N-Substituted Azaaurones: Synthesis and Photochemistry

by
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Abstract

Aurones are a sub-family of natural compounds in the flavonoid family found in plants. Recent research has shown progress in the synthesis of aurones and their therapeutic potential as anti-cancer, anti-fungal, anti-microbial, and anti-inflammatory agents. Observations have found that substituting functional groups and switching between E and Z isomers of aurones can optimize the activity of the compound. This research attempted the synthesis of the azaaurone 2-(4-Methylbenzylidene)-1,2-dihydro-3H-indol-3-one. This parent azaaurone was successfully substituted at the intracyclic nitrogen through N-alkylation and N-acetylation. The possibility of reversibly photoisomerizing between the Z and E isomers of each compound was also studied with the intention of opening new options for photoswitches and to explore the biological application of these isomers. The absorption spectra were identical for each isomer, so it was concluded that this compound was not ideal for reversible isomerization.

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Introduction:

Aurones are a sub-family of natural compounds in the flavonoid family found in plants.[1] They act as secondary metabolites that produce a yellow color in fruits and flowers. They are structural isomers to flavones, but have been less studied for their therapeutic potential.[2] Recent research has shown progress in the synthesis of aurones and their therapeutic potential as anti-cancer, anti-fungal, anti-microbial, and anti-inflammatory agents.[3]

Different aurone analogs can vary in their biological properties. Biological effects of compounds can be predicted by comparing their molecular structure to similar compounds.[4] This structure-activity relationship (SAR) has been applied to aurone analogs, as well. Of note to this work is the observation that SAR analysis has found that aza analogs of aurones, can potentially increase biological properties. These azaaurones, which substitute the intracyclic oxygen atom of aurones with an N—H group, as shown in figure 1, have led to more active analogs. In one key study, the N—H replacement led to an increase in antiplasmodial activity for azaaurones compared to aurones for potential treatment of malaria.[5]

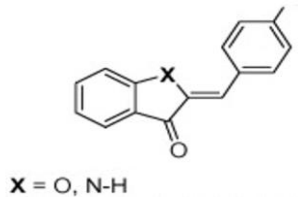


Figure 1. Azaaurone structures contain N—H at the X location instead of an oxygen as found in aurones

Azaaurones can be further substituted to create a variety of derivatives, with the nitrogen allowing for addition of different substituents.[6] This new point of substitution offers a second means to affect the properties of the compound, including solubility, biological activity, and absorption in the electromagnetic spectrum.[5, 7] Synthesizing azaaurones and substituting functional groups has the ability to optimize the activity of an azaaurone.

Although aurones can exist as either E or Z geometric isomers, as shown in figure 2, most aurones are typically formed as Z isomers, making them more abundant.[8] Previous research has calculated the heat of formations for E and Z aurone isomers and found Z-isomers to be generally more thermodynamically stable, making the formation of the Z- isomer more likely.[9] E and Z isomers can usually be distinguished from one another using ^{1}H-NMR. The aurone's NMR signal for the alkene proton in aurones in the Z configuration is more shielded than it is in the E-isomer, shifting its peak upfield.[7]

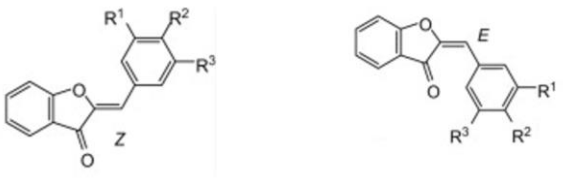


Figure 2. The aurone is more thermodynamically stable as the Z isomer on the left than the E isomer

Azaaurones can potentially be photoisomerized to convert E- or Z- isomers to the other configuration. Photoisomerization converts one isomer to another using light, as shown in figure 3. A compound, like an aurone, absorbs radiation from light, converting the structure from its Z configuration to its E configuration (or from E to Z). [10] Light breaks the pi bond of a double bond to make an unstable diradical intermediate with a single bond between the two carbons and an unpaired electron on each carbon. Free rotation can occur around the single bond and the two unpaired electrons can then pair up to reform the double bond, resulting in isomerization to a different configuration. The process is usually reversible.

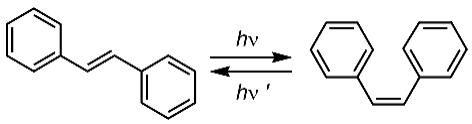


Figure 3. This shows the photoisomerization of one isomer to the other

Being able to photoisomerize azaaurones between their E- and Z-isomers could be used in various applications. In one study, the antioxidant activity of aurone **1** was measured for its E and Z configurations, shown in figure 4. It revealed that the Z-isomer had better activity and higher potency than most commercial antioxidants, like Vitamin C.[8] Photoisomerization can also affect antibacterial activity of aurones. For hydroxyaurones, the Z-configuration had high levels of antibacterial activity in both Gram-positive and Gram-negative bacteria, while the E-isomer had relatively little antibacterial activity.[8]

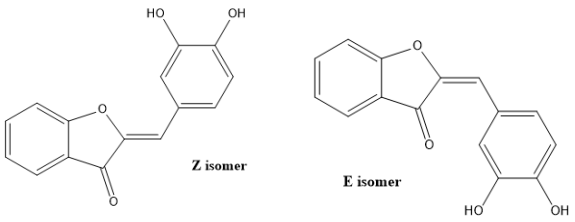


Figure 4. The Z and E isomer of aurone **1**

Photochemical interconversion of aurone isomers has also been studied as a possible method to synthesize photoactivated switches and fluorescent biological probes.[7] A photoswitchable molecule is a molecule that can undergo a reversible change in its structure after being irradiated with light.[11] Synthetic photoswitches are of interest to researchers, because light can easily be spatially and temporally controlled.[12] It is noninvasive (at certain wavelengths) and can have its wavelength and intensity be manipulated.

Recent studies have shown that photoswitches can have multiple uses. In one study, it was shown that the difference in energy between cis- and trans- azobenzenes after photoisomerization in proteins can allow photochemical control of protein folding.[11] Photoswitchable units have also been inserted into DNA strands for reversible control of gene transcription, RNA splicing, and DNA enzymes.[13] Photocontrol of peptide-based materials, like polymers, gels, and fibers has also been studied to change the materials' properties.

Being able to convert an isomer to its more active and useful form through photoisomerization, would make the synthesis of azaaurones more efficient, although most azaaurones are synthesized as primarily the thermodynamically more stable Z-isomer. Should the E-isomer prove to be more beneficial, photoisomerization will be essential and open new treatment options.

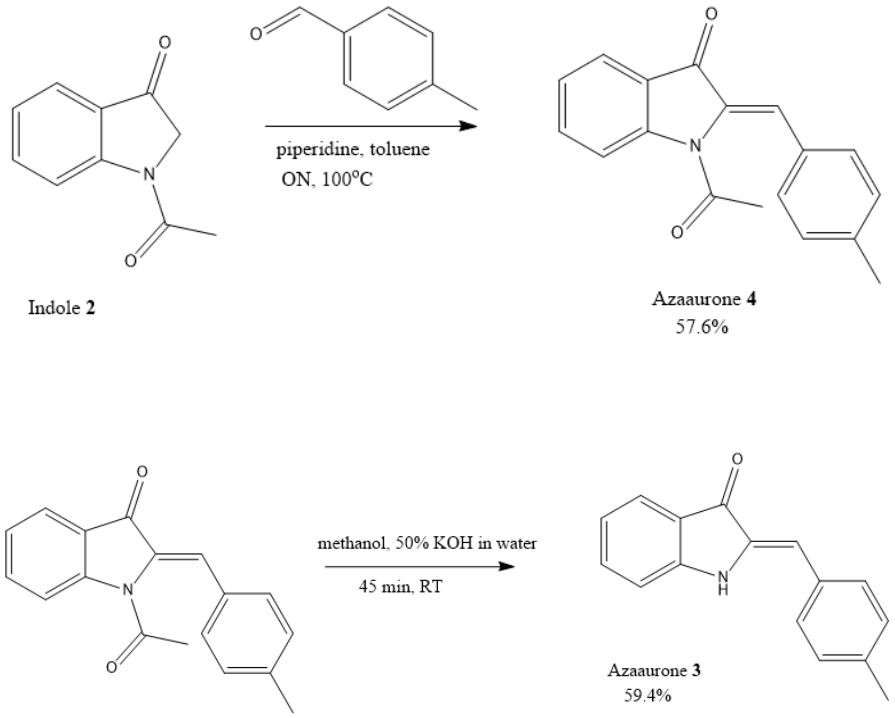
Objective: The goal of this research was to synthesize N-substituted azaaurone analogs that can be studied for reversible photoisomerization between the Z and E isomers with the isomerization being intended to open new options for photoswitches, as well as to explore the biological application of these isomers.

Chapter II: Results and Discussion

The overall goal of this project was the successful synthesis of N-substituted azaaurone analogs, with the intention of studying them for reversible photoisomerization. The synthetic routes taken followed previous literature procedures for N,N'-disubstituted indigo molecules, and were fairly successful for the azaaurone analogs mentioned.[14]

Parent azaaurone synthesis

The parent azaaurone that was synthesized and used to prepare the other N-substituted azaaurones was 2-(4-Methylbenzylidene)-1,2-dihydro-3H-indol-3-one **3**. This azaaurone **3** was prepared in two steps, a condensation step and deacetylation step, from commercially available starting materials. For the condensation, indole **2** and p-tolualdehyde were combined, heated to 100^{o}C in the presence of catalytic piperidene overnight, as shown in scheme 1. The desired product **4** was purified using flash column chromatography on silica with 5 to 10% ethyl acetate in hexane as eluent to afford the product in 59.4% yield. To deacetylate the compound, the literature procedure of Dr. Marta Carrasco was followed using a combination of methanol and 50% potassium hydroxide in water.[15] The mixture was stirred at room temperature for 45 minutes. Following acidification, a standard aqueous work-up, and column chromatography with 5 to 10% ethyl acetate as eluent, the target azaaurone **3** was isolated in 57.6% yield. The identity and purity of the product was confirmed using ^{1}H NMR and ^{13}C NMR. The yields for this compound was probably higher than what was collected after purification as this compound was difficult to purify, and required several rounds of flash column chromatography before pure product was collected.



