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Supplementary Materials for

BCL-X_L–targeting antibody-drug conjugates are active in preclinical models and mitigate on-mechanism toxicity of small-molecule inhibitors

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Supplementary Text

In-vitro Biology

Generation of huEGFR+ *mcl1*$^{-/-}$ mouse embryonic fibroblast cell lines and cell viability assay

Retroviral supernatants were produced through transfection of the GP2-293 packaging cell line (Clontech) with the retroviral construct pLVC-IRES-Hygro (Clontech) containing huEGFR sequence or the empty vector utilizing FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany). After 48 hours of culture, virus-containing supernatant was harvested and applied to *mcl-1*$^{-/-}$ MEFs in 75 cm2 culture flasks (0.5x106 per flask) for a further 48hrs in the presence of polybrene (8 µg/ml; Sigma). *Mcl-1*$^{-/-}$ MEFs were washed and selected after 3 days with 250 µg/ml hygromycin B (Invitrogen) in the full complement of media. The expression of huEGFR was confirmed by flow cytometry and compared to the parental cell line or those transfected with the empty vector.

Mcl-1$^{-/-}$ MEFs expressing huEGFR or the pLVX empty vector (Vct Ctrl) were treated with ADCs or antibodies for 96 hours in DMEM containing 10% FBS. For the assay, the cells were plated at 250 cells per well in 384-well tissue culture plates (Corning, Corning, NY) in a total volume of 25 µL of assay media (DMEM and 10% HI FBS). The plated cells were treated with a 4-fold serial dilution of the Antibody Drug Conjugates of interest from 1 µM to 4 pM dispensed by an Echo 550 Acoustic Liquid Handler (Labcyte). Each concentration was tested in eight replicates for both the *mcl1*$^{-/-}$ MEF huEGFR and *mcl1*$^{-/-}$ MEF vector cell lines. Four replicates each of Staurosporine and of the vehicle (PBS + 0.01% Pluronic F-68) were included as controls in both cell lines. The fraction of viable cells following 96 hours of Antibody Drug Conjugate treatment at 37 °C and 5% CO2 was determined using the CellTiter-Glo® Luminescent Cell Viability Assay according to the manufacturer's recommendations (Promega Corp., Madison, WI). The plates were read in a Perkin Elmer Envision using a Luminescence protocol with 0.5 sec integration time. The replicate values for each dilution point were averaged and the EC_{50} values for the Antibody Drug Conjugates were generated by fitting the data with GraphPad Prism 5 (GraphPad Software, Inc.) to a sigmoidal curve model using linear regression, Y=((Bottom-Top)/(1+((x/K)n)))+Top, where Y is the measured response, x is the compound concentration, n is the Hill Slope and K is the EC_{50} and Bottom and Top are the lower and higher asymptotes respectively. Visual inspection of curves was used to verify curve fit results. *mcl1*$^{-/-}$ MEFs were obtained from David C. S. Huang of the Walter and Eliza Hall Institute of Medical Research.

Chemistry

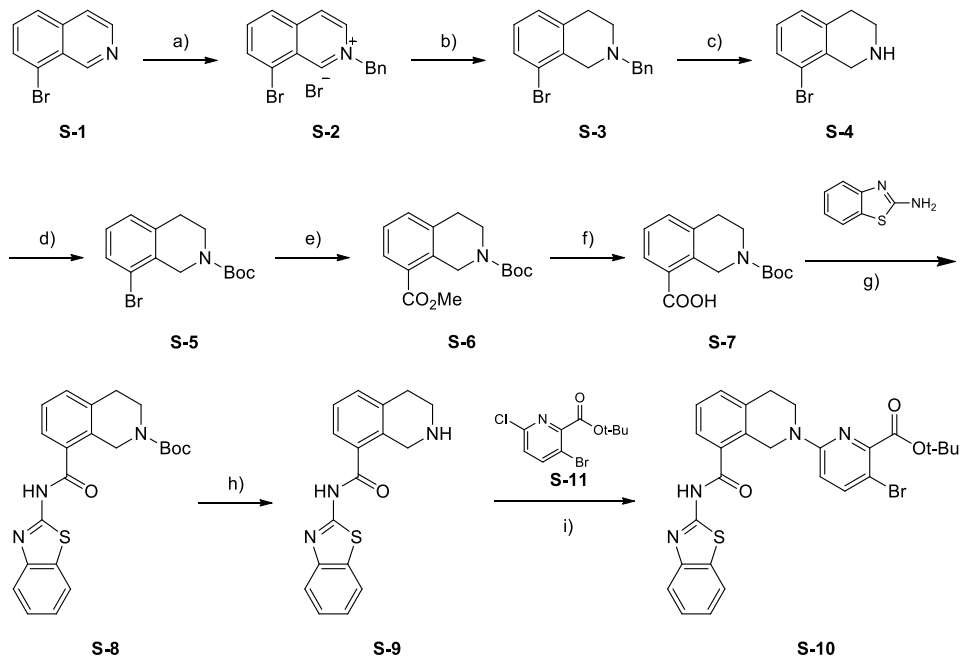
General: ^{1}H NMR spectra were obtained on a Varian UNITY or Inova (500 MHz), Varian UNITY (400 MHz), or Varian UNITY plus or Mercury (300 MHz) instrument. Chemical shifts are reported as values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. Mass spectral analyses were performed on a Finnigan SSQ7000 GC/MS mass spectrometer using different techniques, including electrospray ionization (ESI), desorption chemical ionization (DCI) and atmospheric pressure chemical ionization (APCI), as specified for individual compounds. Exact mass measurements were performed on a Finnigan FTMS Newstar T70 mass spectrometer. The compound is determined to be "consistent" with the chemical formula if the exact mass measurement is within 5.0 ppm relative mass error (RME) of the exact monoisotopic mass.

Analytical LC-MS were performed on a Finnigan Navigator mass spectrometer and Agilent 1100 HPLC system running Xcalibur 1.2 and Open-Access 1.3 software. The mass spectrometer was operated under positive APCI ionization conditions. The HPLC system comprised an Agilent Quaternary pump, degasser, column compartment, autosampler and diode-array detector, with a Sedere Sedex 75 evaporative light-scattering detector. The column used was a Phenomenex Luna Combi-HTS C8(2) 5µm 100Å (2.1mm × 30mm), utilizing Method A or Method B, as detailed below. TFA Method (Method A): A gradient of 10-100% acetonitrile (solvent 1) and 0.1% trifluoroacetic acid in water (solvent 2) was used, at a flow rate of 2 mL/min (0-0.1 min 10% solvent 1, 0.1-2.6 min 10-100% solvent 1, 2.6-2.9 min 100% solvent 1, 2.9-3.0 min 100-10% solvent 1). Ammonium acetate Method (Method B): A gradient of 10-100% acetonitrile (solvent 1) and 10 mM NH_4OAc in water (solvent 2) was used, at a flow rate of 1.5 mL/min (0-0.1 min 10% solvent 1, 0.1-3.1 min 10-100% solvent 1, 3.1¬3.9 min 100% solvent 1, 3.9-4.0 min 100-10% solvent 1). All final analogs were >95% pure as determined by these methods.

Preparative reverse phase HPLC was performed on an automated Gilson HPLC system, using a SymmetryPrep Shield RP18 prep cartridge, 250 mm × 21.20 mm i.d., 10 µm, and a flow rate of 25 mL/min; µ = 214, 245 nm; mobile phase A, 0.1% trifluoroacetic acid in water; mobile phase B, acetonitrile, using a linear gradient 0-70% of B over 40 minutes, unless otherwise stated.

Chemical Structures

Synthesis of Intermediate S-10



Chemical Structures 1. Reagents: a) BnBr, EtOH, 80 °C, 87% yield; b) $NaCNBH_3$, MeOH, 55%; c) 1-chloroethyl carbonochloridate then MeOH, 80 °C, 77% yield; d) di-*tert*-butyl dicarbonate, Na_2CO_3, 81% yield; e) $Pd(dppf)_2$, CO (50 psi), MeOH, 66% yield; f) NaOH, water, 45 °C, 89% yield; g) CDI, DBU, CH_3CN, 60 °C, 87% yield; h) HCl, EtOAc, 99% yield; i) **S-11**, Cs_2CO_3, DMA, 120 °C, 32% yield.

2-benzyl-8-Bromoisoquinolin-2-ium bromide (S-2). To a solution of 8-bromoisoquinoline (25 g, 120 mmol) in ethanol (500 mL) was added (bromomethyl)benzene (25 mL, 211 mmol). The reaction was heated at 80 °C for 12 hrs. The reaction mixture was concentrated under reduced pressure to give a residue, which

was triturated with ethyl acetate to give the title compound (31 g, 87% yield) as a white solid. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 6.15 (s, 2 H) 7.35 - 7.51 (m, 3 H) 7.60 (dd, *J* = 7.7, 1.7 Hz, 2 H) 8.06 - 8.18 (m, 1 H) 8.30 - 8.48 (m, 2 H) 8.59 - 8.71 (m, 1 H) 8.88 (dd, *J* = 6.7, 1.10 Hz, 1 H) 10.24 (s, 1 H).

2-Benzyl-8-bromo-1,2,3,4-tetrahydroisoquinoline (S-3). To a solution of **S-2** (50 g, 132 mmol) in methanol (500 mL) was added sodium cyanoborohydride (16.58 g, 264 mmol), and the mixture was stirred at 25 °C for 12 hrs. The mixture was poured into water (1 L) and extracted with ethyl acetate (3 × 500 mL). The organic layer was washed with brine (500 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to obtain the title compound (21.9 g, 55% yield) as a yellow oil. ^{1}H NMR (400 MHz, chloroform-d) δ ppm 2.63 - 2.74 (m, 2 H) 2.83 - 2.95 (m, 2 H) 3.67 (s, 2 H) 3.75 (s, 2 H) 6.96 - 7.13 (m, 2 H) 7.28 - 7.32 (m, 1 H) 7.33 - 7.44 (m, 5 H).

8-Bromo-1,2,3,4-tetrahydroisoquinoline (S-4). To a solution of **S-3** (30 g, 99 mmol) in 1,2-dichloroethane (300 mL) was added 1-chloroethyl carbonochloridate (28.4 g, 199 mmol) at 0 °C. The reaction was heated to 25°C for 2 hrs. To the reaction mixture was added methanol (500 mL), and the mixture was heated to 80 °C for 1 hr. The reaction mixture was concentrated under reduced pressure. The residue was washed with ethyl acetate (500 mL) to obtain the title compound (16 g, 77% yield) as white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.15 (t, *J* = 6.2 Hz, 2 H) 3.50 (t, *J* = 6.3 Hz, 2 H) 4.34 (s, 2 H) 7.16 - 7.34 (m, 2 H) 7.55 (d, *J* = 7.6 Hz, 1 H).

***tert*-Butyl 8-bromo-3,4-dihydroisoquinoline-2(1H)-carboxylate (S-5).** To a solution of **S-4** (30 g, 141 mmol) in methanol (200 mL) was added sodium carbonate (75.0 g, 707 mmol) in water (200 mL), followed by di-*tert*-butyl dicarbonate (36.1 mL, 156 mmol). The reaction was stirred at 25 °C for 1 hr. The mixture was poured into water (500 mL) and extracted with ethyl acetate (3 × 300 mL). The organic layer was washed with brine (500 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure to give a yellow oil. The oil was triturated with petroleum ether at -78 °C to give the title compound (35.6 g, 81% yield) as white solid. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 1.43 (s, 9 H) 2.79 (br t, *J* = 5.50 Hz, 2 H) 3.50 - 3.60 (m, 2 H) 4.44 (br s, 2 H) 7.11 - 7.24 (m, 2 H) 7.48 (br d, *J* = 7.8 Hz, 1 H). MS (ESI) m/z 256.0, 258.0 (M-*tert*Bu+H)$^+$.

2-*tert*-Butyl 8-methyl 3,4-dihydroisoquinoline-2,8(1H)-dicarboxylate (S-6). To a solution of **S-5** (25 g, 80 mmol) in methanol (200 mL) and N,N-dimethylformamide (100 mL) was added 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium(II) dichloromethane complex (3.27 g, 4.00 mmol) and triethylamine (44.6 mL, 320 mmol). The reaction was heated at 80 °C under an atmosphere of CO (50 psi) for 12 hrs. The reaction was cooled to 25 °C and filtered through celite, eluting with ethyl acetate (200 mL). The organic phase was concentrated under reduced pressure. The residue was diluted with ethyl acetate (800 mL) and washed with 1N aqueous hydrochloride solution (500 mL), brine (500 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to obtain the title compound (15.4 g, 66% yield) as white solid. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 1.41 (s, 9 H) 2.85 (br t, *J* = 5.6 Hz, 2 H) 3.55 (br s, 2 H) 3.84 (s, 3 H) 4.81 (br s, 2 H) 7.27 - 7.35 (m, 1 H) 7.41 (br d, *J* = 7.3 Hz, 1 H) 7.76 (br d, *J* = 7.5 Hz, 1 H). MS (ESI) m/z 236.0 (M-*tert*Bu+H)$^+$.

2-(*tert*-Butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid (S-7). To a solution of **S-6** (30 g, 103 mmol) in methanol (150 mL) was added a solution of sodium hydroxide (16.47 g, 412 mmol) in water (150 mL). The reaction was stirred at 45 °C for 2 hrs. The reaction was cooled to ambient temperature, and the mixture was extracted with ethyl acetate (200 mL). The organic phase was discarded, and the water

layer was adJusted to pH = 5 by 1N aqueous hydrochloride solution. The solid was filtered, washed with water (200 mL) and dried under vacuum to give the title compound (25.4 g, 89% yield). ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 1.21 - 1.58 (m, 9 H) 2.74 - 2.96 (m, 2 H) 3.54 (br s, 2 H) 4.83 (br s, 2 H) 7.19 - 7.43 (m, 2 H) 7.74 (br d, *J* = 7.3 Hz, 1 H). MS (ESI) m/z 222.1 (M-*tert*Bu+H)$^+$.

***tert*-Butyl 8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (S-8).** To a solution of **S-7** (24 g, 87 mmol) in acetonitrile (300 mL) was added carbonyl diimidazole (CDI) (17.54 g, 108 mmol). The reaction was stirred at 25 °C for 30 minutes. To the reaction mixture was added 1,8-diazabicyclo[5.4.0]undec-7-ene (21.08 g, 138 mmol), and the reaction was stirred at 25 °C for another 30 minutes. Benzo[d]thiazol-2-amine (16.25 g, 108 mmol) was added, and the reaction was stirred at 60 °C for 12 hours. The reaction was cooled to ambient temperature, and the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (1 L) and washed with 1N aqueous hydrochloride solution (500 mL), saturated aqueous sodium bicarbonate solution (500 mL) and brine (500 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The solid was washed with methyl *tert*-butyl ether (500 mL) to give the title compound (31.0 g, 87% yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.39 (br s, 9 H) 2.88 (br s, 2 H) 3.57 (br s, 2 H) 4.70 (br s, 2 H) 7.37 (dt, *J* = 15.2, 7.43 Hz, 3 H) 7.47 (br t, *J* = 7.3 Hz, 1 H) 7.59 (br d, *J* = 6.9 Hz, 1 H) 7.79 (br d, *J* = 7.8 Hz, 1 H) 8.03 (br d, *J* = 7.8 Hz, 1 H) 12.85 (br s, 1 H). MS (ESI) m/z 410.1 (M+H)$^+$.

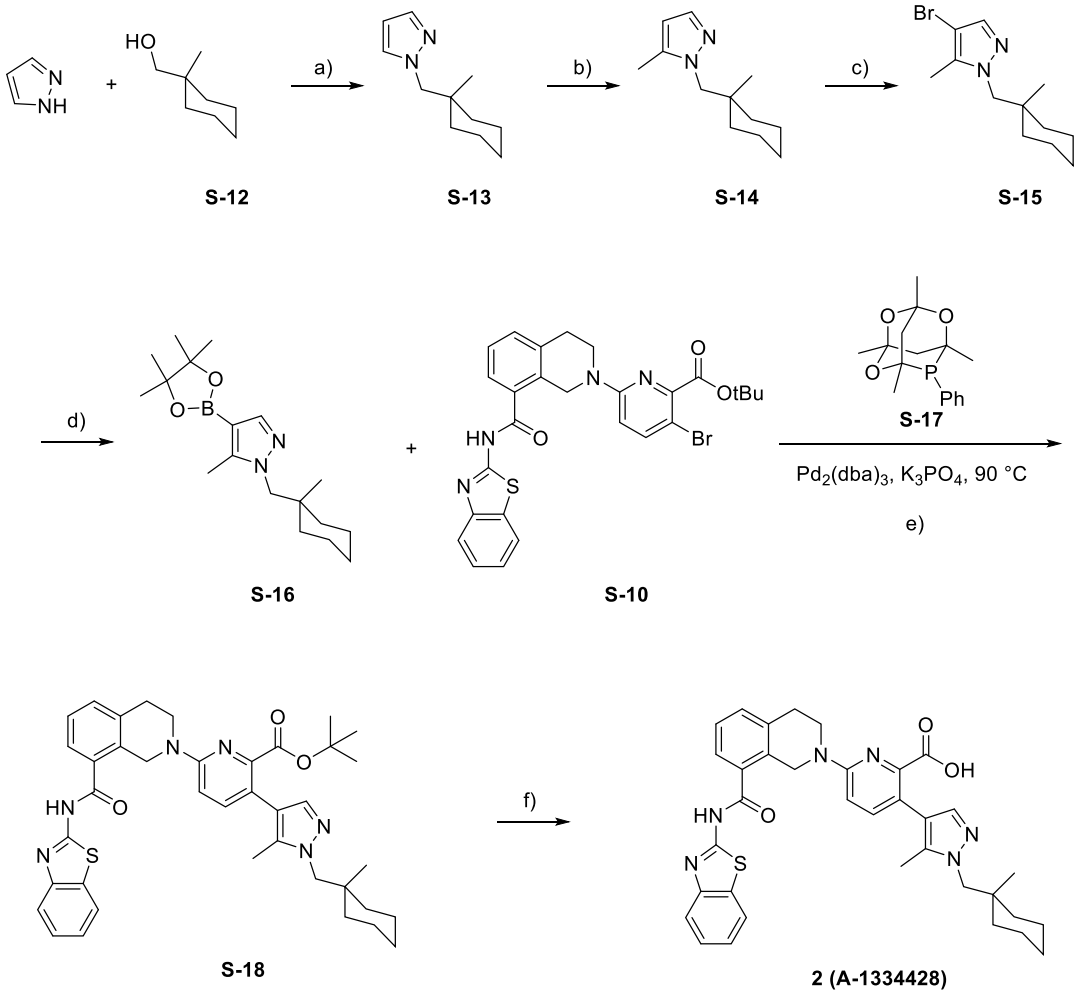
N-(Benzo[d]thiazol-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxamide (S-9), HCl salt. To a solution of **S-8** (30 g, 73.3 mmol) in ethyl acetate (200 mL) was added aqueous hydrochloride solution (200 mL, 800 mmol, 4M in ethyl acetate). The reaction was stirred at 25 °C for 12 hrs. The reaction mixture was filtered, and the filter cake was washed with ethyl acetate (200 mL) and air-dried to give the title compound (25.0g, 99% yield) as white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.16 - 3.28 (m, 2 H) 3.55 (br t, *J* = 6.3 Hz, 2 H) 4.69 (s, 2 H) 7.33 - 7.44 (m, 1 H) 7.46 - 7.62 (m, 3 H) 7.82 (br d, *J* = 8.4 Hz, 2 H) 7.95 (d, *J* = 7.9 Hz, 1 H). MS (ESI) m/z 310.1 (M+H)$^+$.

***tert*-Butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-bromopicolinate (S-10).** To a solution of **S-9** (24 g, 69.4 mmol) in dimethylacetamide (200 mL) were added **S-11** (20.30 g, 69.4 mmol), 4 Angstrom molecular sieves (12 g, 69.4 mmol) and cesium carbonate (22.61 g, 69.4 mmol). The reaction was stirred at 120 °C for 12 hours under nitrogen atmosphere. The reaction was cooled to ambient temperature, and the mixture was filtered through the celite. The filter cake was washed with ethyl acetate (1 L). The filtrate was washed with 10% aqueous citric acid solution (800 mL), brine (500 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The solid was triturated with ethyl acetate (200 mL) and air-dried to give the title compound (12.4 g, 31.6% yield) as a white solid. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 1.33 (s, 9 H) 3.00 (br t, *J* = 5.9 Hz, 2 H) 3.77 (t, *J* = 6.0 Hz, 2 H) 4.93 (s, 2 H) 6.86 (d, *J* = 9.2 Hz, 1 H) 7.30 - 7.39 (m, 2 H) 7.40 - 7.50 (m, 2 H) 7.58 (d, *J* = 7.3 Hz, 1 H) 7.71 - 7.83 (m, 2 H) 8.02 (d, *J* = 7.8 Hz, 1 H) 12.87 (br s, 1 H). MS (ESI) m/z 565.1, 567.2 (M+H)$^+$.

***tert*-Butyl 3-bromo-6-chloropicolinate (S-11)**. To a solution of 3-bromo-6-chloropicolinic acid (20 g, 85 mmol) and pyridine (45.8 mL**,** 567 mmol) in *tert*-butanol (200 mL) was added *p*-toluenesulfonyl chloride (38.7 g, 203 mmol). The mixture was stirred at 25 °C for 12 hrs. The mixture was quenched with aqueous sodium bicarbonate solution (800 mL) and extracted with ethyl acetate (3 × 800 mL). The organic layer was washed with brine (800 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduce pressure to give a residue. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to obtain **S-11** (20 g, 80% yield) as a white solid. ^{1}H NMR (400 MHz,

dimethylsulfoxide- d_6) δ ppm 1.56 (s, 9 H) 7.62 (d, J = 2.6 Hz, 1 H) 8.25(d, J = 8.4 Hz, 1 H). MS(ESI) m/z 237.9 (M-*tert*Bu+H)$^+$.

Synthesis of Compound 2 (A-1334428)



Chemical Structures 2. Reagents: a) Cyanomethylenetributylphosphorane, 75 °C, 80% yield; b) nBuLi, -78 °C then MeI, ~100% yield; c) NBS, N,N-dimethylacetamide, rt, 91% yield; d) nBuLi, $B(O^iPr)_3$, -78 °C then pinacol, 72% yield; e) 2.5 mol% $Pd_2(dba)_3$, 10 mol% **S-17**, K_3PO_4, 1:1 1,4-dioxane:H_2O, 90 °C, 28% yield; f) TFA, DCM, rt, 67% yield.

1-((1-Methylcyclohexyl)methyl)-1H-pyrazole (S-13). To a solution of 1H-pyrazole (0.582 g, 8.55 mmol) and (1-methylcyclohexyl)methanol (0.843 g, 6.58 mmol) in toluene (3 mL) was added tributylcyanomethylphosphorane (1.746g, 7.23 mmol). The reaction was stirred at 75 °C for 16 hrs. The reaction was cooled to 20 °C, and the solution was directly purified by column chromatography on silica gel (80 g), eluting with a gradient of 0.25 to 0.75% acetone in hexanes over 30 minutes (60 mL/min flow rate), to give the title compound (0.937 g, 80 %) as an oil. ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 0.91 (s, 3 H), 1.25 - 1.37 (m, 5 H), 1.38 - 1.61 (m, 5 H), 3.97 (s, 2 H), 6.23 (t, J = 2.0 Hz, 1H), 7.33 (d, J = 2.0 Hz, 1H) and 7.49 (d, J = 2.0 Hz, 1H). MS (ESI) m/z 178.6 (M+H)$^+$.

5-Methyl-1-((1-methylcyclohexyl)methyl)-1H-pyrazole (S-14). To a cold (-50 °C bath) solution of compound **S-13** (5.27 g, 29.3 mmol) in tetrahydrofuran (99 mL) was added *n*-butyl lithium (14.2 mL, 35.5

mmol, 1.6 M in hexane) dropwise. The reaction was stirred for 1.0 h, and methyl iodide (2.22 mL, 35.5 mmol) was added dropwise. The reaction was stirred at 0 °C for 30 minutes and then quenched by the addition of 200 mL ethyl acetate. The organic layer was washed with water (100 mL), brine (100 mL), dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the title compound, which was used in the subsequent step without further purification (5.7 g, ~100%). ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 7.28 (d, J = 1.8 Hz, 1H), 5.99 (q, J = 1.0 Hz, 1H), 3.82 (s, 2H), 2.24 (s, 3H), 1.61 – 1.07 (m, 10H), 0.82 (s, 3H). MS (DCI) m/z 193.2 $(M+H)^+$.

4-Bromo-5-methyl-1-((1-methylcyclohexyl)methyl)-1H-pyrazole (S-15). To a solution of compound **S-14** (5.7 g, 29.5 mmol) in N,N-dimethylacetamide (99 mL) was added *N*-bromosuccinimide (5.54 g, 31.1 mmol). After 30 minutes, the reaction mixture was diluted by the addition of 200 mL ethyl acetate. The organic layer was washed with water (100 mL), brine (100 mL), dried with anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (80 g silica gel), eluting with 0.5% to 7.5% ethyl acetate in hexanes over 30 minutes, to give the title compound (7.5 g, 91 % yield) as a an oil. ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 7.48 (s, 1H), 3.90 (s, 2H), 2.24 (s, 3H), 1.67 – 1.15 (m, 10H), 0.82 (s, 3H). MS (ESI) m/z 271.2 and 273.2 $(M+H)^+$.

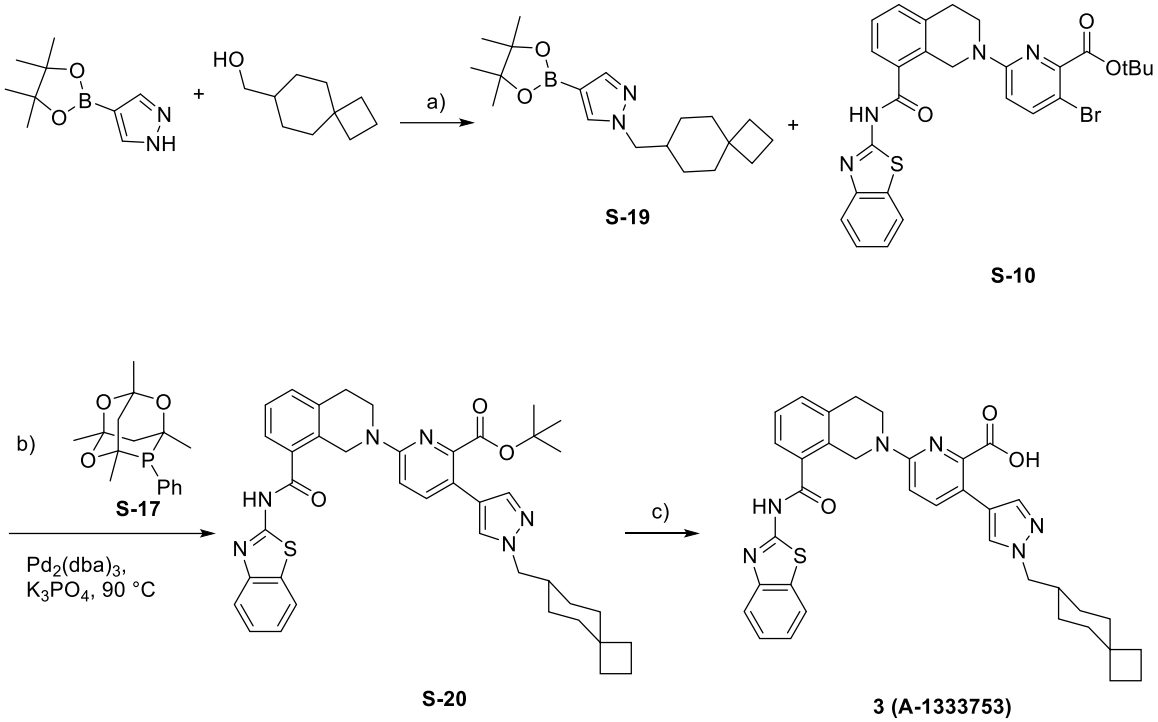
5-Methyl-1-((1-methylcyclohexyl)methyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (S-16). To a cold (-78 °C) solution of compound **S-15** (6.95 g, 25.6 mmol) and triisopropyl borate (7.50 mL, 32.3 mmol) in tetrahydrofuran (64 mL) and toluene (64 mL) was added *n*-butyl lithium (15.38 mL, 38.4 mmol, 2.5 M in hexanes) dropwise, keeping the internal temperature less than -55 °C. The mixture was stirred for 30 min, and pinacol (15.14 g, 128 mmol) in tetrahydrofuran (15 mL) was added. The cooling bath was removed, and the reaction was warmed room temperature and stirred for 2 h. Silica gel (100g) was added, and the mixture was concentrated under reduced pressure. The residue was purified by silica gel chromatography (330 g silica gel), eluting with 0.5% to 25% ethyl acetate in hexanes over 30 minutes, to give the title compound (5.86 g, 72 % yield) as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.69 (s, 1H), 3.84 (s, 2H), 2.43 (s, 3H), 1.61 - 1.14 (m, 22H), 0.92 (s, 3H). MS (ESI) m/z 319.37 $(M+H)^+$.

