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Syntheses of azure-B neuropeptide conjugates and a novel bicyclic triene

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SYNTHESES OF AZURE-B NEUROPEPTIDE CONJUGATES AND A NOVEL BICYCLIC TRIENE

by
MILIND D. CHOUBAL

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To my wife Neeta
&
My Parents

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VITA

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List of Publications

1. Choubal, Milind; Ford, Warren T. "Crosslinked Polystyrene Latexes prepared with 12-(o-Styryloxy)dodecyltrimethylammonium Bromide" J. Polym. Sci.:Polym. Chem. Ed., **1989**, 27, 1873.

2. Choubal, Milind D.; Bassett, Joseph Y. "A New Method for the Synthesis of 3β-Hydroxy-4α-Bromocarane and Cis-3-Carene Epoxide" Org. Prep. Proced. Int., **1991**, 23, 667.

3. Choubal, M. D.; Fernandez, E. J.; Crumrine, D. S. and Pavkovic, S. F. "Structure of Diethyl-cis, cis-3,8-cyclodecadiene-trans-1,6-dicarboxylate" Acta Cryst. (Accepted October-1991).

4. Choubal, Milind D.; Crumrine, David S. and Feigenbaum, Jeff "Synthesis of Dye-Neuropeptide Conjugates" Bioconjugate Chemistry (Submitted July-1991).

5. Feigenbaum, Jeff ; Choubal, Milind D.; Crumrine, David S. "The Unique Method for Inactivating Neurotransmitter Receptors with Exquisite Selectivity" J. of Neurochemistry (To be submitted).

6. Feigenbaum, J.; Simantov, R. and Choubal, Milind "The Comparative Effects of Morphine and Gamma Hydroxybutyrate on Analgesia and Catalepsy in The Rat" Eur. J. Pharmacol. (Accepted).

7. Feigenbaum, J.; Richmond, S. A.; Simantov, R.; Choubal, Milind "The Comparative Effects of Morphine and Gamma Hydroxybutyrate on Respiration in The Rat and Rabbit" Eur. J. Pharmacol. (Accepted).

Patent

Feigenbaum, Jeff; Crumrine, David S. and Choubal, Milind D. "Synthesis and Applications of Dye-Neuropeptide Conjugates" US Patent (Application filed 1/23/92; Serial No. 07/824,295).

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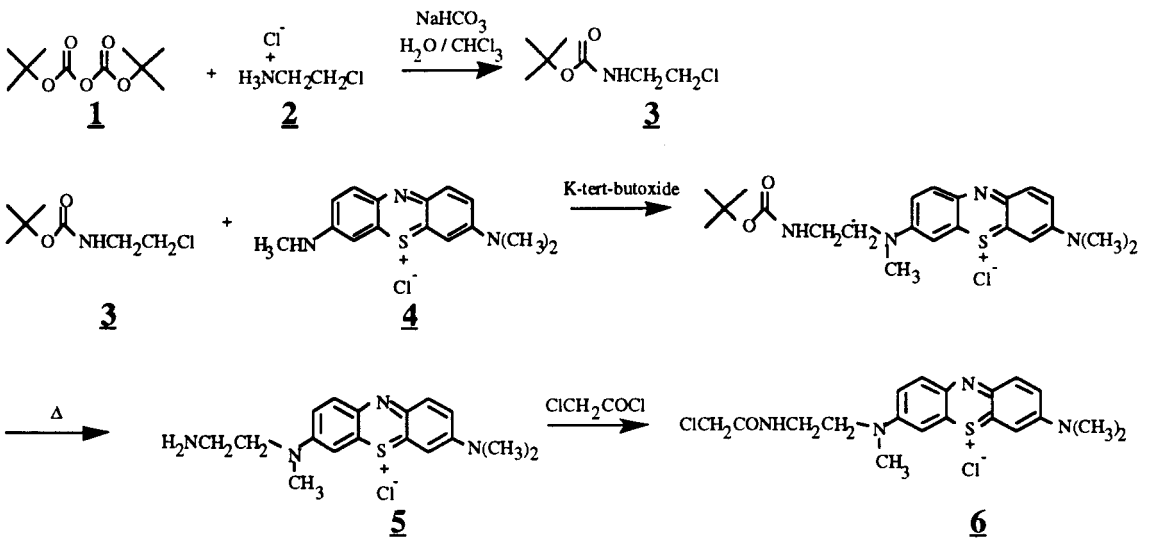
CHAPTER I

STATEMENT OF THE PROBLEM

The objective of our project was to attach a dye molecule to specific peptides. When this dye part is excited photochemically to a triplet state, it can transfer energy to oxygen to form excited singlet oxygen. The singlet oxygen thus formed in situ is highly reactive and can react with C-H, S-H etc. When the dye is attached to a peptide, the peptide can bind to a specific receptor site on a cell. Once the binding takes place, excitation of the dye part can lead to formation of singlet oxygen molecules at the cell surface. These singlet oxygen molecules being very reactive, can react with and destroy the cancerous cell. The receptor site to which the peptide part binds, is a function of the kind of peptide used. Thus by using the right peptide, the dye molecule can be carried to the desired tissue in the body.



1 + 2 → 3

3 + 4 → (K-tert-butoxide) → 5

5 → (Δ) → 6

The dye will be modified in four steps. Di- *tert*-butyl dicarbonate (1) will be reacted with 2-chloroethylamine (2) to form N-*tert*-butoxycarbonyl-2-chloroethylamine (3). It will be coupled with the zwitterion of Azure-B (4) and converted to N-(2-aminoethyl)Azure-B (5) by heating in the appropriate solvent. N-(2-

aminoethyl)Azure-B will be converted to N-(2-chloroacetyl amidoethyl)Azure-B (**6**) by reacting with chloroacetyl chloride. N-(2-Chloroacetyl amidoethyl)Azure-B will be coupled with different peptides in dilute acetic acid.

The conjugates will be characterized by UV, HPLC and ^{1}H-NMR.

CHAPTER II

INTRODUCTION

There are several diseases that take place at receptor levels such as cardiac, neurological or psychiatric disorders. Very high receptor concentrations were found in striatal tissue in Parkinsonism,[1,2] tardive dyskinesia[3] and schizophrenia.[1,3] Drugs that are currently used have several limitations.[4] They are not very specific and hence affect other receptors, thus blocking important functions. The side effects can be more dangerous than the disease itself. The other problems are drug tolerance and overdosing.

It was reported by Raab[5] that acridine dye is toxic to paramecia only when exposed to light. Meyer-Betz[6] reported a development of cutaneous edema upon injection of hematoporphyrin, followed by exposure to sunlight. These observations led to development of the very important technique of photodynamic therapy.

Miller and Selverston[7] were the first to find that single cells could be killed within a tissue without affecting other cells. It was done by filling a single cell with photoabsorptive dye and irradiating the tissue with high-intensity light. They used fluorescent light which has some limitations. It does not penetrate soft tissues and it can damage the cells by itself.

Anderson and Parrish[8-11] studied absorptive properties of human skin, soft tissue, and microvasculature and found that different laser wavelengths could be used to cause specific damage to pigmented tissue. A very brief pulse of laser energy was used. A cell filled with a dye absorbs more laser energy than the surrounding tissue and warms up. It causes the cell temperature to exceed the protein denaturation temperature and thus destroys the cell. After a short period, thermal equilibrium takes place and the whole tissue starts warming up slowly. Hence it is essential that duration of laser exposure should not exceed thermal relaxation of the tissue. They also found that nanosecond laser pulses were necessary to selectively destroy cells without affecting tissue. Higher energy pulses were

used to destroy microvessels and melanosomes several millimeters beneath the stain surface.

For some photosensitizers, the production of singlet oxygen is the mechanism by which the cells are destroyed within a tissue.[12] The cell is filled with a specific photosensitizer and irradiated with a laser beam. The photosensitizer forms singlet oxygen within the cell. Singlet oxygen is very toxic to the cell and can diffuse up to 0.1-0.2 μ, so the technique is highly selective. On the other hand, sometimes cytotoxic agents cause undesirable damage to neighboring cells.

Selection of the chromophore is very important.[13] Most fluroscent dyes require light-wavelengths less than 500 nm which do not penetrate mammalian tissue efficiently. For selective destruction of tissue, a laser of high penetration power is required. Generally the laser wavelength used for these purposes is 650-850 nm. It is necessary to have a chromophore that absorbs in this range. Lasers in this range can penetrate unpigmented tissue up to several millimeters without causing any damage.

Polycyclic aromatic hydrocarbons such as pyrenes and organic dyes (methylene blue or Acridine orange) act as photosensitizers.[14] They catalyze conversion of ground state triplet $^3O_2$ to extremely reactive singlet oxygen $^1O_2$. The photosensitizer molecule first reaches the effective site where it generates singlet oxygen. The lifetime of singlet oxygen is very short. Once singlet oxygen is generated, it decays to the ground state or reacts with a substrate. To cause damage to that cell, it does not have to penetrate inside the cell. It is capable of modifying any component of the biological system. In this respect, its binding to different cell components is not always necessary. It can react with lipids, proteins and nucleic acids. Site of reaction of a photosensitizer depends upon its intracellular location. If the photosensitizer can bind tightly to DNA, followed by breakage of DNA strands, it can cause genetic damage. If a photosensitizer is soluble in fat, it will cause membrane damage. It can alter the structures of mitochondria and endoplasmic

reticulum. It appears that this technique can destroy cells by different mechanisms. In some cases it was found that concentration of a photosensitizer in specific cell parts was critical for cell destruction.[14] In contrast, a photosensitizer bound to polystyrene beads and hence restricted to extracellular space, can inactivate some specific bacteria, but the same photosensitizer was ineffective when injected in solution form.

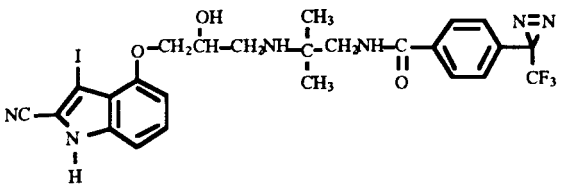
Photodynamic therapy is selective in different aspects.[15]

1. Application - It can be applied to a small group of cells or a single cell using specific carrier molecules.
2. Uptake - The affinity of different cells for the carrier is different. Hence, specific cells such as tumor cells can bind to the carrier molecule more readily than the normal cells. This cell property provides a selectivity for destroying only the tumor cells.
3. Illumination - The modern technology of fiber optics can be used to illuminate specific portions of tissue. Using this technology, it is also possible to irradiate only part of a single cell.
4. Susceptibility - The light will not affect any of the cell processes for normal cells.

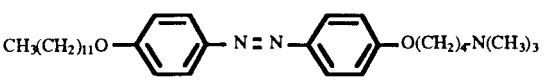
There are different techniques to destroy specific cells.[14]

1. Photosensitizers can be directed towards a particular site.
2. A prodrug can be used that breaks down to release a drug in high concentrations.
3. A drug can be released from a carrier using light. Liposomes can be used as carriers of photosensitizers.

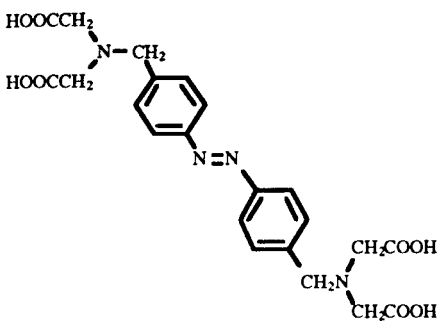
Photoaffinity reagent



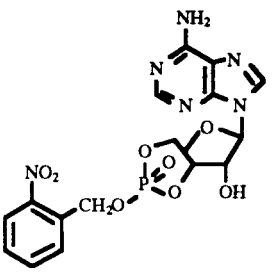
Photoisomerizable lipid



Photosensitive chelator



Photoactivable prodrug



The technique of photodynamic therapy appears to be very useful. Several types of carrier systems have been used in the past, including antibodies and liposomes. Devising a proper system to carry a photosensitizer to the receptor site in specific application is an important achievement in the medical field. A specific and selective delivery of the drugs to the specific cells within the normal tissue will be clinically very useful.

In one report, human T cells were used to test the idea of transporting drugs using monoclonal antibodies.[14] They selectively destroyed human T cells without affecting the B cells. Liposomes were prepared containing pyrene. Pyrene was then attached to a lipid bilayer. A monoclonal antibody was attached to these liposome surfaces. This antibody was bound to 90% T cells in human blood lymphocytes. Liposomes can carry both hydrophilic and hydrophobic molecules trapped in the intravescular space. They were then irradiated with UV light when most of the T cells were destroyed and the B cells stayed untouched.

Hematoporphyrin derivatives are widely used for cancer therapy for lung, bladder and skin tumors.[14] Patients are given hematoporphyrin derivatives several days before the optical therapy is used. Tumors are irradiated with laser beam through fiber optics. The recovery rate is 70-80% for early stages. However, a major limitation of this technique is that it cannot be used in vicinity of liver, spleen or kidneys. There are reports of using hematoporphyrin labeled antibodies to destroy myosarcoma or leukemia.[14]

Selective cell destruction can be carried out both in vitro and in vivo technique. In the case of in vitro studies,[16,17] short laser pulses in 650-700 nm range were effective. The damage was done either by thermal or singlet oxygen mechanisms. The important factors were dye concentration within the cell and the dose of laser irradiation. It was necessary to use a carrier system to carry the dye molecule to the desired tissue. Several different systems were studied without significant success. Different lectins were used for dye conjugation. However, it was not useful for in vivo[18] use. Liposomes with high dye concentration were successful for in vitro studies but three dimensional diffusion limited in vivo studies. One interesting technique reported was the use of polymer beads as a carrier.[19] These beads have a diameter of a few nanometers and they can hold a high concentration of a chromophore. These beads are made from a special type of polymer which is biocompatible and resistent to degradation. Important factors in this technique are particle size, particle surface charge and number of particles at the injection site. One carrier system reported is latex particles. These particles can be loaded with up to 14% (wt/wt) of a dye. This binding did not change the efficiency of singlet oxygen production. In one study chlorin e_6 was incorporated into latex particles.[12] It was found that latex particles do not diffuse at the injection site or form the labelled cells. The cells were labeled in vitro and then transplanted in vivo. Other techniques used earlier were not capable of carrying high concentrations of the photosensitizer.

We are developing a new technique that involves a peptide covalently bound to a dye. The peptide will carry the dye to a specific receptor site. After the receptor binding takes place, the dye will be irradiated with laser. This will lead to a formation of singlet oxygen which will inactivate the neighboring receptors. The important aspects of this technique are: (1) It should have a high quantum yield of singlet oxygen. (2) To have enough concentration of $^1O_2$ at the receptor site, it should not diffuse more than 100 Å. (3) The dye-peptide conjugate must have the same affinity for the receptor site as the peptide.

This technique provides a new tool to cure several diseases of the receptor level. There are several advantages of this technique. Only a narrow area around the intended receptor will be affected. The effect will last much longer than the drugs commonly used. Several side effects caused by regular drugs such as overdosing, drug abuse and drug tolerance problems can be eliminated.

This technique also has several other advantages. It will be possible to study different conjugates coupled to specific receptors and their response in those regions. Thus, based on a specific peptide used, it may be possible to cure the disorders in those particular receptor sites. When this technique is fully developed, it will have advantages in different medical fields such as neurosurgery, neurogenic and musculoskeletal disorders, movement disorders, neuropsychiatric disorders such as schizophrenia.

We are using an analogue of a neuropeptide called FMRF-amide. It was first detected by Price and Greenburg[20] in Lamellibranch Macrocallirta. It was also found in several different species such as Mollusca, Insecta, Crustaca and Mammalia.[21-23] Payza and coworkers[24] reported electrophysiological characterization of effects of FMRF-amide. They also showed its function as a neurotransmitter in Helix brain. It was also found in Helix/auricle and AV junction.[25] Its function in different activities such as cardioexcitatory[26], regulation of feeding[27] and muscle contractibility[28] has been reported.

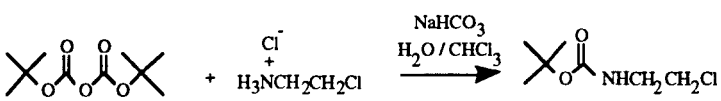
For our work, analogues of FMRF-amide were custom synthesized by Bachem Laboratories. To allow the conjugation of dye to peptide, cysteine was added to FMRF-amide to form CFMRF-amide (Cys-Phe-Met-Arg-Phe-NH_2). Another related peptide from FMRF-amide is FLRF-amide. In this case, phenylalanine was replaced by cysteine and met was replaced by Leu to make CLRF-amide (Cys-Leu-Arg-Phe-NH_2) which is also useful in conjugation.

CHAPTER III

RESULTS AND DISCUSSION

The general objective of our synthetic work was to attach a dye molecule having a suitable λ_{max}, and serving as a good generator of singlet oxygen, to several peptides. The most general procedure would be to prepare derivatives of the dye which could be attached to a variety of peptides. We chose the commercially available Azure-B as a good starting point to make derivatives of methylene-blue, a well known dye for generating singlet oxygen.

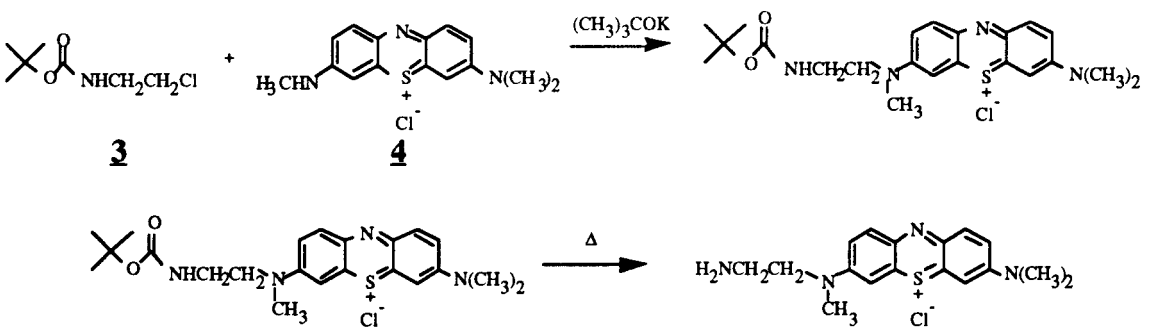
The first synthetic step was the preparation of N-t-butoxycarbonyl-2-chloroethylamine (**3**). A common procedure reported by Tarbell and coworkers[30] was followed involving a reaction of di-t-butyl dicarbonate (**1**) with 2-chloroethylamine hydrochloride (**2**) at RT for 48 h. TLC, IR and ^{1}H NMR of the reaction mixture indicated that product was not formed. When the temperature was changed to reflux, all of the di-t-butyl dicarbonate reacted forming the desired product, but some 2-chloroethylamine remained unreacted. When the reaction was performed with a slight excess of di-t-butyl dicarbonate, IR and ^{1}H NMR indicated that the reaction went to completion. The liquid product was characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy.



1 **2** **3**

The second step was the reaction of N-t-butoxycarbonyl-2-chloroethylamine (**3**) with the zwitterion of Azure-B (**4**). A zwitterion of Azure-B was first prepared with potassium *tert*-butoxide. It was then reacted with N-t-butoxycarbonyl-2-chloroethylamine. The reaction was attempted in different solvents such as THF, acetonitrile, methanol and at

temperatures from RT to reflux. Also, it was essential that the solvent and the base should be non-nucleophilic. The best solvent for the reaction was found to be DMSO, and potassium *tert*-butoxide was used as a base. The temperature for the reaction was critical. It was found that above 150 ^{o}C the decomposition of Azure-B starts, while at 120-130 ^{o}C more by-products form. If the temperature is less than 90 ^{o}C, the reaction does not work. When the reaction was attempted in refluxing DMSO, the reaction mixture was a brownish black liquid. Column chromatography of this mixture yielded traces of the product and the NMR showed the presence of another decomposition product. The best conditions for the reaction were 100 ^{o}C in DMSO for 7 h. Under these conditions the product further decomposes to the desired dye-amine (**5**). HPLC of the reaction mixture showed the presence of three compounds. The most convenient method to remove the DMSO and the undesired decomposition products was column chromatography on silica gel. Fractions from the column chromatography were tested by HPLC. The DMSO and the decomposition products were eluted with methylene chloride; the unreacted Azure-B was eluted with 10% methanol in methylene chloride. The product (**5**) was eluted with 15% methanol in methylene chloride.



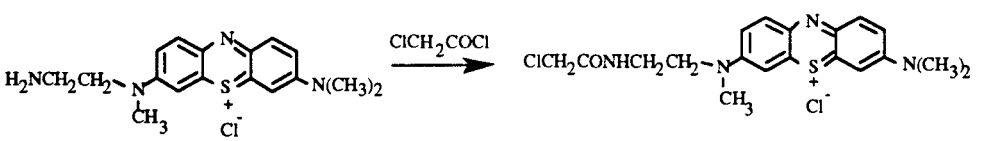
3 **4** **5**

Characterization of the product was based on ^{1}H NMR and reverse phase HPLC. Several attempts were made to find the best solvent system for the HPLC of this reaction

mixture. In one attempt 80% acetonitrile, 20% water and 0.1% TFA was used. This solvent system worked for the reaction mixture in which iodide was counter ion. However, the reaction mixtures with chloride counterion were completely retained by the column. When chloride is a counterion, the dye molecule is bound more tightly to the column due to strong Vander Weal's forces between the dye and the chloride. When the percentage of TFA was increased to 0.2%, the dye came out of the column.

To improve the reaction yield, the same reaction was carried out in the presence of potassium iodide. The reaction mixture was analyzed by HPLC. With the exception of the counterion, the product composition was same as the reaction run without potassium iodide. It was found that conjugates with iodide counterion were not suitable for receptor binding studies, so this technique was not modified further.

The next step was reaction of the dye-amine (5) with chloroacetyl chloride to form the chloroacetyl derivative (6) of the dye. It was essential that the solvent be nonnucleophilic as chloroacetyl chloride is very reactive and can react with a nucleophilic solvent. The best nonnucleophilic solvent used for this reaction was acetonitrile. The HPLC retention time was different than that for the dye-amine and the ^1H NMR confirmed formation of the desired chloroacetamide dye 6.

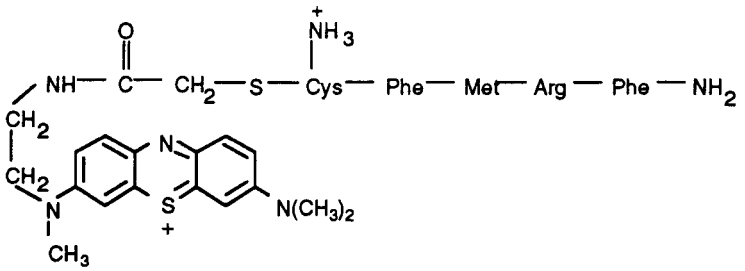


5 6

The chloroacetyl dye derivative (6) was coupled with the pentapeptide amide (7) in 0.1M acetic acid in D_2O at room temperature for 10 h to produce the desired blue dye-peptide amide conjugate (8).

Cys-Phe-Met-Arg-Phe-NH_2

7

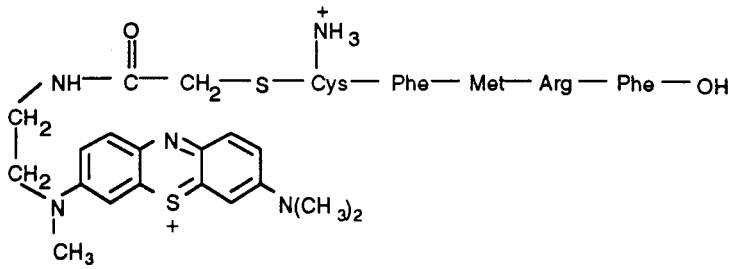


8

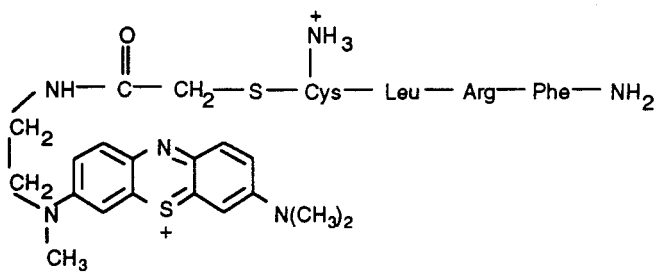
The coupling was done under acidic conditions as the peptides are stable in acidic solution. The cystine thiol group undergoes SN_2 attack on **6**. HPLC analysis using conditions where the chloroacetyl dye (**6**), the pentapeptide amide (**7**), and the conjugate (**8**) could all be distinguished showed only (**8**) after 10 h. A 5 h reaction time showed incomplete coupling. The ^1H NMR in D_2O (see Experimental) showed the presence of methyl and aromatic protons from the dye, protons from the tether, and protons from the peptide in which some of the chemical shifts were different in the original peptide and the dye-peptide conjugate.

Cys-Phe-Met-Arg-Phe-OH	Cys-Leu-Arg-Phe-NH_2
9	**10**

Similar results were obtained when chloroacetyl dye (**6**) was coupled with pentapeptide-OH (**9**) and tetrapeptide-amide (**10**) to obtain the corresponding conjugates (**11 and 12**). All products could be distinguished on the HPLC system (see Table 1 below). HPLC was done on a GOW-MAC instrument (Model No. 080-20) using a 30 cm x 4.6 mm C-18 reverse-phase column, a 254 nm UV detector with 80% acetonitrile, 20% water and 0.2% trifluoroacetic acid as eluant.



11



12

Table 1. HPLC of the Conjugates.

Compound	HPLC retention time in minutes
Azure-B	33
Dye-Amine	18
Dye-chloride	17.5
Pentapeptide-amide	5.3
Pentapeptide-amide conjugate (**8**)	21
Pentapeptide-OH	5.5
Pentapeptide-OH Conjugate (**11**)	19.75
Tetrapeptide-amide	6
Tetrapeptide-amide conjugate (**12**)	20.3

Preliminary data on the efficiency of laser induced production of singlet oxygen shows a pH dependent enhancement of as much as 100% for the conjugates over methylene blue. Specific receptor binding studies in Helix Aspersa shows a 50% greater affinity of the pentapeptide amide conjugate **8** for the FMRFamide receptor site than for FMRFamide itself. The unconjugated pentapeptide amide showed a 2.5 times better binding than FMRFamide. Finally, preliminary electrophysiological experiments[29] confirm earlier reports that the FMRFamide receptor is inactivated by the pentapeptide amide conjugate **8** while it is not inactivated by a conjugate of the same amino acid sequence pentapeptide that is not a C-terminal amide.

CHAPTER IV

CONCLUSION

We have developed a useful technique for covalently attaching a dye, with a chromophore similar to methylene blue, to a small peptide. Using this procedure on the tethered dye derivative **6**, one can now make a number of potentially useful dye-peptide conjugates. We have demonstrated the technique by synthesizing derivatives related to FMRFamide. The utility of these conjugates can be inferred from initial studies in which the dye-peptide conjugates show good enhancement (values vary with pH) in singlet oxygen production over methylene blue and show receptor binding and electrophysiology activities comparable to the native peptides. Thus, we have developed a new tool for control of photodynamic therapy.

CHAPTER V

EXPERIMENTAL

Azure B, di-tert-butyl dicarbonate and 2-chloroethylamine monohydrochloride were obtained from Aldrich. The peptides, Cys-Phe-Met-Arg-Phe-NH_2, Cys-Phe-Met-Arg-Phe-OH and Cys-Leu-Arg-Phe-NH_2, were purchased from Bachem. The infrared spectra were taken on Perkin-Elmer 1310 infrared spectrophotometer, and the NMR spectra were taken on a Varian VXR-300 instrument. Flash grade silica gel from Baker was used for the column chromatography. HPLC was done on a GOW-MAC instrument (Model No. 080-20) using a 30 cm x 4.6 mm C-18 reverse-phase column, a 254 nm UV detector with 80% acetonitrile, 20% water and 0.2% trifluoroacetic acid as eluant. TLC R_f values were measured on silica gel with methanol as eluent. UV spectra were measured on HP 8451 or PE 575 in D_2O.

N-tert-Butoxycarbonyl-2-chloroethylamine (3): A 50 mL 2 neck RB flask fitted with a reflux condenser was charged with 690 mg (6.0 mmol) of 2-chloroethylamine monohydrochloride suspended in 10 mL of chloroform. To this mixture was added 1.2 g of sodium chloride and 504 mg (6.0 mmol) of sodium bicarbonate dissolved in 7.5 mL of water. Di-*t*-butyl dicarbonate (1.09 g, 5.0 mmol) was added, and the reaction mixture was refluxed for 2h. The reaction mixture was cooled to room temperature, and extracted with two 50 mL portions of chloroform. The chloroform extracts were combined, dried over anhydrous magnesium sulfate and the solvent was removed on a rotary evaporator to afford 850 mg of yellow liquid (4.7 mmol, 94 %). ^{1}H-NMR ($CDCl_3$) 5.25 (1H, broad s,

NH) 3.55 (2H, t, CH_2O), 3.4 (2H, q, CH_2N), 1.4 (9H, s, t-Bu); ^{13}C-NMR ($CDCl_3$) 155.6, 79.8, 44.2, 42.5, 28.3; IR 3400, 1680, 1715 cm^{-1}; R_f (TLC) = 0.77.

N-(2-Aminoethyl)Azure-B (5): A 50 mL 2-neck RB flask fitted with a condenser and addition funnel was charged with 62 mg (0.55 mmol) of potassium *t*-butoxide (K-*t*-butoxide) and 10 mL of DMSO. The solution was stirred to dissolve K-tert- butoxide and 170.4 mg (0.56 mmol) of azure-B, and 0.1 g (0.56 mmol) of N-tert-butoxycarbonyl-2-chloroethylamine (**3**) were added to this solution. The solution was maintained at 100 °C for 7h. The color of the solution changed from purple to dark blue. The DMSO was removed using column chromatography. A silica gel (125 g) column was packed using methylene chloride. The reaction mixture (12 mL) was added to the column. 10% Methanol in methylene chloride (875 mL) was used to remove DMSO and other impurities, and the blue solid product (52 mg, 0.17 mmol, 30%) was eluted using 15% methanol (500 mL).^{1}H-NMR (D_2O) 7.25 (2H, m, ArH), 6.8 (1H, m, ArH), 6.7 (1H, m, ArH), 6.55 (1H, s, ArH), 6.4 (1H, s, ArH), 2.93 (4H, m, CH_2N), 2.93 (6H, s, $(CH_3)_2N$), 2.68 (3H, s, CH_3N); IR 3350, 3100 cm^{-1}; λ_{max} = 632 nm; R_f (TLC): R_f (Azure B) = 0.02, R_f (N-tert-butoxycarbonyl-2-chloroethyl amine) = 0.77, R_f (**5**) = 0.02; HPLC: Retention times (min) 18 (5), 33 (Azure-B).

N-(2-Chloroacetyl amidoethyl)Azure-B (6): A 50 mL 3 neck RB flask fitted with a condenser was charged with 4.3 mg (0.013 mmol) of the amine (**5**) and 15 mL of acetonitrile. To this solution was added 0.01 mL (3 mmol) of chloroacetyl chloride. The solution was stirred at room temperature under N_2 for 12 h. The solvent was removed on

a rotary evaporator to obtain a blue solid (5.1 mg, 0.013 mmol, 100%). ^{1}H-NMR ($CDCl_3$) 8.93 (1H, s, ArH), 8.91 (1H, s, ArH), 8.47 (1H, t, J = 7.0, ArH), 7.98 (3H, t, J = 7.0, ArH),3.68 (2H, s, $ClCH_2CO$), 3.38 (4H, m, NCH_2CH_2N), 2.71 (9H, pseudo s, CH_3N); IR 3100, 1710 cm^{-1}; λ_{max} = 629 nm; R_f TLC (Silica gel/methanol): R_f (Dye amine) = 0.02, R_f (chloroacetyl chloride) = 0.78, R_f (Reaction mixture) = 0.04, 0.78. HPLC: Retention time (min) 17.5 (6).

Cys-FMRF-amide analogues: The pentapeptide and tetrapeptides were custom synthesized and analyzed by Bachem Bioscience (Philadelphia). HPLC analysis disclosed a 99% purity of the analogues, and a minimal (<1%) disulfide coupling. A 300 MHz ^{1}H NMR spectra confirmed the structure of the peptides.

Table 2. ^{1}H NMR of Pentapeptide Amide (7) in D_2O

Cys-Phe-Met-Arg-Phe amide

Expected chemical shifts

Residue	αH	βH	Others
Cys	4.69, 1H	3.28, 2.96, 2H	-----
Phe	4.66, 2H	3.22, 2.99, 4H	7.3, 7.39, 7.30, 10H, ArH
Met	4.52, 1H	2.15, 2.01, 2H	2.64, 2.64, 2H, γCH_2
			2.13, 3H, εCH_3
Arg	4.38, 1H	1.89, 1.79, 2H	1.70, 1.70, 2H, γCH_2
			3.32, 3.32, 2H, δCH_2
	5H	10H	19H

Observed chemical shifts

Residue	αH	βH	Others
Cys	4.52, 1H	3.5, 2.7, 2H	-----
Phe	3.56, 2H	2.9, 2.75, 4H	7.0-7.2, 10H, ArH
Met	4.19, 1H	2.2, 2H	2.75, 2H, γCH_2
			1.85, 3H, εCH_3
Arg	4.0, 1H	1.26, 1.09, 2H	1.46, 1.7, 2H, γCH_2
			2.9, 2H, δCH_2
	5H	10H	19H

Table 3. ^{1}H NMR of Pentapeptide-OH (9) in D_2O

Cys-Phe-Met-Arg-Phe-OH

Expected chemical shifts

Residue	αH	βH	Others
Cys	4.69, 1H	3.28, 2.96, 2H	-----
Phe	4.66, 2H	3.22, 2.99, 4H	7.3, 7.39, 7.30, 10H, ArH
Met	4.52, 1H	2.15, 2.01, 2H	2.64, 2.64, 2H, γCH_2
			2.13, 3H, εCH_3
Arg	4.38, 1H	1.89, 1.79, 2H	1.70, 1.70, 2H, γCH_2
			3.32, 3.32, 2H, δCH_2
	5H	10H	19H

Observed chemical shifts

Residue	αH	βH	Others
Cys	4.28, 1H	2.89, 2H	-----
Phe	4.28, 3.12, 2H	2.89, 2.78, 4H	7.0-7.2, 10H, ArH
Met	4.15, 1H	2.22, 2H	2.78, γCH_2
			1.84, 3H, εCH_3
Arg	4.05, 1H	1.3, 2H	1.48, 1.7, 2H, γCH_2
			2.89, 2H, δCH_2
	5H	10H	19H

Table 4. ^{1}H NMR of Tetrapeptide Amide (10) in D_2O

Cys-Leu-Arg-Phe-NH_2

Expected chemical shifts

Residue	αH	βH	Others
Cys	4.69, 1H	3.28, 2.96, 2H	-----
Phe	4.66, 1H	3.22, 2.99, 2H	7.3, 7.39, 7.30, 5H, ArH
Leu	4.38, 1H	1.65, 1.65, 2H	1.64, 1H, γCH_2
			0.94, 0.90, 6H, δCH_3
Arg	4.38, 1H	1.89, 1.79, 2H	1.70, 1.70, 2H, γCH_2
			3.32, 3.32, 2H, δCH_2
	4H	8H	16H

Observed chemical shifts (D_2O)

Residue	αH	βH	Others
Cys	4.4, 1H	2.92, 2H	-----
Phe	4.2, 1H	2.92, 2H	7.05-7.2, 5H, ArH
Leu	4.2, 1H	1.45, 2H	1.33 , 1H, γCH_2
			0.7, 0.75 , 6H, δCH_3
Arg	4.05, 1H	1.45, 2H	1.45, 2H, γCH_2
			3.15 , 2H, δCH_2
	4H	8H	16H

Coupling of dye-chloride (6) with Pentapeptide-amide (7): To a 50 mL RB flask was added 1.2 mL of D_2O and 5.7 μL of acetic acid under N_2. The solution was degassed by repeated freeze pump thaw cycles under vacuum. The dye-chloride (6) (0.55 mg) was then added, followed by 1 mg of pentapeptide-amide (7). The solution was degassed and left under N_2 at room temperature for 10 h. After 10 h, the solution was lyophilized and a blue colored solid (8) was obtained. HPLC, UV spectra (λ_{max} = 628 nm, ε=14090) and 300 MHz ^{1}H-NMR are consistent with the structure. HPLC: Retention times (min) 5.3 (Pentapeptide-amide), 21 (Pentapeptide-amide conjugate).

