SYNTHESIS OF THE AB-RING PORTION OF LAVENDAMYCIN ANALOGS VIA THE DIELS-ALDER CONDENSATION

An Honors Thesis (HONRS 499)

by

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Purpose of Thesis

Lavendamycin analogs have demonstrated potential as cancer-fighting agents but because of their high degree of toxicity and low solubility in pharmacological solvents, new analogs are required which will exhibit selective toxicity for tumor cells and also be soluble in solvents for administration as cancer-fighting drugs. The goal of this research is to produce an analog of Lavendamycin (1) with a high selective toxicity for <u>ras</u>^{K} oncogene transformed cells and low toxicity for normal healthy cells. This can be accomplished through structure-activity relationship studies on a series of lavendamycins. To provide the series of analogs to be used in the studies, means by which various analogs may be produced must be devised.

The goal of this project is to synthesize the AB-ring portion of a 7-N-butyryllavendamycin via a Diels-Alder condensation. The synthesis of 7-butyramido-2-methyl-quinoline-5,8-dione (12) from the precursors 2-butyramido-6-bromobenzoquinone (6) and N-(O-*t*-butyldimethylsiloxy)-1-aza-2-methyl-1,3-butadiene (5) is the goal of this project. The Diels-Alder condensation is a general method for the production of a variety of quinolines and will lead to quinolines which are substituted at the C-7 as well as the C-6 positions. The latter compounds are unavailable through our other synthetic schemes.

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Acknowledgments

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Mass spectral data were obtained by Mr. Robert Guillaud and for his assistance I am very grateful. Without his assistance, such data would have been inaccessible to me without a great deal of trouble.

Mrs. Wen Cai was immensely helpful at every point during this project. She provided a concise synthesis of one of the required compounds (5), provided the NMR spectrum for a compound I had difficulty purifying (6), and was available whenever her experience and expertise were required to propel this project forward. For her guidance and encouragement, I am grateful.

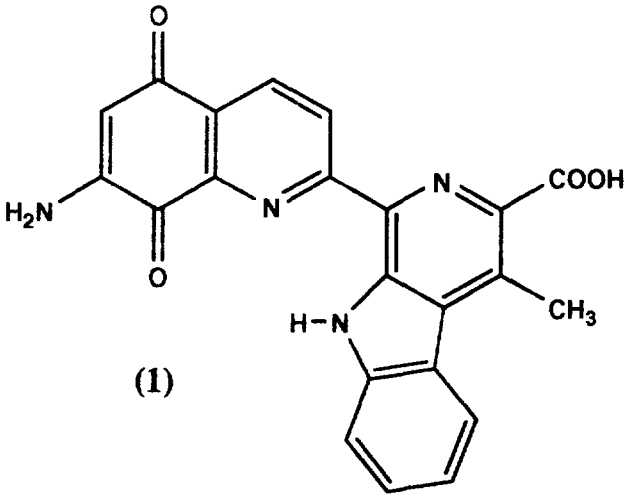
In addition to those individuals who helped with the mechanics of the research and writing of this thesis, I would also like to indicate my gratitude to those persons who made all of this possible. I want to thank my parents, Steven and Donna Scherschel, without whom I wouldn't be, for their financial and moral support throughout the many years of this and other projects. I would also like to recognize Dr. Linda Hanson for her hospitality and generosity with parking space. Without her, I may very well have starved to death before finishing this thesis.

I. Historical Overview

A. Background of Lavendamycin

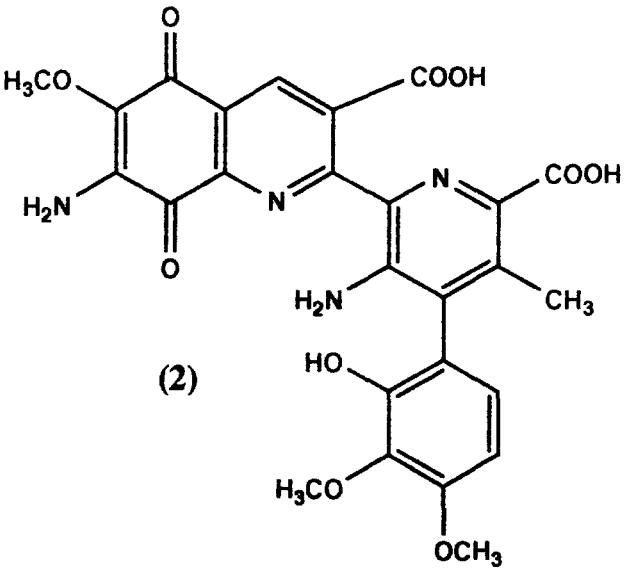
Lavendamycin (1) was first isolated from the fermentation broth of Streptomyces lavendulae strain cc22030 in 1981 by Doyle, Nettleton, Balitz, and Grulich, researchers at Bristol Laboratories.[1] This compound was shown to exhibit antitumor activity but because of its extreme toxicity, lavendamycin cannot be used clinically as an antitumor agent. [2, 3] Lavendamycin is a dark red, crystalline solid with a melting point above 300°C and is insoluble in most organic solvents[1] and is structurally very similar to another potent cytotoxic compound, streptonigrin (2).

Mechanisms have been proposed by several groups to explain the cytotoxicity of streptonigrin and other quinones.[4-10] These mechanisms fall within two distinct classes of effects including those which interfere with the respiratory mechanism, and those which



(1)

LAVENDAMYCIN



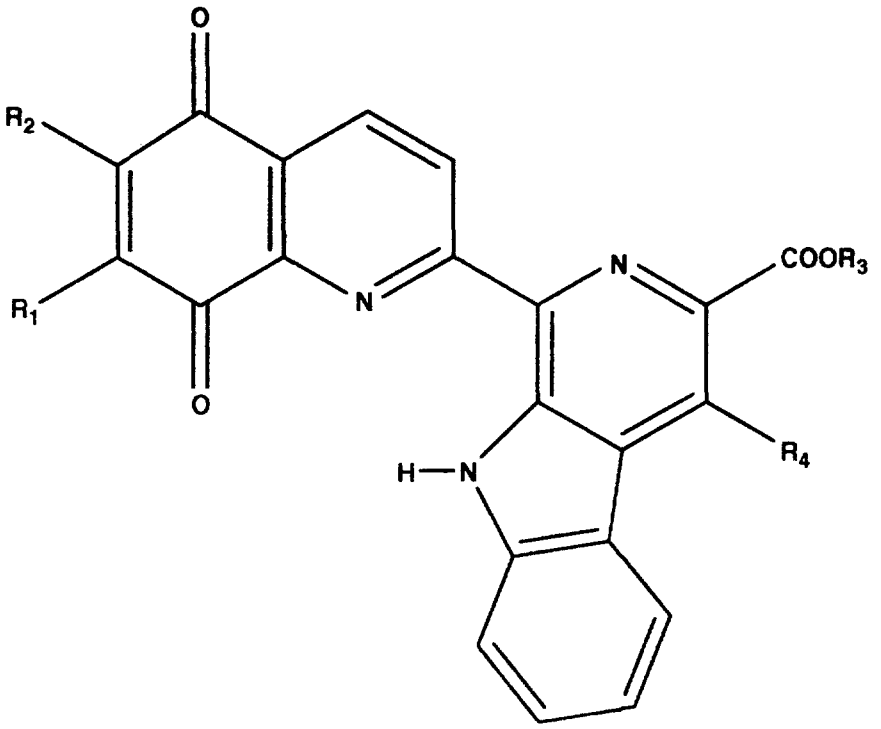
(2)

STREPTONIGRIN

affect the replication mechanisms of the cells.[7] The mechanisms which interfere with cellular respiration affect the electron transport system in the inner mitochondrial membrane.[4-6,9] Quinones have been shown to damage exposed DNA by cleaving the double helix at various places within the base sequence.[7,8,10] At this time no single mechanism seems to explain fully the cytotoxicity of these compounds.

The primary goal of this research is the development of an analog of lavendamycin which possesses a high selective toxicity for tumor cells and which will not injure normal, healthy cells. To accomplish this goal, a series of structure-activity relationship (SAR) studies are in progress to determine the affects of alterations in the lavendamycin parent structure on its cytotoxic and antitumor activities. Streptonigrin has proven to exhibit strong antibiotic as well as antitumor activity. Lavendamycin, although less effective as an antibiotic, has demonstrated promising antitumor capabilities against <u>ras</u>^{K} oncogenic tumors.[11]

Skeletal Structure



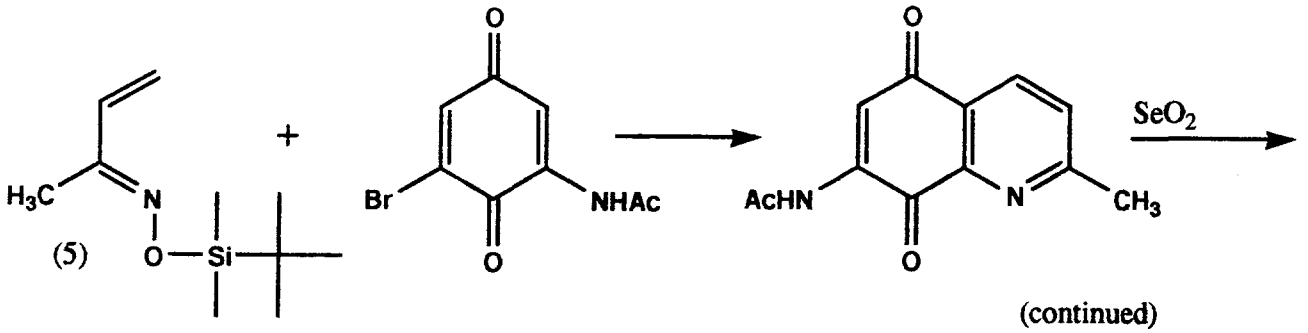
Compound	R_1	R_2	R_3	R_4
1	NH_2	H	H	CH_3
3	NH_2	H	CH_3	CH_3
4	NHR	H	CH_3	CH_3

R=$COCH_2CH_2CH_3$

B. Synthesis of Lavendamycin and Analogs

Lavendamycin methyl ester (3) has been prepared by three groups. The compound was first prepared in 1984 by Kende and Ebetino, researchers at the University of Rochester, using β-methyltryptophan as a precursor.[12] In 1985, Boger and associates reported another method for the preparation of lavendamycin methyl ester (3).[13] These two synthetic methods involved nine and twenty steps respectively and resulted in an overall yield of less than 2%. For the last several years, Mohammad Behforouz of Ball State University has been working to find a more concise synthesis of lavendamycin methyl ester and several analogs using the Pictet-Spengler condensation. In 1993, a group of researchers at Ball State University led by Behforouz reported a very efficient synthesis of lavendamycin methyl ester (3).[14] The method outlined by Behforouz *et al.* provides a practical method for the synthesis of lavendamycin methyl ester and various analogs (Scheme 1). This new method provided short and efficient syntheses for a variety of lavendamycin analogs and enabled researchers at Ball State University in collaboration with investigators at Eli Lilly to perform SAR studies of several N-acyl analogs.[11]

