Supporting Information for

Structure-Based Discovery of Proline-derived Arginase Inhibitors with Improved Oral Bioavailability for Immuno-oncology

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X-Ray Co-Crystal Structures of Enzyme–Inhibitor Complexes

Cloning, Expression, and Purification of Protein for Crystallography. Full-length untagged hArg1 was expressed in E.coli BL21 (DE3) using superbroth media. Expression was induced with 1mM IPTG at OD600 0.8 and cells were grown for 4 hours at 37 °C. Cell pellets were resuspended in lysis buffer (10mM Tris pH 7.5, 5 mM MnCl2, 2 mM BME, 1 mg/mL lysozyme), passed through a microfluidizer 3 times at 15,000 PSI and the soluble fraction was clarified by centrifugation at 11,000 x g. Clarified lysates were heat treated at 60 °C for 20 minutes. Heat treated lysates were passed through a HiTRAP-SP column (GE). Flow through containing hArg1 was diluted to ~40 mM NaCl and reloaded on another HiTrap-SP column. hArg1 was eluted from the column using a linear gradient from 20 mM NaCl to 1 M NaCl. Pooled fractions were concentrated and loaded on a HiLoad Superdex 200 26/60 size exclusion column in 25 mM HEPES pH 7.3, 150 mM NaCl, 1 mM MnCl2. Peak fractions were analyzed by SDS-PAGE, pooled and concentrated. Purification adapted from Strickland (2012) Acta Cryst. (2011). F67, 90–93 (purification adapted from Newman *et al*.[1]).

Crystallization and Structural Analysis. X-ray diffraction-quality crystals of the hArg1 protein were obtained by hanging drop vapor diffusion at 18°C by mixing a 1:1 ratio of the protein solution (10 mg/mL preincubated with 4 mM compound at 4°C for 1hr) and a precipitant solution containing 10% MMT (pH 7.0) 0.1 M ammonium formate, 16-22% PEG 8000, and streak seeding all drops. Crystals were suspended over 0.5 mL of 0.65 M NaCl. Crystals were seen after approximately 24 hours. These crystals diffracted to nominal resolutions of 1.8-2.2 Å and belonged to the space group P21 with six copies of the hArg1 monomer in the asymmetric unit and the following approximate unit cell dimensions: a = 53

Å, b = 285 Å, and c = 67 and Å, $\alpha = \gamma = 90°$, $\beta = 90.3°$. Crystals were harvested directly from drops and plunged into LN2 prior to synchrotron data collection.

Diffraction data for compounds were collected at the IMCA-CAT Beamline at the Advanced Photon Source using a PILATUS 6M (Dectris) detector. All data was processed using autoPROC1, refined using autoBUSTER2, with manual model building using Coot3. Compound geometrical restraints were prepared using grade4. Figures were prepared using PyMOL5.

Crystallography Acknowledgements. Part of the research described in this paper used resources at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) beamline 17-ID, supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with HauptmanWoodward Medical Research Institute. This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

Table S1. Crystal Data Collection and Refinement Statistics Table

PDB code	**7KLK**	**7KLL**	**7KLM**
Compound name	**Compound 3a**	**Compound 18**	**Compound 24a**
Data collection			
Space group	*P*21	*P*21	*P*21
Cell dimensions a, b, c (Å)	53.2, 281.5, 67.1	53.1, 287.1, 67.5	53.7, 289.3, 67.4
Cell dimensions (°)	90.00, 90.06, 90.00	90.00, 90.38, 90.00	90.00, 90.58, 90.00
Resolution (Å)	67.08-1.80 (1.90-1.80)*	95.71-2.22 (2.34-2.22)	72.32-2.27 (2.40-2.27)
Rmerge†	0.06 (0.19)	0.14 (0.52)	0.11 (0.34)
CC(1/2)	1.0 (0.98)	0.99 (0.70)	0.99 (0.86)
I/σI	13.0 (4.7)	7.7 (2.3)	7.5 (2.7)
Completeness (%)	99.2 (98.0)	96.8 (87.1)	98.3 (98.9)
Redundancy	3.4 (3.2)	3.5 (3.4)	3.3 (3.4)
Refinement			
Resolution (Å)	26.35-1.80	33.82-2.31	72.32-2.27
No. reflections	179276	86998	92396
Rwork / Rfree	0.21 / 0.24	0.25 / 0.26	0.20 / 0.26
No. atoms			
Protein	14473	14454	14454
Ligand	96	96	138
Solvent	1076	1035	884
B-factors			
Protein (Å^2)	17.85	24.72	28.68
Ligands (Å^2)	18.72	21.04	26.03
Solvent (Å^2)	23.26	26.37	28.00
R.m.s. deviations			
Bond lengths (Å)	0.008	0.015	0.010
Bond angles (°)	1.01	0.92	1.18

*Values in parentheses are for highest-resolution shell.

†$R_{merge} = \sum hkl \sum i \mid Ihkl,i - < Ihkl> \mid / \sum hkl \sum i\ Ihkl,i$, where <Ihkl> is the average intensity of multiple observations of a reflection with indices hkl.

Synthesis of Arginase Inhibitor Compounds

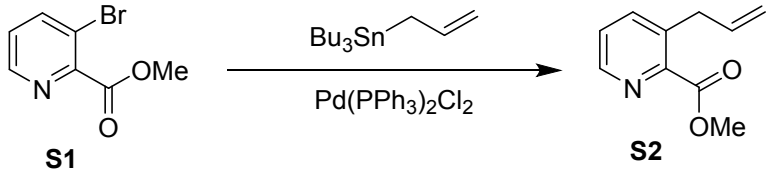
General Synthetic Chemistry Methods. All reactions were performed in dried round-bottomed flasks or glass vials with stirring under a positive pressure of argon or nitrogen as (dried by passage through a column of Drierite calcium sulfate desiccant), unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred by syringe or stainless-steel cannula. When necessary (so noted), solutions were deoxygenated by three cycles of vessel-headspace evacuation and backfilling with inert gas. Organic solutions were concentrated by rotary evaporation (10–100 mbar) at 23–30 °C. Flash-column chromatography was performed using RediSep silica gel cartridges (Teledyne Isco, Lincoln, NE, United States). Reaction monitoring was carried out by analytical liquid chromatography–mass spectrometry (LCMS).

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26; CHD_2OD, δ 3.31; HDO, δ 4.79; DMSO-*d*5, δ 2.50). Data are reported as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, dd = doublet of doublets, td = triplet of doublets, m = multiplet, br = broad, app = apparent), integration, and coupling constant (*J*) in Hertz (Hz). LCMS was performed using a C18 column (30 × 2 mm, 2 μm particle size, beginning with 3% acetonitrile–water containing 0.05% TFA, grading to 98% acetonitrile–water containing 0.05% TFA over 2.3 min, with a flow rate of 0.9 mL/min), with tandem UV detection (254 or 215 nm) and electrospray ionization quadrupole mass spectrometry (ESI-MS).

For clarity, intermediates that have not been assigned numbers in the preceding text are numbered sequentially in the following section, beginning with **S1**. The purity of all compounds screened in biological assays was determined by LCMS (C18 mobile phase, eluting with a gradient of acetonitrile–water containing 20 mM HFBA and 0.1% TFA, with UV detection at 215 nm), and was ≥ 95% in every case, unless otherwise noted.

Synthesis of compound 3, 3a

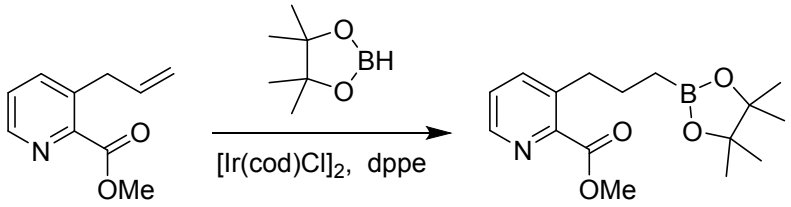
Step 1: methyl 3-allylpicolinate



Allyltributylstannane (31 g, 94 mmol) was added to a mixture of methyl 3-bromopicolinate (18 g, 82 mmol) and dichlorobis(triphenylphosphine)palladium(II) (5.5 g, 8.2 mmol) in DMF (4.0 mL) under nitrogen. The mixture was degassed and backfilled with nitrogen three times. The solution was heated to 120 °C for 2 h and saturated aqueous potassium fluoride (100 mL) was added and stirred for 1 h. The reaction mixture was filtered through CELITE and concentrated under reduced pressure. EtOAc and water were added and the organic phase was separated and dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (EtOAc in hexanes) to give methyl 3-allylpicolinate (13.3 g, 82% yield) as pale yellow oil. LCMS (C10H12NO2+) (ES, m/z): 178.0 [M+H]+.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.55 (d, J = 4.4 Hz, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.36 (dd, J = 4.6, 7.7 Hz, 1H), 6.03 - 5.87 (m, 1H), 5.12 - 4.94 (m, 2H), 3.95 (d, J = 0.9 Hz, 3H), 3.71 (d, J = 6.6 Hz, 2H).

Step 2: methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)picolinate

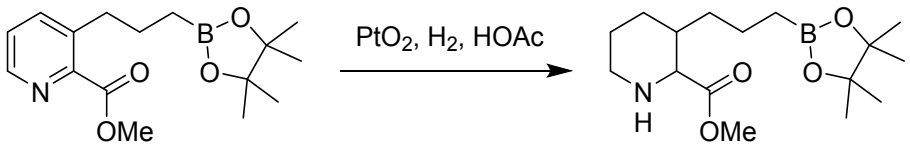


S2 **S3**

A solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (54.0 mL, 372 mmol), chloro(1,5-cyclooctadiene)iridium (I) dimer (2.5 g, 3.7 mmol) and 1,2-bis(diphenylphosphino)ethane (2.1 g, 5.2 mmol) in anhydrous DCM (500 mL) was bubbled with a stream of nitrogen for 3 min. The mixture was stirred at 12 °C for 20 min, and then treated with methyl 3-allylpicolinate (13 g, 75 mmol). The resulting mixture was stirred at 12 °C for 16 h under nitrogen and was directly purified by silica gel column chromatography (EtOAc in hexanes) to give methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)picolinate (33 g, 29.0% yield) as a brown oil. LCMS (C16H25BNO4+) (ES, m/z): 306.1 [M+H]+.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.53 (dd, J = 1.4, 4.5 Hz, 1H), 7.68 - 7.59 (m, 1H), 7.39 - 7.31 (m, 1H), 4.01 - 3.94 (m, 3H), 2.96 - 2.90 (m, 2H), 1.73 (td, J = 8.0, 15.8 Hz, 2H), 1.24 (s, 12H), 0.85 (t, J = 7.9 Hz, 2H).

Step 3: methyl3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-2-carboxylate

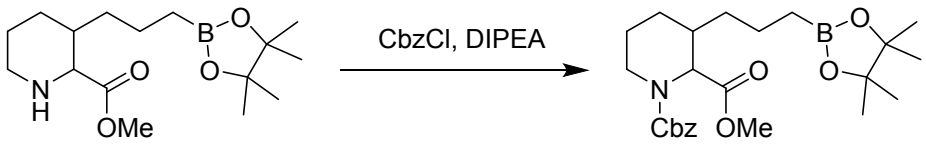


S3 **S4**

Platinum (IV) oxide (0.45 g, 2.0 mmol) was added to a solution of methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)picolinate (6.1 g, 4.0 mmol) in EtOH (10

mL) and HOAc (20 mL) at 10 °C. The solution was degassed with hydrogen three times, and was stirred under 4.5 MPa of hydrogen at 50 °C for 48 h. The mixture was concentrated under reduced pressure and the resulting residue was dissolved in EtOAc, and neutralized to pH 7 by TEA, and the resulting mixture was filtered and concentrated under reduced pressure to give crude methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-2-carboxylate (5.3 g, 85.5% yield) as yellow oil, which was used in the next step without purification. LCMS (C16H31BNO4+) (ES, m/z): 311.8 [M+H]+.

Step 4: 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate



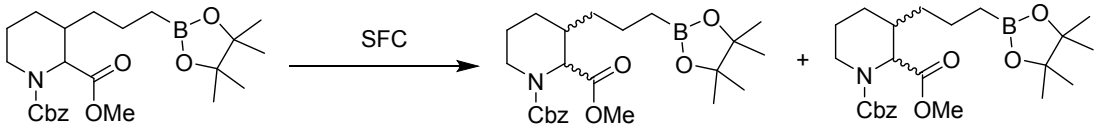
S4 **S5**

Ethyldiisopropylamine (5.3 mL, 32 mmol) was added to a solution of methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-2-carboxylate (5.0 g, 16 mmol) in DCM (30 mL) followed by dropwise addition of benzyl chloroformate (2.487 mL, 17.67 mmol) under nitrogen atmosphere at 0 °C. The mixture was stirred at 20 °C for 16 h then concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate as pale yellow oil (2.8g, 35.2% yield, cis isomer base on chemistry). LCMS (C24H37BNO6+) (ES, m/z): 446.3 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.39 - 7.30 (m, 5H), 5.26 - 5.03 (m, 2H), 4.95 - 4.70 (m, 1H), 4.00 (m, J = 13.6 Hz, 1H), 3.72 - 3.59 (m, 3H), 3.54 - 3.13 (m, 1H), 1.69

(m, 3H), 1.48 (m, 4H), 1.39 - 1.30 (m, 1H), 1.24 (s, 13H), 0.81 - 0.72 (m, 2H).

Step 5: 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate

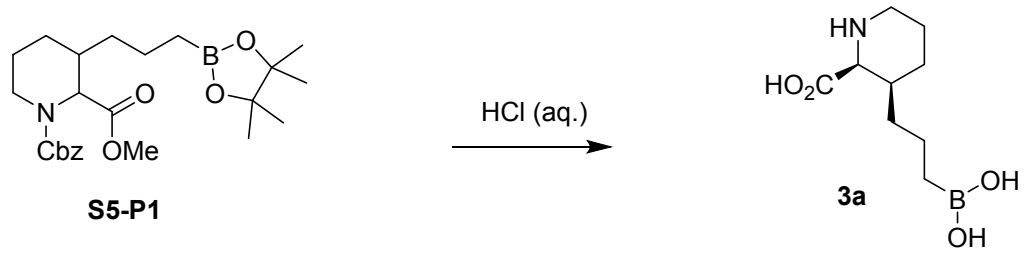


S5 **S5-P1** **S5-P2**

1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate (1.5 g, 3.4 mmol) was resolved by Chiral-SFC [Column: OD (250 mm*50 mm,10 um), Mobile phase: A: CO2, B: IPA (0.1% NH3•H2O), Gradient: 15% of B in 3.5 min, and hold 15% for 1 min, Flow Rate (mL/min) 180, Column temperature: 40 °C] to give 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate (P1, tr = 2.736 min) as the first eluting peak as a pale yellow oil (448.76 mg, 29.3% yield), and 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate (P2, tr = 2.985 min) as the second eluting peak as a pale yellow oil (457.73 mg, 29.6% yield). P1 LCMS (C24H37BNO6+) (ES, m/z): 446.4 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.41 7.28 (m, 5H), 5.27 5.05 (m, 2H), 4.94 4.70 (m, 1H), 4.09 3.91 (m, 1H), 3.76 3.56 (m, 3H), 3.42 3.15 (m, 1H), 1.70 1.62 (m, 3H), 1.48 (m, 4H), 1.40 1.30 (m, 1H), 1.23 (br s, 13H), 0.81 0.72 (m, 2H). P2 LCMS (C24H37BNO6+) (ES, m/z): 446.4 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.42 7.28 (m, 5H), 5.26 5.03 (m, 2H), 4.94 4.72 (m, 1H), 4.09 3.93 (m, 1H), 3.71 3.60 (m, 3H), 3.40 3.15 (m, 1H), 1.79 1.64 (m, 3H), 1.48 (m, 4H), 1.40 1.29 (m, 1H), 1.23 (br s, 13H),

0.83 0.70 (m, 2H).

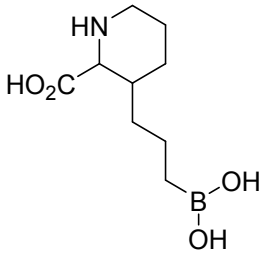
Step 6: (2R,3S)-3-[3-(dihydroxyboranyl)propyl]piperidine-2-carboxylic acid



S5-P1 **3a**

12 N HCl in water (2.5 mL) was added to a stirred suspension of 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate (P1, 425 mg, 0.95 mmol) in water (2.5 mL) in one portion at room temperature. The reaction mixture was heated to 80 °C with stirring overnight, and then cooled to room temperature. The mixture was diluted with water, filtered through a 0.2 um filter and lyophilized to afford (2R,3S)-3-[3-(dihydroxyboranyl)propyl]piperidine-2-carboxylic acid as an HCl salt. HRMS (ESI) calcd for C9H17BNO3 $[M\text{-}H_2O+H]^+$, 198.1303; found, 198.1309. 1H-NMR: (400 MHz, D2O) δ 4.07 (d, J = 3.8 Hz, 1H), 3.41 (d, J = 12.5 Hz, 1H), 2.99 (td, J = 12.5, 3.8 Hz, 1H), 2.41-2.33 (m, 1H), 1.91 (d, J = 12.5 Hz, 1H), 1.81-1.60 (m, 3H), 1.58-1.42 (m, 2H), 1.39-1.26 (m, 1H), 1.21-1.10 (m, 1H), 0.86-0.68 (m, 2H). 13C NMR (151 MHz, D2O) δ 171.27, 60.34, 43.88, 32.95, 28.15, 23.93, 21.25, 16.66, 13.77.

3: 3-[3-(dihydroxyboranyl)propyl]piperidine-2-carboxylic acid



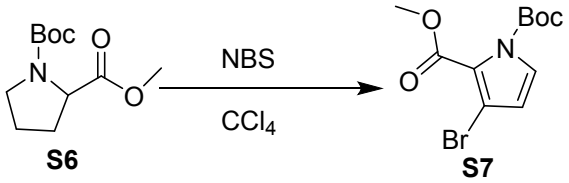
3 was made from **S5** using the same procedure as a white solid. LCMS

(C9H17BNO3+) (ES, m/z): 198.1 [M-H2O+H]+.

1H-NMR (400 MHz, DEUTERIUM OXIDE) δ 3.93 (d, J = 3.5 Hz, 1H), 3.24 (d, J = 12.7 Hz, 1H), 2.90 - 2.74 (m, 1H), 2.30 - 2.13 (m, 1H), 1.74 (d, J = 11.8 Hz, 1H), 1.64 - 1.44 (m, 3H), 1.33 (t, J = 9.9 Hz, 2H), 1.15 (s, 1H), 0.99 (s, 1H), 0.72 - 0.46 (m, 2H).

Synthesis of compound 4, 4a

Step 1: 1-tert-butyl 2-methyl 3-bromo-1H-pyrrole-1,2-dicarboxylate

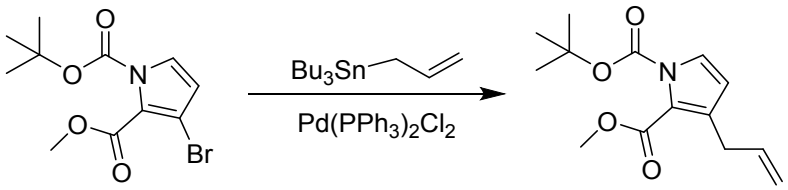


S6 **S7**

To a 10 L round-bottom flask under N_2 were charged 1-tert-butyl 2-methyl pyrrolidine-1,2-dicarboxylate (35 g, 153 mmol), NBS (68 g, 382 mmol), and CCl_4 (7.0 L), and the reaction mixture was stirred at 85 °C for 3 h. (The same reaction was repeated multiple times to obtain more desired products.) The resulting mixture was concentrated, and the residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 3-bromo-1H-pyrrole-1,2-dicarboxylate (9.2 g, 20% yield) as a red oil. LCMS (C7H7BrNO4+) (ES, m/z): 247.8 [M-C4H8+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.21 (d, J = 3.31 Hz, 1H), 6.23 (d, J = 3.31 Hz, 1H), 3.85-3.95 (m, 3H), 1.55 (s, 9H).

Step 2: 1-tert-butyl 2-methyl 3-allyl-1H-pyrrole-1,2-dicarboxylate

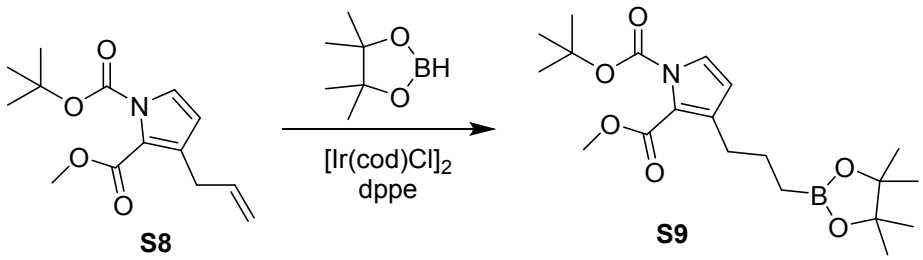


S7 **S8**

Bis(triphenylphosphine)-palladium (II) dichloride (212 g, 0.30 mol) was added to a mixture of 1-tert-butyl 2-methyl 3-bromo-1H-pyrrole-1,2-dicarboxylate (920 g, 3.0 mol), and allyltributylstannane (2521 g, 24 mmol) in DMF (9.2 L) under N_2, and the reaction mixture was stirred at 94 °C for 4 h under nitrogen. The mixture was quenched with 5% aqueous potassium fluoride, and stirred at rt for 1 h, then extracted with EtOAc. The combined organic phase was dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 3-allyl-1H-pyrrole-1,2-dicarboxylate as a red oil (800 g, 100%). LCMS (C10H12NO4+) (ES, m/z): 210.0 [M-C4H8+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.21 (d, J = 3.09 Hz, 1H), 6.06 (d, J = 3.09 Hz, 1H), 5.89 (tdd, J = 6.53, 10.17, 16.90 Hz, 1H), 4.99-5.10 (m, 2H), 3.84 (s, 3H), 3.35 (d, J = 6.62 Hz, 2H), 1.55 (s, 9H).

Step 3: 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1H-pyrrole-1,2-dicarboxylate



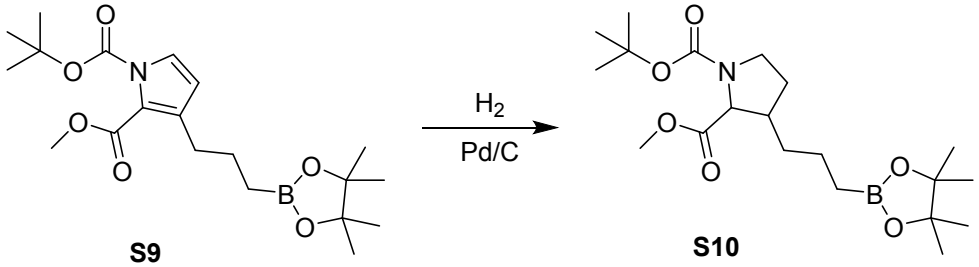
S8 **S9**

1-Tert-butyl 2-methyl 3-allyl-1H-pyrrole-1,2-dicarboxylate (800 g, 3.0 mol) was

added to a solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1158 g, 9.0 mol), chloro(1,5-cyclooctadiene)iridium (I) dimer (142 g, 211 mmol), and bis(diphenylphosphino)ethane (116 g, 302 mmol) in dry DCM (16 L) under N_2, and the reaction mixture was stirred at rt overnight. The mixture was quenched with water/ice and extracted with DCM, and the combined organic phase was dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1H-pyrrole-1,2-dicarboxylate (420 g, 35%) as a colorless oil. LCMS (C16H25BNO6+) (ES, m/z): 338.1 [M-C4H8+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.18 (d, J = 3.09 Hz, 1H), 6.06 (d, J = 3.09 Hz, 1H), 3.83 (s, 3H), 2.56 (t, J = 7.61 Hz, 2H), 1.65 (td, J = 7.97, 15.60 Hz, 2H), 1.55 (s, 9H), 1.23 (s, 12H), 0.80 (t, J = 8.05 Hz, 2H).

Step 4: 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



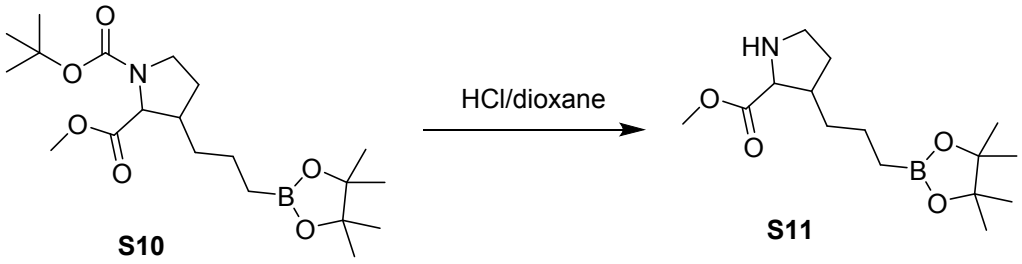
S9 **S10**

Palladium on carbon (10 wt%, 8.0 g) was added to a solution of 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1H-pyrrole-1,2-dicarboxylate (20 g, 5.6 mmol) in MeOH (800 mL) under N_2, and the reaction mixture was degassed and backfilled with hydrogen (three times), and stirred under hydrogen (45-50 psi) at 50 ^oC for 48 h. The mixture was filtered and the filtrate was concentrated under reduced pressure, and the

residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (17 g, 82% yield) as a colorless oil. LCMS (C15H29BNO4+) (ES, m/z): 297.9 [M-CO2C4H8+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 4.18-4.32 (m, 1H), 3.68-3.70 (m, 3H), 3.58-3.67 (m, 1H), 3.20-3.32 (m, 1H), 2.24-2.41 (m, 1H), 1.97 (td, J = 6.28, 12.13 Hz, 1H), 1.63-1.79 (m, 1H), 1.42-1.49 (m, 6H), 1.39 (s, 6H), 1.23 (s, 12H), 1.05-1.17 (m, 1H), 0.72-0.82 (m, 2H).

Step 5: 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate

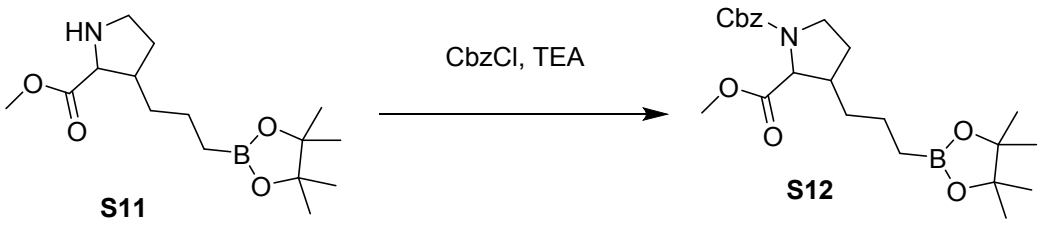


HCl in dioxane (4.0 M, 3.5 L, 14 mol) was added to a solution of 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (350 g, 881 mmol) in DCM (3.5 L) under N_2 at rt, and the reaction mixture was stirred for 3 h at rt. The mixture was concentrated under reduced pressure to give methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate (300 g, 100% yield) as a red oil, which was used in next step without further purification. LCMS (C15H29BNO4+) (ES, m/z): 298.1 [M +H]+.

1H-NMR (400 MHz, CDCl3) δ 4.39 (br s, 1H), 3.83 (s, 3H), 3.60-3.67 (m, 1H), 3.47 (br s, 1H), 2.59 (br s, 1H), 2.19 (br s, 1H), 1.75-1.83 (m, 1H), 1.62 (br s, 2H), 1.46 (br d, J =

7.45 Hz, 2H), 1.23 (s, 12H), 0.78 (br s, 2H).

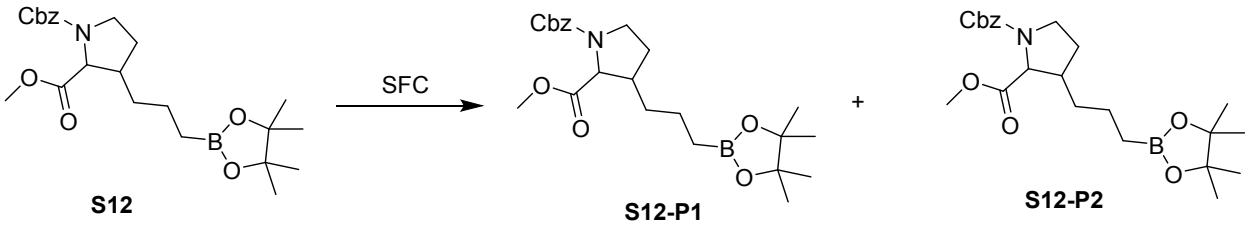
Step 6: 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Benzyl chloroformate (373 g, 2.2 mol) was added dropwise to a stirred solution of methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate (260 g, 874 mmol) and triethylamine (443 g, 4.4 mol) in dry DCM (5.2 L) at 0 °C, and the reaction mixture was stirred overnight at rt. The mixture was quenched with saturated aqueous NH4Cl and extracted with DCM, and the combined organic phase was washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (212 g, 56% yield) as a light brown oil. LCMS (C23H35BNO6+) (ES, m/z): 432.1 [M+H]+.

