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Synthesis and Evaluation of New HIV-1 Protease Inhibitors

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Synthesis and Evaluation of New HIV-1 Protease Inhibitors

A Major Qualifying Project
Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfillment of the requirements for the
Degree in Bachelor of Science in Chemistry

By
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Abstract

In the last year, an estimated 36.7 million people were living with the Human Immunodeficiency Virus (HIV), with 5,700 new infections occurring daily. Currently, there are 26 FDA approved antiretroviral drugs for the treatment of HIV; however, drug resistance still remains a major problem associated with the current treatments. GS-8374 is a novel protease inhibitor that makes use of a diethyl phosphonate group at the P1 position and has a superior resistance profile when compared to that of current FDA approved inhibitors. For this project three new analogues of GS-8374 were synthesized and tested against the I84V mutant, which confers resistance to most protease inhibitors, of HIV protease.
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Introduction

HIV Overview

The Acquired Immunodeficiency Syndrome (AIDS) was first recognized in 1981, with the subsequent recognition of the Human Immunodeficiency Virus (HIV) types 1 and 2 being the causative agents of the disease (Sharp, 2011; Greene, 2007). Since the disease’s discovery over 78 million people have been infected worldwide. In the last year, an estimated 36.7 million people were living with HIV, with 5,700 new infections occurring daily (“AIDS by the numbers”, 2016; “Global Aids Update”, 2016).

HIV destroys the body’s immune system by targeting the CD4 or T-cells. When left untreated, HIV diminishes the number of T-cells in the body leaving patients susceptible to infections. There are three stages of HIV: Acute HIV Infection, Clinical Latency and AIDS. Acute HIV Infection occurs within two to four weeks of the initial infection. At this phase of the infection a patient’s viral load, the concentration of HIV in their blood, is high and they are very contagious. In Clinical Latency, HIV replicates less rapidly allowing T-cell counts to rise. Under treatment, patients are able to remain in stage 2 for several decades. A patient’s viral load once again rises as HIV progresses into AIDS. AIDS is given as a diagnosis once T-cell counts fall below 200 cells/mm. Patients with AIDS will experience an increasing number of illnesses as a result of their severely damaged immune system (“About HIV/AIDS”, 2017).

HIV-1 Genome

HIV-1 is first characterized as a retrovirus, RNA is its genetic material, and then further characterized into the lentivirus subgroup meaning it replicates in non-dividing cells (Sharp, 2011). The entirety of HIV’S genome is encoded by an approximately 9 kb RNA strand. Nine open reading frames that encode for the pol, gag, env, vif, vpr, nef, tat, rer, and vpu genes which give rise to HIV-1’s 15 proteins (Frankel, 1998; Greene, 2007).

The gag and env genes are responsible for the structural polyproteins, Gag and Env. When proteolytically processed Env gives rise to gp120, a surface protein, and gp41, a transmembrane protein, while Gag gives rise to the matrix, capsid, nucleocapsid and p6 proteins. The pol gene gives rise to protease, reverse transcriptase and integrase, which are responsible for carrying out necessary enzymatic functions of the virus. The remaining 6 genes encode for accessory proteins: Vif, Vpr and Nef are proteins found in the viral particle, Tat and Ret are involved in gene regulation and Vpu is involved in assembly of the virion (Frankel, 1998).

HIV-1 Life Cycle

As HIV’s genome only encodes for fifteen proteins it must therefore use the host cells to successfully spread the infection (Frankel, 1998). First, the HIV virion’s gp120 binds to the host T-Cells through the CD4 receptor and either the CXCR4 or CCR5 co-receptors. Once bound gp41 undergoes a conformational change that allows for the fusion of the virion’s lipid bilayer and the T-Cell’s plasma membrane; the viral core is then released into the cytoplasm. Upon entry into the cytoplasm, the capsid is lost and reverse transcriptase synthesizes double stranded DNA from the viral RNA. Integrase then integrates the viral DNA into the host chromosomes. This newly integrated viral DNA then serves as the template for viral RNA synthesis. Env, Gag and Pol polyproteins are synthesized and transported to the plasma membrane. The protein complex induces the formation of a bud in the plasma membrane. Once budding is complete and the immature virion pinches off, viral protease cleaves gag and gag-pol
polyproteins generating the mature infectious virion. (Frankel, 1998; Online Mendelian Inheritance in Man, 2016; Greene, 2007)

**Current HIV Treatments**

With no current cure or vaccine available for HIV, treatment is focused on making HIV a manageable chronic illness. Currently, there are 26 FDA approved antiretroviral drugs approved for the treatment of HIV (“FDA-Approved HIV Medicines”, 2017). These drugs can be classified under five categories: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitor, fusion inhibitors, integrase inhibitors and protease inhibitors. Each class of drug targets a different part of HIV’s life cycle. The current standard for treatment of HIV is High Activity Antiretroviral Therapy (HAART) which is a combination of at least two of the classes of drugs. HAART has been seen to effectively reduce viral loads, increase T-cell count and halt the progression of HIV into AIDS. (Ghosh, 2016; Ali 2010; He, 2011)

In 1987 zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI) became the first FDA approved drug. NRTIs are analogues of the nucleotides used to construct DNA, however, NRTIs lack the 3’-OH group that allows DNA synthesis to continue. In doing this NRTIs competitively inhibit reverse transcriptase and thus interfere with the transcription of viral RNA to viral DNA. (Ghosh, 2016; “FDA-Approved HIV Medicines”, 2017)

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) are similar to NRTIs in the fact that they both inhibit reverse transcriptase; however, the method of doing so is quite different. NNRTIs are not analogues of the nucleotides used by reverse transcriptase, and are therefore not incorporated into the viral DNA strand. NNRTIs bind to the reverse transcriptase enzyme and non-competitively inhibit it. (Ghosh, 2016; “FDA-Approved HIV Medicines”, 2017)

Fusion Inhibitors prevent the entry of HIV into T-cells. Fusion Inhibitors target either HIV’s glycoproteins 120 and 41 or the coreceptors on the healthy T-cell’s surface. Integrate Inhibitors inhibit HIV’s integrase enzyme. This inhibition stops the insertion of the viral genome into the T-cell’s DNA. (Ghosh, 2016 “FDA-Approved HIV Medicines”, 2017)

Introduced in the mid 1990’s protease inhibitors were the second class of FDA approved antiretroviral drugs. Protease Inhibitors are designed to mimic the transition state of the natural substrate, the Gag and Gag-pol polyproteins. There have been two generations of protease inhibitors producing nine FDA approved drugs (see Figure 1). The first generation were highly peptidic and based on hydroxyethylene and hydroxyethylamine isosteres; however, the first generation of protease inhibitors had poor metabolic stability, short half-lives, and poor oral availability requiring them to need frequent doses, which lead to the development of the second generation of protease inhibitors. This second generation was designed in such a way to combat these problems with Atazanavir becoming the first FDA approved second generation protease inhibitor in 2003. (Ghosh, 2016)
HIV Protease

Structure
HIV Protease is a homodimer made up of two 99 residue monomers. Each monomer contains a conserved aspartic acid at residue 25 (See Figure 2). The aspartic acid residues form the dimer interface and the catalytic active site. When natural substrate or inhibitor is bound, two flexible flaps, made up of glycine-dense beta sheets, cover the active site; these flaps open to allow for the entry and release of ligands. (Weber, 2015; Ghosh, 2016)
Mode of Action

HIV-1 Protease is responsible for the processing and maturation of HIV’s structural and nonstructural proteins, including the viral envelope glycoproteins, reverse transcriptase, and integrase. Protease does so with the hydrolysis of gag and gag-pol polyproteins at specific cleavage sites (Ali, 2010)

Protease binds the natural substrate in an extended confirmation with the enzyme interacting with at least 7 amino acid residues. These sites of interactions are labeled as P4, P3, P2, P1, P1’, P2’, P3’ and P4’ (see Figure 3) (Ali, 2010)

![Polyprotein substrate](image)

![Transition state intermediate](image)

**Hydroxyethylamine I**  **Hydroxyaminopentane II**  **Hydroxyethylene III**  **Hydroxyethylamine-sulfonamide IV**  **Aza-hydroxyethylamine V**

**Figure 3: Scissle Bond and Transition State Mimics**

Drug Resistance

Drug resistance is one of the major problems associated with the current drugs used in HIV Treatment. Once highly promising drugs are becoming ineffective as mutant strains of HIV continue to develop. The mutated variants of HIV are the result of the lack of proofreading and low fidelity in DNA synthesis as it is transcribed from RNA through reverse transcriptase. A lack of adherence by patients to their drug program can also cause drug resistant strains to develop.

