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Investigation into the Synthesis of Turmeric Derivatives

Jessica Kisunzu

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Abstract

Curcumin is a dye obtained by extraction from turmeric (*Curcuma Longa L).* Derivatives of curcumin have been synthesized, mainly involving additions at the carbon between the two carbonyl groups. This research attempted to add an indole at both positions β to the ketone carbonyls to form 1,7-bis(indolyl)curcumin. The catalyst free, "on-water" synthesis was implemented and the resulting solid analyzed. A three step synthesis was then executed which involved the formation of an acylindole derivative and its subsequent reaction with a diketone to produce the desired product.

Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (Figure 1) is an oleoresin that is extracted from turmeric (*Curcuma longa L.).* Recently, research has been done with curcumin and its derivatives regarding antitumor and anticancer activities, such as with prostate cancer[1]. Most research regarding curcumin derivatives involves additions of carbon chains between the carbonyls, as in 4-ethoxycarbonylethyl curcumin (ECE Cur) as seen in Figure 1[1].

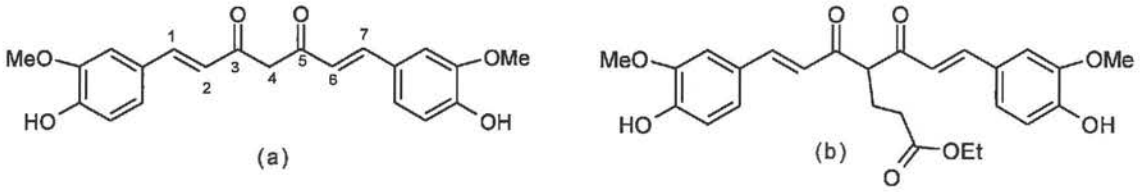

Figure 1: (a) Curcumin and (b) ECE Cur

The current goal is to come up with a synthesis for a derivative that attaches indole in a conjugate addition to the α,β-unsaturated carbonyls to form 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,7-bis(indolyl)-1,6-heptadiene-3,5-dione (Figure 2).

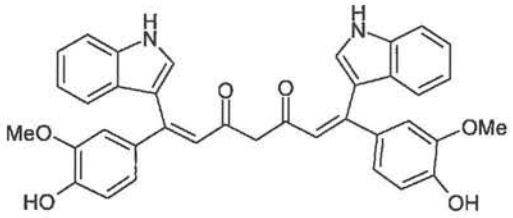

Figure 2: Target product, 1,7-bis(indolyl)curcumin

This study began with a focus on a direct addition of curcumin and indole rather than building up to the product from smaller pieces. Current research has been done using triflic acid to catalyze Michael reactions of indole with α,β-unsaturated ketones[2]. The indole attaches in the desired position, but the double bond, and therefore the stereochemistry, is lost.

The synthetic pathway first attempted was a one step C-C bond formation at the C1 and C7 positions of curcumin with the C3 of the indole. Zhang, *et al* conducted research in which they attached two indoles to 1,4-benzoquinone at points that are both α and β to the carbonyl carbon[3]. The "on-water" procedure in this research was carried out by stirring the two starting products in water for at least 10 hours. The product formed an aqueous suspension, and it was the reaction that was carried out in room temperature water that gave the highest yield. Due to the slight similarity of the structures of benzoquinone and curcumin as they relate to conjugated ketones, this procedure was implemented.

When the one step procedure was unsuccessful, a three step synthesis was devised based on the work described in two patents[4,5]. The three steps proposed complete the synthesis of a compound similar to the target compound and are as follows (all structures are shown in Figure 3):

- Part A: Preparation of N,N-diethyl-4-methoxybenzamide
- Part B: Preparation of 3-(4-methoxybenzoyl)indole

- Part C: Preparation of 1,7-bis(4-methoxyphenyl)-1,7-bis(indolyl)-1,6-heptadiene-3,5-dione

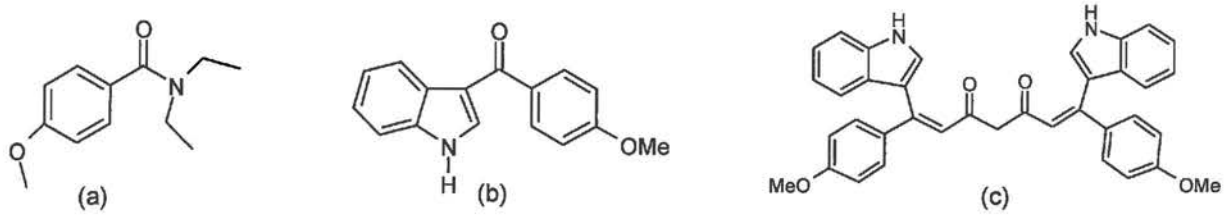

Figure 3: Structures for the products of parts (a) A, (b) B, and (c) C

The first of the studied patents explains the process by which acylindole derivatives of the formula in Figure 4a are synthesized. The R groups may be the same or different and their formula may be any of the following: a hydrogen atom, a lower alkyl group, a lower alkyl group substituted by one or more hydroxy groups, or an acyl group as in Figure 4b. Each alkyl group must have only 1-5 carbon atoms, and the $-OR_2$ must be a substituent at the o- or p-position. One process proposed was the reaction of indole with an amide compound that contained the corresponding acyl residue. The reaction took place in the presence of phosphorus oxychloride and heat[4]. Since the amide compound that corresponds exactly to 1,7-bis(indolyl)curcumin was not readily available or easily synthesized, a similar compound was made in the hope that it would lead to understanding of the actual desired synthesis. The needed amide was N,N-diethyl-4-methoxybenzamide, or N,N-diethyl-p-anisamide, and was synthesized using p-anisic acid (Part A). The acylindole synthesis is Part B. Altering the solvent and other reaction conditions may be necessary depending on the R_1 and R_2 groups that are used.

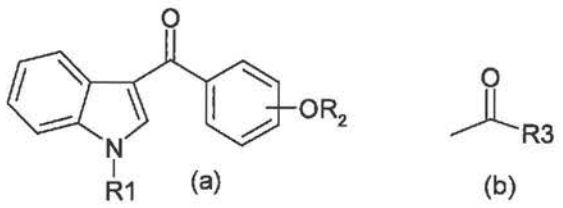

Figure 4: (a) Acylindole derivative structure (b) Possible R group

The second patent used to understand the three step process discussed the synthesis of curcumin-related compounds. The main idea of the invention is to take a 2,4-diketone and react it with an aromatic aldehyde in the presence of a catalyst. The reaction mixture is mixed with acid to precipitate the product[5]. It is proposed that using the acylindole ketone derivative made in Part B instead of the aldehyde will result in the formation of a compound of the formula in Figure 2. The tentative syntheses for Parts B and C are shown in Figure 5.

