

SUPPLEMENTAL INFORMATION – Lab Documentation For On-Line Publication

Two-Step Semi-Microscale Preparation Of A Cinnamate Ester Sunscreen 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 - 7).
- 2. Additional Notes For Instructors** – chemical CAS numbers, equipment needs, synthetic notes and UV spectroscopy sample preparation (pages 8 - 10).
- 3. Spectroscopic Information** – Product $^1\text{H}/^{13}\text{C}$ NMR spectra and assignments, product IR, MS and UV spectra (pages 11 - 21).

Laboratory Notes For Students

Estimated Length Of Experiment: Session 1: 2.5 – 4 hours

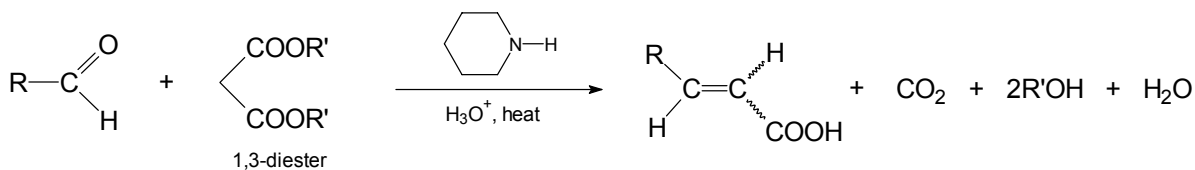
Session 2: 2.5 – 4 hours

Experimental Objectives – Session 1

1. To synthesize a stereoisomer of 4-methoxycinnamic acid (3-(4-methoxyphenyl)-2-propenoic acid) via a Verley-Doebner reaction.
2. To characterize the reaction product by ^1H NMR, IR and UV spectroscopy.
3. To deduce whether the product is the *cis* or *trans* stereoisomer.

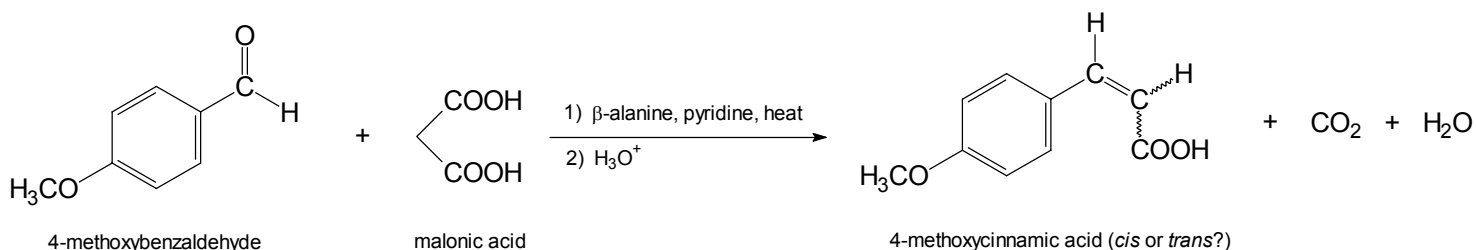
Background

The Knoevenagel condensation reaction dates from 1896 and is a facile one-step method of forming new carbon-carbon bonds under mild reaction conditions. It utilizes a weak amine base such as piperidine to initially form an enolate anion derived from a 1,3-dicarbonyl compound. The enolate subsequently acts as a nucleophile in the condensation with another carbonyl compound. This carbonyl is often an aldehyde and the reaction mechanism bears striking similarity to that of the aldol condensation. The isolated product is generally an α,β -conjugated carboxylic acid, formed via ready dehydration of the intermediate β -hydroxydicarbonyl compound and subsequent saponification and decarboxylation.



A Generalized Knoevenagel Condensation

The Verley-Doebner modification of this condensation employs pyridine as solvent and catalyst and a co-catalyst such as β -alanine. With this procedure malonic acid itself, rather than its diester can be efficiently condensed with 4-methoxybenzaldehyde (*p*-anisaldehyde) to generate 4-methoxycinnamic acid. This compound is an important precursor in the synthesis of UVB light (290 – 320 nm) absorbers found in sunscreens. The reaction is stereoselective in that either the *cis* or the *trans* isomer of 4-methoxycinnamic acid is formed exclusively. You will interpret a combination of physical measurements to conclude which stereoisomer you have generated.



Verley-Doebner Modification Of Knoevenagel Condensation

Safety Notes

Wear eye protection, a laboratory coat and protective gloves during this experiment. All liquid reactants and solvents are highly flammable. Pyridine is foul smelling and toxic by inhalation, ingestion and skin absorption. It is also related to long-term liver, kidney and central nervous system damage. Hydrochloric acid causes severe burns. Malonic acid is harmful if swallowed and poses a risk of serious eye damage. 4-Methoxybenzaldehyde and product 4-methoxycinnamic acid are irritating to the eyes, respiratory system and skin.

CAUTION - PERFORM ALL SYNTHETIC, EXTRACTION 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-methoxybenzaldehyde	136.15	804 μ L	6.61	-1	248	1.119
β -alanine	89.09	100 mg	1.12	205		
malonic acid	104.06	1.75 g	16.8	135-137		
pyridine	79.10	3 mL	37.1	-42	115	0.978
hydrochloric acid	36.46	8 mL				1.200

1. **IN A FUMEHOOD**, place the following in a 25 mL round bottomed flask: 4-methoxybenzaldehyde (804 μ L – automatic delivery pipette); β -alanine (100 mg); malonic acid (1.75 g); and pyridine (3 mL – automatic delivery pipette). Add a boiling chip to prevent bumping.
2. Heat the mixture under reflux (water condenser) for 90 minutes, using a sand bath as heat source. Note the vigorous evolution of a gas (what is it?).
3. Remove the flask from the heat source and allow cooling to room temperature. Cool further in an ice bath for ten minutes.
4. **SLOWLY, AND WITH CARE**, add concentrated HCl (8 mL, in 2 mL portions) to the flask. A white precipitate should form.
5. Collect the product by vacuum filtration using a Buchner funnel. Wash the filter cake with ice cold water (2 x 10 mL). Leave the solid under vacuum on the filter funnel for ten minutes to assist drying.
6. Remove the solid from the funnel and spread onto a watchglass until the next laboratory period to dry. Weigh the product and calculate the percentage yield. Take appropriate physical measurements (mp, UV, IR, ^1H NMR spectra) and identify whether you have synthesized the *cis* or *trans* stereoisomer of 4-methoxycinnamic acid.

SUBMIT A SAMPLE OF YOUR SYNTHESIZED PRODUCT WITH YOUR REPORT

Clean-Up

Dispose of all waste into the appropriately marked containers in the fumehoods. Dismantle and clean all glassware with soap and water.

Laboratory Report

Your report should contain the following points:

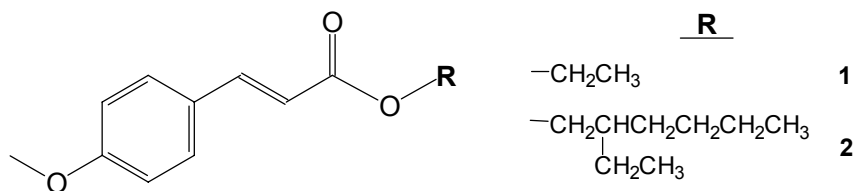
1. Discussion of the Verley-Doebner condensation performed, including
 - (i) a “curved-arrow” reaction mechanism for formation of 4-methoxycinnamic acid, focusing on
 - a) the role of pyridine and β -alanine
 - b) the C-C bond forming reaction step
 - c) the loss of CO_2 (decarboxylation) step
 - (ii) the calculated percent yield
 - (iii) identification of product alkene geometry, based on measured melting point.
2. Discussion of spectral data obtained, including
 - (i) an IR spectrum analysis (in terms of product absorbances and differences from the IR spectrum of 4-methoxybenzaldehyde)
 - (ii) an interpretation of the proton NMR of 4-methoxycinnamic acid (with respect to chemical shifts, spin-spin splitting patterns and coupling constants (J values)). Can you confirm the product alkene geometry from this spectrum? How?