These mutations can then be organized into two groups: primary or secondary. A primary mutation is one that occurs in the active binding site of the enzyme. In HIV-1 Protease there are 15 sites of primary mutations distributed among the active site, flap, and distal regions of the dimer. Secondary mutations take place in all other residues, and often time occur to compensate for inefficiencies caused by primary mutations. Most mutations that occur are single amino acid substitutions and while insertions have been seen they are rare. Strains with high drug resistance are often the result of ten to twenty different mutations. Mutations leading to drug resistance often times do not have an effect on the binding of natural substrate and thus allow for the continued survival of HIV (Ali, 2010).

Primary mutations of HIV-1 protease include D30N, G48V, I50L, I50V, V82A, V82F, V83T, I84V and L90M. The I84V mutation confers resistance to most protease inhibitors, while the other mutations are more specified to specific protease inhibitors. The D30N mutation is seen to
cause failure in patients using nelfinavir, and the I50L/V is seen to cause resistance to APV/darunavir and atazanavir respectively. Resistance to ritonavir and saquinavir is the result of mutations at residue 82, and G48V is responsible for saquinavir and atazanavir resistance (Ali, 2010).

**Substrate Envelope Design Approaches**

When a majority of HIV-1 protease’s substrates are overlaid in the active site they share a conserved volume and shape: the substrate envelope. This is not only hypothesized to be used by the protease to recognize its substrates, but also can be used in the development of protease inhibitors that are less susceptible to drug resistance (Ali, 2010; Nalam, 2013). Just as the substrate envelope exists, so does the inhibitor envelope in which protease inhibitors occupy similar volumes in the active site. Drug resistance is often times the result of mutations that occur outside of the substrate envelope, but still within the inhibitor envelope. (see Figure 4)

![Figure 4: The Substrate and Inhibitor Envelopes](image)

**Figure 4:** The Substrate and Inhibitor Envelopes  
(A) The Substrate Envelope of HIV-1 Protease. (B) The Inhibitor Envelope (C) The substrate envelope (blue) superimposed with the inhibitor envelope (red). Residues that are in the inhibitor envelope, but not the substrate envelope and result in drug resistance are labeled. The inhibitors synthesized in this report are modeled after the basic backbone/scaffold of darunavir, but with side chains changed at the P1, P1’ and P2’ positions. The compound’s P1
group is based upon GS-8374 while the P1’ and P2’ groups are based off of three previous inhibitors synthesized and tested by the Schiffer lab (UMass-2, UMass-4, and UMass-9) (see Figure 5).

**Figure 5:** Structures of designed inhibitors 10a-d in relation to previously synthesized protease inhibitors

GS-8374 is a diethyl phosphonate derivative of TMC-126. First introduced with darunavir, the bis-THF group at the P2 position of both TMC-126 and GS-8374 has provided potent inhibition of both wild type HIV-1 protease as well as many drug resistant strains (Ali, 2010). However, TMC-126 loses inhibition potency for proteases containing the M46I and I50V or 184V and L90M mutations (He, 2011); this has led to further investigations into new protease inhibitors with the ability to combat these mutations. The addition of a diethyl phosphonate methoxy group at P1 sets GS-8374 and TMC-126 apart. The addition of such a group has reduced the $K_i$ of GS-8374 by a factor of 2 to 3 fold when compared to TMC-126. When tested against drug resistant strains M46I/I50V and 184V/L90M, GS-8374 also retained better potency with its $EC_{50}$ fold changes being 2.4 and 0.7, respectively, while TMC-126 ‘s were 72 and 12 (Callebaut, 2010). As all other positions on TMC-126 and G2-8374 are identical, this improved inhibition of both wild type and mutant strains is due to the addition of the diethylphosphonate group at P1. It is believed that the solvation of this phosphonate group is able to anchor the inhibitor and allows for greater flexibility, thus allowing it to accommodate for differences and retain inhibition in mutant binding sites (He, 2011).
UMass-2, UMass-4, and UMass-9 were designed based on the core scaffold of darunavir, to fit within the substrate envelope and to optimize the P1’ and P2’ groups in order to retain contact with the mutation sites of drug resistant variants. Their P1’ moieties were selected as isopentyl and isohexyl, for their greater flexibility and steric volume when compared isobutyl group of darunavir, which loses van der Waals contact with the 150V, V82A, and I84V mutations. The newly synthesized inhibitors’ Ki values were then tested against both wild type, two mutant variants that represent patterns of resistance mutations that occur in patients currently using protease inhibitors (L10I/G48V/154V/L63P/V82A, and L10I/L63P/A71V/G73S), and the signature resistant variant for darunavir and amprenavir (I50V/A71V) (Nalam, 2013).

Of the synthesized compounds UMass-2, which makes use of a (S)-2-methylbutyl group at P1’ and a 4-methoxybenzene at P2’, had the best resistance profile. The Ki of the inhibitor for both wild type and multidrug resistant strains was lower than that of darunavir. While darunavir retains a low nanomolar activity for drug resistant strains 1 and 2, UMass-2 was able to retain subnanomolar potency. For the strain containing I50V UMass-2 was able to keep low nanomolar potency, while darunavir significantly loses its potency (Nalam, 2013).

UMass-4, which differs from UMass-2 only by its P2’ group (1,3-benzodioxolane), also had a stronger resistance profile to that of darunavir. When tested against wild type HIV-protease, the inhibitor Ki value was sub picomolar while darunavir’s Ki was 5 pM. The inhibitor was also able to retain its potency to those mutants containing I50V (Nalam, 2013).

UMass-9 has a 1-ethyl-n-butyl group at P1’ and like UMass-4 has a 1,3-benzodioxolane group at P2’. With wild type HIV protease 11d had a sub picomolar Ki, and with the drug resistant variant containing I50V it was able to retain a Ki of 6 picomolar (Nalam, 2013).
Methods

**Epoxide Synthesis**

**Step 1: Synthesis of ethyl (S)-3-(4-(benzyl oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate**

Commercially available Boc-Tyr(Bzl)-OH (1) (20 g, 53.85 mmol) was dissolved in a 95:5 solution of acetonitrile and water (190 mL and 10 mL respectively). Oxyma (1.49 g, 80.77 mmol), EDC-HCl (15.49 g, 80.77 mmol), ethanol (6.30 mL), and sodium bicarbonate (36.19 g, 430.78 mmol) were added. The reaction mixture was stirred overnight under an argon atmosphere. Solvents were removed, and resulting oil was dissolved in ethyl acetate (500 mL). The organic layer was washed with aqueous sodium bicarbonate (3X). The organic layer was dried over sodium sulfate. The product was purified by flash chromatography on silica gel using 0-80% ethyl acetate in hexanes as an eluent to yield 2 as a clear oil (6.592 g, 61.2%).

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 1.229 (3H, t, $J=7.0$ Hz), 1.42 (9H, s), 2.98-3.07 (2H, m), 4.16 (2H, quart, $J=7.17$ Hz), 4.52 (1H, quart, $J=6.0$ Hz), 4.98 (1H, d, $J=8.0$ Hz), 5.04 (2H, s), 6.90 (2H, d, $J=8.5$ Hz), 7.05 (2H, d, $J=8.5$ Hz), 7.23-7.43 (5H, m). $^{13}$C NMR (CDCl$_3$, 5100 MHz) $\delta$ 14.2, 28.3, 37.5, 8.2, 54.6, 61.3, 70.0, 76.8, 77.1, 77.3, 79.8, 114.9, 127.5, 128.0, 128.4, 128.6, 130.4, 137.0, 155.1, 157.9, 172.0.

