Supplementary Material for

Rapid Directed Evolution of Artificial Metalloenzymes in Whole Cells

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Materials and Methods

1. General Methods

Unless otherwise noted, the chemicals, salts, and solvents used were reagent grade and used as received from commercial suppliers without further purification. Oligonucleotides were obtained from Integrated DNA Technologies. Enzymes and reagents used for cloning were obtained from New England BioLabs and Thermo Fisher Scientific. Mutaflor® was purchased from Pharma-Zentrale GmbH. All expression media and buffers were prepared using ddH_2O (MilliQ A10 Advantage purification system, Millipore). All expression media were sterilized using either an autoclave (30 min, 121 °C) or a sterile syringe filter (0.22 μm). The procedures to prepare Ir(Me)MPIX were reported previously.[1]

2. *E. coli* strains and expression plasmids

Chemically competent BL21(DE3) cells were obtained from New England Biolabs. The plasmids used in this study are listed in the table below. The sequences of CYP119 and outer-membrane transporter were cloned into the respective vectors. Primer sequences are available upon request.

For the ***E. coli* strains** used for the library screening, the pBbS8K plasmid containing hug operon genes were transformed into BL21(DE3) cells. Individual colonies from freshly transformed plates containing 50mg/L Kanamycin were inoculated into 10 mL 2YT media supplemented with 50mg/L Kanamycin for overnight. A 2.5 ml start culture was used to inoculate 1 L of 2YT media, which was then incubated at 37 °C while shaking at 250 rpm for 3 h to an OD of 0.4 to 0.6, followed by shaking occasionally at 4 °C for 45 min. The cells were harvested by centrifuging at 4000 rpm for 10 min and suspended the resulting pellet in 200 ml of ice-cold CCMB80 buffer (10 mM KOAc, 80 mM $CaCl_2.2H_2O$, 20 mM $MnCl_2.4H_2O$, 10 mM $MgCl_2.6H_2O$, 10% glycerol, pH 6.4). The cell mixture was shaking occasionally at 4 °C for 45 min and then centrifuged at 4000 rpm for 10 min. The second cell pellet was suspended in 60 ml ice-cold CCMB80 buffer and further incubated on ice for 20 min before aliquoting into 1.5 mLmicrocentrifuge and stored at -80 °C.

For co-expression of the outer-membrane transporter with CYP 119, the commercial BL21(DE3) from New England Biolabs was used directly.

Name	Marker	Origin	Vector backbone	Genes
CYP119	Amp	ColE1	2BT(Addgene_29666)	CYP119 with T7 promoter
pHug-21	Kan	SC101	pBbS8K	*hug* operon

Sequences of CYP 119 CNH-4B:

MKSSHHHHHHENLYFQSNYDWFSEMRKKDPVYYDGNIWQVFSYRYTKEVLNN
FSKFSSDLTGYHERLEDLRNGKIRFDIPTRYTMLTSDPPLHDELRSMSADIFSPQK
LQTLETFIRETTRSLLDSIDPREDDIVKKLAVPLPIIVISKILGLPIEDKEKFKEWSDL
V**L**FRLGKPGEIFELGKKYLELIGYVKDHLNSGTEVVSRVVNSNLSDIEKLGYIILL
L**F**AGNE**G**TTNLISNSVIDFTRFNLWQRIREENLYLKAIEEALRYSPP**C**M**Y**TVRKTK
ERVKLGDQTIEEGEYVRVWIASANRDEEVFHDGEKFIPDRNPNPHLSFGSGIHL**G**
LGAPLARLEARIAIEEFSKRFRHIEILDTEKVPNEVLNGYKRLVVRLKSNE

3. Preparation of CYP119 mutant library

Combinatorial screening: Libraries generated from combinatorial codon mutagenesis were prepared by a procedure described by Lewis.[2] The CYP119 template was amplified from plasmid **CYPM1**. A library targeting 10 active site residues was prepared with a pool of 10 forward and reverse primers containing degenerate NNK codons at the targeted residues. The mutagenic primers were pooled and used in fragment and joining PCRs followed with Lewis's procedure.[2] The product was purified using agarose gel electrophoresis, and cloned into a 2BT vector using standard Golden Gate assembly methods.

First-round	Primer sequence (5'-3')
insert_for	GCTGTTGGTCTCGAATGAAATCTTCTCACCATCACC
insert_rev	GATGTTGGTCTCAGCGCTCTCATTGCTCTTCAG
69_rev	CGGGTCGCTGGT**MNN**CATGGTATAACG
69_for	CGTTATACCATG**NNK**ACCAGCGACCCG
87_rev	CTTCTGCGGGCT**MNN**GATATCCGCGCTC
87_for	GAGCGCGGATATC**NNK**AGCCCGCAGAAG
152_rev	CCCCAACGAAA**MNN**AACCAGATCGC
152_for	GCGATCTGGTT**NNK**TTTCGTTGGGG
153_rev	GGTTTACCCCAACG**MNN**CGCAACCAGATCG
153_for	CGATCTGGTTGCG**NNK**CGTTGGGGTAAACC
205_rev	CCGCAATCAGCAG**MNN**AATGATGTAACCCA
205_for	TGGGTTACATCATT**NNK**CTGCTGATTGCGG
209_for	GCTGCTGATT**NNK**GGCAACGAAGG
209_rev	CCTTCGTTGCC**MNN**AATCAGCAGC
254_rev	AACGGTACGCAT**MNN**CGGCGGGCTATA
254_for	TATAGCCCGCCG**NNK**ATGCGTACCGTT
282_for	CGTGTTTGG**NNK**GCGAGCGCGAACC
282_rev	GGTTCGCGCTCGC**MNN**CCAAACACG
310_for	GCACCTGAGC**NNK**GGTAGCGGCAT
310_rev	ATGCCGCTACC**MNN**GCTCAGGTGC
353_for	AGGTTCCGAATGAA**NNK**CTGAATGGTTACAAGCGT
353_rev	ACGCTTGTAACCATTCAG**MNN**TTCATTCGGAACCT

Targeted screening: Site-directed mutagenesis was used to introduce mutations into the selected, most selective mutant identified from the previous round of screening. Phusion High-Fidelity DNA polymerase was used to amplify the parent plasmid with primers containing mutations at target sites. After DpnI digestion, the PCR product was purified, concentrated and transformed into XL-1 blue cell.

	Primer sequence (5'-3')
152_153_155 NNK For	AGCGATCTGGTT**NNKNNK**CGT**NNK**GGTAAACCG
152_153_155 NNK-Rev	CGGTTTACC**MNN**ACG**MNNMNN**AACCAGATCGCT

208_F_For	CTGCTGCTGTTTGCGGGCAAC
208_F_Rev	GTTGCCCGCAAACAGCAGCAG
208_L_For	CTGCTGCTGCTTGCGGGCAAC
208_L_Rev	GTTGCCCGCAAGCAGCAGCAG
208_F_For	CTGCTGCTGTTTGCGGGCAAC
208_F_Rev	GTTGCCCGCAAACAGCAGCAG
210_A_For	CTGATTGCGGCCAACGAAGGC
210_A_Rev	GCCTTCGTTGGCCGCAATCAG
210_L_For	CTGATTGCGCTCAACGAAGGC
210_L_Rev	GCCTTCGTTGAGCGCAATCAG
210_F_For	CTGATTGCGTTCAACGAAGGC
210_F_Rev	GCCTTCGTTGAACGCAATCAG
254_L_For	CCGCCGCTGATGCGTACC
254_L_Rev	GGTACGCATCAGCGGCGG
254_F_For	CCGCCGTTTATGCGTACCG
254_F_Rev	CGGTACGCATAAACGGCGG
256_A_For	CCGGCGATGGCTACCGTTCGT
256_A_Rev	ACGAACGGTAGCCATCGCCGG
256_L_For	CCGGCGATGGTTACCGTTCGT
256_L_Rev	ACGAACGGTAACCATCGCCGG
256_F_For	CCGGCGATGTTTACCGTTCGT
256_F_Rev	ACGAACGGTAAACATCGCCGG
353_A_For	GTTCCGAATGAAATTCTGAATGGTTACAAG
353_A_Rev	CTTGTAACCATTCAGAATTTCATTCGGAAC
353_L_For	GTTCCGAATGAACTTCTGAATGGTTACAAG
353_L_Rev	CTTGTAACCATTCAGAAGTTCATTCGGAAC
353_F_For	GTTCCGAATGAATTTCTGAATGGTTACAAG
353_F_Rev	CTTGTAACCATTCAGAAATTCATTCGGAAC
208_Y_For	CTGCTGCTGTATGCGGGCAAC
208_Y_Rev	GTTGCCCGCATACAGCAGCAG
208_W_For	CTGCTGCTGTGGGCGGGCAACG
208_W_Rev	CGTTGCCCGCCCACAGCAGCAG
256_Y_For	CCGGCGATGTATACCGTTCGT
256_Y_Rev	ACGAACGGTATACATCGCCGG
256_W_For	CCGGCGATGTGGACCGTTCGTA
256_W_Rev	TACGAACGGTCCACATCGCCGG
254_NNK_For	AGCCCGCCGNNKATGTATACC
254_NNK_Rev	GGTATACATMNNCGGCGGGCT

4. Media Preparation

Preparation of M9-rich media: Salts (47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 1 g/L NH_4Cl) were dissolved in 1 L ddH_2O and autoclaved to give a medium with pH ~7.

Solutions of glucose (20 w/w%), casamino acids (20 w/w%), $MgSO_4$ (1 M), antibiotics, and $CaCl_2$ (1 M) were sterilized by filtration. 20 mL glucose, 10 mL casamino acids, 2 mL $MgSO_4$, 100 μL $CaCl_2$ were added per liter of sterilized M9 salt solution.

Preparation of M9-N reaction buffer: Salts (47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl) were dissolved in 1 L ddH_2O and autoclaved to give a medium with pH ~7.4. Solutions of $MgSO_4$ (1 M), $CaCl_2$ (1 M) and glucose (20 w/w%) were added to give a final concentration of 2.0 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.8 w/w% glucose.

Preparation of NaPi (pH = 6) reaction buffer: the salts (1.7 g Na_2HPO_4, 12.3 g NaH_2PO_4, 5.85 g NaCl) were dissolved in 1 L ddH_2O and autoclaved to give a medium with pH ~6.0.

5. *In vivo* Expression of Ir-CYP119 in *E. coli* containing hug operon genes for 96-well plates screening

A 2BT plasmid containing the CYP119 gene was transformed into $CaCl_2$ competent BL-21(DE) cells containing a plasmid containing the *hug* operon from *Plesiomonas shigelloides*.[3] Individual colonies from freshly transformed plates were used to inoculate 96-well plates containing 1 mL M9-rich media supplemented with 100 μg/mL ampicillin sodium and 25 ug/ml kanamycin sulfate. The cultures were grown at 37 °C while shaking at 250 rpm for 18 h. The overnight incubated 96-well plate was added 0.3 mL 50% glycerol and stored at - 80 ℃ freezer for further amplification improved mutants. In a new 96-well plate, 1 mL of fresh M9-rich medium was inoculated with 10 μL of this starter culture and incubated at 37 °C and 250 rpm to an OD_{600} of 0.6~0.8. The cultures were then induced by adding a final concentration of 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.1 nM Ir(Me)MPIX (3 μL of 0.033 mM stock solution in DMSO) and further grown at 30 ºC for 18 h with 250 rpm. After harvesting the cells by centrifugation (4 °C, 10 min, 4000 rpm), the cell pellets were resuspended in 200 μL M9-N reaction buffer and used to screen for catalytic activity and selectivity, as described in the following sections.

6. In vivo expression of Ir-CYP119 in *E. coli* BL21(DE3) with co-expression of the outer-membrane transporter

A plasmid containing CYP119 genes was co-transformed with a plasmid encoding the corresponding hug operon into chemically competent BL21(DE3) cells (NEB). Individual colonies from freshly transformed plates were used to inoculate 4 mL of M9-rich media supplemented with 100 μg/mL carbenicillin and 25 μg/mL kanamycin. The cultures were grown at 37 °C while shaking at 250 rpm for 18 h. In a 2 L round bottom flask, 500 mL of M9-rich medium was inoculated with 4 mL of this starter culture and incubated at 37 °C while shaking at 250 rpm to an OD_{600} of 0.6~0.8. The cultures were then induced by adding final concentration of 1 mM IPTG and 0.1 μM Ir(Me)MPIX (17 μL of 3 mM stock solution in DMSO) and was then incubated at 30 ºC for 18 h while shaking at 250 rpm. After harvesting the cells by centrifugation (4 °C, 10 min, 4000 rpm), the cell pellets were resuspended in 4 mL NaPi reaction buffer and dried under normal freeze and dry conditions.

7. Whole-cell Reactions

Whole-cell reactions in glass vials. The cell suspension in M9-N medium (250 μL, OD_{600} ~50) was transferred into a 4 mL screw-capped glass vial, and 2.5 μmol substrate (5 μL of

0.5 M stock solution in DMSO) were added. The vials were capped and shaken at 30 °C and 250 rpm for 1 h. Me-EDA (5 µl of 0.5 M stock solution in DMSO) was added in four batches over 4 hours, and the reaction mixture was shaken at 30 °C for an addition 4 h. The reaction was quenched by adding 300 µL of a 1:1 ratio of ethyl acetate and hexane (containing 0.1 v/v% *N,N*-diethylaniline as internal standard). The mixture was vortexed for 10 s, transferred to a 1.7 mL microcentrifuge tube, and centrifuged at 10 000 rpm for 4 min. After separation of the layers, the organic layer was transferred to a separate vial and analyzed by GC or HPLC.

Whole-cell screening in 96-well plates: To each well of a 96-well plate, *N*-Me aniline (5 µL, 0.5 M in DMSO) was added using a multi-channel pipette. The plate was then sealed with an aluminum film and shaken at 30 °C and 250 rpm for 1 h. The Me-EDA (5 ul*4 of 0.5 M stock solution in DMSO) was added in four batches over 4 h, after which time the reaction mixture was shaken at 30 °C for an addition 4 h. The reaction was quenched by adding 300 µL of a 1:1 ratio of ethyl acetate and hexane (containing 0.1 v/v% *N,N*-diethylaniline as internal standard). The 96-well plate was shaken for 10 min at 300 rpm, and centrifuged at 4000 rpm for 10 min. After freezing the plate at -20 °C overnight, the organic layer in each well of the 96-well plate was transferred to a separate vial and analyzed by GC or HPLC.

If any of the mutants in the library catalyzed the reaction of **1a** with Me-EDA with higher selectivity than any of the prior mutants, new LB plates were inoculated from the corresponding well of the 96-well plate and then expressed on large scale following the methods of Section 8. Only the mutants that reacted with higher er in vivo and in vitro were amplified from the separate cell culture and purified using a Qiagen DNA Miniprep kit (Cat No./ID: 27106) according to the manufacturer's instructions. After sequencing the plasmid, the more selective mutant was used for the next round of evolution.

8. Reactions Catalyzed by Ir-CYP119 Reconstituted *in vitro*

Apo-CYP119 was expressed and purified as described earlier.[1] The apo-protein and Ir(Me)MPIX cofactor were combined in a 10 : 1 molar ratio. The buffer of the protein solution was subsequently exchanged to TRIS buffer (20 mM, pH 7.5) using a NAP-10 column (GE Healthcare). In a 4 ml glass vial, 5 µL of a 0.5 M stock solution of substrate in DMSO was added to 0.5 mL of catalyst stock solution. The vials were capped and shaken at 30 °C and 250 rpm for 1 h. Me-EDA (5 ul*4 of 0.5 M stock solution in DMSO) was added in four batches over 4 h, after which time, the reaction mixture was shaken at 30 °C for an additional 4 h. The reaction was quenched by adding 300 µL of a 1:1 ratio of ethyl acetate and hexane (containing 0.1 v/v% *N,N*-diethylaniline as internal standard). The mixture was vortexed for 10 s, transferred to a 1.7 mL microcentrifuge tube, and centrifuged at 10 000 rpm for 4 min. After separation of the layers, the organic layer was transferred to a separate vial and analyzed by GC or HPLC

9. Organic Synthesis and Characterization

General methods and materials. Unless stated otherwise, all reactions and manipulations were conducted on the laboratory bench in air with reagent grade solvents. Reactions under inert gas atmosphere were conducted with oven dried glassware in a nitrogen-filled glove

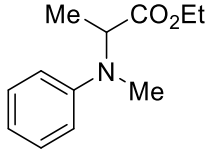
box or by standard Schlenk techniques under nitrogen. Unless noted otherwise, all reagents and solvents were purchased from commercial suppliers and used without further purification. Dry MeCN was purchased from commercial suppliers and used as received. All *N*-Me aniline derivatives were commercially available. Me-EDA was synthesized following the literature report.

NMR spectra were acquired on 400 MHz, Bruker instruments at the University of California, Berkeley. NMR spectra were processed with MestReNova 9.0 (Mestrelab Research SL). Chemical shifts are reported in ppm and referenced to residual solvent peaks. Coupling constants are reported in hertz. Chiral HPLC analysis was conducted on an Agilent 1260 Infinity II Prime LC. GC analyses was obtained on an Agilent 6890 GC equipped with a chiral colunm and an FID detector. HPLC yields and enantioselectivity were calculated using *N,N*-diethylaniline as the internal standard. High-resolution mass spectra were obtained via PerkinElmer AxION II in LBNL catalysis laboratory, Berkeley.

General procedure for the synthesis of authentic products

N-Me aniline (0.5 mmol) and ethyl 2-bromopropanoate (0.6 mmol) in dry MeCN, K_2CO_3 (0.6 mmol) was added to the reaction mixture. The reaction mixture was vigorously stirred at 80 °C for 12 h and cooled to room temperature before adding 20 mL water to quench the reaction. Ethyl acetate (20 ml*3) was added to the reaction mixture, and the combined organic phase was dried with Na_2SO_4. The residue was concentrated under vacuum and purified by silica gel chromatography with EA/hexane (the ratio listed in each substrate) as the elution solvent.

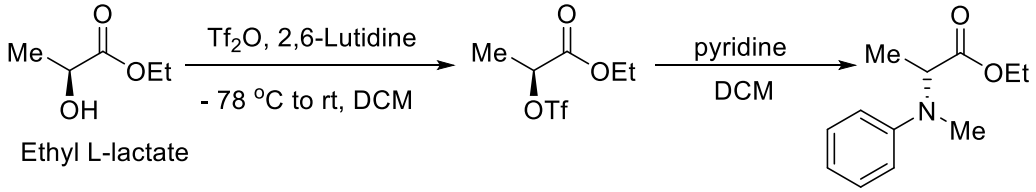
Ethyl N-methyl-N-phenylalaninate (**3a**)[4]



Following the general protocol, a colorless oil (64 mg, 62% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.

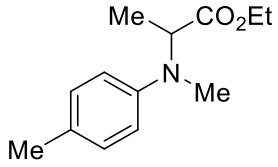
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (t, *J* = 7.1 Hz, 2H), 6.74 (t, *J* = 6.8 Hz, 1H), 6.62 (d, *J* = 7.0 Hz, 2H), 4.22 – 4.11 (m, 3H), 1.47 (d, *J* = 6.5 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.64, 146.60, 129.34, 118.31, 113.45, 61.16, 52.06, 18.96, 14.23.

The synthesis of (R)-enantiomer of **3a**



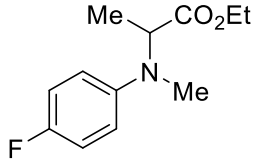
The synthesis of the (*R*)-enantiomer of **3a** was conducted by literature protocols.[5]

Ethyl N-methyl-N-(p-tolyl) alaninate (**3b**)[6]



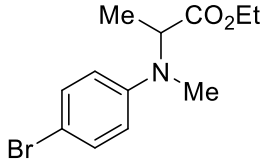
Following the general protocol, a colorless oil (64 mg, 58% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, J = 8.3 Hz, 2H), 6.73 (d, J = 8.5 Hz, 2H), 4.45 (q, J = 7.1 Hz, 1H), 4.22 – 4.09 (m, 2H), 2.87 (s, 3H), 2.25 (s, 3H), 1.45 (d, J = 7.1 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.57, 147.76, 129.78, 127.15, 114.10, 60.85, 57.72, 33.30, 20.40, 15.23, 14.41.

Ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**)[6]



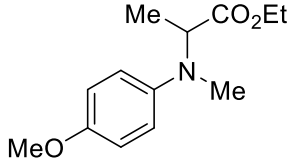
Following the general protocol, a colorless oil (69 mg, 62% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (t, J = 8.7 Hz, 2H), 6.78 (s, 2H), 4.39 (q, J = 7.1 Hz, 1H), 4.22 – 4.10 (m, 2H), 2.86 (s, 3H), 1.46 (d, J = 7.1 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.16, 157.24, 154.88, 146.46, 115.57, 115.35, 115.29, 115.22, 60.81, 58.17, 33.51, 15.23, 14.27.

Ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**)[6]



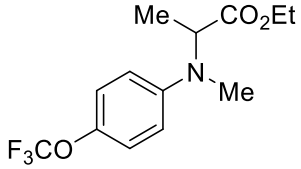
Following the general protocol, white solid (76 mg, 53% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 9.0 Hz, 2H), 6.65 (d, J = 9.0 Hz, 2H), 4.43 (q, J = 7.1 Hz, 1H), 4.22 – 4.09 (m, 2H), 2.86 (s, 3H), 1.47 (d, J = 7.1 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.07, 148.86, 131.92, 115.12, 109.64, 61.07, 57.22, 33.15, 15.38, 14.38.

Ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**)



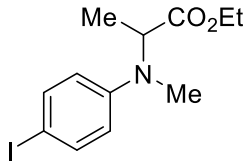
Following the general protocol, light yellow oil (85 mg, 72% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.
^{1}H NMR (400 MHz, CDCl3) δ 6.82 (s, 4H), 4.35 (q, J = 7.1 Hz, 1H), 4.21 – 4.07 (m, 2H), 3.76 (s, 3H), 2.85 (s, 3H), 1.44 (d, J = 7.1 Hz, 1H), 1.22 (t, J = 7.1 Hz, 1H); ^{13}C NMR (101 MHz, CDCl3) δ 173.31, 152.50, 144.32, 116.14, 114.46, 60.56, 58.58, 55.53, 33.65, 15.05, 14.22.
HR MS (ESI): calcd. for $C_{13}H_{20}NO_3$ $[M+H]^+$: 238.1443, found: 238.1448.

Ethyl N-methyl-N-(4-(trifluoromethoxy)phenyl)alaninate (**3f**)[6]



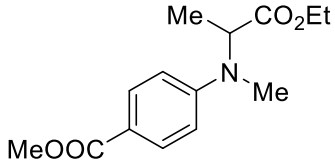
Following the general protocol, a colorless oil (80 mg, 55% yield) was obtained with EA/hexane (4:1 to 3:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 8.5 Hz, 2H), 6.74 (d, J = 8.5 Hz, 2H), 4.50 – 4.40 (m, 1H), 4.16 (dd, J = 6.8, 3.7 Hz, 2H), 2.89 (s, 3H), 1.48 (d, J = 6.9 Hz, 3H), 1.23 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.08, 148.59, 140.75, 122.25, 122.11, 119.57, 113.97, 61.10, 57.46, 33.35, 15.44, 14.37.

Ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**)



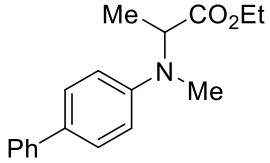
Following the general protocol, white solid (70 mg, 42% yield) was obtained with EA/hexane (4:1 to 3:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 9.0 Hz, 2H), 6.56 (d, J = 9.0 Hz, 2H), 4.43 (q, J = 7.1 Hz, 1H), 4.20 – 4.10 (m, 2H), 2.86 (s, 3H), 1.47 (d, J = 7.1 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 172.93, 149.31, 137.72, 115.58, 78.74, 61.00, 56.94, 32.95, 15.28, 14.29.
HR MS (ESI): calcd. for $C_{12}H_{17}INO_2$ $[M+H]^+$: 334.0304, found: 334.0315.

Ethyl N-(4-acetoxyphenyl)-N-methylalaninate (**3h**)



Following the general protocol, yellow oil (89 mg, 67% yield) was obtained with EA/hexane (2:1 to 1:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 9.0 Hz, 2H), 6.72 (d, J = 9.0 Hz, 2H), 4.58 (q, J = 7.1 Hz, 1H), 4.23 – 4.09 (m, 2H), 3.85 (s, 3H), 2.95 (s, 3H), 1.50 (d, J = 7.1 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.59, 167.28, 152.85, 131.33, 118.28, 111.56, 61.18, 56.43, 51.59, 33.06, 15.37, 14.25.
HR MS (ESI): calcd. for $C_{14}H_{19}NNaO_4$ $[M+Na]^+$: 288.1212, found: 288.1227.

Ethyl N-([1,1'-biphenyl]-4-yl)-N-methylalaninate (**3i**)

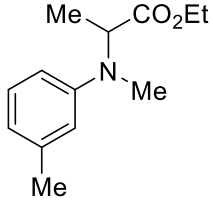


Following the general protocol, yellow oil (81 mg, 57% yield) was obtained with EA/hexane (4:1 to 3:1) as the eluent.

^{1}H NMR (400 MHz, CDCl3) δ 7.59 – 7.49 (m, 4H), 7.40 (t, J = 7.7 Hz, 2H), 7.29 (d, J = 7.2 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 4.56 – 4.51 (m, 1H), 4.22 – 4.14 (m, 2H), 3.01 (s, 3H), 1.54 (d, J = 7.1 Hz, 3H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 173.32, 149.13, 141.14, 130.40, 128.77, 127.89, 126.46, 126.26, 113.65, 61.02, 57.15, 33.18, 15.39, 14.41.

HR MS (ESI): calcd. for $C_{18}H_{22}NO_2$ $[M+H]^+$: 284.1651, found: 284.1643.

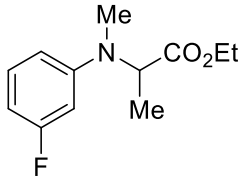
Ethyl N-methyl-N-(m-tolyl)alaninate (**3j**)



Following the general protocol, yellow oil (49 mg, 44% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (t, J = 8.0 Hz, 1H), 6.66 – 6.59 (m, 3H), 4.53 (q, J = 7.1 Hz, 1H), 4.26 – 4.12 (m, 2H), 2.90 (s, 3H), 2.34 (s, 3H), 1.49 (d, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H);^{13}C NMR (101 MHz, $CDCl_3$) δ 173.45, 149.81, 138.84, 129.04, 118.56, 114.25, 110.63, 60.82, 57.12, 32.99, 21.95, 15.22, 14.32.

Ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**) [6]

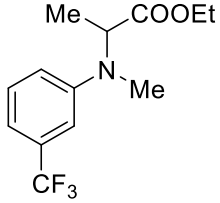


Following the general protocol, yellow oil (48 mg, 43% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, J = 15.8, 7.8 Hz, 1H), 6.53 (d, J = 7.8 Hz, 1H), 6.45 (t, J = 15.8 Hz, 2H), 4.46 (q, J = 7.1 Hz, 1H), 4.23 – 4.10 (m, 2H), 2.88 (s, 3H), 1.48 (d, J = 7.1 Hz, 3H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.04, 164.17 (d, J_{C-F} = 121 Hz), 151.56, 151.45, 130.32, 130.21, 108.79, 104.17, 103.95, 100.54, 100.28, 61.12, 57.08, 33.18, 15.42, 14.38.

HR MS (ESI): calcd. for $C_{12}H_{17}FNO_2$ $[M+H]^+$: 226.1243, found: 226.1247.

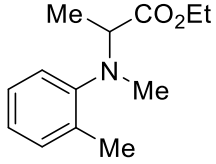
Ethyl N-methyl-N-(3-(trifluoromethyl)phenyl)alaninate (**3l**)



Following the general protocol, yellow oil (52 mg, 38% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.

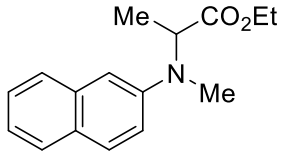
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.3 Hz, 1H), 6.97 (s, 2H), 6.92 (d, *J* = 7.3 Hz, 1H), 4.51 (q, *J* = 7.1 Hz, 1H), 4.25 – 4.10 (m, 2H), 2.93 (s, 3H), 1.51 (d, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H);, 1H), 124.43 (q, *J* = 272.5 Hz, -3H), 113.89 (dd, *J* = 7.7, 3.8 Hz, 5H), 109.55 (dd, *J* = 7.8, 3.9 Hz, 5H).^{13}C NMR (101 MHz, $CDCl_3$) δ 172.77, 149.81, δ 131.45 (q, *J* = 31.4 Hz), 129.55, 124.43 (q, *J* = 272.5 Hz), 116.10, 113.89 (q, *J* =3.8 Hz), 109.55 (q, *J* = 3.9 Hz), 61.06, 56.94, 33.04, 15.32, 14.22.
HR MS (ESI): calcd. for $C_{13}H_{17}F_3NO_2$ $[M+H]^+$: 276.1211, found: 276.1210.

Ethyl N-methyl-N-(o-tolyl)alaninate (**3m**)



Following the general protocol, yellow oil (35 mg, 32% yield) was obtained with EA/hexane (5:1 to 4:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 – 7.08 (m, 3H), 6.96 (t, J = 7.1 Hz, 1H), 4.13 (p, J = 10.8 Hz, 2H), 3.81 (q, J = 7.1 Hz, 1H), 2.77 (s, 3H), 2.31 (s, 3H), 1.39 (d, J = 7.1 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.47, 151.11, 133.14, 131.25, 126.26, 123.33, 121.97, 60.55, 60.49, 35.52, 18.46, 14.91, 14.42.
HR MS (ESI): calcd. for $C_{13}H_{20}NO_2$ $[M+H]^+$: 222.1494, found: 222.1505.

Ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**)



Following the general protocol, yellow oil (53 mg, 41% yield) was obtained with EA/hexane (4:1 to 3:1) as the eluent.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.65 (m, 3H), 7.39 (t, J = 7.5 Hz, 1H), 7.26 – 7.18 (m, 2H), 7.03 (s, 1H), 4.66 (q, J = 7.1 Hz, 1H), 4.25 – 4.12 (m, 2H), 3.01 (s, 3H), 1.54 (d, J = 7.1 Hz, 3H), 1.24 (t, J = 7.1 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.22, 147.63, 134.87, 128.84, 127.42, 126.43, 126.28, 122.57, 116.87, 108.04, 60.91, 57.61, 33.26, 15.24, 14.32
HR MS (ESI): calcd. for $C_{16}H_{20}NO_2$ $[M+H]^+$: 258.1494, found: 258.1500.

Figure S1a. Results from whole cell N-H insertions with additional diazo compounds

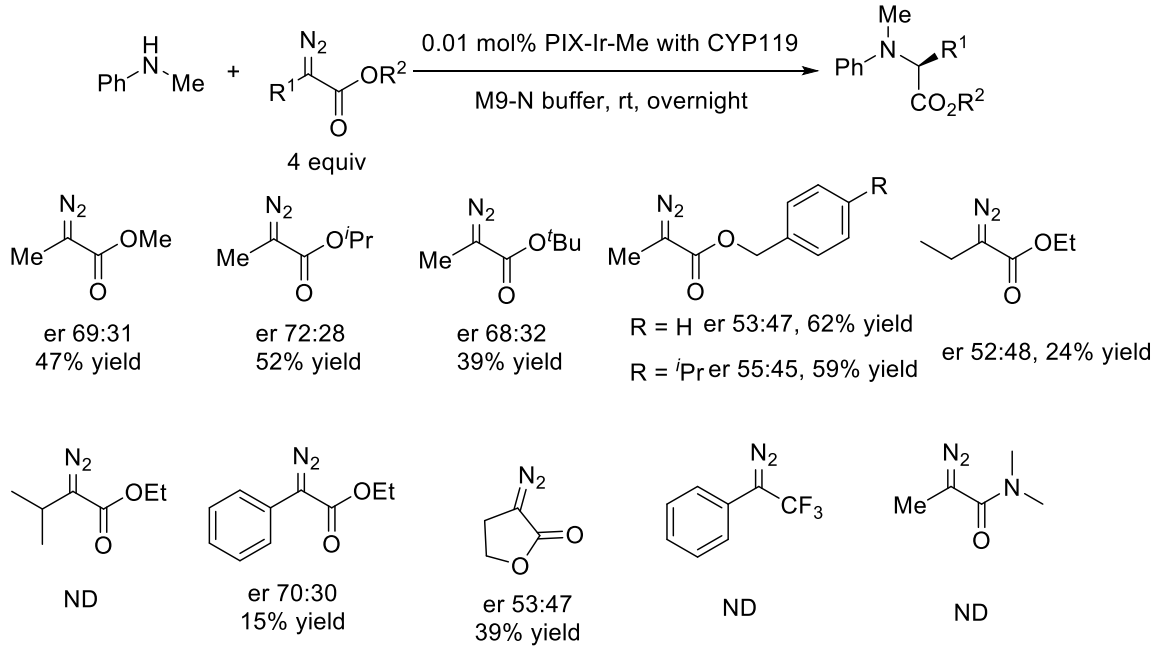
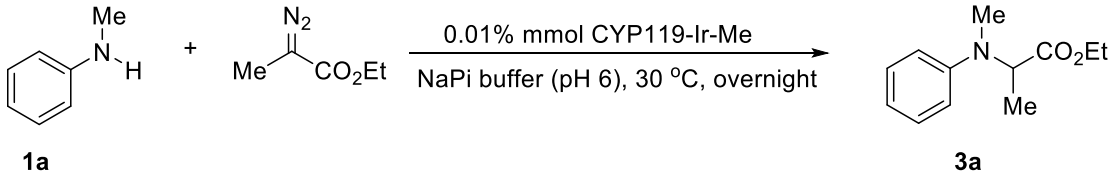


Figure S1b. Results from whole cell N-H insertions catalyzed by five different mutants in fresh whole cells and lyophilized cells.



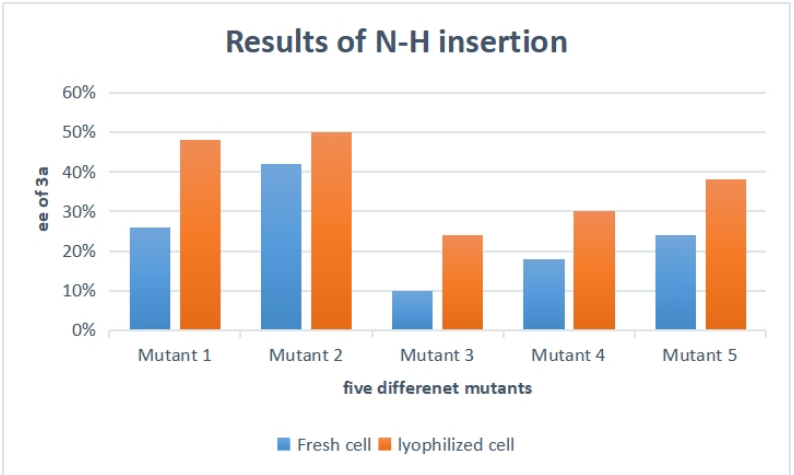
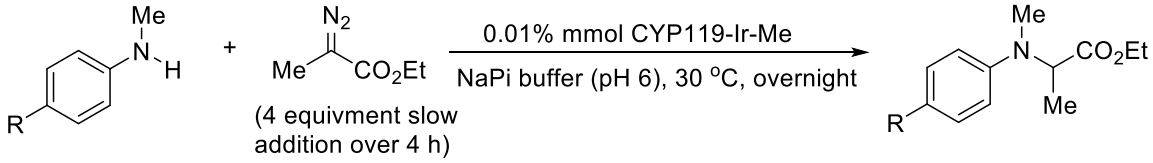


Figure S1c. Results from whole-cell, N-H insertions catalyzed by a series of mutants under optimized conditions.



	CNH-1	CNH-3A	CNH-4B
1a R = H	64.5 ± 0.3 %ee	72.3 ± 0.4 %ee	80.2 ± 0.5 %ee
1e R= OMe	12.3 ± 0.4 %ee	27.1 ± 0.5 %ee	67.5 ± 0.5 %ee

Figure S2. Calibration curve for Ethyl N-methyl-N-phenylalaninate (**3a**)

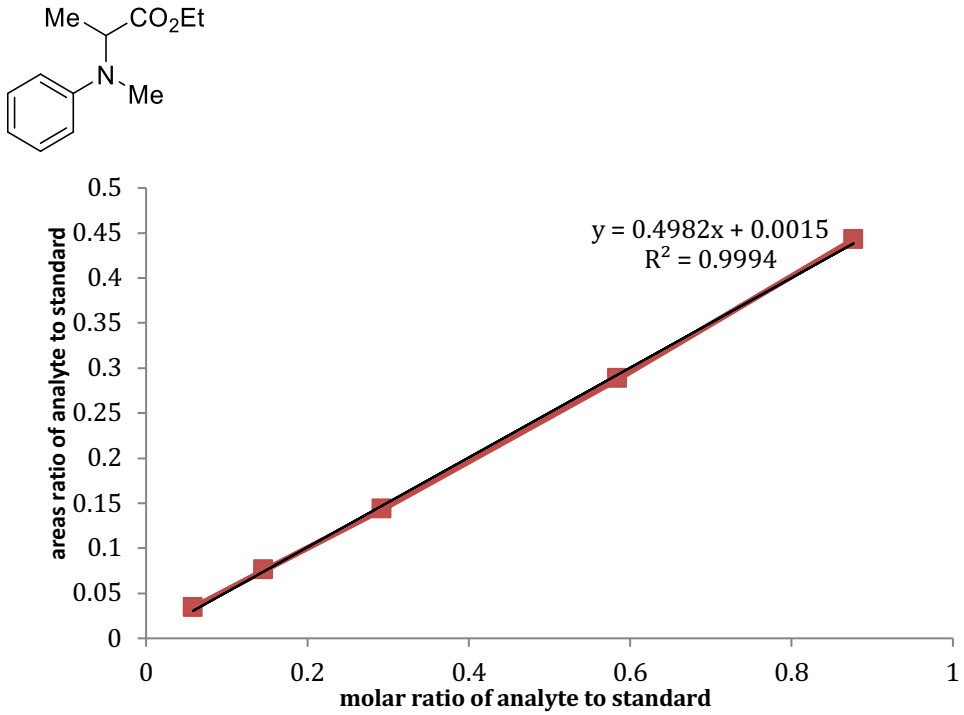


Figure S3. Calibration curve for Ethyl N-methyl-N-(p-tolyl) alaninate (**3b**)

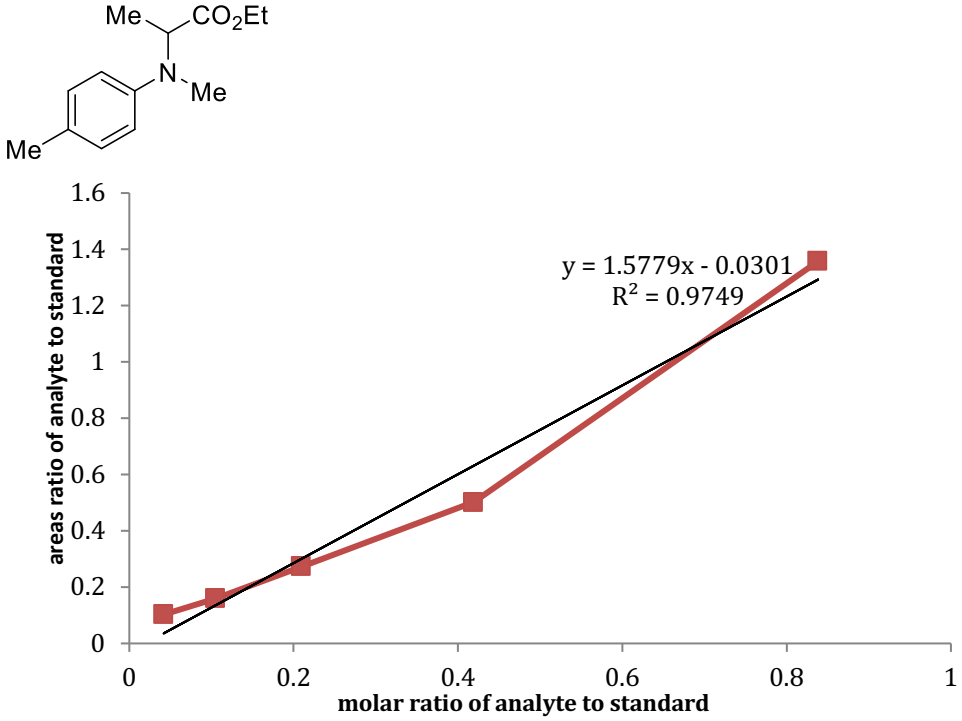


Figure S4. Calibration curve for ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**)

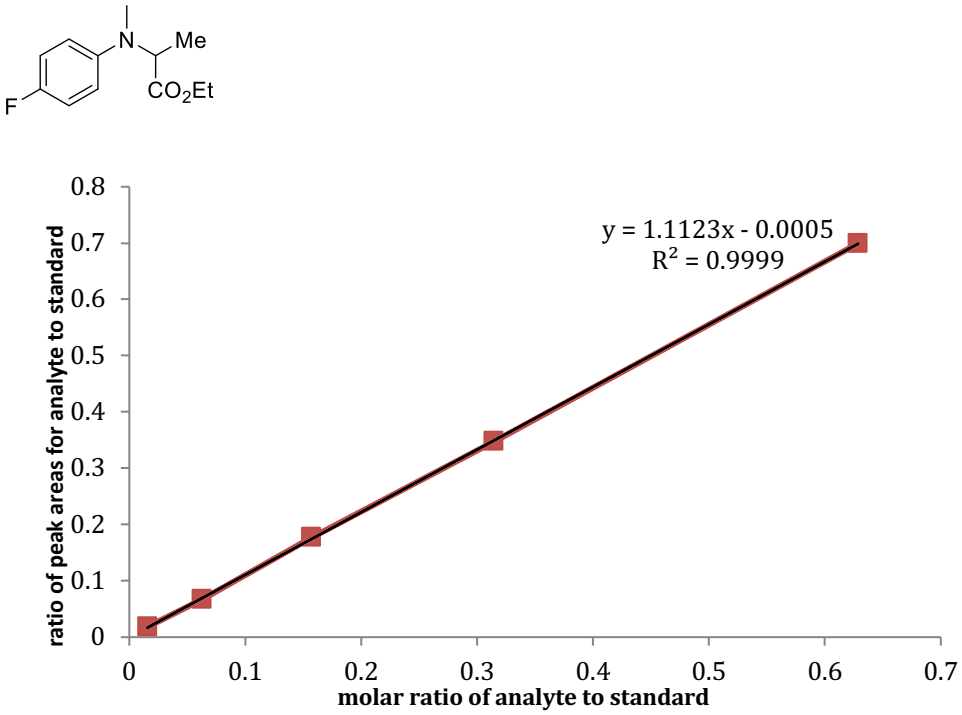


Figure S5. Calibration curve for Ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**)

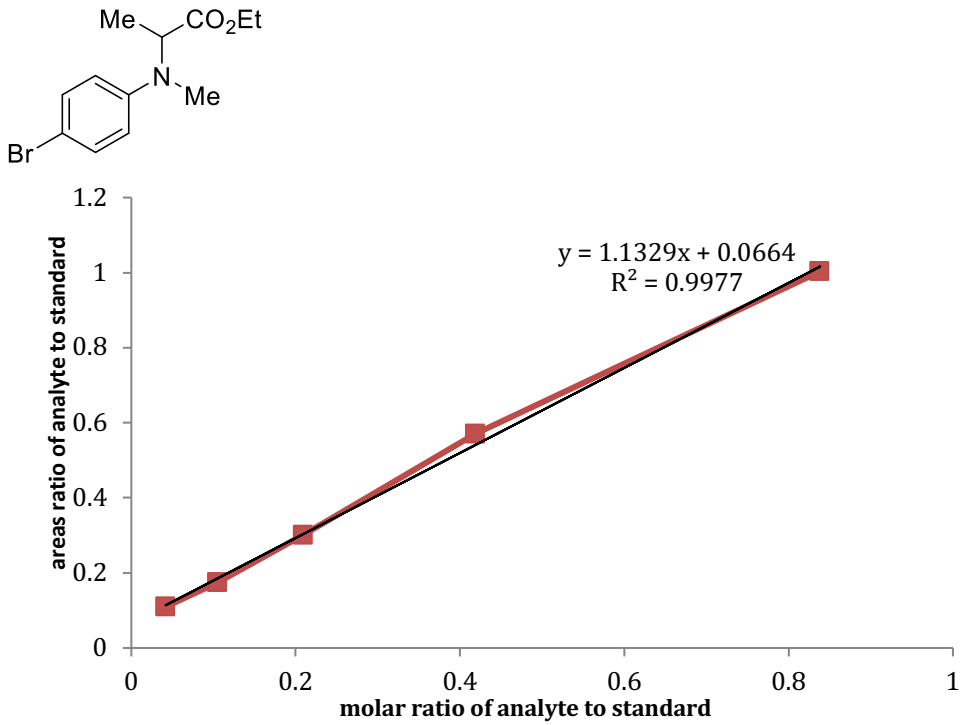


Figure S6. Calibration curve for Ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**)

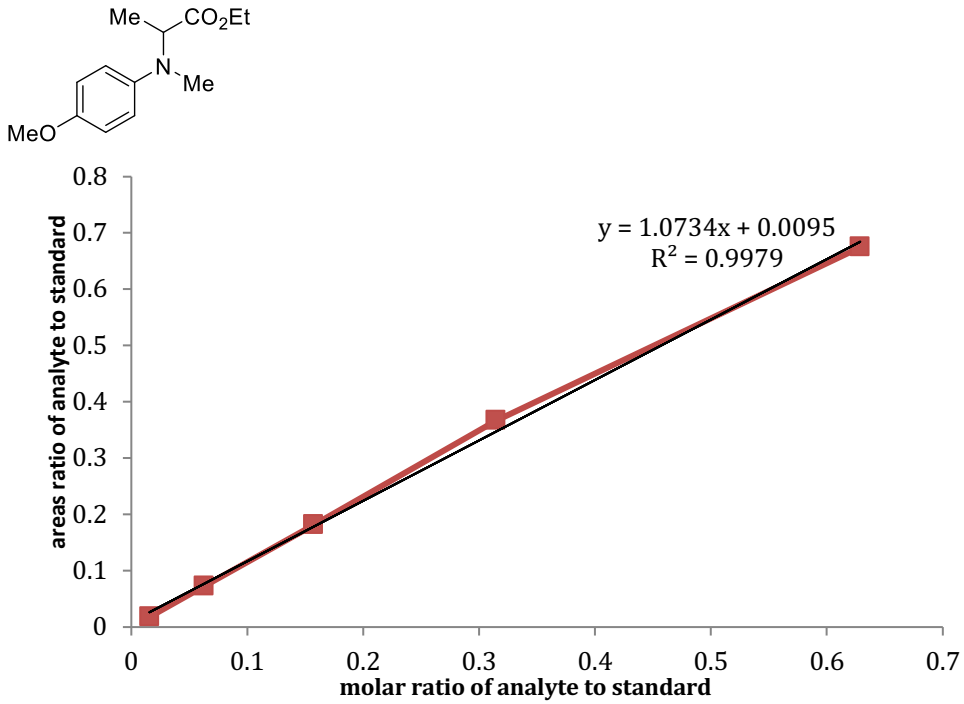


Figure S7. Calibration curve for Ethyl N-methyl-N-(4 (trifluoromethoxy)phenyl)alaninate (**3f**)

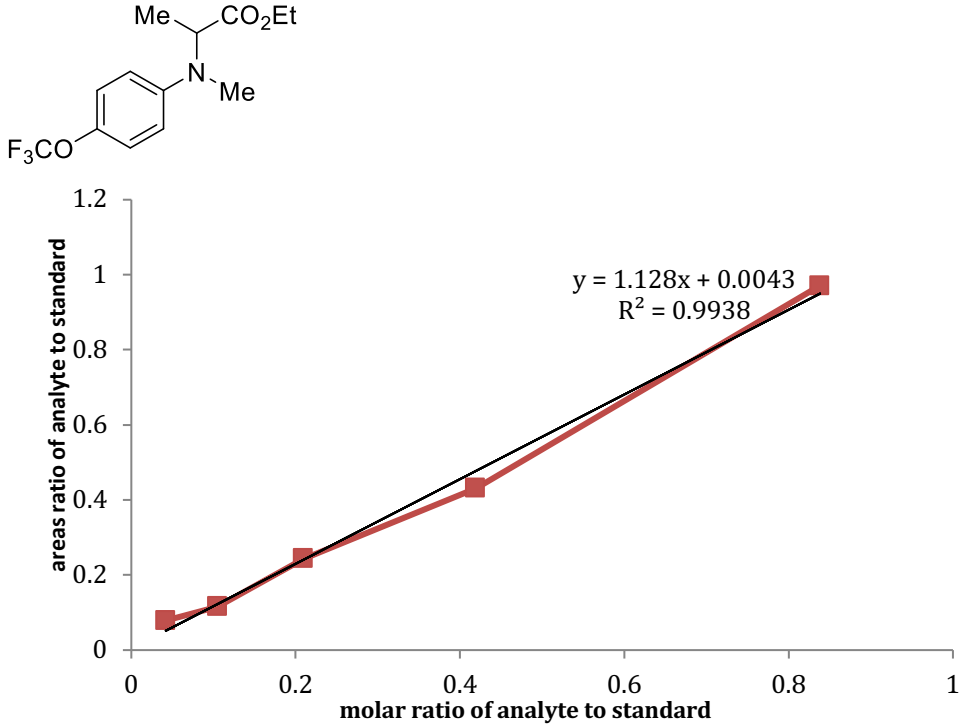


Figure S8. Calibration curve for Ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**)

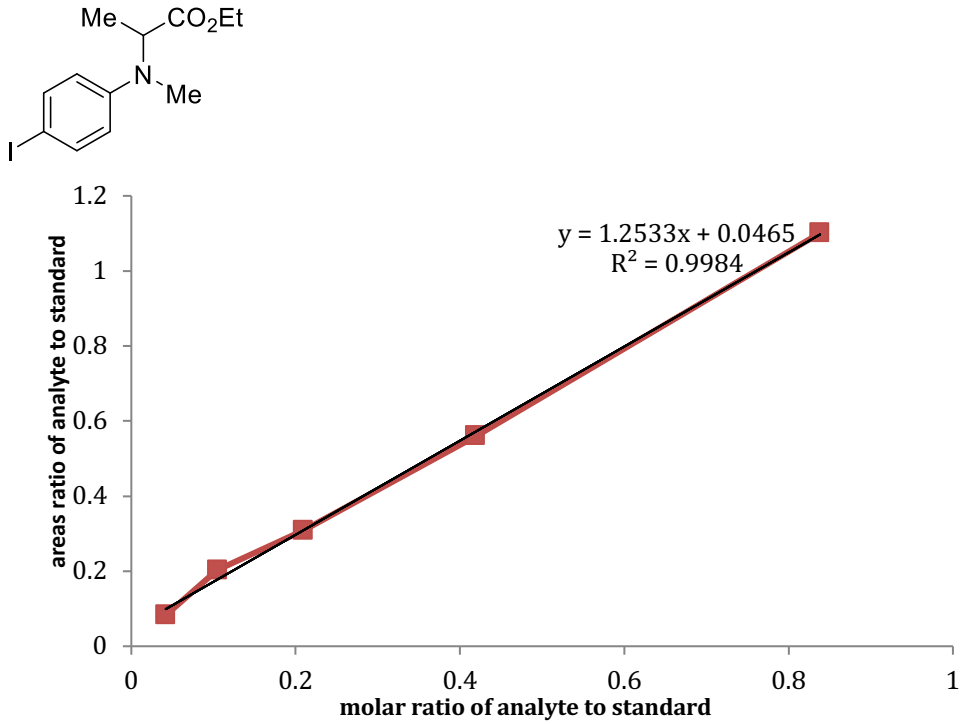


Figure S9. Calibration curve for Ethyl N-(4-acetoxyphenyl)-N-methylalaninate (**3h**)

