Drug Synthesis for HCV

April 26

2012

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Major Qualifying Project Thesis

Acknowledgements

First I would like to graciously thank Celia Schiffer for opening up her lab to me and accepting my project. Second I would like to thank Akbar Ali, without him I could not have made this project possible; he helped me every step of the way and for which I am extremely grateful. I would also like to thank Reinhold Gildemeister for assisting me along the way and making the lab experience that much more interesting. Finally I would like to thank Destin Heilman for his excellent advising and project placement skills. I appreciate both his assistance when I needed it and his stepping back when I needed to figure out solutions to problems on my own. I am very grateful for all of their help in making this a wonderful project and helping me grow as a person.

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Abstract

The hepatitis C virus infects more than 170 million people worldwide. Many of the HCV deaths have been a result of drug resistance. It is essential for researchers to continue to modify the molecules to create a greater variety of drug analogues, and in doing so create one that is the solution to the HCV epidemic. HCV drug analogs that lack a side chain are less hindered and more adaptable in fighting the mutant virus; drugs of this molecular form should be focused on to produce an optimal drug response to the virus. In the non-structural region, protease inhibitors are being created to directly target the virus at the specific sites where the virus is prone to rapid replication. MK-5172 is a drug that directly attacks HCV. Tests have been run on this drug and it was shown MK-5172 produces exemplary results against the drug and the drug-resistant variants. The purpose of this project was to modify the functional groups on the MK-5172 drug and remove the side chain ring. This was done in order for the drug to fit better within the substrate envelope of the virus and to minimize vulnerability to drug resistance; other HCV drugs without a side chain ring have shown to be more adaptable in fighting the mutant variants. The process of drug design by the exchange of function groups on the molecules is essential to eliminating harmful diseases, such as HCV. Scientists need to continue to work together in the drug design field to cure this problematic disease.

Background

The hepatitis C virus is a rapidly expanding problem and needs to be controlled. Hepatitis C is a form of liver inflammation and causes a long- lasting, chronic disease. HCV is a non-cytopathic hepatotropic virus belonging to the *Flaviviridae* family; it is a spherical, enveloped virus 40-60 nm in diameter that infects only humans and chimpanzees (UTMB, 2009). The *Flaviviridae* family consists of enveloped viruses; viral envelopes are made up of cell membranes and glycoproteins (UTMB, 2009). The viral substrate envelope assists HCV in entering the host cells. Hepatitis C is specific to the *Hepacivirus* subgroup. The virus is transmitted through blood by transfusions, hemodialysis and organ transplants. Additionally the virus can be transmitted though the sharing of needles making the spread of the virus more prevalent with drug abusers. Among people who acquired HIV through intravenous drug use in combination with the prevalence of HCV reaches 90%. Co-infection of the two viruses can make treatment all the more difficult; this would require one drug to eliminate both for maximal efficiency (Jamal, 2006). Blood transfusions previously were a leading cause to the spread of HCV; however the technology associated with the practice has advanced, causing transmission of the virus to decrease. Dialysis patients have a higher rate of HCV infection. Possible risk factors of transmission are failure to disinfect devices between patients, sharing of single-use vials for infusions, and poor sterile technique (Jamal, 2006). The hemodialysis risk factor of transmitting the virus can be prevented by using proper techniques when dealing with patients; this means ensuring that the patients are giving a more than sterile treatment. The progression of the virus is acute infection then either clearance (~20 %) or chronic infection (~80 %). Following chronic infection is cirrhosis (~20 %) and hepatocellular carcinoma (Qureshi, 2007). Both cirrhosis and

hepatocellular carcinoma can be fatal. Cirrhosis is the damaging of liver cells and replacing them with scar tissue, eventually leading to the shutdown of the liver. Hepatocellular carcinoma is cancer of the liver, which leads to the spread of cancer to other organs. Since the virus is only present in humans and chimpanzees, it cannot be cultured actively, but a solution to the virus needs to be done retroactively. This makes the process of drug design that much harder; the virus can be attacked directly however it cannot be studied directly therefore, indirect approaches are the only viable option. The virus cannot be studied directly in culture because of the lack of efficient viral culture systems. The virus in culture has a very narrow host range, which prevents it from being a useful culture system. Therefore drugs already in the market and in phases need to be modified for better results in efficacy and safety for the patients.

HCV Genome

The hepatitis C virus is comprised of a 9.6 kb linear single- stranded RNA genome, which contains 10 genes that encode for HCV. It is of positive polarity, compiled of a single uninterrupted open reading frame that is surrounded on either side by 5' and 3' un-translated regions and can be a template for translation or replication. Structural genes on the 5' region of the HCV genome encode a highly basic core protein (C) and two envelope proteins (E1 and E2), followed by the *p7* gene. Then the genes that encode the non-structural proteins are NS2, NS3, NS4A, NS4B, NS5a and NS5B and are closer to the 3' end (Qureshi, 2007). NS represents the nonstructural proteins, as shown in **Figure 1**. The importance in targeting the proteins is that NS3 specifically plays an important role in the hepatocarcinogenesis of HCV (Deng, 2006). The location of the NS3 protein is an essential drug target because it allows for important

replication and spread of the virus. HCV is divided among six genotypes and then divided again into numerous subtypes. These genotypes can differ up to 30% from each other in nucleotide sequence and depending on the HCV genotype, length of treatment can differ greatly (Jamal, 2006). The six genotypes are prevalent in different groups of peoples throughout the world. In the US patients were primarily of genotype 1, patients in China were of genotype 2 and in England, Thailand and India genotype 3 was most prevalent. Genotype 4 is most common in Africa and the Middle East, genotype 5 in South Africa and genotype 6 in south-east Asia (Jamal, 2006). It is imperative to note that genotype 1b is less responsive to alpha-interferon therapy compared to genotypes 2 and 3; this makes it important to track the individual genotypes, so that an appropriate therapy can be made for each (Jamal, 2006).

Protease Inhibitors

A protease inhibitor is a type of drug that disables the enzyme protease. A substance that triggers chemical reactions in the body is an enzyme (Farlex, 2012). The most successful kind of inhibitors used in drug design for HCV are protease inhibitors. In blocking the protease the replication of HCV is stunted, disabling the reproduction of the virus in new cells. In Figure 1, above, at the NS3 protein there is a “metalloprotease, serineprotease, RNA helicase”, which is an example of a protease inhibitor for HCV. Both of the drugs that have been approved for market are protease inhibitors.

Pre-existing Drugs

There are a portion of the drugs that are currently in research, phases and in the market that directly affect HCV, **Figure 2**. One of the leading drugs on the market for HCV treatment is Boceprevir, which is produced by Merck. Boceprevir is currently one of the only drugs that has

been approved and is a protease inhibitor. NS3-NS4A protease is playing a critical role in HCV viral replication and is therefore viewed as an ideal target for the creation of new HCV therapy (Njoroge, 2008). The NS3 region has been seen as an especially key location in HCV viral replication and is a prime location in placing an inhibitor. The difficultly arose when there were no viable lead structures and that the early inhibitors were designed based solely on the substrate-enzyme active site (Njoroge, 2008). Working with the substrate- enzyme active site, Merck was eventually able to create Boceprevir. Extensive tests were performed in order to reach a drug capable of producing positive effects on the virus. This testing showed that Boceprevir, was highly selective toward the HCV serine protease; around 5 years ago it was the best viable option to send into drug phases (Njoroge, 2008). Now that there are more drugs in trial and drug design phases, Boceprevir is no longer one of the best drugs for treatment against HCV. However, it is still one of the only ones approved. Its efficiency needs to be improved due to the negative side effects of the drug which include fatigue, anemia, nausea and dysgeusia; these side effects are less than pleasant to experience (Merck, 2011). Current medical forms of HCV include a pegylated interferon-α (PEG IFN- α) in combination with ribavirin (RBV) treatment in combination with the inhibitors such as Boceprevir (Rehman, 2011). This combination of the two, abbreviated PR is used before the inhibitor is introduced, with the inhibitor and at the end of treatment PR is used alone again (Merck, 2011). The problem faced when having the need to use both is that then the side effects from both drugs affect the patients. It is necessary to create a HCV drug that can be used singularly without PR that directly affects the virus and minimizes adverse effects. Some of the most effective antiviral inhibitors have focused on the following targets, which are the attempts that have been the

main focus so far: NS3-4A serine protease, RNA helicase activity of NS3 and NS5B RNA-dependent RNA polymerase (Rehman, 2011).

