Total Synthesis of 7-N-Acetyl-6-Ethyl-Lavendamycin Methyl Ester

An Honors 499 Thesis
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Abstract

The research detailed within concerns the synthesis of an analog of lavendamycin; 7-N-acetyl-6-ethyl-lavendamycin methyl ester. This synthesis is part of a continuing structure-activity relationship study of various analogs of lavendamycin as possible chemotherapeutic agents. The eventual screening of this analog for biological activity will further the knowledge of the effects of structure on various biological activities. The molecules selected in this SAR study are chosen in hopes that they will show selective toxicity for ras$^k$ oncogene transformed cells.
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I. Acknowledgments

I would first like to thank Dr. Mohammad Behforouz, without whom this project would not have been possible. I have a tremendous amount of respect for Dr. Behforouz and his combined strengths of strong character, vast knowledge, and genuine kindness. Another very special person to thank is Mrs. Wen Cai, whose unselfishness and unwavering work ethic has been key to the completion of this project. I am also grateful to Adrian Adams and MaryAnn Knott for providing me with employment and friendship. Thanks to the Ball State Chemistry Department and all faculty for giving me a quality education that has prepared me for a successful future in chemistry. Recognition goes to the National Institute of Health and the American Cancer Society for providing the major funding for the project. Thank you to the Ball State University Honors College for awarding me an Undergraduate Honors Fellowship, thus allowing me to commit more time to the project. Above all, I would like to thank God for these and the many other blessings in my life.
II. Background Information

Lavendamycin (1), a naturally occurring compound produced by the soil bacterium *Streptomyces lavendulae*, was first isolated and described in 1981 by Doyle and associates at Bristol Laboratories. The similarity in structure of lavendamycin and a known antitumor/antibiotic, streptonigrin (2), was noted. The two compounds also displayed similar biological activities, with lavendamycin showing limited antimicrobial action and significant activity against ras* leukemia cells in mice. 2

![Figure 1: Lavendamycin and Streptonigrin](image)

The prospect of anti-tumor activity, coupled with the extreme difficulty in isolating the natural product, sparked several research groups to begin efforts at a total synthesis of lavendamycin. In 1984, Kende and Ebetino were the first to report synthesis of lavendamycin methyl ester. 3, 4 Their synthesis pathway used a Friedlander condensation to produce the A-B ring portion and a Bischler-Napieriski cyclodehydration to complete the five-ring system. This was followed in 1985 by Hibino reporting the synthesis of lavendamycin methyl ester by first constructing the complete ring system with a Pictet-Spengler condensation between a quinoline analog and β-methyl tryptophan, followed by several steps to functionalize the five ring system. 5 Also in 1985 Boger reported a complicated synthesis involving twenty steps with an overall yield less than 1%. 6 Then in 1993, Behforouz reported a synthesis of only five steps to an overall yield of 33%. 7 This five step route involved a Diels-Alder condensation to produce the A-B ring system, along with a Pictet-Spengler condensation similar to that reported by Hibino, with all functionalizations occurring before the Pictet-Spengler condensation. Behforouz then improved on the method in
1996, producing lavendamycin methyl ester in six steps with a yield of 40%, this time using 8-hydroxyquinoline as the starting material and avoiding the Diels-Alder condensation. Since 1993, Behforouz's group has been conducting a structure-activity relationship (SAR) study on analogs of lavendamycin in order to better understand the mechanism of these compounds in biological activity.