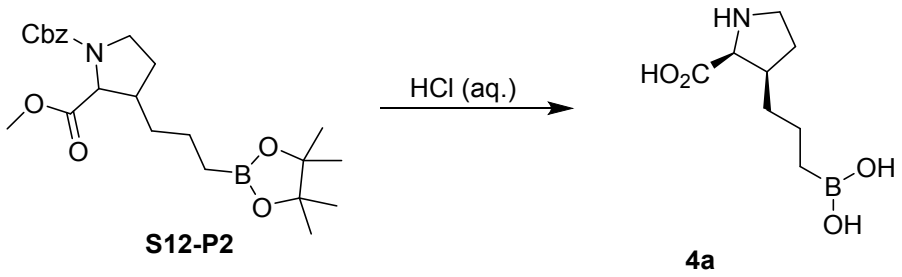
1H-NMR: (400 MHz, Chloroform-d): δ 7.42 – 7.28 (m, 4H), 5.19 (dd, J = 12.5, 2.7 Hz, 1H), 5.08 (dd, J = 22.4, 12.5 Hz, 1H), 4.37 (dd, J = 16.0, 8.3 Hz, 1H), 3.87 – 3.70 (m, 1H), 3.74 (s, 1H), 3.59 (s, 1H), 3.38 (qd, J = 10.3, 6.8 Hz, 1H), 2.39 (dddd, J = 13.8, 10.5, 8.3, 5.3 Hz, 1H), 2.09 – 1.98 (m, 1H), 1.75 (dtdd, J = 19.5, 12.5, 10.5, 8.9 Hz, 1H), 1.56 – 1.39 (m, 1H), 1.26 (d, J = 2.0 Hz, 12H), 1.19 – 1.07 (m, 1H), 0.90 – 0.72 (m, 2H).

Step 7: 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate R-1 and R-2.



1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (3.3 g, 7.6 mmol) was resolved by chiral-SFC [Column: AD (250 mm * 50 mm,10 um), Mobile phase: A: CO2, B: EtOH (0.1% NH3•H2O), Gradient: 25% of B in 3.5 min and hold 25% for 1 min, Flow Rate (mL/min) 200, Column temperature: 40 °C] to give 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (P-1, t_R = 2.355 min) as the first eluting peak, and 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (P-2, t_R = 2.869 min) as the second eluting peak. P-1 LCMS (C23H35BNO6+) (ES, m/z): 432.1 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.39-7.25 (m, 5H), 5.20-4.99 (m, 2H), 4.34 (dd, J = 8.4, 15.7 Hz, 1H), 3.73-3.56 (m, 3H), 3.41-3.30 (m, 1H), 2.41-2.28 (m, 1H), 2.07-1.96 (m, 1H), 1.87-1.62 (m, 2H), 1.54-1.39 (m, 3H), 1.23 (d, J = 1.5 Hz, 12H), 1.16-1.05 (m, 1H), 0.83-0.73 (m, 2H). P-2 LCMS (C23H35BNO6+) (ES, m/z): 432.1 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.38-7.25 (m, 5H), 5.22-4.96 (m, 2H), 4.34 (dd, J = 8.4, 15.7 Hz, 1H), 3.72-3.56 (m, 3H), 3.35 (dq, J = 7.0 , 10.2 Hz, 1H), 2.42-2.27 (m, 1H), 2.03-1.97 (m, 1H), 1.81-1.61 (m, 2H), 1.51-1.41 (m, 3H), 1.23 (d, J = 1.5 Hz, 12H), 1.17-1.06 (m, 1H), 0.82-0.71 (m, 2H).

Step 8: (3R)-3-[3-(dihydroxyboranyl)propyl]-L-proline.

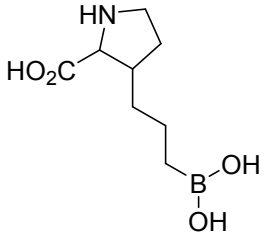


S12-P2 **4a**

12 N HCl in water (3.0 mL) was added to the stirred suspension of 1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)piperidine-1,2-dicarboxylate (R-2, 578 mg, 1.3 mmol) in water (3.0 mL) at room temperature, and the reaction mixture was stirred at 80 °C overnight. The mixture was diluted with water, filtered through a 0.20 µm filter and lyophilized to afford (3R)-3-[3-(dihydroxyboranyl)propyl]-L-proline as an HCl salt. LCMS (C8H15BNO3+) (ES, m/z): 184.1 [M-H2O+H]+.

1H-NMR (500 MHz, D2O) δ 4.30 (d, J = 7.9 Hz, 1H), 3.54-3.45 (m, 1H), 3.30 (dt, J = 12, 7.9 Hz, 1H), 2.64-2.54 (m, 1H), 2.18 (dq, J = 13, 6.7 Hz, 1H), 1.79 (dq, J = 13, 7.8 Hz, 1H), 1.50-1.33 (m, 3H), 1.25-1.16 (m, 1H), 0.82-0.68 (m, 2H).

Compound 4: 3-[3-(dihydroxyboranyl)propyl]-proline

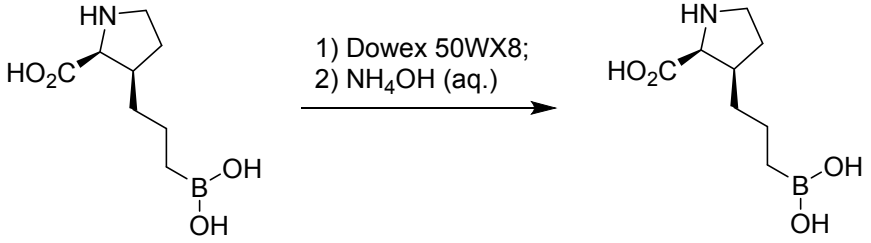


Compound 4 was made from 1-tert-butyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (**S10**) using the same procedure as Compound **4a**. 184.1 [M-H2O+H]+

1H NMR (400 MHz, DEUTERIUM OXIDE) δ 4.16 (d, J = 7.89 Hz, 1H), 3.29-3.43 (m, 1H), 3.16 (td, J = 7.89, 11.84 Hz, 1H), 2.37-2.51 (m, 1H), 2.04 (qd, J = 6.61, 13.48 Hz,

1H), 1.58-1.71 (m, 1H), 1.17-1.40 (m, 3H), 0.99-1.13 (m, 1H), 0.53-0.69 (m, 2H).

Step 9: (3R)-3-[3-(dihydroxyboranyl)propyl]-L-proline (free base).



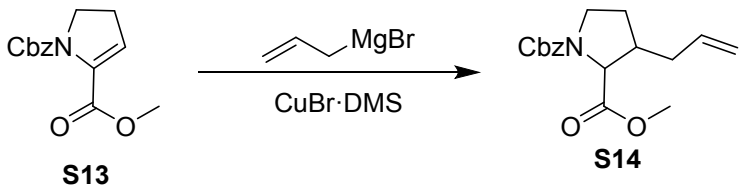
4a **4a-free base**

(3R)-3-[3-(dihydroxyboranyl)propyl]-L-proline (HCl salt, 70 mg) was purified on 2.2 g of Dowex 50WX8 acidic resin (washed with water until pH neutral, then eluted with 2N aqueous ammonium hydroxide) to afford (3R)-3-[3-(dihydroxyboranyl)propyl]-L-proline as a free base. HRMS (ESI) calcd for C8H15BNO3 $[M-H_2O+H]^+$, 184.1147; found, 184.1154.

1H-NMR (500 MHz, D2O) δ 4.05 (d, J = 7.9 Hz, 1H), 3.48 (dt, J = 12, 6.7 Hz, 1H), 3.26 (dt, J = 12, 7.9 Hz, 1H), 2.59-2.46 (m, 1H), 2.15 (dq, J = 13, 6.7 Hz, 1H), 1.79 (dq, J = 13, 7.8 Hz, 1H), 1.54-1.31 (m, 3H), 1.24-1.12 (m, 1H), 0.85-0.68 (m, 2H). 13C NMR (151 MHz, D2O) δ 173.19, 65.09, 44.40, 39.93, 31.48, 28.74, 22.25, 15.10.

Synthesis of compound 4b

Step 1: 1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate

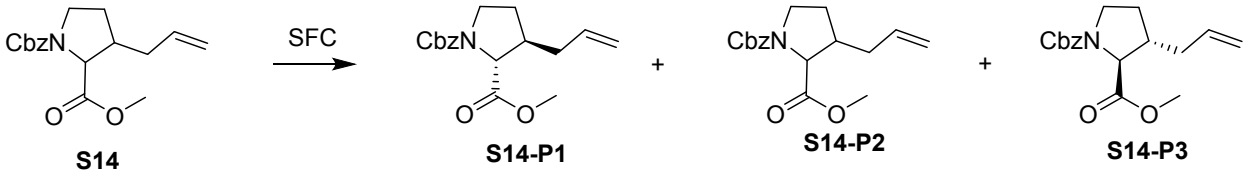


S13 **S14**

Allylmagnesium bromide (1.0 M in Et2O, 11 mL, 11 mmol) was added to a solution of Copper(I) bromide-dimethyl sulfide (787 mg, 3.8 mmol) in diethyl ether (20 mL) at -35

°C, and the resulting mixture was stirred for 1 h at -35 °C. A solution of 1-benzyl 2-methyl 4,5-dihydro-1H-pyrrole-1,2-dicarboxylate (2.0 g, 7.7 mmol) in Et2O (50 mL) was added over 30 min, and the reaction mixture was stirred for 30 min at -35 °C and then for another 30 min at 0 °C. The mixture was quenched with saturated aqueous NH4Cl and extracted with EtOAc, and the combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by RP-HPLC [C18 column, water (0.1%TFA)-CH3CN] to give 1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate (150 mg, 0.470 mmol, 6.14 % yield) as a colorless oil. LCMS (C17H22NO4+) (ES, m/z): 304.1 [M+H]+.

Step 2: 1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate

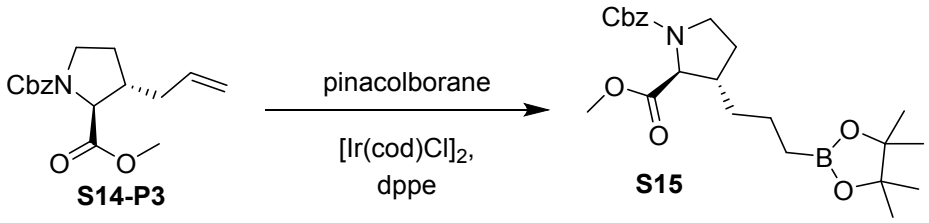


S14 **S14-P1** **S14-P2** **S14-P3**

The 1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate (150 mg, 0.49 mmol) was resolved by chiral-SFC [Column: DAICEL CHIRALPAK AD-H (250 mm*30 mm, 5 um), Mobile phase: A: CO2, B: MeOH (0.1%NH3·H2O), Gradient: 10% of B in 11.5 min and hold 10% for 1 min, Flow Rate (mL/min) 50, Column temperature: 40 °C] to give (2R,3R)-1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate (P-1, t_R = 2.112 min) as the first eluting peak (30 mg, 0.097 mmol, 19.60 % yield), and 1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate (P-2, t_R = 2.412 min) as the second eluting peak (20 mg, 0.065 mmol, 13.07 % yield), and (2S,3S)-1-benzyl 2-methyl 3-allylpyrrolidine-1,2-dicarboxylate (P-3, t_R = 2.706 min) as the third eluting peak (40 mg, 0.129 mmol, 26.1 % yield). P-1 LCMS

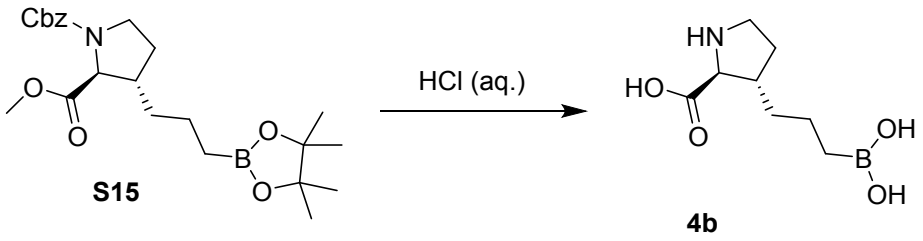
(C17H22NO4+) (ES, m/z): 304.1 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.39-7.24 (m, 5H), 5.82-5.67 (m, 1H), 5.20-4.96 (m, 4H), 4.00 (d, J = 4.4 Hz, 1H), 3.73 (s, 1H), 3.63-3.51 (m, 3H), 2.36-2.24 (m, 2H), 2.17-2.03 (m, 2H), 1.68-1.58 (m, 1H). P-2 LCMS (C17H22NO4+) (ES, m/z): 304.1 [M+H]+. P-3 LCMS (C17H22NO4+) (ES, m/z): 304.1 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.40-7.24 (m, 5H), 5.83-5.66 (m, 1H), 5.22-4.97 (m, 4H), 4.13-3.98 (m, 1H), 3.75 (s, 1H), 3.67-3.53 (m, 4H), 2.40-2.24 (m, 2H), 2.19-2.00 (m, 2H), 1.70-1.57 (m, 1H).

Step 3: 1-benzyl 2-methyl (2S,3S)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (0.029 mL, 0.20 mmol) was added to a solution of bis(1,5-cyclooctadiene)diiridium(I) dichloride (4.4 mg, 6.6 μmol) and 1,2-bis(diphenylphosphino)ethane (5.3 mg, 0.013 mmol) in anhydrous DCM (5.0 mL) under nitrogen and the mixture was stirred for 10 min, followed by addition of 1-benzyl 2-methyl (2S,3S)-3-allylpyrrolidine-1,2-dicarboxylate (P-3, 45 mg, 0.10 mmol). The reaction mixture was stirred at 30 °C for 14 h under nitrogen. The mixture was concentrated under reduced pressure, and the residue was purified by RP-HPLC [C18 column, water (0.1%TFA)-CH3CN] to give (2S,3S)-1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (45 mg, 0.103 mmol, 78 % yield) as colorless oil. LCMS (C17H25BNO6+) (ES, m/z): 350.1 [M-C6H10+H]+.

Step 4: (3S)-3-[3-(dihydroxyboranyl)propyl]-L-proline

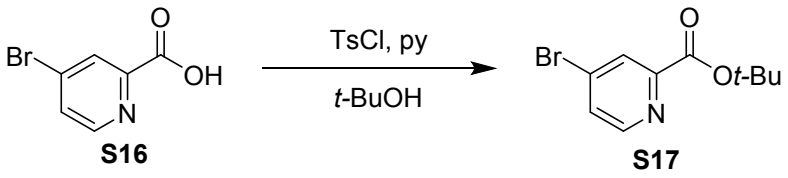


A mixture of (2S,3S)-1-benzyl 2-methyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (40 mg, 0.093 mmol) in 12 N HCl in water (3.0 mL, 36 mmol) was stirred at 100 °C for 16 h, and the mixture was diluted with water and washed with DCM. The combined aqueous phase was concentrated under reduced pressure to give (3S)-3-[3-(dihydroxyboranyl)propyl]-L-proline (19.22 mg, 0.077 mmol, 83 % yield) as a colorless oil. HRMS (ESI) calcd for C8H17BNO4 $[M+H]^+$, 202.1252; found, 202.1256.

1H NMR (400 MHz, D2O) δ 3.76 (d, J = 7. 5 Hz, 1H), 3.34-3.14 (m, 2H), 2.35-2.21 (m, 1H), 2.15-2.02 (m, 1H), 1.63-1.50 (m, 2H), 1.39-1.20 (m, 3H), 0.68-0.55 (m, 2H). 13C NMR (151 MHz, D2O) δ 171.69, 63.71, 45.60, 42.38, 35.01, 29.71, 21.62, 13.94.

Synthesis of compound 5

Step 1: tert-butyl 4-bromopicolinate

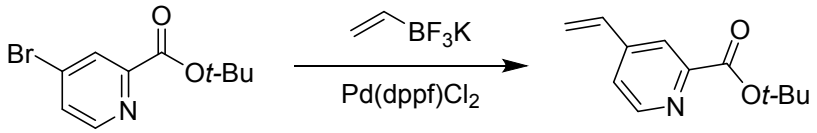


4-methylbenzene-1-sulfonyl chloride (11 g, 59 mmol) was added portion wise to a solution of 4-bromopicolinic acid (5.0 g, 25 mmol) and pyridine (10 mL, 124 mmol) in t-BuOH (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 16 h. The mixture was

quenched with saturated aqueous NaHCO3 and extracted with ethyl acetate. The combined organic phase was dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford tert-butyl 4-bromopicolinate (3.3 g, 49.1 % yield) as colorless oil.

1H NMR (400 MHz, CDCl3) δ 8.55 (d, J = 5.3 Hz, 1H), 8.19 (d, J = 2.2 Hz, 1H), 7.61 (dd, J = 2.0, 5.0 Hz, 1H), 1.64 (s, 9H).

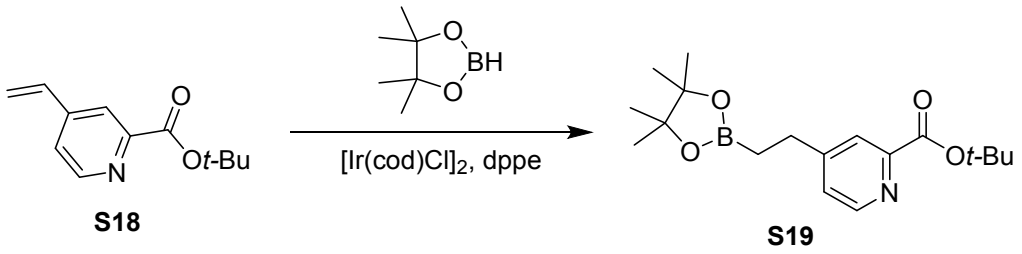
Step 2: tert-butyl 4-vinylpicolinate



S17 **S18**

Potassium vinyltrifluoroborate (3.4 g, 25 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) (0.92 g, 1.3 mmol) and K2CO3 (3.5 g, 25 mmol) were added to a solution of tert-butyl 4-bromopicolinate (3.3 g, 13 mmol) in 1,4-dioxane (40 mL) and water (8.0 mL). The resulting reaction mixture was stirred at 100 °C for 3 h under nitrogen. The mixture was diluted with water and extracted EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give tert-butyl 4-vinylpicolinate (2.2 g, 81 % yield) as a yellow solid. LCMS (C12H16NO2+) (ES, m/z): 206.0 [M+H]+.

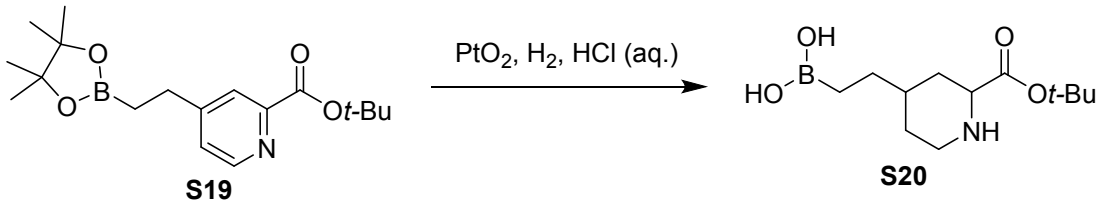
Step 3: tert-butyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)picolinate



S18 **S19**

A solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7.4 mL, 51 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (0.34 g, 0.51 mmol) and 1,2-bis(diphenylphosphino)ethane (0.29 g, 0.72 mmol) in anhydrous DCM (40 mL) was bubbled with a stream of nitrogen for 3 minutes. The resulting mixture was stirred at 12 oC for 20 minutes, followed by addition of tert-butyl 4-vinylpicolinate (2.1 g, 10 mmol). The reaction mixture was stirred at 12 oC for 16 h under nitrogen, then quenched with water and extracted with DCM. The combined organic layer was dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (EtOAc in hexanes) to give the tert-butyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)picolinate (3.5 g, 103 % yield) as a colorless oil. LCMS (C18H29BNO4+) (ES, m/z): 334.2 [M+H]+.

Step 4: (2-(2-(tert-butoxycarbonyl)piperidin-4-yl)ethyl)boronic acid

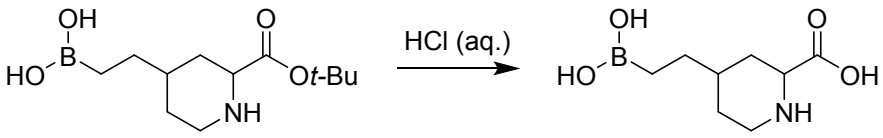


S19 **S20**

Platinum(IV) oxide (4.1 mg, 0.018 mmol) was added to a solution of tert-butyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)picolinate (60 mg, 0.18 mmol) in EtOH (3.0 mL) and 2N HCl in water (2.0 mL, 4.0 mmol) at 10 oC. The reaction mixture was degassed with hydrogen three times then stirred under 50 psi of hydrogen at 50 °C for 16 h.

The mixture was filtered and concentrated under reduced pressure to give the crude (2-(2-(tert-butoxycarbonyl)piperidin-4-yl)ethyl)boronic acid (40 mg, 86 % yield) as a yellowish oil, which was used in the next step without purification. LCMS (C12H25BNO4+) (ES, m/z): 258.1 [M+H]+.

Step 5: 4-[2-(dihydroxyboranyl)ethyl]piperidine-2-carboxylic acid



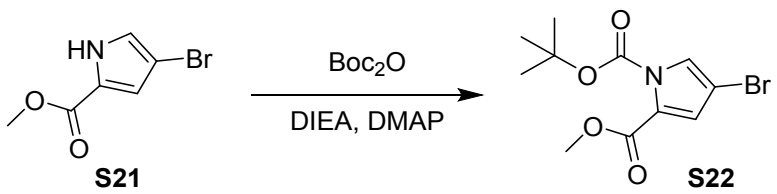
S20 **5**

A mixture of (2-(2-(tert-butoxycarbonyl)piperidin-4-yl)ethyl)boronic acid (40 mg, 0.16 mmol) and 1N HCl in water (1.0 mL, 1.0 mmol) was stirred at 100 °C for 12 h. The mixture was concentrated under reduced pressure to give 4-[2-(dihydroxyboranyl)ethyl]piperidine-2-carboxylic acid (54.87 mg, 158 % yield) as an orange solid. LCMS (C8H17BNO4+) (ES, m/z): 202.1 [M+H]+.

1H NMR (400 MHz, CD3OD) δ 3.92 (br d, J = 12.3 Hz, 1H), 3.42 (br d, J = 10.1 Hz, 1H), 3.06-2.95 (m, 1H), 2.37 (br d, J = 13.6 Hz, 1H), 1.96 (br d, J = 13.2 Hz, 1H), 1.63 (br s, 1H), 1.43-1.20 (m, 4H), 0.83 (br s, 2H).

Synthesis of compound 6

Step 1: 1-tert-butyl 2-methyl 4-bromo-1H-pyrrole-1,2-dicarboxylate

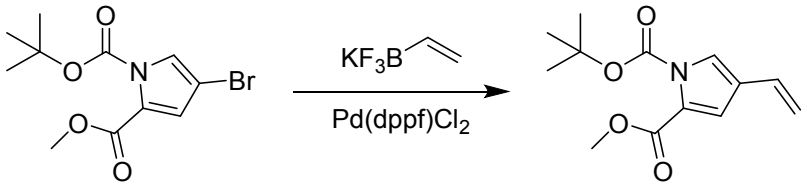


S21 **S22**

N,N-dimethylpyridin-4-amine (0.30 g, 2.5 mmol) was added to a solution of methyl 4-bromo-1H-pyrrole-2-carboxylate (1.0 g, 4.9 mmol), di-tert-butyl dicarbonate (1.4 g, 6.4 mmol) and triethylamine (2.0 mL, 15 mmol) in DCM (20 mL), and the reaction mixture was stirred at 25 oC for 12 h. The mixture was diluted with water and extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 4-bromo-1H-pyrrole-1,2-dicarboxylate (0.8 g, 2.499 mmol, 51.0 % yield) as a colorless oil. LCMS (C7H7BrNO4+) (ES, m/z): 247.9 [M-C4H8+H]+.

1H NMR (400 MHz, CDCl3) δ 7.30 (d, J = 1.98 Hz, 1H), 6.78 (d, J = 1.76 Hz, 1H), 3.81-3.86 (m, 3H), 1.54-1.59 (m, 9H).

Step 2: 1-tert-butyl 2-methyl 4-vinyl-1H-pyrrole-1,2-dicarboxylate



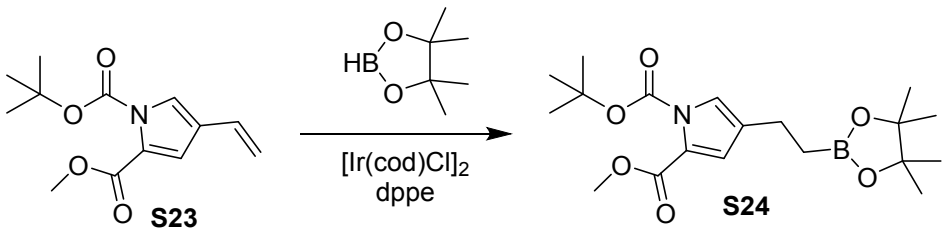
S22 **S23**

A mixture of 1-tert-butyl 2-methyl 4-bromo-1H-pyrrole-1,2-dicarboxylate (350 mg, 1.2 mmol), potassium trifluoro(vinyl)borate (308 mg, 2.3 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (168 mg, 0.23 mmol) and K2CO3 (477 mg, 3.5 mmol) in water (0.50 mL) and 1,4-Dioxane (5.0 mL) was degassed and backfilled with nitrogen (three times), and then heated to 80 oC for 4 h. The mixture was concentrated under reduced pressure, and the crude mixture was diluted with water, and extracted with EtOAc. The combined organic layer was washed with brine, dried over

anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 4-vinyl-1H-pyrrole-1,2-dicarboxylate (250 mg, 0.895 mmol, 78 % yield) as a yellow oil. LCMS (C9H10NO4+) (ES, m/z): 196.0 [M-C4H8+H]+.

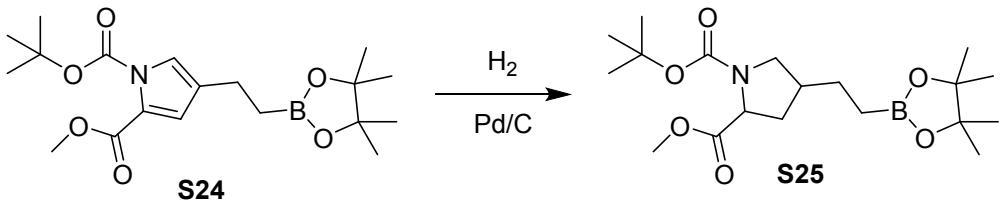
1H NMR (400 MHz, CHLOROFORM-d) δ 7.23-7.28 (m, 1H), 6.96 (d, J = 1.98 Hz, 1H), 6.49 (dd, J = 10.91, 17.53 Hz, 1H), 5.48 (dd, J = 0.88, 17.64 Hz, 1H), 5.13 (dd, J = 1.10, 10.80 Hz, 1H), 3.84 (s, 3H), 1.57 (s, 9H).

Step 3: 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)-1H-pyrrole-1,2-dicarboxylate



A solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.72 mL, 5.0 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (33 mg, 0.050 mmol) and bis(diphenylphosphino)ethane (27 mg, 0.070 mmol) in anhydrous DCM (7.0 mL) was bubbled with a stream of nitrogen for 3 min, and then stirred at 15 oC for 20 min, followed by addition of 1-tert-butyl 2-methyl 4-vinyl-1H-pyrrole-1,2-dicarboxylate (250 mg, 1.0 mmol). The resulting mixture was stirred at 15 oC for 13 h under nitrogen, and the mixture was directly purified by silica gel preparative thin layer chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)-1H-pyrrole-1,2-dicarboxylate (158 mg, 0.321 mmol, 32.2 % yield) as colorless oil. LCMS (C15H23BNO6+) (ES, m/z): 324.1 [M-C4H8+H]+.

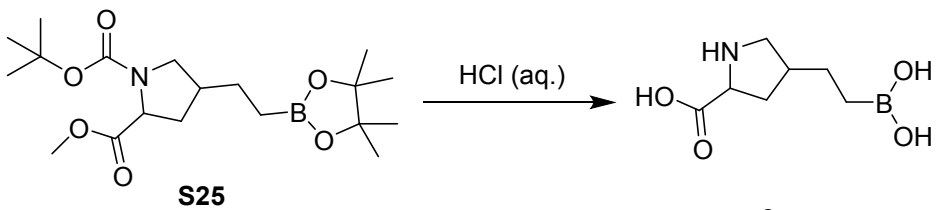
Step 4: 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)pyrrolidine-1,2-dicarboxylate



S24 **S25**

10% palladium on carbon (50 mg, 0.042 mmol) was added to a solution of 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)-1H-pyrrole-1,2-dicarboxylate (158 mg, 0.42 mmol) in MeOH (25 mL) under nitrogen atmosphere, and the mixture was degassed and backfilled with hydrogen (three times). The resulting mixture was stirred under hydrogen (45-50 psi) at 45 oC for 24 h, then filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethyl)pyrrolidine-1,2-dicarboxylate (130 mg, 0.305 mmol, 73.3 % yield) as a colorless oil. HNMR showed that was rotamer. 1H NMR (400 MHz, CDCl3) δ 4.26-4.14 (m, 1H), 3.76 (dd, J = 7.5, 10.1 Hz, 1H), 3.73-3.69 (m, 3H), 2.98 (t, J = 10.4 Hz, 1H), 2.38 (qd, J = 6.4, 12.6 Hz, 1H), 2.15-2.05 (m, 1H), 1.55-1.47 (m, 3H), 1.44 (s, 3H), 1.39 (s, 6H), 1.23 (s, 12H), 0.81-0.73 (m, 2H).