Scheme 1



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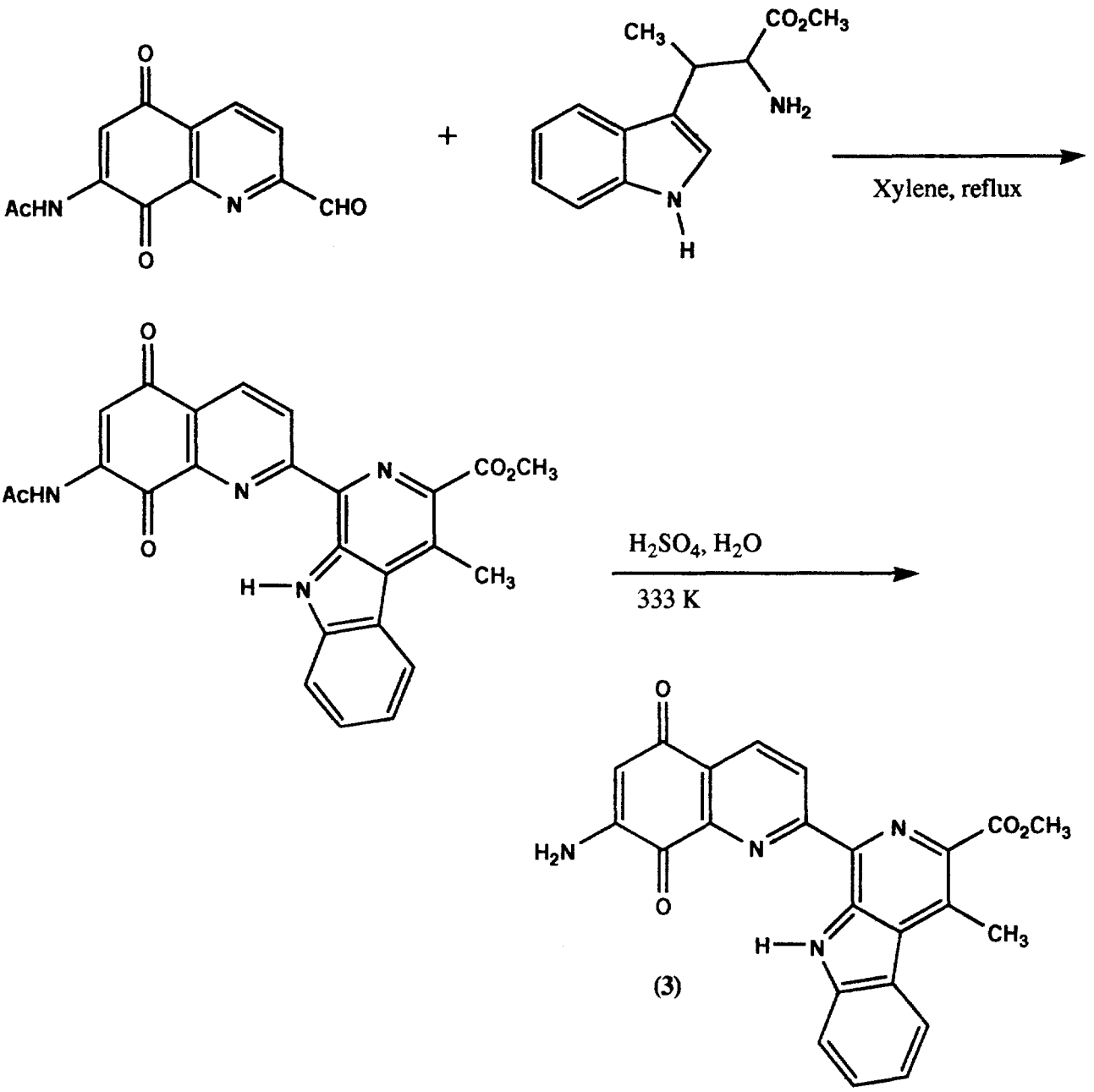
Scheme 1 (Continued)



LAVENDAMYCIN METHYL ESTER

II. Synthesis of the AB-ring System of Various Lavendamycin Analogs Via Diels-Alder Condensations

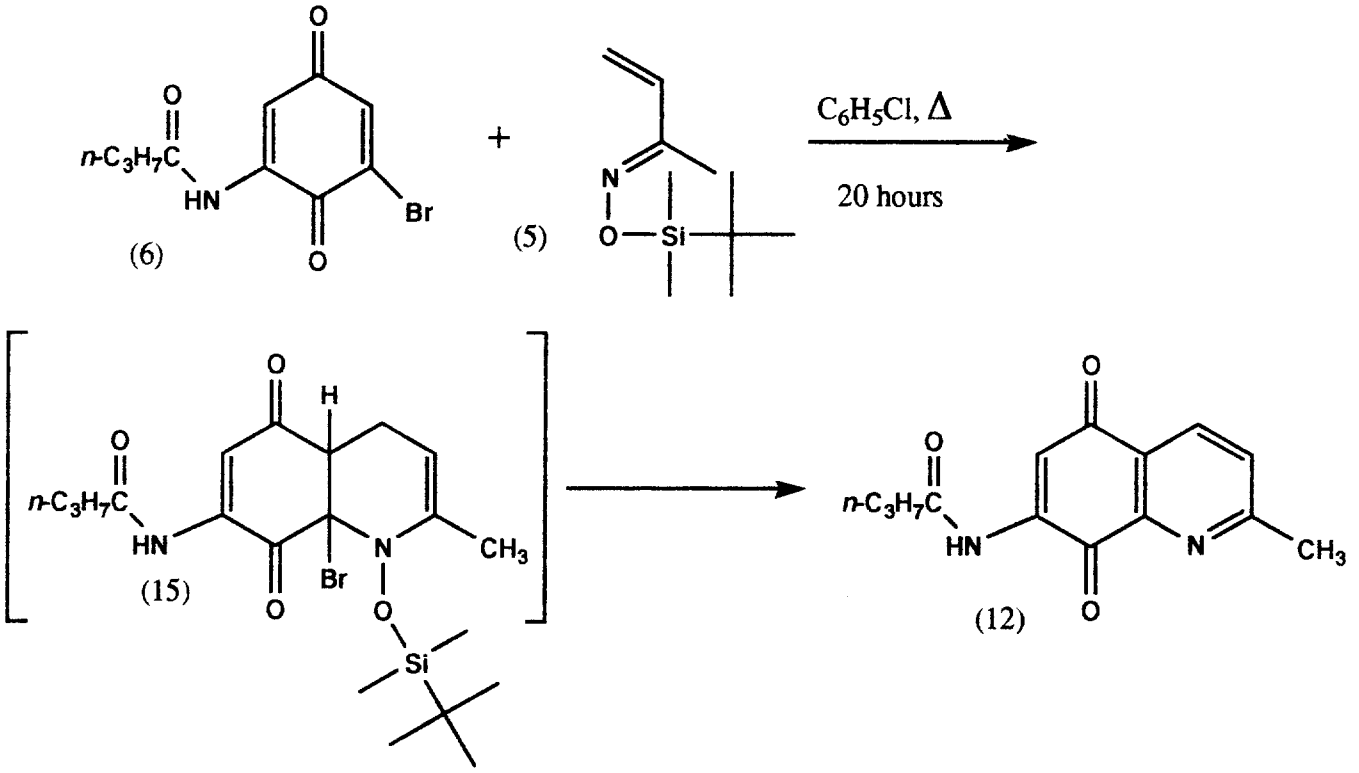
The overall goal of this research is to synthesize derivatives of lavendamycin (1) that are soluble in pharmacological solvents and that exhibit high selective toxicity for tumor cells as compared to normal parent cells. The goal of this project is to synthesize 7-butyramido-2-methylquinoline-5,8-dione via a Diels-Alder condensation of 2-butyramido-6-bromobenzoquinone (6) with N-(O-*t*-butyldimethylsiloxy)-1-aza-2-methyl-1,3-butadiene (5). The 7-butyramido-2-methylquinoline-5,8-dione can then be oxidized to the aldehyde using selenium oxide in dioxane, and a Pictet-Spengler condensation with a tryptophan will provide the 7-N-butyryllavendamycin analog. 7-N-butyryllavendamycin analogs have previously been prepared, though starting with a 8-hydroxy-5,7-dinitro-2-methylquinoline.[15] The method involving the Diels-Alder condensation is superior in that analogs substituted at the 6 position of the quinoline ring system can also be prepared. Compounds prepared by this method will be included in SAR studies to determine the importance of groups at this position in the activity of lavendamycin analogs.

III. Results and Discussion

Synthesis of 7-Butyramido-2-Methylquinoline-5,8-Dione (12)[17]

The preparation of 7-butyramido-2-methylquinoline-5,8-dione was accomplished by the procedure outlined in scheme II.

Scheme II



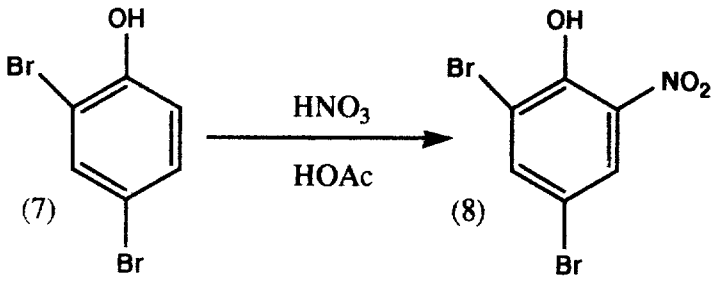
2-butyramido-6-bromobenzoquinone (6) was dissolved in chlorobenzene which had been stored over 4 Angstrom molecular sieves. N-(O-*t*-butyldimethylsiloxy)-1-aza-2-methyl-1,3-butadiene was dissolved in dry chlorobenzene and the two solutions were mixed. The exclusion of water from the reaction mixture is very important, so molecular sieves were

added to the reaction mixture. The flask was immersed in an oil bath at 143°C. The product was obtained via the intermediate shown (15). The reaction mixture was poured directly onto the top of a flash silica gel column and the product isolated. The yield was 30%. The reactants were prepared as reported below.

Synthesis of 2,4-Dibromo-6-Nitrophenol (8)[16,17]

2,4-Dibromo-6-nitrophenol was prepared by the nitration of 2,4-dibromophenol by nitric acid in glacial acetic acid (scheme III).

Scheme III

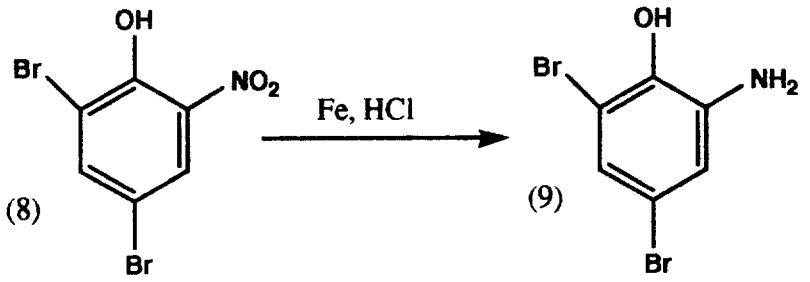


Glacial acetic acid was heated slightly to facilitate the dissolution of the 2,4-dibromophenol on a stirrer/hotplate. The reaction was highly exothermic and proceeded very rapidly. The concentrated nitric acid was added very slowly while the solution was rapidly stirred. If the reaction is allowed to proceed for longer than five minutes, a red oily substance becomes a contaminant and darkens the product. The reaction mixture was poured slowly into 250 ml of water and bright yellow crystals resulted. The solid was filtered out of the suspension to give 95% of the pure product.

Preparation of 2-Amino-4,6-Dibromophenol (9)[17,18]

2-Amino-4,6-dibromophenol (9) was prepared as shown in scheme IV.

Scheme IV

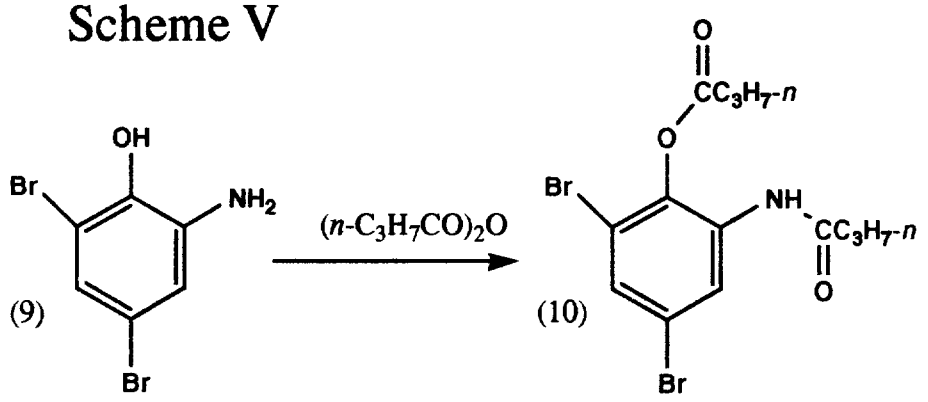


Previous attempts to do this reaction indicated that the quality of the iron powder was of paramount importance in the rate of this reaction. The iron was therefore washed with 3 M HCl, then rinsed with water (3x) and then with acetone (3x) and dried in an oven before addition to the reaction flask. This alteration of the published procedure resulted in a much more rapid reaction. Since the reaction is highly exothermic and is performed near the boiling point of the solvent system, care must be taken when adding iron powder. Several times in previous experiments, some of the reaction mixture was lost when it boiled over. When decanting the reaction mixture from the flask, using a large magnetic stirbar to keep the iron filings in the bottom of the flask helped immensely in preventing contamination of the product. The reaction mixture was poured into water and extracted with ethyl acetate. The extraction is very time consuming because of the formation of an emulsion at the interface between the two phases. The organic extracts were rotoevaporated and when a few milliliters of liquid remained, the residue was triturated with a small amount of water. The result is a very dark solid which must be pressed dry and dried under vacuum to remove water and acetic acid. The yield of this reaction was 88% of the expected yield.

