Supporting Information

Novel quinoline-based P2-P4 macrocyclic derivatives as pan-genotypic HCV NS3/4a protease inhibitors

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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 $KMnO_4$ solution or ethanolic H_2SO_4 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 H_2O 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 $CDCl_3$ 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 YMC-Pack 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 added (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.

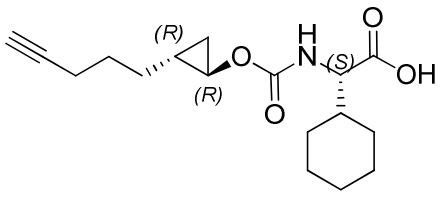
Replicon/Compound Incubation. Cells were seeded at 1,000 cells/well in 384-well collagen I -coated plates containing DMEM supplemented with 500 µg/ml G418 and 5% FBS. Cells were grown at 37°C. Twenty- four hours post-seeding, compounds were added to the cell growth plates. The final concentration of DMSO was 0.5%. Varying concentrations of compound were prepared by a 2-fold dilution scheme in 100% DMSO (20 points; 10 µM top (final) concentration). At harvest, after 72 hours of incubation at 37°C, plates were washed with 50 µl of DPBS (no Ca+2, no Mg+2). 20 µl 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 µl of 40X MultiScribe and RNase Inhibitor (Applied Biosystems #4309169); 60 µl Forward primer (50 µM); 60 µl Reverse primer (50 uM); 18 µl Probe (100 µM): 630 µl RNAse –free water. 8 µl Mastermix was loaded in RT PCR reaction plates (Applied Biosystems #4309849). 4 µl 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.

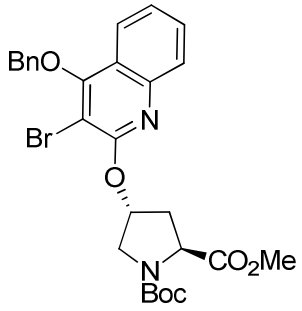
All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories and conform with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life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

(S)-2-cyclohexyl-2-((((1R,2R)-2-(pent-4-yn-1-yl)cyclopropoxy)carbonyl)amino)acetic acid (3).



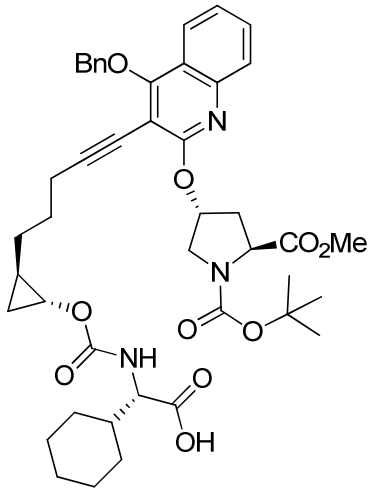
To a solution of commercially purchased (1*R*,2*R*)-2-(pent-4-en-1-yl)cyclopropanol (**1**; see International Patent Application Publication No. WO2008/057209) (17.1 g, 137.70 mmol) in acetonitrile (193 mL) was added *N,N'*-disuccinimidyl carbonate (49.3 g, 192.45 mmol) followed by triethylamine (53.7 mL, 385.27 mmol). The mixture was heated at 40 °C for 18 hours after which it was cooled to room temperature and the solids were removed by filtration. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (ISCO, 10 to 70% ethyl acetate in hexanes) to give 2,5-dioxopyrrolidin-1-yl ((1R,2R)-2-(pent-4-yn-1-yl)cyclopropyl) carbonate (18.0 g, 49.3% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.06-4.03 (m, 1H), 2.87 (s, 4H), 2.28-2.25 (m, 2H), 1.98-1.97 (m, 1H), 1.72-1.63 (m, 2H), 1.44-1.39 (m, 2H), 1.30-1.25 (m, 1H), 1.12-1.08 (m, 1H), 0.72-0.68 (m, 1H). To a solution of the above formed DSC-adduct (14.0 g, 52.8 mmol) in acetonitrile (184 ml) was added commercially purchased (2S)-amino(cyclohexyl)ethanoic acid (10.79 g, 68.6 mmol) followed by triethyl amine (22.0 ml, 158.0 mmol) and water (184 ml). The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with 1N aq. HCl, water and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give intermediate **3**, which was used for the next step without purification. ^{1}H NMR (400 MHz, *$CDCl_3$*): δ 5.15 (d; J = 9.12 Hz; 1 H); 4.31 (dd; J = 9.14; 4.73 Hz; 1 H); 3.74-3.76 (m; 1 H); 2.22 (td; J = 7.04; 2.68 Hz; 2 H); 2.04 (s; 2 H); 1.93 (t; J = 2.64 Hz; 1 H); 1.84 (s; 1 H); 1.56-1.75 (m; 8 H); 1.10-1.27 (m; 4 H); 0.79-0.84 (m; 1 H); 0.52 (q; J = 6.41 Hz; 1 H) (exchangeable protons not observed). LCMS (ESI) *m/z* 308.2 [$(M+H)^+$; calcd for $C_{17}H_{25}NO_4$: 307.18].

(2S,4R)-1-tert-butyl 2-methyl 4-((4-(benzyloxy)-3-bromoquinolin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (6).