***tert*-Butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(5-methyl-1-((1-methylcyclohexyl)methyl)-1H-pyrazol-4-yl)picolinate (S-18).** A mixture of **S-10** (0.240 g, 0.424 mmol), **S-16** (0.135 g, 0.424 mmol), potassium phosphate (0.315 g, 1.49 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.022 g, 0.021 mmol) and (1*R*,3*S*,5*S*,7*R*)-1,3,5,7-tetramethyl-8-phenyl-2,4,6-trioxa-8-phosphaadamantane (0.012 g, 0.042 mmol) were added to N,N-dimethylformamide (0.6 mL), dioxane (0.4 mL) and water (0.2 mL). The reaction was degassed with nitrogen, sealed and heated to 110 °C. After 3 hours the reaction was cooled, diluted with ethyl acetate (50 mL) and washed with water (30 mL) and brine (30 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (40 g), eluting with a gradient of 5 to 45% ethyl acetate in hexane, to give the title compound as a yellow foam (0.081 g, 28%). ^{1}H NMR (400 MHz, dimethyl sulfoxide- d_6) δ ppm 2.84 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 7.4 Hz, 1H), 7.51 – 7.40 (m, 3H), 7.40 – 7.29 (m, 2H), 7.21 (s, 1H), 6.92 (d, J = 8.8 Hz, 1H), 4.96 (s, 2H), 3.92 (s, 2H), 3.88 – 3.79 (m, 4H), 3.02 (t, J = 6.0 Hz, 2H), 2.09 (s, 3H), 1.55 – 1.32 (m, 7H), 1.30 – 1.20 (m, 4H), 1.15 (s, 9H). MS (ESI) m/z 677.5 $(M+H)^+$.

6-[8-(1,3-Benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{5-methyl-1-[(1-methylcyclohexyl)methyl]-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (**A-1334482, 2)**. To an ambient solution of **S-17** (0.078 g, 0.115 mmol) in dichloromethane (0.5 mL) was added trifluoroacetic acid (0.5 mL, 6.39 mmol). The reaction flask was covered with foil and stirred overnight. The reaction mixture was

concentrated under reduced pressure to a thick syrup. The residue was dissolved in dichloromethane, and the solution was loaded onto a silica gel column (12 g). The column was eluted with a gradient of 0.15% to 3% methanol in dichloromethane to give the title compound (0.049 g, 67%) as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.85 (dt, *J* = 6.0, 2.6 Hz, 1H), 7.68 - 7.62 (m, 1H), 7.59 (dd, *J* = 10.5, 3.3 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.45 - 7.30 (m, 5H), 7.01 (d, *J* = 8.8 Hz, 1H), 5.17 (s, 2H), 3.89 (d, *J* = 4.7 Hz, 4H), 3.12 (s, 2H), 2.09 (s, 3H), 1.55 – 1.22 (m, 10H), 0.96 (s, 3H). MS (ESI) m/z 621.2 $(M+H)^+$.

Synthesis of Compound 3



Chemical Structures 3. Reagents: a) Cyanomethylenetributylphosphorane, 70 °C, 79% yield; b) 2.5 mol% $Pd_2(dba)_3$ chloroform adduct, 10 mol% **S-17**, K_3PO_4, 1:1 1,4-dioxane:H_2O, 90 °C, 63% yield; c) TFA, DCM, 82% yield.

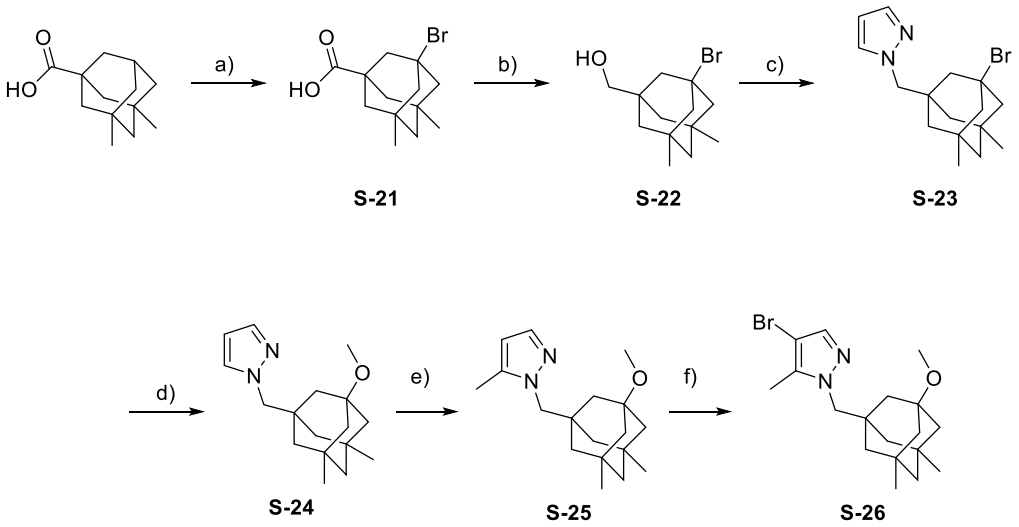
1-(Spiro[3.5]nonan-7-ylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (S-19). To a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (1.439 g, 7.42 mmol) and 7-hydroxymethyl-spiro[3.5]nonane (1.04 g, 6.74 mmol) in toluene (5 mL) was added cyanomethylenetributylphosphorane (2.44 g, 10.11 mmol). The reaction was stirred at 70 °C for 14 hrs. The reaction was cooled to 20 °C, and the solution was directly purified by column chromatography on silica gel (80 g), eluting with a gradient of 0 to 20% ethyl acetate in hexanes over 30 minutes to give the title compound (1.75 g, 79 %) as an oil. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.77 (s, 1H), 7.62 (s, 1H), 3.91 (d, *J* = 7.5 Hz, 2H), 1.88 – 1.61 (m, 7H), 1.48 – 1.37 (m, 3H), 1.32 (s, 12H), 1.24 – 1.15 (m, 3H), 1.06 – 0.96 (m, 2H). MS (ESI) m/z 331.2 $(M+H)^+$.

***tert*-Butyl-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(spiro[3.5]nonan-7-ylmethyl)-1H-pyrazol-4-yl)picolinate (S-20).** A mixture of **S-10** (1.601 g, 2.83 mmol), **S-19** (0.85 g, 2.57 mmol), potassium phosphate (1.64 g, 7.72 mmol), tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (0.133 g, 0.129 mmol) and (1*R*,3*S*,5*S*,7*R*)-1,3,5,7-tetramethyl-8-phenyl-2,4,6-trioxa-8-phosphaadamantane (0.150 g, 0.515 mmol) were added to

tetrahydrofuran (15 mL) and water (5 mL) in a microwavable vial. The vial was degassed with nitrogen, sealed and heated under microwave conditions to 140 °C for 5 minutes. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (30 mL) and brine (30 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (200 g), eluting with a gradient of 10 to 60% ethyl acetate in hexane, to give the title compound as a yellow foam (1.11 g, 63%). ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 12.83 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.68 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.51 – 7.31 (m, 5H), 6.94 (d, *J* = 9.0 Hz, 1H), 4.96 (s, 2H), 3.94 – 3.87 (m, 2H), 3.81 (t, *J* = 6.0 Hz, 2H), 3.07 – 2.97 (m, 2H), 1.86 – 1.57 (m, 10H), 1.34 (d, *J* = 12.9 Hz, 2H), 1.23 (s, 9H), 1.13 (dd, *J* = 12.6, 3.0 Hz, 1H), 1.03 – 0.87 (m, 2H). MS (ESI) m/z 689.0 $(M+H)^+$.

6-(8-(Benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(spiro[3.5]nonan-7-ylmethyl)-1H-pyrazol-4-yl)picolinic acid (**A-1333753, 3)**. To an ambient solution of **S-20** (2.1 g, 3.05 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (8 mL, 104 mmol). The reaction flask was covered with foil and stirred overnight. The reaction mixture was concentrated under reduced pressure to a thick syrup. The residue was dissolved in dichloromethane, and the solution was loaded onto a silica gel column and eluted with a gradient of 0 to 4% methanol in dichloromethane. The purified material was concentrated to a solid, dissolved in dichloromethane (30 mL) and washed with water (6 x 150 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and concentrated. The solid was triturated with ether and dried to give the title compound (1.59 g, 82%) as a solid. ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 13.05 (s, 1H), 12.85 (s, 1H), 8.04 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.64 – 7.58 (m, 1H), 7.53 – 7.32 (m, 5H), 6.93 (d, *J* = 8.9 Hz, 1H), 4.94 (s, 2H), 3.98 – 3.81 (m, 4H), 3.00 (t, *J* = 5.9 Hz, 2H), 1.88 – 1.59 (m, 10H), 1.43 – 1.29 (m, 2H), 1.25 – 1.09 (m, 2H), 1.06 – 0.85 (m, 2H).. MS (ESI) m/z 633.2 $(M+H)^+$.

Synthesis of Intermediate S-26



Chemical Structures 4. Reagents: a) Fe, Br_2, 93% yield; b) borane-THF complex, THF, 95% yield; c) cyanomethylenetributylphosphorane, 70 °C, 87% yield; d) silver sulfate, methanol, microwave irradiation, 100 °C, 47% yield; e) nBuLi, -40 °C then MeI, 65% yield; f) NBS, THF, 73% yield.

3-Bromo-5,7-dimethyladamantanecarboxylic acid (S-21). Into a 50 mL round-bottomed flask at 0 °C, was added bromine (16 mL, 310 mmol). Iron powder (7 g, 125 mmol) was added, and the reaction mixture

was stirred at 0 °C for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g, 57.7 mmol) was added. The mixture was warmed up to room temperature and stirred for 3 days. A mixture of ice and concentrated HCl was poured into the reaction mixture. The resulting suspension was treated twice with Na_2SO (50 g in 200 mL water) and extracted three times with dichloromethane. The combined organic fractions were washed with 1 N aqueous HCl, dried over sodium sulfate, filtered, and concentrated to give the title compound (15.4 g, 93%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.33 (s, 2H), 1.94~1.98 (m, 4H), 1.53~1.62 (m, 4H), 1.21 – 1.22 (m, 2H), 0.93 (s, 3H). MS (DCI) m/z 287 $(M+H)^+$.

1-(((1r,3r)-3-Bromoadamantan-1-yl)methyl)-1H-pyrazole (S-22). To a solution of **S-21** (4.57 g, 15.9 mmol) in tetrahydrofuran (10 mL) was added BH_3 THF (1 M in tetrahydrofuran, 50 mL, 50 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched by adding methanol (20 mL) dropwise. The reaction mixture was concentrated and purified by chromatography on silica gel using ethyl acetate in hexane (0-30%) as eluent to provide the title compound (4.5 g, 95%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 3.30 (s, 2H), 1.99 (s, 3H), 1.97 (s, 3H), 1.36 (s, 1H), 1.14 – 1.1.25 (m, 6H), 0.90 (s, 6H). MS (DCI) m/z 273 $(M+H)^+$.

1-((3-Bromo-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl)methyl)-1H-pyrazole (S-23). To a solution of **S-22** (8.0 g, 29.3 mmol) in toluene (60 mL) was added 1H-pyrazole (1.55 g, 22.8 mmol) and cyanomethylenetributylphosphorane (2.0 g, 29.3 mmol), and the mixture was stirred at 90 °C overnight. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (10:1 heptane:ethyl acetate) to give the title compound (8.3 g, 87%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.49 (d, *J* = 1.7 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 6.24 (d, *J* = 2.0 Hz, 1H), 3.91 (s, 2H), 3.69 – 3.62 (m, 2H), 3.49 (d, *J* = 5.9 Hz, 2H), 1.40 – 1.30 (m, 6H), 1.18 – 1.01 (m, 6H), 0.89 (s, 6H). MS (ESI) m/z 324.2 $(M+H)^+$.

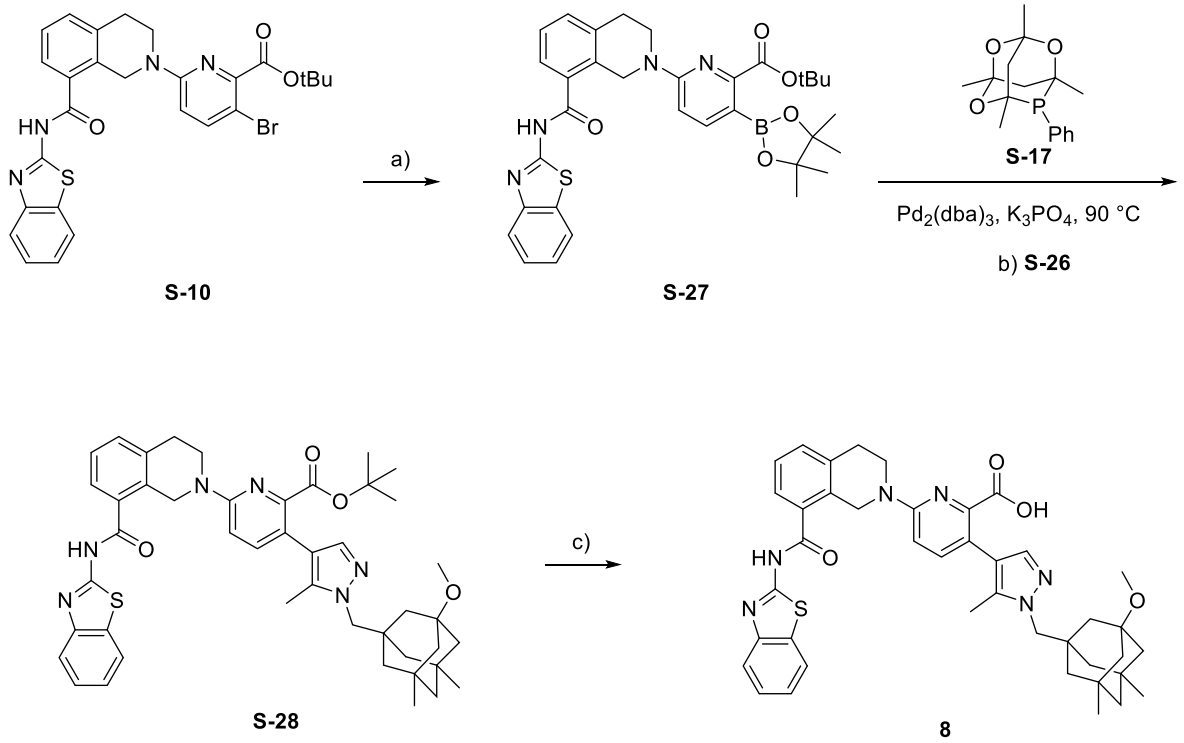
1-(((1r,3r)-3-Methoxy-5,7-dimethyladamantan-1-yl)methyl)-1H-pyrazole (S-24). To a microwave vial equipped with a magnetic stir bar was added **S-23** (0.43 mg, 1.33 mmol), Ag_2SO_4 (1.0 g, 3.21 mmol) and methanol (10 mL). The vial was sealed and heated under microwave conditions at 110 °C for 30 minutes. The reaction was cooled to ambient temperature and diluted with ethyl acetate (5 mL). The mixture was filtered, eluting with additional ethyl acetate (2 x 5 mL). The solvent was removed, and the residue was purified by silica gel chromatography (12 g SiO_2), eluting with 0 to 100% ethyl acetate in hexane to give the title compound (171 mg, 47% yield). ^{1}H NMR (600 MHz, chloroform-*d*) δ ppm 7.50 (dd, *J* = 1.9, 0.7 Hz, 1H), 7.32 (dd, *J* = 2.2, 0.7 Hz, 1H), 3.93 (s, 2H), 3.22 (s, 3H), 1.41 – 1.29 (m, 6H), 1.19 – 1.02 (m, 6H), 0.91 (s, 6H). MS (ESI) m/z 275.3 $(M+H)^+$.

1-(((1r,3r)-3-Methoxy-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazole (S-25). To a cold (-40 °C bath) solution of compound **S-24** (0.16 g, 0.583 mmol) in 1:1 toluene/tetrahydrofuran (4 mL) was added *n*-butyllithium (0.5 mL, 1.25 mmol, 2.5 M in hexane) dropwise. The reaction was stirred for 1.5 h during which time the temperature raised to -20 °C. Methyl iodide (0.1 mL, 1.60 mmol) was added dropwise. The reaction warmed to -5 °C over 90 minutes and was quenched by the addition of 50 mL of water and extracted with ethyl acetate (3 × 50 mL). The combined organics were washed with brine, dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give compound the title compound (0.11 g, 65 % yield), which was used in the subsequent step without further purification. ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 7.30 (d, J = 1.7 Hz, 1H), 6.01 (dd, J = 1.7, 0.8 Hz, 1H), 3.91 (s, 3H), 3.81 (s, 2H), 1.17 – 1.08 (m, 6H), 1.05 – 0.93 (m, 6H), 0.86 (s, 6H). MS (ESI) m/z 289.4 $(M+H)^+$.

4-Bromo-1-(((1r,3r)-3-methoxy-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazole (S-26). To a solution of compound **S-25** (0.100 g, 0.347 mmol) in tetrahydrofuran (2 mL) was added N-bromosuccinimide (0.100 g, 0.562 mmol), and the reaction was stirred for 90 minutes. The reaction mixture

was diluted with ethyl acetate (100 mL) and washed with 10% aqueous sodium carbonate (2 x 300 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography on silica gel (40 g), eluting with 0-50% ethyl acetate in hexane to provide the title compound (0.093 g, 73%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.44 (s, 1H), 3.85 (s, 3H), 3.22 (s, 3H), 2.26 (s, 3H), 1.42 – 1.24 (m, 6H), 1.23 – 1.01 (m, 6H), 0.91 (s, 6H). MS (ESI) m/z 367.4 $(M+H)^+$.

Synthesis of Compound 8



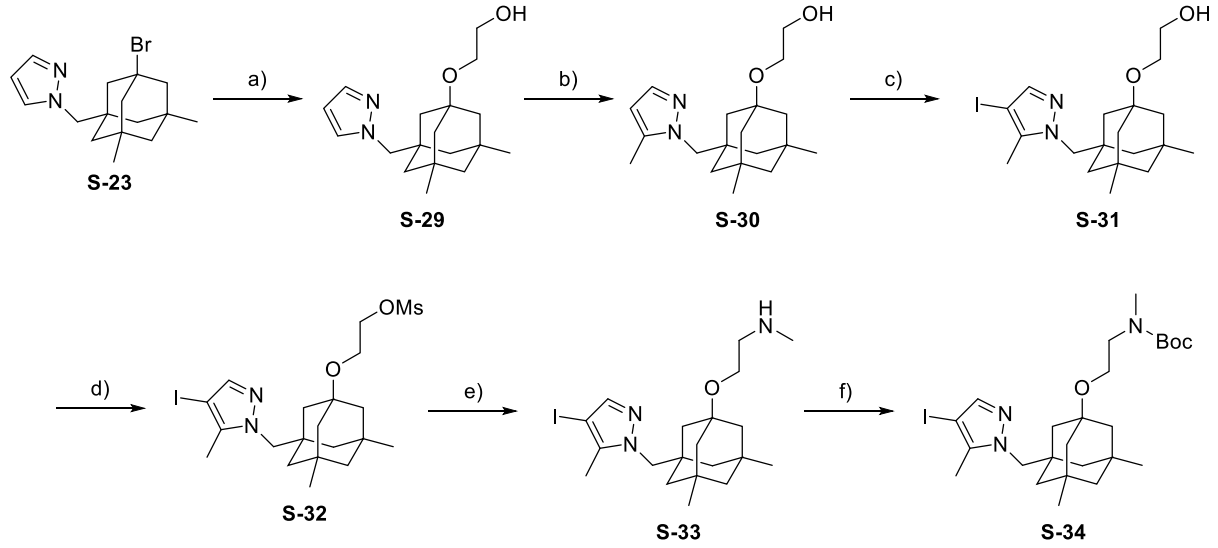
Chemical Structures 5. Reagents: a) pinacol borane, $PdCl_2$(dppf)-CH_2Cl_2, Et_3N, CH_3CN, 1,4-dioxane, 90 °C, 89% yield; b) 2.5 mol% $Pd_2(dba)_3$, 10 mol% **S-17**, K_3PO_4, 3:1 THF:H_2O, microwave irradiation, 140 °C; c) TFA, DCM, rt, 15% yield for 2 steps.

***tert*-Butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinate (S-27).** To a solution **S-10** (15 g, 26.5 mmol) and $PdCl_2$(dppf) dichloromethane adduct (1.2 g, 1.33mmol) in acetonitrile (100mL) and 1,4-dioxane (150 mL) was added triethylamine (16.2 g, 160mmol, 20 mL) and pinacol borane (10 g, 80 mmol). The mixture was stirred under nitrogen at 90 °C overnight. The mixture was diluted with ethyl acetate (600mL) and the organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl actate in hexane to give the title compound (14.5 g, 89%). ^{1}H NMR (300 MHz, dimethyl sulfoxide- d_6) δ ppm 12.82 (s, 1H), 8.07 – 7.97 (m, 1H), 7.77 (ddd, *J* = 8.0, 1.2, 0.6 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.55 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.50 – 7.29 (m, 4H), 6.85 (d, *J* = 8.7 Hz, 1H), 5.00 (s, 2H), 3.81 (t, *J* = 5.9 Hz, 2H), 3.00 (q, *J* = 6.0 Hz, 2H), 1.27 (s, 9H), 1.21 (s, 12H). MS (ESI) m/z 613.0 $(M+H)^+$.

6-(8-(Benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3r)-3-methoxy-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (8). A microwave tube equipped with a stir bar was charged with **S-26** (0.093 g, 0.253 mmol), **S-27** (0.118 g, 0.193 mmol), potassium phosphate (0.10 g, 0.47 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.013 g, 0.014 mmol)

and (1R,3S,5S,7R)-1,3,5,7-tetramethyl-8-phenyl-2,4,6-trioxa-8-phosphaadamantane **S-17** (0.021 g, 0.072 mmol). A mixture of tetrahydrofuran:water (3:1, 4 mL) was added to the vessel containing the solid reagents. The head space was swept with nitrogen gas, and the tube was sealed with a septum. The reaction was heated to 140 °C for 5 minutes under microwave conditions. The reaction was cooled to ambient temperature and quenched by the addition of saturated aqueous bicarbonate solution and ethyl acetate (25 mL each). The layers were separated, and the aqueous was extracted with additional ethyl acetate (2 x 25 mL). The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane (2mL), and trifluoroacetic acid (2 mL) was added. The reaction was stirred overnight at ambient temperature and concentrated under reduced pressure. The residue was purified by RP-HPLC, eluting with 30-100% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound (22 mg, 15%). %). ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 12.88 – 12.71 (m, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.54 – 7.40 (m, 3H), 7.41 – 7.31 (m, 2H), 7.28 (s, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 4.95 (bs, 2H), 3.89 (t, *J* = 5.9 Hz, 2H), 3.82 (bs, 2H), 3.08 (s, 3H), 3.08 – 2.98 (m, 2H), 2.10 (s, 3H), 1.34 (bs, 2H), 1.26 – 0.83 (m, 16H). MS (ESI) m/z 717.0 $(M+H)^+$.

Synthesis of Compound S-34



Chemical Structures 6. Reagents: a) $HOCH_2CH_2OH$, Et_3N, 150 °C, microwave, 80% yield; b) nBuLi, MeI, -78 °C, THF, 55% yield; c) NIS, N,N-DMF, 51% yield; d) CH_3SO_2Cl, Et_3N, DCM; e) 2M $MeNH_2$ in MeOH, 100 °C, microwave; f) di-*tert*-butyl decarbonate, 4-DMAP, THF, 52% yield for 3 steps.

2-{[3,5-Dimethyl-7-(1H-pyrazol-1-ylmethyl)tricyclo[3.3.1.13,7]dec-1-yl]oxy}ethanol (S-29). To a solution of **S-23** (3.85 g, 1.48 mmol) in ethane-1,2-diol (12 mL) was added triethylamine (3 mL). The mixture was stirred at 150 ºC under microwave conditions (Biotage Initiator) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, dried over Na2SO4, and filtered. Evaporation of the solvent gave the crude product, which was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, followed by 5% methanol in dichloromethane, to give the title compound (6.04 g, 80%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.50 (s, 1H), 7.30 (s,1H), 6.25 (s, 1H), 3.89 (s, 2H), 1.88 – 2.04 (m, 6H), 1.08 – 1.25 (m, 6H), 0.89 (s, 6H). MS (ESI) m/z 305.2 $(M+H)^+$.

2-({3,5-Dimethyl-7-[(5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.13,7]dec-1-yl}oxy)ethanol (S-30). To a cooled (-78 ºC) solution of **S-29** (3.69 g, 12.1 mmol) in tetrahydrofuran (50 mL) was added n-BuLi (20 mL, 50 mmol, 2.5M in hexane). The mixture was stirred at -78 ºC for 1.5 hours. Iodomethane (10 mL) was added through a syringe, and the mixture was stirred at -78 ºC for 3 hours. The reaction mixture was then quenched with aqueous NH_4Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was purified by silica gel column chromatography, eluting with 5% methanol in dichloromethane, to give the title compound (3.5, 55%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.39 (d, *J* = 1.6 Hz, 1H), 5.99 (s, 1H), 3.80 (s, 2H), 3.66 (s, 3H), 3.52 – 3.46 (m, 3H), 1.44 (s, 2H), 1.41 – 1.28 (m, 6H), 1.24 – 1.01 (m, 6H), 0.90 (s, 6H). MS (ESI) m/z 319.5 $(M+H)^+$.

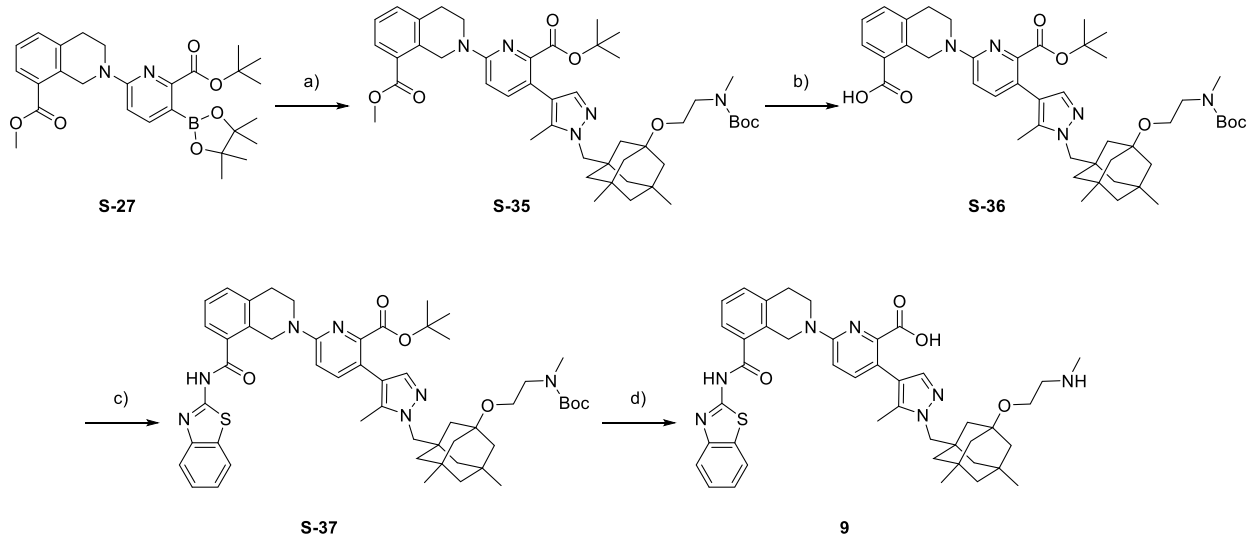
1-({3,5-Dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.13,7]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole (S-31). To a solution of **S-30** (3.5 g, 11 mmol) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g, 14.22 mmol). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with aqueous $NaHSO_3$, water, and brine. After drying over Na_2SO_4, the solution was filtered and concentrated and the residue was purified by silica gel chromatography (20% ethyl acetate in dichloromethane) to give the title compound (2.5 g, 51%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.45 (s, 1H), 3.89 (s, 2H), 3.67 (t, *J* = 5.7 Hz, 2H), 3.50 (t, *J* = 5.7 Hz, 2H), 2.77 (s, 2H), 2.27 (d, *J* = 11.0 Hz, 3H), 1.48 – 1.27 (m, 6H), 1.25 – 1.00 (m, 6H), 0.90 (s, 6H). MS (ESI) m/z 445.3 $(M+H)^+$.

2-({3-[(4-Iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl}oxy)ethyl methanesulfonate (S-32). To a cooled solution of Example 1.1.6 (2.1 g, 4.73 mmol) in dichloromethane (30 mL) was added triethylamine (1.98 mL, 14.2 mmol) followed by methanesulfonyl chloride (0.37 mL, 4.73 mmol). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (300 mL) and washed with water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/z 523.4 (M+H)+ .