Step 5: 4-[2-(dihydroxyboranyl)ethyl]proline



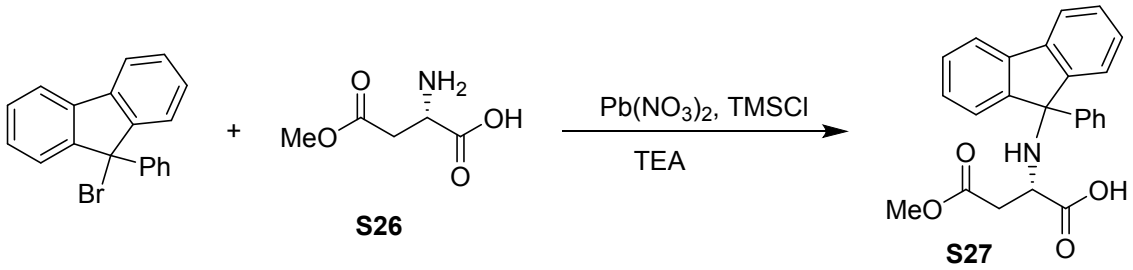
S25 **6**

A mixture of 1-tert-butyl 2-methyl 4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-

yl)ethyl)pyrrolidine-1,2-dicarboxylate (50 mg, 0.13 mmol) and 12 N HCl in water (2.0 mL, 24 mmol) was stirred at 100 oC for 12 h, and the mixture was concentrated to give 4-[2-(dihydroxyboranyl)ethyl]proline (14.14 mg, 0.066 mmol, 50.3 % yield) as an orange solid. LCMS (C7H15BNO4+) (ES, m/z): 188.0 [M+H]+. 1H NMR (400 MHz, D2O) δ 4.25 (t, J = 8.9 Hz, 1H), 3.44 (dd, J = 7.5, 11.5 Hz, 1H), 2.96-2.84 (m, 1H), 2.57-2.46 (m, 1H), 2.31-2.16 (m, 1H), 1.63 (td, J = 9.9, 13.1 Hz, 1H), 1.51-1.33 (m, 2H), 0.74-0.63 (m, 2H).

Synthesis of compound 7b

Step 1: (S)-4-methoxy-4-oxo-2-((9-phenyl-9H-fluoren-9-yl)amino)butanoic acid

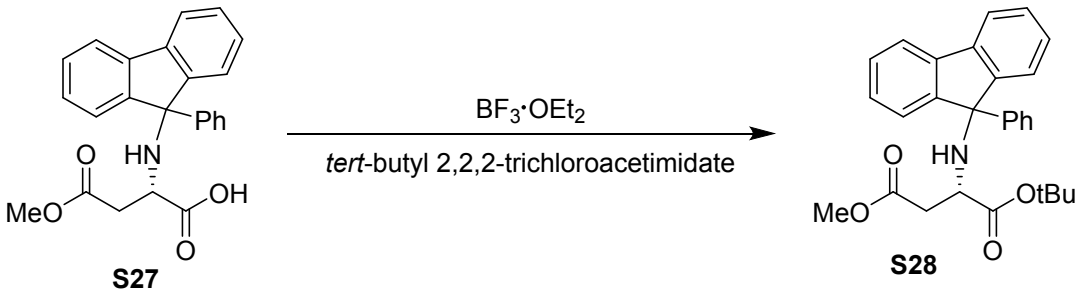


TMS-Cl (3.2 mL, 25 mmol) was added to a stirred suspension of (S)-2-amino-4-methoxy-4-oxobutanoic acid (3.3 g, 22 mmol) in chloroform (100 mL) at 20 °C, followed by addition of TEA (6.6 mL, 47 mmol) after 2 h, then addition of Pb(NO3)2 (4.4 g, 15 mmol) and 9-bromo-9-phenyl-9H-fluorene (9.4 g, 29 mmol) in chloroform (50 mL) after another 15 min. The reaction mixture was stirred vigorously for 72 h, then quenched with MeOH and stirred for another 15 min. The mixture was filtered and concentrated under reduced pressure. The crude mixture was diluted with 5% aqueous citric acid and EtOAc, and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (S)-4-methoxy-4-oxo-2-((9-phenyl-9H-fluoren-

9-yl)amino)butanoic acid (2.5 g, 6.19 mmol, 27.6 % yield) as pale yellow solid. LCMS (C26H24N2O4Na+) (ES, m/z): 451.2 [M+MeCN+Na]+.

1H NMR (400 MHz, CDCl3) δ 7.73 (dd, J = 7.5, 18.9 Hz, 2H), 7.54-7.14 (m, 11H), 3.70-3.56 (m, 3H), 2.87 (t, J = 4.2 Hz, 1H), 2.77 (dd, J = 3.7, 17.3 Hz, 1H), 1.95 (dd, J = 4.8, 17.1 Hz, 1H).

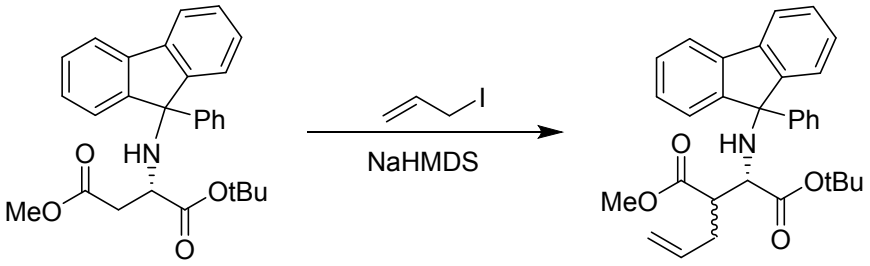
Step 2: (S)-1-tert-butyl 4-methyl 2-((9-phenyl-9H-fluoren-9-yl)amino)succinate



Boron trifluoride etherate (98 μL, 0.77 mmol) was added to the stirred solution of (S)-4-methoxy-4-oxo-2-((9-phenyl-9H-fluoren-9-yl)amino)butanoic acid (1.5 g, 3.9 mmol), tert-butyl 2,2,2-trichloroacetimidate (1.7 g, 7.7 mmol) in THF (50 mL) at 0 °C, and the reaction mixture was stirred at 20 °C for 15 h. The mixture was quenched with saturated aqueous NaHCO3 and extracted with EtOAc, and the combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (S)-1-tert-butyl 4-methyl 2-((9-phenyl-9H-fluoren-9-yl)amino)succinate (800 mg, 1.771 mmol, 45.7 % yield) as a colorless semisolid. LCMS (C28H30NO4+) (ES, m/z): 444.3 [M+H]+.

1H NMR (400 MHz, CHLOROFORM-d) δ 7.79 - 7.60 (m, 2H), 7.45 - 7.08 (m, 11H), 3.65 (s, 3H), 3.37 (br d, J = 12.3 Hz, 1H), 2.87 (t, J = 5.7 Hz, 1H), 2.46 (dd, J = 5.7, 14.9 Hz, 1H), 2.34 - 2.23 (m, 1H), 1.23 (s, 9H).

Step 3: (3S)-4-tert-butyl 1-methyl 2-allyl-3-((9-phenyl-9H-fluoren-9-yl)amino)succinate



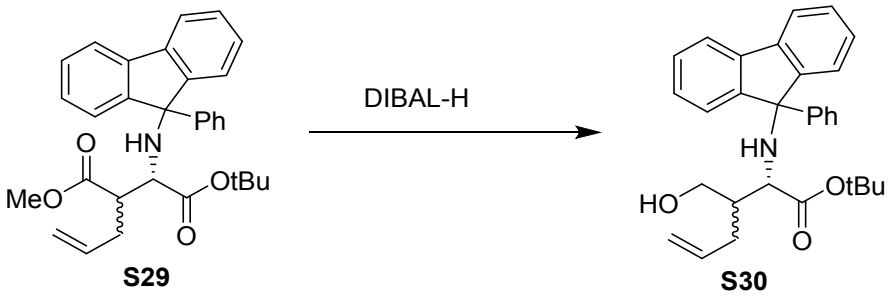
S28 **S29**

Sodium bis(trimethylsilyl)amide (1.0 M in THF, 2.2 mL, 2.2 mmol) was added to the stirred solution of (S)-1-tert-butyl 4-methyl 2-((9-phenyl-9H-fluoren-9-yl)amino)succinate (800 mg, 1.8 mmol) in THF (20 mL) under nitrogen at -78 °C, and the resulting mixture was stirred for 0.5 h at -78 °C, followed by addition of 3-iodoprop-1-ene (909 mg, 5.4 mmol). The reaction mixture was stirred at -78 °C for 1 h, then quenched with saturated aqueous NH4Cl, and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel preparative thin layer chromatography (EtOAc in hexanes) to give (3S)-4-tert-butyl 1-methyl 2-allyl-3-((9-phenyl-9H-fluoren-9-yl)amino)succinate (650 mg, 1.333 mmol, 73.9 % yield, mixture of isomers) as a colorless semisolid. LCMS (C31H34NO4+) (ES, m/z): 484.3 [M+H]+.

1H NMR (400 MHz, METHANOL-d4) δ 7.75 (br t, J = 7.9 Hz, 2H), 7.41 - 7.27 (m, 4H), 7.26 - 7.11 (m, 7H), 5.68 - 5.40 (m, 1H), 4.96 - 4.81 (m, 2H), 3.63 (s, 2.2H), 3.51 (s, 0.8H), 2.83 (br d, J = 6.1 Hz, 0.7H), 2.75 - 2.69 (m, 0.3H), 2.60 - 2.50 (m, 0.7H), 2.49 - 2.36 (m, 0.5H), 2.34 - 2.20 (m, 1H), 2.11 - 1.99 (m, 0.8H), 1.18 (s, 2.5H), 1.14 (s, 6.5H).

Step 4: (2S)-tert-butyl 3-(hydroxymethyl)-2-((9-phenyl-9H-fluoren-9-yl)amino)hex-5-

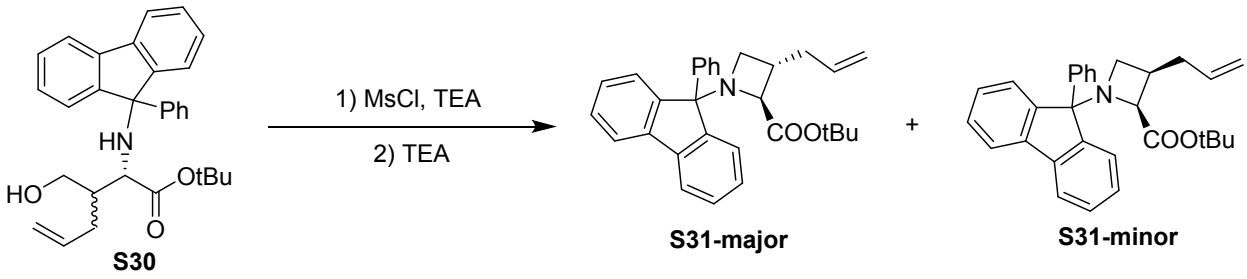
enoate



DIBAL-H (1.0 M in toluene, 5.4 mL, 5.4 mmol) was added to the stirred solution of (3S)-4-tert-butyl 1-methyl 2-allyl-3-((9-phenyl-9H-fluoren-9-yl)amino)succinate (650 mg, 1.3 mmol) in DCM (30 mL) under nitrogen at -78 °C over 5 min, and the reaction mixture was stirred at -78 °C for 1 h. The mixture was quenched with MeOH (0.5 mL) in DCM (10 mL) at -78 °C, then diluted with water, and extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel preparative thin layer chromatography (EtOAc in hexanes) to give (2S)-tert-butyl 3-(hydroxymethyl)-2-((9-phenyl-9H-fluoren-9-yl)amino)hex-5-enoate (250 mg, 0.534 mmol, 39.8 % yield, mixture of isomers) as a colorless oil. LCMS (C30H34NO3+) (ES, m/z): 456.3 [M+H]+.

^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.78 - 7.69 (m, 2H), 7.39 - 7.28 (m, 5H), 7.27 - 7.11 (m, 6H), 5.50 - 5.30 (m, 1H), 4.80 - 4.60 (m, 2H), 3.65 - 3.49 (m, 2H), 2.68 - 2.60 (m, 1H), 2.18 - 2.03 (m, 0.5H), 1.99 - 1.88 (m, 1.5H), 1.73 - 1.56 (m, 1H), 1.18 (s, 9H).

Step 5: (2S,3S)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate and (2S,3R)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate

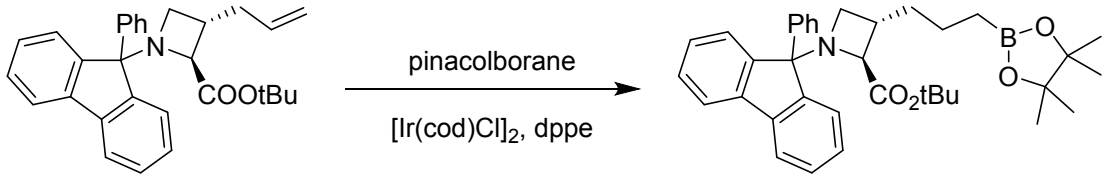


S30 S31-major S31-minor

TEA (1.1 mL, 8.2 mmol) and methanesulfonyl chloride (0.43 mL, 5.5 mmol) were added to the stirred solution of (2S)-tert-butyl 3-(hydroxymethyl)-2-((9-phenyl-9H-fluoren-9-yl)amino)hex-5-enoate (250 mg, 0.55 mmol) in DCM (10 mL) at 0 °C and the reaction mixture was stirred for 1 h at the same temperature. The mixture was quenched with water (10 mL) and neutralized with 12 N HCl in water to pH = 5. The organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The crude mixture was dissolved in DMF (5.0 mL), followed by addition of TEA (0.77 mL, 5.5 mmol), and the resulting mixture was stirred at 80 °C for 15 h. The mixture was quenched with water and extracted with EtOAc, and the combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel preparative thin layer chromatography (EtOAc in hexanes) to give (2S,3S)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate (150 mg, 0.343 mmol, 62.5 % yield, major isomer) as a colorless oil, and (2S,3R)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate (minor isomer). Stereochemistry was confirmed by 2D NMR. Major Isomer LCMS (C30H32NO2+) (ES, m/z): 438.3 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.76 (d, J = 7.5 Hz, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.56 (dd, J = 1.3, 7.9 Hz, 2H), 7.49-7.35 (m, 3H), 7.35-7.27 (m, 2H), 7.26-7.14 (m, 4H), 5.59-5.42 (m, 1H), 4.84 (dd, J = 2.0, 13.8 Hz, 2H), 3.56-3.46 (m, 1H), 3.05 (d, J =

7.0 Hz, 1H), 2.94 (t, J = 7.5 Hz, 1H), 2.53-2.46 (m, 1H), 1.95-1.88 (m, 2H), 1.19 (s, 9H). Minor Isomer LCMS (C30H32NO2+) (ES, m/z): 438 [M+H]+. 1H NMR (400 MHz, CDCl3) δ 7.76 (d, J = 7.5 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.55-7.51 (m, 1H), 7.48-7.40 (m, 2H), 7.37 (d, J = 7.5 Hz, 1H), 7.34-7.32 (m, 1H), 7.28-7.19 (m, 4H), 7.17-7.09 (m, 1H), 5.71-5.59 (m, 1H), 5.02-4.92 (m, 2H), 3.47 (t, J = 8.1 Hz, 1H), 3.35 (d, J = 9.2 Hz, 1H), 3.22 (dd, J = 3.9, 7.5 Hz, 1H), 2.55-2.44 (m, 1H), 2.41-2.30 (m, 1H), 2.26-2.17 (m, 1H), 1.20 (s, 9H).

Step 6: (2S,3S)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate



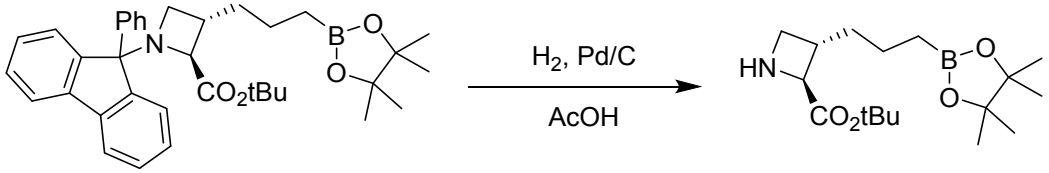
S31-major **S32**

A mixture of (2S,3S)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate (400 mg, 0.91 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (31 mg, 0.046 mmol), and 1,2-bis(diphenylphosphino)ethane (26 mg, 0.064 mmol) in DCM (20 mL) was degassed and backfilled with nitrogen (three times), followed by addition of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (585 mg, 4.6 mmol), and the resulting mixture was stirred at 20 °C for 15 h under nitrogen. The mixture was quenched with water and extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S)-tert-butyl 1-(9-phenyl-9H-fluoren-9-yl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (350 mg, 0.590 mmol, 64.5 % yield) as a colorless oil. LCMS (C36H45BNO4+)

(ES, m/z): 566.4 [M+H]+.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.81 - 7.70 (m, 1H), 7.65 - 7.59 (m, 1H), 7.57 - 7.49 (m, 2H), 7.45 - 7.11 (m, 9H), 3.57 - 3.45 (m, 1H), 3.00 - 2.93 (m, 1H), 2.93 - 2.83 (m, 1H), 2.49 - 2.31 (m, 1H), 1.39 - 1.02 (m, 25H), 0.69 - 0.51 (m, 2H).

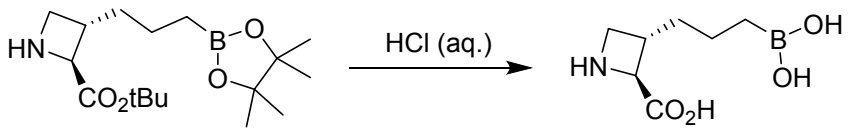
Step 7: (2S,3S)-tert-butyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate



S32 **S33**

Acetic acid (186 mg, 3.1 mmol) and 10% Pd/C (66 mg, 0.062 mmol) was added to a solution of (2S,3S)-tert-butyl 1-(9-phenyl-9H-fluoren-9-yl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (350 mg, 0.62 mmol) in MeOH (20 mL) under nitrogen atmosphere, and the resulting mixture was degassed and backfilled with hydrogen (three times), and stirred under hydrogen (Pressure: 1 atm) at 20 °C for 3 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude (2S,3S)-tert-butyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (350 mg, 1.076 mmol, 174 % yield) as a white semisolid, which was used in next step without further purification. LCMS (C17H33BNO4+) (ES, m/z): 326.2 [M+H]+.

Step 8: (2S,3S)-3-[3-(dihydroxyboranyl)propyl]azetidine-2-carboxylic acid



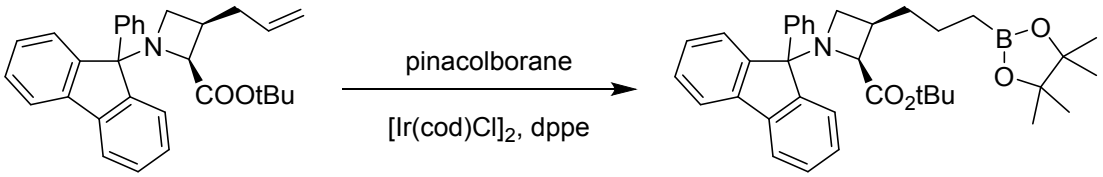
S33 **7b**

A mixture of (2S,3S)-tert-butyl 3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (100 mg, 0.31 mmol) and 12 N HCl in water (2.5 mL, 30 mmol) was stirred at 0 °C for 2 h, and solvent was evaporated in nitrogen stream at 0 °C. The residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3S)-3-[3-(dihydroxyboranyl)propyl]azetidine-2-carboxylic acid (22.87 mg, 0.122 mmol, 39.8 % yield, TFA salt) as a white foam. LCMS (C7H15BNO4+) (ES, m/z): 188.1 [M+H]+.

1H NMR (400 MHz, CD3OD) δ 4.70 (d, J = 7.9 Hz, 1H), 3.97 (t, J = 9.4 Hz, 1H), 3.74 (dd, J = 8.3, 10.1 Hz, 1H), 2.98 (sxt, J = 8.0 Hz, 1H), 1.84 1.63 (m, 2H), 1.57-1.29 (m, 2H), 0.80 (br s, 2H).

Synthesis of compound 7a

Step 1: (2S,3R)-tert-butyl 1-(9-phenyl-9H-fluoren-9-yl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate



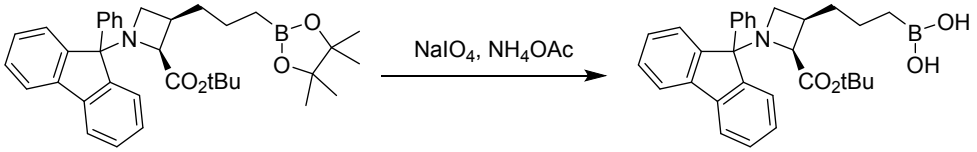
S31 minor **S34**

A mixture of (2S,3R)-tert-butyl 3-allyl-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylate (300 mg, 0.55 mmol), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (351 mg, 2.7 mmol), 1,2-bis(diphenylphosphino)ethane (22 mg, 0.055 mmol), and chloro(1,5-cyclooctadiene)iridium(I) dimer (18 mg, 0.027 mmol) in DCM (10 mL) was degassed and backfilled with nitrogen (three times), and stirred at 20 °C for 15 h. The mixture was quenched with water and extracted with DCM, and the combined organic phase was washed

with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3R)-tert-butyl 1-(9-phenyl-9H-fluoren-9-yl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (240 mg, 0.414 mmol, 75 % yield) as a colorless semi-solid. LCMS (C36H45BNO4+) (ES, m/z): 566.4 [M+H]+.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.75 (d, J = 7.5 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.55 - 7.49 (m, 2H), 7.47 - 7.38 (m, 2H), 7.36 (d, J = 7.5 Hz, 1H), 7.34 - 7.28 (m, 1H), 7.27 - 7.17 (m, 4H), 7.15 - 7.07 (m, 1H), 3.46 (t, J = 8.0 Hz, 1H), 3.31 (d, J = 9.5 Hz, 1H), 3.25 - 3.15 (m, 1H), 2.31 - 2.14 (m, 1H), 1.80 - 1.64 (m, 1H), 1.50 - 1.38 (m, 1H), 1.36 - 1.24 (m, 1H), 1.23 - 1.09 (m, 22H), 0.71 (t, J = 7.8 Hz, 2H).

Step 2: (3-((2S,3R)-2-(tert-butoxycarbonyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidin-3-yl)propyl)boronic acid

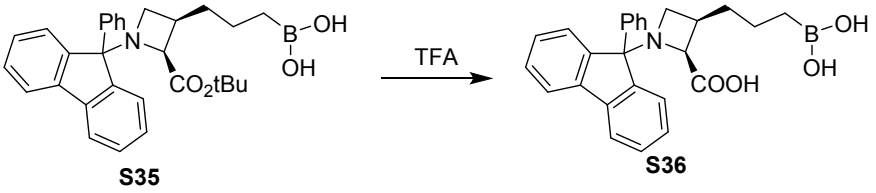


S34 **S35**

Ammonium acetate (327 mg, 4.2 mmol) and sodium periodate (454 mg, 2.1 mmol) were added to the stirred suspension of (2S,3R)-tert-butyl 1-(9-phenyl-9H-fluoren-9-yl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)azetidine-2-carboxylate (120 mg, 0.21 mmol) in THF (5.0 mL) and water (2.5 mL) at 0 °C, and the reaction mixture was stirred at 30 °C for 15 h. The mixture was quenched with water and extracted with EtOAc, and the combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure to give crude (3-((2S,3R)-2-(tert-butoxycarbonyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidin-3-yl)propyl)boronic acid (110 mg, 0.221 mmol, 104 %

yield) as a colorless semisolid, which was used in the next step without further purification. LCMS (C30H35BNO4+) (ES, m/z): 484.3 [M+H]+.

Step 3: (2S,3R)-3-(3-boronopropyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylic acid

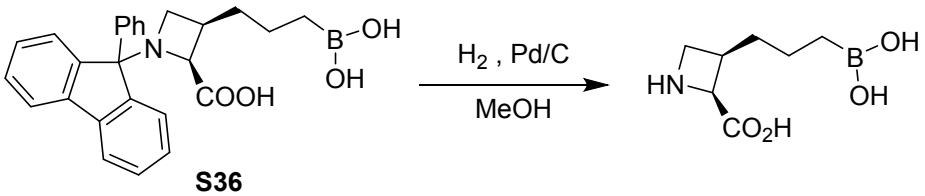


S35 **S36**

TFA (2.0 mL, 26 mmol) was added to the stirred solution of (3-((2S,3R)-2-(tert-butoxycarbonyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidin-3-yl)propyl)boronic acid (110 mg, 0.23 mmol) in DCM (2.0 mL) and the reaction mixture was stirred at 20 °C for 15 h. The mixture was concentrated under reduced pressure, and the residue was purified by RP-HPLC [C18 column, water (0.1%TFA)-CH3CN] to give (2S,3R)-3-(3-boronopropyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylic acid (75 mg, 0.173 mmol, 76 % yield) as a white semisolid. LCMS (C26H27BNO4+) (ES, m/z): 428.3 [M+H]+.

^{1}H NMR (400 MHz, METHANOL-d_4) δ 8.00 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.71 - 7.64 (m, 1H), 7.61 - 7.49 (m, 3H), 7.46 - 7.37 (m, 6H), 4.32 (t, J = 9.9 Hz, 1H), 4.17 (d, J = 10.1 Hz, 1H), 4.07 - 3.98 (m, 1H), 2.71 - 2.47 (m, 1H), 1.62 - 1.53 (m, 2H), 1.36 - 1.19 (m, 2H), 0.74 (br t, J = 7.5 Hz, 2H).

Step 4: (2S,3R)-3-[3-(dihydroxyboranyl)propyl]azetidine-2-carboxylic acid



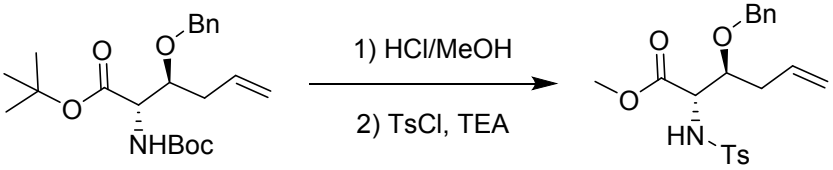
S36 **7a**

10% palladium on carbon (69 mg, 0.064 mmol) was added to the stirred solution of (2S,3R)-3-(3-boronopropyl)-1-(9-phenyl-9H-fluoren-9-yl)azetidine-2-carboxylic acid (55 mg, 0.13 mmol) in MeOH (2.0 mL) under nitrogen atmosphere, and the reaction mixture was degassed and backfilled with hydrogen (three times), and then stirred under hydrogen (Pressure: 1 atm) at 20 °C for 4 h. The mixture was filtered and the filtrate was concentrated under reduced pressure, and the crude mixture was diluted with DCM and extracted with water. The combined aqueous layer was evaporated in nitrogen stream, and the residue was purified by RP-HPLC [C18 column, water (10 mM NH4HCO3)-CH3CN] to give (2S,3R)-3-[3-(dihydroxyboranyl)propyl]azetidine-2-carboxylic acid (10.92 mg, 0.050 mmol, 38.6 % yield) as a white solid. LCMS (C7H13BNO3+) (ES, m/z): 170.1 [M-H2O+H]+.

1H NMR (400 MHz, D2O) δ 4.60 (d, J = 9.7 Hz, 1H), 3.97 (dd, J = 8.8, 10.4 Hz, 1H), 3.48 (dd, J = 6.7, 10.5 Hz, 1H), 3.00-2.74 (m, 1H), 1.56-1.39 (m, 1H), 1.36-1.24 (m, 1H), 1.22-1.03 (m, 2H), 0.63-0.41 (m, 2H).

Synthesis of compound 8

Step 1: (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)hex-5-enoate



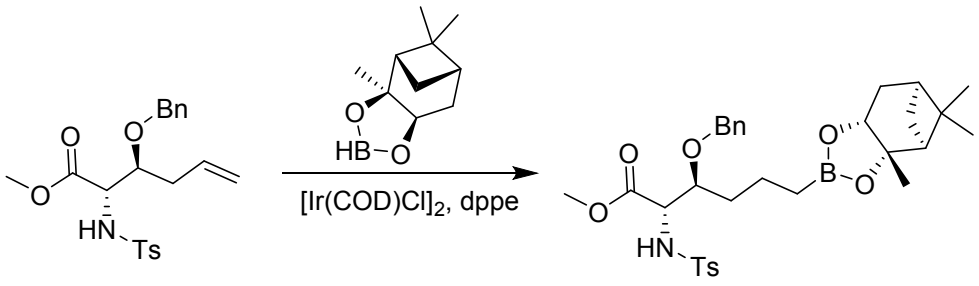
S37 **S38**

A mixture of (2S,3S)-tert-butyl 3-(benzyloxy)-2-((tert-butoxycarbonyl)amino)hex-5-enoate (1.0 g, 2.6 mmol) and 4 N HCl in MeOH (5.0 mL, 20 mmol) in DCM (10 mL) was stirred at 0 °C for 1 h, then allowed to warm to 29 °C and stirred for 14 h. SOCl2 (1.0 mL,

14 mmol) was added and the resulting mixture was stirred for 24 h. The reaction mixture was concentrated, and the crude residue was dissolved in DCM (10 mL) and then treated with Ts-Cl (97 mg, 0.51 mmol) and triethylamine (0.11 mL, 0.77 mmol) at 0 °C. The mixture was allowed to warm to 29 °C and stirred for 73 h under N2. The mixture was concentrated, and the residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)hex-5-enoate (600 mg, 58% yield) as a yellow oil. LCMS (C21H26NO5S+) (ES, m/z): 404.1 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.69 (d, J = 8.3 Hz, 2H), 7.42-7.20 (m, 8H), 5.83-5.73 (m, 1H), 5.29 (d, J = 9.3 Hz, 1H), 5.20-5.08 (m, 2H), 4.61-4.42 (m, 2H), 3.77-3.61 (m, 1H), 3.48 (s, 3H), 2.49-2.27 (m, 5H).

