 16.3, 5.9 , 1H), 2.85 (dd, *J* = 16.3, 7.6 , 1H), 2.74-2.63 (m, 1H), 2.45 (dd, *J* = 15.3, 6.7 , 1H), 2.33 (dd, *J* = 15.3, 7.1 , 1H), 1.06 (d, *J* = 6.8 , 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 199.2, 173.0, 137.0, 133.0, 128.6, 128.1, 51.5, 44.8, 40.8, 26.8, 20.1; **IR** ν_{max}/cm^{-1} 2954 (C-H), 1732 (C=O), 1682 (C=O); **HRMS** calcd. for $C_{13}H_{16}O_3Na$ 243.0992 $[M+Na]^+$, found 243.0992. Data consistent with literature values.[10]

Methyl 4-methyl-5-oxo-5-phenylpentanoate

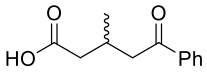


Following General Method 1 with propiophenone (1.00 mL, 7.52 mmol) and methyl acrylate (0.75 mL, 8.27 mmol) and subjecting the crude product to column chromatography (silica, 9:1 cyclohexane:EtOAc, R_f = 0.15), the title compound was afforded as a colorless oil (831 mg, 3.77 mmol, 50%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.00-7.94 (m, 2H), 7.60-7.54 (m, 1H), 7.50-7.44 (m, 2H), 3.65 (s, 3H), 3.63-3.53 (m, 1H), 2.46-2.27 (m, 2H), 2.23-2.11 (m, 1H), 1.84-1.73 (m, 1H), 1.21 (d, *J* = 7.1 , 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 203.5, 173.7, 136.3, 133.0, 128.7, 128.3, 51.5, 39.5, 31.4, 28.2, 17.3; **IR** ν_{max}/cm^{-1} 2966 (C-H), 2951 (C-H), 1733 (C=O), 1679 (C=O); **MS** *m*/*z* 243 $[M+Na]^+$; **HRMS** calcd. for $C_{13}H_{16}O_3Na$ 243.0997 $[M+Na]^+$, found 243.0998. Data consistent with literature values.[11]

5.2.2 General Method 2: Ester Hydrolysis to form Chiral Keto Acids

To a solution of keto ester (1 equiv.) in H_2O:MeOH (3:1, 10 mL/mmol of keto ester) was added LiOH (5 equiv.). The reaction was stirred at room temperature for 18 h and then acidified with 1 M HCl to pH 1.0-2.0. The product was extracted into diethyl ether (3 x), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was subjected to column chromatography or triturated with cyclohexane to afford the keto acid.

3-Methyl-5-oxo-5-phenylpentanoic acid, 5

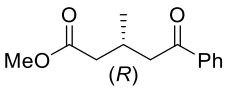


Following General Method 2 with methyl 3-methyl-5-oxo-5-phenylpentanoate (400 mg, 1.82 mmol) and subjecting the crude product to column chromatography (silica, 4:1:0.1 cyclohexane:EtOAc:formic acid, R_f = 0.18) followed by trituration with cyclohexane afforded the title compound as a white solid (343 mg, 1.66 mmol, 91%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.00-7.94 (m, 2H), 7.60-7.54 (m, 1H), 7.51-7.44 (m, 2H), 3.13 (dd, *J* = 16.5, 6.1 , 1H), 2.90 (dd, *J* = 16.4, 7.3, 1H), 2.75-2.64 (m, 1H), 2.52 (dd, *J* = 15.5, 6.4, 1H), 2.37 (dd, *J* = 15.7, 7.2, 1H), 1.10 (d, *J* = 6.8, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 199.2, 178.5, 137.0, 133.1, 128.6, 128.1, 44.7, 40.7, 26.5, 20.1; **IR** ν_{max}/cm^{-1} 2964 (C-H), 2889 (O-H broad), 1703 (C=O), 1679 (C=O); **MS** *m*/*z* 229 $[M+Na]^+$; **HRMS** calcd. for $C_{12}H_{14}O_3Na$ 229.0841 $[M+Na]^+$, found 229.0838. NMR data consistent with literature values.[12]

Separation of enantiomers was achieved using preparative chiral HPLC (Daicel CHIRALCEL®OJH 250 mm x 20 mm x 5 µm semi-prep column, 95:5:0.01, n-hexane:2-propanol:TFA, 15 mLmin^{-1} flow rate).

Determination of absolute configuration was achieved by comparison of the specific rotation of the methyl ester derivative to known literature values.

Formation of methyl ester: To a solution of keto acid (11.6 mg, 0.06 mmol) in methanol (1.5 mL) was added one drop of concentrated aqueous H_2SO_4 and the reaction refluxed for 4 h. The reaction was cooled to room temperature, quenched with $NaHCO_3$ (4 mL, 10% aqueous soln.) and the product extracted into EtOAc (3 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to afford the methyl ester as a colorless oil (12.3 mg, 0.06 mmol, 93%).



$[\alpha]_D^{23}$ +3.11 (*c* = 1.00, benzene). (Lit.,[13] $[\alpha]_D^{20}$ -5.65 (*c* = 1.24, benzene) for (*S*)-isomer)

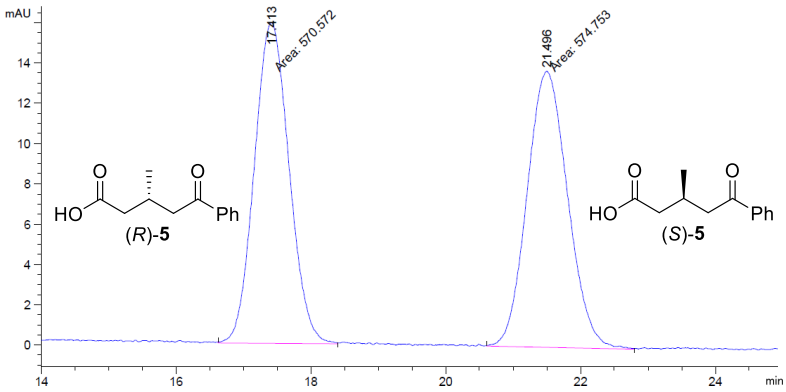
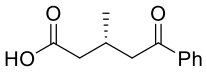
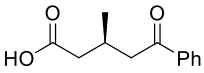
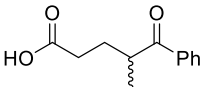


Figure S2: HPLC analysis of **5**. (Daicel CHIRALCEL®OJH 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/trifluoroacetic acid = 95/5/0.1, 1 mL/min, 280 nm).

(*R*)-**5**:  $[\alpha]_D^{23}$ + 6.76 (*c* = 0.30, MeOH)

(*S*)-**5**:  $[\alpha]_D^{23}$ - 9.62 (*c* = 0.42, MeOH)

4-Methyl-5-oxo-5-phenylpentanoic acid, 7



Following General Method 2 with methyl 4-methyl-5-oxo-5-phenylpentanoate (672 mg, 3.05 mmol) and subjecting the crude product to trituration with cyclohexane afforded the title compound as a white solid (625 mg, 3.03 mmol, 99%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.00-7.94 (m, 2H), 7.61-7.55 (m, 1H), 7.52-7.44 (m, 2H), 3.65-3.53 (m, 1H), 2.52-2.31 (m, 2H), 2.25-2.12 (m, 1H), 1.86-1.74 (m, 1H), 1.24 (d, *J* = 7.10, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 203.4, 178.7, 136.2, 133.1, 128.7, 128.3, 39.5, 31.3, 27.8, 17.5; **IR** ν_{max}/cm^{-1} 3065 (C-H), 2961 (C-H), 2995 (O-H broad), 1714 (C=O), 1669 (C=O); **MS** *m/z* 229 $[M+Na]^+$; **HRMS** calcd. for $C_{12}H_{14}O_3Na$ 229.0841 $[M+Na]^+$, found 229.0836.

Separation of enantiomers was achieved using preparative chiral HPLC (Daicel CHIRALCEL®OJH 250 mm x 20 mm x 5 µm semi-prep column, 95:5:0.01, n-hexane:2-propanol:TFA, 15 mLmin^{-1} flow rate).

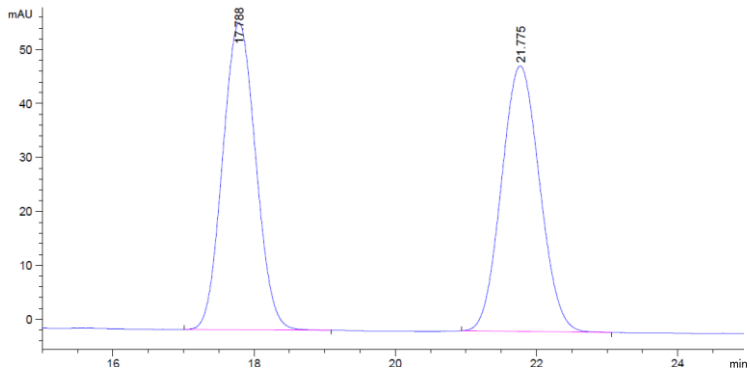


Figure S3: HPLC analysis of **7**. (Daicel CHIRALCEL®OJH 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/trifluoroacetic acid = 95/5/0.1, 1 mL/min, 280 nm).

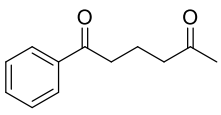
Enantiomer 1 (retention time 17.8 mins): $[\alpha]_D^{23}$ – 37.38 (*c* = 0.43, MeOH)

Enantiomer 2 (retention time 21.8 mins): $[\alpha]_D^{23}$ + 31.81 (*c* = 0.44, MeOH)

5.2.3 General Method 3: Grignard Reaction to form 1,5-Diketones

Under a nitrogen atmosphere, 3,4-dihydro-6-methyl-2*H*-pyran-2-one (1 equiv.) was diluted in dry ether (4 mL/mmol of pyran-2-one) before being cooled to -78°C. The Grignard reagent (1.1 equiv.) was added dropwise and the mixture was stirred overnight at -78°C. The reaction was then warmed to room temperature and quenched by addition of 1 M HCl solution until pH 1.0-2.0. The mixture was then separated and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to yield the crude product. Purification on silica gel afforded the desired product.

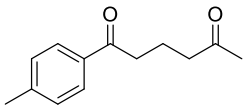
1-Phenylhexane-1,5-dione, 11a



Following General Method 3, pyran-2-one (501 mg, 4.47 mmol) was reacted with phenylmagnesium bromide (1 M solution in THF, 4.92 mL, 4.92 mmol). The crude product was subjected to column chromatography (silica, 92:8 cyclohexane:EtOAc) to afford the title compound **11a** (544 mg, 64% yield) as white crystals. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.98-7.94 (m, 2H, ArH), 7.58-7.54 (m, 1H), 7.49-7.44 (m, 2H), 3.02 (t, *J* = 7.0, 2H), 2.58 (t, *J* = 7.0, 2H, CH_2CH_2), 2.16 (s, 3H), 2.02 (quint, *J* = 7.0, 2H); δ_C **^{13}C NMR** (100 MHz, $CDCl_3$)

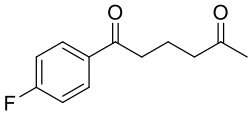
208.5, 199.8, 136.8, 133.1, 128.6, 128.0, 42.6, 37.4, 30.0, 18.2; **MS** *m*/*z* 190 $[M]^+$. **HRMS** calcd. for $C_{12}H_{14}O_2Na$ 213.0866 $[M+Na]^+$, found 213.0975. Spectroscopic data is consistent with literature values.[14]

1-(*p*-Tolyl)hexane-1,5-dione, 11b



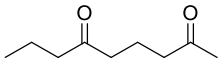
Following General Method 3, pyran-2-one (501 mg, 4.47 mmol) was reacted with (*p*-tolyl)magnesium bromide (0.5 M solution in THF, 9.83 mL, 4.92 mmol). The crude product was subjected to column chromatography (silica, 92:8 cyclohexane:EtOAc) to afford the title compound **11b** (428 mg, 47% yield) as a pale yellow solid. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) δ: 7.88-7.83 (m, 2H), 7.27-7.23 (m, 2H), 2.98 (t, *J* = 7.0, 2H), 2.56 (t, *J* = 7.0, 2H), 2.40 (s, 3H), 2.15 (s, 3H), 2.00 (quint, *J* = 7.0, 2H); **^{13}C NMR** δ_C (100 MHz, $CDCl_3$) 208.8, 199.6, 144.0, 134.5, 129.4, 128.3, 42.8, 37.4, 30.1, 21.8, 18.4; **MS** *m*/*z* 204 $[M]^+$. **HRMS** calcd. for $C_{13}H_{16}O_2Na$ 227.1043 $[M+Na]^+$, found 227.1125. Spectroscopic data is consistent with literature values.[15]

1-(4-Fluorophenyl)hexane-1,5-dione, 11c



Following General Method 3, pyran-2-one (1.1 g, 3.40 mmol) was reacted with (*p*-fluorophenyl)magnesium bromide (1 M solution in THF, 10.8 mL, 10.8 mmol). The crude was subjected to column chromatography (silica, 17:3 cyclohexane:EtOAc) to afford the title compound **11c** (640 mg, 31% yield) as an off-white solid. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.02-7.95 (m, 2H), 7.16-7.09 (m, 2H), 2.99 (t, *J* = 7.0, 2H), 2.57 (t, *J* = 7.0, 2H), 2.15 (s, 3H), 2.00 (quint, *J* = 7.0, 2H); **^{13}C NMR** δ_C (100 MHz, $CDCl_3$) 206.6, 198.3, 165.9 (d, *J* = 255.0), 133.3 (d, *J* = 3.0), 130.8 (d, *J* = 9.0), 115.8 (d, *J* = 22.0), 42.6, 37.5, 30.1, 18.3; **MS** *m*/*z* 208 $[M]^+$. **HRMS** calcd. for $C_{12}H_{13}O_2FNa$ 231.0792 $[M+Na]^+$, found 231.0866.

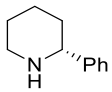
Nonane-2,6-dione, 11d



Following General Method 3, pyran-2-one (501 mg, 4.47 mmol) was reacted with (*n*-propyl)magnesium chloride (2 M solution in THF, 2.46 mL, 4.49 mmol). The crude product was subjected to column chromatography (silica, 9:1 cyclohexane:EtOAc) to afford the title compound **11d** (560 mg, 80% yield) as pale yellow oil, which then spontaneously crystallized to furnish pale yellow crystals. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 2.45 (t, *J* = 7.0, 2H), 2.41 (t, *J* = 7.0, 2H), 2.35 (t, *J* = 7.4, 2H), 2.11 (s, 3H), 1.81 (quint, *J* = 7.0, 2H), 1.57 (sxt, *J* = 7.4, 2H), 0.88 (t, *J* = 7.4, 3H); **^{13}C NMR** δ_C (100 MHz, $CDCl_3$) 210.8, 208.6, 44.8, 42.7, 41.6, 30.0, 17.8, 17.4, 13.8.; **MS** *m*/*z* 156 $[M]^+$. Spectroscopic data is consistent with literature values.[14]

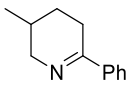
6 Preparative-Scale Biotransformations and Product Synthesis

(*R*)-2-Phenylpiperidine, 2b



Following the CAR-TA-IRED Cascade General Procedure (100 mg, 0.52 mmol) with (*S*)-IRED afforded the title compound (70 mg, 0.43 mmol, 83%) *ee* 93% (*R*). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$); 7.40-7.28 (m, 4H), 7.28-7.21 (m, 1H), 3.64-3.55 (m, 1H), 3.25-3.16 (m, 1H), 2.81 (td, *J* = 11.5, 2.8, 1H), 1.98-1.86 (m, 2H), 1.85-1.74 (m, 1H), 1.74-1.62 (m, 1H), 1.62-1.44 (m, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 145.5, 128.3, 127.0, 126.6, 62.3, 47.8, 34.9, 25.8, 25.4; **HRMS** calcd. for $C_{11}H_{16}N$ 162.1283 $[M+H]^+$, found 162.1273. Data consistent with literature values.[16]

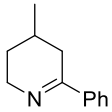
3-Methyl-6-phenyl-2,3,4,5-tetrahydropyridine, (±)-16



Following the TA-IRED Cascade General Procedure for keto aldehydes without the addition of IRED, keto aldehyde **(±)-3** (200 mg, 1.05 mmol) and ATA-113 afforded chiral imine **(±)-16** (120 mg, 0.63 mmol, 60%) as a yellow oil. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.82-7.77 (m, 2H), 7.42-7.36 (m, 3H), 4.02 (ddt, *J* = 17.6, 4.5, 2.1, 1H), 3.32-3.21 (m, 1H), 2.87-2.77 (m,

1H), 2.67-2.56 (m, 1H), 1.98-1.90 (m, 1H), 1.80-1.68 (m, 1H), 1.40 (dtd, *J* =12.9, 11.1, 6.2, 1H), 1.01 (d, *J* = 6.6, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 165.9, 139.5, 129.8, 128.2, 126.1, 57.3, 27.9, 27.5, 27.2, 19.1; **MS** *m*/*z* 173 [$M^{+\cdot}$]

4-Methyl-6-phenyl-2,3,4,5-tetrahydropyridine, 17



Following the CAR-TA-IRED Cascade General Procedure without the addition of IRED, keto acid (*R*)-**5** (33 mg, 0.16 mmol) afforded (*S*)-**17** (26 mg, 0.15 mmol, 94%) and (*S*)-**5** (33 mg, 0.16 mmol) afforded (*S*)-**17** (25 mg, 0.14 mmol, 90%) as yellow oil. **^{1}H NMR** δ_H (400 MHz, CD_3OD of imine hydrochloride salt) 7.94-7.88 (m, 2H), 7.82-7.76 (m, 1H), 7.69-7.63 (m, 2H), 4.03-3.92 (m, 1H), 3.89-3.77 (m, 1H), 3.57-3.47 (m, 1H) 2.90-2.77 (m, 1H), 2.20-2.06 (m, 2H), 1.74-1.61 (m, 1H), 1.21 d, *J* = 6.6, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$ of imine free base); 165.5, 129.5, 128.6, 128.2, 125.9, 50.3, 35.6, 30.2, 26.1, 22.0; **MS** *m*/*z* 174 $[M+H]^+$; **HRMS** calcd. for $C_{12}H_{16}N$ 174.1283 $[M+H]^+$, found 174.1281.

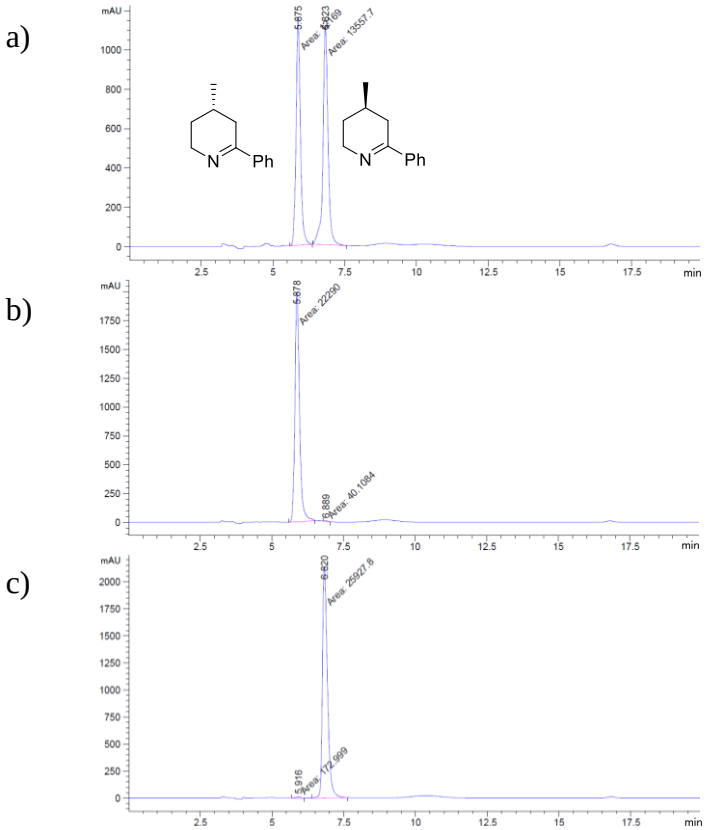
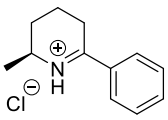


Figure S4: HPLC analysis of 17. (Daicel CHIRALPAK®IB 250 mm × 4.6 mm, 5 µm, 95:5:0.01, n-hexane:2-propanol:diethylamine, 1 mL/min, 254 nm. a) Racemic **17**, b) (*S*)-**17** from biotransformation with (*R*)-**5**, c) (*R*)-**17** from biotransformation of (*S*)-**5**.

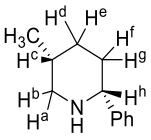
(*S*)-6-methyl-2-phenyl-1-piperidinium hydrochloride, (*S*)-12a



Following the TA-IRED Cascade General Procedure for diketones without the addition of IRED, 1-phenylhexane-1,5-dione, **11a** (100 mg, 0.526 mmol from a 1 M stock solution in DMSO), afforded (*S*)-**12a**. The product was isolated by adjusting the pH of the reaction mixture to pH 2.0 by addition of conc. HCl and the aqueous mixture was washed with ethyl acetate (1 x 60 mL). The pH of the aqueous phase was then adjusted to pH 12.0 with 10 M sodium hydroxide. The aqueous mixture was then extracted with ethyl acetate (3 x 60 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure to yield the crude imine (*S*)-**12a** as yellow oil.

It was found that the chiral imine was unstable in storage, therefore (*S*)-**12a** was dissolved in diethyl ether and then acidified with 2 M HCl/diethyl ether solution (1 mL). The excess diethyl ether was carefully decanted and the remaining liquid was left to dry to yield chiral imine (*S*)-**12a**.HCl (102 mg, 0.486 mmol, 93%, *ee* >98%) as an off-white solid. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.94-7.90 (m, 2H), 7.81-7.76 (m, 1H), 7.69-7.63 (m, 2H), 4.12 (qt, *J* = 7.0, 5.0, 1H), 3.41-3.21 (m, 2H), 2.18 (dddd, *J* = 13.5, 7.5, 5.0, 3.5, 1H), 2.08 (ddd, *J* = 14.0, 7.0, 3.0, 1H), 2.03-1.93 (m, 1H), 1.76 (ddd, *J* = 10.0, 7.5, 3.5, 1H), 1.55 (d, *J* = 7.0, 3H); **^{13}C NMR** δ_C (100 MHz, $CDCl_3$) 185.8, 136.0, 133.2, 130.6, 129.3, 54.0, 40.4, 28.0, 20.0, 16.9; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$ = - 20.0 (*c* = 1.0, MeOH).

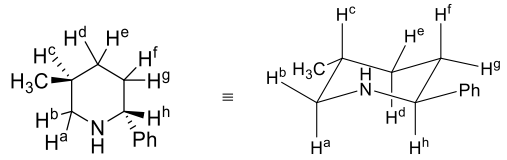
(2*R*,5*R)*-5-Methyl-2-phenylpiperidine, (2*R*,5*R)*-4



Following the TA-IRED Cascade General Procedure for keto aldehydes with (*R*)-**3** (100 mg, 0.53 mmol) and (*S*)-IRED afforded (2*R*,4*R*)-**4**. The crude product was further purified by dissolving the residue in EtOAc (10 mL) and extracting the product into 1 M HCl (3 x 10 mL). The aqueous layers were combined, basified with 10 M NaOH to pH 12.0 and the

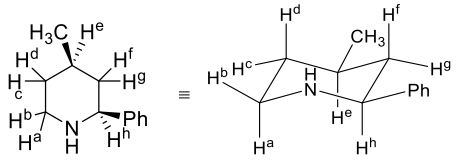
product extracted into CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure to afford (2*R*,4*R*)-**4** (73 mg, 0.42 mmol, 79%, *de* >98%, *ee* 95%) as a pale yellow oil. The *cis*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.43-7.38 (2H, m, ArH), 7.36-7.29 (2H, m, ArH), 7.27-7.21 (1H, m, ArH), 3.66 (1H, dd, *J* = 9.6, 3.0 Hz, H^h), 3.01 (1H, dd, *J* = 11.8, 3.4 Hz, H^a or H^b), 2.88 (1H, ddd *J* = 11.8, 2.9, 1.8 Hz, H^a or H^b), 1.94-1.69 (3H, m, Hc, H^d or H^e and H^f or H^g), 1.67-1.57 (2H, m, H^d or H^e and H^f or H^g), 1.16 (3H, d, *J* =6.9 Hz, CH_3); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 145.4 (ArC), 128.3 (ArC), 126.8 (ArC), 126.6 (ArC), 61.6 (CH^h), 52.3 (CH^a), 30.7 (CH^d or CH^f), 29.5 (CH^d or CH^f), 27.7 (CH^c), 17.2 (CH_3); **IR** ν_{max}/cm^{-1} 3061 (C-H), 3026 (C-H), 2927 (C-H), 2850 (C-H), 2786 (C-H); **HRMS** calcd. for $C_{12}H_{18}N$ 176.1439 $[M+H]^+$, found 176.1381. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$ = + 26.3 (*c* = 1.60, $CHCl_3$). Assignment as the *cis*-diastereomer was based on the absence of *J* values indicative of axial-axial couplings for H^a and H^b with H^c as described by the Karplus equation.

(±)-*cis*/*trans*-5-Methyl-2-phenylpiperidine, (±)-*cis*/*trans*-4



To afford a racemic standard for GC analysis that contained *cis*- and *trans*-isomers, **(±)-3** (50 mg, 0.26 mmol) was subjected to the TA-IRED Cascade General Procedure for keto aldehydes without the addition of IRED. After 24 h $NH_3.BH_3$ (40 mg, 1.30 mmol) was added and the reaction incubated for a further 1 h before extraction as described in the general method. The crude product was further purified by dissolving the residue in EtOAc (5 mL) and extracting the product into 1 M HCl (3 x 5 mL). The aqueous layers were combined, basified with 10 M NaOH to pH 12.0 and the product extracted into CH_2Cl_2 (3 x 10 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure to afford **(±)-4** (25 mg, 0.14 mmol, 55%, *de* 75%) with the major product being the *trans*-diastereomer. The *trans*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.50-7.34 (4H, m, ArH), 7.35-7.28 (1H, m, ArH), 3.62 (1H, dd, *J* = 11.2, 2.6, H^h), 3.22 (1H, ddd, *J* = 11.5, 4.0, 2.0, H^b), 2.49 (1H, t, *J* = 11.2, H^a), 1.99-1.85 (2H, m, H^d or H^e and H^g), 1.81-1.69 (1H, m, H^c), 1.63 (1H, tdd, *J* = 13.1, 11.2, 3.6, H^f), 1.30-1.18 (1H, m, H^d or H^e), 0.98 (3H, d, *J* = 6.6, CH_3); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 145.2 (ArC), 128.3 (ArC), 127.0 (ArC), 126.6 (ArC), 61.9 (CH^h), 55.3 (CH^a), 34.9 (CH^f), 34.2 (CH^d), 31.4 (CH^c), 19.5 (CH_3).

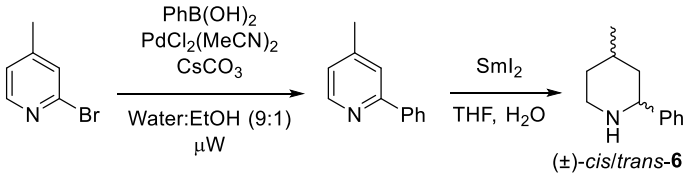
(±)-*cis*-4-Methyl-2-phenylpiperidine, (±)-*cis*-6



Following the CAR-TA-IRED Cascade General Procedure without the addition of IRED, (±)-**5** (100 mg, 0.48 mmol) was subjected to the biotransformation and after 24 h $NH_3.BH_3$ (148 mg, 4.80 mmol) was added. The reaction was incubated for a further 1 h before extraction as described in the general method. The crude product was further purified by dissolving the residue in EtOAc (10 mL) and extracting the product into 1 M HCl (3 x 10 mL). The aqueous layers were combined, basified with 10 M NaOH to pH 12.0 and the product extracted into CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure to afford **(±)-6** (69 mg, 0.39 mmol, 82%, *de* >98%) as a pale yellow oil exclusively as the *cis*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.40-7.28 (4H, m, ArH), 7.27-7.21 (1H, m, ArH), 3.61 (1H, dd, *J* = 11.4, 2.5 Hz, H^h), 3.21 (1H, ddd, *J* = 11.6, 4.0, 2.3, H^b), 2.81 (1H, app td, *J* = 12.0, 2.5, H^a), 1.93 (1H, br s, NH), 1.84-1.77 (1H, m, H^g), 1.73-1.56 (2H, m, H^c or H^d and H^e), 1.26-1.13 (2H, m, H^c or H^d and H^f), 0.97 (3H, d, *J* = 6.6, CH_3); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 145.3 (ArC), 128.3 (ArC), 127.0 (ArC), 126.6 (ArC), 61.9 (CH^h), 47.3 (CH^aH^b), 43.5 (CH^fH^g), 34.4 (CH^cH^d), 32.0 (CH^e), 22.4 (CH_3); **HRMS** calcd. for $C_{12}H_{18}N$ 176.1439 $[M+H]^+$, found 176.1422. Assignment as the *cis*-diastereomer was based on NOESY analysis that showed correlation between H^h and H^e.

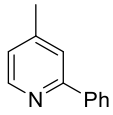
Synthesis of (±)-*cis*/*trans*-6

To afford some of the corresponding *trans*-diastereomer as a standard for GC analysis, the following synthetic route was carried out (Scheme S3).



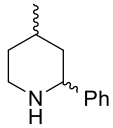
Scheme S3: Route to (±)-*cis/trans*-**6**

4-Methyl-2-phenylpyridine



To a microwave vial with magnetic stirrer was added 2-bromo-4-methylpyridine (250 mg, 1.45 mmol), phenylboronic acid (266 mg, 2.18 mmol), $CsCO_3$ (1.42 g, 4.35 mmol), bis(acetonitrile)dichloropalladium(II) (19 mg, 0.08 mmol) and water:ethanol (15 mL, 9:1). The vial was capped and heated by microwave irradiation at (200 W, 100°C) for 15 mins. The reaction mixture was cooled to room temperature and filtered through Celite® with EtOAc washings (3 x 5 mL). The organic layer was separated and the aqueous layer extracted with EtOAc (3 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was subjected to column chromatography (silica, 9:1 to 4:1 cyclohexane:EtOAc, R_f = 0.40 at 3:1 cyclohexane:EtOAc) to afford the title compound as a colorless oil (171 mg, 1.01 mmol, 70%). **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 8.56 (d, *J* = 5.10, 1H), 8.01-7.96 (m, 2H), 7.57-7.55 (m, 1H), 7.51-7.45 (m, 2H), 7.44-7.39 (m, 1H), 7.09-7.05 (m, 1H), 2.43 (s, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 157.4, 149.4, 147.7, 139.5, 128.8, 128.7, 126.9, 123.1, 121.5, 21.2; **IR** ν_{max}/cm^{-1} 3046 (C-H), 2919 (C-H), 1602 (pyridine ring stretch); **HRMS** calcd. for $C_{12}H_{12}N$ 170.0970 $[M+H]^+$, found 170.0955.