Experimental Objectives – Session 2

1. To synthesize and characterize a sunscreen analog, ethyl *trans*-4-methoxycinnamate ((*E*)-ethyl 3-(4-methoxyphenyl)-2-propenoate) by an esterification reaction.
2. To learn about the “cesium effect” in organic synthesis.
3. To appreciate the relationship between the structure of UV light absorbers and their physical properties.

Background

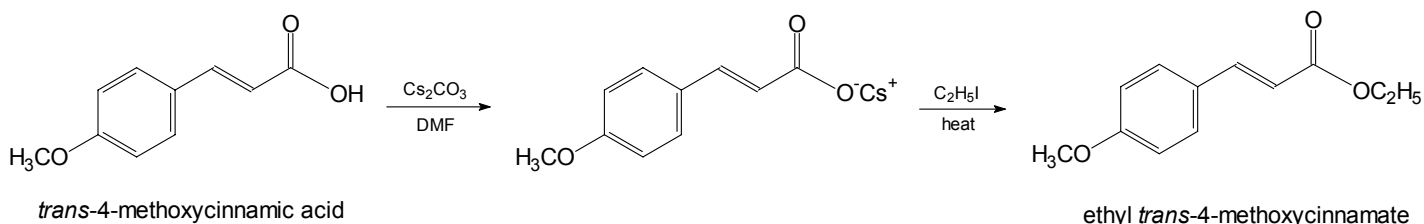
Esters derived from *trans*-4-methoxycinnamic acid (synthesized during the previous laboratory period, $\text{R} = \text{H}$) are effective absorbers of UV radiation.



UV Light Absorbers Based On *trans*-4-Methoxycinnamic acid

The most abundant of these esters is the 2-ethylhexyl derivative (**2**), which is found in many sunscreen preparations such as Bain de Soleil All Day Sunblock, Coppertone Sport and Solbar Shield. This compound is a liquid at room temperature. The structurally similar ethyl ester of *trans*-4-methoxycinnamic acid (**1**, not found in sunscreens) is however a low melting point solid and is synthesized today due to its ease of isolation. The ¹H NMR spectrum of this substance is also particularly interesting, illustrating important concepts of proton deshielding and spin-spin splitting.

One of the many approaches to ester synthesis is the S_N2 reaction of a carboxylate anion with an unhindered alkyl halide. Although sodium salts of these anions are straightforward to prepare, they are often unreactive in this regard. A useful alternative is to form the **cesium** carboxylate by addition of cesium carbonate to the carboxylic acid. These anions often undergo *O*-alkylation smoothly to form esters in good yield. This phenomenon is known as the “cesium effect”. In this experiment, the cesium salt of *trans*-4-methoxycinnamic acid is formed *in situ* and reacted with an alkyl iodide to effect the esterification.



Safety Notes

Wear eye protection, a laboratory coat and protective gloves during this experiment. All liquid reactants and solvents are highly flammable. DMF is toxic by inhalation, ingestion and skin absorption, and is additionally a teratogen. Hydrochloric acid causes severe burns. Iodoethane is harmful by inhalation and a vesicant. Cesium carbonate, *trans*-4-methoxycinnamic acid and product ethyl *trans*-4-methoxycinnamate are irritating to the eyes, respiratory system and skin.

CAUTION - PERFORM ALL SYNTHETIC, EXTRACTION 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)
<i>trans</i> -4-methoxycinnamic acid	178.19	600 mg	3.36	170-173		
cesium carbonate	325.82	1.65 g	5.06			
iodoethane	155.97	1 mL	12.5	-108	69-73	1.95
hydrochloric acid (1M)	36.46	4 mL				1.017
<i>N,N</i> -dimethylformamide (DMF)	73.10	10 mL		-98	153	0.944
3:1 hexanes/ethyl acetate		2 x 10 mL				

- IN A FUMEHOOD**, dissolve *trans*-4-methoxycinnamic acid (600 mg) in dry *N,N*-dimethylformamide (DMF) (10 mL) in a 25 mL round bottomed flask.

2. Add cesium carbonate (1.65 g) to the flask in one portion, followed by iodoethane (1 mL – automatic delivery pipette). Introduce a small magnetic stir bar and cap the flask with a rubber septum.
3. Stir the heterogeneous mixture vigorously at 50°C for one hour (a yellow coloration may develop). After 10 minutes of this reaction time, remove a small aliquot (around five drops), place in a small vial and add five drops of 1 M HCl. Add five drops of 3:1 hexanes/ethyl acetate and mix the layers thoroughly. Perform TLC on the organic layer and starting material (stationary phase, silica gel; eluent, 5:1 hexanes/ethyl acetate; visualization, UV lamp, 254 nm). Run further TLC after 30 minutes and one hour.
4. After one hour reaction time, cool to room temperature and add 1 M HCl (4 mL). Decant the liquid from any solid Cs₂CO₃ remaining into a 50 mL Erlenmeyer flask.
5. Extract the liquid in a separatory funnel with 3:1 hexanes/ethyl acetate (2 x 10 mL). Wash the hexanes/ethyl acetate layer with saturated NaCl (20 mL) and collect in a 50 mL Erlenmeyer flask. Dry the extract with MgSO₄ and remove the drying agent by gravity filtration, collecting the filtrate in a 50 mL round bottomed flask.
6. Evaporate the solvent under vacuum to leave an oil. This oil solidifies on standing to form colorless or pale yellow prisms. Remove the solid from the flask, weigh and calculate the percentage yield.
7. Record the product melting point. Obtain UV, IR and ¹H NMR spectra.

SUBMIT A SAMPLE OF YOUR SYNTHESIZED PRODUCT WITH YOUR REPORT

Clean-Up

Dispose of all waste into the appropriately marked containers in the fumehoods. Dismantle and clean all glassware with soap and water.

Laboratory Report

Your report should contain the following points:

1. Discussion of the cesium base mediated esterification performed, including
 - (i) a “curved-arrow” reaction mechanism for formation of ethyl *trans*-4-methoxycinnamate
 - (ii) the calculated percent yield
 - (iii) reasons behind the “cesium effect” and the utility of cesium salts in organic synthesis (*I*)
 - (iv) two alternative methods of synthesizing ethyl *trans*-4-methoxycinnamate from *trans*-4-methoxycinnamic acid, including an advantage and disadvantage of each approach. What advantages does the cesium carboxylate *O*-alkylation method have?
2. Discussion of spectral data obtained, including
 - (i) an IR spectrum analysis (in terms of product absorbances and differences from the IR spectrum of *trans*-4-methoxycinnamic acid). The C=O stretch absorbance in ethyl acetate is at 1743 cm⁻¹. Can you rationalize any differences from the C=O stretch location in ethyl *trans*-4-methoxycinnamate?

- (ii) identification of each peak in the ^1H NMR product spectrum (calculate spin-spin coupling constants (J values) where appropriate). How do you know that esterification took place?
 - (iii) a comparison of the product UV spectrum with that of one obtained for *trans*-4-methoxycinnamic acid. Explain why, although both substances are potentially effective sunscreen components, the ester is more satisfactory in this role. (Hint – consider the UVB wavelength range).
3. The compound you have synthesized is in fact **not** used in any commercially available sunscreens. 2-Ethylhexyl *trans*-4-methoxycinnamate (octyl methoxycinnamate) is the cinnamate ester most commonly used (2). This chemical is available to you in the laboratory. Devise an experiment to show why this compound is preferred as a sunscreen to the ethyl ester you have synthesized. What beneficial properties does the longer hydrocarbon chain confer?

