Supporting Information for "Discovery of antimalarial azetidine-2-carbonitriles that inhibit *P. falciparum* dihydroorotate dehydrogenase"

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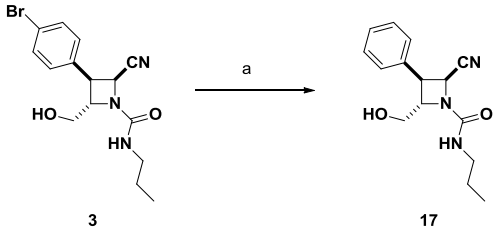
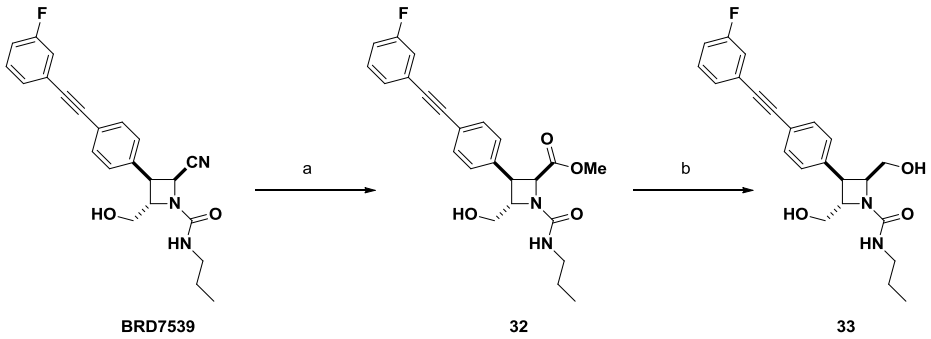
Supplementary Figures and Schemes.

Scheme S1: Synthesis of analogue 17.



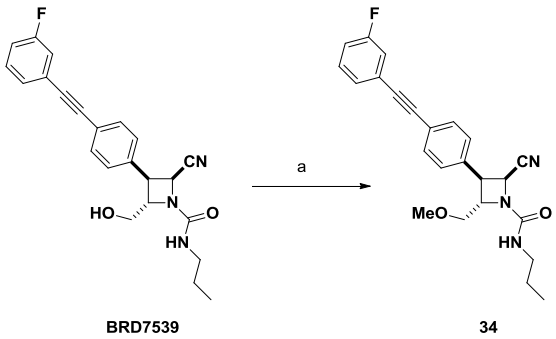
Reagents and conditions: (a) Pd/C (10% w/w), $NaHCO_3$, MeOH, H_2, 2 h, 46%.

Scheme S2: Synthesis of analogues 32 and 33.



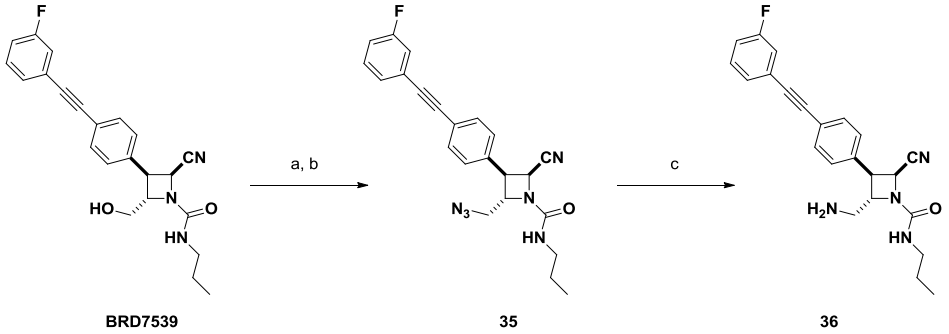
Reagents and conditions: (a) acetyl chloride, MeOH, 23 °C (22 h), then 50 °C (3 h), 74%; (b) $LiBH_4$, THF, 40 °C, 3 h, 49%.

Scheme S3: Synthesis of analogue 34.



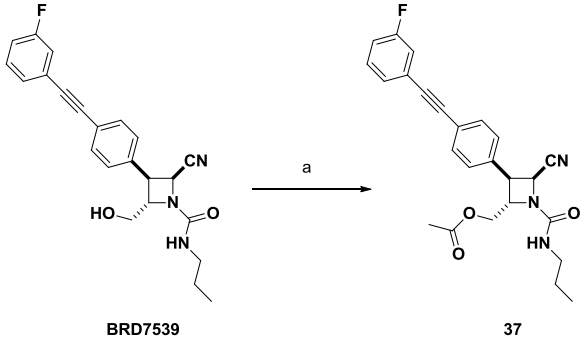
Reagents and conditions: (a) Me_3OBF_4, proton sponge, DCM, 23 °C, 16 h, 31%.

Scheme S4: Synthesis of analogues 35 and 36.



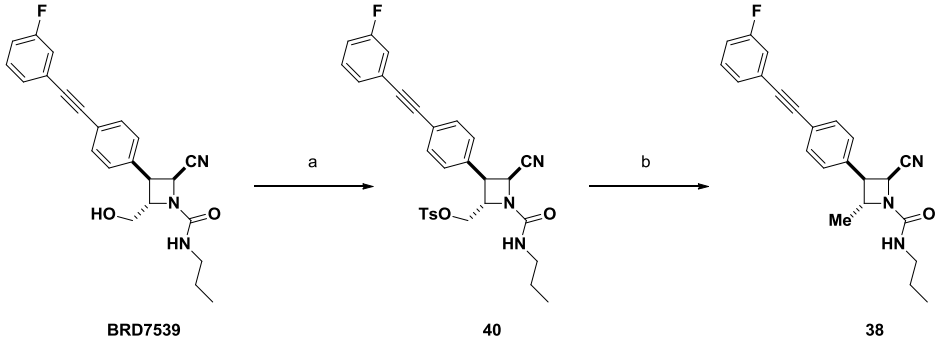
Reagents and conditions: (a) dppa, DBU, THF, 23 °C, 16 h; (b) NaN_3, DMF, 90 °C, 3 h, 88%; (c) PPh_3, H_2O/THF/DCM (20:10:3), 60 °C, 24 h, 41%.

Scheme S5: Synthesis of analogue 37.



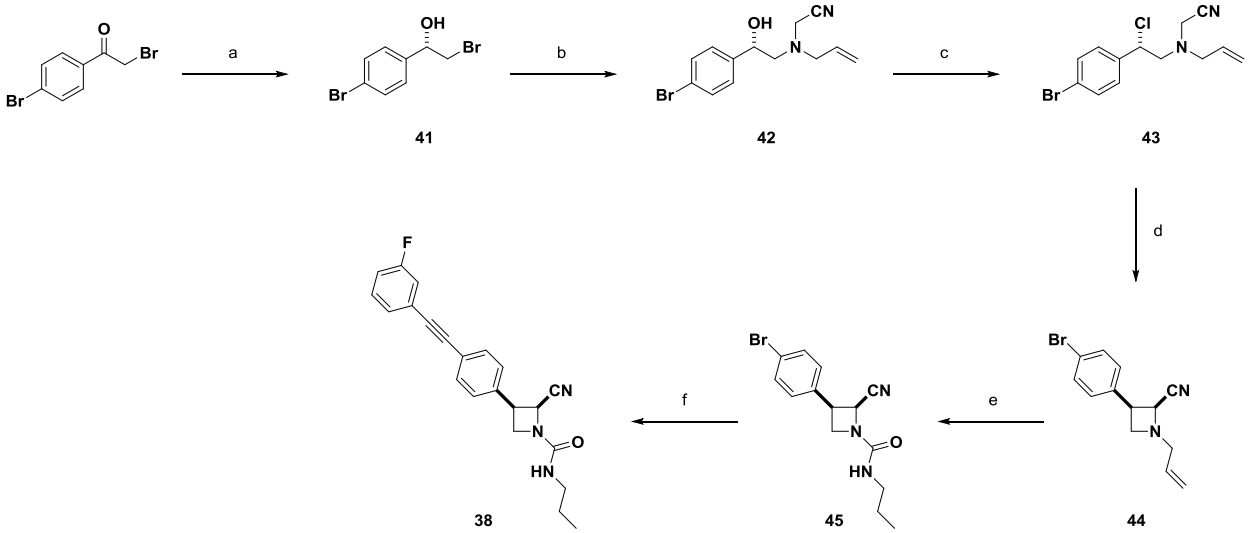
Reagents and conditions: (a) acetyl chloride, pyridine, DCM, 23 °C, 0.5 h, 78%.