Scheme 1. Synthesis of azaaurones **3** and **4**

The procedure for the parent azaaurone synthesis was changed from initial attempts. Previous attempts used reaction conditions that were successful but produced a very low yield of impure products. When this parent aurone was used as the starting material for the other N-substitutions, it resulted in even lower yields of the products. Changing the reaction conditions for the synthesis of azaaurone **2** has improved the yields and impurities for all subsequent alkylation products.

N-substitution of azaaurone

Next, the parent azaaurone was substituted at the intracyclic nitrogen. In this step, the compound gained a functional group through N-acetylation, N-alkylation and N-sulfonation

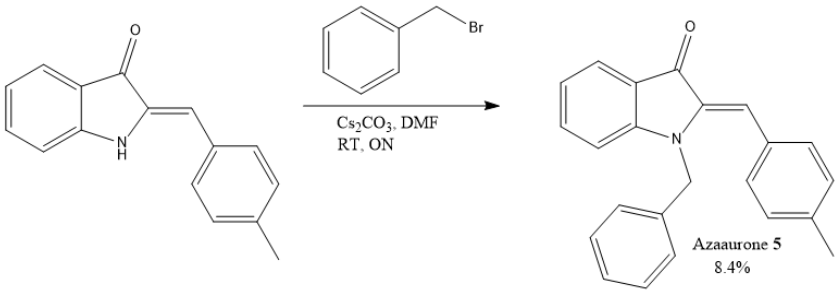
by following literature procedures.[14] The N- groups that were selected were chosen to evaluate their pi stacking and steric interactions.

N-acetylated azaaurone

The N-acetylated azaaurone **4** was obtained as the product of the first step of the method used to synthesize the parent azaaurone **3**, as shown in scheme 1. The azaaurone **4** was isolated in 57.6% yield. The identity and purity of the product was confirmed using ^{1}H NMR and ^{13}C NMR. The acetyl group was studied to evaluate electrostatic and steric pi-pi interactions with the tolyl ring.

N-alkylation of parent azaaurone with benzyl bromide

For alkylation of azaaurone **3**, the desired alkyl bromide as added along with cesium carbonate, and a few drops of DMF for solubility in a vial, and stirred at room temperature overnight, as shown in scheme 2. Following extraction with ethyl acetate and water, the crude product mixture was purified using flash column chromatography with 5-10% ethyl acetate in hexanes as eluent. The product was still not completely pure, so further purification was performed using preparative TLC with 10% ethyl acetate in hexane. At this point, the desired product azaaurone **5** was obtained with an 8.4% yield and in sufficient purity as determined by ^{1}H NMR spectroscopy. The benzyl group was chosen, due to its chance of having pi-pi stacking interactions between the pi electrons in the aromatic rings of the benzyl group and the tolyl ring.



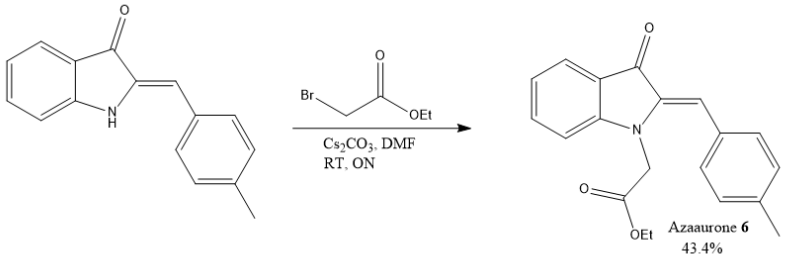
Scheme 2. N-alkylation of parent azaaurone with benzyl bromide for azaaurone **5** synthesis

The yields for the N-benzyl compound was probably higher than what was collected after purification. This compound was difficult to purify, and required several rounds of flash column chromatography before pure product was collected. The percent yield for the N-benzyl yield was 1.6% after column chromatography. The reaction was run again and purified through preparative TLC, which improved the yield slightly to 8.4%.

N-alkylation of parent azaaurone with ethyl acetyl group

A different alkyl group, an ethyl acetyl group, was added in much the same fashion. Azaaurone **3** was combined with ethyl bromo acetate added with cesium carbonate, and a few drops of DMF for solubility in a vial, and stirred at room temperature overnight, as shown in scheme 3. After an extraction using ethyl acetate and water, the crude product mixture was purified using flash column chromatography with 10 to 20% ethyl acetate in hexane as eluent. Then, the desired product azaaurone **6** was obtained with sufficient purity as determined by ^{1}HNMR and ^{13}CNMR spectroscopy with 43.4% yield. The ethyl acetyl

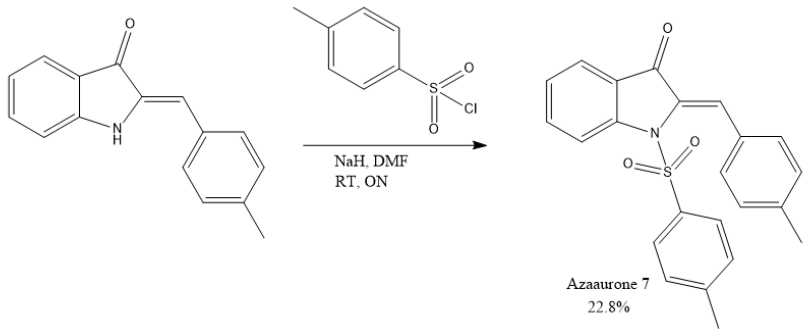
group was selected because of the increase in steric hindrance it would cause in the azaaurone structure.



Scheme 3. N-alkylation of parent azaaurone with ethyl acetyl group for azaaurone **6** synthesis

N-alkylation of parent azaaurone with tosyl group

A different group that still contains an aromatic ring, a tosyl group, was added to the parent azaaurone using similar chemistry. Azaaurone **3** was combined with p-toluenesulfonyl chloride, sodium hydride, and a few drops of DMF for solubility in a vial, and stirred at room temperature overnight, as shown in scheme 4. Following an extraction using ethyl acetate and water, the crude product mixture was purified using flash column chromatography with 5 to 20% ethyl acetate in hexane as eluent. The desired product azaaurone **7** was isolated with 22.8% yield and with sufficient purity as determined by ^{1}HNMR and spectroscopy. The tosyl group was chosen due to the pi-pi stacking interactions that could occur between the pi electrons in the aromatic rings of the tosyl group and the tolyl, although with different geometric preferences than the previous benzyl group.



Scheme 4. N-alkylation of parent azaaurone with tosyl group for azaaurone **7** synthesis

N-acylation of parent azaaurone with benzoyl group

When using the same procedure to perform an acylation reaction using the parent azaaurone and benzoyl chloride, the benzoylated azaaurone was not formed. Several attempts and changes were made to find conditions that would produce the substituted product, but only starting material was recovered after each attempt.

The benzoyl group was chosen again due to the pi-pi stacking interactions that could occur between the pi electrons in the aromatic rings of the functional group and azaaurone. Although the tosyl and benzoyl groups would have similar pi-pi interactions, they differ in their polarization of their pi systems and in their preferred geometries.

Ultraviolet-visible spectroscopy

After the synthesis of each compound, ultraviolet-visible spectroscopy was collected for each sample. A 100 µM stock solution for each compound was prepared in acetonitrile.

One two-fold dilution was done to get a solution of 50 µM. This concentration gave an absorbance within the linear dynamic range when collecting the UV/Vis spectrum.

Photoisomerization

To switch between the E and Z isomer of each sample, some initial efforts were made using sunlight. Although promising, it was rapidly decided that a more reliable and reproducible option would be the use of a blue LED grow lamp. Each sample was dissolved in chloroform-d in an NMR tube and then exposed to selective irradiation at a wavelength range of 450- 460 nm to undergo photoisomerization. The light was placed 13.5 cm from each sample. Isomerization was monitored over time (every 30 minutes initially, and then with longer gaps as determined by the initially observed rate) by ^{1}H NMR to determine when the photostationary state was reached. The signals were observed and compared to see if the signals' strength and integrated intensities changed over time. When the signals no longer changed, the photostationary state of the isomeric mixture was achieved.

For azaaurone **5**, the observed signal at δ7.89 saw an increase in their integration over 2 hours, as shown in table 1. The peak was compared to the peak δ7.77 to see the change in signal strength over that time period, as seen in figure 5. The observed peak shows the signal for proton **A,** shown in figure 6. The change in integration showed that the benzyl azaaurone compound was originally synthesized as a mixture of both Z and E isomers, but irradiation caused the isomerization into the E isomer.