Table 5. ^1H NMR of Pentapeptide-Amide (8) Conjugate in D_2O

Residue	αH	βH	Others
Cys	4.52, 1H	2.9, 2.7, 2H	-----
Phe	4.25, 4.08, 2H	2.9, 2.75, 4H	7.0-7.2, 10H, ArH
Met	4.42, 1H	2.2, 2H	2.75, 2H, γCH_2
			1.85, 3H, εCH_3
Arg	4.01, 1H	1.26, 1.09, 2H	1.46, 1.7, 2H, γCH_2
			3.1, 2H, δCH_2
Dye			7.4, 2H, ArH
			7.1, 1H, ArH
			6.86, 2H, ArH
			6.68, 1H, ArH
			3.07, 6H, CH_3
			2.9, 2.5, 4H, - CH_2CH_2-
			2.8, 3H, CH_3
			2.5, 2H, CH_2S
	5H	10H	40H

Coupling of dye-chloride (6) with Pentapeptide-OH (9): To a 50 mL RB flask was added 1.2 mL of D_2O and 5.7 μL of acetic acid under N_2. The solution was degassed by repeated freeze pump thaw cycles under vacuum. The dye-chloride (**6**) (0.55 mg) was then added, followed by 1 mg of pentapeptide-OH (**9**). The solution was degassed and left under N_2 at room temperature for 10 h. After 10 h, the solution was lyophilized and a

blue colored solid (**11**) was obtained. HPLC, UV spectra (λ_{max} = 628 nm, ε=13290) and 300 MHz ^{1}H-NMR are consistent with the structure. HPLC: Retention times (min) 5.5 (Pentapeptide-OH), 19.75 (Pentapeptide-OH conjugate).

Table 6. ^{1}H NMR of Pentapeptide-OH Conjugate (11) in D_2O

Residue	αH	βH	Others
Cys	4.28, 1H	2.89, 2H	-----
Phe	4.28, 3.12, 2H	2.89, 2.78, 4H	7.0-7.2, 10H, ArH
Met	4.15, 1H	2.22, 2H	2.78, γCH_2
			1.84, 3H, εCH_3
Arg	4.05, 1H	1.3, 2H	1.48, 1.7, 2H, γCH_2
			2.89, 2H, δCH_2
Dye			7.59, 2H, ArH
			7.1, 1H, ArH
			6.89, 2H, ArH
			6.72, 1H, ArH
			3.12, 2.95, 6H, CH_3
			3.15, 2.5, 4H, - CH_2CH_2-
			2.82, 3H, CH_3
			2.53, 2H, CH_2S
	5H	10H	19H

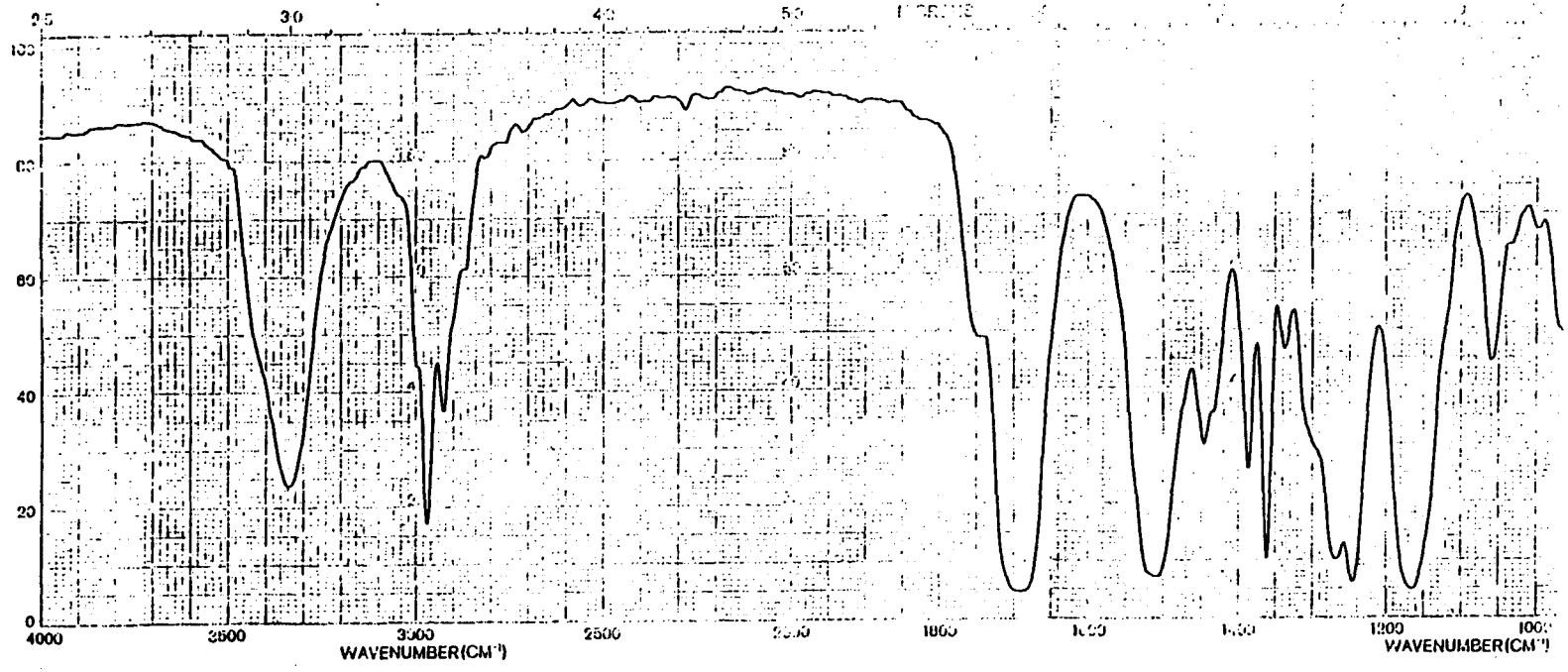
Coupling of dye-chloride (6) with Tetrapeptide-amide (10): To a 50 mL RB flask was added 1.2 mL of D_2O and 5.7 μL of acetic acid under N_2. The solution was degassed by

repeated freeze pump thaw cycles under vacuum. The dye-chloride (**6**) (0.55 mg) was then added, followed by 1 mg of tetrapeptide amide (**10**). The solution was degassed and left under N_2 at room temperature for 10 h. After 10 h, the solution was lyophilized and a blue colored solid (**12**) was obtained. HPLC: Retention times (min) 6 (Tetrapeptide-amide), 20.3 (Tetrapeptide-amide conjugate). HPLC, UV spectra (λ_{max} = 626 nm, ε=14428) and 300 MHz ^{1}H-NMR are consistent with the structure.

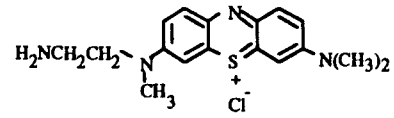
Table 7. ^{1}H NMR of Tetrapeptide-Amide Conjugate (12) in D_2O

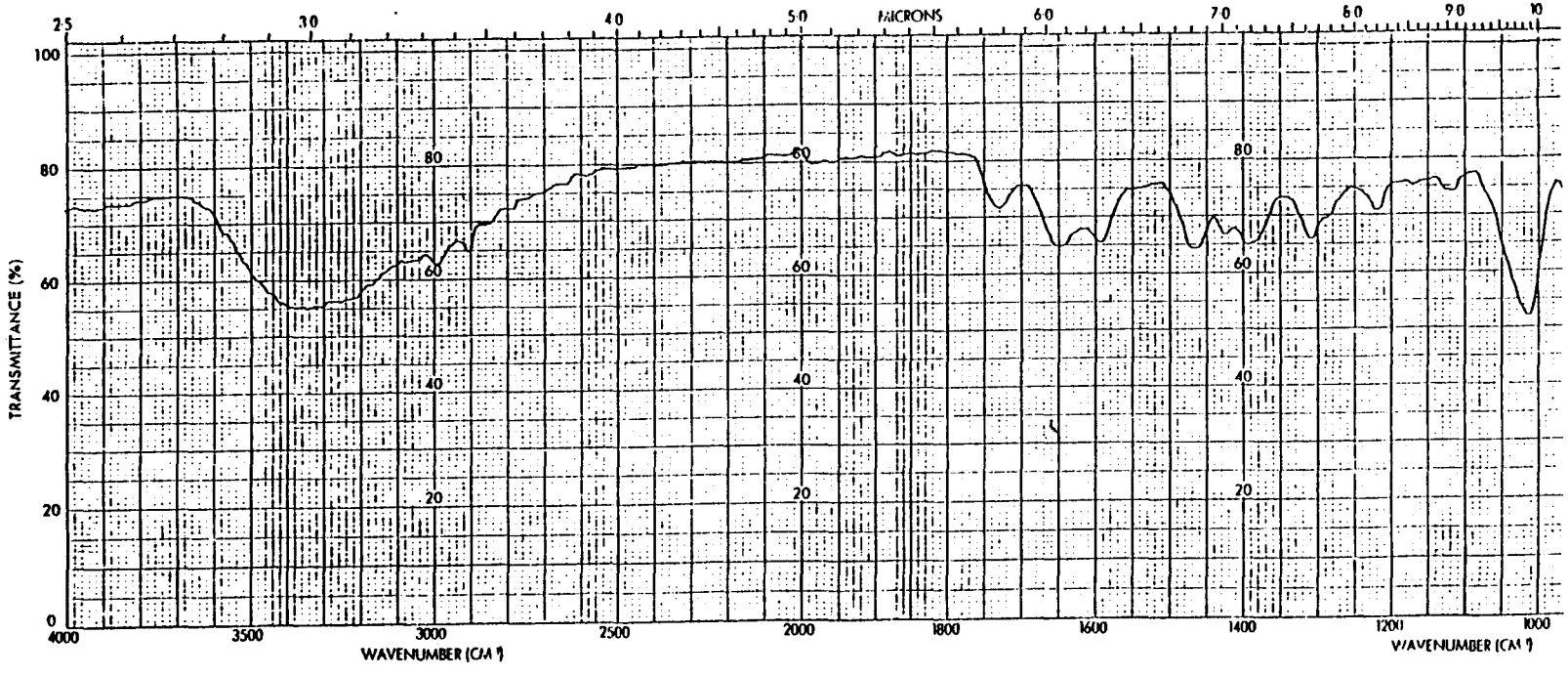
Residue	αH	βH	Others
Cys	4.4, 1H	2.92, 2H	-----
Phe	4.2, 1H	2.92, 2H	7.05-7.2, 5H, ArH
Leu	4.2, 1H	1.45, 2H	1.33 , 1H, γCH_2
			0.7, 0.75 , 6H, δCH_3
Arg	4.05, 1H	1.45, 2H	1.45, 2H, γCH_2
			3.15 , 2H, δCH_2
Dye			7.2, 1H, ArH
			7.49, 2H, ArH
			6.88, 2H, ArH
			6.71, 1H, ArH
			3.1, 2.95, 6H, CH_3
			3.21, 4H, - CH_2CH_2-
			2.9, 3H, CH_3
			2.03, 2H, CH_2S
	4H	8H	37H

SPECTRA

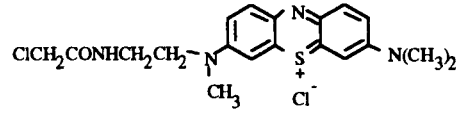


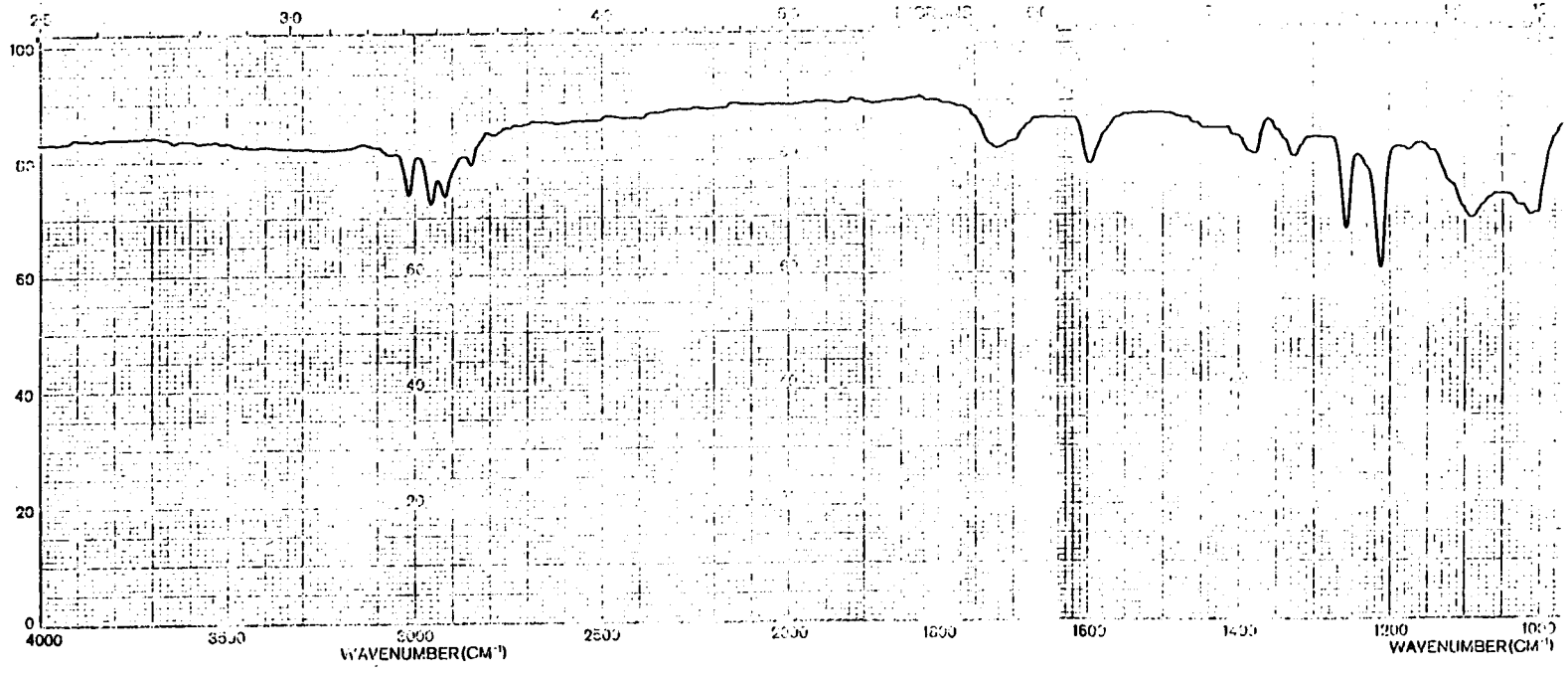
IR of N-tert-Butoxycarbonyl-2-chloroethyl amine (**3**)



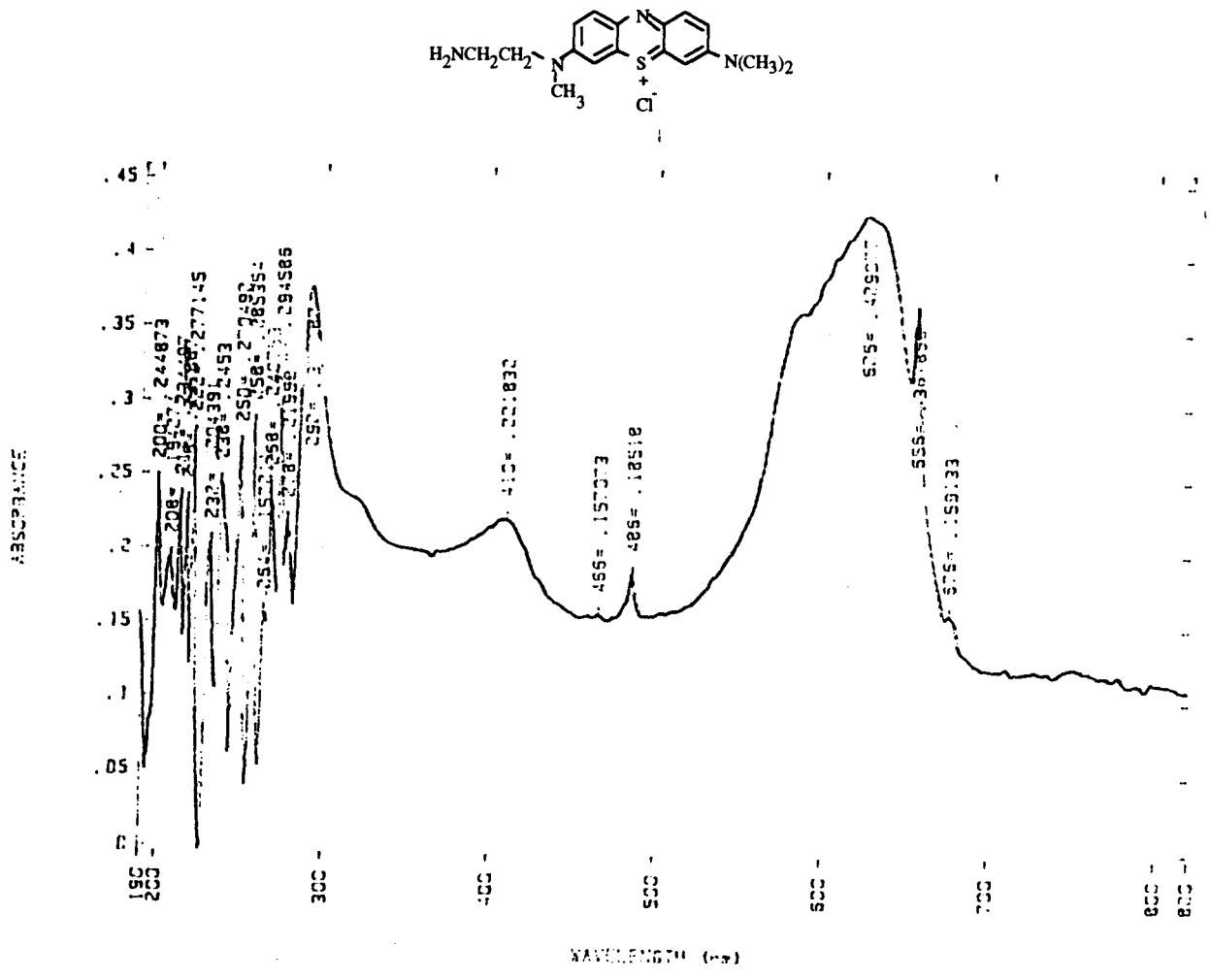


IR of N-(2-Aminoethyl)Azure-B (**5**)

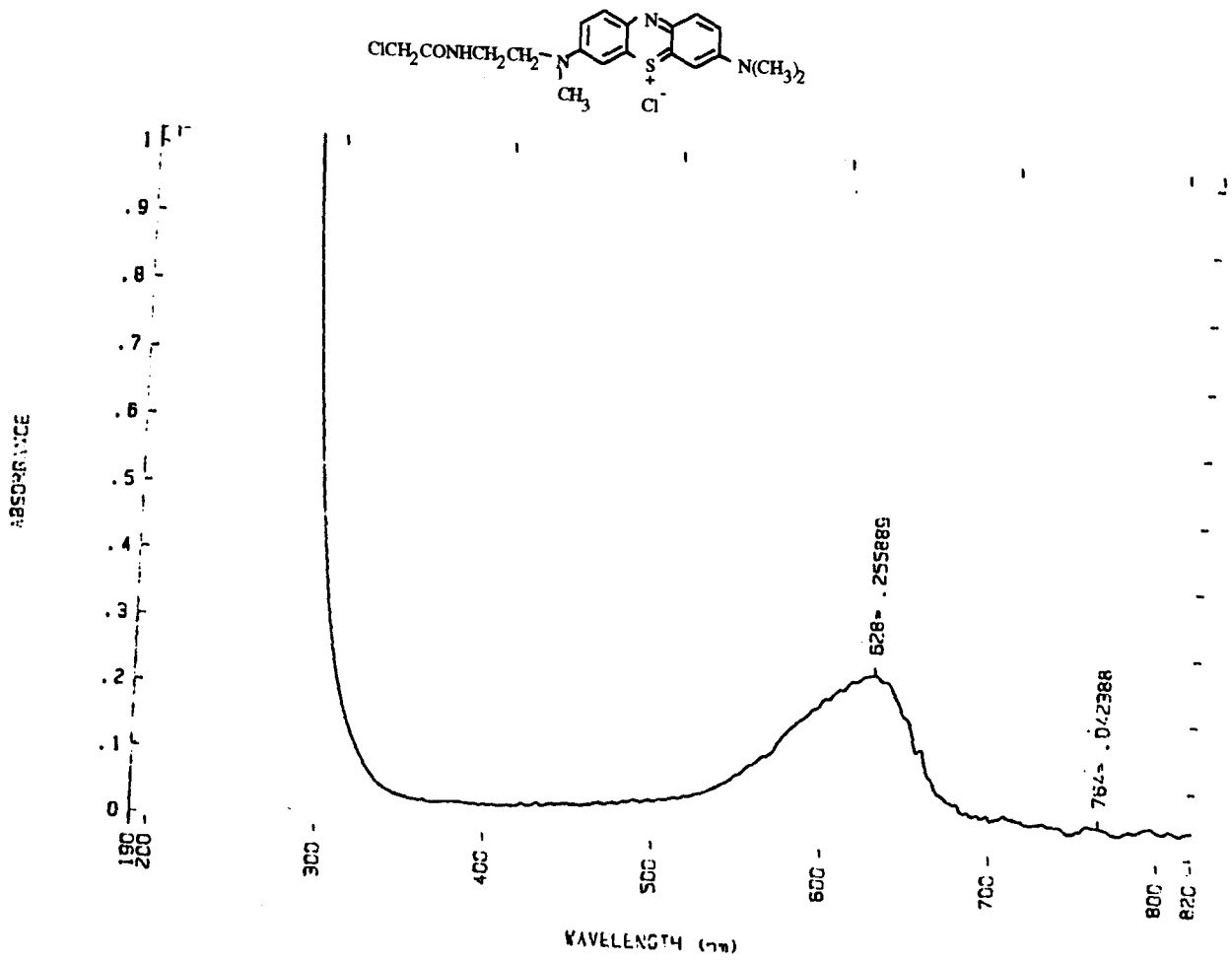




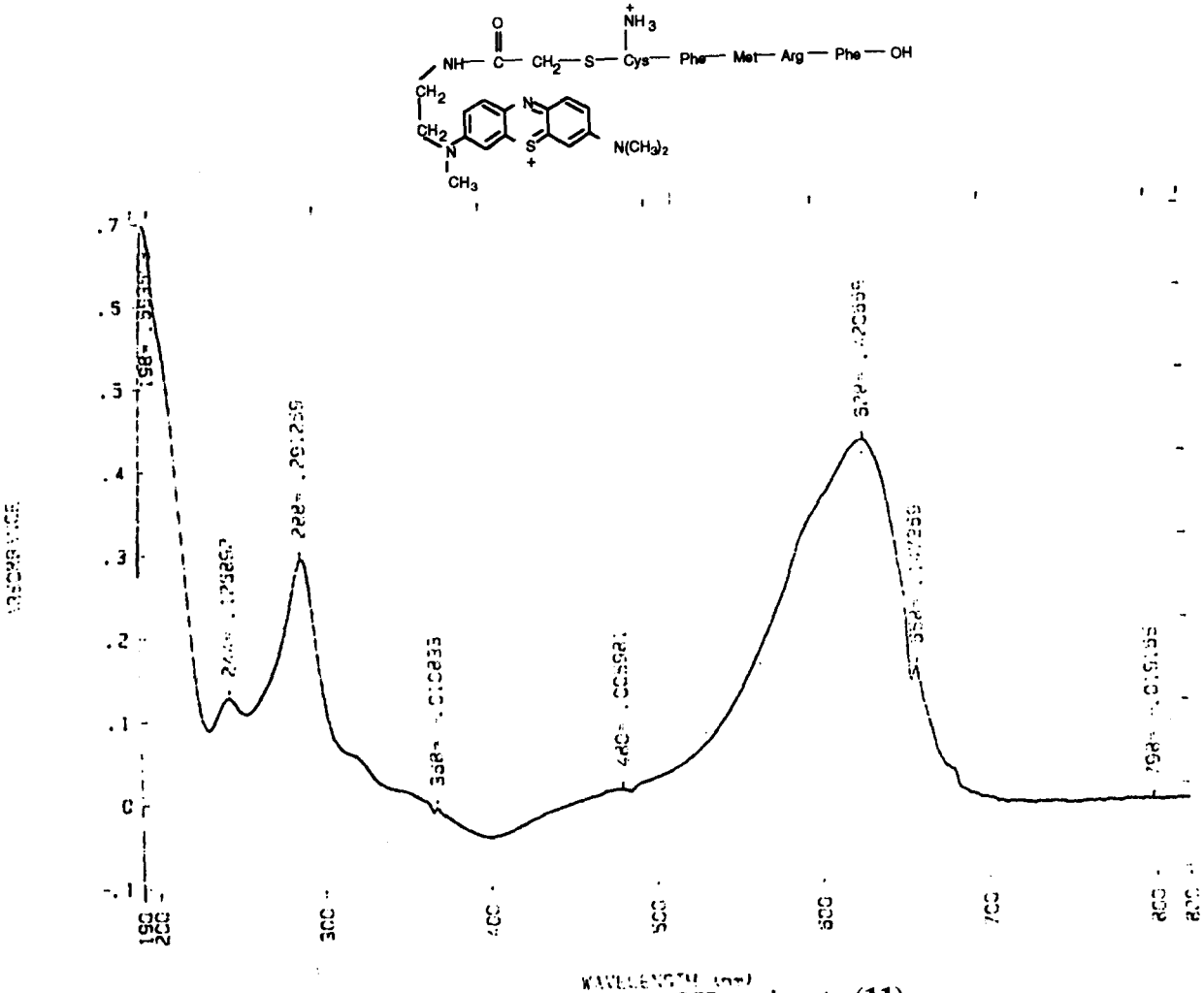
IR of N-(2-Chloroacetyl aminoethyl)Azure-B (**6**)



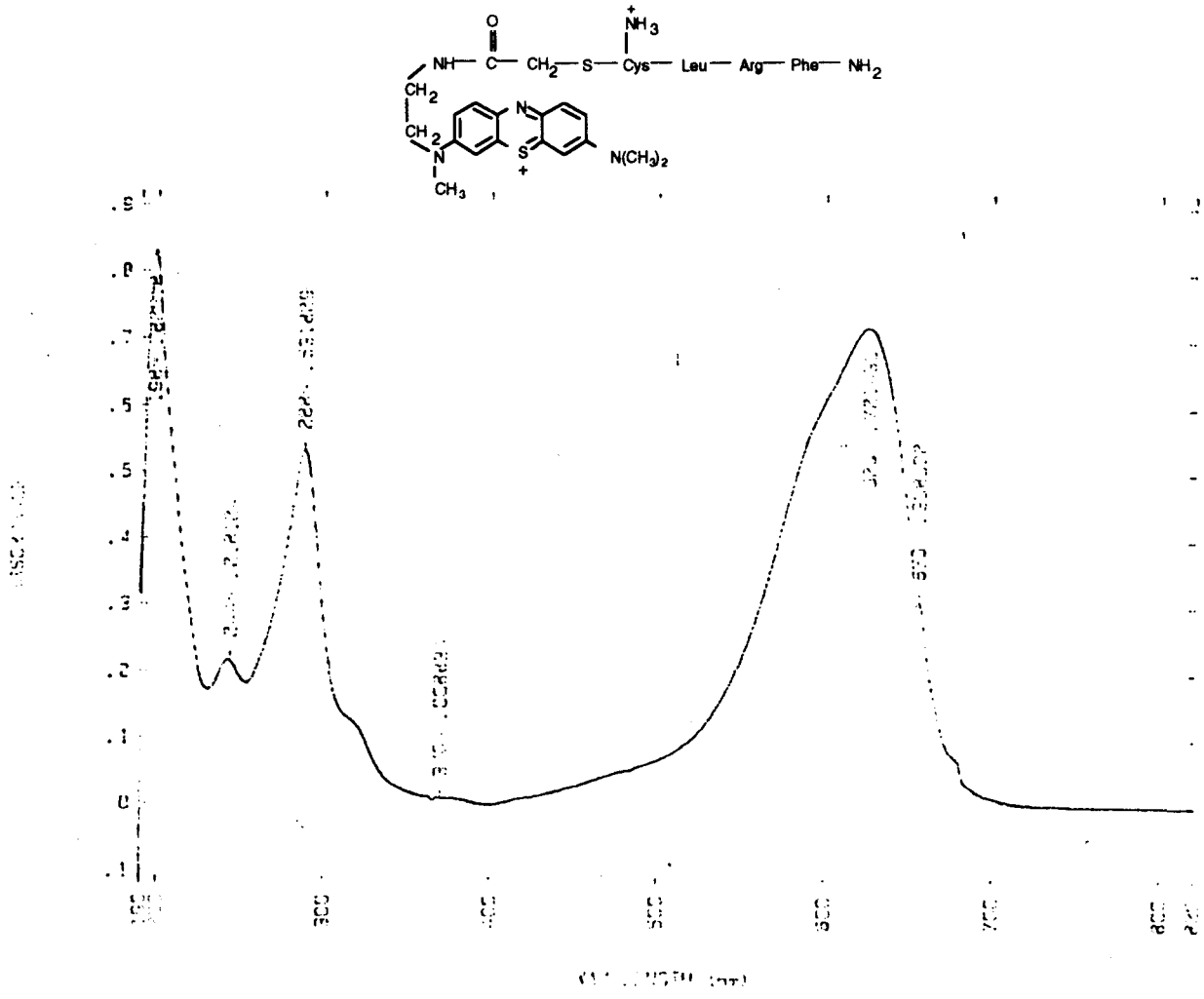
UV of N-(2-Aminoethyl)Azure-B (**5**)



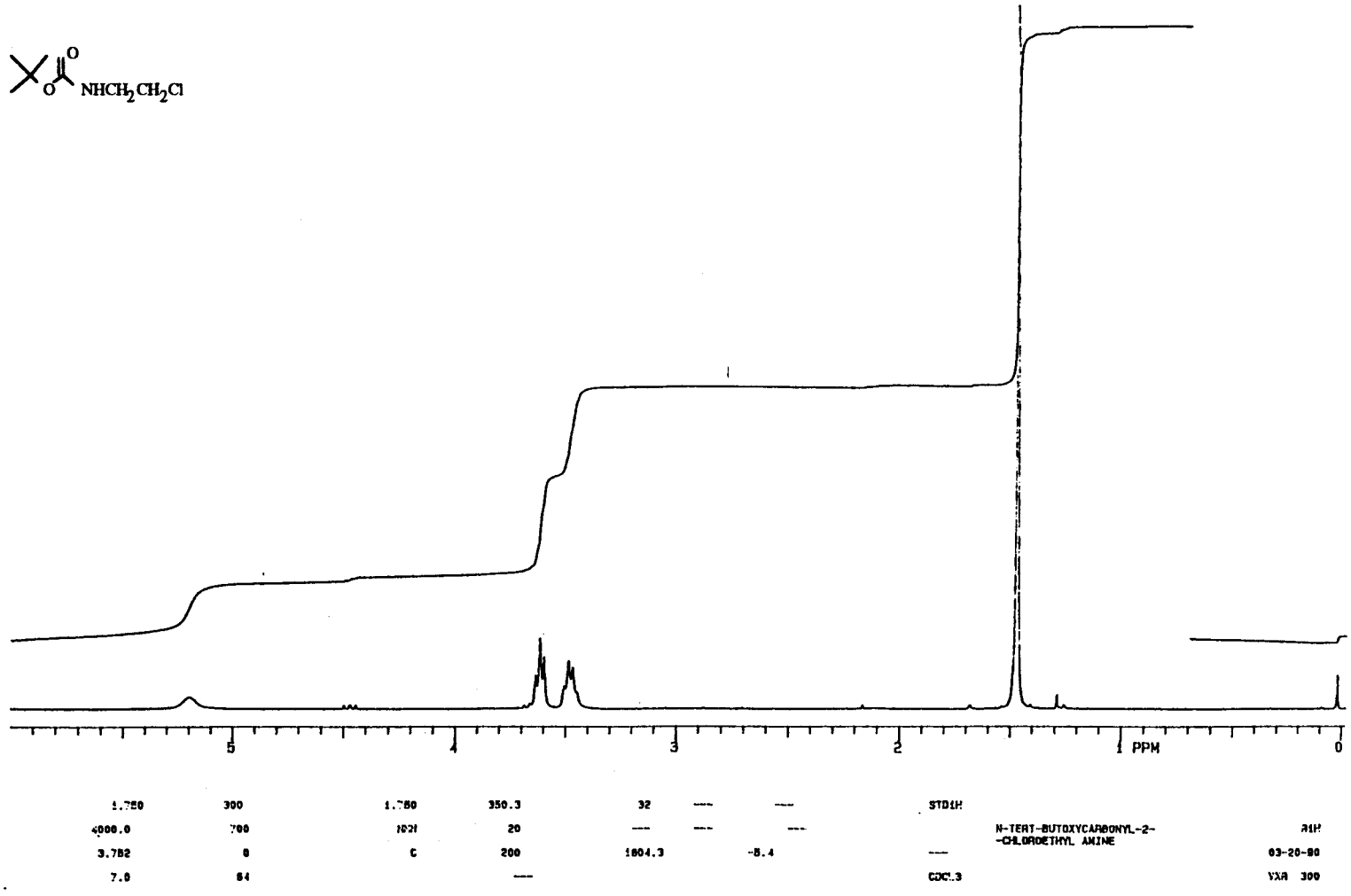
UV of N-(2-Chloroacetyl aminoethyl)Azure-B (**6**)



