Supporting Information for

Discovery and Optimization of Rationally Designed Bicyclic Inhibitors of Human Arginase to Enhance Cancer Immunotherapy

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

Cloning, Expression, and Purification of Protein for Crystallography. Full-length untagged human arginase 1 (hArg1) was expressed in *E. coli* BL21 (DE3) using superbroth media. Expression was induced with 1 mM IPTG at OD_{600} 0.8 and cells were grown for 4 hours at 37 °C. Cell pellets were resuspended in lysis buffer (10 mM Tris pH 7.5, 5 mM $MnCl_2$, 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 × G. Clarified lysates were heat-treated at 60 °C for 20minutes. 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 1M 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 $MnCl_2$. Peak fractions were analyzed by SDS-PAGE, pooled, and concentrated (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. A protein solution (10 mg/mL) was first incubated with inhibitor compound (4 mM) at 4 °C for 1 h before it was mixed with an equal volume of precipitant solution (pH 7, 10% MMT, 0.1 M ammonium formate, 16–22% PEG 8000). Drops of this mixture were streak-seeded, and after approximately 24 h of incubation at 18 °C, crystals were observed. These crystals diffracted to nominal resolutions of 1.8–2.0 Å and belonged to the space group $P2_1$ with six copies of the hArg1 monomer in the

asymmetric unit and the following approximate unit cell dimensions: a = 53.6 Å, b = 285.7 Å, and c = 67.2 Å, $\alpha = \gamma = 90°$, $\beta = 90.05°$. Crystals were harvested directly from drops and plunged into liquid nitrogen prior to synchrotron data collection.

Diffraction data for compound **3** was collected at the Canadian Light Source CLSI Beamline 08ID-1 using a MARMOSAIC 300 mm CCD detector. Diffraction data for Compounds **10**, **12** and **13** were collected at the IMCA-CAT at the Advanced Photon Source using a PILATUS 6M detector (Dectris). All data was processed using autoPROC,[2] refined using autoBUSTER,[3] with manual model building using Coot.[4] Compound geometrical restraints were prepared using grade (Table S1).[5] Figures were prepared using PyMOL.[6]

Crystallography Acknowledgements. Part the research described in this paper was performed at the Canadian Light Source, a national research facility of the University of Saskatchewan, which is supported by the Canada Foundation for Innovation (CFI), the Natural Sciences and Engineering Research Council (NSERC), the National Research Council (NRC), the Canadian Institutes of Health Research (CIHR), the Government of Saskatchewan, and the University of Saskatchewan. This research used resources of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, and was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357, and the Canadian Light Source and its funding partners.

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	**6V7C**	**6V7D**	**6V7E**	**6V7F**
Compound name	**Compound 3**	**Compound 10**	**Compound 12**	**Compound 13**
Data collection				
Space group	*P*21	*P*21	*P*21	*P*21
Cell dimensions a, b, c (Å)	52.98, 281.38, 67.26	53.66, 285.72, 67.23	53.72, 286.49, 67.12	53.51, 285.56, 67.16
Cell dimensions (°)	90.00, 90.08, 90.00	90.00, 90.10, 90.00	90.00, 90.03, 90.00	90.00, 90.04, 90.00
Resolution (Å)	65.42-1.805 (1.811-1.805)*	71.43-1.81 (1.85-1.82)	71.62-1.99 (2.02-1.99)	47.59-2.02 (2.06-2.02)
R_{merge}†	0.11 (0.51)	0.12 (0.48)	0.11 (0.64)	0.17 (0.49)
CC(1/2)	0.99 (0.55)	0.99 (0.78)	0.99 (0.75)	0.97 (0.73)
I/σI	9.7 (2.5)	6.8 (2.0)	8.6 (2.1)	5.1 (2.1)
Completeness (%)	94.5 (95.8)	90.1 (94.3)	98.5 (99.6)	93.0 (81.4)
Redundancy	3.5 (3.1)	3.1 (2.7)	3.4 (3.5)	3.1 (2.6)
Refinement				
Resolution (Å)	35.84-1.80	26.65-1.82	71.62-1.99	47.59-2.02
No. reflections	169488	161956	135880	121305
R_{work} / R_{free}	0.22 / 0.26	0.22 / 0.24	0.21 / 0.25	0.23 / 0.26
No. atoms				
Protein	14473	14553	14454	14547
Ligand	114	210	120	120
Solvent	744	857	1111	806
Ion	12	12	12	12
B-factors				
Protein (Å^2)	16.84	17.92	25.50	16.21
Ligands (Å^2)	15.08	15.60	23.08	13.72
Solvent (Å^2)	18.92	17.45	29.22	11.55
Ion (Å^2)	11.15	9.97	18.11	8.63
R.m.s. deviations				
Bond lengths (Å)	0.010	0.010	0.010	0.009
Bond angles (º)	1.06	1.09	0.91	1.11

*Values in parentheses are for highest-resolution shell.

†R_{merge} = ∑hkl∑i | Ihkl,i – < Ihkl>| / ∑hkl∑i Ihkl,i , where <Ihkl> is the average intensity of multiple observations of a reflection with indices hkl.

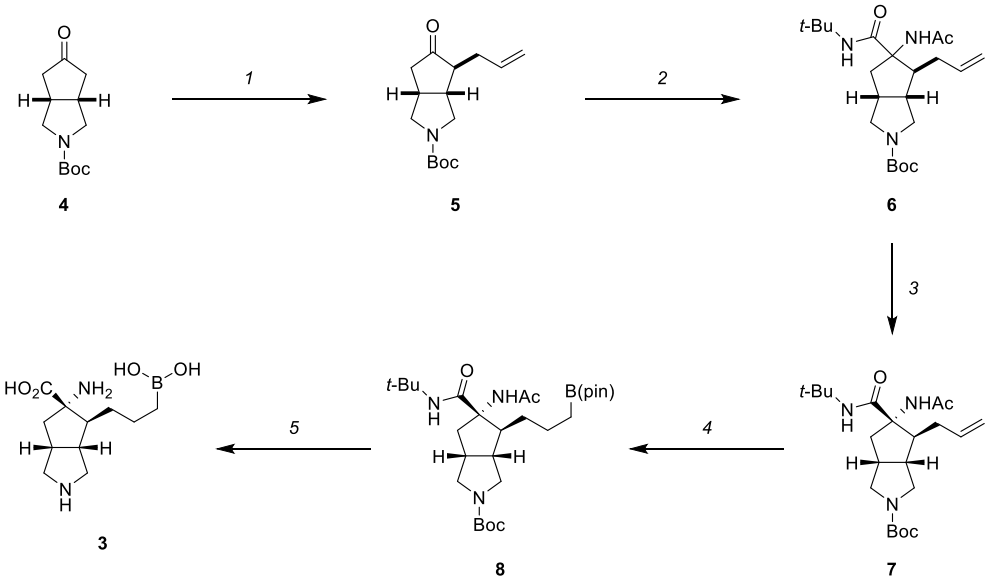
Synthesis of Arginase Inhibitor Compounds

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).

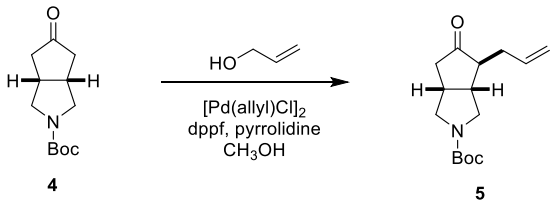
Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26; CHD_2OD, δ 3.31; HDO, δ 4.79; DMSO-*d*5, δ 2.50). Data are 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



Step 1. rac-tert-Butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-oxohexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



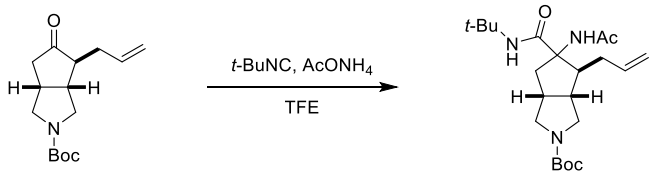
To a mixture of *tert*-butyl 5-oxohexahydrocyclopenta[*c*] pyrrole-2(1*H*)-carboxylate (3.00 g, 13.3 mmol), pyrrolidine (0.330 mL, 3.99 mmol) and 1,1'-bis(diphenylphosphino)ferrocene (0.738 g, 1.33 mmol) in methanol (45 mL) was added allylpalladium(II) chloride dimer (0.244 g, 0.666 mmol) and prop-2-en-1-ol (0.815 mL, 12.0 mmol) under nitrogen gas at 20 °C. The resulting mixture was stirred at 20 °C for 14 h. The solvent was removed under reduced pressure. Water (50 mL) and ethyl acetate (50 mL) were added to the mixture, and the mixture was shaken. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were

dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash-column chromatography (20 g silica gel, eluting with petroleum ether initially, grading to 30% ethyl acetate–petroleum ether) to give the title compound (2.04 g, 6.15 mmol, 46 % yield) as pale-yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84–5.63 (m, 1H), 5.08 (br d, J = 17.9 Hz, 2H), 3.79–3.64 (m, 1H), 3.61–3.51 (m, 1H), 3.47–3.25 (m, 1H), 3.15–2.99 (m, 1H), 2.94–2.83 (m, 1H), 2.68–2.56 (m, 1H), 2.44 (m, 2H), 2.32–2.17 (m, 2H), 2.12 (br s, 1H), 1.46 (s, 9H).

MS (ESI+, m/z): $[M+H–Boc]^+$ calc'd. for $C_{10}H_{16}NO$, 166.1; found 166.1.

Step 2. rac-tert-Butyl (3a*R*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)hexahydro-cyclo-penta[*c*]pyrrole-2(1*H*)-carboxylate

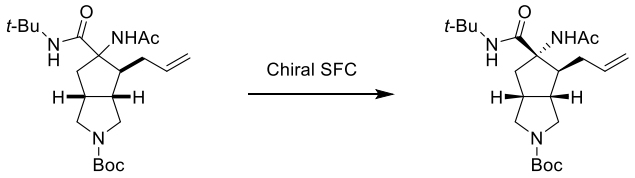


5 **6**

To a solution of *rac-tert*-butyl 4-allyl-5-oxohexa-hydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (2.0 g, 7.7 mmol) in 2,2,2-trifluoroethanol (15 mL) was added *tert*-butyl isocyanide (1.9 g, 23 mmol) and ammonium acetate (2.7 g, 35 mmol). The mixture was stirred at 35 °C for 16 h under nitrogen gas, after which time LCMS analysis indicated that the reaction was complete. Water (50 mL) was added, and the mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (20 g silica gel, eluting with petroleum ether initially, grading to 60% ethyl acetate–petroleum ether) to give the title compound (1.7 g, 4.2 mmol, 54% yield) as white solid.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{22}H_{37}N_3O_4$, 408.3; found 408.3.

Step 3. *tert*-Butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)hexahydro-cyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



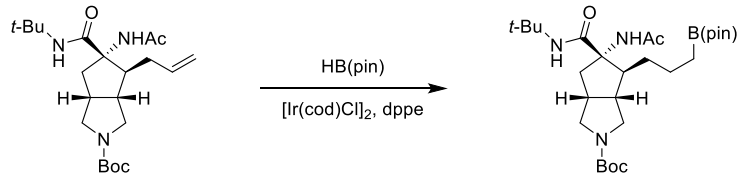
6 **7**

rac-*tert*-Butyl (3a*R*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)hexahydro-cyclopenta[c]pyrrole-2(1*H*)-carboxylate (1.7 g, 4.2 mmol) was separated by chiral supercritical fluid chromatography (ChiralPak IC-3, 150 × 4.6 mm column; 0.05% diethylamine modifier; 20% isopropanol in CO_2 mobile phase; 2.5 mL/min flow rate; 40 °C column temperature) to give the title compound (390 mg, 0.91 mmol, 22 % yield) as the first eluting peak (t_r = 1.623 min).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (br s, 2H), 5.81 (tdd, *J* = 7.2, 10.0, 17.2 Hz, 1H), 5.19–5.06 (m, 2H), 3.44 (br s, 1H), 3.32 (br d, *J* = 6.6 Hz, 2H), 3.23 (dd, *J* = 4.4, 11.4 Hz, 1H), 3.04 (dd, *J* = 9.0, 13.8 Hz, 1H), 2.92 (br s, 1H), 2.55 (br s, 1H), 2.30 (td, *J* = 7.2, 14.1 Hz, 1H), 2.14 (td, *J* = 7.2, 14.1 Hz, 1H), 2.05–1.99 (m, 1H), 1.98 (s, 3H), 1.71–1.59 (m, 2H), 1.44 (s, 9H), 1.33 (s, 9H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{22}H_{38}N_3O_4$), 394.1; found 394.2.

Step 4. tert-Butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



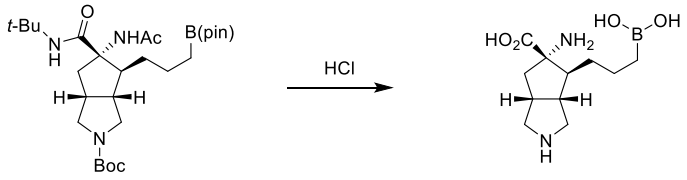
7 **8**

To a mixture of 4Å molecular sieves (86 g) in dichloromethane (430 mL) was added 1,2-bis(diphenylphosphino)ethane (5.9 g, 15 mmol). The headspace of the reaction vessel was flushed with dry nitrogen gas (3 evacuation–backfill cycles). To this solution was then added bis(1,5-cyclooctadiene)diiridium(I) dichloride (7.1 g, 11 mmol), and again the headspace was flushed with nitrogen gas (3 evacuation–backfill cycles). The mixture was cooled to 0 °C in an ice-water bath before 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (130 g, 0.84 mol) was added. Next, a solution of *tert*-butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl) hexahydrocyclopenta[c]pyrrole2(1*H*)-carboxylate (86 g,1.0 eq, 0.21 mol) in dichloromethane (430 mL) was added, and the headspace was again replaced with N_2 (3 evacuation–backfill cycles). The mixture was allowed to warm to 23 °C with constant stirring. After 1 h, TLC and LCMS analyses showed that the reaction was complete. The mixture was filtered, and the filter cake was washed with dichloromethane (2 × 100 mL) and 50% v/v methanol–dichloromethane (2 × 100 mL). The residue was loaded onto a silica gel column, and the product was eluted with dichloromethane initially, grading to 2% methanol–dichloromethane. This semipurified product was then recrystallized from 10 volumes of acetonitrile, and the recrystallized solids were triturated with 10 volumes of methyl *tert*-butyl ether to provide the title compound as a white solid (52 g, 50% yield).

^{1}H-NMR: (400 MHz, CD_3OD) δ 6.45 (s, 1H), 3.50–3.35 (m, 3H), 3.30 (p, *J* = 1.7 Hz, 1H), 3.25–3.06 (m, 2H), 2.96 (s, 1H), 2.42 (dt, *J* = 9.3, 4.5 Hz, 1H), 1.96 (s, 3H), 1.87 (td, *J* = 9.4, 3.9 Hz, 1H), 1.45 (s, 9H), 1.31 (s, 9H), 1.24 (s, 12H), 0.88–0.65 (m, 2H).

MS (ESI+, *m/z*): $[M+Na]^+$ calc'd for $C_{28}H_{50}BN_3O_6$, 558.4; found 558.4.

Step 5. (3a*R*,4*S*,5*S*,6a*R*)-5-Amino-4-(3-boronopropyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid free base



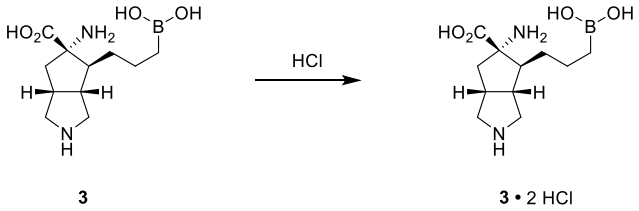
8 **3**

A solution of *tert*-butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate (1.1 g, 2.1 mmol) in 12N aq. hydrochloric acid (20 mL) was stirred at 100 ^{o}C for 15 h. The mixture was then concentrated under reduced pressure, and the residue was redissolved in water (25 mL). The mixture was neutralized with the addition of solid Na_2CO_3 to attain pH = 8. The aqueous phase was washed with dichloromethane (3 × 15 mL) and was then concentrated under reduced pressure to give the crude product. Dowex 50WX8 hydrogen form ion-exchange resin (45 g) was washed with water and MeOH successively. The resin was briefly suction dried. The resin was then added to the solution of the crude product in water at 25 °C. The mixture was stirred for 40 min and aged for 20 min. The mixture was filtered, and the filter cake was washed with MeOH and then water. The filter cake was then eluted with 2N aqueous ammonium hydroxide solution. The aqueous ammonium hydroxide solution was lyophilized to give the title compound as free base (0.32 g, 62% yield).

^{1}H NMR (500 MHz, D_2O) δ 3.14–2.97 (m, 5H), 2.68–2.61 (m, 1H), 2.36–2.31 (m, 1H), 1.58–1.52 (m, 1H), 1.41–1.13 (m, 4H), 1.06–0.98 (m, 1H), 0.60–0.47 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{11}H_{21}BN_2O_4$, 239.2; found 239.1.

*Synthesis of Compound **3** • 2 HCl.*



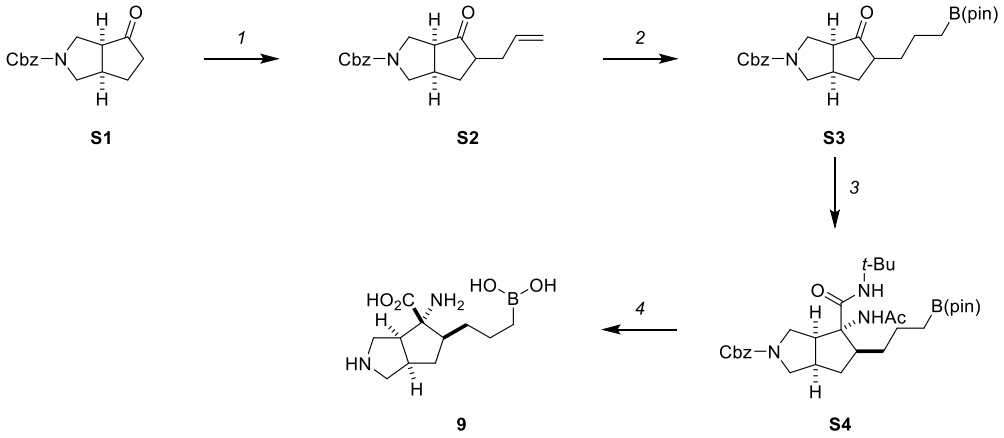
3 **3** • 2 HCl

(3a*R*,4S,5S,6aR)-5-Amino-4-(3-boronopropyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid was dissolved in water and 1N HCl in water (pH<1). The solution was lyophilized to give (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-boronopropyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid hydrochloride.

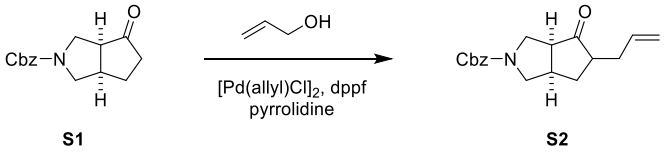
^{1}H NMR (499 MHz, D_2O) δ 3.51 (dd, *J* = 12.2, 8.5 Hz, 1H), 3.43 (dd, *J* = 11.9, 8.4 Hz, 1H), 3.32–3.25 (m, 1H), 3.22–3.11 (m, 2H), 2.89 (ddt, *J* = 14.8, 9.7, 5.0 Hz, 1H), 2.63 (dd, *J* = 13.7, 8.7 Hz, 1H), 2.07 (td, *J* = 10.0, 3.7 Hz, 1H), 1.78 (dd, *J* = 13.8, 9.2 Hz, 1H), 1.57–1.52 (m, 1H), 1.44–1.35 (m, 1H), 1.32–1.18 (m, 2H), 0.77–0.67 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{11}H_{21}BN_2O_4$, 239.2; found 239.1.

Synthesis of Compound 9



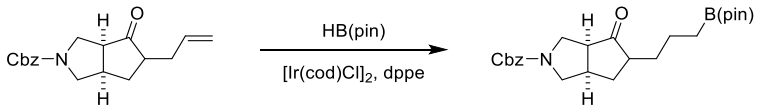
Step 1. *rac*-Benzyl (3a*S*,6a*R*)-5-allyl-4-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate



Allylpalladium(II) chloride dimer (90 mg, 0.25 mmol) and prop-2-en-1-ol (0.37 ml, 5.4 mmol) were added to a solution of *rac*-(3a*S*,6a*R*)-benzyl 4-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate (1.3 g, 4.9 mmol), pyrrolidine (110 mg, 1.5 mmol) and DPPF (270 mg, 0.49 mmol) in methanol (10 ml) under nitrogen gas at 20 °C. The resulting mixture was stirred at 20 °C for 48 h. The solvent was removed under reduced pressure. The residue was suspended in ethyl acetate and the resulting mixture was stirred at room temperature for 10 min before it was filtered through Celite. The filtrate was washed with water, then with brine. The washed organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to afford *rac*-benzyl (3a*S*,6a*R*)-5-allyl-4-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate as a yellow oil (980 mg, 66% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{21}NO_3$, 300.2; found 300.2.

Step 2. rac-Benzyl (3a*S*,6a*R*)-4-oxo-5-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl) hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate

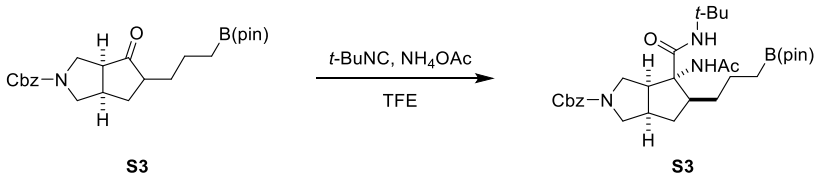


S2 **S3**

4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (1.3 mL, 8.2 mmol) was added to a mixture of bis(1,5-cyclooctadiene)diiridium(I) dichloride (84 mg, 0.16 mmol) and 1,2-bis(diphenylphosphino)ethane (130 mg, 0.33 mmol) in dichloromethane (16 mL) under nitrogen gas. The mixture was degassed by three evacuation–backfill cycles with dry nitrogen gas. After this degassed mixture was stirred at 25 °C for 20 min, a solution of *rac*-(3a*S*,6a*R*)-benzyl 5-allyl-4-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate (977 mg, 3.3 mmol) in dichloromethane (16 mL) was added by syringe. The resulting mixture was stirred at 25 °C for 15 h. The reaction was quenched with the addition of methanol and water. The mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to afford *rac*-benzyl (3a*S*,6a*R*)-4-oxo-5-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclope nta[c]pyrrole-2(1*H*)-carboxylate as a yellow oil (750 mg, 54% yield).

MS (ESI+, *m/z*): $[M+Na]^+$ calc'd for $C_{24}H_{34}BNO_5$, 450.2; found 450.3.

Step 3. *rac*-(3-((3a*S*,6a*R*)-4-Acetamido-2-((benzyloxy)carbonyl)-4-(*tert*-butylcarbamoyl)octahydrocyclopenta[c]pyrrol-5-yl)propyl)boronic acid

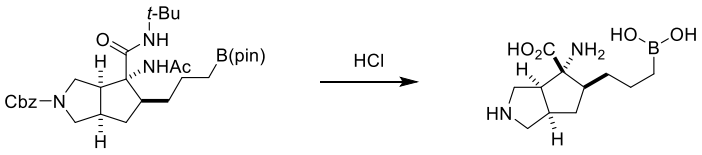


Tert-butyl isocyanide (730 mg, 8.8 mmol) and ammonium acetate (670 mg, 8.8 mmol) were added to a mixture of *rac*-benzyl (3a*S*,6a*R*)-4-oxo-5-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate (750 mg, 1.8 mmol) in 2,2,2-trifluoroethanol (10 ml). The mixture was stirred at 40 °C for 18 h. The reaction was diluted with water and dichloromethane. The organic layer was separated, and the aqueous layer was re-extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–dichloromethane) initially, and then by mass-directed RP-HPLC (eluting with acetonitrile–water containing 0.1% TFA) to give *rac*-(3-((3a*S*,6a*R*)-4-acetamido-2-((benzyloxy)carbonyl)-4-(*tert*-butylcarbamoyl)octahydrocyclopenta[c]pyrrol-5-yl)propyl)boronic acid as a light yellow oil (513 mg, 51% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{31}H_{48}BN_3O_6$, 570.4; found 570.3.

Step 4.

rac-(3a*S*,6a*R*)-4-Amino-5-(3-boronopropyl)octahydrocyclopenta[*c*]pyrrole-4-carbox- ylic acid



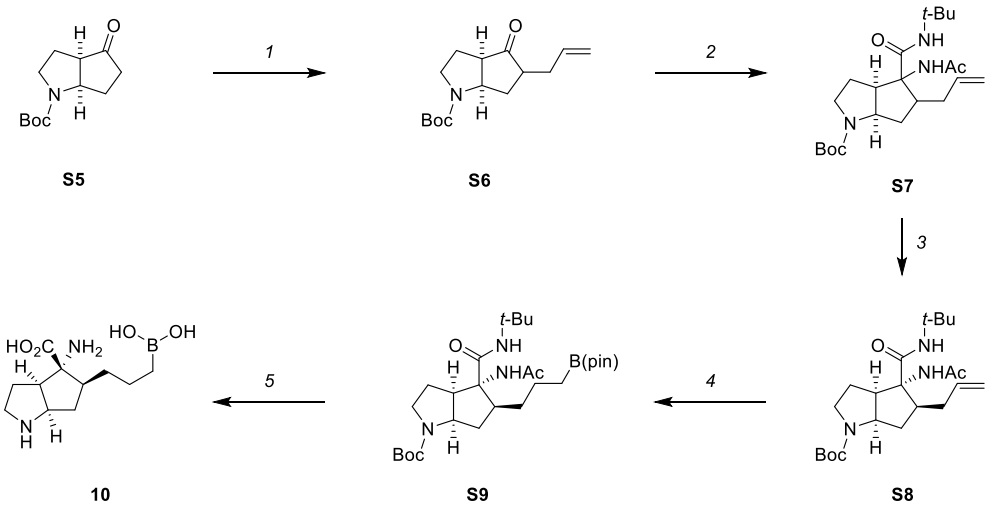
S4 **9**

Aqueous hydrochloric acid (36% w/v, 1.0 mL) was added to a solution of *rac*-(3-((3a*S*,6a*R*)-4-acetamido-2-((benzyloxy)carbonyl)-4-(*tert*-butylcarbamoyl)octahydrocy clopenta[c]pyrrol-5-yl)propyl)boronic acid (70 mg, 0.14 mmol) and 1,4-dioxane (1.0 mL). The vessel was sealed, and the reaction mixture was heated in a microwave reactor at 120 °C for 1 h. The reaction mixture was then concentrated under reduced pressure. Water was added to the residue, and the mixture was washed with dichloromethane. The aqueous layer was concentrated under reduced pressure. The residue was dissolved in water and the resulting solution was freeze-dried to give *rac*-(3a*S*,6a*R*)-4-amino-5-(3-boronopropyl) octahydrocyclopenta[c]pyrrole-4-carboxylic acid hydrochloride as a yellow solid (17 mg, 38% yield).

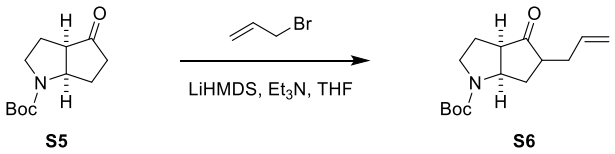
^{1}H NMR (499 MHz, D_2O) 3.87–3.76 (m, 1H), 3.71 (td, J = 12.5, 11.8, 6.6 Hz, 2H), 3.64 (td, J = 11.1, 9.2, 4.5 Hz, 10 2H), 3.58–3.39 (m, 2H), 3.12 (ddt, J = 24.8, 16.9, 8.4 Hz, 2H), 2.85–2.73 (m, 1H), 2.08 (dd, J = 14.2, 7.6 Hz, 1H), 1.81 (td, J = 13.9, 13.5, 9.0 Hz, 1H), 1.57–1.36 (m, 2H), 0.77 (tt, J = 15.4, 8.4 Hz, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{11}H_{21}BN_2O_4$, 239.2; found 239.2.

Synthesis of Compound 10



Step 1: rac-(3a*R*,6a*R*)-*tert*-Butyl 5-allyl-4-oxohexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate



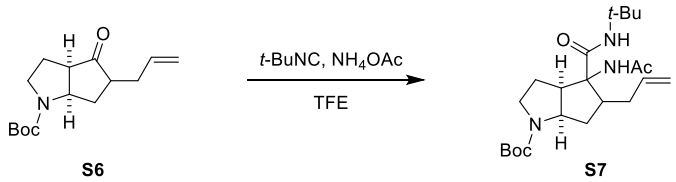
Lithium hexamethyldisilazide (1M solution in tetrahydrofuran, 13 mL, 13 mmol) was added to a solution of *rac*-(3a*R*,6a*R*)-*tert*-butyl 4-oxohexahydrocyclopenta [*b*]pyrrole-1(2*H*)-carboxylate (2.0 g, 8.9 mmol) and triethylamine (2.5 mL, 18 mmol) in tetrahydrofuran (12 mL) at –30 °C. After stirring for 30 min at –30 °C, 3-bromoprop-1-ene (0.77 mL, 8.9 mmol) was added to the reaction mixture dropwise. The mixture was warmed to 0 °C, and was stirred at that temperature for 1.5 h. The reaction mixture was quenched with the addition of water, and the resulting mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-chromatography (eluting with ethyl acetate–hexanes) to give

rac-(3a*R*,6a*R*)-*tert*-butyl 5-allyl-4-oxohexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate as a colorless oil (440 mg, 17% yield).

^{1}H NMR (400 MHz,$CDCl_3$) δ = 5.87–5.61 (m, 1H), 5.23–4.94 (m, 2H), 4.54–4.08 (m, 1H), 3.81–3.36 (m, 1H), 3.25–2.89 (m, 1H), 2.84–2.67 (m, 1H), 2.58–2.21 (m, 3H), 2.18–1.93 (m, 3H), 1.78 (ddd, *J* = 5.3, 12.0, 13.8 Hz, 1H), 1.49 (br s, 9H).

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{15}H_{23}NO_3$, 210.1; found 210.1.

Step 2. rac-(3a*R*,6a*R*)-*tert*-Butyl 4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate

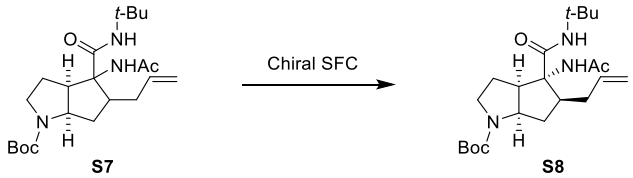


Ammonium acetate (3.3 g, 42 mmol) and *tert*-butyl isocyanide (2.4 mL, 21 mmol) were added to a solution of *rac*-(3a*R*,6a*R*)-*tert*-butyl 5-allyl-4-oxohexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate (1.4 g, 5.3 mmol) in 2,2,2-trifluoroethanol (2.0 mL). The reaction was stirred at 35 °C for 12 h under dry nitrogen gas. The reaction mixture was quenched with the addition of water, and the resulting mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with methanol–dichloromethane). The product thus obtained was then re-purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give *rac*-(3a*R*,6a*R*)-*tert*-butyl 4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate as a white solid (1.1 g, 49% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.00–5.65 (m, 2H), 5.22–4.86 (m, 2H), 4.22–3.88 (m, 1H), 3.86–3.56 (m, 1H), 3.48 (br s, 1H), 3.19 (br s, 1H), 2.85 (br dd, *J* = 3.7, 8.6 Hz, 1H), 2.50 (br d, *J* = 13.2 Hz, 1H), 2.36–2.00 (m, 1H), 2.36–1.96 (m, 4H), 1.93–1.69 (m, 3H), 1.60 (br s, 2H), 1.51–1.39 (m, 10H), 1.38–1.28 (m, 10H)

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{22}H_{38}N_3O_4$, 408.6; found 408.2.

Step 3. *tert*-Butyl (3a*R*,4*R*,5*S*,6a*R*)-4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate

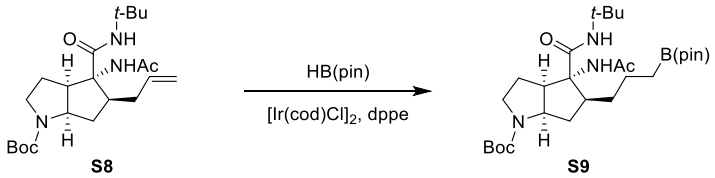


rac-(3a*R*,6a*R*)-*tert*-Butyl 4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate (1.05 g, 2.58 mmol) was resolved by chiral supercritical fluid chromatography (Phenomenex Cellulose-2 [250 × 50mm, 10 μm] column; 0.1% v/v ammonium hydroxide–ethanol modifier; 15% modifier in CO_2 mobile phase; 180 mL/min flow rate; 40 °C column temperature) to give *tert*-butyl (3a*R*,4*R*,5*S*,6a*R*)-4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate (t_r = 2.50 min) as the second eluting peak (140 mg, 12% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 5.87 (qd, J = 8.2, 16.1 Hz, 1H), 5.73 (br s, 1H), 5.23–4.92 (m, 2H), 4.08 (br s, 1H), 3.82–3.63 (m, 1H), 3.53–3.44 (m, 1H), 3.22 (br s, 1H), 2.93–2.80 (m, 1H), 2.53–2.50 (m, 1H), 2.05 (s, 3H), 1.96–1.81 (m, 2H), 1.78–1.58 (m, 3H), 1.46 (s, 9H), 25 1.34 (s, 9H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{22}H_{37}N_3O_4$, 408.3; found 408.2.

