# Discovery of MK-5172, a Macrocyclic Hepatitis C Virus NS3/4a Protease Inhibitor

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**Supporting Information.**

## Experimental

**General.** All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise. All reactions were carried out under an inert atmosphere of nitrogen. $^1$H NMR spectra were obtained on a Varian Unity Inova 400 spectrometer or a Varian Unity Inova 500 spectrometer. Chemical shifts are reported in parts per million relative to TMS as internal standard. Samples provided for accurate mass measurement were taken up in acetonitrile:water (50/50). The solutions were analyzed by use of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on either a Bruker Daltonics 3T or 7T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. External calibration was accomplished with polypropylene glycol (425 or 750). Silica gel chromatography was carried out with an ISCO CombiFlash Sg 100c or ISCO CombiFlash Companion purification system using ISCO silica gel RediSep or Analytical Sales and Products Aspire FlashReady cartridges. Compound purity was determined to be >95% by analytical HPLC analysis on an Agilent 1090 HPLC with binary pump and diode array detector with area quantification performed at 214 nm [Method 1: Zorbax RX-C18, 75 x 4.6 mm, 3.5 μM, 98% A / 2% B to 100% B over 5.5 min then 100% B to 6.0 min (A = 0.1% $H_3PO_4$ / water v/v; B = acetonitrile). Method 2: Luna C8(2), 75 x 4.6 mm, 3 μM, 98% A / 2% B to 100% B over 5.5 min then 100% B to 6.0 min (A = 0.1% $H_3PO_4$ / water v/v; B = acetonitrile)].

**Enzymatic Assays.** Compound inhibitory potencies were determined with use of a time-resolved fluorescence assay for NS3/4A protease activity.[26] The NS3 protease assay was performed in a final volume of 100 μL in assay buffer containing 50 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES), pH 7.5, 150 mM NaCl, 15 % glycerol, 0.15 % Triton X-100, 10 mM dithiothreitol (DTT), and 0.1 % PEG8000. The NS3 protease was pre-incubated with various concentrations of inhibitors in dimethylsulfoxide (DMSO) for 30 minutes. The reaction was initiated by adding the time-resolved fluorescence (TRF) peptide substrate (final concentration 100 nM). NS3 mediated hydrolysis of the substrate was quenched after 1 h at RT with 100 μL of 500 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 5.5. Product fluorescence was detected using either a Victor V2 or Fusion fluorophotometer (Perkin Elmer Life and Analytical Sciences) with excitation at 340 nm and emission at 615 nm with a 400 μs delay. The inhibition constants were derived using a standard four-parameter fit to the data. Full length NS3/4A protease sequences from gt 1b (BK), gt 3a (NZL1), or gt1b encoding amino acid mutations R155K, A156T, A156V, or D168V were expressed and purified from *E. coli*. as his-tagged fusion proteins using a previously described protocol.[26] Protease mutations were engineered into the gt1b expression construct using standard molecular biology techniques.

**Replicon Assay.** Inhibition of viral replication was determined with use of the HCV bicistronic replicon assay[27] adapted for quantitative analysis using *in situ* hybridization.[28] Huh-7 cells that were stably transfected with HCV replicon RNA (gt 1b con1 sequence;[28] gt 2a JFH sequence[29]) were seeded into 96 well plates impregnated with scintillant (Cytostar-T, GE Healthcare) at a density of 20,000 cells per well and incubated at 37 °C/5%$CO_2$ for 24 h in the presence of Dulbecco's modified eagle's medium (DMEM) supplemented with 50% NHS. Compound in DMSO was added to 1%, and incubated for a further 24 h. Cells were fixed by treatment with 10% formaldehyde and permeabilized by treatment with 0.25% Triton X100. A radiolabeled RNA probe that hybridizes to the neomycin resistance gene of the replicon was added, and hybridized at 50 °C for 18 h, followed by RNase A treatment to remove unhybridized probe and washing. The plate was then counted in a Topcount NXT (Packard). The inhibition constants were derived using a standard four-parameter fit to the data.

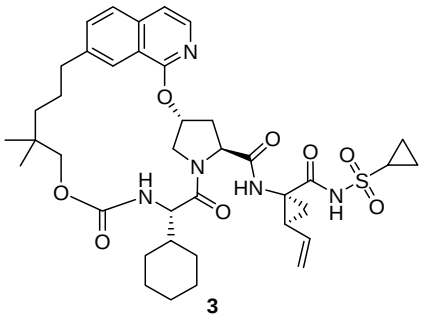
**Pharmacokinetics.** Pharmacokinetic characterization of test agents was conducted in conscious male Sprague-Dawley rats (300-500 g; n = 2-3/study) or male and female beagle dogs (13-15 kg; n = 3/study). Compounds were dosed intravenously to fasted rats and dogs. Compounds in DMSO were administered as a bolus (1.0, 0.1 mL/kg respectively) in DMSO. For oral studies in rat and dog, compounds were dosed as a solution in polyethyleneglycol 400 (PEG400) (2.0 mL/kg). Typical doses were 2 mg/kg IV and 5 mg/kg P.O. to rats and 0.5 mg/kg IV and 1 mg/kg P.O. to dogs. Blood samples for the determination of test agent plasma concentration were obtained at multiple time points up to 24 h after single dose test agent administration. Liver samples were taken at terminal time points for rats and as liver biopsies in dog. Liver samples were homogenized in buffer prior to analysis. Plasma and liver samples were analyzed using liquid-liquid extraction and LC/MS with appropriate standards and QCs. Pharmacokinetic parameters were calculated using Watson software.

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories at West Point and conform with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

**Molecular modeling.** All modeling studies were performed in the full-length NS3/4A active site, employing a published *apo* enzyme structure[30] of a single-chain form of NS3/4A. The six C-terminal residues (DLEVVT) of the helicase domain, which occupy the NS3 protease active site, were removed to allow inhibitor docking. Initially, some protein side-chains, *e.g.* R155 and Q526, were manually adjusted to accommodate the larger P2 substituents in BILN-2061 and vaniprevir. During subsequent energy minimization of docked inhibitors using the MMFF force field[31] with a distance-dependent

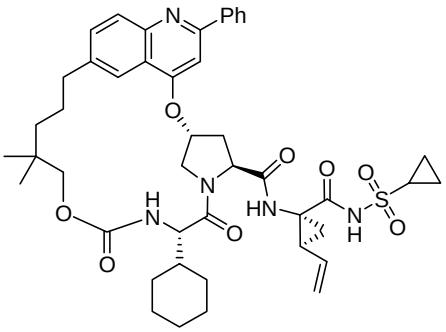
dielectric constant (ε = 2r), all side-chains within 4 Å of any atom of the inhibitor were allowed to relax. Models of the proposed inhibitors were derived from previously published models of BILN-2061 and vaniprevir. Flexibility of the macrocyclic linker and the novel P2 substituents was explored by generating 100 conformers using a distance geometry algorithm.[32] All titratable enzyme residues were charged and all inhibitors were ionized at the acylsulfonamide N. As a general rule, the most favorable pose which resulted in the lowest conformational energy for the ligand was selected. However, because of the flexible nature of the macrocyclic linker, multiple low energy poses were examined.

