Synthesis of Se-Adenosyl-L-Selenohomocysteine Selenoxide and Potential Gram-Negative Antibacterial Analogs

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Dedication

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Abstract

This paper includes work performed on two separate projects. The first project is the synthesis and characterization of a new analog of S-adenosylmethionine (AdoMet or SAM) called Se-adenosyl-L-selenohomocysteine selenoxide (SeAHO). This work outlines the total synthesis starting from adenosine and the characterization data collected along the way. The work culminated with the first successful synthesis of the desired selenoxide analog that can now be studied further for its potential biological activity. The second project involves medicinal chemistry efforts in synthesizing analogs of the oxazolidinone scaffold with desirable characteristics for potential treatment of Gram-negative bacterial infections. The oxazolidinone scaffold has already been shown to be successful in treating Gram-positive bacterial infections and the goal of our research is to be able to convert it to one that also has activity against Gram-negative bacteria. With the goal of developing a novel approach to making compounds with this activity, we look at specific modifications made to certain areas of the scaffold and how these changes impacted the activity against different strains of bacteria through our biological assay.
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Chapter 1: Synthesis and Characterization of Se-adenosyl-L-selenohomocysteine selenoxide

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Introduction

S-adenosylmethionine (SAM or AdoMet) is a well-known, naturally occurring methylating agent that is involved in many chemical processes in the body. S-adenosylmethionine dependent methyltransferases are enzymes that are necessary to catalyze the donation of the methyl group to other compounds, such as proteins or small molecules, and, in the process, form S-adenosylhomocysteine (SAH or AdoHcy). There has been much research in this field on the synthesis of analogs of S-adenosylmethionine at its reactive sulfur center. Some such analogs include keto-AdoMet, propargyl-AdoMet, and Adovin. Developing new analogs is of interest for many reasons which include tagging proteins, studying the functions of methyltransferases, and forming new compounds for the activation or inhibition of certain enzymes.

Another analog that has been synthesized and studied for its role as an inhibitor is S-adenosylhomocysteine sulfoxide (SAHO). This compound was first synthesized in 1970 and has been cited as showing inhibition for enzymes such as mRNA methyltransferase, protein methyltransferase II, and catechol-O-methyltransferase. However, the sulfoxide analog has not shown to be as effective an inhibitor as S-adenosylhomocysteine. While this may be the case, that does not stop analogs of similar structure from being of interest to researchers in this area.

Along with these modifications of the methyl group, another series of analogs that has begun to be explored are those replacing the sulfur with closely related selenium. Selenium plays an important role as a part of many metabolites found in living organisms. Though the amount of selenium present is much less than that of sulfur, these analogs are still of interest because of how little is known about them.
A recent paper published by Willnow et al. outlines a new synthesis for the selenium analog of S-adenosylhomocysteine as they discuss the modification of proteins through copper-catalyzed azide-alkyne cycloaddition (CuAAAC) click chemistry. As part of this work, they synthesized a new selenium AdoMet analog with a propargyl group and showed that the selenium center led to better stability and activity. Compared to S-adenosylmethionine and S-adenosylhomocysteine, not much is known about the biochemical activity and redox chemistry of Se-adenosylselenohomocysteine. Also, the selenoxide analog had not been previously synthesized before our group reported it in a recent publication. In the next chapter, I describe the synthesis and characterization of Se-adenosyl-L-selenohomocysteine selenoxide.

**Synthesis**

*Figure 1.1: Synthetic route for SeAHO.*

**Step 1**

The first step of this synthetic route, preparation of 5'-chloro-5'-deoxyadenosine (CIDA), was run on a 5.0 gram scale using a procedure adapted from the literature. This was a two part
procedure that involved first making a sulfinyl intermediate and then the desired CIDA product. The first part resulted in 5.435 grams (88% yield) of the sulfinyl intermediate. The next step involved removing the protecting group from the intermediate and a recrystallization that yielded 4.68 grams (100% yield) of the desired product.

Melting Point, TLC, and NMR analysis were all performed to determine structure and purity. The product began decomposing between 185-190 °C and this matched up with the literature values. TLC in 3:1 chloroform: methanol, under UV (Figure 1.2), showed one spot for the product and no starting material present.

A proton NMR (Figure 1.6) was run, using a 700 MHz instrument, with the product in deuterated DMSO and the resulting spectrum showed the desired peaks. Some additional solvent peaks showed that there was some residual water and methanol present in the sample. Overall compound characterization proved that we made our desired product with overall 90% yield.

Figure 1.2: TLC under UV of starting material (adenosine), product (CIDA), and a co-spot.

Step 2

The next step of this route was to make the selenium analog of S-adenosylhomocysteine based on a recently published synthesis. In order to carry out this step, however, we first had to
synthesize selenohomocysteine from selenomethionine. This reaction was run on a 700 mg scale and the unpurified product was used for the reaction with 926 mg of CIDA.

The reaction gave crude Se-adenosylselenohomocysteine (SeAH) that was dried under vacuum overnight to give a yellowish white solid. The solid was then purified by two hot filtration/crystallizations in methanol and this resulted in 330 mg (24% yield) of crystals. During the second recrystallization, some of the crystals, about 115 mg, would not dissolve and these were set aside to be analyzed separately.

The melting point of the SeAH crystals was found to be about 204 °C and this is a new value not previously reported in the literature. This step of the synthesis was monitored by a TLC method adapted from the literature. Originally a solvent system of 12:5:3 isopropanol: water: acetic acid was used but was changed to a ratio of 12:1:3 of the same solvent system because it proved to give better separation. TLC of the crystals that would not dissolve showed them to be relatively pure when compared to the crude reaction product (Figure 1.3).

![Figure 1.3: TLC plates stained with potassium permanganate (left) and ninhydin (right). Each has six spots starting from the left: CIDA, selenomethionine, Step 2 before crystallization, crystals that didn’t dissolve during recrystallization, crystals after recrystallization, and methanol mother liquor.](image_url)
Step 3

TLC analysis showed that SeAH was a more polar molecule consistent with the desired product and so a small amount (5 mg) was used in a reaction in order to attempt to make the selenoxide analog. The procedure used for this reaction was one that was adapted from the literature for the synthesis of S-adenosylhomocysteine sulfoxide. An excess of hydrogen peroxide (30%) was used to oxidize selenium and, while the Borchardt paper allowed the reaction with SAH to go overnight, by monitoring with TLC we were able to see the new product spot appear almost instantaneously. While this reaction was going, we also made the sulfoxide analog, SAHO, from SAH in order to compare to the selenoxide analog.

Upon seeing that the selenoxide product, SeAHO, had formed, we tried to precipitate it out of solution with methanol as done in the Borchardt paper for the sulfoxide analog. The sulfoxide successfully precipitated as reported, however, the selenoxide did not and so we had to obtain a solid through lyophilization. The solution was frozen using dry ice and the solvent removed under high vacuum over one hour to give a quantitative yield.

Discussion

Se-adenosyl-L-selenohomocysteine selenoxide (SeAHO) was run on high performance liquid chromatography (HPLC) and compared to samples of SAH, SeAH, and SAHO. These traces appear in Figure 1.4 and we see the product elute around 5 minutes. While we also see some later eluting peaks that would seem to be from degradations products, this analysis shows a clear difference between the starting material and the oxidized product.
Mass Spectrometry data was also collected for these compounds using a liquid chromatography mass spectrometer (LC-MS), but SeAHO was the only product not to give the desired molecular ion, showing an (M-18)+ peak, however.

NMRs of each compound were also taken in acetic acid-$d_4$ and these led to some interesting results. When comparing the NMR for the SeAHO to the other spectra, the movement of the peaks may suggest that a cyclization with the amine and/or carboxylic acid occurred. The structures in Figure 1.5 suggest possible cyclization products that may have formed based on the observed molecular ion.

**Figure 1.4:** HPLC 1 is of $S$-adenosylhomocysteine (SAH) and the peak appears at 11.967 min, HPLC 2 shows $Se$-adenosylselenohomocysteine (SeAH) and this peak elutes at 12.817 min, HPLC 3 is of $S$-adenosylhomocysteine sulfoxide (SAHO) and the peak appears at 5.054, and HPLC 4 is of $Se$-adenosylselenohomocysteine selenoxide (SeAHO) with the peak eluting at 5.114. Column: Alltech Econosil C18, 4.6mm x 250mm.
In order to see if we could observe the desired molecular ion (449) the sample was run on MALDI MS and LC-MS/MS instruments. The data observed did reveal the desired molecular ion along with that observed previously. From this data we see our desired mass of 449 and fragmentation to 431 and 467, which are the M-18 and M+18 peaks respectively. A mass difference of 18 signifies a gain or loss of water. The mass of 467 clearly indicates the hydrated form of the compound or the dihydroxyselenide ion. The 431 ion peak is a little more difficult to explain but, as previously stated, the literature does show precedent for the formation of cyclic analogs. The structures shown in Figure 1.5 are possible cyclization products that may have occurred and their masses are about 431.