Preparation of 2-Butyramido-4,6-Dibromophenyl Butyrate (10)[17]

The preparation of 2-butyramido-4,6-dibromophenyl butyrate was accomplished by the reaction of 9 with butyric anhydride according to scheme V.

Scheme V



2-Propanol was the solvent of choice because of its ability to dissolve both 9 and butyric anhydride. The very foul smelling butyric anhydride must be handled carefully; because of its stench it can make one a social outcast if mishandled. The reaction proceeded for fifty minutes and the solvent was removed by rotoevaporation. The product was washed with petroleum ether. A small amount of 11 was in the product and a small amount of butyric anhydride remained in the product and defied attempts to remove it. The yield was 67%. The small amount of the product (10) was purified by flash column chromatography for analysis, but the mixture can easily be used in the next step, the hydrolysis to phenol 11.

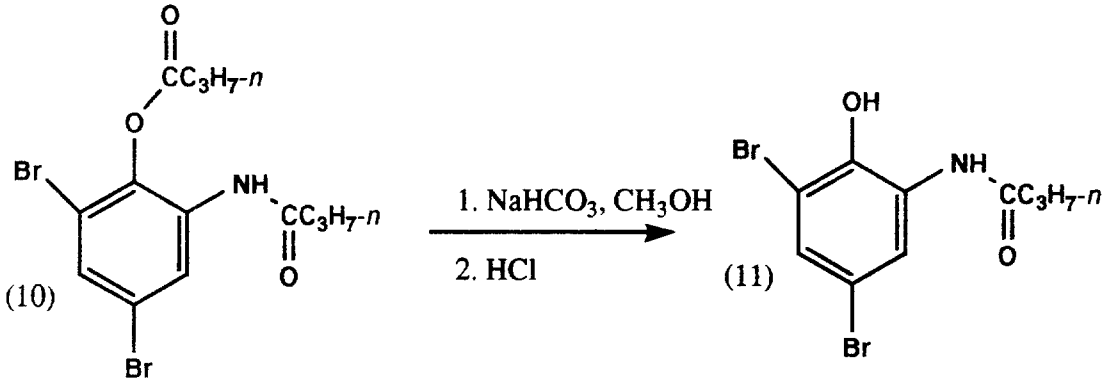
Attempted Synthesis of 2-Butyramido-6-Bromobenzoquinone (6)

Several attempts were made for the oxidation of the phenyl butyrate 10 in the presence of ceric ammonium nitrate. The ester at the C-1 position prevented the formation of the benzoquinone 6 in each of these cases. Other attempts were made involving potassium dichromate as the oxidizing agent, but these, too, were unsuccessful. Hydrolysis of the ester to the phenol 11 became an obvious necessity before the oxidation could be performed.

Preparation of 2-Butyramido-4,6-Dibromophenol (11)[17,18]

The hydrolysis of the ester at the C-1 position of the ring was accomplished in the presence of sodium bicarbonate in methanol (scheme VI).

Scheme VI



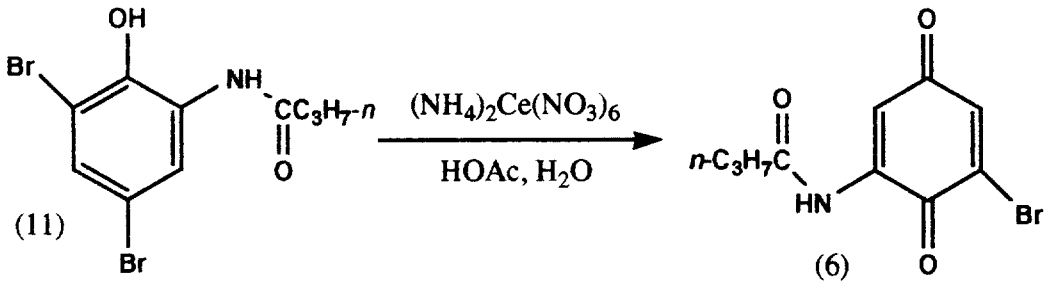
The addition of saturated sodium bicarbonate to a solution of 10 and 11 in methanol resulted in the precipitation of the basic salt. The removal of the excess sodium bicarbonate prior to the acidification of the reaction mixture is a vast improvement on the earlier method. The addition of a layer of petroleum ether on the top of the mixture before acidification allows the butyric acid formed to be removed from the product. The product

forms as a precipitate at the interface between the two layers. The yield of this reaction was 77% and the structure of the product was confirmed by NMR and mass spectroscopy.

Preparation of 2-Butyramido-6-Bromobenzoquinone (6)[18]

Scheme VII shows the process by which the preparation of 6 was accomplished.

Scheme VII

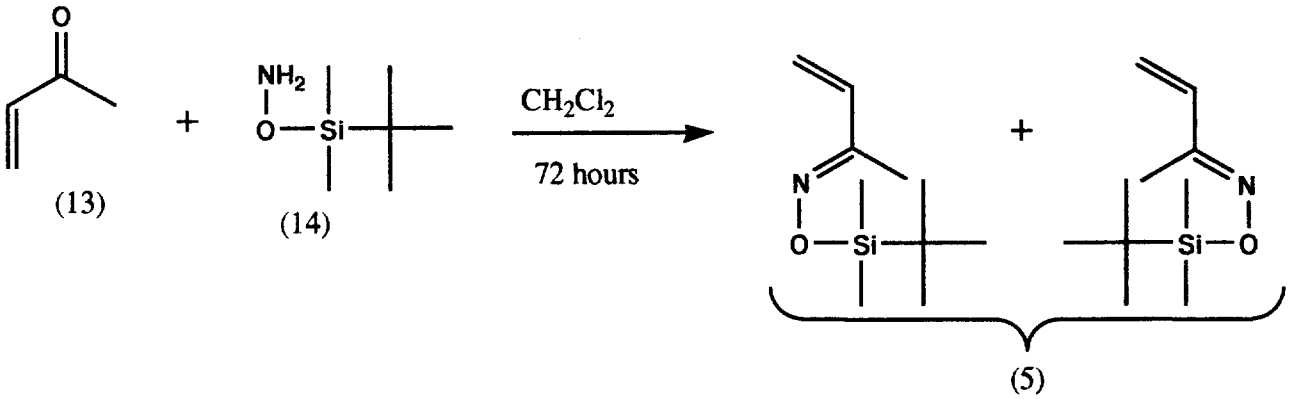


Phenol 11 was dissolved in glacial acetic acid at low temperature, near the freezing point of the solvent. A solution of ceric ammonium nitrate was added dropwise and slowly. The reaction mixture changed from a light brown color to orange as the oxidizing agent was added. The reaction mixture was poured into ice water and extracted with ethyl acetate. The product was a mixture of the benzoquinone 6 and some other, less soluble product. The less soluble contaminant can be removed by filtering the reaction mixture after adding it to the cold water. The resulting filtrate can then be extracted to remove the product. The yield was low, primarily because the product is unstable and subject to nucleophilic substitution of the bromine by nucleophiles. The yield was 56% of the dark orange crystalline solid.

Preparation of N-(O-t-Butyldimethylsiloxy)-1-Aza-2-Methyl-1,3-Butadiene (5)[19]

The butadiene required for the Diels-Alder condensation (5) was prepared by the reaction of methyl vinyl ketone (13) with O-*t*-butyl-dimethylsilyl hydroxylamine (14) (scheme VIII).

Scheme VIII

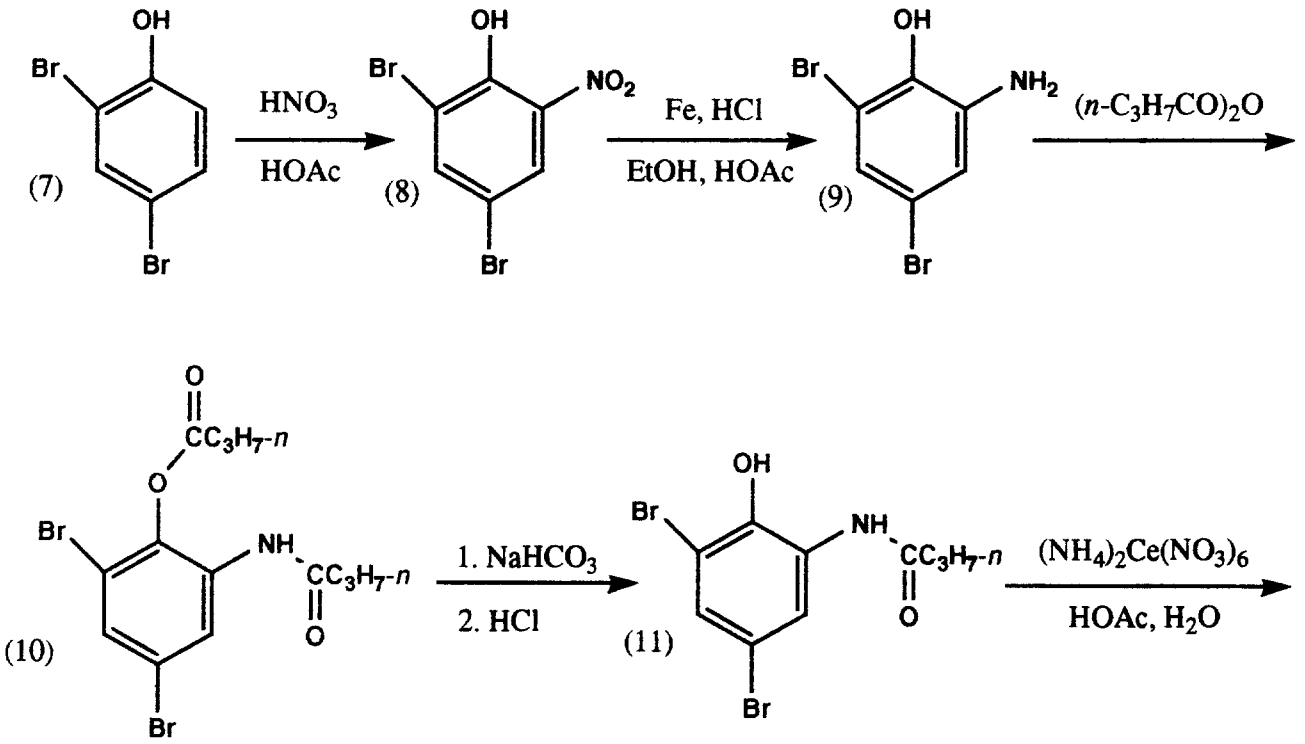


Methyl vinyl ketone (13) was stirred for 72 hours with a solution of O-*t*-butyldimethylsilyl hydroxyamine in dry dichloromethane. After 72 hours, the product was isolated by flash column chromatography and was found to consist of a mixture of E and Z. Interconversion between the two enantiomers is rapid at the high temperatures found in the reaction forming (12), so the mixture was used as though it were only the Z isomer. The yield of the reaction was 61%.