MK-5172

One of Merck's newer HCV drug inhibitors is MK-5172, **Figure 3**, and is currently in phase 2 of testing. MK-5172 is a new generation, competitive inhibitor of the HCV NS3/4a protease with a broad HCV genotypic activity spectrum, and it produces potent *in vitro* activity against viral variants that are resistant to other protease inhibitors in development (Levin, 2011). Multiple tests have been run for MK-5172 and it has shown to produce astounding results against the virus itself and the mutants that arise from it. In tests performed, MK-5172 demonstrated potent and rapid viral load reductions with 7 days of mono therapy, with 75% of patients with chronic GT1 infection achieved reductions in plasma HCV RNA to below the limit of possible study (Levin, 2011). Antiviral activity persisted for several days beyond the treatment period in GT1 patients, which is extremely beneficial. MK-5172 was generally well-tolerated with no serious adverse experiences and no discontinuations due to adverse experiences; it is key to lower the adverse affects (Levin, 2011). As compared to drugs currently in the market, a new drug, MK-5172, shown in Figure 3, producing little to no adverse effects is a serious improvement. If it is possible to further increase the positive effectiveness of this drug, series of tests should be run and the drug appropriately modified.

MK-5172 Derivative

Drug design is all about modifying current drugs in order to get the most useful one as possible; this is done by changing side groups on the molecule or changing the molecule all together to get the best product possible, demonstrated in **Figure 4**. The purpose of this product was to

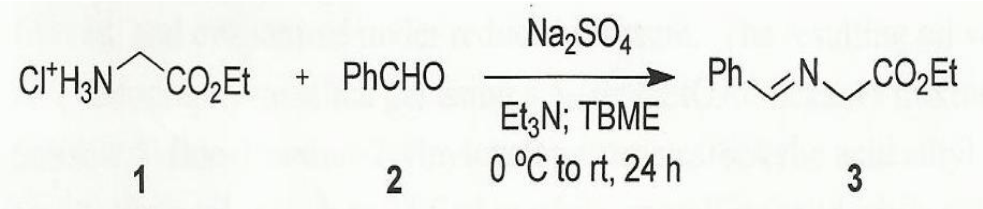
manipulate and change the MK-5172 drug in order to make it as efficient as possible for the patients using it. A side group change was made in transitioning from a ring system to a chlorine atom. This was performed in hopes that the drug would fit better in the substrate envelope and then from there perform better against the multiple drug variants. Nuclear magnetic resonance tests were performed to ensure that the final products synthesized were in the right conformation and were the right products that were sought. Further tests need to be run such as an enzymatic kinetic evaluation to see that the new derivative performs better against the drug resistant variants.

Methods

Synthesis of HCV NS3/4A Protease Inhibitor MK-5172 Analogues

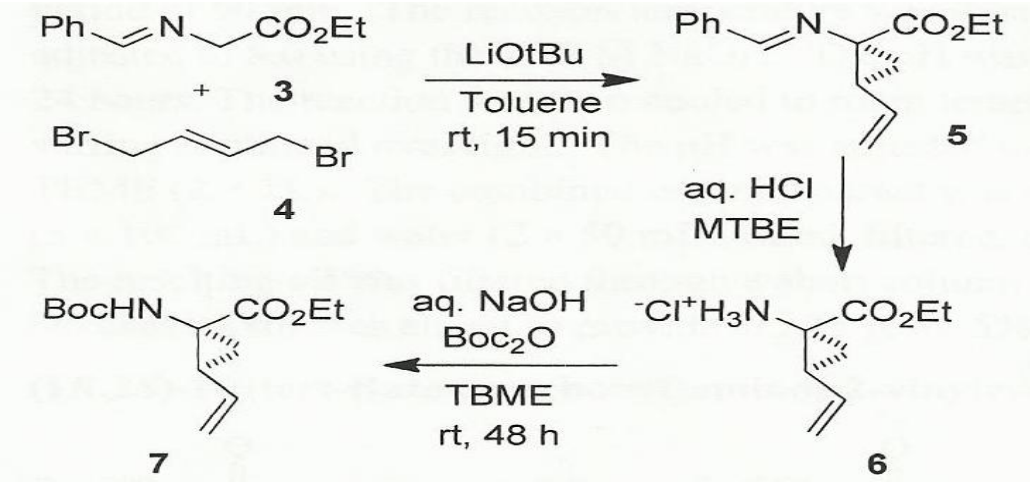
Synthesis of Vinyl-ACCA-OEt and the P1-P1' Fragment:

(E)-Ethyl 2-(benzylideneamino) actetate



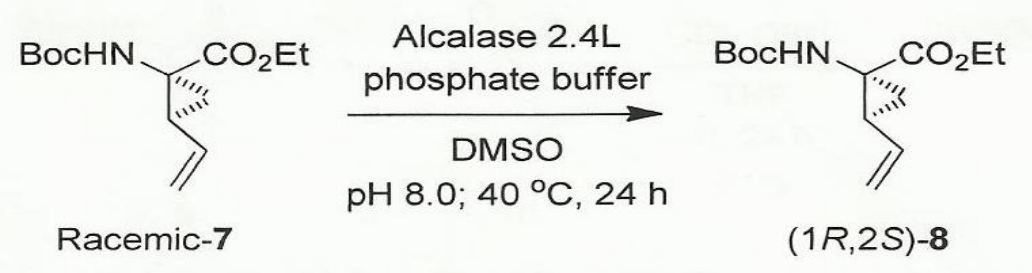
Benzaldehyde (27.63 mL, 28.88 g, 0.27 mmol) and anhydrous sodium sulfate (19.38 g, 0.14 mmol) were added to s suspension of glycine ethyl ester hydrochloride (38 g, 0.27 mmol) in *tert*-butylmethyl ether (TBME) (200 mL), and the mixture was cooled to 0^{O}C. Triethylamine (56.88 mL, 0.41 mmol) was slowly added over the course of 30 minutes, the resulting mixture was stirred at 0^{O}C for 10 minutes, warmed to room temperature and then stirred for 24 hours. The reaction mixture was quenched with ice-cold water (125 mL), layers were separated and the aqueous layer was further extracted with TBME (150 mL). The combined organic extract was washed with a 1:1 mixture of saturated aqueous$NaHCO_3$ (125 mL) and NaCl (125 mL) solutions, dried with Na_2SO_4, and filtered. It was then evaporated under reduced pressure to provide the *N*-benzyl imine (50.6 g, 98.15 %) as thick yellow oil.

(±)-(1R,2S)-Ethyl 1-((*tert*-butoxycarbonyl)amino)-2-vinylcyclopropanecarboxylate



To a suspension of lithium *tert*-butoxide (42.05 g, 0.503 mmol) in dry toluene (600 mL) at room temperature was added to a mixture of the *N*- benzyl imine (50.6 g, 0.265 mmol) and *trans*- 1,4-dibromo-2-butene (53.5 g, 0.25 mmol) in dry toluene (300 mL) over 90 minutes. After 15 minutes at room temperature, the reaction was quenched by an addition of water (500 mL). TBME (500 mL) was added and layers were separated, the aqueous phase was further extracted with TBME (2 x 500 mL). The organic portion was mixed with 2 M HCl (500 mL) and the mixture was stirred at room temperature over –night [2 hours enough]. The organic phase was separated and extracted with water (400 mL). The combined aqueous phase was saturated with NaCl (350 g), TBME (500 mL) was added and the mixture was cooled to 0^{O}C. The mixture was then basified to pH 14 by the drop-wise addition of 10 M NaOH (~ 150 mL), keeping the temperature below 5^{O}C. The organic layer was separated and the aqueous phase was extracted with TBME (2 x 250 mL). The combine organic extract was dried, Na_2SO_4, filtered, and concentrated to a volume of 500 mL. Di-*tert*-butyldicarbonate (Boc_2O) (57 g, 0.261 mmol) was added to the solution and the mixture was stirred at room temperature over the weekend. The reaction mixture was dried again, filtered and evaporated under reduced pressure. The resulting oil was purified by flash column chromatography on silica gel using a 5-20% EtOAc-hexanes mixture as eluent to provide the racemic *N*-Boc- 1 –amino-2 –vinylcyclopropane carboxylic acid ethyl ester (36.93 g, 54.7%) as an off-white oil, which solidified to white crystalline solid while sitting in the refrigerator.

