Total Synthesis of Demethyllavendamycin Amides

An Honors 499 Thesis

By

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I. Abstract

This thesis involves the total synthesis of three analogs of lavendamycin, a chemotherapeutic agent: 7-N-chloroacetyldemethyllavendamycin Amide, 7-N-chloroacetyldemethyllavendamycin Amide of Pyrrolidine, and 7-N-butyryldemethyllavendamycin Amide of Piperazine. This research is part of an ongoing project involving synthesis and structure-activity relationship studies of various analogs of lavendamycin. These analogs contain the amide functional group in the C-2' position. The C-2' amides have proved to be very active anti-cancer agents.
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III. Background Information

Lavendamycin (1) was discovered in the fermentation broth of the microorganism *Steptomyces lavendulae* by researchers at Bristol Laboratories in 1981.\(^1\) Lavendamycin closely resembled a previously discovered antitumor agent, streptonigrin (2), in both structure and biological activity. Studies have shown that both lavendamycin and streptonigrin exhibit potent antitumor and anticancer activities, but are unusable thus far due to their high cytotoxicity and low solubility.\(^2,3,4\)

![Structural formulas of lavendamycin (1) and streptonigrin (2)](image)

The exact mechanism for antitumor activity and cytotoxicity exhibited by lavendamycin and streptonigrin is unknown. The quinone structure alone has shown activities such as DNA cleavage, which is related to the quinone reduction potential.\(^5\) Other proposed mechanisms deal with the effects of the compound on the electron transport system within the mitochondria.\(^6-10\) No single mechanism has been able to explain the cytotoxic activity of these compounds thus far.

Lavendamycin analogs have exhibited encouraging antitumor activities against \(^{\text{ras}}^\text{K}\) oncogenic tumors.\(^{11,12}\) Although lavendamycin has proven to be less potent than the
compound streptonigrin, lavendamycins are more potent than their quinolinedione analogs (A-B ring portion of lavendamycin). The additional three ring system (C-E) in lavendamycin that make up the pentacyclic structure moderates the cytotoxic effects of the quinoline structure and increase the overall antitumor activity of the system compared to the corresponding quinolinedione.

For necessary structure-activity relationship studies to be performed, an efficient method of synthesis for lavendamycin had to be developed. After the discovery of lavendamycin in 1981, several research groups began studying the synthesis of the compound. Kende and Ebetino at the University of Rochester published the first total synthesis of lavendamycin in 1984. This synthesis involved the use of a Friedlander condensation to produce the A-B portion of the compound, and a Bischler-Napieralski cyclodehydration to complete the pentacyclic structure. In 1985 Hibino and group reported a total synthesis of lavendamycin methyl ester through the use of a Pictet-Spengler condensation between β-methyl-tryptophan and a quinoline analog to produce a pentacyclic intermediate. This intermediate was modified to the final lavendamycin product through a series of transformations. Also in 1985, Boger’s research group reported a total synthesis pathway containing twenty steps and an overall yield of less than 1%.

In 1993, Behforouz and group at Ball State University reported a total synthesis of lavendamycin methyl ester with five steps and an overall yield of 33%.
This synthesis involved using a novel azadiene Diels-Alder reaction followed by a Pictet-Spengler condensation between a formylquinolinedione and a β-methyl tryptophan. Behforouz's highly concise procedure is much more practical than the previously reported synthesis routes due to the reduced number of intermediates, intermediate stabilities, and the increased overall yield.\textsuperscript{16}
In 1996, Behforouz’s group reported a new method of synthesis of lavendamycin methyl ester with an overall yield of 40%. This new method of efficient synthesis has allowed for the production of many lavendamycin analogs such as the ones involved in this thesis. With the ability for efficient synthesis, the analogs of the antitumor agent lavendamycin are readily produced for structure-activity relationship tests to be performed. These studies focus on finding a direct relationship between the biological activities of the antitumor agents and their structure.

IV. Biological Activity

The efficient synthesis of Lavendamycin developed by Behforouz’s group, has allowed for profound structure-activity relationship studies on a variety of analogs. The analogs of Lavendamycin consist of five main positions for the addition or deletion of functional groups as shown below.
Structure activity relationship studies have shown that an acetyl group at the C-7 position exhibits the most selective toxicity against tumor cells compared to parent cells. Several other groups such as the butyryl also exhibit biological activity and selective toxicity against ras^k oncogene transformed cells. The SAR studies have also shown that an amide or ester functional group is needed at the C-2' position for increased biological activity.

The purpose of this thesis project was to synthesize three analogs with different substituents at the C-7 and C-2' positions to analyze the activity against oncogene transformed cells, the solubility of the compound, and the selectivity of the compound. All three of the analogs synthesized for this thesis involve an amide functional group in the C-2' position. Amides in this position have shown good activity when tested against different tumor lines, therefore we have synthesized three different amides in this project. Pyrrolidine was chosen because of the high activity and selectivity it has previously exhibited, and piperazine was chosen for increased water solubility. The extra N-H on the piperazine is a position available for making a salt to increase solubility. In the C-7 position, two compounds synthesized exhibit a chloroacetyl group, and one compound exhibits a butyryl group. The chloroacetyl group was added to compare the biological activity of the analog when adding a chloro group verses an acetyl group. The butyryl group in the C-7 position was chosen because of the increased anti-tumor activity it has exhibited. A comparison of these compounds with other amide analogs previously synthesized should provide additional information on the role of the substituents in relation to activity and solubility.
V. Total Synthesis of Lavendamycin Analogs