**Conclusion**

From the NMR and mass spectral data, we are able to say confidently that we successfully synthesized Se-adenosyl-L-selenohomocysteine selenoxide for the first time. This compound is of interest as a possible inhibitor of certain S-adenosylmethionine dependent methyltransferases, other enzymes, and proteins due to its close similarity to the sulfoxide analog of SAM. Further studies of this compound have shown its stability in aqueous environments and
its ability to be reduced by glutathione and cysteine. This work offers further opportunities for selenium analogs of SAM to be studied in order to gain a better understanding of selenium’s biological functionality compared to sulfur.
Data

Se-adenosylselenohomocysteine (SeAH) in HOAc-\textit{d}_4

\textbf{Figure 1.6:} Proton NMR of SeAH in HOAC-\textit{d}_4.
*Se*-adenosyl-L-selenohomocysteine selenoxide (SeAHO) in HOAc-d4

**Figure 1.7:** a) Proton NMR of SeAHO in HOAC-d4. b) COSY of SeAHO.
Se-adenosylselenohomocysteine (SeAH)

Figure 1.8: LC-MS of SeAH
Figure 1.9: LC-MS of SeAHO.
SeAHO Sample (Full scan):

Figure 1.10: Total ion chromatography (TIC) of SeAHO.
**Figure 1.11:** Extracted ion chromatography (XIC) of 431.0, 449.0 and 467.0 m/z in SeAHO sample.

**Figure 1.12:** MS at 2.16, 2.25 and 4.74 min in SeAHO sample.
Experimental

General Data

TLC was carried out on plastic-backed silica gel 60, PE SIL G Whatman Plates, UV\textsubscript{254}. HPLC used an Agilent/Hewlett Packard Series 1100. NMR spectra were recorded on a Bruker 700 MHz spectrometer. Mass Spectrometry Instrumentation used included: (1) LC-MS-ToF Waters LCT Premier ToF MS; (2) LCQ-MS: Finnegan LCQ ESI ion trap MS; (3) MALDI-MS: AB SCIEX 5800 TOF/TOF

1. Synthesis of 5’-chloro-5’-deoxyadenosine (CIDA)

The procedure for this step was taken directly from a paper by Scovill et al.\textsuperscript{17} A suspension of adenosine (5.00 g, 19 mmol) in 19 mL of acetonitrile was treated drop wise with thionyl chloride (4 mL, 6.6 g, 56 mmol) keeping the temperature between 0 to 5 °C. Pyridine (3 mL) was then added and the mixture was stirred at 0 °C for 3 hours. Stirring was continued overnight, and the mixture allowed to warm to room temperature. The precipitate that formed was dissolved by adding 56 mL of water to the reaction mixture. The solution was neutralized, to pH 5-6, with 5.5 g of sodium bicarbonate, and the solid that precipitated was collected, washed with cold water, and dried under vacuum to yield 5.435 g (90\%) of the sulfinyladenosine intermediate.

The sulfinyladenosine mixture converted to 5’-chloro-5’-deoxyadenosine, without further purification, as follows: a solution containing 5.435 g (16 mmol) of the sulfinyl ester in 50 mL of
methanol was treated with 5 mL of concentrated ammonium hydroxide. Crystals formed upon standing for an hour at room temperature. These were collected, washed with cold methanol/ammonium hydroxide solution, and dried under vacuum to yield 4.68 g (99%) of colorless needles of 5’-chloro-5’-deoxyadenosine. $^1$H NMR in Appendix A1.

2a. Synthesis of L-selenohomocysteine from L-selenomethionine

\[
\text{Se-} \text{NH}_2 \text{COOH} \quad \quad \quad \quad \quad \text{HSe-} \text{NH}_2 \text{COOH}
\]

For this step, we used a procedure that was outlined in a paper published previously by our group. A round bottom flask was prepared with inert atmosphere and to this was added selenomethionine (~700 mg). This was cooled to -80 °C in a dry ice/acetone bath and 30 mL of ammonia was added. This reaction was stirred and to this was added small pieces of sodium metal until the reaction remained blue (260 mg). This was stirred, in darkness, for 1 hour and then ammonium chloride (590 mg) was added slowly in order to neutralize any sodium amide present. The reaction was then removed from the dry ice/acetone both and nitrogen was blown over it to remove solvent. This was then dried under vacuum to give a white solid.

2b. Synthesis of Se-adenosyl-L-selenohomocysteine (SeAH)

\[
\text{NH}_2 \text{Cl} \text{O} \text{OH} \quad \quad \quad \quad \quad \text{HO-} \text{NH}_2 \text{SeH} \quad \quad \quad \quad \quad \text{HO} \text{NH}_2 \text{Se} \text{O} \text{OH}
\]

\(\text{NaOH}\) 15 hr, 80°C
The procedure for this step was adapted from a paper by Willnow et al.\textsuperscript{7} A fresh batch of L-selenohomocysteine (700 mg) was dissolved in water (4 ml) and to this was added 5'-chloro-5'-deoxyadenosine (926 mg). To this mixture was then added 2.5 mL of a 10% sodium hydroxide solution and more water (5 mL). This reaction was let stir for 1.5 hours at 80 °C. The solution remained cloudy for 15 minutes before becoming homogeneous. Acetic acid (~1.25 mL) was then added drop wise until the solution pH was between 5 and 6. The reaction was put under vacuum and let dry overnight to give a yellowish white solid. This solid was dissolved in an ethanol/toluene solution and again dried under vacuum to remove residual solvent.

The reaction was monitored by TLC using a 12:5:3 (from literature) and 12:3:3 isopropanol:water:acetic acid solvent system. The product was purified through recrystallization with hot filtration in methanol. As much of the product as possible was dissolved in boiling methanol. The solid that didn’t dissolve was filtered off and the remaining solution was boiled down until crystals started forming. This sat overnight before the mother liquor was filtered off and the crystals were washed with cold methanol.

A second recrystallization was run in the same way. The mother liquor was also saved as partially purified SeAH. TLC of the final crystals showed a single UV spot lower than the ClDA starting material spot. Additional plates were run and stained with potassium permanganate and ninhydrin. The adenine group was visible by UV254 TLC plates with a hand-held short wave UV lamp, potassium permanganate staining showed thioether, selenoether, and ribosyl groups, and the ninhydrin stain showed the amino group. The pure crystals of SeAH acetate salt were isolated in a final yield of 330 mg (24%). MP 204-205 °C. TLC: 12:1:3 isopropanol:water:acetic acid: ClDA \( R_f \) 0.80, selenomethionine \( R_f \) 0.73, and SeAH \( R_f \) 0.44. TLC 12:5:3 isopropanol:water:acetic acid: SeAH \( R_f \) 0.72. DAD UV spectrum \( \lambda_{max} \) 258 nm. \(^1\)H NMR (700
MHz, CD$_3$CO$_2$D) δ 2.22-2.30 (m, 1H, βb), 2.32-2.39 (m, 1H, βa), 2.74-2.84 (m, 2H, γ), 3.03 (dd, J=13.2, 6.2 Hz, 1H, 5b’), 3.05 (dd, J=13.2, 5.7 Hz, 1H, 5a’), 4.16 (m, 1H, α), 4.38 (ddd, J=4.3, 5.7, 6.2 Hz, 1H, 4’), 4.49 (dd, J=4.3, 5.4 Hz, 1H, 3’), 4.86 (dd, J=4.4, 5.4 Hz, 1H, 2’), 6.16 (d, J=4.4 Hz, 1H, 1’), 8.44 (s, 1H), 8.50 (s, 1H, Ar) (Figure 1.6)

3. Synthesis of Se-adenosyl-L-selenohomocysteine selenoxide (SeAHO)

![Diagram of SeAHO synthesis](image)