Scheme S6: Synthesis of analogue 38.



Reagents and conditions: (a) TsCl, pyridine, 40 °C, 8 h, 49%; (b) $NaBH_4$, DMSO, 70 °C, 1 h, 23%.

Scheme S7: Synthesis of analogue 39.



Reagents and conditions: (a) (*S*)-CBS, BH_3·THF, 2-bromo-1-(4-bromophenyl)ethanone, THF, 23 °C, 0.5 h, 89%; (b) allylamine, I_2, 23 °C, 16 h; then bromoacetonitrile, K_2CO_3, MeCN, 85 °C, 40 h, 74%; (c) pyridine, $SOCl_2$, DCM, 0 °C, 0.25 h, 70%; (d) benzyl chloride, LiHMDS, THF, -60 °C, 1 h, 56%; (e) 1,3-dimethylbarbituric acid, $Pd(PPh_3)_4$, EtOH/DCM (2:1), 40 °C, 16 h; then propylisocyanate, DIPEA, DCM, 23 °C, 0.5 h, 61%; (f) 1-ethynyl-3-fluorobenzene, Et_3N, XPhos Pd-G3, MeCN, 70 °C, 3 h, 29%.

Figure S1. Inhibition of *P. falciparum* DHODH activity after incubation with compound **27**. Single experiment performed in triplicate.

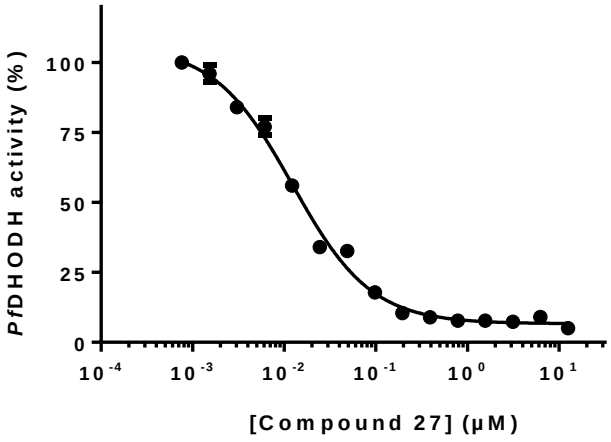
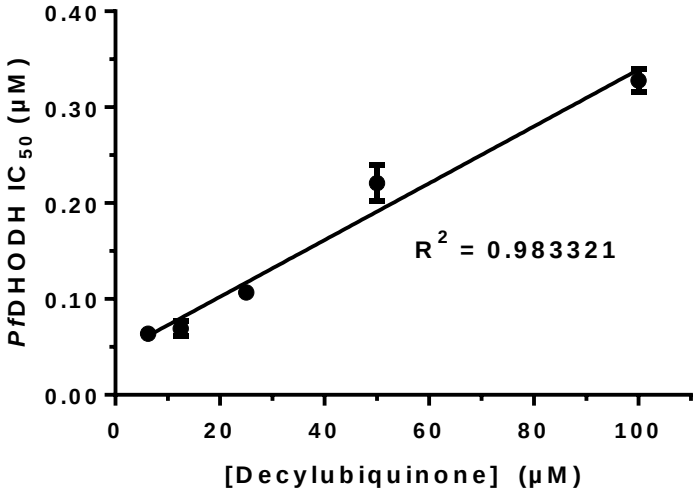


Table S1. Activity of selected BRD7539 analogues against recombinant *Pf*DHODH[a]

Cmpd	IC_{50} (μM)
3	0.286
4	0.064
5	0.060
10	0.041
15	0.031
18	0.017
19	0.023
20	>25
25	0.043
26	0.109
29	0.036
30	0.035

[a]EC_{50} values are the mean of a single experiment performed in triplicate.

Figure S2. Compound **27** IC_{50} versus varying concentration of decylubiquinone for *P. falciparum* DHODH. Single experiment in triplicate.



Biological and Biochemical Experimental Procedures.

In vitro P. falciparum **blood-stage culture and assay**. *P. falciparum* (Dd2 strain) parasites were obtained from the Malaria Research and Reference Reagent Resource Center (MR4). Parasites isolates were maintained with O-positive human blood in an atmosphere of 93% N_2, 4% CO_2, 3% O_2 at 37 °C in complete culturing medium (10.4 g/L RPMI 1640, 5.94 g/L HEPES, 5 g/L albumax II, 50 mg/L hypoxanthine, 2.1 g/L sodium bicarbonate, 10% human serum and 43 mg/L Gentamicin) and were cultured in medium until parasitemia reached 3–8%. Parasitemia was determined by checking at least 500 red blood cells from a Giemsa-stained blood smear. For the compound testing, a parasite dilution at 2.0% parasitemia and 2.0% hematocrit was created with medium. 25 μL of medium was dispensed into 384-well black, clear-bottom plates and 100 nL of each compound in DMSO was transferred into assay plates along with the control compound (Mefloquine). Next, 25 μL of the parasite suspension in medium was dispensed into the assay plates giving a final parasitemia of 1% and a final hematocrit of 1%. The assay plates were incubated for 72 h at 37 °C. 10 μL of detection reagent consisting of 10x SYBR Green I (Invitrogen; supplied in 10,000× concentration) in lysis buffer (20 mM Tris-HCl, 5mM EDTA, 0.16% (w/v) Saponin, 1.6% (v/v) Triton X-100) was dispensed into the assay plates. For optimal staining, the assay plates were left at room temperature for 24 h in the dark. The assay plates were read with 505 dichroic mirrors with 485 nm excitation and 530 nm emission settings in an Envision (PerkinElmer).

Animal welfare. All animal experiments were conducted in compliance with institutional policies and appropriate regulations and were approved by the institutional animal care and use committees for the Broad Institute (0016-09-14). No method of randomization or blinding was used in this study.

In vivo P. berghei **blood-stage assay**. CD-1 mice (n = 4 for control and compound **27** groups, n = 2 for artesunate group; female; 6–7-week-old; 20–24 g, Charles River) were intravenously inoculated with approximately 1×10^5 *P. berghei* (ANKA GFP-luc) blood-stage parasites 24 h before treatment. Compound **27**, artesunate, or vehicle solution were administered orally at 0, 24 h, and 48 h. Compound **27** and artesunate were formulated in 70% PEG300 and 30% (5% dextrose in H_2O) at 6.6 mg/mL. Formulation solution was used to treat control (vehicle) group. Parasitemia was monitored by the *in vivo* imaging system (IVIS SpectrumCT, PerkinElmer) to acquire the bioluminescence signal (150 mg/kg of luciferin was intraperitoneally injected approximately 10 min before imaging). In addition, blood smear samples were obtained from each mouse periodically, stained with Giemsa, and viewed under a microscope for visual detection of blood parasitemia. Animals with parasitemia exceeding 25% were humanely euthanized.

Mouse pharmacokinetics assay. Pharmacokinetics of **27** were performed following single intravenous and oral administrations to female CD-1 mice. **27** was formulated in 70% PEG300 and 30% (5% dextrose in H_2O) at 0.5 mg/mL for oral dosing, and 5% DMSO, 10% cremophor, and 85% H_2O at 0.25 mg/mL for intravenous formulation. Pharmacokinetic parameters were estimated by non-compartmental model using WinNonlin 6.2.

Plasma protein binding assays. Plasma protein binding was determined by equilibrium dialysis using the Rapid Equilibrium Dialysis (RED) device (Pierce Biotechnology, Rockford, IL) for both human and mouse plasma. Each compound was prepared in duplicate at 5 µM in plasma (0.95% acetonitrile, 0.05% DMSO) and added to one side of the membrane (200 µL) with PBS pH 7.4 added to the other side (350 µL). Compounds were incubated at 37 ºC for 5 h with a 350-rpm orbital shaker. After incubation, samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer.