II. Synthesis of Lavendamycin Analogs

The potent antitumor capacity of both lavendamycin and streptonigrin was originally overshadowed by their high toxicity and lavendamycin's low solubility in pharmaceutical solvents. An SAR study of lavendamycin analogs allows researchers to select for more soluble analogs and search for compounds with more selective cytotoxicity. Quinones often are highly cytotoxic, resulting in DNA cleavage, and many have shown anti-cancer potential. It has also been suggested that the quinone toxicity is due to effects on the electron transport system in mitochondria. In hopes of gaining a better understanding of these mechanisms and possibly discovering a useful chemotherapeutic agent, Behforouz and his group at Ball State University began an SAR study on lavendamycin analogs, using the five step syntheses reported in 1993 and 1996. It is the concise and practical nature of these synthesis routes that has allowed the SAR study to take place. In 1993 (Scheme 1a) Behforouz reported the use of a Diels-Alder condensation between the bromoquinone (3) and the azadiene (4), to produce the quinolinedione (5) portion of lavendamycin. Oxidation of the methyl group on the quinolinedione provided the aldehyde (6) used in a Pictet-Spengler condensation with β-methyl tryptophan methyl ester (7) to produce 7-N-acetyllavendamycin methyl ester (8), which was then hydrolyzed to give the final product (9).
In 1996, Behforouz modified the method (Scheme 1b) and increased the yield from 33% to 40% by using 8-hydroxyquinaldine (10) as starting.
In 1996, Behforouz modified the method (Scheme 1b) and increased the yield from 33% to 40% by using 8-hydroxyquinaldine (10) as starting material for the A-B portion of the ring system, and thus avoiding the relatively low-yield Diels-Alder reaction. It is this improved method that has been used for the research presented in this thesis.

IV. 7-N-acetyllavendamycin esters

It has been discovered in the SAR study that the 7-N-acetyl analogs of lavendamycin proved a much more selective toxicity against the ras\(^k\) tumor cells than the amino counterparts. The 7-N-acetyl compound 8 displayed 9-fold selectivity against the tumor cells compared to the much lower 0.5-fold selectivity displayed by 9. Because the 7-N-acetyl analogs are more selective and are produced in one less step than the amino counterpart, the target molecule for this project was chosen to be a 7-N-acetyl analog.

V. Total Synthesis:

A. 7-N-Acetyl-6-ethyl-lavendamycin methyl ester

The key reaction in Behforouz's synthesis route to lavendamycin analogs is the Pictet-Spengler condensation of a formylquinolinedione and a tryptophan. The novel work presented in this thesis involves new functionalization on the quinolinedione portion of the lavendamycin molecule. The final product, 7-N-acetyl-6-ethyl-lavendamycin methyl ester was produced as follows. 8-hydroxyquinaldine (10), available commercially, was nitrated with a 70:30 mixture of concentrated nitric and sulfuric acids. The reaction was quite exothermic, so an ice bath was used to maintain room temperature. The hydroxy group on the 8-hydroxyquinaldine acts as an ortho-para director, and the nitrogen on the opposite ring deactivates the positions on that ring, so the nitro groups are added only ortho and para to the hydroxy group.
The resulting yellow 5,7-dinitro-8-hydroxy-2-methylquinoline (11) was then hydrogenated using a Parr hydrogenator. The dinitro is mixed with 5% Pd-C and a 10% HCl solution, and shaken under H₂ at 30 psi for 20 hours to give the dark red dihydrochloride salt 12. This salt, which protects the sensitive amino group, is not isolated, but is reacted immediately with sodium sulfite, sodium acetate, and a slow addition of acetic anhydride to give the diacetamido compound 13. The reaction proceeds by nucleophilic attack of the amino groups to the carbonyl carbons in the acetic anhydride. There was possibly a mixture of the hydroxy and acetoxy groups at the 8 position, but these do not need to be separated as they do not affect the next step.

This white acetoxy/hydroxy mixture was then oxidized with K₂Cr₂O₇ in acetic acid solution, extracted with dichloromethane, and neutralized with 5% NaHCO₃ to give the bright yellow quinolinedione 5. The mechanism for this reaction is unknown.

The remainder of the steps in the synthesis can be seen in Scheme 2. Compound 5 was reacted with a 1M diethylaluminum cyanide/toluene solution to give, surprisingly, a major product of the ethylated compound 14. This most likely occurred by way of a 1,4-Michael addition, rather than the previously reported 1,2-addition resulting in 17₁⁹, or the originally expected 1,4-conjugate addition of HCN. There was also recovery of a smaller amount of starting material.