	δ7.89 : δ7.77
0 min	0.6:1
30 min	1.7:1
60 min	1.9:1
90 min	1.8:1
120 min	1.9:1

Table 1. Benzyl azaaurone **5** integration ratios of observed signals during photoisomerization

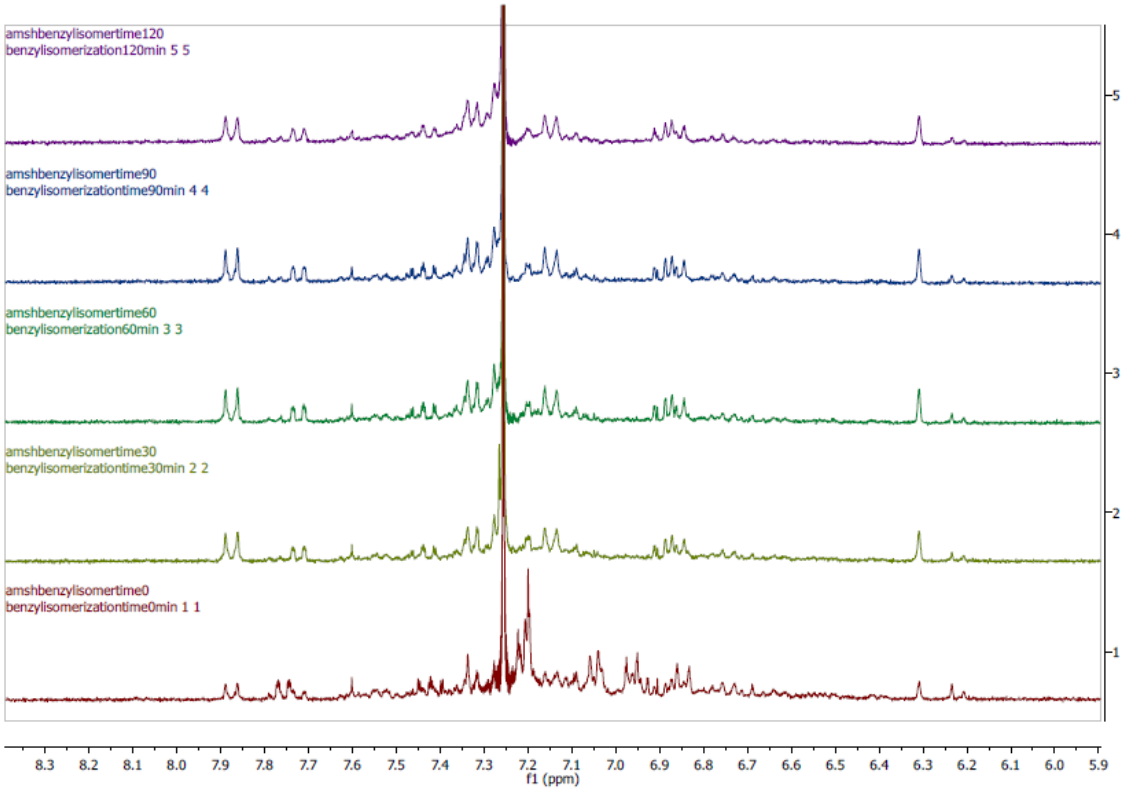


Figure 5. ^{1}H NMR of benzyl azaaurone **5** during photoisomerization

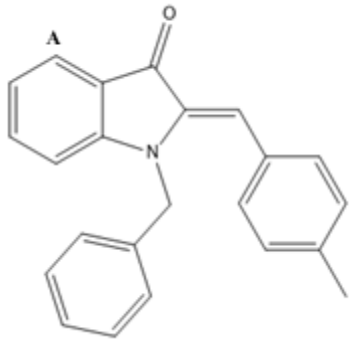


Figure 6. Observed proton on benzyl azaaurone **5** during isomerization

For azaaurone **6**, the observed signal at δ7.94 saw an increase in its integration over 2 hours, as shown in table 2. The peak was compared to the peak δ7.78 to see the change in signal strength over that time period, as seen in figure 7. This peak shows the signal for proton B in figure 8. The change in integration showed that the ethyl acetyl azaaurone compound was originally synthesized as mostly the Z isomer, but irradiation caused the majority of the product to isomerize into the E isomer.

	δ7.94 : δ7.78
0 min	0.4:1
30 min	0.9:1
60 min	1.5:1
90 min	2.1:1
120 min	2.2:1

Table 2. Ethyl acetyl azaaurone **6** integration ratios of observed signals during photoisomerization

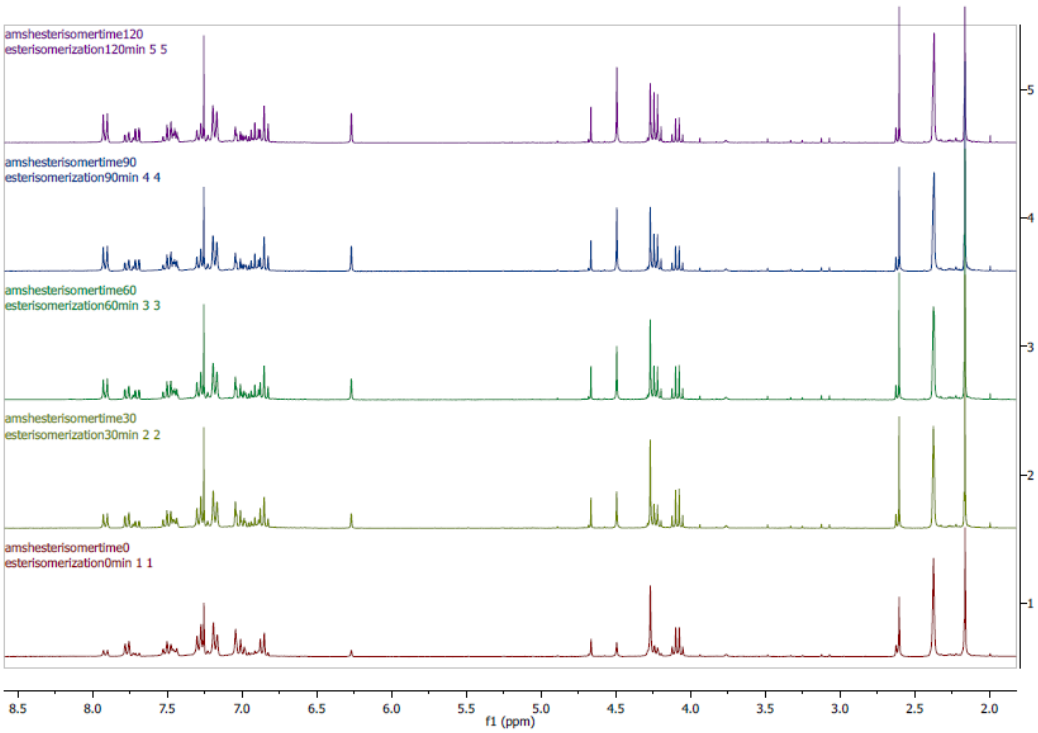


Figure 7. ^{1}H NMR of ethyl acetyl azaaurone **6** during photoisomerization

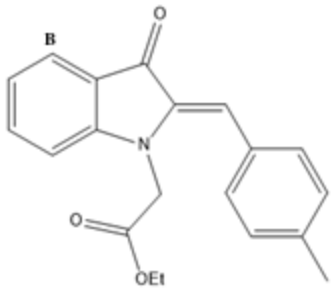


Figure 8. Observed proton on benzyl azaaurone **6** during isomerization

For azaaurone **7**, the observed signals at saw a decrease in their integration over 2 hours, as shown in table 3. The peaks were compared to the peaks at δ7.02 and δ8.10, to see the change in signal strength over time, as seen in figure 9. The peaks at δ7.11 and δ8.17 show the signals for protons **C** and **D**, respectively, in figure 10.The change in integration showed that the toscyl azaaurone compound was originally synthesized as mostly the Z isomer, but irradiation caused the majority of product to isomerize into the E configuration.

	δ7.11 : δ7.02	δ8.17 : δ8.10
0 min	16.3:1	8.3:1
30 min	3.5:1	2.3:1
60 min	2.3:1	1.7:1
90 min	1.6:1	1.4:1
120 min	1.5:1	1.4:1

Table 3. Tosyl azaaurone **7** integration ratios of observed signals during photoisomerization