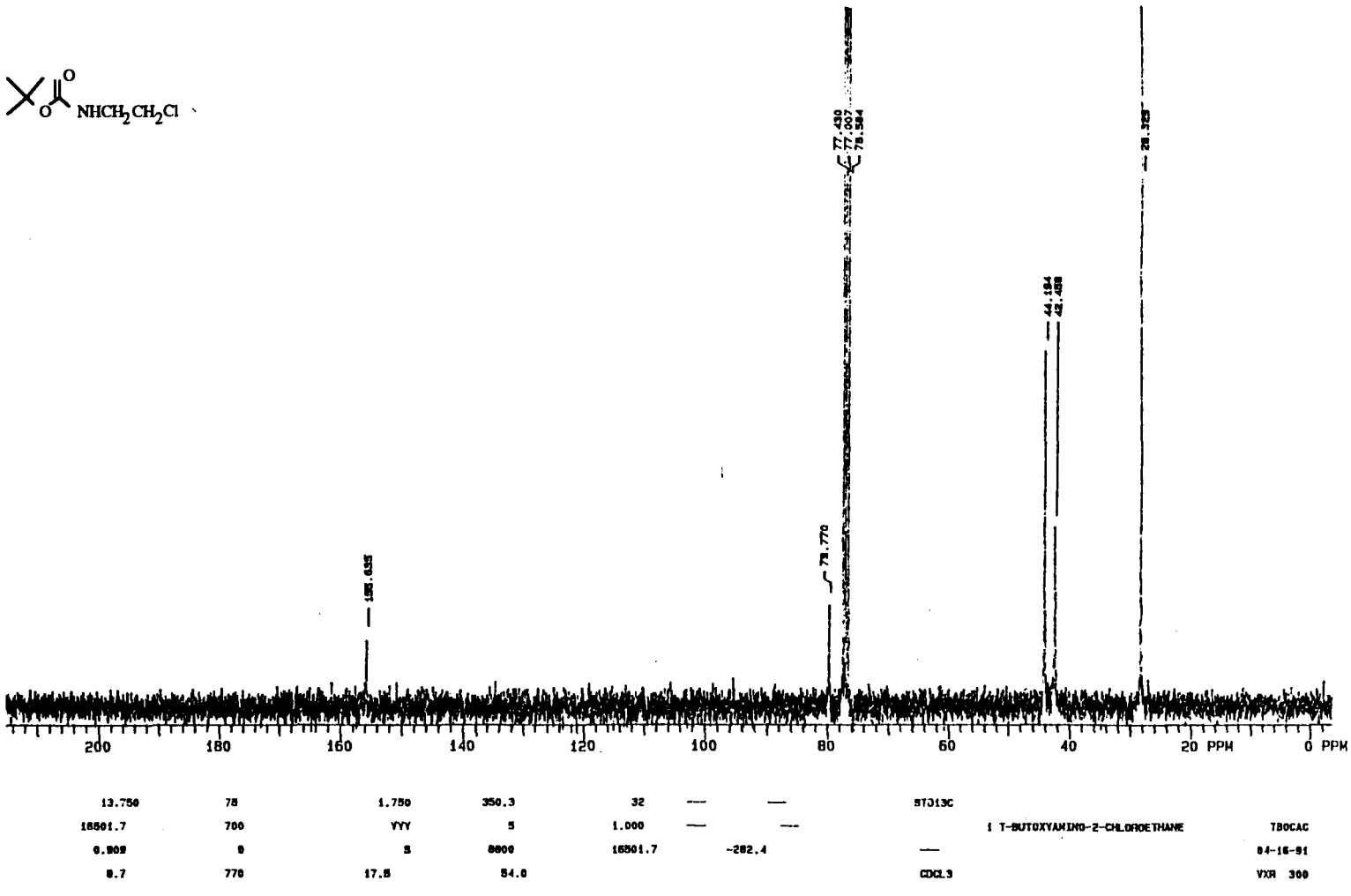
UV of Pentapeptide-OH conjugate (**11**)



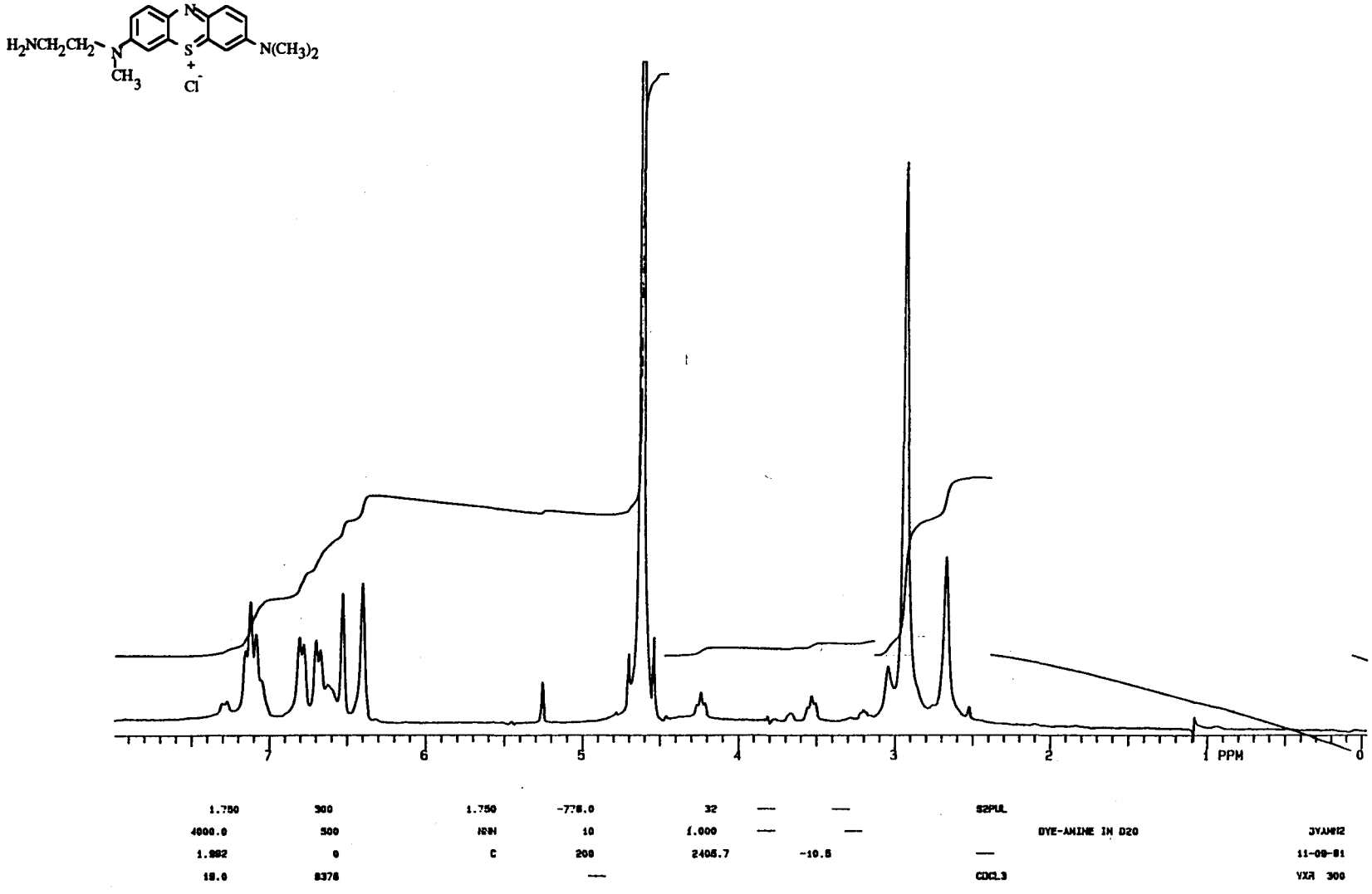
UV of Tetrapeptide amide conjugate (**12**)



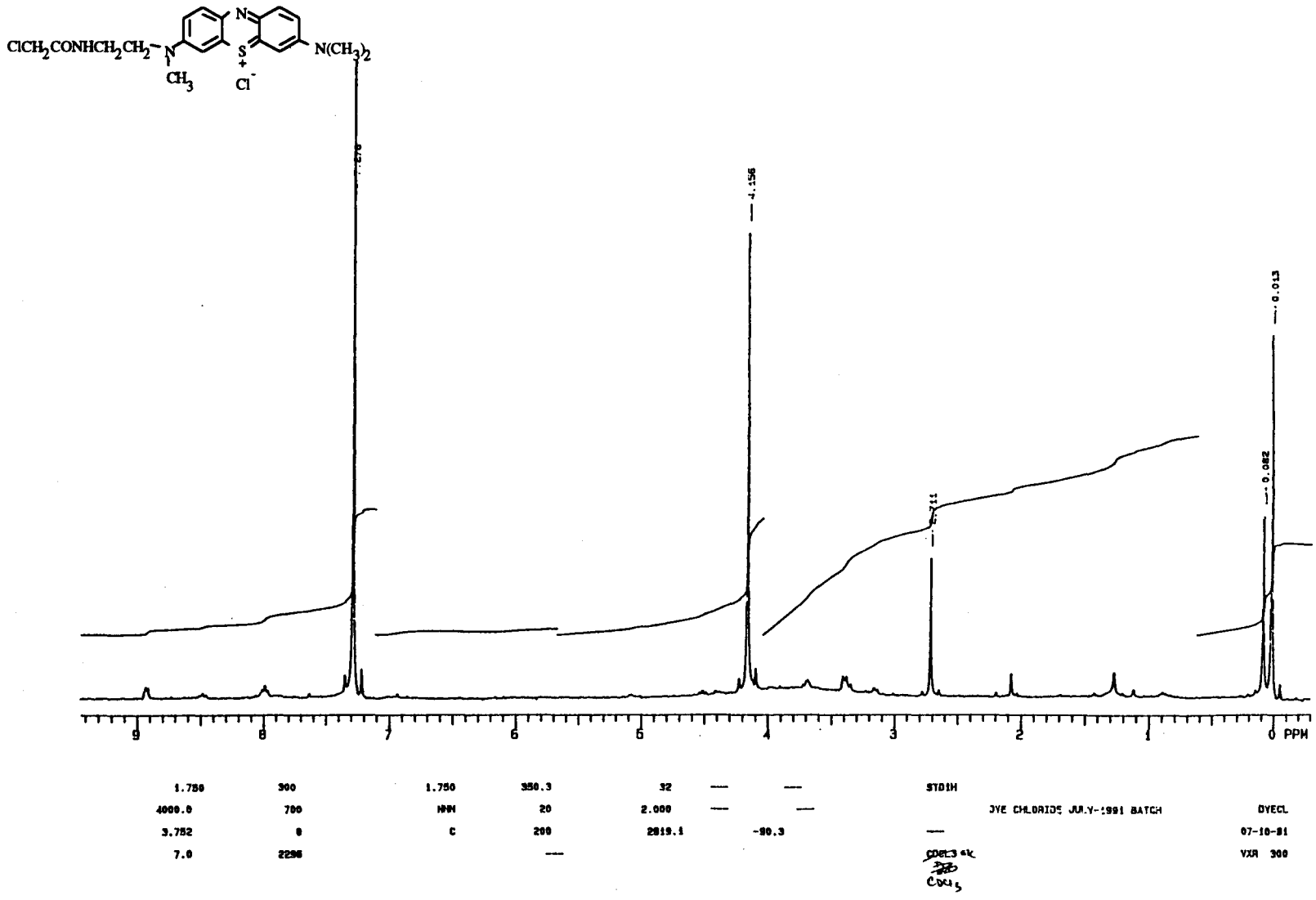
^{1}H NMR of N-tert-Butoxycarbonyl-2-chloroethyl amine (**3**)



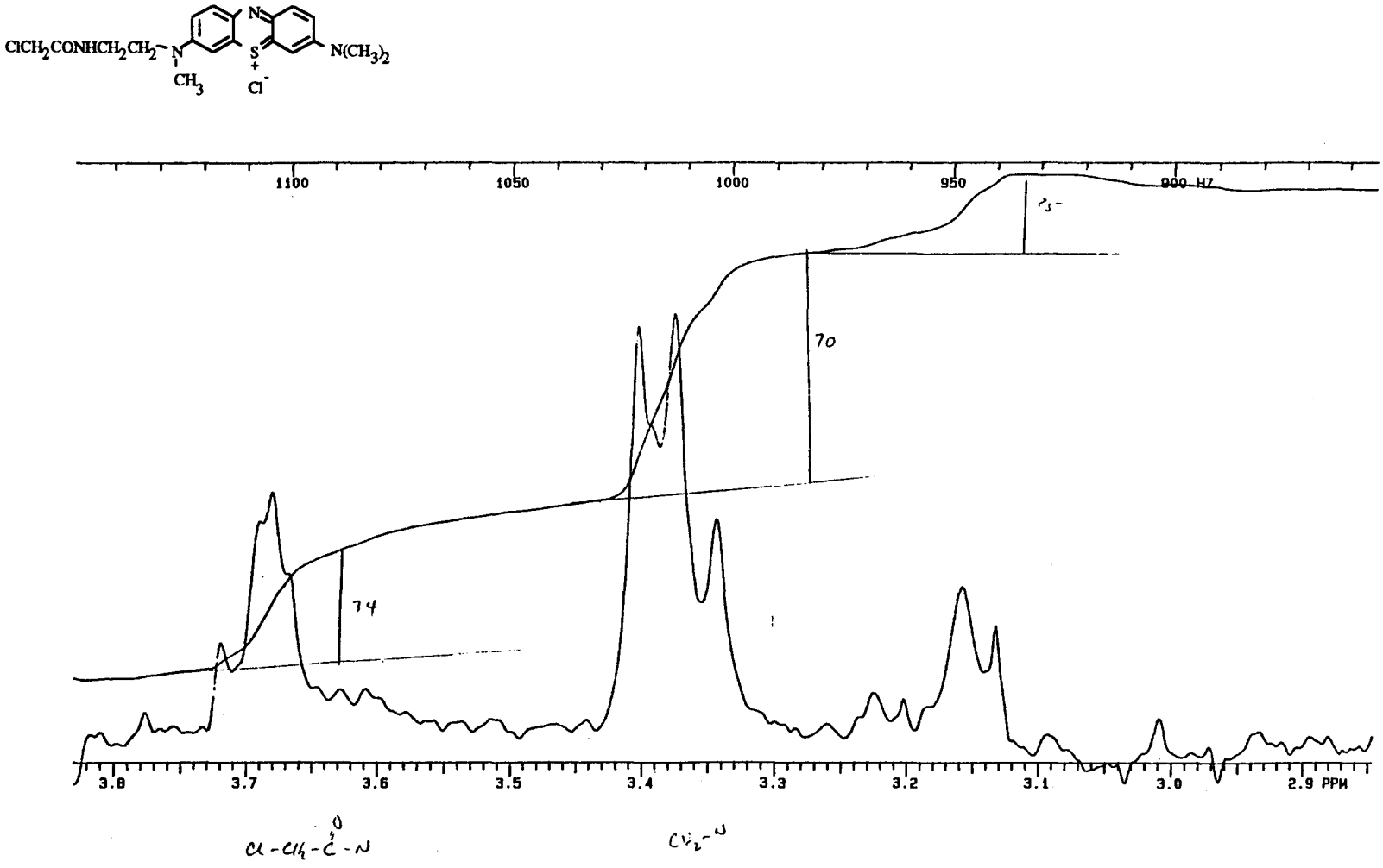
^{13}C NMR of N-tert-Butoxycarbonyl-2-chloroethyl amine (**3**)



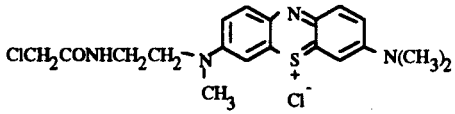
^{1}H NMR of N-(2-Aminoethyl)Azure-B (**5**)

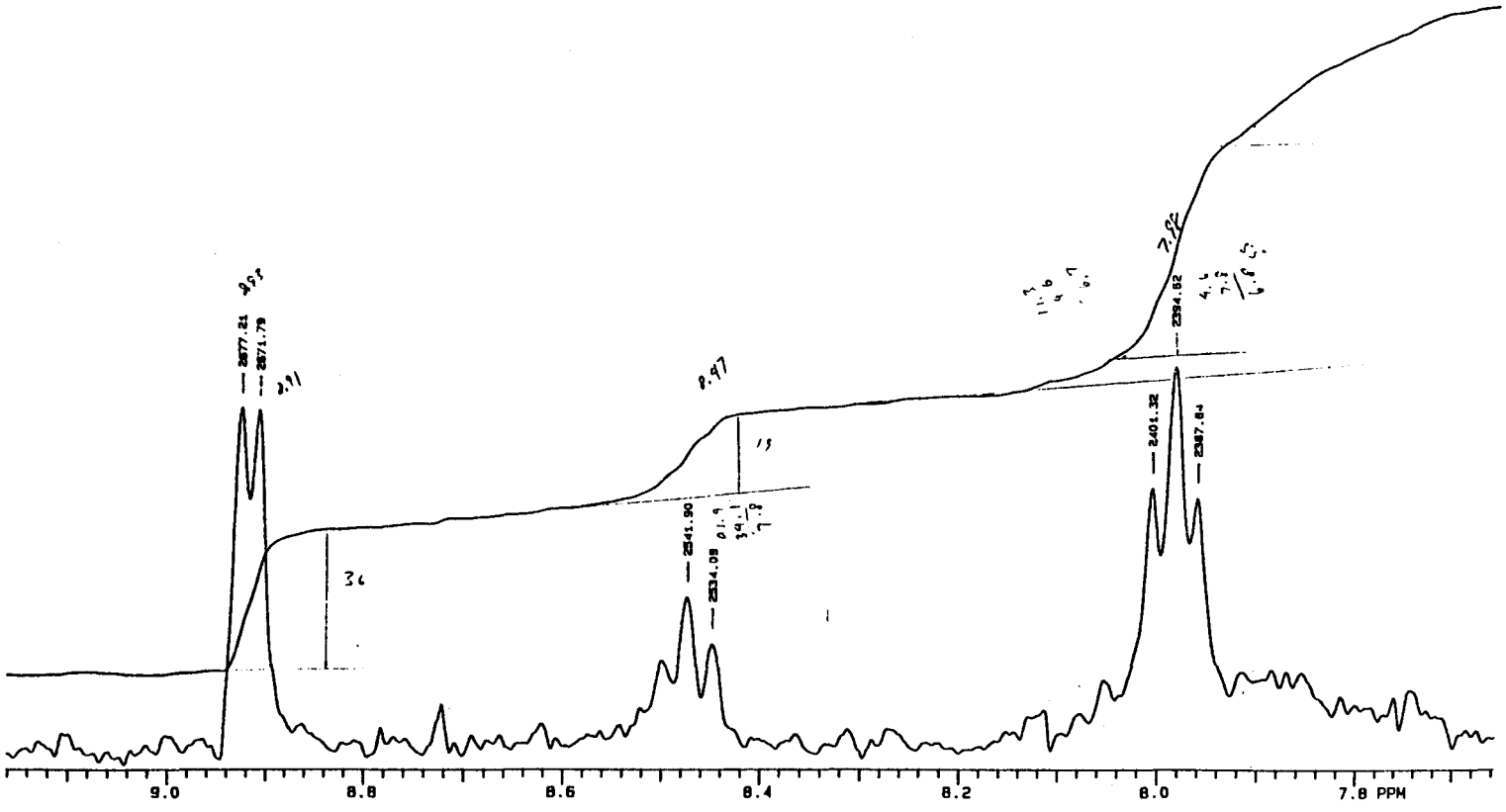


^{1}H NMR of N-(2-Chloroacetyl aminoethyl)Azure-B (**6**)



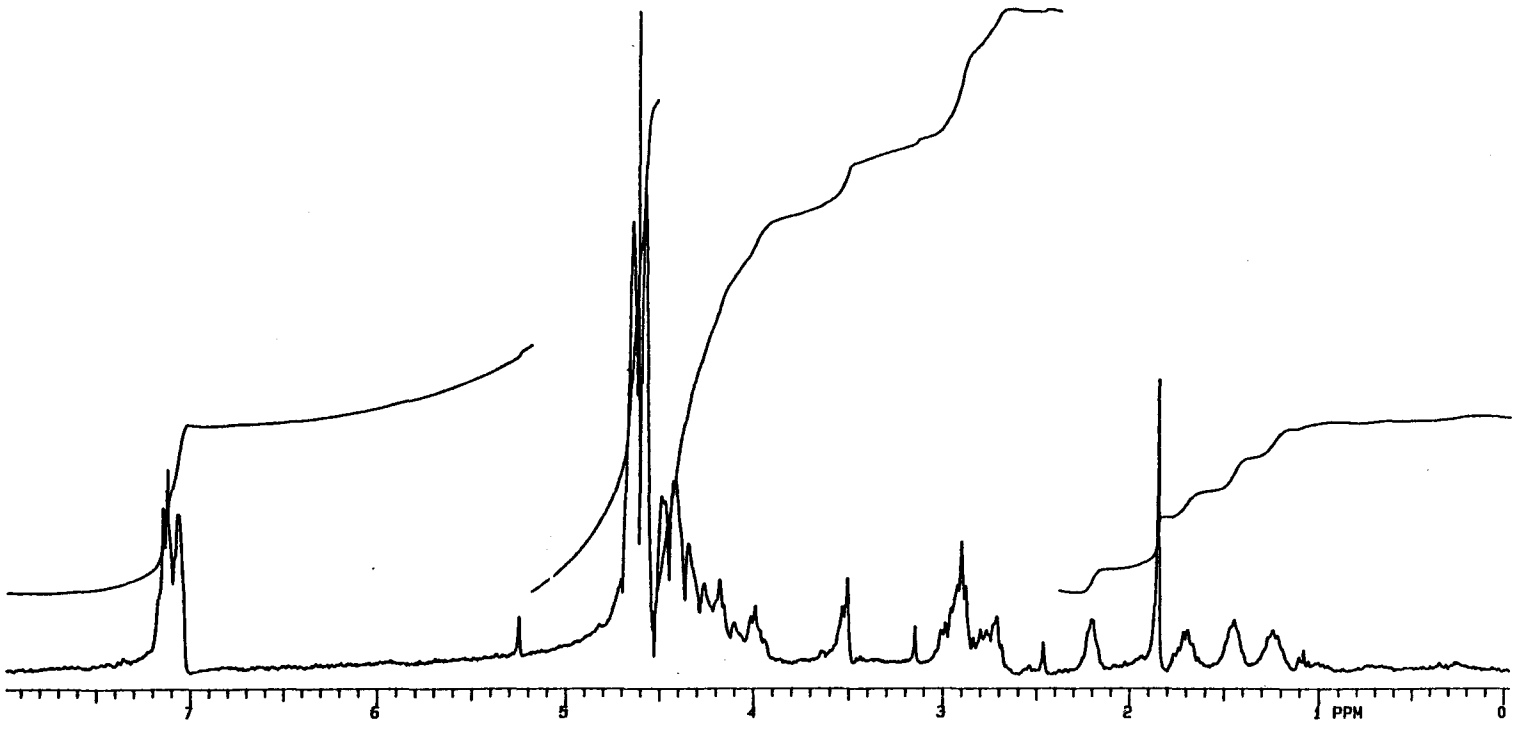
^{1}H NMR of N-(2-Chloroacetyl aminoethyl)Azure-B (**6**) (continued)





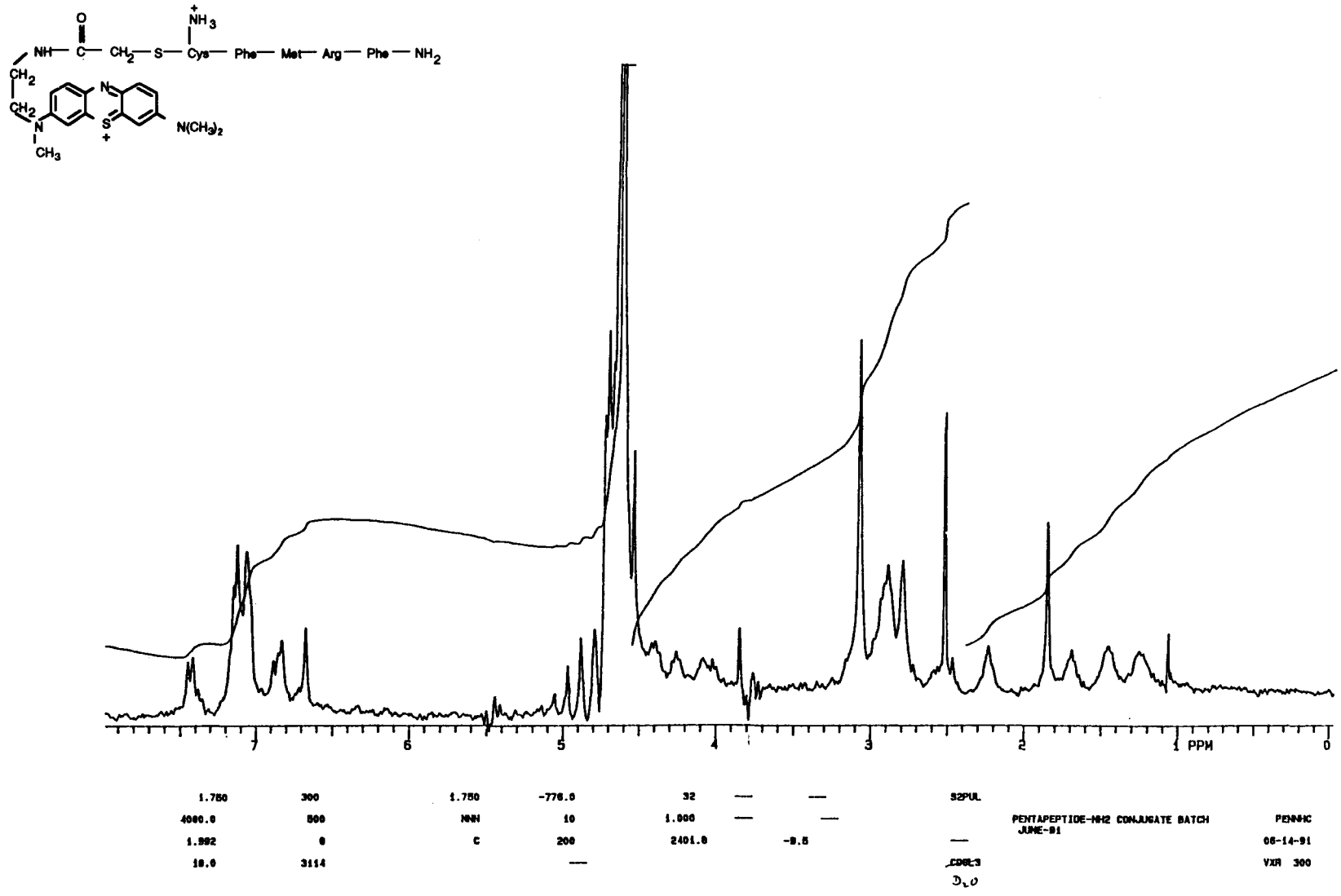
^{1}H NMR of N-(2-Chloroacetyl aminoethyl)Azure-B (**6**) (continued)

Cys-Phe-Met-Arg-Phe-NH_2

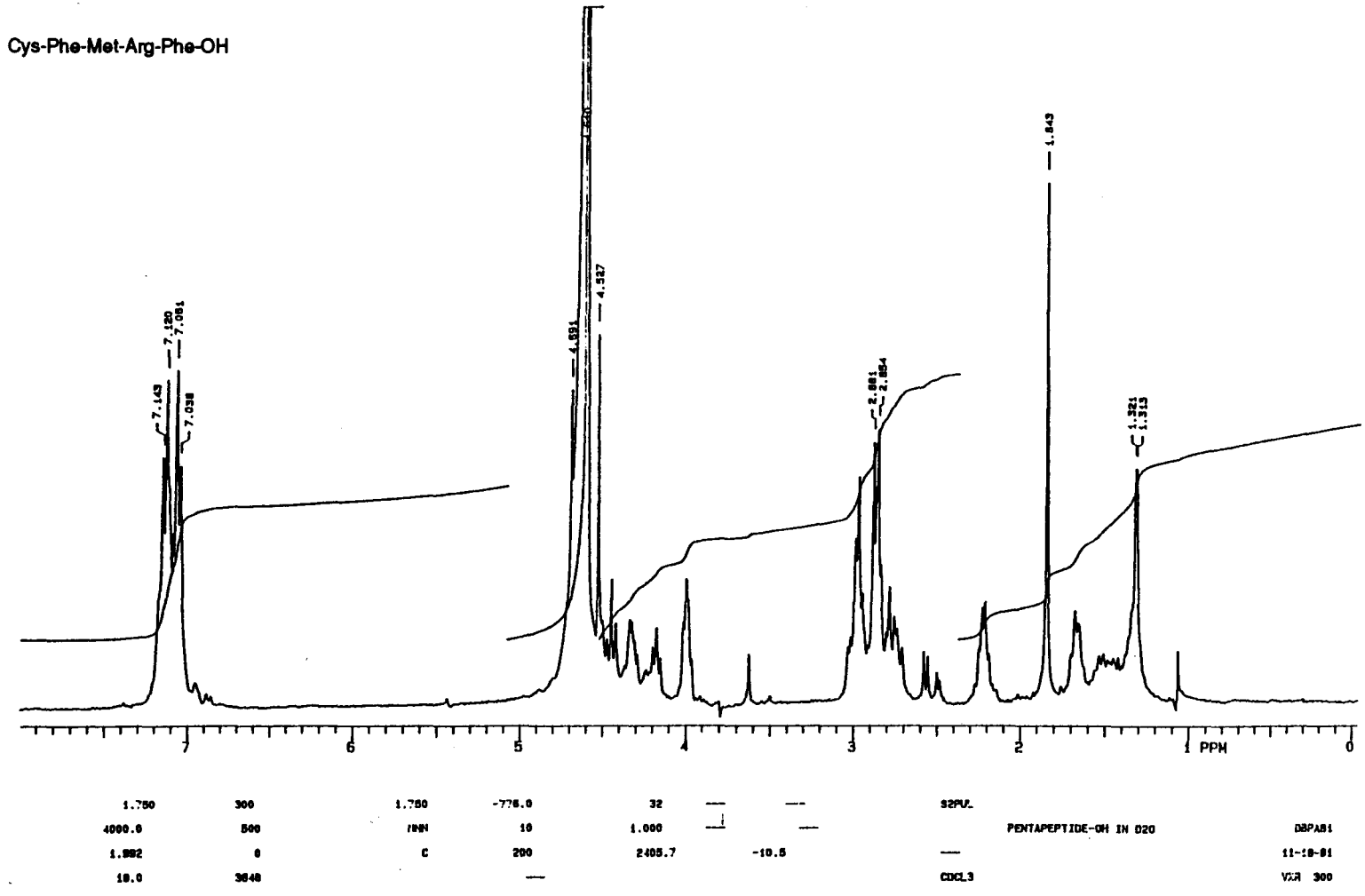


1.750	300	1.750	1029.3	32	---	---	S2PUL		
4000.0	500	YNN	12	1.000	---	---		PENTAPEPTIDE AMIDE IN D20	PPANE
1.992	8.000	C	200	2401.8		-8.5	---		11-26-91
18.0	8136		---				CDCL3		YXR 300

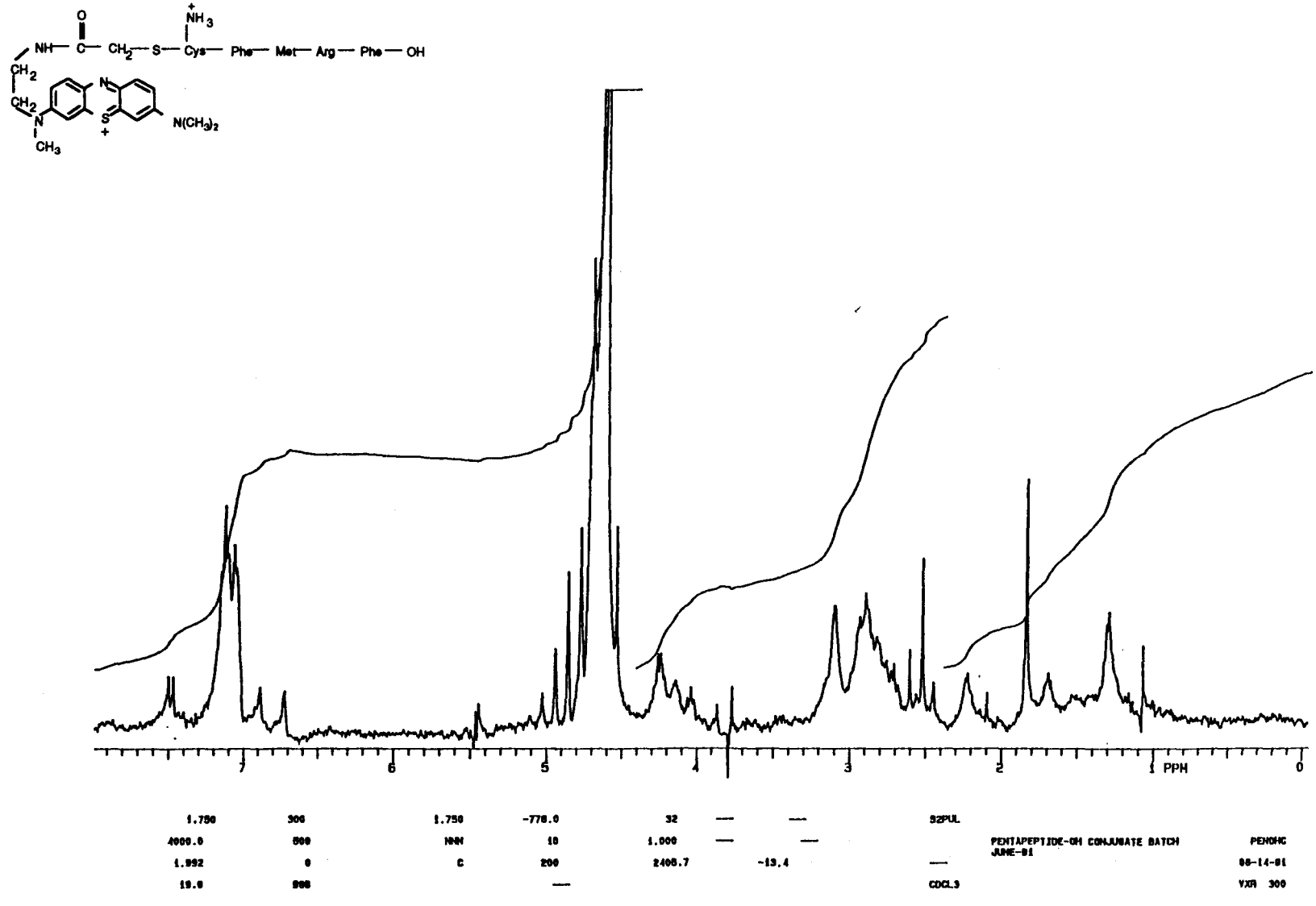
^{1}H NMR of Pentapeptide amide (7)