1-({3,5-Dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.13,7]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole (S-33). A solution of **S-32** (2.5 g, 4.79 mmol) in 2M methylamine in methanol (15 mL) was stirred at 100 ºC for 20 minutes under microwave conditions (Biotage Initiator). The reaction mixture was concentrated under vacuum. The residue was then diluted with ethyl acetate (400 mL) and washed with aqueous NaHCO3, water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/z 458.4 $(M+H)^+$.

tert-Butyl [2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl}oxy)ethyl]methylcarbamate (S-34). To a solution of **S-33** (2.2 g, 4.81 mmol) in tetrahydrofuran (30 mL) was added di-*tert*-butyl dicarbonate (1.26 g, 5.77 mmol) and a catalytic amount of 4-dimethylaminopyridine (0.59 g, 4.81 mmol). The mixture was stirred at room temperature for 1.5 hours and diluted with ethyl acetate (300 mL). The solution was washed with saturated aqueous $NaHCO_3$, water (60 mL), and brine (60 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound (1.4 g, 52% 3 steps). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 7.46 (q, *J* = 0.4 Hz, 1H), 3.89 (s, 2H), 3.48 (d, *J* = 6.0 Hz, 2H), 3.29 (t, *J* = 5.8 Hz, 2H), 2.90 (d, *J* = 0.8 Hz, 3H), 2.28 (t, *J* = 0.4 Hz, 3H), 1.46 (d, *J* = 0.8 Hz, 9H), 1.41 – 0.99 (m, 11H), 0.89 (d, *J* = 0.8 Hz, 6H). MS (ESI) m/z 558.5 $(M+H)^+$.

Synthesis of Compound 9



Chemical Structures 7. Reagents: a) **S-34**, 2.5 mol% $Pd_2(dba)_3$, 10 mol% 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane, K_3PO_4, 3:1 THF:H_2O, reflux 24 h, 88% yield; b) LiOH, methanol, water, THF, rt, ~100% yield; c) TFFH, iPr_2NEt, benzo[d]thiazol-2-amine, 60 °C, 73% yield; d) TFA, DCM, rt, 97% yield.

Methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (S-35). To a solution of **S-27** (4.94 g, 9.99 mmol) in tetrahydrofuran (60 mL) and water (20 mL) was added **S-34** (5.57 g, 9.99 mmol), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (412 mg, 0.999 mmol), tris(dibenzylideneacetone)dipalladium(0) (457 mg, 0.500 mmol), and K_3PO_4 (11 g, 50.0 mmol). The mixture was stirred at reflux for 24 hours. The reaction mixture was cooled, diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na2SO4. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound (7.0 g, 88%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.88 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.43 – 7.31 (m, 4H), 7.29 – 7.22 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 1H), 5.04 (s, 2H), 4.86 (s, 1H), 4.03 (t, *J* = 5.9 Hz, 2H), 3.93 (s, 3H), 3.82 (d, *J* = 12.7 Hz, 3H), 3.45 (t, *J* = 5.3 Hz, 3H), 3.23 (d, *J* = 5.7 Hz, 4H), 3.03 (t, *J* = 5.9 Hz, 2H), 2.14 (s, 3H), 2.04 (s, 5H), 1.46 – 1.39 (m, 18H), 1.36 – 1.01 (m, 12H), 0.90 (d, *J* = 4.1 Hz, 6H). MS (ESI) m/z 784.1 $(M+H)^+$.

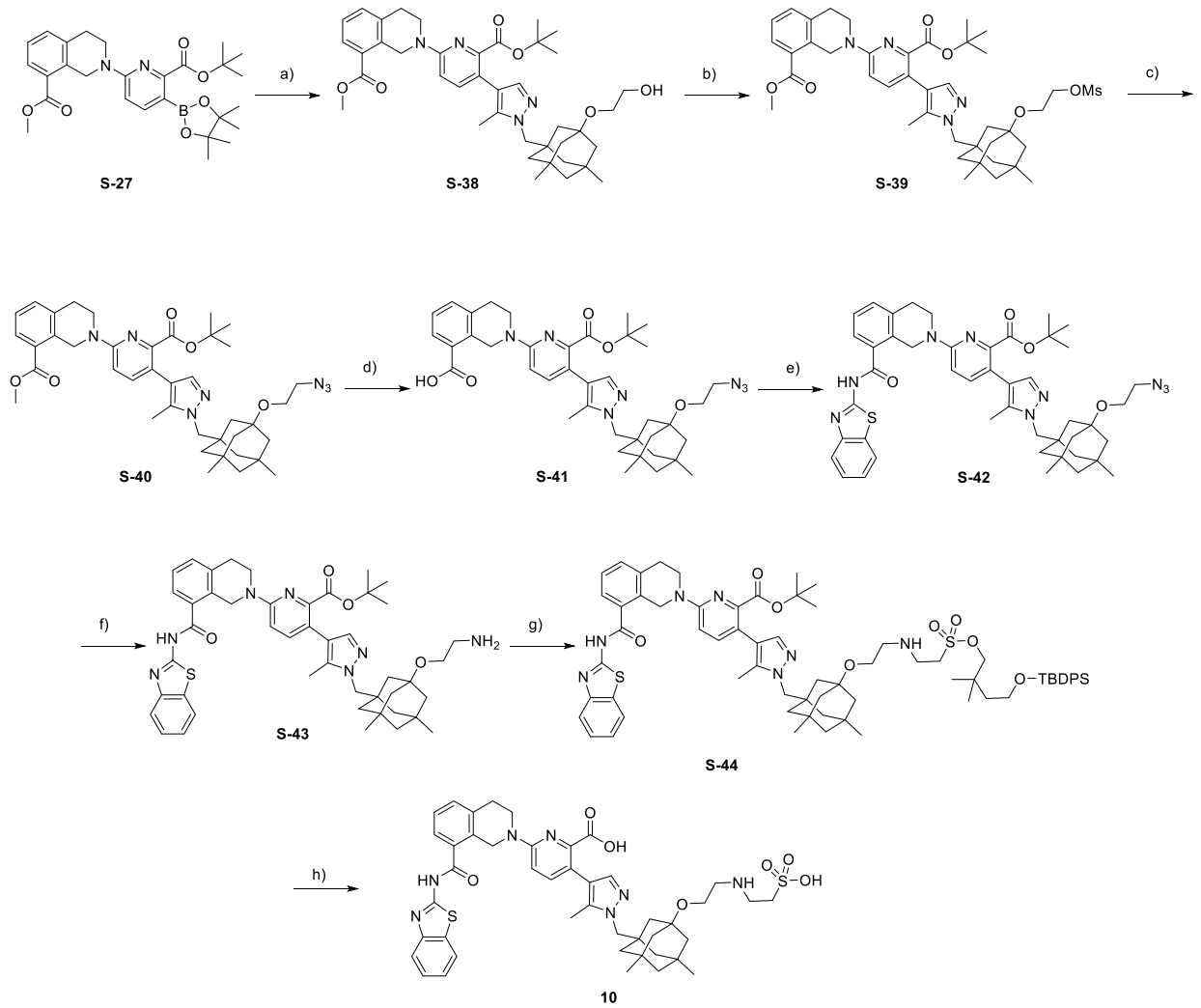
2-(6-(*tert*-Butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid (S-36). To a solution of **S-35** (10 g, 12.5 mmol) in tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g, 28.6 mmol). The mixture was stirred at room temperature for 24 hours. The reaction mixture was neutralized with 2% aqueous HCl and concentrated under vacuum. The residue was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the title compound (10g, ~100%), which was used without further purification. MS (ESI) m/z 785.1 $(M+H)^+$.

***tert*-Butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(tert-butoxycarbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.13,7]dec-1-yl)methyl]-5-methyl-**

1H-pyrazol-4-yl}pyridine-2-carboxylate (S-37). To a solution of **S-36** (3.16 g, 4.03 mmol) in N,N-dimethylformamide (20 mL) was added benzo[d]thiazol-2-amine (1.82 g, 12.09 mmol), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (2.13 g, 8.06 mmol) and N,N-diisopropylethylamine (2.28 mL, 16.1 mmol). The mixture was stirred at 60 ºC for 3 hours. The reaction mixture was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound (2.7 g, 73%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.84 (s, 1H), 8.02 (d, *J* = 7.4 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.50 – 7.41 (m, 3H), 7.35 (q, *J* = 7.3 Hz, 2H), 7.21 (s, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 4.96 (s, 2H), 3.82 (d, *J* = 6.1 Hz, 4H), 3.40 (t, *J* = 5.8 Hz, 2H), 3.18 (t, *J* = 5.8 Hz, 2H), 3.02 (t, *J* = 6.0 Hz, 2H), 2.78 (d, *J* = 12.6 Hz, 3H), 2.08 (s, 3H), 1.36 (s, 11H), 1.30 – 0.92 (m, 18H), 0.82 – 0.81 (m, 7H). MS (ESI) m/z 915.5 $(M+H)^+$.

6-[8-(1,3-Benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.13,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (9). To a solution of **S-37** (1.9 g, 2.07 mmol) in dichloromethane (20 mL) was added trifluoroa-cetic acid (10 mL). The mixture was stirred overnight. The solvent was evaporated under vacuum, and the residue was dissolved in dimethyl sulfoxide/methanol (1:1, 10 mL), and chromatographed via reverse-phase using an Analogix system and a C18 cartridge (300 g), eluting with 10-85% acetonitrile and 0.1% trifluoroacetic acid in water, to give the title compound as a TFA salt (1.75 g, 97%). ^{1}H NMR (300 MHz, dimethylsulfoxide-d6) □ ppm 12.85 (s, 1H), 8.13-8.30 (m, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.32-7.54 (m, 3H), 7.28 (d, 1H), 6.96 (d, 1H), 4.96 (dd, 1H), 3.80-3.92 (m, 4H), 3.48-3.59 (m, 1H), 2.91-3.11 (m, 2H), 2.51-2.59 (m, 4H), 2.03-2.16 (m, 2H), 1.21-1.49 (m, 6H), 0.97-1.20 (m, 4H), 0.87 (s, 6H). MS (ESI) m/z 760.4 $(M+H)^+$.

Synthesis of Compound 10



Chemical Structures 8. Reagents: a) 2.5 mol% $Pd_2(dba)_3$, 10 mol% **S-17**, **S-31**, K_3PO_4, 3:1 THF:H_2O, 90 °C, 78% yield; b) CH_3SO_2Cl, Et_3N, DCM, c) NaN_3, N,N-DMF, 80 °C, 77% yield for 2 steps; d) LiOH, methanol, water, THF, rt; e) TFFH, iPr_2NEt, benzo[d]thiazol-2-amine, 60 °C, 66% yield for 2 steps; f) 1 atm H_2, 10% Pd/C, THF, rt, 92% yield; g) 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutylethenesulfonate, DCM, rt, 71% yield; h) TFA, DCM, rt, 91% yield.

Methyl 2-[6-(tert-butoxycarbonyl)-5-(1-{[3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (S-43). To a solution of S-27 (10.78 g, 21.8 mmol) in tetrahydrofuran (120 mL) and water (40 mL) was added **S-31** (9.69 g, 21.8 mmol), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane (900 mg, 2.18 mmol), tris(dibenzylideneacetone)dipalladium(0) (999 mg, 1.1 mmol) and potassium phosphate tribasic (23.2 g, 109 mmol). The mixture was refluxed overnight, cooled, and diluted with ethyl acetate (500 mL). The resulting mixture was washed with water and brine, and the organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluted with 20% ethyl acetate in heptanes followed by 5% methanol in dichloromethane, to provide the title compound (11.6 g, 78%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.88 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.38 (dd, *J* = 17.5, 8.7 Hz, 3H), 7.29 – 7.22 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 1H), 5.04 (s, 2H), 4.03 (t, *J* = 5.9 Hz, 2H), 3.93 (s, 3H), 3.85 (s, 2H),

3.73 – 3.63 (m, 3H), 3.51 (dd, *J* = 5.3, 4.0 Hz, 3H), 3.03 (t, *J* = 5.9 Hz, 2H), 2.14 (s, 3H), 2.04 (s, 4H), 1.42 (s, 10H), 1.25 (d, *J* = 7.6 Hz, 16H), 0.91 (d, *J* = 4.1 Hz, 8H). MS (ESI) m/z 685.03 $(M+H)^+$.

Methyl 2-[6-(tert-butoxycarbonyl)-5-{1-[(3-{2-[(methanesulfonyl)oxy]ethoxy}-5,7-dimethyladamantan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridin-2-yl]-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (S-44). To a cold solution of **S-43** (14.94 g, 21.8 mmol) in dichloromethane (100 mL) in an ice-bath was sequentially added triethylamine (6.08 mL, 43.6 mmol) and methanesulfonyl chloride (2.55 mL, 32.7 mmol). The reaction mixture was stirred at room temperature for 1.5 hours, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound, which was used in the next step without further purification. MS (ESI) m/z 763.16 $(M+H)^+$.

Methyl 2-[5-(1-{[3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (S-45). To a solution of **S-44** (16.5 g, 21.6 mmol) in N,N-dimethylformamide (120 mL) was added sodium azide (4.22 g, 94.9 mmol). The mixture was heated at 80 °C for 3 hours, cooled, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluted with 20% ethyl acetate in heptanes, to provide the title compound (11.8 g, 77% 2 steps). ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 7.89 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.45 – 7.31 (m, 3H), 7.30 – 7.23 (m, 2H), 6.85 (d, *J* = 8.7 Hz, 1H), 5.30 (s, 3H), 5.04 (s, 2H), 4.03 (t, *J* = 5.9 Hz, 2H), 3.93 (s, 3H), 3.85 (s, 2H), 3.61 (t, *J* = 5.1 Hz, 2H), 3.29 (t, *J* = 5.1 Hz, 2H), 3.03 (t, *J* = 5.8 Hz, 2H), 2.14 (s, 3H), 1.50 (s, 2H), 1.42 (s, 9H), 1.35 – 1.02 (m, 8H), 0.91 (s, 6H). MS (ESI) m/z 710.2 $(M+H)^+$.

2-[5-(1-{[3-(2-Azidoethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid (S-46). To a solution of **S-45** (10 g, 14.09 mmol) in a mixture of tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g, 42.3 mmol). The mixture was stirred at room temperature overnight and neutralized with 2% aqueous HCl. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate (800 mL) and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound (9.7 g, 97%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.01 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.50 – 7.36 (m, 3H), 7.34 – 7.23 (m, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 5.08 (s, 2H), 4.03 (t, *J* = 5.9 Hz, 2H), 3.88 (s, 2H), 3.60 (t, *J* = 5.0 Hz, 2H), 3.28 (t, *J* = 5.0 Hz, 2H), 3.04 (t, *J* = 5.9 Hz, 2H), 2.13 (d, *J* = 6.8 Hz, 3H), 1.51 (s, 2H), 1.40 (d, *J* = 15.1 Hz, 13H), 1.31 – 1.01 (m, 8H), 0.91 (s, 7H). MS (ESI) m/z 696.1 $(M+H)^+$.

tert-Butyl 3-(1-{[3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylate (S-47). A mixture of S-46 (10 g, 14.4 mmol), benzo[d]thiazol-2-amine (3.24 g, 21.6 mmol), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (5.69 g, 21.6 mmol) and N,N-diisopropylethylamine (5.57 g, 43.1 mmol) in N,N-dimethylformamide (20 mL) was heated at 60 °C for 3 hours, cooled and diluted with ethyl acetate. The resulting mixture was washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluted with 20% ethyl acetate in dichloromethane to give the title compound (7.9 g, 66% for 2 steps). ^{1}H NMR (500 MHz, chloroform-*d*) δ ppm 7.82 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.52 (ddd, *J* = 22.3, 7.6, 1.2 Hz, 1H), 7.40 – 7.33 (m, 2H), 7.32 – 7.16 (m, 6H), 7.16 – 7.06 (m, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 5.01 (s, 2H), 4.12 (q, *J* = 7.1 Hz, 1H), 3.99 (t, *J* = 6.0 Hz, 2H), 3.83 (s, 2H), 3.59 (dd, *J* = 5.5, 4.6 Hz, 2H), 3.35 – 3.24 (m,

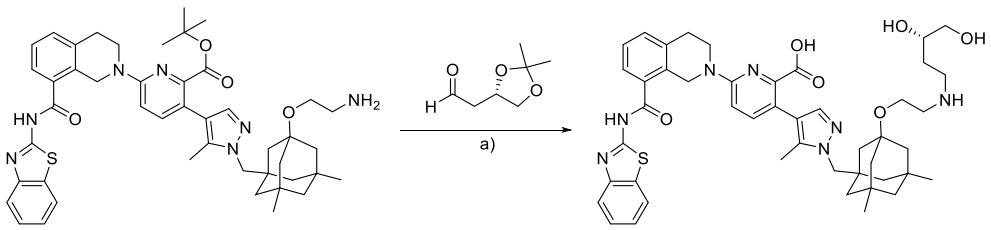
2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.11 (s, 3H), 2.04 (s, 1H), 1.48 (s, 2H), 1.36 (d, *J* = 8.8 Hz, 13H), 1.30 – 0.98 (m, 7H), 0.90 (s, 6H). MS (ESI) m/z 828.1 $(M+H)^+$.

tert-Butyl 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylate (S-48). To a solution of **S-47** (2.0 g, 2.41 mmol) in tetrahydrofuran (30 mL) was added Pd/C (10%, 200 mg). The mixture was stirred under a hydrogen atmosphere (18 psi) overnight. The insoluble material was filtered off and the filtrate was concentrated to provide the title compound (1.78 g, 92%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 7.89 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.72 – 7.60 (m, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.40 – 7.16 (m, 5H), 6.90 (d, *J* = 8.8 Hz, 1H), 5.75 (s, 1H), 5.02 (s, 2H), 3.93 – 3.78 (m, 4H), 3.33 (t, *J* = 5.6 Hz, 2H), 3.00 (t, *J* = 6.0 Hz, 2H), 2.66 (t, *J* = 5.6 Hz, 2H), 2.50 (p, *J* = 1.9 Hz, 2H), 2.08 (s, 3H), 1.39 (s, 2H), 1.29 – 0.92 (m, 21H), 0.83 (s, 6H). MS (ESI) m/z 802.2 $(M+H)^+$.

tert-Butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[(2,2,7,7-tetramethyl-10,10-dioxido-3,3-diphenyl-4,9-dioxa-10□6-thia-13-aza-3-silapentadecan-15-yl)oxy]tricyclo[3.3.1.13,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylate (S-49). To a solution of **S-48** (1.0 g, 1.25 mmol) in dichloromethane (6 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutylethenesulfonate (670 mg, 1.50 mmol). The reaction was stirred at room temperature for 2 days. The reaction was applied directly to a silica gel column (75 g SiO_2) and eluted with 1.5 to 3.0 % methanol in dichloromethane to provide the title compound (1.1 g, 71%). 1H NMR (500 MHz, chloroform-*d*) δ ppm 7.88 – 7.82 (m, 1H), 7.73 – 7.65 (m, 4H), 7.55 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.49 – 7.37 (m, 9H), 7.34 – 7.28 (m, 3H), 7.28 – 7.20 (m, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 5.06 (s, 2H), 4.06 (t, *J* = 6.0 Hz, 2H), 3.98 (s, 2H), 3.85 (s, 2H), 3.76 (t, *J* = 6.6 Hz, 2H), 3.52 (t, *J* = 5.2 Hz, 2H), 3.27 (t, *J* = 6.8 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H), 3.06 (t, *J* = 5.9 Hz, 2H), 2.75 (t, *J* = 5.2 Hz, 2H), 2.14 (s, 3H), 1.63 (t, *J* = 6.7 Hz, 2H), 1.42 – 1.35 (m, 14H), 1.29 – 1.20 (m, 4H), 1.08 (s, 12H), 0.99 (s, 6H), 0.92 (s, 6H). MS (ESI+) m/z 1248.4 $(M+H)^+$.

6-[8-(1,3-Benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.13,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (10). S-49 (4.0 g, 3.2 mmol) in dichloromethane (40 mL) was treated with trifluoroacetic acid (9.4 mL) overnight. The reaction mixture was concentrated and purified by reverse phase chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound (2.5 g, 91%). ^{1}H NMR (500 MHz, dimethyl sulfoxide-d6) δ ppm 12.86 (s, 1H), 8.32 (s, 2H), 8.02 (d, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.51 (d, 1H), 7.40-7.49 (m, 2H), 7.31-7.39 (m, 2H), 7.27 (s, 1H), 6.95 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.15-3.25 (m, 2H), 3.03-3.13 (m, 2H), 3.00 (t, 2H), 2.79 (t, 2H), 2.09 (s, 3H), 1.39 (s, 2H), 1.22-1.34 (m, 4H), 0.94-1.18 (m, 6H), 0.85 (s, 6H). MS (ESI) m/z 854.1 $(M+H)^+$.

Synthesis of Compound 11

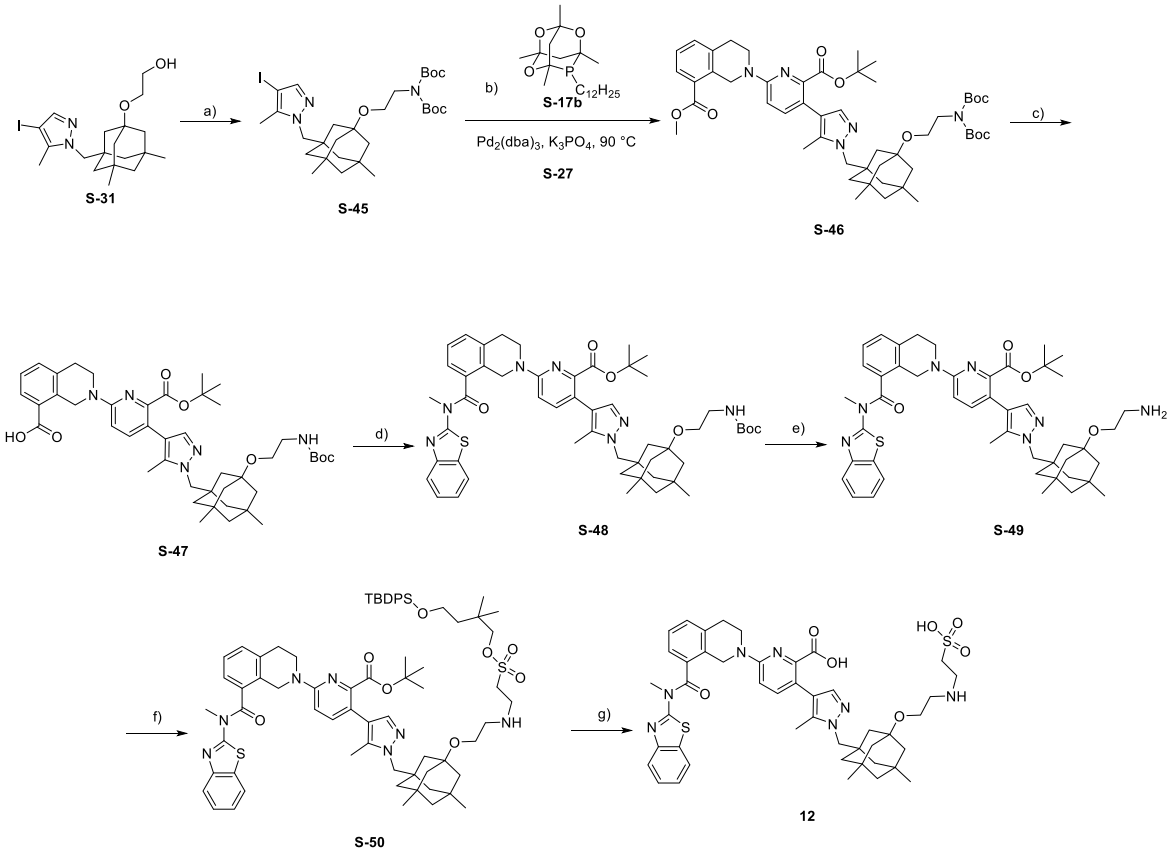


S-43 **11**

Chemical Structures 9. Reagents: a) i. $NaBH(OAc)_3$, dichloromethane, rt; ii. trifluoroacetic acid, rt, 29 % yield.

6-{8-[(1,3-Benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-3-(1-{[3-(2-{[(3S)-3,4-dihydroxybutyl]amino}ethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (11). To a solution of **S-48** (213 mg, 0.266 mmol) in dichloromethane (2 mL) was added (S)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (42 mg, 0.291 mmol). After stirring at room temperature for 30 minutes, sodium triacetoxyborohydride (144 mg, 0.679 mmol) was added. The reaction mixture was stirred at room temperature overnight. Trifluoroacetic acid (2 mL) was added and stirring was continued overnight. The reaction mixture was concentrated, and the residue was purified by reverse-phase HPLC using a Gilson system (Phenomenex® Luna ® C18 250 × 50 mm column), eluted with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid (100 mL/minute). The desired fractions were combined and freeze-dried to provide the title compound as a TFA salt (65 mg, 29% yield). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (s, 1H), 8.22 (d, 2H), 8.05 – 8.01 (m, 1H), 7.79 (d, 1H), 7.61 (d, 1H), 7.53 – 7.41 (m, 3H), 7.36 (td, 2H), 7.28 (s, 1H), 6.95 (d, 1H), 4.95 (s, 2H), 3.88 (t, 2H), 3.82 (s, 2H), 3.26 – 2.94 (m, 7H), 2.10 (s, 3H), 1.84 – 1.75 (m, 1H), 1.52-1.63 (m, 1H), 1.45 – 1.23 (m, 6H), 1.19 – 0.96 (m, 7H), 0.86 (s, 6H); MS (ESI) m/z 834.3 $(M+H)^+$.

Synthesis of Compound 12



Chemical Structures 10. Reagents: a) i. $MeSO_2Cl$, Et_3N, DCM; ii. $HN(Boc)_2$, Cs_2CO_3, CH_3CN, reflux, 97 % yield; b) 2.5 mol% $Pd_2(dba)_3$, 10 mol% **S-17b**, K_3PO_4, 1:1 1,4-dioxane:H_2O, 90 °C, 55% yield; c) LiOH•H_2O, THF, methanol, water, 91% yield; d) N-methylbenzo[d]thiazol-2-amine, DMAP, EDCI, DCM,

77%; e) Trifluoroacetic acid, DCM; f) 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutylethenesulfonate, iPr_2NEt, 58% yield, 2 steps; g) Trifluoroacetic acid, DCM, rt, 76% yield.

tert-Butyl (tert-butoxycarbonyl)(2-(((1s,3r,5R,7S)-3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)carbamate (S-45). To a cold (0 °C) solution of **S-31** (5.0 g, 11.25 mmol) in dichloromethane (50 mL) was added triethylamine (2.125 mL, 15.25 mmol) and methanesulfonyl chloride (1.150 mL, 14.76 mmol) dropwise. The reaction was warmed to room temperature and quenched by the addition of 50 mL saturated aqueous $NaHCO_3$ solution. The layers were separated, and the organic was washed with brine (50 mL). The combined aqueous layer was extracted with 50 mL dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in acetonitrile (50 mL). $HN(Boc)_2$ (2689 mg, 12.38 mmol) and Cs_2CO_3 (7332 mg, 22.50 mmol) were added, and the mixture was heated to reflux overnight. The reaction was cooled and diluted with diethyl ether (100 mL) and water (100 mL). The layers were separated, and the organic was washed with brine (100 mL). The combined aqueous layer was extracted with diethyl ether (100 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 10% ethyl acetate in heptanes to give the title compound (6.99 g, 97%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.45 (s, 1H), 3.87 (d, J = 3.4 Hz, 2H), 3.71 (t, J = 6.1 Hz, 2H), 3.53 (t, J = 6.1 Hz, 2H), 2.29 (s, 2H), 1.52 (s, 18H), 1.40 – 1.25 (m, 6H), 1.23 – 1.00 (m, 6H), 0.90 (s, 6H). MS (DCI+) m/z 644.2 $(M+H)^+$.

Methyl 2-(5-(1-(((1r,3s,5R,7S)-3-(2-(bis(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (S-46). To a solution of **S-27** (1.23 g, 2.49 mmol) and **S-45** (1.59 g, 2.47 mmol) in tetrahydrofuran (24 mL) in a 3-neck 100 mL flask fitted with with a condenser, septum and nitrogen inlet was added K_3PO_4 (2.7 g, 12.72 mmol), (1S,3R,5R,7S)-1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (105 mg, 0.254 mmol) and water (7.5 mL). The headspace of the reaction vessel was flushed with nitrogen gas, and $Pd_2(dba)_3$ (120 mg, 0.131 mmol) was added. The reaction was heated to 65 °C under nitrogen overnight. The reaction was cooled to room temperature and partitioned between ethyl ether (100 mL) and water (100 mL). The layers were separated, and the organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (100 g SiO_2), eluting with a gradient of 25 % to 35% ethyl acetate in heptane to give the title compound as a foam (1.20 g, 55%). ^{1}H NMR (501 MHz, chloroform-*d*) δ ppm 7.88 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.40 – 7.37 (m, 2H), 7.35 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.28 – 7.24 (m, 1H), 6.84 (d, *J* = 8.8 Hz, 1H), 5.04 (s, 2H), 4.03 (t, *J* = 5.9 Hz, 2H), 3.93 (s, 3H), 3.82 (s, 2H), 3.70 (t, *J* = 6.3 Hz, 2H), 3.53 (t, *J* = 6.2 Hz, 2H), 3.03 (t, *J* = 5.9 Hz, 2H), 2.13 (s, 3H), 1.50 (s, 18H), 1.45 (d, *J* = 9.1 Hz, 2H), 1.41 (s, 9H), 1.38 – 1.29 (m, 4H), 1.29 – 1.18 (m, 5H), 1.08 (dt, *J* = 12.7, 1.9 Hz, 1H), 1.02 (d, *J* = 12.4 Hz, 1H), 0.89 (s, 6H). MS (ESI -) m/z 882.3 $(M-H)^-$.