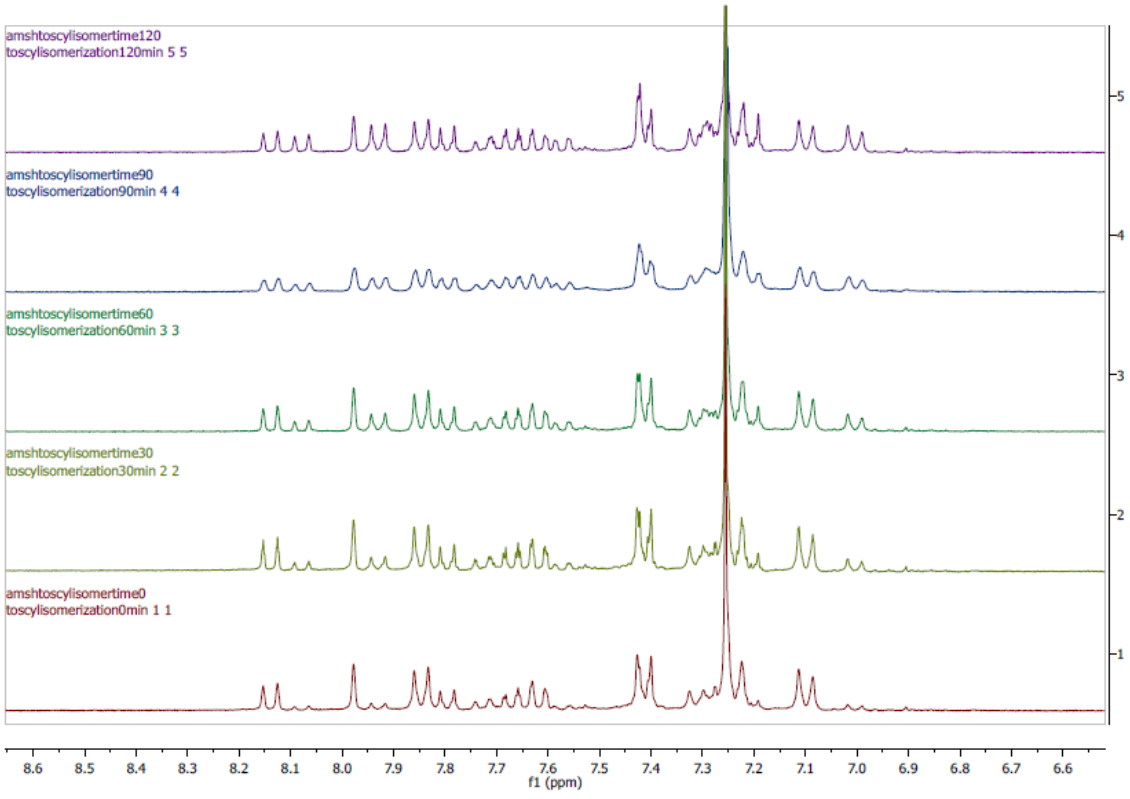


Figure 9. ^{1}H NMR of tosyl azaaurone **7** during photoisomerization

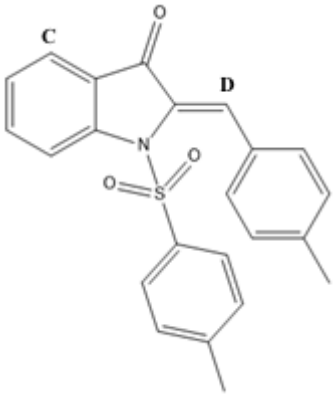


Figure 10. Observed protons on toscyl azaaurone **7** during isomerization

For the compounds that did successfully photoequalibrate based upon 1H NMR, TLC was used to determine whether the isomers in the isomeric mixture could be separated. Unfortunately, the isomers for each compound had essentially identical Rf values and would be extremely difficult to separate using column chromatography. Given this challenge, the UV/Vis spectra of the isomeric mixtures were taken to see if any shift in the wavelength of the maximum absorption could be observed. Unfortunately, the UV/Vis spectra were identical for each compound and the equilibrated mixtures. The benzyl isomeric mixes for azaaurone **5** both had maximum absorptions at 312 nm and 490 nm, as seen in figure 8. The ethyl acetyl azaaurone **6** isomeric mixes both had maximum absorptions at 308 nm and 472 nm, shown in figure 9. The tosyl isomeric mixtures for azaaurone **7** both had maximum absorptions at 324 nm and 394 nm, shown in figure 10.

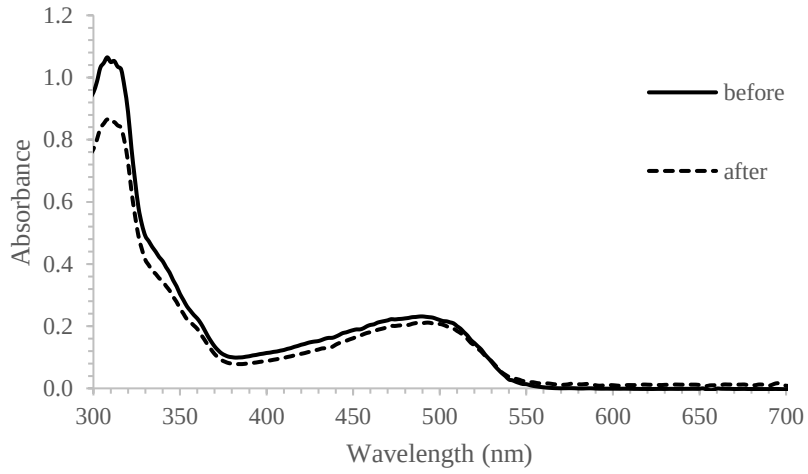


Figure 11. UV/Vis spectrum for benzyl azaaurone **5** before and after irradiation

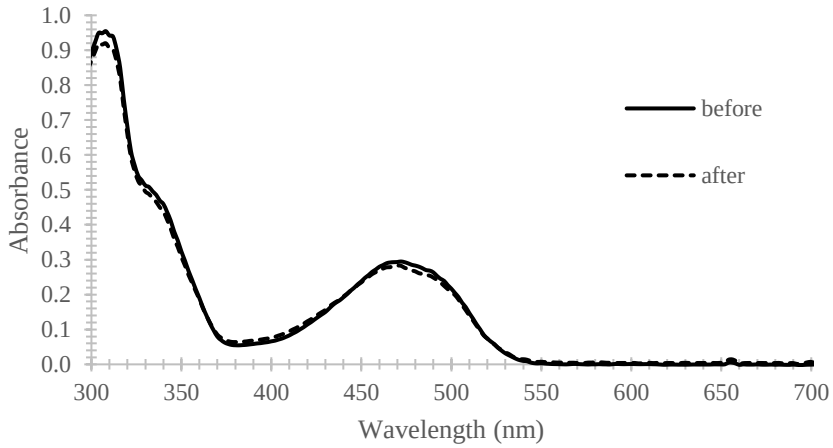


Figure 12. UV/Vis spectrum for ethyl acetyl azaaurone **6** before and after irradiation

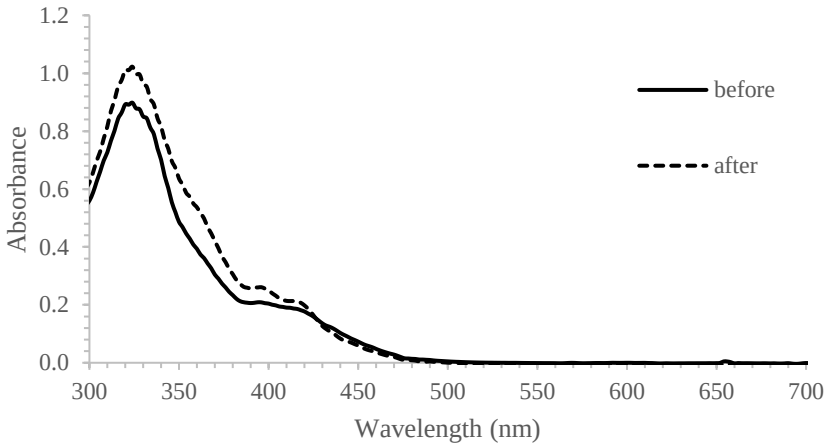


Figure 13. UV/Vis spectrum for tosyl azaaurone **7** before and after irradiation

Had a shift been observed, then studies could be performed using selective irradiation at different wavelengths that are better absorbed by new isomer than the original isomer, allowing for equilibration back to the original isomer.

Heat of formation calculations

To help explain the UV/Vis results, simple heat of formation calculations were performed on the cis and trans isomers using Spartan modeling, as shown in table 1, to determine the approximate energy difference between the two isomers. The heat of formation was calculated with Spartan using Equilibrium Geometry with Semi-Empirical PM3. Following the pattern of other aurone molecules, the cis form of each compound was found to be more stable than the trans isomer. In cases with a large difference in the heat of formations of the two isomers, it was expected that photoequilibration would largely afford the more stable isomer, and not provide much of an opportunity for

photoswitching. Isomers with small differences in the heat of formation would be better candidates for reversible switching between the two isomeric forms.

Azaaurone	Cis	Trans	ΔHeat of formations
Parent 3	109.488 kJ/mol	121.320 kJ/mol	11.832 kJ/mol
N-acetyl 4	-46.716 kJ/mol	-40.195 kJ/mol	6.521 kJ/mol
N-benzyl 5	224.911 kJ/mol	231.390 kJ/mol	6.479 kJ/mol
N-ethyl acetyl 6	-232.963 kJ/mol	-224.098 kJ/mol	8.865 kJ/mol
N-tosyl 7	-43.014 kJ/mol	-30.419 kJ/mol	12.595 kJ/mol

Table 4. Heat of formation calculations for each azaaurone cis and trans isomer

The parent azaaurone **3** had a relatively large difference between the heats of formation of the two isomers, suggesting it would be difficult to isomerize into its less stable trans configuration. This may explain why the parent azaaurone was unable to be photoisomerized after its synthesis. The N-benzyl and N-ethyl acetyl azaaurones have much smaller differences in heat of formations, suggesting that they should be better able to photoisomerize into the trans isomer, since it is only slightly less stable than the cis configuration. Although this did occur, the compounds were not candidates for reversible photoisomerization, because there was no change in their UV/Vis spectra.

Based upon the successful isomerization of aurones and hemiindigo compounds in prior studies, there was hope that photoisomerization would be successful in the

synthesized compounds. In one study, when a hemiindigo compound with formyl groups on the pyrrole rings, shown in figure 11, was photoisomerized from its Z to E isomer, there was a shift in their absorption spectrum.[16] The E isomers had absorption maxima at longer wavelengths than the Z isomers, with an absorption band shifting from 470 nm to 524 nm. It was concluded the shift in the absorption was likely due to the intramolecular hydrogen bonding that could occur in the new Z configuration that would lower the excitation energy of the Z isomer.