Step 4. (3a*R*,6a*R*)-*tert*-Butyl 4-acetamido-4-(*tert*-butylcarbamoyl)-5-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate

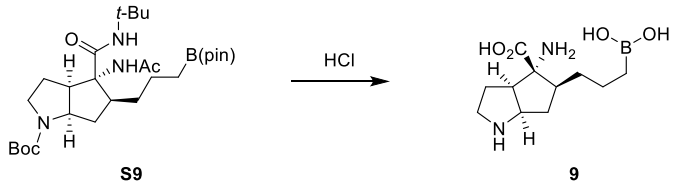


A solution of (3a*R*,6a*R*)-*tert*-butyl 4-acetamido-5-allyl-4-(*tert*-butylcarbamoyl) hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate (140 mg, 0.34 mmol), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (66 mg, 0.52 mmol) and 1,2-bis(diphenylphosphino)ethane (14 mg, 0.034 mmol) in anhydrous dichloromethane (3.0 mL) was degassed by bubbling dry nitrogen gas through it for 3 min. The mixture was stirred at 25 °C for 10 min before it was treated with bis(1,5-cyclooctadiene)diiridium(I) dichloride (12 mg, 0.017 mmol). The resulting mixture was stirred at 25 °C for 10 h under N_2. The reaction was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (3a*R*,6a*R*)-*tert*-butyl 4-acetamido-4-(*tert*-butylcarbamoyl)-5-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate as a white solid containing the corresponding boronic acid as a minor impurity (85 mg, 42% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.33 (br s, 1H), 8.01 (br s, 1H), 5.64 (br s, 1H), 4.15–4.01 (m, 1H), 3.73 (br s, 1H), 3.48 (br d, *J* = 12.0 Hz, 1H), 3.21 (br s, 1H), 2.89–2.72 (m, 1H), 2.43 (br s, 2H), 2.17 (s, 2H), 2.05 (s, 3H), 1.89–1.70 (m, 1H), 1.67–1.48 (m, 4H), 1.45 (s, 9H), 1.31 (d, *J* = 4.9 Hz, 9H), 1.27–1.16 (m, 8H), 1.07–0.92 (m, 1H), 0.89–0.76 (m, 2H)

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{50}BN_3O_6$, 536.4; found 536.4.

Step 5. (3a*R*,6a*R*)-4-Amino-5-(3-boronopropyl)octahydrocyclopenta[*b*]pyrrole-4-carboxylic acid

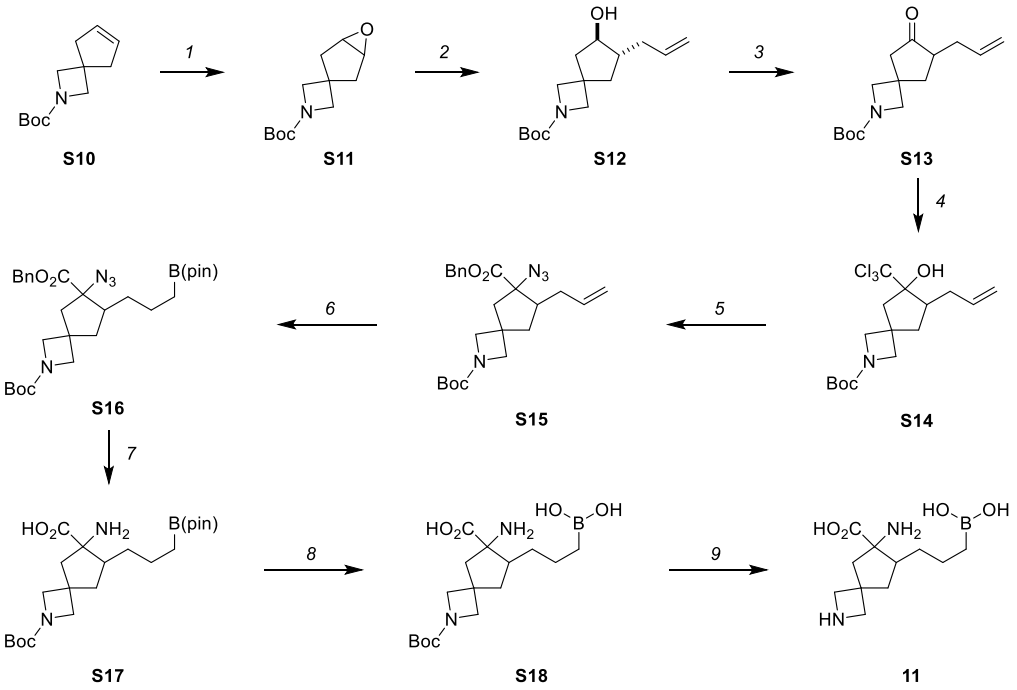


(3a*R*,6a*R*)-*tert*-Butyl 4-acetamido-4-(*tert*-butylcarbamoyl)-5-(3-(4,4,5,5-tetramethyl-1,3,2-20 dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*b*]pyrrole-1(2*H*)-carboxylate (85 mg, 0.16 mmol) was combined with 12N aq. hydrochloric acid (3.0 mL, 36 mmol), and the resulting mixture was stirred at 100 °C for 12 h. The mixture was concentrated under reduced pressure. Saturated aq. sodium bicarbonate solution was added to the residue to attain pH ~8. The basified aqueous layer was then washed with dichloromethane. The aqueous layer was concentrated under reduced pressure. The residue was then treated with 2N aq. hydrochloric acid until pH ~6 was achieved. The acidified mixture was concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.05% hydrochloric acid) to give (3a*R*,6a*R*)-4-amino-5-(3-boronopropyl)octahydrocyclopenta[*b*]pyrrole-4-carboxylic acid hydrochloride as a white solid (22 mg, 45% yield).

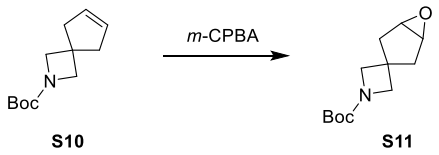
^{1}H NMR (400 MHz, D_2O) δ 4.33 (br t, J = 8.2 Hz, 1H), 3.48–3.33 (m, 1H), 3.23–3.06 (m, 2H), 2.91–2.73 (m, 1H), 2.36 (br 10 dd, J = 7.1, 15.4 Hz, 1H), 2.26–2.15 (m, 1H), 2.11–1.89 (m, 2H), 1.41 (br d, J = 3.7 Hz, 2H), 1.31–1.22 (m, 2H), 0.83–0.63 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{11}H_{21}BN_2O_4$, 257.1; found 257.0.

Synthesis of Compound 11



Step 1. tert-Butyl 6'-oxaspiro[azetidine-3,3'-bicyclo[3.1.0]hexane]-1-carboxylate

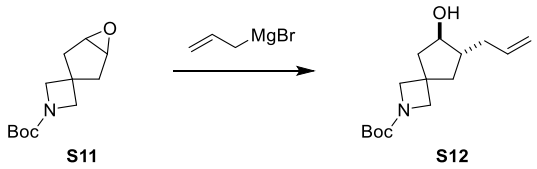


m-Chloroperbenzoic acid (80% w/w with *m*-chlorobenzoic acid, 3.7 g, 17 mmol) was added to a solution of *tert*-butyl 2-azaspiro[3.4]oct-6-ene-2-carboxylate (3.0 g, 14 mmol) in dichloromethane (50 mL) at 0 °C. The mixture was stirred at 25 °C for 2 h. The reaction was treated with sat. aq. Na_2CO_3 and the resulting mixture was extracted with dichloromethane. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give *tert*-butyl 6'-oxaspiro[azetidine-3,3'-bicyclo[3.1.0]hexane]-1-carboxylate as a white solid (3.1 g, 86% yield), which was used in the next step without purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.80 (tdd, *J* = 7.0, 10.1, 17.1 Hz, 1H), 5.13–4.95 (m, 2H), 3.87 (br d, *J* = 8.4 Hz, 2H), 3.79 (d, *J* = 6.4 Hz, 2H), 3.76–3.73 (m, 1H), 2.22–2.12 (m, 3H), 2.07–2.02 (m, 1H), 1.93–1.82 (m, 2H), 1.48–1.39 (m, 10H)

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{12}H_{19}NO_3$, 170.1; found 170.0.

Step 2. rac-tert-Butyl (6*R*,7*R*)-6-allyl-7-hydroxy-2-azaspiro[3.4]octane-2-carboxylate



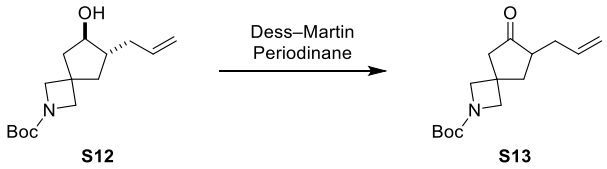
S11 **S12**

Allylmagnesium bromide (1M solution in diethyl ether, 24 mL, 24 mmol) was added dropwise to a solution of *tert*-butyl 6'-oxaspiro[azetidine-3,3'-bicyclo[3.1.0]hexane]-1-carboxylate (2.7 g, 12 mmol) in diethyl ether (100 mL) at 0 °C under dry nitrogen gas. The reaction was stirred at 20 °C for 12 h. before sat. aq. NH_4Cl and ethyl acetate were added. The layers were shaken then separated. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residual was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give *rac-tert*-butyl (6*R*,7*R*)-6-allyl-7-hydroxy-2-azaspiro[3.4]octane-2-carboxylate as a colorless oil (1.7 g, 53% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.80 (tdd, *J* = 7.0, 10.1, 17.1 Hz, 1H), 5.13–4.95 (m, 2H), 3.87 (br d, *J* = 8.4 Hz, 2H), 3.79 (d, *J* = 6.4 Hz, 2H), 3.76–3.73 (m, 1H), 2.22–2.12 (m, 3H), 2.07–2.02 (m, 1H), 1.93–1.82 (m, 2H), 1.48–1.39 (m, 10H).

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{15}H_{25}NO_3$, 212.1; found 212.1.

Step 3. tert-Butyl 6-allyl-7-oxo-2-azaspiro[3.4]octane-2-carboxylate



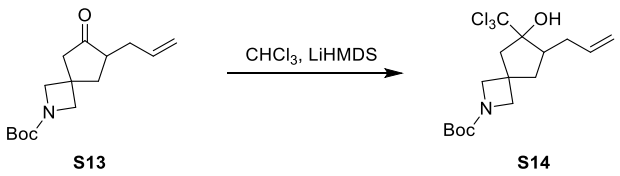
S12 **S13**

Dess–Martin periodinane (3.2 g, 7.6 mmol) was added to a solution of *tert*-butyl 6-allyl-7-hydroxy-2-azaspiro[3.4]octane-2-carboxylate (1.7 g, 6.4 mmol) in dichloromethane (30 mL) at 0 °C under dry nitrogen gas. The reaction was stirred at 20 °C for 2 h before sat. aq. sodium bicarbonate and dichloromethane were added to the reaction mixture. The layers were shaken, then separated. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The residual was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give the *tert*-butyl 6-allyl-7-oxo-2-azaspiro[3.4]octane-2-carboxylate as a colorless oil (1.2 g, 70% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.72 (tdd, *J* = 7.0, 10.1, 17.1 Hz, 1H), 5.20–4.92 (m, 2H), 4.00–3.71 (m, 4H), 2.62 (br d, *J* = 18.8 Hz, 1H), 2.55–2.46 (m, 1H), 2.44–2.32 (m, 2H), 2.28 (br d, *J* = 7.5 Hz, 1H), 2.14–2.03 (m, 1H), 1.79 (t, *J* = 12.1 Hz, 1H), 1.47–1.41 (m, 9H).

MS (ESI+, *m/z*): $[M+H–Boc]^+$ calc'd for $C_{15}H_{23}NO_3$, 166; found 166.

Step 4. tert-Butyl 7-allyl-6-hydroxy-6-(trichloromethyl)-2-azaspiro[3.4]octane-2-carboxylate

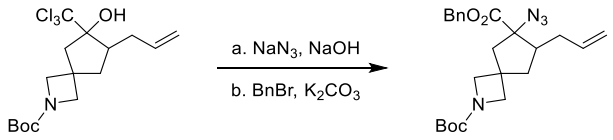


S13 **S14**

Lithium bis(trimethylsilyl)amide (1M solution in THF, 5.7 mL, 5.7 mmol) was added dropwise to a mixture of *tert*-butyl 6-allyl-7-oxo-2-azaspiro[3.4]octane-2-carboxylate (0.50 g, 1.9 mmol) and redistilled chloroform (0.76 mL, 9.4 mmol) in tetrahydrofuran (10 mL) at –78 °C under nitrogen gas. The mixture was stirred for 60 min at –78 °C. Then the mixture was warmed to 20 °C for 60 min. The mixture was poured into sat. aq. NH_4Cl, and the resulting mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the crude *tert*-butyl 7-allyl-6-hydroxy-6-(trichloromethyl)-2-azaspiro[3.4]octane-2-carboxylate as a brown oil (0.80 g, 110% yield). This material was used in the next step without further purification.

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{16}H_{24}Cl_3NO_3$, 328; found 328.

Step 5. 6-Benzyl 2-(*tert*-butyl) 7-allyl-6-azido-2-azaspiro[3.4]octane-2,6-dicarboxylate



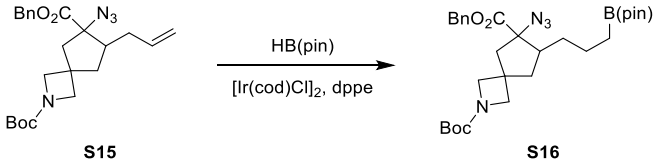
S14 **S15**

A solution of *tert*-butyl 7-allyl-6-hydroxy-6-(trichloromethyl)-2-azaspiro[3.4] octane-2-carboxylate (0.80 g, 2.1 mmol) in 1,4-dioxane (5.0 mL) was added to a solution of sodium azide (0.67 g, 10 mmol) and sodium hydroxide (0.25 g, 6.2 mmol) in water (5.0 mL) at 0 °C. The mixture was stirred for12 h at 20 °C. Acetic acid was added to acidify to pH ~6. The mixture was poured into water and extracted with ethyl acetate. The separated organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the corresponding azido acid, which was dissolved in *N*,*N*-dimethylformamide (8.0 mL). This solution was then treated with K_2CO_3 (1.4 g, 10 mmol) and (bromomethyl)benzene (0.37 mL, 3.1 mmol) at 20 °C. The resulting mixture was stirred for 2 h. The mixture was poured into brine and extracted with ethyl acetate. The separated organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give 6-benzyl 2-*tert*-butyl 7-allyl-6-azido-2-azaspiro[3.4]octane-2,6-dicarboxylate as a pale-yellow oil (100 mg, 11% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.42–7.32 (m, 5H), 5.77–5.48 (m, 1H), 5.32–5.13 (m, 2H), 5.07–4.86 (m, 2H), 3.97–3.72 (m, 4H), 2.55 (d, *J* = 14.3 Hz, 1H), 2.36–2.09 (m, 4H), 1.88–1.64 (m, 2H), 1.46–1.39 (m, 9H).

MS (ESI+, *m/z*): $[M–N]^+$ calc'd for $C_{23}H_{30}N_4O_4$, 412; found 412.

Step 6. 6-Benzyl 2-(*tert*-butyl) 6-azido-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)-2-azaspiro[3.4]octane-2,6-dicarboxylate

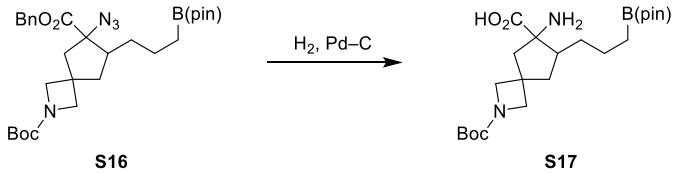


Dry nitrogen gas was bubbled through a solution of 1,2-bis(diphenylphosphino)ethane (7.5 mg, 0.019 mmol), bis(1,5-cyclooctadiene)diiridium(I) dichloride (7.9 mg, 0.012 mmol) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.17 mL, 1.2 mmol) in anhydrous dichloromethane (2 mL) for 3 min. The mixture was stirred at 20 °C for 20 min before a solution of 6-benzyl 2-*tert*-butyl 7-allyl-6-azido-2-azaspiro[3.4] octane-2,6-dicarboxylate (100 mg, 0.23 mmol) in dichloromethane (1.0 mL) was added. The resulting mixture was stirred at 20 °C for 15 h under dry nitrogen gas before it was concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give 6-benzyl 2-*tert*-butyl 6-azido-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)-2-azaspiro[3.4]octane-2,6-dicarboxylate as a colorless oil (80 mg, 59% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39–7.34 (m, 5H), 5.32–5.01 (m, 2H), 3.90–3.74 (m, 4H), 2.52 (d, *J* = 14.2 Hz, 1H), 2.26 (dd, *J* = 7.1, 13.0 Hz, 1H), 2.17–2.06 (m, 2H), 1.74 (dd, *J* = 9.6, 13.0 Hz, 1H), 1.43 (s, 9H), 1.39–1.27 (m, 3H), 1.22 (s, 11H), 1.05–0.93 (m, 1H), 0.79–0.52 (m, 2H).

MS (ESI+, *m/z*): $[M+H–C_4H_8–N_2]^+$ calc'd for $C_{29}H_{43}BN_4O_6$, 471; found 471.

Step 7. 6-Amino-2-(*tert*-butoxycarbonyl)-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)-2-azaspiro[3.4]octane-6-carboxylic acid

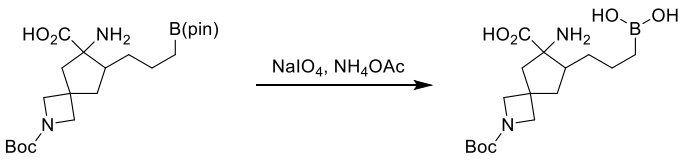


S16 **S17**

Palladium on carbon (10% w/w, 38 mg, 0.036 mmol) was added to a solution of 6-benzyl 2-*tert*-butyl 6-azido-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)-2-azaspiro[3.4]octane-2,6-dicarboxylate (40 mg, 0.072 mmol) in methanol (4.0 mL) under an atmosphere of nitrogen gas. The mixture was degassed under reduced pressure, and the reaction flask was backfilled with hydrogen gas. This evacuation–backfill cycle was repeated three times. The resulting mixture was stirred under 15 psi of hydrogen gas at 15 °C for 3 h. The mixture was then filtered through a pad of Celite to remove the catalyst, and the filtrate was concentrated under reduced pressure to give 6-amino-2-(*tert*-butoxycarbonyl)-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-azaspiro[3.4]octane-6-carboxylic acid as a colorless, glassy solid (33 mg, 58% yield). This material was used in next step without purification.

MS (ESI+, *m/z*): $[M+H–C_4H_8–H_2O]^+$ calc'd for $C_{22}H_{39}BN_2O_6$, 283; found 283.

Step 8. 6-Amino-7-(3-boronopropyl)-2-(*tert*-butoxycarbonyl)-2-azaspiro[3.4]octane-6-carboxylic acid

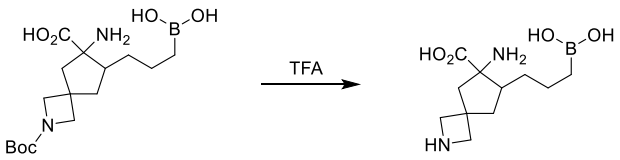


S17 **S18**

Ammonium acetate (170 mg, 2.1 mmol) and sodium periodate (230 mg, 1.1 mmol) were added a mixture of 6-amino-2-(*tert*-butoxycarbonyl)-7-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-azaspiro[3.4]octane-6-carboxylic acid (47 mg, 0.11 mmol), tetrahydrofuran (2.0 mL) and water (1.0 mL) at 20 °C. The milky suspension was stirred at 20 °C for 15 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give the crude 6-amino-7-(3-boronopropyl)-2-(*tert*-butoxycarbonyl)-2-azaspiro[3.4]octane-6-carboxylic acid as a yellow oil (43 mg, 113% yield), which was used in the next step without further purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{16}H_{29}BN_2O_6$, 357; found 357.

Step 9. 6-Amino-7-(3-boronopropyl)-2-azaspiro[3.4]octane-6-carboxylic acid



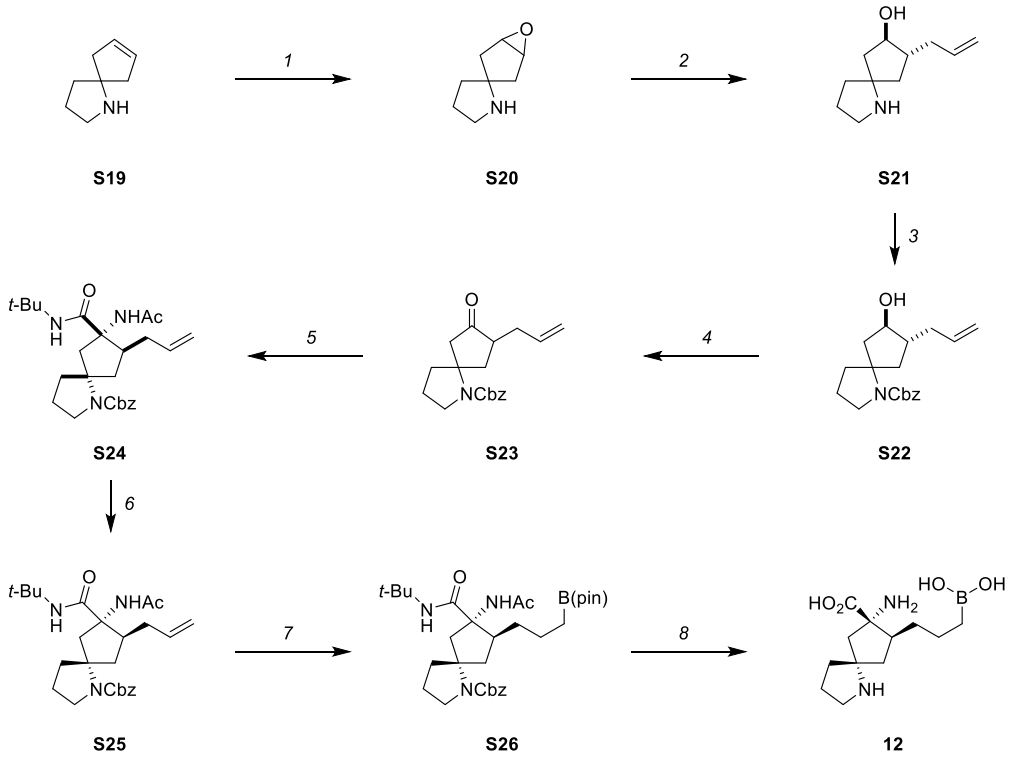
S18 **11**

A mixture of 6-amino-7-(3-boronopropyl)-2-(*tert*-butoxycarbonyl)-2-azaspiro[3.4]octane-6-carboxylic acid (43 mg, 0.12 mmol), trifluoroacetic acid (0.50 mL), and dichloromethane (2.5 mL) was stirred at 0 °C for 1 h. The mixture was concentrated under a stream of nitrogen gas. The residue was purified by RP-HPLC (eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA) to give 6-amino-7-(3-boronopropyl)-2-azaspiro[3.4]octane-6-carboxylic acid as its HFBA salt (27 mg, 84% yield).

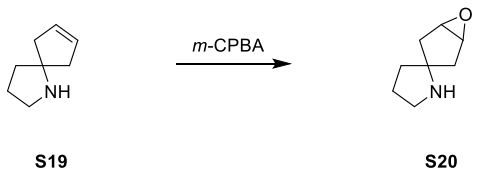
^{1}H NMR (500 MHz, D_2O) δ 4.16–4.13 (m, 1H), 4.04–3.90 (m, 3H), 2.70 (d, J = 14.8 Hz, 1H), 2.56–2.39 (m, 1H), 2.26–2.11 (m, 2H), 1.98–1.86 (m, 1H), 1.56–1.32 (m, 2H), 1.31–1.16 (m, 1H), 1.11–0.98 (m, 1H), 0.77–0.58 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{11}H_{21}BN_2O_4$, 239.2; found 239.2.

Synthesis of Compound 12



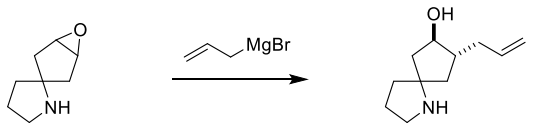
Step 1. 6-Oxaspiro[bicyclo[3.1.0]hexane-3,2'-pyrrolidine



A solution of Oxone monopersulfate compound (10 g, 16 mmol) in water (50 mL) was added to a solution of 1-azaspiro[4.4]non-7-ene (4.0 g, 33 mmol) in water (50 mL) over 30 min at 0 °C. The reaction solution was stirred at 25 °C for 30 min. Powdered sodium bicarbonate (4.1 g, 49 mmol) was added to the reaction to adjust to pH = 6. The solvent was removed by lyophilization to give crude product, which was used in the next step directly without further purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_8H_{13}NO$, 140.1; found 140.1.

Step 2. rac-(7*R*,8*R*)-8-allyl-1-azaspiro[4.4]nonan-7-ol

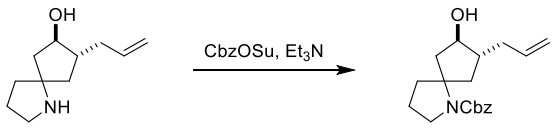


S20 **S21**

Crude 6-oxaspiro[bicyclo[3.1.0]hexane-3,2'-pyrrolidine (theoretically 13 mmol) was added to allylmagnesium bromide (1M solution in diethyl ether, 52mL, 52 mmol) at 0 °C. The resulting gray suspension was stirred under dry nitrogen gas at 25 °C for 15 h. The reaction mixture was then quenched with water. The mixture was concentrated under reduced pressure, and the residue was suspended in methanol. This suspension was filtered, and the filtrate was concentrated under reduced pressure to give crude *rac*-(7*R*,8*R*)-8-allyl-1-azaspiro [4.4]nonan-7-ol as a pale-yellow oil (2.5 g, 107% yield). This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{11}H_{19}NO$, 182.2; found 182.1.

Step 3. *rac*-Benzyl (7*R*,8*R*)-7-allyl-8-hydroxy-1-azaspiro[4.4]nonane-1-carboxylate

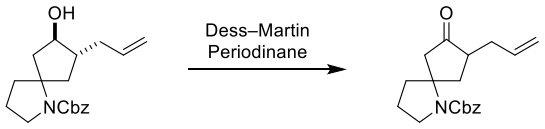


S21 **S22**

Triethylamine (14 mL, 100 mmol) and benzyl (2,5-dioxopyrrolidin-1-yl) carbonate (Cbz-OSu, 15 g, 61 mmol) were added to a solution of *rac*-(7*R*,8*R*)-8-allyl-1-azaspiro[4.4] nonan-7-ol (5.3 g, 21 mmol) in dichloromethane (100 mL). The resulting white suspension was stirred at 25 °C for 2 h. The reaction mixture was quenched with the addition of water, and the mixture was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give *rac*-benzyl (7*R*,8*R*)-7-allyl-8-hydroxy-1-azaspiro[4.4]nonane-1- carboxylate as a colorless oil (3.6 g, 46% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{19}H_{25}NO_3$, 316.2; found 316.4.

Step 4. Benzyl 7-allyl-8-oxo-1-azaspiro[4.4]nonane-1-carboxylate



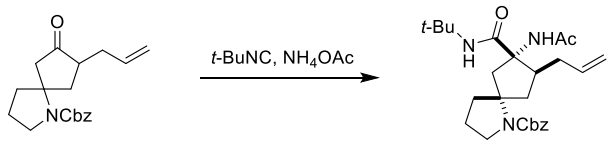
S22 **S23**

Dess–Martin periodinane (9.7 g, 23 mmol) was added to a solution of *rac*-benzyl (7*R*,8*R*)-7-allyl-8-hydroxy-1-azaspiro[4.4]nonane-1-carboxylate (3.6 g, 11 mmol) in dichloromethane (100 mL). The resulting white suspension was stirred at 25 °C for 1 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give benzyl 7-allyl-8-oxo-1-azaspiro[4.4]nonane-1-carboxylate as a colorless oil (2.9 g, 71% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.19 (m, 5H), 5.83–5.48 (m, 1H), 5.32–4.91 (m, 4H), 3.63–3.40 (m, 2H), 3.17–2.80 (m, 2H), 2.73–2.46 (m, 2H), 2.37–2.20 (m, 1H), 2.13–1.62 (m, 6H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{19}H_{23}NO_3$, 314.2; found 314.2.

Step 5. *rac*-Benzyl (5*S*,7*S*,8*S*)-7-acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4] nonane-1-carboxylate

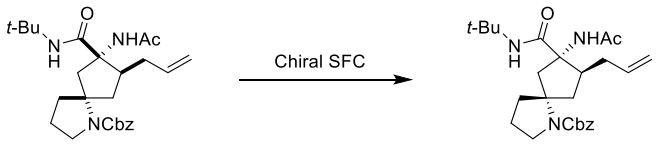


S23 **S24**

Ammonium acetate (1.2 g, 16 mmol) and *tert*-butyl isocyanide (1.8 mL, 16 mmol) were added to a solution of benzyl 7-allyl-8-oxo-1-azaspiro[4.4]nonane-1-carboxylate (1.0 g, 3.2 mmol) in 2,2,2-trifluoroethanol (5.0 mL). The reaction mixture was stirred at 35 °C for 15 h. Water (20 mL) was then added to the reaction mixture, and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by RP-HPLC (C18 column, eluting with water–acetonitrile containing 0.1% TFA) to give *rac*-benzyl (5*S*,7*S*,8*S*)-7-acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4] nonane-1-carboxylate as the second-eluting isomer (170 mg, 12% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{26}H_{37}N_3O_4$, 456.3; found 456.2.

Step 6. (5*S*,7*S*,8*S*)-7-Acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4]nonane-1-carboxylate



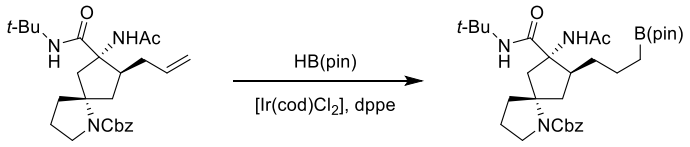
S24 **S25**

rac-Benzyl 7-acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4]nonane-1-carboxylate (530 mg, 1.2 mmol) was resolved by chiral supercritical-fluid chromatography (OD column, 250 × 50 mm, 10 μm; 0.1% ammonium hydroxide in ethanol modifier; 15% modifier in CO_2 mobile phase; 50 mL/min flow rate; 40 °C column temperature) to give benzyl (5*S*,7*S*,8*S*)-7-acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4]nonane-1- carboxylate, the first-eluting peak (t_r = 2.3 min), as a colorless, viscous oil (190 mg, 30% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.56–8.24 (m, 2H), 7.60–7.04 (m, 5H), 5.84–5.55 (m, 1H), 5.18–4.69 (m, 4H), 3.48–3.31 (m, 2H), 2.39–2.28 (m, 1H), 2.26–2.06 (m, 3H), 2.06–1.87 (m, 7H), 1.81–1.62 (m, 3H), 1.31 (s, 9H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{26}H_{37}N_3O_4$, 456.2; found 456.2.

Step 7. Benzyl (5*S*,7*S*,8*S*)-7-acetamido-7-(*tert*-butylcarbamoyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1-azaspiro[4.4]nonane-1-carboxylate



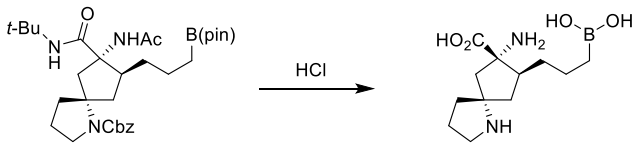
S25 **S26**

A mixture of 1,2-bis(diphenylphosphino)ethane (26 mg, 0.066 mmol) and bis(1,5-cyclooctadiene)diiridium(I) dichloride (22 mg, 0.033 mmol) in dichloromethane (50 mL) was stirred at 0 °C under dry nitrogen gas for 5 min. Next, benzyl (5*S*,7*S*,8*S*)-7-acetamido-8-allyl-7-(*tert*-butylcarbamoyl)-1-azaspiro[4.4]nonane-1- carboxylate (150 mg, 0.33 mmol) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.30 mL, 1.6 mmol) were added to the reaction mixture. The reaction was stirred at 25 °C under nitrogen gas for 15 h. The mixture was concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 Column, eluting with acetonitrile–water containing 0.1% TFA) to give (5*S*,7*S*,8*S*)-7-acetamido-7-(*tert*-butylcarbamoyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan -2-yl)propyl)-1-azaspiro[4.4]nonane-1-carboxylate as a colorless, viscous semisolid (55 mg, 21% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.80–7.92 (m, 2H), 7.59–7.06 (m, 5H), 5.13–4.96 (m, 2H), 3.58–3.29 (m, 2H), 3.08–2.77 (m, 2H), 2.47–2.27 (m, 1H), 2.26–2.14 (m, 1H), 2.13–1.93 (m, 5H), 1.85–1.62 (m, 3H), 1.52–1.39 (m, 2H), 1.31 (s, 9H), 1.26–1.19 (m, 4H), 0.91–0.59 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{32}H_{50}BN_3O_6$, 584.4; found 584.6.

