ChemBioChem

Supporting Information

Scalable and Selective β-Hydroxy-α-Amino Acid Synthesis Catalyzed by Promiscuous L-Threonine Transaldolase ObiH

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Part I. Substrate synthesis information

General Information: Chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, VWR, Chem-Impex International, Combi-blocks, Alfa Aesar, New England Biolabs, Zymo Research, Bio-Rad) and used without further purification unless otherwise noted. All small molecules were commercially available with exception of 4-OPiv-benzaldehyde and 4-OTf-benzaldehyde, which were previously reported.[1,2] BL21 (DE3) E. coli cells were electroporated with a Bio-Rad MicroPulser electroporator at 2500 V. New Brunswick I26R, 120 V/60 Hz shaker incubators (Eppendorf) were used for cell growth. Optical density and UV-vis measurements were collected on a UV-2600 Shimadzu spectrophotometer (Shimadzu). UPLC-MS data were collected on an Acquity UHPLC with an Acquity QDa MS detector (Waters) using an ACQUITY UPLC CSH BEH C18 column (Waters) or an Intrada Amino Acid column (Imtakt). Preparative flash chromatographic separations were performed on an Isolera One Flash Purification system (Biotage). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III-500 MHz spectrometer equipped with a DCH cryoprobe or a Bruker AVANCE-400 MHz spectrometer equipped with a BBFO probe. ^{1}H chemical shifts are reported in ppm (δ) relative to the solvent resonance (i.e., HOD, δ 4.79 ppm, δ DMSO 2.50 ppm, or δ MeOH 3.31 ppm). ^{13}C NMR data were acquired with ^{1}H decoupling and chemical shifts are reported in ppm (δ) relative to the solvent resonance (δ DMSO 39.52 ppm, δ MeOD 49.00 ppm, δ CD_3CN 1.32 ppm and 118.26 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), doublet of doublets (dd), multiplet (m)], coupling constants [Hz], integration). All NMR spectra were recorded at ambient temperature (20–25 °C).

Part II. Plasmid and protein information

Protein sequence of *N-His-ObiH* (Uniprot accession code: A0A1X9LWZ7):

MGSSHHHHHHSSMSNVKQQTAQIVDWLSSTLGKDHQYREDSLSLTANENYPSALVRLTSGSTAGAFYHCS
FPFEVPAGEWHFPEPGHMNAIADQVRDLGKTLIGAQAFDWRPNGGSTAEQALMLAACKPGEGFVHFAHRD
GGHFALESLAQKMGIEIFHLPVNPTSLLIDVAKLDEMVRRNPHIRIVILDQSFKLRWQPLAEIRSVLPDS
CTLTYDMSHDGGLIMGGVFDSPLSCGADIVHGNTHKTIPGPQKGYIGFKSAQHPLLVDTSLWVCPHLQSN
CHAEQLPPMWVAFKEMELFGRDYAAQIVSNAKTLARHLHELGLDVTGESFGFTQTHQVHFAVGDLQKALD
LCVNSLHAGGIRSTNIEIPGKPGVHGIRLGVQAMTRRGMKEKDFEVVARFIADLYFKKTEPAKVAQQIKE
FLQAFPLAPLAYSFDNYLDEELLAAVYQGAQR

Protein sequence of *N-His-PSDH* (Phenylserine dehydratase; Uniprot accession code: Q10725)***:***

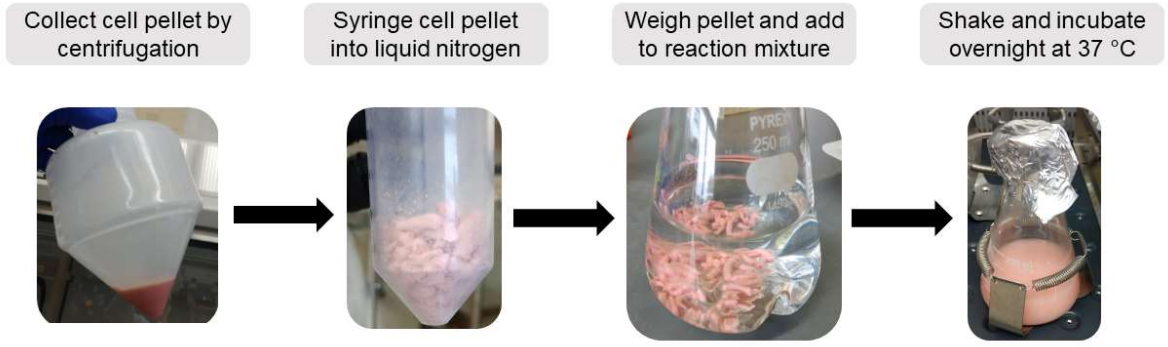
MGSSHHHHHHSSMTQLDTTTLPDLSAIAGLRARLKQWVRTTPVFDKTDFEPVPGTAVNFKLELLQASGTF
KARGAFSNLLALDDDQRAAGVTCVSAGNHAVGVAYAAMRLGIPAKVVMIKTASPARVALCRQYGAEVVLA
ENGQTAFDTVHRIESEEGRFFVHPFNGYRTVLGTATLGHEWLEQAGALDAVIVPIGGGGLMAGVSTAVKL
LAPQCQVIGVEPEGADAMHRSFETGGPVKMGSMQSIADSLMAPHTEQYSYELCRRNVDRLVKVSDDELRA
AMRLLFDQLKLATEPACATATAALVGGLKAELAGKRVGVLLCGTNTDAATFARHLGLG

Cloning and expression of ObiH

A codon-optimized copy of the ObiH gene was purchased as a gBlock from Integrated DNA Technologies. This DNA fragment was inserted into a pET-28b(+) vector by the Gibson Assembly method.[3] BL21 (DE3) *E. coli* cells were subsequently transformed with the resulting cyclized DNA product via electroporation. After 45 min of recovery in Luria-Burtani (LB) media containing 0.4% glucose at 37 °C, cells were plated onto LB plates with 50 µg/mL kanamycin (Kan) and incubated overnight. Single colonies were used to inoculate 5 mL LB + 50 µg/mL Kan, which were grown overnight at 37 °C, 200 rpm. Expression cultures, typically 1 L of Terrific Broth (TB) + 50 µg/mL Kan (TB-Kan), were inoculated from these starter cultures and shaken (180 rpm) at 37 °C. After 3 hours (OD_{600} = ~0.6), the expression cultures were chilled on ice. After 30 min on ice, ObiH expression was induced with 0.5 mM IPTG, and the cultures were expressed for 16 hours at 20 °C with shaking at 180 rpm. Cells were then harvested by centrifugation at 4,300×*g* at 4 °C for 10 min. Cell pellets were pink in color and were used immediately for preparation of freeze-dried cells. Cell pellets were frozen and stored at -20 °C if cells were used for protein purification.

Preparation of freeze-dried ObiH cells

Cell pellets were transferred into a 10 mL syringe and injected into liquid nitrogen to flash freeze cells as thin 'cell noodles' which will facilitate easy weighing of the biocatalyst when setting up reactions. The flash frozen cells were stored at -80 °C.



Purification of ObiH

To purify ObiH, cell pellets were thawed on ice and then resuspended in lysis buffer (50 mM potassium phosphate buffer buffer (pH = 8.0), 500 mM NaCl, 1 mg/ml Hen Egg White Lysozyme (GoldBio), 0.2 mg/ml DNaseI (GoldBio), 1 mM $MgCl_2$, 1 X BugBuster Protein extraction reagent (Novagen), and 400 µM pyridoxal 5'-phosphate (PLP)). A volume of 4 mL of lysis buffer per gram of wet cell pellet was used. After 45 min of shaking at 37 °C, the resulting lysate was then spun down at 75,600×*g* to pellet cell debris. The pellet was colorless whereas the supernatant was pink in color. Ni/NTA beads (GoldBio) were added to the supernatant and incubated on ice for 45 min prior to purification by Ni-affinity chromatography with a gravity column. The column was washed with 5 column volumes of 20 mM imidazole, 500 mM NaCl, 10% glycerol, 50 mM potassium phosphate buffer (pH = 8.0). Washing with higher concentrations of imidazole resulted in slow protein elution. ObiH was eluted with 250 mM imidazole, 500 mM NaCl, 10% glycerol, 50 mM potassium phosphate buffer, pH 8.0. Elution of the desired protein product was monitored by the disappearance of its bright red color (resulting from the release of ObiH) from the column. The protein

product was dialyzed to < 1 µM imidazole in 100 mM Tris buffer, pH 8.5 containing 2 mM DTT. Purified enzyme was flash frozen in pellet form by pipetting enzyme dropwise into a crystallization dish filled with liquid nitrogen. The enzyme was transferred to a plastic conical and stored at -80 °C until further use. Frozen pellets were thawed at room temperature and centrifuged before use. The concentration of protein was determined by Bradford assay using bovine serum albumin for a standard concentration curve. Generally, this procedure yielded 200 – 250 mg per L culture. Protein purity was analyzed by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis using 12% polyacrylamide gels.

Cloning, expression, and purification of phenylserine dehydratase (PSDH)

A codon-optimized copy of the PSDH gene was purchased as a gBlock from Integrated DNA Technologies. This DNA fragment was inserted into a pET-28b(+) vector by the Gibson Assembly method.[3] BL21 (DE3) *E. coli* cells were subsequently transformed with the resulting cyclized DNA product via electroporation. After 45 min of recovery in Luria-Burtani (LB) media containing 0.4% glucose at 37 °C, cells were plated onto LB plates with 50 µg/mL kanamycin (Kan) and incubated overnight. Single colonies were used to inoculate 5 mL LB + 50 µg/mL Kan, which were grown overnight at 37 °C, 200 rpm. Expression cultures, typically 1 L of Terrific Broth (TB) + 50 µg/mL Kan (TB-Kan), were inoculated from these starter cultures and shaken (180 rpm) at 37 °C. After 3 hours (OD_{600} = ~0.6), the expression cultures were chilled on ice. After 30 min on ice, PSDH expression was induced with 0.5 mM IPTG, and the cultures were expressed for 16 hours at 20 °C with shaking at 180 rpm. Cells were then harvested by centrifugation at 4,300×*g* at 4 °C for 10 min. Cell pellets were frozen and stored at -20 °C if cells were used for protein purification.

To purify PSDH, cell pellets were thawed on ice and then resuspended in lysis buffer (50 mM potassium phosphate buffer buffer (pH = 8.0), 500 mM NaCl, 1 mg/ml Hen Egg White Lysozyme (GoldBio), 0.2 mg/ml DNaseI (GoldBio), 1 mM $MgCl_2$, 1 X BugBuster Protein extraction reagent (Novagen), and 400 µM pyridoxal 5'-phosphate (PLP)). A volume of 4 mL of lysis buffer per gram of wet cell pellet was used. After 45 min of shaking at 37 °C, the resulting lysate was then spun down at 75,600×*g* to pellet cell debris. The pellet was colorless whereas the supernatant was yellow in color. Ni/NTA beads (GoldBio) were added to the supernatant and incubated on ice for 45 min prior to purification by Ni-affinity chromatography with a gravity column. The column was washed with 5 column volumes of 20 mM imidazole, 500 mM NaCl, 10% glycerol, 50 mM potassium phosphate buffer (pH = 8.0). PSDH was eluted with 250 mM imidazole, 500 mM NaCl, 10% glycerol, 50 mM potassium phosphate buffer, pH 8.0. Elution of the desired protein product was monitored by the disappearance of its bright yellow color (resulting from the release of PSDH) from the column. The protein product was dialyzed to < 1 µM imidazole in 100 mM Tris buffer, pH 8.5 containing 2 mM DTT. Purified enzyme was flash frozen in pellet form by pipetting enzyme dropwise into a crystallization dish filled with liquid nitrogen. The enzyme was transferred to a plastic conical and stored at -80 °C until further use. Frozen pellets were thawed at room temperature and centrifuged before use. The concentration of protein was determined by Bradford assay using bovine serum albumin for a standard concentration curve. Generally, this procedure yielded 125 – 150 mg per L culture. Protein purity was analyzed by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis using 12% polyacrylamide gels.

Preparation of phototreated ObiH

ObiH stock solutions (150 – 400 μM) or diluted samples in quartz cuvettes were placed on ice directly under an 8 Watt, green LED bulb for 10 min. The protein solutions were subsequently kept on ice or in the UV-spectrophotometer for 45 min, followed by a second round of green light treatment for 10 minutes which ensured complete abolishment of the 515 nm band.

Kinetics and UV-Vis Spectroscopy

Data were collected between 600 and 250 nm on a UV-2600 Shimadzu spectrophotometer (Shimadzu) with a semi-micro quartz cuvette (Starna Cells) at 25 °C (unless stated otherwise). ObiH stock solutions were diluted to 20 μM in 100 mM Tris-HCl, pH 8.5 and phototreated. To monitor product reentry, 20 μM ObiH samples were prepared in 100 mM Tris-HCl, pH 8.5 and phototreated. Stocks of 4-chlorophenylserine were prepared in at 100 mM concentration. Products were added to a final concentration of 5 mM and spectra were gathered after a two-minute incubation period at 30 °C.

Part III. Biocatalytic reaction conditions and products

Stock solutions: Stock solutions of each aldehyde (500 mM) were prepared by dissolving the substrate in MeOH or DMSO (analytical grade). 500 mM L-threonine (Thr) stock solutions were prepared in 100 mM Tris pH 8.5. Aliquots of purified ObiH were stored at -80 °C.

Calculation of UPLC yield using Marfey's Derivatization

General Procedure: All reactions were done in duplicate on analytical scale (50 μL). Stocks of Thr were made in 50 mM Tris pH 8.5 and aldehydes were prepared in MeOH. All samples were analyzed following Marfey's derivatization by Waters Acquity UPLC-PDA-MS using a BEH C18 column (Waters). Derivatized amino acid product quantitation was performed by PDA analysis, integrating the area under the product curve and correcting by dividing by the internal standard (tryptamine) peak area. To calculate product concentrations, a standard curve was generated by subjecting stock solutions of L-Phe in buffer using the identical procedure used to process and derivatize enzymatic reaction solutions, in duplicate. These curves were used to calculate the concentrations of β-hydroxy-α-amino acid product in solution.

General Marfey's Procedure: A Marfey's derivatization reaction was performed to assess UPLC yield of all the compounds in the substrate scope. In a microcentrifuge tube, 25 μL of quenched reaction mix (1 equiv., 1.0 mM final total amines from unreacted Thr and formed and β-hydroxy-α-amino acid product) was added to a solution of 125 μL of 15 mM $NaHCO_3$ (10 equiv., 5 mM final concentration) followed by addition of 150 μL 10 mM L-FDAA dissolved in ACN (4 equiv., 5 mM final concentration) to bring the total reaction volume to 300 μL. Each reaction was placed in a dark 37 °C incubator for 12 h, then quenched with 300 μL of 1:1 ACN:60 mM HCl (15 mM post-quench) before analyzing by UPLC-MS.

General procedure for whole cell biocatalytic reactions

A 500 mL glass bottle was charged with 100 mM Tris buffer (pH 8.5) and Methanol (4% v/v). Then, the corresponding aldehyde (1 equiv., 20 mM final concentration) and Thr (5.0 equiv., 100 mM final concentration) were added to the solution. Water was added to adjust the final reagent concentration to the appropriate amounts. The reaction was initiated upon the addition of freeze-dried *E. coli* cells harboring expressed ObiH (10-20 mg/mL, 1-2% w/v). Reaction vessel was placed in the shaking incubator at 37 °C for 20 h. Product formation was monitored by UPLC-MS. After reaction completion, the reaction mixture was quenched with an equivalent volume of acetonitrile (ACN) and centrifuged (4,300×*g*, 15 min) to remove aggregated protein. Supernatant was transferred to a clean beaker and pellets were resuspended with 1 equivalent volume of ACN, centrifuged and supernatant collected. The decanted supernatant was then concentrated to ~10 mL by rotary evaporation and loaded onto a preparative reverse-phase C18 column pre-equilibrated with water. Purification was performed via gradient elution on an Isolera One Flash Purification system (Biotage). Fractions bearing product (confirmed by UPLC-MS sampling of fraction tubes) were pooled and dried by rotary evaporation. The product was then resuspended in a minimal quantity of water, transferred to a pre-weighed 20 mL vial, frozen, and lyophilized.

Analytical Scale Biocatalytic Reactions

Time course of ObiH reaction

Reactions were done in duplicate on analytical scale (250 µL) for each time point. Stocks of Thr were made in 50 mM Tris pH 8.5 and 4-chlorobenzaldhyde in MeOH. 0.5-dram glass vials were charged with 50.0 µL of 500 mM Thr, 10.0 µL of 500 mM 4-chlorobenzaldhyde, 2.5 µL of 20 mM PLP and 162.9 µL of 50 mM Tris pH:8.5. Reactions were initiated by the addition of 24.6 µL of 400 µM of purified ObiH (Final concentrations: 100 mM Thr, 25 µmol; 20 mM 4-chlorobenzaldhyde, 5 µmol; 40 µM ObiH, 0.2 mol% cat., 500 max TON; 20 µM PLP; 4% Methanol). Reactions were allowed to proceed in a 37 °C incubator for 0.02 h, 0.08 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h and 20 h prior to quenching with 250 µL ACN with 2.5 mM benzophenone as the internal standard. Quenched reactions were then centrifuged at 15,000 xg to remove aggregated protein, and 1:10 dilution of the quenched reaction mixture in water were made. Quantification was performed by UPLC-MS analysis on a BEH C18 column (Waters). Measurement of internal standard and product concentrations was done by measurement of the corresponding 254 nm UV peak areas and using positive mode single ion readout for the M+H mass peak. Variability in injection volumes were corrected by dividing peak areas by the observed internal standard peak area for each injection. To calculate product concentrations, a standard curve was generated by subjecting stock solutions of **18** (4-chlorobenzaldehyde product) (0.05 mM – 1 mM) to UPLC-MS analysis in triplicate, with internal standards. These curves were used to calculate the concentrations of both the *threo* and erythron *isomer of* **18**, and subsequently yields after dilution factor correction. Note: We observed significant stochasticity with respect to the stability of ObiH in solution over time between replicates. Consequently, when ObiH precipitated from solution, the diastereomeric excess ceased to change, resulting in larger apparent errors in results. Nevertheless, repetition always resulted in the same trend: As yields increased, diastereomeric excess decreased.

Hydration State NMR Experiments

Two 0.75 mL ampules of DMSO-d_6 were opened and combined in a 1-dram glass vial. Two 700 µL aliquots of appropriate solvent were added to a 0.5-dram glass vial containing product and a blank 0.5 dram glass vial, respectively. After the product dissolved fully, 700 µL of the product and blank solution were transferred to two oven dried NMR tubes. NMR spectra were acquired on either a Bruker Avance-400 or 500 MHz magnet, both equipped with a BBFO probe. T1 measurements were made on representative product containing solutions using a standard inversion recovery pulse sequence, and subsequent experiment relaxation delays (60 s for DMSO-d_6 samples) were set to ≥5x the maximum measured T1 value to ensure reestablishment of equilibrium magnetism. For hydration state quantification, a standard ^{13}C-decoupled ^{1}H pulse sequence was used. Initially, the product-containing NMR sample was inserted into the NMR spectrometer and analyzed. For the blank sample, the same receiver gain value measured for the product-containing sample was used. The water content contributed by the dissolved product was measured by subtracting the blank spectra water peak integration from the product-containing sample water peak integration and comparing the resulting value to peaks with assigned proton counts from the product.

Part IV. Supplemental figures 1 and 2

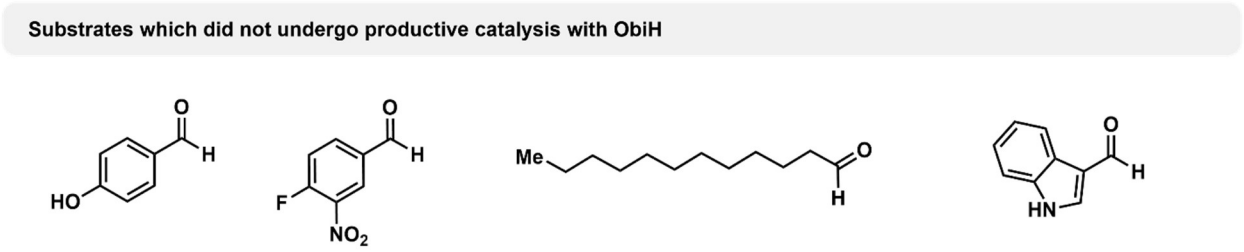


Figure S1. Substrates tested in ObiH reaction which did not undergo productive catalysis

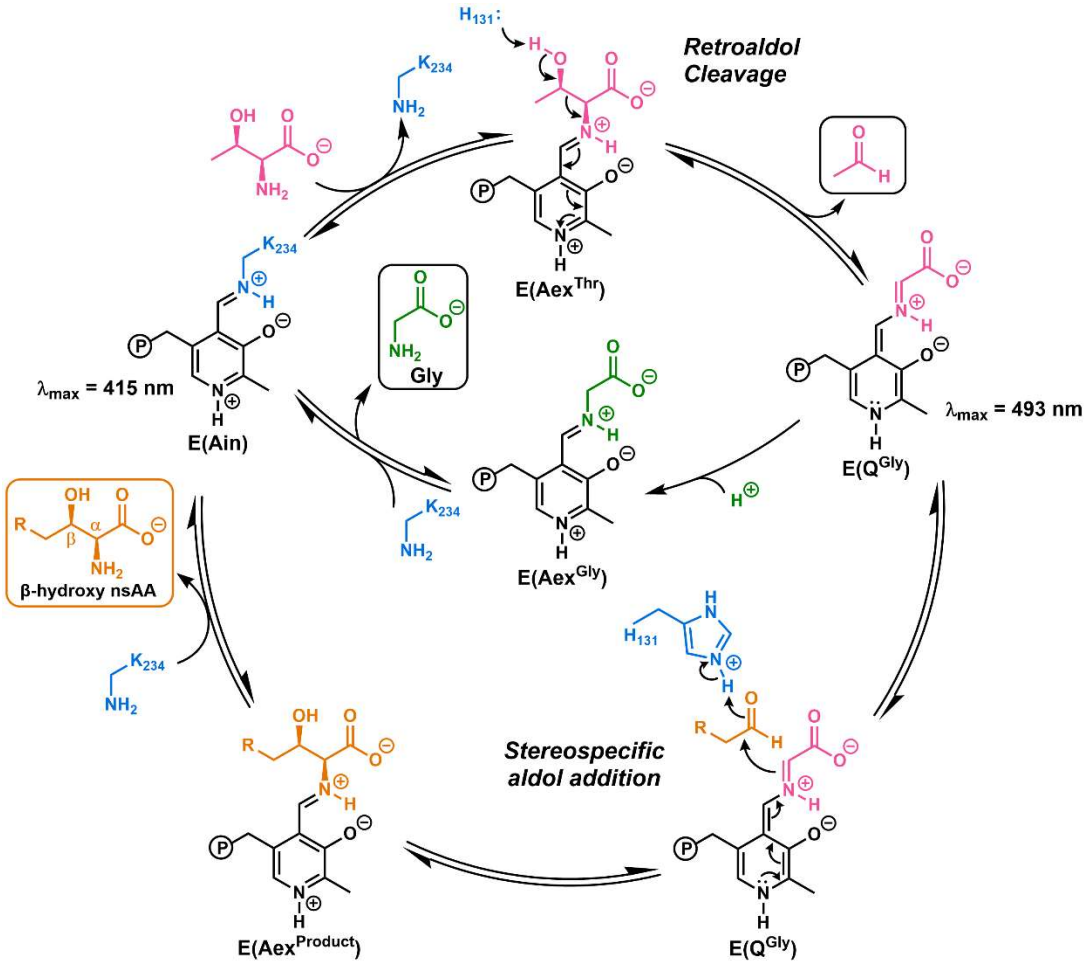
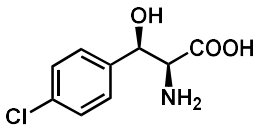


Figure S2. Catalytic Mechanism of ObiH. The mechanism of ObiH catalysis with Thr and an aldehyde substrate (outer cycle) along with the disfavored shunt pathway (inner cycle). The PLP cofactor (black) is shown covalently bound either to the substrates/intermediates (pink) or product (orange) or to the relevant catalytic residues of the protein (blue). Adapted from Kumar et al.[4]

Part V. Biocatalytic reaction products and synthetic procedures

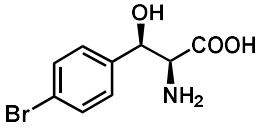
(2*S*,3*R*)- 2-amino-3-(4-chlorophenyl)-3-hydroxypropanoic acid (18)



18

Thr (2977.5 mg, 25.00 mmol, 100 mM final conc.), p-chloro-benzaldehyde (700 mg, 5.0 mmol, 20 mM final conc.), 10.00 mL MeOH (4% v/v), and 240.0 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 10 mg/mL (1% w/v). The total reaction volume was 250 mL. The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (680.7 mg isolated). **Hydration analysis:** $C_9H_{10}ClNO_3 \bullet 0.5H_2O$, 61% yield. **^{1}H NMR** (400 MHz, MeOD)) δ 7.51 – 7.45 (m, 2H), 7.42 – 7.36 (m, 2H), 5.22 (d, J = 3.8 Hz, 1H), 3.60 (d, J = 3.8 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 172.45, 140.31, 133.03, 130.50, 71.18, 62.09, 62.08, 61.09, 58.27; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 214.0276, found: 214.0276.

(2*S*,3*R*)- 2-amino-3-(4-bromophenyl)-3-hydroxypropanoic acid (19)

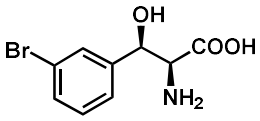


19

Thr (2977.5 mg, 25.00 mmol, 100 mM final conc.), p-bromo-benzaldehyde (920 mg, 5.0 mmol, 20 mM final conc.), 2.00 mL MeOH (4% v/v), and 48.0 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 10 mg/mL (1% w/v). The total reaction volume was 250 mL. The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred

to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (777.9 mg isolated). **Hydration analysis:** $C_9H_{10}BrNO_3 \bullet 0.5H_2O$, 58% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.60 – 7.51 (m, 2H), 7.47 – 7.39 (m, 2H), 5.22 (d, J = 3.7 Hz, 1H), 3.62 (d, J = 3.8 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 171.91, 140.71, 131.17, 127.81, 121.09, 70.96, 61.83, 60.98, 58.58; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 257.9771, found: 257.9771.

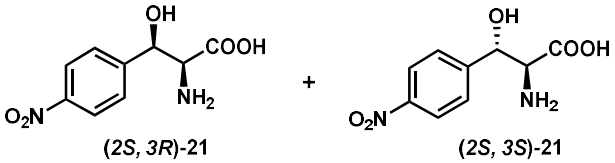
(2*S*,3*R*)- 2-amino-3-(3-bromophenyl)-3-hydroxypropanoic acid (20)



20

Thr (595.6 mg, 5.00 mmol, 100 mM final conc.), m-bromo-benzaldehyde (184 mg, 1.0 mmol, 20 mM final conc.), 2.00 mL MeOH (4% v/v), and 48.0 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 10 mg/mL (1% w/v). The total reaction volume was 50 mL. The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (93.6 mg isolated). **Hydration analysis:** $C_9H_{10}BrNO_3 \bullet H_2O$, 34% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.72 (d, J = 2.0 Hz, 1H), 7.47 (dt, J = 7.7, 2.0 Hz, 2H), 7.31 (t, J = 7.8 Hz, 1H), 5.26 (d, J = 3.4 Hz, 1H), 3.64 (d, J = 3.5 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 171.77, 144.14, 130.43, 129.93, 128.91, 124.67, 122.25, 70.72, 61.70, 60.93, 58.74; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 257.9771, found: 257.9771.

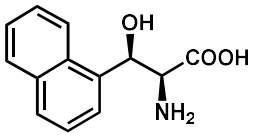
(2*S*,3*R*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid ((*2S, 3R*)-21) and **(2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid ((*2S, 3S*)-21)**



(*2S, 3R*)-21 (*2S, 3S*)-21

Thr dissolved in 100 mM Tris pH 8.5 (2.63 g, 22.08 mmol, 100 mM final conc.), *p*-nitro-benzaldehyde (1.0 g, 6.62 mmol, 30 mM final conc.), 8.84 mL MeOH (4% v/v), and 66.3 mL 100 mM Tris-HCl, pH 8.5 buffer (final buffer concentration: 50 mM) and were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 221 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding an off-white solid (898 mg isolated). **Hydration analysis:** $C_9H_{10}N_2O_5 \bullet H_2O$, 55% yield. Major diastereomer (**xx**): **^{1}H NMR** (400 MHz, D_2O) δ 8.32-8.29 (m, 2H), 7.72-7.67 (m, 2H), 5.40 (d, $J_{H\text{-}H}$ = 4.5 Hz, 1H), 3.95 (d, $J_{H\text{-}H}$ = 3.9 Hz, 1H); Minor diastereomer (**xx**): **^{1}H NMR** (400 MHz, D_2O) δ 8.29-8.25 (m, 2H), 7.64-7.59 (m, 2H), 5.45 (d, $J_{H\text{-}H}$ = 3.9 Hz, 1H), 4.12 (d, $J_{H\text{-}H}$ = 4.4 Hz, 1H); Both diastereomers: **^{13}C{^{1}H} NMR** (125 MHz, D_2O) δ 171.41, 170.44, 147.28, 147.21, 146.73, 144.98, 126.98, 126.76, 123.66, 123.41, 70.49, 70.30, 60.18, 59.86; **HR-ESI-MS**: m/z calcd for $C_9H_{10}N_2O_5$ $[M\text{-}H]^-$: 225.0517, found: 225.0518.

(2*S*,3*R*)-2-amino-3-hydroxy-3-(naphthalen-1-yl)propanoic acid (22)

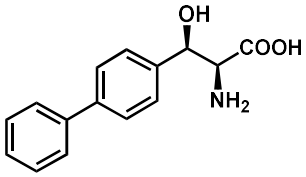


22

Thr dissolved in 100 mM Tris pH 8.5 (953 mg, 8.01 mmol, 100 mM final conc.), napthaldehyde (250 mg, 1.6 mmol, 20 mM final conc.), 3.72 mL MeOH (4% v/v), and 24.0 mL 100 mM Tris-HCl, pH 8.5 buffer (final buffer concentration: 50 mM) and were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 93 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while

shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (69 mg isolated). **Hydration analysis:** $C_{13}H_{13}NO_3 \bullet H_2O$, 17% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.96 (d, $J_{H\text{-}H}$ = 1.7 Hz, 1H), 7.93-7.80 (m, 3H), 7.57 (dd, $J_{H\text{-}H}$ = 8.5 Hz, 1.8 Hz, 1H), 7.52-7.42 (m, 2H), 5.35 (d, $J_{H\text{-}H}$ = 3.7 Hz, 1H), 3.74-3.65 (m, 1H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 176.19, 140.04, 134.59, 134.35, 129.22, 129.04, 128.60, 127.22, 126.99, 126.01, 125.26, 78.28, 62.39; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M\text{-}H]^-$: 230.0823, found: 230.0823.

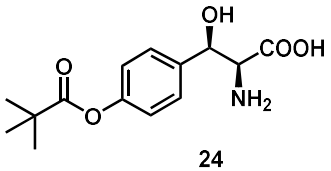
(2*S*,3*R*)-3-([1,1'-biphenyl]-4-yl)-2-amino-3-hydroxypropanoic acid (23)



23

Thr (3.27 g, 27.46 mmol, 100 mM final conc.) dissolved in 100 mM Tris pH 8.5, *p*-phenyl-benzaldehyde (1.0 g, 5.49 mmol, 30 mM final conc.), 11.0 mL MeOH (4% v/v), and 82.5 mL 100 mM Tris-HCl, pH 8.5 buffer (final buffer concentration: 50 mM) and were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 275 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (530 mg isolated). **Hydration analysis:** $C_{15}H_{15}NO_3 \bullet H_2O$, 35% yield.**^{1}H NMR** (500 MHz, d_6-DMSO) δ 7.76-7.56 (m, 4H), 7.54-7.31 (m, 5H), 5.09 (d, $J_{H\text{-}H}$ = 3.9 Hz, 1H), 3.40 (d, $J_{H\text{-}H}$ = 3.9 Hz, 1H). **^{13}C{^{1}H} NMR** (125 MHz, d_6-DMSO) δ 168.65, 141.62, 140.06, 138.93, 129.86, 129.02, 128.93, 127.29, 127.14, 126.88, 126.54, 126.27, 70.68, 69.77, 59.13; **HR-ESI-MS**: m/z calcd for $C_{15}H_{15}NO_3^+$ $[M\text{-}H]^-$: 256.0979, found: 256.0982.

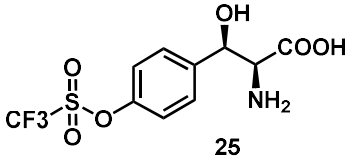
2-amino-3-hydroxy-3-(4-(pivaloyloxy)phenyl)propanoic acid (24)



24

Thr (595.6 mg, 5.00 mmol, 100 mM final conc.), 4-OPiv-benzaldehyde (184 mg, 1.0 mmol, 20 mM final conc.), 2.00 mL MeOH (4% v/v), and 48.0 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 10 mg/mL (1% w/v). The total reaction volume was 50 mL. The reaction mixture was incubated at 37 °C for 18 h while shaking at 220 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (75.8 mg isolated). **Hydration analysis:** $C_{14}H_{19}NO_5.H_2O$, 25% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.61 – 7.47 (m, 2H), 7.08 (d, J = 8.6 Hz, 2H), 5.35 (s, 0H), 3.73 – 3.62 (m, 1H), 1.35 (s, 9H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 177.63, 171.15, 150.69, 138.87, 126.85, 121.38, 114.93, 70.38, 61.05, 38.68, 26.05; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 280.1190, found: 280.1190.

2-amino-3-hydroxy-3-(4-(((trifluoromethyl)sulfonyl)oxy)phenyl)propanoic acid (25)

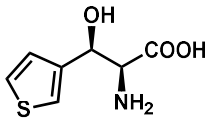


25

Thr (595.6 mg, 5.00 mmol, 100 mM final conc.), 4-OTf-benzaldehyde (254 mg, 1.0 mmol, 20 mM final conc.), 2.00 mL MeOH (4% v/v), and 48.0 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 10 mg/mL (1% w/v). The total reaction volume was 50 mL. The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product

containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (75.1 mg isolated). **Hydration analysis:** $C_{10}H_{10}F_3NO_6S.H_2O$, 22% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.72 – 7.64 (m, 2H), 7.44 – 7.36 (m, 2H), 5.34 (d, J = 3.6 Hz, 1H), 3.69 (d, J = 3.7 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 171.11, 149.09, 142.35, 128.82, 128.05, 121.09, 120.82, 120.05, 117.51, 70.51, 70.39, 60.84; **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 328.0108, found: 328.0109.

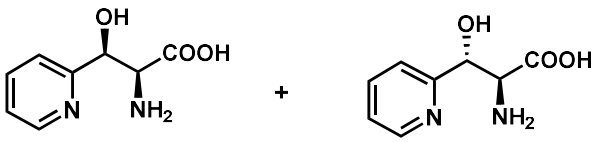
(2*S*,3*R*)-2-amino-3-hydroxy-3-(thiophen-3-yl)propanoic acid (26)



26

Thr dissolved in 100 mM Tris pH 8.5 (1.06 g, 8.9 mmol, 100 mM final conc.), thiophene-3-carboxaldehyde (200 mg, 1.78 mmol, 20 mM final conc.), 3.6 mL MeOH (4% v/v), and 26.7 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 89.2 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding an off-white solid (82 mg isolated). **Hydration analysis:** $C_7H_9NO_3S \bullet H_2O$, 23% yield. **^{1}H NMR** (500 MHz, D2O/MeOD) δ 7.44 (dd, J_{H-H} = 5.1 Hz, 3.0 Hz, 1H), 7.35 (dt, J_{H-H} = 3.0 Hz, 1.1 Hz, 1H), 7.05 (dd, J_{H-H} = 5.0, 1.4 Hz, 1H), 5.27 (d, J_{H-H} = 4.1 Hz, 1H), 3.81 (d, J_{H-H} = 4.1 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, D_2O) δ 172.64, 142.78, 127.48, 125.72, 122.60, 68.83, 60.49; **HR-ESI-MS**: m/z calcd for $C_7H_9NO_3S$ $[M-H]^-$: 186.0230, found: 186.0230.

(2*S*,3*S*)-2-amino-3-hydroxy-3-(pyridin-2-yl)propanoic acid ((*2S,3R*)-27) and **(2*S*,3*R*)-2-amino-3-hydroxy-3-(pyridin-2-yl)propanoic acid ((*2S,3S*)-27)**

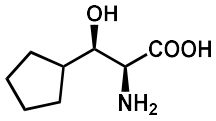


(*2S,3R*)-27 (*2S,3S*)-27

Thr dissolved in 100 mM Tris pH 8.5 (1.1 g, 9.29 mmol, 100 mM final conc.), pyridine-2-carboxaldehyde (200 mg, 1.87 mmol, 20 mM final conc.), 3.72 mL MeOH (4% v/v), and 27.9 mL 100 mM Tris-HCl, pH 8.5

buffer (final buffer concentration: 50 mM) and were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 93 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a yellow solid (228.5 mg isolated). **Hydration analysis:** $C_8H_{10}N_2O_3 \bullet 2H_2O$, 59% yield. Major diasteromer (**xx**): **^{1}H NMR** (400 MHz, D_2O) δ 8.52-8.46 (m, 1H), 7.88 (td, $J_{H\text{-}H}$ = 7.8 Hz, 1.8 Hz, 1H), 7.56 (d, $J_{H\text{-}H}$ = 7.9 Hz, 1H), 7.38 (ddd, $J_{H\text{-}H}$ = 7.6 Hz, 4.9 Hz, 1.2 Hz, 1H), 5.25 (d, $J_{H\text{-}H}$ = 3.7 Hz, 1H), 3.97 (d, $J_{H\text{-}H}$ = 3.7 Hz, 1H); Both diastereomers **^{13}C{^{1}H} NMR** (125 MHz, D_2O) δ 170.48, 164.36, 161.84, 161.39, 151.55, 148.21, 142.32, 136.67, 122.22, 121.76, 121.19, 100.12, 71.66, 61.62, 58.57, 57.72; **HR-ESI-MS**: m/z calcd for $C_{10}H_{10}N_2O_3$ $[M\text{-}H]^-$: 181.0619 , found: 181.0619.