**Step 2: Synthesis of tert-butyl ((2S,3S)-1-(4-(benzyl oxy)phenyl)-4-chloro-3-hydroxybutan-2-yl)carbamate**

2 (6.59 g, 0.0165 mol) was dissolved in anhydrous tetrahydrofuran (150 mL). Iodochloromethane (6 mL) and lithium diisopropylamide (83 mL) were added. The reaction mixture was stirred at -78°C for 15 minutes. A 1:1 mixture of acetic acid and tetrahydrofuran (15 mL each) was added dropwise and the reaction mixture was stirred for 15 minutes. Toluene (200 mL) then a 1% Hydrochloric acid solution (100 mL) was added.
The resulting organic phase was washed with 0.5M sodium bicarbonate (10 mL) and separated. Ethanol (100 mL) was added and the reaction mixture was cooled to -78°C. Sodium borohydride (6.3 g, 0.167 mol) was added and the reaction mixture was stirred for 1 hour at -78°C. The reaction was quenched with saturated potassium bisulfate (100 mL), and then the organic phase was washed with water and dried over sodium sulfate. The reaction is concentrated on the rotovap and washed with hexanes. The resulting solid is then dissolved in hot ethyl acetate, filtered, cooled to room temperature and placed in the fridge to yield 4.

**Step3: Synthesis of tert-butyl (S)-2-(4-(benzyloxy)phenyl)-1-((S)-oxiran-2-yl)ethyl)carbamate**

<chem>
\[
\begin{array}{c}
\text{Boc}^+ \text{N} \text{HO} \text{Cl} \\
\text{4}
\end{array}
\text{KOH, EtOH} \\
\begin{array}{c}
\text{Boc}^+ \text{N} \text{HO} \text{C} \\
\text{5}
\end{array}
\]

4 (220 mg, 0.542 mmol) was suspended in ethanol (8 mL) and 1M potassium hydroxide (0.82 mL) was added. The reaction mixture was stirred under an argon atmosphere for 1 hour at room temperature. The reaction was concentrated on the rotovap then dissolved in dichloromethane. The organic layer was then separated using dichloromethane and water. The organic layer was then dried over sodium sulfate, filtered and concentrated on the rotovap to yield 5.

**Synthesis of (diethoxyphosphoryl)methyl trifluoromethanesulfonate**

<chem>
\[
\begin{array}{c}
\text{HO} \text{PO} \text{OEt} \\
\text{2,6-Lutidine, CH}_2\text{Cl}_2
\end{array}
\text{2,7ML, 80 mmol}
\begin{array}{c}
\text{F} \text{F} \text{F} \\
\text{S} \text{S} \text{O} \\
\text{OEt}
\end{array}
\rightarrow
\begin{array}{c}
\text{F} \text{F} \text{F} \\
\text{S} \text{S} \text{O} \\
\text{OEt}
\end{array}
\text{LR2-76 as a yellow oil (3.19 g, 78%)}
\]

Freshly distilled dichloromethane(25 mL) was added to a round bottom flask under argon, and cooled to -50°C. Diethyl(hydroxymethyl)phosphonate (2.28g, 13.56 mmol) and 2,6-lutidine ( 1.9 mL) were added. Triflic acid ( 2.7ML, 15.60 mmol) was added dropwise. Over the following 2 hours, the reaction mixture was allowed to warm up to 0°C, and then stirred for hour at 0°C. Diethyl ether (150 mL) was added and the reaction mixture was stirred for 15-30 minutes at 0°C. Lutidium triflate was filtered out using gravity filtration and the filtrate was washed with water (50 mL), hydrochloric acid (1M, 50 mL), and brine (50mL) and dried over magnesium sulfate. The magnesium sulfate was filtered out using gravity filtration. The pale pink solution was concentrated on the rotovap to yield LR2-76 as a yellow oil (3.19 g, 78%).
Synthesis of Inhibitor 10a

**Step 1: Synthesis of tert-butyl ((2S, 3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(isobutylamino)butan-2-yl)carbamate**

5 (1.00 g, 2.707 mmol) was dissolved in isopropanol (20 mL) and isobutyl amine (807 μL, 8.12 mmol). The reaction mixture is stirred for 6 hours at 80°C under reflux and an argon atmosphere. Solvents are removed using the rotovap and 6a is dissolved in chloroform, dried on the rotovap and placed on the high vacuum for one hour.

**Step 2: Synthesis of tert-butyl ((2S, 3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamide)butan-2-yl)carbamate**

6a is dissolved in a 1:1 mixture of ethyl acetate and water (40 mL each). Anhydrous sodium carbonate (0.58 g, 5.414 mmol), and 4-methoxybenzenesulfonyl chloride (0.69 g, 3.248 mmol) were added. The reaction mixture is stirred vigorously overnight; an empty balloon was set up to relieve pressure buildup. The aqueous and organic layers were separated, and the organic layer was extracted with ethyl acetate (3 x 50 mL). The combined organic extract was rinsed with brine and dried over sodium sulfate, and evaporated on the rotovap. The product was purified by flash chromatography on silica gel using 0-80% ethyl acetate in hexanes as an eluent to yield 7a (1.5 g, 90.36%).

$^1$H NMR(CDCl$_3$, 500 MHz) δ 1.30 (6H, t, J = 7.0 Hz), 2.16 (4H, dt, J = 15.5, 7.25 Hz), 4.56 (2H, d, J = 8.5 Hz). F NMR(CDCl$_3$, 500 MHz) δ 78.89, 75.35, 74.19. $^{31}$P NMR(CDCl$_3$, 500 MHz) δ 11.35, 12.19.
Step 3: (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzylxyloxy)phenyl)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)butan-2-yI)carbamate

7a was dissolved in a 1:1 mixture of trifluoroacetic acid and dichloromethane (18 mL each). The reaction mixture was stirred for one hour under an argon atmosphere. The reaction mixture was concentrated (1x reaction mixture, 2x dissolved in dichloromethane, 1x dissolved in toluene). The resulting deprotected amine was placed under high vacuum for one hour.

The deprotected amine was dissolved in anhydrous acetonitrile (25 mL) in an ice bath. Bis-tetrahydrofuran (730 mg, 2.693 mmol) and n,n-diisopropylethylamine (1.28 mL, 7.344 mmol) were added and stirred overnight under an argon atmosphere. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield impure 8a. The impure 8a was purified by flash chromatography on silica gel using 0-100% acetone in hexanes as an eluent to yield 8a (1.20 g, 73.17%).

Step 4: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-3-hydroxy-1-(4-hydroxyphenyl)-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)butan-2-yI)carbamate

8a was dissolved in ethanol (10 mL) and ethyl acetate (6 mL). Palladium on activated charcoal was added (120 mg). The reaction mixture was stirred under a hydrogen balloon overnight. The Palladium catalyst was filtered out of the reaction mixture. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 9a.

1H NMR( CDCl3, 500 MHz) δ 0.88 (d, 3H, J=6.5 Hz), 0.92 (d, 3H, J= 6.5 Hz), 1.51-1.54 (m, 1H), 1.64-1.72 (m, 1H), 1.81-1.87 (m, 1H), 2.70-2.84 (m, 2H), 2.88-3.04 (m, 4H), 3.09-3.19 (m, 1H), 3.68-3.74 (m, 2H), 3.82-3.87 (m, 6H), 3.94-3.96 (m, 1H), 5.03 (d, 2H, J= 7.5 Hz), 5.50 (s, 1H), 5.648(d, 1H), 6.73 (d, 2H, J= 8.5 Hz), 6.99 (d, 2H, J= 8.5 Hz), 7.06 (d, 2H, J= 8.0 Hz), 7.71 (d, 2H, J= 8.5 Hz).
Step 5: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)butan-2-yl)carbamate

