# Diversity-Oriented Synthesis Yields a Novel Lead for the Treatment of Malaria

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## Supporting Information

## General Information

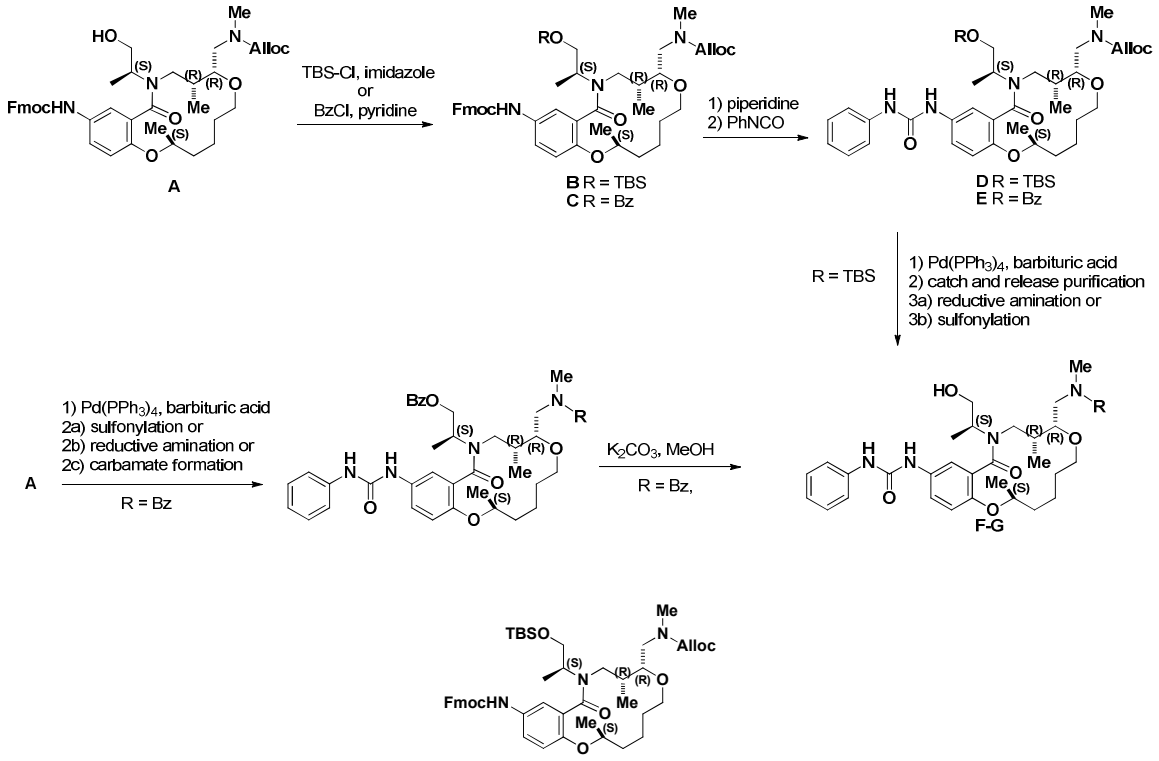
All oxygen and/or moisture sensitive reactions were carried out under $N_2$ atmosphere in glassware that had been flame-dried under vacuum (~0.5 mmHg) and purged with $N_2$ prior to use. All reagents and solvents were purchased from commercial vendors and used as received, or synthesized according to the footnoted references. NMR spectra were recorded on a Bruker 300 (300 MHz $^1$H, 75 MHz $^{13}$C) or Varian UNITY INOVA 500 (500 MHz $^1$H, 125 MHz $^{13}$C) spectrometer. Proton chemical shifts are reported in ppm (δ) referenced to the NMR solvent.[1] Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet; coupling constant(s) in Hz; integration). Unless otherwise indicated NMR data were collected at 25 °C. Flash chromatography was performed using 40-60 µm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash $R_f$. For purity analysis, purity was measured by UV absorbance at 210 nm for all examples, and identity was determined on a SQ mass spectrometer by positive electrospray ionization. The following methods were used: Method A: UPLC-MS (Waters, Milford, MA). Mobile phase A consisted of either 0.01% ammonium hydroxide or 0.01% formic acid in water, while mobile phase B consisted of the same additives in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 0.8 minutes at 0.45 mL/min. An Acquity BEH C18, 1.7 um, 1.0x50 mm column was used with column temperature maintained at 65 $^o$C. Method B: Tandem Liquid Chromotography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 15 minutes at 1 mL/min. An XBridge C18, 3.5 um, 4.6x30 mm column was used with column temperature maintained at 40 $^o$C. 5 uL of sample solution were injected. Method C: Tandem Liquid Chromotography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 7.5 minutes at 1.75 mL/min. An Agilent Poroshell 120 EC-C18, 2.7 um, 3.0x30 mm column was used with column temperature maintained at 40 $^o$C. 2.1 uL of sample solution were injected. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. High-resolution mass spectra were obtained at the Boston University Mass Spectrometry Facility.

[1] Gottlieb, H. E., Kotlyar, V., Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

## Experimental Procedures

Schemes 1-5 outline the various protocols that were developed to prepare the products **1**-**27**. The synthesis of compounds **A** and **2**-**10** were previously reported.[2]

### Scheme 1. Initial synthesis of derivatives of compound 1

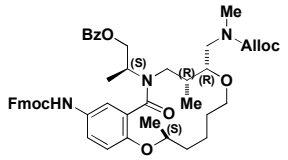



**Allyl (((2*S*,8*R*,9*R*)-14-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-11-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (B):**

To a solution of alcohol **A** (0.494 g, 0.692 mmol) in dry DMF (2.77 mL) was added imidazole (0.141 g, 2.076 mmol) followed by TBSCl (0.146 g, 0.692 mmol) under $N_2$ atmosphere at room temperature. The resulting mixture was stirred at room temperature for 16 h. The reaction was diluted with DCM (10 mL) and washed with water (2 X). The organic phase was then separated, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using ethyl acetate / hexanes to give 0.37 g (65%) of the desired product. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, 2H), 7.60 (d, 2H), 7.53 – 7.20 (m, 4H), 7.09 (s, 1H), 6.82 (m, 1H), 6.55 (s, 1H), 5.94 (m, 1H), 5.47 – 5.06 (m, 1H), 4.75 – 4.39 (m, 4H), 4.26 (m, 1H), 4.10 (m, 1H), 3.99 – 3.62 (m, 4H), 3.49 –

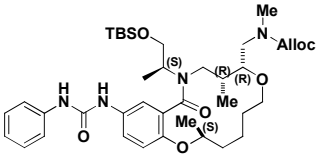
[2] Marcaurelle, L. A.; Comer, E.; Dandapani, S.; Duvall, J. R.; Gerard, B.; Kesavan, S.; Lee, M. D, IV; Liu, H.; Lowe, J. T.; Marie, J.-C.; Mulrooney, C. A.; Pandya, B. A.; Rowley, A.; Ryba, T. D.; Suh, B.-C.; Wei, J.; Young, D. W.; Akella, L. B.; Ross, N. B.; Zhang, Y.-L.; Fass, D. M.; Reis, S. A.; Zhao, W.-N.; Haggarty, S. J.; Palmer, M.; Foley, M. A. *J. Am. Chem. Soc.* **2010**, *132*, 16962-16976.

3.10 (m, 2H), 3.00 (m, 2H), 2.88 – 2.66 (m, 1H), 2.19 (s, 1H), 2.01 (s, 1H), 1.83 (m, 1H), 1.63 (s, 4H), 1.32 (m, 4H), 1.16 – 1.02 (m, 2H), 1.02 – 0.57 (m, 9H), 0.15 – -0.15 (m, 5H). HRMS (ESI) calcd for $C_{47}H_{66}N_3O_8Si$ $[M + H]^+$: 828.4619. Found: 828.4620.




**(*S*)-2-((2*S*,8*R*,9*R*)-14-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-8-((((allyloxy)carbonyl)(methyl)amino)methyl)-2,9-dimethyl-12-oxo-3,4,5,6,9,10-hexahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-11(2H,8H,12H)-yl)propyl benzoate (C)**

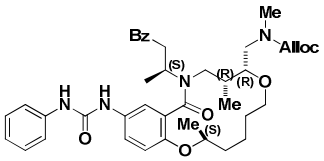
To a solution of alcohol **A** (0.509 g, 0.713 mmol) in dry DCM (5.94 mL) was added pyridine (0.577 mL, 7.13 mmol) followed by benzoyl chloride (0.248 mL, 2.139 mmol) under $N_2$ atmosphere at room temperature. The resulting mixture was stirred at room temperature for 4 h. The reaction was diluted with DCM (10 mL) and washed with water (2 X 10 mL). The organic phase was then separated, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using ethyl acetate / hexanes to give 0.54 g (93%) of the desired product. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, 1H), 8.00 (d, 1H), 7.77 (d, 2H), 7.66-7.50 (m, 3H), 7.48 – 7.37 (m, 4H), 7.36-7.28 (m, 2H), 7.22-7.11 (m, 1H), 6.89-6.66 (m, 2H), 6.03-5.78 (m, 1H), 5.26 – 5.06 (m, 1H), 4.72-4.40 (m, 4H), 4.32-4.22 (m, 1H), 4.18 – 4.02 (m, 1H), 4.00-3.84 (m, 1H), 3.80-3.50 (m, 1H), 3.47 (d, 2H), 3.41 – 3.15 (m, 1H), 3.12-2.73 (m, 3H), 2.19 (m, 1H), 2.04 (m, 1H), 1.91-1.69 (m, 2H), 1.67-1.45 (m, 3H), 1.37-1.08 (m, 9H), 0.99-0.80 (m, 6H), 0.77 (m, 2H). HRMS (ESI) calcd for $C_{48}H_{56}N_3O_9$ $[M + H]^+$: 818.4017. Found: 818.4017.




**Allyl (((2*S*,8*R*,9*R*)-11-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (D)**

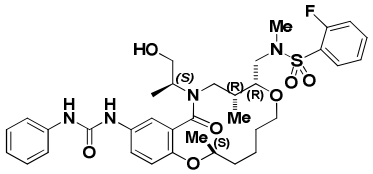
To a solution of Fmoc protected aniline **B** (0.330 g, 0.398 mmol) in dry DMF (3.98 mL) was added piperidine (0.079 mL, 0.797 mmol). The reaction mixture was stirred for 30 min, then phenyl isocyanate (0.174 mL, 1.594 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. The reaction was diluted with water and EtOAc, the phases were separated, and the organic phase was washed with $H_2O$ 6 X. The organic phase was then dried ($Na_2SO_4$) and evaporated to dryness.
This material was chromatographed on silica, using MeOH / DCM to yield 0.200 g (69%) of the desired product. $^1$H NMR (300 MHz, CDCl3) δ 7.91 (m, 1H), 7.70 (m, 1H), 7.47 (m, 2H), 7.37 – 7.08 (m, 1H), 6.94 (m, 1H), 6.67 (m, 2H), 5.91 (m, 1H), 5.43 – 5.08 (m, 2H), 4.58 (m, 3H), 3.86 (m, 4H), 3.49 (m, 4H), 3.18 – 2.92 (m, 4H), 2.85 (m, 1H), 2.21

(m, 1H), 1.73 (m, 2H), 1.49 (m, 5H), 1.23 (m, 2H), 1.20 – 1.06 (m, 2H), 1.04 – 0.77 (m, 11H), 0.22 – -0.08 (m, 5H). HRMS (ESI) calcd for $C_{39}H_{61}N_4O_7Si$ $[M + H]^+$: 725.4310. Found: 725.4316.