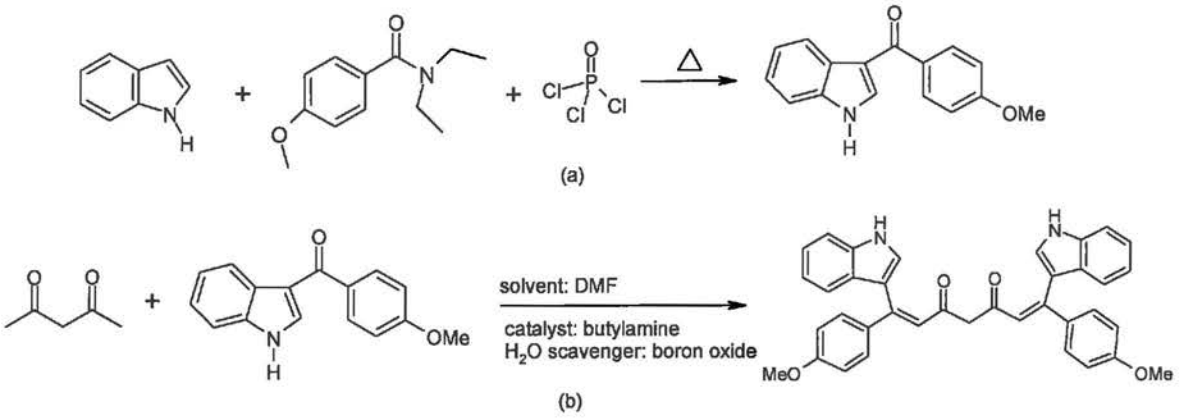

Figure 5: Syntheses for (a) Part B and (b) Part C of the 3 step procedure

Experimental

The Infrared (IR) spectra referenced were taken using a Thermo Electron Corporation Nicolet IR200 FT-IR. Mass spectrum data was collected using an Agilent Technologies 6890N Network GC System coupled with a 5973 inert Mass Selective Detector.

Procedure JK1

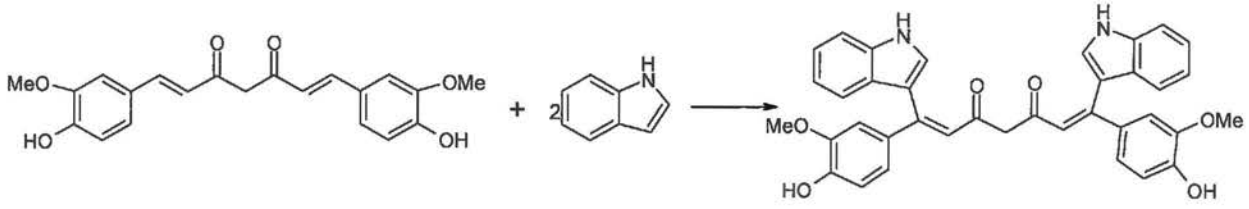

Figure 6: Procedure JK1

The one-step synthesis attempt (Figure 6) involved a 1:2 ratio of curcumin to indole. To a 50-mL Erlenmeyer flask were added 0.5523 g (1.50 mmol) curcumin and 0.3518 g (3.00 mmol) indole and 25 mL distilled water along with a magnetic stirbar. The flask was stoppered, sealed with Parafilm, and set stirring on a heating plate/magnetic stirrer. The solution was still light yellow with orange solid. The reaction was stopped 74.5 hours later. The orange solid settled to the bottom of the flask. The mixture was then filtered using vacuum filtration and a Büchner funnel and dried in air (product mass = 0.7198, 80.16% yield). IR peaks for the starting materials and product are as follows: curcumin (3500, 1509, 1280 cm^{-1}), indole (3402, 1337, 746, 724 cm^{-1}), product (3500, 3400, 1509, 1280, 745 cm^{-1}). When measuring the melting point of the product, part of the solid melted at 48-50°C. The rest of the solid dissolved in the melt and it was not possible to obtain the melting point. The crude product was washed in about 50-70 mL of water and dried by vacuum filtration (recovered product mass = 0.7194 g). An IR spectrum was taken for the rewashed product giving peaks at 3500, 3400, 1509, 1280, and 745 cm^{-1}.

In order to obtain a suitable solvent for the recrystallization of the product, the solubility of the starting materials was tested in ethanol, dichloromethane, water, and acetone.

Small samples of the starting materials were put into separate test tubes followed by a few drops of each solvent. The results are presented in Table I.

	Ethanol (EtOH)	**CH_2Cl_2**	**Acetone**	**Water**
Curcumin	mostly soluble; yellow; flakes on bottom; cloudy	Slightly soluble; Very cloudy, yellow	Soluble (mostly) more than EtOH; color change	No interaction w/ solvent; No color change; no dissolving
Indole	Soluble @ room temp	Soluble @ room temp	Soluble @ room temp	Hydrophobic; wouldn't mix

Table I: Results of solvent testing for recrystallization

The samples in ethanol and dichloromethane were placed in a hot water bath. When heated, the CH_2Cl_2 boiled off and left the cloudy solution. The ethanol solution became clear and all the curcumin dissolved. Based on the results in Table I, ethanol was solvent of choice for recystallization of the crude product. Hot ethanol was added, and the solution was a dark orange-red color. A small amount of solid initially dissolved, but even after much heating and addition of hot solvent, the solid did not dissolve further. This solution was left to sit at room temperature for about 8 hours and the entire solid precipitated out. The orange solid was filtered using a Büchner funnel. It was lightly washed with a few mL of cold ethanol and allowed to dry (0.2670 g). The IR spectrum was taken of this solid, showing major peaks at 3512, 1508, 1429, 1280, 1154, 964 cm^{-1} (See Appendix I).