Results and Discussion

The Pictet-Spengler condensation of a quinolinedione aldehyde and a tryptophan results in the formation of the pentacyclic structure of lavendamycin. The quinolinedione aldehyde and tryptophan are fully functionalized prior to the condensation, as well as purified and dried. The Pictet-Spengler condensation is believed to involve a spiroindolenine intermediate.\(^\text{19}\)

Preparation of the quinoline aldehyde proceeds in the manner diagramed below (refer to Scheme 1). The starting compound, 8-hydroxy-2-methylquinoline 1 is commercially available, and a nitration yielded a dinitro product 2. The nitration is accomplished using a mixture of concentrated nitric and sulfuric acids. The nitro groups add in an ortho-para fashion because of the hydroxyl group on the starting compound. The reaction is exothermic, and therefore performed in an ice bath.

Scheme 1

\[ \text{HNO}_3-\text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{Pd}/\text{C} \]

\[ \text{NaOAc/Na}_2\text{SO}_3 \rightarrow \text{RCOHN} \]

\[ \text{K}_2\text{Cr}_2\text{O}_7 \rightarrow \text{KOH} \]

\[ \text{R(a) = CH}_3(\text{CH}_2)_2 \quad \text{R(b) = ClCH}_2 \]
The next step involves the reduction of the dinitro compound 2 to an ammonium chloride salt. This reaction is performed on the Parr Hydrogenator at a pressure of 43 psi utilizing H₂ over 5% palladium charcoal as a catalyst. The nitro groups are reduced to amino groups, and then HCl changes these amino groups to ammonium chloride salts. Since amino compounds are easily oxidized, the ammonium salts act as protecting groups. The ammonium chloride salt compound was not isolated. Instead, the reaction mixture with the salt was immediately placed into a solution with sodium acetate, sodium sulfite, and either chloroacetic anhydride or butyric anhydride. The sodium acetate acted as a base, and the sodium sulfite acted as a base and an antioxidant. This reaction is a nucleophilic attack of the amino groups to the carbonyl carbons of the anhydride, resulting in the dibutrylamido (3a) or dichloroacetamido (3b) compounds.

Compound 3 was then oxidized using potassium dichromate, in a solution of glacial acetic acid and water, to form the quinolinedione 4. The reaction mixture was extracted with dichloromethane, and the organic phase was neutralized with 3% NaHCO₃. The quinolinedione 4, was then further oxidized with selenium dioxide in a solution of wet dioxane. The water acts to catalyze the reaction, forming a reactive oxide of selenium. This oxidizes the methyl group of the quinolinedione to an aldehyde. The quinoline aldehyde 5 is used in the Pictet-Spengler condensation to form the final lavendamycin product.

The preparation of tryptophan amide 7 involves a simple neutralization of the commercially available tryptophan amide hydrochloride 6, purchased from Aldrich (refer to scheme 2). The hydrochloride salt of the tryptophan amide was neutralized by the dropwise addition of 14% ammonium hydroxide to form the tryptophan 7.
The preparation of the tryptophan amide of pyrrolidine and the tryptophan amide of piperazine was performed in the following manner (refer to Scheme 3). N-Cbz tryptophan 9, purchased from Aldrich, was reacted with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide. This reaction involved an esterification, yielding Cbz tryptophan succinimide ester 10. This reaction involved the formation of urea, which was difficult to remove. The reaction mixture was allowed to sit over two nights in the refrigerator to allow the urea to precipitate out of solution. Compound 10 was reacted with either pyrrolidine or piperazine in the presence of triethylamine to afford the Cbz-tryptophan amide of pyrrolidine 11a or the Cbz-tryptophan amide of piperazine 11b. The Cbz protecting group of the amide was then cleaved off in a deprotection reaction to
yield the fully functional tryptophan amide 12. This reaction involved the use of ammonium formate in the presence of 10% palladium on charcoal and a argon balloon.

The reaction mixture containing compound 12a was washed 3% NaHCO₃ solution after the reaction was complete and the palladium on charcoal was filtered off to remove the ammonium formate from the solution. The water layer was extracted with 5 X 20 mL dichloromethane, and the pure Tryptophan amide of pyrrolidine was removed to the organic phase. Since the ammonium formate remained in the aqueous phase, the resulting compound was much cleaner than previous procedures. The deprotection reaction of Cbz-tryptophan amide of piperazine (11b) required a large amount of catalyst was well as a hydrogen balloon to help the reaction go to completion. The extra amino group on the piperazine ring acted to poison the catalyst, thus a 4:1 ratio was utilized. Compound 12b is very soluble in water, therefore washing with 3% NaHCO₃ resulted in a huge loss of compound. Instead, the ammonium formate was sublimed off at high temperatures on the rotary evaporator and vacuum pump. The NMR of Tryptophan amide of piperazine was also very difficult to interpret. The compound is totally insoluble in CDCl₃, so DMSO-d₆ was used as the solvent. The peaks that correspond to the hydrogens on the piperazine ring come at the same ppm as the water peak, therefore the ring hydrogens were difficult to interpret.

