Synthesis and Evaluation of New HCV NS3/4A Protease Inhibitors

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Abstract

The Hepatitis C virus infects an estimated 170 million people worldwide, and is the major cause of hepatitis, cirrhosis and liver cancer. The available treatment is a combination therapy with two non-specific antiviral agents, pegylated interferon (Peg-IFN) and ribavirin (RBV), which is effective for only half of the treated patients and has severe side effects. Recent addition of HCV NS3/4A protease inhibitors, Telaprevir and Boceprevir, to the therapy has improved HCV treatment outcomes. However, the severe side effects associated with Peg-IFN/RBV and the rapid emergence of drug resistance against protease inhibitors limit the effectiveness of this new therapy. In addition, the new therapies are mostly developed for genotype 1 patients, and may not be affective against other genotypes. Therefore, new HCV therapies must be developed that are effective against a broader spectrum of resistant viral variants and HCV genotypes. This project involved following the multi-step synthesis of a new HCV NS3/4A protease inhibitor, MK-5172, evaluated the synthesis plan, and provided the University of Massachusetts Medical School with a new variant to test activity in biochemical assays against wild-type and drug resistant NS3/4A protease variants.
Background

Hepatitis C continues to affect 170 million people worldwide; nearly 3% of the global population. This disease develops from an infection of the Hepatitis C Virus (HCV), and primarily attacks and inflames the liver. While some treatments have become available, there is no vaccine yet that can combat this disease that affects both humans and chimpanzees. Chronic HCV infections lead to cirrhosis, as well as liver failure or liver cancer. Hepatitis C accounts for 30-40% of orthotopic liver transplants in many western countries,¹ and results in an estimated 476,000 deaths annually as a result of end-stage liver disease and its complications.²

The spread of Hepatitis C has increased in the 20th century, and about 4 million people are infected each year.³ This is primarily due to unsafe and unclean medical practices and equipment, which is also a factor in the spread of HCV by intravenous drug use and needle sharing. However, this is not the only way to transmit the virus. As seen in Figure 1, drug use only accounts for about 60% of the continued spread.⁴ HCV screenings for blood used in transfusions and organs for transplants have not always been performed, and are still not done in some countries due to the cost. The requiring of these screenings in 1992 by the United States reduced the risk of infection from 1 in 200 to 1 in 10,000,000 per unit of blood,⁵ but there was still a great for risk anyone who received a blood transfusion prior. HCV is also believed to be spread through sexual intercourse, but that remains a controversial subject. An association between infections and high-risk sexual behavior exists, but that may “be because of a high rate of sexual transmission or… unacknowledged drug use.”⁴ Other ways to transmit the virus include body tattoos and piercings, due to unclean needles or contaminated dyes, and vertical transmission from mother to fetus.
The map shown in Figure 2 shows the major areas of the globe that are affected by HCV. It is easy to spot the high and low prevalence areas; for example about 2% of residents in countries like the US, Australia, Spain, and Japan are infected. That number has decreased over the past years because of improved screenings of blood and organs before use. The greatest prevalence of HCV is in Egypt, and certain areas of the country have reported infection rates as high as 24% of the local population.³

The Hepatitis C Virus, discovered in 1989, is a member of the Flaviviridae family of viruses, and the Hepacivirus genus. The virus’ structure is an enveloped, positive single-stranded RNA molecule 9.6 kilobases long, and encodes about 3,000 amino acids. The virus generates 10 viral proteins: the viral core, Envelope 1 (E1), E2, p7, and nonstructural (NS) 2, NS3, NS4A and NS4B, and NS5A and NS5B.⁶ These proteins spread the virus throughout the host, and also affect different cellular functions. HCV has nine major genotypes, numbered one through nine, but to date more than 30 have been identified. Genotype 1 accounts for 72% of cases worldwide, Genotype 2 accounts for 16-19%, 8-10% for Genotype 3, and 1-2% for the remaining.⁷ Apart from the United States, Genotype 1 is also the most common is South America and Europe.⁸ The virus can remain dormant for decades after initial infection, and when active primarily causes cirrhosis and liver cancer.³ About 15% of those infected by HCV develop acute symptoms, and 80% of cases develop chronic infections.