9a was dissolved in acetonitrile (20 mL). Diethoxyphosphoryl)methyl trifluoromethanesulfonate (561 mg, 1.869 mmol) was added to the solution at 0°C. Over a 20 minute period, cesium carbonate (703 mg, 2.157 mmol) was added to the reaction mixture. The reaction mixture was first stirred at 0°C for an hour and then stirred for an additional hour at room temperature. The solvent was removed using the rotovap, and the resulting residue was partitioned between ethyl acetate and brine. The resulting organic layer was concentrated and the resulting product was purified by column chromatography on silica gel using 0-10% methanol in dichloromethane as an eluent to yield 10a (860 mg, 100%)

\[
\begin{align*}
\text{H NMR (CDCl}_3, 500 MHz) &\delta 0.87 (3H, d, J = 6.5 Hz), 0.91 (3H, d, J = 6.5 Hz), 1.36 (6H, t, J = 7.0 Hz), 1.53-1.57 (1H, m), 1.63-1.71 (1H, m), 1.82 (1H, sept, J = 7.0 Hz), 2.75-2.89 (2H, m), 2.89-2.99 (4H, m), 3.88-3.83 (3H, m), 3.93-3.97 (1H, m), 4.23 (6H, quint, J = 7.38 Hz), 4.46-5.03 (2H, m), 5.2 (1H, d, J = 5.5 Hz), 6.87 (2H, d, J = 8.5 Hz), 6.98 (2H, d, J = 9.0 Hz), 7.10 (2H, d, J = 8.5 Hz), 7.70 (2H, d, J = 9.0 Hz). \\
\text{C NMR (CDCl}_3, 100 MHz) &\delta 16.6, 16.6, 20.0, 20.2, 26.0, 27.4, 34.8, 45.5, 53.9, 55.3, 55.8, 59.0, 61.8, 63.0, 63.1, 63.1, 69.7, 71.0, 72.8, 73.6, 76.9, 77.2, 77.4, 109.4, 114.5, 114.7, 129.6, 129.8, 130.6, 131.0, 155.6, 157.6, 157.7, 163.3. \\
\end{align*}
\]

Synthesis of Inhibitor 10b

Step 1: Synthesis of tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(((S)-2-methylbutyl)amino)butan-2-yl)carbamate

5 (1.00 g, 2.707 mmol) was dissolved in isopropanol (12 mL) and (S)-(-)-2-methylbutylamine (380 μL, 3.248 mmol). The reaction mixture is stirred for 6 hours at 80°C under reflux and an argon
atmosphere. Solvents are removed using the rotovap and 6b is dissolved in chloroform, dried on the rotovap and placed on the high vacuum for one hour.

**Step 2: Synthesis of tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(N-((S)-2-methylbutyl)benzo[d][1,3]dioxole-5-sulfonamido)butan-2-yl)carbamate**

6b is dissolved in a 1:1 mixture of ethyl acetate and water (30 mL each). Anhydrous sodium carbonate (0.58 g, 5.414 mmol), and 1-benzodioxole-5-sulfonyl chloride (0.72 g, 3.248 mmol) were added. The reaction mixture is stirred vigorously overnight; an empty balloon was set up to relieve pressure build up. The aqueous and organic layers were separated, and the organic layer was extracted with ethyl acetate (2 x 50 mL). The combined organic extract was rinsed with brine and dried over sodium sulfate, and evaporated on the rotovap. The product was purified by flash chromatography on silica gel using 0-80% ethyl acetate in hexanes as an eluent to yield 7b (1.24 g, 71.68%).

\[^1\]H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 0.85 (t, 6H, \(J = 6.5\) Hz), 1.07 (quint, 1H, \(J = 7\)Hz), 1.36 (s, 9H), 1.46-1.50 (m, 1H), 1.59-1.63 (m, 2H), 2.78-2.93 (m, 3H), 2.99-3.10 (m, 3H), 3.73 (t, 2H, \(J = 7.0\) Hz), 3.84 (s, 1H), 4.63 (d, 1H, \(J = 7.0\) Hz), 5.04 (s, 2H), 6.06 (s, 2H), 6.88 (d, 1H, \(J = 8.0\) Hz), 6.92 (d, 2H, \(J = 8.5\) Hz), 7.16-7.18 (m, 3H), 7.31-7.33 (m, 2H), 7.37 (t, 2H, \(J = 8.0\) Hz), 7.43 (d, 2H, \(J = 7.0\) Hz).

\[^{13}\]C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 11.2, 17.0, 26.5, 28.4, 33.5, 34.7, 53.8, 54.8, 57.4, 70.1, 72.7, 76.9, 79.8, 102.4, 107.7, 108.4, 115.0, 123.2, 127.5, 128.0, 128.7, 130.7, 137.2, 148.4, 151.5, 156.2, 157.6.

**Step 3: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-(4-methoxy-N-((S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

7b was dissolved in a 1:1 mixture of trifluoroacetic acid and dichloromethane (18 mL each). The reaction mixture was stirred for one hour under an argon atmosphere. The reaction mixture was concentrated
(1x reaction mixture, 2x dissolved in dichloromethane, 1x dissolved in toluene). The resulting deprotected amine was placed under high vacuum for one hour.

The deprotected amine was dissolved in anhydrous acetonitrile (20 mL) in an ice bath. Bis-tetrahydrofuran (0.58 g, 2.129 mmol) and n,n-diisopropylethylamine (1.0 mL, 5.805 mmol) were added and stirred overnight under an argon atmosphere. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 8b (920 mg, 70.47%).

1H NMR( CDCl₃, 500 MHz) δ 0.87 (t, 6H, J= 6.5 Hz), 1.05-1.14 (m, 1H), 1.46-1.70 (m, 7H), 2.78 (q, 2H, J= 6.75 Hz), 2.90-3.14 (m, 5H), 3.55 (s, 1H), 3.69-3.74 (m, 2H), 3.83-3.86 (m, 3H), 3.96-3.99 (m, 1H), 4.89(d, 1H, J = 9Hz), 5.00-5.06 (m, 3H), 5.66 (d, 1H, J= 5.0 Hz), 6.07(s, 2H), 6.90( dd, 3H, J= 2.5 Hz, 8.5 Hz), 7.13 (d, 2H, J= 8.5 Hz), 7.17 (d, 1H, J= 1.5 Hz), 7.33 (dd, 2H, J= 2.0Hz, 8.0 Hz), 7.37-7.43 (m, 4H). 13C NMR ( CDCl₃, 100 MHz) δ11.2, 17.1, 26.0, 26.5, 33.6, 34.9, 45.5, 53.9, 55.3, 57.6, 69.7, 70.1, 70.9, 72.8, 73.6, 76.9, 77.2, 77.4, 102.5, 107.7, 108.5, 115.1, 123.3, 127.5, 128.2, 128.7, 129.3, 130.5, 131.7, 148.5, 151.7, 155.6, 157.7.

Step 4: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-3-hydroxy-1-(4-hydroxyphenyl)-4-(4-methoxy-N-((S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate

8b was dissolved in ethanol (7 mL) and ethyl acetate (4 mL). Palladium on activated charcoal was added to be (90 mg, 10% by mass). The reaction mixture was stirred under a hydrogen balloon overnight. The Palladium catalyst was filtered out of the reaction mixture. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 9b.

1H NMR( CDCl₃, 500 MHz) δ 0.87 (t, 6H, J= 7.0 Hz), 1.09 (sept, 1H, J= 7.25 Hz), 1.46-1.53 (m, 1H), 1.57-1.60 (m, 2H), 1.67-1.75 (m, 1H), 2.74-2.80 (m, 2H), 2.93-3.12 (m, 5H), 3.53 (s, 1H), 3.70-3.75 (m, 2H), 3.82 (s, 2H), 3.88-3.90 (m, 1H), 3.96-3.99 (m, 1H), 4.92-4.95 (m, 2H), 5.05 (dd, 1H, J= 6.25Hz, 13.0 Hz), 5.66 (d, 1H, J= 5.0 Hz), 6.09 (s, 2H), 6.75 (d, 2H, J= 8.5 Hz), 6.90 (d, 1H, J=8.0 Hz), 7.08 (d, 2H, J= 8.0 Hz), 7.33 (dd, 1H, J= 1.25 Hz, 8.25 Hz). 13C NMR ( CDCl₃, 100 MHz) δ 11.2, 17.1, 26.0, 26.6, 33.6, 34.9, 45.5, 53.9, 55.3, 57.6, 69.7, 70.1, 70.9, 72.8, 73.6, 76.9, 77.2, 77.4, 102.5, 107.7, 108.5, 115.1, 123.3, 127.5, 128.2, 128.7, 130.5, 131.7, 148.5, 151.7, 154.6, 155.7.
**Step 5: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-((diethoxyphosphoryl)methoxy)phenyl)-3-hydroxy-4-((4-methoxy-N-((S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

9b was dissolved in acetonitrile (20 mL) under an argon atmosphere. (Diethoxyphosphoryl)methyl trifluoromethanesulfonate (444 mg, 1.478 mmol) was added to the solution at 0°C. Over a 20 minute period, cesium carbonate (703 mg, 2.157 mmol) was added to the reaction mixture. The reaction mixture was first stirred at 0°C for 40 minutes and then stirred for one hour at room temperature. The solvent was removed using the rotovap, and the resulting residue was partitioned between ethyl acetate and brine. The resulting organic layer was concentrated and the resulting product was purified by column chromatography on silica gel using 0-10% methanol in dichloromethane as an eluent to yield 10b.