2-(6-(*tert*-Butoxycarbonyl)-5-(1-(((1r,3s,5R,7S)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid (S-47). To a solution of **S-46** (1.18 g, 1.335 mmol) in tetrahydrofuran (5 mL) was added methanol (2.5 mL), water (2.5 mL), and $LiOH•H_2O$ (161 mg, 3.83 mmol). The reaction was stirred overnight at room temperature and then quenched by the addition of water (25 mL) and 2N aqueous HCl solution (1.92 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (40 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (50 g SiO_2), eluting with a gradient of 10 to 50% ethyl acetate in dichloromethane containing 1% by volume

acetic acid to give the title product as a foam (740 mg, 91%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.00 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.44 (s, 1H), 7.42 – 7.35 (m, 2H), 7.32 – 7.22 (m, 1H), 7.21 – 7.12 (m, 1H), 6.84 (d, *J* = 8.8 Hz, 1H), 5.08 (s, 2H), 4.90 (s, 1H), 4.04 (t, *J* = 5.9 Hz, 2H), 3.86 (s, 2H), 3.46 (t, *J* = 5.2 Hz, 2H), 3.23 (q, *J* = 5.4 Hz, 2H), 3.03 (t, *J* = 5.9 Hz, 2H), 2.35 (s, 2H), 2.14 (s, 3H), 1.44 (s, 9H), 1.42 (s, 9H), 1.39 – 1.28 (m, 4H), 1.29 – 1.14 (m, 4H), 1.14 – 1.00 (m, 2H), 0.89 (s, 6H). MS (ESI-) m/z 768.4 $(M-H)^-$.

***tert*-Butyl-6-(8-(benzo[d]thiazol-2-yl(methyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate (S-48)**. To a solution of **S-47** (390 mg, 0.463 mmol) and N-methylbenzo[d]thiazol-2-amine (73 mg, 0.445 mmol) in dichloromethane (6 mL) was added DMAP (97 mg, 0.794 mmol) and EDCI (153 mg, 0.798 mmol). The reaction was stirred at room temperature overnight and then concentrated under reduced pressure to an oil. The residue was purified by silica gel chromatography (20 g SiO2), eluting with 15% to 25% ethyl acetate in dichloromethane to give the title compound as a foam (330 mg, 77%). ^{1}H NMR (501 MHz, chloroform-*d*) δ ppm 7.90 – 7.81 (m, 2H), 7.46 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), 7.38 (s, 1H), 7.36 – 7.28 (m, 4H), 7.22 (dd, *J* = 6.3, 2.5 Hz, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 4.85 (s, 1H), 4.76 (s, 2H), 3.97 (t, *J* = 5.9 Hz, 2H), 3.81 (s, 2H), 3.67 (s, 3H), 3.44 (t, *J* = 5.2 Hz, 2H), 3.22 (q, *J* = 5.4 Hz, 2H), 3.04 (t, *J* = 5.9 Hz, 2H), 2.11 (s, 3H), 1.46 – 1.41 (m, 11H), 1.31 (s, 13H), 1.24 – 1.15 (m, 4H), 1.11 – 1.00 (m, 2H), 0.88 (s, 6H). MS (ESI+) m/z 916.1 $(M+H)^+$.

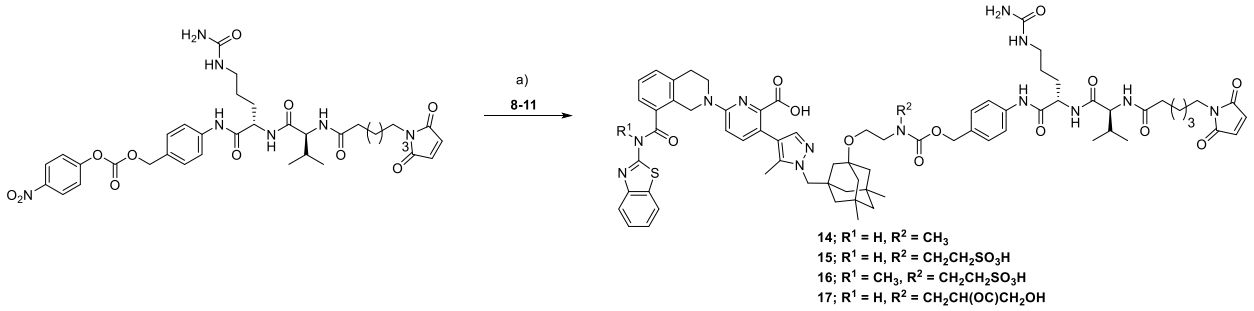
***tert*-Butyl-3-(1-(((1r,3s,5R,7S)-3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl(methyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate (S-49)**. To a solution of **S-48** (320 mg, 0.349 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (5 mL, 64.9 mmol). The reaction was stirred for 20 minutes at room temperature and was then concentrated under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and washed with saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the title product as a foam (275 mg), which was used in the subsequent step without further purification. ^{1}H NMR (501 MHz, chloroform-*d*) δ ppm 7.88 – 7.83 (m, 2H), 7.46 (td, *J* = 8.2, 7.7, 1.3 Hz, 1H), 7.38 – 7.28 (m, 5H), 7.22 (dd, *J* = 6.4, 2.5 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 4.76 (s, 2H), 3.97 (t, *J* = 5.9 Hz, 2H), 3.80 (s, 2H), 3.67 (s, 3H), 3.41 (t, *J* = 5.2 Hz, 2H), 3.04 (t, *J* = 5.9 Hz, 2H), 2.80 (t, *J* = 5.2 Hz, 2H), 2.10 (s, 3H), 1.31 (d, *J* = 10.7 Hz, 13H), 1.23 – 1.14 (m, 4H), 1.12 – 1.00 (m, 3H), 0.87 (s, 7H). MS (ESI+) m/z 816.2 $(M+H)^+$.

***tert*-Butyl-6-(8-(benzo[d]thiazol-2-yl(methyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((2-((4-((-butyldiphenylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate (S-50)**. To a solution of **S-49** (224 mg, 0.274 mmol) in dichloromethane (3 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutylethenesulfonate (192 mg, 0.430 mmol) and N-ethyl-N-isopropylpropan-2-amine (200 µL, 1.145 mmol). The reaction was stirred overnight, after which additional 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutylethenesulfonate (92 mg, 0.206 mmol) was added. The reaction stirred for 4 days and was then concentrated under reduced pressure to an oil. The residue was purified by silica gel chromatography (20 g SiO2), eluting with 1% to 2.5% methanol in dichloromethane to give the title compound (200 mg, 58%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.87 (t, *J* = 7.8 Hz, 2H), 7.69 – 7.64 (m, 4H), 7.50 – 7.29 (m, 12H), 7.23 (dd, *J* = 6.1, 2.7 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 1H), 4.77 (s, 2H), 4.01 – 3.93 (m, 4H), 3.82 (s, 2H), 3.73 (t, *J* = 6.7 Hz, 2H), 3.68 (s, 3H), 3.48 (t, *J* = 5.1 Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 3.07 (dt, *J* = 15.0, 6.4 Hz, 4H), 2.71 (t, *J* = 5.2 Hz, 2H), 2.11 (s, 3H),

1.60 (t, *J* = 6.7 Hz, 2H), 1.33 (d, *J* = 10.7 Hz, 13H), 1.25 – 1.15 (m, 4H), 1.12 – 1.00 (m, 11H), 0.96 (s, 7H), 0.89 (s, 7H). MS (DCI+) m/z 464.2 $(M+NH_4)^+$.

6-(8-(Benzo[d]thiazol-2-yl(methyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3R,5S,7s)-3,5-dimethyl-7-(2-((2-sulfoethyl)amino)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (12). To a solution of **S-50** (190 mg, 0.150 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (10 mL, 130 mmol). The reaction was stirred overnight and then concentrated under reduced pressure to an oil. The residue was dissolved in a solution of 3 mL MeOH, 2.5 mL N,N-dimethylformamide and 2.5 mL water. The solution was purified by RP-HPLC on a Phenomenex Luna 250 x 50 mm column, eluting with a gradient of 5 to 75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The fractions containing the product were lyophilized to give the title compound as a solid (113 mg, 76%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.33 (s, 2H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.54 – 7.47 (m, 2H), 7.44 – 7.33 (m, 4H), 7.28 (s, 1H), 7.03 (d, *J* = 8.9 Hz, 1H), 3.92 (s, 2H), 3.83 (s, 2H), 3.61 – 3.51 (m, 5H), 3.22 (q, *J* = 6.2 Hz, 2H), 3.10 (t, *J* = 5.8 Hz, 2H), 2.99 (d, *J* = 6.1 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H), 2.10 (s, 3H), 1.41 (s, 2H), 1.35 – 1.24 (m, 4H), 1.19 – 0.97 (m, 6H), 0.86 (s, 6H). MS (ESI+) m/z 868.2 $(M+H)^+$.

Synthesis of Payloads 14-17



Chemical Structures 11. Reagents: a) iPr_2NEt, N,N-DMF, rt, 49%-75% yields.

General Synthesis of Payloads 14-17. BCL-X_L inhibitor (117 mmol, **8-11**) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate (purchased from Synchem, 155 mmol) in N,N-dimethylformamide (7 mL) was cooled in an water-ice bath, and N,N-diisopropylethylamine (0.15 mL) was added. The mixture was stirred at 0 °C for 30 minutes and then at room temperature overnight. The reaction was purified by a reverse phase HPLC using a Gilson system, eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound as a TFA salt.

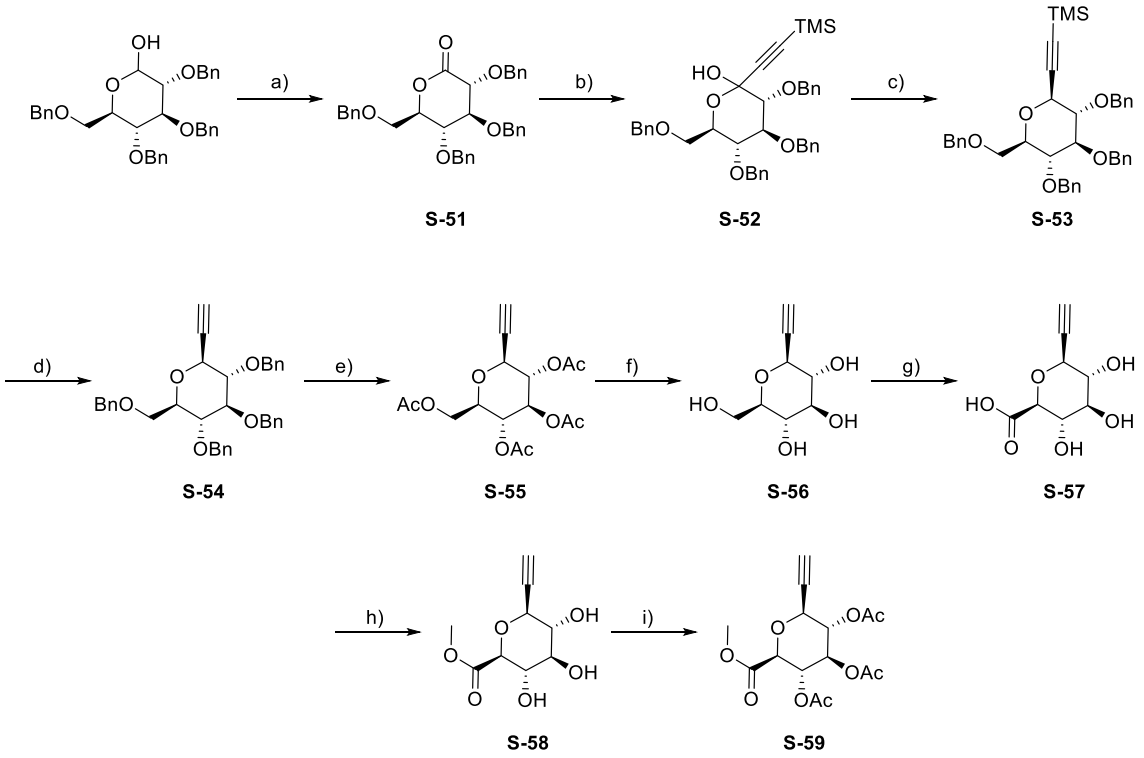
6-(8-(Benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (14). Isolated in 56% yield. ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (d, 1H), 9.98 (s, 1H), 7.96-8.10 (m, 2H), 7.74-7.83 (m, 2H), 7.54-7.64 (m, 3H), 7.31-7.52 (m, 6H), 7.24-7.29 (m, 3H), 6.99 (s, 2H), 6.94 (d, 1H), 4.96 (d, 4H), 4.33-4.43 (m, 2H), 4.12-4.24 (m, 2H), 3.22-3.42 (m, 7H), 2.77-3.07 (m, 7H), 1.86-2.32 (m, 7H), 0.92-1.70 (m, 22H), 0.72-0.89 (m, 13H). MS (ESI) m/z 1358.2 $(M+H)^+$.

6-(8-(Benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (15). Isolated in 75% yield. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 9.99 (s, 1H), 8.04 (t, 2H), 7.75-7.82 (m, 2H), 7.40-7.63 (m, 6H), 7.32-7.39 (m, 2H), 7.24-7.29 (m, 3H), 6.99 (s, 2H), 6.95 (d, 1H), 6.01 (s, 1H), 4.83-5.08 (m, 4H), 4.29-4.48 (m, 1H), 4.19 (t, 1H), 3.84-3.94 (m, 2H), 3.80 (d, 2H), 3.14-3.29 (m, 2H), 2.87-3.06 (m, 4H), 2.57-2.69 (m, 2H), 2.03-2.24 (m, 5H), 1.89-2.02 (m, 1H), 1.53-1.78 (m, 2H), 1.26-1.53 (m, 8H), 0.89-1.27 (m, 12H), 0.75-0.88 (m, 12H). MS (ESI) m/e 1452.2 $(M+H)^+$.

6-(8-(Benzo[d]thiazol-2-yl(methyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (16). Isolated in 69 % yield**.** ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 9.99 (s, 1H), 8.12 – 8.02 (m, 2H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.79 (dd, *J* = 8.8, 3.6 Hz, 1H), 7.59 (t, *J* = 8.2 Hz, 2H), 7.55 – 7.47 (m, 2H), 7.45 – 7.35 (m, 4H), 7.31 (s, 1H), 7.27 (d, *J* = 8.3 Hz, 2H), 7.07 – 6.97 (m, 3H), 5.15 – 4.57 (m, 4H), 4.40 (q, *J* = 7.4 Hz, 1H), 4.19 (q, *J* = 6.8 Hz, 1H), 3.92 (s, 2H), 3.81 (d, *J* = 10.2 Hz, 2H), 3.57 (s, 3H), 3.54 – 3.31 (m, 4H), 3.25 (t, *J* = 5.9 Hz, 2H), 3.09 – 2.91 (m, 3H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.23 – 2.06 (m, 5H), 2.02 – 1.92 (m, 1H), 1.69 (s, 1H), 1.59 (s, 1H), 1.53 – 1.28 (m, 8H), 1.27 – 1.01 (m, 12H), 1.01 – 0.89 (m, 1H), 0.88 – 0.77 (m, 12H). MS (ESI) m/z 1466.3 $(M+H)^+$.

6-(8-(Benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-(((S)-3,4-dihydroxybutyl)(((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (17). Isolated in 49% yield. ^{1}H NMR (600 MHz, Pyridine-d_5) δ 10.93 – 10.81 (m, 2H), 9.55 (d, *J* = 8.1 Hz, 1H), 8.16 – 8.07 (m, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 8.00 – 7.95 (m, 2H), 7.80 (dd, *J* = 7.3, 1.5 Hz, 1H), 7.55 – 7.48 (m, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.38 – 7.33 (m, 1H), 7.32 – 7.25 (m, 2H), 7.01 – 6.93 (m, 2H), 6.79 (s, 2H), 5.93 (s, 8H), 5.43 (s, 1H), 5.34 – 5.21 (m, 4H), 5.03 (dd, *J* = 8.7, 7.5 Hz, 1H), 4.15 (s, 1H), 4.02 – 3.90 (m, 3H), 3.90 – 3.64 (m, 5H), 3.58 (s, 2H), 3.46 (t, *J* = 7.2 Hz, 2H), 3.25 – 3.16 (m, 1H), 2.89 (t, *J* = 5.9 Hz, 2H), 2.47 – 2.33 (m, 3H), 2.30 (s, 3H), 2.24 (s, 2H), 2.07 – 1.89 (m, 2H), 1.77 (dqd, *J* = 44.1, 16.2, 13.7, 7.7 Hz, 4H), 1.60 (s, 2H), 1.56 – 1.46 (m, 2H), 1.33 – 1.16 (m, 11H), 1.14 (d, *J* = 6.7 Hz, 3H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.93 – 0.72 (m, 8H). MS (ESI) m/z 1434.5 $(M+H)^+$.

Synthesis of Intermediate S-59



Chemical Structures 12. Reagents: a) Ac_2O, DMSO, 97% yield; b) HCCTMS, nBuLi, -70 °C; c) Et_3SiH, BF_3•OEt_2, CH_3CN, DCM, -15 °C, 66% yield 2 steps; d) NaOH, methanol, DCM, 93% yield; e) Ac_2O, BF_3•OEt_2, 74% yield; f) NaOMe, methanol, 87% yield; g) nBu_4Br, TEMPO, saturated aqueous $NaHCO_3$, 10% w/w aqueous NaOCl solution; h) SO_2Cl_2, methanol, -1 °C, 27% yield over 2 steps; i) Ac_2O, 4-DMAP, N,N-DMF, 87% yield.

(3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-6-[(benzyloxy)methyl]oxan-2-one (S-51). To a mixture of (3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-[(benzyloxy)methyl]oxan-2-ol (75 g, 139 mmol) in dimethyl sulfoxide (400 mL) at 0 °C was added acetic anhydride (225 mL, 2380 mmol). The mixture was stirred for 16 hours at room temperature before it was cooled to 0 °C. A large volume of water was added and stirring was stopped allowing the reaction mixture to settle for 3 hours (the crude lactone migrated to the bottom of the flask). The supernatant was removed, and the crude mixture was diluted with ethyl acetate and was washed 3 times with water, neutralized with a saturated aqueous mixture of $NaHCO_3$, and washed again twice with water. The organic layer was then dried over magnesium sulfate, filtered, and concentrated to give the title compound (73.2 g, 97%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 2.05 (d, *J*=1.47 Hz, 6H), 2.10 (s, 3H), 3.38 (s, 3H), 3.40-3.51 (m, 2H), 3.53-3.60 (m, 4H), 3.61-3.74 (m, 27H), 4.00 (d, *J* = 9.90 Hz, 1H), 4.54 (d, *J* = 9.41 Hz, 1H), 4.82-4.93 (m, 2H), 5.17-5.34 (m, 3H), 6.99 (br t, *J* = 5.75 Hz, 1H), 7.77-7.82 (m, 1H), 8.24 (dq, *J* = 4.49, 2.29 Hz, 2H). MS (ESI) *m/z* 561 $(M+Na)^+$.

(3R,4S,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-2-((trimethylsilyl)ethynyl)tetrahydro-2H-pyran-2-ol (S-52). To a mixture of ethynyltrimethylsilane (18.23 g, 186 mmol) in tetrahydrofuran (400 mL) under nitrogen and chilled in a dry ice/acetone bath (internal temp -65 °C) was added 2.5 M butyllithium in hexane (55.7 mL, 139 mmol) dropwise, keeping the temperature below -60 °C. The mixture was stirred in a cold bath for 40 minutes, followed by an ice-water bath (internal temperature rose to 0.4 °C) for 40 minutes, and finally cooled to -75 °C again. A mixture of **S-51** (50 g, 93 mmol) in tetrahydrofuran (50 mL) was added dropwise, keeping the internal temperature below -70 °C. The mixture was stirred in a dry

ice/acetone bath for an additional 3 hours. The reaction was quenched with saturated aqueous $NaHCO_3$ (250 mL). The mixture was allowed to warm to room temperature, extracted with ethyl acetate (3 × 300 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give the title compound (58.2 g), which was used in the next step without further purification ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 0.18 - 0.26 (m, 9H), 3.66 - 3.81 (m, 4H), 3.84 - 3.96 (m, 1H), 4.00 - 4.06 (m, 1H), 4.50 - 4.59 (m, 2H), 4.61 - 4.70 (m, 1H), 4.76 - 4.96 (m, 4H), 5.03 - 5.11 (m, 1H), 7.16 (dd, *J* = 6.80, 2.85 Hz, 2H), 7.24 - 7.45 (m, 18H). MS (ESI) *m/z* 659 $(M+Na)^+$.

Trimethyl({(2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-[(benzyloxy)methyl]oxan-2-yl}ethynyl)silane (S-53). To a mixed mixture of **S-52** (60 g, 94 mmol) in acetonitrile (450 mL) and dichloromethane (150 mL) at -15 °C in an ice-salt bath was added triethylsilane (81 mL, 508 mmol) dropwise, followed by addition of boron trifluoride diethyl ether complex (40.6 mL, 320 mmol) at such a rate that the internal temperature did not exceed -10 °C. The mixture was then stirred at -15 °C to -10 °C for 2 hours. The reaction was quenched with saturated aqueous $NaHCO_3$ (275 mL) and stirred for 1 hour at room temperature. The mixture was then extracted with ethyl acetate (3 × 550 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography eluted with a gradient of 0% to 7% ethyl acetate/petroleum ether to give the title compound (46.5 g, 66 % yield, 2 steps). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.37 – 7.19 (m, 18H), 7.12 – 7.06 (m, 2H), 5.00 (d, *J* = 10.4 Hz, 1H), 4.87 (d, *J* = 11.0 Hz, 1H), 4.82 – 4.73 (m, 3H), 4.58 (d, *J* = 12.2 Hz, 1H), 4.49 (dd, *J* = 11.5, 6.6 Hz, 2H), 4.02 (d, *J* = 8.9 Hz, 1H), 3.74 – 3.52 (m, 5H), 3.38 (d, *J* = 5.1 Hz, 1H), 0.14 (s, 9H). MS (ESI) *m/z* 643 $(M+Na)^+$.

(2*R*,3*R*,4*R*,5*S*,6*S*)-3,4,5-Tris(benzyloxy)-2-[(benzyloxy)methyl]-6-ethynyloxane (S-54). To a mixture of **S-53** (80 g, 129 mmol) in dichloromethane (200 mL) and methanol (1000 mL) was added 1 N aqueous NaOH (258 mL, 258 mmol). The mixture was stirred at room temperature for 2 hours and then concentrated. The residue was then partitioned between water and dichloromethane. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give the title compound (66 g, 93%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.39 – 7.22 (m, 18H), 7.13 (dd, *J* = 6.5, 2.9 Hz, 2H), 5.00 (d, *J* = 10.4 Hz, 1H), 4.91 (d, *J* = 11.0 Hz, 1H), 4.85 – 4.77 (m, 3H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 2H), 4.09 – 4.00 (m, 1H), 3.78 – 3.57 (m, 5H), 3.44 (s, 1H), 2.53 (d, *J* = 2.1 Hz, 1H). MS (ESI) *m/z* 571 $(M+Na)^+$.

(2*R*,3*R*,4*R*,5*S*,6*S*)-2-[(Acetyloxy)methyl]-6-ethynyloxane-3,4,5-triyl triacetate (S-55). To a mixture of **S-54** (66 g, 120 mmol) in acetic anhydride (500 mL) cooled by an ice/water bath was added boron trifluoride diethyl ether complex (152 mL, 1203 mmol) dropwise. The mixture was stirred at room temperature for 16 hours, cooled with an ice/water bath and neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with ethyl acetate (3 × 500 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluted with a gradient of 0% to 30% ethyl acetate/petroleum ether to give the title compound (33 g, 74%). ^{1}H NMR (chloroform-*d*, 400 MHz): d ppm 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.53 (d, *J* = 2.0 Hz, 1H), 3.68-3.72 (m, 1H), 4.12-4.16 (m, 1H), 4.20-4.28 (m, 2H), 5.09-5.23 (m, 3H). MS (ESI) *m/z* 357.1 $(M+H)^+$.

(2*S*,3*R*,4*R*,5*S*,6*R*)-2-Ethynyl-6-(hydroxymethyl)oxane-3,4,5-triol (S-56). To a mixture of **S-55** (25 g, 70.2 mmol) in methanol (440 mL) was added sodium methanolate (2.1 g, 39.9 mmol). The mixture was stirred at room temperature for 2 hours, and then neutralized with 4 M HCl in dioxane. The mixture was concentrated, and the residue was adsorbed onto silica gel and loaded onto a silica gel column. The column was eluted with a gradient of 0 to 100% ethyl acetate/petroleum ether then 0% to 12% methanol/ethyl acetate to give the title compound (12.8 g, 87%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.95 – 3.87 (m, 1H),

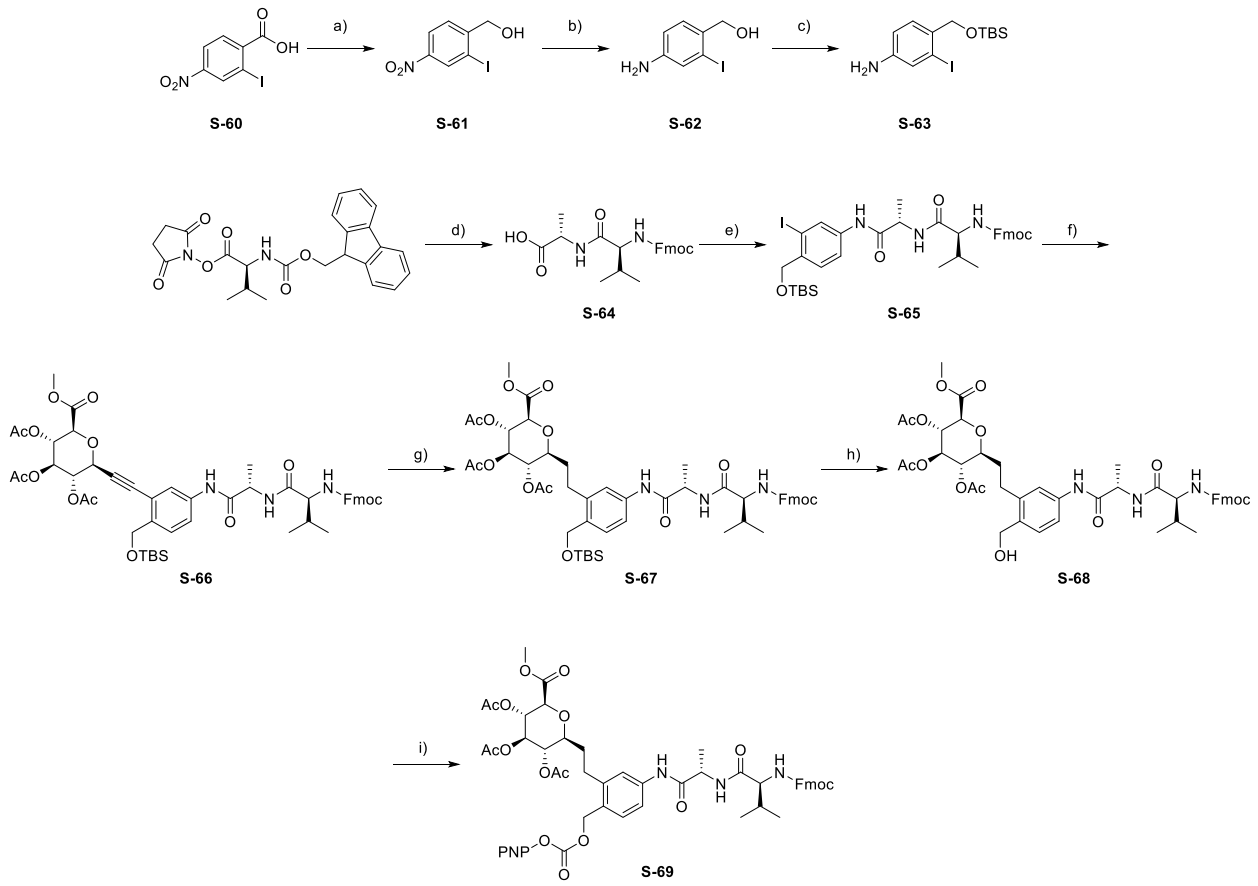
3.84 (dd, *J* = 12.1, 1.8 Hz, 1H), 3.63 (dd, *J* = 12.1, 5.3 Hz, 1H), 3.33 – 3.20 (m, 4H), 2.86 (d, *J* = 2.1 Hz, 1H). MS (ESI) *m/z* 211 $(M+Na)^+$.

