SUPPLEMENTAL INFORMATION – Lab Documentation For On-Line Publication

Convenient Microscale Synthesis Of A Coumarin Laser Dye Analog

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The information contained within this document is organized into three sections-

1. **Laboratory Notes For Students** – background information, hazards and safety, experimental procedures and post-lab questions (pages 2 - 6).

2. **Additional Notes For Instructors** – chemical CAS numbers, equipment needs, synthetic notes and UV-visible/fluorescence spectroscopy sample preparation (pages 7 - 8).

3. **Spectroscopic Information** – useful $^{1}H/^{13}C$ NMR spectra and assignments, product IR, MS, UV-visible and fluorescence spectra (pages 9 - 15).

CONVENIENT MICROSCALE SYNTHESIS OF A COUMARIN LASER DYE ANALOG

Laboratory Notes For Students

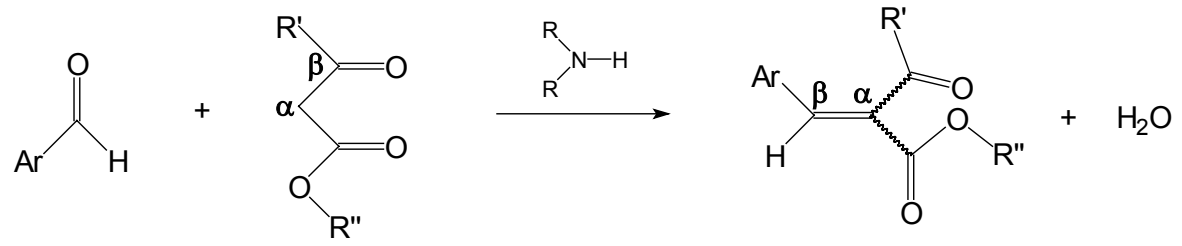
Estimated Length of Experiment: 2 hours

Experimental Objectives

1. To synthesize a fluorescent laser dye analog (3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one) via a Knoevenagel condensation reaction.
2. To characterize the reaction product by ^{1}H NMR and IR spectroscopy.
3. To record and interpret the UV-visible and fluorescence spectra of the product.
4. To learn about the use of coumarins as laser dyes.

Background

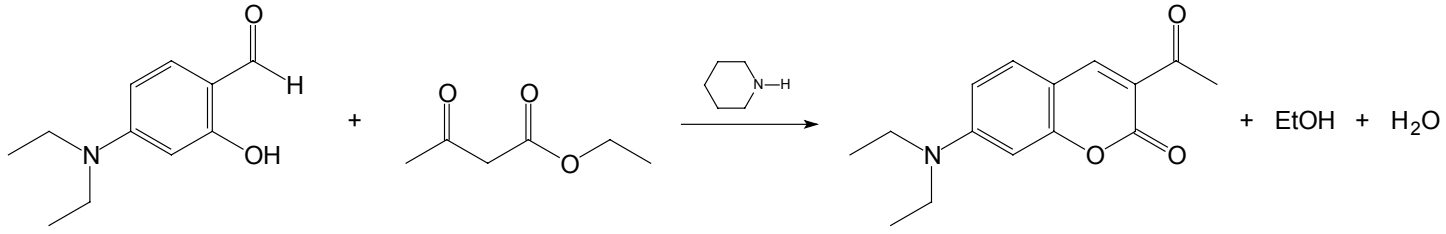
Dating from the late nineteenth century, the Knoevenagel condensation remains a favoured approach to form new carbon-carbon bonds under mild reaction conditions. This reaction employs a weak amine base to form an enolate anion derived from a 1,3-dicarbonyl compound (e.g. a β-ketoester, below). The enolate generated acts as a nucleophile in condensation with another carbonyl compound (commonly an aromatic aldehyde). The reaction mechanism bears striking similarity to that of the aldol condensation. The isolated product in this instance is a conjugated α,β-unsaturated ester, formed via facile dehydration of the initially formed β-hydroxydicarbonyl intermediate.



β–ketoester **α,β–unsaturated ester**

A Typical Knoevenagel Condensation

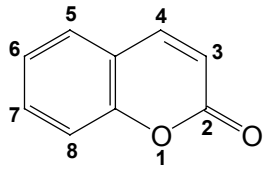
In today's experiment, 4-(diethylamino)salicylaldehyde (4-(diethylamino)-2-hydroxybenzaldehyde) is condensed with ethyl acetoacetate in the presence of a weak base (piperidine) to synthesize a heterocyclic *coumarin* product (below).



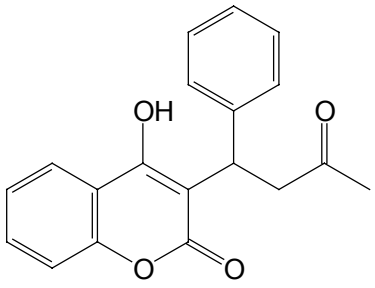
4-(diethylamino)salicylaldehyde ethyl acetoacetate 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one

Synthesis of a Coumarin Fluorescent Laser Dye Analog

Coumarins are compounds containing the 2*H*-1-benzopyran-2-one ring (below) (*1*). A very familiar coumarin is Warfarin, which has historically seen great usage as a rat poison. Warfarin is known to act as a blood anti-coagulant by antagonism (inhibition) of vitamin K 2,3-epoxide (*2*). It has subsequently been prescribed as a treatment for blood clots, and was administered to President Eisenhower when he suffered a heart attack in 1956.



