SYNTHESIS OF SUBSTITUTED 2-IODOANILINE AND 2-IODOACETANILIDE

A DISSERTATION SUBMITTED TO THE PARTIAL FULFILMENT OF THE REQUIREMENT FOR DEGREE OF MASTER OF PHILOSOPHY (M.PHIL) IN CHEMISTRY

SUBMITTED

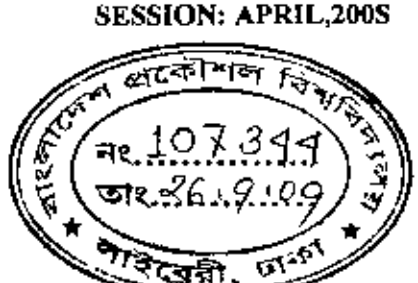
BY

MOHAMMAD MIZANUR RAHMAN

STUDENT NO. 040503103 F

REGISTRATION NO. 0405028

SESSION: APRIL,200S



ORGANIC RESEARCH LABORATORY
DEPARTMENT OF CHEMISTRY
BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET),
DHAKA-1000, BANGLADESH

September, 2009

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET), DHAKA-1000, BANGLADESH.
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THESIS ACCEPTANCE LETTER

The thesis entitled "Synthesis of substituted 2-iodoaniline and 2-iodoacetanilide" submitted by Mohammad Mizanur Rahman, Roll No. 040503103F, Registration No. 0405028, Session April, 2005 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Master of Philosophy (M.Phil) in chemistry on September 14, 2009.

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   Department of Chemistry, University of Dhaka

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET), DHAKA-1000, BANGLADESH.

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It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

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(Mohammad Mizanur Rahman)

ACKNOWLEDGMENT

All praise and admiration for almighty Allah, the most kind and merciful who enable me in carrying out the research work presented in this dissertation.

I am extremely delighted to express my deepest gratitude to my advisor and supervisor, Prof. Dr. Md. Wahab Khan, for his helpful advice and encouragement offered throughout this thesis. He has given me the chance to participate in several interesting research projects.

I am highly grateful to all my respected teachers of this department, particularly Dr. Enamul Huq, Dr. A. K. M. Matior Rahman, Dr. Md. Abdur Rashid, Dr. Monimul Huque, Dr. Manwarul Islam, Dr. Nazrul Islam, Dr.Al-Nakib Chowdhury, Dr. Shakila Rahman and Mrs. Tahera Saad for their helpful suggestions at different stages of studies in Chemistry.

I desire to offer my pleasant thanks to Dr. M.A. Rashid, Dean of Pharmacy, University of Dhaka for his kind help to carry out biological test of various synthesized compounds.

I would like to thanks Md. Kamruzzaman, Md. Abdul Bari, Md. Enamul Haque, Laila Arifun Nahar, Md. Ismail Hossain, Md. Faiaz Ahmed and Md. Mazaharul Islam for their help and co-operation in the laboratory.

I acknowledge to the University of Engineering and Technology (BUET), Dhaka and Ministry of National Science Information and Communication Technology (NSICT), Bangladesh for giving me the financial support to carry out my research work and I am also grateful to the Department of Chemistry to give me opportunity to do M.Phil program.

I convey thanks to all staffs in the Department of Chemistry, BUET for their kind co-operations. In fine, I am highly thankful to all of my well-wishers, in home and abroad.

I am also grateful to my younger sisters Rumana Binte Habib, Sumona Binte Habib, Jhumona Binte Habib and Jannatul Ferdoush Binte Habib for their constant inspiration and help during the period of my study.

There are two people to whom I owe more than I can possibly repay in lifetime, I would like to express my deepest gratitude for constant support, understanding and love that I received from them. They are my father Mohammad Habibur Rahman and my mother Farida Begum. They are my greatest advisors, confidants and supporters. In times of uncertainty, their love and guidance always steered me in the right direction.

14.09.2009

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(Mohammad Mizanur Rahman)

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LIST OF ABBREVIATIONS

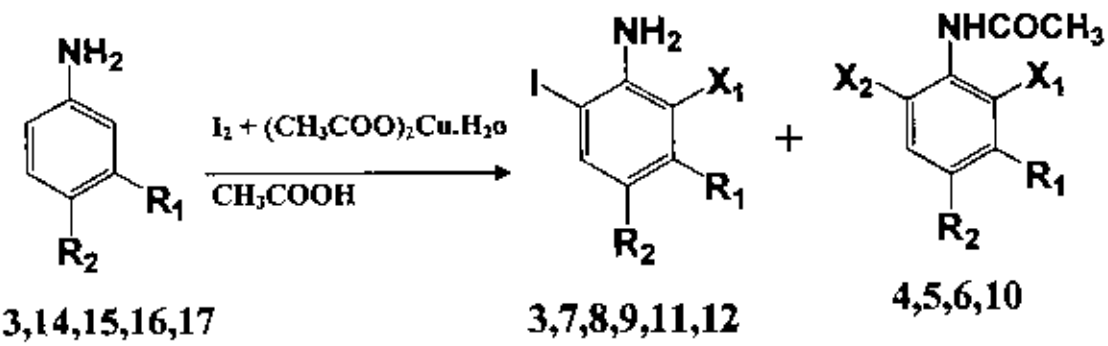
NMR	nuclear magnetic resonance
IR	infrared (spectrum)
mp.	melting point
TLC	thin layer chromatography
^0C	degree celsius
hr	hour
mmol	millimole
UV	ultra violet
J	coupling constant
Hz	hertz
s	singlet
d	doublet
dd	double doublet
t	triplet
M.F.	molecular formula
T	temperature
µg	microgram
Δ	heat at reflux
δ	chemical shift
λ_{max}	ultraviolet absorption in nm
ν_{max}	infrared absorption in per centimeter
KAN	kanamycin
mm	millimeter
cm	centimeter
mg	milligram
mL	milliliter
sq	square
lbs.	pounds
TH	thyroid hormone
T_3	triiodothyronine

T_4	thyroxine
T_1a	iodothyronamine
T_0a	thyronamine
NIS	N-iodosuccinimide
NCS	N-chlorosuccinimide
NBS	N-bromosuccinimide
GC	Gas chromatography

Title: "Synthesis of substituted 2-iodoaniline and 2-iodoacetanilide"

Abstract

In view of the extensive natural occurrence and biological importance of the 2-iodoaniline and 2-iodoacetanilide compound a general and facile method for the synthesis of aryl-iodide derivatives through iodine-cupper(II) acetate in acetic acid, afforded the corresponding iodo compound in the better yield. In this purpose different 2-iodoaniline or acetanilide compounds were synthesized from *p* or *m*-substituted aryl amines by iodination reaction (**Scheme 1**).



13,14,15,16,17 **3,7,8,9,11,12** **4,5,6,10**

Scheme 1

Where,

	Substrates		Product
13	$R_2 = CH_3$, R_1=H	**3**	X_1 = I, R_1= H, R_2 = CH_3
		4	X_1 = H, X_2 = I, R_1= H, R_2 = CH_3
		5	X_1 = H, X_2 = H, R_1= H, R_2 = CH_3
14	$R_1 = CH_3$, R_2=H	**6**	X_1 = H, X_2 = I, R_1= CH_3, R_2 = H
15	R_2 = Cl, R_1=H	**7**	X_1 = I, R_1= H, R_2 = Cl
		8	X_1 = H, R_1= H, R_2 = Cl
16	$R_2 = NO_2$, R_1=H	**9**	X_1 = I, R_1= H, R_2 = NO_2
		10	X_1 = I, X_2=H, R_1= I, R_2 = NO_2
		11	X_1 = I, R_1= H, R_2 = NO_2
17	R_1 = OH, R_2=H	**12**	X_1 = H, R_1= OH, R_2 = H

All the synthesized compounds were characterized by using analytical data obtained from M.P., UV, IR, ^{1}H NMR and ^{13}C NMR.

SUMMARY

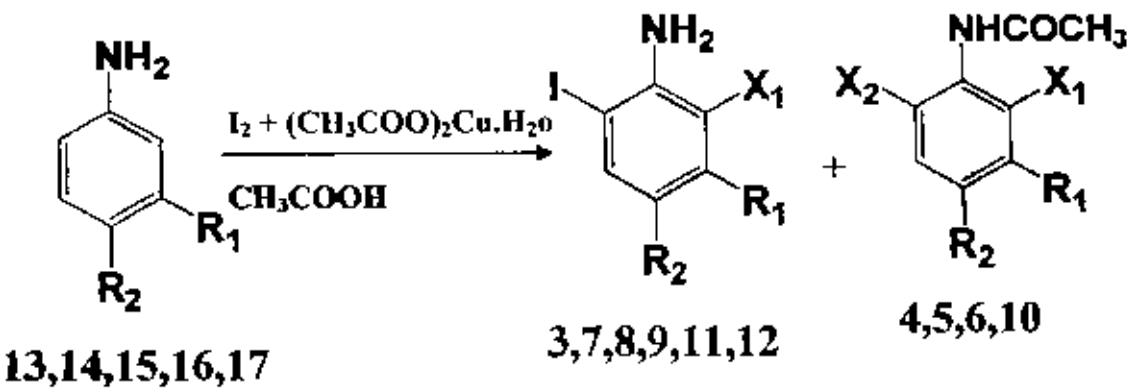
Investigation incorporated in this dissertation titled "Synthesis of substituted 2-iodoaniline and 2-iodoacetanilide" have been presented in four chapters. The first is the introductory section, in which the background, biological action and the important synthesis are presented. Chapter two deals with results and discussion of the synthesis of substituted aryl iodide. Chapter three was deals with the detailed methodologies and experimental procedures for the synthesis of 2-substituted aniline or acetanilide derivatives, spectra and references. Chapter four also deals with the biological test of the synthesized products.

Chapter I

It represents the importance and synthesis of aryl iodide derivatives. Aryl iodide is the first door of synthesis to biological and pharmaceutical active compound. So it was important in synthetic and pharmaceutical chemistry. Aryl iodide presence in nature, its derivative has proved considerable interest due to their pharmacological activities. Various methods are known for the synthesis of aryl iodide derivatives but iodine-copper (II) acetate in acetic acid procedures for the synthesis of 2-iodoaniline of acetanilide derivatives are limited in number.

Chapter II

In Chapter II, result and discussion of the synthesis of 2,6-diiodo-4-methylaniline, 2-iodo-4-methylacetanilide, 4-methylacetanilide **3, 4, 5** and 2-iodo-5-methylacetanilide, 4-chloro-2,6-ditodoaniline, 4-chloro-2-iodoaniline **6, 7, 8** and also 2-iodo-4-mitroaniline, 2,3-diiodo-4-nitroacetanilide, 2,6-diiodo-4-nitroaniline, 3-amino-4-iodophenol **9, 10, 11, 12** were described as shown in the **scheme** 1.Reactions were carried out through iodine-copper (II) acetate in acetic acid at 120^0C-130^0C for 6-11hs. Structure of all of these synthesized 2-iodoaniline and 2-iodoacetanilide derivatives have been established on the basis of their UV, IR and NMR spectral evidences.



Scheme 1

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17	R_1 = OH, R_2=H	**12**	X_1 = H, R_1= OH, R_2 = H

Chapter III

In chapter III, all the experimental procedure and analytical data are reported. This chapter also contains references and spectra of the synthesized compounds.

Chapter IV

In Chapter IV, introduction, methodology, results and discussion, references and conclusion of the biological test of the synthesized compounds are presented. Nine synthesized 2-iodoaniline and 2-iodoacetanilide derivatives compounds have been tested for antimicrobial activity against five gram-positive and eight gram-negative bacteria as well as three human fungal pathogens. Among tested compound 8,9,10 exhibited relatively greater or moderate (8-25mm) inhibition of growth of the microorganism.

CHAPTER-1

INTRODUCTION

INTRODUCTION

1.1. Aromatic iodide

Aromatic iodides, known for ca.150 year, are generally more reactive than the respective bromides and chlorides. There are a considerable number of different methods, direct or indirect, for their synthesis, and they are widely used in chemical laboratories and sometimes, also in chemical industry and medicine[1]. Moreover, they are able to form a large variety of stable, aromatic polyvalent iodine compounds, which have found increasing applications in modern organic synthesis[2].

Aromatic iodo compounds are an important class of compounds in synthetic organic chemistry. They are useful for the preparation of organometallic reagents and some are potential intermediates for the synthesis of pharmaceutical and bioactive materials. They are useful in metal catalyzed coupling reactions which are widely applied in the preparation of complex molecules.

(a) Iodide

An iodide ion is an iodine atom with a −1 charge[3]. Compounds with iodine in formal oxidation state−1 are called iodides. This can include ionic compounds such as caesium iodide or covalent compounds such as phosphorus triiodide. This is the same naming scheme as is seen with chloride sand bromides. The chemical test for an iodide compound is to acidify the aqueous compound by adding some drops of acid, to dispel any carbonate ions present, then adding lead(II) nitrate, yielding a bright yellow precipitate of lead iodide. Most ionic iodides are soluble, with the exception of yellow silver iodide and yellow lead iodide. Aqueous solutions of iodide dissolve iodine better than pure water due to the formation of complex ions:

$I^-(aq) + I_2(s) \rightleftharpoons I_3^-(aq)$

The color of new triiodide ions formed is brown.

(b) Iodide as an antioxidant

Iodide can function as an antioxidant as it is a reducing species that can detoxify reactive oxygen species such as hydrogen peroxide. Over three billion years ago, blue-green algae were the most primitive oxygenic photosyntheticorganisms and are the ancestors of multicellular

eukaryoticalgae. Algae that contain the highest amount of iodine (1-3 % of dry weight) and peroxidase enzymes, were the first living cells to produce poisonous oxygen in the atmosphere[4, 5] . Therefore algal cells required a protective antioxidant action of their molecular components, in which iodides, through peroxidase enzymes, seem to have had this specific role[6,7, 8]. In fact, iodides are greatly present and available in the sea, where algal phytoplankton, the basis of marine food-chain, acts as a biological accumulator of iodides, selenium and n-3 fatty acids[9, 10, 11] .

(c) Antioxidant biochemical mechanism of iodides[12]

$2\ I^- \rightarrow I_2 + 2\ e^-$(electrons) = −0.54 Volt ;

$2\ I^- +$ Peroxidase + $H_2O_2 +$ 2 Tyrosine → 2 Iodo-Tyrosine + H_2O + 2 e^-(antioxidants);

$2\ e^- + H_2O_2 + 2\ H^+$(of intracellular water-solution) → 2 H_2O

Antioxidant biochemical mechanism of iodides, probably one of the most ancient mechanisms of defense from poisonous reactive oxygen species:

$2\ I^- +$ Peroxidase + $H_2O_2 +$ Tyrosine, Histidine, Lipids, Carbons ⟶ Iodo-Compounds + H_2O + 2 e^- (antioxidants)

Clinical Use

Iodide (>6mg/day) can be used to treat patients with hyperthyroidism due to its ability to block the release of thyroid hormone(TH), known as the Wolff-Chaikoff Effect, from the thyroid gland. In fact, prior to 1940, iodides were the predominant antithyroid agents. In large doses, iodides inhibit proteolysis of thyroglobulin. This permits TH to be synthesized and stored in colloid, but not released into the bloodstream.

Of note, this treatment is seldom used today as a stand-alone therapy despite the rapid improvement of patients immediately following administration. The major disadvantage of iodide treatment lies in the fact that excessive stores of TH accumulate, slowing the onset of action of thioamides (TH synthesis blockers). Additionally, the functionality of iodides fade after the initial treatment period. An "escape from block" is also a concern, as extra stored TH may spike following discontinuation of treatment [13].

1.2. Some Aromatic iodide compound and their importance.

(a) The Halogenated hydroxyquinolines[14]

Diiodohydroxyquin (Iodoquinol), Iodochlorhydroxyquin (Clioquinol). The halogenated hydroxyquinolines were among the first synthetic drugs active in amebiasis. Iodochlorhydroxyquin (introduced in 193 1) and diiodohydroxyquin (introduced in 1936) are effective against organisms in the bowel lumen but not against trophozoites in the intestinal wall or extraintestinal tissues.

Iodoquinol, also known generically as Diiodohydroxyquinon. Iodine is the active ingredient, used for generations as a treatment for amoebic infections and similar large cell organisms, fungi, algae and protozoa."

(i) Chemistry

Halogen-substituted 8-hydroxyquinolines have had extensive clinical use, chiniofon (8-hydroxy-7-iodoquinoline-5-sulfoniacc id), iodachlorhydroxyquin (clioquinol) (5-chloro-8-hydroxy-7-iodoquinoline) and diiodohydroryquin (iodoquinol) (8-hydroxy-5,7-diiodoquinoline). Iodochlorhydroxyquin contains approximately 408 iodine and 12% chlorine, and diiodohydroxyquin contains approximately 64% iodine **(Fig. 1)**.

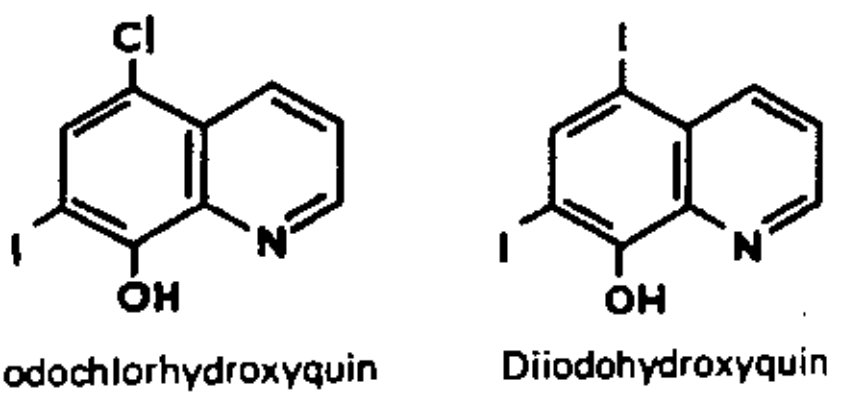


Figure 1: Structure of Iodochlorhydroxyquin and Diiodohydroxyquin

Absorption, Metabolism & Excretion knowledge is incomplete on the pharmacokinetics of the hydroxyquinolines. Iodochlorhydroxyquin is more readily absorbed than diiodohydroxyquin. Metabolic studies in humans using 14C iodochlorhydroxyquin indicated that maximal plasma

concentrations were reached at 4 hr after administration of a single dose and then decreased, with an apparent half-life of between 11 and 14 hr. Approximately 25% of a single 750-mg oral dose was excreted in the urine over 72 hr. Use of radioactive iodochlorhydroxyquin in animals showed high uptake of the drug in visceral tissues. The drugs may interfere with certain thyroid function tests by increasing protein-bound serum iodine levels, leading to a decrease in ^{131}I uptake.

(ii) Antiamebic Effects

The mechanism of action of diiodohydroxyquin and iodochlorhydroxyquin against amebas is not known. Opinions vary on whether the drugs act only against trophozoites or against cysts as well.

(iii) Clinical Uses

A. Intestinal Amebiasis: Diiodohydroxyquin and iodochlorhydroxyquin are alternative drugs for the treatment of asymptomatic or mild to moderate intestinal amebiasis. However, until the question of the association of iodochlorhydroxyquin with the **SMON** syndrome is resolved, only diiodohydroxyquin should be used in therapy. The drugs are not effective in the initial treatment of severe intestinal disease but are used in the subsequent 2radication of the infection. They are not effective against amebomas or extraintestinal forms of the disease, including hepatic amebiasis.

(b) 123I-labeled bicyclic nucleoside analogue (BCNA)[15]

An iodine-123 labeled bicyclic nucleoside analogue ([123I]-4) **(Fig. 2)** has been synthesized and evaluated as a potential single photon emission tomography (SPECT) reporter probe for the non-invasive imaging of expression of the varicella zoster virus thymidine kinase (VZV-tk) reporter gene. In vitro enzymatic assays revealed that the non-radioactive mono-iodo derivative 4 has good affinity for VZV-TK (IC50: 4.2 μM). Biodistribution of [123I]-4 was examined in normal mice. Evaluation of [123I]-4 in HEK-293T cells showed 1.74-fold higher accumulation in VZV-TK-expressing cells compared to control cells.

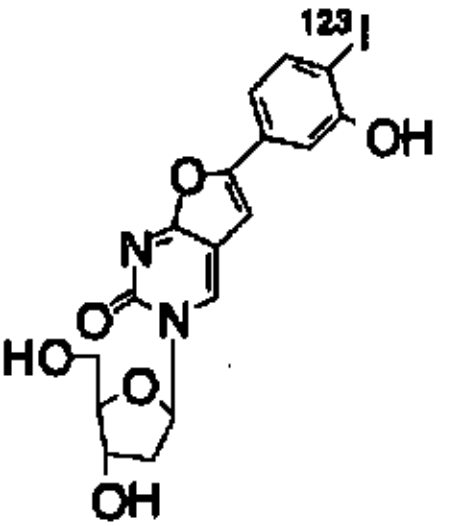


Figure 2: 123I-labeled bicyclic nucleoside analogue (BCNA)

(c) Thyroid hormones[16,17,18]

(1) Thyroxine

Thyroxine **(Fig. 3,4)**, or 3, 5, 3', 5'-tetraiodothyronine (often abbreviated as T_4), a form of thyroid hormones is the major hormone secreted by the follicular cells of the thyroid gland.

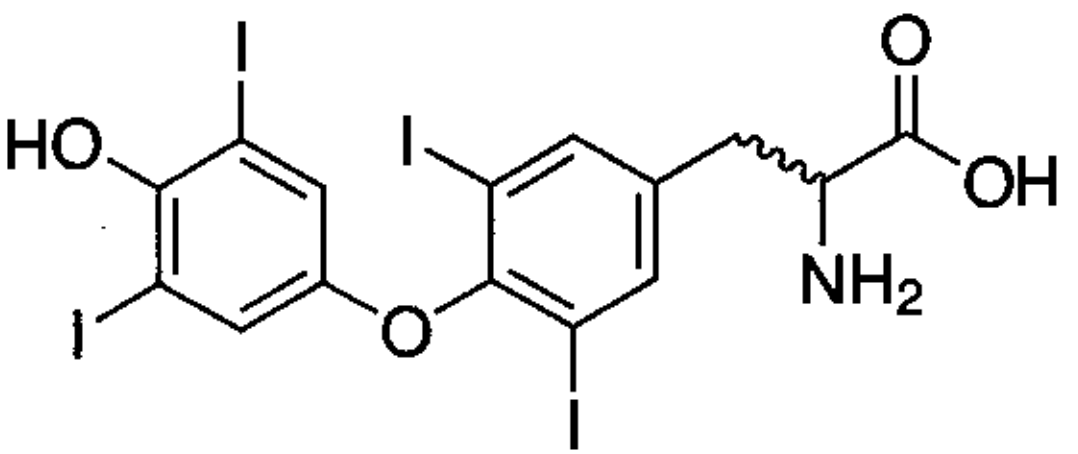


Figure 3: Structure of Thyroxine (3, 5, 3', 5'-tetraiodothyronine)

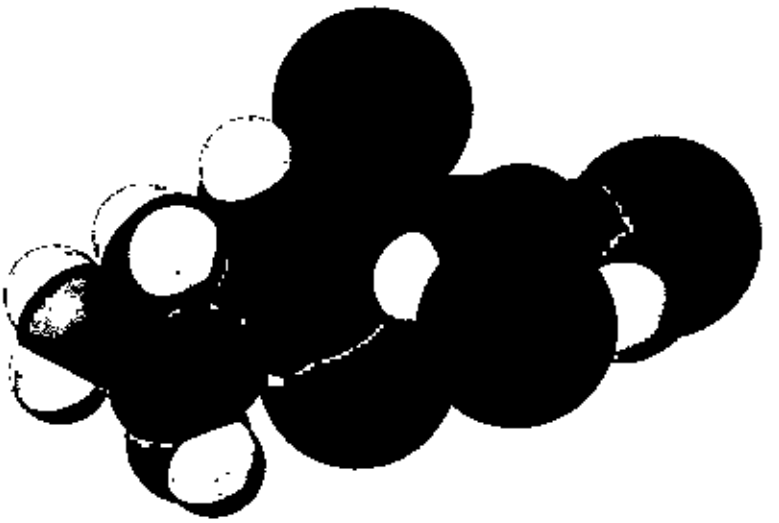


Figure 4: Structure of 3D- vdW model of Thyroxine, T_4.

T_4 is transported in blood, with 99.95% of the secreted T_4 being protein bound, principally to thyroxine-binding globulin (TBG), and, to a lesser extent, to transthyretin and serum albumin. T_4 is involved in controlling the rate of metabolic processes in the body and influencing physical development. Administration of thyroxine has been shown to significantly increase the concentration of nerve growth factor in the brains of adult mice.

Thyroxine is a prohormone and a reservoir for the active thyroid hormone triiodothyronine (T_3) which is about four times more potent. T_4 is converted in the tissues by deiodinases to T_3. The half-life of thyroxine once released into the blood circulatory system is about 1 week.

(2) Triiodothyronine (T_3)

Triiodothyronine (T_3) **(Fig. 5, 6, 7)**, are tyrosine-based hormones produced by the thyroid gland. An important component in the synthesis of thyroid hormones is iodine. The major form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half life than T_3. The ratio of T_4 to T_3 released in the blood is roughly 20 to 1. Thyroxine is converted to the active T_3 (three to four times more potent than T_4) within cells by deiodinases (5'-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T_1a) and thyronamine (T_0a).

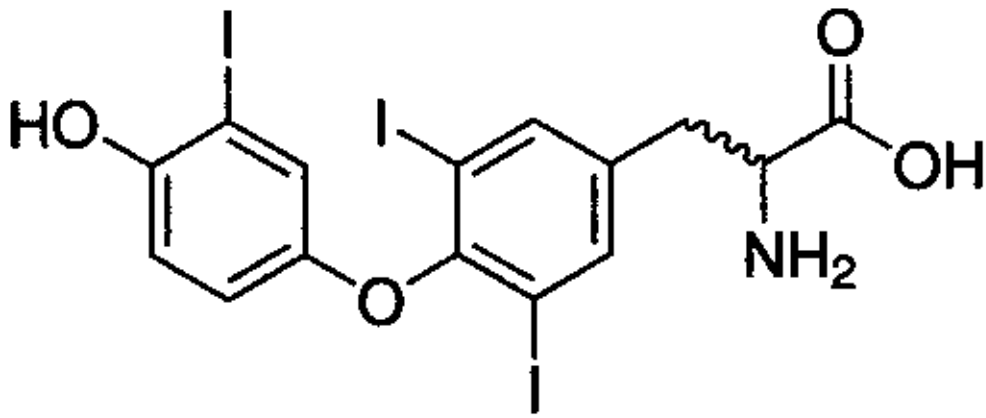


Figure 5: Structure of Triiodothyronine (T_3).

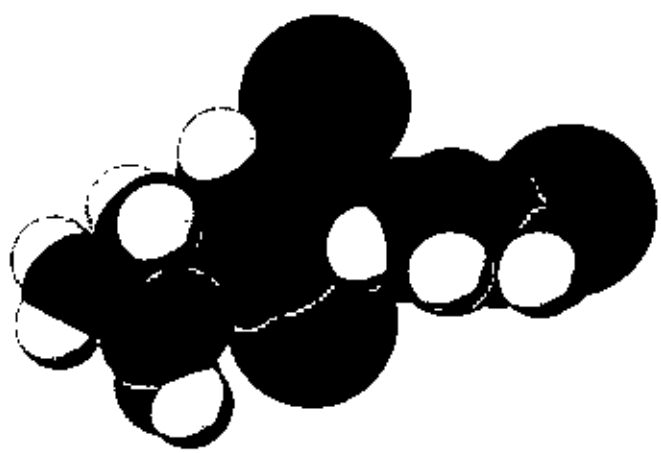


Figure 6: 3D structure of Triiodothyronine, T_3 (3, 3', 5-triiodo-L-thyronine).

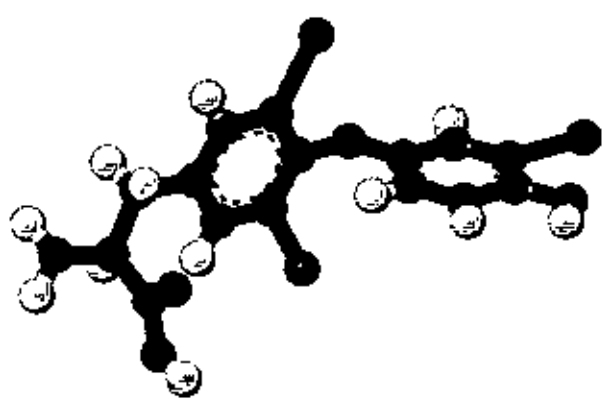


Figure 7: 3D-balls structure of Triiodothyronine, T_3 (3, 3', 5-triiodo-L-thyronine).

(i) Circulation

Most of the thyroid hormone circulating in the blood is bound to transport proteins. Only a very small fraction of the circulating hormone is free (unbound) and biologically active, hence measuring concentrations of free thyroid hormones is of great diagnostic value.

When thyroid hormone is bound, it is not active, so the amount of free T_3/T_4 is what is important. For this reason, measuring total thyroxine in the blood can be misleading (**Table 1**).

Table 1: Measuring total thyroxine in the blood.

Type	Percent
bound to thyroxine-binding globulin (TBG)	70%
bound to transthyretin or "thyroxine-binding prealbumin" (TTR or TBPA)	10-15%
paraalbumin	15-20%
unbound T_4 (fT_4)	0.03%
unbound T_3 (fT_3)	0.3%

T_3 and T_4 cross the cell membrane, probably via amino acid importins, and function via a well-studied set of nuclear receptors in the nucleus of the cell, the thyroid hormone receptors.

T_1a and T_0a are positively charged and do not cross the membrane; they are believed to function via the trace amine-associated receptor TAAR1 (TAR1, TA1), a G-protein-coupled receptor located in the cell membrane.

Another critical diagnostic tool is measurement of the amount of thyroid-stimulating hormone (TSH) that is present.

(ii) Function

The thyronines act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone), neuronal maturation and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also

stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis.

Thyroid hormone leads to heat generation in humans. However, the thyronamines function via some unknown mechanism to inhibit neuronal activity; this plays an important role in the hibernation cycles of mammals and the moulting behaviour of birds. One effect of administering the thyronamines is a severe drop in body temperature.

(iii) Related diseases

Both excess and deficiency of thyroxine can cause disorders.

- Thyrotoxicosis or hyperthyroidism (an example is Graves Disease) is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. It is a common disorder that affects approximately 2% of women and 0.2% of men.

- Hypothyroidism (an example is Hashimoto's thyroiditis) is the case where there is a deficiency of thyroxine, triiodiothyronine, or both.

- Clinical depression can sometimes be caused by hypothyroidism. Some research has shown that T_3 is found in the junctions of synapses, and regulates the amounts and activity of serotonin, norepinephrine, and Gamma-aminobutyric acid (GABA) in the brain.

(iv) Medical use of thyroid hormones

Both T_3 and T_4 are used to treat thyroid hormone deficiency (hypothyroidism). They are both absorbed well by the gut, so can be given orally. Levothyroxine, the most commonly used synthetic thyroxine form, is a stereoisomer of physiological thyroxine, which is metabolised more slowly and hence usually only needs once-daily administration. Natural desiccated thyroid hormones, also under the commercial name Armour Thyroid, is derived from pig thyroid glands, it is a "natural" hypothyroid treatment containing 20% T_3 and traces of T_2, T_1

and calcitonin. Also available are synthetic combinations of T3/T4 in different ratios (such as Thyrolar) and pure-T_3 medications (Cytomel).

Thyronamines have no medical usages yet, though their use has been proposed for controlled induction of hypothermia which causes the brain to enter a protective cycle, useful in preventing damage during ischemic shock.

Synthetic thyroxine was first successfully produced by Charles Robert Harington and George Barger in 1926.

(v) Production of the thyroid hormones

Thyroid hormones (T_4 and T_3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotrophs of the anterior pituitary gland. Because the effects of T_4 in vivo are mediated via T_3 (T_4 is converted to T_3 in target tissues; T_3 is 3- to 5- fold more active than T_4).

Thyroxine (3,5,3',5'-tetraiodothyronine) is produced by follicular cells of the thyroid gland. It is produced as the precursor thyroglobulin (this is *not* the same as TBG), which is cleaved by enzymes to produce active T_4.

Thyroxine is produced by attaching iodine atoms to the ring structures of tyrosine molecules. Thyroxine (T_4) contains four iodine atoms. Triiodothyronine (T_3) is identical to T_4, but it has one less iodine atom per molecule.

Iodide is actively absorbed from the bloodstream by a process called iodide trapping. In this process, sodium is cotransported with iodide from the apical side of the membrane into the cell and then concentrated in the thyroid follicles to about thirty times its concentration in the blood. Via a reaction with the enzyme thyroperoxidase, iodine is bound to tyrosine residues in the thyroglobulin molecules, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). Linking two moieties of DIT produces thyroxine. Combining one particle of MIT and one particle of DIT produces triiodothyronine.

- DIT + MIT → r-T_3 (biologically inactive)
- MIT + DIT → triiodothyronine (usually referred to as T_3)
- DIT + DIT → thyroxine (referred to as T_4)

Proteases digest iodinated thyroglobulin, releasing the hormones T_4 and T_3, the biologically active agents central to metabolic regulation. Thyroxine is supposedly a prohormone and a reservoir for the most active and main thyroid hormone T_3. T_4 is converted as reqnired in the tissues by deiodinases. Deficiency of deiodinase can mimic an iodine deficiency. T_3 is more active than T_4 and is the final form of the hormone, thongh it is present in less qnantity than T_4.

(vi) Effect of iodine deficiency on thyroid hormone synthesis

If there is a deficiency of dietary iodine, the thyroid will not be able to make thyroid hormone. The lack of thyroid hormone will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid stimnlating hormone, which canses the thyroid to enlarge (goiter). This has the effect of increasing the thyroid's ability to trap more iodide, compensating for the iodine deficiency and allowing it to produce adequate amounts of thyroid hormone.

(vii) Effects of thyroxine

- Increases cardiac output
- Increases heart rate
- Increases ventilation rate
- Increases basal metabolic rate
- Potentiates the effects of catecholamines (i.e increases sympathetic activity)
- Potentiates brain development
- Thickens endometrium in females

(3) 3-Iodothyronamine (T_1MA)

3-iodothyronamine (T_1AM) is an endogenous thyronamine **(Fig. 8)**. T_1AM is a high-affinity ligand for the trace amine-associated receptor TAAR1 (TAR1, TA1), a G protein-coupled receptor[19,20]. When injected into rodents, T_1AM causes a rapid drop in body temperature and alterations in blood pressure and heart rate[21].

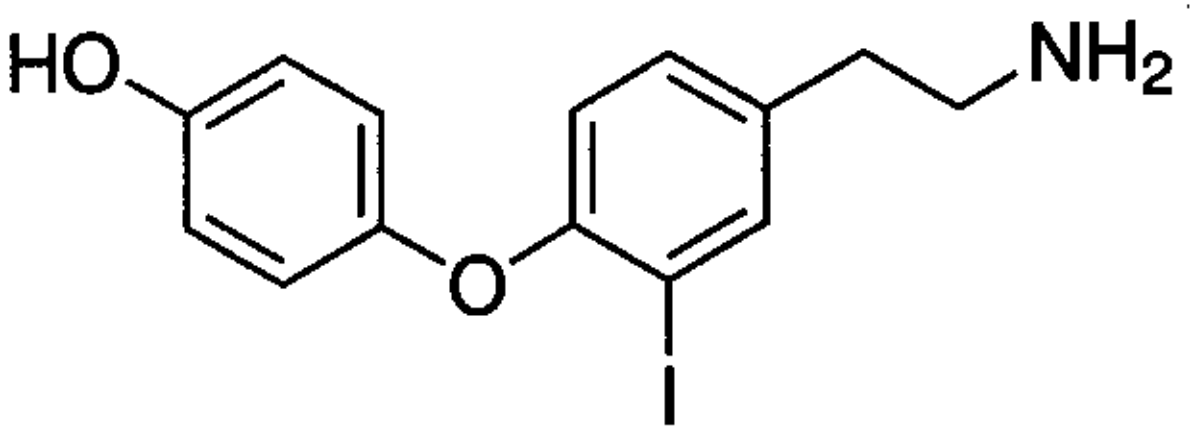


Figure 8: Structure of 3-iodothyronamine (T_1AM)

T_1AM may be part of a signaling pathway to modulate cardiac function as the compound can induce negative inotropic effects and decrease cardiac output[22].

(4) Monoiodotyrosine

Monoiodotyrosine **(Fig. 9)** an iodinated amino acid and a precursor of thyroid hormone. Two units can combine to form 3, 3'-diiodothyronine. It is abbreviated "MIT"[23].

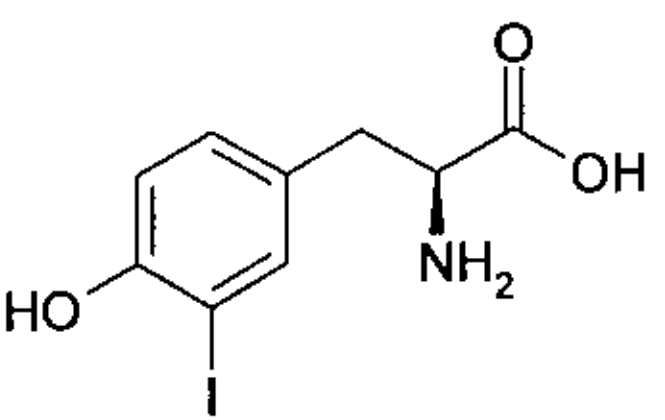


Figure 9: Structure of Monoiodotyrosine

(5) Diiodotyrosine

Diiodotyrosine **(Fig. 10)** is a modulator of thyroid peroxidase[24].

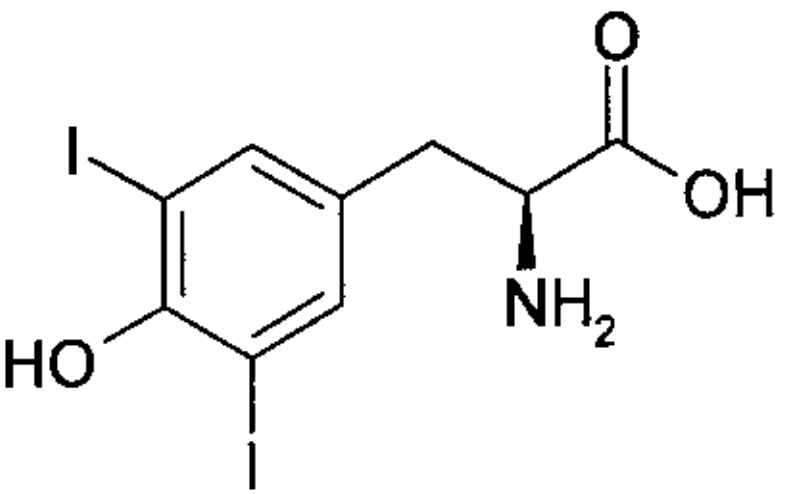


Figure 10: Structure of Diiodotyrosine.

(6) 3,3'-Diiodothyronine

3, 3'-Diiodothyronine **(Fig. 11)** is a metabolite of thyroid hormone. It is formed from the breakdown of tri-iodothyronine (T_3) **(Scheme 2)**. Levels can be affected in certain disease states[25].

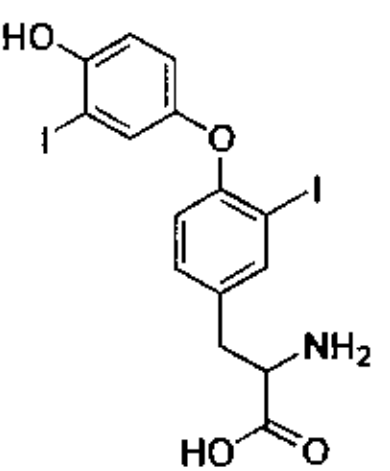
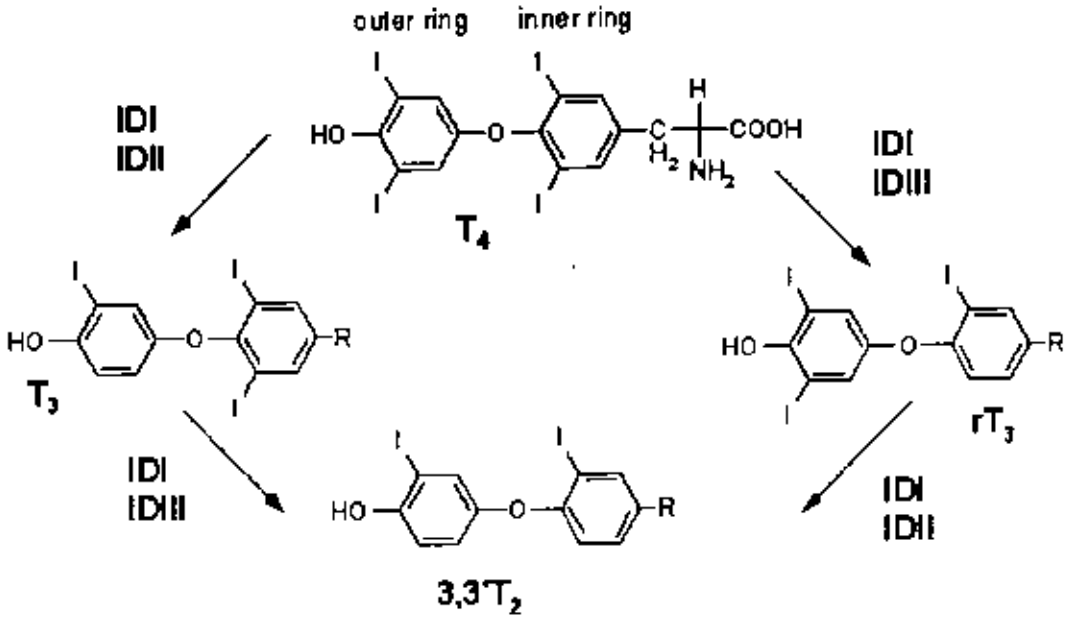


Figure 11: Structure of 3,3'-Diiodothyronine.



Scheme 2: 3, 3'-Diiodothyronine made from T_4 (Thyroxine).

(7) 2-[4-(4-hydroxy-3-iodophenoxy)-3, 5-diiodophenyl]acetic acid[26]

2-[4-(4-hydroxy-3-iodophenoxy)-3, 5-diiodophenyl]acetic acid (**Fig. 12**) was use as a Thyroid hormone receptor beta-1.

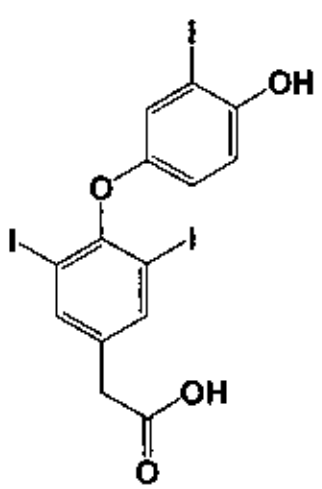


Figure 12: Structure of [4-(4-Hydroxy-3-Iodo-Phenoxy)-3, 5-Diiodo-Phenyl]-Acetic Acid.

Function of this compounds:

1. Thyroid hormone receptor activity
2. Signal transducer activity
3. Receptor activity
4. Ligand-dependent nuclear receptor activity
5. Steroid hormone receptor activity
6. Binding
7. Nucleic acid binding
8. DNA binding
9. Transcription factor activity

1.3. Synthesis of organic iodo-compound and their derivatives by various methods:

Iodination of aromatic compounds has been the subject of numerous studies due to the interest of iodo derivatives as substrates for reactions involving C-C bond formation mainly mediated by transition metals. These derivatives have been used in the synthesis of many interesting natural products and bioactive compounds. Concerning iodination procedures, the moderate reactivity of iodine with aromatic substrates determined the use of different activating agents to effectively succeed in the goal. Iodonium donating reagents and other more sophisticated procedures have also been employed. The wide range of methods described so far revealed the lack of an efficient and general enough procedure.

1.3a. Iodination of Methoxybenzenes with N-Iodosuccinimide in Acetonitrile

In 1996, M. C. Carreno *et al.*[27] reported that N-iodosuccinimide (NIS) as iodination agent. The ability of NIS to achieve nuclear iodination of activated aromatic compounds under very mild conditions and in good to excellent yields. Whereas NCS (N-chlorosuccinimide) and NBS (N-bromosuccinimide) have been extensively used for many years as halogenating agents for aromatic substrates under different conditions, aromatic iodinations using N-iodoamides have been used in a lesser extent.

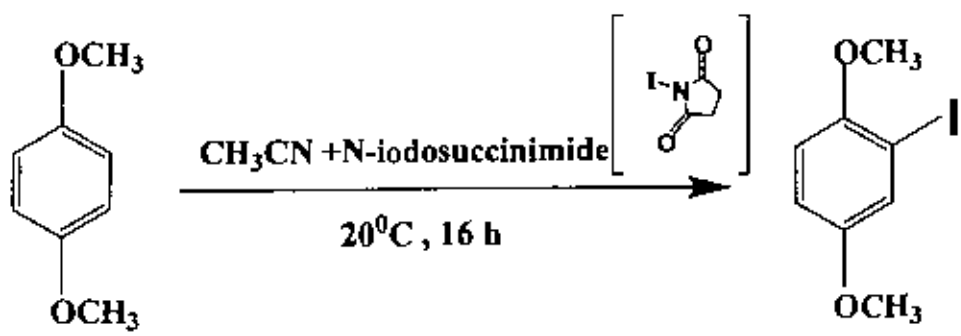
Methoxy aromatic derivatives used in this study were commercially available. These compounds were submitted to reaction with NIS in CH_3CN at different temperatures. The results are collected in the **Table 2.**

Representative procedure

To a solution of 1 mmol of the aromatic compound in 4 ml of CH_3CN, 1.5 mmol NIS was added and the reaction was stirred at the desired reaction temperature. After the time required in each case, the solvent was evaporated and ether added. The etheral phase was washed with aqueous $NaHSO_3$ solution followed by water. The ether layer was dried over $MgSO_4$ and evaporated to give the crude compound.