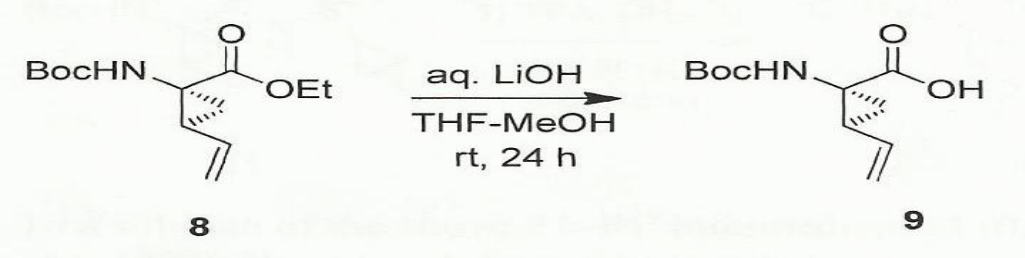
(1R, 2S)-Ethyl 1-((*tert*-butoxycarbonyl)amino)-2-vinylcyclopropanecarboxylate



To 1.85 L of 0.1 M phosphate buffer (pH 8.0) maintained at 40 ^{O}C was added Alcalase 2.4L (Novazyme) (275 mL). The temperature was brought to 38^{O}C and the pH was adjusted to 8.0 by slowly adding 10 M NaOH. A solution of the racemic *N*-Boc- 1- amino-2- vinylcyclopropane carboxylic acid ethyl ester (36.9 g, 0.145 mmol) in DMSO (370 mL) was slowly added over a period of 90 min. The reaction temperature was the raised to 40^{O}C, after 3 hours the pH was adjusted to 8.0 using the 10.0 M NaOH. The pH was adjusted periodically to 8.0 over the next 24 hours. The reaction was then cooled to room temperature, the pH was again adjusted to 8.0, and stirring continued overnight. The pH was adjusted to 8.5 and the mixture was extracted with TBME (2 x 1L). The combined organic extract was washed with a saturated aqueous $NaHCO_3$ (3 x 100 mL) and water (2 x 50 mL), dried, filtered and evaporated under reduced

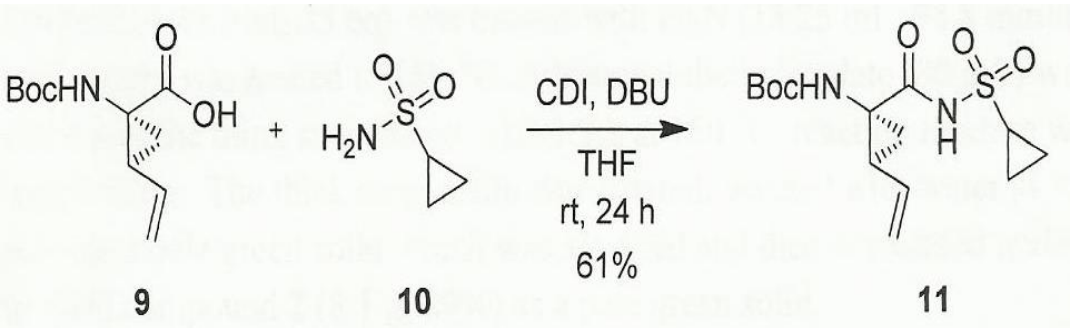
pressure. The resulting oil was filtered through a short column of silica gel using a 20-25% EtOAc-hexanes mixture as eluent to provide (15.75 g, 42.5%) of the compound as pale yellow oil.

(1R, 2S)-1-((tert-Butoxycarbonyl)amino)-2-vinylcyclopropanecarboxylic acid



To a solution of (1R, 2S)-ethyl 1-((*tert*-butoxycarbonyl)amino)-2-vinylcyclopropanecarboxylate (11.25 g, 44.1 mmol) in THF (33.3 mL) and MeOH (33.3 mL) was added to a solution of LiOH (7.87 g, 187.54 mmol). The resulting mixture was stirred at room temperature for 24 hours. The reaction mixture was separated using 1M NaOH (75 mL), water (75 mL) and EtOAc (90 mL). The mixture was shaken and the layers were separated using a separatory funnel. The organic layer was further extracted with 0.5M NaOH (75 mL). The combined aqueous portion was cooled to 0 ^{O}C, acidified with 1 M HCl (~150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic portion was washed with saturated aqueous NaCl solution, dried with Na_2SO_4, filtered and evaporated to dryness. This provides the acid as a light yellow gummy solid. The crude acid was dissolved in a 15% methanol-chloroform mixture and was evaporated with a high vacuum twice. The solid was then dissolved in chloroform and evaporated again under high vacuum. The gummy residue (10.51 g, 93.4%)was then used in the subsequent reaction.

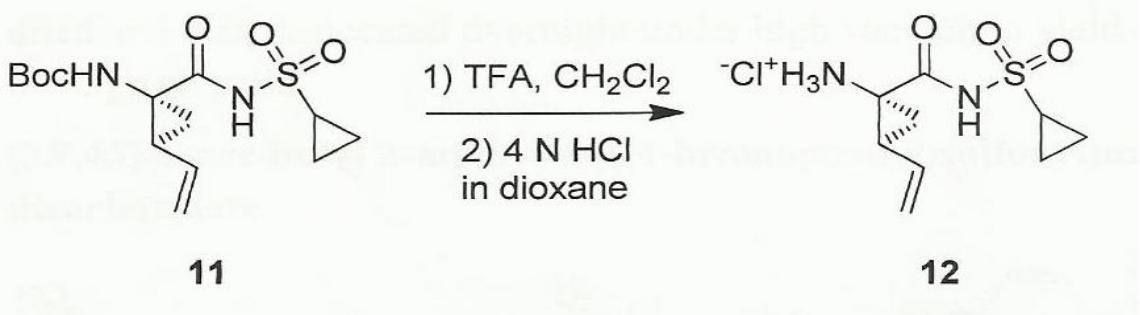
***tert*-Butyl (1R,2S)-1-(cyclopropylsulfonylcarbamoyl)-2-vinylcyclopropylcarbamate**



A solution of acid (10.51 g, 46.24 mmol) and CDI (10.54 g, 65.0 mmol, 1.4 eq.) in anhydrous THF (115 mL) was heated at reflux for 1 h under dry nitrogen. The solution was cooled to room temperature. Then the solution was cooled to room temperature and transferred via cannula to a solution of cyclopropanesulfonamide (7.87 g, 65.0 mmol, 1.4 eq) in anhydrous THF (25 mL).

DBU (10.4 mL, 64.6 mmol, 1.4 eq) was added slowly to the resulting solution and stirring continued for 24 hrs. The reaction was quenched with 1N HCl to about pH of 1 and THF evaporated under reduced pressure. EtOAc was then used to extract the resulting suspension (3 x 350 mL); the combined organic portion was dried (Na_2SO_4), filtered, and evaporated to yield a yellowish solid. The crude material was purified by flash chromatography, eluting 3-8% acetone in CH_2Cl_2 to afford the compound as a white crystalline solid (10.96 g, 78%).

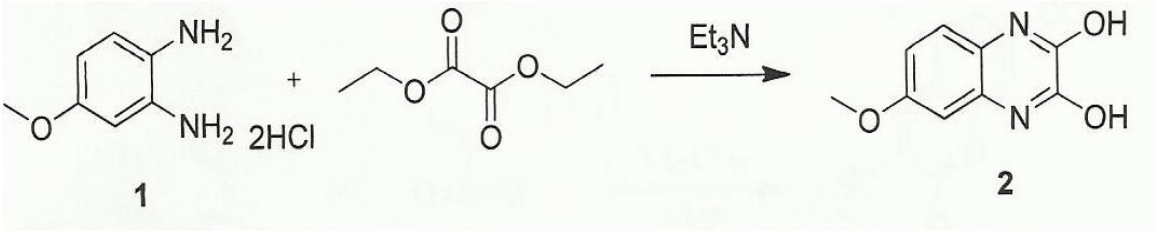
(1R, 2S)-1-amino-N-(cyclopropylsulfonyl)-2-vinylcyclopropanecarboxamide hydrochloride



11 12

Dichloromethane (5mL) was added to the product from the previous synthesis (0.41g, 1.234 mmol), under nitrogen and stirred. TFA (5mL) was then added and stirring was continued for 2 hours. The solvents were then evaporated under reduced pressure. The residue was treated with 4N HCl in 1,4-dioxane (2mL) and evaporated to dryness. The residue was then treated again with 4N HCl in 1,4-dioxane (2mL) and evaporated to dryness under a high vacuum. The resulting P1-P1' amine-HCl salt was used as such in the following coupling reaction.