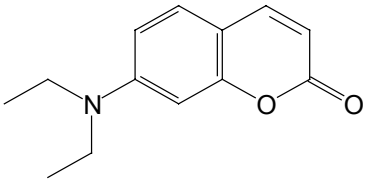
Coumarin
2*H*-1-benzopyran-2-one



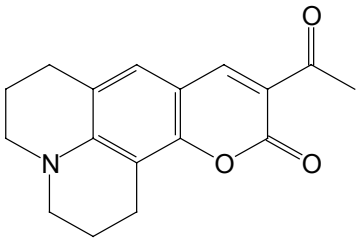
Warfarin

Structures of Coumarin (Parent Compound) and Warfarin

In addition to the Knoevenagel condensation occurring in today's reaction, transesterification subsequently takes place forming the cyclic ester (lactone) component of the coumarin. The specific compound generated (3-acetyl-7-(diethylamino)-2H-1-benzopyran-2-one) is a close structural analog of two commercially available materials that have applications as fluorescent laser dyes (Coumarin 110 and Coumarin 334, sold by Sigma-Aldrich).



Coumarin 110



Coumarin 334

Structures of Synthetic Coumarin Laser Dyes

Coumarin derivatives with an amino group in the 7-position lase easily in the blue and green regions of the visible spectrum when flash-lamp pumped. This characteristic makes them common components of dye lasers. A *dye* laser is one that uses an organic dye as a lasing medium, usually as a liquid solution. Compared to gases and most solid-state lasing media, a dye can usually be used for a much wider range of wavelengths. Coumarins are particularly suitable for use in tunable lasers (where the laser output frequency is adjusted to fall within a specific range of values). In addition the coumarin dye may be replaced by a close structural derivative in order to generate different wavelengths with the same laser, although this may require replacing other optical components.

Safety Notes

Wear eye protection, a laboratory coat and protective gloves during this experiment. All liquid reactants and solvents are highly flammable. 4-(Diethylamino)salicylaldehyde and ethyl acetoacetate are skin irritants. Piperidine is foul smelling and corrosive towards the eyes, respiratory system and skin. **The reaction product is very brightly coloured and will readily dye any exposed skin!**

CAUTION - PERFORM ALL SYNTHETIC AND PURIFICATION OPERATIONS IN A FUMEHOOD

Experimental Procedure

A table of the reactant/solvent physical properties is detailed below:

Compound	GMW	Amount Added	mmol	mp (°C)	bp (°C)	d (g/mL)
4-(diethylamino)salicylaldehyde	193.25	400 mg	2.07	62-64		
ethyl acetoacetate	130.14	530 μL	4.16	-43	181	1.021
piperidine	85.15	3 drops		-13	106	0.860
absolute ethanol	46.07				78	0.790

1. **IN A FUMEHOOD**, place the following in a 10 mL Erlenmeyer flask: 4-(diethylamino)salicylaldehyde (400 mg); ethyl acetoacetate (530 μL – automatic delivery pipette); and piperidine (3 drops).

2. Introduce a small magnetic stir bar and stir **VIGOROUSLY** on a stirrer/hotplate until a yellow/brown viscous solution is formed (usually about 20 – 30 minutes). **NOTE** – warm very gently if all the solid 4-(diethylamino)salicylaldehyde does not dissolve immediately, then turn the heat off. After 30 minutes, perform TLC on the viscous solution (stationary phase, silica gel; eluent, 1:1 hexanes/ethyl acetate).

3. Remove the flask from the stirrer/hotplate and add absolute ethanol to the reaction flask (5 mL). This will cause formation of a fine precipitate that is difficult to filter satisfactorily.

4. Heat the precipitate on a stirrer/hotplate until it dissolves in the ethanol solvent, and gradually bring to the boil.

5. Remove the reaction flask from the heat source and allow to cool slowly to room temperature. A crystalline solid should form. Cool further in ice for ~ 10 – 15 minutes.

6. Collect the product by vacuum filtration using a Hirsch funnel. Wash the filter cake with ice cold absolute ethanol (1 x 5 mL) to remove any traces of aldehyde starting material. Leave the solid under vacuum on the filter funnel for ten minutes to assist drying.

7. The crude product is easily recrystallized from absolute ethanol to leave a bright yellow solid. Weigh the product and calculate the percentage yield.

8. Record the product melting point. Obtain UV-visible, fluorescence, IR and ^{1}H NMR spectra.

SUBMIT A SAMPLE OF YOUR SYNTHESIZED PRODUCT WITH YOUR REPORT

Clean-Up

Dispose of all waste into the appropriately marked container in the fumehood. Rinse all apparatus used with acetone, discard the rinsings in the waste container and allow the glassware to dry.

Laboratory Report

Your report should contain the following points:

1. Discussion of the Knoevenagel condensation undertaken, including:

   (i) the calculated percent yield and comment on purity based on the product literature melting point (*3*).

   (ii) A "curved-arrow" reaction mechanism for formation of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one, showing the steps of enolate anion formation, nucleophilic addition, dehydration and transesterification.