**(*R*)-2-((2*S*,8*R*,9*R*)-8-((((Allyloxy)carbonyl)(methyl)amino)methyl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-3,4,5,6,9,10-hexahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-11(2H,8H,12H)-yl)propyl benzoate (E)**

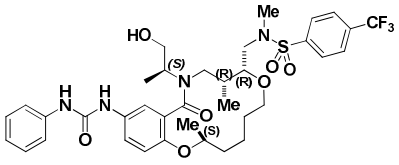
A mixture of Fmoc-protected aniline **C** (1.61 g, 1.968 mmol) and piperidine (0.390 mL, 3.94 mmol) in dry DMF (19.7 mL) was stirred under $N_2$ atmosphere at room temperature for 40 min. Then phenyl isocyanate (0.209 mL, 1.914 mmol) was introduced to the reaction mixture, which was stirred at room temperature for 3 h. The reaction was diluted with water/EtOAc (10 mL) and extracted with EtOAc. The organic phase was separated, washed with water, brine, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using ethyl acetate / hexanes to afford the desired product (0.77 g, 60% over 2 steps). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.06 (m, 1H), 8.04 – 7.89 (m, 1H), 7.81 (m, 1H), 7.59 (m, 2H), 7.51 – 7.30 (m, 2H), 7.23 (m, 2H), 6.96 (m, 1H), 6.82 (m, 1H), 6.69 (m, 1H), 5.89 (m, 1H), 5.45 – 5.08 (m, 2H), 4.83 (m, 1H), 4.55 (m, 3H), 4.18 – 4.02 (m, 1H), 3.93 (m, 2H), 3.50 (m, 1H), 3.34 (m, 1H), 3.26 – 2.95 (m, 3H), 2.88 (m, 3H), 2.26 (m, 1H), 2.04 (m, 1H), 1.77 (m, 2H), 1.62 (m, 2H), 1.38 (m, 3H), 1.24 (m, 5H), 1.14 – 1.00 (m, 2H), 0.99 – 0.85 (m, 2H), 0.77 (m, 2H). HRMS (ESI) calcd for $C_{40}H_{51}N_4O_8$ $[M + H]^+$: 715.3707. Found: 715.3718.



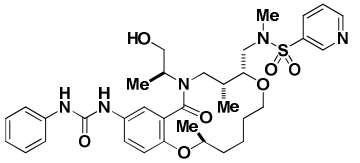

**2-Fluoro-N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-N-methylbenzenesulfonamide (13)**

To a solution of alloc protected amine **D** (0.157 g, 0.217 mmol) in DCM (2.5 mL) under $N_2$ was added 1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (0.483 g, 3.09 mmol) followed by $Pd(PPh_3)_4$ (0.143 mg, 0.124 mmol). The reaction mixture was stirred for 30 min, then purification via siliabond catch and release was performed to yield the product of the TBS deprotection. The crude material was dissolved in DCM (2.5 mL) and to this solution was added 2,6-lutidine (43 μL, 0.373 mmol) followed by 2-fluorobenzene-1-sulfonyl chloride (31 mg, 0.16 mmol). The reaction mixture was stirred 16 h. Purification of the crude mixture was accomplished with $SiO_2$ chromatography to yield 10 mg (12% yield) of desired product. LC/MS: Method A, RT 0.84 min, 95% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.01 – 7.71 (m, 2H), 7.71 – 7.48 (m, 3H), 7.39 (m, 1H), 7.37 – 7.10 (m, 3H), 7.10 – 6.86 (m, 2H), 6.86 – 6.63 (m, 1H), 4.64 (s, 1H), 4.40 (m,

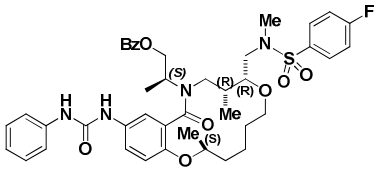
1H), 4.22 – 4.03 (m, 1H), 3.91 (m, 3H), 3.76 (m, 1H), 3.65 – 3.39 (m, 2H), 3.32 (m, 1H), 3.09 (m, 1H), 2.99 – 2.71 (m, 4H), 2.67 (m, 1H), 2.04 (m, 1H), 1.90 – 1.59 (m, 4H), 1.47 (m, 4H), 1.39 – 1.22 (m, 2H), 1.22 – 1.09 (m, 2H), 1.08 – 0.89 (m, 2H), 0.77 (d, 2H). MS calculated for $C_{35}H_{45}FN_4O_7S$ $[M+H]^+$: 685. Found: 685. HRMS (ESI) calcd for $C_{35}H_{46}FN_4O_7S$ $[M + H]^+$: 685.3071. Found: 685.3071.



**3-[(2*S*,8*R*,9*R*)-11-[(2*S*)-1-Hydroxypropan-2-yl]-2,9-dimethyl-8-({N-methyl[4-(trifluoromethyl)benzene]sulfonamido}methyl)-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (14)** was synthesized in 10% yield using the above protocol. LC/MS: Method A, RT 0.91 min, 100% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.98-7.91 (m, 1H), 7.90-7.83 (d, *J* = 8.4 Hz, 1H), 7.82 – 7.71 (m, 3H), 7.71-7.55 (m, 1H), 7.47 – 7.30 (m, 3H), 7.10 – 6.95 (m, 2H), 6.95 – 6.82 (m, 1H), 6.80 – 6.67 (m, 1H), 4.68 (br s, 1H), 4.40 (br s, 1H), 4.10 – 3.63 (m, 5H), 3.61-3.38 (m, 2H), 3.35 – 2.97 (m, 3H), 2.95 – 2.66 (m, 4H), 2.01 (br s, 1H), 1.86-1.58 (m, 2H), 1.57-1.42 (m, 3H), 1.37 (d, *J* = 6.1 Hz, 3H), 1.32 – 1.11 (m, 3H), 1.11 – 0.93 (m, 3H), 0.90-0.75 (m, 3H). HRMS (ESI) calcd for $C_{36}H_{46}F_3N_4O_7S$ $[M + H]^+$: 735.3039. Found: 735.3034.



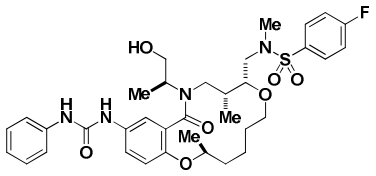
**3-[(2*S*,8*R*,9*R*)-11-[(2*S*)-1-Hydroxypropan-2-yl]-2,9-dimethyl-8-[(N-methylpyridine-3-sulfonamido)methyl]-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (15)** was synthesized in 21% yield using the above protocol. LC/MS: Method B, RT 8.48 min, 96% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 9.12 – 8.92 (m, 1H), 8.8-8.76 (m, 1H), 8.15-7.96 (m, 2H), 7.82 – 7.52 (m, 2H), 7.52 – 7.33 (m, 3H), 7.33 – 7.17 (m, 1H), 7.15 – 6.94 (m, 2H), 6.94 – 6.80 (m, 1H), 6.77-6.69 (m, 1H), 4.65-4.22 (m, 1H), 4.00-3.62 (m, 4H), 3.60-3.33 (m, 2H), 3.33 – 2.95 (m, 3H), 2.95 – 2.75 (m, 4H), 2.71-2.34 (m, 1H), 2.11-1.70 (m, 1H), 1.59-1.43 (m, 4H), 1.42-1.30 (m, 2H), 1.28-1.07 (m, 3H), 1.05 – 0.93 (m, 3H), 0.83-0.74 (m, 3H). HRMS (ESI) calcd for $C_{34}H_{46}N_5O_7S$ $[M + H]^+$: 668.3118. Found: 688.3112.



**(2*S*)-2-[(2*S*,8*R*,9*R*)-2,9-Dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-14-[(phenylcarbamoyl)amino]-**

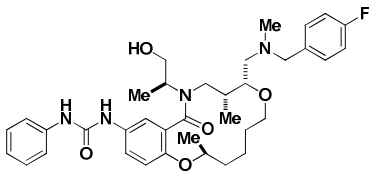
**2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-11-yl]propyl benzoate (22)**

A mixture of Alloc-protected amine **E** (0.77 g, 1.077 mmol), 1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)-trione (1.261 g, 8.08 mmol) and $Pd(PPh_3)_4$ (0.249 g, 0.215 mmol) in dry DCM (15.4 mL) was stirred under $N_2$ atmosphere at room temperature for 1 h. The reaction mixture was diluted with DCM (25 mL), washed with Sat. $NaHCO_3$ (2 X 25 mL) and water (2 X 25 mL). The organic phase was separated, dried ($Na_2SO_4$) and evaporated to dryness. The crude product was then re-dissolved in dry DCM (11 mL), then 2,6-lutidine (0.878 mL, 7.54 mmol) was added, followed by 4-fluorobenzene-1-sulfonyl chloride (0.419 g, 2.154 mmol) under $N_2$ atmosphere. The resulting mixture was stirred at room temperature for 15 h. The reaction was diluted with DCM (25 mL) and washed with water (2 X 25 mL). The organic layer was separated, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using ethyl acetate / hexanes to give 0.53 g (62%) of the title product as colorless resin. LC/MS: Method B, RT 10.90 min, 96% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.11 – 7.84 (m, 2H), 7.79 (m, 1H), 7.62 (m, 3H), 7.37 (m, 2H), 7.34 – 7.16 (m, 2H), 7.04 (m, 4H), 6.87 – 6.63 (m, 2H), 6.52 (m, 1H), 4.89 – 4.48 (m, 1H), 4.45 – 4.18 (m, 1H), 4.06 (m, 1H), 3.77 (m, 2H), 3.50 (m, 1H), 3.35 (m, 1H), 2.94 (m, 2H), 2.64 (m, 5H), 1.88 (m, 1H), 1.56 (m, 2H), 1.43 (m, 2H), 1.31 (m, 4H), 1.04 (m, 3H), 0.88 (m, 2H), 0.75 (m, 1H), 0.60 (m, 2H), 0.37 (m, 1H). HRMS (ESI) calcd for $C_{42}H_{50}FN_4O_8S$ $[M + H]^+$: 789.3333. Found: 789.3336.