The methyl group of this quinolinedione was then oxidized with selenium dioxide in 1,4-dioxane and a small amount of water to give the aldehyde 15. The water and SeO₂ react, forming a reactive selenium oxide, which then reacts selectively to oxidize only the methyl group on the quinolinedione.
Next, the Pictet-Spengler condensation was performed with the aldehyde and the commercially available β-methyl tryptophan methyl ester (7), refluxing in xylene, to yield the final product 16. In the Pictet-Spengler condensation, the aldehyde of the quinolinedione and the amine group of the tryptophan react, are thought to form a spiroidolenine intermediate, and result in a new carbon-carbon bond.

B. Reaction of Diethylaluminum Cyanide with α,β-Unsaturated Ketones

For many years, diethylaluminum cyanide has been known to produce β-cyano carbonyl compounds by way of a conjugate addition to α,β-unsaturated ketones. In early 1998 Behforouz reported from our own research group a reaction in which diethylaluminum cyanide added to the quinolinedione 5 in a 1,2 fashion, resulting in the quinoline quinol 17 and a small amount of recovered starting material.
Quinoline quinols have shown promise as chemotherapeutic agents, so the project was begun intending to produce a lavendamycin analog with a quinoline quinol portion. Instead, working under the same reaction conditions but with a different reagent bottle, I repeated the published reaction with compound 7 and diethylaluminum cyanide and obtained a major product of compound 14, along with a small amount of recovered starting material 7. The proposed mechanism for this reaction would be a 1,4 conjugate addition, but with an ethyl group acting as the nucleophile rather than the expected cyano group.

VI. Experimental

A. General Information

Reagents: 8-hydroxyquinaldine, selenium dioxide, diethylaluminum cyanide (1M in toluene), and β-methyl ester were purchased from the Aldrich Chemical Co.

Solvents: All solvents used were reagent grade, excluding 1,4-dioxane, xylene, and in specified instances, dichloromethane.

NMR Spectra: The $^1$H NMR spectra were recorded with a Varian Gemini 200 spectrometer, and the samples were prepared in CDCl$_3$ (w/TMS as internal standard) or DMSO.

IR Spectra: The IR spectra were recorded with a Perkin-Elmer Spectrum 1000 FT-IR Spectrometer and the samples were prepared in KBr pellets.
B. Solvent Purification

For the reaction with diethylaluminum cyanide, dichloromethane was distilled over CaH₂ and stored under argon. 1,4-dioxane was first refluxed with potassium hydroxide, decanted, refluxed with benzophenone and sodium spheres until dry, and then distilled. The potassium hydroxide breaks down the dioxane into monomers, and the sodium is a drying agent. The benzophenone acts as an indicator of complete dryness, and the solution turns blue at such time. The xylene is also purified by refluxing with sodium spheres and benzophenone, followed by distillation.

C. Procedures

Preparation of 8-Hydroxy-2-methyl-5,7-dinitroquinoline (11)

In a 1L erlenmeyer flask cooled by ice, concentrated nitric acid (140 ml) and concentrated sulfuric acid (60 ml) were combined and stirred. In small portions over a one hour period was added 8-hydroxyquinaldine (10; 20.00 g, 0.125 mol), while keeping the solution at room temperature. Once the addition was complete, the reddish solution was allowed to stir in the ice bath for an additional two hours. This solution was then poured into a 2L beaker containing ice water(1:1, 1200 ml), and stirred with a glass rod. The solution immediately precipitated a bright yellow solid, which was then vacuum filtered, washed with water and diethyl ether to yield 17.68 g (56%) of product 11. mp 292-296 °C. ¹H NMR(DMSO): δ 9.68(1H, d,J=9.1Hz,C-4H), 9.22(1H,s, C-6H), 8.15(1H,d,J=9.1Hz,C-3H), 2.95(3H,s,CH₃).