2. Discussion of spectral data obtained, including:

   (i) IR spectral analysis (in terms of product absorbances and differences from the IR spectrum of 4-(diethylamino)salicylaldehyde). What new functional groups have been created in the reaction product and where might they be apparent in the IR spectrum of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one?

   (ii) identification of each peak in the ^{1}H NMR product spectrum (calculate spin-spin coupling constants (*J* values) where appropriate). Decide which peaks tell you that the reaction has definitely taken place.

   (iii) calculation of the molar extinction coefficient (ε) at the wavelength of maximal absorption (λ_{max}) in the UV-visible spectrum of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one. State/explain the similarities and differences between the UV-visible spectrum and fluorescence spectrum of this compound. How would you expect the laser emission spectrum to compare with the fluorescence spectrum? (*4*).

3. Discussion of the phenomena of fluorescence and laser emission, including:

   (i) why and how 7-aminocoumarins are generally suitable as dyes for tuning lasers.

(ii) the meaning of the term "quantum yield", and why knowledge of its numerical value for a laser dye is important.

(iii) why a coumarin dye with a "rigidized" structure (such as Coumarin 334) affords enhanced fluorescence efficiency (*5*).

References

1. O'Kennedy, R.; Thornes, R. D. In *Coumarins – Biology, Applications and Mode of Action*; Wiley: Chichester, NY, **1997**, p. 321 and cited references.

2. Gringauz, A. In *Introduction to Medicinal Chemistry: How Drugs Act and Why*; Wiley: New York, **1997**, pp. 501-507.

3. Czerney, P.; Hartmann, H. *J. Prakt. Chem*., **1982**, *324*, 21-28.

4. Hair, S. R. *J. Chem. Educ*., **1996**, *73*, A7-A9.

5. Reynolds, G. A.; Drexhage, K. H. *Opt. Commun*., **1975**, *13*, 222-25.

Additional Notes For Instructors

CAS Numbers

4-(Diethylamino)-2-hydroxybenzaldehyde (4-(diethylamino)salicylaldehyde) [*17754-90-4*]
Ethyl 3-oxobutanoate (ethyl acetoacetate, acetoacetic ester) [*141-97-9*]
3-Acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one [*74696-96-1*]
Piperidine [*110-89-4*]
Ethanol [*64-17-5*]
Hexanes [*73513-42-5*]
Ethyl acetate [*141-78-6*]

Equipment Needs (Per Student)

1 x 10 mL Erlenmeyer flask
2 x 25 mL Erlenmeyer flasks
1 x 10 mL measuring cylinder
Hirsch funnel and flask
Magnetic stirrer/hotplate
Magnetic stir bar
Ice bath
3 x Pasteur pipettes
Automatic delivery pipette (set to 530 μL, can be shared)
Silica gel TLC plates with fluorescent indicator (Sigma-Aldrich, product no. Z19,329-1)
UV lamp (254 nm) - optional

Notes Regarding Synthesis of 3-Acetyl-7-(diethylamino)-2H-1-benzopyran-2-one

1. Dependent on the rate of stirring, the reactant mixture may need to be warmed slightly for all the 4-(diethylamino)salicylaldehyde to dissolve. If heating is required the hotplate is turned off after complete dissolution and rapid stirring maintained.

2. TLC is run using 1:1 hexanes/ethyl acetate as the eluent on silica gel plates. The product 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one is evident as a yellow spot, $R_f = 0.5$, whereas 4-(diethylamino)salicylaldehyde appears as a purple spot, $R_f = 0.75$. This spot becomes more evident on the plate as time passes but can be observed immediately under a UV lamp.

3. The authors have tried filtering the fine precipitate initially formed on addition of ethanol, with little success. Heating and cooling the ethanolic solution helps to isolate the product in a more crystalline form.

4. A recrystallization volume of ~10 ml absolute ethanol is typically required.

5. After recrystallization and drying, typical product masses range from 0.24 g (45%) to 0.32 g (60%).

6. If the filtrate from the crude product is refrigerated for a week (until the next laboratory period), a further crop of crystals can be collected (up to 0.1 g) which can be recrystallized.

7. The authors have performed a scaled-up reaction (x 20) to synthesize material for student recrystallization purposes only. After stirring the reaction mixture for one hour, 8.9 g of coumarin product was isolated in the usual manner (83%).

8. In order to see the blue fluorescence of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one, dissolve 1 – 2 mg of solid in a few drops of a relatively non-polar solvent (e.g. THF, chloroform).

9. The authors obtain the synthetic starting material (4-(diethylamino)salicylaldehyde) from Sigma-Aldrich, product number 22,568-1. We have noticed that this solid varies from being a purple colour to a tan colour depending on the sample purchased. However, each sample permits synthesis of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one that exhibits spectral characteristics and a melting point in keeping with published literature.

Preparation of Samples for UV-visible Analysis

The UV-visible absorption spectrum (p. 15) of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one can be measured using the following procedure. A stock solution of the compound is firstly prepared by dissolving 29 mg 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one in 10 mL acetone (volumetric flask). This solution (20 μL) is then dissolved in 10 mL acetone (volumetric flask). This solution should have an absorbance of ~ 1.0 at λ_{max} = 426 nm ($\varepsilon = 4.51 \times 10^4$ M^{-1} cm^{-1}) (*1*).