The procedure for this step was adapted from a paper by Borchardt and Wu where they make the corresponding sulfoxide analog. $^{12}$ Se-adenosylselenocysteine, SeAH, (6 mg, 1.4 x $10^{-5}$ mol) in a 4 mL vial was suspended in acetic acid (0.2 mL) and stirred magnetically. Excess 30% hydrogen peroxide solution (65 µL) was added, the suspended grey solid became soluble giving a homogeneous, clear, and colorless solution. In order to obtain a solid, the solution needed to be frozen by shell freezing with dry ice and the solvent removed under high vacuum. TLC (silica gel 60 on plastic, F254) was used to monitor the reaction and showed almost instantaneous complete formation of the corresponding selenoxide product (Se-adenosyl selenohomocysteine selenoxide, SeAHO. TLC (12:1:3 isopropanol/water/acetic acid, SeAH Rf 0.46, SeAHO Rf 0.26), TLC (12:5:3 isopropanol/water/acetic acid, SeAH Rf 0.68, SeAHO Rf 0.41), HPLC (Alltech Econosil C18, 4.6 x 250 mm, 1.00 ml min$^{-1}$, λ 280 and 215 nm, 2:98 CH$_3$CN/H$_2$O for two minutes, then gradient to 40:60 over 20 min, SeAH tR tailing peak at 12.82
min, SeAHO tR 9.3 min as a broader peak with shoulders at 8.9 min and 10.2 min for the more polar compound), HPLC-ESI MS 431, 402, 348, 250, DAD UV spectrum λmax 260 nm, NMR: 1H NMR (700 MHz, CD$_3$CO$_2$D) δ 2.60-2.70 (m, 1 H, βa), 2.75-2.85 (m, 1 H, βb), 3.65 (br d, 1 H, J = 10.7 Hz, 5a’), 3.84-3.95 (m, 3 H, 5b’ and both γ), 4.67-4.58 (m, 2 H, α, 4’), 4.72 (dd, J = 5.7, 4.7 Hz, 1 H, 3’), 4.94 (dd, J = 4.7, 3.7 Hz, 1 H, 2’), 6.20 (d, J = 3.7 Hz, 1 H, 1’), 8.45 (s, 1 H, Ar) 8.48 (s, 1 H, Ar). (Figure 1.7)
Chapter 2: Introduction to Multidrug Resistant Gram-Negative Bacteria

Finding compounds that act as potent antibacterial agents has long been an arduous task of scientists and, in particular, medicinal chemists. In the middle of the 20th century, this area of research reached its peak success as many of the antibiotics still used today, such as penicillin and vancomycin, were discovered. The “golden age” of antibiotics produced many antibacterial agents that led to a revolution in the field of medicine. Unfortunately, in the half a century or so since that time period, the number of antibiotics developed has severely plummeted and the current pipeline is not all too encouraging.\textsuperscript{20-21} This is mainly to do with a lack of urgency and financial incentive for major pharmaceutical companies; many of whom have already left behind this area of drug development. Both of these factors need to change (and seem to be heading in that direction) in the immediate future as the development of bacterial resistance is an area of growing concern.\textsuperscript{22}

In the years since the golden age of antibiotics, bacteria have evolved to fight off all of the antibiotics we have at our disposal. The consistent use, and over prescription, of the same antibiotics have led certain strains of bacteria to mutate and develop mechanisms of resistance against all antibacterial agents we have on the market. From 1999 to 2010, the resistance levels of \textit{Acinetobacter baumannii}, a Gram-negative bacterium, against carbapenems (a common class of antibiotic used as the last line of defense against multidrug-resistant bacteria) rose from 5\% to 40\% (as seen in Figure 2.1) and the assumption is that it has only increased further in the five years since then. A 2013 report by the Centers for Disease Control and Prevention stated that two million Americans are infected with resistant bacteria each year and 23,000 of them die as a direct result.\textsuperscript{23} This is not to say that all bacteria are resistant to the antibiotics we currently have, but the numbers are growing and the problem must be addressed.
Figure 2.1: Graph of growing bacterial resistance to carbapenem antibiotics from 1999-2010. (Data used to generate graph was obtained from the Center for Disease Dynamics, Economics & Policy website)  

The biggest threat posed by these strains comes from multidrug resistant Gram-negative bacteria (GNB) as these are responsible for the majority of hospital acquired infections. Unlike Gram-positive bacteria (GPB), GNB have an additional layer of protection to ward against antibiotics. This extra layer is a lipopolysaccharide containing outer membrane that does not allow compounds with antibacterial activity (or anything) to penetrate inside to the bacteria. These bacteria do need certain nutrients in order to survive, however, and so this membrane does have small entrance points along it called porins. These porins represent an area to exploit when developing Gram-negative antibiotics.

That being said, these porins limit the size and properties of the compounds that can pass through and are not the final obstacle potential antibiotics need to overcome. Within the membrane of these bacteria are efflux pumps that effectively pump out any undesired compounds that happen to find their way through the porins. A potential Gram-negative antibiotic needs to have properties that allow it to pass through the porins and get to the bacteria.
without being transported back outside the membrane by the efflux pumps (Figure 2.2). The lack of development of potential Gram-negative antibiotics to this point may be due to an incomplete understanding of these bacteria. A rational approach to making these antibiotics does not currently exist and the goal of our group is to change that.

The approach is two-fold as we want to: 1) develop compounds that show they are able to penetrate the outer membrane and remain inside and 2) develop a biochemical assay that can not only show this but also be an effective predictor of how the compound will work once inside whole cells. The assay being used currently is a minimum inhibitory concentration (MIC) assay and involves comparing the activity of compounds against gram positive bacteria vs. strains of wild type GNB (*Escherichia coli*) and modified mutants. The mutants are efflux pump knockout strains and membrane permeabilized variations that allow us to see how modifications made to our target scaffold affect its activity against each individual barrier.

![Diagram of the outer membrane of gram-negative bacteria.](image)

**Figure 2.2**: Diagram of the outer membrane of gram-negative bacteria.

Our idea to target compounds with only desired physicochemical properties so that they can penetrate and remain in the cell means we should be able to take a scaffold already used
against GPB and apply it toward this problem. The oxazolidinone class is a good starting point because of the amount of literature already published on its SAR activity and toxicity. This class derives its activity from its ability to disrupt protein synthesis by blocking aminoacyl tRNA substrate binding at the A-site of the peptidyl transferase center through competitive inhibition at the 50S ribosomal subunit.\textsuperscript{26} Linezolid and tedezolid phosphate are the two drugs of this class that have market approval as GPB antibiotics (shown in Figure 2.3). Radezolid is a compound that has shown activity against weaker strains of GNB (\textit{H. influenzae}) and proves that this class has potential as an antibacterial against GNB.

Looking at the structure, we can divide the oxazolidinone scaffold into an A, B, and C ring. The A ring contains the center of the scaffold in the oxazolidinone ring and an R group where modifications are typically made. The C ring is the other area of this scaffold where modifications well tolerated. Minimal modifications can be made to the B ring due to tolerability issues but there are some that can be explored. Based on previous work done, the ideal candidate will have a low molecular weight ($< 400$), high polarity (Log P $\sim 0$), and multi-charge character at physiological pH. Here I include the compounds that I have synthesized and the biological assay MIC results associated with them.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures and properties of oxazolidinones linezolid, radezolid, and tedezolid.}
\end{figure}
Chapter 3: Synthesis and Analysis of Potential Gram-Negative Antibacterials

**Figure 3.1:** General scheme for oxazolidinone compound synthesis.

**General Synthesis**

The general procedure for the synthesis of these oxazolidinone structures requires 3-4 main steps (Figure 3.1). Depending on the modifications in functionalities that we are looking at this can change but for the compounds that I made this outlines the common synthetic scheme. We begin by purchasing an aniline with the desired components and protecting it with benzyl chloroformate. This is done by adding benzyl chloroformate dropwise to a solution of the desired aniline starting material and potassium carbonate in tetrahydrofuran (THF) at 0°C. After being stirred for two hours the reaction is quenched with water and extracted with ethyl acetate. The reaction yields the desired CBz protected compound (1) at about 95% and is ready for direct use in step two.

From the protected aniline can be formed the oxazolidinone ring. Compound 1 is reddissolved in THF and cooled to -78 °C in a dry ice acetone bath. To this is slowly added n-
butyl lithium or lithium bis(trimethylsilyl)amide (LHMDS) and the reaction is stirred for an hour at the same temperature. After this point, (R)-(-)-glycidyl butryate is added dropwise and let warm to room temperature. It is important to use this form of glycidyl butryate in order to generate the desired stereochemistry of the tail coming off the A ring. Upon completion of this step (as determined by LC-MS) the reaction is quenched with water and stirred for an hour before being worked up by the same conditions used in step 1. The reaction does not always go all the way to completion as it has been found to stall with some intermediate still remaining. Also, depending on the compound, a recrystallization in 20% ethyl acetate/hexanes may be necessary in order to get a solid after concentrating the organic layer down.