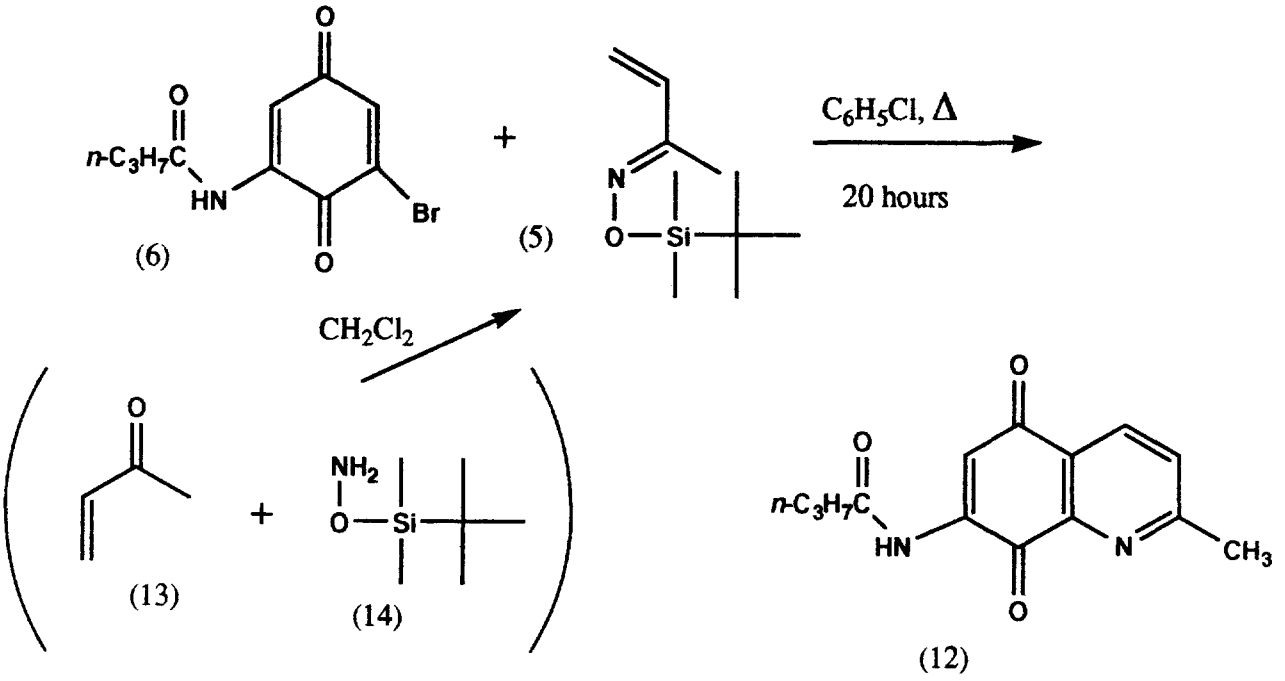
Summary of Synthetic Process

Scheme IX is a summary of the synthetic method used to produce 7-butyramido-2-methylquinoline-5,8-dione (12) from commercially available compounds (7), (13), and (14). The overall yield of the procedure was nearly 11%.

Scheme IX



Scheme IX (continued)



The Diels-Alder condensation is the primary source of this low overall yield and should be the focus of attempts to improve the synthesis. The oxidation of phenol 11 to quinone 6 is another source of a low yield in the synthesis. A method of preventing the formation of decomposition products of quinone 6 will also be useful for the application of this procedure to a larger scale.

IV. Experimental

A. General Information

Solvents: Glacial acetic acid, 2-propanol, dry dichloromethane (dried over 4 Angstrom molecular sieve), Absolute ethanol, dry chlorobenzene distilled over calcium hydride, 70% aqueous ethanol, ethyl acetate, petroleum ether.

Melting Points: Melting points determinations were performed with a Thomas Hoover capillary melting point apparatus and are not corrected.

Thin Layer Chromatography: TLCs were performed on Kodak silica gel sheets containing a fluorescent indicator.

Mass Spectroscopy: Mass spectra were obtained on an Extrel ELQ 400 mass spectrometer.

Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR): NMR spectra were collected on a Varian Gemini 200 in deuterated chloroform with TMS as the internal reference.

B. Procedures

Preparation of 2,4-Dibromo-6-Nitrophenol (8)[16,17]

Using a mortar and pestle, 25.03 g (99.5 mmol) of 2,4-dibromophenol (7) was crushed to a fine powder, and added to 50 ml of glacial acetic acid in a 250 ml beaker. The solvent was stirred over a stirrer/hot plate at room temperature until a clear solution was formed. When the 2,4-dibromophenol had completely dissolved, 11 ml of concentrated nitric acid were added. The reaction mixture was stirred at 20 °C for five minutes and then poured into 250 ml of distilled water in a 500 ml beaker. The yellow crystalline precipitate was collected by vacuum filtration and dried under vacuum to yield 28.1 g of 2,4-dibromo-6-nitrophenol (94.5 mmol, 95% yield). The structure of the compound was verified by NMR spectroscopy. ^{1}H NMR data ($CDCl_3$) δ 11.08 (s, 1, -OH); δ 8.27 (d, 1, J=2.3 Hz, C-3 H); δ 8.01 (d, 1, J=2.3 Hz, C-5 H)

Preparation of 2-Amino-4,6-Dibromophenol (9)[17,18]

To a 500 ml three necked flask equipped with a mechanical stirrer, reflux condenser and thermometer, 200 ml of a 1:1 80% ethanol/acetic acid were added. 2,4-dibromo-6-nitrophenol (8) (10.0 g, 33.7 mmol) was added and the mixture was stirred with gentle heating (60 °C) until a yellow solution was formed. Iron powder[19] (15.4 g, 0.276 mol) was added to the reaction vessel portionwise over about 5 minutes (1 g each 20 sec) while vigorously stirring with the mechanical stirrer. The reaction mixture was heated to reflux for 15 minutes, at which time 0.2 ml of 12 N HCl were added. After an additional 15 minutes, the reaction mixture was decanted into 500 ml of distilled water. The aqueous mixture was extracted with ethyl acetate (3x 250 ml). The organic extracts were then washed three times with 250 ml of distilled water. The organic layer was then rotoevaporated in a 45°C water bath until 10 ml of liquid remained. The remaining liquid was poured into 100 ml of distilled water. The precipitate was filtered and pressed. The filtrate was then extracted with 50 ml of ethyl acetate and the organic layer was rotoevaporated until 3-5 ml of liquid remained. The residue was then poured into 40 ml of distilled water and the precipitate was filtered and pressed dry. The combined product was then dried under vacuum to yield 7.92 grams (88% yield) of 2-amino-4,6-dibromophenol (9).

The structure of the 2-amino-4,6-dibromophenol (9) was confirmed by NMR spectroscopy. ^{1}H NMR data ($CDCl_3$) δ 6.98 (d, 1, J=2.1 Hz, C-5 H); δ 6.79 (d, 1, J=2.0 Hz, C-3 H); δ 5.38 (s, 1, -OH); δ 3.94 (s, 2, $-NH_2$)

Preparation of 2-Butyramido-4,6-Dibromophenyl Butyrate (10)

2-amino-4,6-dibromophenol (9) (4.0 g, 15 mmol) was dissolved in 30 ml of 2-propanol in a 100 ml round-bottomed flask equipped with a reflux condenser. Butyric anhydride (5.0 ml,) was added to the flask. The reaction mixture was stirred by magnetic stirrer while heating (reflux) for 50 minutes. The reaction mixture was rotoevaporated to remove the solvent (60 °C water bath) until 3-4 ml of liquid remained. The residue was then transferred to a 10 ml beaker and cooled in an ice bath. Light colored crystals formed and were removed from the liquid by vacuum filtration and washed with petroleum ether. The filtrate was then rotoevaporated (60 °C water bath) to condense it further and when 2-3 ml of liquid remained, the residue was again transferred to a 10 ml beaker and cooled in an ice bath. The resulting crystals were filtered and washed with petroleum ether. The washed crystals were then dried under vacuum to give 4.1 g of 2-butyramido-4,6-dibromophenyl butyrate containing a small amount of 2-butyramido-4,6-dibromophenol contaminant. A small amount of 10 was purified by flash column chromatography (8 cm x 1 cm) eluted with 5:1 pet. ether/ethyl acetate.

The structure of 2-butyramido-4,6-dibromophenyl butyrate (10) was confirmed by NMR and mass spectroscopic data. ^{1}H NMR data ($CDCl_3$) δ 8.47 (s, 1, **NH**); δ 7.50 (d, 1, J=2.1 Hz, C-5 **H**); δ 7.16 (d, 1, J=2.1 Hz, C-3 **H**); δ 2.68 (t, 2, J=7.3 Hz, -$OCOCH_2$-); δ 2.33 (t, 2, J=7.3 Hz,-$NHCOCH_2$-); δ 1.88 (m, 2, J=7.3, -$OCOCH_2CH_2$-); δ 1.72 (m, 2, J=7.3 Hz, -$NHCOCH_2CH_2$-); δ 1.10 (t, 3, J=7.4, ester-CH_3); δ 1.04 (t, 3, J=7.3, amido-CH_3). EIMS data, m/e (relative intensity), 405 (46), 406 (61), 407 (70), 408 (100), 409 (73), 410 (78).

Attempted Synthesis of 2-Butyramido-6-Bromobenzoquinone

Oxidation of the phenyl butyrate (10) was attempted prior to hydrolysis of the ester at the C-1 position. 2-Butyramido-4,6-dibromophenyl butyrate (10) (100 mg, .246 mmol) was dissolved in 5 ml of glacial acetic acid in a 10 ml two-necked, roundbottom flask with argon balloon and rubber membrane attached. A solution of cerium ammonium nitrate (.746 g, 1 mmol) in 1,0 ml of distilled water was added dropwise over a period of two minutes. The solution was stirred at 20 °C for five minutes. One drop of concentrated HCl was added to the reaction mixture, and the solution was stirred for an additional ten minutes. The reaction mixture was then poured into 50 ml of distilled water, filtered to remove a particulate solid and extracted with ethyl acetate (100 ml, 50 ml). The organic extracts were washed with water and dried over sodium sulfate. The solvent was then removed by rotoevaporation. The product was not obtained.

Preparation of 2-Butyramido-4,6-Dibromophenol (11)[17,18]

The 2-butyramido-4,6-dibromophenyl butyrate (10) prepared in the last step was hydrolyzed to 2-butyramido-4,6-dibromophenol (11) in the presence of sodium bicarbonate. To a 150 ml beaker equipped with a magnetic stirrer, 1.75 g (4.3 mmol) of 2-butyramido-4,6-dibromophenyl butyrate and 40 ml of methanol were added and stirred until a clear solution formed. Saturated sodium bicarbonate solution (15 ml) was added in 2 ml portions over a period of 5 minutes with vigorous stirring. The resulting, slightly basic (pH 8.5) suspension was stirred for 30 minutes at room temperature. The suspension was then heated to boiling for 15 minutes. The mixture was allowed to cool to room temperature and the solid was then removed from the suspension by vacuum filtration and discarded (this solid material is excess sodium bicarbonate). The filtrate was transferred to a 150 ml beaker and 15 ml of petroleum ether were added to create a two phase system. Water (50 ml) was then added and the resulting system was allowed to sit for fifteen minutes. The two phase system was then acidified using 3 M HCl to pH 4. A

precipitate formed at the interface between the two phases. The two phase system was stirred for five minutes and then allowed to sit for an additional five minutes. The solid material was then collected by vacuum filtration and washed with petroleum ether to give 0.97 g of the phenol. The filtrate was then transferred to a 500 ml separatory funnel and the aqueous layer was drained into a 150 ml beaker. The aqueous layer was then cooled in an ice bath and white crystals formed which were filtered out by vacuum filtration. The remaining filtrate was then transferred to a separatory funnel and extracted with ethyl acetate (2x 100 ml). The organic extracts were dried over sodium sulfate and the solvent removed by rotoevaporation. The combined mass of the three samples was 1.12 g (77% yield) of pure 2-butyramido-4,6-dibromophenol (11). ^{1}H NMR data ($CDCl_3$) δ 7.97 (d, 1, J=1.8 Hz, C-5 H); δ 7.77 (s, 1, -NH-); δ 7.42 (d, 1, J=2.0 Hz, C-3 H); δ 2.43 (t, 2, J=7.4 Hz, -NHCOC**H**$_2$-); δ 1.78 (m, 2, J=7.3 Hz, -NHCOCH$_2$C**H**$_2$-); δ 1.03 (t, 3, J=7.4 Hz, amido-C**H**$_3$). EIMS data, m/e (relative intensity), 335 (47), 336 (12), 337 (M^+, 100), 338 (11), 339 (46), 340 (12).