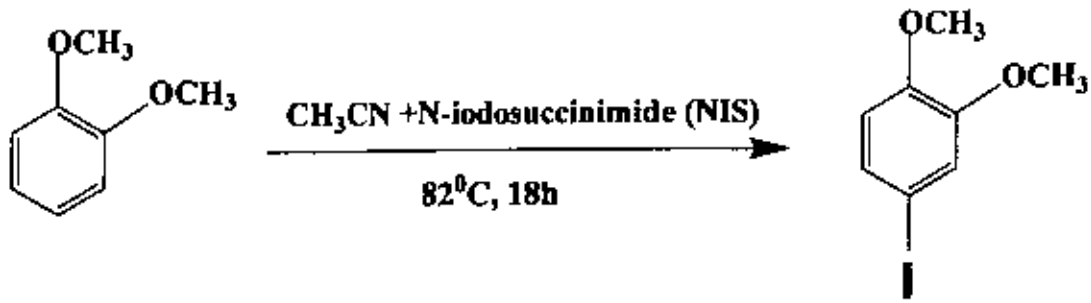
Table 2: Iodination of Aromatic ring with CH_3CN/ N-iodosuccinimide (NIS)

Substrate	Temp.	Time	Yiel (%)	Productc	Scheme No
1,4-MeO-benzene	20°C	16h	95%	2-iodo-1,4-MeO-benzene	3
1,2-MeO-benzene	82°C	18h	85%	4-iodo-1,2- MeO-benzene	4
Anisole	82°C	6h	95%	4-iodoanisole	5
1,2,4-MeO-benzene	20°C	4h	95%	5-iodo-1,2,4-MeO-benzene	6



1, 4-MeO-benzene **2-iodo-1, 4-MeO-benzene**

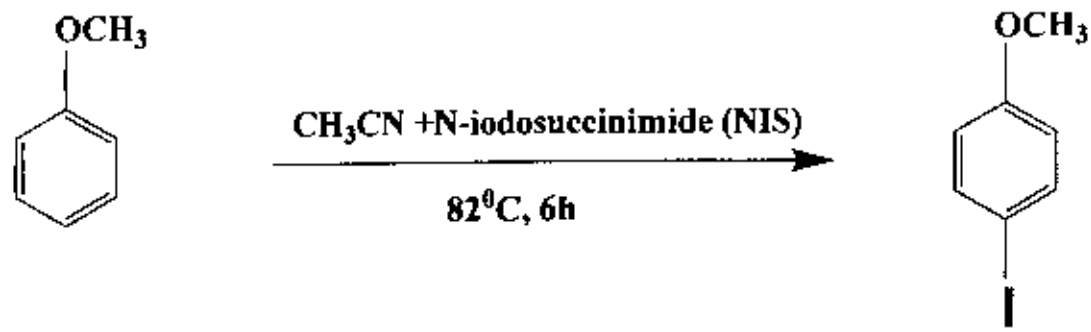
Scheme 3



1, 2-MeO-benzene

4-iodo-1, 2- MeO-benzene

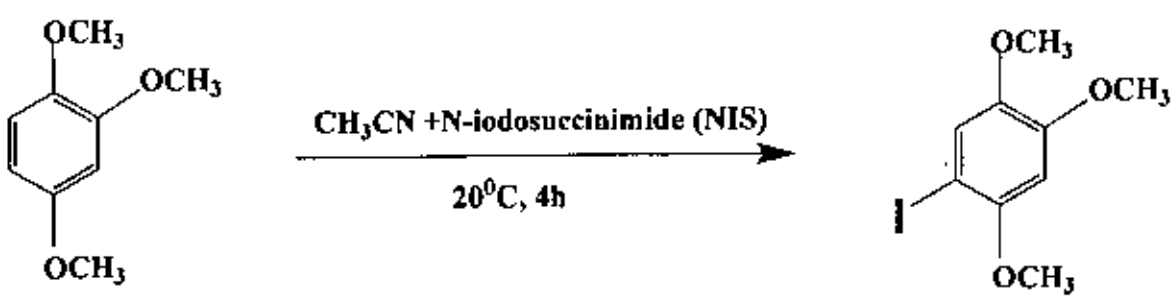
Scheme 4



Anisole

4-iodoanisole

Scheme 5



1, 2, 4-MeO-benzene

5-iodo-1, 2, 4-MeO-benzene

Scheme 6

1.3b. A Novel Iodination of Aromatic Rings Using Iodine/Metallic Nitrate

In 1998, Mehnan S. *et. al.*[28] reported a new and convenient synthetic method for the iodination of an aromatic ring using I_2 and nitrate.To a solution of anisole (3 mmol) and I_2 (1.7 mmol) in acetic acid (10 mL) was added sodium nitrate (0.3 mmol) at room temperature. The reaction mixture in a condenser-attached flask was heated at 85^0C for 6 hrs under a deoxygenated argon atmosphere. During the reaction, three additional portions of sodium nitrate (3x 0.3 mmol) were added respectively at the first every hour. The resulting mixture was treated with 10% aqueous $NaHSO_3$ solution (20 mL) and extracted with ether (3x 30 mL). The organic extracts were washed with brine (20 mL) and dried with Na_2SO_4. Evaporation of solvents in vacuo gave the crude iodination product. The crude product was purified by flash chromatography (hexaneibenzene) with silica gel to afford 4-iodoanisole in 92% yield.

The new method, various aromatic rings with an electron-donating group, they (Mehnan S. *et. al.*) can be successfully iodinated in good yield in the absence of strong acid (**Table 3**). It is quite peculiar that neither a strong acid nor acetic anhydride is necessary for the iodination. And the reaction needs only cheap and readily available chemicals. Moreover, the directional selectivity of iodination is excellent to produce para-iodoaromatic ring except for toluene.

A change of the counter-cations of nitrate ($NaN0_3$, $KN0_3$ or $Ca(NO_3)_2$) makes little effect on the result of iodination. Thus, it is apparont that I_2 is activated by the action of NO_3^- not by any metallic cations.

Table 3. Iodination of Aromatic Rings with I_2/NO_3^-[a]

Substrate	Nitrate	mmol of Nitrate	Temp. (°C)	Time (hr)	Yield[b] (%)	Product[c]
anisole	$NaNO_3$	1.2	85	6	92	4-iodoanisole
anisole	$NaNO_3$	0.6	85	4	92[d]	4-iodoanisole
anisole	KNO_3	1.2	85	6	90	4-iodoanisole
anisole	$Ca(NO_3)_2{\cdot}4H_2O$	0.6	85	6	92	4-iodoanisole
anisole	-	0	85	6	0	4-iodoanisole
diphenylether	$NaNO_3$	2.4	85	21	70[e]	4,4'-diiodo-diphenylether
9-methylcarbazole	$NaNO_3$	0.6	30	21	87[e]	3,6-diiodo-9-methylcarbazole
9-benzylcarbazole	$NaNO_3$	0.6	30	10	88,	9-benzyl-3,6-diiodocarbazole[e]
aniline	$Ca(NO_3)_2{\cdot}4H_2O$	0.6	65	30	37	4-iodoacetanilide
acetanilide	$Ca(NO_3)_2{\cdot}4H_2O$	0.6	105	24	47[e]	4-iodoacetanilide
durene	$NaNO_3$	1.2	116	6	65	1-iodo-2,3,5,6-tetramethylbenzene
mesitylene	$NaNO_3$	1.2	116	4	81	1-iodo-2,4,6-trimethylbenzene
m-xylene	$Ca(NO_3)_2{\cdot}4H_2O$	1.2	116	2	90	1-iodo-2,4-dimethylbenzene
toluene	$Ca(NO_3)_2{\cdot}4H_2O$	3.0	116	2.5	61[f]	iodotoluenes
chlorobenzene	$Ca(NO_3)_2{\cdot}4H_2O$	3.0	116	13	0	1-chloro-4-iodobenzene

[a]3.0 mmol of substrate, 1.7 mmol of I_2, nitrate and 10 mL of acetic acid were used under an argon atmosphere unless otherwise specified. [b]yields of pure and isolated products. [c]All the structures were identified by IR, NMR data and melting points. [d]Oxygewn as bubbled through the reaction mixture.[e]3.3 mmol of I_2 were used. [f]GC yield. [g]para: ortho=61: 39.[4] mp 174-175 ^{0}C (heptane /benzene); IR (KBr) 554 cm^{-1};^{1}H NMR (100 MHz, $CDCl_3$ /TMS)δ 5.36 (s, 2H), 7.00-7.22 (m, 7H), 7.62 (d, 2H), 8.28 (s, 2H); C 44.82%, H 2.57%, N 2.75% for $C_{19}H_{13}NI_2$ found C 44.68%, H 2.63%, N 2.46%.

1.3c. Oxidative Iodination of Aromatic Amides Using Sodium Perborate or Hydrogen Peroxide with Sodium Tungstate

Philipp Beinker *et al* [29]in 1998, had reported the *Iodination using Sodium Perborate*.- Sodium perborate (3 g) was dissolved in a mixture of glacial acetic acid (15 cm^3) and acetic anhydride (10 cm^3). A solution of potassium iodide (1.4 g) and sodium tungstate (300 mg) in water (10 cm^3) was added together with conc. sulfuric acid (5 cm^3). The substrate (7.5 mmol) in glacial acetic acid (10 cm^3) was added and the solution was warmed to 50^{0}C over 1 h. The mixture was poured into water (100 cm^3) and the excess iodine was destroyed with sodium thiosulfate and the solution was neutralized with sodium hydroxide. The product was filtered and recrystallized from ethanol. The products (**Table 4**) were identified by their ^{1}H NMR spectra and melting point data. Occasionally it was necessary to extract the product with chloroform in this last step. The extract was then washed with water, dried over sodium sulfate and the solvent evaporated to give the product.

Also reported the Iodination Using Hydrogen Peroxide. - Hydrogen peroxide (30%, 3 cm^3) was dissolved in glacial acetic acid (20 cm^3), cooled in ice and treated with conc. sulfuric acid (1 cm^3). A solution of potassium iodide (1.6 g) and sodium tungstate (300 mg) in water (10 cm3) was added. After 15 min a solution of the substrate (10 mmol) in glacial acetic acid (10 cm3) was added and the mixture was warmed to 50°C for 1 h. The mixture was poured into water (100 cm^3) and the excess iodine was destroyed with sodium sulfite. The solution was neutralized with sodium hydroxide and the iodo derivative filtered and recrystallized. The products (**Table 4**) were identified by their ^1H NMR spectra and mp data.

Table 4. Iodination of aromatic amides

		Sodium perborate		Hydrogen peroxide		Lit. mp*
Substrate	Product	Yield (%)	Mp/°c	Yield (%)	Mp/°C	
Acetanilide	4-Iodoacetanilide	83	180	96	175	180
2-Methylacetanilide	4-Iodo-2-methylacetanilide	51	165	87	165	162
3-Methylacetanilide	4-Iodo-3-methylacetanilide	71	138	87	135	134
4-Methylacetanilide	2-Iodo-4-methylacetanilide	35	130	40	125	131
2,3-Dimethylacetanilide	2,3-Dimethyl-4-iodoacetanilide	43	155	69	158	
2,4-Dimethylacetanilide	2,4-Dimethyl-6-iodoacetanilide	30	86	56	85	85
2,6-Dimethylacetanilide	2,6-Dimethyl-3-iodoacetanilide	51	190	72	190	
2-Nitroacetanilide	4-Iodo-2-nitroaniline	45	121	-	-	123
4-Nitroacetanilide	2-Iodo-4-nitroaniline	38	114	-	-	115
	2-Iodo-4-nitroacetanilide			77	200	202
Methyl N-acetylanthranilate	Methyl 2-acetylamino-5-iodobenzoate	31	110	37	110	

*Dictionary of Organic Compounds, ed J. Buckingha1 and F. Macdonald, Chapman and Hall, London 6th edn., 1996.

1.3d. Oxidative Iodination of Aromatic Amines and Other Arenes, with Sodium Percarbonate as the Oxidant

Agnieszka Zielinska and Lech Skulski[30] reported that Four easy eco-friendly laboratory procedures are presented for the oxidative iodination of various arenes with molecular iodine, in the presence of sodium percarbonate as the oxidant. The purified mono- or diiodinated products were obtained in 60-92% yields. The four general procedures given below:

General Procedure 1, applicable for some arylamines (**Table 5**). Powdered diiodine (0.51 g, 2.0 mmol; 0% excess) was suspended in a mixture made of ethyl acetate (8 mL) and glacial acetic acid (10 mL), then sodium percarbonate (0.31 g, 3.0 mmol; 50% excess) was slowly added portionwise, with stirring, within 20-30 min., next followed by an *aromatic amine* (4.4 mmol; 10% excess)[when *aniline* was diiodinated, only 2.2 mmol of aniline was added]. The stirring was continued for 30 min at r.t., next the temperature was raised to 45-50 °C, and the stirring was continued for a further 3.5 h. After cooling, the reaction mixture was slowly added to an aq. Na_2SO_3 solution (1 g Na_2SO_3 dissolved in 70 mL cold water), with stirring. The precipitated crude products were collected by filtration, washed well with cold water until the filtrates were neutral, dried preliminarily by the suction, an next air-dried in the dark; they were recrystallized from either hexane or anhydr. ethanol. If the semisolid crude products could not be efficiently isolated, they were extracted with $CHCl_3$ (3 x 10 mL), the combined extracts were dried over anhydr. $MgSO_4$, filtered, the solvent was distilled off, and the solidified residues were recrystallized from hexane or anhydr. EtOH.

General Procedure 2, applicable for easily oxidizable arylamines (**Table 5**). Powdered diiodine (0.51 g, 2.0 mmol; 0% excess) was suspended in a mixture made of ethyl acetate (20 mL) and glacial acetic acid (0.11 mL), and sodium percarbonate (0.31 g, 3.0 mmol; 50% excess) was slowly added portionwise, with stirring, within 20-30 min., next followed by an *aromatic amine* (4.4 mmol; 10% excess). The reaction mixture was stirred at 45-50 °C for 4 h. After cooling, it was slowly added, with stirring, to an aq. Na_2SO_3 solution (1 g Na_2SO_3 dissolved in 70 mL cold water). The following workups were the same as above. The crude solid products were recrystallized from anhydr. ethanol or hexane.

General Procedure 3, applicable for benzene and some weakly activated arenes (**Table 5**). Powdered diiodine (0.51 g, 2.0 mmol; 0% excess) was suspended in a mixture made of glacial acetic acid (6 mL) and acetic anhydride (3 mL), and sodium percarbonate (0.42 g, 4 mmol; 100% excess) was slowly added portionwise, with stirring, within 20-30 min. The stirred reaction mixture was slowly warmed up to 30-35 °C, and an *activated arene* (4.2 mmol; 5% excess) or *benzene* (2.1 mmol; 5% excess – for the diiodination of benzene) were added. After cooling the reaction mixtures to 5-10 °C, the *varied amounts* of concd. (95%) H_2SO_4 were slowly added dropwise with stirring and keeping the given temperature:

- 5.27 mL H_2SO_4 (9.68 g; 98 mmol) for the iodination of 4-MeC_6H_4COOH and 4-MeC_6H_4COOMe;
- 5.30 mL H_2SO_4 (9.74 g ; 99 mmol) for the iodination of 4-$O_2NC_6H_4OMe$;
- 5.50 mL H_2SO_4 (10.11 g ; 102 mmol) for the iodination of 4-$O_2NC_6H_4Me$;
- 7.50 mL H_2SO_4 (13.8 g ; 140 mmol) for the diiodination of PhH.

The reaction mixture was stirred at 45-50 °C for 2 h. After cooling, it was slowly added, with stirring, to an aq. Na_2SO_3 solution (1g Na_2SO_3 dissolved in 50 mL cold water). The precipitates were collected by filtration, washed well with cold water until the filtrates were neutral, air-dried in the dark, and recrystallized from appropriate organic solvents to give the purified solid iodinated products.

General Procedure 4, applicable for benzene and some deactivated arenes (**Table 5**). Powdered diiodine (0.56 g, 2.2 mmol; 10% excess) was suspended in a mixture made of glacial acetic acid (8 mL) and acetic anhydride (5 mL), and sodium percarbonate (1.25 g, 12 mmol; 100% excess) was slowly added portionwise, with stirring, within 20-30 min. The reaction mixture was slowly warmed up to 30-35 °C, and a deactivated arene (4.0 mmol; 0% excess) or benzene (2.0 mmol; 0% excess – for the diiodination of benzene) were added. After cooling the reaction mixtures to 5-10 °C, the varied amounts of concd (95%) H_2SO_4 were slowly added drop wise with stirring and keeping the given temperature:

- 3.60 mL H_2SO_4 (6.60 g ; 67.5 mmol) for the iodination of PhCOOH;
- 4.67 mL H_2SO_4 (8.58 g ; 87.6 mmol) for the iodination of PhI, PhCOOMe, and 4-MeC_6H_4COOH;
- 4.93 mL H_2SO_4 (9.06 g ; 92 mmol) for the iodination of PhH and 4-$O_2NC_6H_4Me$.

Next, the reaction mixtures were stirred at 35-40 °C for a further 2 h. After cooling, they were slowly added to stirred aq. Na_2SO_3 solutions (2 g Na_2SO_3 dissolved in 50 mL cold water). The precipitates were collected by filtration, washed well with cold water until the filtrates were neutral, dried preliminarily by the suction, air-dried in the dark, and recrystallized from appropriate organic solvents to give the purified solid iodinated products.

The final yields given in the **Table 5** for the purified iodinated products were calculated from the amounts of those reactants (arene or diiodine) which were used in the oxidative iodination reactions in strictly stoichiometric quantities (0% excess).

Table 5: Pure Iodinated Products Prepared

Substrate	Procedure	Product	Yield (%)	MP/(S)* (°C)	Lit, mp[31] (°C)
$PhNH_2$	1	$4\text{-}IC_6H_4NH_2$	68	63-65/(H)	62-63
$PhNH_2$	1	$2,4\text{-}I_2C_6H_3NH_2$	85	93-94/(H)	95-96
$4\text{-}IC_6H_4NH_2$	1	$2,4\text{-}I_2C_6H_3NH_2$	78	96-97/(H)	95-96[32]
$2\text{-}BrC_6H_4NH_2$	1	$4\text{-}I\text{-}2\text{-}BrC_6H_3NH_2$	67	71-74/(G)	71-72
$2\text{-}MeC_6H_4NH_2$	1	$4\text{-}I\text{-}2\text{-}MeC_6H_3NH_2$	86	86-87/(H)	86-88
$PhNMe_2$	2	$4\text{-}IC_6H_4NMe_2$	60	81-83/(E)	81-82
$2\text{-}ClC_6H_4NH_2$	2	$4\text{-}I\text{-}2\text{-}ClC_6H_3NH_2$	73	60-61/(H)	62-63
PhH	3	$1,4\text{-}I_2C_6H_4$	83	128-130/(L)	129
PhH	4	$1,4\text{-}I_2C_6H_4$	83	128-130/(L)	129
PhI	4	$1,4\text{-}I_2C_6H_4$	94	128-129/(L)	129
PhCOOH	4	$3\text{-}IC_6H_4COOH$	93	185-187/(C)	187-188[32]
$4\text{-}MeC_6H_4COOH$	4	$3\text{-}I\text{-}4\text{-}MeC_6H_3COOH$	79	208-209/(C)	208-210[32]
PhCOOMe	4	$3\text{-}IC_6H_4COOMe$	60	52-53/(L)	50-52
$4\text{-}NO_2C_6H_4Me$	3	$2\text{-}I\text{-}4\text{-}NO_2C_6H_3Me$	75	51-52/(N)	54-55
$4\text{-}NO_2C_6H_4Me$	4	$2\text{-}I\text{-}4\text{-}NO_2C_6H_3Me$	87	51-53/(N)	53-54
$4\text{-}NO_2C_6H_4OMe$	3	$2\text{-}I\text{-}4\text{-}NO_2C_6H_3\ OMe$	92	95-96/(L)	96-97
$4\text{-}MeOC_6H_4COOMe$	3	$3\text{-}I\text{-}4\text{-}MeOC_6H_4COOMe$	85	93-95/(N)	94-96

*Solvent used for recrystallization: C: CCl_4; E: EtOH; G: heptane; H: hexane; L: EtOH-H_2O (4:1); N: EtOH-H_2O (3:2).

CHAPTER-2

PRESENT WORK
RESULTS AND DISCUSSION

Present work: Synthesis of substituted 2-iodoaniline and 2-iodoacetanilide

2.1. Rationale

Aromatic iodides are a considerable number of different methods, direct or indirect, for their synthesis and they are widely used in chemical laboratories and sometimes, also in chemical industry and medicine[1]. Moreover, they are able to form a large variety of stable, aromatic polyvalent iodine compounds, which have found increasing applications in modern organic synthesis[2].

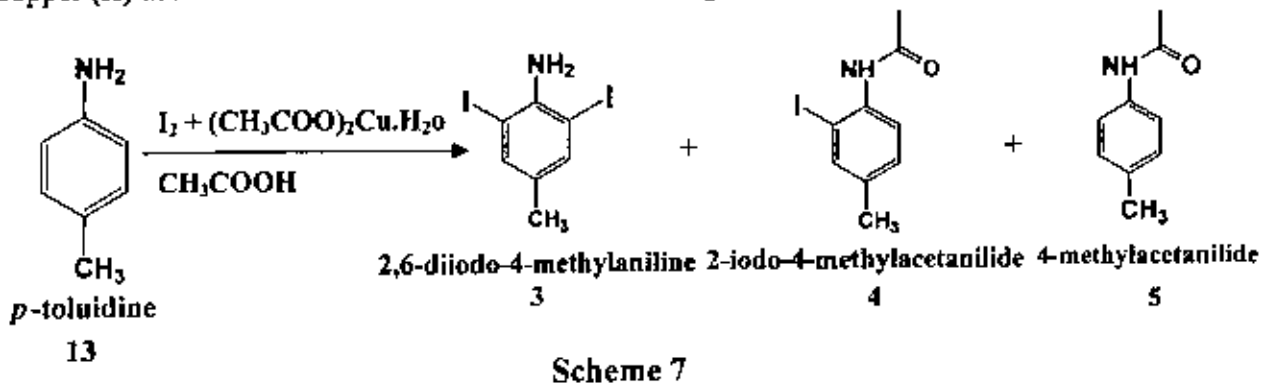
Much work has been reported on the bromination and chlorination of aromatic compounds. However, since it is known that iodination is usually difficult accomplish due to the reducing effect of the hydrogen iodide produced, the iodination of aromatic compounds has been carried out in the presence of an oxidizing reagent such as nitric acid, iodic acid, sulfur trioxide or hydrogen peroxide[33]. Many procedures are available for the iodination of activated aryl compounds by iodine-thallium(I) acetate[34], iodine-mercury(II) acetate[35], iodine-silver(I) acetate[36], iodine-ammonium hexanitratocerate(IV)[37] and alkali metal iodide-ammonium hexanitratocerate(IV)[37]. Moreover, Bird and Surridge recently reported[38] that the iodination of aromatic compound with iodine and copper(II) halide gave the aryl iodides. Still more recently, it was reported the iodination of the less reactive substrates with iodine-aluminum(III) and copper(II) chloride[39] gave the corresponding aryl halide.

Due to the natural occurrence and biological importance of the aromatic iodide derivatives and lack of convenient aromatic iodination precursors, we were interested in developing a general and facile method for the synthesis of aromatic iodide derivatives. We became interested in the synthesis of substituted 2-iodoaniline and 2-iodoacetanilide derivatives through iodine and copper(II) acetate in acetic acid reactions.

RESULTS AND DISCUSSION

2.2. Synthesis of substituted 2-iodoaniline, 2-iodoacetanilide and acetanilide from *p*-toluidine

Compound **3, 4, 5** were prepared by refluxing the solution mixture of *p*-toluidine **13**, iodine, copper (II) acetate and acetic acid in oil bath with stirring 9 hr at 120°C as shown in the **Scheme 7**.



p-toluidine
13

2,6-diiodo-4-methylaniline
3

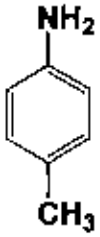
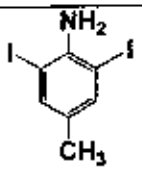
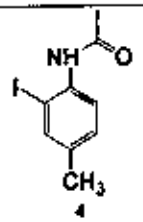
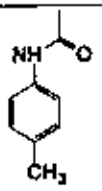
2-iodo-4-methylacetanilide
4

4-methylacetanilide
5

Scheme 7

After usual workup and purification by column chromatography on silica gel, iodo-*p*-toluidine derivatives **3, 4, 5** were obtained **(Table 6)**.

Table 6: Preparation of disubstituted or monosubstituted aniline or acetanilide.

Entry	Substrate	Product	Yield (%)
1	 13	 3	35
		 4	26
		 5	22

2.2a. Characterization of 2, 6-diiodo-4-methylaniline 3

A light yellowish crystal was obtained (yield 35%), mp. 110^{0}C, which was not moisture sensitive. The structure of compound **3** was established by spectral data. In UV (**Fig. 13a**) spectrum, the value was found in the range of λ_{max} 400.35, 202.00 nm indicated amino group.

The IR spectrum (KBr) (**Fig.13b**) of this compound exhibited absorption bands at ν_{max}3406.1 and 3317.3 cm^{-1} for Stretching of amine proton (-NH_2), 3037.7 and 2898.8 cm^{-1} for stretching of methyl and aromatic (methyne) protons (-CH_3, -CH), 1608.5,1460 and 1566.1 cm^{-1} for stretching and bending of aromatic ring and primary aromatic amine respectively (C-C and N-H), 700.1 and 846.7 cm^{-1} for waging of primary aromatic amine (N-H).

The ^{1}H NMR spectrum (**Fig. 13c**) (400 MHz, $CDCl_3$) of the compound **3** explained the chemical shift position δ_H 7.45 (s, 2H, C-3 & C-5) two methyne group in aromatic ring (-CH) which are chemically equivalent, δ 4.19 (s, 2H) for the presence of primary amine (-NH_2) group, δ 2.15 (s, 3H) for presence of methyl group (Ar-CH_3) that is assured structure of the compound **3**.

The structure of the compound **3** was further confirmed by its ^{13}C NMR data (**Fig. 13d**) (100 MHz, $CDCl_3$) at the chemical shift position of δ 143.83 due to the presence of (C-NH_2) carbon, at δ 139.77 designed for C-3 & C-5 was similar chemical environment, δ130.97 and 19.22 due to the presence of (C-CH_3) and (Ar-CH_3) carbon respectively and δ 81.45 also indicated that similar chemical environment (C-2 & C-6) of Ar-I carbon.

2.2b. Characterization of 2-iodo-4-methylacetanilide 4

A brownish colored crystal was obtained (yield 26%) and mp. 125-130^{0}C. The structure of compound **4** was recognized from its spectral data. In UV spectrum (**Fig.14a**) the value was found in the range of 401.85 & 199.99 nm.

The IR spectrum (KBr) (**Fig. 14b**) demonstrated following characteristic peaks: ν_{max} 3265.3cm^{-1} (Stretching secondary amine, -NH-), 1654.8 cm^{-1} for (stretching C=O), 1523.7 cm^{-1} for stretching of (-C=C-, aromatic ring) and 1290.3 cm^{-1} for stretching of aromatic amine (C-N).

In the ^{1}H NMR spectrum (**Fig. 14c**) (400 MHz, $CDCl_3$) of this compound **4** the chemical shift was observed at δ 7.99 (d, 1H, *J*=7.96 Hz) and 7.12 (d, 1H, *J* = 8.09Hz) for one proton doublet (-CH-) of C-6 & C-5 respectively, 7.59 (s, 1H) for secondary amine group of (-NH-), 7.32 (s, 1H) for –CH- of (C-3), 2.26 (s, 3H) for three proton of methyl (Ar-CH_3) group in aromatic ring and 2.20 (s, 3H) for three proton of -CO-CH_3 group.

The compound was further established from its ^{13}C NMR spectrum (**Fig. 14d**) (100 MHz, $CDCl_3$). The chemical shifts of this compound were showed following characteristic peak: δ_C 168.15 (-C=O), 145.98 (C-NH), 138.96 (C-6), 135.79 (C-CH_3, C-4), 129.92 (C-3), 122.12 (C-5), 90.29 (Ar-I, C-2), 24.67 (-CO-CH_3) and 20.30 (Ar-CH_3). The ^{13}C NMR spectrum indicated the presence of nine carbons in the molecule corresponding to the molecular formula $C_9H_{10}INO$, thereby suggesting the formation of the compound **4**.

On the basis of complete analysis of the UV, IR, ^{1}H NMR and ^{13}C NMR spectra, the structure of this compound was accorded as 2-iodo-4-methylacetanilide **4**.

2.2c. Characterization of 4-methylacetanilide 5

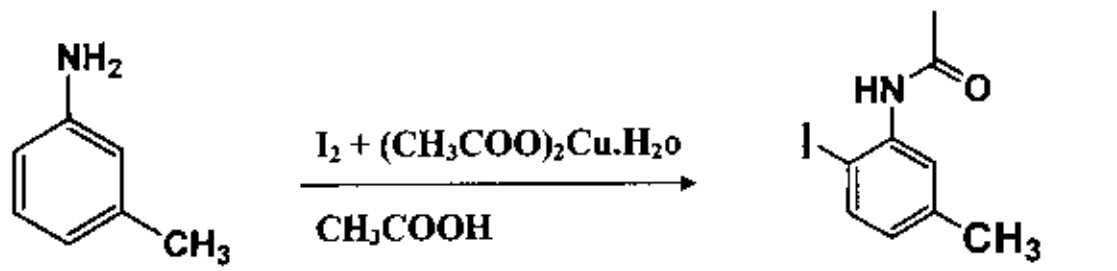
The product **5** was a brownish crystal (yield 22%), mp. 148-151^{0}C. The structure of compound **5** was assigned by spectral data. In the UV spectrum (**Fig. 15a**) the absorption was found in the range of λ_{max} 201.04 nm.

The IR spectrum (KBr) (**Fig. 15b**) showed 3292.3 cm^{-1} (-NH-) of the acetanilide group, and 1662.5 cm^{-1} due to the presence of (-C=O) group in the acetanilide.

The ^{1}H NMR spectrum (**Fig. 15c**) (400 MHz, $CDCl_3$)of this compound **5** showed the chemical shift at δ_H 7.52 (s, 1H) for secondary amine group (–NH-), δ 7.35 (d, 2H, J=8.23 Hz) for –CH- of C-2 & C-6 proton, δ 7.08 (d, 2H, J = 8.07 Hz) for C-3 & C-5 proton and δ 2.28 (s, 3H) & δ 2.21 (s, 3H) displayed methyl groups (Ar-CH_3 and -$COCH_3$) respectively, which confirmed the structure of compound **5.** The presence of eleven hydrogen atoms was in good agreement with the molecular formula of $C_9H_{11}NO$.

2.3. Synthesis of 2-iodo-5-methylacetanilide from *m*-toluidine

Compound **6** were prepared by refluxing the solution mixture of *m*-toluidine **14**, iodine, copper(II) acetate and acetic acid in oil bath with stirring 10 hr at 130^{0}C as shown in the **Scheme 8.**



***m*-toluidine**

14

2-iodo-5-methylacetanilide

6

Scheme 8

After usual workup and purification by column chromatography on silica gel, iodo-*m*-toluidine derivative **6** (55%, yield) was obtained.

2.3a. Characterization of 2-iodo-5-methylacetanilide 6

A brownish crystalline product was obtained (yield 55%). The structure of compound **6** was confirmed by UV, IR, and NMR data. In UV spectrum (**Fig. 16a**) the λ_{max} value was found in the range of 400.99 & 201.08 nm.

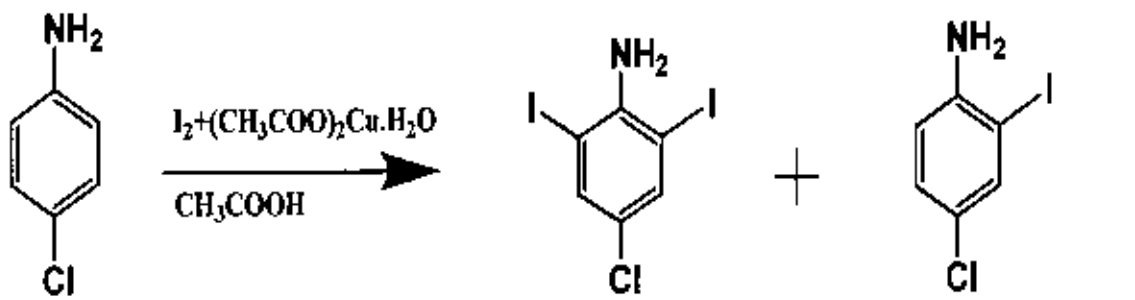
The IR spectrum (**Fig. 16b**) of the compound **6** showed the following absorption bands: ν_{max} 3315.4 (-NH) cm^{-1} due to the ν_{NH} of the acetanilide group, and a band at 1668.3 cm^{-1} due to the presence of (-C=O) group in the acetanilide.

The ^1H NMR spectrums (**Fig. 16c**) (400 MHz, $CDCl_3$) of the compound **6** revealed that one proton doublet at δ 7.67 (d, 1H, J = 8.5 Hz, C-3) and δ 7.42 (d, 1H, J = 1.94, C-6) respectively due to aromatic proton. δ 7.46 (s, 1H) indicted the secondary amine –NH-, δ 7.03 (dd, 1H, J = 2.1 Hz, 8.3 Hz,) for one proton double doublet (-CH-) of C-4, δ 2.36 (s, 3H, Ar-CH_3) and δ 2.13 (s, 3H, $COCH_3$) indicated the presence of three protons singlet of terminal (-CH_3) methyl groups which confirmed the structure of product **6**.

The structure of the compound further confirmed by its ^{13}C NMR spectrum (**Fig. 16d**) (100 MHz, $CDCl_3$). It was observed that the chemical shift at δ_C 168.52 (C=O), 142.03 (C-NH), 139.11 (C-3), 138.11 (C-CH_3), 21.11 (C-6), 119.02 (C-4), 94.43 (Ar-I), 28.13 ($COCH_3$) and 24.58 (Ar-CH_3). So the ^{13}C NMR spectrum indicated the presence of nine carbon atoms in the molecule corresponding to the molecular formula $C_9H_{10}IN0$, thereby suggesting the formation of compound **6**.

2.4. Synthesis of 4-chloro-2,6-diiodoaniline and 4-chloro-2-iodoaniline from 4-chloroaniline

Compound **7, 8** were prepared by refluxing the solution mixture of 4-chloroaniline **15**, iodine, copper(II) acetate and acetic acid in oil bath with stirring 11 hr at 130^{0}C as shown in the **Scheme 9.**



4-chloroaniline **15**

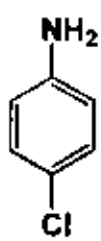
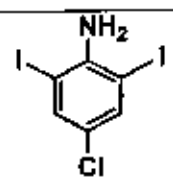
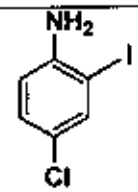
4-chloro-2,6-diiodoaniline **7**

4-chloro-2-iodoaniline **8**

Scheme 9

After usual workup and purification by column chromatography on silica gel, iodo-4-chloroaniline derivative **7, 8** was obtained **(Table 7).**

Table 7: Preparation of iodo-4-chloroaniline derivatives 7, 8.

Entry	Substrate	Product	Yield (%)
1	 **15**	 **7**	30
		 **8**	45

2.4a. Characterization of 4-chloro-2, 6-diiodoaniline 7

Yellowish amorphous powder was obtained (yield 30%), mp. 127-129^{0}C. The structure of compound 7 was interpreted by spectral data. In UV spectrum (**Fig. 17a**) the λ_{max} value was found in the range of 400.89 & 199.92 nm.

The IR spectrum (KBr) (**Fig. 17b**) of this compound assigned the following characteristic absorption peaks: ν_{max} 3408.0 and 3317.3 cm^{-1} for stretching of primary amine (-NH_2) group. A strong band at 860.2 cm^{-1} represents the chlorine-carbon stretching.

The ^{1}H NMR spectrum (**Fig. 17c**) (400 MHz, $CDCl_3$) of this compound expressed following chemical shift at δ_H 7.60 (s, 2H) indicated that two proton of similar chemical environment of C-3 & C-5 carbon in the benzene ring and δ 4.59 (s, 2H) also expressed primary amine (-NH_2) group with benzene ring as a terminal amine.

The compound **7** also established by following characteristic ^{13}C NMR spectrum (**Fig. 17d**) (100 MHz, $CDCl_3$): δ_C 145.16 (C-NH_2), 138.33 (C-3 & C-5), 123.25 (C-Cl) and 80.25 (C-I). So the ^{13}C NMR spectrum indicated the presence of nine carbons in the molecule corresponding to the molecular formula $C_9H_4ClI_2N$, thereby suggesting the formation of compound **7**.

2.4b. Characterization of 4-chloro-2-iodoaniline 8

The brownish crystalline product was obtained (yield, 45%), mp. 40-42^{0}C. The structure of compound **8** was interpreted by spectral data. In UV spectrum (**Fig. 18a**) the λ_{max} value was found in the range of 240.65 & 200.51 nm.

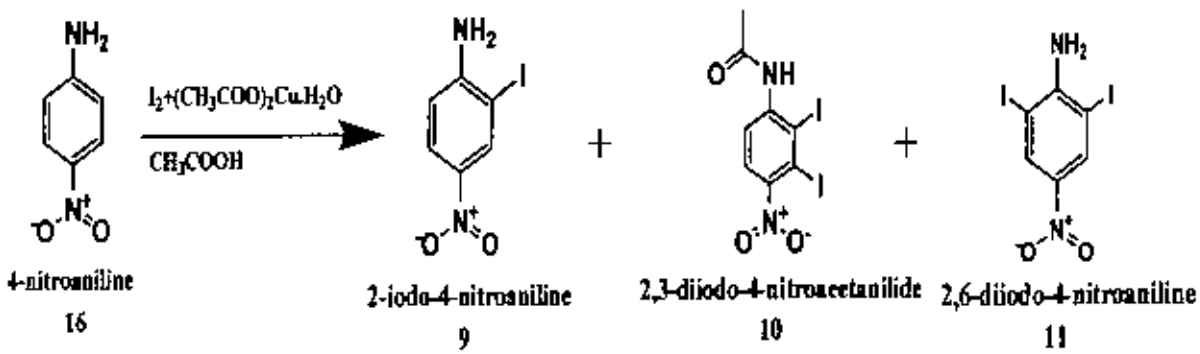
The IR spectrum (KBr) (**Fig.18b**) of the compound **8** exhibited the absorption at ν_{max}3408.0 and 3317.3 cm^{-1} for stretching of primary amine (-NH_2) group. A strong band at 868.0 cm^{-1} represents the chlorine-carbon stretching.

The ^{1}H NMR spectrum (**Fig. 18c**) (400 MHz, $CDCl_3$) of this product **8** showed that a one proton doublet at δ_H 7.58 (d, 1H, J = 2.3Hz, C-3) & 6.64 (d, 1H, J= 7.1Hz, C-6) respectively and one proton double doublet at 7.08 (dd, 1H, J= 2.3Hz, 8.5Hz, C-5), which attached in benzene ring. δ 4.07 (s, 2H) also indicated primary amine (-NH_2), attached with benzene ring as a terminal. The spectra displayed the presence of five hydrogen corresponding to be confirmed the structure of product **8**.

Further analysis the structure of **8** confirmed by its ^{13}C NMR spectrum (Fig.18d) (100 MHz, $CDCl_3$). It was observed that the chemical shift at δ_C 145.57 (C-NH_2), 137.78 (C-3), 129.26 (C-5), 123.18 (Ar-Cl, C-4), 114.99 (C-6) and 83.46 (Ar-I, C-2). So the ^{13}C NMR spectrum indicated the presence of nine carbon atoms in the molecule corresponding to the molecular formula C_9H_5ClIN, thereby suggesting the formation of compound **8**.

2.5. Synthesis of compound 9, 10, 11 from 4-nitroaniline

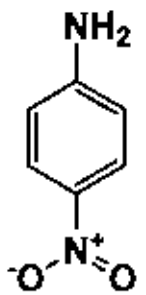
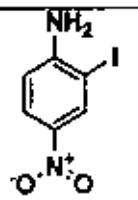
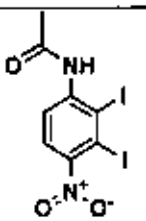
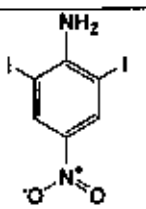
Compound **9, 10, 11** were prepared by refluxing the solution mixture of 4-nitroaniline **16**, iodine, copper(II) acetate and acetic acid in oil bath with stirring 10 hr at 130^{0}C as shown in the **Scheme 10.**



Scheme 10

After usual workup and purification by column chromatography on silica gel, iodo-4-nitroaniline derivative **9, 10, 11** was obtained **(Table 8)**.

Table 8: Preparation of iodo-4-nitroaniline derivatives 9, 10, 11.

Entry	Substrate	Product	Yield (%)
1	 16	 9	30
		 10	26
		 11	38

2.5a. Characterization of 2-iodo-4-nitroaniline 9

A yellowish crystaline product was obtained (yield 30%), mp. 105-109^{0}C. The structure of compound 9 was interpreted by spectral data. In UV spectrum (**Fig.19a**) the λ_{max} value was found in the range of 240.00 & 200.00 nm.

The IR spectrum (KBr) (**Fig. 19b**) of this compound assigned the following characteristic absorption peaks: ν_{max} 3477.4 and 3371.3 cm^{-1} (str. Ar-NH_2), 1608.5 and 1488.9 cm^{-1} (str. Ar-NO_2).

The ^{1}H NMR spectrum (**Fig. 19c**) (400 MHz, $CDCl_3$) of this compound expressed following chemical shift at δ 6.68 (d, 1H, Ar-H, *J*=8.932 Hz), δ 8.70 (d, 1H, Ar-H, *J*=2.36 Hz) and δ 8.64 (dd, 1H, Ar-H, *J*=2.37Hz, 8.91Hz,) indicated the presence of one protons doublet and double doublet of C-6, C-3 and C-5 respectively. Two hydrogen singlet at the position of δ_H 4.83 (s, 2H) showed of primary amine ($-NH_2$) group as a terminal in benzene ring.

The compound was further established from its ^{13}C NMR spectrum (**Fig. 19d**) (100 MHz, $CDCl_3$). The chemical shifts of this compound were showed following characteristic peak: δ_C 152.33 ($C-NH_2$), 139.33 ($C-NO_2$), 80.53 (Ar-I, C-2), 135.51 (C-3), 125.72 (C-5) and 112.27 (C-6) for remaining carbon atoms. The ^{13}C NMR spectrum indicated the presence of six carbons in the molecule was corresponding the molecular formula $C_6H_5IN_2O_2$, thereby suggesting the formation of the compound 9.

2.5b. Characterization of 2, 3-diiodo-4-nitroacetanilide 10

A yellowish crystaline compound **10** was obtained (yield, 26%). It was recognized from its spectral data. In UV spectrum (**Fig. 20a**) the value λ_{max} was found in the range of 320.00, 241.08 & 202.14 nm.

The IR spectrum (KBr) (**Fig.20b**) of this compound illustrated following characteristic absorption bands: ν_{max} 3276.8 cm^{-1} stretching for aromatic secondary amine (-NH-) group in acetanilide, 1346.2 and 1506.3 cm^{-1} stretching for aryl-nitro ($-NO_2$) group, where as a band at 1683.7 cm^{-1} due to the presence of keto (-C=O) group in the acetanilide.

The ^{1}H NMR spectrum (**Fig. 20c**) (400 MHz, $CDCl_3$) assigned the chemical shift for the determination of molecular structure of compound **10**. It is observed that a three protons singlet at δ_H 2.23 (s, 3H) for methyl group of (CH_3), a one proton singlet at δ 7.47 (s, 1H) for secondary amine group of (-NH-), a one proton doublet at δ 7.68 (d, 1H, Ar-H, *J*=8.9Hz,) showed Ar-H of C-6 and also one proton doublet at δ 8.19 (d, 1H, *J*=9.01 Hz) for Ar-H of C-5.

The compound was further established from its ^{13}C NMR spectrum (**Fig. 20d**) (100 MHz, $CDCl_3$). The chemical shifts of this compound showed following characteristic peak: δ_C 168.528 (C=O), 143.631 (C-NH), 142.039 (C-NO_2), 125.138 (C-5), 118.988 (C-6), 94.431 (Ar-I, C-2), 90.291 (Ar-I, C-3), and 24.837 ($COCH_3$) for remaining carbon atoms. The ^{13}C NMR spectrum indicated the presence of eight carbons in the molecule was corresponding the molecular formula $C_8H_6I_2N_2O_3$, thereby suggesting the formation of the compound 10.