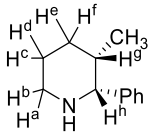
(±)-*cis*/*trans*-4-Methyl-2-phenylpiperidine, (±)-*cis*/*trans*-6



4-Methyl-2-phenylpyridine (100 mg, 0.59 mmol) was dissolved in a solution of SmI_2 in THF (0.1 M, 11.7 mL) under nitrogen and then water (0.19 mL, 10.7 mmol) added. The reaction was stirred at room temperature for 15 mins. The reaction mixture was cooled to 0°C and 3 M HCl (10 mL) added slowly. The aqueous layer was separated and washed with Et_2O (3 x 20 mL). The aqueous layer was basified with 10 M NaOH to pH 10.0 and the product extracted into CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was subjected to column chromatography (silica, 9:1:0.1 CH_2Cl_2, CH_3OH, Et_3N, R_f = 0.24 and 0.15 diastereomers) to afford the title compound as a yellow oil

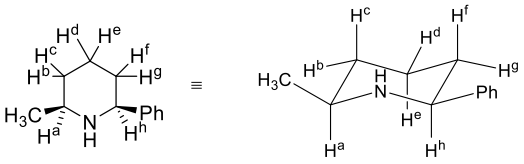
(16 mg, 0.09 mmol, 16%). The compound was isolated as ~2:1 mixture of *trans*:*cis* isomers after chromatography. GC-MS analysis of crude reaction showed ~1:1 ratio before chromatography. Clearly separated diagnostic peaks of minor *trans*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 4.02 (dd, *J* = 10.2, 3.2, 1H), 3.04-2.95 (m, 1H), 2.95-2.87 (m, 1H), 2.17-1.99 (2H, m), 1.98-1.87 (m, 1H), 1.12 (d, *J* = 7.1, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 141.6, 128.6, 127.5, 127.1, 55.4, 41.1, 38.4, 30.5, 25.9, 18.3.

(2*R*,3*S*)-3-Methyl-2-phenylpiperidine, (2*R*,3*S*)-10



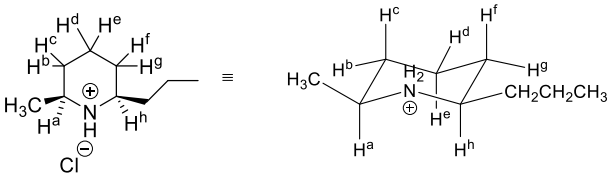
Following the CAR-TA-IRED Cascade General Procedure with **(±)-7** (100 mg, 0.48 mmol) and (*S*)-IRED afforded (2*R*,3*S*)-**10** as the major product (*de* 80%). The crude product was further purified by column chromatography (silica. 9:1:0.1 CH_2Cl_2:CH_3OH:Et_3N, R_f = 0.23) to afford the title compound as a pale yellow oil (64 mg, 0.37 mmol, 76%, *de* 50%, *ee* 81%). The *cis*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.35-7.26 (4H, m, ArH), 7.25-7.19 (1H, m, ArH), 3.90 (1H, d, *J* = 3.2, H^h), 3.28-3.20 (1H, m, H^a or H^b), 2.86-2.72 (1H, m, H^a or H^b), 2.06-1.95 (1H, m, H^g), 1.87-1.67 (4H, m, H^e, H^f, NH and H^c or H^d), 1.50-1.39 (1H, m, H^c or H^d), 0.77 (3H, d, *J* = 7.1, CH_3); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 144.3 (ArC), 128.0 (ArC), 126.5 (ArC), 126.4 (ArC), 64.3 (CH^h), 48.2 (CH^aH^b), 34.3 (H^g), 32.2 (CH^eH^f), 20.5 (CH^cH^d), 11.8 (CH_3); **IR** ν_{max}/cm^{-1} 3060 (C-H), 3025 (C-H), 2930 (C-H), 2851 (C-H), 277 (C-H); **HRMS** calcd. for $C_{12}H_{18}N$ 176.1439 $[M+H]^+$, found 176.1416. Clearly separated diagnostic peaks of minor *trans*-diastereomer: **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 3.13 (d, *J* = 9.9, 1H) 0.63 (d, *J* = 6.7, 3H); **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 143.7, 128.3, 127.9, 127.3, 69.8, 47.7, 37.4, 26.5, 18.9; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$ = + 11.9 (*c* = 1.01, $CHCl_3$). Assignment as the (2*R*) center was based on the assumption that the (*S*)-IRED affords the (*R*)-center in high *ee* in the formation of the parent compound **2b**.

(2*S*,6*S*)-2-methyl-6-phenylpiperidine, (2*S*,6*S*)-13a



A non-baffled conical flask was charged with the hydrochloride salt of (*S*)-**12a** (60 mg, 0.286 mmol), (*R*)-IRED whole-cells (11.4 g), 50 mM glucose and the mixture was made up to 57 mL with 100 mM pH 7.0 sodium phosphate buffer. The reaction mixture was incubated at 30°C, 250 rpm for 24 h and the biotransformation was monitored by GC-MS. The pH of the reaction mixture was then adjusted to pH 12.0 by addition of 10 M sodium hydroxide and the aqueous mixture was extracted with ethyl acetate (3 x 60 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure to yield (2*S*,6*S*)-**13a** (46 mg, 0.262 mmol, 92%, *de* >98%) as pale yellow oil. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 7.33-7.27 (m, 2H, ArH), 7.27-7.21 (m, 2H, ArH), 7.19-7.13 (m, 1H, ArH), 3.58 (dd, *J* = 10.5, 2.5, 1H, H^h), 2.73 (dqd, *J* = 11.0, 6.5, 2.5, 1H, H^a), 1.84 (m, 1H, H^e), 1.72-1.65 (m, 1H, H^g), 1.64-1.54 (m, 2H, NH and H^b), 1.50-1.32 (m, 2H, H^d and H^f), 1.14-1.04 (m, 1H, H^c), 1.04 (d, *J* = 6.5, 3H, CH_3); **^{13}C NMR** δ_C (100 MHz, $CDCl_3$) 145.7 (ArC), 128.5 (ArC), 127.1 (ArC), 126.9 (ArC), 62.6 (CH^h), 53.2 (CH^a), 34.4 (CH^f), 34.0 (CH^b), 25.5 (CH^e), 23.2 (CH_3); $[\alpha]_D^{23}$ = - 40.0 (c = 1.0, $CHCl_3$) [Lit. ref. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = - 35.6 (*c* = 1.38, $CHCl_3$).[17] Spectroscopic data is consistent with literature values.[17] Assignment as the *cis*-diastereomer was based on the large *J* value for coupling between H^a and H^c, indicative of axial-axial coupling according to the Karplus equation. NOESY analysis also showed correlation between H^a and H^h.

(2*S*,6*R*)-2-methyl-6-propylpiperidine hydrochloride, (-)-dihydropinidine hydrochloride, (2*S*,6*R*)-13d HCl



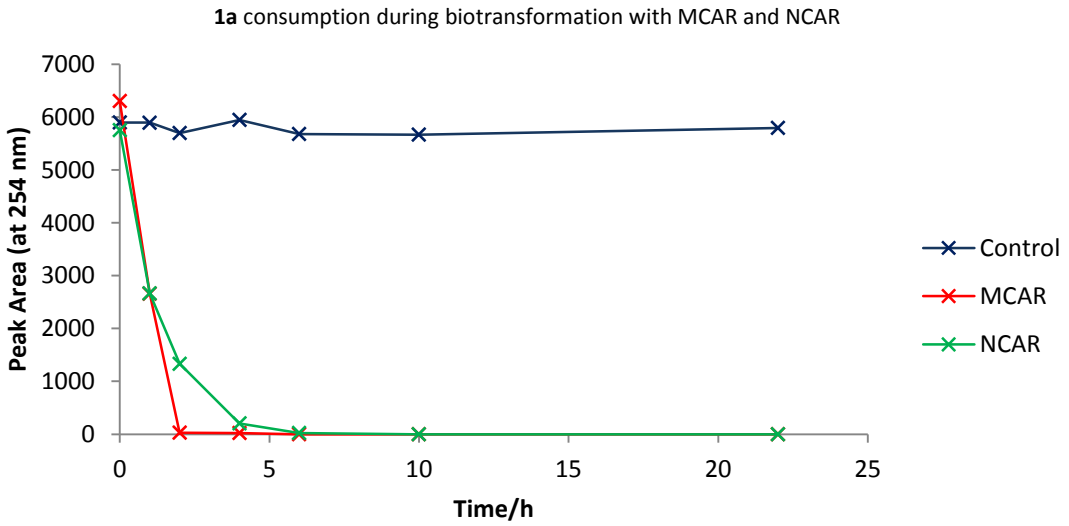
Nonane-2,6-dione, **11d** (50 mg, 0.320 mmol from a 1 M stock solution in DMSO), was subjected to the TA-IRED Cascade General Procedure for diketones with ATA-113 and (*R*)-IRED. The pH of the reaction mixture was then adjusted to pH 12.0 with 10 M sodium hydroxide. The aqueous mixture was then extracted with diethyl ether (3 x 60 mL). The organic phases were combined and dried over $MgSO_4$, before addition of 2 M HCl/diethyl ether solution (1 mL). The excess diethyl ether was carefully decanted and the remaining liquid was allowed to dry in air to yield amine (2*S*, 6*R*)-**13d** (51 mg, 0.298 mmol, 90%, *de* >98%, *ee* >98%) as a white solid. **^{1}H NMR** δ_H (400 MHz, CD_3OD) 3.28-3.14 (m, 1H, H^a), 3.07 (d, *J* = 8.0, 1H, H^h), 2.01 (d, *J* = 13.9, 1H, H^g), 1.96-1.83 (m, 2H, H^b and H^d), 1.78-1.65 (m, 1H, $CH_2CH_2CH_3$), 1.65-1.52 (m, 2H, $CH_2CH_2CH_3$ and H^e), 1.51-1.28 (m, 4H, $CH_2CH_2CH_3$,H^c and H^f) 1.35 (d, *J* = 6.5, 3H, CH_3), 0.98 (t, *J* = 7.5, 3H, $CH_2CH_2CH_3$); **^{13}C NMR** δ_C (100 MHz, CD_3OD) 58.7 (CH^h), 54.9 (CH^a), 36.9 ($CH_2CH_2CH_3$), 31.6 (CH^b), 29.0 (CH^f), 23.5 (CH^d), 19.6 (CH_3), 19.5 ($CH_2CH_2CH_3$), 14.1 ($CH_2CH_2CH_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$ = - 40.0(*c* = 1.0, EtOH) [Lit. ref. $[\alpha]_D^{20}$ = - 12.2 (*c* = 0.5, EtOH).[18] Spectroscopic data is consistent with literature values.[18]

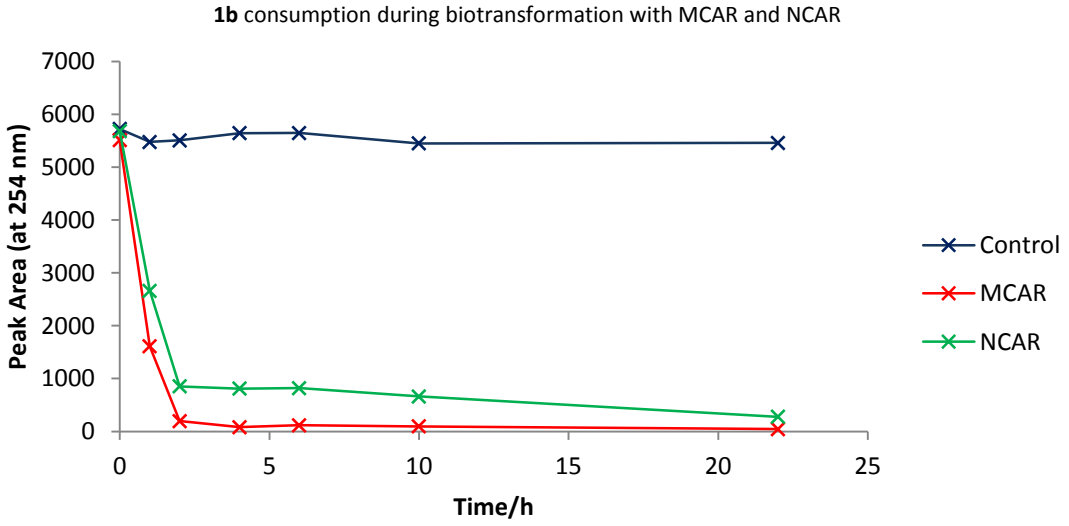
7 Cascade Parameters Investigation

7.1 Time Course Assays of Keto Acid Consumption with MCAR or NCAR

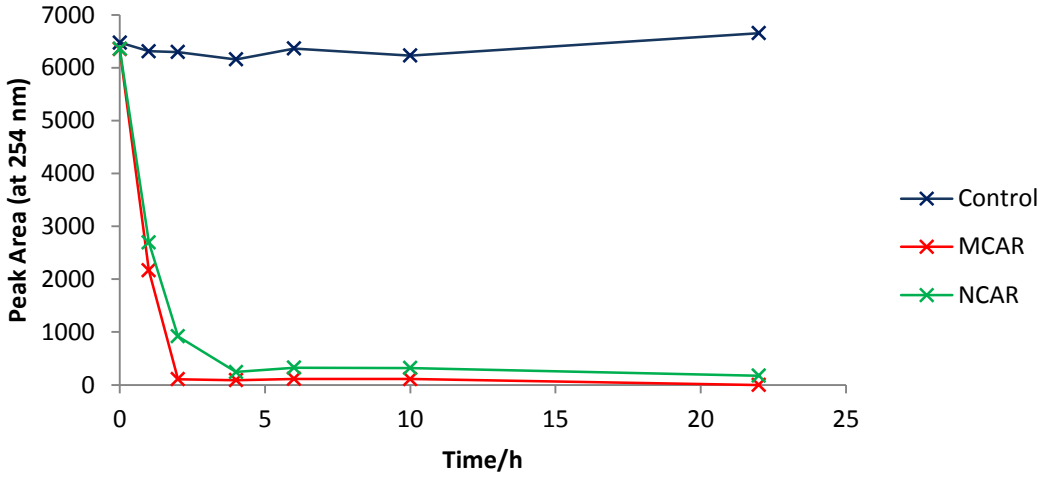
Consumption of starting keto acids **1a-d** by MCAR and NCAR were investigated. Both CARs performed similarly for each substrate, however, MCAR typically afforded higher conversions in a shorter time compared to NCAR.

Reaction conditions: 5 mM substrate , 70 mg/mL CAR wet whole cells, 2.5 mg/mL ATA-113, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM DL-alanine, 100 mM glucose, 1.5 mM, NAD+, 1 mM PLP, 100 mM pH 7.0 NaP_i buffer

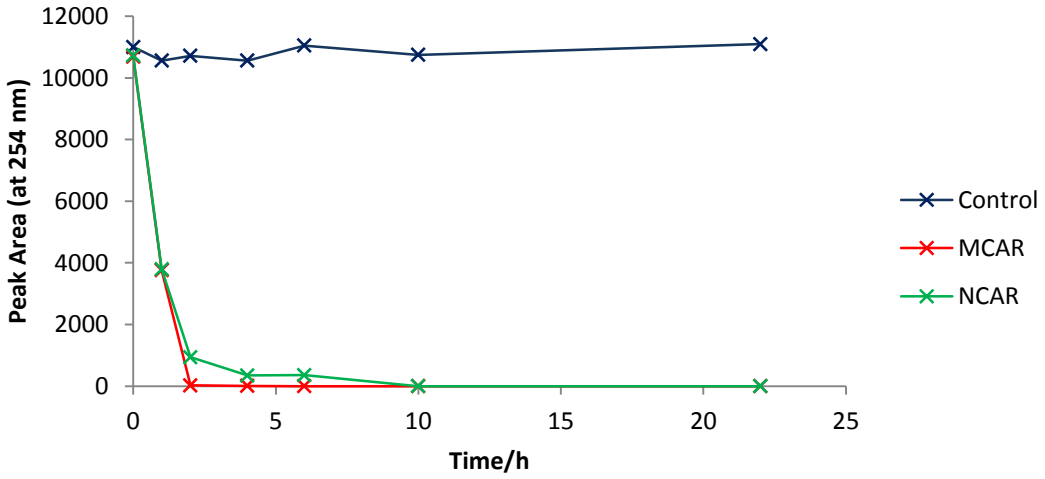




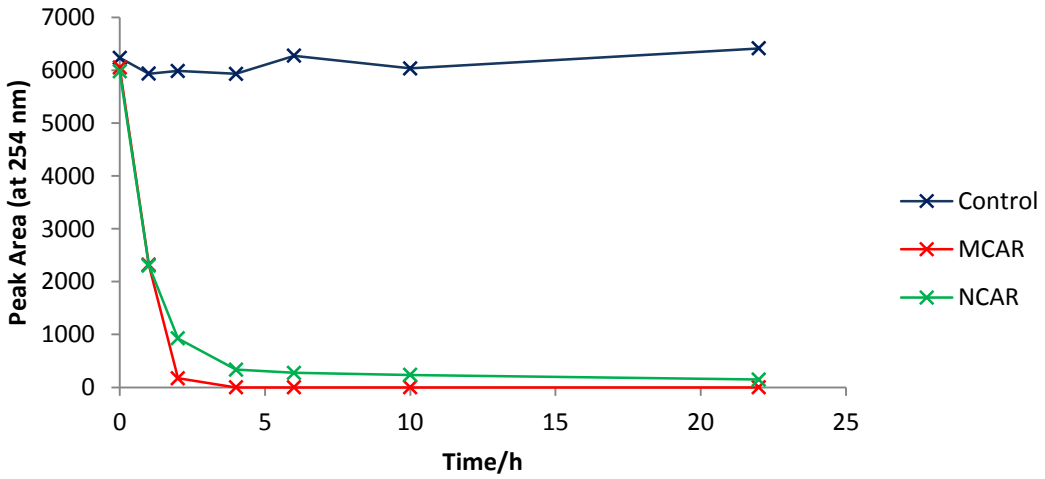
1c consumption during biotransformation with MCAR and NCAR



1d consumption during biotransformation with MCAR and NCAR



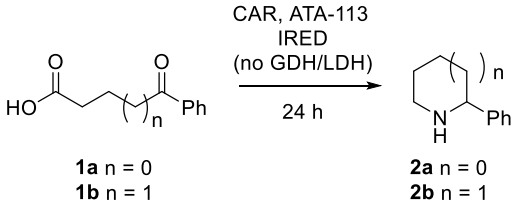
1e consumption during biotransformation with MCAR and NCAR



7.2 Effect of the GDH/LDH Recycling System on Conversion

The CAR-TA-IRED cascade was investigated without the addition of the GDH/LDH system for substrate **1a** and **1b** to assess if the IRED alone is capable of driving the transaminase equilibrium by removing imine. The CAR-TA-IRED Cascade General Procedure was employed on an analytical scale without GDH, LDH or NAD^+ and the results summarized in Table S1. As described in the main paper, without the addition of this driving system for the transaminase, significant keto alcohol by-product was formed.

Table S1: Results of CAR-TA-IRED cascade without GDH/LDH transaminase driving system.



1a n = 0
1b n = 1

2a n = 0
2b n = 1

Substrate	IRED	Amine /%	Imine/%	Keto alcohol/%
1a	(*R*)-IRED	40	7	54
1a	(*S*)-IRED	8	27	65
1b	(*R*)-IRED	83	2	15
1b	(*S*)-IRED	59	6	35

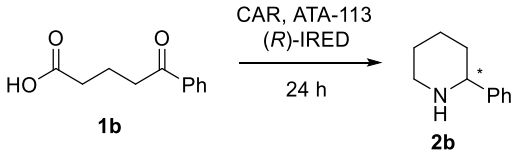
Analysis by GC-MS on an HP1-MS column (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temp 270°C, Method; 50°C-175°C, 5°C/min^{-1}, 175°C-250°C, 10°C/min^{-1}). Retention times for reaction with **1a**: amine (12.3 mins), imine (13.3 mins), keto alcohol (17.1 mins). Retention times for reaction with **1b**: amine (13.9 mins), imine (16.4 mins), keto alcohol (19.7 mins).

7.3 Comparison of Amine Donors in Cascade Reactions

A comparison of DL-alanine and isopropylamine as amine donors for the transaminase were investigated in the CAR-TA-IRED cascade reactions of **1b**. The CAR-TA-IRED Cascade General Procedure was employed on an analytical scale with (*R*)-IRED as described or modified by adding isopropylamine (250 mM) instead of DL-alanine, GDH, LDH and NAD^+. An internal standard was used to measure the amount of product amine as it was observed that some keto acid remained unconverted in the reaction with isopropylamine. For this test, biotransformations were extracted into 1 mL of CH_2Cl_2 containing decane (1 mg/mL) followed by GC-FID analysis. Amount of amine product was then calculated using the ratio of the peak area for amine relative to decane and comparison to a calibration curve using

commercially available **2b**. Isopropylamine afforded a lower assayed yield than the DL-alanine system in the cascade (Table S2).

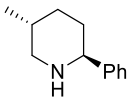
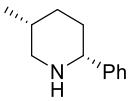
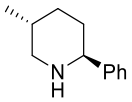
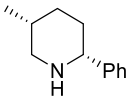
Table S2: Comparison of DL-alanine and isopropylamine in the cascade reaction of **1b**.



Transaminase Amine Donor	Amine Product/%
DL-alanine	77
isopropylamine	63

A further test with (*R*)-**3** as substrate in the TA-IRED cascade was investigated. The TA-IRED Cascade General Procedure for keto aldehydes was employed on an analytical scale as described or modified by adding isopropylamine (1 M) instead of DL-alanine, GDH, LDH and NAD^+. Higher isopropylamine was investigated for this substrate in an attempt to ensure rapid conversion to the imine to minimize racemization of the alpha-chiral center of **3**. For both the DL-alanine and IPA reactions, full conversion of the starting material to the imine was observed. However, subsequent reduction by the IREDs was considerably lower with the IPA reactions although the similar product *de* and *ee* were observed (Table S3).

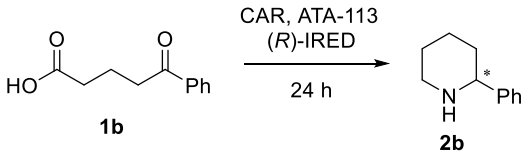
Table S3: Comparison of DL-alanine and isopropylamine in the cascade reaction of (*R*)-**3**.

Transaminase Amine Donor	(*R*)-IRED				(*S*)-IRED			
	Product	Conversion/%	*de*/%	*ee*/%	Product	Conversion/%	*de*/%	*ee*/%
DL-alanine	 (2*S*,5*R*)-**4**	83	72	88	 (2*R*,5*R*)-**4**	95	>98	>98
isopropylamine	 (2*S*,5*R*)-**4**	22	72	82	 (2*R*,5*R*)-**4**	46	>98	>98

7.4 Cascade Substrate Loading Test

Increased substrate loading in the CAR-TA-IRED cascade reactions of **1b** was investigated. The CAR-TA-IRED Cascade General Procedure was employed on an analytical scale with (*R*)-IRED as described, however, the substrate concentration was varied. To account for unreacted keto acid, an internal standard was used to measure the amount of product amine. For this investigation, biotransformations were extracted into 1 mL of CH_2Cl_2 containing decane (1 mg/mL) followed by GC-FID analysis. Amount of amine product was then calculated using the ratio of the peak area for amine relative to decane and comparison to a calibration curve using commercially available **2b**. The results (Table S4) show that upon increasing the substrate loading to 10 mM and above still affords product, however, the assayed yield of **2b** decreases significantly

Table S4: Substrate loading investigation in CAR-TA-IRED cascade with **1b**.

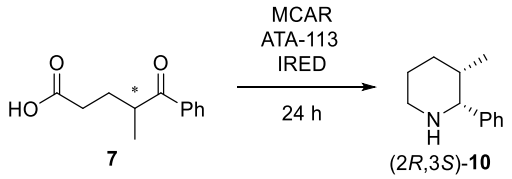


Substrate Conc./mM	Amine Product/%
5	77
10	67
20	36
50	5

7.5 Investigation of effect of supplying single enantiomers of keto acid 7 to CAR-TA-IRED Cascade

Single enantiomers of **7** were subjected to the CAR-TA-IRED Cascade General Procedure and the results are shown in Table S5. The conversions and product distributions when a single enantiomer was used were comparable to when racemic **7** was supplied as substrate.

Table S5: Comparison of (±)-**7**, (-)-**7** and (+)-**7** as substrates in the CAR-TA-IRED Cascade



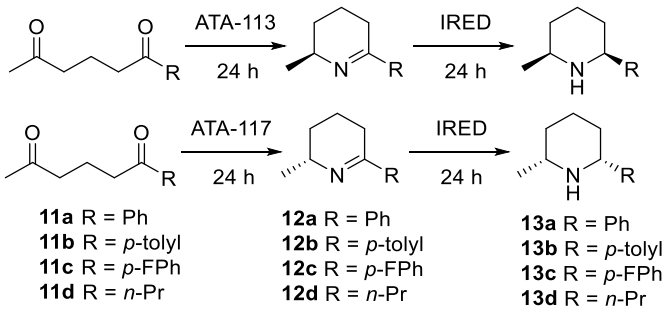
7 (2*R*,3*S*)-**10**

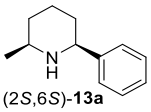
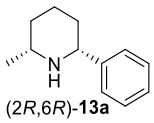
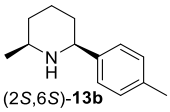
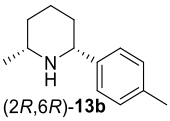
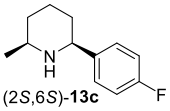
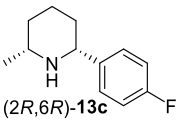
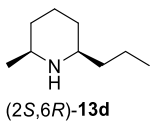
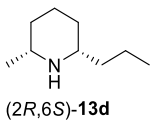
Substrate	*IRED*	*Conv./%*	*de/%*	*ee/%*
(±)-**7**	(*R*)-IRED	89	95	18
(-)-**7**	(*R*)-IRED	94	92	13
(+)-**7**	(*R*)-IRED	98	91	20
(±)-**7**	(*S*)-IRED	86	81	81
(-)-**7**	(*S*)-IRED	86	75	78
(+)-**7**	(*S*)-IRED	95	75	80

7.6 Alternative ATA and IRED Combinations in TA-IRED Cascade of Diketones

The lower yielding combinations of ATA and IRED in the TA-IRED cascade of diketones **11a-11d** are presented in Table S6. The TA-IRED Cascade General Procedure for diketones on an analytical scale was employed. Conversion to the imine **12** was complete in all cases and, therefore, the cause of poor overall conversions was the IRED-catalysed step. These entries (excepting entry 8) correspond to chiral imine substrates for the IREDs that result in an unfavorable 1,3-steric interaction between methyl substituent and incoming NADPH as described in the main paper.

Table S6: Alternative combinations of ATA and IRED in the TA-IRED cascade of diketones **11a-11d**.



Entry	*Substrate*	*ATA*	*Conv. to **12**/%*	*IRED*	*Amine product*	*Conv./%*	*de/%*	*ee/%*
1	**11a**	113	>98	(*S*)-IRED	 (2*S*,6*S*)-**13a**	15	90	>98
2	**11a**	117	>98	(*R*)-IRED	 (2*R*,6*R*)-**13a**	23	96	>98
3	**11b**	113	>98	(*S*)-IRED	 (2*S*,6*S*)-**13b**	33	96	>98
4	**11b**	117	>98	(*R*)-IRED	 (2*R*,6*R*)-**13b**	7	93	>98
5	**11c**	113	>98	(*S*)-IRED	 (2*S*,6*S*)-**13c**	19	97	>98
6	**11c**	117	>98	(*R*)-IRED	 (2*R*,6*R*)-**13c**	25	98	>98
7	**11d**	113	>98	(*S*)-IRED	 (2*S*,6*R*)-**13d**	5	95	>98
8	**11d**	117	>98	(*S*)-IRED	 (2*R*,6*S*)-**13d**	3	87	>98

8 HPLC Conditions for Keto Acid Analysis

Keto acids **1a-1e** were assayed for consumption by HPLC (Daicel CHIRALPAK®IC 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/trifluoroacetic acid = 90/10/0.1, 1 mL/min, 265 nm) and monitored at 265 nm. Retention times are given in Table S7.

Table S7: Keto acid HPLC retention times

Keto Acid	Retention Time/mins
1a	15.4
1b	12.2
1c	11.2
1d	14.8
1e	16.5

9 Imine and Amine Standards for Analytical-Scale Reactions

Standards of imines were achieved by running the CAR-TA-IRED or TA-IRED cascades with each substrate on an analytical-scale without the addition of IRED and extracting as described in the general procedure.

Amine standards were prepared in the same manner, however, after extracting the imine, the solvent was removed under reduced pressure and redissolved in ethanol or methanol (100 µL) followed by addition of $NaBH_4$ or $NH_3.BH_3$ (excess). After 1 h, water was added (100 µL), the pH adjusted to 10.0-12.0, the product extracted into methyl-tert-butyl ether or CH_2Cl_2 (1 mL) and dried over $MgSO_4$. Products were confirmed by GC-MS analysis prior to analysis by GC-FID or HPLC.