Step 2: (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)-6-((3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)hexanoate



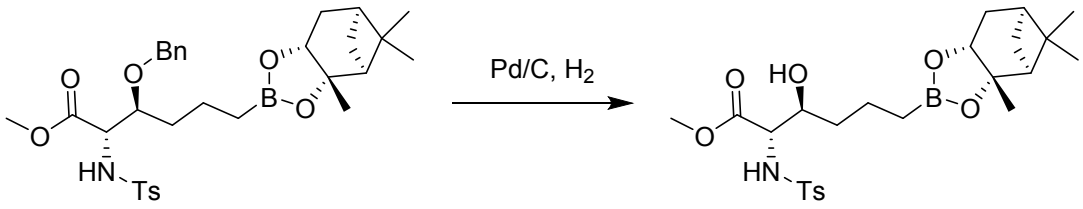
S38 **S39**

A solution of (3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]-dioxaborole (0.34 g, 1.9 mmol) and (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)hex-5-enoate (0.30 g, 0.74 mmol) in DCM (2.0 mL) was added to the stirred solution of 1,2-bis(diphenylphosphino)ethane (50 mg, 0.13 mmol) and [Ir(cod)Cl]2 (50 mg, 0.074 mmol) in DCM (5.0 mL) at 26 °C under N2. The reaction mixture was stirred at 26 °C for 15 h, then quenched with water and extracted with DCM. The combined organic

phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)-6-((3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)hexanoate (0.28 mg, 65% yield) as a brown oil. LCMS (C31H43BNO7S+) (ES, m/z): 584.0 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.70 (d, J = 8.3 Hz, 2H), 7.35-7.22 (m, 8H), 5.29 (d, J = 9.2 Hz, 1H), 4.54-4.41 (m, 2H), 4.27-4.24 (m, 1H), 4.16-4.13 (m, 1H), 3.70-3.60 (m, 1H), 3.49 (s, 3H), 2.40 (s, 3H), 2.38-2.29 (m, 1H), 2.26-2.18 (m, 1H), 2.04 (t, J = 5.7 Hz, 1H), 1.95-1.90 (m, 1.5H), 1.84-1.79 (m, 0.5H), 1.71-1.59 (m, 1H), 1.57-1.40 (m, 3H), 1.38 (s, 3H), 1.29 (s, 3H), 0.85 (s, 3H), 0.78 (br t, J = 7.2 Hz, 2H).

Step 3: (2S,3S)-methyl 4-tosyl-2-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)morpholine-3-carboxylate

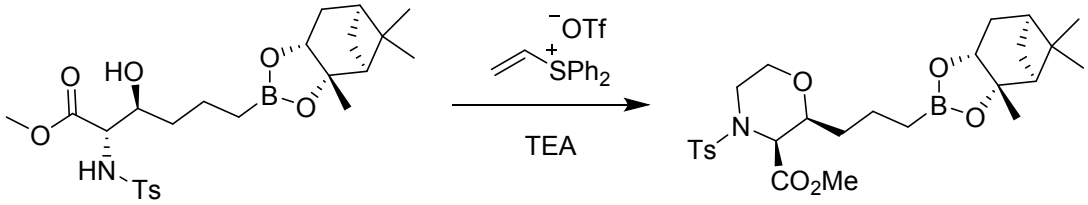


S39 **S40**

Pd-C (10 wt%, 0.50 g) was added to the stirred solution of (2S,3S)-methyl 3-(benzyloxy)-2-(4-methylphenylsulfonamido)-6-((3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)hexanoate (0.28 g, 0.48 mmol) in MeOH (15 mL) under Ar. The mixture was degassed and backfilled with H2 (three times), and stirred under H2 (Pressure: 15 psi) at 33 °C for 1 h. The reaction mixture was filtered and concentrated to give crude (2S,3S)-methyl 3-hydroxy-2-(4-methylphenylsulfonamido)-6-((3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)hexanoate (0.15 g) as a

colorless oil, which was used in the next step directly without further purification. LCMS (C24H37BNO7S+) (ES, m/z): 494.1 [M+H]+.

Step 4: (2S,3S)-methyl 4-tosyl-2-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)morpholine-3-carboxylate



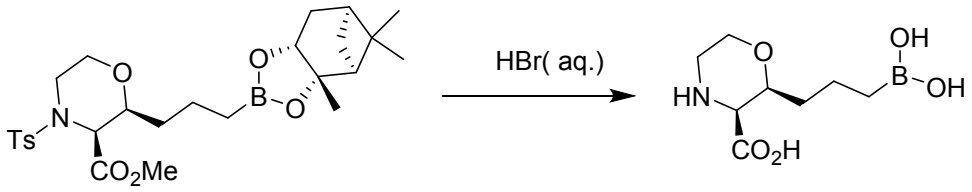
S40 **S41**

Triethylamine (0.13 mL, 0.93 mmol) was added to a solution of (2S,3S)-methyl 3-hydroxy-2-(4-methylphenylsulfonamido)-6-((3aS,4S,6S)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)hexanoate (0.15 g, 0.30 mmol) in DCM (4.0 mL) at 0 °C. After 10 min, diphenylvinylsulfonium triflate (0.13 g, 0.37 mmol) in DCM (1.0 mL) was added dropwise to the mixture at 0 °C and the resulting mixture was stirred for 12 h at 0 °C. The reaction mixture was then quenched with saturated aqueous ammonium chloride solution, extracted with DCM, washed with brine, dried over MgSO4, filtered and concentrated. The residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3S)-methyl 4-tosyl-2-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)morpholine-3-carboxylate (150 mg, 95% yield) as a white gum. LCMS (C26H39BNO7S+) (ES, m/z): 520.0 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.62 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 4.37-4.36 (m, 1H), 4.26-4.24 (m, 1H), 4.04-3.96 (m, 1H), 3.71-3.55 (m, 4H), 3.35 (s, 3H), 2.43 (s, 3H), 2.36-2.30 (m, 1H), 2.24-2.18 (m, 1H), 2.04 (t, J = 5.5 Hz, 1H), 1.94-1.88 (m, 1H), 1.88-1.81 (m, 1H), 1.60-1.44 (m, 3H), 1.38 (br s, 4H), 1.29 (s, 3H), 1.10-1.08

(m, 1H), 0.88-0.77 (m, 5H).

Step 5: (2S,3S)-2-(3-boronopropyl)morpholine-3-carboxylic acid



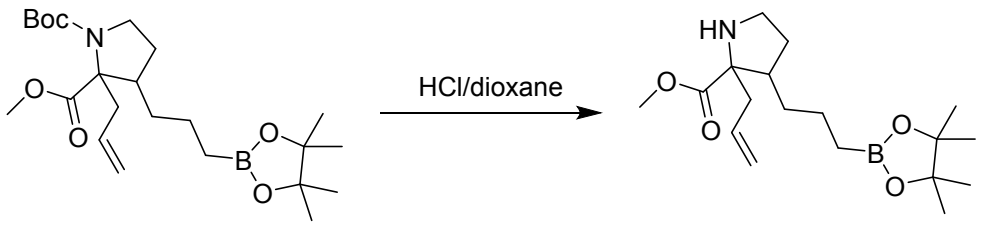
S41 **8**

A mixture of (2S,3S)-methyl 4-tosyl-2-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)morpholine-3-carboxylate (0.10 g, 0.19 mmol) and 48% HBr in water (5.0 mL, 0.19 mmol) was stirred at 130 °C for 2 h, and concentrated. The residue was purified by RP-HPLC [C18 column, water (20 mM HFBA and 0.1% TFA)-CH3CN] to give (2S,3S)-2-(3-boronopropyl)morpholine-3-carboxylic acid (54 mg, 65% yield, HFBA salt) as a brown oil. LCMS (C8H15BNO4+) (ES, m/z): 200 [M+H-H2O]+.

1H-NMR (400MHz, D2O) δ 4.21 - 4.16 (m, 1H), 4.14 - 4.07 (m, 1H), 3.97-3.92 (m, 1H), 3.82-3.76 (m, 1H), 3.54-3.49 (m, 1H), 3.21-3.16 (m, 1H), 1.88 - 1.74 (m, 1H), 1.58 - 1.32 (m, 3H), 0.84 - 0.65 (m, 2H).

Synthesis of compound 9

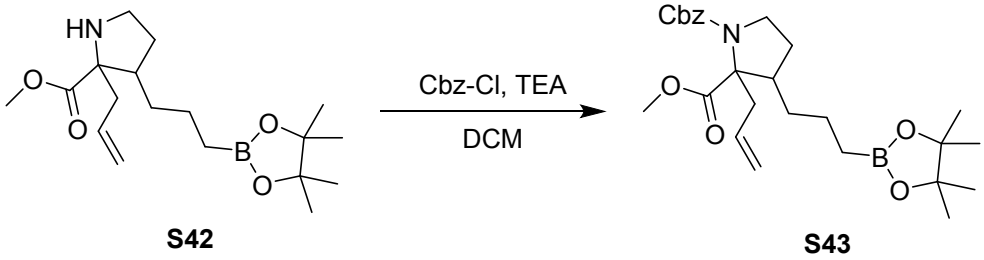
Step 1: methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate



S10 **S42**

A solution of 1-tert-butyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (2 g, 4.57 mmol) in 4 M HCl/dioxane (1.143 mL, 4.57 mmol) was stirred for 3 h. LCMS showed the reaction was completed. The reaction mixture was and concentrated under reduced pressure to give methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate (1.623 g, 4.57 mmol, 100 % yield) as a colorless oil, which was used directly in the next step without further purification. LCMS (C18H34BNO4) (ES, m/z): 338.1 [M+H]+.

Step 2: 1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



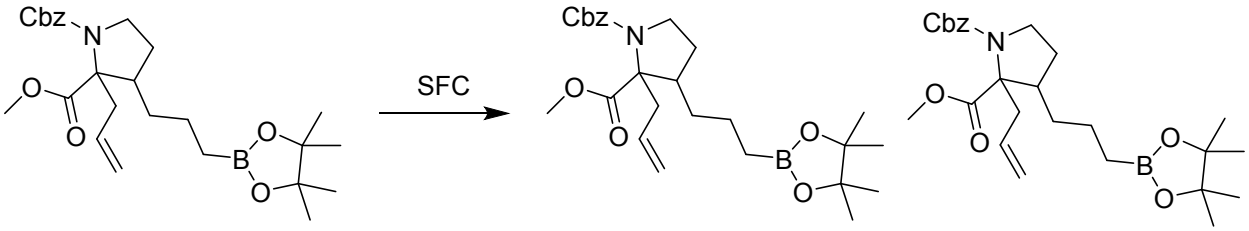
S42 **S43**

To a stirred solution of methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylate (1.6 g, 4.74 mmol) and TEA (1.984 mL, 14.23 mmol) in dry DCM (25 mL) at 0 °C was added benzyl carbonochloridate (1.104 mL, 7.12 mmol). And the reaction was stirred for 12 h at 25 °C. LCMS showed the desired compound was formed and the material was remained. Another batch of benzyl carbonochloridate (0.736 mL, 4.74 mmol) was added. And the reaction was stirred at 25 °C for 12 h. LCMS showed the desired

compound was formed and the material was remained. CbzOSu (1.419 g, 5.69 mmol) was added and the reaction was stirred for 12 h at 25 °C. LCMS and TLC showed the reaction was completed. The organics were washed with sat. NH4Cl (20 mL) and brine (10 mL), dried (Na2SO4), filtered and concentrated. The residue was purified by flash silica gel chromatography (ISCO®, 12 g SepaFlash® Silica Flash Column, eluent of 0-20% ethyl acetate/pet. ether gradient @ 18 mL/min) to give 1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (1.2 g, 2.291 mmol, 48.3 % yield) as a colorless oil. LCMS (C26H40BNO6) (ES, m/z): 472.3 [M+H]+.

1H NMR (400 MHz, CHLOROFORM-d) δ 7.26-7.42 (m, 5H), 5.53-5.70 (m, 1H), 4.99-5.24 (m, 4H), 3.77-3.99 (m, 1H), 3.42-3.70 (m, 3H), 2.97-3.28 (m, 2H), 2.49 (ddd, J = 8.77, 14.58, 18.75 Hz, 1H), 2.17-2.32 (m, 1H), 1.93 (td, J = 6.08, 11.95 Hz, 1H), 1.30-1.55 (m, 4H), 1.23 (d, J = 3.07 Hz, 13H), 0.68-0.98 (m, 2H).

*Step 3: 1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (**P2**)*

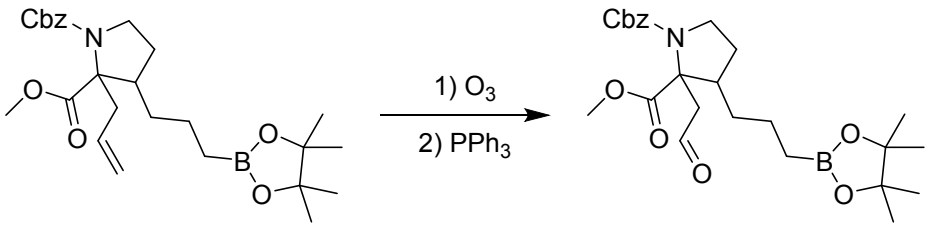


S43 **S43-P1** **S43-P2**

1-Benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (400 mg, 0.849 mmol) was resolved by Chiral-SFC (Column: DAICEL CHIRALCEL OD-H (250mm*30mm,5um), Condition: 0.1%NH3·H2O EtOH Begin B 25%, End B 25% Gradient Time (min), 100%B Hold Time (min) Flow Rate

(mL/min) 50) to give (1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (150 mg, 0.270 mmol, 31.9 % yield) (t_R = 2.084 min, P1) as a colorless oil and 1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (150 mg, 0.270 mmol, 31.9 % yield) (t_R = 2.431 min, P2) as a colorless oil. P1 LCMS (C26H40BNO6) (ES, m/z): 472.3 [M+H]+. 1H NMR (400 MHz, CHLOROFORM-d) δ 7.21-7.39 (m, 5H), 5.52-5.75 (m, 1H), 4.94-5.25 (m, 4H), 3.77-3.97 (m, 1H), 3.42-3.69 (m, 3H), 2.95-3.29 (m, 2H), 2.48 (ddd, J = 8.55, 14.47, 18.64 Hz, 1H), 2.15-2.29 (m, 1H), 1.92 (td, J = 6.25, 12.06 Hz, 1H), 1.54-1.68 (m, 1H), 1.38-1.52 (m, 3H), 1.22 (d, J = 3.51 Hz, 13H), 0.62-0.86 (m, 2H). P2 LCMS (C26H40BNO6) (ES, m/z): 472.3 [M+H]+. 1H NMR (400 MHz, CHLOROFORM-d) δ 7.19-7.42 (m, 5H), 5.51-5.67 (m, 1H), 4.97-5.24 (m, 4H), 3.75-4.02 (m, 1H), 3.39-3.72 (m, 3H), 2.90-3.28 (m, 2H), 2.48 (ddd, J = 8.77, 14.47, 18.86 Hz, 1H), 2.16-2.29 (m, 1H), 1.92 (td, J = 6.08, 11.95 Hz, 1H), 1.60 (dq, J = 3.07, 11.84 Hz, 1H), 1.42-1.52 (m, 3H), 1.22 (d, J = 3.07 Hz, 13H), 0.66-0.85 (m, 2H).

Step 4: 1-benzyl 2-methyl 2-(2-oxoethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

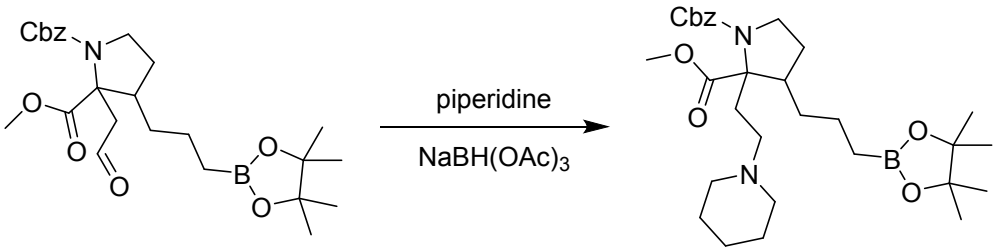


S43-P2 **S44**

To a solution of 1-benzyl 2-methyl 2-allyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (150 mg, 0.318 mmol, 3-P2) in DCM (10 mL) was bubbled with a stream of O3 at -78 °C for 10 min to give a blue solution. Then

the solution was bubbled with O2 for 5 min to give colorless solution. PPh3 (167 mg, 0.636 mmol) was added to the mixture at 0 °C under N2, then the reaction mixture was stirred at 25 °C for 16 h. LCMS showed the reaction was completed. Then the mixture reaction was concentrated in vacuo. The residue was purified by flash silica gel chromatography (ISCO®, 4 g SepaFlash® Silica Flash Column, eluent of 0-30 % ethyl acetate/pet. ether gradient @ 18 mL/min) to give 1-benzyl 2-methyl 2-(2-oxoethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (100 mg, 0.201 mmol, 63.1 % yield) as a colorless oil. LCMS (C25H38BNO7) (ES, m/z): 474.2 [M+H]+.

Step 5: 1-benzyl 2-methyl 2-(2-(piperidin-1-yl)ethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

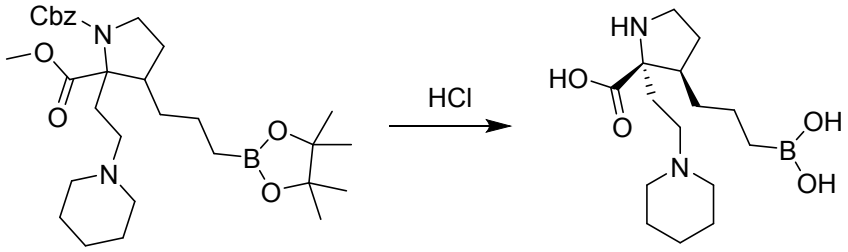


S44 **S45**

To a solution of 1-benzyl 2-methyl 2-(2-oxoethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (100 mg, 0.211 mmol) and piperidine (54.0 mg, 0.634 mmol) in 1,2-Dichloroethane (5 mL) was added NaBH(OAc)3 (66.8 mg, 0.317 mmol).The reaction mixture was stirred at 25 oC for 2 h under N2. LCMS showed that the desired compound was formed and the reaction was stirred for another 12h. LCSM showed the reaction was completed. The reaction was quenched with saturated NH4Cl (10 mL), and extracted with DCM (8 mL*3). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na2SO4, filtered and concentrated under reduced

pressure. The residue was purified by Pre-HPLC (Column: YMC-Actus Pro C18 150*30 5u, Condition water (0.1%TFA)-ACN Begin B 36, End B 66 Gradient Time (min) 11, 100%B Hold Time (min) 1.1, Flow Rate (mL/min) 40) to give 1-benzyl 2-methyl 2-(2-(piperidin-1-yl)ethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (80 mg, 0.140 mmol, 66.3 % yield) as a colorless oil. LCMS (C30H49BN2O6) (ES, m/z): 543.1 [M+H]+.

Step 6: 3-(3-boronopropyl)-2-(2-(piperidin-1-yl)ethyl)pyrrolidine-2-carboxylic acid



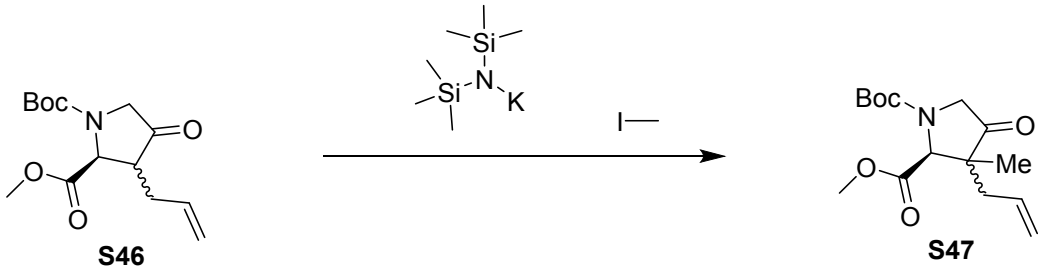
S45 **9**

A mixture of 1-benzyl 2-methyl 2-(2-(piperidin-1-yl)ethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (80 mg, 0.147 mmol) in 12 M HCl (5 mL, 60 mmol) was stirred at 100 °C for 72 h to give colorless mixture. LCMS showed that the reaction was completed. The solvent was removed to give 3-(3-boronopropyl)-2-(2-(piperidin-1-yl)ethyl)pyrrolidine-2-carboxylic acid hydrochloride (29.13 mg, 0.084 mmol, 56.7 % yield) as a white solid. LCMS (C15H31NO2O4) (ES, m/z): 295.1 [M-H2O+H]+.

^{1}H-NMR (400 MHz, DEUTERIUM OXIDE) δ 3.34-3.51 (m, 3H), 3.18-3.30 (m, 1H), 2.98-3.16 (m, 2H), 2.74-2.88 (m, 2H), 2.39-2.51 (m, 1H), 2.06-2.26 (m, 3H), 1.79 (br d, J = 14.33 Hz, 2H), 1.48-1.69 (m, 4H), 1.16-1.47 (m, 4H), 0.98-1.11 (m, 1H), 0.54-0.73 (m, 2H).

Synthesis of compound 10

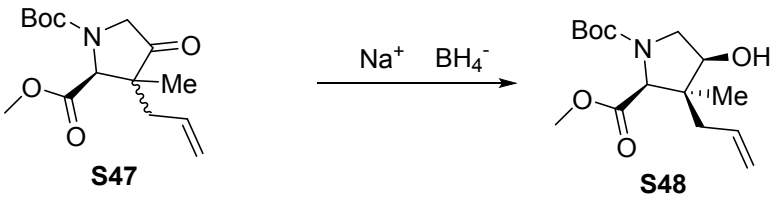
Step 1: 1-tert-butyl 2-methyl (2S)-3-methyl-4-oxo-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate



Potassium bis(trimethylsilyl)amide (0.5 M in Toluene, 15 mL, 7.5 mmol) was added dropwise to the stirred solution of 1-tert-butyl 2-methyl (2S)-4-oxo-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (2.1 g, 7.5 mmol) in THF (23 mL) at -78°C, and the reaction mixture was stirred for 1 h at -78°C, followed by addition of iodomethane (0.47 mL, 7.5 mmol) at -78 °C, and the resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was quenched with saturate aqueous NH4Cl, and extracted with DCM. The combined organic layer was dried over Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-tert-butyl 2-methyl (2S)-3-methyl-4-oxo-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (1.2 g, 54% yield, mixture of diastereomers) as a yellow oil. LC-MS (C10H16NO3+) (ES, m/z): 198.1 [M-CO2C4H8+H]+.

1H-NMR: (400 MHz DMSO-d6) δ: 5.71-5.75 (m, 1H), 5.01-5.16 (m, 2H), 4.30-4.40 (m, 1H), 4.03-4.10 (m, 1H), 3.70-3.82 (m, 1H), 3.61-3.68 (m, 3H), 2.23-2.30 (m, 1H), 1.80-1.98 (m, 1H), 1.38 (s, 9H), 1.15-1.20 (m, 2H), 0.91-0.95 (m, 1H).

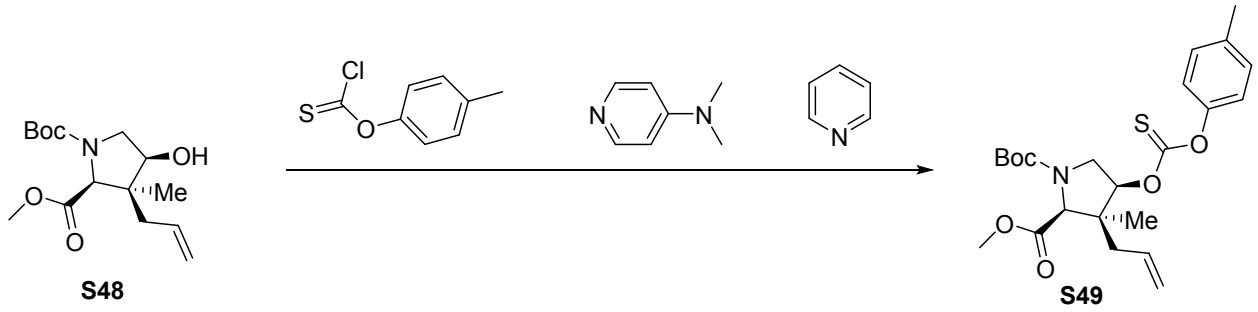
Step 2: 1-tert-butyl 2-methyl (2S,3S,4S)-4-hydroxy-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate



Sodium borohydride (0.23 g, 6.1 mmol) was added in two portions to the stirred solution of 1-tert-butyl 2-methyl (2S)-3-methyl-4-oxo-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (1.2 g, 4.0 mmol) in MeOH (12 mL) within 10 min at 0 °C, and the reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture was quenched with saturated aqueous NH4Cl, and extracted with EtOAc. The combined organic layer was dried over Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-tert-butyl 2-methyl (2S,3S,4S)-4-hydroxy-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate as the first eluting peak (686 mg, 57% yield, existed as two rotamers) as a yellow oil. LC-MS (C10H18NO3+) (ES, m/z): 200.1 [M-CO2C4H8+H]+.

1H-NMR (600MHz, DMSO-d6): δ 5.82-5.72 (m, 1H), 5.08-4.99 (m, 2H), 4.92 (dd, J = 12.6, 4.5 Hz, 1H), 3.83 (s, 0.37H), 3.81 (s, 0.63H), 3.76-3.71 (m, 1H), 3.62 (s, 1.90H), 3.60 (s, 1.10H), 3.60-3.53 (m, 1H), 3.33-3.30 (m, 1H), 2.27-2.20 (m, 1H), 2.05-1.96 (m, 1H), 1.39 (s, 3.30H), 1.31 (s, 5.70H), 0.97 (s, 1.90H), 0.95 (s, 1.10H). LC-MS (C10H18NO3+) (ES, m/z): 200 [M-CO2C4H8+H]+.

Step 3: 1-tert-butyl 2-methyl (2S,3S,4S)-3-methyl-4-{[(4-methylphenoxy)carbonothioyl]oxy}-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate



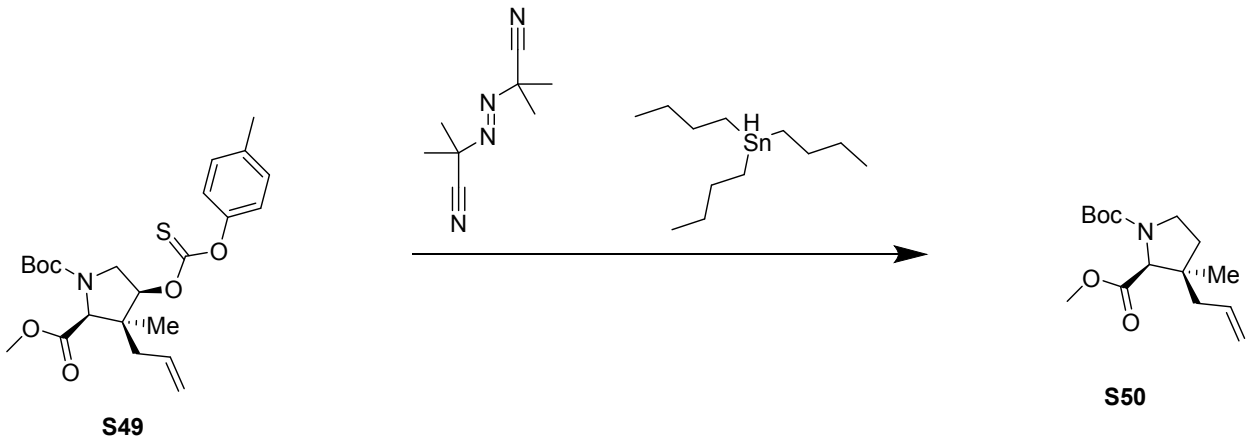
S48 **S49**

Pyridine (927 µL, 11 mmol) was added to the stirred solution of 1-tert-butyl 2-methyl (2S,3S,4S)-4-hydroxy-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (686 mg, 2.3 mmol) and 4-dimethylaminopyridine (28 mg, 0.23 mmol) in DCM (6.0 mL), followed by dropwise addition of p-tolyl chlorothionoformate (873 µL, 5.7 mmol) at 0 °C, and the reaction mixture was allowed to warm to room temperature and stirred overnight. The resulting mixture was cooled to 0 °C, and pyridine (927 µL, 11 mmol) was added in one portion, followed by dropwise addition of p-tolyl chlorothionoformate (873 µL, 5.7 mmol). The reaction mixture was allowed to warm to room temperature and stirred for another 8 h. The mixture was quenched with saturated aqueous NaHCO3, and extracted with DCM. The combined organic layer was dried over Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-tert-butyl 2-methyl (2S,3S,4S)-3-methyl-4-{[(4-methylphenoxy)carbonothioyl]oxy}-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (452 mg, 44% yield) as a yellow oil. LC-MS (C23H31NNaO6S+) (ES, m/z): 472.1 [M+Na]+.

