Synthesis of β-branched tryptophan analogs using an engineered subunit of tryptophan synthase

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Supporting Information

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Experimental Procedures:

General

Chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, VWR, Chem-Impex International, Alfa Aesar) and used without further purification unless otherwise noted. Multitron shakers (Infors) were used for cell growth. UV-vis spectra were collected on a UV1800 Shimadzu spectrophotometer (Shimadzu). LC-MS data were collected on an Agilent 1290 UHPLC with 6140 MS detector (Agilent Technologies). NMR data were collected on a Varian 300 MHz or 500 MHz spectrometer, or a Bruker 400 MHz spectrometer with a Prodigy broadband cryoprobe. High resolution mass data were collected with a JMS-600H (JEOL) instrument with the samples ionized by fast atom bombardment or by time-of-flight mass spectroscopy with an LCT Premier XE with UPLC (Waters).

Cloning, expression, and purification of *Pf*TrpB

The gene encoding *Pf*TrpB^{WT} (UNIPROT ID Q8U093) was previously codon-optimized for *Escherichia coli* and cloned into pET22(b)+ with a C-terminal his6-tag.[1] Expression and purification protocols for the variants reported here were similar to those reported previously.[1] Briefly, a single colony of *E.coli* BL21 *E. cloni* Express cells (Lucigen) harboring the *Pf*TrpB plasmid was used to inoculate a 5-mL culture of Terrific Broth with 100 µg/mL ampicillin (TB$_{amp}$) and incubated overnight at 37 °C and 250 rpm. This culture was used to inoculate a 500-mL TB$_{amp}$ expression culture, which was incubated at 250 rpm and 37 °C for ~ 3 h or until an OD$_{600}$ of 0.8 was reached. Cultures were chilled on ice for 20 min and expression was induced by the addition of 500 mM isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Cells continued to grow shaking at 250 rpm and 20 °C for another 20 h. Cells were harvested by centrifugation at 4 °C and 5,000g for 10 min; the pellets were frozen at -20 °C until further use.

For preparation as a heat treated lysate, frozen cell pellets were thawed at room temperature and resuspended in 50 mM potassium phosphate buffer, pH 8.0, with 200 µM PLP, 1 mg/mL hen egg white lysozyme, and 0.02 mg/mL DNAse. After vortexing, cells were lysed with BugBuster (Novagen) according to the manufacturer's recommendations, and the cleared lysates were centrifuged at 20,000g and 4 °C for 10 min. The lysates were then incubated at 75 °C for 10 min, centrifuged again as described above and buffer exchanged into 50 mM potassium phosphate buffer pH 8.0, snap-frozen in liquid N$_2$ and stored at -80 °C until further use.

For purification, frozen cell pellets where thawed and treated as above with modification of the lysis buffer: 50 mM phosphate buffer, pH 8.0, with 20 mM imidazole and 100 mM NaCl (buffer A), with 200 µM PLP. The heat-treated lysate was applied to a 1-mL Histrap HP column (GE

Healthcare). The purification was performed with an AKTA purifier FPLC system (GE Healthcare). *Pf*TrpB eluted during a linear gradient from buffer A to buffer B (50 mM phosphate buffer with 500 mM imidazole and 100 mM NaCl, pH 8.0) at 140 mM imidazole. Purified *Pf*TrpB was desalted into 50 mM phosphate buffer, pH 8, frozen in liquid N_2, and stored at -80 °C until further use.

Purified protein concentrations were determined via the Bradford assay (Bio-Rad). The concentration of *Pf*TrpB[2B9] in heat-treated lysates was determined by specific activity. Tryptophan formation was monitored at 75 °C over 1 min at 290 nm using $\Delta\varepsilon_{290} = 1.89$ mM^{-1} cm^{-1}.[2] The assay buffer contained 200 mM potassium phosphate pH 8.0, and 5 μM PLP. Using purified *Pf*TrpB[2B9], we recorded an activity of 0.9 s^{-1} with 400 μM indole and 20 mM Ser. Using this parameter, we calculated that 375 mg of *Pf*TrpB[2B9] active catalyst was obtained per liter of culture as a heat-treated lysate.

Library construction

The error-prone PCR library was constructed with 200, 300 and 400 μM MnCl$_2$ as the mutagen using the gene for *Pf*TrpB[4D11] as the parent. The recombination library of hits found in the random mutagenesis studies (I16V, Q89L, F95L, L182P, Y192H, and V384A) was constructed using site-directed mutagenesis by overlap extension (SOE) PCR.[3] The library was constructed from fragments that were *Dpn*I digested, gel purified, and used as template for the subsequent assembly PCR using the flanking primers only. The process is described in greater detail in Buller et al.[1] Each library was cloned into pET22(b)+ between restriction sites *Nde*I and *Xho*I in frame with the *C*-terminal his-tag for expression in *E. coli* BL21 *E. cloni* Express cells.

High-throughput screening

For high-throughput expression, BL21 *E. cloni* Express cells carrying *Pf*TrpB[4D11] and variant plasmids were grown in 96-well deep well plates in 300 µL TB$_{amp}$ at 37 °C and 80 % humidity with shaking at 250 rpm overnight. TB$_{amp}$ expression cultures (630 µL) were inoculated with 20 µL of the overnight cultures and continued to grow at 37 °C and 80 % humidity with shaking at 250 rpm for 3 h. Expression was induced with the addition of ITPG to a final concentration of 1 mM to pre-chilled (20 min on ice-water bath) cultures. The expression continued for another 20 h at 20 °C with shaking at 250 rpm. Cells were then centrifuged at 4000g for 10 min and frozen at – 20 °C overnight. For screening, cells were allowed to thaw at room temperature and then lysed in buffer consisting of 200 mM phosphate buffer, pH 8.0, with 1 mg/mL lysozyme and ~0.05 mg/mL DNaseI. To increase the enzyme concentration, 350 µL/ well was used for the error-prone PCR library and 400 µL/ well was used for the recombination library. Cells were lysed via incubation at 37 °C for 1 h. After centrifugation at 5,000g for 20 min, 180-µL aliquots of the lysates (160-µL aliquot for recombination libraries) were transferred into PCR plates (USA Scientific, Ocala, USA), heat-treated for 1 h at 75 °C, and then spun again at 1,000g and 4 °C for 30 min. After the addition of 120-µL assay buffer (50 mM phosphate buffer, 41.6 mM L-threonine, 0.833 mM indole, pH 8.0) to 80 µL of cleared, heat-treated lysates in UV-transparent assay plates (Evergreen Scientific), the plates were sealed and incubated at 75 °C for up to 45 min (generation 1: 30 min, generation 2: 45 min). The reactions were then arrested by incubation in an ice-water bath, and the amount of β-MeTrp formed was recorded at 290 nm with a plate reader (Tecan Infinite M200).

Low enzyme activity in the first generation led to a relatively high error in the assay (CV ~50 %) and only the nine hits with activity 2.5-fold greater than parent were selected for subsequent

sequencing and rescreening (Figure S2). One of the hits was revealed to be the parent sequence, and three contained only silent mutations. Cultures of each hit containing missense mutations were grown in triplicate on 5-mL scale, and expression and lysis proceeded in the same manner as described in the Cloning, Expression and Purification section above. Reactions using 10 μM of protein as heat-treated lysate (determined by Bradford assay) and 20 mM of indole and Thr were run for 1 h at 75 °C and quenched by dilution to 50% acetonitrile (ACN). Samples were analyzed by quantitation of product on HPLC, and the increase in activity relative to parent is shown in Table S3.

For the second generation, each of the silent mutations identified through error-prone were added to the PfTrpB[4D11] plasmid and the resultant construct was found to have higher expression under the growth conditions of the high-throughput screen, but not when grown at larger scale in a shaking flask. Nonetheless, this construct was used as the parent for the recombination libraries (described above), which were screened under similar conditions as the random mutagenesis libraries. Many potentially activated proteins were identified from the recombination library but rescreening the top five hits showed that only a single protein, PfTrpB[2B9] had activity that was greater than the most active variant from the random mutagenesis library (Table S4).

UV-Vis spectroscopy

Spectra were collected between 550 and 250 nm on a UV1800 Shimadzu spectrophotometer (Shimadzu) using 20 μM of enzyme in 200 mM potassium phosphate pH 8.0 in a quartz cuvette. Samples were incubated at 75 °C for > 3 min to ensure a stable temperature was reached. Stage I of the reaction was initiated by addition of 20 mM L-threonine, and the spectra were measured in

< 15 s to limit production of α-ketobutyrate from deamination of L-threonine, which absorbs at 320 nm.

Specificity Measurement

We measured the selectivity of PfTrpB^{2B9} for L-serine vs. L-threonine by direct competition. A 200 µL reaction containing 1 µM enzyme, 5 mM indole, 10 mM L-serine, 10 mM L-threonine in 0.2 M potassium phosphate buffer pH 8.0 was allowed to proceed overnight in a water bath at 75 °C. Reactions were quenched with 200 µL ACN and analyzed via LC-MS, which showed only the canonical L-tryptophan product within the limits of detection, suggesting > 1000-fold specificity for L-serine.

Total Turnover Number (TTN) determination

Measurements were made using HPLC and LC-MS instruments. The amount of Trp formed was measured at 280 nm using a standard curve with trichlorobenzene as internal standard. Measurement of the UV-absorption spectrum of β-MeTrp showed an identical spectrum (Figure S9), and the same curve was employed for both products while determining the TTN for the catalysts engineered for activity with Ser. Later, it was found that the conversion could be measured by monitoring at 277 nm, the isosbestic point for the conversion of indole to L-Trp or β-MeTrp, and calculating the ratio of product peak to the sum of the substrate and product peaks. This method is insensitive to loading volume, and results agreed with measurements using a standard curve. This method of TTN determination is only accurate when there are no competing reactions with indole or product, which we observed for the conditions employed here. We used this technique to determine the TTN of the PfTrpB^{2B9} reaction with different nucleophiles. The

isosbestic point for the conversion of substrate to product was measured using a time course UV-vis analysis of the reaction catalyzed by PfTrpB[2B9] using L-Ser as the amino acid source because the reactions are significantly faster than with L-Thr and the β-Me group does not change the absorbance spectrum of the product (Table S5). Next, reactions were set up in triplicate using a total volume of 150 µL with 25 mM nucleophile and 250 mM L-Thr in 200 mM potassium phosphate pH 8.0 in borosilicate glass vials, as plastics can absorb indoles at high temperatures. Before addition of enzyme, all reagents were transferred into a Coy anaerobic chamber with < 1 ppm O_2 to rapidly exchange the atmosphere in the headspace of the vials to 98/2% N_2/H_2, which limits competing non-catalytic oxidation of indoles. Enzyme was added in the anaerobic environment with varied catalyst loading to ensure a robust product signal in subsequent analysis. Reaction vessels were capped before being removed from the anaerobic chamber and incubated in a 75 °C water bath for 24 h. Reactions were quenched the next day by addition of 1 volume (150 µL) of 100% ACN, mixing, and allowing to rest at room temperature for 10 min. Quenched solutions were then diluted 5-fold with 40% ACN in water and analyzed via LC-MS. In all cases, auxiliary peaks that would indicate non-catalytic degradation of substrate were present at only low levels, contributing to < 1% of the total integration signal for a given wavelength. For 7-azaindole, a second peak with m/z = 220 was observed that was also found in larger scale reactions and whose properties are consistent with an N-alkylated product.

Different conditions were needed to ascertain the TTN with thiophenol because of competing oxidation by molecular oxygen. Use of sparged buffers and addition of dithiothreitol (DTT) helped to limit oxidation. A 5-mL reaction containing 25 mM thiophenol, 250 mM Thr, 12.5 mM DTT, and 0.02 mol % catalyst loading was prepared in the Coy anaerobic chamber. The reaction was allowed to proceed for 55 hours, during which the solution slowly became cloudy as the

thiophenol oxidized. The reaction was analyzed via LC-MS and integration of the product peak at 254 nm after 55 hrs was compared to the total integration at 20 hrs, before significant oxidation had occurred. This ratio indicated 24 % conversion for 1,200 turnovers.

Protein Crystallography

*Pf*TrpB^{WT} crystallization conditions were identified previously,[1] and crystals routinely grew in sitting drops against a 1-mL reservoir of 15-25% PEG3350 and 0.1 M Na HEPES pH 7.85 with mother liquor comprised of 1.5 µL of 8.0 mg/mL *Pf*TrpB^{WT} and 1.5 µL of well solution. Ligand-bound structures were determined by soaking crystals of *Pf*TrpB^{WT} with 100 mM L-threonine for 2 min. Crystals were cryo-protected through oil immersion in Fomblin Y (Sigma) and flash frozen in liquid N$_2$ until diffraction. Diffraction data were collected remotely at the Stanford Synchrotron Radiation Laboratories on beamline 12-2. Crystals routinely diffracted at or below 2.0 Å, and the data were integrated and scaled using XDS [4] and AIMLESS. [5] A resolution cutoff of CC1/2 > 0.3 was applied along the strongest axis of diffraction.[5, 6] These data contributed to model quality as judged by R$_{free}$ in the final bin < 0.4. The structure was solved using molecular replacement with PHASER, as implemented in CCP4.[7, 8] The search model comprised a single monomer of *Pf*TrpB^{WT} (PDB ID: 5DW3) subjected to 10 cycles of geometric idealization in Refmac5 and removal of all ligands. Model building was performed in Coot [9] beginning with data processed at 2.4 Å, followed by subsequent inclusion of increasingly higher resolution shells of data with relaxed geometric constraints. Refinement was performed using REFMAC5.[10] The MolProbity server was used to identify rotamer flips and clashes.[11] After the protein, ligand, and solvent atoms were built, TLS operators were added to refinement, which resulted in

substantial improvements in R_{free} for the models. Crystallographic and refinement statistics are reported in Table S2. The structure is deposited with PDB ID: 5IXJ.