(2*S*,3*S*,4*R*,5*R*,6*S*)-6-Ethynyl-3,4,5-trihydroxyoxane-2-carboxylic acid (S-57). A three-necked round bottom flask was charged with **S-56** (10.0 g, 53.1 mmol), KBr (0.506 g, 4.25 mmol), tetrabutylammonium bromide (0.685 g, 2.126 mmol) and 100 mL of saturated aqueous $NaHCO_3$. TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl, 0.249 g, 1.59 mmol) in dichloromethane (100 mL) was added. The mixture was stirred vigorously and cooled in an ice-salt bath to -2 °C internal temperature. A mixture of brine (12 mL), saturated aqueous $NaHCO_3$ (24 mL) and 13 weight % aqueous NaOCl (256 mL, 532 mmol) solution was added dropwise such that the internal temperature was maintained below 2 °C. The pH of the reaction mixture was maintained in the 8.2-8.4 range with the addition of solid Na_2CO_3. After a total of 6 hours, the reaction mixture was cooled to 3 °C internal temperature and ethanol (~20 mL) was added dropwise. The mixture was stirred for ~ 30 minutes. The mixture was transferred to a separatory funnel, and the dichloromethane layer was discarded. The pH of the aqueous layer was adjusted to 2-3 using 1 M aqueous HCl. The aqueous layer was then concentrated to dryness to afford a solid. Methanol (100 mL was) added to the dry solid, and the slurry was stirred for ~30 minutes. The mixture was filtered over a pad of diatomaceous earth, and the residue in the funnel was washed with ~100 mL of methanol. The filtrate was concentrated under reduced pressure to obtain the title compound (11 g), which was used in the next step without further purification. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.96 (dt, *J* = 6.6, 2.4 Hz, 1H), 3.56 (d, *J* = 9.5 Hz, 1H), 3.46 – 3.39 (m, 1H), 3.39 – 3.35 (m, 2H), 2.91 (d, *J* = 2.2 Hz, 1H). MS (ESI-) m/z 201.0 $(M-H)^-$.

Methyl (2*S*,3*S*,4*R*,5*R*,6*S*)-6-ethynyl-3,4,5-trihydroxyoxane-2-carboxylate (S-58). A 500 mL three-necked round bottom flask was charged with a suspension of **S-57** (11.0 g, 54.4 mmol) in methanol (100 mL), and the mixture was cooled in an ice-salt-bath with internal temperature of -1 °C. Neat thionyl chloride (4.05 mL, 55.5 mmol) was carefully added. The internal temperature kept rising throughout the addition but did not exceed 10 °C. The reaction was allowed to slowly warm up to 15-20 °C over 2.5 hours. After 2.5 hours, the reaction was concentrated. The residue was purified by silica gel chromatography, eluting with 10:1 ethyl acetate:methanol to give the title compound (3.3 g, 27% over two steps). ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 4.01 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.84 (d, *J* = 9.8 Hz, 1H), 3.78 (s, 3H), 3.56 – 3.51 (m, 1H), 3.40 – 3.34 (m, 2H), 2.96 (d, *J* = 2.1 Hz, 1H).

Methyl (2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-tris(acetyloxy)-6-ethynyloxane-2-carboxylate (S-59). To **S-58** (3.3 g, 15.26 mmol) as a mixture in *N*,*N*-dimethylformamide (75 mL) was added 4-(dimethylamino)pyridine (0.056 g, 0.458 mmol) and acetic anhydride (14.4 mL, 153 mmol). The suspension was cooled in an ice-bath and pyridine (7.41 mL, 92 mmol) was added via syringe over 15 minutes. The ice-bath was removed, and reaction warmed to room temperature and stirred overnight. The reaction was cooled in an ice-bath and 150 mL of saturated aqueous $NaHCO_3$ solution was added and stirred for 1 hour. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The organic extract was washed twice with saturated $CuSO_4$ mixture, dried, filtered, and concentrated. The residue was purified by flash chromatography, eluted with 50% ethyl acetate/petroleum ether to give the title compound (4.8 g, 87%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 5.30 (t, *J* = 9.5 Hz, 1H), 5.08 (td, *J* = 9.7, 3.1 Hz, 2H), 4.48 (dd, *J* = 9.9, 2.1 Hz, 1H), 4.23 (d, *J* = 10.0 Hz, 1H), 3.72 (s, 2H), 3.04 (d, *J* = 2.1 Hz, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H). MS ESI(+) m/z 359.9 $(M+NH_4)^+$.

Synthesis of Intermediate S-69



Chemical Structures 13. Reagents: a) BH_3•THF complex, 0 to 65 °C, THF, 90% yield; b) Fe, NH_4Cl, EtOH, 80 °C; c) TBS-Cl, 1*H*-imidazole, DCM, 0 °C, 65% yield for 2 steps; d) *S*-Alanine, $NaHCO_3$, water, THF, rt; 76% yield; e) **S-63**, EDQ, MeOH, DCM, 39% yield; f) **S-59**, CuI, $Pd(Ph_3P)_2Cl_2$, iPr_2NEt, N,N-DMF, rt, 73% yield; g) 5% Pt/C, 50 psi H_2, THF, 76% yield; h) AcOH, water, THF, rt, ~100% yield; i) bis(4-nitrophenyl) carbonate, iPr_2NEt, CH_3CN, rt, 46% yield.

2-Iodo-4-nitrobenzoic acid (S-60). A 3-L fully jacketed flask equipped with a mechanical stirrer, temperature probe and an addition funnel under a nitrogen atmosphere, was charged with 2-amino-4-nitrobenzoic acid (50 g, 275 mmol) and 1.5 M aqueous (500 mL) sulfuric acid. The resulting suspension was cooled to 0 °C internal temperature, and a mixture of sodium nitrite (20.83 g, 302 mmol) in water (250 mL) was added dropwise over 43 minutes with the temperature kept below 1 °C. The reaction was stirred at ca. 0 °C for 1 hour. A mixture of sodium iodide (70.0 g, 467 mmol) in water (250 mL) was added dropwise over 44 minutes with the internal temperature kept below 1 °C. (Initially addition was exothermic and there was gas evolution). The reaction was stirred 1 hour at 0 °C. The temperature was raised to 20 °C and then stirred at ambient temperature overnight. The reaction mixture became a suspension. The reaction mixture was filtered, and the collected solid was washed with water. The wet solid (~108 g) was stirred in 10% sodium sulfite (350 mL, with ~200 mL water used to wash in the solid) for 30 minutes. The suspension was acidified with concentrated hydrochloric acid (35 mL), and the solid was collected by filtration and washed with water. The solid was slurried in water (1 L) and re-filtered, and the solid was left to dry in the funnel overnight. The solid was then dried in a vacuum oven for 2 hours at 60 °C. The resulting solid was triturated with dichloromethane (500 mL), and the suspension was filtered and washed with additional

dichloromethane. The solid was air-dried to give the title compound (47.5 g, 59%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.63 (d, *J* = 2.0 Hz, 1H), 8.27 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H).

(2-Iodo-4-nitrophenyl)methanol (S-61). A flame-dried 3-L 3-necked flask was charged with **S-60** (54.78 g, 187 mmol) and tetrahydrofuran (800 mL). The mixture was cooled in an ice bath to 0.5 °C, and borane-tetrahydrofuran complex (467 mL, 467 mmol, 1 M in tetrahydrofuran) was added dropwise (gas evolution) over 50 minutes, reaching a final internal temperature of 1.3 °C. The reaction mixture was stirred for 15 minutes, and the ice bath was removed. The reaction was left to come to ambient temperature over 30 minutes. A heating mantle was installed, and the reaction was heated to an internal temperature of 65.5 °C for 3 hours, and then allowed to cool to room temperature while stirring overnight. The reaction mixture was cooled in an ice bath to 0 °C and quenched by dropwise addition of methanol (400 mL). After a brief incubation period, the temperature rose quickly to 2.5 °C with gas evolution. After the first 100 mL are added over ~30 minutes, the addition was no longer exothermic, and the gas evolution ceased. The ice bath was removed, and the mixture was stirred at ambient temperature under nitrogen overnight. The mixture was concentrated to a solid, dissolved in dichloromethane/methanol and adsorbed on to silica gel (~150 g). The residue was loaded on a plug of silica gel (3000 mL) and eluted with dichloromethane to give the title compound (46.9 g, 90%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.63 (d, *J* = 2.0 Hz, 1H), 8.24 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 4.72 (s, 2H), 2.43 (br s, 1H). MS (DCI) m/z 296.8 $(M+NH_4)^+$.

(4-Amino-2-iodophenyl)methanol (S-62). A 5-L flask equipped with a mechanical stirrer, heating mantle controlled by a *J*KEM temperature probe and a condenser was charged with **S-61** (98.83 g, 354 mmol) and ethanol (2 L). The reaction was stirred rapidly, and iron (99 g, 1771 mmol) was added, followed by a mixture of ammonium chloride (20.84 g, 390 mmol) in water (500 mL). The reaction was heated over the course of 20 minutes to an internal temperature of 80.3 °C, where it began to reflux vigorously. The mantle was dropped until the reflux calmed. Thereafter, the mixture was heated to 80 °C for 1.5 hours. The reaction was filtered hot through a membrane filter, and the iron residue was washed with hot 50% ethyl acetate/methanol (800 mL). The eluent was passed through a diatomaceous earth pad, and the filtrate was concentrated. The residue was partitioned between 50% brine (1500 mL) and ethyl acetate (1500 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (400 mL × 3). The combined organic layers were dried over sodium sulfate, filtered, and concentrated to give the title compound, which was used without further purification (88.9 g, 100%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 4.28 (d, *J*=5.26 Hz, 2 H) 4.98 (t, *J*=5.44 Hz, 1 H) 5.17 (br s, 2 H) 6.52 - 6.58 (m, 1 H) 7.03 - 7.10 (m, 2 H) 8.57 - 8.60 (m, 1 H). MS (DCI+) 266.9 $(M+NH_4)^+$.

4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-3-iodoaniline (S-63). A 5-L flask with a mechanical stirrer was charged with **S-62** (88 g, 353 mmol) and dichloromethane (2 L). The suspension was cooled in an ice bath to an internal temperature of 2.5 °C, and *tert*-butylchlorodimethylsilane (53.3 g, 353 mmol) was added portion-wise over 8 minutes. After 10 minutes, 1*H*-imidazole (33.7 g, 495 mmol) was added portionwise to the cold reaction. The reaction was stirred for 90 minutes while the internal temperature rose to 15 °C. The reaction mixture was diluted with water (3 L) and dichloromethane (1 L). The layers were separated, and the organic layer was dried over sodium sulfate, filtered, and concentrated to an oil. The residue was purified by silica gel chromatography (1600 g silica gel), eluted with a gradient of 0 - 25% ethyl acetate in heptane, to give the title compound (83.2 g, 65% 2 steps). %). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.23 (d, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 2.3 Hz, 1H), 6.69 (dd, *J* = 8.0 and 2.2 Hz, 1H), 4.58 (s, 2 H), 3.61 (s, 2 H), 0.96 (s, 9 H), and 0.13 (s, 6 H).

(2*S*)-2-{[(2*S*)-2-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-3-methylbutanoyl]amino}propanoic acid (S-64). To a mixture of (9*H*-fluoren-9-yl)methyl {(2*RS*)-1-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-methyl-1-oxobutan-2-yl}carbamate (5.5 g, 12.6 mmol) in dimethoxyethane (40 mL) was added (2*S*)-2-aminopropanoic acid (1.18 g, 13.2 mmol) and sodium bicarbonate (1.11 g, 13.2 mmol) in water (40 mL). Tetrahydrofuran (20 mL) was added to aid solubility. The resulting mixture was stirred at room temperature for 16 hours. Aqueous citric acid (15%, 75 mL) was added, and the mixture was extracted with 10% 2-propanol in ethyl acetate (2 × 100 mL). A precipitate formed in the organic layer. The combined organic layers were washed with water (2 × 150 mL). The organic layer was concentrated under reduced pressure and then triturated with diethyl ether (80 mL). After brief sonication, the title compound was collected by filtration and air-dried (4.0 g, 76%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 0.87 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 1.27 (d, *J* = 7.6 Hz, 3H), 1.94 - 2.00 (m, 1H), 3.90 (dd, *J* = 7.2, 9.2 Hz, 1H), 4.17 - 4.31 (m, 4H), 7.30 - 7.44 (m, 5H), 7.75 (t, *J* = 6.6 Hz, 2H), 7.89 (d, *J* = 7.6 Hz, 2H), 8.21 (d, *J* = 7.2 Hz, 1H), 12.48 (bs, 1H). MS (ESI) *m/z* 411 $(M+H)^+$.

(9*H*-Fluoren-9-yl)methyl [(2*S*)-1-({(2*S*)-1-[4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-iodoanilino]-1-oxopropan-2-yl}amino)-3-methyl-1-oxobutan-2-yl]carbamate (S-65). To a mixture of **S-64** (5.44 g, 14.98 mmol) and **S-70** (6.15 g, 14.98 mmol) in a mixture of dichloromethane (70 mL) and methanol (35.0 mL) was added ethyl 2-ethoxyquinoline-1(2*H*)-carboxylate (4.08 g, 16.48 mmol), and the reaction mixture was stirred overnight. The reaction mixture was concentrated, and the residue was loaded onto silica gel, eluted with a gradient of 10% to 95% ethyl acetate in heptane followed by 5% methanol in dichloromethane. The product-containing fractions were concentrated, and the residue was dissolved in 0.2% methanol in dichloromethane (50 mL) and loaded onto silica gel eluted with a gradient of 0.2% to 2% methanol in dichloromethane. The product containing fractions were collected to give the title compound (4.4 g, 39%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 10.01 (s, 1H), 8.16 (d, *J* = 1.9 Hz, 1H), 7.88 – 7.83 (m, 2H), 7.70 (dd, *J* = 7.4, 5.4 Hz, 2H), 7.52 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.42 – 7.34 (m, 3H), 7.33 – 7.25 (m, 4H), 4.52 (s, 2H), 4.40 – 4.23 (m, 2H), 4.22 – 4.15 (m, 2H), 3.87 (dd, *J* = 8.8, 7.1 Hz, 1H), 1.95 (dp, *J* = 14.1, 7.0 Hz, 1H), 1.27 (d, *J* = 7.2 Hz, 3H), 0.92 – 0.79 (m, 15H), 0.06 (s, 6H). MS (ESI) *m/z* 756.0 $(M+H)^+$.

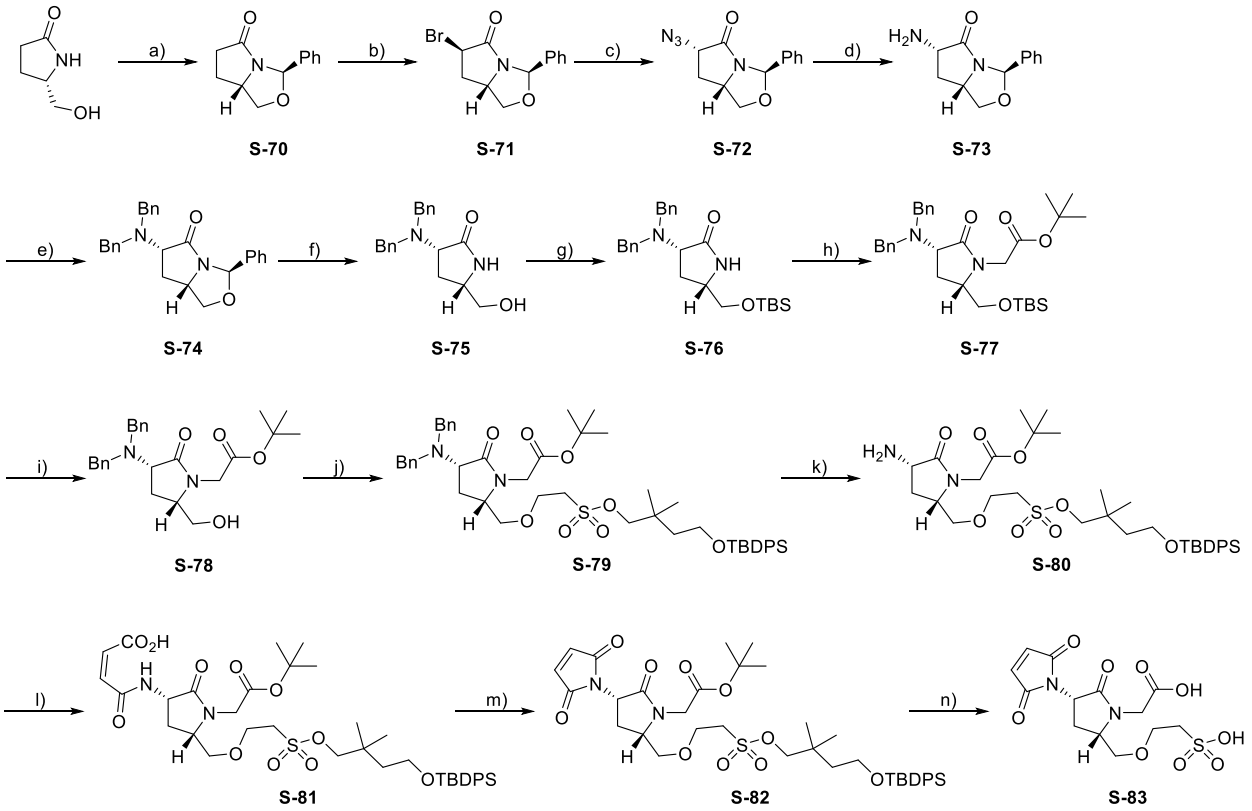
Methyl (2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-tris(acetyloxy)-6-{[2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-{[(2*S*)-2-{[(2*S*)-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-3-methylbutanoyl]amino}propanoyl]amino}phenyl]ethynyl}oxane-2-carboxylate (S-66). A mixture of **S-59** (4.50 g, 13.15 mmol), **S-65** (6.62 g, 8.76 mmol), copper(I) iodide (0.083 g, 0.438 mmol) and bis(triphenylphosphine)palladium(II) dichloride (0.308 g, 0.438 mmol) were combined in vial and degassed. *N*,*N*-Dimethylformamide (45 mL) and *N*-ethyl-*N*-(propan-2-yl)propan-2-amine (4.55 mL) were added, and the reaction vessel was flushed with nitrogen and stirred at room temperature overnight. The reaction was partitioned between water (100 mL) and ethyl acetate (250 mL). The layers were separated, and the organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluted with a gradient of 5% to 95% ethyl acetate in heptane. The product containing fractions were collected and concentrated. The residue was purified by silica gel chromatography, eluted with a gradient of 0.25% to 2.5% methanol in dichloromethane to give the title compound (6.23 g, 73%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 10.05 (s, 1H), 8.16 (d, J = 6.8 Hz, 1H), 7.87 – 7.82 (m, 2H), 7.75 – 7.66 (m, 3H), 7.52 (dd, J = 8.5, 2.2 Hz, 1H), 7.42 – 7.33 (m, 4H), 7.28 (tt, J = 7.4, 1.4 Hz, 2H), 5.38 (t, J = 9.5 Hz, 1H), 5.11 – 4.90 (m, 3H), 4.69 – 4.59 (m, 2H), 4.48 (d, J = 10.0 Hz, 1H), 4.37 (h, J = 6.9 Hz, 1H), 4.31 – 4.14 (m, 3H), 3.88 (dd, J = 9.0, 7.1 Hz, 1H), 3.61 (s, 3H), 2.00 (s, 3H), 1.95 (d, J = 1.7 Hz, 6H), 1.27 (d, J = 7.2 Hz, 3H), 0.91 – 0.79 (m, 16H), 0.05 (s, 6H). MS (ESI) *m/z* 970.4 $(M+H)^+$.

Methyl (2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-tris(acetyloxy)-6-{2-[2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-{[(2*S*)-2-{[(2*S*)-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-3-methylbutanoyl]amino}propanoyl]amino}phenyl]ethyl}oxane-2-carboxylate (S-67). **S-66** (4.7 g) and tetrahydrofuran (95 mL) were added to 5% Pt/C (2.42 g, wet) in a 50 mL pressure bottle and shaken for 90 minutes at room temperature under 50 psi of hydrogen. The reaction mixture was filtered and concentrated to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.84 (s, 1H), 8.10 (d, J = 7.0 Hz, 1H), 7.85 (d, J = 7.6 Hz, 2H), 7.70 (t, J = 7.6 Hz, 2H), 7.44 – 7.33 (m, 4H), 7.32 – 7.24 (m, 3H), 7.20 (d, J = 8.4 Hz, 1H), 5.27 (t, J = 9.5 Hz, 1H), 4.93 (t, J = 9.8 Hz, 1H), 4.75 (t, J = 9.6 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.42 – 4.31 (m, 2H), 4.31 – 4.14 (m, 3H), 3.87 (dd, J = 8.9, 7.0 Hz, 1H), 3.72 (td, J = 9.2, 2.8 Hz, 1H), 3.63 – 3.53 (m, 4H), 2.77 – 2.63 (m, 1H), 2.59 – 2.49 (m, 1H), 2.01 – 1.87 (m, 10H), 1.75 – 1.53 (m, 1H), 1.32 (s, 0H), 1.26 (d, J = 7.1 Hz, 3H), 0.91 – 0.77 (m, 15H), 0.02 (d, J = 3.5 Hz, 6H). MS (ESI) *m/z* 974.6 $(M+H)^+$.

Methyl (2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-tris(acetyloxy)-6-{2-[5-{[(2*S*)-2-{[(2*S*)-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-3-methylbutanoyl]amino}propanoyl]amino}-2-(hydroxymethyl)phenyl]ethyl}oxane-2-carboxylate (S-68). A mixture of **S-67** (5.4 g, 5.54 mmol) in tetrahydrofuran (7 mL), water (7 mL) and glacial acetic acid (21 mL) was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL). The organic fraction was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluted with a gradient of 0.5% to 5% methanol in dichloromethane, to give the title compound (5.0g, ~100%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.83 (s, 1H), 8.12 (d, *J* = 7.0 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.72 (t, *J* = 7.6 Hz, 2H), 7.46 – 7.19 (m, 9H), 5.28 (t, *J* = 9.5 Hz, 1H), 4.96 – 4.91 (m, 2H), 4.77 (t, *J* = 9.6 Hz, 1H), 4.45 – 4.16 (m, 8H), 3.89 (dd, *J* = 8.9, 7.0 Hz, 1H), 3.73 (td, *J* = 9.9, 9.3, 2.5 Hz, 1H), 2.73 – 2.63 (m, 1H), 2.56 (td, *J* = 13.8, 12.1, 6.1 Hz, 1H), 1.98 – 1.95 (m, 7H), 1.95 – 1.90 (m, 3H), 1.79 – 1.65 (m, 1H), 1.65 – 1.52 (m, 1H), 1.28 (d, *J* = 7.1 Hz, 3H), 0.93 – 0.78 (m, 7H). MS (ESI) *m/z* 860.4 $(M+H)^+$.

Methyl-(2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-tris(acetyloxy)-6-{2-[5-{[(2*S*)-2-{[(2*S*)-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-3-methylbutanoyl]amino}propanoyl]amino}-2-({[(4-nitrophenoxy)carbonyl]oxy}methyl)phenyl]ethyl}oxane-2-carboxylate (S-69). To a mixture of **S-68** (4.00 g, 4.65 mmol) and bis(4-nitrophenyl) carbonate (2.83 g, 9.30 mmol) in acetonitrile (80 mL) was added *N*-ethyl-*N*-(propan-2-yl)propan-2-amine (1.22 mL, 6.98 mmol) at room temperature. After stirring overnight, the reaction was concentrated, dissolved in dichloromethane (250 mL) and washed with saturated aqueous $NaHCO_3$ mixture (4 × 150 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The resulting foam was purified by silica gel chromatography, eluted with a gradient of 5% to 75% ethyl acetate in hexanes to give the title compound (2.2 g, 46%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.98 (s, 1H), 8.31 – 8.24 (m, 2H), 8.14 (d, *J* = 6.9 Hz, 1H), 7.88 – 7.83 (m, 2H), 7.70 (t, *J* = 7.4 Hz, 2H), 7.55 – 7.49 (m, 2H), 7.46 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.43 – 7.34 (m, 4H), 7.34 – 7.25 (m, 3H), 5.30 – 5.18 (m, 4H), 4.93 (t, *J* = 9.8 Hz, 1H), 4.76 (t, *J* = 9.6 Hz, 1H), 4.43 – 4.33 (m, 2H), 4.32 – 4.15 (m, 4H), 3.88 (dd, *J* = 8.9, 7.0 Hz, 1H), 3.74 (td, *J* = 9.2, 2.6 Hz, 1H), 2.83 – 2.69 (m, 1H), 2.69 – 2.55 (m, 1H), 2.01 – 1.93 (m, 4H), 1.93 – 1.89 (m, 7H), 1.74 (td, *J* = 12.6, 11.1, 7.3 Hz, 1H), 1.67 – 1.55 (m, 1H), 1.27 (d, *J* = 7.1 Hz, 3H), 0.85 (dd, *J* = 13.4, 6.7 Hz, 6H). MS (ESI) *m/z* 1025.5 $(M+H)^+$.

Synthesis of S-83



Chemical Structures 14. Reagents: a) PhCHO, *p*-TsOH•H_2O, $PhCH_3$, Dean-Stark trap, reflux, 80% yield; b) LiHMDS, Br_2, THF, -78 °C, 31% yield; c) NaN_3, N,N-DMF, 60 °C, 81% yield; d) PS-PPh_3, water, THF, 94% yield; e) BnBr, NaI, K_2CO_3, N,N-DMF, rt, 69% yield; f) *p*-TsOH•H_2O, water, THF, 65 °C, 92% yield; g) TBS-Cl, 1*H*-imidazole, N,N-DMF, rt, 100% yield; h) NaH, *t*-butyl 2-bromoacetate, 0 °C to rt, THF, 92% yield; i) TBAF, THF, rt, 100% yield; *J*) 4-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate, K_2CO_3, DMSO, water, 60 °C, 55% yield; k) 20% $Pd(OH)_2$/C, 30 psi H_2, MeOH, EtOAc, rt to 50 °C, 96% yield; l) Maleic anhydride, 40 °C, DCM, 93% yield; m) Et_3N, $NaSO_4$, $PhCH_3$, reflux, 29% yield; n) TFA, 70 °C, 42% yield.

(3*R*,7a*S*)-3-Phenyltetrahydro-3*H*,5*H*-pyrrolo[1,2-*c*][1,3]oxazol-5-one (S-70). A mixture of (5*S*)-5-(hydroxymethyl)pyrrolidin-2-one (25 g, 217 mmol), benzaldehyde (25.5 g, 240 mmol) and *para*-toluenesulfonic acid monohydrate (0.50 g, 2.63 mmol) in toluene (300 mL) was heated to reflux using a Dean-Stark trap under a drying tube for 16 hours. The reaction mixture was cooled to room temperature, and the solvent was decanted from the insoluble materials. The decanted organic layer was washed with saturated aqueous sodium bicarbonate mixture (twice) and brine (once). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluted with 35/65 heptane/ethyl acetate, to give the title compound (35.3 g, 80%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.47 – 7.41 (m, 2H), 7.39 – 7.28 (m, 3H), 6.33 (s, 1H), 4.23 (dd, *J* = 7.9, 6.3 Hz, 1H), 4.14 (ddd, *J* = 13.8, 7.7, 5.7 Hz, 1H), 3.48 (t, *J* = 8.0 Hz, 1H), 2.87 – 2.74 (m, 1H), 2.55 (ddd, *J* = 17.3, 10.0, 3.8 Hz, 1H), 2.37 (dddd, *J* = 13.8, 10.2, 7.6, 3.8 Hz, 1H), 1.94 (dtd, *J* = 13.4, 9.4, 5.3 Hz, 1H). MS (DCI) *m/z* 204.0 $(M+H)^+$.

(3*R*,6*R*,7a*S*)-6-Bromo-3-phenyltetrahydro-3*H*,5*H*-pyrrolo[1,2-*c*][1,3]oxazol-5-one (S-71). To a cold (-77 °C) mixture of **S-70** (44.6 g, 219 mmol) in tetrahydrofuran (670 mL) was added lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 250 mL, 250 mmol) dropwise over 40 minutes, keeping the

reaction mixture temperature less than -73 °C. The reaction was stirred at -77 °C for 2 hours, and bromine (12.5 mL, 243 mmol) was added dropwise over 20 minutes, keeping the reaction mixture temperature less than -64 °C. The reaction was stirred at -77 °C for 75 minutes and was quenched by the addition of cold 10% aqueous sodium thiosulfate (150 mL) to the -77 °C reaction. The mixture was warmed to room temperature and partitioned between half-saturated aqueous ammonium chloride and ethyl acetate. The layers were separated, and the organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with gradients of 80/20, 75/25, and 70/30 heptane/ethyl acetate to give the title compound. (19.3 g, 31%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.47 – 7.42 (m, 2H), 7.42 – 7.32 (m, 3H), 6.32 (s, 1H), 4.51 (dd, *J* = 6.9, 1.3 Hz, 1H), 4.45 – 4.36 (m, 1H), 4.28 (dd, *J* = 8.4, 6.4 Hz, 1H), 3.62 (dd, *J* = 8.4, 7.2 Hz, 1H), 2.66 (ddd, *J* = 14.7, 6.2, 1.3 Hz, 1H), 2.56 – 2.40 (m, 1H). MS (DCI) *m/z* 299.0 and 301.0 $(M+NH_4)^+$.