On the basis of analysis of the UV, IR, ^1H NMR, ^{13}C NMR spectra, the structure of compound 10 was accorded as 2, 3-diiodo-4-nitroacetanilide.

2.5c. Characterization of 2, 6-diiodo-4-nitroaniline 11

A light yellow crystalline product was obtained (yield 38%), mp. 249-250^{0}C. The structure of compound **11** was interpreted by spectral data. In UV spectrum (**Fig.21a**) the λ_{max} value was found in the range of 243.05 & 204.09 nm.

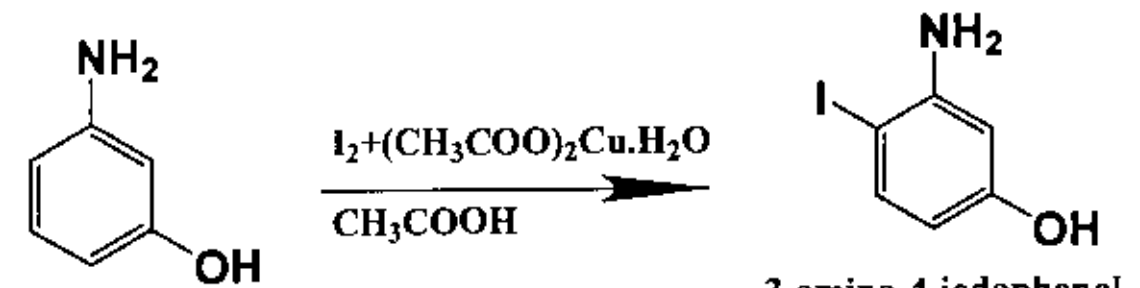
The IR spectrum (KBr) (**Fig. 21b**) of this compound assigned the following characteristic absorption peaks: ν_{max} 3479.5 and 3373.1 cm^{-1} (str. Ar-NH_2), 1609.6, 1489.5 cm^{-1} (str. Ar-NO_2).

The ^1H NMR spectrum (**Fig. 21c**) (400 MHz, $CDCl_3$) of this compound expressed following chemical shift at δ 5.33 (s, 2H) indicated two hydrogen singlet of primary amine (-NH_2) group as a terminal in benzene ring. δ 8.54 (s, 2H) showed same chemical environment at C-3 and C-5 in benzene ring. Its peaks confirmed the structure of product **11**.

The compound was further established from its ^{13}C NMR spectrum (**Fig. 21d**) (100 MHz, $CDCl_3$). The chemical shifts of this compound were showed following characteristic peak: δ_C 152.338 (C-NH_2), 143.832 (C-NO_2), 135.315 (C-3 & C-5) and 94.431 (Ar-I, C-2 & C-6) for remaining carbon atoms. The ^{13}C NMR spectrum indicated the presence of six carbons in the molecule was corresponding the molecular formula $C_6H_4I_2N_2O_2$, thereby suggesting the formation of the compound **11**.

2.6. Synthesis of 3-amino-4-iodophenol 12 from 3-Aminophenol

Compound **12** were prepared by refluxing the solution mixture of 3-aminophenol **17**, iodine, copper(II) acetate and acetic acid in oil bath with stirring 6 hr at 120^{0}C as shown in the **Scheme 11.**



3-aminophenol
17

3-amino-4-iodophenol
12

Scheme 11

After usual workup and purification by column chromatography on silica gel, iodo-3-aminophenol derivative **12** (yield, 60%) was obtained.

2.6a. Characterization of 3-amino-4-iodophenol 12

A bright yellow crystalline compound was obtained, yield % 60. The structure of compound 12 was recognized by spectral data. The UV spectrum (**Fig. 22a**) of the compound showed at absorption bands at 239.99 & 202.55 nm.

The IR spectrum (KBr)(**Fig. 22b**) of this compound expressed following absorption band at ν_{max}3477.4 and 3371.3 cm^{-1} indicted stretching of aromatic primary amine (-NH_2) group, 3198.2 cm^{-1} for phenolic OH group and 1606.6 for C=C.

The ^1H NMR spectrum (**Fig. 22c**) (400 MHz, $CDCl_3$) of this compound revealed an one proton singlet at δ_H 1.57 (s, 1H, Ar-OH), two proton singlet at δ 4.84 (s, 2H, NH_2), an one proton doublet at δ 6.68 (d, 1H, *J*=8.94Hz, C-5) & δ 8.54 (d, 1H, *J*=2.38, C-2) respectively, and δ 8.03 (dd, 1H, *J*= 2.39Hz, 8.9Hz) indicated the presence of one proton double doublet for Ar-H of C-6. The presence of six hydrogen atoms was in good agreement with the compound **12**.

The compound **12** was further analysis by ^{13}C NMR spectrum (**Fig. 22d**) (100 MHz, $CDCl_3$). It was observed that the chemical shift at δ_C 152.36 (Ar-OH, C-1), 139.27 (Ar-NH_2, C-3), 135.50 (C-5), 125.71 (C-6), 112.26 (C-2) and 80.52 (Ar-I, C-4). So the ^{13}C NMR spectrum indicated the presence of six carbon atoms in the molecule corresponding to the molecular formula C_6H_6INO, thereby suggesting the formation of compeund **12**.

Dept (**Fig. 22d**) spectrum showed that there were three tertiary carbons present at δ135.50, 125.71 and 112.26 in the ring.

DISCUSSION

Table 9: Distinction among some spectral data of 2, 6-Diiodo-4-methylaniline; 2-iodo-4-methylacetanilide; 4-methylacetanilide; 2-iodo-5-methylacetanilide 3, 4, 5, 6.

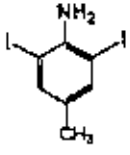
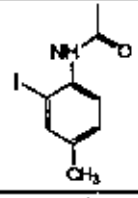
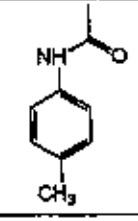
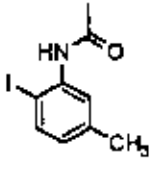
Comp. No.	Structure	UV (nm) λ_{max}	IR spectrum in ν_{max} cm^{-1}	^1H NMR (δ_H)	^{13}C NMR (δ_C)
3		202.00	3406.1, 3317.3 (-NH_2), 3037.7, 2898.8 (-CH_3, -CH), 1608.5, 1460, 1566.1,700.1 and 846.7	7.45 (s, 2H, C-3 & C-5), 4.19 (s, 2H, NH_2), 2.15 (s, 3H, Ar-CH3).	143.83 (C-NH_2), 139.77 (C-H, C-3 & C-5), 130.97 (C-CH_3), 81.45 (Ar-I, C-2 & C-6), 19.22 (Ar-CH_3).
4		425.32, 240.05 & 199 99	3265.3, 1654.8, 1523.7 and 1290.3	7.99 (d, 1H, *J*=7 96 Hz, C-6), 7.59 (s, 1H, NH), 7.32 (s, 1H, C-3), 7.12 (d, 1H, *J* = 8.09Hz, C-5), 2.26 (s, 3H, Ar-CH_3), 2.20 (s, 3H, -CO-CH_3).	168.15 (C=O), 145.98 (C-NH), 138.96 (C-6), 135.79 (C-CH_3, C-4), 129.92 (C-3), 122.12 (C-5), 90.29 (Ar-I, C-2), 24.67 (-CO-CH_3), 20.30 (Ar-CH_3).
5		241.36 & 201.04	3292.3 (-NH-), 3255.6, 1662.5 (-C=O)1602.7, 1550.7, 1510.2, 1454.2, 1402.2, 1365.5, 1321.1, 819.7	7.52 (s, 1H, NH), 7.35 (d, 2H, CH, *J*=8.23 Hz, C-2 & C-6), 7.08 (d, 2H, CH, *J* = 8.07 Hz, C-3 & C-5), 2.28 (s, 3H, Ar-CH_3), 2.21 (s, 3H, -$COCH_3$)	
6		240.67 & 201.08	3315.4 (-NH-), 1668.3 (-C=O),1606.6, 1581.5, 1533.3, 1471.6, 1396.4, 1311.5, 819.7.	7.67 (d, 1H, *J* = 8.5 Hz, C-3), 7.46 (s, 1H, NH), 7.42 (d, 1H, *J* = 1.94, C-6), 7.03 (dd, 1H, *J* = 2.1 Hz, 8.3 Hz, C-4), 2.36 (s, 3H, Ar-CH_3), 2.13 (s, 3H, $COCH_3$).	168.52 (C=O), 142.03 (C-NH). 139.11 (C-3), 138.11 (C-CH_3),121.11 (C-6), 119.02 (C-4), 94.43 (Ar-I), 28.13 ($COCH_3$), 24.58 (Ar-CH_3).

Table 10: Distinction among some spectral data of 4-chloro-2, 6-diiodoaniline & 4-chloro-2-iodoaniline 7, 8.

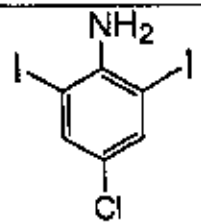
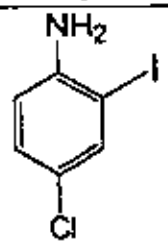
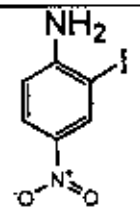
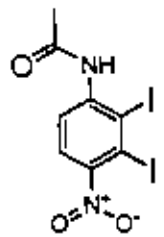
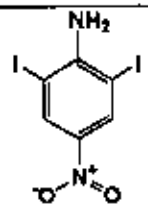
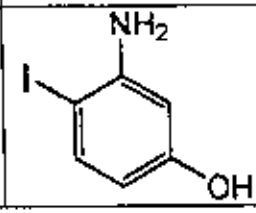
Comp. No.	Structure	UV (nm) λ_{max}	IR spectrum in ν_{max} cm^{-1}	^1H NMR (δ_H)	^{13}C NMR (δ_C)
7		199.92	3408.0 & 3317.3(-NH_2), 1604.7, 1442.7, 860.2 (Ar-Cl).	7.60 (s, 2H, C-3 & C-5), 4.59 (s, 2H, NH_2).	145.16 (C-NH_2), 138.33 (C-3 & C-5), 123.25 (Ar-Cl, C-4), 80.25 (Ar-I, C-2 & C-6).
8		200.51	3408.1 &3317.3 (-NH_2), 1603.7, 1443.6, 868.0 (Ar-Cl).	7.58 (d, 1H, J = 2.3Hz, C-3), 7.08 (dd, 1H, J= 2.3Hz, 8.5Hz, C-5), 6.64 (d, 1H, J= 7.1Hz, C-6), 4.07 (s, 2H, NH_2).	145.57 (C-NH_2), 137.78 (C-3), 129.26 (C-5), 123.18 (Ar-Cl, C-4), 114.99 (C-6), 83.46 (Ar-I, C-2).

Table 11: Distinction among some spectral data of 2-iodo-4-nitroaniline; 2, 3-diiodo-4-nitroacetanilide; 2, 6-diiodo-4-nitroaniline; 3-amino-4-iodophenol 9, 10, 11 & 12.

Comp. No.	Structure	UV (nm) λ_{max}	IR spectrum in ν_{max} cm^{-1}	^1H NMR (δ_H)	^{13}C NMR (δ_C)
9		240.00 & 200.00	3477.4 & 3371.3 (-NH_2), 1608.5, 1488.9 (-NO_2), 1301.9, 1257.5, 1114.8.	4.83 (s, 2H, NH_2), 6.68 (d, 1H, Ar-H, *J*=8.932 Hz, C-6), 8.64 (dd, 1H, Ar-H, *J*=2.37Hz, 8.91Hz, C-5), 8.70 (d, 1H, Ar-H, *J*=2.36, C-3).	152.33 (Ar-NH_2, C-1), 139.33 (Ar-NO_2, C-4), 135.51 (C-3), 125.72 (C-5), 112.27 (C-6). 80.53 (Ar-I, C-2).
10		320.00, 241 08 & 202.14	3276(-NH-), 1683.7 (-C=O), 1618.2, 1596.9, 1566.1, 1506.3 (-NO_2), 1346.2, 1332.7, 1301.9, 1267.1, 1112.9, 848.6, 750.3.	2.23 (s, 3H, -CH_3), 7.47 (s, 1H, -NH), 7.68 (d, 1H, Ar-H, *J*=8.9Hz, C-6), 8.19 (d, 1H, Ar-H, *J*=9.01, C-5)	168.528 (C=O), 143.631 (Ar-NH, C-1), 142.039 (Ar-NO_2, C-4), 125.138 (C-5), 118.988 (C-6), 94.431 (Ar-I, C-2), 90.291 (Ar-I, C-3), 24.837 ($COCH_3$).
11		322.01 & 204.09	3479.5 & 3373.1 (-NH_2), 1609.6, 1489.5 (-NO_2), 1302.7, 1257.5, 1118.5.	5.336 (s, 2H, -NH_2), δ 8.545 (s, 2H, C-3 & C-5).	152.338 (Ar-NH_2, C-1), 143.832 (Ar-NO_2, C-4), 135.315 (C-3 &C-5), 94.431 (Ar-I, C-2 & C-6).
12		239.99 & 202.55	3477.4&3371.3 (-NH_2), 3198.2 (Ar-OH), 3107.0, 1606.6, 1488.9, 1301.9, 1257.5, 1114.8.	1.57 (s, 1H, Ar-OH), 4.84 (s, 2H, NH_2), 6 68 (d, 1H, *J*=8.94Hz, C-5), 8.03 (dd, 1H, *J*=2.39Hz, 8.9Hz, C-6), 8.54 (d, 1H, *J*=2.38, C-2)	152.36 (Ar-OH, C-1), 139.27 (Ar-NH_2, C-3), 135.50 (C-5), 125.71 (C-6), 112.26 (C-2), 80.52 (Ar-I, C-4).

CHAPTER-3

EXPERIMENTAL

EXPERIMENTAL

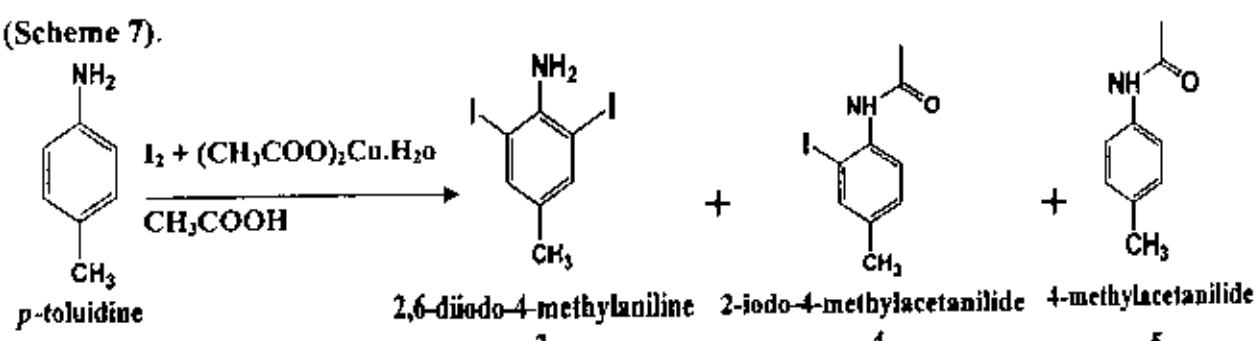
3.1 Chemical reagents and experimental instruments

All commercial reagents were purchased from E. Merck (Germany) and were used without further purification. Thin layer Chromatography (TLC) plate made by Merck silica gel coated was used and visualized by UV lamp (254-365nm). Column chromatography was used for the separation by Merck silica gel (60-120) mesh. All evaporations were carried out with the help of rotary vacuum evaporator (Buchii, Switzerland) at bath temperature 40^0-55^0C. Melting points were determined on Gallenkamp (England) melting point apparatus. Infrared (IR) spectra were obtained in cm^{-1} and recorded by SHIMADZU FTIR Spectrometer using KBr pellet. UV spectra were recorded in dry $CHCl_3$ with Shimatzu visible spectrophotometer and ^1H NMR spectra and ^{13}C NMR spectra were recorded by Bruker Model DPX 400 MHz spectrometer in $CDCl_3$ using as the solvent.

3.2. Iodination of *p*-toluidine

A mixture of *p*-toluidine **13** (5.014g, 0.0468 mol), iodine (12.424g, 0.0489 mol), cupper(II) acetate (9.339g, 0.0468 mol) and acetic acid 220 ml was taken in 500 ml one-neck round-bottom flask and put on the oil-bath and was stirred and refluxed for 9 hr at 120^0C. The completion of the reaction was monitored by TLC. After completion of the reaction, acetic acid was evaporated to dryness and residue was extracted with chloroform (50x3). The product was neutralized by saturated solution of sodium hydrogen carbonate ($NaHCO_3$), free iodine was removed by using sodium thiosulfate ($Na_2S_2O_3$) solution, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure.

The crude product was purified by column chromatography on silica gel with hexane: chloroform in different ratio. Three compounds (**3**, **4**, **5**) were isolated in different ratio (**Scheme 7**).



p-toluidine
13

2,6-diiodo-4-methylaniline
3

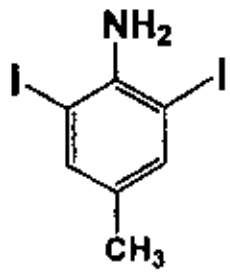
2-iodo-4-methylacetanilide
4

4-methylacetanilide
5

Scheme 7

3.2.1. Study of 2, 6-diiodo-4-methylaniline 3

Structure:



2,6-diiodo-4-methylaniline

3

Compound name: 2, 6-diiodo-4-methylaniline

Synonym: 2,6-diiodo-p-toluidine

Chemical Formula: $C_7H_7I_2N$

Molecular Weight: 358.946

Yield (%): 35

Physical stage: Yellowish amorphous powder.

mp. 110^0C

R_f value: 0.94 n-hexane:chloroform (2:1)

UV($CHCl_3$): λ_{max} 400.35, 202 nm

IR (KBr): ν_{max} 3406.1, 3317.3 (-NH_2), 3037.7, 2898.8 (-CH_3, -CH), 1608.5, 1460, 1566.1, 7001.1 and 846.7 cm^{-1}.

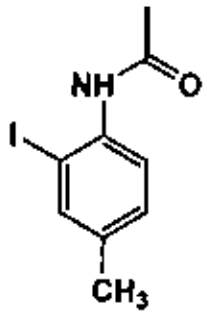
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.45 (s, 2H, C-3 & C-5), δ 4.19 (s, 2H, NH_2), δ 2.15 (s, 3H, Ar-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 143.83 (Ar-NH_2), 139.77 (C-H, C-3 & C-5), 130.97 (C-CH_3, C-4), 81.45 (Ar-I, C-2 & C-6), 19.22 (Ar-CH_3).

Dept-135: 139.77 (C-H, C-3 & C-5), 19.22 (Ar-CH_3).

3.2.2. Study of 2-iodo-4-methylacetanilide 4

Structure:



2-iodo-4-methylacetanilide

4

Compound name: 2-iodo-4-methylacetanilide

Synonym: *N*-(2-iodo-4-methylphenyl)acetamide

Chemical Formula: $C_9H_{10}IN0$

Molecular Weight: 275.086

Yield (%): 26

Physical stage: Brownish crystal

mp. 125-130^{0}C

R_f value: 0.77 n-hexane:chloroform (2:1)

UV($CHCl_3$): λ_{max} 401.85 & 199.99 nm

IR: ν_{max}3265.3 (-NH-), 1654.8 (C=O), 1523.7 (-C=C-) and 1290.3 (C-N) cm^{-1}.

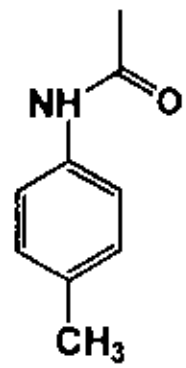
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.99 (d, 1H, *J*=7.96 Hz, C-6), δ 7.59(s, 1H, NH), δ 7.32 (s, 1H, C-3), δ 7.12(d, 1H, *J* = 8.09Hz, C-5), δ 2.26(s, 3H, Ar-CH_3), δ 2.20 (s, 3H, -CO-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 168.15 (C=O), 145.98 (C-NH), 138.96 (C-3), 135.79 (C-CH_3, C-4),129.92 (C-5), 122.12 (C-6), 90.29 (Ar-I, C-2), 24.67 (-CO-CH_3), 20.30 (Ar-CH_3).

Dept-135: 129.92 (C-3), 122.12 (C-5), 138.96 (C-6), 24.67(-CO-CH_3), 20.30 (Ar-CH_3).

3.2.3. Study of 4-methylacetanilide 5

Structure:



4-methylacetanilide

5

Compound name: 4-methylacetanilide.

Synonyms: *p*-acetotoluidide; *N*-(4-methylphenyl)acetamide; *N*-*p*-tolylacetamide.

Chemical Formula: $C_9H_{11}N0$

Molecular Weight: 149.190

Yield (%): 22

Physical state: Brownish crystal

mp. : 148-151^{0}C

R_f value: 0.30 *n*-hexane:chloroform (2:1)

UV($CHCl_3$): λ_{max} 201.04 nm

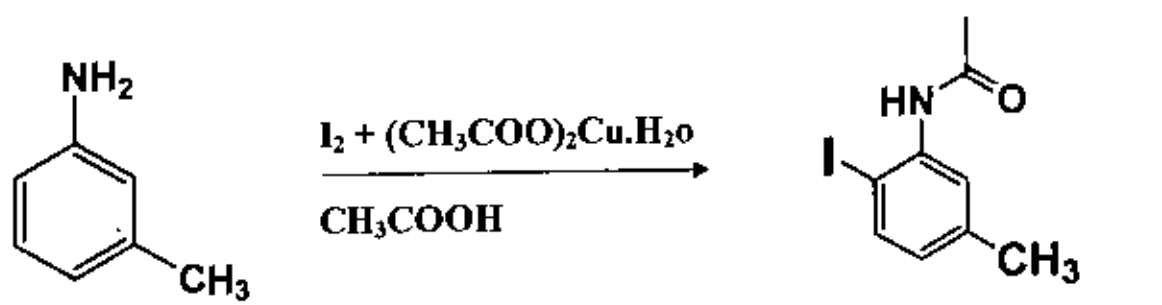
IR (KBr): ν_{max} 3292.3 (-NH-), 3255.6, 1662.5 (-C=O), 1602.7, 1550.7, 1510.2, 1454.2, 1402.2, 1365.5, 1321.1 and 819.7 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.52 (s, 1H, NH), δ 7.35 (d, 2H, CH, *J*=8.23 Hz, C-2 & C-6), δ 7.08 (d, 2H, CH, *J* = 8.07 Hz, C-3 & C-5), δ 2.28 (s, 3H, Ar-CH_3), δ 2.21 (s, 3H, -$COCH_3$).

3.3. Synthesis of 2-iodo-5-methylacetanilide 6

A mixture of *m*-toluidine 14 (5.418g, 0.0506 mol), iodine (12.934g, 0.0509 mol), cupper(II) acetate (10.468g, 0.0524 mol) and acetic acid 230 ml was taken in 500 ml one-neck round-bottom flask and put on the oil-bath and was stirred with refluxed for 10 hr at 130^{0}C. The completion of the reaction was monitored by TLC and completion of the reaction, acetic acid was evaporated to dryness and was extracted with chloroform. The product was neutralized by a saturated solution of sodium hydrogen carbonate ($NaHCO_3$), free iodine was removed by using sodium thiosulfate solution, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure.

The crude product was purified by column chromatography on silica gel with hexane: chloroform in different ratio. One compound **6** was isolated (**Scheme 8**).



***m*-toluidine**

14

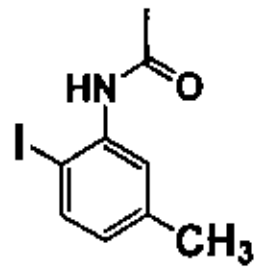
2-iodo-5-methylacetanilide

6

Scheme 8

3.3.1. Study of 2-iodo-5-methylacetanilide 6

Structure:



2-iodo-5-methylacetanilide

6

Compound name: 2-iodo-5-methylacetanilide.

Synonyms: *N*-(2-iodo-5-methylpheny)acetamide.

Chemical Formula: $C_9H_{10}IN0$

Molecular Weight: 275.086

Yield (%): 55

Physical state: Brownish crystal

R_f value: 0.73 n-hexane:chloroform (2:1)

UV($CHCl_3$): λ_{max} 400.99 & 201.08 nm

IR (KBr): ν_{max}3315.4 (-NH-), 1668.3 (-C=O),1606.6, 1581.5, 1533.3, 1471.6, 1396.4, 1311.5, 819.7.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.67 (d, 1H, J = 8.5 Hz, C-3), δ 7.46 (s, 1H, NH), δ 7.42 (d, 1H, J = 1.94 Hz, C-6), δ 7.03 (dd, 1H, J = 2.1 Hz, 8.3 Hz, C-4), δ 2.36 (s, 3H, Ar-CH_3), δ 2.13 (s, 3H, $COCH_3$).

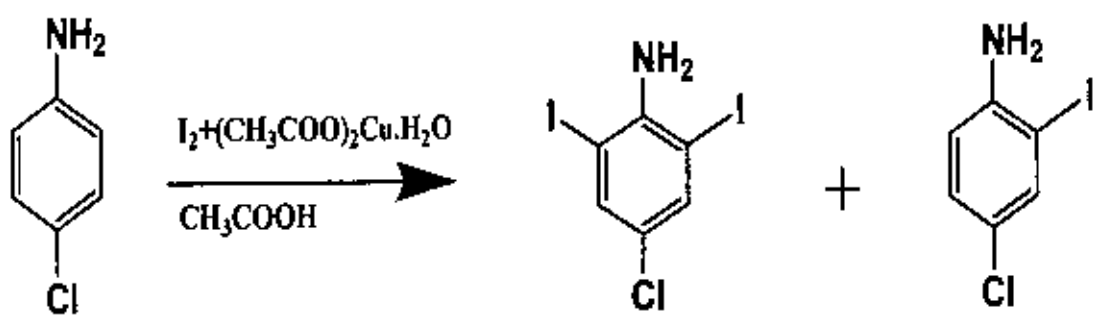
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 168.52 (C=O), 142.03 (C-NH). 139.11 (C-3), 138.11 (C-CH_3),121.11 (C-6), 119.02 (C-4), 94.43 (Ar-I), 28.13 ($COCH_3$), 24.58 (Ar-CH_3).

Dept-135: 139.11 (C-3), 121.11 (C-6), 119.02 (C-4), 28.13 ($COCH_3$), 24.58 (Ar-CH_3).

3.4. Synthesis of 4-chloro-2, 6-diiodoaniline & 4-chloro-2-iodoaniline 7,8

A mixture of 4-chloroaniline **15** (10.152g, 0.0796 mol), iodine (20.256g, 0.0797 mol), cupper(II) acetate (16.896g, 0.0846 mol) and acetic acid 170 ml were taken in 500 ml one-neck round-bottom flask and put on the oil-bath and was stirred with reflux for11 hr at 130^{0}C. The progress of the reaction was monitored by TLC (n-hexane/chloroform 3:1). After completion of the reaction, the mixture was then evaporated to dryness under reduced pressure and the residue was then evaporated to dryness under reduced pressure and the residue was extracted with chloroform (3x50 mL). The chloroform extract was neutralized by a saturated solution of sodium hydrogen carbonate ($NaHCO_3$), free iodine was removed by using sodium thiosulfate solution, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain brownish black semisolid.

The latter was purified by chromatography on a column of silica gel with n-hexane, n-hexane /chloroform 2:1 and two compounds (**7, 8**) were isolated (**Scheme 9**).



4-chloroaniline **15**

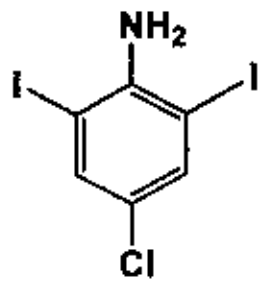
4-chloro-2,6-diiodoaniline **7**

4-chloro-2-iodoaniline **8**

Scheme 9

3.4.1. Study of 4-chloro -2, 6-diiodoaniline 7

Structure:



4-chloro-2,6-diiodoaniline

7

Compound name:. 4-chloro-2, 6-diiodoaniline

Chemical Formula: $C_9H_4ClI_2N$

Molecular Weight: 379.365

Yield (%): 30

mp. : 127-129 ^{0}C

Physical state: yellowish amorphous powder

R_f value: 0.98 n-hexane:chloroform (3:1)

UV($CHCl_3$): λ_{max} 400.89 and 199.92 nm

IR (KBr): ν_{max}3408.0 & 3317.3 (-NH_2), 1604.7, 1442.7, 860.2 (Ar-Cl).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.60 (s, 2H, C-3 & C-5), δ 4.59 (s, 2H, NH_2).

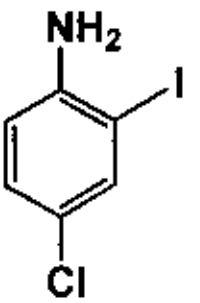
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 145.16 (C-NH_2), 138.33 (C-3 & C-5), 123.25 (C-Cl),

80.25 (C-I).

Dept-135: 138.33 (C-3 & C-5).

3.4.2. Study of 4-chloro-2-iodoaniline 8

Structure:



4-chloro-2-iodoaniline

8

Compound name:.4-chloro-2-iodoaniline

Chemical Formula: C_9H_5ClIN

Molecular Weight: 253.468

Yield (%): 45

mp. : 40-42 ^{0}C

Physical state: Light brownish white ash crystal

R_f value: 0.87 n-hexane:chloroform (3:1)

UV($CHCl_3$): λ_{max} 400.75 and 200.51 nm

IR (KBr): ν_{max}3408.1 &3317.3 (-NH_2), 1603.7, 1443.6, 868.0 (Ar-Cl).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.58 (d, 1H, J = 2.3Hz, C-3), 7.08 (dd, 1H, J= 2.31Hz, 8.5Hz, C-5), 6.64 (d, 1H, J= 7.1Hz, C-6), 4.07 (s, 2H, NH_2).

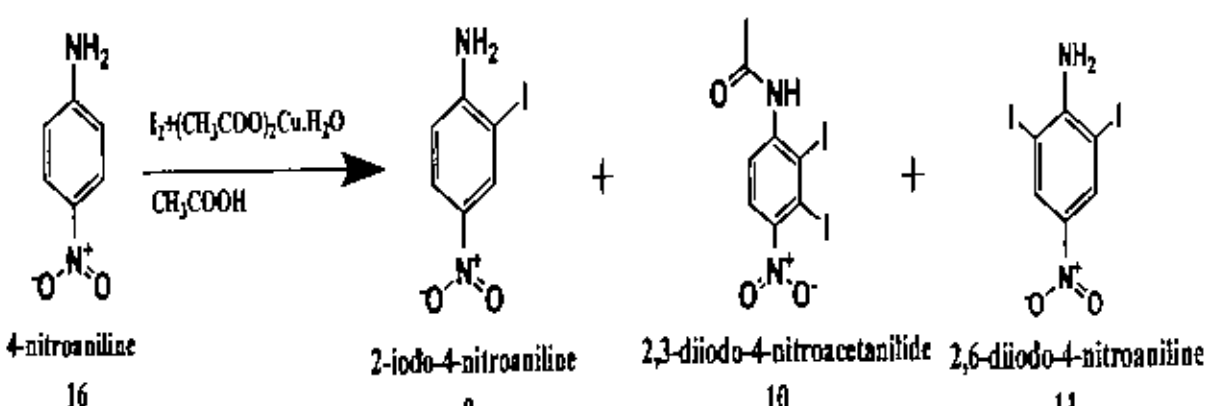
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 145.57 (C-NH_2), 137.78 (C-3), 129.26 (C-5), 123.18 (C-Cl), 114.99 (C-6), 83.46 (C-I).

Dept-135: 137.78 (C-3), 129.26 (C-5), 114.99 (C-6).

3.5. Synthesis of 2-iodo-4-nitroaniline &2, 3-diiodo-4-nitroacetanilide 9, 10, 11

A mixture of 4-nitroaniline **16** (7.865g, 0.0569 mol), iodine (20.078 g, 0.0790 mol), cupper(II) acetate (12.052g, 0.0604 mol) and acetic acid 300 ml was taken in 500 ml one-neck round-bottom flask and put on the oil-bath and was stirred with reflux for 10hr at 130^{0}C. The progress of the reaction was monitored by TLC (n-hexane/chloroform 1:2). After completion of the reaction, the mixture was then evaporated to dryness under reduced pressure and the residue was extracted with chloroform (3x50 mL). The chloroform extract was neutralized by a saturated solution of sodium hydrogen carbonate ($NaHCO_3$), free iodine was removed by using sodium thiosulfate solution, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain brownish black semisolid.

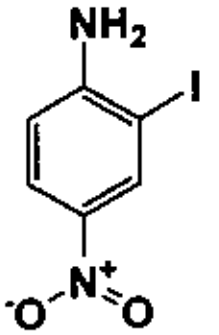
The latter was purified by chromatography on a column of silica gel with n-hexane, n-hexane /chloroform 2:1, chloroform, acetone and two compound (**9, 10, 11**) were isolated **(Scheme 10)**.



Scheme 10

3.5.1. Study of 2, 3-diiodo-4-nitroacetanilide 9

Structure:



2-iodo-4-nitroaniline

9

Compound name: 2-iodo-4-nitroaniline

Synonyms: 2-iodo-4-nitrobenzenamine

Chemical Formula: $C_6H_5IN_2O_2$

Molecular Weight: 264.021

Yield (%): 30

mp. : 105-109^{0}C

Physical state: Yellowish crystal

R_f value: 0.83 n-hexane : chloroform (1:2)

UV($CHCl_3$): λ_{max} 240.00 & 200.00 nm

IR (KBr): ν_{max} 3477.4 & 3371.3 (-NH_2), 1608.5,1488.9 (-NO_2), 1301.9, 1257.5, 1114.8.

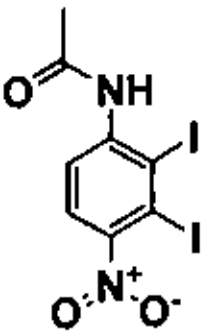
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.70 (d, 1H, Ar-H, *J*=2.36Hz, C-3), δ 8.64 (dd, 1H, Ar-H, *J*=2.37 Hz, 8.91 Hz, C-5), δ 6.68 (d, 1H, Ar-H, *J*=8.932 Hz, C-6), δ 4.83 (s, 2H, NH_2).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 152.33 (C-NH_2), 139.33 (C-NO_2), 80.53 (C-I), 135.51 (C-3), 125.72 (C-5), 112.27 (C-6).

Dept-135: 135.51 (C-3), 125.72 (C-5), 112.27 (C-6).

3.5.2. Study of 2, 3-diiodo-4-nitroacetanilide 10

Structure:



2,3-diiodo-4-nitroacetanilide
10

Compound name:. 2, 3-diiodo-4-nitroacetanilide

Synonyms: *N*-(2, 3-diiodo-4-nitrophenyl)acetamide

Chemical Formula: $C_8H_6I_2N_2O_3$

Molecular Weight: 431.954

Yield (%): 26

Physical state: Yellowish crystal

R_f value: 0.47 n-hexane : chloroform (1:2)

UV($CHCl_3$): λ_{max} 320.00 & 202.14 nm

IR (KBr): ν_{max}3276(-NH-), 1683.7 (-C=O), 1618.2, 1596.9, 1566.1, 1506.3 ($-NO_2$), 1346.2, 1332.7, 1301.9, 1267.1, 1112.9, 848.6, 750.3.

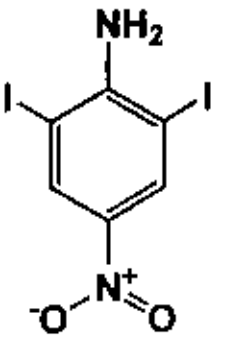
^{1}H NMR (400 MHz, $CDCl_3$): δ_H δ 8.19 (d, 1H, Ar-H, *J*=9.01Hz, C-5), δ 7.68 (d, 1H, Ar-H, *J*=8.9Hz, C-6), δ 7.47 (s, 1H, -NH), 2.23 (s, 3H, $-CH_3$) .

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 168.528 (-C=O), 143.631 (C-NH), 142.039 (C-NO_2), 125.138 (C-5), 118.988 (C-6), 94.431 (C-I, C-2), 90.291 (C-I, C-3), 24.837 ($COCH_3$)

Dept-135: 125.138 (C-5), 118.988 (C-6), 24.837 ($COCH_3$).

3.5.3. Study of 2, 6-diiodo-4-nitroacetanilide 11

Structure:



2,6-diiodo-4-nitroaniline

11

Compound name:. 2, 6-diiodo-4-nitroaniline

Chemical Formula: $C_6H_4I_2N_2O_2$

Molecular Weight: 389.917

Yield (%): 38

Physical state: Yellow crystal

mp.: 249-250^{0}C

R_f value: 0.38 n-hexane : chloroform (1:2)

UV($CHCl_3$): λ_{max} 322.01 & 204.09 nm

IR (KBr): ν_{max} 3479.5 & 3373.1 (-NH_2), 1609.6, 1489.5 (-NO_2), 1302.7, 1257.5, 1118.5.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.336 (s, 2H, -NH_2), δ 8.545 (s, 2H, C-3 &C-5).

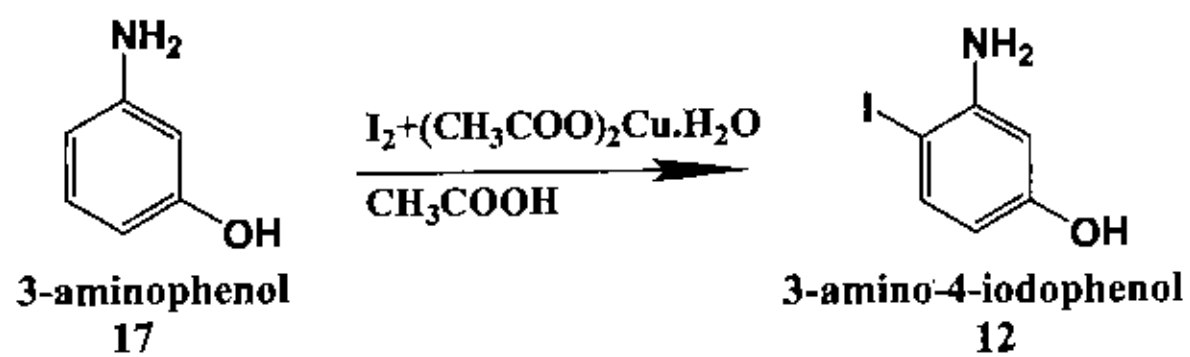
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 152.338 (C-NH_2), 143.832 (C-NO_2), 135.315 (C-3 &C-5)
94.431 (Ar-I, C-2 & C-6)

Dept-135: 135.315 (C-3 &C-5)

3.6. Synthesis of 3-amino-4-iodophenol 12

A mixture of 3-aminophenol **17** (8.908g, 0.0816 mol), iodine (23.366g, 0.0920 mol), cupper(II) acetate (18.438g, 0.0924 mol) and acetic acid 120 ml was taken in 500 ml one-neck ronnd-bottom flask and put on the oil-bath and was stirred with refluxed for 6 hr at 120°C. The progress of the reaction was monitored by TLC (n-hexane/chloroform 1:3). After completion of the reaction, the mixture was then evaporated to dryness under reduced pressure and the residue was extracted with chloroform (3x50 mL). The chloroform extract was neutralized by a saturated solution of sodium hydrogen carbonate ($NaHCO_3$), free iodine was removed by using sodium thiosulfate solution, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain brownish black semisolid.

The latter was purified by chromatography on a column of silica gel with n-hexane, chloroform and one compound (**12**) was isolated **(Scheme 11)**.



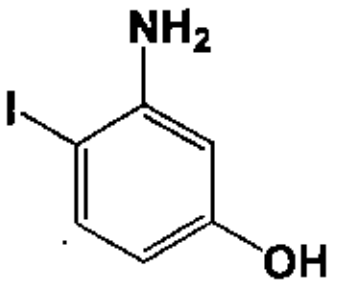
3-aminophenol
17

3-amino-4-iodophenol
12

Scheme 11

3.6.1. Study of 3-amino-4-iodophenol 12

Structure:



3-amino-4-iodophenol

12

Compound name: 3-amino-4-iodophenol

Chemical Formula: C_6H_6INO

Molecular Weight: 235.022

Yield (%): 60

Physical state: Bright yellow crystal

R_f value: 0.68 n-hexane : chloroform (1:2)

UV($CHCl_3$): λ_{max} 401.87 & 202.55 nm

IR (KBr): ν_{max} 3477.4&3371.3 (-NH_2), 3198.2 (Ar-OH), 3107.0, 1606.6, 1488.9, 1301.9, 1257.5, 1114.8.

^{1}H NMR (400 MHz, $CDCl_3$): 1.57 (s, 1H, Ar-OH), 4.84 (s, 2H, NH_2), 6.68 (d, 1H, *J*=8.94 Hz, C-5), 8.03 (dd, 1H, *J*=2.39Hz, 8.9Hz, C-6), 8.54 (d, 1H, *J*=2.38 Hz, C-2).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 152.36 (C-OH), 139.27 (C-NH_2), 135.50 (C-5), 125.71 (C-6), 112.26 (C-2), 80.52 (C-I).

Dept-135: 135.50 (C-5), 125.71 (C-6), 112.26 (C-2).