(2*S*,3*R*)-2-amino-3-cyclopentyl-3-hydroxypropanoic acid (29)

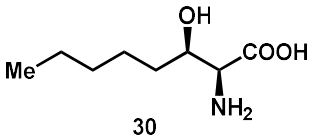


29

Thr dissolved in 100 mM Tris pH 8.5 (606.8 mg, 5.09 mmol, 100 mM final conc.), cyclopentane carboxaldehyde (100 mg, 1.01 mmol), 2.04 mL MeOH (4% v/v), and 15.3 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 51.0 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (90 mg isolated). **Hydration analysis:** $C_8H_{15}NO_3 \bullet H_2O$, 46% yield. **^{1}H NMR** (500 MHz, D_2O) δ 3.83 (d, $J_{H\text{-}H}$ = 9.5 Hz, 1H), 3.63 (s, 1H), 1.94 (q, $J_{H\text{-}H}$ = 8.6 Hz, 1H), 1.84-1.74 (m, 1H), 1.72-1.42 (m, 6H), 1.34 (dd, $J_{H\text{-}H}$ = 12.6 Hz, 7.6 Hz, 1H), 1.19 (q, $J_{H\text{-}H}$ = 9.3 Hz, 1H); **^{13}C{^{1}H} NMR** (125 MHz, D_2O/MeOD) δ 181.47,

81.45, 68.77, 64.86, 35.87, 35.40, 31.77, 31.69; **HR-ESI-MS**: m/z calcd for $C_8H_{15}NO_3$ $[M-H]^-$: 172.0979, found: 172.0980.

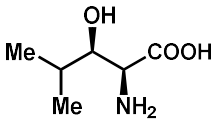
(2*S*,3*R*)-2-amino-3-hydroxyoctanoic acid (30)



30

Thr dissolved in 100 mM Tris pH 8.5 (19.96 mL, 1.19 g, 9.97 mmol, 100 mM final conc.), hexanal (200 mg, 1.99 mmol, 20 mM final conc.), 4.0 mL MeOH (4% v/v), and 29.94 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 99.8 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (84.2 mg isolated). **Hydration analysis:** $C_8H_{17}NO_3 \bullet H_2O$, 22% yield. **^{1}H NMR** (500 MHz, D_2O) δ 4.09 (dt, J_{H-H} = 9.1 Hz, 4.7 Hz, 1H), 3.64 (d, J_{H-H} = 4.5 Hz, 1H), 1.70-1.27 (m, 8H), 0.96-0.82 (m, 3H); **^{13}C{^{1}H} NMR** (125 MHz, D_2O/MeOD) δ 173.36, 69.93, 59.46, 30.77, 24.70, 21.96, 13.38; **HR-ESI-MS**: m/z calcd for $C_8H_{17}NO_3$ $[M-H]^-$: 174.1135, found: 174.1136.

(2*S*,3*R*)-2-amino-3-hydroxy-4-methylpentanoic acid (31)

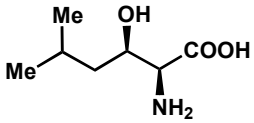


31

Thr dissolved in 100 mM Tris pH 8.5(5.51 g, 43.37 mmol, 100 mM final conc.), isobutyraldehyde (1.0 g, 13.86 mmol, 30 mM final conc.), 18.5 mL MeOH, and 138.9 mL of 100 mM Tris-HCl, pH 8.5 buffer were added to a 1.0 L glass bottle with a sealable lid. MQ water was added to a final volume of 463 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells.

The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (1.065 g isolated). **Hydration analysis:** $C_6H_{13}NO_3 \bullet 2H_2O$, 42% yield. NMR spectra and HRMS of the purified compound matched previously reported results.[4] **^{1}H NMR** (400 MHz, D_2O) δ 3.74 (d, $J_{H\text{-}H}$ = 3.8 Hz, 1H), 3.69 (dd, $J_{H\text{-}H}$ = 7.9 Hz, 3.8 Hz, 1H). 1.69 (dq, $J_{H\text{-}H}$ = 7.8 Hz, 6.6 Hz, 1H), 0.95 (d, $J_{H\text{-}H}$ = 6.6 Hz, 3 H), 0.90 (d, $J_{H\text{-}H}$ = 6.8 Hz, 3H).

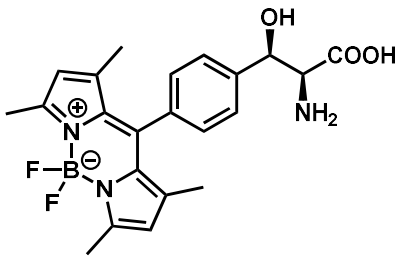
(2*S*,3*R*)-2-amino-3-hydroxy-5-methylhexanoic acid (32)



32

Thr dissolved in 100 mM Tris pH 8.5 (690.8 mg, 5.80 mmol, 100 mM final conc.), isovaleraldehyde (100 mg, 1.16 mmol, 20 mM final conc.), 2.32 mL MeOH (4% v/v), and 17.4 mL 100 mM Tris-HCl, pH 8.5 buffer (final buffer concentration: 50 mM) and were added to a 0.5 L glass bottle with a sealable lid. MQ water was added to a final volume of 58 mL. Whole *E. coli* cells containing overexpressed wild-type ObiH were added to a final concentration of 20 mg/mL (2% w/v). The reaction mixture was incubated at 37 °C for 18 h while shaking at 250 rpm to ensure thorough mixing. The reaction mixture was then quenched by the addition of 1 volume equivalent of acetonitrile, followed by freeze-thaw to improve material recovery from cells. The thawed mixture was transferred to 50 mL conical vials and centrifuged at 4,300×g for 10 minutes to pellet insoluble whole cells. The supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a white solid (127.2 mg isolated). **Hydration analysis:** $C_7H_{15}NO_3 \bullet 2H_2O$, 55% yield. **^{1}H NMR** (400 MHz, D_2O) δ 4.12 (dt, $J_{H\text{-}H}$ = 4.3 Hz, 1H), 3.55 (d, $J_{H\text{-}H}$ = 4.6 Hz, 1H), 1.72 (dddd, $J_{H\text{-}H}$ = 1.33 Hz, 9.2 Hz, 6.7 Hz, 5.1 Hz, 1H), 1.50 (ddd, $J_{H\text{-}H}$ = 14.7 Hz, 9.8 Hz, 5.1 Hz, 1H), 1.35 (ddt, $J_{H\text{-}H}$ = 14.6 Hz, 9.4 Hz, 4.7 Hz, 1H), 0.91 (dd, $J_{H\text{-}H}$ = 12.5 Hz, 6.6 Hz, 6H). **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 173.59, 69.12, 62.38, 61.16, 44.01, 25.41, 23.82, 21.87; **HR-ESI-MS**: m/z calcd for $C_7H_{15}NO_3$ $[M\text{-}H]^-$: 160.0979, found: 160.0980.

(2S,3R)-2-amino-3-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)-3-hydroxypropanoic acid (34)

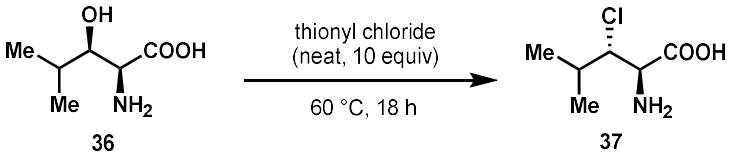


34

Thr (1116.6 mg, 9.37 mmol, 125 mM final conc.), Bodipy-aldehyde (258 mg, 1.5 mmol, 20 mM final conc.), 7.50 mL DMSO (10% v/v), and 59.6 mL 100 mM Tris-HCl, pH 8.5 buffer were added to a 0.5 L glass bottle with a sealable lid. 10 molar equivalents of pyridoxal-5'-phosphate (PLP) relative to final ObiH concentration were then added, followed by addition of ObiH (7.5 mL of 400 µM ObiH, 0.2% mol cat). The total reaction volume was 75 mL. The reaction flask was placed in the dark at 37 °C for 20 h. After reaction completion, the reaction mixture was quenched with an equivalent volume of acetonitrile (ACN) and centrifuged (4,000 rpm, 10 min) to remove aggregated protein. The decanted supernatant was transferred to a 1.0 L round bottom flask and concentrated by rotary evaporation. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 30g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions were pooled, concentrated by rotary evaporation, and dried via lyophilization, yielding a red solid (46.0 mg isolated). **Hydration analysis:** $C_{22}H_{24}BF_2N_3O_3 \bullet 0.25H_2O$, 7% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.67 (d, J = 7.9 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 6.06 (s, 2H), 5.26 (d, J = 4.7 Hz, 1H), 3.70 (d, J = 4.7 Hz, 1H), 2.49 (s, 6H), 1.44 (s, 6H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 172.15, 156.66, 144.83, 144.06, 143.35, 135.81, 132.64, 129.42, 128.43, 122.19, 72.61, 62.53, 61.21, 14.97, 14.53; **^{19}F{^{1}H} NMR** (400 MHz, MeOD) δ 147.13 (dd, J = 64.9, 32.2 Hz). **HR-ESI-MS**: m/z calcd for $C_{13}H_{13}NO_3$ $[M-H]^-$: 426.1810, found: 426.1812.

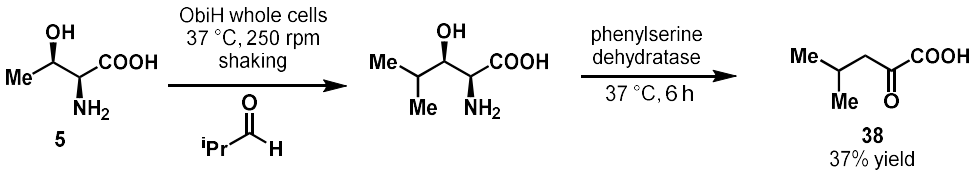
Functionalization of ObiH-generated β-OH-α-amino acids

(2*R*,3*S*)-2-amino-3-chloro-4-methylpentanoic acid (37)



An oven-dried 10 mL round bottom flask was charged with 100 mg (0.68 mmol) β-hydroxyleucine and a dry stir bar. 493 µL (808 mg, 6.8 mmol, 10 equiv) of thionyl chloride was added via syringe and the reaction was sealed with a rubber stopper. The reaction mixture was rapidly stirred and heated at 60 °C for 18 h forming a dark orange oil. The overnight reaction was quenched by adding the resulting oil dropwise to a chilled (4 °C) round bottom flask containing a 4 mL mixture of acetone and water (1:1). The solvent was removed using rotary evaporation into a chilled trap containing 50 mL MeOH and water (1:1) to avoid corrosive contaminants inside the vacuum. For purification, the resulting reaction mixture was loaded onto a Biotage SNAP Ultra 18g C18 column and purified on a Biotage flash purification system using a water/methanol gradient. Fractions were analyzed by UPLC-MS to identify product containing fractions. All product containing fractions from both rounds of purification were then pooled, concentrated by rotary evaporation, and dried via lyophilization to yield a white solid. Yield: 54 mg (48%). **^{1}H NMR** (400 MHz, D_2O) δ 4.28 (d, J_{H-H} = 2.8 Hz, 1H), 4.08 (dd, J_{H-H} = 9.2 Hz, 2.8 Hz, 1H), 2.28 (dp, J_{H-H} = 9.2 Hz, 6.6 Hz, 1H), 1.10 (t, J_{H-H} = 6.2 Hz, 6H); **^{13}C{^{1}H} NMR** (125 MHz, MeOD) δ 169.92, 69.36, 59.52, 33.30, 21.20, 20.81; **HR-ESI-MS**: m/z calcd for $C_6H_{12}NO_2Cl$ $[M-H]^-$: 164.0484, found: 164.0484.

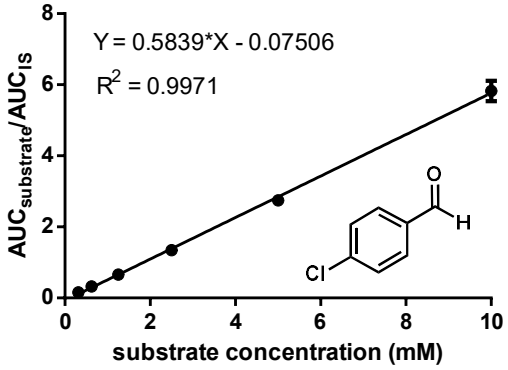
4-methyl-2-oxopentanoic acid (38)

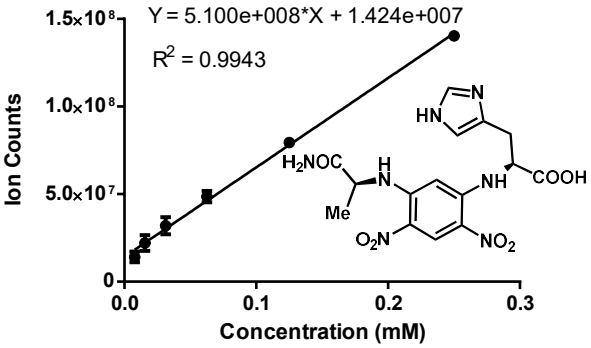


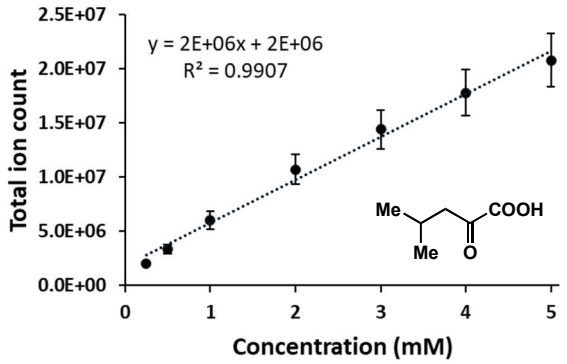
Reactions were done in triplicate on analytical scale (250 µL). Stocks of Thr were made in 50 mM Tris pH 8.5 and Isobutyraldehyde in MeOH. 0.5-dram glass vials were charged with 50.0 µL of 500 mM Thr, 10.0 µL of 500 mM Isobutyraldehyde, 2.5 µL of 20 mM PLP and 153.4 µL of 50 mM Tris pH:8.5. Reactions were initiated by the addition of 25.0 µL of 10% whole *E. coli* cells containing overexpressed wild-type ObiH (Final concentrations: 100 mM Thr, 25 µmol; 20 mM Isobutyraldehyde, 5 µmol; 1% ObiH cells, 200 µM PLP; 4% Methanol). Reactions were allowed to proceed in a 37 °C incubator for 20 h and then centrifuged to remove cells. The supernatant was transferred to a new 0.5-dram glass vial and 9.1 µL of 550 µM phenylserine dehydratase (PSDH) was added and dehydration reactions were allowed to proceed in a 37 °C incubator for 6 h (Final concentration: 20 µM PSDH, 0.1 mol% cat., 1000 max TON). Reaction mixture was quenched with 250 µL ACN with 2.5 mM benzophenone as the internal standard. Quenched reactions

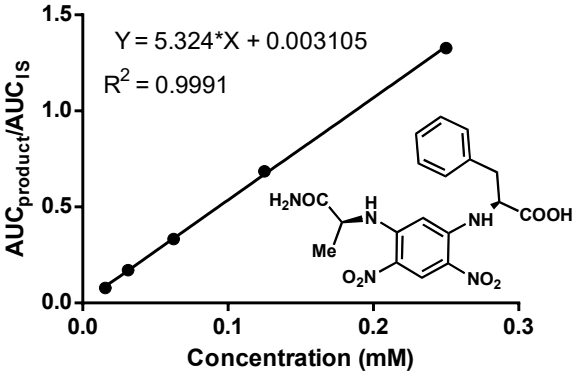
were then centrifuged at 15,000 xg to remove aggregated protein, amount of **38** were quantified by performing UPLC-MS analysis of the undiluted supernatant on a BEH C18 column (Waters). Measurement of internal standard and product concentrations was done by measurement of the corresponding 254 nm UV peak areas and using negative mode single ion readout for the M-H mass peak. Variability in injection volumes were corrected by dividing peak areas by the observed internal standard peak area for each injection. To calculate product concentrations, a standard curve was generated by subjecting stock solutions of **38** (1 mM – 5 mM; **38** was purchased from from Sigma-Aldrich) to UPLC-MS analysis similar to enzymatic reaction, in triplicate, with internal standards. These curves were used to calculate the concentrations of 3**8**, and subsequently yields.

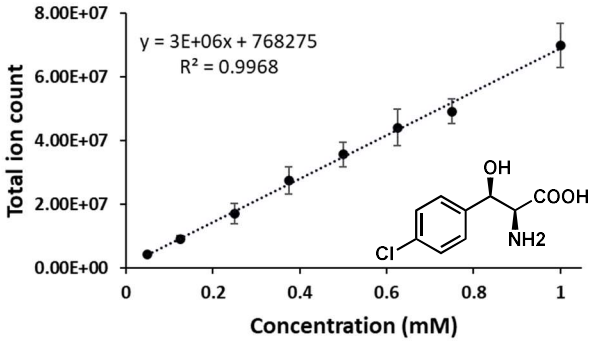
Part VI. Standard curves and LC/MS traces



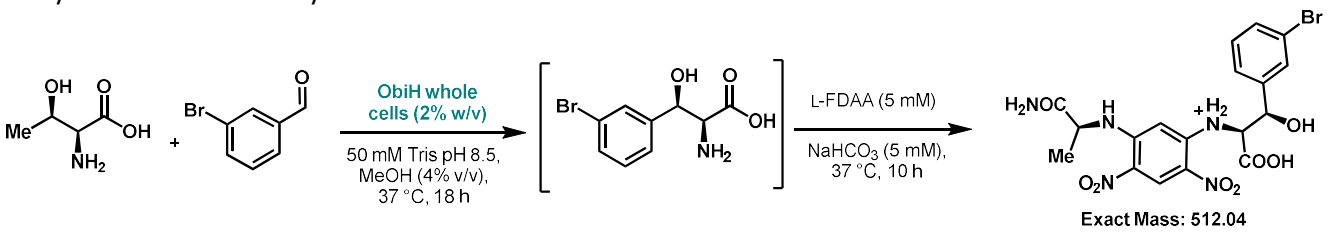


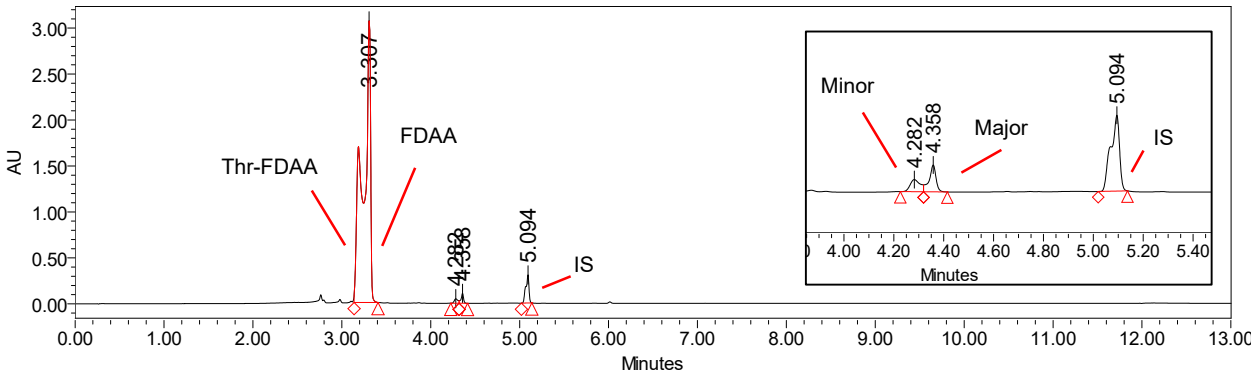




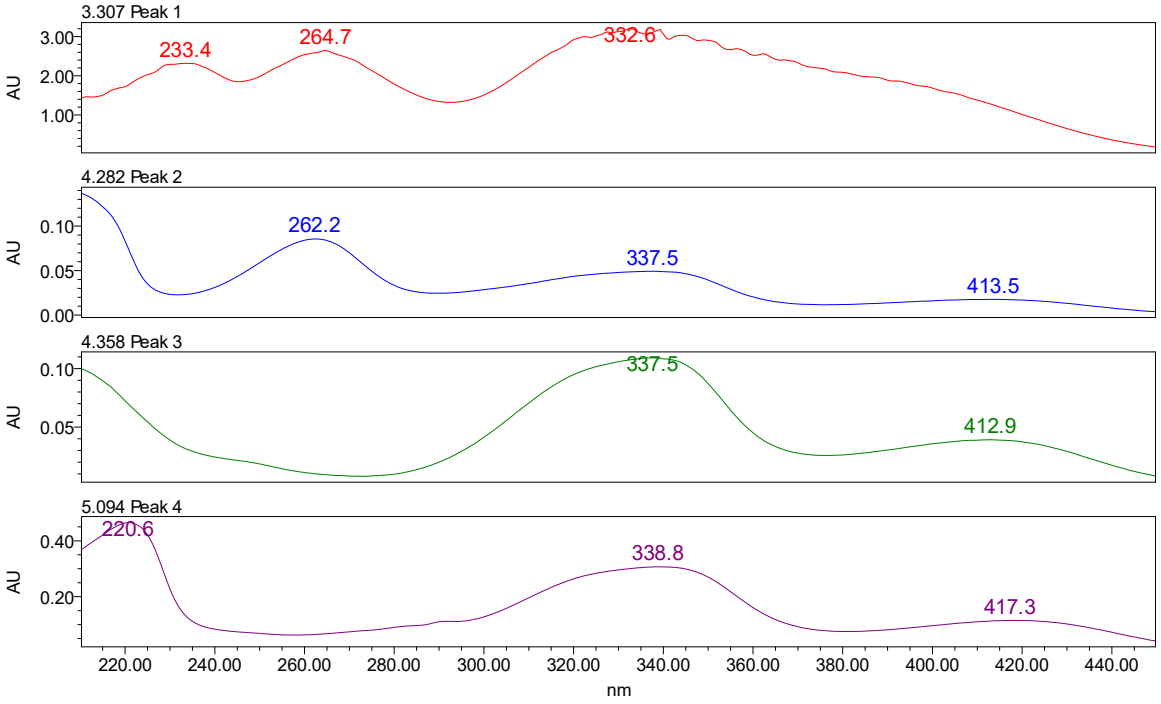


Supplemental Figure S3. ObiH-catalyzed reaction with 3-bromobenzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

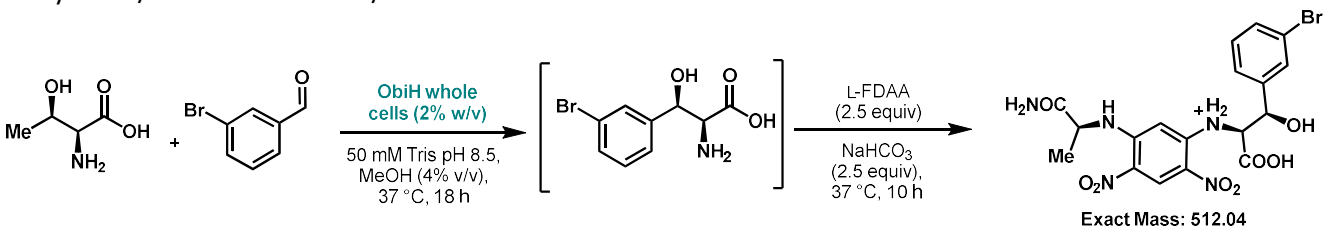


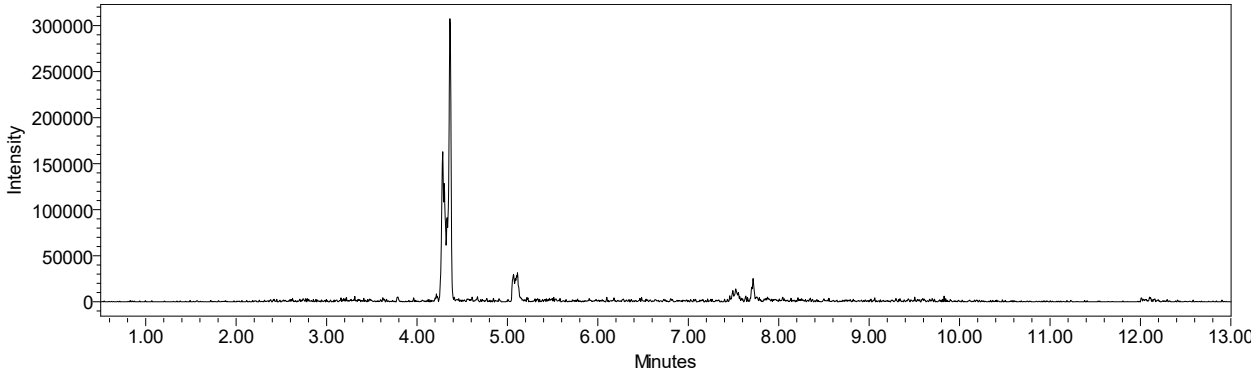


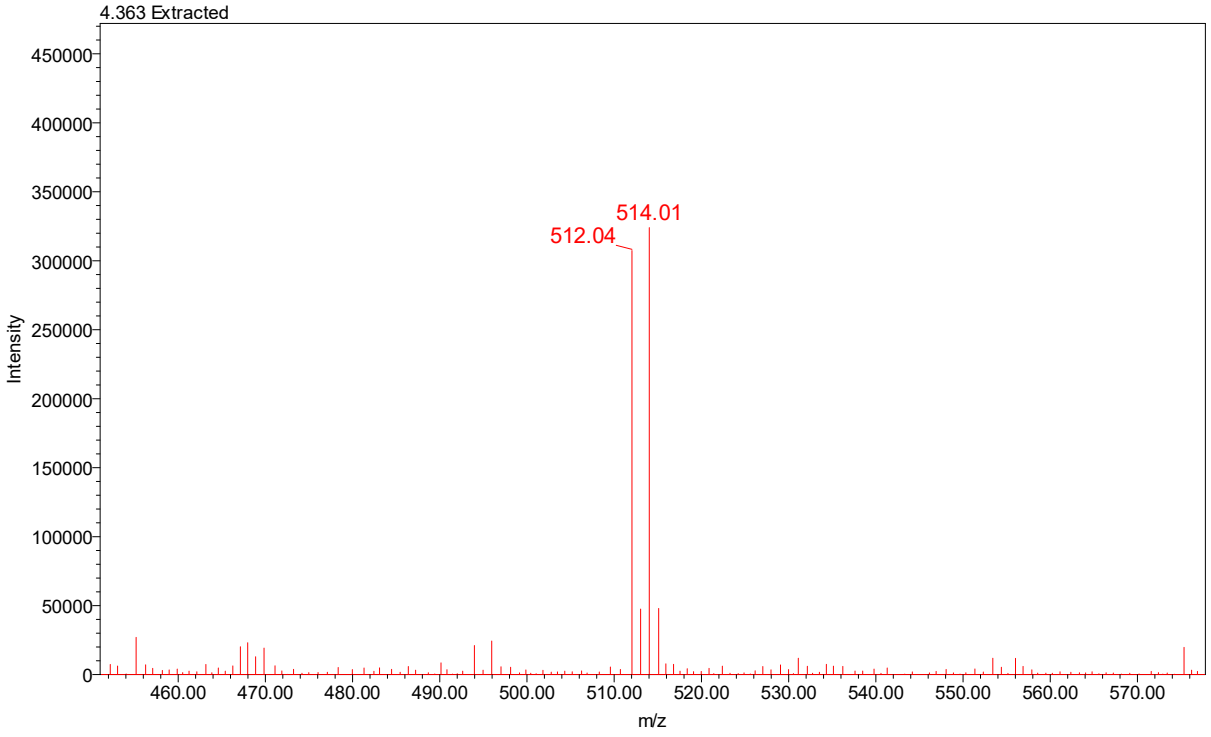
	Retention Time	Area	% Area	Height
1	3.307	16842379	93.88	3065920
2	4.282	137657	0.77	48805
3	4.358	205973	1.15	108438
4	5.094	753451	4.20	306496



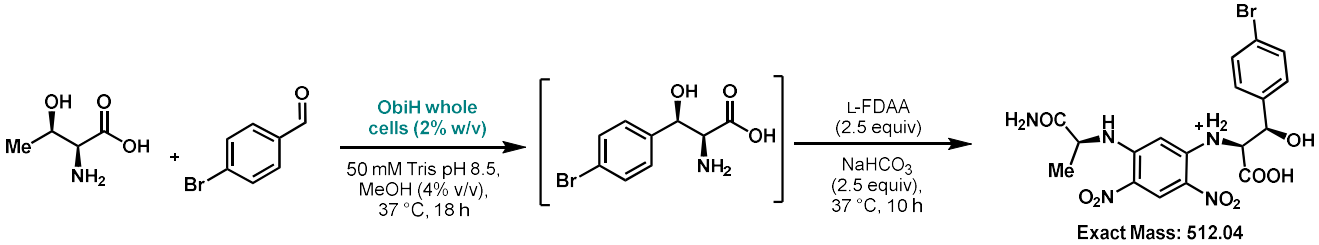
Supplemental Figure S4. ObiH-catalyzed reaction with 3-bromobenzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 512.

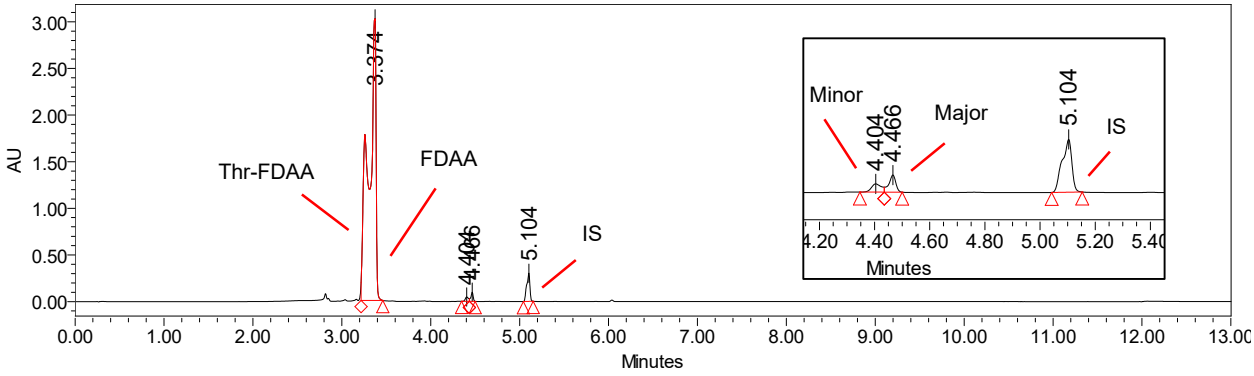




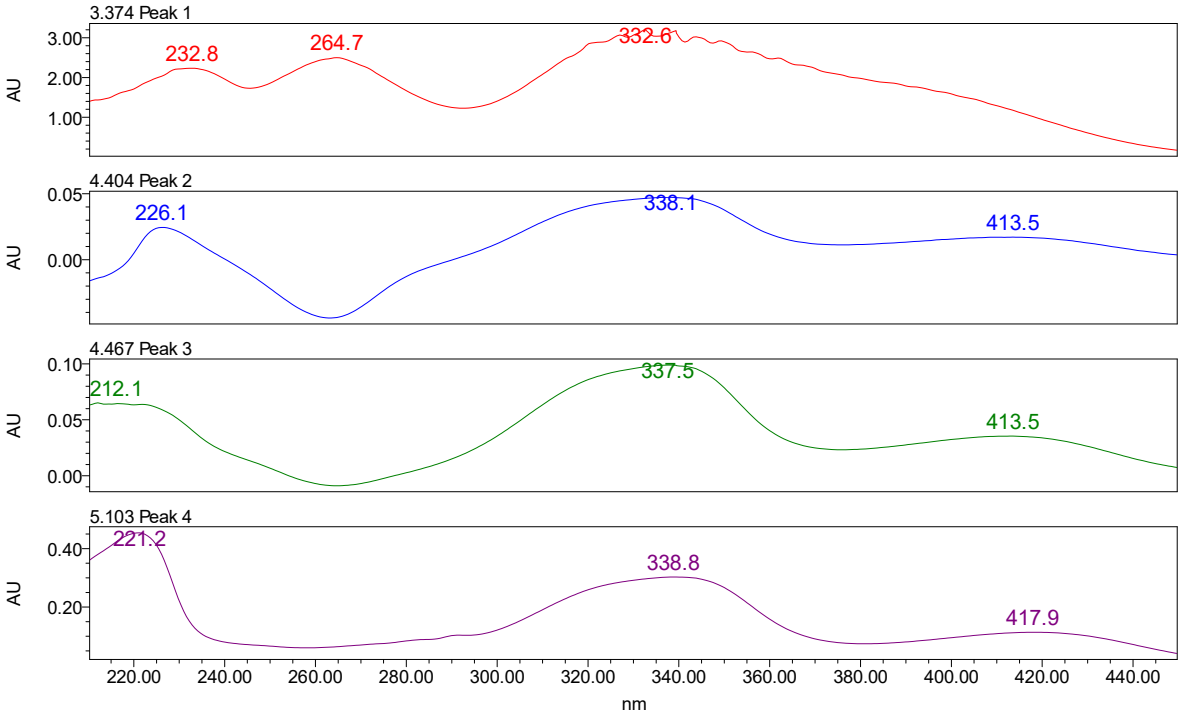


Supplemental Figure S5. ObiH-catalyzed reaction with 4-bromobenzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

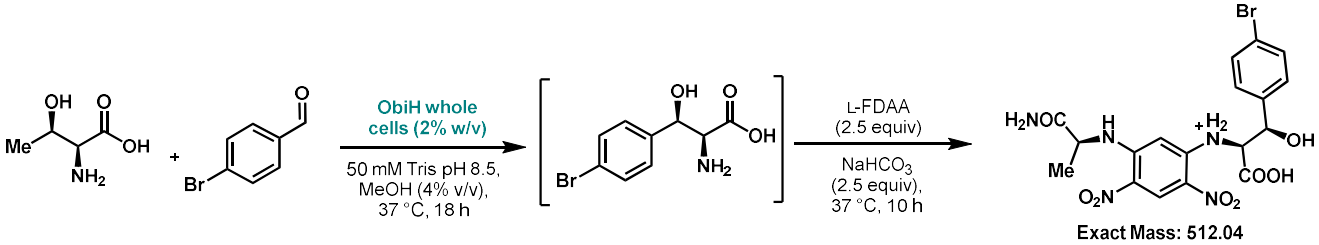


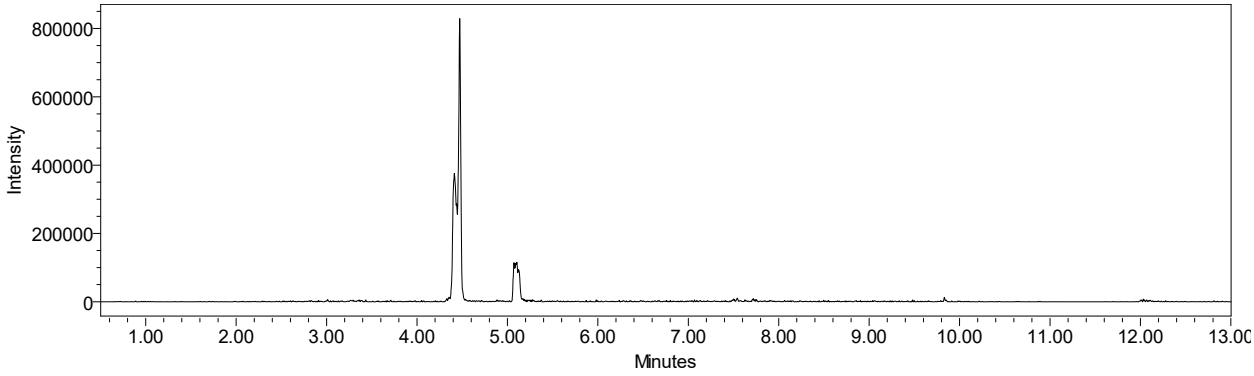


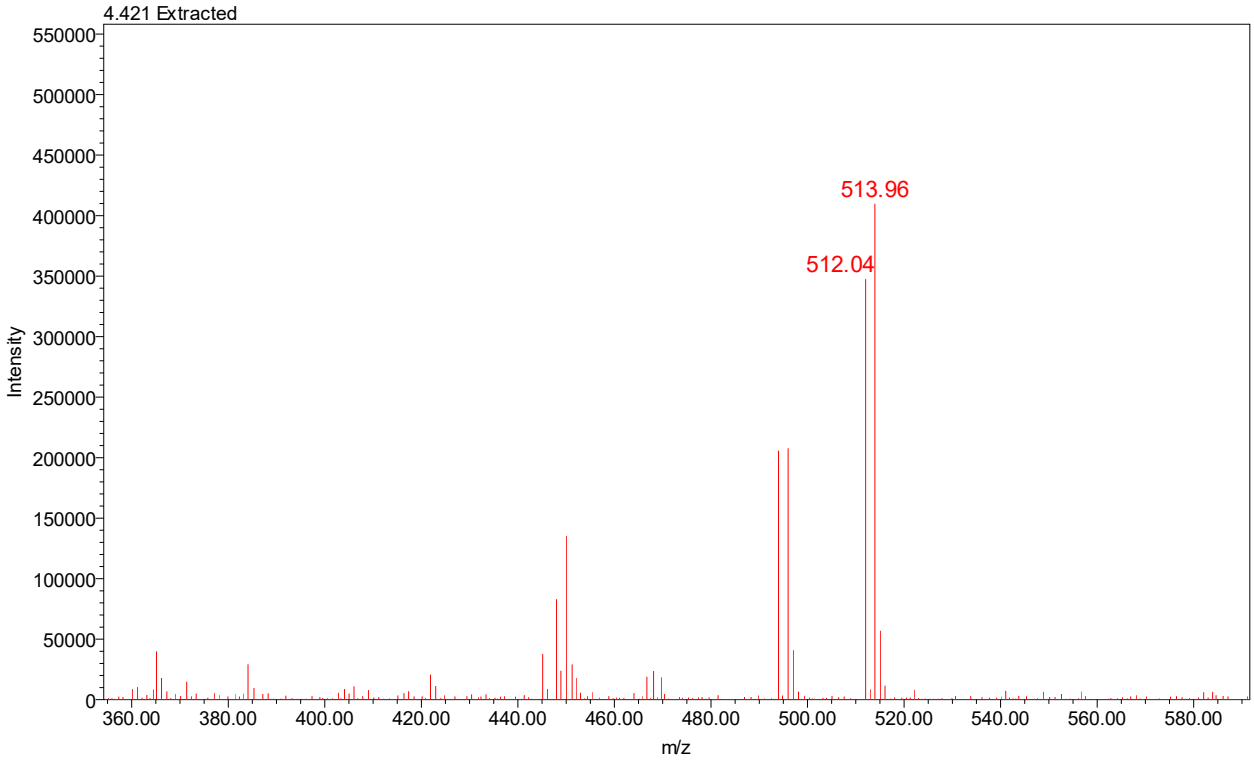
	Retention Time	Area	% Area	Height
1	3.374	16752043	94.21	3020997
2	4.404	117767	0.66	46961
3	4.466	171584	0.96	98265
4	5.104	740491	4.16	302544



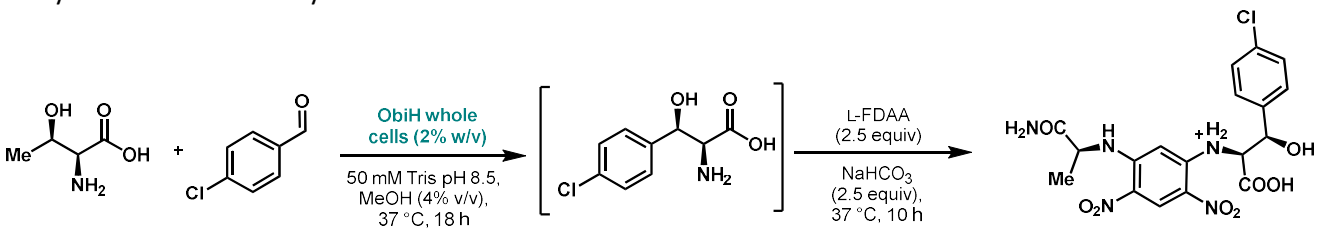
Supplemental Figure S6. ObiH-catalyzed reaction with 4-bromobenzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 512.