$^1$H NMR( CDCl$_3$, 500 MHz) δ 0.89 (6H, t, J= 6.5 Hz), 1.08-1.14 (1H,m), 1.39 (6H, t, J= 7.35 Hz), 1.49-1.1.54 (1H, m), 1.62 (2H, s), 1.62- 1.76 (1H, m), 2.78-2.84 (2H, m), 2.95-3.15 (5H,m), 3.59 (1H,s), 3.71-3.91 (5H,m), 3.92-4.00 (1H, m), 4.260 (6H, pent, J= 14.8), 4.94 (1H, d, J= 8.5 Hz), 6.06 (1H, dd, J = 6.5 Hz, 14.0 Hz), 5.68 (1H, d, J = 5.0 Hz), 6.12 (2H, d, J= 1.0 Hz), 6.92 (3H, t, J = 7.0 Hz), 7.18-7.18 (3H, m), 7.34 (1H, dd , J= 1.75 Hz, 8.25 Hz). $^{13}$C NMR ( CDCl$_3$, 100 MHz) δ 11.2, 16.6, 16.7, 17.1, 26.0, 26.6,33.6, 34.9, 45.4, 54.0, 55.2, 57.6, 61.8, 63.0, 63.0, 63.1, 69.7, 70.9, 72.8,76.9, 77.2, 77.4, 102.6, 107.7, 108.6, 109.4, 123.3 ,130.6, 130.9, 131.4, 148.5, 151.8, 155.6, 157.7, 157.8, 163.3. $^{31}$P NMR( CDCl$_3$, 500 MHz) δ 19.14, 19.18, 19.23, 19.27, 19.31.

**Synthesis of Inhibitor 10c**

**Step 1: tert-butyl ((2S,3R)-1-(4-(benzyl)phenyl)-3-hydroxy-4-((S)-2-methylbutyl)amino)butan-2-yl)carbamate**

5 (347.4 mg, 0.940 mmol) was dissolved in isopropanol (12 mL) and (S)(-)-2-methylbutylamine (140 μL, 1.128 mmol). The reaction mixture is stirred for 6 hours at 80°C under reflux and an argon atmosphere.
atmosphere. Solvents are removed using the rotovap and 6c is dissolved in chloroform, dried on the rotovap and placed on the high vacuum for one hour.

**Step 2: Synthesis of tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-((4-methoxy-N-((S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

6c is dissolved in a 1:1 mixture of ethyl acetate and water (15 mL each). Anhydrous sodium carbonate (0.69 g, 6.442 mmol), and 4-methoxybenzenesulfonyl chloride (0.80 g, 3.865 mmol) were added. The reaction mixture is stirred vigorously overnight; an empty balloon was set up to relieve pressure build up. The aqueous and organic layers were separated, and the organic layer was extracted with ethyl acetate (2 x 50 mL). The combined organic extract was rinsed with brine and dried over sodium sulfate, and evaporated on the rotovap. The product was purified by flash chromatography on silica gel using 0-80% ethyl acetate in hexanes as an eluent to yield 7c (0.35 g, 59.0%).

**Step 3: Synthesis of (3R,3aS,6aR)-hexahydrofurano[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzyloxy)phenyl)-3-hydroxy-4-((4-methoxy-N-((S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

7c was dissolved in a 1:1 mixture of trifluoroacetic acid and dichloromethane (6 mL each). The reaction mixture was stirred for one hour under an argon atmosphere. The reaction mixture was concentrated (1x reaction mixture, 2x dissolved in dichloromethane, 1x dissolved in toluene). The resulting deprotected amine was placed under high vacuum for one hour.

The deprotected amine was dissolved in anhydrous acetonitrile (10 mL) in an ice bath. Bis-tetrahydrofuran (0.17 g, 0.6138 mmol) and n,n-diisopropylethylamine (0.30 mL, 1.674 mmol) were added and stirred overnight under an argon atmosphere. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 8c (300 mg, 78.74%).
\^1H NMR (CDCl\textsubscript{3}, 500 MHz) \( \delta \) 0.86 (dd, 6H, \( J=7.25 \) Hz), 1.05-1.11 (m, 1H), 1.47-1.60 (m, 6H), 2.74-3.15 (m, 7H), 3.63 (s, 1H), 3.69-3.74 (m, 2H), 3.81-3.86 (m, 6H), 3.97 (m, 1H), 3.96 (dd, 1H, \( J=6.5 \) Hz, 9.5 Hz), 6.90 (d, 2H, 8.5 Hz), 6.99 (d, 2H, \( J=9.0 \) Hz), 7.13 (d, 2H, \( J=8.0 \) Hz), 7.33 (t, 1H, \( J=7.25 \) Hz), 7.40 (quint, 4H, \( J=7.5 \) Hz), 7.70 (d, 2H, \( J=9.0 \) Hz). \^{13}C NMR (CDCl\textsubscript{3}, 100 MHz) \( \delta \) 11.2, 17.1, 26.5, 33.6, 34.9, 45.5, 53.9, 55.2, 55.8, 57.6, 69.8, 70.1, 70.9, 73.6, 76.9, 77.2, 77.4, 109.4, 114.5, 115.1, 127.6, 128.2, 128.8, 129.5, 129.8, 130.9, 137.0, 155.6, 157.7, 163.3.

**Step 4: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-3-hydroxy-1-(4-hydroxyphenyl)-4-((4-methoxy-N-(S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

\[ \text{8c} \]

\[ \text{9c} \]

8c was dissolved in a 1:1 mixture of methanol and ethyl acetate (5mL of each). Palladium on activated charcoal was added to be (130 mg, 20% by mass). The reaction mixture was stirred under a hydrogen balloon overnight. The Palladium catalyst was filtered out of the reaction mixture. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 9c (320mg, 61.42%).

**Step 5: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-((diethoxyphosphoryl)methoxy)phenyl)-3-hydroxy-4-((4-methoxy-N-(S)-2-methylbutyl)phenyl)sulfonamido)butan-2-yl)carbamate**

\[ \text{9c} \]

\[ \text{10c} \]

9c was dissolved in acetonitrile (10 mL). (Diethoxyphosphoryl)methyl trifluoromethanesulfonate, (217 mg, 0.724 mmol) was added to the solution at 0°C. Over a 20 minute period, cesium carbonate (703 mg, 2.157 mmol) was added to the reaction mixture. The reaction mixture was then stirred for one hour at room temperature. The solvent was removed using the rotovap, and the resulting residue was partitioned between ethyl acetate and brine. The resulting organic layer was concentrated and the
resulting product was purified by column chromatography on silica gel using 0-10% methanol in dichloromethane as an eluent to yield 10c.

$^1$H NMR (CDCl$_3$, 500 MHz) δ 0.89 (6H, t, J= 7.25), 1.07 (1H, sext, J= 7.20 Hz), 1.36 (6H, t, J= 7.0 Hz), 1.46-1.59 (3H, m), 1.64-1.72 (1H, m), 2.75-2.81 (2H, m), 2.90-3.01 (4H, m), 3.02-3.13 (1H, m), 3.64-3.74 (3H, m), 3.81-3.90 (6H, m), 3.94-3.97 (1H, m), 4.23 (6H, quint, J= 7.38 Hz), 4.93 (1H, d, J= 8.5 Hz), 5.02 (1H, dd, J= 6.25 Hz, 14.0 Hz), 5.65 (1H, d, J= 11 Hz), 6.87 (2H, d, J= 8.5 Hz), 6.98 (2H, d, J= 9.0 Hz), 7.14 (1H, d, J= 8.5 Hz), 7.70 (2H, d, J= 9.0 Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 16.1, 16.6, 17.1, 26.0, 26.6, 33.6, 34.8, 45.5, 53.8, 55.2, 55.8, 57.5, 61.8, 63.0, 63.1, 63.1, 69.7, 70.9, 72.7, 76.9, 77.2, 77.4, 109.4, 114.5, 114.8, 129.6, 129.7, 130.6, 130.9, 155.6, 157.6, 1633. Pd NMR (CDCl$_3$, 500 MHz) 18.790, 19.10, 19.215. $^{31}$P NMR (CDCl$_3$, 500 MHz) δ 19.08, 19.12, 19.17, 19.22, 19.26, 19.30.