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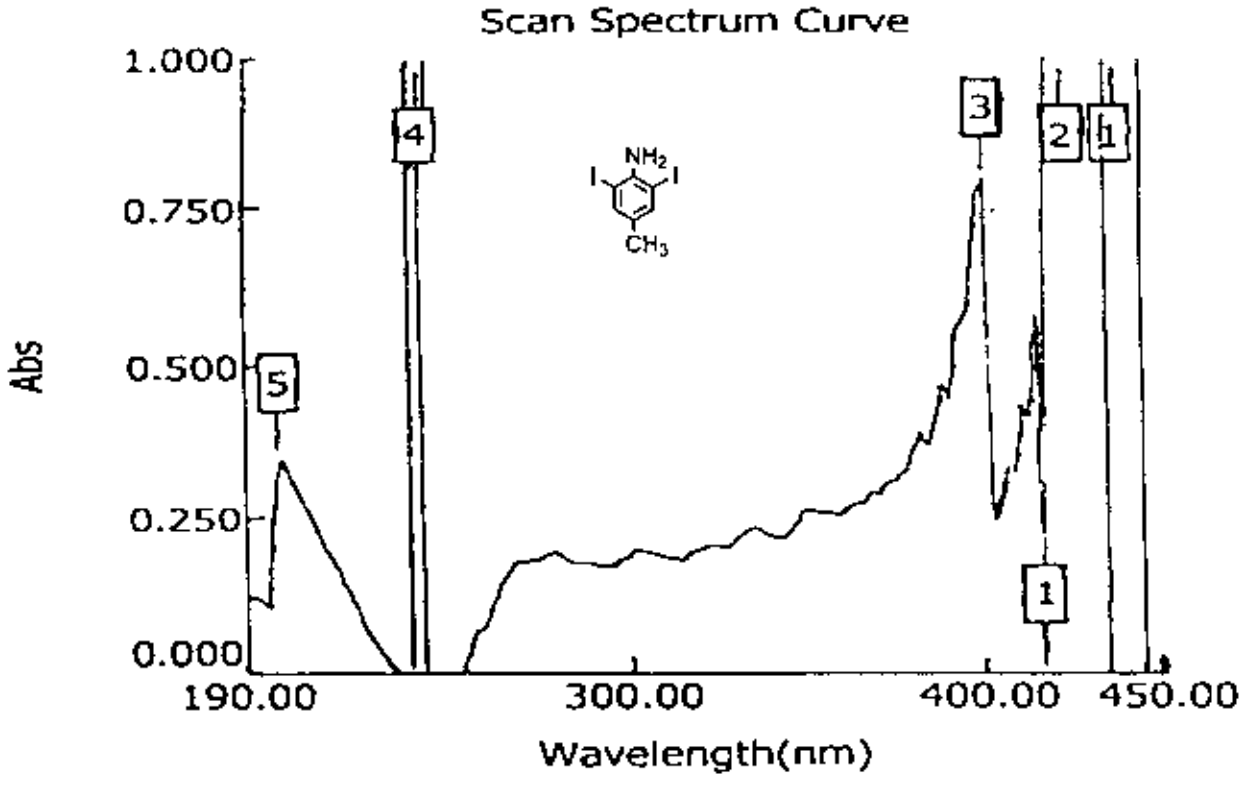

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- Analyse Note
  Analyser : Administrator
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  Comment :
- No. P/V Wavelength(nm) Abs Comment

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3	Peak	400.35	0.798
4	Peak	239.98	9.999
5	Peak	202.00	0.350
1	Valley	415.99	0.000

Figure 13a: UV spectrum of the compound 3

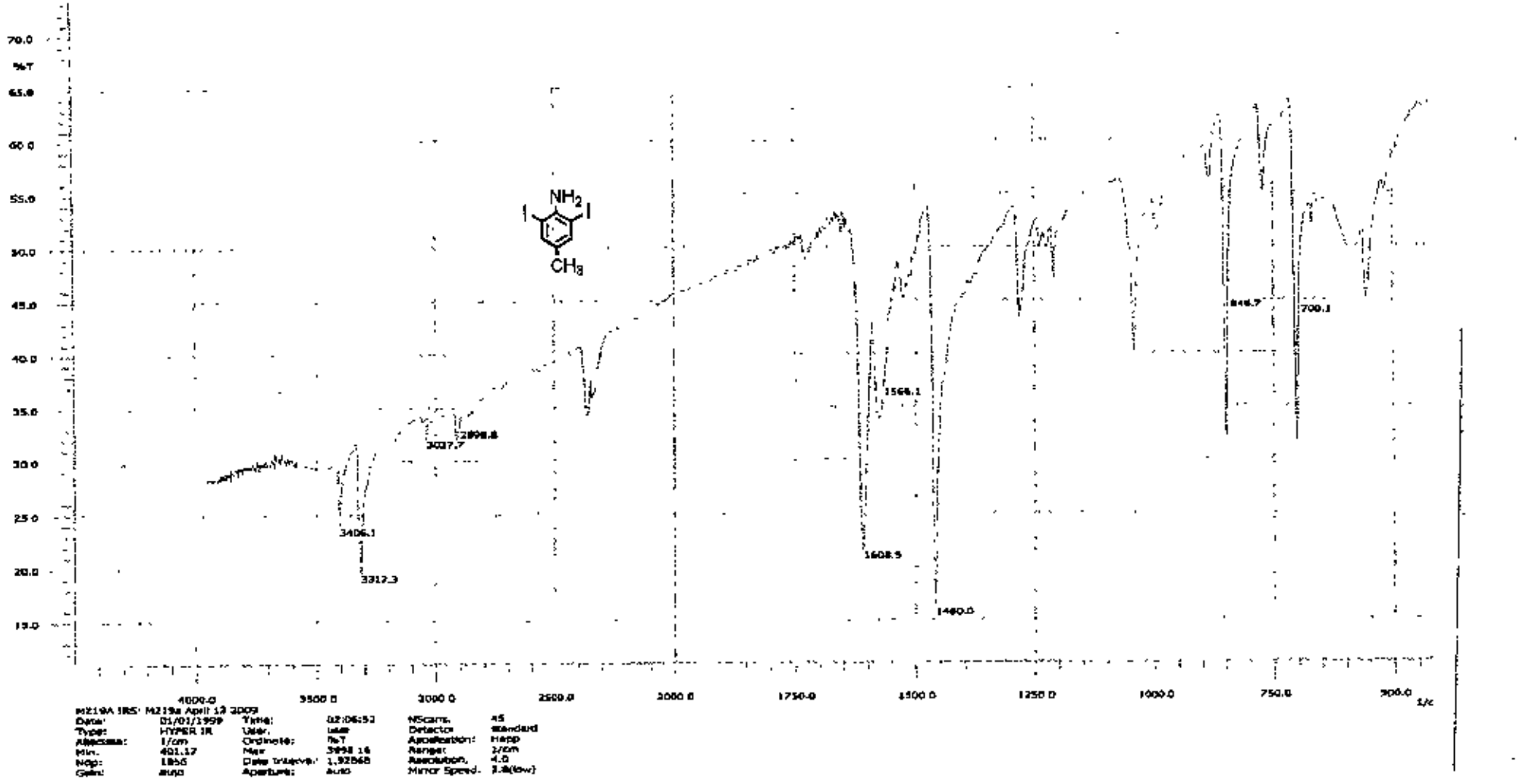
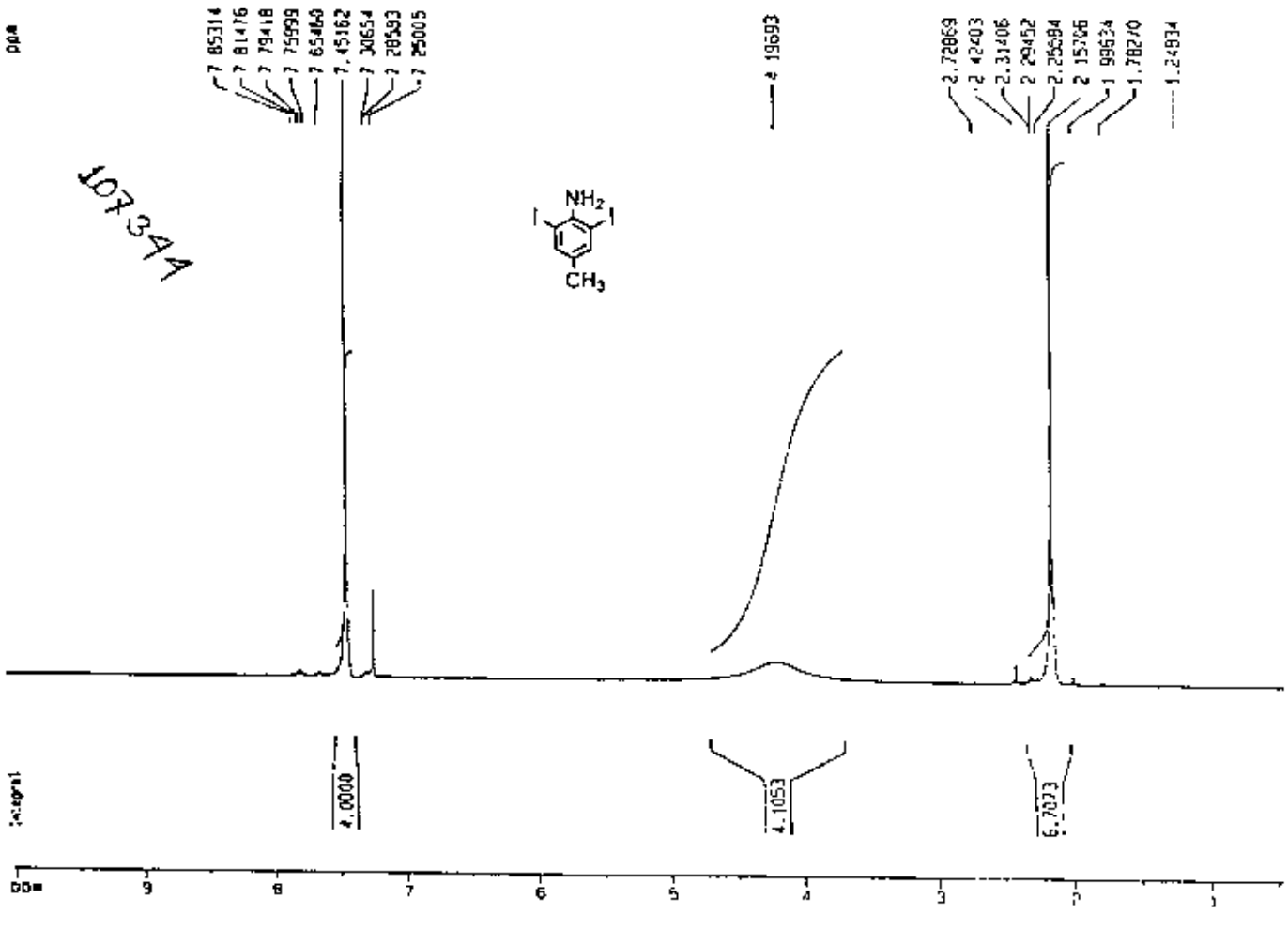


Figure 13b: IR spectrum of the compound 3

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P1                      8.30 usec
PL1                    -6.00 dB
SFO1              400.1428010 MHz

F2 - Processing parameters
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WDW                       EM
SSB                        0
LB                   0.30 Hz
GB                         0
PC                      1.40

1D NMR plot parameters
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F1                  4024.56 Hz
F2P                   0.448 ppm
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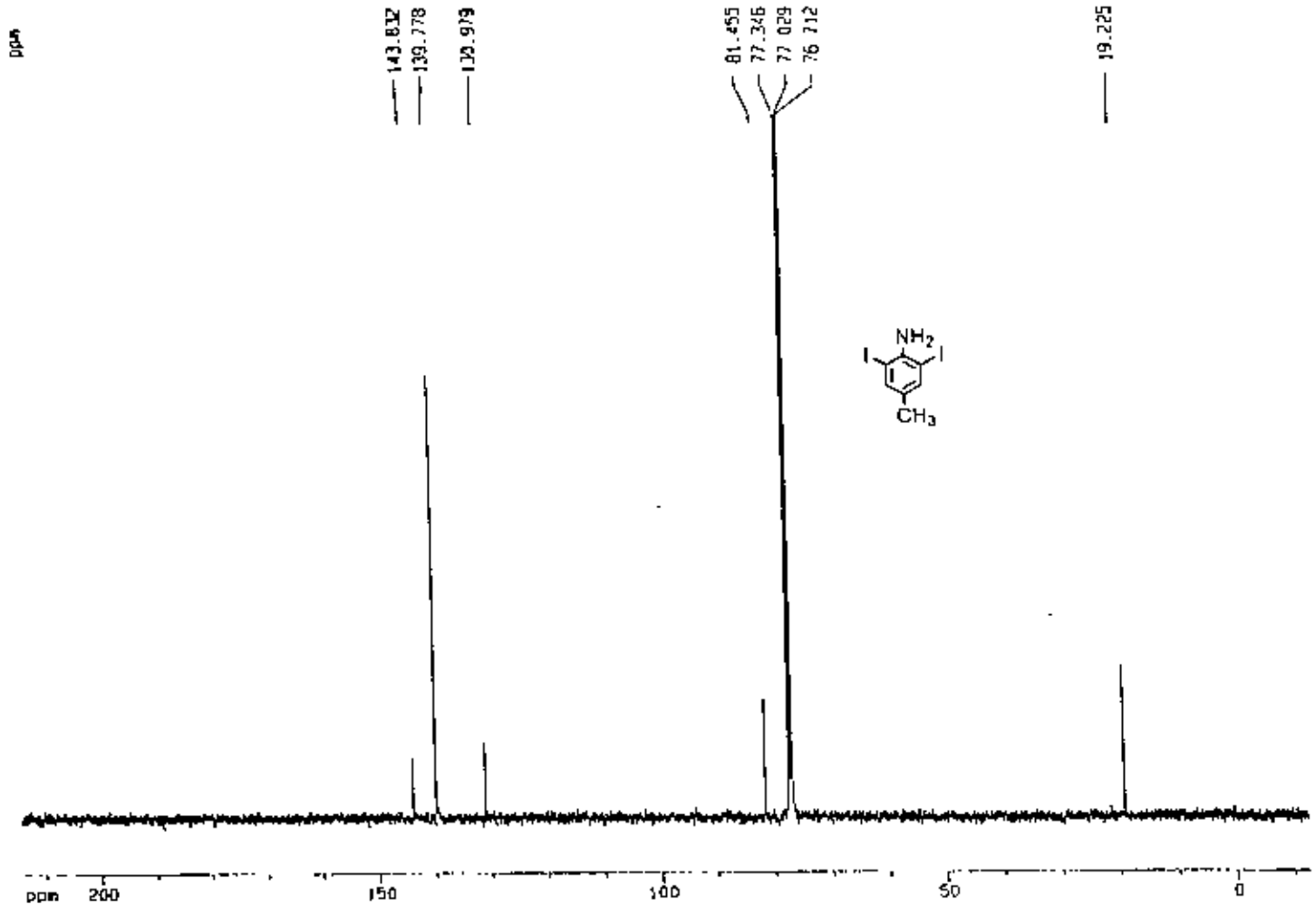
Figure 13c: ^{1}H NMR spectrum of the compound 3



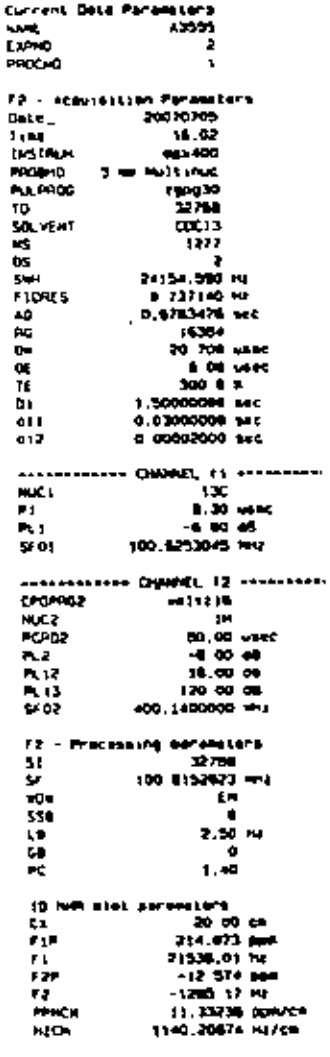
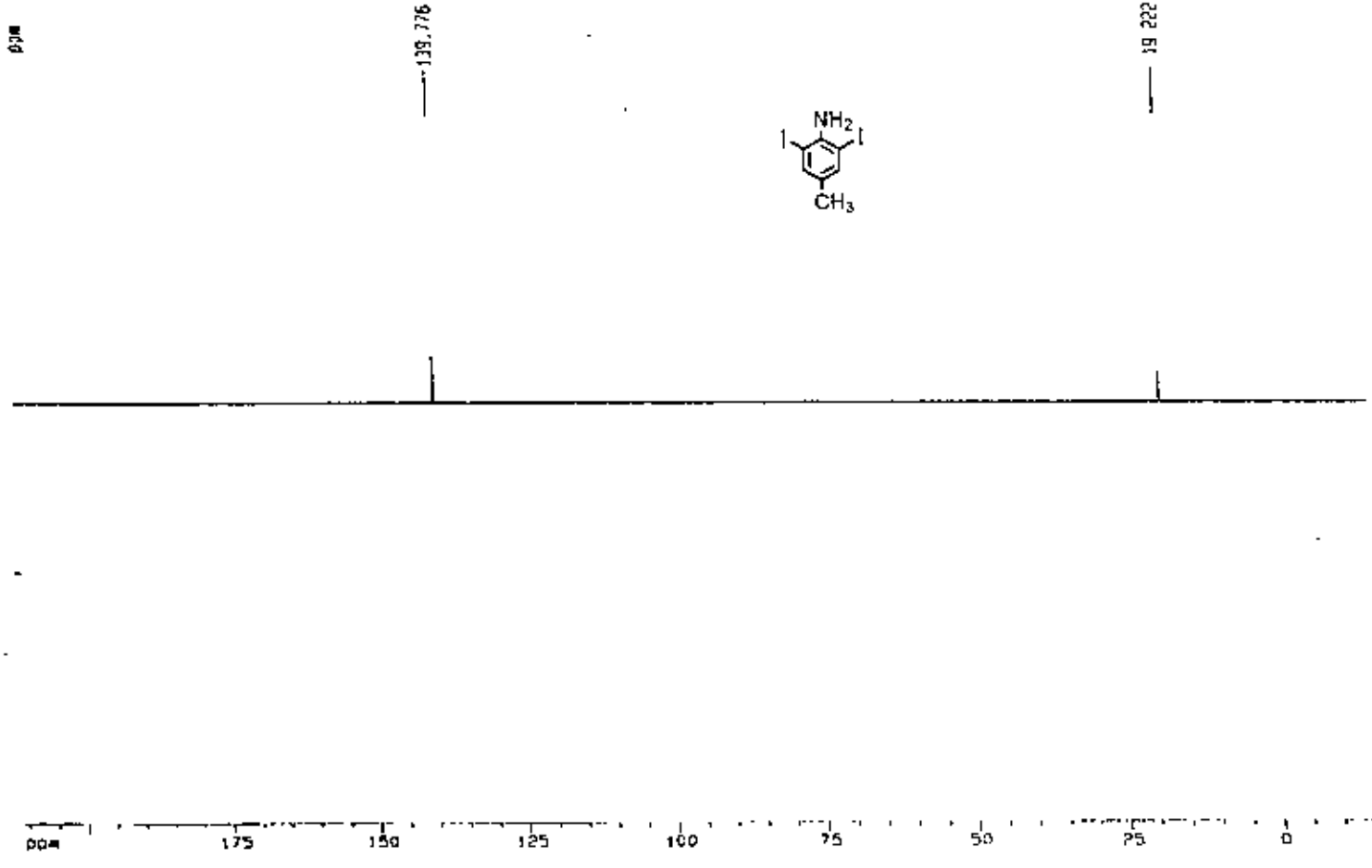


Figure 13d: ^{13}C NMR spectrum of the compound 3



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AQ 0.6783476 sec
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DE 6.00 usec
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d2 0.00344828 sec
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PL1 -6.00 dB
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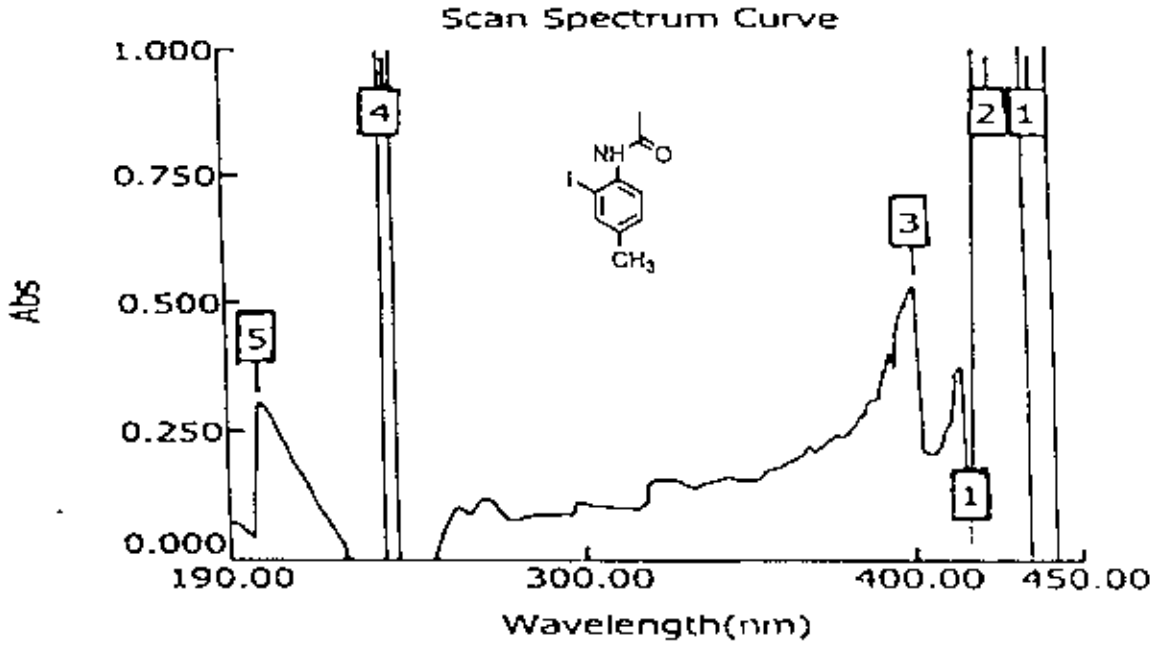
P3 8.50 usec
P4 16.60 usec
PCPD2 80.00 usec
PL2 -6.00 dB
PL12 16.00 dB
SFO2 400.1420007 MHz

F2 - Processing parameters
SI 32768
SF 100.6152827 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 211.838 ppm
F1 21233.88 Hz
F2P -16.857 ppm
F2 -1694.66 Hz
PPMCM 11.29437 ppm/cm
HZCM 1136.38672 Hz/cm

Figure 13d: Dept-135 NMR spectrum of the compound 3



- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 450.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled10.sod
  Create Date/Time : Monday, August 24, 2009 4:01:30 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**

1	Peak	437.21	9.999
2	Peak	425.32	9.999
3	Peak	401.85	0.534
4	Peak	240.05	9.999
5	Peak	199.99	0.307
1	Valley	416.89	0.000

Figure 14a: UV spectrum of the compound 4

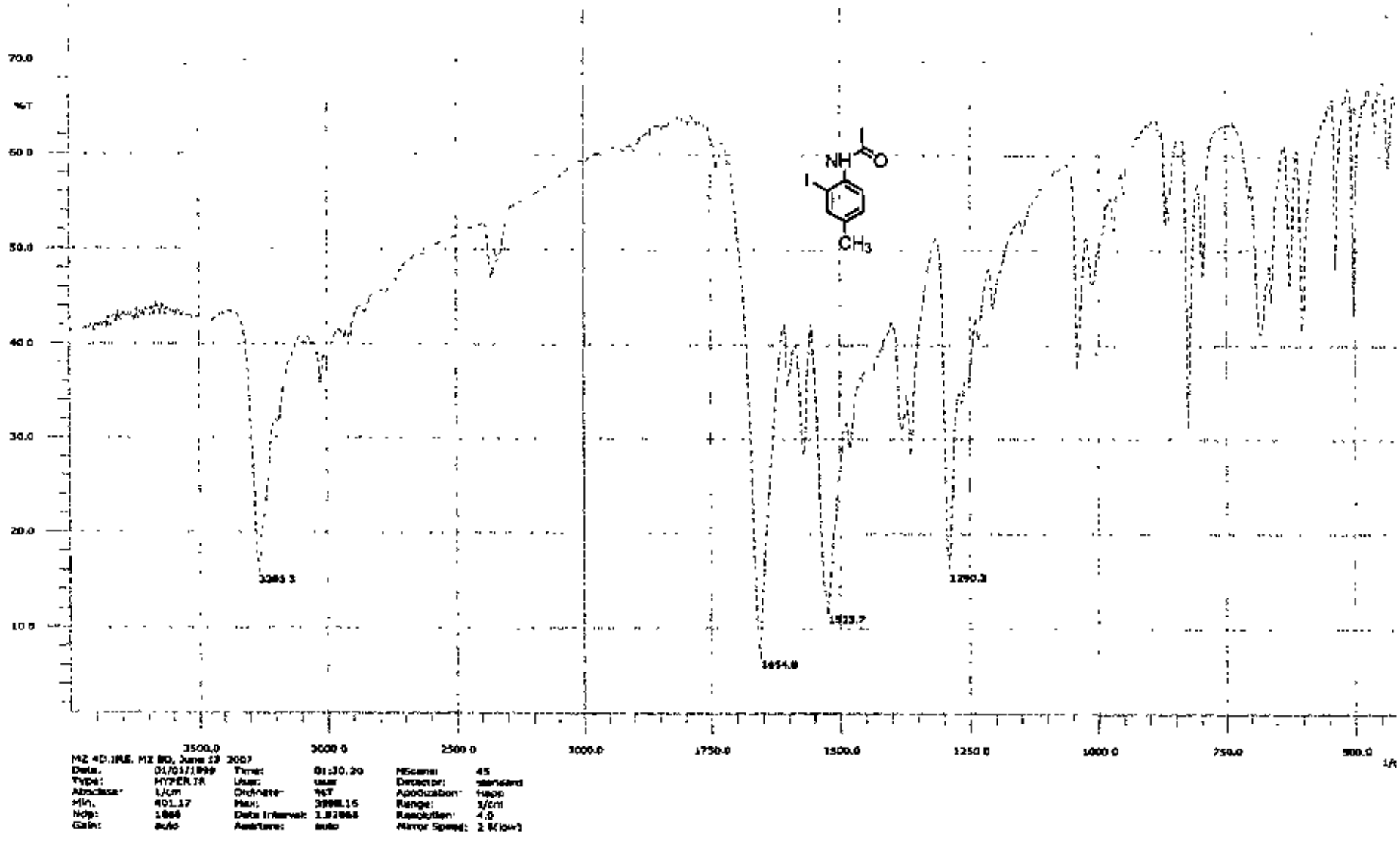
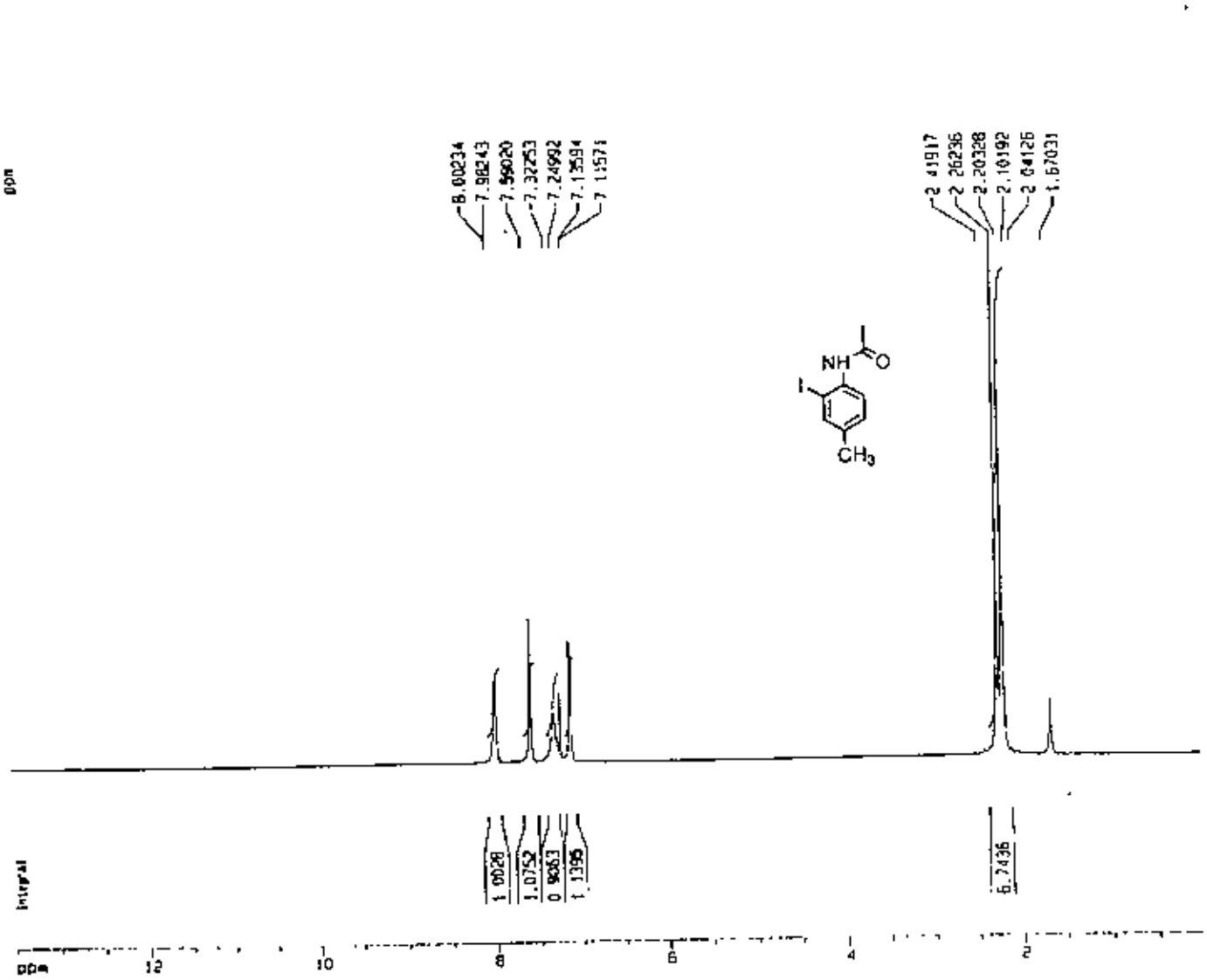


Figure 14b: IR spectrum of the compound 4

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Current Data Parameters
NAME                  A3529
EXPNO                     1
PROCNO                    1

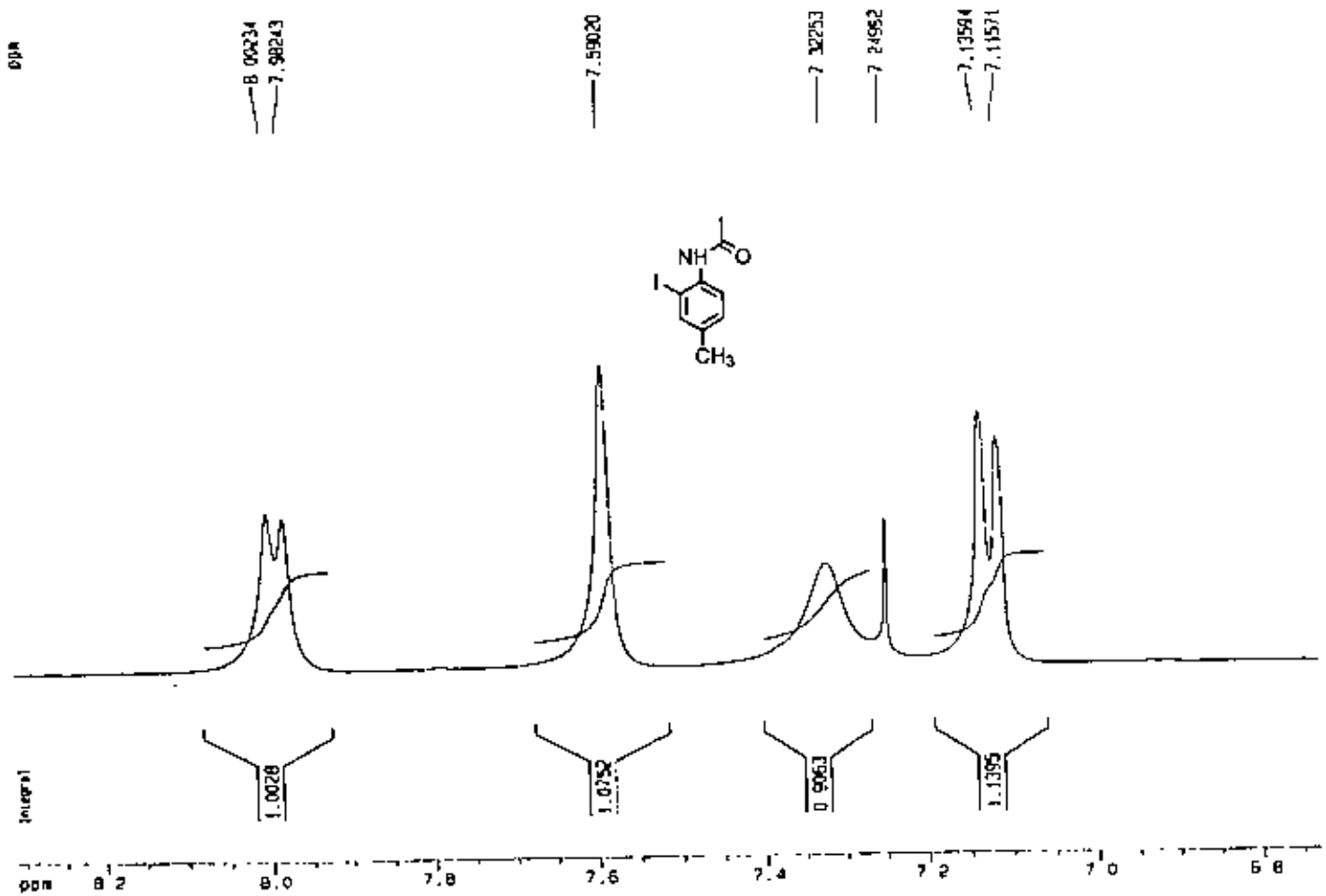
F2 - Acquisition Parameters
Date_              20070811
Time                  11.17
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                      128
DS                        2
SWH               6410.256 Hz
FIDRES            0.195825 Hz
AQ                2.5559540 sec
RG                      181
DW                 78.000 usec
DE                  6.00 usec
TE                 310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 -------
NUC1                      1H
P1                     8.30 usec
PL1                   -6.00 dB
SFO1              400.1428010 MHz

F2 - Processing parameters
SI                        32768
SF                400.1400126 MHz
WDW                          EM
SSB                           0
LB                     0.30 Hz
GB                            0
PC                         1.40

1D NMR plot parameters
CX                    20.00 cm
F1P                   13.525 ppm
F1                  5411.94 Hz
F2P                   0.023 ppm
F2                    9.20 Hz
PPMCM                 0.67511 ppm/cm
HZCM                270.13687 Hz/cm
```

Figure 14c: ^{1}H NMR spectrum of the compound 4




```
Current Data Parameters
NAME                  A3529
EXPNO                     1
PROCNO                    1

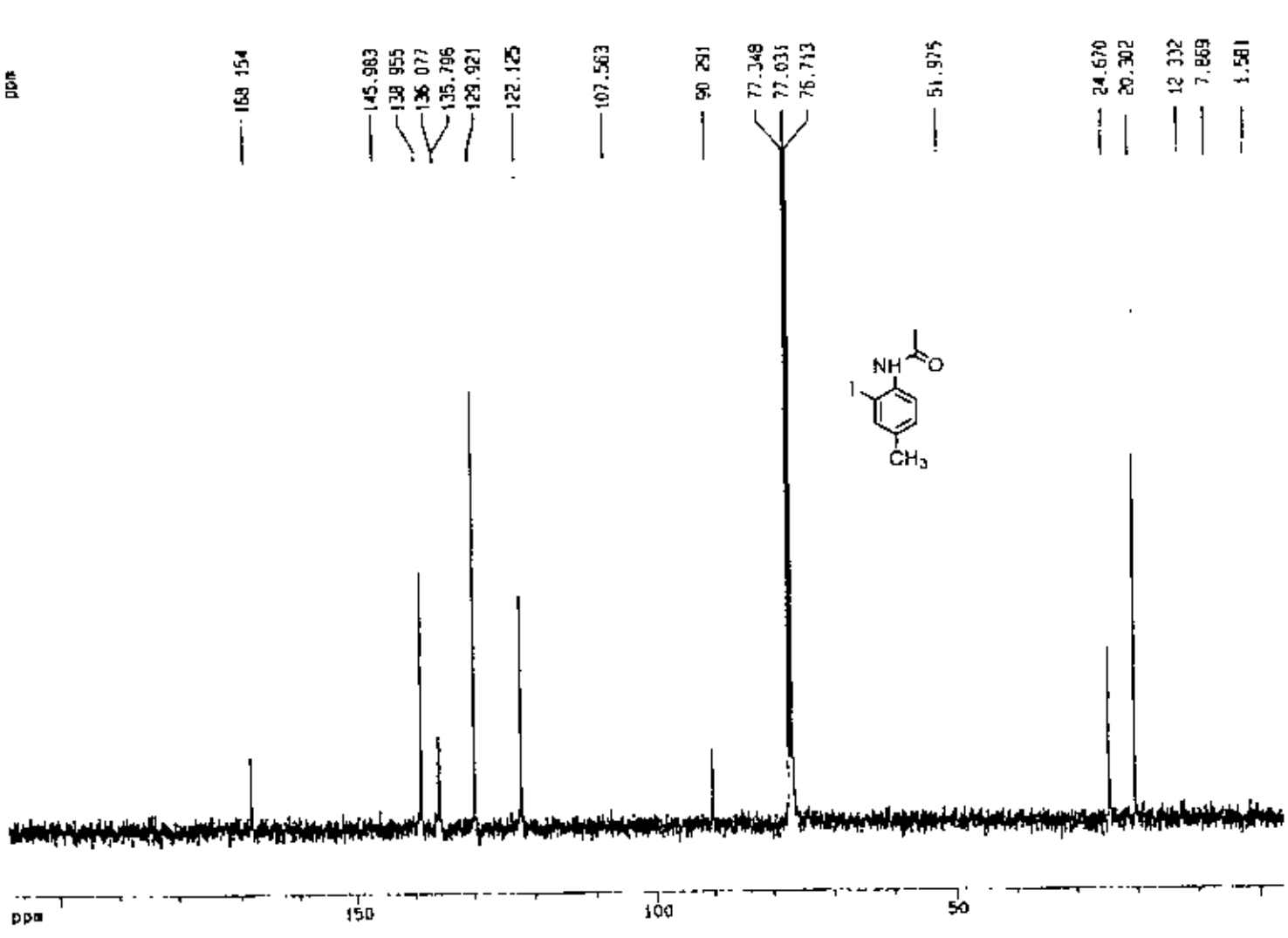
F2 - Acquisition Parameters
Date_              20070611
Time                 11.17
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                     181
DW                 78.000 usec
DE                    6.00 usec
TE                   310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 --------
NUC1                     1H
P1                    8.30 usec
PL1                  -5.00 dB
SFO1            400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF              400.1400126 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                  8.312 ppm
F1                3328.08 Hz
F2P                  5.731 ppm
F2                2293.48 Hz
PPMCM             0.07905 ppm/cm
HZCM             31.62995 Hz/cm
```

Figure 14c: ^{1}H NMR spectrum of the compound 4 (Expansion)



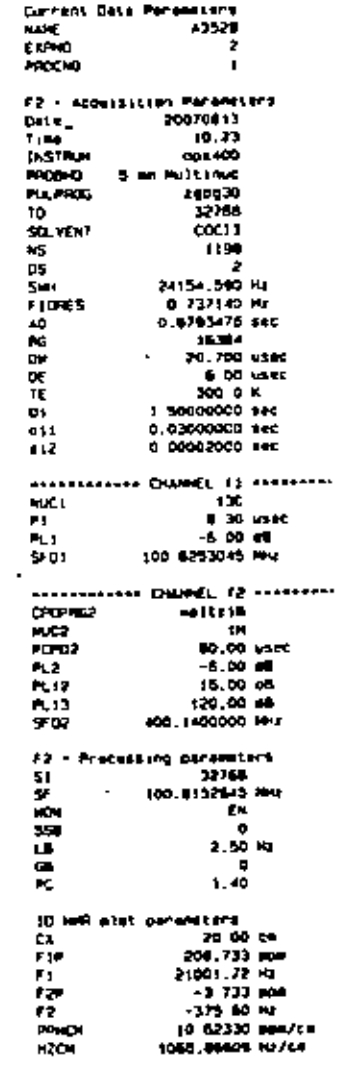


Figure 14d: ^{13}C NMR spectrum of the compound 4

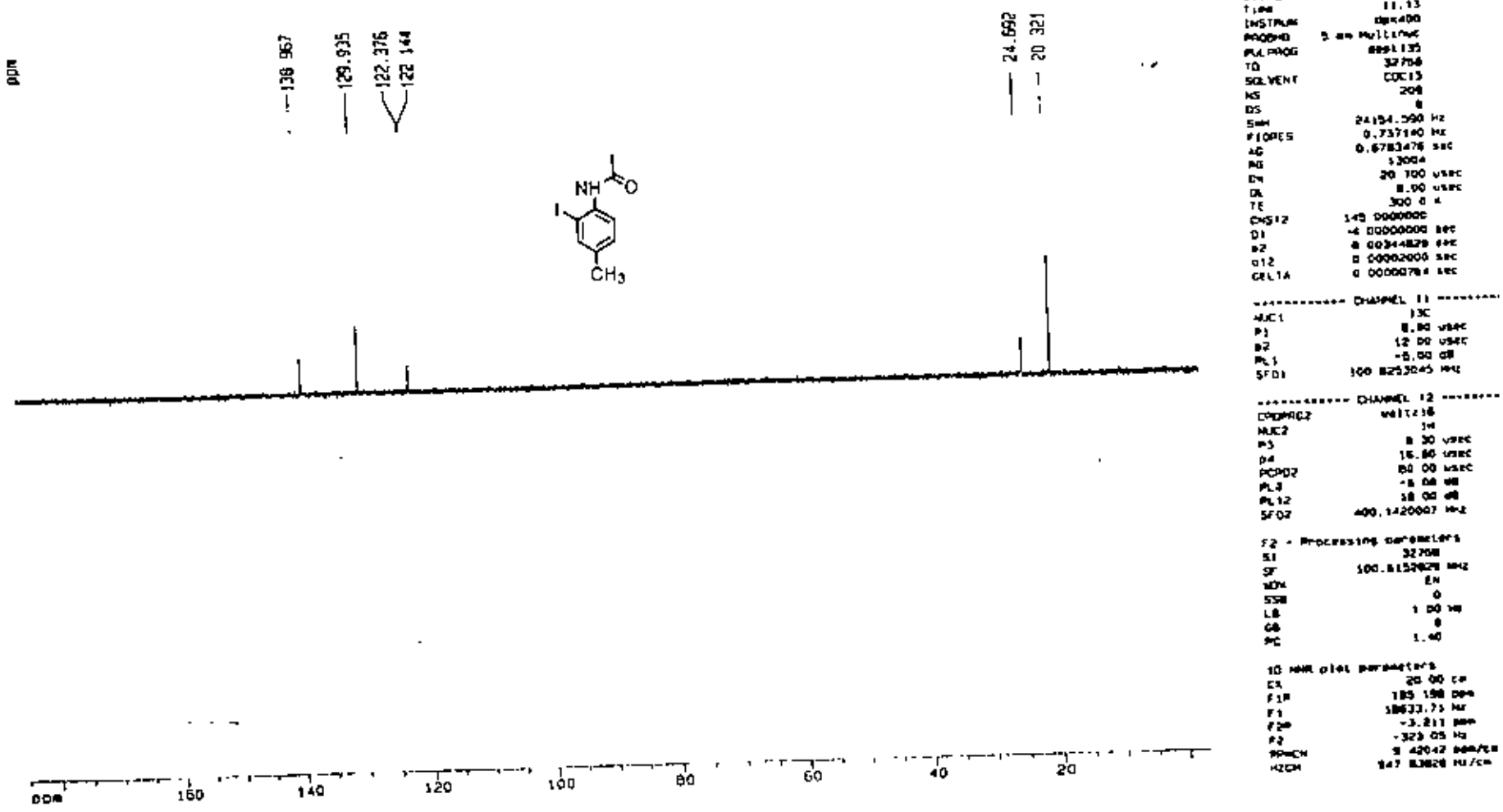
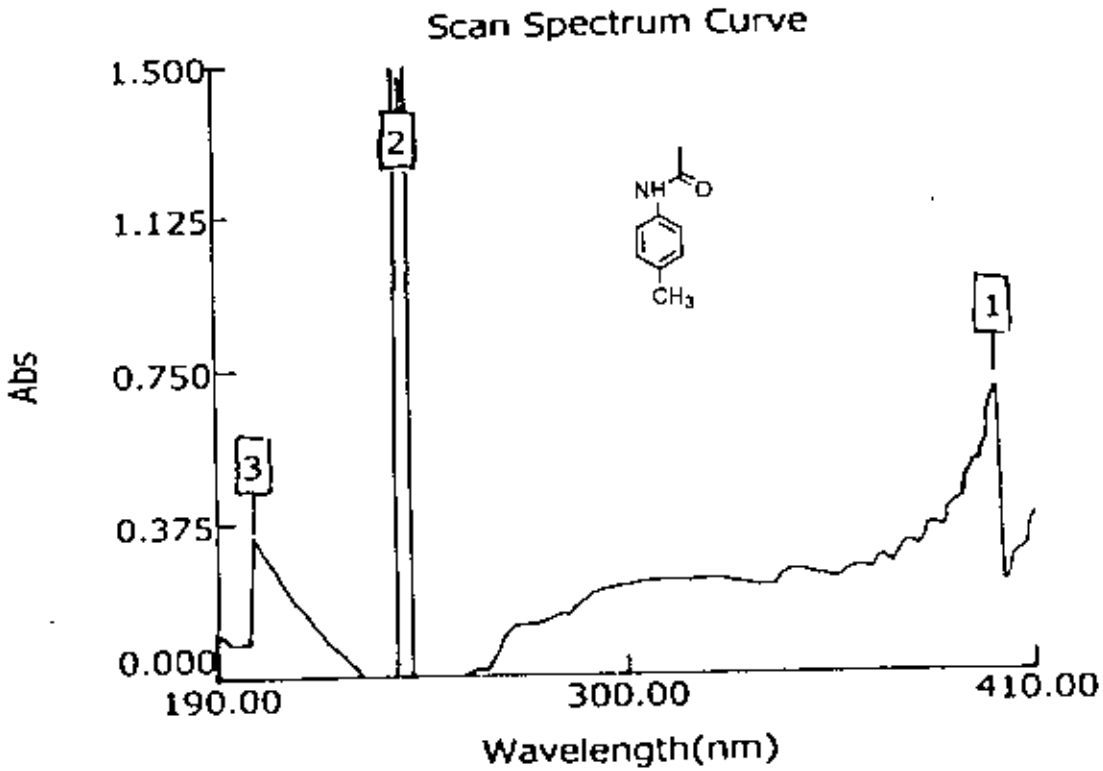


Figure 14d: Dept-135 NMR spectrum of the compound 4



- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 410.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled4.spd
  Create Date/Time : Monday, August 24, 2009 3:48:48 PM
  Data Type : Original
  Method File.
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	401.09	0.695	
2	Peak	241.36	9.999	
3	Peak	201.04	0.337	

Figure 15a: UV spectrum of the compound 5

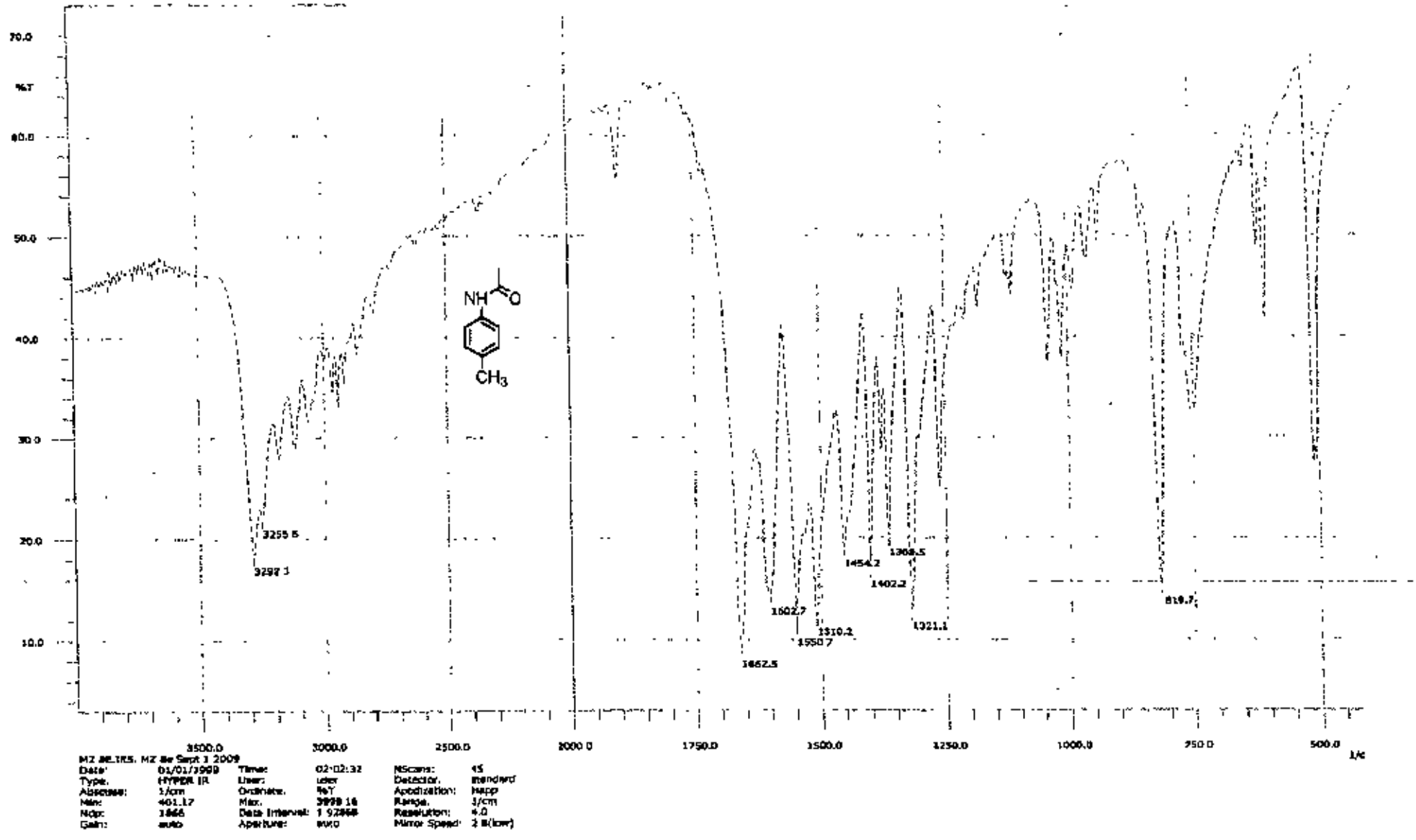
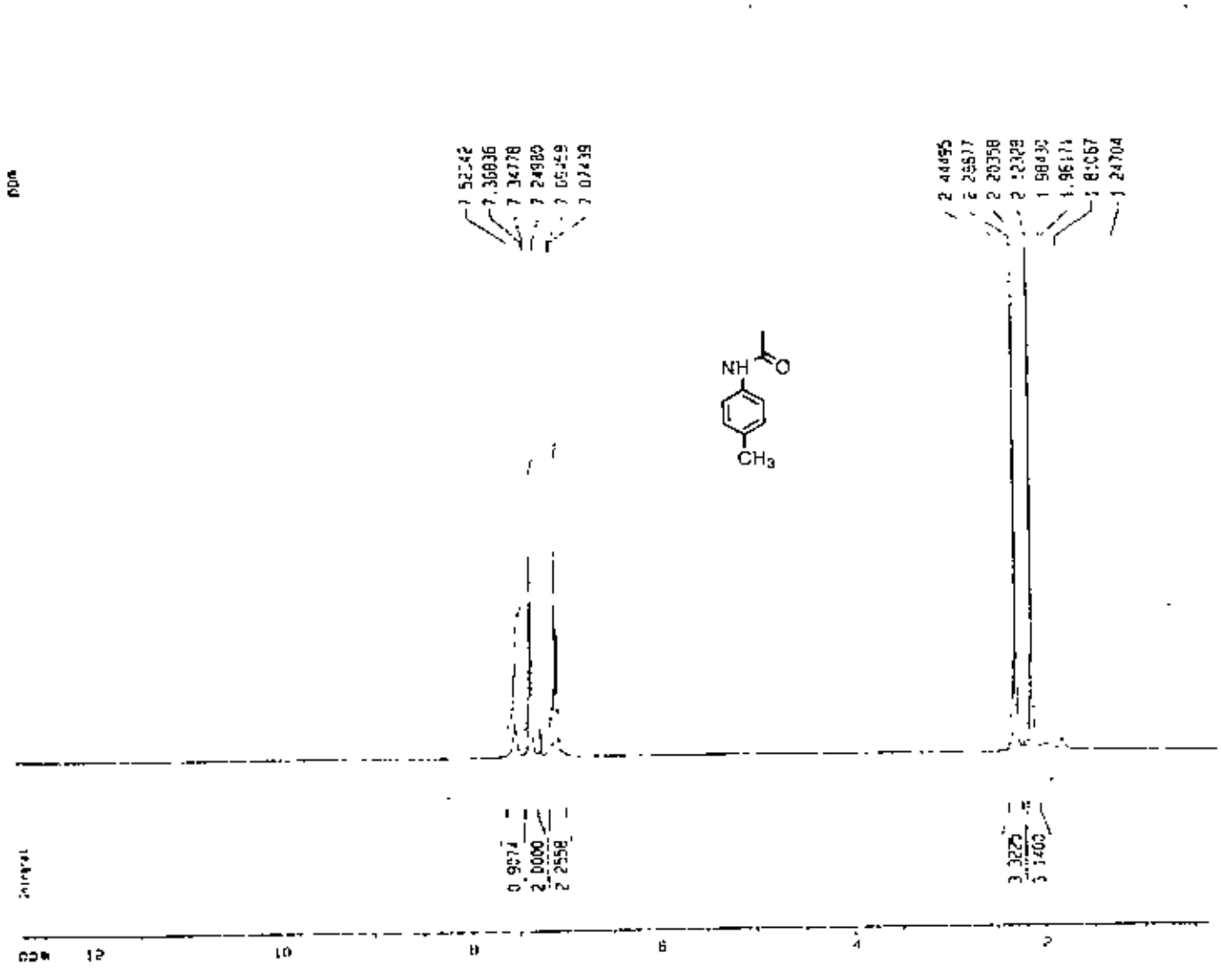


Figure 15b: IR spectrum of the compound 5



Current Data Parameters
NAME A3530
EXPNO 1
PROCNO 1

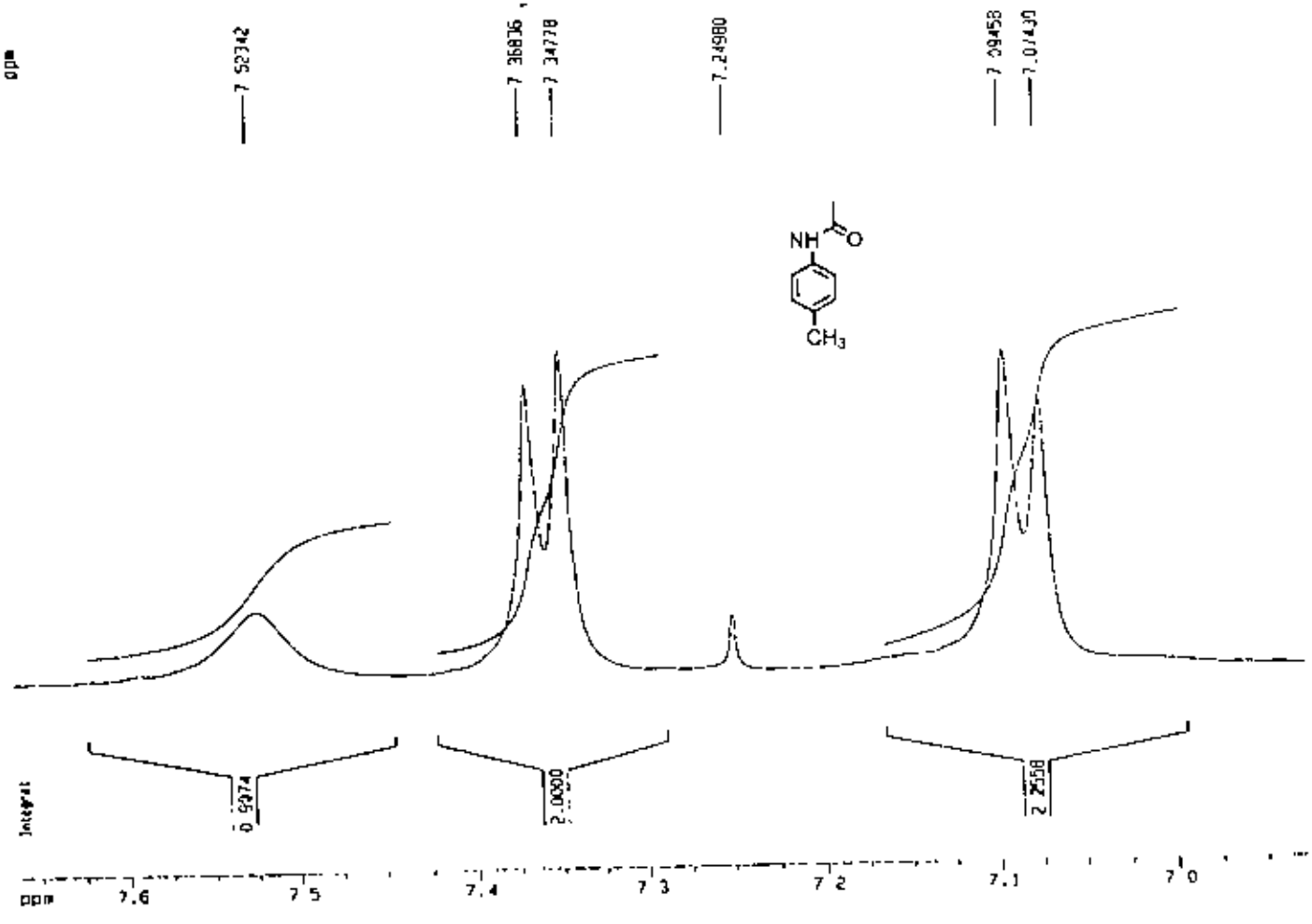
F2 - Acquisition Parameters
Date_ 20070611
Time 11.29
INSTRUM spx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT1 CDCl3
NS 128
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 181
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

---------- CHANNEL f1 ----------
NUC1 1H
P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1428010 MHz

F2 - Processing parameters
SI 32768
SF 400.1400126 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 12.782 ppm
F1 5114.42 Hz
F2P 0.243 ppm
F2 97.11 Hz
PPMCM 0.62894 ppm/cm
HZCM 250.86552 Hz/cm

Figure 15c: ^{1}H NMR spectrum of the compound 5