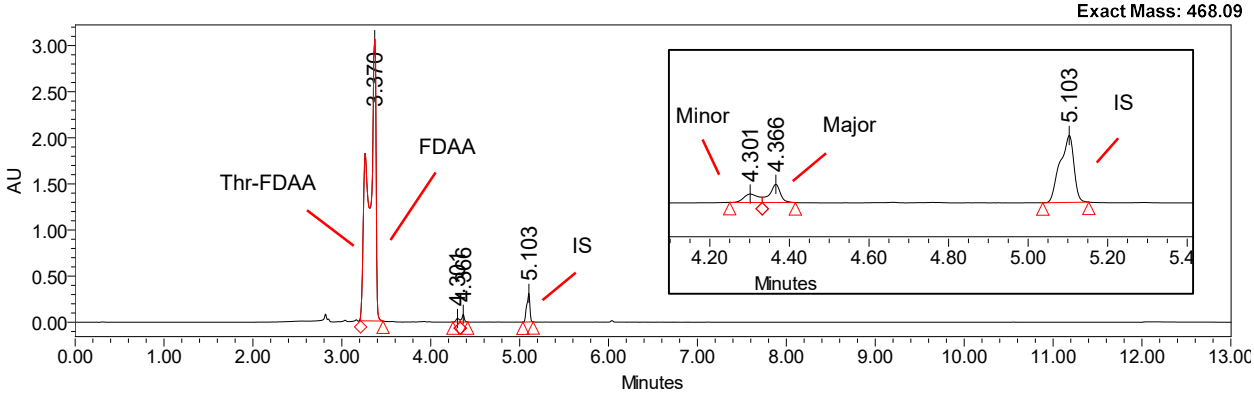




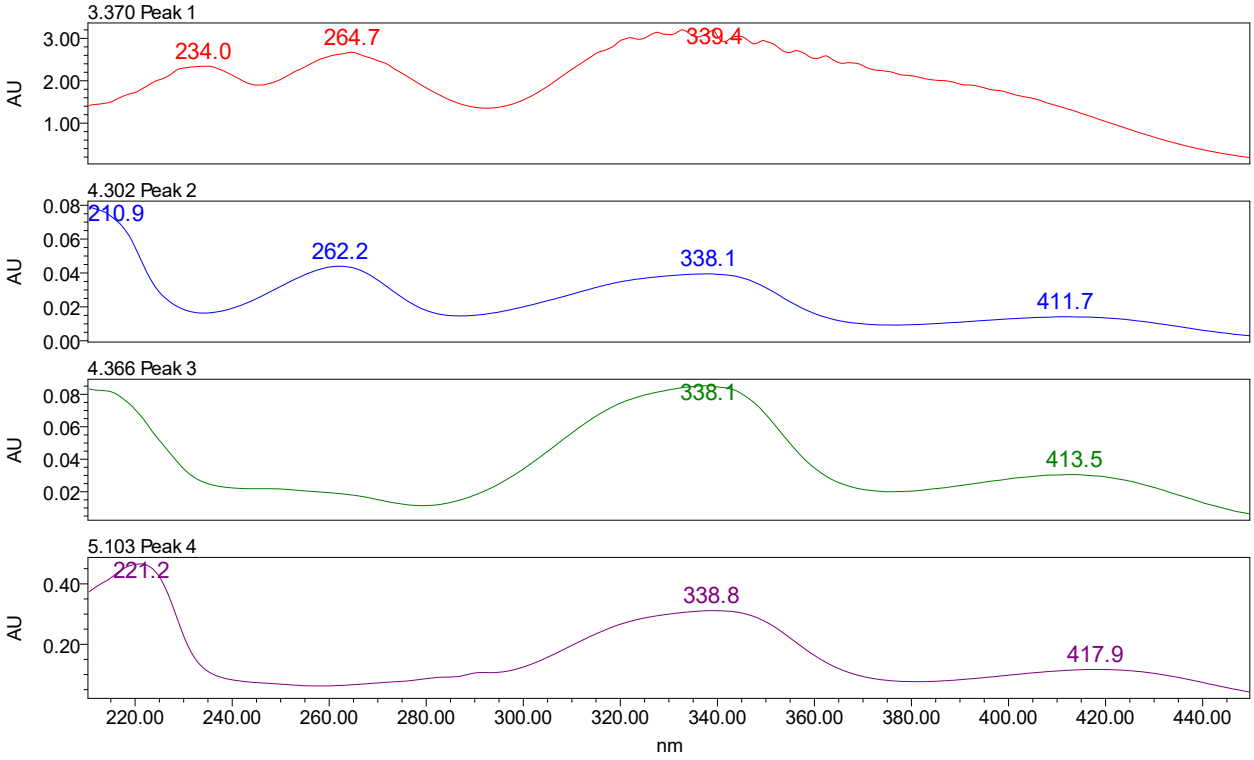


Supplemental Figure S7. ObiH-catalyzed reaction with 4-chlorobenzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

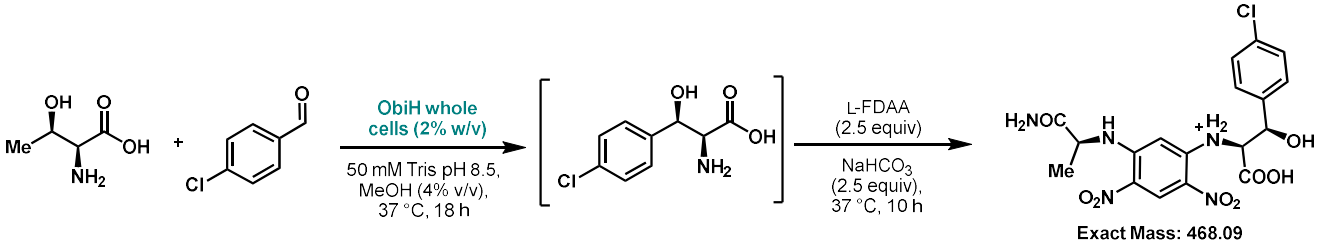


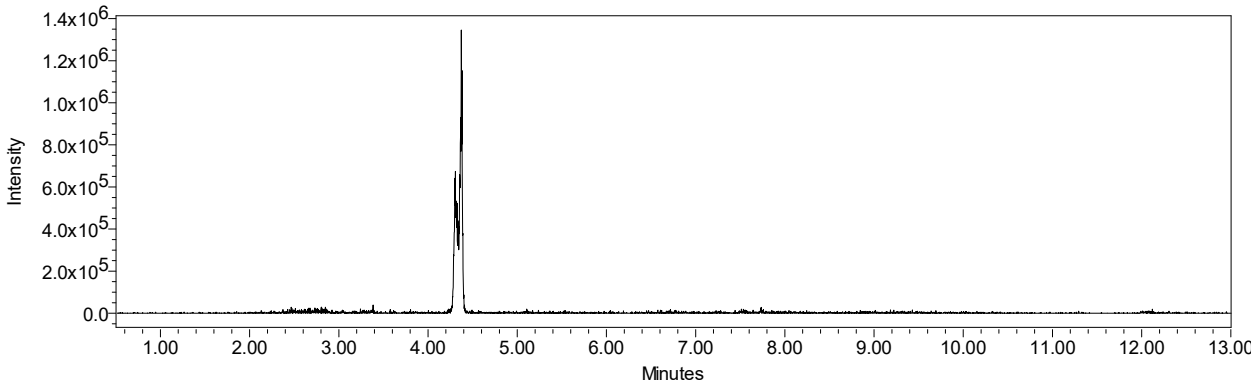


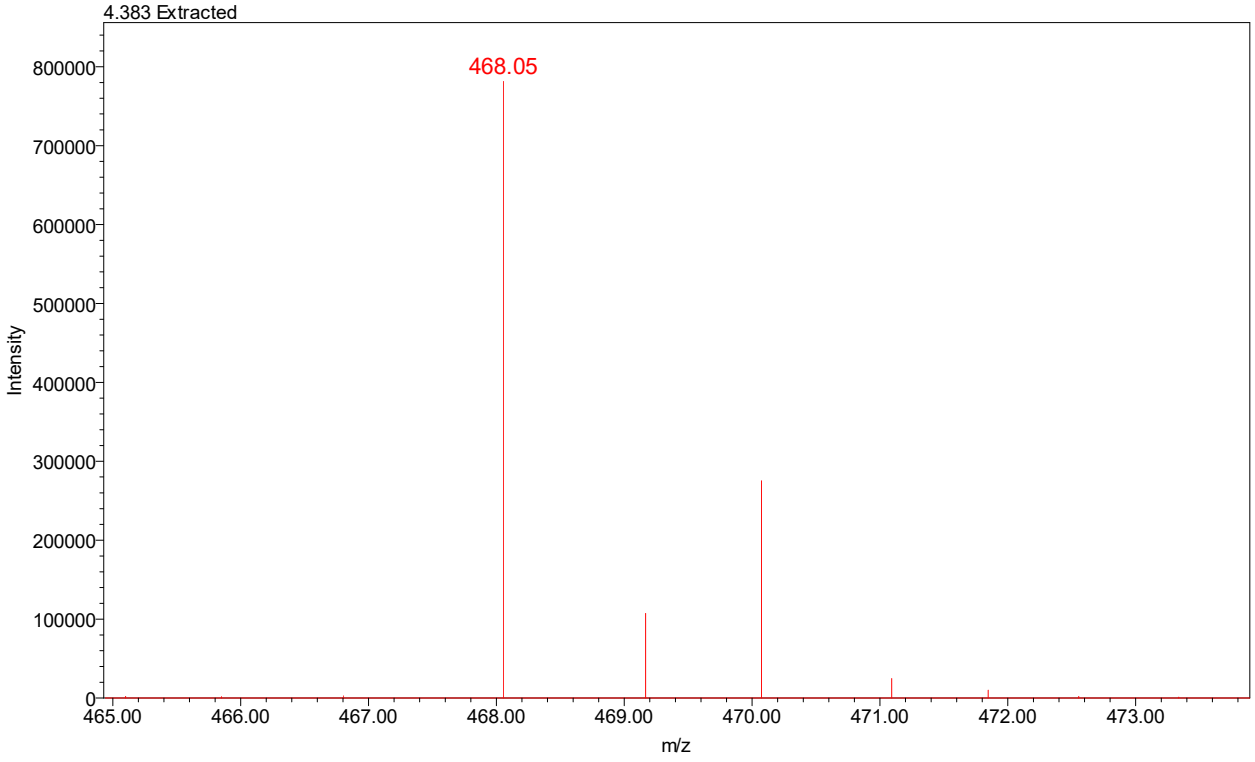
	Retention Time	Area	% Area	Height
1	3.370	16768271	94.31	3056364
2	4.301	100252	0.56	39190
3	4.366	158834	0.89	84523
4	5.103	752201	4.23	310883



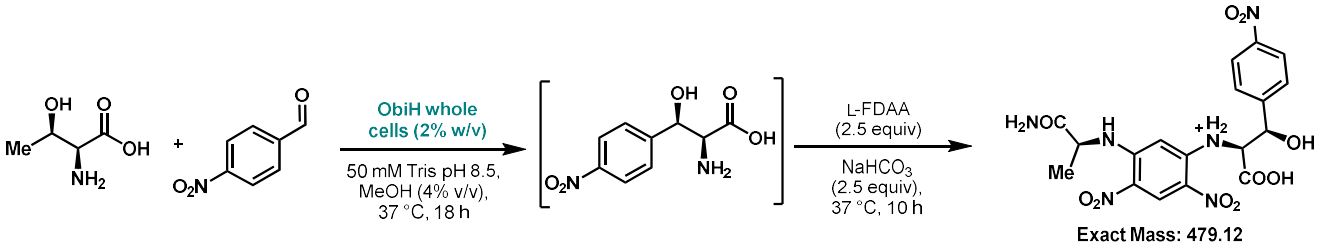
Supplemental Figure S8. ObiH-catalyzed reaction with 4-chlorobenzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 468.1

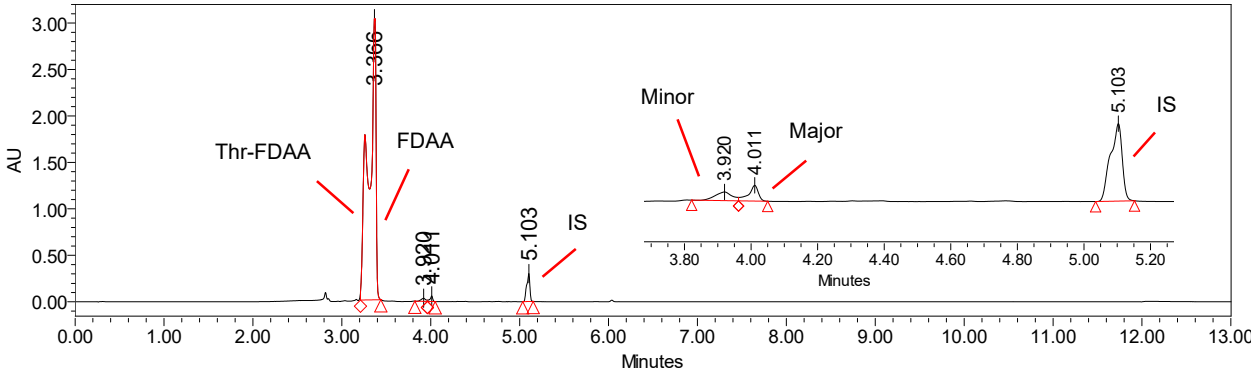




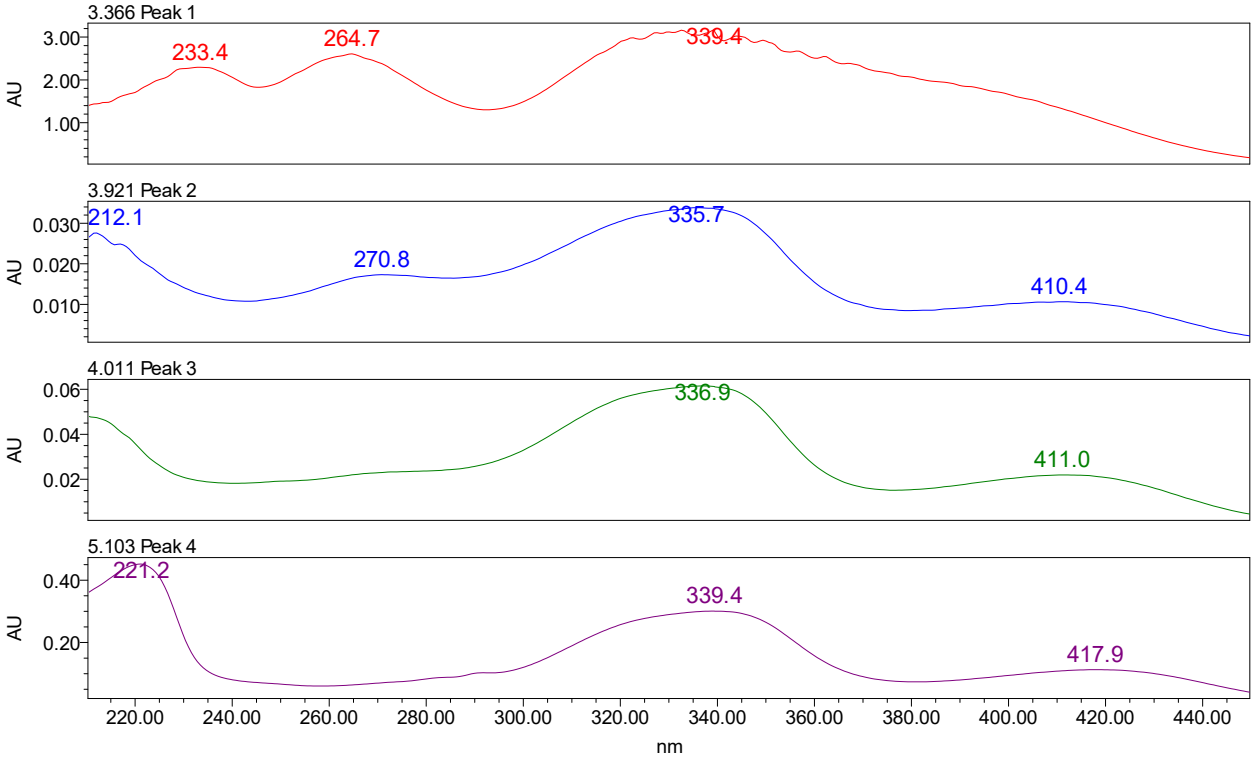


Supplemental Figure S9. ObiH-catalyzed reaction with 4-nitrobenzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

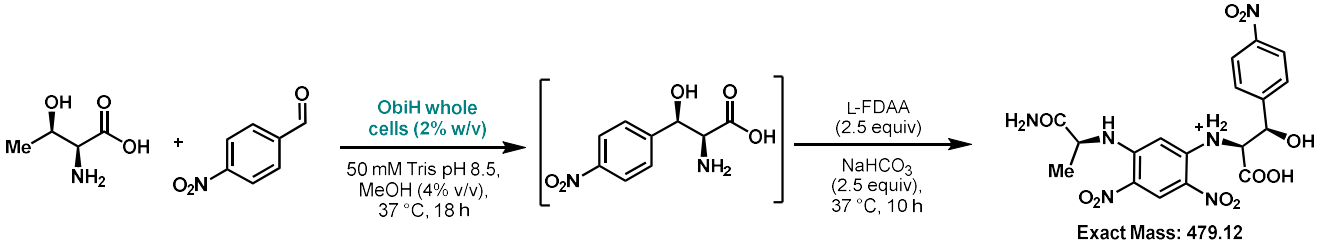


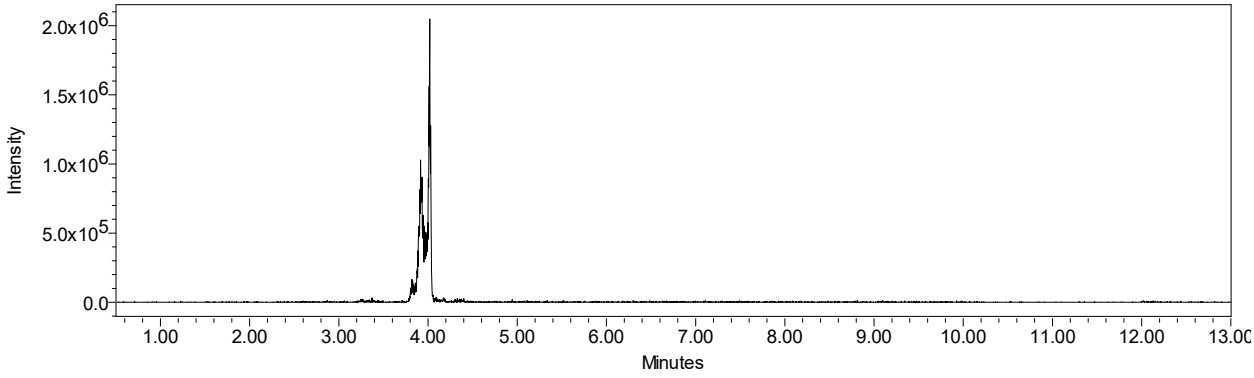


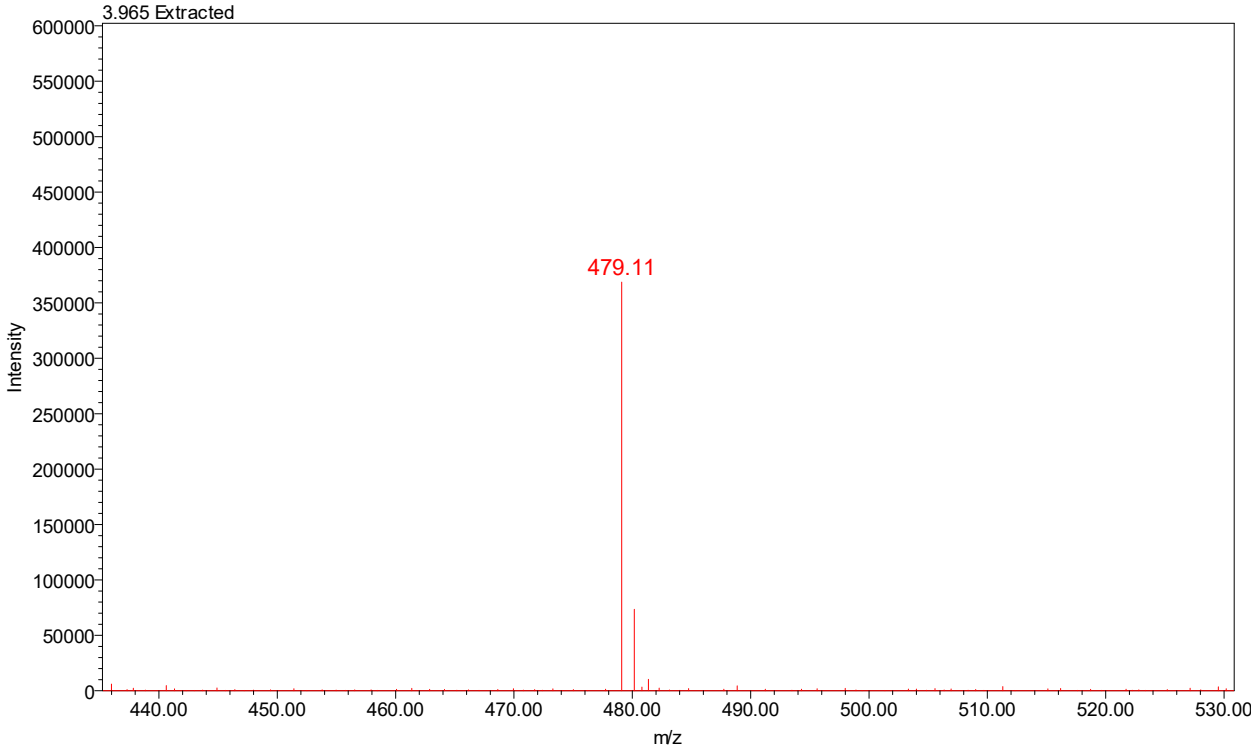
	Retention Time	Area	% Area	Height
1	3.366	16762801	94.48	3026896
2	3.920	119081	0.67	33483
3	4.011	124234	0.70	60910
4	5.103	736483	4.15	300411



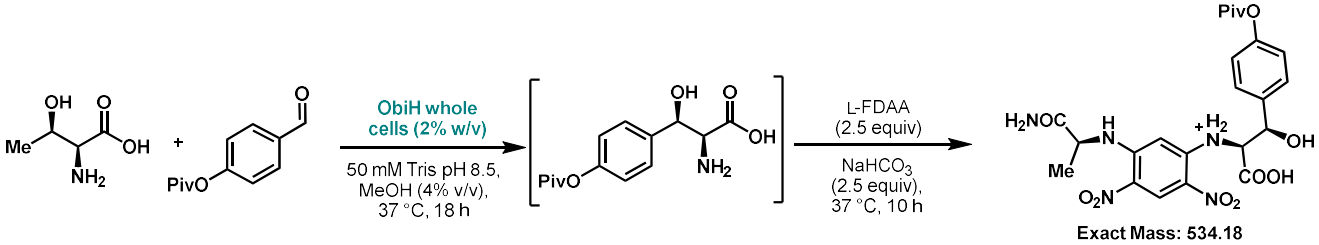
Supplemental Figure S10. ObiH-catalyzed reaction with 4-nitrobenzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 479.1

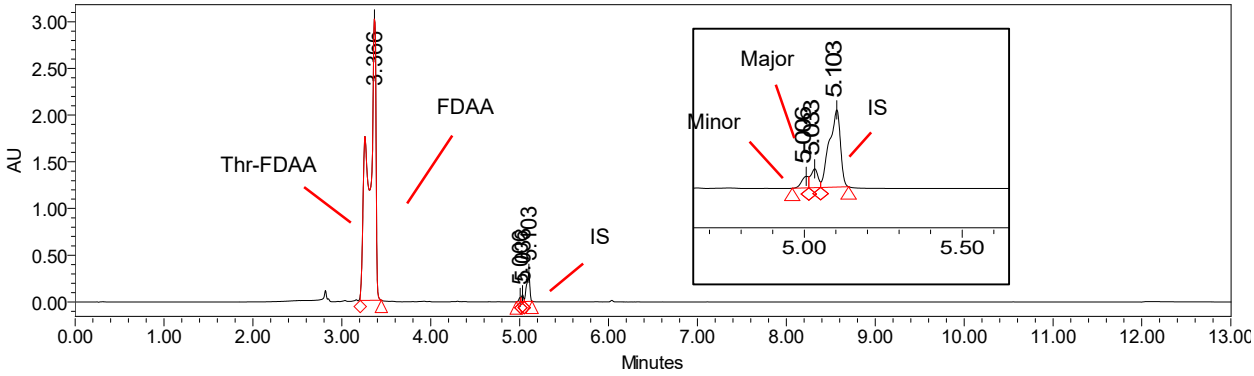




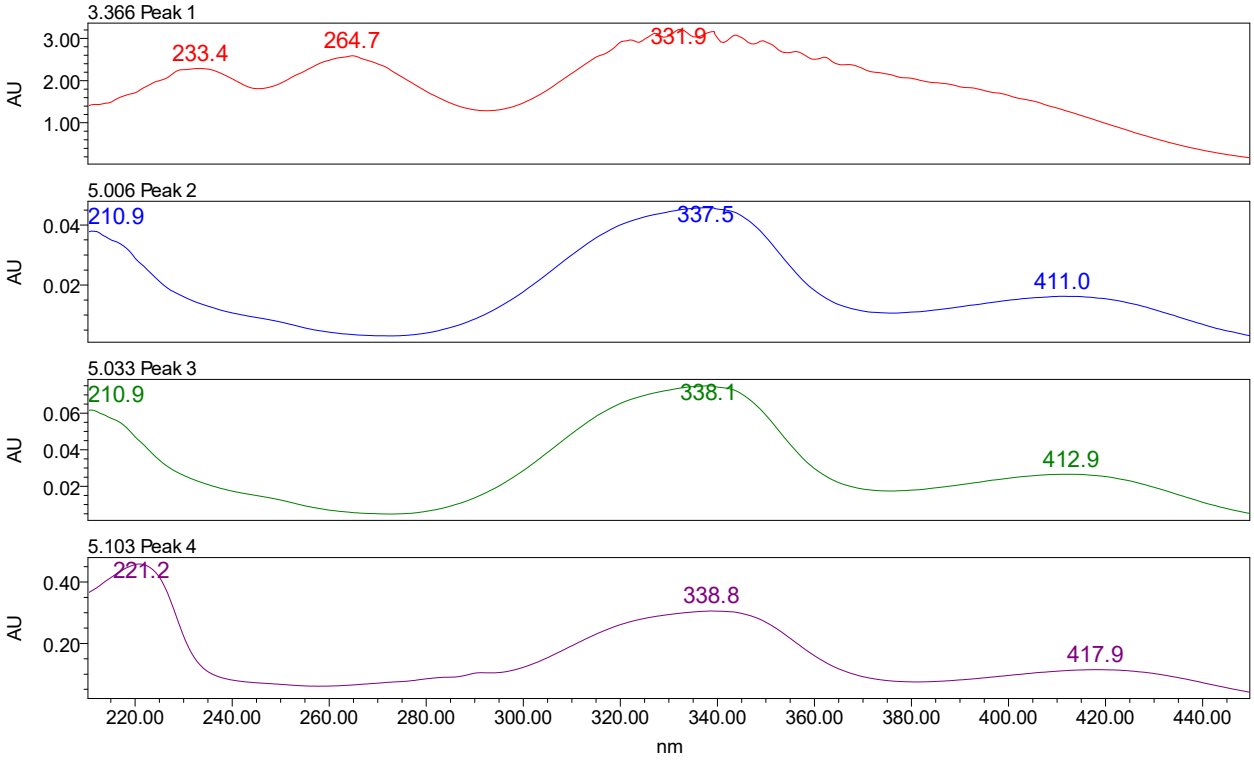


Supplemental Figure S11. ObiH-catalyzed reaction with 4-(OPiv)-benzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

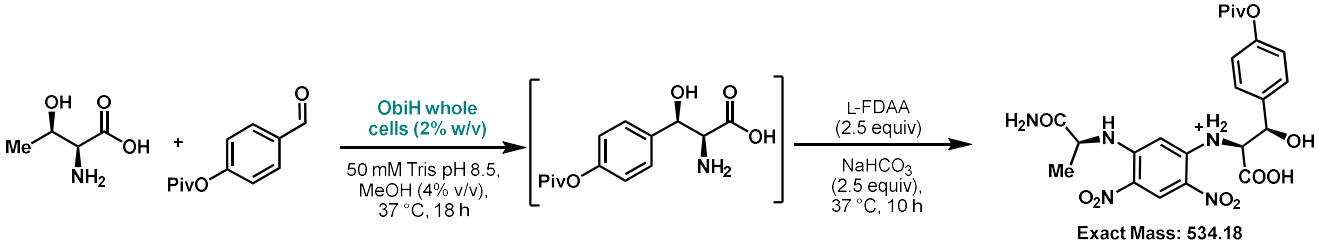


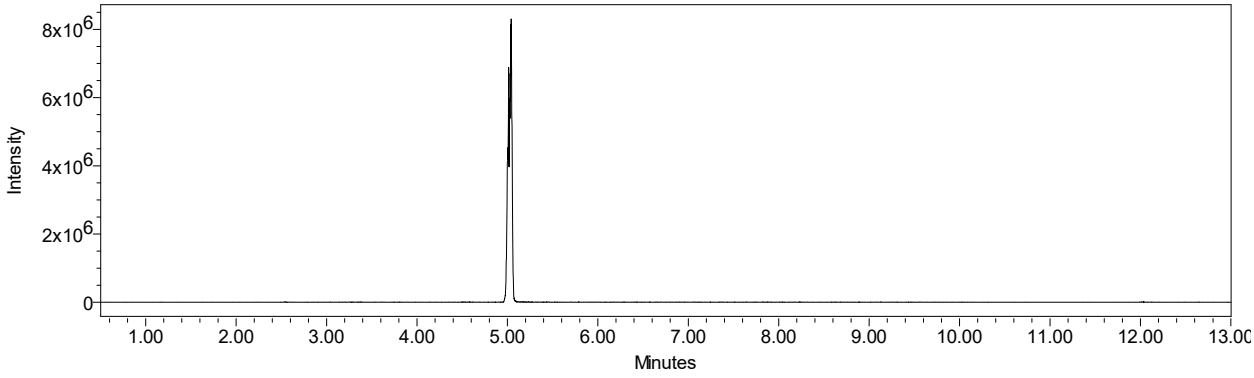


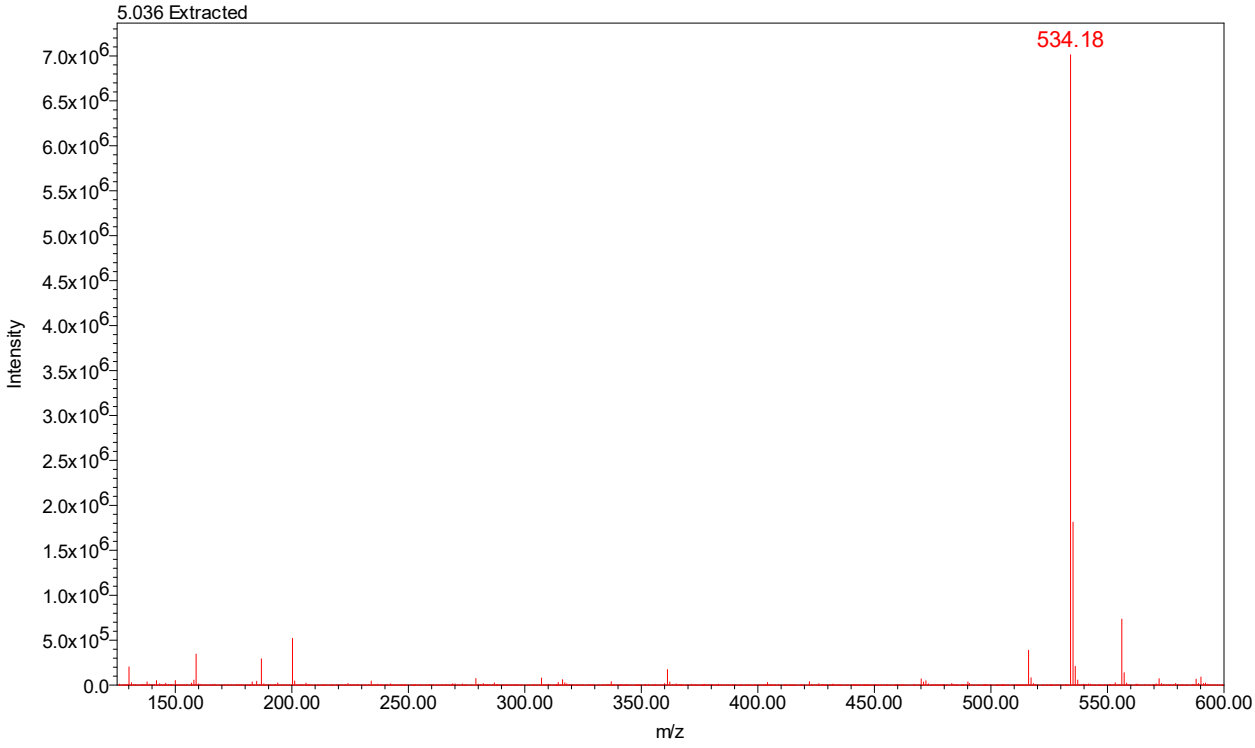
	Retention Time	Area	% Area	Height
1	3.366	16527399	94.69	3012410
2	5.006	68563	0.39	45333
3	5.033	118687	0.68	74290
4	5.103	739612	4.24	305037



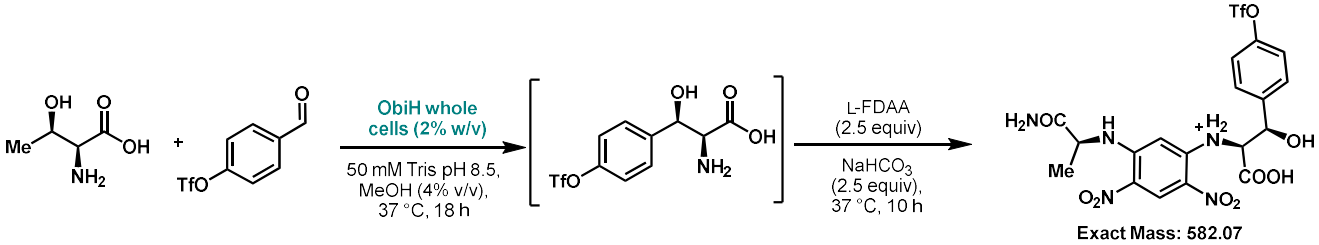
Supplemental Figure S12. ObiH-catalyzed reaction with 4-(OPiv)-benzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 534.2

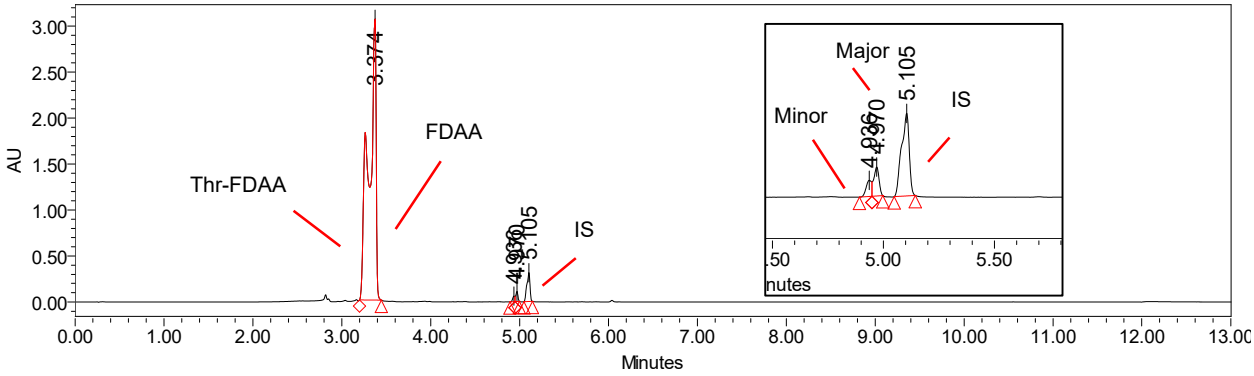




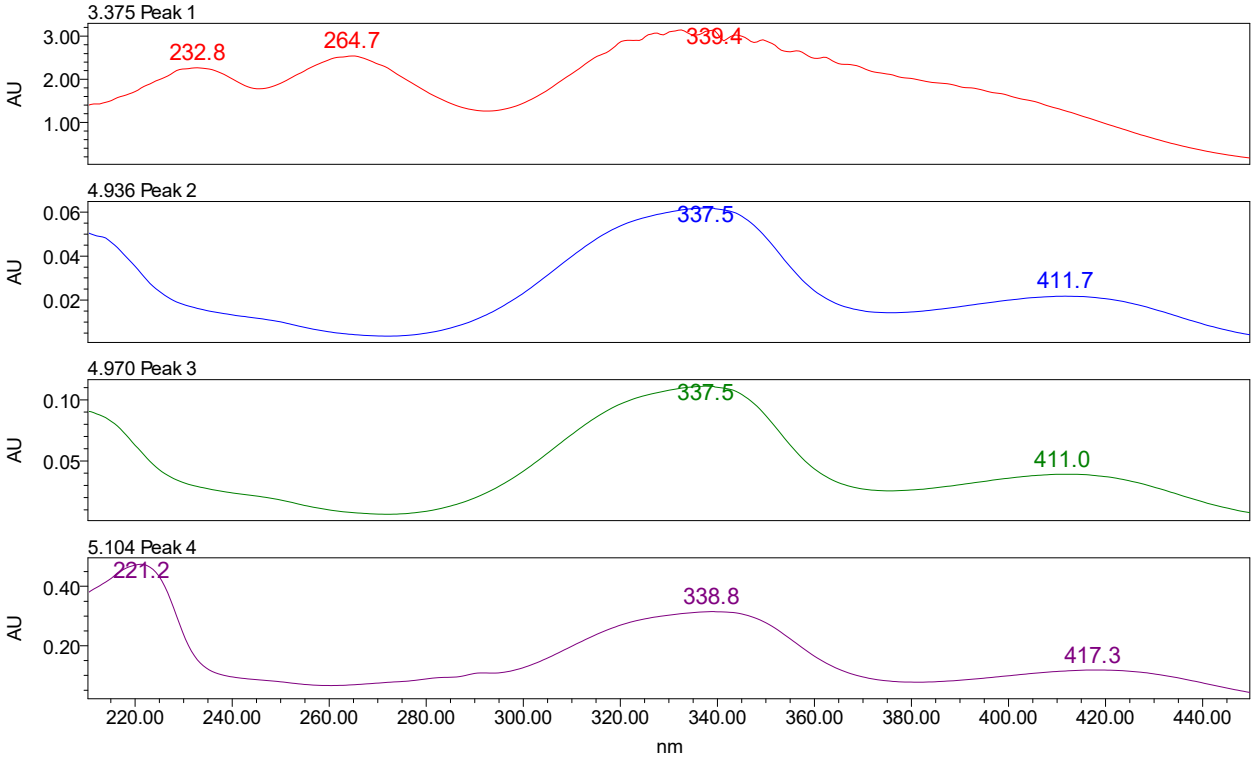


Supplemental Figure S13. ObiH-catalyzed reaction with 4-(OTf)-benzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