**3-[(2*S*,8*R*,9*R*)-11-[(2*S*)-1-Hydroxypropan-2-yl]-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (1)**

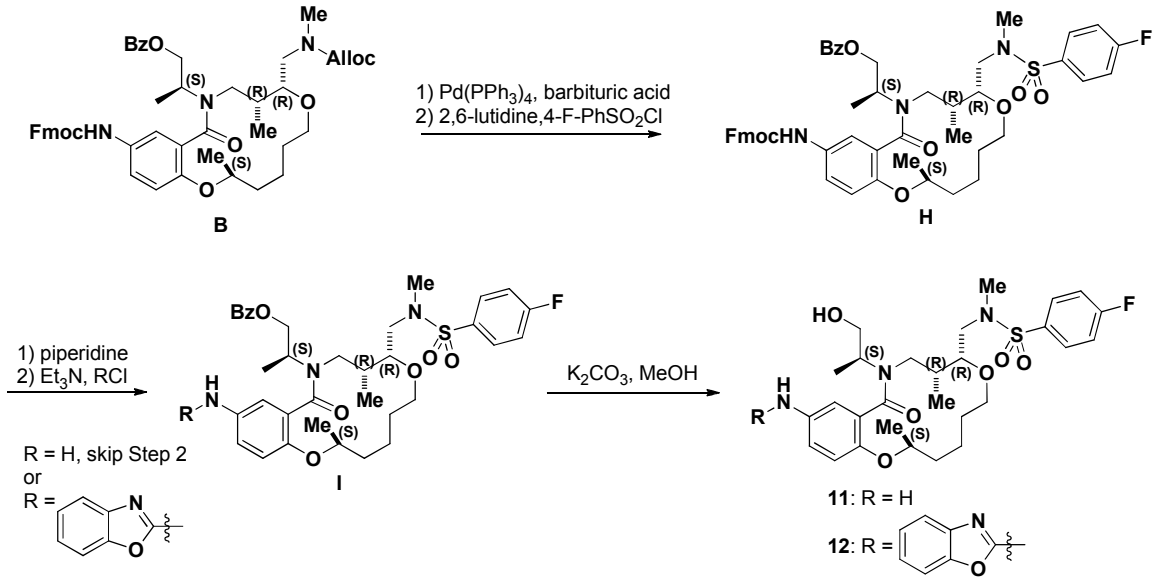
To a solution of Bz-protected alcohol **22** (0.49 g, 0.621 mmol) in methanol (12.4 mL) was added potassium carbonate (0.472 g, 3.42 mmol) under $N_2$ atmosphere. The suspension was stirred at room temperature for 15 h. The reaction mixture was then quenched with Sat. $NH_4Cl$ (15 mL) and extracted with EtOAc. The phases were separated, the organic phase was washed with water and brine, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using methanol / dichloromethane to afford the desired product (0.4 g, 94%) as colorless resin. LC/MS: Method A, RT 0.85 min, 96% purity. $^1$H NMR (300 MHz, CDCl3) δ 7.95 – 7.51 (m, 3H), 7.39 (m, 1H), 7.34 – 7.07 (m, 5H), 7.07 – 6.91 (m, 1H), 6.91 – 6.60 (m, 2H), 4.65 (s, 1H), 4.33 (s, 1H), 3.93 (m, 4H), 3.56 (m, 2H), 3.10 (m, 2H), 2.96 – 2.55 (m, 5H), 2.47 – 2.27 (m, 1H), 2.04 (m, 1H), 1.64 (m, 3H), 1.47 (m, 4H), 1.38 – 1.22 (m, 2H), 1.22 – 1.09 (m, 3H), 1.09 – 0.90 (m, 2H), 0.79 (m, 2H). HRMS (ESI) calcd for $C_{35}H_{46}FN_4O_7S$ $[M + H]^+$: 685.3071. Found: 685.3065.

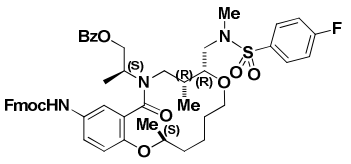


**3-[(2*R*,8*R*,9*R*)-8-({[(4-Fluorophenyl)methyl](methyl)amino}methyl)-11-[(2*S*)-1-hydroxypropan-2-yl]-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (16)**

A mixture of crude amine (deprotected **E**, 0.0484 g, 0.077 mmol) and magnesium sulfate (7.37 mg, 0.077 mmol) in dry DCM (0.77 mL) was stirred under $N_2$ and to this mixture was added 4-fluorobenzaldehyde (0.024 mL, 0.230 mmol). The suspension was stirred for 1 h followed by the addition of sodium triacetoxyborohydride (0.101 g, 0.537 mmol). The resulting mixture was stirred at room temperature for 4 h. The reaction was diluted with DCM (10 mL) and water (10 mL). The aqueous phase was separated and washed with DCM (10 mL). Then the combined organic layers were washed with water (10 mL), separated, dried ($Na_2SO_4$) and evaporated to dryness. Crude product was carried on to the benzoyl deprotection step, following the same procedure as for compound **(1).** 11 mg (23% over 2 steps) of the desired compound were obtained as colorless resin. LC/MS: Method B, RT 6.39 min, 98% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.18 – 7.79 (m, 2H), 7.46 (m, 4H), 7.37 – 7.17 (m, 3H), 7.17 – 6.89 (m, 2H), 6.76 (m, 1H), 4.69 – 4.44 (m, 1H), 4.24 (m, 1H), 3.84 (m, 2H), 3.52 (m, 2H), 3.20 – 3.00 (m, 1H), 2.77 (m, 1H), 2.64 (m, 6H), 2.17 (m, 2H), 2.03 – 1.87 (m, 1H), 1.61 (m, 4H), 1.40 (m, 4H), 1.31 – 1.16 (m, 4H), 1.08 (m, 2H), 0.98 – 0.84 (m, 2H), 0.80 – 0.62 (m, 2H). HRMS (ESI) calcd for $C_{36}H_{48}FN_4O_5$ $[M + H]^+$: 635.3609. Found: 635.3616.

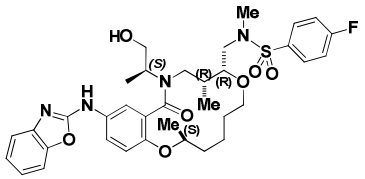
**Scheme 2. An alternate synthesis to provide compounds 11 and 12**






**(*S*)-2-((2*S*,8*R*,9*R*)-14-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-8-((4-fluoro-N-methylphenylsulfonamido)methyl)-2,9-dimethyl-12-oxo-3,4,5,6,9,10-hexahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-11(2H,8H,12H)-yl)propyl benzoate (H)**

A mixture of Alloc-protected amine **B** (0.268 g, 0.328 mmol), 1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (0.384 g, 2.46 mmol) and $Pd(PPh_3)_4$ (76 mg, 0.066 mmol) in dry $CH_2Cl_2$ (4.7 mL) was stirred under $N_2$ atmosphere at room temperature for 1 h. The reaction mixture was diluted with $CH_2Cl_2$, washed with Sat. $NaHCO_3$ and water, dried ($Na_2SO_4$) and evaporated to dryness. The crude product was then re-dissolved in dry $CH_2Cl_2$ (3.3 mL), then 2,6-lutidine (0.27 mL, 2.3 mmol) was added, followed by 4-fluorobenzene-1-sulfonyl chloride (0.127 g, 0.654 mmol) under $N_2$ atmosphere. The resulting mixture was stirred at room temperature for 15 h. The reaction was diluted with $CH_2Cl_2$ and washed with water, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using ethyl acetate / hexanes to give 0.20 g (70%) of the title product as colorless resin. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.08-7.95 (m, 2H), 7.86 – 7.67 (m, 4H), 7.65-7.49 (m, 3H), 7.48-7.37 (m, 4H), 7.36 – 7.23 (m, 3H), 7.22-7.09 (m, 3H), 6.88 – 6.74 (m, 1H), 6.68 (m, 1H), 4.89 – 4.58 (m, 1H), 4.57 – 4.40 (m, 3H), 4.30-4.17 (m, 1H), 4.06 (m, 1H), 3.99-3.80 (m, 2H), 3.78-3.40 (m, 2H), 3.20-2.87(m, 2H), 2.86-2.68 (m, 3H), 2.28-2.15 (m, 1H), 2.07-1.89 (m, 1H), 1.86-1.52 (m, 4H), 1.52-1.29 (m, 6H), 1.23-1.08 (m, 3H), 1.01-0.70 (m, 3H). HRMS (ESI) calcd for $C_{50}H_{55}FN_3O_9S$ $[M + H]^+$: 892.3643. Found: 892.3648.



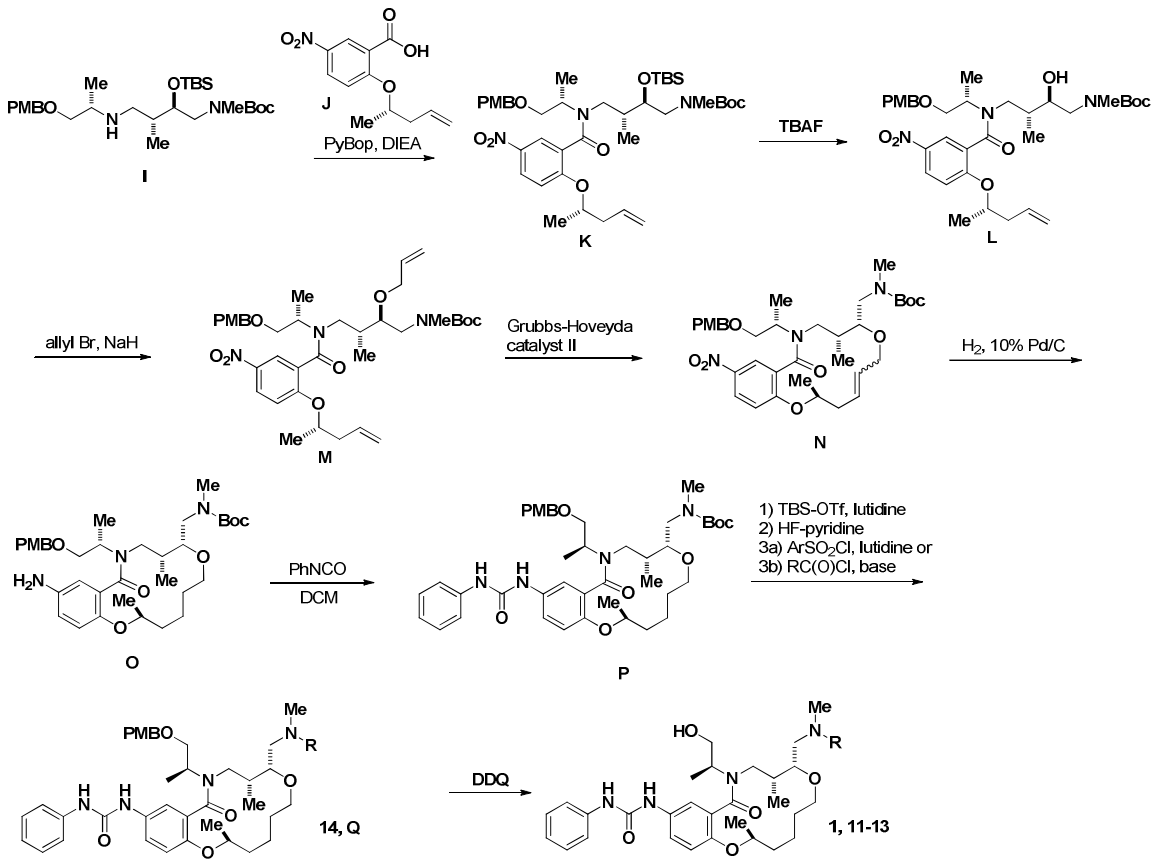

**N-{[(2*S*,8*R*,9*R*)-14-[(1,3-Benzoxazol-2-yl)amino]-11-[(2*S*)-1-hydroxypropan-2-yl]-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-8-yl]methyl}-4-fluoro-N-methylbenzene-1-sulfonamide (12)**