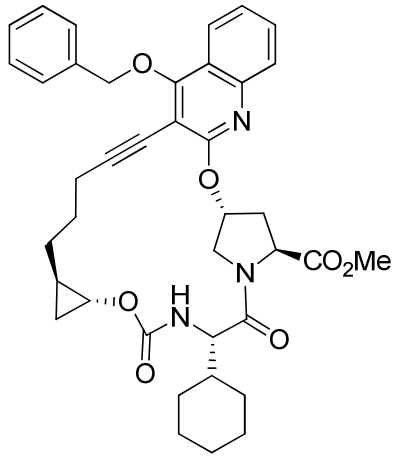
To a suspension of triphenylphophine (2.30 g, 8.77 mmol), hydroxy-proline derivative **4** (2.080 g, 8.48 mmol) and quinolinone **5** (2.0 g, 6.06 mmol; synthesized as described in WO 2013074386) in THF (40 mL) was added DIAD (1.7 ml, 8.74 mmol) dropwise at 0°C under a stream of nitrogen. After 15 min of stirring at 0°C, the solution was allowed to warm up slowly to room temperature. After 2 hours, the solvents were evaporated and the crude residue was dry loaded over silica gel and purified by silica gel chromatography (ISCO, 80 g column, 10-30% ethyl acetate in hexanes) to furnish intermediate **6** (2.6g, 4.66 mmol, 77 % yield) as a colorless foam. 1H NMR (400 MHz, *CDCl$_3$*): δ 7.95 (t; J = 7.92 Hz; 1 H); 7.82 (dd; J = 12.02; 8.40 Hz; 1 H); 7.58-7.65 (m; 3 H); 7.35-7.46 (m; 4 H); 5.19-5.27 (m; 2 H); 4.55 (t; J = 8.01 Hz; 1 H); 3.95-3.99 (m; 2 H); 3.79 (s; 3 H); 2.68-2.70 (br m; 1 H); 2.37-2.43 (m; 1 H); 1.44 (s; 9 H); 1.32-1.34 (m; 1 H). LCMS (ESI) *m/z* 557.0 [$(M+H)^+$; calcd for $C_{27}H_{29}BrN_2O_6$: 556.12].

(S)-2-((((1R,2R)-2-(5-(4-(benzyloxy)-2-(((3R,5S)-1-(tert-butoxycarbonyl)-5-(methoxycarbonyl)pyrrolidin-3-yl)oxy)quinolin-3-yl)pent-4-yn-1-yl)cyclopropoxy)carbonyl)amino)-2-cyclohexylacetic acid (7).



In a pressure tube were added intermediate **3** (0.855 g, 2.78 mmol), K_2CO_3 (0.874 g, 6.32 mmol), bis(acetonitrile)dichloropalladium(II) (0.033 g, 0.126 mmol) and tri-tert-butylphosphonium tetrafluoroborate (0.110 g, 0.379 mmol) followed by 10 mL CH_3CN and dibenzylamine (0.499 g, 2.53 mmol). The reaction mixture was evacuated and filled with nitrogen (x3), and then intermdeidate **6** (1.41 g, 2.53 mmol) was added as a solution in 5 mL CH_3CN. The reaction was purged with N_2 for another 5 minutes and then stirred at 75 °C overnight. After bringing to room temperature, 1% aq. HCl was added and the mixture was extracted with EtOAc (x3). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to provide a brown oily residue. Purification by silica gel chromatography (ISCO, 0-5% MeOH in CH_2Cl_2) afforded intermediate **7** (1.5 g, 1.913 mmol, 76 % yield). ^{1}H NMR (400 MHz, *$CDCl_3$*): δ 7.98 (t; J = 8.25 Hz; 1 H); 7.72 (dd; J = 11.01; 8.37 Hz; 1 H); 7.48-7.57 (m; 3 H); 7.29-7.43 (m; 4 H); 5.76 (s; 1 H); 5.56-5.63 (m; 2 H); 5.15-5.17 (m; 1 H); 4.48 (t; J = 7.55 Hz; 1 H); 4.23-4.26 (m; 2 H); 3.91-4.03 (m; 2 H); 3.73-3.77 (m; 6 H); 2.34-2.72 (m; 4H); 1.29-1.52 (m; 21 H); 0.82-0.89 (m; 2 H); 0.52 (d; J = 6.60 Hz; 1 H). LCMS (ESI) *m/z* 784.7 [$(M+H)^+$; calcd for $C_{44}H_{53}N_3O_{10}$: 783.37].

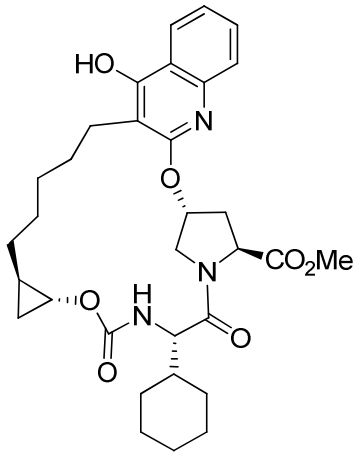
Synthesis of intermediate 8.