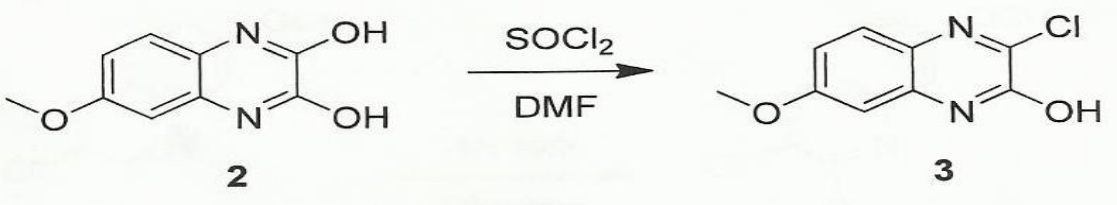
6-Methoxyquinoxaline-2,3-diol



1 2

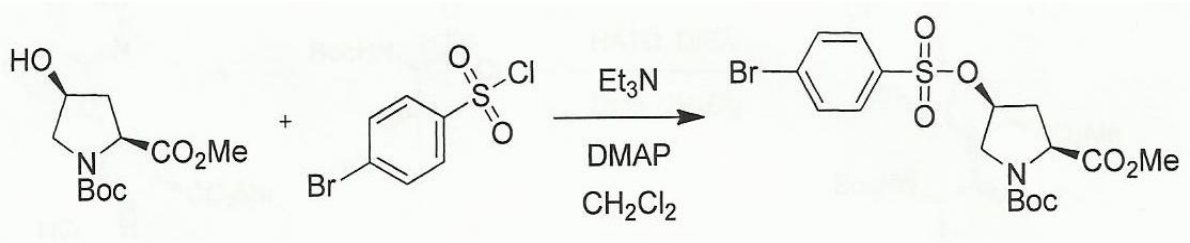
A suspension of 4-methoxy-1, 2-phenylenediamine-2HCl (10 g, 47.4 mmol) in diethyloxalate (140 mL, 1.18 mmol, 25 eq) was treated with Et_3N (13.25 mL, 94.8 mmol, 2 eq). The resulting thick slurry was heated to 150^{o}C. Additional diethyloxalate (80 mL) was added to assist stirring of the thick suspension. After 3 h at 150^{o}C, the reaction mixture was cooled to room temperature. The thick suspension was filtered, washed with water 4 times and EtOH 4 times to provide a pale green solid. The solid was air dried and then desiccated under high vacuum over night to yield the compound (8.1g, 89%) as a pale green solid.

3-Chloro-7-methoxyquinoxaline-2-ol



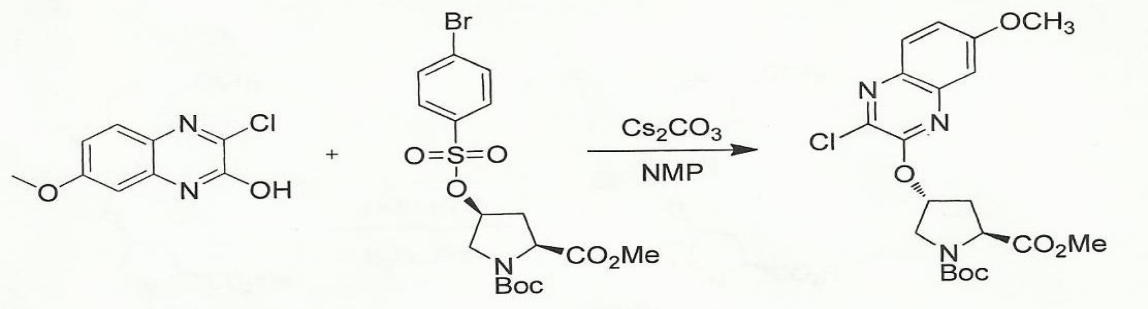
To a thick slurry of the compound (7.45 g, 38.77 mmol), from the previous synthesis, in anhydrous DMF (40 mL) was slowly added $SOCl_2$ (2.85 mL, 39 mmol) over 45 minutes. The reaction mixture gradually became less thick, the more $SOCl_2$ was added. After the addition was complete, the reaction mixture was stirred at room temperature for 15 minutes and then heated to 110-115^{o}C and stirred for 2 hours. The reaction mixture was then cooled to room temperature and an aqueous solution of 1 N HCl (100 mL) was added in one portion. The mixture was stirred for 30 minutes and the product precipitated. The precipitate was filtered and washed with water (4x) and Et_2O (4x) to provide an olive green solid. The product was then air dried and desiccated overnight under high vacuum to yield an olive green solid (7.30 g. 89%).

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



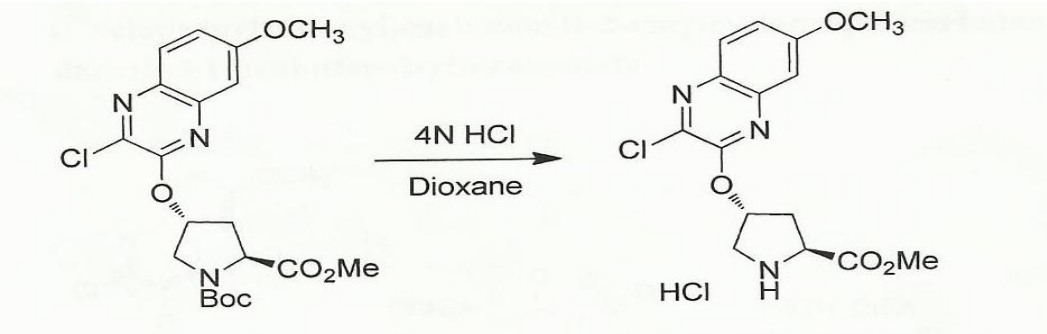
A solution of cis-proline (4 g, 16.31 mmol) in dry CH_2Cl_2 (30 mL) was cooled to 0^{O}C and then Et_3N (11.3 mL, 81.061 mmol, 4.97 eq) was added slowly. After 15 minutes solid 4-Br-Ph-SOCl (8.34 g, 32.61 mmol, 2 eq) was added followed by the addition of DMAP (≈ 100 mg). The reaction mixture was then stirred at 30 minutes at 0^{O}C and then stored in the refrigerator (5 ^{O}C) for 24 hours. The reaction mixture was slowly warmed to room temperature (ice water, cold water, water and then room temperature). Then it was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution and 10% aqueous citric acid solution. Organic phase was dried and evaporated. The product was purified by flash column chromatography to produce a light yellow gummy solid.

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



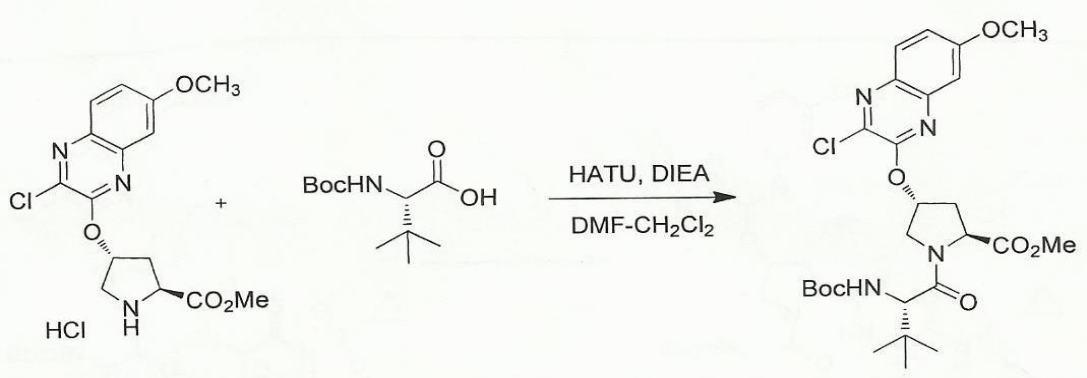
Half of the NMP (30 mL) was added to the quinoxaline under nitrogen and was stirred. Then $CsCo_3$ (7.98 g, 24.51 mmol, 1.5 eq) was added and stirring continued. The other half of the NMP (30 mL) was added to the Bos-Proline-OMe and rotated at 40^{O}C for 30 minutes. The resulting Bos-Proline-OMe solution was then added to the quinoxaline solution; it was then refluxed for over 24 hours. Flash column chromatography was then used to separate the layers at 15-20% EtOAc in hexanes to produce a yellow gummy solid.

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



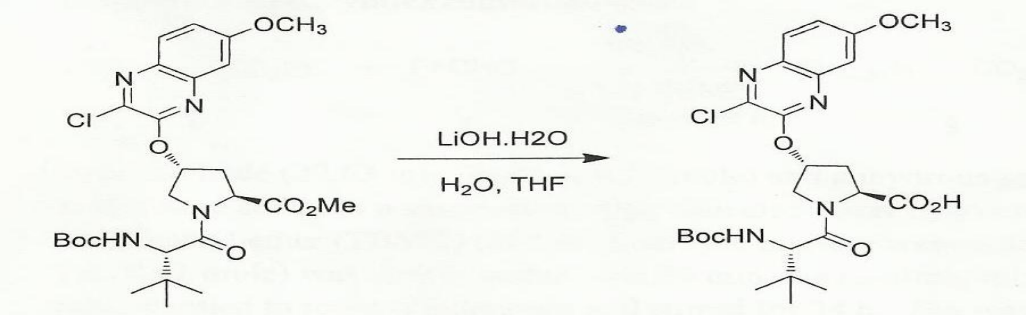
Dry CH_2Cl_2 (6 mL) and 4N HCl were added to the Boc-Proline under nitrogen. The resulting solution was stirred for 4 hours. After 4 hours the resulting solution was evaporated to dryness to produce the compound to be used in the next synthesis.