To a solution of Fmoc protected compound **H** (181 mg, 0.203 mmol) in DMF (2.0 mL) was added piperidine (40 μL, 0.406 mmol). The reaction mixture was stirred 1 h, then diluted with aqueous sat. $NH_4Cl$ and DCM. The phases were separated, the aqueous was washed with DCM, and the combined organics were washed with $H_2O$, dried ($Na_2SO_4$) and the solvent evaporated. The crude material was carried on to the next step without further purification. The aniline (28.6 mg, 0.086 mmol) was dissolved in anhydrous DMF (0.43 mL) and to this solution under Ar was added triethylamine (24 μL, 0.170 mmol) followed by 2-cholrobenzo[d]oxazole (20 μL, 0.170 mmol). The reaction mixture was stirred at 40 $^o$C for 72 h. The reaction mixture was cooled, diluted with DCM and

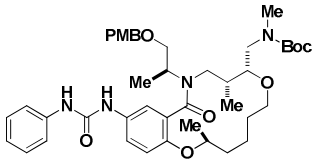
washed with $H_2O$. The organic phase was dried ($Na_2SO_4$) and the solvent evaporated. The residue was chromatographed on silica using MeOH / DCM and the resultant impure product was carried on to the next step without further purification.

To a solution of this crude benzoyl protected alcohol (53 mg, 0.067 mmol) in MeOH (0.67 mL) was added potassium carbonate (51 mg, 0.37 mmol). The reaction mixture was stirred for 24 h, then quenched with aqueous sat. $NH_4Cl$ and diluted with DCM. The aqueous phase was washed with DCM, and the combined organics were washed with $H_2O$ 2X, dried ($Na_2SO_4$) and the solvent evaporated. The residue was chromatographed on silica using MeOH / DCM to yield 7.1 mg (16% yield) of the title product as a colorless resin. LC/MS: Method B, RT 9.96 min, 99% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.93 – 7.79 (m, 2H), 7.78-7.66 (m, 1H), 7.50-7.42 (m, 1H), 7.35–7.28 (m, 3H), 7.23-7.08 (m, 4H), 7.04 – 6.86 (m, 1H), 4.76 – 4.46 (m, 1H), 4.13 – 3.70 (m, 3H), 3.68-3.46 (m, 3H), 3.18-2.94(m, 2H), 2.92-2.76 (m, 3H), 2.71-2.62 (m, 1H), 2.28-2.15 (m, 1H), 1.92-1.73 (m, 1H), 1.68-1.47 (m, 3H), 1.47-1.36 (m, 6H), 1.34-1.20 (m, 3H), 1.01-0.77 (m, 3H). HRMS (ESI) calcd for $C_{35}H_{44}FN_4O_7S$ $[M + H]^+$: 683.2915. Found: 683.2919.

**Scheme 3. Alternate synthesis of compound 1 and synthesis of compounds 15, 17-20, 28-31 from an early stage intermediate.**

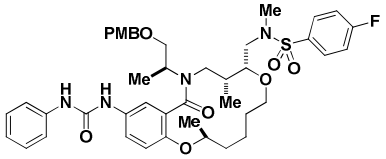


Intermediates **I-O** were synthesized using the procedures previously reported.[2]




***tert*-Butyl (((2*S*,8*R*,9*R*)-11-((*S*)-1-((4-methoxybenzyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (P)**

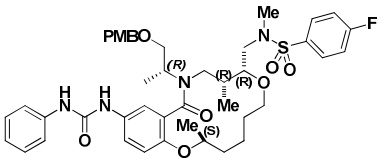
To a solution of aniline **O** (570 mg, 0.908 mmol) in DCM (9.08 ml) under $N_2$ was added phenyl isocyanate (0.198 ml, 1.816 mmol). The reaction was stirred for 16 h. The crude reaction mixture was evaporated under reduced pressure and purified by column chromatography using Ethyl acetate/hexane to afford the desired product (568 mg, 84%) $^1$HNMR (300 MHz, $CDCl_3$) δ 8.32–7.74 (m, 1H), 7.72–7.56 (m, 1H), 7.54-7.41 (m, 2H), 7.36-7.17 (m, 5H), 7.08–6.64 (m, 2H), 6.79–6.59 (m, 1H), 4.70–4.25 (m, 3H), 4.24–4.03 (m, 2H), 4.03–3.88 (m, 1H), 3.88–3.71 (m, 3H), 3.71–3.56 (m, 2H), 3.54–3.21 (m, 3H), 3.21–2.66 (m, 5H), 1.94–1.72 (m, 1H), 1.72–1.55 (m, 3H), 1.55–1.31 (m, 12H), 1.33–0.99 (m, 8H), 0.96–0.86 (m, 1H), 0.83–0.65 (m, 2H). HRMS (ESI) calcd for $C_{42}H_{59}N_4O_8$ $[M + H]^+$: 747.4333. Found: 747.4335.




**4-Fluoro-N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-((4-methoxybenzyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-N-methylbenzenesulfonamide (20)**

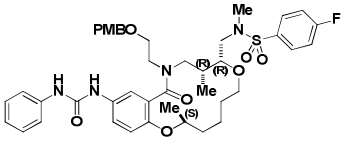
A solution of boc-protected amine **P** (2.30 g, 3.08 mmol) in dry DCM (62 mL) under $N_2$ was chilled in a ice-$H_2O$ bath and to this was added 2,6-dimethylpyridine (1.44 mL, 12.32 mmol) and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.82 mL, 7.70 mmol) dropwise. The reaction was stirred at 0 ºC for 30 min, and was then stirred at room temperature for a further 3 h. To the solution was added 2,6-lutidine (1.4 mL) and the reaction was quenched with sat. $NH_4Cl$. The layers were separated, and the aqueous layer was extracted with DCM. The combined organic extracts were washed with sat. $NH_4Cl$ and brine, dried ($MgSO_4$), filtered and the solvent evaporated. The residue was dissolved in THF (30 mL), transferred to a teflon bottle, then a solution of 70% pyridine hydrofluoride in pyridine (0.382 mL, 3.08 mmol) was added. The reaction was stirred for 30 min. and to this was added sat. $NaHCO_3$, the organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the crude product which was carried onto the next step. To a solution of the residue (1.992 g, 3.08 mmol) in DCM (30.8 ml) was added 2,6-dimethylpyridine (0.717 ml, 6.16 mmol) was added 4-fluorobenzene-1-sulfonyl

chloride (0.899 g, 4.62 mmol). The reaction was stirred at room temperature under $N_2$ overnight. To the solution was added $H_2O$, the layers were separated and aqueous layer extracted with DCM, washed with $H_2O$, dried ($Na_2SO_4$) and evaporated under reduced pressure. The reaction was purified by column chromatography using Ethyl acetate/hexane to afford the desired product (2.14 g, 86%). LC/MS: Method B, RT 6.39 min, 98% purity. LC/MS: Method C, RT 3.65 min, 100% purity. $^1$HNMR (300 MHz, $CDCl_3$) δ 8.74–7.86 (m, 1H), 7.81–7.42 (m, 5H), 7.37-6.98 (m, 7H), 6.98-6.74 (m, 1H), 6.77–6.54 (m, 2H), 5.57–5.38 (m, 1H), 4.66–4.37 (m, 1H), 4.35–3.98 (m, 1H), 3.96–3.19 (m, 7H), 3.19–2.87 (m, 2H), 2.87–2.53 (m, 6H), 2.14–1.82 (m, 2H), 1.82–1.30 (m, 8H), 1.29–0.84 (m, 7H), 0.84–0.47 (m, 2H). HRMS (ESI) calcd for $C_{43}H_{53}FN_4O_8S$ $[M + H]^+$: 805.3646. Found: 805.3652.



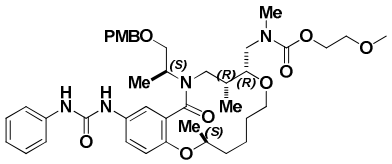

**3-[(2*S*,8*R*,9*R*)-11-[(2*R*)-1-[(4-Methoxyphenyl)methoxy]propan-2-yl]-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (29)**

This derivative was synthesized in 55% yield from the corresponding diastereomer of intermediate **O** following the methods described for compound **20**. LC/MS: Method B, RT 11.02 min, 96% purity. $^1$HNMR (300 MHz, $CDCl_3$) δ 8.72–7.86 (m, 1H), 7.80–7.42 (m, 5H), 7.38-6.98 (m, 7H), 6.94-6.74 (m, 1H), 6.77–6.54 (m, 2H), 5.57–5.30 (m, 1H), 4.63–4.37 (m, 1H), 4.32–3.97 (m, 1H), 3.94–3.20 (m, 7H), 3.19–2.87 (m, 2H), 2.87–2.53 (m, 6H), 2.14–1.82 (m, 2H), 1.79–1.33 (m, 8H), 1.29–0.84 (m, 7H), 0.86–0.49 (m, 2H). HRMS (ESI) calcd for $C_{43}H_{53}FN_4O_8S$ $[M + H]^+$: 805.3646. Found: 805.3656.