^{1}H NMR of Pentapeptide amide Conjugate (**8**)

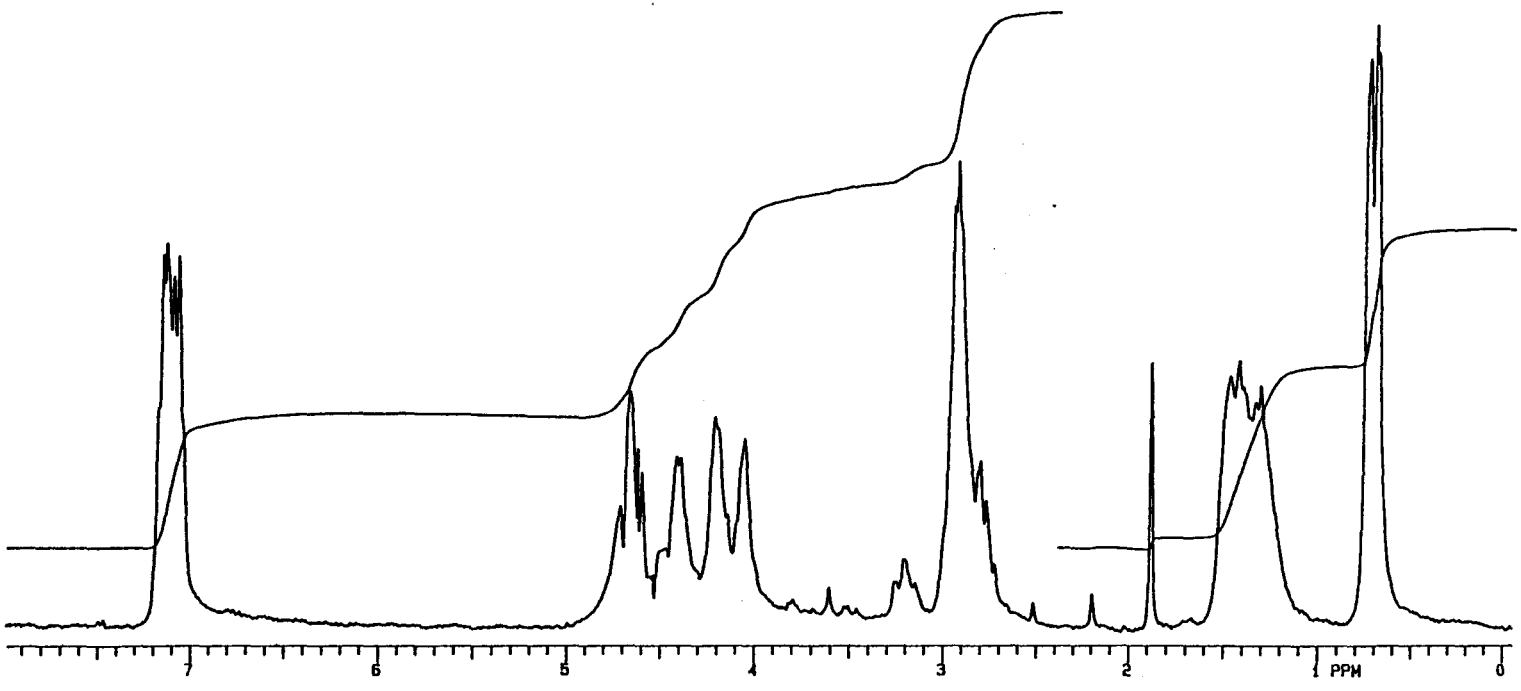


^{1}H NMR of Pentapeptide-OH (**9**)



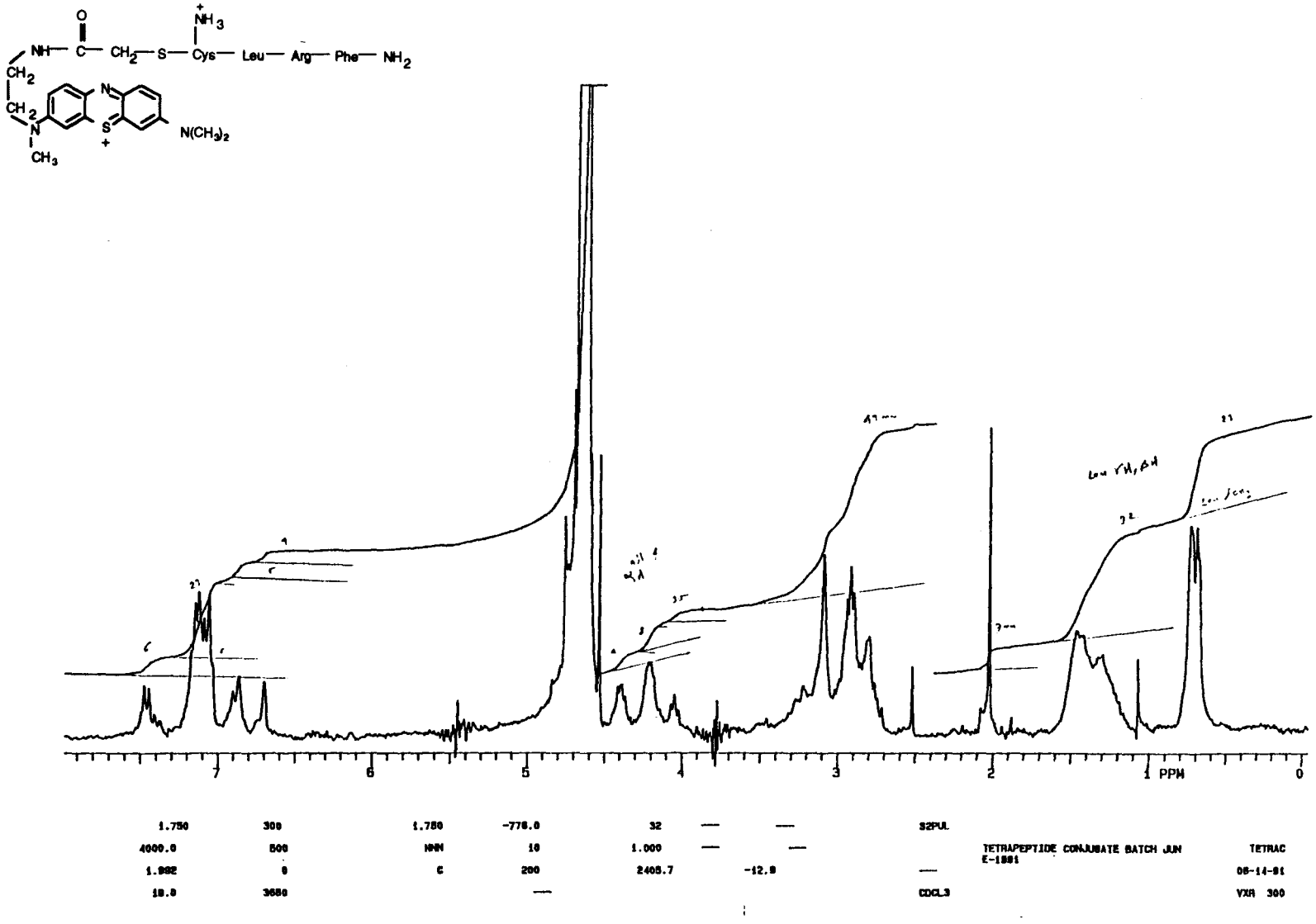
^{1}H NMR of Pentapeptide-OH conjugate (**11**)

Cys-Leu-Arg-Phe-NH_2



1.750	300	1.750	1028.2	32	—	—	S2PUL		
4000.0	500	YNN	12	1.000	—	—		WATER ELIMINATION OF TETRAPEPTIDE IN D2O	XETP
1.892	8.000	C	200	2409.6	-17.6		—		11-21-91
19.0	1776		—				CDCL3		VXR 300

^{1}H NMR of Tetrapeptide amide (**10**)



^{1}H NMR of Tetrapeptide amide conjugate (**12**)

REFERENCES

1. Kandel, E. R., "Principles of Neuroscience"; Editor: Kandel, E. R.; Schwartz, J. H. and Teffell, T. M., Elsevior, NY Science Publisher, pp 854-868.
2. Calne, D. B.,"Biochemistry & Neurology", Editor: Bradford, H. S. and Marzden C. D., Acadamic Press, London, 1976, pp 21-26.
3. Marzden,"Biochemistry & Neurology", Editor: Bradford, H. S. and Marzden C. D., Acadamic Press, London, 1976, pp 3-11.
4. Goodman, L. S. and Gilman, A.,"Pharmacological Basis of Therapeutics", McMilan, London, 1970, pp 1-34.
5. Raab, O. (1990), Cited in Blum, H. F., "Photodynamic Action and Diseases caused by Light"; Hafner, New York, 1964.
6. Meyer-Betz, Dtsh. Arch. Klin. Med., **1913**, 112, 476.
7. Miller, J. P. and Selverston, A. I. Science, **1979**, 206, 702.
8. Anderson, J. P. and Parrish J. A. Intl. Adv. in Sur. Onc., **1982**, 5, 341.
9. Anderson, J. P. and Parrish J. A. J. Invest. Derm., **1981**, 77, 13.
10. Anderson, J. P. and Parrish J. A. Lasers in Surg. amd Med., **1981**, 1, 263.
11. Anderson, J. P. and Parrish J. A. Science, **1983**, 220, 524.
12. Madison, R. and Macklis, J. D. "Selective Lesioning Techniques" Society of Neuroscience, Short Course 1989.
13. Wan, S.; Parrish, J. A.; Anderson R. R. and Madden M. Photochem. Photobiol., **1981**, 34, 679.
14. Bayly, H.; Gasparro, F. and Edelson, R. TIPS, **1987**, 8, 138.
15. Pooler, J. P. and Valenzno, D. P. Med. Phys., **1981**, 8, 614.
16. Macklis, J. D. and Madison, R. Brain Res., **1985**, 359, 158.
17. Madison, R. D.; Macklis, J. D. and Frosch, M. P. Brain Res., **1988**, 445, 101.

18. Gregoriadis, G. Liposome Technology III: Targeted drug delivery and biological interactions CRC Press Inc., Boca Raton, Florida, 1984.

19. Kraljic, I. and Mohsni, S.E. Photochem. and Photobiol, **1978**, 28, 577.

20. Price, D. A.; Greenberg, M. J., Science, **1977**, 197, 670.

21. Price, D. A., Am. Zool. Sci., **1986**, 26, 157.

22. Veenstra, J. A., Neurosci. Lett.,**1984**, 48, 185.

23. Dockray, G. J.; Williams, R. G. Brain Res., 266, 295.

24. Payza, K.; Greenberg, M. J.; Price, D. A., Peptides,**1989**, 10, 657.

25. Lehman, H. K.; Greenberg, M. J., J. Exp. Biol., **1987**, 131, 55.

26. Cottrell, G. A.; Davis, N. W.; Green, K. A., J. Physiol., **1984**, 356, 315.

27. Lehman, H. K.; Price, D. A., J. Exp. Biol., **1987**, 131, 37.

28. Lehman, H. K.; Price, D. A.; Greenberg, M., J. Biol. Bull., **1984**, 167, 460.

29. Arber,S.; Crumrine, D. and Reymer, W. Z., Biomedical Optics Conference, January 1991, Los Angeles, CA, 1428.

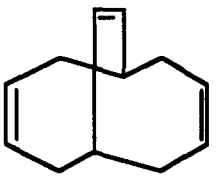
30. Tarbell D. S.; Yamamoto Y; Pope B., Proc. Nat. Acad. Sci., **1972**, 69, 730.

Part B
Synthesis of A Novel Bicyclic Triene

CHAPTER I

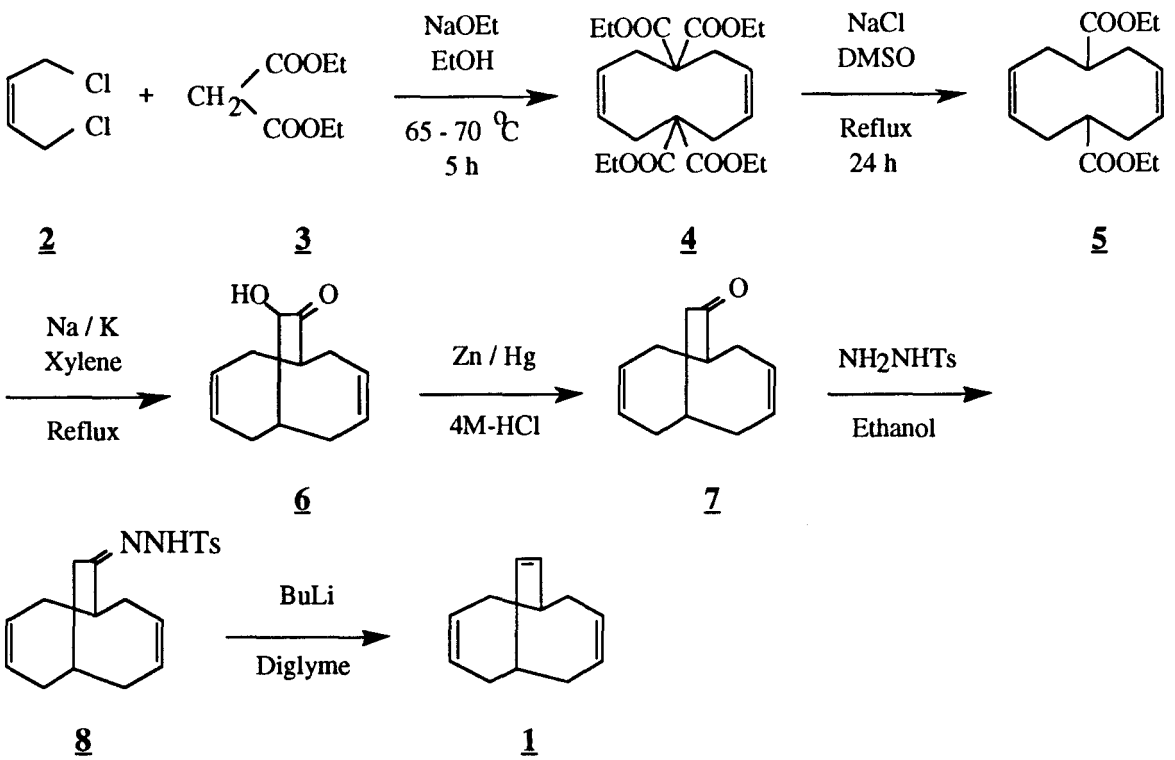
STATEMENT OF THE PROBLEM

The objective of this project is to synthesize bicyclo[4.4.2]dodeca-3,8,11-triene (**1**).



1

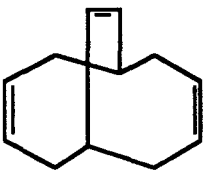
The synthesis will be achieved in six steps from known tetraethyl cis,cis-3,8-cyclododecadiene-1,1,6,6-tetracarboxylate (**4**).



CHAPTER II

INTRODUCTION

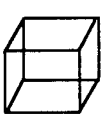
The objective of our research is to synthesize bicyclo[4.4.2]dodeca-3,8,11-triene (**1**).



1

A synthesis of this compound has never been reported, but syntheses of other compounds with similar carbon skeletons have been reported (see below).

Scientists have been interested in synthesizing molecules that have interesting shapes such as cubane (**9**), adamantane (**10**), buckminsterfullerene (**11**) etc. Buckminsterfullerene[1] has 60 carbon atoms. The atoms are arranged in 12 pentagons and 20 hexagons. It has a soccerball like symmetrical structure, and was isolated from soot.



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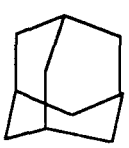

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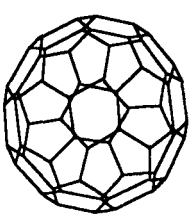


11

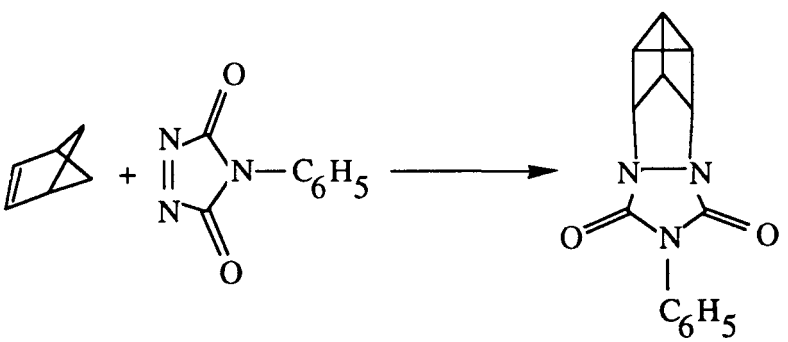
As the name suggests, Cubane is a cube like structure and can be synthesized from cyclobutadiene irontricarbonyl in four steps.[2] The syntheses of **10** and **11** are much less

straightforward. Our idea was to synthesize a molecule that had an interesting shape and could be used to synthesize other molecules that have interesting shapes and symmetries.

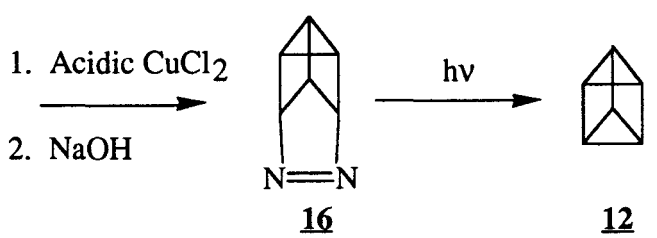
Syntheses of several interesting compounds with a general formula $(CH)_n$ have been reported. One important compound from the C_6H_6 family is prismane (**12**).

12

Hexamethylprismane was synthesized by irradiating hexamethylbicyclo[2.2.0]hexa-2,5 diene.[3] Prismane is a molecule in which six sp^3 carbon atoms are present at corners of a triangular prism. It was synthesized from benzvalene (**13**) in three steps.[4] Benzvalene was reacted with 4-phenyltriazolinedione (**14**) to obtain an adduct. It gave an azo compound by treatment with acidic $CuCl_2$ followed by reaction with aqueous NaOH. Photolysis of the azo compound led to the formation of prismane.

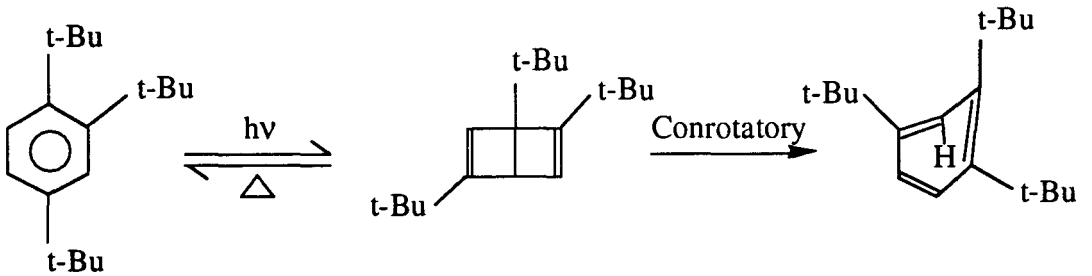


13 **14** **15**



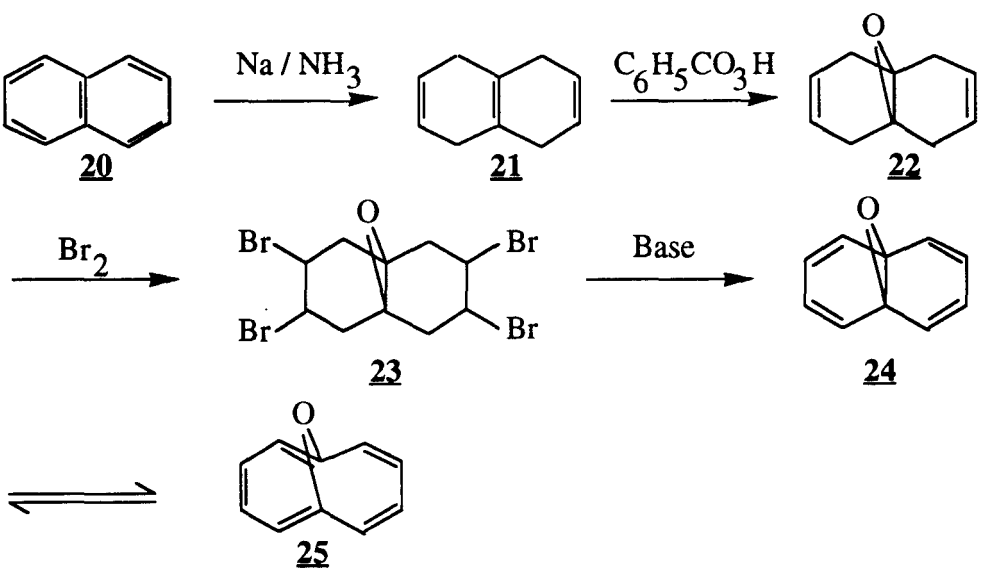
16 **12**

Another interesting C_6H_6 hydrocarbon is Dewar benzene. 1,2,5-tri-t-butylbicyclo[2.2.0]hexa-2,5-diene (a Dewar benzene) (**18**) was synthesized by a photolysis of 1,2,4-tri-t-butylbenzene (**17**).[5] In this case the product cannot revert to the starting material as it violates orbital-symmetry rules.



17 **18** **19**

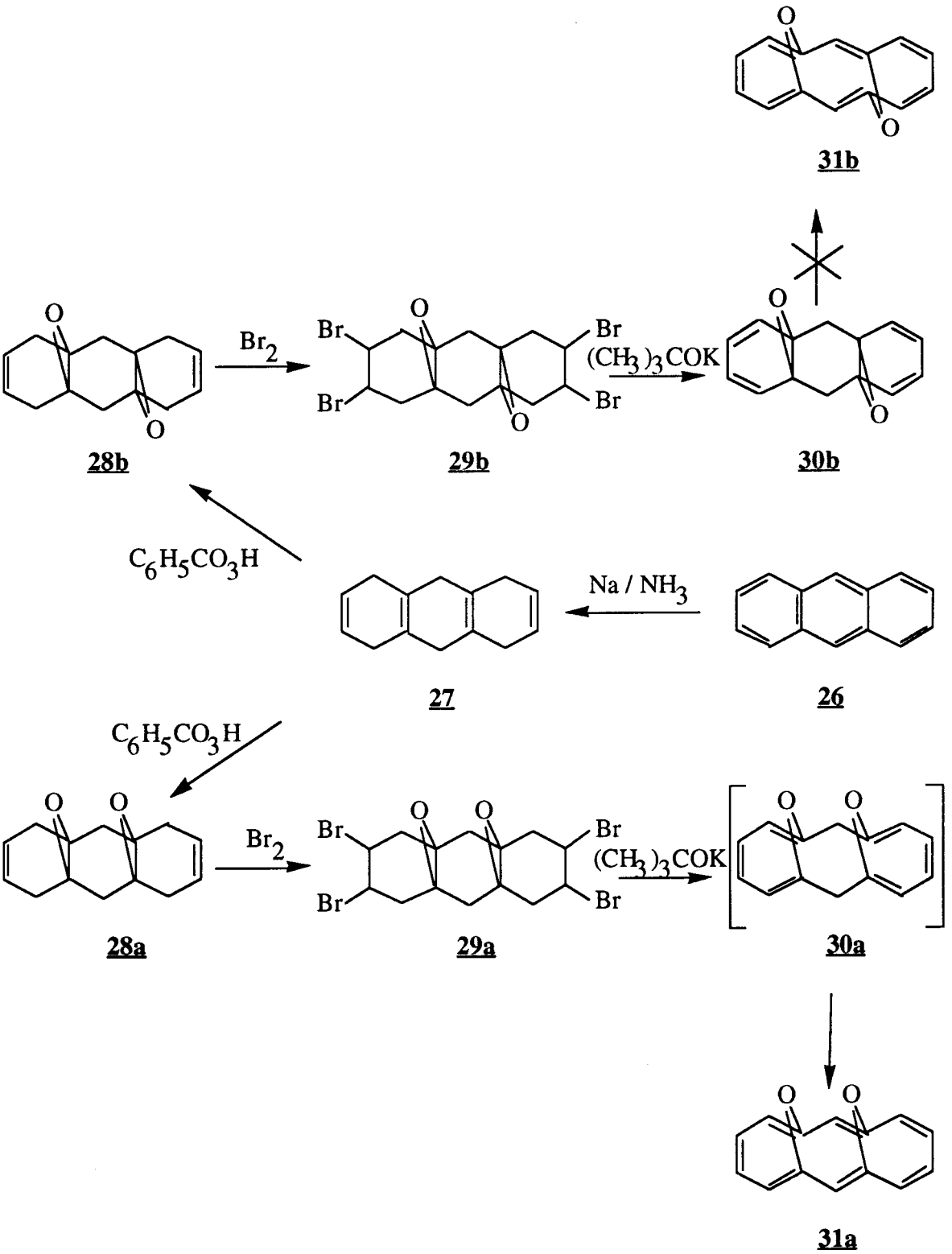
Vogel reported a synthesis of 1,6-methano[10]annulene and 1,6-oxido[10]annulene (**25**) from naphthalene (**20**).[6] Naphthalene was reduced by Birch reduction to obtain tetrahydronaphthalene (**21**). Selective oxidation of the double bond gave an epoxide (**22**) which gave 1,6-oxido[10]annulene (**25**) by Vogel's technique. Vogel brominated a diene, followed by dehydrobromination to yield a bicyclic tetraene **24** which rearranged to **25**.



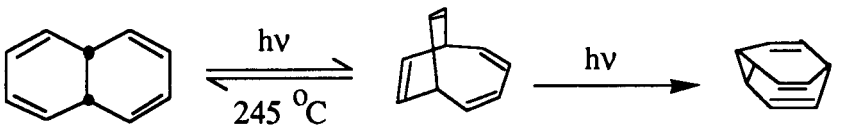
20 **21** **22** **23** **24** **25**

The aromatic nature of the product is confirmed by its chemical nature. It is stable towards heat, oxygen and light. It undergoes substitution reactions with electophiles.

Vogel also reported syntheses of 1,6,8,13-bisoxido[14]annulenes (**31a, b**).[6] 1,4,5,8,9,10-hexahydroanthracene was prepared by Birch reduction of anthracene (**26**). On peracid oxidation, it gave two isomeric diepoxides (**28a, b**) which on bromination gave tetrabromo adducts (**29a, b**). Base treatment of these adducts gave 1,6,8,13-bisoxido[14]annulenes.

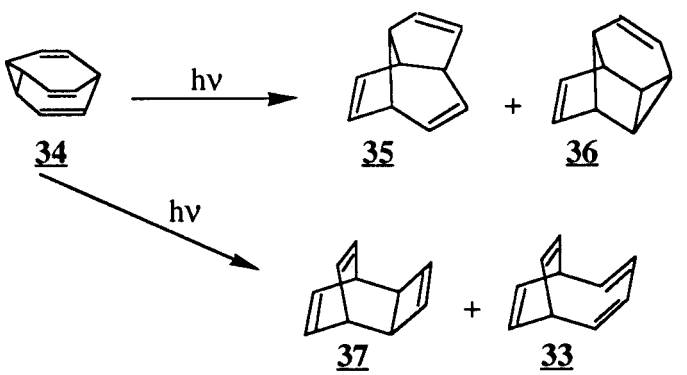

O
O
31b
Br2
Br
Br
(CH3)3COK
28b
29b
30b
C6H5CO3H
Na / NH3
27
26
C6H5CO3H
Br2
28a
29a
30a
31a

When 9,10-dihydronaphthalene (**32**) was irradiated with UV light, it rearranged to form two compounds.[7] One is bicyclo[4.2.2]deca-2,4,7,9-tetraene (**33**), an important intermediate in the formation of bullvalene. It rearranged to form bullvalene (**34**) in high yield on ultraviolet irradiation.



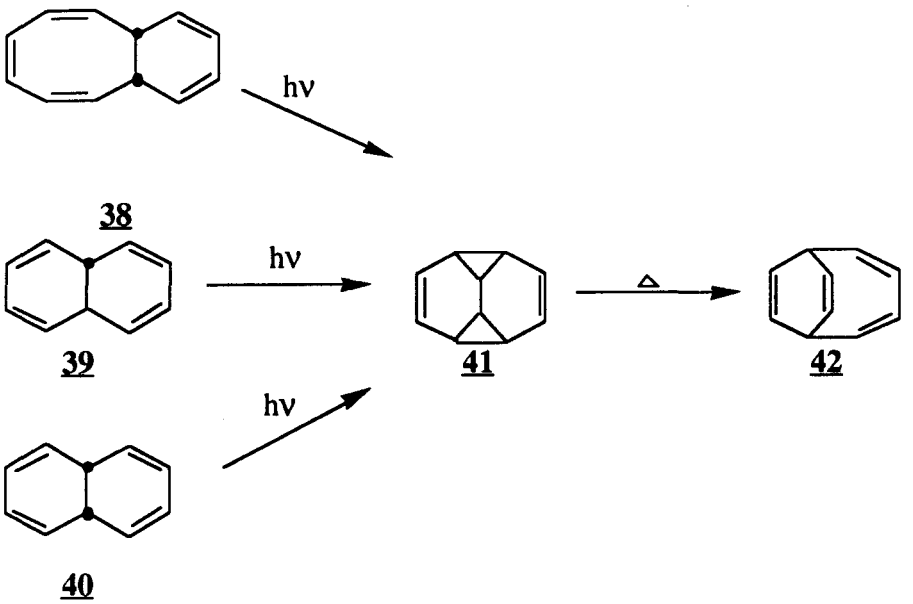
32 **33** **34**

Ultraviolet irradiation of bullvalene (**34**) formed different products.[9]

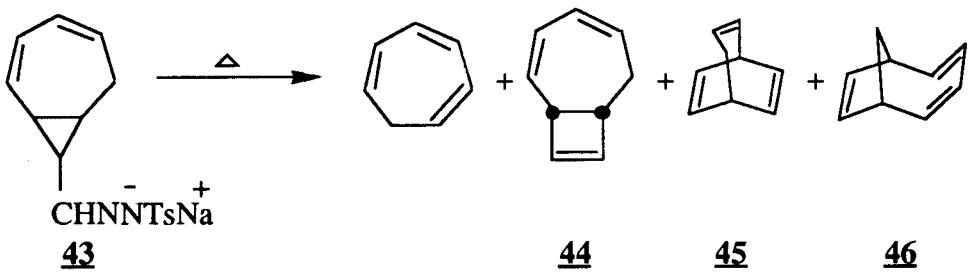


34 **35** **36** **37** **33**

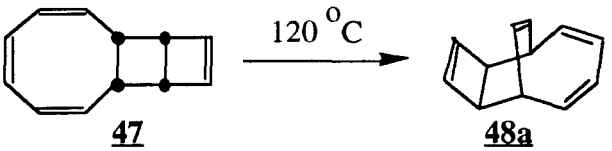
Photolysis of **38**, **39** and **40** gives tetracyclo[4.4.0.0^{2,10}.0^{5,7}]deca-3,8-diene (**41**) which is an important intermediate discussed in many important $(CH)_{10}$ hydrocarbons.[17]



Thermal decomposition of (**43**) gave bicyclo[5.2.0]nona-2,4,8-triene (**44**), bicyclo[4.2.1]nona-2,4,7-triene (**45**) and bicyclo[3.2.2]nona-2,6,8-triene (**46**).[8]

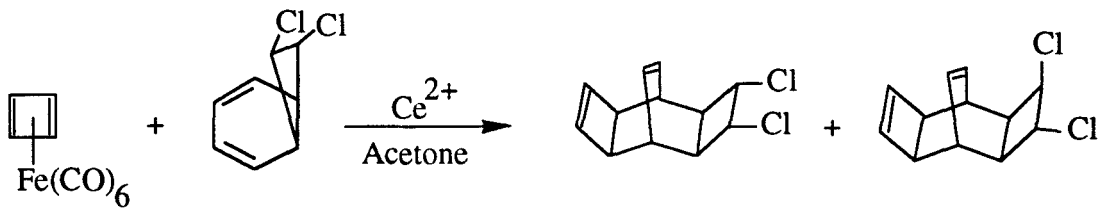


Paquette[10] was interested in the interconversion of $(CH)_{12}$ hydrocarbons. It is an interesting family of compounds with theoretical significance and several electrocyclic and sigmatropic processes are observed for different isomers. However, not much work has been reported due to the unavailability of good synthetic routes for these compounds. Schroder and Paquette have reported[11] a synthesis of tetraene **48a** from cis,syn,cis tricyclo[8.2.0.0 ^{2,9}]dodeca-3,5,7,11-tetraene (**47**) by thermal rearrangement.

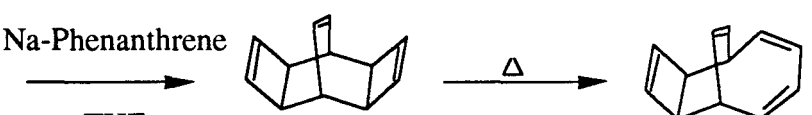


47 48a

The same compound, exo-tricyclo[4.4.2.0.^{7,10}]dodecatetraene (**48a**) was synthesized[10] by reacting cyclobutadieneiron tricarbonyl with ceric ammonium nitrate in the presence of a cis, trans mixture of 7, 8-dichlorobicyclo[4.2.0]octadiene (**51a, b**) to obtain Diels-Alder adducts. The adducts were reacted with sodium phenanthrene to obtain a triene which on pyrolysis at 500 °C yields a tetraene.