Identification of β-methyl amino acid products

The identities of the amino acid products were confirmed by ^{1}H, ^{13}C, and ^{19}F NMR and high-resolution mass spectrometry (HRMS). Proton NMR spectra were recorded on a Varian 300 or 500 MHz or Brucker 400 MHz spectrometer. Proton chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using the residual solvent resonance (CD_3OD, δ 3.31 ppm; D_2O, δ 4.79 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), triplet of doublets (td)], coupling constants [Hz], integration). Carbon NMR spectra were recorded on a Varian 300 or 500 MHz or Brucker 400 MHz spectrometer with proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using the residual solvent resonance (CD_3OD, δ 49.00). Fluorine NMR spectra were recorded on a 300 MHz (282 MHz) spectrometer without proton decoupling. Fluorine chemical shifts are reported in ppm relative to $FCCl_3$ (δ 0.00 ppm) and were calibrated automatically by the spectrometer using the solvent deuterium lock signal. High-resolution mass spectra were obtained on a JMS-600H HRMS (JEOL) using fast atom bombardment.

Reaction with heat-treated lysate: Reactions were conducted using *Pf*TrpB[2B9], which was prepared by heat-treatment as described above. The protein was used as a solution in potassium phosphate buffer (50 mM, pH 8.0) and the concentration varied between preps between 97 μM and 670 μM, as determined by specific activity. Threonine was added as a solid and PLP was used as a 15 mM aqueous solution.

Generally, reactions were conducted in 2–5 mL volume with the following reagents added sequentially: 0.1 M nucleophile, 1.0 M threonine, 0.2 M potassium phosphate buffer pH 8.0 with dimethyl sulfoxide (5% of final volume), and a 5-fold molar excess of PLP relative to enzyme. Finally, the enzyme solution was added and the vial was capped and immersed in a water bath that had been equilibrated to 75 °C. After 16–24 h, the reaction mixture was allowed to cool to room temperature, then purified directly on C18 silica (20-mL column volume) using an Isolera automated column instrument (Biotage) with 0% to 100% ACN/H$_2$O or 0% to 100% MeOH/H$_2$O. The method holds for 5-7 column volumes at 1% ACN (or MeOH) to wash the majority of the DMSO from the column before initializing the gradient, which runs over 10 column volumes. From this procedure the vast majority of DMSO is removed, however a trace amount remains. Product can be re-columned with the above procedure to remove this residual solvent.

Analysis of diastereoselectivity: Diastereoselectivity of each product is assessed at the conclusion of the reaction via LC-MS analysis and later during isolation. In all but two instances, a single peak with the predicted mass is observed. The reaction with 7-azaindole yielded two peaks,. however isolation of this second compound and analysis by ^{1}H-NMR revealed a clear quartet of doublets at 5.2 ppm not consistent with a second diastereomer, but rather an *N*-alkylated regioisomer (spectra are provided below). A similar second peak was inconsistently observed with reactions using 4-fluoroindole. While we cannot unequivocally rule out that this is a second diastereomer, the above observations suggest this is also a trace *N*-alkylation product.

Analysis of enantioselectivity: The TrpS enzyme system has been extensively studied and does not produce D-Trp at a measurable level, consistent with its biological function. The configuration of the (2*S*,3*S*)-β-methyl-tryptophan product, which was a known compound,[12] was

assigned based on the ^{1}H-NMR chemical shift of the β-methyl group, for which there is a clear difference between the (2*S*,3*R*) compound. This retention of configuration matches the known stereochemical outcome of the TrpS reaction performed using H,D-labelled L-serine.[13] The configuration of the β-Me-Trp analogs is inferred by analogy to the parent compound. To further assess the stereochemical purity of the products, we derivatized the amino acid products with both racemic and enantiopure *N*-(5-fluoro-2,4-dinitrophenyl)alanamide (FDNP-alanamide) for comparison.[14] Approximately 1.0 μmol of amino acid product was resuspended in 10 μL of 1.0 N aqueous HCl and aliquoted into 2 glass HPLC vials containing 97 μL of 1 M aqueous NaHCO$_3$. Two equivalents of FDNP-alanamide were added to each vial as a 33 mM solution in acetone. In one reaction the reagent added is enantiopure and in the other a racemate is added. This solution was incubated at 37 °C for 1 h, diluted by the addition of 500 μL 50 % ACN/H$_2$O and analyzed by LC-MS, which indicated >99 % ee for each compound tested.

Supplemental Tables

Table S1. Total turnovers catalyzed by TrpB enzymes prior to evolution for activity with L-threonine.

Entry	Enzyme	Turnovers
1	*Pf*TrpB^{WT}	66
2	*Pf*TrpB^{T292S}	420
3	*Pf*TrpB^{4D11}	660
4	*Pf*TrpB^{0B2}	180

Activity measured using 1 µM of purified enzyme in 0.2 M potassium phosphate buffer pH 8.0 with 20 mM of each reactant, L-threonine and indole, with a 24-h reaction at 75 °C.

Table S2. Crystallographic data collection and refinement statistics

Protein		*Pf*TrpB^{WT}
Ligand		L-Threonine
Data Collection		
Space group		P2$_1$2$_1$2$_1$
Cell dimensions (Å)		a,b,c = 86.2, 112.4, 160.3
Cell angles		$\alpha = \beta = \gamma = 90°$
Wavelength (Å)		0.9795
Beamline		SSRL 12.2
Resolution (Å)		40 – 1.54
Last bin (Å)		(1.57 – 1.54)
No. observations		2,230,262
Completeness (%)		98.9 (78.1)
R$_{pim}$ (%)		0.021 (0.577)
CC(1/2)		1.000 (0.587)
I/σI		19.4 (1.3)
Redundancy		9.8 (5.5)
Refinement		
Total no. of reflections		214,599
Total no. of atoms		12,924
Final bin (Å)		(1.54 – 1.58)
R$_{work}$ (%)		17.5 (30.7)
R$_{free}$ (%)		19.5 (31.7)
Average B factor (Å^2)		25.1
Ramachandran plot Favored, %		97.9
Allowed, %		99.8
Outliers, %		0.2

Values in parenthesis are for the highest resolution shell

R$_{merge}$ is $\Sigma|Io - I| / \Sigma Io$, where Io is the intensity of an individual reflection, and I is the mean intensity for multiply recorded reflections

R$_{work}$ is $\Sigma||Fo - Fc|| / Fo$, where Fo is an observed amplitude and Fc a calculated amplitude;

R$_{free}$ is the same statistic calculated with a 5% subset of the data that was included in refinement.

Ramachandran statistics calculated by the MolProbity server.

Table S3. Activity of hits from random mutagenesis library relative to parent

Variant	Fold Improvement	Mutations
*Pf*TrpB[4D11]	1.0 (parent)	
*Pf*TrpB[3C7]	1.5	I16V, Q89L
*Pf*TrpB[4H5]	0.9	L182P
*Pf*TrpB[4G1]	2.4	F95L
*Pf*TrpB[4E9]	1.2	V384A
*Pf*TrpB[2A9]	1.3	Y192H

Improvement measured as increase in β-MeTrp formation using heat-treated lysate after 60-min reaction at 75 °C. Concentration of the protein was normalized for all variants. See SI Experimental Procedures for details.

Table S4. Activity of hits from recombination library relative to parent

Variant	Fold Improvement	Mutations
*Pf*TrpB[4G1]	1.0 (parent)	F95L
*Pf*TrpB[1B5]	0.3	I16V, F95L, L182P, V384A
*Pf*TrpB[1F4]	0.8	I16V, F95L, Y192H, V384A
*Pf*TrpB[3H2]	< 0.01	I16V, A93V, F95L, Y192H, V384A
*Pf*TrpB[1E4]	< 0.01	I16V, Q89L, F95L, L182P, V384A
*Pf*TrpB[2B9]	1.7	I16V, F95L, V384A

Improvement measured as increase in β-MeTrp formation after 90-min reaction at 75 °C without normalization for protein expression. See SI Experimental Procedures for details.

Table S5. TTN of β-substitution reactions with L-Thr and different indole nucleophiles

Nucleophile	Isosbestic Point (nm)	Catalyst Loading (%)	TTN
Indole	277	0.01	8200 ± 1600
2-Methylindole	279	0.01	6400 ± 1400
6-Methylindole	273	0.01	1100 ± 100
4-Fluoroindole	267	0.1	380 ± 10
5-Fluoroindole	282	0.01	1300 ± 280
Indazole	276	0.04	500 ± 40
7-Azaindole	292	0.1	*C*-alkylation - 220 ± 40 *N*-alkylation - 32 ± 3

Activity measured via LC-MS and conversion as the ratio of substrate to total peak intensity at the isosbestic point after 24-h reaction at 75 °C. See SI Experimental Procedures for details.

Supplemental Figures
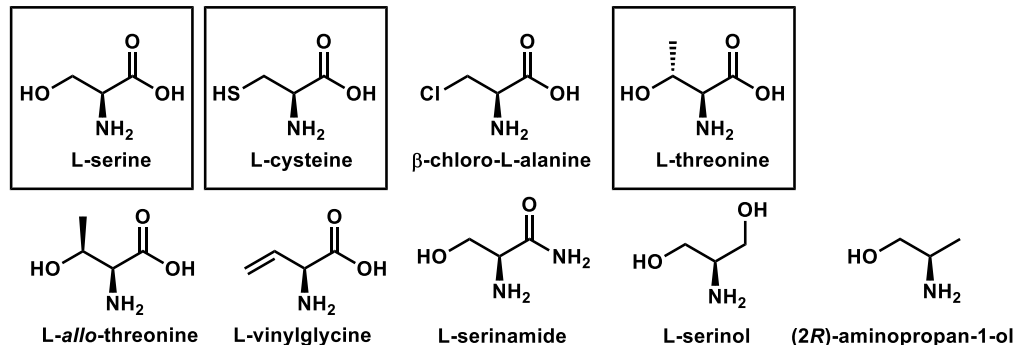
Figure S1. Different amines tested for reactivity with *Pf*TrpB[0B2]. Product formation was assessed by LC-MS analysis. Boxes indicate substrates for which the expected product mass was observed.
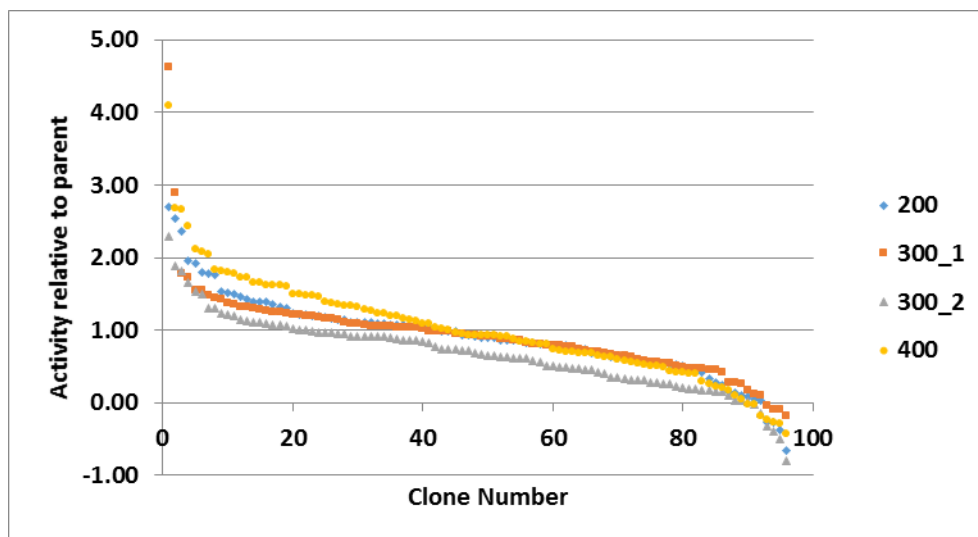
Figure S2. Retention of function curves for *Pf*TrpB[4D11] random mutagenesis library for activity with Thr and indole. Legend refers to the concentration of MgCl$_2$ (in μM) used to introduce mutations; two plates with 300 μM MnCl$_2$ were assayed. The variance in the assay is attributed