A solution of intermediate **7** (3.5 g, 4.46 mmol) in dichloromethane (20 ml) was treated with TFA (20 ml, 260 mmol) and the reaction was stirred at room temperature for 1h. The reaction was concentrated under reduced pressure and azeotroped twice with toluene to provide a brown oily residue which was used for the next step without purification (LRMS (ES+) m/z 684.8 $(M+H)^+$). A solution of this residue in DMF (20 mL) was added to a stirred solution of HATU (3.39 g, 8.92 mmol) and DIPEA (3.12 ml, 17.85 mmol) in DMF (20 ml) at 0° C over 1 h. After the addition was complete, the reaction was stirred at room

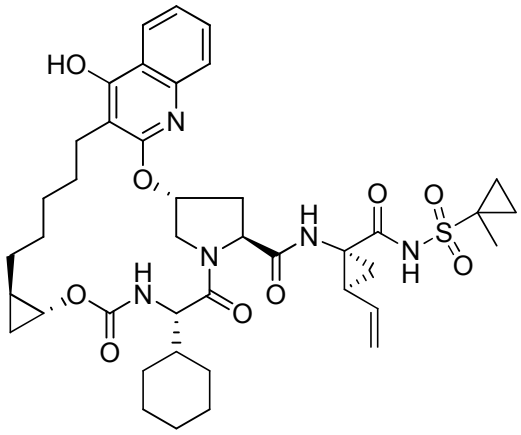
temperature for 2h at which stage it was judged to be complete by LCMS. The reaction was quenched with water and extracted with EtOAc (x3). The combined organic layers were washed successively with 1N aq. HCl, saturated aq. NaHCO3, and brine. The organic fraction was dried (Na_2SO_4), concentrated under reduced pressure and purified by silica gel chromatography (ISCO, 0-30% ethylacetate in hexane) to afford intermediate **8** (2.10 g, 3.15 mmol, 70.7 % yield). ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 8.00 (dd; J = 8.26; 1.41 Hz; 1 H); 7.70 (d; J = 8.33 Hz; 1 H); 7.51-7.59 (m; 3 H); 7.31-7.41 (m; 4 H); 5.94 (t; J = 4.32 Hz; 1 H); 5.65 (d; J = 1.49 Hz; 2 H); 5.08 (d; J = 9.46 Hz; 1 H); 4.62 (dd; J = 10.49; 7.15 Hz; 1 H); 4.51 (d; J = 11.47 Hz; 1 H); 4.32 (t; J = 9.25 Hz; 1 H); 4.02 (dd; J = 11.47; 4.50 Hz; 1 H); 3.82-3.85 (m; 1 H); 3.67-3.80 (m; 4 H); 2.68-2.82 (m; 2 H); 2.49-2.57 (m; 1 H); 2.30 (ddd; J = 14.11; 10.52; 4.43 Hz; 1 H); 1.96 (t; J = 14.13 Hz; 2 H); 1.67-1.77 (br m; 6 H); 1.03-1.32 (m; 5 H); 0.93 (d; J = 4.15 Hz; 2 H); 0.77 (br s; 1 H); 0.47 (d; J = 7.01 Hz; 1 H); LCMS (ESI) *m/z* 666.4 [(M+H)$^+$; calcd for $C_{39}H_{43}N_3O_7$: 665.31].

Synthesis of intermediate 9.



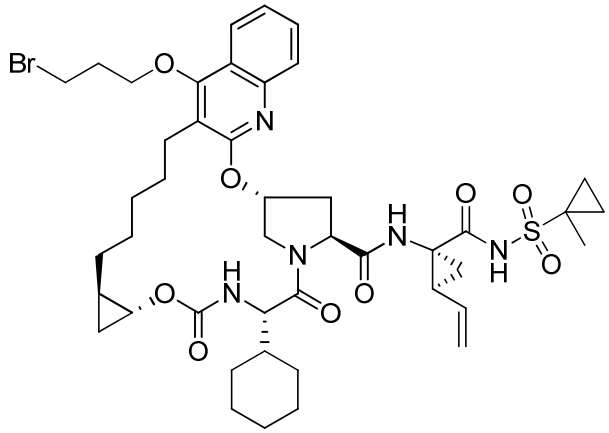
To a solution of intermediate **8** (640 mg, 0.961 mmol) in a mixture of THF (10 ml) and MeOH (20 ml) was added 10% Pd-C (200 mg). The reaction was hydrogenated overnight under atmospheric pressure (balloon), 1.879 mmol). The reaction was filtered through celite, washed with ethylacetate and concentrated. Purification by silica gel chromatography (0-40% ethylacetate in hexanes) afforded intermediate 9 (560 mg, 0.966 mmol, 100 % yield). ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 8.00 (d; J = 8.22 Hz; 1 H); 7.71 (d; J = 8.37 Hz; 1 H); 7.55 (t; J = 7.64 Hz; 1 H); 7.34 (t; J = 7.57 Hz; 1 H); 6.49 (s; 1 H); 6.05 (s; 1 H); 5.12 (d; J = 9.59 Hz; 1 H); 4.53 (dd; J = 10.75; 6.97 Hz; 1 H); 4.44 (d; J = 11.45 Hz; 1 H); 4.31 (t; J = 9.54 Hz; 1 H); 3.98 (dd; J = 11.40; 4.26 Hz; 1 H); 3.62-3.82 (m; 4 H); 2.52-2.71 (m; 3 H); 2.21-2.28 (m; 1 H); 1.96 (t; J = 14.24 Hz; 2 H); 0.85-1.73 (m; 18 H); 0.59-0.65 (m; 1 H); 0.43 (q; J = 6.12 Hz; 1 H); LCMS (ESI) *m/z* 580.4 [(M+H)$^+$; calcd for $C_{32}H_{41}N_3O_7$: 579.29].

Synthesis of intermediate 11.