Procedure JK2

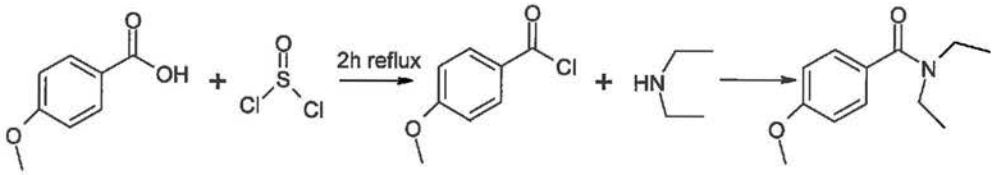

Figure 7: Procedure JK2A - Preparation of N,N-diethylanisamide

The next synthesis was a three step process, but only the first step (Figure 7) has been attempted. It was attempted four times: trials JK2A1 – JK2A4. In reaction JK2A1, 1.9711 g (12.95 mmol) p-anisic acid (white solid) and 8.00 mL (0.1097 mol) $SOCl_2$ were measured into a 25-mL round-bottom flask. A condenser was attached and the apparatus was set on a heater/stirrer. As the mixture refluxed, the solid disappeared and a clear liquid was left. In a separate beaker, 10.00 mL (0.0967 mol) of diethylamine were set to cool in an ice bath. After the beaker was cool, the refluxing mixture was taken and added to the amine. As it was added, the mixture reacted vigorously with a cloud of black/gray smoke. There was a black solid/mixture in the beaker and on the flask.

The second time the reaction was attempted (JK2A2), 1.9794 g (13.01 mmol) p-anisic acid was placed in a 100-mL round-bottom flask and approximately 15-20 mL methylene chloride was added. The mixture was a thick milky white. While stirring the mixture, 8.00 mL (0.1097 mol) $SOCl_2$ was added slowly. The condenser was then set up, and the mixture was heated to reflux. During that time, a separate beaker with 10.00 mL (0.0967 mol) diethylamine and 15 mL CH_2Cl_2 was set in ice to cool. After 35 minutes of refluxing, the mixture in the flask was slightly less cloudy. The reflux mixture was cooled to room temperature and added dropwise to the beaker of cold solution. White smoke evolved with each drop, and a hiss was heard as well. While the mixture was being added, yellow/green crystals formed on the side of

the beaker. A white solid formed as well. Another 10 mL CH_2Cl_2 was added. The solution turned a brown/green. After the presumed acid chloride solution was added, the resulting mixture was stirred and the solid was collected using vacuum filtration. It was washed with cold CH_2Cl_2. The mass of the crude product was 2.4253 g (assumed yield: 89.93%). The IR spectra of the starting material and of the product gave the following peaks: p-Anisic acid (broad 3000-2500, 1682, 1603, 1299, 1259 cm^{-1}), crude solid (2973, 2822, 2776, 2482, 1466 cm^{-1}). To test if the solid was the hydrochloride salt of the desired amide, a small portion was dissolved in water in a test tube. The pH of the solution was between 6 and 7.

The product was divided into two 100-mL beakers with 1.5027 g in one (beaker 1) and 0.8357 g in the other (beaker 2). To beaker 1 was added 30 mL of water but the solid did not completely dissolve. The pH of the solution was approximately 5. An attempt was made to recrystallize the solid in beaker 2 using water but the solid would not completely dissolve in hot solvent. The product in the beaker 1 sample was extracted using CH_2Cl_2. This organic layer was dried with anhydrous sodium sulfate and the solvent was evaporated leaving nothing in the flask. The reaction was then restarted because neither the recrystallization nor the extraction was successful.

For the following two reaction attempts, the syntheses that were implemented resembled those in two articles in which the same amide was synthesized[6,7]. For reaction JK2A3, the solvent used was CH_2Cl_2. To a 100-mL round-bottom flask was added 1.0252 g (6.74 mmol) p-anisic acid, 1.00 mL (13.74 mmol) $SOCl_2$, and 20 mL CH_2Cl_2. The milky white mixture was refluxed for 2 hours. In a separate beaker, 2.10 mL (20.31 mmol) diethylamine was mixed with 15 mL CH_2Cl_2 and set to cool. The refluxed solution was cooled to room temperature and

the amine mixture was added dropwise to the flask. White smoke evolved and the solution turned a clear, light yellow. After stirring overnight, 15 mL CH_2Cl_2 was added to the flask and the solution was washed 3 times with 50 mL portions of water, once each with 50 mL of 0.1 M HCl, 50 mL of 0.1 M NaOH, and 50 mL saturated NaCl. The organic layer was dried with anhydrous sodium sulfate and the solvent was then evaporated off. A dark yellow/brown liquid remained but did not solidify at room temperature. The flask was stoppered and placed in a refrigerator overnight. The IR spectra of the product gave the following peaks: Liquid product (1768, 1736, 1600, 1508, 1252, 1166, 1027 cm^{-1}). After three days, a white product appeared in the flask. The mass spectra for the compounds detected in the sample contained the following peaks:

Peak	Time (min)	M^+	Significant mass/charge peaks
1	13.953	166.1	77.1, 92.0, 107.1, 123.1, 135.1
2	14.101	170.1	77.0, 92.0, 107.1, 135.1, 142.0, 152.0
3	14.657	152.0	77.0, 92.0, 107.0, 128.9, 135.0
4	14.868	180.1	77.0, 121.0, 135.0, 152.0, 165.0
5	18.513	206.2	77.1, 120.1, 135.1, 164.1, 178.1, 192.1

Table II: Mass Spectrum Data for the Solid Product in Trial JK2A3 Dissolved in Methanol (See Appendix I)

The fourth attempt at the synthesis (JK2A4) used diethyl ether as the solvent. To a 100-mL flask was added 1.0193 g (6.70 mmol) p-anisic acid, 1.00 mL (13.71 mmol) $SOCl_2$, and 20 mL ether. The mixture was refluxed for 2 hours and had a white solid in it. Into a 100-mL beaker, 2.10 mL (20.31 mmol) diethylamine was mixed with 15 mL ether and cooled. The amine solution was added dropwise to the flask after both were cool. White smoke evolved and a large amount of white solid was seen. The mixture was transferred to a 150-mL beaker using ether. First, 50 mL of water were added and the mixture stirred, then 50 mL of ether were

added until the solid dissolved. The product was extracted from the aqueous layer with ether. The combined ether extracts were washed with 0.1 M HCl, 0.1 M NaOH, and saturated NaCl. The organic layer was then dried with anhydrous sodium sulfate and the ether was evaporated off. No solid was present in the beaker once the ether had evaporated. A white solid precipitated out in the aqueous washes.