The Pictet-Spengler condensation was utilized to produce the final lavendamycin analogs. Analog 8 involved the condensation of 7-Chloroacetamido-2-formylquinoline-5,8-dione (5b) and tryptophan amide (7) in anisole. The reaction mixture was heated at 155°C over a period of 20 hours, and the pure compound 8 fell out of solution (49%). The NMR of this compound exhibited two extra singlets at 7.5 ppm and 8.0 ppm due to
the chloro in the C-7 position, as well as DMSO-d$_6$ as the solvent (the compound is insoluble in CDCl$_3$). Analog 13a involved the condensation of 7-Chloroacetamidoo-2-formylquinoline-5,8-dione (5b) and tryptophan amide of pyrrolidine (12a) in anisole. The reaction mixture was heated at 155°C over a period of 21 hours. This reaction is very sensitive, as the hydrolyzed form of the compound is also formed during the reaction. A column was utilized to separate these two forms of the compound, yielding pure 7-N-Chloroacetetyldemethylavendamycin Amide of Pyrrolidine (20%).

**Scheme 3**
VI. Experimental

A. General Information

Reagents: 8-hydroxy-2-methylquinoline, selenium dioxide, L-tryptophan amide hydrochloride salt, N-carbobenzoxyltryptophan, N-hydroxysuccinimide, and N-dicyclohexylcarbodiimide were purchased from the Aldrich Chemical Company.

Solvents: All solvents utilized in the reactions were reagent grade (except for 1,4-dioxane, chloroform, ethanol, and methanol which were dried before use, see below).

NMR Spectra: $^1$H NMR Spectra were recorded on a Varian Gemini 200 Spectrometer and an JEOL Eclipse 400 Spectrometer using CDCl$_3$ and DMSO.

Low and High Resolution Mass Spectra: EI and FAB Mass Spectra were obtained at the Chemistry Department of the University of Illinois.

Thin-Layer Chromatography: Eastman silica gel strips with fluorescent indicator were used to determine purity of all products.

B. Solvent Purification

The solvent used in the oxidation reaction to the aldehyde and in the preparation of Cbz-tryptophan succinimide ester, 1,4-dioxane, was dried and distilled before use. The solvent was refluxed with sodium spheres for 2-3 hours (until the spheres appeared metallic), and then benzophenone was added to the mixture. 1,4-dioxane has a tendency to polymerize, thus this procedure also purified the solvent.

Chloroform, absolute ethanol, and methanol were all utilized in the preparation of the tryptophan amide derivatives. These reactions required that the solvents be completely dry prior to use. The solvents were refluxed for several hours with calcium hydride (until the mixture appeared milky white) and then distilled.
C. Procedures

Preparation of 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2)

A solution of concentrated nitric acid (210 mL) and concentrated sulfuric acid (90 mL) was placed in a 500 mL Erlenmeyer flask equipped with a magnetic stir bar in an ice bath. Commercially available 8-Hydroxy-2-methylquinoline (1; 40.60 g, 0.25 mol) was added to the flask drop wise over a period of 45 minutes while the mixture was being stirred. A brownish-yellow solution resulted, and red fumes were emitted. The solution was then allowed to stir for 2 hours in an ice-bath. After 2 hours, the solution was poured into a 2 L Erlenmeyer flask containing 1 L of ice water while still stirring. A bright yellow precipitate was immediately formed. The precipitate was then filtered and washed with ice water (500 mL) and diethyl ether (300 mL). The bright yellow product was pressed dry with paper towel and allowed to air dry overnight. The reaction yielded 45.03 g (72%) of 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2). $^1$H NMR (CDCl$_3$): δ 9.63 (1H, d, J=8.9 Hz, C-4H), 9.20 (1H, s, C-6H), 8.13 (1H, d, J=8.9 Hz, C-3H), 2.92 (3H, s, C-2CH$_3$).

Preparation of 5,7-Dibutyramido-2-methyl-8-butyroxyquinoline (3a)

To a thick-walled 500 ml hydrogenation bottle, was added finely ground 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2; 5.98 g, 0.24 mol) and 10% HCl solution (100 ml). Next, 2.0 g of 5% Pd/C was added as a catalyst. The solution was then hydrogenated on a Parr Hydrogenator overnight at a pressure of 30 psi. When the reaction was completed the mixture was filtered and washed with water. The filtrate was then placed in a 1000 ml round-bottomed flask equipped with a magnetic stir bar. To this solution was added
sodium sulfite (18 g), sodium acetate (24 g), and butyric anhydride (97.5 ml) as quickly as possible. The stirred solution immediately formed a white precipitate, and it was then allowed to stir for 3 hours. The precipitate was filtered off and washed with 800 ml water until the orange tint was gone. The white product (3a) was allowed to air dry overnight and yielded 9.82 g (80.%). ¹H NMR (DMSO-d₆): δ 9.94 (1H, s, C-7NH), 9.65 (1H, s, C-5NH), 8.24 (1H, s, C-6H), 8.21 (1H, d, J=8.8 Hz, C-4H), 7.37 (1H, d, J=8.8 Hz, C-3H), 2.70 (2H, t, J=8.0 Hz, OCOCH₂CH₂CH₃), 2.58 (3H, s, C-2CH₃), 2.30-2.40 (4H, m, 2NHCOCH₂CH₂CH₃), 1.73-1.89 (2H, m, OCOCH₂CH₂CH₃), 1.52-1.72 (4H, m, 2NHCOCH₂CH₂CH₃), 1.08 (3H, t, J=8.0 Hz, OCOCH₂CH₂CH₃), 0.96 (3H, t, J=8.0 Hz, NHCOCH₂CH₂CH₃), 0.92 (3H, t, J=8.0 Hz, NHCOCH₂CH₂CH₃).

