**Supporting Information**

# Identification of Potent Tricyclic S1P$_1$ Prodrug Receptor Modulators

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**Biology Protocols.**

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 2011).

**SIP1 Binding Assay:**

Membranes were prepared from CHO cells expressing human $S1P_1$. Cells pellets (l x $10^9$ cells/pellet) were suspended in buffer containing 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.5, 50 mM NaCl, 2 mM EDTA (Ethylenediaminetetraacetic acid) and Protease Inhibitor cocktail (Roche), and disrupted on ice using the Polytron homogenizer. The homogenate was centrifuged at 20,000 rpm (48,000g) and the supernatant was discarded. The membrane pellets were resuspended in buffer containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 2 mM EDTA and stored in aliquots at -80 °C after protein concentration determination.

Membranes (2 µg/well) and 0.03 nM final concentration of $^{33}$P-S1P ligand (1 mCi/ml, Perkin elmer or American Radiolabeled Chemicals) diluted in assay buffer (50 mM HEPES, pH7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% fatty acid free BSA (bovine serum albumin), 1 mM NaF) were added to the compound plates (384 Falcon v-bottom plate (0.5 µL/well in a 11 point, 3-fold dilution). Binding was performed for 45 minutes at room temperature, terminated by collecting the membranes onto 384-well Millipore FB filter plates, and radioactivity was measured by TOPCOUNT®. The competition data of the test compounds over a range of concentrations was plotted as percentage inhibition of radioligand specific binding.

**Receptor [$^{35}$S] GTPγS Binding Assays: ($S1P_1$ GTPγS / $S1P_3$ GTPγS):**

Compounds were loaded in a 384 Falcon v-bottom plate (0.5 µL/well in a 11 point, 3-fold dilution). Membranes prepared from $S1P_1$/CHO cells or EDG3-Ga15-bla HEK293T cells (EDG3 equivalent $S1P_3$) were added to the compound plate (40 µl/well, final protein 3 µg/well) with MULTIDROP®. [$^{35}$S]GTP (1250 Ci/mmol, Perkin

Elmer) was diluted in assay buffer: 20 mM HEPES, pH7.5, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA (ethylene glycol tetraacetic acid), 1 mM DTT (Dithiothreitol), 10 µM GDP, 0.1% fatty acid free BSA, and 10 µg/ml Saponin to 0.4 nM. 40 µl of the [$^{35}$S] GTP solution was added to the compound plate with a final concentration of 0.2 nM. The reaction was kept at room temperature for 45 min. At the end of incubation, all the mixtures in the compound plate were transferred to Millipore 384-well FB filter plates via the VELOCITY 11® Vprep liquid handler. The filter plate was washed with water 4 times by using the manifold Embla plate washer and dried at 60 °C for 45 min. MicroScint 20 scintillation fluid (30 µL) was added to each well for counting on the Packard TOPCOUNT®. $EC_{50}$ is defined as the agonist concentration that corresponds to 50% of the Ymax (maximal response) obtained for each individual compound tested.

A smaller value for GTPγS S1P1 $EC_{50}$ value indicated greater activity for the compound in the GTPγS $S1P_1$ binding assay. A larger value for the GTPγS $S1P_3$ $EC_{50}$ value indicated less activity in the GTPγS $S1P_3$ binding assay.

**Blood Lymphocyte Reduction (BLR) assay in rodent:**

Lewis rats were dosed orally with vehicle alone (polyethylene glycol 300, "PEG300") or with test compounds. Compounds were dosed as a solution or suspension in the vehicle, adjusted to reflect the free amount of test article in the event that salt forms are utilized. Blood was drawn at different time points and blood lymphocyte counts were determined on an ADVIA 120 Hematology Analyzer (Siemens Healthcare Diagnostics). The results were measured as a reduction in the percentage of circulating lymphocytes as compared to the vehicle treated group at the time of measurement. The results represent the average results of all animals within each treatment group (n = 2).

**Protein levels in the bronchoalveolar lavage (BAL) fluid:**

Post study mice were euthanized with intraperitoneal barbiturate overdose. The animals were placed in a supine position, a skin incision was made and blunt dissection followed to expose the trachea. The trachea was incised and a catheter was inserted 4-6 mm into

the trachea. Phosphate-buffered saline (PBS; 1mL/mouse) was infused into the lungs and then aspirated. The concentration of the protein in the recovered BAL fluid was determined on an Advia 1800 Chemistry Analyzer (Siemens Healthcare Diagnostics).

**Multi-Electrode Array (MEA) Electrophysiology Studies in Human-Inducible Pluripotent Stem Cell-Derived Cardiomyocytes :**

Human-inducible pluripotent stem cell-derived cardiomyocytes (hiPSC CMs) were purchased from Cellular Dynamics International (Madison, WI). qPCR analysis showed similar RNA expression levels of $S1P_1$, $S1P_2$ and $S1P_3$ in hiPSC CMs as in adult human heart tissue (results not shown). hiPSC CMs were cultured with 7% $CO_2$ on 0.1% gelatin treated 6-well culture plates for 7 days, then trypsinized and diluted with cardiac fibroblasts (10%). Suspensions of hiPSC CMs and fibroblasts were then co-cultured on laminin-coated 9-well multi-electrode arrays (MEA) plates (256-9 well MEA300/30iR-ITO-mq; Multichannel Systems; Atlanta, GA). After 7 days culture on MEA plates, cells formed a spontaneously beating monolayer over recording electrodes imbedded in each well. Spontaneous extracellular field potentials (FPs) were recorded from 28 electrodes/well at a sampling frequency of 10 kHz using an USB-MEA256-System and MC Rack acquisition software (Multi Channel Systems). Following a 20-60 minute equilibration period in a humidified environment at 37$^{o}$C with constant 5% $CO_2$ and 95% $O_2$ supply, compounds were added to each well in 300 μL maintenance medium with final DMSO or NaOH vehicle concentration less than 0.1% or 30 μM. Dilute NaOH (30 μM was used as control in these studies and had no significant effect on beating rate of hiPSC CMs. Effects of test agents on field potentials were evaluated for at least 2 hours. Data were analyzed with MC_DataTool and custom software written in MatLab (Mathworks; Natick, MA). Data are reported as the mean change in the beating rate relative to control and represent the mean ± SD of at least 3 separate experiments

**General Synthetic Methods**.

All commercially available chemicals and solvents were used without further purification. Reactions were performed under an atmosphere of nitrogen. All new compounds gave satisfactory $^{1}$H NMR, LC/MS, and mass spectrometry results. $^{1}$H NMR spectra were obtained on a Bruker 400 MHz or a JEOL 500 MHz NMR spectrometer using the residual

TMS signal of deuterated NMR solvent as internal reference. Mass spectral analysis was performed on Waters Acquity ultraperformance liquid chromatography. The purity of tested compounds determined by analytical HPLC was >95%. HPLC traces are provided as a sample in a methanol solution. Blank spectrums, injection with methanol only, are also provided due to the low UV absorption of these compounds.

**HPLC Conditions**

Condition A: (Analytical)

Waters Acquity UPLC, BEH C18 2.1x50mm, 1.7um particles; MP A 98:2 water:ACN 0.05%TFA; MP B acetonitrile 0.05%TFA, Column temp 50C; Gradient 2-98%B over 1 min then 0.5 min hold at 100%B. Flow 0.8 mL/min; UV 200 nm.

Condition B: (Preparative)

Shimatzu prep HPLC, Luna® $C_{18}$ 30 x 100 mm, 5 µm (Phenomenex Inc.); 2 mL injection; Mobile Phase : 0.1% TFA in Water; Mobile Phase B: 0.1% TFA in MeCN; Temperature: 50 °C; Gradient: 20-100% B over 5 min, then a 10 min hold at 100% B, Flow: 30 mL/min; Detection: UV at 220 nm.

Condition C: (Analytical)

Waters Acquity UPLC BEH C18 (2.1 x 50) mm, 1.7-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0-100% B over 2 minutes, then a 0.5-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 220 nm.

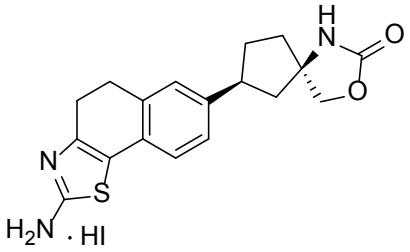
Condition D: (Purity Long Run)

Shimatzu prep HPLC, Sunfire C18 (3.0 x 150) mm, 3.5 µm particles; Mobile Phase A: 5:95 acetonitrile:water with 0.05% TFA; Mobile Phase B: 95:5 acetonitrile:water with 0.05 TFA; Temperature: 25 °C; Gradient: 0-100% B over 12 minutes, then a 3-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 254 nm

Condition E: (Purity Long Run)

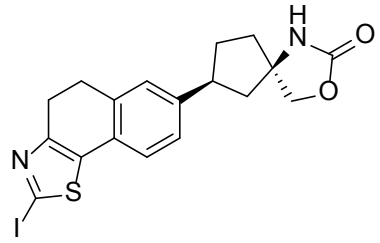
Shimatzu prep HPLC, Xbridge Phenyl (3.0 x 150) mm, 3.5 µm particles; Mobile Phase A: 5:95 acetonitrile:water with 0.05% TFA; Mobile Phase B: 95:5 acetonitrile:water with 0.05 TFA; Temperature: 25 °C; Gradient: 0-100% B over 12 minutes, then a 3-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 254 nm

(5R,7S)-7-(2-amino-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one, hydroiodinic acid salt (**6**)



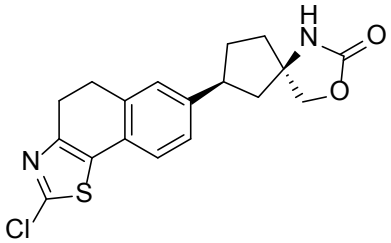

(5R,7S)-7-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (200 mg, 0.7 mmol) (see WO 2006/028959 A1) was dissolved in Ethanol (1.4 mL) in a screw cap vial. Thiourea (187 mg, 2.5 mmol) and iodine (196 mg, 0.77 mmol) were then added at room temperature when the tube was sealed and heated to 100°C for 2h. LCMS analysis revealed full conversion of starting material. The tube was unsealed allowing the reaction mixture to concentrate for 15 min at 100°C. Upon cooling to room temperature, the desired product precipitated out. Addition of 1 mL of water, stirring an additional 15 min followed by filtration afforded the desired compound (5R,7S)-7-(2-amino-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one, hydroiodinic acid salt (200 mg, 61%) as a brown solid. LCMS (M+H): 342.3; LC retention time: 0.64 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.23 (s, 1H), 7.21 - 7.15 (m, 1H), 7.06 (d, *J*=7.9 Hz, 1H), 4.40 (d, *J*=8.6 Hz, 1H), 4.31 (d, *J*=8.6 Hz, 1H), 3.17 - 3.03 (m, 3H), 2.93 - 2.81 (m, 2H), 2.34 (dd, *J*=13.0, 7.3 Hz, 1H), 2.22 - 2.08 (m, 2H), 2.04 - 1.76 (m, 3H).