References

1. Aspects of the “cesium effect” are discussed in the following article:
Dijkstra, G.; Kruizinga, W. H.; Kellogg, R. M. *J. Org. Chem.*, **1987**, *52*, 4230-34.
2. A very informative article pertaining to the history and physical properties of sunscreens is published in the July 2001 issue of *Chemistry In Britain*, p. 58 – 61.

Additional Notes For Instructors

CAS Numbers - Verley-Doebner Synthesis

4-Methoxybenzaldehyde (*p*-anisaldehyde) [123-11-5]
β-Alanine (3-aminopropanoic acid) [107-95-9]
Malonic acid (1,3-propanedioic acid) [141-82-2]
Pyridine [110-86-1]
Hydrochloric acid [7647-01-0]
trans-4-Methoxycinnamic acid ((*E*)-3-(4-methoxyphenyl)-2-propenoic acid) [830-09-1]

CAS Numbers - Esterification Synthesis

trans-4-Methoxycinnamic acid ((*E*)-3-(4-methoxyphenyl)-2-propenoic acid) [830-09-1]
Cesium carbonate [534-17-8]
Iodoethane [75-03-6]
Hydrochloric acid [7647-01-0]
N,N-Dimethylformamide (DMF) [68-12-2]
Hexanes [73513-42-5]
Ethyl acetate [141-78-6]
Magnesium sulfate [7487-88-9]
Methanol [67-56-1]
Ethyl *trans*-4-methoxycinnamate ((*E*)-ethyl 3-(4-methoxyphenyl)-2-propenoate) [1929-30-2]
Octyl *trans*-4-methoxycinnamate ((*E*)-2-ethylhexyl 3-(4-methoxyphenyl)-2-propenoate) [83834-59-7]

Equipment Needs (Per Student) - Verley-Doebner Synthesis

1 x 25 mL round bottomed flask
1 x 10 mL measuring cylinder
Watchglass
Buchner funnel and flask
Reflux condenser
Heat source
Ice bath
Automatic delivery pipettes (set to 804 μL and 3 mL, can be shared)

Equipment Needs (Per Student) - Esterification Synthesis

1 x 25 mL round bottomed flask
1 x 50 mL round bottomed flask
4 x 50 mL Erlenmeyer flasks
50 mL separatory funnel
1 x 10 mL measuring cylinder
Glass funnel and filter paper
4 x small glass vials
4 x Pasteur pipettes
Magnetic stir bar
Silica gel TLC plates with fluorescent indicator (Sigma-Aldrich, product no. Z19,329-1)
UV lamp (254 nm)
Heat source
Automatic delivery pipette (set to 1 mL, can be shared)

Notes Regarding Synthesis Of *trans*-4-Methoxycinnamic acid

1. The methodology described for synthesizing this compound assumes that the product alkene geometry is unknown – this adds a nice investigative feature to the experiment. Simple modifications can be made to remove this aspect of the procedure.
2. The product is difficult to dry thoroughly - it is important that the solid is spread out on a watchglass or filter paper until the following laboratory session, when characterization can be performed. The product should be dry for the esterification reaction.
3. The product is usually of sufficient purity after washing with ice cold water on the Buchner funnel. Recrystallization can be undertaken if necessary using absolute ethanol (~ 20 mL).
4. “Authentic” *trans*-4-methoxycinnamic acid is available from Sigma-Aldrich, catalogue no. M1,380-7. We purchase this material as a stand-by in case the Verley-Doebner synthesis fails, although it is an extremely reliable reaction.

Notes Regarding Synthesis Of Ethyl *trans*-4-methoxycinnamate

1. The reaction may be performed under nitrogen gas with a slight improvement in yield. If this is undertaken a multi-necked reaction vessel is recommended.
2. The product is easily visualized under a 254 nm UV lamp with an R_f value of 0.48 (5:1 hexanes/ethyl acetate). Several TLC plates are run to monitor reaction progress.
3. The oil remaining after removal of the extraction solvent does not solidify immediately at times. This does not pose a problem as UV, IR and ^1H NMR spectra can be obtained from the oil without any difficulty.
4. The product (oil or solid) appears to be very pure at the end of the extraction. If a solid is obtained, and recrystallization practice is desired (!), ethyl *trans*-4-methoxycinnamate may be recrystallized from a small volume of methanol (< 1 mL).

Preparation Of Samples For UV Analysis

The UV absorption spectrum of *trans*-4-methoxycinnamic acid, ethyl *trans*-4-methoxycinnamate and 2-ethylhexyl *trans*-4-methoxycinnamate can be measured using the following procedure.

A stock solution of the appropriate compound is firstly prepared by dissolving the quantities below **SEPARATELY** in 10 mL 95% ethanol (volumetric flask):

trans-4-Methoxycinnamic acid: 14.5 mg
Ethyl *trans*-4-methoxycinnamate: 16.8 mg
2-Ethylhexyl *trans*-4-methoxycinnamate: 23.4 μL

50 μL of this stock solution is then dissolved in 10 mL 95% ethanol (volumetric flask). This corresponds to a concentration of 4.08×10^{-5} M for each compound.

Typical λ_{max} values and absorbances:

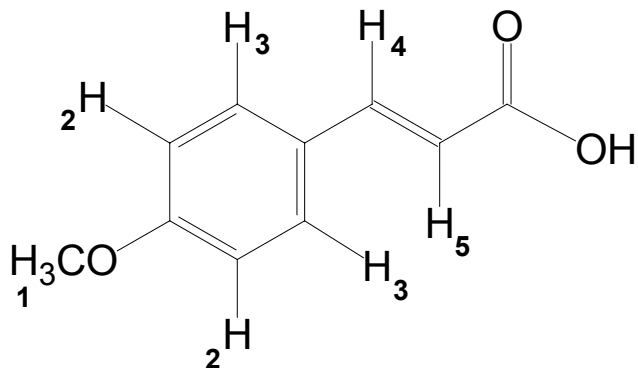
trans-4-Methoxycinnamic acid: $\lambda_{\text{max}} = 288$ nm, absorbance ~ 0.83 , $\epsilon = 2.03 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
Ethyl *trans*-4-methoxycinnamate: $\lambda_{\text{max}} = 310$ nm, absorbance ~ 1.01 , $\epsilon = 2.47 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (1)
2-Ethylhexyl *trans*-4-methoxycinnamate: $\lambda_{\text{max}} = 312$ nm, absorbance ~ 1.23 , $\epsilon = 3.01 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (2)

References

1. UV absorption literature values for ethyl *trans*-4-methoxycinnamate:
 $\lambda_{\text{max}} = 310 \text{ nm}$, $\epsilon = 2.67 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
Bloomfield, J. J.; Fuchs, R. *J. Org. Chem.*, **1961**, *26*, 2991-93.
2. UV absorption literature values for 2-ethylhexyl *trans*-4-methoxycinnamate:
 $\lambda_{\text{max}} = 303 \text{ nm}$, $\epsilon = 3.10 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
Tran, C.; Sorg, O.; Carraux, P.; Didierjean, L.; Siegenthaler, G.; Falson, F.; Saurat, J-H. *Photochem. Photobiol.*, **2002**, *75*, 1-5.