Preparation of 2-Butyramido-6-Bromobenzoquinone (6) [17,18]

Ceric ammonium nitrate (3.3 g, 6 mmol) was dissolved in 6 ml of distilled water and cooled in an ice bath. 2-butyramido-4,6-dibromophenol (11) (1.02 g, 3 mmol) was dissolved in 15 ml of acetic acid in a 50 ml three-necked, round-bottomed flask. Argon was streamed through the flask and out through a reflux condenser at the middle neck. The flask was cooled in an ice bath to 18 °C and 7.5 ml of the ceric ammonium nitrate solution was added dropwise over a period of two minutes with vigorous stirring. The one phase solution was stirred while cooling in an ice bath to 10 °C for five minutes after the addition of the first half of the $(NH_4)_2Ce(NO_3)_6$ solution. The second half of the ceric ammonium nitrate solution was then added dropwise over a period of two additional minutes and the mixture was then stirred for 10 minutes at 10 °C. The reaction mixture was then poured

into 60 ml of ice water and extracted with ethyl acetate (2x 120 ml). After the organic layer had been washed with cooled, saturated NaCl solution (2x 100 ml) and dried over Na_2SO_4 for thirty minutes, the mixture was concentrated by rotoevaporation until 5-6 ml remained. The residue was then cooled to 5 °C and the yellow crystals which formed were separated by vacuum filtration. The filtrate was further concentrated by rotoevaporation and cooled to give even more of the yellow-orange crystalline solid. The crystals were washed with 5 ml of a 1:2 mixture of ethyl acetate and petroleum ethers and dried under vacuum at room temperature.

The product was confirmed by NMR spectroscopy. The yield was 430 mg (56%). ^{1}H NMR data ($CDCl_3$) δ 8.09 (s, 1, -**NH**-); δ 7.65 (d, 1, J=2.3 Hz, C-5 H); δ 7.23 (d, 1, J=2.3 Hz, C-3 H); δ 2.91 (t, 2, J=7.3 Hz, -NHCO**CH**$_2$-); δ 1.73 (m, 2, J=7.3 Hz, -NHCOCH$_2$**CH**$_2$-); δ 0.98 (t, 3, J=7.3 Hz, amido-**CH**$_3$). EIMS data m/e (relative intensity) 270 (75), 271 (88), 272 (75), 273 (100).

Preparation of N-(O-t-Butyldimethylsiloxy)-1-Aza-2-Methyl-1,3-Butadiene (5)[20]

Freshly distilled methyl vinyl ketone (0.525 g, 7.5 mmol) was added to 5 ml of dry dichloromethane in a 20 ml two-necked round-bottom flask. Molecular sieve (4 Angstrom, 2.65 g) was dried at 130 °C for three days, cooled under vacuum and added to the flask. A solution of O-*t*-butyldimethylsilyl) hydroxyamine (1.38 g, 9.4 mmol) in 5 ml of dry dichloromethane was added to the flask and the mixture was stirred at room temperature for 72 hours. The reaction mixture was filtered and the filtrate was distilled under decreased pressure (20 mmHg, aspirator) from a 40 °C oil bath. Dichloromethane was collected at 28°C. The residue was then purified by flash silica gel column chromatography (12 g silica gel, 1.5 cm x 16 cm column), using 350 ml of hexane as the eluent. Fractions containing either or both of the enantiomers were combined and the hexane was removed by rotoevaporation to yield 0.91 g (61 %) of a mixture of the E and Z isomers of the product as confirmed by NMR spectroscopic data (see appendix).

Preparation of 7-Butyramido-2-Methyl-Quinoline-5,8-Dione (12)[17]

2-butyramido-6-bromobenzoquinone (6) (70 mg, 0.26 mmol) was dissolved in 5 ml of dry chlorobenzene. A sample (102 mg, 0.52 mmol) of N-(O-*t*-butyldimethylsiloxy)-1-aza-2-methyl-1,3-butadiene (5) was dissolved in 1.0 ml of dry chlorobenzene. A 10 ml two-necked, round-bottom flask with magnetic stirbar, reflux condenser and all of the glass syringes, glassware and spatulas required for this synthesis were dried in an oven (130°C for 2 days) and cooled in a desiccator. Rubber membranes were installed in one of the two necks of the flask and in the top of the reflux condenser. The apparatus was assembled and flushed with dried argon for two hours prior to the reaction. The solution of 2-butyramido-6-bromobenzoquinone was injected into the flask by argon filled syringe. The solution of

N-(O-*t*-butyldimethylsiloxy)-1-aza-2-methyl-1,3-butadiene was then added. The flask was covered with aluminum foil to exclude light from the apparatus and lowered into a 143 °C oil bath and stirred for 20 hours. The reaction mixture was then poured onto the top of a flash silica gel column (1 cm x 8 cm) and eluted with 1:1 ethyl acetate and petroleum ether. Fractions containing 7-butyramido-2-methylquinoline-5,8-dione (12) were combined and rotoevaporated to remove the solvent. The result was 20.2 mg of the pure product (30% yield).

The structure of the product was confirmed by ^{1}H NMR spectroscopy. ^{1}H NMR data ($CDCl_3$) δ 8.38 (s, 1, -NH-); δ 8.31 (d, 1, J=8.1 Hz, C-4 H); δ 7.93 (s, 1, C-6 H); δ 7.56 (d, 1, J=7.9 Hz, C-3 H); δ 2.78 (s, 3, C-2 -CH_3); δ 2.50 (t, 2, J=7.3 Hz, -$NHCH_2$-); δ 1.78 (m, 2, J=7.3 Hz, -$NHCH_2CH_2$-); δ 1.02 (t, 3, J=6.0, -$NHCH_2CH_2CH_3$)

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19. Iron powder was purified by washing with 3 M hydrochloric acid for 30 seconds, followed by rinsing (3x) with distilled water and with acetone (3x). The filings were then dried in an oven and allowed to cool in a desiccator before use.

20. W. Cai, unpublished results.

Appendix

Index of Spectra

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DATE 01-26-96
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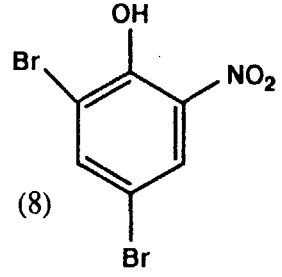

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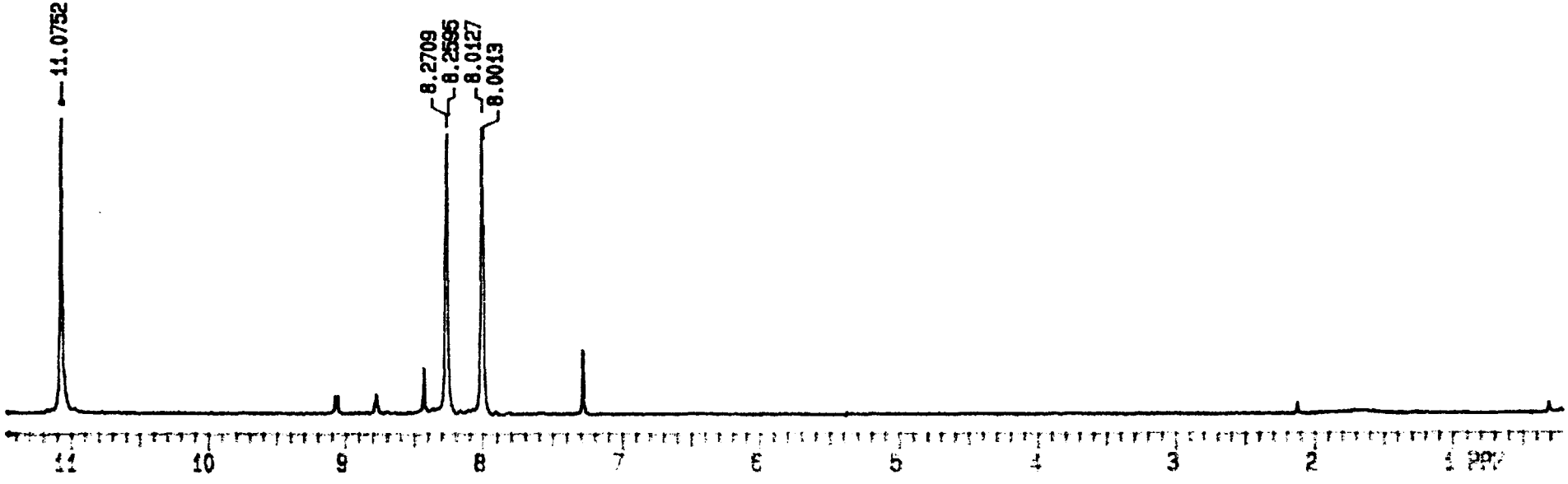

JOHN A SCHERSCHEL

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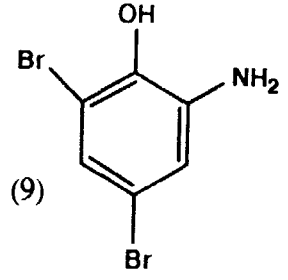

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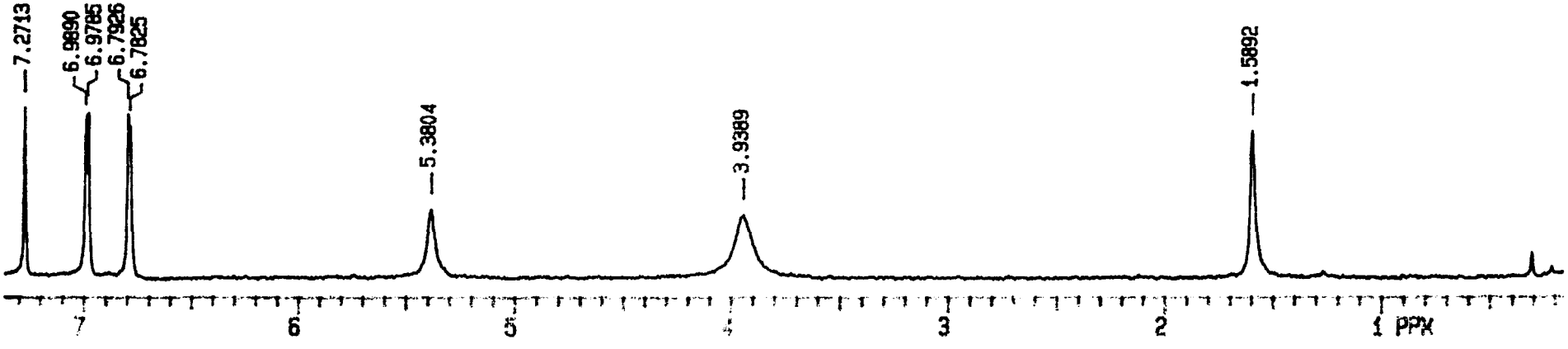

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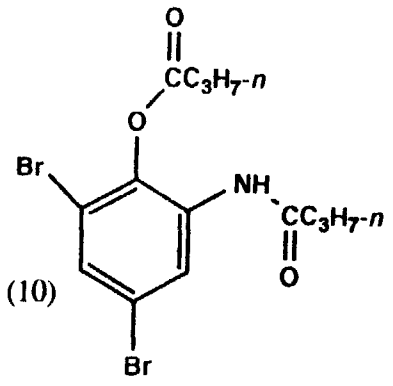

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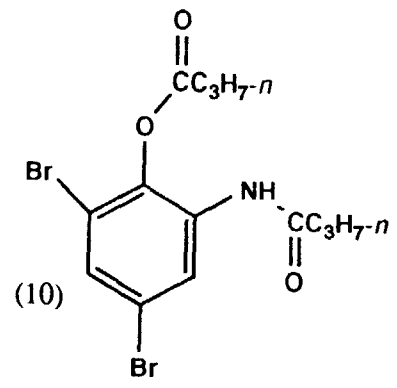