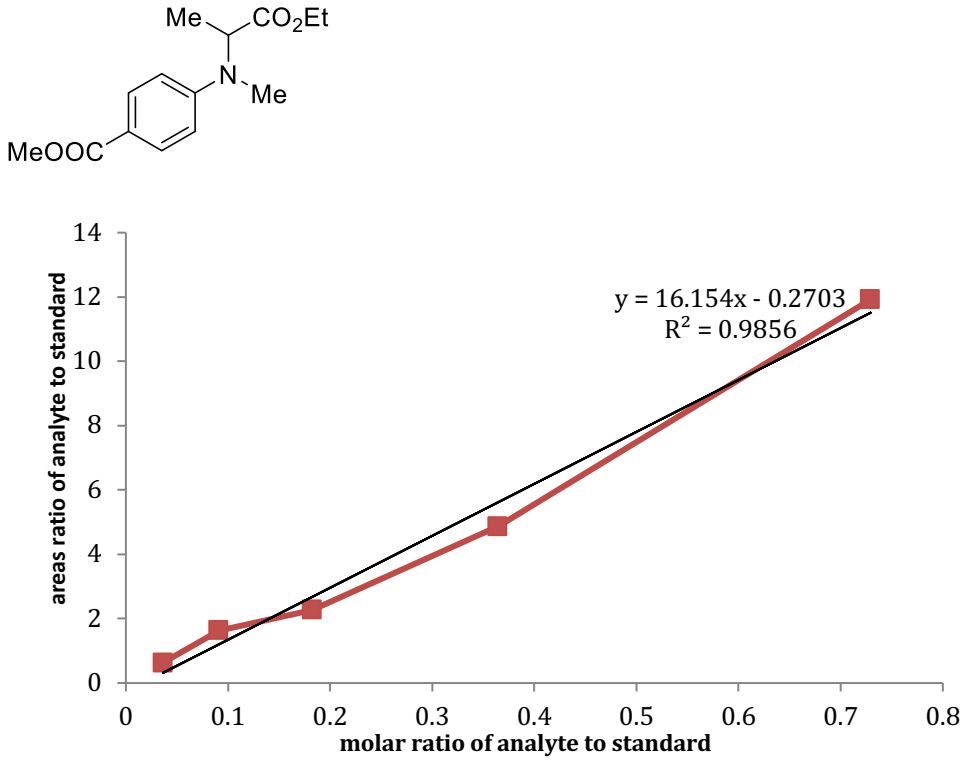


Figure S10. Calibration curve for Ethyl N-(4-acetoxyphenyl)-N-methylalaninate (**3i**)

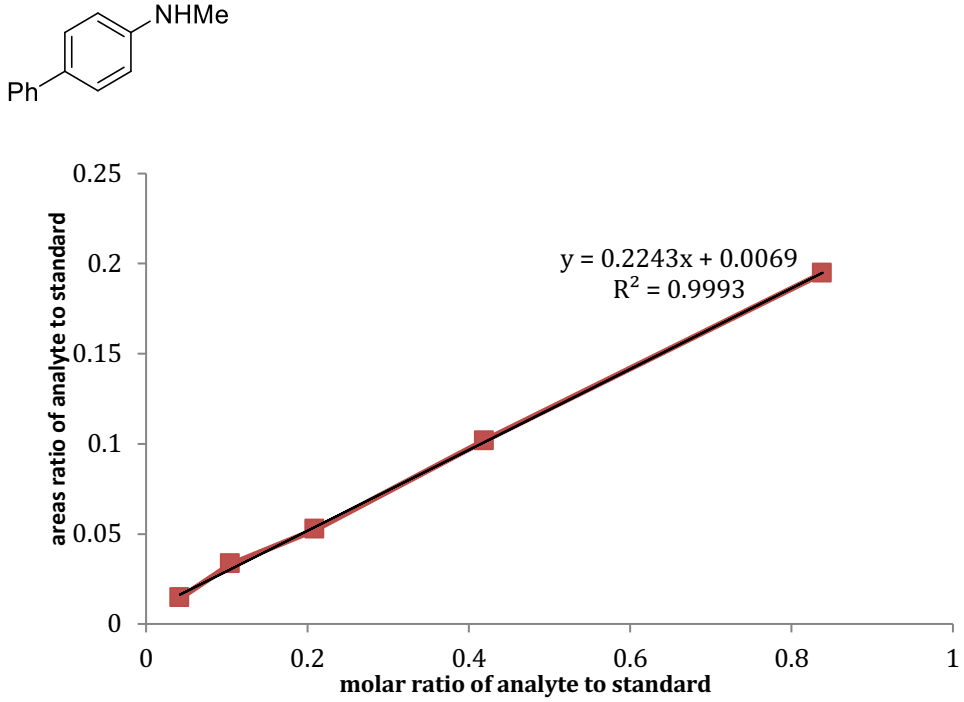
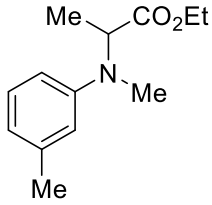


Figure S11. Calibration curve for ethyl N-methyl-N-(m-tolyl)alaninate (**3j**)



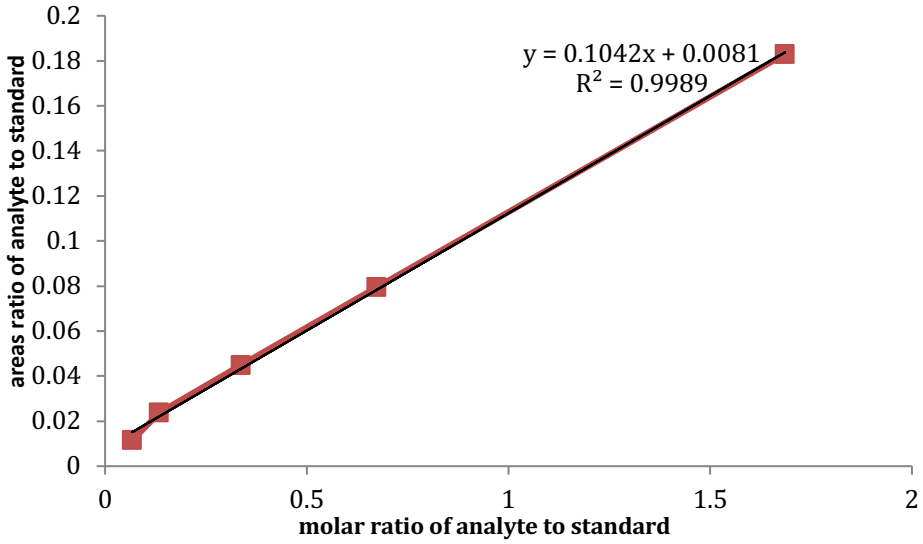
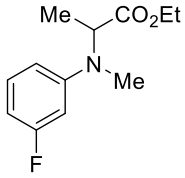


Figure S12. Calibration curve for ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**)



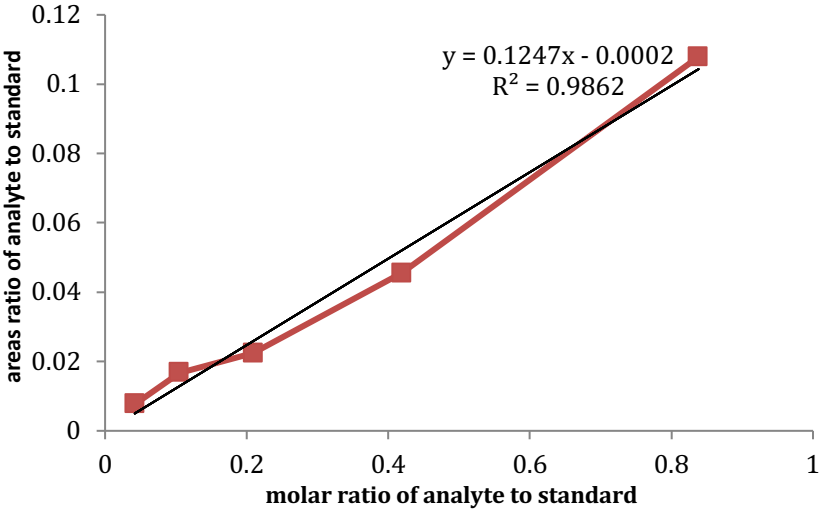
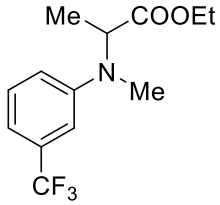


Figure S13. Calibration curve for ethyl N-(3-fluorophenyl)-N-methylalaninate (**3l**)



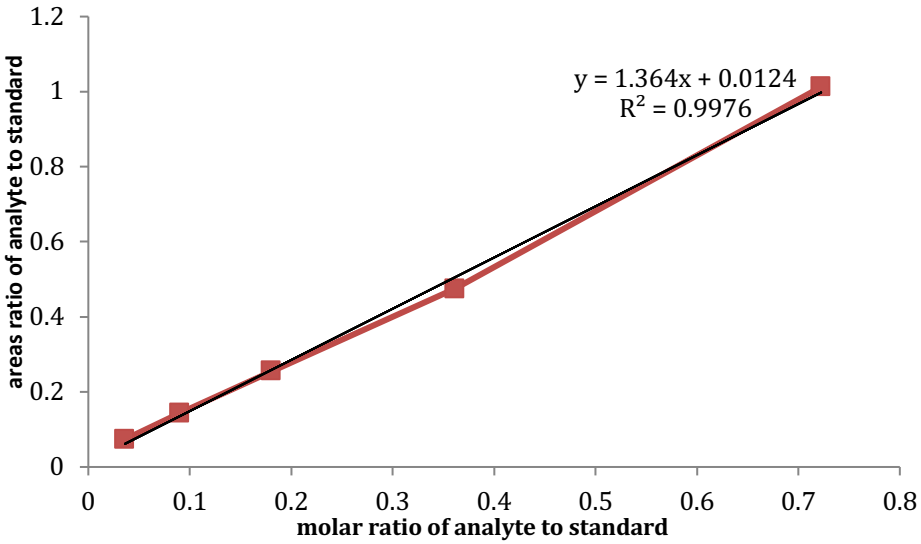
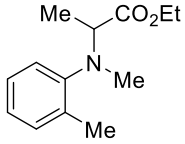


Figure S14. Calibration curve for ethyl N-methyl-N-(o-tolyl)alaninate (**3m**)



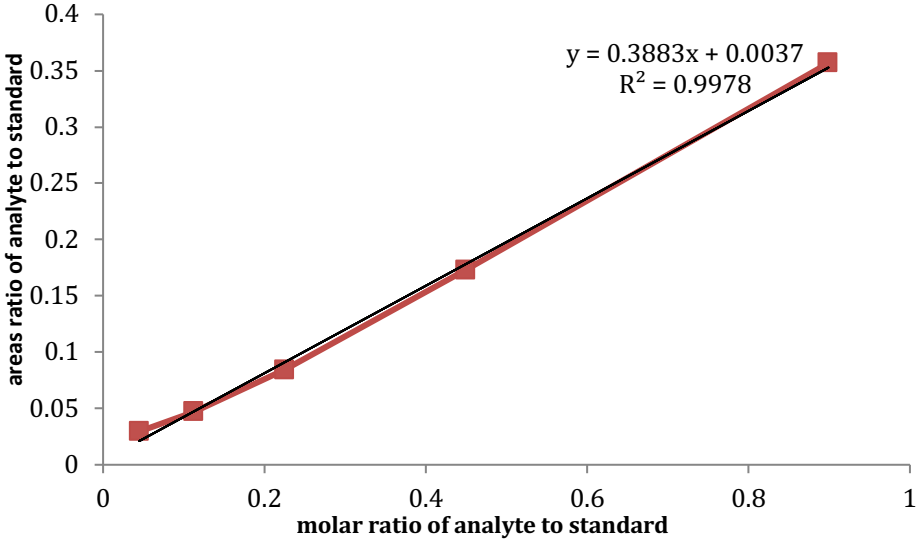


Figure S15. Calibration curve for ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**)

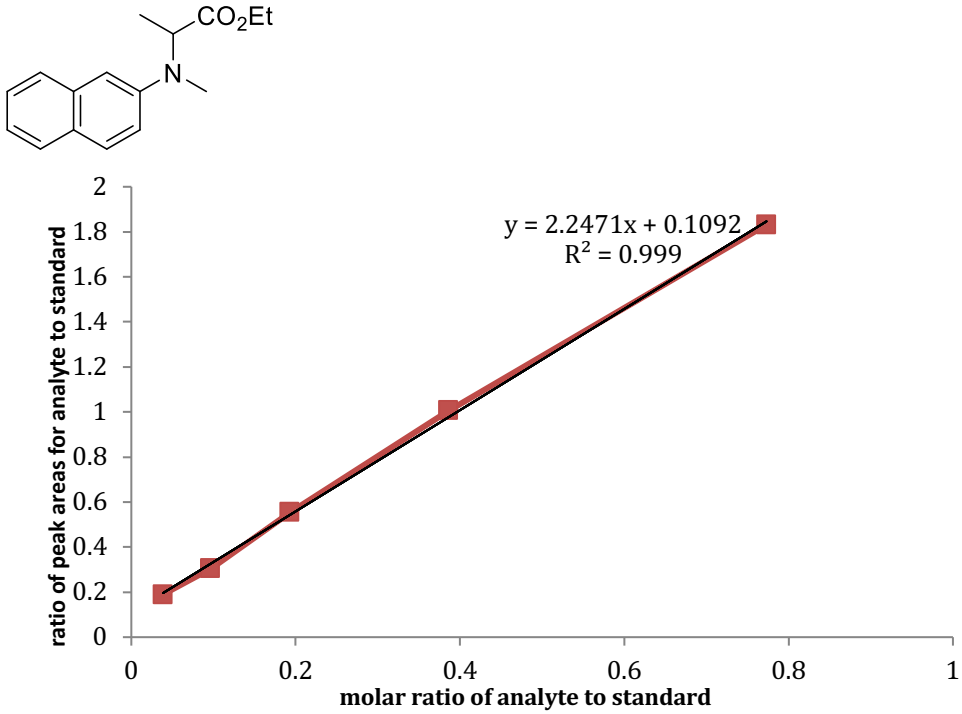


Figure S16. HPLC trace of racemic ethyl N-methyl-N-phenylalaninate (**3a**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)

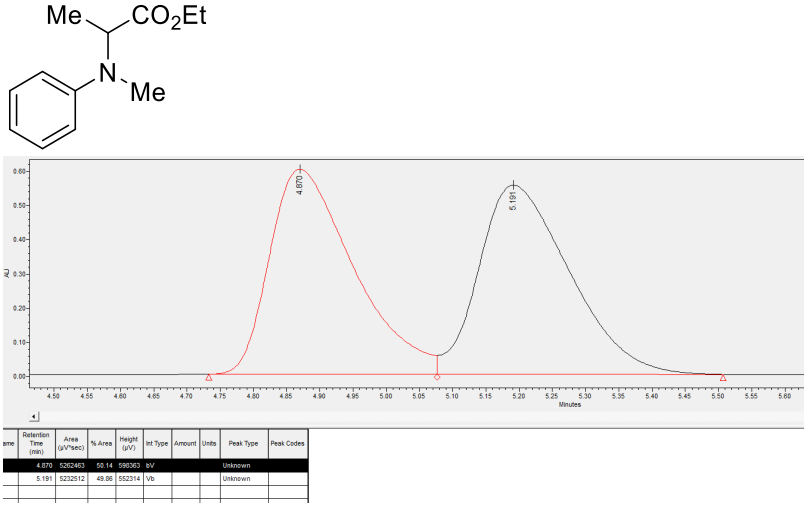
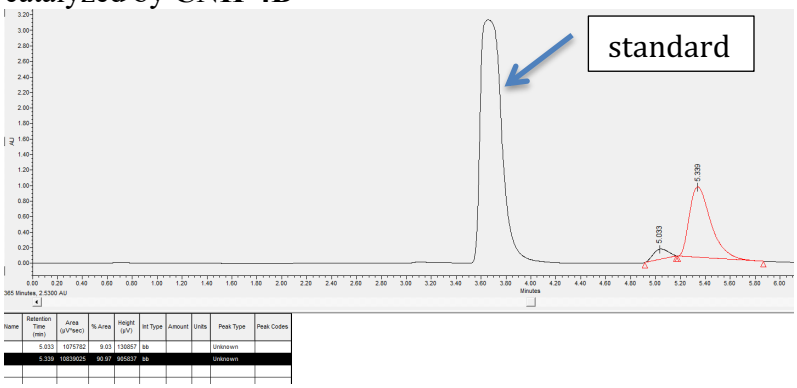
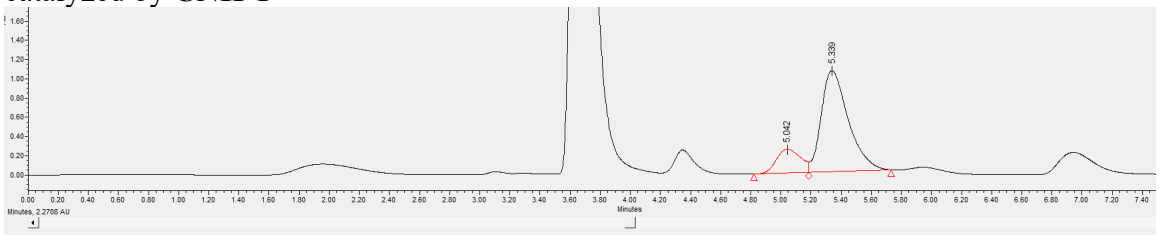


Figure S17a. HPLC trace of ethyl N-methyl-N-phenylalaninate (**3a**) from the reaction catalyzed by **CNH-4B**



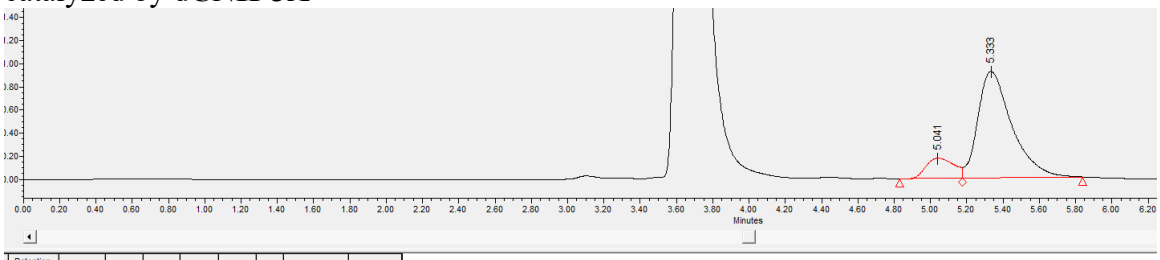
Name	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
	5.033	1075782	9.03	130857	bb			Unknown	
	5.339	10839625	90.97	965837	bb			Unknown	

Figure S17b. HPLC trace of Ethyl N-methyl-N-phenylalaninate (**3a**) from the reaction catalyzed by **CNH-1**



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.042	2743578	17.27	245669	bV			Unknown	
5.339	13140028	82.73	1049985	Vb			Unknown	

Figure S17c. HPLC trace of Ethyl N-methyl-N-phenylalaninate (**3a**) from the reaction catalyzed by d**CNH-3A**



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.041	1867594	13.49	174048	bV			Unknown	
5.333	11978558	86.51	920896	Vb			Unknown	

Figure S17d. Peaks of Ethyl N-methyl-N-phenylalaninate (**3a**) from the reaction catalyzed by **CNH-4B** with the two peaks fits to a combination of Gaussian curves.

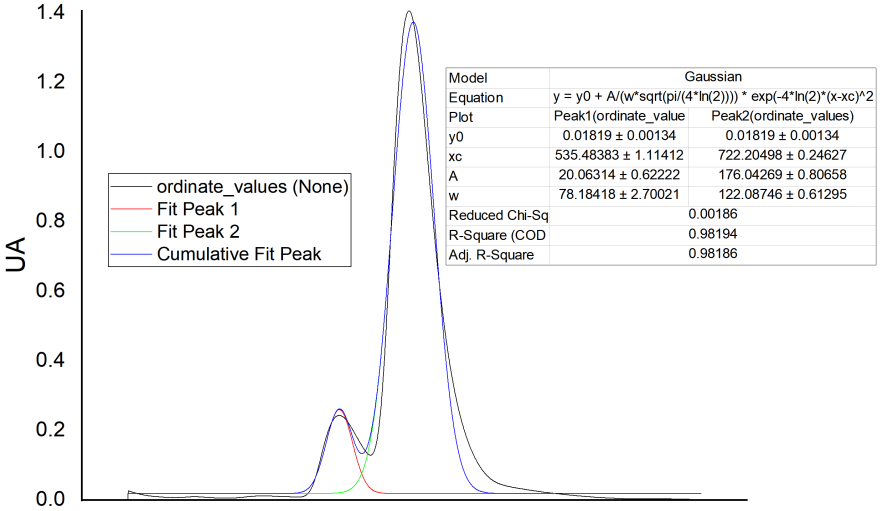


Figure S18. HPLC trace of (R)-Ethyl N-methyl-N-phenylalaninate (**3a**) synthesized from ethyl L-lactate

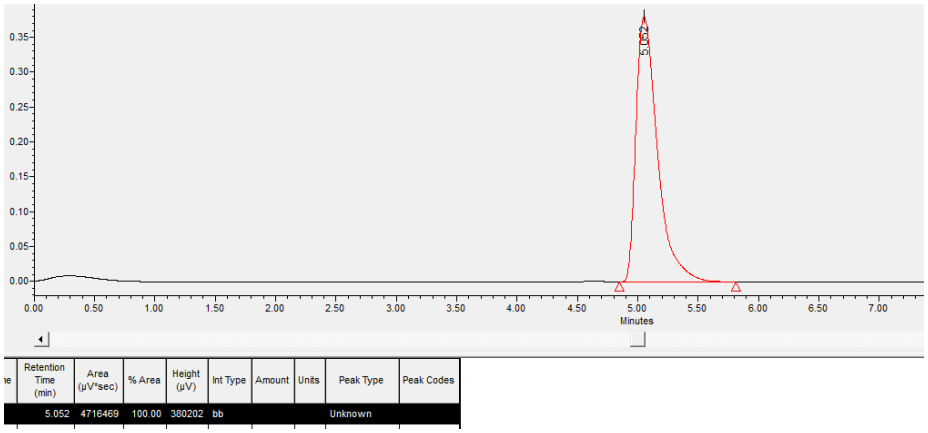
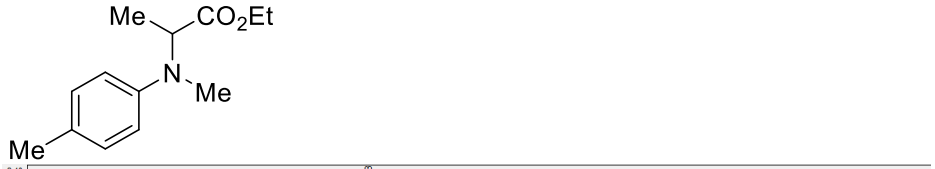
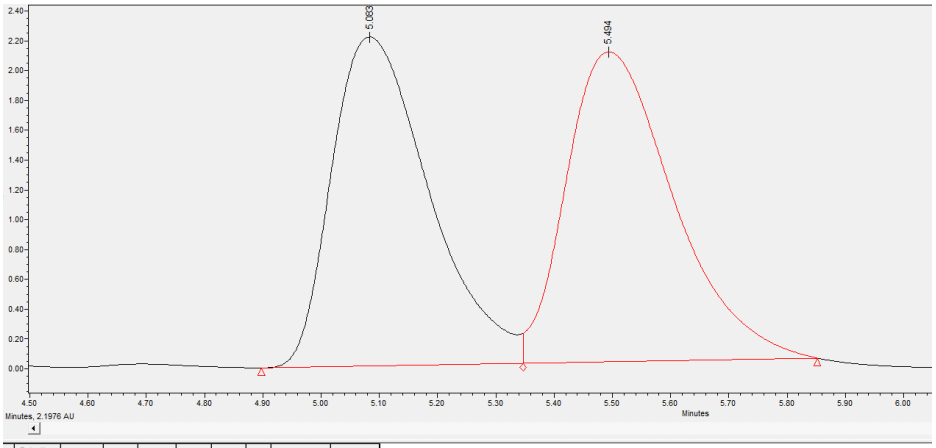


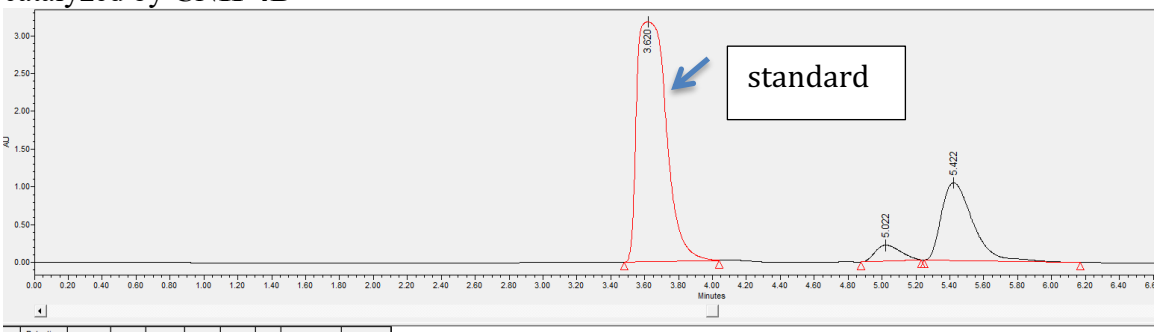
Figure S19. HPLC trace of racemic ethyl N-methyl-N-(p-tolyl) alaninate (**3b**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow



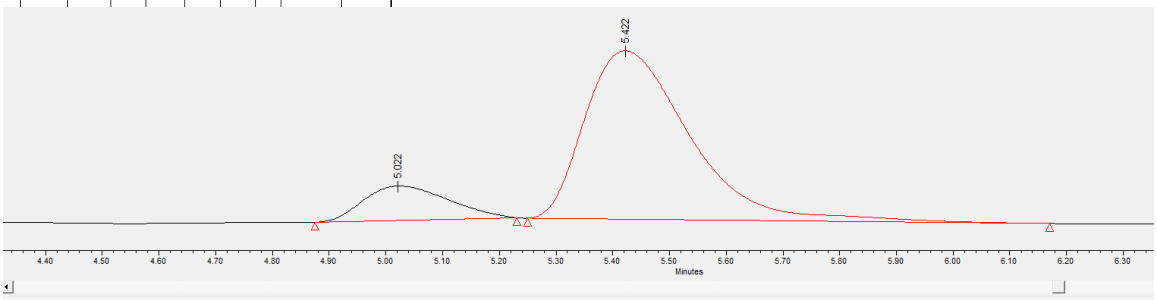


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.083	25664863	49.15	2205945	bv			Unknown	B7
5.494	26550859	50.85	2077896	vb			Unknown	B7