Reference

1. UV-visible absorption literature values for 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one:
λ_{max} = 428 nm, $\varepsilon = 4.36 \times 10^4$ M^{-1} cm^{-1} (acetone)
λ_{max} = 432 nm, $\varepsilon = 4.47 \times 10^4$ M^{-1} cm^{-1} (chloroform)

Gao, F.; Li, H-R.; Yang, Y. *Dyes and Pigments*, **2000**, *47*, 231-38.

Preparation of Samples for Fluorescence Analysis

The fluorescence spectrum (p. 15) of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one can be measured by diluting the solution used for UV-visible analysis (above) by a factor of 1000 with acetone (10 μL dissolved in 10 mL acetone, volumetric flask). This solution has an absorption at λ_{max} = 426 nm and fluorescence at λ_{max} = 471 nm.

This fluorescence spectrum was obtained using a Perkin Elmer luminescence spectrometer (model LS50B, with FL Winlab Version 3.00 software).

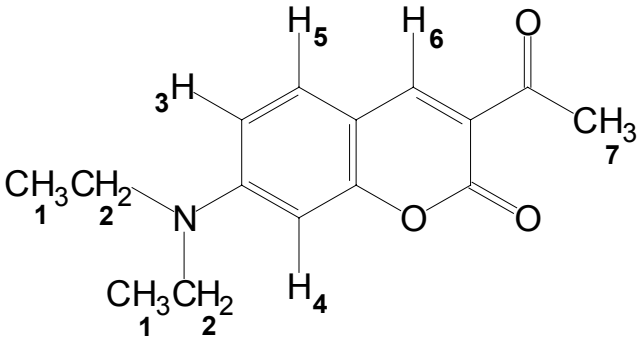
Reference

1. Fluorescence literature values for 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one:
λ_{max} = 472 nm (acetone), 464 nm (chloroform)

Gao, F.; Li, H-R.; Yang, Y. *Dyes and Pigments*, **2000**, *47*, 231-38.

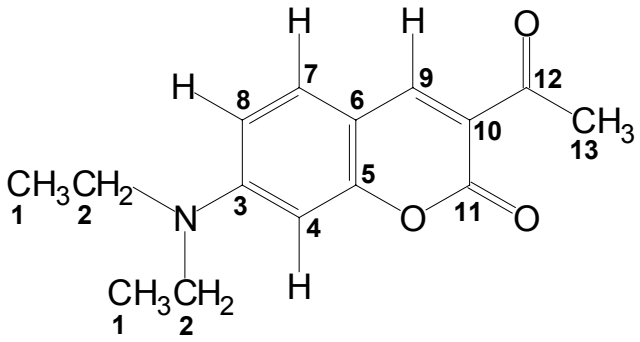
Spectroscopic Information

^{1}H NMR assignments for 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one



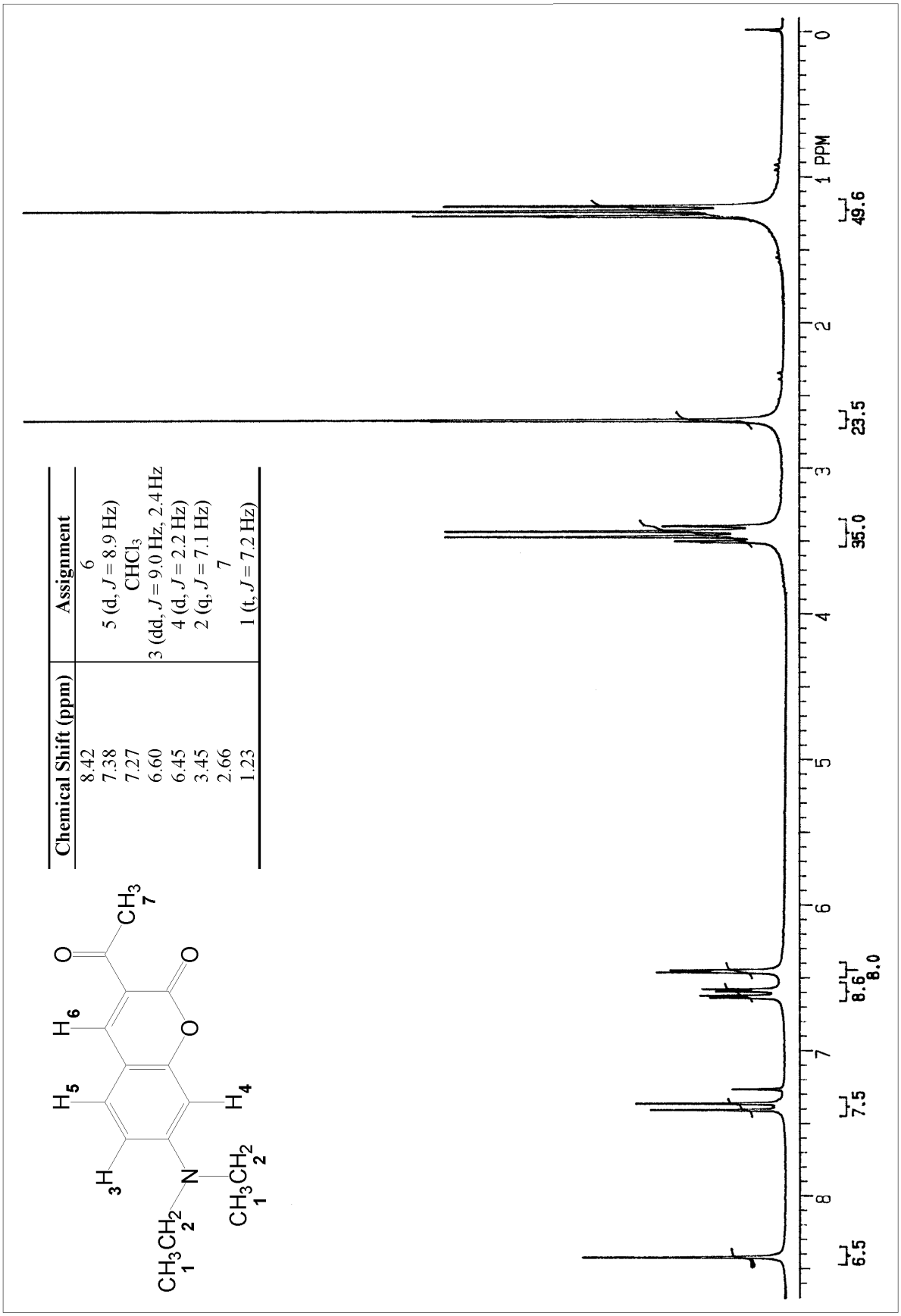
Chemical Shift (ppm)	Assignment
8.42	6
7.38	5 (d, *J* = 8.9 Hz)
7.27	$CHCl_3$
6.60	3 (dd, *J* = 9.0 Hz, 2.4 Hz)
6.45	4 (d, *J* = 2.2 Hz)
3.45	2 (q, *J* = 7.1 Hz)
2.66	7
1.23	1 (t, *J* = 7.2 Hz)