Step 8. (5*S*,7*S*,8*S*)-7-Amino-8-(3-boronopropyl)-1-azaspiro[4.4]nonane-7-carboxylic acid



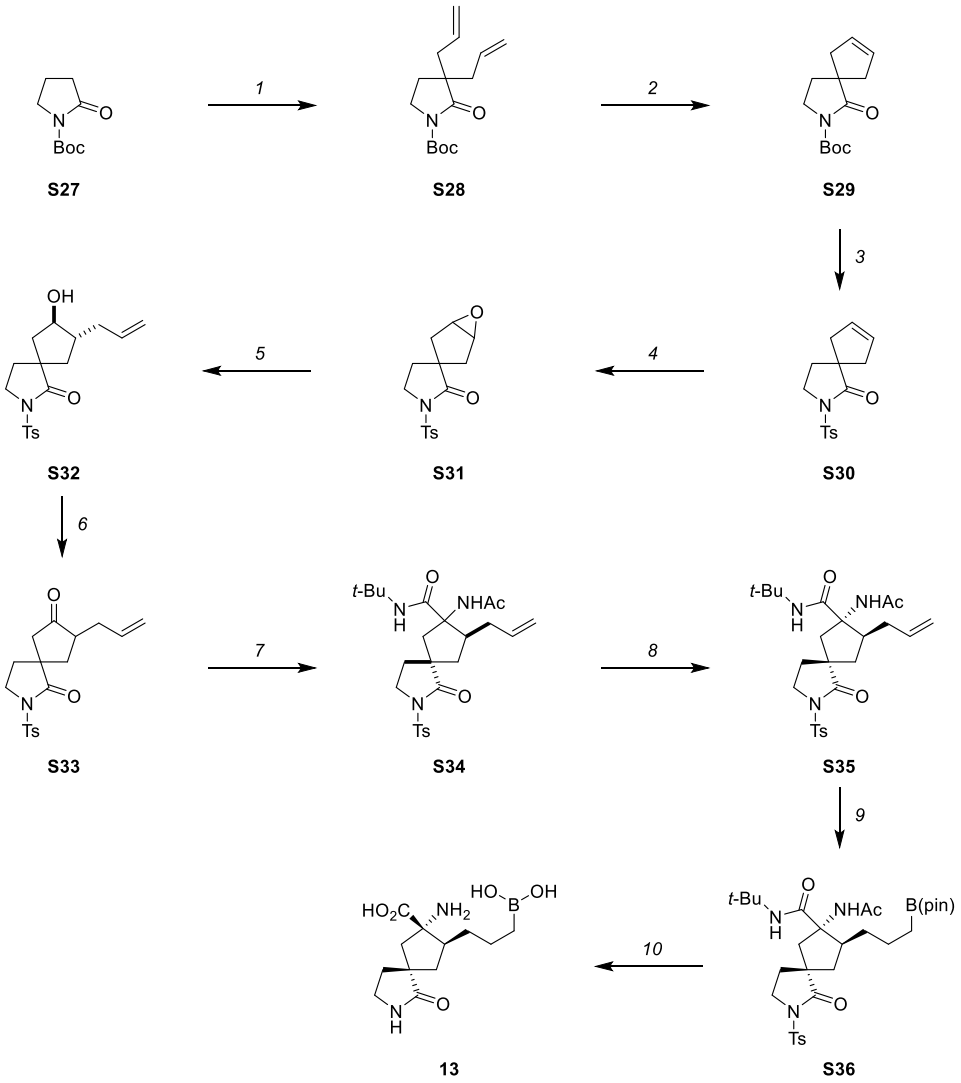
S26 **12**

A mixture of benzyl (5*S*,7*S*,8*S*)-7-acetamido-7-(*tert*-butylcarbamoyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1-azaspiro[4.4]nonane-1-carboxylate (55 mg, 0.11 mmol) and 12N aq. hydrochloric acid solution (15 mL, 180 mmol) was stirred at 110 °C for 12 h. The mixture was concentrated under reduced pressure. The residue was dissolved in water (3 mL) and the pH was adjusted to 8 with the addition of solid sodium bicarbonate. The basified mixture was washed with dichloromethane. The aqueous layer was then acidified to pH = 2 with the addition of 12N aq. hydrochloric acid solution. The acidified product mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA) to provide (5*S*,7*S*,8*S*)-7-amino-8-(3-boronopropyl)-1-azaspiro[4.4]nonane-7-carboxylic acid as a viscous oil (HFBA salt, 28 mg, 105% yield).

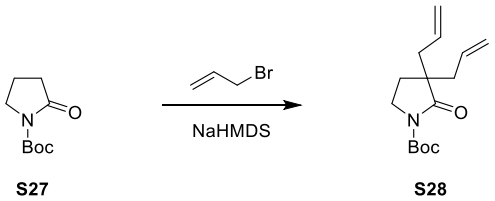
^{1}H NMR (400 MHz, D_2O) δ 3.32 (t, J = 7.6 Hz, 2H), 2.84-2.80 (m, 1H), 2.51-2.30 (m, 3H), 2.18-2.05 (m, 2H), 2.03-1.89 (m, 3H), 1.63-1.49 (m, 1H), 1.45-1.32 (m, 1H), 1.32-1.18 (m, 1H), 1.18-1.06 (m, 1H), 0.78-0.60 (m, 2H).

MS (ESI+, *m/z*): $[M+H-H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.2.

Synthesis of Compound 13



Step 1. tert-Butyl 3,3-diallyl-2-oxopyrrolidine-1-carboxylate



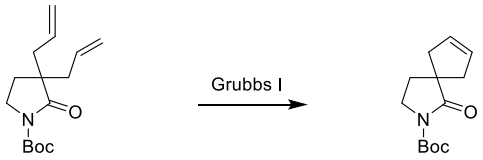
Sodium hexamethyldisilazide (1M solution in tetrahydrofuran, 320 mL, 320 mmol) was added dropwise to a solution of *tert*-butyl 2-oxopyrrolidine-1-carboxylate (30 g, 160 mmol) and allyl bromide (49 g, 410 mmol) in tetrahydrofuran (300 mL) at –78 °C over 30 min.

The reaction was stirred at –78 °C for 2 h. The reaction mixture was then neutralized with the addition of sat. aq. ammonium chloride solution (550 mL), and the resulting mixture was extracted with ethyl acetate (3 × 250 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give *tert*-butyl 3,3-diallyl-2-oxopyrrolidine-1-carboxylate as a colorless oil (38 g, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.88–5.63 (m, 2H), 5.23–5.03 (m, 4H), 3.61 (t, *J* = 7.3 Hz, 2H), 2.40–2.31 (m, 2H), 2.30–2.21 (m, 2H), 1.90 (t, *J* = 7.2 Hz, 2H), 1.54 (d, *J* = 0.6 Hz, 9H).

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{15}H_{23}NO_3$, 210.1; found 210.1.

Step 2. tert-Butyl 1-oxo-2-azaspiro[4.4]non-7-ene-2-carboxylate



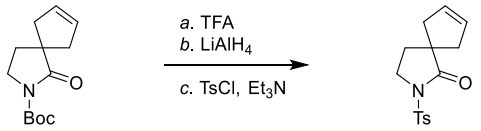
S28 **S29**

A solution of *tert*-butyl 3,3-diallyl-2-oxopyrrolidine-1-carboxylate (17 g, 64 mmol) and benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (Grubbs I, 2.7 g, 3.2 mmol) in dichloromethane (250 mL) was deoxygenated by three evacuation–backfill cycles with dry nitrogen gas. The mixture was stirred at 25 °C for 192 h. Water was then added (100 mL), and the resulting was extracted with dichloromethane (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to give *tert*-butyl 1-oxo-2-azaspiro[4.4]non-7-ene-2-carboxylate as a pale gray solid (8.0 g, 50% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.62 (s, 2H), 3.67 (t, *J* = 6.8 Hz, 2H), 2.85 (d, *J* = 14.5 Hz, 2H), 2.31 (d, *J* = 14.9 Hz, 2H), 1.96 (t, *J* = 6.8 Hz, 2H), 1.53 (s, 9H).

MS (ESI+, *m/z*): $[M+Na]^+$ calc'd for $C_{13}H_{19}NO_3$, 260.1; found 260.1.

Step 3. 2-Tosyl-2-azaspiro[4.4]non-7-ene



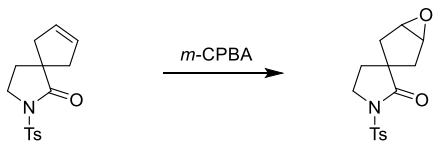
S29 **S30**

tert-Butyl 1-oxo-2-azaspiro[4.4]non-7-ene-2-carboxylate (8.0 g, 34 mmol) was added portionwise over 10 min to ice-cold trifluoroacetic acid (2.6 mL, 34 mmol). The mixture was warmed to 25 °C, and it was stirred at this temperature for 2 h. The solvent was removed under reduced pressure, and the residue was treated with sat. aq. sodium carbonate solution (20 mL). The resulting mixture was extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to provide a grey solid (4.7 g), which was dissolved in tetrahydrofuran (50 mL). This solution was chilled to 0 °C before it was treated with a solution of lithium aluminum hydride (1.5 g, 41 mmol) in tetrahydrofuran (30 mL). The mixture was then heated to 60 °C, at which temperature it was stirred for 2 h. After cooling to ambient temperature, excess reductant was quenched with the careful addition of 30% w/v aq. potassium hydroxide solution (30 mL). The resulting mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to leave a brown oil (~5 g). This residue was dissolved in dichloro-methane (100 mL), and the resulting solution was treated with *para*-toluenesulfonyl chloride (13 g, 67 mmol) and triethylamine (14 mL, 100 mmol). The brown suspension was stirred at 25 °C for 16 h. The mixture was then diluted with water (30 mL), and the resulting mixture was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue thus

obtained was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to afford 2-tosyl-2-azaspiro[4.4]non-7-ene as a pale brown solid (8.4 g, 73% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 5.57 (s, 2H), 3.35 (t, *J* = 6.9 Hz, 2H), 3.15 (s, 2H), 2.43 (s, 3H), 2.14 (s, 4H), 1.73 (t, *J* = 6.9 Hz, 2H). MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{15}H_{19}NO_2S$, 278.1; found 278.1.

Step 4. 1'-Tosyl-6-oxaspiro[bicyclo[3.1.0]hexane-3,3'-pyrrolidine]



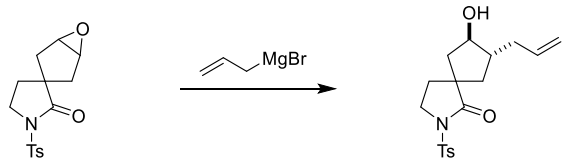
S30 **S31**

To a solution of 2-tosyl-2-azaspiro[4.4]non-7-ene (8.4 g, 30 mmol) in dichloromethane (100 mL) was added *meta*-chloroperbenzoic acid (80% w/w with *meta*-chlorobenzoic acid, 7.8 g, 36 mmol) at 0 °C. The reaction was warmed to 25 °C, and the mixture was stirred at this temperature for 5 h. The reaction was treated with 4M aq. sodium hydroxide solution (100 mL), and the resulting mixture was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with brine (25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide 1'-tosyl-6-oxaspiro[bicyclo[3.1.0]hexane-3,3'-pyrrolidine] as a pale-yellow oil (4.8 g, 49% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.72–7.64 (m, 2H), 7.35–7.28 (m, 2H), 3.50–3.37 (m, 2H), 3.30–3.17 (m, 2H), 3.09–3.00 (m, 2H), 2.48–2.39 (m, 3H), 1.82–1.73 (m, 2H), 1.71–1.62 (m, 2H), 1.61–1.50 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{15}H_{19}NO_3S$, 294.1; found 294.1.

Step 5. rac-(7*R*,8*R*)-7-Allyl-8-hydroxy-2-tosyl-2-azaspiro[4.4]nonan-1-one



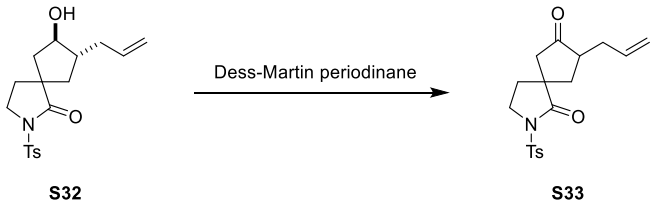
S31 **S32**

Allylmagnesium bromide (1M solution in diethyl ether, 33 mL, 33 mmol) was added to a solution of 1'-tosyl-6-oxaspiro[bicyclo[3.1.0]hexane-3,3'-pyrrolidine] (4.8 g, 16 mmol) in tetrahydrofuran (20 mL) over 5 min at 25 °C. The reaction mixture was stirred at ambient temperature for 2 h under dry nitrogen gas. The resulting milky suspension was carefully treated with sat. aq. ammonium chloride solution (100 mL), and the resulting mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to provide crude *rac*-(7*R*,8*R*)-7-allyl-8-hydroxy-2-tosyl-2-azaspiro[4.4]nonan-1-one as a pale yellow oil, suitable for use in the next step without purification (5.5 g, 89% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.83–7.62 (m, 2H), 7.41–7.16 (m, 2H), 5.87–5.49 (m, 1H), 5.19–4.91 (m, 2H), 4.37–4.25 (m, 0.1H), 4.06–3.95 (m, 0.2H), 3.88–3.75 (m, 0.7H), 3.37–2.99 (m, 4H), 2.69–2.46 (m, 0.4H), 2.41 (s, 3H), 2.30–2.12 (m, 1.3H), 2.01–1.73 (m, 4.2H), 1.72–1.36 (m, 3.4H), 1.10–0.94 (m, 0.8H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{25}NO_3S$, 336.2; found 336.2.

Step 6. 8-Allyl-2-tosyl-2-azaspiro[4.4]nonan-7-one



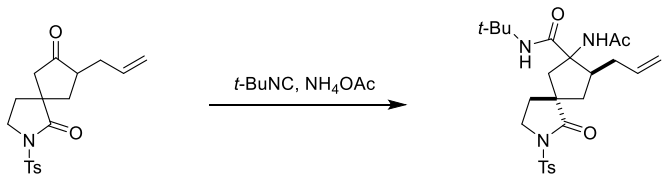
S32 **S33**

Dess–Martin periodinane (10 g, 25 mmol) was added portionwise to an ice-cold solution of *rac*-(7*R*,8*R*)-7-allyl-8-hydroxy-2-tosyl-2-azaspiro[4.4]nonan-1-one (5.5 g, 16 mmol) in dichloromethane (100 mL). The resulting suspension was warmed to 25 °C. The mixture was stirred at this temperature for 15 h before water (100 mL) was added. The biphasic mixture was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide 8-allyl-2-tosyl-2-azaspiro[4.4]nonan-7-one as a colorless, viscous oil (4.3 g, 66% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 (dd, *J* = 3.1, 8.3 Hz, 2H), 7.39–7.30 (m, 2H), 5.75–5.43 (m, 1H), 5.08–4.91 (m, 2H), 3.47 (s, 0.5H), 3.46–3.34 (m, 1H), 3.30 (br d, *J* = 6.6 Hz, 1H), 3.23 (s, 0.5H), 3.15 (m, 0.5H), 3.08 (m, 0.5H), 2.53–2.45 (m, 1H), 2.43 (s, 3H), 2.35–2.20 (m, 1H), 2.19–1.97 (m, 3H), 1.96–1.87 (m, 1H), 1.81 (dt, *J* = 3.5, 7.0 Hz, 1H), 1.77–1.62 (m, 1H), 1.57–1.41 (m, 1H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{23}NO_3S$, 334.1; found 334.1.

Step 7. *rac*-(5*R*,8*S*)-7-Acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4] nonane-7-carboxamide

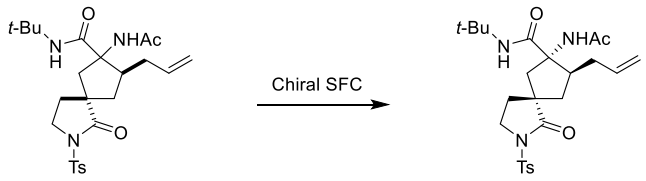


S33 **S34**

A solution of 8-allyl-2-tosyl-2-azaspiro[4.4]nonan-7-one (4.3 g, 11 mmol) in 2,2,2-trifluoroethanol (20 mL) was treated with ammonium acetate (8.4 g, 110 mmol) and *tert*-butyl isocyanide (4.5 g, 54 mmol). The reaction mixture was stirred at 35 °C for 15 h under dry nitrogen gas. The mixture was then diluted with water (50 mL), and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to provide *rac*-(5*R*,8*S*)-7-acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4]nonane-7-carboxamide, the first-eluting peak, as a white solid (1.0 g, 19% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{25}H_{37}N_3O_4S$, 476.3; found 476.3.

Step 8. (5*R*,7*S*,8*S*)-7-Acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4] nonane-7-carboxamide



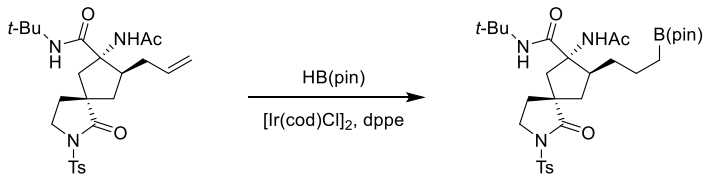
S34 **S35**

rac-(5*R*,8*S*)-7-Acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4]non ane-7-carboxamide was resolved by chiral supercritical-fluid chromatography (Daicel Chiralpak AD column, 250 × 50 mm, 10 μm; 0.1% v/v ammonium hydroxide–ethanol modifier; 20% modifier in CO_2 mobile phase; 180 mL/min flow rate) to give (5*R*,7*S*,8*S*)-7-acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4]nonane-7-car boxamide, the fourth-eluting peak (t_r = 3.75 min), as a pale orange solid (420 mg, 28% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.69 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 7.9 Hz, 2H), 6.05 (s, 1H), 6.01 (s, 1H), 5.72–5.51 (m, 1H), 5.06–4.98 (m, 2H), 3.34–3.18 (m, 3H), 3.01 (d, *J* = 9.2 Hz, 1H), 2.75 (d, *J* = 14.9 Hz, 1H), 2.43 (s, 3H), 2.32–2.20 (m, 1H), 2.19–2.09 (m, 1H), 1.99 (s, 3H), 1.95–1.87 (m, 1H), 1.87–1.77 (m, 2H), 1.72 (br d, *J* = 14.5 Hz, 1H), 1.61–1.53 (m, 1H), 1.52–1.46 (m, 1H), 1.30 (s, 9H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{25}H_{37}N_3O_4S$, 476.3; found 476.3.

Step 9. (5*R*,7*S*,8*S*)-7-Acetamido-*N*-(*tert*-butyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-tosyl-2-azaspiro[4.4]nonane-7-carboxamide

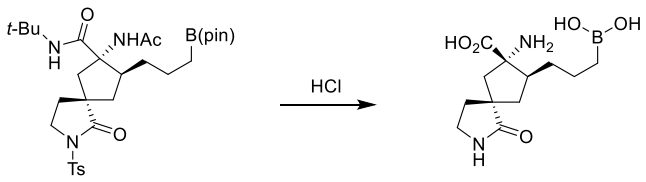


S35 **S36**

To a mixture of (5*R*,7*S*,8*S*)-7-acetamido-8-(but-3-en-1-yl)-*N*-(*tert*-butyl)-2-tosyl-2-azaspiro[4.4]nonane-7-carboxamide (100 mg, 210 mmol), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (270 mg, 2.1 mmol), and cyclohexene (86 mg, 1.1 mmol) in dichloromethane (10 mL) was added bis(1,5-cyclooctadiene)diiridium(I) dichloride (9.9 mg, 0.015 mmol) and 1,2-bis(diphenylphosphino)ethane (8.4 mg, 0.021 mmol). The reaction mixture was deoxygenated by 3 evacuation–backfill cycles with dry nitrogen gas. The reaction mixture was stirred at 20 °C for 15 h under dry nitrogen gas. The mixture was then treated with water (10 mL), and the resulting mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with brine (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to provide (5*R*,7*S*,8*S*)-7-acetamido-*N*-(*tert*-butyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-tosyl-2-azaspiro[4.4]nonane-7-carboxamide as a white solid containing the corresponding boronic acid as an impurity(70 mg, 20% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{31}H_{50}BN_3O_6S$, 604.4; found 604.5.

Step 10. (5*R*,7*S*,8*S*)-7-Amino-8-(3-boronopropyl)-2-azaspiro[4.4]nonane-7-carboxylic acid

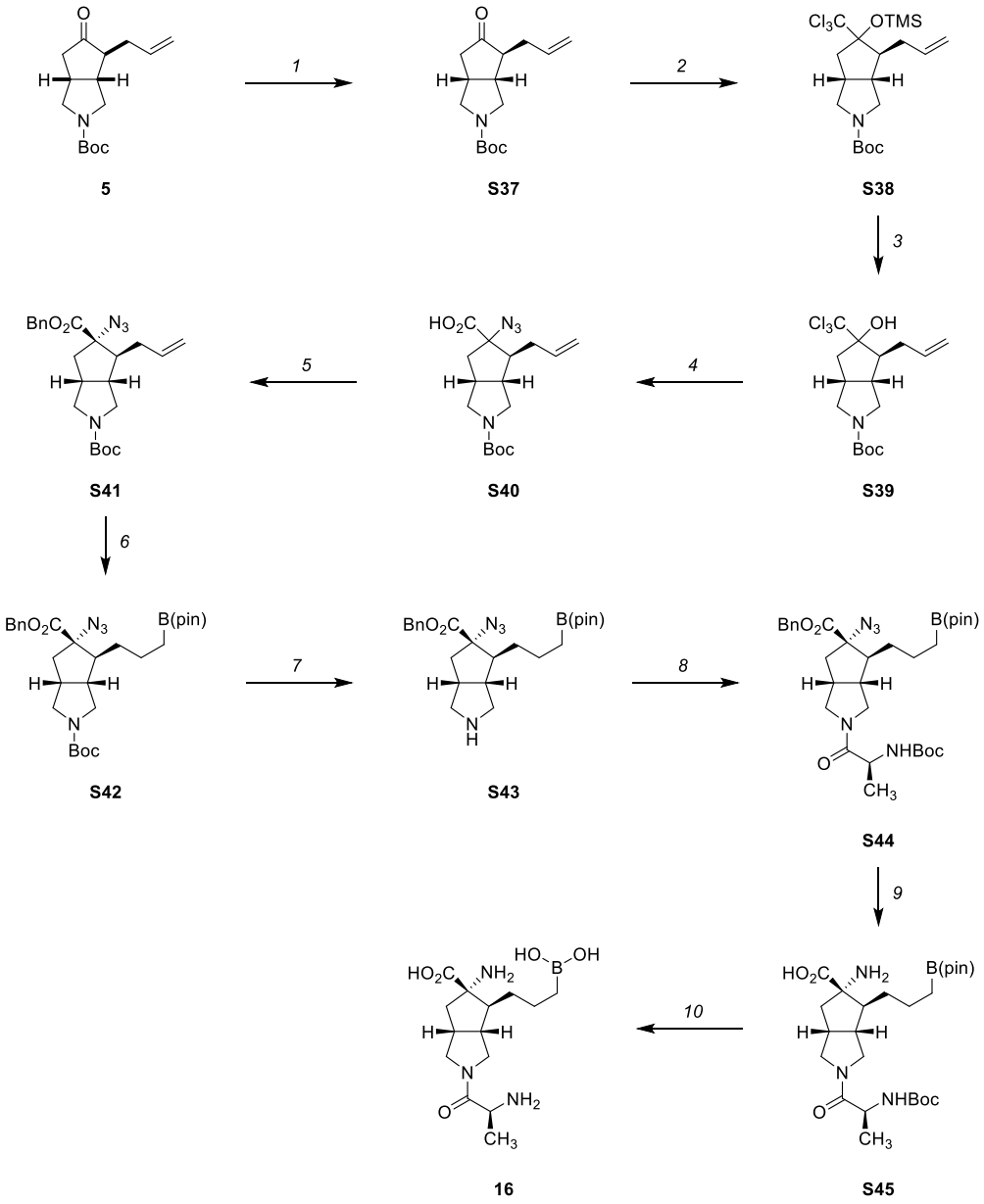


A solution of (5*R*,7*S*,8*S*)-7-acetamido-*N*-(*tert*-butyl)-8-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-tosyl-2-azaspiro[4.4]nonane-7-carboxamide (60 mg, 0.99 mmol) in 37% w/v aq. hydrochloric acid (5.0 mL) was stirred at 120 °C for 15 h. The solvent was then removed under reduced pressure, and the pale-yellow residue was dissolved in 4M aq. sodium hydroxide solution (5.0 mL). The solvent was again removed under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.05% hydrochloric acid) to give (5*R*,7*S*,8*S*)-7-amino-8-(3-boronopropyl)-2-azaspiro[4.4] nonane-7-carboxylic acid as a pale-yellow semisolid (11 mg, 32% yield).

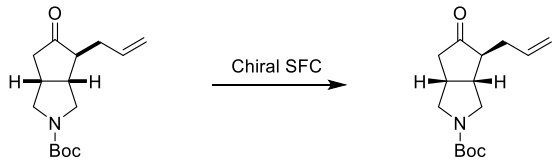
^{1}H NMR (400 MHz, D_2O) δ = 3.34–3.14 (m, 3H), 3.07 (d, *J* = 11.7 Hz, 1H), 2.56 (d, *J* = 15.4 Hz, 1H), 2.28–2.14 (m, 1H), 2.08–1.91 (m, 4H), 1.75–1.61 (m, 1H), 1.44 (ddd, *J* = 3.4, 6.0, 12.6 Hz, 1H), 1.38–1.28 (m, 1H), 1.27–1.13 (m, 1H), 1.12–0.99 (m, 1H), 0.77–0.53 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$ 253.2; found 253.2.

Synthesis of Compound 16



Step 1. tert-Butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-oxohexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



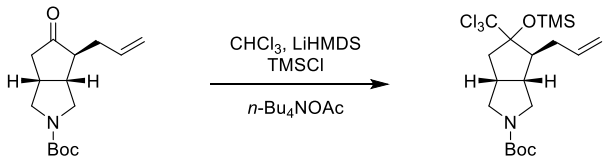
5 **S37**

rac-tert-Butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate (110 g, 0.43 mol)was resolved by chiral supercritical-fluid chromatography (Chiralpak AD-H column, 5 × 25 cm, 5 μm; 20% methanol in CO_2 mobile phase) to give *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-oxohexahydrocyclopenta[c]pyrrole-2(1*H*)-carboxylate, the first-eluting peak, as a colorless oil (40 g, 35% yield, 88% ee). This material could be re-purified using the same method as before to produce the product in enantiomerically pure form (100% ee).

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (ddt, *J* = 17.2, 10.1, 7.1 Hz, 1H), 5.19–5.02 (m, 2H), 3.73 (dd, *J* = 11.4, 8.4 Hz, 1H), 3.60 (dd, *J* = 11.5, 6.8 Hz, 1H), 3.39 (s, 1H), 3.17–3.01 (m, 1H), 2.97–2.84 (m, 1H), 2.62 (qd, *J* = 7.3, 2.9 Hz, 1H), 2.54–2.39 (m, 2H), 2.34–2.20 (m, 2H), 2.17–2.08 (m, 1H), 1.48 (s, 9H).

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{11}H_{15}NO_3$, 210; found 210.

Step 2. tert-Butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-(trichloromethyl)-5-((trimethylsilyl)oxy)hexahydro-cyclopenta[*c*]pyrrole-2(1*H*)-carboxylate

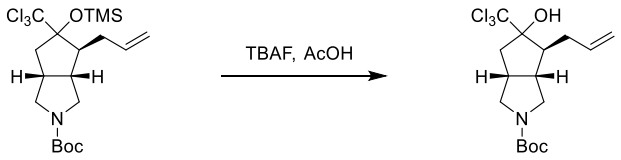


S37 **S38**

To a mixture of anhydrous chloroform (4.6 mL, 56 mmol) and redistilled chlorotrimethylsilane (5.3 mL, 42 mmol) in tetrahydrofuran (75 mL) was added lithium bis(trimethylsilyl)amide (1.0M solution in THF, 50 mL, 50 mmol) at –78 °C. The mixture was stirred under dry nitrogen gas at –78 °C for 30 min before it was warmed to –30 °C, at which temperature it was maintained for 60 min further. Next, solutions of *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-oxohexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (4.4 g, 17 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) and tetrabutylammonium acetate (0.50 g, 1.658 mmol) in *N*,*N*-dimethylformamide (10 mL) were added dropwise at –30 °C. The mixture was warmed slowly to 0 °C and stirred at that temperature for 12 h. The mixture was then poured into sat. aq. ammonium chloride solution (300 mL), and the resulting mixture was extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-(trichloromethyl)-5-((trimethylsilyl)oxy)hexahydrocyclopenta[*c*]pyrro le-2(1*H*)-carboxylate as a red oil (8.0 g, 106% yield). This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H–C_4H_8]^+$ calc'd for $C_{19}H_{32}Cl_3NO_3Si$, 400.1; found 400.0.

Step 3. tert-Butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-hydroxy-5-(trichloromethyl)hexahydrocyclopenta[*c*] pyrrole-2(1*H*)-carboxylate

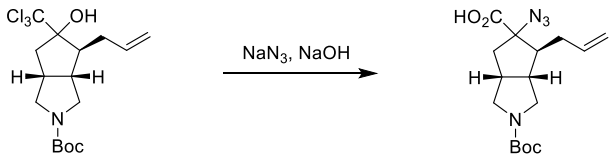


S38 **S39**

Acetic acid (6.0 mL, 110 mmol) and tetra-*n*-butylammonium fluoride (1M solution tetrahydrofuran, 63 mL, 63 mmol) were added sequentially, dropwise, to a solution of *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-(trichloromethyl)-5-((trimethylsilyl)oxy)hexahydrocyclo-penta[c]pyrrole-2(1*H*)-carboxylate (24 g, 53 mmol) in tetrahydrofuran at 0 °C. The mixture was aged at 0 °C for 30 min before it was warmed to 20 °C, at which temperature it was stirred for an additional 5 h. The mixture was diluted with sat. aq. sodium bicarbonate solution, and the resulting mixture was extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-hydroxy-5-(trichloromethyl)hexahydrocyclopenta[c]pyrrole-2(1*H*)-car boxylate as a brown oil (18 g, 89% yield). This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{16}H_{24}Cl_3NO_3$, 384.1; found 384.0.

Step 4. (3aR,4S,6aR)-4-allyl-5-azido-2-(tert-butoxycarbonyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid

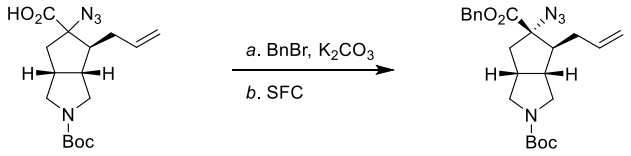


S39 **S40**

To an ice-cold solution of *tert*-butyl (3a*R*,4*S*,6a*R*)-4-allyl-5-hydroxy-5-(trichloromethyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (18 g, 47 mmol) in 1,4-dioxane (90 mL) was added a solution of sodium azide (8.6 g, 130 mmol) and sodium hydroxide (6.6 g, 160 mmol) in water (90 mL) at 0 °C. The mixture was warmed to 25 °C and was stirred at that temperature for 20 h. Next, acetic acid was added to acidify the mixture to pH ~6. The mixture was diluted with sat. aq. sodium chloride solution (100 mL), and the resulting mixture was extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to provide (3a*R*,4*S*,6a*R*)-4-allyl-5-azido-2-(*tert*-butoxycarbonyl)octahydro-cyclopenta[*c*]pyrrole-5-carboxylic acid as a brown oil (17 g, 108% yield), which was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{16}H_{24}N_4O_4$, 337.2; found 337.1.

Step 5. 5-Benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-4-allyl-5-azidohexahydrocyclopenta[*c*]pyrrole-2,5(1*H*)-dicarboxylate



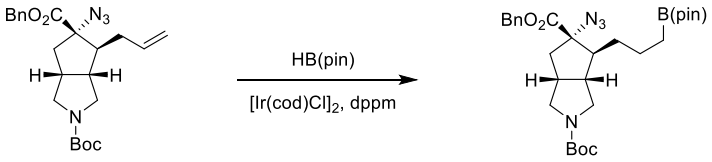
S40 **S41**

A mixture of (3a*R*,4*S*,6a*R*)-4-allyl-5-azido-2-(*tert*-butoxycarbonyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid (17 g, 51 mmol) and potassium carbonate (10 g, 76 mmol) in *N*,*N*-dimethylformamide (100 mL) was treated with benzyl bromide (6.6 mL, 56 mmol) at 25 °C. The reaction mixture was stirred at this temperature for 2.5 h before it was diluted with 1M aq. sodium chloride solution (500 mL). The resulting mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide crude product (~4 g) as a pale-yellow oil. The stereoisomeric components of this product mixture were separated by supercritical-fluid chromatography (Daicel Chiralpak IC column, 250 × 30 mm, 5 μm; 0.1% v/v ammonium hydroxide in ethanol modifier; 15% modifier in CO_2 mobile phase; 180 mL/min flow rate) to give 5-benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-4-allyl-5-azidohexahydrocyclopenta[*c*]pyrrole-2,5(1H)-dicarboxylate, the first-eluting peak (t_r = 1.86 min), as a light orange oil (3.5 g, 21% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.44 (m, 5H), 5.61–5.75 (m, 1H), 5.13–5.30 (m, 2H), 4.87–5.02 (m, 2H), 3.36–3.44 (m, 1H), 3.30 (br d, *J* = 4.9 Hz, 3H), 2.81–2.93 (m, 1H), 2.52–2.60 (m, 1H), 2.44–2.52 (m, 1H), 2.21–2.30 (m, 1H), 1.92–2.00 (m, 1H), 1.83–1.91 (m, 1H), 1.73–1.82 (m, 1H), 1.45 (s, 9H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{23}H_{30}N_4O_4$, 427.2; found 427.2.