Figure S20. HPLC trace of Ethyl N-methyl-N-(p-tolyl) alaninate (**3b**) from the reaction catalyzed by **CNH-4B**

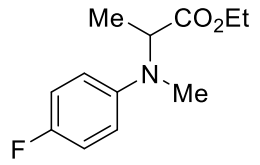


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
3.620	38325821	71.34	3171867	bb			Unknown	
5.022	2179983	4.06	210281	bb			Unknown	
5.422	13219213	24.61	1028614	bb			Unknown	



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.022	2179983	14.16	210281	bb			Unknown	
5.422	13219213	85.84	1028614	bb			Unknown	

Figure S21. GC chromatograms for racemic ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**) (Column: CP-chirasil-Dex CB, start 100 °C, hold 3 min, then rise to 130 °C with ramp 0.5°C/min; hold 4min and rise to 200 °C with ramp 45 °C/min, hold 2 min)



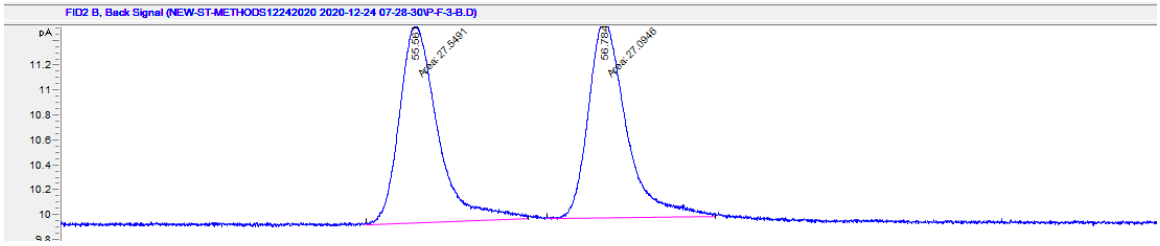
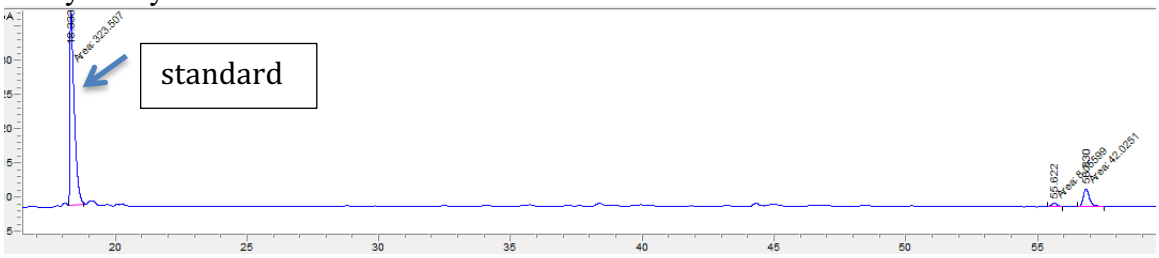


Figure S22. GC chromatograms for ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**) catalyzed by **CNH-4B**



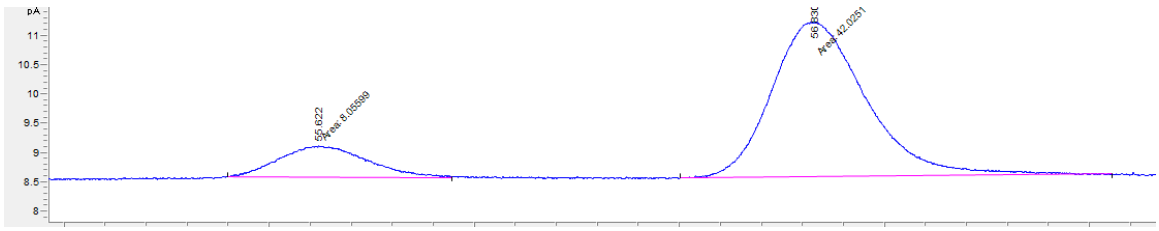
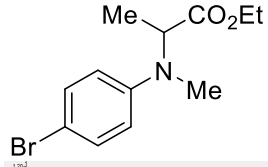


Figure S23. HPLC trace of racemic Ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)



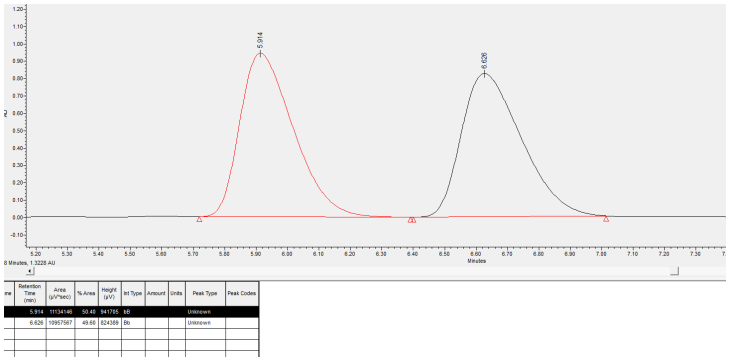
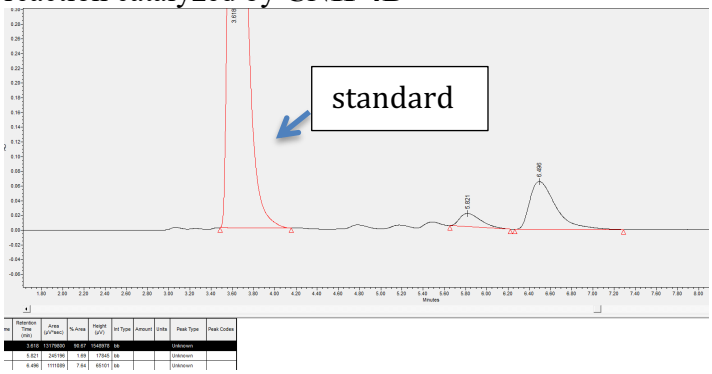
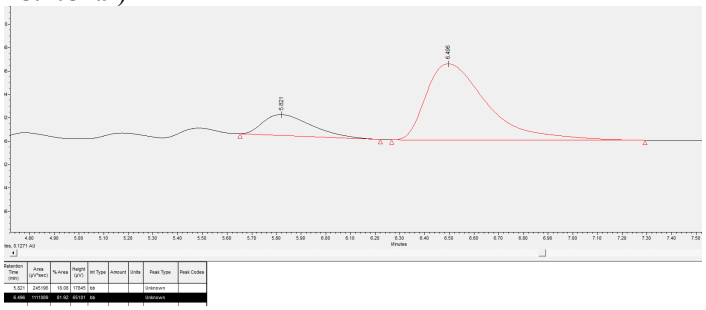


Figure S24. HPLC trace of Ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**) from the reaction catalyzed by **CNH-4B**



HPLC traces of the reaction to form **3d** acquired with 210 nm wavelengths (left peak:right peak = **18.1:81.9**)



HPLC traces of the reaction to form **3d** acquired with 254 nm UV wavelengths (left peak:right peak = **19.0:81.0**)

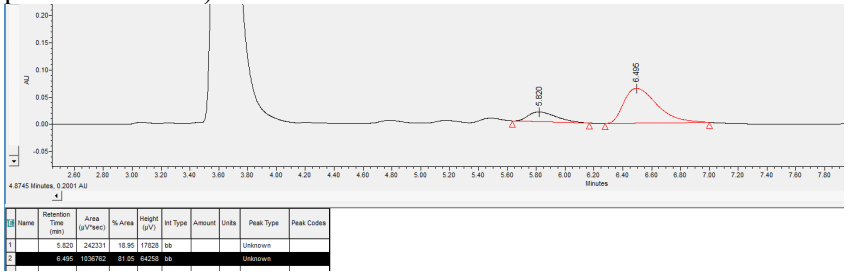
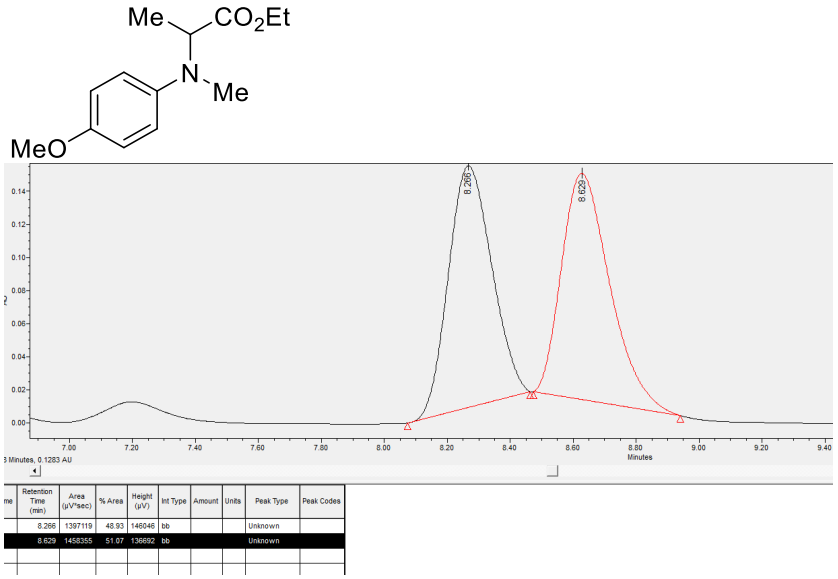
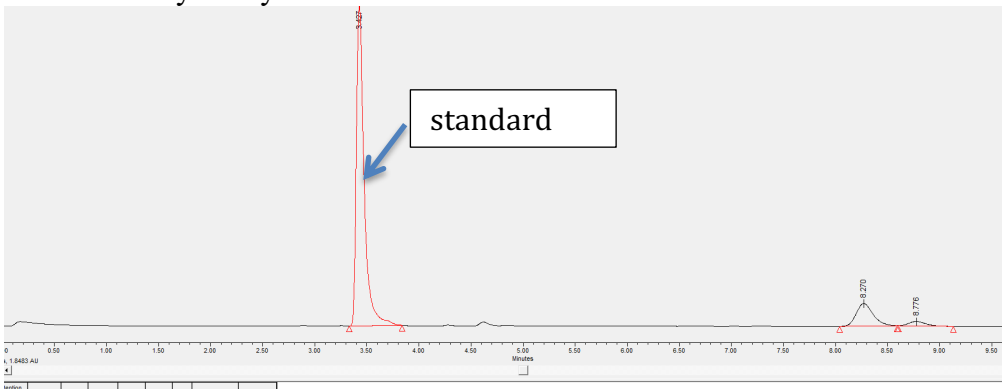


Figure S25. HPLC trace of racemic Ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**) (Chiracel OJ-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)

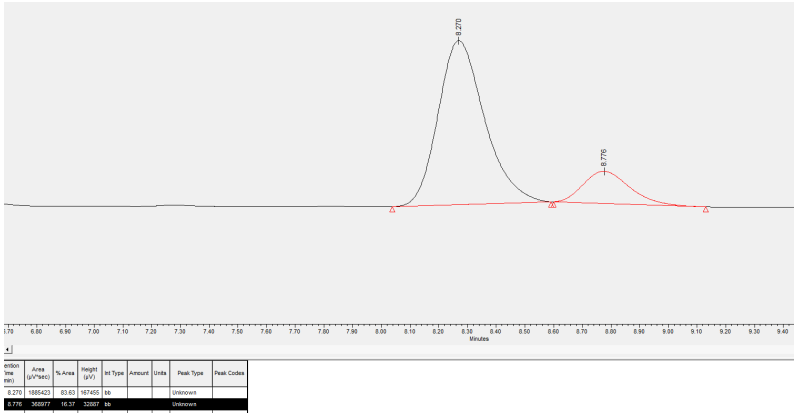


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.266	1397119	48.93	146046	bb			Unknown	
8.629	1458355	51.07	136692	bb			Unknown	

Figure S26a. HPLC trace of ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**) from the reaction catalyzed by **CNH-4B**

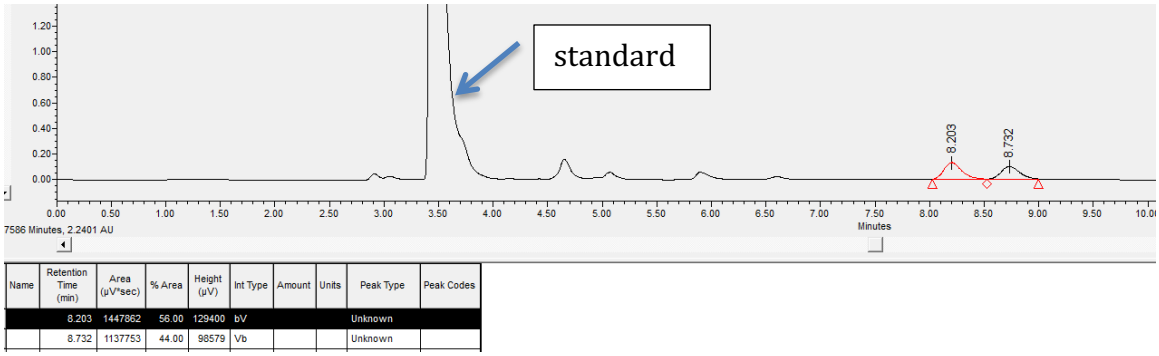


Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
12399962	84.61	2381627	bb			Unknown	
1885423	12.87	167455	bb			Unknown	
368977	2.52	32887	bb			Unknown	



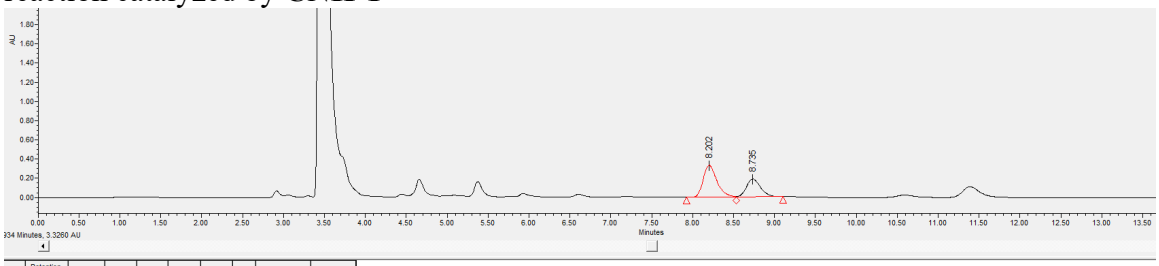
Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
1885423	83.63	167455	bb			Unknown	
368977	16.37	32887	bb			Unknown	

Figure S26b. HPLC trace of ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**) from the reaction catalyzed by **CNH-1**



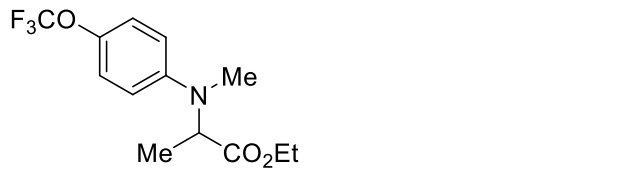
Name	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
	8.203	1447862	56.00	129400	bV			Unknown	
	8.732	1137753	44.00	98579	Vb			Unknown	

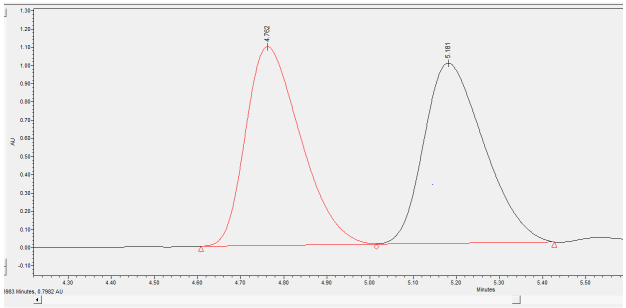
Figure S26c. HPLC trace of ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**) from the reaction catalyzed by **CNH-1**



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.202	3819268	63.55	332271	bV			Unknown	
8.735	2190559	36.45	185845	Vb			Unknown	

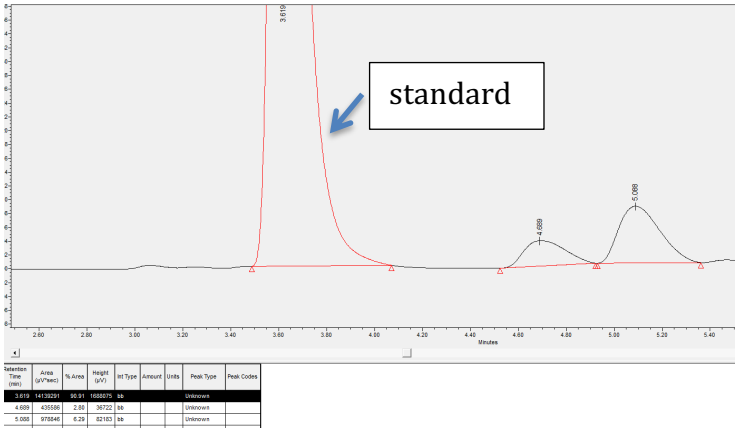
Figure S27. HPLC trace of racemic Ethyl N-methyl-N-(4-(trifluoromethoxy)phenyl)alaninate (**3f**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)





Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
4.762	9636135	50.19	1891577	bV			Unknown	
5.181	9561321	49.81	988146	Vb			Unknown	

Figure S28. HPLC trace of Ethyl N-methyl-N-(4-(trifluoromethoxy)phenyl)alaninate (**3f**) from the reaction catalyzed by **CNH-4B**



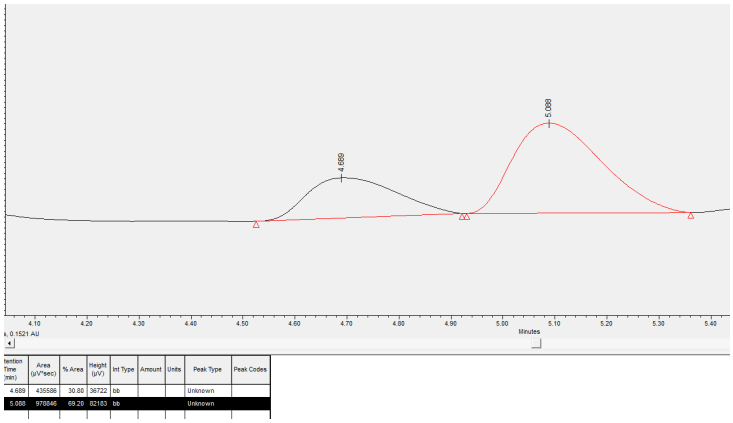
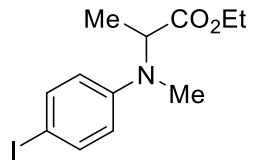


Figure S29. HPLC trace of racemic ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)



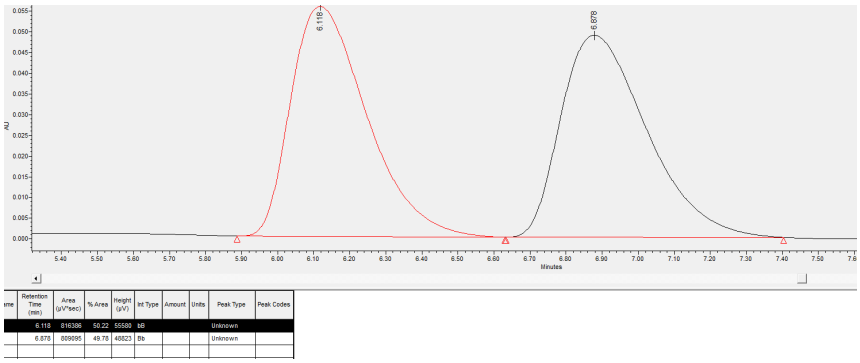
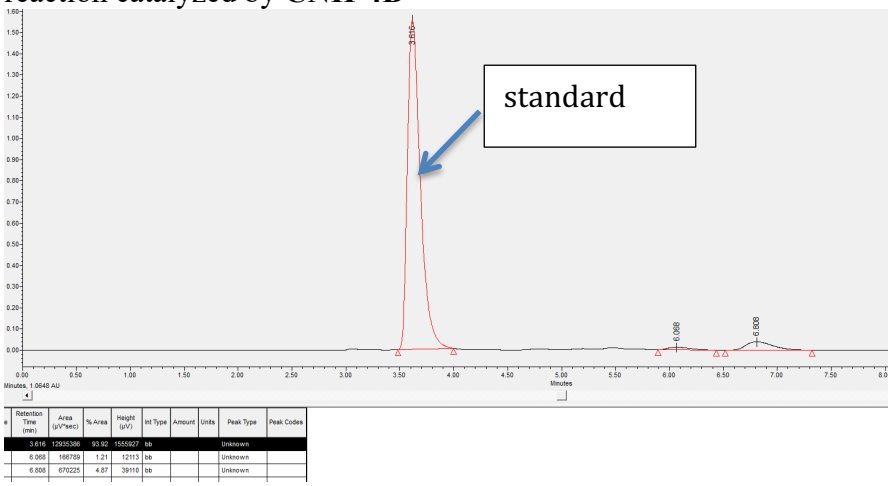
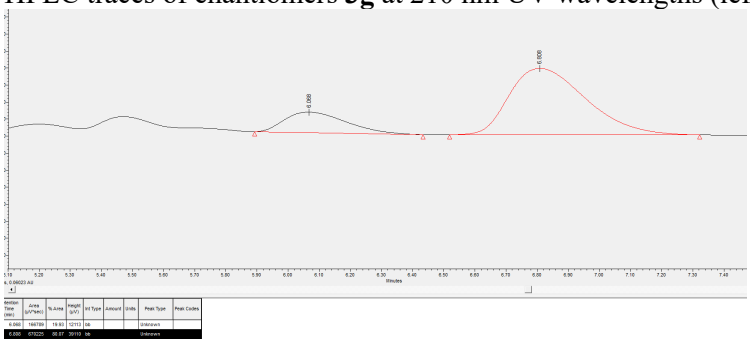


Figure S30. HPLC trace of ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**) from the reaction catalyzed by **CNH-4B**

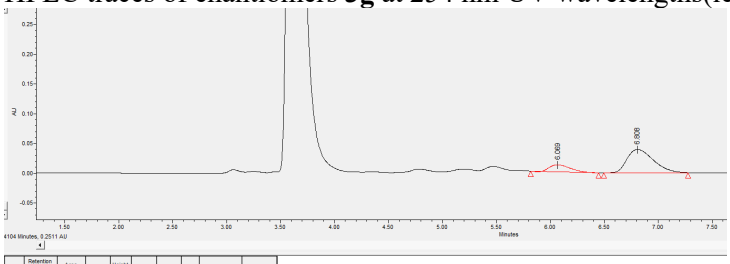


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
3.616	12935386	93.92	1555927	bb			Unknown	
6.068	166789	1.21	12113	bb			Unknown	
6.808	670225	4.87	39110	bb			Unknown	

HPLC traces of enantiomers **3g** at 210 nm UV wavelengths (left peak:right peak = **19.9:80.1**):



HPLC traces of enantiomers **3g** at 254 nm UV wavelengths(left peak:right peak = **19.4:80.6**):



Name	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
	6.069	165495	19.41	11731	bB			Unknown	
	6.808	686467	80.59	39065	Bb			Unknown	

Figure S31. HPLC trace of racemic ethyl N-(4-acetoxyphenyl)-N-methylalaninate (**3h**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)

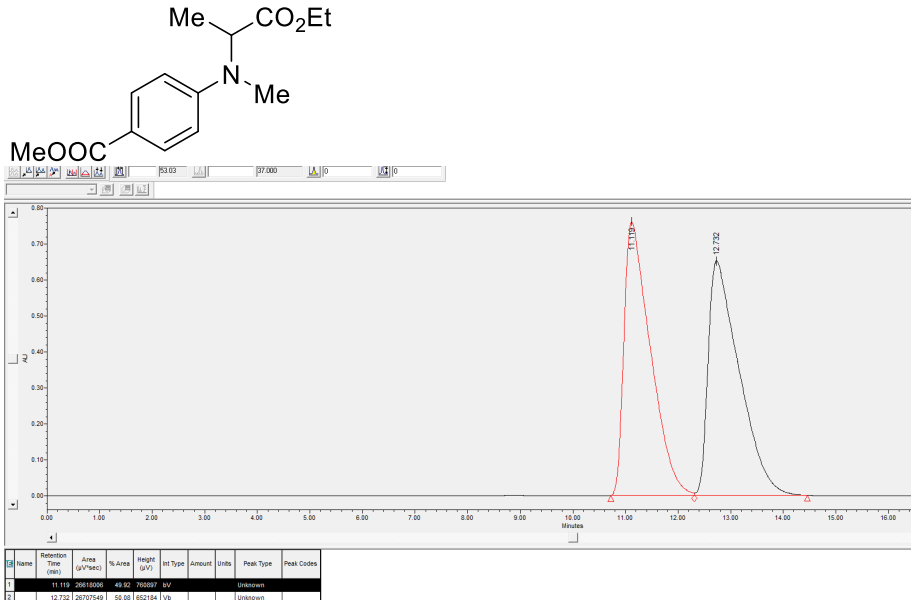


Figure S32. HPLC trace of ethyl N-(4-acetoxyphenyl)-N-methylalaninate (**3h**) from the reaction catalyzed by **CNH-4B**