(3*R*,6*S*,7a*S*)-6-Azido-3-phenyltetrahydro-3*H*,5*H*-pyrrolo[1,2-*c*][1,3]oxazol-5-one (S-72). To a mixture of **S-71** (19.3 g, 68.4 mmol) in *N*,*N*-dimethylformamide (100 mL) was added sodium azide (13.5 g, 208 mmol). The reaction was heated to 60 °C for 2.5 hours. The reaction was cooled to room temperature and quenched by the addition of water (500 mL) and ethyl acetate (200 mL). The layers were separated, and the organic layer was washed brine. The combined aqueous layers were back-extracted with ethyl acetate (50 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with 78/22 heptane/ethyl acetate, to give the title compound (13.5 g, 81%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.46 – 7.41 (m, 2H), 7.40 – 7.31 (m, 3H), 6.35 (s, 1H), 4.53 (dd, *J* = 10.5, 8.5 Hz, 1H), 4.30 (dd, *J* = 8.3, 6.2 Hz, 1H), 4.07 – 3.98 (m, 1H), 3.59 (t, *J* = 8.0 Hz, 1H), 2.75 (ddd, *J* = 13.0, 8.5, 6.6 Hz, 1H), 1.78 (ddd, *J* = 13.1, 10.5, 7.4 Hz, 1H). MS (DCI) *m/z* 262.0 $(M+NH_4)^+$.

(3*R*,6*S*,7a*S*)-6-Amino-3-phenyltetrahydro-3*H*,5*H*-pyrrolo[1,2-*c*][1,3]oxazol-5-one (S-73). To a mixture of **S-72** (13.5 g, 55.3 mmol)) in tetrahydrofuran (500 mL) and water (50 mL) was added polymer-supported triphenylphosphine (55 g, Aldrich catalog #366455, loading - 3 mmol/g). The reaction mixture was mechanically stirred overnight at room temperature. The reaction mixture was filtered through diatomaceous earth, eluted with ethyl acetate and toluene. The mixture was concentrated under reduced pressure, dissolved in dichloromethane (100 mL), dried with sodium sulfate, then filtered and concentrated to give the title compound, which was used in the subsequent step without further purification (11.3 g, 94%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.46 – 7.41 (m, 2H), 7.40 – 7.30 (m, 3H), 6.33 (s, 1H), 4.27 (dd, *J* = 8.3, 6.2 Hz, 1H), 4.07 – 3.94 (m, 2H), 3.59 (dd, *J* = 8.3, 7.1 Hz, 1H), 2.81 (ddd, *J* = 12.5, 8.2, 6.4 Hz, 1H), 1.62 (td, *J* = 12.0, 8.1 Hz, 1H). MS (DCI) *m/z* 219.0 $(M+H)^+$.

(3*R*,6*S*,7a*S*)-6-(Dibenzylamino)-3-phenyltetrahydro-3*H*,5*H*-pyrrolo[1,2-*c*][1,3]oxazol-5-one (S-74). To a mixture of Example **S-73** (11.3 g, 51.8 mmol) in *N*,*N*-dimethylformamide (100 mL) was added potassium carbonate (7.0 g, 50.6 mmol), potassium iodide (4.2 g, 25.3 mmol), and benzyl bromide (14.5 mL, 122 mmol). The reaction mixture was stirred at room temperature overnight and quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with a gradient of 10 to 15% ethyl acetate in heptane to give a solid that was triturated with heptane to give the title compound (14.3 g, 69%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.43 (ddd, *J* = 10.0, 8.1, 1.6 Hz, 6H), 7.37 – 7.27 (m, 7H), 7.26 – 7.21 (m, 2H), 6.35 (s, 1H), 4.26 (dd, *J* = 8.3, 6.1 Hz, 1H), 4.09 (dd, *J* = 11.3, 8.7 Hz, 1H), 4.03 (d, *J* = 13.9 Hz, 2H), 3.93 – 3.84 (m, 1H), 3.71 (d, *J*

= 13.9 Hz, 2H), 3.50 (t, *J* = 8.0 Hz, 1H), 2.47 (ddd, *J* = 12.8, 8.7, 7.0 Hz, 1H), 1.89 (ddd, *J* = 12.8, 11.2, 7.4 Hz, 1H). MS (DCI) *m/z* 399.1 $(M+H)^+$.

(3*S*,5*S*)-3-(Dibenzylamino)-5-(hydroxymethyl)pyrrolidin-2-one (S-75). To a mixture of **S-74** (13 g, 32.6 mmol) in tetrahydrofuran (130 mL) was added *para*-toluene sulfonic acid monohydrate (12.4 g, 65.2 mmol) and water (50 mL), and the reaction was heated to 65 °C for 6 days. The reaction was cooled to room temperature and quenched by the addition of saturated aqueous sodium bicarbonate and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The waxy solids were triturated with heptane (150 mL) to give the title compound (9.3 g, 92%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.40 (d, *J* = 8.2 Hz, 4H), 7.27 (t, *J* = 7.5 Hz, 4H), 7.20 (t, *J* = 7.2 Hz, 2H), 7.07 (s, 1H), 3.87 (d, *J* = 13.7 Hz, 2H), 3.76 – 3.48 (m, 5H), 3.39 (dd, *J* = 11.0, 7.1 Hz, 1H), 2.10 (ddd, *J* = 12.7, 8.9, 6.9 Hz, 1H), 1.73 (dt, *J* = 12.9, 9.5 Hz, 1H). MS (DCI) *m/z* 311.1 $(M+H)^+$.

(3*S*,5*S*)-5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-3-(dibenzylamino)pyrrolidin-2-one (S-76). To a mixture of **S-75** (9.3 g, 30.0 mmol) and 1*H*-imidazole (2.2 g, 32.3 mmol) in *N*,*N*-dimethylformamide was added *tert*-butylchlorodimethylsilane (11.2 mL, 32.2 mmol, 50 weight % in toluene), and the reaction mixture was stirred overnight. The reaction mixture was quenched by the addition of water and diethyl ether. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with 35% ethyl acetate in heptane, to give the title compound (13.5 g, ~100%). ^{1}H NMR (501 MHz, chloroform-*d*) δ ppm 7.45 – 7.36 (m, 4H), 7.32 – 7.23 (m, 4H), 7.24 – 7.15 (m, 2H), 5.93 (s, 1H), 3.94 (d, *J* = 13.8 Hz, 2H), 3.67 – 3.60 (m, 4H), 3.55 – 3.45 (m, 1H), 3.34 (dd, *J* = 10.2, 8.0 Hz, 1H), 2.13 (ddd, *J* = 12.8, 9.0, 6.7 Hz, 1H), 1.68 – 1.57 (m, 1H), 0.83 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). MS (DCI) *m/z* 425.1 $(M+H)^+$.

***tert*-Butyl [(3*S*,5*S*)-5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl]acetate (S-77).** To a cold (0 °C) mixture of **S-76** (4.5 g, 10.6 mmol) in tetrahydrofuran (45 mL) was added 95% sodium hydride (320 mg, 12.7 mmol) in two portions. The cold mixture was stirred for 40 minutes, and *tert*-butyl 2-bromoacetate (3.2 mL, 21.7 mmol) was added. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with a gradient of 5-12% ethyl acetate in heptane, to give the title compound (5.3 g, 92%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.47 – 7.37 (m, 4H), 7.30 – 7.23 (m, 4H), 7.22 – 7.16 (m, 2H), 4.36 (d, *J* = 17.5 Hz, 1H), 3.93 (d, *J* = 13.8 Hz, 2H), 3.79 (d, *J* = 17.4 Hz, 1H), 3.72 – 3.53 (m, 6H), 2.12 (ddd, *J* = 12.8, 9.3, 6.8 Hz, 1H), 1.72 – 1.62 (m, 1H), 1.40 (s, 9H), 0.83 (s, 9H), 0.02 – 0.00 (m, 6H). MS (DCI) *m/z* 539.2 $(M+H)^+$.

***tert*-Butyl [(3*S*,5*S*)-3-(dibenzylamino)-5-(hydroxymethyl)-2-oxopyrrolidin-1-yl]acetate (S-78).** To a mixture of **S-77** (5.3 g, 9.84 mmol)) in tetrahydrofuran (25 mL) was added tetrabutylammonium fluoride (11 mL, 11.0 mmol, 1.0 M in 95/5 tetrahydrofuran/water). The reaction mixture was stirred at room temperature for one hour and then quenched by the addition of saturated aqueous ammonium chloride, water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back extracted with ethyl acetate. The combined organic layers were dried with sodium

sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with 35% ethyl acetate in heptane, to give the title compound (4.2 g, 100%). ^{1}H NMR (501 MHz, chloroform-*d*) δ ppm 7.49 – 7.41 (m, 4H), 7.32 – 7.27 (m, 4H), 7.24 – 7.19 (m, 2H), 4.37 (d, *J* = 17.6 Hz, 1H), 3.93 (d, *J* = 13.8 Hz, 2H), 3.75 (d, *J* = 13.7 Hz, 3H), 3.68 (t, *J* = 9.8 Hz, 1H), 3.61 – 3.54 (m, 1H), 3.51 – 3.43 (m, 2H), 3.37 (ddt, *J* = 9.7, 8.1, 2.1 Hz, 1H), 2.33 (ddd, *J* = 13.0, 9.9, 8.4 Hz, 1H), 2.09 (ddd, *J* = 13.1, 9.7, 7.6 Hz, 1H), 1.48 (s, 9H). MS (DCI) *m/z* 425.1 $(M+H)^+$.

***tert*-Butyl [(3*S*,5*S*)-3-(dibenzylamino)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxo-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradecan-1-yl)pyrrolidin-1-yl]acetate (S-79)**. To a mixture of **S-78** (4.7 g, 11.1 mmol) in dimethyl sulfoxide (14 mL) was added a mixture of 4-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (14.5 g, 32.5 mmol) in dimethyl sulfoxide (14 mL). Potassium carbonate (2.6 g, 18.81 mmol) and water (28 µL, 1.55 mmol) were added, and the reaction mixture was heated at 60 °C under nitrogen for one day. The reaction was cooled to room temperature and then quenched by the addition of brine, water and diethyl ether. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluted with a gradient of 15-25% ethyl acetate in heptane to give the title compound (5.3 g, 55%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.55 – 7.47 (m, 4H), 7.31 – 7.21 (m, 10H), 7.19 – 7.11 (m, 4H), 7.10 – 7.05 (m, 2H), 4.04 (d, *J* = 17.4 Hz, 1H), 3.85 – 3.69 (m, 4H), 3.68 – 3.63 (m, 2H), 3.62 – 3.50 (m, 6H), 3.39 – 3.33 (m, 2H), 3.11 (t, *J* = 6.5 Hz, 2H), 2.02 (ddd, *J* = 12.9, 9.3, 7.2 Hz, 1H), 1.53 – 1.40 (m, 4H), 1.29 (s, 9H), 0.90 (s, 9H), 0.81 (s, 6H). MS (ESI+) *m/z* 871.2 $(M+H)^+$.

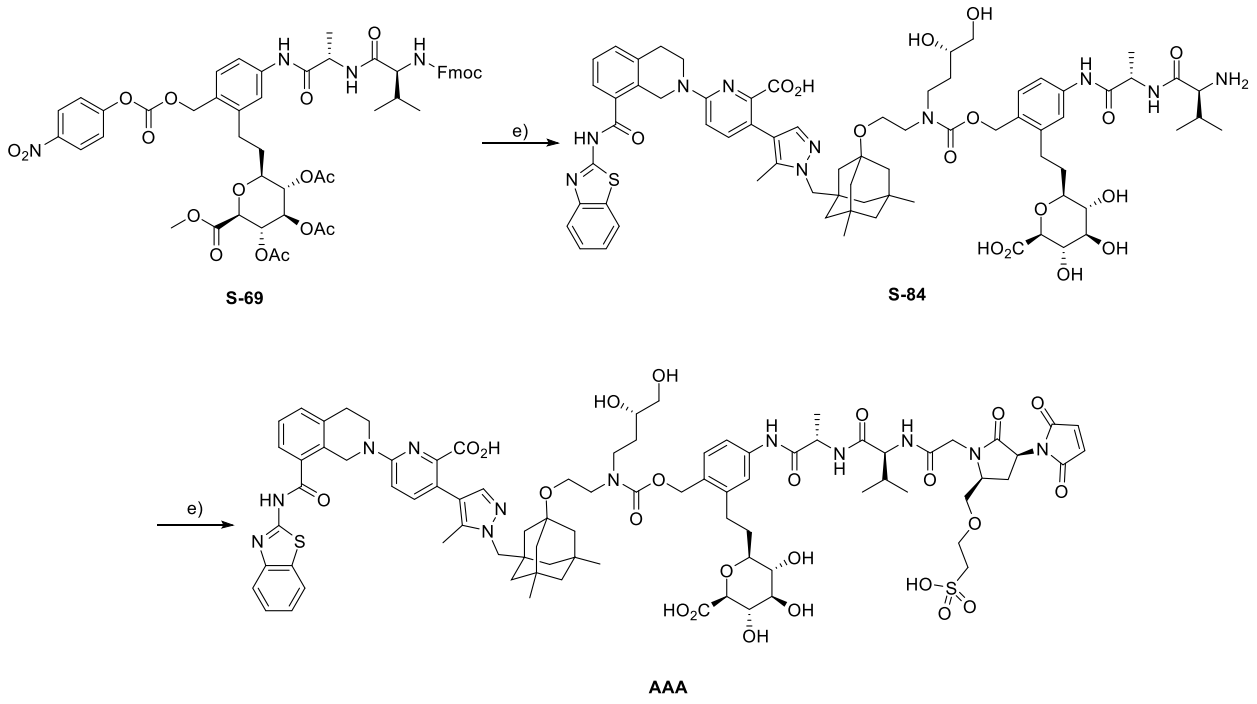
***tert*-Butyl [(3*S*,5*S*)-3-amino-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxo-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradecan-1-yl)pyrrolidin-1-yl] (S-80). S-79** (870 mg, 0.99 mmol) was dissolved in ethyl acetate (5 mL) and methanol (15 mL), and palladium hydroxide on carbon, 20% by weight (100 mg, 0.99 mmol) was added. The reaction mixture was stirred under a hydrogen atmosphere (30 psi) at room temperature for 30 hours, then at 50 °C for one hour. The reaction mixture was cooled to room temperature, filtered, and concentrated to give the title compound (0.665 g, 96%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.75 – 7.63 (m, 4H), 7.52 – 7.37 (m, 6H), 4.39 – 4.27 (m, 1H), 3.99 (s, 2H), 3.93 – 3.80 (m, 5H), 3.76 (t, *J* = 6.6 Hz, 2H), 3.62 – 3.49 (m, 3H), 3.32 (t, *J* = 6.4 Hz, 2H), 2.50 (ddd, *J* = 12.5, 8.7, 6.8 Hz, 1H), 1.62 (t, *J* = 6.7 Hz, 2H), 1.48 (s, 9H), 1.07 (s, 9H), 0.99 (s, 6H). MS (ESI+) *m/z* 691.0 $(M+H)^+$.

(2*Z*)-4-{[(3*S*,5*S*)-1-(2-*tert*-Butoxy-2-oxoethyl)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxo-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradecan-1-yl)pyrrolidin-3-yl]amino}-4-oxobut-2-enoic acid (S-81). Maleic anhydride (340 mg, 3.47 mmol) was dissolved in dichloromethane (3 mL), and a mixture of **S-80** (2.2 g, 3.18 mmol) in dichloromethane (3 mL) was added dropwise followed by heating at 40 °C for 2 hours. The reaction mixture was directly purified by silica gel chromatography, eluted with a gradient of 1.0-2.5% methanol in dichloromethane containing 0.2% acetic acid. After concentrating the product-bearing fractions, toluene (40 mL) was added, and the mixture was concentrated again to give the title compound (2.33 g, 93%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.74 (d, *J* = 7.2 Hz, 1H), 7.57 – 7.50 (m, 4H), 7.35 – 7.23 (m, 6H), 7.17 – 7.10 (m, 1H), 7.08 – 7.01 (m, 1H), 6.38 – 6.11 (m, 2H), 4.58 (dt, *J* = 9.8, 7.4 Hz, 1H), 4.24 (d, *J* = 17.6 Hz, 1H), 3.87 (s, 2H), 3.74 (dp, *J* = 11.0, 5.5 Hz, 2H), 3.62 (dd, *J* = 12.0, 5.4 Hz, 3H), 3.48 (dd, *J* = 10.3, 2.3 Hz, 1H), 3.38 (dd, *J* = 10.3, 4.5 Hz, 1H), 3.24 – 3.08 (m, 2H), 2.65 (ddd, *J* = 13.4, 9.8, 7.8 Hz, 1H), 1.64 (dt, *J* = 13.7, 7.0 Hz, 1H), 1.47 (t, *J* = 6.6 Hz, 2H), 1.34 (s, 9H), 0.93 (s, 9H), 0.85 (s, 6H). MS (ESI-) *m/z* 787.3 $(M-H)^-$.

***tert*-Butyl [(3*S*,5*S*)-3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxo-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradecan-1-yl)pyrrolidin-1-yl]acetate (S-82). S-81** (530 mg, 0.672 mmol) was slurried in toluene (7 mL), and triethylamine (220 µL, 1.51 mmol) and sodium sulfate (500 mg, 3.52 mmol) were added. The reaction was heated at reflux under a nitrogen atmosphere for 6 hours, and the reaction mixture was stirred at room temperature overnight. The mixture was filtered, and the solids were rinsed with ethyl acetate. The eluent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluted with 45/55 heptane/ethyl acetate to give the title compound (0.152 g, 29%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.78 – 7.66 (m, 4H), 7.43 (qd, *J* = 8.5, 7.6, 3.5 Hz, 6H), 6.74 (s, 2H), 4.84 (t, *J* = 10.1 Hz, 1H), 4.43 (d, *J* = 17.4 Hz, 1H), 4.08 – 3.98 (m, 3H), 3.93 – 3.86 (m, 3H), 3.79 – 3.68 (m, 3H), 3.59 (dd, *J* = 10.0, 3.0 Hz, 1H), 3.35 (t, *J* = 6.5 Hz, 2H), 2.43 (ddd, *J* = 12.5, 9.6, 7.0 Hz, 1H), 1.97 (ddd, *J* = 12.5, 10.5, 8.9 Hz, 1H), 1.66 – 1.61 (m, 2H), 1.49 (s, 9H), 1.07 (s, 9H), 1.00 (s, 6H). MS (ESI+) 771.5 $(M)^+$.

{(3*S*,5*S*)-3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetic acid (S-83). S-82 (0.180g, 0.233 mmol) was dissolved in trifluoroacetic acid (5 mL) and heated to 65-70 °C under nitrogen overnight. The trifluoroacetic acid was removed under reduced pressure. The residue was dissolved in acetonitrile (2.5 mL) and purified by preparative reverse-phase high-pressure liquid chromatography on a Phenomenex® Luna® C18(2) AXIA™ column (250 × 50 mm, 10 µm particle size) using a gradient of 5-75% acetonitrile containing 0.1% trifluoroacetic acid in water (70 mL/minute) over 30 minutes, to give the title compound (37 mg, 42%). ^{1}H NMR (501 MHz, Methanol-d_4) δ ppm 6.86 (s, 2H), 4.87 (d, *J* = 11.3 Hz, 1H), 4.36 – 4.16 (m, 2H), 4.01 (ttd, *J* = 7.8, 6.1, 4.7, 2.9 Hz, 1H), 3.90 – 3.77 (m, 2H), 3.69 (ddd, *J* = 11.6, 8.0, 3.6 Hz, 1H), 3.63 (dd, *J* = 10.1, 3.0 Hz, 1H), 3.16 – 3.05 (m, 2H), 2.47 (ddt, *J* = 12.6, 9.8, 5.9 Hz, 1H), 1.95 (tdd, *J* = 12.5, 9.0, 2.8 Hz, 1H). MS (ESI-) *m/z* 375.2 $(M-H)^-$.

Synthesis of 25 (AAA Payload)



Chemical Structures 15. Reagents: a) **11**, *i*Pr$_2$NEt, 1-HOBt, N,N-DMF then LiOMe, THF, 46% yield; b) **S-83,** HATU, *i*Pr$_2$NEt, N,N-DMF, 41% yield.

3-[1-({3-[2-({[(4-{[(2*S*)-2-{[(2*S*)-2-Amino-3-methylbutanoyl]amino}propanoyl]amino}-2-{2-[(2*S*,3*R*,4*R*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxyoxan-2-yl]ethyl}phenyl)methoxy]carbonyl}[(3*S*)-3,4-dihydroxybutyl]amino)ethoxy]-5,7-dimethyladamantan-1-yl}methyl)-5-methyl-1*H*-pyrazol-4-yl]-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1*H*)-yl}pyridine-2-carboxylic acid (S-84). To a solution of **S-69** (0.085 g, 0.083 mmol), compound **11** (0.065 g, 0.078 mmol), *N*,*N*-dimethylformamide (0.5 mL) and *N*,*N*-diisopropylethylamine (0.6 mL, 0.344 mmol). After the solids were dissolved, 1-hydroxybenzotriazole hydrate (0.013 g, 0.085 mmol) was added, and the reaction progress was monitored by HPLC (Ascentis® Express® C18, 4.6 × 150 mm, 2.7 µm, 1.5 mL/minute flow rate, eluted with a gradient of 40 to 100% acetonitrile in 0.05% $HClO_4$ in water over 18 minutes). After coupling was determined to be complete, tetrahydrofuran (0.5 mL) was added, and the reaction mixture was cooled to 0 °C. Lithium methoxide (1.2 mL, 1.2 mmol, 1.0 M solution in methanol) was charged, and the reaction mixture was allowed to warm to ambient temperature. The reaction progress was monitored by HPLC (Ascentis® Express® C18, 4.6 × 150 mm, 2.7 µm, 1.5 mL/minute flow rate, eluted with a gradient of 40 to 100% acetonitrile in 0.05% $HClO_4$ in water over 18 minutes), and after hydrolysis was determined to be complete, the reaction was concentrated under reduced pressure. The residue was dissolve in methanol:water (1:1) and purified via reverse phase chromatography (Phenomenex® Luna® C18, 50 × 250 mm, 10 µm, 80 mL/minute flow rate, 20 to 100% acetonitrile in water containing 0.1 % trifluoroacetic acid), and the desired fractions were lyophilized to give the title compound as an trifluoroacetate salt (49 mg, 46%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.80 (s, 2H), 10.06 (s, 1H), 8.64 (d, J = 7.1 Hz, 1H), 8.08 – 7.96 (m, 5H), 7.76 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.51 – 7.38 (m, 5H), 7.38 – 7.27 (m, 3H), 7.27 – 7.18 (m, 2H), 6.91 (d, J = 8.9 Hz, 1H), 4.98 (s, 2H), 4.92 (s, 2H), 4.47 (t, J = 7.2 Hz, 1H), 3.85 (t, J = 6.0 Hz, 2H), 3.81 – 3.70 (m, 2H), 3.67 – 3.15 (m, 11H), 3.14 – 3.03 (m, 2H), 3.01 – 2.95 (m, 3H), 2.90 (t, J = 9.0 Hz, 1H), 2.81 – 2.66 (m, 1H), 2.65 – 2.52 (m, 1H), 2.11 – 1.94 (m, 5H), 1.71 – 1.59 (m, 1H), 1.59 – 1.47 (m, 1H), 1.41 – 1.15 (m, 8H), 1.15 – 0.97 (m, 6H), 0.96 – 0.84 (m, 9H), 0.83 – 0.70 (m, 8H). MS (ESI) *m/z* 1357.5 $(M+H)^+$.

6-{8-[(1,3-Benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1*H*)-yl}-3-[1-({3-[2-({[(2-{2-[(2*S*,3*R*,4*R*,5*S*,6*S*)-6-carboxy-3,4,5-trihydroxyoxan-2-yl]ethyl}-4-{[(2*S*)-2-{[(2*S*)-2-(2-{(3*S*,5*S*)-3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetamido)-3-methylbutanoyl]amino}propanoyl]amino}phenyl)methoxy]carbonyl}[(3*S*)-3,4-dihydroxybutyl]amino)ethoxy]-5,7-dimethyladamantan-1-yl}methyl)-5-methyl-1*H*-pyrazol-4-yl]pyridine-2-carboxylic acid (25, payload AAA). S-89 (16 mg, 0.043 mmol) was dissolved in *N*,*N*-dimethylformamide (0.2 mL), and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (16.0 mg, 0.042 mmol) and *N*,*N*-diisopropylethylamine (17 µL, 0.097 mmol) were added. The mixture was stirred for 3 minutes at room temperature and then added to a mixture of **S-90** (48.0 mg, 0.033 mmol) and *N*,*N*-diisopropylethylamine (20 µL, 0.115) in *N*,*N*-dimethylformamide (0.2 mL). After 1 hour, the reaction was diluted with *N*,*N*-dimethylformamide/water 1/1 (1.0 mL) and purified by reverse-phase HPLC (Phenomenex® Luna® C18 250 × 50 mm column), eluted with 5-75% acetonitrile in 0.1% trifluoroacetic acid/water (100 mL/minute), to provide the title compound (0.023 g, 41%). ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 9.86 (br d, 1H), 8.17 (br d, 1H), 8.04 (m, 2H), 7.78 (d, 1H), 7.61 (d, 1H), 7.51 (br d, 1H), 7.49-7.39 (m, 4H), 7.36 (m, 2H), 7.29 (s, 1H), 7.21 (d, 1H), 7.07 (s, 2H), 6.95 (d, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.64 (t, 1H), 4.36 (m, 1H), 4.19 (m, 1H), 4.16 (d, 1H), 4.01 (d, 1H), 3.88 (br t, 2H), 3.82 (br m, 3H), 3.75 (br m, 1H), 3.64 (t, 2H), 3.54 (d, 2H), 3.47 (m, 4H), 3.43 (br m, 4H), 3.23 (br m, 5H), 3.13 (t, 1H), 3.10 (br m, 1H), 3.01 (br m, 2H), 2.93 (t, 1H), 2.83-2.68 (m, 3H), 2.37 (m, 1H), 2.08 (s, 3H), 1.99 (br m, 2H), 1.85 (m, 1H), 1.55 (br m, 1H), 1.37 (br m, 1H), 1.28 (br m, 6H), 1.10 (br m, 7H), 0.93 (br m, 1H), 0.88-0.69 S(m, 12H); MS (ESI) *m/z* 1713.6 $(M-H)^-$.

Crystallography Methods

Protein

The following clone was used for structure studies [BCL-XL (1-25)-GGGGGGG-(83-209) W24A, E158K, D189A]-LE-6His. In this form of the protein, an extended loop, residues 26-82 has been deleted and replaced with seven glycine residues. The protein was expressed in E.coli. After cell lysis, the protein was purified by Ni-NTA chromatography (buffer 20 mM Tris, 300 mM NaCl, 0.5 mM TCEP, with a gradient of imidazole from 20 mM to 500 mM, pH 8.0). Prior to compound complexation and sample concentration, glycerol was added to prevent protein precipitation on concentration (buffer 20 mM Tris, 300 mM NaCl, 10 (v/v)% glycerol, 0.5 mM TCEP, pH 8.0).

Compound complex

The compound was dissolved in DMSO. Compound solution was added to the protein solution at 3.7 mg/ml to give a final DMSO concentration of 2% (v/v). After incubation the sample was concentrated to 12.4 mg/ml for use in crystallization.

Crystallization

Crystals were grown by sitting drop vapor diffusion at 290K using a 1:1 ratio of the protein solution and the reservoir solution (1 M sodium acetate, 0.1 M HEPES pH 7.5, 0.05 M cadmium sulfate). They were harvested using a cryo-protectant made by adding propylene glycol to the reservoir solution to give 20% (v/v) propylene glycol and cryo-cooled in liquid nitrogen. Diffraction data were collected at the Advanced Photon Source (Argonne National Laboratory, IL) at the 17D beamline under gaseous nitrogen at 100K.

Structure solution and refinement

Diffraction intensities were processed using autoPROC [55] and the structure was solved by molecular replacement using MOLREP [56] from the CCP4 program suite [57]. Models were rebuilt using COOT [58] and refined against structure factors using the programs REFMAC5 [59] and autoBUSTER [60]. Figures were prepared using the program PYMOL (DeLano Scientific, Schroedinger Inc.).

Safety Pharmacology and Toxicology

Beagle dogs for anesthetized safety pharmacology studies were male beagles 9 months to 2 years old obtained from Marshall BioResources, North Rose, NY. Dogs were pair housed in stainless steel caging prior to use and fed Teklad 2025C Global 25% Protein Dog Diet, Envigo Teklad Diets, Madison, WI and had access to reverse osmosis-purified water ad libitum. Cynomolgus monkeys were obtained through Charles River Laboratories/Envigo or Covance Research Animals and were of Chinese origin, aged 2-6 years (2-6 kg) at study initiation and were housed in groups up to 4. Animals were fed a commercially available balanced and complete monkey diet (Harlan 2055 Teklad Global 25% protein primate diet, Envigo Teklad Diets, Madison, WI or Lab Diet Certified Primate Diet #5048, PMI Nutrition International, Inc., St. Louis, MO), had free access to an automatic watering system supplied with tap water, or reverse osmosis water that had been chlorinated for some studies. Animals were judged to be healthy by physical examination and serum chemistry and hematology analysis by a veterinarian. Animals were provided enrichment, including in the form of food enrichment, and were maintained at 64-77°F on a 12-hour light/dark cycle. Animals were randomized into dose groups using a measured value randomization procedure that ensured balanced body weights between groups at study initiation.