The next step is determined by the commercial availability of desired C-rings as boronic acids or bromides. The boronic acid form can be coupled directly to compound 2 by a Suzuki (also known as a Suzuki-Miyaura) coupling. However, sometimes these are not readily available for purchase and the bromide must be bought instead. In this case, the boronic ester/acid (3) must be synthesized first. For this step, compound 2 is dissolved in dry DMSO and mixed with, bis(pinacolato)diboron, potassium acetate, and 1,1’-[bis-(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl₂). This is heated to 80 °C under nitrogen and let stir overnight before being cooled to room temperature upon completion. The product is then recrystallized by adding methanol and water to the mixture and letting it sit at 4 °C.

As it was just mentioned, the final step of our general synthesis of these oxazolidinone compounds is a suzuki coupling to add the desired C-ring. The literature is overflowing with hundreds of possible conditions with which to run a suzuki cross-coupling reaction and often times chemists must try multiple procedures before finding the most efficient for their
compounds. Our laboratory has found a procedure that is efficient in a majority of our suzuki couplings with these oxazolidinone intermediates. Exceptions exist to every rule, however, and sometimes the conditions need to be tweaked or a new procedure attempted depending on the functionalities contained on our C-rings.

The typical conditions for our suzuki couplings involve the use of Pd(dppf)Cl₂ (0.05-0.1 equiv) as our catalyst and potassium carbonate (4 equiv) as our base. These reagents along with the boronic ester/acid (1.2 equiv) are added to the arylbromide starting material (1 equiv) dissolved in 9:1 dioxane:water. These reactions are stirred between 85-90 °C and typically are done after 1-2 hours (occasionally being run longer or even overnight). LC-MS is used to determine when the reactions are done and, upon completion, they are quenched with water and worked up in ethyl acetate. The crude final product is then purified by reverse-phase HPLC (RP-HPLC) using 0.1% TFA in water and 0.1% TFA in acetonitrile as the solvents. Fractions containing the purified material are collected, combined, and frozen in a dry ice/acetone bath before being lyophilized (freeze-dried). Once dry, the material is tested by analytical HPLC to determine purity and then 2 mg/mL samples in 12% DMSO/water are made for submission to be tested.

Reasoning behind making chosen analogs

The structures of the compounds that I synthesized along with their antibacterial activity generated from our assay are listed in Table 3.1. The focus of the modifications I made to the oxazolidinone scaffold were at the B and C rings. Changes at the C ring may be the easiest to make of the three areas mentioned earlier because of the wealth of commercially available arylbromides and boronic acids for coupling. In some instances (such as DP-305 and 314), however, additional synthetic steps were required in order to get the desired functional groups in
position. C ring modifications at the positions ortho to the B ring were not attempted as these have proven ineffective in the past. Previous work has identified that having a hydroxyl group at the meta position seems to be beneficial to the efflux activity of the molecule so DP-304 was synthesized to see the effect of having them at both meta positions. Substitutions at the para position also seems to be well tolerated with methylamine giving the most positive results to date. In order to improve baseline potency, a cyclopropyl amine group was installed in DP-315. Adding charge character to our scaffold is also important and synthesizing the N-oxides, DP-305 and DP-314, was of major interest. DP-281 was made in order to see the effect of a strongly electron withdrawing group on the activity.

B ring changes require a little more effort as the synthesis must be started from the beginning with the aniline that contains the desired change. As of this writing, our main scaffold contains a fluorine on the B ring at the ortho position relative to the C ring. DP-64, 65, 293, 296, 318, 327, 328, and 329 were compounds that were of interest in exploring how the activity was affected by replacing this fluorine. Decreasing hydrophobicity to improve activity against efflux was the goal with these modifications. By combining these new B rings with C rings that had previously shown the best activity we were able to get a good sense of how the changes impacted the molecules ability to penetrate the lipopolysaccharide membrane and avoid being effluxed.

Analyzing the biological data

As mentioned in the previous chapter, the compounds were tested in a biological assay against an initial panel of bacteria that include a wild type Gram-negative strain of E. coli, a modified version of this strain with the outer membrane destabilized, a mutation of the wild type strain with the efflux pumps knocked out, and a control Gram-positive strain of Staphylococcus aureus. From this assay, we generate MIC values and then use these to calculate efflux and
permeability ratios that allow us to see how well each compound is performing against these individual barriers. This data is shown in Table 3.1 and we can calculate each ratio through a simple formula that involves dividing the MIC value generated against the bacterial strain by the MIC value for the Gram-positive bacterial strain. Understanding this we can then analyze the modifications made to our scaffold as I do below.

Attempts at modifying the B ring provided us with some interesting results. We learned that changing the B-ring to a pyridine appears to obliterate the antibacterial activity. This is based on the data from DP-65, which has the pyridyl nitrogen ortho to the C-ring, and data we saw when its analog was synthesized by a colleague. Oxidizing the nitrogen on this ring to form the N-oxide doesn’t reverse this trend either as can be seen by the data for DP-293. Using oxygen to replace the fluorine was tried in DP-327 and DP-328 with replacements by a methoxy and hydroxyl respectively. As with the previous changes, these modifications completely obliterated all antibacterial activity. Replacing the fluorine with hydrogen, on the otherhand, showed some promise starting with my intial compound DP-64.

When looking at the MIC data, comparing the ratios between the mutants and our baseline Gram-positive strain is what we are interested in. We want to show that we can convert a Gram-positive bacteria antibacterial scaffold to a Gram-negative antibacterial. DP-64 appears to show a nice improvement against efflux and because of this we pursued other “des-fluoro” compounds. Next we looked at making a compound with a fluorine at the ortho position on the C-ring in order to determine if giving the compound torque at that bond was important for its activity. DP-296 lost the improved efflux ratio but did see a much improved permeability ratio. With our previous work showing that a hydroxyl group at the meta C ring position is good for
efflux, and DP-64 seemingly backing that up, and the data from DP-296 we decided to make DP-318 in hopes that we would see additive effects.

While we didn’t see the improvements we hoped for in terms of efflux and permeability we did see DP-296 and DP-318 give us a nice return to the baseline activity against our *S. aureus* strain as compared to DP-64. This was important because it may suggest that having a fluorine (or possibly a similar sized functional group) at the ortho position on the C ring can work as a substitution for the fluorine on the B ring. This may be due to the torque effect on the rotatable bond caused by having a group adjacent to it or to the possibility of a binding pocket located in the target that a substituent at either position can fit into.

Other compounds that I synthesized looked at potential modifications of interest on the C ring. DP-281 looked at the effect of having a strong electron withdrawing group at the para position with a nitro group. While the compound showed strong activity against the *S. aureus* strain it had solubility issues and failed to do anything against the Gram-negative strains. Putting a hydroxyl group at both of the meta positions, DP-304, also failed to give us any positive trend in activity. DP-315 was made to compare to a similar compound without the cyclopropane group and we found them similar but the addition of that ring slightly lowered the activity of the compound in terms of efflux.

One group that did interest us was the N-oxide when placed on the C ring. While it showed no activity when place on the B ring, compounds with zwitterionic character at physiological pH have already shown positive results against Gram-negative bacteria. DP-305 and DP-314 are two isomers containing the N-oxide functionality and both show good baseline activity. Based on the aforementioned trend of reduced efflux with the presence of a hydroxyl group at the meta position, we expected DP-314 might be a better compound. Instead, we found
both had the same activity against the permeabilized membrane strain and that DP-305 appeared to be better at penetrating the lipopolysaccharide membrane.

**Conclusions**

While none of the compounds I synthesized have led to the desired activity numbers our group is looking for, they have revealed some trends that can be used in future work. From the modifications made to the B-ring of our oxazolidinone scaffold, we can conclude that changes in this area are not highly tolerated. Substitutions of the fluorine, on this ring, with anything other than hydrogen appears to make the compound totally inactive against all bacteria. Replacing the fluorine with hydrogen gave us some interesting data and the compounds I synthesized showed potential in improving permeability or efflux. However, combining modifications that gave these improvements individually did not provide us with the desired additive effects we expected to see.

In regards to the C-ring modifications I made, only the introduction of an N-oxide appeared to have interesting potential. Particularly at the para position, the N-oxide showed decent permeability and good baseline potency and this may be attributed to its charge character. Further work with the n-oxide has not been attempted at this point in time.