Microsomal stability assays. Microsomal stability was determined at 37 °C at 60 minutes in both human and mouse microsomes. Each compound was prepared in duplicate at 1 µM with 0.3 mg/mL microsomes in PBS pH 7.4 (1% DMSO). Compounds were incubated at 37 °C for 60 minutes with a 350 rpm orbital shake with time points taken at 0 minutes and 60 minutes. Samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by selected ion recording detection on a single quadrupole mass spectrometer.

Recombinant production of human and *Plasmodium falciparum* DHODH. *Hs*DHODH[1] and *Pf*DHODH[2] were obtained as previously described.

Human and *Plasmodium falciparum* DHODH activity assay. Inhibition assays were performed as previously described.[1,2]

Competition experiments with decylubiquinone. The experiments were performed in a 96-well microplate reader, containing the assay buffer: 60 µM DCIP, 50 mM Tris pH 8.15, 150 mM KCl, 0.1% Triton X-100, 500 µM L-DHO, CoQ_D varying from 6.25 to 100 µM, and compound **27** concentrations varying from 11.7 to 6000 nM (serial dilution). Stock enzyme buffer was composed of 50 mM HEPES pH 7.7, 400 mM NaCl, 10 % glycerol and 0.05% Thesit. To start the reaction, 200 µL assay buffer was added in 5 µL *Pf*DHODH (1.6 µM in enzyme buffer), to a final enzyme concentration of 40 nM. As a blank, 200 µL assay buffer was added in 5 µL enzyme buffer without the presence of enzyme. Additionally, 200 µL buffer was added in 5 µL *Pf*DHODH, without the presence of the inhibitors to determine the activity for each substrate concentration. The reactions were monitored every 3 s over a period of 60 s, in triplicate, for each concentration and each tested compound. The inhibition mechanism was determined through the graph of IC_{50} versus CoQ_D concentration using linear data fitting obtained in the software Origin 2016.

Chemistry Experimental Procedures.

General Chemistry

Oxygen and/or moisture sensitive reactions were carried out in oven or flame-dried glassware under nitrogen atmosphere. All reagents and solvents were purchased and used as received from commercial vendors or synthesized according to cited procedures. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Flash chromatography was performed using 20-40 μm silica gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. Analytical thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60-F plates and visualized by UV light (254 nm) and/or iodine vapor.

NMR spectra were recorded on Bruker 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers. NMR solvents were purchased from Cambridge Isotope Laboratories, Inc., and NMR data were collected in $CDCl_3$ at ambient temperature unless otherwise noted. Chemical shifts are reported in parts per million (δ, ppm) relative to $CDCl_3$ (^{1}H, 7.26; ^{13}C, 77.16) or MeOD-d_4 (^{1}H, 3.31; ^{13}C, 49.00). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (br = broad, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (J) in Hz, and integration. Purity was measured by LC-MS on a Waters 2795 separations module by UV absorbance at 210 nm and identity was determined on a SQ mass spectrometer by positive $(M+H)^+$ or negative $(M-H)^-$ electrospray ionization. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.5, 5.0, or 7.5 minutes at 1.75 mL/min. An Agilent Poroshell 120 ECC18, 2.7 um, 3.0x30 mm column was used with column temperature maintained at 40 °C.

(2S,3S,4S)-1-allyl-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (1) was prepared according to the method of Lowe *et al*.[3]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.43 – 7.23 (m, 16H), 7.20 – 7.15 (m, 2H), 5.77 (dddd, J = 17.2, 10.3, 7.0, 5.1 Hz, 1H), 5.34 (d, J = 16.8 Hz, 1H), 5.18 (dq, J = 10.2, 1.3 Hz, 1H), 4.64 (d, J = 6.7 Hz, 1H), 3.88 – 3.74 (m, 2H), 3.53 (ddt, J = 13.8, 5.2, 1.7 Hz, 1H), 3.41 – 3.20 (m, 3H). Molecular formula: $C_{33}H_{29}BrN_2O$. Calculated mass: 548.15. ESIMS m/z 549.0 $(M+H)^+$.

(2S,3S,4S)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (2) was prepared according to the method of Kato *et al*.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.44 – 7.38 (m, 6H), 7.34 – 7.23 (m, 9H), 7.21 – 7.15 (m, 2H), 5.77 (dddd, J = 17.2, 10.3, 7.0, 5.1 Hz, 1H), 5.34 (dq, J = 17.2, 1.6 Hz, 1H), 5.23 – 5.15 (m, 1H), 4.64 (d, J = 6.7 Hz, 1H), 3.87 – 3.77 (m, 2H), 3.53 (ddt, J = 13.8, 5.2, 1.7 Hz, 1H), 3.39 – 3.31 (m, 2H), 3.31 – 3.24 (m, 1H). Molecular formula: $C_{30}H_{25}BrN_2O$. Calculated mass: 508.12. ESIMS m/z 553.4 $(M+HCOO^-)^-$.

(2S,3S,4S)-3-(4-bromophenyl)-2-cyano-N-propyl-4-((trityloxy)methyl)azetidine-1-carboxamide (3) was prepared according to the method of Kato *et al*.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 7.8 Hz, 2H), 7.20 (d, J = 7.7 Hz, 2H), 6.54 (s, 1H), 4.97 (d, J = 8.4 Hz, 1H), 4.89 – 4.70 (m, 1H), 4.70 – 4.61 (m, 1H), 3.96 – 3.62 (m, 3H), 3.13 (m, 2H), 1.57 – 1.40 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). Molecular formula: $C_{34}H_{32}BrN_3O_2$. Calculated mass: 593.17. ESIMS m/z 638.8 (M+ $HCOO^-$)$^-$.

(2S,3S,4S)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (BRD7539) was prepared according to the method of Kato *et al*.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 7.7 Hz, 2H), 7.38 – 7.28 (m, 4H), 7.21 (dd, J = 9.5, 3.0 Hz, 1H), 7.04 (dq, J = 7.8, 3.7 Hz, 1H), 6.27 (s, 1H), 5.01 (d, J = 8.4 Hz, 1H), 4.74 (t, J = 7.8 Hz, 1H), 4.02 (s, 1H), 3.92 (d, J = 10.9 Hz, 1H), 3.88 – 3.80 (m, 1H), 3.74 (t, J = 8.1 Hz, 1H), 3.18 (m, 2H), 1.51 (d, J = 7.2 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 163.73, 161.28, 158.06, 134.35, 132.25, 130.10 (d, J = 8.6 Hz), 129.48, 128.51, 127.66 (d, J = 3.0 Hz), 124.92 (d, J = 9.5 Hz), 123.45, 118.62, 118.39, 116.05, 115.79 (d, J = 10.1 Hz), 89.59, 89.29 (d, J = 3.4 Hz), 68.12, 65.81, 52.73, 42.29, 39.54, 29.82, 23.27, 11.46. ESIMS m/z 392 $(M+H)^+$. HRMS (ESI) calculated for $C_{23}H_{22}FN_3O_2$ $[M+H]^+$: 392.1774. Found: 392.1781.

General Protocol 1 for addition of R_1 group: Heck alkynylation.

Aryl bromide **3** (1.0 equiv.) and appropriate alkyne (5.0 equiv.) were dissolved in degassed MeCN (0.1 M). XPhos Pd-G3 (0.15 equiv.) and Et_3N (20 equiv.) were added in sequence, and the reaction was heated to 70 ^oC for 2-3 hours or until completion. The reaction was then cooled to ambient temperature, diluted with pH 7 buffer, and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM).

General Protocol 2 for addition of R_1 group: Suzuki coupling.

Aryl bromide **3** (1.0 equiv.) and appropriate boronic acid (3.0 equiv.) were dissolved in degassed 2:1 THF/0.5 M K_3PO_4 (0.17 M) before the addition of XPhos Pd-G3 (0.15 equiv). The reaction was heated to 50 ^oC for 2-5 hours or until completion, at which point the mixture was cooled to ambient temperature, diluted with pH 7 buffer, and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM).