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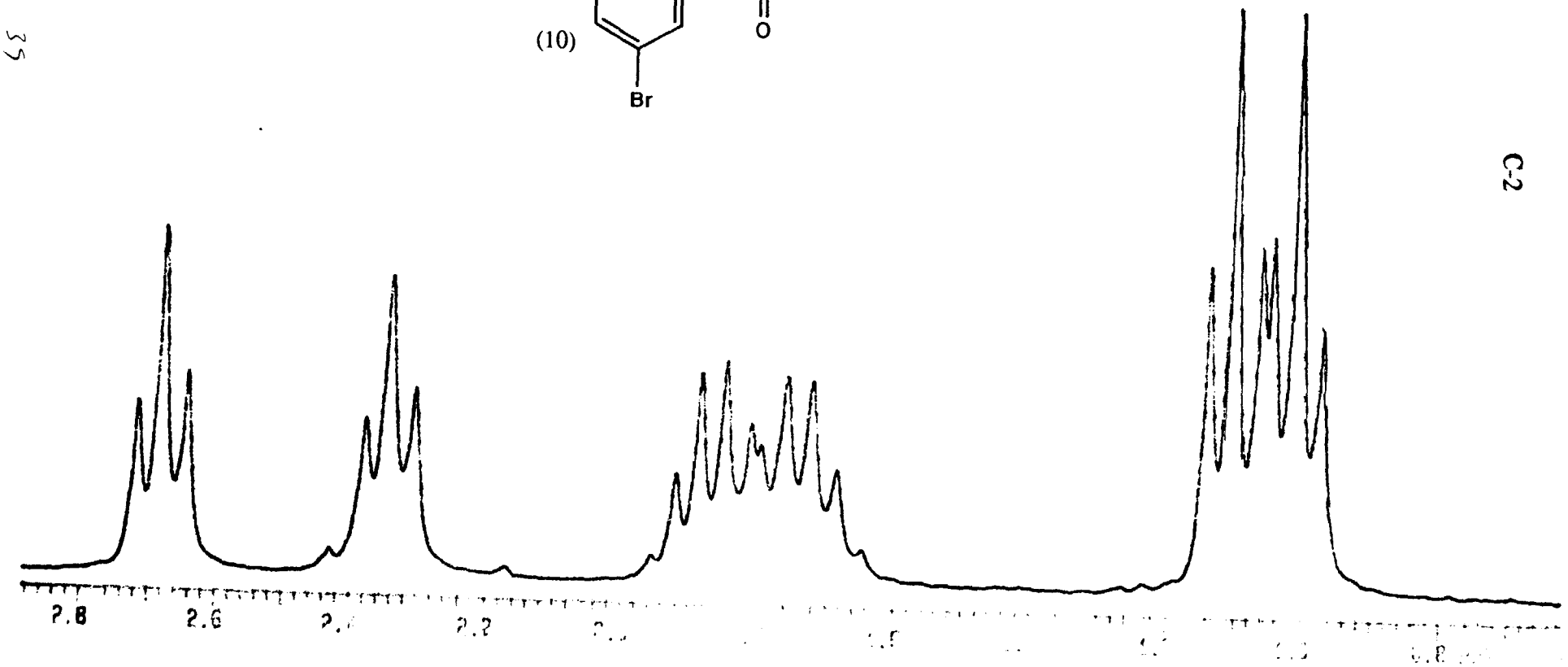

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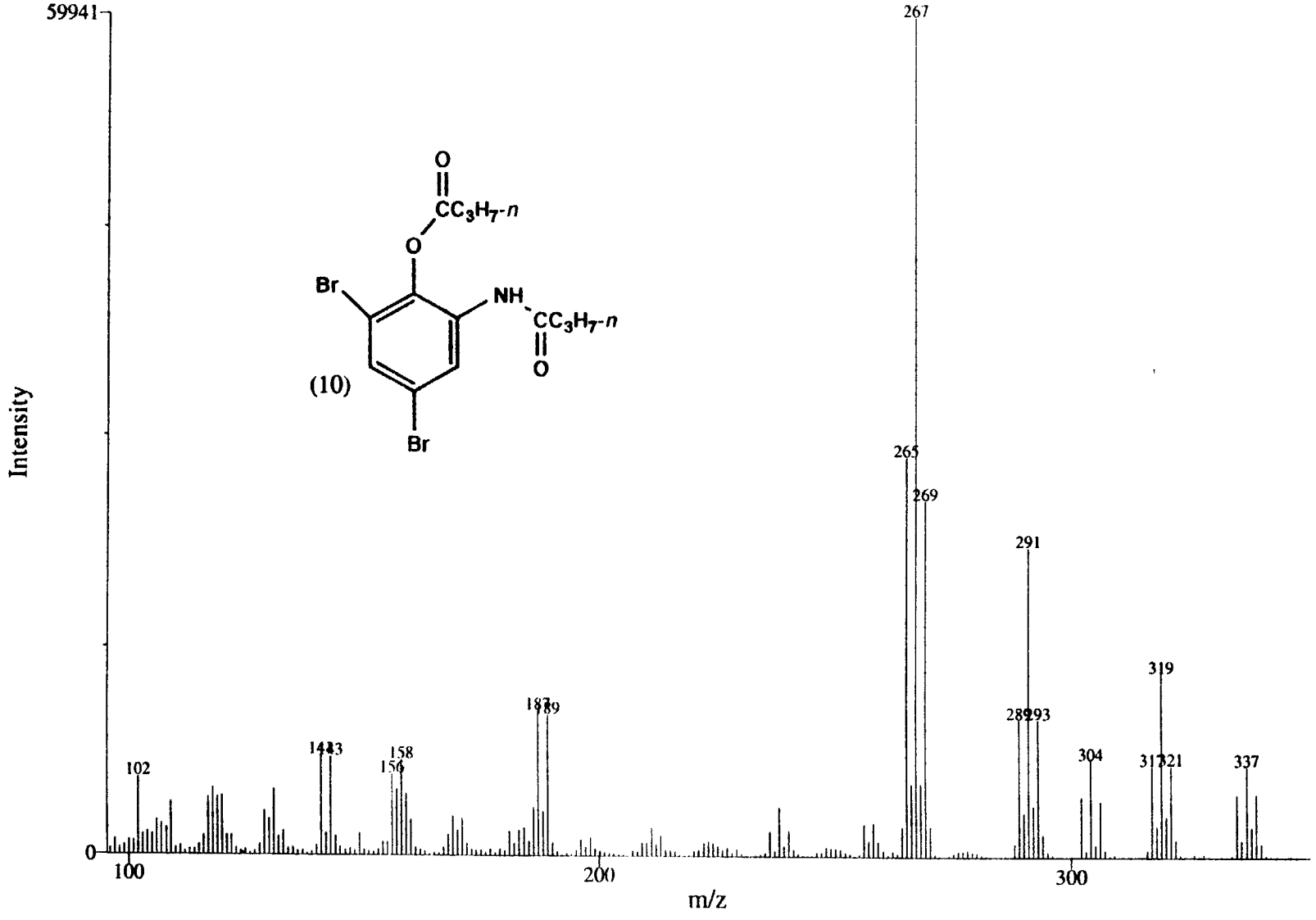
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C-3

JOHN SCHEUSCHEL
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3-13-96

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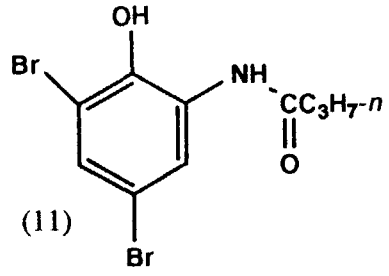