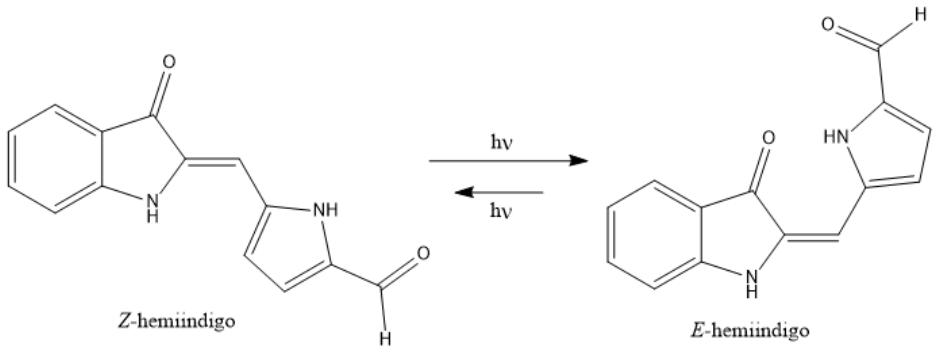


Figure 14. Photoisomerization of hemiindigo compound to *Z* to *E* isomer

In another study by Dr. Natasha Shanker, an aminoaurone, shown in figure 12, was photoisomerized from its Z isomeric configuration into a mixture of Z and E isomers with irradiation using 400 nm light.[17] Absorption spectra showed that the absorption maximum with the E isomer was 9 nm higher than the Z isomer alone. Irradiation at a higher wavelength of 480 nm reversibly isomerized the mixture to the Z isomer.

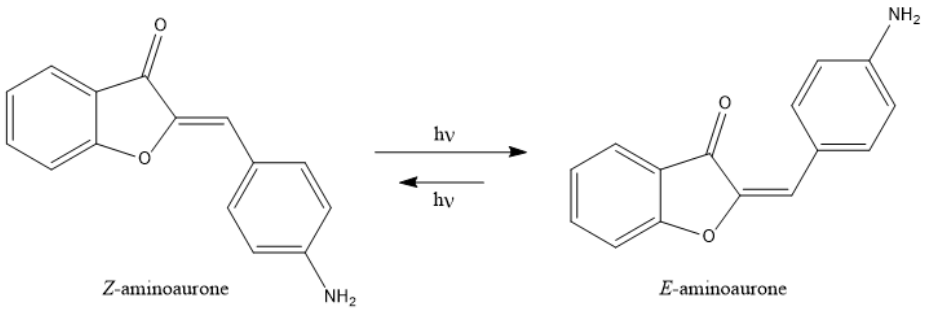


Figure 15. Photoisomerization of aminoaurone compound from *Z* to *E* isomer

Because of the change in absorption in the previous aurone isomerization study, a shift in the absorption of the UV/Vis spectra for the synthesized azaaurones was also expected. However, like the hemiindigo compounds, the aurone that was examined by Dr. Shanker would also experience hydrogen bonding. The N-substituted groups of the azaaurones in this experiment had been selected because of the expectation that the changes and breaking of pi-pi stacking and electrostatic pi-pi interactions after isomerization would have changed the excitation energy of the isomer, causing a change in the UV/Vis absorption. However, it is possible that these changes in pi-pi electron interactions are not sufficient to provide sufficient electronic differences to create and observable change in the absorption spectra and also result in little difference in terms of chromatographic features.

Chapter III: Conclusion

The objective of this research project was to synthesize N-substituted azaaurones and reversibly photoisomerize between the Z and E isomers of the analogs. Although there was some success synthesizing the azaaurones and photoisomerizing the molecules to get their isomeric mixtures, the compounds cannot be reversibly isomerized at this point.

In terms of future efforts, testing new procedures to successfully produce an acylated azaaurone would be attempted, perhaps by varying the acyl donor. Also, methods other than conventional column chromatography should be explored to separate isomeric mixtures of the compounds that photoisomerized. If this was successfully done, the UV/Vis spectra would be collected again for each isomer to determine in greater detail whether the wavelength where maximum absorption occurs does change. Even if such change was modest, then photoisomerization would be reattempted on each isomer to see if the azaaurones are photoswitchable compounds. Also, the biological properties of the azaaurones and their isomers could be tested. Previous studies have shown that the isomers of aurones can differ in their biological activity and potency.[8] The isomers would be tested to see whether there is a difference in their biological properties or if they have any biological properties at all.

Chapter IV: Experimental

Instrumentation:

The NMR data were obtained on a 300 MHz FT-NMR model ECA-500 JEOL or on a 500 MHz FT-NMR model ECA-300 JEOL, with $CDCl_3$ a solvent. All chemical shifts are reported in parts per million using tetramethylsilane (TMS) as a standard. All UV/Visible spectra were collected using a Hewlett Packard diode array spectrophotometer model 8452A. The lamp used for the photoisomerization studies was an Esbaybulbs 36W blue LED lamp.

Synthetic Methods:

***1-acetyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one* 4**

1-(3-hydroxy-1H indole) ethanone (175 mg, 1 mmol) was combined with p-tolualdehyde (132 mg, 1.1 mmol) and piperidine (2-3 drops) in a round bottom flask. The mixture was dissolved in toluene (2 mL). The solution was stirred at 100^{o}C overnight. The resulting mixture was purified using flash column chromatography on silica with dichloromethane as eluent. The acetylated product was collected and solvent was removed *in vacuo* to afford 100.8 mg (57.6%) of compound **4** as yellow-green solid. ^{1}HNMR (300 MHz, $CDCl_3$), δ8.27 (d, 1H, J=8.4 Hz, ArH), δ8.10 (d, 1H, J=8.4 Hz, ArH), δ7.80 (dd, 1H, J=7.5 Hz, ArH), δ7.63 (dd, 1H, J=7.2 Hz, ArH), δ7.40 (d, 2H, J=6.9 Hz, tolyl ArH), δ7.29 (s, 1H, C=CH), δ7.23 (d, 2H, J=8.1 Hz, toyl ArH), δ2.62 (s, 3H, tolyl CH_3), δ1.96 (s, 3H, acetyl CH_3). ^{13}CNMR (500 MHz, $CDCl_3$), δ186.4, δ171.9, δ165.7, δ140.6, δ136.1, δ131.2, δ130.2, δ129.9, δ128.6, δ124.8, δ124.5, δ123.9, δ122.6, δ117.7, δ30.8, δ25.0.

***2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one* 2**

138 mg of previous product **4** (0.5 mmol) was dissolved in methanol (5 mL) and a solution of 50% potassium hydroxide in water (750 µL) was added. The mixture was stirred at room temperature for 45 minutes. After the reaction was completed, the mixture was extracted using ethyl acetate and water. The organic layer was collected and dried using anhydrous magnesium sulfate. The crude product was collected and purified on silica using 5 to 10% ethyl acetate in hexane as eluent. The fractions containing the product were collected to afford 81.9 mg (59.4%) of compound **2** as orange solid. ^{1}HNMR (300 MHz, $CDCl_3$), δ7.75 (d, 1H, J=7.5 Hz, ArH), δ7.49 (d, 1H, J=3.0 Hz, ArH), δ7.43 (d, 2H, J=3.0 Hz, tolyl ArH), δ7.27 (d, 2H, J=8.4 Hz, tolyl ArH), δ7.01 (dd, 2H, J=6.0 Hz, ArH), δ6.85 (s, 1H, C=CH), δ2.62 (s, 3H, tolyl CH_3), δ1.58 (s, 1H, NH). ^{13}CNMR (500 MHz, $CDCl_3$), δ187.4, δ153.3, δ138.9, δ136.1, δ135.8, δ132.1, δ130.0, δ129.6, δ128.1, δ125.0, δ121.8, δ120.5, δ112.1, δ21.5.

***1-benzyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one* 5**

2-(4-Methylbenzylidene)-1,2-dihydro-3H-indol-3-one **2** (50 mg, 0.21mmol) was combined with benzyl bromide (0.05 mL, 0.42 mmol), cesium carbonate (77 mg), and dissolved with DMF (5-6 drops) for solubility in a vial. The mixture was stirred overnight at room temperature. The mixture was worked up and extracted using ethyl acetate and water. The organic layer was dried using anhydrous magnesium sulfate. The crude product was collected and purified on silica with 5 to 10% ethyl acetate in hexane as eluent. The product was collected and solvent was removed *in vacuo*. Further purification

was needed, so preparative thin-layer chromatography was done using 10% ethyl acetate in hexane. The product was collected to afford 4.3 mg (8.4%) of compound **5** as a yellow solid. ^{1}HNMR (300 MHz, $CDCl_3$), δ7.88 (d, 1H, J=6.6 Hz, ArH), δ7.74 (d, 1H, J=7.5 Hz, ArH), δ7.43 (dd, 1H, J=4.8 Hz, ArH), δ7.38 (dd, 1H, J=6.5 Hz, ArH), δ7.29 (d, 2H, J=4.5 Hz, tolyl ArH), δ7.21 (d, 2H, J=4.8 Hz, benzyl ArH), δ7.06 (d, 2H, J=5.2 Hz, tolyl ArH), δ6.93 (dd, 2H, J=3.8 Hz, benzyl ArH), δ6.87 (dd, 1H, J=3.1 Hz, ArH), δ6.31 (s, 1H, C=CH), δ4.82 (s, 2H, benzyl CH_2), δ2.32 (s, 3H, tolyl CH_3).