10 HPLC and GC Traces

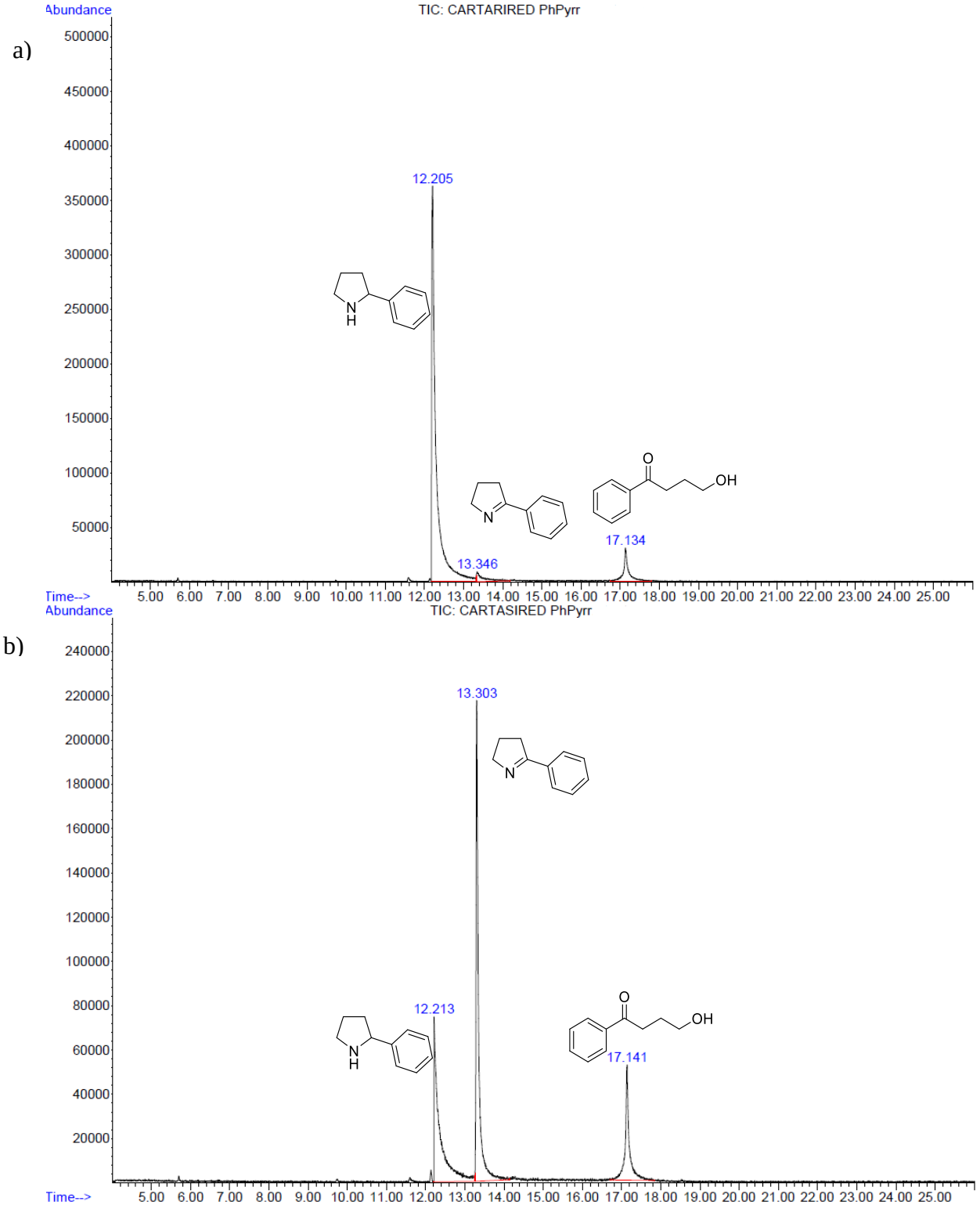


Figure S5: GC trace from GC-MS analysis of cascade biotransformation of **1a** to determine conversion. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temp 270°C, Method; 50°C-175°C, 5°C/min^{-1}, 175°C-250°C, 10°C/min^{-1}). a) CAR-TA-(*R*)-IRED biotransformation of **1a**, b) CAR-TA-(*S*)-IRED biotransformation of **1a**.

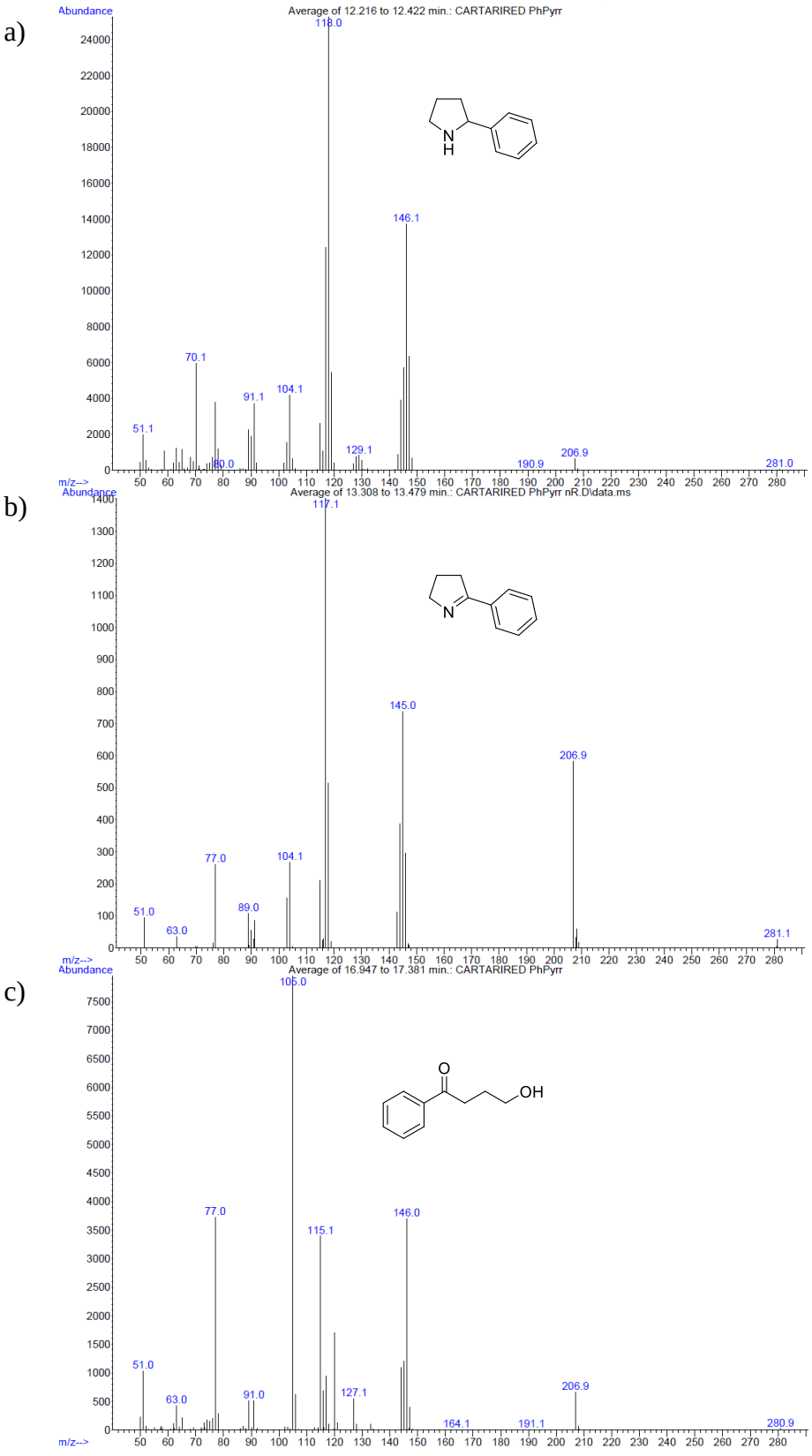


Figure S6: MS data from GC-MS analysis of cascade biotransformation of **1a**. a) MS of amine, b) MS of imine, c) MS of keto alcohol.

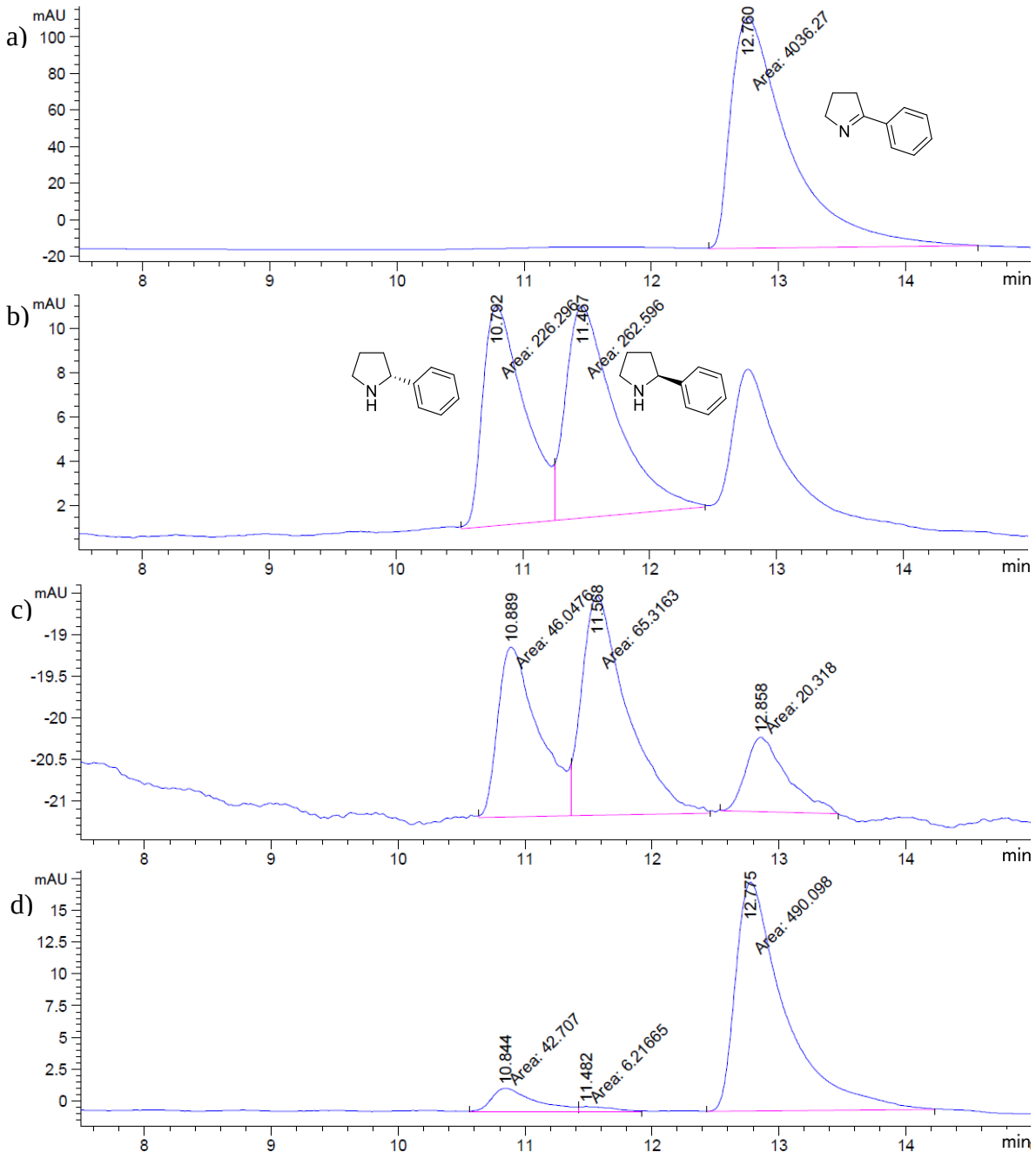


Figure S7: HPLC analysis of cascade biotransformation of **1a** to determine *ee*. (Daicel CHIRALPAK®IE 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/diethylamine = 97/3/0.1, 1 mL/min, 265 nm). a) Imine standard, b) racemic amine standard, c) CAR-TA-(*R*)-IRED biotransformation of **1a**, d) CAR-TA-(*S*)-IRED biotransformation of **1a**. Absolute configuration based on literature retention times.[1]

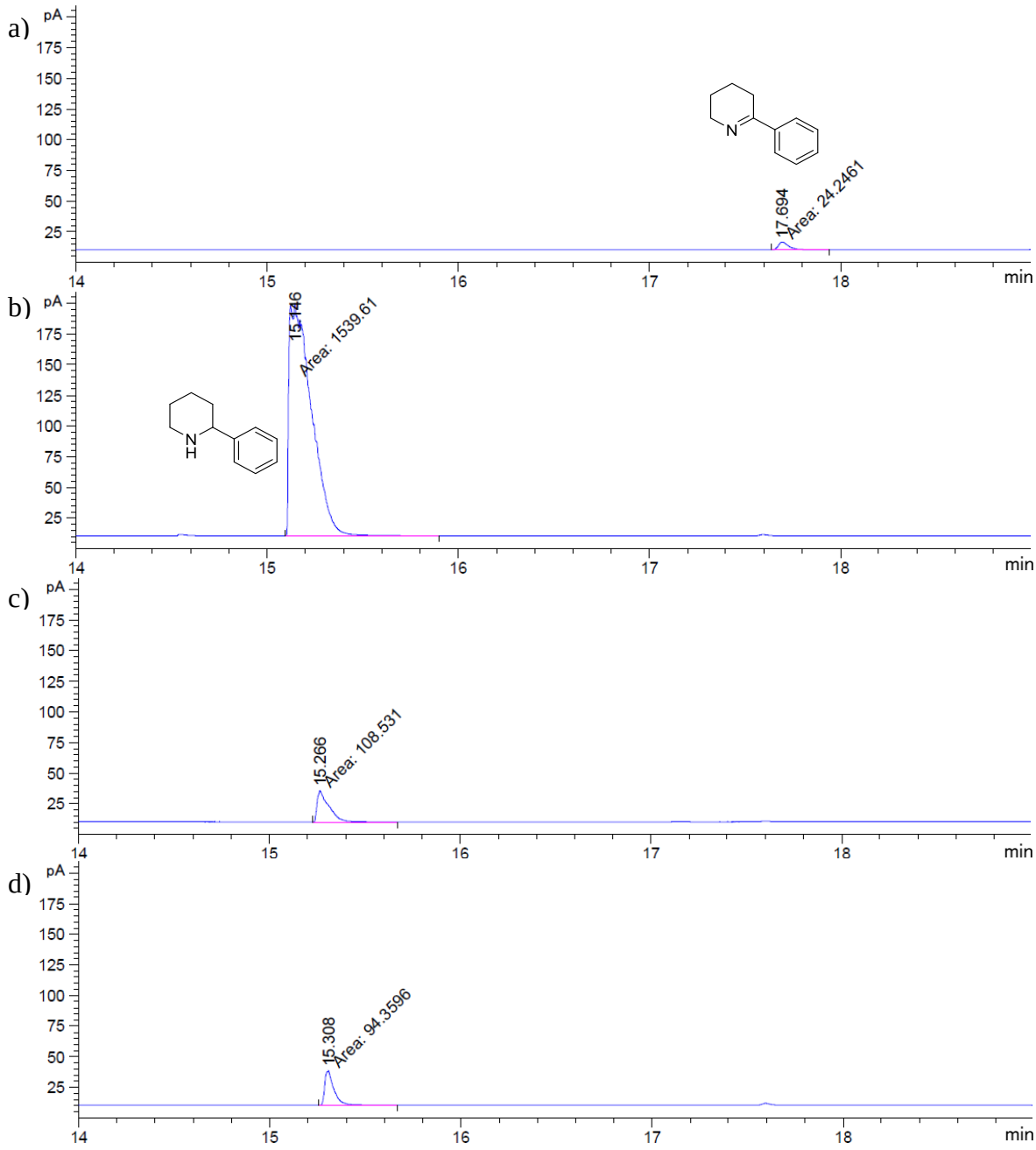


Figure S8: GC-FID analysis of cascade biotransformation of **1b** to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). a) Imine standard, b) racemic amine standard, c) CAR-TA-(*R*)-IRED biotransformation of **1b**, d) CAR-TA-(*S*)-IRED biotransformation of **1b**.

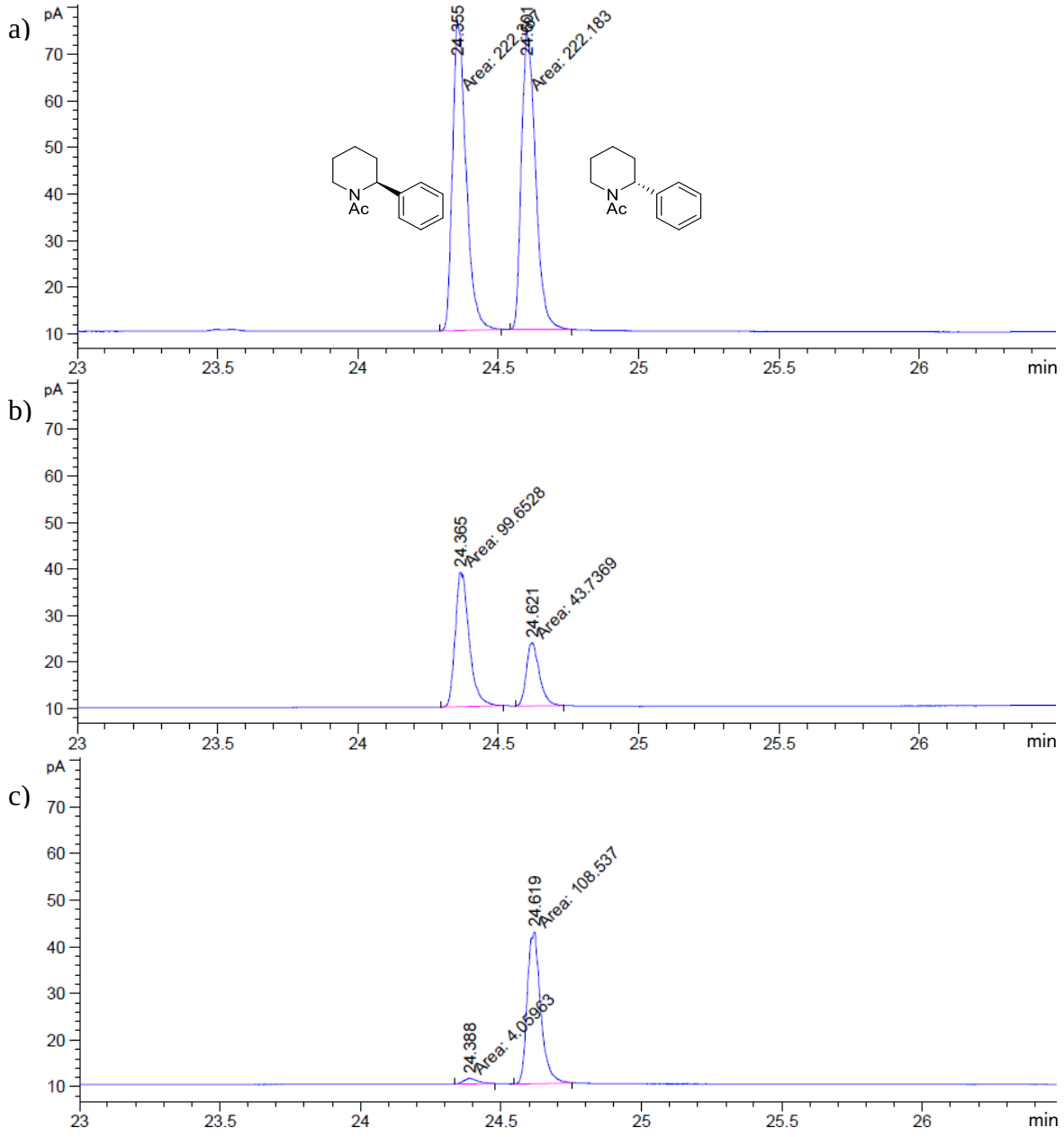


Figure S9: GC-FID analysis of cascade biotransformation of **1b** to determine *ee*. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 90°C - 200°C, 4°C/min, then hold for 5 min). All samples were derivatized with acetic anhydride prior to analysis. a) racemic amine standard, b) CAR-TA-(*R*)-IRED biotransformation of **1b**, c) CAR-TA-(*S*)-IRED biotransformation of **1b**. Absolute configuration based on literature retention times.[1]

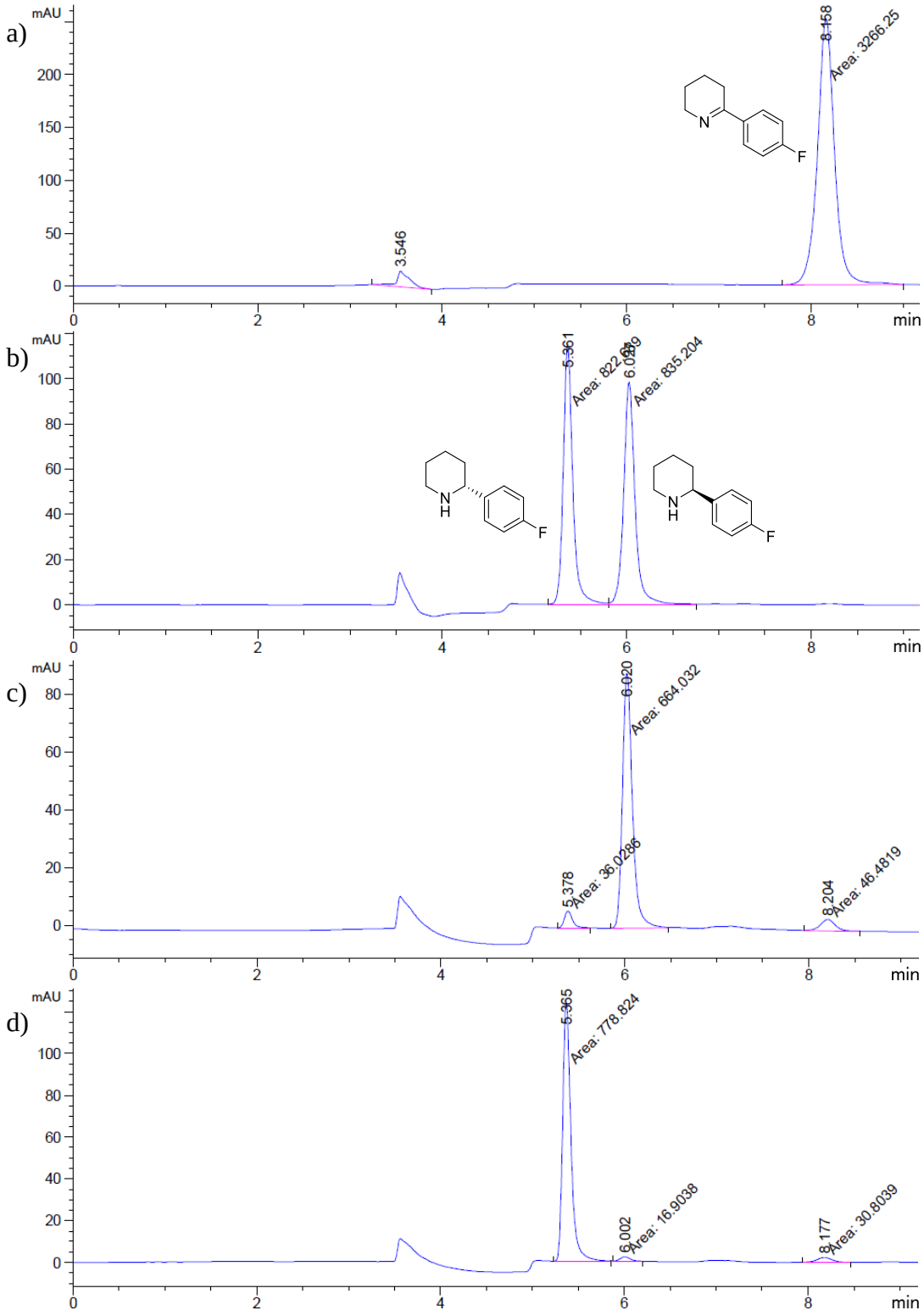


Figure S10: HPLC analysis of cascade biotransformation of **1c** to determine conversion and *ee*. (Daicel CHIRALPAK®IC 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/diethylamine = 98/2/0.1, 1 mL/min, 265 nm). a) Imine standard, b) racemic amine standard, c) CAR-TA-(*R*)-IRED biotransformation of **1c**, d) CAR-TA-(*S*)-IRED biotransformation of **1c**. Literature[1] relative response factor (A_{imine}/A_{amine}) of 8.3 used in conversion calculation. Absolute configuration based on literature retention times.[1]

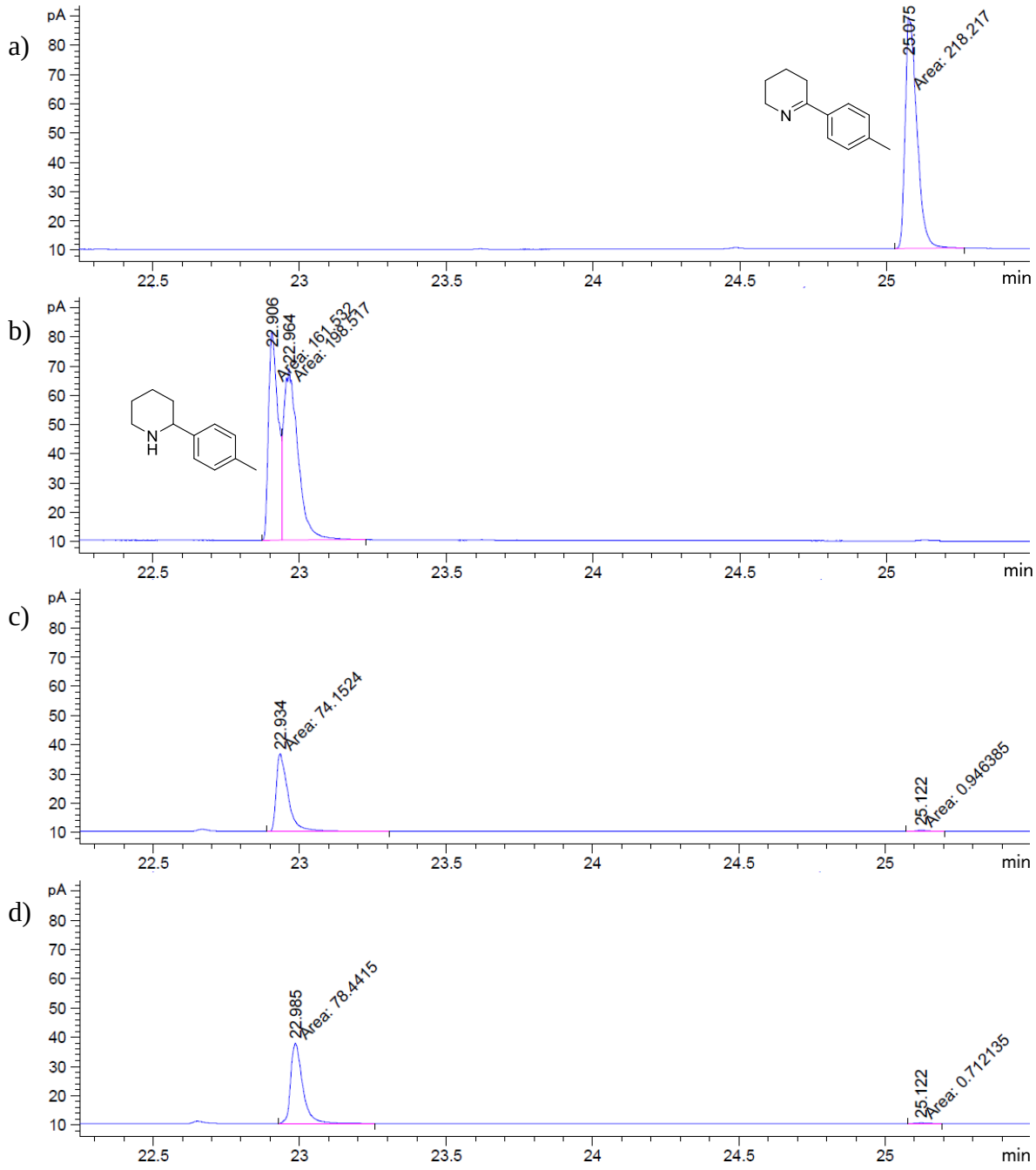


Figure S11: GC-FID analysis of cascade biotransformation of **1d** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) Imine standard, b) racemic amine standard, c) CAR-TA-(*R*)-IRED biotransformation of **1d**, d) CAR-TA-(*S*)-IRED biotransformation of **1d**.