All final compounds were prepared using a procedure similar to that used to prepare compound **15** using the appropriate P2 heterocycle and linker acid.



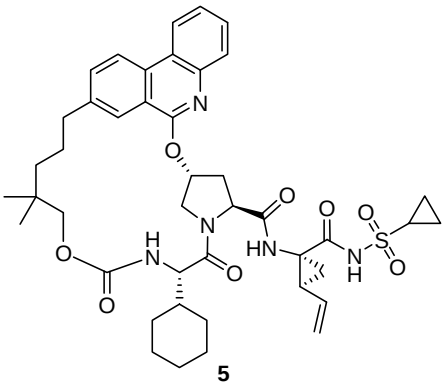
**3**

**(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12,12-dimethyl-6,9-dioxo-3,4,6,7,8,9,12,13,14,15-decahydro-2*H*,11*H*-16,18-etheno-2,5-methanopyrido[2,3-*k*][1,10,3,6]dioxadiazacyclononadecine-4-carboxamide (3).** $^1$H NMR (500 MHz, $CD_3OD$) δ 9.26 (s, 1 H), 7.87 (m, 2 H), 7.71 (d, *J* = 8.3 Hz, 1 H), 7.54 (dd, *J* = 8.3, 1.7 Hz, 1 H), 7.27 (d, *J* = 5.9 Hz, 1 H), 6.04 (apparent t, *J* = 2.5 Hz, 1 H), 5.75 (ddd, *J* = 17.3, 10.3, 9.0 Hz, 1 H), 5.27 (dd, *J* = 17.1, 1.5 Hz, 1 H), 5.09 (dd, *J* = 10.5, 1.7 Hz, 1 H), 4.53-4.42 (m, 2 H), 4.31 (m, 2 H), 4.01 (dd, *J* = 11.5, 2.9 Hz, 1 H), 3.27 (d, *J* = 10.7 Hz, 1 H), 2.95 (ddd, *J* = 12.7, 8.0, 4.6 Hz, 1 H), 2.80 (ddd, *J* = 14.2, 8.3, 6.3 Hz, 1 H), 2.62 (m, 1 H), 2,54 (dd, *J* = 13.7, 6.4 Hz, 1 H), 2.25 (ddd, *J* = 13.9, 11.0, 3.4 Hz, 1 H), 2.17 (dd, *J* = 17.5, 8.8 Hz, 1 H), 1.94-1.84 (m, 3 H), 1.82-1.64 (m, 6 H), 1.56 (m, 1 H), 1.44-1.36 (m, 2 H), 1.33-1.19 (m, 6 H), 1.12 (m, 8 H), 0.78 (s, 3 H) ppm; HRMS (ESI) *m/z* 750.3509 [$(M+H)^+$; calcd for $C_{39}H_{52}N_5O_8S$: 750.3531].



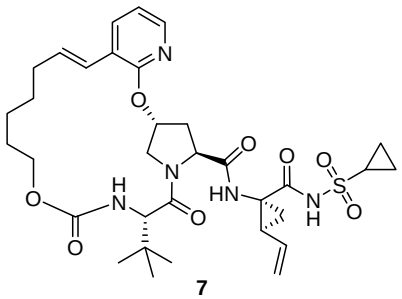
**4**

**(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12,12-dimethyl-6,9-dioxo-20-phenyl-3,4,6,7,8,9,12,13,14,15-decahydro-2*H*,11*H*-16,18-etheno-2,5-methanopyrido[4,3-*k*][1,10,3,6]dioxadiazacyclononadecine-4-carboxamide (4).** $^1$H NMR (500 MHz, $CD_3OD$) δ 9.30 (s, 1 H), 8.07 (m, 4 H), 7.96 (dd, *J* = 8.8, 2.0 Hz, 1 H), 7.84 (s, 1 H), 7.75 (m, 3 H), 6.02 (br s, 1 H), 5.76 (m, 1 H), 5.26 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.11 (dd, *J* = 10.0, 1.6 Hz, 1 H), 4.84 (m, 1 H), 4.72 (d, *J* = 12.0 Hz, 1 H), 4.48 (dd, *J* = 10.8, 6.4 Hz, 1 H), 4.30 (m, 2 H), 4.13 (dd, *J* = 12.0, 2.8 Hz, 1 H), 3.29 (m, 1 H), 2.96 (m, 2 H), 2.70 (m, 2 H), 2.40 (m, 1 H), 2.16 (dd, *J* = 17.6, 8.8 Hz, 1 H), 1.90 (m, 3 H), 1.85-1.55 (m, 6 H), 1.38 (dd, *J* = 9.6, 5.2 Hz, 1 H), 1.35-1.20 (m, 8 H), 1.08 (s, 3 H), 1.15-0.95 (m, 4 H), 0.79 (s, 3 H) ppm; HRMS (ESI) *m/z* 826.3818 [$(M+H)^+$; calcd for $C_{45}H_{56}N_5O_8S$: 826.3844].



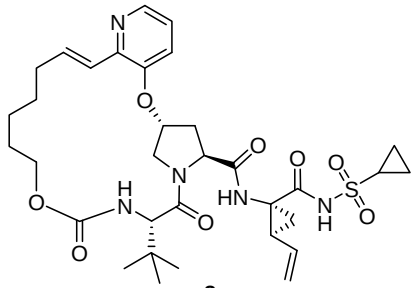
**5**

**(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12,12-dimethyl-6,9-dioxo-3,4,6,7,8,9,12,13,14,15-decahydro-2*H*,11*H*-16,18-etheno-2,5-methano[1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoline-4-carboxamide (5).** $^1$H NMR (500 MHz, $CD_3OD$) δ 8.49 (apparent t, *J* = 8.4 Hz, 2 H), 8.00 (s, 1 H), 7.81 (d, *J* = 7.8 Hz, 1 H), 7.69 (d, *J* = 8.3 Hz, 1 H), 7.60 (apparent t, *J* = 7.3 Hz, 1 H), 7.49 (apparent t, *J* = 7.3 Hz, 1 H), 7.43 (d, *J* = 9.5 Hz, 1 H), 6.23 (s, 1 H), 5.74 (m, 1 H), 5.48 (s, 1 H), 5.24 (d, *J* = 17.1 Hz, 1 H), 5.19 (d, *J* = 10.3 Hz, 1 H), 4.57 (d, *J* = 11.5 Hz, 1 H), 4.48 (m, 1 H), 4.34 (d, *J* = 10.5 Hz, 2 H), 4.05 (d, *J* = 10.3 Hz, 1 H), 2.96 (m, 1 H), 2.83 (m, 1 H), 2.65 (m, 3 H), 2.31 (m, 1 H), 2.16 (m, 1 H), 1.90 (m, 3 H), 1-85-1.55 (m, 6 H), 1.52-1.18 (series of m, 9 H), 1.17-0.95 (m, 6 H), 0.80 (s, 3 H) ppm; MS (ESI) *m/z* 800.6 [$(M+H)^+$; calcd for $C_{43}H_{54}N_5O_8S$: 800.4].