***1-ethylacetyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one* 6**

2-(4-Methylbenzylidene)-1,2-dihydro-3H-indol-3-one **2** (50 mg, 0.21mmol) was combined with ethyl bromoacetate (0.046 mL, 0.42 mmol), cesium carbonate (77 mg), and dissolved with DMF (5-6 drops) for solubility in a vial. The mixture was stirred at room temperature overnight. The mixture was worked up and extracted using ethyl acetate and water. The organic layer was dried using anhydrous magnesium sulfate. The crude product was collected and purified on silica with 10 to 20% ethyl acetate in hexane as eluent. The product was collected and solvent was removed *in vacuo* to afford 21.7 mg (43.4%) of compound **6** as a yellow solid. ^{1}HNMR (500 MHz, $CDCl_3$), δ7.92 (d, 1H, J=15.0 Hz, ArH), δ7.76 (d, 1H, J=13.5 Hz, ArH), δ7.69 (dd, 1H, J=11.5 Hz, ArH), δ7.47 (dd, 1H, J=13.0 Hz, ArH), δ7.29 (d, 2H, J=13.5 Hz, tolyl ArH), δ7.17 (d, 2H, J=13.0 Hz, tolyl ArH), δ7.05 (s, 1H, C=CH), δ4.28 (s, 2H, acetyl CH_2), δ4.11 (q, 2H, J=11.5 Hz, ethyl CH_2), δ2.38 (s, 3H, tolyl CH_3), δ1.14 (t, 3H, ethyl CH_3, J=10.0 Hz). ^{13}CNMR (500

MHz, $CDCl_3$), δ187.2, δ168.6, δ155.8, δ138.4, δ136.1, δ130.2, δ129.9, δ129.4, δ129.2, δ124.8, δ122.7, δ120.9, δ113.3, δ110.3, δ61.3, δ21.3, δ14.0.

***1-tosyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one* 7**

2-(4-Methylbenzylidene)-1,2-dihydro-3H-indol-3-one **2** (50 mg, 0.21 mmol) was combined with p-toluenesulfonyl chloride (80 mg, 0.42 mmol), sodium hydride (9.1 mg, 0.23 mmol), and dissolved with DMF (5-6 drops) for solubility in a vial. The mixture was stirred at room temperature overnight. The mixture was worked up and extracted using ethyl acetate and water. The organic layer was dried using anhydrous magnesium sulfate. The crude product was collected and purified using silica with 5 to 20% ethyl acetate in hexane as eluent. The product was collected and solvent was removed *in vacuo* to afford 11.4 mg (22.8%) of compound **7** as a yellow solid. ^{1}HNMR (300 MHz, $CDCl_3$), δ8.12 (d, 1H, J=8.4 Hz, ArH), δ7.97 (s, 1H, C=CH), δ7.78 (d, 1H, J=8.1 Hz, ArH), δ7.66 (dd, 1H, J=7.2 Hz, ArH), δ7.59 (d, 2H, J=7.8 Hz, tolyl ArH), δ7.40 (d, 2H, J=7.8 Hz, tosyl ArH), δ7.32 (dd, 1H, J=8.1 Hz, ArH), δ7.22 (d, 2H, J=7.8 Hz, tosyl ArH), δ7.11 (d, 2H, J=7.8 Hz, tolyl ArH), δ2.41 (s, 3H, tosyl CH_3), δ2.31 (s, 3H, tolyl CH_3).

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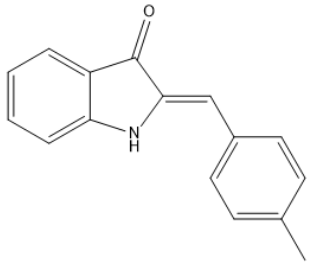
APPENDIX

2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one-2



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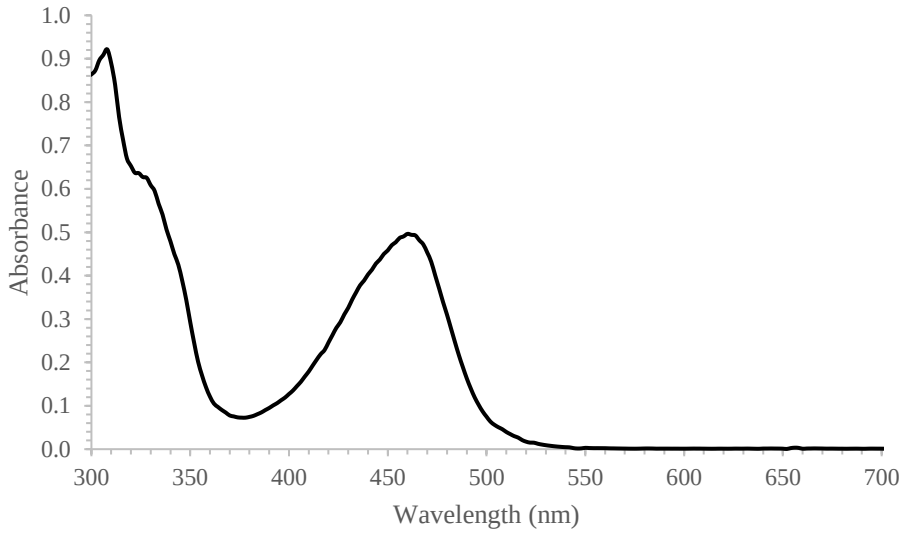
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400
450
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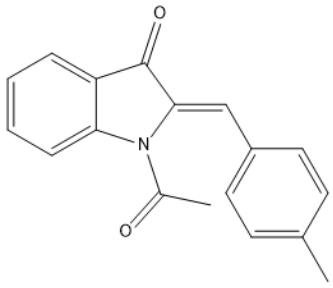
550
600
650
700
Wavelength (nm)

1-acetyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one



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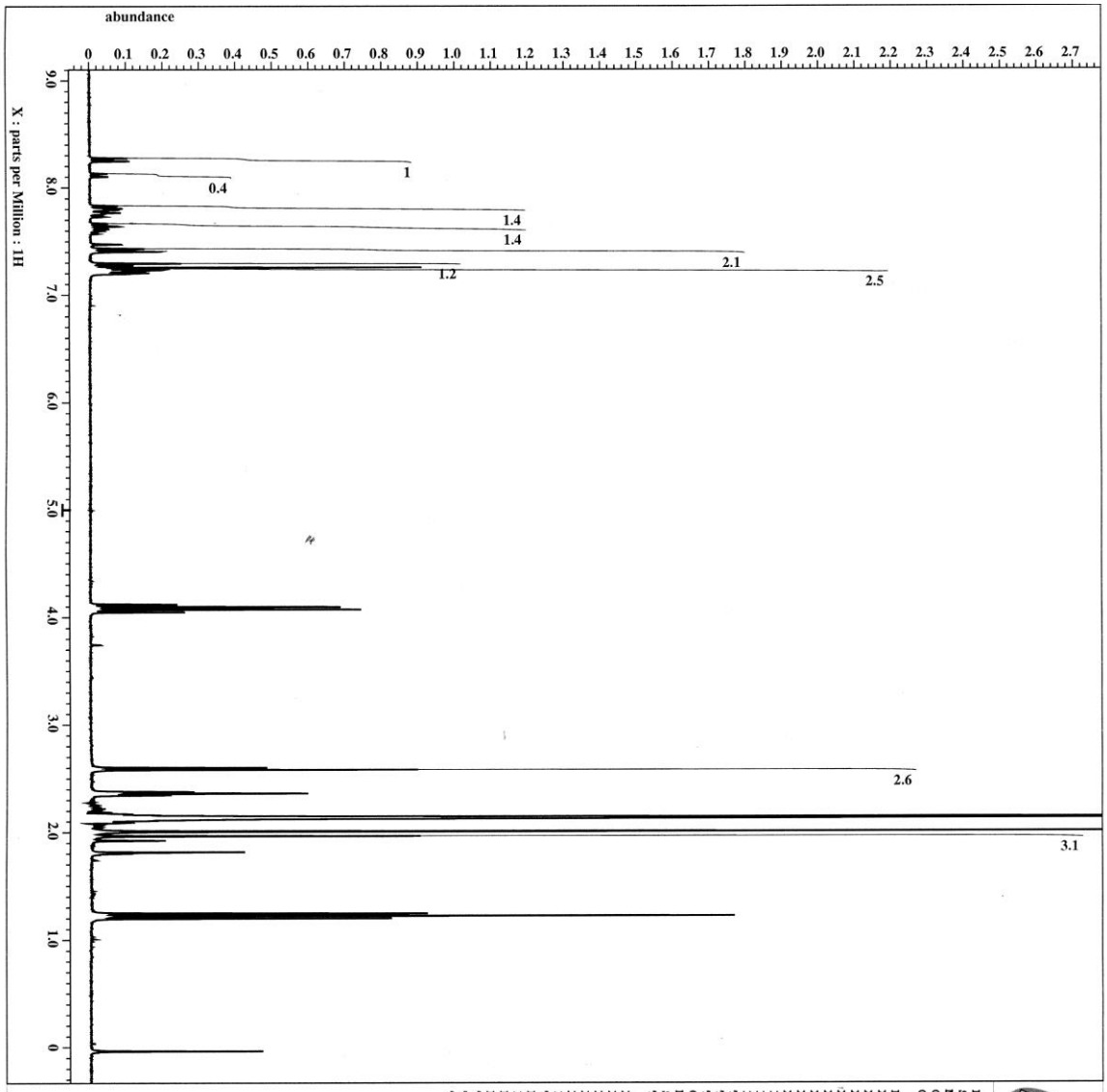
- ^{13}CNMR
- UV/Vis