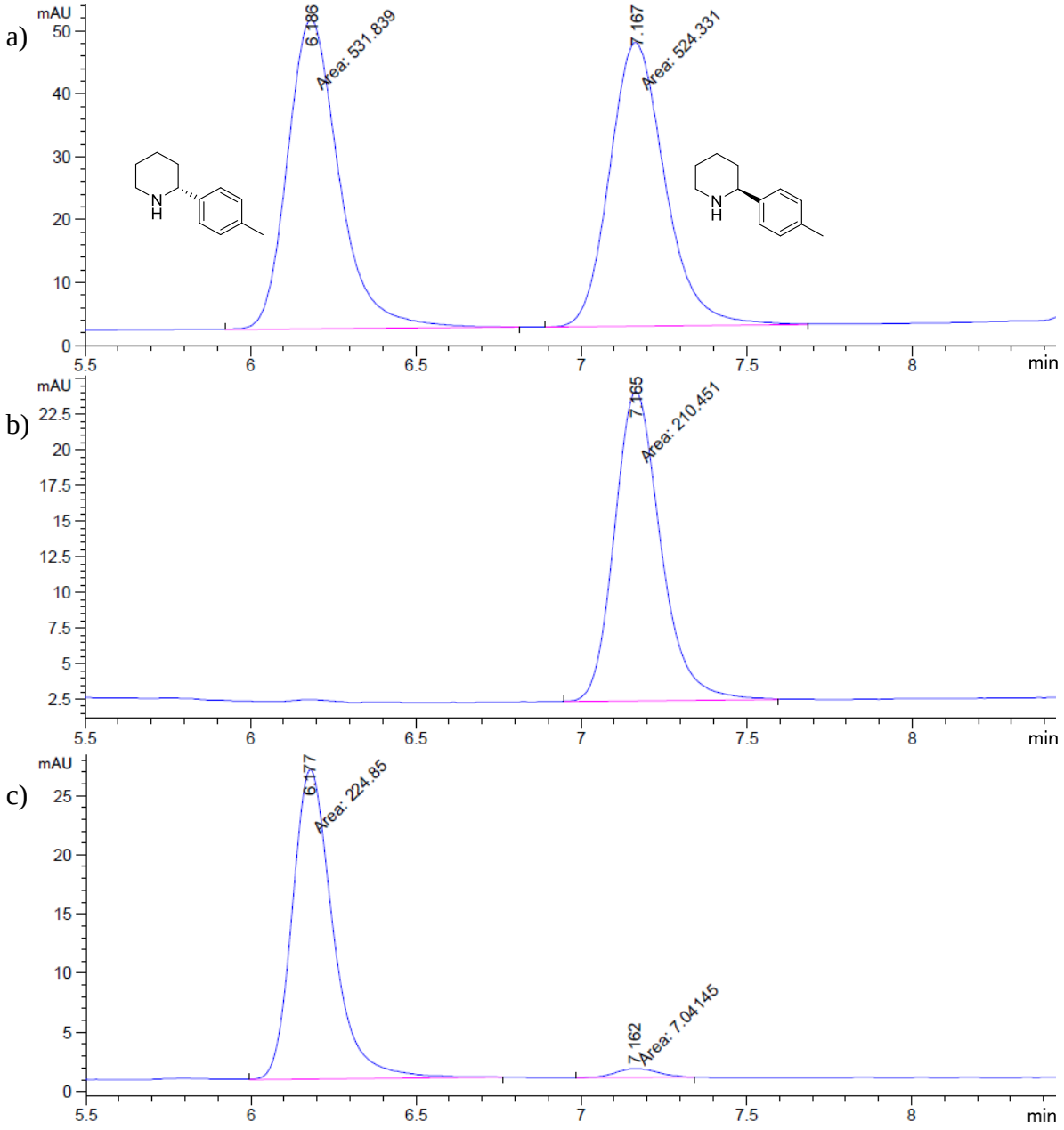


Figure S12: HPLC analysis of cascade biotransformation of **1d** to determine *ee*. (Daicel CHIRALPAK®IC 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/isopropanol/diethylamine = 90/10/0.1, 1 mL/min, 265 nm). a) Racemic amine standard, b) CAR-TA-(*R*)-IRED biotransformation of **1d**, c) CAR-TA-(*S*)-IRED biotransformation of **1d**. Absolute configuration based on literature retention times.[1]

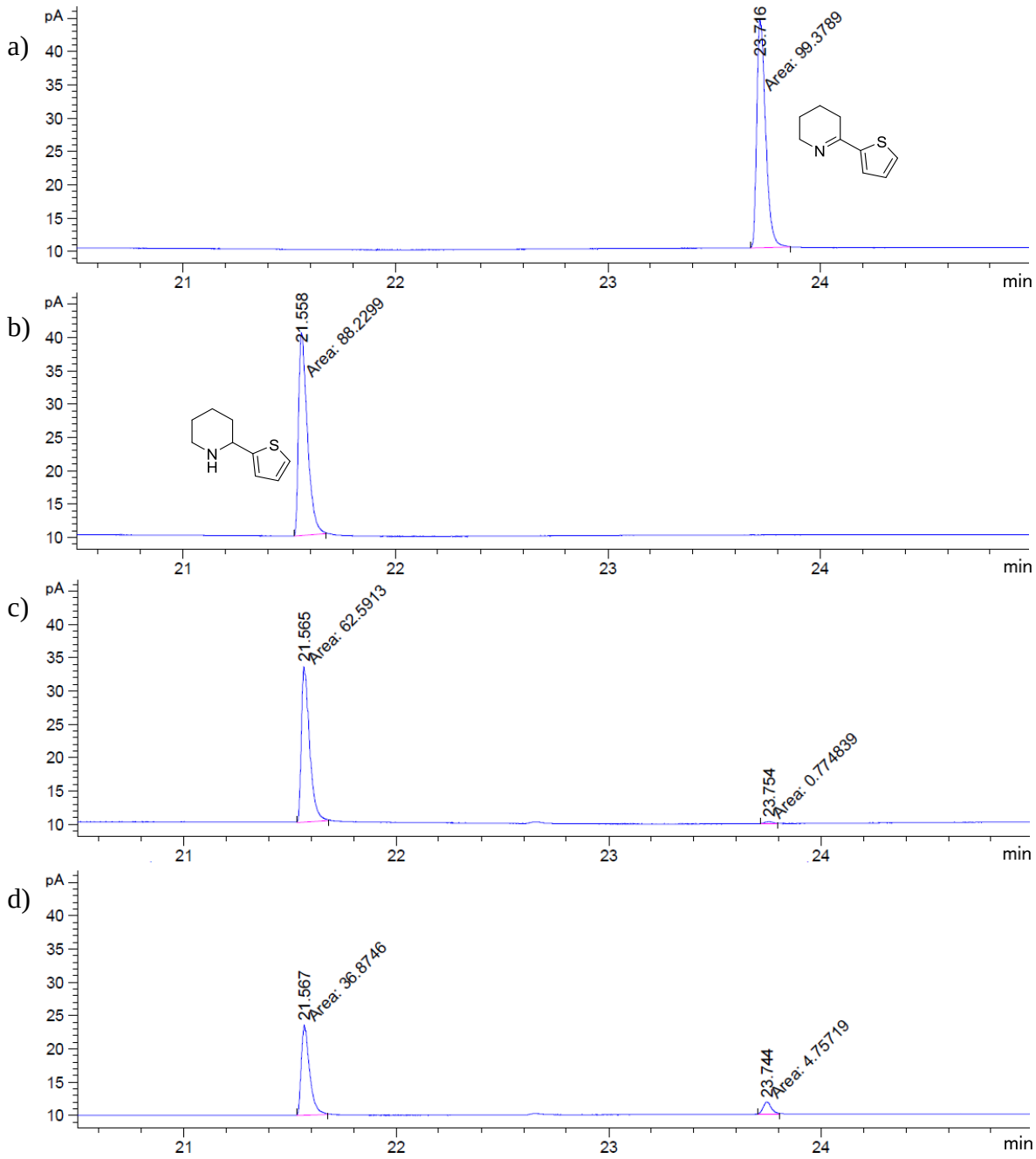


Figure S13: GC-FID analysis of cascade biotransformation of **1e** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) Imine standard, b) racemic amine standard, c) CAR-TA-(*R*)-IRED biotransformation of **1e**, d) CAR-TA-(*S*)-IRED biotransformation of **1e**.

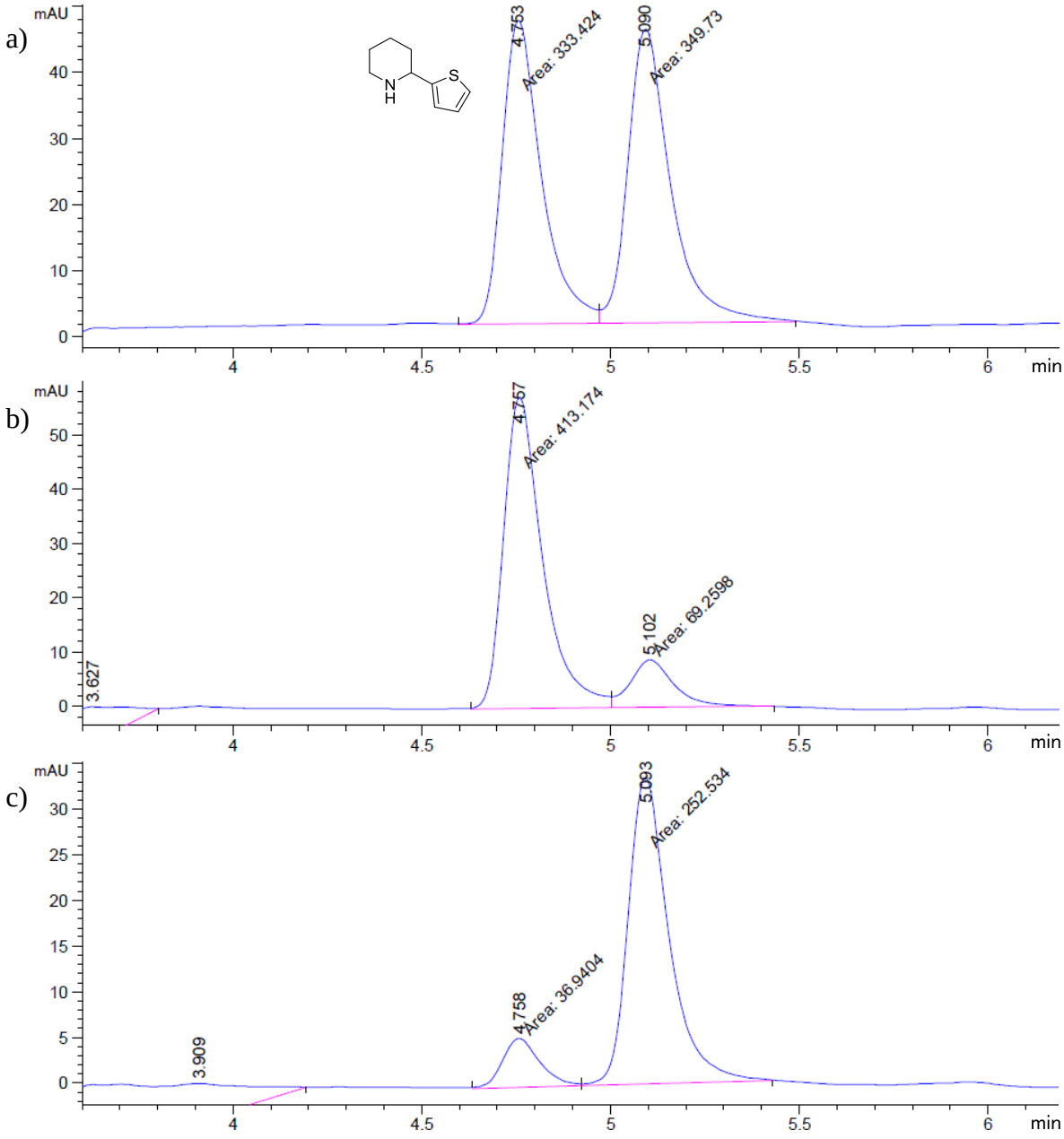


Figure S14: HPLC analysis of cascade biotransformation of **1e** to determine *ee*. (Daicel CHIRALPAK®IB 250 mm × 4.6 mm, 5 µm, solvent: n-hexane/ethanol/diethylamine = 90/10/0.1, 1 mL/min, 265 nm). a) Racemic amine standard, b) CAR-TA-(*R*)-IRED biotransformation of **1d**, c) CAR-TA-(*S*)-IRED biotransformation of **1d**.

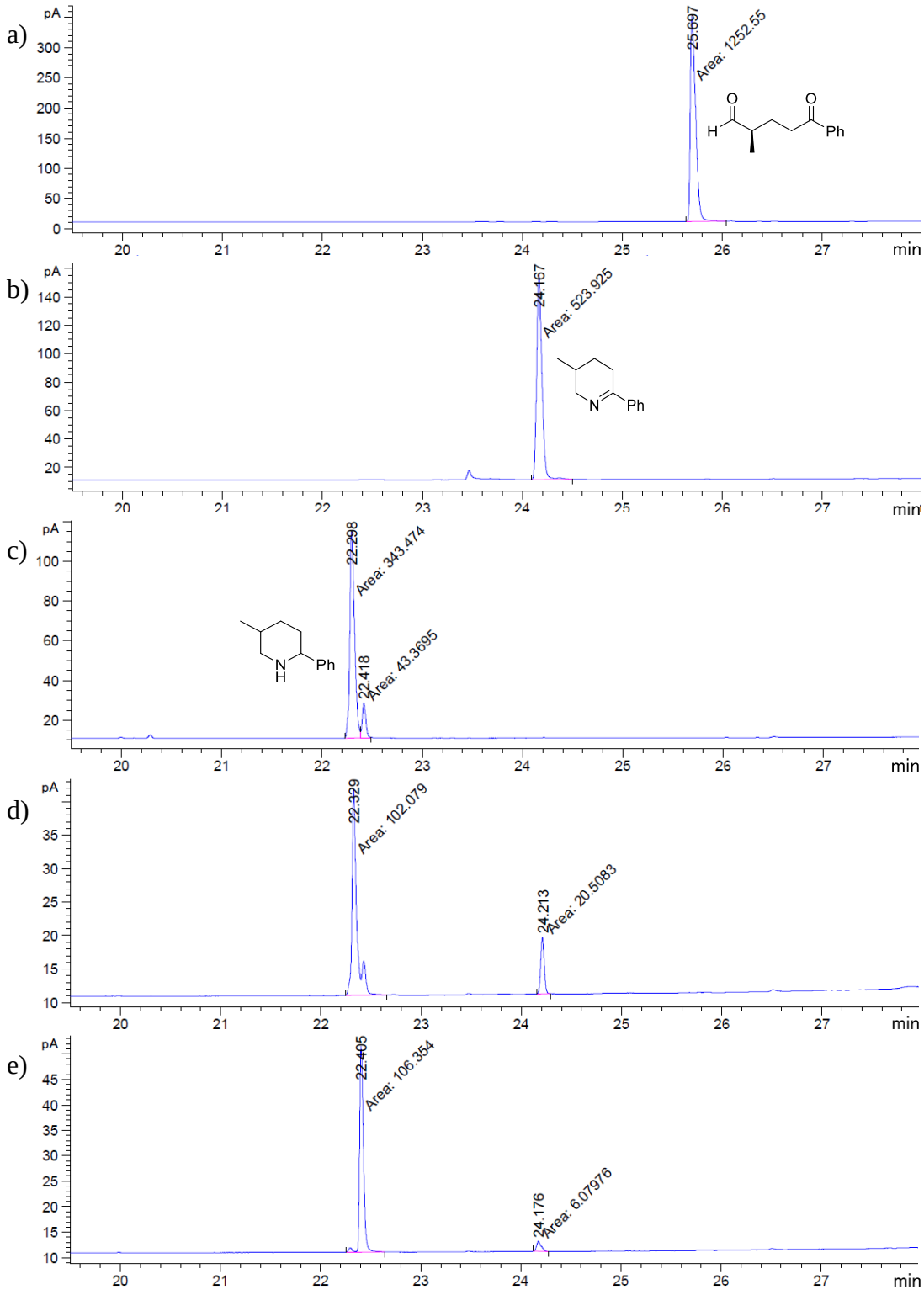


Figure S15: GC-FID analysis of cascade biotransformation of (*R*)-**3** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) (*R*)-**3**, b) imine standard, c) racemic amine standard, d) TA-(*R*)-IRED biotransformation of (*R*)-**3**, e) TA-(*S*)-IRED biotransformation of (*R*)-**3**.

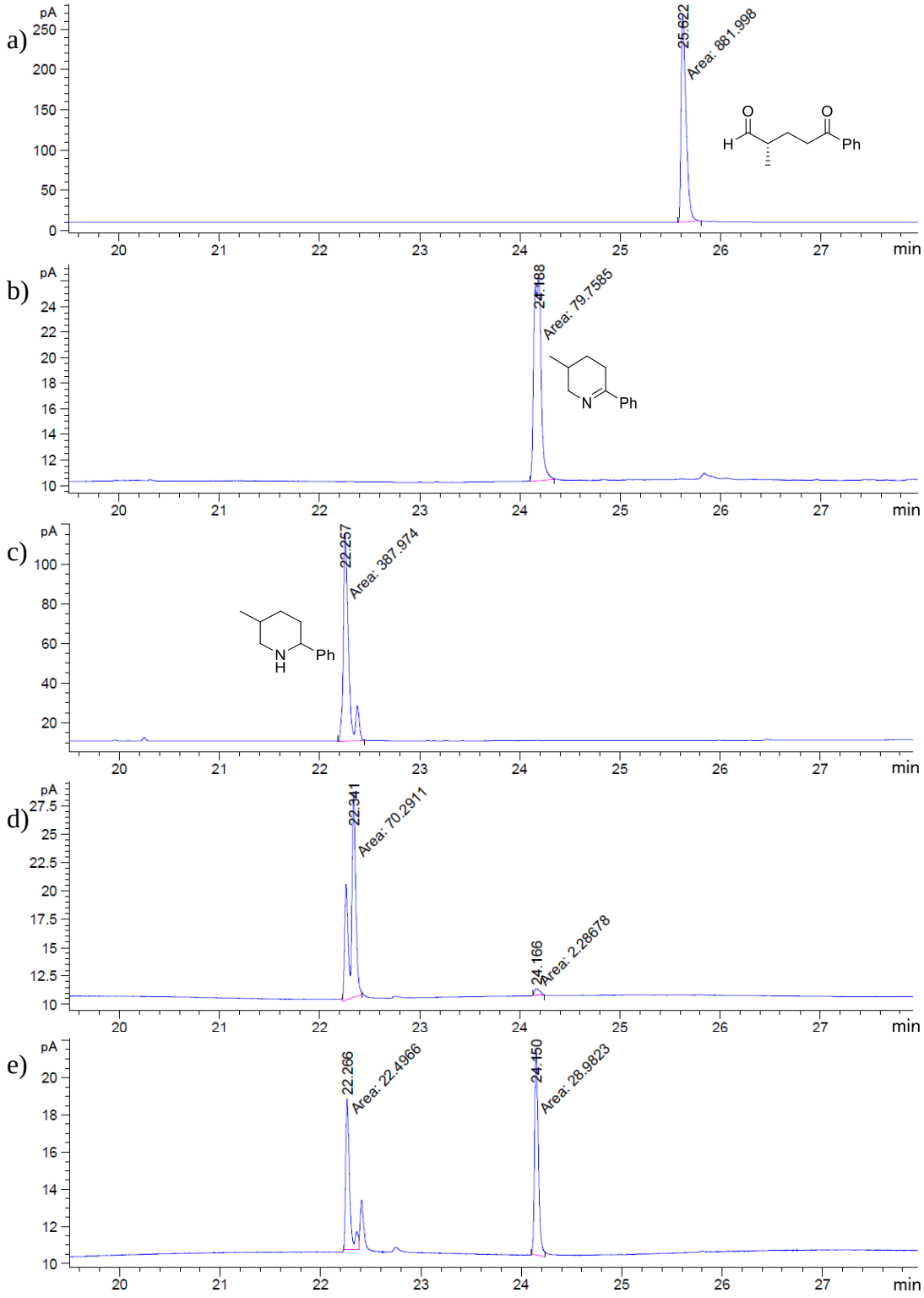


Figure S16: GC-FID analysis of cascade biotransformation of (*S*)-**3** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) (*S*)-**3**, b) imine standard, c) racemic amine standard, d) TA-(*R*)-IRED biotransformation of (*S*)-**3**, e) TA-(*S*)-IRED biotransformation of (*S*)-**3**.

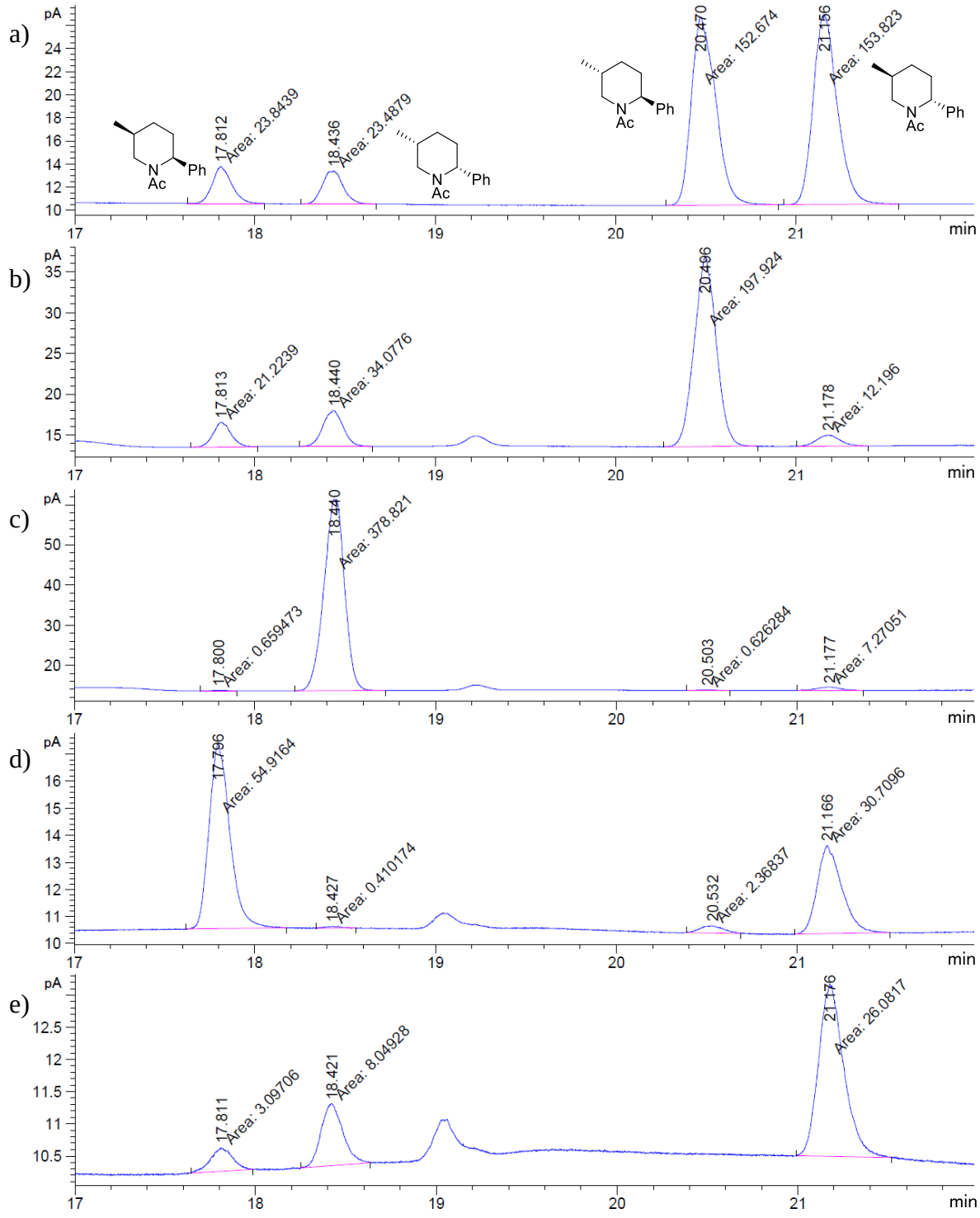


Figure S17: GC-FID analysis of cascade biotransformation of (*R*)-**3** and (*S*)-**3** to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min, then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) racemic amine standard (from $NaBH_4$ reduction of the imine), b) TA-(*R*)-IRED biotransformation of (*R*)-**3**, c) TA-(*S*)-IRED biotransformation of (*R*)-**3**, d) TA-(*R*)-IRED biotransformation of (*S*)-**3**, e) TA-(*S*)-IRED biotransformation of (*S*)-**3**.

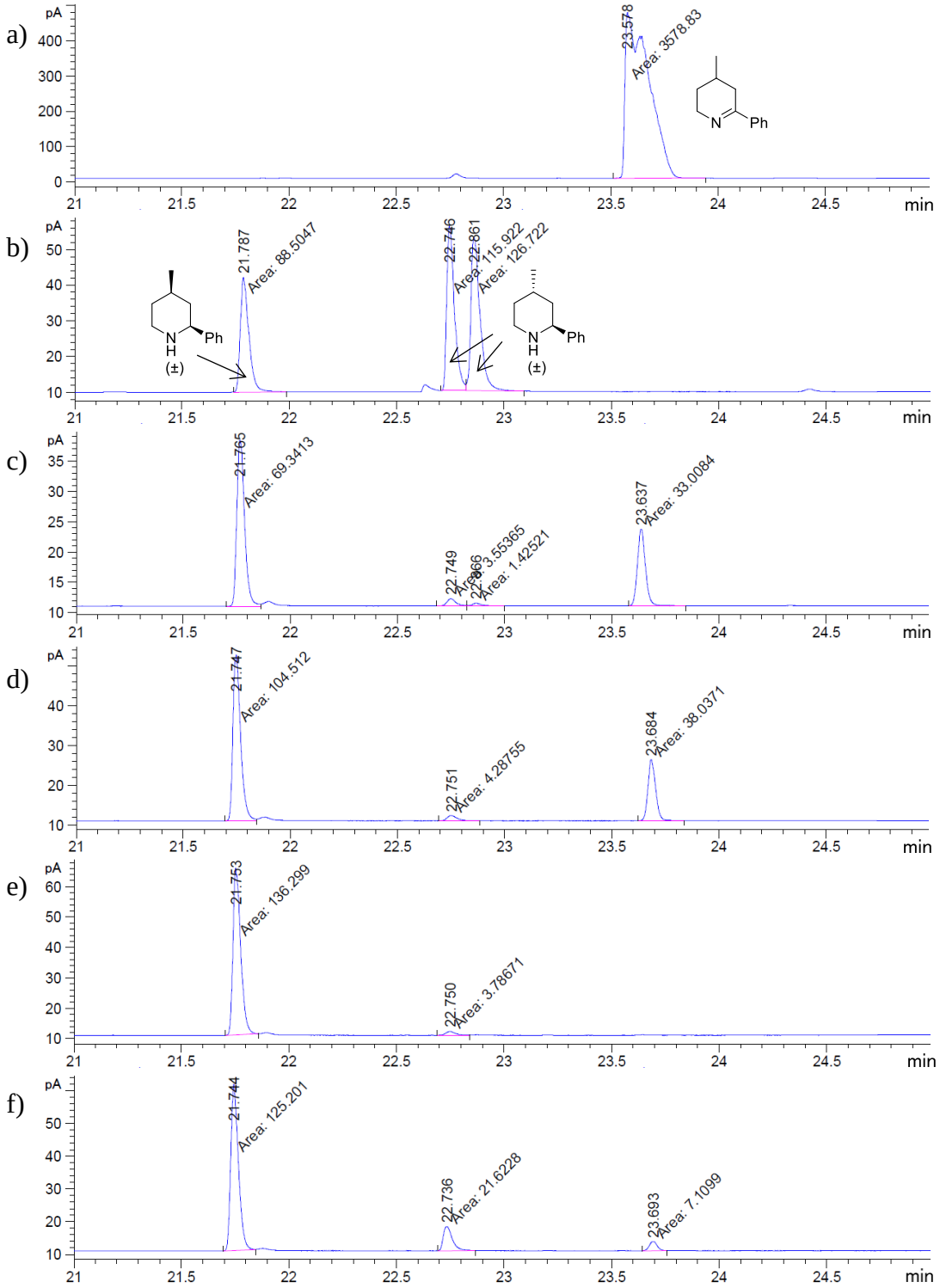


Figure S18: GC-FID analysis of cascade biotransformation of (*R*)-**5** and (*S*)-**5** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) Imine standard, b) racemic amine standard (from SmI_2 reduction of 4-methyl-2-phenylpyridine), c) CAR-TA-(*R*)-IRED biotransformation of (*R*)-**5**, d) CAR-TA-(*R*)-IRED biotransformation of (*S*)-**5**, e) CAR-TA-(*S*)-IRED biotransformation of (*R*)-**5**, f) CAR-TA-(*S*)-IRED biotransformation of (*S*)-**5**.

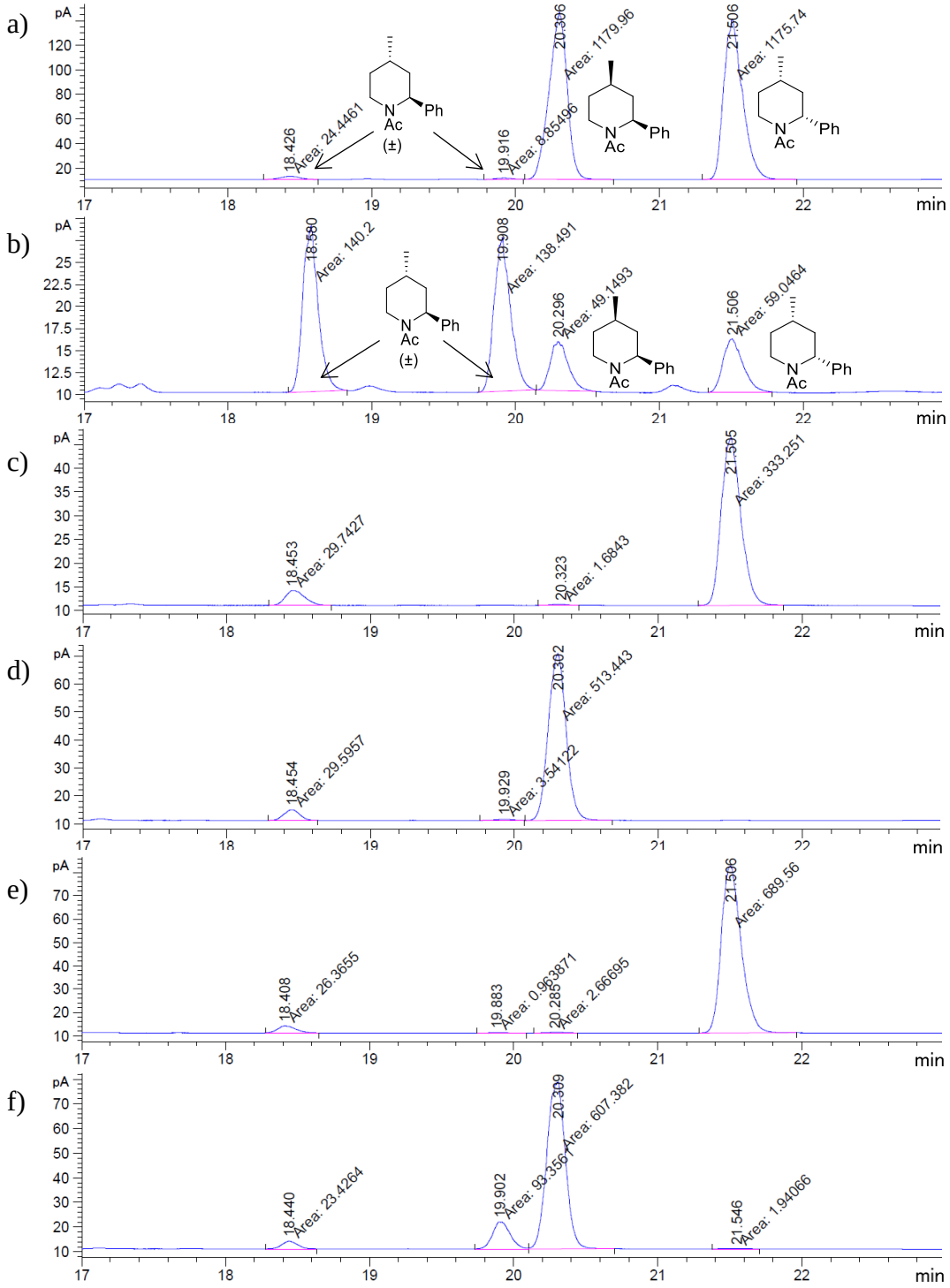


Figure S19: GC-FID analysis of cascade biotransformation of (*R*)-**5** and (*S*)-**5** to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min, then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine), b) racemic amine standard (from SmI_2 reduction of 4-methyl-2-phenylpyridine), c) CAR-TA-(*R*)-IRED biotransformation of (*R*)-**5**, d) CAR-TA-(*R*)-IRED biotransformation of (*S*)-**5**, e) CAR-TA-(*S*)-IRED biotransformation of (*R*)-**5**, f) CAR-TA-(*S*)-IRED biotransformation of (*S*)-**5**.