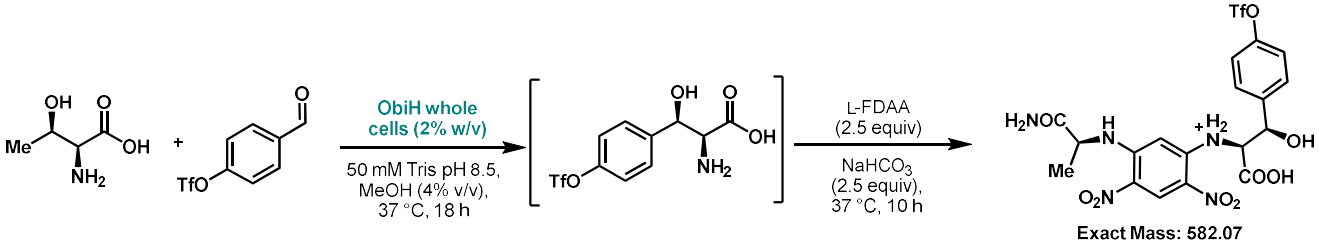


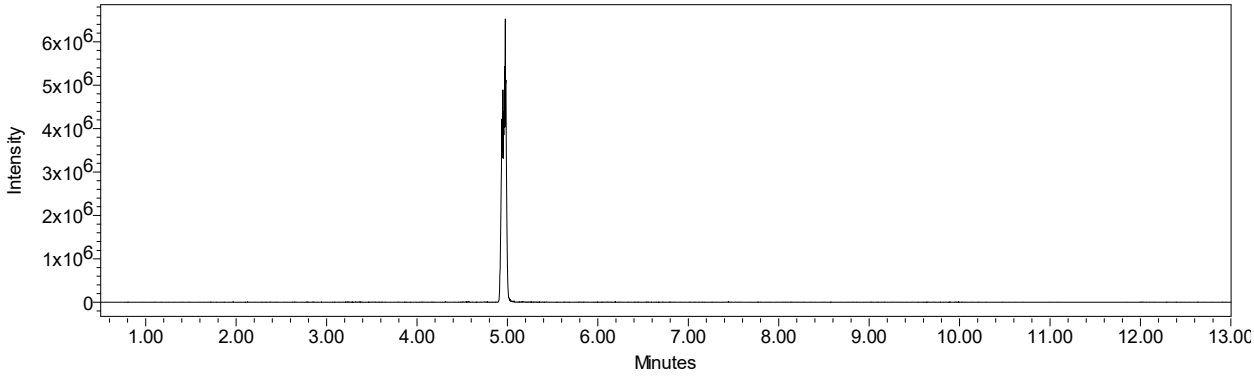


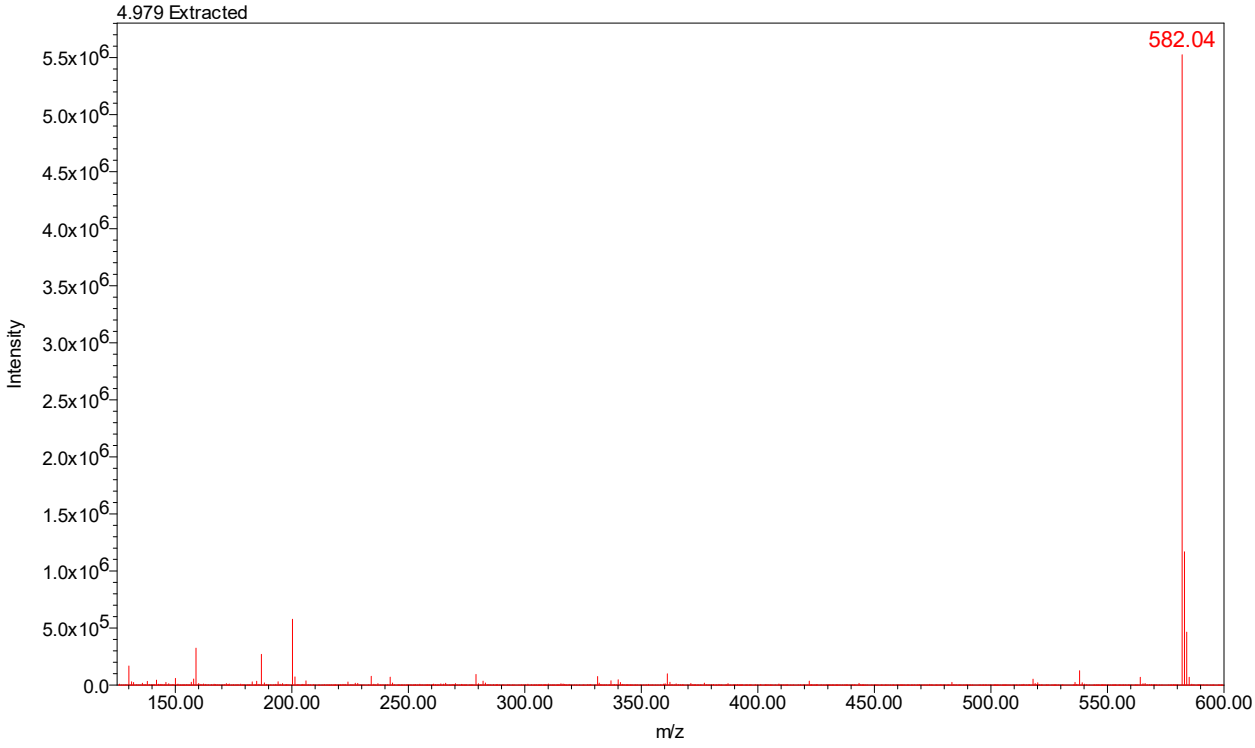
	Retention Time	Area	% Area	Height
1	3.374	16824164	94.19	3055574
2	4.936	102323	0.57	61406
3	4.970	181920	1.02	110274
4	5.105	754346	4.22	314816



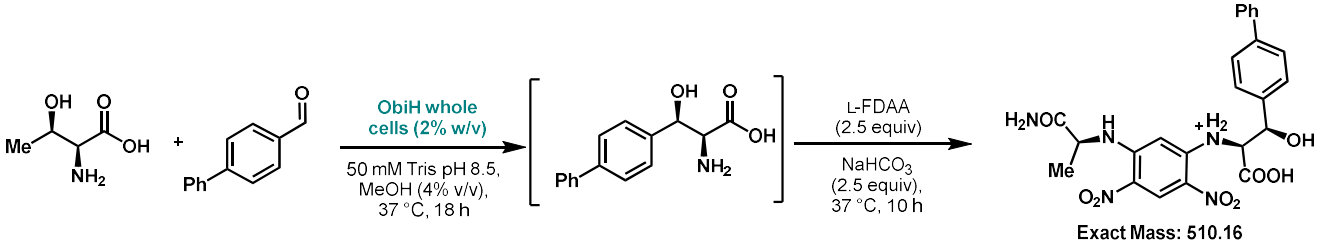
Supplemental Figure S14. ObiH-catalyzed reaction with 4-(OTf)-benzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 582.1

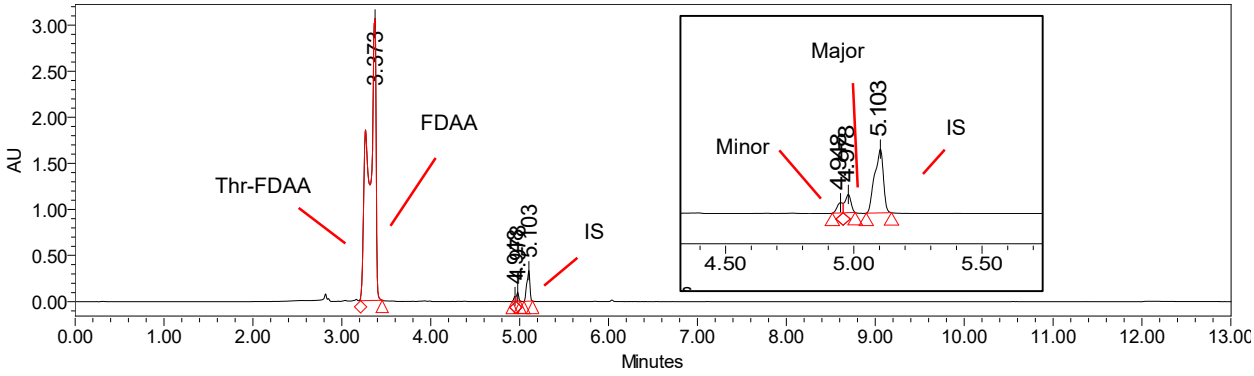




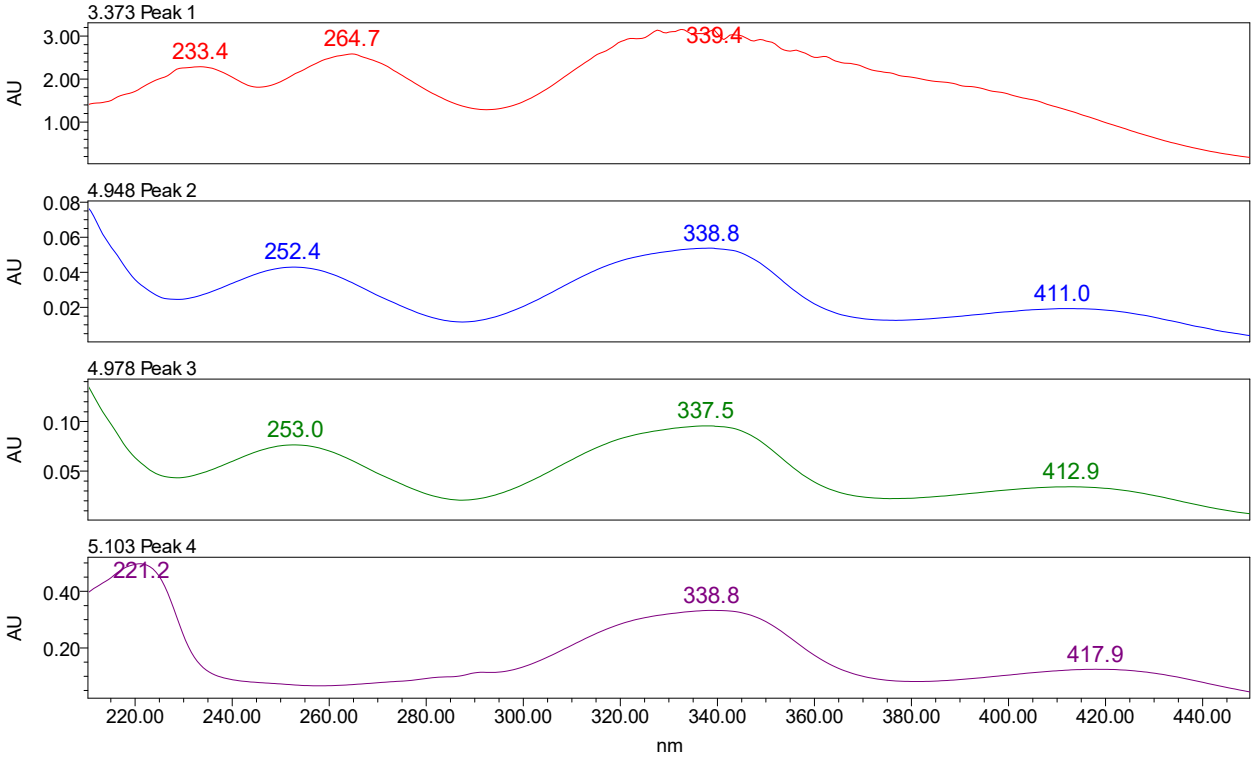


Supplemental Figure S15. ObiH-catalyzed reaction with 4-phenylbenzaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

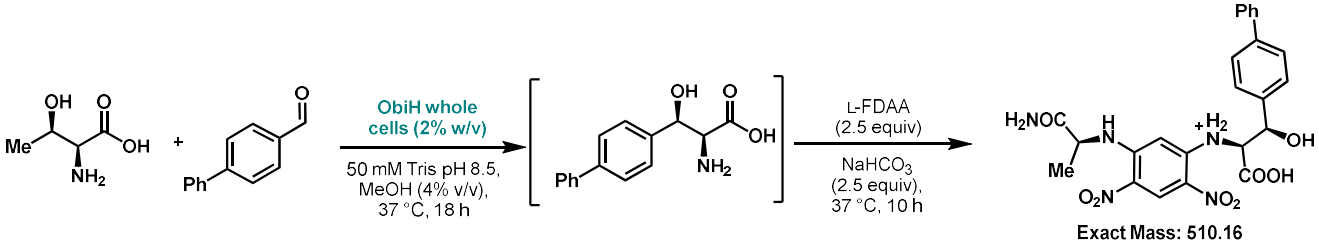


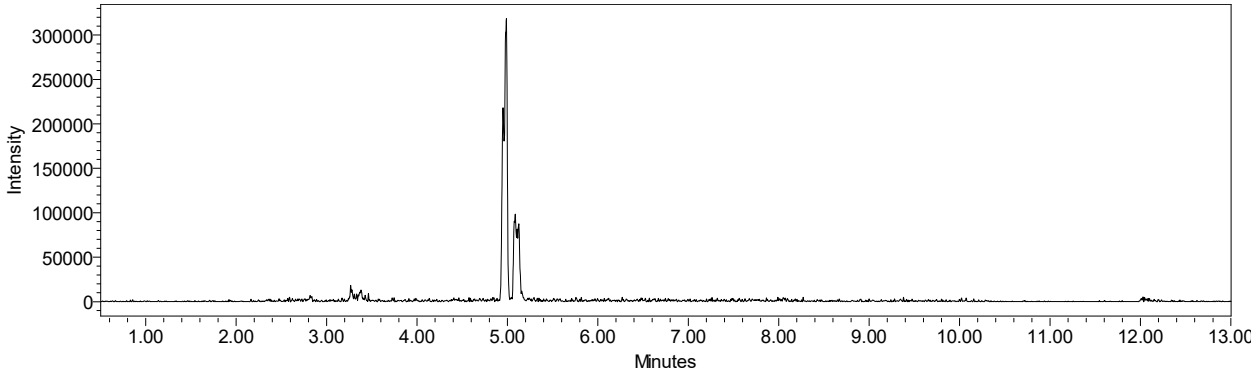


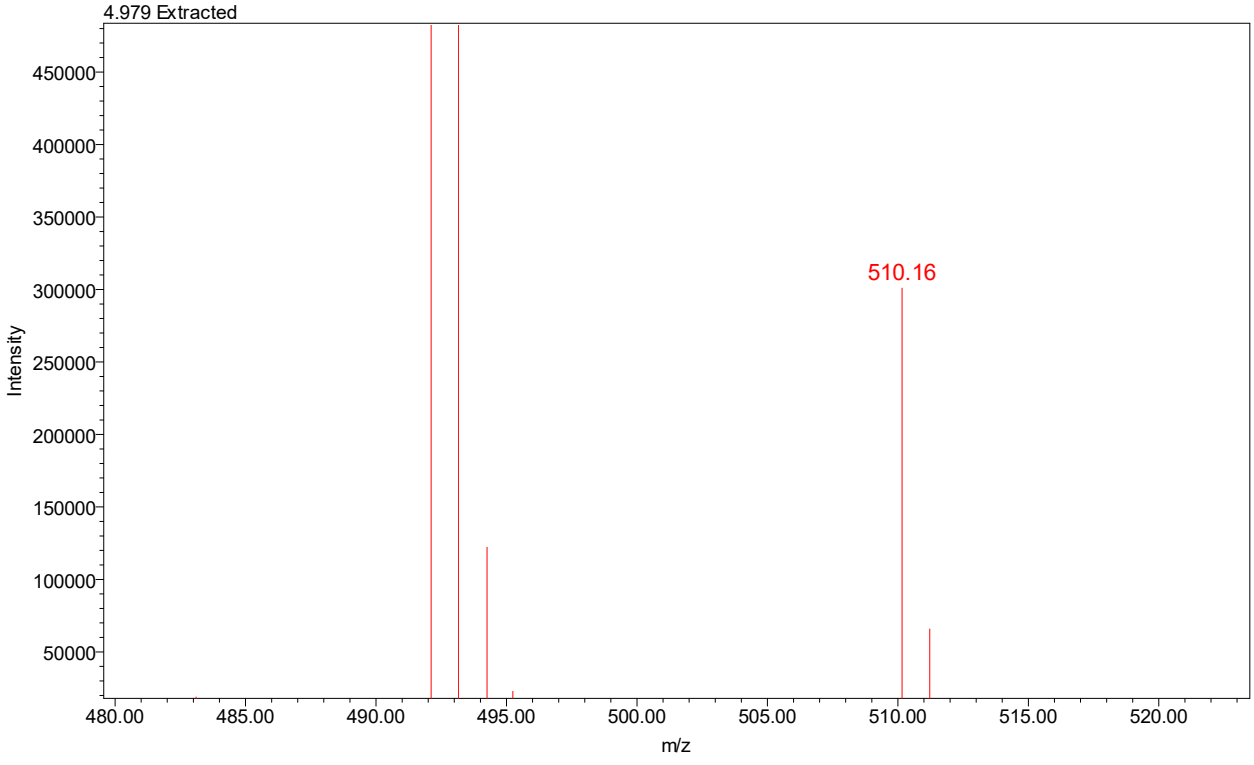
	Retention Time	Area	% Area	Height
1	3.373	16680649	94.24	3056483
2	4.948	81770	0.46	53427
3	4.978	152633	0.86	95065
4	5.103	784796	4.43	332081



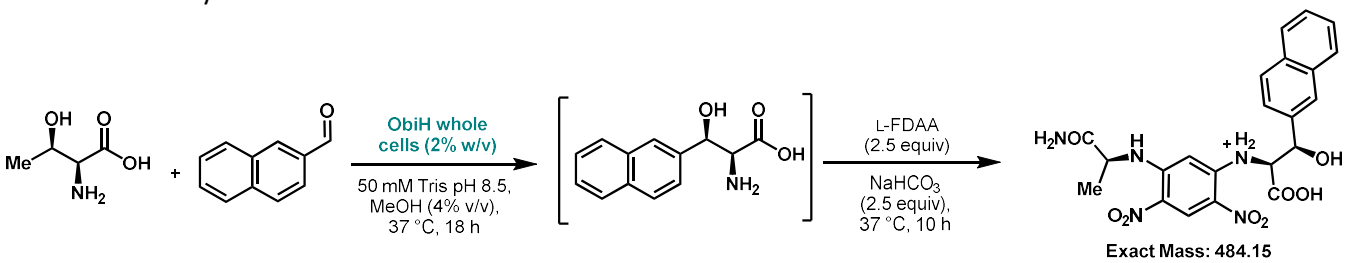
Supplemental Figure S16. ObiH-catalyzed reaction with 4-phenylbenzaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 510.2

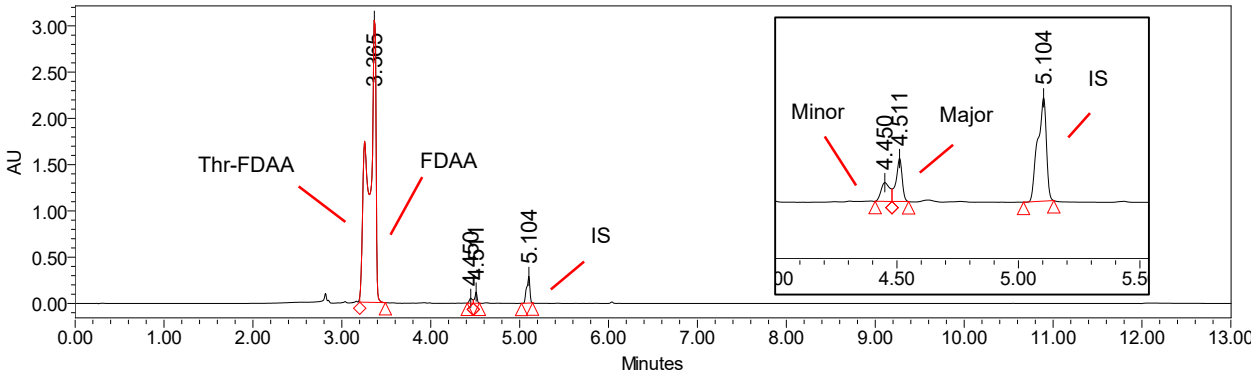




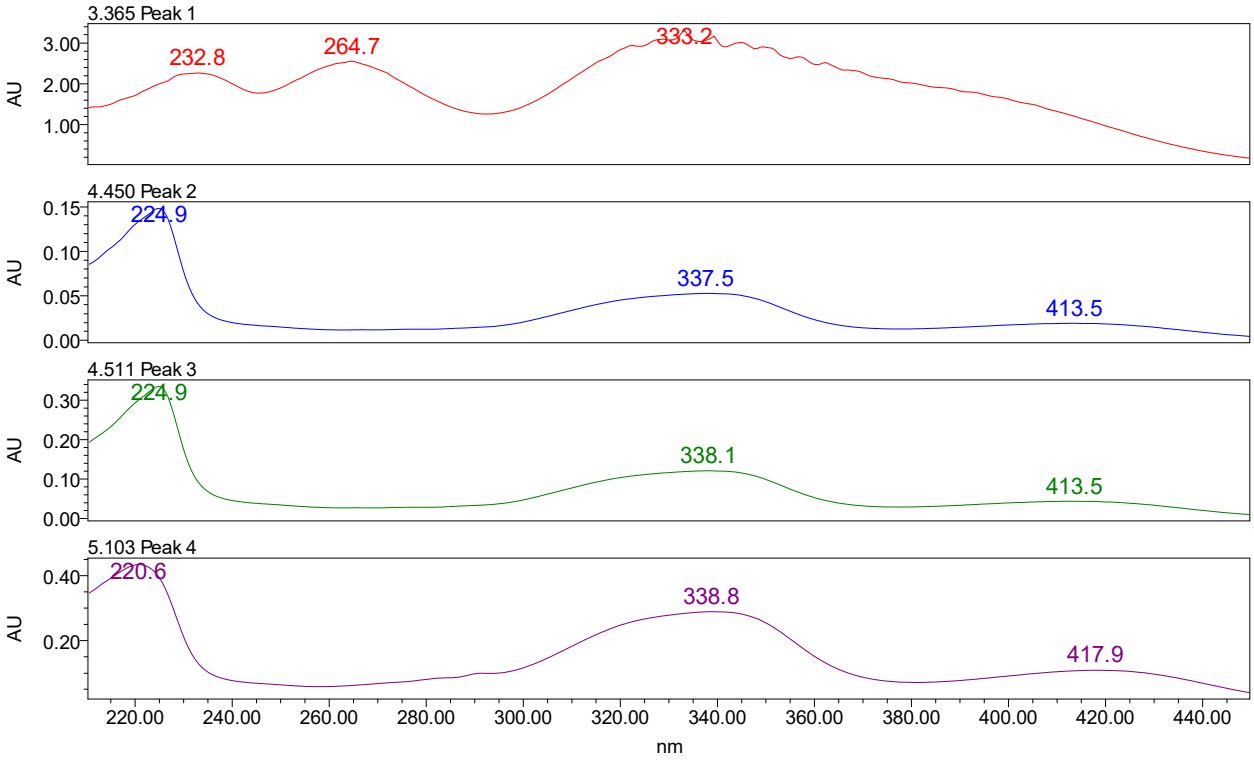


Supplemental Figure S17. ObiH-catalyzed reaction with 2-napthaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

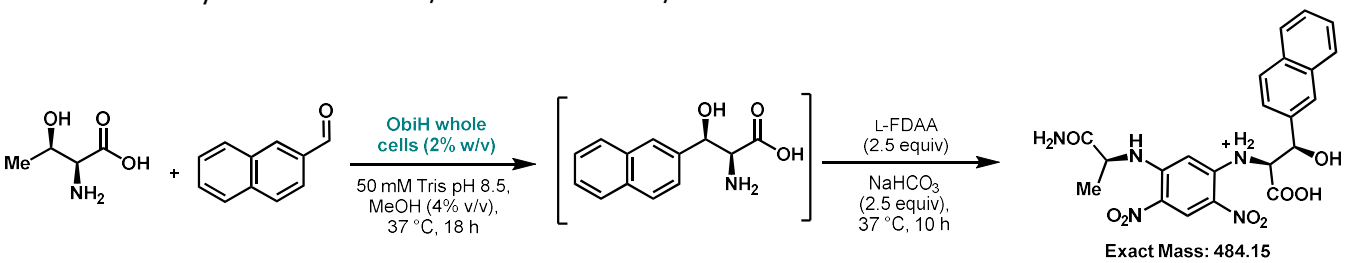


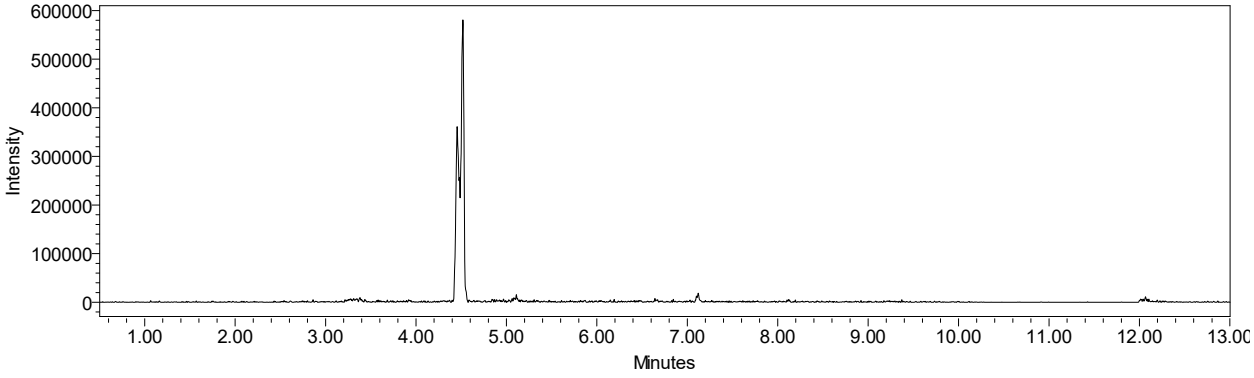


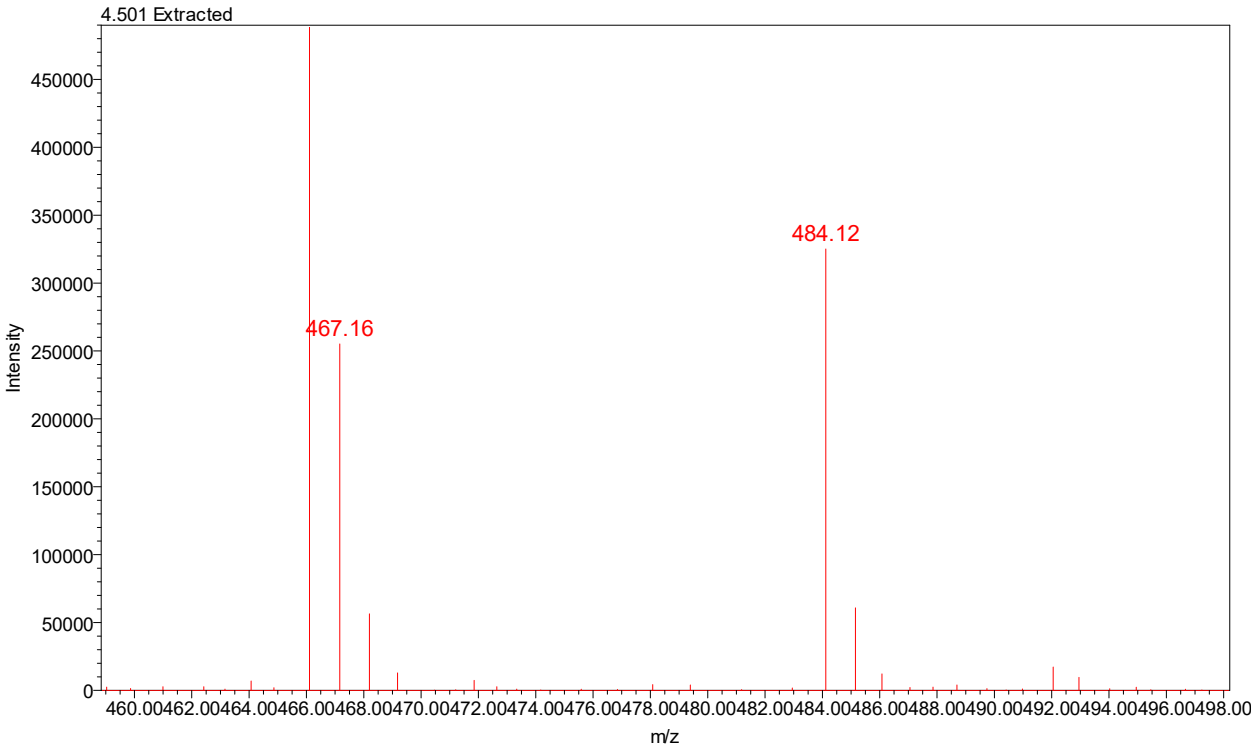
	Retention Time	Area	% Area	Height
1	3.365	16973561	94.12	3050139
2	4.450	131952	0.73	52404
3	4.511	212477	1.18	120261
4	5.104	715261	3.97	288490



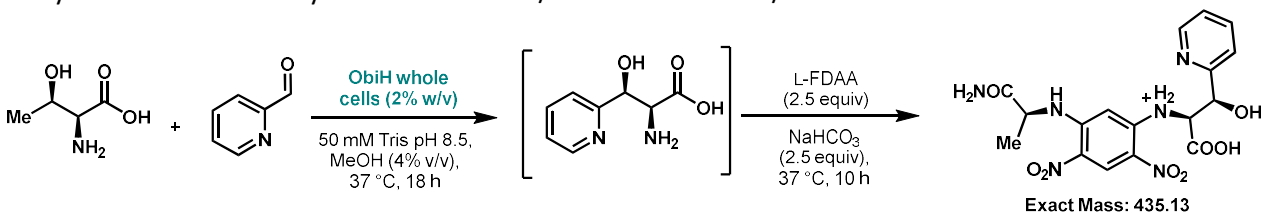
Supplemental Figure S18. ObiH-catalyzed reaction with 2-napthaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm. LC/MS traces of ESI M/Z 484.2

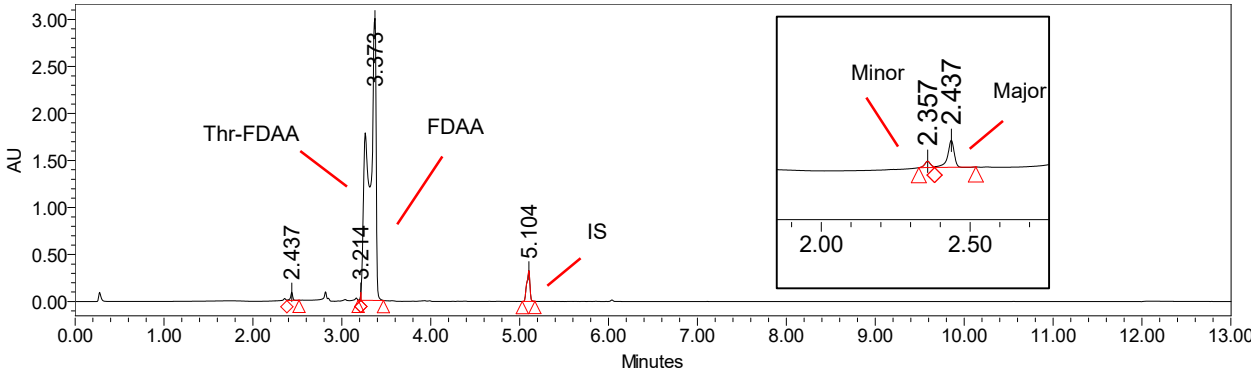




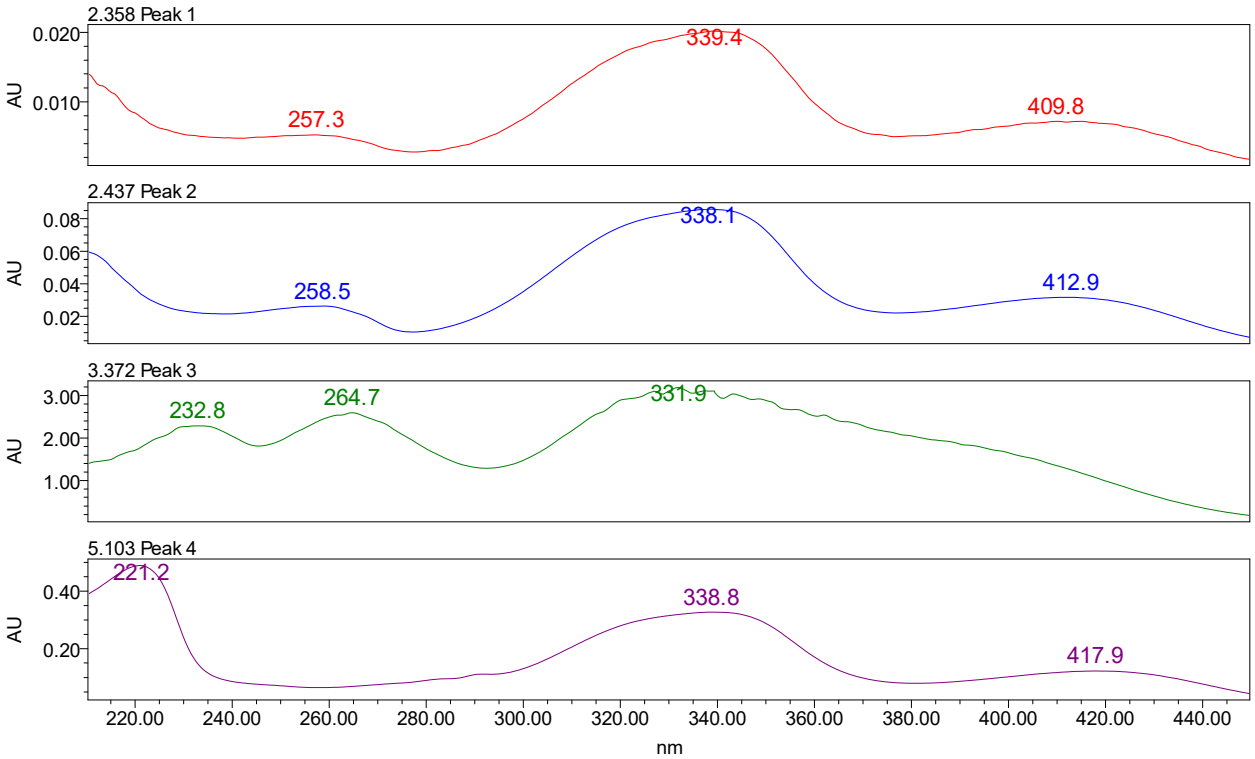


Supplemental Figure S19. ObiH-catalyzed reaction with 2-pyridinecarboxaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm. LC/MS traces of ESI M/Z 435.13