I have reviewed the compounds that I synthesized personally and the data that was produced from the biological assays that these targets were tested in. None of these compounds reached the desired levels of potency (4 µg/mL) against the Gram-negative bacterial strains we tested against. However, our group has had success with other analogs that have not been included in this paper. We hope to include this work in future publications.
<table>
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<th>E Coli</th>
<th>Mg1665 Perm.</th>
<th>Efflux Ratio</th>
<th>Mg1665 ΔacrAB Ratio</th>
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<td></td>
<td>&gt;256</td>
<td>64</td>
<td>16</td>
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</table>

Table 3.1: Structures and minimum inhibitory concentration (MIC, µg/mL) data shown for submitted compounds. Strains include a Gram-negative wild type (E. coli), a membrane permeabilized mutant (PMB-n), an efflux knockout strain (ΔacrAB), and a strain of Gram-positive bacteria as a baseline (S. aureus). Compounds are prepared in a 12% DMSO/Water solution normally. Solubility issues lead some to be prepared in 100% DMSO (marked with an *) and these can not be tested at as high a concentration.
Experimental

Step 1: Benzyl chloroformate protection of 4-bromo-3-fluoroaniline (1)\textsuperscript{28}

\[
\begin{array}{c}
\text{F} \quad \text{NH}_2 \\
\text{Br} \\
\text{Br}
\end{array}
\xrightarrow{\text{CBzCl, NaHCO}_3, \text{THF, 2 hrs}}
\begin{array}{c}
\text{F} \quad \text{H} \\
\text{Br} \quad \text{O} \\
\text{N} \\
\text{O}
\end{array}
\]

Molecular Weight: 324.15

A solution of 3-fluoroaniline (18.7 g, 168.3 mmol) in tetrahydrofuran (THF, 150 mL) was treated with sodium bicarbonate (NaHCO\textsubscript{3}, 46.45 g, 336.6 mmol, 2.0 equiv) and cooled to ~0 °C in an ice bath before a solution of benzyl chloroformate (CBzCl, 31.58 g, 185.1 mmol, 26.1 mL, 1.1 equiv) in THF (50 mL) was dropwise added into the reaction mixture under N\textsubscript{2}. The resulting reaction mixture was allowed to warm to room temperature and then stirred for 2 hours. When the reaction was judged to be complete by LC-MS analysis, the reaction mixture was partitioned between water (100 mL) and ethyl acetate (EtOAc, 100 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (2×100 mL). The combined organic extracts were washed with water (2×100 mL) and saturated aqueous sodium chloride (1x100 mL), dried over magnesium sulfate (MgSO\textsubscript{4}), and concentrated \textit{in vacuo}. The residue was further dried \textit{in vacuo} to afford the desired 1 (39.2 g, 95% yield) as pale-yellow oil. Observed mass M+H = 326.0 amu (Appendix B1). This product was directly used in subsequent reactions without further purification.

1a. Protection of 4-bromoaniline

\[
\begin{array}{c}
\text{Br} \quad \text{NH}_2 \\
\text{Br}
\end{array}
\xrightarrow{\text{CBzCl, NaHCO}_3, \text{THF, 2 hrs}}
\begin{array}{c}
\text{Br} \\
\text{H} \\
\text{O} \\
\text{N} \\
\text{O}
\end{array}
\]

Molecular Weight: 306.16
Compound 1a was synthesized using the step 1 conditions. Beginning with 2 grams of 4-bromoaniline, we were able to produce the CBzCl protected aniline in 90% yield (3.198 g). Observed mass M-H = 305.8 amu (Appendix B2).

1b. Protection of 6-bromopyridin-3-amine

![Chemical structure of 6-bromopyridin-3-amine and its CBzCl protected form with molecular weight 307.15 amu.]

Compound 1b was synthesized using the step 1 conditions. Beginning with 2 grams of 6-bromopyridin-3-amine, we were able to produce the CBzCl protected aniline in 100% yield (3.55 g). Observed mass M+H = 308.0 amu (Appendix B3).

1c. Protection of 4-bromo-3-methoxyaniline

![Chemical structure of 4-bromo-3-methoxyaniline and its CBzCl protected form with molecular weight 336.19 amu.]

Compound 1c was synthesized using the step 1 conditions. Beginning with 2 grams of 4-bromo-3-methoxyaniline, we were able to produce the CBzCl protected aniline in 100% yield (3.33 g). Observed mass M+H = 335.9 amu (Appendix B4).
A solution of 1 (39.2 g, 160.0 mmol) in anhydrous THF (300 mL) was cooled to -78 °C in a dry-ice/acetone bath before a solution of LHMDS (1 M solution in THF, 176 mmol, 1.1 equiv) was added dropwise under N₂. The resulting reaction mixture was subsequently stirred at -78 °C for 1 hour before a solution of (R)-(-)-glycidyl butyrate (25.37 g, 24.6 mL, 176 mmol, 1.1 equiv) in anhydrous THF (100 mL) was added dropwise into the reaction mixture at -78 °C under N₂. The resulting reaction mixture was stirred at -78 °C for 30 min before being gradually warmed to room temperature for 12 h under N₂. When LC-MS showed the reaction was complete, the reaction mixture was quenched with H₂O (200 mL), and the resulting mixture was stirred at room temperature for 1 hour before ethyl acetate (200 mL) was added. The two layers were separated, and the aqueous layer was extracted with ethyl acetate (2×100 mL). The combined organic extracts were washed with water (2×100 mL) and saturated aqueous sodium chloride (1×100 mL), dried over MgSO₄, and concentrated in vacuo. White crystals precipitated from the concentrated solution when most of the solvent was evaporated. The residue was then treated with 20% EtOAc/hexane (100 mL) and the resulting slurry was stirred at room temperature for 30 min. The solids were collected by filtration and washed with 20% ethyl acetate/hexane (2×50 mL) to afford the desired compound 2 (24.4 g, 72.3% yield) as white crystals. Observed mass M+H = 290.0 amu (Appendix B5). This product was directly used in subsequent reactions without further purification. $^1$H NMR (399 MHz, DMSO-$d_6$) $\delta$ ppm 3.52 -
3.60 (m, 1 H) 3.63 - 3.73 (m, 1 H) 3.77 - 3.88 (m, 1 H) 4.08 (t, *J*=9.16 Hz, 1 H) 4.67 - 4.77 (m, 1 H) 5.24 (t, *J*=5.50 Hz, 1 H) 7.36 (d, *J*=8.79 Hz, 1 H) 7.62 - 7.75 (m, 2 H)

2a. Des-Fluoro B-Ring

![Diagram of compound 2a](image)

Compound 2a was synthesized using the step 2 conditions. Beginning with 3.198 grams of compound 1a, we were able to form the oxazolidinone 2a in 76% yield (2.15 g). Observed mass M+H = 271.8 amu (Appendix B7). $^1$H NMR (400 MHz, DMSO-d$_6$) δ ppm 3.50 - 3.60 (m, 1 H) 3.61 - 3.69 (m, 1 H) 3.79 (m, 1 H) 4.05 (t, *J*=9.16 Hz, 1 H) 4.63 - 4.72 (m, 1 H) 5.19 (t, *J*=5.50 Hz, 1 H) 7.47 - 7.58 (m, 4 H)

2b. Pyridyl B-Ring

![Diagram of compound 2b](image)

Compound 2b was synthesized using the step 2 conditions. Beginning with 3.55 grams of compound 1b, we were able to form the oxazolidinone 2b in 50% yield (1.572 g). Observed mass M+H = 273.0 amu (Appendix B9). $^1$H NMR (400 MHz, DMSO-d$_6$) δ ppm 3.50 - 3.62 (m, 1 H) 3.66 - 3.77 (m, 1 H) 3.81 - 3.93 (m, 1 H) 4.11 (t, *J*=8.79 Hz, 1 H) 4.76 (d, *J*=3.66 Hz, 1 H) 5.24 (t, *J*=5.50 Hz, 1 H) 7.67 (d, *J*=8.79 Hz, 1 H) 7.94 - 8.16 (m, 1 H) 8.45 - 8.65 (m, 1 H)
2c. N-oxide B-ring

\[
\text{Br} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \rightarrow \quad \text{Br} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{O} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{OH}
\]

Molecular Weight: 289.09

Compound 2c was synthesized from 2b using a procedure found in the literature for the \textit{in situ} preparation of the oxidizing agent trifluoroperacetic acid.\textsuperscript{29} Urea hydrogen peroxide (0.344 g, 3.66 mmol) and potassium carbonate (1.012 g, 7.32 mmol) were stirred in dry dioxane (36 mL) for 1 hour. After an hour, the mixture was cooled in an ice bath and to this was added trifluoroacetic anhydride (1.16 mL, 3.66 mmol) dropwise. This solution was warmed to room temperature and compound 2b (0.100 g, 0.366 mmol) was added in dioxane (1 mL). This reaction was heated to 50 °C overnight and LC-MS confirmed complete conversion to product. The dioxane was removed by rotary evaporation and the crude product (65 mgs, 65% crude yield) was taken forward to the next step. Observed mass M+H = 288.8 amu (Appendix B11).