Preparation of 7-Butyramido-2-methylquinoline-5,8-Dione (4a)

In a 1000 mL round-bottomed flask containing a magnetic stir bar, 5,7-Dibutyramido-2-methyl-8-butyroxyquinoline (3a; 3.29 g, 8.25 mmol) was added along with glacial acetic acid (122 mL). While stirring vigorously, a solution of Potassium dichromate (8.80 g, 0.03 mol) and distilled water (115 mL) was added. After 2 hours, dichloromethane (70 mL) was added. This two-phase solution was then poured into a separatory funnel, and the organic layer was removed into a 1000 mL Erlenmeyer flask. The water layer was extracted with 12 X 50 mL dichloromethane, and saturated NaCl solution (50 mL) was added for the last 5 extractions. The organic layer was a reddish-orange color, and the water layer was very dark red. The combined organic layers were then washed with 3 X 200 mL 3% sodium bicarbonate solution. The organic layer was kept, and the water layer was washed with 2 X 50 mL dichloromethane. The new organic phase was
extracted with 200 mL 3% sodium bicarbonate, and then added to the previous organic layer. The organic solution was dried over anhydrous magnesium sulfate and rotary evaporated to a red-orange precipitate (4a). After drying overnight on the vacuum pump, the reaction yielded 1.56 g (73%). $^1$H NMR (CDCl$_3$): $\delta$ 8.32 (1H, s, C-7NH), 7.92 (1H, s, C-6H), 7.55 (1H, d, J=8.1 Hz, C-3H), 2.79 (3H, s, C-2CH$_3$), 2.50 (2H, t, J=7.8 Hz, C-7NHCOCH$_2$CH$_2$CH$_3$), 1.64-1.82 (2H, m, C7-NHCOCH$_2$CH$_2$CH$_3$), 1.0 (3H, t, J=7.8 Hz, C-7NHCOCH$_2$CH$_2$CH$_3$).

Preparation of 7-Butyramido-2-formylquinoline-5,8-dione (5a)

To a 25 mL round-bottomed flask equipped with a reflux condenser, argon balloon, and magnetic stir bar was added, 7-Butyramido-2-methylquinoline-5,8-dione (4a; 0.516 g, 2 mmol), selenium dioxide (0.3104 g, 2.8 mmol), 12 mL dried, distilled dioxane, and 0.25 mL water. The reaction mixture was heated at 120-130°C and monitored by TLC. The reaction was completed after 25 hours. To the final reaction mixture was added 12 mL dioxane, and then was refluxed for an additional 10 minutes. The mixture was then filtered hot on a Buchner funnel with the aid of a pipette. The filter cake and remaining selenium were then refluxed in 10 mL dichloromethane for 10 minutes and filtered off. The combined filtrates were then rotary evaporated until nearly dry. The reddish-brown precipitate was dissolved in dichloromethane and washed with 2 X 50 mL 3% sodium bicarbonate. The organic layers were saved, and the water layers were extracted with 3 X 50 mL dichloromethane. The combined organic phases were then dried over magnesium sulfate. The bright yellow filtrate was rotary evaporated to yield 0.21 g (39%) pure bright yellow product (5a). $^1$H NMR (CDCl$_3$): $\delta$ 10.31 (1H, s, C-2CHO), 8.65 (1H, d,
J=7.68 Hz, C-4H), 8.40 (1H, s, C-7NH), 8.32 (1H, d, J=8.1 Hz, C-3H), 8.08 (1H, s, C-6H), 2.54 (2H, t, J=7.34 Hz, C-7NHCOCH₂CH₂CH₃), 1.62-1.80 (2H, m, C7-NHCOCH₂CH₂CH₃), 1.04 (3H, t, J=7.37 Hz, C-7NHCOCH₂CH₂CH₃).

Preparation of 5,7-Bis(Chloroacetamido)-8-hydroxy-2-methylquinoline (3b)

To a thick-walled 500 mL hydrogenation bottle was added finely ground 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2; 5.25 g, 0.21 mol) and 10% HCl solution (100 ml). Next, 2.0 g of 5% Pd/C was added as a catalyst. The solution was then hydrogenated on a Parr Hydrogenator overnight at a pressure of 30 psi. When the reaction was completed the mixture was filtered and the filter cake was washed with water (20 mL). The filtrate was then placed in a 250 mL round-bottomed flask equipped with a magnetic stir bar. To this solution was added sodium sulfite (12 g), sodium acetate (16 g), and chloroacetic anhydride (65 g) as quickly as possible. Heat was evolved and it took several minutes for the chloroacetic anhydride to dissolve. The reaction mixture was then stirred for one hour during which a precipitate was formed. The mixture was then added to 100 mL of ice water and filtered. The filter cake was then washed with cold ethanol (10 mL). The filtrate was allowed to stand over night, and the additional precipitate was filtered. The reaction yielded 3.61 g (50%) pure product (3b). \(^1\)H NMR (DMSO-d₆): \(\delta\) 10.19 (1H, s, C-5NH), 9.92 (1H, s, C-7NH), 8.16 (1H, s, C-6H), 8.15 (1H, d, J=9.16 Hz, C-4H), 7.43 (1H, d, J=8.8 Hz, C-3H), 4.47 (2H, s, CHCH₂Cl), 4.38 (2H, s, COCH₂Cl), 2.71 (3H, s, C-2CH₃).
**Preparation of 7-Chloroacetamido-2-methylquinoline-5,8-dione (4b)**