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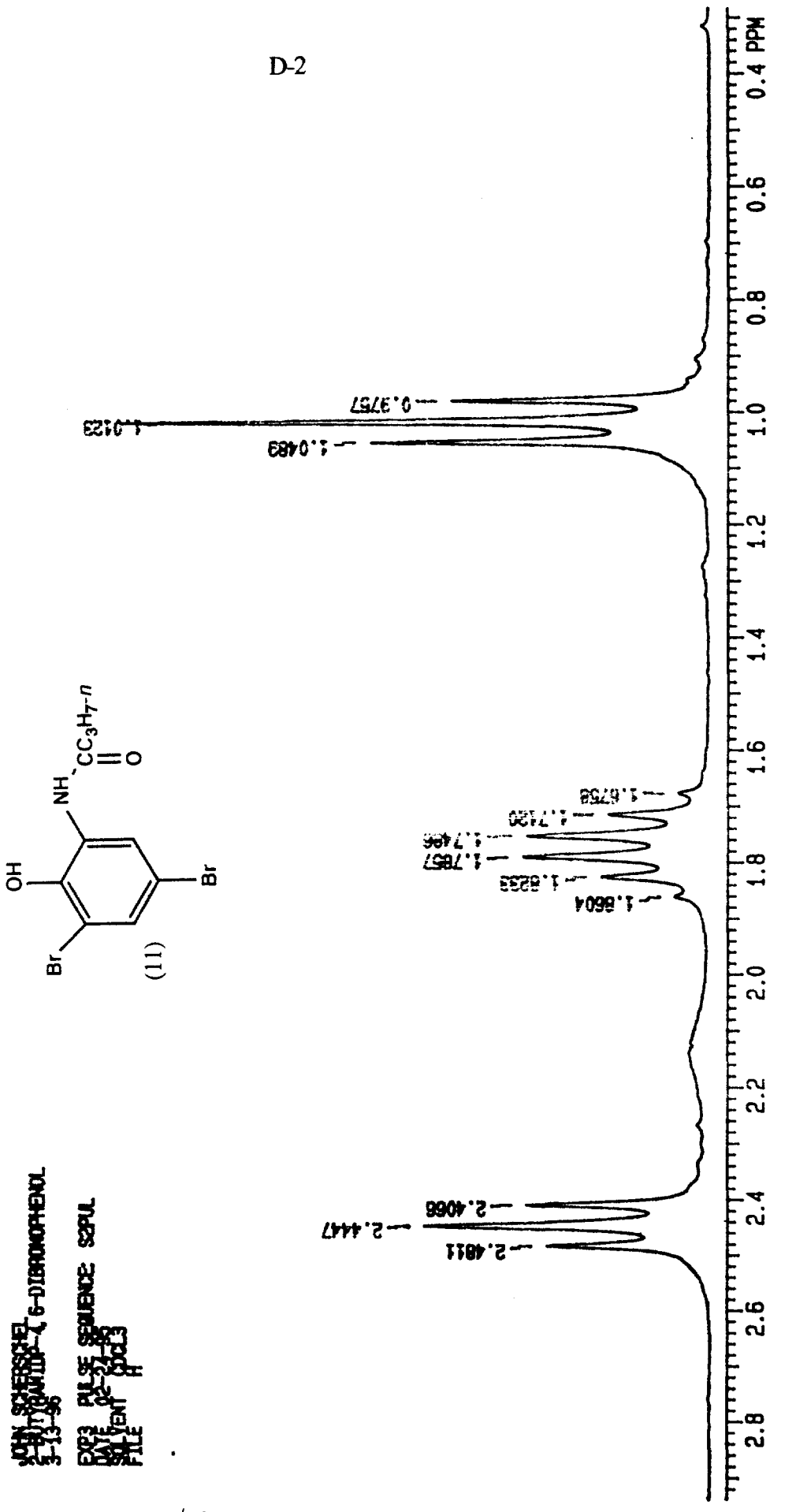
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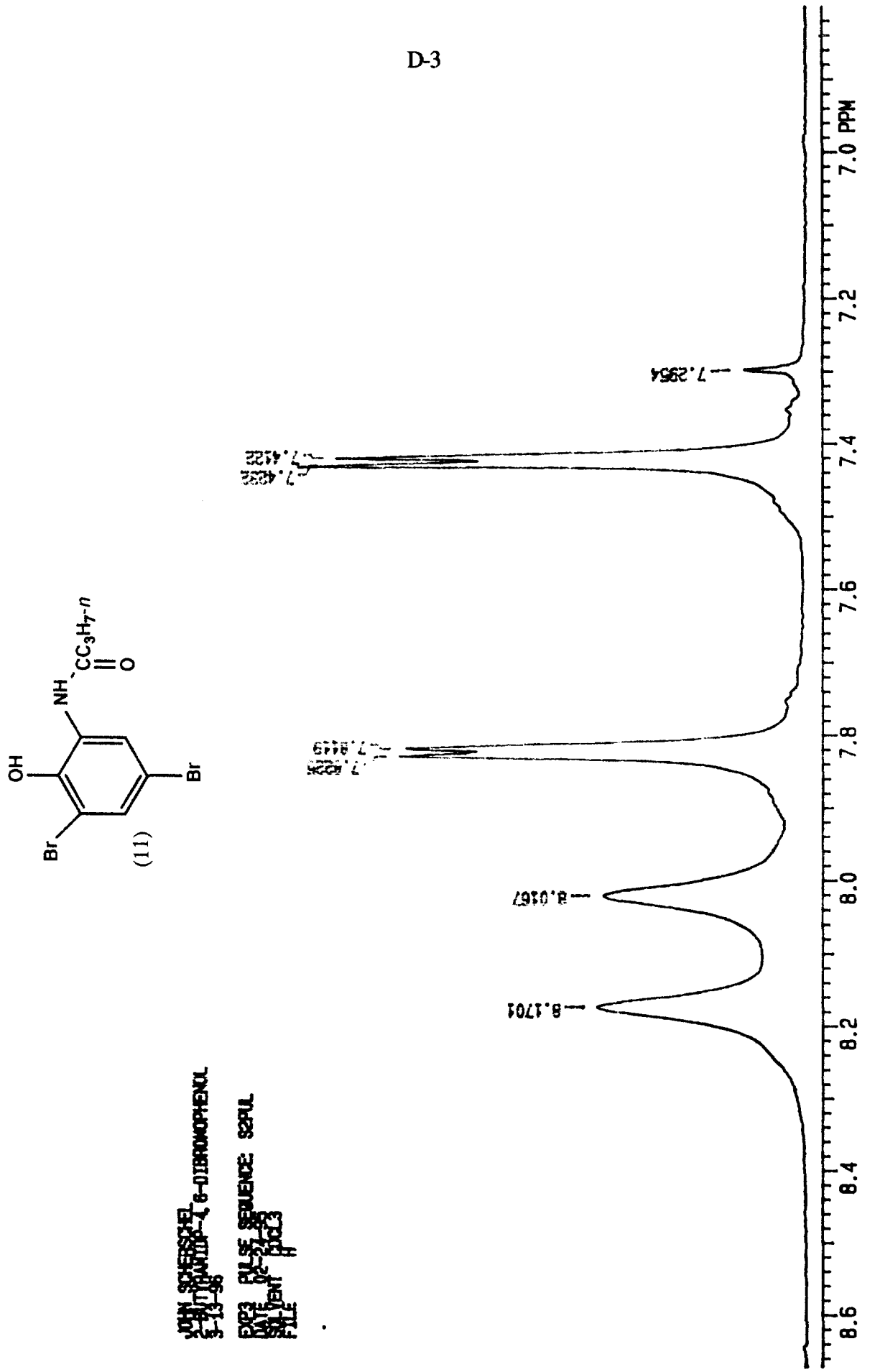
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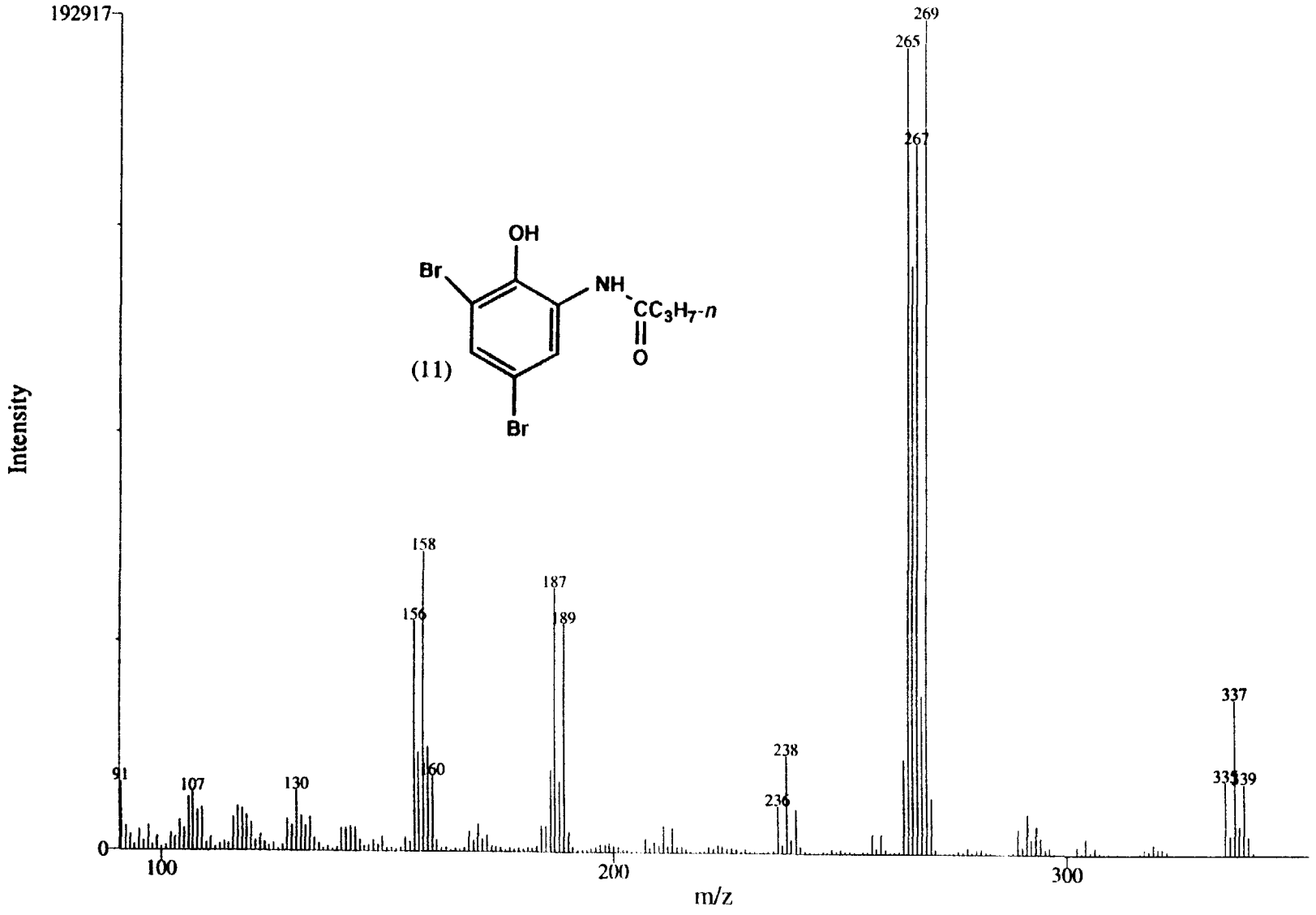
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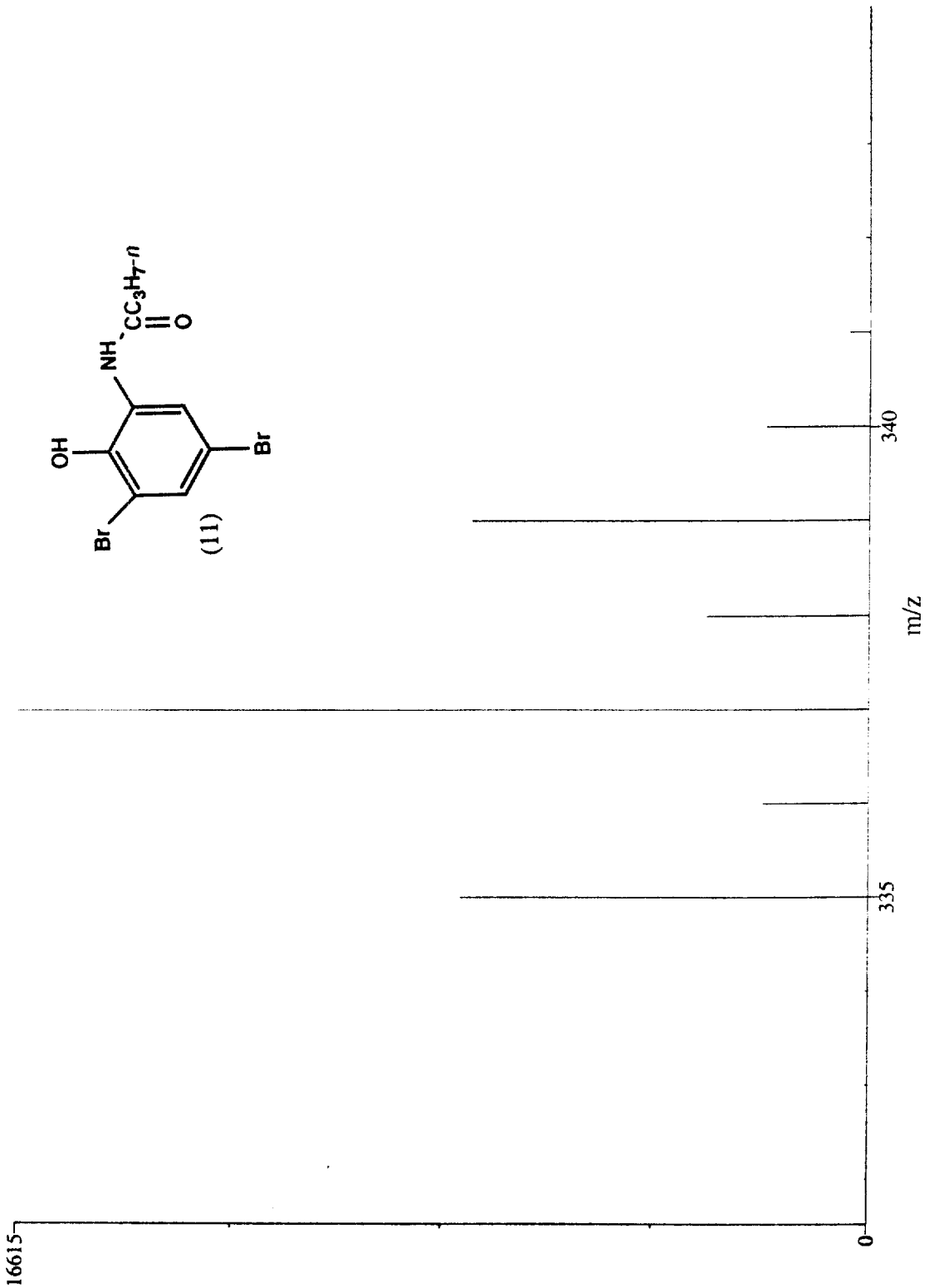
D-4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JOHN A SCHERSCHEL

2-BUTYRAMIDO-
6-BROMOBENZOQUINONE

5-8-96

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DATE 01-15-96
SOLVENT CDCL3
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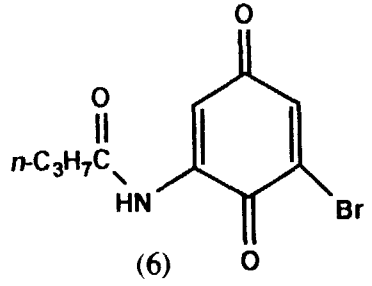

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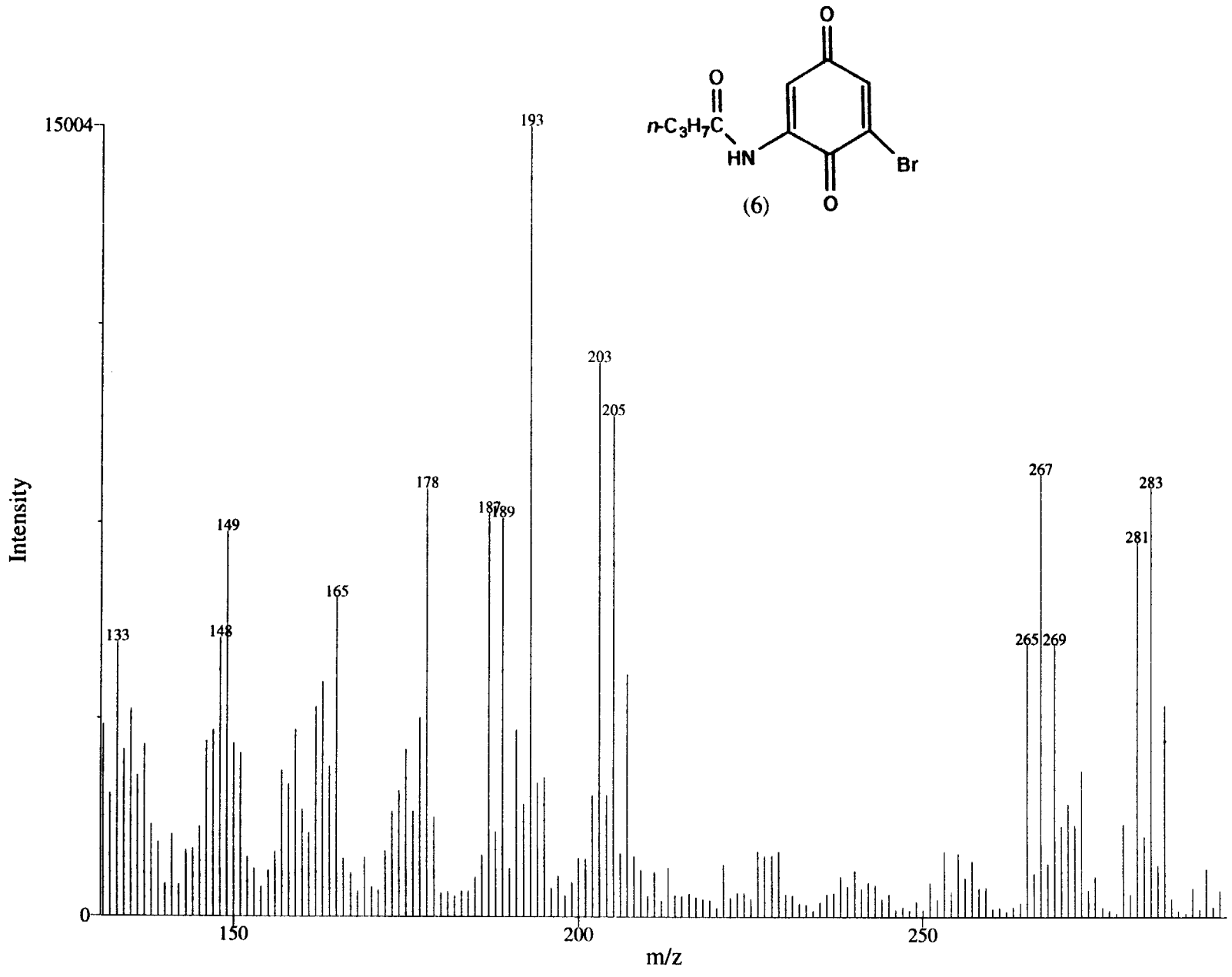
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E-2