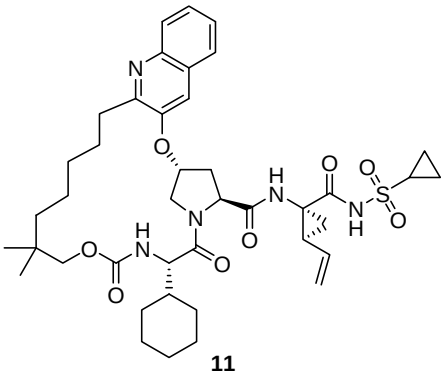
**7**

**(2*R*,4*S*,7*S*,16*E*)-7-*tert*-Butyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-6,9-dioxo-3,4,6,7,8,9,12,13,14,15-decahydro-2*H*,11*H*-2,5-methanopyrido[2,3-*k*][1,10,3,6]dioxadiazacyclononadecine-4-carboxamide (7).** $^1$H NMR (500 MHz, $CD_3OD$) δ 7.97 (m, 1 H), 7.75 (dd, *J* = 7, 1.5 Hz, 1 H), 7.04 (dd, *J* = 10, 1.5 Hz, 1 H), 6.93 (dd, *J* = 7.5, 5 Hz, 1 H), 6.43 (d, *J* = 16 Hz, 1 H), 6.28 (m, 1 H), 6.01 (s, 1 H), 5.76 (m, 1 H), 5.28 (dd, *J* = 17.5, 1.5 Hz, 1 H), 5.11 (dd, *J* = 10.5, 1.5 Hz, 1 H), 4.56 (m, 1 H), 4.45 (dd, *J* = 11, 7 Hz, 1 H), 4.39 (d, *J* = 10 Hz, 1 H), 4.26 (d, *J* = 12 Hz, 1 H), 3.96 (dd, *J* = 11.5, 3.5 Hz, 1 H), 3.75 (m, 1 H), 2.94 (m, 1 H), 2.43 (dd, *J* = 13, 7 Hz, 1 H), 2.31 (m, 1 H), 2.21-2.09 (m, 3 H), 1.86 (dd, *J* = 8, 5.5 Hz, 1 H), 1.72 (m, 1 H), 1.60-1.20 (series of m, 8 H), 1.10-0.96 (m, 10 H), 0.88 (m, 2 H) ppm; HRMS (ESI) *m/z* 672.3028 [$(M+H)^+$; calcd for $C_{33}H_{46}N_5O_8S$: 672.3062].



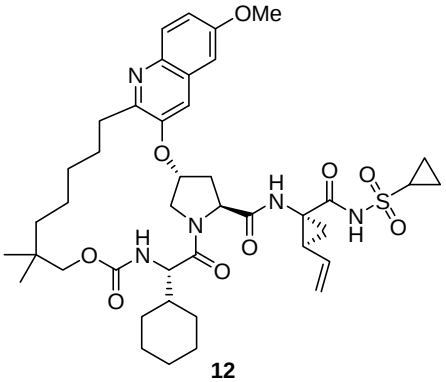
**8**

**(2*R*,4*S*,7*S*,16*E*)-7-*tert*-Butyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-6,9-dioxo-3,4,6,7,8,9,12,13,14,15-decahydro-2*H*,11*H*-2,5-methanopyrido[3,2-*k*][1,10,3,6]dioxadiazacyclononadecine-4-carboxamide (8).** $^1$H NMR (400 MHz, $CD_3OD$) δ 8.06 (m, 1 H), 7.52 (d, *J* = 7.6 Hz, 1 H), 7.22 (m, 1 H), 7.04 (d, *J* = 10 Hz, 1 H), 6.69 (m, 2 H), 5.73 (m, 1 H), 5.38 (s, 1 H), 5.27 (d, *J* = 17.2 Hz, 1 H), 5.10 (s, *J* = 10.4 Hz, 1 H), 4.57 (m, 1 H), 4.43 (dd, *J* = 10.8, 6.8 Hz, 1 H), 4.36 (d, *J* = 9.6 Hz, 1 H), 4.30 (d, *J* = 11.6 Hz, 1 H), 3.96 (m, 1 H), 3.76 (m, 1 H), 2.94 (m, 1 H), 2.46-2.32 (m, 2 H), 2.18 (m, 3 H), 1.86 (dd, *J* = 8.4, 5.6 Hz, 1 H), 1.72 (m, 1 H), 1.64-1.46 (m, 3 H), 1.44-1.20 (series of m, 6 H), 1.05 (m, 11 H) ppm; HRMS (ESI) *m/z* 672.3063 [$(M+H)^+$; calcd for $C_{33}H_{46}N_5O_8S$: 672.3062].



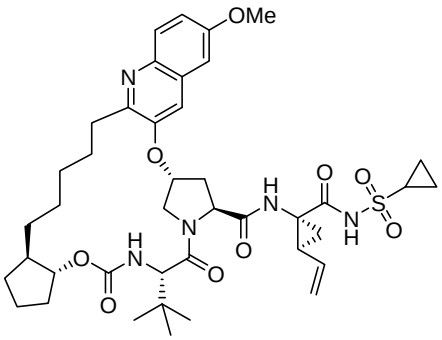
**11**

**(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12,12-dimethyl-6,9-dioxo-3,4,6,7,8,9,12,13,14,15,16,17-dodecahydro-2*H*,11*H*-2,5-methano[1,10,3,6]dioxadiazacyclononadecino[12,11-*b*]quinoline-4-carboxamide (11).** $^1$H NMR (400 MHz, $CD_3OD$) δ 8.10 (br s, 1 H), 7.97 (apparent t, *J* = 8.8 Hz, 2 H), 7.72 (apparent t, *J* = 6.8 Hz, 1 H), 7.64 (apparent t, *J* = 7.6 Hz, 1 H), 5.77 (m, 1 H), 5.53 (br s, 1 H), 5.28 (dd, *J* = 17.6, 1.6 Hz, 1 H), 5.11 (dd, *J* = 10.4, 1.6 Hz, 1 H), 4.85 (m, 1 H), 4.42 (m, 3 H), 4.23 (d, *J* = 9.2 Hz, 1 H), 4.11 (dd, *J* = 12.0, 3.6 Hz, 1 H), 3.19 (m, 1 H), 2.97 (m, 3 H), 2.51 (dd, *J* = 14.0, 7.6 Hz, 1 H), 2.25 (m, 2 H), 1.88 (dd, *J* = 8.4, 5.6 Hz, 1 H), 1.88-1.65 (m, 9 H), 1.39 (m, 4 H), 1.35-1.18 (m, 9 H), 1.11 (m, 3 H), 1.00 (s, 3 H), 0.79 (s, 3 H) ppm; HRMS (ESI) *m/z* 778.3845 [$(M+H)^+$; calcd for $C_{41}H_{56}N_5O_8S$: 778.3844].