1H-NMR: (400 MHz DMSO-d6) δ: 7.23-7.29 (m, 2H), 7.03-7.06 (m, 2H), 5.72-5.79 (m, 1H), 5.11-5.20 (m, 2H), 4.00-4.07 (m, 4H), 3.60-3.70 (m, 5H), 2.23-2.33 (m, 3H), 2.15-2.18 (m, 2H), 1.39 (s, 9H), 1.15-1.19 (m, 3H).

Step 4: 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-

dicarboxylate

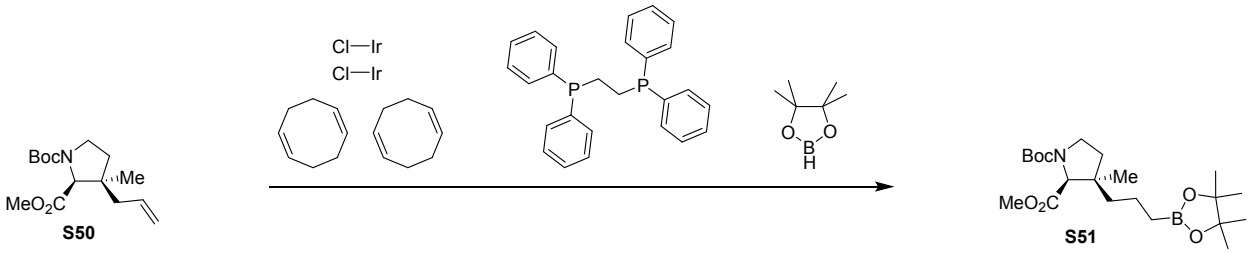


Tri-n-butyltin hydride (0.60 mL, 2.2 mmol) was added to the stirred solution of 1-tert-butyl 2-methyl (2S,3S,4S)-3-methyl-4-{[(4-methylphenoxy)carbonothioyl]oxy}-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (452 mg, 1.0 mmol) and 2,2'-azobis(2-methylpropionitrile) (17 mg, 0.10 mmol) in toluene (7.0 mL) under nitrogen atmosphere at room temperature, and the reaction mixture was heated to 110 oC and stirred for 2.5 h, then cooled to room temperature and purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (185 mg, 65% yield) as a yellow oil. LC-MS (C10H18NO2+) (ES, m/z): 184.1 [M-CO2C4H8+H]+.

1H-NMR (600 MHz, DMSO-d6) δ 5.82 – 5.72 (m, 1H), 5.10 – 5.05 (m, 2H), 3.82 (d, J = 15.1 Hz, 1H), 3.65 (d, J = 17.3 Hz, 3H), 3.50 – 3.42 (m, 1H), 3.38 – 3.27 (m, 2H), 2.02 (ddd, J = 17.0, 13.7, 7.0 Hz, 1H), 1.90 (ddd, J = 17.3, 13.7, 7.6 Hz, 1H), 1.78 (ddt, J = 14.9, 12.2, 8.9 Hz, 1H), 1.57 (tdd, J = 12.3, 7.1, 3.0 Hz, 1H), 1.39 (s, 3H), 1.32 (s, 6H), 1.05 (d, J = 10.5 Hz, 3H).

Step 5: 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-[3-(4,4,5,5-tetramethyl-1,3,2-

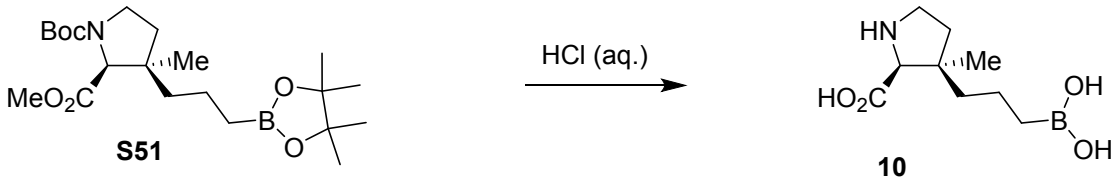
dioxaborolan-2-yl)propyl]pyrrolidine-1,2-dicarboxylate



A pre-formed solution of bis(1,5-cyclooctadiene)diiridium(I) dichloride (22 mg, 0.032 mmol), 1,2-bis(diphenylphosphino)ethane (26 mg, 0.064 mmol), and pinacolborane (500 uL, 3.1 mmol) in DCM (2.5 mL) was added to the stirred solution of 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-(prop-2-en-1-yl)pyrrolidine-1,2-dicarboxylate (182 mg, 0.64 mmol) in DCM (2.5 mL) under nitrogen atmosphere at room temperature, and the reaction mixture was stirred at room temperature for 24 h, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]pyrrolidine-1,2-dicarboxylate (256 mg, 97% yield) as a yellow oil. LC-MS (C16H31BNO4+) (ES, m/z): 312.2 [M-CO2C4H8+H]+.

1H-NMR (600 MHz, DMSO-d6) δ 3.76 (d, J = 14.2 Hz, 1H), 3.62 (d, J = 16.9 Hz, 3H), 3.46 (q, J = 9.4 Hz, 1H), 3.30 – 3.24 (m, 1H), 1.70 (dt, J = 20.9, 10.3 Hz, 1H), 1.59 (q, J = 11.9 Hz, 1H), 1.38 (s, 4H), 1.31 (s, 5H), 1.26 (d, J = 8.3 Hz, 1H), 1.23 (s, 1H), 1.21 (s, 1H), 1.16 (d, J = 5.7 Hz, 15H), 1.10 (s, 1H), 1.07 (s, 2H), 1.03 (d, J = 10.1 Hz, 3H), 0.62 (dt, J = 16.2, 7.1 Hz, 2H).

Step 6: (3R)-3-[3-(dihydroxyboranyl)propyl]-3-methyl-L-proline

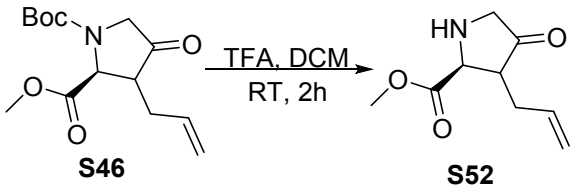


12 N HCl in water (1.3 mL) was added to the stirred suspension of 1-tert-butyl 2-methyl (2S,3R)-3-methyl-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]pyrrolidine-1,2-dicarboxylate (256 mg, 0.62 mmol) in water (1.3 mL) at room temperature, and the reaction mixture was heated to 90 °C with stirring overnight, then cooled to room temperature. The mixture was diluted with water, filtered through a 0.25 um filter and lyophilized to afford (3R)-3-[3-(dihydroxyboranyl)propyl]-3-methyl-L-proline (86 mg, 55% yield) as an off-white solid. HRMS (ESI) calcd for C9H17BNO3 $[M-H_2O+H]^+$, 198.1303; found, 198.1308.

1H-NMR (500 MHz, DMSO-d6) δ 3.91 (s, 1H), 3.50-3.38 (m, 2H), 2.14 (ddd, J = 12.9, 7.3, 5.1 Hz, 1H), 1.87 (dt, J = 13.4, 8.9 Hz, 1H), 1.54-1.28 (m, 4H), 1.28 (s, 3H), 0.79 (t, J = 7.5 Hz, 2H). 13C NMR (151 MHz, D2O) δ 172.37, 70.81, 44.24, 43.10, 36.53, 35.20, 23.04, 18.65, 14.85.

Synthesis of compound 11

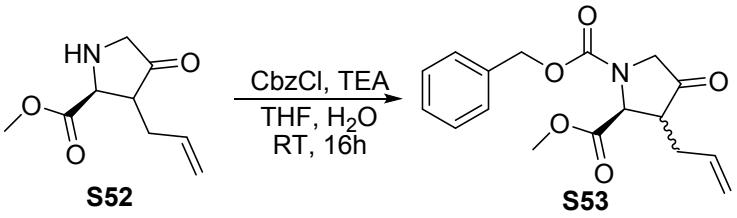
Step 1: methyl (2S)-3-allyl-4-oxopyrrolidine-2-carboxylate



TFA (3.0 L) was added dropwise to a stirred solution of 1-tert-butyl 2-methyl (2S)-4-oxopyrrolidine-1,2-dicarboxylate (590 g, 2.4 mol) in DCM (3.0 L) at 0 °C. The resulting

solution was stirred for 2 h at rt, then concentrated to yield crude methyl (2S)-3-allyl-4-oxopyrrolidine-2-carboxylate (355 g, 80% yield) as a brown oil, which was used directly in the next step without further purification.

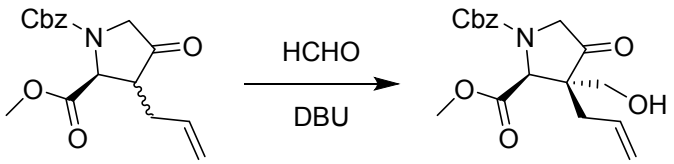
Step 2: (2S)-1-benzyl 2-methyl 3-allyl-4-oxopyrrolidine-1,2-dicarboxylate



TEA (588 g, 5.8 mol) was added dropwise to the stirred solution of methyl (2S)-3-allyl-4-oxopyrrolidine-2-carboxylate (355 g, 1.9 mol), and CbzCl (298 g, 1.7 mol) in THF (2.0 L) and water (2.0 L) at 0 °C, and the resulting mixture was stirred for 16 h at rt. The reaction mixture was extracted with EtOAc three times, and the combined organic layers were concentrated, and the residue was purified by Flash-Prep-HPLC (Column, C18; mobile phase, ACN/H2O=20 increasing to ACN/H2O=100 within 30 min) to give (2S)-1-benzyl 2-methyl 3-allyl-4-oxopyrrolidine-1,2-dicarboxylate (204 g, 33% yield) as a brown oil. LCMS (C17H20NO5+) (ES, m/z): 318.1 [M+H]+.

1H-NMR (300 MHz, DMSO-d6, ppm) δ 7.38-7.30 (m, 5H), 5.81-5.70 (m, 1H), 5.22-5.03 (m, 4H), 4.93-4.38 (m, 1H), 4.20-3.70 (m, 2H), 3.67-3.54 (m, 3H), 3.10-2.60 (m, 1H), 2.50-2.35 (m, 2H).

Step 3: (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(hydroxymethyl)-4-oxopyrrolidine-1,2-dicarboxylate

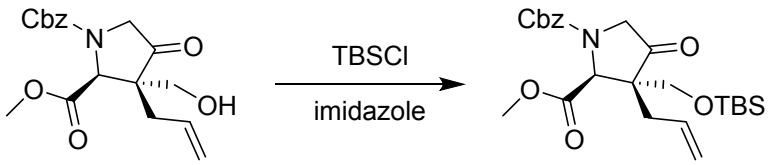


S53 **S54**

DBU (0.14 mL, 0.95 mmol) was added to the stirred solution of (2S)-1-benzyl 2-methyl 3-allyl-4-oxopyrrolidine-1,2-dicarboxylate (3.0 g, 9.5 mmol) and formaldehyde (37% in water, 0.70 mL, 9.5 mmol) in THF (45 mL) at 0 °C, and the resulting mixture was stirred for 12 h at 0 °C. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(hydroxymethyl)-4-oxopyrrolidine-1,2-dicarboxylate (2.4 g, 73% yield) as a colorless oil. The stereochemistry was assigned by 2D NMR. LCMS (C18H22NO6+) (ES, m/z): 348.0 [M+H]+.

1H-NMR (400MHz, CDCl3) δ 7.44 - 7.28 (m, 5H), 5.91 - 5.69 (m, 1H), 5.33 - 5.02 (m, 4H), 4.77 (br d, J = 12.3 Hz, 1H), 4.06 - 3.98 (m, 2H), 3.75 (s, 3H), 3.70 (br s, 1H), 3.57 (s, 1H), 2.53 - 2.43 (m, 1H), 2.17-2.09 (m, 1H).

Step 4: (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-oxopyrrolidine-1,2-dicarboxylate



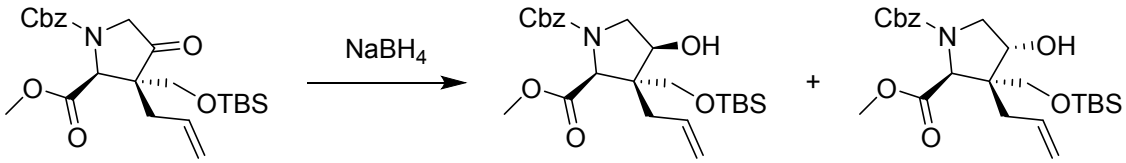
S54 **S55**

TBS-Cl (3.5 g, 23 mmol) was added to the stirred solution of (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(hydroxymethyl)-4-oxopyrrolidine-1,2-dicarboxylate (4.0 g, 12 mmol) and imidazole (2.4 g, 35 mmol) in DCM (50 mL) and the mixture was stirred at 20 °C for 5 h.

The reaction mixture was quenched with saturated aqueous NH4Cl and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-oxopyrrolidine-1,2-dicarboxylate (4.5 g, 95% yield) as a colorless oil. LCMS (C24H36NO6Si+) (ES, m/z): 462.0 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.38-7.28 (m, 5H), 5.75-5.68 (m, 1H), 5.27-4.99 (m, 4H), 4.78 (br d, J = 14.5 Hz, 1H), 4.02-3.89 (m, 2H), 3.76-3.53 (m, 5H), 2.47-2.33 (m, 1H), 2.18-2.08 (m, 1H), 0.81-0.80 (m, 9H), -0.00--0.02 (m, 6H).

Step 5: ((2S,3S,4S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1,2-dicarboxylate as the first eluting peak (4-P1)

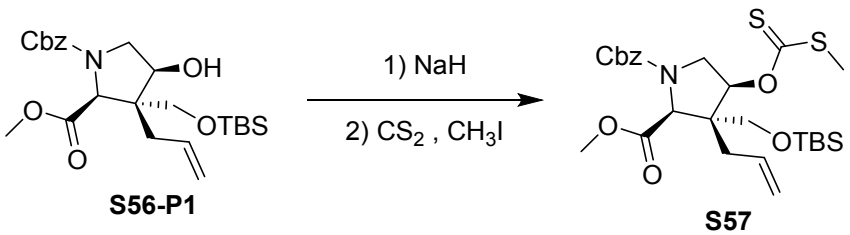


S55 **S56-P1** **S56-P2**

NaBH4 (1.1 g, 29 mmol) was added to the stirred solution of (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-oxopyrrolidine-1,2-dicarboxylate (4.5 g, 9.8 mmol) in MeOH (50 mL), and the mixture was stirred at 0 °C for 0.5 h. The reaction mixture was diluted with acetone and stirred for 10 min at 0 °C, then concentrated. The residue was quenched with saturated aqueous NH4Cl and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S,4S)-1-benzyl 2-methyl 3-allyl-3-(((tert-

butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1,2-dicarboxylate as the first eluting peak (P1, 2.1 g, 47% yield) and (2S,3S,4R)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1,2-dicarboxylate as the second eluting peak (P2, 1.2 g, 27% yield). The stereochemistry was assigned by 2D NMR. P1: LCMS (C24H38NO6Si+) (ES, m/z): 464 [M+H]+; 1H NMR (400MHz, CDCl3) δ 7.42 - 7.26 (m, 5H), 5.89 - 5.71 (m, 1H), 5.24 - 4.99 (m, 4H), 4.30-4.29 (m, 1H), 4.00 - 3.84 (m, 1H), 3.82 (s, 1.5H), 3.79-3.75 (m, 2H), 3.62 (s, 1.5H), 3.51 - 3.42 (m, 1H), 3.35 - 3.23 (m, 1H), 2.34 - 2.23 (m, 2H), 0.94 - 0.82 (m, 9H), 0.05 - -0.03 (m, 6H); P2: LCMS (C24H38NO6Si+) (ES, m/z): 464 [M+H]+; 1H NMR (400MHz, CDCl3) δ 7.39 - 7.23 (m, 5H), 5.89 - 5.71 (m, 1H), 5.22 - 4.99 (m, 4H), 4.41 - 4.31 (m, 2H), 3.94 - 3.78 (m, 3H), 3.74 (s, 1.5H), 3.53 (s, 1.5H), 3.50 - 3.39 (m, 1H), 2.31 - 2.18 (m, 1H), 2.14 - 2.04 (m, 1H), 0.92 - 0.85 (m, 9H), 0.12 - 0.04 (m, 6H).

Step 6: 1-benzyl 2-methyl (2S,3S,4S)-3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-(((methylthio)carbonothioyl)oxy)pyrrolidine-1,2-dicarboxylate

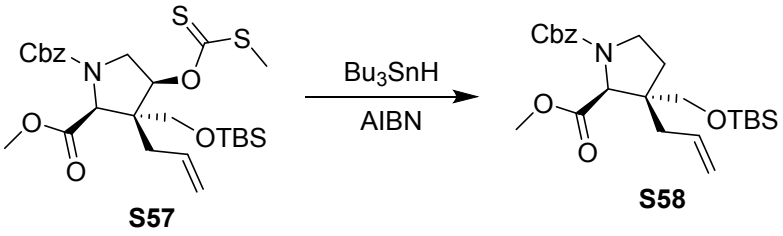


NaH (60% in mineral oil, 65 mg, 1.6 mmol,) was added to the stirred solution of (2S,3S,4S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1,2-dicarboxylate (0.50 g, 1.1 mmol) in THF (15 mL) under N2 and the mixture was stirred at 0 °C for 30 min. Carbon disulfide (0.21 g, 2.7 mmol) and iodomethane (0.64 mL, 10 mmol) were added at 0 °C and the resulting mixture was stirred for another 30

min. The reaction mixture was quenched with saturated aqueous NH4Cl and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to give (2S,3S,4S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-(((methylthio)carbonothioyl)oxy)pyrrolidine-1,2-dicarboxylate (200 mg, 33% yield) as a yellowish oil. LCMS (C26H40NO6S2Si+) (ES, m/z): 554.1 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.38-7.25 (m, 5H), 5.77-5.58 (m, 2H), 5.24-5.00 (m, 4H), 4.49-4.35 (m, 1H), 4.30-4.02 (m, 1H), 3.92-3.84 (m, 1H), 3.82-3.61 (m, 3H), 3.55-3.34 (m, 2H), 2.56-2.55 (m, 3H), 2.46-2.35 (m, 1H), 2.32-2.16 (m, 1H), 0.88 (s, 9H), 0.06-0.04 (m, 6H).

Step 7: (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1,2-dicarboxylate



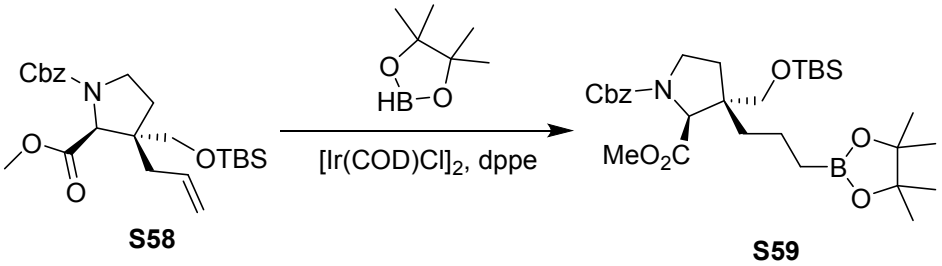
S57 **S58**

Tributylstannane (0.83 mL, 3.6 mmol) was added to the stirred solution of (2S,3S,4R)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)-4-(((methylthio)carbonothioyl)oxy)pyrrolidine-1,2-dicarboxylate (1.0 g, 1.8 mmol) and AIBN (0.030 g, 0.18 mmol) in toluene (10 mL) at 15 °C under N2. The reaction mixture was stirred at 110 °C for 2 h, then cooled to rt and quenched with saturated aqueous KF, and extracted with EtOAc. The combined organic phase was dried over anhydrous Na2SO4, filtered and

concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to give (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1,2-dicarboxylate (700 mg, 87% yield). LCMS (C24H38NO5Si+) (ES, m/z): 448.2 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.36-7.28 (m, 5H), 5.74-5.67 (m, 1H), 5.15-5.05 (m, 4H), 4.32 (s, 1H), 3.74-3.70 (m, 2.5H), 3.57 (s, 1.5H), 3.43-3.39 (m, 2H), 2.37-2.31 (m, 1H), 1.93-1.87 (m, 1H), 1.77-1.72 (m, 1H),1.33-1.31 (m, 1H), 1.13-1.11 (m, 1H), 0.89 (s, 9H), 0.04-0.01 (m, 6H).

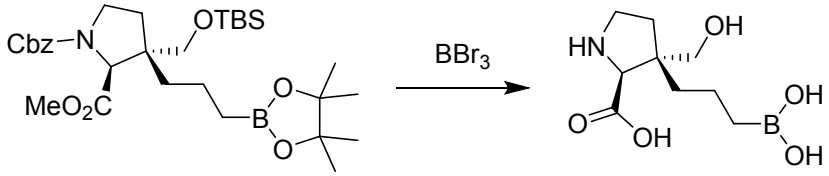
Step 8: (2S,3S)-1-benzyl 2-methyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (0.049 mL, 0.34 mmol) was added to the stirred solution of [Ir(cod)Cl]2 (5.3 mg, 7.8 μmol) and 1,2-bis(diphenylphosphino)ethane (6.7 mg, 0.017 mmol) in CH2Cl2 (2.0 mL) under N2 and the mixture was stirred for 20 min, followed by addition of (2S,3S)-1-benzyl 2-methyl 3-allyl-3-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidine-1,2-dicarboxylate (50 mg, 0.11 mmol) and the resulting mixture was stirred at 26 °C for 12 h under N2. The reaction mixture was concentrated and the residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3S)-1-benzyl 2-methyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (50 mg, 78% yield) as a colorless oil. LCMS (C30H51BNO7Si+) (ES, m/z): 576.1 [M+H]+.

1H-NMR (400 MHz, CHLOROFORM-d) δ 7.35-7.26 (m, 5H), 5.15-5.10 (m, 2H), 4.29-4.26 (m, 1H), 3.74-3.37 (m, 7H), 1.85-1.82 (m, 2H), 1.43-1.40 (m, 2H),1.24-1.21 (m, 13H), 1.10-1.08 (m, 1H), 0.89 (s, 9H), 0.74-0.71(m, 2H), 0.03-0.01 (m, 6H).

Step 9: (2S,3S)-3-(3-boronopropyl)-3-(hydroxymethyl)pyrrolidine-2-carboxylic acid



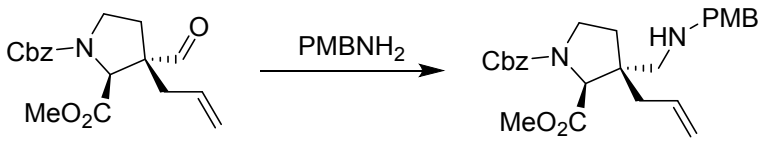
S59 **11**

BBr3 (0.20 mL, 2.1 mmol) was added to the stirred solution of (2S,3S)-1-benzyl 2-methyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (50 mg, 0.087 mmol) in DCM (1.0 mL) at -78 °C, and the resulting mixture was stirred for 12 h at 26 °C. The reaction mixture was diluted with H2O and the aqueous layer was purified by RP-HPLC [C18 column, water (20 mM HFBA and 0.1% TFA)-CH3CN] to give ((2S,3S)-3-(3-boronopropyl)-3-(hydroxymethyl)pyrrolidine-2-carboxylic acid (19 mg, 50% yield, HFBA salt) as a white solid. HRMS (ESI) calcd for C9H17BNO4 $[M-H_2O+H]^+$, 214.1252; found, 214.1260.

1H-NMR (400MHz, D2O) δ 4.00 (s, 1H), 3.59 - 3.48 (m, 2H), 3.36 - 3.27 (m, 1H), 3.27 - 3.17 (m, 1H), 1.93 - 1.82 (m, 2H), 1.36 - 1.10 (m, 4H), 0.68 - 0.54 (m, 2H). 13C NMR (151 MHz, D2O) δ 170.95, 65.40, 63.92, 49.45, 43.63, 33.27, 30.46, 18.20, 14.63.

Synthesis of compound 12

Step 1: (2S,3R)-1-benzyl 2-methyl 3-allyl-3-(((4-methoxybenzyl)amino)methyl)pyrrolidine-1,2-dicarboxylate

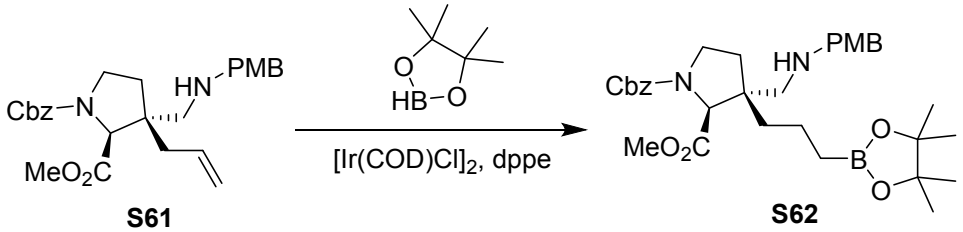


S60 **S61**

Titanium(IV) isopropoxide (0.43 g, 1.5 mmol) was added to the stirred solution of (2S,3S)-1-benzyl 2-methyl 3-allyl-3-formylpyrrolidine-1,2-dicarboxylate (0.10 g, 0.30 mmol) and (4-methoxyphenyl)methanamine (83 mg, 0.60 mmol) in THF (4.0 mL) at 25 °C under N2, and the mixture was stirred at 25 °C for 4 h. The reaction mixture was diluted with MeOH, followed by addition of NaBH4 (11 mg, 0.30 mmol) and the resulting mixture was stirred at 25 °C for 2 h. The reaction mixture was filtered and concentrated. The residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3R)-1-benzyl 2-methyl 3-allyl-3-(((4-methoxybenzyl)amino)methyl)pyrrolidine-1,2-dicarboxylate (45 mg, 33% yield) as a yellowish oil. LCMS (C26H33N2O5+) (ES, m/z): 452.9 [M+H]+.

1H-NMR (400MHz, CHLOROFORM-d) δ 7.42-7.15 (m, 7H), 6.88 (br s, 2H), 5.55-5.22 (m, 1H), 5.15-4.80 (m, 3H), 4.39-4.25 (m, 1H), 4.14 (br t, J = 11.8 Hz, 1H), 3.91-3.88 (m, 1H), 3.76 (s, 3H), 3.67 (br s, 3H), 3.45 (br s, 2H), 3.03 (br d, J = 12.3 Hz, 1H), 2.90-2.77 (m, 1H), 2.44-2.34 (m, 1H), 2.29-2.27 (m, 1H), 2.11-1.88 (m, 2H), 1.79-1.78 (m, 1H).

Step 2: (2S,3R)-1-benzyl 2-methyl 3-(((4-methoxybenzyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



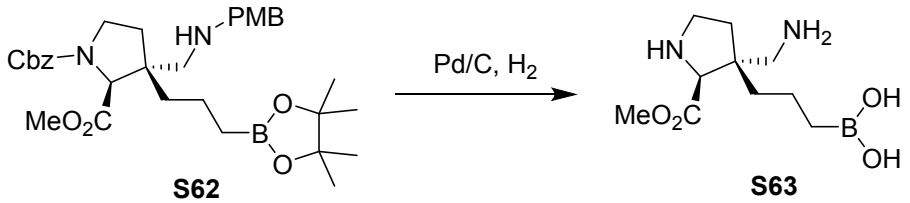
S61 **S62**

[Ir(cod)Cl]2 (4.1 mg, 6.1 μmol) and 1,2-bis(diphenylphosphino)ethane (4.8 mg, 0.012

mmol) were added to the stirred solution of 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.088 mL, 0.61 mmol) in CH2Cl2 (5.0 mL), and the mixture was stirred at 25 °C under N2 for 20 min, followed by addition of (2S,3R)-1-benzyl 2-methyl 3-allyl-3-(((4-methoxybenzyl)amino)methyl)pyrrolidine-1,2-dicarboxylate (55 mg, 0.12 mmol) and the resulting mixture was stirred at 25 °C for 12 h under N2. The reaction mixture was concentrated and the residue was purified by RP-HPLC [C18 column, water (0.1% TFA)-CH3CN] to give (2S,3R)-1-benzyl 2-methyl 3-(((4-methoxybenzyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (30 mg, 42% yield, contained minor corresponding boronic acid) as a white solid. LCMS (C32H46BN2O7+) (ES, m/z): 581.0 [M+H]+.