(5R,7S)-7-(2-iodo-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**8**)




To a suspension of (5R,7S)-7-(2-amino-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one, hydroiodinic salt (50 mg, 0.1 mmol) and copper(I) iodide (31 mg, 0.16 mmol) in Acetonitrile (2.2 mL) was added tert-butyl nitrite (20 μL, 0.15 mmol) at 0 °C. The reaction was stirred at this temperature for 15 min and at room temperature overnight when LCMS analysis showed complete consumption of starting material. The mixture was diluted with EtOAc and filtered through Celite. The resulting solution was concentrated under reduced pressure and used as is. LCMS (M+H): 453.1; LC retention time: 1.01 min (analytical HPLC Method A).

(5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (**7**)

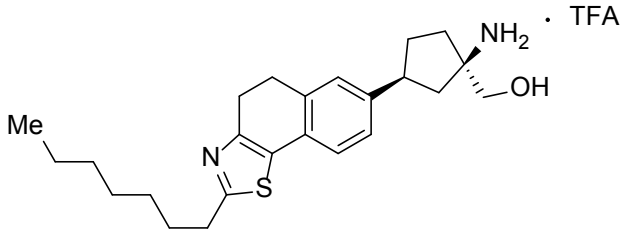



A solution (5R,7S)-7-(2-amino-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one, iodinic acid (38 mg, 0.08 mmol) in EtOAc (25 mL) was washed once with 1N NaOH (25 mL). The org layer was dried over sodium sulfate and filtered. To the organic solution was added TFA (100 μL) and the solution was concentrated under reduced pressure. The residue was dissolved in acetonitrile (1.7 mL). To this solution was added copper(I) chloride (12 mg, 0.13 mmol) followed by tert-butyl nitrite (15 μL, 0.12 mmol) at 0 °C. The reaction was stirred at this temperature for 15 min and at room temperature overnight when LCMS analysis showed complete consumption of starting material. The mixture was diluted with 2mL of MeOH and purified by HPLC using

condition B affording (5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (12 mg, 0.033 mmol, 40% yield) as a brown solid which was used as is.

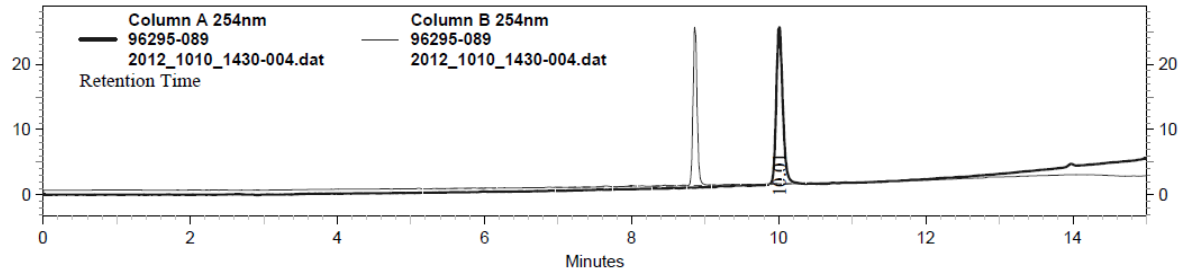
**General procedure for the Fe-catalyzed coupling reaction**

((1R,3S)-1-amino-3-(2-heptyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**14**)




To a solution of (5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (14 mg, 0.04 mmol) and Iron(III) acac (1.4 mg, 3.9 µmol) in a mixture of THF (0.5 mL) and N-Methyl-2-pyrrolidinone (0.1 mL) was added a 1M solution of heptylmagnesium bromide (0.12 mL, 0.12 mmol) at room temperature. Analysis of the reaction by LCMS after 15 min showed full conversion. The reaction was diluted with diethyl ether and was quenched by the addition of 1N HCl. The aqueous layer was back extracted twice with EtOAc. The organic layer were combined, dried with MgSO4 and concentrated under reduced pressure. The resulting oil was dissolved in dioxane (1 mL) followed by the addition of NaOH (0.56 mL, 0.56 mmol). The solution was warmed to 100 °C and stirred for 3h when LCMS showed complete conversion. The solution was injected on HPLC using Condition B providing ((1R,3S)-1-amino-3-(2-heptyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA (3 mg, 5.6 µmol, 15 % yield, 2 steps) as a yellow solid. Retention time: 10.00 min and 8.86 (long run HPLC D and E); LCMS (M+H): 399.5; LC retention time: 0.94 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.26 - 7.13 (m, 3H), 3.65 (dd, *J*=14.7, 12.1 Hz, 2H), 3.26 - 3.12 (m, 1H), 3.12 - 2.91 (m, 4H), 2.47 (dd, *J*=13.4, 7.0 Hz, 1H), 2.25 - 2.11 (m, 1H), 2.08 - 1.91 (m, 3H), 1.90 - 1.70 (m, 2H), 1.53 - 1.25 (m, 10H), 0.93 (t, *J*=6.8 Hz, 3H).

**Sample HPLC:**

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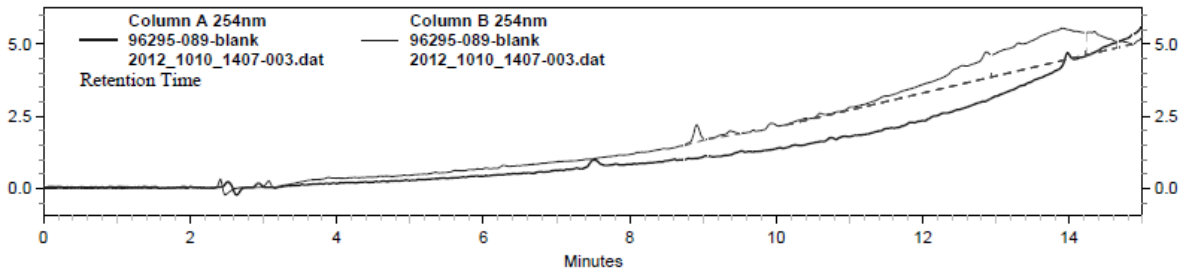
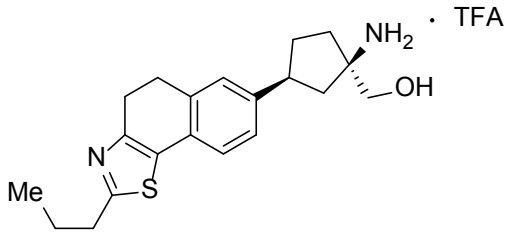
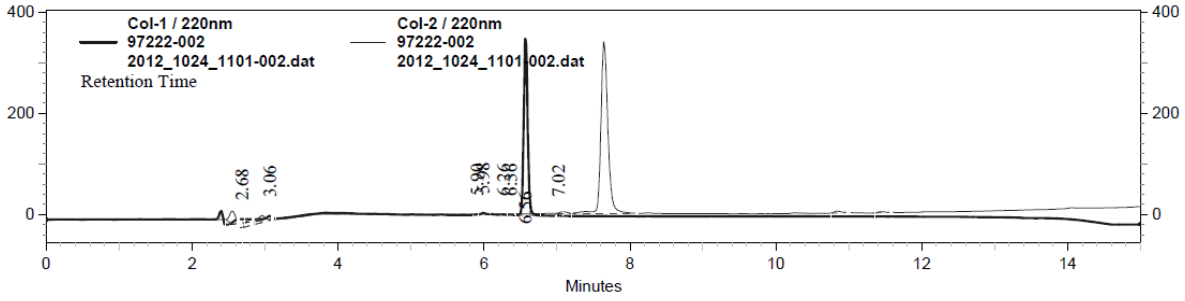



((1R,3S)-1-amino-3-(2-propyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**10**)




Retention time: 6.56 min and 7.56 (long run HPLC D and E); LCMS (M+H): 343.2; LC retention time: 0.76 min (analytical HPLC Method A), $^{1}$H NMR (400MHz, METHANOL-$d_4$) δ 7.27 - 7.13 (m, 3H), 3.65 (dd, *J*=15.0, 11.4 Hz, 2H), 3.22 - 3.14 (m, 1H), 3.11 - 3.04 (m, 2H), 3.04 - 2.93 (m, 4H), 2.52 - 2.43 (m, 1H), 2.24 - 2.11 (m, 1H), 2.05 - 1.92 (m, 3H), 1.91 - 1.80 (m, 2H), 1.76 (t, *J*=12.8 Hz, 1H), 1.06 (t, *J*=7.3 Hz, 3H).

**Sample HPLC:**



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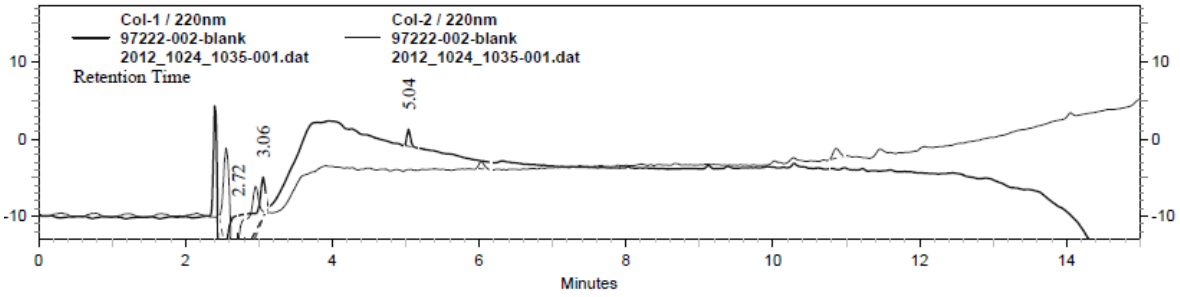
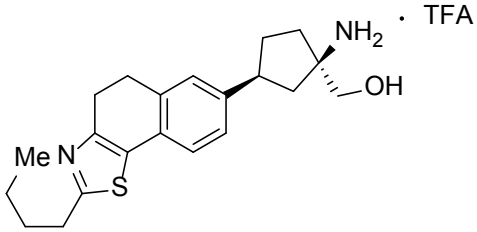
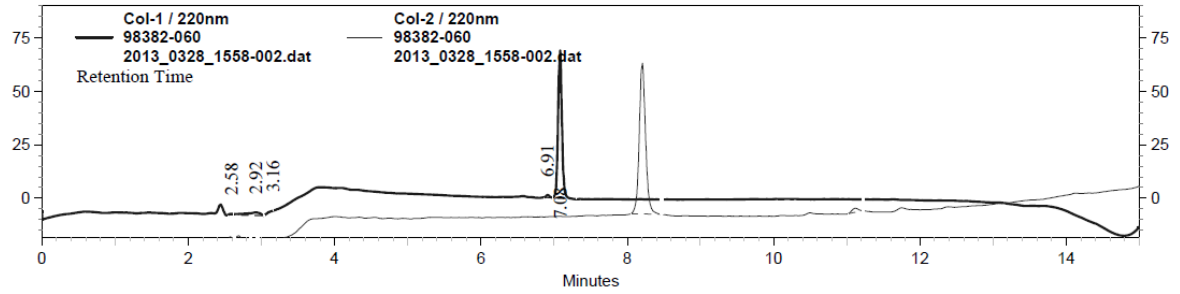



((1R,3S)-1-amino-3-(2-butyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**11**)




Retention time: 7.08 min and 8.11 (long run HPLC D and E); LCMS (M+H): 357.3; LC retention time: 0.80 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.26 - 7.13 (m, 3H), 3.74 - 3.59 (m, 2H), 3.26 - 3.13 (m, 1H), 3.12 - 2.90 (m, 6H), 2.47 (ddd, *J*=13.4, 7.1, 1.1 Hz, 1H), 2.24 - 2.12 (m, 1H), 2.05 - 1.92 (m, 3H), 1.87 - 1.70 (m, 3H), 1.48 (dq, *J*=14.9, 7.4 Hz, 2H), 1.01 (t, *J*=7.4 Hz, 3H).