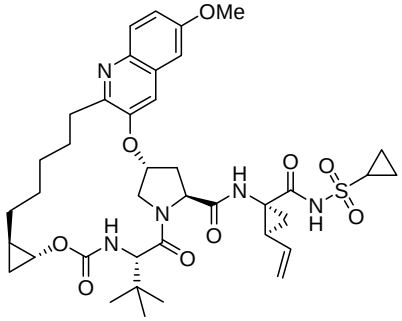
**12**

**(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-21-methoxy-12,12-dimethyl-6,9-dioxo-3,4,6,7,8,9,12,13,14,15,16,17-dodecahydro-2*H*,11*H*-2,5-methano[1,10,3,6]dioxadiazacyclononadecino[12,11-*b*]quinoline-4-carboxamide (12).** $^1$H NMR (400 MHz, $CD_3OD$) δ 8.17 (br s, 1 H), 7.90 (d, *J* = 9.2 Hz, 1 H), 7.40 (m, 2 H), 5.77 (m, 1 H), 5.53 (br s, 1 H), 5.29 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.12 (dd, *J* = 10.0, 1.6 Hz, 1 H), 4.85 (m, 1 H), 4.44 (m, 3 H), 4.22 (d, *J* = 9.6 Hz, 1 H), 4.12 (dd, *J* = 12.0, 3.6 Hz, 1 H), 3.97 (s, 3 H), 3.19 (m, 1 H), 2.94 (m, 3 H), 2.51 (dd, *J* = 14.0, 6.8 Hz, 1 H), 2.31 (m, 1 H), 2.20 (m, 1 H), 1.88 (dd, *J* = 8.4, 5.2 Hz, 1 H), 1.88-1.64 (m, 9 H), 1.39 (m, 4 H), 1.33-1.16 (m, 9 H), 1.11 (m, 3 H), 1.00 (s, 3 H), 0.79 (s, 3 H) ppm; HRMS (ESI) *m/z* 808.3941 [$(M+H)^+$; calcd for $C_{42}H_{58}N_5O_9S$: 808.3950].



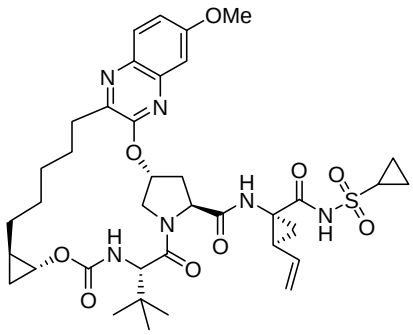
**13**

**(3a*R*,7*S*,10*S*,12*R*,24a*R*)-7-*tert*-Butyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-16-methoxy-5,8-dioxo-1,2,3,3a,5,6,7,8,11,12,20,21,22,23,24,24a-hexadecahydro-10*H*-9,12-methanocyclopenta[18,19][1,10,3,6]dioxadiazacyclononadecino[12,11-*b*]quinoline-10-carboxamide (13).** $^1$H NMR (400 MHz, $CD_3OD$) δ 8.03 (br s, 1 H), 7.87 (d, *J* = 9.2 Hz, 1 H), 7.36 (m, 2 H), 5.75 (m, 1 H), 5.47 (br s, 1 H), 5.27 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.11 (dd, *J* = 10.4, 1.6 Hz, 1 H), 4.89 (m, 2 H), 4.42 (dd, *J* = 11.2, 6.4 Hz, 1 H), 4.35 (m, 2 H), 4.14 (dd, *J* = 12.0, 3.6 Hz, 1 H), 3.96 (s, 3 H), 3.12 (m, 2 H), 2.95 (m, 1 H), 2.83 (m, 1 H), 2.55 (dd, *J* = 14.4, 7.2 Hz, 1 H), 2.28 (m, 1 H), 2.19 (m, 1 H), 2.00-1.89 (m, 2 H), 1.87 (dd, *J* = 8.4, 5.6 Hz, 1 H), 1.85-1.76 (m, 2 H), 1.74-1.46 (m, 5 H), 1.42 (dd, *J* = 9.6, 5.6 Hz, 1 H), 1.42-1.21 (m, 9 H), 1.06 (m, 10 H) ppm; HRMS (ESI) *m/z* 794.3786 [$(M+H)^+$; calcd for $C_{41}H_{56}N_5O_9S$: 794.3793].



**14**

**(1a*R*,5*S*,8*S*,10*R*,22a*R*)-5-*tert*-Butyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[12,11-*b*]quinoline-8-carboxamide (14).** $^1$H NMR (400 MHz, $CD_3OD$) δ 7.94 (br s, 1 H), 7.85 (d, *J* = 9.2 Hz, 1 H), 7.32 (m, 2 H), 5.72 (m, 1 H), 5.46 (br s, 1 H), 5.24 (dd, *J* = 16.8, 1.6 Hz, 1 H), 5.09 (dd, *J* = 10.4, 1.6 Hz, 1 H), 4.53 (d, *J* = 11.6 Hz, 1 H), 4.40-4.34 (m, 3 H), 4.17 (dd, *J* = 11.6, 4.0 Hz, 1 H), 3.94 (s, 3 H), 3.78 (m, 1 H), 3.25 (m, 2 H), 2.94 (m, 2 H), 2.51 (dd, *J* = 14.0, 6.0 Hz, 1 H), 2.20 (m, 3 H), 1.84 (dd, *J* = 8.4, 5.6 Hz, 1 H), 1.83-1.50 (m, 7 H), 1.39 (dd, *J* = 9.6, 5.6 Hz, 1 H), 1.24 (m, 3 H), 1.08 (m, 8 H), 0.97 (m, 3 H), 0.72 (m, 1 H), 0.50 (m, 1 H) ppm; HRMS (ESI) *m/z* 766.3468 [$(M+H)^+$; calcd for $C_{39}H_{52}N_5O_9S$: 766.3480].