to the relatively low activity of the parent enzyme, whose activity is close to the limit of detection in the assay used here.
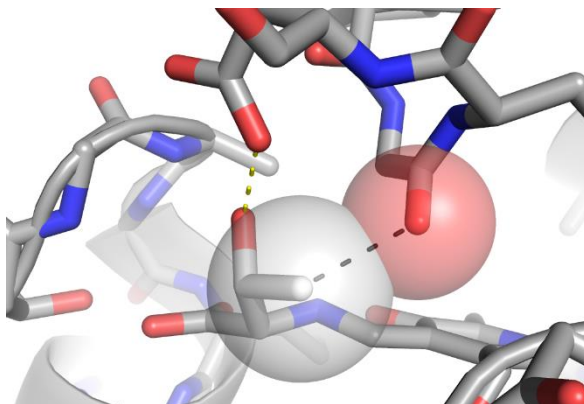
Figure S3. Modeling the Thr external aldimine of *Pf*TrpB based on the 2.0-Å X-ray crystal structure of the Ser external aldimine with *Pf*TrpB (PDB ID: 5DW0). Spheres shown represent the group van der Waals radii and their intersection indicates a steric clash between the Thr β-methyl group and the backbone of Gly298, black dashes. Yellow dashes indicate a hydrogen bond between Thr and the sidechain of Asp300.
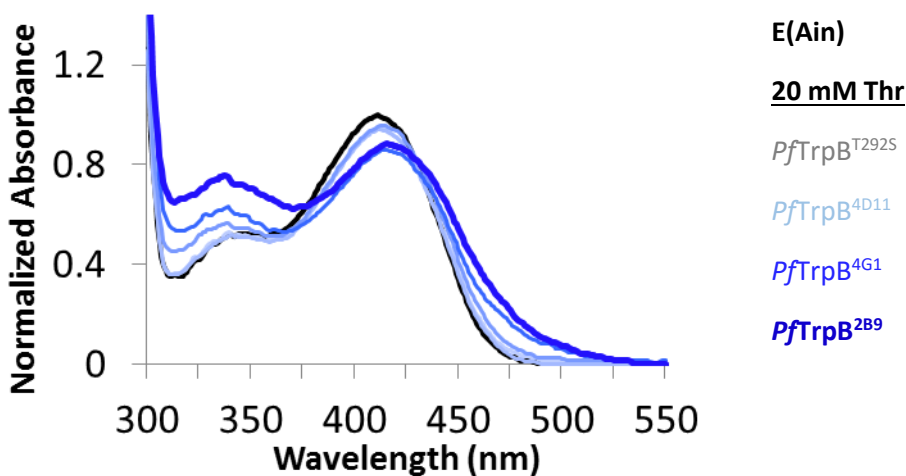
Figure S4. UV-vis analysis of the reaction between Thr and engineered TrpB proteins. The black trace shows the internal aldimine resting state of each catalyst and the traces in increasingly dark

shades of blue are engineered proteins with the addition of 20 mM Thr. The increase in absorbance at 350 nm and broad shoulder extending past 500 nm are consistent with steady-state accumulation of an E(A-A) intermediate. The slight shift to the right of the peak at 412 nm suggests a mixed population $\quad$ E(A-A) $\quad$) and E(Aex$_1$) is also present in solution. Spectra were collected at 75 °C using 20 µM purified protein.
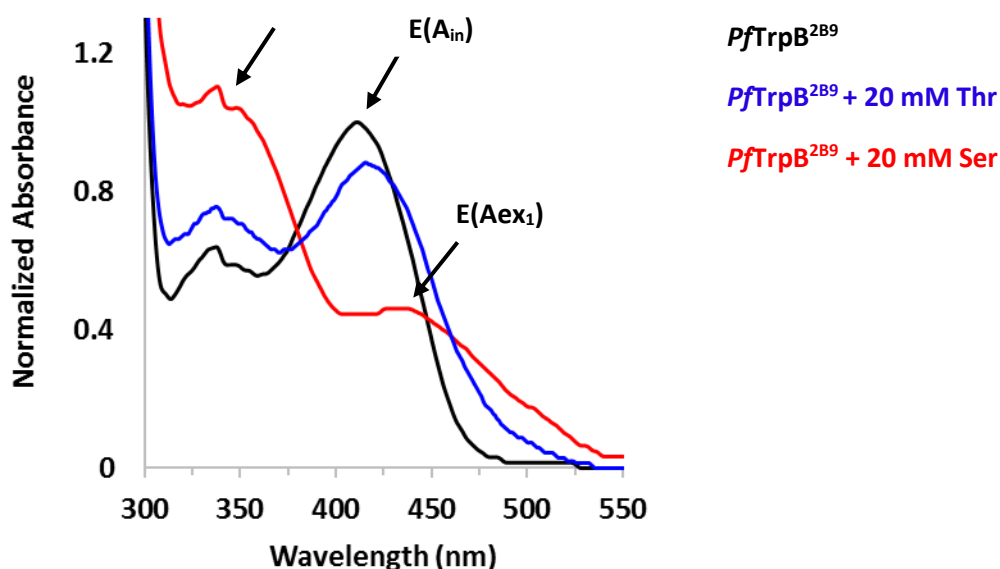
Figure S5. Comparison of steady state intermediates of *Pf*TrpB[2B9] in a reaction with either Thr or Ser. In its resting state, the PLP of *Pf*TrpB[2B9] exists as the E(Ain) (black). When 20 mM Ser is added as the amino acid source the prominent λ_{max} at 350 nm with a broad shoulder to 550 nm is indicative of E(A-A) formation (red). The residual peak at ~420 nm indicates some E(Aex$_1$) is also present in solution. Addition of 20 mM Thr also shows a λ_{max} at 350 nm, however it is less prominent than the E(Aex$_1$) peak (blue). Spectra were collected at 75 °C using 20 µM purified protein.
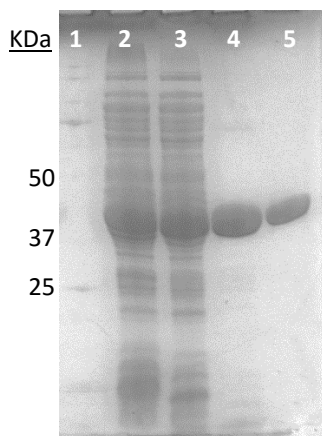
Figure S6. Any KD gradient SDS-PAGE gel of *Pf*TrpB^{2B9}. Lane 1 = Protein Ladder (Bio-Rad). Lane 2 = Whole cells expressing *Pf*TrpB^{2B9}. Lane 3 = Soluble fraction upon lysis. Lane 4 = Heat treated lysate. Lane 5 = Purified via His-trap.
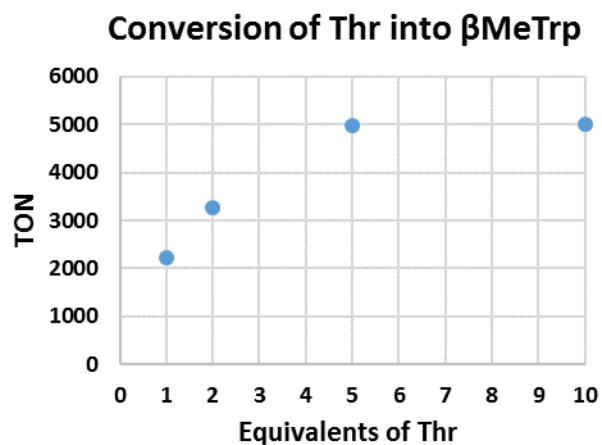
Figure S7. Effect of Thr:indole stoichiometry on β-substitution reaction. Reactions conducted using 25 mM indole in 0.2 M potassium phosphate pH 8.0 with 0.02 mol% *Pf*TrpB^{2B9} catalyst. The maximum of 5,000 TTN was achieved with five equivalents of Thr. Additional Thr was not deleterious to the reaction.
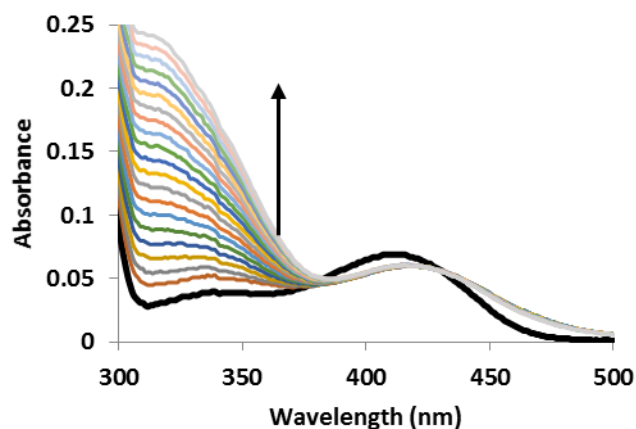
Figure S8. UV-vis analysis of *Pf*TrpB[2B9] Thr deamination activity. Black trace shows the enzyme E(Ain) spectrum before addition of substrate. Upon addition of 20 mM Thr, the spectrum shifts to reflect the steady-state distribution of stage I intermediates of the catalytic cycle. The features of the spectrum past 390 nm remain constant while there is a linear increase in broad absorbance at 320 nm that is consistent with deamination of Thr to form α-ketobutyrate and ammonium. The initial spectrum upon addition of Thr was collected within 5 s and each subsequent trace is separated by 1 min. Reaction conducted with 20 μM *Pf*TrpB[2B9].
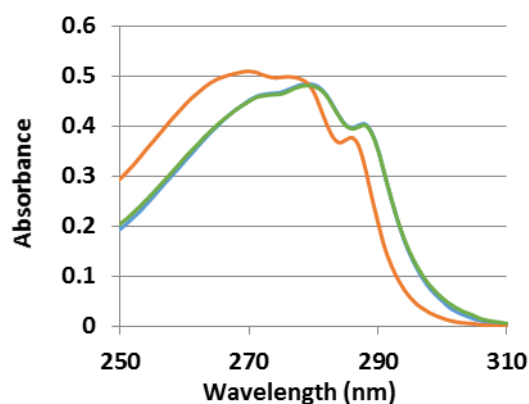
Figure S9. Absorbance spectra of indole (red), Trp (blue), and β-MeTrp (green). Each sample was prepared as 100 μM solution in H_2O.

β-Alkyl Amino Acid Synthesis
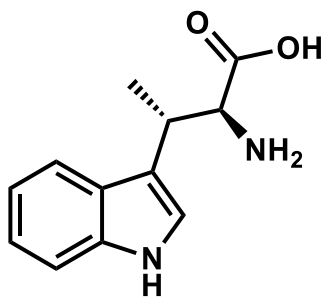
(2*S*,3*S*)-2-amino-3-(1H-indol-3-yl)butanoic acid (**5**). A 20 mL vial was charged with indole (59.3 mg) and L-Thr (595.6 mg). The substrates were suspended in 3.97 mL of aq. potassium phosphate (0.2 M, pH 8.0) with 250 μL DMSO (5% final volume). Aq. PLP was added from a 15 mM stock in H_2O to achieve a final concentration of 100 μM. Lastly, 625 μL of *Pf*TrpB[2B9] (160 μM) was added as a heat-treated lysate for a final reaction volume of 5.0 mL with 0.02 mol % catalyst loading with respect to indole. The reaction was allowed to proceed overnight at 75 °C in an oil bath. (A slight orange color developed overnight, consistent with non-catalytic oxidation of indole.) After 16.7 hours, approximately 90 % of indole was converted to product, as indicated by the UV absorbance at 280 nm from LC-MS analysis of the reaction mixture. The product was purified by flash chromatography using C_{18}-silica, which was first prepared as a gel with MeOH and then equilibrated to 100 % H_2O. The reaction mixture was applied to the column and washed with 2 column volumes (CVs) of 100 % H_2O. The product was subsequently eluted with 2 CVs of 50% MeOH/H_2O. Fractions were assessed for the presence of product using LC-MS, and those that contained it were pooled and concentrated with a rotary evaporator. The product was dissolved in H_2O, frozen on dry ice, and lyophilized overnight. From this procedure 85.0 mg of a pale brown solid product were obtained that, correcting for trace DMSO that was carried over during the purification, indicate an isolated yield of 75 %. This trace DMSO was removed by further purification using automated flash chromatography using a C18-silica

column with a gradient from 0 to 100% methanol and subsequent lyophilization of the pooled product fractions. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

¹H NMR (300 MHz, Methanol-d_4) δ 7.72 (dt, J = 8.0, 1.0 Hz, 1H), 7.35 (dt, J = 8.2, 1.0 Hz, 1H), 7.18 (s, 1H), 7.10 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H), 3.72 (d, J = 7.6 Hz, 1H), 3.52 (p, J = 7.3 Hz, 1H), 1.53 (d, J = 7.2 Hz, 3H). **¹³C NMR** (101 MHz, Methanol-$d4$) δ 175.37, 138.78, 127.80, 124.27, 122.86, 120.38, 120.13, 115.66, 112.67, 61.94, 35.71, 19.85. **MS** (FAB) (*m/z*) for [M+H]$^+$ $C_{12}H_{15}N_2O_2$ requires 219.1134, observed 219.1120.
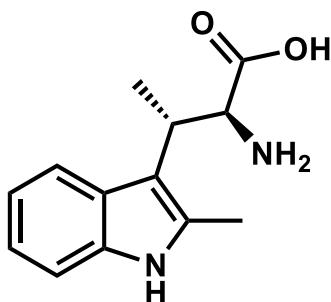
(2S,3S)-2-amino-3-(2-methyl-1H-indol-3-yl)butanoic acid. A 5 mL vial was charged with 2-methylindole (27.3 mg) and L-Thr (238.2 mg). The substrates were suspended in 1.78 mL 0.2 M potassium phosphate pH 8.0 with 100 μL DMSO (5 % final) and PLP was added from a 15 mM stock in H_2O to a final concentration of 130 μM. Lastly, 111 μL of 240 μM *Pf*TrpB^{2B9} was added as a heat-treated lysate for a final reaction volume of 2.0 mL with 0.013 mol % catalyst loading with respect to 2-methyl-indole. The reaction was allowed to proceed overnight in an water bath at 75 °C. Approximately 93 % of 2-methyl-indole was converted to product, as indicated by the UV absorbance at 279 nm from LC-MS analysis of the reaction mixture, however this may be an overestimate, as the substrate was sampled as a suspension. The product was purified using

automated flash chromatography using a C_{18}-silica column with a gradient from 0 to 100% methanol. The fractions containing product, as indicated by LC-MS, were pooled and concentrated by rotary evaporation. From this procedure 35 mg of a pale yellow solid product were obtained that, correcting for trace DMSO that was carried over during the purification, indicate an isolated yield of 72 %. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