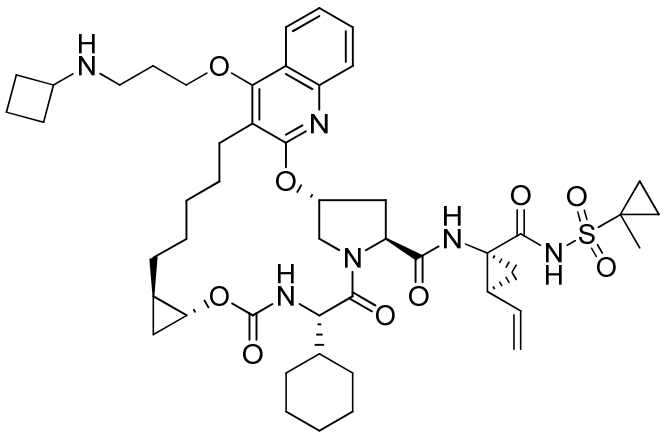
To a solution of intermediate **9** (1.57 g, 2.71 mmol) in MeOH (10 ml) and THF (20 ml) was added lithium hydroxide (1M aq.; 6.0 mL, 12.0 mmol). After stirring for 4h at room temperture, the reaction was quenched with 1M aq. HCl and extracted with EtOAc (x3). The organic fraction was dried (Na_2SO_4), filtered and concentrated under reduced pressure to provide the crude acid (1.42 g, 2.51 mmol, 93 % yield), which was used for the next step without purification. To a solution of the above prepared acid (1.4219 g, 2.51 mmol) in DMF (20 ml) was added intermediate **10** (0.74 g, 3.02 mmol; synthesized as described in US Patent 7,135,462) and HATU (1.05 g, 2.77 mmol) followed by DIPEA (1.32 ml, 7.54 mmol). After stirring overnight at room temperature under N_2 the reaction was poured into water and extracted with EtOAc (x3). The combined organic fractions were dried (MgSO4), filtered, concentrated and purified by silica gel chromatography (ISCO, 0-80% EtOAc in hexanes) to afford (1.35 g, 1.71 mmol, 67.9 % yield). ^{1}H NMR (400 MHz, *$CDCl_3$*): δ 9.80 (s; 1 H); 8.00 (d; J = 8.23 Hz; 1 H); 7.70 (d; J = 8.37 Hz; 1 H); 7.52 (t; J = 7.65 Hz; 1 H); 7.12 (s; 1 H); 6.03 (s; 1 H); 5.66-5.75 (m; 1 H); 5.56 (d; J = 10.01 Hz; 1 H); 5.19 (d; J = 17.13 Hz; 1 H); 5.09 (d; J = 10.53 Hz; 1 H); 4.02-4.46 (m; 5 H); 3.77-3.79 (m; 1 H); 2.52-2.63 (m; 2 H); 2.35-2.43 (m; 2 H); 0.70-2.24 (m, 30 H); 0.59 (br s; 1 H); 0.43-0.47 (m; 1 H); LCMS (ESI) *m/z* 792.2 [$(M+H)^+$; calcd for $C_{41}H_{53}N_5O_9S$: 791.36].

Synthesis of intermediate 12.



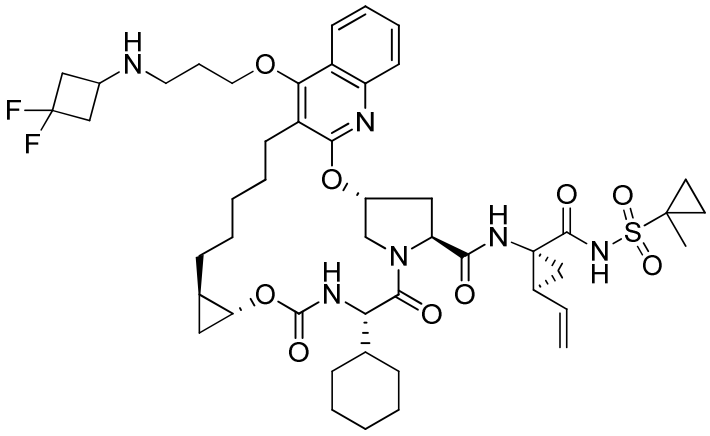
To a solution of intermediate **11** (1.35 g, 1.71 mmol) in DMF (5 ml) was added Cs_2CO_3 (2.22 g, 6.83 mmol) followed by 1,3-dibromopropane (1.74 ml, 17.07 mmol). The reaction was stirred at 50 °C for 1h after which it was brought to room temperature, poured into water and extracted with EtOAc (x3). Purification by silica gel chromatography (0-70% ethyl acetate in hexanes) provided intermediate **12** (1.21 g, 1.32 mmol, 77 % yield). ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 9.74 (s; 1 H); 7.88-7.90 (m; 1 H); 7.78 (d; J = 8.35 Hz; 1 H); 7.54-7.58 (m; 1 H); 7.35-7.39 (m; 1 H); 6.98 (s; 1 H); 6.06 (t; J = 4.01 Hz; 1 H); 5.64-5.73 (m; 2 H); 5.18 (d; J = 17.14 Hz; 1 H); 5.07 (d; J = 10.45 Hz; 1 H); 4.49 (t; J = 12.03 Hz; 2 H); 4.32 (dd; J = 10.73; 6.54 Hz; 1 H); 4.07-4.19 (m; 3 H); 3.77-3.81 (m; 3 H); 2.78-2.85 (m; 1 H); 0.90-2.66 (m; 33 H); 0.71-0.81 (m; 3 H); 0.47-0.51 (m; 1 H); LCMS (ESI) *m/z* 912.1 [$(M+H)^+$; calcd for $C_{44}H_{58}BrN_5O_9S$: 911.31].

Synthesis of compound 13.