**Sample HPLC:**

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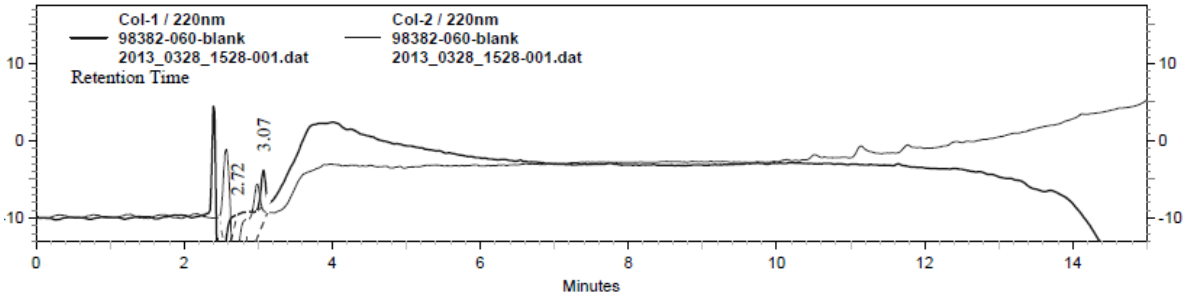
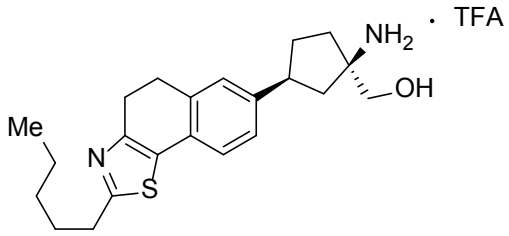
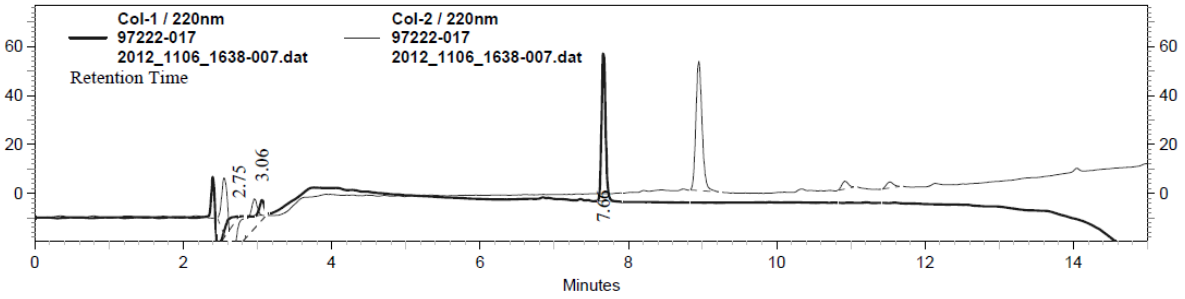


((1R,3S)-1-amino-3-(2-pentyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**12**)




Retention time: 7.66 min and 8.94 (long run HPLC D and E); LCMS (M+H): 371.2; LC retention time: 0.83 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.27 - 7.13 (m, 3H), 3.74 - 3.57 (m, 2H), 3.27 - 3.11 (m, 1H), 3.10 - 2.90 (m, 6H), 2.45 (ddd, *J*=13.4, 7.1, 1.1 Hz, 1H), 2.24 - 2.12 (m, 1H), 2.05 - 1.92 (m, 3H), 1.87 - 1.70 (m, 3H), 1.56 – 1.29 (m, 4H), 0.99 (t, *J*=7.4 Hz, 3H).

**Sample HPLC:**

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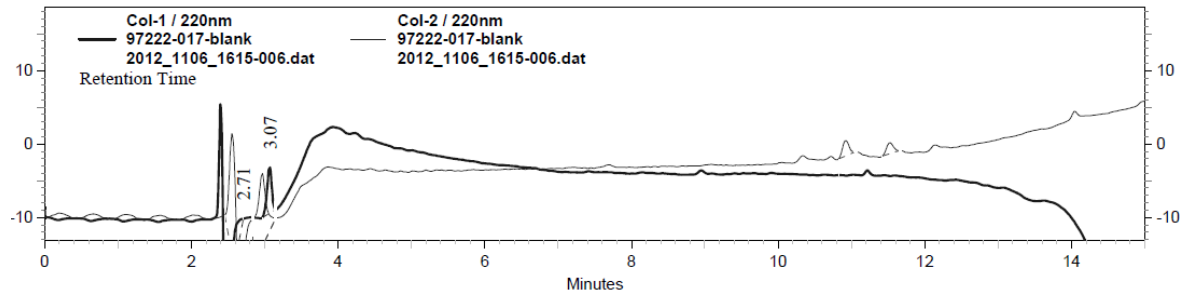
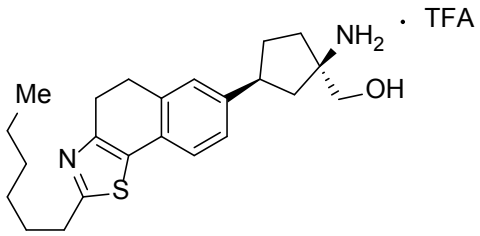
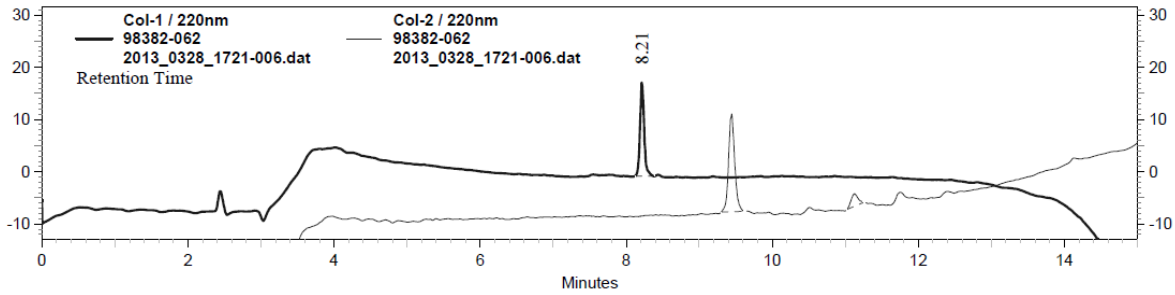


((1R,3S)-1-amino-3-(2-hexyl-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**13**)




Retention time: 8.22 min and 9.44 (long run HPLC D and E); LCMS (M+H): 385.6; LC retention time: 0.89 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.27 - 7.12 (m, 3H), 3.74 - 3.58 (m, 2H), 3.17 (d, *J*=11.2 Hz, 1H), 3.11 - 2.92 (m, 6H), 2.47 (ddd, *J*=13.3, 7.2, 1.1 Hz, 1H), 2.17 (d, *J*=4.8 Hz, 1H), 2.06 - 1.91 (m, 3H), 1.89 - 1.70 (m, 3H), 1.53 - 1.41 (m, 2H), 1.41 - 1.26 (m, 4H), 1.00 - 0.87 (m, 3H).

**Sample HPLC:**

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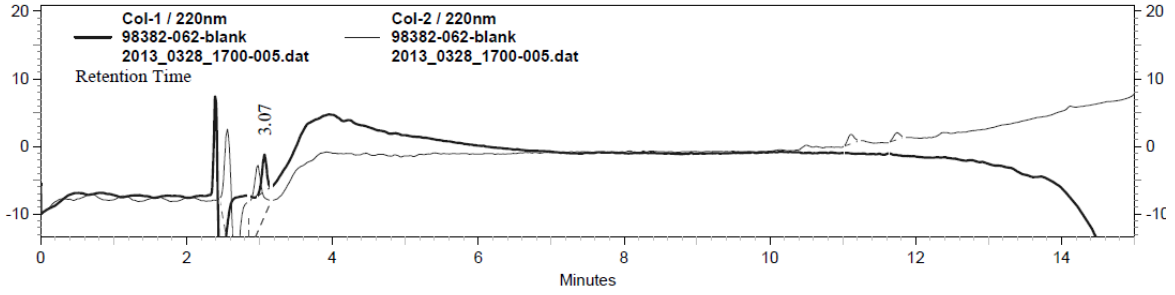
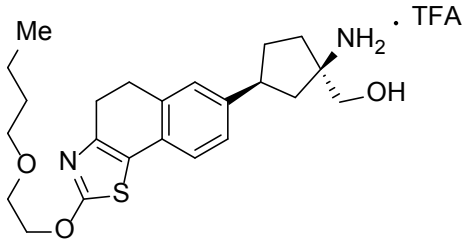


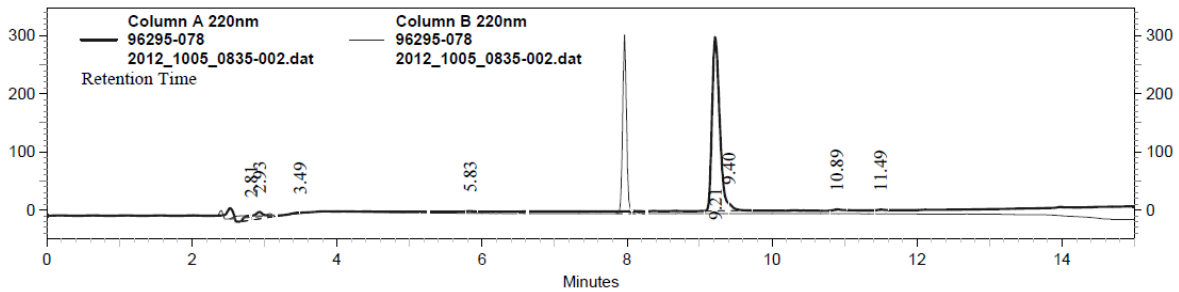
**General condition for alkoxy and thio thiazole formation**

((1R,3S)-1-amino-3-(2-(2-butoxyethoxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**18**)

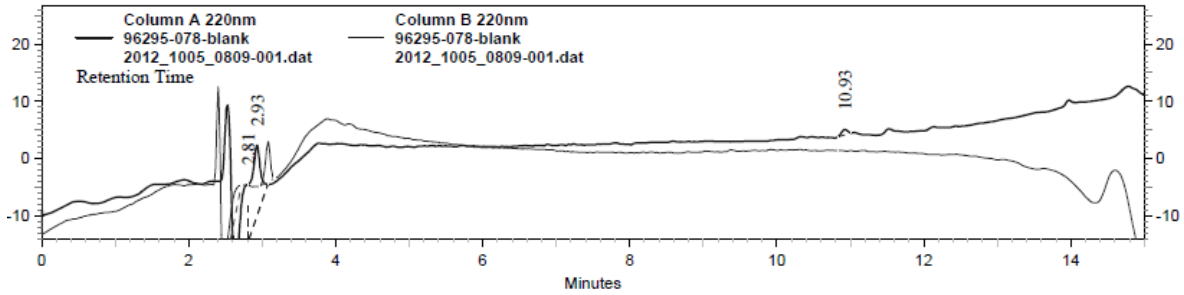



To a solution of (5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (3 mg, 8 µmol) in dioxane (0.5 mL) was added 2-butoxyethanol (20 mg, 0.17 mmol) followed by potassium tert-butoxide (9 mg, 0.08 mmol) at room temperature. The mixture was stirred at 70 °C for 2h when LCMS showed complete consumption of starting material. To this mixture was added a 1M NaOH solution (0.5 mL, 0.5 mmol) at room temperature. The mixture was heated to 70°C and stirred for 14h. LCMS showed complete consumption of the intermediate. The solution was injected on the HPLC prep and purified using condition B affording ((1R,3S)-1-amino-3-(2-(2-butoxyethoxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA (3 mg, 5.5 µmol, 66 % yield) as a white solid. Retention time: 9.21 min and 7.97 (long run HPLC D and E); LCMS (M+H): 417.3; LC retention time: 0.87 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-d$_4$) δ 7.17 (s, 1H), 7.13 (dd, *J*=7.8, 1.4 Hz, 1H), 7.03 (d, *J*=7.9 Hz, 1H), 4.60 - 4.49 (m, 2H), 3.87 - 3.77 (m, 2H), 3.65 (dd, *J*=13.2, 10.8 Hz, 2H), 3.56 (t, *J*=6.5 Hz, 2H), 3.24 - 3.10 (m, 1H), 3.03 (t, *J*=7.0 Hz, 2H), 2.83 (t, *J*=8.4 Hz, 2H), 2.51 - 2.41 (m, 1H), 2.24 - 2.09 (m, 1H), 2.05 - 1.89 (m, 3H), 1.75 (t, *J*=12.7 Hz, 1H), 1.68 - 1.52 (m, 2H), 1.49 - 1.33 (m, 2H), 0.95 (t, *J*=7.4 Hz, 3H).