Step 6. 5-Benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2,5(1*H*)-dicarboxylate



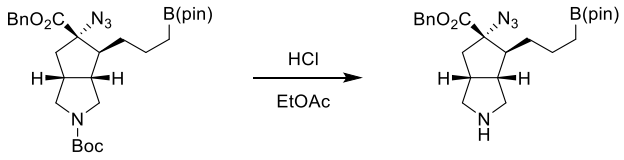
S41 **S42**

A solution of bis(diphenylphosphino)methane (0.10 g, 0.26 mmol), bis(1,5-cyclooctadiene)diiridium(I) dichloride (0.13 g, 0.19 mmol) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.7 mL, 19 mmol) in anhydrous dichloromethane (30 mL) was stirred at 25 °C for 20 min under nitrogen gas. A solution of 5-benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-4-allyl-5-azidohexahydrocyclopenta[c]pyrrole-2,5(1*H*)-dicarboxylate (1.6 g, 3.8 mmol) in anhydrous dichloromethane (10 mL) was then added into the mixture. The reaction mixture was stirred at 25 °C for 12 h before it was concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide 5-benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydro cyclopenta[*c*]pyrrole-2,5(1*H*)-dicarboxylate as a yellow oil (1.8 g, 82% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.42 (m, 5H), 5.15–5.26 (m, 2H), 3.17–3.47 (m, 4H), 2.87 (br d, J = 4.4 Hz, 1H), 2.39–2.55 (m, 2H), 1.90 (br t, J = 9.4 Hz, 1H), 1.75 (br dd, J = 7.5, 13.6 Hz, 1H), 1.45 (s, 10H), 1.34–1.43 (m, 3H), 1.22 (s, 12H), 1.19–1.22 (m, 3H), 0.68 (br s, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{43}BN_4O_6$, 555.3; found 555.3.

Step 7. Benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate



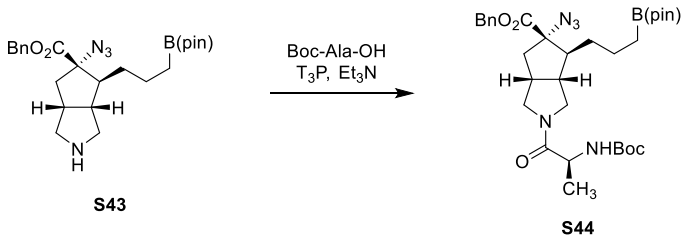
S42 **S43**

To a solution of 5-benzyl 2-(*tert*-butyl) (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2,5(1*H*)-dicarboxylate (2.0 g, 3.6 mmol) in dichloromethane (21 mL) was added anhydrous 4M hydrochloric acid in ethyl acetate (3.0 mL, 12 mmol) at 25 °C. The mixture was stirred at 25 °C for 12 h. The solution was then concentrated under reduced pressure (at ≤ 25 °C) to give benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate (hydrochloride salt, 1.8 g, 92 % yield) as white solid, which was used in the next step without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 7.31–7.49 (m, 5H), 5.23–5.31 (m, 2H), 3.35–3.42 (m, 1H), 3.15–3.23 (m, 3H), 2.74 (dq, J = 4.0, 8.9 Hz, 1H), 2.63 (dd, J = 8.3, 13.6 Hz, 1H), 1.95–2.00 (m, 1H), 1.85 (dd, J = 7.9, 13.6 Hz, 1H), 1.32–1.49 (m, 3H), 1.23 (s, 13H), 1.06–1.18 (m, 1H), 0.63–0.70 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{24}H_{35}BN_4O_4$, 455.3; found 455.5.

Step 8. Benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-2-((*tert*-butoxycarbonyl)-L-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate



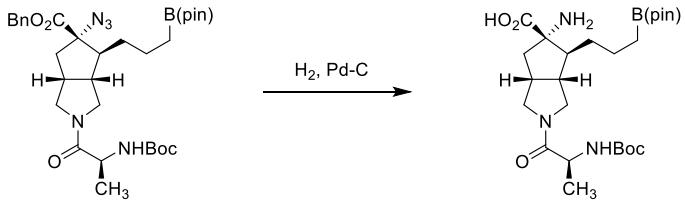
To a solution of benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxylate (180 mg, 0.37 mmol) and (*S*)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (Boc-Ala-OH, 140 mg, 0.73 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was added triethylamine (0.256 mL, 1.834 mmol) at 25 °C. The mixture was stirred at this temperature for 2 min before propanephosphonic acid anhydride (T3P, 1.6M solution in *N*,*N*-dimethylformamide, 0.46 mL, 0.73 mmol) was added. The mixture was stirred at 25 °C for 12 h. The reaction was then diluted with 1M aq. sodium chloride solution (15 mL), and the resulting mixture was extracted with ethyl acetate (3 × 10 mL). The separated organic layers were dried over sodium sulfate, filtered, and concentrated under reduced pressure to give crude product. This residue was then purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to provide (3a*R*,4*S*,5*S*,6a*R*)-5-azido-2-((*tert*-butoxycarbonyl)-L-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate as a white solid (110 mg, 24% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 7.30–7.47 (m, 5H), 5.25 (s, 2H), 4.24–4.44 (m, 1H), 3.63–3.81 (m, 2H), 3.52–3.62 (m, 1H), 3.39–3.52 (m, 1H), 3.32–3.38 (m, 1H), 2.89–3.08 (m, 1H),

2.46–2.72 (m, 2H), 1.80–1.98 (m, 2H), 1.75 (br dd, *J* = 6.8, 13.8 Hz, 1H), 1.43 (d, *J* = 4.8 Hz, 10H), 1.31–1.40 (m, 3H), 1.18–1.30 (m, 11H), 0.64 (br d, *J* = 7.0 Hz, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{32}H_{48}BN_5O_7$, 626.3; found 626.4.

Step 9. (3a*R*,4*S*,5*S*,6a*R*)-5-Amino-2-((*tert*-butoxycarbonyl)-l-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid

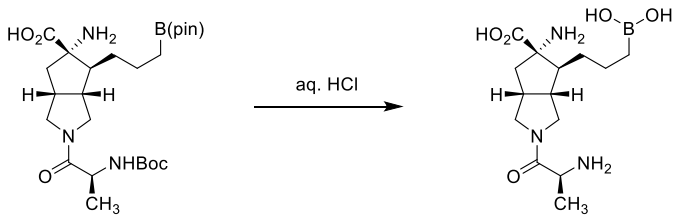


S44 **S45**

Palladium on carbon (10% w/w, 30 mg, 0.028 mmol) was added to a solution of benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-2-((*tert*-butoxycarbonyl)-L-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate (170 mg, 0.15 mmol) in methanol (6.0 mL) under a blanket of nitrogen gas. The headspace of the reaction vessel was then flushed with hydrogen gas using three evacuation–backfill cycles. The resulting mixture was stirred under 15 psi of hydrogen gas at 25 °C for 1 h. The mixture was then filtered, and the filtrate was concentrated under reduced pressure to give (3a*R*,4*S*,5*S*,6a*R*)-5-amino-2-((*tert*-butoxycarbonyl)-L-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid together with the corresponding boronic acid (~1:1 molar ratio, 140 mg, ~97 % yield) as a yellow oil. This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H–Boc]^+$ calc'd for $C_{25}H_{44}BN_3O_7$, 410.3; found 410.3.

Step 10. (3a*R*,4*S*,5*S*,6a*R*)-2-(L-Alanyl)-5-amino-4-(3-boronopropyl)octahydrocyclopenta[*c*] pyrrole-5-carboxylic acid



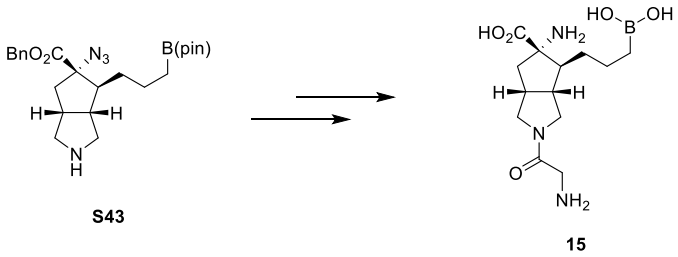
S45 **16**

A mixture of (3a*R*,4*S*,5*S*,6a*R*)-5-amino-2-((*tert*-butoxycarbonyl)-L-alanyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid and the corresponding boronic acid (~1:1 molar ratio, 140 mg, ~0.14 mmol) was dissolved in 6M aq. hydrochloric acid solution (4.0 mL, 24 mmol), and the resulting solution was stirred at 20 °C for 13 h. The reaction was concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (3a*R*,4*S*,5*S*,6a*R*)-2-(L-alanyl)-5-amino-4-(3-boronopropyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid as a white solid (TFA salt, 68 mg, 102% yield).

^{1}H NMR (400 MHz, D_2O) δ 4.18–4.34 (m, 1H), 3.58–3.80 (m, 2H), 3.30–3.53 (m, 2H), 3.04–3.24 (m, 1H), 2.66–2.87 (m, 1H), 2.61 (ddd, J = 4.2, 8.7, 13.5 Hz, 1H), 1.88–2.05 (m, 1H), 1.70 (br dd, J = 8.6, 12.1 Hz, 1H), 1.49 (br dd, J = 4.0, 11.0 Hz, 1H), 1.41 (br dd, J = 6.8, 16.0 Hz, 4H), 1.22–1.36 (m, 2H), 0.63–0.79 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{14}H_{26}BN_3O_5$, 310.1, found, 310.0.

*Synthesis of Compound **15**.*



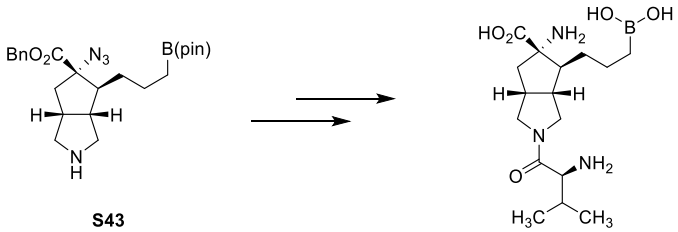
S43 **15**

Compound **15** was prepared from **S43** by a sequence analogous to that described above for compound **16**.

^{1}H NMR (400 MHz, D_2O) δ = 4.00–3.81 (m, 2H), 3.76–3.57 (m, 1H), 3.76–3.56 (m, 2H), 3.50–3.33 (m, 1H), 3.29–3.09 (m, 1H), 2.90–2.73 (m, 1H), 2.67 (dd, *J* = 8.3, 13.6 Hz, 1H), 2.14–1.97 (m, 1H), 1.78 (td, *J* = 9.0, 13.6 Hz, 1H), 1.61–1.26 (m, 4H), 0.82–0.68 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{24}BN_3O_5$, 296.2; found 296.0.

*Synthesis of Compound **17**.*



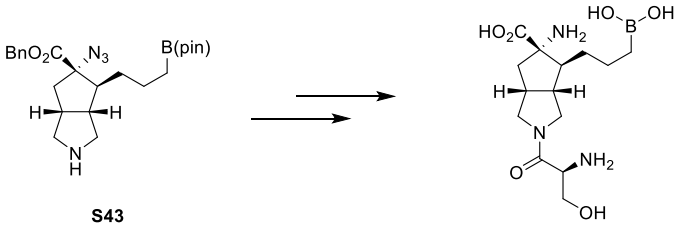
S43 **17**

Compound **17** was prepared from **S43** by a sequence analogous to that described above for compound **16**. (*tert*-Butoxycarbonyl)-L-valine (Boc-Val-OH) was used as the coupling partner in Step 8.

^{1}H NMR (500 MHz, D_2O) δ 4.27–3.98 (m, 1H), 3.95–3.61 (m, 2H), 3.59–3.28 (m, 2H), 3.27–3.04 (m, 1H), 2.95–2.50 (m, 2H), 2.30–2.12 (m, 1H), 2.10–1.89 (m, 1H), 1.85–1.66 (m, 1H), 1.64–1.19 (m, 4H), 1.11–0.83 (m, 6H), 0.80–0.63 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{16}H_{28}BN_3O_5$, 338.2; found 338.2.

*Synthesis of Compound **18**.*

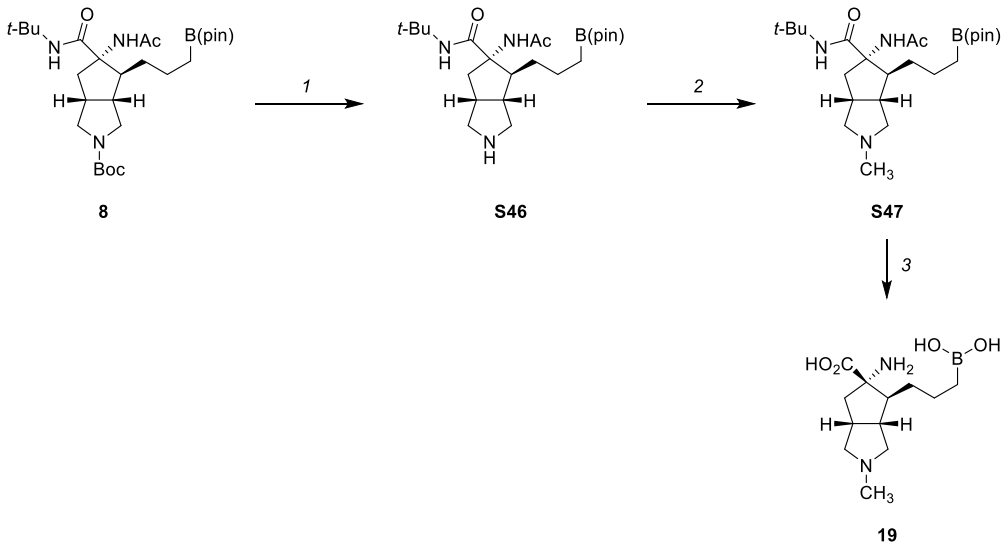


Compound **18** was prepared from **S43** by a sequence analogous to that described above for compound **16**. *N*-(*tert*-Butoxycarbonyl)-*O*-(*tert*-butyl)-L-serine (Boc-Ser[O*t*-Bu]-OH) was used as the coupling partner in Step 8.

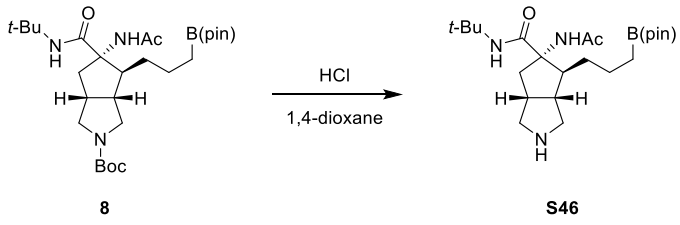
^{1}H NMR (400 MHz, D_2O) δ 4.47–4.32 (m, 1H), 3.98–3.73 (m, 3H), 3.72–3.65 (m, 1H), 3.59–3.37 (m, 2H), 3.29–3.07 (m, 1H), 2.90–2.69 (m, 1H), 2.69–2.59 (m, 1H), 2.09–1.92 (m, 1H), 1.75 (br dd, *J* = 13.2, 8.8 Hz, 1H), 1.57–1.42 (m, 2H), 1.33 (m 2H), 0.82–0.68 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{14}H_{26}BN_3O_6$, 326.2; found 326.0.

Synthesis of Compound 19



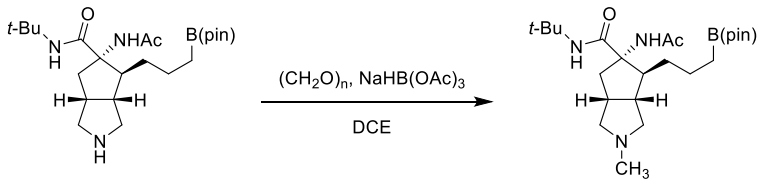
Step 1. (3a*R*,4*S*,5*S*,6a*R*)-5-Acetamido-*N*-(*tert*-butyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide



A mixture of *tert*-butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (1.0 g, 1.9 mmol) and 4M HCl in dioxane (8 mL) was stirred at 20 °C for 20 min. The mixture was concentrated under reduced pressure to give (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-*N*-(*tert*-butyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide as a white solid (HCl salt, 1.0 g, 113% yield). This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{23}H_{42}BN_3O_4$, 436.4; found 436.4.

Step 2. (3a*R*,4*S*,5*S*,6a*R*)-5-Acetamido-*N*-(*tert*-butyl)-2-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide



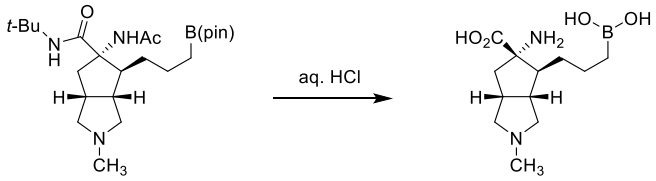
S46 **S47**

Sodium triacetoxyborohydride (610 mg, 2.9 mmol) was added to a mixture of (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-*N*-(*tert*-butyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxamide (500 mg, 1.1 mmol) and paraformaldehyde (500 mg, 16 mmol) in 1,2-dichloroethane (10 mL). The mixture was heated to 70 °C and stirred for 12 h at that temperature. The mixture was then cooled to ambient temperature before methanol (10 mL) was added. The mixture was concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to afford (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-*N*-(*tert*-butyl)-2-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxamide as a colorless oil (TFA salt, 160 mg, 30% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 3.96–3.69 (m, 1H), 3.55–3.35 (m, 2H), 3.29–3.05 (m, 3H), 2.97–2.85 (m, 3H), 2.73–2.49 (m, 1H), 2.03 (s, 1H), 2.02–1.96 (m, 3H), 1.72–1.53 (m, 1H), 1.47–1.37 (m, 2H), 1.37–1.03 (m, 18H), 0.93–0.63 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{24}H_{44}BN_3O_4$, 450.3; found 450.3.

Step 3. (3a*R*,4*S*,5*S*,6a*R*)-5-Amino-4-(3-boronopropyl)-2-methyloctahydrocyclopenta[*c*] pyrrole-5-carboxylic acid



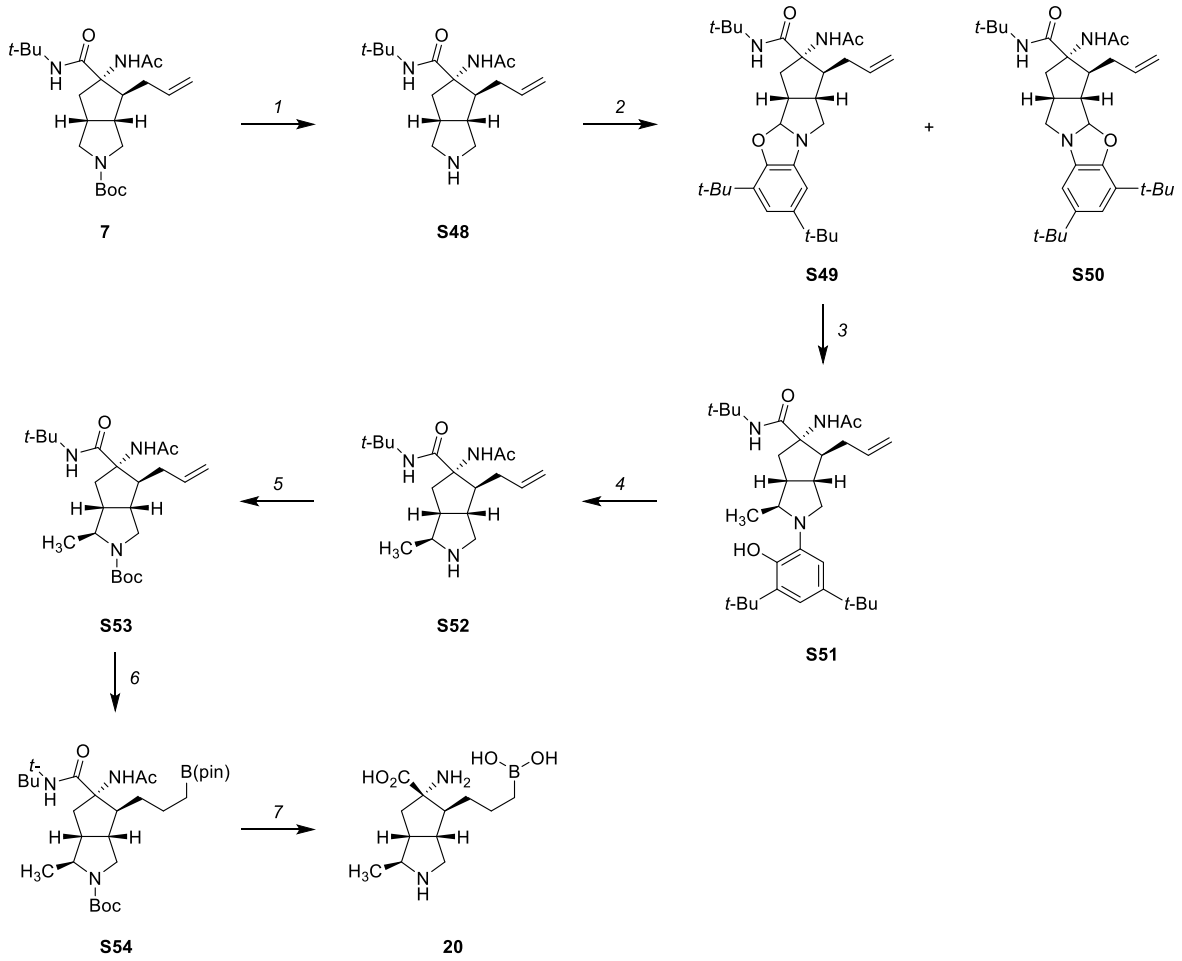
S47 19

A mixture of (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-*N*-(*tert*-butyl)-2-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[c]pyrrole-5-carboxamide (140 mg, 0.31 mmol) in 37% w/v aq. hydrochloric acid (3.0 mL) was stirred at 100 °C for 12 h. The mixture was then concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-boronopropyl)-2-methyloctahydrocyclopenta[c]pyrrole-5-carboxylic acid as a colorless oil (TFA salt, 39 mg, 32% yield).

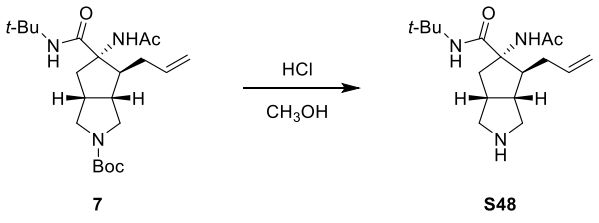
^{1}H NMR (400 MHz, D_2O) δ 3.96–3.75 (m, 1H), 3.55–3.37 (m, 2H), 3.32–3.12 (m, 2H), 3.03–2.92 (m, 1H), 2.85 (br d, J = 4.6 Hz, 3H), 2.68–2.50 (m, 1H), 2.25–1.96 (m, 1H), 1.91–1.69 (m, 1H), 1.53 (br d, J = 10.8 Hz, 1H), 1.41–1.13 (m, 3H), 0.69 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.1.

Synthesis of Compounds 20 and 21



Step 1. (3a*R*,4*S*,5*S*,6a*R*)-5-Acetamido-4-allyl-*N*-(*tert*-butyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide

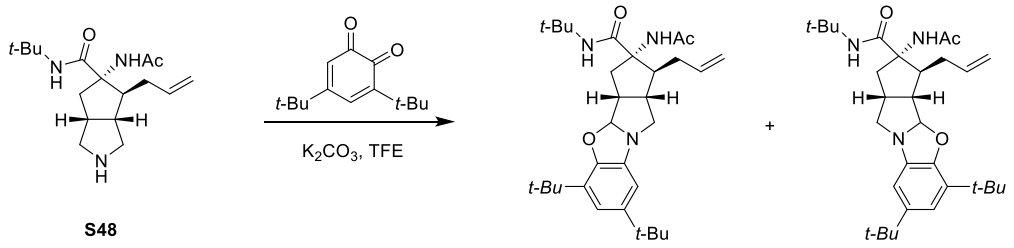


A mixture of *tert*-butyl (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (1.0 g, 2.5 mmol) and 4M hydrochloric acid in methanol (10 mL, 40 mmol) was stirred at 15 °C for 12 h. The mixture was then concentrated under reduced pressure to give

(3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide as a white solid (HCl salt, 850 mg, 91% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{17}H_{29}N_3O_2$, 308.3; found 308.2.

Step 2. (1*S*,2*S*,3a*R*,10a*S*)-2-acetamido-1-allyl-*N*,5,7-tri-*tert*-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide and (2*S*,3*S*,3a*R*,10a*R*)-2-acetamido-3-allyl-*N*,5,7-tri-*tert*-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide



Potassium carbonate (510 mg, 3.7 mmol) and 3,5-di-*tert*-butylcyclohexa-3,5-diene-1,2-dione (550 mg, 2.5 mmol) were added sequentially to a solution of (3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)octahydrocyclopenta[*c*]pyrrole-5-carboxamide hydrochloride (850 mg, 2.5 mmol) in 2,2,2-trifluoroethanol (15 mL) at 20 °C under a blanket of nitrogen gas. The mixture was stirred at ambient temperature for 3 h before it was concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide an orange solid comprising a mixture (molar ratio 42:58) of (1*S*,2*S*,3a*R*,10a*S*)-2-acetamido-1-allyl-*N*,5,7-tri-*tert*-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide and (2*S*,3*S*,3a*R*,10a*R*)-2-acetamido-3-allyl-*N*,5,7-*tri*-tert-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide (1.0 g, 80% combined yield).

This mixture (1.0 g, 491 mmol) was then separated by supercritical-fluid chromatography (Phenomenex Cellulose 2 column, 250 × 50 mm, 10 μm; 0.1% v/v ammonium hydroxide in methanol modifier; 15% modifier in CO_2 mobile phase; 200 mL/min flow rate). The first-eluting peak (t_r = 2.60 min) corresponded to

(1*S*,2*S*,3a*R*,10a*S*)-2-acetamido-1-allyl-*N*,5,7-tri-*tert*-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide (**S49**), which was obtained as a pale orange oil (330 mg, 26% overall yield). The second-eluting peak (t_r = 2.81 min) corresponded to (2*S*,3*S*,3a*R*,10a*R*)-2-acetamido-3-allyl-*N*,5,7-*tri*-tert-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide (**S50**), which was obtained as a pale orange oil (510 mg, 40% overall yield).

Compound **S49**

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (d, *J* = 1.8 Hz, 1H), 6.78–6.81 (m, 1H), 6.43 (s, 1H), 6.10 (s, 1H), 5.72–5.86 (m, 1H), 5.08–5.15 (m, 1H), 3.46 (s, 1H), 3.29 (d, *J* = 9.7 Hz, 1H), 3.17–3.25 (m, 1H), 3.10–3.15 (m, 1H), 2.73–2.87 (m, 1H), 2.30 (td, *J* = 6.4, 13.1 Hz, 1H), 2.05–2.20 (m, 2H), 1.96–2.04 (m, 3H), 1.41 (d, *J* = 4.4 Hz, 1H), 1.21–1.39 (m, 27H).

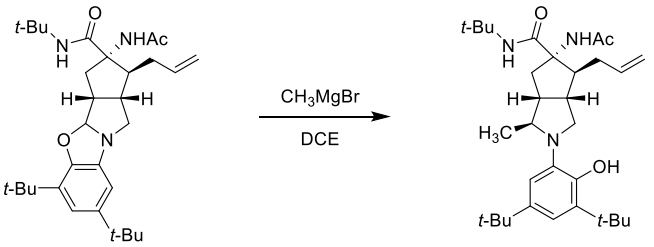
MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{31}H_{47}N_3O_3$, 510.7; found 510.6.

Compound **S50**

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82–6.87 (m, 1H), 6.72–6.77 (m, 1H), 6.62 (s, 1H), 6.30 (br s, 1H), 5.78–5.93 (m, 1H), 5.11–5.15 (m, 1H), 3.29–3.39 (m, 1H), 3.23 (dd, *J* = 4.6, 10.7 Hz, 1H), 2.99–3.12 (m, 1H), 2.82–2.99 (m, 2H), 2.42–2.55 (m, 1H), 2.30–2.42 (m, 1H), 2.10–2.22 (m, 1H), 1.95–2.05 (m, 3H), 1.73–1.84 (m, 1H), 1.24–1.40 (m, 27H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{31}H_{47}N_3O_3$, 510.7; found 510.6.

Step 3. (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-Acetamido-4-allyl-*N*-(*tert*-butyl)-2-(3,5-di-*tert*-butyl-2-hydroxyphenyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide



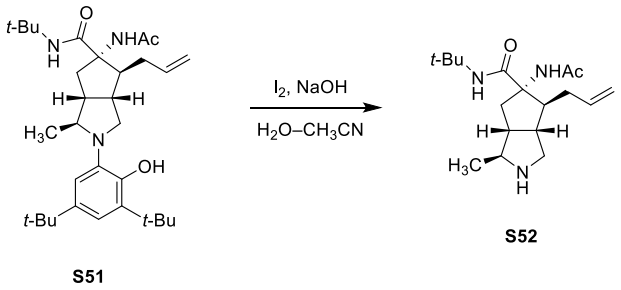
S49 **S51**

Methylmagnesium bromide (3M solution diethyl ether, 1.1 mL, 3.2 mmol) was added to a solution of (1*S*,2*S*,3a*R*,10a*S*)-2-acetamido-1-allyl-*N*,5,7-tri-*tert*-butyl-2,3,3a,3b,10,10a-hexahydro-1*H*-benzo[*d*]cyclopenta[3,4]pyrrolo[2,1-*b*]oxazole-2-carboxamide in 1,2-dichloroethane (5.0 mL) at 0 °C. The mixture was stirred at this temperature for 4 h before excess organometallic reagent was quenched with the careful addition of sat. aq. ammonium chloride solution (8.0 mL). The resulting mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with brine (5.0 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to provide (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)-2-(3,5-di-*tert*-butyl-2-hydroxyphenyl) -1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide as a white solid (250 mg, 62% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 1.8 Hz, 1H), 7.02 (d, *J* = 2.2 Hz, 1H), 6.49 (s, 1H), 5.83 (s, 1H), 5.70 (dt, *J* = 7.5, 16.9 Hz, 1H), 5.06 (br d, *J* = 17.1 Hz, 1H), 4.98 (br d, *J* = 10.1 Hz, 1H), 3.41–3.49 (m, 1H), 2.99–3.07 (m, 1H), 2.67 (dd, *J* = 8.6, 13.8 Hz, 1H), 2.44–2.63 (m, 4H), 2.22–2.34 (m, 2H), 2.04 (s, 3H), 1.90–2.00 (m, 1H), 1.39 (m, 17H), 1.28 (s, 9H), 0.90 (d, *J* = 6.1 Hz, 3H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{32}H_{51}N_3O_3$, 526.3; found 526.5.

Step 4. (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-Acetamido-4-allyl-*N*-(*tert*-butyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide

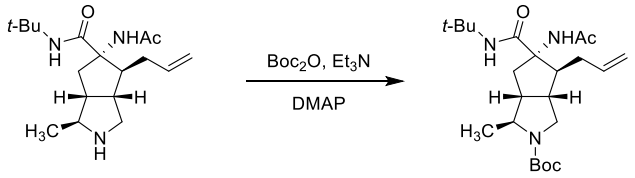


S51 **S52**

Crystalline iodine (160 mg, 0.62 mmol) was added to a mixture of (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)-2-(3,5-di-*tert*-butyl-2-hydroxyphenyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide (310 mg, 0.59 mmol), acetonitrile (30 mL), and 1.0M aq. sodium hydroxide solution (15 mL, 15 mmol) under a blanket of argon gas at 0 °C. The mixture was stirred at this temperature for 1 h before it was extracted with dichloromethane (3 × 15 mL). The combined organic extracts were extracted with 2M aq. hydrochloric acid solution (2 × 10 mL). The combined aqueous acid extracts were washed with petroleum ether (10 mL), and the aqueous product solution was then concentrated under reduced pressure to afford (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide as a light orange solid (300 mg, 158% yield). The product was suspected to contain sodium chloride as an impurity, accounting for the additional product mass.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_8H_{31}N_3O_2$, 322.2; found 322.2.

Step 5. *tert*-Butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)-1-methylhexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



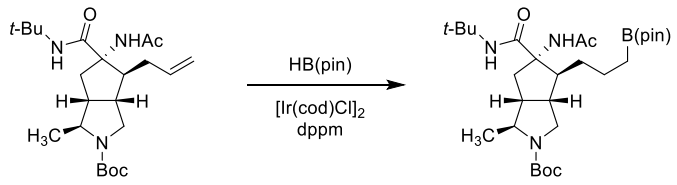
S52 **S53**

Triethylamine (520 μL, 380 mg, 3.7 mmol), di-*tert*-butyl dicarbonate (220 mg, 1.0 mmol) and 4-(dimethylamino)pyridine were added sequentially to a solution of (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxamide in tetrahydrofuran (10 mL) at 0 °C. The mixture was warmed gradually to 15 °C, and the mixture was stirred at this temperature for 40 min. The reaction mixture was then diluted with sat. aq. ammonium chloride solution (10 mL). This mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to give *tert*-butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)-1-methylhexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate as a white solid (130 mg, 29% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.28 (br s, 1H), 5.90 (br s, 1H), 5.87–5.74 (m, 1H), 5.18–5.04 (m, 2H), 3.46–3.30 (m, 2H), 3.17 (br dd, *J* = 9.0, 13.7 Hz, 1H), 2.63 (br s, 1H), 2.52 (br d, *J* = 8.3 Hz, 1H), 2.32 (td, *J* = 7.1, 14.0 Hz, 1H), 2.22–2.09 (m, 1H), 1.98 (s, 3H), 1.92–1.80 (m, 1H), 1.93–1.79 (m, 1H), 1.54–1.48 (m, 1H), 1.45 (s, 9H), 1.32 (s, 9H), 1.28–1.22 (m, 1H), 1.14–1.03 (m, 3H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{23}H_{39}N_3O_5$, 422.3; found 422.3.