(2S, 4R)-Methyl 1-((S)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate



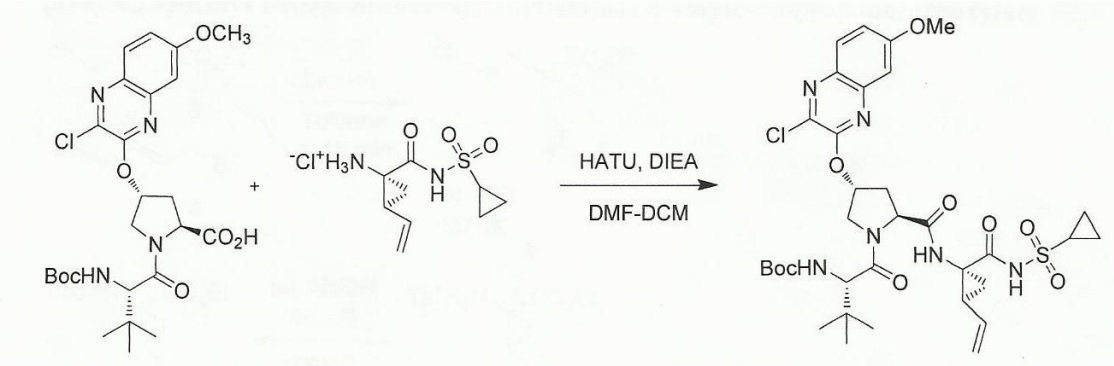
Boc-Tle-OH (0.5 g, 2.11 mmol, 1.3 eq) was added to the proline derivative (0.61 g, 1.63 mmol) under nitrogen and stirred. Both dry CH_2Cl_2 (10 mL) and dry DMF (7 mL) were added and the resulting solution was cooled to 0^{o}C. At 0^{o}C DIEA (1.62 mL, 9.78 mmol, 6.0 eq) was added and stirred for 15 minutes. Solid HATU (0.93 mL, 2.445 mmol) was added, stirred for 15 minutes and then the ice bath was removed. The solution was stirred over the weekend. The solution was then washed with EtAc (50 mL), HCl (0. 25 M, 15 mL), Sodium bicarbonate (15 mL) and NaCl (15 mL). Then the resulting mixture was high vacuumed to produce a thick red liquid (0.68 g, 89.7%).

(2S, 4R)-1-((S)-2-((*tert*-Butoxycarbonyl)amino)-3,3dimethylbutanol)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylic acid



Under nitrogen, THF (6 mL) was added to $C_{26}H_{35}ClN_4O_7$ (0.68 g, 1.234 mmol). Water (6 mL) was then added to the mixture. The mixture was stirred, LiOH (0.16 g, 3.7026 mL, 3 eq) was added and stirring continued for 24 hours. Then 0.2 N HCl was added to the mixture until it reached a pH of 3. The solution was washed twice with both EtOH (2 x100mL) and then NaCl (20 mL). The solution was evaporated to dryness under high vacuum to produce a yellow gummy solid (0.70 g, 97.1%).

***Tert*-Butyl ((S)-1-((2S, 4R)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-2-(((1R,2S)-1-((cyclopropylsulfornyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate**



Proline (0.70 g, 1.234 mmol) was added to the Boc (0.41 g, 1.234 mmol, 1 eq) under nitrogen and stirred. Dry DMF (10 mL) and dry DCM (7 mL) were added and the resulting mixture was cooled to 0^{O}C. At 0^{O}C DIEA (2.14 mL, 7.404 mmol, 6.0 eq) was added and stirring continued. HATU (1.23 g, 1.85 mmol, 1.5 eq) was then added, stirring continued for 15 minutes and ice was removed. The solution was stirred for 24 hours. After stirring overnight, the solution was washed with EtAc (100 mL), HCl (0.25 M, 30 mL), $NaHCO_3$ (15 mL) and NaCl (15 mL). The solution was then evaporated to dryness under high vacuum. A column was run at 50% EtAc in hexanes to further purify the product, which was a yellow solid (0.63 g, 90%).

Results

The purpose of this project was to modify the pre-existing compound MK-5172 and remove the side chain ring in order for it to fit better in the substrate envelope to minimize vulnerability to drug resistance, **Figure 4**. HCV drugs without a side chain ring are less hindered and therefore are more adaptable in fighting the mutant variants. A final compound was developed and synthesized. Instead of the side chain ring, the ring was removed and a chlorine atom was attached to the upper ring system; this enabled the molecule to fit better within the substrate envelope, **Figure 5**.

Synthesis Evaluation

The final compound was synthesized though a serious of steps involving the piecewise addition of smaller molecules. The initial six syntheses went according to plan producing a very efficient yield and there were no problems encountered in the actual lab work. Synthesis two, **(±)-(1R,2S)-Ethyl 1-((*tert*-butoxycarbonyl)amino)-2-vinylcyclopropanecarboxylate**, had a low yield; this matched the yield seen in the assisting synthesis. However during the seventh synthesis, **6-Methoxyquinoxaline-2,3-diol**, problems arose. As the suspension was prepared, the mixture immediately turned into thick slurry that was difficult to work with. The hard to handle, slurry was then placed in a flask and stirred. The flask used was insufficient in containing the solution and it overflowed. Additional solvent was used to aid in mixing. Nevertheless the synthesis produced a decent yield. Additionally the eighth synthesis, **(2R, 4S)-1-*tert*-butyl 2-methyl 4-(((4-bromophenyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate**, had problems with yield and so it had to be ran multiple times to produce enough product to move on to the next step. This was

a result of using a different brand of reagents than before, which greatly hindered the synthesis. Overall, the syntheses produced adequate yields.

NMR

After gaining the desired final compound, tests need to be run to ensure the validity of the compound that was experimentally created. A nuclear magnetic resonance test was run on the final product to ensure that the functional groups were in the correct places and the product reflected the predicted one. A proton NMR is done with respect to hydrogen-1 nuclei. The spectrum produces peaks based on the location of hydrogen atoms in the molecule. It is run to ensure that the molecule has all of the function groups that it is predicted to have. In the NMRs performed on the final product you will see the entire spectrum as a whole and two sections of it viewed from a closer perspective, **Figures 6-8**. This is done to see where and how many peaks there were in total, making the analysis of the NMR easier.

NMR Assignments: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.8 (d, 1H), 7.25 (s, 5H), 7.2 (s, 1H), 7.05 (s, 1H), 5.85 (s, 1H), 5.78 (m, 1H), 5.20 (m, 3H), 4.55 (t, 1H), 4.4 (d, 1H), 4.2 (d, 1H), 4.15 (m, 1H), 3.98 (s, 3H), 2.14 (q, 1H), 1.98 (t, 1H), 1.65 (s, 2H), 1.48 (m, 1H), 1.32 (s, 12H), 1.04 (s, 12H).

The peak in the 10.1 ppm region represents a COH bond, at 7.8-7.0 ppm region are the aromatic and amide hydrogen and in the 6.0-5.0 ppm region are the hydrogen on the Boc group. The peaks in the 4.0 ppm represent esters, the 2.0 ppm region ketones and nitro groups, and in the 1.0-2.0 ppm region are the saturated alkanes, alcohols and double bonds. As a whole the NMR supports the location of the hydrogens on the predicted final product, verifying it to

match the predicted molecule. Additional testing needs to be run on the final product, such the enzymatic test run on MK-5172. This is done to fully ensure the final product is the desired compound.

Enzymatic Assay

Tests were run on MK-5172, **Figure 9** and compared to various drugs on the market or in phases against the wild type of the virus and three of the drug resistant variants. Against the wild type MK-5172 produced low K_i values. K_i is the dissociation constant for inhibitor binding and therefore the lower the K_i, the more beneficial the protease inhibitor. Additionally MK-5172 produced excellent results against the drug-resistant variants, which is important because the mutants are so variable. Finding a compound that is able to combat three of the most common mutant sites is very essential in creating a worthy drug for HCV. The results of those tests showed that MK-5172 was superior at combating the virus compared to the other drugs. The lower the enzymatic activity, the more efficient the drug is in combating the virus directly. Therefore it can be predicted that in switching the side chain ring to a chlorine atom would simultaneously produce more enhanced results against the drug resistant variants; tests still need to be run to verify this prediction. These tests would involve the same ones run for the MK-5172 drug.