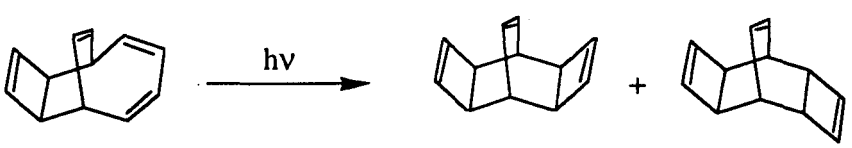


49 50 51a 51b



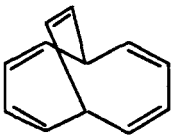
52a 48a

Photoisomerization of this tetraene in ether gave a mixture of two isomeric trienes (**52a, b**) in equal amounts.[10]



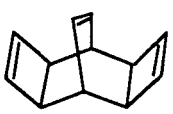
48a 52a 52b

Formation of the above tetraene is speculated to take place via a strained pentaene (**53**) intermediate. However, this pentaene was never isolated or synthesized by a different route.

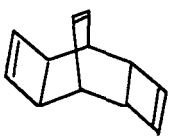


53

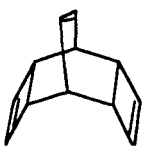
Formation of the cyclobutene ring relieves strain in the tetraene. Erhardt[12] reported a synthesis of these isomeric trienes (**52a, b, c**) from a carbonate in four steps.



52a

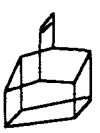


52b



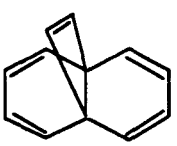
52c

On benzophenone sensitized irradiation in ether, the third triene gave a cage compound (**54**).



54

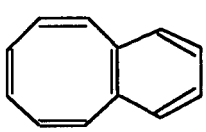
The following pentaene on heating gave benzocyclotetraene (**56**).



55

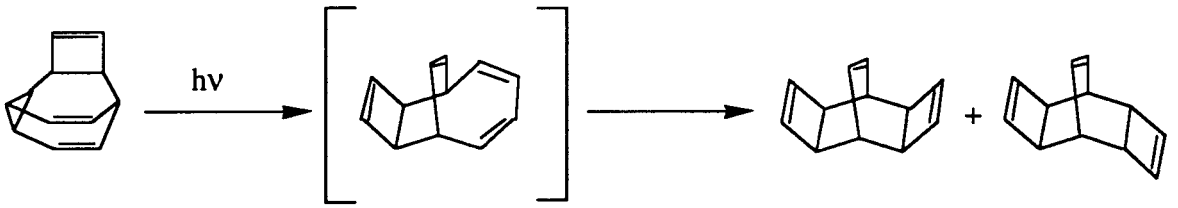
$430\,^{o}C$

Gas phase



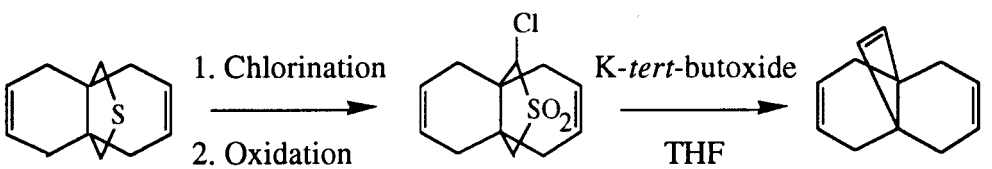
56

The following cyclobutene derivative (**57**) on photoisomerization in pentane gave exo-tricyclo[4.4.2.0.^{7,10}] dodecatetraene (**48a**) which resulted in a formation of two isomeric trienes (**52a, b**).[13]



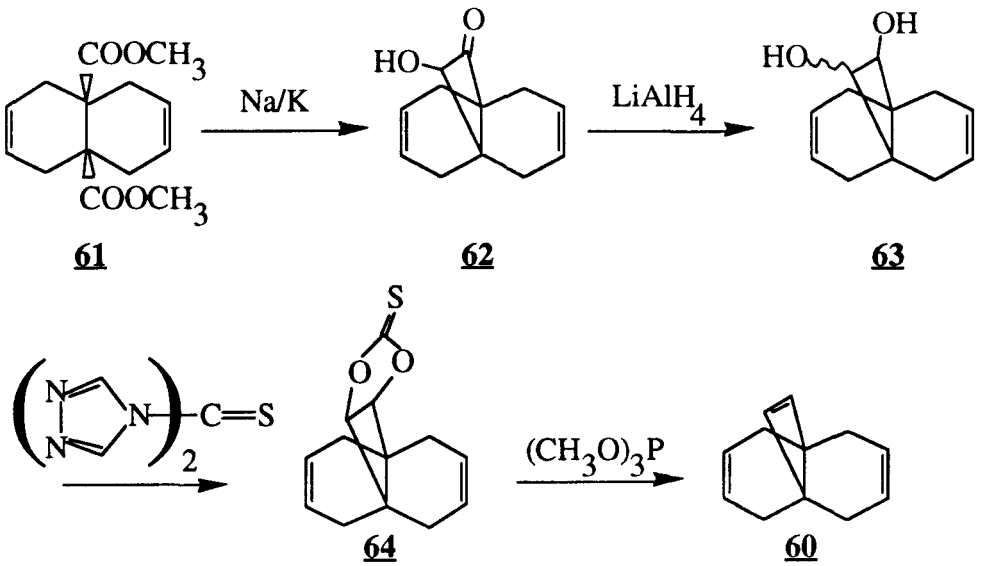
57 **48a** **52a** **52b**

Paquette[14,15] has reported a synthesis of [4.4.2]propella-3,8,11-triene (**60**) by Ramberg-Backlund rearrangement of α–chlorosulfone (**59**) with K-tert-butoxide. The α-chlorosulfone was prepared by chlorination and subsequent oxidation of the corresponding sulfide (**58**).

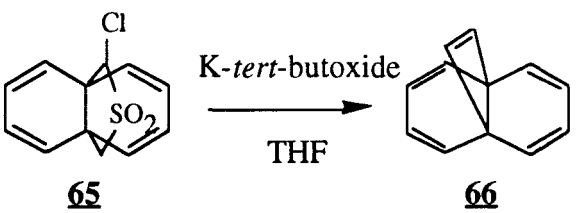


58 **59** **60**

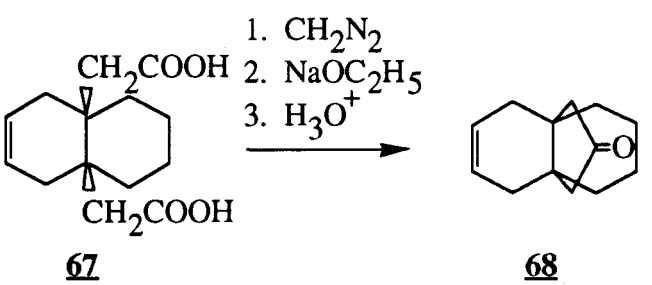
An alternate route[15] was used for the synthesis of [4.4.2]propella-3,8,11-triene (**65**). In this approach, dimethyl $\Delta^{2,6}$-hexalin-9,10-dicarboxylate (**61**) was reacted with Na/K alloy to form the corresponding hydroxy-ketone (**62**). It was reduced to a mixture of cis and trans diols (**63**) by $LiAlH_4$. The cis-diol was converted to cis-thiocarbonate ester (**64**) which was then reacted with trimethyl phosphite to form the triene (**60**).



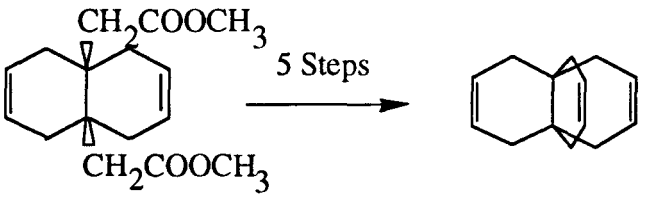
A synthesis of [4.4.2]propella-2,4,7,9,11-pentaene (**66**) has been reported[16] from the corresponding α–chlorosulfone (**65**).



Another interesting synthesis of a tricyclic compound has been reported. A series of three reactions was used to make the third ring (**68**).

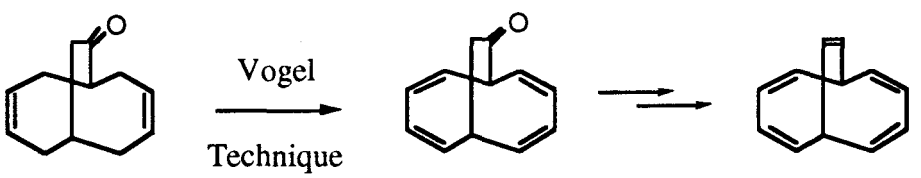


Ginsburg[17] has reported a synthesis of a trienic[4.4.4]propellane from a diester. The bicyclic diester (**69**) was converted into a triene (**70**) in five steps.



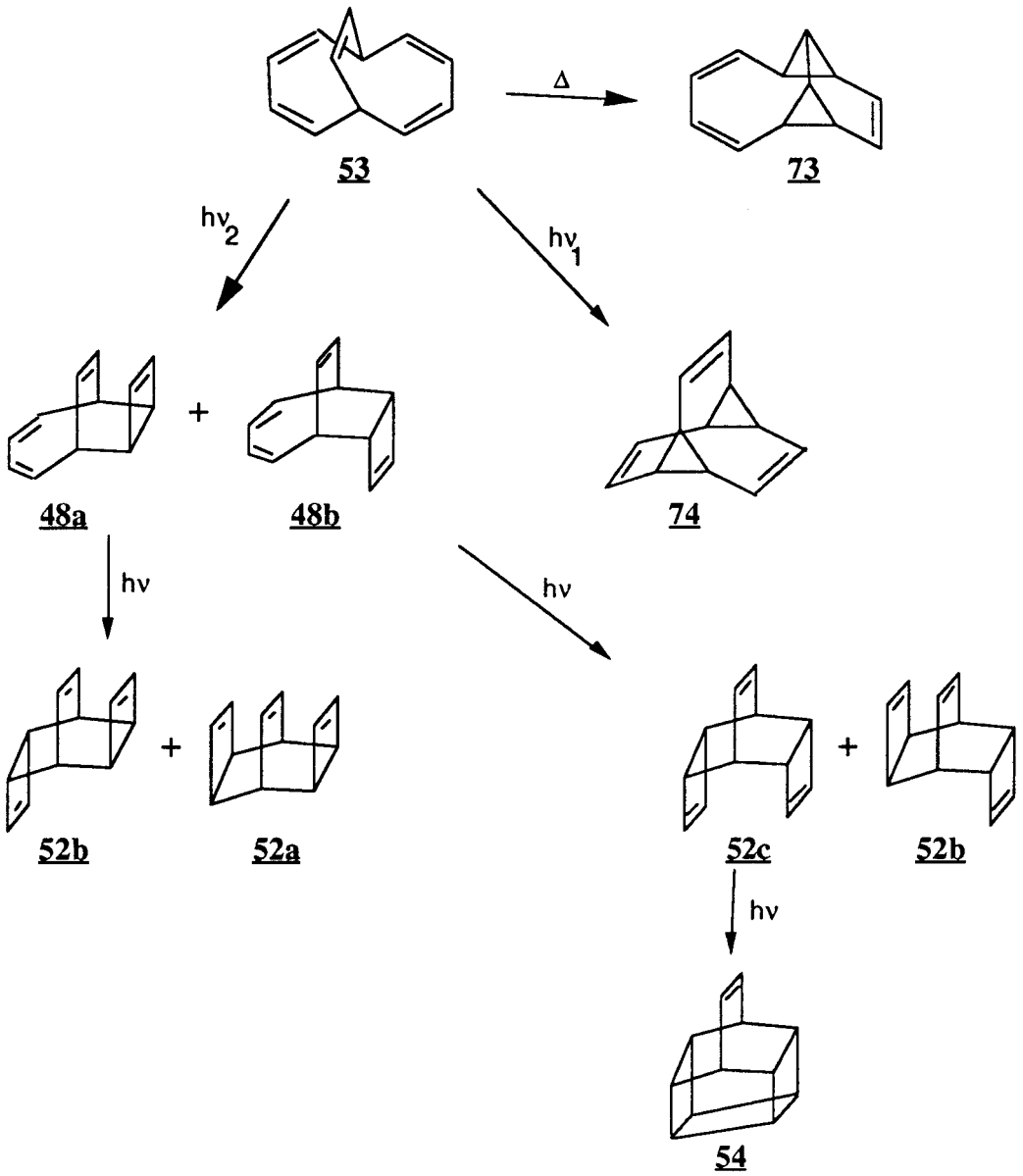
69 **70**

Bicyclo[4.4.2]dodeca-1,3,8,11,13-pentaene (**53**) can also be synthesized from the ketone (**7**) which is a precursor of the triene (**1**), using Vogel's technique. Bromination of diene double bonds followed by dehydrobromination of the tetrabromide to obtain a tetraeneone (**71**) which can be converted into a pentaene using the same technique that was used to make the triene.



7 **71** **53**

This pentaene (**53**) could have several synthetic uses. It could undergo thermal [4+2] cycloaddition to form unsymmetrical bicyclopropane derivative (**73**). Photochemical [4+4] addition in this compound should lead to the formation of a symmetrical bicyclopropane derivative (**74**), while [2+2] photochemical cycloaddition should give a mixture of two tetraenes (**48a, b**). One of the isomeric trienes (**52a**) has a geometry such that the two double bonds can undergo 2+2 photochemical cycloaddition to form basketene compound (**54**) as previously reported (see below).

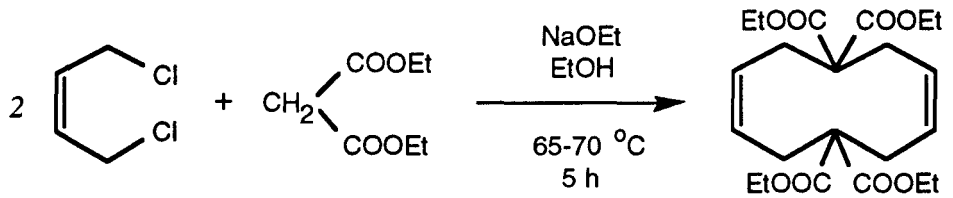


Synthesis of some of these products has been reported in the literature and was discussed before. Since these compounds are already characterized, it will be easy to identify the reaction products of the pentaene.

CHAPTER III

RESULTS & DISCUSSION

A literature procedure[18,19] was used for the synthesis of the tetraethyl cis,cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (4).



2 3 4

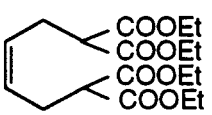
The major limitation of this procedure is the 2.6% yield of tetraester, and several attempts were made to improve the yield. To find whether slow addition would improve the reaction yield, 1,4-dichloro-2-butene was added to the sodium salt of diethyl malonate over 5 hr and 12 hr. The yield stayed the same. In another attempt, the solvent was changed to xylene and sodium carbonate was used as base. When the reaction was done at reflux, no product was obtained due to the insolubility of sodium carbonate in xylene. The same result was obtained twice. In one reaction, it was found that the yield of the tetraester decreased when the reaction temperature was lower than 65 to 75 °C. However, when the reaction was carried out in refluxing ethanol, the yield did not increase. An attempt was made to improve the yield of tetraester by adding 1,4-dichloro-2-butene to the sodium salt of diethyl malonate, but the yield did not improve. Thus it appears that for the tetraester synthesis, the best set of conditions is the one reported in the literature.[18,19] However, we found that the best yield was obtained only after modifying that procedure. A heating tape

was used to keep the sodium salt of diethyl malonate above its melting point. A mechanical stirrer was used to stir the solution at high speed to allow immediate mixing of the components. The best yield was 2.6%, and the research was continued using that yield of tetraester (4).

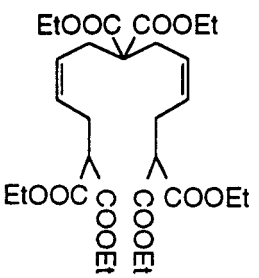
The literature[19] used methanol for recrystallization of tetraester (4). However, we found that a chloroform/petroleum ether mixture makes the recrystallization considerably easier.

This tetraester is an important starting material in our research. Major products of the reaction were diethyl cyclopent-3-ene-1,1-dicarboxylate (74) and diethyl 2-vinylcyclopropane-1,1-dicarboxylate (75) (67% total). We investigated the reaction to find other unreported products (33%) of the reaction.

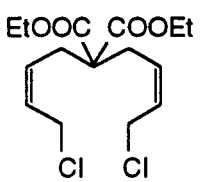
During the workup, the major volatile products were removed by vacuum distillation. The residue was washed with heptane to obtain crude tetraester. Compounds in the heptane washing were separated by column chromatography and two pure compounds were isolated. One of them was diethyl 2,7-dicarboethoxy-octa-4-enedioate (76) and the other is diethyl 2,7,7,12-tetracarboethoxy trideca-4,9-dienedioate (77).



76



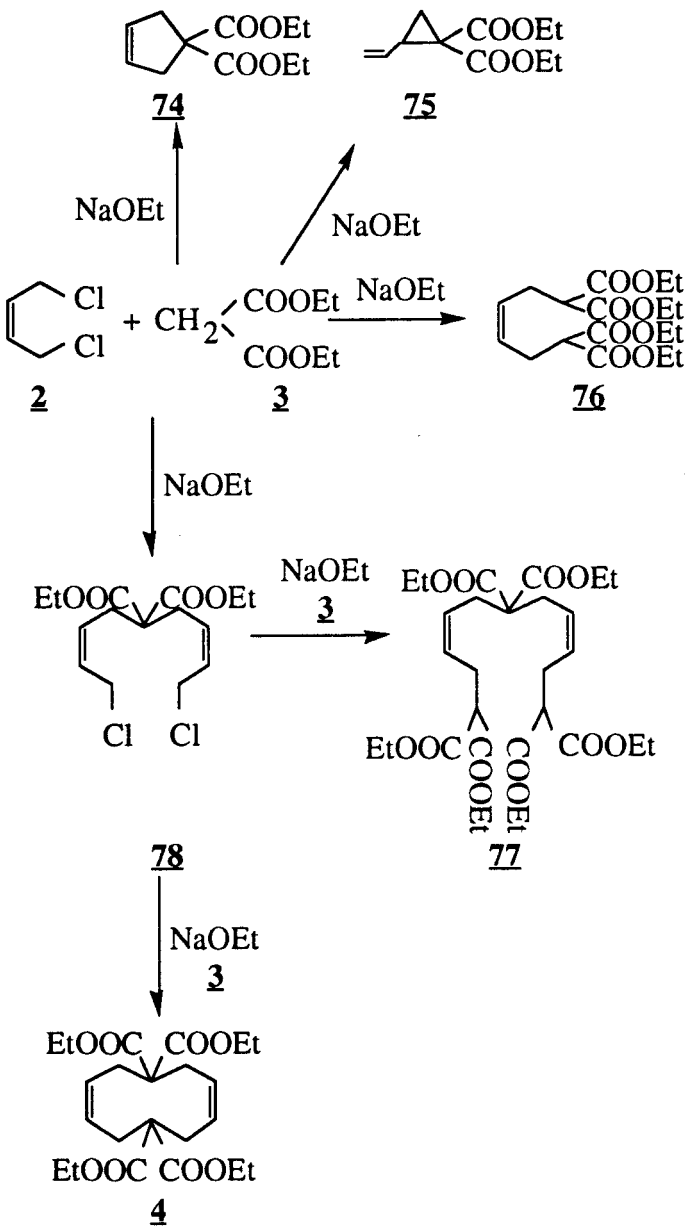
77



78

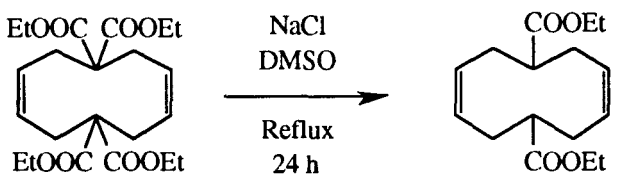
Characterization of these products suggests the presence of diethyl 1,9-dichlorononа-2,8-diene-5,5-dicarboxylate (78) as a reaction intermediate. This intermediate further reacts with diethyl malonate dianion to form the desired tetraester. Isolation of these intermediates clarifies the mechanism of tetraester (4) formation. As seen in the

proposed mechanism, there are several competing reactions. Formation of the two major products **74** and **75** are kinetically favored, hence they form in high yield. Two competing reactions are formation of diethyl 1,9-dichloronona-2,8-diene-5,5-dicarboxylate (**78**) and diethyl 2,7-dicarobethoxy-octa-4-enedioate (**76**). Out of these two, the dichloride (**78**) is an intermediate for tetraester (**4**). When this dichloride reacts with one equivalent of diethyl malonate, it forms the desired tetraester by ring closure. Reaction with two equivalents of the diethyl malonate forms the undesired hexaester **77**.



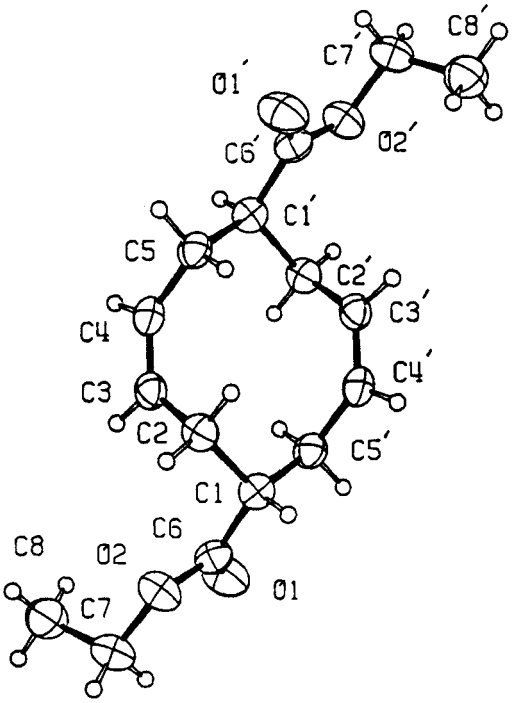
Due to the high boiling points of the components in tetraester washings, GC cannot be used for its analysis. The tetraester washing was decarboalkoxylated using the Krapcho method.[20] The GC of this reaction mixture showed the presence of two major products in 56 and 44% yield in the tetraester washing.

Tetraester **4** was decarboalkoxylated using Krapcho's technique[20] to obtain diester **5**. When the tetraester was refluxed in NaCl/DMSO for 24 hours under a nitrogen atmosphere, the desired diester **5** was obtained. Further modifications in the workup procedure gave 78 % yield of the diester **5**. Although the spectra clearly identified the cyclic diester, the stereochemistry of the crystals was uncertain.



4 **5**

Good quality diester crystals were obtained by recrystallizing from DMSO. Dimensions of these crystals are 4mm x 1mm x 0.5mm. The quality of these crystals was suitable for X-Ray crystallography. Structure elucidation was done in collaboration with Dr. Pavkovic. X-Ray crystallography indicated that the two ester groups are in the trans conformation.

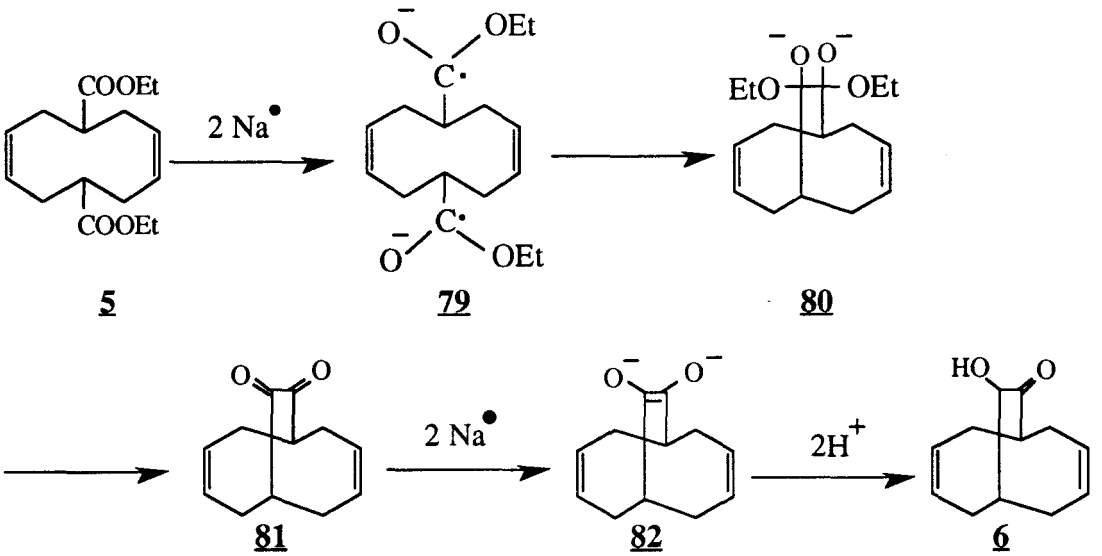


For the acyloin reaction to work, these two ester groups should be cis. The gas chromatographic analysis of the solution part of the acyloin reaction mixture revealed the presence of two isomers of the diester. The GC-mass spectra showed that these two peaks have the same molecular weight and the same fragmentation pattern. This confirms that two isomers of the diester were present in the reaction mixture of the acyloin reaction. However, the initial diester showed only one peak in GC under the same conditions. It appears that epimerization of the *trans*-diester takes place during the acyloin reaction to form a *cis*-diester which then reacts to form the corresponding hydroxy-ketone.

Two low energy conformations were computed with PC model using trans-1,10-dimethyl-3,8-cyclodecane as a model. The minimum energy conformation agreed with the conformation of the diester **5** obtained by X-ray crystallography. Similar calculations done on the *cis*-diene show three minimum energy wells, the lowest of which has an energy

similar to *trans*-diene. This result clarifies the epimerization of diester **5**. Minimum energy conformations tend to be staggered. Examining the eclipsed conformation which would be intermediate in the acyloin closure, one finds a 9 to 12 Kcal/mole barrier.

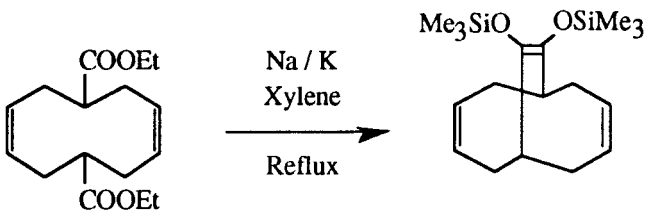
The next reaction was the acyloin reaction of this diester (**5**) to form a disilyl ether (**83**).[21] Acyloin reaction involves a reaction of a diester with Na or Na/K in inert solvent to form a hydroxy-ketone. The mechanism of this reaction is as follows.



This reaction can be done with or without TMSCl. In the presence of TMSCl, the intermediate dianion (**82**) is trapped to form a disilyl ether (**83**), which can be hydrolyzed to form the hydroxy-ketone (**6**). In the absence of TMSCl, hydroxy-ketone is obtained directly.

To test the technique of the reaction, dimethyl adipate was refluxed with Na/toluene, TMSCl under nitrogen for 5 h. The reaction proceeded smoothly and the yield of the silyl-

ether was 60%. Further reaction of this silyl-ether with methanol gave 2-hydroxy-cyclohexanone in 98% yield.



5 83

However, when the diester (5) was reacted under the same conditions, hardly any of the diester underwent reaction to form the disilyl-ether (83). To obtain the desired product in this reaction, it is essential to have anhydrous conditions. The reaction was carried out under the same conditions with a bigger quantity of the diester and more careful drying of toluene and TMSCl. The yield did not change. Since this reaction takes place at the metal surface, particle size of the suspended sodium metal is important. The smaller the particle size of the sodium suspension, the larger the area of sodium surface, and the faster the reaction should go to completion. Hence the reaction was again carried out using a 50% suspension of sodium in mineral-oil, available from Aceton Associates. The yield did not change. These facts indicated that the problem was not with the anhydrous nature of the toluene and TMSCl or with the suspension of the sodium metal.

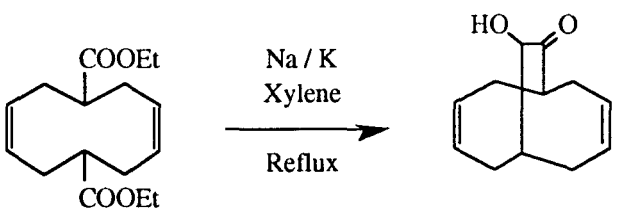
To find the root of the problem, tetraester 4 was reacted under the same conditions. If the problem is with the conformation of the diester (5), the tetraester 4 should react under these conditions, as it definitely has two cis ester linkages.[19] However, if the problem is with the reaction conditions, the tetraester will not react. It was found that the tetraester

also barely reacted. This clearly indicated that problem was the reaction conditions. When the tetraester was reacted in refluxing xylene, NMR analysis of the reaction mixture showed increased formation of the desired product. This indicated that the probable cause of failure of the silylation was the reaction conditions. Hence the diester was refluxed again with Na/TMSCl in xylene and the yield of the desired product significantly improved. When the reaction was carried out with Na/K, the yield increased some more. When Na/K alloy was prepared with more care, almost all of the diester reacted. The IR spectrum of the reaction mixture shows a weak carbonyl stretch at 1740 cm^{-1} indicating a trace of the unreacted diester.

When the disilyl-ether (**83**) was reacted with methanol under N_2 at RT, a yellow colored liquid was obtained. The IR spectrum of this liquid showed the presence of a carbonyl stretch at 1700 cm^{-1} and the hydroxy stretch at 3300 cm^{-1}. The literature[4] indicates that the disilyl-ether should be distilled before reaction with methanol and the reactivity of this compound decreases with the age of the compound. The distillation was unsuccessfully attempted twice.

The acyloin reaction mixture was separated using column chromatography. The fraction collected with ethyl acetate showed the presence of hydroxyl and carbonyl in the IR spectra. However impurities of silylated compounds were found in all the fractions. The same result was obtained in four different attempted separations. Probably an excess of TMSCl formed undesired silyl compounds which interfered with the separation. Hence the reaction was run in absence of TMSCl. The reaction mixture was separated by column chromatography. The IR spectrum of the 20% ethyl acetate in petroleum ether fraction showed a hydroxy peak at 3300 cm^{-1} and a carbonyl stretch at 1700 cm^{-1}. Since the diester

absorbs at 1725 cm^{-1}, this must be the hydroxy-ketone. ^{1}H-NMR spectrum of this fraction showed a peak at 3.62 ppm. ^{13}C NMR analysis of this reaction mixture showed a ketone carbonyl peak at 221 ppm. High resolution mass spectrum of the hydroxy-ketone, purified by column chromatography, showed a mass of 192.1142 while calculated molecular weight of the desired hydroxy-ketone is 192.1150. This proves formation of the hydroxy-ketone in the acyloin reaction without TMSCl. Although the reaction did not go to completion, pure hydroxy-ketone (**6**) was obtained when the acyloin reaction was run in the absence of TMSCl. Hence the best choice was to run this reaction in the absence of TMSCl.



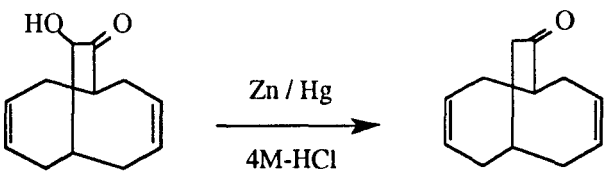
5 **6**

The diester was reacted with Na/K in refluxing xylene for 24 h to obtain the hydroxy-ketone (**6**). In an attempt to improve the yield of the product, the reaction was carried out for 6 h and 12 h. However, a larger quantity of the diester stayed unreacted. Hence, 22 h reflux is the best condition for this reaction. Purity of the hydroxy-ketone **6** was confirmed by TLC.

The next reaction of this hydroxy-ketone (**6**) is a Clemmensen reaction[22,23], to selectively remove the hydroxy group without affecting the ketone (**7**). Clemmensen reduction is generally used to reduce a carbonyl to a methylene group. The selective

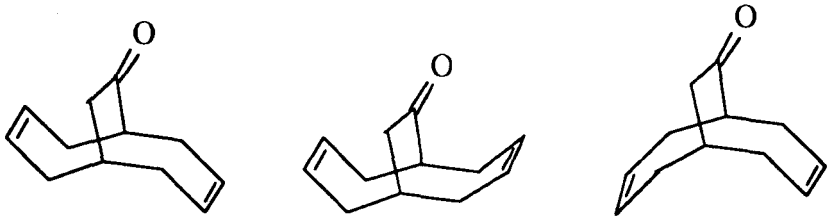
reduction of the alpha hydroxy group in a hydroxy-ketone can be achieved by using 4M-HCl and Zn/Hg. The reaction was carried out on a model compound of 2-hydroxycyclohexanone. This model compound was reacted with Zn/Hg in 4M-HCl under reflux for two hours. Comparison of the IR spectrum of the hydroxy-ketone with the reaction mixture showed loss of the hydroxy peak and formation of a new carbonyl peak at 1710 cm^{-1}.

The hydroxy-ketone (**6**) was selectively reduced to the ketone **7** using Zn/Hg and 4M-HCl for 2 1/2 h. ^{1}H-NMR and GC analysis indicated that the reaction worked. ^{1}H NMR analysis indicated a change in the 3.62 ppm region. IR spectrum showed a less intense hydroxy peak as compared to the hydroxy-ketone (**6**). The product was not separated due to the small quantity of material involved.



6 **7**

Energy minimization calculations were performed on the three possible ketone conformations using PC model. It indicated that they have comparable stabilities.

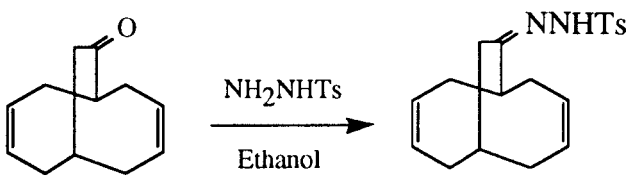


7a **7b** **7c**

mmx Energy	37.06 Kcal/mole	35.38 Kcal/mole	36.08 Kcal/mole
Strain Energy	32.43 Kcal/mole	30.86 Kcal/mole	32.01 Kcal/mole
Heat of Formation	3.59 Kcal/mole	1.92 Kcal/mole	2.61 Kcal/mole

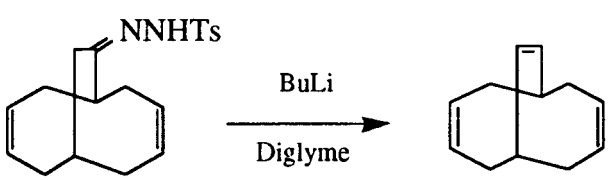
X-ray crystallography has indicated that conformation of the diester (5) is trans with the two rings pointing away from each other. The ketone should also have the same conformation as the diester. The orientation of the carbonyl group in the ketone (7a) is such that it pushes the neighboring hydrogens in the shielding zone. As a result, these protons absorb at 3.6 and 4.1 ppm in ^{1}H NMR.

The next step was conversion of the ketone to an alkene by the Shapiro reaction.[24] In this reaction, a ketone is first converted to its tosylhydrazone derivative. The tosylhydrazone is then reacted with butyllithium to obtain the alkene.