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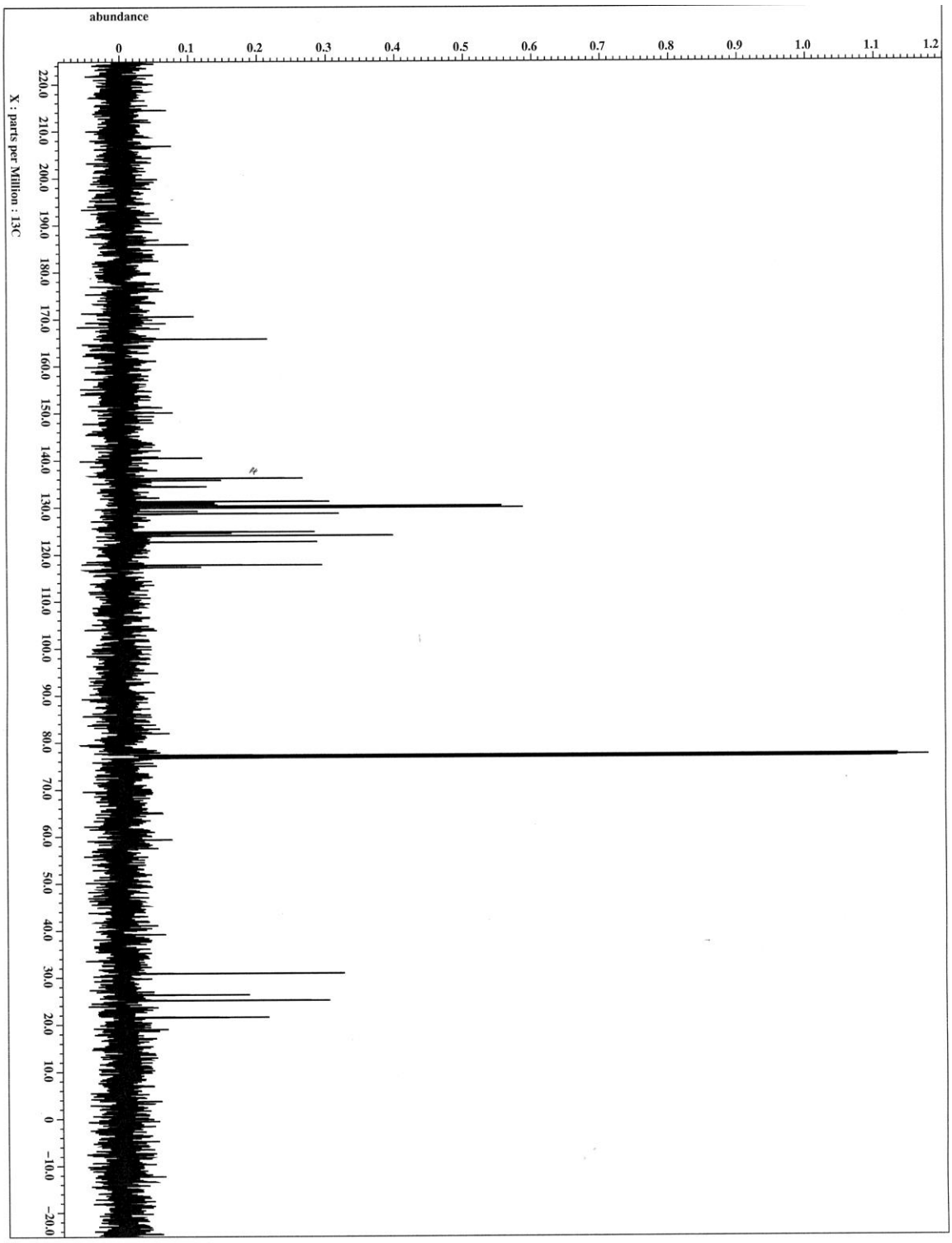
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1.1
1.2
X : parts per Million : 13C
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190.0
180.0
170.0
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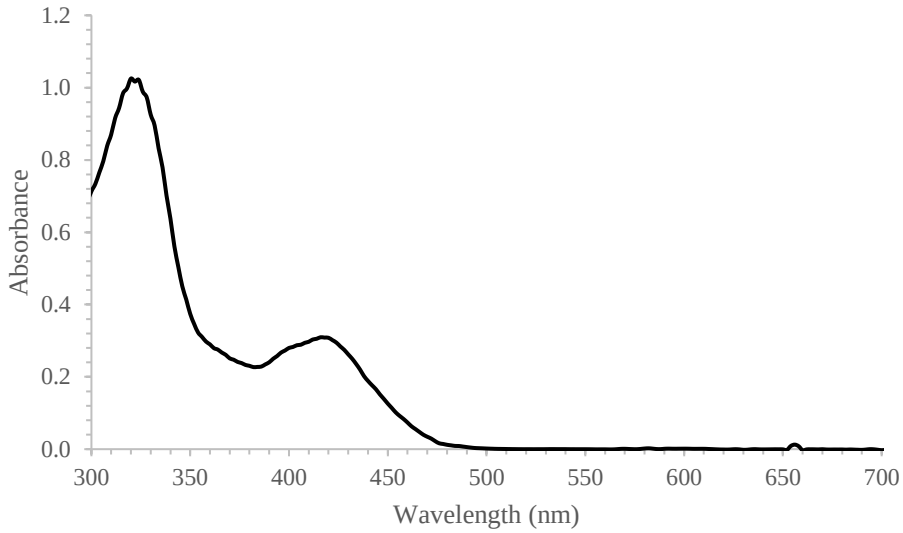
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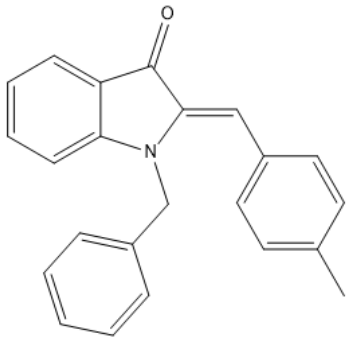
Wavelength (nm)

1-benzyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one



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abundance
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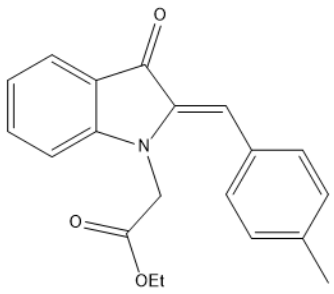
1-ethylacetyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one