Conclusions

The purpose of this project was to modify the functional groups on the MK-5172 drug and remove the side chain ring. This was done in order for the drug to fit better within the substrate envelope of the virus and to minimize vulnerability to drug resistance; other HCV drugs without a side chain ring have shown to be more adaptable in fighting the mutant variants. As a result of this project a new MK-5172 derivative was developed; 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 compound, therefore is predicted to fit easier in the substrate envelope and protect more proficiently against drug resistance. In future studies it would be beneficial to exchange the chlorine atom with other groups of atoms, such as methyl, ethyl or amine groups, **Figure 10**. Also, by changing the functional groups on a molecule it can have positive or negative effects on inhibition. Hence, it is the hope that this new derivative produces positive inhibition effects.

In the future, new derivatives and analogs should be tested to see their efficacy against HCV, in hopes that one of them will eliminate the virus all together. This can be done by exchanging the functional groups on pre-existing drugs, or by creating new drugs all together by combining the portions of previous ones that are the most effective. Drugs that are able to fit in the substrate envelope will produce the best results; inhibitors that bind to the NS3-NS4A proteins will also produce results. Standard testing such as NMR and an enzymatic kinetic evaluation should be run on the drugs that are the most promising in order to move them further into the drug design phases and eventually on to the market.

The hope, after synthesizing a drug to eliminate HCV, would be to create one that has the capability to eliminate HIV simultaneously. In doing this people who have both diseases would be able to get a concise, operational drug; combining drugs is a difficult task but once it is created patients will have a lot simpler, safer treatment. Treatments should not be the only type of drug used to combat these viruses. Prevention drugs need to be created once a solution to the treatment of the virus is synthesized. Once drugs are capable of preventing a virus, the virus becomes a thing of the past and can no longer harm people.

Additionally in working with difficult to culture diseases, such as HCV, new techniques should be created that can be universally used for other disease. It should be the goal when techniques are difficult to use in drug design, new ones should be found in order to prevent that problem from happening in the future.

Difficult to solve viruses, like HCV, should drive scientists to work hard to create a solution to the problem. The more difficult the disease is in solving, the more interesting the solution that comes out of it. In working with a difficult disease, new techniques arise that can be beneficial to others along the line. Additionally it is amazing creating massive molecules and drugs out of simple functional groups. Scientists are able to create a drug to eliminate a harmful virus, by the addition of small molecules; this in itself is an incredible concept.

Hopefully scientists will continue to work hard at creating a new drug to eliminate HCV altogether.

Appendix

Figure 1 HCV structure: HCV enclosing a single stranded RNA of 9.6 kb. The genome carries a single long open reading frame (ORF) which on processing forms a polyprotein that is proteolytically cleaved into distinctive products. The HCV polyprotein is cleaved co- and posttranslationally by cellular and viral proteinases into 10 different products, with the structural proteins located in the amino- terminal one-third and the nonstructural (NS) replicative proteins in the rest. NS3/NS4A have been focused on in particular. (Rehman, 2011)

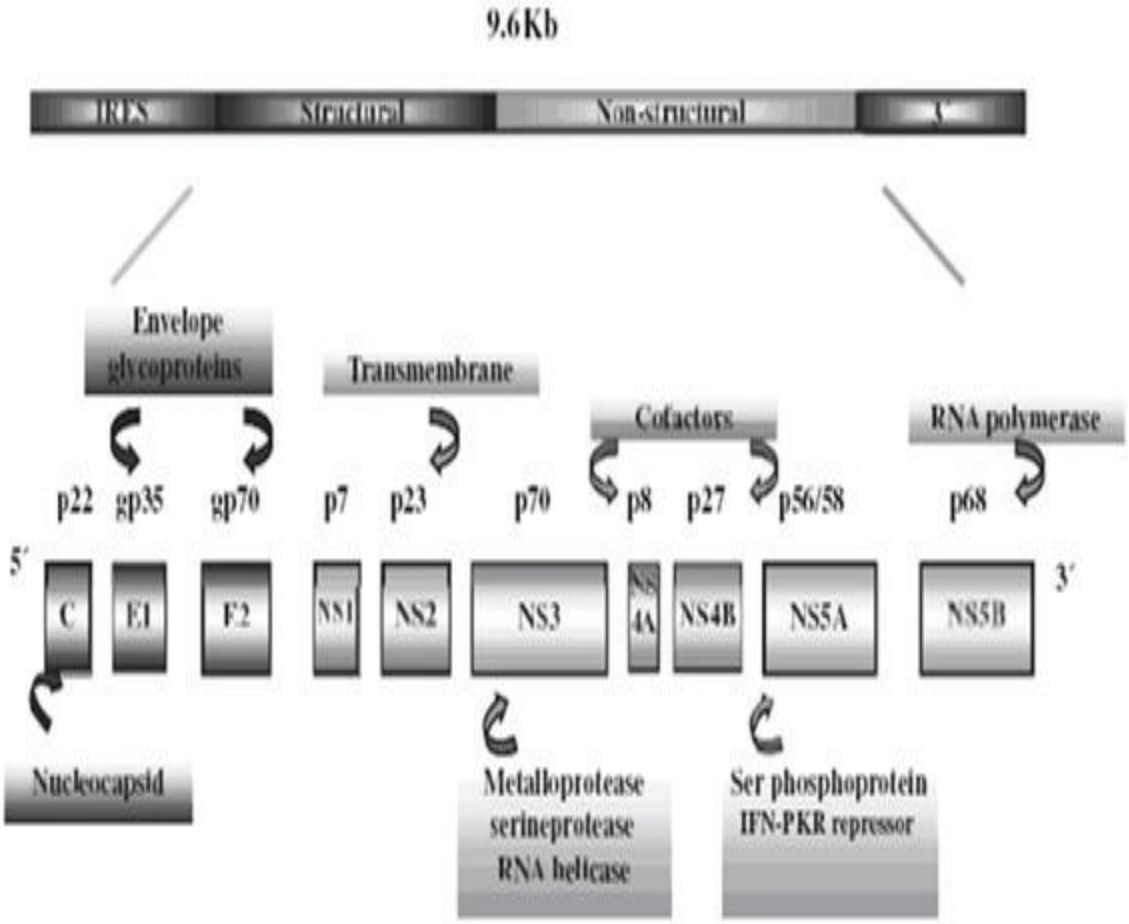


Figure 2: A portion of the drugs in phases and in the market that directly affect HCV. As you can see Merck's approved product is Victrelis, Boceprevir. Every drug has to undergo a series of phases and testing before it reaches the market. The above figure shows some of the drugs undergoing this process. MK-5172 is another drug created by the Merck Company. The benefits in making the MK-5172 drug is that it has shown better potential in combating drug-resistant variants than Boceprevir. (HCV drugs).

Figure 3: MK-5172; as shown the side chain is the line connecting the top of the molecule to the bottom. In that side chain elimination it is predicted to increase the efficacy against the virus, by fitting well within the substrate envelope.

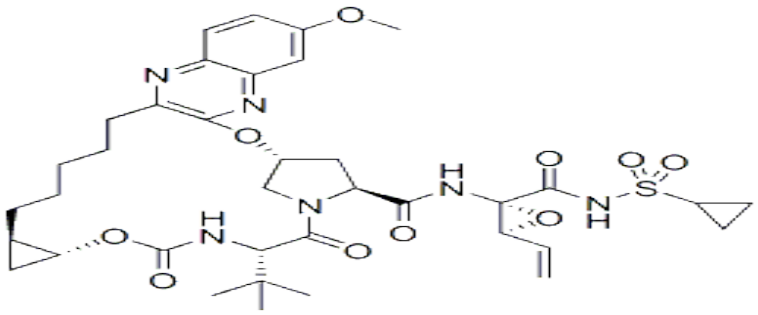


Figure 4: Drugs along with their derivatives. MK-5172 is shown along with the final compound demonstrated as the first derivative and the R group being a chlorine atom. Side chains are manipulated, focusing on minimizing the size of the compounds.