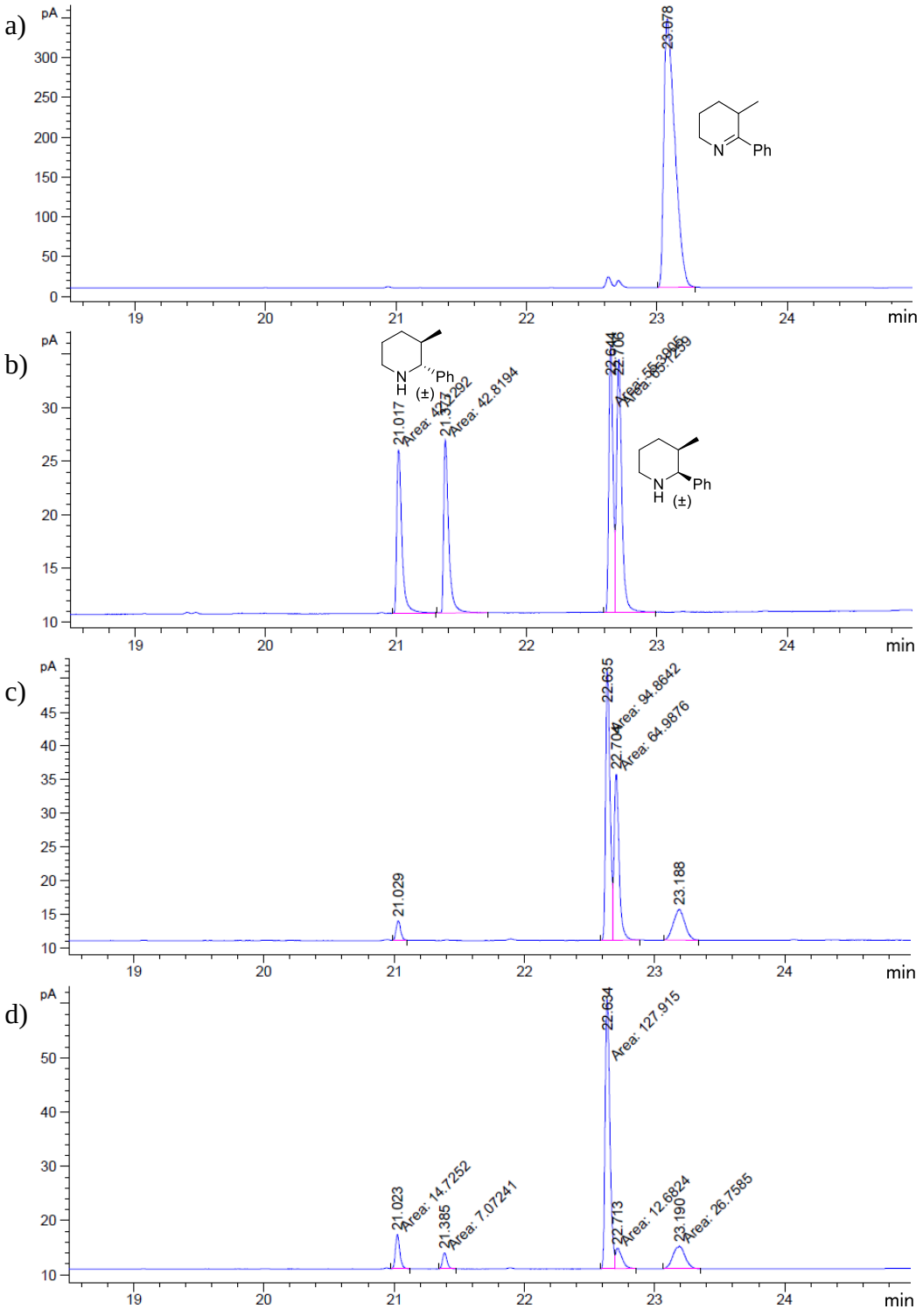


Figure S20: GC-FID analysis of cascade biotransformation of (±)-**7** to determine conversion. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 200°C, 5°C/min then hold for 2 min). a) Imine standard, b) racemic amine standard (from $NH_3.BH_3$ reduction of imine), c) CAR-TA-(*R*)-IRED biotransformation of (±)-**7**, d) CAR-TA-(*R*)-IRED biotransformation of (±)-**7**.

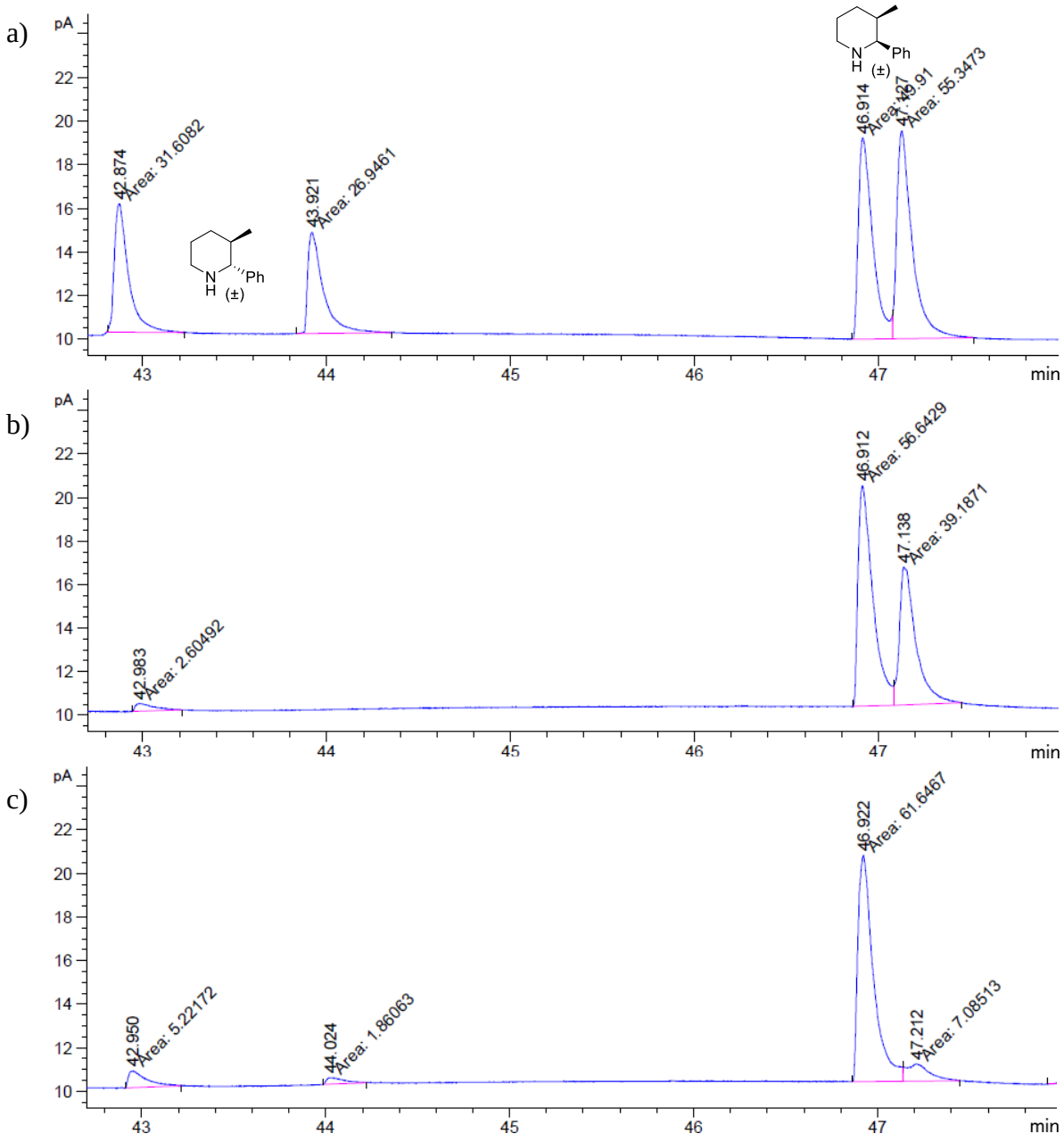


Figure S21: GC-FID analysis of cascade biotransformation of (±)-**7** to determine *de* and *ee*. (Carrier gas helium, 1.2 mL/min, injector temp. 200°C, detector temp. 250°C, programmed temperature: 50°C- 154°C, 2°C/min then 10°C/min to 200°C). a) Racemic amine standard (from $NH_3.BH_3$ reduction of imine), b) CAR-TA-(*R*)-IRED biotransformation of (±)-**7**, c) CAR-TA-(*R*)-IRED biotransformation of (±)-**7**.

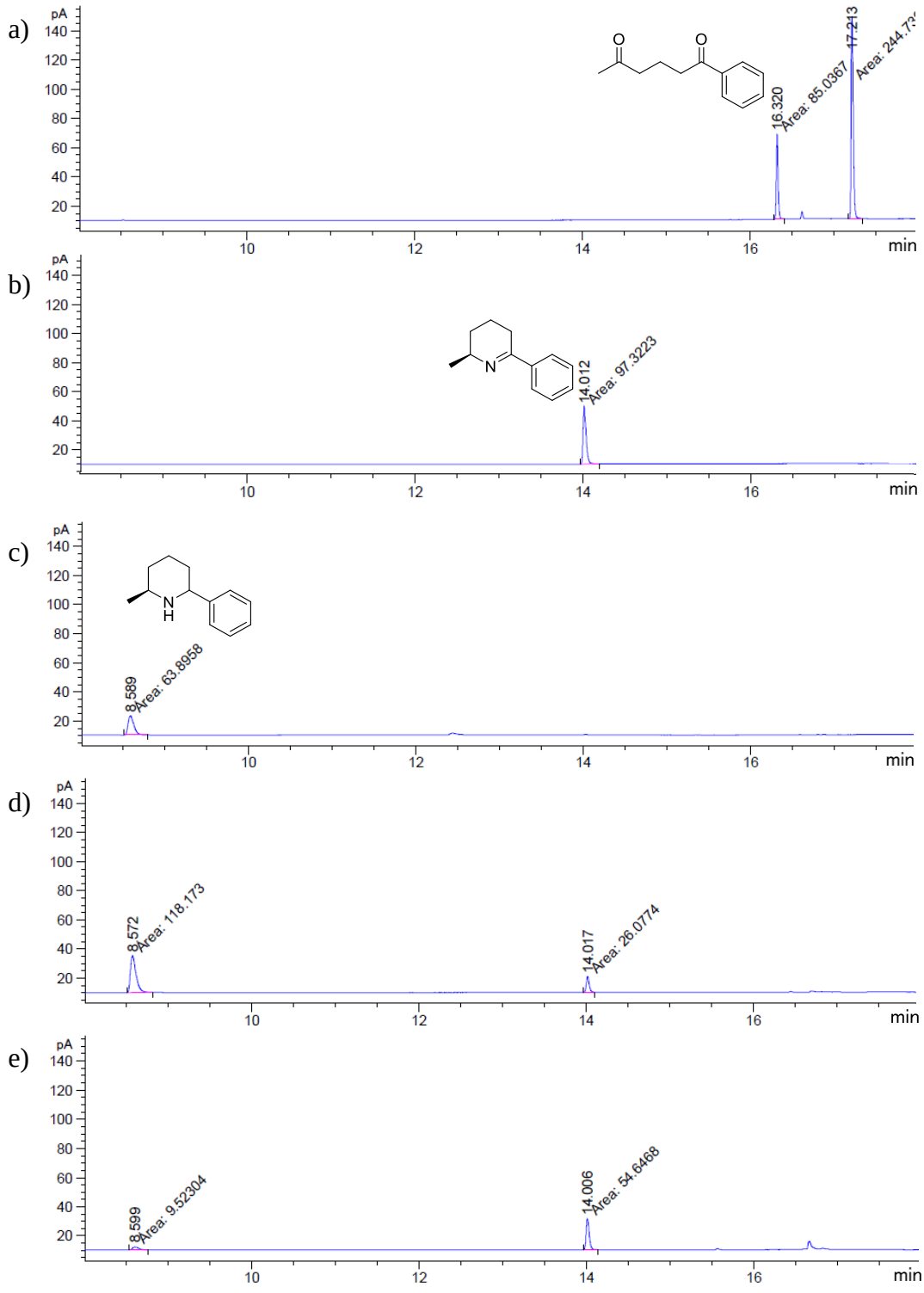


Figure S22: GC-FID analysis of cascade biotransformation of **11a** with ATA-113 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). a) diketone standard (extracted after basification), b) imine (*S*)-**12a** standard, c) amine **13a** standard, d) ATA-113/(*R*)-IRED biotransformation of **11a**, e) ATA-113/(*S*)-IRED biotransformation of **11a**.

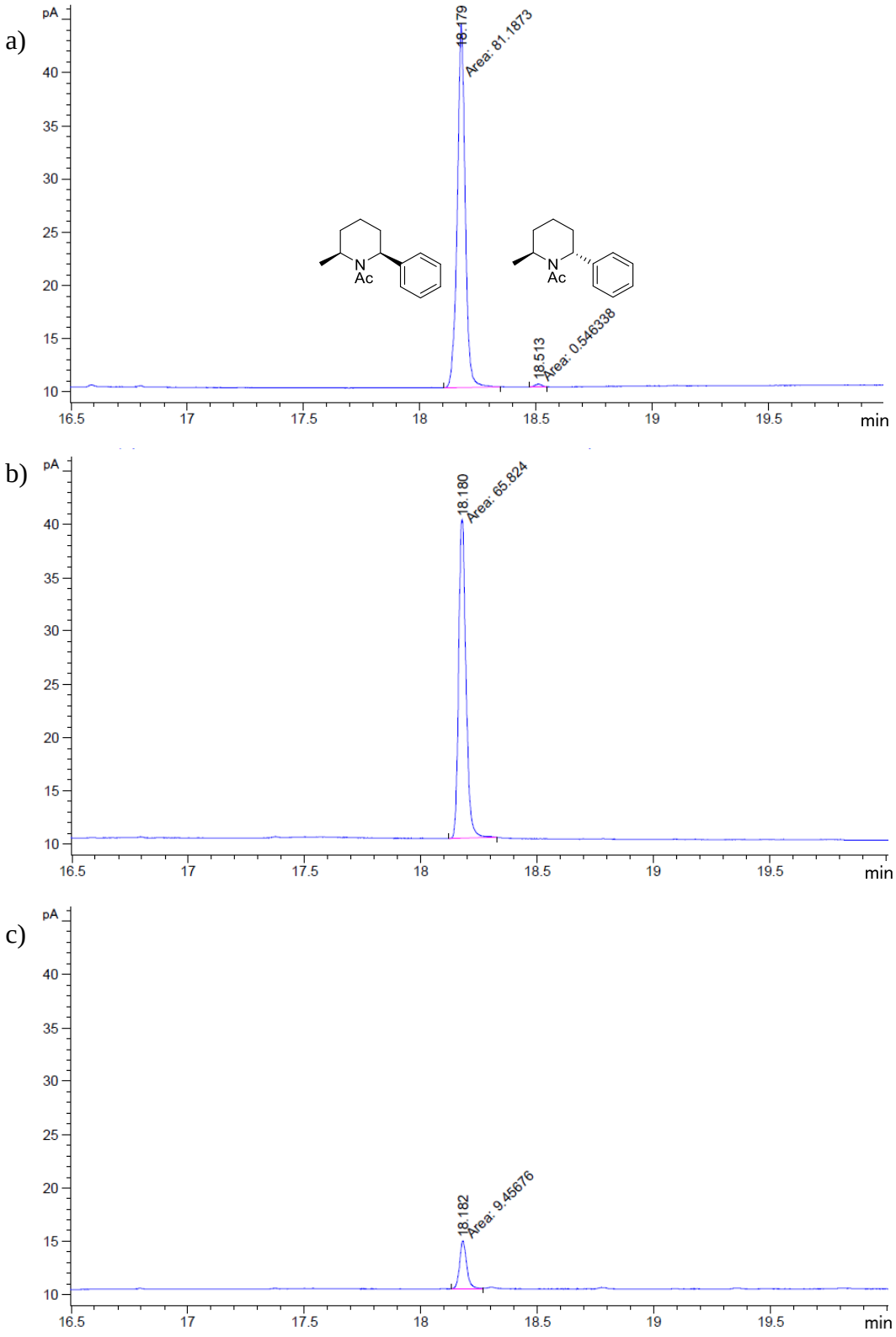


Figure S23: GC-FID analysis of cascade biotransformation of **11a** with ATA-113 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*S*)-**12a**), b) ATA-113/(*R*)-IRED biotransformation of **11a**, c) ATA-113/(*S*)-IRED biotransformation of **11a.**

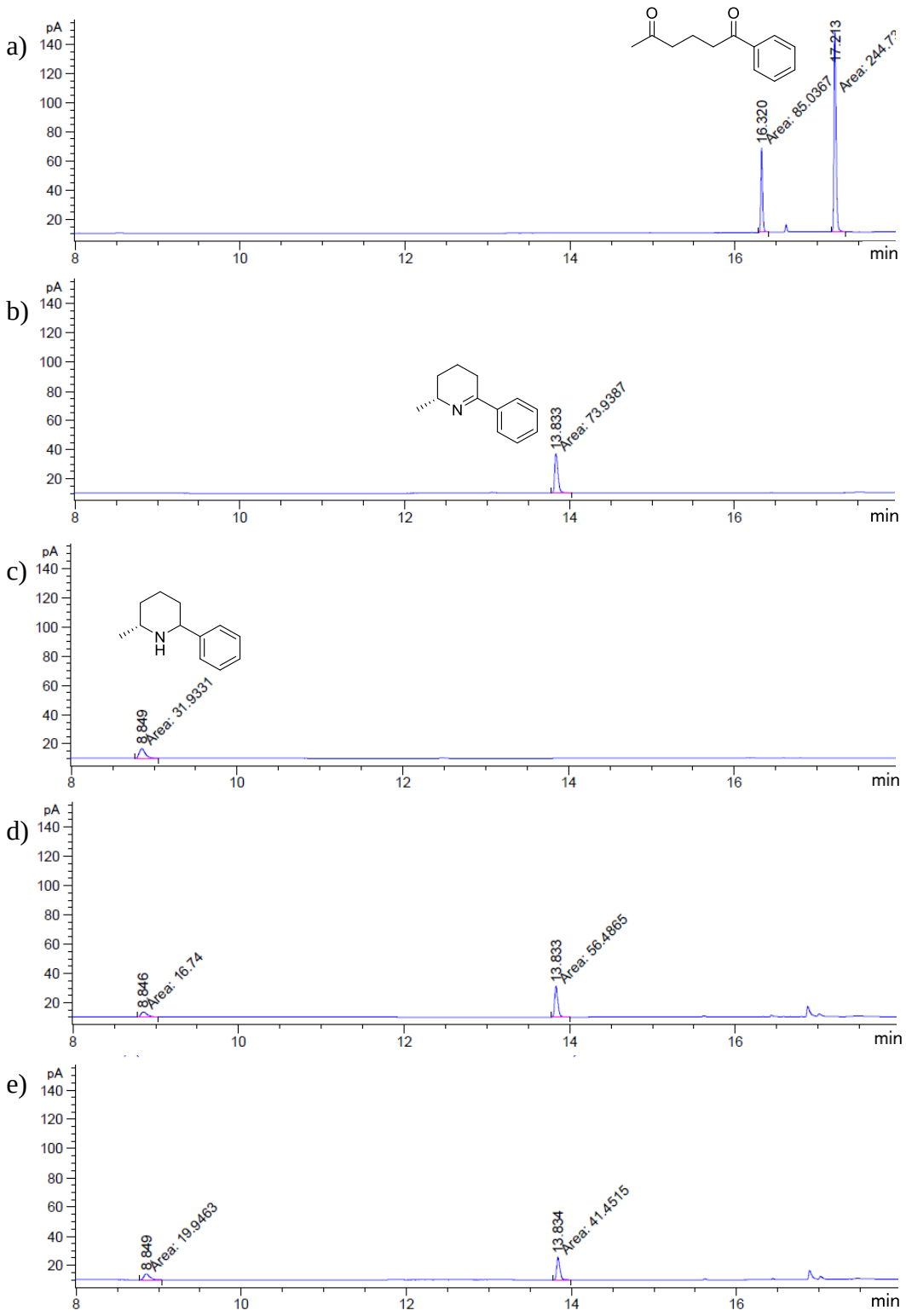


Figure S24: GC-FID analysis of cascade biotransformation of **11a** with ATA-117 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). a) diketone standard (extracted after basification), b) imine (*R*)-**12a** standard, c) amine **13a** standard, d) ATA-117/(*R*)-IRED biotransformation of **11a**, e) ATA-117/(*S*)-IRED biotransformation of **11a**

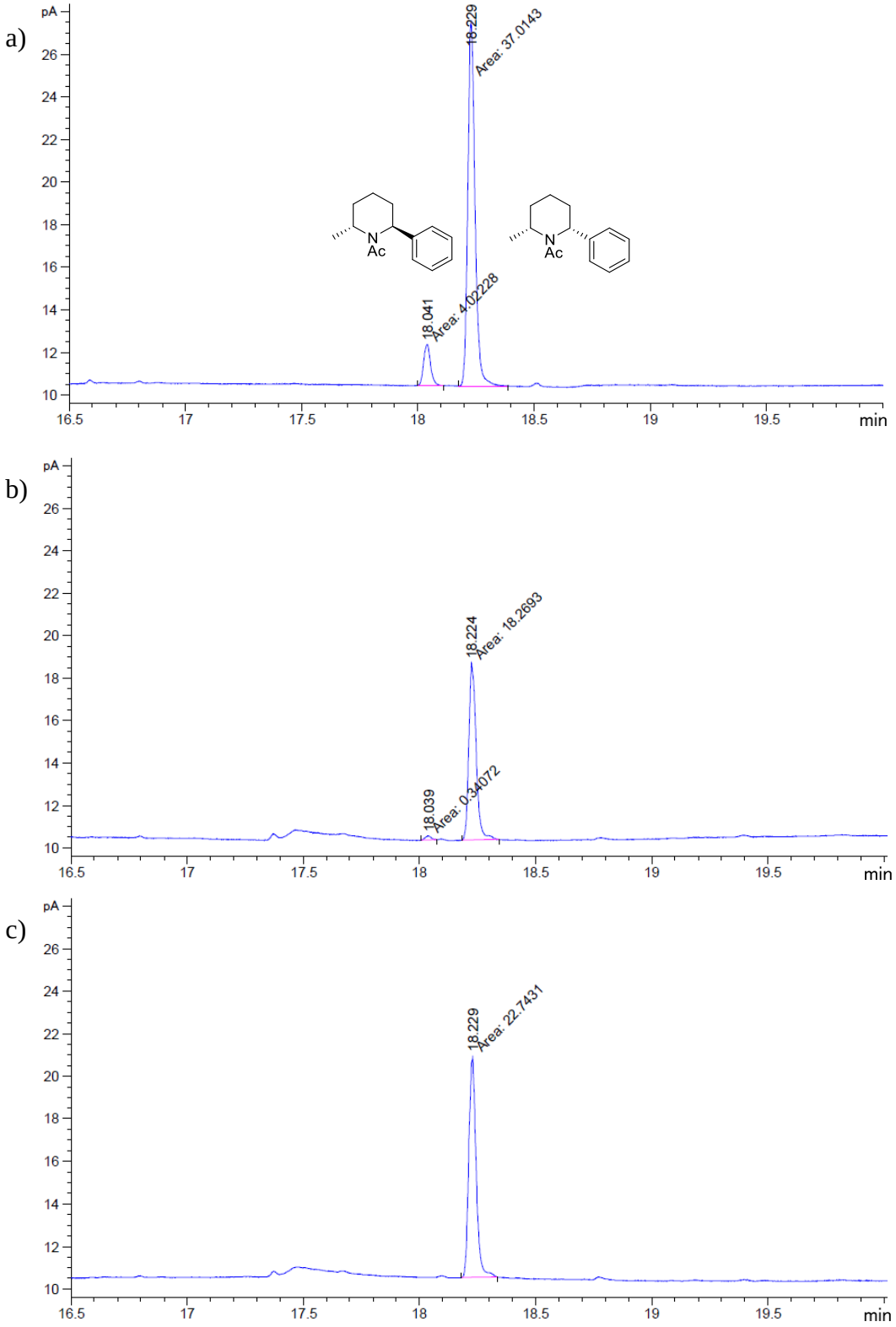


Figure S25: GC-FID analysis of cascade biotransformation of **11a** with ATA-117 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*R*)-**12a**),, b) ATA-117/(*R*)-IRED biotransformation of **11a**, c) ATA-117/(*S*)-IRED biotransformation of **11a.**

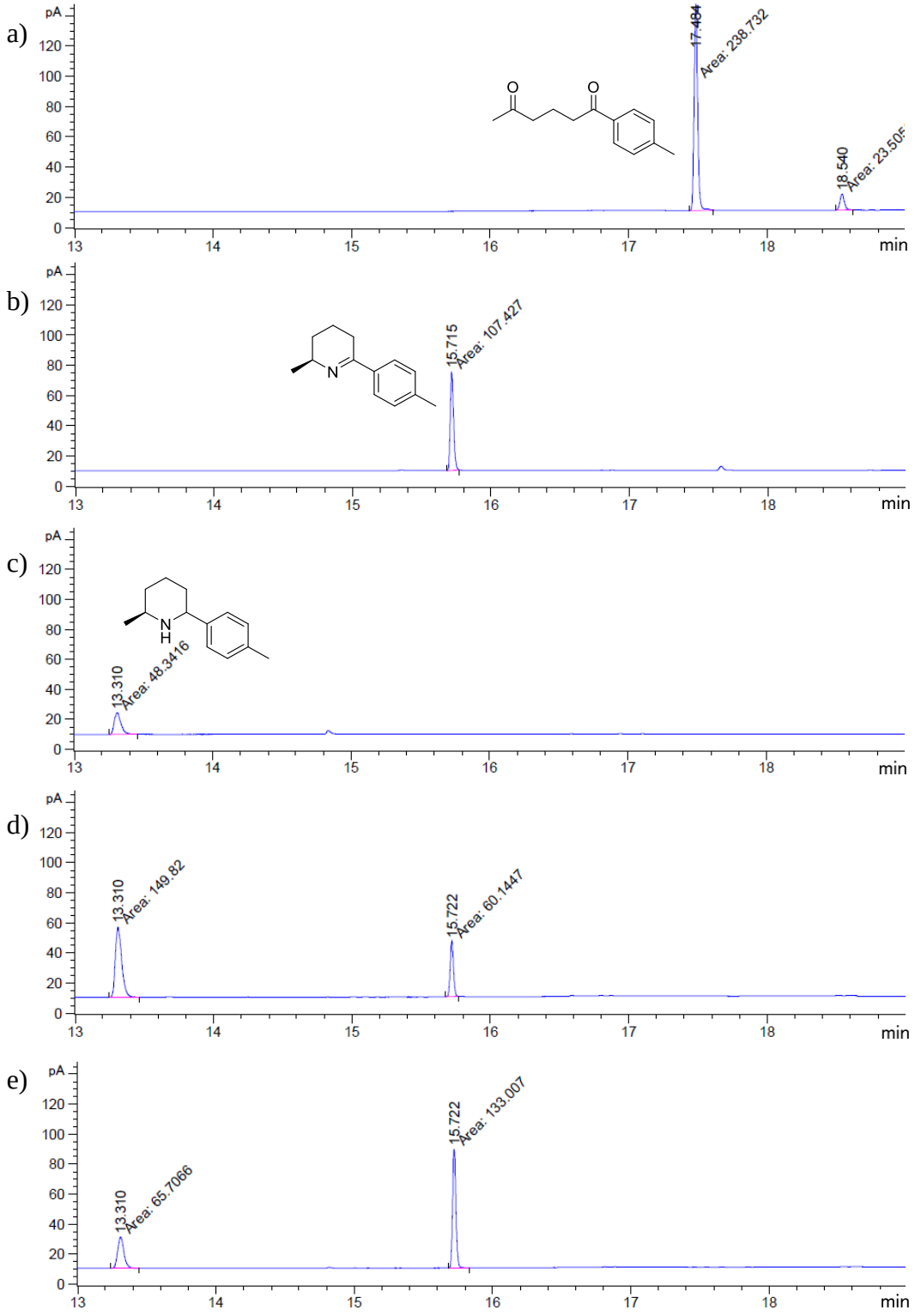


Figure S26: GC-FID analysis of cascade biotransformation of **11b** with ATA-113 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). a) diketone standard (extracted after basification), b) imine (*S*)-**12b** standard, c) amine **13b** standard, d) ATA-113/(*R*)-IRED biotransformation of **11b**, e) ATA-113/(*S*)-IRED biotransformation of **11b**.