**Synthesis of Inhibitor 10d**

**Step 1: Synthesis of tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-4-((2-ethylbutyl)amino)-3-hydroxybutan-2-yl)carbamate**

5 (1.19 g, 3.221 mmol) was dissolved in isopropanol (20 mL) and 2-ethylbutylamine (0.45 mL, 3.543 mmol). The reaction mixture is stirred for 6 hours at 80°C under reflux and an argon atmosphere. Solvents are removed using the rotovap and 6d is dissolved in chloroform, dried on the rotovap and placed on the high vacuum for one hour.

**Step 2: Synthesis of tert-butyl ((2S,3R)-1-(4-(benzyloxy)phenyl)-4-(N-(2-ethylbutyl)benzo[d][1,3]dioxole-5-sulfonamido)-3-hydroxybutan-2-yl)carbamate**

6d was dissolved in a 1:1 mixture of ethyl acetate and water (15 mL of each). Anhydrous sodium carbonate (0.69 g, 6.442 mmol), and benzo[d][1,3]dioxole-5-sulfonyl chloride (0.85g, 3.865 mmol) were added. The reaction mixture is stirred vigorously overnight; an empty balloon was set up to relieve pressure build up. The aqueous and organic layers were separated, and the organic layer was extracted.
with ethyl acetate (2 x 50 mL). The combined organic extract was rinsed with brine and dried over sodium sulfate, and evaporated on the rotovap. The product was purified by flash chromatography on silica gel using 0-80% ethyl acetate in hexanes as an eluent to yield 7d (1.04 g, 51.48%).

1H NMR( CDCl$_3$, 500 MHz) δ 0.82 (q, 7H, J= 7.25), 1.24-1.36 (m, 17H), 2.84-3.71 (m, 7H), 3.73 (s, 2H), 3.93 (s, 1H), 4.61 (d, 1H, J= 7.5 Hz), 5.04 (s, 2H), 6.06 (s, 2H), 6.88 (d, 1H, J= 8.5 Hz), 6.92 (d, 2H, 8.5 Hz), 7.16-7.18 (m, 3H), 7.31-7.34 (m, 2H), 7.38 (t, 2H, J= 7.75), 7.43 (d, 2H, J= 7.5 Hz).

Step 3: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-(benzyloxy)phenyl)-4-(N-(2-ethylbutyl)benzo[d][1,3]dioxole-5-sulfonamido)-3-hydroxybutan-2-yl)carbamate

7d was dissolved in a 1:1 mixture of trifluoroacetic acid and dichloromethane (18 mL each). The reaction mixture was stirred for one hour under an argon atmosphere. The reaction mixture was concentrated (1x reaction mixture, 2x dissolved in dichloromethane, 1x dissolved in toluene). The resulting deprotected amine was placed under high vacuum for one hour.

The deprotected amine was dissolved in anhydrous acetonitrile (15 mL) in an ice bath. Bis-tetrahydrofuran (0.48 g, 1.747 mmol) and n,n-diisopropylethylamine (1.1 mL, 6.352 mmol) were added and stirred overnight under an argon atmosphere. The reaction was concentrated and purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 8d (717 mg, 63.50%).

1H NMR( CDCl$_3$, 500 MHz) δ 0.81-0.84 (m, 6H), 1.24-1.35 (m, 3H), 1.42-1.47 (m, 2H), 1.51-1.56 (m, 6H), 1.60-1.69 (m, 1H), 2.74-2.85 (m, 2H), 2.97-3.13 (m, 5H), 3.67-3.73 (m, 3H), 3.79-3.85 (m, 3H), 3.96 (dd,1H, J= 6.25Hz,9.75 Hz), 6.90 (d, 3H, J= 9.0 Hz), 7.13 (d, 2H, J= 8.0 Hz), 7.17 (d, 1H, J= 1.5 Hz), 7.31-7.34 (m, 2H), 7.37-7.43 (m, 4H). 13C NMR ( CDCl$_3$, 100 MHz) δ 10.4, 10.8, 22.9, 23.2, 25.9, 35.1, 39.3, 45.5, 54.0, 55.2, 69.7, 70.2, 70.8, 73.2, 73.6, 76.9, 77.2, 77.4, 102.6, 107.7, 108.6, 109.4, 115.1, 123.4, 127.6, 128.8, 130.5, 137.1, 148.5, 151.8, 155.6, 157.8.
Step 4: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-4-(N-(2-ethylbutyl)benzo[d][1,3]dioxole-5-sulfonamido)-3-hydroxy-1-(4-hydroxyphenyl)butan-2-yl)carbamate

8d was dissolved in ethyl acetate (10 mL). Palladium on activated charcoal was added to be (90 mg, 10% by mass). The reaction mixture was stirred under a hydrogen balloon overnight. The Palladium catalyst was filtered out of the reaction mixture. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 9d.

1H NMR (CDCl₃, 500 MHz) δ 0.75-0.86 (m, 6H), 1.24-1.33 (m,5H), 1.35-1.49 (m, 3H), 1.61-1.69 (m, 1H), 1.94 (s, 1H), 2.69 (dd, 1H, J= 9.25 Hz, 13.75 Hz), 2.86-2.92 (m, 2H), 2.97-3.10 (m, 4H), 3.65-3.74 (m, 3H), 3.79-3.83 (m, 3H), 3.94 (dd, 1H, J= 6.0 Hz, 9.5 Hz), 5.03 (dd, 1H, J= 6.0 Hz, 14.0 Hz), 5.14 (d, 1H, 8.5 Hz), 5.64 (d, 1H, J= 5.5 Hz), 6.08 (s, 2H), 6.27 (s, 1H), 6.72 (d, 2H, J= 8.5 Hz), 6.89 (d, 1H, J= 8.0 Hz), 7.04 (d, 2H, J= 8.0 Hz), 7.17 (d, 1H, J= 3.0 Hz), 7.33 (dd, 1H, J= 1.5 Hz, 8.5 Hz).

Step 5: Synthesis of (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(4-((diethoxyphosphoryl)methoxy)phenyl)-4-(N-(2-ethylbutyl)benzo[d][1,3]dioxole-5-sulfonamido)-3-hydroxybutan-2-yl)carbamate