Step 6. tert-Butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-1-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate



S53 **S54**

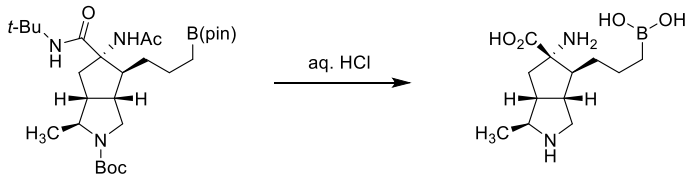
A solution of bis(diphenylphosphino)methane (7.0 mg, 0.018 mmol), bis(1,5-cyclooctadiene)diiridium(I) dichloride (8.8 mg, 0.013 mmol), and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (110 μL, 100 mg, 0.78 mmol) in anhydrous dichloromethane (4.0 mL) was stirred under dry nitrogen gas at 15 °C for 20 min before a solution of *tert*-butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)-1-methylhexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (110 mg, 0.26 mmol) in anhydrous dichloromethane (4.0 mL) was added. The resulting reaction mixture was stirred at 15 °C for 12 h under an atmosphere of nitrogen gas. The mixture was then concentrated under reduced pressure, and the residue was purified by flash-column chromatography (eluting with ethyl acetate–petroleum ether) to give *tert*-butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-1-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate as a white solid (170 mg, 92% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.32–6.05 (m, 1H), 5.95 (br s, 1H), 3.61 (br s, 1H), 3.40–3.26 (m, 2H), 3.09 (dd, *J* = 8.8, 13.4 Hz, 1H), 2.70–2.52 (m, 1H), 2.49–2.36 (m, 1H), 1.95 (s,

3H), 1.91 (br s, 1H), 1.74 (br s, 1H), 1.38 (s, 12H), 1.24 (s, 9H), 1.20–1.19 (m, 6H), 1.18 (s, 9H), 1.02 (br d, J = 4.9 Hz, 3H), 0.84–0.62 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{52}BN_3O_6$, 550.4; found 550.4.

Step 7. (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-Amino-4-(3-boronopropyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxylic acid



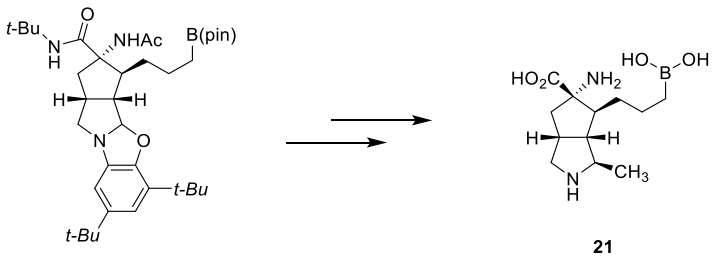
S54 **20**

A mixture of 37% w/v aq. hydrochloric acid solution (4.0 mL, 48 mmol) and *tert*-butyl (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-acetamido-5-(*tert*-butylcarbamoyl)-1-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate was stirred at 100 °C for 13 h. The mixture was then concentrated under reduced pressure, and the residue was treated with sat. aq. sodium carbonate solution until pH ~9–10 was achieved. The basified aqueous mixture was washed with dichloromethane (3 × 3 mL). The aqueous layer was then concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.2 mM HTBA and 0.1% TFA) to give (1*S*,3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-boronopropyl)-1-methyloctahydrocyclopenta[*c*]pyrrole-5-carboxylic acid as a white solid (HFBA salt, 49 mg, 60% yield).

^{1}H NMR (400 MHz, D_2O) δ = 3.68 (dd, *J* = 8.3, 12.0 Hz, 1H), 3.55–3.40 (m, 1H), 3.12 (dd, *J* = 6.7, 12.1 Hz, 1H), 3.01–2.79 (m, 2H), 2.64 (dd, *J* = 8.6, 13.7 Hz, 1H), 2.15 (dt, *J* = 3.3, 9.8 Hz, 1H), 1.86–1.71 (m, 1H), 1.60–1.47 (m, 1H), 1.43–1.35 (m, 1H), 1.33 (d, *J* = 6.4 Hz, 3H), 1.31–1.14 (m, 2H), 0.80–0.65 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.2.

*Synthesis of Compound **21**.*



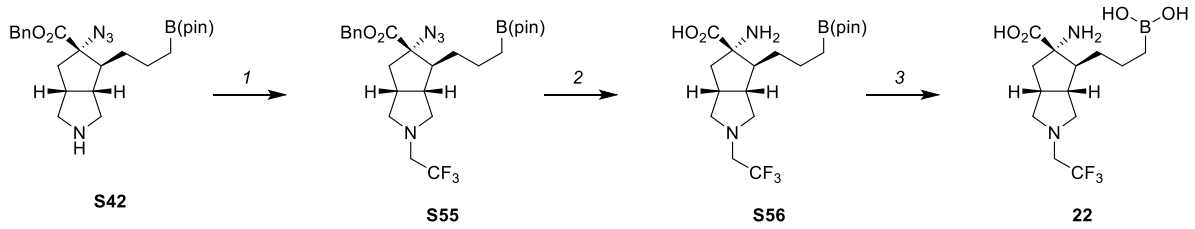
S50 **21**

Compound **21** was synthesized from intermediate **S50** by a sequence analogous to that described above for the synthesis of **20**.

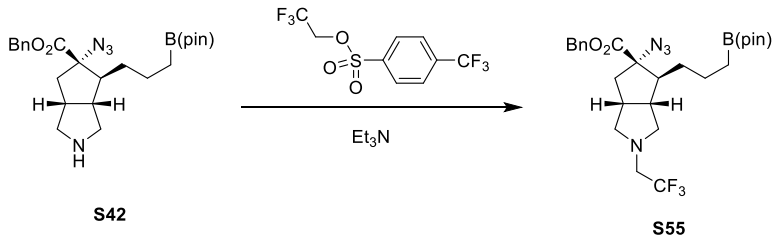
^{1}H NMR (400 MHz, D_2O) δ = 3.51 (br dd, *J* = 2.4, 6.8 Hz, 1H), 3.28 (dd, *J* = 8.3, 11.8 Hz, 1H), 3.09–2.97 (m, 1H), 2.93 (dd, *J* = 3.5, 11.8 Hz, 1H), 2.33–2.21 (m, 2H), 1.64–1.55 (m, 1H), 1.38 (br dd, *J* = 5.9, 13.8 Hz, 1H), 1.29 (br d, *J* = 7.9 Hz, 1H), 1.26–1.17 (m, 2H), 1.14 (d, *J* = 7.0 Hz, 3H), 1.10–0.99 (m, 1H), 0.61–0.46 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.2.

Synthesis of Compound 22



Step 1. Benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate



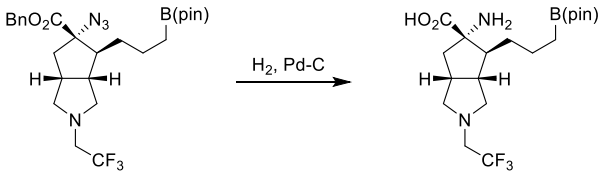
2,2,2-Trifluoroethyl 4-(trifluoromethyl)benzenesulfonate (280 mg, 0.92 mmol) was added to a solution of benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydrocyclopenta[*c*]pyrrole-5-carbox ylate hydrochloride (150 mg, 0.31 mmol) and triethylamine (0.21 mL, 160 mg, 1.5 mmol) in *N*,*N*-dimethylacetamide (2.0 mL) at 15 °C. The mixture was stirred at 115 °C for 12 h. The mixture was then concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to provide benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate as an orange-colored oil (90 mg, 47% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.41 (m, 5H), 5.19–5.26 (m, 2H), 3.37–3.58 (m, 2H), 3.16–3.31 (m, 2H), 3.01–3.10 (m, 1H), 2.89–3.00 (m, 2H), 2.65 (td, *J* = 7.6, 15.0 Hz,

1H), 2.48 (dd, *J* = 8.7, 13.6 Hz, 1H), 1.98–2.07 (m, 1H), 1.84 (dd, *J* = 7.1, 13.7 Hz, 1H), 1.27–1.47 (m, 4H), 1.22 (s, 11H), 0.99–1.10 (m, 1H), 0.64–0.73 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{26}H_{36}BF_3N_4O_4$, 537.3; found 537.5.

Step 2. (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid

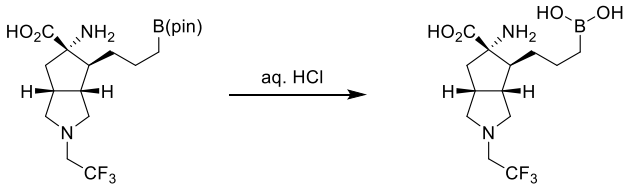


S55 **S56**

Palladium on carbon (10% w/w, 15 mg, 0.014 mmol) was added to a solution of benzyl (3a*R*,4*S*,5*S*,6a*R*)-5-azido-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylate (90 mg, 0.17 mmol) in methanol (6.0 mL) under a blanket of nitrogen gas. The headspace of the reaction vial was replaced with hydrogen gas by three evacuation–backfill cycles, and the reaction mixture was stirred under 15 atm of hydrogen gas at 25 °C for 30 min. The mixture was then filtered, and the filtrate was concentrated under reduced pressure to give (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[c]pyrrole-5-carboxylic acid as a colorless oil (60 mg, 85% yield). This material was used in the next step without purification.

MS(ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{19}H_{32}BF_3N_2O_4$, 421.3; found 421.2.

Step 3. (3a*R*,4*S*,5*S*,6a*R*)-5-Amino-4-(3-boronopropyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid



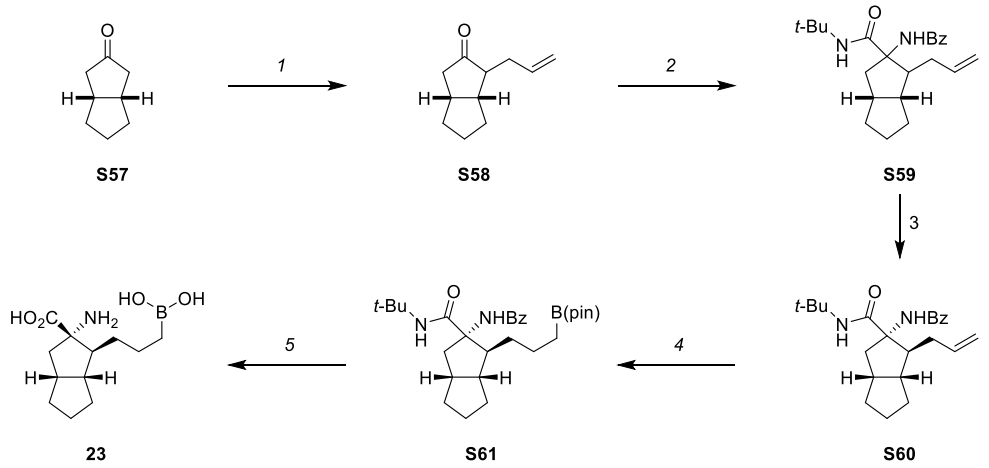
S56 **22**

A mixture of (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid (60 mg, 0.14 mmol) and 6N aq. hydrochloric acid solution (4.0 mL, 24 mmol) was stirred at 20 °C for 13 h. The reaction mixture was then concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (3a*R*,4*S*,5*S*,6a*R*)-5-amino-4-(3-boronopropyl)-2-(2,2,2-trifluoroethyl)octahydrocyclopenta[*c*]pyrrole-5-carboxylic acid as a white solid (TFA salt, 28 mg, 41% yield).

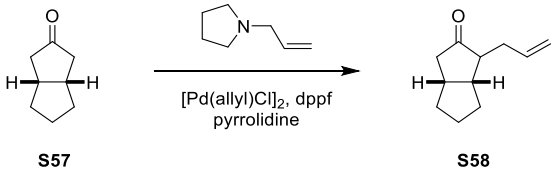
^{1}H NMR (400 MHz, D_2O) δ 4.16 (q, J = 8.5 Hz, 2H), 4.02–3.92 (m, 1H), 3.91–3.80 (m, 1H), 3.41–3.24 (m, 3H), 3.02–2.89 (m, 1H), 2.67–2.57 (m, 1H), 2.23–2.12 (m, 1H), 1.92–1.80 (m, 1H), 1.60–1.49 (m, 1H), 1.44–1.32 (m, 1H), 1.31–1.18 (m, 2H), 0.80–0.67 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{22}BF_3N_2O_4$, 321.2; found 321.0.

Synthesis of Compound 23



Step 1. *rac*-(3a*R*,6a*R*)-1-Allylhexahydropentalen-2(1*H*)-one

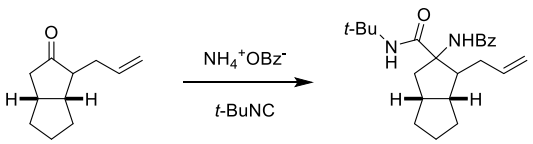


Under an atmosphere of dry nitrogen gas, allylpalladium(II) chloride dimer (0.31 g, 0.85 mmol), 1,1'-bis(diphenylphosphino)ferrocene (0.94 g, 1.7 mmol) were dissolved in anhydrous methanol (30 mL) at 20 °C. A pale brown solution resulted. To this solution was added a solution of *N*-allylpyrrolidine (1.9 g, 17 mmol) in methanol (5.0 mL), and the mixture was stirred at ambient temperature for an additional 10 min. Next, pyrrolidine (0.25 mL, 0.24 g, 3.4 mmol) was added, followed by a solution of *cis*-bicyclo[3.3.0]-octan-2-one (2.1 g, 17 mmol) in methanol (10 mL). The reaction was stirred at 20 °C for 15 h. The mixture was then concentrated under reduced pressure, and the residue was re-dissolved in dichloromethane (20 mL). This solution was washed with water (15 mL). The aqueous wash phase was extracted with fresh dichloromethane (2 × 15 mL). The organic layers were then combined, and the combined solution was washed with brine (20 mL), dried over anhydrous sodium sulfate,

filtered, and concentrated under reduced pressure to provide crude *rac*-(3a*R*,6a*R*)-1-allylhexahydropentalen-2(1*H*)-one (2.2 g), which was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{11}H_{16}O$, 165.1; found 165.2.

Step 2. rac-(3a*R*,6a*R*)-1-Allyl-2-benzamido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide

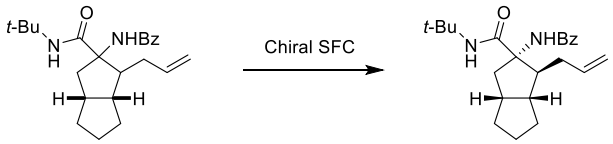


S58 **S59**

Ammonium benzoate (5.3 g, 38 mmol) and *tert*-butyl isocyanide (4.3 mL, 3.2 g, 38 mmol) were added to a solution of *rac*-(3a*R*,6a*R*)-1-allylhexahydropentalen-2(1*H*)-one (crude, 2.1 g, theoretically 13 mmol) in 2,2,2-trifluoroethanol (10 mL). The mixture was stirred at 60 °C for 14 h before the solvent was removed under reduced pressure. The residue was suspended in a mixture of water (50 mL) and ethyl acetate (60 mL). The biphasic mixture was shaken, and the layers were separated. The aqueous layer was extracted with additional portions of ethyl acetate (3 × 30 mL). The organic fractions were pooled, and the pooled solution was washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to provide *rac*-(3a*R*,6a*R*)-1-allyl-2-benzamido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide as a brown oil (1.3 g, 26% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{23}H_{32}N_2O_2$ 369.2; found 369.2.

Step 3. (1*S*,2*S*,3a*R*,6a*R*)-1-Allyl-2-benzamido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide



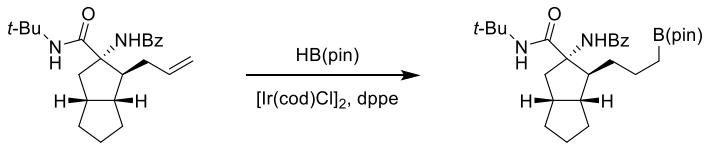
S59 **S60**

rac-(3a*R*,6a*R*)-1-Allyl-2-benzamido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide (1.3 g, 3.5 mmol) was resolved by chiral supercritical-fluid chromatography (Regis [*S,S*]-Whelk-O1 column, 250 × 50 mm, 10 μm; 0.1% v/v ammonium hydroxide–methanol modifier; 25% modifier in CO_2 mobile phase; 180 mL/min flow rate). The third-eluting peak (t_r = 8.7 min) corresponded to (1*S*,2*S*,3a*R*,6a*R*)-1-allyl-2-benzamido-*N*-(*tert*-butyl)octahydro-pentalene-2-carboxamide (160 mg, 11% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 7.6 Hz, 2H), 7.53–7.40 (m, 3H), 5.96 (br s, 1H), 5.84 (br dd, J = 10.0, 17.2 Hz, 1H), 5.18–4.74 (m, 2H), 2.65 (br d, J = 9.5 Hz, 1H), 2.54–2.47 (m, 2H), 2.34–2.30 (m, 3H), 2.14 (td, J = 6.9, 13.9 Hz, 1H), 1.90–1.81 (m, 1H), 1.65 (br d, J = 10.5 Hz, 2H), 1.57–1.51 (m, 2H), 1.40 (s, 9H),

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{23}H_{32}N_2O_2$, 369.2; found 369.2.

Step 4. (1*S*,2*S*,3a*R*,6a*R*)-2-Benzamido-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide

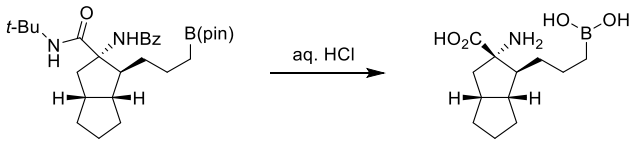


S60 **S61**

Dry nitrogen gas was bubbled through a solution of (1*S*,2*S*,3a*R*,6a*R*)-1-allyl-2-benzamido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide (150 mg, 0.41 mmol), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (160 mg, 1.2 mmol), and 1,2-bis(diphenylphosphino)ethane (16 mg, 0.041 mmol) in anhydrous dichloromethane (3.0 mL) for 3 min. Bis(1,5-cyclooctadiene)diiridium(I) dichloride (14 mg, 0.020 mmol) was added under a blanket of nitrogen gas, and the resulting mixture was stirred at 25 °C for 10 h. The mixture was then concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (1*S*,2*S*,3a*R*,6a*R*)-2-benzamido-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide as a yellow oil containing the corresponding boronic acid as a minor impurity (100 mg, 49% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{45}BN_2O_4$, 497.3; found 497.3.

Step 5. (1*S*,2*S*,3a*R*,6a*R*)-2-Amino-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid



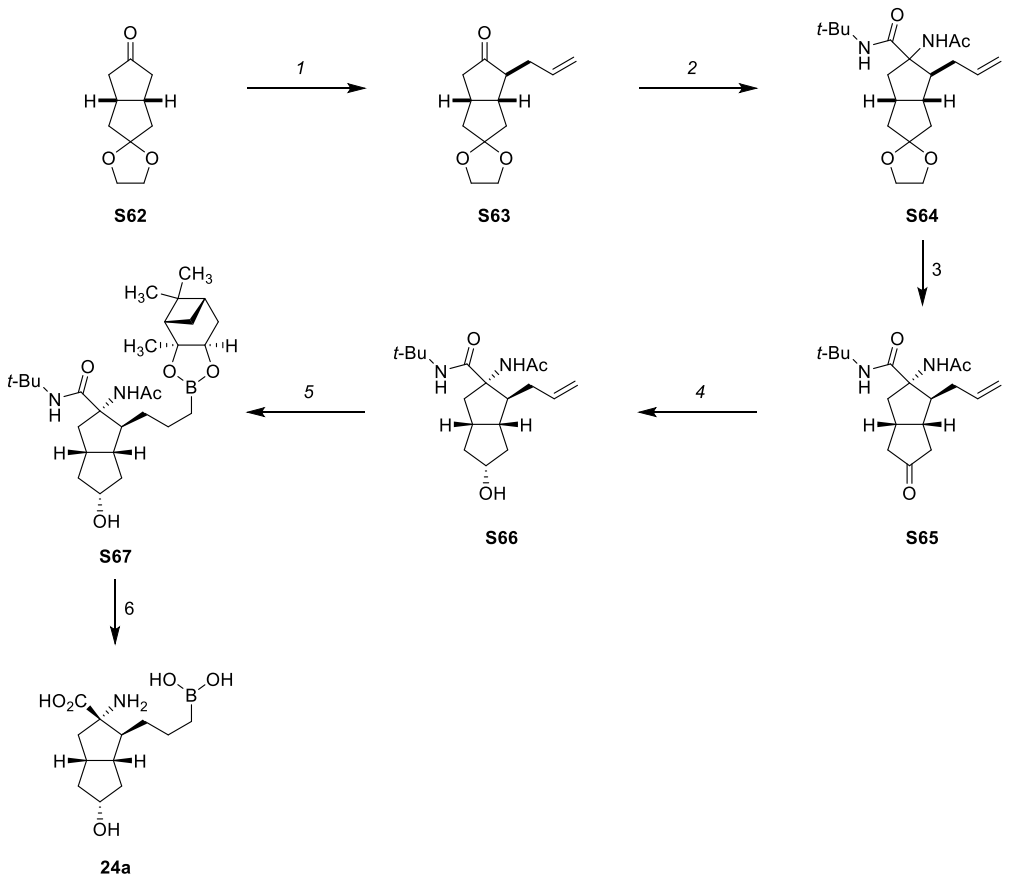
S61 **23**

A mixture of (1*S*,2*S*,3a*R*,6a*R*)-2-benzamido-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide (100 mg, 0.20 mmol), 37% w/v aq. hydrochloric acid solution (1.0 mL), acetic acid (0.50 mL), and water (0.50 mL) was heated to 130 °C for 30 min in a microwave reactor. After the mixture was allowed to cool to ambient temperature, it was concentrated under reduced pressure, and the residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA) to give (1*S*,2*S*,3a*R*,6a*R*)-2-amino-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid as a colorless oil (HFBA salt, 52 mg, 96% yield).

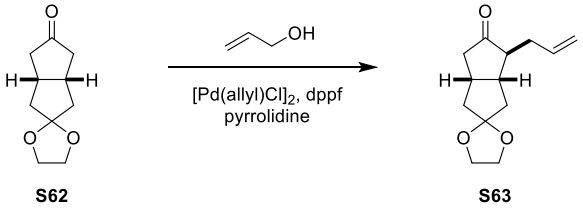
^{1}H NMR (400 MHz, D_2O) δ 2.92–2.66 (m, 1H), 2.55–2.26 (m, 2H), 1.74 (br t, J = 8.4 Hz, 1H), 1.66–1.39 (m, 8H), 1.33 (br s, 2H), 1.28–1.11 (m, 1H), 0.87–0.54 (m, 2H).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{12}H_{22}BNO_4$, 256.2; found 256.2.

Synthesis of Compound 24a



Step 1: rac-(3a*R*,4*S*,6a*S*)-4-Allyltetrahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5(3*H*)-one

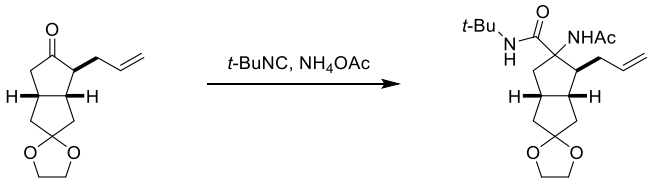


Methanol (80.0 mL) was added to 1,1′-ferrocenediyl-bis(diphenylphosphine) (3.57 g, 6.43 mmol) and allylpalladium(II) chloride dimer (1.18 g, 3.22 mmol), and the mixture was stirred at 23 °C for 1 h, during which time the mixture became homogeneous. Allyl alcohol (4.81 mL, 4.11 g, 70.8 mmol) was added dropwise next, and the mixture was aged for another 10 min. Pyrrolidine (1.60 mL, 1.37 g, 19.3 mmol) and

(3a*R*,6a*S*)-tetrahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5(3*H*)-one (11.7 g, 64.3 mmol) were then added sequentially, and the reaction mixture was stirred at 23 °C for 17 h. The mixture was then diluted with sat. aq. NH_4Cl solution (150 mL) and ethyl acetate (100 mL). The diluted mixture was stirred at 23 °C for 10 min before the layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were washed with sat. aq. sodium chloride solution, and the washed organic solution was then dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluting with ethyl acetate–hexanes) to give the title compound (8.19 g, 57%).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{13}H_{18}O_3$, 223.1; found 223.2.

Step 2. *rac*-(3a*R*,4*S*,6a*S*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)hexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolane]-5-carboxamide

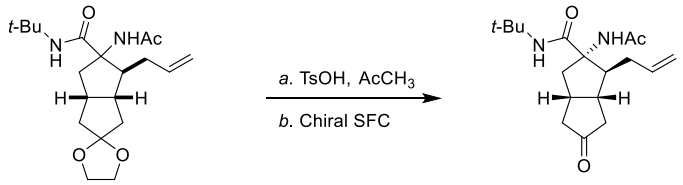


S63 **S64**

Ammonium acetate (8.51 g, 110 mmol) and *tert*-butyl isocyanide (12.5 mL, 9.18 g, 110 mmol) were added sequentially to a solution of (3a*R*,4*S*,6a*S*)-4-allyltetrahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5(3*H*)-one (8.18 g, 36.8 mmol) in 2,2,2-trifluoroethanol (123 mL) at 23 °C. The mixture was stirred at this temperature for 22 h before it was diluted with ethyl acetate (250 mL) and water (250 mL). The resulting mixture was shaken, and the layers were separated. The aqueous phase was extracted with ethyl acetate, the combined organic extracts were washed with sat. aq. sodium chloride solution, and the washed organic solution was dried, filtered, and concentrated to provide (3a*R*,4*S*,6a*S*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)hexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolane]-5-carboxamide, which was used without further purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{20}H_{32}N_2O_4$ 365.2; found 365.2.

Step 3: (1*S*,2*S*,3a*S*,6a*R*)-2-Acetamido-1-allyl-*N*-(*tert*-butyl)-5-oxooctahydropentalene-2-carboxamide

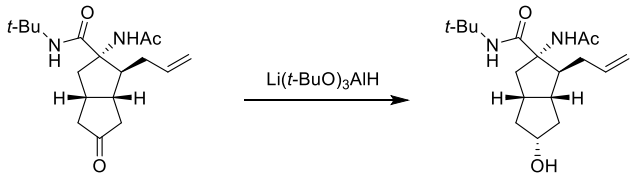


S64 **S65**

p-Toluenesulfonic acid monohydrate (700 mg, 3.68 mmol) was added to a suspension of (3a*R*,4*S*,6a*S*)-5-acetamido-4-allyl-*N*-(*tert*-butyl)hexahydro-1*H*-spiro[pentalene-2,2'-[1,3] dioxolane]-5-carboxamide (36.8 mmol theoretical) in acetone (92.0 mL). The mixture was heated to reflux for 30 min before it was diluted with toluene (50.0 mL). The diluted mixture was concentrated to dryness under reduced pressure, and the dried residue was re-suspended in acetone (92.0 mL). The mixture was again brought to reflux for 30 min, diluted with toluene (50.0 mL), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluting with ethyl acetate–hexanes) to provide a racemic ~1:1 mixture (^{1}H NMR analysis) of epimers. This mixture was resolved by SFC (column: IC, 21 × 250 mm; modifier: 0.1% v/v NH_4OH/MeOH; mobile phase: 15% modifier in CO_2; flow rate: 70 mL/min) to provide (1*S*,2*S*,3a*S*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-oxooctahydropentalene-2-carboxamide (1.75 g, 15% over 2 steps) as the first-eluting peak (t_r = 3.1 min).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{28}N_2O_3$, 321.2; found 321.2.

Step 4. (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-hydroxyoctahydropentalene-2-carboxamide

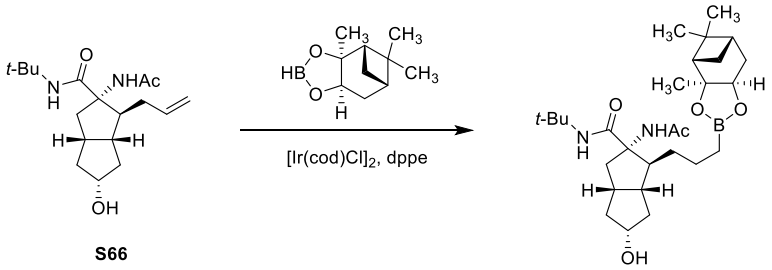


S65 **S66**

Lithium tri-*tert*-butoxyaluminum hydride solution (1.0 M in tetrahydrofuran, 2.6 mL, 2.6 mmol) was added dropwise to a solution of (1*S*,2*S*,3a*S*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-oxooctahydropentalene-2-carboxamide (411 mg, 1.28 mmol) in tetrahydrofuran (6.41 mL) at –40 °C, and the reaction mixture was stirred for 2.5 h at this temperature. The reaction mixture was then diluted with ethyl acetate (40 mL), and excess reductant was quenched with the careful, dropwise addition of 0.1 M aq. sodium hydroxide solution (30 mL). The resulting white, biphasic slurry was stirred for 5 min; then the layers were separated. The aqueous layer was extracted with 5% v/v methanol–ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to give (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-hydroxyoctahydropentalene-2-carboxamide as a white solid (380 mg, 93% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{30}N_2O_3$, 323.2; found 323.2.

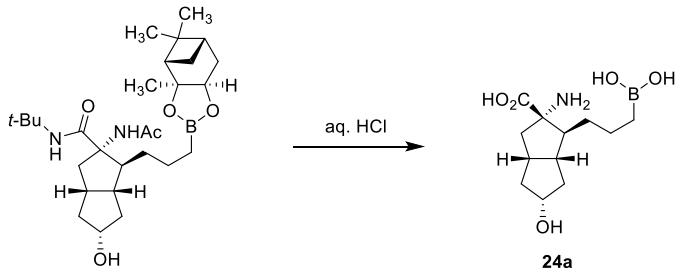
Step 5. (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide



A solution of (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-hydroxyoctahydropentalene-2-carboxamide (384 mg, 1.19 mmol) in dichloromethane (6.00 mL) was added to a solution of (+)-pinanediol borane (1.07 g, 5.95 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (40.0 mg, 0.0600 mmol), and 1,2-bis(diphenylphosphino)ethane (47.0 mg, 0.119 mmol) in dichloromethane (6.00 mL) at 23 °C. The reaction mixture was stirred at this temperature for 1.5 h under nitrogen gas. Excess reductant was quenched with the addition of methanol (2 mL), and the mixture was stirred for 10 min, by which time bubbling had ceased. Half-saturated aq. sodium chloride solution (10 mL) was then added, the mixture was shaken, and the layers were separated. The aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash-column chromatography (eluting with ethyl acetate–dichloromethane) to give (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide as a white solid (280 mg, 47% yield).

MS (ESI+, m/z): [M+H]+ calc'd for $C_{28}H_{47}BN_2O_5$, 503.4; found 503.4.

Step 6. (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-amino-1-(3-boronopropyl)-5-hydroxyoctahydropentalene-2-carboxylic acid

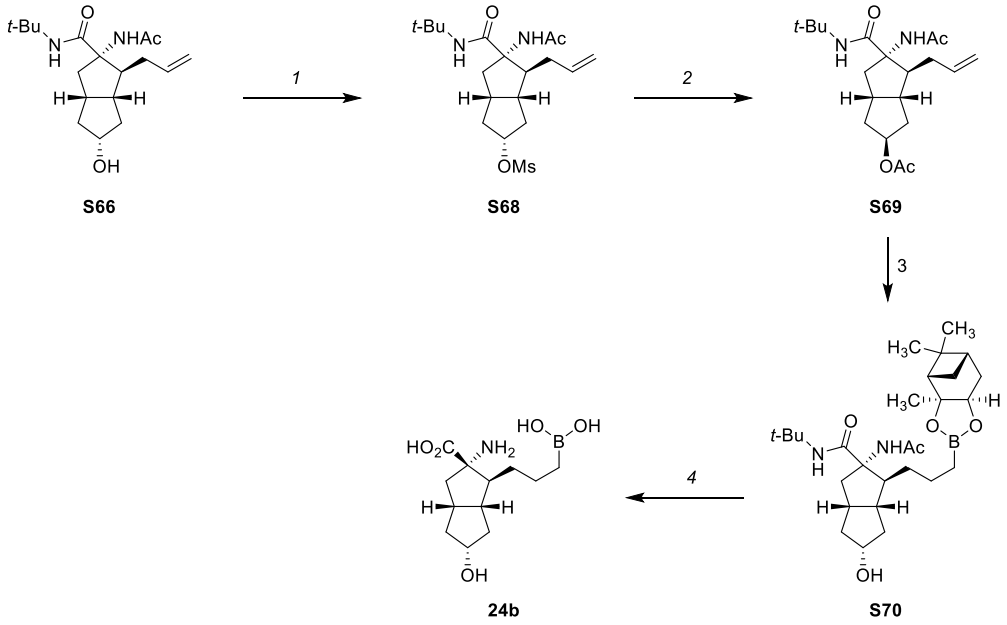


(1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (100 mg, 0.199 mmol) and 6N aq. hydrochloric acid solution (3.98 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to 23 °C and was diluted with water (20 mL). The diluted aqueous mixture was washed with dichloromethane until both the aqueous solution and the organic washes were colorless. The aqueous solution was then diluted with acetonitrile (20 mL), and the resulting mixture was concentrated under reduced pressure. The residue was purified by RP-HPLC (Column: Waters Atlantis T3 19 × 250 mm, 5 μm; eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA). Fractions containing product were concentrated under reduced pressure to provide (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-amino-1-(3-boronopropyl)-5-hydroxyoctahydropentalene-2-carboxylic acid as a white solid (HFBA salt, 20 mg, 21% yield).