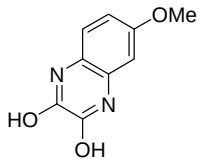


**15** MK-5172

**(1a*R*,5*S*,8*S*,10*R*,22a*R*)-5-*tert*-Butyl-*N*-((1*R*,2*S*)-1-{[(cyclopropylsulfonyl)amino] carbonyl}-2-vinylcyclopropyl)-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-carboxamide (MK-5172, 15).** A solution of methyl (1a*R*,5*S*,8*S*,10*R*,22a*R*)-5-*tert*-butyl-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-carboxylate (**22**, 8.03 g, 14.1 mmol) in $H_2O$ (70.6 mL)/THF (70.6 mL) was treated with LiOH-$H_2O$ (1.78 g, 42.4 mmol). The resulting mixture was stirred at 20 °C for 18 h, acidified with aqueous HCl (0.2 M) and diluted with EtOAc. The organic phase was separated, washed with aqueous HCl (0.2 M) and brine then dried. Removal of the solvent afforded the corresponding carboxylic acid (7.8 g, 100% yield) which was used with no further purification. MS (ESI) *m/z* 555.3 [$(M+H)^+$; calcd for $C_{29}H_{39}N_4O_7$: 555.3].

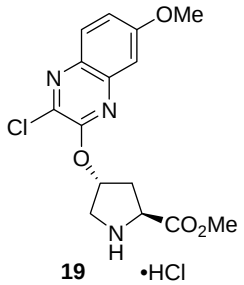
A solution of the above carboxylic acid (7.8 g, 14.1 mmol) in $CH_2Cl_2$ (141 mL) was treated with (1R,2S)-1-{[(cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropanaminium chloride[25] (**23**, 4.9 g, 18.36 mmol), DIPEA (5.47 g, 42.4 mmol), DMAP (0.863 g, 7.06 mmol) and TBTU (6.57 g, 20.47 mmol). The resulting mixture was stirred at 20 °C for 15 h and then diluted with EtOAc. The solution was washed with aqueous HCl (0.2 M), saturated aqueous $NaHCO_3$ and brine. The organic phases were dried and concentrated to give a residue that was purified by flash-chromatography (eluent 2.5% MeOH/$CH_2Cl_2$) to give **15** (9.6 g, 89% yield) as a white powder. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.79 (dd, *J* = 9.6, 1.8 Hz, 1 H), 7.23 (s, 1 H), 7.22 (m, 1 H), 7.10 (d, *J* = 9.6 Hz, 1 H), 6.01 (apparent t, *J* = 3.6 Hz, 1 H), 5.74 (m, 1 H), 5.24 (dd, *J* = 17.0 Hz, 1.6 Hz, 1 H), 5.11 (dd, *J* = 10.4 Hz, 1.6 Hz, 1 H), 4.49 (d, *J* = 11.2 Hz, 1 H), 4.40 (m, 2 H), 4.13 (dd, *J* = 12.0 Hz, 4.0 Hz, 1 H), 3.92 (s, 3 H), 3.76 (m, 1 H), 2.92 (m, 2 H), 2.85 (m, 1 H), 2.55 (dd, *J* = 13.6 Hz, 6.4 Hz, 1 H), 2.28 (m, 1 H), 2.18 (apparent q, *J* = 8.8 Hz, 1 H), 1.85 (dd, *J* = 8.0 Hz, 5.6 Hz, 1 H), 1.73 (m, 2 H), 1.5 (m, 2 H), 1.40 (dd, *J* = 9.6 Hz, 5.6 Hz, 1 H), 1.3 (m, 2 H), 1.23 (m, 4 H), 1.08 (s, 9 H), 0.99 (m, 2 H), 0.89 (m, 3 H), 0.73 (m, 1 H), 0.49 (m, 1 H) ppm; HRMS (ESI) *m/z* 767.3411 [$(M+H)^+$; calcd for $C_{38}H_{51}N_6O_9S$: 767.3433].

**Potassium {[(1*R*,2*S*)-1-({[(1a*R*,5*S*,8*S*,10*R*,22a*R*)-5-*tert*-butyl-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxalin-8-yl]carbonyl}amino)-2-ethenylcyclopropyl]carbonyl}(cyclopropylsulfonyl)azanide (15 K-salt).** To a solution of **15** (400 mg, 0.52 mmol) in EtOH (10 mL) at 0 °C was added a solution of potassium *t*-butoxide (76 mg, 0.68 mmol) in EtOH (2 mL). Upon addition, a white solid formed and the mixture was stirred overnight at RT. After cooling in an ice bath, the solid was isolated by filtration. The solid was then washed with cold EtOH and vacuum dried to give the potassium salt of compound **15** (393 mg, 94% yield) as a white crystalline solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.91 (br s, 1 H), 7.75 (d, $J$ = 8.3 Hz, 1 H), 7.15 (m, 1 H), 7.04 (m, 1 H), 5.97 (m, 1 H), 5.73 (br s, 1 H), 4.96 (m, 1 H), 4.79 (apparent q, $J$ = 9.3 Hz, 1 H), 4.26 (dd, $J$ = 9.7, 7.7 Hz, 1 H), 4.20 (d, $J$ = 11.3 Hz, 1 H), 4.14 (d, $J$ = 8.8 Hz, 1 H), 3.90 (dd, $J$ = 11.1, 3.2 Hz, 1 H), 3.86 (s, 3 H), 3.62 (m, 1 H), 2.86-2.60 (m, 3 H), 2.38 (m, 1 H), 2.21 (m, 1 H), 1.80-1.48 (m, 6 H), 1.42 (m, 5 H), 1.14 (m, 1 H), 0.95 (m, 10 H), 0.81 (m, 2 H), 0.72-0.50 (m, 3 H), 0.41 (m, 1 H) ppm.



**17**

**6-Methoxyquinoxaline-2,3-diol (17).** A suspension of 4-methoxybenzene-1,2-diamine dihydrochloride (**16**, 14.0 g, 66.3 mmol) in diethyl oxalate (72.5 mL, 531 mmol) was treated with TEA (18.5 mL, 133 mmol) and then heated at 150 °C for 2 h. The mixture was cooled and filtered, and the collected solid was washed with $H_2O$ and EtOH. The residue was dried to give **17** (8.85 g, 69% yield). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 11.85 (s, 1 H), 11.75 (s, 1 H), 7.05 (d, $J$ = 7.5 Hz, 1 H), 6.65 (m, 2 H), 3.70 (s, 3 H) ppm. MS (ESI) *m/z* 193.1 [$(M+H)^+$; calcd for $C_9H_9N_2O_3$: 193.1].