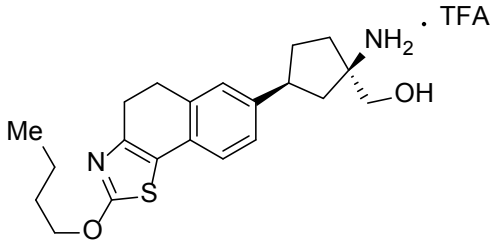
**Sample HPLC:**



**Blank HPLC:**

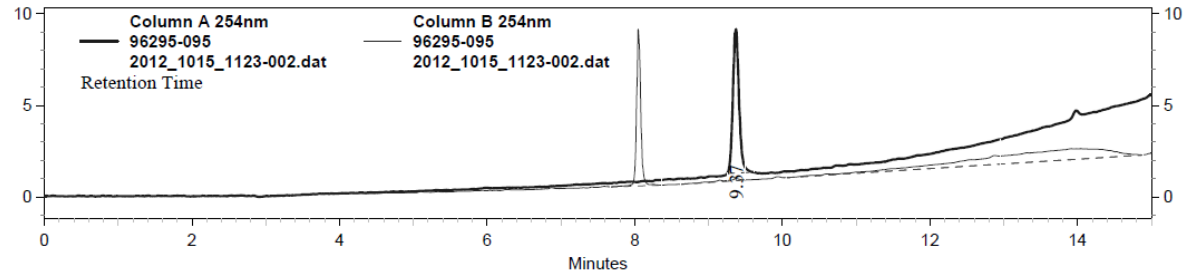



((1R,3S)-1-amino-3-(2-butoxy-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**15**)

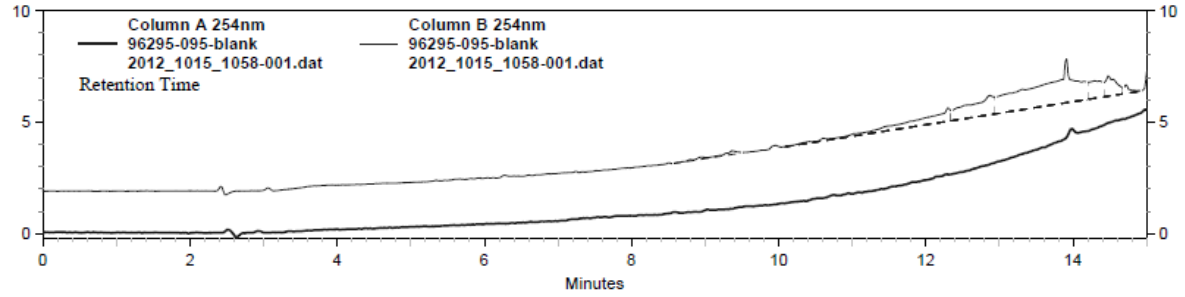



Retention time: 9.37 min and 8.05 (long run HPLC D and E); LCMS (M+H): 373.3; LC retention time: 0.86 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.17 (s, 1H), 7.13 (d, *J*=7.7 Hz, 1H), 7.03 (d, *J*=7.9 Hz, 1H), 4.43 (t, *J*=6.5 Hz, 2H), 3.65 (dd, *J*=15.0, 11.7 Hz, 2H), 3.23 - 3.10 (m, 1H), 3.03 (t, *J*=7.9 Hz, 2H), 2.83 (t, *J*=8.0 Hz, 2H), 2.46 (dd, *J*=12.9, 6.5 Hz, 1H), 2.17 (br. s., 1H), 2.04 - 1.89 (m, 3H), 1.89 - 1.79 (m, 2H), 1.75 (t, *J*=12.7 Hz, 1H), 1.59 - 1.45 (m, 2H), 1.02 (t, *J*=7.4 Hz, 3H).

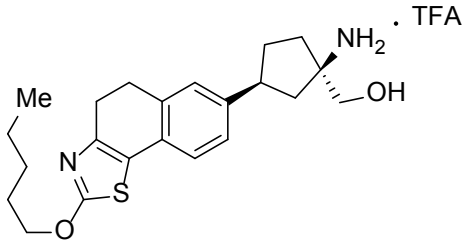
**Sample HPLC:**



**Blank HPLC:**

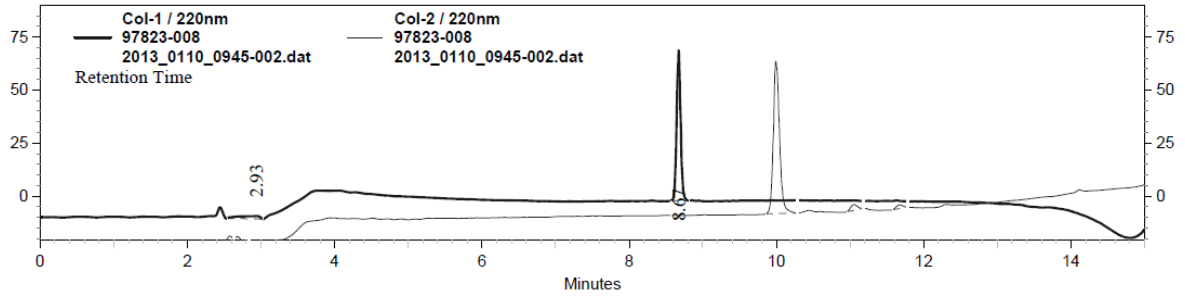



((1R,3S)-1-amino-3-(2-pentoxy-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**16**)

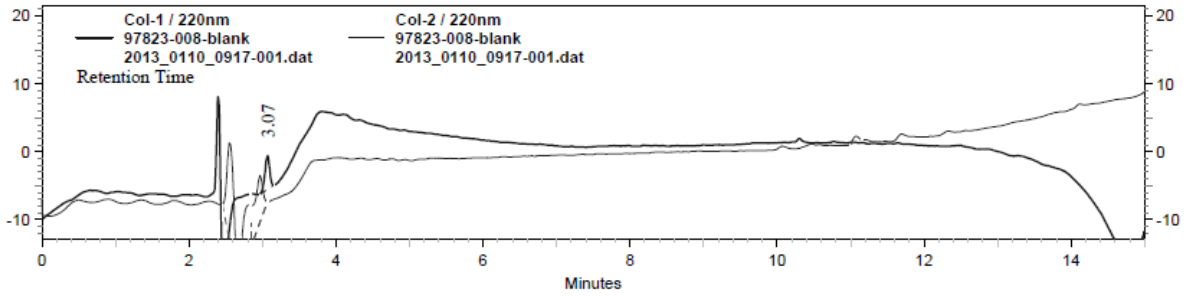



Retention time: 8.67 min and 9.99 (long run HPLC D and E); LCMS (M+H): 387.2; LC retention time: 0.90 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.17 (s, 1H), 7.13 (d, *J*=7.7 Hz, 1H), 7.03 (d, *J*=7.7 Hz, 1H), 4.42 (t, *J*=6.5 Hz, 2H), 3.65 (dd, *J*=15.2, 11.2 Hz, 2H), 3.15 (dt, *J*=3.3, 1.7 Hz, 1H), 3.08 - 2.97 (m, 2H), 2.88 - 2.78 (m, 2H), 2.46 (dd, *J*=14.0, 7.6 Hz, 1H), 2.17 (br. s., 1H), 2.06 - 1.91 (m, 3H), 1.91 - 1.79 (m, 2H), 1.75 (t, *J*=12.8 Hz, 1H), 1.56 - 1.36 (m, 4H), 1.04 - 0.92 (m, 3H).

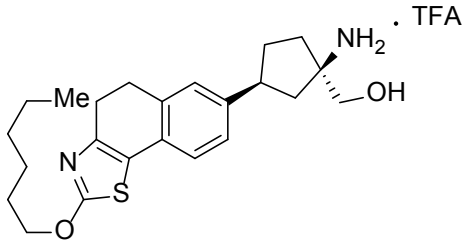
**Sample HPLC:**



**Blank HPLC:**

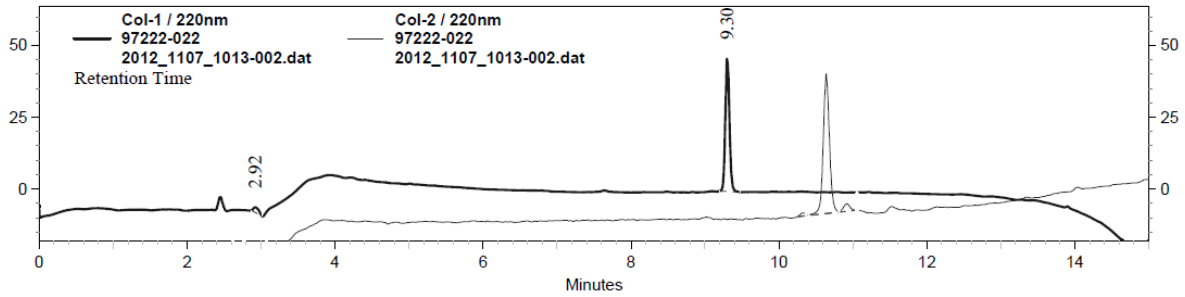



((1R,3S)-1-amino-3-(2-(hexyloxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**17**)

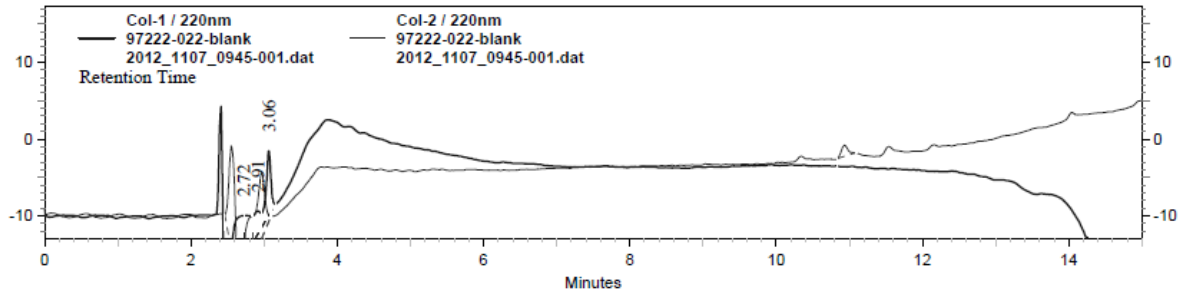



Retention time: 9.30 min and 10.64 (long run HPLC D and E); LCMS (M+H): 401.2; LC retention time: 0.98 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.17 (s, 1H), 7.16 - 7.10 (m, 1H), 7.03 (d, *J*=7.7 Hz, 1H), 4.42 (t, *J*=6.5 Hz, 2H), 3.65 (dd, *J*=14.5, 11.7 Hz, 2H), 3.24 - 3.10 (m, 1H), 3.09 - 2.97 (m, 2H), 2.88 - 2.77 (m, 2H), 2.46 (dd, *J*=13.3, 6.9 Hz, 1H), 2.17 (br. s., 1H), 2.05 - 1.91 (m, 3H), 1.91 - 1.79 (m, 2H), 1.75 (t, *J*=12.7 Hz, 1H), 1.57 - 1.44 (m, 2H), 1.39 (dq, *J*=7.3, 3.6 Hz, 4H), 1.03 - 0.88 (m, 3H).