A solution of intermediate **12** (0.085 g, 0.093 mmol) and cyclobutylamine (0.120 mL, 1.397 mmol) in DMF (2 mL) was stirred at 50 °C for 2h. After cooling to room temperature, the reaction was treated with water and with ethyl acetate. The combined organic fractions were dried ($Na_2SO_)$, filtered and concentrated under reduced pressure. Purification by preparative TLC (5% MeOH in CH_2Cl_2) provided compound **13** (59 mg, 0.062 mmol, 67% yield). A formate salt was prepared by adding 5 mL MeCN containing 0.1% HCO_2H; the volatiles were removed under reduced pressure and the residue was dried under vacuum. ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 9.69 (br s; 1 H); 8.26 (s; 2 H); 8.07 (s; 1 H); 7.73 (dd; J = 24.16; 8.30 Hz; 2 H); 7.48 (t; J = 7.64 Hz; 1 H); 7.23-7.26 (m; 1 H); 6.02 (s; 1 H); 5.81 (dt; J = 17.15; 9.54 Hz; 1 H); 5.28 (d; J = 9.21 Hz; 1 H); 5.15 (d; J = 17.15 Hz; 1 H); 5.05 (d; J = 10.55 Hz; 1 H); 4.53 (d; J = 11.56 Hz; 1 H); 4.22-4.33 (m; 2 H); 4.07-4.11 (m; 3 H); 3.69 (t; J = 7.28 Hz; 2 H); 3.03-3.20 (m; 2 H); 2.64 (t; J = 12.09 Hz; 1 H); 2.48 (dd; J = 13.91; 6.08 Hz; 1 H); 0.77-2.37 (m; 39 H); 0.65-0.67 (br m; 1 H); 0.43-0.48 (m; 1 H); LCMS (ESI) *m/z* 903.4 [(M+H)$^+$; calcd for $C_{48}H_{66}N_6O_9S$: 902.46].

Synthesis of compound 14.



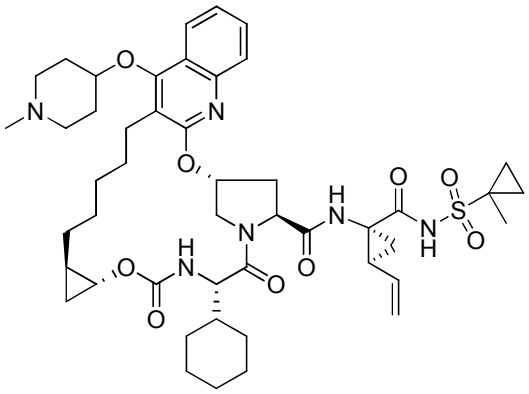
A solution of intermediate **12** (50.0 mg, 0.055 mmol), 3,3-difluorocyclobutanamine hydrochloride 5 (79 mg, 0.548 mmol), Et_3N (0.076 mL, 0.548 mmol) and KI (91 mg, 0.548 mmol) in DMF (2 mL) was stirred at 55 °C for 4 h. After cooling to room temperature, the reaction was diluted with ethyl acetate and washed with water and brine. The organic fraction was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by preparative TLC (5% MeOH/CH_2Cl_2) provided compound **14** (22 mg, 0.021 mmol, 38.9 % yield). ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 7.90 (d; J = 8.16 Hz; 1 H); 7.78 (d; J = 8.39 Hz; 1 H); 7.57 (ddd; J = 8.42; 6.87; 1.53 Hz; 1 H); 7.36-7.40 (m; 1 H); 6.63 (s; 1 H); 6.07 (t; J = 3.89 Hz; 1 H); 5.76-5.85 (m; 1 H); 5.17-5.26 (m; 2 H); 5.08-5.11 (m; 1 H); 4.56 (d; J = 11.53 Hz; 1 H); 4.19-4.29 (m; 2 H); 4.04-4.13 (m; 3 H); 3.68-3.71 (m; 1 H); 3.30-3.34 (m; 1 H); 2.78-2.92 (m; 5 H); 2.34-2.58 (m; 5

H); 0.72-2.10 (m; 32 H); 0.62-0.69 (m; 2 H); 0.48 (q; J = 6.40 Hz; 1 H); LCMS (ESI) *m/z* 939.4 [$(M+H)^+$; calcd for $C_{48}H_{64}F_2N_6O_9S$: 938.44].

Synthesis of compounds 15-18.

Compounds **15-18** were synthesized in 30-60% yield from intermediate **12** and the corresponding commercially available amines following the procedure described for the synthesis of compound **14**.

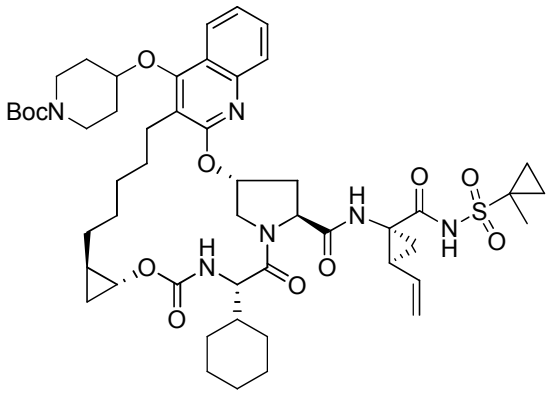
Synthesis of compound 21.