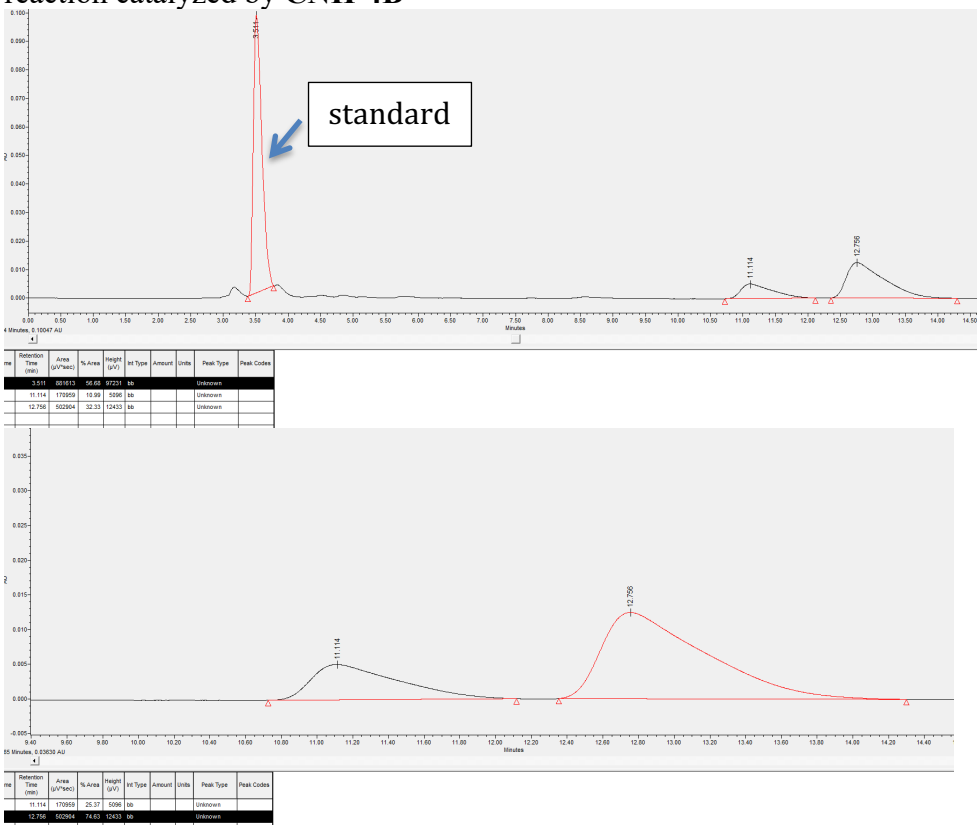
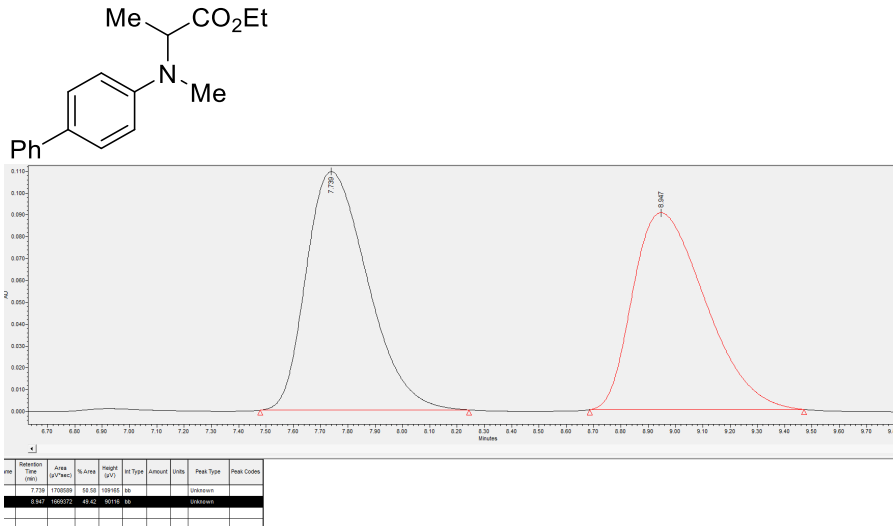
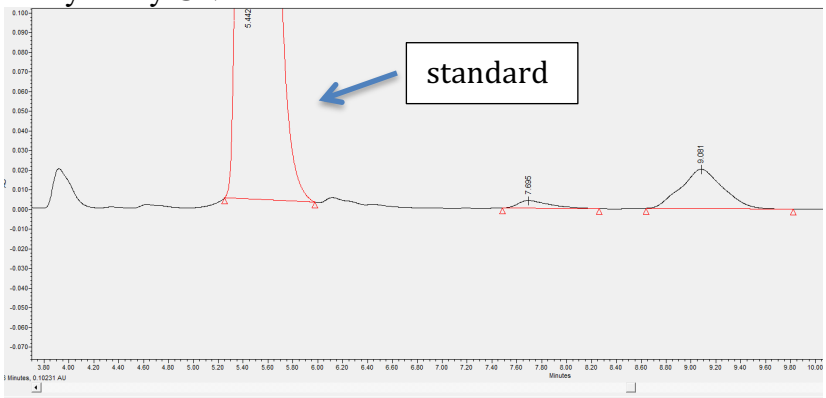


Figure S33. HPLC trace of racemic Ethyl N-([1,1'-biphenyl]-4-yl)-N-methylalaninate (**3i**) (Chiracel OD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)

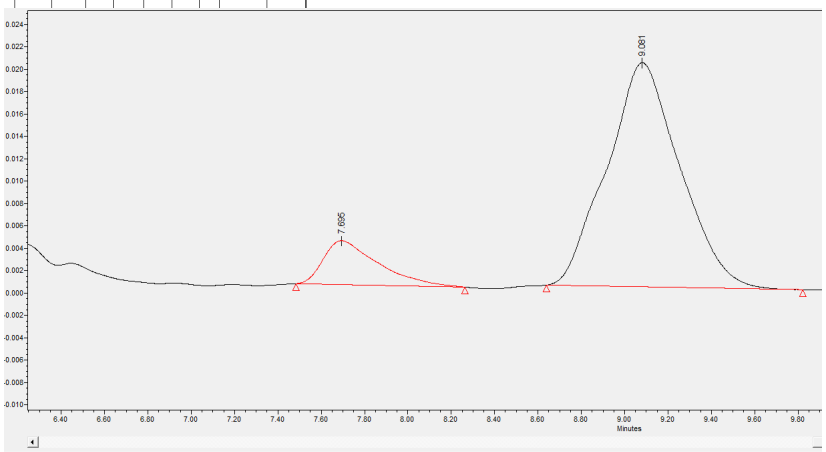


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
7.736	1700589	50.58	109165	bb			Unknown	
8.947	1660972	49.42	90116	bb			Unknown	

Figure S34. HPLC trace of ethyl N-([1,1'-biphenyl]-4-yl)-N-methylalaninate (**3i**) catalyzed by **CNH-4B**

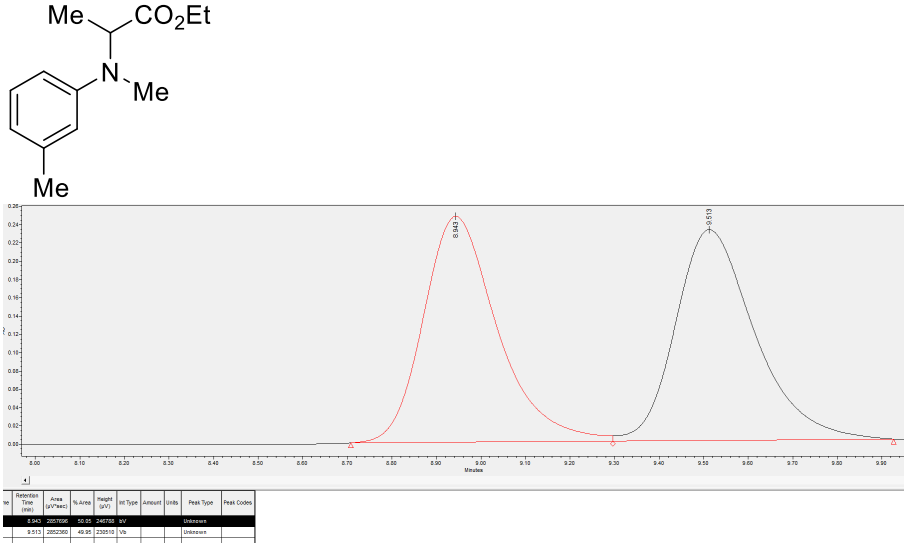


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.442	19196734	97.28	1516104	bb			Unknown	
7.695	68936	0.35	3928	bb			Unknown	
9.081	468026	2.37	20023	bb			Unknown	



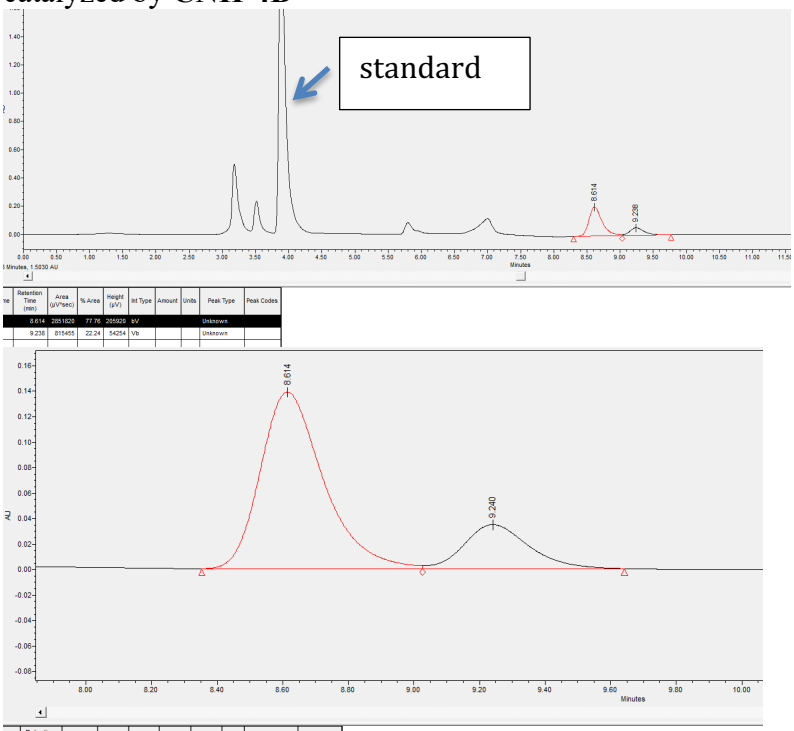
Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
7.695	68936	12.84	3928	bb			Unknown	
9.081	468026	87.16	20023	bb			Unknown	

Figure S35. HPLC trace of racemic ethyl N-methyl-N-(m-tolyl)alaninate (**3j**) (Chiracel IE, 0.5% *i*-PrOH in hexane with 1.0 ml/min flow)



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.943	2857696	50.95	246788	bV			Unknown	
9.513	2852560	49.95	230510	Vb			Unknown	

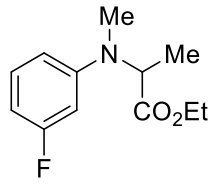
Figure S36. HPLC trace of ethyl N-methyl-N-(m-tolyl)alaninate (**3j**) from the reaction catalyzed by **CNH-4B**

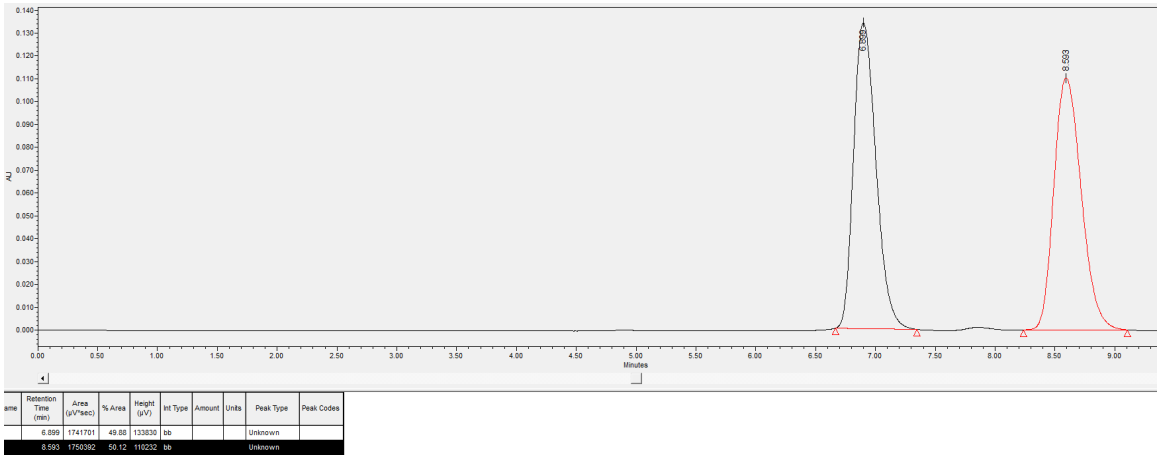


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.614	2851629	77.76	265929	bV			Unknown	
9.238	815455	22.24	54254	Vb			Unknown	

Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.614	1853389	79.69	138703	bV			Unknown	
9.240	472449	20.31	34539	Vb			Unknown	

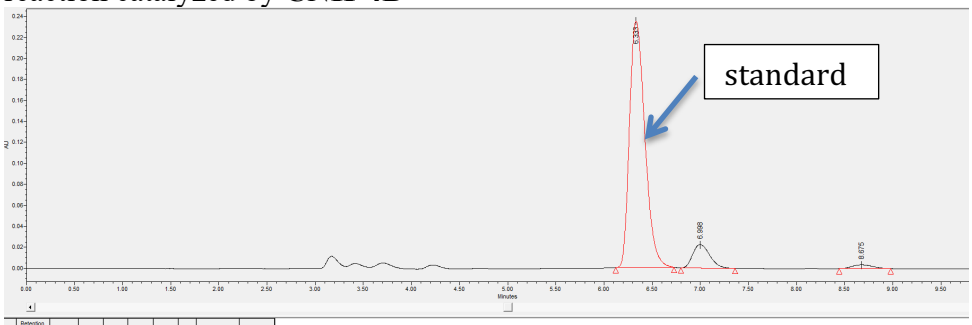
Figure S37. HPLC trace of racemic ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**) (Chiracel OD-H, 1.0% *i*-PrOH in hexane with 1.0 ml/min flow)



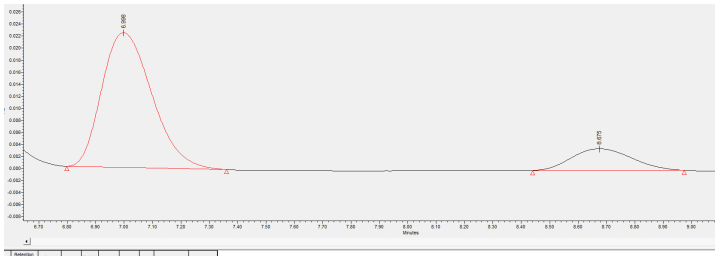


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
6.899	1741701	49.88	133830	bb			Unknown	
8.593	1750392	50.12	110232	bb			Unknown	

Figure S38. HPLC trace of ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**) from the reaction catalyzed by **CNH-4B**

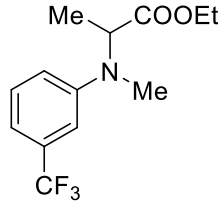


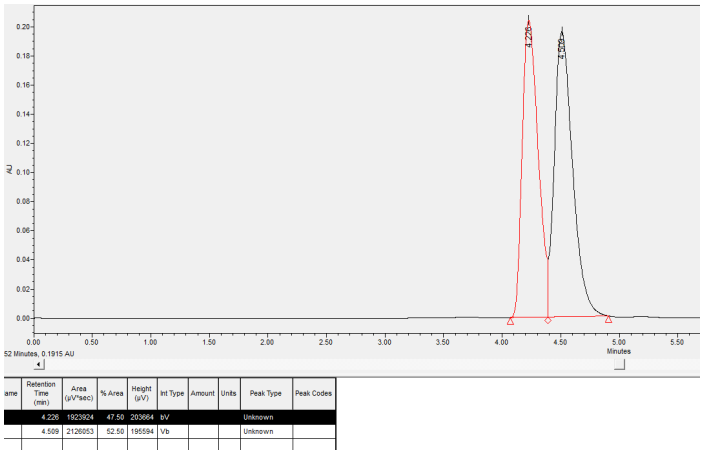
Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
6.333	2609533	88.84	234664	bb			Unknown	
6.990	274819	9.36	22431	bb			Unknown	
8.675	52841	1.80	3600	bb			Unknown	



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
6.990	274819	83.87	22431	bb			Unknown	
8.675	52841	16.13	3600	bb			Unknown	

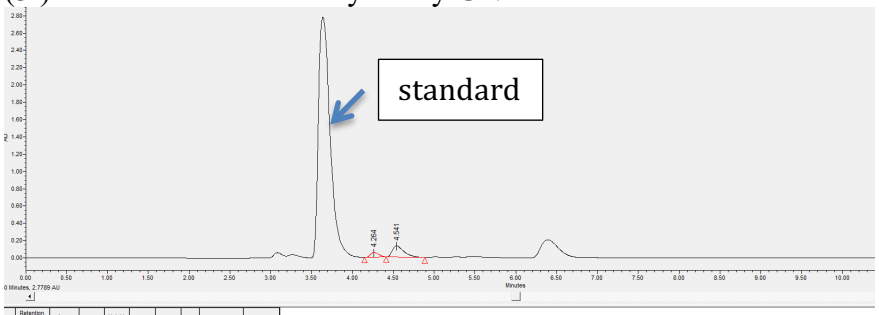
Figure S39. HPLC trace of racemic ethyl N-methyl-N-(3-(trifluoromethyl)phenyl)alaninate (**3l**) (Chiracel AD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)



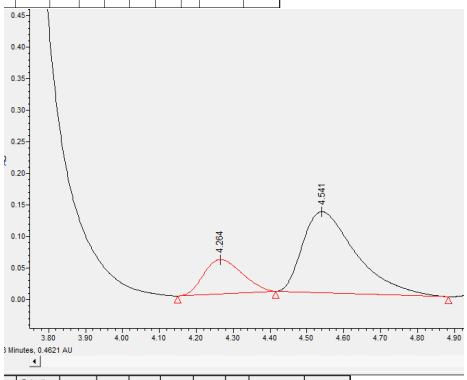


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
4.226	1923924	47.50	203664	bV			Unknown	
4.509	2126053	52.50	195594	Vb			Unknown	

Figure S40. HPLC trace of ethyl N-methyl-N-(3-(trifluoromethyl)phenyl)alaninate (**3l**) from the reaction catalyzed by **CNH-4B**

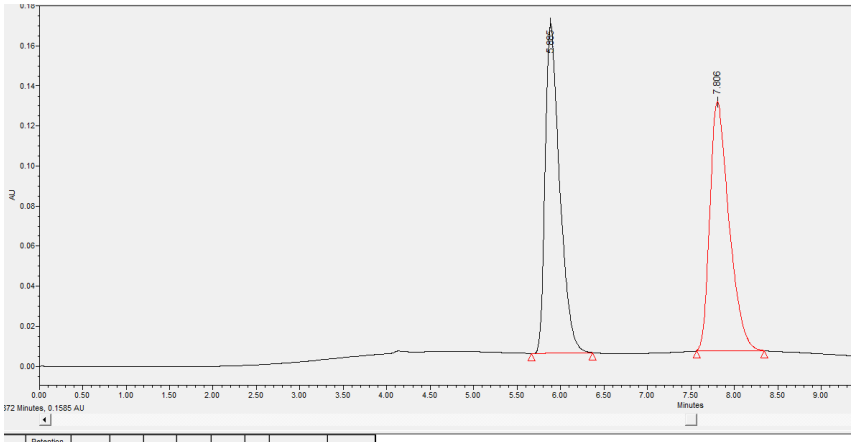


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
4.264	392731	23.38	54488	bb			Unknown	
4.541	1287112	76.62	128442	BB			Unknown	



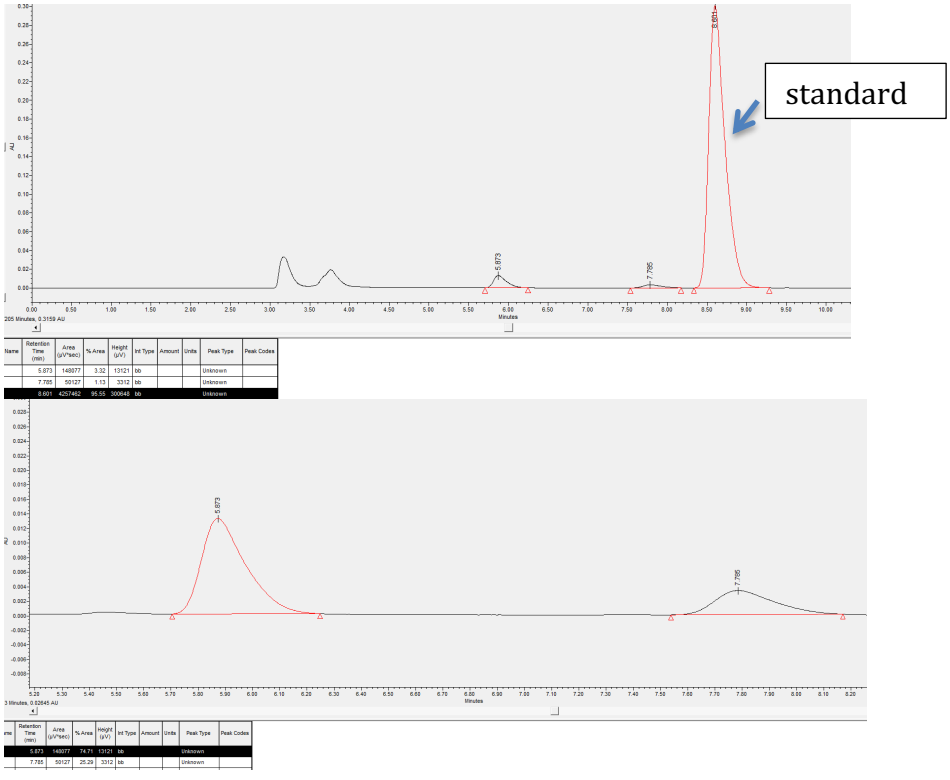
Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
4.264	392731	23.38	54488	bb			Unknown	
4.541	1287112	76.62	128442	BB			Unknown	

Figure S41. HPLC trace of racemic ethyl N-methyl-N-(o-tolyl)alaninate (**3m**) (Chiracel OJ-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)



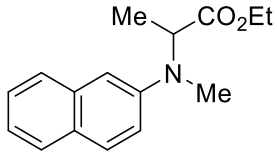
Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.885	1902311	49.98	164854	bb			Unknown	
7.806	1903533	50.02	124287	bb			Unknown	

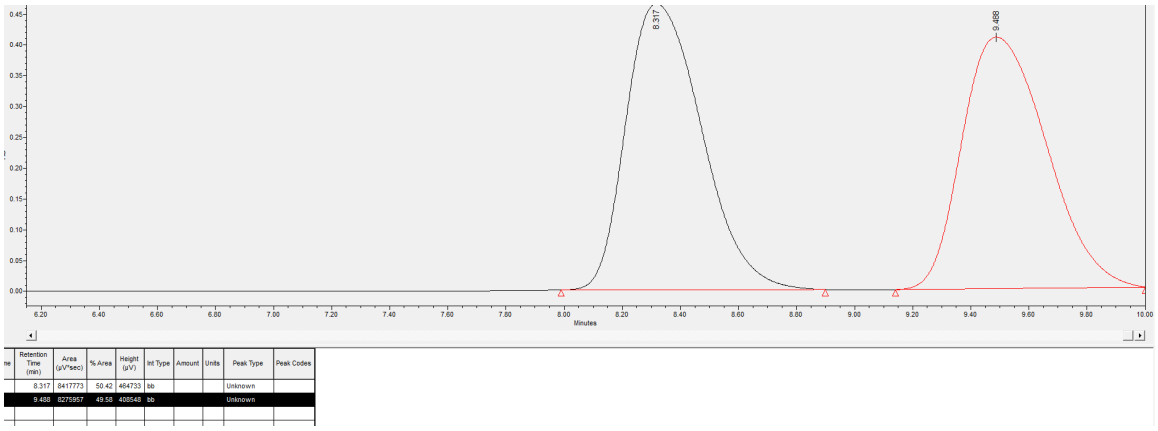
Figure S42. HPLC trace of ethyl N-methyl-N-(o-tolyl)alaninate (**3m**) from the reaction catalyzed by **CNH-4B**



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.873	148077	3.32	13121	bb			Unknown	
7.785	50127	1.13	3312	bb			Unknown	
8.691	4257462	95.55	300848	bb			Unknown	

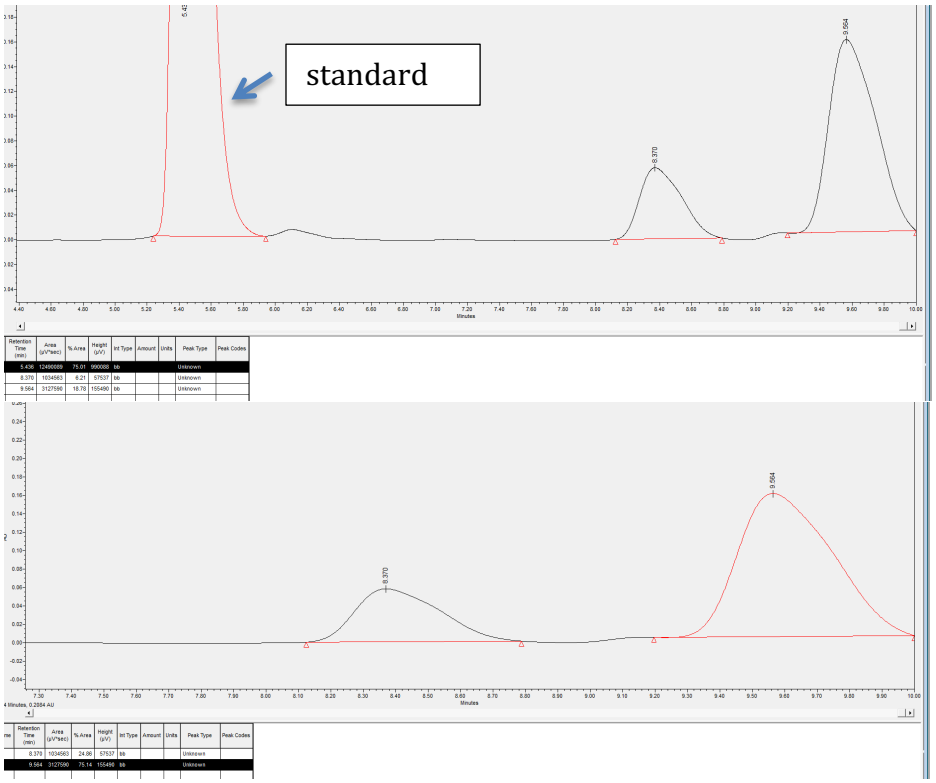
Figure S43. HPLC trace of racemic Ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**) (Chiracel OD-H, 2.0% *i*-PrOH in hexane with 1.0 ml/min flow)





Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.317	8417773	50.42	464733	bb			Unknown	
9.488	8275957	49.58	408548	bb			Unknown	

Figure S44. HPLC trace of ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**) from the reaction catalyzed by **CNH-4B**



Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
5.436	1249669	75.01	990888	bb			Unknown	
8.370	1034563	6.21	57537	bb			Unknown	
9.564	3127590	18.78	155490	bb			Unknown	

Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
8.370	1034563	24.86	57537	bb			Unknown	
9.564	3127590	75.14	155490	bb			Unknown	

Figure S45. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl N-methyl-N-phenylalaninate (**3a**)

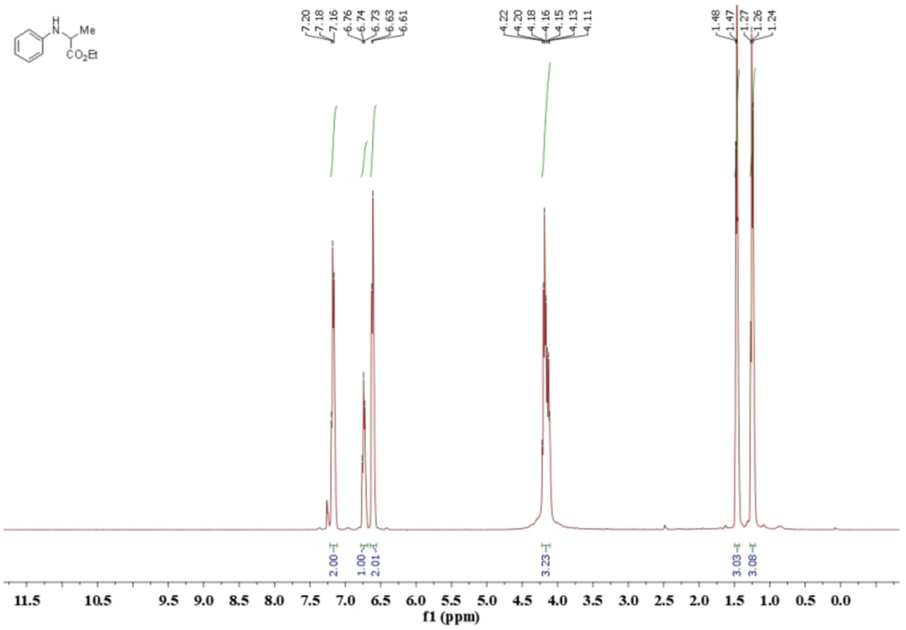


Figure S46. ^{13}C NMR (101 MHz, $CDCl_3$) of Ethyl N-methyl-N-phenylalaninate (**3a**)

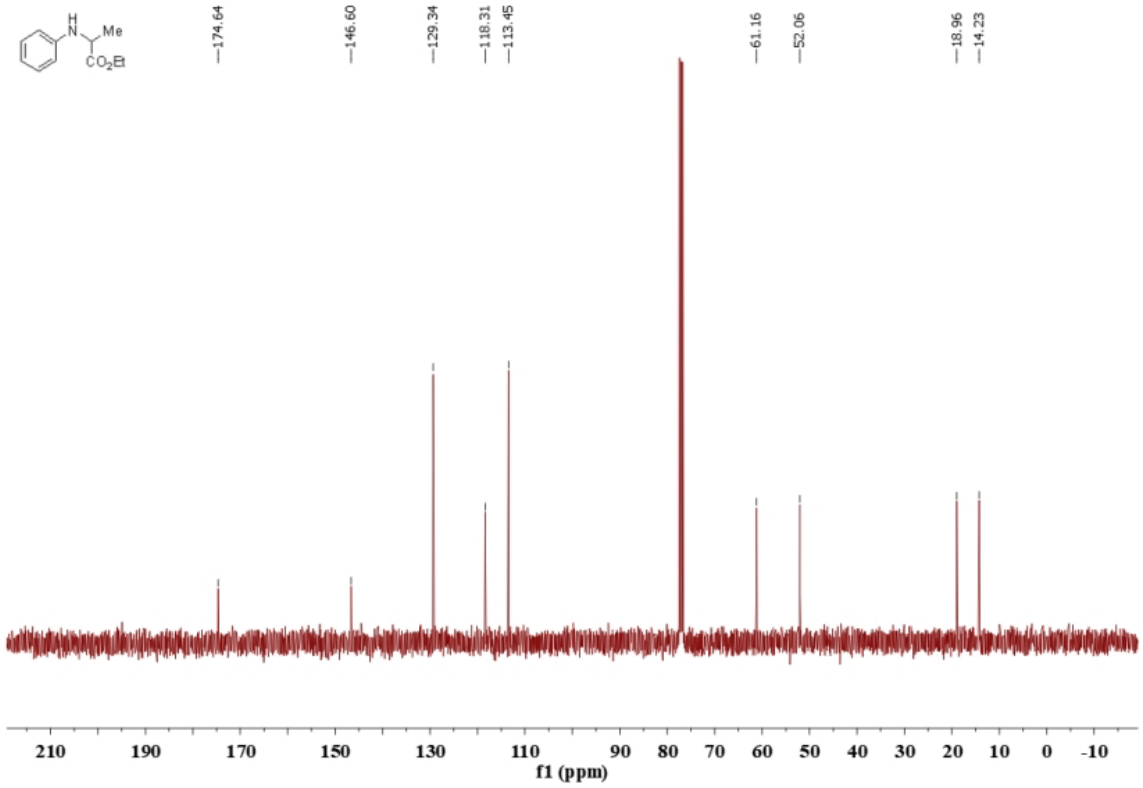


Figure S47. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-methyl-N-(p-tolyl) alaninate (**3b**)

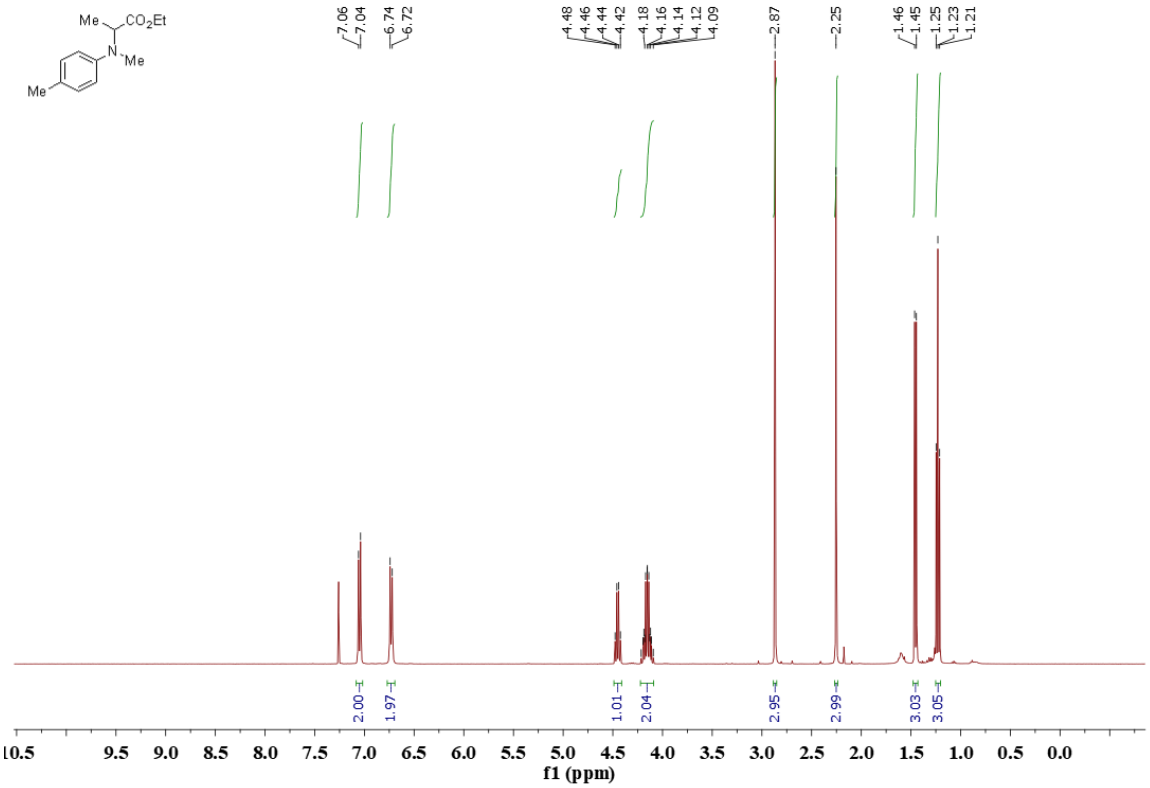


Figure S48. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-methyl-N-(p-tolyl) alaninate (**3b**)

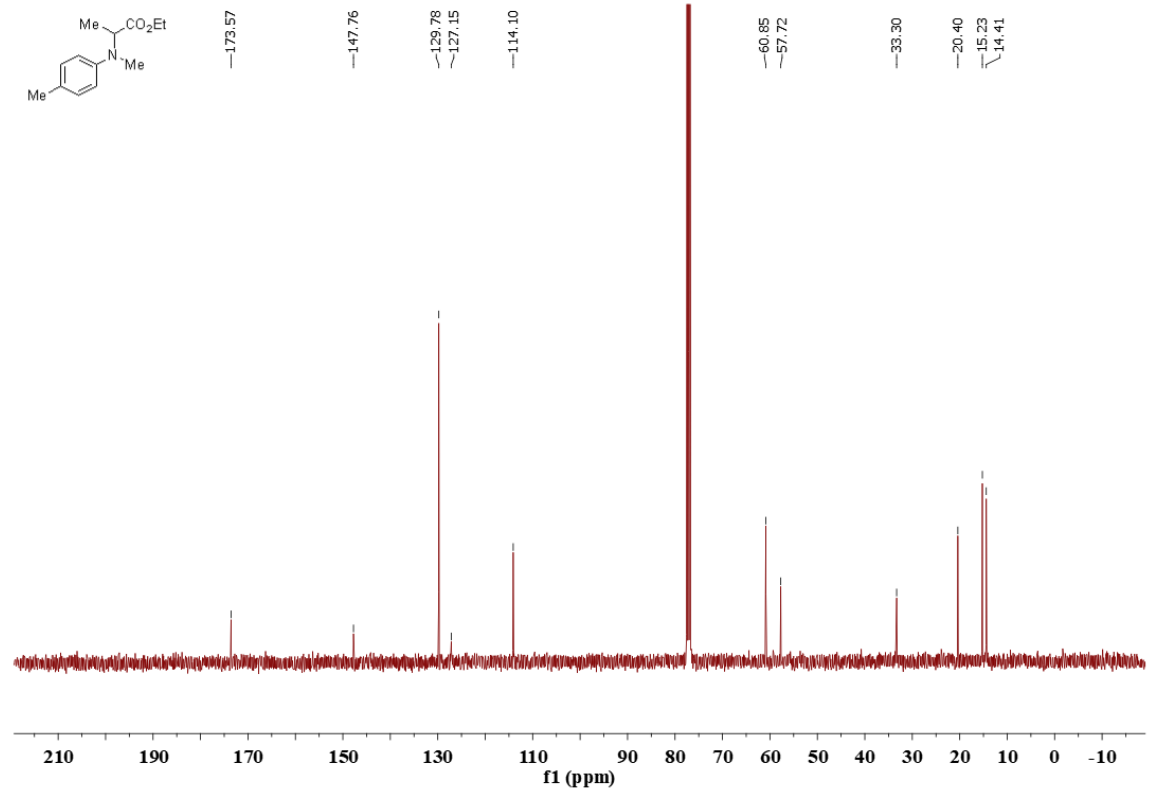


Figure S49. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**)

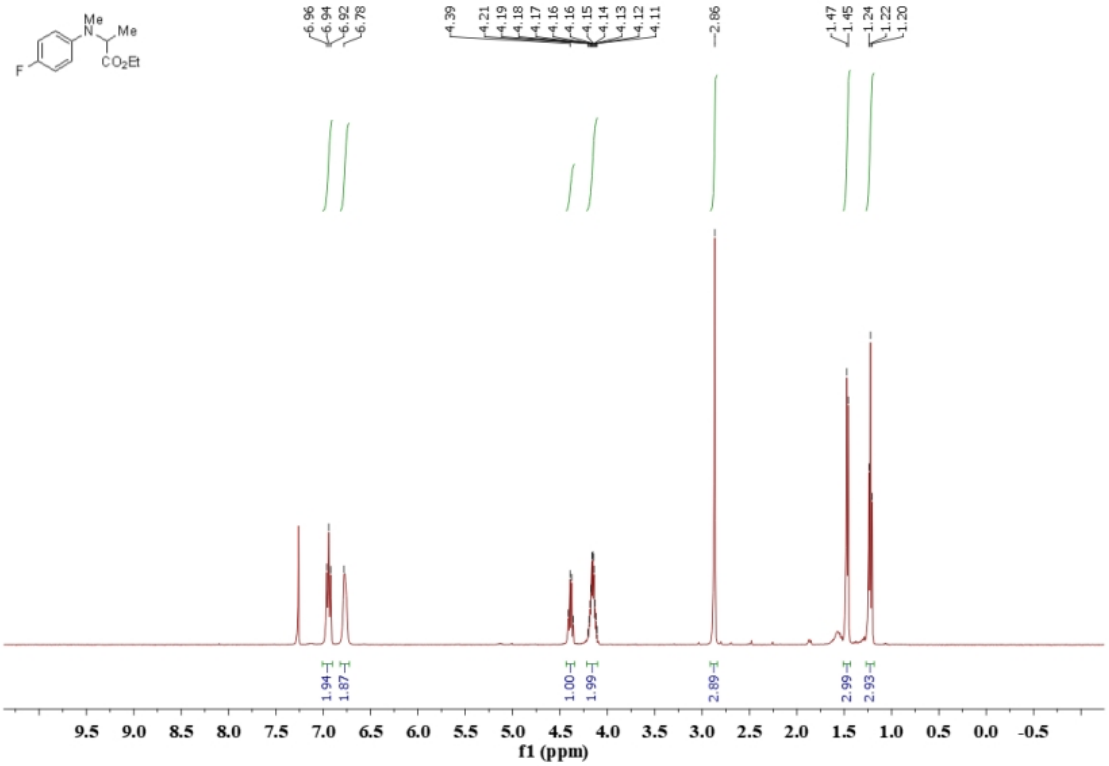


Figure S50. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-(4-fluorophenyl)-N-methylalaninate (**3c**)

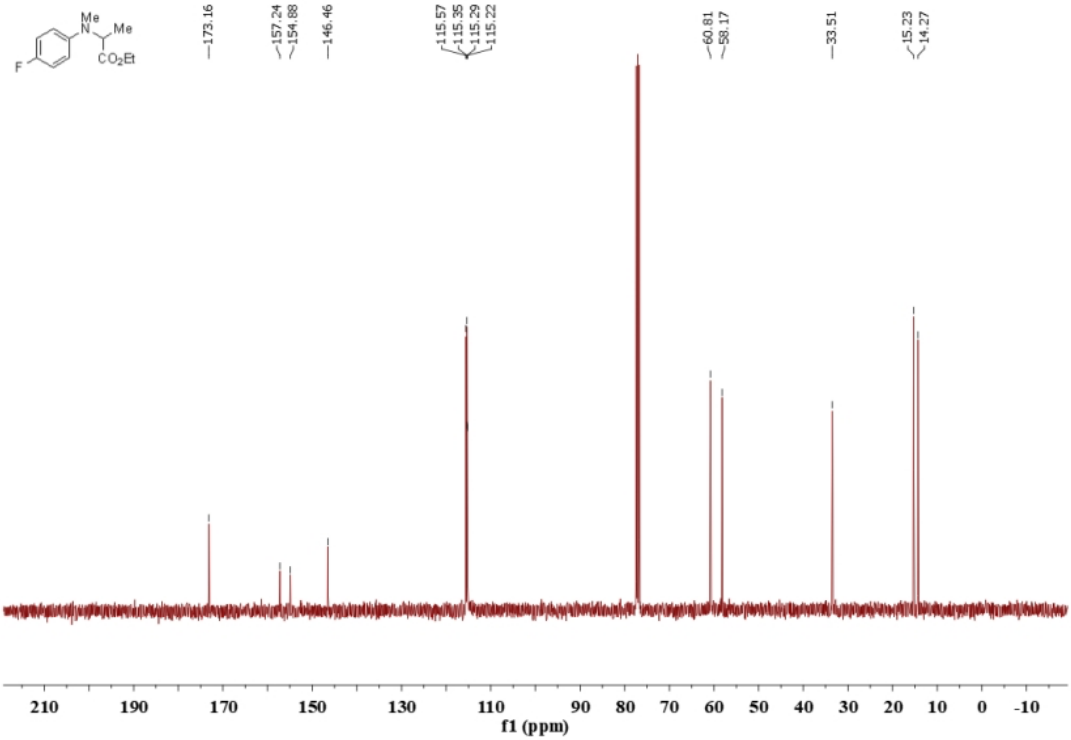


Figure S51. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**)

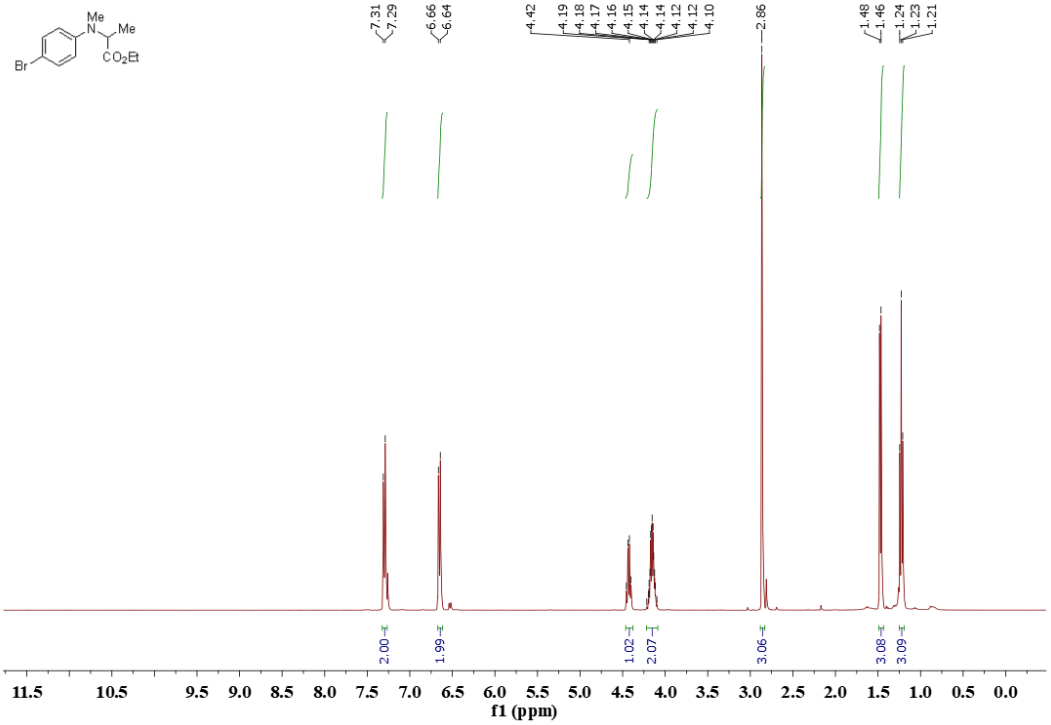


Figure S52. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-(4-bromophenyl)-N-methylalaninate (**3d**)

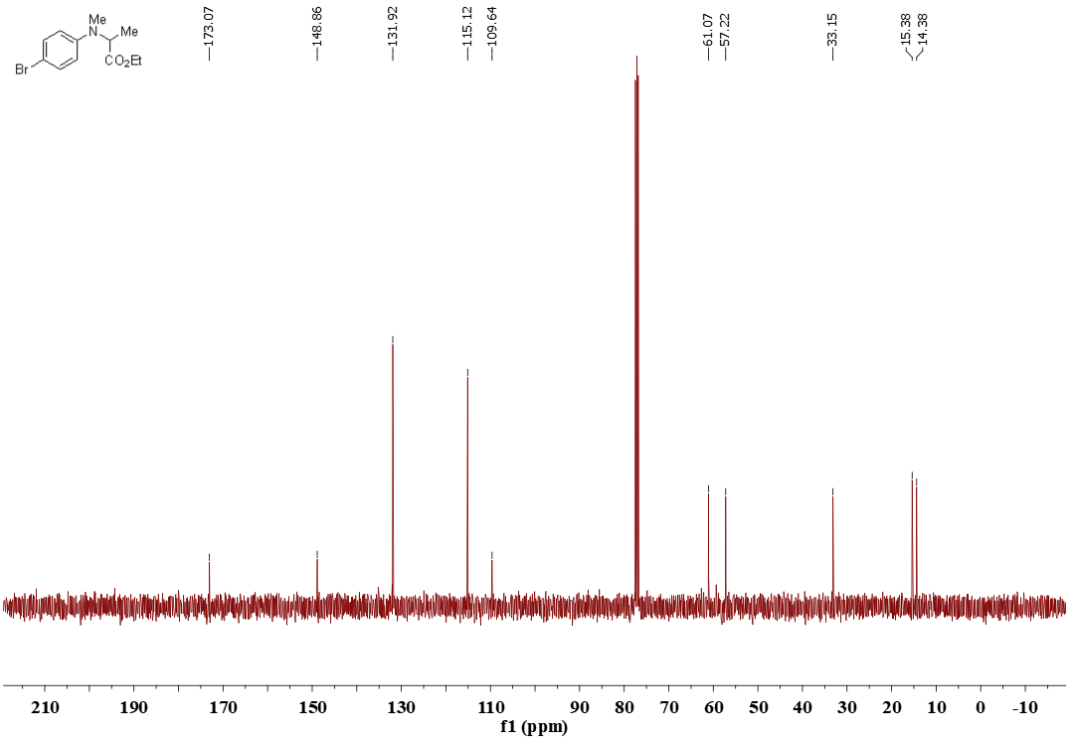


Figure S53. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**)

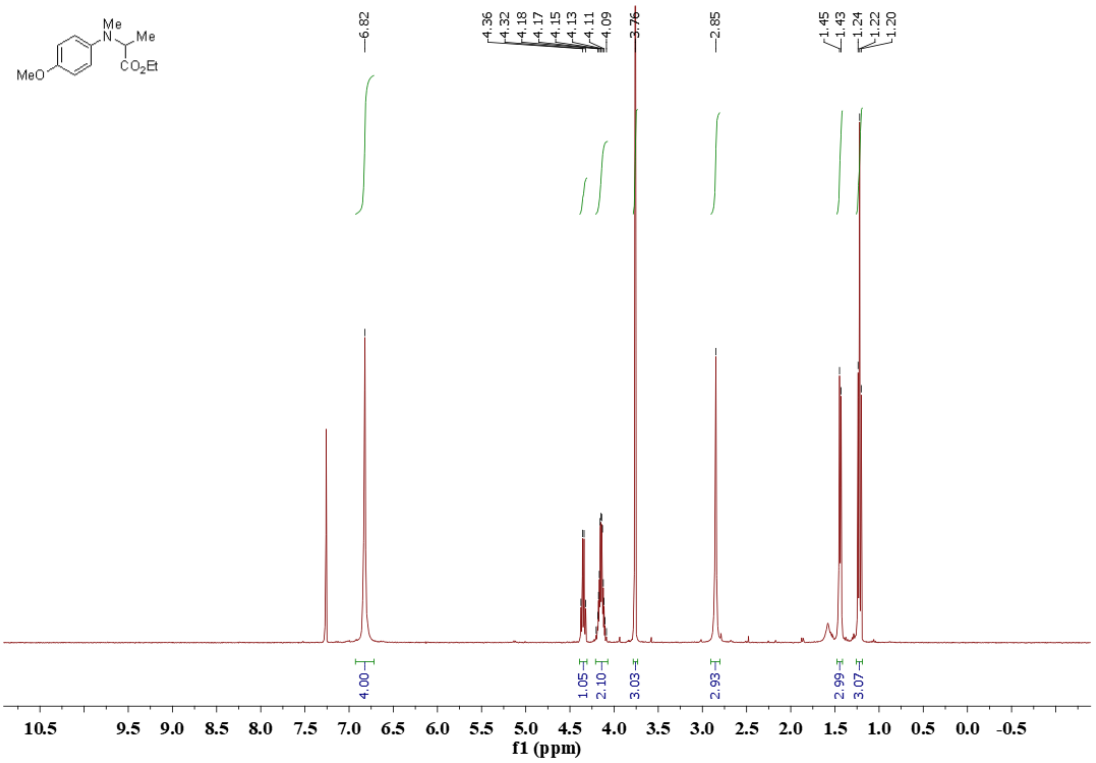


Figure S54. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-(4-methoxyphenyl)-N-methylalaninate (**3e**)