In a 500 mL round-bottomed flask equipped with a magnetic stir bar, 5,7-
Bis(Chloroacetamido)-8-hydroxy-2-methylquinoline (3b; 3.42 g, 0.01 mol) was dissolved in glacial acetic acid (122 mL). To this solution Potassium dichromate (8.8 g, 0.03 mol) in distilled water (115 mL) were added. This dark brown solution was stirred vigorously overnight at room temperature with a watch glass covering the top of the flask. The solution was then extracted with 12 X 50 mL dichloromethane. The organic extracts were washed with 2 X 200 mL 3% sodium bicarbonate solution and added together. The solution was dried over anhydrous magnesium sulfate and rotary evaporated to yield a bright yellow solid. After vacuum drying the pure product weighed 1.64 g (62%). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 9.54 (1H, s, C-7NH), 8.32 (1H, d, J=16.88 Hz, C-4H), 7.90 (1H, s, C-6H), 7.57 (1H, d, J=8.04 Hz, C-3H), 4.24 (2H, s, COCH\(_2\)Cl), 2.77 (3H, s, C-2CH\(_3\)).

**Preparation of 7-Chloroacetamido-2-formylquinoline-5,8-dione (5b)**

In a two-neck 25 mL round-bottomed flask equipped with a magnetic stir bar, water-cooled condenser, and an argon balloon was added 7-Chloroacetamido-2-methylquinoline-5,8-dione (4b; 0.529 g, 2 mmol), selenium dioxide (0.332 g, 3 mmol), dried, distilled dioxane (12 mL), and distilled water (0.32 mL). The reaction mixture was stirred to a reflux over a two hour period. The reaction was monitored by TLC, and after 17 hours it was completed. An additional 12 mL dioxane was added to the reaction mixture and refluxed for an additional 10 minutes. Next, the solution was vacuum filtered hot using pipettes. The filter cake was then added back to the reaction flask and refluxed for 10 minutes with 10 mL dichloromethane and filtered. The filtrates were
placed on the rotary evaporator until dry. The dry precipitate was dissolved in 50 mL dichloromethane and placed in a separatory funnel. The mixture was then washed with 2 X 50 mL 3% sodium bicarbonate solution. The water layers were extracted with 3 X 50 mL dichloromethane, and all the organic layers were combined and dried over anhydrous magnesium sulfate. The solution was rotary evaporated to a yellow-brown solid weighing 0.25 g (45%). \( ^1 \text{H} \text{NMR (CDCl}_3): \delta 10.29 (1H, s, C-2\text{CHO}), 9.56 (1H, s, C-7\text{NH}), 8.64 (1H, d, J=1.12 \text{ Hz}, C-4\text{H}), 8.33 (1H, d, J=8.08 \text{ Hz}, C-3\text{H}), 8.05 (1H, s, C-6\text{H}), 4.26 (2H, s, CO\text{CH}_2\text{Cl}).

**Preparation of Tryptophan Amide (7)**

In a 125 mL Erlenmeyer flask was added commercially available tryptophan amide hydrochloride salt (6; 0.4794 g, 2 mmol) and 35 mL ethyl acetate. To this cloudy solution was added 14% ammonium hydroxide solution dropwise until the solution was clear. The resulting solution was placed in a separatory funnel, and the water layer was separated from the organic layer. The organic layer was then washed with 5 X 6 mL saturated sodium chloride solution. The organic layer was dried over anhydrous magnesium sulfate, and rotary evaporated to a thick gel. The reaction mixture was allowed to dry several days on the vacuum pump (89%). \( ^1 \text{H} \text{NMR (CDCl}_3): \delta 8.05 (1H, s, -\text{NH}), 7.68 (1H, d, J=8.2 \text{ Hz}, C-4\text{H}), 7.39 (1H, d, J=8.5 \text{ Hz}, C-7\text{H}), 7.07-7.22 (2H, m, C-2\text{H} C-5\text{H}, C-6\text{H}), 5.32 (2H, s, CONH\text{H}_2), 3.71 (1H, dd, J=8.1 \text{ Hz}, J=7.9 \text{ Hz}, C-2'\text{H}), 2.84-3.42 (2H, m, C-3'\text{H}), 1.4 (2H, s, C-2''\text{NH}_2).
Preparation of Benzyloxycarbonyltryptophan Succinimide Ester (10)
In a 25 mL round-bottomed flask equipped with a magnetic stir bar and an argon filled balloon, N-carbobenzyloxytryptophan (9; 0.338 g, 1 mmol), N-hydroxysuccinimide (0.155 g, 1 mmol) and 5 mL dried, distilled dioxane were added. The reaction mixture was stirred in a 12°C ice bath until the solution was clear. To this clear solution was added N-dicyclohexylcarbodimide (0.206 g, 1 mmol), and a white precipitate formed after 1 minute. The reaction mixture was stirred at 15°C to 20°C for 2 hours, and then stirred for 2 additional hours at room temperature. The reaction flask was then placed in the refrigerator over 2 nights. The solid was filtered and rinsed with 5 mL dioxane. The filtrate was then rotary evaporated until a thick colorless gel was formed. The product was placed on the vacuum pump for two days where it foamed up into a white solid (10) weighing 0.4215 g (97%). $^1$H NMR (CDCl$_3$): δ 8.09 (1H, s, -NH), 7.54 (1H, d, J=7.7 Hz, C-4H), 7.32-7.37 (6H, m, C-7H, C$_6$H$_5$), 7.19 (1H, t, J=7.5 Hz, C-5H), 7.09 (1H, t, J=7.32 Hz, C-6H), 5.5.11-5.21 (3H, m, CHCOOCH$_2$), 5.05 (1H, d, J=12.48 Hz, C-2'NH), 3.5 (2H, m, C-3'CH), 2.85 (4H, s, COCH$_2$CH$_2$).

