Discovery of MK-8831, A Novel Spiro-proline Macrocycle as a Pan-Genotypic HCV-NS3/4a Protease Inhibitor

Santhosh F. Neelamkavil,* Sony Agrawal, Thomas Bara, Chad Bennett, Sathesh Bhat, Dipshikha Biswas, Linda Brockunier, Nicole Buist, Duane Burnette, Mark Cartwright, Samuel Chackalamannil, Robert Chase, Mariappan Chelliah, Austin Chen, Martin Clasby, Vincent Colandrea, Ian W. Davies, Keith Eagen, Zhuyan Guo, Yongxin Han, John Howe, Charles Jayne, Hubert Josien, Stacia Kargman, Karen Marcantonio, Shouwu Miao, Randy Miller, Andrew Nolting, Patrick Pinto, Murali Rajagopalan, Rebecca T. Ruck, Unmesh Shah, Aileen Soriano, Donald Sperbeck, Francisco Velazquez, Jin Wu, Yan Xia and Srikanth Venkatraman

Merck Research Laboratories, Kenilworth, New Jersey 07033, United States

KEYWORDS: Antiviral, HCV, NS3/4a, genotype 3a, pan-genotypic, macrocycle, MK-5172, MK-8831

Supporting Information

Experimental Section

General. Reagents and solvents, including anhydrous THF, dichloromethane and DMF, were purchased from Aldrich, Acros or other commercial sources and were used without further purification. Reactions that were moisture sensitive or that required the use of anhydrous solvents were performed under either nitrogen or argon atmosphere. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic KMnO4 solution or ethanolic H2SO4 solution. Compounds were purified by flash chromatography using an automated purification system (ISCO, Biotage, Analogix) using disposable silica gel prepacked cartridges. Alternatively, compounds were purified by preparative reverse-phase HPLC using a Gilson 215 liquid handler and a Phenomenex Luna C18 column (150 x 20 mm I.D.) with a linear gradient over 15 minutes (95:5 to 0:100 H2O containing 0.1% trifluoroacetic acid:acetonitrile or 0.1% formic acid:acetonitrile). NMR spectra were recorded on 400 or 500 MHz for 1H and at 75, 100 or 125 MHz for 13C on a Bruker or Varian spectrometer with CDCl3 or DMSO-d6 as solvent. The chemical shifts are given in ppm, referenced to the deuterated solvent signal. Purity of target compounds were determined using LC-MS and HPLC. LC/MS analyses were performed using an Applied Biosystems API-150 mass spectrometer and Shimadzu SCL-10A LC system. Column: Phenomenex Gemini C18, 5 micron, 50 mm x 4.6 mm ID, gradient: from 90% water, 10% CH3CN, 0.05%TFA, 5 min to 5% water, 95% CH3CN, 0.05% TFA in 5 minutes, UV detection: 254nm. Analytical HPLC were done using YMCPack Diol NP column, 150x3 mm; 6% 8% [CH3CN (0.3), *i*-PrOH (1.7), DCM (2)] in Hexanes; 0.8-1.0 mL/min, UV detection: 254 nm. Purity of targets compounds were ≥95%.

NS3/4A Enzyme Inhibition Assay (*IC*50 Measurement). NS3/4A (full-length NS3 with tethered NS4A peptide) protease activity was measured by cleavage of a peptide substrate (Ac-C(Eu)DDMEEAbu[COO]ASK(QSY7)-amide) in an endpoint time-resolved fluorescence (TRF) assay. The peptide substrate was labeled with europium cryptate (Eu) and a quencher, QSY7. Cleavage of the substrate by NS3/4A, via the ester bond (COO), separates the quencher and europium cryptate resulting in an increased TRF signal. Enzyme and substrate solutions were prepared in buffer containing 50 mM HEPES, 150 mM NaCl, 0.1% PEG-8000, 15% glycerol, 0.15% Triton X-100, 1mM DTT, pH 7.6 For*IC*50 measurements, varying compound concentrations were prepared by a 3-fold dilution scheme (20 concentrations, 1mM top concentration) in 100 % DMSO. NS3/4A (10 ul) was pre-incubated with each compound solution

(0.2 ul) for 30 minutes at room temperature in a 384-well plate. To start the reaction, 10 ul of substrate was ad ed (final concentration, 25 nM) to each well. The plate was sealed and incubated for 2.5 hours (away from light) at room temperature. The product fluorescence was detected using PheraStar Plus plate reader (excitation at 340 nm; emission at 620 nm). *IC*50 values were calculated by fitting the data to a 4-parameter dose-response equation using PRISM software.