9d was dissolved in acetonitrile (15 mL). (Diethoxyphosphoryl)methyl trifluoromethanesulfonate (352 mg, 1.172 mmol) was added to the solution at 0°C. Over a 20 minute period, cesium carbonate (703 mg, 2.157 mmol) was added to the reaction mixture. The reaction mixture was first stirred at 0°C for 40 minutes and then stirred for one hour at room temperature. The solvent was removed using the rotovap, and the resulting residue was partitioned between ethyl acetate and brine. The resulting organic layer was concentrated and the resulting product was purified by column chromatography on silica gel using 0-10% methanol in dichloromethane as an eluent to yield 10d.
\[^1\text{H}\ \text{NMR}\ (\text{CDCl}_3,\ 500\ \text{MHz})\ \delta\ 0.825\ (6\text{H},\ t,\ J=7.25\ \text{Hz}),\ 1.28-1.33\ (3\text{H},\ m),\ 1.36\ (5\text{H},\ t,\ J=7.0\ \text{Hz}),\ 1.41-1.44\ (2\text{H},\ m),\ 1.56-1.59\ (1\text{H},\ m),\ 1.64-1.73\ (1\text{H},\ m),\ 2.75-2.86\ (2\text{H},\ m),\ 2.90-3.11\ (5\text{H},\ m),\ 3.68-3.80\ (5\text{H},\ m),\ 3.82\ (1\text{H},\ dt,\ J=2.5\ \text{Hz},\ 8.0\ \text{Hz}),\ 3.94\ (1\text{H},\ m),\ 4.23\ (6\text{H},\ quint,\ J=7.38\ \text{Hz}),\ 4.90\ (1\text{H},\ d,\ J=8.5\ \text{Hz}),\ 5.03\ (1\text{H},\ dd,\ J=6.25\ \text{Hz},\ 14.25\ \text{Hz})\ 5.65\ (1\text{H},\ d,\ J=5.0\ \text{Hz}),\ 6.90\ (2\text{H},\ s),\ 6.89\ (3\text{H},\ t,\ J=9.0\ \text{Hz}),\ 7.14-7.16\ (3\text{H},\ m),\ 7.33\ (1\text{H},\ dd,\ J=1.75\ \text{Hz},\ 8.25\ \text{Hz}).\ \]^13\text{C}\ \text{NMR}\ (\text{CDCl}_3,\ 100\ \text{MHz})\ \delta\ 10.4,\ 10.7,\ 16.6,\ 16.6,\ 22.9,\ 23.2,\ 26.0,\ 35.0,\ 39.3,\ 45.4,\ 54.0,\ 55.1,\ 55.2,\ 61.7,\ 63.0,\ 63.1,\ 69.7,\ 70.8,\ 73.2,\ 73.6,\ 76.9,\ 77.2,\ 77.4,\ 102.6,\ 107.7,\ 108.6,\ 109.4,\ 123.3,\ 130.6,\ 130.9,\ 131.2,\ 148.5,\ 151.8,\ 155.6,\ 157.6,\ 157.74.\ ^{31}\text{P}\ \text{NMR}\ (\text{CDCl}_3,\ 500\ \text{MHz})\ \delta\ 19.10,\ 19.13,\ 19.18,\ 19.22,\ 19.27,\ 19.31.\ \text{Pd}\ \text{NMR}\ (\text{CDCl}_3,\ 500\ \text{MHz})\ \delta\ 19.22.

**Synthesis of Inhibitor 12a (TMC-126)**

\(11a\) (500 mg, 0.988 mmol) was dissolved in a one to one mixture of TFA and dichloromethane (6 mL each). The reaction mixture was stirred under argon for one hour. The reaction mixture was concentrated on the rotovap. The product was dissolved in dichloromethane and concentrated twice, and then dissolved in toluene, concentrated and placed on the high vacuum for one hour. The resulting deprotected amine was dissolved in acetonitrile (10 mL) in an ice bath. Bis-tetrahydrofuran (0.30 g, 1.09 mmol) and n,n-diisopropylethylamine (0.52 mL, 2.97 mmol) were added and the reaction mixture was stirred overnight. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield impure 12a. The impure product was further purified by flash chromatography on silica gel using 0-100% dichloromethane in methanol, and then again using 0-100% acetone in hexanes as an eluent to yield 12a.

JRF-03 (1): \[^1\text{H}\ \text{NMR}\ (\text{CDCl}_3,\ 500\ \text{MHz})\ \delta\ 0.88\ (d,\ 3\text{H},\ J=6.5\ \text{Hz}),\ 0.93\ (d,\ 3\text{H},\ J=6.5\ \text{Hz}),\ 1.47\ (dd,\ 1\text{H},\ J=5.5\ \text{Hz},\ 13.5\ \text{Hz}),\ 1.59-1.65\ (m,\ 1\text{H}),\ 1.83\ (quint,\ 1\text{H},\ J=7.0\ \text{Hz}),\ 2.77-2.83\ (m,\ 2\text{H}),\ 2.87-2.92\ (m,\ 1\text{H}),\ 2.96-3.00\ (m,\ 1\text{H}),\ 3.08\ (dd,\ 1\text{H},\ J=4.25\ \text{Hz},\ 14.25\ \text{Hz}),\ 3.14-3.19\ (m,\ 1\text{H}),\ 3.66-3.72\ (m,\ 3\text{H}),\ 3.83-3.97\ (m,\ 7\text{H}),\ 4.94\ (d,\ 1\text{H},\ J=9.0\ \text{Hz}),\ 4.99-5.03\ (m,\ 1\text{H}),\ 5.64\ (d,\ 1\text{H},\ J=5.0\ \text{Hz}),\ 6.99\ (d,\ 2\text{H},\ J=9.0\ \text{Hz}),\ 7.21\ (t,\ 3\text{H},\ J=7.5\ \text{Hz}),\ 7.26-7.29\ (m,\ 3\text{H}),\ 7.71\ (d,\ 2\text{H}, J=9.0\ \text{Hz}).\ \]^13\text{C}\ \text{NMR}\ (\text{CDCl}_3,\ 100\ \text{MHz})\ \delta\ 19.9,\ 20.2,\ 25.8,\ 27.3,\ 35.7,\ 45.3,\ 53.8,\ 55.1,\ 55.7,\ 58.9,\ 69.6,\ 70.8,\ 72.8,\ 73.5,\ 76.8,\ 77.0,\ 77.2,\ 77.3\ 109.3,\ 114.4,\ 126.6,\ 128.6,\ 129.4,\ 129.5,\ 129.7,\ 137.6,\ 155.5,\ 163.1.\)
Synthesis of Inhibitor 12b

11b (500 mg, 0.960 mmol) was dissolved in a one to one mixture of TFA and dichloromethane (6 mL each). The reaction mixture was stirred under argon for one hour. The reaction mixture was concentrated on the rotovap. The product was dissolved in dichloromethane and concentrated twice, and then dissolved in toluene, concentrated and placed on the high vacuum for one hour. The resulting deprotected amine was dissolved in acetonitrile (10 mL) in an ice bath. Bis-tetrahydrofuran (286 mg, 1.06 mmol) and n,n-diisopropylethylamine (0.5 mL, 2.99 mmol) were added and the reaction mixture was stirred overnight. The reaction was concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as an eluent to yield 12b (480 mg, 86.71%)

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 0.89 (d, 3H, $J=6.5$ Hz), 0.94 (t, 3H, $J=7.0$ Hz), 1.48 (dd, 1H, $J=5.0$ Hz, 9 Hz), 1.64 (t, 1H, $J=11.0$ Hz), 1.83 (quint, 1H, $J=7.0$ Hz), 2.78-3.01 (m, 6H), 3.04-3.17 (m, 2H), 3.60 (s, 1H), 3.66-3.72 (m, 2H), 3.83-3.90 (m, 3H), 4.92 (d, 1H, $J=8.5$ Hz), 5.08 (q, 1H, $J=7.0$ Hz), 5.65 (d, 1H, $J=5.5$ Hz), 6.09 (s, 2H), 6.90 (d, 1H, $J=8.5$ Hz), 7.09-7.35 (m, 9H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.0, 20.3, 25.9, 27.5, 35.8, 45.5, 54.0, 55.3, 59.1, 69.7, 70.9, 73.1, 73.6, 76.9, 77.2, 77.4, 77.4, 102.5, 107.7, 108.5, 109.4, 123.3, 126.8, 128.7, 129.5, 131.5, 137.7, 148.5, 151.7, 155.6.