Preparation of Benzyloxycarbonyltryptophan Amide of Pyrrolidine (11a)
In a 100 mL round-bottomed flask equipped with a magnetic stir bar and an argon filled balloon, benzyloxycarbonyltryptophan succinimide ester (10; 0.87 g, 2 mmol), pyrrolidine (0.166 mL, 2 mmol), dried, distilled triethylamine (0.28 mL, 2 mmol), dried, distilled absolute ethanol (26 mL), and dried, distilled chloroform (24 mL), were added. The reaction mixture was allowed to stir at room temperature, while being monitored by TLC. The reaction was complete after 30 minutes. The reaction mixture was then rotary
evaporated to dry. This solid was dissolved in 3 X 60 mL ethyl acetate. This solution was allowed to sit overnight in the refrigerator so the salts from the triethylamine could precipitate out of solution. The solution was immediately filtered when removed from the refrigerator, and the filtrate was washed with 60 mL of distilled water. The organic phase was then washed with 2 X 60 mL 10% citric acid solution, followed by 30 mL 1 N NaHCO$_3$ solution. The neutralized organic phases were then washed with 5 X 10 mL brine solution and dried over anhydrous sodium sulfate overnight. The solution was rotary evaporated to a thick gel and placed on the vacuum pump overnight where it foamed up into a white solid weighing 0.6935 % (89%). $^1$H NMR (CDCl$_3$): δ 7.97 (1H, s, -NH), 7.62 (1H, d, J=8.04 Hz, C-4H), 7.32-7.35 (5H, m, C$_6$H$_5$), 7.29 (1H, d, J=5.84 Hz, C-7H), 7.17 (1H, t, J=7.5 Hz, C-5H), 7.10 (1H, t, J=7.72 Hz, C-6H), 7.07 (1H, s, C-2H), 5.74 (1H, d, J=8.4 Hz, C-2'NH), 5.10 (2H, s, COOCH$_2$), 4.69-4.73 (1H, m, C-2'H), 3.19-3.37 (5H, m, C-3' cis to the C-1 amide group, -pyrrolidine-C-2H, C-5H), 2.49-2.53 (1H, m, C-3'H trans to the C-1 amide group), 1.26-1.62 (4H, m, -pyrrolidine-C-3H, C-4H).

**Preparation of Tryptophan Amide of Pyrrolidine (12a)**

In a 50 mL two necked, round-bottomed flask equipped with a stir bar and an argon filled balloon were added benzyloxycarbonyltryptophan amide of pyrrolidine (11a; 0.336 g, 0.86 mmol) and dried, distilled methanol (17.5 mL). To this stirred suspension, vacuum dried ammonium formate (0.1595 g, 2.53 mmol) and 10% palladium on charcoal (0.1595 g) were added. The reaction was allowed to stir at room temperature and was monitored by TLC. The reaction was found to be complete after 30 minutes. The reaction mixture
was then filtered and the palladium on charcoal filter cake was washed with 10 mL methanol. The filtrate was then rotary evaporated to concentrate (about one half the volume). To this concentrated organic solution was added 15 mL 3% NaHCO₃ solution, forming a white precipitate. The solution was then extracted with 5 X 20 mL dichloromethane. The organic phase was dried overnight on anhydrous sodium sulfate, and it was rotary evaporated to dry yielding 0.1836 g (83%) of pure compound 12a. ^1H NMR (CDCl₃): δ 8.01 (1H, s, -NH), 7.59 (1H, d, J=7.7 Hz, C-4H), 7.35 (1H, d, J=8.04 Hz, C-7H), 7.19 (1H, t, J=7.52 Hz, C-5H), 7.11 (1H, t, J=7.68 Hz, C-6H). 7.08 (1H, s, C-2H), 3.99 (2H, s, -NH₂), 3.82 (1H, t, J=6.96 Hz, C-2'H), 2.86-3.48 (6H, m, C-3'H, pyrrolidine-C-2H₁, C-5H₁), 1.67 -1.95 (4H, m, -pyrrolidine-C-3H₁, C-4H₁).