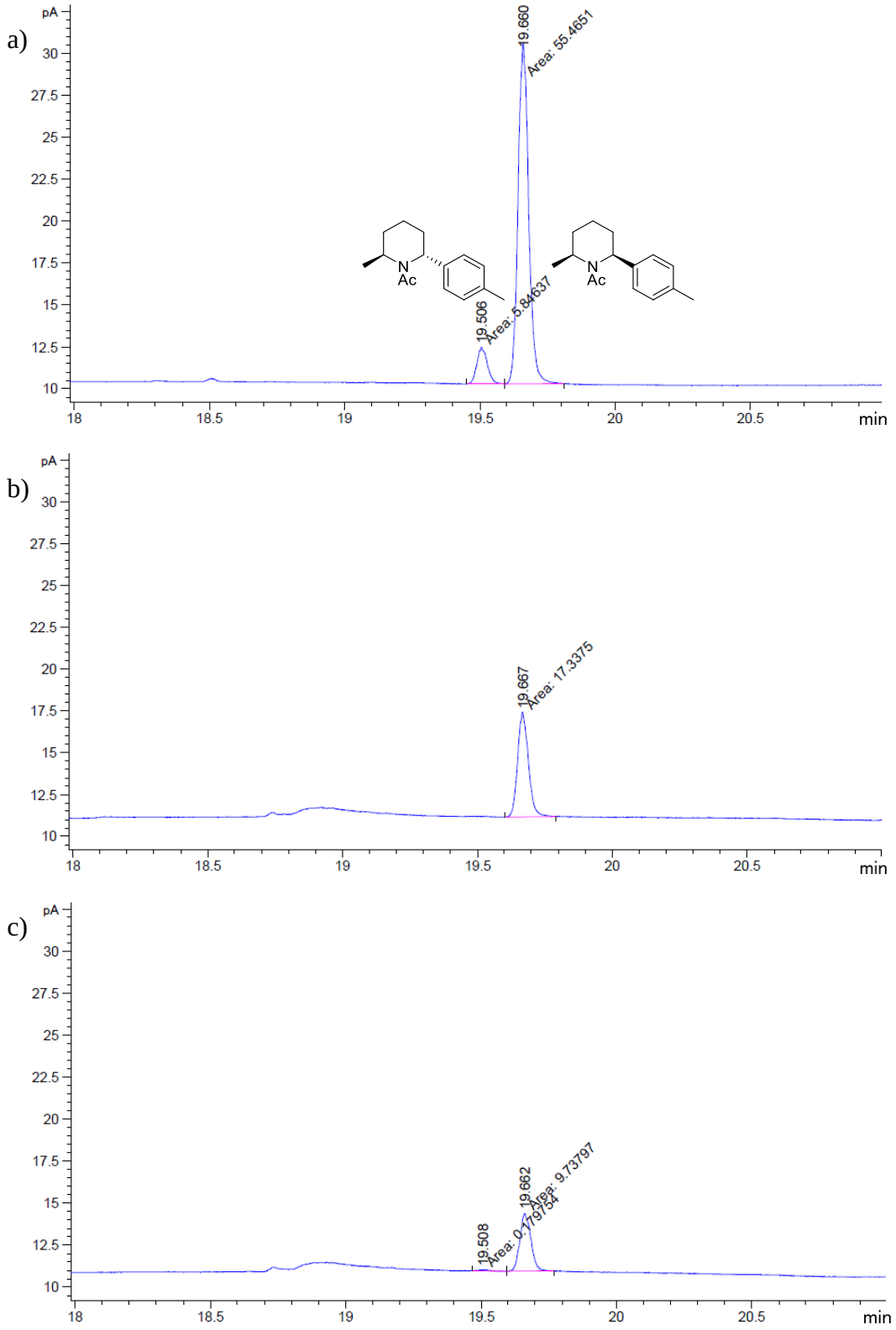


Figure S27: GC-FID analysis of cascade biotransformation of **11b** with ATA-113 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*S*)-**12b**), b) ATA-113/(*R*)-IRED biotransformation of **11b**, c) ATA-113/(*S*)-IRED biotransformation of **11b.**

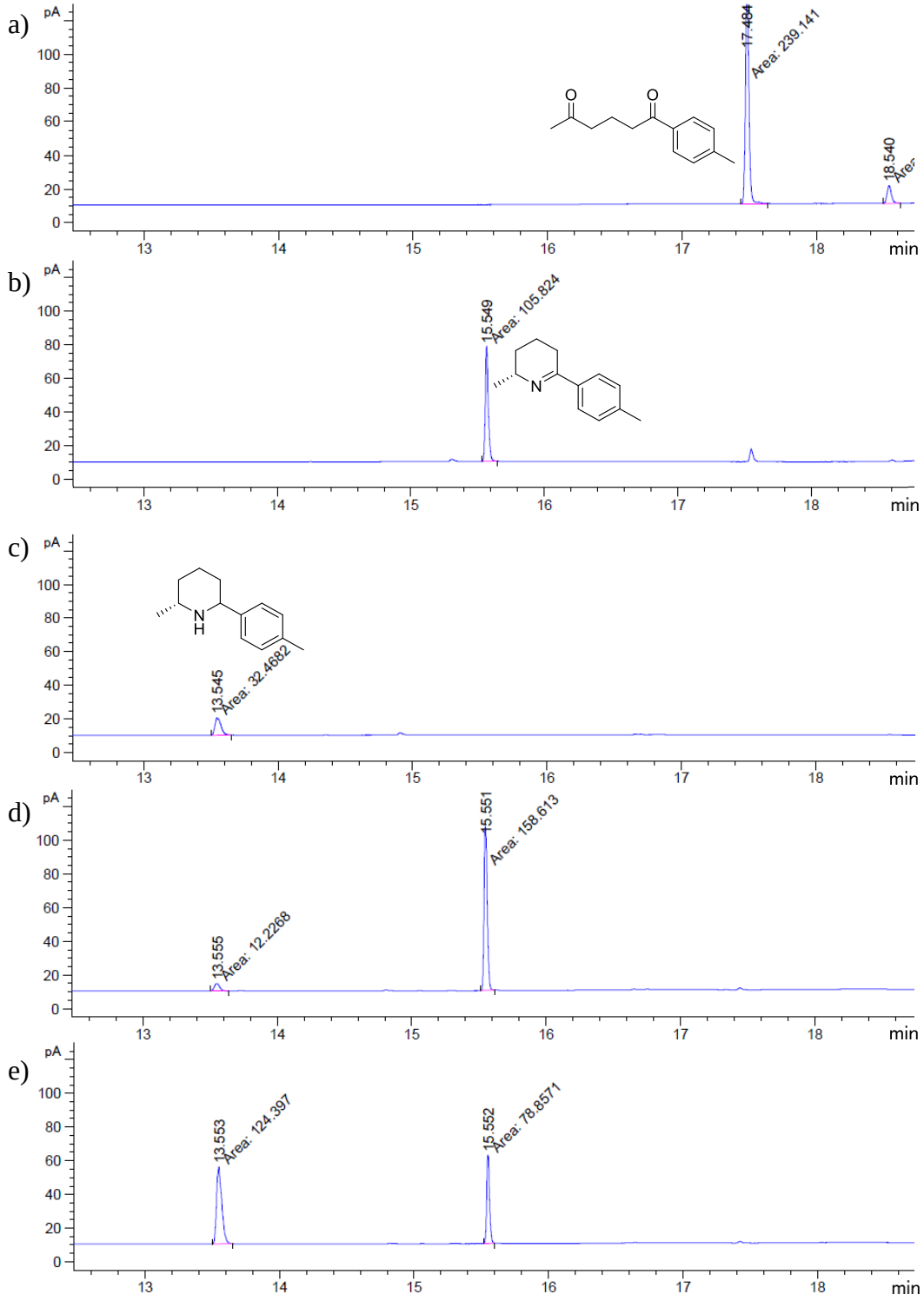


Figure S28: GC-FID analysis of cascade biotransformation of **11b** with ATA-117 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). a) diketone standard (extracted after basification), b) imine (*R*)-**12b** standard, c) amine **13b** standard, d) ATA-113/(*R*)-IRED biotransformation of **11b**, e) ATA-117/(*S*)-IRED biotransformation of **11b**.

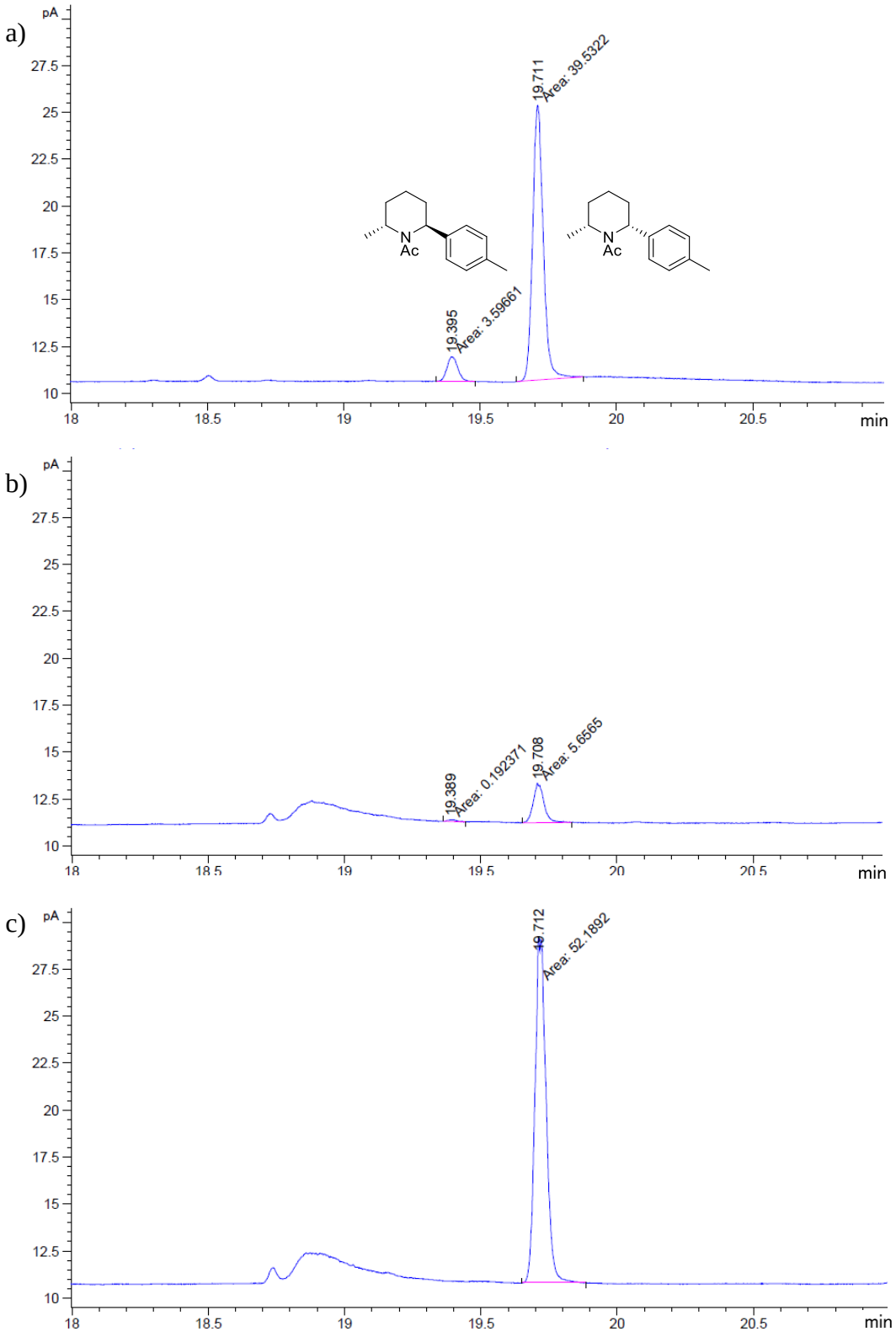


Figure S29: GC-FID analysis of cascade biotransformation of **11b** with ATA-117 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 250°C, detector temp. 275°C, programmed temperature: 130°C hold for 12 min, then 15°C/min ramp to 200°C, hold for 5 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*R*)-**12b**), b) ATA-117/(*R*)-IRED biotransformation of **11b**, c) ATA-117/(*S*)-IRED biotransformation of **11b**.

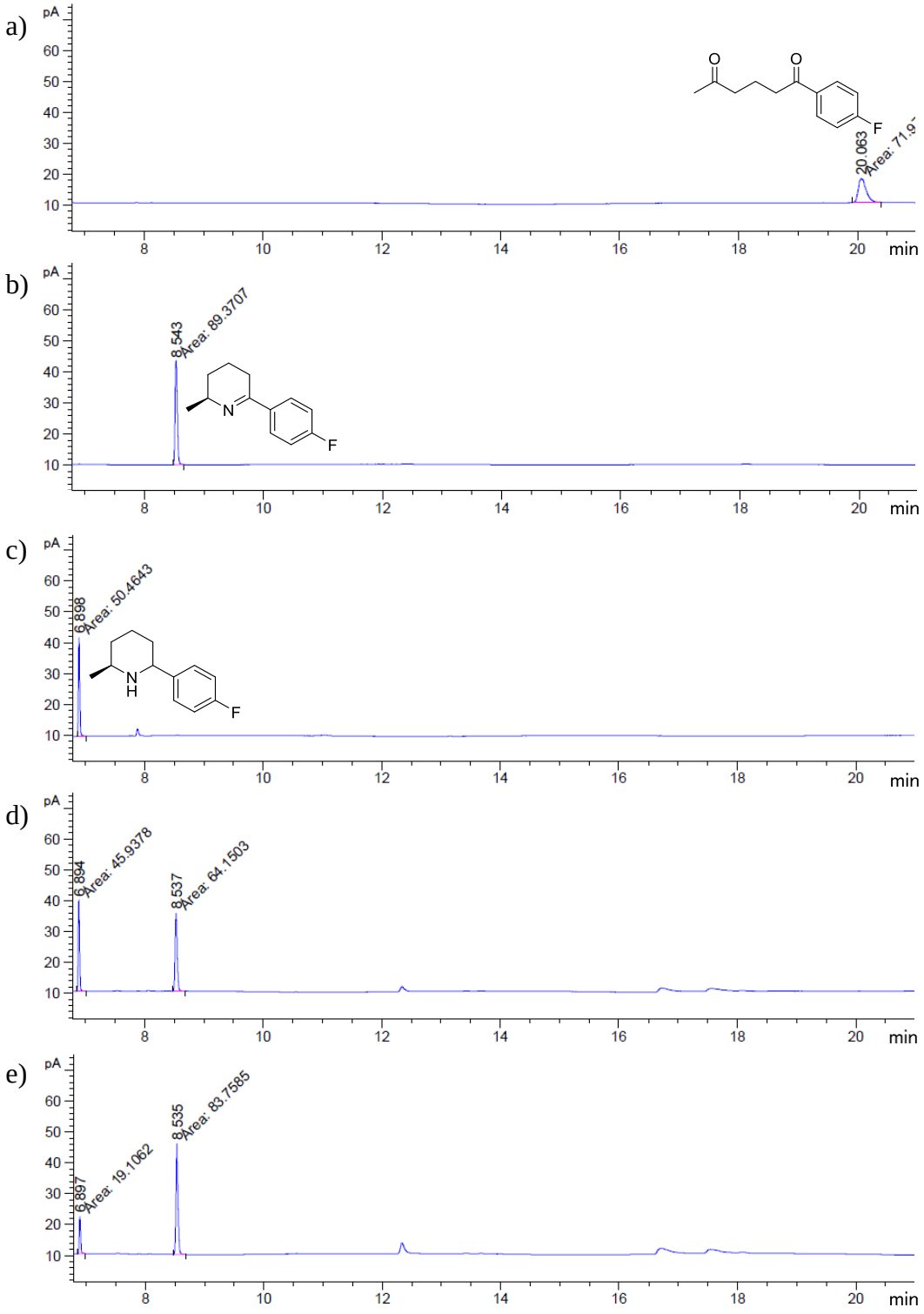


Figure S30: GC-FID analysis of cascade biotransformation of **11c** with ATA-113 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*S*)-**12c** standard, c) amine **13c** standard, d) ATA-113/(*R*)-IRED biotransformation of **11c**, e) ATA-113/(*S*)-IRED biotransformation of **11c**.

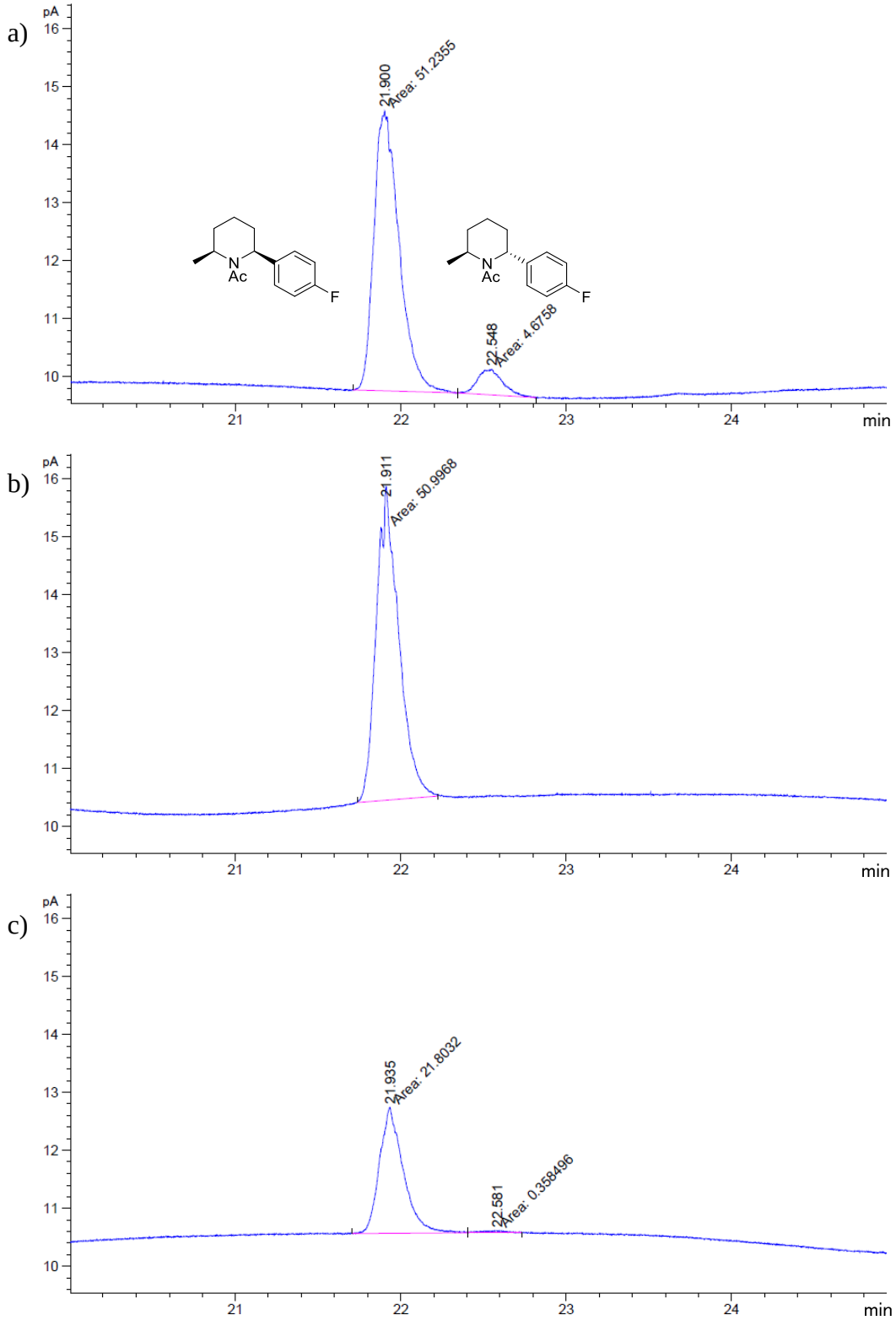


Figure S31: GC-FID analysis of cascade biotransformation of **11c** with ATA-113 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*S*)-**12c**), b) ATA-113/(*R*)-IRED biotransformation of **11c**, c) ATA-113/(*S*)-IRED biotransformation of **11c.**

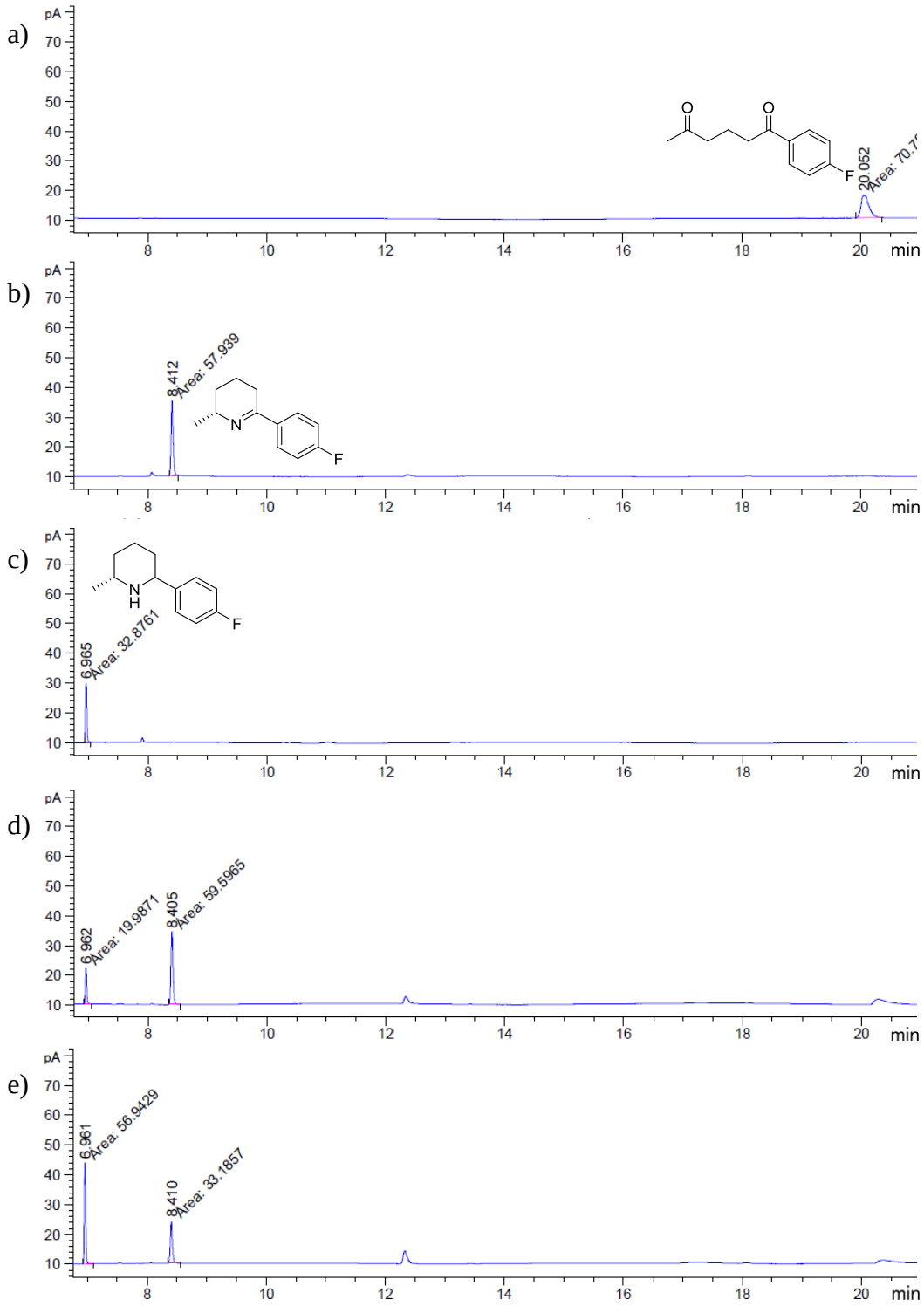


Figure S32: GC-FID analysis of cascade biotransformation of **11c** with ATA-117 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*R*)-**12c** standard, c) amine **13d** standard, d) ATA-117/(*R*)-IRED biotransformation of **11c**, e) ATA-117/(*S*)-IRED biotransformation of **11c**.

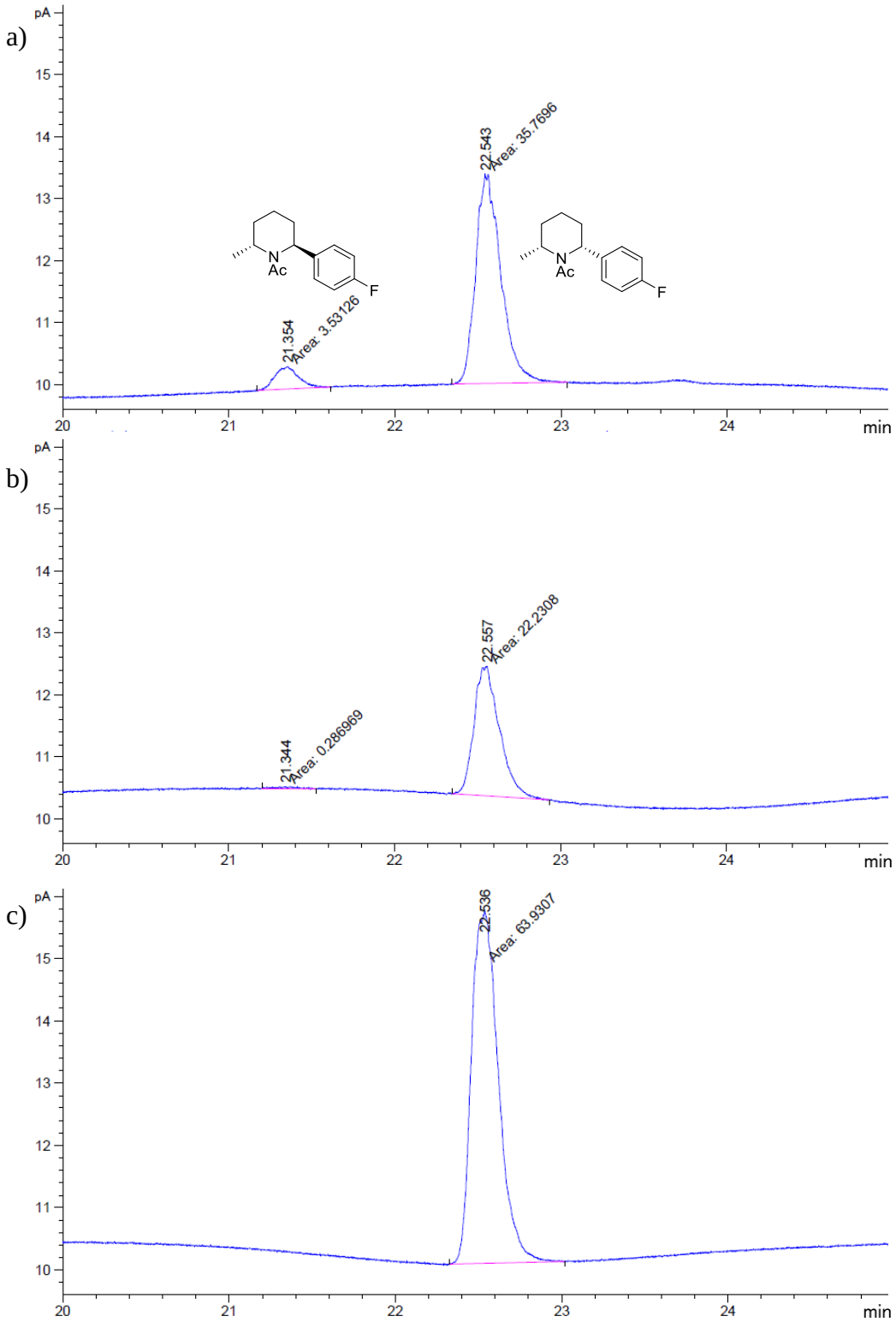


Figure S33: GC-FID analysis of cascade biotransformation of **11c** with ATA-117 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*R*)-**12c**), b) ATA-117/(*R*)-IRED biotransformation of **11c**, c) ATA-117/(*S*)-IRED biotransformation of **11c.**

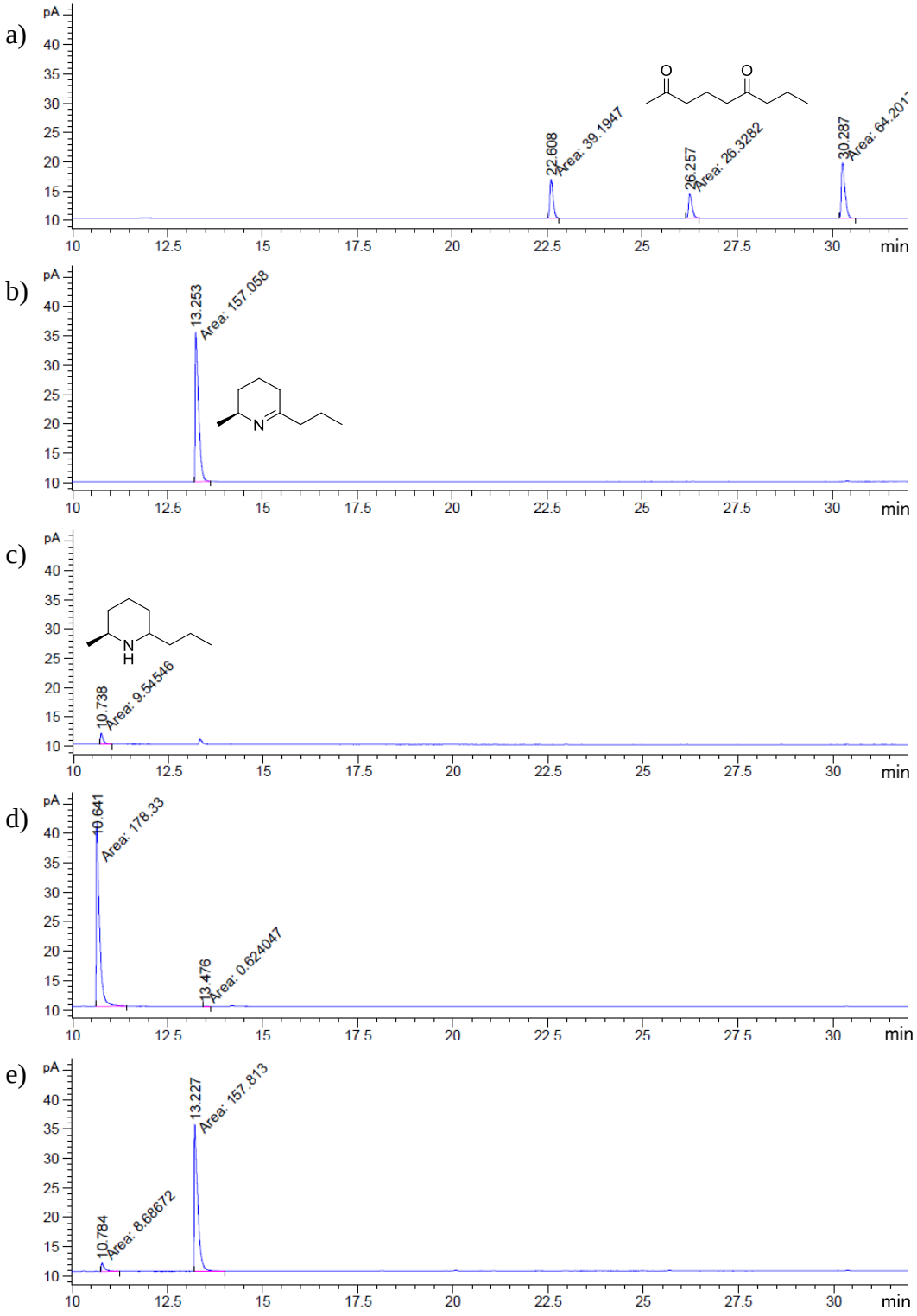


Figure S34: GC-FID analysis of cascade biotransformation of **11d** with ATA-113 and IRED to determine conversion. (Carrier gas helium, 1.1 mL/min, injector temp. 250°C, detector temp. 250°C, programmed temperature: 80°C hold for 2 min, then 1.5°C/min ramp to 140°C, hold for 3 min then 4°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*S*)-**12d** standard, c) amine **13d** standard, d) ATA-113/(*R*)-IRED biotransformation of **11d**, e) ATA-113/(*S*)-IRED biotransformation of **11d**.