^{13}C NMR assignments for 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one

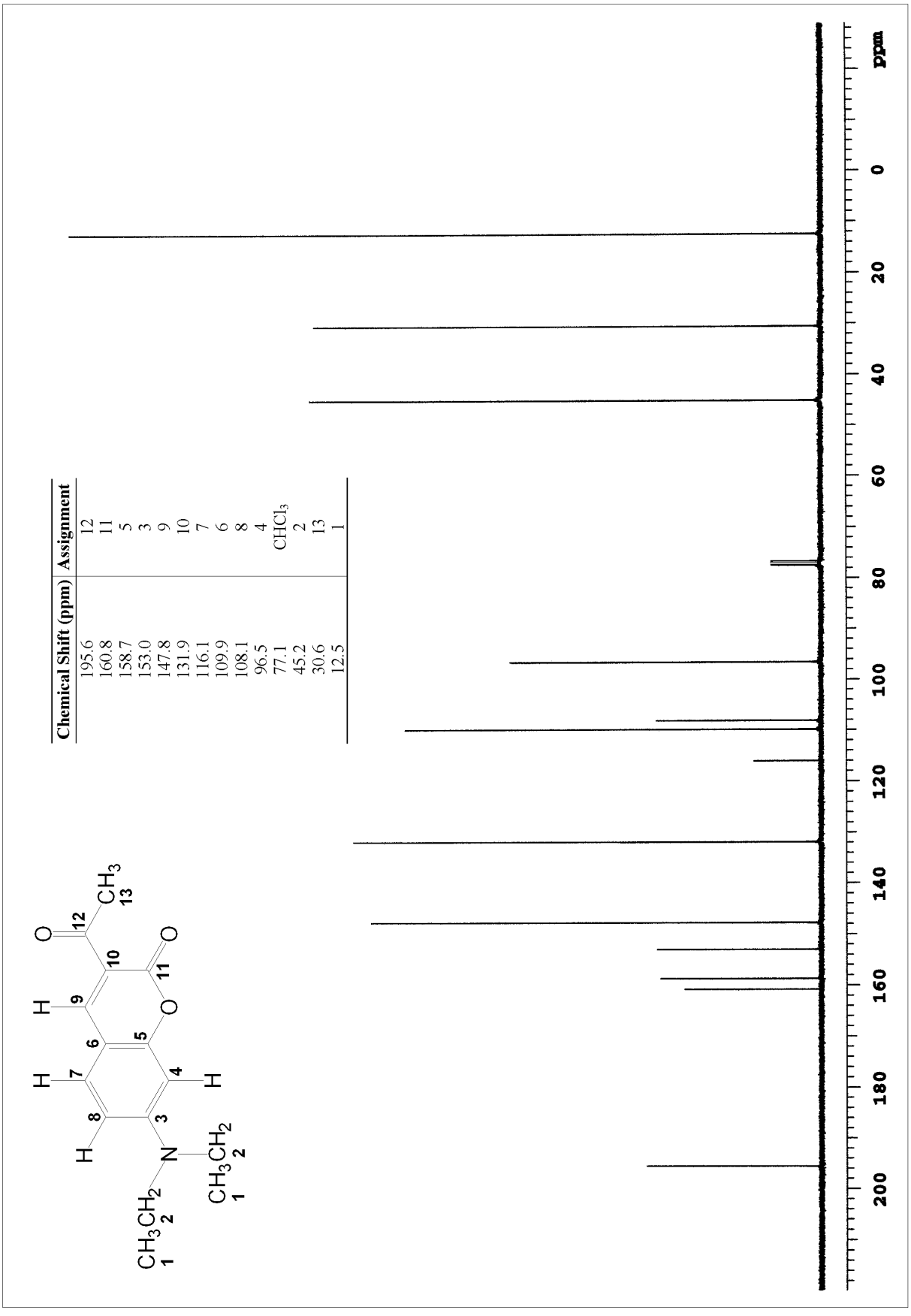


Chemical Shift (ppm)	Assignment
195.6	12
160.8	11
158.7	5
153.0	3
147.8	9
131.9	10
116.1	7
109.9	6
108.1	8
96.5	4
77.1	$CHCl_3$
45.2	2
30.6	13
12.5	1