Current HCV treatments aim to slow the progression of the virus, as well as preventing cirrhosis and other symptoms from developing, and have been found to clear as high as 80% of chronically infected persons.⁹ However the treatments vary by genotype, and new ones focus mainly on Genotype 1, which may not be effective when treating any of the others. The recommended treatment for Genotype 1 lasts as long as 48 weeks, and patients are required to
take 1000mg ribavirin (RBV) daily if they are 75kg or less, and 1200mg daily if their weight is greater than 75kg. Pegylated interferon α-2a or 2b (PEG-IFN-α), a 165 amino acid pegylated interferon, is also administered intravenously, and helps trigger cell immune responses. The viral responses to these treatments are about 45-50% for Genotype 1, and with slight alterations to RBV quantities for Genotypes 2-4 the response is 70-80%.4

**Protease Inhibitors**

Protease inhibitors are drugs that are used to treat and prevent viral infections such as the Hepatitis C Virus and HIV. These inhibitors work by binding to and inhibiting the activity of enzyme proteases, and have proven to be effective at treating viral infections. Specifically, HCV inhibitors target the NS3 and NS4A proteases, as they are essential for viral replication and circumventing the host immune response.

There are a number of protease inhibitors in development, seen in Figure 3, and two have already been approved by the FDA for adult Hepatitis C treatment; Telaprevir and Boceprevir. They are both from a class of keto-amide compounds that target and bind to the active site serine, and are used in addition to RBV and PEG-IFN-α therapy. However, the severe side effects associated with PEG-IFN-α/RBV, and the rapid emergence of drug resistance against protease inhibitors limit the effectiveness of this new triple combination therapy.

**Telaprevir**

Telaprevir, C_{36}H_{53}N_{7}O_{6}, is a drug developed by Vertex and Johnson & Johnson used to treat Hepatitis C. The compound is in the class of protease inhibitors and specifically binds and inhibits the NS3 and NS4A serine proteases. Seen in Figure 3, the drug has only been found to be effective on Genotype 1. Due to the lower responses from current therapies on this genotype, it is
a great addition to treating the virus. The most common side effect of the drug is a rash, which during testing occurred in 51% of patients, and 5% developed a severe case.\textsuperscript{10}

**Boceprevir**

Boceprevir, $\text{C}_{27}\text{H}_{45}\text{N}_{5}\text{O}_{5}$, is another protease inhibitor used to treat HCV Genotype 1. It also targets and binds to the NS3 active site.\textsuperscript{11} Shown by Figure 3, and marketed as Victrelis, it is being developed by Merck, and has been approved by the FDA for oral use after 4 weeks of regular PEG-IFN-\(\alpha\)/RBV treatments. It has more noted side effects than Telaprevir, which include nausea, hair loss, and rash outbreaks. Boceprevir has also been found to cause more severe effects like fainting, dizziness, shortness of breath, and patients can exhibit possible signs of infections.\textsuperscript{12}

**MK-5172**

One of the drugs currently in development is the MK-5172 inhibitor, seen in Figure 3, and was the compound focused on for this project. It is a non-covalent acyl sulfonamide inhibitor and contains P2–P4 macrocycles. Within each inhibitor series of same P2 moiety, the drug resistance profiles are similar, indicating that the identity of P2 moiety largely determines susceptibility to drug resistance. The P2–P4 macrocyclic inhibitors also display higher fold-losses.\textsuperscript{13} Unlike other inhibitors, MK-5172 contains a short ether linkage between its P2 proline and the quinoxaline moiety, which can be seen in Figure 4, and past studies have shown that MK-5172 retains relatively better potency against HCV. Both the linear and P1–P3 macrocyclic analogues of MK-5172 retain better potency against drug resistant variants compared to the original drug structure. The research conducted for this drug attempt to decrease it in size, in order to better fit the substrate the envelope and increase its overall effectiveness. These results suggest that even in the absence of P2–P4 macrocycle, the P2 quinoxaline moiety maintains the
unique binding conformation observed for MK-5172. The linear structure, seen in Figure 5, was the focus for this synthesis.