Characterization

(2S,3S,4S)-2-cyano-3-(4-((2-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (4). Prepared from **3** following **General Protocol 1** (41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.7 Hz, 2H), 7.51 (td, *J* = 7.4, 1.8 Hz, 1H), 7.39 – 7.28 (m, 3H), 7.12 (q, *J* = 8.5 Hz, 2H), 5.03 (d, *J* = 8.3 Hz, 1H), 4.76 (t, *J* = 7.3 Hz, 1H), 3.98 – 3.91 (m, 1H), 3.85 (dd, *J* = 11.4, 7.6 Hz, 1H), 3.76 (d, *J* = 8.1 Hz, 1H), 3.21 (dtd, *J* = 27.1, 13.4, 7.0 Hz, 2H), 1.54 (p, *J* = 7.0 Hz, 2H), 0.99 – 0.86 (m, 3H). Molecular formula: $C_{23}H_{22}FN_3O_2$. Calculated mass: 391.17. ESIMS m/z 392.5 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4-((4-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (5). Prepared from **3** following **General Protocol 1** (34% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 – 7.46 (m, 4H), 7.40 – 7.29 (m, 2H), 7.10 – 6.99 (m, 2H), 6.14 (t, J = 5.6 Hz, 1H), 5.01 (d, J = 8.5 Hz, 1H), 4.74 (td, J = 8.0, 2.2 Hz, 1H), 3.97 – 3.69 (m, 3H), 3.31 – 3.06 (m, 2H), 1.54 (p, J = 7.3 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{22}FN_3O_2$. Calculated mass: 391.17. ESIMS m/z 392.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(4-(phenylethynyl)phenyl)-N-propylazetidine-1-carboxamide (6). Prepared from **3** following **General Protocol 1** (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.54 (m, 2H), 7.54 – 7.50 (m, 2H), 7.39 – 7.29 (m, 5H), 6.08 (t, *J* = 5.6 Hz, 1H), 5.00 (d, *J* = 8.5 Hz, 1H), 4.74 (td, *J* = 8.2, 2.2 Hz, 1H), 4.00 – 3.78 (m, 3H), 3.74 (t, *J* = 8.1 Hz, 1H), 3.30 – 3.08 (m, 2H), 1.54 (h, *J* = 7.3 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{23}N_3O_2$. Calculated mass: 373.18. ESIMS m/z 374.8 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-3-ylethynyl)phenyl)azetidine-1-carboxamide (7). Prepared from **3** following **General Protocol 1**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.57 (d, J = 4.9 Hz, 1H), 7.90 (dt, J = 8.0, 1.8 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.38 (dd, J = 8.0, 4.5 Hz, 3H), 5.58 (s, 1H), 5.05 (d, J = 8.5 Hz, 1H), 4.77 (t, J = 7.2 Hz, 1H), 3.99 – 3.74 (m, 4H), 3.24 (ddd, J = 13.9, 12.2, 6.7 Hz, 2H), 1.58 (p, J = 7.2 Hz, 4H), 0.98 – 0.91 (m, 3H). Molecular formula: $C_{22}H_{22}N_4O_2$. Calculated mass: 374.17. ESIMS m/z 375.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-2-ylethynyl)phenyl)azetidine-1-carboxamide (8). Prepared from **3** following **General Protocol 1**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (dt, *J* = 5.0, 1.3 Hz, 1H), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H), 7.56 (td, *J* = 8.1, 1.4 Hz, 3H), 7.36 – 7.27 (m, 3H), 6.28 (t, *J* = 5.6 Hz, 1H), 5.01 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.2, 2.1 Hz, 1H), 3.98 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.85 (dd, *J* = 11.4, 8.4 Hz, 1H), 3.74 (t, *J* = 8.2 Hz, 1H), 3.30 – 3.11 (m, 2H), 1.54 (h, *J* = 7.3 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}N_4O_2$. Calculated mass: 374.17. ESIMS m/z 375.2 $(M+H)^+$.

(2S,3S,4S)-3-(4-((3-chlorophenyl)ethynyl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (9). Prepared from **3** following **General Protocol 1** (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 2H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.33 (dt, *J* = 7.2, 1.5 Hz, 1H), 7.31 – 7.18 (m, 4H), 5.76 (t, *J* = 5.8 Hz, 1H), 4.95 (d, *J* = 8.5 Hz, 1H), 4.68 (td, *J* = 8.0, 2.1 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.78 (dd, *J* = 11.4, 8.1 Hz, 1H), 3.69 (t, *J* = 8.1 Hz, 1H), 3.24 – 3.04 (m, 2H), 1.46 (d, *J* = 7.2 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{22}ClN_3O_2$. Calculated mass: 407.14. ESIMS m/z 408.3 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4-((3,4-difluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (10). Prepared from **3** following **General Protocol 1** (42% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.52 (m, 2H), 7.42 – 7.30 (m, 3H), 7.25 (dd, *J* = 3.8, 1.8 Hz, 1H), 7.14 (dt, *J* = 10.3, 8.3 Hz, 1H), 5.84 (s, 1H), 5.03 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.0, 2.1 Hz, 1H), 3.94 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.86 (dd, *J* = 11.4, 8.1 Hz, 1H), 3.77 (t, *J* = 8.1 Hz, 1H), 3.21 (dtd, *J* = 27.4, 13.4, 7.0 Hz, 2H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{21}F_2N_3O_2$. Calculated mass: 409.16. ESIMS m/z 410.7 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4-((E)-3-fluorostyryl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (11). Prepared from **3** following **General Protocol 2** (45% yield). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.62 – 7.56 (m, 2H), 7.42 – 7.37 (m, 2H), 7.37 – 7.29 (m, 2H), 7.25 (ddd, *J* = 10.5, 2.5, 1.4 Hz, 1H), 7.14 (s, 2H), 6.98 (dddd, *J* = 8.7, 7.5, 2.6, 1.5 Hz, 1H), 5.94 – 5.82 (m, 1H), 5.01 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.1, 2.3 Hz, 1H), 3.97 – 3.81 (m, 2H), 3.77 (t, *J* = 8.1 Hz, 1H), 3.45 – 3.36 (m, 1H), 3.20 (qtd, *J* = 13.0, 7.0, 5.6 Hz, 2H), 1.54 (p, *J* = 7.2 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{24}FN_3O_2$. Calculated mass: 393.19. ESIMS m/z 394.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4-(3-fluorophenethyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (12). To a solution of **BRD7539** (12.0 mg, 0.031 mmol) in MeOH (310 µL) was added 10% Pd/C (w/w). The atmosphere was purged 3×15 seconds with a H_2 balloon, and the reaction was maintained under a H_2 atmosphere for 16 hours. Upon completion, the reaction mixture was filtered over Celite to afford pure product as a white solid (11.6 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.20 (m, 4H), 7.00 – 6.85 (m, 3H), 5.95 (d, *J* = 5.9 Hz, 1H), 5.03 (d, *J* = 8.5 Hz, 1H), 4.78 (t, *J* = 7.5 Hz, 1H), 4.00 – 3.84 (m, 3H), 3.75 (t, *J* = 8.2 Hz, 1H), 3.34 – 3.13 (m, 2H), 2.94 (s, 4H), 1.58 (h, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{26}FN_3O_2$. Calculated mass: 395.20. ESIMS m/z 396.9 $(M+H)^+$.