¹H NMR (400 MHz, Deuterium Oxide) δ 7.68 (dt, J = 7.7, 1.0 Hz, 1H), 7.42 (dt, J = 8.1, 1.0 Hz, 1H), 7.17 (ddd, J = 8.1, 7.1, 1.3 Hz, 1H), 7.11 (ddd, J = 8.2, 7.1, 1.2 Hz, 1H), 3.97 (d, J = 10.0 Hz, 1H), 3.34 (dq, J = 10.0, 7.2 Hz, 1H), 2.39 (s, 3H), 1.50 (d, J = 7.2 Hz, 3H). **¹³C NMR** (101 MHz, Deuterium Oxide) δ 175.07, 135.60, 134.63, 125.76, 121.05, 119.15, 118.39, 111.28, 108.27, 59.88, 33.46, 17.09, 10.77. **MS** (FAB) (*m/z*) for [M+H]⁺ $C_{13}H_{17}N_2O_2$ requires 233.1290, observed 233.1286.
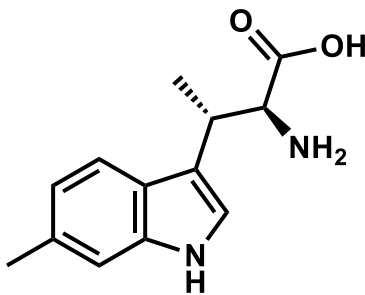
(2*S*,3*S*)-2-amino-3-(6-methyl-1H-indol-3-yl)butanoic acid. A 20 mL vial was charged with 6-methylindole (21.7 mg) and L-Thr (71.0 mg). The substrates were suspended in 1.27 mL aq. potassium phosphate (0.2 M, pH 8.0) with 82.5 µL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 100 µM. Lastly, 199 µL of 671 µM *Pf*TrpB[2B9] was added as a heat-treated lysate for a final reaction volume of 1.65 mL with 0.1 mol % catalyst loading with respect to 6-methyl-indole. The reaction was allowed to

proceed in a water bath at 75 °C. After 1 h, 46 mg additional Thr was added. Because the 6-methyl-indole was not fully dissolved in solution an additional 350 μL of buffer were added to decrease the concentration. After 2 h, 80 mg of Thr were added for a final amount of 196.5 mg Thr (10 eq). The reaction was allowed to proceed overnight at 75 °C. Approximately 92 % of 6-methyl-indole was converted to product, as indicated by the UV absorbance at 274 nm from LC-MS analysis of the reaction mixture, however this may be an overestimate, as the substrate was sampled as a suspension. The product was purified using automated flash chromatography using a C18-silica column with a gradient from 0 to 100% acetonitrile. The fractions containing product, as indicated by LC-MS, were pooled and concentrated by rotary evaporation. The light yellow solid was dissolved in 2 mL H_2O, frozen on dry ice, and lyophilized overnight. This procedure afforded 20.1 mg of product for an isolated yield of 52%. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.39 (d, J = 8.1 Hz, 1H), 6.96 (s, 1H), 6.91 (s, 1H), 6.67 (dd, J = 8.2, 1.4 Hz, 1H), 3.55 (d, J = 7.5 Hz, 1H), 3.12 (p, J = 1.6 Hz, 1H), 1.33 (d, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, Methanol-d_4) δ 174.4, 139.1, 132.4, 125.4, 123.6, 121.8, 119.9, 114.9, 112.4, 61.4, 35.1, 21.8, 19.6. **MS** (FAB) (*m/z*) for $[M+H]^+$ $C_{13}H_{17}N_2O_2$ requires 233.1290, observed 233.1289.
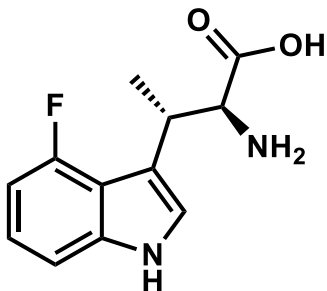
(2*S*,3*S*)-2-amino-3-(4-fluoro-1H-indol-3-yl)butanoic acid. A 6 mL vial was charged with 4-fluoroindole (28.6 mg) and L-Thr (75.6 mg). The substrates were suspended in 0.58 mL of aq. potassium phosphate (0.2 M, pH 8.0) with 100 μL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 100 μM. The mixture was then sparged with $Ar_{(g)}$ to remove O_2. Lastly, 1250 μL of 160 μM *Pf*TrpB[2B9] was added as a heat-treated lysate for a final reaction volume of 2.0 mL with 0.1 mol % catalyst loading with respect to 4-fluoro-indole. The reaction was allowed to proceed in an oil bath at 75 °C. After 1 h, 79.8 mg additional Thr was added. After 2 h, 82.8 mg of Thr were added for a final amount of 238.2 mg Thr (10 eq). The reaction was allowed to proceed overnight at 75 °C and monitored the following day by LC-MS analysis. The product peak continued to increase until 1585 min after initiation, however it was not allowed to proceed for a second night. Approximately 47 % of 4-fluoro-indole was converted to product, as indicated by the UV absorbance at 280 nm, however this wavelength overestimates the product formed. The product was purified using the same protocol as described for β-methyltryptophan. In the late-eluting fractions from the column, a minor peak with m/z = 237 was observed, suggestive of *N*-alkylation, however this compound could not be isolated in sufficient quantity for characterization. From this procedure 9.4 mg of purified product were obtained, indicating 19 % isolated yield. We were unable to complete Marfey's analysis for this product. However, as described above, the appearance of a single peak in the LC-MS indicating >99 % dr. Coupled with the fact that the engineered *Pf*TrpB catalysts

are highly enantioselective with 4-F-indole and Ser,[1] there is strong support for assigning the stereochemistry of this product.

¹H NMR (500 MHz, Methanol-d_4) δ 7.20 (s, 1H), 7.18 (d, J = 8.2 Hz, 1H), 7.05 (td, J = 8.0, 5.0 Hz, 1H), 6.71 (dd, J = 11.7, 7.7 Hz, 1H), 3.81 (d, J = 9.0 Hz, 1H), 3.46 (p, J = 7.2 Hz, 1H), 1.49 (d, J = 6.9 Hz, 3H). **¹⁹F NMR** (282 MHz, Methanol-d_4) δ -120.84 (d, J = 11.7 Hz). **¹³C NMR** (126 MHz, Methanol-d_4) δ 175.1, 158.2 (d, J = 243.1 Hz), 142.0 (d, J = 12.2 Hz), 126.1–122.2 (m), 116.1 (d, J = 20.3 Hz), 115.3 (d, J = 3.6 Hz), 109.3 (d, J = 3.4 Hz), 105.3 (d, J = 20.7 Hz), 62.2, 36.6, 19.9 (d, J = 3.4 Hz). **MS** (FAB) (m/z) for [M+H]⁺ $C_{12}H_{14}FN_2O_2$ requires 237.1039, observed 237.1032.
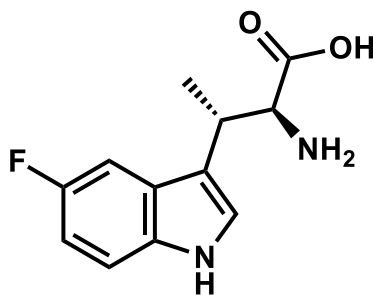
(2S,3S)-2-amino-3-(5-fluoro-1H-indol-3-yl)butanoic acid. A 6 mL vial was charged with 5-fluoroindole (27.1 mg) and L-Thr (63.6 mg). The substrates were suspended in 1.24 mL of aq. potassium phosphate (0.2 M, pH 8.0) with 100 µL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to a achieve a final concentration of 100 µM. The mixture was then sparged with Ar$_{(g)}$ to remove O$_2$. Lastly, 1250 µL of 160 µM PfTrpB^{2B9} was added as a heat-treated lysate for a final reaction volume of 2.0 mL with 0.1 mol % catalyst loading with respect to 5-fluoro-indole. The reaction was allowed to proceed in an oil bath at 75 °C. After 1 h, 63.6 mg additional Thr was added. After 2 h, 58.0 mg of Thr were added. After 4 h, 59.0 mg of Thr were added for a final amount of 244.2 mg Thr (10 eq). The reaction was

allowed to proceed overnight at 75 °C and monitored the following day by LC-MS analysis. Approximately 77 % of 5-fluoro-indole was converted to product, as indicated by the UV absorbance at 280 nm. The product was purified using the same protocol as described for β-methyl-tryptophan. From this procedure 31.1 mg of a white solid were obtained, indicating 64 % isolated yield. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

¹H NMR (300 MHz, Methanol-d_4) δ 7.41 (ddd, J = 10.2, 2.5, 0.6 Hz, 1H), 7.31 (ddd, J = 8.9, 4.5, 0.6 Hz, 1H), 7.25 (s, 1H), 6.87 (td, J = 9.1, 2.5 Hz, 1H), 3.71 (d, J = 6.9 Hz, 1H), 3.54 (p, J = 7.2 Hz, 1H), 1.52 (d, J = 7.2 Hz, 3H). **¹⁹F NMR** (282 MHz, Methanol-d_4) δ –127.22 (td, J = 9.7, 4.6 Hz). **¹³C NMR** (101 MHz, Methanol-d_4) δ 172.9, 157.5 (d, J = 232.2 Hz), 133.6, 126.5 (d, J = 9.8 Hz), 124.7, 114.00 (d, J = 4.9 Hz), 111.8 (d, J = 9.7 Hz), 109.4 (d, J = 26.6 Hz), 103.5 (d, J = 24.0 Hz), 60.0, 33.5, 18.1. **MS** (FAB) for [M+H]⁺ $C_{12}H_{14}FN_2O_2$ requires 237.1039, observed 237.1046.
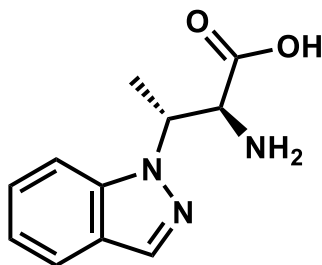
(2S,3R)-2-amino-3-(1H-indazol-1-yl)butanoic acid. A 20 mL vial was charged with indazole (22.4 mg) and L-Thr (74.2 mg). The substrates were suspended in 0.14 mL of aq. potassium phosphate (0.2 M, pH 8.0) with 100 μL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 100 μM. Lastly, 1667 μL of 160 μM PfTrpB²ᴮ⁹ was added as a heat-treated lysate for a final reaction volume of 2.0 mL with 0.13 mol % catalyst loading with respect to indazole. The reaction was allowed to proceed in a water bath at 75 °C. After 1 h, 77.2 mg additional Thr was added. After 2 h, 86.3 mg of Thr were

added for a final amount of 238.2 mg Thr (10 eq). The reaction was allowed to proceed overnight at 75 °C. Approximately 30 % of indazole was converted to product, as indicated by the UV absorbance at 280 nm from LC-MS analysis of the reaction mixture. The product was purified using automated flash chromatography using a C18-silica column with a gradient from 0 to 100% acetonitrile. The fractions containing product, as indicated by LC-MS, were pooled and concentrated by rotary evaporation. The white solid was dissolved in 2 mL H_2O, frozen on dry ice, and lyophilized overnight. This procedure afforded 14.0 mg of product for an isolated yield of 33%. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

¹H NMR (300 MHz, Methanol-d_4) δ 8.07 (d, J = 0.8 Hz, 1H), 7.73 (dt, J = 8.1, 1.0 Hz, 1H), 7.62 (dt, J = 8.6, 0.9 Hz, 1H), 7.40 (ddd, J = 8.5, 6.9, 1.1 Hz, 1H), 7.15 (ddd, J = 8.0, 6.9, 0.8 Hz, 1H), 5.43 (qd, J = 7.0, 4.7 Hz, 1H), 4.06 (d, J = 4.7 Hz, 1H), 1.62 (d, J = 7.1 Hz, 4H). **¹³C NMR** (101 MHz, Methanol-d4) δ 172.6, 141.6, 135.8, 128.2, 125.3, 122.5, 122.3, 110.9, 61.0, 54.9, 19.2. **MS** (FAB) (*m/z*) for [M+H]⁺ $C_{11}H_{14}N_3O_2$ requires 220.1086, observed 220.1114.
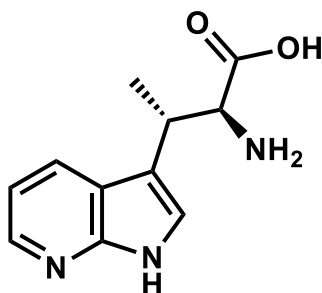
(2*S*,3*S*)-2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)butanoic acid. A 6 mL vial was charged with 7-azaindole (23.6 mg) and L-Thr (238.2 mg). The substrates were suspended in 0.144 mL aq. potassium phosphate (0.2 M, pH 8.0) with 100 µL DMSO (5% of the final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 660 µM. Lastly, 1667 µL of 160 µM *Pf*TrpB[2B9] was added as a heat-treated lysate for a final reaction volume of 2.0 mL with 0.13 mol % catalyst loading with respect to 7-azaindole. The mixture was then

sparged with $Ar_{(g)}$ to remove O_2. The reaction was allowed to proceed overnight at 75 °C in a water bath. The product was purified using the same protocol as described for 6-methyl-β-methyltryptophan. In the late-eluting fraction from the column, a second peak with m/z = 220 was observed, suggestive of *N*-alkylation. This fraction was a mixture of products and was kept separate for characterization. ^{1}H-NMR later indicated a product consistent with *N*-alkylation, however the compound was not characterized further. The fractions containing the anticipated *C*-alkylation product were pooled and concentrated by rotary evaporation. The white solid was dissolved in 2 mL H_2O, frozen on dry ice, and lyophilized overnight. This procedure afforded 7.6 mg of product for an isolated yield of 17%, from which the *N*-alkylated compound was incompletely removed. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee.