2d. Methoxy B-Ring

\[
\text{Br} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \rightarrow \quad \text{Br} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{OH}
\]

Molecular Weight: 302.12

Compound 2d was synthesized using the step 2 conditions. Beginning with 3.33 grams of compound 1c, we were able to form the oxazolidinone 2d in 76% yield (2.27 g). Observed mass M+H = 302.0 amu (Appendix B12). \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \textit{δ} ppm 3.56 (m, 1 H) 3.64 (m, 1 H) 3.83 (m, 4 H) 4.08 (t, \textit{J}=9.16 Hz, 1 H) 4.68 (m, 1 H) 5.07 (s, 1 H) 7.34 (m, 3 H)
2e. Hydroxy B-Ring

Compound 2e was synthesized from compound 2d using a general procedure for deprotection of methyl ethers. Compound 2d (300 mgs, 0.993 mmol) was dissolved in DCM (~5 mL) and cooled to -78 °C in a dry ice/acetone bath. The reaction was kept under N₂ and boron tribromide (0.160 mL, 1.688 mmol) in DCM was then added slowly. The reaction went overnight forming a brown precipitate that would prove to be the desired product. The solvent was removed by filtration and the product was collected to give 257 mgs (90% yield). Observed mass M+H = 287.8 amu (Appendix B14). This was taken on to the next step without further purification. \(^1\)H NMR (400 MHz, DMSO-d₆) δ ppm 3.55 (dd, J=12.46, 2.93 Hz, 1 H) 3.67 (dd, J=12.46, 2.20 Hz, 1 H) 3.76 (t, J=7.33 Hz, 1 H) 4.03 (t, J=8.79 Hz, 1 H) 4.60 - 4.80 (m, 1 H) 6.82 (d, J=8.79 Hz, 1 H) 7.30 - 7.49 (m, 2 H) 10.39 (br s, 1 H)

Step 3: Making the boronic ester/acid (3 and 3')

To a solution of 2 (3.37 g, 10 mmol) in 20 mL of dry DMSO, bis(pinacolato)diboron (20 mmol, 2 equiv), potassium acetate (50 mmol, 5 equiv), and Pd(dppf)Cl₂ (0.5 mmol, 0.05 equiv)
were added. The mixture was heated at 80 °C under nitrogen overnight. When HPLC/MS showed the reaction was complete, it was cooled to room temperature. Then 20 mL of methanol were added and the mixture was filtered through celite. To this filtrate was added 20 mL of water and this was allowed to stand overnight at 4 °C. The precipitate formed was isolated by filtration with a Buchner funnel and washed with water. The filtrate was further concentrated to get a second crop of precipitate. The solids were combined and recrystallized in methanol/water. The formed precipitate was filtered and washed with water and dried under vacuum to give a mixture of products 3 and 3' as a brown powder. Observed masses M+H = 255.9 (3.166 min) amu and 338.0 (4.644 min) amu (Appendix B16). This was used in subsequent reactions without further purification. \(^1\)H NMR (400 MHz, DMSO-\textit{d}_6) \(\delta\) ppm 1.28 (br s, 12 H) 3.57 (d, \(J=9.53\) Hz, 1 H) 3.66 (d, \(J=10.99\) Hz, 1 H) 3.84 (m, 1 H) 4.09 (t, \(J=9.16\) Hz, 1 H) 4.73 (br s, 1 H) 7.36 (d, \(J=6.60\) Hz, 1 H) 7.47 (d, \(J=6.60\) Hz, 1 H) 7.63 (t, \(J=7.69\) Hz, 1 H)

3a: Des-fluoro boronic ester/acid

\[
\begin{align*}
\text{Br} & \quad \text{O} \quad \text{N} \quad \text{O} \quad \text{OH} \\
\text{O} \quad \text{B} & \quad \text{OH} \\
\text{Molecular Weight: 319.16}
\end{align*}
\]

Compound 3a was synthesized using the conditions outlined in step 3. Crystallization in water/methanol proved unsuccessful, however, and so the product was extracted in ethyl acetate. Ethyl acetate was removed by rotary evaporation and then the product was place under vacuum to complete drying. Beginning with 905 mgs of compound 2a, we yielded 514 mgs (48%) of flakey, brown material which was used in subsequent reactions without further purification.
Observed mass M+H = 320.0 amu (Appendix B18). $^1$H NMR (400 MHz, DMSO- $d_6$) $\delta$ ppm 1.27 (s, 12 H) 3.49 - 3.60 (m, 1 H) 3.61 - 3.71 (m, 1 H) 3.82 (dd, $J$=8.43, 623 Hz, 1 H) 4.02 - 4.12 (t, $J$=9.16 Hz, 1 H) 4.63 - 4.73 (m, 1 H) 5.13 - 5.26 (m, 1 H) 7.50 - 7.62 (m, 2 H) 7.62 - 7.71 (m, 2 H)

**Step 4: General Suzuki Coupling reaction conditions (4)**

The desired bromide (100mgs, 1.0 equiv) was dissolved in 2-3 mL of 9:1 dioxane: water. To this solution was added the corresponding boronic acid/ester (1.2 equiv), Pd(dppf)Cl$_2$ (0.1 equiv), and potassium carbonate (4 equiv). This mixture was then heated to 85-90°C and let stir. The reaction was monitored by LC-MS and, generally, was complete within 1-2 hours. Upon completion, the reaction was quenched with water (5 mL) and the product extracted with ethyl acetate (1x10 mL). The aqueous layer was back-extracted with ethyl acetate (1x5 mL) and the organic layers were combined. These were washed with saturated sodium chloride (1x10 mL), dried over magnesium sulfate, and concentrated to dryness by rotary evaporation. The crude product was dissolved in 1 mL of DMF and purification was performed using reverse phase HPLC (water and acetonitrile with 0.1% TFA as solvents for mobile phase). Those fractions containing pure product were pooled and lyophilized to give the pure product as a white solid.
DP-64 was synthesized by Suzuki coupling 4-fluoro-3-hydroxy boronic acid with compound 2a using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~30% B. Analytical HPLC using method 2 to 10 in 30 (Table 3.4) showed the compound was 96% pure at 254 nm with a retention time of 10.879 minutes. Observed mass M+H = 304.1 amu. HPLC and LC-MS chromatograms are available in the Appendix (B20 and B21). ¹H NMR: not available due to lack of material after submission for testing.

DP-65 was synthesized by Suzuki coupling 4-fluoro-3-hydroxy boronic acid with compound 2b using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~17% B. Analytical HPLC using method 2 to 50 in 30 (Table 3.3) showed the compound was 95% pure at 254 nm with a retention time of 12.126 minutes. Observed mass M+H = 305.1 amu. HPLC and LC-MS chromatograms are available in the Appendix (B22 and
DP-281

was synthesized by Suzuki coupling 5-bromo-2-nitrophenol with compound 3 using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~38% B. Analytical HPLC using method 2to50in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 12.692 minutes. Observed mass M+H = 348.8 amu. HPLC and LC-MS chromatograms are available in the Appendix (B25 and B6). ¹H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.59 (m, 1H) 3.70 (m, 1H) 3.88 (m, 1H) 4.14 (t, \(J=8.79\) Hz, 1H) 4.76 (m, 1H) 5.25 (br s, 9H) 7.17 (m, 1H) 7.31 (br s, 1H) 7.50 (d, \(J=8.06, 11\) Hz, 1H) 7.58 - 7.72 (m, 2H) 8.00 (d, \(J=8.06, 11\) Hz, 1H)

DP-293
DP-293 was synthesized by Suzuki coupling 4-fluoro-3-hydroxy boronic acid with compound 2c using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~18%B. Analytical HPLC using method 2to100in30 (Table 3.4) showed the compound was 90% pure at 254 nm with a retention time of 6.646 minutes. The impurity present was known to be DP-65. Prior testing showed that compound to be inactive so we moved forward with submission at this point. Observed mass M+H = 320.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B28 and B29). \(^{1}H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) ppm 3.59 (m, 1 H) 3.68 (m, 1 H) 3.88 (m, 1 H) 4.12 (m, 1 H) 4.78 (br s, 1 H) 7.19 (br s, 1 H) 7.24 (s, 1 H) 7.55 - 7.64 (m, 3 H) 8.66 (s, 1 H) 10.09 (br s, 1 H)

**DP-296**

![Diagram of DP-296 synthesis](image)

Molecular Weight: 287.29

DP-296 was synthesized by Suzuki coupling 2-fluoro boronic acid with compound 2a using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~40%B. Analytical HPLC using method 2to50in30 (Table 3.4) showed the compound was 95% pure at 254 nm with a retention time of 13.590 minutes. Observed mass M+H = 287.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B31 and B32). \(^{1}H\) NMR: not available due to lack of material after submission for testing.
DP-304 was synthesized by Suzuki coupling 5-bromoresorcinol with compound 2 using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~17% B. Analytical HPLC using method 2to50in30 (Table 3.4) showed the compound was 95% pure at 254 nm with a retention time of 12.545 minutes. Observed mass M+H = 319.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B33 and B34). $^1$H NMR: not available due to lack of material after submission for testing.