Spectroscopic Information

¹H NMR assignments for *trans*-4-methoxycinnamic acid

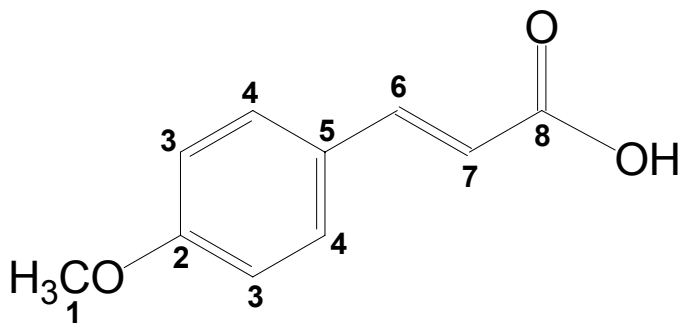


Chemical Shift (ppm)	Assignment
7.75	4 (d, $J = 16.0$ Hz)
7.51	3 (d, $J = 8.8$ Hz)
7.27	CHCl ₃
6.92	2 (d, $J = 8.8$ Hz)
6.33	5 (d, $J = 15.8$ Hz)
3.85	1

(signal for -COOH proton not observed)

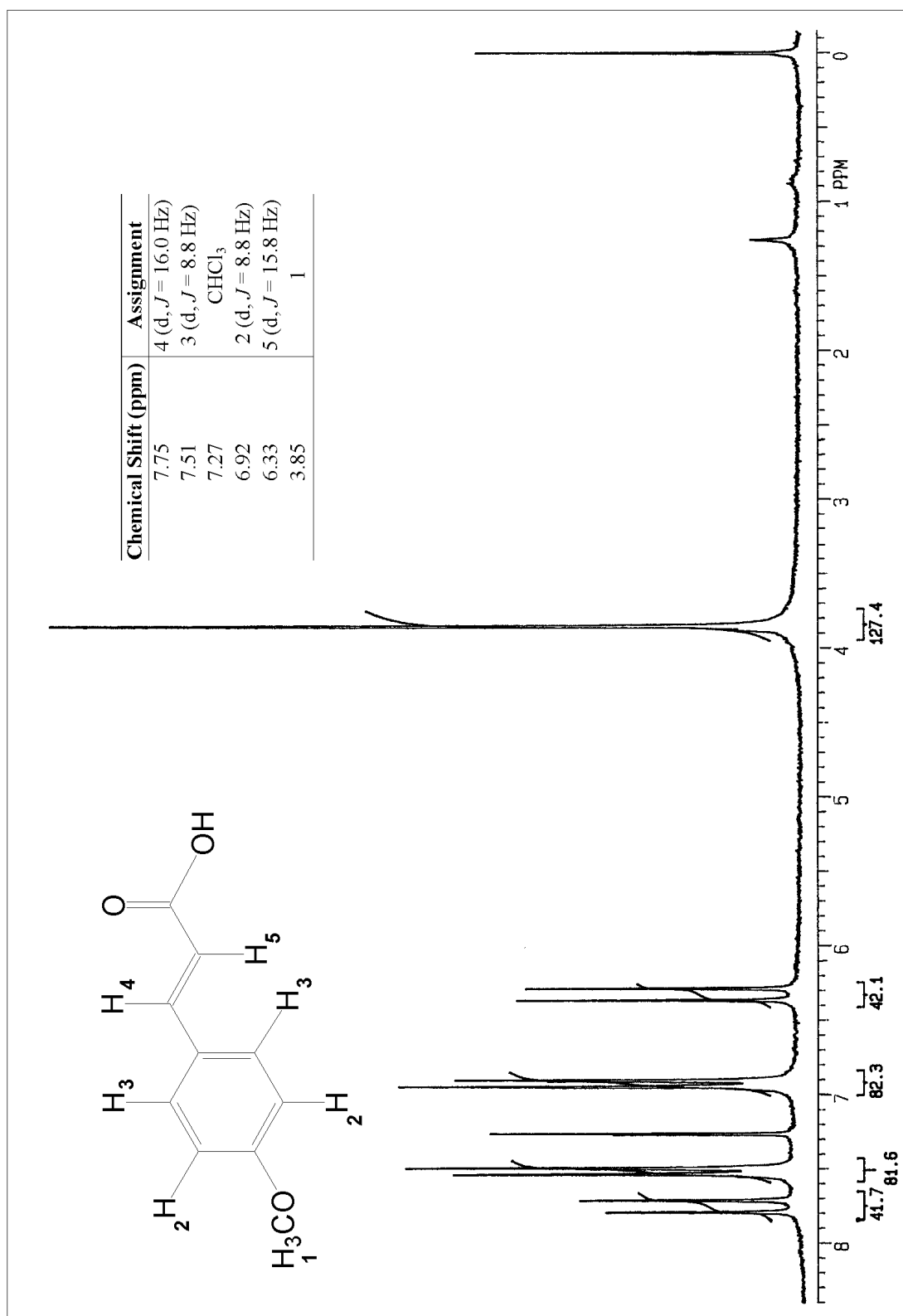
¹³C NMR assignments for *trans*-4-methoxycinnamic acid

(see SDBSWeb: <http://www.aist.go.jp/RIODB/SDBS/> (accessed September 2003))