1H-NMR (400MHz, METHANOL-d4) δ 7.42 (d, J = 8.1 Hz, 2H), 7.38-7.24 (m, 5H), 7.01-6.99 (m, 2H), 5.25-5.04 (m, 2H), 4.25-4.21 (m, 3H), 3.81-3.79 (m, 3H), 3.66-3.57 (m, 1H), 3.73-3.53 (m, 3H), 3.44-3.33 (m, 1H), 3.04-3.02 (m, 2H), 2.02 (m, 1H), 2.00-1.90 (m, 1H), 1.89-1.79 (m, 1H), 1.50-1.34 (m, 1H), 1.33-1.19 (m, 9H), 1.19-1.04 (m, 2H), 0.78-0.54 (m, 2H).

Step 3: (3-((2S,3R)-3-(aminomethyl)-2-(methoxycarbonyl)pyrrolidin-3-yl)propyl)boronic acid

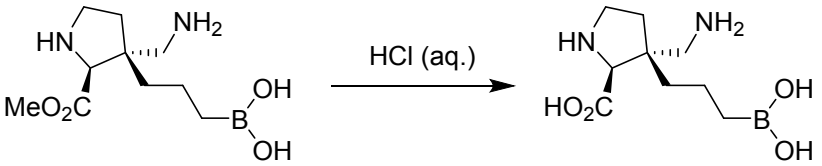


S62 **S63**

A mixture of 10 wt% Pd-C and 20 wt% Pd(OH)2 (1:1, 37 mg, 0.034 mmol) was added to the stirred solution of (2S,3R)-1-benzyl 2-methyl 3-(((4-

methoxybenzyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (20 mg, 0.034 mmol) in MeOH (8.0 mL) under N2 atmosphere. The mixture was degassed and backfilled with H2 (three times), and stirred under H2 (15 psi) at 25 °C for 6 h. The reaction mixture was filtered and concentrated to give crude (3-((2S,3R)-3-(aminomethyl)-2-(methoxycarbonyl)pyrrolidin-3-yl)propyl)boronic acid (10 mg) as a yellow oil, which was used in the next step directly without further purification. LCMS (C10H22BN2O4+) (ES, m/z): 245.1 [M+H]+.

Step 4: (2S,3R)-3-(aminomethyl)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid



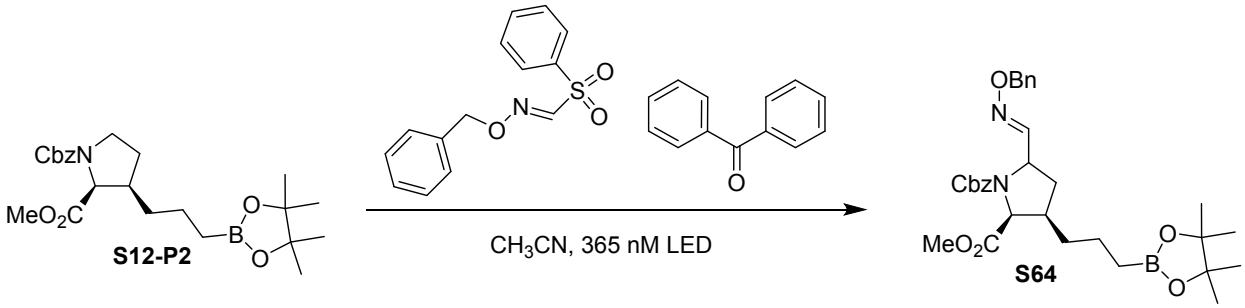
S63 **12**

A mixture of (3-((2S,3R)-3-(aminomethyl)-2-(methoxycarbonyl)pyrrolidin-3-yl)propyl)boronic acid (10 mg, 0.041 mmol) in 12 N HCl in water (10 mL, 0.12 mol) was stirred at 105 °C for 13 h. The reaction mixture was concentrated and the residue was purified by RP-HPLC [C18 column, water (20 mM HFBA and 0.1% TFA)-CH3CN] to give (2S,3R)-3-(aminomethyl)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid (3 mg, 32% yield, HFBA salt) as a white solid. LCMS (C9H18BN2O3+) (ES, m/z): 213.2 [M+H-H2O]+.

1H-NMR (500MHz, D2O) δ 4.12 (s, 1H), 3.49 - 3.39 (m, 3H), 3.11 (d, J = 13.7 Hz, 1H), 2.29-2.26 (m, 1H), 1.93 - 1.82 (m, 1H), 1.48 - 1.32 (m, 4H), 0.82-0.80 (m, 2H).

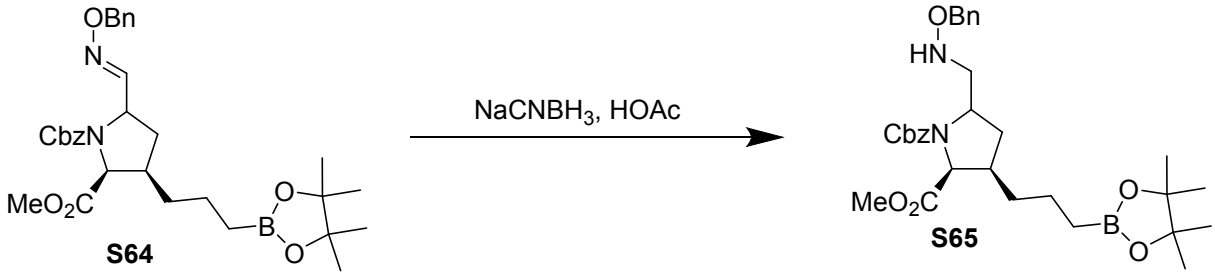
Synthesis of compound 15

Step 1: 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)imino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



(Phenylsulfonyl)methanal O-benzyl oxime (1.6 g, 5.8 mmol) and benzophenone (0.71 g, 3.9 mmol) were added to a scintillation vial containing R-2 (1.7 g, 3.9 mmol) under N2, followed by addition of acetonitrile (40 mL). The reaction mixture was stirred under N2 for 5 min, then irradiated at 365 nm for 24 h at 1000 rpm in Gen2 Merck Photoreactor (100% intensity). The resulting mixture was filtered and concentrated, and the residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)imino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (2.8 g, inseparable mixture containing unreacted starting material) as a yellowish oil, which was used directly in the next step without further purification. LC-MS (C31H42BN2O7+) (ES, m/z): 565.3 [M+H]+.

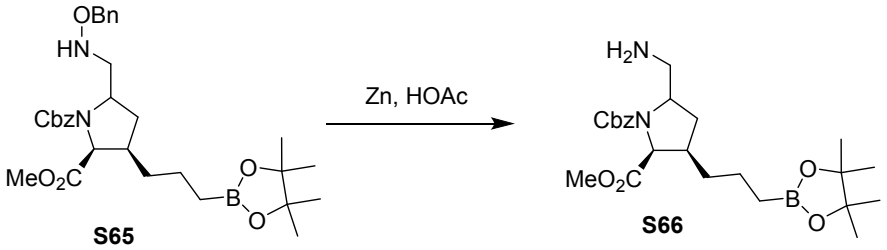
Step 2: 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Sodium cyanoborohydride (1.0 g, 16 mmol) was added in one portion to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)imino)methyl)-3-(3-(4,4,5,5-

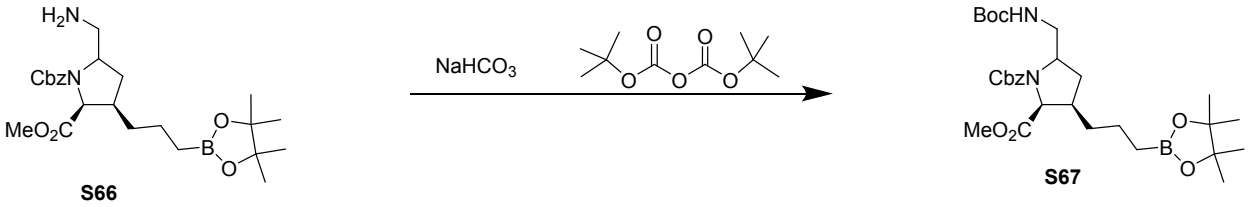
tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (2.8 g, crude mixture) in acetic acid (12 mL) at rt, and the reaction mixture was stirred overnight. The resulting mixture was diluted with EtOAc, quenched with saturated aqueous Na2CO3 to pH ~ 7, and extracted with EtOAc. The combined organic phase was dried over Na2SO4, filtered and concentrated to afford crude 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (1.6 g) as a colorless oil, which was used directly in the next step without further purification. LC-MS (C31H44BN2O7+) (ES, m/z): 567.2 [M+H]+.

Step 3: 1-benzyl 2-methyl (2S,3R)-5-(aminomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Zinc powder (0.64 g, 5.3 mmol) was added in one portion to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.64 g, crude) in acetic acid (7.4 mL), the resulting slurry was sonicated for 30 s and then stirred at rt for 5 h. The reaction mixture was diluted with MeOH, filtered and the filter cake was rinsed with EtOAc. The combined filtrate was concentrated to yield crude 1-benzyl 2-methyl (2S,3R)-5-(aminomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.23 g), which was used without further purification in the next step. LC-MS (C24H38BN2O6+) (ES, m/z): 461.2 [M+H]+.

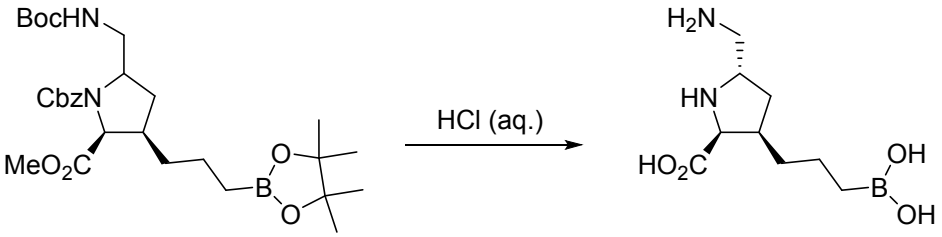
Step 4: 1-benzyl 2-methyl (2S,3R)-5-(((tert-butoxycarbonyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Saturated aqueous NaHCO3 (1.2 mL) was added to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(aminomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.12 g, 0.26 mmol) in THF (3.7 mL), followed by di-tert-butyl dicarbonate (0.31 g, 1.4 mmol) in one portion at rt. The reaction mixture was stirred at rt overnight, then concentrated, and extracted with EtOAc. The combined organic phase was dried over Na2SO4, pinacol (150 mg, 1.3 mmol) was added. The resulting mixture was aged for 30 min at rt, filtered and concentrated, the residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-benzyl 2-methyl (2S,3R)-5-(((tert-butoxycarbonyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.15 g, 97% yield) as a colorless oil. LC-MS (C29H45BN2NaO8+) (ES, m/z): 583.0 [M+Na]+.

1H-NMR (499 MHz, Chloroform-d) δ 7.38 – 7.25 (m, 6H), 5.22 – 5.08 (m, 1H), 4.97 (d, J = 12.3 Hz, 1H), 4.34 (dd, J = 14.6, 8.3 Hz, 1H), 4.12 (dd, J = 40.0, 6.8 Hz, 1H), 3.70 (s, 1H), 3.47 (s, 2H), 3.30 (t, J = 5.5 Hz, 1H), 2.50 (d, J = 6.3 Hz, 1H), 1.86 (ddt, J = 40.1, 25.3, 9.5 Hz, 4H), 1.47 – 1.33 (m, 12H), 1.23 (d, J = 8.6 Hz, 22H), 1.07 (ddt, J = 30.1, 15.3, 7.8 Hz, 1H), 0.74 (dh, J = 15.8, 7.8 Hz, 2H).

Step 5: (2S,3R,5S)-5-(aminomethyl)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid



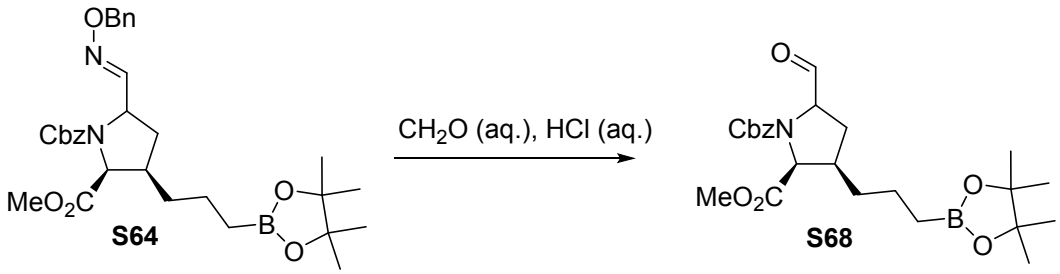
S67 **15**

12N HCl (4.0 mL, 48 mmol) was added to the stirred suspension of 1-benzyl 2-methyl (2S,3R)-5-(((tert-butoxycarbonyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (156 mg, 0.26 mmol) in water (4.0 mL) at rt, and the reaction mixture was heated to 90 °C with stirring overnight, then cooled to room temperature. The mixture was diluted with water, filtered through a 0.25 um filter and lyophilized to afford (2S,3R,5S)-5-(aminomethyl)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid (45 mg, 58% yield) as a yellow solid, relative stereochemistry determined by 2D NMR. HRMS (ESI) calcd for C9H18BN2O3 $[M-H_2O+H]^+$, 213.1412; found, 213.1420.

1H-NMR (500 MHz, D2O) δ 4.47 (d, J = 7.2 Hz, 1H), 4.24 (quintet, J = 7.4 Hz, 1H), 3.49 (dd, J = 13.7, 7.0 Hz, 1H), 3.38 (dd, J = 13.7, 7.0 Hz, 1H), 2.79-2.72 (m, 1H), 2.27 (ddd, J = 13.3, 7.5, 5.0 Hz, 1H), 2.09 (dt, J = 13.8, 7.2 Hz, 1H), 1.56-1.38 (m, 3H), 1.32-1.23 (m, 1H), 0.87-0.74 (m, 2H).

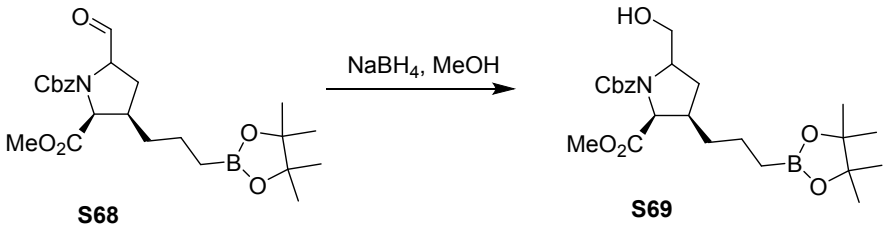
Synthesis of compound 14

Step 1: 1-benzyl 2-methyl (2S,3R)-5-formyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Formaldehyde solution (37 wt% in water, 11 mL, 142 mmol) was added in one portion to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(((benzyloxy)imino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (50 wt%, 2.9 g, 2.5 mmol) in THF (42 mL) at rt, followed by addtion of 1N HCl in water (11 mL, 11 mmol), and the reaction mixture was stirred overnight. The resulting mixture was quenched with saturated aqueous Na2CO3 to pH ~ 7, and extracted with EtOAc. The combined organic phase was dried over Na2SO4, filtered and concentrated to afford crude 1-benzyl 2-methyl (2S,3R)-5-formyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (1.2 g) as a colorless oil, which was used in the next step without further purification. LC-MS (C24H34BNNaO7+) (ES, m/z): 482.1 [M+Na]+.

Step 2: 1-benzyl 2-methyl (2S,3R)-5-(hydroxymethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

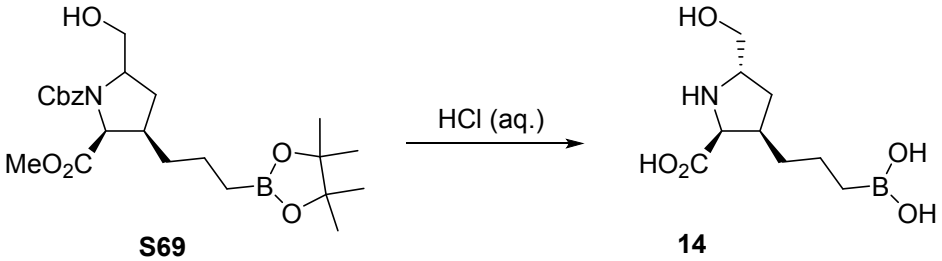


NaBH4 (0.53 g, 14 mmol) was added in one portion to the stirred solution of crude 1-benzyl 2-methyl (2S,3R)-5-formyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (1.2 g, 2.5 mmol) in MeOH (25 mL) at -15 °C, the

resulting slurry was allowed to warm to rt and then stirred at rt overnight. The reaction mixture was quenched with saturated aqueous NH4Cl solution, and extracted with EtOAc. The combined organic phase was dried Na2SO4, filtered and concentrated, the residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-benzyl 2-methyl (2S,3R)-5-(hydroxymethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.91 g, 82% yield) as a colorless oil. LC-MS (C24H3BNO7+) (ES, m/z): 462.2 [M+H]+.

1H-NMR (499 MHz, Chloroform-d) δ 7.37 – 7.24 (m, 7H), 5.20 (dd, J = 12.3, 6.0 Hz, 1H), 5.04 (dd, J = 47.6, 12.3 Hz, 1H), 4.35 (d, J = 8.2 Hz, 1H), 4.30 – 4.07 (m, 1H), 3.74 – 3.63 (m, 2H), 3.51 (s, 2H), 2.48 (dq, J = 12.6, 7.0, 6.2 Hz, 1H), 1.93 – 1.77 (m, 4H), 1.42 (tq, J = 15.3, 8.1, 7.3 Hz, 3H), 1.23 (d, J = 5.8 Hz, 25H), 1.13 – 0.98 (m, 1H), 0.74 (dp, J = 15.9, 8.2, 7.5 Hz, 2H).

Step 3: (2S,3R,5S)-3-(3-boronopropyl)-5-(hydroxymethyl)pyrrolidine-2-carboxylic acid



S69 **14**

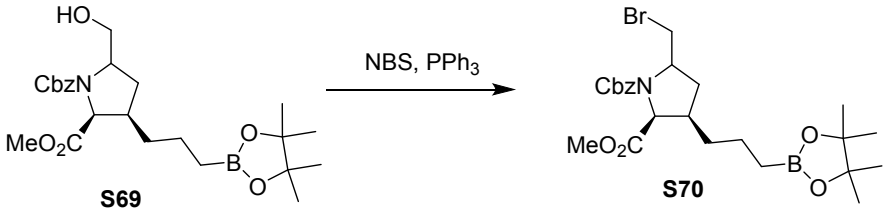
12N HCl (89 mL, 1.1 mol) was added to the stirred suspension of 1-benzyl 2-methyl (2S,3R)-5-(((tert-butoxycarbonyl)amino)methyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (1.9 g, 4.0 mmol) in water (89 mL) at rt, and the reaction mixture was heated to 90 °C with stirring for 24 h, then cooled to room temperature. The mixture was diluted with water, filtered through a 0.25 um filter and

lyophilized to afford (2S,3R,5S)-3-(3-boronopropyl)-5-(hydroxymethyl)pyrrolidine-2-carboxylic acid as a HCl salt. LCMS (C9H17BNO4+) (ES, m/z): 214.1 [M-H2O+H]+.

1H-NMR (500 MHz, D2O) δ 4.39 (d, J = 7.0 Hz, 1H), 4.10-4.05 (m, 1H), 3.86 (dd, J = 12.4, 3.9 Hz, 1H), 3.69 (dd, J = 12.4, 7.2 Hz, 1H), 2.69 (septet, J = 5.6, 1H), 2.08-1.97 (m, 2H), 1.58-1.37 (m, 3H), 1.31-1.22 (m, 1H), 0.86-0.74 (m, 2H).

Synthesis of compound 13

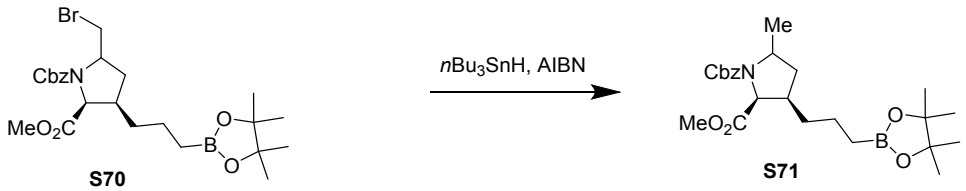
Step 1: 1-benzyl 2-methyl (2S,3R)-5-(bromomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



Triphenylphosphine (0.41 g, 1.6 mmol) was added in one portion to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(hydroxymethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.14 g, 0.31 mmol) in THF (3.1 mL), followed by addtion of N-bromosuccinimide (0.27 g, 1.5 mmol) in one portion at 0 °C. The reaction mixture was allowed to warm to rt and diluted with CH2Cl2 (3.1 mL) and then stirred at rt overnight. The resulting mixture was concentrated and the residue was purified by silica gel chromatography (EtOAc in hexanes) to yield crude 1-benzyl 2-methyl (2S,3R)-5-(bromomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.11 g, 67% yield) as a yellowish oil. LC-MS (C24H36BBrNO6+) (ES, m/z): 524.1 [M+H]+.

1H-NMR (499 MHz, Chloroform-d) δ 7.40 – 7.25 (m, 6H), 5.21 (dd, J = 12.4, 2.2 Hz, 1H), 5.04 (dd, J = 73.8, 12.4 Hz, 1H), 4.39 – 4.27 (m, 2H), 3.73 – 3.66 (m, 2H), 3.50 (s, 2H), 3.42 (dd, J = 9.7, 8.6 Hz, 1H), 2.67 – 2.53 (m, 1H), 2.12 (ddd, J = 17.0, 12.9, 6.5 Hz, 1H), 1.90 (dtd, J = 34.6, 12.9, 8.7 Hz, 1H), 1.49 – 1.34 (m, 3H), 1.24 (d, J = 3.2 Hz, 12H), 1.14 – 1.02 (m, 1H), 0.85 – 0.69 (m, J = 7.8 Hz, 2H).

Step 2: 1-benzyl 2-methyl (2S,3R)-5-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

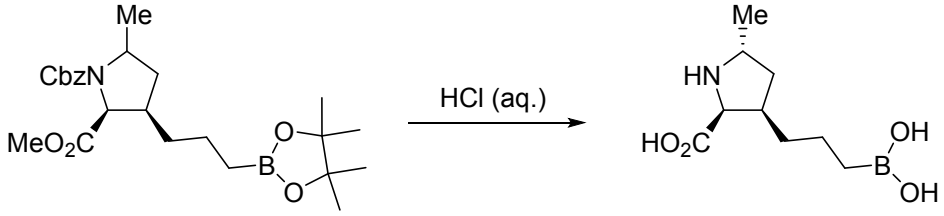


Tri-n-butyltin hydride (66 µL, 0.25 mmol) was added to the stirred solution of 1-benzyl 2-methyl (2S,3R)-5-(bromomethyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.11 g, 0.20 mmol) in toluene (0.82 mL), followed by 2,2'-azobis(2-methylpropionitrile) (1.7 mg, 10 µmol) in one portion at rt. The reaction mixture was stirred at 85 °C for 3 h, then diluted with CH2Cl2, and purified by silica gel chromatography (EtOAc in hexanes) to afford 1-benzyl 2-methyl (2S,3R)-5-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (81 mg, 80% yield) as a colorless oil. LC-MS (C24H37BNO6+) (ES, m/z): 446.2 [M+H]+.

1H-NMR (499 MHz, Chloroform-d) δ 7.37 – 7.26 (m, 6H), 5.19 (d, J = 12.5 Hz, 1H), 5.02 (dd, J = 63.0, 12.5 Hz, 1H), 4.33 (dd, J = 12.6, 8.3 Hz, 1H), 4.19 (dp, J = 21.1, 6.6 Hz, 1H), 3.71 (s, 1H), 3.50 (s, 2H), 2.52 (dd, J = 13.3, 5.3 Hz, 1H), 1.89 (dtd, J = 28.7, 12.5, 8.4 Hz, 1H), 1.71 – 1.59 (m, 1H), 1.43 (tdd, J = 27.3, 15.4, 7.2 Hz, 3H), 1.23 (d, J = 2.9 Hz,

14H), 1.18 (d, J = 6.4 Hz, 1H), 1.10 – 1.02 (m, 1H), 0.77 (dh, J = 16.0, 8.3, 7.8 Hz, 2H).

Step 3: (2S,3R,5R)-3-(3-boronopropyl)-5-methylpyrrolidine-2-carboxylic acid



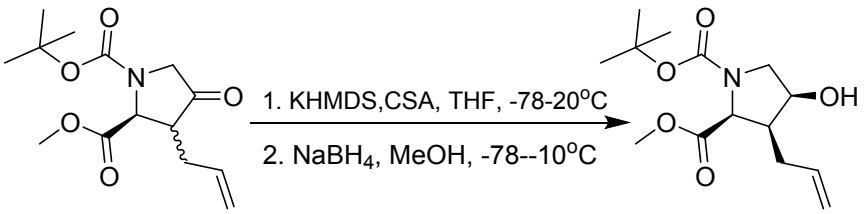
S71 **13**

12N HCl in water (3.0 mL, 36 mmol) was added to the stirred suspension of 1-benzyl 2-methyl (2S,3R)-5-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (81 mg, 0.16 mmol) in water (3.0 mL) at rt, and the reaction mixture was heated to 90 °C with stirring overnight, then cooled to room temperature. The mixture was diluted with water, filtered through a 0.25 um filter and lyophilized to afford (2S,3R,5R)-3-(3-boronopropyl)-5-methylpyrrolidine-2-carboxylic acid as a HCl salt. HRMS (ESI) calcd for C9H17BNO3 $[M\text{-}H_2O+H]^+$, 198.1303; found, 198.1307.

1H-NMR (500 MHz, D2O) δ 4.37 (d, J = 7.4 Hz, 1H), 4.04-4.00 (m, 1H), 2.74-2.68 (m, 1H), 2.13 (ddd, J = 13.2, 7.0, 5.0 Hz, 1H), 1.90 (dt, J = 13.2, 7.2 Hz, 1H), 1.54-1.34 (m, 3H), 1.40 (d, J = 6.8 Hz, 3H), 1.28-1.20 (m, 1H), 0.86-0.74 (m, 2H).

Synthesis of compound 18

Step 1: 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate



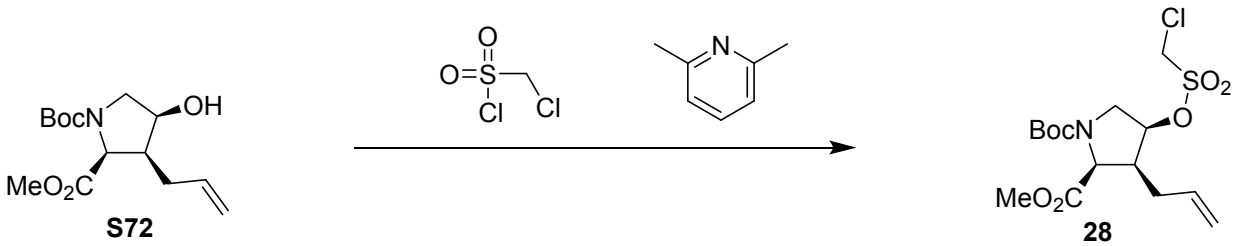
S46 **S72**

KHMDS (1.0 M, 247 mL, 247 mmol) was added dropwise to a solution of 1-(tert-butyl) 2-methyl (2S)-3-allyl-4-oxopyrrolidine-1,2-dicarboxylate (70 g, 247 mmol) in THF (2000 mL) at -78 °C under N_2. The reaction mixture was held at -78 °C for 10 minutes then warmed to -20 °C for 30 minutes, followed by dropwise addition of a solution of CSA (69 g, 296 mmol) in THF (230 mL) at -78 °C. The reaction mixture was held at -78 °C for 10 minutes then warmed to rt for 1 h, before addition of pre-cooled MeOH (-78 °C, 660 mL) at -78 °C, followed by addition of NaBH4 (13 g, 346 mmol) at -78 °C. The resulting mixture was allowed to warm to -10 °C slowly over 1 h, then diluted with EtOAc, and poured into saturated aqueous NH_4Cl slowly at 0 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine three times, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford a mixture of stereoisomers with desired product present, which was purified again by silica gel chromatography (EtOAc in DCM) to afford 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate as a black oil (32 g, 15% yield, single isomer). LCMS (C9H16NO3+) (ES, m/z): 186.1 [M-CO2C4H8+H]+.

1H-NMR (499 MHz, methanol-d4) δ 5.99 – 5.83 (m, 1H), 5.21 – 5.11 (m, 1H), 5.10 – 5.02 (m, 1H), 4.84 (s, 1H), 4.34 (d, J = 9.0 Hz, 1H), 4.25 – 4.18 (m, 1H), 3.76 (s, 1.8H), 3.75 (s, 1.2H), 3.63 – 3.49 (m, 2H), 2.58 – 2.41 (m, 1H), 2.32 – 2.18 (m, 2H), 1.48 (s, 3.6H), 1.43

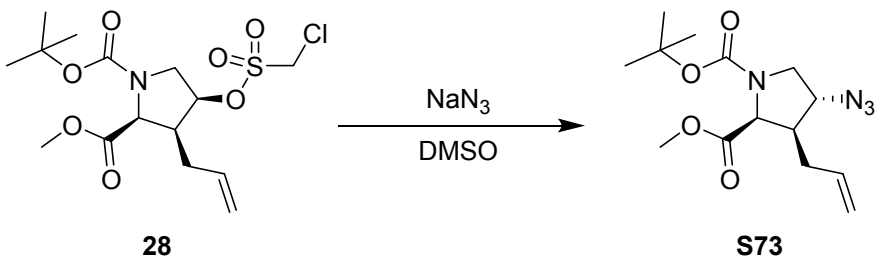
(s, 5.4H).