HCV Replicon Assay: Tracking HCV replication inhibition using Reverse Transcription/Real-

Time PCR [TaqMan] Analysis. Inhibition of HCV replication in the replicon cell (Huh-7 cells harboring self-amplifying HCV RNA containing non-structural genes) was tracked by measuring HCV RNA levels after incubation with varying concentrations of compound.

Replicon/Compound Incubation. Cells were seeded at 1,000 cells/well in 384-well collagen I – coated plates containing DMEM supplemented with 500 Mg/ml G418 and 5% FBS. Cells were grown at 37°C. Twenty- four hours post-seeding, compounds were added to the cell growth plates. The final concentration of DMSO was 0.5%. Varying concentrations of compound were prepared by a 2-fold dilution scheme in 100% DMSO (20 points; 10 MM top (final) concentration). At harvest, after 72 hours of incubation at 37°C, plates were washed with 50 Ml of DPBS (no Ca+2, no Mg+2). 20 Ml of cell processing buffer (FCP, Qiagen #1062731) was then added to lyse the cells in a 5-min. incubation at room temperature. The cell lysates were then used for PCR amplification.

Reverse-Transcription/Real-Time PCR (TaqMan) Analysis. Cell lysates were used directly as template for reverse transcription-PCR and TaqMan analysis to measure replicon RNA levels. For genotype 1a replicon cells (1AT1), the amplicon was located in the IRES region. The PCR primers used were: Forward primer, TGCGGAACCGGTGAGTACA; Reverse primer, GCGGGTTTATCCAAGAAAGGA. The probe sequence was 6FAM-CGGAATTGCCAGGACGACCGG-TAMRA. For genotype 1b replicon cells (clone16-1b), the amplicon was located in NS5B. The PCR primers used were the following: Forward primer, ATGGACAGGCGCCCTGA; Reverse primer, TTGATGGGCAGCTTGGTTTC. The probe sequence was 6FA-CACGCCATGCGCTGCGG-TAMRA. For gt 2a and gt 2b replicon cells (JFH1_2a; JFH1-2b; chimeric gt 3 NS3 in gt 2a background), the

amplicon was located in the IRES region. The PCR primers used were the following: Forward primer, CGCAAGACTGCTAGCCGAG; Reverse primer, GCCCTATCAGGCAGTACCACA. The probe

sequence was 6FAM-AGCGTTGGGTTGCGAAAGGCC –TAMRA. To start the analysis, a primer/probe

Mastermix was prepared as follows: 2.4 ml TaqMan 2X master mix (Applied Biosystems #4309169), 120 Ml of 40X MultiScribe and RNase Inhibitor (Applied Biosystems #4309169); 60 Ml Forward primer (50 MM); 60 Ml Reverse primer (50 uM); 18 Ml Probe (100 MM): 630 Ml RNAse –free water. 8 Ml Mastermix was loaded in RT PCR reaction plates (Applied Biosystems #4309849). 4 Ml of cell lysate (pre-diluted 5-fold) was then added. The real-time reverse transcription-PCRs were run on the ABI PRISM 7900HTS Sequence Detection System using the following program: 48°C for 30 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. The cyclic threshold (CT) values were plotted against drug concentration and fitted to the sigmoid dose response model using PRISM software (Graphpad Software Inc.). The 50% effective inhibition concentration (*EC*50) was the compound concentration necessary to achieve an increase of 1 in CT over the estimated baseline. *EC*90 was the compound concentration necessary to achieve an increase of 3.2 in CT over the baseline. 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 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

Molecular Dynamics (MD) simulations were run using the AMBER 9 suite of programs.[1]The starting structure for MD simulations of compound 2 was based on the solved crystal structure of compound 1. The AMBER force field for the protein was augmented with the generalized amber force field (GAFF)[2], with atom types for inhibitors generated using the antechamber module of AMBER. Partial charges of the inhibitors were calculated by the AM1-BCC method.[3]

Each enzyme−inhibitor complex conformation was solvated in a truncated octahedron TIP3P water box.[4] Select solvent exposed charge residues were neutralized to generate a net neutral charge for the system. Energy minimization, applying harmonic restraints with force constants of 10 kcal/mol/Å2 to all solute atoms, was carried out, followed by initial annealing of solvent and then heating from 0 to 300 K over 20 ps in the isothermal isobaric ensemble (NPT). After a 50 ps equilibration simulation, a 1 ns production NPT run was obtained with snapshots collected every 1 ps. For all simulations, a 2 fs time-step and 9 Å nonbonded cutoff were used. The particle mesh Ewald (PME) method[5] was used to treat long-range electrostatic interactions. Bond lengths involving hydrogen atoms were constrained by SHAKE.[6]

References for the molecular modeling section.