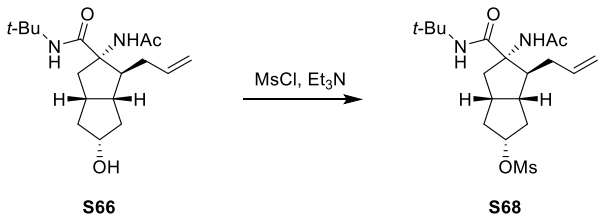
^{1}H NMR (499 MHz, D_2O) δ 4.31 (app p, *J* = 6.1 Hz, 1H), 2.88 (app h, *J* = 8.8 Hz, 1H), 2.61 (dd, *J* = 13.4, 8.8 Hz, 1H), 2.46 (app qd, *J* = 9.4, 6.4 Hz, 1H), 2.29–2.18 (m, 2H), 2.11 (ddd, *J* = 13.2, 8.4, 5.4 Hz, 1h), 1.91 (dd, *J* = 13.4, 9.2 Hz, 1H), 1.60–1.51 (m, 3H), 1.46 (app dt, *J* = 13.3, 6.9 Hz, 1H), 1.41–1.38 (m, 1H), 1.30–1.22 (m, 1H), 0.86–0.74 (m, 2H).

MS (ESI+, *m/z*): $[M+H-H_2O]^+$ calc'd for $C_{12}H_{22}BNO_5$, 254.2; found 254.2.

Synthesis of Compound 24b



Step 1. (2*R*,3a*R*,4*S*,5*S*,6a*S*)-5-Acetamido-4-allyl-5-(*tert*-butylcarbamoyl)octahydropentalen-2-yl methanesulfonate

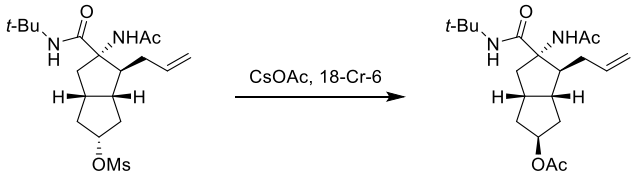


Methanesulfonyl chloride (146 μL, 214 mg, 1.87 mmol) was added dropwise to a suspension of (1*S*,2*S*,3a*S*,5*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-hydroxyoctahydro-pentalene-2-carboxamide (402 mg, 1.25 mmol) and triethylamine (521 μL, 378 mg, 3.74 mmol) in dichloromethane (12.5 mL) at 0 °C. As this addition was performed, the cloudy reaction mixture clarified to form a homogeneous solution. After 5 min at 0 °C, excess methanesulfonyl chloride was quenched with the addition of sat. aq. sodium bicarbonate solution (25 mL). The resulting biphasic mixture was allowed to warm to 23 °C with stirring, and once warmed, the layers were separated. The aqueous phase was extracted with

dichloromethane, and the combined organic layers were dried over sodium sulfate. The dried organic solution was filtered and concentrated to give (2R,3aR,4S,5S,6aS)-5-acetamido-4-allyl-5-(tert-butylcarbamoyl)octahydropentalen-2-yl methanesulfonate as a white solid (497 mg, 100% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{19}H_{32}N_2O_5S$, 401.2; found 401.2.

Step 2. (2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-Acetamido-4-allyl-5-(*tert*-butylcarbamoyl)octahydropentalen-2-yl acetate

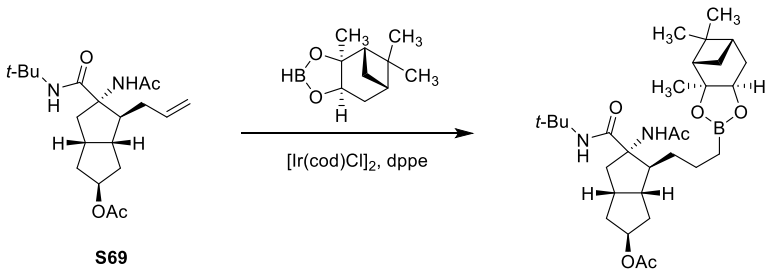


S68 **S69**

1,4,7,10,13,16-Hexaoxacyclooctadecane (18-crown-6, 16.5 mg, 62.0 mmol) was added to a suspension of (2*R*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl) octahydropentalen-2-yl methanesulfonate (50.0 mg, 125 mmol) and cesium acetate (71.9 mg, 375 mmol) in toluene (1.25 mL) at 23 °C. The mixture was heated to 80 °C for 3 days. The mixture was then cooled to 23 °C, and the cooled mixture was diluted with sat. aq. sodium bicarbonate solution (10 mL) and ethyl acetate (10 mL). The layers were shaken and were separated. The aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to provide (2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)octahydropentalen-2-yl acetate as an off-white solid. This material was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{20}H_{32}N_2O_4$, 365.2; found 365.3.

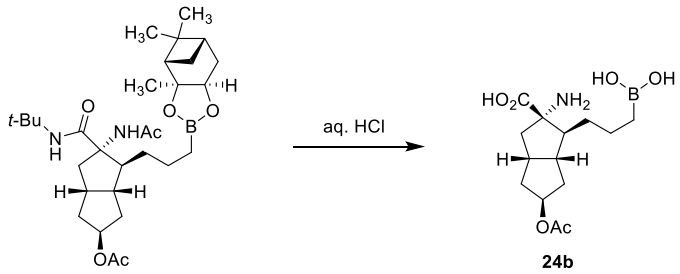
Step 3. (2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-5-(*tert*-butylcarbamoyl)-4-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)octahydropentalen-2-yl acetate



A solution of (2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl) octahydropentalen-2-yl acetate (theoretically 125 mmol) in dichloromethane (750 μL) was added to a solution of (+)-pinanediol borane (113 mg, 0.625 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (4.2 mg, 6.3 μmol), and 1,2-bis(diphenylphosphino)ethane (5.0 mg, 13 μmol) in dichloromethane (750 μL) at 23 °C. The reaction mixture was stirred at 23 °C for 2.5 h under nitrogen gas. Excess reductant was quenched with the addition of methanol (500 μL), and the mixture was stirred for 10 min, by which time bubbling had ceased. Water (5 mL) and dichloromethane (5 mL) were added, the mixture was shaken, and the layers were separated. The aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-5-(*tert*-butylcarbamoyl)-4-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)octahydropentalen-2-yl acetate (31.5 mg, 46% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{30}H_{49}BN_2O_6$, 545.4; found 545.3.

Step 4. (1*S*,2*S*,3a*S*,5*S*,6a*R*)-2-Amino-1-(3-boronopropyl)-5-hydroxyoctahydropentalene-2-carboxylic acid

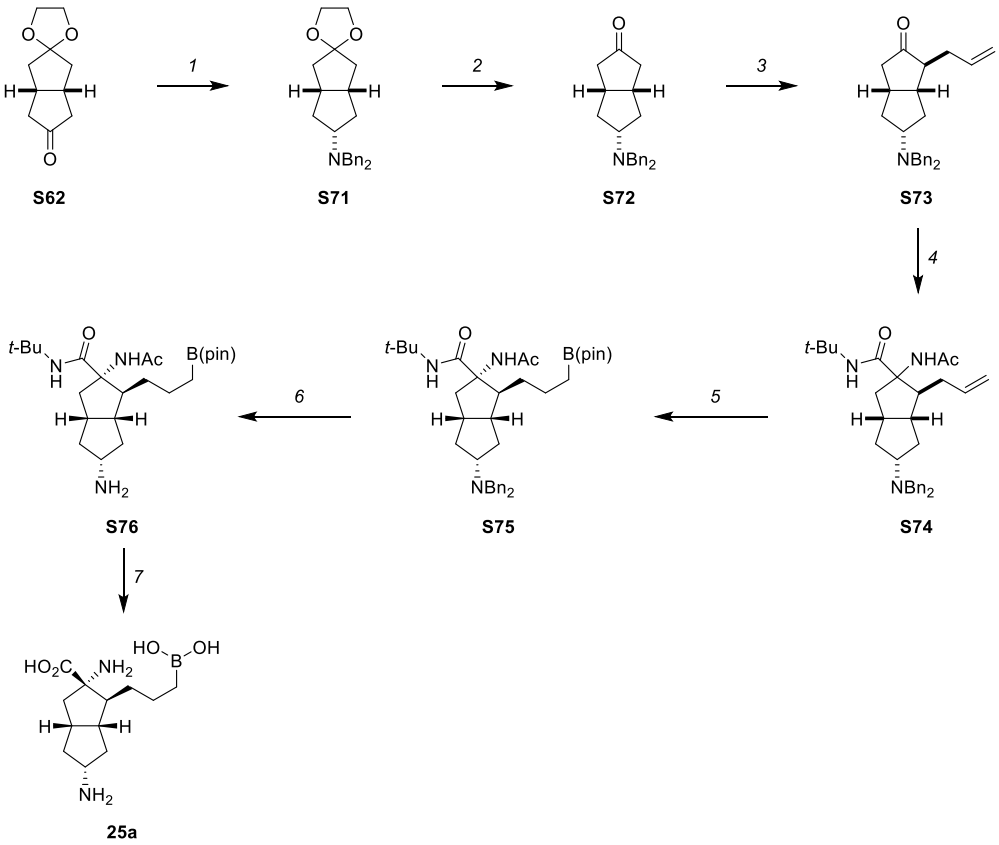


(2*S*,3a*R*,4*S*,5*S*,6a*S*)-5-Acetamido-5-(*tert*-butylcarbamoyl)-4-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)propyl)octahydropentalen-2-yl acetate (31.5 mg, 58.0 μmol) and 6N aq. hydrochloric acid solution (1.15 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to 23 °C and was diluted with water (9 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (10 mL) and the diluted mixture was concentrated under reduced pressure. The residue was purified by RP-HPLC (Column: Waters Atlantis T3 19 × 250 mm, 5 μm; eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA). Fractions containing product were concentrated under reduced pressure to provide (1*S*,2*S*,3a*S*,5*S*,6a*R*)-2-amino-1-(3-boronopropyl)-5-hydroxyoctahydropentalene-2-carboxylic acid as a white solid (HFBA salt, 8.5 g, 30% yield).

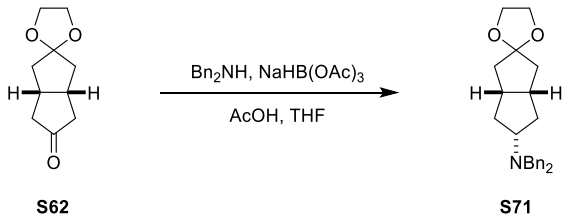
^{1}H NMR (499 MHz, D_2O) δ 4.43 (app p, *J* = 5.0 Hz, 1H), 3.03 (app dq, *J* = 18.0, 9.0 Hz, 1H), 2.64–2.56 (m, 2H), 1.98–1.86 (m, 2H), 1.85–1.74 (m, 2H), 1.70–1.64 (m, 3H), 1.57–1.54 (m, 1H), 1.43–1.35 (m, 1H), 1.33–1.24 (m, 1H), 0.85–0.74 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{22}BNO_5$, 254.2; found 254.2

Synthesis of Compound 25a



Step 1. *meso*-(3a*R*,5*s*,6a*S*)-*N*,*N*-Dibenzylhexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5-amine

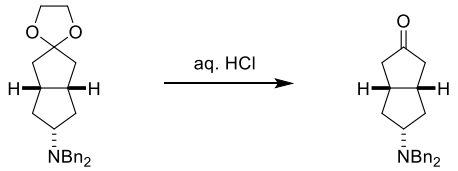


Dibenzylamine (13.9 mL, 14.3 g, 72.4 mmol) and acetic acid (12.0 mL) were added to a solution of *meso*-(3a*R*,6a*S*)-tetrahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5(3*H*)-one (11.0 g, 60.4 mmol) in tetrahydrofuran (180 mL) at room temperature. The mixture was stirred for 30 min before sodium triacetoxyborohydride (19.2 g, 91.0 mmol) was added. The mixture was stirred at room temperature for 24 h longer before it was concentrated under reduced

pressure. The residue was suspended in dichloromethane, and the organic suspension was washed sequentially with sat. aq. sodium bicarbonate solution and brine. The organic solution was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–hexanes) to give *meso*-(3a*R*,5*s*,6a*S*)-*N*,*N*-dibenzylhexahydro-1*H*-spiro [pentalene-2,2'-[1,3]dioxolan]-5-amine as a colorless oil (17.6 g, 80% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{24}H_{29}NO_2$, 364.2; found 364.2.

Step 2. meso-(3a*R*,5*s*,6a*S*)-5-(Dibenzylamino)hexahydropentalen-2(1*H*)-one

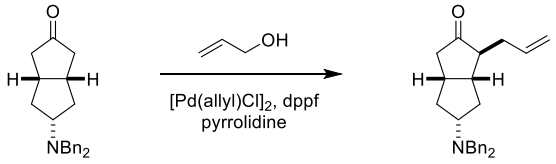


S71 **S72**

A solution of *meso*-(3a*R*,6a*S*)-*N*,*N*-dibenzylhexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxolan]-5-amine (12.0 g, 33.0 mmol) in tetrahydrofuran (120 mL) was treated with 3M aq. hydrochloric acid solution (33.0 mL, 99.0 mmol). The mixture was heated to 75 °C for 12 h. The mixture was cooled to 0 °C, and its pH was adjusted to 8 with the portionwise addition of aq. sodium hydroxide solution. The basified mixture was then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (eluting with ethyl acetate–heptane) to give *meso*-(3a*R*,6a*S*)-5-(dibenzylamino)hexahydropentalen-2(1*H*)-one as a yellow solid (5.06 g, 48% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{22}H_{25}NO$, 320.2; found 320.2.

Step 3. rac-(1*S*,3a*R*,5*R*,6a*R*)-1-Allyl-5-(dibenzylamino)hexahydropentalen-2(1*H*)-one

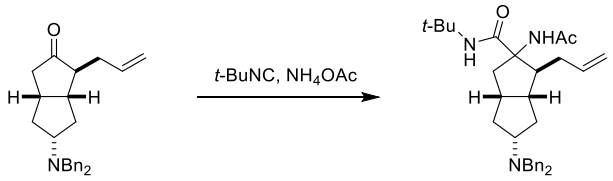


S72 **S73**

Allylpalladium(II) chloride dimer (617 mg, 1.69 mmol) and prop-2-en-1-ol (2.52 mL, 2.15 g, 37.1 mmol) were added to a mixture of *meso*-(3a*R*,6a*S*)-5-(dibenzylamino)hexahydropentalen-2(1*H*)-one (10.8 g, 33.7 mmol), pyrrolidine (719 mg, 10.1 mmol) and 1,1'-bis(diphenylphosphino)ferrocene (1.87 g, 3.37 mmol) in methanol (25.0 mL) under an atmosphere of nitrogen gas at 20 °C. The resulting mixture was stirred at 20 °C for 14 h. The solvent was removed under reduced pressure. The residue was suspended in ethyl acetate, and this suspension was stirred at ambient temperature for 10 min. The mixture was filtered through a pad of Celite. The filtrate was washed sequentially with water and brine before it was dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash-column chromatography (eluting with ethyl acetate–heptane) to provide *rac*-(1*S*,3a*R*,5*R*,6a*R*)-1-allyl-5-(dibenzylamino) hexahydropentalen-2(1*H*)-one as a yellow oil (4.85 g, 40% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{25}H_{29}NO$, 360.2; found 360.2.

Step 4. rac-(1*S*,3a*R*,5*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide

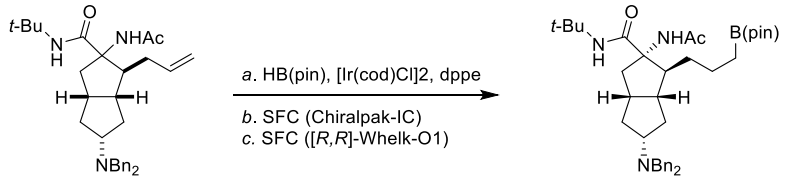


S73 **S74**

tert-Butyl isocyanide (3.5 g mg, 42 mmol) and ammonium acetate (3.2 g, 42 mmol) were added to a mixture of *rac*-(3aR,6aR)-1-allyl-5-(dibenzylamino)hexahydropentalen-2(1H)-one (5.0 g, 14 mmol) in 2,2,2-trifluoroethanol (20 mL) at room temperature. The reaction mixture was stirred at 40 °C for 18 h. The reaction was diluted with water and dichloromethane, and the layers were shaken. The organic layer was separated, and the aqueous layer was extracted with fresh portions of dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash-column chromatography (eluting with ethyl acetate–dichloromethane) to provide *rac*-(1*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide as a yellow oil (3.0 g, 43% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{32}H_{43}N_3O_2$, 502.3; found 502.3.

Step 5. (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide



S74 **S75**

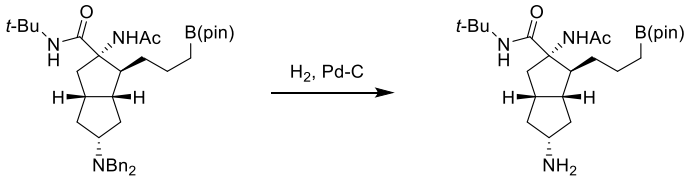
4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (7.0 g, 55 mmol) was added to a deoxygenated solution of bis(1,5-cyclooctadiene)diiridium(I) dichloride (280 mg, 0.55 mmol) and 1,2-bis(diphenylphosphino)ethane (440 mg, 1.1 mmol) in dichloromethane (10 mL). The mixture was stirred under nitrogen gas for 20 min at 25 °C. A solution of *rac*-(1*S*,3a*R*,5*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-(dibenzylamino)octahydropentalene-2-carboxamide (5.5 g, 11 mmol) in dichloromethane (10 mL) was added by syringe, and the mixture was stirred at ambient temperature for 48 h. Excess reductant was then quenched with the dropwise addition of methanol (1.0 mL). The mixture was concentrated under reduced pressure, and the residue was purified by flash-column chromatography (eluting with ethyl acetate–dichloromethane) to provide a racemic, stereoisomeric mixture of (1*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide as a yellow oil (6.6 g).

This mixture was subjected to two rounds of supercritical-fluid chromatography to produce the desired stereoisomer in pure form. In the first, the product mixture (6.6 g) was purified using a Chiralpak-IC column (21 × 250 mm; 0.1% v/v ammonium hydroxide in isopropanol modifier; 25% modifier in CO_2 mobile phase; 70 mL/min flow rate). The second-eluting peak (t_r = 4.1 min) was collected, providing a mixture of the desired (1*S*,2*S*,3a*R*,5*R*,6a*R*) isomer alongside one undesired stereoisomer (1.7 combined). This sample

was then resolved by supercritical-fluid chromatography using an (*R*,*R*)-Whelk-O1 column (21 × 250 mm; 0.1% v/v ammonium hydroxide in isopropanol modifier; 30% modifier in CO_2 mobile phase; 70 mL/min flow rate). The second-eluting peak (t_r = 5.0 min) was collected, providing (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide in isomerically pure form (0.81 g, 12% overall yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{38}H_{56}BN_3O_4$, 629.4; found 630.4

Step 6. (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-Acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide

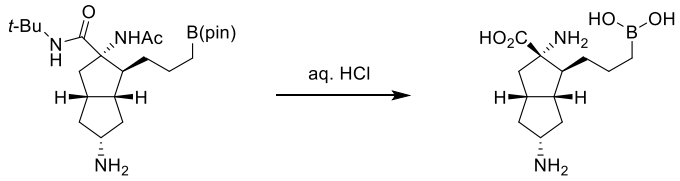


S75 **S76**

A solution of (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(dibenzylamino)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide (810 mg, 1.3 mmol) in methanol (10 mL) was deoxygenated by three evacuation–backfill cycles with nitrogen gas. Palladium on carbon (10% w/w, 410 mg, 0.39 mmol) was added to this mixture, and the headspace in the flask was replaced with hydrogen gas. The mixture was stirred under 50 psi of hydrogen gas for 48 h at 25 °C. The mixture was then filtered through a pad of Celite to remove the heterogeneous catalyst, and the filtrate was concentrated under reduced pressure to afford (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide as a white solid (700 mg, 120% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{24}H_{44}BN_3O_4$, 450.4; found 450.4.

Step 7. (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2,5-Diamino-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid



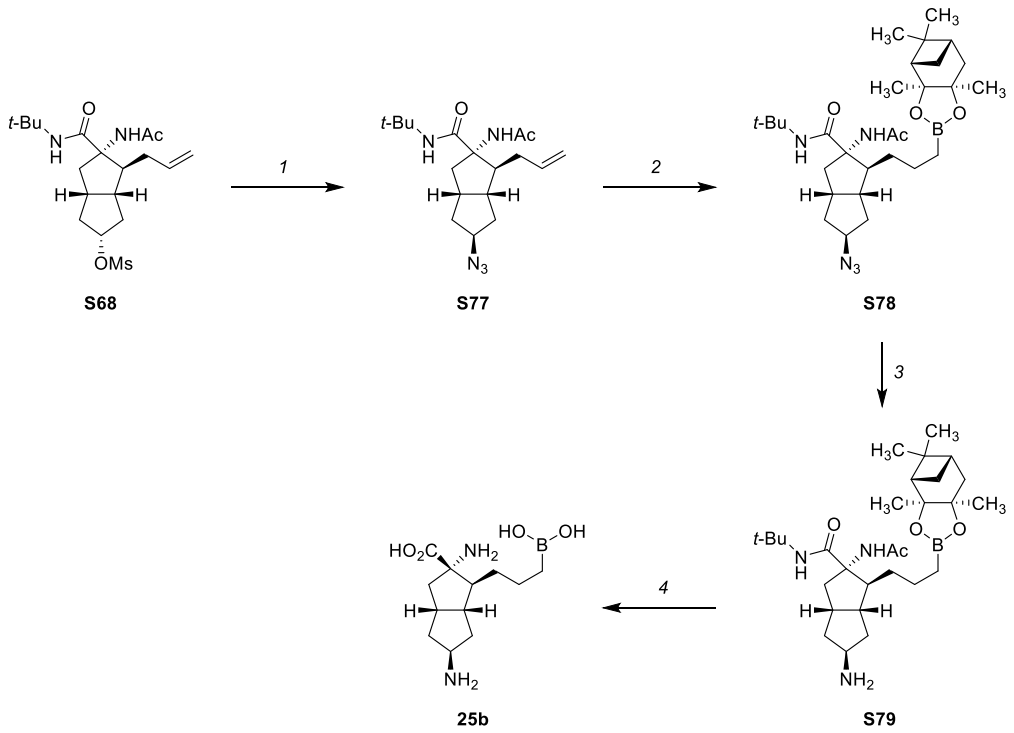
S76 **25a**

A mixture of (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)octahydropentalene-2-carboxamide (100 mg, 0.22 mmol), 37% w/v aq. hydrochloric acid solution (1.0 mL), acetic acid (0.50 mL), and water (0.50 mL) was heated to 130 °C for 30 min in a microwave reactor. The aqueous reaction mixture was then cooled to ambient temperature, diluted with water (4.0 mL), and washed with dichloromethane (3 × 4.0 mL). The aqueous solution was then concentrated under reduced pressure. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA) to provide (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2,5-diamino-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid as a white solid (HFBA salt, 32 mg, 45% yield).

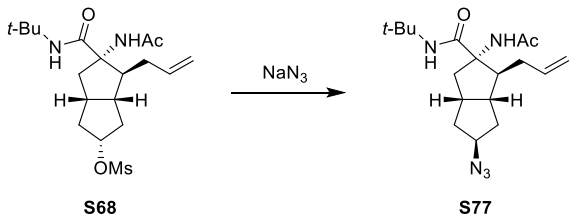
^{1}H NMR (500 MHz, D_2O) δ 3.60 (tt, *J* = 12.2, 6.3 Hz, 1H), 2.91 (app h, *J* = 8.8 Hz, 1H), 2.63 (dd, *J* = 13.6, 8.6 Hz, 1H), 2.57–2.45 (m, 2H), 2.39 (dt, *J* = 12.5, 6.8 Hz, 1H), 2.11 (td, *J* = 10.8, 2.8 Hz, 1H), 1.81 (dd, *J* = 13.7, 8.7 Hz, 1H), 1.57–1.39 (m, 4H), 1.26–1.18 (m, 2H), 0.81–0.70 (m, 2H).

MS (ESI, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.0.

Synthesis of Compound 25b



Step 1. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Acetamido-1-allyl-5-azido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide

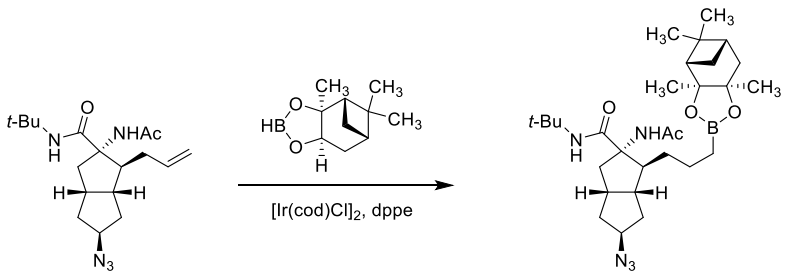


N,*N*-Dimethylformamide (11.4 mL) was added to a mixture of (2*R*,3a*R*,4*S*,5*S*,6a*S*)-5-acetamido-4-allyl-5-(*tert*-butylcarbamoyl)octahydropentalen-2-yl methanesulfonate (457 mg, 1.14 mmol) and sodium azide (111 mg, 1.71 mmol). The mixture was heated at 60 °C with magnetic stirring for 2.5 h, and was then allowed to cool to 23 °C. Once cooled, the reaction mixture was diluted with ethyl acetate (30 mL), and the diluted solution was washed with sat. aq. sodium bicarbonate solution (20 mL). The aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with sat. aq.

sodium chloride solution (20 mL), dried over sodium sulfate, filtered, and concentrated to provide (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-1-allyl-5-azido-*N*-(*tert*-butyl)octahydropentalene-2-carboxamide as a beige solid (374 mg, 94% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{29}N_5O_2$, 348.2; found 348.3.

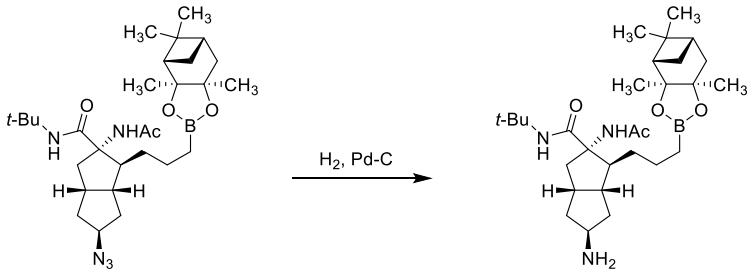
Step 2. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Acetamido-5-azido-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide



A solution of (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-1-allyl-5-azido-*N*-(*tert*-butyl) octahydropentalene-2-carboxamide (374 mg, 1.08 mmol) in dichloromethane (3.00 mL) was added to a solution of (+)-pinanediol borane (969 mg, 5.38 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (36.2 mg, 54.0 μmol), and 1,2-bis(diphenylphosphino)ethane (42.9 mg, 108 μmol) in dichloromethane (21.0 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 3 h under nitrogen gas. Excess reductant was quenched with the addition of methanol (1.0 mL), and the mixture was stirred for 10 min, by which time bubbling had ceased. Water (30 mL) was added, the layers were shaken, and the phases were separated. The aqueous layer was extracted with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluting with ethyl acetate–hexanes) to give (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-5-azido-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide as a white solid (478 mg, 84% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{46}BN_5O_4$, 528.4; found 528.4.

Step 3. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide

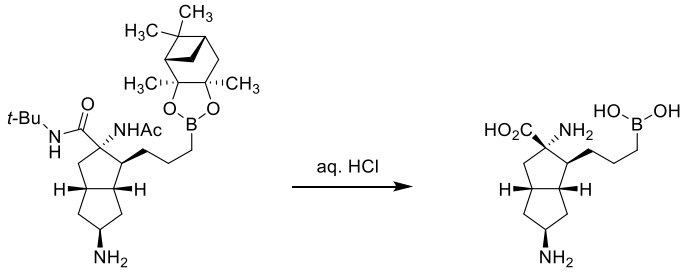


S78 **S79**

Palladium on carbon (10% w/w, 48 mg, 45 μmol) was added to a solution of (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-5-azido-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (478 mg, 0.906 mmol) in 50% v/v ethyl acetate–methanol (18.0 mL). The headspace within the reaction flask was replaced with hydrogen gas via three evacuation–backfill cycles, and the reaction mixture was stirred under an atmosphere of hydrogen gas at 23 °C for 6 h. The mixture was filtered through a pad of Celite to remove the heterogeneous catalyst, the filtrate was concentrated, and the residue was purified by supercritical fluid chromatography (column: Polar-RP, 21 × 250 mm; modifier: 0.1% v/v NH_4OH/MeOH; mobile phase: 15% modifier in CO_2; flow rate: 70 mL/min) to give (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide as a brilliant white solid (265 mg, 58% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{48}BN_3O_4$, 502.4; found 502.4.

Step 4. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2,5-Diamino-1-(3-boronopropyl)octahydropentalene-2-carboxylate



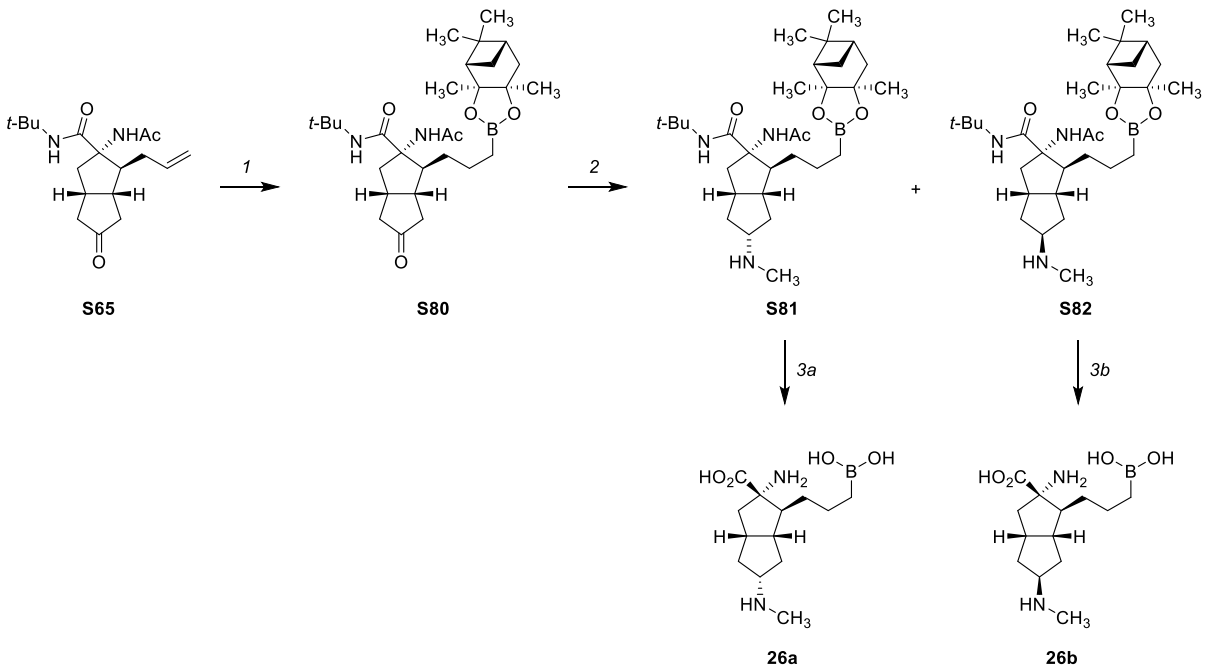
S79 **25b**

(1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Acetamido-5-amino-*N*-(*tert*-butyl)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (265 mg, 0.528 mmol) and 6N aq. hydrochloric acid solution (10.5 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to 23 °C and was diluted with water (50 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (50 mL) and the diluted mixture was concentrated under reduced pressure to provide (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2,5-diamino-1-(3-boronopropyl)octahydropentalene-2-carboxylate as a 1:1:3 salt with *tert*-butylamine and HCl (235 mg, 98% yield).