(2S,3S,4S)-3-([1,1'-biphenyl]-4-yl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (13). Prepared from **3** following **General Protocol 2** (87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.61 (m, 2H), 7.61 – 7.55 (m, 2H), 7.48 – 7.40 (m, 4H), 7.40 – 7.34 (m, 1H), 5.84 (s, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.80 (td, *J* = 8.0, 2.2 Hz, 1H), 3.95 (dd, *J* = 11.4, 2.2 Hz, 1H), 3.86 (dd, *J* = 11.4, 8.2 Hz, 1H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.21 (ddt, *J* = 27.6, 13.5, 6.8 Hz, 3H), 1.56 (q, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{23}N_3O_2$. Calculated mass: 349.18. ESIMS m/z 350.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(3'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (14). Prepared from **3** following **General Protocol 2** (57% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.51 – 7.33 (m, 4H), 7.29 (t, *J* = 5.4 Hz, 1H), 7.11 – 7.02 (m, 1H), 5.67 – 5.56 (m, 1H), 5.05 (d, *J* = 8.4 Hz, 1H), 4.80 (t, *J* = 7.1 Hz, 1H), 4.01 – 3.93 (m, 1H), 3.88 (dd, *J* = 11.4, 7.9 Hz, 1H), 3.81 (t, *J* = 8.1 Hz, 1H), 3.24 (dp, *J* = 26.0, 6.5 Hz, 2H), 1.57 (h, *J* = 7.1 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. ESIMS m/z 368.6 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (15). Prepared from **3** following **General Protocol 2** (77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.51 – 7.33 (m, 4H), 7.29 (t, *J* = 5.4 Hz, 1H), 7.11 – 7.02 (m, 1H), 5.67 – 5.56 (m, 1H), 5.05 (d, *J* = 8.4 Hz, 1H), 4.80 (t, *J* = 7.1 Hz, 1H), 4.01 – 3.93 (m, 1H), 3.88 (dd, *J* = 11.4, 7.9 Hz, 1H), 3.81 (t, *J* = 8.1 Hz, 1H), 3.24 (dp, *J* = 26.0, 6.5 Hz, 2H), 1.57 (h, *J* = 7.1 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. ESIMS m/z 368.6 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(2'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (16). Prepared from **3** following **General Protocol 2** (80% yield). ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.63 (d, *J* = 7.6 Hz, 2H), 7.47 (d, *J* = 7.4 Hz, 3H), 7.41 – 7.31 (m, 1H), 7.24 (t, *J* = 7.4 Hz, 1H), 7.18 (dd, *J* = 10.9, 8.0 Hz, 1H), 5.74 (s, 1H), 5.08 (d, *J* = 8.4 Hz, 1H), 4.89 – 4.77 (m, 1H), 3.98 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.89 (dd, *J* = 11.5, 8.0 Hz, 1H), 3.82 (t, *J* = 8.1 Hz, 1H), 3.25 (dt, *J* = 23.8, 8.8 Hz, 2H), 1.66 – 1.52 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. ESIMS m/z 368.8 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-phenyl-N-propylazetidine-1-carboxamide (17). To a flask containing aryl bromide **3** (12 mg, 0.034 mmol), 10% Pd/C (w/w), and sodium bicarbonate (17 mg, 0.204 mmol) was added a solution of MeOH (340 µL). The atmosphere was purged 3×15 seconds with a H_2 balloon, and the reaction was maintained under a H_2 atmosphere for 2 hours. The reaction was then filtered over Celite and purified *via* flash column chromatography to afford product as a colorless residue (4.2 mg, 46%). ^{1}H NMR (400 MHz, MeOD) δ 7.42 (d, *J* = 4.4 Hz, 4H), 7.34 (ddd, *J* = 8.5, 5.1, 3.8 Hz, 1H), 5.14 (d, *J* = 8.6 Hz, 1H), 4.67 (td, *J* = 6.9, 2.7 Hz, 1H), 3.98 (dd, *J* = 8.6, 7.2 Hz, 1H), 3.91 (dd, *J* = 11.8, 2.7 Hz, 1H), 3.74 (dd, *J* = 11.8, 6.4 Hz, 1H), 3.21 (dt, *J* = 13.7, 7.0 Hz, 1H), 3.11 (dt, *J* = 13.3, 6.9 Hz, 1H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{15}H_{19}N_3O_2$. Calculated mass: 273.15. ESIMS m/z 274.1 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(4'-phenoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (18). Prepared from **3** following **General Protocol 2**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 2H), 7.58 – 7.52 (m, 2H), 7.46 – 7.33 (m, 4H), 7.19 – 7.01 (m, 5H), 5.70 (s, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.80 (td, *J* = 7.9, 2.2 Hz, 1H), 4.00 – 3.73 (m, 3H), 3.22 (s, 2H), 1.56 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{27}H_{27}N_3O_3$. Calculated mass: 441.41. ESIMS m/z 442.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(4-(1-phenyl-1H-pyrazol-4-yl)phenyl)-N-propylazetidine-1-carboxamide (19). Prepared from **3** following **General Protocol 2** (31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.00 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 7.7 Hz, 2H), 7.50 (q, *J* = 8.0, 6.9 Hz, 2H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 5.52 (s, 1H), 5.04 (d, *J* = 8.3 Hz, 1H), 4.79 (t, *J* = 7.6 Hz, 1H), 4.00 – 3.91 (m, 1H), 3.87 (dd, *J* = 11.5, 7.9 Hz, 1H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.35 – 3.15 (m, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{24}H_{25}N_5O_2$. Calculated mass: 415.20. ESIMS m/z 417.6 $(M+2H)^{2+}$.

(2S,3S,4S)-3-(4-(1H-indol-2-yl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (20). Prepared from **3** following **General Protocol 2**. ^{1}H NMR (300 MHz, MeOD) δ 7.53 (dd, *J* = 8.4, 2.5 Hz, 2H), 7.31 – 7.24 (m, 1H), 7.17 – 6.96 (m, 4H), 6.86 (ddt, *J* = 8.4, 6.8, 1.4 Hz, 1H), 6.75 (tdd, *J* = 8.7, 5.6, 3.4 Hz, 1H), 6.58 – 6.53 (m, 1H), 4.00 (dt, *J* = 8.0, 3.9 Hz, 1H), 3.96 – 3.86 (m, 1H), 3.79 – 3.59 (m, 3H), 3.01 – 2.88 (m, 2H), 2.75 (ddd, *J* = 11.0, 8.3, 5.7 Hz, 2H), 1.01 – 0.92 (m, 2H), 0.31 (td, *J* = 6.8, 6.2, 2.1 Hz, 3H). Molecular formula: $C_{23}H_{24}N_4O_2$. Calculated mass: 388.19. ESIMS m/z 389.2 $(M+H)^+$.

(2S,3S,4S)-3-(4-(benzofuran-2-yl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (21). Prepared from **3** following **General Protocol 2** (80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.0 Hz, 2H), 7.63 – 7.57 (m, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.45 (d, *J*

= 8.1 Hz, 2H), 7.34 – 7.27 (m, 1H), 7.23 (d, *J* = 7.3 Hz, 1H), 7.06 (d, *J* = 0.9 Hz, 1H), 5.51 (s, 1H), 5.05 (d, *J* = 8.5 Hz, 1H), 4.85 – 4.76 (m, 1H), 4.00 – 3.77 (m, 4H), 3.25 (tq, *J* = 13.3, 6.5 Hz, 2H), 1.57 (q, *J* = 7.3 Hz, 3H), 0.96 (dd, *J* = 7.9, 6.9 Hz, 3H). Molecular formula: $C_{23}H_{23}N_3O_2$. Calculated mass: 389.19. ESIMS m/z 390.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-4-yl)phenyl)azetidine-1-carboxamide (22). Prepared from **3** following **General Protocol 2** (80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 – 8.63 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.60 – 7.47 (m, 4H), 5.67 (s, 1H), 5.07 (d, *J* = 8.5 Hz, 1H), 4.85 – 4.75 (m, 1H), 4.02 – 3.78 (m, 4H), 3.25 (dp, *J* = 18.9, 6.3 Hz, 2H), 1.57 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{20}H_{22}N_4O_2$. Calculated mass: 350.17. ESIMS m/z 351.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (23). Prepared from **3** following **General Protocol 2** (43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.98 (m, 2H), 7.82 – 7.73 (m, 2H), 7.69 – 7.63 (m, 2H), 7.53 – 7.45 (m, 2H), 5.64 (t, *J* = 5.7 Hz, 1H), 5.07 (d, *J* = 8.6 Hz, 1H), 4.80 (td, *J* = 7.9, 2.2 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.93 – 3.87 (m, 1H), 3.87 – 3.79 (m, 1H), 3.48 (t, *J* = 3.4 Hz, 1H), 3.33 – 3.16 (m, 2H), 3.10 (s, 3H), 1.57 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{23}N_3O_2S$. Calculated mass: 427.16. ESIMS m/z 428.1 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(2'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (24). Prepared from **3** following **General Protocol 2** (32% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.72 (m, 1H), 7.61 – 7.54 (m, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.40 (s, 4H), 7.34 (d, *J* = 7.6 Hz, 1H), 5.87 (s, 1H), 5.10 (d, *J* = 8.5 Hz, 1H), 4.84 (td, *J* = 7.9, 2.0 Hz, 1H), 4.04 (dd, *J* = 11.6, 2.1 Hz, 1H), 3.92 (dd, *J* = 11.4, 8.2 Hz, 1H), 3.84 (t, *J* = 8.1 Hz, 1H), 3.24 (tp, *J* = 13.4, 7.0 Hz, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. ESIMS m/z 462.3 $(M+HCOO^-)^-$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (25). Prepared from **3** following **General Protocol 2** (59% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.70 – 7.44 (m, 7H), 5.61 (t, *J* = 5.5 Hz, 1H), 5.06 (d, *J* = 8.5 Hz, 1H), 4.81 (t, *J* = 7.7 Hz, 1H), 4.04 – 3.94 (m, 1H), 3.94 – 3.87 (m, 1H), 3.83 (q, *J* = 7.9 Hz, 1H), 3.23 (dh, *J* = 25.7, 6.4 Hz, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. ESIMS m/z 418.8 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (26). Prepared from **3** following **General Protocol 2** (35% yield).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 2H), 7.68 – 7.61 (m, 2H), 7.52 – 7.45 (m, 2H), 5.63 (s, 1H), 5.06 (d, *J* = 8.6 Hz, 1H), 4.81 (td, *J* = 7.8, 2.2 Hz, 1H), 4.02 – 3.78 (m, 3H), 3.35 – 3.15 (m, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. ESIMS m/z 418.5 $(M+H)^+$.