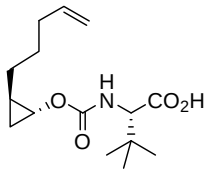
**19** •HCl

**Methyl (4*R*)-4-[(3-chloro-7-methoxyquinoxalin-2-yl)oxy]-L-prolinate hydrochloride (19).** A solution of 6-methoxyquinoxaline-2,3-diol (**17**, 20.0 g, 104 mmol) in DMF (100 mL) was treated with $SOCl_2$ (7.6 mL, 104 mmol) and heated at 110 °C. After 1.5 h, the reaction mixture was cooled and

poured into aqueous HCl (1 N). The resulting precipitate was filtered and washed with $H_2O$ and $Et_2O$. The dried solid (19 g) contained predominantly 3-chloro-7-methoxyquinoxalin-2-ol as a mixture with 3-chloro-6-methoxyquinoxalin-2-ol, 6-methoxyquinoxaline-2,3-diol and 2,3-dichloro-6-methoxyquinoxaline. This material was used directly in the subsequent step.

A solution of the above crude 3-chloro-7-methoxyquinoxalin-2-ol (16 g, 76 mmol) in NMP (217 mL) was treated with $Cs_2CO_3$ (37.1 g, 114 mmol) and 1-*tert*-butyl 2-methyl (2S,4S)-4-{[(4-bromophenyl)sulfonyl]oxy}pyrrolidine-1,2-dicarboxylate (**18**,[23] 31.7 g, 68.4 mmol). The resulting mixture was stirred at 50 °C for 4 h then a further portion of **18** (1.7 g, 3.66 mmol) was added. After stirring for 2 h, the mixture was cooled and diluted with 1 N HCl and EtOAc. The organic phases were washed with aqueous HCl (1 N), saturated aqueous $NaHCO_3$ and brine. The dried organic phase was concentrated to a residue that was purified by flash-chromatography (0-60% gradient elution, EtOAc/petroleum ether) to give (2*S*,4*R*)-1-*tert*-butyl 2-methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (23.3 g, 70% yield) as a solid. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.87 (d, $J$ = 7.5 Hz, 1 H), 7.32 (d, $J$ = 7.5 Hz, 1 H), 7.30 (s, 1 H), 5.65 (br s, 1 H), 4.40 (m, 1 H), 3.90 (s, 3 H), 3.70 (m, 1 H), 3.70 (s, 3 H), 3.65 (m, 1 H), 2.65 (m, 1 H), 2.40 (m, 1 H), 1.32 (s, 9 H) ppm MS (ESI) *m/z* 338.1 [(M-Boc+H)$^+$; calcd for $C_{15}H_{17}ClN_3O_4$: 338.1].

A solution of 1-*tert*-butyl 2-methyl (2*S*,4*R*)-4-[(3-chloro-7-methoxyquinoxalin-2-yl)oxy]pyrrolidine-1,2-dicarboxylate (12.0 g, 27.4 mmol) was treated with a solution (4 M) of HCl in dioxane (82 mL, 328 mmol). After 2 h, the reaction was concentrated under reduced pressure. The residue was triturated with $Et_2O$ to give **19** (10.2 g, 99% yield) as a solid. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.90 (d, $J$ = 9.3 Hz, 1 H), 7.34 (d, $J$ = 9.3 Hz, 1 H), 7.30 (s, 1 H), 5.77 (s, 1 H), 4.67 ( apparent t, $J$ = 7.5 Hz, 1 H), 3.9 (s, 3 H), 3.85 (m, 1 H), 3.81 (s, 3 H), 3.62 (m, 1 H), 2.70 (m, 1 H), 2.60 (m, 1 H) ppm MS (ESI) *m/z* 338.1 [(M+H)$^+$; calcd for $C_{15}H_{17}ClN_3O_4$: 338.1].




**20**

**3-Methyl-*N*-({[(1*R*,2*R*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-L-valine (20).** A solution of butenyl magnesium bromide (487 mL, 0.5 M, 244 mmol) in THF (75 mL) was treated at -78 °C with Cu(I)Br-$SMe_2$ (1.83 g, 8.9 mmol) and DMPU (51.6 mL, 428 mmol). The mixture was stirred for 10 min, then a solution of acrolein (11.9 mL, 178 mmol) and TMSCl (45.6 mL, 357 mmol) in THF (150 mL) was added over 1 h such that the internal temperature remained below -68 °C. The resulting mixture was stirred at -78 °C for 2 h then treated with excess TEA (45 mL) and diluted with hexane.

After reaching RT the mixture was treated with a small portion of $H_2O$ and filtered through celite. The filtrate was washed 10 times with $H_2O$ and then with brine. The organic layer was dried over $Na_2SO_4$ and the volatiles were removed to give a residue that was distilled under reduced pressure (20 mbar). The fraction collected at 80-86 °C contained (*E*)-(hepta-1,6-dien-1-yloxy)trimethylsilane (18 g, 55% yield) as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$) δ 6.19 (d, *J* = 11.6 Hz, 1 H), 5.85-5.75 (m, 1 H), 5.02-4.92 (m, 3 H), 2.08-2.02 (m, 2 H), 1.94-1.88 (m, 2 H), 1.46-1.38 (m, 2 H), 0.18 (s, 9 H) ppm.

A solution of (*E*)-(hepta-1,6-dien-1-yloxy)trimethylsilane (40.3 g, 219 mmol) in hexane (486 mL) was treated with $Et_2Zn$ (1.1 M in toluene, 278 mL, 306 mmol) and the resulting solution was cooled in an ice bath. Diiodomethane (24.7 mL, 306 mmol) was added dropwise and the solution was stirred for 1 h before being warmed to 20 °C. Pyridine (145.8 mL,1.3 mol) was added, the slurry was stirred for 15 min and poured onto petroleum ether. The mixture was filtered repeatedly through celite until a transparent solution was obtained. This mixture was concentrated at 100 mbar and the solution which remained (that contained trimethyl{[(*trans*)-2-pent-4-en-1-ylcyclopropyl]oxy}silane, toluene and pyridine) was further diluted with THF (1 L). The mixture was cooled to 0 °C then treated dropwise with TBAF (1 M in THF, 260 mL, 260 mmol). After 10 min, the mixture was allowed to warm to 20 °C, and after a further 1 h was poured into $H_2O$. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with brine then dried over $Na_2SO_4$. Removal of the volatiles afforded a residue that was purified by flash chromatography (0-66% gradient elution, $Et_2O$/petroleum ether) to furnish racemic *trans* 2-(pent-4-en-1-yl)cyclopropanol (24.8 g, 91% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.85-5.75 (m, 1 H), 5.00 (dd, *J* = 17.1, 1.6 Hz, 1 H), 4.94 (br d, *J* = 10.4 Hz, 1 H), 3.20 (apparent dt, *J* = 6.4, 2.5 Hz, 1 H), 2.10-2.04 (m, 2 H), 1.52-1.44 (m, 2 H), 1.29-1.19 (m, 1 H), 1.15-1.07 (m, 1 H), 0.95-0.87 (m, 1 H), 0.71-0.66 (m,1 H), 0.31 (apparent q, *J* = 6.0 Hz, 1 H) ppm.