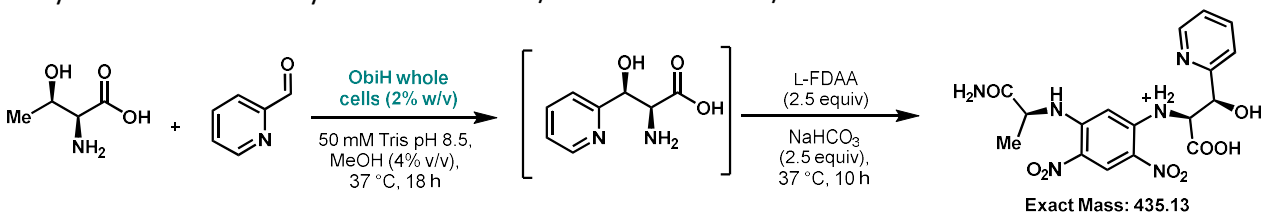


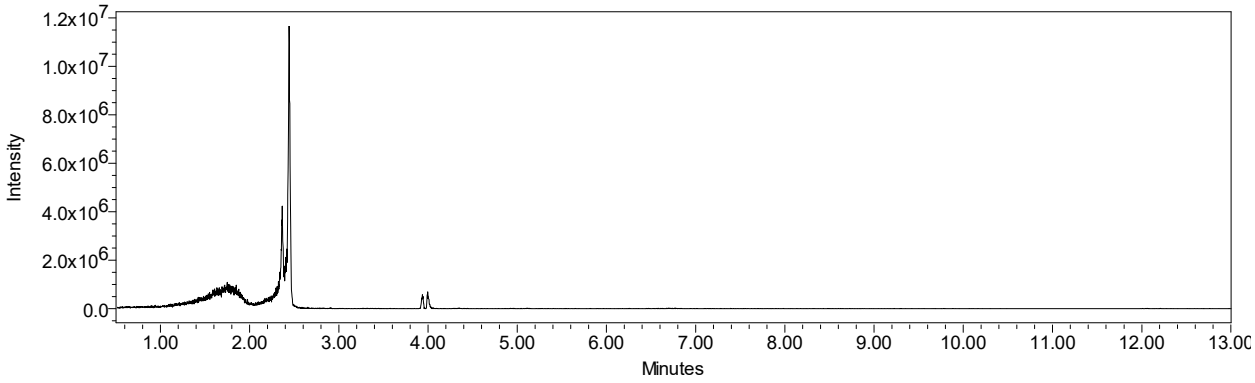


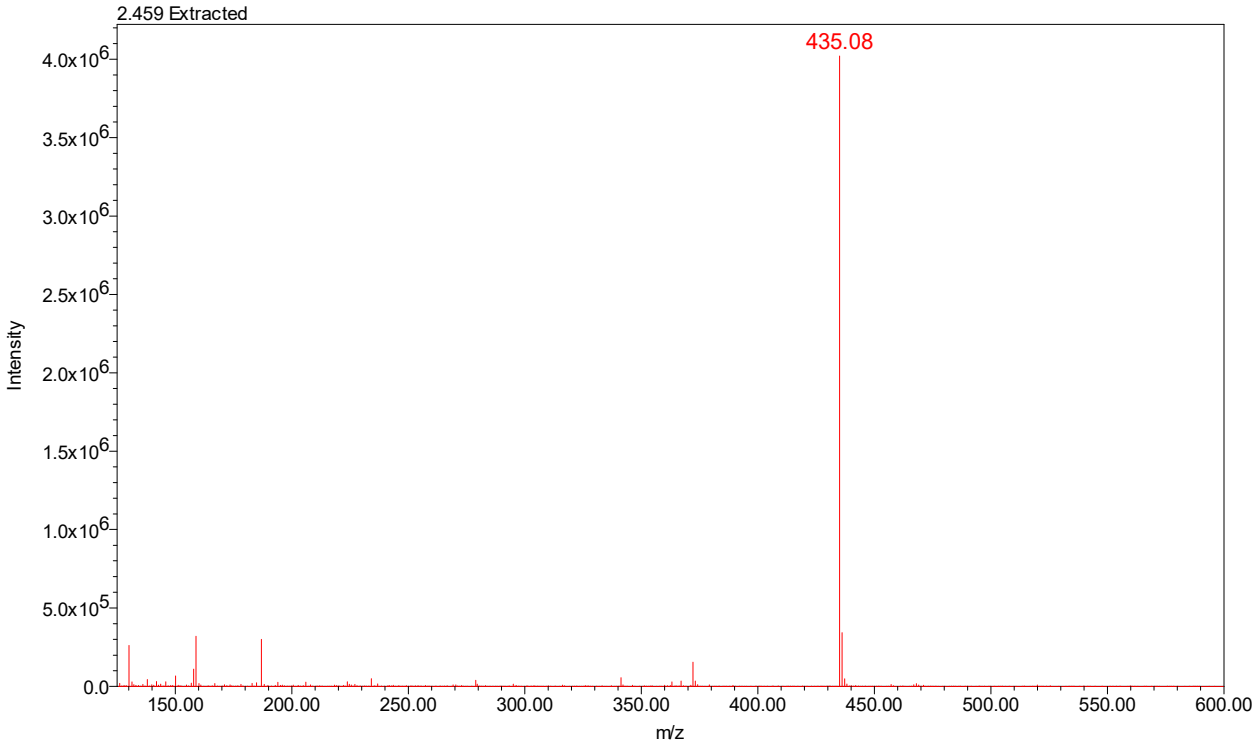
	Retention Time	Area	% Area	Height
1	2.357	27822	0.16	20174
2	2.437	133696	0.77	85595
3	3.373	16476123	94.51	2998869
4	5.104	795034	4.56	326781



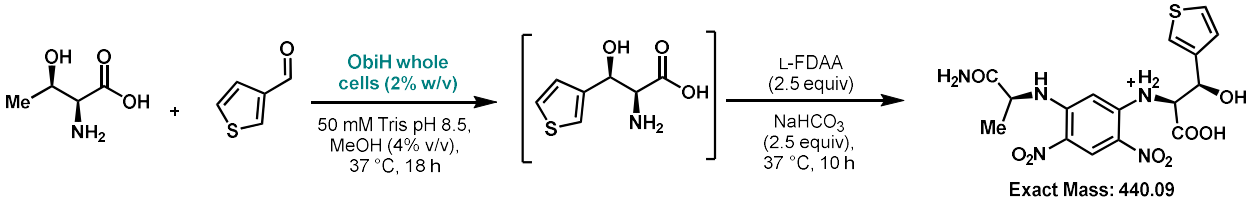
Supplemental Figure S20. ObiH-catalyzed reaction with 2-pyridinecarboxaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm. LC/MS traces of ESI M/Z 435.13

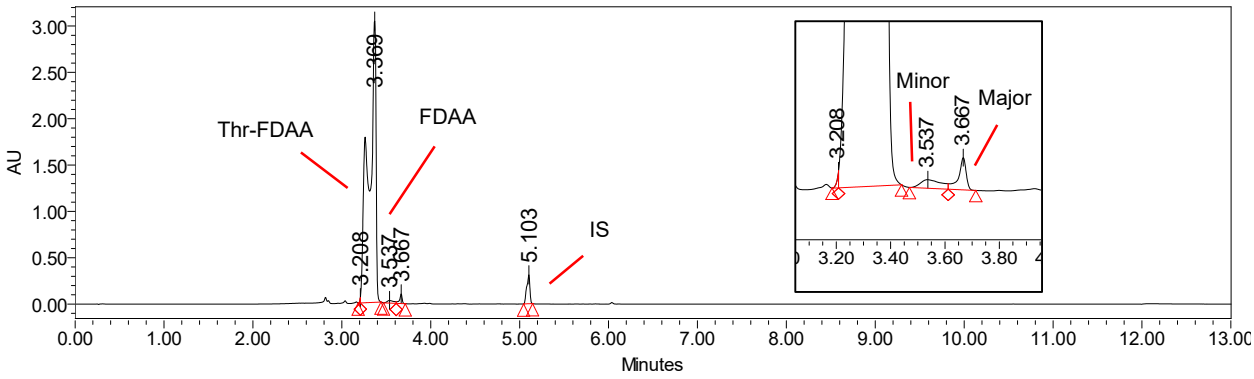




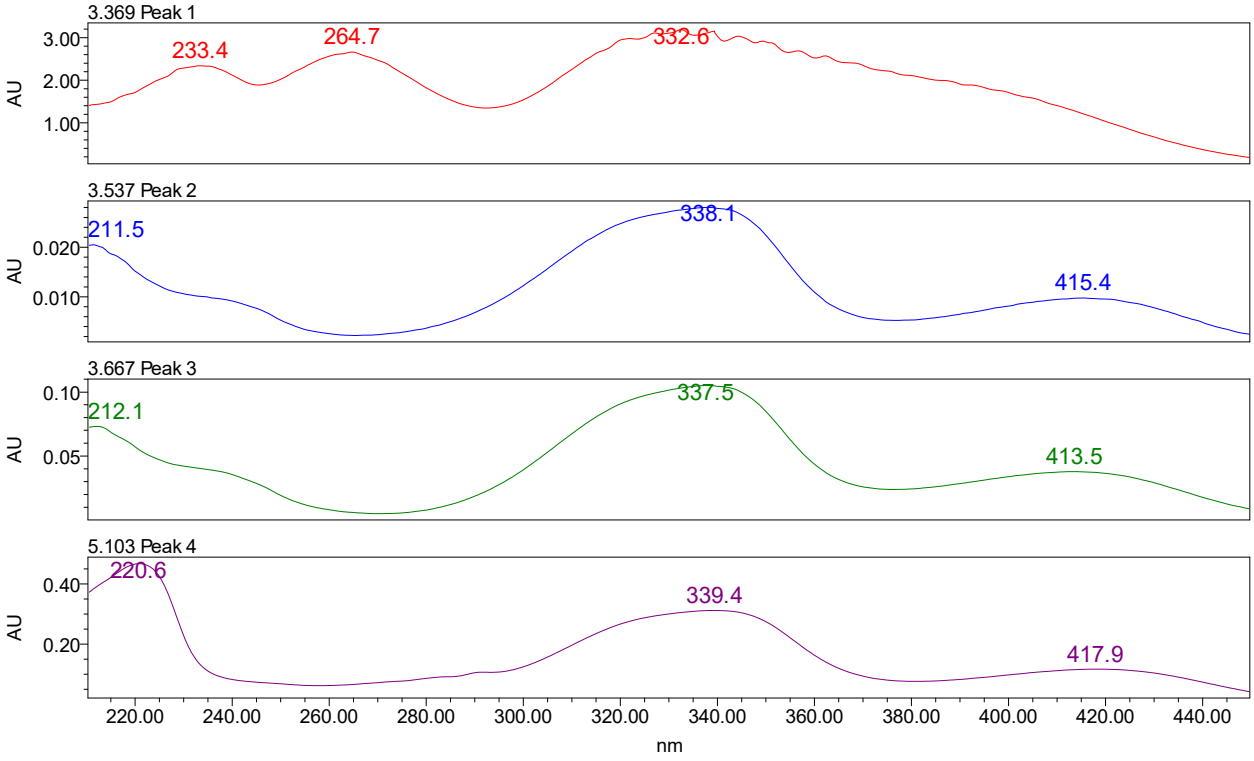


Supplemental Figure S21. ObiH-catalyzed reaction with 3-thiophenecarboxaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm. LC/MS traces of ESI M/Z 440.1

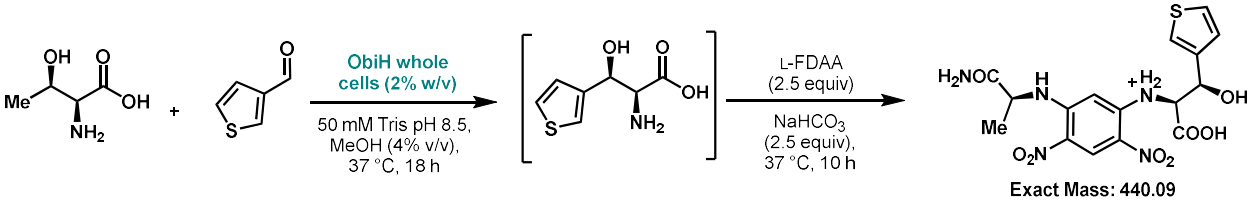


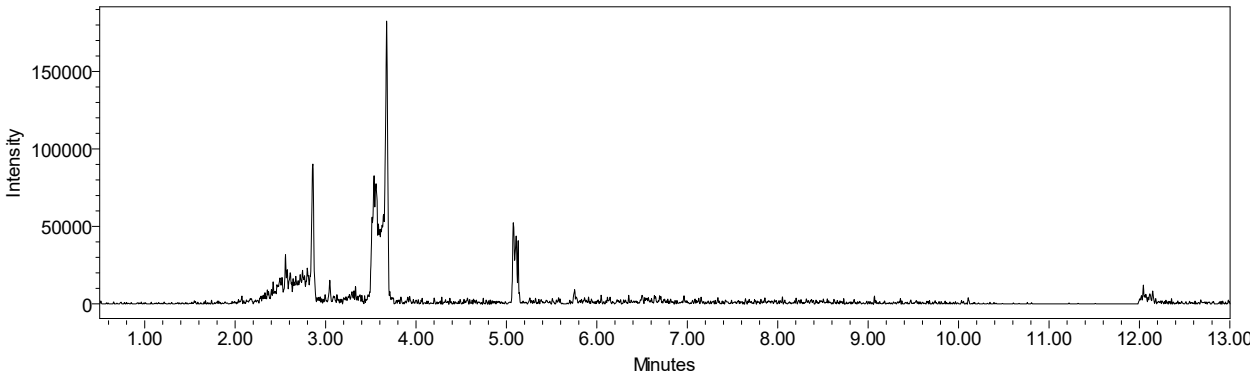


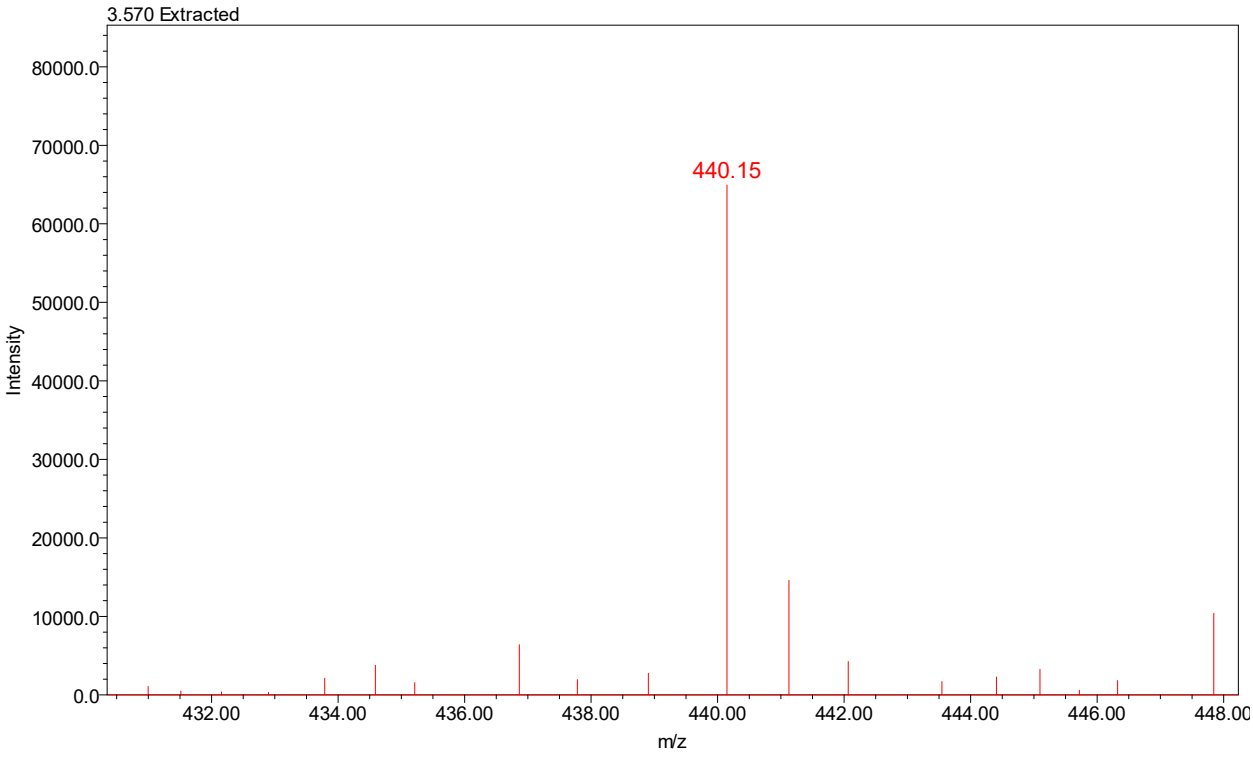
	Retention Time	Area	% Area	Height
1	3.369	16685878	93.77	3033942
2	3.537	149229	0.84	27845
3	3.667	204299	1.15	104430
4	5.103	754291	4.24	311782



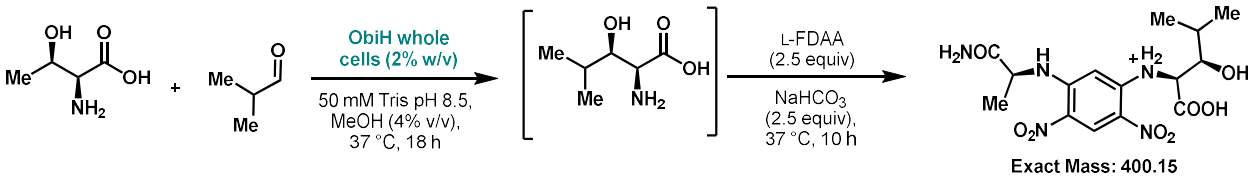
Supplemental Figure S22. ObiH-catalyzed reaction with 3-thiophenecarboxaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm. LC/MS traces of ESI M/Z 440.1

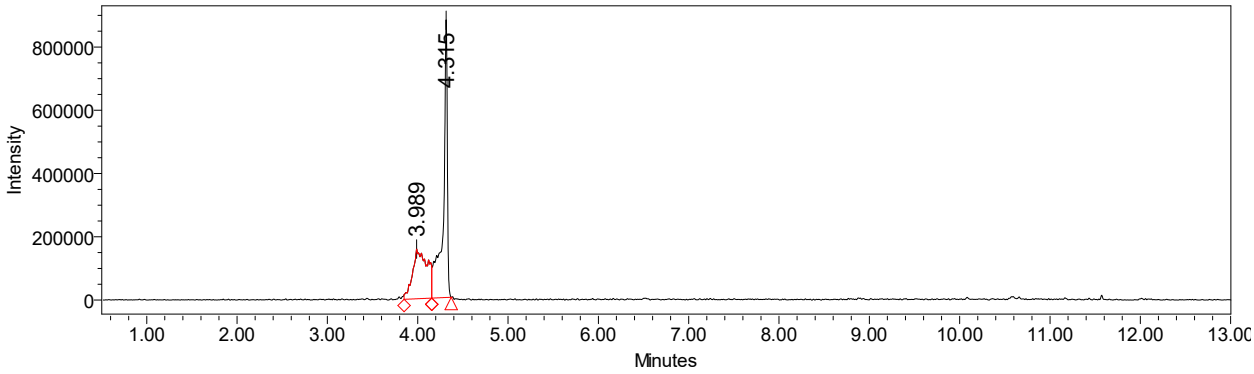




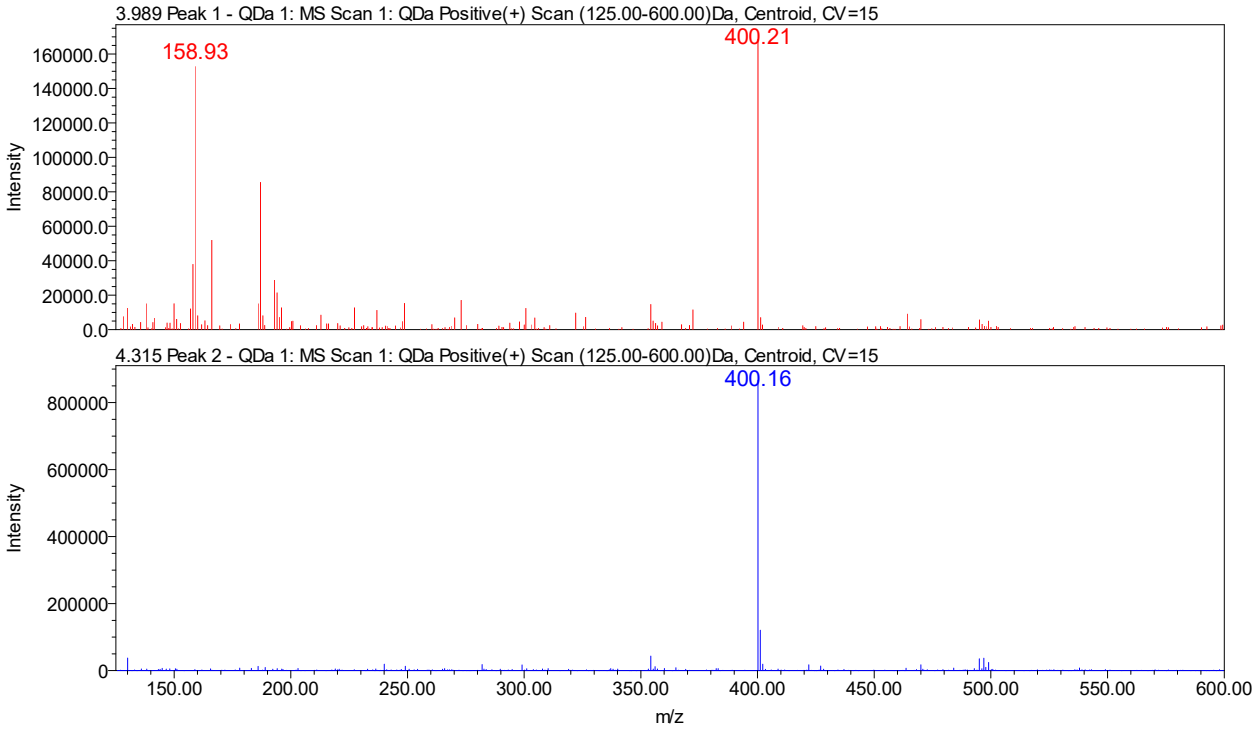


Supplemental Figure S23. ObiH-catalyzed reaction with isobutyraldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 440.1

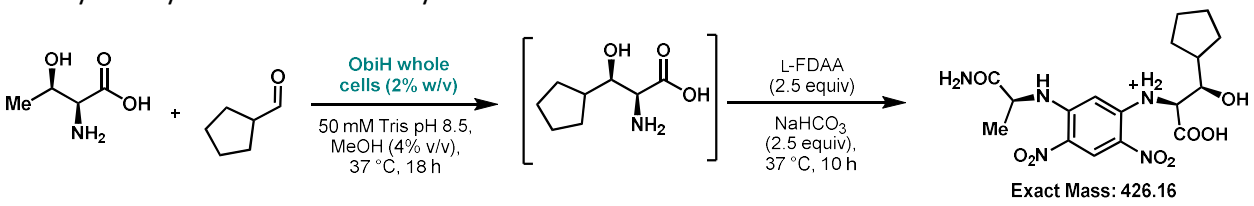


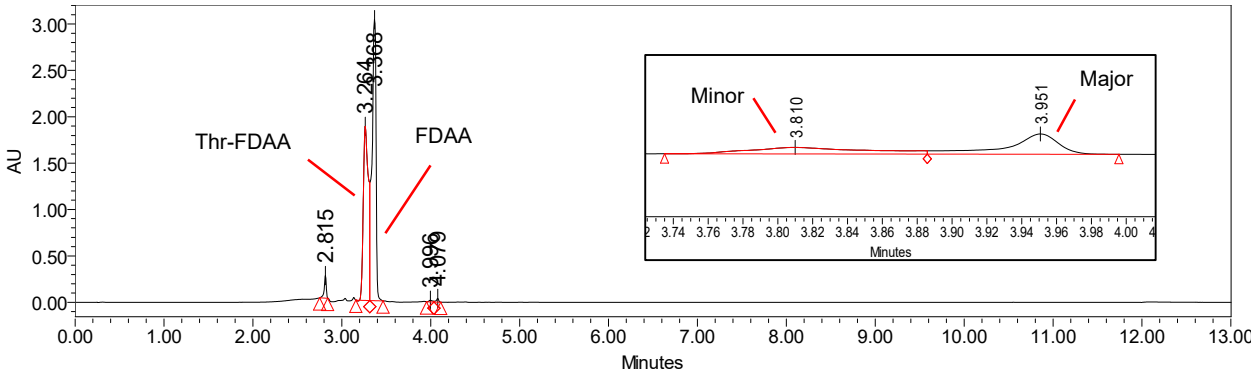


	Retention Time	Area	% Area	Height
1	3.989	1761489	38.57	157275
2	4.315	2805694	61.43	877725

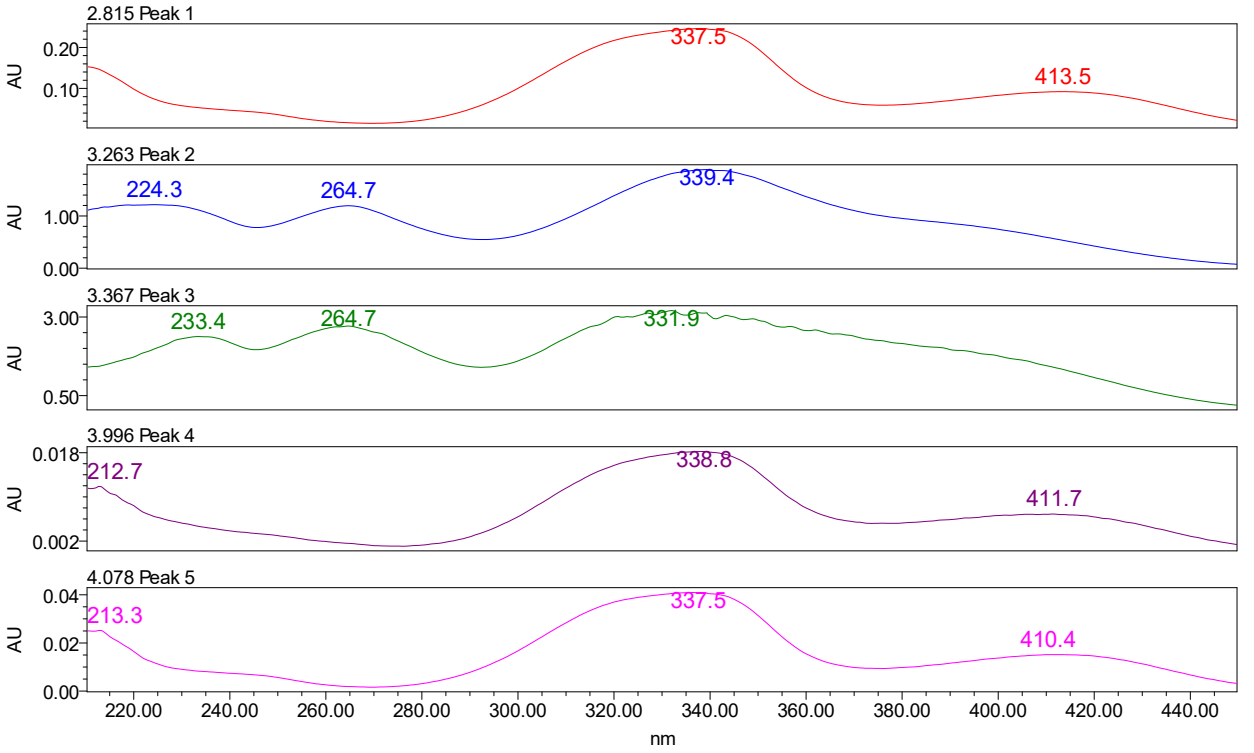


Supplemental Figure S24. ObiH-catalyzed reaction with cyclopentanecarboxaldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

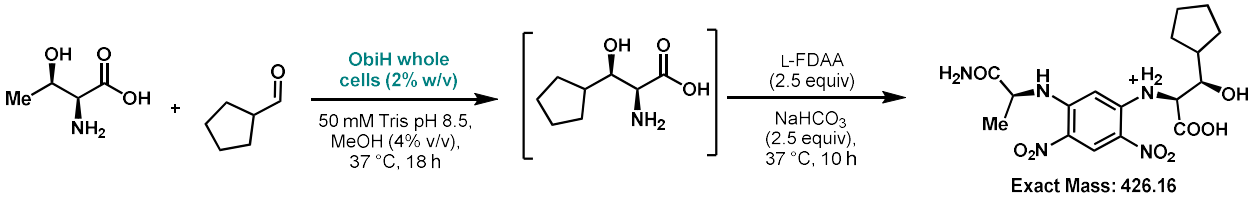


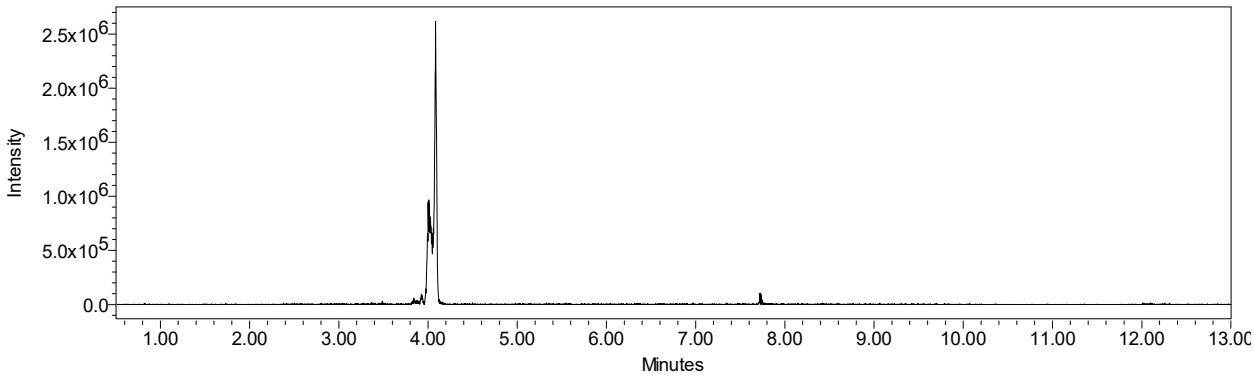


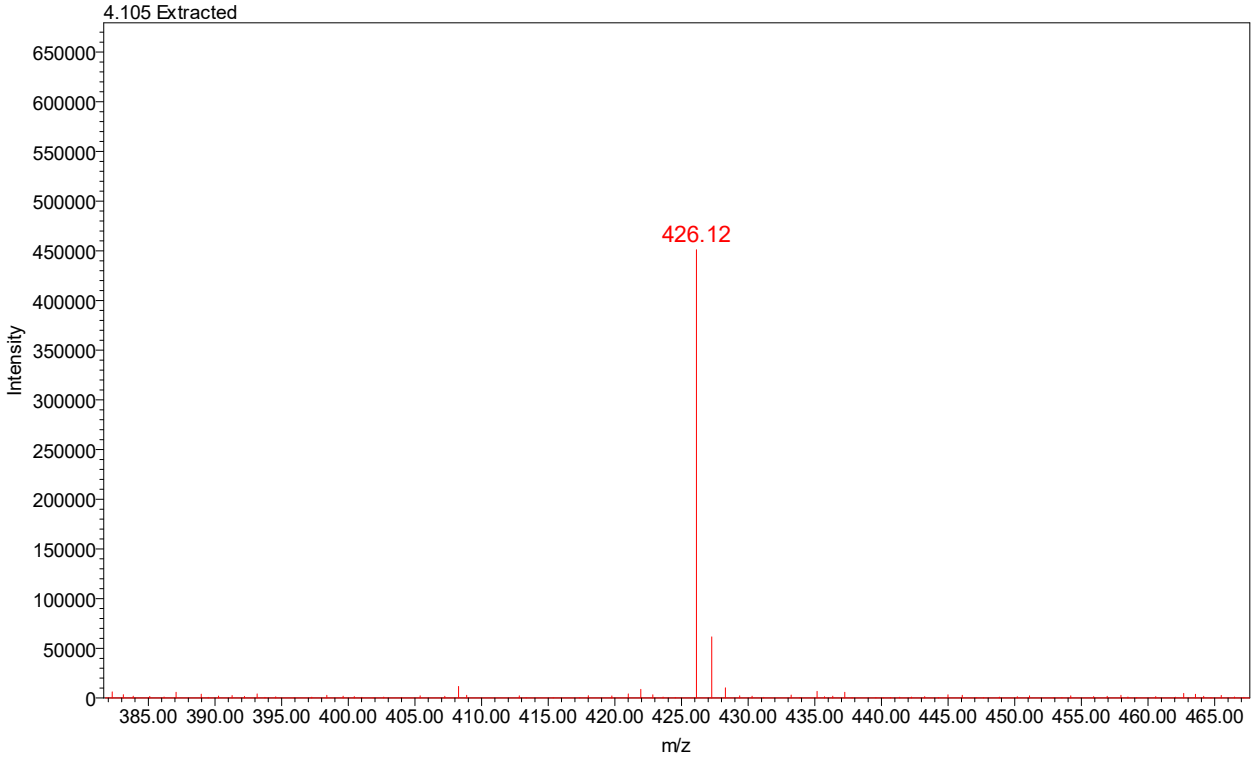
	Retention Time	Area	% Area	Height
1	2.815	377861	2.14	244158
2	3.264	7528590	42.61	1877081
3	3.368	9635761	54.54	3032394
4	3.996	49883	0.28	18090
5	4.079	75280	0.43	40480



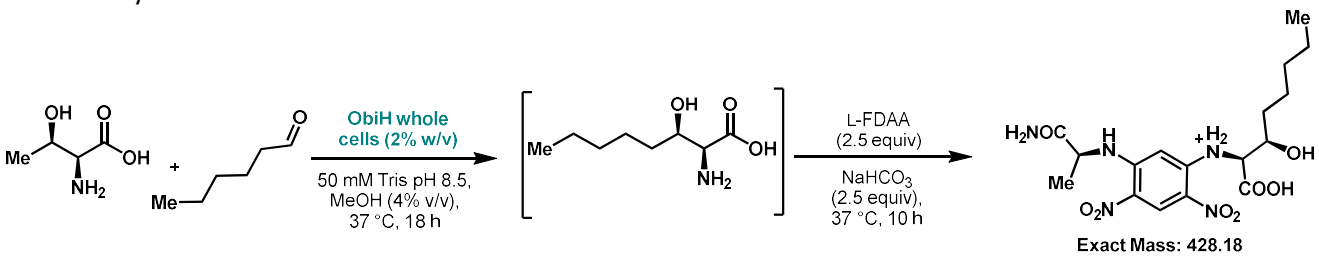
Supplemental Figure S25. ObiH-catalyzed reaction with cyclopentanecarboxaldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 426.2

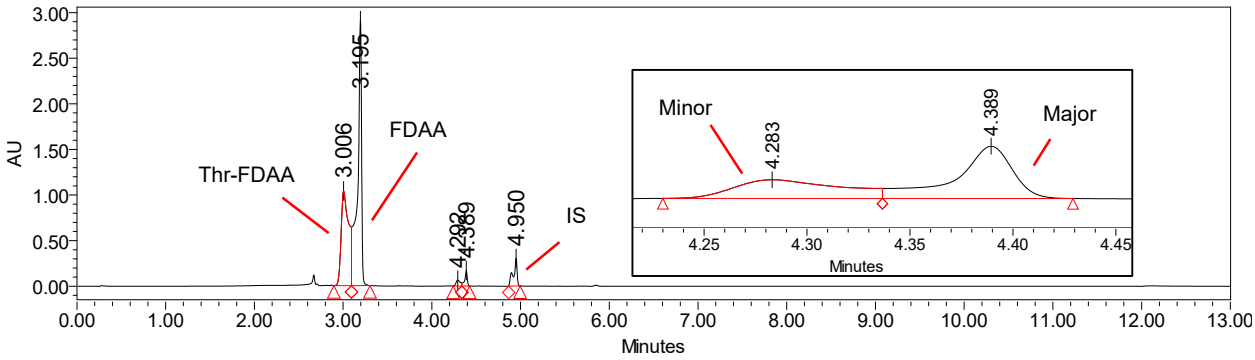




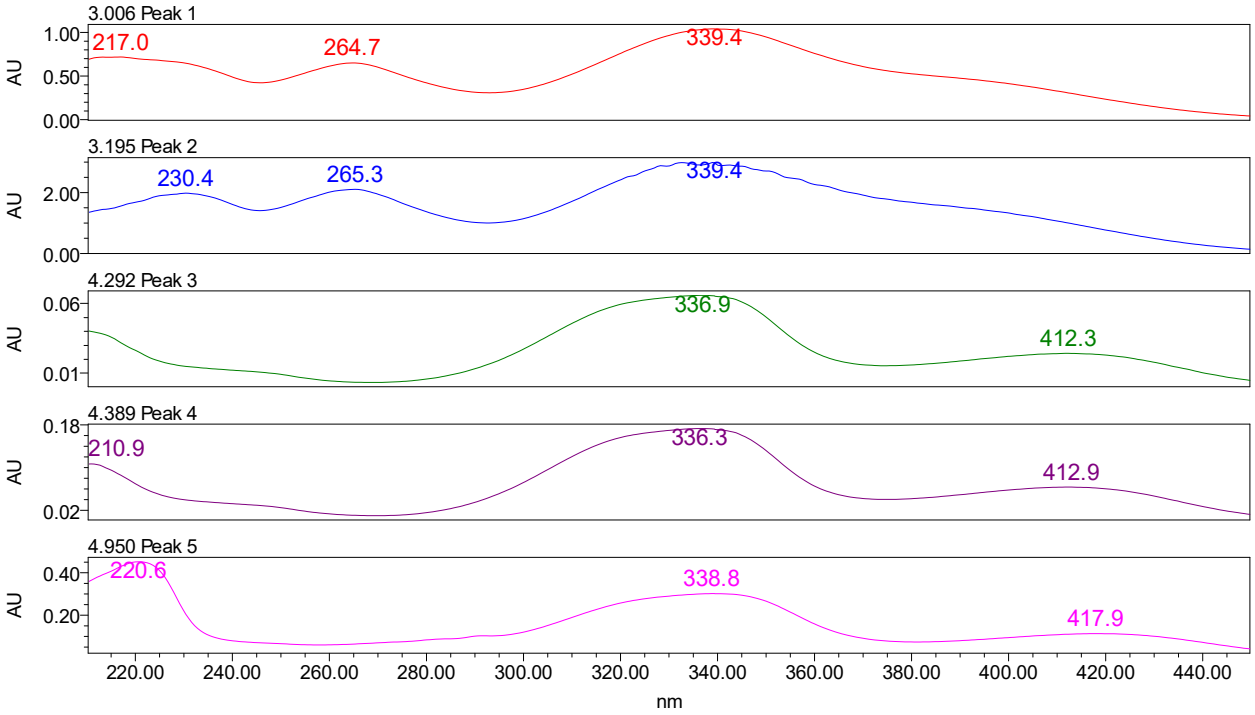


Supplemental Figure S26. ObiH-catalyzed reaction with hexanal analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