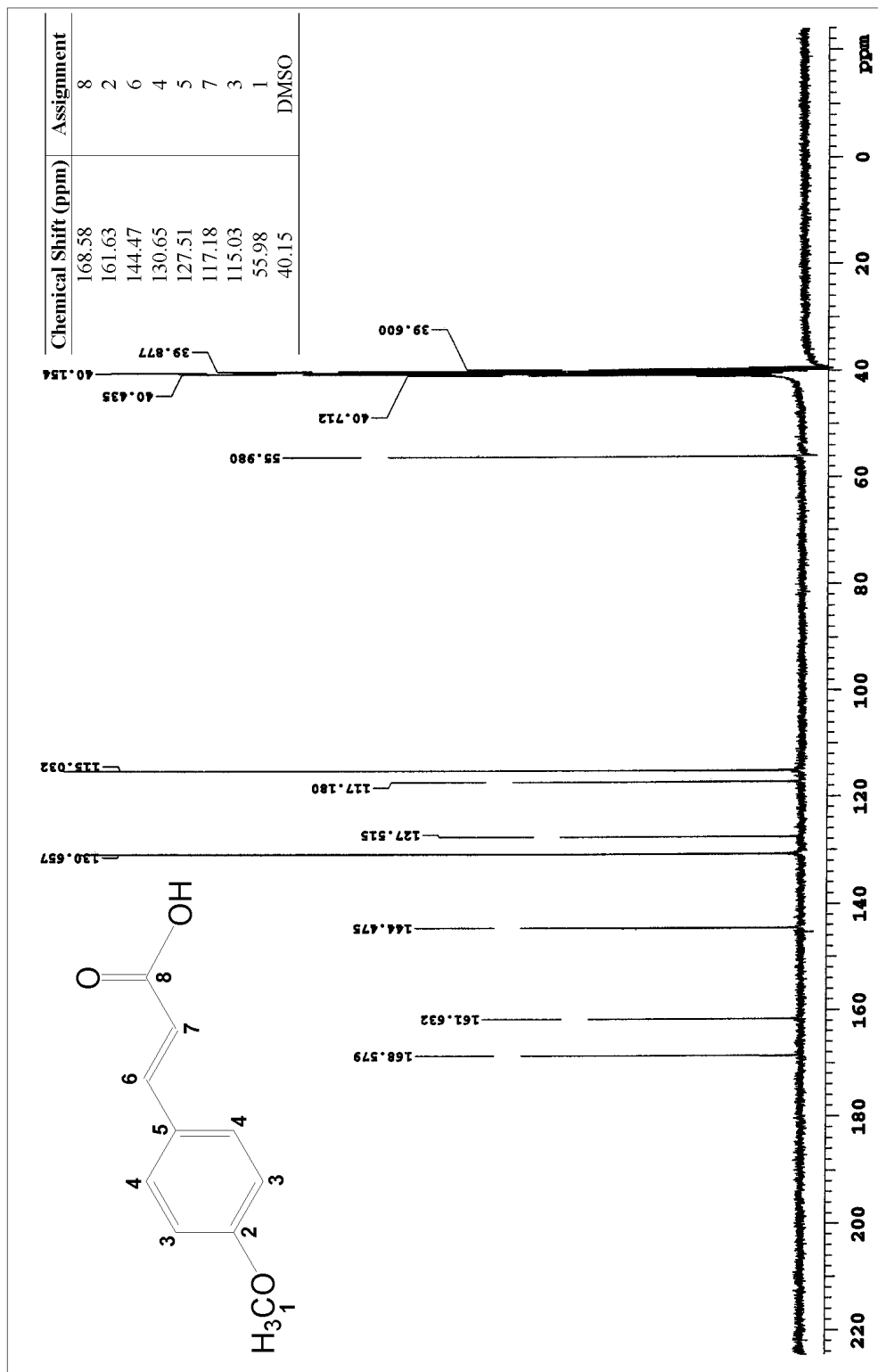


Chemical Shift (ppm)	Assignment
168.58	8
161.63	2
144.47	6
130.65	4
127.51	5
117.18	7
115.03	3
55.98	1
40.15	DMSO

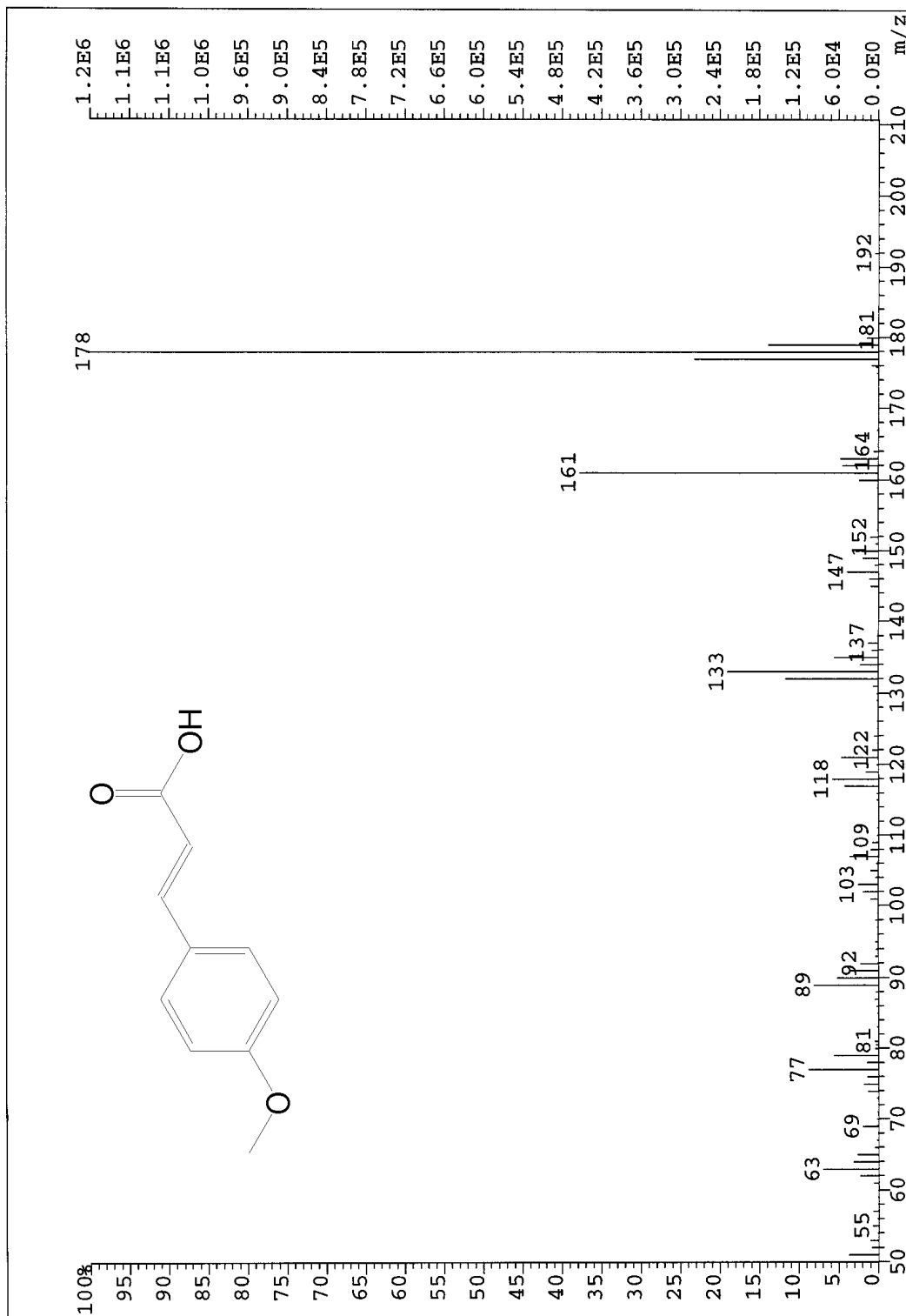
¹H NMR (200 MHz) of *trans*-4-methoxycinnamic acid (CDCl₃)



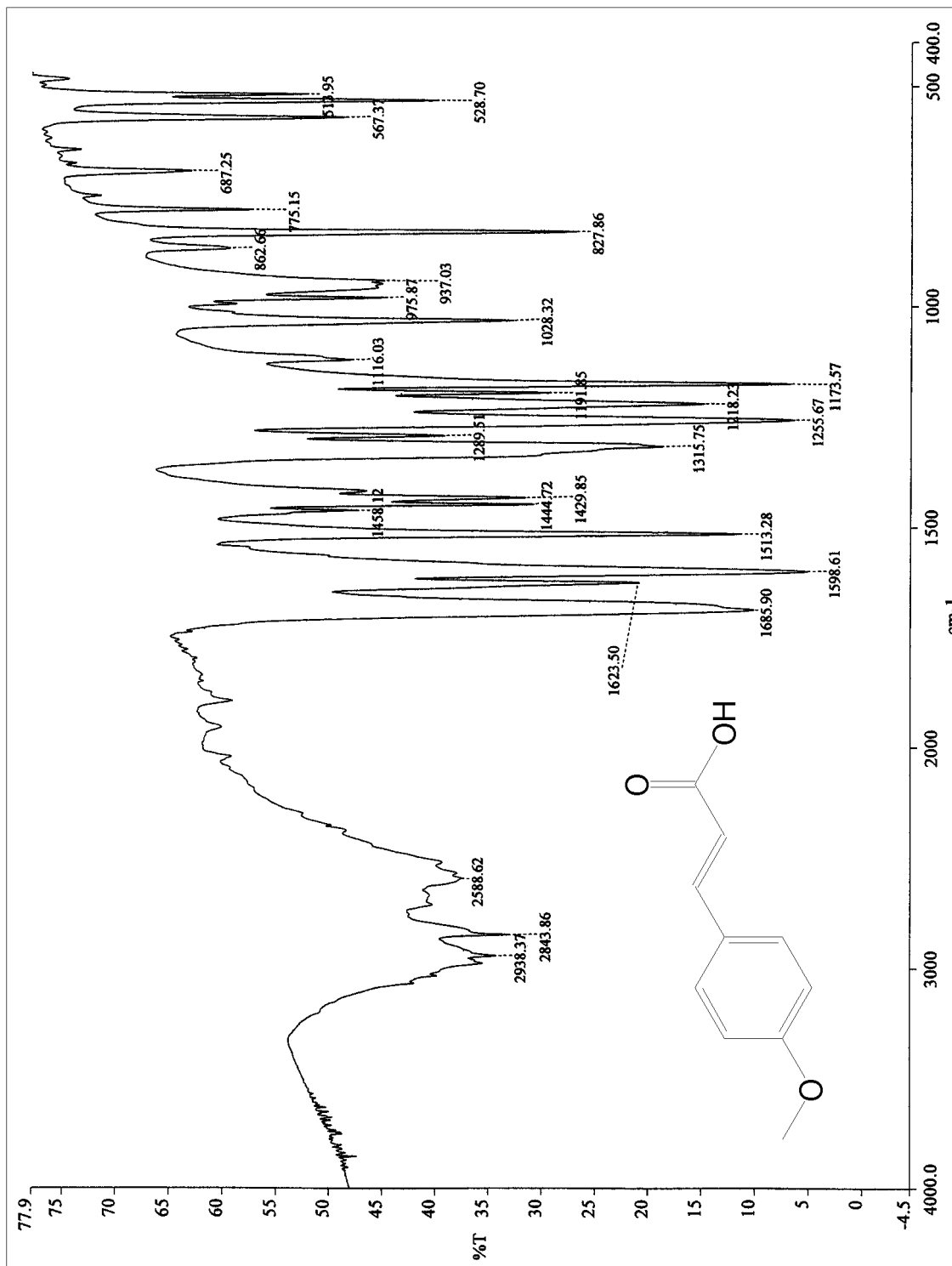
¹³C NMR (300 MHz) of *trans*-4-methoxycinnamic acid (DMSO-*d*₆)