To a solution of racemic *trans* 2-(pent-4-en-1-yl)cyclopropanol (25.1 g, 199 mmol) and vinyl acetate (73.4 mL, 796 mmol) in diethyl ether (995 mL) was added Amano lipase PS (90 g). After stirring at RT for 7 h, the solids were removed by filtration, and the mixture was concentrated *in vacuo*. The crude material was then purified by silica gel flash chromatography (0-10% gradient elution, $Et_2O$/petroleum ether) to give (1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropyl acetate (18 g, 54% yield, 50% ee). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.78 (m, 1 H), 5.00 (dd, *J* = 16.8, 1.2 Hz, 1 H), 4.94 (dd, *J* = 10.0, 0.8 Hz, 1 H), 3.81 (m, 1 H), 2.08 (m, 2 H), 2.01 (s, 3 H), 1.5 (m, 2 H), 1.25 (m, 2 H), 1.0 (m, 1 H), 0.8 (m, 1 H), 0.52 (q, *J* = 6.8 Hz, 1 H) ppm.

To a solution of (1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropyl acetate (17.5 g, 104 mmol, 50% ee) in MeOH (1.2 L) was added sodium methoxide (43.5 mL, 25%, 190 mmol). After 1 h at RT, dowex 50WX8-100 ion exchange resin (methanol washed) was added unitl the reaction mixture reached neutral pH. The resin was filtered off and methanol was evaporated under reduced pressure. The residue was dissolved

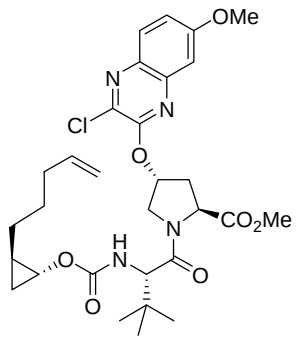
in diethyl ether and washed with water and brine. The organic layer was dried over $Na_2SO_4$, filtered and concentrated to obtain (1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropanol (12.8 g, 98 % yield, 50% ee). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.85-5.75 (m, 1 H), 5.00 (dd, *J* = 17.1, 1.6 Hz, 1 H), 4.94 (br d, *J* = 10.4 Hz, 1 H), 3.20 (apparent dt, *J* = 6.4, 2.5 Hz, 1 H), 2.10-2.04 (m, 2 H), 1.52-1.44 (m, 2 H), 1.29-1.19 (m, 1 H), 1.15-1.07 (m, 1 H), 0.95-0.87 (m, 1 H), 0.71-0.66 (m, 1 H), 0.31 (apparent q, *J* = 6.0 Hz, 1 H) ppm.

A solution of methyl 3-methyl-L-valinate hydrochloride (20.0 g, 110 mmol) in a 2:1 mixture of saturated aqueous $NaHCO_3$ (875 mL) and $CH_2Cl_2$ (451 mL) was cooled in an ice bath and stirred rapidly. The mixture was treated with triphosgene (13.1 g, 44 mmol) in one portion and the resulting mixture was stirred for 2 h. The reaction was diluted with $CH_2Cl_2$ and the layers were separated. The aqueous phase was extracted with $CH_2Cl_2$ then the combined organics were washed with brine and dried over $Na_2SO_4$. Removal of the solvent gave methyl 3-methyl-*N*-(oxomethylene)-L-valinate (19.2 g, 100% yield) as clear oil that was kept for 12 h under vacuum (0.1 mbar) then used directly in the subsequent step. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.79 (s, 3 H), 3.75 (s, 1 H), 1.00 (s, 9 H) ppm.

To a solution of (1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropanol (12.8 g, 101 mmol, 50% ee) in toluene (225 mL) was added methyl 3-methyl-*N*-(oxomethylene)-L-valinate (19.1 g, 112 mmol) and DMAP (12.4 g, 101 mmol). After heating to reflux for 6 h, the mixture was cooled to RT. The solvent was removed in vacuo, EtOAc was added, and the organic layer was washed with 1 N HCl, brine, filtered and concentrated in vacuo. The crude material was purified on silica gel flash chromatography (0-30% gradient elution, $Et_2O$/petroleum ether) to give the first eluting methyl 3-methyl-*N*-({[(1*R*,2*R*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-L-valinate (13.6 g, 60% yield) as a single diastereomer. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.43 (d, *J* = 8.5 Hz, 1 H), 5.80 (m, 1 H), 5.00 (d, *J* = 18 Hz, 1 H), 4.95 (d, *J* = 12 Hz, 1 H), 3.9 (d, *J* = 8.7 Hz, 1 H), 3.65 (m, 1 H), 3.63 (s, 3 H), 2.03 (m, 2 H), 1.50 (m, 2 H), 1.20 (m, 2 H), 0.98 ( br s, 10 H), 0.7 (m, 1 H), 0.48 (m, 1 H) ppm. MS (ESI) *m/z* 298.2 [$(M+H)^+$; calcd for $C_{16}H_{28}NO_4$: 298.2]. The later fractions contained methyl 3-methyl-*N*-({[(1*S*,2*S*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-L-valinate (2.7 g, 35% yield). MS (ESI) *m/z* 298.2 [$(M+H)^+$; calcd for $C_{16}H_{28}NO_4$: 298.2].

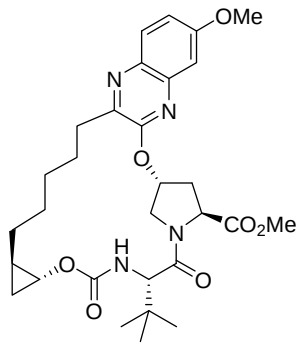
To a solution of methyl 3-methyl-*N*-({[(1*R*,2*R*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-L-valinate (13.6 g, 45.6 mmol) in 2:1 mixture of MeOH/$H_2O$ (456 mL) was added LiOH-hydrate (7.65 g, 182 mmol). After heating at 60 °C for 4 h, the mixture was cooled and concentrated to half volume then diluted with EtOAc and acidified to pH 5 with aqueous HCl (1 N). The organic layer was separated, washed with brine, and then dried with $Na_2SO_4$. Removal of the solvent afforded **20** (12.7 g, 98% yield) as an oil. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.20 (d, *J* = 8.5 Hz, 1 H), 5.80 (m, 1 H), 4.98 (d, *J* = 18 Hz, 1 H), 4.93 (d, *J* = 12 Hz, 1 H), 3.85 (d, *J* = 8.7 Hz, 1 H), 3.65 (m, 1 H), 2.1 (m, 2 H), 1.5 (m, 2 H), 1.2

(m, 2 H), 0.98 ( br s, 10 H), 0.7 (m, 1 H), 0.48 (m, 1 H) ppm. MS (ESI) *m/z* 284.2 [$(M+H)^+$; calcd for $C_{15}H_{26}NO_4$: 284.2].