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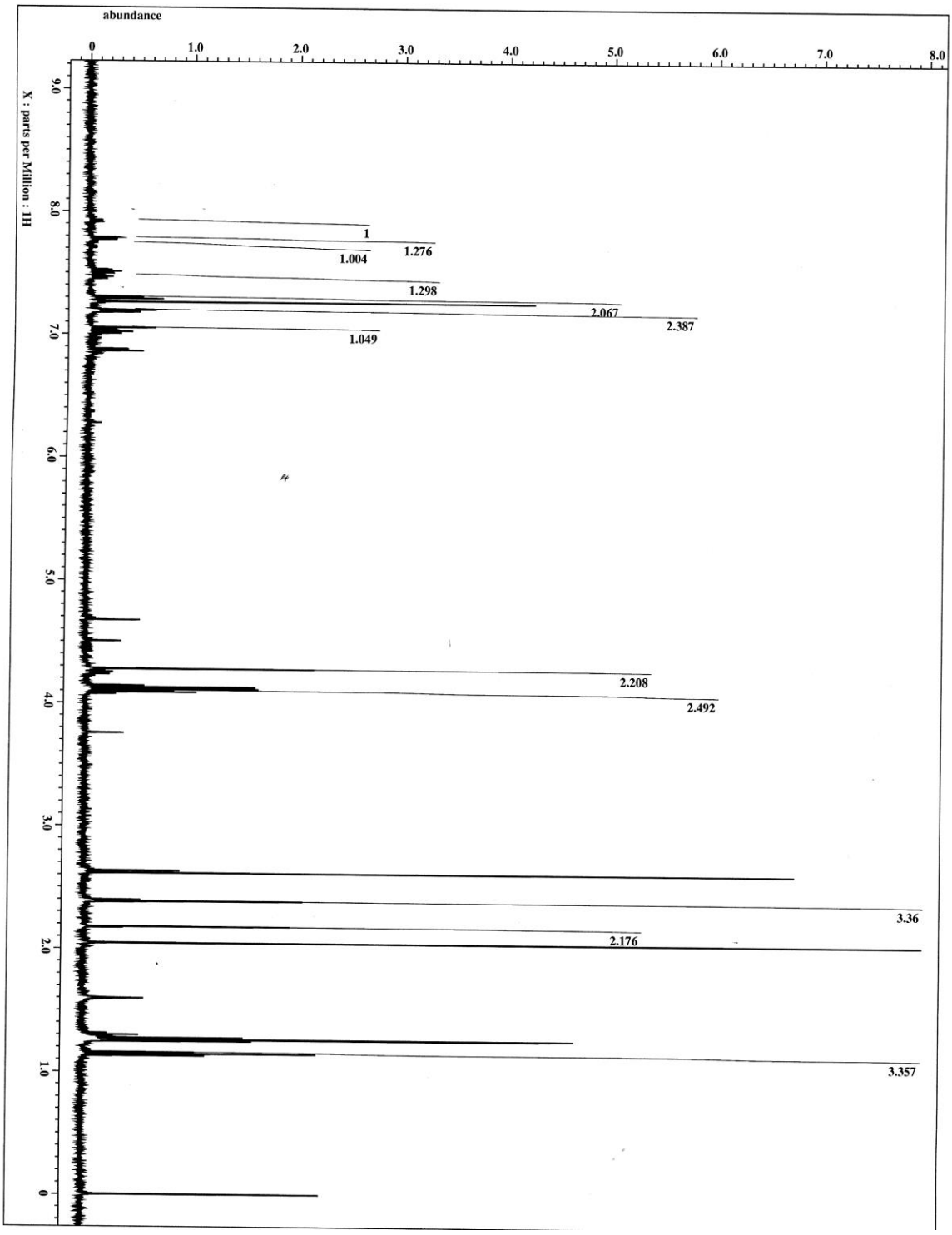
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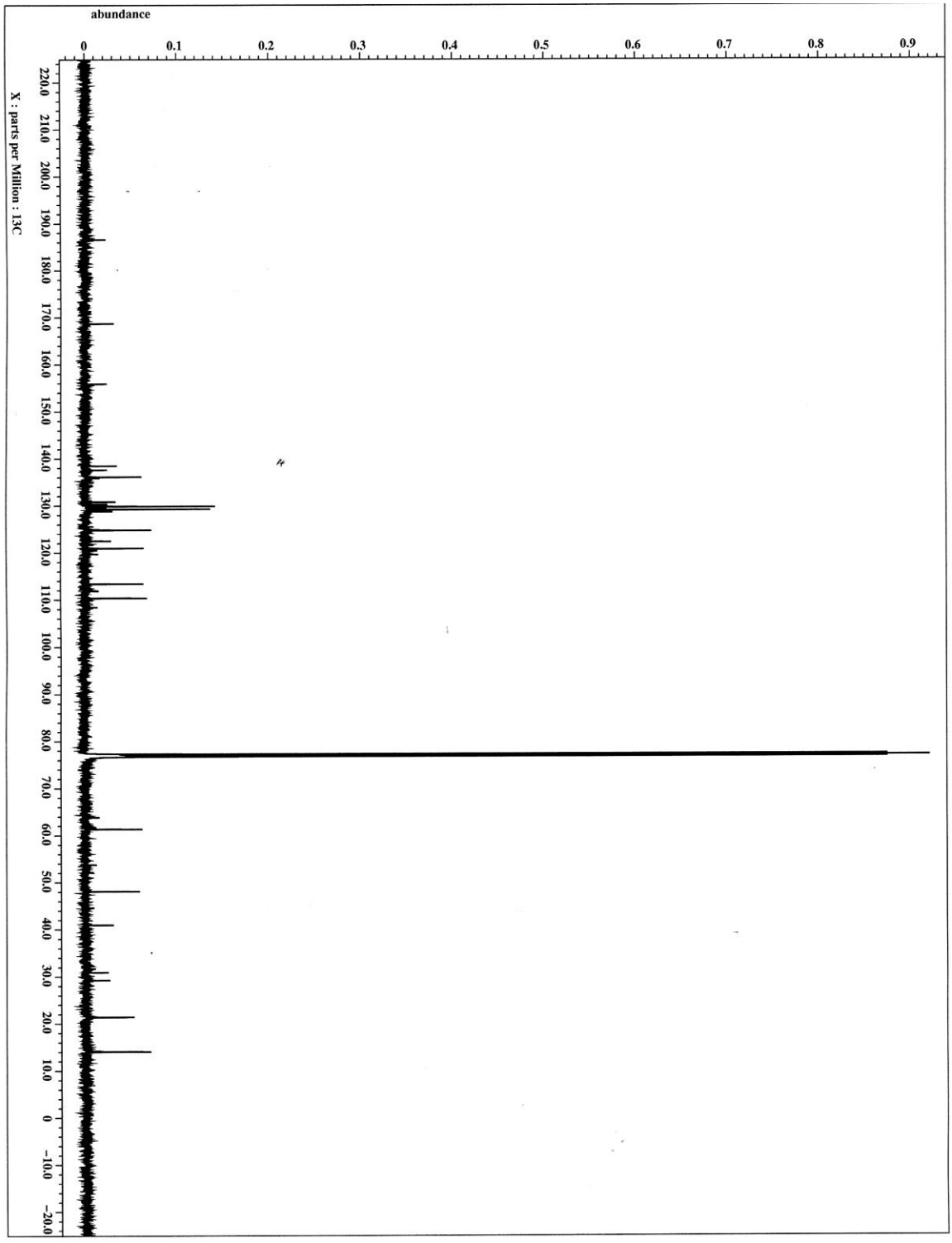
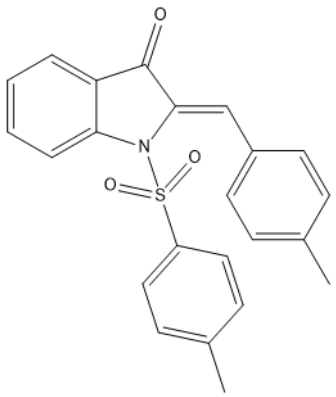
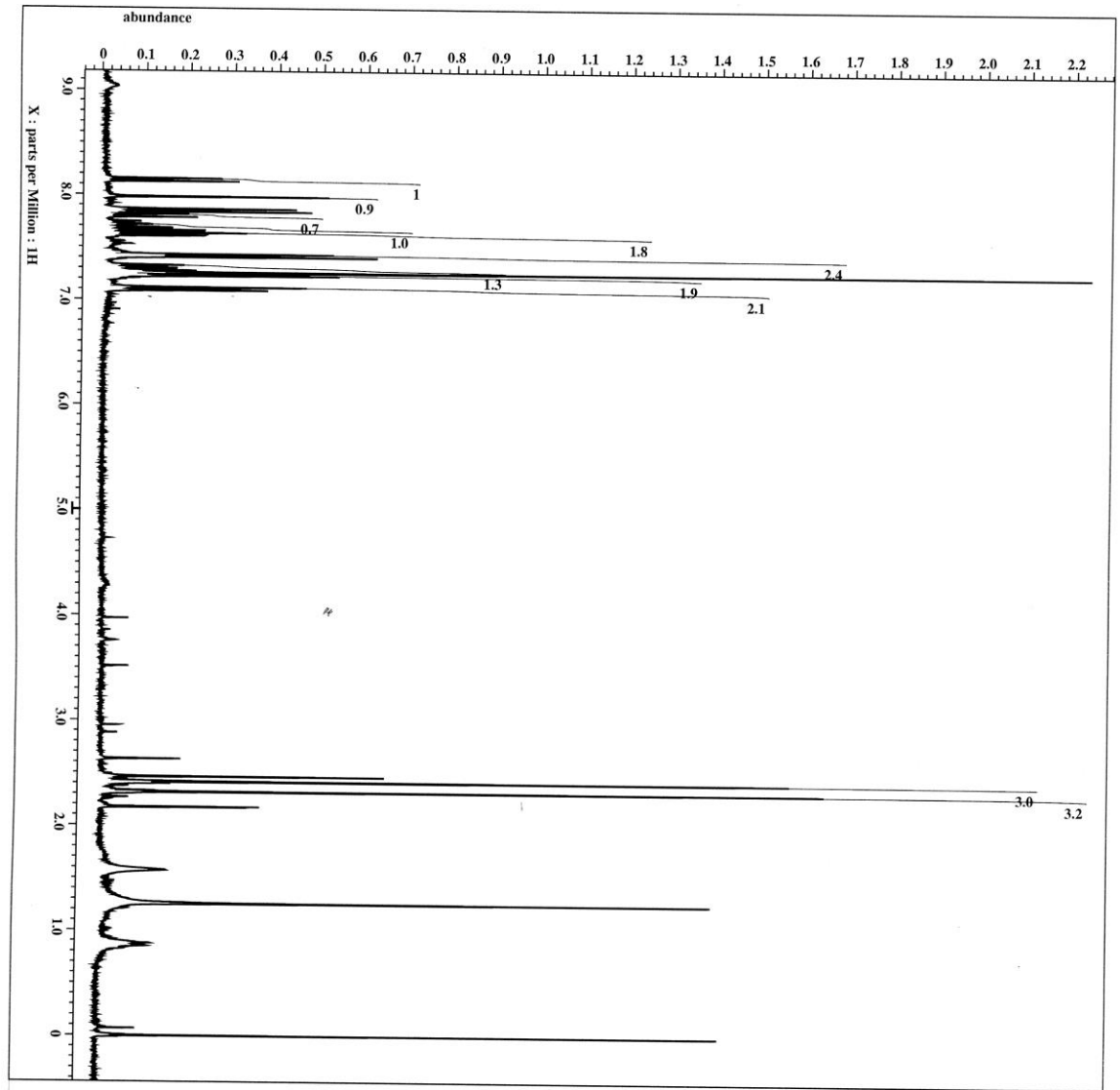
X : parts per Million : 1H
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
1.004
1.276
1.298
2.067
2.387
1.049
2.208
2.492
3.36
2.176
3.357


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
X : parts per Million : 13C
220.0
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
-10.0
-20.0

1-tosyl-2-(4-methylbenzylidene)-1,2-dihydro-3H-indol-3-one



- ^{1}HNMR



JEOL

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Filename                = amshtcosylcrude022119
Sample_id               = amshtcosylcrude022119
Machine                 = mtsu300sp
Creation_time           = 21-FEB-2019 15:59:35
Comment                 = tosylsynthesisupper

Field_strength          = 7.0586013[T] (300[MHz
X_acq_duration          = 2.90717696[s]
X_domain                = 1H
X_freq                  = 300.52965592[MHz]
X_offset                = 5[ppm]
X_points                = 16384
X_prescans              = 1
X_resolution            = 0.34397631[Hz]
X_sweep                 = 5.63570784[kHz]
Irr_domain              = 1H
Irr_freq                = 300.52965592[MHz]
Irr_offset              = 5[ppm]
Tri_domain              = 1H
Tri_freq                = 300.52965592[MHz]
Tri_offset              = 5[ppm]
Clipped                 = FALSE
Mod_return              = 1
Scans                   = 16
Total_scans             = 16

X_90_width              = 11.09[us]
X_acq_time              = 2.90717696[s]
X_angle                 = 45[deg]
X_atn                   = 4[dB]
X_pulse                 = 5.545[us]
Irr_mode                = Off
Tri_mode                = Off
Dante_presat            = FALSE
Initial_wait            = 1[s]
Relaxation_delay        = 4[s]
Repetition_time         = 6.90717696[s]
Temp_state              = TEMP OFF
Temp_set                = 25[dC]
Temp_get                = 419.9[dC]
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