Preparation of 5,7-Diacetamido-8-acetoxy-2-methylquinoline (13)

In a thick walled 500 ml hydrogenation flask, 2.0 g 5% Pd-C catalyst was added to a suspension of finely powdered 8-hydroxy-2-methylquinoline-5,7-dinitroquinoline (11; 5.98 g, .024 mol) in
100 ml 10% hydrochloric acid. This mixture was placed on a Parr hydrogenator and shaken under 30 psi for 21 hours. The resulting red solution was immediately filtered to remove the Pd-C, and the filter cake rinsed with 10-20 ml water. Immediately, 20.0 g sodium acetate and 10.0 g sodium sulfite was added to the dark red filtrate and stirred. Then 67 ml acetic anhydride was added dropwise while stirring and cooling in an ice bath. The solution turned yellow as precipitate formed. Once the addition was complete, the solution was allowed to stir for an additional 30 minutes, then the precipitate was filtered off and rinsed with 20 ml water. The filtrate was then concentrated to approximately 25-30 ml, and an extra 13 ml acetic anhydride was added dropwise over fifteen minutes, causing more precipitate to form. The solution was filtered again, and the filter cakes were combined and dried to give 6.10 g (81%) of 13.

Preparation of 7-Acetamido-2-methylquinoline-5,8-dione(5)

In a 500 ml erlenmeyer flask, 5,7-diacetamido-8-acetoxy-2-methylquinoline (13; 3.15 g, .01 mol) was dissolved and stirred in 120 ml glacial acetic acid. Potassium dichromate (8.75 g, .03 mol) was then dissolved in 100 ml water and added to the acetic acid solution. This solution was then stirred at room temperature for 24 hours. The resulting black solution was poured into 900 ml water in a 2L separatory funnel, and extracted with dichloromethane (4 x 250 ml). The yellow organic extracts were then combined and washed with 5% sodium carbonate in saturated sodium chloride solution (3 x 200 ml) to neutralize the acid. The organic solution was then dried over magnesium sulfate overnight. The next day, the solution was rota-evaporated to dryness and dried under vacuum to yield 1.18 g (52%) of yellow solid 5. mp 216-218 °C. $^1$H NMR(CDCl₃): δ 8.41(1H,s,C-7NH), 8.32 (1H,d,J=8.1Hz,C-4H), 7.92(1H,s,C-6H), 7.57(1H,d,J=8.1Hz,C-3H), 2.78(3H,s,C-2CH₃), 2.33(3H,s,NHCOCH₃).
Preparation of 7-Acetamido-6-ethyl-2-methylquinoline-5,8-dione (14)

7-acetamido-2-methylquinoline-5,8-dione (5; 230 mg, 1 mmol) was dissolved and stirred in 10 ml of dichloromethane (distilled over CaH₂). To this bright yellow solution was added a 1M solution of diethylaluminum cyanide in Toluene (5 ml, 5 mmol, 5 eq), which immediately turned the solution to a thick brown mixture. The mixture was then stirred in an argon atmosphere for one hour. Next, 40 ml of ice-cooled saturated sodium potassium tartrate was added and stirred for 20 minutes. Then 100 ml dichloromethane and another 40 ml saturated sodium potassium tartrate was added and stirred for an additional 20 minutes. The resulting mixture was extracted with dichloromethane (3 x 100 ml) and the yellow organic layers combined, washed with 200 ml water, and dried over magnesium sulfate. The extracts were then concentrated to about 5 ml. Flash chromatography (silica gel 40μm, 2.5 x 15 cm, EtOAc eluent) afforded 21 mg (9%) of recovered starting material 3, and 76.4 mg (30%) of 14 as a dull yellow solid. (CH₂Cl₂-Pet. Ether) m.p. 159-162 °C. IR(KBr) 3445(s, br), 3265, 2964, 2935, 1670, 1655, 1588, 1508, 1304, 1256, cm⁻¹; ¹H NMR (CDCl₃) δ 8.32 (1H, d, J=8.1 Hz, C-4H), 7.75 (1H, s, C-7N-H), 7.50 (1H, d, J=8.1 Hz, C-3H), 2.74 (3H, s, COCH₃), 2.25 (3H, s, COCH₃), 1.14 (3H, t, J=7.47 Hz, CH₂CH₃)