Results & Discussion

When compared to the spectrum for curcumin, the crude product JK1's IR spectrum had 2 major differences: added peaks at 3400 and 745 cm^{-1}. These two wavenumbers correspond to the two main peaks in the indole spectrum, indicating that the solid tested had indole in it. Two possibilities were that the solid was indeed the target product or that the unreacted indole had not been completely washed out. When attempting to measure the melting point, it was found that the product was a mixture because the indole melted first (at about 48-50°C) and then the curcumin seemed to dissolve in the indole making it impossible to obtain correct data. After being washed with water, the product's spectrum didn't change from the original crude product.

During the recrystallization, the solid didn't completely dissolve in the ethanol because the solubility in hot solvent was less than what was anticipated. The yellow solution and the light fluorescent green on the test tube were characteristic of curcumin[8]. The indole that was mixed in the solid did dissolve in the ethanol and allowed for a higher purity sample. The IR spectrum of this more pure solid was noticeably missing the peaks at 3400, 746, and 724 cm^{-1} that distinguish indole. Therefore, it was determined that procedure JK1 was unsuccessful as far as forming 1,7-bis(indolyl)curcumin.

Part A of the three step synthesis, preparation of N,N-diethylanisamide, was the only part that was physically implemented. The first two tries failed due to technique problems. In the first attempt, the reaction was carried out without solvent, and with reactants in excess. This caused a more vigorous reaction. The second attempt also had reactants in excess which caused the product that was formed to be completely hidden by side reactions. The peaks for the white solid product didn't match what should appear if N,N-diethylanisamide was actually formed. It lacked a significant carbonyl/amide peak as well as the peak for the sp^3-hybridized hydrogens on the ethyl groups. The 1682 and 1259 cm^{-1} peaks on the p-anisic acid spectrum, which correspond to the carboxylic acid and methoxy functional groups are both gone in the spectra for the second and third products (both solid and liquid samples). Testing the pH of the JK2A2 product dissolved in water would indicate whether or not it was a salt. If an ammonium salt had been formed, the solution would have been more acidic than it was.

The mass spectrum for the JK2A3 sample (Appendix I) indicates that there was a little starting material present as well as its corresponding acyl chloride. Three peaks that were common at every elution time were those with a mass to charge ratio (m/z) of approximately 77.0, 107.1, and 135.1. These peaks correspond to possible fragments of p-anisic acid and the desired product as seen in Figure 8. The mass spectrum for peaks 1 and 4 most closely match those of the methyl and ethyl esters of p-anisic acid, respectively. The second peak has a mass spectrum that resembles that of p-anisoyl chloride, the acid chloride that is formed during the reaction with $SOCl_2$. This indicates that the intermediate reaction did occur, and that any complications that arose were related to the wrong molecules reacting with the acyl chloride. The third spectrum matched p-anisic acid, which shows that the reaction did not completely

take place, and starting material was left in the sample. The M^+ ion for peak 5 is close to the molecular weight of N,N-diethylanisamide (207.2748 g/mol), but it is slightly too low, and the other peaks do not allow for the fragmentation that would be expected.

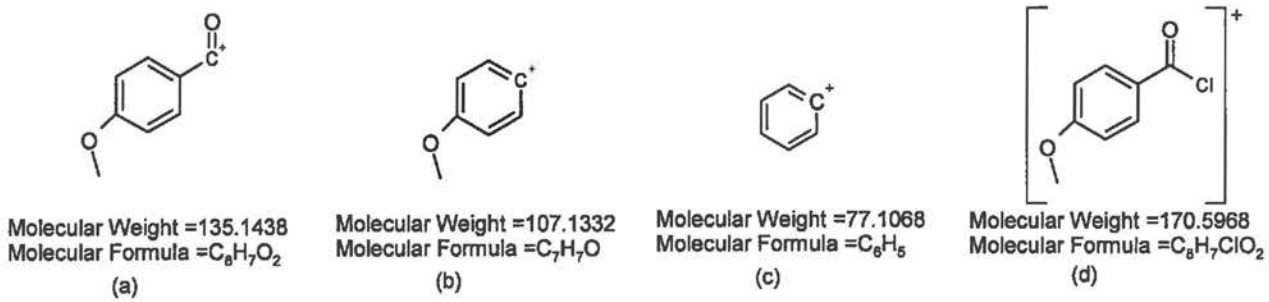

Figure 8: (a), (b), (c) Possible fragments of p-anisic acid and (d) the M^+ ion of anisoyl chloride

In the last two trials, a white solid precipitated out during the aqueous washes. A possible explanation for this occurrence is that the reaction did not take place at all, and the assumed product was actually the starting material. If the solid that was washed was a carboxylic acid, its movement from the organic layer to the aqueous layer during the washes with HCl and NaOH would be explained.

Further research can be done to try and form 1,7-bis(indolyl)curcumin directly from curcumin and indole. Utilizing the triflic acid catalyzed procedure[2] yields a product with no double bonds at carbons 1 and 6. If a leaving group, such as a halogen, was attached to either carbon 1 or 2 and either carbon 6 or 7, then two elimination reactions would have a 50% chance of forming the bis product (Figure 9). Other progress could be made in examining the reactivity of both reactants in acidic and basic environments. Using a deprotonated indole as a nucleophile might have some relevance. If a one step synthesis is not viable, then study should move to a multistep synthesis starting from smaller building blocks such as indole-2-carboxylic acid and 4-chloro-2-methoxyphenol (Figure 10).

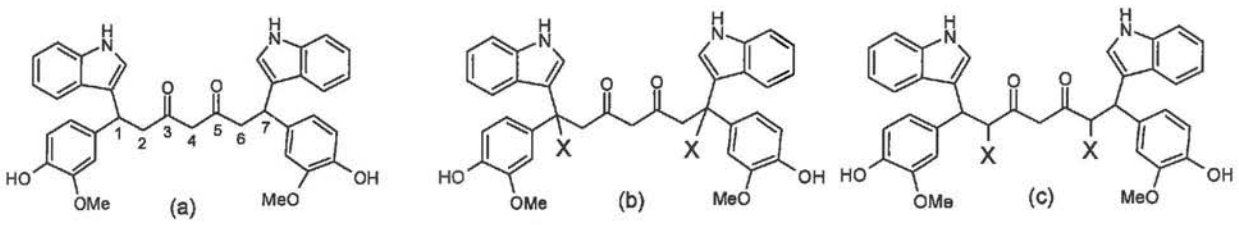

Figure 9: (a) 1,7-bis(indolyl)curcumin without double bonds at carbons 1 and 6. Elimination reactions are possible with halogens at (b) carbons 1 and 6 or (c) carbons 2 and 7.