7 8

To test the technique of the Shapiro reaction, 1'-acetonaphthone was reacted with tosylhydrazide in ethanol under reflux. Within one hour the flask was filled with white solid as the product precipitated out of solution. The ketone (7) was refluxed with tosylhydrazide in ethanol for six hours to obtain a solid. ^{1}H NMR spectrum showed the presence of tosylhydrazide peaks along with tosyl hydrazone peaks. ^{1}H NMR spectrum of 8 also showed upfield shift of protons that were at 3.6 ppm in the ketone (7). As nitrogen is less electronegative than oxygen, this shift confirms the formation of tosylhydrazone. Most of the unreacted tosylhydrazide was removed by washing with chloroform.



8 1

The tosylhydrazone (**8**) was reacted with BuLi in diglyme at 60 ^{o}C for 4h. The reaction mixture was separated by column chromatography. The desired triene (**1**) was eluted with petroleum ether. ^{1}H NMR shows some confirming evidence for the formation of **1**.

CHAPTER IV

CONCLUSIONS

Tetraethyl cis,cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (4) was prepared in 2.6% yield using the literature procedure. Two products of the reaction of 1,4-dichloro-2-butene and diethyl malonate are isolated for the first time. Characterization of these products explains the mechanism of the formation of tetraethyl cis,cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (4). Some modifications in the apparatus and the workup that made the procedure considerably easier, are also reported. Krapcho decarboalkoxylation of this tetraester stereospecifically gave a trans isomer of the corresponding diester (5). Acyloin reaction of this diester gave the hydroxy-ketone (6) via cis-diester which was formed by epimerization of the trans-diester under the reaction conditions. Hydroxy-ketone 6 has been completely characterized. The pure hydroxy-ketone was selectively reduced to obtain the corresponding ketone (7). The ketone was reacted with tosylhydrazide to obtain the tosylhydrazone (8). The tosyl-hydrazone was reacted with BuLi to give the final triene (1). There is spectral evidence of the formation of 7, 8 and 1 but characterization was not completed due to the small quantity of material.

CHAPTER V

EXPERIMENTAL

Diethyl malonate, and 1,4-dichloro-2-butene were obtained from Aldrich. Toluene and xylene were dried over anhydrous CaH_2 and distilled. Trimethylsilyl chloride was purified by mixing it with a few drops of water to hydrolyze dichlorodimethylsilane impurity, followed by drying over anhydrous CaH_2 and distillation. Petroleum ether, and ethyl acetate were distilled to remove the non-volatile impurity. The infrared spectra were taken on a Perkin-Elmer 1310 Infrared Spectrophotometer, and the NMR spectra were taken on a Varian VXR-300 Instrument. ^1H NMR were run in deuterochloroform unless otherwise stated. Flash-grade silica gel available from Baker was used for the column chromatography.

Tetraethyl cis,cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (**4**): A 2 L four-neck r.b. flask, fitted with a reflux condenser, two addition funnels, and a thermometer was charged with 100 g (0.80 mol) of 1,4-dichloro-2-butene. Then 300 mL ethanol was added to each addition funnel followed by 18.9 g (0.84 mol) of sodium spheres. After all the sodium reacted, slow addition of 63.9 g (0.40mol) of diethyl malonate was added to each addition funnel over 5 min. The solution in the addition funnels was kept hot by a heating tape. This solution was slowly added to 1,4-dichloro-2-butene in the flask over a period of 1 h. The temperature was maintained between 65 and 70 °C during the addition and for additional 5h of mechanical stirring.

The reaction mixture was cooled to room temperature and the solution was vacuum-filtered. The solvent was removed on a rotary evaporator, and the yellow oil was vacuum distilled at 65 °C and 0.3 mmHg. When the residue from the distillation was washed with heptane, white solid was left after washing which on recrystallization from chloroform/petroleum-ether gave 4.3 g (0.708 mmol, 2.6 %) of white crystals of the tetraester (4). mp 160-162 °C; lit mp 160-161 °C.[18] ^{1}H-NMR: δ 5.3 (4H, m, vinylic CH), 4.25 (8H, q, OCH_2), 2.79 (4H, t, CH_2), 2.5 (4H, d, CH_2), 1.28 (12H, t, CH_3); ^{13}C-NMR:δ 171.3, 128.2, 62, 61.9, 57.2, 30.4, 14.6, 0.5; IR: 3100 (C=CH), 1700 (C=O), 1650 (C=C) cm^{-1}.

Column chromatography of the tetraester washing was done using a silica gel column. Diethyl 2,7-dicarboethoxy-octa-4-enedioate (76) eluted with 4.5% ethyl acetate in petroleum ether while diethyl 2,7,7,12-tetracarboethoxy-trideca-4,9-dienedioate (77) eluted with 8% ethyl acetate in petroleum ether. Diethyl 2,7-dicarboethoxy-octa-4-enedioate (76): ^{1}H-NMR: δ 5.44 (2H, t, vinylic CH), 4.19 (8H, m, OCH_2), 3.35 (2H, t, CH), 2.68 (4H, dd, CH_2), 1.27 (12H, t, 4-CH_3); ^{13}C-NMR:δ 168.9, 127.9, 61.4, 51.8, 26.6, 14.1, 14.0; IR: 3100 (C=CH), 1700 (C=O), 1650 (C=C) cm^{-1}. Diethyl 2,7,7,12-tetracarboethoxy-trideca-4,9-dienedioate (77): ^{1}H-NMR: δ 5.43 (4H, m, vinylic CH) 4.21 (12H, mixed q, OCH_2), 3.33 (2H, t, CH), 2.65 (8H, q, CH_2), 1.27 (18H, mixed t, CH_2); ^{13}C-NMR:δ 171.0, 169.0, 128.8, 126.0, 57.8, 51.9, 30.1, 26.8, 14.1; IR: 3100 (C=CH), 1700 (C=O), 1650 (C=C) cm^{-1}.

Diethyl trans-3,8-cyclodecadiene-1,6-dicarboxylate (5): A 250 mL three neck r.b. flask fitted with a reflux condenser was charged with 85 mL of DMSO, 0.8 g NaCl and 0.6 mL of deionized water. The mixture was stirred and degassed at RT by vigorously bubbling

nitrogen gas through it for 20 min. The system was maintained under an atmosphere of nitrogen and 2.12 g (5.0 mmol) of the tetraester (**4**) was added to it. The solution was refluxed under nitrogen for 24 h. The reaction mixture was cooled to RT and extracted with pentane (5x). The pentane extracts were combined and dried over anhydrous $MgSO_4$ for 15 min. The solvent was removed on the rotary evaporater to obtain a yellow solid. This solid was washed with ice-cold methanol to remove DMSO and passed through silica gel, to afford 1.1 g (3.9 mmol, 78 %) of white solid. mp 41-42 °C; ^{1}H-NMR: δ 5.45 (4H, m, vinylic CH), 4.21 (4H, 2 q, OCH_2), 2.6 (2H, m, CH), 2.1 to 2.7 (8H, m, CH_2), 1.32 (6H, t, CH_3); ^{13}C NMR: δ 175.3, 130.5, 129.9, 128.2, 127.6, 60.9, 42.8, 42.7, 27.1, 27.0, 26.9, 14.8, 0.5; IR: 3010 (C=CH), 1720 (C=O), 1650 (C=C) cm^{-1}. Mass Spectra: 280 (16.0%), 235 (25.8%), 206(59.3%), 177 (5.6%), 161 (30.8%), 133 (100%), 105 (19.3%), 91 (46.0%), 79 (38.9%), 67 (39.9%), 55 (12.0%). R_f (**5**) = 0.62.

Bicyclo[4.4.2]dodeca-11,12-bis(trimethylsilyloxy)-3,8,11-triene (**83**): A 50 mL three neck r.b. flask fitted with a reflux condenser and an addition funnel was charged with 0.1324 g (5.8 mmol) of sodium metal, and 0.2244 g (5.8 mmol) of potassium metal under nitrogen. The flask was heated until the metals melted and fused. The flask was cooled, and 10 mL of xylene was added to it. The solution was then vigorously stirred with a high speed stirrer to obtain a fine dispersion of the melted metal-alloy. The stirrer speed was reduced and 0.10 g (0.35 mmol) of the diester (**5**) and freshly distilled TMSCl (1 mL) was added to this suspension over 1 h at reflux temp., and the heating was continued for 6 h. The reaction mixture was then filtered in a dry bag under nitrogen. The solvent was removed

on a rotary evaporator to yield a yellow oil. ^{1}H-NMR: δ 5.4 (4H, m, vinylic CH), 2.8 (2H, m, CH), 1.3-2.2 (8H, m, CH_2), 0.25 (18H, s, $OSi(CH_3)_3$).

The disilyl-ether (**84**) was mixed with 5 mL of methanol and stirred under a N_2 atmosphere at RT for 24 h. The solvent was removed on a rotary evaporator to obtain a yellow oil. IR: 3400 (O-H), 3010 (C=CH), 1700 (C=O), 1630 (C=C) cm^{-1}.

Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**): A 50 mL three neck r.b. flask fitted with a reflux condenser and an addition funnel was charged with 132 mg (5.8 mmol) of sodium metal, and 224 mg (5.8 mmol) of potassium metal under nitrogen. The flask was heated until the metals melted and fused. The flask was cooled, and 10 mL of xylene was added to it. The solution was then vigorously stirred with a high speed stirrer to obtain a fine dispersion of the melted metal-alloy. The stirrer speed was reduced and 0.10 g (0.35 mmol) of the diester (**5**) was added to this suspension over 1 h reflux temp., and the reflux was continued for 22 h. The reaction mixture was then filtered in a dry bag under nitrogen. The solvent was removed on a rotary evaporator to yield a yellow oil. Pure hydroxy-ketone (**6**) (3 mg, 0.015 mmol, 4%) was eluted with 20% ethyl acetate in petroleum ether. ^{1}H-NMR: δ 5.42 (4H, m, CH), 3.62 (3H, m, CH), 1.34-2.74 (8H, m, CH_2); ^{13}C-NMR: 129.5, 127.2, 65.9, 40.2, 40.1, 29.7, 27.5, 25.5, 25.5, 0.01; The carbonyl carbon was seen in the impure **6** at 221 ppm before the chromatographic separation, however the quantitty of the pure hydroxy-ketone **6** was not enough to show carbonyl carbon in ^{13}C. IR: 3400 (O-H), 3010 (C=C), 1700 (C=O), 1630 (C=C) cm^{-1}. Mass spectra: 192, 178, 166, 153, 136, 121, 105, 91, 77, 67, 55. Mass calculated for $C_{12}H_{16}O_2$ = 192.1150, found 192.1142. R_f (**6**) = 0.21.

Bicyclo[4.4.2]dodeca-3,8-diene-12-one (7): A three neck r. b. flask fitted with a reflux condenser was charged with 3 mg of the hydroxy-ketone (6) and 2 mL of toluene. It was mixed with 1.2 g of freshly prepared Zn/Hg and 2 mL of 4M-HCl. Zinc amalgam was prepared by stirring 1.2 g of mossy zinc, 0.12 g of $HgCl_2$, 1.5 mL of H_2O and 0.06 mL of conc. HCl. The reaction mixture was refluxed for 2 1/2 h. The two layers were separated and the aqueous layer was extracted with 10 mL of methylene chloride. The organic layers were dried over anhydrous magnesium sulfate and the solvent was removed on the rotary evaporator to obtain 1 mg of yellow oil (0.0058 mmol, 37%). ^{1}H-NMR: δ 5.48 (4H, m, vinylic CH), 4.25 (1H, m, CH), 3.72 (2H, m, CH_2), 1.5-2.93 (9H, m, CH_2 and CH).

Bicyclo[4.4.2]dodeca-3,8-diene-12-tosylhydrazone (8): To a 50 mL three neck r. b. flask was added 1 mg of the ketone, 2 mg of tosylhydrazide and 10 mL of absolute ethanol. The reaction mixture was refluxed for 6 h. The solvent was removed on the rotary evaporator to obtain < 1 mg solid. It was washed with 2 mL of chloroform. A ^{1}H-NMR was recorded on a mixture of tosylhydrazone and tosylhydrazide. ^{1}H-NMR: δ 7.85 (2H, m, ArH), 7.2 (2H, m, ArH), 5.44 (4H, m, vinylic CH), 2.47 (3H, s, CH_3), 1.52-2.72 (9H, m, CH_2 and CH).

Bicyclo[4.4.2]dodeca-3,8,11-triene (1): To a 25 mL three neck flask was added 1 mg of tosylhydrazone suspended in 5 mL of diglyme. An excess of 2.5M butyllithium in hexane was slowly added to this solution at -78 ^{o}C under a nitrogen atmosphere. The solution was stirred for 10 min and then allowed to warm to room temperature. The reaction mixture was maintained at 60 ^{o}C for next 6 h. The solution was cooled to room temperature, mixed with 2 mL of water and extracted with 50 mL of diethyl ether. The

solvent was removed on a rotary evaporator to obtain an oil which was separated by column chromatography on Florisil. Concentration of the early fraction with petroleum ether gave a hydrocarbon (<1mg) which appeared to be triene 1 by ^{1}H-NMR. ^{1}H-NMR: δ 5.7 (6H, m, vinylic H), 3.5-4.3 (6H, m, CH_2 and CH), 1.85-2.7 (4H, m, CH_2).

Since some of the reactions used for the synthesis of the triene require special techniques, model reactions[21] were run starting from dimethyl adipate.

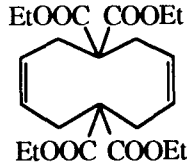
1,2-Disilyloxy-1-cyclohexene: A 250 mL three neck round-bottom flask fitted with a reflux condenser, and mechanical stirrer was charged with 1.9 g (80 mmoles) of sodium metal and 150 mL of toluene under nitrogen. The mixture was refluxed and vigorously stirred with a mechanical stirrer to obtain a fine dispersion of the sodium metal. To this mixture was slowly added a mixture of 3.05 g (20 mmoles) of dimethyl adipate and 9 g (80 moles) of TMSCl over a period of 1 h. The reaction mixture was maintained at reflux for 5 h. At the end of the reaction, the reaction mixture was filtered in the dry-box under an atmosphere of nitrogen. The solvent was removed on the rotary evaporator to obtain 4 g (12 mmol, 60 %) of yellow liquid. ^{1}H-NMR: δ 2.05 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.25 (2H, m, CH_2), 0.85 (2H, m, CH_2), 0.2 (18H, s, $OSi(CH_3)$).

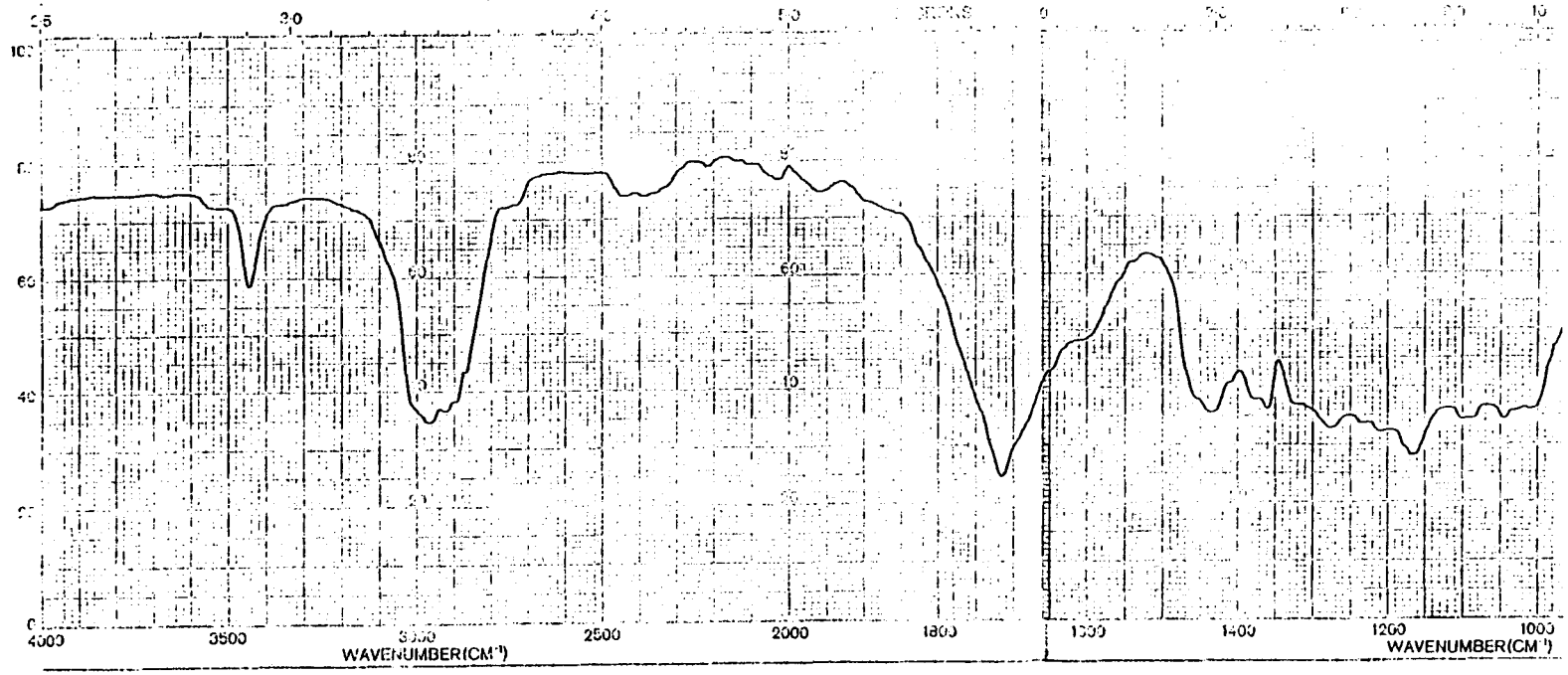
2-Hydroxycyclohexanone: A 250 mL three neck flask, fitted with a reflux condenser and an addition funnel was charged with 100 mL of reagent grade methanol. The methanol was degassed by bubbling nitrogen gas for a period of 30 min. The disilyl-ether was slowly added to methanol and the reaction mixture was stirred at a room temperature over a period of 26 h. The solvent was removed on a rotary evaporator to yield 1.3 g (11.8

mmol, 98 %) of dark brown liquid. ^{1}H-NMR:δ 3.8 (1H, s, CH_2), 3.3 (1H, t, CH_2), 1.2-2.8 (5H, m, CH_2 and OH), 1.0 (1H, m, CH_2). IR: cm^{-1} 3475 (OH), 1715 (C=O).

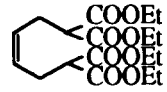
Cyclohexanone: A 100 mL r.b. flask was charged with 1.78 g (27 mmol) of mossy zinc, 2.3 mL water, and 0.1 mL of conc HCl. The solution was stirred, and 178 mg (0.65 mmoles) of $HgCl_2$ was slowly added to the mixture. The solution was stirred for the next 10 min. The solution was then decanted to remove the liquid part, and the zinc was mixed with 0.3 g (2.6 mmol) of hydroxy-ketone dissolved in 5 mL of toluene, and 15 mL of 4M-HCl. The reaction mixture was refluxed for 2 h. The solution was cooled and extracted with diethyl ether (3x). The ether extracts were combined, and dried over anhydrous $MgSO_4$. The solvent was removed on a rotary evaporator to yield 0.2 g of yellow liquid. IR: cm^{-1} weak band at 3400 (OH), 1705 (C=O)

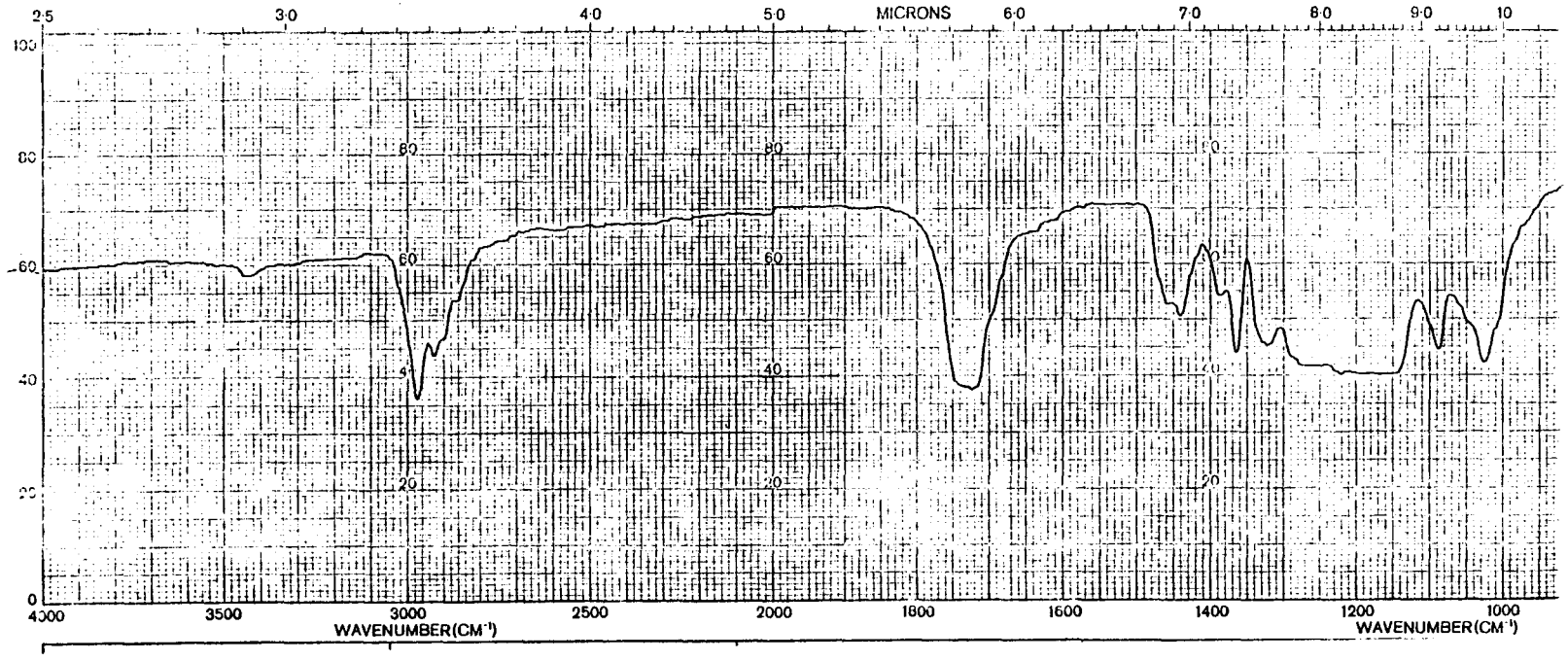
SPECTRA



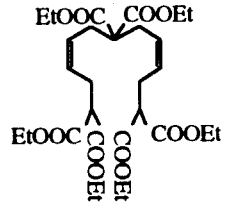


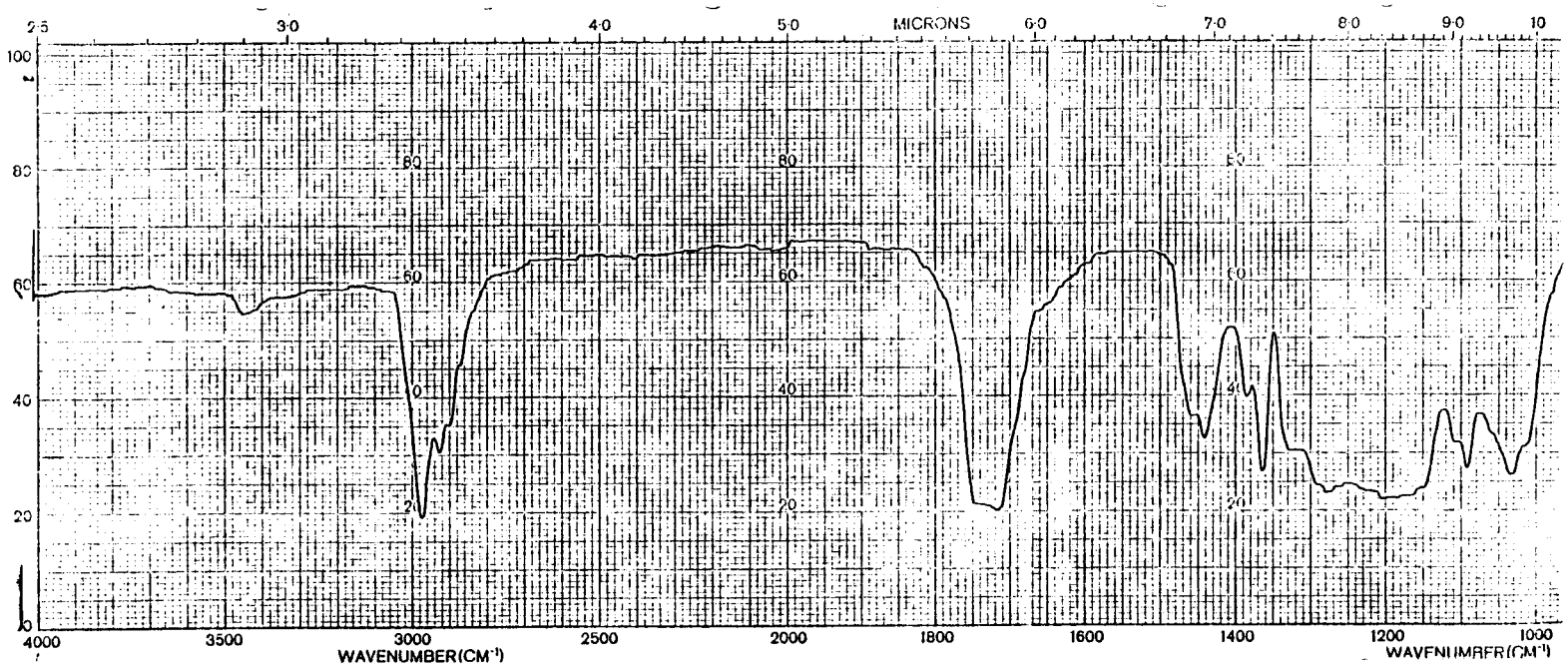
IR of Tetraethyl-cis, cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (**4**)



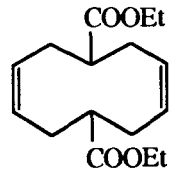


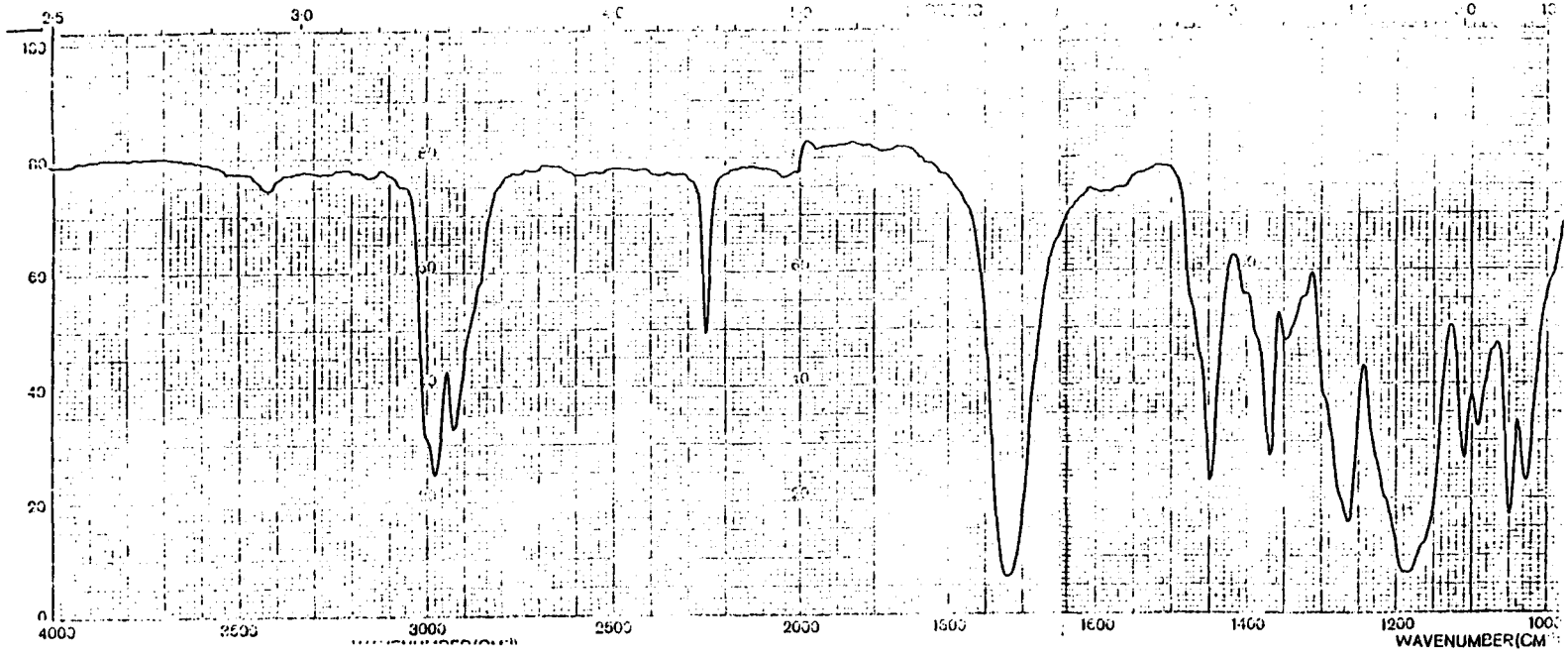
IR of Diethyl 2,7-dicarboethoxy-octa-4-enedioate (**76**)



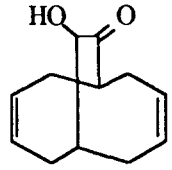


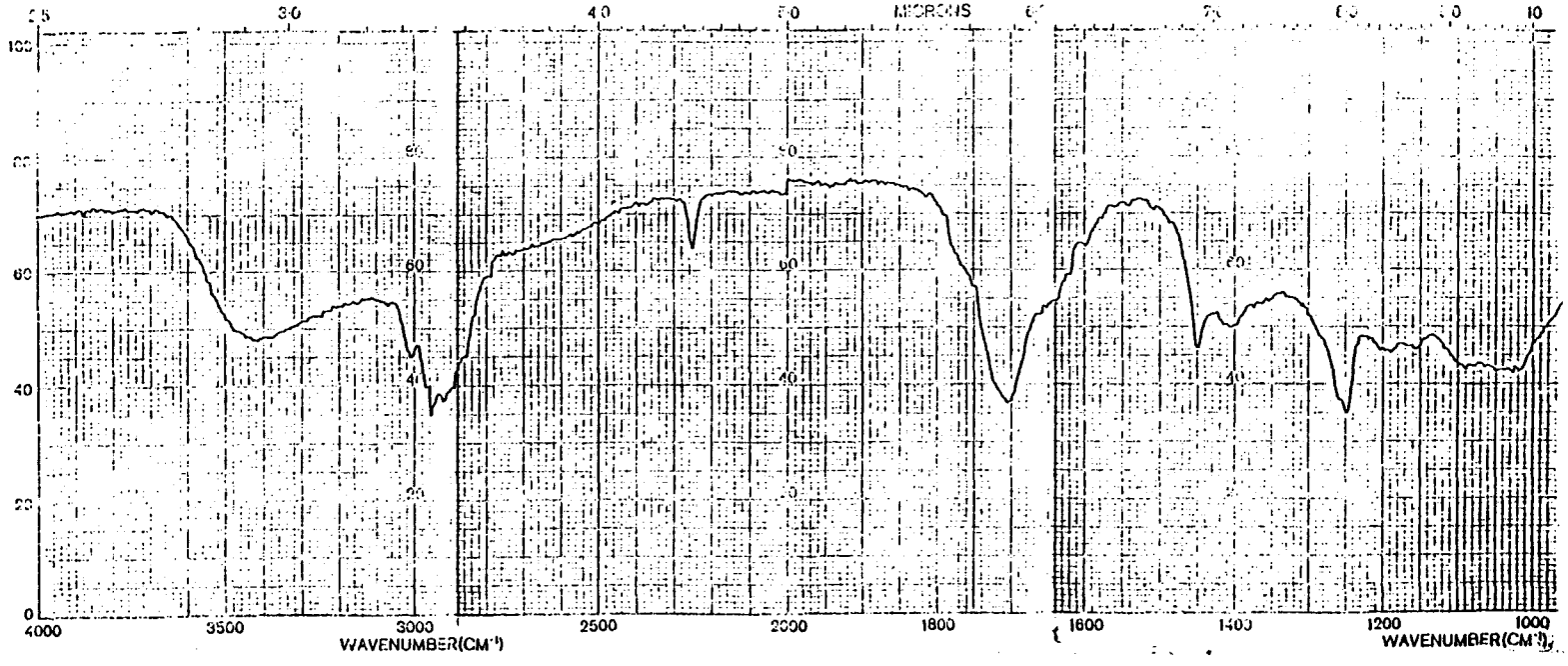
IR of Diethyl 2,7,7,12-tetracarboethoxy trideca-4,9-dienedioate (77)



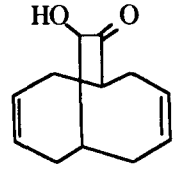


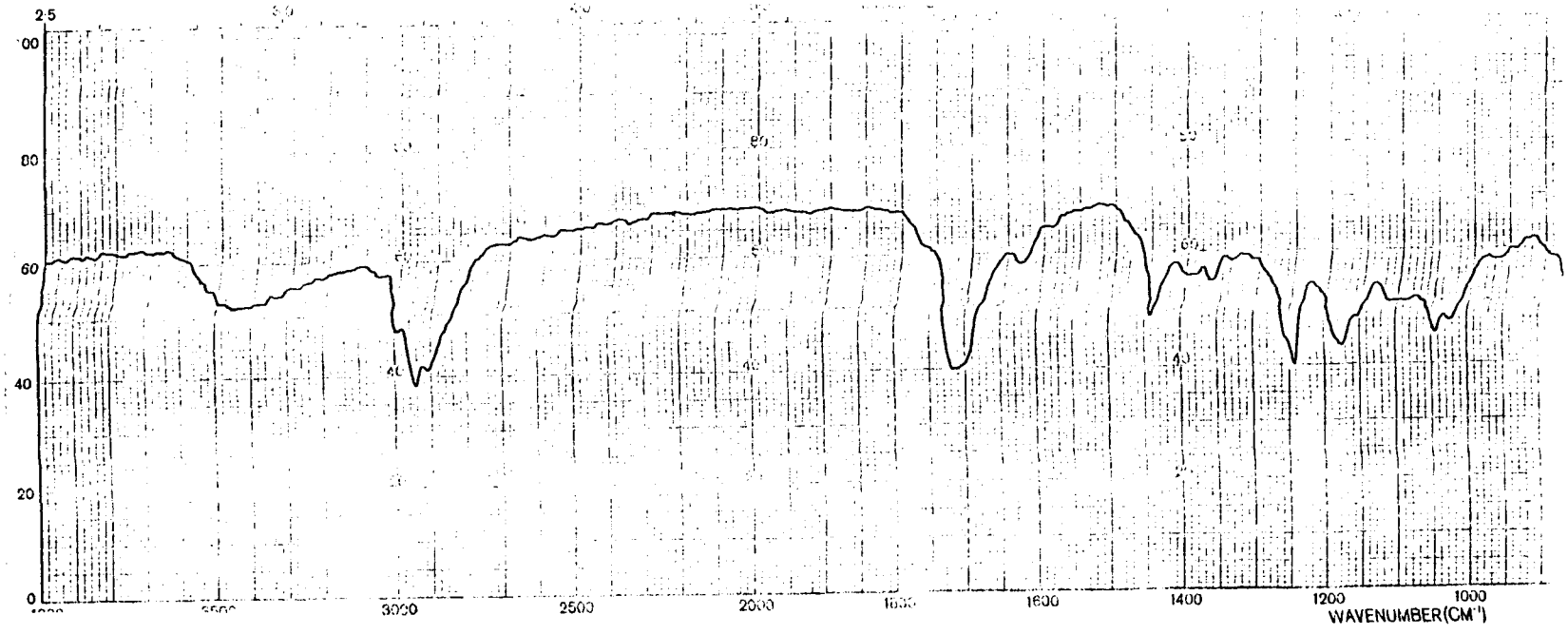
IR of Diethyl-trans-3,8-cyclodecadiene-1,6-dicarboxylate (**5**)