Combatting HCV’s rapid mutation and drug resistance rates is necessary when researching new drugs and treatments, so further development of these compounds and treatments is essential. The introduction of new protease inhibitors to the PEG-IFN-α/RBV therapy may also increase effectiveness against the varying genotypes of HCV, which make it difficult to treat. This research will also allow for better managing the severe side effects and symptoms caused by HCV and its treatments.
Methods

This section details the synthesis of the NS3/4A inhibitor derivative in Figure 6. All reactions were carried out in standard glassware. When purifying with column chromatography, unless otherwise stated thin layer chromatography (TLC) tests were run in 30% EtOAc in hexanes. Nuclear Magnetic Resonance (NMR) tests were run at 400 MHz and the solvents were CDCl₃. Chemical shift multiplicity data are presented as follows: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet. Ethyl acetate is abbreviated EtOAc; 4-Dimethylaminopyridine is abbreviated DMAP; N-Methyl-2-pyrrolidone is abbreviated NMP; Dimethylformamide is abbreviated DMF; (O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate) is abbreviated HATU; N,N-Diisopropylethylamine is abbreviated DIEA; Diethyl ether is abbreviated Et₂O.

1. (2R,4S)-1-tert-butyl 2-methyl 4-(((4-bromophenyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate

![Chemical structure](image)

A solution of N-Boc-cis-4-hydroxy-L-proline methyl ester (10.17g, 41.4 mmol) in dry CH₂Cl₂ (70 mL) was cooled to 0 °C and Et₃N (28.7 mL, 205.8 mmol) was slowly added. After 15 minutes, solid 4-bromobenzesulfonyl chloride (21.16g, 82.8 mmol) was added, followed by the addition of DMAP (.3 g, cat.). The reaction mixture was stirred at 0 °C for 30 minutes and then stored in a refrigerator (5 °C) for 24 hours. The reaction mixture was slowly warmed to room temperature (ice water, cold water, water, and then RT), diluted with CH₂Cl₂ (200 mL), and
washed with saturated aqueous NaHCO₃ solution (100 mL) and 10% aqueous citric acid solution (100 mL). The organic portion was dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by flash column chromatography, eluting with 2-4% EtOAc in CH₂Cl₂ mixture to yield a light yellow gummy solid (16.35 g), which solidified on standing in a refrigerator.

2. (2R,4R)-1-tert-butyl 2-methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate

To an ice-cooled solution of 3-chloro-7-methoxyquinoxalin-2-ol (1g, 4.75 mmol) in NMP (15 mL), Cs₂CO₃ (2.32g, 7.12 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 15 minutes, warmed to 15 °C and treated with solid bosylated-cis proline-OMe (2.2 g, 4.75 mmol). The resulting mixture was stirred at room temperature for 30 minutes, heated to 50 °C and stirred overnight. The reaction mixture was cooled to room temperature, diluted with EtOAc (150 mL) and H₂O (50 mL) and layers were separated. The organic portion was washed with aqueous 1N HCL solution (50 mL), saturated aqueous NaHCO₃ solution (50 mL) and saturated aqueous NaCl solution (50 mL). The organic portion was dried (Na₂SO₄), filtered and evaporated to dryness under reduced pressure. The residue was purified
by flash column chromatography, using 15-20% EtOAc in hexanes as eluent, to provide the desired product (.58g) as a pale yellow foamy solid.

3. (2S,4R)-Methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate hydrochloride

To a solution of proline derivative (2R,4R)-1-tert-butyl 2-methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (.58g, 1.32 mmol) in anhydrous CH$_2$Cl$_2$ was added a solution of 4N HCL in dioxane and the mixture was stirred at room temperature overnight. Solvents were removed by rotary evaporation and the residue was dried under high vacuum. The solid residue was washed with THF (10 mL), then triturated with anhydrous Et$_2$O (30 mL), transferred, washed with Et$_2$O (2 x 10 mL), and dried under high vacuum to provide the product (.48 g, 97%) as a pale yellow solid.

4. (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate
A mixture of proline derivative (2S,4R)-Methyl 4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate hydrochloride (.48g, 1.28 mmol) and Boc-Tle-OH (.38g, 1.66 mmol, 1.3 eq) in dry DMF(10 mL) was cooled to 0 °C under a dry nitrogen atmosphere. DIEA (1.3 mL, 7.68 mmol, 6.0 eq) was added and the mixture was stirred at 0 °C for 15 minutes. Solid HATU (.73g, 1.94 mmol, 1.5 eq) was added and after 15 minutes the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was diluted with EtOAc (150 mL) and washed with aqueous 1N HCL solution (40 mL), saturated aqueous NaHCO₃ solution (40 mL), and saturated aqueous NaCl (40 mL). The organic portion was dried (Na₂SO₄), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography, using 20% EtOAc in hexanes as eluent, to provide the desired product (.38g, 54%) as an off-white foamy solid.