Preparation of Benzyloxy carbonyltryptophan Amide of Piperazine (11b)

In a 100 mL round-bottomed flask equipped with a magnetic stir bar and an argon filled balloon, benzyloxy carbonyltryptophan succinimide ester (10; 1.007 g, 2.31 mmol), piperazine (0.1949, 2.31 mmol), dried, distilled triethylamine (0.32 mL, 2.31 mmol), dried, distilled absolute ethanol (26 mL), and dried, distilled chloroform (24 mL), were added. The reaction mixture was allowed to stir at room temperature, while being monitored by TLC. The reaction was complete after 4 hours. To the reaction mixture was added 10% citric acid solution drop wise until a pH of 6 was reached. This solution was then neutralized to a pH of 7-8 with 3% NaHCO₃ solution (added drop wise). The neutralized reaction mixture was then washed with 3 X 2 mL brine solution and dried over anhydrous sodium sulfate overnight. The solution was rotary evaporated to a thick gel and placed on the vacuum pump where it foamed up yielding 0.7268 g (72%) of the
pure compound 11a. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.89 (1H, s, -NH), 7.62 (1H, d, J=9.88 Hz, C-4H), 7.51 (1H, d, J=13.6, C-7H), 7.29-7.35 (5H, m, -C$_6$H$_5$), 7.15 (1H, s, C-2H), 7.06 (1H, t, J=7.68 Hz, C-5H), 6.96 (1H, t, 8.04 Hz, C-6H), 4.99 (3H, s, NHCOOCH$_3$), 4.35 (1H, s, C-2’H), 3.04-3.33 (4H, m, -piperazine-C-2H, C-6H), 2.90-3.08 (2H, m, C-3’H), 2.59 (4H, s, -piperazine-C-3H, C-4H), 2.34 (1H, s, -piperazine-NH)

Preparation of Tryptophan Amide of Piperazine (12b)

In a 50 mL two necked, round-bottomed flask equipped with a stir bar and an argon filled balloon were added benzyloxycarbonyltryptophan amide of piperazine (11b; 0.4065 g, 1 mmol) and dried, distilled methanol (25 mL). To this stirred suspension, vacuum dried ammonium formate (0.189 g, 3 mmol) and 10% palladium on charcoal (0.472 g, 4 mmol) were added. After all of the reactants were added to the flask, the argon balloon was replaced by a hydrogen balloon. The reaction was allowed to stir at room temperature and was monitored by TLC. The reaction was complete after 1.5 hours. The palladium on charcoal was then filtered off, and the filter cake was washed with 10 mL methanol. The filtrate was then rotary evaporated to dry, and left on the rotary evaporator for an hour at a high temperature (90°C) to sublime the ammonium formate. The product, 12b, was then placed on the vacuum pump at a temperature of 40°C for two days yielding 0.241 g (89%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.85 (1H, s, -NH), 7.48 (1H, d, J=7.36 Hz, C-4H), 7.32 (1H, d, J=6.6, C-7H), 7.14 (1H, s, C-2H), 7.06 (1H, t, J=6.22 Hz, C-5H), 6.97 (1H, t, J=7.14 Hz, C-6H), 4.04 (3H, m, C-2’H, NH$_2$), 3.04-3.33 (4H, m, -piperazine-C-2H, C-6H), 2.90-3.08 (2H, m, C-3’H), 2.59 (4H, s, -piperazine-C-3H, C-4H), 2.34 (1H, s, -piperazine-NH).
Preparation of 7-N-Chloroacetyldemethyllavendamycin Amide (8)

In a 250 mL 3 necked round-bottomed flask equipped with a magnetic stir bar, argon flow, and a dean stock condenser, 7-Chloroacetamido-2-formylquinoline-5,8-dione (5b; 0.082569 g, 0.3 mmol), tryptophan amide (7; 0.0609 g, 0.03 mmol), and anisole (120 mL) were added. The reaction mixture was allowed to heat to 155°C over the course of three hours and was monitored by TLC. TLC showed the reaction complete after 20 hours. The reaction mixture was allowed to cool to room temperature, and it was then filtered to give a solid yellow precipitate. The precipitate was washed with petroleum ether and allowed to dry overnight on the vacuum pump. The reaction yielded 0.0677 g (49%) pure compound (8). \(^1\)H NMR (DMSO-d6): δ 12.26 (1H, s, 8'-NH), 10.19 (1H, s, C7-NH), 9.93 (1H, s, C-7NH), 9.75 (2H, s, CONH\(_2\)), 9.12 (1H, d, J=9.16 Hz, C-4H), 9.02 (1H, s, C-3'H), 8.61 (1H, d, J=8.8 Hz, C-3'H), 8.49 (1H, d, J=8.08 Hz, C-12'H), 8.49 (1H, s, C-6H), 7.82 (1H, d, J=8.04 Hz, C-9'H), 7.70 (1H, t, J=8.4 Hz, C-10'H), 7.39 (1H, t, J=8.4 Hz, C-11'H), 4.55 (2H, s, ClCH\(_2\)CO).