**3-[(2*S*,8*R*,9*R*)-11-{2-[(4-Methoxyphenyl)methoxy]ethyl}-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (31)**

This derivative was synthesized in 60% yield from the corresponding des-methyl analog of intermediate **O** following the methods described for compound **20**. LC/MS: Method B, RT 10.77 min, 96% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.05 – 7.59 (m, 2H), 7.49 – 7.30 (m, 2H), 7.30 – 7.08 (m, 4H), 7.08 – 6.92 (m, 1H), 6.92 – 6.77 (m, 1H), 6.76 – 6.47 (m, 1H), 4.67 – 4.35 (m, 1H), 4.34-4.16 (m, 1H), 4.12-4.06 (m, 1H), 4.00-3.91 (m, 1H), 3.88-3.70 (m, 3H), 3.69-3.28 (m, 3H), 3.28 – 2.96 (m, 2H), 2.94 – 2.60 (m, 3H), 2.35-2.06 (m, 1H), 1.93-1.66 (m, 1H), 1.67 – 1.36 (m, 3H), 1.35-1.14 (m, 3H), 1.13 – 0.68 (m, 5H). HRMS (ESI) calcd for $C_{42}H_{52}FN_4O_8S$ $[M + H]^+$: 791.3490. Found: 7.488.

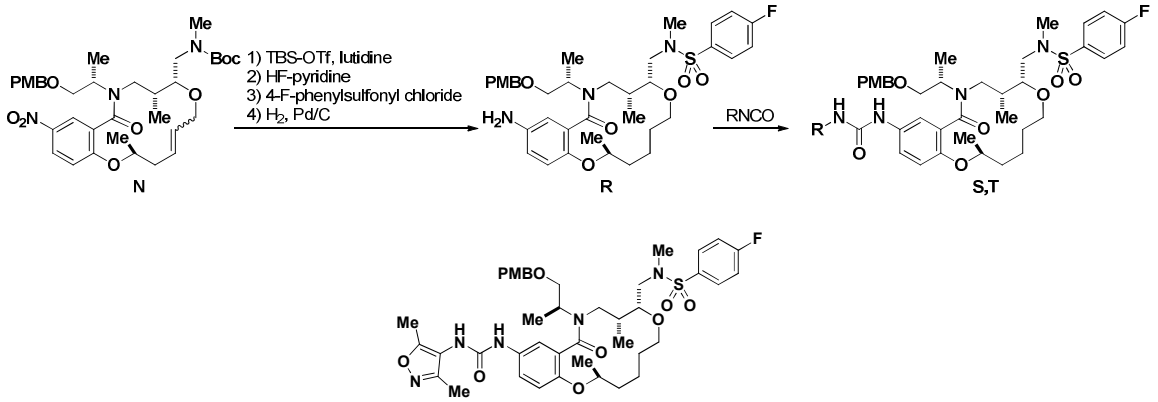


**2-Methoxyethyl (((2*S*,8*R*,9*R*)-11-((*S*)-1-((4-methoxybenzyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (Q)**

To a solution of crude amine (0.22 g, 0.340 mmol) in dioxane (8.5 mL) under $N_2$ was added a 10% Sodium Bicarbonate (1.648 mL, 2.041 mmol) aqueous solution, followed by 2-methoxyethyl chloroformate (0.079 mL, 0.680 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction was diluted with EtOAc and water. The aqueous phase was separated and washed with EtOAc, then the combined organic layers were washed with water and brine, dried over $Na_2SO_4$ and evaporated to dryness. This material was chromatographed on silica, using methanol / dichloromethane to give 0.15 g (59%) of the final product as colorless resin.

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.76 – 7.57 (m, 2H), 7.49 – 7.29 (m, 2H), 7.23 – 7.17 (m, 6H), 7.02 – 6.81 (m, 3H), 6.80 – 6.65 (m, 4H), 4.64-4.37 (m, 2H), 4.37 – 4.09 (m, 3H), 4.06-3.86 (m, 2H), 3.81-3.63 (m, 3H), 3.62-3.41 (m, 5H), 3.43 – 3.19 (m, 4H), 3.12-2.87 (m, 3H), 1.89-1.70 (m, 1H), 1.49-1.36 (m, 2H), 1.36-1.14 (m, 7H), 1.13 – 0.96 (m, 3H), 0.95-0.65 (m, 3H). MS (ESI) calcd for $C_{41}H_{57}N_4O_9$ : 749 $[M+H]^+$. Found 749.

**Scheme 4. Alternate synthesis of compounds 5 and 6 from intermediate N**




**N-(((2*S*,8*R*,9*R*)-14-(3-(3,5-Dimethylisoxazol-4-yl)ureido)-11-((*S*)-1-((4-methoxybenzyl)oxy)propan-2-yl)-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-4-fluoro-N-methylbenzenesulfonamide (S)**

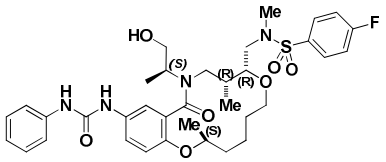
To a solution of the mixture of diastereomers of RCM product **N** (1.10 g, 1.68 mmol) in DCM (17 mL) under $N_2$ chilled to 0 $^oC$ was added 2,6-lutidine (0.78 mL, 6.7 mmol) followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (0.99 mL, 4.2 mmol). The solution was stirred at 0 $^oC$ for 30 min, then the bath was removed and the solution was stirred an additional 3 h at room temperature. An additional 0.78 mL of 2,6-lutidine was

then added to the reaction vessel, and the solution was quenched with sat. aqueous $NaHCO_3$. The aqueous phase was washed with DCM and the organic phases were combined and washed with $H_2O$ 2X. The organic phase was dried over $Na_2SO_4$ and the solvent evaporated. The residue was dissolved in THF (17 mL) and HF-pyridine (70% solution in pyridine, 0.21 mL, 1.68 mmol) was added. The solution was stirred 45 min, then quenched with sat. aqueous $NaHCO_3$ and extracted into EtOAc. The organic phase was washed with $H_2O$, brine, dried ($Na_2SO_4$) and the solvent evaporated. The residue was dissolved in DCM (17 mL) and to this solution under $N_2$ was added 2,6-lutidine (0.12 mL, 1.1 mmol) followed by 4-fluorobenzenesulfonyl chloride (156 mg, 0.801 mmol). The solution was stirred 16 h, then diluted with DCM and $H_2O$. The phases were separated, the aqueous extracted with DCM and the combined organics were washed with $H_2O$ 2X, dried ($Na_2SO_4$) and the solvent evaporated. The residue was chromatographed on $SiO_2$ (EtOAc/hexanes) to remove 2,6-lutidine and excess sulfonyl chloride, and the crude mixture of diastereomers was carried on to the next step without further purification.

To a solution of this PMB ether (1.064 g, 1.49 mmol) in EtOH ( 149 mL) was added 10% Pd on carbon (159 mg) and the suspension was stirred under $H_2$ at 1 atm for 24 h. The suspension was then filtered through celite, the solvent evaporated, and the residue was used without further purification for the next step of urea formation.

To a solution of the above aniline **R** (458 mg, 0.668 mmol) in DCM (6.7 mL) was added 4-isocyanato-3,5-dimethylisoxazole (0.15 mL, 1.34 mmol). The solution was stirred for 2 h at room temperature, then the solvent was evaporated and the residue chromatographed on $SiO_2$ to yield 0.40 g (73%) of colorless resin.

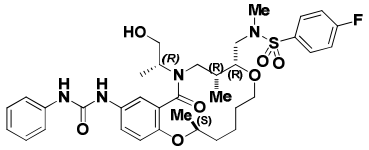
1H NMR: $^1$H NMR (300 MHz, $CDCl_3$) δ 8.01 – 7.65 (m, 2H), 7.59-7.46 (m, 1H), 7.39 – 7.09 (m, 4H), 7.06-6.95 (m, 1H), 6.92-6.79 (m, 3H), 6.79-6.70 (m, 1H), 6.48-6.10 (m, 1H), 4.72 – 4.49 (m, 1H), 4.49 – 4.27 (m, 2H), 4.19 – 3.82 (m, 2H), 3.81-3.73 (m, 3H), 3.72-3.59 (m, 2H), 3.57-3.38 (m, 2H), 3.35-3.17 (m, 1H), 3.16-2.97 (m, 2H), 2.91 – 2.62 (m, 4H), 2.33 – 2.22 (m, 3H), 2.21 – 1.95 (m, 4H), 1.84-1.65 (m, 2H), 1.65 – 1.37 (m, 4H), 1.36 – 1.12 (m, 6H), 1.07-1.00 (m, 2H), 0.97-0.81 (m, 2H), 0.78-0.62 (m, 2H). HRMS (ESI) calcd for $C_{42}H_{55}FN_5O_9S$ $[M + H]^+$: 824.3705. Found: 824.3704.




**4-Fluoro-N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-N-methylbenzenesulfonamide (1)**

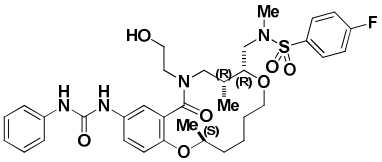
To a solution of PMB-protected alcohol **14** (1.99 g, 2.472 mmol) in DCM (25 mL) was added 2.5 mL pH 7 buffer. The reaction mixture was cooled to 0 ºC in an ice-$H_2O$ bath. To this mixture was added 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile (0.730 g, 3.21 mmol) and the mixture was stirred under $N_2$ for 16 h, slowly warming to room temperature. The reaction was quenched with sat. $NaHCO_3$, and stirred for 1 h. The

phases were separated and the aqueous phase was washed with DCM 2X. The organic phases were combined and washed with saturated $NaHCO_3$ and $H_2O$. The organic components were dried ($Na_2SO_4$) and evaporated under reduced pressure to afford crude material. The reaction was purified by column chromatography using Ethylacetate/hexane to afford the desired product **1** (1.41 g, 83%). LC/MS: Method B, RT 9.49 min, 100% purity.



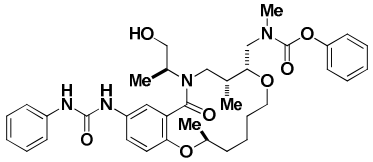
**3-[(2*S*,8*R*,9*R*)-11-[(2*R*)-1-Hydroxypropan-2-yl]-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (28)**

The –PMB deprotection of compound **29** was achieved in 9% yield following the method used for the synthesis of compound **1**. LC/MS: Method B, RT 9.56 min, 99% purity.
$^1$H NMR (300 MHz, CDCl3) δ 7.94 – 7.51 (m, 3H), 7.38-7.32 (m, 1H), 7.34 – 7.07 (m, 5H), 7.07 – 6.91 (m, 1H), 6.91 – 6.60 (m, 2H), 4.62 (s, 1H), 4.32 (s, 1H), 3.94 (m, 4H), 3.57 (m, 2H), 3.10 (m, 2H), 2.96 – 2.55 (m, 5H), 2.47 – 2.27 (m, 1H), 2.04 (m, 1H), 1.64 (m, 3H), 1.47 (m, 4H), 1.38 – 1.22 (m, 2H), 1.22 – 1.09 (m, 3H), 1.07 – 0.89 (m, 2H), 0.79 (m, 2H). HRMS (ESI) calcd for $C_{35}H_{46}FN_4O_7S$ $[M + H]^+$: 685.3071. Found: 685.3077.