To a solution of intermediate **9** (75 mg, 0.129 mmol) and 4-hydroxy-1-methylpiperidine (0.152 ml, 1.294 mmol) in dry THF (2 ml) was added triphenylphosphine (339 mg, 1.294 mmol) followed by diisopropyl azodicarboxylate (0.252 ml, 1.294 mmol). The reaction was stirrred under N_2 for 3 h at 40 °C after which the solvent was removed under reduced pressure and the crude product was partially purified by preparative TLC (5% MeOH in CH_2Cl_2) to afford compound **19** (88 mg, 0.129 mmol, 100 % yield). LRMS (ES+) m/z 677.4 $(M+H)^+$. To a solution of the above prepared compound **19** (88 mg, 0.130 mmol) in THF (3 ml) and MeOH (1.5 ml) was added lithium hydroxide monohydrate (27.3 mg, 0.650 mmol) and 0.3 mL H_2O. The reaction was complete after 3 h and was quenched with acetic acid (0.074 ml, 1.300 mmol). Water was added and the mixture was extracted with EtOAc (3x). The organic fractions were combined, dried (Na_2SO_4) and concentrated to provide the corresponding acid as an oily residue. To a solution of this residue in DMF (3 mL) was added intermediate **10** (43.2 mg, 0.154 mmol; synthesized as described in US Patent 7,135,462) followed by DIPEA (0.090 ml, 0.513 mmol) and HATU (58.5 mg, 0.154 mmol). The reaction was stirred overnight at room temperature after which it was quenched with

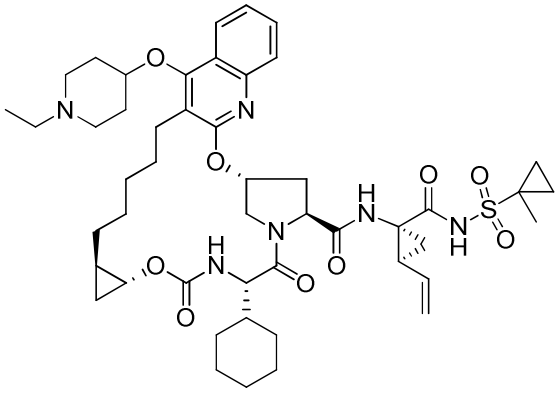
water and extracted with EtOAc. The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue thus obtained was purified by reverse phase CombiFlash (0% to 100% ACN in water, 0.1% HCO_2H) to provide Compound **21** (58 mg, 0.065 mmol, 51% yield). ^{1}H NMR (599 MHz, *DMSO-d6*): δ 8.35 (br s, 2H), 7.90 (d, J = 8.31 Hz, 1H), 7.68 (d, J = 8.31 Hz, 1H), 7.57 (t, J = 7.58 Hz, 1H), 7.38 (t, J = 7.58 Hz, 1H), 7.34 (d, J = 7.34 Hz, 1H), 5.78 - 5.90 (m, 2H), 5.04 (d, J = 17.36 Hz, 1H), 4.89 (d, J = 11.00 Hz, 1H), 4.31 (d, J = 11.25 Hz, 1H), 4.17 (t, J = 8.30 Hz, 1H), 4.10 - 4.14 (m, 1H), 3.99 - 4.07 (m, 2H), 3.60 (s, 1H), 2.77 (s, 3H), 2.41 - 2.52 (m, 2H), 2.32 - 2.39 (m, 1H), 2.15 - 2.30 (m, 4H), 1.44 - 2.13 (m, 18H), 1.03 - 1.43 (m, 11H), 0.75 - 1.01 (m, 4H), 0.52 - 0.67 (m, 2H), 0.42 (d, J = 5.87 Hz, 1H). ^{13}C NMR (151 MHz, *DMSO-d6*): δ 172.1, 171.2, 171.0, 160.5, 159.2, 157.0, 145.0, 136.6, 129.1, 126.8, 123.9, 122.3, 121.7, 118.2, 114.9, 80.3, 74.4, 59.1, 57.5, 53.7, 53.4, 52.9, 45.2, 42.1, 39.9, 39.8, 39.6, 39.4, 39.2, 39.1, 38.7, 35.5, 32.8, 31.6, 31.4, 30.2, 29.7, 29.2, 29.0, 28.3, 27.9, 26.0, 25.6, 25.5, 25.0, 21.7, 18.6, 18.4, 12.4, 11.8, 11.2. LCMS (ESI) *m/z* 889.2 [$(M+H)^+$; calcd for $C_{47}H_{64}N_6O_9S$: 888.45]

Synthesis of compound 22.



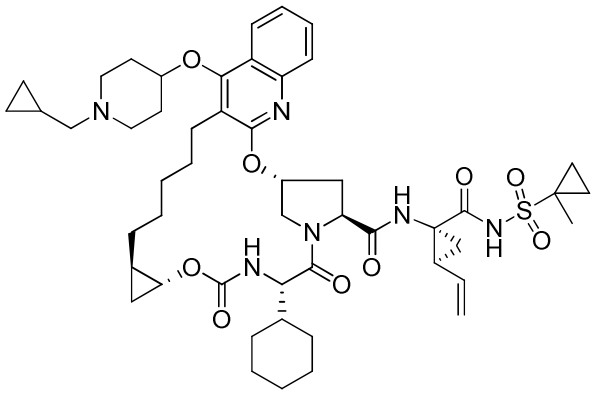
Compound **22** was synthesized in 71% yield from intermediate **9** and N-BOC-4-hydroxypiperidine following the procedure described for the synthesis of compound **21**. ^{1}H NMR (400 MHz, *$CDCl_3$*): δ 10.11 (s; 1 H); 7.89-7.94 (m; 1 H); 7.78 (d; J = 8.33 Hz; 1 H); 7.58 (t; J = 7.74 Hz; 1 H); 7.37 (t; J = 7.65 Hz; 1 H); 6.72 (s; 1 H); 6.08 (t; J = 3.88 Hz; 1 H); 5.77-5.86 (m; 1 H); 5.38 (d; J = 9.20 Hz; 1 H); 5.21 (d; J = 17.17 Hz; 1 H); 5.11 (d; J = 10.48 Hz; 1 H); 4.98 (dt; J = 12.55; 6.28 Hz; 1 H); 4.56 (d; J = 11.58 Hz; 1 H); 4.20-4.29 (m; 3 H); 4.03-4.09 (m; 3 H); 3.69-3.71 (m; 1 H); 2.82-2.97 (m; 3 H); 2.40-2.59 (m; 3 H); 1.28-1.53 (m; 42 H); 0.68 (d; J = 19.85 Hz; 1 H); 0.46-0.51 (m; 1 H). LCMS (ESI) *m/z* 975.4 [$(M+H)^+$; calcd for $C_{51}H_{70}N_6O_{11}S$: 974.48].