```
Current Data Parameters
NAME                  A3530
EXPNO                     1
PROCNO                    1

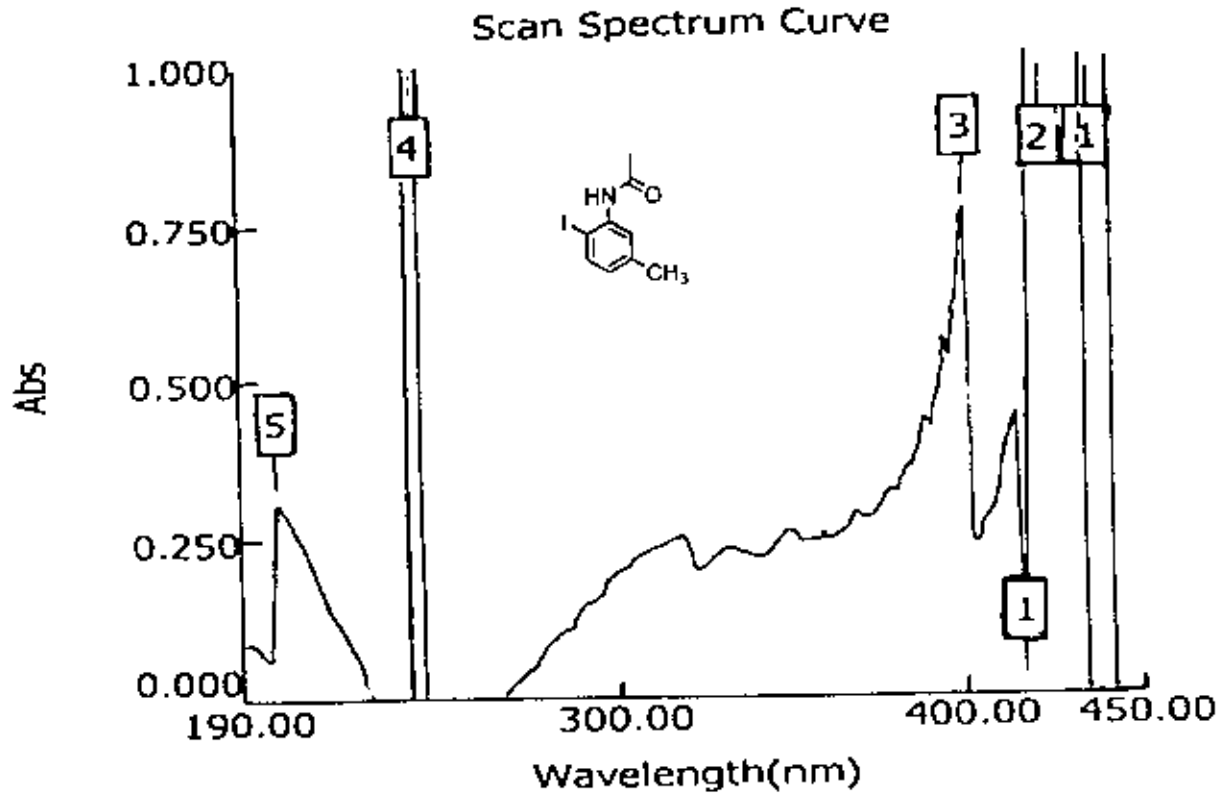
F2 - Acquisition Parameters
Date_              20070611
Time                  11.29
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                     161
DW                 78.000 usec
DE                  6.00 usec
TE                 310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 --------
NUC1                   1H
P1                  8.30 usec
PL1                -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400126 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 7.664 ppm
F1                3066.79 Hz
F2P                 6.927 ppm
F2                2771.68 Hz
PPMCM            0.03685 ppm/cm
HZCM            14.74649 Hz/cm
```

Figure 15c: ^{1}H NMR spectrum of the compound 5 (Expansion)



- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 450.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled1.spd
  Create Date/Time : Monday, August 24, 2009 4:03:48 PM
  Data Type : Original
  Method File
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment
- **No. P/V Wavelength(nm) Abs Comment**

1	Peak	436.58	9.999
2	Peak	422.35	9.999
3	Peak	400.99	0.770
4	Peak	240.67	9.999
5	Peak	201.08	0.313
1	Valley	416.20	0.000

Figure 16a: UV spectrum of the compound 6

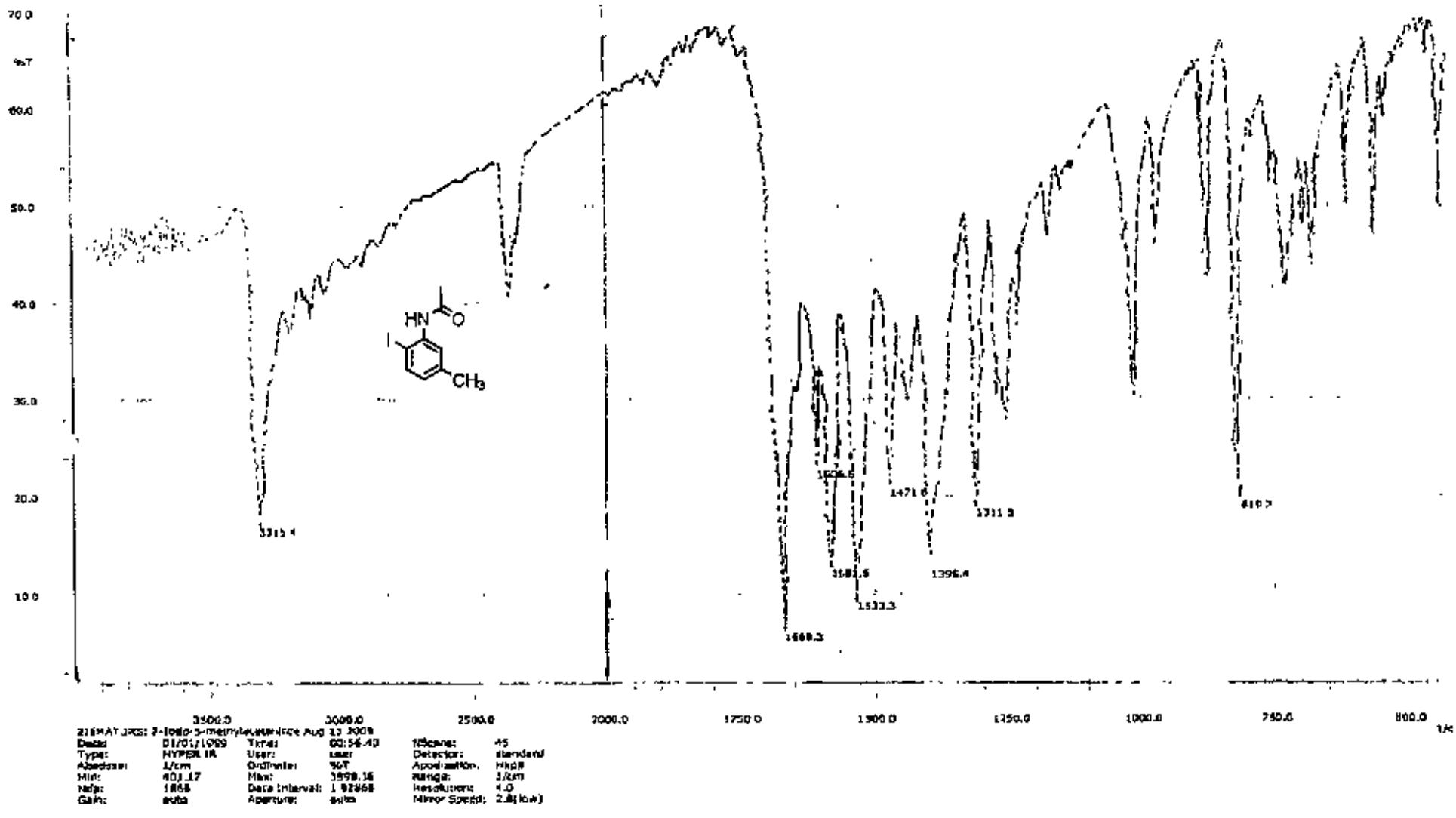
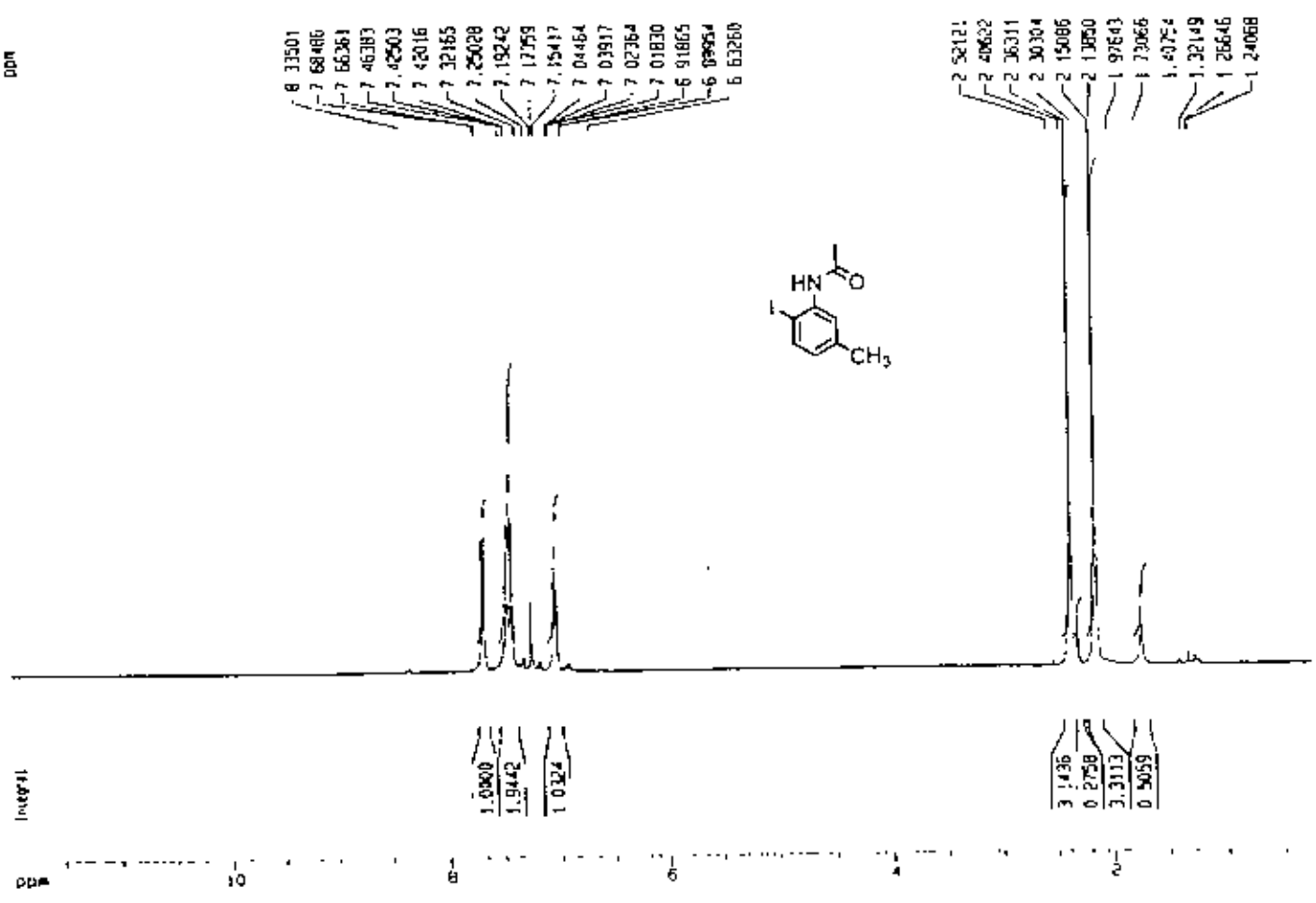


Figure 16b: IR spectrum of the compound 6




```
Current Data Parameters
NAME                  A3665
EXPNO                     1
PROCNO                    1

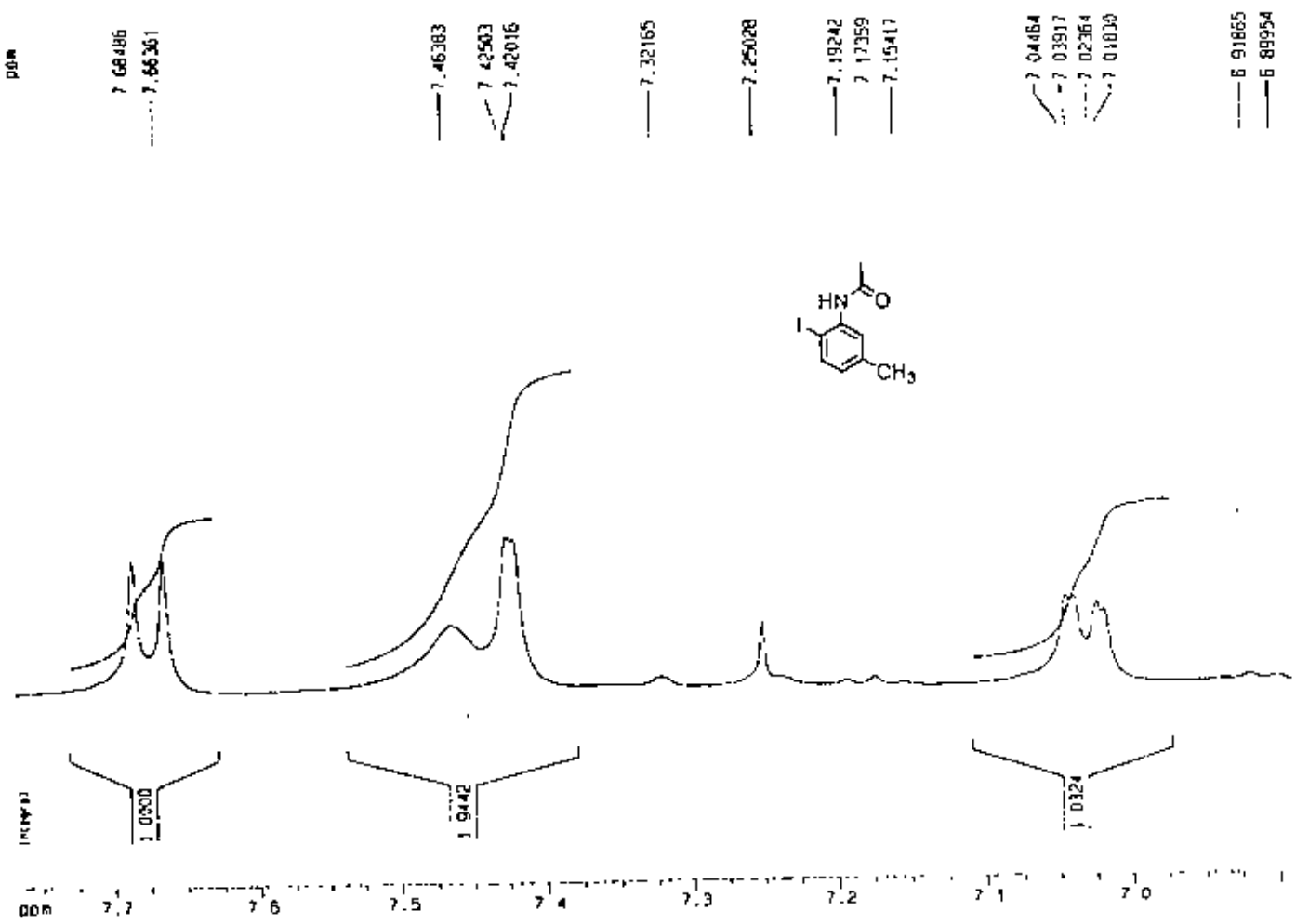
F2 - Acquisition Parameters
Date_              20070725
Time                  12 51
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       28
DS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                      256
DW                78.000 usec
DE                   6.00 usec
TE                  310.0 K
D1                1.00000000 sec

============ CHANNEL f1 ========
NUC1                      1H
P1                     8.30 usec
PL1                   -6.00 dB
SFO1            400.1426010 MHz

F2 - Processing parameters
SI                    32768
SF              400.1400128 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                    20.00 cm
F1P                   11.970 ppm
F1                  4789.65 Hz
F2P                   0.242 ppm
F2                   96.91 Hz
PPMCM              0.58639 ppm/cm
HZCM             234.63702 Hz/cm
```

Figure 16c: ^{1}H NMR spectrum of the compound 6




```
Current Data Parameters
NAME                  A3666
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20070725
Time                  12.51
INSTRUM             spect400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       28
DS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                      256
DW                 78.000 usec
DE                  6.00 usec
TE                 310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 --------
NUC1                      1H
P1                     6.30 usec
PL1                   -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                        32768
SF                400.1400128 MHz
WDW                       EM
SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.40

1D NMR plot parameters
CX                     20.00 cm
F1P                    7.769 ppm
F1                  3108.70 Hz
F2P                    6.891 ppm
F2                  2757.27 Hz
PPMCM              0.04391 ppm/cm
HZCM               17.57172 Hz/cm
```

Figure 16c: ^{1}H NMR spectrum of the compound 6 (Expansion)

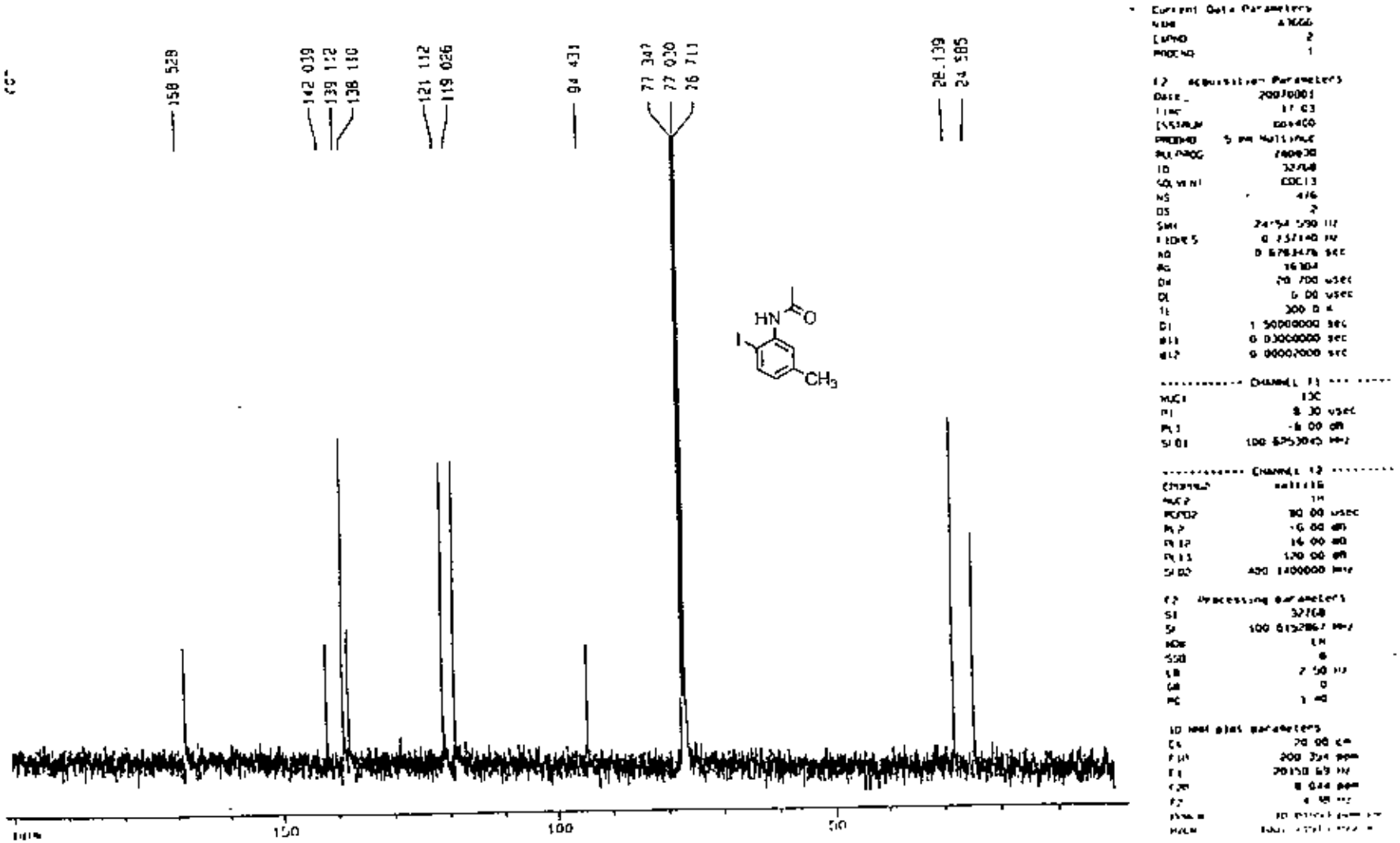


Figure 16d: ^{13}C NMR spectrum of the compound 6

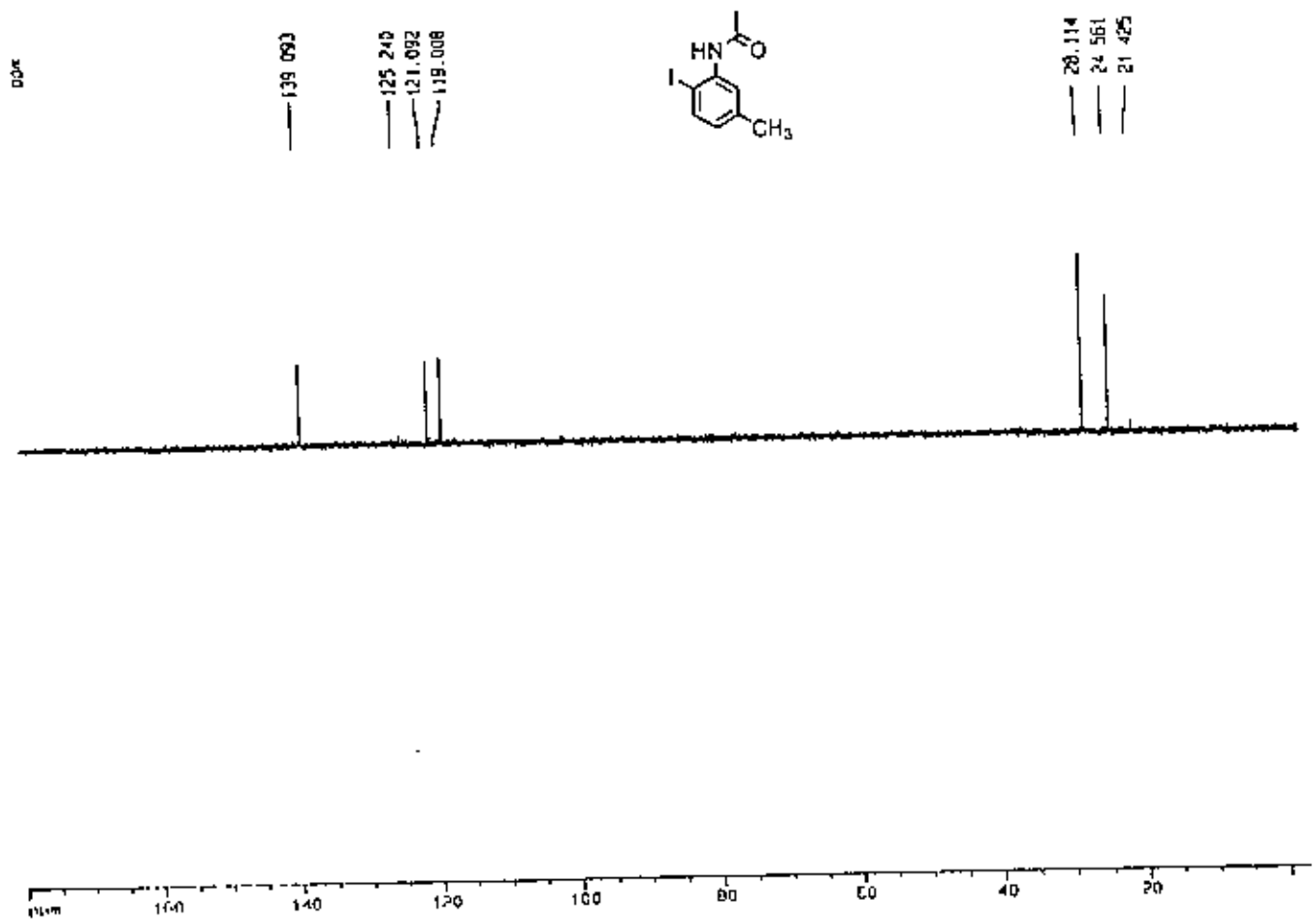
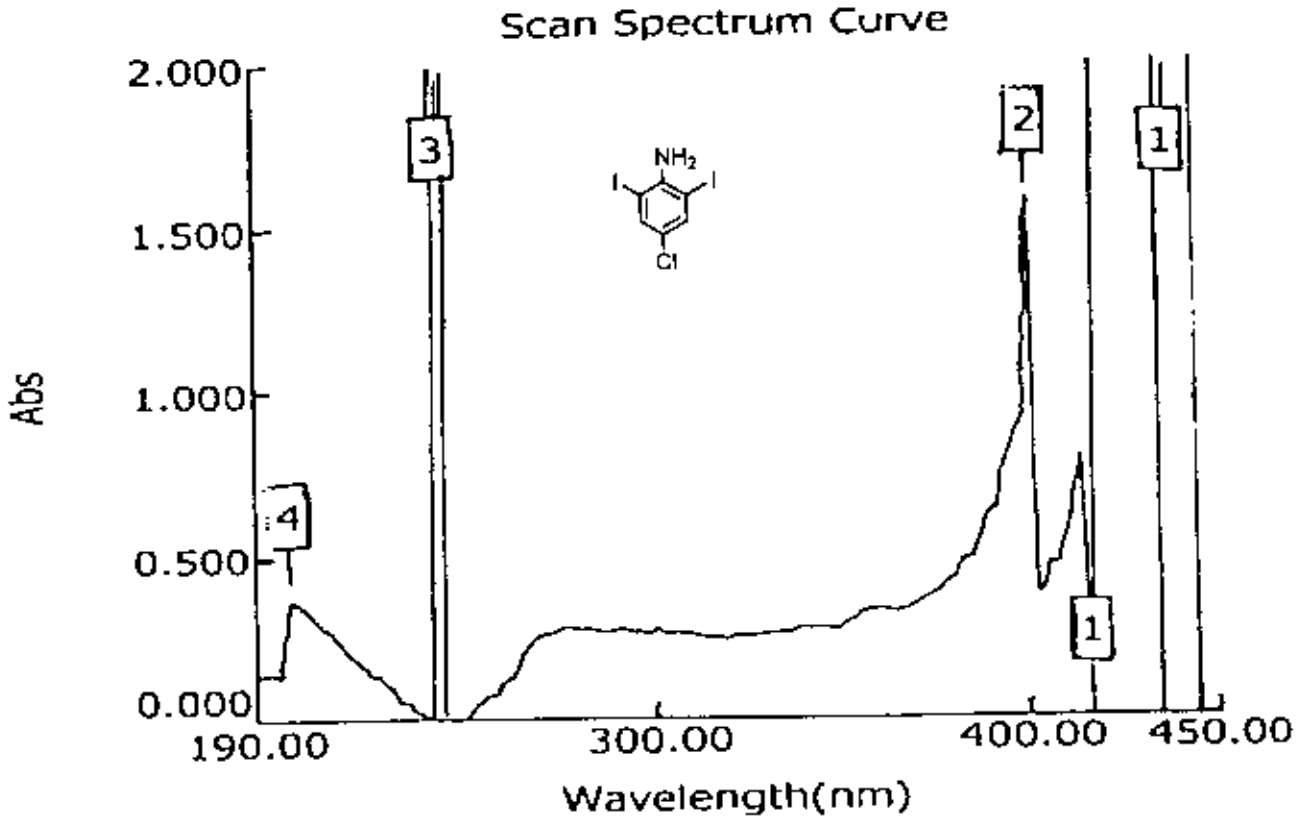


Figure 16d: Detp-135 NMR spectrum of the compound 6



- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range . 190.00 to 450.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed . Fast
  Data File : Untitled7.spd
  Create Date/Time : Monday, August 24, 2009 3:53:57 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	436.28	9.999	
2	Peak	400.89	1.566	
3	Peak	240.21	9.999	
4	Peak	199.92	0.379	
1	Valley	416.86	0.000	

Figure 17a: UV spectrum of the compound 7

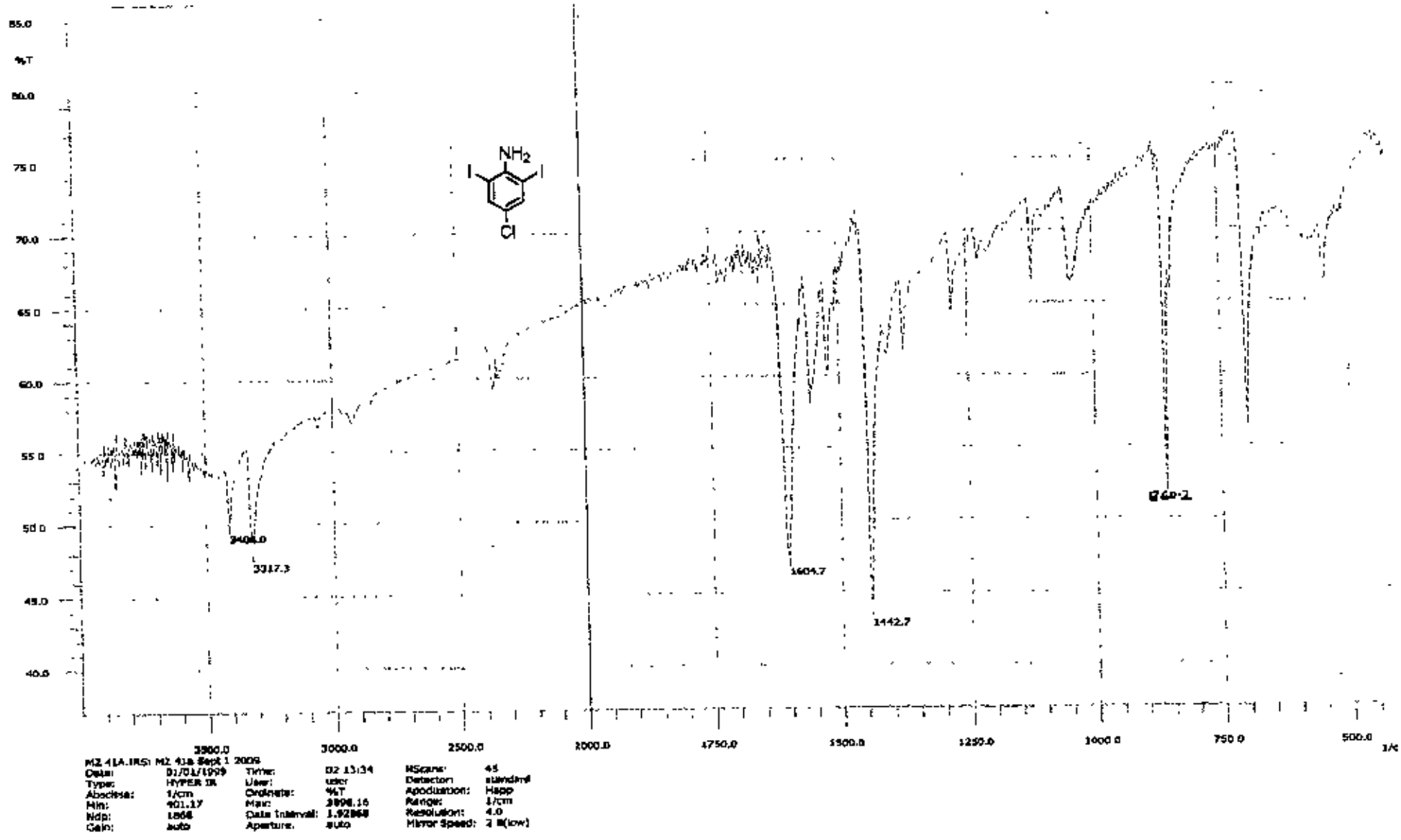


Figure 17b: IR spectrum of the compound 7

Current Data Parameters

NAME	A3667
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20070725
Time	13 03
INSTRUM	cdx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	79
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	456.1
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 =======

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400130 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	11.767 ppm
F1	4708.32 Hz
F2P	-0.164 ppm
F2	-65.57 Hz
PPMCM	0.59653 ppm/cm
HZCM	238.69415 Hz/cm

Figure 17c: ^{1}H NMR spectrum of the compound 7

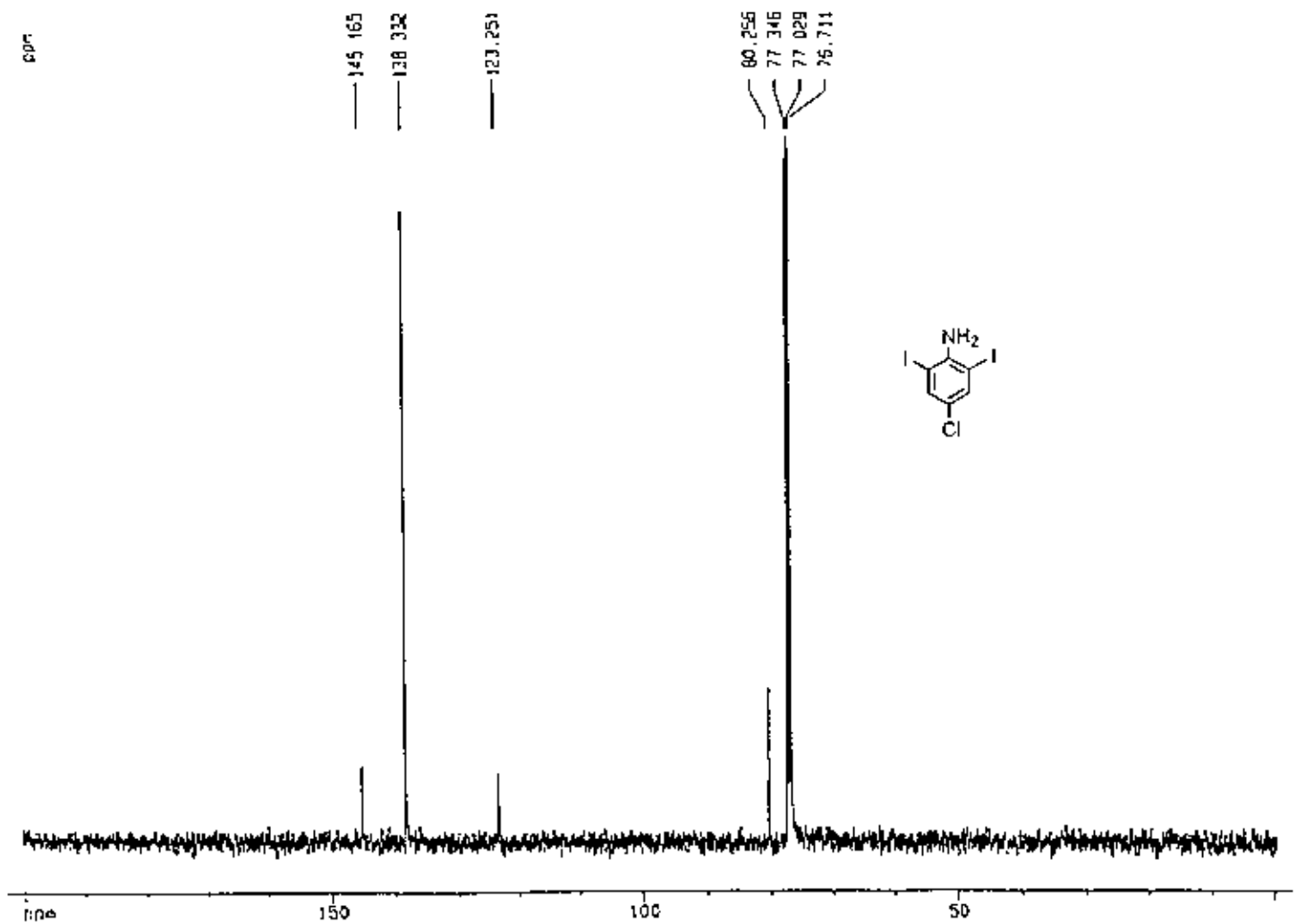


Figure 17d: ^{13}C NMR spectrum of the compound 7

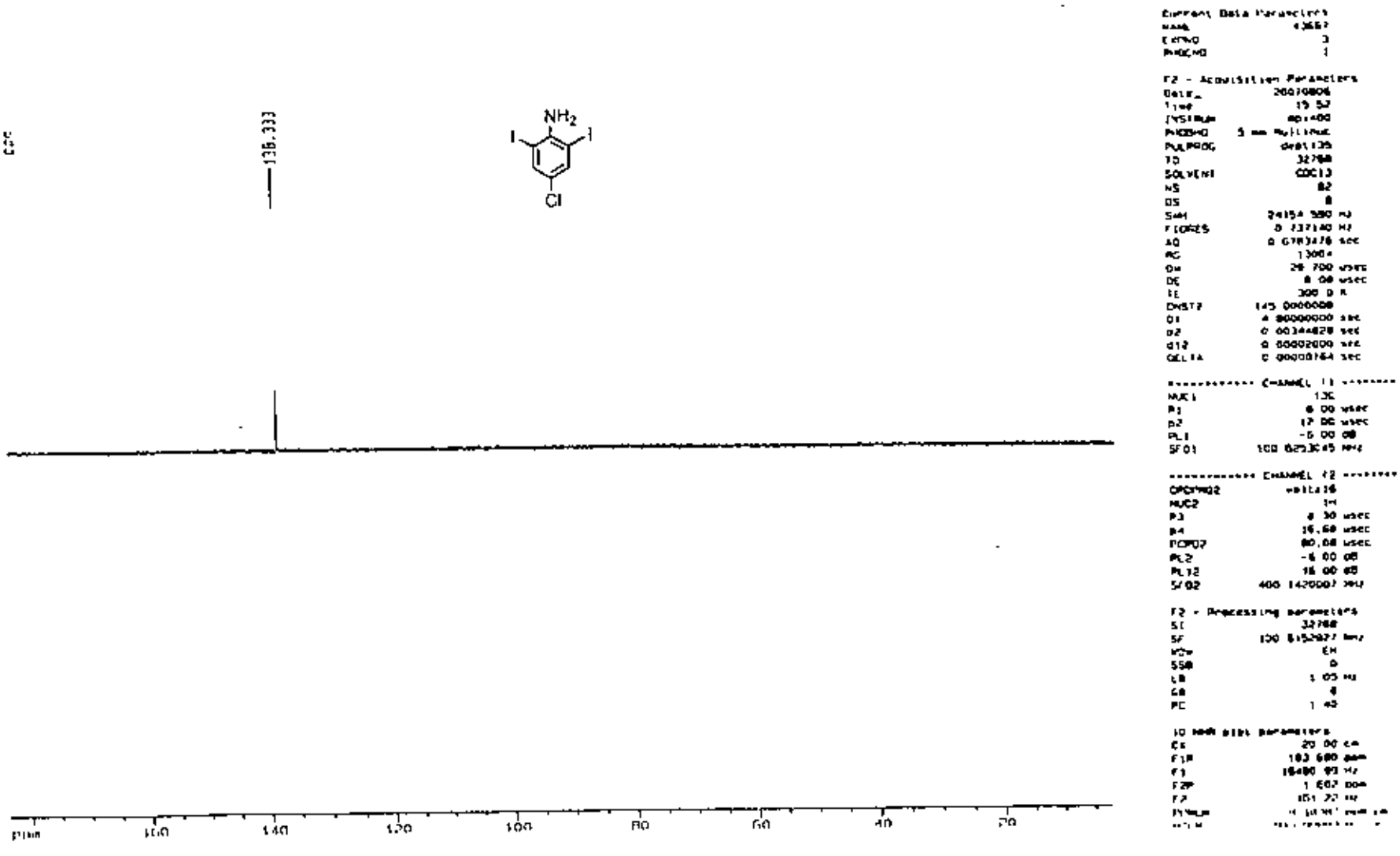
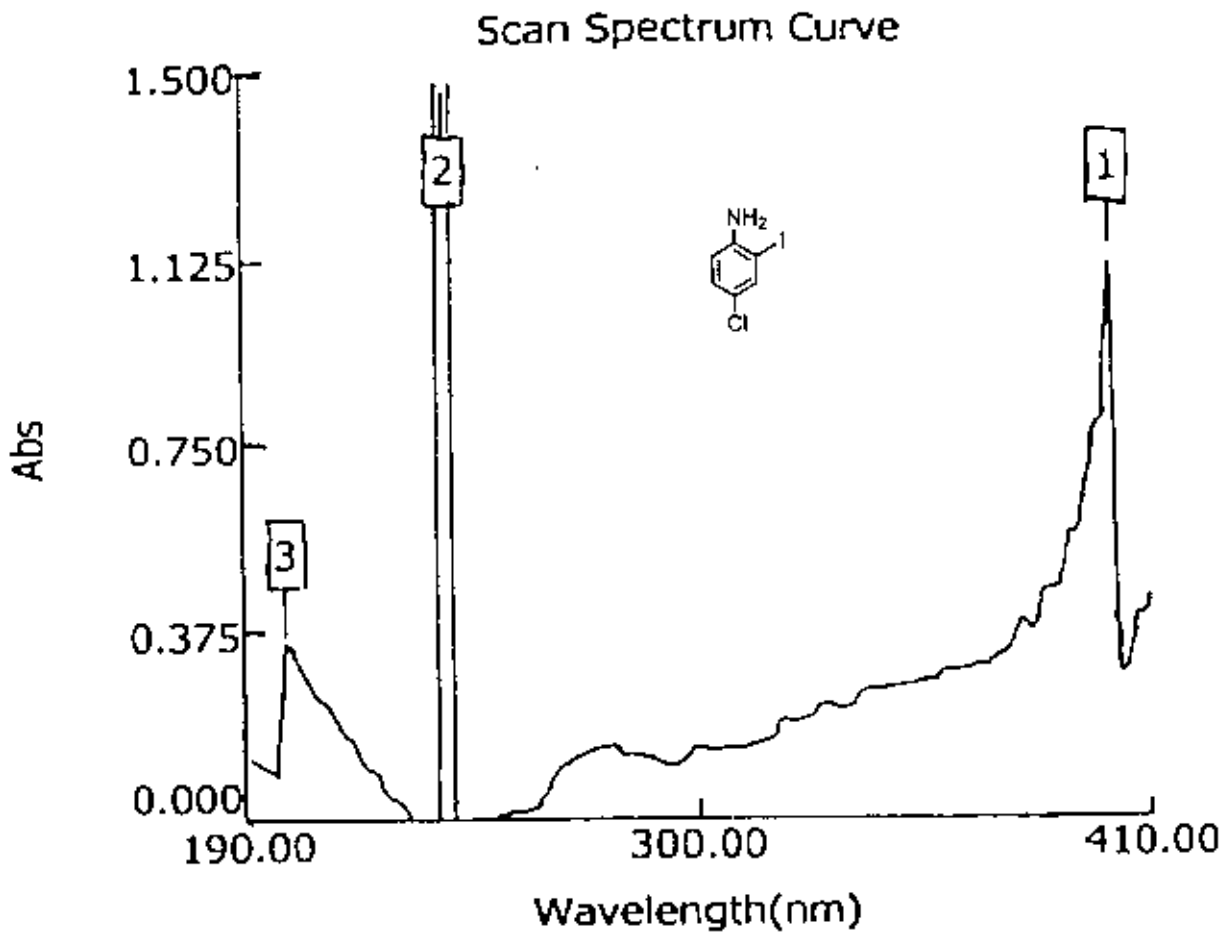


Figure 17d: Dept-135 NMR spectrum of the compound 7



- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 410.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled5.spd
  Create Date/Time : Monday, August 24, 2009 3:51:04 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**

1	Peak	400.75	1.121
2	Peak	240.65	9.999
3	Peak	200.51	0.348

Figure 18a: UV spectrum of the compound 8

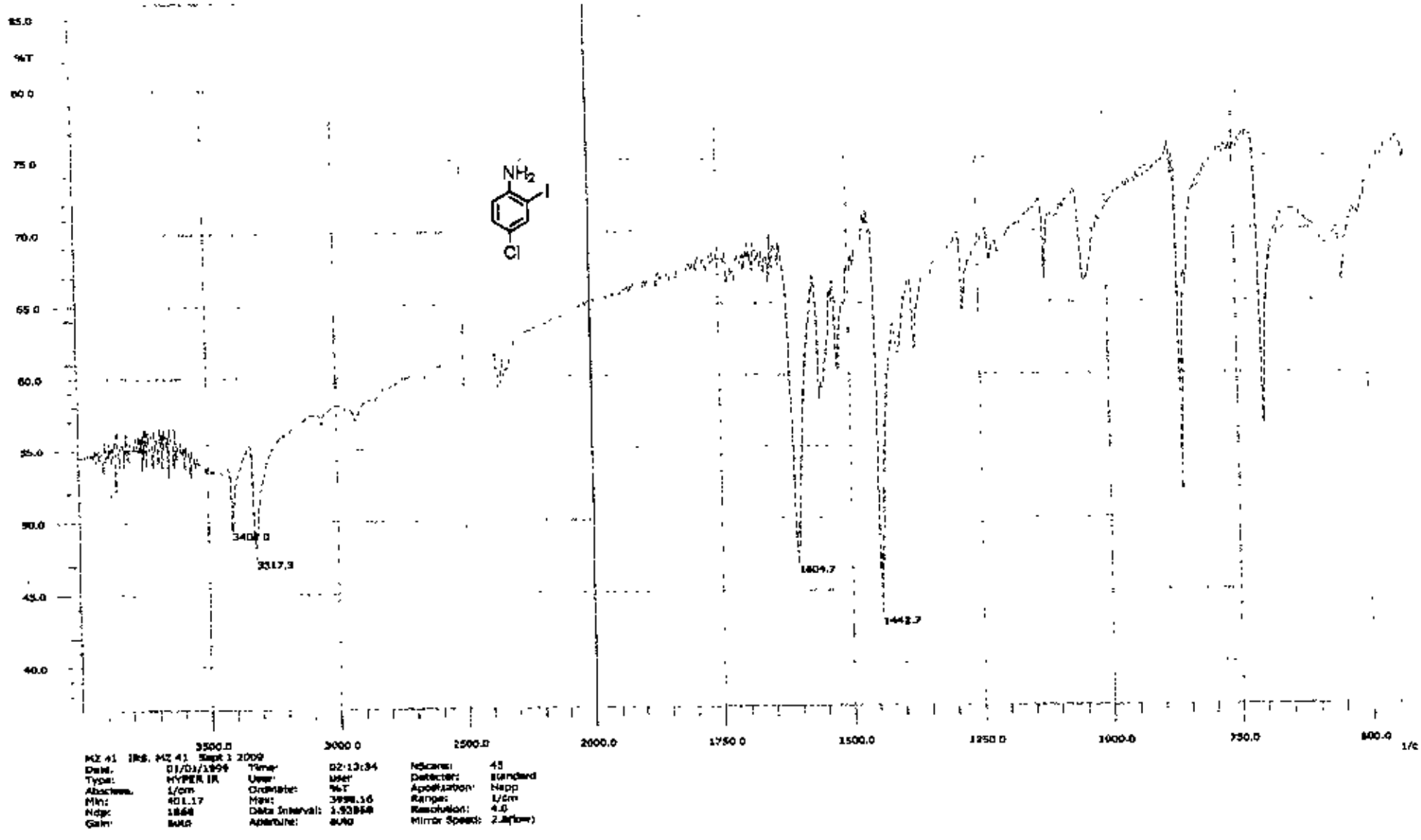
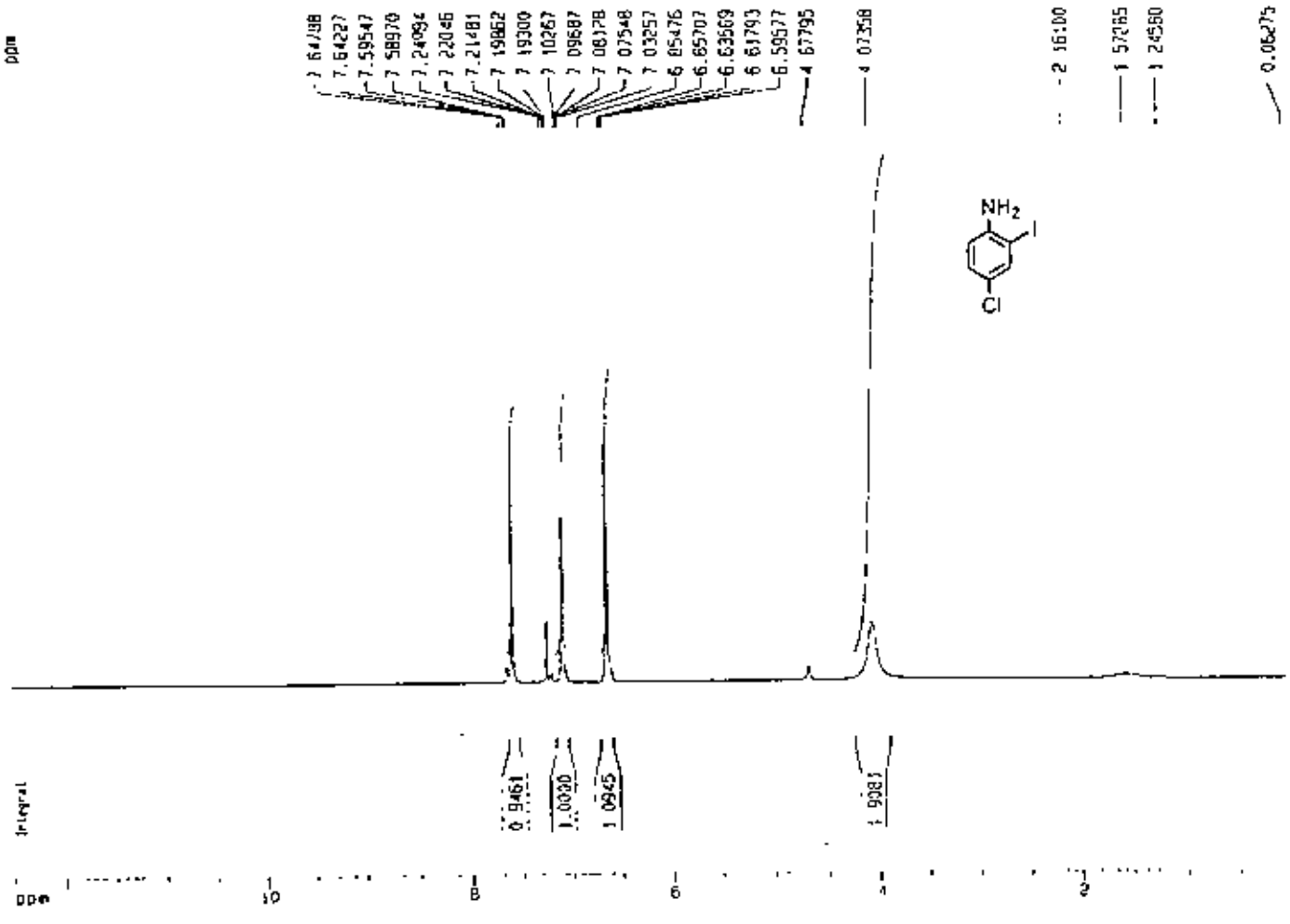


Figure 18b: IR spectrum of the compound 8