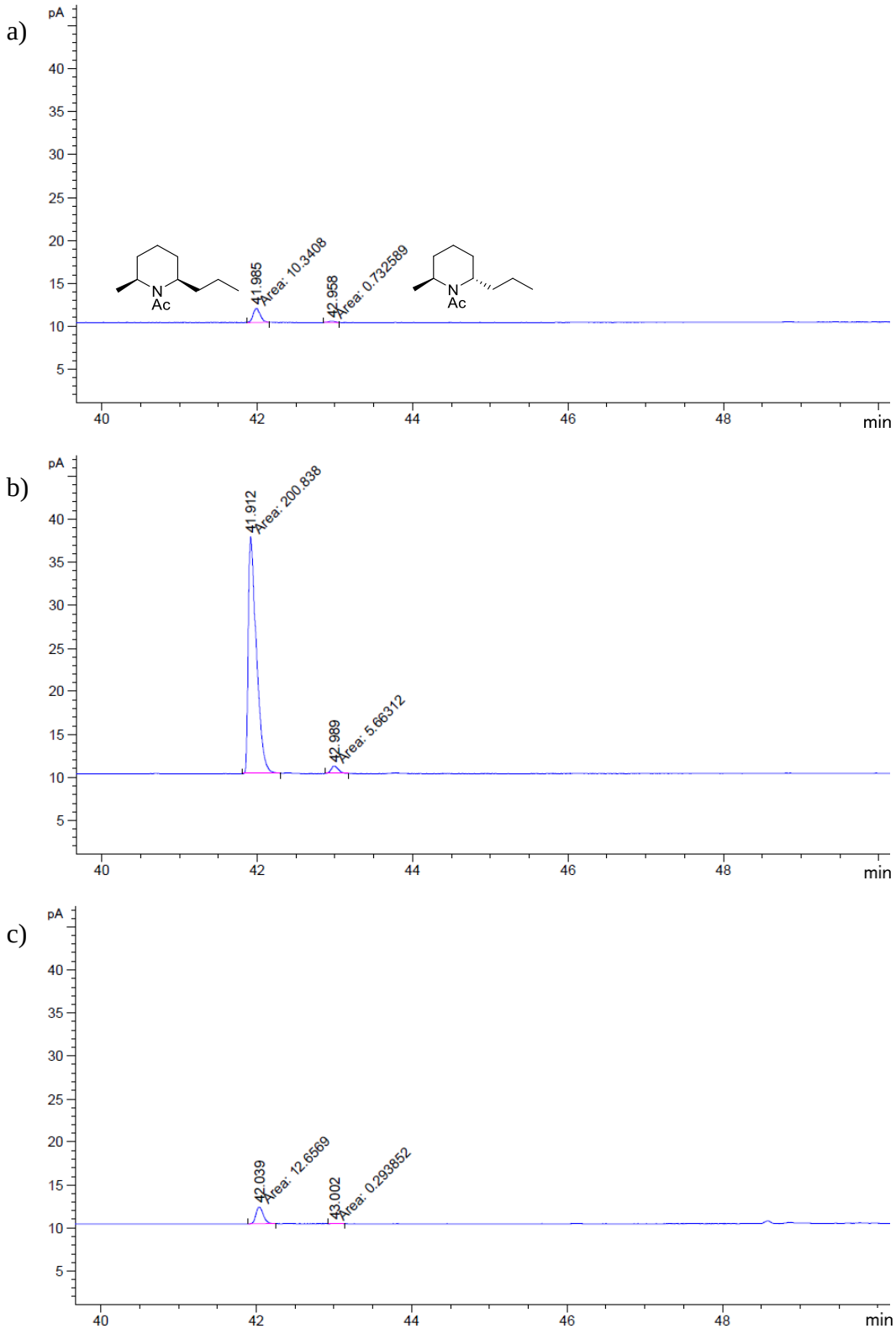


Figure S35: GC-FID analysis of cascade biotransformation of **11d** with ATA-113 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.1 mL/min, injector temp. 250°C, detector temp. 250°C, programmed temperature: 80°C hold for 2 min, then 1.5°C/min ramp to 140°C, hold for 3 min then 4°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*S*)-**12d**), b) ATA-113/(*R*)-IRED biotransformation of **11d**, c) ATA-113/(*S*)-IRED biotransformation of **11d.**

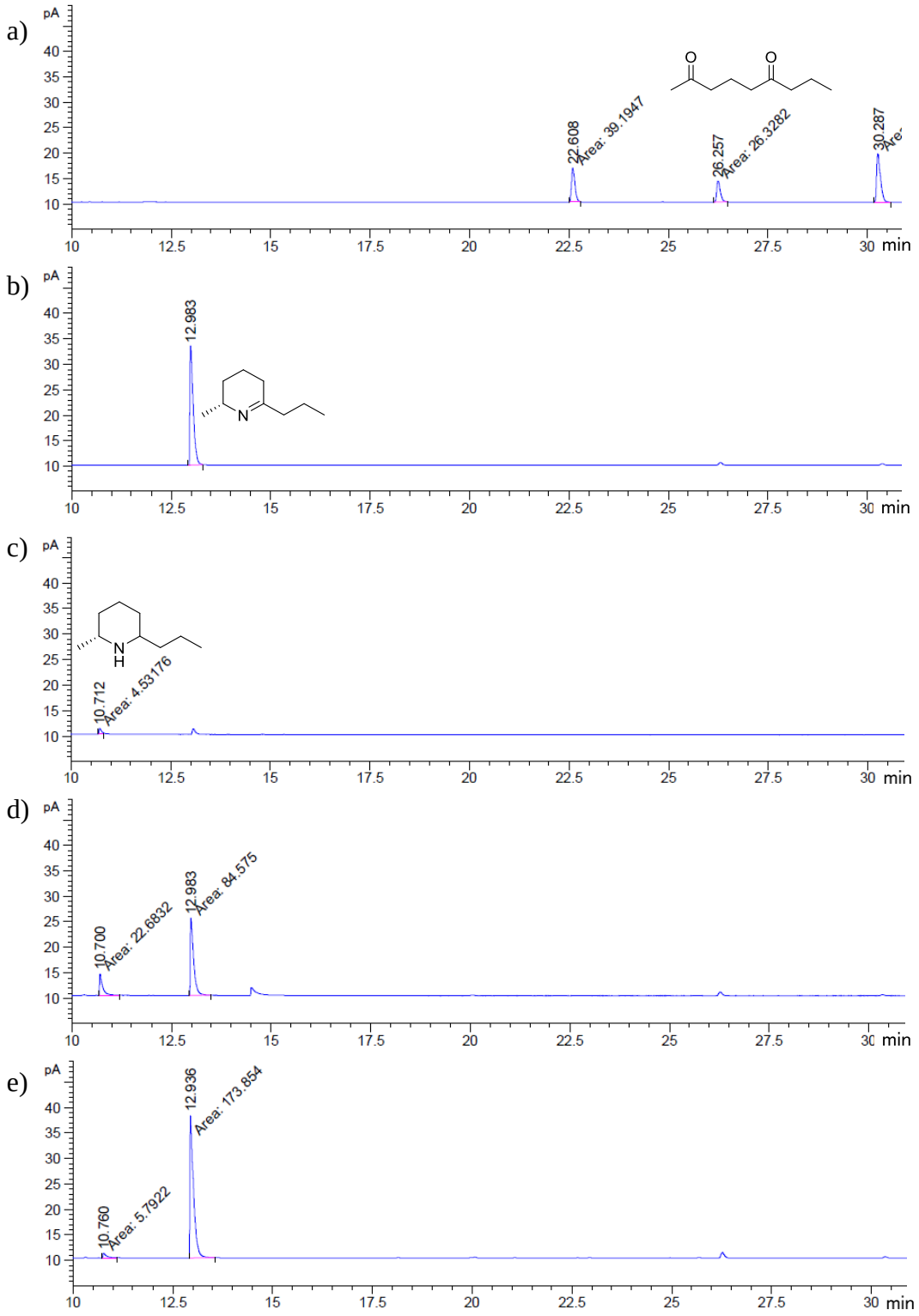


Figure S36: GC-FID analysis of cascade biotransformation of **11d** with ATA-117 and IRED to determine conversion. (Carrier gas helium, 1.1 mL/min, injector temp. 250°C, detector temp. 250°C, programmed temperature: 80°C hold for 2 min, then 1.5°C/min ramp to 140°C, hold for 3 min then 4°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*R*)-**12d** standard, c) amine **13d** standard, d) ATA-117/(*R*)-IRED biotransformation of **11d**, e) ATA-117/(*S*)-IRED biotransformation of **11d**.

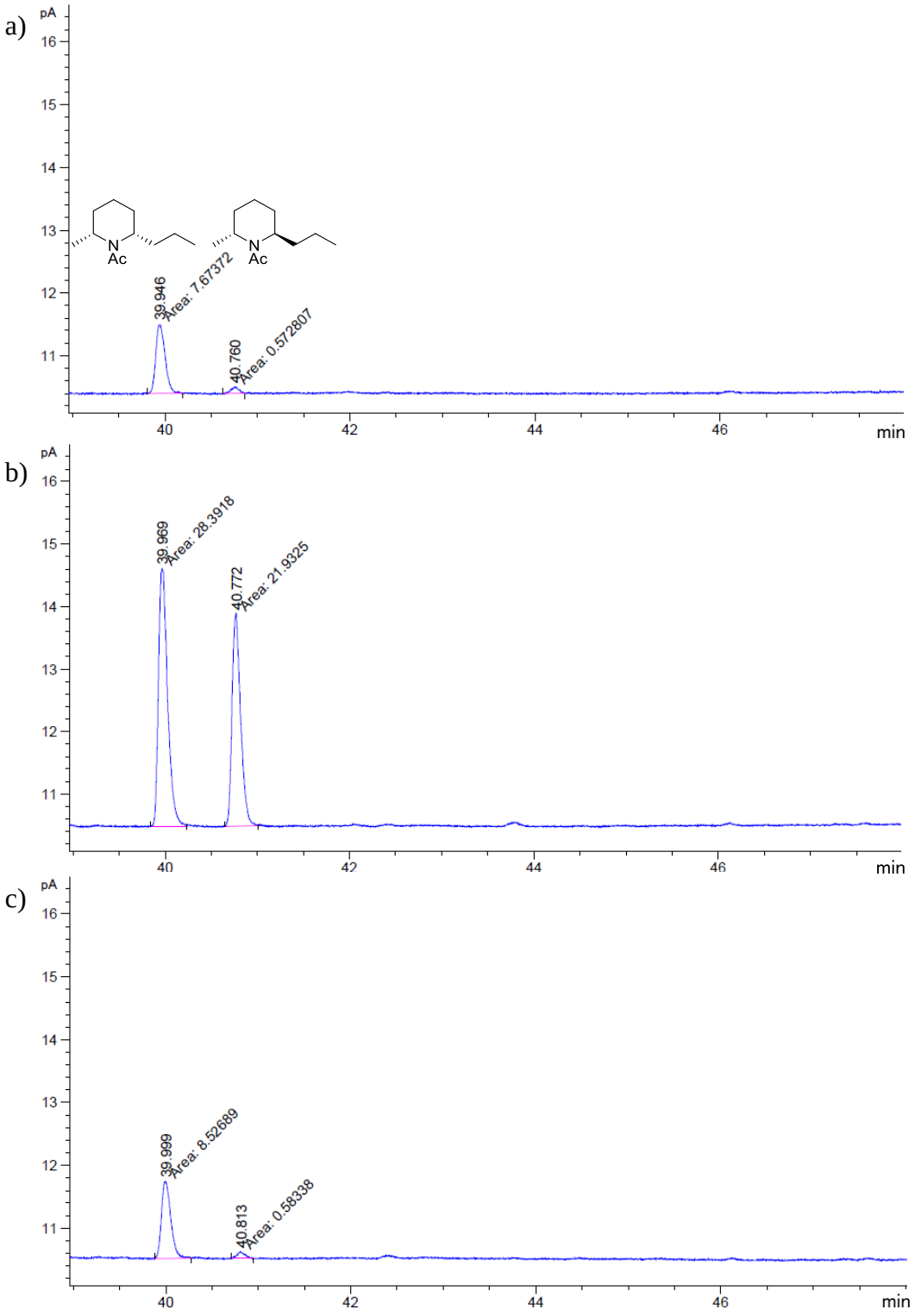


Figure S37: GC-FID analysis of cascade biotransformation of **11d** with ATA-117 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.1 mL/min, injector temp. 250°C, detector temp. 250°C, programmed temperature: 80°C hold for 2 min, then 1.5°C/min ramp to 140°C, hold for 3 min then 4°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*R*)-**12d**), b) ATA-117/(*R*)-IRED biotransformation of **11d**, c) ATA-117/(*S*)-IRED biotransformation of **11d.**

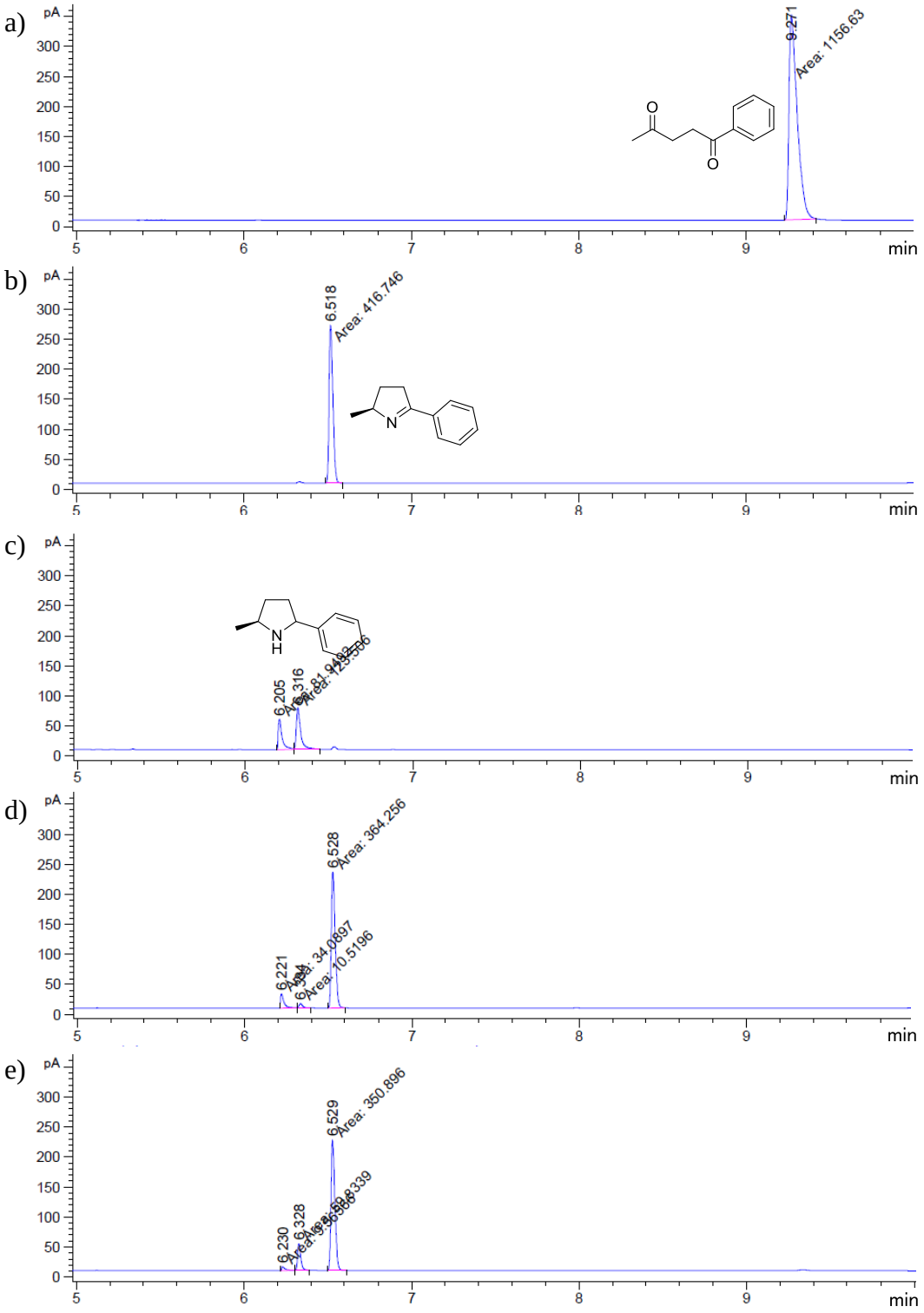


Figure S38: GC-FID analysis of cascade biotransformation of **14** with ATA-113 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*S*)-**15** standard, c) amine **16** standard, d) ATA-113/(*R*)-IRED biotransformation of **14**, e) ATA-113/(*S*)-IRED biotransformation of **14**.

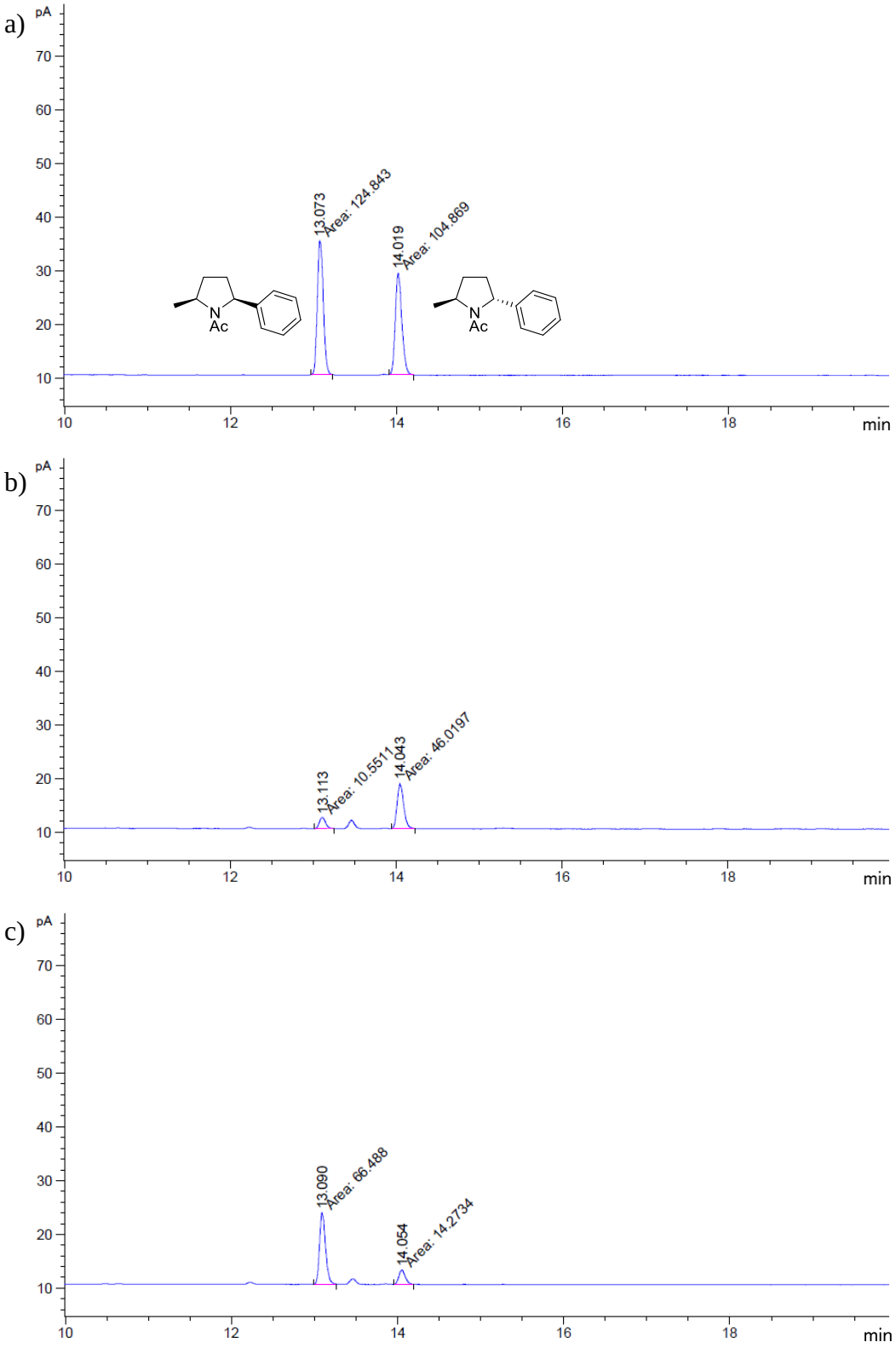


Figure S39: GC-FID analysis of cascade biotransformation of **14** with ATA-113 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*S*)-**15**), b) ATA-113/(*R*)-IRED biotransformation of **14**, c) ATA-113/(*S*)-IRED biotransformation of **14**.

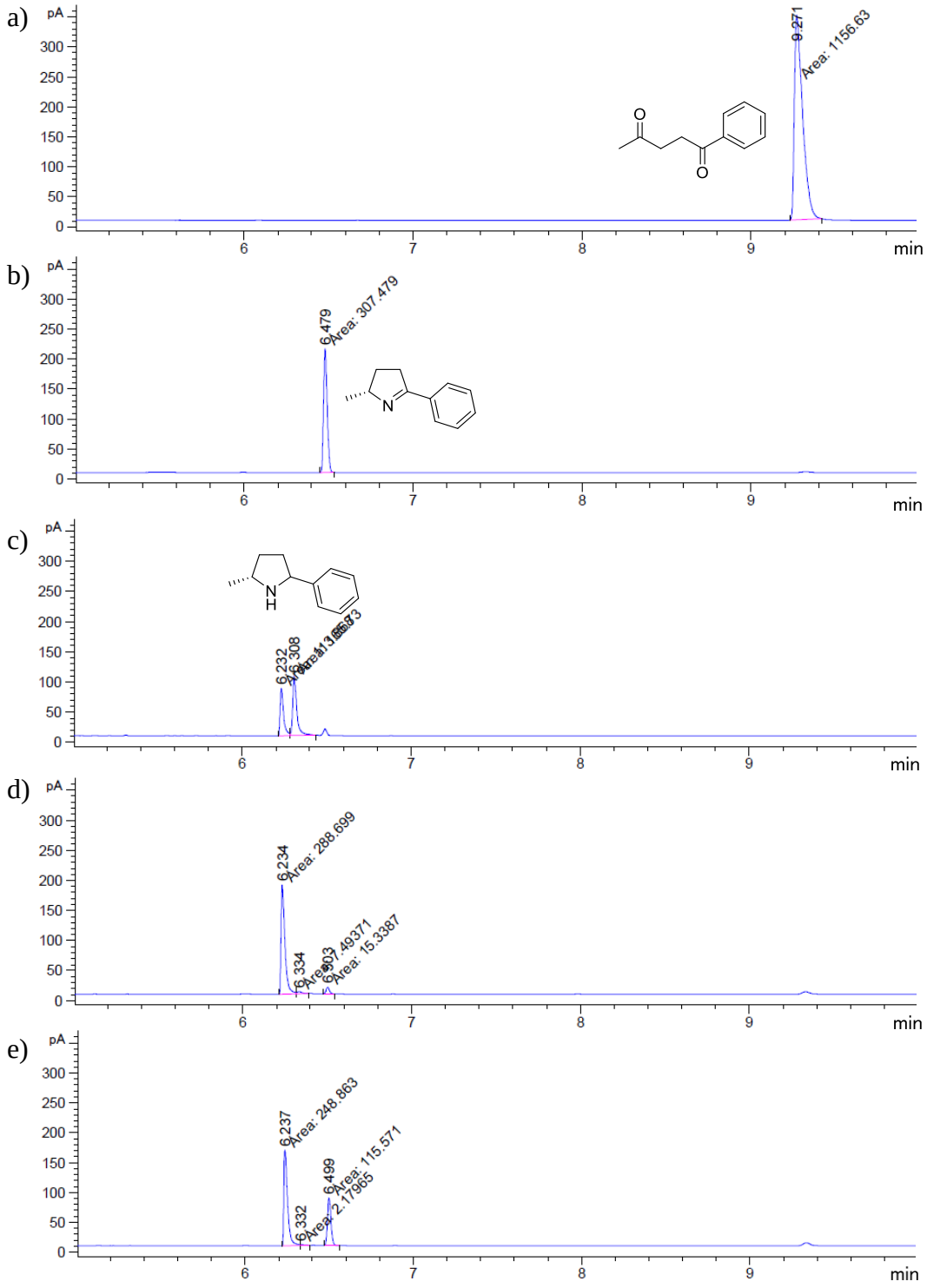


Figure S40: GC-FID analysis of cascade biotransformation of **14** with ATA-117 and IRED to determine conversion. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). a) diketone standard (extracted after basification), b) imine (*R*)-**15** standard, c) amine **16** standard, d) ATA-117/(*R*)-IRED biotransformation of **14**, e) ATA-117/(*S*)-IRED biotransformation of **14**.

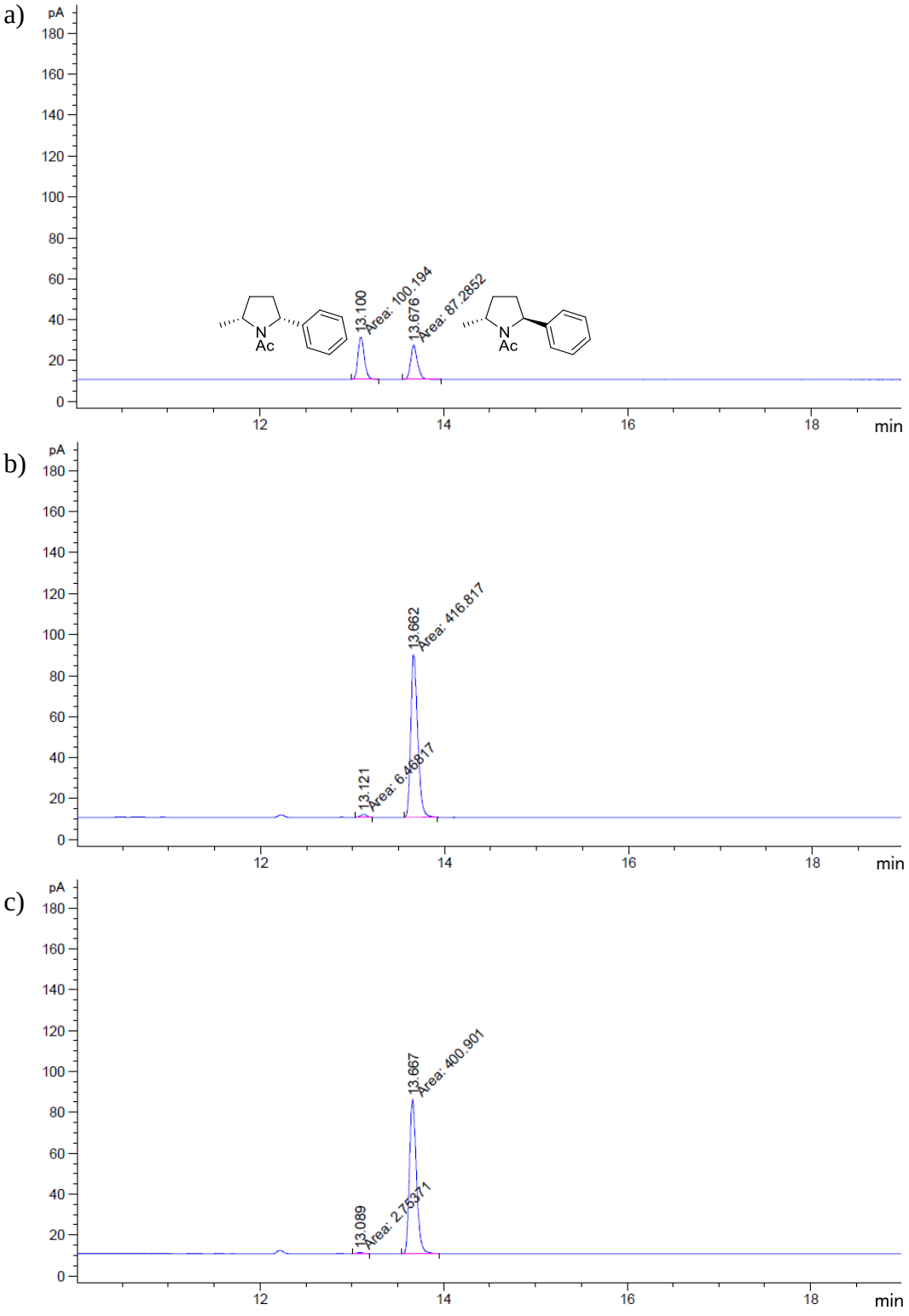
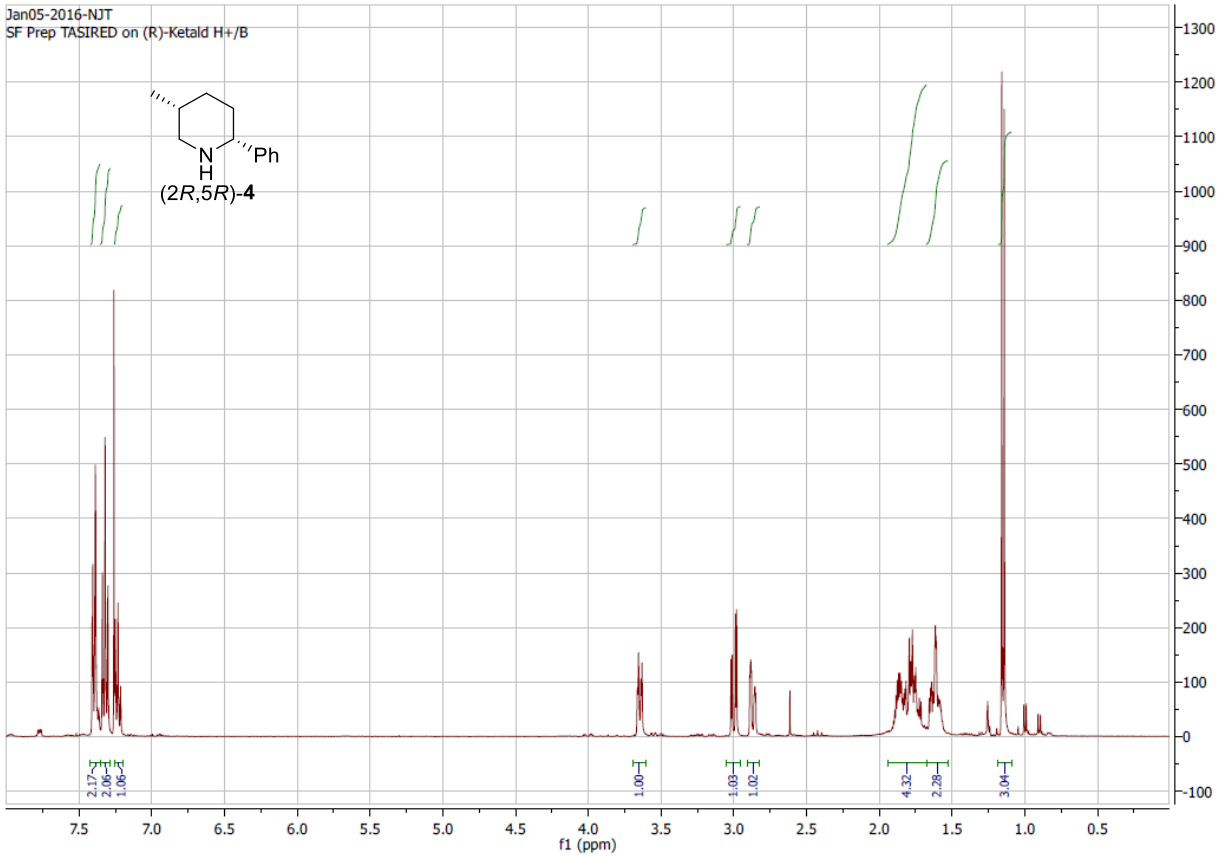
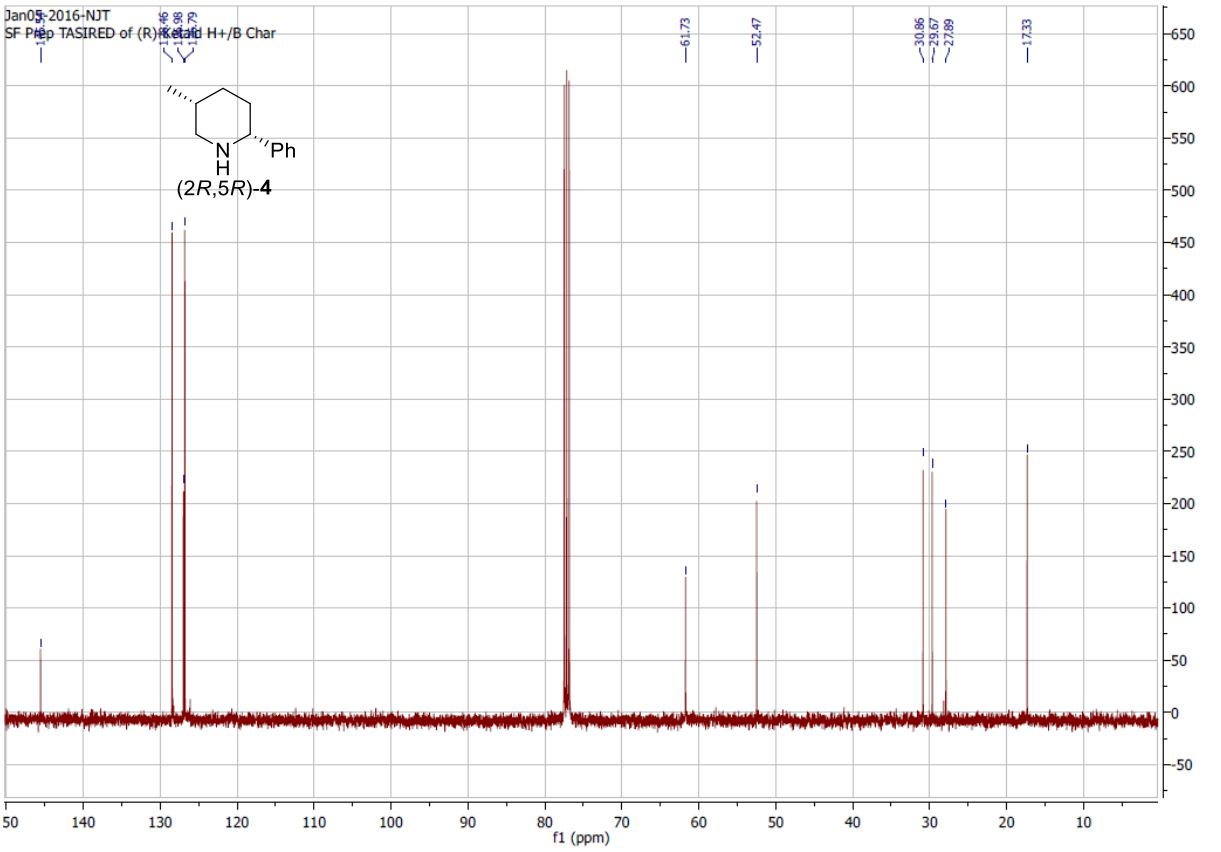
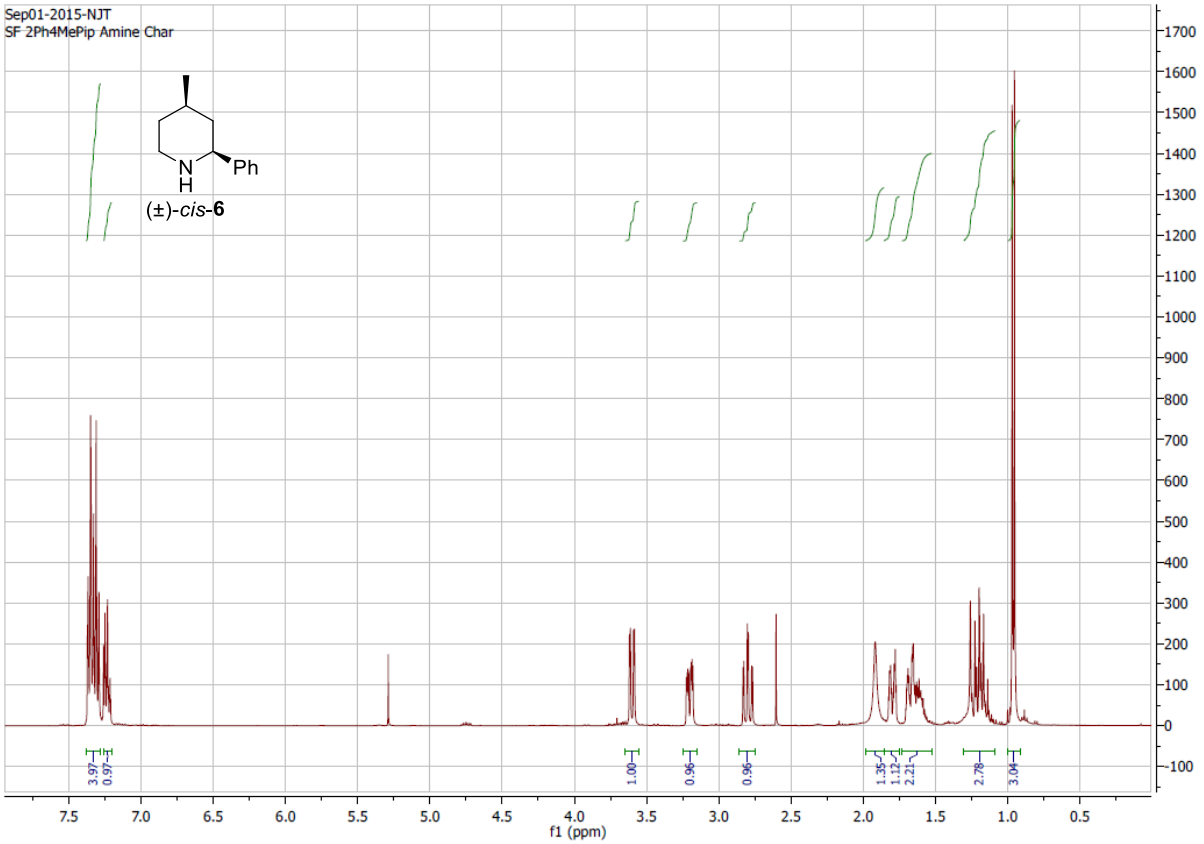