5. (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((7-methoxy-3-vinylquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate
To a solution of the above intermediate (.38g, .69 mmol) in EtOH (15 mL) was added solid potassium vinyltrifluoroborate (.14g, 1.035 mmol, 1.3 eq) at room temperature. The reaction vessel was filled with dry N₂ and Et₃N (.105 mL, 1.04 mmol) was slowly added. Dry N₂ was bubbled through the reaction mixture for 10 minutes, then catalyst PdCl₂(dppf)-CH₂Cl₂ (.06g, .07 mmol) was added, and the reaction mixture was heated at 80 °C for 2 hours. Reaction mixture was cooled to room temperature, diluted with H₂O (40 mL) and EtOAc (100 mL) and layers were separated. The organic portion was washed with H₂O (40 mL) and saturated aqueous NaCl solution (40 mL), dried (Na₂SO₄), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography, using 20% EtOAc in hexanes as eluent, to provide the desired product (.03g, 10%) as an orange gummy solid.

6. (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylate
The above compound (.87 g) was dissolved in a mixture of MeOH-1,4-dioxane (14 mL, 1:1), and the solution was degassed using a vacuum and was filled with dry N₂ (three cycles). Pd/C (212 mg) was added, and the degassing process was repeated for another three cycles. The reaction was degassed a final time and then filled with Hydrogen using three large balloons, and left to stir overnight. The solution was filtered by vacuum filtration through Celite, which was thoroughly washed with Methanol and Ethyl Acetate. It was then evaporated under reduced pressure and purified by flash column chromatography, using 25% EtOAc + .05% Et₃N in hexanes as eluent, to provide the desired product (.51g, 59%) as a yellow foamy solid.

7. (2S,4R)-Methyl 4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate hydrochloride
A solution of proline derivate (0.18 g) in anhydrous CH2Cl2 (2-3 mL) was added to a solution of 4N HCl in dioxane (5 mL) and the mixture was stirred at room temperature for 6 hours. Solvents were removed by rotary evaporation, and the residue was dried under high vacuum. It was then washed with THF and dried under high vacuum (2 x 5 mL), and was washed a final time with Et2O (5 mL) and dried to provide the product (0.18 g, 100%) as a pale yellow solid.

8. (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylate
A mixture of proline derivative (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylate hydrochloride (.18g, .5 mmol) and Boc-Tle-OH (.16g, .7 mmol, 1.3 eq) in dry DMF(5 mL) was cooled to 0°C under a dry nitrogen atmosphere. DIEA (.53 mL, 3.03 mmol, 6.0 eq) was added and the mixture was stirred at 0 oC for 15 minutes. Solid HATU (.29g, .75 mmol, 1.5 eq) was added and after 15 minutes the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was diluted with EtOAc (50 mL) and washed with aqueous 1N HCL solution (30 mL), saturated aqueous NaHCO3 solution (30 mL), and saturated aqueous NaCl (30 mL). All layers were rewashed with clean EtOAc (100 mL), and the organic portions were combined. The organic portion was dried (Na2SO4), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography, using 20% EtOAc in hexanes as eluent, to provide the desired product (.14g, 81%) as a yellow foamy solid. 1H NMR (400 MHz,CDCl3) δ 8.87 (d, J=9.2 Hz, 1H), 7.19 (dd, J=8.8, 2.8 Hz, 1H) 7.13 (d, J=2.8 Hz, 1H), 5.18 (d, J=9.2 Hz, 1H), 4.73 (t, J=8.4 Hz, 1H), 4.24 (m, 2H), 4.07 (m, 1H), 3.94 (s, 3H), 3.78 (s, 3H), 2.89 (m, 2H), 2.68 (m, 1H), 2.35 (m, 1H), 1.33 (s, 9H), 1.27 (t, J=7.2 Hz, 1H), 1.05 (s, 9H), 0.99 (s, 1H).