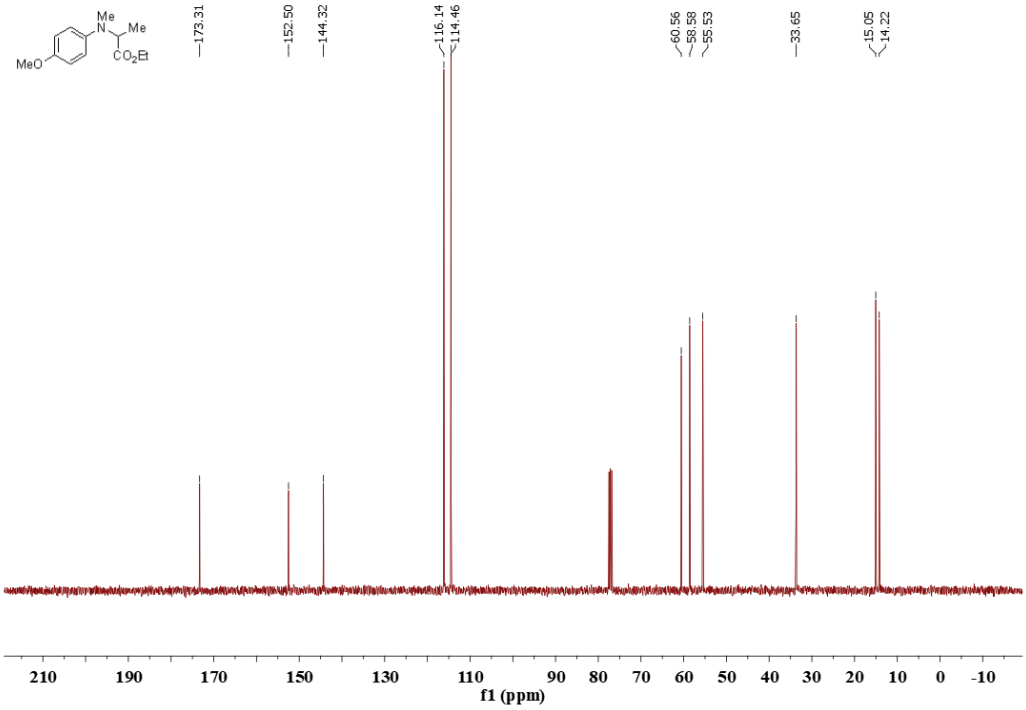


Figure S55. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-methyl-N-(4-(trifluoromethoxy)phenyl)alaninate (**3f**)

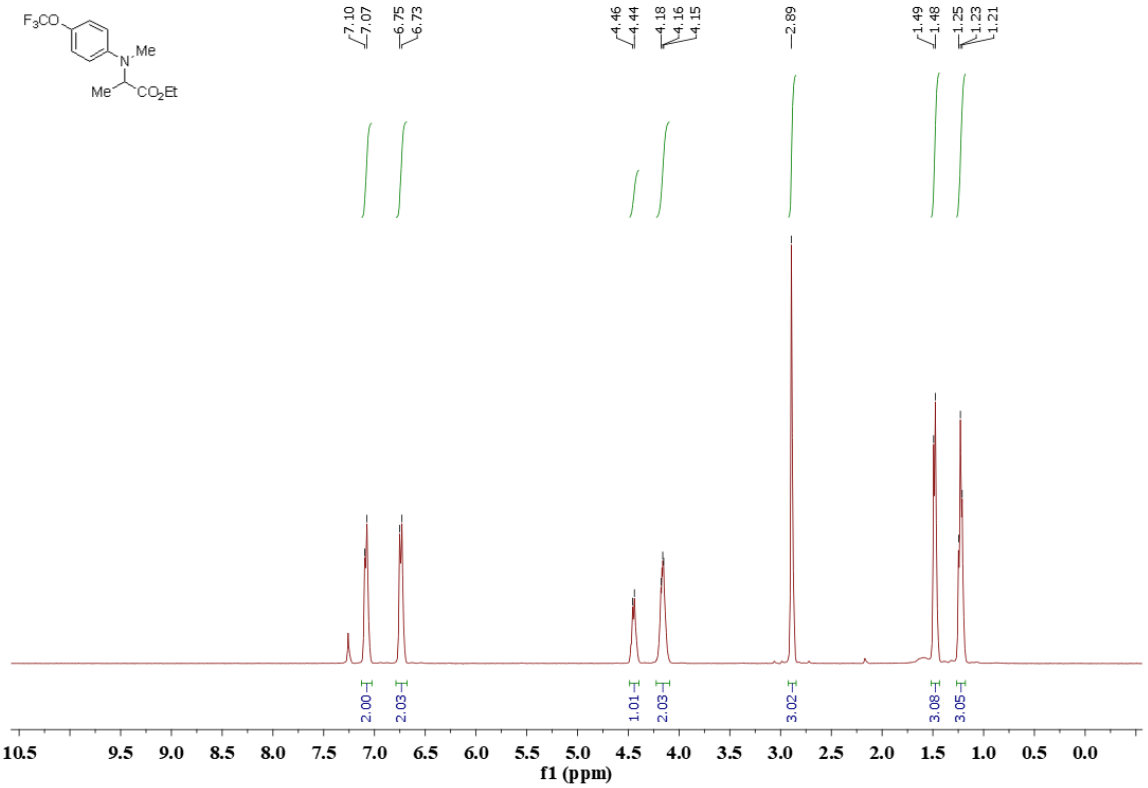


Figure S56. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-methyl-N-(4-(trifluoromethoxy)phenyl)alaninate (**3f**)

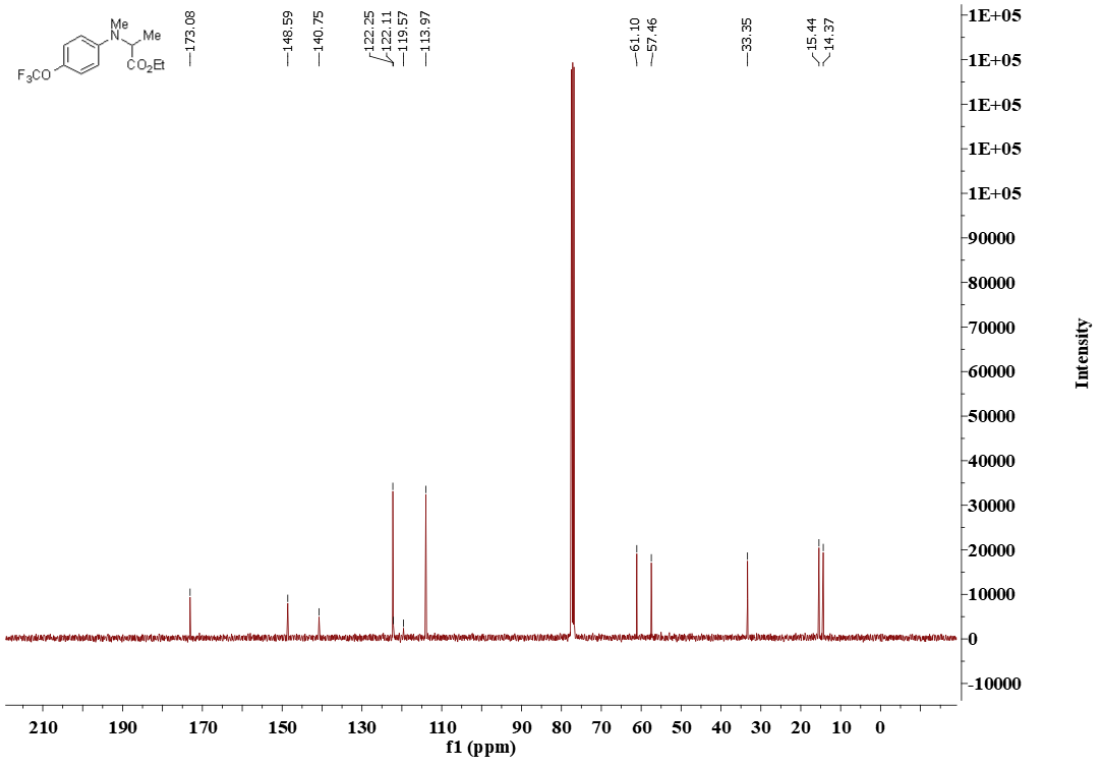


Figure S57. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**)

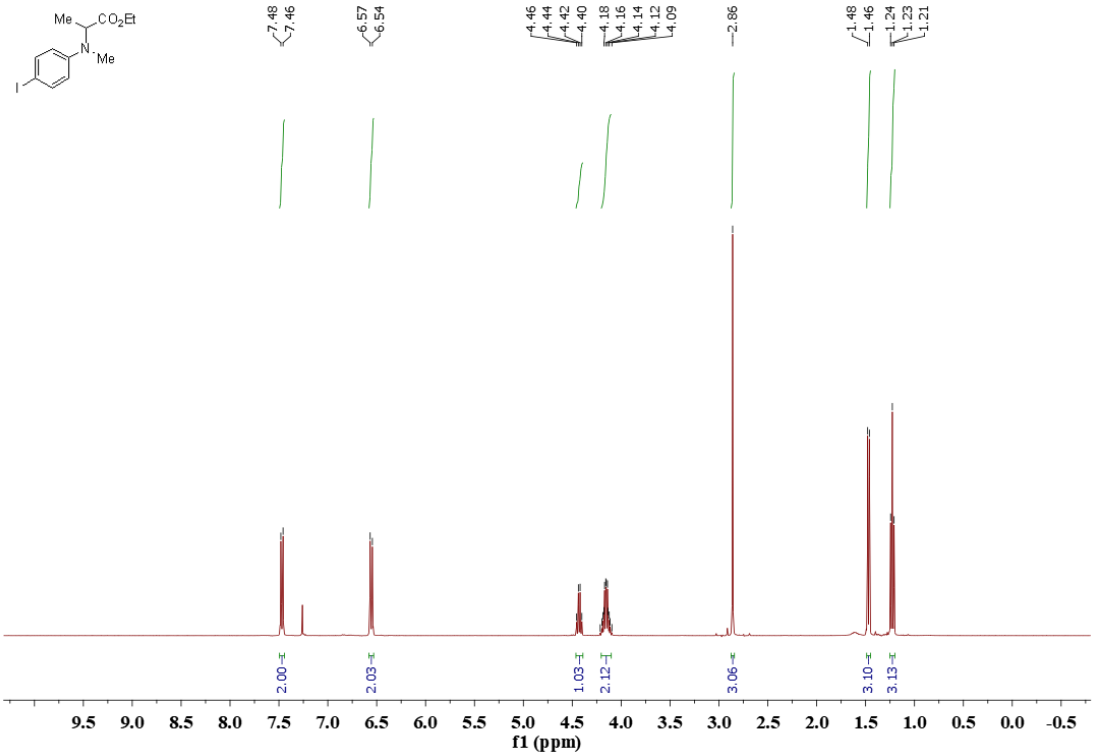


Figure S58. ^{13}C NMR (101 MHz, $CDCl_3$) of Ethyl N-(4-iodophenyl)-N-methylalaninate (**3g**)

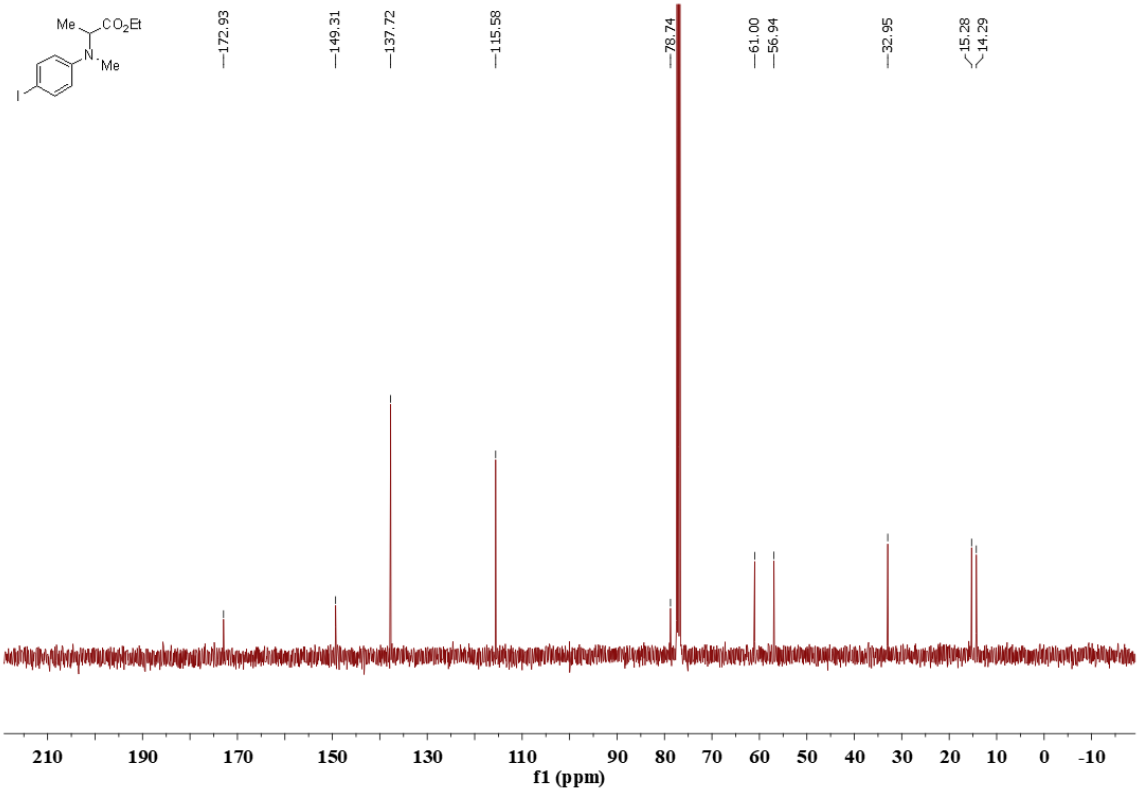


Figure S59. ^{1}HNMR(400 MHz, $CDCl_3$)of methyl 4-((1-ethoxy-1-oxopropan-2-yl)(methyl)amino)benzoate (**3h**)

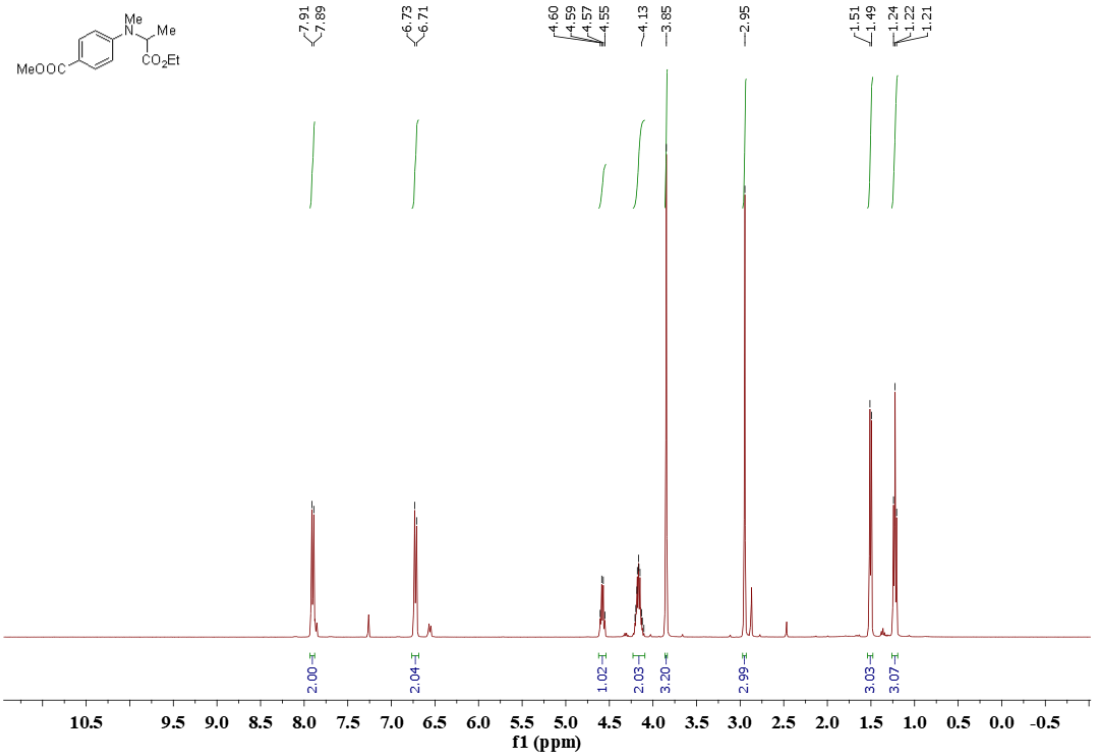


Figure S60. ^{13}C NMR(101 MHz, $CDCl_3$)of methyl 4-((1-ethoxy-1-oxopropan-2-yl)(methyl)amino)benzoate (**3h**)

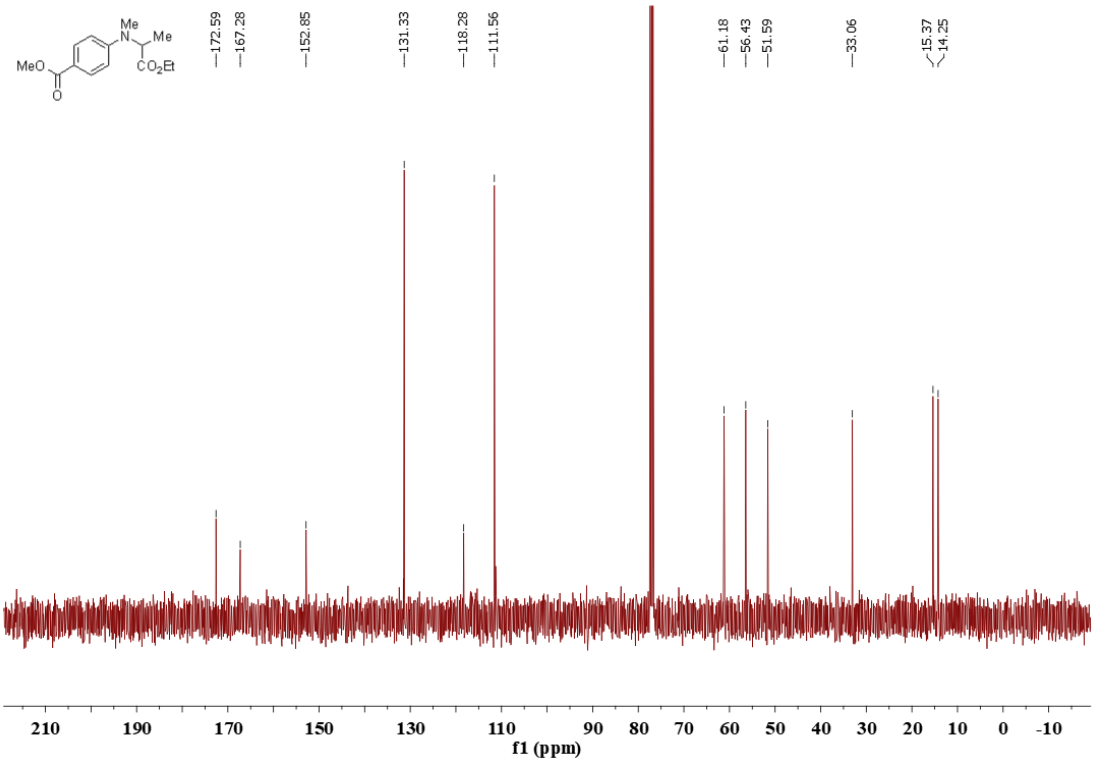


Figure S61. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-([1,1'-biphenyl]-4-yl)-N-methylalaninate (**3i**)

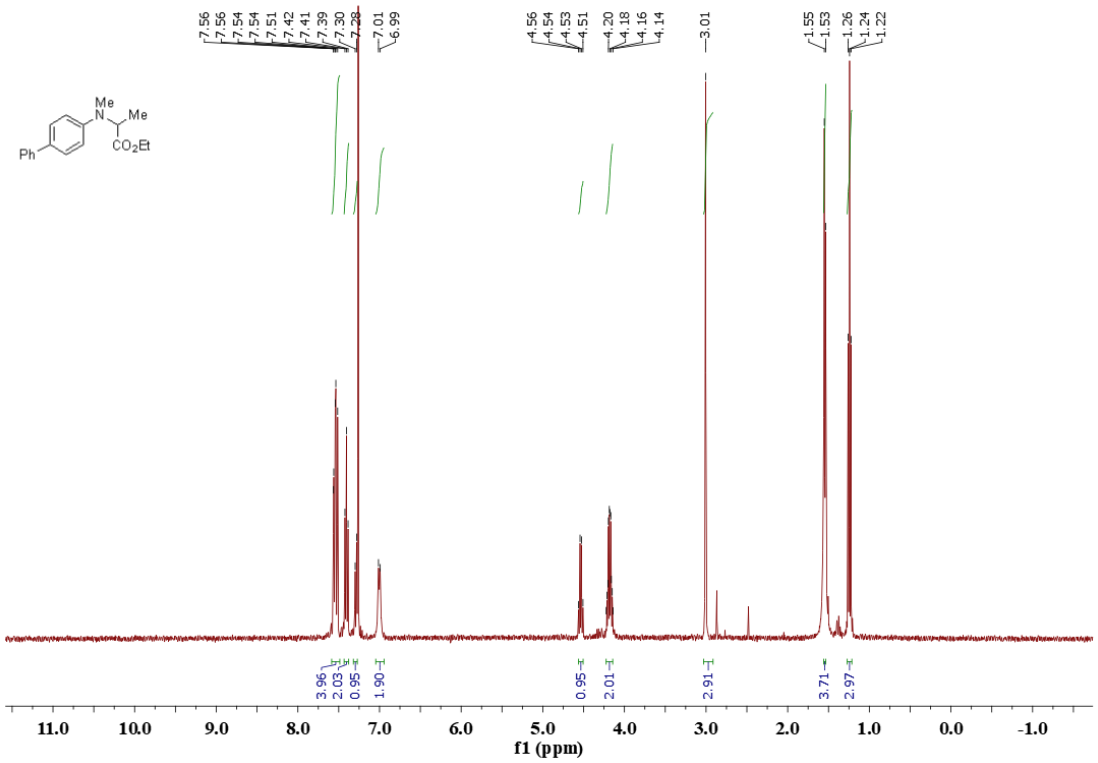


Figure S62. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-([1,1'-biphenyl]-4-yl)-N-methylalaninate (**3i**)

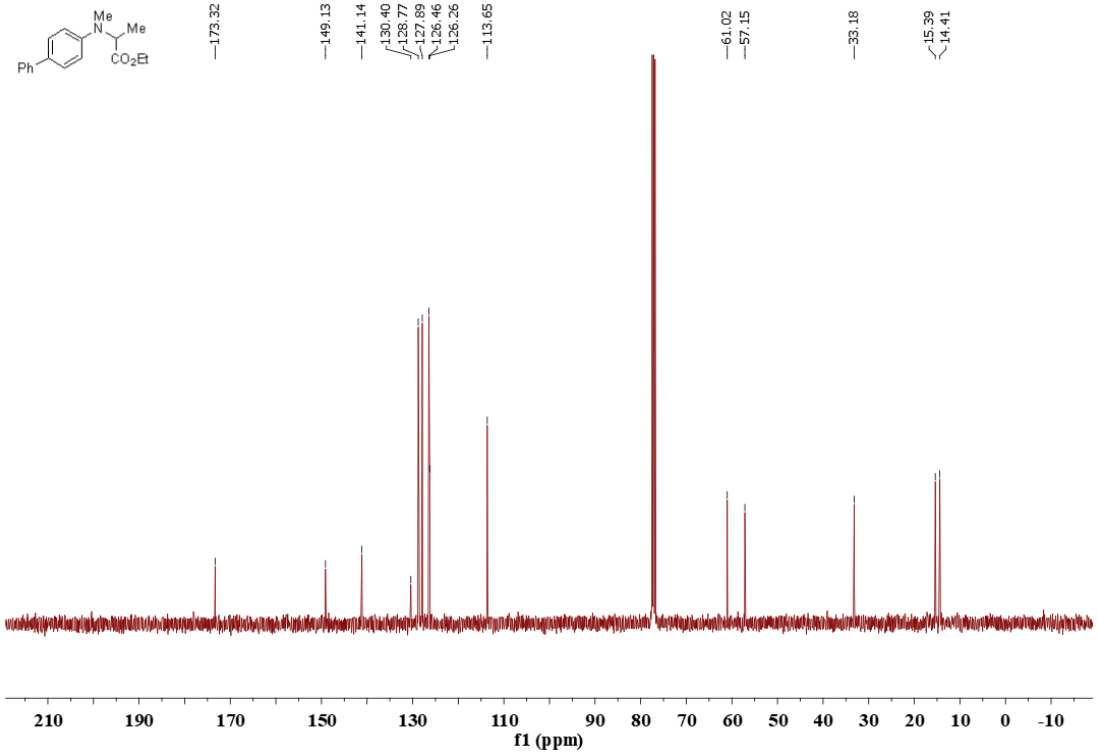


Figure S63. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-methyl-N-(m-tolyl)alaninate (**3j**)

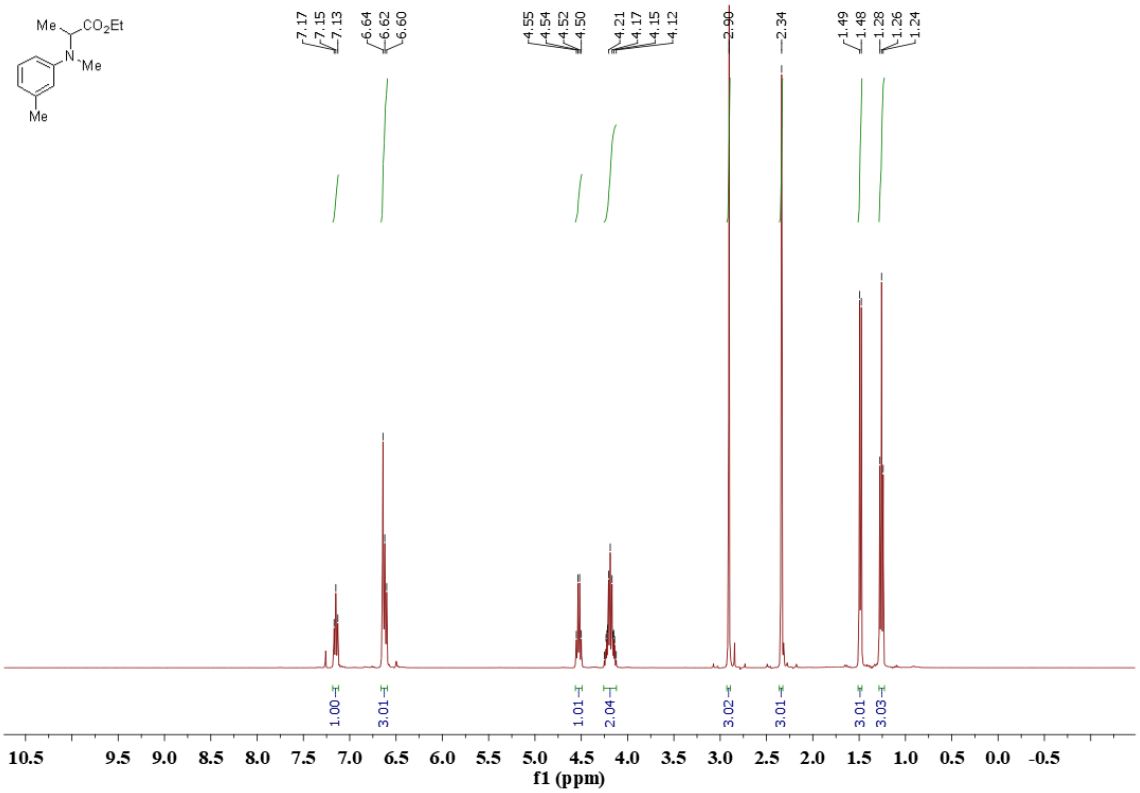


Figure S64. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-methyl-N-(m-tolyl)alaninate (**3j**)