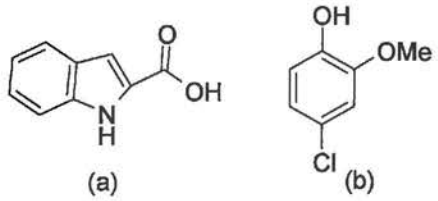

Figure 10: (a) indole-2-carboxylic acid and (b) 4-chloro-2-methoxyphenol

As far as a multistep procedure, the one proposed by this research, if successful, can be used to make a variety of curcumin derivatives. Part C, the reaction with 2,4-pentanedione, can be carried out with different aldehydes and ketones that contain acyl and indole residues that match the desired product. Derivatives with different alkoxy and acyl substituents can also be prepared. Further study must be done into the conditions necessary for these syntheses. Because most of these derivatives have not been made previously, it is necessary for the conditions of the documented reactions to be changed slightly to account for the different functional groups and structures that are being formed. Also, if the reaction in step three is as successful with a ketone as with an aldehyde, the door is opened for another class of curcumin derivatives.

References

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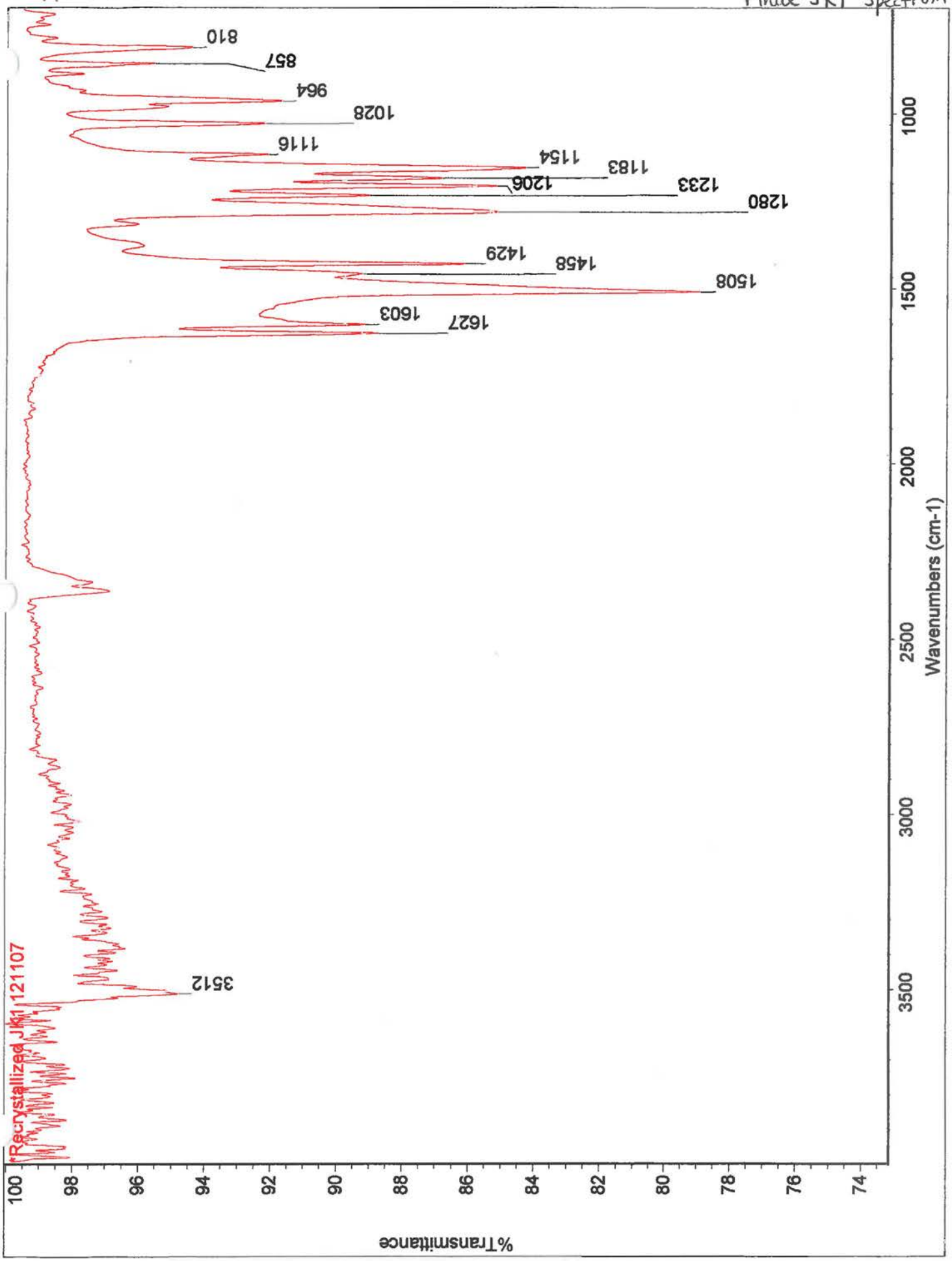
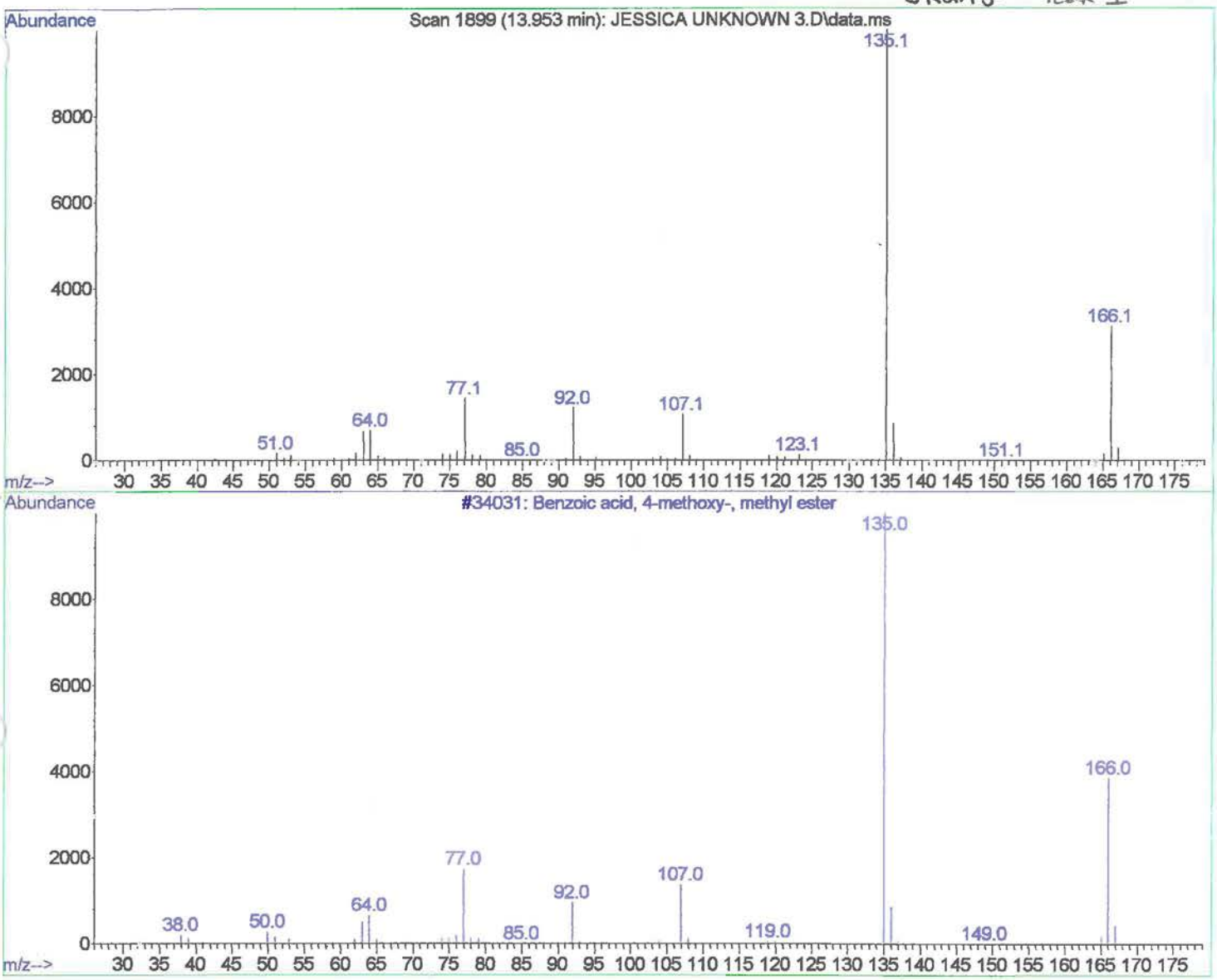
Appendix I