```
Current Data Parameters
NAME                  A3670
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20070730
Time                  18 26
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH              6410.256 Hz
FIDRES           0.195625 Hz
AQ               2.5559540 sec
RG                   456.1
DW                  78.000 usec
DE                   6.00 usec
TE                  310.0 K
D1               1.00000000 sec

========== CHANNEL f1 =======
NUC1                   1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1          400.1429010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400136 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                12.509 ppm
F1                 5005.25 Hz
F2P                 0.054 ppm
F2                  21.75 Hz
PPMCM              0.62272 ppm/cm
HZCM             249.17506 Hz/cm
```

Figure 18c: ^{1}H NMR spectrum of the compound 8

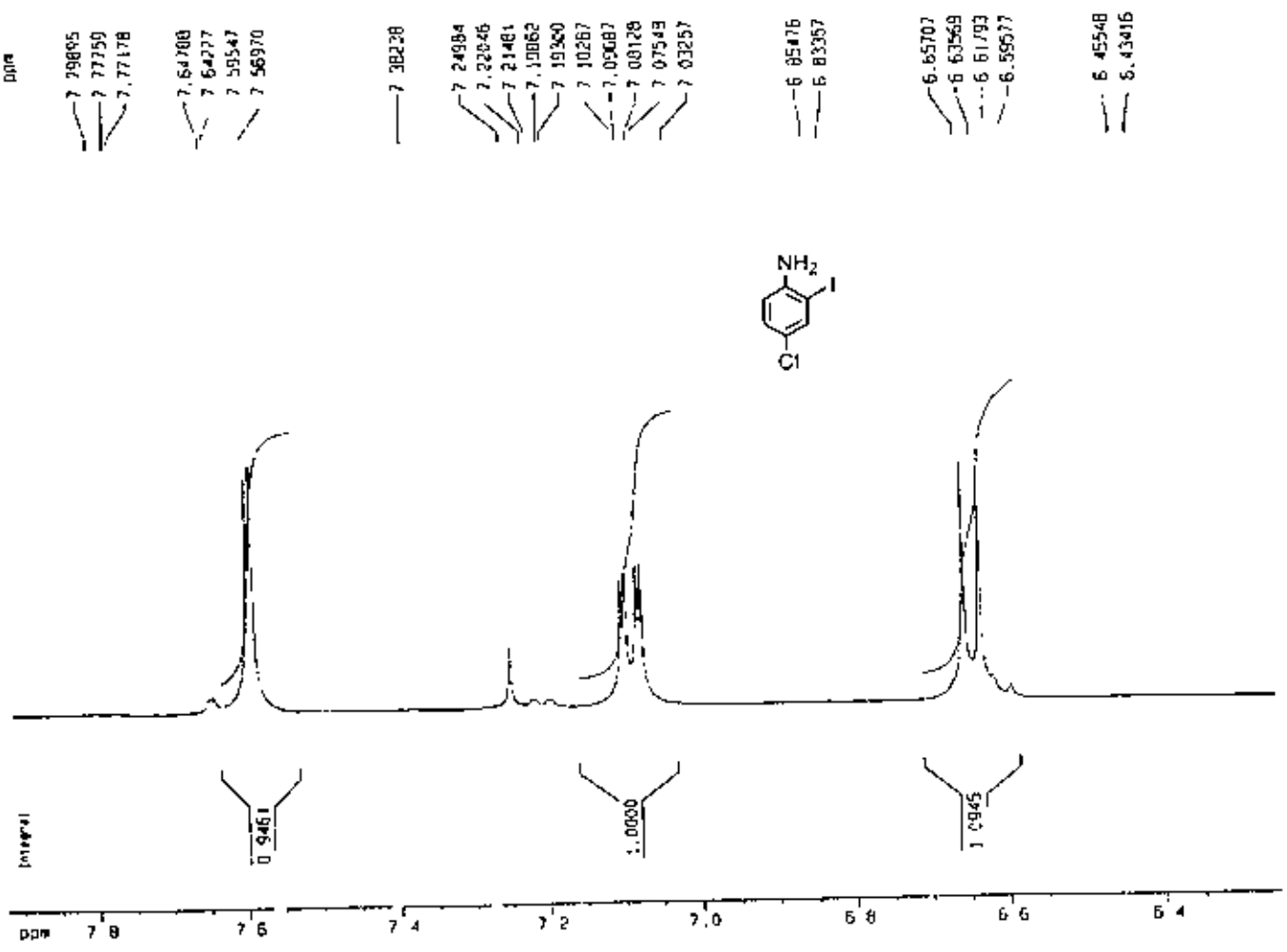


Figure 18c: ^{1}H NMR spectrum of the compound 8 (Expension)

```
Current Data Parameters
NAME                  A3670
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20070730
Time                  18.26
INSTRUM               dpx400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                      128
DS                        2
SWH               6410.255 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                    456.1
DW                78.000 usec
DE                    6.00 usec
TE                   310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 --------
NUC1                     1H
P1                    8.30 usec
PL1                  -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                    32768
SF          400.1400136 MHz
WDW                      EM
SSB                       0
LB                 0.30 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                  7.911 ppm
F1                3165.35 Hz
F2P                  6.255 ppm
F2                2502.99 Hz
PPMCM             0.08277 ppm/cm
HZCM             33.11820 Hz/cm
```

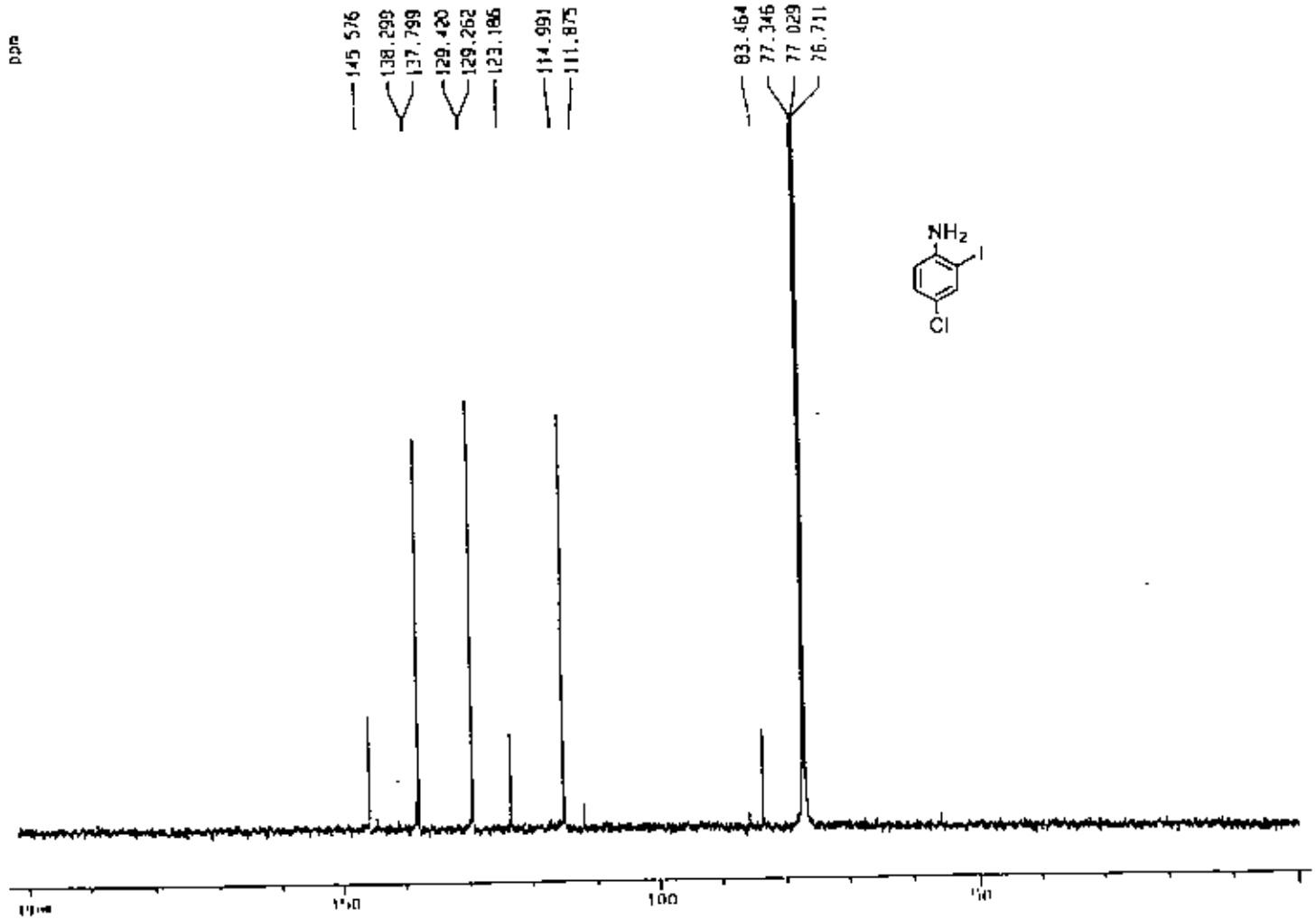


Figure 18d: ^{13}C NMR spectrum of the compound 8

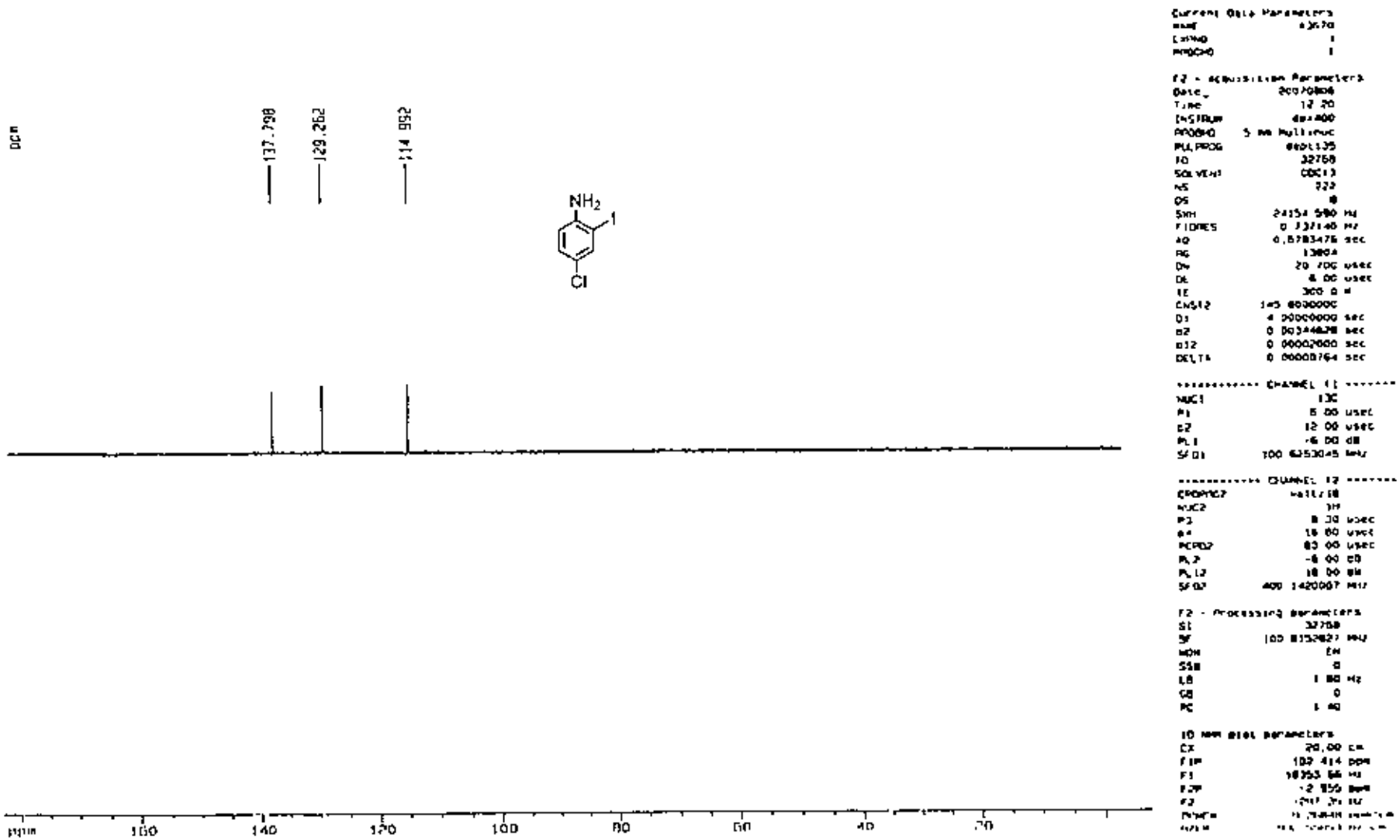
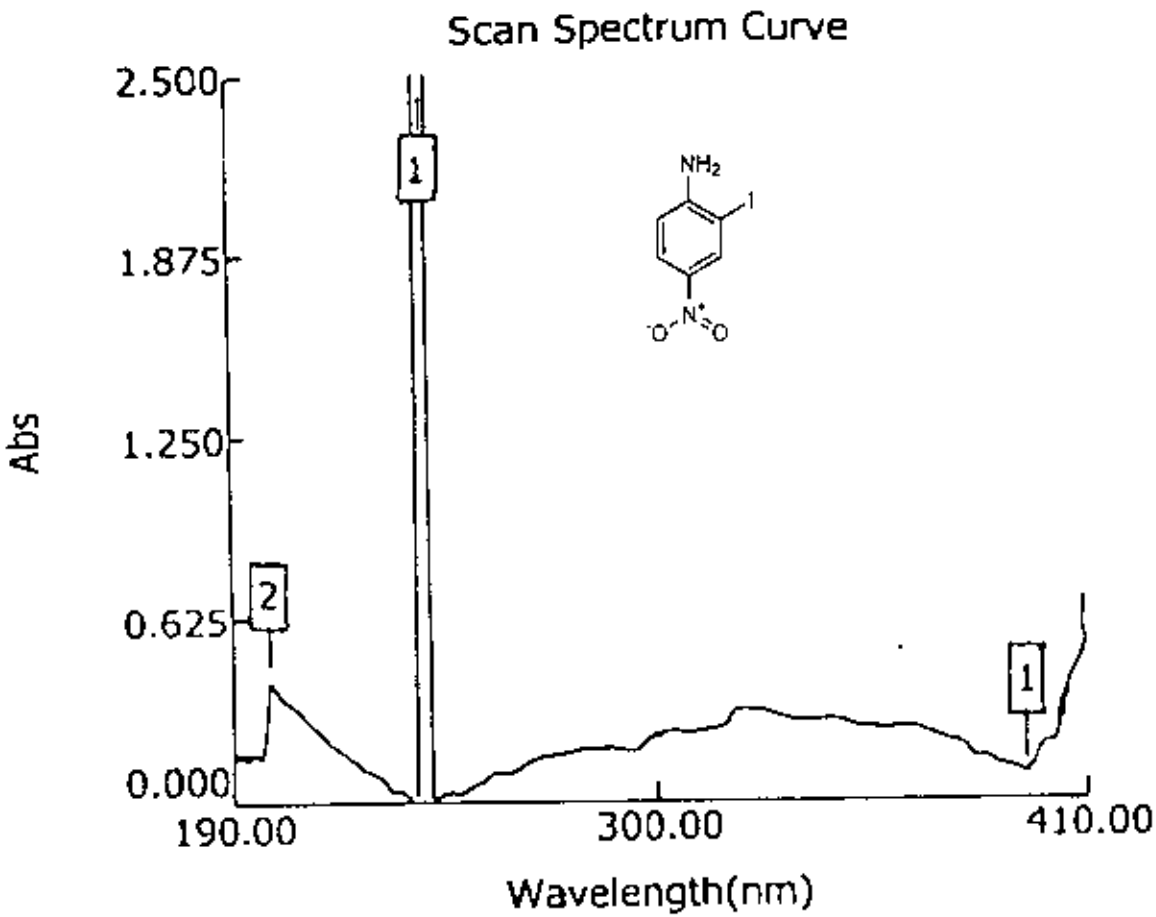


Figure 18d: Detp-135 NMR spectrum of the compound 8



- **Instrument Performance**
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  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 410.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File . Untaled2.spd
  Create Date/Time : Monday, August 24, 2009 3:45:27 PM
  Data Type Original
  Method File
- **Analyse Note**
  Analyser : Administrator
  Sample Name .
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**

1	Peak	240.00	9.999
2	Peak	200.00	0.405
1	Valley	394.00	0.084

Figure 19a: UV spectrum of the compound 9

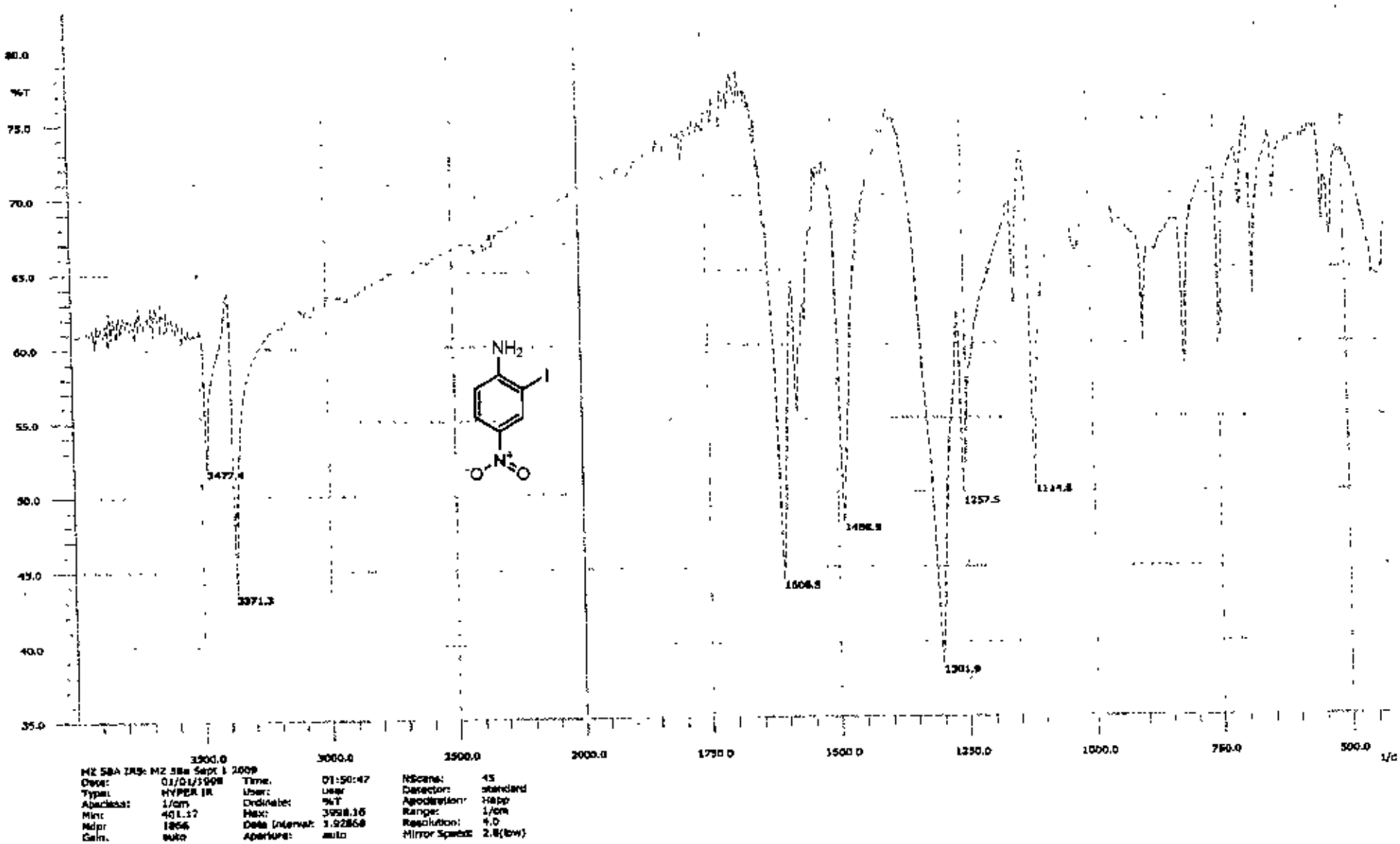
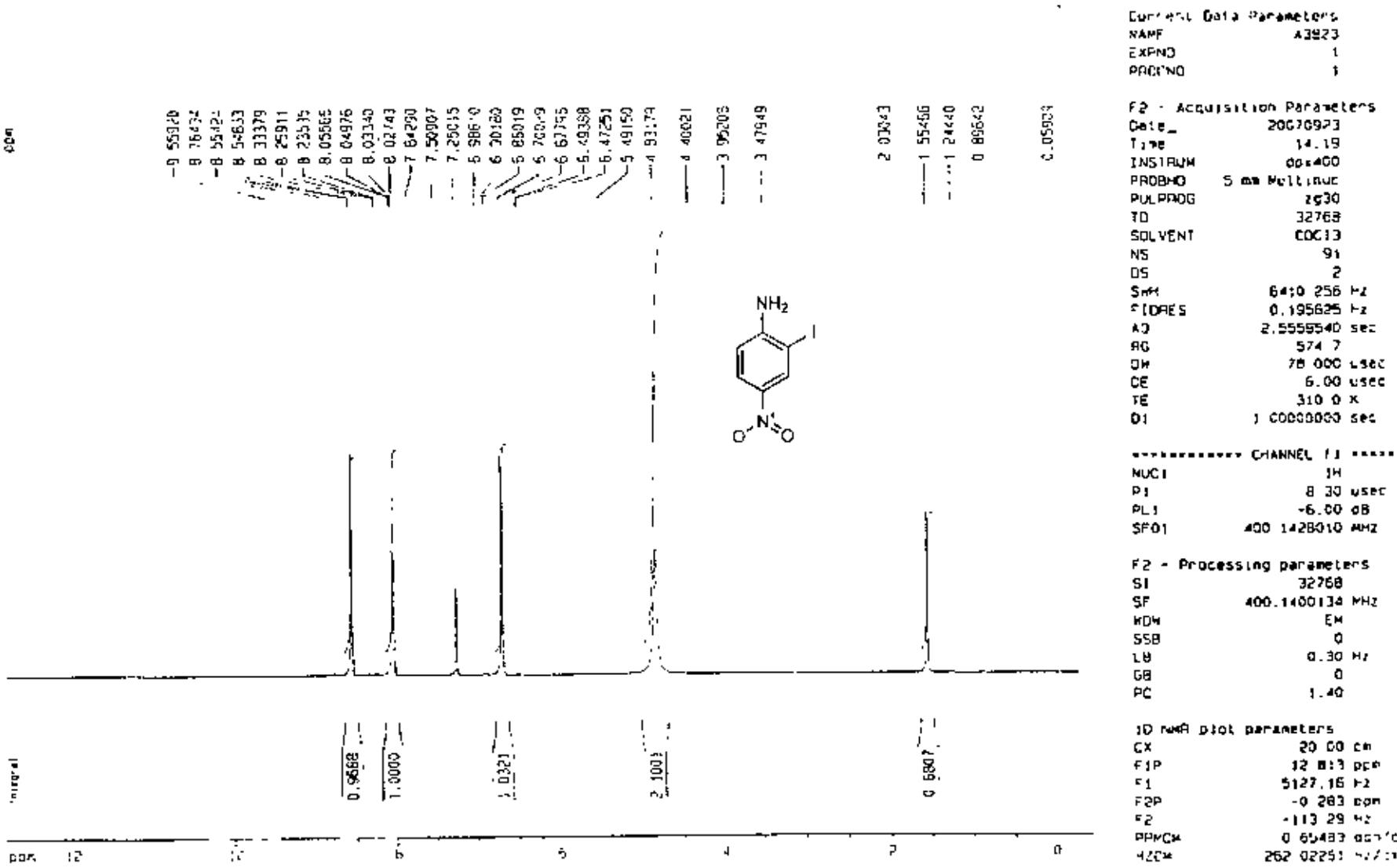


Figure 19b: IR spectrum of the compound 9

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PROCNO                    1

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PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       91
DS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5555540 sec
RG                    574.7
DW                   78.000 usec
DE                    6.00 usec
TE                   310.0 K
D1                1.00000000 sec

======== CHANNEL f1 =======
NUC1                      1H
P1                     8.30 usec
PL1                   -6.00 dB
SFO1           400.1428010 MHz

F2 - Processing parameters
SI                    32768
SF              400.1400134 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                  12.813 ppm
F1                 5127.16 Hz
F2P                 -0.283 ppm
F2                  -113.29 Hz
PPMCM              0.65483 ppm/cm
HZCM               262.02251 Hz/cm
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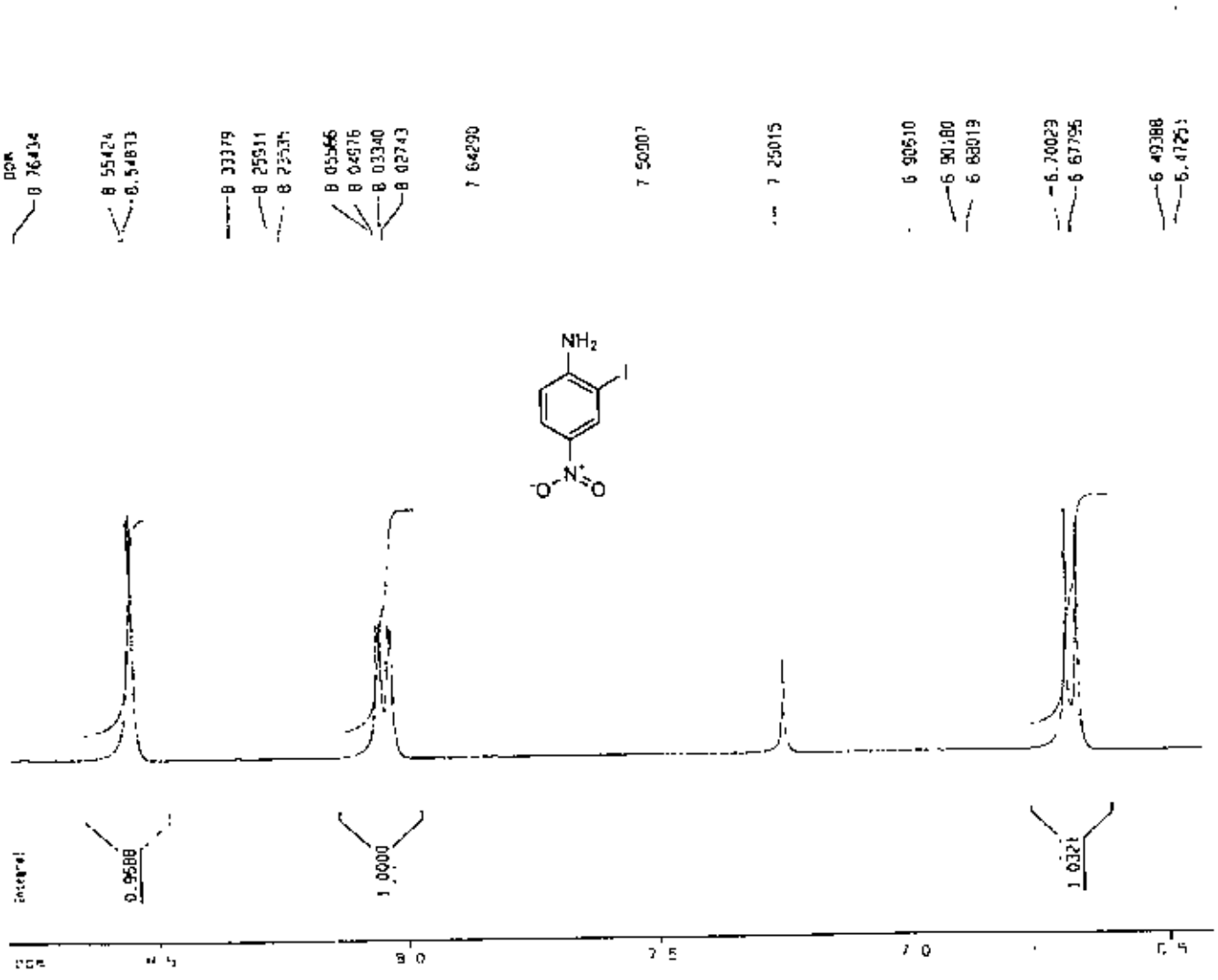
Figure 19c: ^{1}H NMR spectrum of the compound 9

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PROCNO                    1

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PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       91
DS                        2
SWH               6410.256 Hz
FIDRES            0.195525 Hz
AQ                2.5555540 sec
RG                    574.7
DW                  78.000 usec
DE                   6.00 usec
TE                  310.0 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1           400.1298010 MHz

F2 - Processing parameters
SI                     32768
SF                400.1400134 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                      1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                  8.793 ppm
F1                3518.54 Hz
F2P                  6.431 ppm
F2                2573.27 Hz
PPMCM             0.11812 ppm/cm
HZCM             47.26357 Hz/cm
```

Figure 19c: ^{1}H NMR spectrum of the compound 9 (Expansion)