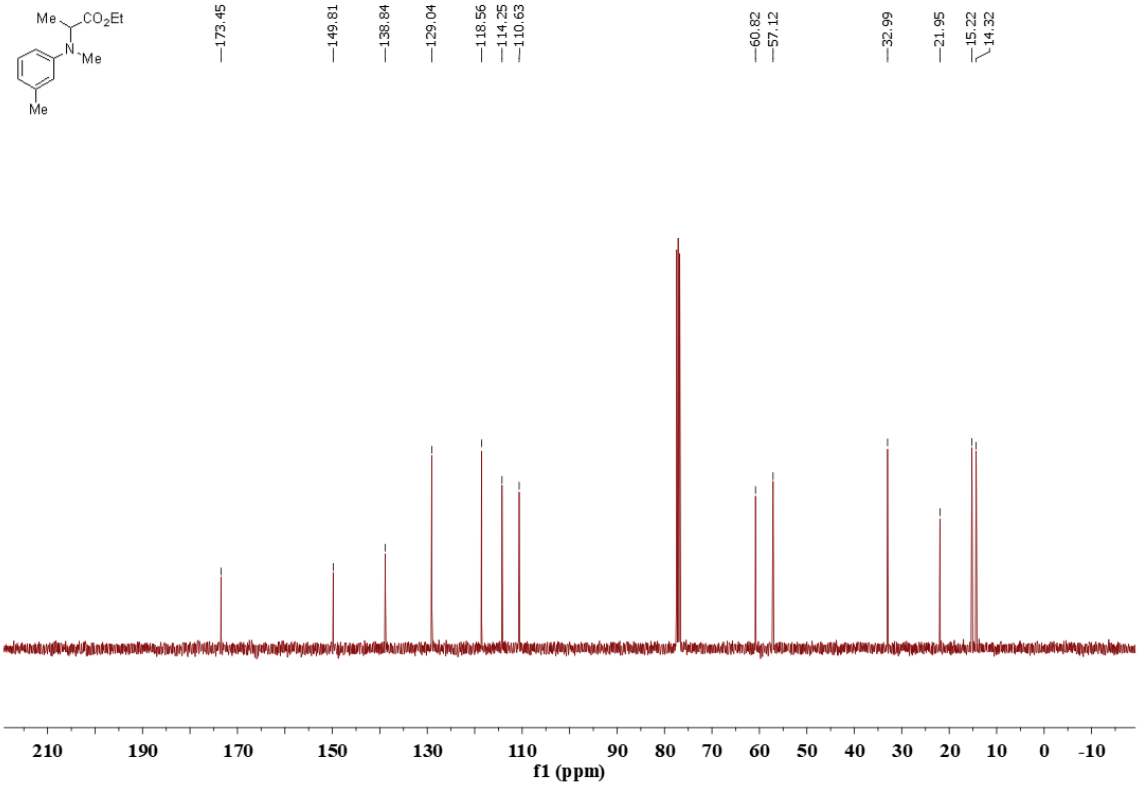


Figure S65. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**)

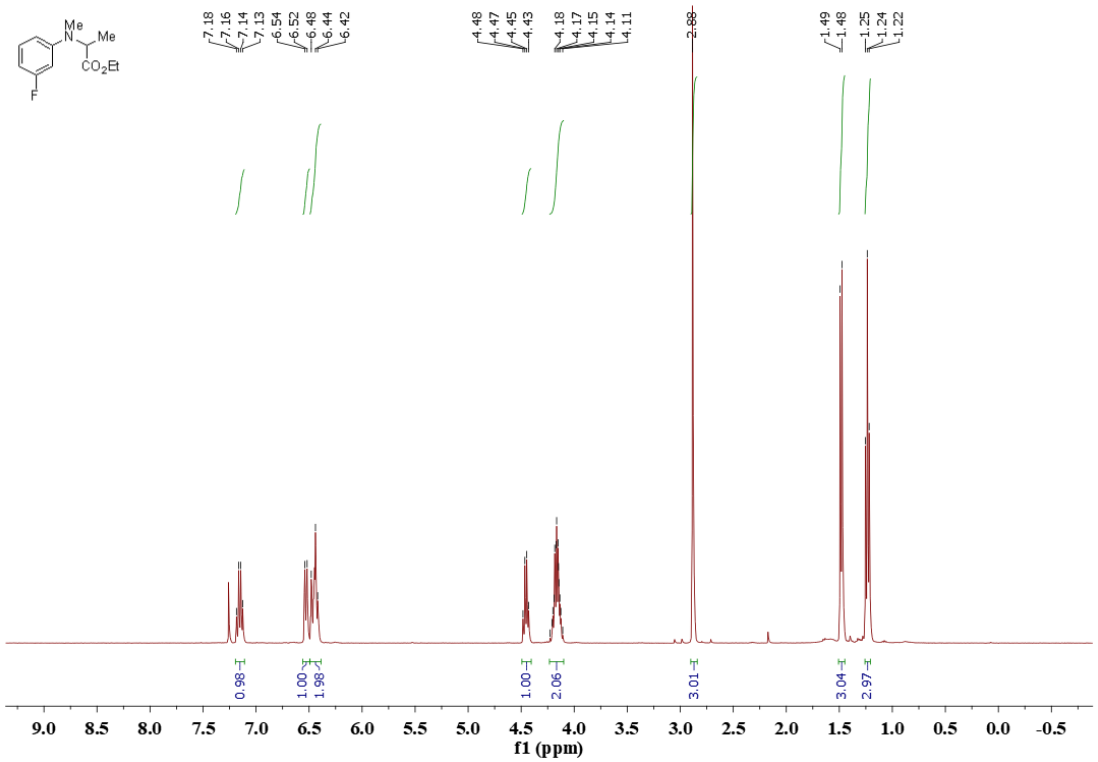


Figure S66. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-(3-fluorophenyl)-N-methylalaninate (**3k**)

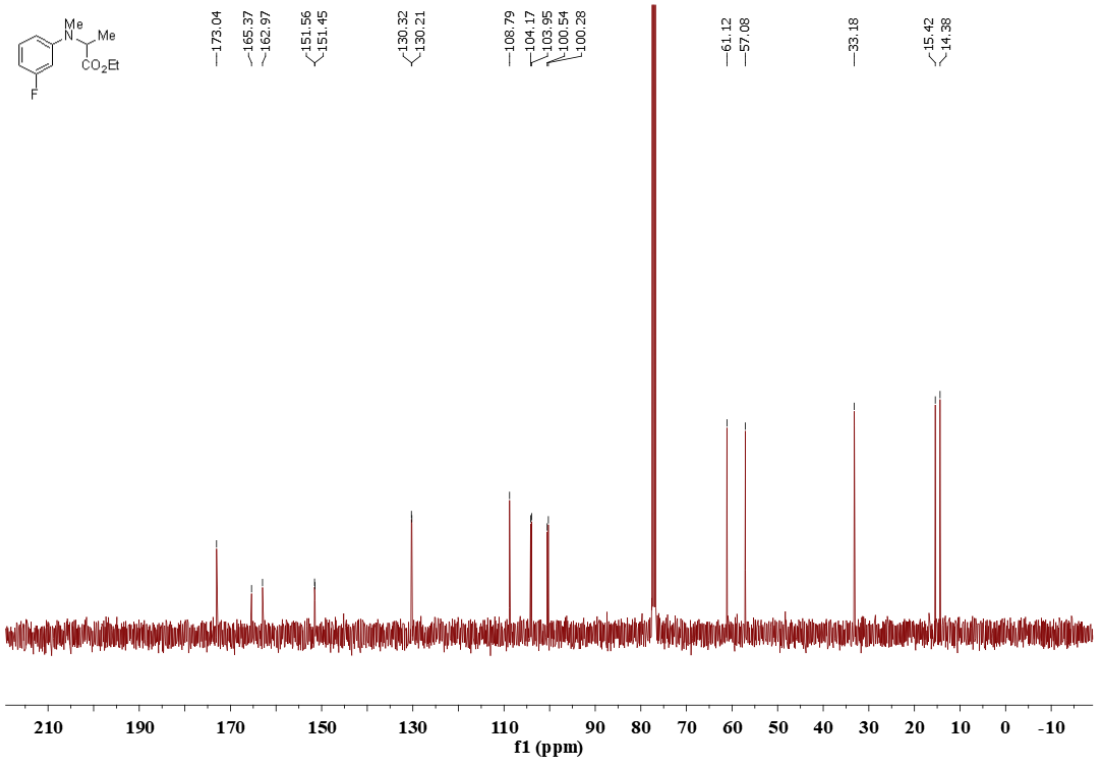


Figure S67. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-methyl-N-(3-(trifluoromethyl)phenyl)alaninate (**3l**)

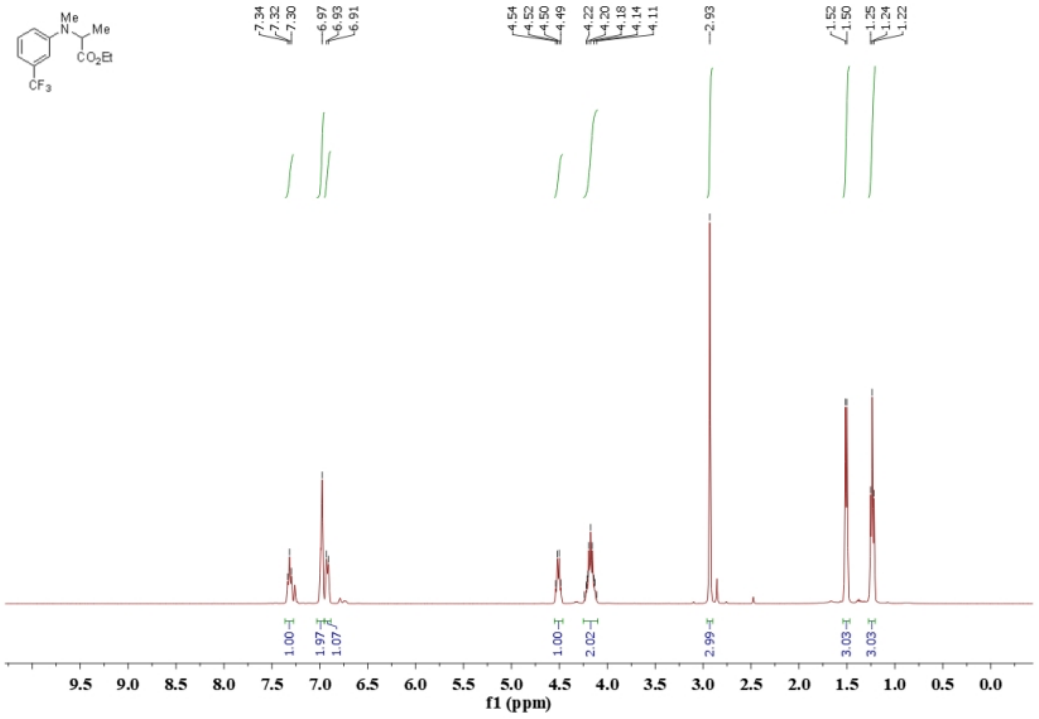


Figure S68. ^{13}C NMR (101 MHz, $CDCl_3$) of ethyl N-methyl-N-(3-(trifluoromethyl)phenyl)alaninate (**3l**)

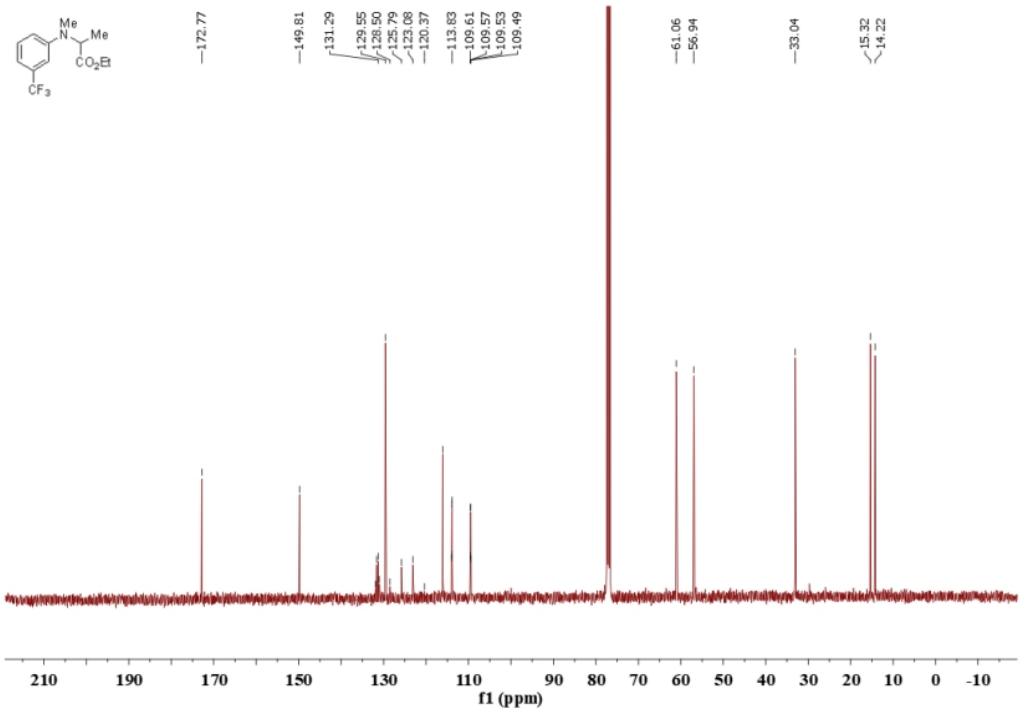


Figure S69. ^{1}H NMR (400 MHz, $CDCl_3$) of ethyl N-methyl-N-(o-tolyl)alaninate (**3m**)

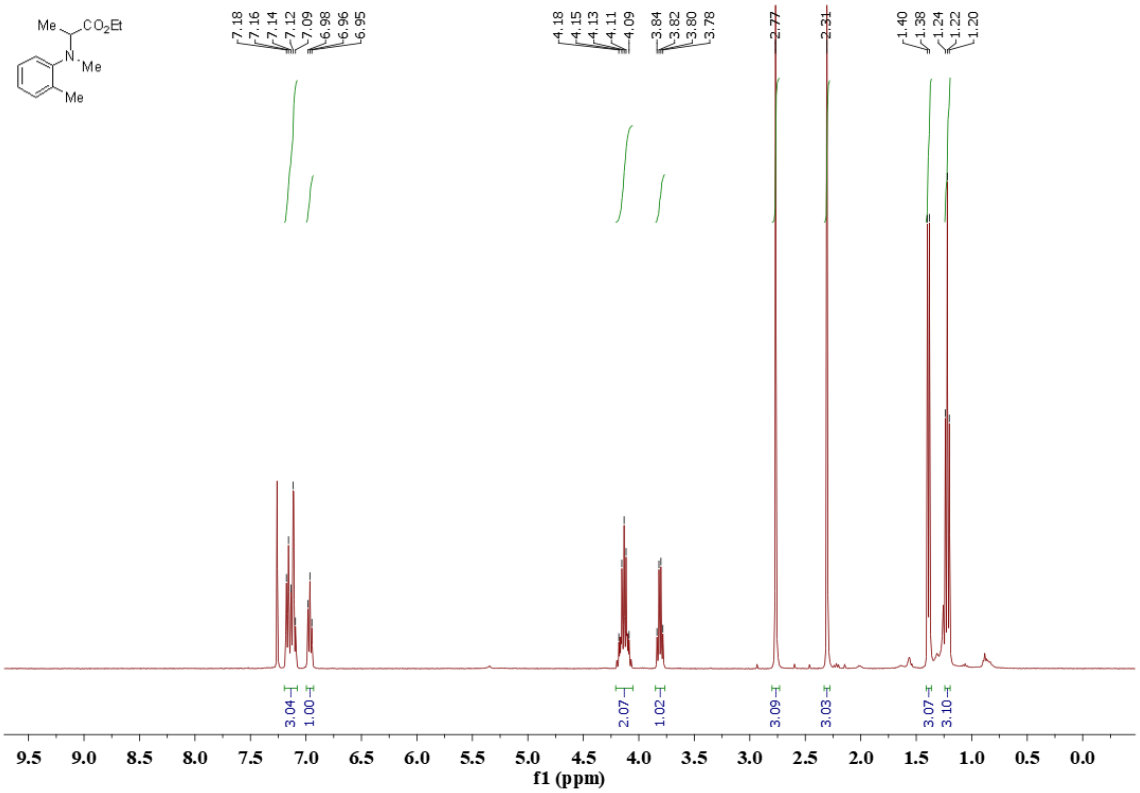


Figure S70. ^{13}CNMR(101 MHz, $CDCl_3$)of ethyl N-methyl-N-(o-tolyl)alaninate (**3m**)

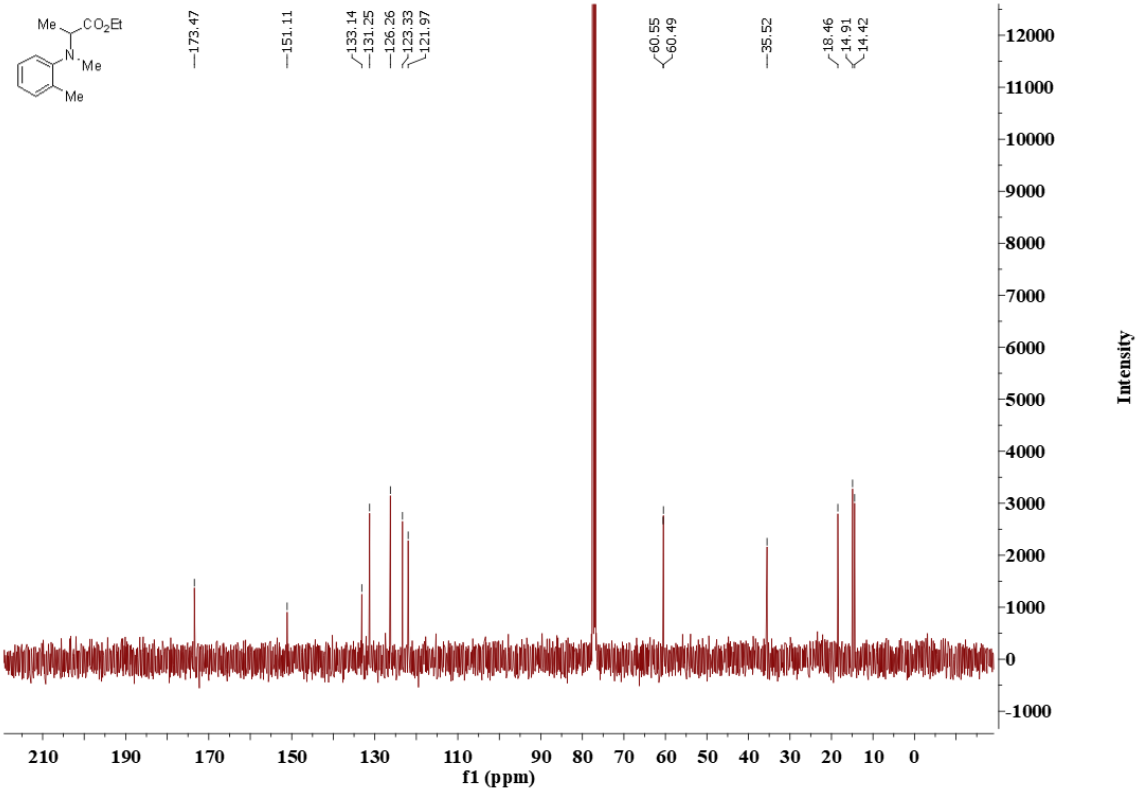


Figure S71. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**)

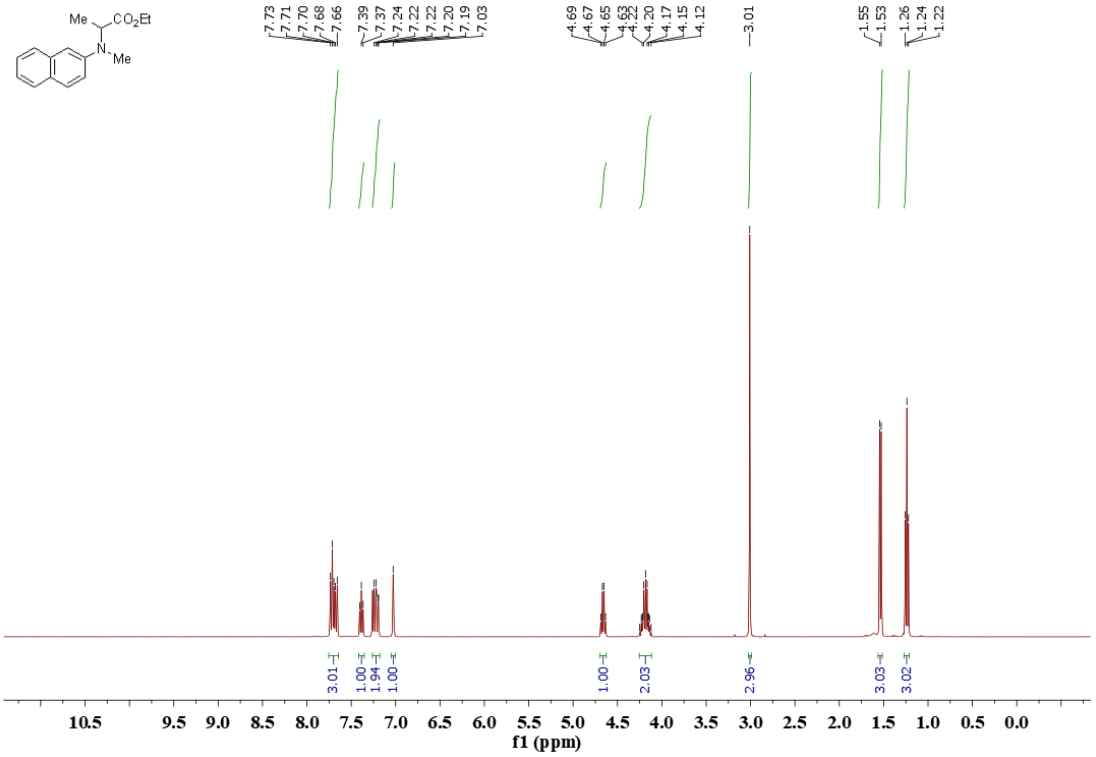
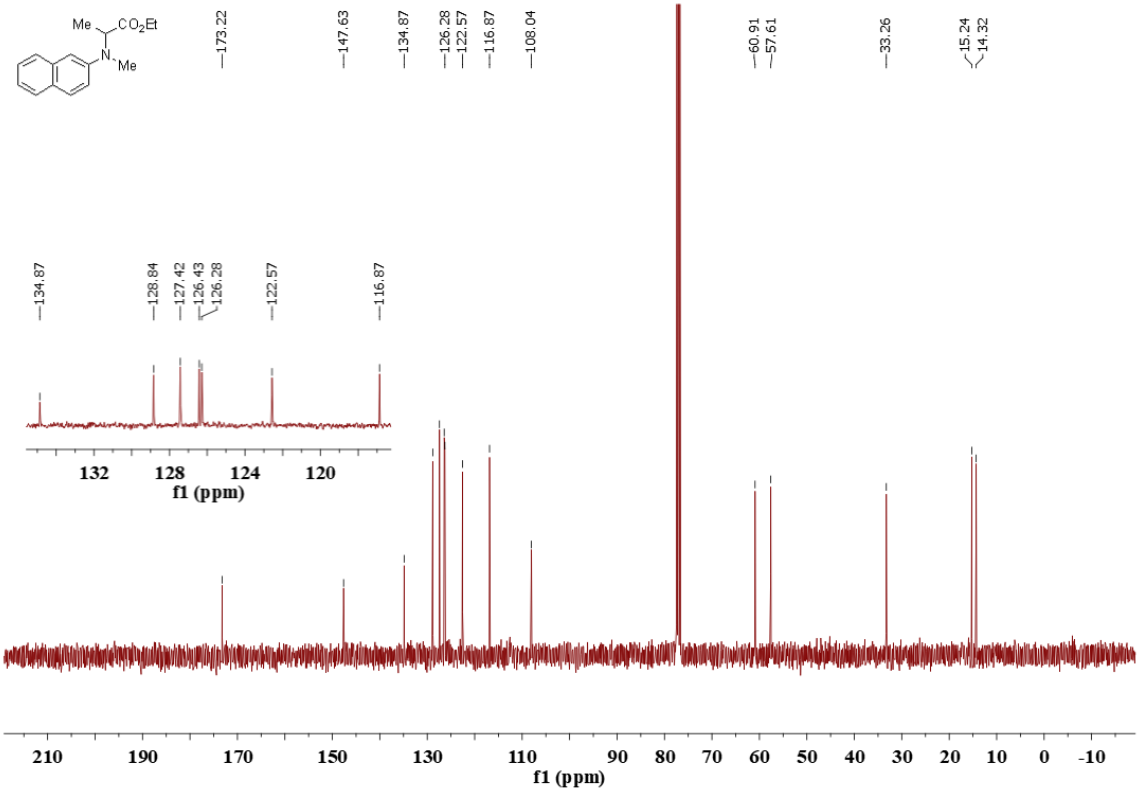


Figure S72. ^{13}C NMR (101 MHz, $CDCl_3$) of Ethyl N-methyl-N-(naphthalen-2-yl)alaninate (**3n**)



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