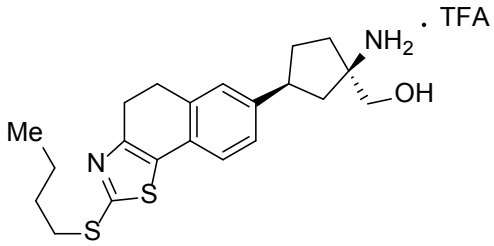
**Sample HPLC:**



**Blank HPLC:**

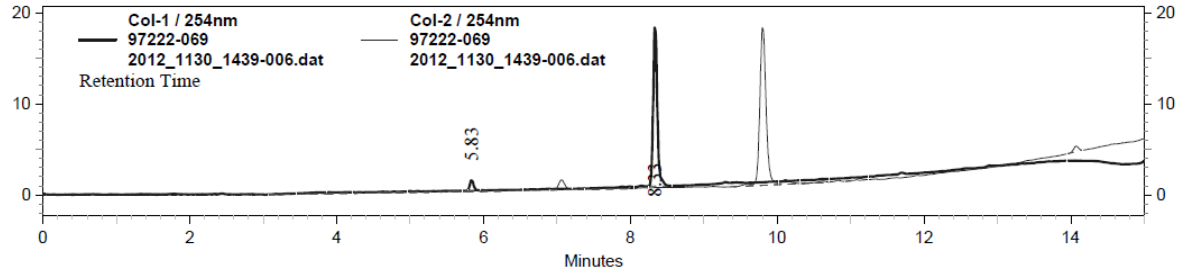



((1R,3S)-1-amino-3-(2-(butylthio)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**19**)

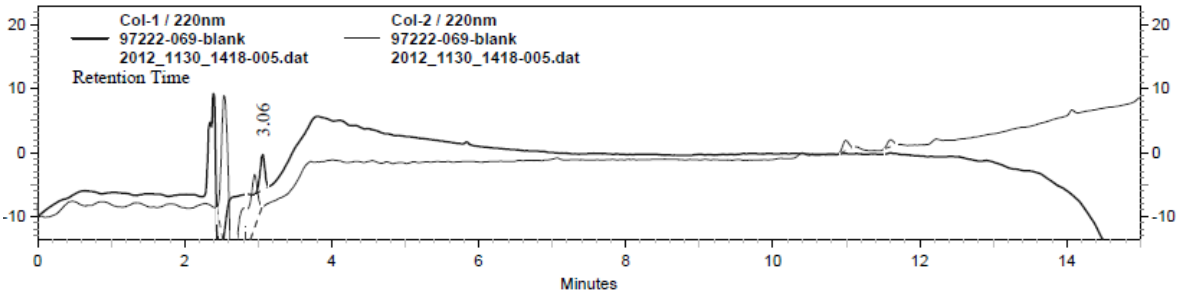



Retention time: 8.34 min and 9.80 (long run HPLC D and E); LCMS (M+H): 389.3; LC retention time: 0.89 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.22 (s, 1H), 7.17 (d, *J*=1.1 Hz, 2H), 3.65 (dd, *J*=13.4, 12.1 Hz, 2H), 3.28 - 3.22 (m, 2H), 3.11 - 3.02 (m, 2H), 3.01 - 2.90 (m, 2H), 2.53 - 2.41 (m, 1H), 2.25 - 2.11 (m, 1H), 2.05 - 1.91 (m, 3H), 1.83 - 1.73 (m, 3H), 1.53 (dq, *J*=15.0, 7.4 Hz, 2H), 0.99 (t, *J*=7.4 Hz, 3H).

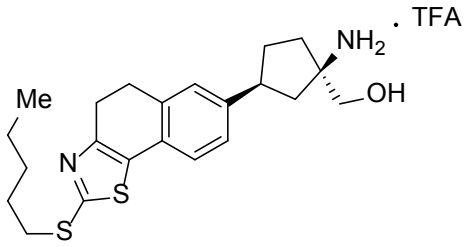
**Sample HPLC:**




**Blank HPLC:**

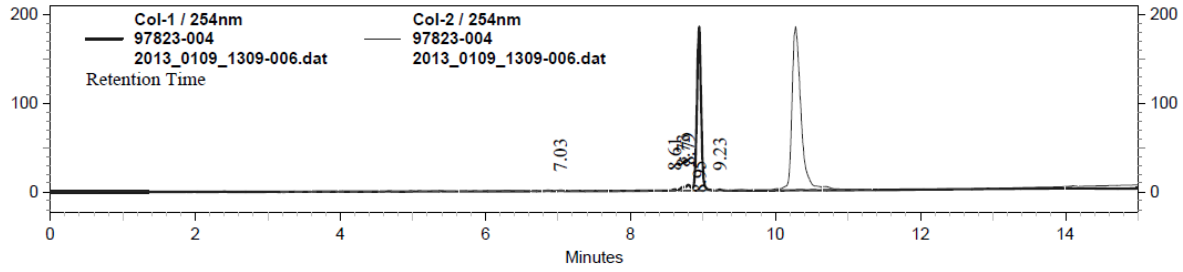


((1R,3S)-1-amino-3-(2-(pentylthio)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**20**)

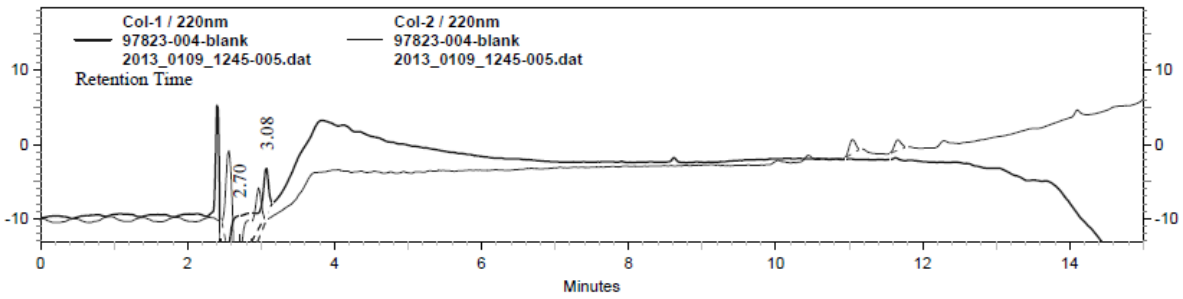



Retention time: 8.94 min and 10.27 (long run HPLC D and E); LCMS (M+H): 403.2; LC retention time: 0.93 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.22 (s, 1H), 7.17 (d, *J*=0.9 Hz, 2H), 3.65 (dd, *J*=14.3, 11.7 Hz, 2H), 3.27 - 3.20 (m, 2H), 3.20 - 3.12 (m, 1H), 3.10 - 3.02 (m, 2H), 3.01 - 2.91 (m, 2H), 2.47 (ddd, *J*=13.4, 7.1, 1.1 Hz, 1H), 2.23 - 2.10 (m, 1H), 2.06 - 1.90 (m, 3H), 1.87 - 1.70 (m, 3H), 1.56 - 1.33 (m, 4H), 0.96 (t, *J*=6.6 Hz, 3H).

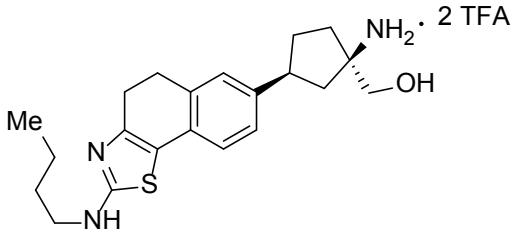
**Sample HPLC:**




**Blank HPLC:**

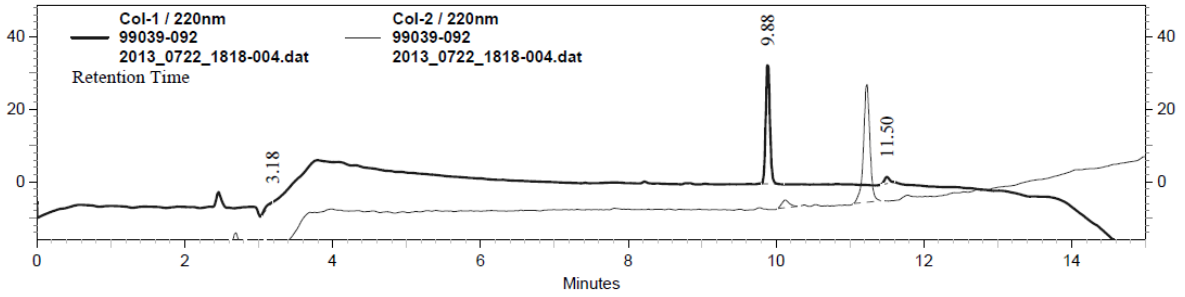


((1R,3S)-1-amino-3-(2-(butylamino)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, bis TFA salt (**21**)

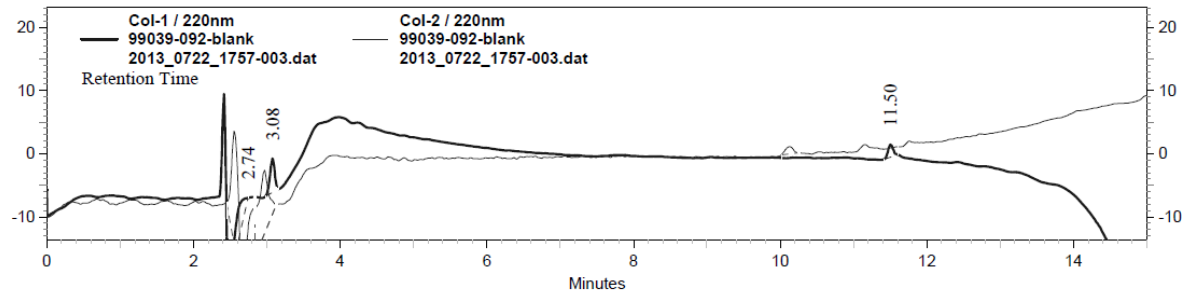



To a solution of (5R,7S)-7-(2-amino-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (50 mg, 0.15 mmol) in MeOH (15 mL) was added butyraldehyde (53 mg, 0.73 mmol). The reaction was heated to 70 °C for 1h and cooled down to room temperature. Sodium borohydride (55 mg, 1.5 mmol) was then added and the solution was stirred for 30 min when LCMS showed complete consumption of starting material. LCMS (M+H): 398.4; LC retention time: 0.75 min (analytical HPLC Method A). The solvent was removed under reduced pressure and diluted in EtOAc. The organic layer was washed with 1N NaOH, dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting oil was dissolved in dioxane (0.7 mL) followed by the addition of sodium hydroxide (1M, 150 µL, 0.15 mmol). The solution was warmed to 100 °C and stirred for 2h when LCMS showed complete conversion. The solution was injected on HPLC prep and purified using Condition B providing ((1R,3S)-1-amino-3-(2-(butylamino)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, 2 TFA (12 mg, 0.019 mmol) as a white solid. LCMS (M+H): 372.3; LC retention time: 0.61 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-d$_4$) δ 7.23 (s, 1H), 7.22 - 7.16 (m, 1H), 7.08 (d, *J*=7.7 Hz, 1H), 3.71 - 3.58 (m, 2H), 3.47 (t, *J*=7.2 Hz, 2H), 3.23 - 3.14 (m, 1H), 3.11 (t, *J*=8.1 Hz, 2H), 2.89 (t, *J*=8.1 Hz, 2H), 2.52 - 2.42 (m, 1H), 2.23 - 2.09 (m, 1H), 2.05 - 1.91 (m, 3H), 1.83 - 1.68 (m, 3H), 1.50 (dq, *J*=15.0, 7.5 Hz, 2H), 1.03 (t, *J*=7.4 Hz, 3H).