Synthesis of Inhibitor 12c

11c (500 mg, 0.937 mmol) was dissolved in 4M hydrochloric acid in dioxane (8 mL) and the reaction mixture was stirred for 2 hours under an argon atmosphere. Solvents are removed on the rotovap. Diethyl ether is added and removed using a pipet, twice, to remove any excess hydrochloric acid. The resulting deprotected amine is placed on the high vacuum for one hour. The resulting deprotected amine is dissolved in acetonitrile (10mL) in and ice bath. Bis-tetrahydrofuran (280 mg, 1.03 mmol), and n,n-diisopropylethylamine (0.65 mL, 3.748 mmol) were added and the reaction mixture is stirred overnight. The reaction is concentrated and the product was purified by flash chromatography on silica gel using 0-100% ethyl acetate in hexanes as the eluent to yield 12c (400 mg, 72.39%)
$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 0.91 (d, 3H, J = 6.5 Hz), 0.97 (d, 3H, J = 6.5 Hz), 1.5 (dd, 1H, J = 6.0 Hz, 12.5 Hz), 1.62 (s, 1H), 1.86-1.92 (m, 1H), 2.82-2.86 (m, 1H), 2.90-2.95 (m, 2H), 3.06-3.14 (m, 3H), 3.25 (dd, 1H, J = 8.5 Hz, 15.0 Hz), 3.64 (d, 1H, J = 3 Hz), 3.68-3.74 (m, 2H), 3.87 (td, 1H, J = 2.5 Hz, 8.0 Hz), 3.91-3.98 (m, 3H), 4.98 (d, 1H, J = 8.0 Hz), 5.02-5.06 (m, 1H), 5.67 (d, 1H, J = 5.0 Hz), 7.24 (d, 3H, J = 7.5 Hz), 7.28-7.32 (m, 3H), 7.91 (dd, 1H, J = 2 Hz, 6.5 Hz), 8.28 (d, 1H, J = 9.0 Hz), 8.48 (d, 1H, J = 1.5 Hz), 9.24 (s, 1H).

$^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.0, 20.3, 26.0, 27.5, 35.8, 45.5, 53.9, 55.4, 59.0, 69.7, 70.9, 73.0, 73.9, 109.5, 122.4, 124.6, 124.9, 126.8, 128.8, 129.5, 134.6, 135.7, 137.6, 155.67, 155.8, 158.2.
The goal of this project was to synthesize three new analogues of the protease inhibitor GS-8374 with the hope of improving its resistance profile against multidrug resistant strains of HIV-1 protease. Additionally, four previously synthesized inhibitors were also synthesized to be used in the comparison of how effective the P1’ and P2’ side chain changes were to the potency of the inhibitors against the I84V mutant of HIV-1 protease.

**Synthetic**

**Synthesis of Epoxide**

Epoxide 5, the starting material for inhibitors 10a-d, was synthesized through a four step synthesis starting with commercially available ethyl (S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate 1. (See Scheme 1)

![Scheme 1: Synthetic Scheme for Epoxide 5 Synthesis](image)

Carboxylic acid 1 was reacted with oxyma, EDC∙Hydrochloric acid, ethanol and sodium bicarbonate in acetonitrile and water to yield ester 2. Ester 2 was reduced through the treatment of lithium diisopropylamide and iodochloromethane in anhydrous tetrahydrofuran at -78°C. The resulting chloroketone 3 was further reduced with sodium borohydride in ethanol at -78°C to yield chlorohydrin 4. 4 was reacted with 1M potassium hydroxide in ethanol for one hour to yield epoxide 5.

**Synthesis of Inhibitors 10a-d**

The synthesis of inhibitors 10a-d was achieved through a six step synthesis starting from a common epoxide intermediate 5, derived from a tyrosine derivative (See Scheme 2).
Scheme 2: Synthetic Scheme for Inhibitors 10a-d

To introduce the P2’ moiety, epoxide 5 was opened via the nucleophilic substitution of an amine [(a) isobutylamine, (b/c) (S)-(-)-2-methylbutylamine, (d) 2-ethylbutylamine] in isopropanol at reflux. Amino alcohol 6 was reacted with sulfonyl chloride [(a/c) 4-methoxybenzene sulfonyl chloride (b/d) 1-benzodioxole-5-sulfonyl chloride] in sodium carbonate to introduce the sulfonamide at the P1’ position of 7 with yields between 51.48% and 90.36%. The amine was treated with trifluoroacetic acid and dichloromethane for one hour at room temperature and the resulting deprotected amine was reacted with bis-tetrahydrofuran and n,n-diisopropylethylamine in acetonitrile overnight to add the bis-THF moiety at P2 on compound 8 with percent yields between 63.50% and 78.74%. The phenyl ether at the P1 position of 8 was reduced with catalytic palladium in methanol and ethylacetate, The resulting compound 9 was reacted with (diethoxyphosphoryl)methyl trifluoromethanesulfonate in acetonitrile with cesium carbonate being added over a 20 minute period to incorporate the diethyl phosphonate moiety to the p2 position of inhibitors 10a-d.

Synthesis of Inhibitors 12a-c
The synthesis of inhibitors 12a-c was achieved through a one-step synthesis starting from compounds 11a-c, which were previously synthesized in the lab.

Scheme 3: Synthetic Scheme for Inhibitors 12a-c
The Boc protected amines were treated with trifluoroacetic acid and dichloromethane for one hour at room temperature and the resulting deprotected amine was reacted with bis-tetrahydrofuran and n,n-diisopropylethylamine in acetonitrile overnight to produce compounds 12a-c with yields between 72.39% and 86.71%.

### 184V Mutant Assays

The 7 synthesized inhibitors’, their UMass parent inhibitors’ and darunavir’s, the current leading FDA approved protease inhibitor, $K_i$ values were calculated for the I84V mutant of HIV-1 protease (See Table 1). All synthesized inhibitors had $K_i$ values in the low picomolar range and were lower than that of darunavir’s.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ (pM) for I84V Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a (GS-8374)</td>
<td><img src="10a.png" alt="Image" /></td>
</tr>
<tr>
<td>10b</td>
<td><img src="10b.png" alt="Image" /></td>
</tr>
<tr>
<td>10c</td>
<td><img src="10c.png" alt="Image" /></td>
</tr>
<tr>
<td>10d</td>
<td><img src="10d.png" alt="Image" /></td>
</tr>
<tr>
<td>Compound</td>
<td>Assay Results</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
</tr>
<tr>
<td>12a (TMC-126)</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>12b</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>12c</td>
<td>2.6 ± 0.08</td>
</tr>
<tr>
<td>UMass-2</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>UMass-4</td>
<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>UMass-9</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>DRV</td>
<td>25.6 ± 5.6</td>
</tr>
</tbody>
</table>

Table 1: I84V Assay Results
Discussion

The goal of this report was to synthesize and evaluate the resistance profile of new optimized protease inhibitors against the I84V mutant of HIV-1 protease. The three synthesized inhibitors were modeled after the scaffold for darunavir while optimizing the P1, P1’ and P2’ positions. The inhibitor’s P1 group was based upon GS-8374 while the P1’ and P2’ groups are based off previously synthesized inhibitors from the Schiffer lab.

Of the synthesized inhibitors, 10a-d and 12a-c, all were found to have low picomolar Ki values ranging from 1.8 ± 0.6 to 5.6 ± 1.5 pM. Darunavir had the least favorable resistance profile with a Ki of 25.6 ± 5.6, showing that all seven combinations of altered P1, P1’ and P2’ groups had a positive impact on the resistance profile.

Inhibitor 10c was found to have the most favorable resistance profile against the I84V mutation with a Ki of 1.8 ± 0.6; this finding is consistent with those of the previously synthesized UMass compounds in which the inhibitors containing a (S)-2-methylbutyl group at P1’ and a 4-methoxybenezene at P2’ had the most favorable resistance profiles. Inhibitor 12a (TMC-126) had the second most favorable resistance profile, with a Ki of 1.9 ± 0.7 pM. Both 12a and 10c have the same P2’ group, though their P1 and P1’ groups differ. Inhibitor 10a had a higher Ki value than both 12a and 10c, suggesting that the larger P2’ moiety of 10c or the overall specific combination of all side groups are responsible for 10c’s more favorable resistance profile.

When compared to their UMass parent compounds, UMASS-2, UMass-4, and UMass-9, inhibitors 10a-b’s Ki values were nearly half that of the non-phosphonate compounds. Such a change proves that the use of the diethylphosphonate moiety at the P1 position is indeed beneficial in improving the resistance profile of already established inhibitors against the I84V mutant of HIV-1 protease.
References


2. AIDS by the numbers - AIDS is not over, but it can be [PDF]. (2016, November 21). Geneva, Switzerland: UNAIDS


Appendix

Appendix A: Maestro Models of Inhibitors 10a-d in WT HIV Protease

Co-crystal structure of **GS-8374** (magenta) in WT HIV-1 protease PDBID: 2I4W

Energy-minimized model of **10b** (green) in WT HIV-1 protease
Energy-minimized model of 10c (cyan) in WT HIV-1 protease

Energy-minimized model of 10d (pink) in WT HIV-1 protease