**21**

**(2*S*,4*R*)-Methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-1-((*S*)-3,3-dimethyl-2-((((1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropoxy)carbonyl)amino)butanoyl)pyrrolidine-2-carboxylate (21).** A solution of methyl (4*R*)-4-[(3-chloro-7-methoxyquinoxalin-2-yl)oxy]-*L*-prolinate hydrochloride (**19**, 10.2 g, 27.3 mmol) in DMF (136 mL) was treated with 3-methyl-*N*-({[(1*R*,2*R*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-L-valine (**20**, 7.72 g, 27.3 mmol), DIPEA (28.6 mL, 164 mmol) and HATU (12.44 g, 32.7 mmol). The resulting mixture was stirred at 20 °C overnight then diluted with EtOAc. The organic layer was separated and washed with aqueous 1N HCl, saturated aqueous $NaHCO_3$ and brine. The dried ($Na_2SO_4$) organic phase was concentrated under reduced pressure to give a residue that was purified by silica flash chromatography (10-30% gradient elution, EtOAc/petroleum ether) to furnish **21** (12.8 g, 78% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.82 (d, $J$ = 9.6 Hz, 1 H), 7.32 (d, $J$ = 8.8 Hz, 1 H), 7.3 (s, 1 H), 7.18 (d, $J$ = 8.8 Hz, 1 H), 5.8 (m, 1 H), 4.99 (d, $J$ = 17.2 Hz, 1 H), 4.94 (d, $J$ = 11.2 Hz, 1 H), 4.58 (d, $J$ = 8.8 Hz, 1 H), 4.35 (d, $J$ = 9.6 Hz, 1 H), 4.08 (m, 2 H ), 3.99 (s, 3 H), 3.72 (s, 3 H), 3.45 (m, 1 H), 2.65 (m, 1 H), 2.38 (m, 1 H), 2.05 (m, 1 H), 1.45 (m, 2 H), 1.2 (m, 4 H), 1.0 (s, 9 H), 0.9 (m, 1 H), 0.6 (m, 1 H), 0.3 (m, 1 H) ppm. MS (ESI) *m/z* 603.2 [$(M+H)^+$; calcd for $C_{30}H_{40}ClN_4O_7$: 603.2].



22

**Methyl (1a*R*,5*S*,8*S*,10*R*,22a*R*)-5-*tert*-butyl-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-carboxylate (22).** A solution of (2*S*,4*R*)-methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-1-((*S*)-3,3-dimethyl-2-((((1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropoxy)carbonyl)amino)butanoyl)pyrrolidine-2-carboxylate (**21**, 12.8 g, 21.3 mmol) in EtOH (213 mL) was treated with potassium trifluoro(vinyl)borate (4.28 g, 32.0 mmol) and TEA (4.46 mL, 32.0 mmol). The resulting mixture was degassed with nitrogen, then $PdCl_2$(dppf)-$CH_2Cl_2$ adduct (1.74 g, 2.1 mmol) was added. The mixture was heated at reflux for 1 h, then cooled to RT and diluted with $H_2O$ and EtOAc. The organic phase was separated, washed with $H_2O$ and brine then dried over $Na_2SO_4$. Removal of the volatiles afforded a residue that was purified by silica flash chromatography (20-30% gradient elution, EtOAc/petroleum ether) to give methyl 3-methyl-N-({[(1*R*,2*R*)-2-pent-4-en-1-ylcyclopropyl]oxy}carbonyl)-*L*-valyl-(4*R*)-4-[(7-methoxy-3-vinylquinoxalin-2-yl)oxy]-L-prolinate which was used with no further purification. MS (ESI) *m/z* 595.3 [$(M+H)^+$; calcd for $C_{32}H_{43}N_4O_7$: 595.3].

To a solution of the above bis-olefin (10.8 g, 18.1 mmol) in DCE (906 mL) was added Zhan 1b catalyst[24] (1.2 g, 1.81 mmol). The resulting mixture was stirred at 90 °C for 1 h then cooled to RT and concentrated under reduced pressure. The residue was purified by flash chromatography (0-50% gradient elution, EtOAc/ petroleum ether) to give methyl (1a*R*,5*S*,8*S*,10*R*,18*E*,22a*R*)-5-*tert*-butyl-14-methoxy-3,6-dioxo-1,1a,3,4,5,6,9,10,20,21,22,22a-dodecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-carboxylate (2.6 g, 25% yield, 2 steps). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.82 (d, $J$ = 9 Hz, 1 H), 7.28 (m, 2 H), 7.18 (s, 1 H), 7.15 (m, 1 H), 6.9 (d, $J$ = 15.8 Hz, 1 H), 5.82 (br s, 1 H), 4.45 (m, 2 H), 4.25 (d, $J$ = 8.3 Hz, 1 H), 3.92 (m, 1 H), 3.9 (s, 3 H), 3.75 (m, 1 H), 3.63 (s, 3 H), 2.65 (m, 1 H), 2.45-2.25 (m, 3 H), 2.88 (m, 1 H), 2.68 (m, 2 H), 1.03 (s, 9 H), 0.9 (m, 2 H), 0.75 (m, 1 H), 0.52 (m, 1 H) ppm. MS (ESI) *m/z* 567.3 [$(M+H)^+$; calcd for $C_{30}H_{39}N_4O_7$: 567.3].

A solution of the above olefin (8.0 g, 14.1 mmol) in MeOH (141 mL)/dioxane (141 mL) was treated with Pd/C (10%, 0.346 g). The resulting mixture was stirred under an atmosphere of hydrogen for 2 h and then an additional Pd/C (10%, 0.3 g) was added and stirring under hydrogen was continued for 2 h. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give compound **22** (8.3 g, 99% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.82 (d, $J$ = 9 Hz, 1 H), 7.2 (m, 3 H), 5.87 (br s, 1 H), 4.43 (m, 1 H), 4.37 (d, $J$ = 11.8 Hz, 1 H), 4.20 (d, $J$ = 8 Hz, 1 H), 4.0 (m, 1 H), 3.9 (s, 3 H), 3.65 (m, 1 H), 3.63 (s, 3 H), 2.9 (m, 1 H), 2.75 (m, 1 H), 2.6 (m, 1 H), 2.2 (m, 1 H), 1.9-1.4 (m, 7 H),1.03 (s, 9 H), 0.85 (m, 2 H), 0.65 (m, 1 H), 0.48 (m, 1 H) ppm. MS (ESI) *m/z* 569.3 [$(M+H)^+$; calcd for $C_{30}H_{41}N_4O_7$: 569.3].

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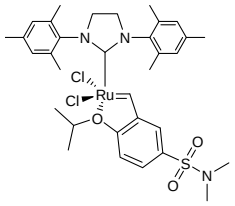


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