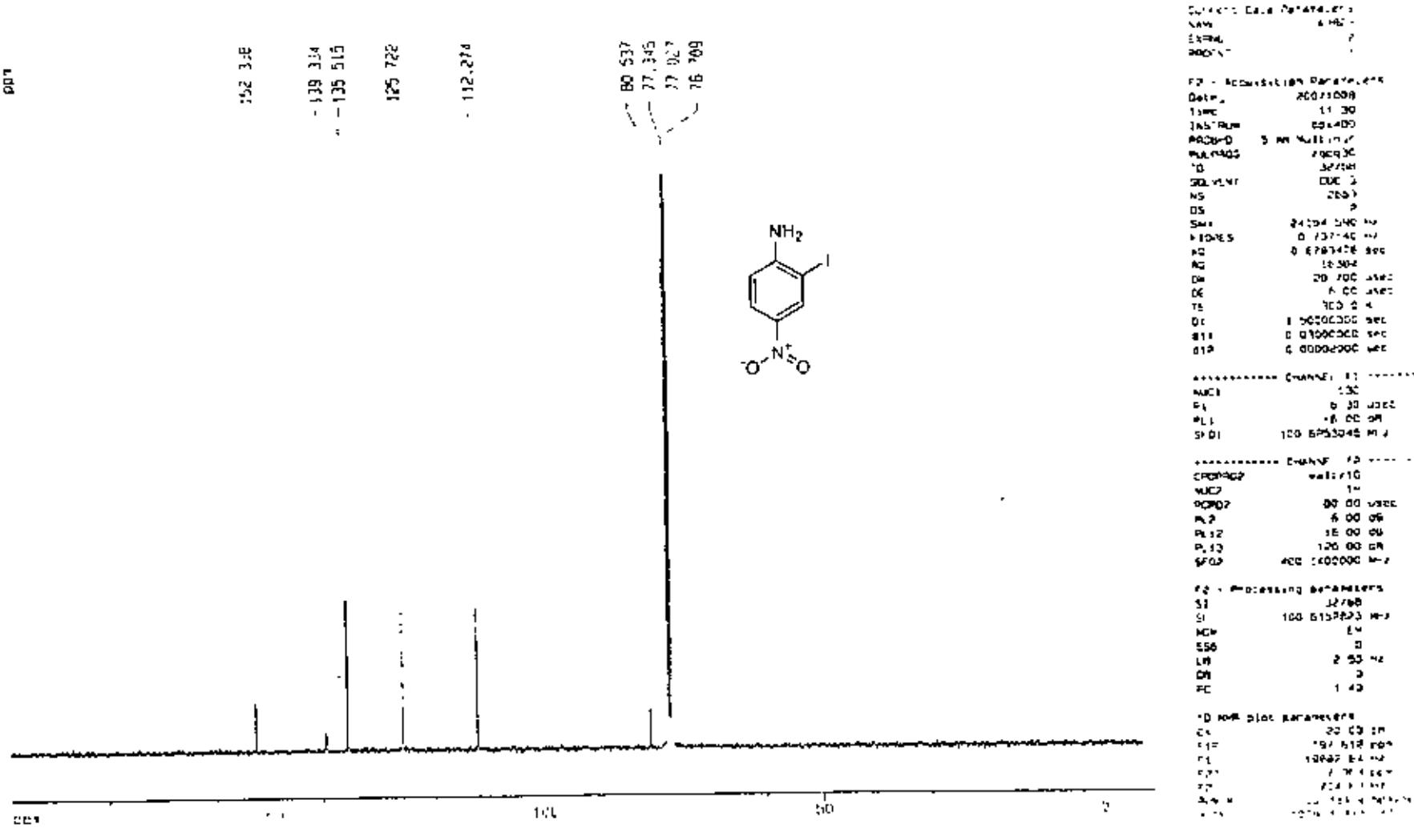


Figure 19d: ^{13}C NMR spectrum of the compound 9

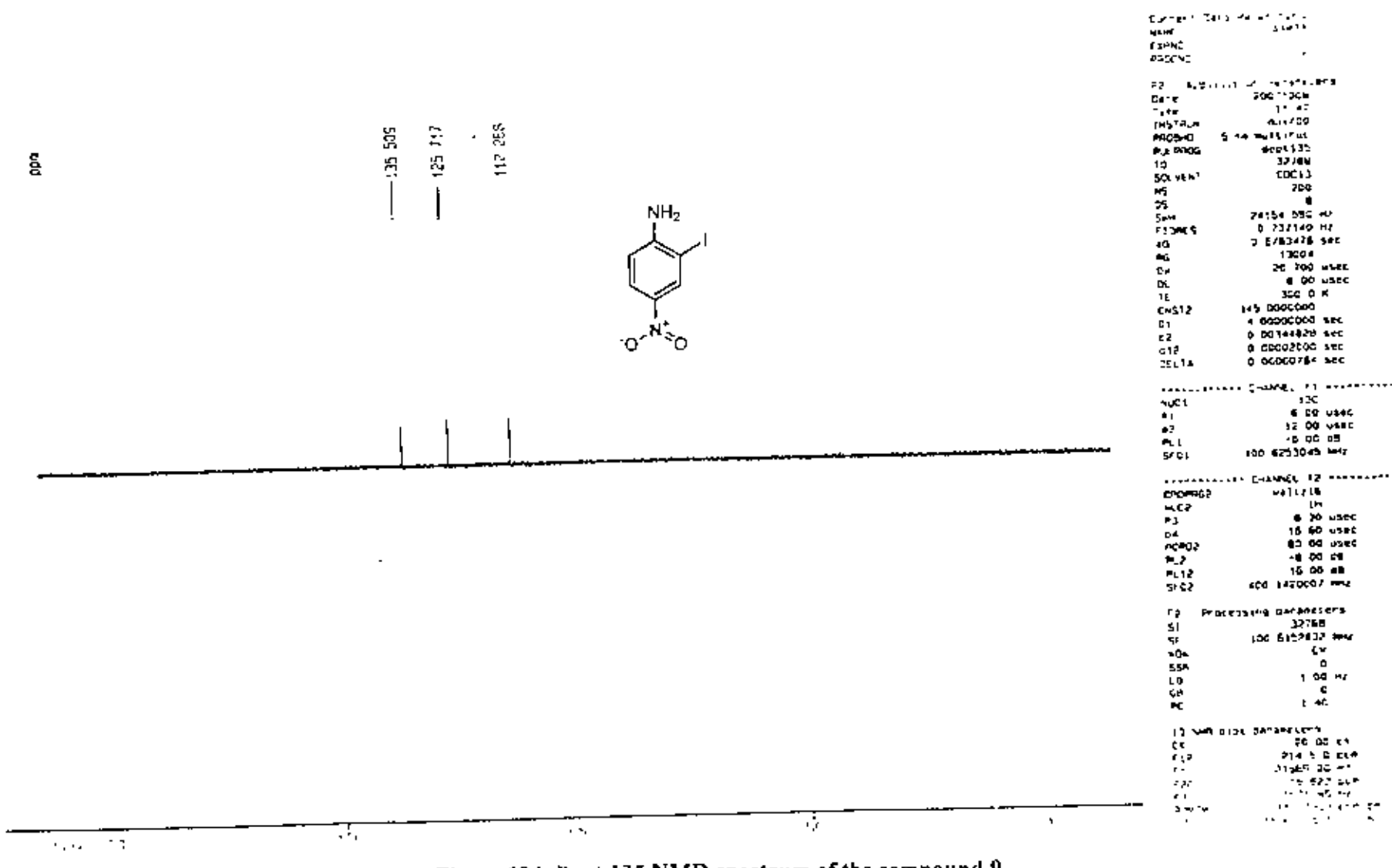
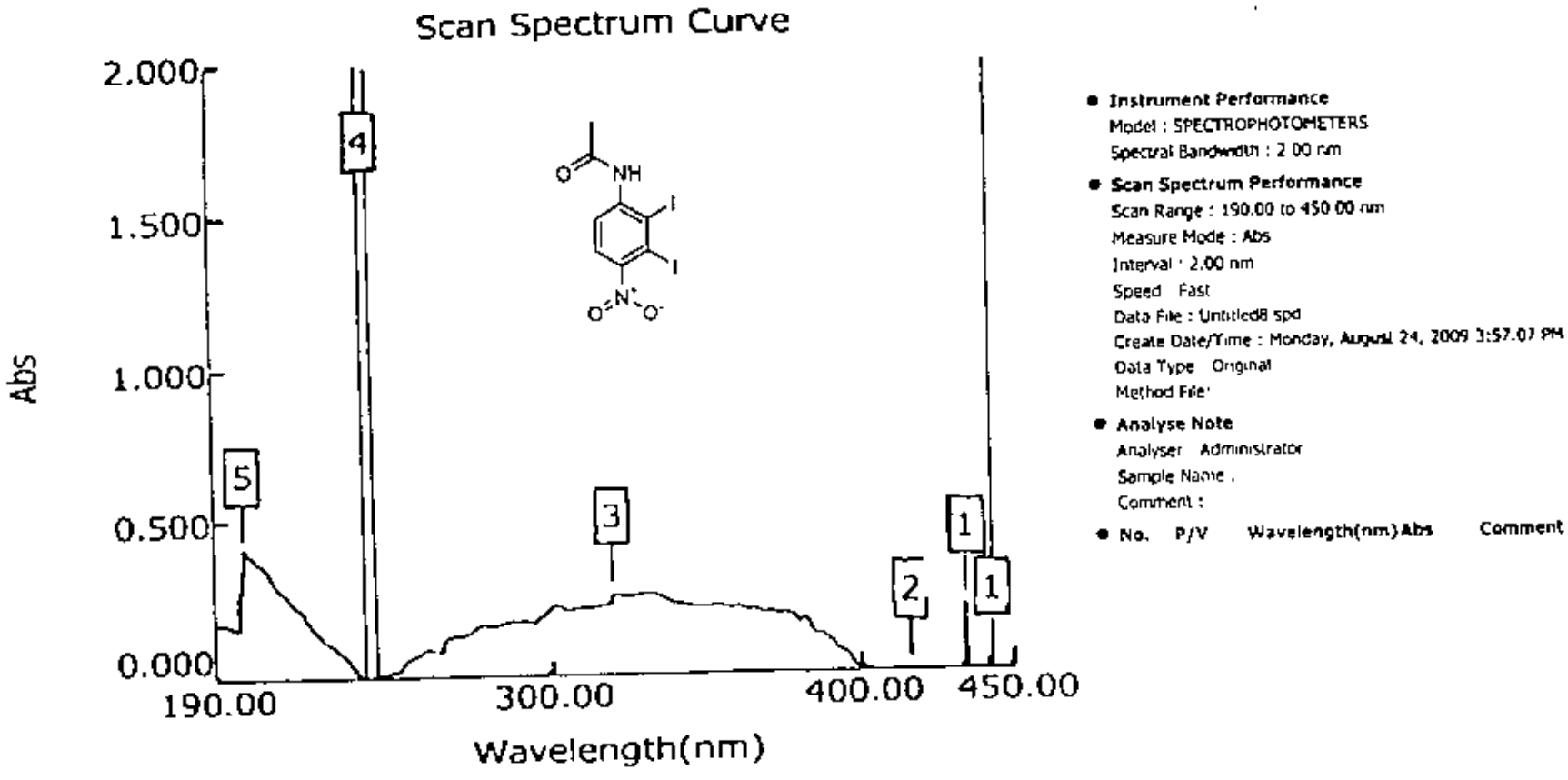


Figure 19d: Dept-135 NMR spectrum of the compound 9



1	Peak	434.01	0.202
2	Peak	416.21	0.000
3	Peak	320.00	0.253
4	Peak	241.08	9.999
5	Peak	202.14	0.408
1	Valley	442.00	-1.329

Figure 20a: UV spectrum of the compound 10

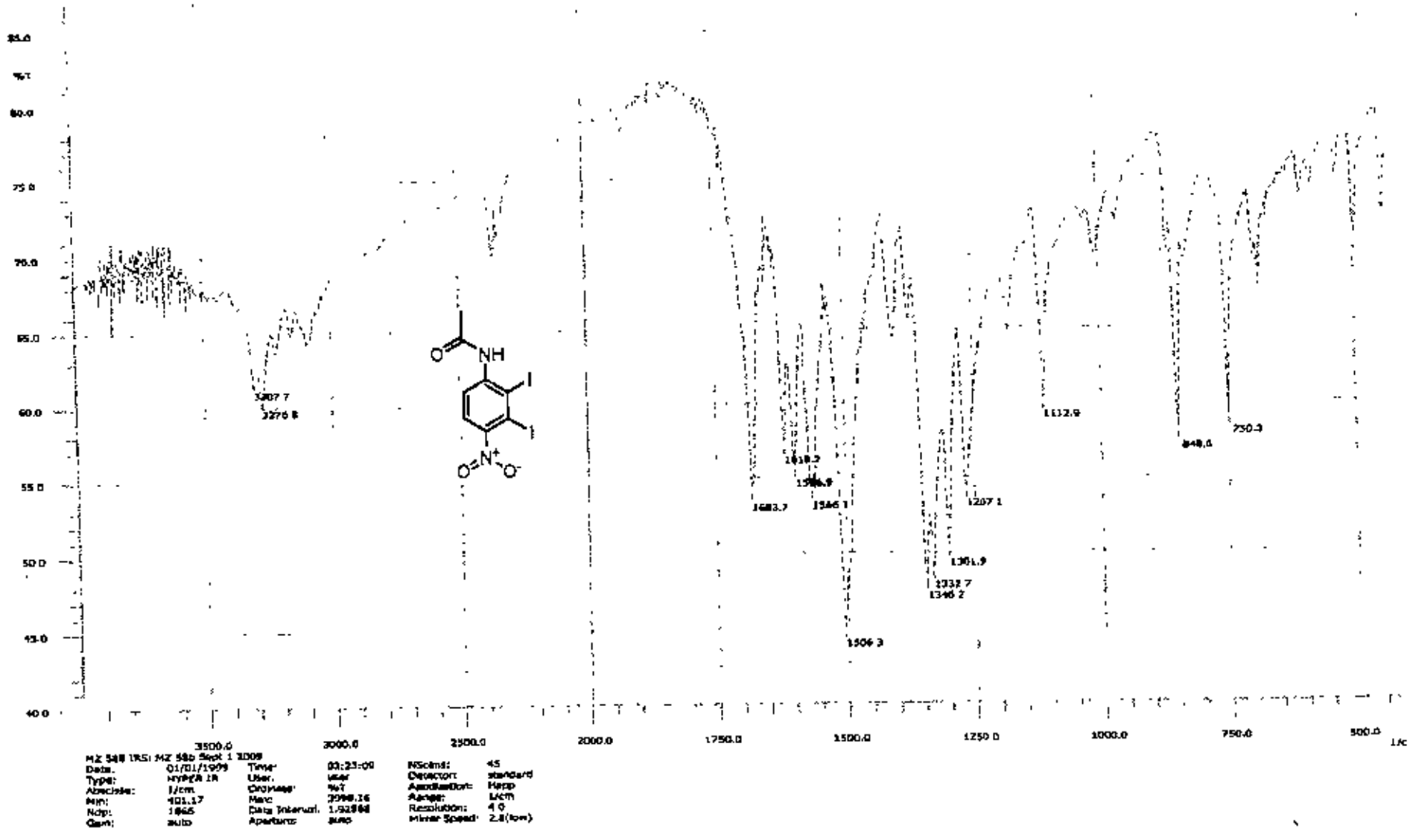
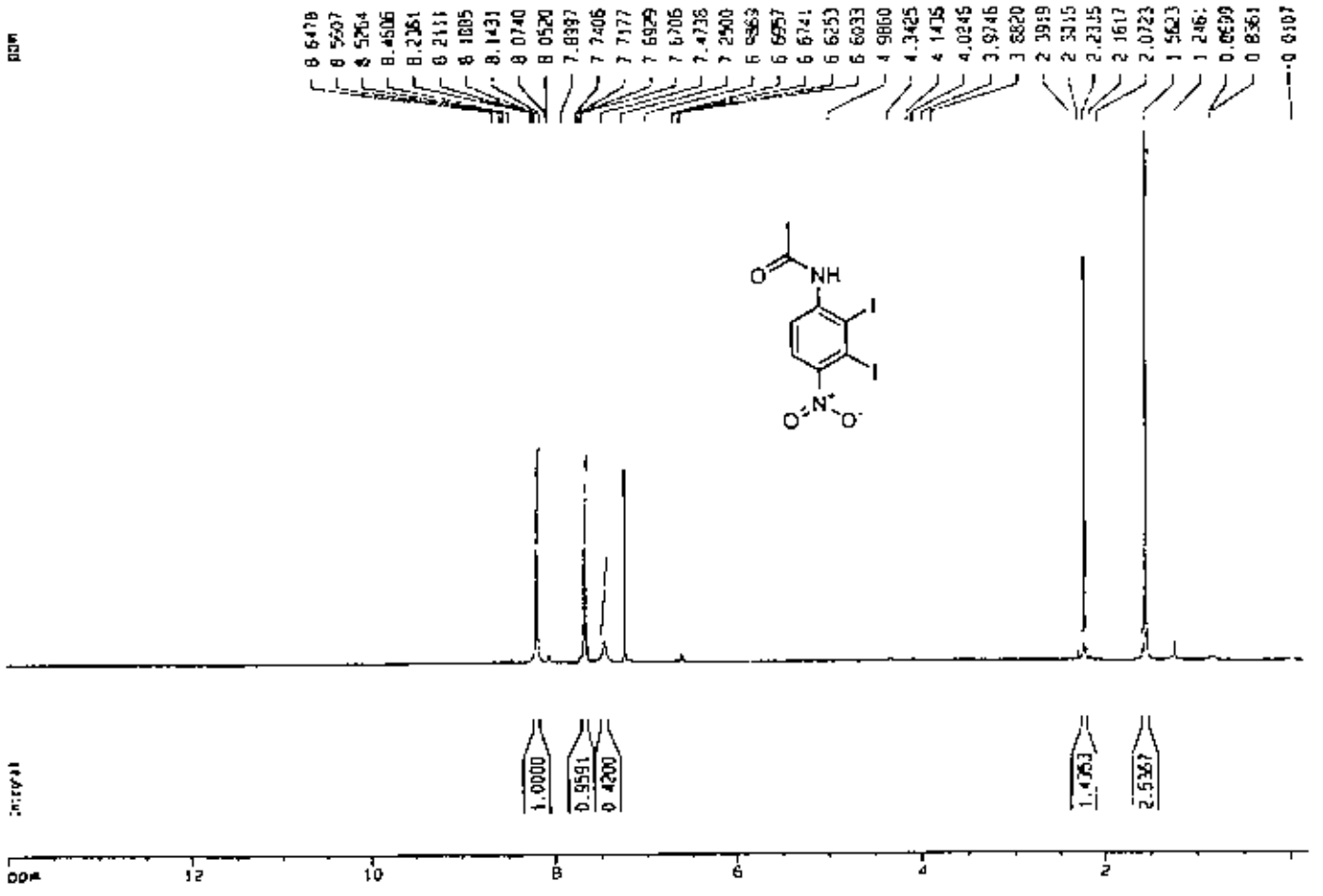


Figure 20b: IR spectrum of the compound 10

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EXPNO                     1
PROCNO                    1

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Time                  13 12
INSTRUM             qs400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                      89
DS                       2
SWH               6410 256 Hz
FIDRES            0 195625 Hz
AQ                2.5555540 sec
RG                   574.7
DW                 78 000 usec
DE                  6.00 usec
TE                 310.0 K
D1                 1 00000000 sec

========== CHANNEL f1 ========
NUC1                    1H
P1                   8 30 usec
PL1                 -6 00 dB
SFO1           400 1428010 MHz

F2 - Processing parameters
SI                   32768
SF              400.1400124 MHz
WDW                     EM
SSB                      0
LB                   0 30 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                  20 00 cm
F1P                 14 013 ppm
F1                5607 00 Hz
F2P                -0.125 ppm
F2                 -50.06 Hz
PPMCM             0 70669 ppm/cm
HZCM            282 85309 Hz/cm
```

Figure 20c: ^{1}H NMR spectrum of the compound 10

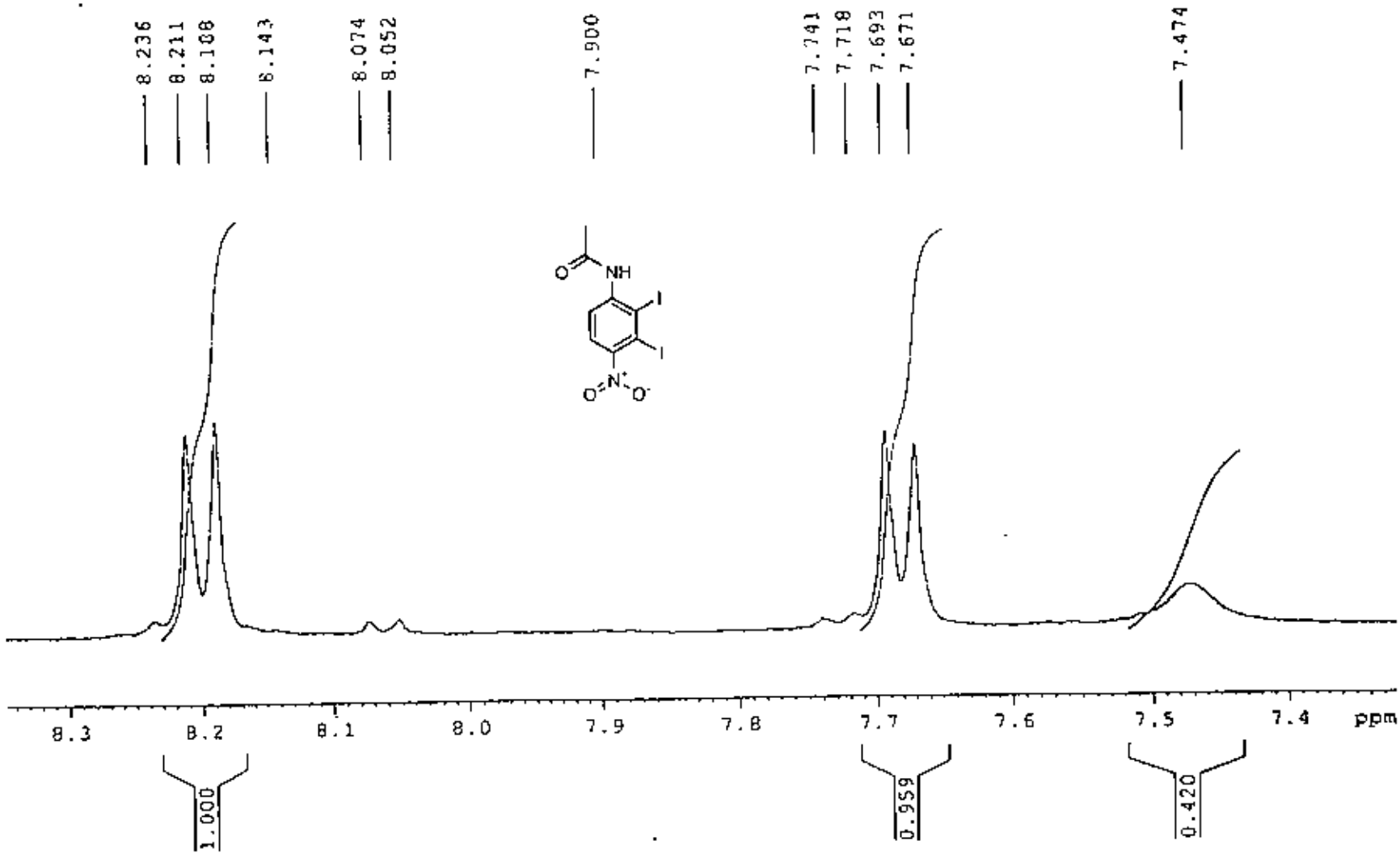


Figure 20c: ^{1}H NMR spectrum of the compound 10 (Expansion)

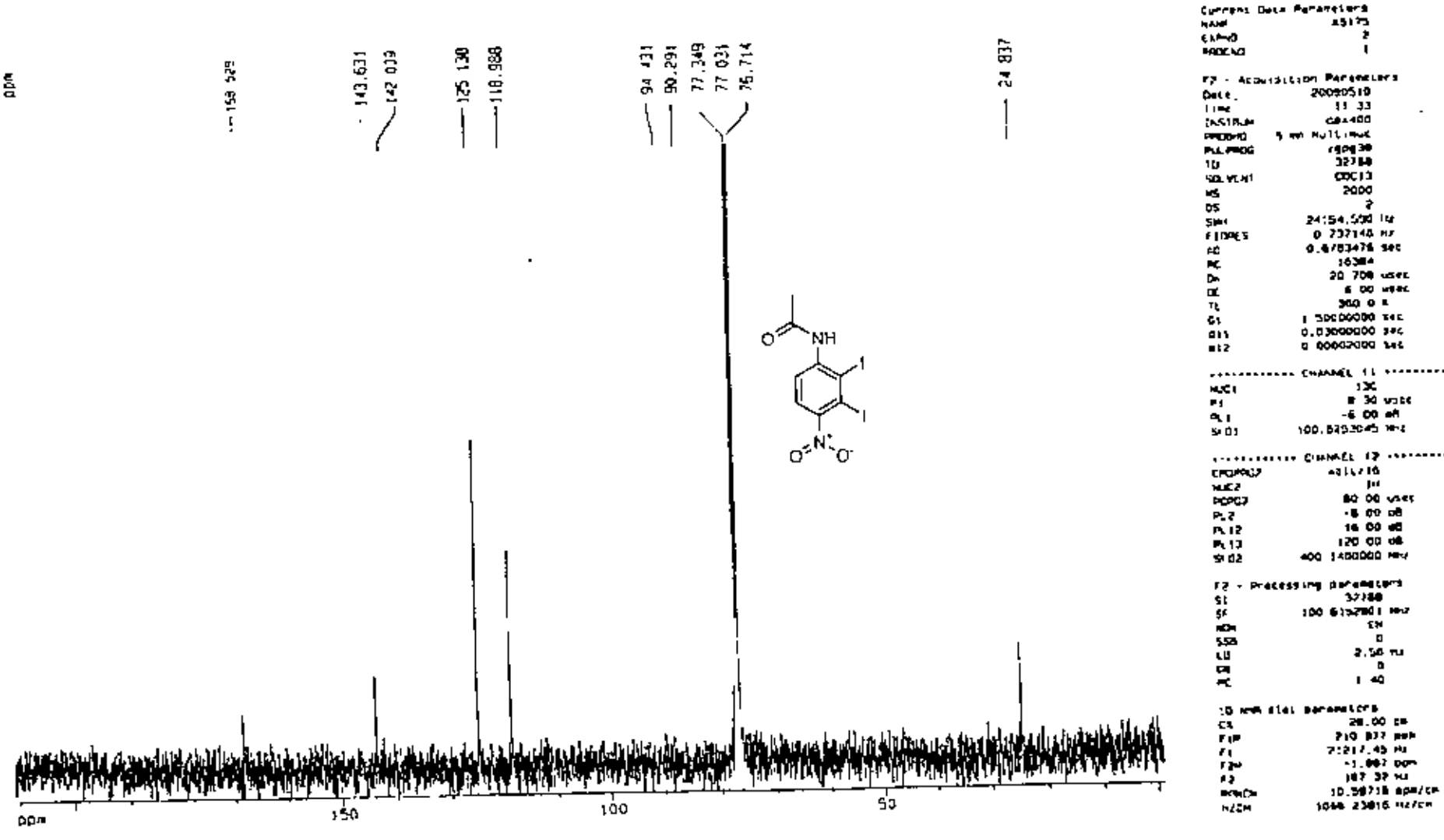


Figure 20d: ^{13}C NMR spectrum of the compound 10

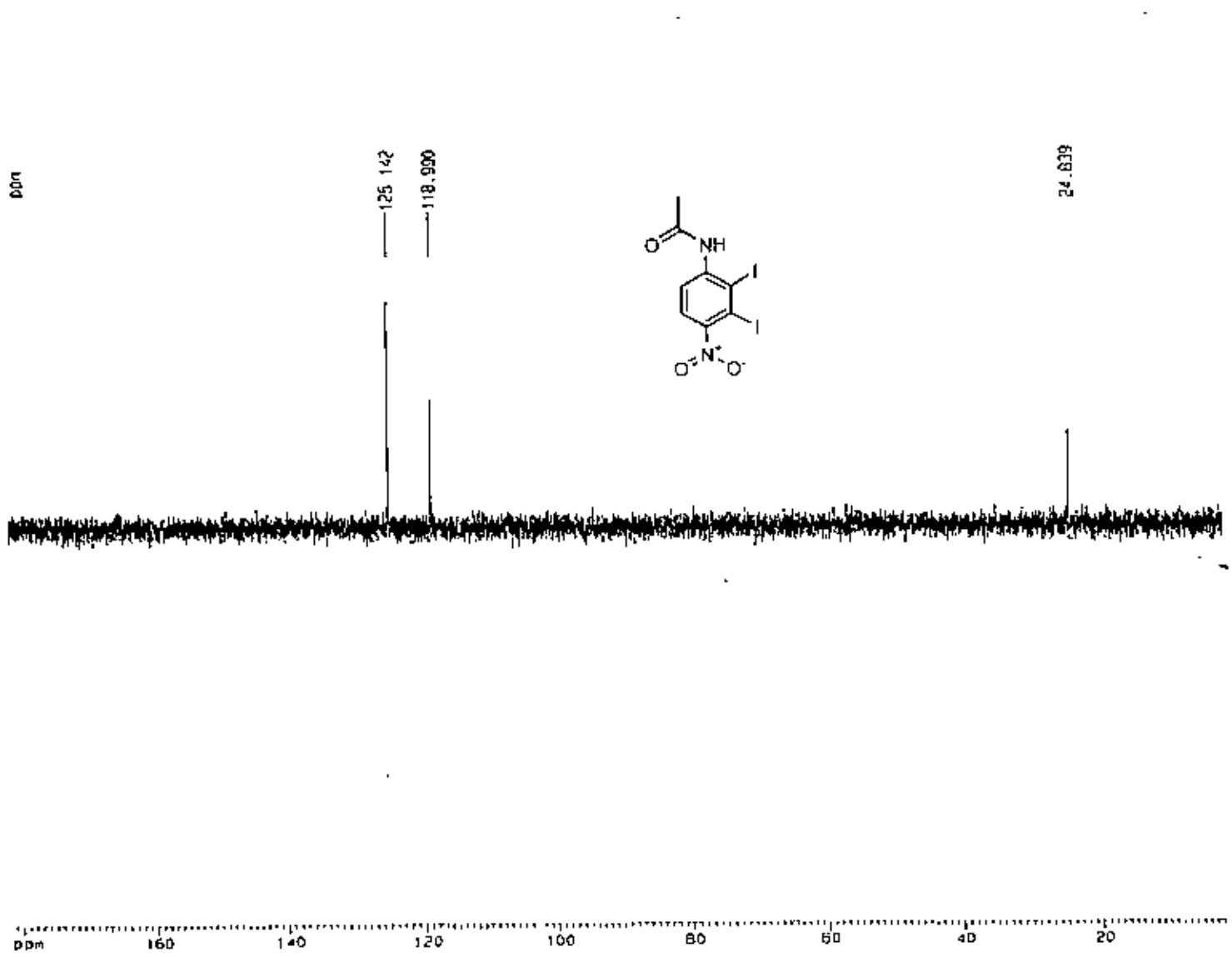
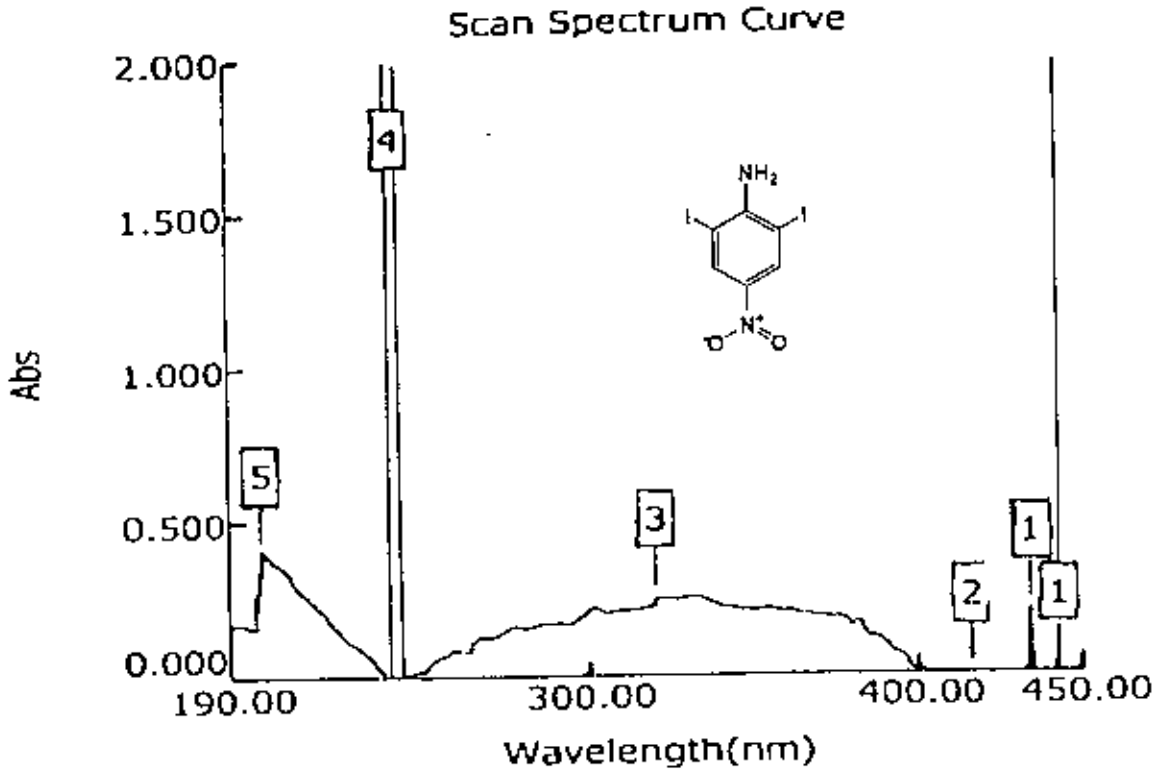


Figure 20d: Dept-135 NMR spectrum of the compound 10



1	Peak	437.00	0.202
2	Peak	415.31	0.000
3	Peak	322.01	0.253
4	Peak	243.05	9.999
5	Peak	204.09	0.408
1	Valley	444.10	-1.329

Figure 21a: UV spectrum of the compound 11

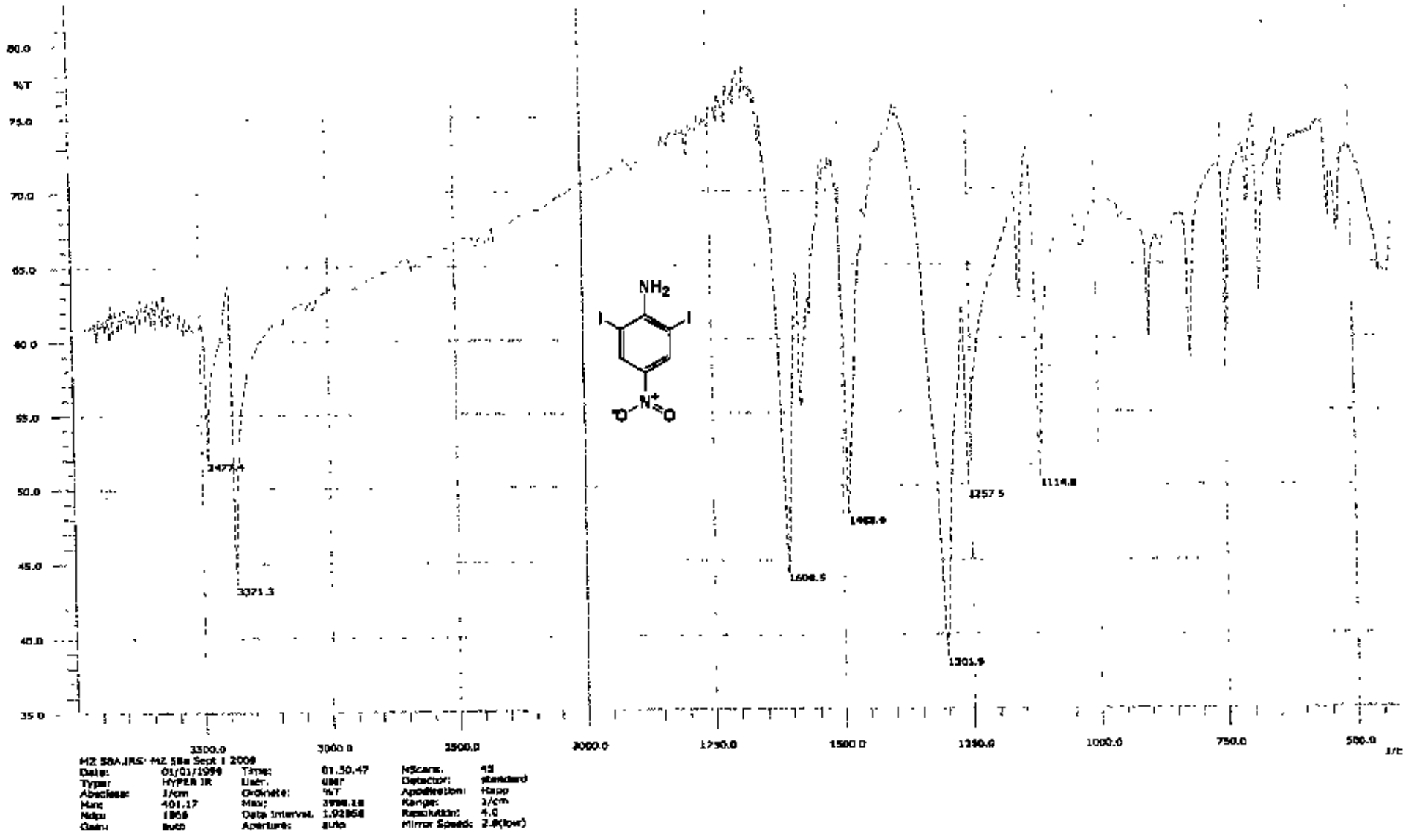
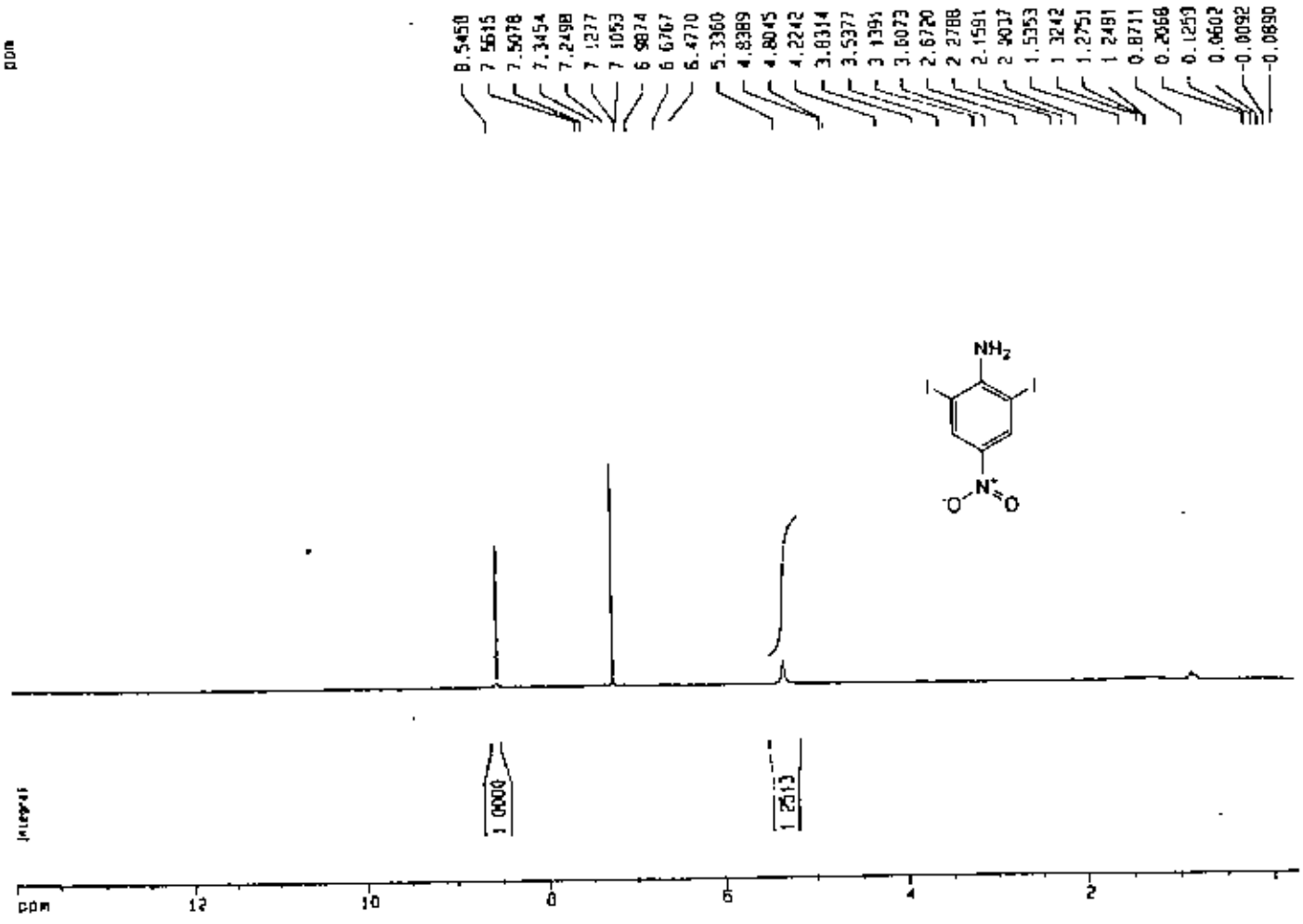


Figure 21b: IR spectrum of the compound 11



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PROCNO 1

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PULPROG zg30
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SOLVENT CDCl3
NS 32
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 512
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

..... CHANNEL f1 .....
NUC1 1H
P1 8.30 usec
PL1 -5.00 dB
SFO1 400.1428010 MHz

F2 - Processing parameters
SI 32768
SF 400.1400124 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.007 ppm
F1 5604.75 Hz
F2P -0.237 ppm
F2 -94.74 Hz
PPMCM 0.71219 ppm/cm
HZCM 284.97479 Hz/cm

Figure 21c: ^{1}H NMR spectrum of the compound 11

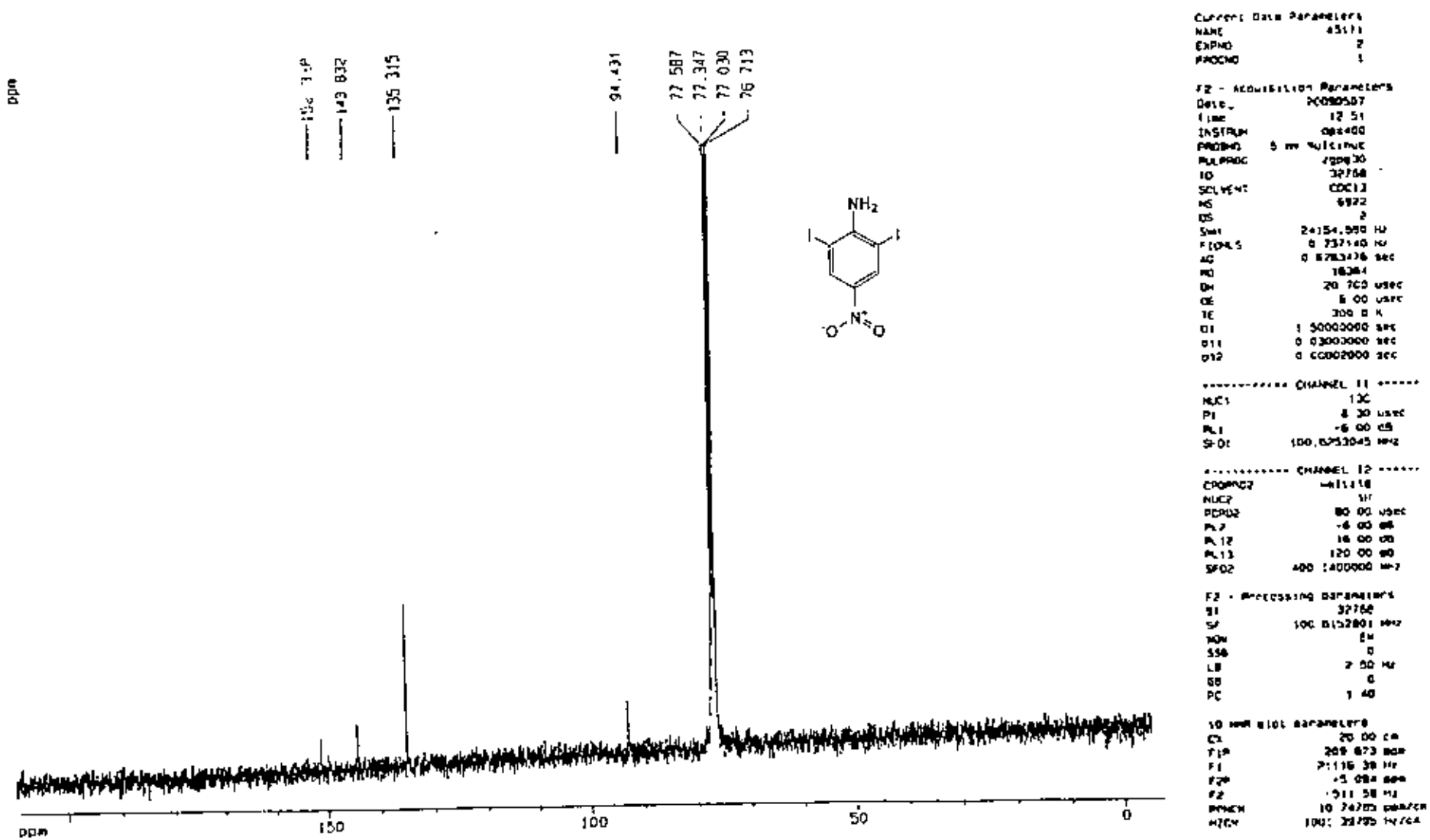


Figure 21d: ^{13}C NMR spectrum of the compound 11

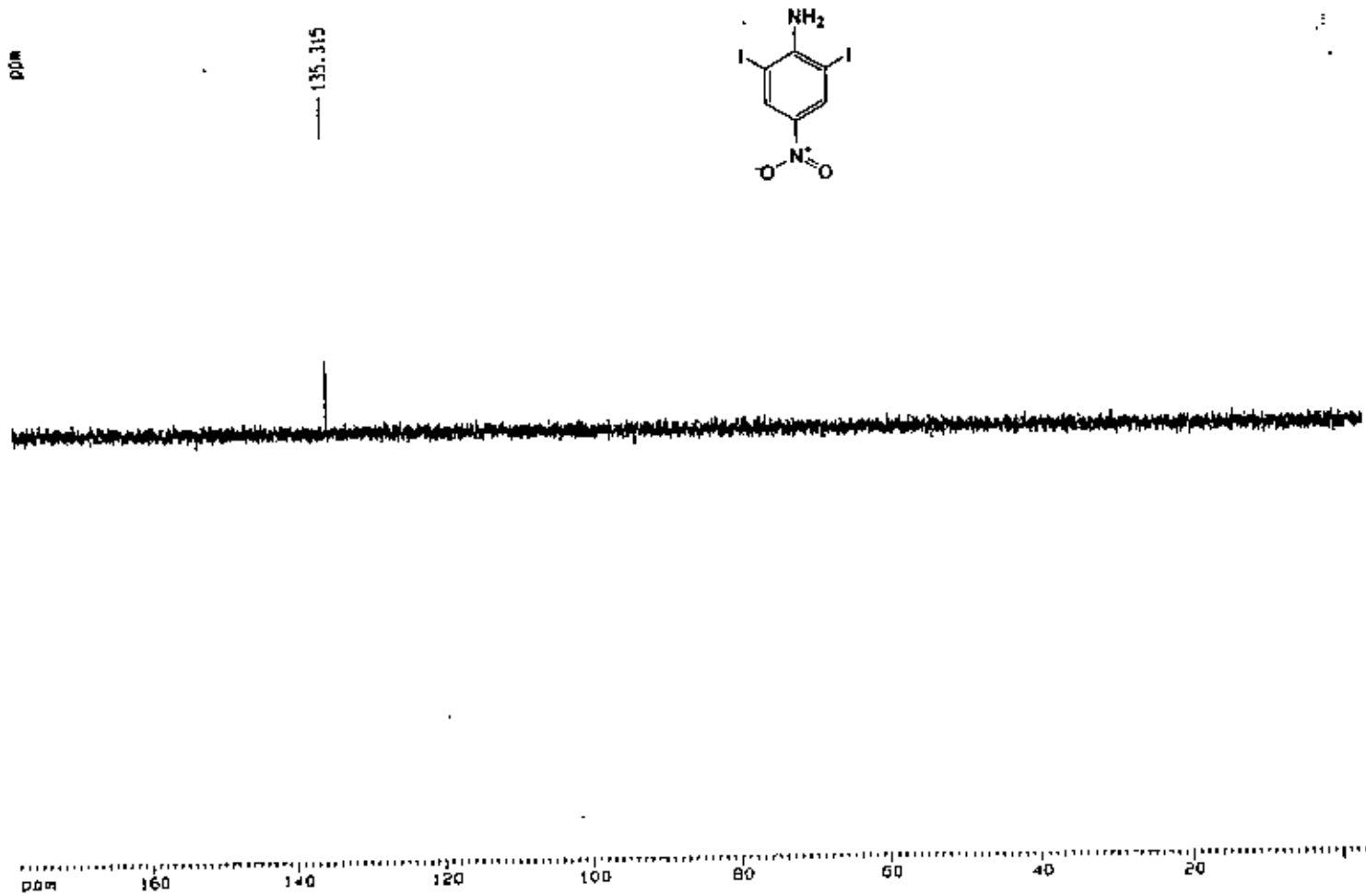
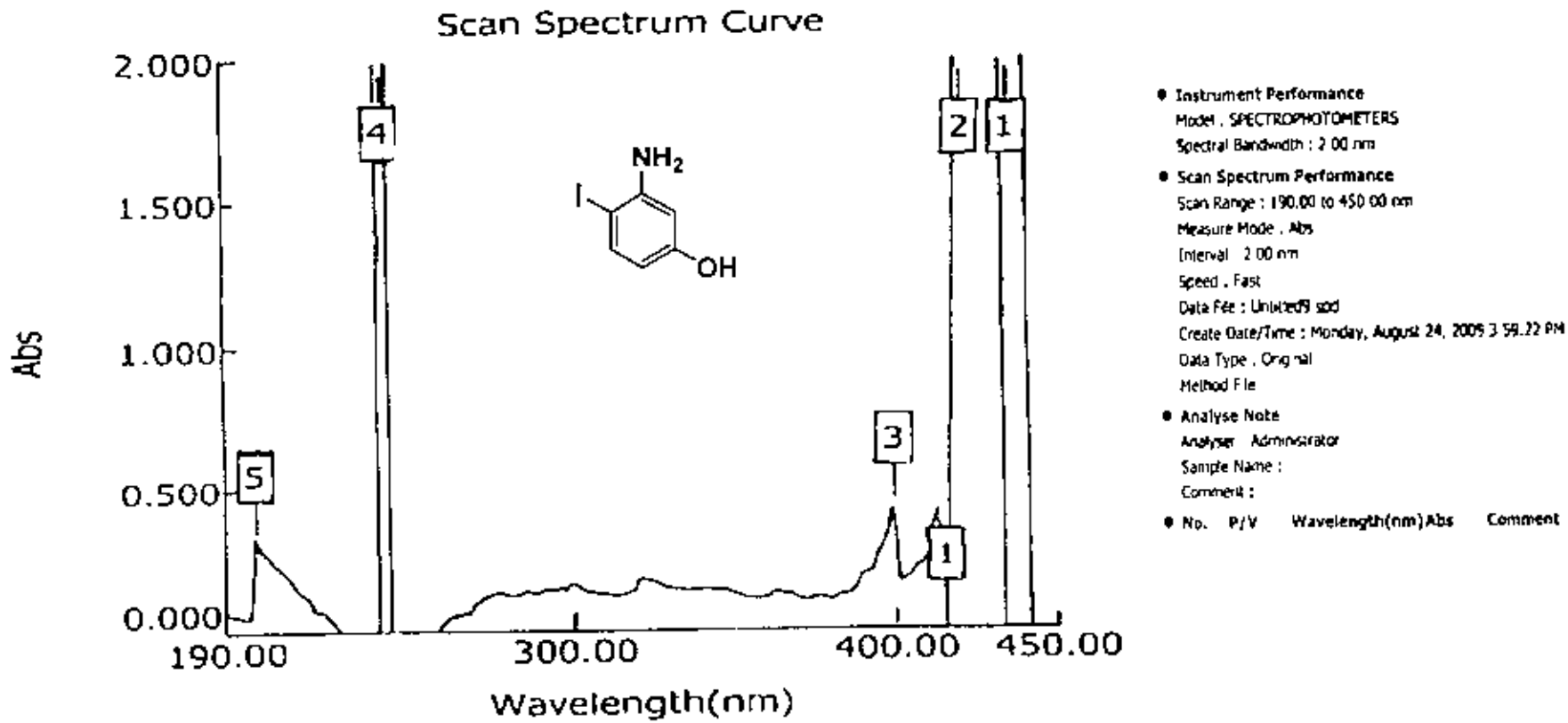


Figure 21d: Dept-135 NMR spectrum of the compound 11



1	Peak	435.98	9.999
2	Peak	423.01	9.999
3	Peak	401.87	0.420
4	Peak	239.99	9.999
5	Peak	202.55	0.306
1	Valley	415.94	0.000

Figure 22a: UV spectrum of the compound 12

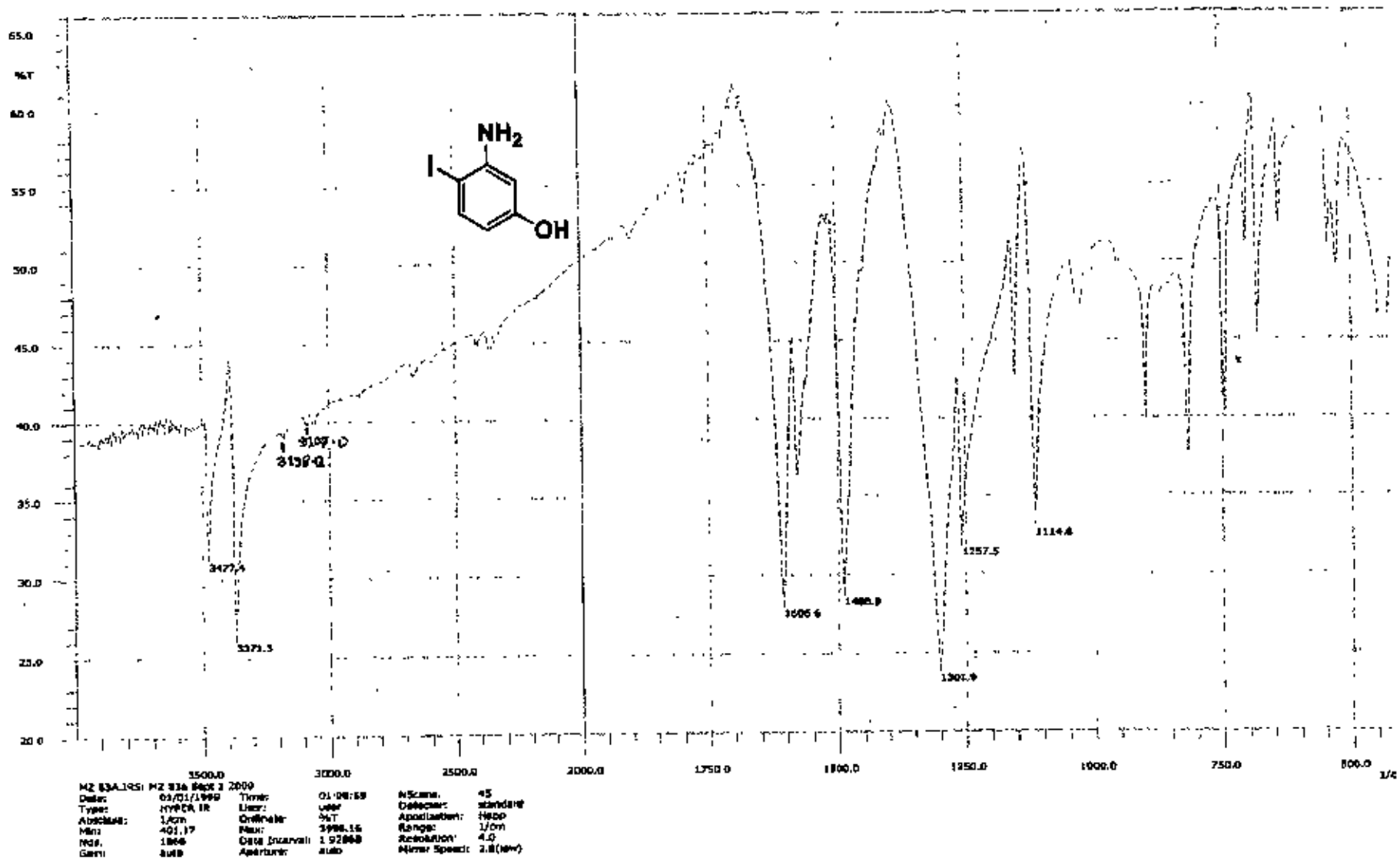
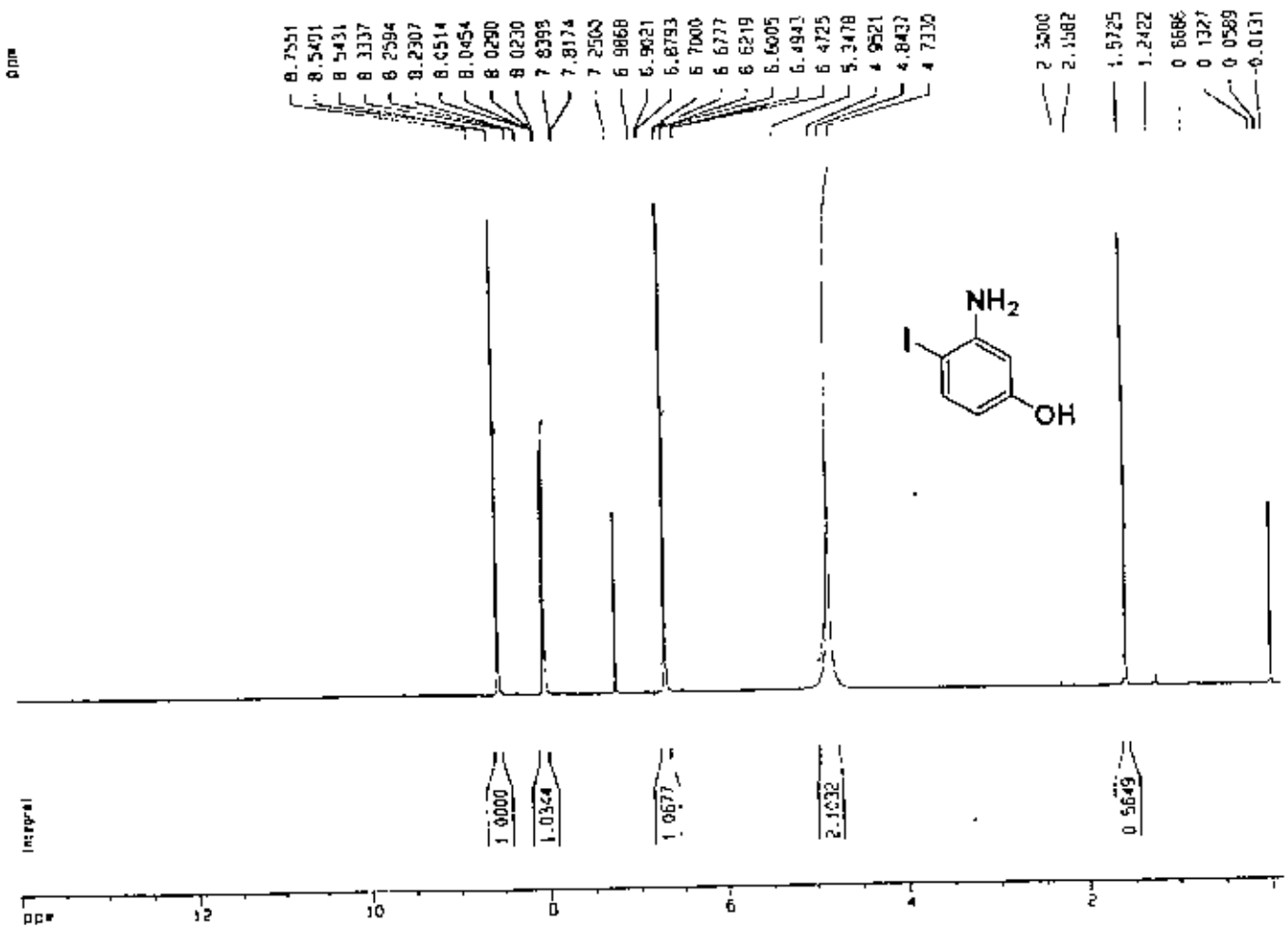


Figure 22b: IR spectrum of the compound 12

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PROCNO                    1

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TD                   32768
SOLVENT              CDCl3
NS                       16
DS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                      256
DW                 78.000 usec
DE                  6.00 usec
TE                 310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 --------
NUC1                      1H
P1                     8.30 usec
PL1                   -6.00 dB
SFO1            400.1426010 MHz

F2 - Processing parameters
SI                       32768
SF                400.1400126 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.40

1D NMR plot parameters
CX                       20.00 cm
F1P                      14.006 ppm
F1                     5604.56 Hz
F2P                     -0.120 ppm
F2                     -46.00 Hz
PPMCM                 0.70632 ppm/cm
HZCM                282.62793 Hz/cm
```