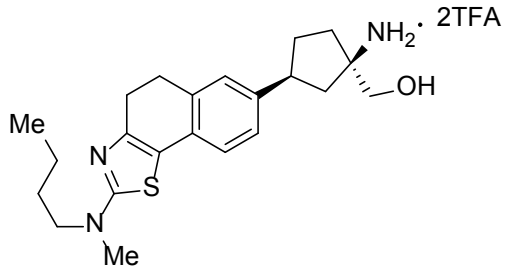
**Sample HPLC:**



**Blank HPLC:**



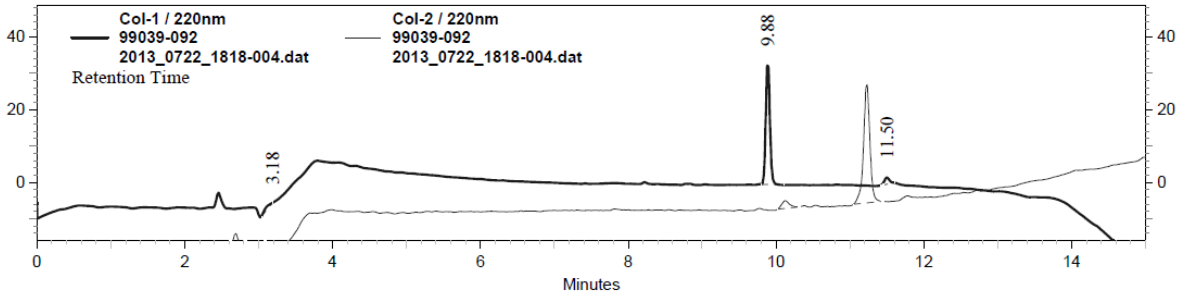

((1R,3S)-1-amino-3-(2-(butyl(methyl)amino)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, bis TFA salt (**22**)



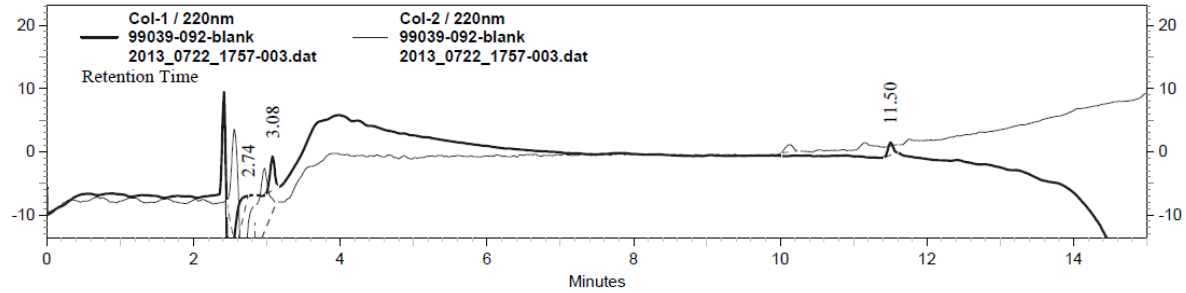

To a suspension of (5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (20 mg, 0.055 mmol) in toluene (500 µL) was added N-methylbutan-1-amine (13 µL, 0.11 mmol) followed by potassium tert-butoxide (19 mg, 0.17 mmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (5 mg, 0.011 mmol), and $Pd_2(dba)_3$ (3 mg, 0.003 mmol) at room temperature. The mixture was heated to 70 °C and stirred for 1h when LCMS showed complete consumption of starting material. The mixture was partitioned between water and EtOAc. The aqueous solution was back extract twice with EtOAc. The organic layers were combined, dried and concentrated under reduced pressure. LCMS (M+H): 412.3; LC retention time: 0.79 min (analytical HPLC Method A). The resulting oil was dissolved in dioxane (0.5 mL) and NaOH (705 µl, 0.705 mmol) was added. The mixture was heated at 100 °C for 3h when LCMS showed complete consumption of starting material. The solution was worked up using EtOAc and water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting oil was solubilized in MeOH and injected on the HPLC prep and purified using condition B affording ((1R,3S)-1-amino-3-(2-(butyl(methyl)amino)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, 2 TFA (5 mg, 7.9 µmol, 11 % yield) as a white solid. LCMS (M+H): 386.2; LC retention time: 0.64 min (analytical

HPLC Method A), $^1$H NMR (400MHz, METHANOL-d$_4$) δ 7.20 (s, 1H), 7.18 (d, *J*=7.7 Hz, 1H), 7.07 (d, *J*=7.7 Hz, 1H), 3.73 - 3.57 (m, 4H), 3.28 (s, 3H), 3.23 - 3.14 (m, 1H), 3.08 (t, *J*=8.0 Hz, 2H), 2.88 (t, *J*=8.0 Hz, 2H), 2.52 - 2.41 (m, 1H), 2.17 (br. s., 1H), 2.05 - 1.90 (m, 3H), 1.84 - 1.69 (m, 3H), 1.45 (dd, *J*=15.2, 7.5 Hz, 2H), 1.03 (t, *J*=7.4 Hz, 3H).

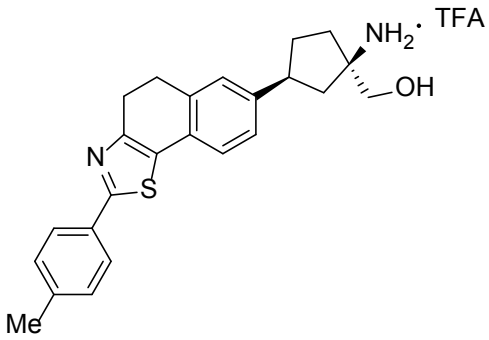
**Sample HPLC:**




**Blank HPLC:**



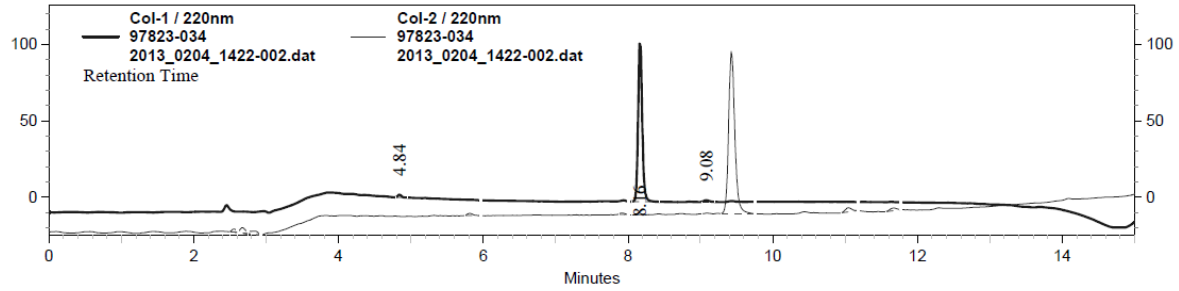

<u>((1R,3S)-1-amino-3-(2-(p-tolyl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, trifluoroacetic acid salt (**23**)</u>



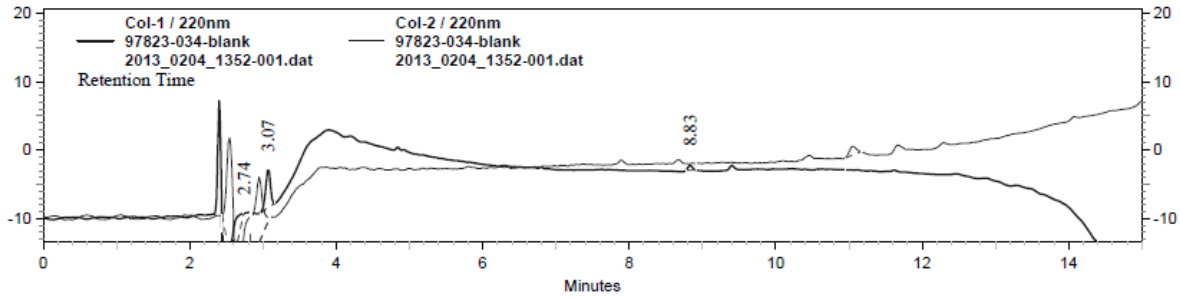

To a solution of (5R,7S)-7-(2-chloro-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)-3-oxa-1-azaspiro[4.4]nonan-2-one (20 mg, 0.055 mmol) in dioxane (550 µL) was added p-tolylboronic acid (38 mg, 0.28 mmol) and sodium carbonate (55 µL, 0.11 mmol). The reaction was purged with nitrogen followed by the addition of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II) (2 mg, 2.78 µmol). The reaction was

heated to 100 °C and LCMS showed complete conversion after 1h. LCMS (M+H): 417.1; LC retention time: 1.12 min (analytical HPLC Method A). The solution was cooled to room temperature and NaOH (1M, 554 µL, 0.554 mmol) was then added. The reaction was heated to 100 °C and LCMS showed complete conversion after 6h. The solution was diluted with EtOAc and water. The aqueous layer was back extracted with EtOAc twice. The organic fraction were combined, dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting solid was solubilized in MeOH and purified on HPLC prep using condition B affording ((1R,3S)-1-amino-3-(2-(p-tolyl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA (8 mg, 0.015 mmol, 27 % yield). LCMS (M+H): 391.2; LC retention time: 0.87 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.86 (d, *J*=8.1 Hz, 2H), 7.32 (dd, *J*=7.8, 5.4 Hz, 3H), 7.28 - 7.17 (m, 2H), 3.66 (dd, *J*=15.4, 11.4 Hz, 2H), 3.14 - 3.01 (m, 4H), 2.49 (dd, *J*=13.3, 7.2 Hz, 1H), 2.43 (s, 3H), 2.20 (br. s., 1H), 2.08 - 1.92 (m, 3H), 1.77 (t, *J*=12.8 Hz, 1H).