**3-[(2*S*,8*R*,9*R*)-11-(2-Hydroxyethyl)-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (30)**

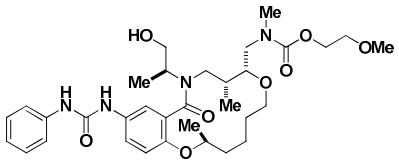
The –PMB deprotection of compound **31** was achieved in 71% yield following the method used for the synthesis of compound **1**. LC/MS: Method B, RT 9.26 min, 97% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.59 (m, 3H), 7.51 – 7.29 (m, 2H), 7.29 – 7.10 (m, 4H), 7.10 – 6.92 (m, 1H), 6.92 – 6.78 (m, 2H), 6.78 –6.49 (m, 2H), 4.64-4.37 (m, 2H), 4.37 – 4.17 (m, 1H), 4.05-3.94 (m, 1H), 3.90 – 3.69 (m, 4H), 3.70-3.58 (m, 1H), 3.56 – 3.26 (m, 2H), 3.26-2.97 (m, 1H), 2.97 – 2.66 (m, 4H), 2.40-2.11 (m, 1H), 1.91-1.70 (m, 2H), 1.65-1.40 (m, 2H), 1.38-1.16 (m, 2H), 1.15 – 0.68 (m, 6H).
HRMS (ESI) calcd for $C_{34}H_{44}FN_4O_7S$ $[M + H]^+$: 671.2915. Found: 671.2910.



**Phenyl (((2*S*,8*R*,9*R*)-11-((*S*)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-**

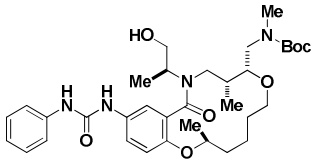
**decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (18)**

Compound **18** was prepared from intermediate **P** in 38% overall yield following the methods used to prepare intermediate **Q** and compound **1**. LC/MS: Method A, RT 0.85 min, 99% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.84 – 7.51 (m, 2H), 7.42 – 7.28 (m, 4H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.14 (dd, *J* = 17.5, 9.1 Hz, 2H), 7.06 (dd, *J* = 14.1, 7.1 Hz, 2H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.93 – 6.80 (m, 1H), 6.69 (dd, *J* = 23.1, 7.8 Hz, 1H), 4.75 – 4.27 (m, 2H), 4.16 – 3.63 (m, 6H), 3.62-3.29 (m, 3H), 3.26 – 2.96 (m, 5H), 1.89 (br s, 1H), 1.85-1.62 (m, 2H), 1.58-1.34 (m, 5H), 1.33-1.09 (m, 3H), 1.09-0.91 (m, 2H), 0.81 (d, *J* = 3.5 Hz, 3H). HRMS (ESI) calcd for $C_{36}H_{47}N_4O_7$ $[M + H]^+$: 647.3445. Found: 647.3447.



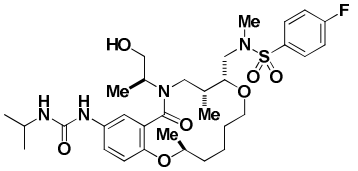

**2-Methoxyethyl (((2S,8R,9R)-11-((S)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (19)**

Compound **19** was prepared from intermediate **Q** in 94% yield following the method used to prepare compound **1**. LC/MS: Method A, RT 0.91 min, 100% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.29 – 7.52 (m, 3H), 7.47 – 7.27 (m, 2H), 7.27 – 7.06 (m, 2H), 6.95 (t, *J* = 7.0 Hz, 1H), 6.88-6.79 (m, 1H), 6.75-6.57 (m, 1H), 4.71-4.28 (m, 2H), 4.18 (s, 2H), 4.05-3.67 (m, 4H), 3.64-3.43 (m, 4H), 3.41 – 3.27 (m, 3H), 3.28-2.98 (m, 3H), 2.96-2.51 (m, 3H), 2.30 (br s, 1H), 1.98 – 1.55 (m, 2H), 1.46 (s, 3H), 1.38-1.19 (m, 2H), 1.13 (d, *J* = 6.0 Hz, 3H), 1.05-0.88 (m, 2H), 0.89 – 0.56 (m, 3H). HRMS (ESI) calcd for $C_{33}H_{49}N_4O_8$ $[M + H]^+$: 629.3550. Found: 629.3558.



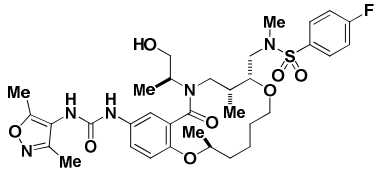

***tert*-Butyl (((2*S*,8*R*,9*R*)-11-((*S*)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)(methyl)carbamate (17)**

Compound **17** was prepared from intermediate **P** in 67% yield following the method used to prepare compound **1**. LC/MS: Method B, RT 9.32 min, 100% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.83-7.55 (m, 3H), 7.45-7.30 (m, 2H), 7.29 – 7.14 (m, 2H), 7.06-6.92 (m, 1H), 6.85 – 6.58 (m, 2H), 4.72-4.33 (m, 2H), 4.02-3.86 (m, 3H), 3.73 (br s, 2H), 3.58 (br s, 2H), 3.51-3.26 (m, 2H), 3.25-2.78 (m, 9H), 2.69-2.33 (m, 1H), 1.94-1.62 (m, 2H), 1.56 – 1.32 (m, 13H), 1.20 – 1.08 (m, 3H), 1.07-0.91 (m, 3H), 0.79 (d, *J* = 6.1 Hz, 3H). HRMS (ESI) calcd for $C_{34}H_{50}N_4O_7Na$ $[M + Na]^+$: 649.3577. Found: 649.3580.



**4-Fluoro-N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-hydroxypropan-2-yl)-14-(3-isopropylureido)-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-N-methylbenzenesulfonamide (6)**

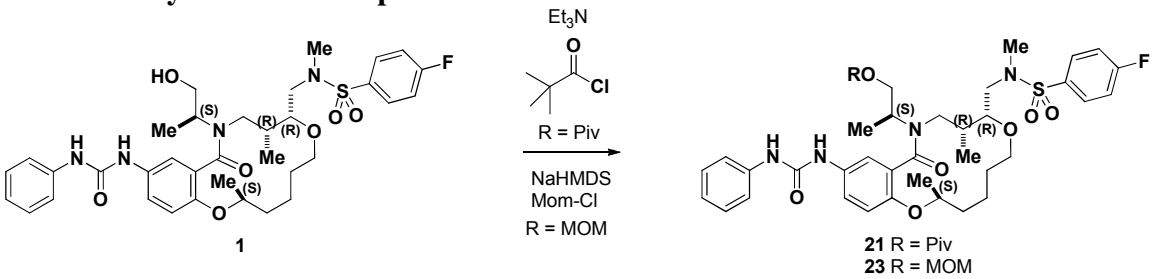
Compound **6** was prepared from intermediate **N** in 40% yield following the methods used to prepare intermediate **S** and compound **1**. LC/MS: Method B, RT 8.76 min, 94% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.89-7.69 (m, 2H), 7.63 – 7.26 (m, 1H), 7.24 – 7.07 (m, 3H), 6.99-6.67 (m, 2H), 6.34 (s, 1H), 5.10 – 4.42 (m, 2H), 4.11 – 3.79 (m, 2H), 3.63-3.43 (m, 1H), 3.33 – 2.93 (m, 1H), 2.93 – 2.74 (m, 4H), 2.74 – 2.59 (m, 1H), 2.44-1.93 (m, 1H), 1.85-1.71 (m, 1H), 1.67-1.51 (m, 2H), 1.50 – 1.34 (m, 3H), 1.33-1.19 (m, 2H), 1.12 (t, *J* = 7.1 Hz, 6H), 1.07-0.92 (m, 4H), 0.78 (d, *J* = 6.6 Hz, 3H). HRMS (ESI) calcd for $C_{32}H_{47}FN_4O_7S$ $[M + H]^+$: 651.3228. Found: 651.3223.

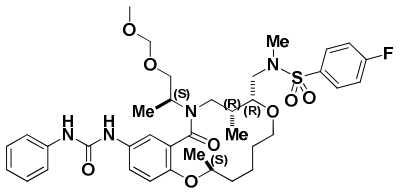


**N-(((2*S*,8*R*,9*R*)-14-(3-(3,5-Dimethylisoxazol-4-yl)ureido)-11-((*S*)-1-hydroxypropan-2-yl)-2,9-dimethyl-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-4-fluoro-N-methylbenzenesulfonamide (7)**

Compound 6 was prepared from the intermediate **S** in 80% yield following the method for compound **7**. LC/MS: Method C, RT 3.25min, 98% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.11 – 7.62 (m, 3H), 7.41 – 7.27 (m, 1H), 7.24-7.06 (m, 3H), 7.03 – 6.88 (m, 1H), 6.87-6.70 (m, 1H), 6.63 (s, 1H), 4.75 – 4.35 (m, 2H), 4.09 – 3.65 (m, 5H), 3.64-3.27 (m, 3H), 3.24 – 2.96 (m, 3H), 2.96 – 2.62 (m, 6H), 2.52 – 1.98 (m, 7H), 1.86-1.68 (m, 2H), 1.67-1.47 (m, 2H), 1.37 (d, *J* = 4.2 Hz, 2H), 1.30 – 1.09 (m, 3H), 1.09 – 0.89 (m, 3H), 0.82 (d, *J* = 6.4 Hz, 3H). HRMS (ESI) calcd for $C_{34}H_{47}FN_5O_8S$ $[M + H]^+$: 704.3129. Found: 704.3128.