Final JK1 Spectrum



Library Searched : C:\Database\NIST05a.L
Quality : 97
ID : Benzoic acid, 4-methoxy-, methyl ester

JK2A3: Peak 1



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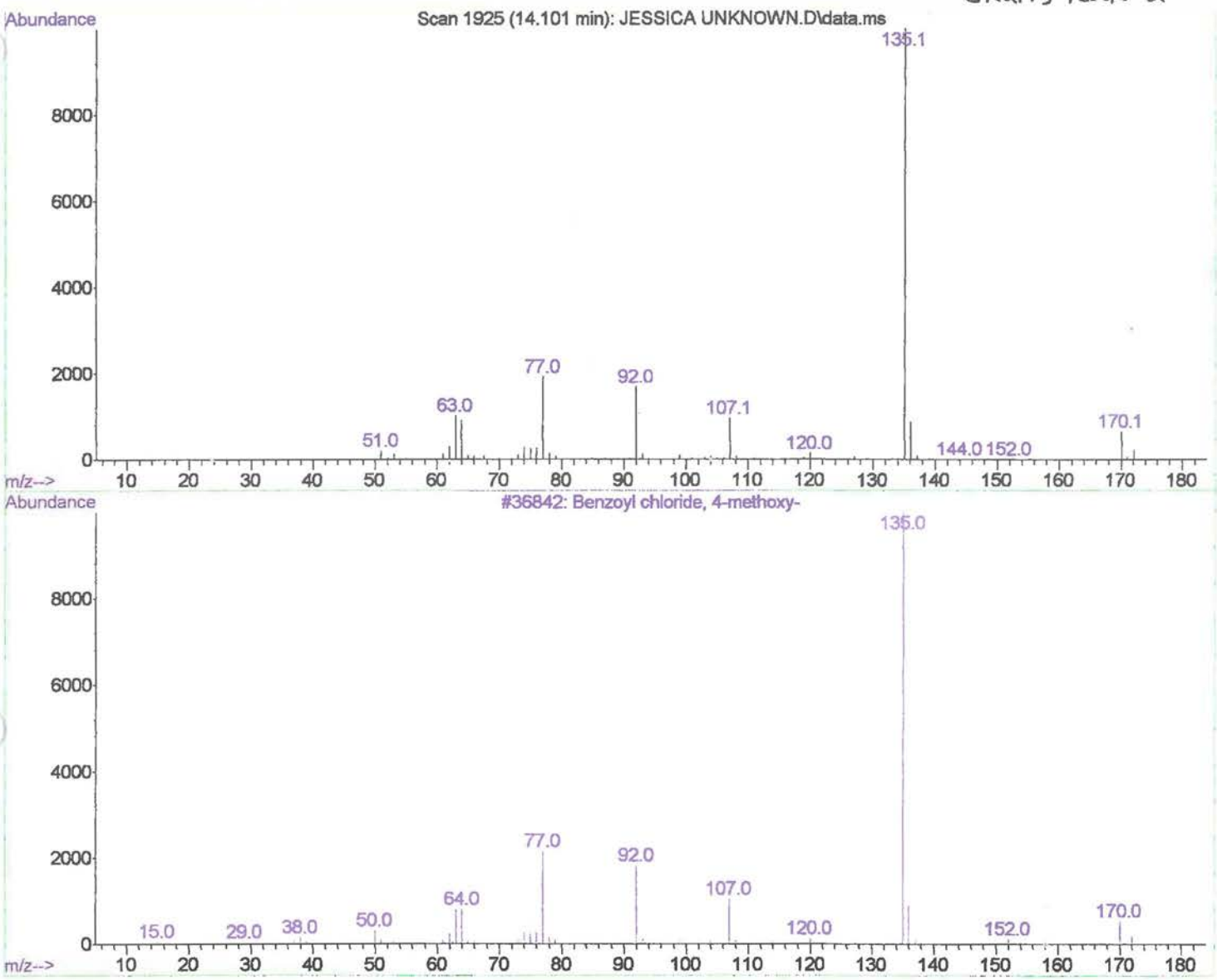
Quality : 95
ID : Benzoyl chloride, 4-methoxy-