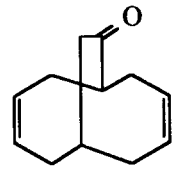


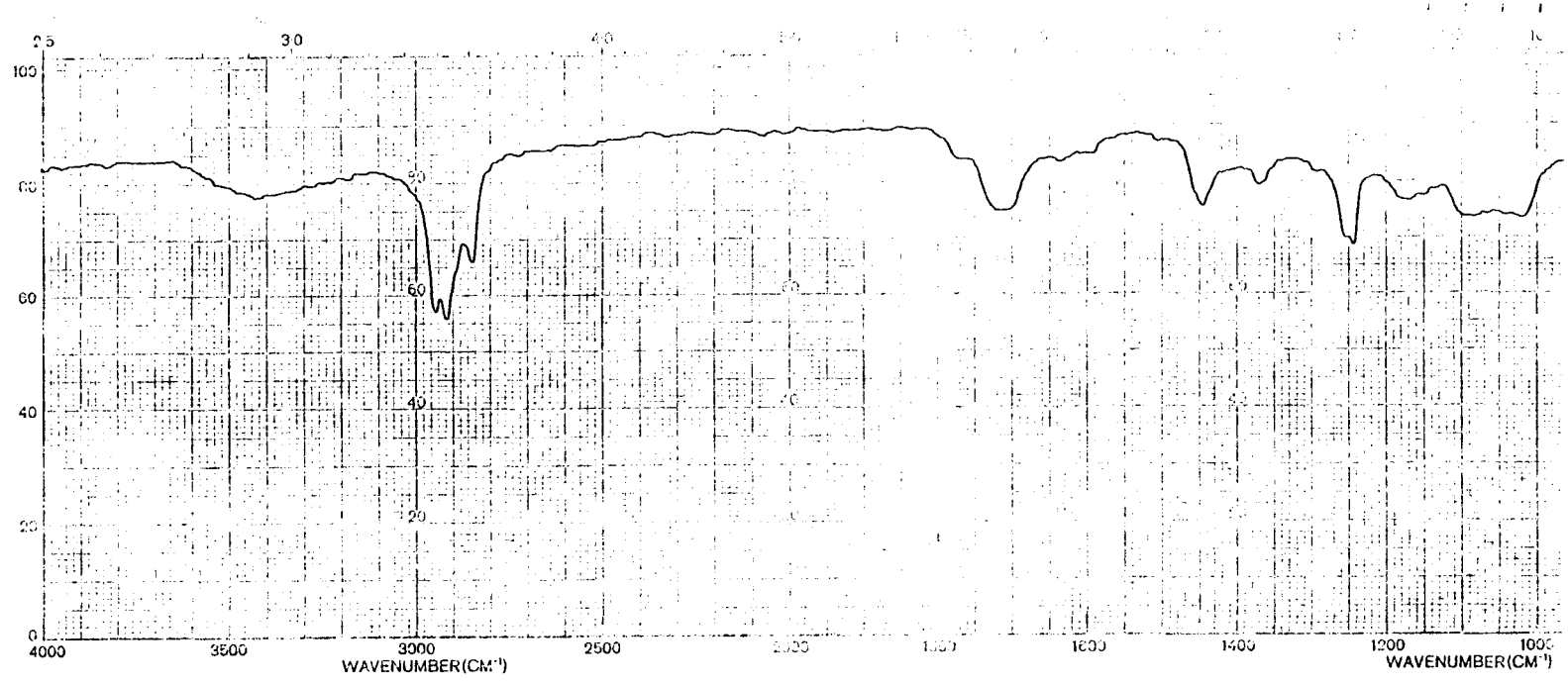
IR of pure Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**)



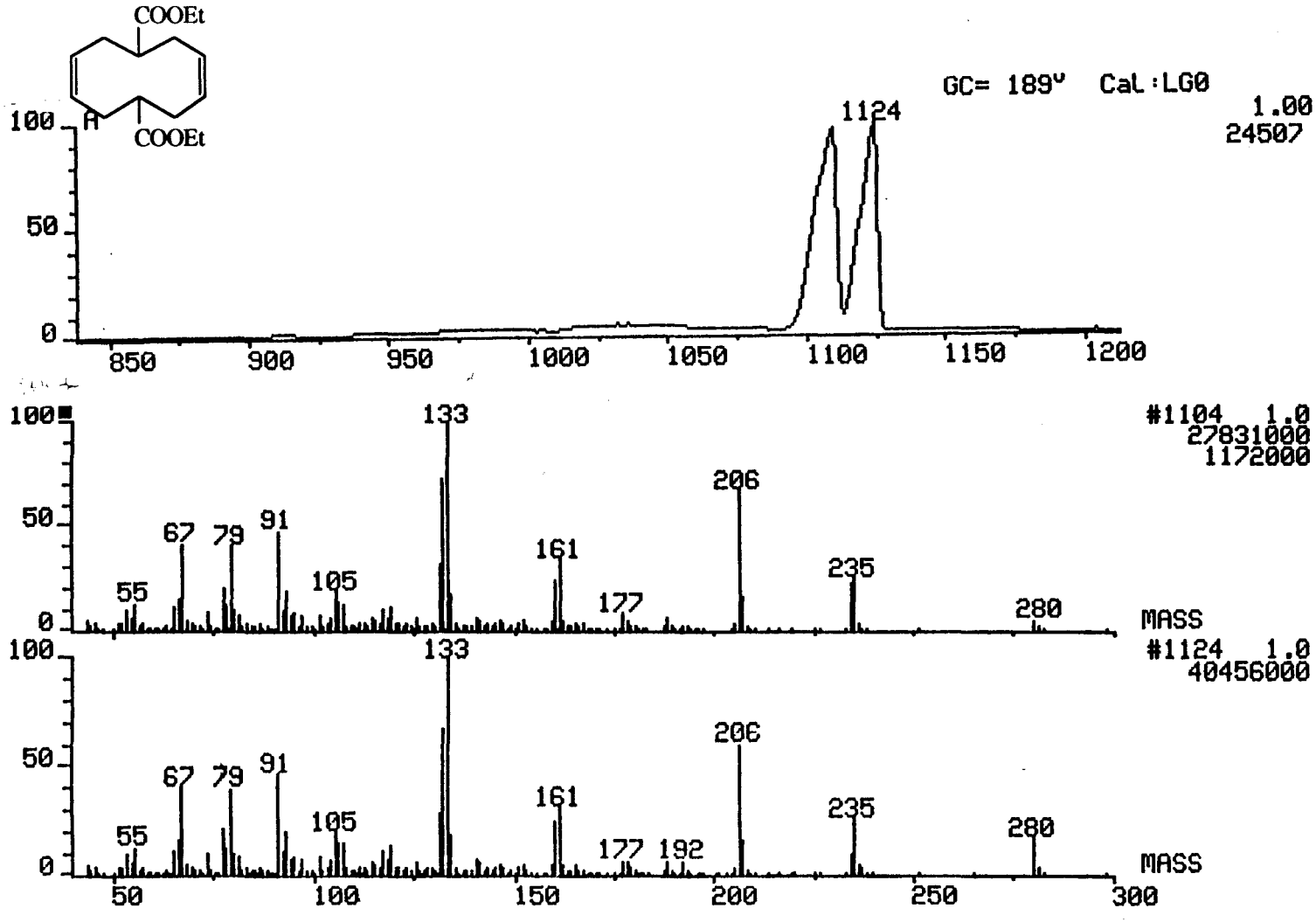


IR of impure Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**) obtained by hydrolysis of bicyclo[4.4.2]dodeca-11,12-bis(trimetylsilyloxy)-3,8,11-triene

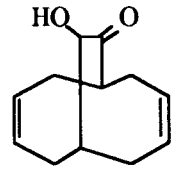


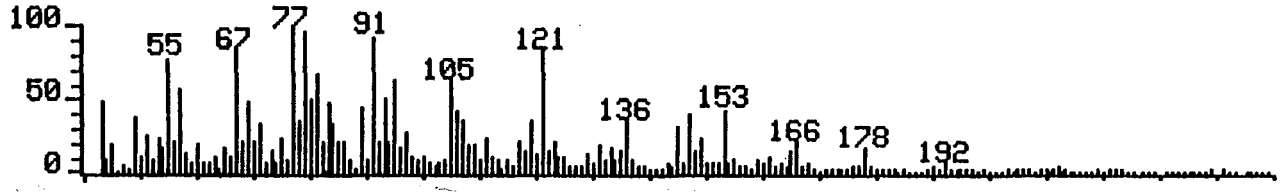


IR of Bicyclo[4.4.2]dodeca-3,8-diene-12-one (7)

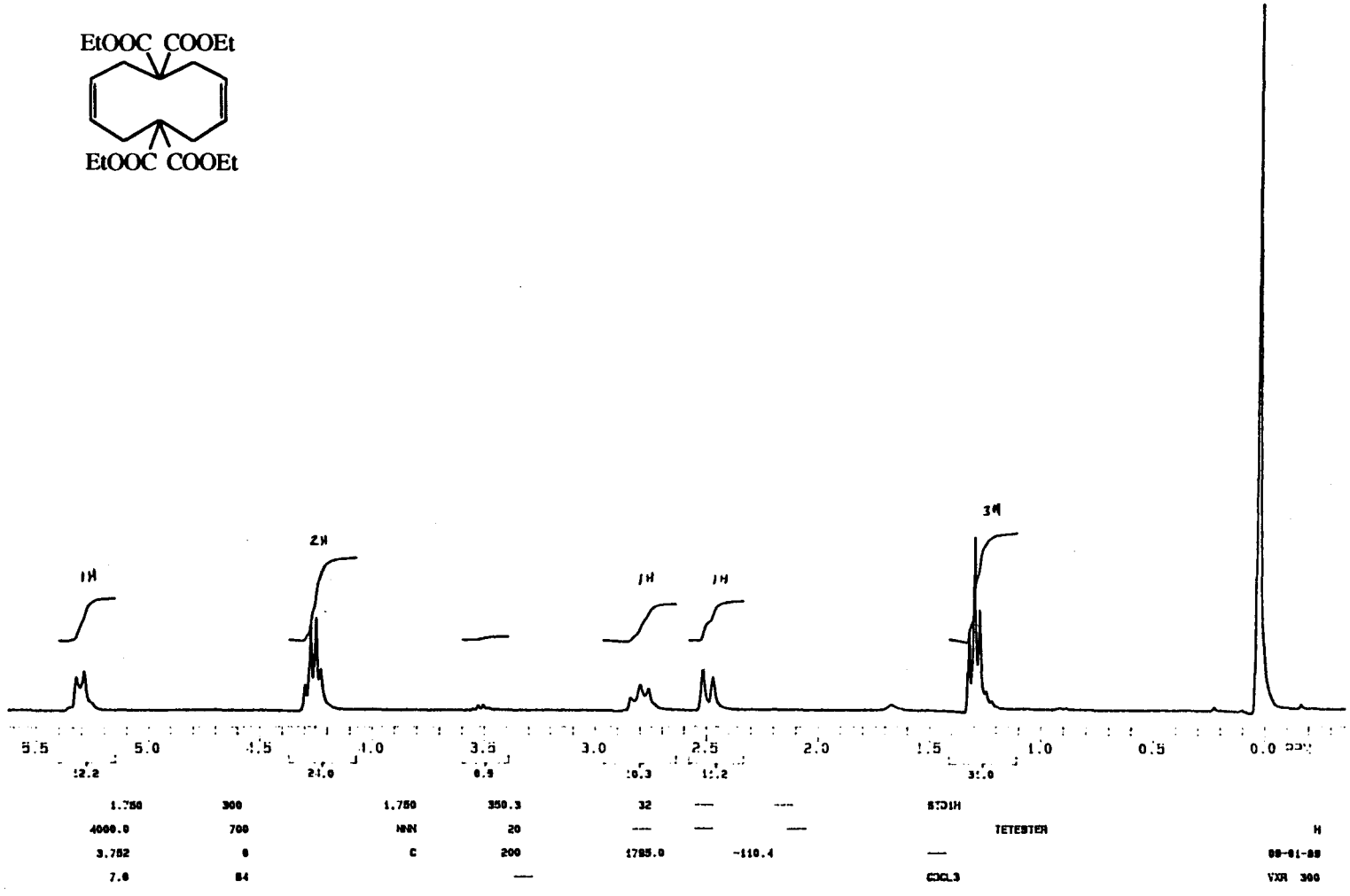


Mass spectrum of cis and trans Diethyl-3,8-cyclodecadiene-1,6-dicarboxylate (**5**)

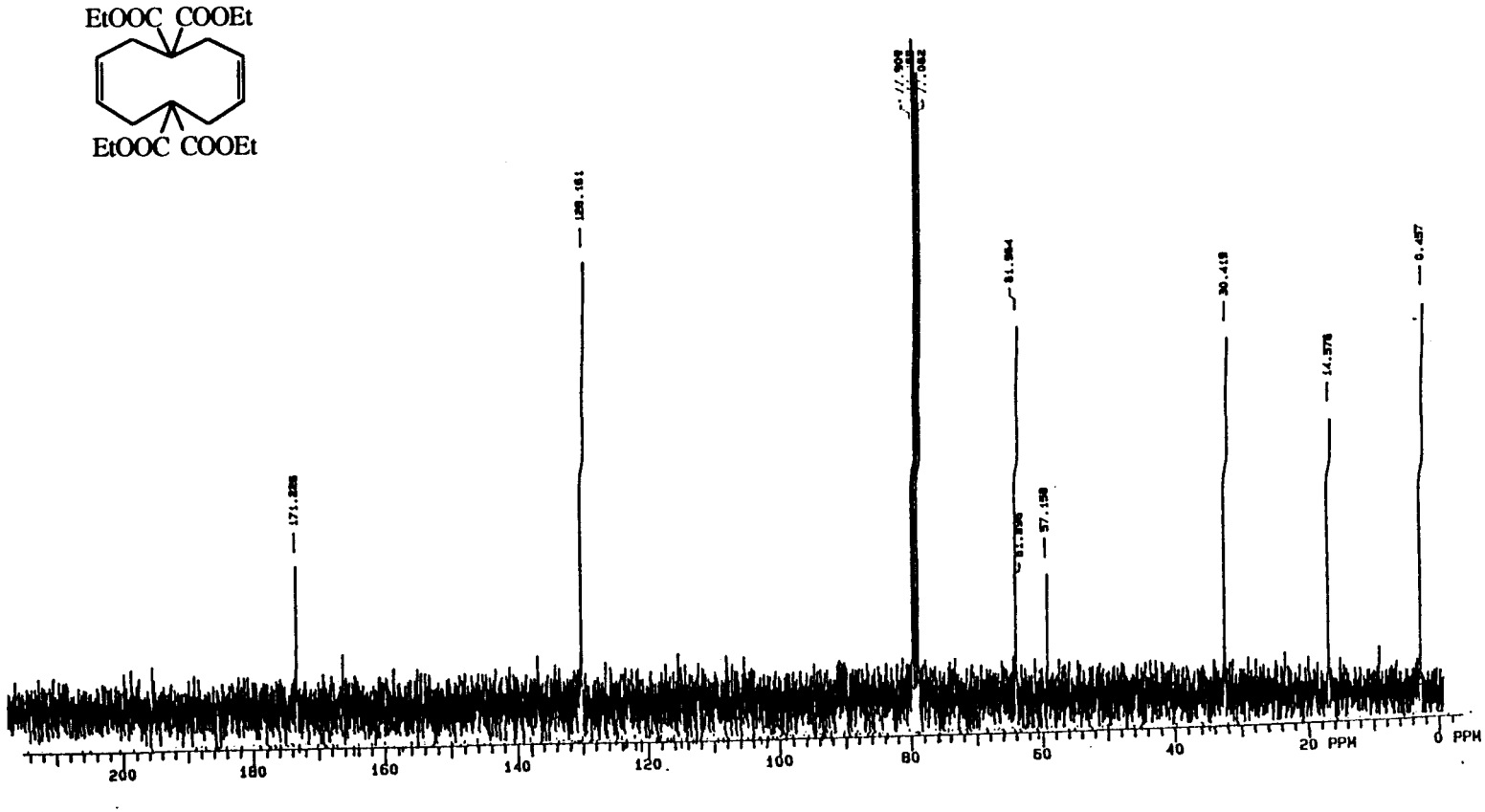




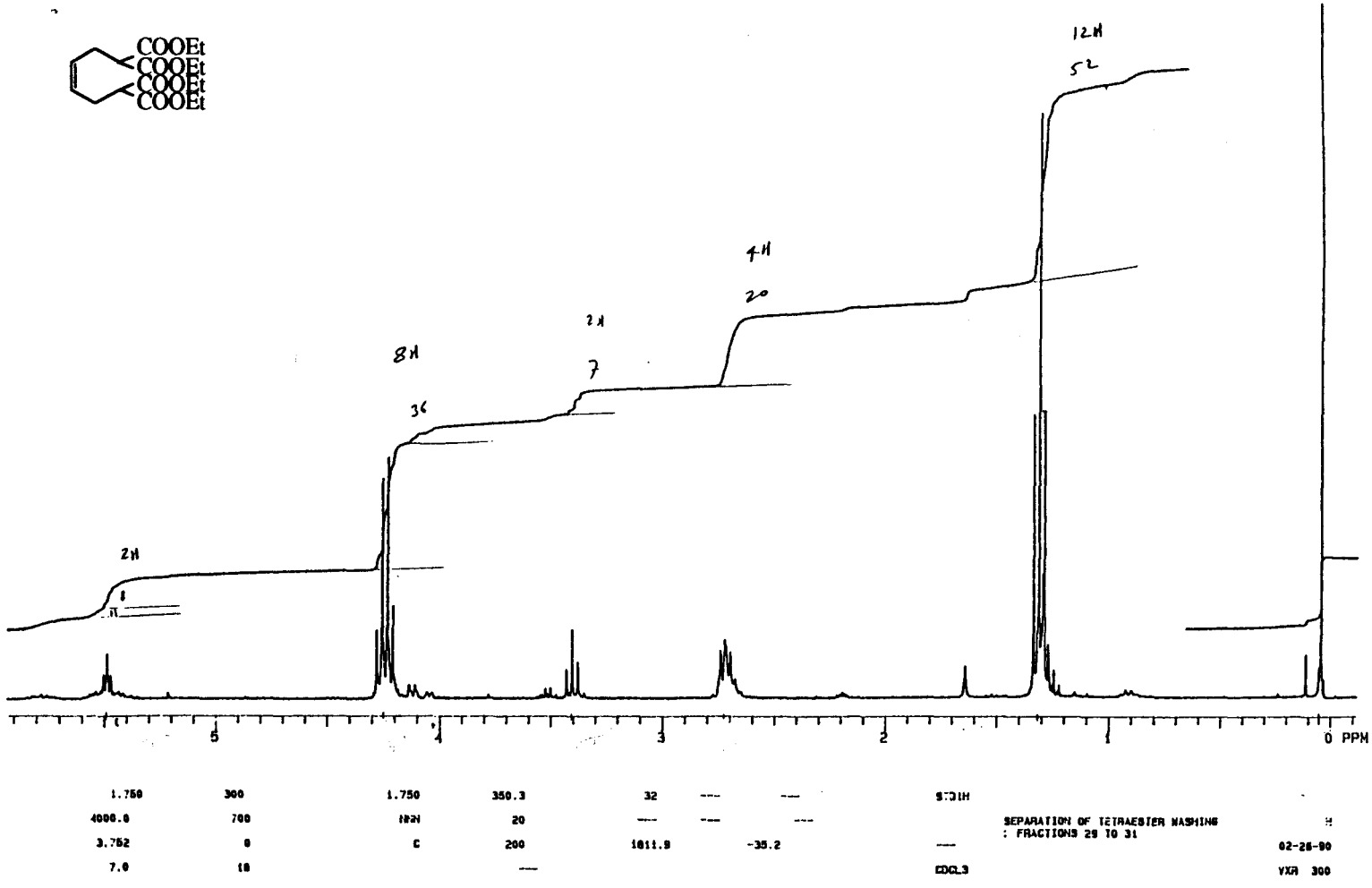
Mass spectrum of Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one(**6**)



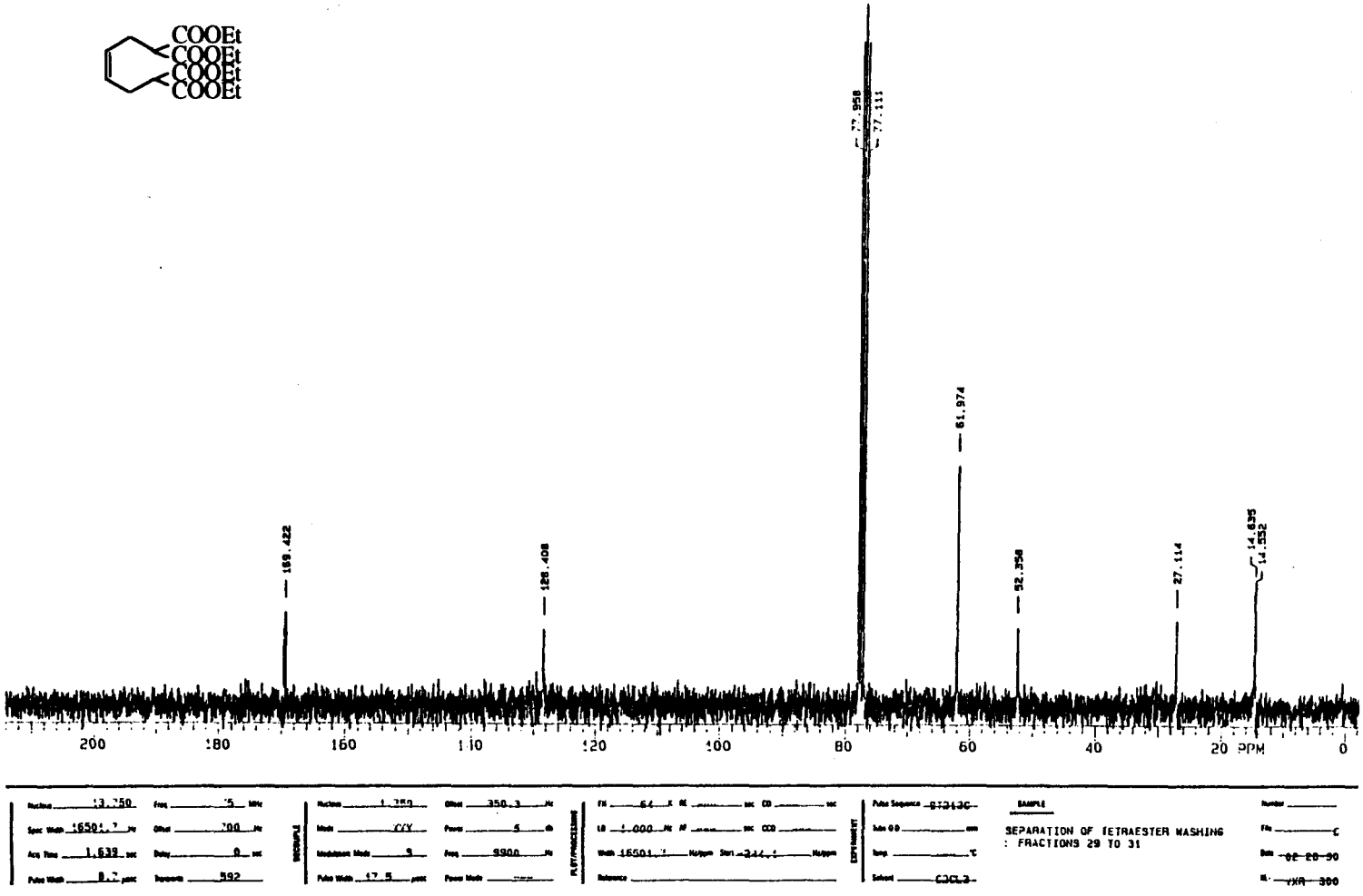
^{1}H NMR of Tetraethyl-cis, cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (**4**)



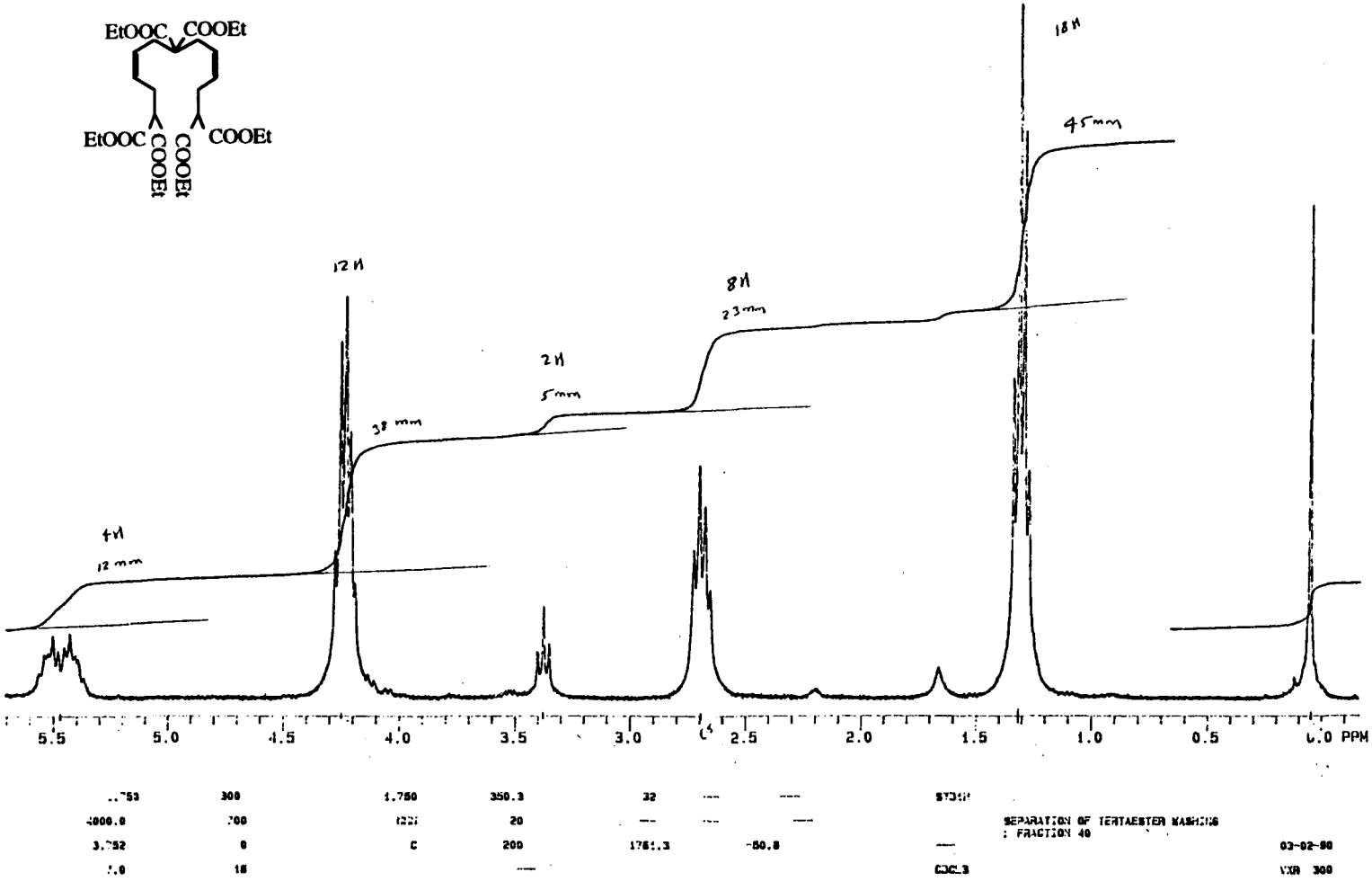
^{13}C NMR of Tetraethyl-cis, cis-3,8-cyclodecadiene-1,1,6,6-tetracarboxylate (4)



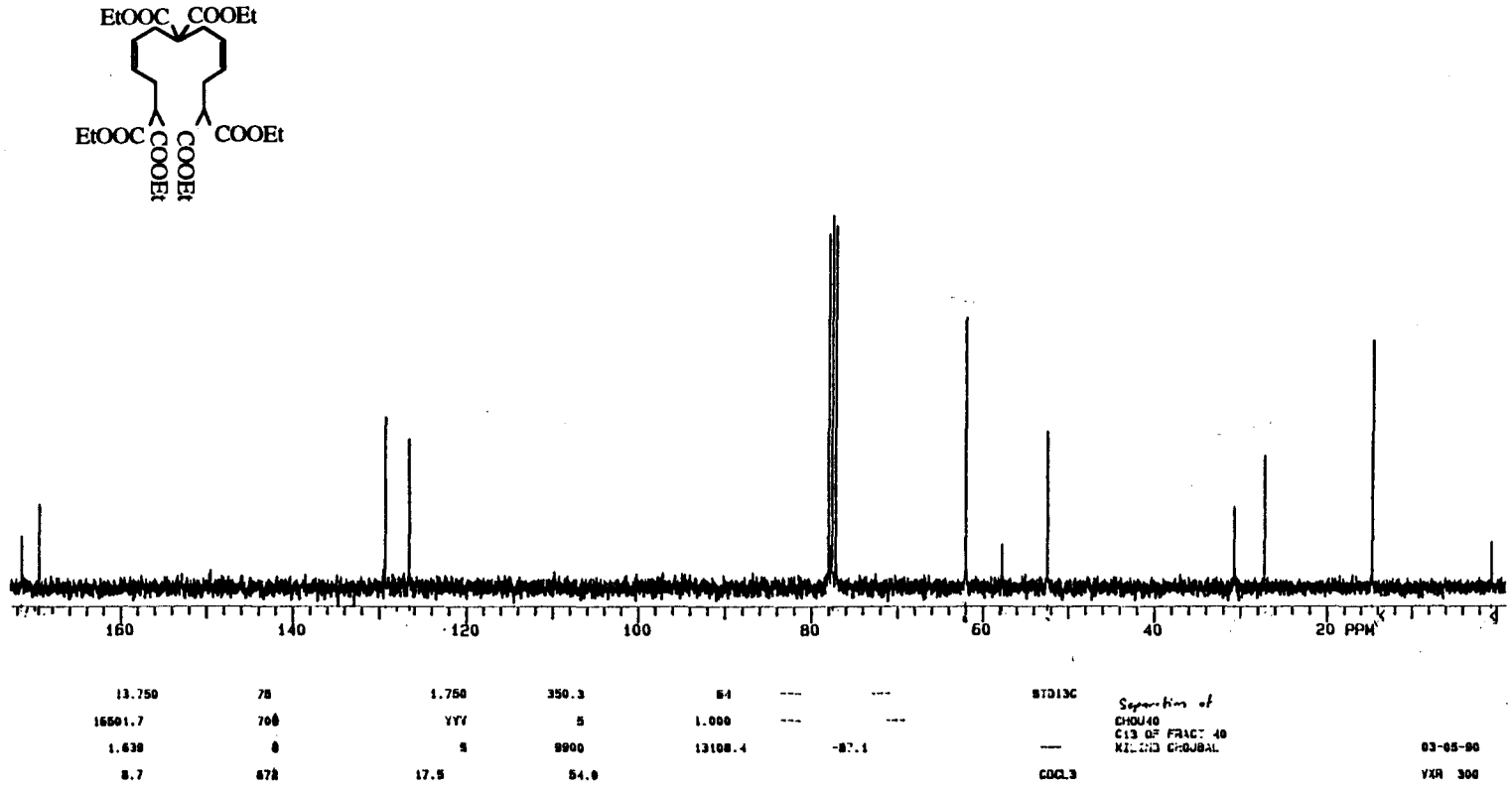
^{1}H NMR of Diethyl 2,7-dicarboethoxy-octa-4-enedioate (**76**)



^{13}C NMR of Diethyl 2,7-dicarboethoxy-octa-4-enedioate (**76**)



^{1}H NMR of Diethyl 2,7,7,12-tetracarboethoxy trideca-4,9-dienedioate (77)