9. (2S,4R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylic acid
A solution of the above intermediate (.64g, 1.17 mmol) in H₂O-THF (1:1) mixture (30 mL) was treated with LiOH (.17g, 4.05 mmol), and the mixture was stirred at room temperature for 24 hours. The reaction mixture was acidified to pH 3.0 by slowly dripping aqueous .2N HCl, and was extracted with EtOAc (2 x 150 mL). The organic portion was washed with saturated aqueous NaCl solution (50 mL), dried (Na₂SO₄), filtered, and evaporated under reduced pressure. The residue was dried under high vacuum to provide the acid (.64g, 100%) as a clear gummy solid. It was used in the next coupling step.

10. (1R,2S)-1-amino-N-(cyclopropylsulfonyl)-2-vinylcyclopropanecarboxamide hydrochloride

A solution of the above intermediate (.5g, 1.51 mmol) in anhydrous CH₂CL₂ (5 mL) under dry N₂ was treated with TFA (5 mL) and the resulting solution was stirred at room temperature for 1
hour. After the solvents were evaporated under reduced pressure, the residue was treated with toluene (5 mL) and the solution was evaporated to dryness using high vacuum. The residue was then treated with 4N HCL in 1,4-dioxane (4 mL) and the solution was evaporated to dryness. The residue was dissolved in anhydrous THF (5 mL) and the solution was evaporated to dryness using high vacuum (2x) to provide the product (.5g, 100%) as a brown gummy solid.

11. tert-Butyl ((S)-1-((2S,4R)-2-(((1R,2S)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (5172-linear)

A solution of (2S,4R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylic acid (.64g, 1.17 mmol) and (1R,2S)-1-amino-N-(cyclopropylsulfonyl)-2-vinylcyclopropanecarboxamide hydrochloride (.5g, 1.35 mmol) in CH₂Cl₂-DMF (20 mL, 10:1 mixture) was cooled to 0 °C under a dry N₂ atmosphere. DIEA (1.02 mL, 5.85 mmol) was slowly added, and after 15 minutes at 0 °C solid HATU (.67g, 1.76 mmol) was added, followed by DMAP (.14g, 1.17 mmol). The resulting mixture was stirred for 15 minutes at 0 °C, warmed to room temperature, and stirred overnight. The solvents were partially evaporated under reduced pressure, and the residue was diluted with EtOAc (100 mL).
The solution was washed with aqueous .5N HCl solution (35 mL), saturated aqueous NaHCO₃ (35 mL), and saturated aqueous NaCl solution (35 mL). The organic portion was dried (Na₂SO₄), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography, using 50% EtOAc in hexanes as eluent, to provide the desired product (.5g) as a white solid. TLC tests were run in 65% EtOAc. ³¹H NMR (400 MHz,CDCl₃) δ 10.08 (s, 1H), 7.83 (d, J=9.2 Hz, 1H), 7.19 (dd, J=8.4,2.4 Hz, 1H), 7.14 (s, 1H), 7.07 (s, 1H), 5.90 (s br, 1H), 5.76 (m, 1H), 5.28-5.22 (m, 2H), 5.14 (d, J=10.4 Hz, 1H), 4.84 (t, J=8.4 Hz, 1H), 4.30 (d, J=12 Hz, 1H), 4.25 (d, J=9.6 Hz, 1H), 4.04 (dd, J=11.6, 3.2 Hz, 1H), 3.94 (s, 3H), 2.94-2.83 (m, 3H), 2.79 (s, 1H), 2.55 (m, 2H), 2.12 (q, J=17.2, 8.8 Hz, 2H), 1.97 (t, J=6.4 Hz, 1H), 1.47 (m, 1H), 1.33 (m, 9H), 1.27 (t, J=7.2 Hz, 3H), 1.11-0.97 (m, 12H).

12. A solution of tert-Butyl ((S)-1-((2S,4R)-2-(((1R,2S)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (.25g) in anhydrous CH₂Cl₂ (3 mL) was added to a solution of 4N HCl in dioxane (5 mL) and the mixture was stirred at room temperature overnight. The solvents were removed by rotary evaporation, and the residue was dried under high vacuum. It
was then washed with THF and dried under high vacuum (2 x 5 mL), and was washed a final time with Et$_2$O (5 mL) and dried to provide the product (.25 g, 100%) as a pale white solid.