1. Case, D. A.; Cheatham, T. E., III; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M., Jr.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J.The Amber Biomolecular Simulation Programs J. Comput. Chem. 2005, 26, 1668– 1688

2. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A.Development and testing of a general amber force field J. Comput. Chem. 2004, 25, 1157– 1174
3.Jakalian, A.; Jack, D. B.; Bayly, C. I.Fast, Efficient Generation of High-Quality Atomic Charges. AM1-BCC Model: II. Parameterization and Validation J. Comput. Chem. 2002, 23, 1623– 1641
4. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L.Comparison of simple potential functions for simulating liquid water J. Chem. Phys. 1983, 79, 926– 935
5. Darden, T.; York, D.; Pedersen, L.Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems J. Chem. Phys. 1993, 98, 10089– 10092
6. Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C.Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes J. Comput. Phys. 1977, 23, 327– 341

PK

Table 1: Pharmacokinetic parameters for MK-8831 in rat and dog compared to MK-5172[a]

	Species	Dose mpk (iv/po)	Cl[b]	V_d (L/kg)	$T_{1/2}$ (h)	AUC (μM.h)
MK-5172	Rat	2/5	28	3.1	1.4	0.7
	Dog	0.5/1	5	0.7	3.0	0.4
MK-8831	Rat	2/5	8	0.8	7.7	0.8
	Dog	0.5/1	0.2	0.04	6	16.4

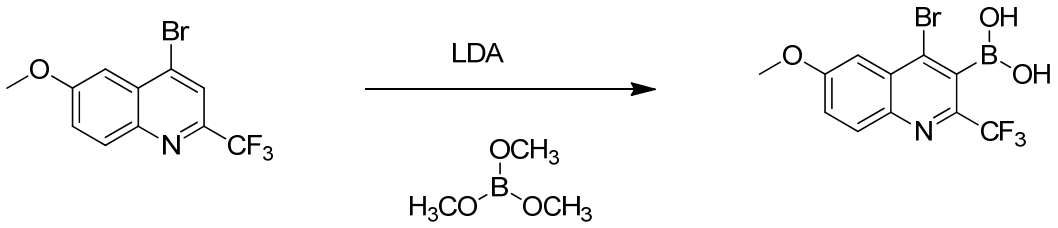
[a]Rat iv (n = 3, DMSO), dog iv (n=3, DMSO), rat po (n =3, PEG400), and dog po (n=3, PEG400). [b]mL/min/kg

Note: All the spirocyclic macrocycles can be made by following the general procedure described below for the synthesis of MK-8831

Example 10: Isobutyl ((2R,6S,13aS,14aR,16aS,Z)-9'-methoxy-14a-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-5,16-dioxo-5'-(trifluoromethyl)-1',2',3,5,6,7,8,9,10,11,13a,14,14a,15,16,16a-hexadecahydro-1H-spiro[cyclopropa[e]pyrrolo[1,2-a][1,4]diazacyclopentadecine-2,3'-pyrano[2,3-c]quinolin]-6-yl)carbamate

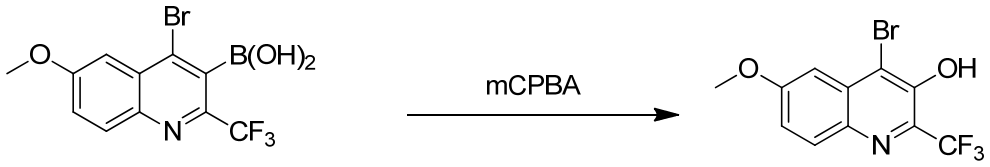
MK-8831 synthesis

Step 1:



To a solution of diisopropyl amine (7.16 ml, 50.6 mmol) in 35 mL of THF at -20 °C was treated with n-butyl lithium (19.60 ml, 49.0 mmol) and stirred at -20 °C for 20 minutes and 0 °C for 10 minutes. The reaction mixture was then cooled to -78 °C (30 mins) and was treated dropwise with 2-trifluoromethyl-4-bromo-6-methoxy quinoline (5.0g, 16.34 mmol) in THF (20.00 mL) and stirred at -78°C for 1 h. The reaction mixture was then treated with trimethyl borate (14.57ml, 131 mmol) and stirred at -78°C for an additional 3 h. The colour of the reaction mixture changed from brown to very dark green (almost black) to green to light green to yellow over 3 hours. The reaction mixture was then quenched with 1N HCl till the system was acidic. The aqueous solution was extracted with EtOAc (2X), combined organic layers were washed with water, dried (Na_2SO_4), filtered, concentrated in vacuo to obtain the product (5.3g, 93%) and used as it is in next step. MS m/e = 350.0 (MH^+)