JOHN A SCHERSCHEL

N-(O-TERT-BUTYLDIMETHYLSILOXY)-
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MAY 21, 1996

EXP1 PULSE SEQUENCE: S2PUL
DATE 01-29-96
SOLVENT CDCL3
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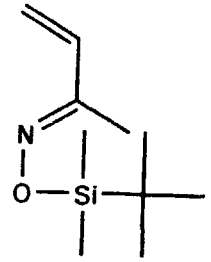

F-1

JOHN A SCHERSCHEL

7-BUTYRAMIDO-2-METHYL-
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5-14-96

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DATE 01-22-96
SOLVENT CDCL3
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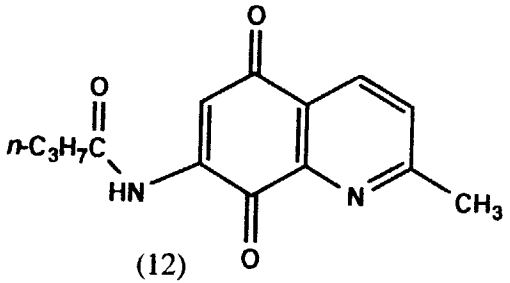

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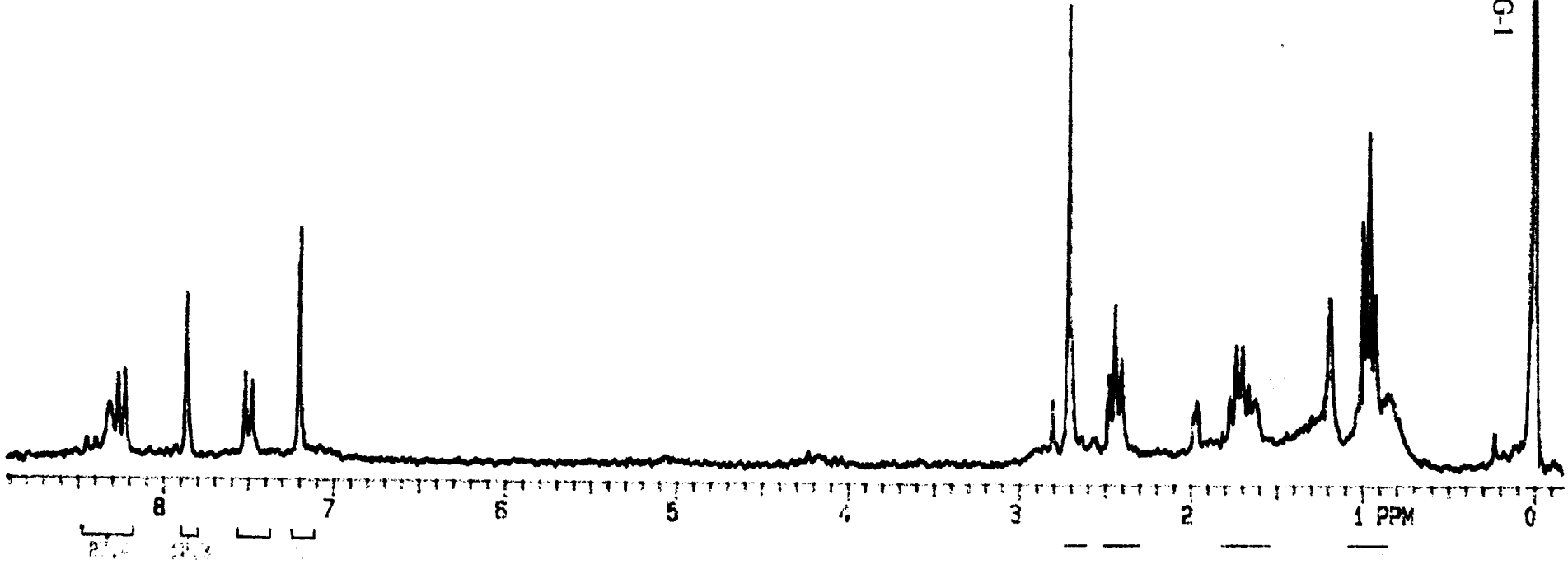

JOHN A SCHERSCHEL

7-BUTYRAMIDO-2-METHYL-
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5-14-96

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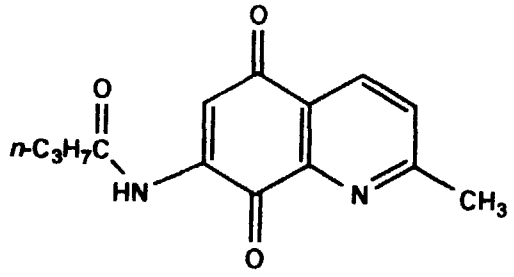

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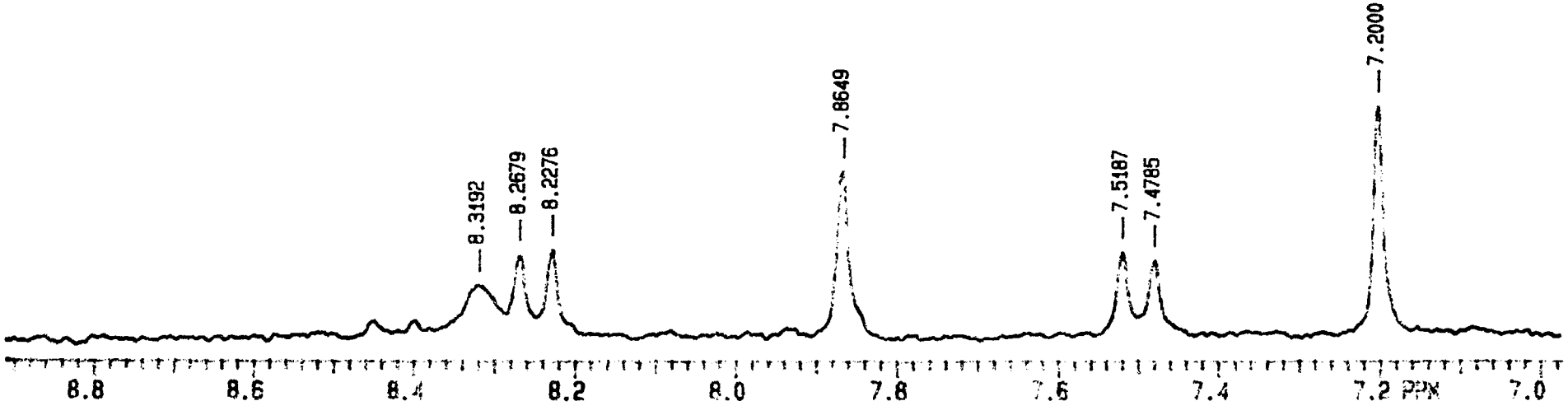

G-2

JOHN A SCHERSCHEL

7-BUTYRAMIDO-2-METHYL-
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5-14-96

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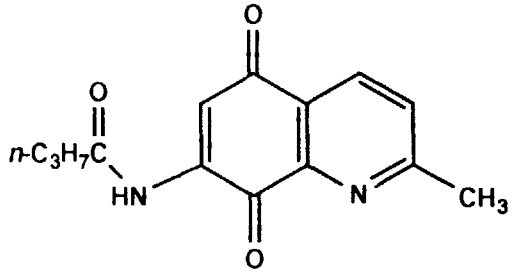

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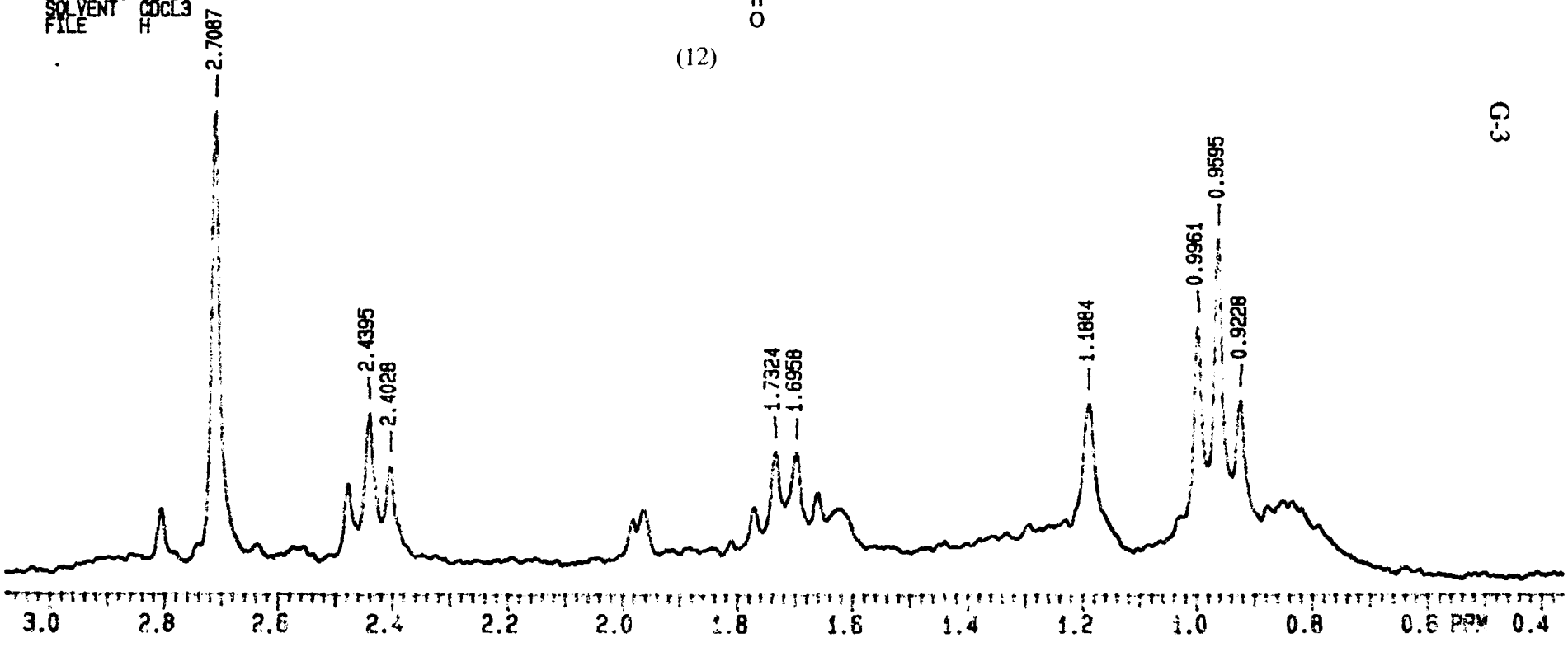

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