^{1}H NMR (300 MHz, Methanol-d_4) δ 8.19 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.15 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.32 (s, 1H), 7.09 (dd, *J* = 7.9, 4.9 Hz, 1H), 3.74 (d, *J* = 6.2 Hz, 1H), 3.66 (p, *J* = 7.0 Hz, 1H), 1.55 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, Methanol-d_4) δ 174.5, 149.9, 143.7, 130.2, 125.6, 121.3, 116.7, 114.7, 61.6, 35.1, 19.5. **MS** (FAB) (*m/z*) for [M+H]$^+$ $C_{11}H_{14}N_3O_2$ requires 220.1086, observed 220.1088.
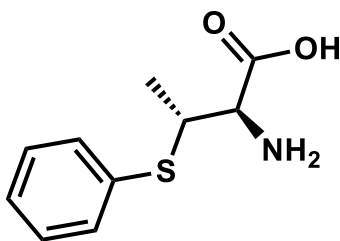
**(2R,3R)-2-amino-3-(phenylthio)butanoic acid.** A 20 mL vial was charged with 12.8 μL of thiophenol (9.77 M, > 99 % pure), followed by L-Thr (148.9 mg), and 1,4-dithiothreitol (96.4 mg). The reactants were suspended in 5.808 mL of $Ar_{(g)}$-sparged aq. potassium phosphate (0.2

M, pH 8.0) with 312.5 µL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 50 µM. Lastly, 93.1 µL of 671 µM of *Pf*TrpB^{2B9} prepared as a heat-treated lysate was added for a final reaction volume of 6.25 mL with 0.05 mol % catalyst loading with respect to thiophenol. To reduce exposure to oxygen, the reaction was transferred into a Coy anaerobic chamber. The reaction was allowed to proceed for 1 week at room temperature until all of the thiophenol was either converted to product or oxidized. The reaction mixture was purified using the same protocol as described for 6-methyl-β-methyltryptophan. A prominent peak was observed close to the product during purification that we attribute to the oxidized form of DTT. The fractions believed to contain the *S*-alkylation product, as indicated by LC-MS, were pooled and concentrated in a rotary evaporator. The white solid was dissolved in 2 mL H$_2$O, frozen on dry ice, and lyophilized overnight. This procedure afforded 8.4 mg of product that was determined to be in a 2:1 mixture with oxidized DTT that was carried through during the purification. Marfey's analysis of the product indicated the reaction proceeded with > 99 % ee. A high-resolution mass was obtained to confirm the production of (2*R*,3*R*)-2-amino-3-(phenylthio)butanoic acid, which was converted to its Boc-derivative for further purification and characterization.

MS (TOF) (MS ES+) for [M+H]$^+$ C$_{10}$H$_{14}$NO$_2$S requires 212.0745, observed 212.0734.
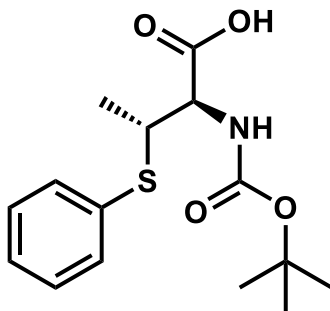
(2*R*,3*R*)-2-((tert-butoxycarbonyl)amino)-3-(phenylthio)butanoic acid. A 20 mL vial was charged with 25.6 µL of thiophenol (9.77 M, > 99 % pure), followed by L-Thr (297.8 mg), and

1,4-dithiothreitol (19.3 mg). The reactants were suspended in 9.037 mL of aq. potassium phosphate (0.2 M, pH 8.0) with 100 μL DMSO (5% of final volume), then aq. PLP was added from a 15 mM stock solution to achieve a final concentration of 83 μM. Lastly, 125 μL of 650 μM of purified PfTrpB^{2B9} was added for a final reaction volume of 10.0 mL with 0.03 mol % catalyst loading with respect to thiophenol. The mixture was then sparged with Ar$_{(g)}$ to remove O_2. The reaction was allowed to proceed overnight at 37 °C. (Reactivity was also observed at higher temperature, however for isolation it was beneficial to conduct the reaction at lower temperature to increase the time window between consumption of starting material and enzyme-catalyzed β-elimination of the product.)