AbbVie is committed to ensuring the humane care and use of laboratory animals in the company's research and development programs. Our programs exceed regulatory agency standards, and we are committed to the internationally accepted principles of the 3Rs (refinement, reduction, replacement). For safety pharmacology studies and toxicology studies conducted by Abbvie, all animal studies were reviewed and approved by AbbVie's Institutional Animal Care and Use Committee or Oversight Body (in accordance with national regulations). Animal studies were conducted under an AAALAC accredited program, where veterinary care and oversight was provided to ensure optimal animal care. For toxicology studies conducted by contract research organizations (Charles River Laboratories/Envigo), our partner organizations meet regulatory agency standards and are committed to the internationally accepted principles of the 3Rs (refinement, reduction, replacement). All externally executed animal studies occur at sites vetted and approved by our Global Animal Welfare Committee and study protocols are approved by the relevant local Institutional Animal Care and Use Committee. For dog safety pharmacology studies conducted at Abbvie, protocol numbers were 1402B00003 and 1701B00020. For the anesthetized monkey safety pharmacology study, the protocol number was 1308C00187.

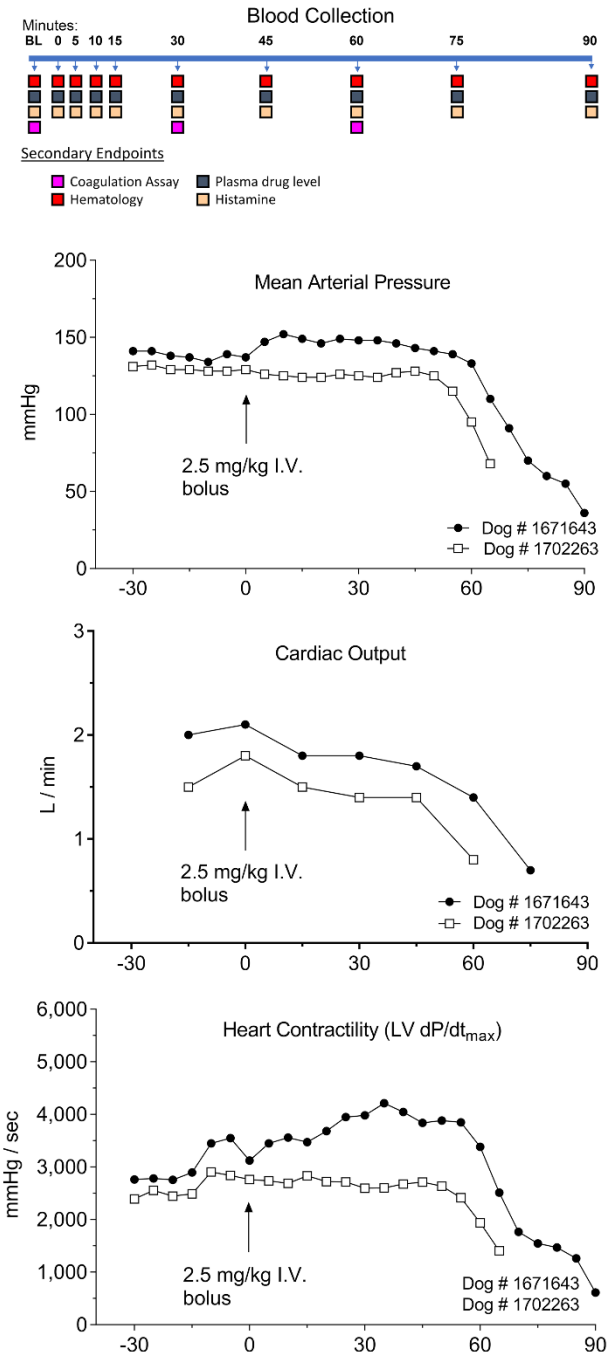


Figure S1. Evaluation of A-1331852 in two anesthetized dogs. Instrumented anesthetized beagle dogs (Koshman, 2020) were continuously monitored for cardiovascular parameters including cardiac output (CO), mean arterial pressure (MAP) and contractility (dp/dt50) with intermittent blood collection for hematology, histamine, coagulation, and plasma drug level determination over a 90-minute period. A-1331852 adminstered as an intravenous bolus dose at time 0 resulted in cardiovascular collapse at 60 to 90 minutes after dosing.

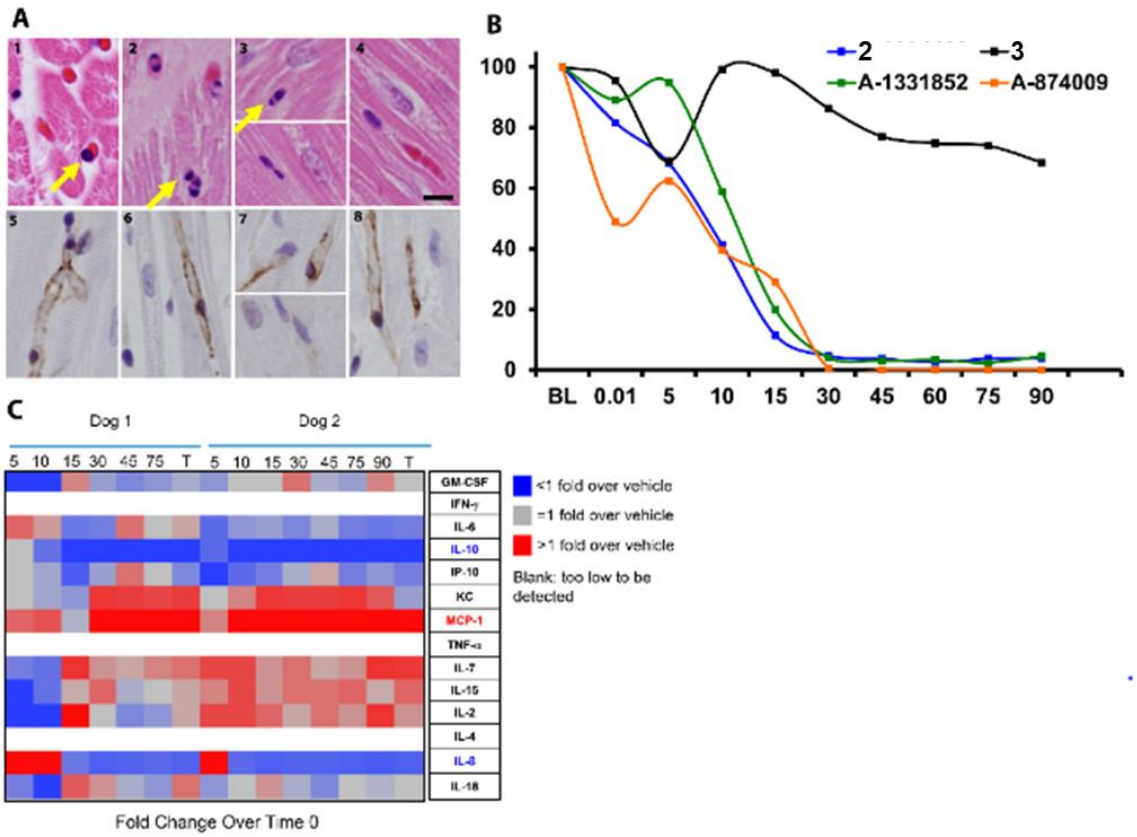
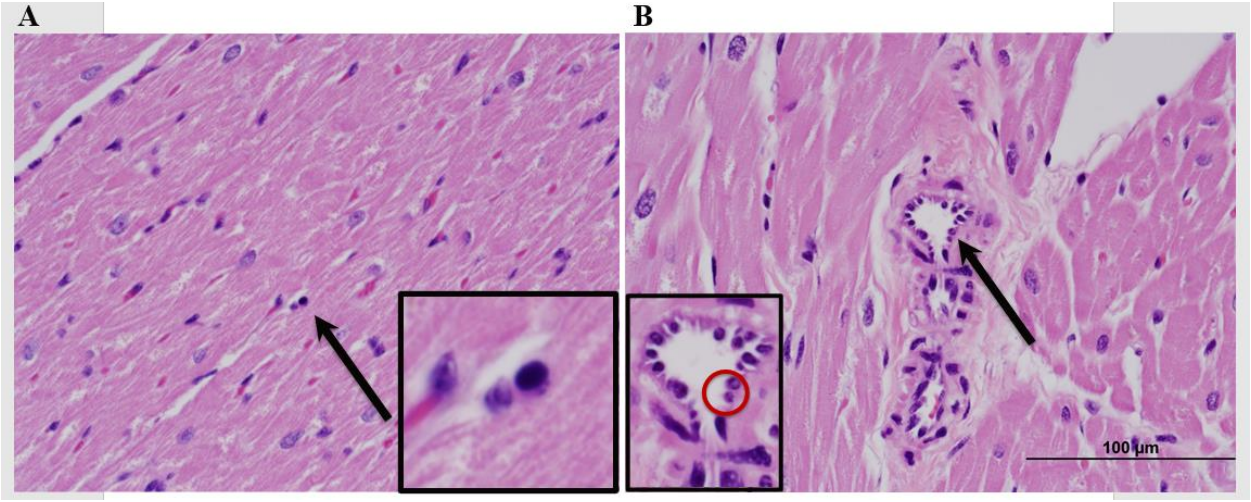


Figure S2. (A) Representative heart histology from dogs treated with compounds in an anesthetized instrumented cardiovascular model. Panels 1-4 are hematoxylin and eosin stained; arrows indicate apoptotic nuclei. Panels 5-8 are immunostaining for activated caspase 3. Treatment by panel number: 1,5 A-1331852; 2,6 Compound 2; 3,7 A-874009; 4,8 Compound 3. Of two dogs treated with A-874009, one dog (bottom panels) had minimal apoptosis relative to the other dog (top panel). Magnification is the same in all images. Scale bar in panel 4 represents 10 μm. (**B**) Graph of platelet counts in anesthetized dogs following bolus infusion of either compound 2, 3, A-1331852 or A-874009. (**C**) Heatmap of selected cytokines through a timecourse after treatment with A-1331852 relative to vehicle control. IFN-γ, IL-4, and TNF-α were undetected in all samples examined.



C Plasma concentration (µg/mL)

Monkey	Dose Level (mg/kg)	Outcome	0	15m	30m	60m	120m	180m
1	2.5	Survival	0	28.5	24.0	7.7	4.9	2.4
2	2.5	Survival	0	20.9	12.8	8.4	3.4	1.5
3	5.0	Mortality	0	36.8	12.9	6.0	1.7	2.2
4	5.0	Survival	0	21.3	14.8	8.4	4.7	3.0

Figure S3. (A) Apoptotic nuclei (H&E) in monkey #1 treated with 2.5 mg/kg. (**B**) apoptotic nuclei in monkey #3 treated with 5 mg/kg. **C**, outcome and plasma concentration in aged, anesthetized cynomolgous monkeys dosed with A-1331852 via IV bolus.

Protocol. Cynomolgus monkeys were fasted overnight the day before the experiment. The animals were anesthetized with sevoflurane in oxygen following premedication with ketamine. Aortic pressure was measured via a micromanometer catheter inserted into the right carotid artery for measurement of aortic pressure. Polyethylene catheters were inserted into the right femoral vein and artery for infusion of tested compounds or vehicle and collection of blood samples, respectively. The primary hemodynamic variables (HR and MAP) were computed using commercial software and a signal processing workstation (Ponemah; Gould Instrument Systems, Inc., Valley View, OH). At the end of the data collection period, the monkeys were euthanized under anesthesia and tissues were collected into neutral buffered formalin and processed for routine histology.

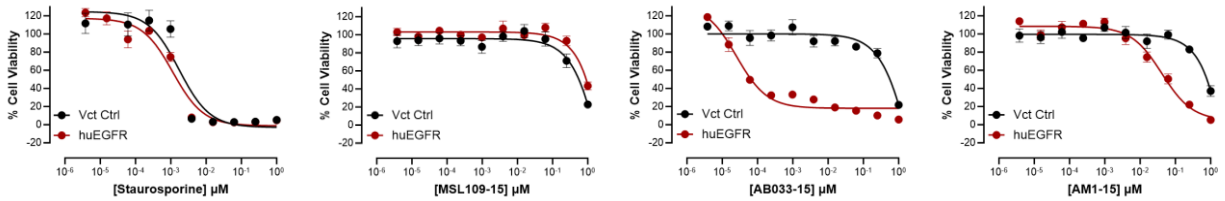


Figure S4. EGFR-targeted XL-ADCs induce antigen-dependent cell death in *mcl1*$^{-/-}$ MEFs. MEFs deficient in *mcl1* expressing huEGFR or the pLVX empty vector (Vct Ctrl) were treated with MSL109-15, AM1-15, AB033-15 or Straurosporine at the indicated concentrations for 96 hours. The impact on cell viability was subsequently determined using CellTiter-Glo (Promega Corp).

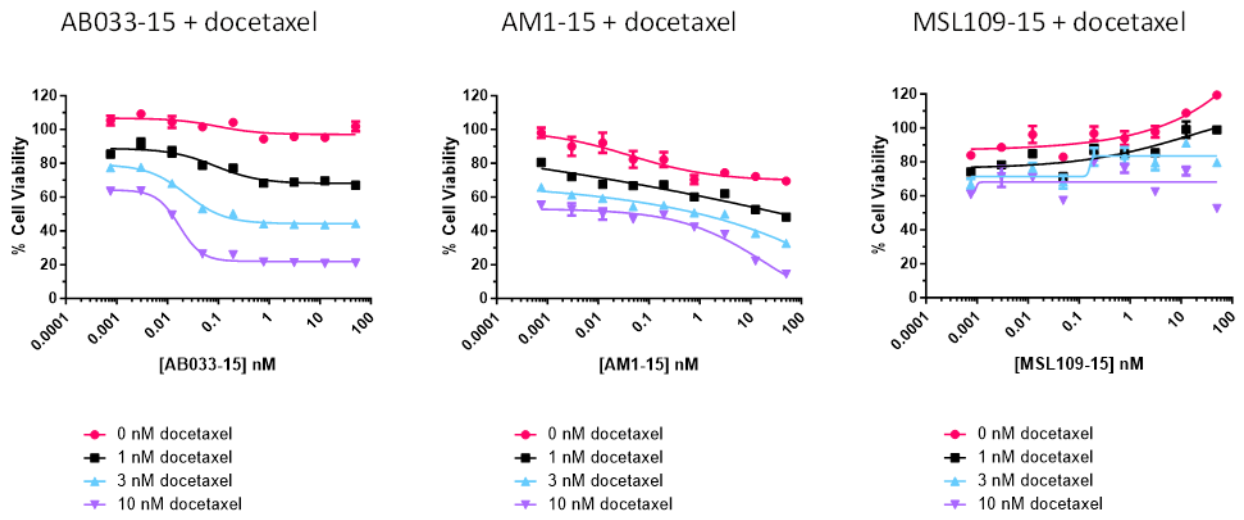


Figure S5. Docetaxel (DTX) enhanced the activity of AB033-15 and AM1-15 in NCI-H1650 lung cancer cell line. NCI-H1650 cell line was treated with AB033-15, AM1-15 or MSL109-15 (the negative control ADC) in combination with DTX at the indicated concentrations. Increasing concentrations of DTX sensitized cell killing by AB033-15 and AM1-15, but not by MSL109-15.

Table S1. Plasma concentration in dogs dosed with 2.5 mg/kg A-1331852 (IV, PO).

		Plasma concentration (µg/mL)					
	Dog #	**0.1 hr**	**0.25 hr**	**0.5 hr**	**1 hr**	**2 hr**	**Terminal**
IV dosing	1	27.1	18.6	14.6	9.2		
	2	25.5	17.7	12.8	8.4	2.5	1.9
	3	26.9	20.6	15.1			
Mean		26.5	19.0	14.2	8.8		
SD		0.9	1.5	1.2	0.6		
SEM		0.5	0.8	0.7	0.4		
Oral dosing	4		0.3	0.5	1.4	2.7	1.7
	5		0.2	0.5	1.5	3.3	1.5
	6		0.1	0.4	3.0	2.8	0.6
Mean			0.2	0.5	2.0	3.0	1.3
SD			0.1	0.1	0.9	0.3	0.6
SEM			0.0	0.0	0.5	0.2	0.3

10:90 (v/v) DMSO:PEG-400 dosed at 1 mL/kg - Day 1 Fasted.

Table S2. Plasma concentration, cardiovascular parameters and platelets in anesthetized rats (n=3) administered 3 step infusion of A-1331852.

Infusion (mg/kg)	Plasma Conc (µg/ml)	MAP	P-value	HR	P-value	dP/dt_{50}	P-value	%platelet/baseline
10	57.2 ± 6.5	-10	0.10	+1	0.84	-2	0.72	NT
30	194.0 ± 12.8	+1	0.91	+2	0.56	+5	0.43	16.7
100 (@ 85 minutes)	331.6 ± 17.2	-21*	<0.01	+5	0.74	+5	0.45	5.4

Male Sprague-Dawley rats were anesthetized with Inactin, instrumented, and allowed to stabilize. Following a 30 minute baseline period, A-1331852 was infused at 10, 30, and 100 mg/kg/30 minutes in 20% DMA/40% PG/40% PEG-400 vehicle (1 mL/kg). Peak plasma concentrations at the end of each dose are shown above. MAP = mean arterial pressure: HR = heart rate; dP/dt@50 = cardiac contractility. * Statistically significant difference from vehicle.

Table S3. Drug concentrations, hemodynamic parameters and platelets in anesthetized dogs (n=2) treated with a bolus of inhibitor 9 at 0.03 mg/kg.

Mean Change From Time Zero

Timepoint	[Drug] ng/mL ± SEM	MAP (%)	HR (%)	dp/dt@50 (%)	SVR (%)	CO (%)	QTcV (ms)	QRS (ms)	PR (ms)	Platelets $10^3/\mu L$
0	0									188
5	93 ± 21	-2	8	13			-15	1	-8	205
10	36 ± 0.5	-5	5	10			-7	1	-6	167
15	14 ± 1.2	-2	9	10	-9	4	-0	0	-9	71
30	3.3 ± 0.4	-3	3	9	9	-8	15	2	-6	28
45	1.6 ± 0.1	-5	-1	1	10	-14	12	-1	-3	23
60	1.1 ± 0.1	-2	-1	0	19	-20	13	-2	-3	21
75	0	0	-5	-3	26	-23	13	-2	-4	20
90	0	1	-4	-5	38	-29	13	0	-5	18
105		-1	-1	-4	67	-41	9	-2	-9	NS
120	0	-3	-2	-10	89	-47	10	1	-9	21
135		-8	-1	-10	155	-67				NS
150		-15	-1	-20						NS
180	0	-22	1	-24						18
210	0	-33	0	-29						28

15% change from vehicle for MAP, HR, SVR, CO considered biologically relevant. 20% change from vehicle for dP/dt@50 is considered biologically relevant. 10 ms increase in QTcV is cause for concern (ICH E14 criteria). A single intravenous bolus of inhibitor 9 delivered over one minute at 0.03 mg/kg was followed for 210 minutes. All values shown in the table are expressed as changes from time zero in the drug-treated dogs. MAP and dP/dt@50 decreased to -33 and -29%, respectively at 210 minutes. Cardiac output decreased and SVR increased -67 and 155%, respectively at 135 minutes (the final timepoint these endpoints were measured). ECG endpoints are n=1 after 120 minutes. QTcV showed a transient decrease (-15 ms) followed by a sustained increase from 30 (15 ms) to 120 minutes (10 ms) post dosing. At 120 minutes there were clear reductions in the QRS amplitude. This effect occurs prior to changes in hemodynamics. MAP = mean arterial pressure; HR = heart rate; SVR= systemic vascular resistance; CO= cardiac output; dP/dt@50 = cardiac contractility; QTcV= QT interval corrected using Van de Water formula; PR= interval from P to R on electrocardiogram.

Table S4. Drug concentrations, hemodynamic parameters and platelets in anesthetized dogs (n=2) treated with a bolus of inhibitor 10 at a dose of 2.5 mg/kg.

Mean Change Relative to Vehicle

Timepoint	[Drug] µg/mL ± SEM	MAP (%)	HR (%)	dp/dt@50 (%)	SVR (%)	CO (%)	QTcV (ms)	PR (ms)	Platelets 10^3/µL
0	0								267
5	9.2 ± 1.2	-1	-3	2	0	0	1	0	212
10	1.7 ± 0.2	1	-2	-1	0	0	-1	1	205
15	0.2 ± 0.04	0	-3	-4	0	-1	3	0	188
30	0.04 ± 0.0	3	-3	-5	9	-6	7	-1	221
45	0.02 ± 0.0	5	-7	-1	24	-17	4	-1	234
60	0.01 ± 0.0	11	3	7	23	-9	-2	-6	237
75	0.0 ± 0.0	11	2	6	33	-14	-8	-6	233
90	0.0 ± 0.0	14	2	7	31	-10	-10	-6	214
120	0.0 ± 0.0	15	-7	1	47	-21	-3	-1	221
180	0.0 ± 0.0	18	8	17			-6	-6	225

15% change from vehicle for MAP, HR, SVR, CO considered biologically relevant. 20% change from vehicle for dP/dt@50 is considered biologically relevant. 10 ms increase in QTcV is cause for concern (ICH E14 criteria). 2.5 mg/kg-1' infusion of 10 produced minimal to moderate cardiovascular effects on mean arterial pressure (MAP), heart rate (HR), myocardial contractility (dP/dt), cardiac output (CO), QTcV and PR-interval. Apparent changes in systemic vascular resistance (SVR) and cardiac output (CO) may be the result of baseline shifts in one animal. MAP = mean arterial pressure; HR = heart rate; SVR= systemic vascular resistance; CO= cardiac output; dP/dt@50 = cardiac contractility; QTcV= QT interval corrected using Van de Water formula; PR= interval from P to R on electrocardiogram.

Table S5. High molecular weight species (HMWS) of ADCs Ab033-14, Ab033-15, AM1-AAA and Adcetris (R) in buffer.

ADC	DAR	% HMWS in Buffer[a]
Ab033-14	4	45
Ab033-15	4	3.3
AM1-15	4	2.9
AM1-15	2	1.6
AM1-AAA	4	2.9
AM1-AAA	2	1.2
Adcetris(R)	4	2.5

[a]DPBS buffer, pH7.4

Table S6. Select Characterization of DAR 4 AM1-15.

Property	Result
Solubility, 15 mM histidine buffer, pH 6.0	> 45 mg/mL
Heat stress stability (DSC)	Stable up to 57 °C
In-vitro human stability (7 days at 37°C)	<0.05% warhead release, but observed aggregation in serum (See Table S10)
Accelerated stability @ 1 mg/mL in buffer pH 6 up to 14 days (5 and 40°C)	< 1% monomer loss, no change in DAR, no changes by IEX
Accelerated stability @ 50 mg/mL in buffer pH 6 up to 14 days (5 and 40°C)	< 1% monomer loss, no change in DAR, no changes by IEX
4x Freeze/thaw cycles in buffer	< 1% increase in aggregated proteins

Table S7. Mean Toxicokinetic Parameters of Total Antibody (TAb) and payload following a single intravenous dose of AM1-15 in cynomolgus monkeys.

Dose	Analyte	C_{max}	T_{max}	$T_{1/2}$	MRT	$AUC_{0\text{-}504hr}$	V_{ss}
3	Tab	97	-	5.6	5.6	6.1	63
	Payload	3.0	4	-	-	45	-
6	Tab	235	-	7.3	7.9	17.1	61
	Payload	6.0	4	-	-	85	-
10	Tab	309	-	5.2	6.2	23.7	59
	Payload	9.4	14	-	-	312	-
15	Tab	454	-	7.3	7.5	35.1	71
	Payload	11.3	4	-	-	564	-
20	Tab	666	-	6.5	7.6	52.6	64
	Payload	17.3	4	-	-	976	-
30	Tab	1210	-	7.0	8.9	100.4	57
	Payload	26.1	14	-	-	1780	-

C_{max}: Maximal concentration after dosing; AUC: area under cover; Units for total antibody: AUC (µg•hr/mL); $T_{1/2}$ and MRT (days, harmonic mean); V_{ss} (mL/kg); Units for payload: C_{max} (ng/mL); AUC (ng•hr/mL); Tmax (hours).

Supplementary Table S8. Mean Cmax and AUC of total and conjugate antibody and released payload following intravenous doses of AM1-15 and AM1-25 in cynomolgus monkeys.

Construct	Dose Regimen	First Dose							Last Dose					
		Total		Conjugate		Payload			Total		Conjugate		Payload	
		C_{max}	AUC_{0-168}	C_{max}	AUC_{0-168}	C_{max}	AUC_{0-168}		C_{max}	AUC_{0-168}	C_{max}	AUC_{0-168}	C_{max}	AUC_{0-168}
AM1-15, DAR4	10 mg/kg; Q1wx3	283	14	NA	NA	2.1	25		365	25	NA	NA	2	24
	30 mg/kg; Q1wx3	818	46	NA	NA	17	843		1231	93	NA	NA	18	1146
	30 mg/kg; Q3wx2	958	48	NA	NA	20	1036		1019	56	NA	NA	15	985
	30 mg/kg; Q1wx3	868	69	1149	65	NA	NA		1339	131	1415	104	1.1	156
AM1-15, DAR2	30 mg/kg; Q1wx3	970	50	1014	50	6.9	205		1185	85	1112	81	6.3	625
AM1-25, DAR2	10 mg/kg; Q3wx2	245	18	242	15	0.75	61		268	23	308	22	1.5	182
	30 mg/kg; Q3wx2	790	62	744	53	1.9	279		840	83	741	64	3.7	439
	10 mg/kg; Q1wx3	245	18	362	22	NA	NA		368	36	539	40	1.8	216
AM1-16, DAR2	10 mg/kg; Q1wx3	211	20.6	220	19	NA	NA		323	34.3	367	31	NA	NA
MSL109-15, DAR2	10 mg/kg; Q1wx3	278	30	230	17.6	NA	NA		725	80.7	404	37	NA	NA
CD98-15, DAR2	10 mg/kg; Q1wx3	289	11.6	273	9.61	1.19	93.6		296	9.06	286	7.98	2.61	157

C_{max}: Maximal concentration after dosing; NA: Not applicable; Units for TAB and ADC μg/mL and μg•hr/mL for C_{max} and AUC_{0-168}, respectively; Units for payload ng/mL and ng•hr/mL

Supplementary Table S9. Dose-normalized Cmax and AUC of total and conjugate antibody and released payload following intravenous doses of AM1-15, AM1-25, and all test item ADCs utilized in mechanistic studies in cynomolgus monkeys.

Construct	Dose Regimen	First Dose							Last Dose					
		Total		Conjugate		Payload			Total		Conjugate		Payload	
		C_{max}/D	AUC_{0-168}/D	C_{max}/D	AUC_{0-168}/D	C_{max}/D	AUC_{0-168}/D		C_{max}/D	AUC_{0-168}/D	C_{max}/D	AUC_{0-168}/D	C_{max}/D	AUC_{0-168}/D
AM1-15, DAR4	10 mg/kg; Q1wx3	28	1.4	NA	NA	0.05	0.6		37	2.5	NA	NA	0.05	0.6
AM1-15, DAR4	30 mg/kg; Q1wx3	27	1.5	NA	NA	0.15	7		41	3.1	NA	NA	0.15	9.5
AM1-15, DAR4	30 mg/kg; Q3wx2	32	1.6	NA	NA	0.16	8.6		34	1.9	NA	NA	0.13	8.2
AM1-15, DAR4	30 mg/kg; Q1wx3	29	2.3	38	2.2	NA	NA		45	4.4	47	3.5	0.02	2.6
AM1-15, DAR2	30 mg/kg; Q1wx3	32	1.7	34	1.7	0.06	1.7		40	2.8	37	2.7	0.05	5.2
AM1-25, DAR2	10 mg/kg; Q3wx2	25	1.8	24	1.5	0.04	3.1		27	2.3	31	2.2	0.07	9.1
AM1-25, DAR2	30 mg/kg; Q3wx2	26	2.1	25	1.8	0.03	4.7		28	2.8	25	2.1	0.06	7.3
AM1-25, DAR2	10 mg/kg; Q1wx3	25	1.8	36	2.2	NA	NA		37	3.6	54	4	0.09	11
AM1-16, DAR2	10 mg/kg; Q1wx3	21.1	2.06	22	1.9	NA	NA		32	3.4	37	3.1	NA	NA
MSL109-15, DAR2	10 mg/kg; Q1wx3	27.8	3	23	1.8	NA	NA		73	8.1	40	3.7	NA	NA
CD98-15, DAR2	10 mg/kg; Q1wx3	28.9	1.2	27	1.0	0.06	4.7		30	0.91	29	0.80	0.13	7.9

Table S10. High Molecular Weight Species (HMWS) of ADCs AM1-15 and AM1-25 in Buffer and Across Species Plasma.