^{1}H NMR (200 MHz) of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one ($CDCl_3$)

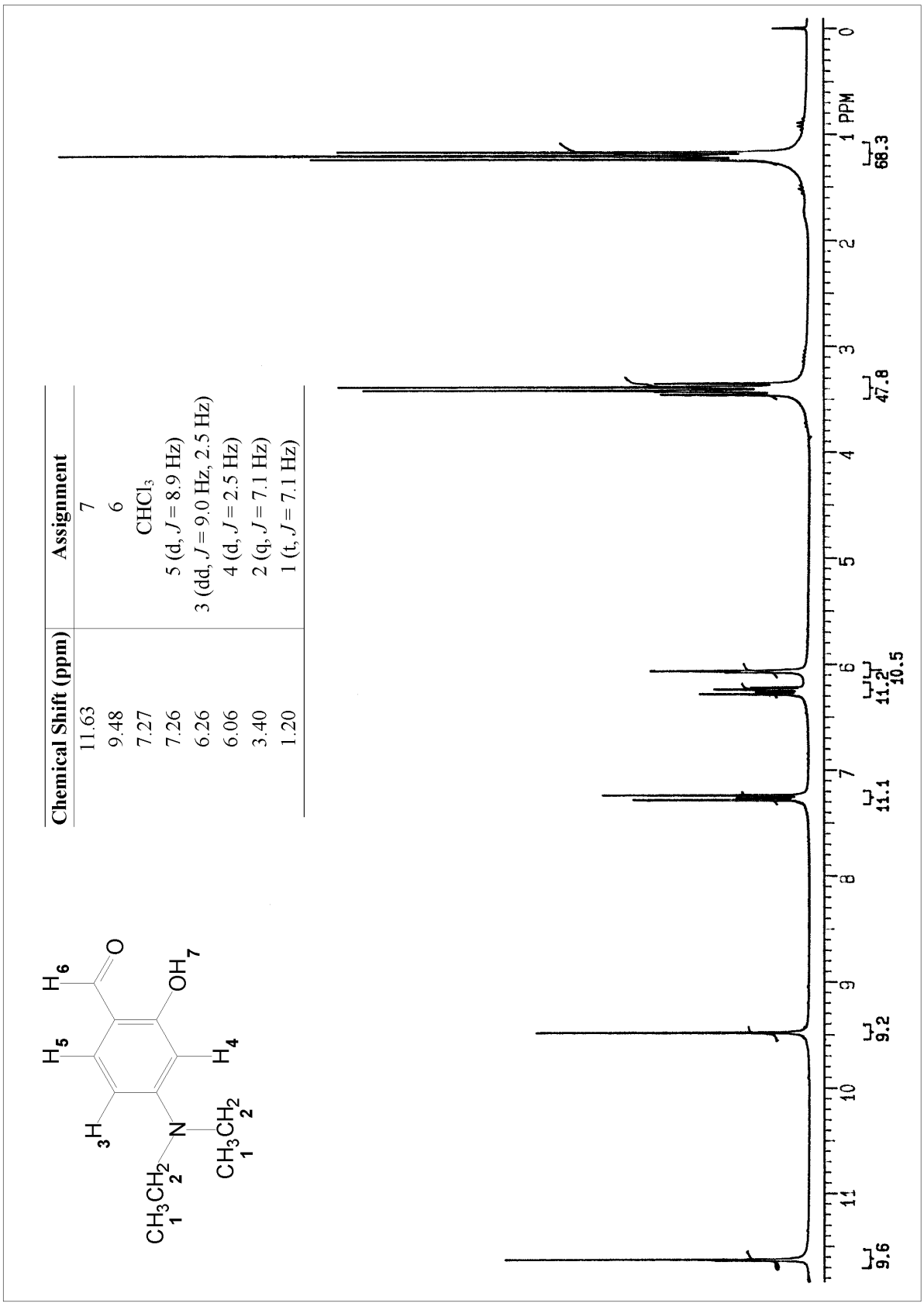


^{13}C NMR (400 MHz) of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one ($CDCl_3$)