(2S,3S,4S)-3-(3',4'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (27, BRD9185). Prepared from **3** following **General Protocol 2** (84% yield). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.08 (d, *J* = 1.6 Hz, 2H), 7.90 (s, 1H), 7.72 (d, *J* =

8.4 Hz, 2H), 7.56 – 7.51 (m, 2H), 5.98 (s, 1H), 5.07 (d, *J* = 8.6 Hz, 1H), 4.79 (td, *J* = 8.0, 2.2 Hz, 1H), 3.98 – 3.82 (m, 3H), 3.20 (qq, *J* = 12.8, 6.6 Hz, 2H), 1.56 (p, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DCM-*d*2) δ 157.72, 142.39, 138.36, 135.04 (d, J = 7.3 Hz), 132.12, 131.79, 130.79, 129.31, 127.44 (d, J = 26.5 Hz), 124.78, 122.07, 121.66 – 121.08 (m), 115.75, 67.89, 65.81, 53.33, 53.13, 52.86, 52.55, 42.11, 39.13, 23.21, 11.07. HRMS (ESI) calcd for $C_{23}H_{21}F_6N_3O_2$ $[M+H]^+$: 486.1608. Found: 486.1618.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(2'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (28). Prepared from **3** following **General Protocol 2** (70% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 8.5 Hz, 2H), 7.09 – 6.94 (m, 2H), 5.74 – 5.62 (m, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.87 – 4.73 (m, 1H), 3.94 (d, *J* = 12.4 Hz, 1H), 3.90 – 3.84 (m, 1H), 3.81 (s, 4H), 3.24 (dh, *J* = 25.6, 6.5 Hz, 2H), 1.56 (h, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. ESIMS m/z 380.8 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(3'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (29). Prepared from **3** following **General Protocol 2** (58% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 7.7 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.35 (q, *J* = 6.7, 5.6 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 3.0 Hz, 1H), 6.91 (dd, *J* = 8.4, 2.9 Hz, 1H), 5.71 – 5.53 (m, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.86 – 4.76 (m, 1H), 3.95 (d, *J* = 10.8 Hz, 1H), 3.87 (s, 4H), 3.79 (t, *J* = 8.1 Hz, 1H), 3.23 (dh, *J* = 27.1, 7.2, 6.7 Hz, 2H), 1.56 (h, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. ESIMS m/z 379.6 $(M+H)^+$.

(2S,3S,4S)-2-cyano-4-(hydroxymethyl)-3-(4'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (30). Prepared from **3** following **General Protocol 2** (61% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 7.7 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 5.71 – 5.62 (m, 1H), 5.03 (d, *J* = 8.4 Hz, 1H), 4.79 (td, *J* = 8.0, 7.4, 2.6 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.86 (d, *J* = 4.1 Hz, 4H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.63 (d, *J* = 31.9 Hz, 1H), 3.23 (dp, *J* = 26.3, 6.5 Hz, 2H), 1.56 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. ESIMS m/z 380.2 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4'-cyclopropyl-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (31). Prepared from **3** following **General Protocol 2** (31% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.59 (m, 2H), 7.51 – 7.46 (m, 2H), 7.44 – 7.39 (m, 2H), 7.17 – 7.13 (m, 2H), 5.51 (t, *J* = 5.7 Hz, 1H), 5.04 (d, *J* = 8.6 Hz, 1H), 4.80 (td, *J* = 8.0, 2.1 Hz, 1H), 3.95 (dd, *J* = 11.2, 4.5 Hz, 1H), 3.91 – 3.82 (m, 1H), 3.79 (t, *J* = 8.1 Hz, 1H), 3.45 (s, 1H), 3.32 – 3.17 (m, 2H), 1.94 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.62 – 1.51 (m, 3H), 1.02 – 0.98 (m, 2H), 0.95 (td, *J* = 7.4, 4.1 Hz, 4H), 0.74 (dt, *J* = 6.7, 4.7 Hz, 2H). Molecular formula: $C_{24}H_{27}N_3O_2$. Calculated mass: 389.21. ESIMS m/z 390.4 $(M+H)^+$.

(2S,3R,4S)-methyl 3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-1-(propylcarbamoyl)azetidine-2-carboxylate (32). To a vial containing MeOH (80 µL, 0.1 M) at 0 ^{o}C was added acetyl chloride (6.5 µL, 0.92 mmol) and stirred for 10 min. The resulting solution was transferred into a secondary vial containing **BRD7539** (3 mg, 0.008 mmol). The

reaction was stirred for 22 hours at ambient temperature, after which point the temperature was raised to 50 ^{o}C. Three hours later, the solvent was removed *in vacuo* and crude residue purified *via* flash column chromatography (MeOH/DCM) to afford product as white, waxy residue (2.4 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.45 (m, 2H), 7.33 – 7.28 (m, 2H), 7.28 – 7.19 (m, 3H), 7.05 (ddq, *J* = 11.0, 6.0, 3.2, 2.6 Hz, 1H), 5.45 (d, *J* = 6.1 Hz, 1H), 4.96 (dd, *J* = 9.3, 1.7 Hz, 1H), 4.82 – 4.74 (m, 1H), 4.38 – 4.26 (m, 1H), 3.98 – 3.88 (m, 2H), 3.71 – 3.63 (m, 1H), 3.35 (d, *J* = 1.7 Hz, 3H), 3.24 (dtd, *J* = 13.1, 6.6, 6.1, 1.9 Hz, 1H), 3.19 – 3.08 (m, 1H), 1.53 (qd, *J* = 7.4, 1.7 Hz, 2H), 0.93 (td, *J* = 7.4, 1.7 Hz, 3H). Molecular formula: $C_{24}H_{25}FN_2O_4$. Calculated mass: 424.18. ESIMS m/z 425.3 $(M+H)^{+}$.

(2S,4S)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-2,4-bis(hydroxymethyl)-N-propylazetidine-1-carboxamide (33). To a solution of methyl ester **32** (10 mg, 0.024 mmol) in THF (200 µL) was added $LiBH_4$ (1.3 mg, 0.059 mmol). The reaction was heated to 40 ^{o}C for 3 hours, after which point one drop of water was added to quench the reaction. The mixture was extracted 3x with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue (4.5 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.9 Hz, 2H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 2H), 7.05 (tq, *J* = 10.4, 3.8, 3.1 Hz, 1H), 6.55 (d, *J* = 4.7 Hz, 1H), 4.74 (t, *J* = 7.5 Hz, 1H), 4.56 (td, *J* = 9.8, 2.0 Hz, 1H), 3.97 (t, *J* = 10.2 Hz, 1H), 3.84 – 3.77 (m, 1H), 3.60 (t, *J* = 10.6 Hz, 1H), 3.46 (dd, *J* = 9.5, 6.3 Hz, 1H), 3.27 – 3.20 (m, 1H), 3.14 (dq, *J* = 16.5, 6.7 Hz, 2H), 1.56 – 1.46 (m, 2H), 0.92 (td, *J* = 7.6, 2.7 Hz, 3H). Molecular formula: $C_{23}H_{25}FN_2O_3$. Calculated mass: 396.18. ESIMS m/z 397.6 $(M+H)^{+}$.