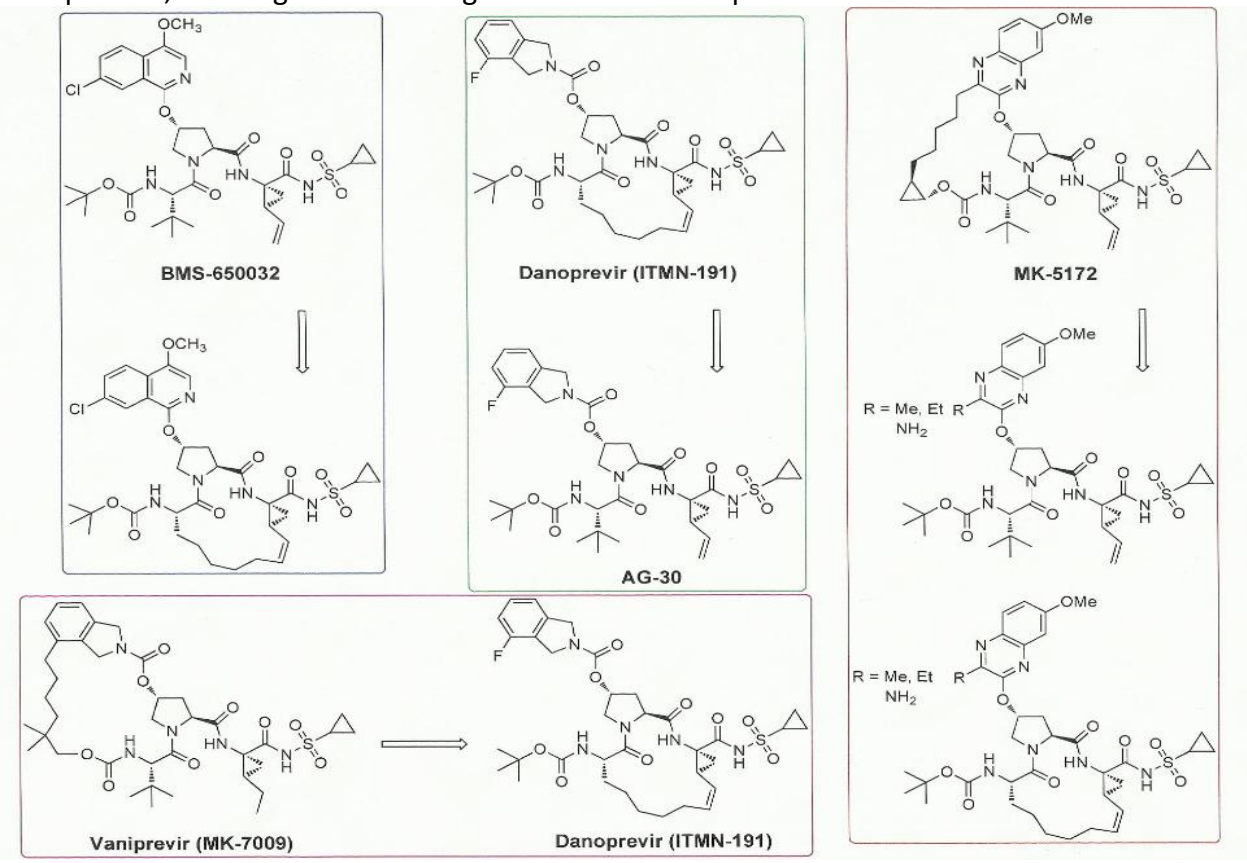


Figure 5: Final step in the drug synthesis. In the original MK-5172 molecule the location of the chlorine atom would be attached to the lower Boc group. The elimination of the side chain ring is predicted to produce better results against the virus and the drug resistant variants.

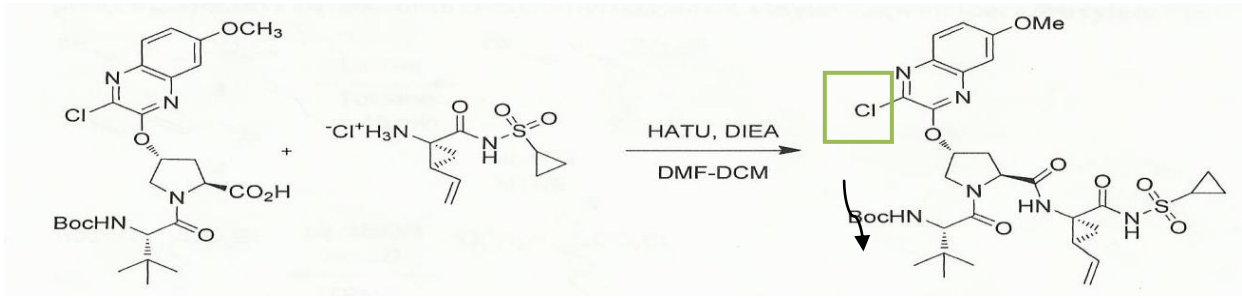


Figure 6: Full Proton NMR spectrum for the final product. The NMR shows peaks where the hydrogen are located on the final compound.

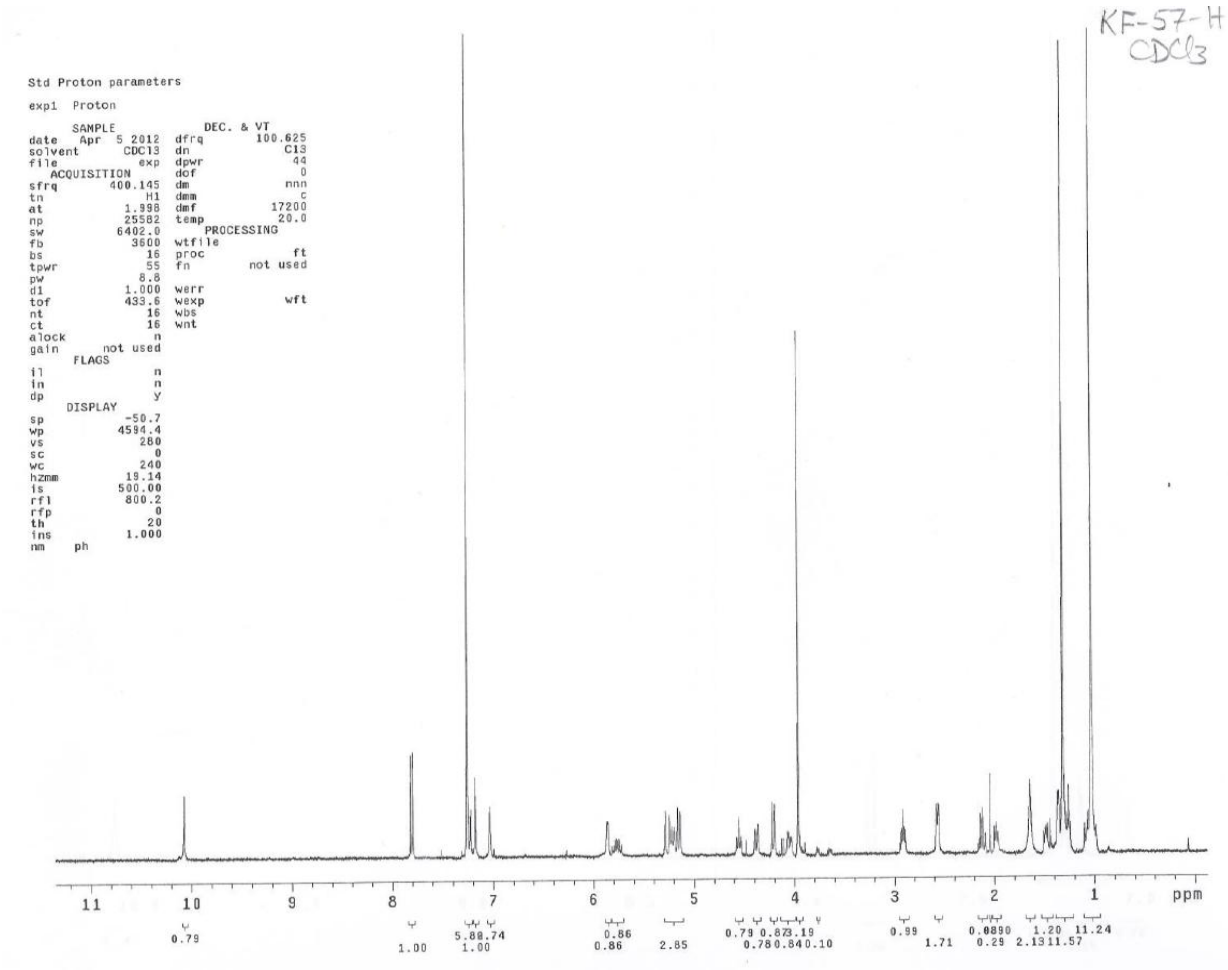


Figure 7: Proton NMR from the 6.2 - 3.6 ppm region. A closer look allows for better interpretation of the NMR peaks.