Synthesis of compound 24



To a solution of compound **23** (74 mg, 0.075 mmol) in DMF (2 mL) was added triethylamine (0.031 mL, 0.224 mmol) and iodoethane (23.34 mg, 0.150 mmol). The reaction was stirred at 55 °C for 1h at which stage LCMS indicated no starting material and a major peak corresponding to the product mass. After cooling to room temperature, water was added and the reaction was extracted with ethyl acetate (x2). The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated to give an oily residue. The crude was purified by preparative TLC (5% MeOH in CH_2Cl_2) and the purified product was treated with 2 mL of MeCN containing 0.1% formic acid. The volatiles were removed under reduced pressure and the resulting residue was vacuum dried to furnish compound **24** as a formate salt (33.6 mg, 0.035 mmol, 46.4 % yield). ^{1}H NMR (599 MHz, *DMSO-d6*): δ 8.52 - 8.87 (m, 1H), 7.92 (d, J = 8.07 Hz, 1H), 7.71 (d, J = 8.31 Hz, 1H), 7.59 (t, J = 7.30 Hz, 1H), 7.35 - 7.48 (m, 2H), 5.80 - 5.86 (m, 1H), 5.69 (br. s, 1H), 5.11 (d, J = 17.12 Hz, 1H), 4.98 (d, J = 10.27 Hz, 1H), 4.38 (d, J = 11.00 Hz, 1H), 4.13 - 4.23 (m, 2H), 3.95 - 4.07 (m, 2H), 3.55 - 3.59 (m, 1H), 2.87 - 2.99 (m, 2H), 2.73 - 2.83 (m, 1H), 2.31 - 2.39 (m, 1H), 2.09 - 2.27 (m, 3H), 1.77 - 2.08 (m, 8H), 1.48 - 1.74 (m, 10H), 1.25 - 1.45 (m, 7H), 1.17 - 1.24 (m, 2H), 1.05 - 1.14 (m, 3H), 1.00 (t, J = 6.80 Hz, 3H), 0.59 - 0.95 (m, 7H), 0.40 - 0.49 (m, 1H). ^{13}C NMR (151 MHz, *DMSO-d6*) δ 171.4, 171.3, 169.7, 163.6, 160.5, 159.1, 156.9, 145.0, 134.88, 129.2, 126.9, 124.0, 122.4, 121.7, 118.1, 116.42, 80.4, 74.3, 58.9, 57.6, 53.6, 53.4, 51.1, 50.2, 41.4, 40.4, 40.1, 38.5, 35.9, 35.2, 33.5, 31.4, 31.2, 30.3, 29.7, 29.3, 29.2, 28.1, 27.9, 26.0, 25.5, 25.1, 22.3, 18.4, 18.0, 13.0, 12.2, 12.0, 11.7, 11.2. LCMS (ESI) *m/z* 903.3 [$(M+H)^+$; calcd for $C_{48}H_{66}N_6O_9S$: 902.46].