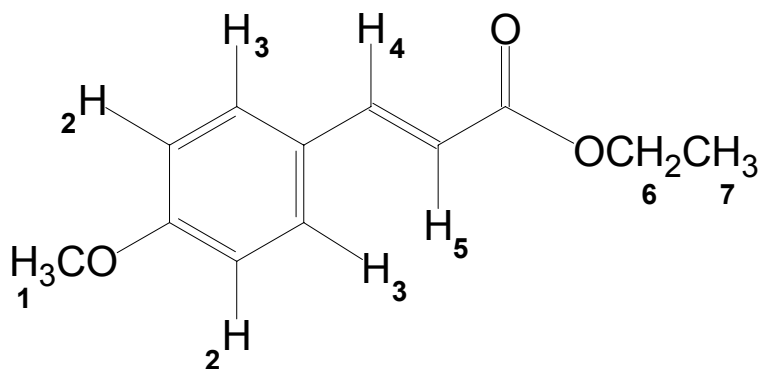
Mass spectrum (electron impact) of *trans*-4-methoxycinnamic acid



Infra-red spectrum of *trans*-4-methoxycinnamic acid (KBr disk)

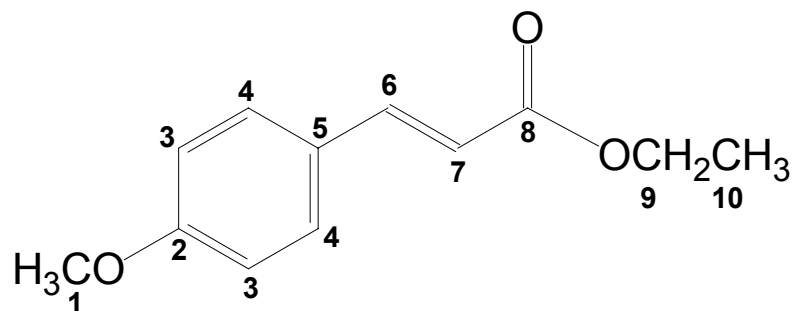


¹H NMR assignments for ethyl *trans*-4-methoxycinnamate



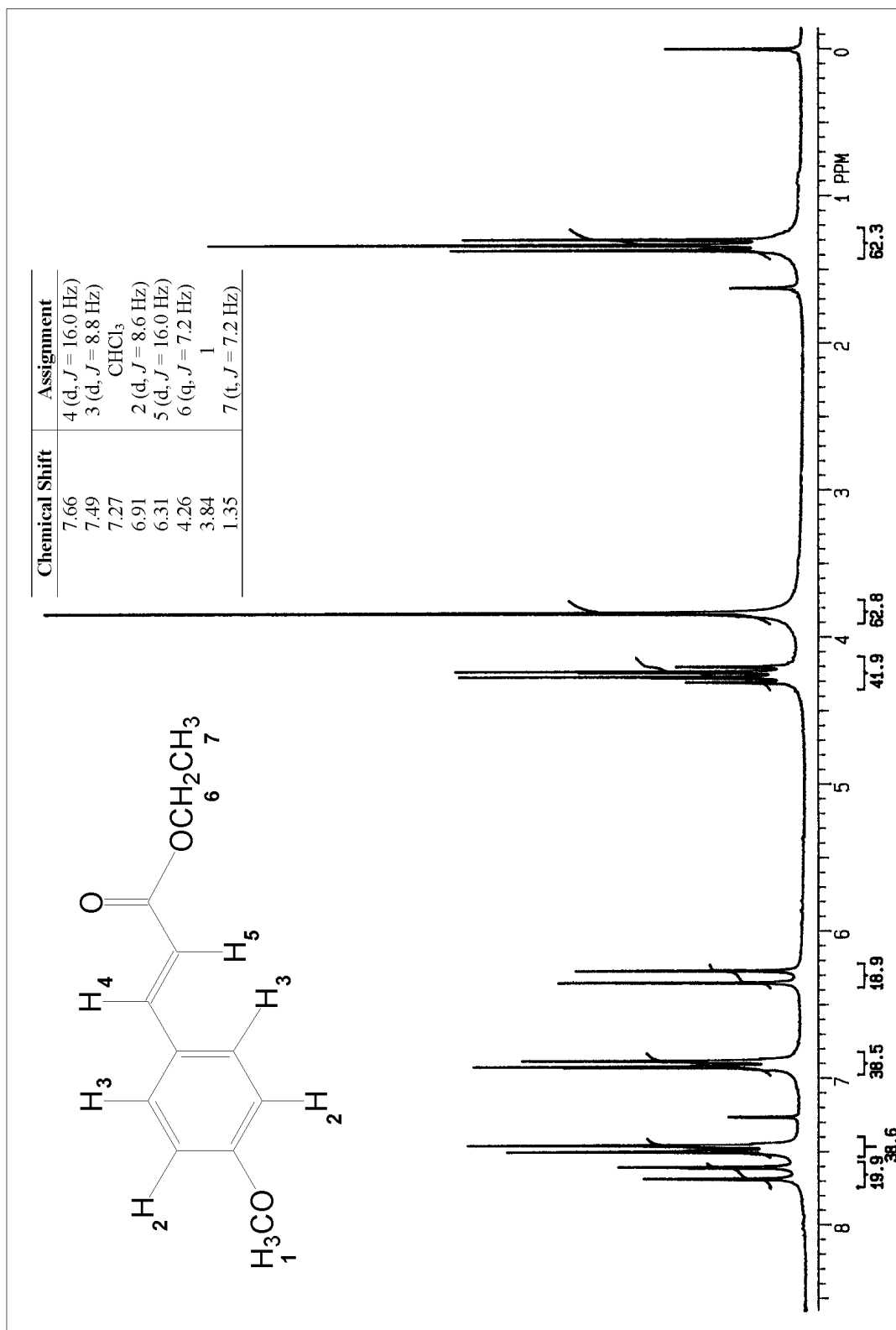
Chemical Shift (ppm)	Assignment
7.66	4 (d, $J = 16.0$ Hz)
7.49	3 (d, $J = 8.8$ Hz)
7.27	CHCl ₃
6.91	2 (d, $J = 8.6$ Hz)
6.31	5 (d, $J = 16.0$ Hz)
4.26	6 (q, $J = 7.2$ Hz)
3.84	1
1.35	7 (t, $J = 7.2$ Hz)