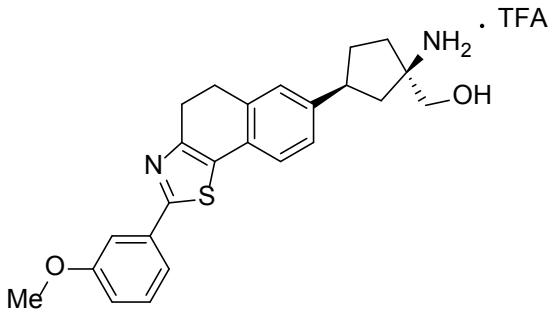
**Sample HPLC:**




**Blank HPLC:**

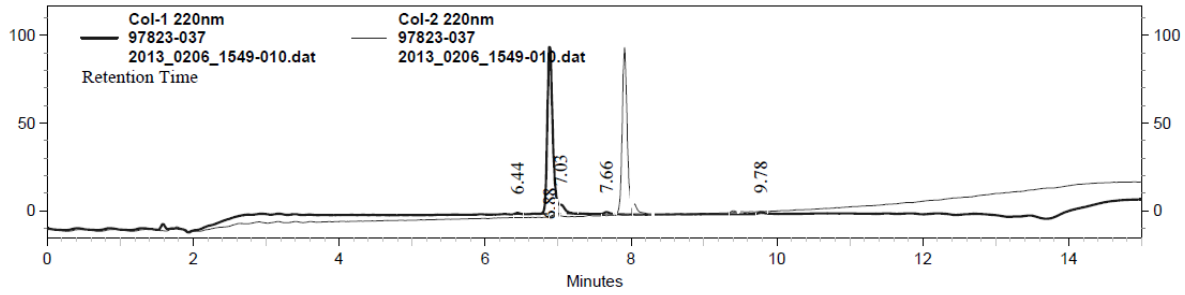


((1R,3S)-1-amino-3-(2-(3-methoxyphenyl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**21**)

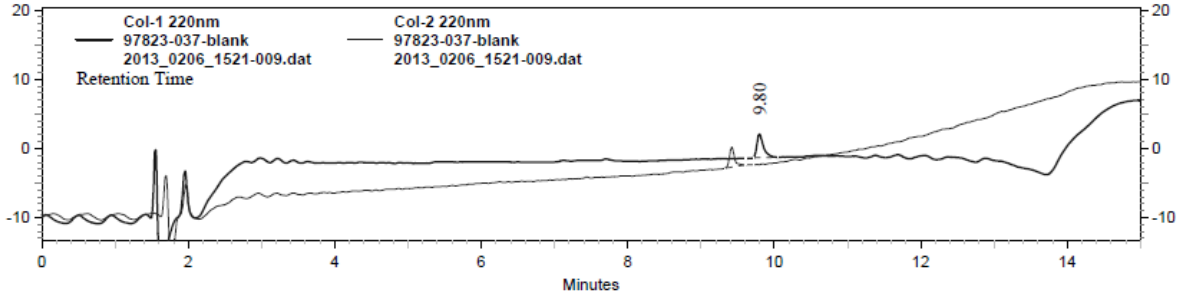



Retention time: 6.88 min and 7.91 (long run HPLC D and E); LCMS (M+H): 407.3; LC retention time: 0.82 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.58 - 7.50 (m, 2H), 7.41 (t, *J*=8.0 Hz, 1H), 7.33 (d, *J*=7.9 Hz, 1H), 7.26 (s, 1H), 7.22 (d, *J*=7.9 Hz, 1H), 7.06 (ddd, *J*=8.3, 2.5, 0.9 Hz, 1H), 3.90 (s, 3H), 3.66 (dd, *J*=14.3, 1.1 Hz, 2H), 3.26 - 3.17 (m, 1H), 3.17 - 3.03 (m, 4H), 2.49 (dd, *J*=14.0, 6.5 Hz, 1H), 2.25 - 2.10 (m, 1H), 2.07 - 1.91 (m, 3H), 1.78 (t, *J*=12.8 Hz, 1H).

**Sample HPLC:**

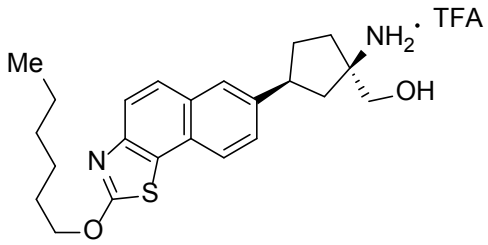



**Blank HPLC:**



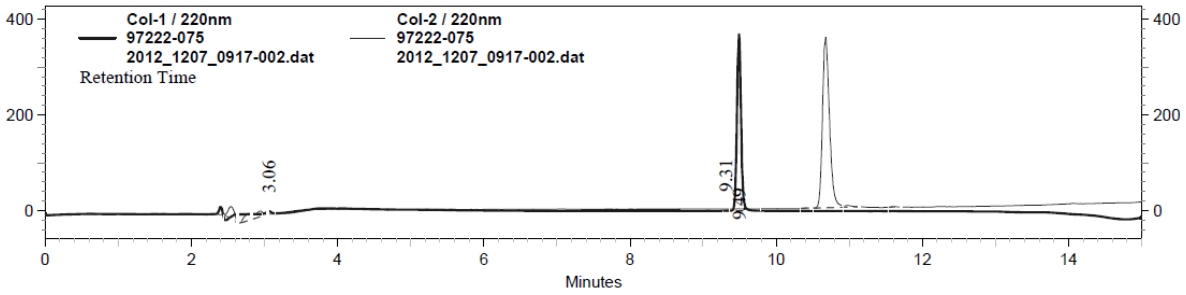
**General procedure for the tricyclic oxidation**

((1R,3S)-1-amino-3-(2-(hexyloxy)naphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**27**)

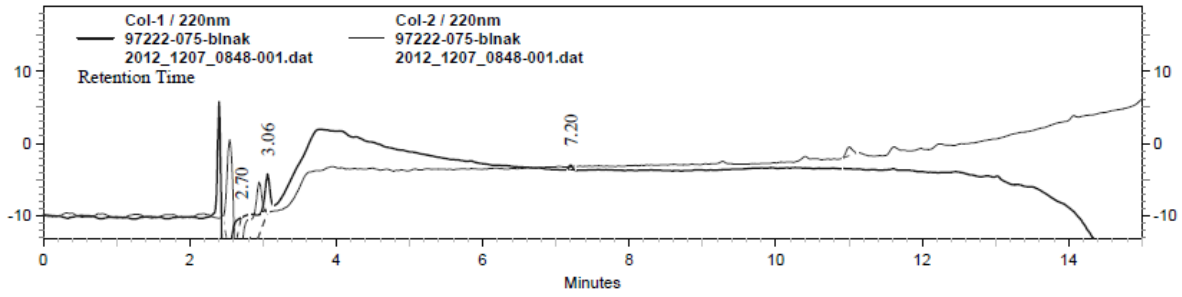



To a solution of ((1R,3S)-1-amino-3-(2-(hexyloxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA (8 mg, 0.016 mmol) in MeCN (1.6 mL) was added copper(II) chloride (21 mg, 0.16 mmol). The reaction was stirred at room temperature overnight open to air when LCMS showed full conversion. The reaction was diluted with MeOH and injected on HPLC prep and purified using condition B affording ((1R,3S)-1-amino-3-(2-(hexyloxy)naphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA (5 mg, 9.3 µmol, 60 % yield). Purity: 97%; retention time: 9.49 min and 10.68 (long run HPLC D and E); LCMS (M+H): 399.2; LC retention time: 1.00 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.90 - 7.79 (m, 3H), 7.76 (d, *J*=8.6 Hz, 1H), 7.58 (dd, *J*=8.6, 1.8 Hz, 1H), 4.61 (t, *J*=6.6 Hz, 2H), 3.70 (dd, *J*=16.3, 11.9 Hz, 2H), 3.47 - 3.37 (m, 1H), 2.58 (dd, *J*=13.2, 6.2 Hz, 1H), 2.35 - 2.24 (m, 1H), 2.17 - 1.97 (m, 3H), 1.97 - 1.81 (m, 3H), 1.62 - 1.49 (m, 2H), 1.49 - 1.35 (m, 4H), 0.96 (t, *J*=7.0 Hz, 3H).

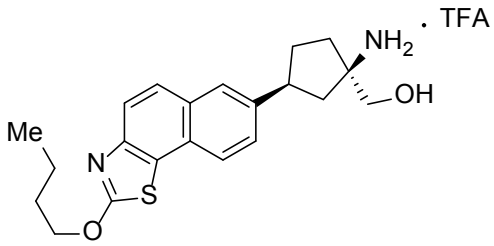
**Sample HPLC:**



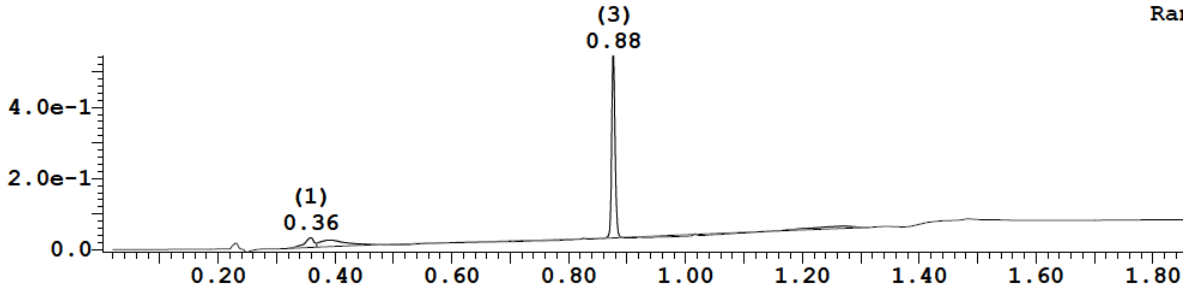
**Blank HPLC:**



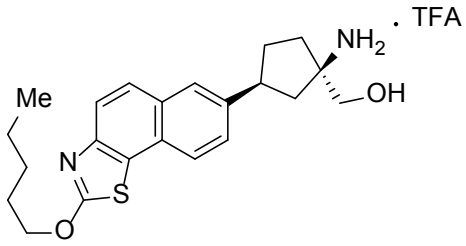

((1R,3S)-1-amino-3-(2-butoxynaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**25**)




LCMS (M+H): 371.2; LC retention time: 0.88 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-d$_4$) δ 7.86 (d, *J*=8.4 Hz, 2H), 7.83 (s, 1H), 7.80 - 7.72 (m, 1H), 7.58 (dd, *J*=8.5, 1.7 Hz, 1H), 4.62 (t, *J*=6.5 Hz, 2H), 3.70 (dd, *J*=16.5, 11.4 Hz, 2H), 3.50 - 3.38 (m, 1H), 2.58 (dd, *J*=12.9, 6.3 Hz, 1H), 2.28 (d, *J*=8.1 Hz, 1H), 2.18 - 1.98 (m, 3H), 1.97 - 1.80 (m, 3H), 1.65 - 1.50 (m, 2H), 1.05 (t, *J*=7.4 Hz, 3H).