JK2A3: Peak 2



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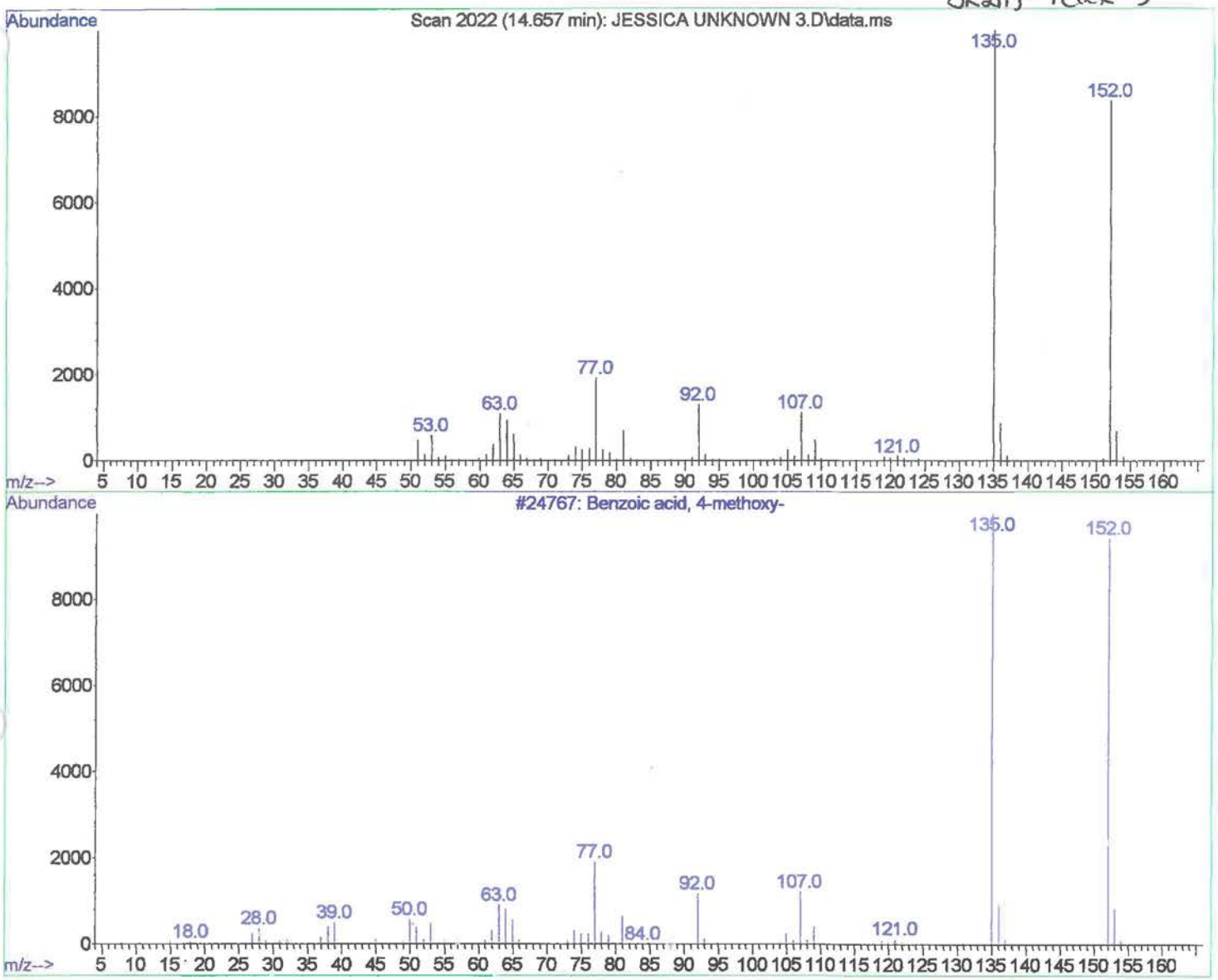
ID : Benzoic acid, 4-methoxy-

JK2A3: Peak 3



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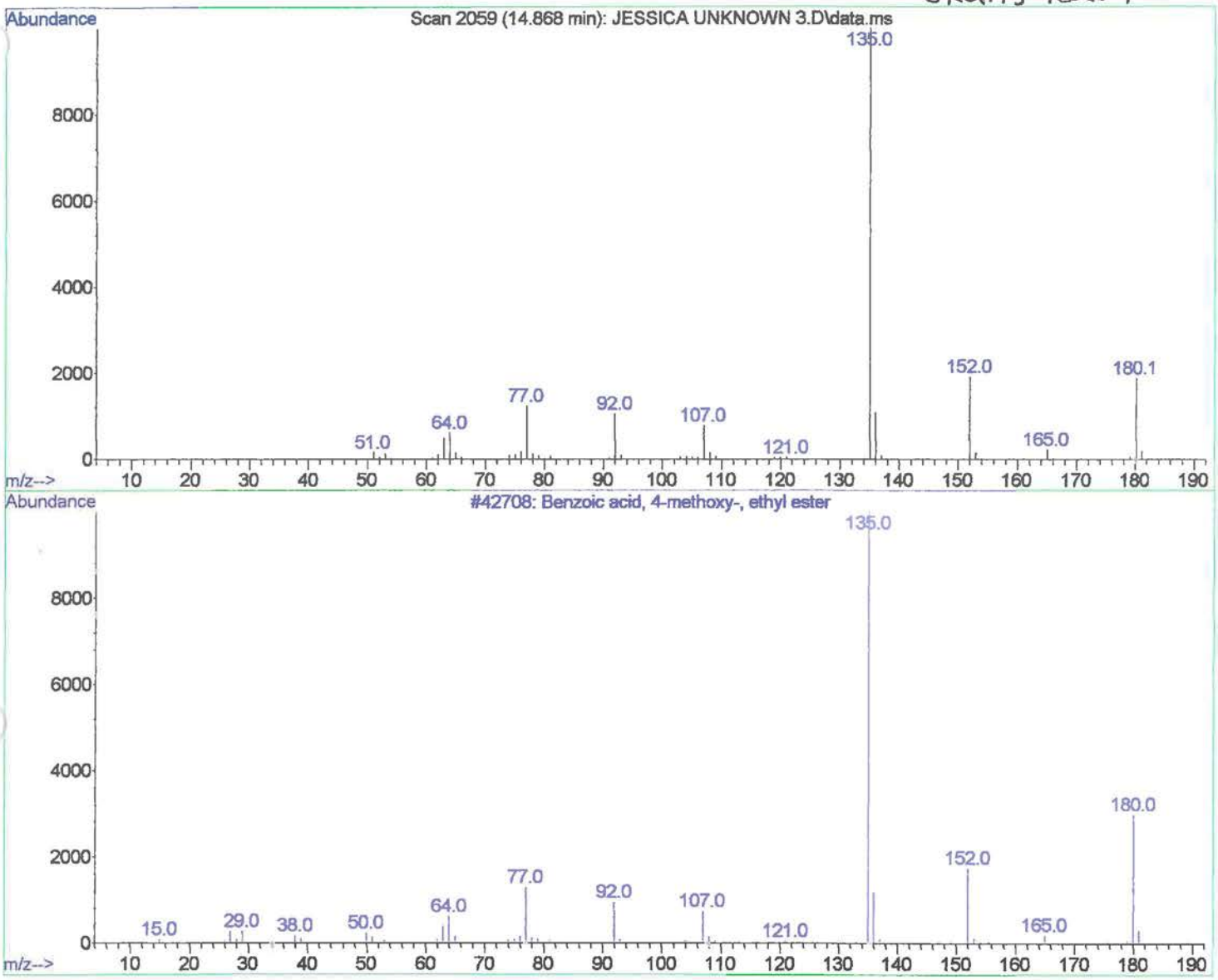
JK2A3: Peak 4