¹³C NMR assignments for ethyl *trans*-4-methoxycinnamate

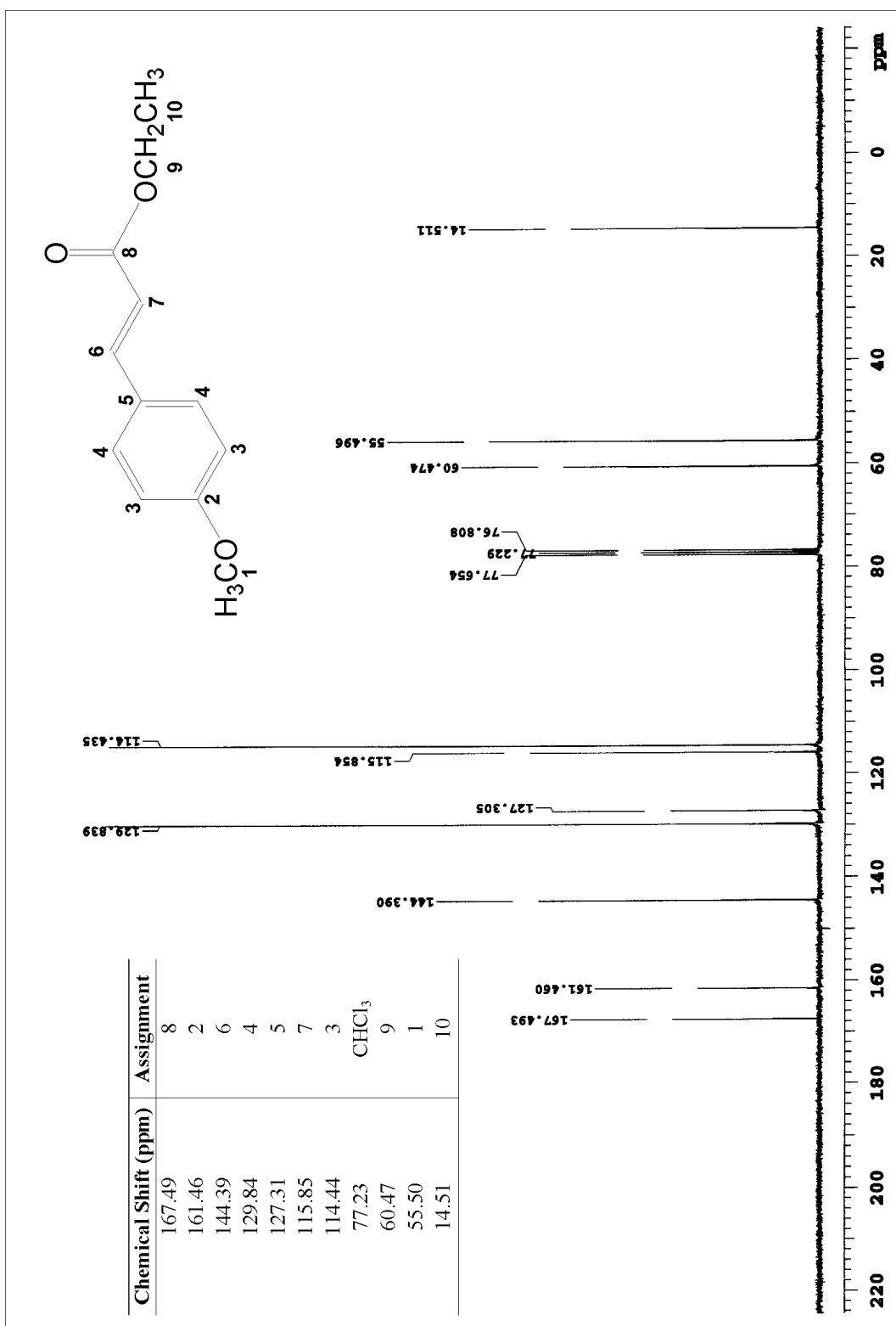


Chemical Shift (ppm)	Assignment
167.49	8
161.46	2
144.39	6
129.84	4
127.31	5
115.85	7
114.44	3
77.23	CHCl ₃
60.47	9
55.50	1
14.51	10

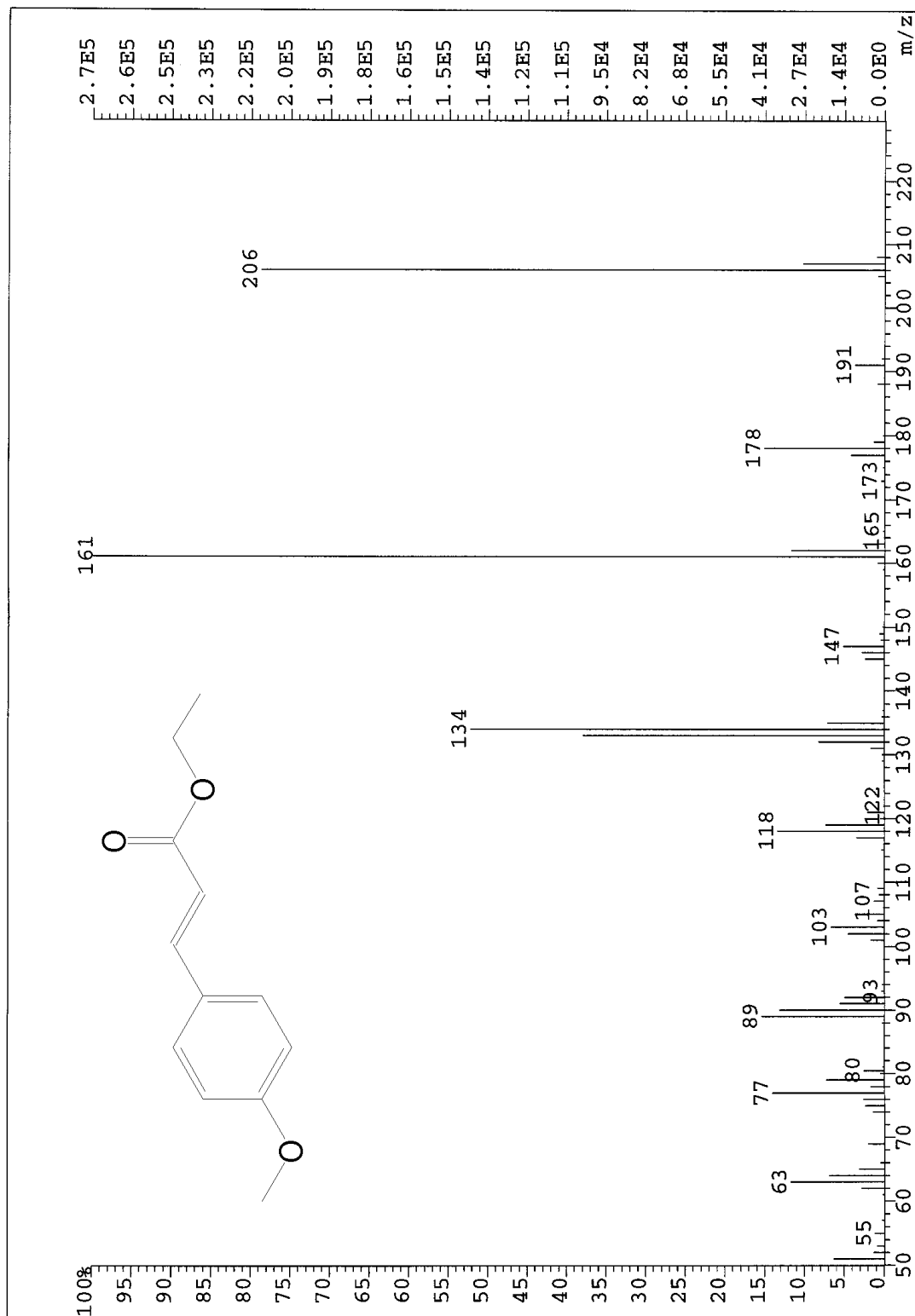
¹H NMR (200 MHz) of ethyl *trans*-4-methoxycinnamate (CDCl₃)