Of this crude reaction mixture, 5 mL were subjected to Boc-derivatization as follows: Boc$_2$O (21.8 mg) was added along with 1 mL 1,4-dioxane. The mixture was allowed to sir for 5 h, after which there was an apparent 26% conversion by LC-MS. Additional Boc$_2$O (110 mg) was added in 40, 50, and 20 mg increments over the course of 4 days while the derivatization proceeded to a final apparent conversion of 71 % by LC-MS. The product was purified by automated C$_{18}$-silica column chromatography and the fractions containing the anticipated Boc-protected product were pooled and concentrated by rotary evaporation. The resulting white solid was dissolved in 1 mL H$_2$O, frozen on dry ice, and lyophilized overnight. This procedure afforded 5.0 mg of product for an isolated yield of 14% over two steps.
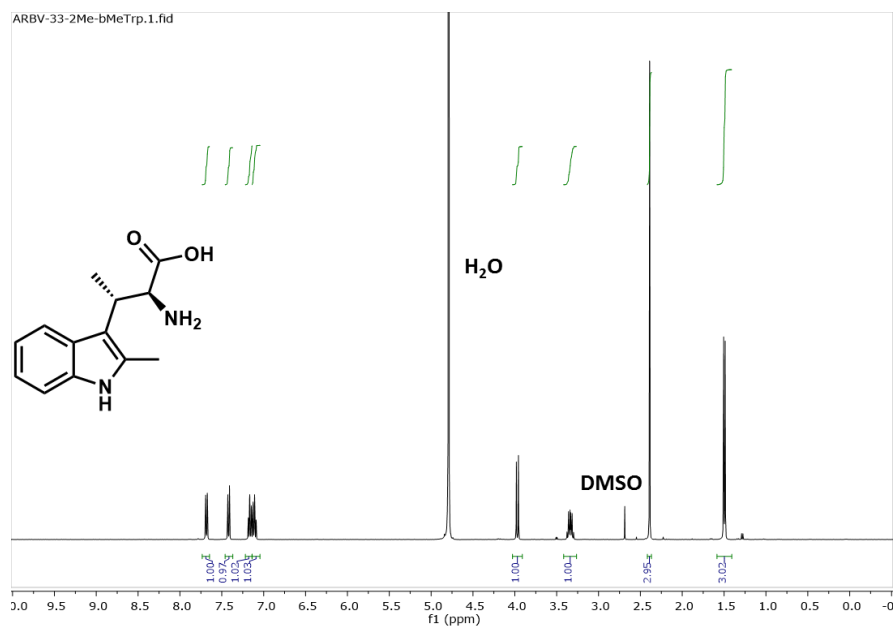
^{1}H NMR (400 MHz, Methanol-d4) δ 7.50–7.43 (m, 2H), 7.32–7.18 (m, 3H), 4.17 (d, J = 4.9 Hz, 1H), 3.69 (dt, J = 10.8, 5.6 Hz, 1H), 1.45 (s, 9H), 1.30 (d, J = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, Methanol-d_4) δ 177.1, 157.7, 136.7, 133.4, 129.8, 127.9, 80.2, 61.9, 40.4, 28.7, 20.3.
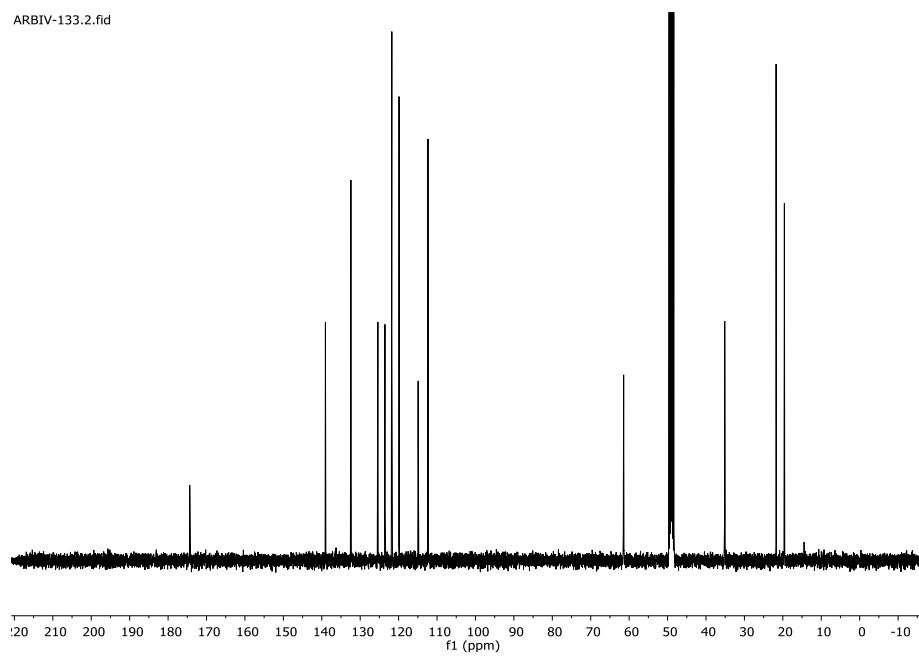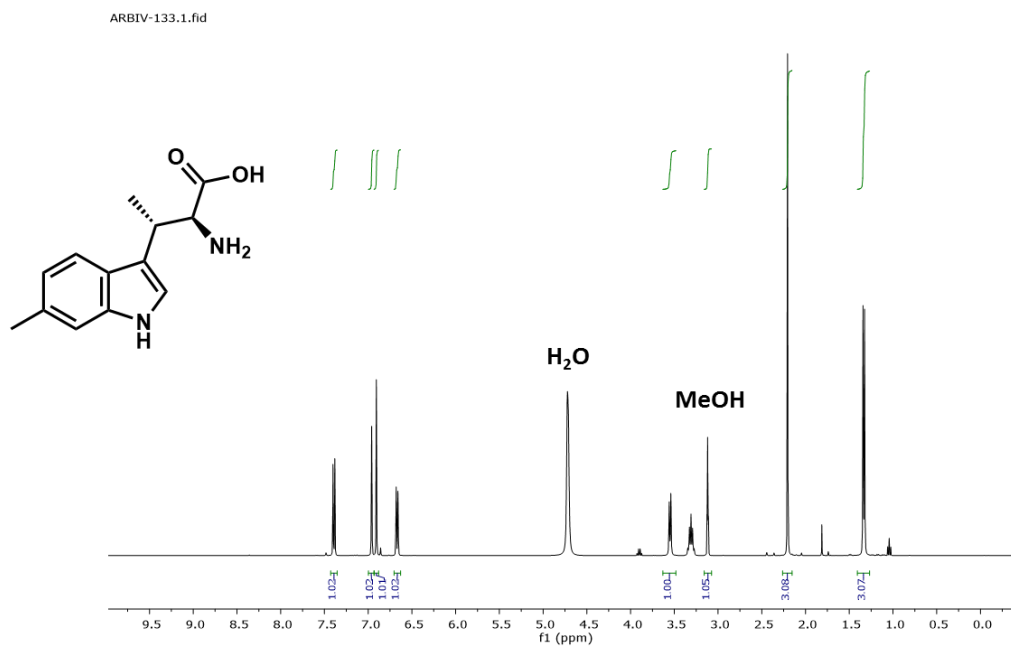
NMR Spectra
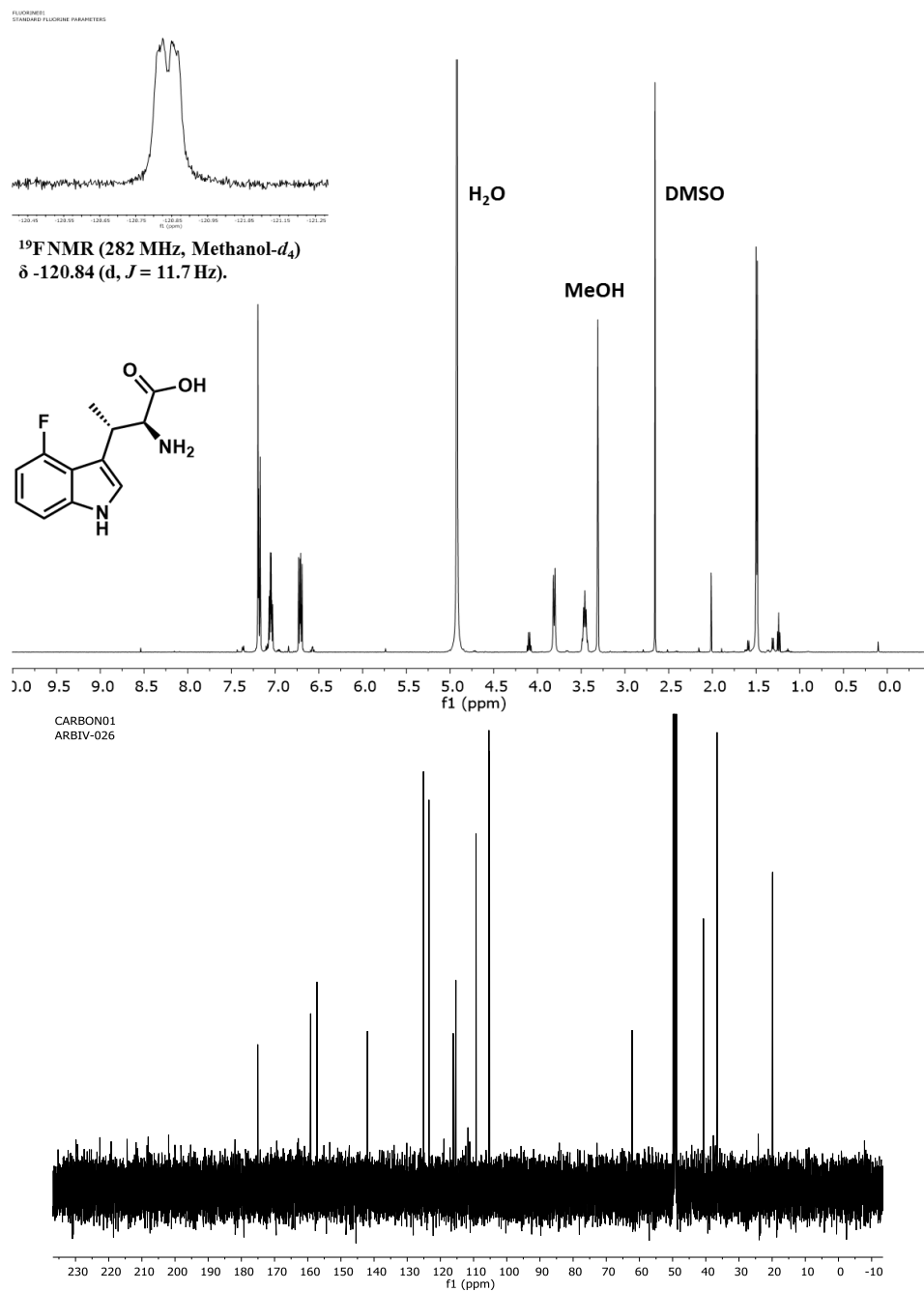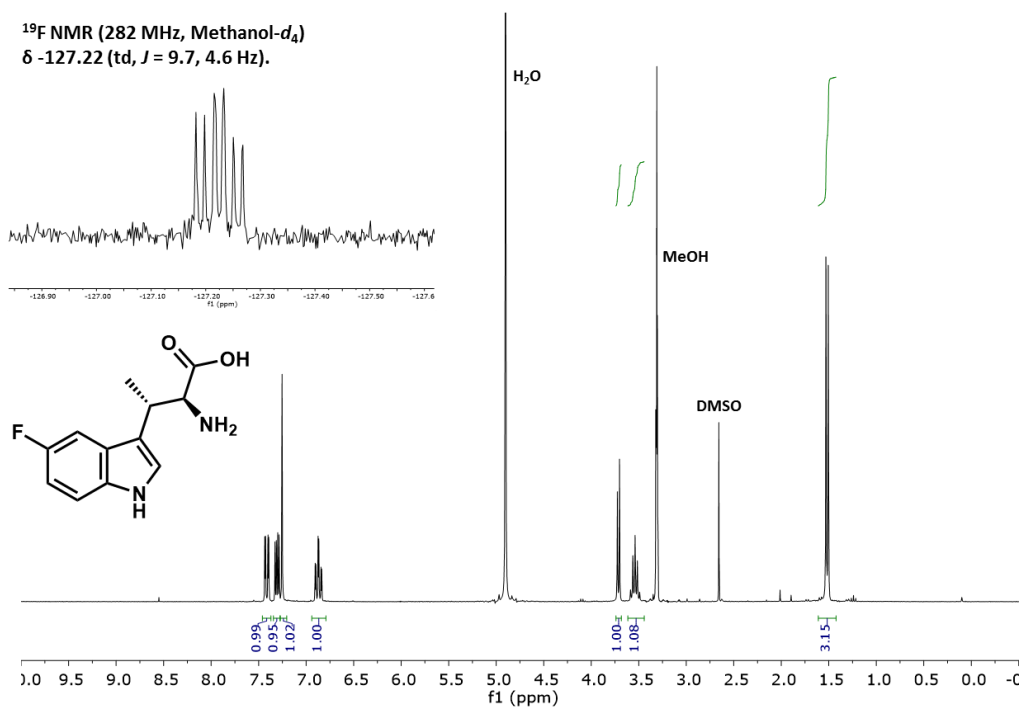
(2*S*,3*S*)-2-amino-3-(1H-indol-3-yl)butanoic acid
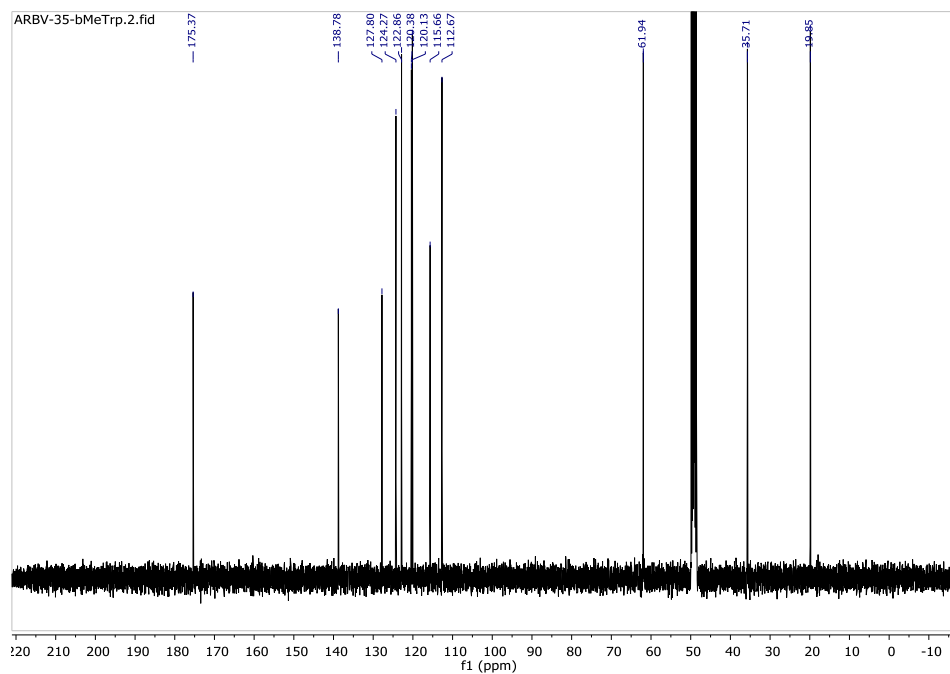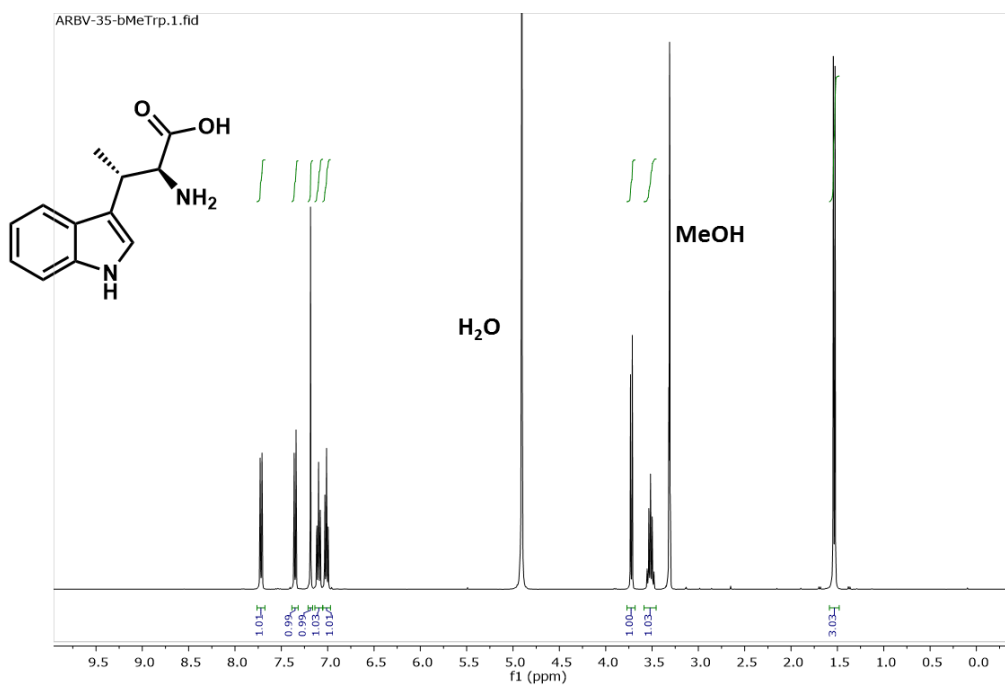
(2*S*,3*S*)-2-amino-3-(2-methyl-1H-indol-3-yl)butanoic acid
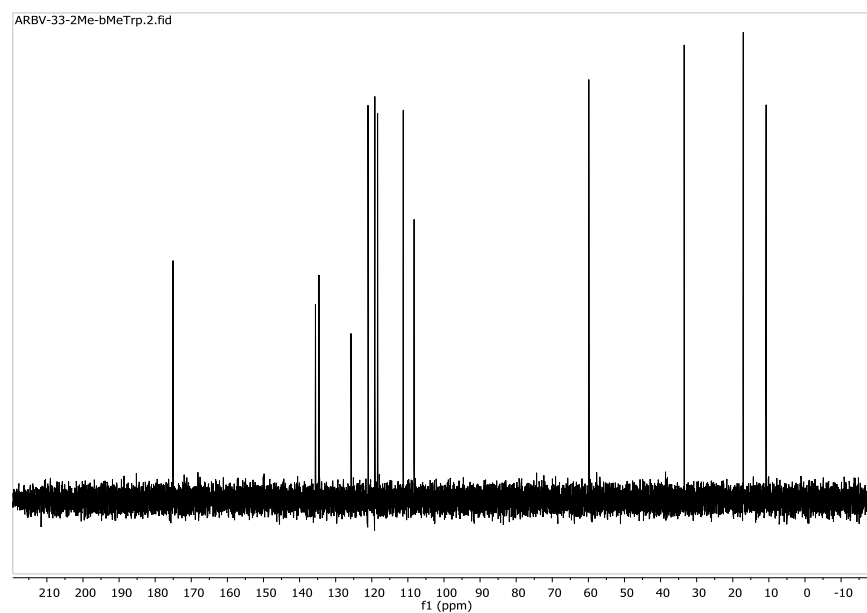
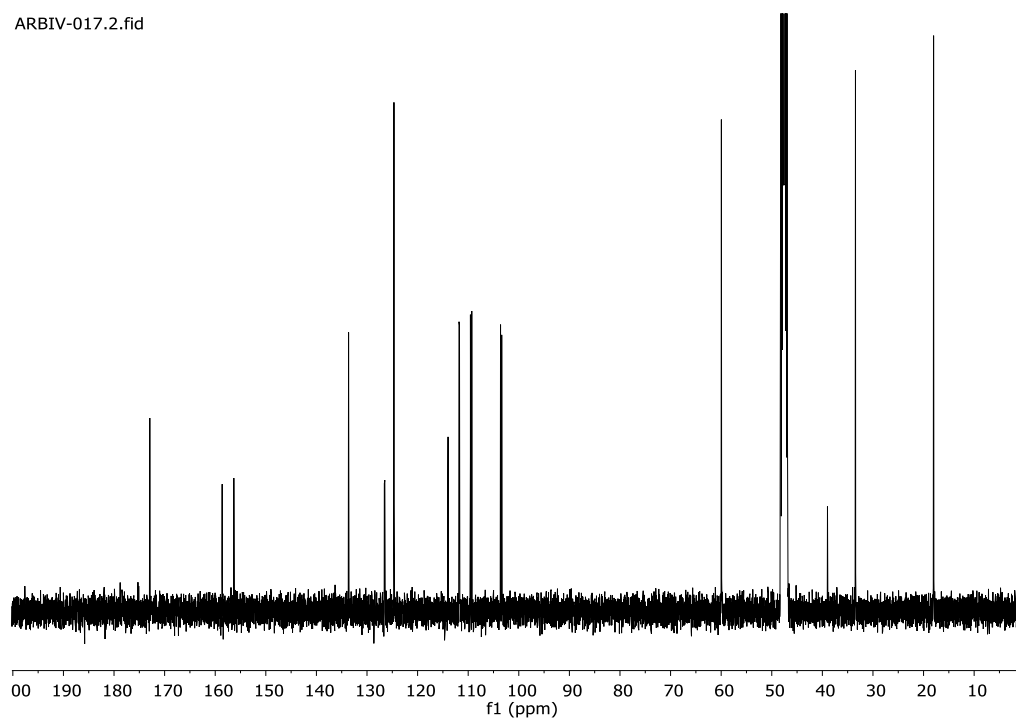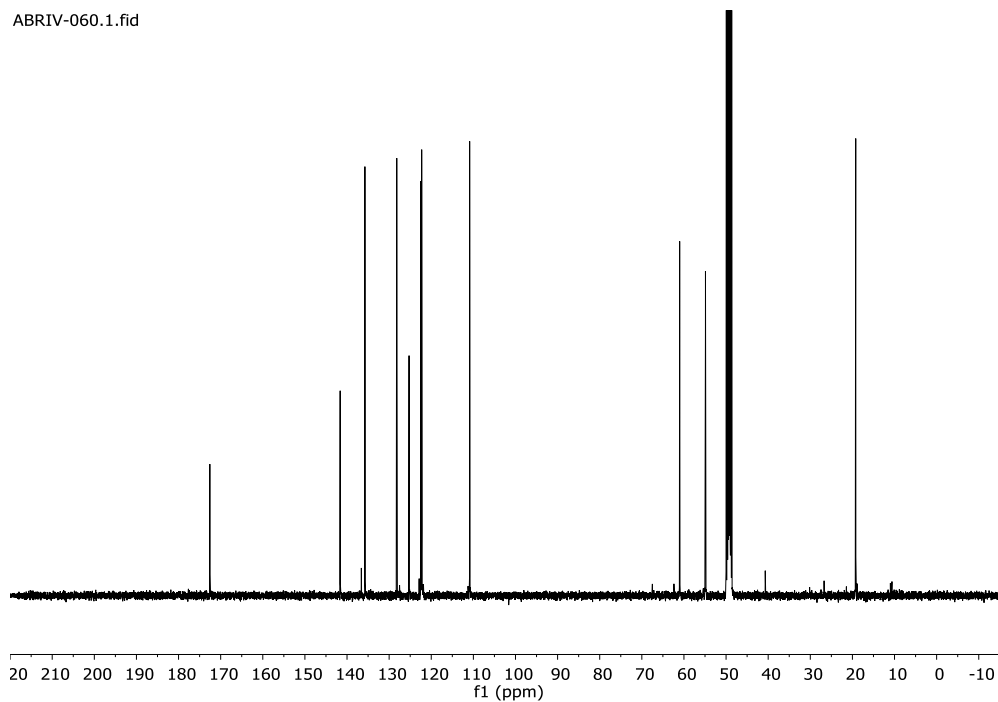## (2*S*,3*S*)-2-amino-3-(6-methyl-1H-indol-3-yl)butanoic acid.