DP-305 was synthesized by first Suzuki coupling 4-bromopyridine with compound 3 using the reaction conditions outlined in step 4. This was followed by oxidation by trifluoroperoacetic acid that was generated in situ. Urea hydrogen peroxide (0.483 g, 5.14 mmol) and potassium carbonate (1.421g, 10.28 mmol) were stirred in dry dioxane (40mL) for 1
hour. After an hour, the mixture was cooled in an ice bath and to this was added trifluoroacetic anhydride (1.4 mL, 5.14 mmol) dropwise. This solution was warmed to room temperature and then our previously coupled intermediate was added in dioxane (1 mL). This reaction was heated to 50°C overnight and LC-MS confirmed complete conversion to product. The dioxane was removed by rotary evaporation and the crude product was then dissolved in water and purified. Purification resulted in the desired compound eluting at ~11%B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 8.187 minutes. Observed mass M+H = 304.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B35 and B36). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.52 - 3.63 (dd, $J$=12.09, 4.03 Hz, 1 H) 3.69 (dd, $J$=12.46, 4.03 Hz, 1 H) 3.91 (m, 1 H) 4.14 (t, $J$=9.16 Hz, 1 H) 4.75 (m, 2 H) 7.51 (dd, $J$=8.06, 2.20 Hz, 1 H) 7.68 (m, 2 H) 7.72 (m, 2 H) 8.33 (d, $J$=6.60 Hz, 2 H)

*DP-314*

![Chemical Structure]

DP-314 was synthesized by the same procedure as used for DP-305. First a Suzuki coupling of 3-bromopyridine with compound 3 using the reaction conditions outlined in step 4 was performed. This was followed by oxidation to form the N-oxide. Purification resulted in the desired compound eluting at ~12%B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 8.313 minutes.
Observed mass M+H = 304.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B38 and B39). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.59 (d, $J=3.66$ Hz, 1 H) 3.68 (d, $J=3.66$ Hz, 2 H) 3.89 (m, 1 H) 4.14 (t, $J=9.16$ Hz, 1 H) 4.74 (m, 1 H) 7.54 (m, 3 H) 7.69 (m, 2 H) 8.26 (d, 5.13 Hz, 1 H) 8.44 (s, 1 H)

$DP\text{-}315$

![Molecular structure of DP-315](image)

Molecular Weight: 342.37

DP-315 was synthesized by Suzuki coupling 4-bromo styrene oxide with compound 3 using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~23% B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 11.601 minutes. Observed mass M+H = 343.2 amu. HPLC and LC-MS chromatograms are available in the Appendix (B41 and B42). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.27 (m, 2 H) 1.36 (m, 2 H) 3.59 (m, 1 H) 3.69 (m, 1 H) 3.89 (m, 1 H) 4.13 (t, $J=9.16$ Hz, 1 H) 4.76 (m, 1 H) 5.26 (br s, 1 H) 7.43 - 7.54 (m, 3 H) 7.54 - 7.69 (m, 4 H) 8.69 (br s, 2 H)

$DP\text{-}318$

![Molecular structure of DP-318](image)

Molecular Weight: 303.29
DP-318 was synthesized by Suzuki coupling 4-fluoro-3-bromo phenol with compound 3a using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~33%B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 11.601 minutes. Observed mass M+H = 303.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B44 and B45). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.59 (dd, $J=$12.46, 3.66 Hz, 1 H) 3.68 (dd, $J=$12.46, 2.93 Hz, 1 H) 3.87 (m, 1 H) 4.13 (t, $J=$8.79 Hz, 1 H) 4.72 (m, 1 H) 5.22 (br s, 1 H) 6.75 (m, 1 H) 6.84 (m, 1 H) 7.09 (m, 1 H) 7.54 (d, $J=$7.33 Hz, 2 H) 7.66 (d, $J=$8.79 Hz, 2 H) 9.50 (s, 1 H)

DP-327

DP-327 was synthesized by Suzuki coupling 4-(tertbutoxycarbonylaminomethyl)phenyl boronic acid with compound 2d using the reaction conditions outlined in step 4. In this case the crude needed to be taken one step further in order to deprotect the methyl amine. This was done simply by stirring in 1:1 DCM:TFA for 30 minutes. The DCM was removed by rotary evaporation and the compound was purified. Purification resulted in the desired compound eluting at ~19%B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 9.855 minutes. Observed mass M-NH$_2$ = 311.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (B47 and B48). $^1$H NMR
(400 MHz, DMSO-$d_6$) δ ppm 3.58 (m, 1 H) 3.70 (m, 1 H) 3.76 (s, 3 H) 3.88 (m, 1 H) 4.05 (br s, 2 H) 4.12 (m, 1 H) 4.73 (m, 1 H) 5.26 (br s, 1 H) 7.12 (m, 2 H) 7.29 (m, 2 H) 7.35 - 7.57 (m, 3 H) 8.26 (br s, 2 H)

**DP-328**

![Chemical Structure](image)

DP-328 was synthesized similarly to DP-327. First a Suzuki coupling was performed with 4-(tertbutoxycarbonylaminomethyl)phenyl boronic acid and compound 2e using the reaction conditions outlined in step 4. This was followed by a boc-deprotection to give us our desired product. Purification resulted in the desired compound eluting at ~19% B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 8.685 minutes. Observed mass $M$-$\text{NH}_2 = 298.1$ amu and $M+H = 315.1$ amu. HPLC and LC-MS chromatograms are available in the Appendix (B50 and B51). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.52 (m, 2 H) 3.66 (m, 1H) 3.79 (m, 1 H) 4.02 (m, 2 H) 4.69 (m, 1 H) 5.28 (br s, 1 H) 6.99 (d, $J=8.06$ Hz, 1 H) 7.27 (d, $J=8.79$ Hz, 1 H) 7.37(m, 1 H) 7.48 (m, 2 H) 7.58 (m, 2 H) 8.45 (br s, 2 H) 9.93 (s, 1 H)
DP-329 was synthesized by Suzuki coupling 5-bromo-2,4-difluoro phenol with compound 3a using the reaction conditions outlined in step 4. Purification resulted in the desired compound eluting at ~35%B. Analytical HPLC using method 2to30in30 (Table 3.2) showed the compound was 95% pure at 254 nm with a retention time of 17.575 minutes. Observed mass M+H = 321.9 amu. HPLC and LC-MS chromatograms are available in the Appendix (53 and B54). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.51 - 3.62 (m, 1 H) 3.66 - 3.74 (m, 1 H) 3.83 - 3.92 (m, 1 H) 4.13 (t, $J=9.16$ Hz, 1 H) 4.66 - 4.77 (m, 1 H) 5.24 (t, $J=5.50$ Hz, 1 H) 7.03 (t, $J=8.79$ Hz, 1H) 7.24 - 7.34 (m, 1 H) 7.51 (d, $J=8.79$ Hz, 2 H) 7.67 (d, $J=8.06$ Hz, 2 H) 9.97 (br s, 1 H)

Characterization

The raw data generated for the characterization of the oxazolidinone analogs detailed in this paper are included in the Appendix. HPLC chromatograms from our HP 1100 analytical HPLC system are included and used to determine the purity (goal of 95%) of the final compounds. These are thirty minute methods that use one of the following gradients dependent on retention time of the compound:
Table 3.2: Gradient of HPLC runs using 2to30in30 method. Mobile phase A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>25.01</td>
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</tr>
<tr>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td>27.01</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.3: Gradient of HPLC runs using 2to50in30 method. Mobile phase A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
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<tr>
<td>25.01</td>
<td>100</td>
</tr>
<tr>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td>27.01</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.4: Gradient of HPLC runs using 2to100in30 method. Mobile phase A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>27.01</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.5: Gradient of LC-MS runs of 2to100in8min-PosM and 2to100in8min-NegM methods. Mobile phase A: 0.1% FA in water and B: 0.1%FA in acetonitrile.