Synthesis of compound 25

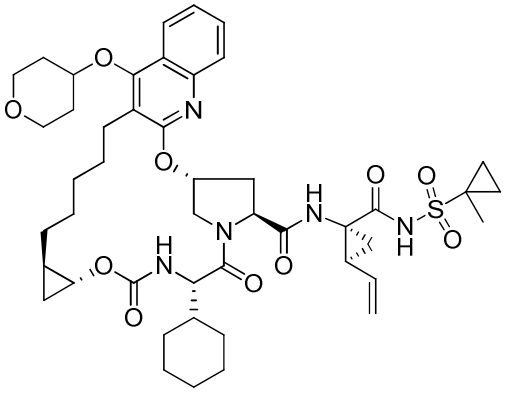


To a solution of compound **23** (70 mg, 0.071 mmol) in DMF (3 mL) was added triethylamine (0.049 mL, 0.354 mmol), potassium iodide (2.350 mg, 0.014 mmol) and (bromomethyl)cyclopropane (28.7 mg, 0.212 mmol). The reaction was heated to 55 °C and stirred for 3h at which stage LCMS indicated no starting material. After cooling to room temperature, water was added and the reaction was extracted with ethyl acetate (x2). The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated to give an oily residue. The crude was purified by preparative TLC (5% MeOH in CH_2Cl_2) and the purified product was treated with 2 mL of MeCN containing 0.1% formic acid. The volatiles were removed under reduced pressure and the resulting residue was vacuum dried to furnish compound **25** as a formate salt (36.4 mg, 0.071 mmol, 52% yield). ^{1}H NMR (400 MHz, *$CDCl_3$*): δ 8.47 (s; 1 H); 7.82 (dd; J = 16.27; 8.32 Hz; 2 H); 7.57-7.61 (m; 1 H); 7.39 (t; J = 7.61 Hz; 1 H); 7.33 (s; 1 H); 6.07 (t; J = 3.84 Hz; 1 H); 5.83 (dt; J = 17.15; 9.52 Hz; 1 H); 5.19-5.25 (m; 2 H); 5.10 (d; J = 10.52 Hz; 1 H); 4.57 (d; J = 11.55 Hz; 1 H); 4.39 (s; 1 H); 4.25-4.30 (m; 2 H); 4.08 (dd; J = 11.51; 4.31 Hz; 1 H); 3.70-3.72 (m; 1 H); 3.38 (br s; 2 H); 2.77 (d; J = 7.33 Hz; 3 H); 2.57 (dd; J = 13.77; 6.06 Hz; 1 H); 2.40-2.46 (m; 2 H); 2.32 (s; 2 H); 2.19 (s; 2 H); 2.08 (q; J = 8.84 Hz; 1 H); 1.98 (dd; J = 8.73; 5.22 Hz; 2 H); 1.89 (d; J = 11.86 Hz; 1 H); 0.80-1.84 (m; 29 H); 0.70-0.72 (m; 3 H); 0.49 (q; J = 6.40 Hz; 1 H); 0.33 (d; J = 5.13 Hz; 2 H). ^{13}C NMR (151 MHz, DMSO-d6) δ 172.0, 171.7, 160.9, 159.5, 157.3, 145.4, 129.6, 127.3, 124.4, 122.8, 122.1, 118.5, 80.6, 74.8, 62.3, 59.3, 58.0, 54.1, 53.9, 50.9, 41.7, 40.9, 40.5, 38.9, 36.4, 35.6, 34.0, 31.7, 31.6, 30.8, 30.1, 29.7, 29.6, 28.5, 28.3, 26.4, 25.9, 25.5, 22.8, 18.8, 18.3, 13.5, 12.5, 11.7, 8.3, 4.3, 4.2. LCMS (ESI) *m/z* 929.4 [$(M+H)^+$; calcd for $C_{50}H_{68}N_6O_9S$: 928.48].

Synthesis of Compounds 26-30

Compounds **26-30** were synthesized in 20-60% yields from compound **23** following the procedure described for the synthesis of compound **25**.

Synthesis of Compound 31

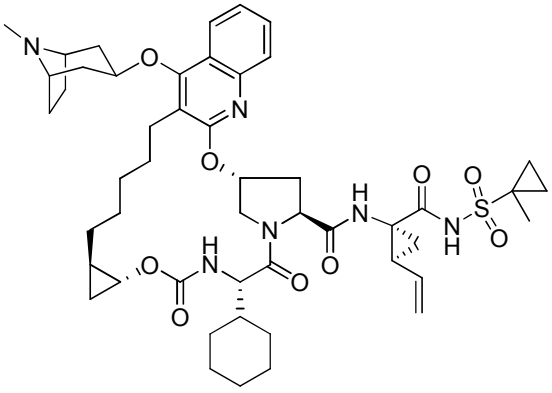


Compound **31** was synthesized from intermediate **9** following the procedure described for the synthesis of compound **21**.

Synthesis of Compounds 32-36, 38

Compounds **32-36** and **38** were synthesized in 20-60% yields using procedures described for the synthesis of compounds described in Scheme 1. The required endo and exo NBoc-nor-tropanol derivatives were prepared as described *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3290.

Synthesis of Compound 37



Compound **37** was synthesized from intermediate **9** and tropine in 55% yield following the procedure described for the synthesis of compound **21**. ^{1}H NMR (400 MHz, *CDCl$_3$*): δ 7.94 (d; J = 8.10 Hz; 1 H);

7.74 (d; J = 8.33 Hz; 1 H); 7.55 (t; J = 7.60 Hz; 1 H); 7.35 (t; J = 7.60 Hz; 1 H); 6.82 (s, 1 H); 6.04 (s; 1 H); 5.76-5.85 (m; 1 H); 5.35 (d; J = 9.18 Hz; 1 H); 5.20 (d; J = 17.13 Hz; 1 H); 5.09 (d; J = 10.46 Hz; 1 H); 4.55 (d; J = 11.50 Hz; 1 H); 4.36-4.41 (m; 1 H); 4.21-4.28 (m; 2 H); 4.06 (dd; J = 11.47; 4.31 Hz; 1 H); 3.69 (s; 1 H); 3.48 (q; J = 7.02 Hz; 2 H); 3.32 (s; 1 H); 2.79 (t; J = 12.48 Hz; 1 H); 2.57 (dd; J = 13.88; 6.11 Hz; 1 H); 0.63-2.50 (m, 43 H); 0.48 (d; J = 6.47 Hz; 1 H). ^{13}C NMR (151 MHz, *CDCl$_3$*) δ 172.8, 171.9, 168.3, 160.4, 159.8, 157.2, 145.3, 133.0, 129.0, 127.1, 124.0, 122.3, 122.2, 118.2, 118.0, 73.9, 59.9, 57.5, 54.9, 54.0, 41.4, 41.0, 39.8, 38.5, 36.5, 35.5, 35.5, 30.6, 30.4, 30.0, 29.7, 28.8, 28.3, 26.2, 26.1, 25.9, 25.5, 24.0, 18.5, 14.3, 13.1, 11.4. LCMS (ESI) *m/z* 915.2 [$(M+H)^+$; calcd for $C_{49}H_{66}N_6O_9S$: 914.46].