**Scheme 5: Synthesis of compounds 21 and 23.**

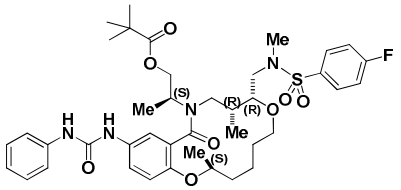




**3-[(2*S*,8*R*,9*R*)-11-[(2*S*)-1-(Methoxymethoxy)propan-2-yl]-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (23)**

To a chilled (0 $^{o}$C) solution of alcohol **1** (33.8 mg, 0.049 mmol) and chloro(methoxy)methane (0.072 mL, 0.987 mmol) in dry THF (0.494 mL) under $N_2$ was added NaHMDS (0.054 ml, 0.054 mmol). The solution was stirred for 16 h, letting slowly warm to room temperature. The reaction mixture was quenched with sat. aqueous $NH_4Cl$, and the aqueous phase was washed with EtOAc. The organic phase was washed with $H_2O$, brine, dried ($Na_2SO_4$) and the solvent evaporated. Purification was accomplished with $SiO_2$ chromatography to yield 7.1 mg (20%) of a colorless resin. LC/MS: Method B, RT 10.27 min, 97% purity. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.85-7.69 (m, 3H), 7.64-7.44 (m, 1H), 7.44 – 7.29 (m, 3H), 7.24 – 7.12 (m, 4H), 7.12 – 7.00 (m, 1H), 7.00 – 6.82 (m, 1H), 6.82-6.67 (m, 1H), 4.72-4.53 (m, 3H), 4.17 – 3.74 (m, 3H), 3.74 – 3.46 (m, 3H), 3.46 – 3.30 (m, 3H), 3.28 – 2.94 (m, 2H), 2.94 – 2.70 (m, 5H), 2.27-1.94 (m, 1H), 1.70 – 1.42 (m, 3H), 1.38-1.14 (m, 3H), 1.16 – 1.02 (m, 3H), 0.98 (d, *J* = 7.1 Hz, 3H), 0.82 (d, *J* = 6.3 Hz, 3H).

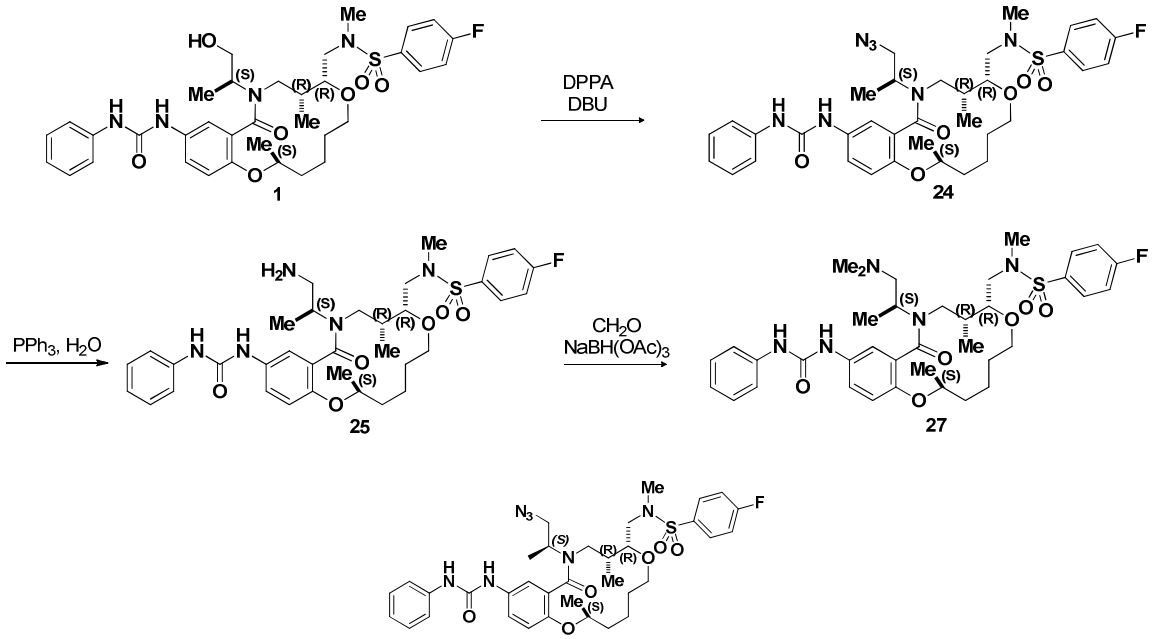
HRMS (ESI) calcd for $C_{37}H_{50}FN_4O_8S$ $[M + H]^+$: 729.3333. Found: 729.3337.



**(2*S*)-2-[(2*S*,8*R*,9*R*)-2,9-Dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-12-oxo-14-[(phenylcarbamoyl)amino]-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-11-yl]propyl 2,2-dimethylpropanoate (21)**

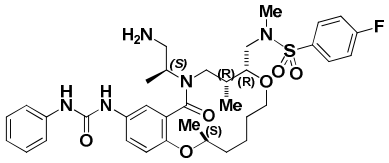
To a solution of alcohol **1** (0.041 g, 0.060 mmol) in dry DCM (0.60 mL) was added pyridine (0.024 mL, 0.299 mmol) followed by pivaloyl chloride (17.84 µL, 0.148 mmol) under $N_2$ atmosphere. The resulting mixture was stirred at room temeperature for 15 h. The reaction was diluted with DCM (10 mL) and washed with water (10 mL) and brine. The organic phase was then separated, dried ($Na_2SO_4$) and evaporated to dryness. This material was chromatographed on silica, using methanol / dichloromethane to afford 30 mg (15%) of the desired product. LC/MS: Method B, RT 10.96 min, 95% purity. $^1$H NMR (300 MHz, CDCl3) δ 7.79 (m, 2H), 7.65 – 7.45 (m, 1H), 7.37 (m, 2H), 7.29 – 7.03 (m, 4H), 6.98 (s, 1H), 6.75 (m, 2H), 4.56 (m, 2H), 4.46 – 4.31 (m, 1H), 4.26 – 4.03 (m, 2H), 3.93 (s, 3H), 3.78 – 3.62 (m, 1H), 3.14 (m, 2H), 2.88 (m, 3H), 2.75 (m, 3H), 1.75 (m, 2H), 1.53 (m, 6H), 1.39 – 1.11 (m, 4H), 1.04 (m, 5H), 0.92 – 0.60 (m, 7H). HRMS (ESI) calcd for $C_{40}H_{54}FN_4O_8S$ $[M+H]^+$: 769.3646. Found: 769.3652.

**Scheme 6: Synthesis of compounds 24, 25 and 27.**



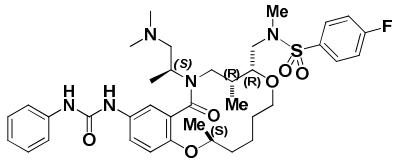

**N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-Azidopropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-4-fluoro-N-methylbenzenesulfonamide (24)**

To a solution of alcohol **1** in dry THF (2.9 mL) under $N_2$ was added DBU (0.27 mL, 1.76 mmol) followed by diphenyl phosphorazidate (0.19 mL, 0.88 mmol). The reaction was stirred at room temperature for 16 h. The reaction solvent was evaporated under reduced pressure to afford crude material which was purified by column chromatography using MeOH/DCM to afford the desired product (360 mg, 87%). LC/MS: Method C, RT 4.48 min, 100% purity. $^1$HNMR (300 MHz, $CDCl_3$) δ 8.24–7.64 (m, 2H), 7.57–7.33 (m, 2H), 7.31-7.05 (m, 4H), 7.03–6.77 (m, 3H), 6.77–6.53 (m, 1H), 4.67–4.27 (m, 3H), 4.23–4.09 (m, 1H), 4.01–3.84 (m, 2H), 3.83–3.61 (m, 4H), 3.61–3.29 (m, 2H), 3.25–3.02 (m, 2H), 3.06–2.55 (m, 4H), 2.27–1.94 (m, 1H), 1.92–1.32 (m, 6H), 1.30–1.14 (m, 3H), 1.14–1.01 (m, 2H), 0.98–0.85 (m, 1H), 0.83–0.62 (m, 1H). HRMS (ESI) calcd for $C_{35}H_{45}FN_7O_6S$ $[M + H]^+$: 7103136. Found: 710.3142.



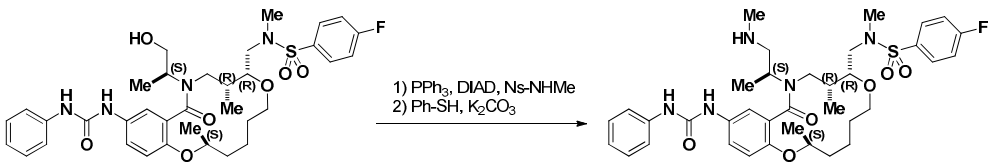
**N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-Aminopropan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-4-fluoro-N-methylbenzenesulfonamide (25)**

To a solution of azide **24** (0.33 g, 0.465 mmol) in THF (14.6 mL) under $N_2$ was added $H_2O$ (0.86 mL) followed by triphenylphosphine (0.305 g, 1.162 mmol). The reaction mixture was stirred for 16 h at room temperature. The reaction was diluted with EtOAc, dried ($Na_2SO_4$) and solvent evaporated to afford a crude material. This material was purified by column chromatography using MeOH/DCM to afford the desired product (45 mg, 76%). $^1$H NMR (300 MHz, CDCl3) δ 8.96–8.20 (m, 2H), 7.92–7.65 (m, 2H), 7.58–7.33 (m, 2H), 7.33–7.05 (m, 4H), 7.08–6.85 (m, 1H), 6.84–6.62 (m, 1H), 4.73–4.32 (m, 1H), 4.16–3.53 (m, 4H), 3.48–3.24 (m, 1H), 3.21–2.92 (m, 4H), 2.91–2.79 (m, 2H), 2.80–2.51 (m, 4H), 2.31–1.89 (m, 2H), 1.89–1.37 (m, 6H), 1.37–1.14 (m, 6H), 1.10–0.89 (m, 2H), 0.67–0.32 (m, 2H). HRMS (ESI) calcd for $C_{35}H_{47}FN_5O_6S$ $[M + H]^+$:684.3231. Found: 684.3228.