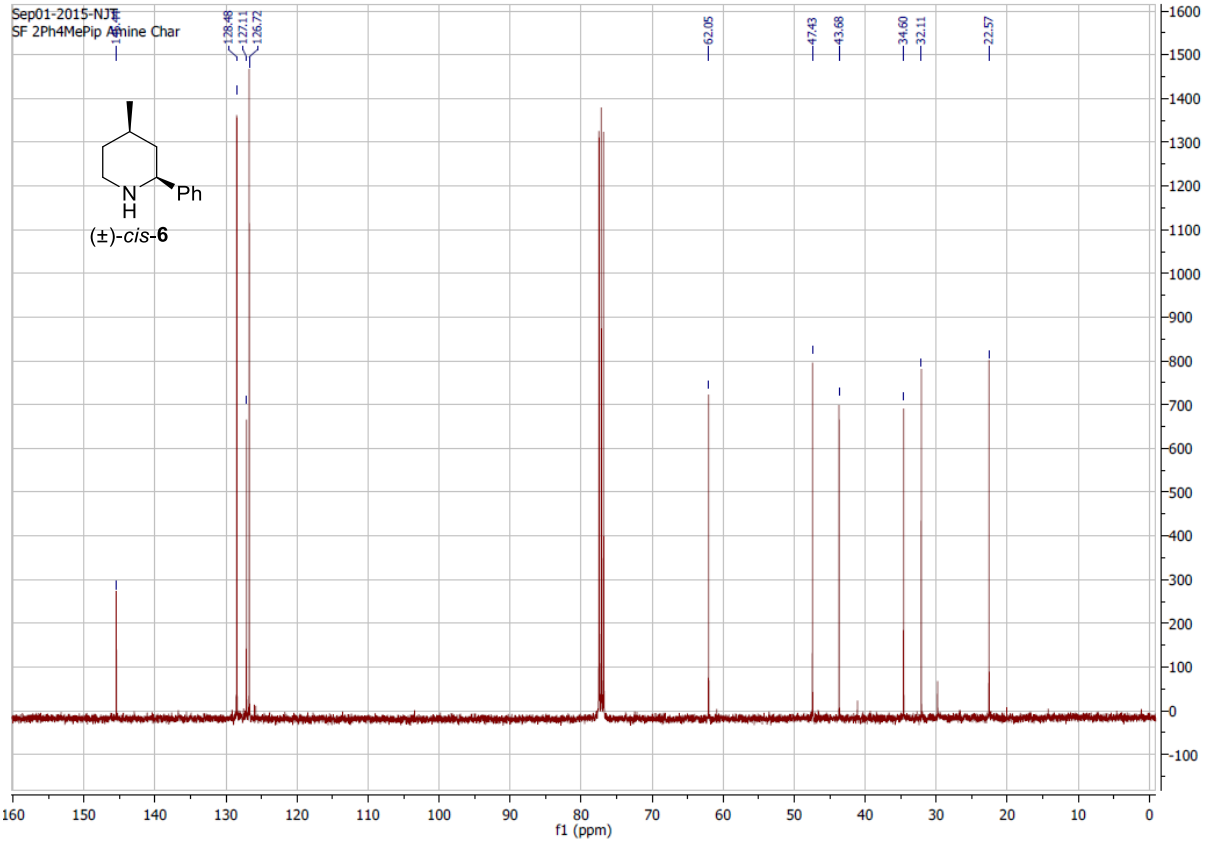
Figure S41: GC-FID analysis of cascade biotransformation of **14** with ATA-117 and IRED to determine *de* and *ee*. (Carrier gas helium, 1.7 mL/min, injector temp. 220°C, detector temp. 250°C, programmed temperature: 100°C hold for 3 min, then 40°C/min ramp to 160°C, hold for 20 min then 20°C/min ramp to 200°C, hold for 2 min). All samples were derivatized with acetic anhydride prior to analysis. a) Amine standard (from $NaBH_4$ reduction of imine (*R*)-**15**), b) ATA-117/(*R*)-IRED biotransformation of **14**, c) ATA-117/(*S*)-IRED biotransformation of **14**.

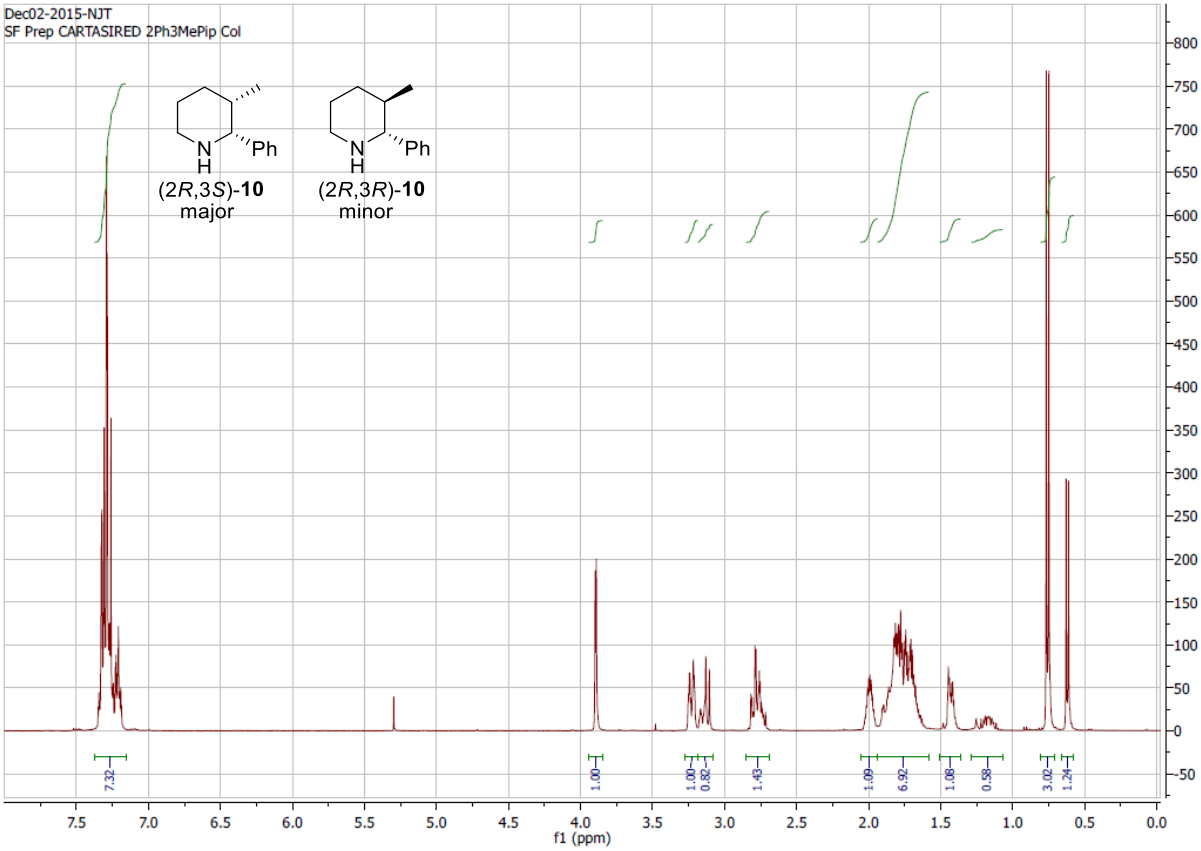
11 NMR of Amine Products From Preparative Biotransformations

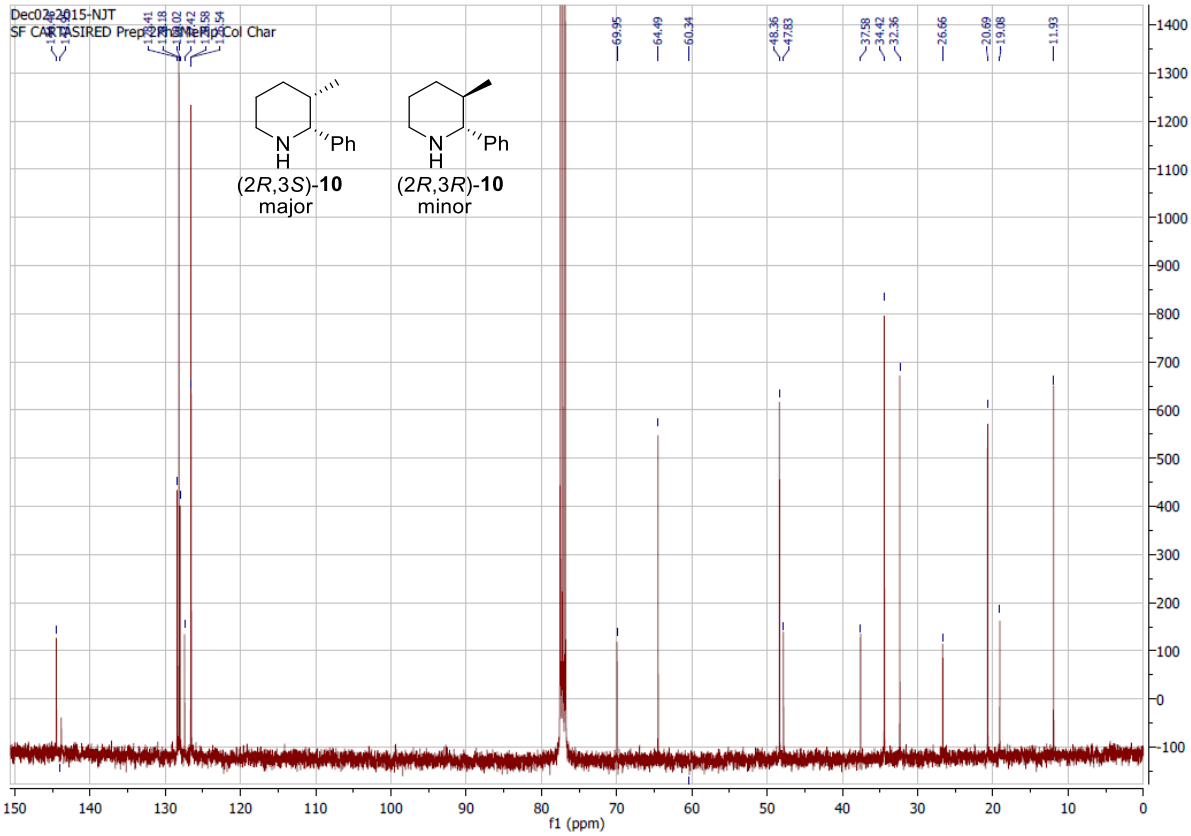


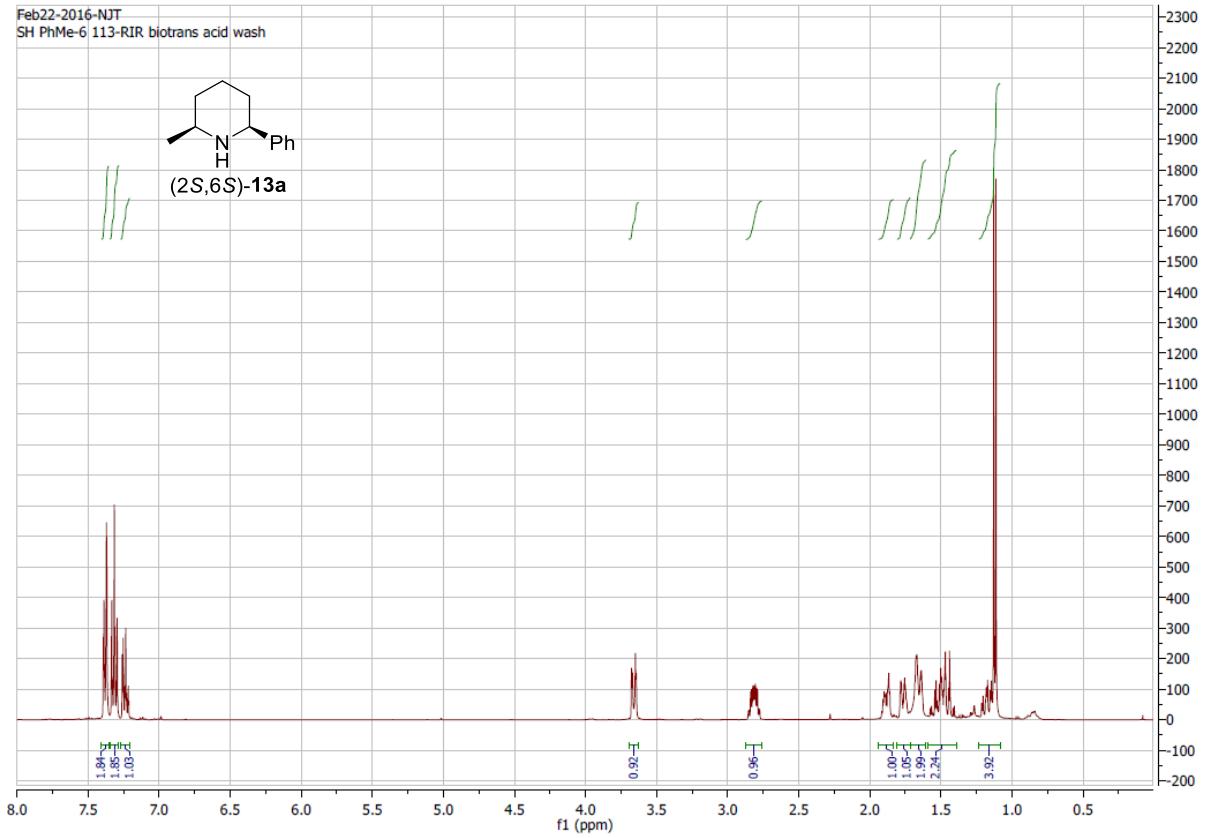


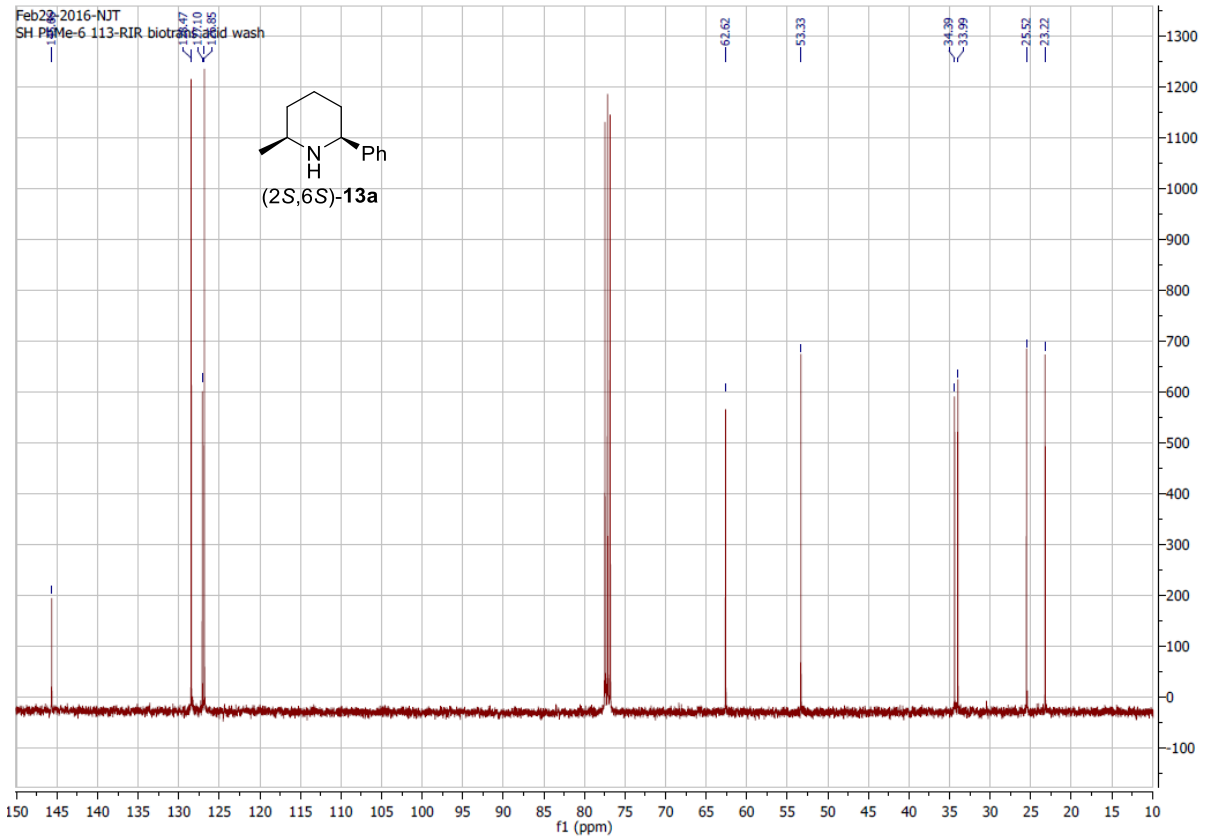

Sep01-2015-NJT
SF 2Ph4MePip Amine Char
Ph
N
H
(±)-cis-6
f1 (ppm)

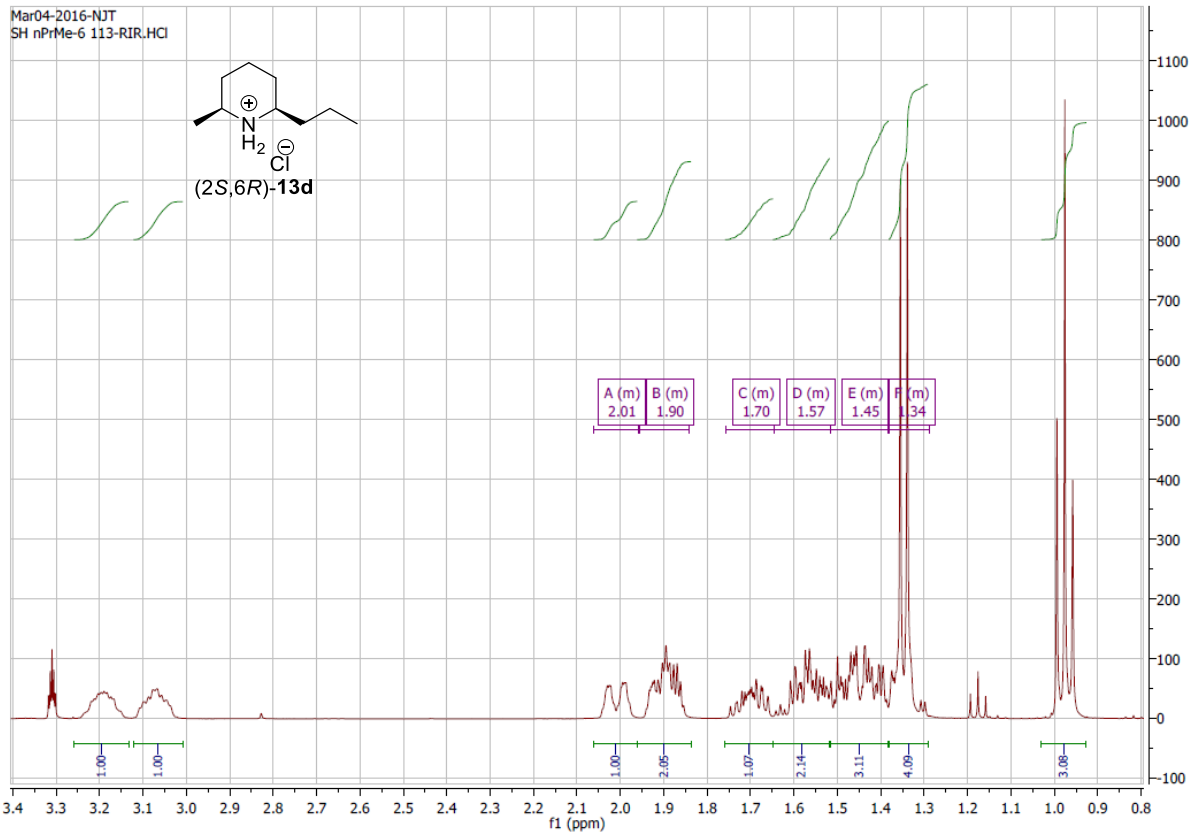

Sep01-2015-NJT
SF 2Ph4MePip Amine Char
Ph
N
H
(±)-cis-6
62.05
47.43
43.68
34.60
32.11
22.57
f1 (ppm)

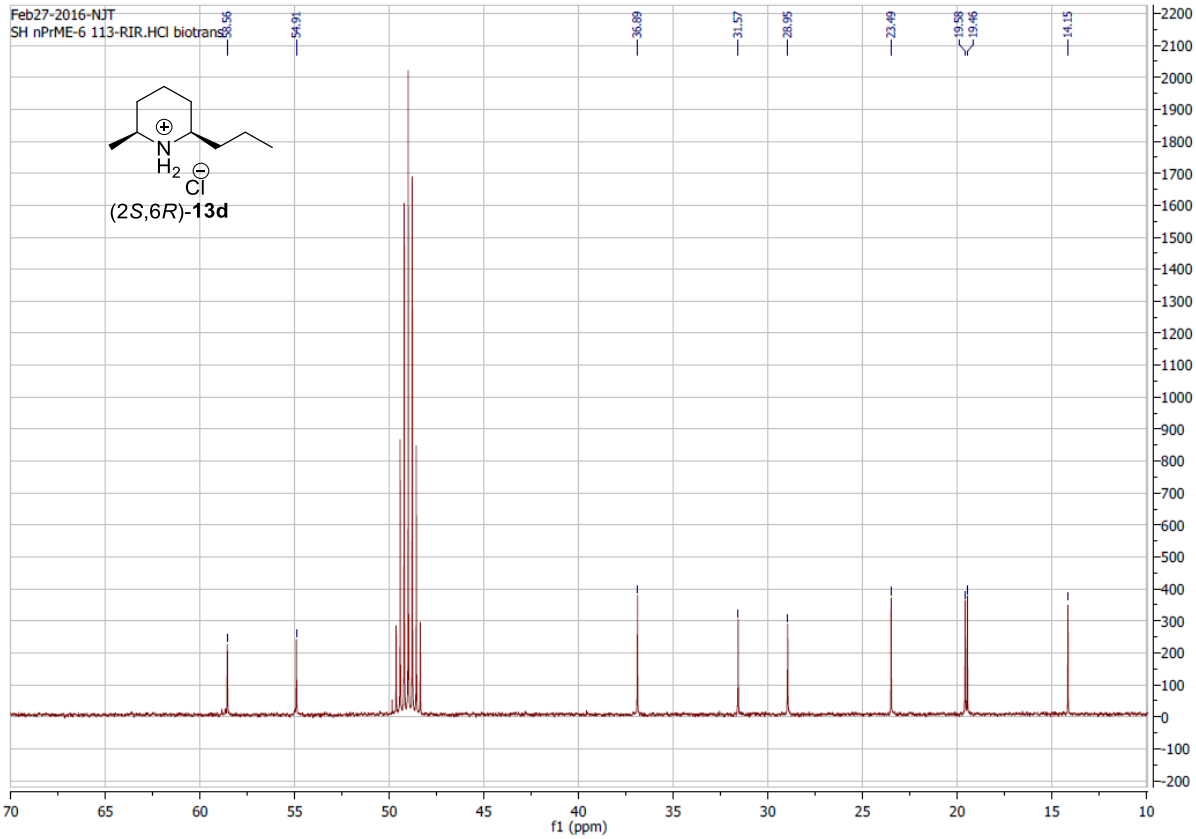

Dec02-2015-NJT
SF Prep CARTASIRED 2Ph3MePip Col
(2R,3S)-10
major
(2R,3R)-10
minor
f1 (ppm)


Dec02-2015-NJT
(2R,3S)-10
major
(2R,3R)-10
minor
f1 (ppm)


Feb22-2016-NJT
SH PhMe-6 113-RIR biotrans acid wash
Ph
N
H
(2S,6S)-13a
f1 (ppm)


Feb22-2016-NJT
SH PhMe-6 113-RIR biotrans acid wash
62.62
53.33
34.39
33.99
25.52
23.22
Ph
N
H
(2S,6S)-13a
f1 (ppm)


Mar04-2016-NJT
SH nPrMe-6 113-RIR.HCl
(2S,6R)-13d
A (m) 2.01
B (m) 1.90
C (m) 1.70
D (m) 1.57
E (m) 1.45
F (m) 1.34
f1 (ppm)


Feb27-2016-NJT
SH nPrME-6 113-RIR.HCl biotrans
58.56
54.91
36.89
31.57
28.95
23.49
19.58
19.46
14.15
(2S,6R)-13d
f1 (ppm)

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