Preparation of 7-Acetamido-6-ethyl-2-formylquinoline-5,8-dione (15)

In a 10 ml pear-shaped flask, 4 ml of dried and purified 1,4-dioxane and 0.025 ml of water were added to a mixture of 7-acetamido-6-ethyl-2-methylquinoline-5,8-dione (14, 51.6 mg, 0.2 mmol) and selenium dioxide (27.8 mg, 0.25 mmol, white crystal only). The resulting mixture was gently stirred under an argon atmosphere and slowly heated to reflux at 115-120 °C over a 2 hour period. The now dark orange-black solution was allowed to
reflux for 16 hours, until the reaction is complete when checked by TLC (1:1 CH₂Cl₂:Acetone). At this time 4 ml more 1,4-dioxane was added to the solution and refluxed for 15 minutes. The solution was decanted from the black selenium and vacuum filtered while hot. 5 ml of dichloromethane was added to the residue in the reaction flask and refluxed for 10 minutes. This solution was then filtered hot, the filtrates combined, and rotary-evaporated to dryness, leaving an orange-yellow solid which is dried to yield 38.6 mg (71%) of 15. (CH₂Cl₂:Pet. Ether). m.p. 180-185 °C IR(KBr) 3455(br), 3280, 1675, 1652, 1581, 1500, 1240; ¹H NMR (CDCl₃) δ 10.33 (1H, s, CHO), 8.67 (1H, d, J=8.1 Hz, C-4H), 8.34 (1H, d, J=8.1 Hz, C-3H), 7.83 (1H, s, C-7NH), 2.72 (2H, q, J=7.5 Hz, CH₂CH₃), 2.37 (3H, s, COCH₃), 1.22 (3H, t, J=7.5 Hz, CH₂CH₃).

Preparation of 7-N-Acetyl-6-ethyl-lavendamycin methyl ester (16)

In a 100 ml round bottom flask, 7-acetamido-6-ethyl-2-formylquinoline-5,8-dione (15, 35.3 mg, 0.13 mmol) was dissolved in 42 ml of dried xylene and stirred under argon flow. β-methyl tryptophan methyl ester (30.1 mg, 0.13 mmol) was added and the mixture heated slowly to reflux at 160 °C. The pale yellow solution was refluxed for 21 hours, and completion of reaction was then checked by TLC (6ml CH₂Cl₂: 4 drops MeOH). The solution was concentrated to one-fifth its original volume, vacuum filtered and the yellow crystals were washed with petroleum ether to remove the xylene. The solid is dried and weighed to yield 41.4 mg (66%) of rather impure 16. Flash chromatography (1 x 7.5 cm, 40 μm silica gel, 50:1 CH₂Cl₂:MeOH eluent) results in pure 16 as dark orange solid. IR(KBr) 3350, 3244, 2962, 2925, 1717, 1690, 1662, 1589, 1508, 1334, 1262, 1099, 1077, 1020, 802 cm⁻¹; ¹H NMR (CDCl₃) δ 11.82 (1H, s, NH), 9.04 (1H, d, J=8.1 Hz, C-4H), 8.50 (1H, d, J=8.1 Hz, C-3H), 8.33 (1H, d, J=8.1 Hz), 7.75 (1H, d), 7.72 (1H, br s, NHCOCH₃), 7.60 (1H, t, J=8.1 Hz), 7.35 (1H, t, J=8.1 Hz), 4.03 (3H, s,
$\text{COOCH}_3$, 3.17 (3H, s, $-\text{CH}_3$), 2.64 (2H, q, $\text{CH}_2\text{CH}_3$), 2.29 (3H, s, NCOCH$_3$), 1.18 (3H, t, $\text{CH}_2\text{CH}_3$).
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\[ \text{CH}_3\text{CH}_2 - \text{AcHN} - \text{CHO} \]
7-N-acetyl-6-ethyl-lavendamycin methyl ester
Works Cited