(2S,3S,4S)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(methoxymethyl)-N-propylazetidine-1-carboxamide (34). To a solution of **BRD7539** (26 mg, 0.066 mmol) in DCM (266 µL) was added 1,8-bis(dimethylamino)naphthalene (28 mg, 0.133 mmol) and trimethyloxonium tetrafluoroborate (12 mg, 0.078 mmol). The mixture was stirred for ambient temperature for 16 hours. The solvent was then removed *in vacuo* and the crude residue was purified by flash column chromatography (MeOH/DCM) to afford product as a pale yellow residue (8.2 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.54 (m, 2H), 7.38 – 7.28 (m, 4H), 7.25 – 7.20 (m, 1H), 7.05 (dtd, *J* = 9.1, 4.8, 2.6 Hz, 1H), 6.23 (s, 1H), 4.96 (d, *J* = 8.4 Hz, 1H), 4.71 (td, *J* = 7.9, 2.6 Hz, 1H), 3.78 – 3.60 (m, 3H), 3.44 (s, 3H), 3.33 – 3.21 (m, 1H), 3.21 – 3.12 (m, 1H), 1.53 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{24}H_{24}FN_3O_2$. Calculated mass: 405.19. ESIMS m/z 406.2 $(M+H)^{+}$.

(2S,3R,4S)-2-(azidomethyl)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (35). To a solution of **BRD7539** (11 mg, 0.028 mmol) in THF (300 µL) was added DBU (22 µL, 0.225 mmol), then diphenylphosphoryl azide (30 µL, 0.141 mmol). The mixture was stirred at ambient temperature for 16 hours. The reaction solvent was then removed in vacuo, and crude material purified briefly via flash column chromatography (MeOH/DCM) to afford *intermediate ((2S,3S,4S)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl diphenyl phosphate.* The intermediate was dissolved in DMF (300 µL), and NaN_3 (8.9 mg, 0.137 mmol) was added in one portion. The mixture was stirred at 90 ^{o}C for 3 hours, after which point the reaction was

quenched with H_2O. The resulting mixture was extracted 3x with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a pale yellow solid (8.3 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.28 (m, 2H), 7.25 – 7.19 (m, 1H), 7.09 – 7.02 (m, 1H), 5.09 (d, *J* = 8.5 Hz, 1H), 5.01 (t, *J* = 5.8 Hz, 1H), 4.64 (ddd, *J* = 6.5, 4.7, 3.0 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.90 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.56 (dd, *J* = 13.1, 4.7 Hz, 1H), 3.34 – 3.16 (m, 2H), 1.59 (dt, *J* = 14.6, 7.5 Hz, 2H), 0.99 – 0.92 (m, 3H). Molecular formula: $C_{23}H_{21}FN_6O$. Calculated mass: 416.18. ESIMS m/z 417.7 $(M+H)^+$.

(2S,3R,4S)-2-(aminomethyl)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (36). To a solution of azide **35** (6.7 mg, 0.016 mmol) in DCM (300 µL) was added triphenylphosphine (7.0 mg, 0.024 mmol). The mixture was stirred at ambient temperature for 4 hours, after which point 2 mL of H_2O and 1 mL of THF were added to the reaction. The reaction was then heated to 60 ^{o}C for 24 hours. Upon completion, solvent was removed *in vacuo* and crude residue purified by flash column chromatography (MeOH/DCM) to afford product as colorless solid (2.6 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.61 – 7.53 (m, 2H), 7.42 – 7.29 (m, 4H), 7.24 – 7.19 (m, 1H), 7.05 (ddt, *J* = 9.2, 8.1, 3.2 Hz, 1H), 4.94 (d, *J* = 8.4 Hz, 1H), 4.49 (ddd, *J* = 9.9, 8.2, 1.8 Hz, 1H), 3.69 (t, *J* = 8.2 Hz, 1H), 3.21 (dddd, *J* = 18.6, 16.8, 9.8, 4.0 Hz, 3H), 2.94 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.66 – 1.50 (m, 5H), 0.98 – 0.89 (m, 3H). Molecular formula: $C_{23}H_{23}FN_4O$. Calculated mass: 390.19. ESIMS m/z 391.9 $(M+H)^+$.

((2S,3S,4S)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl acetate (37). To a solution of **BRD7539** (6 mg, 0.015 mmol) in DCM (300 µL) was added pyridine (1.6 µL, 0.020 mmol) and acetyl chloride (1.4 µL, 0.018 mmol) in sequence. The reaction was stirred at ambient temperature for 30 min. Upon completion, the solvent was removed *in vacuo* and crude residue purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue (5.2 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.2 Hz, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.28 (m, 2H), 7.25 – 7.20 (m, 1H), 7.15 – 7.02 (m, 1H), 5.05 (dd, *J* = 7.5, 4.6 Hz, 2H), 4.71 (ddd, *J* = 7.0, 5.4, 3.1 Hz, 1H), 4.59 (dd, *J* = 12.3, 3.3 Hz, 1H), 4.23 (dd, *J* = 12.3, 5.4 Hz, 1H), 3.89 (dd, *J* = 8.5, 7.0 Hz, 1H), 3.35 – 3.14 (m, 2H), 2.12 (s, 3H), 1.69 – 1.51 (m, 3H), 0.96 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{25}H_{24}FN_3O_3$. Calculated mass: 433.18. ESIMS m/z 435.0 $(M+2H)^{2+}$.

(2S,3R,4R)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-methyl-N-propylazetidine-1-carboxamide (38). To a solution of **40** (6 mg, 0.011 mmol) in DMSO (220 µL) was added $NaBH_4$ (1.6 mg, 0.044 mmol). The reaction was heated to 70 ^{o}C and stirred for 1 hour. Upon completion, the mixture was quenched with H_2O and extracted 4x with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue (1.0 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 8.1, 5.9 Hz, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.29 (m, 2H), 7.22 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.09 – 7.02 (m, 1H), 5.11 (d, *J* = 8.5 Hz, 1H), 5.00 (d, *J* = 9.3 Hz, 1H), 4.89 (t, *J* = 8.0 Hz, 1H), 4.62 (p, *J* = 6.1 Hz, 1H), 4.45 (t, *J* = 8.7

Hz, 1H), 4.28 (t, *J* = 5.7 Hz, 1H), 3.75 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.62 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.48 (dd, *J* = 10.2, 2.0 Hz, 1H), 3.40 – 3.14 (m, 3H), 1.60 – 1.55 (m, 2H), 0.96 (td, *J* = 7.4, 4.1 Hz, 3H). Molecular formula: $C_{23}H_{22}FN_3O$. Calculated mass: 375.17. ESIMS m/z 376.0 $(M+H)^+$.

(2S,3R)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (39). Prepared from **45** following **General Protocol 1** (29% yield).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 – 7.56 (m, 2H), 7.47 – 7.41 (m, 2H), 7.35 – 7.29 (m, 2H), 7.25 – 7.18 (m, 1H), 7.11 – 7.00 (m, 1H), 5.19 (d, *J* = 8.3 Hz, 1H), 4.48 (t, *J* = 5.9 Hz, 1H), 4.30 (dd, *J* = 8.4, 7.2 Hz, 1H), 4.14 – 3.97 (m, 2H), 3.22 (dt, *J* = 7.8, 6.3 Hz, 2H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{20}FN_3O$. Calculated mass: 361.16. ESIMS m/z 362.5 $(M+H)^+$.