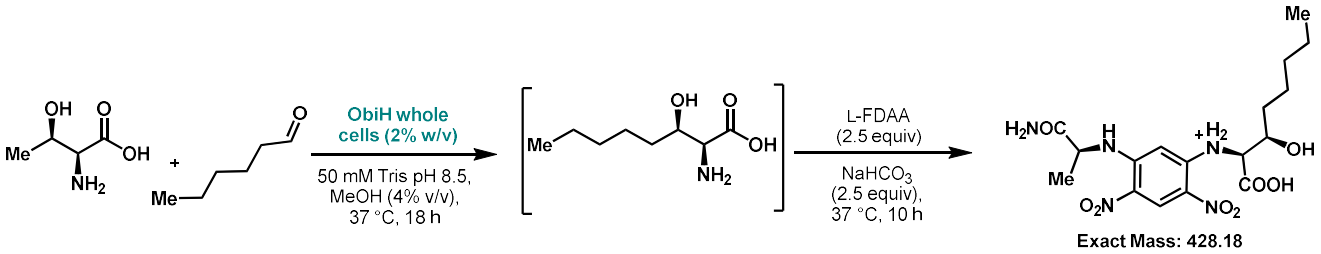


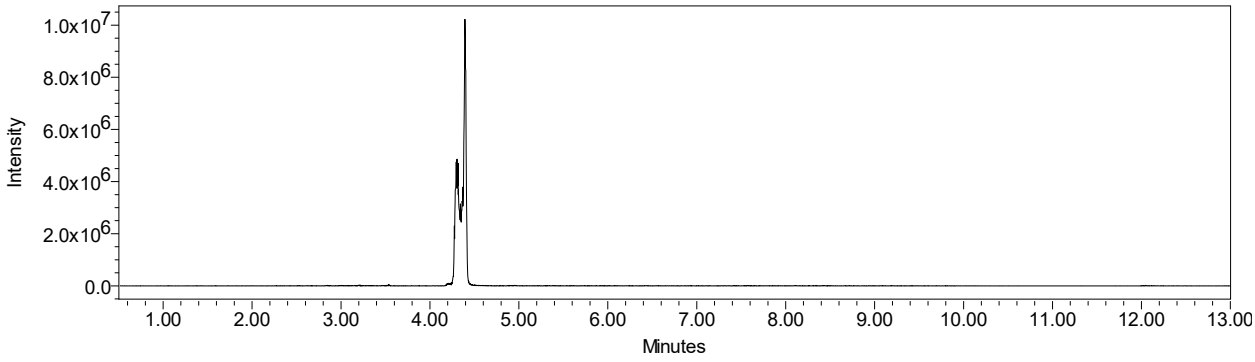


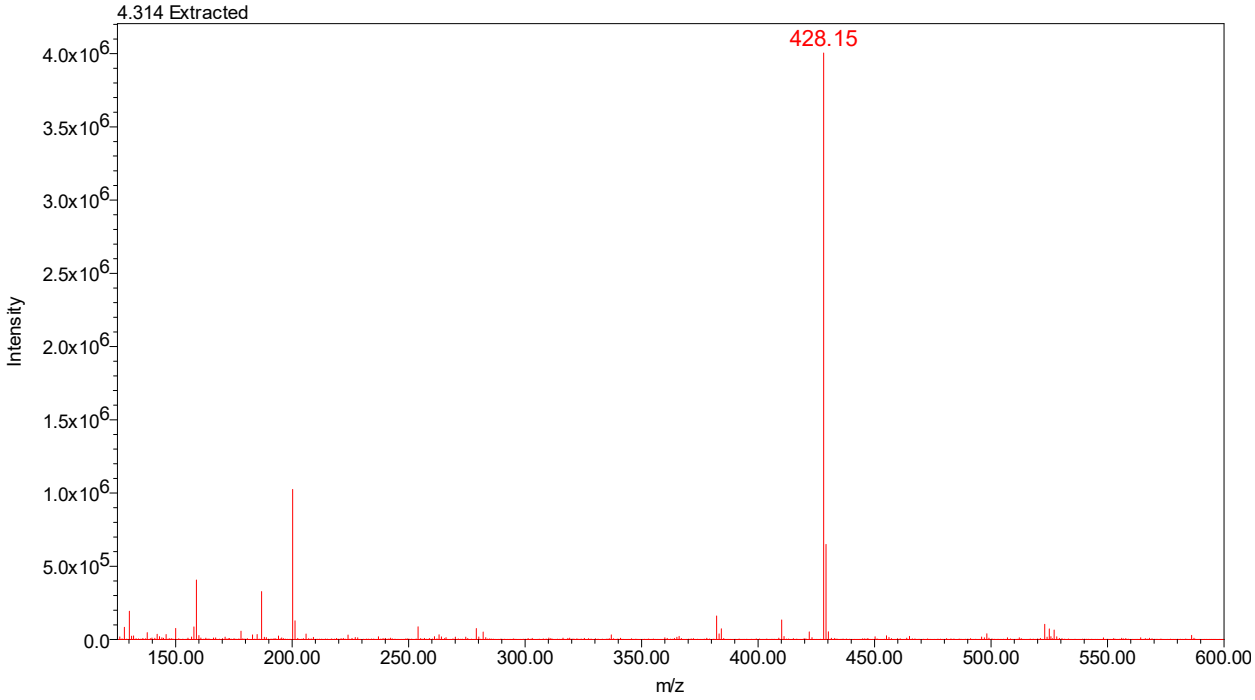
	Retention Time	Area	% Area	Height
1	3.006	6178427	36.85	1038715
2	3.195	9185948	54.79	2907050
3	4.292	214715	1.28	64879
4	4.389	325726	1.94	171109
5	4.950	860016	5.13	301205



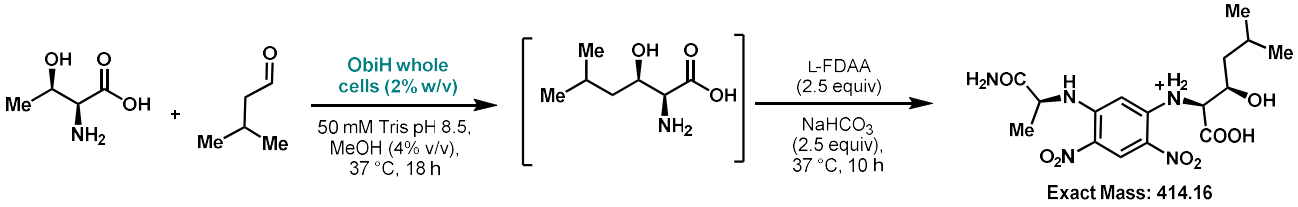
Supplemental Figure S27. ObiH-catalyzed reaction with hexanal analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 428.2

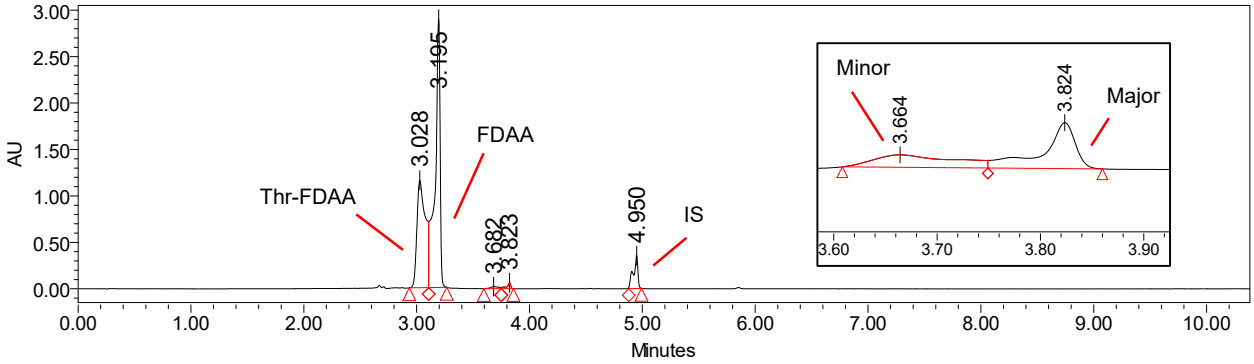




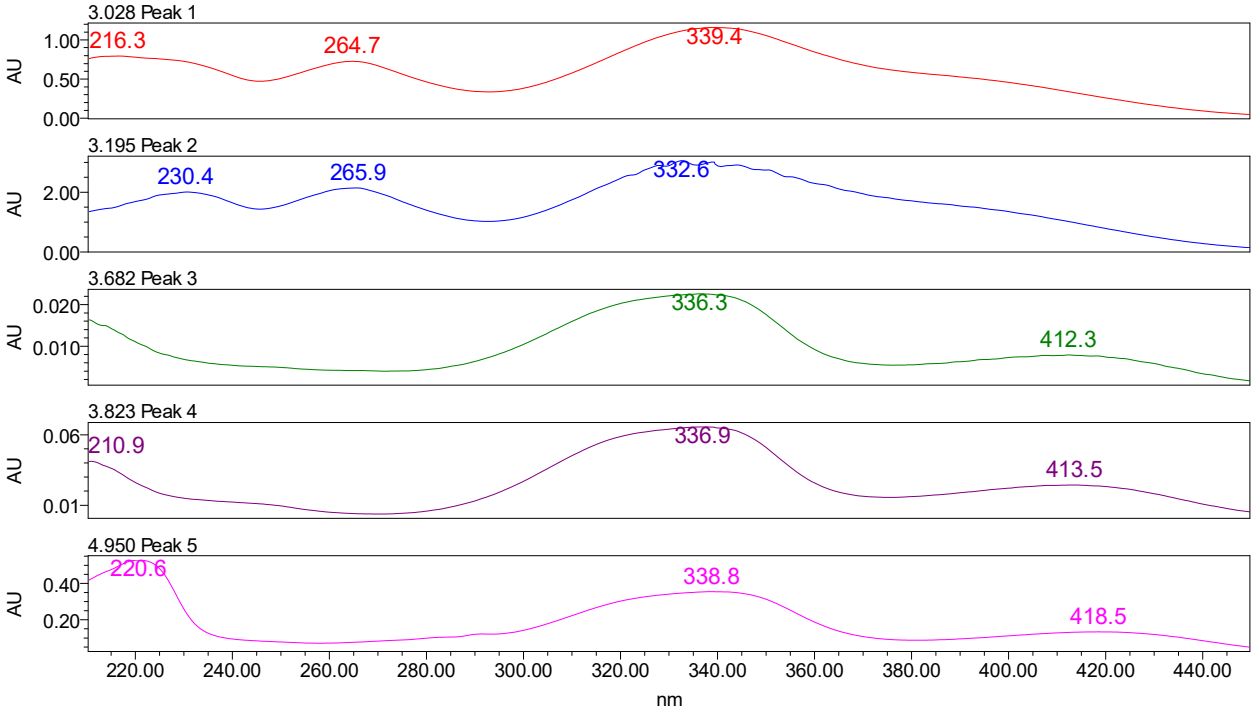


Supplemental Figure S28. ObiH-catalyzed reaction with isovaleraldehyde analyzed via Marfey's analysis. UPLC traces analyzed at 340 nm.

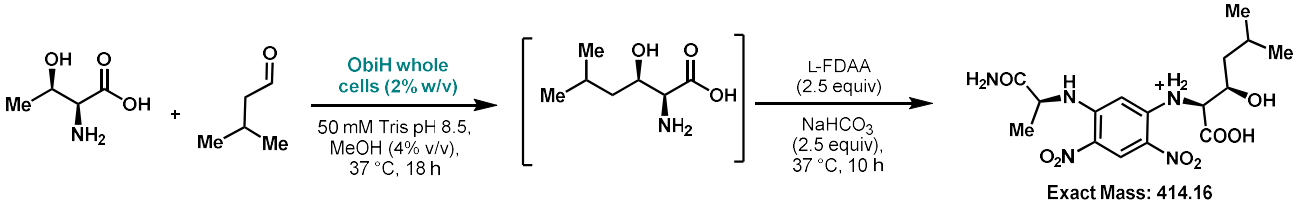


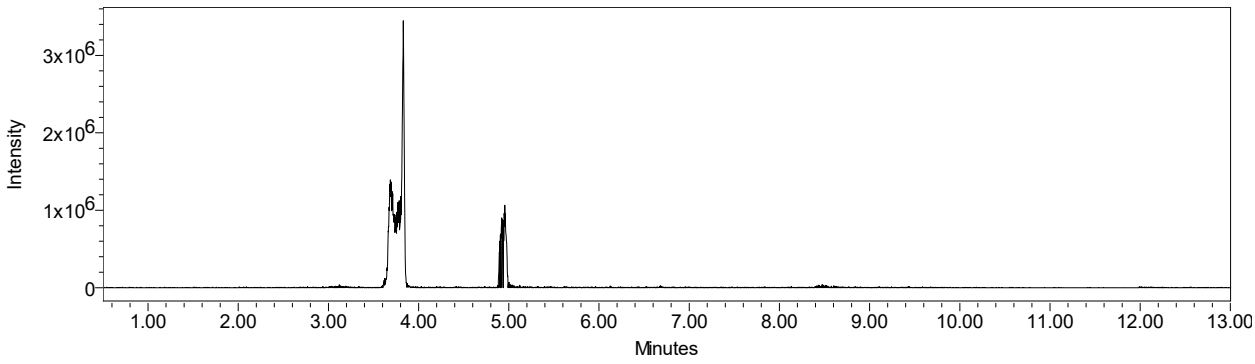


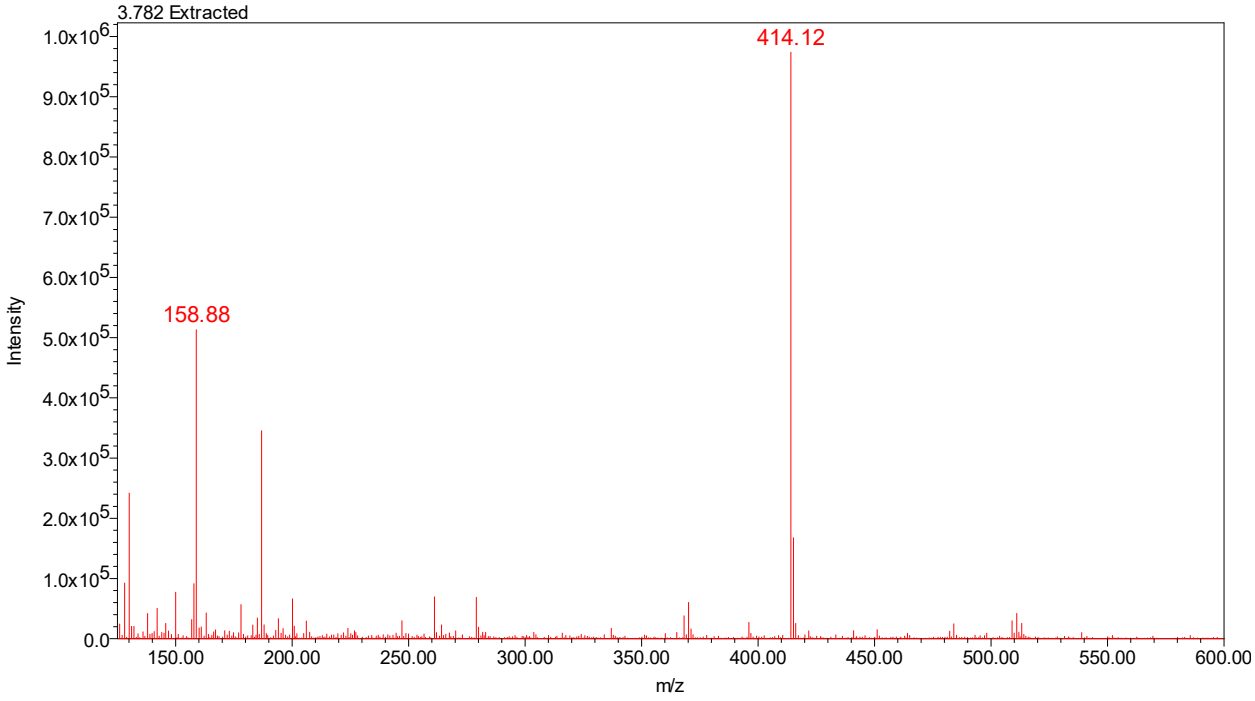
	Retention Time	Area	% Area	Height
1	3.028	6077234	37.29	1157821
2	3.195	8994834	55.20	2888605
3	3.682	110666	0.68	22327
4	3.823	147876	0.91	64950
5	4.950	965675	5.93	354270



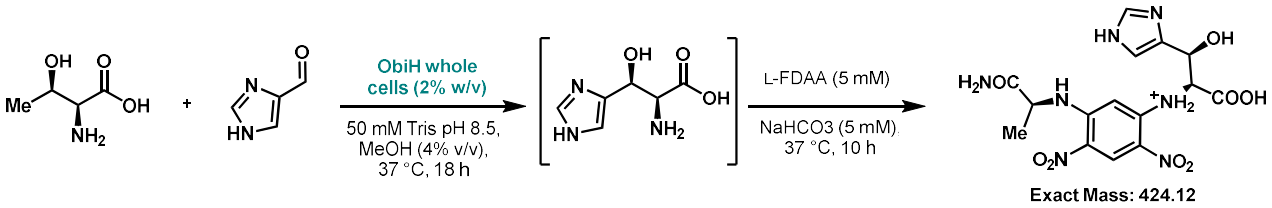
Supplemental Figure S29. ObiH-catalyzed reaction with isovaleraldehyde analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 414.8



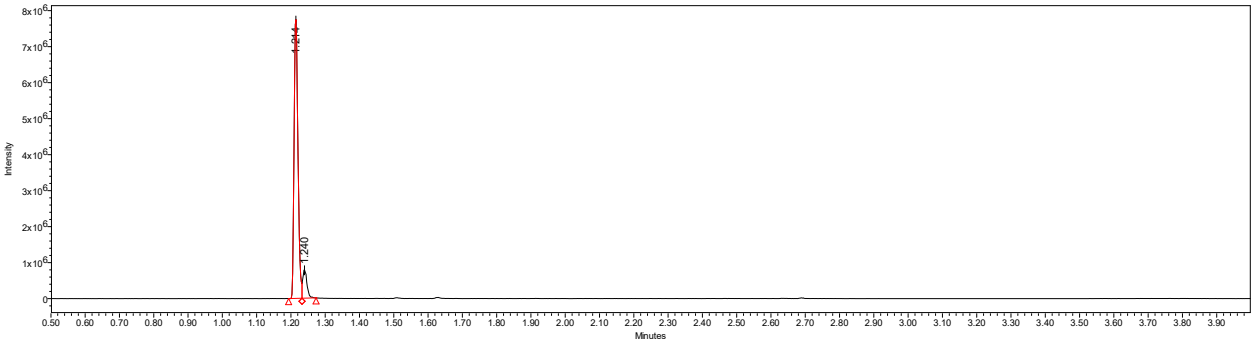




Supplemental Figure S30. ObiH-catalyzed reaction with imidazole-4-carboxaldehyde to generate β-hydroxy-histidine (**xx**) analyzed via Marfey's analysis. LC/MS traces of ESI M/Z 424.1

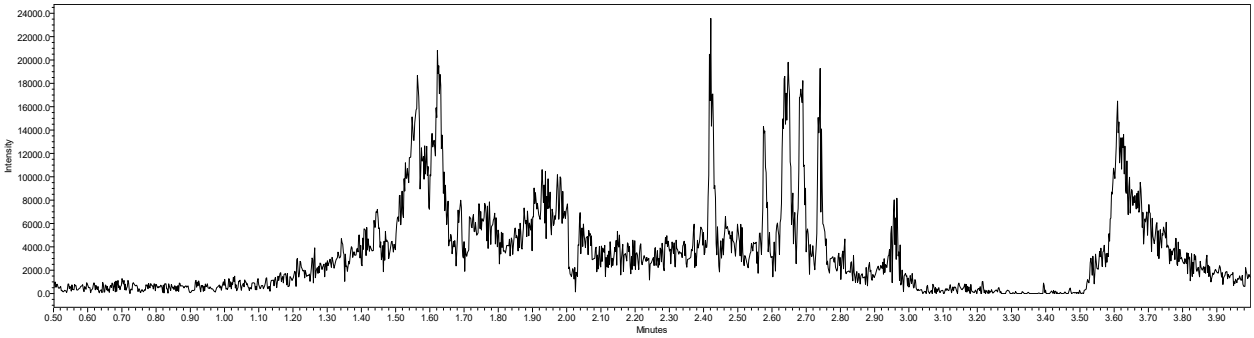


Reaction with ObiH whole cells

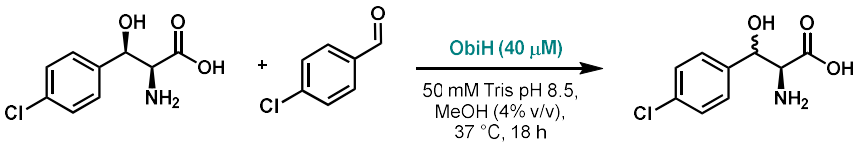


	Retention Time	Area	% Area	Height
1	1.214	6105356	90.20	7840083
2	1.240	663639	9.80	775838

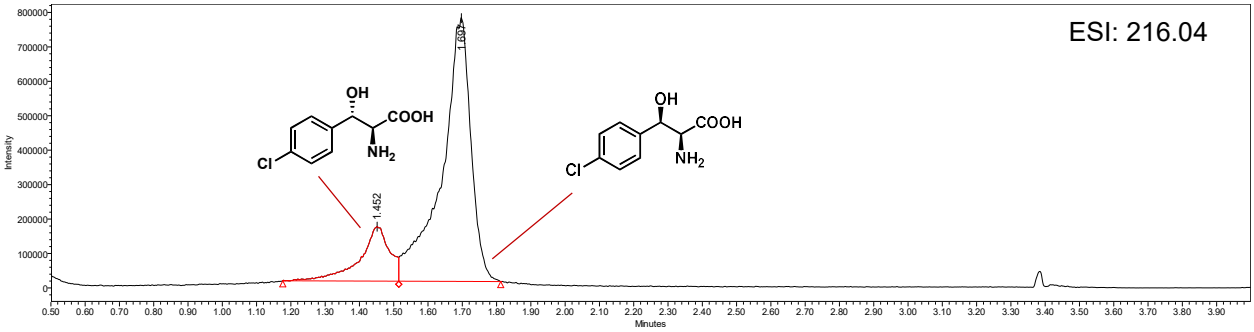
No enzyme control



Supplemental Figure S31. ObiH-catalyzed racemization of 4-chloro-phenylserine (**xx**). LC/MS traces of ESI M/Z 216.04

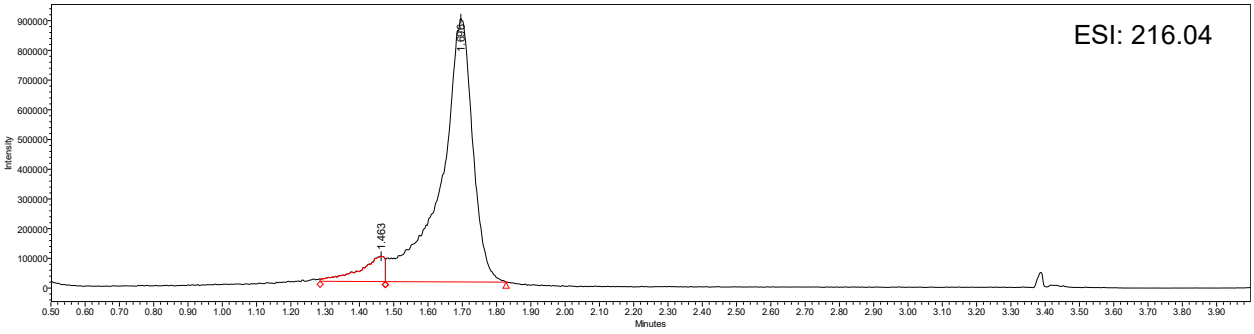


Reaction with ObiH after 3 h



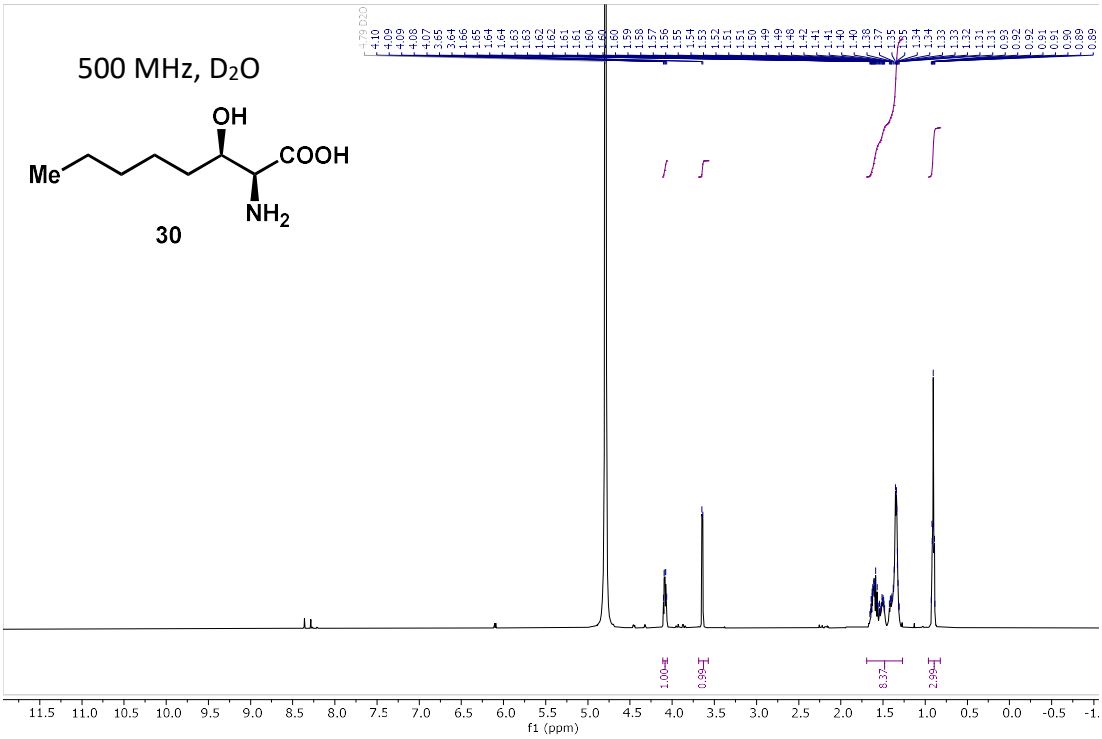
	Retention Time	Area	% Area	Height
1	1.452	959054	17.53	158069
2	1.697	4511958	82.47	765549

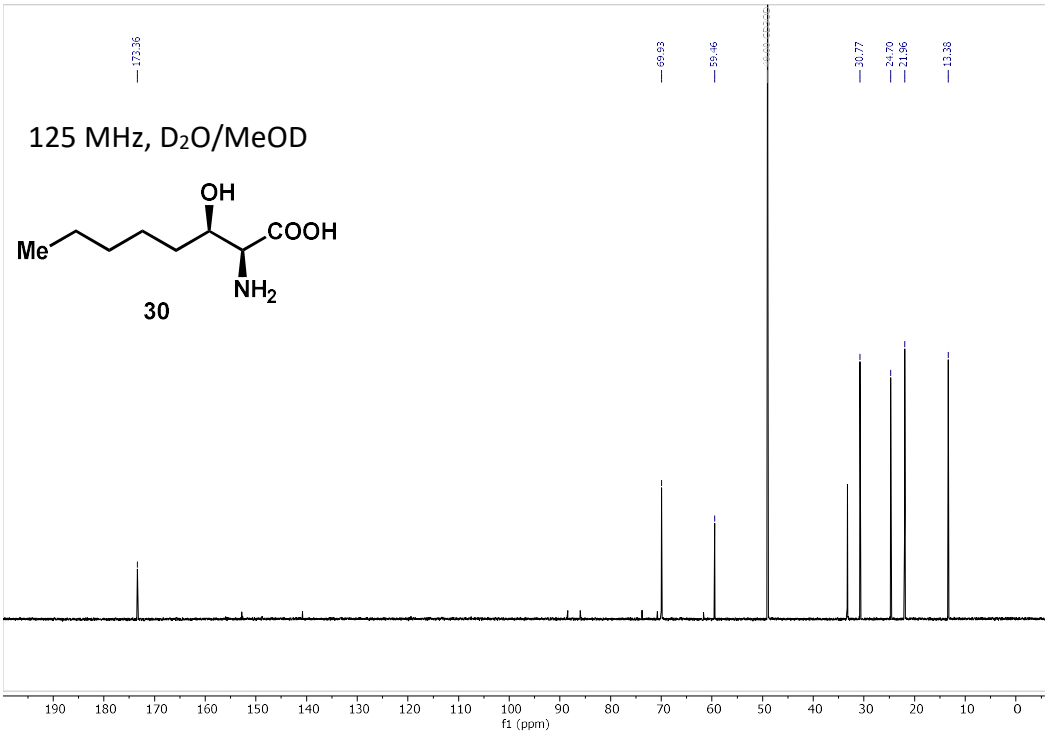
No enzyme control



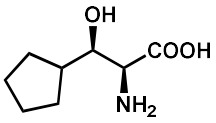
	Retention Time	Area	% Area	Height
1	1.463	429785	7.04	85368
2	1.696	5674406	92.96	887965

Part VII. NMR spectra of isolated products



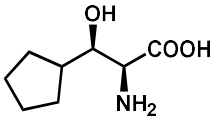


500 MHz, D_2O

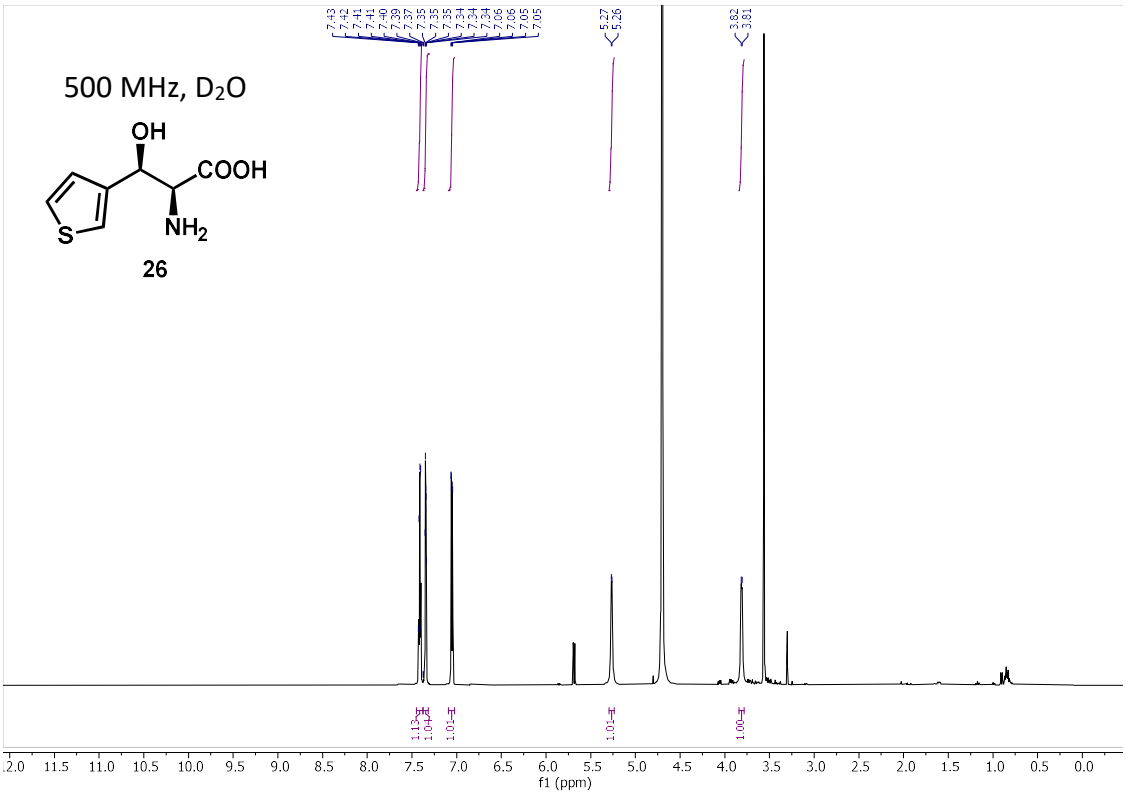


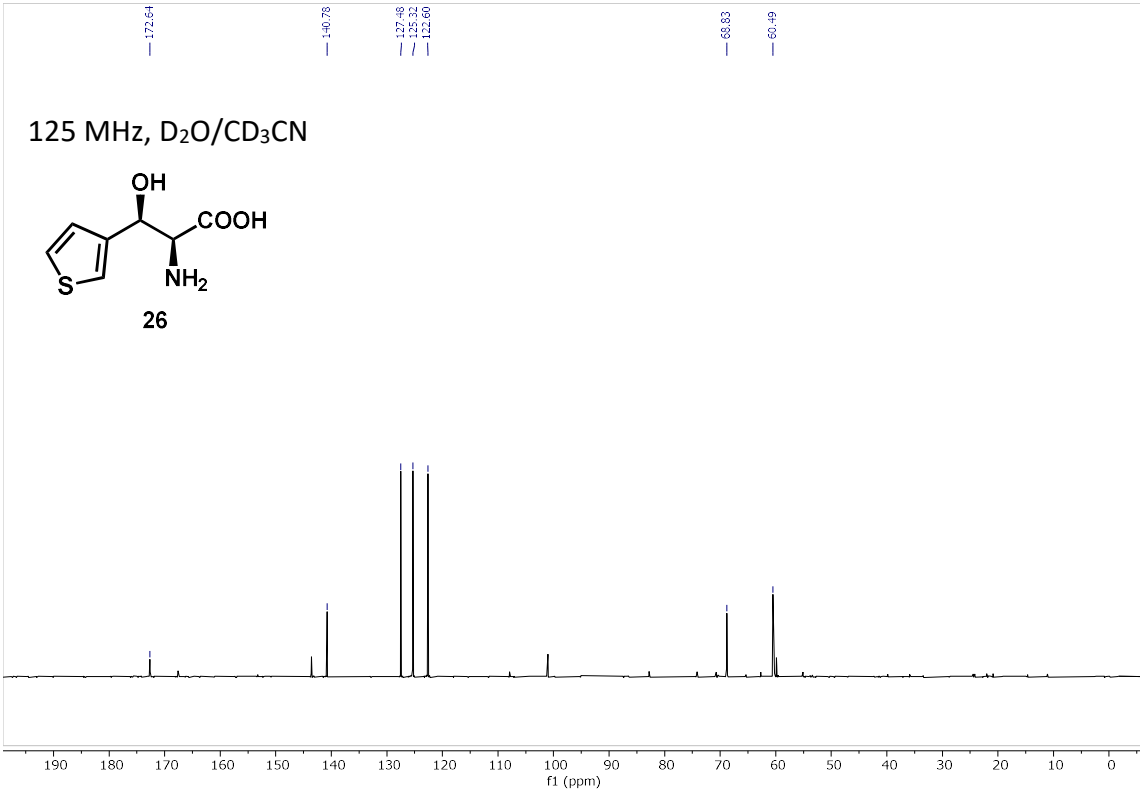
29

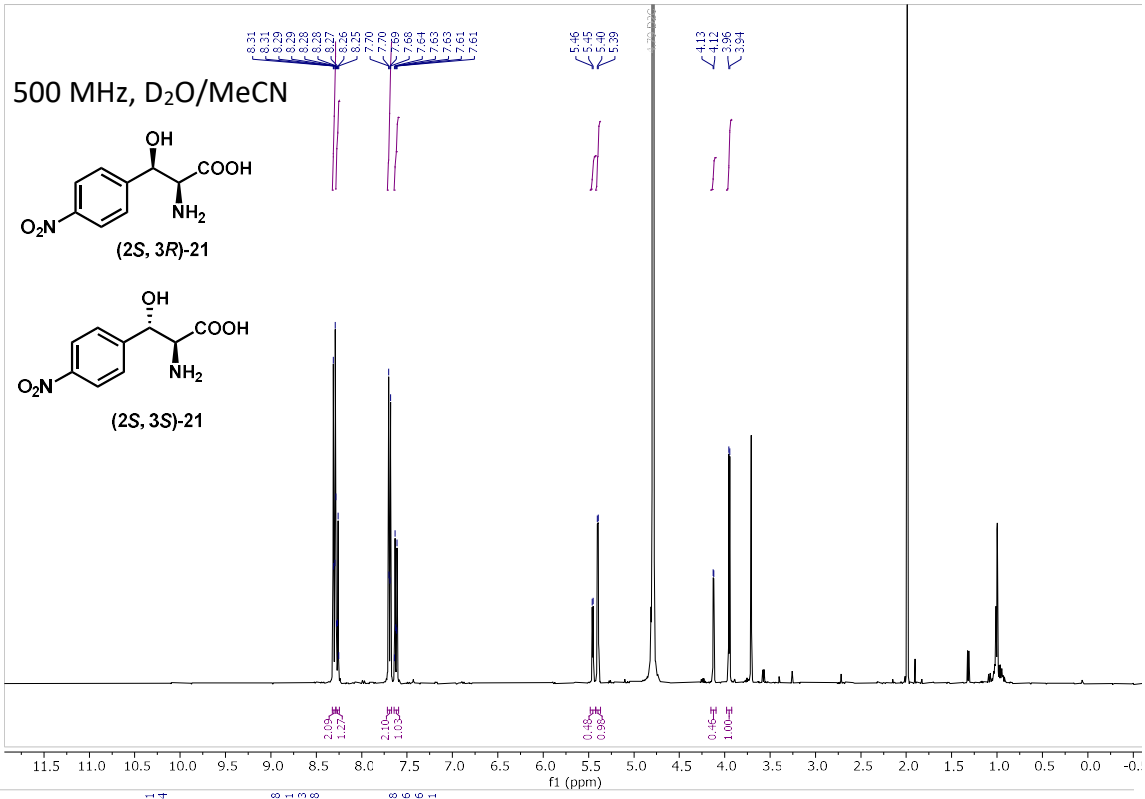
125 MHz, D_2O/MeOD

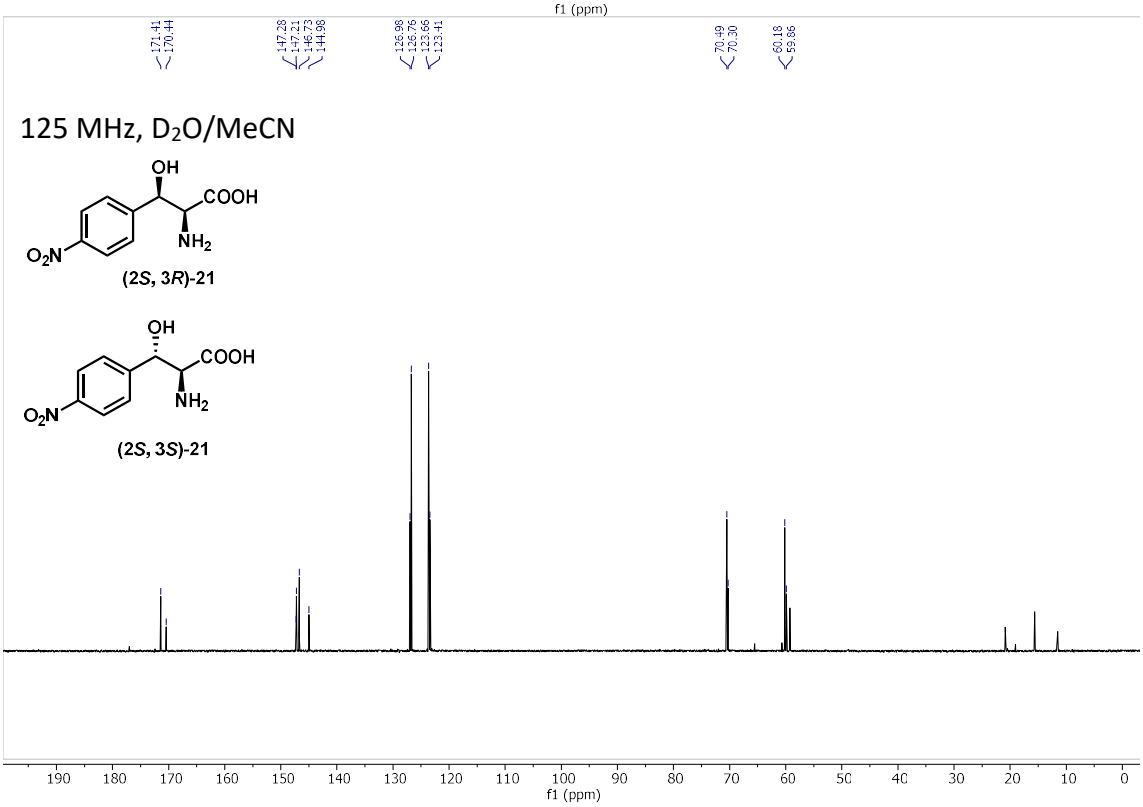


29

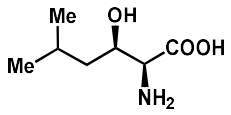

500 MHz, D2O
OH
COOH
NH2
S
26
7.43
7.42
7.41
7.41
7.40
7.39
7.37
7.35
7.35
7.35
7.34
7.34
7.34
7.06
7.06
7.06
7.05
7.05
5.27
5.26
3.82
3.81
1.13
1.04
1.01
1.01
1.00
f1 (ppm)