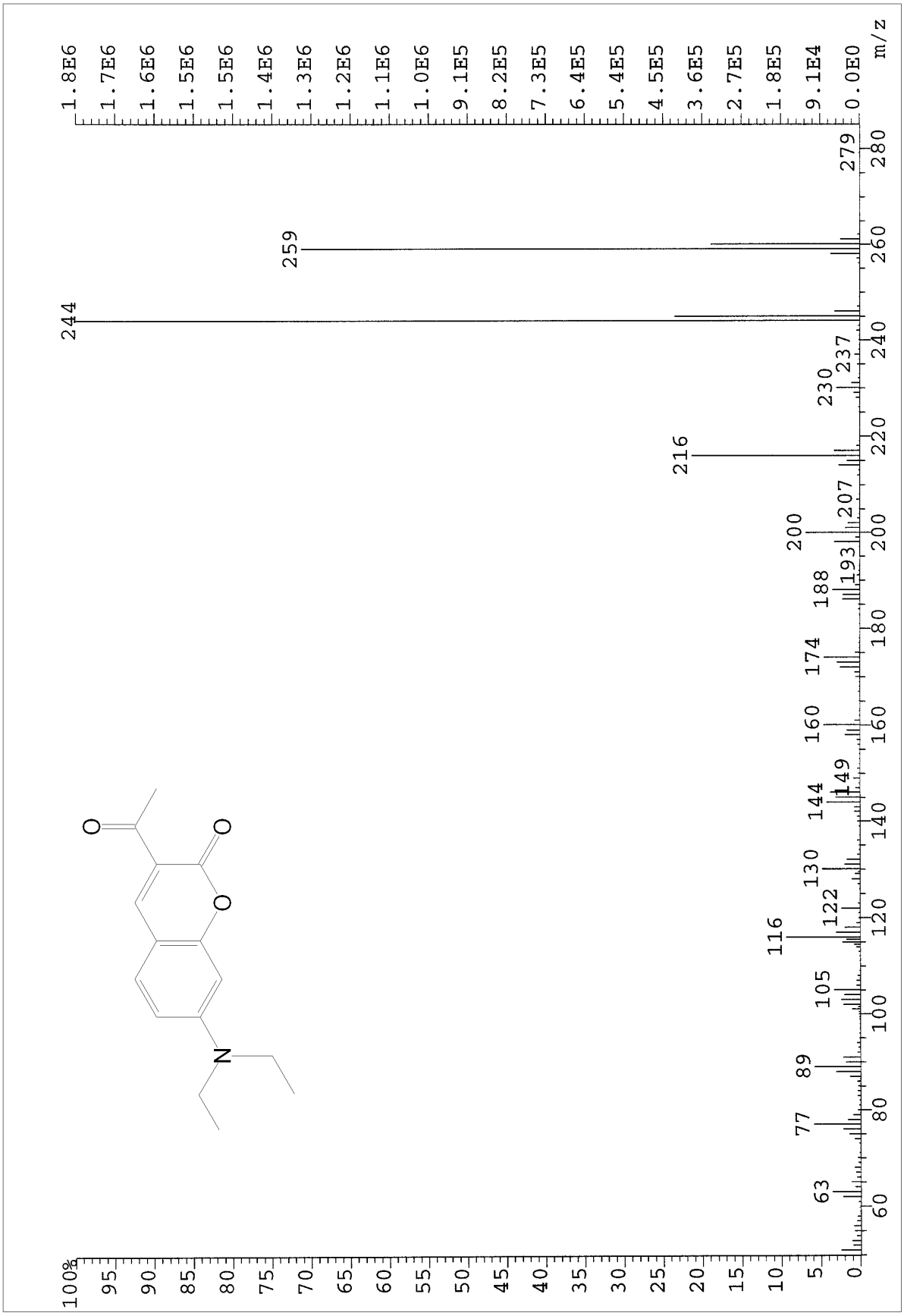


Chemical Shift (ppm)	Assignment
195.6	12
160.8	11
158.7	5
153.0	3
147.8	9
131.9	10
116.1	7
109.9	6
108.1	8
96.5	4
77.1	$CHCl_3$
45.2	2
30.6	13
12.5	1