**N-(((2*S*,8*R*,9*R*)-11-((*S*)-1-(Dimethylamino)propan-2-yl)-2,9-dimethyl-12-oxo-14-(3-phenylureido)-2,3,4,5,6,8,9,10,11,12-decahydrobenzo[b][1,9,5]dioxaazacyclotetradecin-8-yl)methyl)-4-fluoro-N-methylbenzenesulfonamide (27)**

To a solution of amine **25** (39 mg, 0.058 mmol) in DCM (1.15 mL) was added magnesium sulfate (0.069 g, 0.577 mmol) followed by a solution of formaldehyde 30% in $H_2O$ (0.026 mL, 0.346 mmol). This mixture was stirred for 1 h at room temperature. After this period sodium triacetoxyborohydride (0.147 g, 0.692 mmol) was added to the reaction flask and the mixture stirred overnight. The reaction solvent was evaporated under reduced pressure to afford crude mixture which was purified by column chromatography using MeOH/DCM to afford the desired product (360 mg, 87%). LC/MS: Method B, RT 2.94 min, 100% purity. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.89 – 7.82 (m, 1H), 7.80 – 7.73 (m, 1H), 7.70 – 7.60 (m, 1H), 7.47 – 7.35 (m, 2H), 7.34 – 7.11 (m, 4H), 7.03 – 6.87 (m, 1H), 6.85 – 6.65 (m, 2H), 4.67 – 4.47 (m, 1H), 4.46 – 4.20 (m, 1H), 4.19 – 3.97 (m, 1H), 3.96 – 3.54 (m, 2H), 3.52 – 3.34 (m, 1H), 3.33 – 3.18 (m, 1H), 3.17 – 2.99 (m, 1H), 2.91 (s, 1H), 2.84 – 2.69 (m, 4H), 2.69 – 2.35 (m, 1H), 2.32 (s, 3H), 2.19 – 2.01 (m, 3H), 1.96 – 1.65 (m, 2H), 1.64 – 1.48 (m, 2H), 1.48 – 1.34 (m, 3H), 1.35 – 1.18 (m, 2H), 1.18 – 1.07 (m, 2H), 1.7 – 0.92 (m, 2H), 0.91 – 0.75 (m, 2H). **$^{13}$C NMR** (125 MHz, $CDCl_3$) δ 172.7, 172.4, 166.0, 164.0, 153.3, 147.5, 139.4, 133.3, 130.0, 128.8, 128.6, 126.1, 122.1, 119.8, 119.4, 119.0, 118.8, 116.6, 116.4, 116.2, 113.4, 111.9, 83.2, 74.3, 71.6, 71.2, 69.8, 69.4, 68.4, 63.6, 63.1, 62.7, 53.6, 53.1, 51.9, 51.2, 51.0, 50.7, 50.4, 46.4, 46.0, 45.8, 45.2, 43.4, 43.1, 40.8, 38.4, 37.3, 36.7, 34.6, 34.4, 30.5, 29.3, 28.8, 18.4, 18.3, 18.2, 17.9, 17.2, 16.9, 15.8, 14.7, 13.6, 12.7. HRMS (ESI) calcd for: $C_{37}H_{50}FN_5O_6S$ $[M + H]^+$: 712.3539. Found: 712.3511.

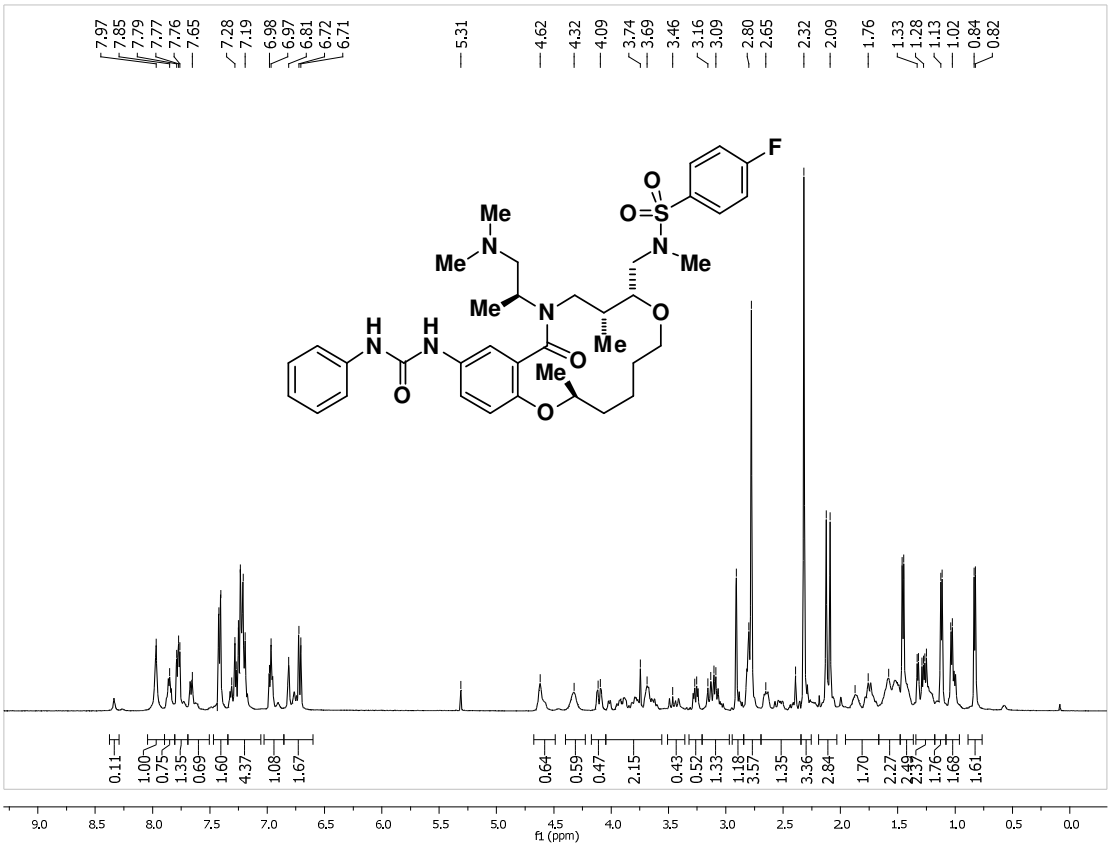


**3-[(2S,8R,9R)-2,9-dimethyl-8-{[N-methyl(4-fluorobenzene)sulfonamido]methyl}-11-[(2S)-1-(methylamino)propan-2-yl]-12-oxo-2,3,4,5,6,8,9,10,11,12-decahydro-1,7,11-benzodioxazacyclotetradecin-14-yl]-1-phenylurea (26)**

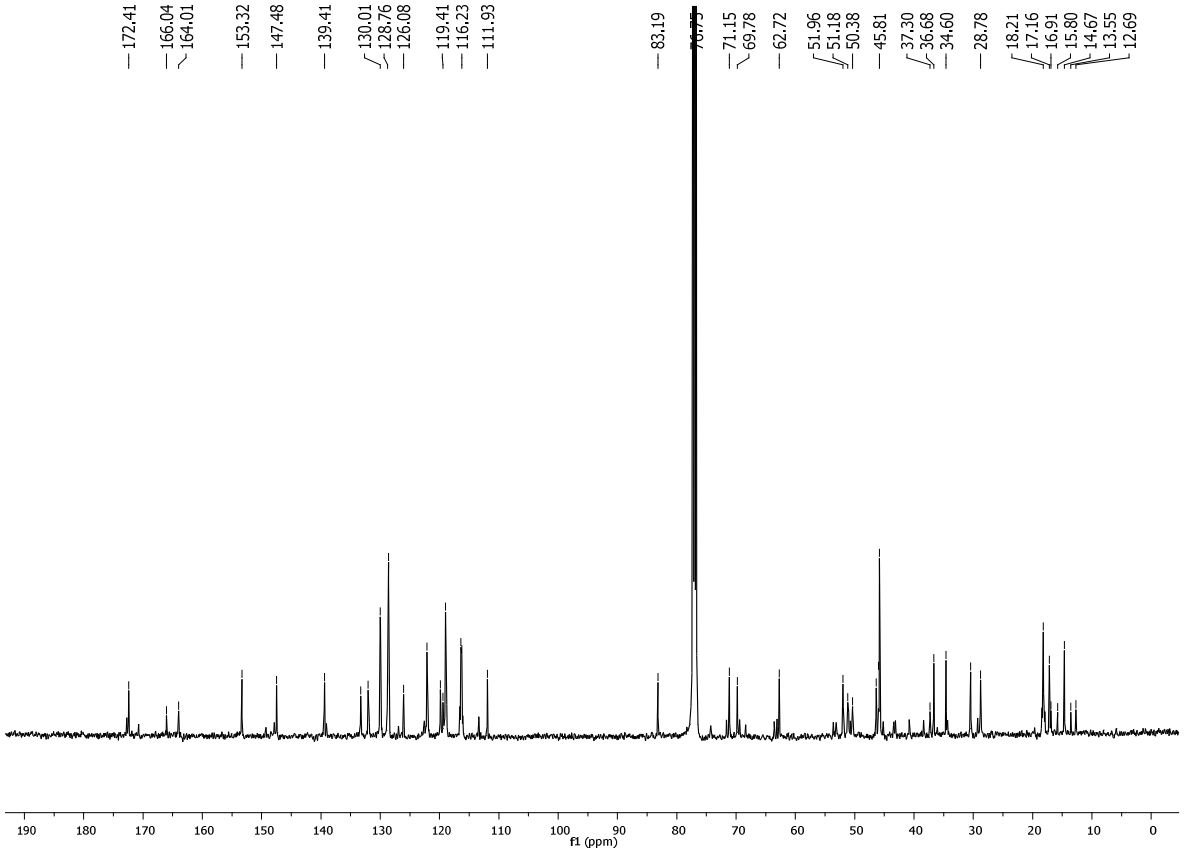
To a solution of alcohol **1** (0.081 g, 0.118 mmol) in dry THF (2.4 ml) was added triphenylphosphine (0.155 g, 0.591 mmol) and N-methyl-2-nitrobenzenesulfonamide (0.051 g, 0.237 mmol) under $N_2$ atmosphere at room temperature. This stirring solution was then cooled to 0°C and DIAD (0.115 ml, 0.591 mmol) was added. The resulting mixture was stirred for 15 h, letting slowly warm to room temperature. The reaction was concentrated in vacuo, then crude product was re-dissolved in dry DMF (0.67 ml) and potassium carbonate (0.030 g, 0.215 mmol) was introduced to the reaction mixture, followed by benzenethiol (0.011 ml, 0.108 mmol). The resulting mixture was stirred under Ar at room temperature for 15 h. This material was then evaporated to dryness and chromatographed on silica , using methanol / dichloromethane to give 2.9 mg (4%) of the desired compound. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, 2H), 7.95 – 7.67 (m, 3H), 7.59 (d, 1H), 7.40 (m, 2H), 7.33 – 7.05 (m, 2H), 6.95 (m, 1H), 6.70 (m, 1H), 4.61 (s, 1H), 4.37 – 3.97 (m, 1H), 3.97 – 3.72 (m, 2H), 3.64 (m, 2H), 3.31 – 3.09 (m, 2H), 2.99 (m, 2H), 2.85 (m, 1H), 2.74 (m, 4H), 2.45 (m, 4H), 1.97 (s, 2H), 1.71 (m, 2H), 1.50 (m, 2H), 1.36 (m, 3H), 1.18 (m, 4H), 0.96 (m, 2H), 0.66 (m, 2H).

## Spectral Data

$^1$H NMR Spectrum ($CDCl_3$, 500 MHz) of Compound **27**



$^{13}C$ NMR Spectrum ($CDCl_3$, 500 MHz) of Compound **27**




LC/MS Chromatogram of Compound **27**