13.

A solution of the above intermediate (.25g, 0.34 mmol) and the above dicarbamate (.082g, 1.35 mmol) in anhydrous DMF (6 mL) was cooled to 0 °C under a dry N$_2$ atmosphere. DIEA (0.35 mL, 1.75 mmol) was slowly added, and after 15 minutes the resulting mixture was warmed to room temperature, and stirred overnight. The solvents were evaporated under reduced pressure and the residue was purified by flash column chromatography, using 50% EtOAc in hexanes as eluent, to provide the final product as a white solid. TLC tests were run in 65% EtOAc in hexanes. $^1$H NMR (400 MHz,CDCl$_3$) $\delta$ 9.94 (s, 1H), 7.60 (d, J=9.2 Hz, 1H), 7.19 (s, 2H), 7.12 (d, J=8.4, 2.4 Hz, 1H), 7.07 (s, 1H), 6.94 (s, 1H), 5.83 (s, 1H), 5.7 (q, J= 12 Hz, 4H), 5.22 (s, 1H), 5.17 (t, J=9.2 Hz, 2H), 5.10 (d, J=10.4 Hz, 1H), 4.82 (q, J=8.8 Hz, 3H), 4.43 (t, J=16 Hz, 2H), 4.22 (dd, J=11.6 Hz, 10 Hz, 1H), 3.99 (d, J=3.6 Hz, 1H), 3.87 (s, 3H), 2.85 (m, J=18, 5H), 2.50 (m, J=25.6, 5H), 2.00 (dd, J=8.8 Hz, 1H) 1.91 (t, J=6.4 Hz, 2H), 1.50 (s, 1H), 1.29 (d, 1H), 1.2 (t, J=14.8 Hz, 2H), 1.14 (dd, J=2.4 Hz, 3H) 0.96 (s, 12H).
Results

The Hepatitis C virus causes a range of symptoms, and the side effects from current treatment methods are equally severe. The development of new drugs is necessary because of HCV’s rapid mutation and drug resistance rates. The goal of this project was to successfully synthesize the linear structure of the 5172 NS3/4A inhibitor, seen in Figure 5. The targeted groups, marked “A” and “B” in the figure, were then modified from the original drug structure, seen in Figure 3, in an attempt to increase enzymatic activity and effectiveness. The structure of the new compound can be seen in Figure 6.

Evaluation of the Synthesis

The synthesis plan of the MK-5172 inhibitor was developed in order to produce the most efficient yields, and involves a series of steps to build the compound from a collection of smaller molecules. Building the molecule begins with the synthesis of the quinoxaline and proline moiety, and to provide a better product yield in step 1, the 3-chloro-7-methoxyquinoxalin-2-ol was purchased from an outside company, instead of being synthesized in the lab. As expected, this compound provided a greater yield than previous experiments where the compound had been made on site. Beginning with these steps builds the compound while keeping the product yields consistent for the Boc removal in step 3, and the coupling in step 4. The purification process of step 4 resulted in an unusually low yield, and but still provided enough product to continue. A challenging part of the compound synthesis came between steps 5 and 6. The structure and instability of the product from 5 was observed as the yield dropped significantly after the compound was stored at 4°C for 10-12 days. The following step 6 gave an extremely low product yield, approximately 10%, confirming the decomposition, and was not enough to continue to the next step in the synthesis. To obtain more of the product (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-
oxy)pyrrolidine-2-carboxylate, the steps seen in steps 7 and 8 were taken, due to the availability of the starting material in 7. These reactions were carried out in order to attain enough of the (2S,4R)-Methyl 1-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)-oxy)pyrrolidine-2-carboxylate to use in experiment 9. Steps 7 and 8 provided better yields than the reactions in steps 3 and 4, and after discussing these results with Dr. Ali, it was confirmed that they are consistently higher when the group on the quinoxaline moiety was an ethyl instead of the chlorine before the removal of the Boc group and addition of the Boc-Tle-OH. To get a good yield with a sample less than 300 mg in steps 7 and 3, the trituration and transfer with Et₂O is left out, and the workup follows the procedure as written in step 7. The availability of the compounds in step 10 and 12 are a factor in the total synthesis plan, as they are necessary for the completion of the molecule. Overall each step in the synthesis provided the expected products, and produced 500 mg of a new derivative of the MK-5172 inhibitor.