Step 2: 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-(((chloromethyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate



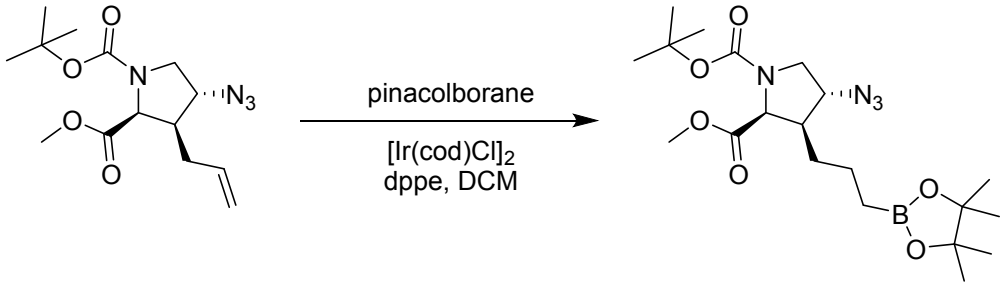
2,6-Dimethylpyridine (19 g, 179 mmol) followed by chloromethanesulfonyl chloride (11 g, 69 mmol) were added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate (8.5 g, 30 mmol) in DCM (99 mL) and 0 °C. The resulting solution was allowed to warm to rt and stirred overnight. Reaction mixture was diluted with water and EtOAc. The organic layer was separated then washed with 1 N HCl in water twice, saturated aqueous NaHCO3 and brine. The resulting organic layer was dried over Na2SO4, filtered and concentrated to afford crude 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-(((chloromethyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (12 g, 100% yield) as a brownish oil, which was used directly in the next step without further purification. LCMS (C10H17ClNO5S+) (ES, m/z): 298.0 [M-CO2C4H8+H]+.

Step 3: 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-azidopyrrolidine-1,2-dicarboxylate



Sodium azide (33 mg, 0.50 mmol) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-(((chloromethyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (100 mg, 0.25 mmol) in DMSO (1.7 mL). The reaction mixture was stirred at 80 °C for 1 h. After cooling to rt the reaction was diluted with saturated aqueous NaHCO3 and EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated to afford crude 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-azidopyrrolidine-1,2-dicarboxylate (77 mg, 99% yield) as a bronze oil, which was used directly in the next step without further purification. LCMS (C9H15N4O2+) (ES, m/z): 211.2 [M-CO2C4H8+H]+.

Step 4: 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-azido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

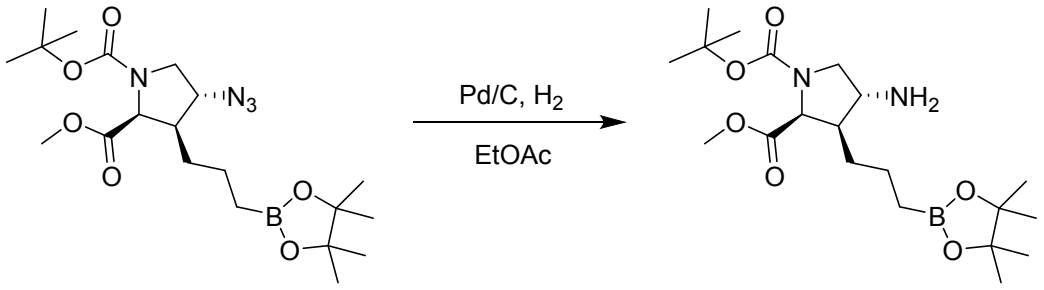


S73 **S74**

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 µL, 0.58 mmol), chloro(1,5-cyclooctadiene)Iridium(I) dimer (7.8 mg, 0.012 mmol) and 1,2-bis(diphenylphosphino)ethane (9.2 mg, 0.023 mmol) in anhydrous DCM (3 mL) was placed under an atmosphere of argon and the resulting mixture was stirred at ambient temperature for 20 minutes, followed by addition of a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-azidopyrrolidine-1,2-dicarboxylate (72 mg, 0.23 mmol) in DCM (1.5 mL). The reaction mixture was stirred at ambient temperature for 21 hours under argon. Reaction was quenched by slow addition of methanol then diluted with water and DCM. The organic layer was separated, washed with

brine, dried over Na2SO4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-azido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (15 mg, 15% yield) as a colorless oil. LCMS (C15H28BN4O4+) (ES, m/z): 339.2 [M-CO2C4H8+H]+.

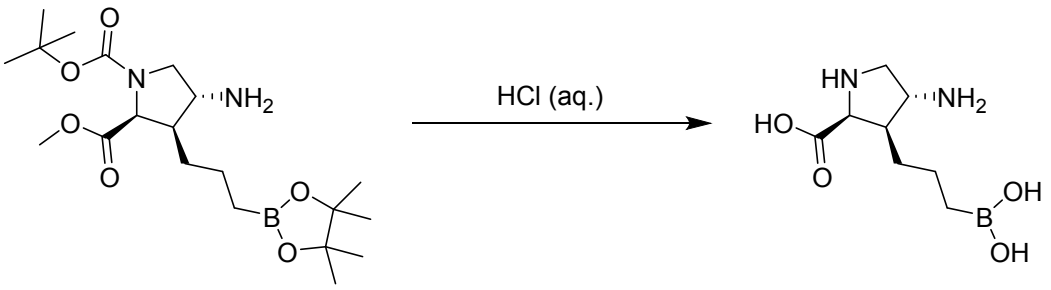
Step 5: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-amino-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



S74 **S75**

Pd/C (10 wt%, 25 mg) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-azido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (104 mg, 0.24 mmol) in EtOAc (5.0 mL). The reaction mixture was degassed and backfilled with H2 three times then stirred under H2 for 16 h. The mixture was filtered and concentrated to give crude 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-amino-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate as a dark oil (98 mg, 100% yield), which was used directly for the next step without further purification. LCMS (C15H30BN2O4+) (ES, m/z): 313.1 [M-CO2C4H8+H]+.

Step 6: (2S,3R,4R)-4-amino-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid



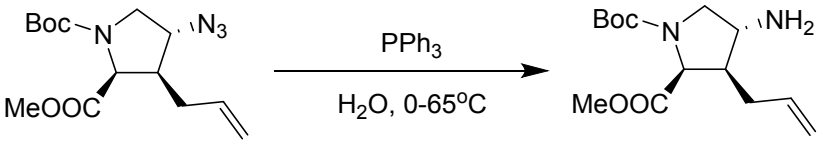
S75 **18**

A mixture of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-amino-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (26 mg, 0.064 mmol) and 6 N HCl in water (1.5 mL, 9.0 mmol) was heated in a microwave reactor with stirring at 120 °C for 1 h. The reaction mixture was concentrated to give (2S,3R,4R)-4-amino-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid (16 mg, 88%) as a pale tan solid. HRMS (ESI) calcd for C8H16BN2O3 $[M-H_2O+H]^+$, 199.1255; found, 199.1264.

1H-NMR (499 MHz, D2O) δ 4.52 (d, J = 7.2 Hz, 1H), 4.02 – 3.88 (m, 2H), 3.44 (dd, *J* = 13.0, 4.3 Hz, 1H), 2.74 – 2.61 (m, 1H), 1.54 – 1.31 (m, 4H), 0.81 – 0.66 (m, 2H).

Synthesis of compound 19

Step 1: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate



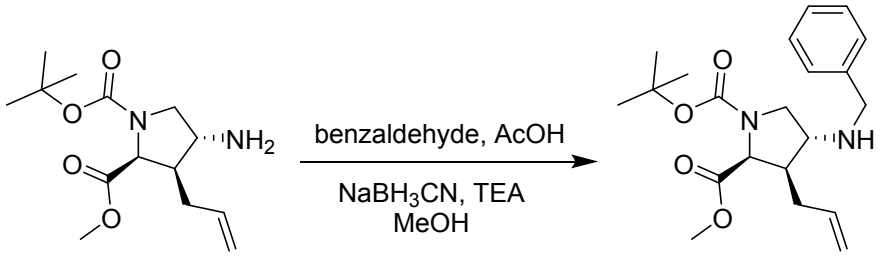
S73 **S76**

Crude 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-azidopyrrolidine-1,2-dicarboxylate (35 g, 112 mmol) was added to water (400 mL) at rt, followed by portionwise addition of PPh_3 (59 g, 224 mmol) at 0 °C. The resulting mixture was stirred at 65 °C for 16 h, then cooled to rt and extracted by EtOAc three times. The combined organic layers were dried

over Na2SO4, filtered, and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate (6.0 g, 17% yield) as a pale yellow oil. LCMS (C9H17N2O2+) (ES, m/z): 185.2 [M-CO2C4H8+H]+.

1H-NMR: (400 MHz CDCl3) δ: 5.82-5.92 (m, 1H), 5.04-5.12 (m, 2H), 4.36-4.45 (m, 1H), 3.84-3.94 (m, 1H), 3.70-3.71 (m, 3H), 3.32-3.42 (m, 1H), 2.94-3.02 (m, 1H), 2.18-2.27 (m, 1H), 1.98-2.13 (m, 2H), 1.39-1.47 (m, 11H).

Step 2: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzylamino)pyrrolidine-1,2-dicarboxylate

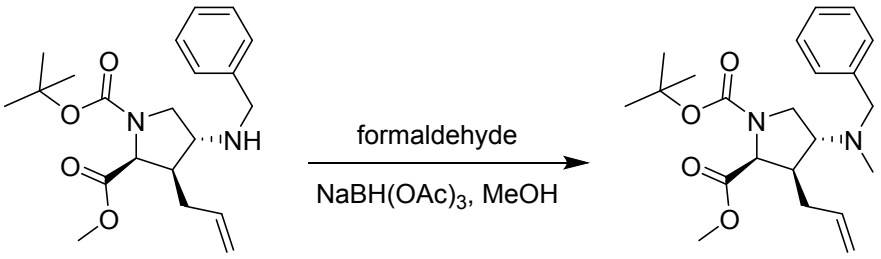


S76 **S77**

Benzaldehyde (33 μL, 0.32 mmol) and acetic acid (17 μL, 0.29 mmol) were added to a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate (83 mg, 0.29 mmol) in MeOH (2.0 mL). The resulting solution was stirred at rt for 1 h then brought to 0 °C. Sodium cyanoborohydride (22 mg, 0.35 mmol) was added to the cooled solution which was allowed to warm to rt with stirring for 18 h. Triethylamine (81 μL, 0.58 mmol) was added to the reaction mixture which was then concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzylamino)pyrrolidine-1,2-dicarboxylate (96 mg, 87% yield) as a colorless oil. LCMS (C21H31N2O4+) (ES, m/z): 375.3 [M+H]+.

1H-NMR (499 MHz, Acetonitrile-d3) δ 7.36 – 7.28 (m, 4H), 7.24 (ddt, J = 8.6, 5.5, 2.7 Hz, 1H), 5.91 – 5.79 (m, 1H), 5.10 – 4.97 (m, 2H), 4.34 – 4.29 (m, 1H), 3.82 – 3.64 (m, 5H), 3.62 (s, 1H), 3.13 – 2.98 (m, 2H), 2.28 (dtt, J = 15.4, 11.0, 6.1 Hz, 2H), 2.13 (s, 1H), 1.92 – 1.82 (m, 1H), 1.38 (d, J = 31.4 Hz, 10H).

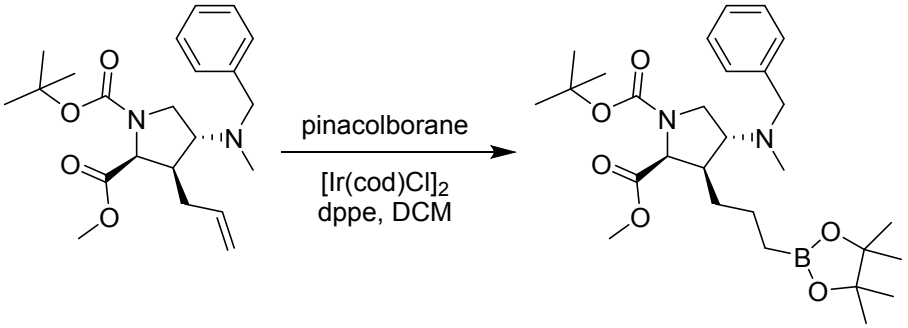
Step 3: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzyl(methyl)amino)pyrrolidine-1,2-dicarboxylate



S77 **S78**

Formaldehyde (37 wt % in water) (38 □L, 0.51 mmol) followed by sodium triacetoxyborohydride (81 mg, 0.38 mmol) were added to a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzylamino)pyrrolidine-1,2-dicarboxylate (95 mg, 0.25 mmol) in MeOH (2.5 mL). The reaction mixture was stirred at ambient temperature for 17 hours. Reaction was concentrated under reduced pressure and purified directly by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzyl(methyl)amino)pyrrolidine-1,2-dicarboxylate (73 mg, 74%) as a colorless oil. LCMS (C22H33N2O4+) (ES, m/z): 389.2 [M+H]+.

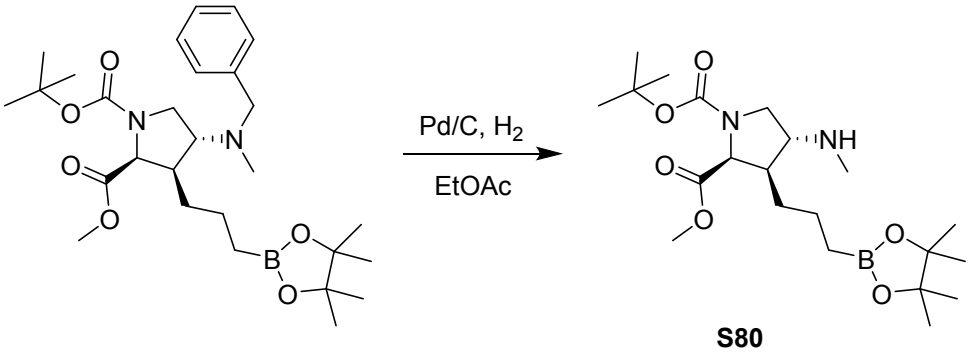
Step 4: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(benzyl(methyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



S78 **S79**

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (68 μL, 0.47 mmol), chloro(1,5-cyclooctadiene)Iridium(I) dimer (6.3 mg, 0.0094 mmol) and 1,2-bis(diphenylphosphino)ethane (7.5 mg, 0.019 mmol) in anhydrous DCM (2.5 mL) was placed under argon and the resulting mixture was stirred at rt for 20 minutes, followed by addition of a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-(benzyl(methyl)amino)pyrrolidine-1,2-dicarboxylate (73 mg, 0.19 mmol) in DCM (1.3 mL). The reaction mixture was stirred at rt for 1.5 h under argon. Reaction was quenched by slow addition of methanol then diluted with water and EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(benzyl(methyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (72 mg, 74% yield) as a colorless oil. LCMS (C28H46BN2O6+) (ES, m/z): 517.3 [M+H]+.

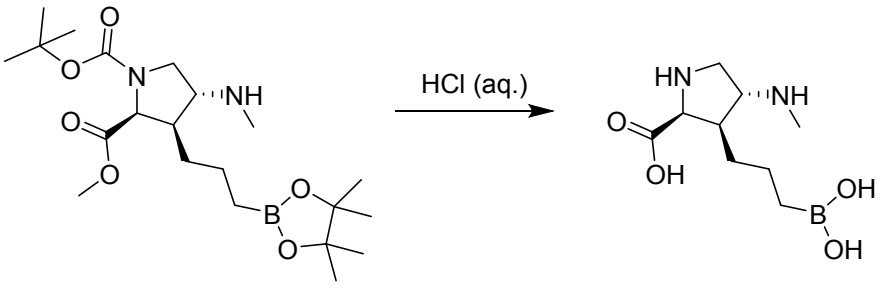
Step 5: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(methylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



S79 **S80**

Pd/C (10 wt%, 15 mg) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(benzyl(methyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (72 mg, 0.14 mmol) in EtOAc (5.0 mL). The reaction mixture was degassed and backfilled with H2 three times then stirred under H2 for 19 h. The mixture was filtered and concentrated. The residue was purified by silica gel chromatography (MeOH in DCM) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(methylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (41 mg, 68% yield) as a colorless oil. LCMS (C21H40BN2O6+) (ES, m/z): 427.3 [M+H]+.

Step 6: (2S,3R,4R)-3-(3-boronopropyl)-4-(methylamino)pyrrolidine-2-carboxylic acid



S80 **19**

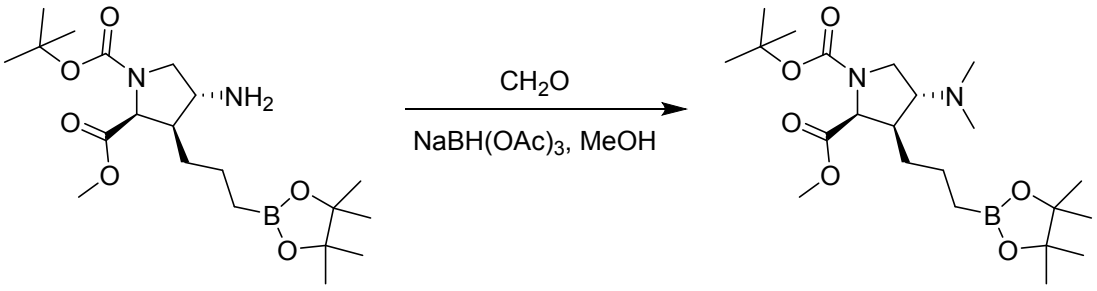
A mixture of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(methylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (41 mg, 0.095 mmol) and 6 N HCl in water (2.0 mL, 12 mmol) was heated in a microwave reactor with

stirring at 120 °C for 2 h. The reaction mixture was concentrated to give (2S,3R,4R)-3-(3-boronopropyl)-4-(methylamino)pyrrolidine-2-carboxylic acid (24 mg, 83%) as a white solid. LCMS (C9H18BN2O3+) (ES, m/z): 213.1 [M-H2O+H]+.

1H-NMR (499 MHz, D2O) δ 4.52 – 4.43 (m, 1H), 3.98 (dd, J = 14.0, 7.9 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.61 – 3.48 (m, 1H), 2.82 – 2.69 (m, 4H), 1.55 – 1.28 (m, 4H), 0.82 – 0.65 (m, 2H).

Synthesis of compound 20

Step 1: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(dimethylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

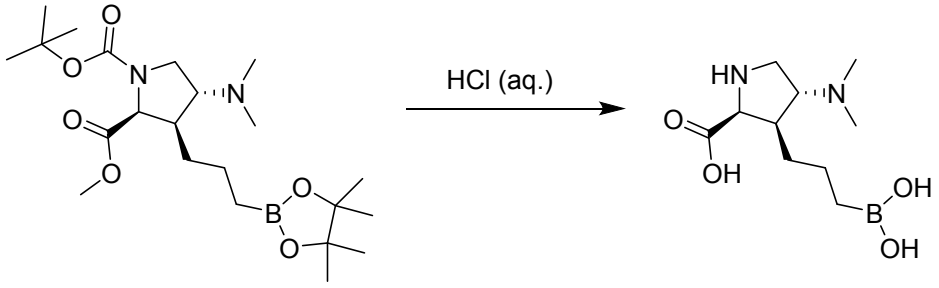


S81 **S82**

Formaldehyde (37 wt % in water, 36 μL, 0.49 mmol) followed by sodium triacetoxyborohydride (77 mg, 0.36 mmol) were added to a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-amino-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (50 mg, 0.12 mmol) in MeOH (1.2 mL). The reaction mixture was stirred at rt for 1.5 h. Reaction was concentrated and the residue was purified by silica gel chromatography (MeOH in DCM) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(dimethylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (28 mg, 52% yield) as a colorless oil. LCMS (C22H42BN2O6+) (ES, m/z):

441.3 [M+H]+.

Step 2: (2S,3S,4R)-3-(3-boronopropyl)-4-(dimethylamino)pyrrolidine-2-carboxylic acid



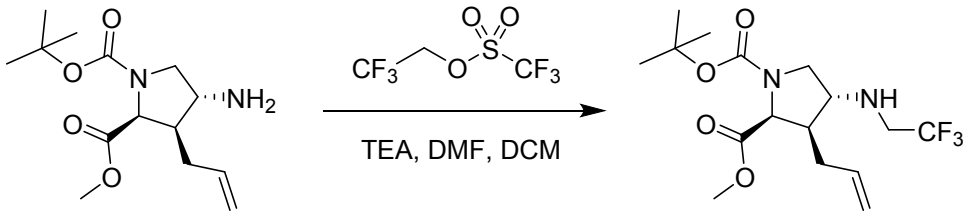
S82 **20**

A mixture of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-(dimethylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (28 mg, 0.063 mmol) and 6 N HCl in water (1.5 mL, 9.0 mmol) was heated in a microwave reactor with stirring at 120 °C for 2 hours. The reaction mixture was concentrated to give (2S,3S,4R)-3-(3-boronopropyl)-4-(dimethylamino)pyrrolidine-2-carboxylic acid (18 mg, 89% yield) as a off-white solid. LCMS (C10H22BN2O4+) (ES, m/z): 245.2 [M+H]+.

1H-NMR (499 MHz, D2O) δ 4.51 (d, J = 7.3 Hz, 1H), 4.04 – 3.97 (m, 2H), 3.70 – 3.61 (m, 1H), 2.97 – 2.83 (m, 7H), 1.52 – 1.36 (m, 4H), 0.79 – 0.68 (m, 2H).

Synthesis of compound 21

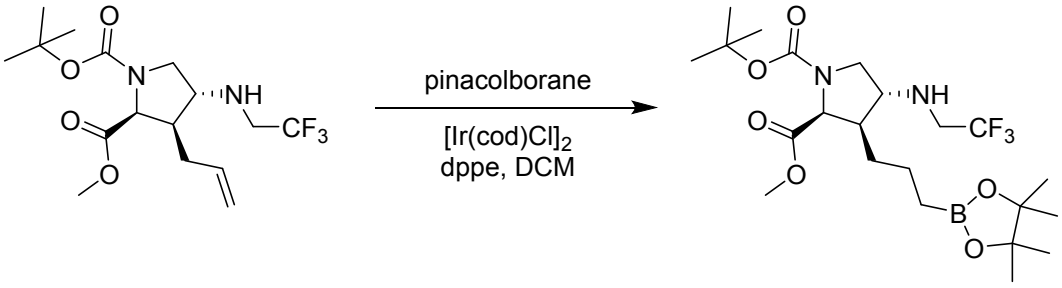
Step 1: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate



S76 **21a**

Triethylamine (98 μL, 0.70 mmol) followed by 2,2,2-trifluoroethyl trifluoromethanesulfonate (63 μL, 0.44 mmol)was added to a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate (50 mg, 0.18 mmol) in DMF (0.60 mL) and DCM (0.60 mL). Reaction was stirred at 50 °C for 2 h then at rt for 18 h. The reaction mixture was diluted with water and EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate (42 mg, 65% yield) as a colorless oil. LCMS (C11H18F3N2O2+) (ES, m/z): 267.0 [M-CO2C4H8+H]+.

Step 2: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate

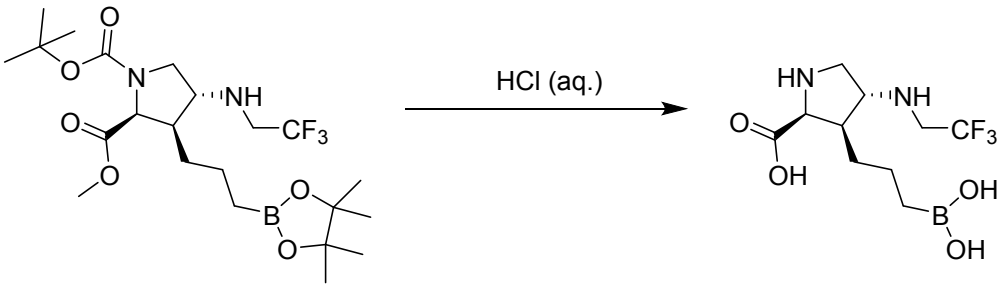


21a **S83**

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1 M in THF, 0.28 mL, 0.28 mmol) was added to a solution of chloro(1,5-cyclooctadiene)Iridium(I) dimer (3.8 mg, 0.0056 mmol) and 1,2-bis(diphenylphosphino)ethane (4.5 mg, 0.011 mmol) in anhydrous DCM (1.5 mL). The resulting solution was placed under argon and stirred at rt for 20 min, followed by addition of a solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate (41 mg, 0.11 mmol) in DCM (0.75 mL). The reaction mixture was stirred at rt for 1.5 h under argon. Reaction was quenched by slow

addition of methanol then diluted with water and EtOAc. The organic layer was separated, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate (40 mg, 73% yield) as a colorless oil. LCMS (C17H31BF3N2O4+) (ES, m/z): 395.2 [M-CO2C4H8+H]+.

Step 3: (2S,3R,4R)-3-(3-boronopropyl)-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-2-carboxylic acid



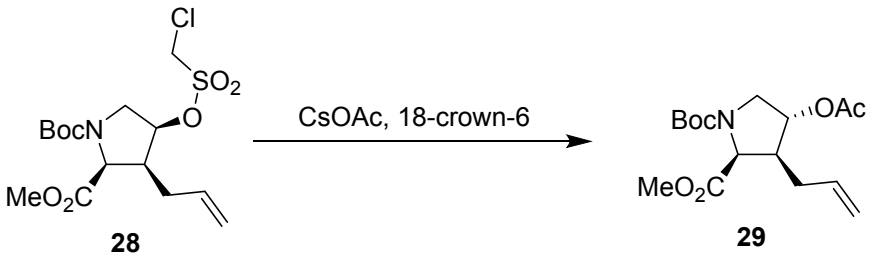
S83 **21**

A mixture of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-1,2-dicarboxylate (40 mg, 0.081 mmol) and 6 N HCl in water (1.5 mL, 9.0 mmol) was heated in a microwave reactor with stirring at 120 °C for 1 h. The reaction mixture was concentrated to afford (2S,3R,4R)-3-(3-boronopropyl)-4-((2,2,2-trifluoroethyl)amino)pyrrolidine-2-carboxylic acid (26 mg, 86% yield) as a white solid. LCMS (C10H19BF3N2O4+) (ES, m/z): 299.4 [M+H]+.

1H-NMR (499 MHz, D2O) δ 4.61 – 4.51 (m, 1H), 3.89 – 3.79 (m, 1H), 3.79 – 3.71 (m, 1H), 3.71 – 3.54 (m, 2H), 3.42 – 3.31 (m, 1H), 2.73 – 2.57 (m, 1H), 1.53 – 1.21 (m, 4H), 0.81 – 0.66 (m, 2H).

Synthesis of compound 16

Step 1: 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-allylpyrrolidine-1,2-dicarboxylate (4)

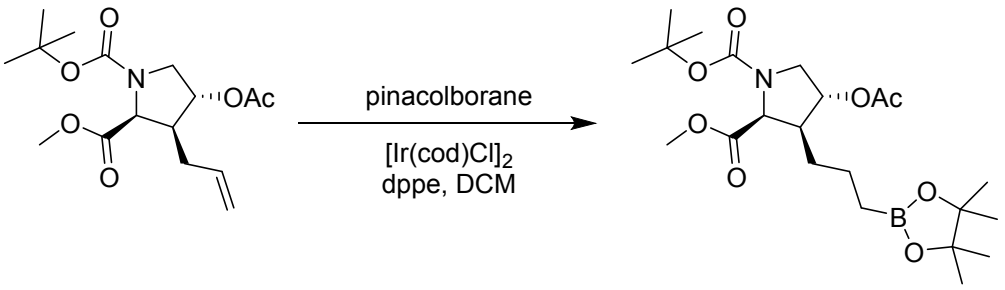


28 **29**

Cesium acetate (17 g, 89 mmol) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4S)-3-allyl-4-(((chloromethyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (12 g, crude) in toluene (0.20 L), followed by addition of 18-crown-6 (3.9 g, 15 mmol) in one portion under N2 at rt. The resulting mixture was sonicated for 5 min, and then stirred at 80 °C for 4 h. After cooling to rt the reaction mixture was diluted with saturated aqueous NaHCO3 and EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-allylpyrrolidine-1,2-dicarboxylate (4.5 g, 46% yield) as a colorless oil. LCMS (C11H18NO4+) (ES, m/z): 228.1 [M-CO2C4H8+H]+.

1H-NMR (499 MHz, Chloroform-d) δ 5.77 (tq, J = 15.2, 7.1 Hz, 1H), 5.12 – 5.02 (m, 3H), 3.95 (ddd, J = 18.1, 11.6, 6.4 Hz, 1H), 3.73 (d, J = 1.5 Hz, 3H), 3.36 (dd, J = 11.8, 4.3 Hz, 1H), 2.58 (dp, J = 15.4, 7.6 Hz, 1H), 2.09 (q, J = 6.1, 4.8 Hz, 2H), 2.05 (s, 3H), 1.43 (d, J = 19.0 Hz, 9H).