172.64
140.76
127.48
125.32
122.60
68.83
60.49
125 MHz, D2O/CD3CN
OH
COOH
NH2
S
26
f1 (ppm)


500 MHz, D2O/MeCN
OH
COOH
NH2
O2N
(2S, 3R)-21
OH
COOH
NH2
O2N
(2S, 3S)-21
f1 (ppm)

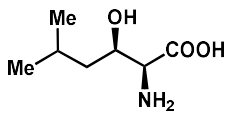

125 MHz, D2O/MeCN
OH
COOH
NH2
O2N
(2S, 3R)-21
OH
COOH
NH2
O2N
(2S, 3S)-21
f1 (ppm)

500 MHz, D_2O/MeCN

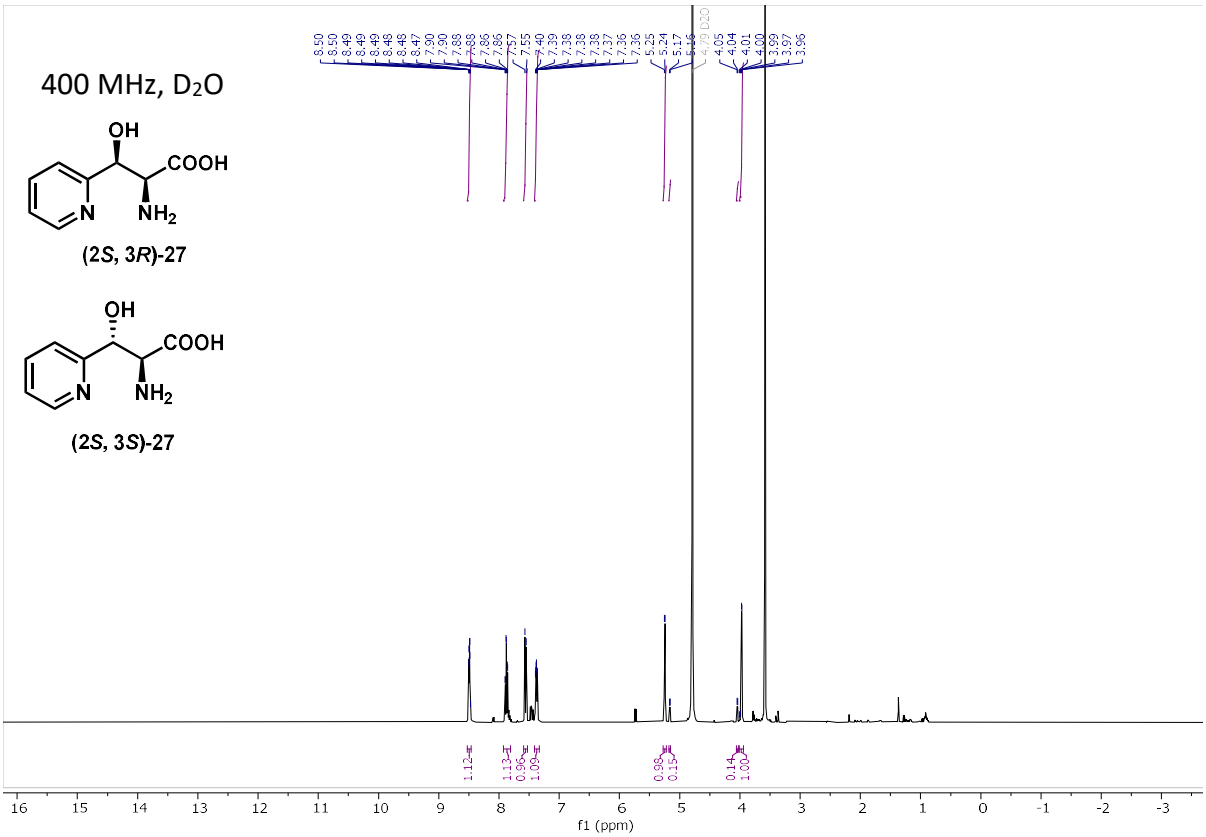


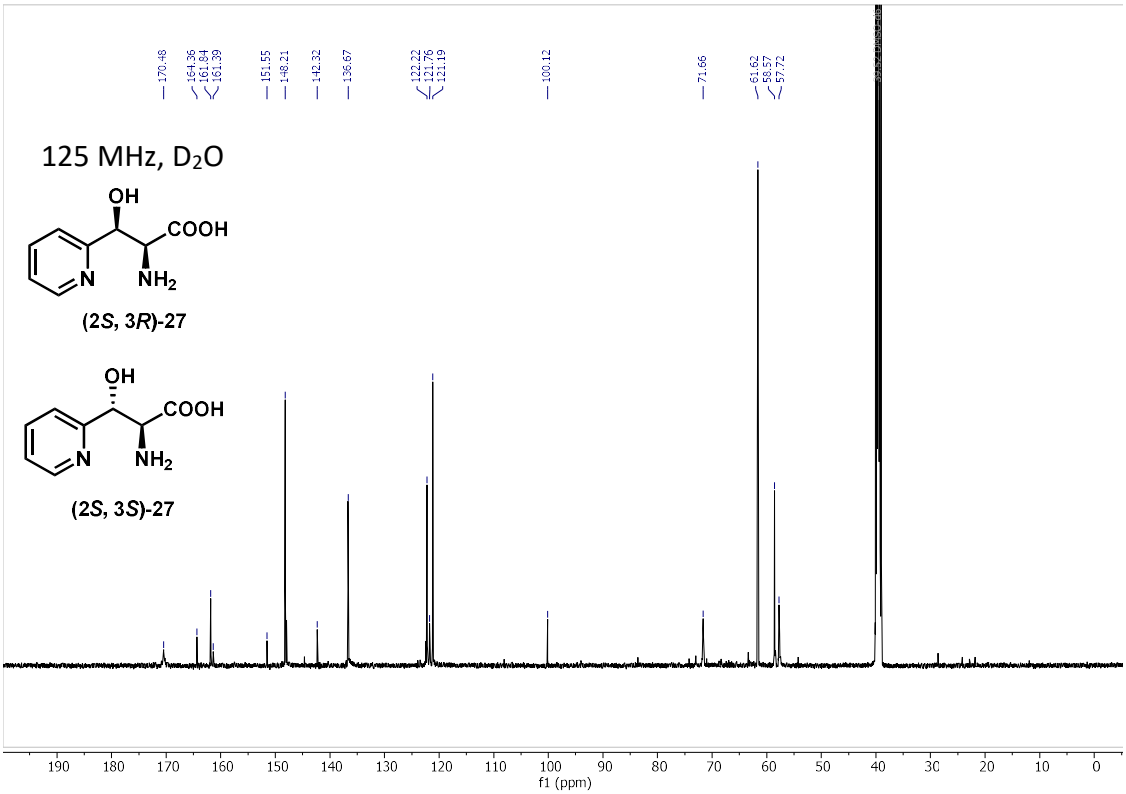
32

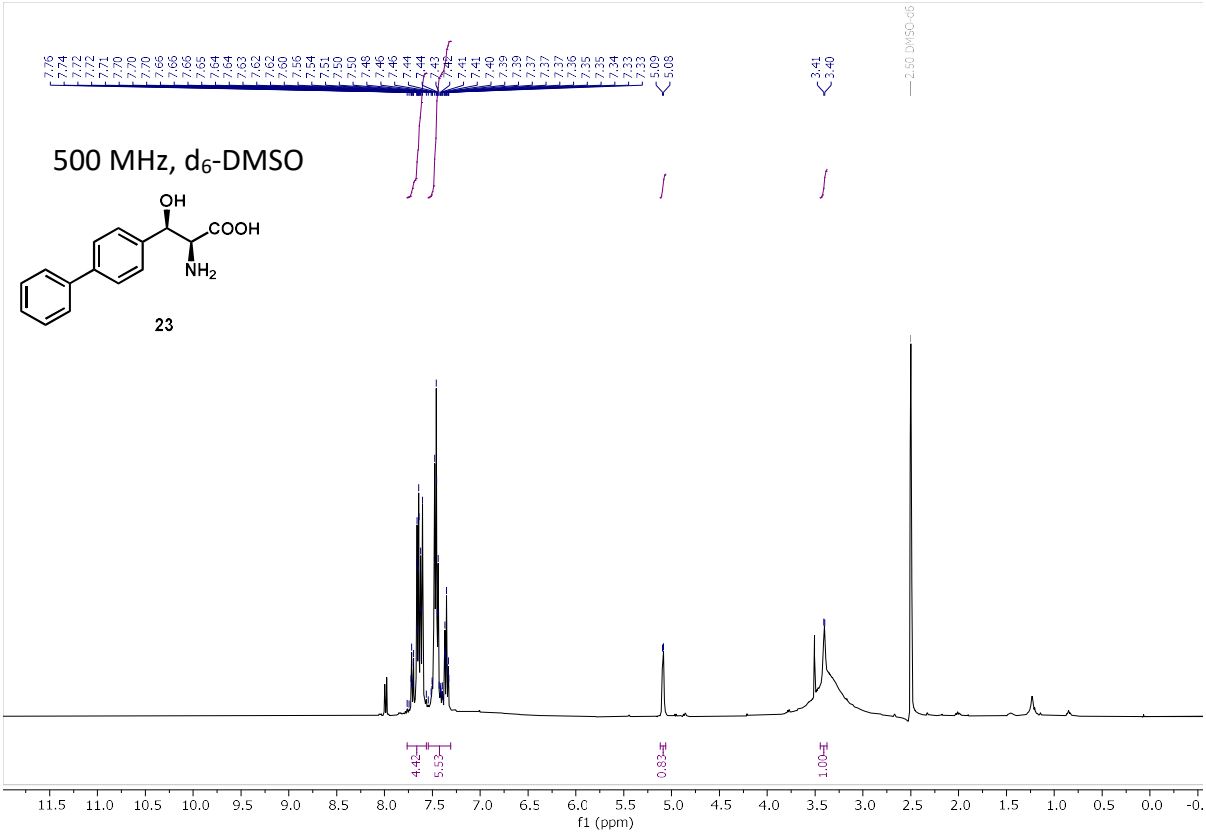
125 MHz, MeOD

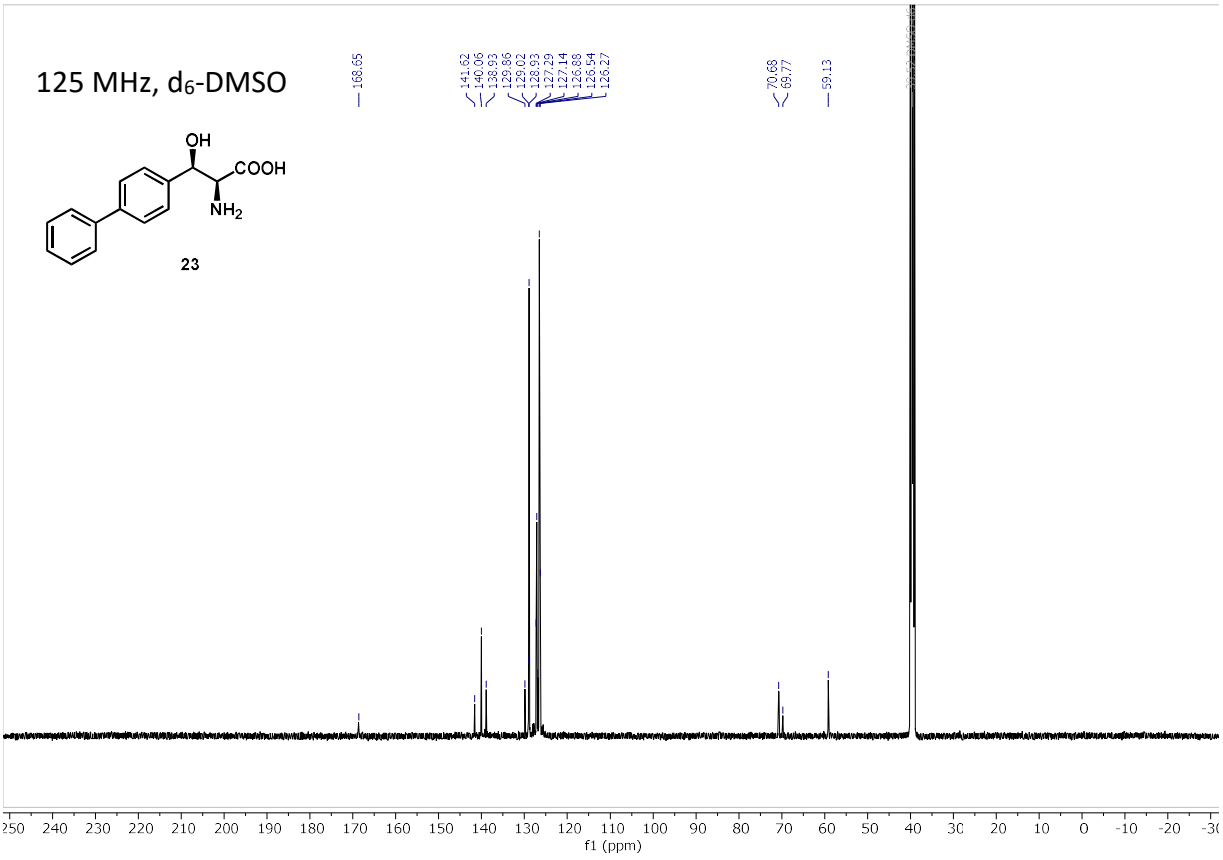


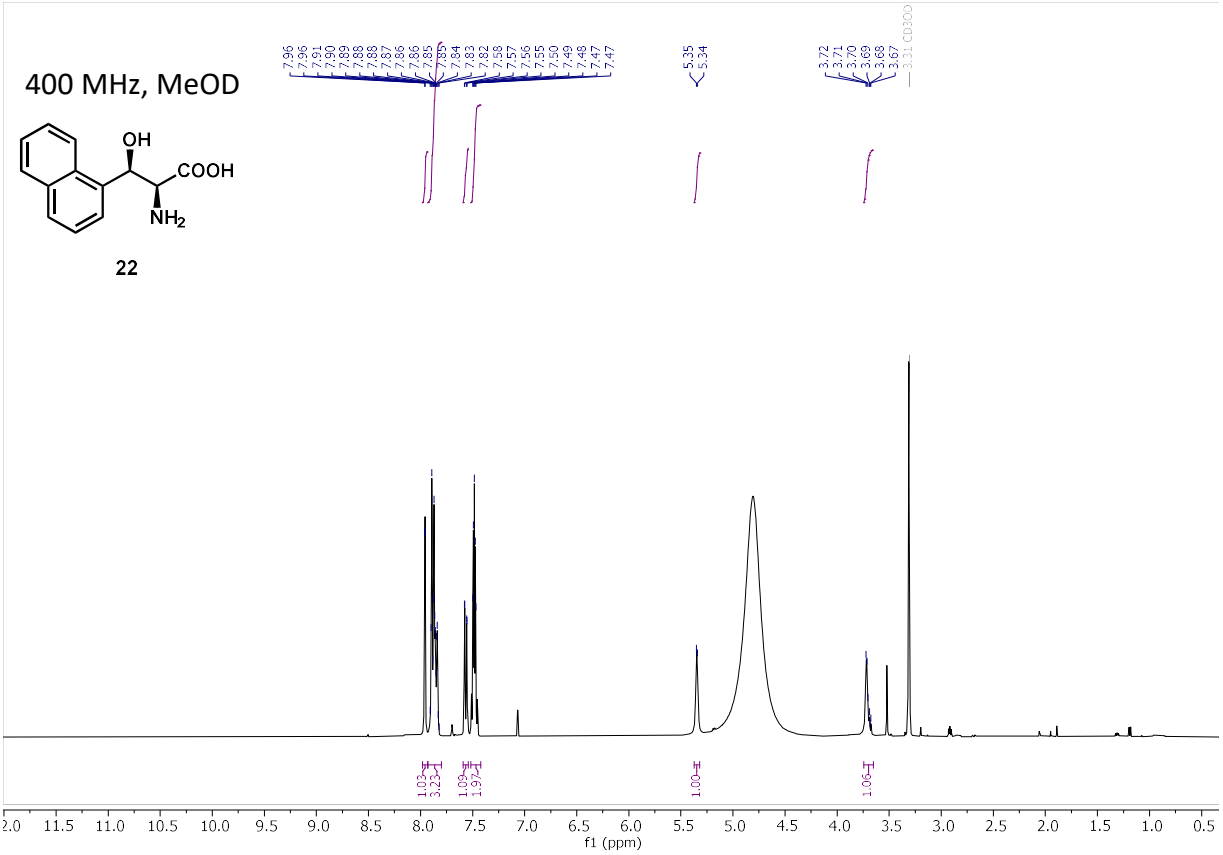
32

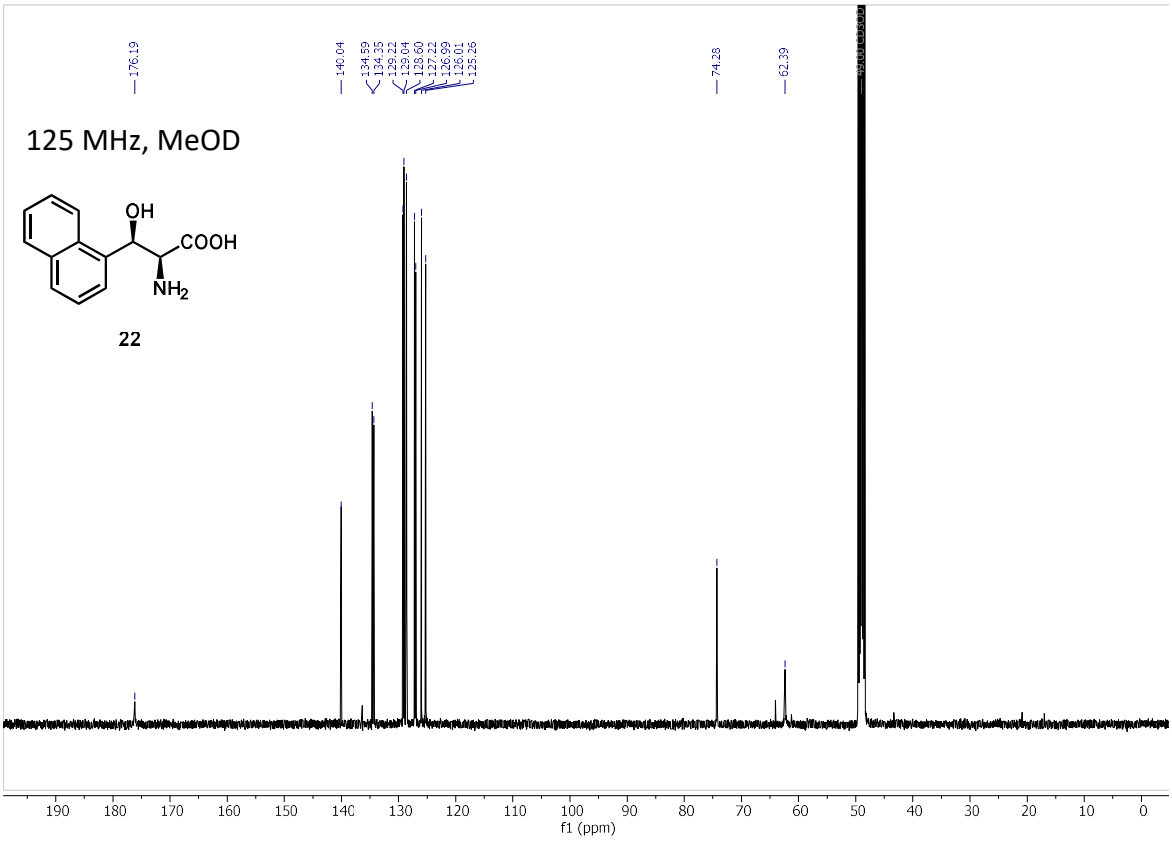

400 MHz, D2O
OH
COOH
N
NH2
(2S, 3R)-27
OH
COOH
N
NH2
(2S, 3S)-27
f1 (ppm)

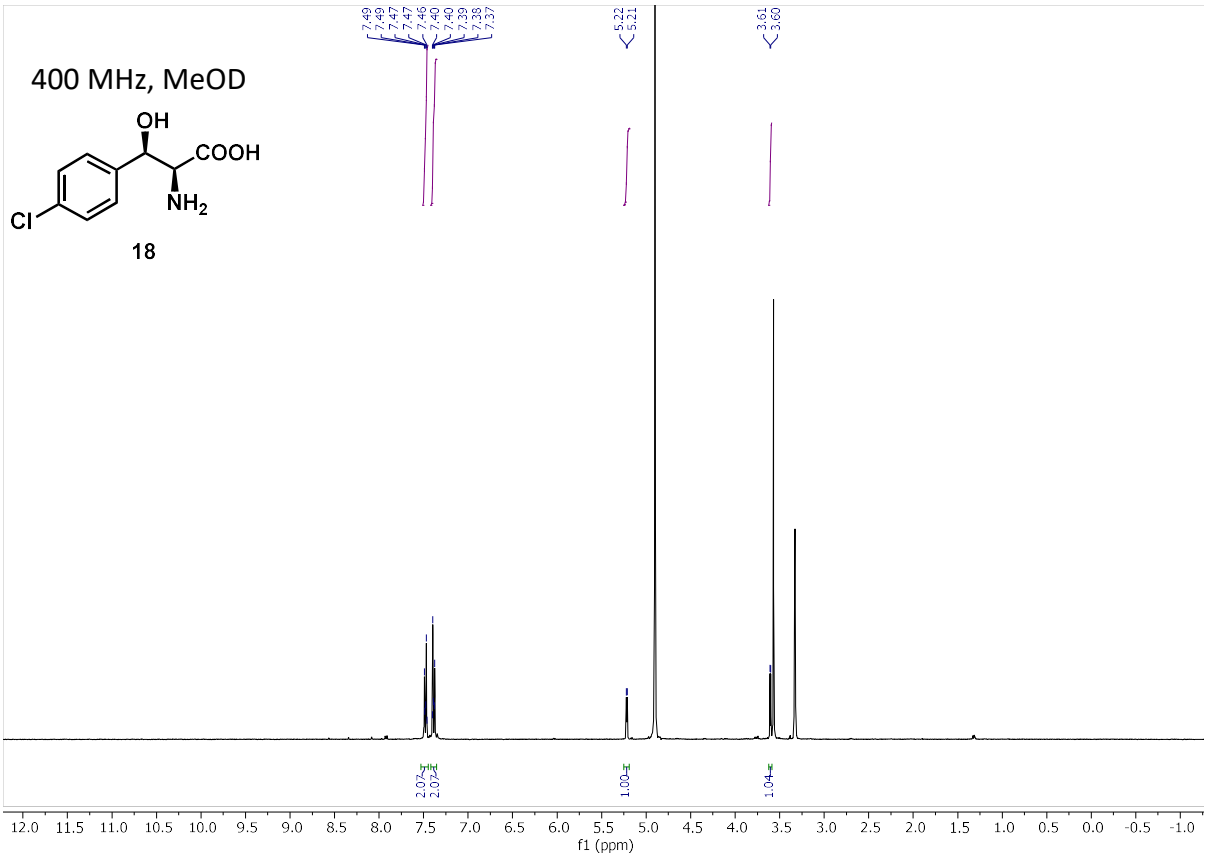

125 MHz, D2O
OH
COOH
N
NH2
(2S, 3R)-27
OH
COOH
N
NH2
(2S, 3S)-27
f1 (ppm)

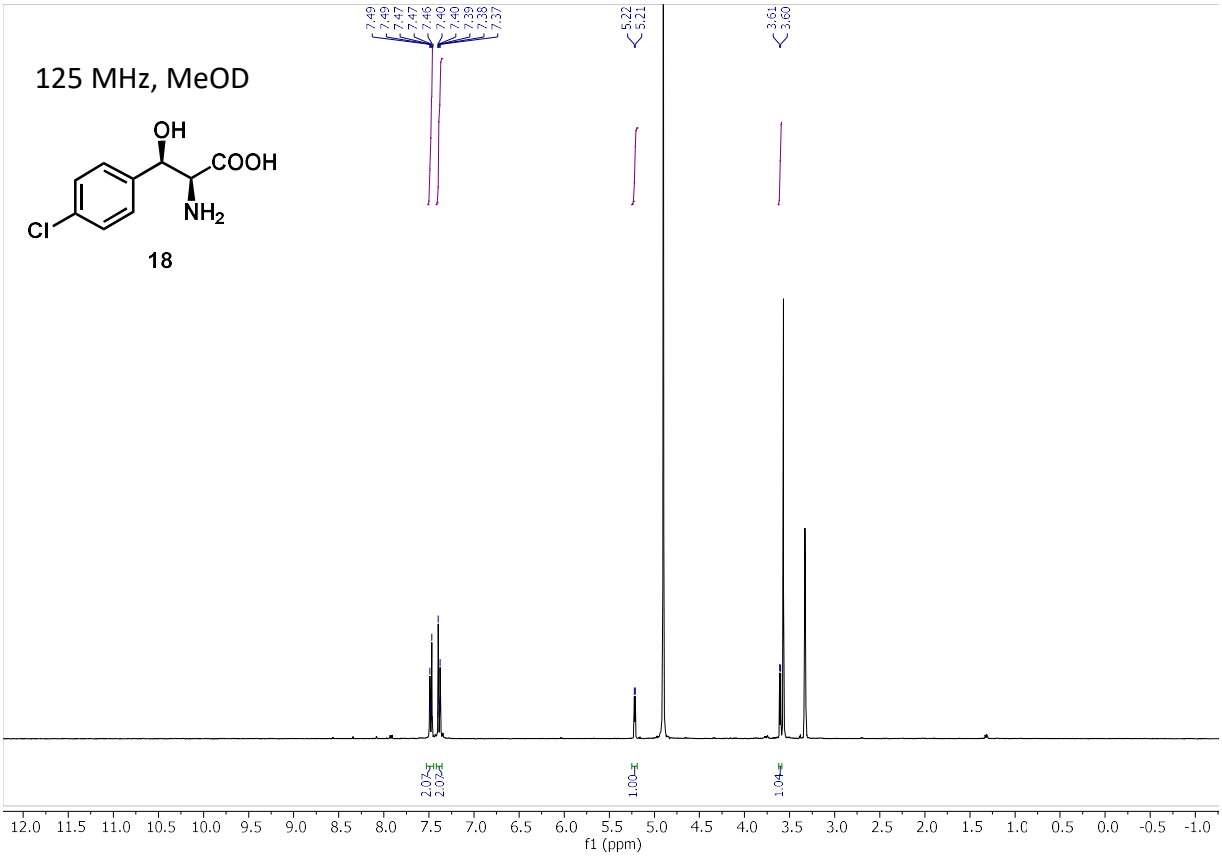

500 MHz, d6-DMSO
OH
COOH
NH2
23
f1 (ppm)

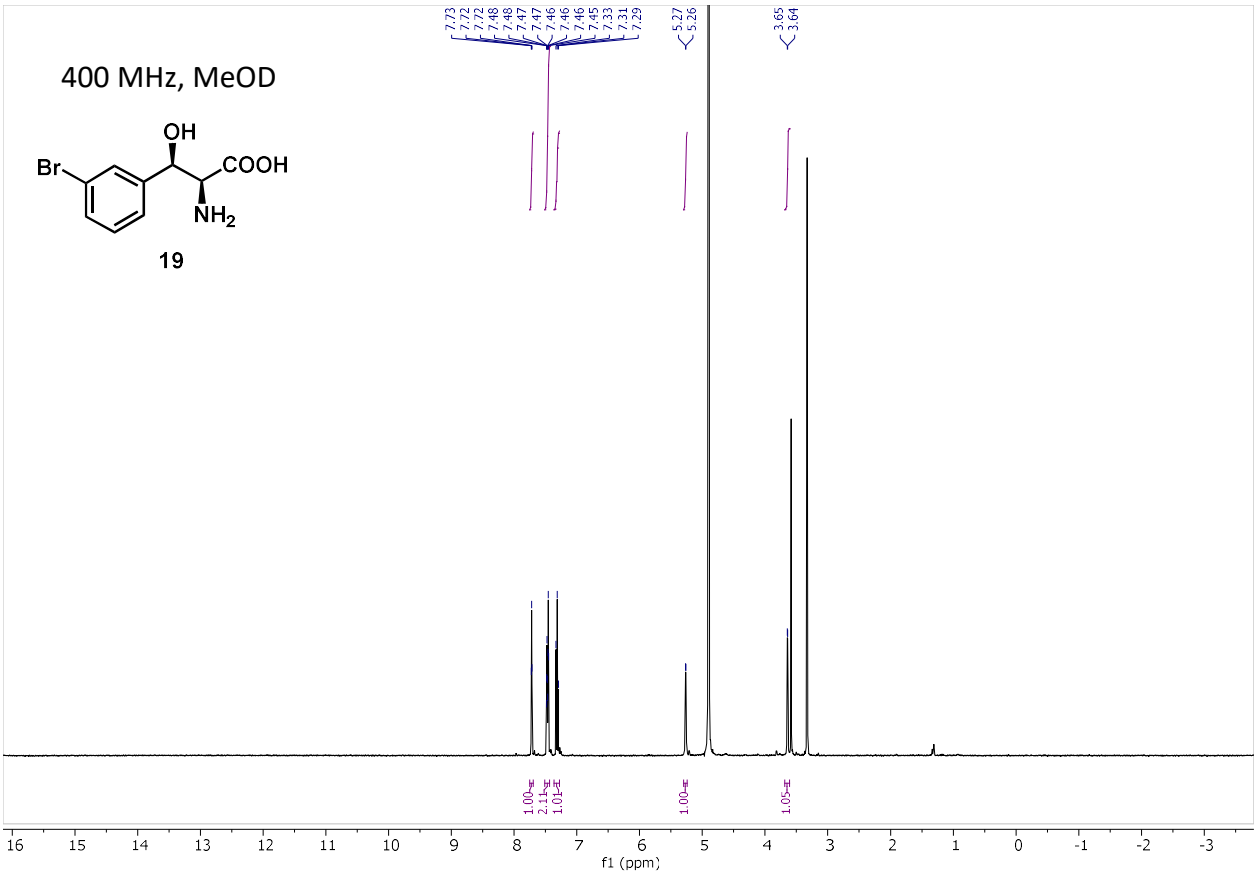

125 MHz, d6-DMSO
OH
COOH
NH2
23
f1 (ppm)

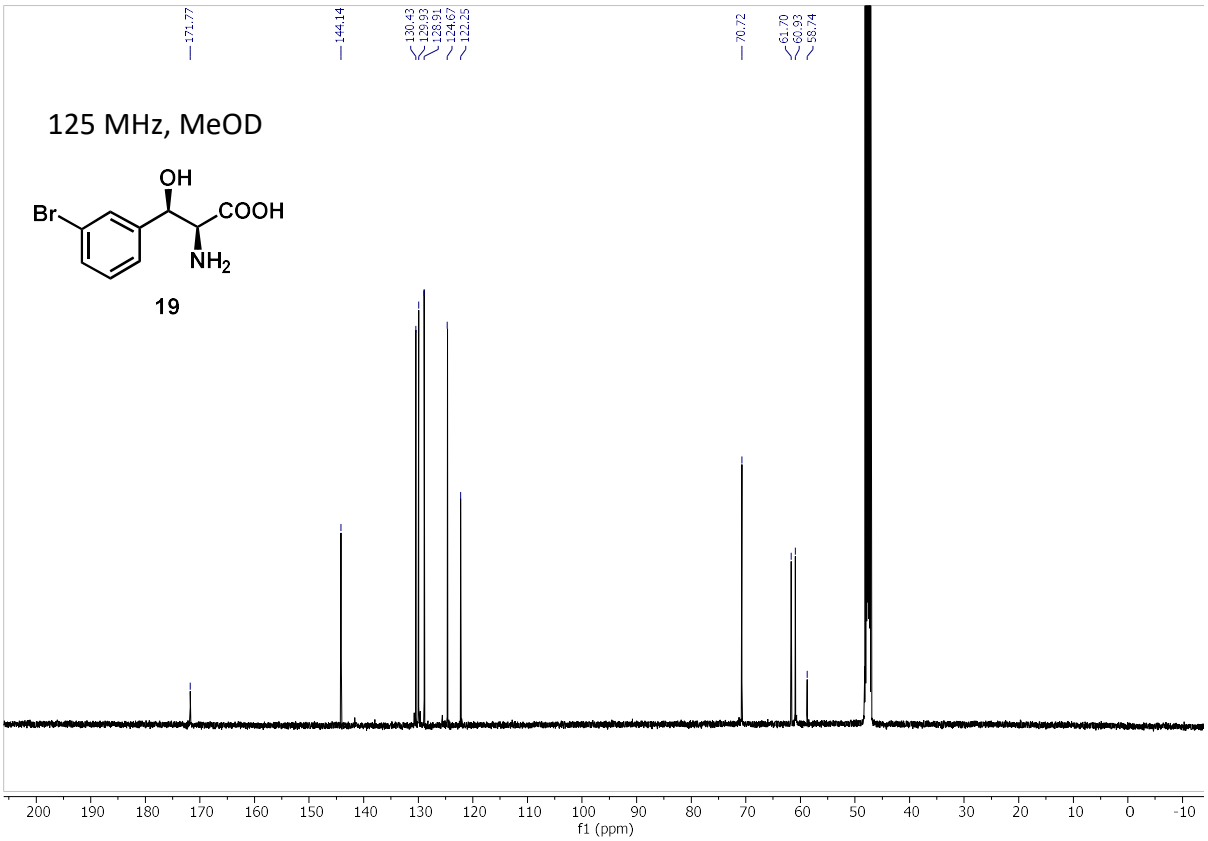

400 MHz, MeOD
OH
COOH
NH2
22
f1 (ppm)

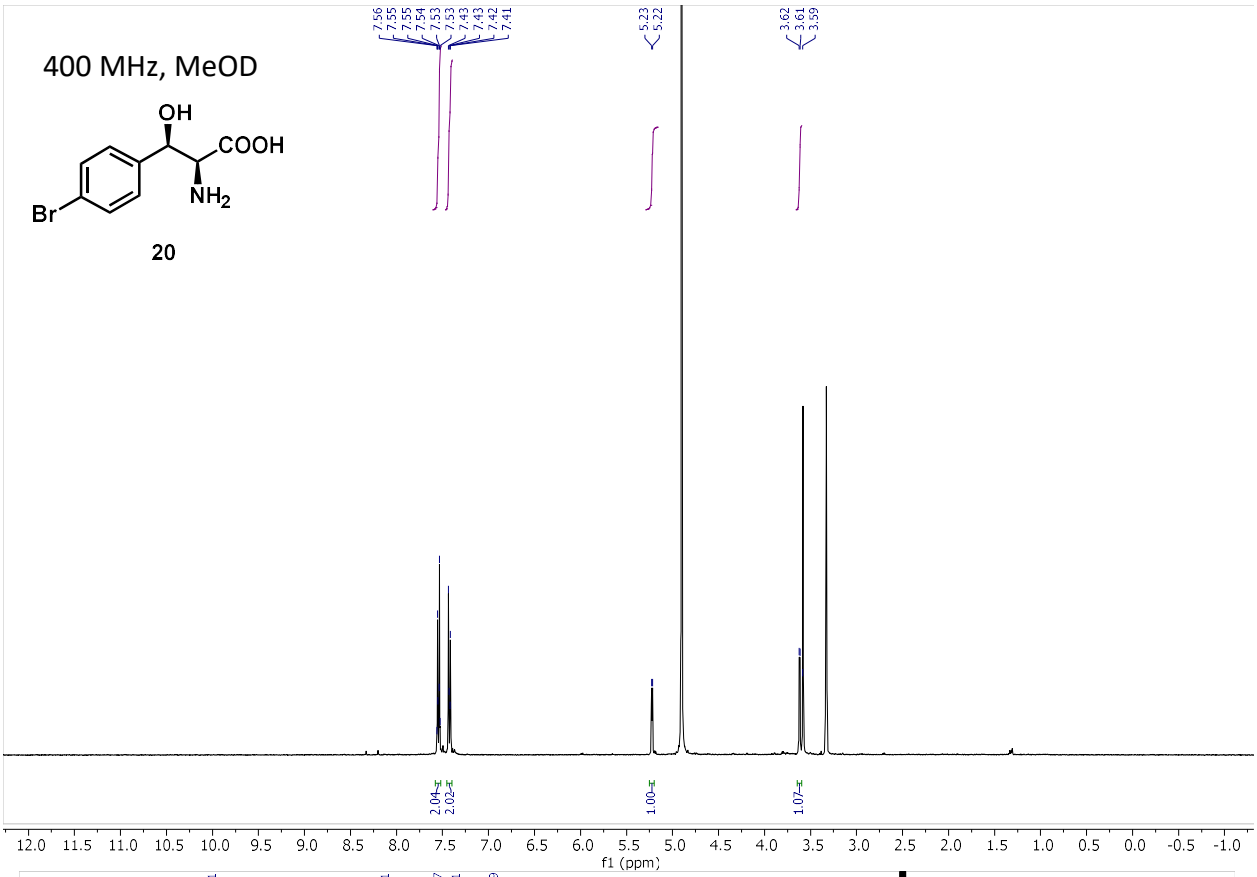

125 MHz, MeOD
OH
COOH
NH2
22
f1 (ppm)

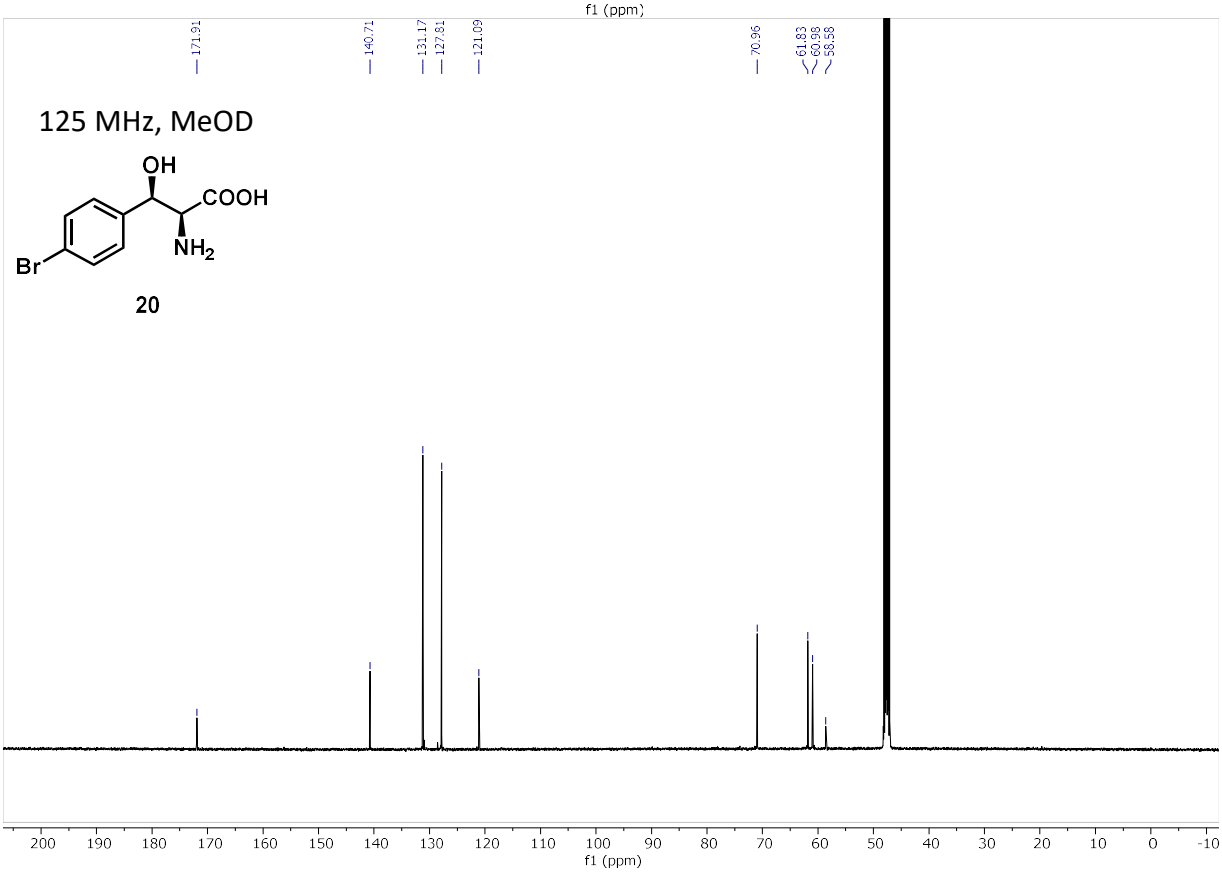

400 MHz, MeOD
OH
COOH
NH2
Cl
18
7.49
7.49
7.47
7.47
7.46
7.40
7.40
7.39
7.38
7.37
5.22
5.21
3.61
3.60
2.07
2.07
1.00
1.04
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