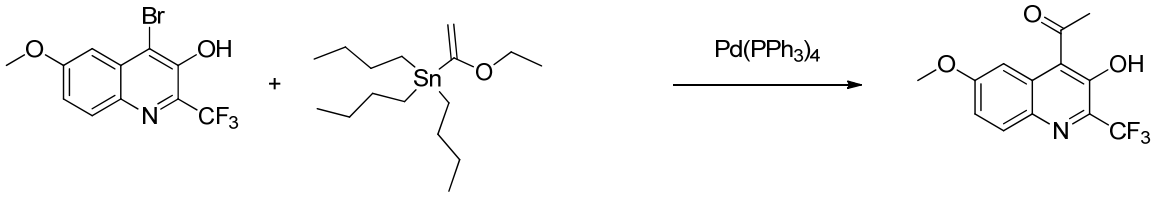
Step 2:



To the product from step 1 (1g, 2.8 mmol) dissolved in acetonitrile (5 ml) was added mCPBA (0.773 g, 3.36 mmol) and stirred at room temperature for 1 hour. The reaction mixture was almost homogeneous after addition of mCPBA and then turned to a slurry. LCMS after one hour showed ~10% product formation and rest was starting material. Solid $NaHCO_3$ (0.282 g, 3.36 mmol) was added to the mixture and the resulting mixture was stirred overnight at room temperature. LCMS after over-night stirring showed ~90% of product formation with very little starting material. Sodium bisulfite (2.040 ml, 1.961 mmol) was added to the mixture and stirred

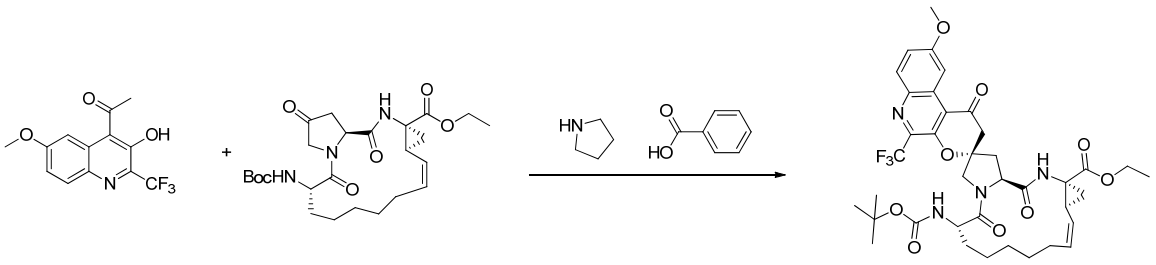
for 1 minute. The solid obtained was filtered and washed with 1:1 ACN/H_2O, dried in vacuum to get the product (453 mg, 1.406 mmol, 50.2 % yield) which was used as is in the next step. MS m/e = 322, 324 (MH^+)

Step 3:



A mixture of product from step 2 (4.42 g, 13.72 mmol), tributyl(1-ethoxyvinyl)tin (9.27 ml, 27.4 mmol) and Tetrakis (1.586 g, 1.372 mmol) in dioxane (46.5 ml) was heated at reflux overnight at 119 ^oC. The reaction mixture was then cooled to rt, added ~35 mL of 1N HCl and stirred at rt for ~30 minutes. LCMS indicated hydrolysis of vinyl ether. The reaction mixture was diluted with water, extracted 3x with EtOAc, combined organic layer washed with brine, dried over MgSO4, filtered, concentrated and a lot of solid was obtained. 20 ml of 10% DCM/Hexanes was added to the solid, filtered to get yellow solid which was the desired product (2.79 g, 9.78 mmol, 71.3 % yield). MS m/e = 286.1 (MH^+)