((2S,3S,4S)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl 4-methylbenzenesulfonate (40). To a solution of **BRD7539** (25 mg, 0.064 mmol) in pyridine (650 µL) was added 4-toluenesulfonyl chloride (18 mg, 0.096 mmol). The reaction was heated to 40 ^oC for 8 hours. Upon completion, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a pale yellow residue (17 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.74 (m, 2H), 7.60 – 7.54 (m, 2H), 7.41 – 7.28 (m, 6H), 7.24 – 7.19 (m, 1H), 7.05 (ddt, *J* = 9.3, 8.2, 3.3 Hz, 1H), 5.08 (d, *J* = 8.5 Hz, 1H), 4.81 (t, *J* = 5.7 Hz, 1H), 4.69 (ddd, *J* = 6.7, 4.1, 2.8 Hz, 1H), 4.50 (dd, *J* = 11.1, 2.9 Hz, 1H), 4.21 (dd, *J* = 11.1, 4.1 Hz, 1H), 3.98 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.28 – 3.07 (m, 2H), 2.46 (s, 3H), 1.52 (q, *J* = 7.3 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{30}H_{28}FN_3O_4S$. Calculated mass: 545.18. ESIMS m/z 546.3 $(M+H)^+$.

(S)-2-bromo-1-(4-bromophenyl)ethanol (41). To a solution of (*S*)-(−)-2-Methyl-CBS-oxazaborolidine (250 mg, 0.90 mmol) in THF (3 mL) was added borane tetrahydrofuran complex solution (9.0 mL, 1.0 M in THF). After 5 min, a solution of 2-bromo-1-(4-bromophenyl)ethanone (5.00 g, 17.99 mmol) in THF (15 mL) was added dropwise over 10 min. The reaction was stirred at ambient temperature for 30 min. Upon completion, the reaction was quenched with MeOH and solvent was removed *in vacuo*. Crude residue was purified by flash column chromatography (EtOAc/hexanes) to afford product as a white crystalline solid (4.51 g, 89%, 92% ee by chiral HPLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 2H), 4.91 (dd, *J* = 8.9, 3.5 Hz, 1H), 3.63 (dd, *J* = 10.6, 3.5 Hz, 1H), 3.51 (t, *J* = 9.7 Hz, 1H). Molecular formula: $C_8H_8Br_2O$. Calculated mass: 277.89. ESIMS m/z 278.7 $(M+H)^+$.

(S)-2-(allyl(2-(4-bromophenyl)-2-hydroxyethyl)amino)acetonitrile (42). To a flask containing alcohol **41** (1.00 g, 3.57 mmol) was added neat allylamine (5.35 mL, 71.4 mmol) and I_2 (54 mg, 0.36 mmol). The reaction was stirred at ambient temperature for 16 hours. Upon completion, allylamine was removed *in vacuo*. The resulting crude residue was dissolved in MeCN (18 mL), and bromoacetonitrile (746 µL, 10.71 mmol) and K_2CO_3 (740 mg, 5.36 mmol) were added in sequence. The reaction was heated at 85 ^oC for 40 hours. Upon completion, the solvent was removed *in vacuo* and the resulting crude residue was suspended in H_2O. The aqueous layer was extracted 3x with Et_2O, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a yellow crystalline solid (778 mg, 74% over two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 4.2 Hz, 2H), 5.81 (ddt, *J* = 16.9, 10.1, 6.5 Hz, 1H), 5.34 (dd, *J* = 21.8, 13.7 Hz, 2H), 4.74 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.68 (q, *J* = 17.4 Hz, 2H), 3.38 (dd, *J* = 13.6, 6.2 Hz, 1H), 3.27 – 3.16 (m, 2H), 2.82 (dd, *J* = 13.3, 3.4 Hz, 1H), 2.63 (dd, *J* = 13.3, 10.0 Hz, 1H). Molecular formula: $C_{13}H_{15}BrN_2O$. Calculated mass: 294.04. ESIMS m/z 296.5 $(M+H)^+$.

(S)-2-(allyl(2-(4-bromophenyl)-2-chloroethyl)amino)acetonitrile (43). To a solution of pyridine (1.06 mL, 13.09 mmol) in DCM (26 mL) was added thionyl chloride (380 µL, 5.24 mmol). The mixture was cooled to 0 ^oC, and a solution of alcohol **42** (773 mg, 2.62 mmol) in DCM (2 mL) was added in dropwise over ten minutes. After 15 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a yellow oil (572 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 4.1 Hz, 2H), 5.73 (ddt, *J* = 16.6, 9.8, 6.2 Hz, 1H), 5.37 – 5.21 (m, 2H), 4.86 (t, *J* = 7.0 Hz, 1H), 3.60 (d, *J* = 17.6 Hz, 1H), 3.49 (d, *J* = 17.6 Hz, 1H), 3.23 (dd, *J* = 6.6, 2.9 Hz, 2H), 3.15 (dd, *J* = 14.1, 7.5 Hz, 1H), 3.05 (dd, *J* = 14.1, 6.5 Hz, 1H). Molecular formula: $C_{13}H_{14}BrClN_2$. Calculated mass: 312.00. ESIMS m/z 315.3 $(M+H)^+$.

(2S,3R)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile (44). A solution of benzyl chloride **43** (572 mg, 1.824 mmol) in THF (18 mL) was cooled to -60 ^oC, and lithium bis(trimethylsilyl)amide (2.2 mL, 1.0 M in THF) was added dropwise over 25 minutes. After 60 min, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a separable mixture of diastereomers (1.8:1). (2*S*,3*R*) - 282 mg, 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 5.83 (ddt, *J* = 16.5, 9.9, 4.9 Hz, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.24 (d, *J* = 10.2 Hz, 1H), 4.37 (d, *J* = 7.8 Hz, 1H), 3.84 (td, *J* = 7.7, 4.8 Hz, 1H), 3.53 (dt, *J* = 23.0, 6.8 Hz, 2H), 3.28 (d, *J* = 6.1 Hz, 2H). Molecular formula: $C_{13}H_{13}BrN_2$. Calculated mass: 276.03. ESIMS m/z 276.7 $(M+H)^+$. (2*R*,3*R*) - 158 mg, 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.1 Hz, 2H), 7.17 (s, 2H), 5.84 (ddt, *J* = 16.6, 10.0, 6.1 Hz, 1H), 5.28 (dd, *J* = 28.5, 13.7 Hz, 2H), 3.93 (q, *J* = 7.5 Hz, 1H), 3.87 – 3.74 (m, 2H), 3.26 (d, *J* = 6.2 Hz, 2H), 3.18 (t, *J* = 7.2 Hz, 1H). Molecular formula: $C_{13}H_{13}BrN_2$. Calculated mass: 276.03. ESIMS m/z 277.0 $(M+H)^+$.

(2S,3R)-3-(4-bromophenyl)-2-cyano-N-propylazetidine-1-carboxamide (45). To a solution of nitrile **44** (154 mg, 0.556 mmol) in EtOH (3.7 mL) and DCM (1.85 mL) was added 1,3-dimethylbarbituric acid (130 mg, 0.833 mmol) and $Pd(PPh_3)_4$ (64 mg, 0.056 mmol). The reaction was heated to 40 ^oC for 16 hours. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated product. This material was then directly dissolved in DCM (5.56 mmol), followed by the addition of DIPEA (968 µL, 5.56 mmol) and propylisocyanate (78 µL, 0.834 mmol). The reaction was allowed to stir at ambient temperature. After 30 min, solvent was removed *in vacuo* and crude material purified *via* flash column chromatography to afford product as a yellow solid (110 mg, 61% over two steps). Molecular formula: $C_{14}H_{16}BrN_3O$. Calculated mass: 321.05. ESIMS m/z 362.6 $(M+HCOOH)^+$.

Abbreviations

CoQ_D - decylubiquinone

DBU – 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCIP – 2,6-dichloroindophenol

DCM – dichloromethane

DHO – dihydroorotate

DHODH – dihydroorotate dehydrogenase

DIPEA – *N*,*N*-Diisopropylethylamine

DMF – dimethylformamide

DMSO – dimethylsulfoxide

dppa – diphenylphosphoryl azide

EtOH – ethanol

GFP-luc – green fluorescent protein - luciferase

Hs – Homo sapiens

IVIS – *in vivo* imaging system

LiHMDS – lithium bis(trimethylsilyl)amide

MeCN – acetonitrile

PEG300 – poly(ethylene glycol) 300

PCR – polymerase chain reaction

Pf – Plasmodium falciparum

(*S*)-CBS – (*S*)-(−)-2-methyl-CBS-oxazaborolidine

THF – tetrahydrofuran

TsCl – 4-tosyl chloride

UPLC-MS – ultra performance liquid chromatography – mass spectrometry

XPhos Pd-G3 - (2-Dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate

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