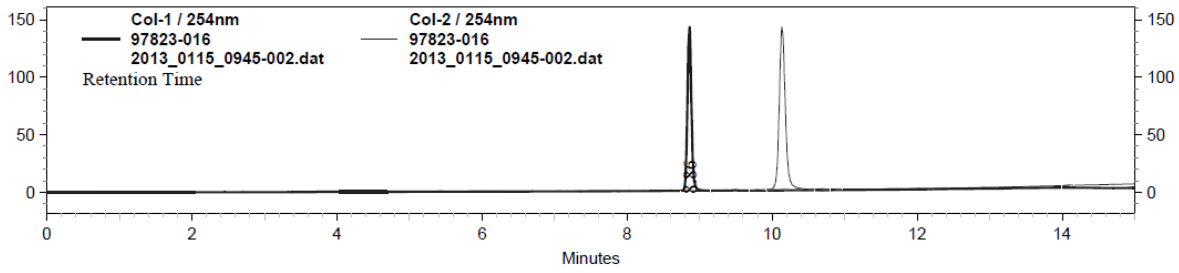



((1R,3S)-1-amino-3-(2-pentoxynaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**26**)

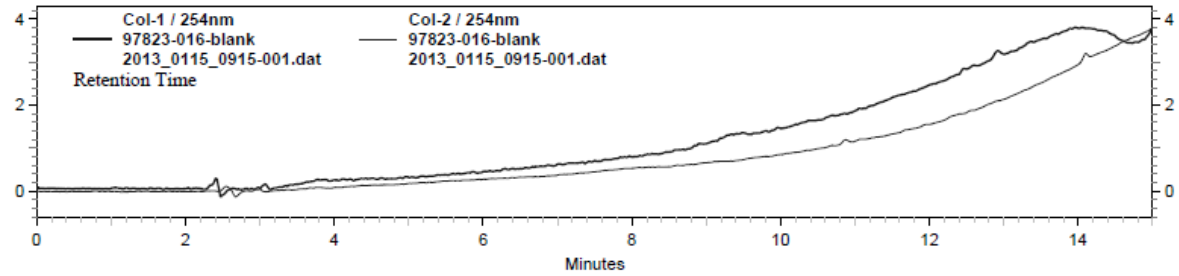


Retention time: 8.85 min and 10.14 (long run HPLC D and E); LCMS (M+H): 385.2; LC retention time: 0.92 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.91 - 7.84 (m, 2H), 7.83 (s, 1H), 7.80 - 7.73 (m, 1H), 7.58 (dd, *J*=8.6, 1.8 Hz, 1H), 4.61 (t, *J*=6.6 Hz, 2H), 3.70 (dd, *J*=16.1, 11.4 Hz, 2H), 3.47 - 3.38 (m, 1H), 2.58 (dd, *J*=13.4, 5.9 Hz, 1H), 2.36 - 2.22 (m, 1H), 2.19 - 2.00 (m, 3H), 1.99 - 1.82 (m, 3H), 1.58 - 1.38 (m, 4H), 0.99 (t, *J*=7.2 Hz, 3H).

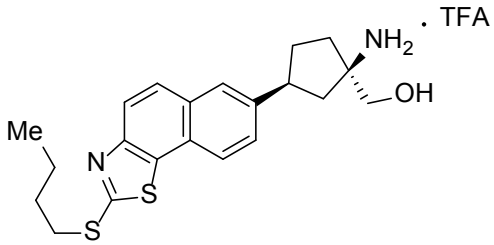
**Sample HPLC:**




**Blank HPLC:**

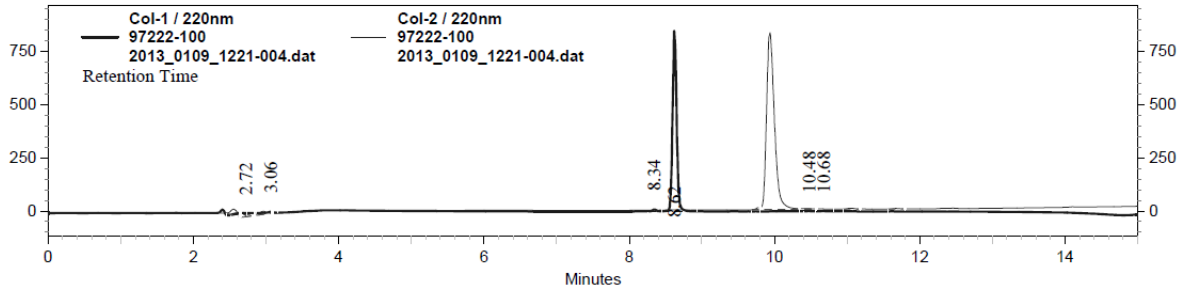



<u>((1R,3S)-1-amino-3-(2-(butylthio)naphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol, TFA salt (**28**)</u>

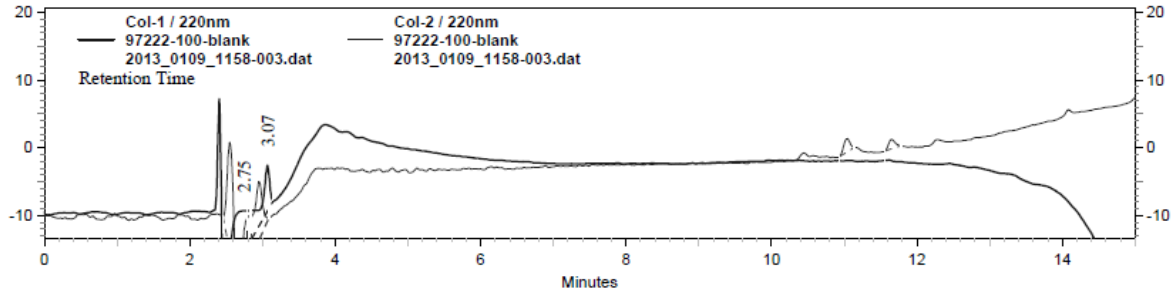



Retention time: 8.62 min and 9.94 (long run HPLC D and E); LCMS (M+H): 387.2; LC retention time: 0.90 min (analytical HPLC Method A), $^1$H NMR (400MHz, METHANOL-$d_4$) δ 7.98 - 7.83 (m, 4H), 7.62 (dd, *J*=8.6, 1.8 Hz, 1H), 3.71 (dd, *J*=16.9, 15.6 Hz, 2H), 3.47 - 3.39 (m, 3H), 2.64 - 2.53 (m, 1H), 2.37 - 2.27 (m, 1H), 2.19 - 2.08 (m, 1H), 2.06 - 1.94 (m, 2H), 1.93 - 1.81 (m, 3H), 1.65 - 1.50 (m, 2H), 1.02 (t, *J*=7.4 Hz, 3H).

**Sample HPLC:**

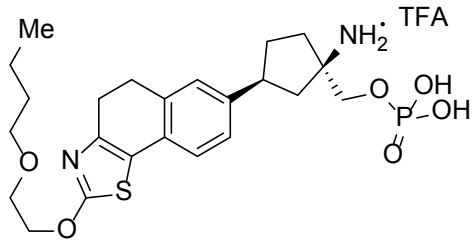



**Blank HPLC:**



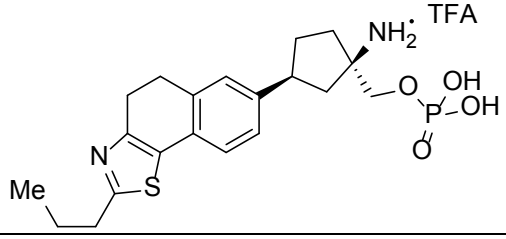
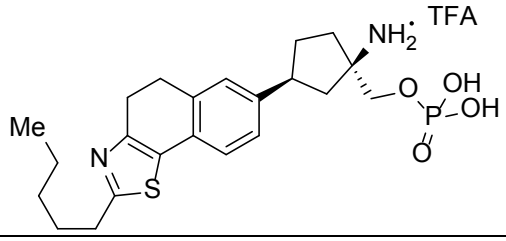
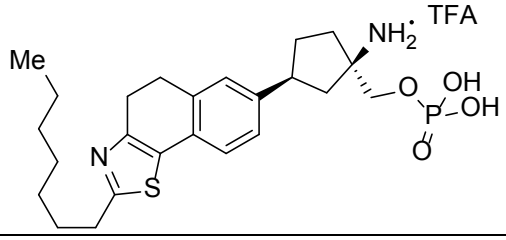
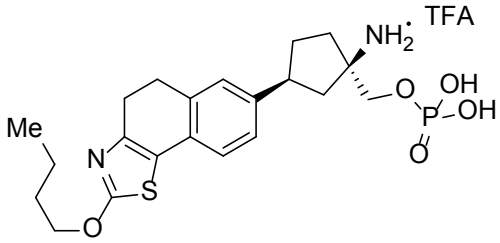
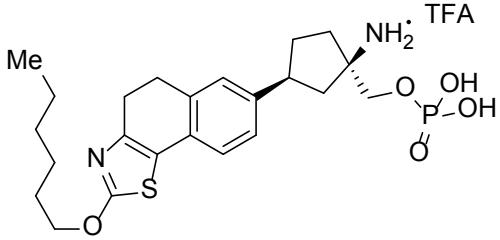
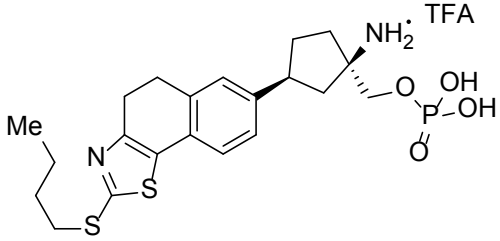

**General procedure for phosphates formation**

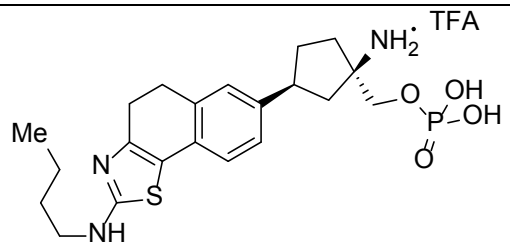
((1R,3S)-1-amino-3-(2-(2-butoxyethoxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methyl dihydrogen phosphate, TFA salt (**18-P**)




To a solution of ((1R,3S)-1-amino-3-(2-(2-butoxyethoxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methanol (4 mg, 9.6 µmol) in acetonitrile (0.5 mL) was added pyrophosphoryl chloride (0.013 mL, 0.096 mmol) at 0°C. After 5 min, the cold bath is removed and the reaction is allowed to reach room temperature. The reaction was stirred at this temperature for 1.5h. LCMS showed full conversion. The reaction was quenched by the addition of 0.2 mL of water and after stirring for 15 min, the solution was injected on HPLC and purified using Condition B affording ((1R,3S)-1-amino-3-(2-(2-butoxyethoxy)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)cyclopentyl)methyl dihydrogen phosphate, TFA (1.2 mg, 1.9 µmol, 19 % yield) as a white solid. LCMS (M+H): 497.2; LC retention time: 0.82 min (analytical HPLC Method A).

**Table 1**

| Example number | Structure | MS observed (M+1) | HPLC ret. time (min.) | HPLC method |
|---|---|---|---|---|
| **10-P** |  | 423.2 | 0.67 | A |
| **12-P** |  | 451.2 | 0.77 | A |
| **14-P** |  | 479.2 | 0.82 | A |
| **15-P** |  | 453.2 | 0.72 | A |
| **17-P** |  | 481.2 | 0.91 | A |
| **19-P** |  | 469.2 | 0.83 | A |

| Example number | Structure | MS observed (M+1) | HPLC ret. time (min.) | HPLC method |
|---|---|---|---|---|
| **21-P** |  | 452.1 | 0.57 | A |