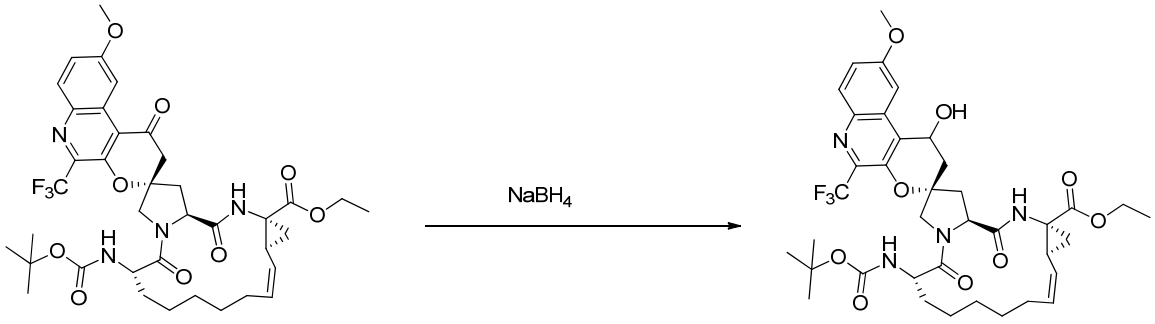
Step 4:



Product from step 3 (5.25g, 18.41 mmol), macrocyclic ketone (10g, 20.34 mmol), benzoic acid (4.5g, 36.8 mmol), pyrrolidine (0.61 mL, 7.36 mmol) and powdered 4A molecular sieves (1.7g) were added in dry 2-propanol (108 mL). The reaction mixture was purged with N_2 and heated to 75°C for 20 hours. LCMS shows complete consumption of starting quinoline. Cooled, filtered through a minimum amount of celite, washing the filter cake well with MeOH and CH2Cl2.

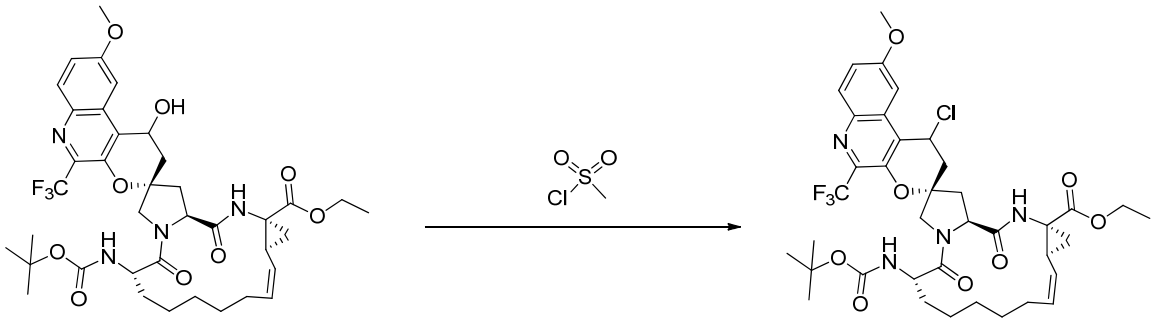
Concentrated in vacuo and purified by silica gel column chromatography using a gradient of 0-100% EtOAc/Hexanes. Further impurtites after the column purification were removed by triturating the product with acetone. Collected the solid by filtration and dried under vacuum to provide the product as a free-flowing tan solid (7.0g, 50% yield). MS m/e = 759.3 (MH^+)

Step 5:



The ketone from step 4 (5 g, 6.59 mmol) was taken up in MeOH (50 ml) / THF (17 ml) and cooled to 0 °C under N_2. Sodium brohydride (0.374 g, 9.88 mmol) was then added and the mixture stirred for 15 minutes. Upon completion reaction was quenched with saturated NH_4Cl solution and diluted with EtOAc. Extracted the aquesous layer with EtOAc (3X) and washed the combined organic extracts with brine, dried over $MgSO_4$ and concentrated in vacuo to obtain the product (5.01g, 100%). MS m/e = 761.3 (MH^+)

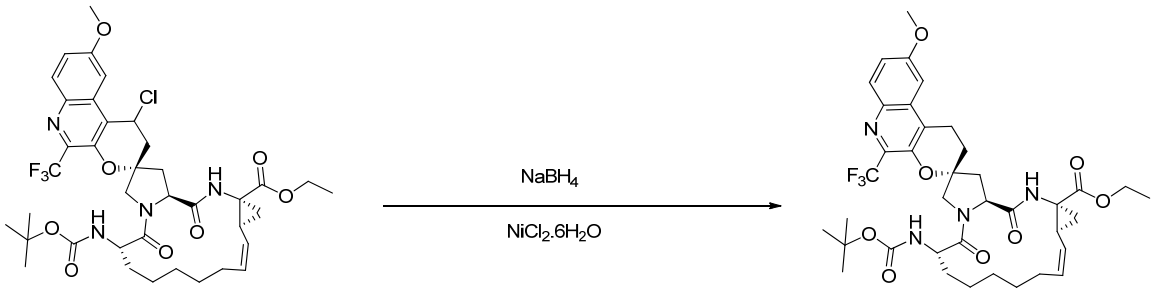
Step 6:



The alcohol from Step 5 (5.01 g, 6.59 mmol) was suspended in DCM (65 ml) and cooled to 0 °C under N_2. After 15 min added methanesulfonyl chloride (1.284 ml, 16.48 mmol) and stirred at 0 °C for 15 minutes. Added the triethylamine (2.76 ml, 19.77 mmol) and allowed the resultant

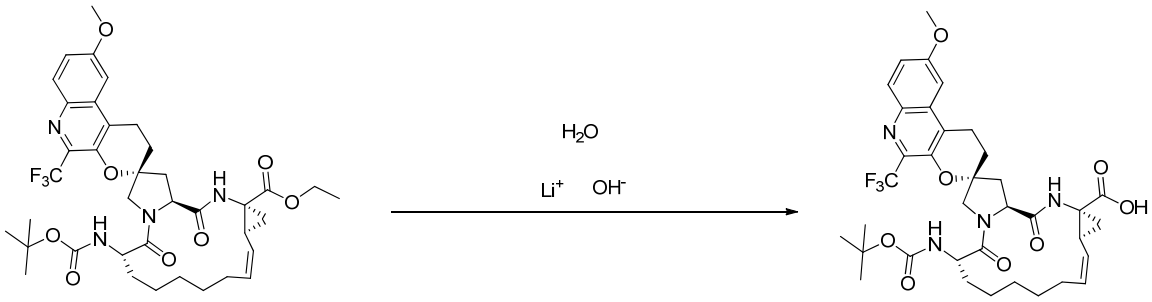
mixture to warm to ambient temperature overnight. LCMS indicated only about 20% conversion to chloride so cooled to 0 °C and added an additional mesyl chloride (1.284 ml, 16.48 mmol) and 3 equiv. of triethylamine (2.76 ml, 19.77 mmol). LCMS shows reaction complete. Diluted with EtOAc, washed with saturated $NaHCO_3$ sol'n (2X) and brine. Dried over $MgSO_4$, concentrated in vacuo, and purified by column chromatography (ISCO system, 50% EtOAC/hexanes) to give product (3.9 g, 5.00 mmol, 76 % yield). MS m/e = 779.3 (MH^+)

Step 7:



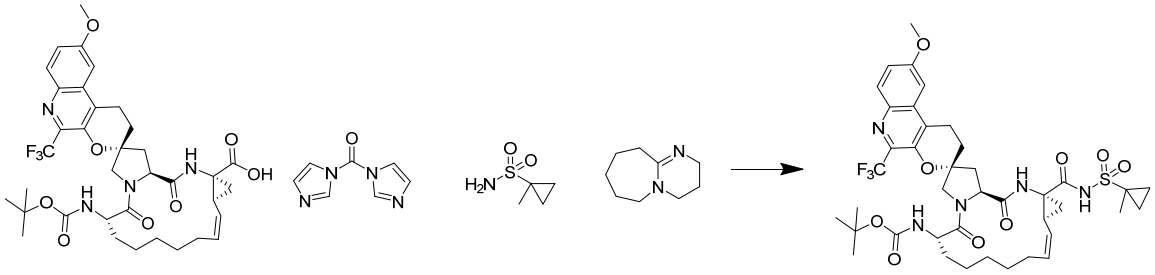
The chloride from Step 6 (3.9 g, 5.00 mmol) was suspended in MeOH and the nickel(II) chloride hexahydrate (3.57 g, 15.01 mmol) was added. The reaction mixture was allowed for the Ni catalyst to fully dissolve (light green color). The mixture was then cooled to -78°C and the sodium borohydride (0.473 g, 12.51 mmol) was added in one portion. After stirring the light green solution at -78 °C for 1h an aliquot was taken and quenched into HCl-CH_3CN for LCMS. The LCMS showed completion, then quenched by dumping the cold mixture into a stirring mixture of saturated $NaHCO_3$ (aq) solution (200 mL) and EtOAc (100 mL). Diluted with EtOAc and extracted the aqueous layer with EtOAc (3X). Washed the combined organic extracts with sat'd $NaHCO_3$ sol'n (3X) and brine (1X). Dried over MgSO4, filtered, and concentrated in vacuo to give the product (3.5 g, 4.70 mmol, 94 % yield). This was carried onto next step without further purification. MS m/e = 745.4 (MH^+)

Step 8:



The ester from Step 7 (3.5 g, 4.70 mmol) was suspended in MeOH (20 mL)/THF (72 mL)/THF (72 mL) and the lithium hydroxide monohydrate (1.18 g, 28.2 mmol) was added. Heated the yellow solution to 60 °C. LCMS after 1.5 hours shows hydrolysis complete so acidified to pH 2 with 1N HCl. Diluted with EtOAC and H_2O. Separated layers and extracted the aqueous layer with EtOAc. Combined the organic layers and washed with brine (4X) ensuring that last brine wash was not acidic. Dried over $MgSO_4$, concentrated in vacuo, and azeotroped with toluene (4X). Placed the white solid under high vacuum and dried to obtain the product (3.37 g, 4.70 mmol, 100 % yield). MS m/e = 717.4 (MH^+)

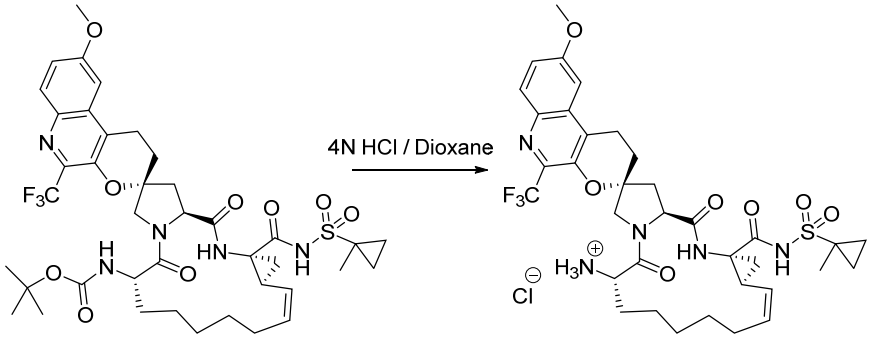
Step 9:



Compound 9

A solution of product from step 8 (3.37 g, 4.70 mmol) and CDI (1.32 g, 8.14 mmol) in THF (64 ml) was stirred in an oil-bath kept at 70°C for 3hr, then cooled to rt. To this was added 1-methylcyclopropane-1-sulfonamide (1.589 g, 11.75 mmol) and DBU (11.75 ml, 11.75 mmol) in THF(1M) and the mixture was stirred in an oil-bath kept at 50°C for overnight. LCMS indicates that the major peak is desired product. The reaction mixture was diluted with EtOAc (200 ml), washed with aq. NH_4Cl (2x50ml), brine, water (2x50 mL) dried over $MgSO_4$, filtered, concentrated. The residue was first purified by 30% EtOAc/DCM and then again clean fractions was further purified (dry loading with silica gel) with 0-30% EtOAc/hexanes 10 min to 30% hexanes / EtOAc over 20 min-30-75% EtOAc/hexanes over 20 min to give the tert-butyl ((2R,6S,13aS,14aR,16aS,Z)-9'-methoxy-14a-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-5,16-dioxo-5'-(trifluoromethyl)-1',2',3,5,6,7,8,9,10,11,13a,14,14a,15,16,16a-hexadecahydro-1H-spiro[cyclopropa[e]pyrrolo[1,2-a][1,4]diazacyclopentadecine-2,3'-pyrano[2,3-c]quinolin]-6-yl)carbamate (2.2 g, 2.64 mmol, 56.1 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H), 8.01-7.99 (d, J = 9.2 Hz, 1H), 7.29-7.26 (m, 1H), 6.99 (d, J = 2.6 Hz, 1H), 6.89 (br s, 1H), 5.74-5.67 (m, 1H), 5.18 (d, J = 8.7Hz, 1H), 4.99 (t, J= 10.0 Hz, 1H), 4.61-4.57 (m, 1H), 4.43 (d, J= 11.0 Hz, 1H), 4.23-4.17 (m, 1H), 3.96 (s, 3H), 3.71 (d, J = 11.0Hz, 1H), 3.21-3.09 (m, 2H), 2.61-2.53 (m, 2H), 2.44-2.32 (m, 4H), 1.92-0.8 (m, 27H). ^{19}F NMR (400 MHz, $CDCl_3$) δ -70.14. MS m/e = 834.2 (MH^+)