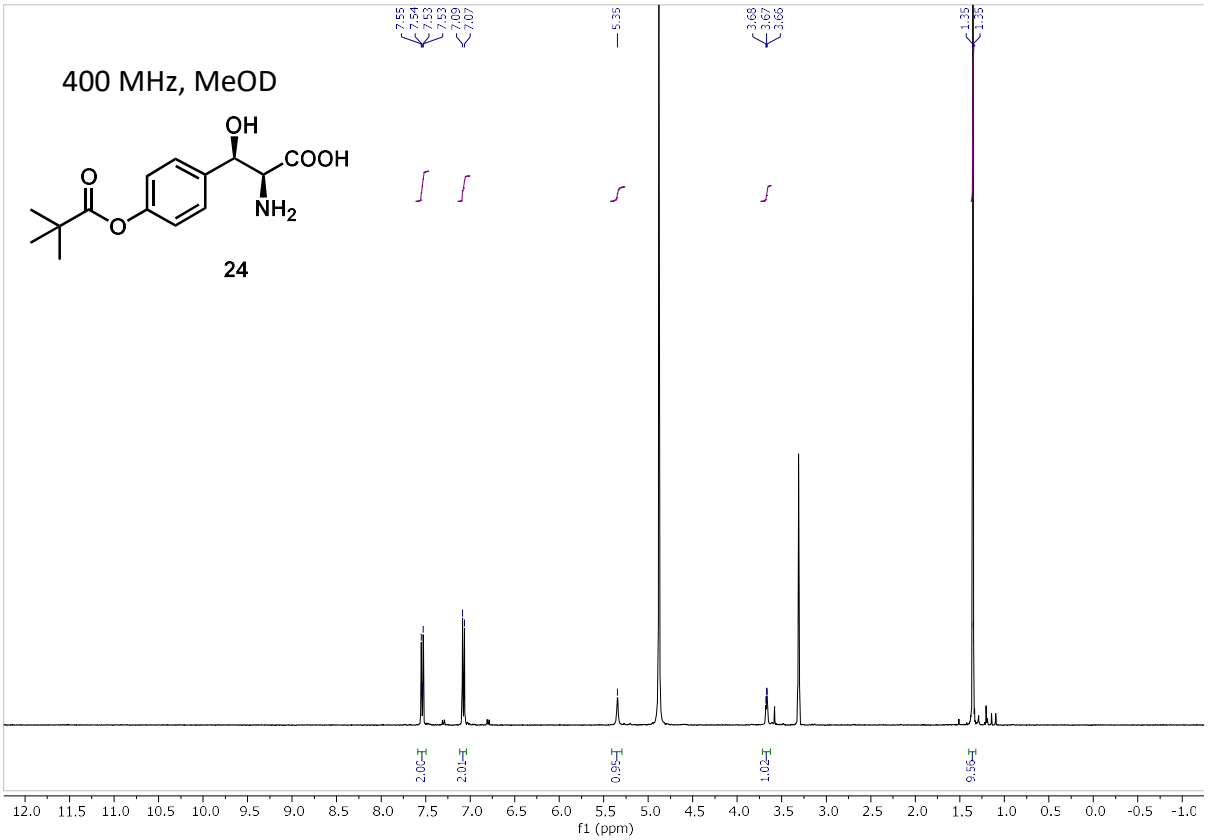

125 MHz, MeOD
OH
COOH
NH2
Cl
18
7.49
7.49
7.47
7.47
7.46
7.40
7.40
7.39
7.38
7.37
5.22
5.21
3.61
3.60
2.07
2.07
1.00
1.04
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

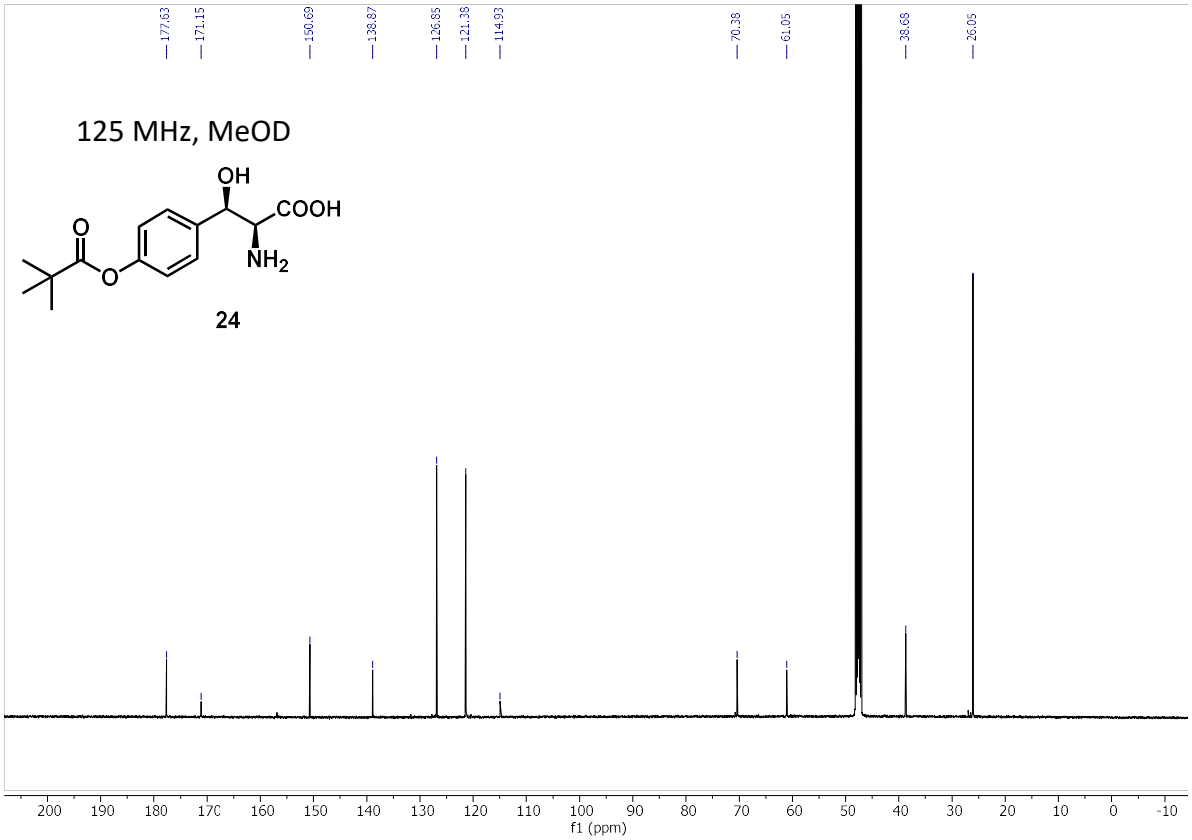

400 MHz, MeOD
OH
Br
COOH
NH2
19
7.73
7.72
7.72
7.48
7.48
7.47
7.47
7.46
7.46
7.46
7.45
7.33
7.31
7.29
5.27
5.26
3.65
3.64
1.00
2.11
1.01
1.00
1.05
f1 (ppm)

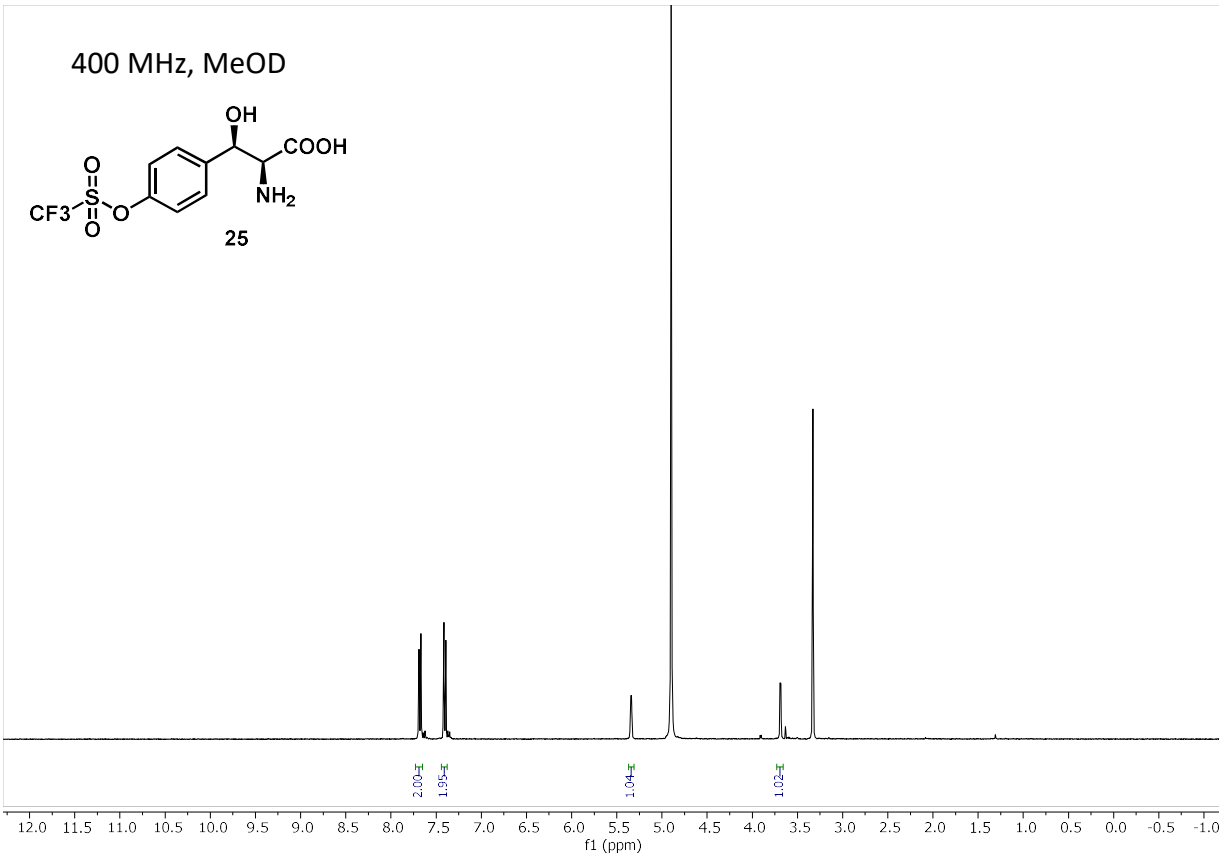

125 MHz, MeOD
OH
Br
COOH
NH2
19
171.77
144.14
130.43
129.93
128.91
124.67
122.25
70.72
61.70
60.93
58.74
f1 (ppm)

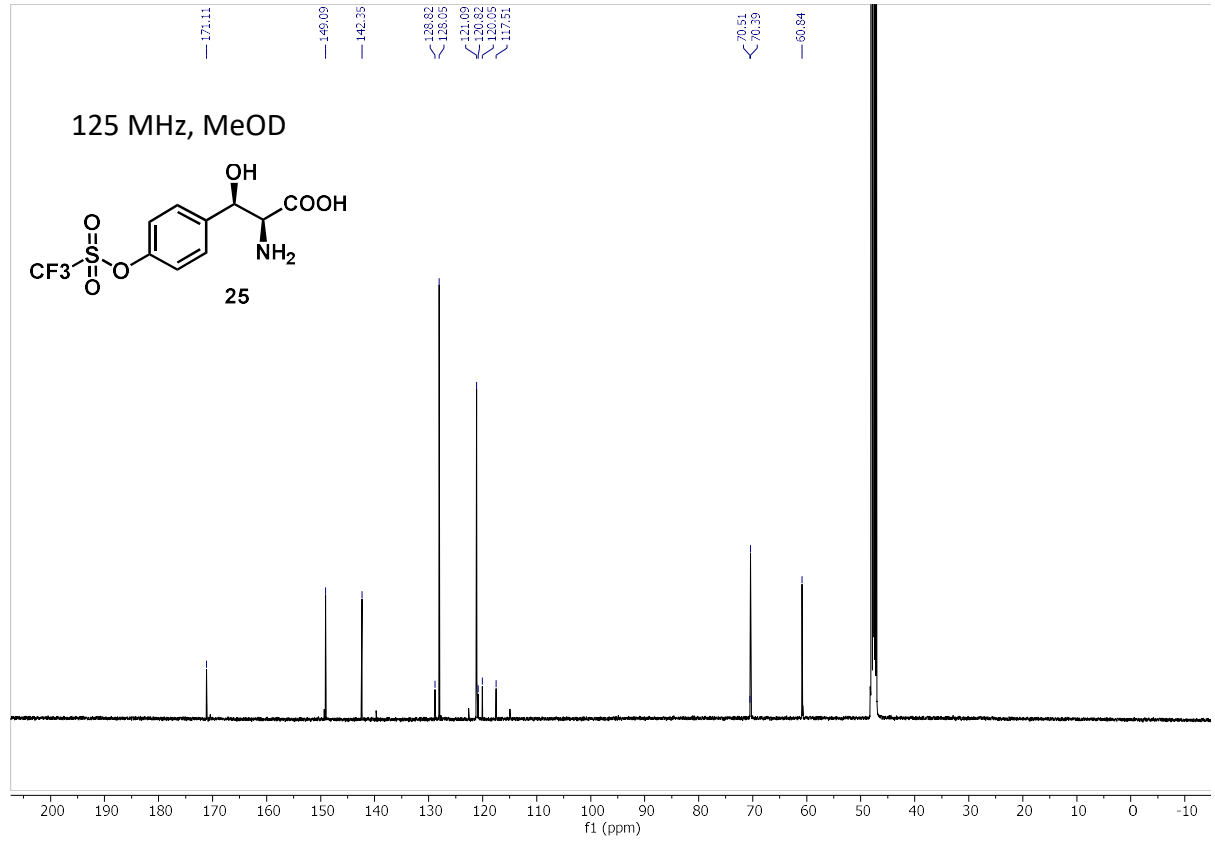

400 MHz, MeOD
OH
COOH
NH2
Br
20
7.56
7.55
7.55
7.54
7.53
7.53
7.43
7.43
7.42
7.41
5.23
5.22
3.62
3.61
3.59
2.04
2.02
1.00
1.07
f1 (ppm)

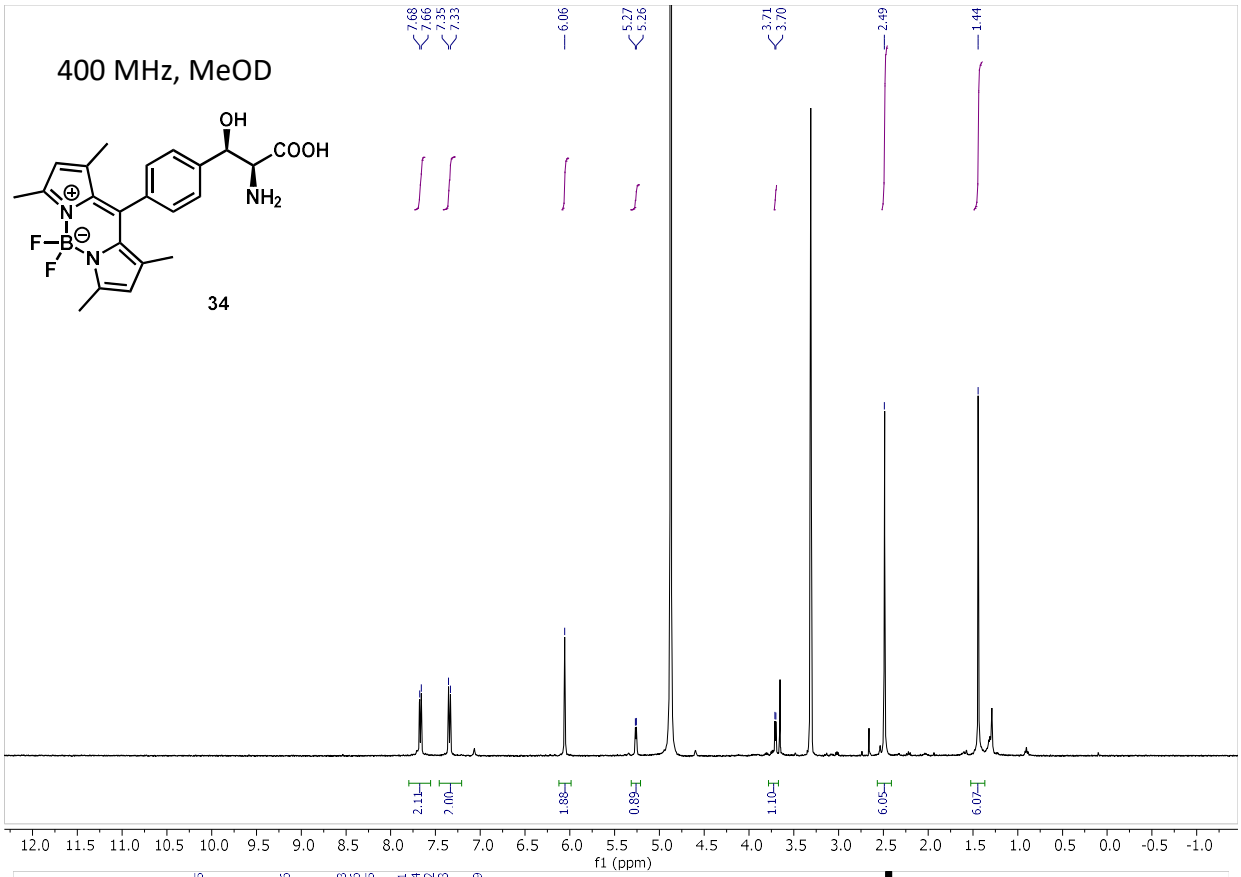

125 MHz, MeOD
OH
COOH
NH2
Br
20
171.91
140.71
131.17
127.81
121.09
70.96
61.83
60.98
58.58
f1 (ppm)

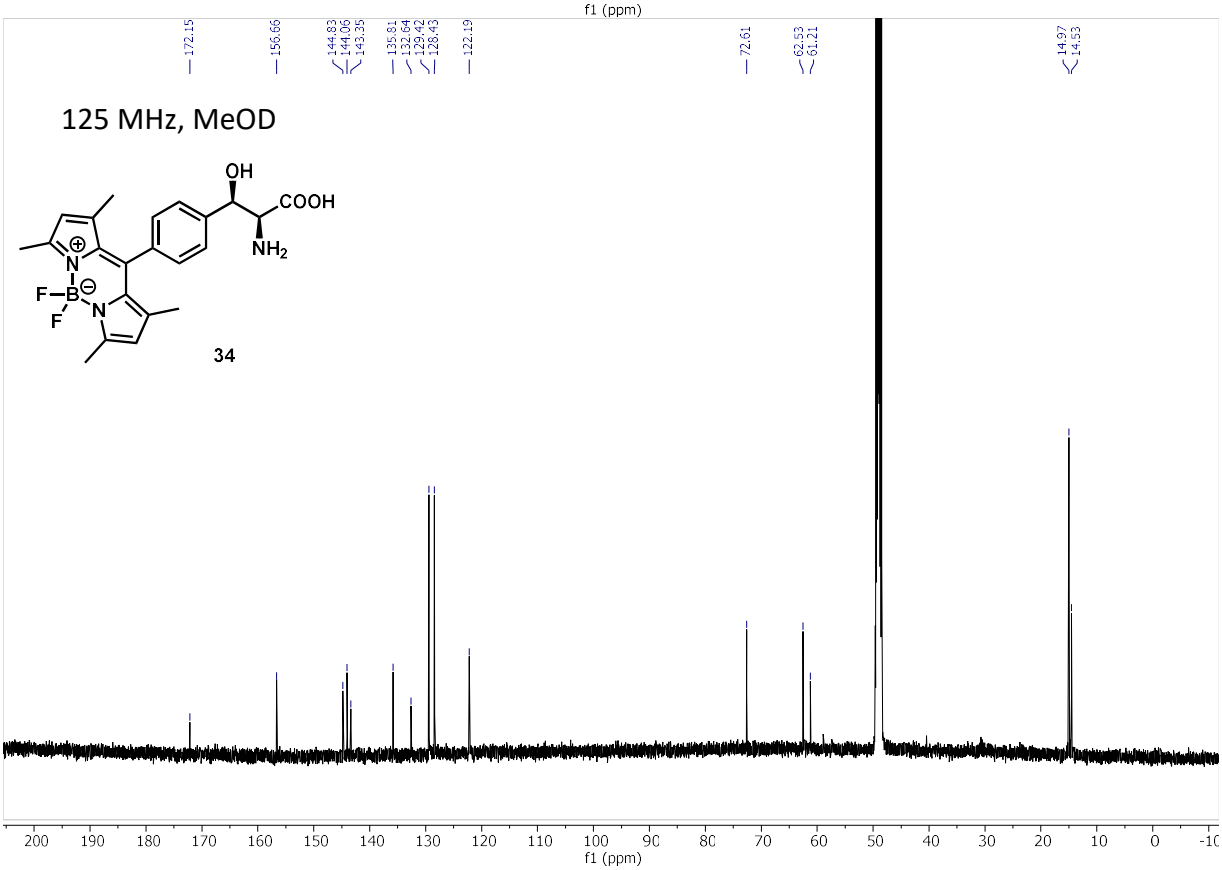

400 MHz, MeOD
OH
COOH
NH2
O
O
24
7.55
7.54
7.53
7.53
7.09
7.07
5.35
3.68
3.67
3.66
1.35
1.35
2.00
2.01
0.99
1.02
9.96
f1 (ppm)

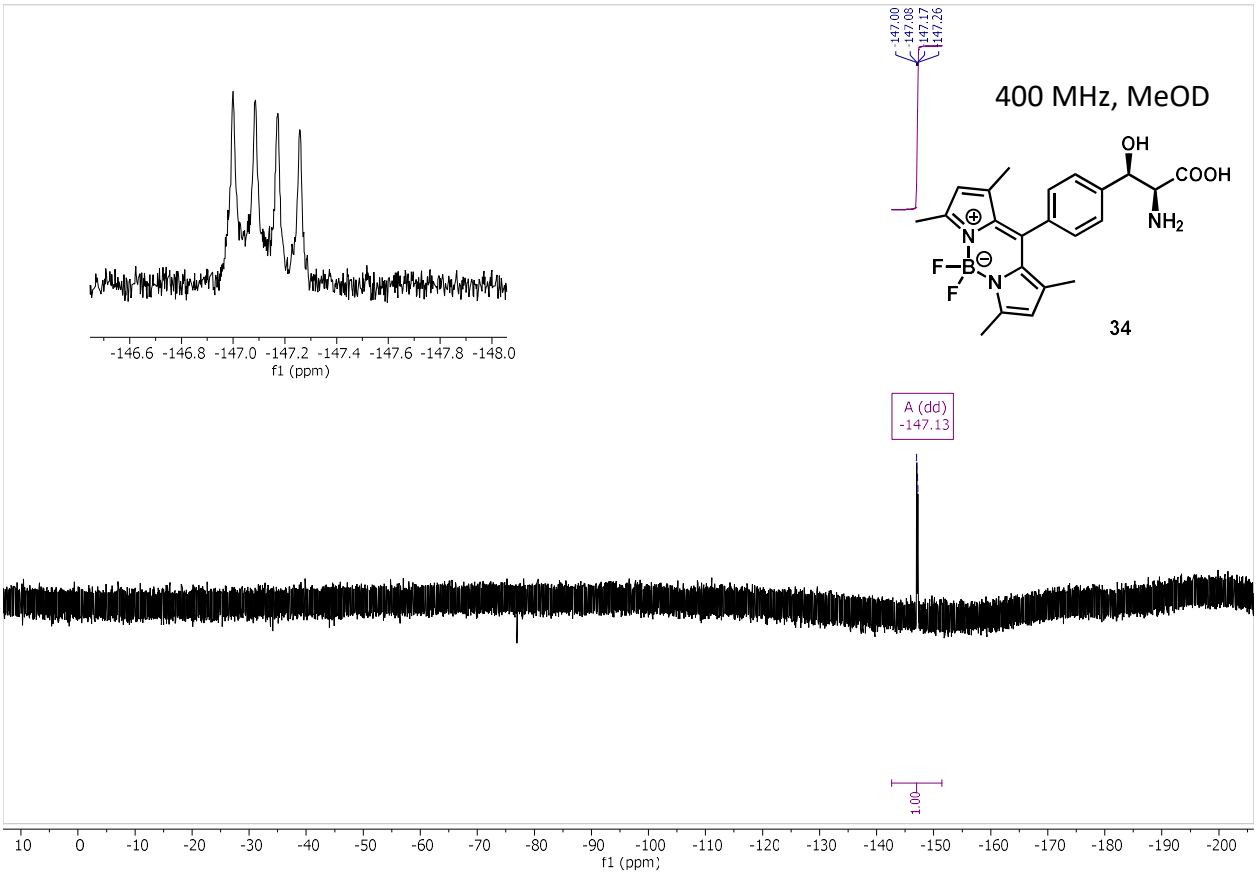

125 MHz, MeOD
OH
COOH
NH2
O
O
24
177.63
171.15
150.69
138.87
126.85
121.38
114.93
70.38
61.05
38.68
26.05
f1 (ppm)

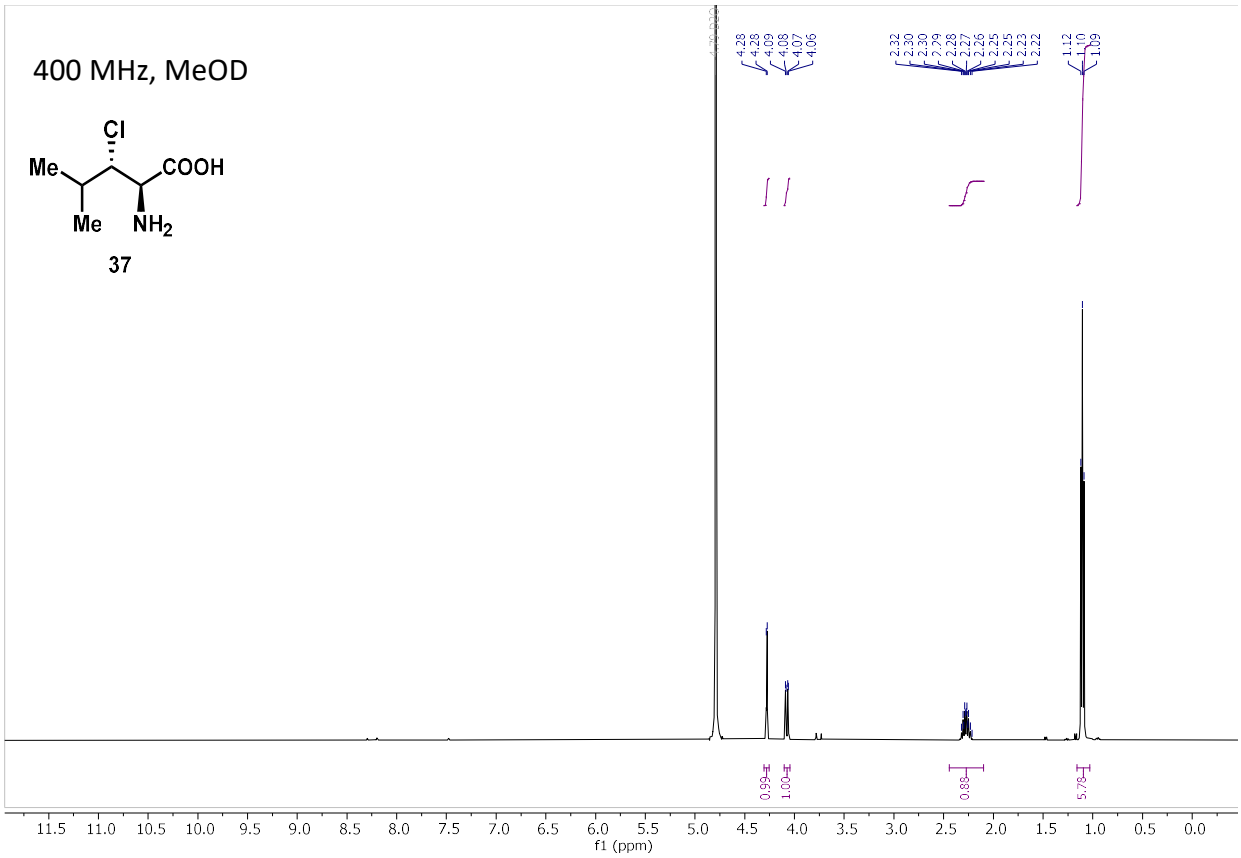

400 MHz, MeOD
OH
COOH
NH2
CF3
O
S
O
O
25
2.00
1.95
1.04
1.02
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

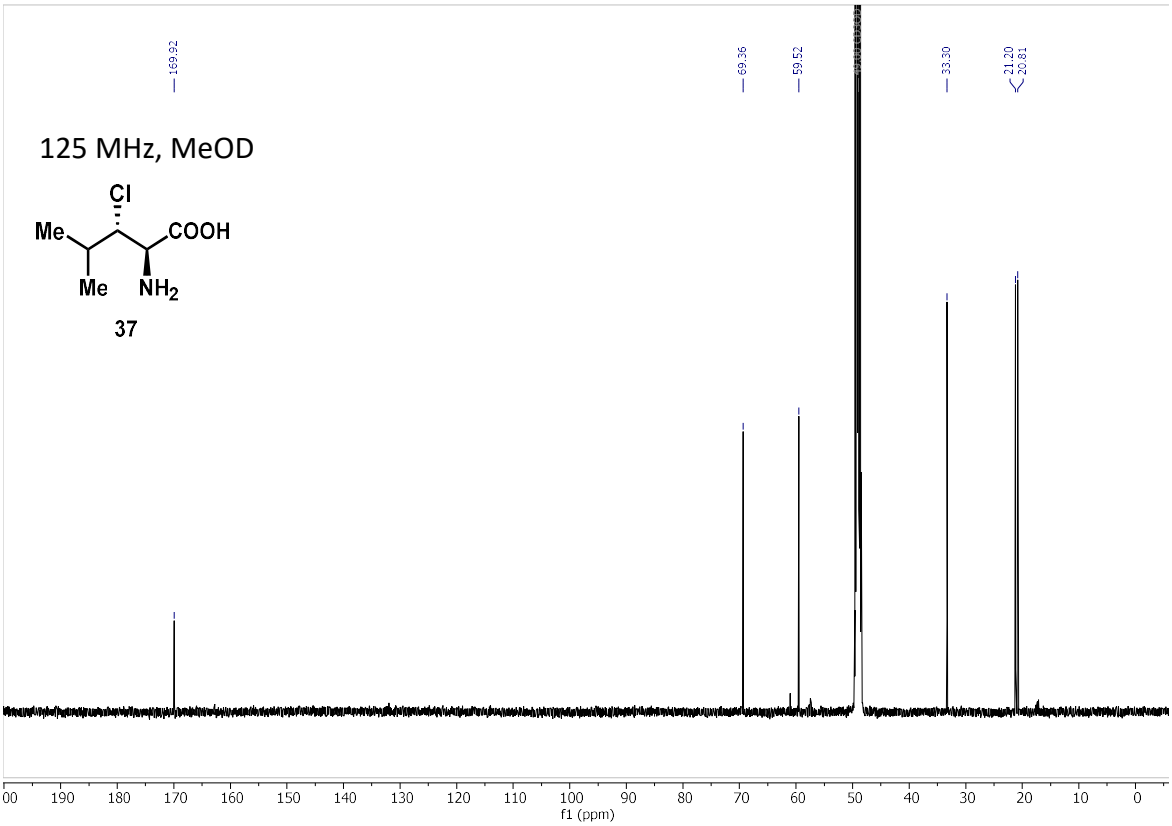

171.11
149.09
142.35
128.82
128.05
121.09
120.82
120.05
117.51
70.51
70.39
60.84
125 MHz, MeOD
OH
COOH
NH2
CF3
O
S
O
O
25
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


400 MHz, MeOD
OH
COOH
NH2
F
B
N
F
34
7.68
7.66
7.35
7.33
6.06
5.27
5.26
3.71
3.70
2.49
1.44
2.11
2.00
1.88
0.89
1.10
6.05
6.07
f1 (ppm)


125 MHz, MeOD
OH
COOH
NH2
F
B
N
F
34
172.15
156.66
144.83
141.06
135.35
135.81
132.64
129.42
128.43
122.19
72.61
62.53
61.21
14.97
14.53
f1 (ppm)


400 MHz, MeOD
34
A (dd)
-147.13
-147.00
-147.08
-147.17
-147.26
1.00
f1 (ppm)


400 MHz, MeOD
Cl
Me
COOH
Me
NH2
37
4.28
4.28
4.09
4.08
4.07
4.06
2.32
2.30
2.30
2.29
2.28
2.27
2.26
2.25
2.25
2.23
2.22
1.12
1.10
1.09
0.99
1.00
0.88
5.78
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


169.92
69.36
69.52
33.30
21.20
20.81
125 MHz, MeOD
Cl
Me
COOH
Me
NH2
37
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Part VIII. Small molecule crystallography

Data Collection

A colorless crystal with approximate dimensions 0.30 x 0.20 x 0.20 mm^3 was selected under oil under ambient conditions and attached to the tip of a MiTeGen MicroMount©. The crystal was mounted in a stream of cold nitrogen at 100(1) K and centered in the X-ray beam by using a video camera. The crystal evaluation and data collection were performed on a Bruker D8 VENTURE PhotonIII four-circle diffractometer with Cu Kα (λ = 1.54178 Å) radiation and the detector to crystal distance of 4.0 cm. The initial cell constants were obtained from a 180° ϕ scan conducted at a 2θ = 50° angle with the exposure time of 1 second per frame. The reflections were successfully indexed by an automated indexing routine built in the APEX3 program. The final cell constants were calculated from a set of 9890 strong reflections from the actual data collection. The data were collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.80 Å. A total of 25108 data were harvested by collecting 23 sets of frames with 0.5–1.0° scans in ω and ϕ with an exposure time 0.5–3 sec per frame. These highly redundant datasets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements.[5]

Structure Solution and Refinement

The systematic absences in the diffraction data were uniquely consistent for the space group $P2_12_12_1$ that yielded chemically reasonable and computationally stable results of refinement. A successful solution by the direct methods provided most non-hydrogen atoms from the E-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms not participating in hydrogen-bonding interactions were included in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients. There is also one molecule of solvent water in the asymmetric unit. **The absolute configuration of the 2-amino-3-(4-chlorophenyl)-3-hydroxypropanoic acid was unequivocally established by anomalous dispersion effects: C2 – *S*, C3 – *R***. The crystal chosen for the experiment proved to be an inversion twin with the minor component contribution of 5(2)%. The final least-squares refinement of 155 parameters against 2298 data resulted in residuals R (based on F^2 for I≥2σ) and wR (based on F2 for all data) of 0.0316 and 0.0898, respectively. The final difference Fourier map was featureless.

Summary

Crystal Data for $C_9H_{12}ClNO_4$ (M=233.65 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 5.5401(5) Å, b = 6.3588(7) Å, c = 30.308(3) Å, V = 1067.72(19) $Å^3$, Z = 4, T = 100.0 K, μ(CuKα) = 3.164 mm^{-1}, D_{calc} = 1.453 g/cm^3, 25108 reflections measured (5.832° ≤ 2Θ ≤ 158.884°), 2298 unique (R_{int} = 0.0478, R_{sigma} = 0.0206) which were used in all calculations. The final R_1 was 0.0316 (I > 2σ(I)) and wR_2 was 0.0898 (all data).

Part IX. References

References

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