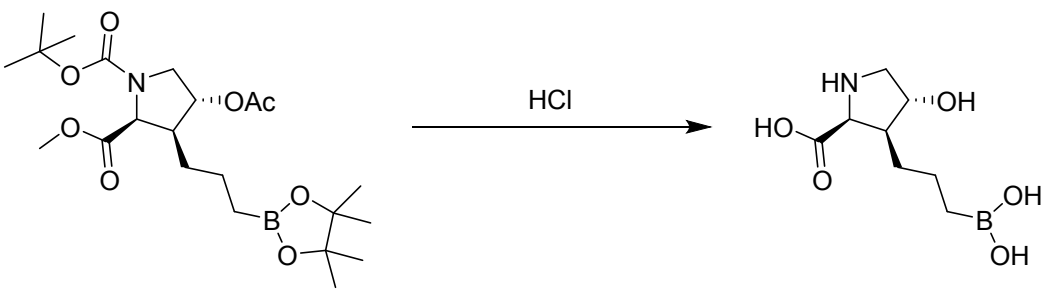
Step 2: 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



29 **S84**

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5.9 mL, 41 mmol), chloro(1,5-cyclooctadiene)Iridium(I) dimer (0.46 g, 0.68 mmol) and 1,2-bis(diphenylphosphino)ethane (0.53 g, 1.4 mmol) in anhydrous DCM (36 mL) was placed under N2 and the resulting mixture was stirred at rt for 20 minutes, followed by addition of a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-allylpyrrolidine-1,2-dicarboxylate (4.5 g, 14 mmol) in DCM (18 mL). The reaction mixture was stirred at rt overnight under N2. The reaction mixture was quenched with saturated aqeuous NH4Cl and extracted with EtOAc. The combined organic phase was washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (6.2 g, 100% yield). LCMS (C17H31BNO6+) (ES, m/z): 356.1 [M-CO2C4H8+H]+.

Step 3: (2S,3S,4R)-3-(3-boronopropyl)-4-hydroxypyrrolidine-2-carboxylic acid



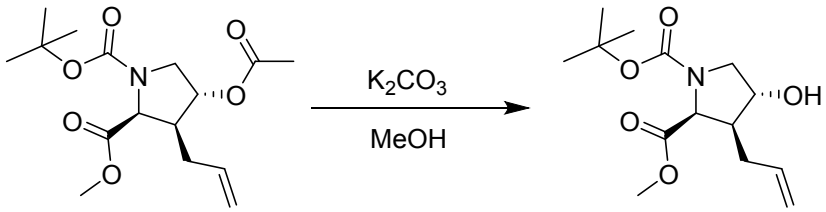
S84 **16**

12N HCl (40 mL, 0.48 mol) was added to the stirred suspension of 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (5.5 g, 12 mmol) in water (40 mL) at rt, and the reaction mixture was heated to 95 °C with stirring for 24 h, then cooled to room temperature. The mixture was diluted with water, filtered through a 0.25 um filter and lyophilized to give (2S,3S,4R)-3-(3-boronopropyl)-4-hydroxypyrrolidine-2-carboxylic acid (3.1 g, 100% yield) as a yellowish solid. HRMS (ESI) calcd for C8H15BNO4 [M-H_2O+H]$^+$, 200.1096; found, 200.1096.

1H-NMR (500 MHz, D2O) δ 4.66 (d, J = 6.8 Hz, 1H), 4.50 – 4.82 (m, 1H), 3.69 (dd, J = 13.1, 4.5 Hz, 1H), 3.31 (d, J = 13.1 Hz, 1H), 2.61 – 2.56 (m, 1H), 1.59 – 1.40 (m, 2H), 1.36-1.27 (m, 1H), 1.21-1.14 (m, 1H), 0.85 – 0.73 (m, 2H). 13C NMR (151 MHz, D2O) δ 172.34, 73.37, 64.22, 50.88, 47.79, 29.15, 21.78, 14.06.

Synthesis of compound 17

Step 1: 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate



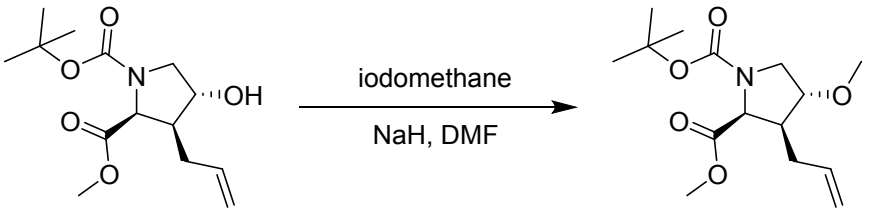
29 **S85**

Potassium carbonate (0.22 g, 1.6 mmol) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-acetoxy-3-allylpyrrolidine-1,2-dicarboxylate (0.49 g, 1.5 mmol) in

MeOH (6.2 mL). The resulting mixture was stirred at rt for 1.5 h then filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate (0.42 g, 99% yield) as a yellowish oil. LCMS (C9H16NO3+) (ES, m/z): 186.2 [M-CO2C4H8+H]+.

1H-NMR (499 MHz, Chloroform-d) δ 5.86 (dddd, J = 17.7, 13.7, 6.3, 4.2 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.24 (tt, J = 11.4, 7.0 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.71 (d, J = 4.3 Hz, 3H), 3.22 (ddd, J = 26.3, 10.9, 6.4 Hz, 1H), 2.36 (dp, J = 28.9, 7.9 Hz, 1H), 2.21 – 2.02 (m, 3H), 1.42 (d, J = 25.3 Hz, 9H).

Step 2: 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-methoxypyrrolidine-1,2-dicarboxylate

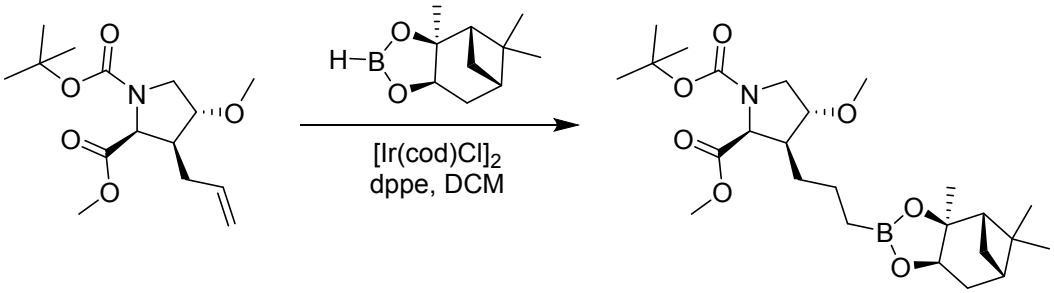


S85 **30**

Sodium hydride (60 wt% in mineral oil, 22 mg, 0.55 mmol) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-hydroxypyrrolidine-1,2-dicarboxylate (78 mg, 0.27 mmol) in DMF (2.5 mL). The resulting solution was stirred for 15 minutes at rt followed by addition of iodimethane (68 □L, 1.1 mmol). The reaction mixture was stirred at rt for 1 h then quenched by addition of saturated aqueous NH4Cl and diluted with EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to

afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-methoxypyrrolidine-1,2-dicarboxylate. LCMS (C10H18NO3+) (ES, m/z): 200 [M-CO2C4H8+H]+.

Step 3: 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

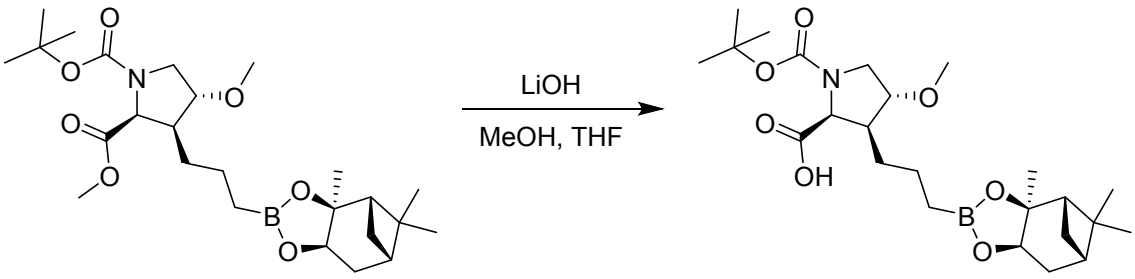


30 **S86**

(3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborole (68 mg, 0.38 mmol), chloro(1,5-cyclooctadiene)Iridium(I) dimer (5.0 mg, 0.0075 mmol) and 1,2-bis(diphenylphosphino)ethane (6.0 mg, 0.015 mmol) in anhydrous DCM (2.0 mL) was placed under argon and the resulting mixture was stirred at rt for 20 minutes, followed by addition of a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-3-allyl-4-methoxypyrrolidine-1,2-dicarboxylate (45 mg, 0.15 mmol) in DCM (1.0 mL). The reaction mixture was stirred at rt for 19 h under argon. Reaction was quenched by slow addition of methanol then diluted with water and EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-1,2-dicarboxylate. LCMS (C20H35BNO5+) (ES, m/z): 380 [M-CO2C4H8+H]+.

Step 4: (2S,3S,4R)-1-(tert-butoxycarbonyl)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-

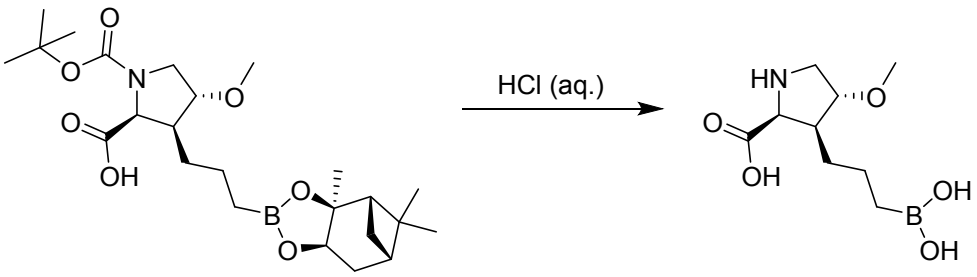
trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-2-carboxylic acid



S86 **S87**

Lithium hydroxide (61 mg, 1.5 mmol) was added to a solution of 1-(tert-butyl) 2-methyl (2S,3S,4R)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (70 mg, 0.15 mmol) in a mixture of THF (3.5 mL) and MeOH (0.5 mL). The reaction mixture was stirred at rt for 89 h. Reaction mixture was acidified to pH ~ 4 by addition of 1 N HCl then diluted with EtOAc. The organic layer was separated, washed with brine, dried over Na2SO4, filtered and concentrated. The resulting residue was purified by silica gel chromatography (EtOAc in hexanes) to afford (2S,3S,4R)-1-(tert-butoxycarbonyl)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-2-carboxylic acid. LCMS (C19H33BNO5+) (ES, m/z): 366 [M-CO2C4H8+H]+.

Step 5: (2S,3S,4R)-3-(3-boronopropyl)-4-methoxypyrrolidine-2-carboxylic acid

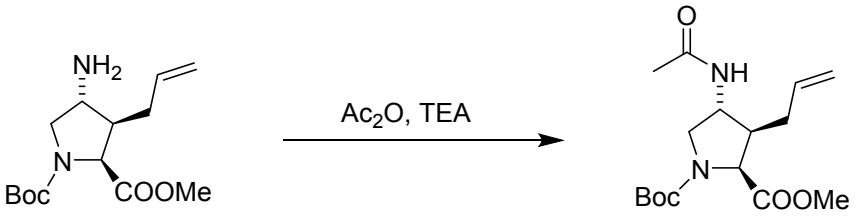


S87 **17**

A mixture of (2S,3S,4R)-1-(tert-butoxycarbonyl)-4-methoxy-3-(3-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)pyrrolidine-2-carboxylic acid (18 mg, 0.038 mmol) and 6 N HCl in water (0.80 mL, 4.8 mmol) was heated to 35 °C with stirring for 18 h. The reaction mixture was concentrated to give (2S,3S,4R)-3-(3-boronopropyl)-4-methoxypyrrolidine-2-carboxylic acid as an HCl salt. LCMS (C9H19BNO5+) (ES, m/z): 232 [M+H]+. 1H NMR (499 MHz, D2O) δ 4.44 (d, J = 6.7 Hz, 1H), 4.05 (d, J = 4.1 Hz, 1H), 3.58 (dd, J = 13.4, 4.4 Hz, 1H), 3.38 (d, J = 13.5 Hz, 1H), 3.30 (s, 3H), 2.72 – 2.62 (m, 1H), 1.55 – 1.43 (m, 1H), 1.42 – 1.31 (m, 1H), 1.31 – 1.21 (m, 1H), 1.13 – 1.03 (m, 1H), 0.79 – 0.65 (m, 2H).

Synthesis of compound 22

Step 1: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-allylpyrrolidine-1,2-dicarboxylate



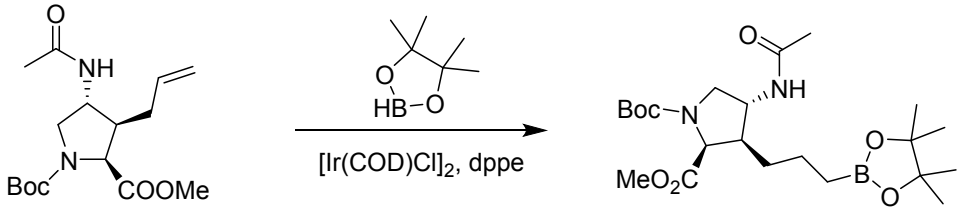
S76 **22a**

Triethylamine (0.22 mL, 1.5 mmol) was added to a solution of 1-(tert-butyl) 2-methyl

(2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate (0.22 g, 0.77 mmol) in DCM (3.9 mL), followed by addition of acetic anhydride (80 μL, 0.85 mmol) dropwise at rt. The resulting mixture was stirred at rt for 1 h, then quenched with saturated aqueous NaHCO3 solution, and extracted with EtOAc. The combined organic phase was washed with brine, dried over Na2SO4, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-allylpyrrolidine-1,2-dicarboxylate (0.20 g, 80% yield) as a colorless oil. LCMS (C16H26N2NaO5+) (ES, m/z): 349.1 [M+Na]+.

1H-NMR (499 MHz, Acetonitrile-d3) δ 6.44 (s, 1H), 5.90 – 5.76 (m, 1H), 5.04 (dd, J = 27.9, 13.7 Hz, 2H), 4.33 (d, J = 8.2 Hz, 1H), 4.30 – 4.19 (m, 1H), 3.75 (ddd, J = 13.7, 10.4, 7.9 Hz, 1H), 3.69 (s, 2H), 3.66 (s, 1H), 3.02 – 2.95 (m, 1H), 2.42 – 2.29 (m, 1H), 2.13 (s, 5H), 1.93 – 1.86 (m, 1H), 1.84 (d, J = 1.3 Hz, 3H), 1.39 (d, J = 25.9 Hz, 9H).

Step 2: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate

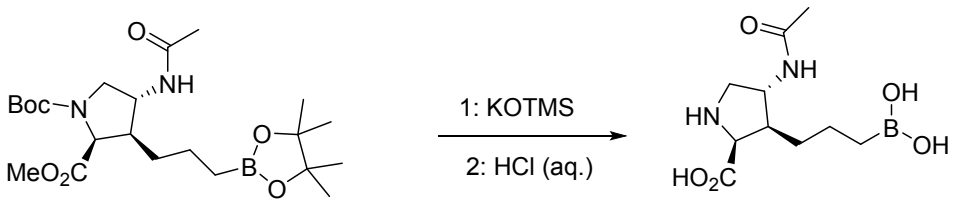


22a **S88**

4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (0.36 mL, 2.5 mmol) was added to the stirred solution of chloro(1,5-cyclooctadiene)iridium(I)dimer (41 mg, 0.061 mmol) and DPPE (49 mg, 0.12 mmol) in DCM (0.50 mL) at rt under N2. The resulting solution was added to the stirred solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-allylpyrrolidine-1,2-dicarboxylate (0.20 g, 0.61 mmol) in DCM (2.0 mL) at rt under N2, and the reaction mixture

was stirred for 2 h, then concentrated and the residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.20 g, 70% yield) as a colorless oil. LCMS (C22H39BN2NaO7+) (ES, m/z): 477.3 [M+Na]+.

Step 3: (2S,3R,4R)-4-acetamido-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid



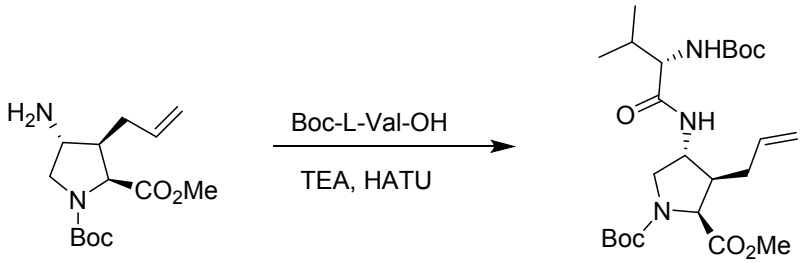
S88 **22**

Potassium trimethylsilanolate (0.11 g, 0.86 mmol) was added to the stirred solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-acetamido-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (0.20 g, 0.43 mmol) in THF (4.3 mL) at rt. The resulting mixture was stirred at rt overnight, and concentrated. The crude residue was treated with 6 N HCl in water (2.0 mL, 12 mmol) and stirred at 60 °C for 60 min. The reaction mixture was cooled to rt, diluted with water and washed with DCM. The aqueous layer was concentrated and the residue was purified by RP-HPLC [C18 column, water (0.2 mM heptafluorobutyric acid/0.1% TFA)-CH3CN] to afford (2S,3R,4R)-4-acetamido-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid (46 mg, 23% yield, HFBA salt) as a white solid. LCMS (C10H18BN2O4+) (ES, m/z): 241.2 [M-H2O+H]+.

1H-NMR (500 MHz, D2O) δ 4.42 (d, J = 7.7 Hz, 1H), 4.22 (q, J = 5.8 Hz, 1H), 3.76 (dd, J = 12.6, 7.4 Hz, 1H), 3.18 (dd, J = 12.6, 5.4 Hz, 1H), 2.49 (p, J = 7.5, 7.0 Hz, 1H), 1.93 (s, 3H), 1.51 – 1.38 (m, 2H), 1.33 (qt, J = 13.6, 6.4 Hz, 2H), 0.78 – 0.65 (m, 2H).

Synthesis of compound 24a

Step 1: 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)pyrrolidine-1,2-dicarboxylate

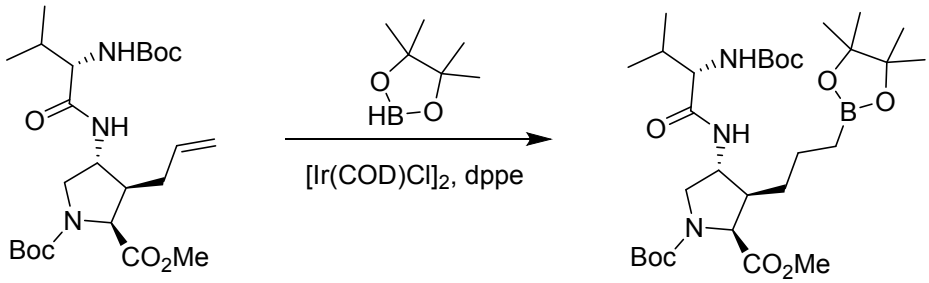


S76 **24**

(Tert-butoxycarbonyl)-L-valine (Boc-L-Val-OH, 20 g, 92 mmol), Et3N (33 mL, 0.23 mol) and HATU (33 g, 86 mmol) were adde sequentially to the stirred solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-aminopyrrolidine-1,2-dicarboxylate (22 g, 78 mmol) in DMF (0.26 L) at rt. The reaction mixture was stirred at rt for 1 h, then diluted with saturated aqueous NaHCO3 solution, and extracted with ether. The combined organic phase was washed with brine, dried over MgSO4, and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)pyrrolidine-1,2-dicarboxylate (36 g, 95% yield) as a white solid. LCMS (C24H41N3NaO7+) (ES, m/z): 506.3 [M+Na]+.

1H-NMR (499 MHz, Acetonitrile-d3) δ 6.69 (d, J = 7.6 Hz, 1H), 5.88 – 5.75 (m, 1H), 5.06 (d, J = 17.1 Hz, 1H), 5.01 (d, J = 10.1 Hz, 1H), 4.34 (dd, J = 8.2, 4.4 Hz, 1H), 4.30 – 4.18 (m, 1H), 3.82 – 3.72 (m, 2H), 3.68 (d, J = 13.2 Hz, 3H), 3.04 (d, J = 12.8 Hz, 1H), 2.41 (s, 1H), 2.13 (s, 3H), 2.00 (d, J = 6.3 Hz, 1H), 1.89 (dt, J = 15.6, 7.9 Hz, 1H), 1.41 (s, 13H), 1.36 (s, 5H), 0.91 (d, J = 6.8 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H).

Step 2: 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate



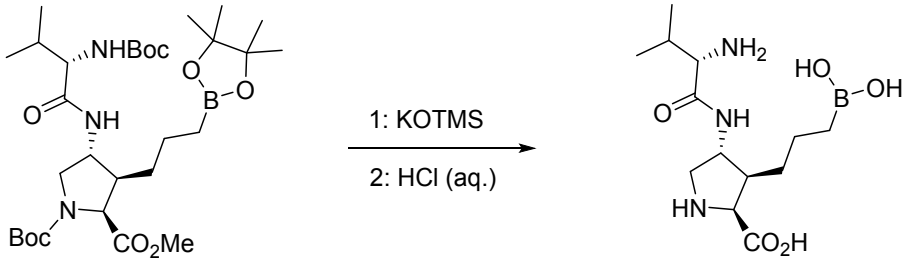
24 **S89**

4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (7.5 mL, 51 mmol) was added to a solution of chloro(1,5-cyclooctadiene)iridium(I)dimer (1.7 g, 2.6 mmol) and DPPE (2.1 g, 5.1 mmol) in DCM (69 mL) at rt under N2. The resulting solution was stirred at rt for 10 min, then added to the solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-3-allyl-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)pyrrolidine-1,2-dicarboxylate (12 g, 26 mmol) in DCM (34 mL) at rt under N2. The reaction mixture was stirred at rt overnight, then quenched with saturated aqueous NH4Cl solution and extracted with EtOAc. The combined organic phase was washed with brine, dried over MgSO4, and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (4.5 g, 29% yield) as a colorless oil. LCMS (C30H54BN3NaO9+) (ES, m/z): 634.4 [M+Na]+.

1H-NMR (499 MHz, Acetonitrile-d3) δ 6.65 (d, J = 7.1 Hz, 1H), 5.41 (s, 1H), 4.35 (d, J = 8.3 Hz, 1H), 4.25 – 4.11 (m, 1H), 3.81 – 3.72 (m, 2H), 3.69 (d, J = 13.3 Hz, 3H), 3.10 – 2.91 (m, 1H), 2.31 (d, J = 6.4 Hz, 1H), 1.99 (s, 1H), 1.51 (dd, J = 15.9, 7.0 Hz, 1H), 1.41 (s,

13H), 1.36 (s, 7H), 1.20 (s, 14H), 1.14 (s, 4H), 0.91 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.66 (q, J = 6.9 Hz, 2H).

Step 3: (2S,3R,4R)-4-((S)-2-amino-3-methylbutanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid



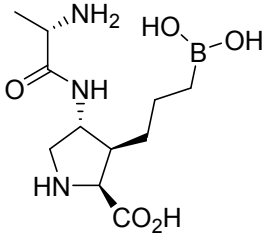
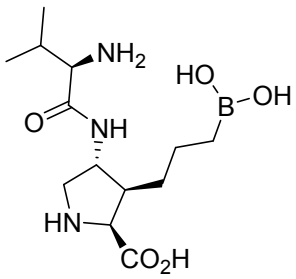
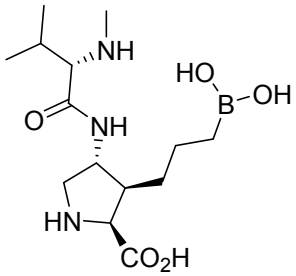
S89 **24a**

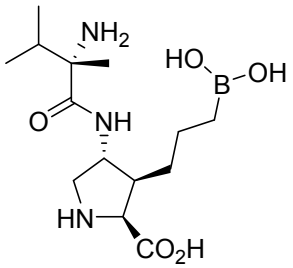
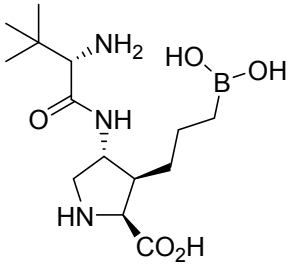
Potassium trimethylsilanolate (2.7 g, 21 mmol) was added to the stirred solution of 1-(tert-butyl) 2-methyl (2S,3R,4R)-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1,2-dicarboxylate (4.3 g, 7.0 mmol) in THF (47 mL) at rt. The reaction mixture was stirred at rt overnight, then diluted with water and extracted with ether. The aqueous layer was acidified with 2N HCl in water to pH ~ 3 and then extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na2SO4, and concentrated. The residue was purified by silica gel column chromatography (EtOAc in hexanes) to afford (2S,3R,4R)-1-(tert-butoxycarbonyl)-4-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-2-carboxylic acid, which was treated with 6 N HCl in water (20 mL, 0.12 mol) and stirred at 60 °C for 1.5 h, then cooled to rt, diluted with water and washed with DCM. The aqueous layer was concentrated to afford (2S,3R,4R)-4-((S)-2-amino-3-methylbutanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid (3.6 g, 85% yield) as a white solid. HRMS (ESI)

calcd for C13H25BN3O4 [M-H2O+H]+, 298.1941; found, 298.1947.

1H-NMR (500 MHz, D2O) δ 4.44 (d, J = 7.8 Hz, 1H), 4.39 (q, J = 6.9 Hz, 1H), 3.92 (dd, J = 12.7, 7.8 Hz, 1H), 3.80 (d, J = 5.7 Hz, 1H), 3.25 (dd, J = 12.7, 6.1 Hz, 1H), 2.59 – 2.53 (m, 1H), 2.27 – 2.21 (m, 1H), 1.56 – 1.38 (m, 4H), 1.05 (dd, J = 8.5, 7.1 Hz, 6H), 0.88 – 0.75 (m, 2H). 13C NMR (151 MHz, D2O) δ 174.07, 172.39, 64.22, 59.60, 52.90, 48.32, 45.20, 31.21, 30.39, 21.73, 18.25, 17.03, 14.08.

Using the general methodology for **24a**, compounds **23**-**27** in the following Table S1 were prepared.

Compound	Structure	Chemical Name	Mass [M-H2O+H]+
23		(2S,3R,4R)-4-((S)-2-aminopropanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid	270.1
24b		(2S,3R,4R)-4-((R)-2-amino-3-methylbutanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid	298.1
25		(2S,3R,4R)-3-(3-boronopropyl)-4-((S)-3-methyl-2-(methylamino)butanamido)pyrrolidine-2-carboxylic acid	312.0

26		(2S,3R,4R)-4-((S)-2-amino-2,3-dimethylbutanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid	312.2
27		(2S,3R,4R)-4-((S)-2-amino-3,3-dimethylbutanamido)-3-(3-boronopropyl)pyrrolidine-2-carboxylic acid	HRMS (ESI) calcd for C14H27BN3O4 [M-H2O+H]+, 312.2097; found, 312.2098.

Arginase Thioornithine Generating Assay (TOGA)

Compounds were serially diluted in ten 3-fold steps in DMSO starting from 10 mM DMSO stocks. Compound dilutions or DMSO alone were then dispensed from the dilution plate into a Greiner black 384-well assay plate (catalog #781086) using an Echo 555 acoustic liquid handler (Labcyte).
Arginase protein was recombinantly expressed in Escherichia coli. Purified protein was then 5 diluted in assay buffer (50 mM Tris pH 7.5, 50 mM NaCl, 1mM manganese chloride, 0.05% bovine serum albumin to obtain a final Arginase concentration of 1.88 nM. Arginase solution (20 µL) or buffer alone (20 µL) were dispensed to wells of the assay plate using a BioRAPTR liquid dispenser (Beckman Coulter). Assay plates containing compound and arginase enzyme were incubated at room temperature for 30 minutes. Afterwards, 5µL of 2.5 mM thioarginine (Cayman Chemicals) in 10 assay buffer were added to each well of the assay plate using a BioRAPTR liquid dispenser. Plates were incubated at room temperature for 60 minutes and reactions were quenched by addition of 15 µL of 200 uM 7-Diethylamine-3-(4-maleimidophenyl)-4-methylcoumarin (Sigma Chemical) in 70% ethanol. Plates were briefly shaken to mix and the fluorescence was measured in an Spectramax plate reader (Molecular Devices) with a 410 nm excitation wavelength and an 490 nm emission 15 wavelength.
The fluorescence intensity of each well was corrected for the background observed in wells that did not receive arginase and was expressed as a fraction of the intensity observed in wells that received arginase enzyme and DMSO only. Potencies were calculated by linear least squares fit to the four parameter logistic IC50 equation.

References

1) Vonrhein C., Flensburg C., Keller P., Sharff A., Smart O., Paciorek W., Womack T. & Bricogne G. (2011). Data processing and analysis with the autoPROC toolbox. Acta Cryst. D67, 293-302.
2) Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2017). BUSTER. Cambridge, United Kingdom: Global Phasing Ltd.
3) Emsley P., Lohkamp B., Scott W. G. & Cowtan K. (2010) Features and Development of Coot. Acta Cryst. D66, 486-501.
4) Smart O. S., Womack T. O., Sharff A., Flensburg C., Keller P., Paciorek W., Vonrhein C. & Bricogne G. (2011) grade. Cambridge, United Kingdom, Global Phasing Ltd.
5) The PyMOL Molecular Graphics System, Schrödinger, LLC.