NMR

In order to ensure the structure of the product for each reaction, Proton Nuclear Magnetic Resonance (NMR) tests were run following the completion of each step in the synthesis. These tests detect the presence of \(^1\)H protons, and measure their absorption of electromagnetic radiation. The spectrum it produces shows peaks based on the locations of these protons on the molecule, as well as their relative locations to other protons. The analysis of the NMR spectra for the final product is detailed below, and all of the spectra for the products obtained throughout the synthesis can be seen in Figures 7-13. These tests were used to verify that the desired compounds had been made in each step before moving on, as well as confirming the structure of the final compound.
The analysis of the final product synthesized is detailed below:

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.94 (s, 1H), 7.60 (d, \(J=9.2\) Hz, 1H), 7.19 (s, 2H), 7.12 (d, \(J=8.4, 2.4\) Hz, 1H), 7.07 (s, 1H), 6.94 (s, 1H), 5.83 (s, 1H), 5.7 (q, \(J=12\) Hz, 4H), 5.22 (s, 1H), 5.17 (t, \(J=9.2\) Hz, 2H), 5.10 (d, \(J=10.4\) Hz, 1H), 4.82 (q, \(J=8.8\) Hz, 3H), 4.43 (t, \(J=16\) Hz, 2H), 4.22 (dd, \(J=11.6\) Hz, 10 Hz, 1H), 3.99 (d, \(J=3.6\) Hz, 1H), 3.87 (s, 3H), 2.85 (m, \(J=18, 5H\)), 2.50 (m, \(J=25.6, 5H\)), 2.00 (dd, \(J=8.8\) Hz, 1H) 1.91 (t, \(J=6.4\) Hz, 2H), 1.50 (s, 1H), 1.29 (d, 1H), 1.2 (t, \(J=14.8\) Hz, 2H), 1.14 (dd, \(J=2.4\) Hz, 1H) 0.96 (s, 12H).

The single peak at 9.94 ppm shows the carbonyl. The quinoxaline group is in the 7.8-7.0 ppm region. The range of peaks between 6.0-4.0 ppm show the additional Hydrogen on the carbamate, the esters, and double bonds. The peaks between 3.0-2.0 ppm are the sulfone. At approximately 2.0 ppm are the peaks for the ketones, and in the 2.0-1.0 ppm region are the remaining alkanes. This spectrum provides evidence of the locations of Hydrogen on the final compound and confirms the predicted structure.
Conclusions

The goal of this project was to synthesize the linear structure of the MK-5172 HCV drug, and modify the functional groups in order for it to fit better within the substrate envelope of HCV and minimize vulnerability to drug resistance. Past studies have shown HCV drugs without a side chain ring to be more adaptable in fighting the mutant variants. As a result of this project a new MK-5172 derivative was developed, and the compound is predicted to produce more efficient results against both the virus itself and its mutant variants. The derivative is smaller than the original drug, and is therefore predicted to fit in the substrate envelope and protect more proficiently against drug resistance.

The synthesis plan was developed for this drug in order to produce the most efficient yields, and the results of the synthesis show this. These steps provided consistent yields and, after further research of this drug and its variants, will allow for development on a large scale for treating HCV. In future development of the drug, different variations to the modified side groups is recommended, which may allow for better fitting in the substrate envelope and increase the inhibitor’s effectiveness. Following the current synthesis plan will provide the most efficient results, but it is recommended that step 5 is completed before steps 3 and 4, which will provide the intermediate used in step 7, (2S,4R)-Methyl 4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-dicarboxylate. This synthesis showed steps 7 and 8 consistently gave more products, which will affect the development if the drug is to be produced on a larger scale. The current plan for the new MK-5172 derivate also results in the expected compounds without impurities. This was observed by the visible spots on the TLC tests, and the low amount of impurities seen in the NMR spectra of the products in Figures 7-13.
The Hepatitis C virus continues to affect the global population, and while current treatments are effective at combatting its symptoms and complications, there are still many severe side effects associated with them. HCV’s rapid mutations and drug resistance rates also make it increasingly difficult to treat. The continued development of new drugs, like MK-5172, will play a crucial role in combatting this virus and others like it. Researching new derivatives can result in greater drug effectiveness against HCV, and potentially a vaccine to prevent any further spread of the virus. The research should also focus on treating the different genotypes that have been identified, in an effort to combat the virus as a whole, and not just one variant. As new drugs are developed, researchers should explore exchanging functional groups on pre-existing drugs, like the ones seen in this project. Continued development of these new derivatives or new compounds will help to combat drug resistance, and are able to fit in the substrate envelope and bind to the NS3/4A proteins. It is the hope that in finding a solution to HCV, researchers will be able to use their information to treat other viruses, such as HIV, in an attempt to eliminate them and the diseases they cause.
References