LC-MS data is also included from our HP 1100 HPLC-MS system that we use to monitor our reactions by providing us with the desired molecular weight of our target compounds. These are eight minute methods run in the positive mode that use the following gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Mobile Phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>6.1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>
LC-MS and HPLC data were used as our main sources of characterization. When material was available, proton NMR, using a 400 MHz Varian NMR spectrometer, were run to confirm the structures. For all structures, d6-DMSO with 0.03% TMS was used.
References


Appendix A

Additional Characterization Data of SeAHQ Intermediates and Comparators

A2: Proton NMR of SAH in DMSO-d6.
A3: Proton NMR of SAHO in DMSO-d6

A4: COSY of SAHO
A5: LC-MS data for SAH

A6: LC-MS of SAHO

Exact Mass: 384.12
Molecular Weight: 384.41
Exact Mass: 400.12
Molecular Weight: 400.41
Appendix B

LC-MS Data for Intermediates

B1: Compound 1 HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B2: Compound 1a HPLC-MS chromatogram and mass fragment using method 2 to 100 in 8 min - Neg M
B3: Compound 1b HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B4: Compound 1c HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B5: Compound 2 HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B6: Compound 2 $^1H$ NMR

![NMR Spectrogram](B6-C2-HNMR.png)

Chemical Shift (ppm):
- 7.73
- 7.71(9,12)
- 7.69
- 7.69
- 7.66
- 7.37(13)
- 7.35
- 5.25
- 5.24(7)
- 5.22
- 4.73
- 4.72(2)
- 4.71
- 4.11
- 4.08(3)
- 3.85
- 3.83(3)
- 3.66(6)
- 3.57(6)
- 3.56
- 3.54

Normalized Intensity:
- 1.00
- 0.97
- 0.96
- 0.87
- 0.83
- 1.06
- 1.57
B7: Compound 2a HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B8: Compound 2a $^1$H NMR
B9: Compound 2b HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B10: Compound 2b $^1$H NMR
B11: Compound 2c HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B12: Compound 2d HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
The NMR was taken of the crude product. Additional peaks present are due to the presence of a reaction intermediate (structure below) that did not go to completion. The peak can be seen in the LC-MS above at retention time 4.07 minutes.

Molecular Weight: 372.22
B14: Compound 2e HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B15: Compound 2e $^1$H NMR

Chemical Shift (ppm)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Normalized Intensity

DC-43-hydroxy.esp

Chemical Shift (ppm)

0.76
1.09
1.14
1.46
1.56
1.97
2.85
3.00

TMS
DMSO
B16: Compounds 3/3’ HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B17: Compound 3/3' $^1$H NMR

[Diagram of NMR spectrum with chemical shifts and assignments]
B18: Compounds 3a HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
B19: Compound 3a $^1$H NMR
Analytical Data for compound DP-64

B20: HPLC chromatogram using method 2to100in30

B21: HPLC-MS chromatogram and mass fragment using method 2to100in8min-PosM
Analytical Data for compound DP-65

**B22: HPLC chromatogram using method 2to50in30**

**B23: HPLC-MS chromatogram and mass fragment using method 2to100in8**
B24: $^1H$ NMR

Chemical Shift (ppm)

Normalized Intensity

DMSO

TMS
Analytical Data for compound DP-281

**B25: HPLC chromatogram using method 2to50in30**

![HPLC chromatogram using method 2to50in30](image1)

**B26: HPLC-MS chromatogram and mass fragment using method 2to100in8**

![HPLC-MS chromatogram and mass fragment using method 2to100in8](image2)
B27: $^1$H NMR
Analytical Data for compound DP-293

B28: HPLC chromatogram using method 2to100in30

B29: HPLC-MS chromatogram and mass fragment using method 2to100in8
Analytical Data for compound DP-296

B31: HPLC chromatogram using method 2to50in30

B32: HPLC-MS chromatogram and mass fragment using method 2to100in8
Analytical Data for compound DP-304

B33: HPLC chromatogram using method 2to50in30

B34: HPLC-MS chromatogram and mass fragment using method 2to100in8
Analytical Data for compound DP-305

B35: HPLC chromatogram using method 2to30in30

B36: HPLC-MS chromatogram and mass fragment using method 2to100in8
B37: $^1$H NMR

Chemical Shift (ppm):
- 8.33 (18,20)
- 7.72 (17,21)
- 7.68 (9,12)
- 7.51 (13)
- 4.75 (2,7)
- 4.14 (3)
- 3.91 (3)
- 3.69 (6)
- 3.59
- 1.43

Normalized Intensity:
- 1.07
- 0.99
- 0.96
- 1.00
- 1.57
- 0.83
- 2.33
- 0.63
- 1.43

TMS
DMSO
Analytical Data for compound DP-314

B38: HPLC chromatogram using method 2to30in30

B39: HPLC-MS chromatogram and mass fragment using method 2to100in8
Analytical Data for compound DP-315

B41: HPLC chromatogram using method 2to30in30

B42: HPLC-MS chromatogram and mass fragment using method 2to100in8
B43: $^1$H NMR

- Chemical Shift (ppm)
- Normalized Intensity
- DC-37.esp
- TMS
- Water
- DMSO

Normalized Intensity

Chemical Shift (ppm)

- 2.31, 8.68 (25)
- 3.42, 2.76
- 5.26 (7)
- 4.76 (2)
- 4.13 (3)
- 3.89 (3)
- 3.69 (6)
- 3.59 (6)
- 1.36 (23, 24)
- 1.27 (23, 24)
- 0.71

Normalized Intensity

0.00
Analytical Data for compound DP-318

B44: HPLC chromatogram using method 2to30in30

B45: HPLC-MS chromatogram and mass fragment using method 2to100in8
B46: $^1H$ NMR
Analytical Data for compound DP-327

B47: HPLC chromatogram using method 2to30in30

B48: HPLC-MS chromatogram and mass fragment using method 2to100in8
B49: $^1H$ NMR

Chemical Shift (ppm)

<table>
<thead>
<tr>
<th>Chemical Shift (ppm)</th>
<th>Normalized Intensity</th>
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<tbody>
<tr>
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<td>2.26</td>
</tr>
<tr>
<td>7.93</td>
<td>0.82</td>
</tr>
<tr>
<td>7.50</td>
<td>0.82</td>
</tr>
<tr>
<td>7.49(13,9)</td>
<td>0.68</td>
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<tr>
<td>7.48</td>
<td>0.38</td>
</tr>
<tr>
<td>7.47(12)</td>
<td>0.50</td>
</tr>
<tr>
<td>7.42</td>
<td>0.63</td>
</tr>
<tr>
<td>7.30(19,21)</td>
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<tr>
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<td>2.07</td>
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<tr>
<td>4.12(3)</td>
<td>2.06(7)</td>
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<tr>
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<td>1.17</td>
</tr>
<tr>
<td>3.89(3)</td>
<td>1.12</td>
</tr>
<tr>
<td>3.76</td>
<td>0.38</td>
</tr>
<tr>
<td>3.75(16)</td>
<td>0.50</td>
</tr>
<tr>
<td>3.67(6)</td>
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<td>0.38</td>
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<tr>
<td>3.55</td>
<td>0.50</td>
</tr>
<tr>
<td>3.58(6)</td>
<td>0.63</td>
</tr>
<tr>
<td>2.87</td>
<td>0.38</td>
</tr>
<tr>
<td>2.71</td>
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<td>0.38</td>
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<td>2.07</td>
<td>0.50</td>
</tr>
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</table>

DP-327.esp
Analytical Data for compound DP-328

**B50: HPLC chromatogram using method 2to30in30**

**B51: HPLC-MS chromatogram and mass fragment using method 2to100in8**
$B52$: $^1H$ NMR

Chemical Shift (ppm)

0

0.05

0.10

Normalized Intensity

Water

DMSO

TMS

10 9 8 7 6 5 4 3 2 1 0

1.02 2.36 1.59 1.72 1.09 0.75 1.00 0.82 0.73 1.88 0.35 0.04 1.51

99
Analytical Data for compound DP-329

B53: HPLC chromatogram using method 2to30in30

B54: HPLC-MS chromatogram and mass fragment using method 2to100in8
B55: $^1H$ NMR

Chemical Shift (ppm)

0
0.05
0.10
0.15
0.20
Normalized Intensity

0.86
0.81
0.88
0.92
0.99
0.92
1.00
0.97
1.93
2.19
1.01

9.97 (23)
7.68
7.66 (9, 13)
7.52 (12, 10)
7.49
7.29 (20)
7.06
7.03 (17)
7.01
5.25
5.24
5.23 (7)
4.74
4.73
4.72 (2)
4.15
4.13 (3)
4.11
3.88 (3)
3.67
3.60
3.55
3.32
3.30

10 9 8 7 6 5 4 3 2 1 0

DP-329.esp