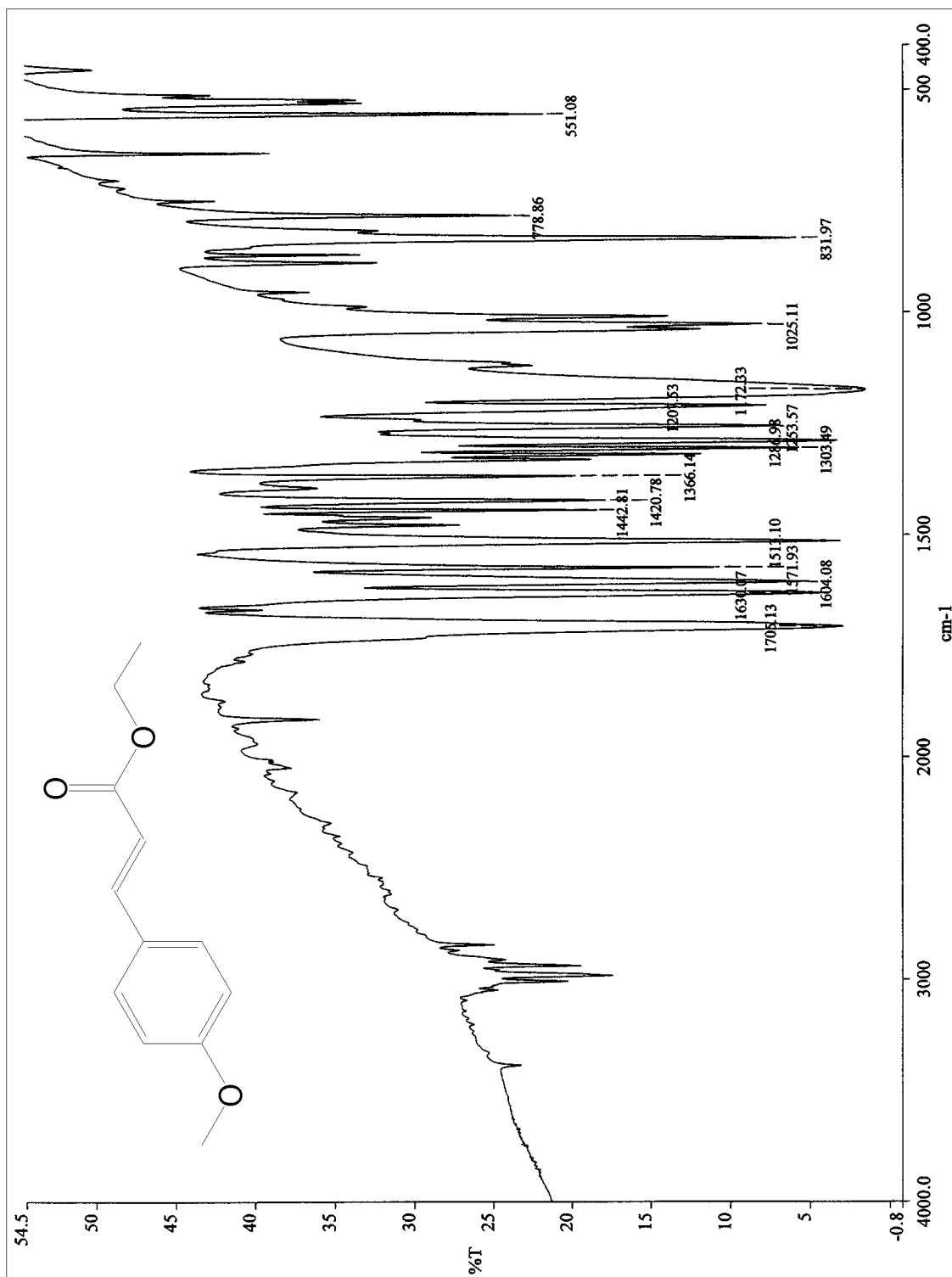
¹³C NMR (300 MHz) of ethyl *trans*-4-methoxycinnamate (CDCl₃)



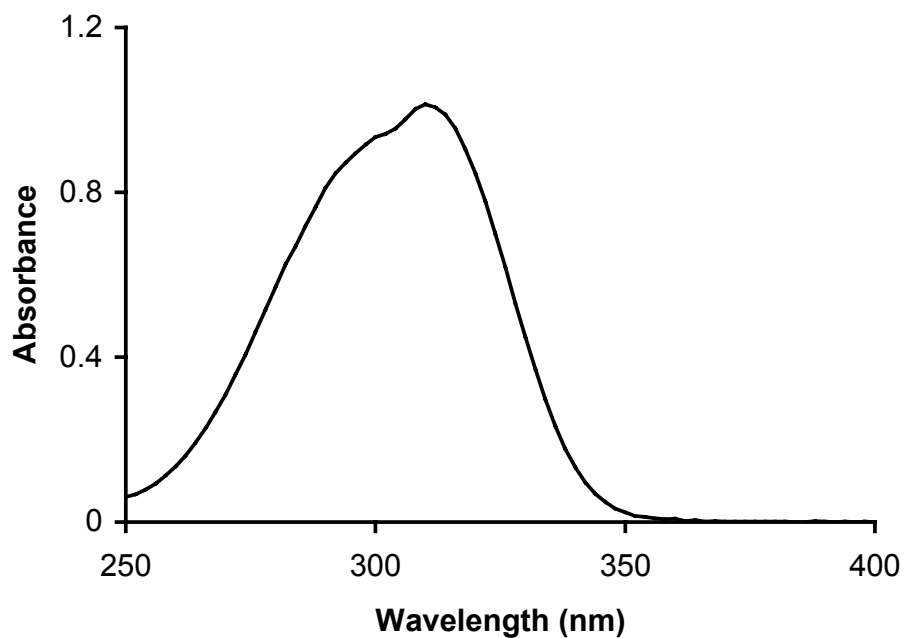
Mass spectrum (electron impact) of ethyl *trans*-4-methoxycinnamate



Infra-red spectrum of ethyl *trans*-4-methoxycinnamate (KBr disk)



UV spectrum of ethyl *trans*-4-methoxycinnamate (95% C₂H₅OH, 4.08 x 10⁻⁵ M, 1 cm path length)



UV spectrum of ethyl *trans*-4-methoxycinnamate, 2-ethylhexyl *trans*-4-methoxycinnamate and *trans*-4-methoxycinnamic acid (95% C₂H₅OH, all 4.08 x 10⁻⁵ M, 1 cm path length)

Acid: $\lambda_{\max} = 288 \text{ nm}$, $\epsilon = 2.03 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
Ethyl ester: $\lambda_{\max} = 310 \text{ nm}$, $\epsilon = 2.47 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
2-Ethylhexyl ester: $\lambda_{\max} = 312 \text{ nm}$, $\epsilon = 3.01 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$