Preparation of 7-N-Chloroacetyldemethyllavendamycin Amide of Pyrrolidine (13a)

In a 100 mL 3 necked round-bottomed flask equipped with a magnetic stir bar, argon flow, and a dean stock condenser were added, 7-Chloroacetamido-2-formylquinoline-5,8-dione (5b; 0.04247 g, 0.1525 mmol), tryptophan amide of pyrrolidine (12a; 0.03925 g, 0.1525 mmol), and anisole (55 mL). The reaction was heated to 155°C over a period of 3 hours. The reaction was monitored by TLC and found to be complete after 18 hours. The reaction mixture was then filtered hot, and the filter cake was washed with 4 mL
dichloromethane and 4 mL chloroform. The filtrate was rotary evaporated to dry. Column chromatography was needed to purify the filter cake and the filtrate. The product was dissolved in 20 mL dichloromethane and 4 mL methanol, and the developing solvent was ethyl acetate. After column chromatography, the reaction yielded 0.011 g pure product (13a) (21%). \( ^1 \text{H} \text{NMR (DMSO-d}_6 \): } \delta 11.87 (1H, s, 8'-NH), 10.65 (1H, s, C-7NH), 8.94 (1H, d, J=8.4 Hz, C-4H), 8.88 (1H, s, C-3'H), 8.57 (1H, d, J=8.04 Hz, C-3H), 8.48 (1H, d, J=8.04 Hz, C-12'H), 7.81 (1H, s, C-6H), 7.11 (2H, d, t, C-9'H, C-11'H), 7.41 (1H, t, J=8.1 Hz, C-10'H), 4.66 (2H, s, ClCH\(_2\)CO), 3.98 (2H, s, -pyrrolidine-C-2H), 3.64 (2H, s, -pyrrolidine-C-5H), 1.96 (4H, s, -pyrrolidine-C-3H\(_2\), C-4H\(_2\)).

**Preparation of 7-N-butryldemethylavendamycin Amide of Piperazine (13b)**

In a 50 mL 3 necked round-bottomed flask equipped with a magnetic stir bar, argon flow, and a dean stock condenser were added, 7-Butrylamido-2-formylquinoline-5,8-dione (5a; 0.02721 g, 0.1 mmol) and anisole (35 mL). This solution was heated to 70°C, until all of the starting compound (5a) had dissolved. To this reaction mixture was added tryptophan amide of piperazine (12b; 0.02724 g, 0.1 mmol) dissolved in 0.83 mL dried, distilled pyridine. The reaction mixture was then heated to 155°C over a period of 3 hours. The reaction was monitored by TLC and was stopped after 26 hours. The reaction mixture was then filtered hot, and the filter cake was washed with 4 mL ethyl acetate and 4 mL petroleum ether. The product was impure, and column chromatography was needed for purification.
Appendix A

Index of Spectra

8-Hydroxy-2-methyl-5,7-dinitroquinoline ........................................... ($^1$H NMR) ............ (2)
5,7-Dibutrylamido-8-butyroxy-2-methylquinoline ........................................ ($^1$H NMR) ............ (3a)
7-Butyramido-2-methylquinoline-5,8-dione ........................................... ($^1$H NMR) ............ (4a)
7-Butyramido-2-formylquinoline-5,8-dione ........................................... ($^1$H NMR) ............ (5a)
5,7-Bis(Chloroacetamido)-8-hydroxy-2-methylquinoline ................................ ($^1$H NMR) ............ (3b)
7-Chloroacetamido-2-methylquinoline-5,8-dione ........................................ (7a) ($^1$H NMR) ............ (4b)
7-Chloroacetamido-2-formylquinoline-5,8-dione ........................................ (7b) ($^1$H NMR) ............ (5b)
Tryptophan Amide ........................................................................ ($^1$H NMR) ............ (7)
Benzyloxy carbonyltryptophan Succinimide Ester ........................................ ($^1$H NMR) ............ (10)
Benzyloxy carbonyltryptophan Amide of Pyrrolidine ................................ ($^1$H NMR) ............ (11a)
Tryptophan Amide of Pyrrolidine ......................................................... ($^1$H NMR) ............ (12a)
Benzyloxy carbonyltryptophan Amide of Piperazine ................................ ($^1$H NMR) ............ (11b)
Tryptophan Amide of Piperazine ......................................................... ($^1$H NMR) ............ (12b)
7-N-Chloroacetyldemethyllavendamycin Amide ........................................ ($^1$H NMR) ............ (8)
(Mass Spec) ............ (8)
7-N-Chloroacetyldemethyllavendamycin Amide of Pyrrolidine ........ ($^1$H NMR) ............ (13a)
H₃CH₂CH₂COCHN

\[ \text{H₃CH₂CH₂COCHN} \]

4a

JEN/DIONE2

EXPERIMENT: S25UL

DATE: 01-01-97

SOLVENT: CDCl₃

FILE: H

PPM

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0.43 1.22 5.08 1.41

0.77
X: parts per Million: 1H
Phenylamide of Piperazine
Heteroatom Max: 40

Ion: Both Even and Odd

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90%   136.1  232.1  214.1  289.1  307.2  341.2  367.2  417.2
80%   60  462.2
70%   55  40  35
60%   44  30  25  20  15  10  5
50%   39  24  19  14  9  4
40%   28  13  8  3
30%   27  12  7  2
20%   26  11  6  1
10%   25  10  5
0%    24  9  4  3  2  1
Appendix B – Research Presentations

The author of this thesis presented the research conducted for this paper on two different occasions. One presentation was for the Ball State University Summer Research Program. The other presentation was at the Indiana Academy of Science Annual Meeting November 4, 1999 in Evansville, Indiana.
VII. Works Cited


12) Behforouz, M. Grant Proposal to the NIH, 1997, unpublished.