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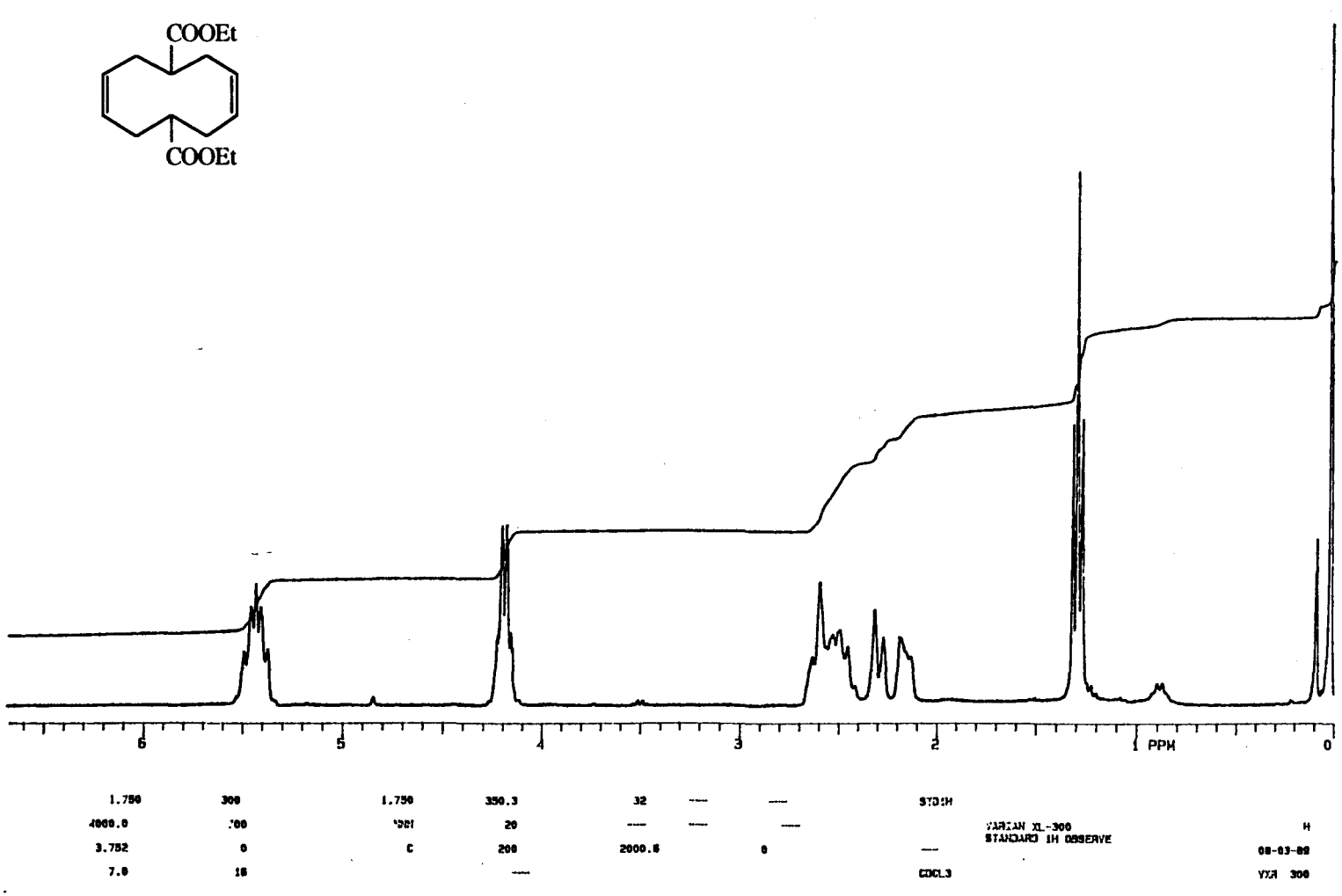
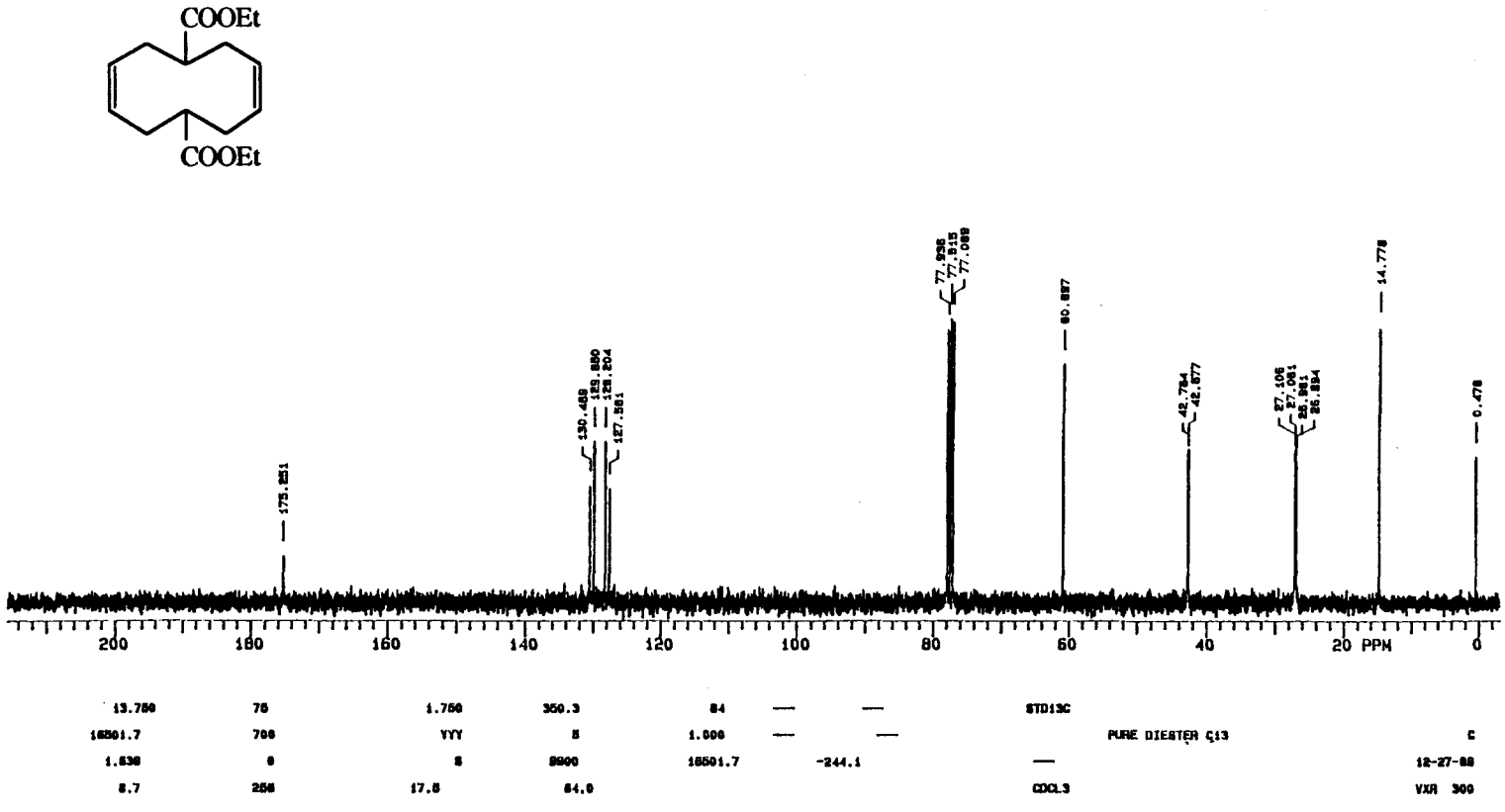
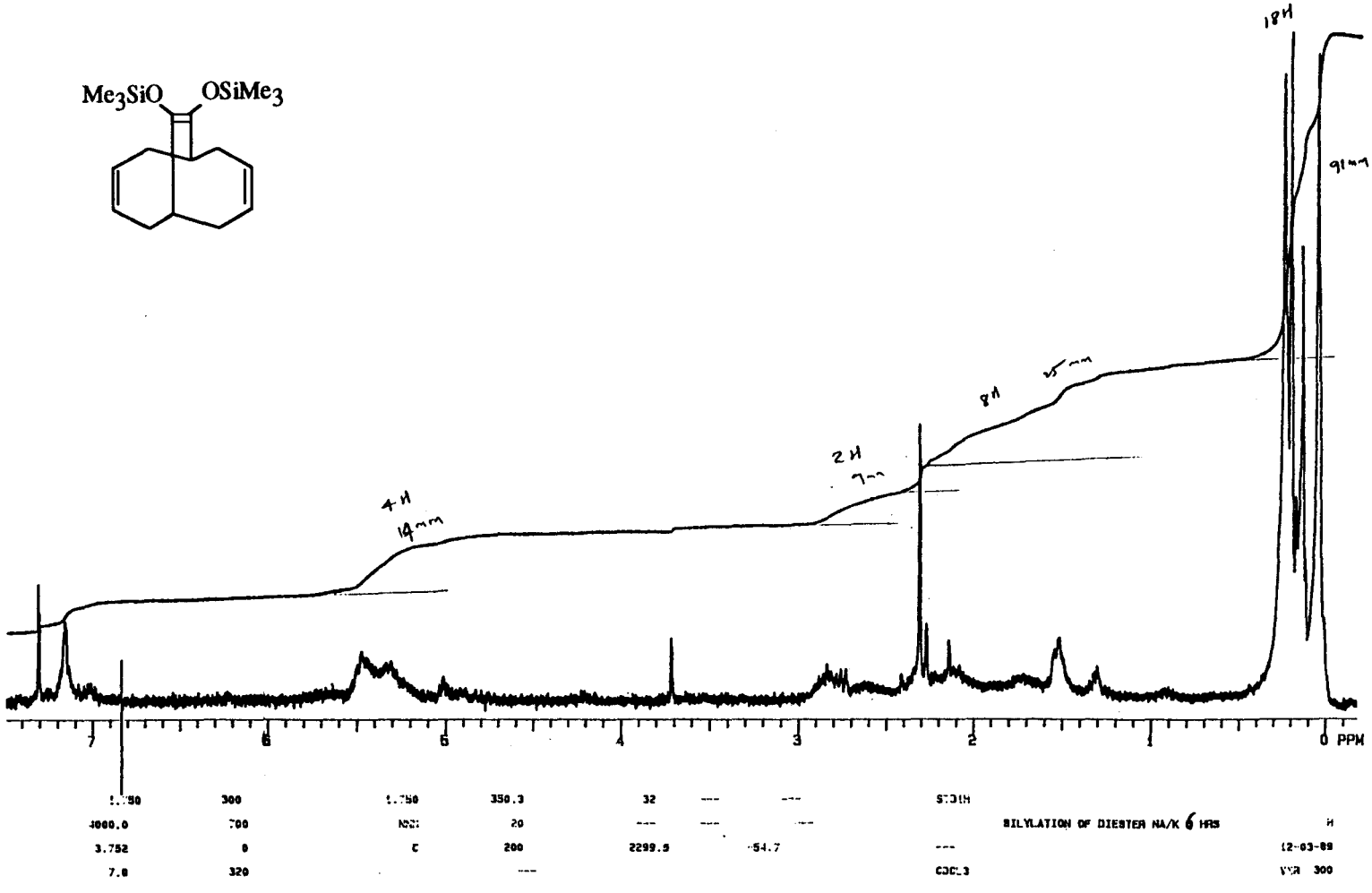


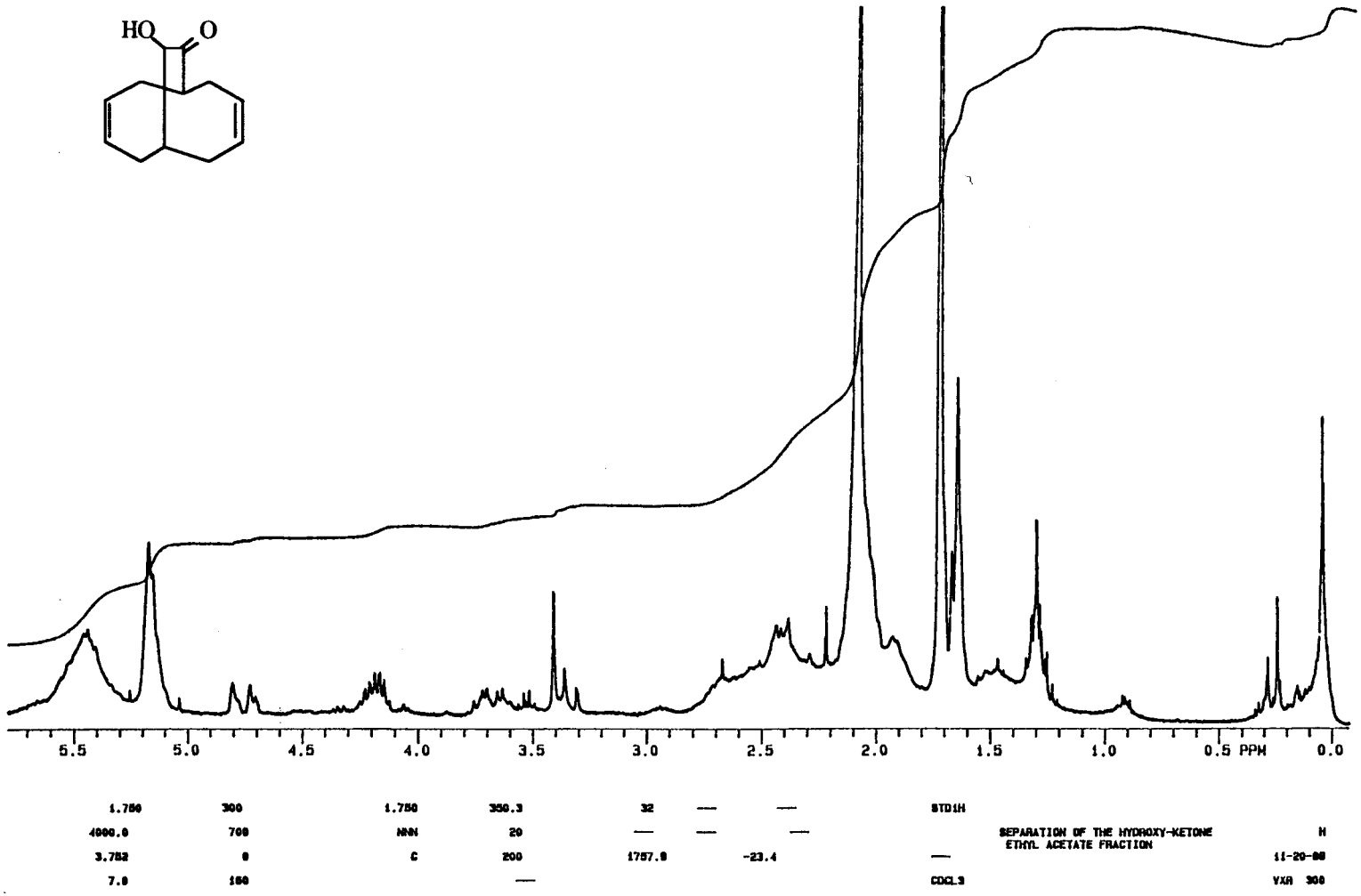
^{1}H NMR of Diethyl-trans-3,8-cyclodecadiene-1,6-dicarboxylate (**5**)



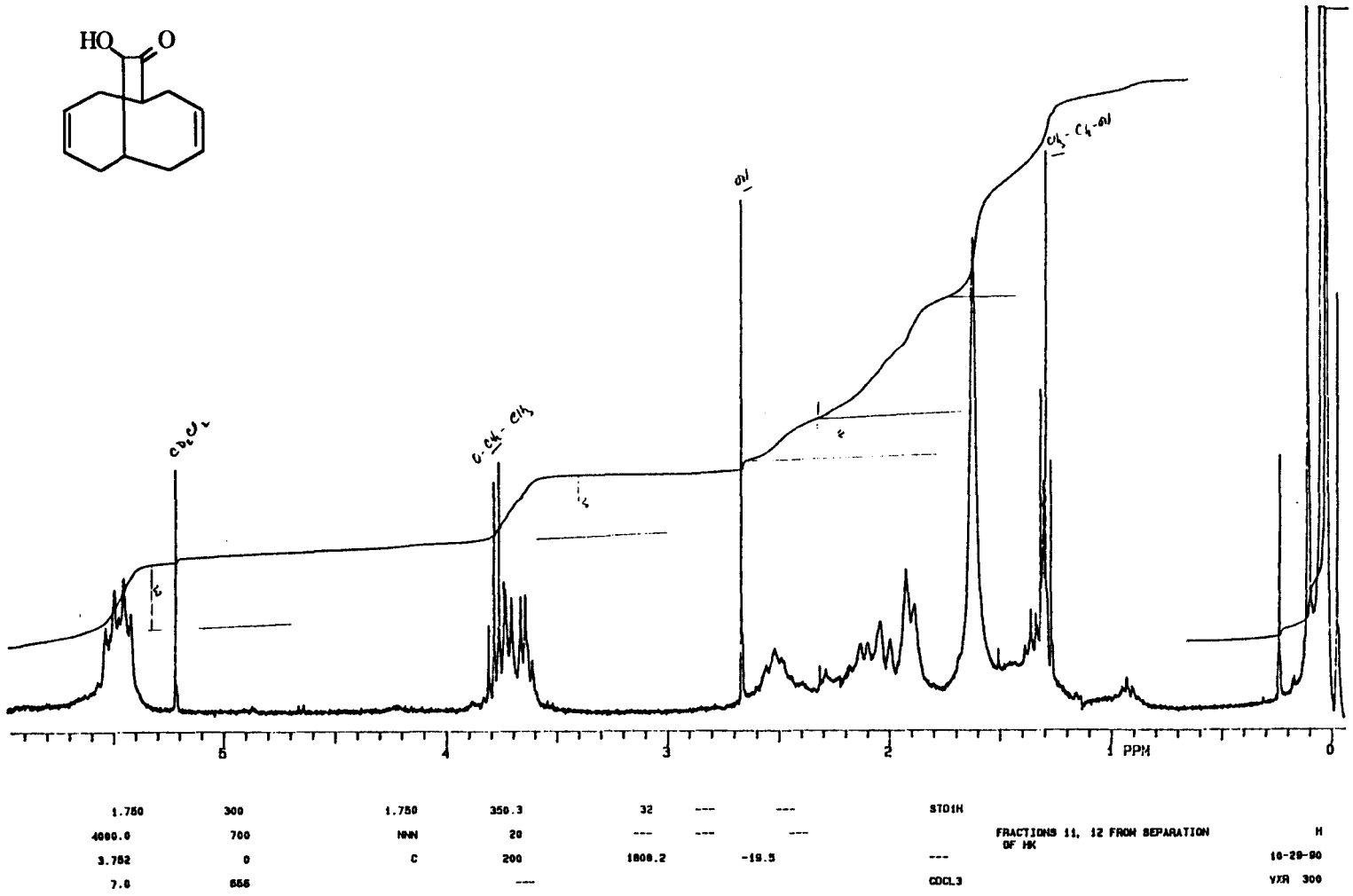
^{13}C NMR of Diethyl-trans-3,8-cyclodecadiene-1,6-dicarboxylate (**5**)



^{1}H NMR of Bicyclo[4.4.2]dodeca-11,12-bis(trimetylsilyloxy)-3,8,11-triene (**83**)

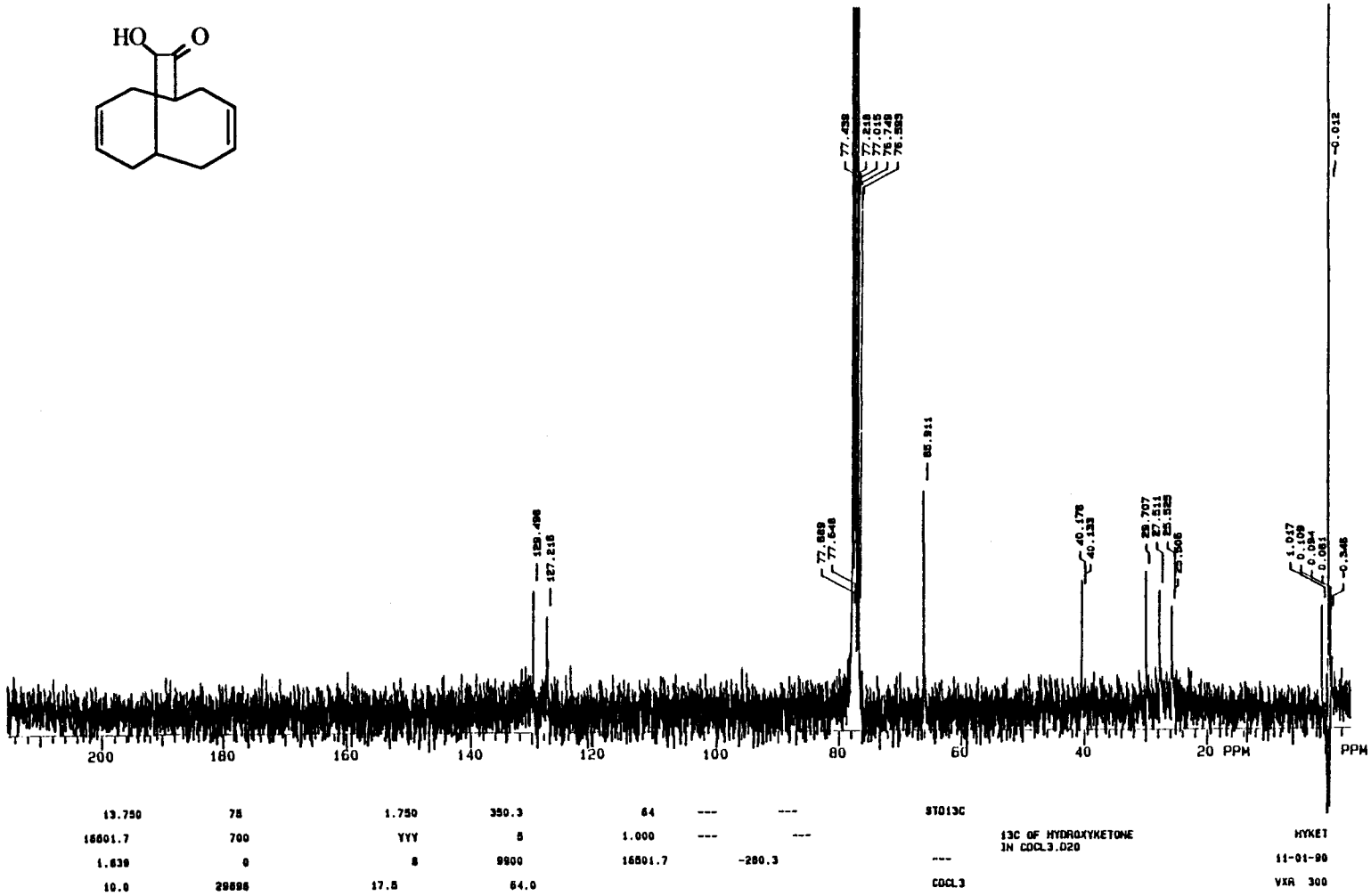


^{1}H NMR of impure Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**) obtained by hydrolysis of bicyclo[4.4.2]dodeca-11,12-bis(trimetylsilyloxy)-3,8,11-triene

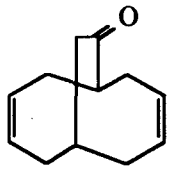


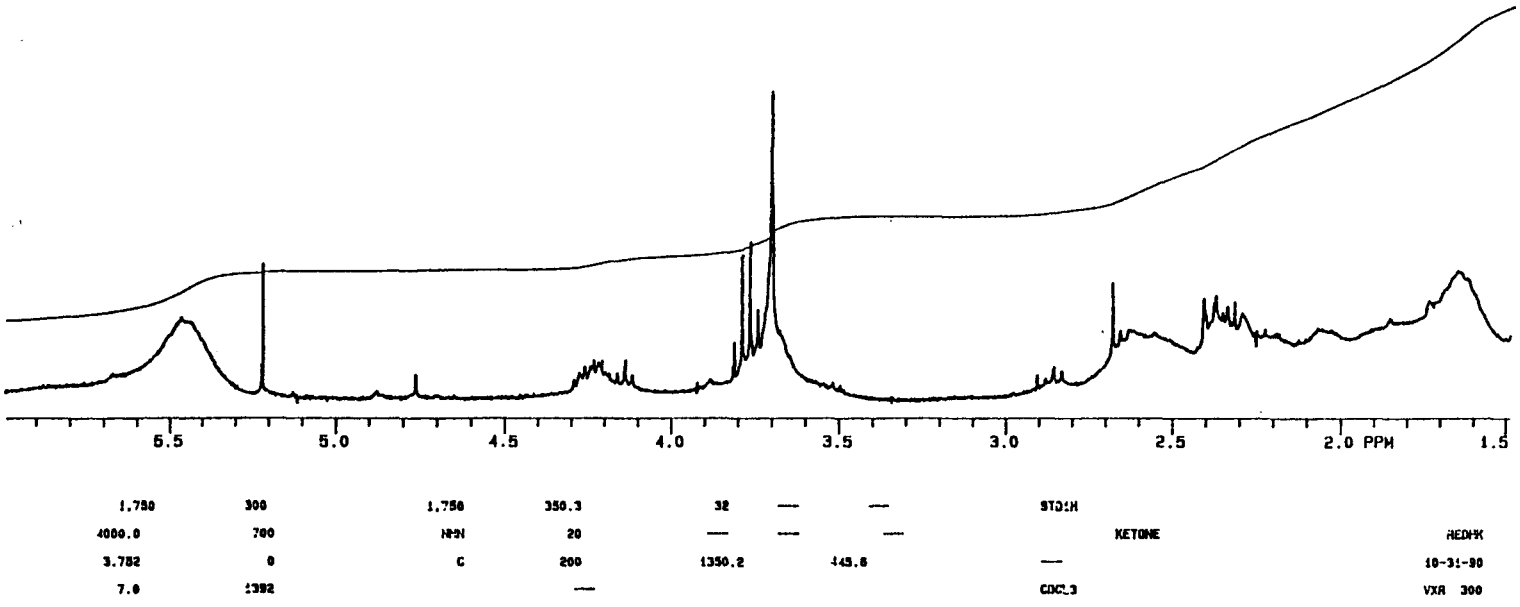
^{1}H NMR of Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**)

(Contains a trace of ethanol from $CDCl_3$)

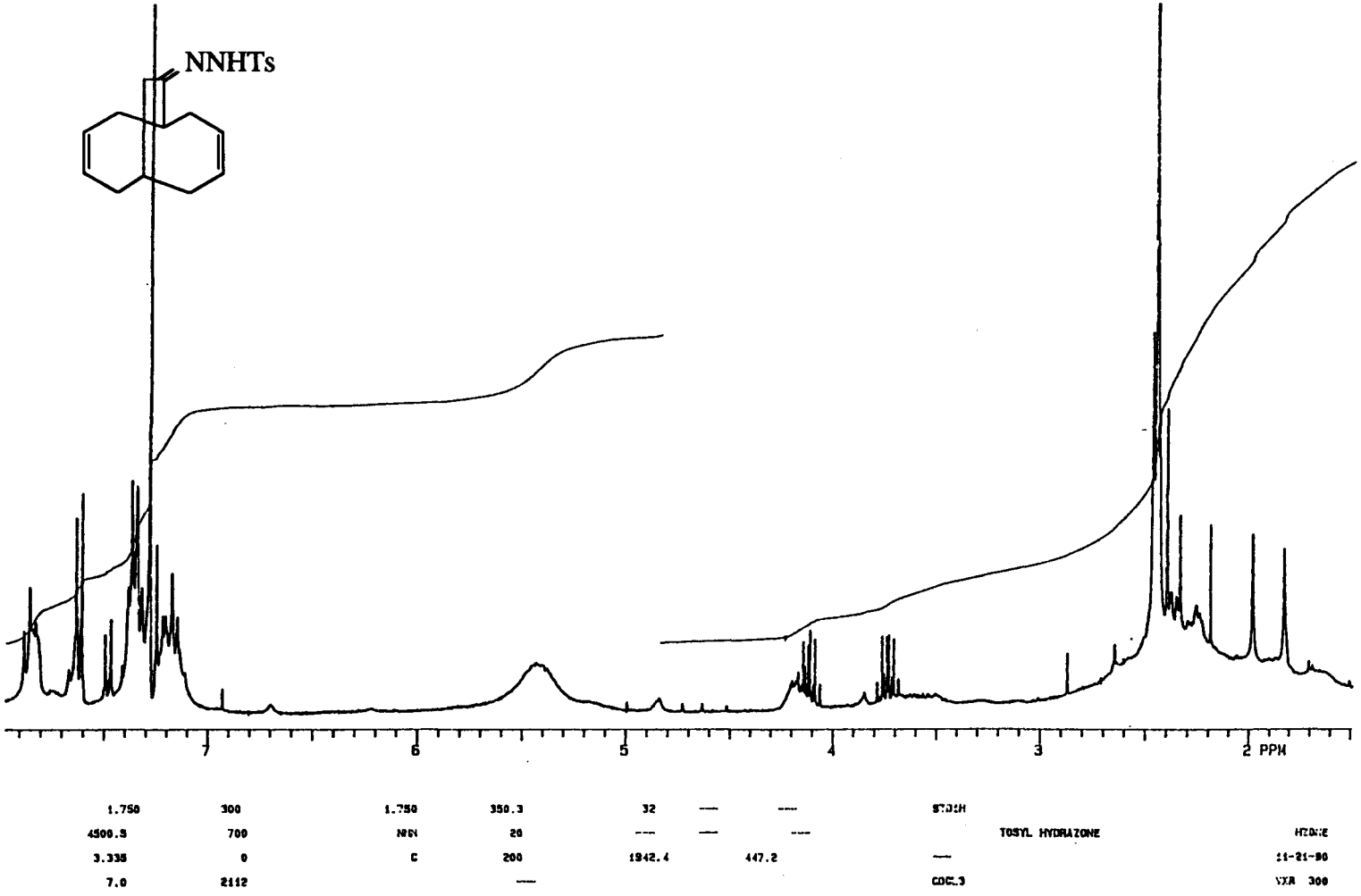


^{13}C NMR of pure Bicyclo[4.4.2]dodeca-11-hydroxy-3,8-diene-12-one (**6**)

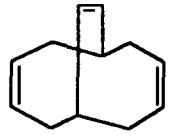


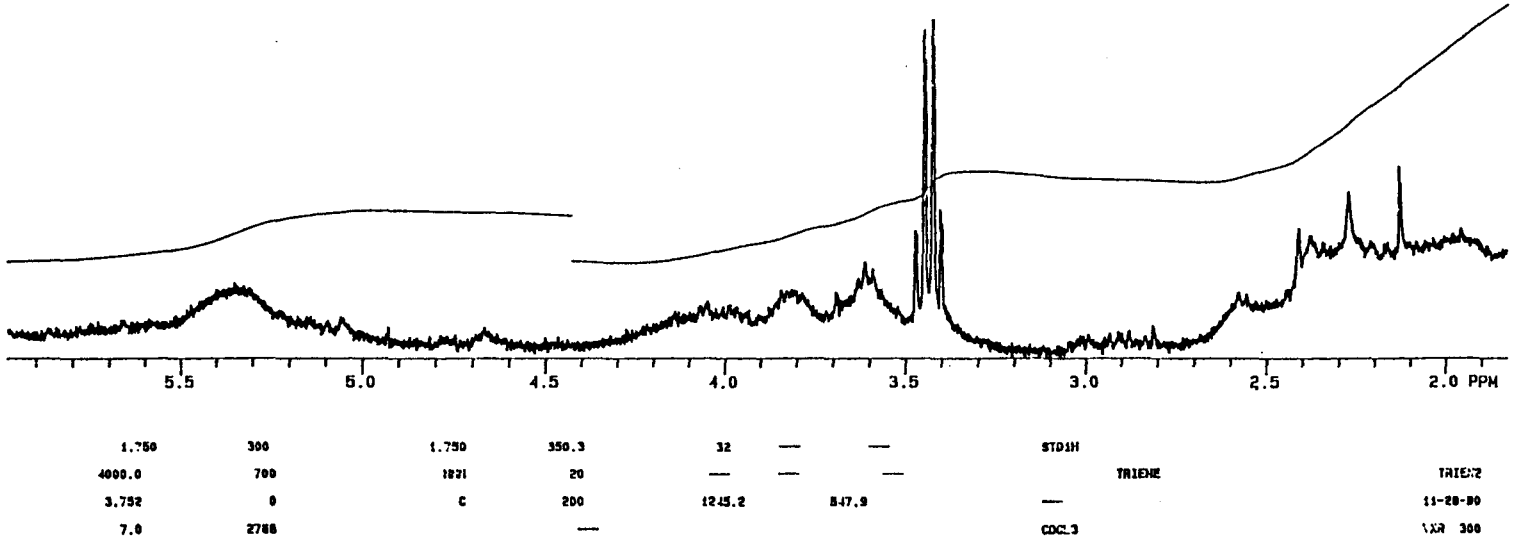


^{1}H NMR of Bicyclo[4.4.2]dodeca-3,8-diene-12-one (7)



^{1}H NMR of Bicyclo[4.4.2]dodeca-3,8-diene-12-tosyl-hydrazone (**8**)





^{1}H NMR of Bicyclo[4.4.2]dodeca-3,8,11-triene (**1**)

REFERENCES

1(a). Curl, R. F.; Smalley, R. E. Scientific America, **1991**, 54. (b). Edelson, E. Popular Science, **1991**, 52.

2. Barborak, J. C.; Watts, L.; Pettit, R. J. Am. Chem. Soc., **1966**, 88, 1328.
3. Wiberg, K. B.; Lowry, B. R.; Colby, T. H. J. Am. Chem. Soc., **1961**, 83, 1398.
4. Katz, T. J., Acton, N. J. Am. Chem. Soc., **1973**, 95, 2738.
5. Tamelen, E. E.; Pappas, S. P.; Kirk, K. L. J. Am. Chem. Soc., **1971**, 93, 6092.
6. Vogel, E. and Gunther, H. Angew. Chem. Int. Ed., **1967**, E6, 385.
7. Doering, W.E. and Rosenthal, J. W., Tet. Lett., **1967**, 349.
8. Jones, M. and Reich, S. D. J. Am. Chem. Soc., **1967**, 89, 3935.
9. Jones, M., J. Am. Chem. Soc., **1967**, 89, 4236.
10. Paquette L. A. and Stowell J. C., J. Am. Chem. Soc., **1971**, 93, 5735.
11. Lobows, J. N.;Meiwald, J.; Rottele, H. and Schroder, G. J. Am. Chem. Soc., **1967**, 89, 612.
12. Erhardt U. and Daub J., J. Chem. Soc.: Chem. Comm., **1974**, 83.
13. Paquette L. A. and Kekula M. J., J. Chem. Soc.:Chem. Comm., **1973**, 409.
14. Paquette, L.A.; Wingard, R. E.; Philips, J. C.; Tompson, G. L.; Read, L.K. and Clardy, J. J. Am. Chem. Soc., **1971**, 93, 4508.
15. Paquette L. A.; Philips, J. C. and Wingard, R. E., J. Am. Chem. Soc.,**1971**, 93, 4516.
16. Paquette L.A.; Meisinger, R. H. and Wingard, R. E., J. Am. Chem. Soc., **1973**, 95, 2230.
17. Ginsburg D., Acc. Chem. Res., **1969**, 121.
18. Murdock K. C; Angier R. B., J. Org. Chem., **1962**, 27, 2395.
19. Gipson, R. M.; Guin, H. W.; Simonsen, S. H.; Skinner, C. G. and Shive, W., J. Am. Chem. Soc., **1966**, 88, 5366.

20. Krapcho, A. P., Synthesis, **1982**, 805.

21. Bloomfield, J. J.; Owsley, D.C.; Nelke, J. M. Org. React., **1976**, 23, 259.

22. Organic Synthesis, Vol. 57, pp. 1-7.

23. Fieser & Fieser, Reagents for Organic Synthesis; John Wiley and Sons Inc. 1967, pp. 1287-1289.

24. Shapiro, R. H., Org. React., **1972**, 94, 7118.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to the content and form.

The dissertation is, therefore, accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

15 April 1992
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