ADC	DAR	HMWS in Buffer		HMWS in Mouse Plasma		HMWS in Monkey Plasma		HMWS in Human Plasma	
		% at Time = 0	% incr. per day	% at Time = 0	% incr. per day	% at Time = 0	% incr. per day	% at Time = 0	% incr. per day
AM1-15	4	1.97	-0.18	16.1	7.09	8.79	6.59	5.93	6.27
AM1-15	2	0.76	0.14	0.94	5.59	0.41	7.73	0.15	8.03
AM1-25	2 (1.4)	1.20	-0.22	1.47	1.10	1.07	1.33	1.10	1.20

Table S11: List of BCL-XL ADCs administered to cynomolgus monkeys in non-GLP toxicity studies.

ADC	Antibody Target	Payload (inhibitor)	Purification (DAR)	Dose level (in mg/kg)	Dose schedule (no of doses)	Animals/ group
AM1-15	EGFR	15 (10)	No (broad DAR 4)	3, 6, 10, 15, 20, 30	Single dose	N = 2
AM1-15	EGFR	15 (10)	No (broad DAR 4)	10, 30	Q1W (3)	N = 4
				30	Q3W (2)	
AM1-15	EGFR	15 (10)	Yes (DAR 2)	30	Q1W (3)	N = 3
CD98-15	CD98	15 (10)	Yes (DAR 2)	10	Q1W (3)	N = 3
MSL109-15	cytomegalovirus	15 (10)	Yes (DAR 2)	10	Q1W (3)	N = 2
AM1-16	EGFR	16 (12)	Yes (DAR 2)	10	Q1W (3)	N = 2
AM1-25	EGFR	25 (11)	Yes (DAR 2)	10, 30	Q3W (2)	N = 3
				10	Q1W (3)	

Table S12. **Investigative toxicology studies reveal mechanism-based mesangial cell effect of BCL-X_L inhibition.** Tabular synopsis of results and conclusions from toxicity studies conducted with various BCL-X_L ADCs to investigate the potential role of ADC aggregation, antibody target and BCL-XL inhibition in the observed kidney lesions in monkey.

Objective	**Role of aggregation in kidney finding**	**Role of antigen in kidney finding**	**Role of SMI in kidney finding**
Compound	DAR purified ADC AM1-15 (DAR2)	Non-EGFR ADC MSL109-15, CD98-15	AM1-16 (inactive warhead)
Dose in mg/kg (schedule)	10, 30 (Q1Wx3)	10 (Q1Wx3)	10 (Q1Wx3)
Results	Increased glomerular matrix	Increased glomerular matrix	No increase in glomerular matrix
Conclusion	ADC aggregation not cause of kidney finding	Kidney findings not antigen-dependent	Kidney findings dependent on BCL-XL activity

Table S13. Drug concentrations, hemodynamic parameters and platelets in anesthetized dogs (n=1) treated with a bolus of inhibitor 11 at 0.01 or 0.1 mg/kg/min.

Mean Change from Time Zero

Timepoint	[Drug] ng/mL 0.01 mg/kg/min	MAP 0.01	Platelets 10^3/µL	[Drug] ng/mL 0.1 mg/kg/min	MAP 0.1 mg/kg	Platelets 10^3/uL
0	<2	0	281	<4	0	231
5	29	-3	249	386	1	221
10	6	-1	273	85	-2	253
15	<2	0	258	29	1	247
30	<2	1	256	7	-2	262
45	<2	-1	266	<4	0	248
60	<2	-1	260	<4	-1	247
75	<2	0	***222***	<4	2	251
90	<2	1	260	<4	-1	247
105	<2	3	264	<4	-5	246
120	<2	4	261	<4	-8	***225***
135	<2	6	264	<4	-9	223
150	<2	9	261	<4	-13	***255***
180	<2	NC*	-	<4	-14	***221***

2 dogs evaluated in each dose group. *NC = Not collected. MAP = Mean Arterial Pressure (mmHg). Italicized bolded platelet and drug concentration values are n=1 due to sample issues. Individual platelet values for the 1 mg/kg dose are shown in the far right in the table. Drug concentration values with < denote Lowest Limit of Quantitation.

Table S14. Drug concentrations, hemodynamic parameters and platelets in anesthetized dogs (n=2) treated with a bolus of inhibitor 11 at 0.3 mg/kg/min.

Mean Change Relative to Vehicle

Timepoint	[Drug] ng/mL ± SEM animal 1	[Drug] ng/mL ± SEM animal 2	Platelets $10^3/\mu L$ animal 1	Platelets $10^3/\mu L$ animal 2	MAP (%)	HR (%)	dp/dt@50 (%)	SVR (%)	CO (%)	QTcV (ms)	QRS (ms)	PR (ms)
0			133	158								
5	1300	730	113	101								
10	422	133	154	184								
15	133	57.3	179	175	-5	0	1	12	-14	1	0	-6
30	19	9.12	162	195	-18	-5	-16	2	-20	10	1	-1
45	9.48	3.84	148	202	-24	-9	-23	7	-31	14	0	-2
60	5.35	3.34	120	190	-26	-7	-26	-11	-20	11	1	-4
75	4.58	1.18	120	178	-28	-10	-25	-14	-16	14	0	-3
90	2.49	0.4	127	193	-30	-15	-30	-7	-26	16	-1	0
105	1.44	0	141	228	-35	-16	-33	-8	-50	16	-2	0
120	1.35		145		-36	-16	-35	-9	-45	15	-2	1
135												
150												

† indicates $p<0.05$ change from baseline, treated vs. vehicle (n=4-6 studies only). No statistical analyses done on $n \leq 3$. 15% change from vehicle for MAP, HR, SVR, CO considered biologically relevant. 20% change from vehicle for dP/dt@50 is considered biologically relevant. 10 ms increase in QTcV is cause for concern (ICH E14 criteria). Decreases in QTcV less well characterized, warrants caution. PR is a measure of AV conduction time as such prolongation or shortening is undesirable. QRS represents ventricular depolarization time as such prolongation or shortening is undesirable. MAP = mean arterial pressure; HR = heart rate; SVR = systemic vascular resistance; CO = cardiac output; dP/dt@50 = cardiac contractility; QTcV= QT interval corrected using Van de Water formula; PR = interval from P to R on electrocardiogram.

Table S15. Procedures and experimental details, results for ADCs.

Compound	Procedure	DAR	Aggregation (%)	HIC purification*	Hydrolysis
AB033-14 broad DAR 4	1	3.2	45	No	No
AB033-15 broad DAR 4	1	3.7	3.3	No	No
AM1-15 broad DAR 4	1	4	3.3	No	No
AM1-15 DAR 2	2	2	1.6	Yes	No
CD98-15 DAR 2	2	2	0.5	Yes	No
MSL109-15 DAR 2	2	2	0.4	Yes	No
AM1-16 DAR 2	2	2	1.1	Yes	No
AM1-25 DAR 2	2	2	0.7	Yes	Yes

*HIC, hydrophobic interaction chromatography.

Table S16. Observed mass of ADCs.

ADC	MW LC+0 (height)	MW LC+1 (height)	MW HC+0 (height)	MW HC+1 (height)	MW HC+2 (height)	MW HC+3 (height)	DAR (MS)	DAR (HIC)
AB033-14[#]	23424 (95806)	24782 (84557)	52285 (4943)	53644 (5856)	55003 (3476)	56365 (1835)	3	4
AB033-15[#]	23424 (86248)	24876 (68110)	52285 (4783)	53738 (9397)	55190 (6104)	56644 (4040)	4	4
AM1-15[#]	23409 (283678)	24862 (324990)	50345 (177068)	51798 (538000)	53250 (311592)	54703 (229956)	4	4
AM1-15*	23409 (465055)	24862 (388567)	50346 (1046694)	51798 (1152227)	0	0	2	2
CD98-15*	24289 (436756)	25741 (357354)	50707 (232044)	52160 (265881)	53617 (1090)	0	2	2
MSL109-15*	23981 (176079)	25433 (284044)	51716 (218563)	53169 (222967)	0	0	2	2
AM1-16*	23409 (180378)	24876 (130957)	50345 (388249)	51812 (409877)	0	0	2	2
AM1-25* (AM1-AAA)	23410 (16105)	25143 (6145)	50345 (18215)	52080 (14480)	0	0	2	2

[#] ADC sample is a broad distribution of DARs with average DAR noted in the table. *Purified by hydrophobic interaction chromatography to a homogenous sample with DAR species indicated in table, see Supplementary Table S10.

Table S17. X-ray diffraction statistics for the complex of BCL-X_L with A-1331852 (PDB code 9AQZ).

Property	**Value**
Space group	I 21 21 21
Cell constants a, b, c (Å) α, β, γ (deg)	 33.03, 78.39, 144.77 90.00, 90.00, 90.00
Resolution (Å)	72.4 – 1.93
% Data completeness	99.9 (72.4 – 1.93Å)
R_{merge}	0.085
$< I/\sigma(I) >$	2.0 (at 1.93 Å)
R, R_{free} (%)	22.3, 25.8
Number of atoms (protein/heterogen/water)	1127/52/70
Mean B-factor ($Å^2$)	31.81
r.m.s.d bonds (Å)	0.008
r.m.s.d angles (deg)	0.812
Ramachandran Preferred (%) Allowed (%) Outlier (%)	 95.68 2.88 1.44

REFERENCES AND NOTES

1. D. Hanahan, R. A. Weinberg, Hallmarks of cancer: The next generation. *Cell* **144**, 646–674 (2011).

2. A. Frenzel, F. Grespi, W. Chmelewskij, A. Villunger, Bcl2 family proteins in carcinogenesis and the treatment of cancer. *Apoptosis* **14**, 584–596 (2009).

3. N. Khan, B. Kahl, Targeting BCL-2 in hematologic malignancies. *Target. Oncol.* **13**, 257–267 (2018).

4. S. A. Amundson, T. G. Myers, D. Scudiero, S. Kitada, J. C. Reed, A. J. Fornace Jr., An informatics approach identifying markers of chemosensitivity in human cancer cell lines. *Cancer Res.* **60**, 6101–6110 (2000).

5. M. Vogler, Targeting BCL2-proteins for the treatment of solid tumours. *J. Adv. Med.* **2014**, 1–14 (2014).

6. C. Tse, A. R. Shoemaker, J. Adickes, M. G. Anderson, J. Chen, S. Jin, E. F. Johnson, K. C. Marsh, M. J. Mitten, P. Nimmer, L. Roberts, S. K. Tahir, Y. Xiao, X. Yang, H. Zhang, S. Fesik, S. H. Rosenberg, S. W. Elmore, ABT-263: A potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* **68**, 3421–3428 (2008).

7. C. M. Park, M. Bruncko, J. Adickes, J. Bauch, H. Ding, A. Kunzer, K. C. Marsh, P. Nimmer, A. R. Shoemaker, X. Song, S. K. Tahir, C. Tse, X. Wang, M. D. Wendt, X. Yang, H. Zhang, S. W. Fesik, S. H. Rosenberg, S. W. Elmore, Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J. Med. Chem.* **51**, 6902–6915 (2008).

8. A. W. Roberts, J. F. Seymour, J. R. Brown, W. G. Wierda, T. J. Kipps, S. L. Khaw, D. A. Carney, S. Z. He, D. C. S. Huang, H. Xiong, Y. Cui, T. A. Busman, E. M. McKeegan, A. P. Krivoshik, S. H. Enschede, R. Humerickhouse, Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: Results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **30**, 488–496 (2012).

9. T. J. Kipps, H. Eradat, S. Grosicki, J. Catalano, W. Cosolo, I. S. Dyagil, S. Yalamanchili, A. Chai, S. Sahasranaman, E. Punnoose, D. Hurst, H. Pylypenko, A phase 2 study of the BH3 mimetic BCL2

inhibitor navitoclax (ABT-263) with or without rituximab, in previously untreated B-cell chronic lymphocytic leukemia. *Leuk. Lymphoma* **56**, 2826–2833 (2015).

10. H. Zhang, P. M. Nimmer, S. K. Tahir, J. Chen, R. M. Fryer, K. R. Hahn, L. A. Iciek, S. J. Morgan, M. C. Nasarre, R. Nelson, L. C. Preusser, G. A. Reinhart, M. L. Smith, S. H. Rosenberg, S. W. Elmore, C. Tse, Bcl-2 family proteins are essential for platelet survival. *Cell Death Differ.* **14**, 943–951 (2007).

11. L. Gandhi, D. R. Camidge, M. R. de Oliveira, P. Bonomi, D. Gandara, D. Khaira, C. L. Hann, E. M. McKeegan, E. Litvinovich, P. M. Hemken, C. Dive, S. H. Enschede, C. Nolan, Y. L. Chiu, T. Busman, H. Xiong, A. P. Krivoshik, R. Humerickhouse, G. I. Shapiro, C. M. Rudin, Phase I study of navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors. *J. Clin. Oncol.* **29**, 909–916 (2011).

12. A. J. Souers, J. D. Leverson, E. R. Boghaert, S. L. Ackler, N. D. Catron, J. Chen, B. D. Dayton, H. Ding, S. H. Enschede, W. J. Fairbrother, D. C. S. Huang, S. G. Hymowitz, S. Jin, S. L. Khaw, P. J. Kovar, L. T. Lam, J. Lee, H. L. Maecker, K. C. Marsh, K. D. Mason, M. J. Mitten, P. M. Nimmer, A. Oleksijew, C. H. Park, C. M. Park, D. C. Phillips, A. W. Roberts, D. Sampath, J. F. Seymour, M. L. Smith, G. M. Sullivan, S. K. Tahir, C. Tse, M. D. Wendt, Y. Xiao, J. C. Xue, H. Zhang, R. A. Humerickhouse, S. H. Rosenberg, S. W. Elmore, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* **19**, 202–208 (2013).

13. C. N. Harrison, J. S. Garcia, R. A. Mesa, T. C. P. Somervaille, R. S. Komrokji, N. Pemmaraju, C. Jamieson, N. Papadantonakis, J. M. Foran, C. L. O'Connell, L. Holes, J. Jia, J. Harb, J. Hutti, J. T. Prchal, Results from a phase 2 study of navitoclax in combination with ruxolitinib in patients with primary or secondary myelofibrosis. *Blood* **134** (suppl. 1), 671 (2019).

14. E. M. Bertino, R. D. Gentzler, S. Clifford, J. Kolesar, A. Muzikansky, E. B. Haura, Z. Piotrowska, D. R. Camidge, T. E. Stinchcombe, C. Hann, J. Malhotra, L. C. Villaruz, C. P. Paweletz, C. L. Lau, L. Sholl, N. Takebe, J. A. Moscow, G. I. Shapiro, P. A. Jänne, G. R. Oxnard, Phase IB study of osimertinib in combination with navitoclax in EGFR-mutant NSCLC following resistance to initial EGFR therapy (ETCTN 9903). *Clin. Cancer Res.* **27**,1604–1611 (2021).

15. M. Puglisi, L. R. Molife, M. J. de Jonge, K. H. Khan, L. van Doorn, M. D. Forster, M. Blanco, M. Gutierrez, C. Franklin, T. Busman, J. Yang, F. A. Eskens, Phase I safety and pharmacokinetic (PK) study of navitoclax (N) in combination with docetaxel (D) in patients (pts) with solid tumors. *J. Clin. Oncol.* **29**, 2518 (2011).

16. J. D. Leverson, D. C. Phillips, M. J. Mitten, E. R. Boghaert, D. Diaz, S. K. Tahir, L. D. Belmont, P. Nimmer, Y. Xiao, X. M. Ma, K. N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T. J. Magoc, K. S. Vaidya, D. H. Albert, J. M. Tarrant, N. La, L. Wang, Z. F. Tao, M. D. Wendt, D. Sampath, S. H. Rosenberg, C. Tse, D. C. S. Huang, W. J. Fairbrother, S. W. Elmore, A. J. Souers, Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci. Transl. Med.* **7**, 279ra4027ra40 (2015).

17. L. Wang, G. A. Doherty, A. S. Judd, Z. F. Tao, T. M. Hansen, R. R. Frey, X. Song, M. Bruncko, A. R. Kunzer, X. Wang, M. D. Wendt, J. A. Flygare, N. D. Catron, R. A. Judge, C. H. Park, S. Shekhar, D. C. Phillips, P. Nimmer, M. L. Smith, S. K. Tahir, Y. Xiao, J. Xue, H. Zhang, P. N. Le, M. J. Mitten, E. R. Boghaert, W. Gao, P. Kovar, E. F. Choo, D. Diaz, W. J. Fairbrother, S. W. Elmore, D. Sampath, J. D. Leverson, A. J. Souers, Discovery of A-1331852, a first-in-class, potent and orally-bioavailable BCL-X_L inhibitor. *ACS Med. Chem. Lett.* **11**, 1829–1836 (2020).

18. H. Xiong, R. S. Pradhan, A. Nada, A. P. Krivoshik, K. D. Holen, J. W. Rhodes, G. B. Gordon, R. Humerickhouse, W. M. Awni, Studying navitoclax, a targeted anticancer drug, in healthy volunteers—Ethical considerations and risk/benefit assessments and management. *Anticancer Res* **34**, 3739–3746 (2014).

19. P. Khongorzul, C. J. Ling, F. U. Khan, A. U. Ihsan, J. Zhang, Antibody-drug conjugates: A comprehensive review. *Mol. Cancer Res.* **18**, 3–19 (2020).

20. S. Coats, M. Williams, B. Kebble, R. Dixit, L. Tseng, N. S. Yao, D. A. Tice, J. C. Soria, Antibody-drug conjugates: Future directions in clinical and translational strategies to improve the therapeutic index. *Clin. Cancer Res.* **25**, 5441–5448 (2019).

21. J. C. Masters, D. J. Nickens, D. Xuan, R. L. Shazer, M. Amantea, Clinical toxicity of antibody drug conjugates: A meta-analysis of payloads. *Invest. New Drugs* **36**, 121–135 (2018).

22. A. Wolska-Washer, T. Robak, Safety and tolerability of antibody-drug conjugates in cancer, *Drug Saf.* **42**, 295–314 (2019).

23. Y. J. Wang, Y. Y. Li, X. Y. Liu, X. L. Lu, X. Cao, B. H. Jiao, Marine antibody-drug conjugates: Design strategies and research progress. *Mar. Drugs* **15**, 18 (2017).

24. W. J. Fairbrother, J. D. Leverson, D. Sampath, A. J. Souers, Discovery and development of venetoclax, a selective antagonist of BCL-2. *Success. Drug Discov.* **4**, 225–245 (2019).

25. A. W. Tolcher, B. A. Carneiro, A. Dowlati, A. Ryan A. Razak, Y. K. Chae, J. A. Villella, S. Coppola, S. Englert, A. C. Phillips, A. J. Souers, Z. Salman, S. Penugonda, J. D. Powderly, P. LoRusso, A first-in-human study of mirzotamab clezutoclax as monotherapy and in combination with taxane therapy in relapsed/refractory solid tumors: Dose escalation results. *J. Clin. Oncol.* **39**, 15 (2021).

26. Y. E. Koshman, A. S. Wilsey, B. M. Bird, A. L. Endemann, S. Sadilek, J. Treadway, R. L. Martin, J. S. Polakowski, G. A. Gintant, S. W. Mittelstadt, Drug-induced QT prolongation: Concordance of preclinical anesthetized canine model in relation to published clinical observations for ten CiPA drugs. *J. Pharmacol. Toxicol. Methods* **103**, 106871 (2020).

27. K. S. Weber, P. J. Nelson, H. J. Gröne, C. Weber, Expression of CCR2 by endothelial cells: Implications for MCP-1 mediated wound injury repair and In vivo inflammatory activation of endothelium. *Arterioscler. Thromb. Vasc. Biol.* **19**, 2085–2093 (1999).

28. H. Morimoto, M. Takahashi, Role of monocyte chemoattractant protein-1 in myocardial infarction. *Int. J. Biomed. Sci.* **3**, 159–167 (2007).

29. Z. F. Tao , X. Wang , J Chen, J. P. Ingram, S. Jin, R. A. Judge, P. J. Kovar, C. Park, C. Sun, B. D. Wakefield, L. Zhou, H. Zhang, S. W. Elmore, D. C. Phillips, A. S. Judd, J. D. Leverson, A. J. Souers, Structure-based design of A-1293102, a potent and selective BCL-XL inhibitor. *ACS Med. Chem. Lett.* **12**, 1011–1016 (2021).

30. J. Ryan, A. Letai, BH3 profiling in whole cells by fluorimeter or FACS. *Methods* **61**, 156–164 (2013).

31. P. Senter, E. Sievers, The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat. Biotechnol.* **30**, 631–637 (2012).

32. K. R. Durbin, C. Phipps, X. Liao, Mechanistic modeling of antibody-drug conjugate internalization at the cellular level reveals inefficient processing steps. *Mol. Cancer Ther.* **17**, 1341–1351 (2018).

33. L. Mazzarella, A. GuidA, G. Curigliano, Cetuximab for treating non-small cell lung cancer. *Expert Opin. Biol. Ther.* **18**, 483–493 (2018).

34. G. Lessene, P. E. Czabotar, B. E. Sleebs, K. Zobel, K. N. Lowes, J. M. Adams, J. B. Baell, P. M. Colman, K. Deshayes, W. J. Fairbrother, J. A. Flygare, P. Gibbons, W. J. A. Kersten, S. Kulasegaram, R. M. Moss, J. P. Parisot, B. J. Smith, I. P. Street, H. Yang, D. C. S. Huang, K. G. Watson, Structure-guided design of a selective BCL-X_L inhibitor. *Nat. Chem. Biol.* **9**, 390–397 (2013).

35. A. E. Fouts, L. Comps-Agrar, K. F. Stengel, D. Ellerman, A. J. Schoeffler, S. Warming, D. L. Eaton, B. Feierbach, Mechanism for neutralizing activity by the anti-CMV gH/gL monoclonal antibody MSL-109. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 8209–8214 (2014).

36. A. C. Phillips, E. R. Boghaert, K. S. Vaidya, H. D. Falls, M. J. Mitten, P. J. DeVries, L. Benatuil, C. M. Hsieh, J. A. Meulbroek, S. C. Panchal, F. G. Buchanan, K. R. Durbin, M. J. Voorbach, D. R. Reuter, S. R. Mudd, L. I. Loberg, S. L. Ralston, D. Cao, H. K. Gan, A. M. Scott, E. B. Reilly, Characterization of ABBV-221, a tumor-selective EGFR-targeting antibody drug conjugate. *Mol. Cancer Ther.* **17**, 795–805 (2018).

37. I. E. Wertz, S. Kusam, C. Lam, T. Okamoto, W. Sandoval, D. J. Anderson, E. Helgason, J. A. Ernst, M. Eby, J. Liu, L. D. Belmont, J. S. Kaminker, K. M. O'Rourke, K. Pujara, P. B. Kohli, A. R. Johnson, M. L. Chiu, J. R. Lill, P. K. Jackson, W. J. Fairbrother, S. Seshagiri, M. J. C. Ludlam, K. G. Leong, E. C. Dueber, H. Maecker, D. C. S. Huang, V. M. Dixit, Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. *Nature* **471**, 110–114 (2011).

38. “Common terminology criteria for adverse events (CTCAE)”; https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

39. J. L. Rojko, M. G. Evans, S. A. Price, B. Han, G. Waine, M. DeWitte, J. Haynes, B. Freimark, P. Martin, J. T. Raymond, W. Evering, M. C. Rebelatto, E. Schenck, C. Horvath, Formation, clearance, deposition, pathogenicity, and identification of biopharmaceutical-related immune complexes: Review and case studies. *Toxicol. Pathol.* **42**, 725–764 (2014).

40. L. Boysen, B. M. Viuff, L. H. Landsy, J. Lykkesfeldt, J. T. Raymond, S. A. Price, H. Pelzer, B. Lauritzen, Formation and glomerular deposition of immune complexes in mice administered human antibodies: Evaluation of dose, frequency, and biomarkers. *Toxicol. Pathol.* **48**, 570–585 (2020).

41. G. M. Hayes, L. Chinn, J. M. Cantor, B. Cairns, Z. Levashova, H. Tran, T. Velilla, D. Duey, J. Lippincott, J. Zachwieja, M. H. Ginsberg, E. H. van der Horst, Antitumor activity of an anti-CD98 antibody. *Int. J. Cancer* **137**, 710–720 (2015).

42. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD, Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **45**, 2615–2623 (2002).

43. T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, B. A. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, S. Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Connor, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. Wang, M. D. Wendt, H. Zhang, S. W. Fesik, S. H. Rosenberg, An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature.* **435**, 677–681 (2005).

44. N. Tan, M. Malek, J. Zha, P. Yue, R. Kassees, L. Berry, W. J. Fairbrother, D. Sampath, L. D. Belmont, Navitoclax enhances the efficacy of taxanes in non-small cell lung cancer models. *Clin. Cancer Res.* **17**, 1394–1404 (2010).

45. J. Chen, S. Jin, V. Abraham, X. Huang, B. Liu, M. J. Mitten, P. Nimmer, X. Lin, M. Smith, Y. Shen, A. R. Shoemaker, S. K. Tahir, H. Zhang, S. L. Ackler, S. H. Rosenberg, H. Maecker, D. Sampath, J. D. Leverson, C. Tse, S. W. Elmore, The Bcl-2/Bcl-X_L/Bcl-w inhibitor, navitoclax, enhances the activity of chemotherapeutic agents in vitro and in vivo. *Mol. Cancer Ther.* **10**, 2340–2349 (2011).

46. R. B. Corcoran, K. A. Cheng, A. N. Hata, A. C. Faber, H. Ebi, E. M. Coffee, P. Greninger, R. D. Brown, J. T. Godfrey, T. J. Cohoon, Y. Song, E. Lifshits, K. E. Hung, T. Shioda, D. Dias-Santagata, A. Singh, J. Settleman, C. H. Benes, M. Mino-Kenudson, K. Wong, J. A. Engelman, Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* **23**, 121–128 (2013).

47. M. G. Scioli, G. Storti, F. D'Amico, R. R. Guzmán, F. Centofanti, E. Doldo, E. M. C. Miranda, A. Orlandi, Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: Potential diagnostic biomarkers and therapeutic Targets. *J. Clin. Med.* **9**, 1995 (2020).

48. S. Göser, R. Ottl, A. Brodner, T. J. Dengler, J. Torzewski, K. Egashira, N. R. Rose, H. A. Katus, Z. Kaya, Critical role for monocyte chemoattractant protein-1 and macrophage inflammatory protein-1alpha in induction of experimental autoimmune myocarditis and effective anti-monocyte chemoattractant protein-1 gene therapy. *Circulation* **112**, 3400–3407 (2005).

49. J. Noireaud, R. Andriantsitohaina, Recent insights in the paracrine modulation of cardiomyocyte contractility by cardiac endothelial cells. *Biomed. Res. Int.* **2014**, 923805 (2014).

50. S. Khan, X. Zhang, D. Lv, Q. Zhang, Y. He, P. Zhang, X. Liu, D. Thummuri, Y. Yuan, J. S. Wiegand, J. Pei, W. Zhang, A. Sharma, C. R. McCurdy, V. M. Kuruvilla, N. Baran, A. A. Ferrando, Y. Kim, A. Rogojina, P. J. Houghton, G. Huang, R. Hromas, M. Konopleva, G. Zheng, D. Zhou, A selective BCL-X_L PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* **25**, 1938–1947 (2019).

51. K. Brinkmann, P. Waring, S. P. Glaser, V. Wimmer, D. L. Cottle, M. S. Tham, D. Nhu, L. Whitehead, A. R. Delbridge, G. Lessene, I. M. Smyth, M. J. Herold, G. L. Kelly, S. Grabow, A. Strasser, BCL-XL exerts a protective role against anemia caused by radiation-induced kidney damage. *EMBO J.* **39**, e105561 (2020).

52. Z. X. Wang, An exact mathematical expression for describing competitive binding of two different ligands to a protein molecule. *FEBS Lett.* **360**, 111–114 (1995).

53. S. L. Ackler, N. B. Bennett, E. R. Boghaert, S. C. Cullen, G. Doherty, R. R. Frey, A. R. Haight, A. S. Judd, A. R. Kunzer, V. L. Marin, X. Shen, X. Song, A. J. Souers, G. M. Sullivan, Z. Tao, X. Wang, D.

S. Welch, M. D. Wendt, BCL-XL inhibitory compounds having low cell permeability and antibody drug conjugates including the same. US2016339117A1 (2016).

54. L. Benatuil, M. Bruncko, A. S. Judd, Y. Li, A. Mccluskey, A. C. Phillips, D. C. Phillips, J. Seagal, A. J. Souers, Anti-CD98 antibodies and antibody drug conjugates. WO2017214458 (2017).

55. C. Vonrhein, C. Flensburg, P. Keller, A. Sharff, O. Smart, W. Paciorek, T. Womack, G. Bricogne, Data processing and analysis with the autoPROC toolbox. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 293–302 (2011).

56. A. Vagin, A. Teplyakov, MOLREP: An automated program for molecular replacement. *J. Appl. Cryst.* **30**, 1022–1025 (1997).

57. M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin, K. S. Wilson, Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **67** (Part 4), 235–242 (2011).

58. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66** (Part 4), 486–501 (2010).

59. G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long, A. A. Vagin, REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr. D Biol. Crystallogr.* **67** (Part 4), 355–367 (2011).

60. G. Bricogne, E. Blanc, M. T. Brandl, C. Flensburg, P Keller, W. Paciorek, P. Roversi, A. Sharff, O. S. Smart, C. Vonrhein. BUSTER, Version 2.10.0, Global Phasing Ltd. (2011).