Figures

**Sources of Infection for HCV**

- Injection/Drug Use (60%)
- Sexual Transmission (15%)
- Blood Transfusion (10%)
- Other (5%)
- Unknown (10%)

Figure 1: This figure represents a graphical representation of the major causes of HCV infection. Information obtained from: http://www.cdc.gov/hepatitis/Statistics/index.htm

Figure 2: This figure shows the global estimated prevalence of Hepatitis C Virus based on regions as of 2011. The darker regions represent higher percentages Graphic from: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-c.htm
Figure 3: The chemical structures of the HCV NS3/4A protease inhibitors. Telaprevir, danoprevir, vaniprevir and MK-5172 are some of the protease inhibitors in development. Telaprevir, which is approved for clinical use, is an acyclic ketoamide inhibitor that forms a reversible, covalent bond with the protease. Danoprevir, currently in phase II clinical trials, is a non-covalent acyl sulfonamide inhibitor with a P1–P3 macrocycle. Vaniprevir and MK-5172 are also non-covalent acyl sulfonamide inhibitors, but contain P2–P4 macrocycles. Vaniprevir and MK-5172 differ in the construction of their P2 moieties: vaniprevir contains a carbamate linkage between the P2 proline and the isoindoline moiety, whereas MK-5172 contains a shorter ether linkage between its P2 proline and the quinoxaline moiety.14

Figure 4: This figure shows the locations and variations of the P1-P3 and P2-P4 macrocycles for certain protease inhibitors.
Figure 5: This figure shows the MK-5172-linear structure. The side-groups marked “A” and “B” were modified for this project to determine if other chemical groups would increase enzymatic activity and effectiveness. The Chlorine in “A” was removed and an ethyl group was added in its place, and the t-butyl in “B” was removed and replaced with a cyclopentyl ring.

Figure 6: This figure shows the new MK-5172 derivative. This new compound is predicted to produce more efficient results against both the virus itself and its mutant variants. By changing the functional groups on a molecule it can have positive or negative effects on inhibition, and it is the hope that this new derivative produces positive effects.
Figure 7: This figure shows the proton NMR spectra of the product in reaction 6. The peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃. This product was synthesized through steps 1-6. This sample was 145mg and was combined with the product from reaction 8 in order to continue with the synthesis.
Figure 8: This figure shows the proton NMR spectra of the product in reaction 8. The peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃. This product was synthesized starting with the intermediate starting material in reaction 7. This sample was 500mg and was combined with the product from reaction 6 in order to continue with the synthesis of the entire drug.
Figure 9: This figure shows the proton NMR spectra of the product in reaction 11. The peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl$_3$. 
Figure 10: This figure shows the proton NMR spectra of the final compound. The structure of the compound can be seen in Figure 5. The peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃.
Figure 11: This figure shows the proton NMR spectra of the final compound within the range of 0.5 ppm to 3.0 ppm. The structure of the compound can be seen in Figure 5. The highlighted peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃.
Figure 12: This figure shows the proton NMR spectra of the final compound within the range of 6.0 ppm to 3.0 ppm. The structure of the compound can be seen in Figure 5. The highlighted peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃.
Figure 13: This figure shows the proton NMR spectra of the final compound within the range of 10.5 ppm to 6.0 ppm. The structure of the compound can be seen in Figure 5. The highlighted peaks represent the locations of Hydrogen on the compound, and the solvent used is CDCl₃.