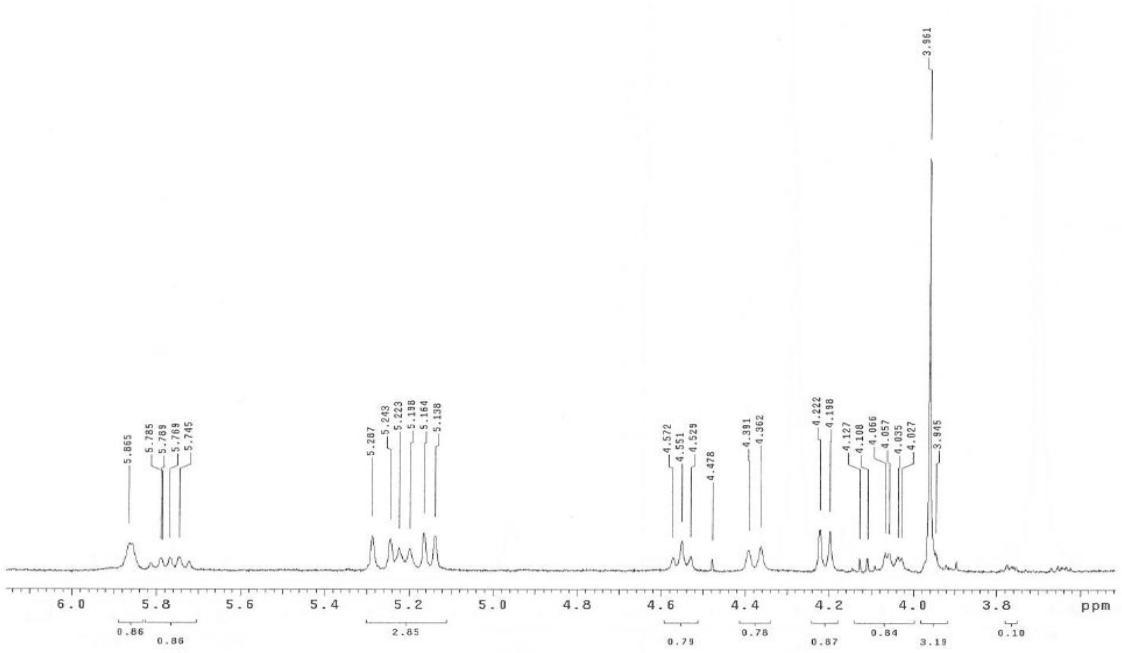


Figure 8: Proton NMR from the 2.2 – 0 ppm region. Again a closer look enables the view to see the peaks in an enhanced fashion.

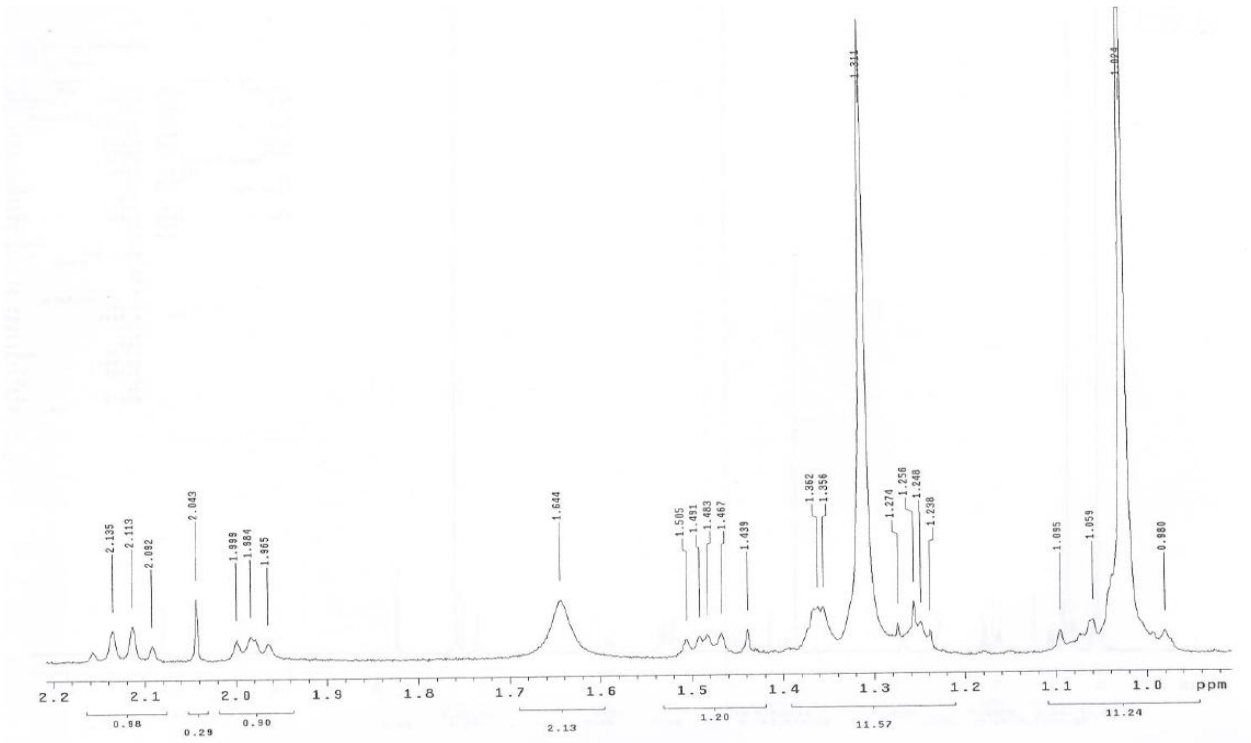


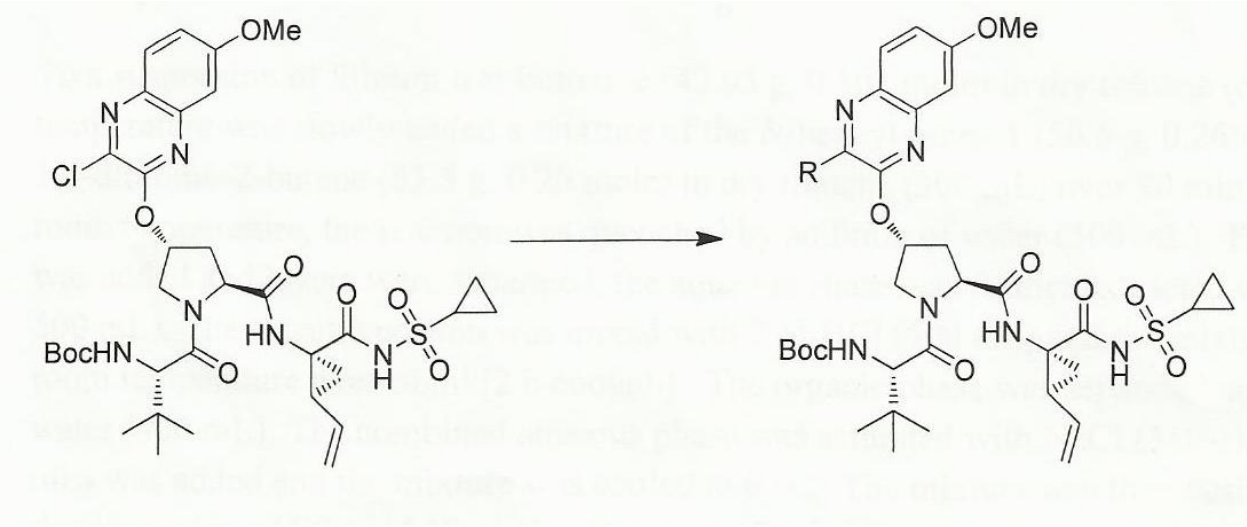
Figure 9: Activity against wild-type and drug resistant variants. MK-5172 produced the lowest K_i values as compared to the wild type virus and drug resistant variants; the lower the K_i the more effective the drug is in combating the virus (Akbar Ali).

Enzymatic and antiviral activity of HCV NS3/4A protease inhibitors against wild-type and drug-resistant variants

Drug	K_i (nM)	Antiviral - IC_{50} (nM) (fold change)			
	WT	WT	R155K	D168A	A156T
Telaprevir	66 ± 3	1030	5300 (5.1)	420 (0.4)	>50,000 (>49)
Danoprevir	0.46 ± 0.03	0.24	>100 (>416)	48 (200)	5.7 (24)
AG-30	1.5	-	-	-	-
Vaniprevir	0.13 ± 0.02	0.34	>400 (>1176)	>400 (>1176)	176 (518)
AG-53	0.50	-	-	-	-
MK-5172	0.0024 ± 0.005	0.11	0.55 (5)	13 (118)	108 (982)

Numbers in parentheses reflect fold-change relative to wild-type.

Figure 10: Final product exchanged to a new drug derivative involving other side groups, represented by R. The R groups would be functional groups smaller than a ring system such as methyl, ethyl or amine groups. The ring system has trouble fitting in the substrate envelope decreasing the efficacy of the drug, therefore the smaller the functional groups the better the drug.



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