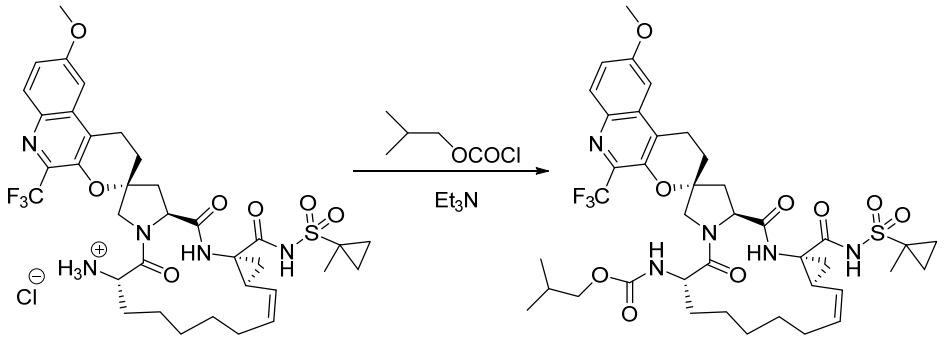
Step 10



Compound 9

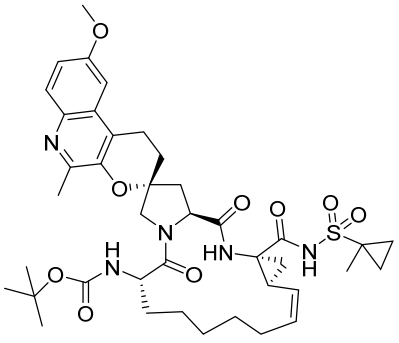
To a solution of the compound 9 (70mg, 0.080mmol) in DCM (1ml) was added 4M HCl in dioxane (0.020ml, 0.080mmol). The resulting mixture was allowed to stir at RT for 3h. The volatiles were then removed *in vacuo* to furnish crude product as a white solid (62mg, 0.080mmol, 100% yield). MS m/e = 734.2 (MH^+). This material was used without further purification in the next step.

Step 11



A solution of the product of Step 10 (35mg, 0.045mmol), TEA (0.019ml, 0.136mmol) and isobutyl chloroformate (0.012ml, 0.091mmol) in DCM (2ml) was stirred at RT for 1h. The reaction was then quenched with NH_4Cl and extracted with DCM. The organics were washed twice with water, brine and dried over Na_2SO_4. It was then filtered, concentrated and chromatographed with 0-40% acetone-hexanes to give the compound **10** (**MK-8831**, 25mg, 0.030mmol, 66%). MS m/e = 834.2 (MH^+). ^{1}H NMR δ (ppm)($CHCl_3$-d): 10.17 (1 H, s), 8.03 (1 H, d, J = 9.21 Hz), 7.33-7.28 (2 H, m), 7.04 (1 H, d, J = 2.66 Hz), 6.89 (1 H, s), 5.74 (1 H, q, J = 8.88 Hz), 5.28 (1 H, d, J = 8.78 Hz), 5.03 (1 H, t, J = 9.45 Hz), 4.65 (1 H, dd, J = 9.65, 7.08 Hz), 4.55 (1 H, d, J = 11.06 Hz), 4.22 (1 H, t, J = 9.47 Hz), 3.99 (3 H, s), 3.70 (1 H, d, J = 10.94 Hz), 3.65-3.55 (2 H, m), 3.24-3.19 (1 H, m), 3.15 (1 H, t, J = 8.26 Hz), 2.60 (2 H, dd, J = 13.53, 7.08 Hz), 2.49-2.33 (5 H, m), 2.20 (1 H, s), 1.94 (1 H, t, J = 7.11 Hz), 1.89-1.72 (4 H, m), 1.42-1.25 (7 H, m), 0.93-0.78 (10 H, m).

Compound 6 prepared same as above with the appropriate quinoline ketone.



^{1}H-NMR (400MHz, acetone-d_6) δ 10.56 (br s, 1H), 8.42 (s, 1H), 7.75 (d, J = 838 Hz, 1H), 7.16-7.13 (m, 2H), 6.04 (d, J = 7.1 Hz, 1H), 5.72-5.66 (m, 1H), 4.99 (t, J = 9.5 Hz, 1H), 4.71 (dd, J =

10.2, 6.9 Hz, 1H), 4.50 (d, J = 11.3 Hz, 1H), 4.11-4.07 (m, 1H), 3.94 (s, 3H), 3.79 (d, J = 10.9 Hz, 1H), 3.77 (s, 1H), 3.20-3.07 (m, 2H), 2.60-2.26 (m, 3H), 2.08-2.04 (m), 1.93-1.86 (m), 1.73 (dd, J = 5.5, 8.1 Hz, 1H), 1.65-1.61 (m, 1H), 1.58-1.54 (m, 2H), 1.48 (s, 9H), 1.46-1.26 (m), 0.89-0.820 (m). MS m/e = 780.2 (MH+)