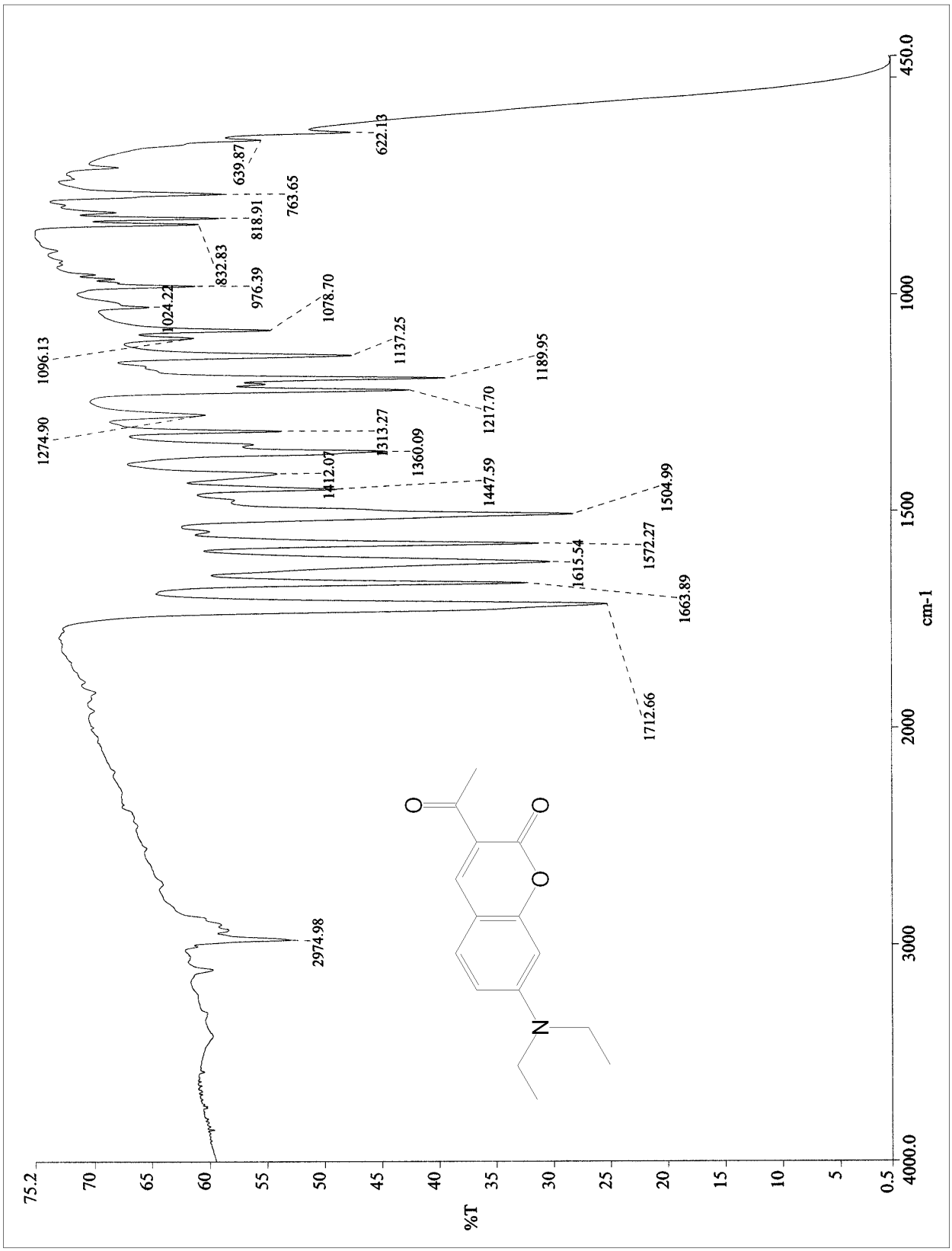
^{1}H NMR (200 MHz) of 4-(diethylamino)salicylaldehyde ($CDCl_3$)



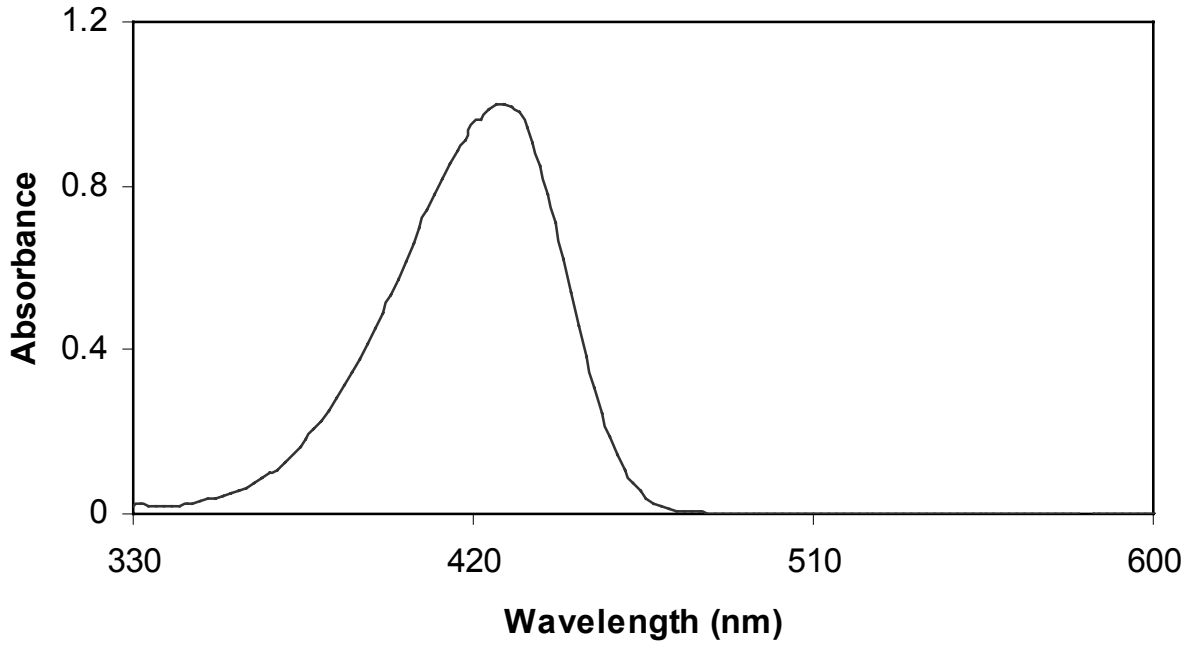
Mass spectrum (electron impact) of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one



Infra-red spectrum of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one (thin film from $CHCl_3$)



UV-visible spectrum of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one (acetone, 2.24 x 10^{-5} M, 1 cm path length)



Absorption and fluorescence spectra of 3-acetyl-7-(diethylamino)-2*H*-1-benzopyran-2-one (acetone, 2.24 x 10^{-9} M, 1 cm path length)