ARBIV-133.1.fid

H₂O
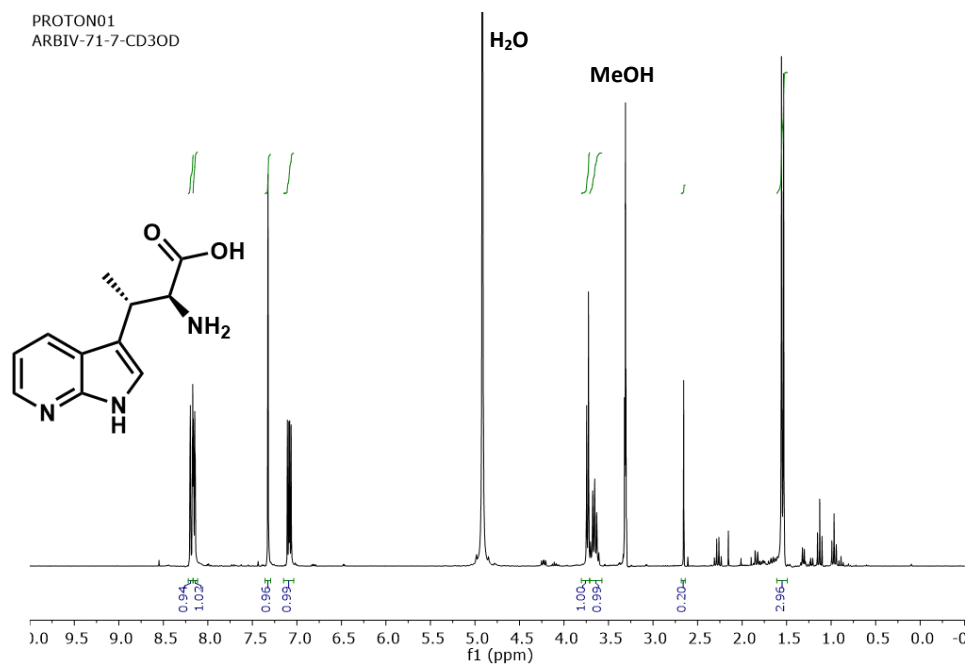
MeOH

ARBIV-133.2.fid

(2*S*,3*S*)-2-amino-3-(4-fluoro-1H-indol-3-yl)butanoic acid

FLUORINE01
STANDARD FLUORINE PARAMETERS

H$_2$O

DMSO

MeOH

^{19}F NMR (282 MHz, Methanol-*d$_4$*)
δ -120.84 (d, *J* = 11.7 Hz).

CARBON01
ARBIV-026

(2*S*,3*S*)-2-amino-3-(5-fluoro-1H-indol-3-yl)butanoic acid

¹⁹F NMR (282 MHz, Methanol-*d₄*)
δ -127.22 (td, *J* = 9.7, 4.6 Hz).

ARBIV-017.2.fid

(2*S*,3*R*)-2-amino-3-(1H-indazol-1-yl)butanoic acid

PROTON01
ARBIV-60-CD3OD

H₂O

MeOH

DMSO

ABRIV-060.1.fid

(2*S*,3*S*)-2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)butanoic acid

PROTON01
ARBIV-71-7-CD3OD

H$_2$O

MeOH

f1 (ppm)

ARBIV-071-7.1.fid

f1 (ppm)
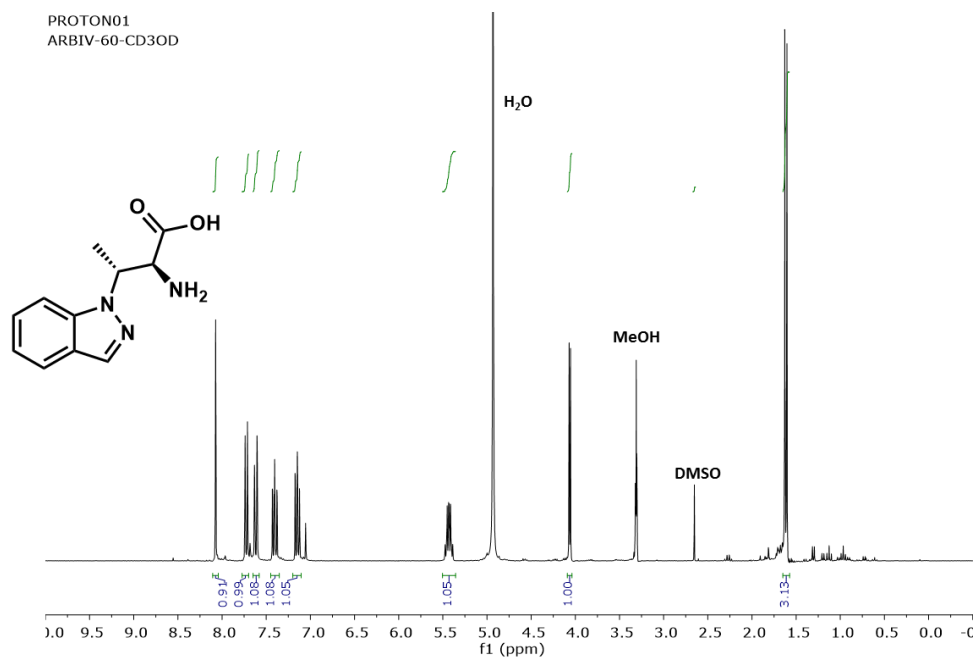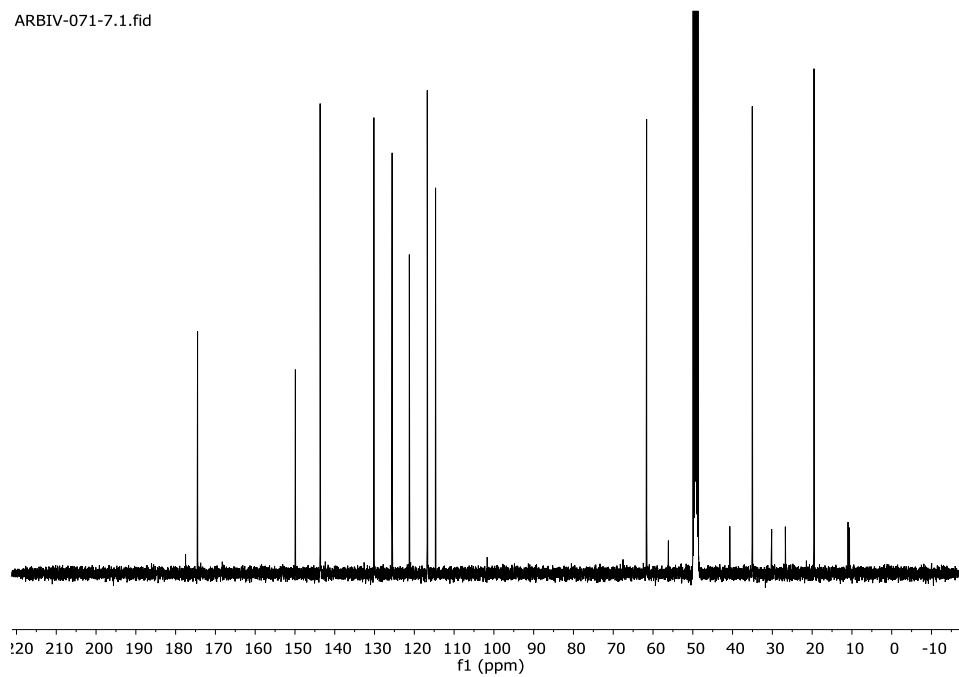
Putative: (2*S*,3*R*)-2-amino-3-(1H-pyrrolo[2,3-b]pyridin-1-yl)butanoic acid.

Peak at 5.2 ppm indicative of *N*-alkylation[1].

ARBIV-078-8.1.fid
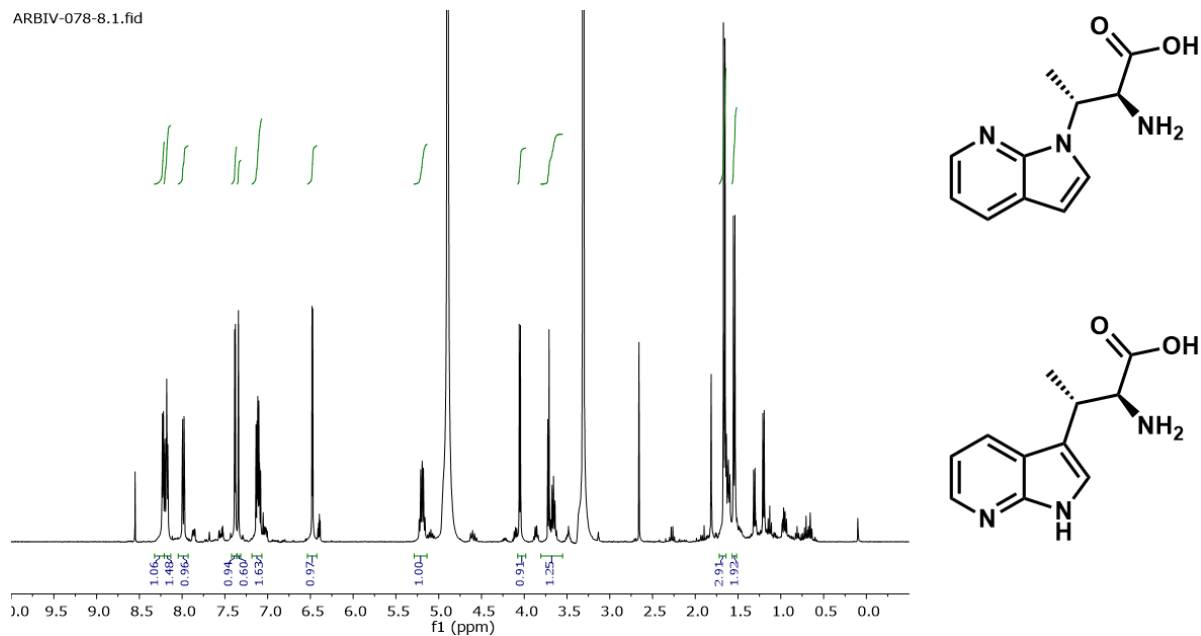
¹H NMR (400 MHz, Methanol-*d₄*) δ 8.23 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.99 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.38 (d, *J* = 3.5 Hz, 1H), 6.48 (d, *J* = 3.5 Hz, 1H), 5.20 (qd, *J* = 7.2, 5.3 Hz, 1H), 4.05 (d, *J* = 5.4 Hz, 1H), 1.66 (d, *J* = 7.3 Hz, 3H).