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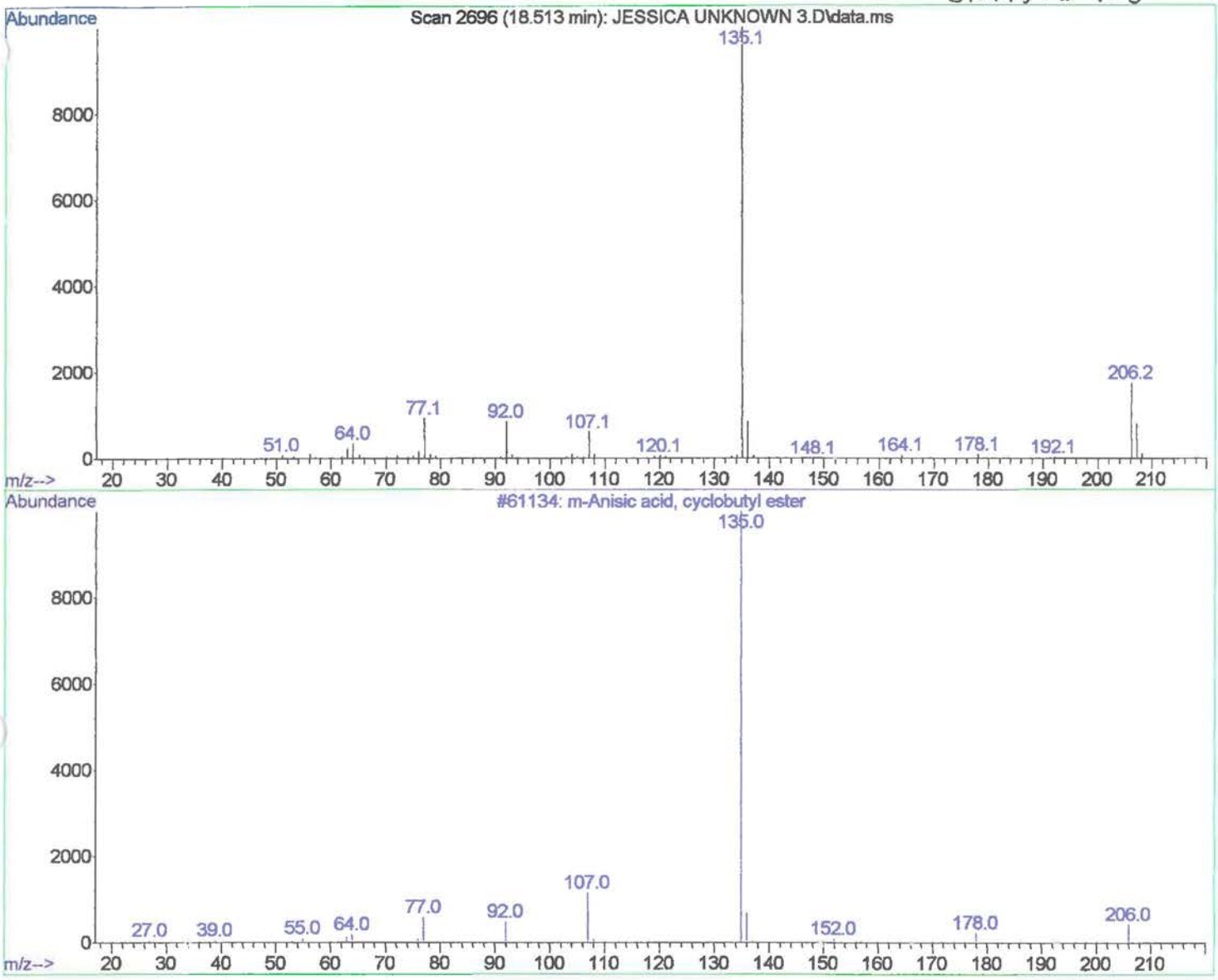
ID : m-Anisic acid, cyclobutyl ester

JK2A3: Peak 5



Southern Scholars Honors Program
Senior Project Proposal Information Sheet

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Name Jessica Kisunzu Date 9/21/07

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A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. The project is expected to be of sufficiently high quality to warrant a grade of "A" and to justify public presentation.

Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor four weeks prior to the last day of class for the semester the project is turned in. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is usually done as directed study or in a research class.

NOTE-Senior Project Proposal Due Date: The senior project proposal is due in the Honors Program Director's office two weeks after the beginning of the semester the project will be completed. The proposal should be a detailed description of the Honors Project's purpose and proposed methodology.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. Attach a separate sheet of paper.

Signature of faculty advisor Brent Hamrick

Expected date of completion 3/17/08

NOTE: An advisor's final project approval does not guarantee that the Honors Faculty Committee will automatically approve the project. The Honors Faculty Committee has the final vote.

Approval to be signed by faculty advisor when the project is completed:

This project has been completed as planned (date) 4/1/08

This is an "A" project yes

This project is worth 2-3 hours of credit yes

Advisor's Final Signature Brent Hamrick Date: 4/1/08

Chair, Honors Committee Mark Peach Date Approved: 1 May '08

Dear Advisor,

(1) Please write your **final** evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.

(2) Please include a paragraph explaining your specific academic credentials for advising this Senior Project.