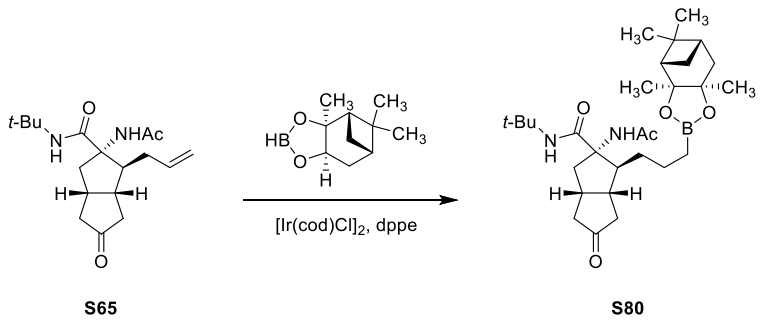
^{1}H NMR (499 MHz, D_2O) δ 3.74 (ddd, *J* = 15.4, 9.3, 6.3 Hz, 1H), 3.06 (app pd, *J* = 9.5, 2.5 Hz, 1H), 2.64–2.57 (m, 2H), 1.98–1.94 (m, 2H), 1.92–1.88 (m, 2H), 1.86–1.77 (m, 1H), 1.66 (dd, *J* = 13.6, 9.8 Hz, 1H), 1.57–1.48 (m, 2H), 1.38–1.35 (m, 1H), 1.28–1.19 (m, 1H), 0.81–0.70 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{23}BN_2O_4$, 253.2; found 253.2.

Synthesis of Compounds 26a and 26b



Step 1. (1*S*,2*S*,3a*S*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide

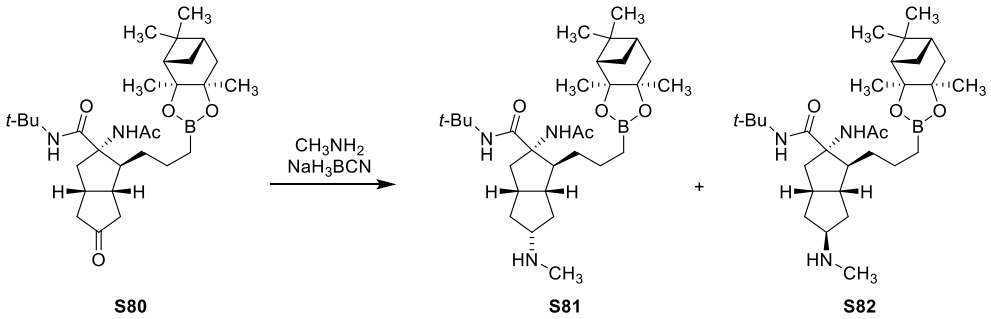


A solution of (1*S*,2*S*,3a*S*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-5-oxooctahydropentalene-2-carboxamide (200 mg, 0.624 mmol) in dichloromethane (4.5 mL) was added to a solution of (+)-pinanediolborane (225 mg, 1.25 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (21.0 mg, 31.3 μmol), and 1,2-bis(diphenylphosphino)ethane (25.0 mg, 62.8 μmol) in dichloromethane (8.0 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 18 h under nitrogen gas. Excess reductant was quenched with the addition of methanol (1.0 mL), and the

mixture was stirred for 10 min, by which time bubbling had ceased. Water (20 mL) was added, the layers were shaken, and the phases were separated. The aqueous layer was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The crude residue, constituting (1*S*,2*S*,3a*S*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, was suitable for use in subsequent reductive amination reactions without further purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{45}BN_2O_5$, 501.4; found 501.3.

Step 2. (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide and (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide

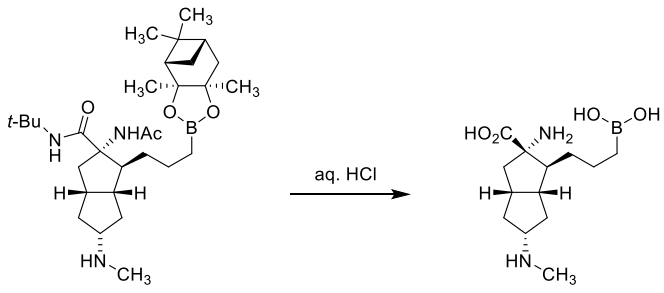


Methylamine solution (2.0 M in tetrahydrofuran, 625 μL, 1.25 mmol) was added to a solution of (1*S*,2*S*,3a*S*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (crude residue from hydroboration, 0.250 mmol theoretical) in absolute ethanol (5.00 mL) at 0 °C. After 30 min, sodium cyanoborohydride (47.1 mg, 0.750 mmol) was added in one portion. Stirring was maintained at 0 °C for 24 h. The reaction mixture was then diluted with sat. aq. sodium bicarbonate solution (10 mL), sat. aq. sodium chloride solution (10 mL), and ethyl acetate (10 mL). The layers were shaken, and the phases were separated. The aqueous layer was extracted with ethyl acetate. The combined extracts were dried over sodium sulfate, filtered, and concentrated. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give a ~80:20 mixture (^{1}H-NMR analysis) of epimeric products, **S81** and **S82**, as their corresponding TFA salts. These stereoisomeric products were separated by SFC (Lux-2 column, 21 x 250 mm; 0.1% v/v ammonium hydroxide in methanol modifier; 30% modifier in CO_2 mobile phase; 70 mL/min flow rate) to provide (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,

5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalen e-2-carboxamide (**S81**, t_r = 3.1 min, 44.7 mg, 35% over 2 steps) and (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5, 5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalen e-2-carboxamide (**S82**, t_r = 3.8 min, 26.6 mg, 21% over 2 steps).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{50}BN_3O_4$, 516.4; found 516.4.

Step 3a. (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-amino-1-(3-boronopropyl)-5-(methylamino)octahydropentalene-2-carboxylic acid



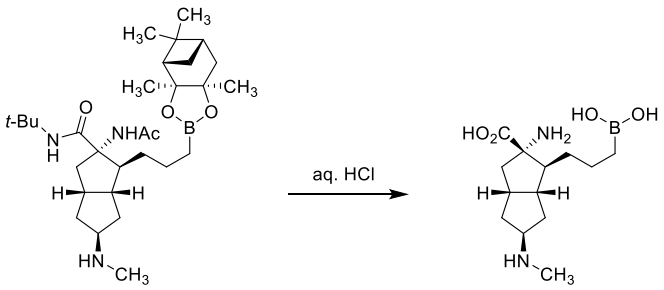
(1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (44.7 mg, 86.7 μmol) and 6N aq. hydrochloric acid solution (1.73 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to 23 °C and was diluted with water (10 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (10 mL) and the diluted mixture was concentrated under reduced pressure. The residue was purified by RP-HPLC (Column: Waters Atlantis T3 19 × 250 mm, 5 μm; eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA). Fractions containing product were concentrated under reduced pressure to provide (1*S*,2*S*,3a*R*,5*R*,6a*R*)-2-amino-1-(3-boronopropyl)-5-(methylamino)octahydropentalene-2-carboxylic acid as a white solid (HFBA salt, 40.4 mg, 65%).

^{1}H NMR (499 MHz, D_2O) δ 3.57 (tt, *J* = 12.1, 5.9 Hz, 1H), 2.94 (app h, *J* = 9.3 Hz, 1H), 2.71 (s, 3H), 2.66 (dd, *J* = 13.6, 8.7 Hz, 1H), 2.59–2.54 (m, 2H), 2.48 (app dt, *J* = 12.9, 7.1 Hz, 1H), 2.13 (app t, *J* = 8.5 Hz, 1H), 1.83 (dd, *J* = 13.6, 8.7 Hz, 1H), 1.61–1.50 (m, 3H),

1.44 (app q, J = 11.7 Hz, 1H), 1.37–1.32 (m, 1H), 1.29–1.23 (m, 1H), 0.84–0.73 (m, 2H).

MS (ESI+, *m/z*): $[M+H-H_2O]^+$ calc'd for $C_{13}H_{25}BN_2O_4$, 267.2; found 267.2.

Step 3b. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Amino-1-(3-boronopropyl)-5-(methylamino)octahydropentalene-2-carboxylate



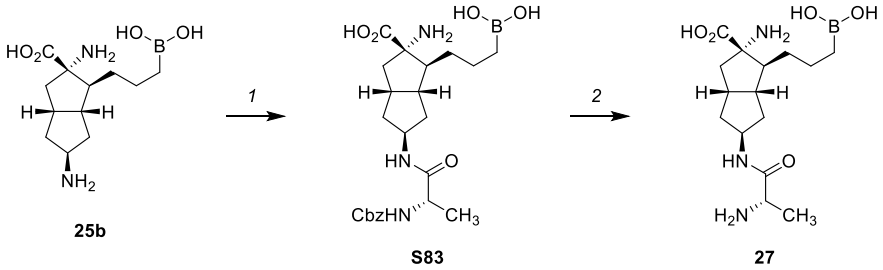
S82 **26b**

(1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (26.6 mg, 51.6 μmol) and 6N aq. hydrochloric acid solution (1.03 mL) were combined. The resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to ambient temperature and was diluted with water (10 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (10 mL) and the diluted mixture was concentrated under reduced pressure to provide (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-amino-1-(3-boronopropyl)-5-(methylamino)octahydropentalene-2-carboxylate as a white solid (1:1:3 salt with *t*-butylamine and HCl, 16.9 mg, 70% yield).

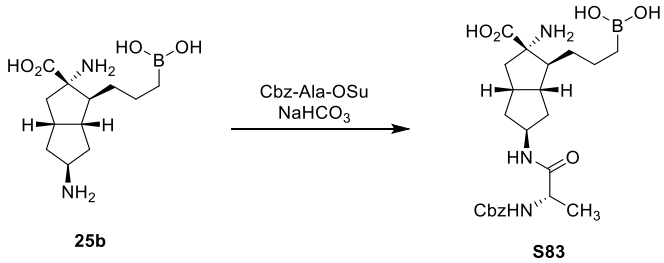
^{1}H NMR (499 MHz, D_2O) δ 3.68 (ddd, *J* = 15.5, 9.7, 6.2 Hz, 1H), 3.12 (app p, *J* = 9.0 Hz, 1H), 2.74 (s, 3H), 2.69–2.60 (m, 2H), 2.10–1.92 (m, 4H), 1.89–1.83 (m, 1H), 1.67 (dd, *J* = 13.5, 9.8 Hz, 1H), 1.62–1.52 (m, 2H), 1.45–1.40 (m, 1H), 1.34–1.28 (m, 1H), 0.86–0.76 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{25}BN_2O_4$, 267.2; found 267.3.

Synthesis of Compounds 27 and 28



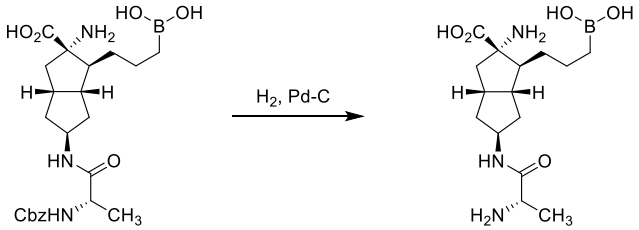
Step 1. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Amino-5-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid



Saturated aq. sodium bicarbonate solution (ca. 200 μL) was added to a solution of (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2,5-diamino-1-(3-boronopropyl)octahydropentalene-2-carboxylate (1:1:3 salt with *t*-butylamine and HCl, 61 mg, 0.14 mmol) in water (880 μL) and acetonitrile (1.0 mL) at 23 °C, until pH = 9 was achieved. A solution of 2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-alaninate (Cbz-Ala-OSu, 65 mg, 0.20 mmol) in acetonitrile (2.5 mL) was added next. Additional sat. aq. sodium bicarbonate solution was added as required in order to maintain pH = 9. The mixture was stirred at 23 °C for 16 h before it was concentrated under reduced pressure. The crude residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-amino-5-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid as a white solid (TFA salt, 66 mg, 83% yield).

MS (ESI+, *m/z*): $[M+H-H_2O]^+$ calc'd for $C_{23}H_{34}BN_3O_7$, 458.3; found 458.3.

Step 2. (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-Amino-5-((*S*)-2-aminopropanamido)-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid



S83 **27**

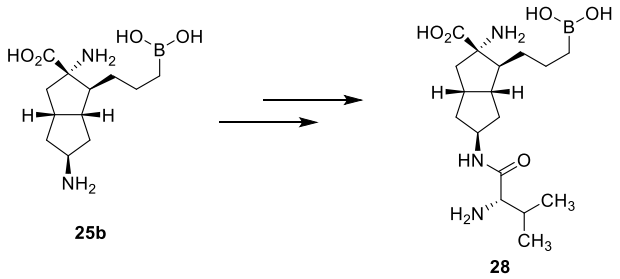
Palladium on carbon (10% w/w, 3.0 mg, 2.8 μmol) was added to a solution of (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-amino-5-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid (1:1 salt with TFA, 28.5 mg, 48.0 μmol) in water (1.93 mL) at 23 °C. The headspace of the reaction vessel was replaced with hydrogen gas via three evacuation/backfill cycles, and the reaction mixture was stirred under an atmosphere of hydrogen gas at 23 °C for 45 min. The reaction mixture was then filtered through a 0.2 μm PTFE syringe filter in order to remove the heterogeneous catalyst, and the filtrate was purified by RP-HPLC (Waters Atlantis T3 column, 19 x 250 mm, 5 μm; eluting with acetonitrile–water containing 20 mM HFBA and 0.1% TFA). Fractions containing product were concentrated under reduced pressure to provide (1*S*,2*S*,3a*R*,5*S*,6a*R*)-2-amino-5-((*S*)-2-aminopropanamido)-1-(3-boronopropyl)octahydropentalene-2-carboxylic acid as a white solid (HFBA salt, 24.6 mg, 66% yield).

^{1}H NMR (499 MHz, D_2O) δ 4.24 (ddd, *J* = 15.5, 9.6, 6.2 Hz, 1H), 4.00 (q, *J* = 7.1 Hz, 1H), 3.03 (app pd, *J* = 9.6, 2.5 Hz, 1H), 2.60 (dd, *J* = 13.6, 8.9 Hz, 1H), 2.60–2.52 (m, 1H), 1.95 (td, *J* = 10.1, 3.0 Hz, 1H), 1.88 (dd, *J* = 11.6, 5.7 Hz, 1H), 1.83–1.75 (m, 2H), 1.70–1.64

(m, 2H), 1.61–1.53 (m, 2H), 1.50 (d, J = 7.1 Hz, 3H), 1.44–1.36 (m, 1H), 1.32–1.26 (m, 1H), 0.86–0.74 (m, 2H).

MS (ESI+, *m/z*): $[M+H-H_2O]^+$ calc'd for $C_{15}H_{28}BN_3O_4$, 324.2; found 324.2.

*Synthesis of Compound **28***

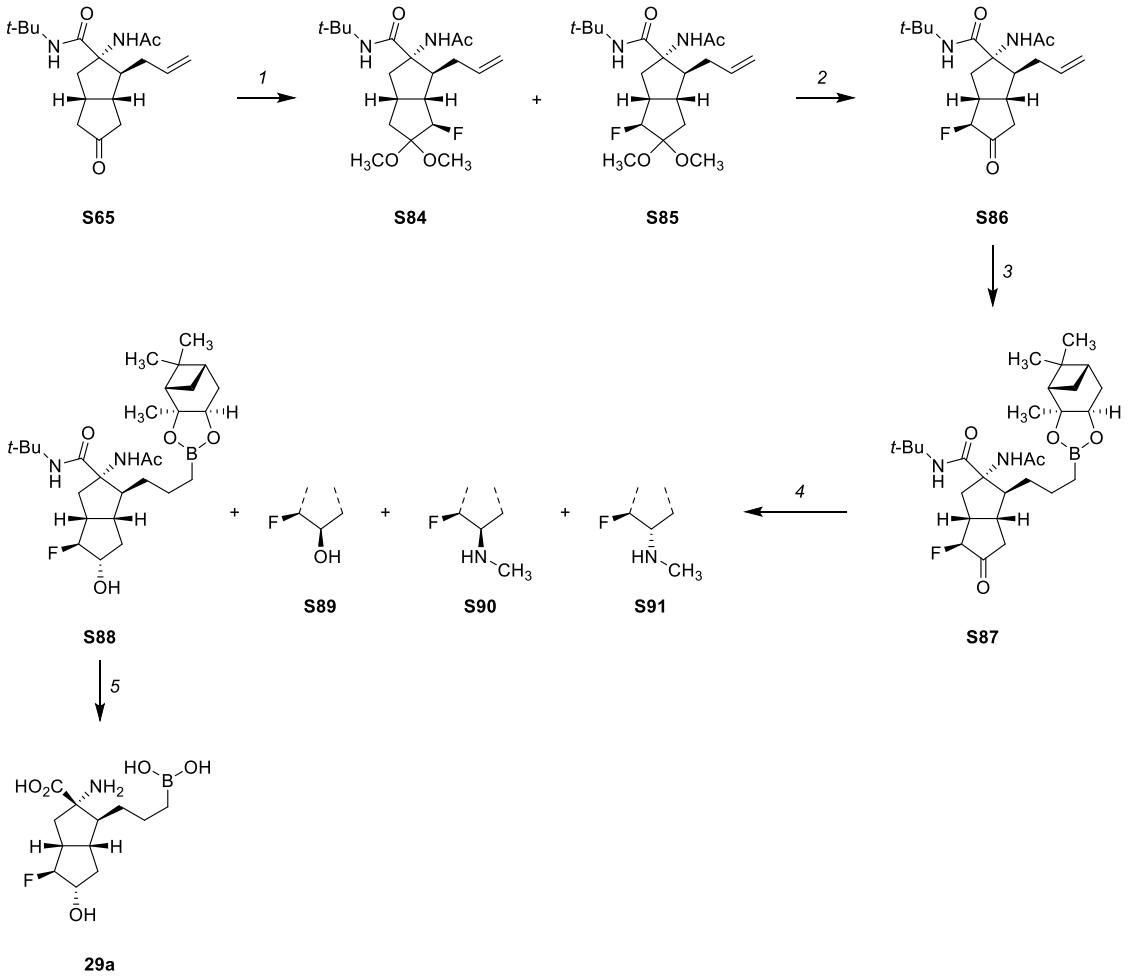


Compound **28** was prepared from intermediate **25b** by a sequence analogous to that used to prepare compound **27**.

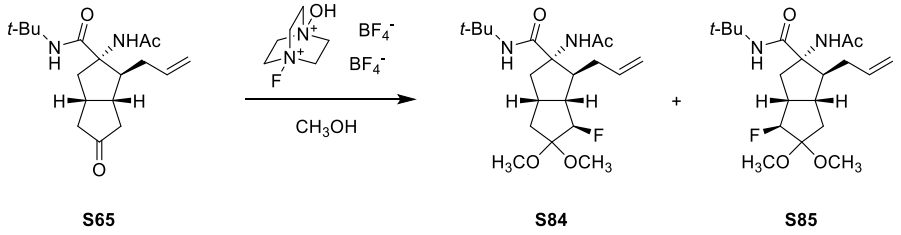
^{1}H NMR (499 MHz, D_2O) δ 8.52 (d, *J* = 7.3 Hz, 1H), 4.28 (app h, *J* = 5.9 Hz, 1H), 3.69 (d, *J* = 6.5 Hz, 1H), 3.04 (app pd, *J* = 9.8, 3.0 Hz, 1H), 2.62–2.53 (m, 2H), 2.18 (oct, *J* = 7.0 Hz, 1H), 1.96–1.87 (m, 2H), 1.83–1.77 (m, 2H), 1.74–1.65 (m, 2H), 1.59–1.53 (m, 2H), 1.42–1.36 (m, 1H), 1.32–1.25 (m, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.86–0.74 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{17}H_{32}BN_3O_5$, 352.2; found 352.3.

Synthesis of Compounds 29a, 29b, 30a, and 30b.



Step 1. (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-Acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5,5-dimethoxyoctahydropentalene-2-carboxamide and (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5,5-dimethoxyoctahydropentalene-2-carboxamide



(1*S*,2*S*,3a*S*,6a*R*)-2-Acetamido-1-allyl-*N*-(*tert*-butyl)-5-oxooctahydropentalene-2-carboxamide (400 mg, 1.25 mmol), 1-fluoro-4-hydroxy-1,4-diazoniabicyclo[2,2,2]octane bis(tetrafluoroborate) (~50% w/w on aluminum oxide, 1.2 g, ~1.9 mmol), and methanol (25

mL) were combined, and the mixture was heated to 65 °C for 40 min. The mixture was then cooled to room temperature before it was diluted with dichloromethane (50 mL). The diluted mixture was filtered through a pad of Celite, and the filter cake was rinsed with dichloromethane (25 mL). The filtrate was transferred to a separatory funnel, where it was washed with water. The washed organic solution was then dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by supercritical-fluid chromatography (Lux-4 column, 21 x 250 mm; 0.1% v/v ammonium hydroxide in methanol modifier; 10% modifier in CO_2 mobile phase; 70 mL/min flow rate) to provide (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5,5-dimethoxyoctahydropentalene-2-carboxamide (**S84**, t_r = 4.1 min, 177 mg, 37% yield) as the first-eluting peak, and (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5,5-dimethoxyoctahydropentalene-2-carboxamide (**S85**, t_r = 5.4 min, 151 mg, 32% yield) as the second-eluting peak.

S84

^{1}H NMR (499 MHz, $CDCl_3$) δ 6.12 (s, 1H), 6.10 (s, 1H), 5.86 (ddd, *J* = 17.1, 10.0, 5.3 Hz, 1H), 5.18 (d, *J* = 17.2 Hz, 1H), 5.12 (d, *J* = 10.0 Hz, 1H), 4.52 (d, *J* = 51.1 Hz, 1H), 3.31 (s, 3H), 3.24 (s, 3H), 2.93 (p, *J* = 10.8, 9.9 Hz, 1H), 2.85 (dd, *J* = 12.8, 9.1 Hz, 1H), 2.53 (dt, *J* = 30.2, 10.0 Hz, 1H), 2.37 (d, *J* = 24.6 Hz, 1H), 2.30–2.16 (m, 2H), 2.02–1.95 (m, 1H), 1.99 (s, 3H), 1.88 (d, *J* = 14.2 Hz, 1H), 1.70 (dd, *J* = 12.9, 9.7 Hz, 1H), 1.34 (s, 9H).

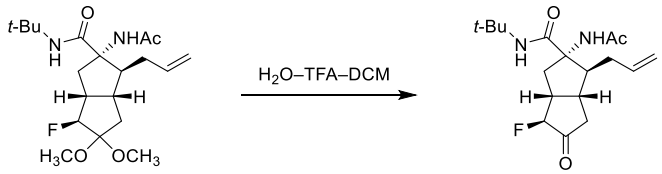
MS (ESI+, *m/z*): $[M+H–CH3OH]^+$ calc'd for $C_{20}H_{33}FN_2O_4$, 353.2; found 353.3.

S85

^{1}H NMR (499 MHz, Chloroform-*d*) δ 6.09 (s, 1H), 5.97 (s, 1H), 5.82 (ddt, *J* = 17.2, 10.1, 7.3 Hz, 1H), 5.12 (dd, *J* = 17.1, 1.5 Hz, 1H), 5.08 (d, *J* = 10.1 Hz, 1H), 4.50 (d, *J* = 51.5 Hz, 1H), 3.29 (s, 3H), 3.22 (s, 3H), 3.01–2.93 (m, 2H), 2.43 (q, *J* = 9.3 Hz, 1H), 2.27 (dt, *J* = 14.4, 7.4 Hz, 1H), 2.10 (dt, *J* = 14.1, 7.2 Hz, 1H), 2.02–1.95 (m, 1H), 1.97 (s, 3H), 1.83 (d, *J* = 14.3 Hz, 1H), 1.78–1.73 (m, 1H), 1.32 (s, 9H).

MS (ESI+, *m/z*): $[M+H–CH_3OH]^+$ calc'd for $C_{20}H_{33}FN_2O_4$, 353.2; found 353.3.

Step 2. (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-Acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5-oxooctahydropentalene-2-carboxamide

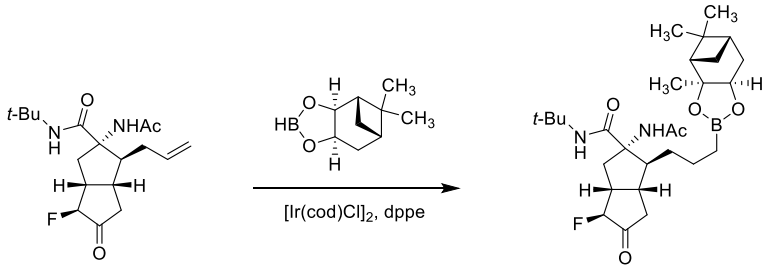


S85 **S86**

Water (1.96 mL) and trifluoroacetic acid (3.93 mL) were added sequentially to a solution of (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5,5-dimethoxyoctahydropentalene-2-carboxamide (151 mg, 0.393 mmol) in dichloromethane (1.96 mL) at 23 °C. The mixture was stirred for 30 min before it was poured into sat. aq. sodium bicarbonate solution (100 mL). The resulting mixture was stirred at 23 °C until bubbling ceased (ca. 10 min). This mixture was then extracted with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to provide (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5-oxooctahydropentalene-2-carboxamide as a white solid (128 mg, 96% yield).

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{27}FN_2O_3$, 339.2; found 339.3.

Step 3. (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-Acetamido-*N*-(*tert*-butyl)-4-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide

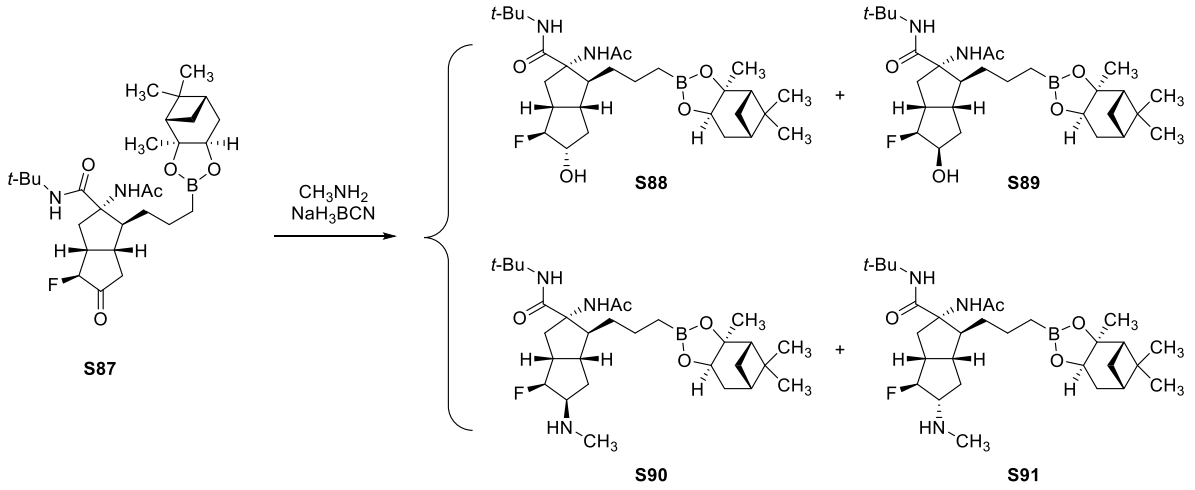


S86 **S87**

A solution of (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-4-fluoro-5-oxooctahydropentalene-2-carboxamide (128 mg, 0.378 mmol) in dichloromethane (3.5 mL) was added to a solution of (+)-pinanediolborane (341 mg, 1.89 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (12.7 mg, 18.9 μmol), and 1,2-bis(diphenylphosphino)ethane (15.1 mg, 37.9 μmol) in dichloromethane (4.0 mL) at 23 °C. The reaction mixture was stirred at this temperature for 25 min under nitrogen gas. Excess reductant was quenched with the addition of methanol (1.0 mL), and the mixture was stirred for 10 min, by which time bubbling had ceased. Water (10 mL) was added, the layers were shaken, and the phases were separated. The aqueous layer was extracted with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated. The crude residue, containing (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{44}BFN_2O_5$, 519.3; found 519.3.

Step 4. (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-Acetamido-*N*-(*tert*-butyl)-4-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, (1*S*,2*S*,3a*R*,4*S*,5*R*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, (1*S*,2*S*,3a*R*,4*S*,5*R*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, and (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide



Methylamine solution (2.0 M in THF, 945 μL, 1.89 mmol) was added to a solution of (1*S*,2*S*,3a*R*,4*S*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (crude residue from hydroboration, theoretically 0.378 mmol) in absolute ethanol (7.56 mL) at 0 °C. After 30 min, sodium cyanoborohydride (88.0 mg, 1.40 mmol) was added in one portion. Stirring was maintained while the temperature was allowed to warm gradually to 23 °C over 2 h. The reaction mixture was then diluted with sat. aq. sodium bicarbonate solution (20 mL), sat. aq. sodium chloride solution (20 mL), and ethyl acetate (20 mL). The layers were shaken, and the phases were separated. The aqueous layer was extracted with ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by RP-HPLC (C18 column, eluting with

acetonitrile–water containing 0.1% TFA) to give a ~3:1 mixture of epimeric methylamino compounds **S90** and **S91** as the first-eluting peak; (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S88**, 33.8 mg, 17% yield over 2 steps) as the second-eluting peak; and (1*S*,2*S*,3a*R*,4*S*,5*R*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S89**, 28.4 mg, 14% yield over 2 steps) as the third-eluting peak. The mixture of **S90** and **S91** was then resolved by supercritical-fluid chromatography ([*R*,*R*]-Whelk-O column , 21 x 250 mm; 0.1% v/v ammonium hydroxide in methanol modifier; 20% modifier in CO_2 mobile phase; 70 mL/min flow rate) to provide the minor epimer **S90** as the first-eluting peak (t_R = 4.6 min), and the major epimer **S91** as the second-eluting peak (t_R = 5.3 min). The purity of these samples was further boosted by re-subjecting them separately to RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to provide (1*S*,2*S*,3a*R*,4*S*,5*R*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S90**, TFA salt, 10.2 mg, 4% yield over 2 steps), and (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-acetamido-*N*-(*tert*-butyl)-4-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S91**, TFA salt, 34.7 mg, 14% yield over 2 steps).

S88

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{46}BFN_2O_5$, 521.4; found 521.3.

S89

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{46}BFN_2O_5$, 521.4; found 521.3.

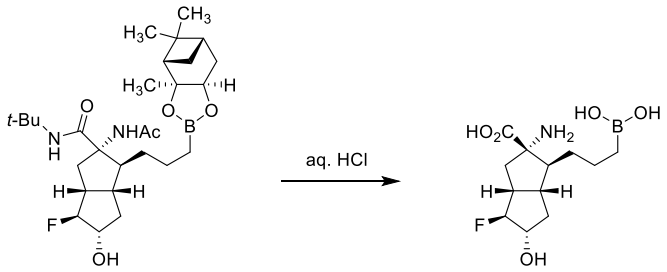
S90

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{49}BFN_3O_4$, 534.4; found 534.4.

S91

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{49}BFN_3O_4$, 534.4; found 534.4.

Step 5. (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-Amino-1-(3-boronopropyl)-4-fluoro-5-hydroxyoctahydro-pentalene-2-carboxylate



S88 **29a**

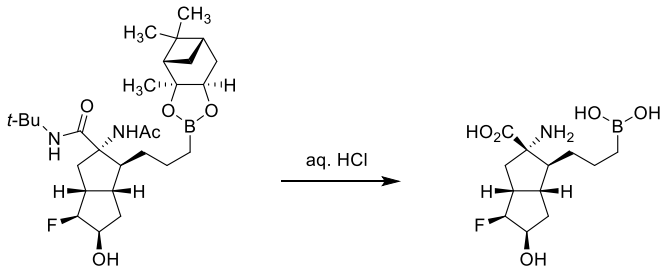
(1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-Acetamido-*N*-(*tert*-butyl)-4-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (33.8 mg, 64.9 μmol) and 6N aq. hydrochloric acid solution (1.30 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to room temperature and was diluted with water (10 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (10 mL) and the diluted mixture was concentrated under reduced pressure to provide (1*S*,2*S*,3a*R*,4*S*,5*S*,6a*S*)-2-amino-1-(3-boronopropyl)-4-fluoro-5-hydroxyoctahydropentalene-2-carboxylate as a white solid (salt with *t*-butylamine and HCl, 1:1:2 molar ratio, 23.8 mg, 84% yield).

^{1}H NMR (500 MHz, D_2O) δ 4.38 (dddd, *J* = 23.7, 9.7, 6.3, 3.0 Hz, 1H), 3.09 (app dq, *J* = 28.2, 10.0 Hz, 1H), 2.69–2.59 (m, 2H), 2.04 (app q, *J* = 11.2 Hz, 1H), 1.96 (app td, *J* = 9.4, 2.7 Hz, 1H), 1.86 (ddd, *J* = 13.3, 6.7, 1.1 Hz, 1H), 1.66 (dd, *J* = 13.4, 10.7 Hz, 1H), 1.61–1.53 (m, 2H), 1.46–1.40 (m, 1H), 1.30–1.24 (m, 1H), 0.86–0.74 (m, 2H). The

α-fluoro methine signal at δ ~ 4.72 was partially obscured by the residual solvent signal, HDO.

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{21}BFNO_5$, 272.2; found 272.2.

*Synthesis of Compound **29b**.*



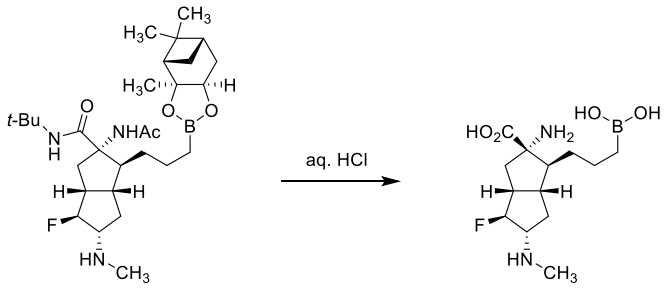
S89 **29b**

Compound **29b** was prepared from intermediate **S89** in an analogous fashion. The product was isolated as a white solid (salt with *t*-butylamine and HCl, 1:1:2 molar ratio).