N-alkylated product is in a 2:1 ratio with the *C*-alkylated product.

(2R,3R)-2-amino-3-(phenylthio)butanoic acid in a 2:1 ratio with 1,2-dithiane-4,5-diol
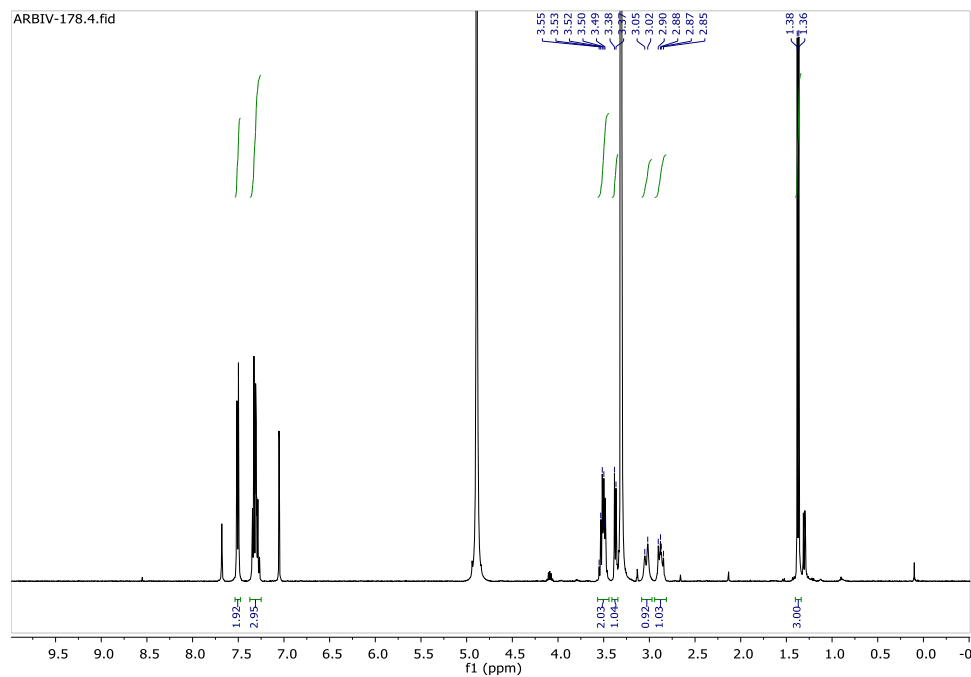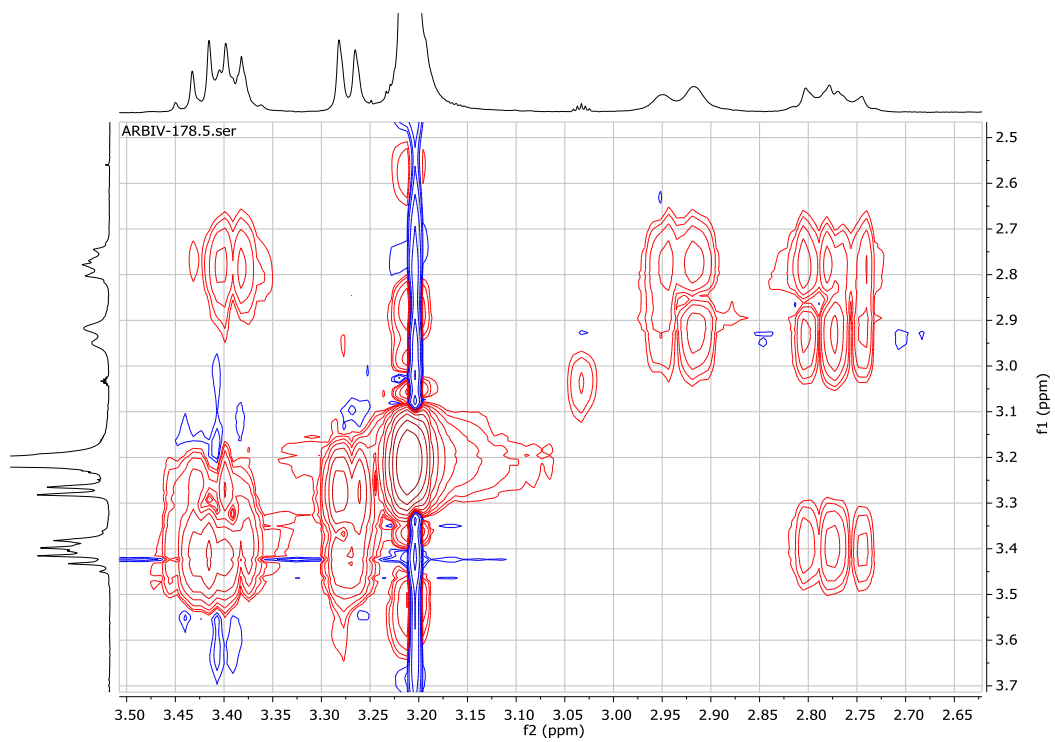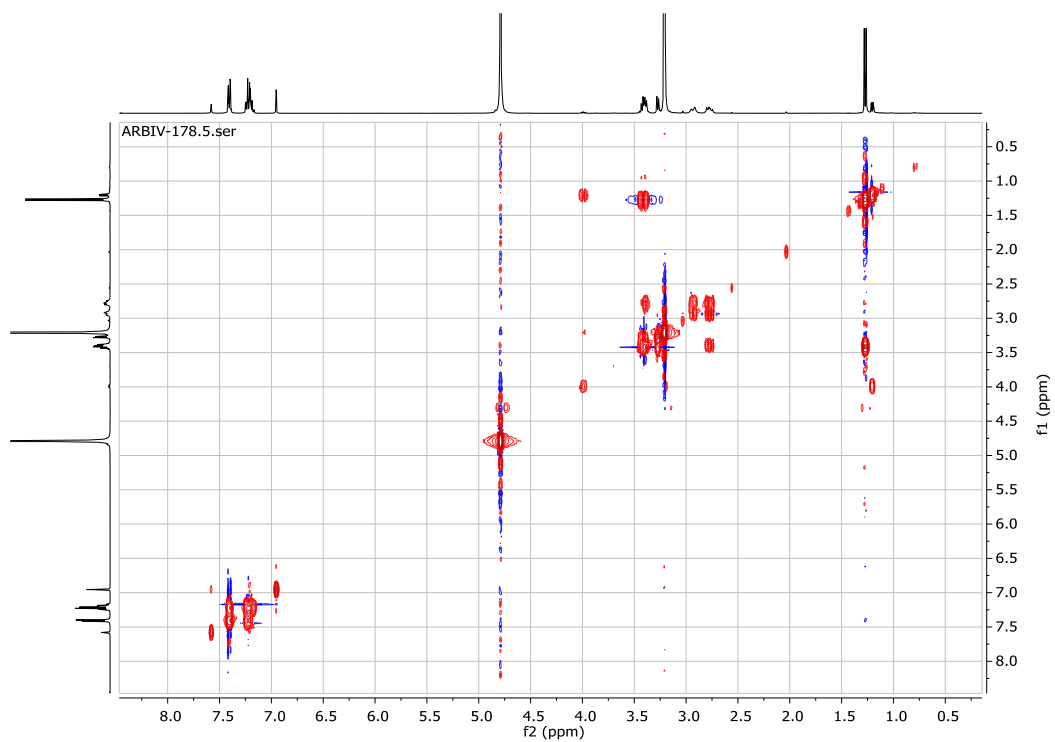

(2R,3R)-2-amino-3-(phenylthio)butanoic acid

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 – 7.48 (m, 2H), 7.36 – 7.26 (m, 3H), 3.52 (dt, J = 11.7, 5.9 Hz, 1H), 3.37 (d, J = 6.7 Hz, 1H), 1.37 (d, J = 7.0 Hz, 3H).
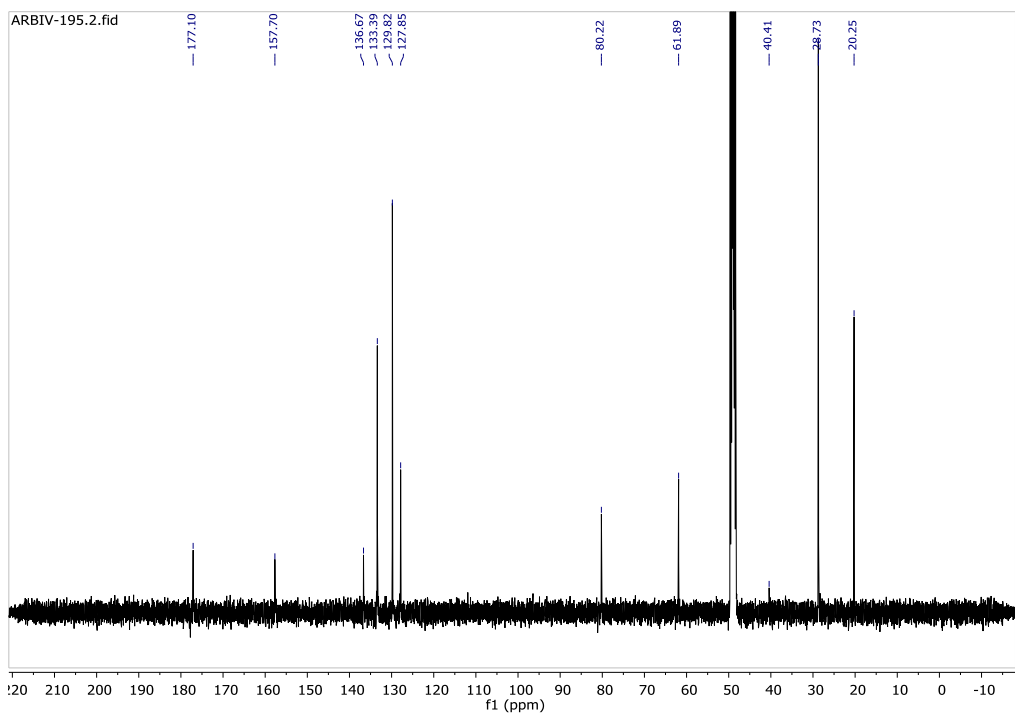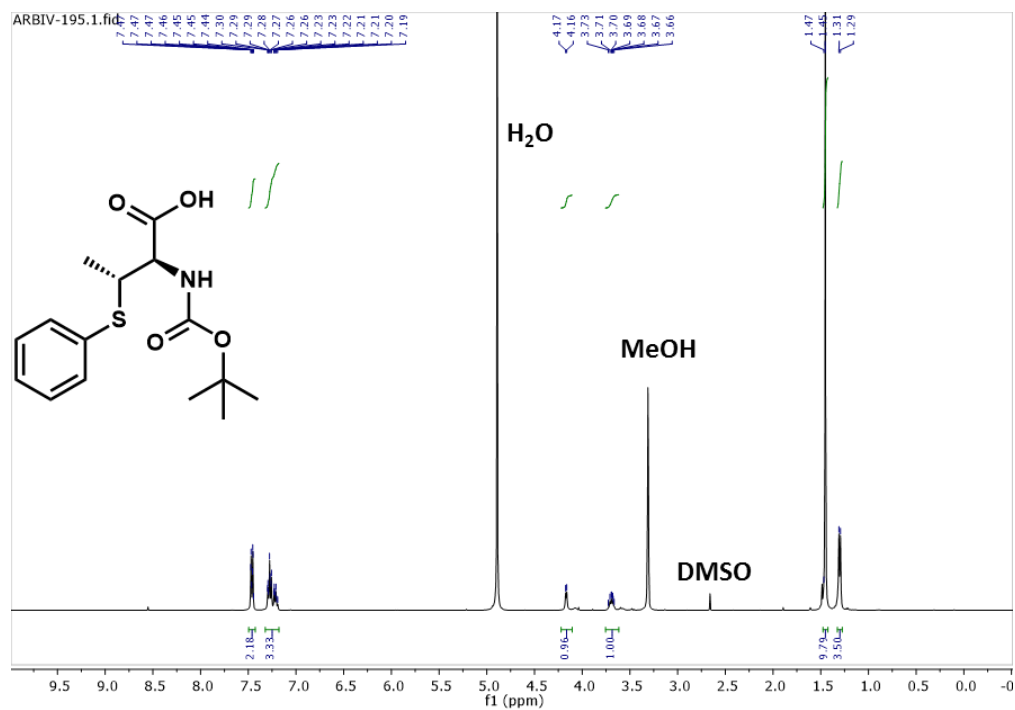
1,2-dithiane-4,5-diol

^{1}H NMR (400 MHz, Methanol-d_4) δ 3.52 (m, 1H), 3.03 (d, J = 13.0 Hz, 1H), 2.87 (dd, J = 13.3, 9.8 Hz, 1H)

(2*R*,3*R*)-2-amino-3-(phenylthio)butanoic acid in a 2:1 ratio with 1,2-dithiane-4,5-diol

(2*R*,3*R*)-2-((tert-butoxycarbonyl)amino)-3-(phenylthio)butanoic acid

ARBIV-195.1.fid

H_2O

MeOH

DMSO

ARBIV-195.2.fid

References

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