^{1}H NMR (500 MHz, D_2O) δ 4.37 (app dq, *J* = 12.9, 6.4 Hz, 1H), 3.01 (app dqd 25.1, 9.6, 4.8 Hz, 1H), 2.70 (dd, *J* = 13.5, 9.3 Hz, 1H), 2.66–2.60 (m, 1H), 2.36–2.30 (m, 1H), 2.18 (app td, *J* = 10.0, 2.6 Hz, 1H), 2.04 (dd, *J* = 13.5, 9.5 Hz, 1H), 1.65–1.54 (m, 3H), 1.42–1.37 (m, 1H), 1.24 (app q, *J* = 10.6 Hz, 1H), 0.86–0.74 (m, 2H). The α-fluoro methine signal at δ ~ 4.74 was partially obscured by the residual solvent signal, HDO.

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{21}BFNO_5$, 272.2; found 272.2.

*Synthesis of Compound **30a**.*



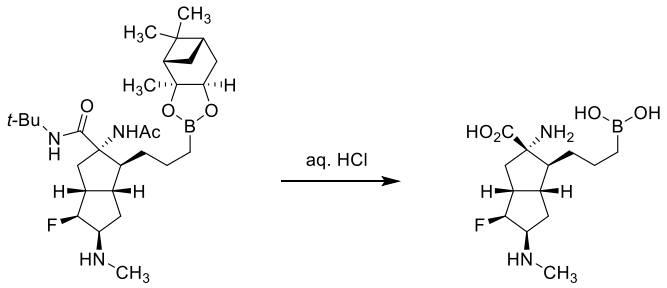
S91 **30a**

Compound **30a** was prepared analogously to **29a**, beginning with intermediate **S91**. The product was obtained as a white solid (salt with *t*-butylamine and HCl, 1:1:3 molar ratio).

^{1}H NMR (499 MHz, D_2O) δ 5.07 (app dt, *J* = 54.7, 8.0 Hz, 1H), 3.92 (app tt, *J* = 15.5, 8.1 Hz, 1H), 3.13 (app ddt, *J* = 24.3, 20.3, 8.6 Hz, 1H), 2.82–2.73 (m, 2H), 2.77 (s, 3H), 2.67–2.61 (m, 1H), 2.14 (app td, *J* = 10.7, 3.2 Hz, 1H), 2.10 (dd, *J* = 13.8, 8.8 Hz, 1H), 1.70–1.60 (m, 1H), 1.60–1.48 (m, 2H), 1.39–1.32 (m, 1H), 1.24 (app qd, *J* = 11.3, 4.0 Hz, 1H), 0.84–0.74 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{24}BFN_2O_4$, 285; found 285.

Synthesis of Compound 30b.



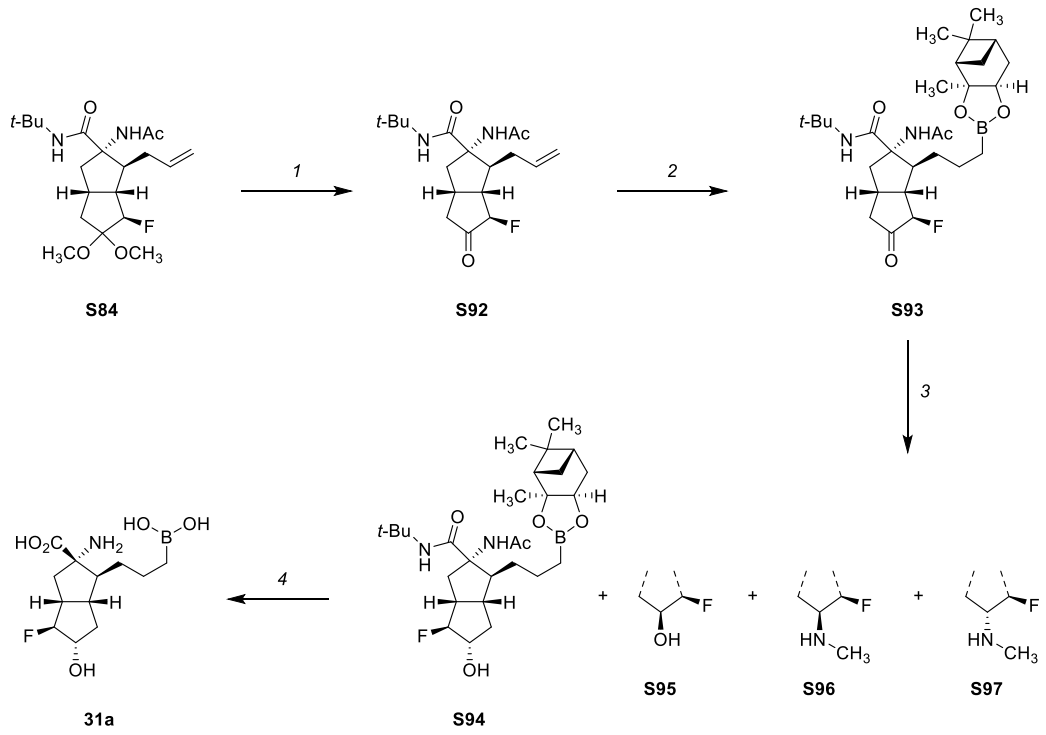
S90 **30b**

Compound **30b** was prepared analogously to **29a**, beginning with intermediate **S90**. The product was obtained as a white solid (salt with *t*-butylamine and HCl, 1:1:3 molar ratio).

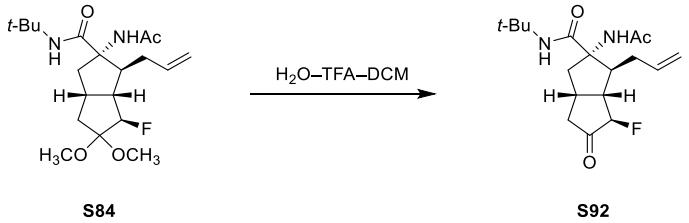
^{1}H NMR (500 MHz, D_2O) δ 5.13 (dd, *J* = 52.7, 3.0 Hz, 1H), 3.86 (dddd, *J* = 27.5, 11.2, 7.0, 2.7 Hz, 1H), 3.29 (app dq, *J* = 29.1, 9.8 Hz, 1H), 2.86–2.77 (m, 1H), 2.83 (s, 3H), 2.66 (dd, *J* = 13.7, 9.6 Hz, 1H), 2.24–2.12 (m, 2H), 1.95 (app td, *J* = 9.8, 3.3 Hz, 1H), 1.65–1.55 (m, 3H), 1.47–1.40 (m, 1H), 1.35–1.29 (m, 1H), 0.85–0.79 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{24}BFN_2O_4$, 285; found 285.

Synthesis of Compounds 31a, 31b, 32a, and 32b



Step 1. (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-Acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5-oxooctahydropentalene-2-carboxamide

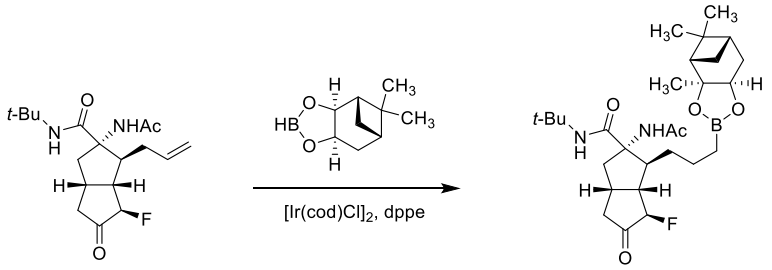


Water (2.30 mL) and trifluoroacetic acid (4.58 mL) were added sequentially to a solution of (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5,5-dimethoxy-octahydropentalene-2-carboxamide (0.176 g, 0.458 mmol) in dichloromethane (2.30 mL) at 23 °C. The mixture was stirred for 1 h before it was poured into sat. aq. sodium bicarbonate solution (100 mL). The resulting mixture was stirred at 23 °C until bubbling ceased (ca. 10 min). This mixture was then extracted with dichloromethane, and the combined extracts were dried over sodium sulfate, filtered, and concentrated to provide

(1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5-oxooctahydropentalene-2-carboxamide as a white solid (159 mg, 103% yield)

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{18}H_{27}FN_2O_3$, 339.2; found 339.2.

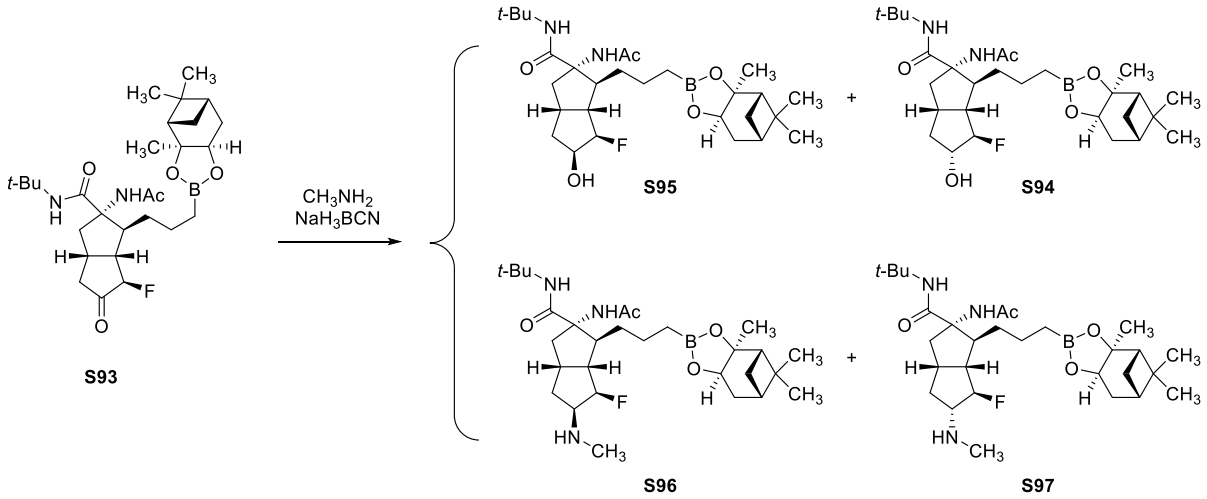
Step 2. (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-6-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide



A solution of (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-1-allyl-*N*-(*tert*-butyl)-6-fluoro-5-oxooctahydropentalene-2-carboxamide (158 mg, 0.467 mmol) in dichloromethane (3.3 mL) was added to a solution of (+)-pinanediolborane (420 mg, 2.33 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (15.7 mg, 23.3 μmol), and 1,2-bis(diphenylphosphino)ethane (18.6 mg, 46.7 μmol) in dichloromethane (6.0 mL) at 23 °C. The reaction mixture was stirred at this temperature for 20 min under nitrogen gas. Excess reductant was quenched with the addition of methanol (1.0 mL), and the mixture was stirred for 10 min, by which time bubbling had ceased. Water (10 mL) was added, the layers were shaken, and the phases were separated. The aqueous layer was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The crude residue, containing (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, was used in the next step without purification.

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{44}BFN_2O_5$, 519.3; found 519.3.

Step 3. (1*S*,2*S*,3a*R*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-(3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, (1*S*,2*S*,3a*S*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, (1*S*,2*S*,3a*R*,5*S*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide, and (1*S*,2*S*,3a*R*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide



Methylamine solution (2.0 M in tetrahydrofuran, 1.17 mL, 2.34 mmol) was added to a solution of (1*S*,2*S*,3a*S*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-oxo-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (crude residue from hydroboration, 0.467 mmol theoretical) in absolute ethanol (9.34 mL) at 0 °C. After 30 min, sodium cyanoborohydride (88.0 mg, 1.40 mmol) was added in one portion. Stirring was maintained while the temperature was allowed to warm gradually to 23 °C over 18 h. The reaction mixture was then diluted with sat. aq. sodium bicarbonate solution (20 mL), sat. aq. sodium chloride solution (20 mL), and ethyl acetate (20 mL). The layers were shaken, and the phases were separated. The aqueous layer was extracted with ethyl acetate. The combined extracts were dried over sodium sulfate, filtered, and

concentrated. The residue was purified by RP-HPLC (C18 column, eluting with acetonitrile–water containing 0.1% TFA) to give a ~2:1 mixture of epimeric methylamino compounds **S96** and **S97** as the first-eluting peak; (1*S*,2*S*,3a*R*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-(3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S95**, 35.9 mg, 15% yield over 2 steps) as the second-eluting peak; and (1*S*,2*S*,3a*S*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S94**, 30.4 mg, 13% yield over 2 steps) as the third-eluting peak. The mixture of **S96** and **S97** was then resolved by supercritical-fluid chromatography (CCA column, 21 × 250 mm; 0.1% v/v ammonium hydroxide–methanol modifier; 8% modifier in CO_2 mobile phase; 70 mL/min flow rate) to provide (1*S*,2*S*,3a*R*,5*S*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S96**, t_r = 4.1 min, 11.9 mg, 5% yield over 2 steps) as the first-eluting peak, and (1*S*,2*S*,3a*R*,5*R*,6*R*,6a*R*)-2-acetamido-*N*-(*tert*-butyl)-6-fluoro-5-(methylamino)-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (**S97**, t_r = 5.3 min, 34.5 mg, 14% yield over 2 steps) as the second-eluting peak.

S95

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{46}BFN_2O_5$, 521.4; found 521.3.

S94

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{28}H_{46}BFN_2O_5$, 521.4; found 521.3.

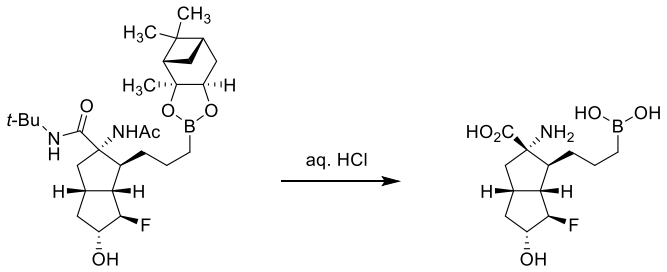
S96

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{49}BFN_3O_4$, 534.4; found 534.4.

S97

MS (ESI+, *m/z*): $[M+H]^+$ calc'd for $C_{29}H_{49}BFN_3O_4$, 534.4; found 534.4

Step 4. (1*S*,2*S*,3a*S*,5*R*,6*R*,6a*R*)-2-Amino-1-(3-boronopropyl)-6-fluoro-5-hydroxyoctahydropentalene-2-carboxylate



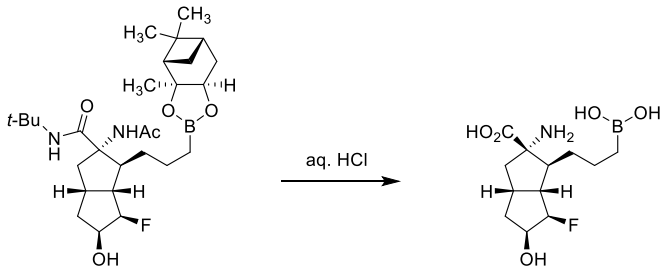
S94 **31a**

(1*S*,2*S*,3a*S*,5*R*,6*R*,6a*R*)-2-Acetamido-*N*-(*tert*-butyl)-6-fluoro-5-hydroxy-1-(3-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)propyl)octahydropentalene-2-carboxamide (30.4 mg, 58.4 μmol) and 6N aq. hydrochloric acid solution (1.17 mL) were combined, and the resulting mixture was heated at 125 °C for 1 h. The mixture was then cooled to room temperature and was diluted with water (10 mL). The diluted solution was washed with dichloromethane until both the aqueous layer and the organic washes were colorless. The washed aqueous layer was then diluted with acetonitrile (10 mL) and the diluted mixture was concentrated under reduced pressure to provide (1*S*,2*S*,3a*S*,5*R*,6*R*,6a*R*)-2-amino-1-(3-boronopropyl)-6-fluoro-5-hydroxyoctahydropentalene-2-carboxylate as a white solid (salt with *t*-butylamine and HCl, 1:1:3 molar ratio, 24.7 mg, 97% yield).

^{1}H NMR (500 MHz, D_2O) δ 4.79 (dd, *J* = 53.1, 2.9 Hz, 1H), 4.37 (dddd, *J* = 24.4, 10.0, 6.4, 3.2 Hz, 1H), 3.12 (app p, *J* = 8.8 Hz, 1H), 2.69–2.58 (m, 2H), 1.97–1.91 (m, 2H), 1.79 (ddd, *J* = 12.8, 6.4, 1.5 Hz, 1H), 1.67 (dd, *J* = 13.6, 10.0 Hz, 1H), 1.64–1.58 (m, 2H), 1.47–1.36 (m, 2H), 0.86–0.77 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{21}BFNO_5$, 272.2; found 272.1.

*Synthesis of Compound **31b**.*



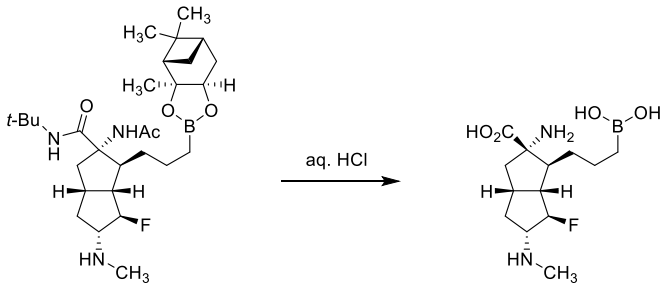
S95 **31b**

Compound **31b** was prepared analogously to **31a**, beginning with intermediate **S95**. The product was obtained as a white solid (salt with *t*-butylamine and HCl, 1:1:2 molar ratio).

^{1}H NMR (500 MHz, D_2O) δ 4.78 (dt, *J* = 52.4, 4.4 Hz, 1H), 4.38 (app dq, *J* = 12.9, 6.0 Hz, 1H), 3.07 (app h, *J* = 8.8 Hz, 1H), 2.61 (dd, *J* = 13.4, 8.9 Hz, 1H), 2.56 (td, *J* = 10.4, 3.8 Hz, 1H), 2.34 (td, *J* = 10.2, 2.8 Hz, 1H), 2.27–2.21 (m, 1H), 1.86 (dd, *J* = 13.5, 9.2 Hz, 1H), 1.67–1.53 (m, 3H), 1.49–1.42 (m, 1H), 1.39–1.33 (m, 1H), 0.87–0.75 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{12}H_{21}BFNO_5$, 272.2; found 272.2.

*Synthesis of Compound **32a**.*



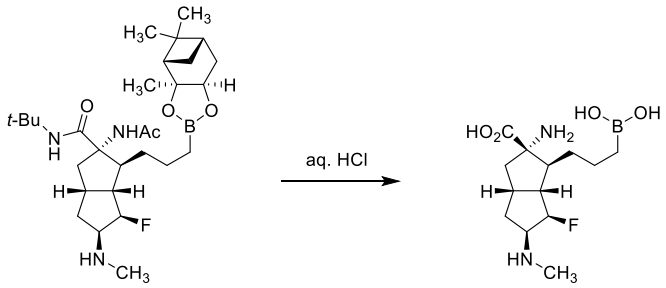
S97 **32a**

Compound **32a** was prepared analogously to **31a**, beginning with intermediate **S97**. The product was obtained as a white solid (salt with *t*-butylamine and HCl, 1:1:3 molar ratio).

^{1}H NMR (500 MHz, D_2O) δ 5.09 (ddd, *J* = 53.9, 8.0, 6.2 Hz, 1H), 3.93 (app ddt, *J* = 15.5, 12.8, 7.5 Hz, 1H), 3.17 (app dq, *J* = 19.4, 8.7 Hz, 1H), 2.79 (s, 3H), 2.78–2.70 (m, 1H), 2.66 (dd, *J* = 13.7, 8.7 Hz, 1H), 2.57 (app dt, *J* = 12.8, 6.4 Hz, 1H), 2.35 (app td, *J* = 10.1, 2.5 Hz, 1H), 1.79 (dd, *J* = 13.7, 8.6 Hz, 1H), 1.64–1.56 (m, 3H), 1.46–1.34 (m, 2H), 0.87–0.75 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{24}BFN_2O_4$, 285.2; found 285.2.

*Synthesis of Compound **32b**.*



S96 **32b**

Compound **32b** was prepared analogously to **31a**, beginning with intermediate **S96**. The product was obtained as a white solid (salt with *t*-butylamine and HCl, 1:1:3 molar ratio).

^{1}H NMR (500 MHz, D_2O) δ 5.14 (dd, *J* = 52.5, 2.9 Hz, 1H), 3.85 (dtd, *J* = 27.7, 10.6, 2.6 Hz, 1H), 3.34–3.26 (m, 1H), 2.88–2.78 (m, 1H), 2.84 (s, 3H), 2.63 (dd, *J* = 13.6, 9.0 Hz, 1H), 2.11–2.08 (m, 2H), 1.91 (app t, *J* = 9.7 Hz, 1H), 1.68–1.62 (m, 3H), 1.47–1.40 (m, 2H), 0.84–0.81 (m, 2H).

MS (ESI+, *m/z*): $[M+H–H_2O]^+$ calc'd for $C_{13}H_{24}BFN_2O_4$, 285.2; found 285.2.

Detailed NMR Characterization of Compound 3

Analysis of ^{15}N HMBC data

Proton–nitrogen correlations were observed from the major peaks of compound **3** to both nitrogen atoms, N9 and N4 (Figure S1). As expected, no HMBC correlations were observed from the α-boron methylene protons (green H12, resonating at 0.74 ppm) to any nitrogen atom. However, the minor α-boron methylene protons (blue H12) – as well as protons H6 and H8", did show HMBC correlation to nitrogen N9. These observations were the first indication that the minor peaks of compound **3** observed at pH=4 were related to boro-amino intramolecular interactions leading to the cyclo-**3** compound.

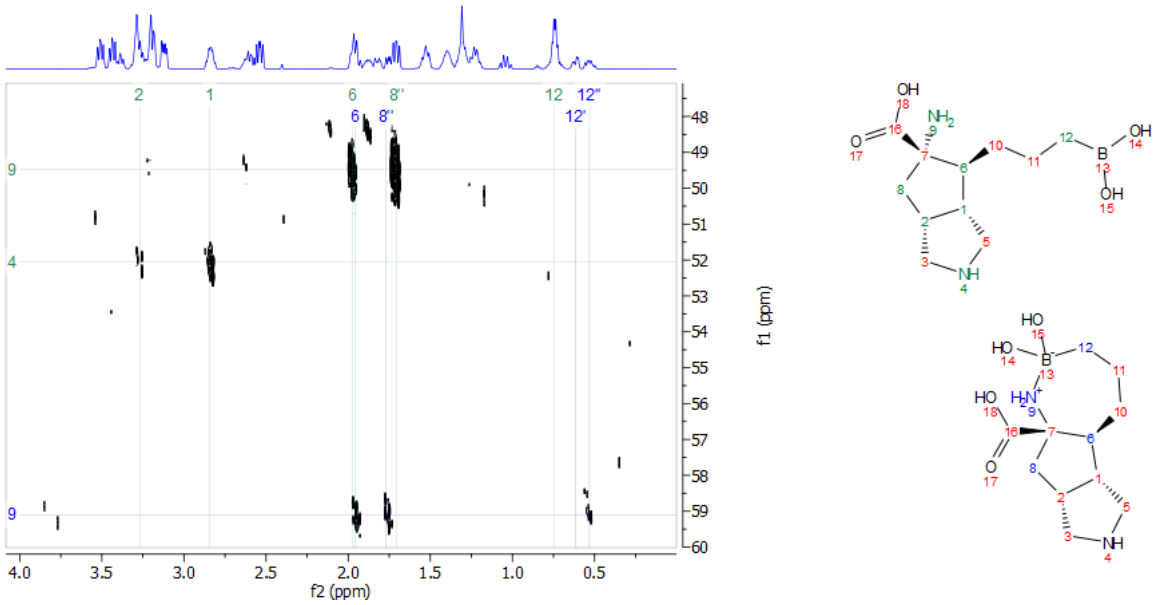


Figure S1. ^{15}N HMBC spectrum of **3** (atom assignments in green) in D_2O at pH = 4, revealing a key correlation between methylene protons 12 and nitrogen N9 of the minor species, *cyclo*-**3** (atom assignments in blue).

Analysis of 2D DOSY data

Diffusion-ordered spectroscopy (DOSY) experiments aim to separate the NMR signals of compounds in a mixture based on their different diffusion coefficients, which in

turn depend on the size and shape of the molecules. The DOSY spectrum of **3** at pH = 4 revealed different diffusion coefficients for **3** and its cyclic constitutional isomer, with a larger diffusion coefficient observed for *cyclo*-**3** due to its compact shape (Figure S2). These results further supported the assignment of the minor peaks to *cyclo*-**3**.

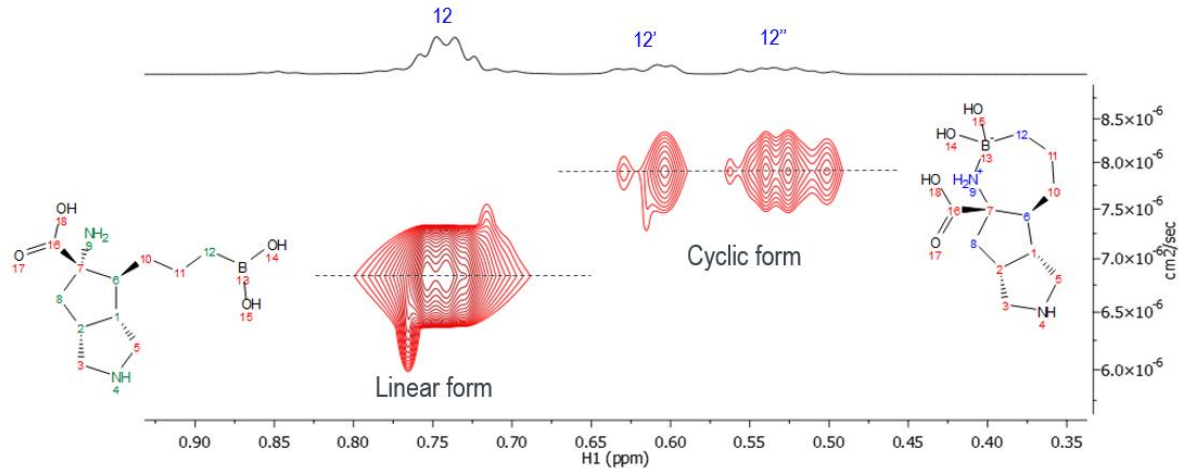


Figure S2. Expansion of 2D DOSY spectra of 3 in D_2O at pH = 4.

Analysis of ^{11}B NMR data

Additional evidence for the identity of *cyclo*-**3** was gathered by analysis of ^{11}B NMR spectra of compound **3** at pH = 1 and pH = 4 (Figure S3). As shown in Figure 2 in the main manuscript, ^{1}H NMR spectrum of **3** at pH = 1 showed one set of singals only, while ^{1}H NMR spectrum at pH = 4 showed minor species present. ^{11}B NMR spectra of these two samples also indicated the presence of one single compound at pH = 1 with a unique boron chemical shift (32.7 ppm) while two boron signals were observed at pH = 4 (32.7 ppm and 7.8 ppm). These ^{11}B chemical shifts results supported the presence of *cyclo*-**3** at pH = 4.

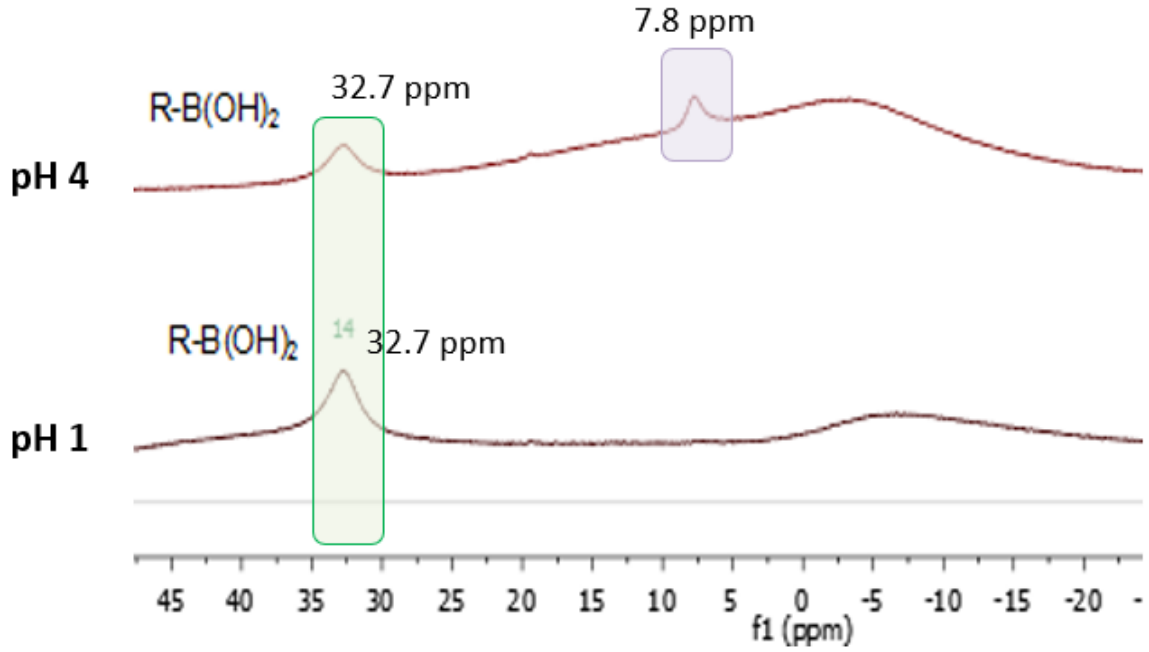


Figure S3. Bottom trace: ^{11}B NMR spectrum of **3** in D_2O at pH = 1. Top trace: ^{11}B NMR spectrum of **3** in D_2O at pH=4, revealing the presence of a minor component with upshifed ^{11}B resonance consistent with *cyclo*-**3**.

Titration with KCl:

In order to determine whether the observed spectral features of *cyclo*-**3** were attributable to covalent, versus electrostatic (e.g., hydrogen-bonding), interaction of the boronic acid and amine groups, titration with potassium chloride solution was performed. Over 5 concentrations of KCl electrolyte, we observed no significant changes in ^{1}H NMR chemical shifts (D_2O, pH = 4), allowing us to conclude that *cyclo*-**3** features a covalently bonded, aminoboronate structure (Figure S4).

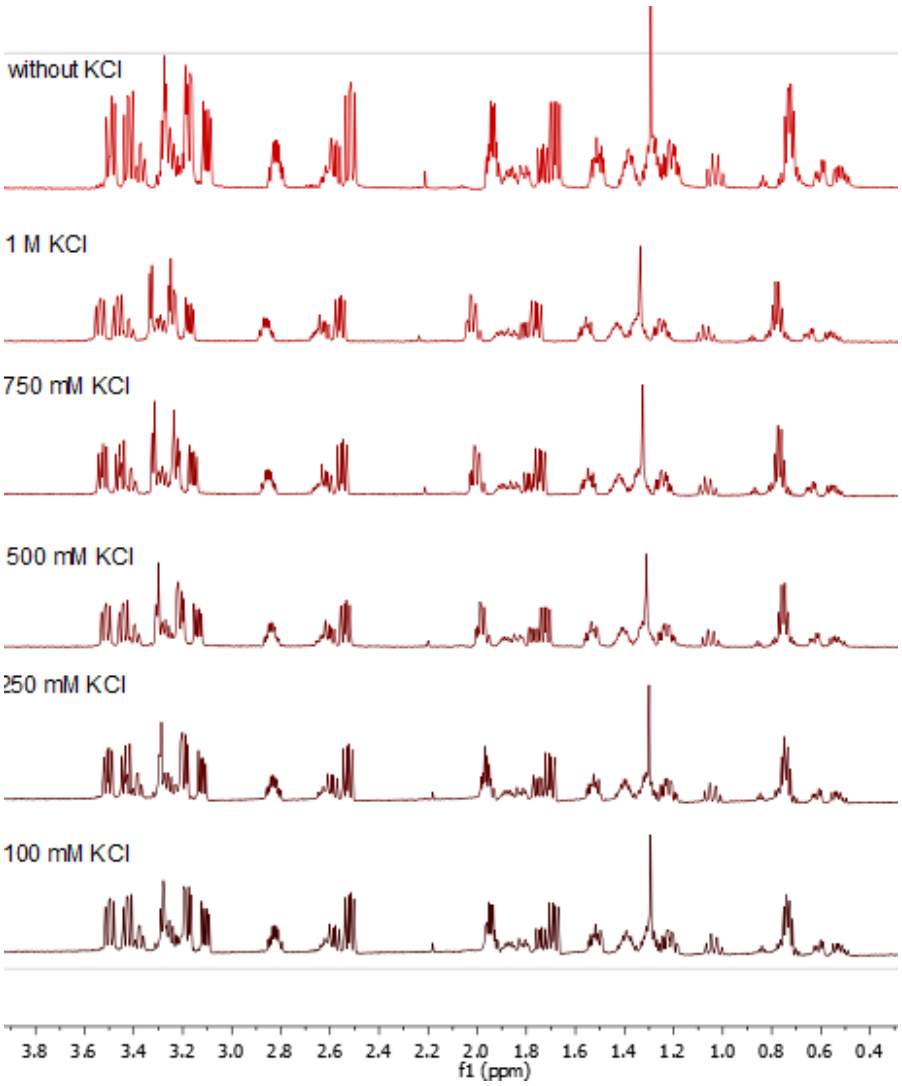


Figure S4. Titration of KCl caused no changes in the ^{1}H NMR spectra of **3** in D_2O at pH = 4, establishing the covalent nature of the boronic acid–amine bond within *cyclo*-**3**.

References

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