Figure 22c: ^{1}H NMR spectrum of the compound 12

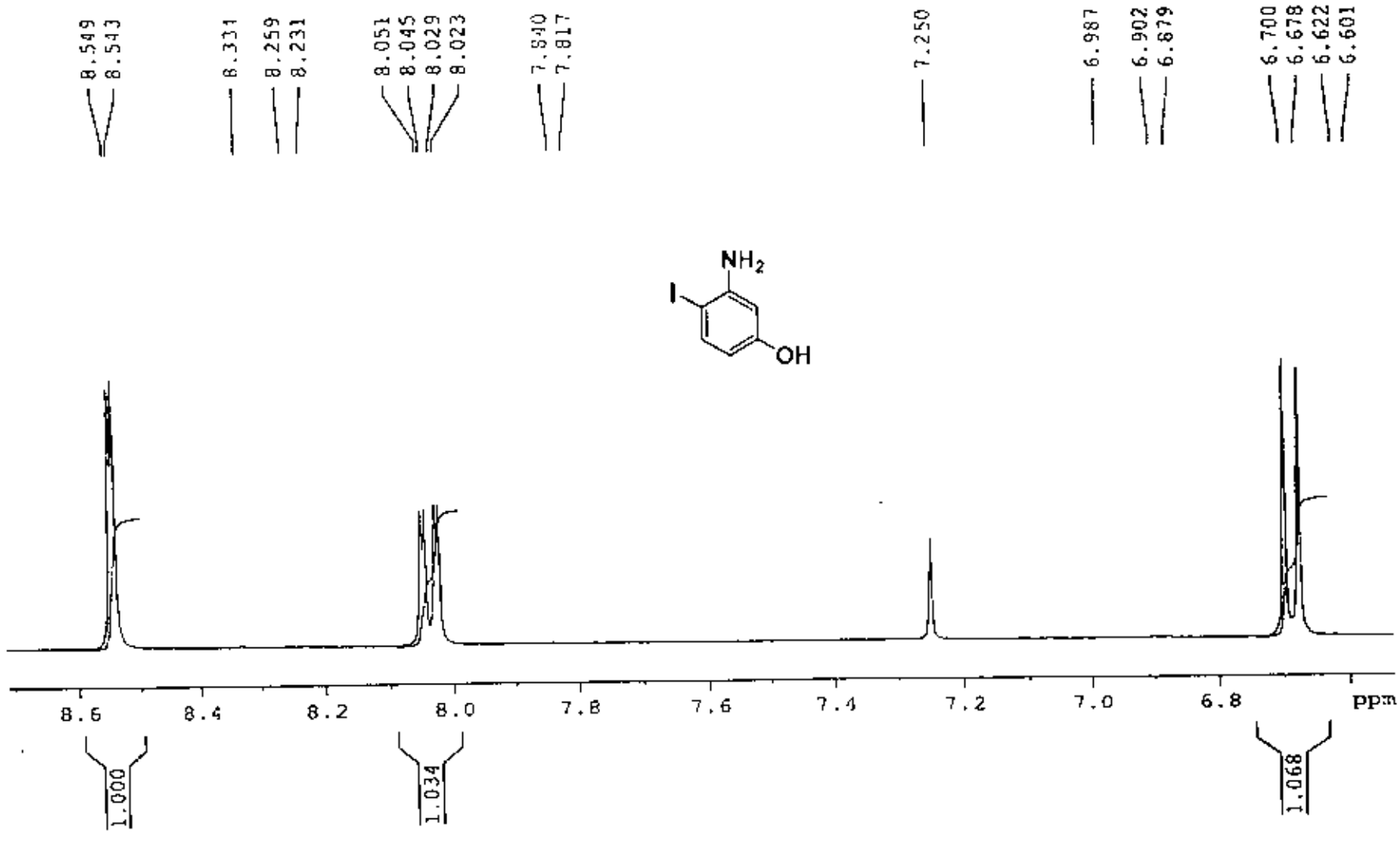


Figure 22c: ^{1}H NMR spectrum of the compound 12 (Expansion)

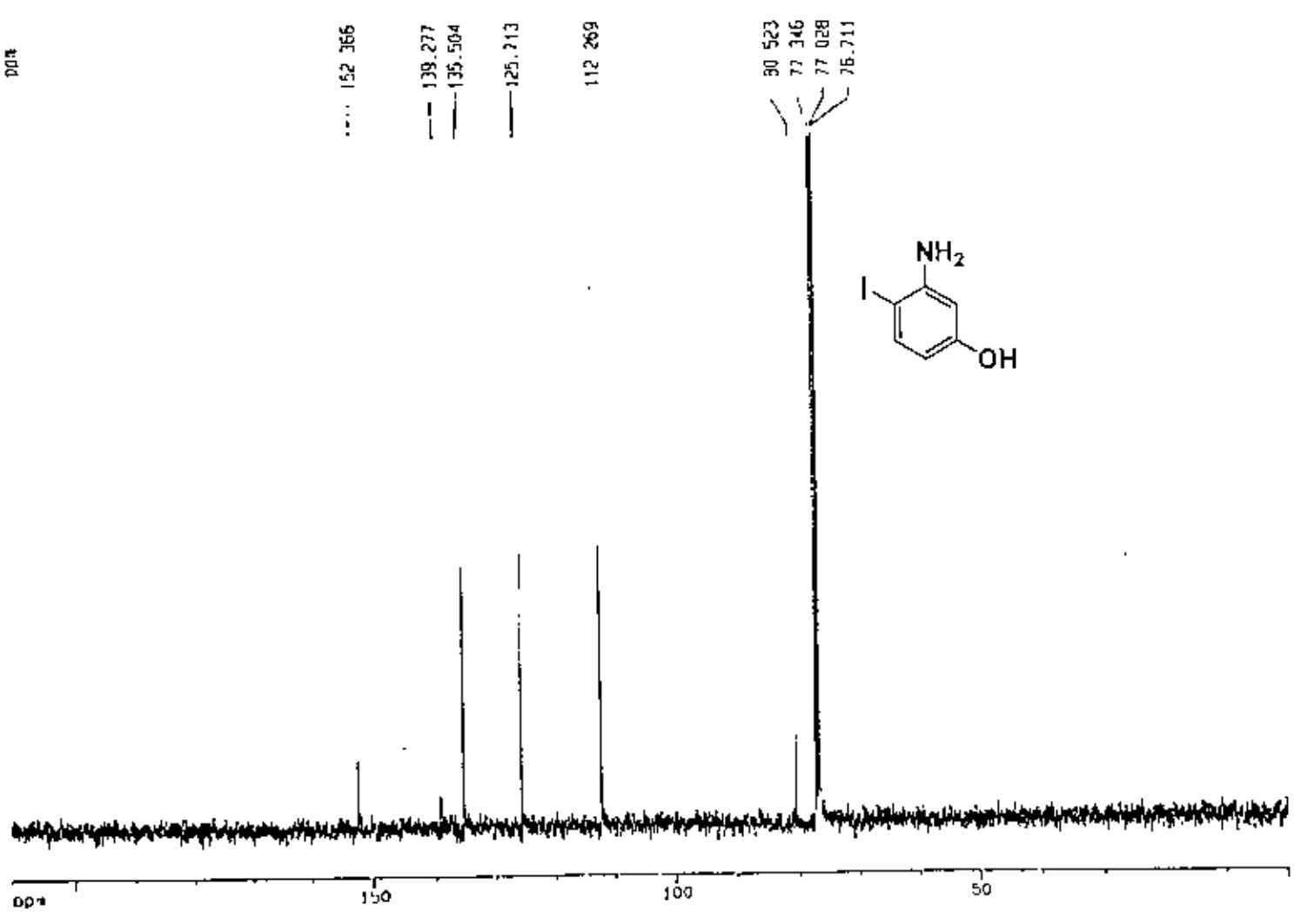


Figure 22d: ^{13}C NMR spectrum of the compound 12

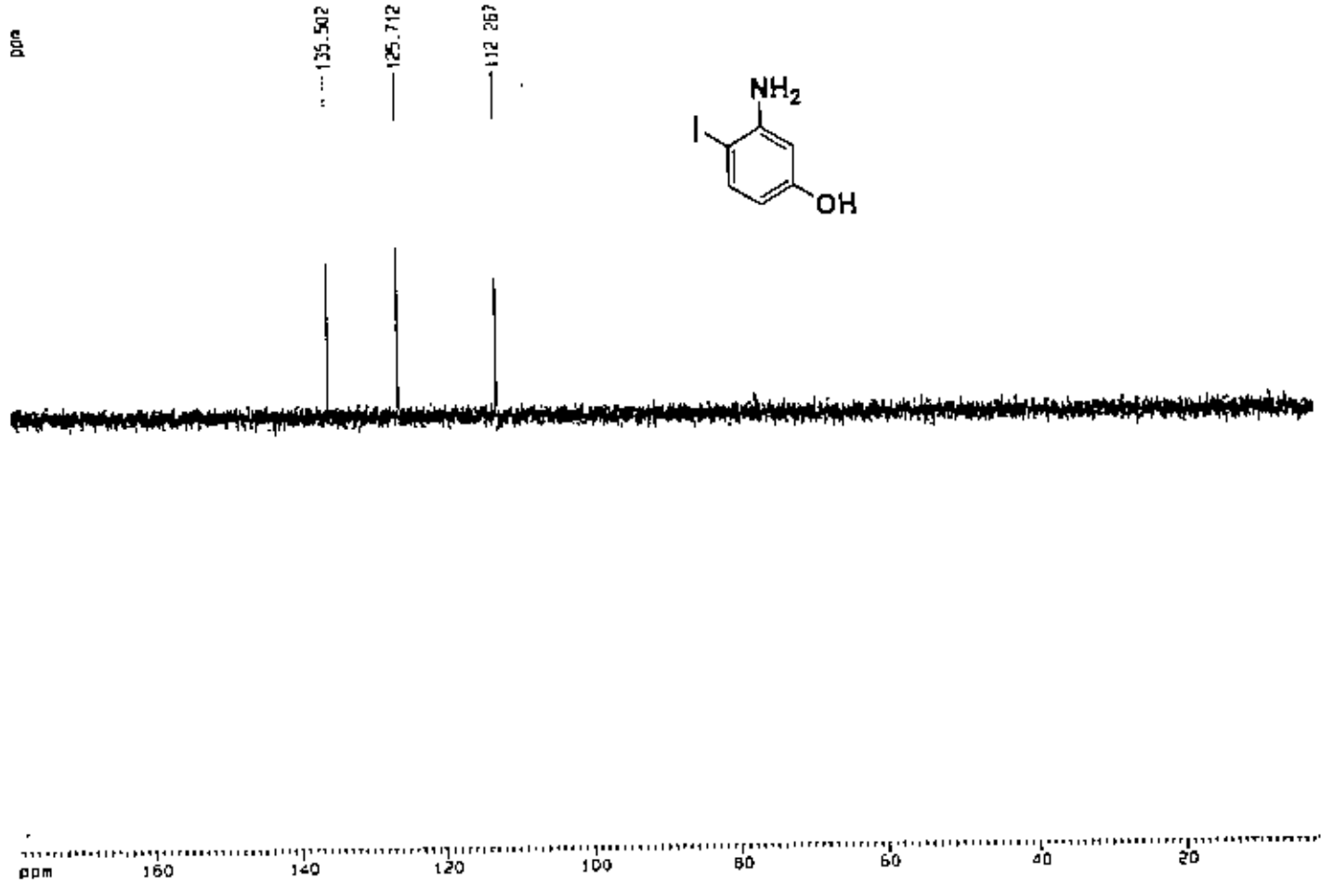


Figure 22d: Dept-135 NMR spectrum of the compound 12

CHAPTER-4

ANTIMICROBIAL SCREENING

INTRODUCTION

Bacteria and fungi are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The deterioration of human population due to enhance of prevalence of infections diseases is becoming a global problem[1]. It was found from the literature that nitrogen and sulfur containing compounds showed marked microbial activities[2-6]. When heterocyclic part of the compounds, such as; imidazole, nitroimidazole etc. become attached to carbohydrates[7],their efficiency to inhibit bacteria of fungus sharply increased. It was also found that a large number of biologically active compounds possesses aromatic and heteroaromatic molecule may possess greater potential for biological activity[8]. In *vitro* antimicrobial activities of fused pyrimidines were successfully evaluated in our laboratory[9].

M. shaheb[10] a post graduate student carried out in *vitro* antimicrobial activates of fused pyrimidine derivatives. M. S. Rabman[11] showed that antimicrobial activities of alkaloids plants leaves. The alkaloids were screened against several pathogenic bacteria.

S. M. Shahed[12, 13] a former research student of organic laboratory carries out antifungal activities of a series of acylated D-Mannose derivatives.

M. Fakruddin[14] also a research student of organic laboratory carries out antifungal activities of a series of fused pyrimidine derivatives. He used five human pathogenic bacteria *viz. Bacillus cereus, Bacillus megaterium, Bacillus, Vibrio parahemolyticus, Aspergillus niger and panicillum sp.* S. M. Abe Kawsar[15, 16] also a former research student of organic laboratory carried out in *vitro* antibacterial activities of a series of acylated uridine derivatives.

Recently, our groups synthesized 2-substituted banzofurans[17], isoindonone and isoquinolinone[18] and tested their antibacterial and antifungal activities. Plants are the natural reservoir of many antimicrobial agents. In recent times, traditional medicine as an alternative form of health care and to overcome microbial resistance has led the researchers to investigate the antimicrobial activity of medicinal plants.

The present work was under taken to select the chemicals (iodide derivatives) that have not been studied before pathogenic microorganisms of animals and plants.

4.1. Materials and methods

The anti bacterial activities of furan derivatives were studied against **thirteen bacteria** and the activities of the same compounds were also studied against three fungi. For the detection of antibacterial activities the disc diffusion method[19] was followed.

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by any of the following three methods.

a) Disc diffusion method
b) Serial dilution method
c) Bioautographic method

Among the above mentioned techniques the disc diffusion[19] is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method[20].

4.2. Principle of disc diffusion method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (Kanamycin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4^0C) for 24 hours to allow maximum diffusion of the test materials to the

surrounding media. The plates are then inverted and incubated at 37^0C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimetre.

In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method (**Fig. 22**). The experiment is carried out more than once and the mean of the readings is required[19].

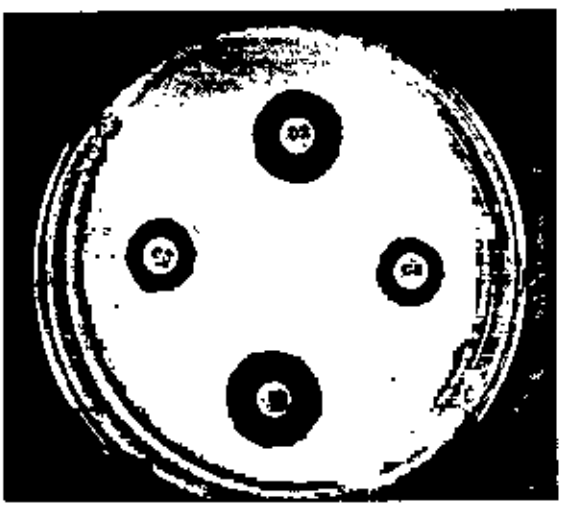


Figure 22: Disc diffusion method

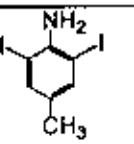
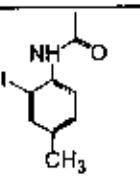
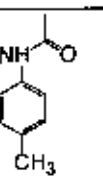
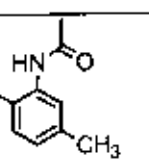
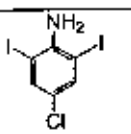
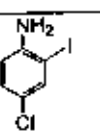
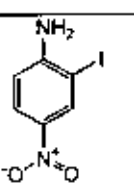
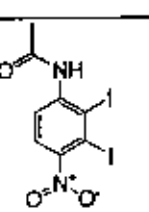
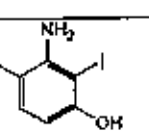
4.3. Experimental

4.3a. Apparatus and reagents:

Filter paper discs	Petri dishes	Inoculating loop
Sterile cotton	Sterile forceps	Spirit burner
Micropipette	Screw cap test tubes	Nose mask and Hand gloves
Laminar air flow hood	Autoclave	Incubator
Refrigerator	Nutrient agar medium	Ethanol
Chloroform		

4.3b. Test materials

Table 12: List of compounds used for antimicrobial activities

Comp. No.	Name of the test chemicals	Molecular structure
3	2,6-Diiodo-4-methylaniline	
4	2-Iodo-4-methylacetanilide	
5	4-Methylacetanilide	
6	*N*-(2-iodo-5-methylpheny)acetamide	
7	4-chloro-2,6-diiodoaniline	
8	4-chloro-2-iodoaniline	
9	2-Iodo-4-nitroaniline	
10	2,3-diiodo-4-nitroacetanilide	
12	3-amino-2,4-diiodophenol	

4.3c. Test organisms

The microbial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in the **Table 13.**

Table 13: List of test microorganisms

Gram positive Bacteria	Gram negative Bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Aspergillus niger*
Bacillus subtilis	*Salmonella paratyphi*	*Sacharomyces cerevaceae*
Staphylococcus aureus	*Salmonella typhi*	
Sarcina lutea	*Shigella boydii*	
	Shigella dysenteriae	
	Vibrio mimicus	
	Vibrio parahemolyticus	

4.3d. Composition of culture medium

Nutrient agar medium (DIFCO) **(Table 14)** was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

Table 14: Composition of nutrient agar medium.

a. Nutrient agar medium

Ingredients	Amounts
Bacto peptone	0.5 gm/litter
Sodium chloride	0.5 gm/litter
Bacto yeast extract	1.0 gm/litter
Bacto agar	2.0 gm/litter
Distilled water q.s.	100 ml
pH	7.2-7.6 at 25^{0}C

b. Nutrient both medium

Ingredients	Amounts
Bacto beef extract	0.3 gm/litter
Bacto peptone	0.5 gm/litter
Distilled water q.s.	100 ml
pH	7.2±0.1 at 25^{0}C

c. Muller-Hunton medium

Ingredients	Amounts
Beef infusion	30 gm/litter
Casamino acid	1.75 gm/litter
Starch	0.15 gm/litter
Bacto agar	1.70 gm/litter
Distilled water q.s.	100 ml
pH	7.3±0.2 at 25^{0}C

d. Tryptic soya both medium

Ingredients	Amounts
Bacto tryptone	1.7 gm/litter
Bacto soytone	0.3 gm/litter
Bacto dextrose	0.25 gm/litter
Sodium chloride	0.5 gm/litter
Di potassium hydrogen phosphate	0.25 gm/litter
Distilled water q.s.	100 ml
pH	7.3±0.2 at 25^{0}C

Nutrient agar medium (DIFCO) is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

4.4. Preparation of medium

Amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25^{0}C) was adjusted at 7.2-7.6 using NaOH or HCl 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs pressure at 121^{0}C for 20 minutes. The slants were used for making fresh culture of microorganisms that were in turn used for sensitivity study.

4.5. Sterilization procedure

To avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were strictly maintained. UV light was switched on an hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121^{0}C and a pressure of 15-lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

4.6. Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^{0}C for their optimum growth. These fresh cultures were used for the sensitivity test.

4.7. Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The microbial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

4.8. Preparation of discs

Three types of discs were used of antibacterial screening. They were:

(a) Standard Discs

(b) Blank Discs and

(c) Sample Discs

The descriptions of these discs were given below:

(a) Standard Discs

These were used as positive control to ensure the activity of activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the know antibacterial agent with that of produced by the test sample. In this investigation , Kanamycin, (30 µg/ disc) standard disc was used as the reference.

(b) Blank Discs

These were used as negative control which ensures that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

(c) Preparation of Sample Discs with Test Sample

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Then discs were soaked with solution of test samples and dried.

4.9. Diffusion and incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test microorganisms. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours.

4.10. Determination of the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition.

After incubation, the antimicrobial activity of the test materials was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

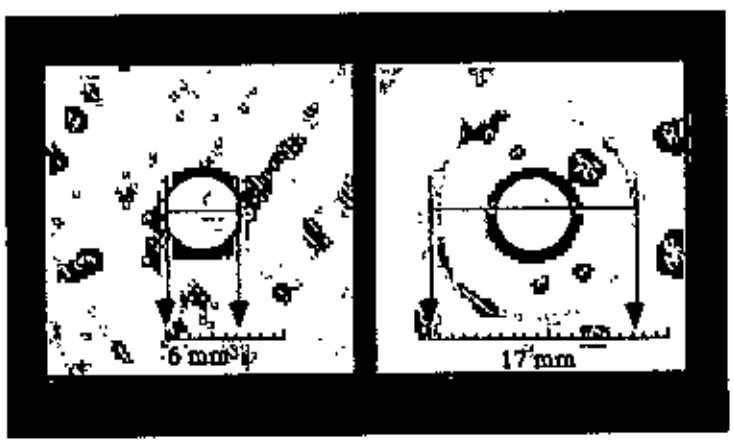


Figure 23: Determination of the zone of inhibition

4.11. RESULTS AND DISCUSSION

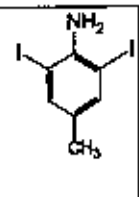
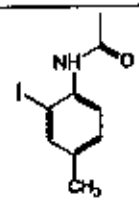
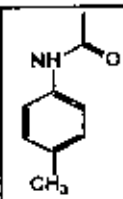
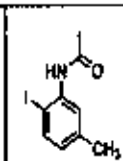
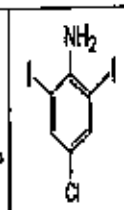
The antimicrobial activities of new aryl iodo derivatives were examined in the present study. The antibacterial activities of 2-iodoanilin or acetanilide derivatives were studied against thirteen bacteria such as *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus, Vibrio parahemolyticus* and the activities of the same compounds were also studied against three fungi such as *Candida albicans, Aspergillus niger, Sacharomyces cerevaceae*. All compounds were soluble in chloroform and some compounds were showed mild to sensitive inhibitory activity against microbial growth & the average zone of inhibition produced by them 8-25 mm. The result of the diameter of inhibition zone and percentage of inhibition of microbial growth due to the effect of chemicals, are presented in **table 15** to **table 16.**

The antibacterial activities were measured in terms of diameters of zone of inhibition in (mm). All experiments were performed thrice to minimize the experimental plus individual errors. The mean value of the diameters of zone inhibition (M.DIZ) was taken as in disc for determining antimicrobial spectra. Sensitivity test results are in **table 15 to 16** and were compared with a standard antibiotic kanamycin (30-40 µg/disc).

The gram positive and gram negative as well as pathogenic fungi used in the present investigation, three synthesized compounds **(8, 9 and 10)** were found comparatively good inhabitant activity against most of the tested organisms, at a dose of 400 µg/disc shown in **tables 15 and 16**. There were completely no activities of the synthesized compounds **3, 4, 5, 6, 7** and **12 (Table 15 & Table 16)**.

Two compounds 2-iodo-4-nitroaniline and 2,3-diiodo-4-nitroacetanilide (**9, 10**) showed mild (M.DIZ 8-10 mm) and sensitive (M.DIZ 17-25 mm, but the fungi *Aspergillus niger* showed mild activities, M.DIZ 13 mm) activities all tested organisms respectively (**Table 16**). One the other hand, 4-chloro-2-iodoaniline, **8** showed sensitive activities (M.DIZ 16-19 mm) all the tested organisms except the fungi *Aspergillus niger* (M.DIZ 13 mm). But the compound 4-chloro-2,6-diiodoaniline, 7 was showed full resistance against all the tested organisms. Therefore, it is not possible to determine the essential structure feature for antimicrobial action of this series of compound properly.

Table 15: Antimicrobial activities of test samples 3-7

Test microorganisms	Diameter of zone of inhibition(mm)*					
	 3	 4	 5	 6	 7	KAN
Gram positive bacteria						
Bacillus cereus	-	-	-	-	-	30
Bacillus megaterium	-	-	-	-	-	31
Bacillus subtilis	-	-	-	-	-	31
Staphylococcus aureus	-	-	-	-	-	32
Sarcina lutea	-	-	-	-	-	32
Gram negative bacteria						
Escherichia coli	-	-	-	-	-	31
Pseudomonas aeruginosa	-	-	-	-	-	32
Salmonella paratyphi	-	-	-	-	-	32
Salmonella typhi	-	-	-	-	-	32
Shigella boydii	-	-	-	-	-	32
Shigella dysenteriae	-	-	-	-	-	31
Vibrio mimicus	-	-	-	-	-	32
Vibrio parahemolyticus	-	-	-	-	-	32
Fungi						
Candida albicans	-	-	-	-	-	32
Aspergillus niger	-	-	-	-	-	32
Sacharomyces cerevacae	-	-	-	-	-	32

*potency per disc 400 µg

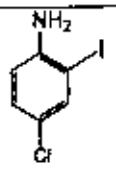
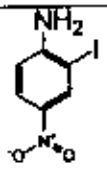
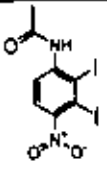
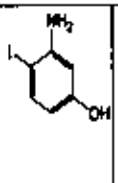
Interpretation of sensitivity test results:

Gram (+) Bacteria:	**Gram (-) bacteria**
18mm (M.DIZ) = Sensitive	>16mm (M.DIZ) = Sensitive
14-18 mm (M.DIZ) = Intermediate	13-16 mm (M.DIZ) = Intermediate
>14 mm (M DIZ) = resistant	>13 mm (M.DIZ) = resistant

KAN: Standard kanamycin disc

"-" indicated no sensitivity or zone of inhibition lower than 6 mm.

Table 16: Antimicrobial activities of test samples 8, 9, 10 & 12

Test microorganisms	Diameter of zone of inhibition(mm)*				
	 8	 9	 10	 12	KAN
Gram positive bact.					
Bacillus cereus	16	8	17	-	30
Bacillus megaterium	17	8	18	-	31
Bacillus subtilis	16	12	25	-	31
Staphylococcus aureus	17	10	22	-	32
Sarcina lutea	17	10	19	-	32
Gram negative bact.					
Escherichia coli	17	10	21	-	31
Pseudomonas aeruginosa	17	10	17	-	32
Salmonella paratyphi	16	10	20	-	32
Salmonella typhi	17	9	20	-	32
Shigella boydii	19	10	19	-	32
Shigella dysenteriae	17	9	18	-	31
Vibrio mimicus	16	10	20	-	32
Vibrio parahemolyticus	16	10	16	-	32
Fungi					
Candida albicans	17	10	20	-	32
Aspergillus niger	13	8	13	-	32
Sacharomyces cerevacae	17	10	20	-	32

*potency per disc 400 µg

Interpretation of sensitivity test results:

Gram (+) Bacteria:	**Gram (-) bacteria**
18mm (M.DIZ) = Sensitive	>16mm (M.DIZ) = Sensitive
14-18 mm (M.DIZ) = Intermediate	13-16 mm (M.DIZ) = Intermediate
>14 mm (M.DIZ) = resistant	>13 mm (M.DIZ) = resistant

KAN: Standard kanamycin disc

"-" indicated no sensitivity or zone of inhibition lower than 6 mm.

4.12. Conclusion

Nine synthesized iodo compounds have been tested for in antimicrobial activity against five gram-positive and eight gram-negative bacteria as well as three human fungal pathogens. Some of this compound demonstrated mild to sensitive antimicrobial activity against most of the test organism. Form these structures we found that the aryl-nitro group causes relatively microbial growth inhibition.

Two compounds 8, and 9 were showed intermediate and mild activity, could probably bed due to their chlorine, iodine and nitro and acetanilide group of C-4, C-2 and C-4, C-1 position of Benzene ring respectively. The higher activity of the compound 10 could probably bed due to their nitro and acetanilide group of C-4 & C-1 position of benzene ring. Which subsequently facilitated the diffusion of the chemical entities through the microbial cell wall? Among the tested compound 3, 4, 5, 6, 7, 12 were showed resistant the entire tested microorganism.

4.13. REFERENCES

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CONCLUSIONS

- In this thesis, we demonstrated a convenient and facile method for the synthesis of 2-iodoaniline and 2-iodoacetanilide from *o* or *p*-substituted amine.

- The one pot iodination reaction of *o* or *p*-substituted amine by iodine, copper(II) acetate in acetic acid afforded 2,6-diiodo-4-methylaniline, 2-iodo-4-methylacetanilide, 4-methylacetanilide, 2-iodo-5-methylacetanilide, 4-chloro-2,6-diiodoaniline, 4-chloro-2-iodoaniline, 2-iodo-4-nitroaniline, 2,3-diiodo-4-nitroacetanilide, 2,6-diiodo-4-nitroaniline and 3-amino-4-iodophenol is also establish successfully.

- Finally, all synthesized compounds (except the compound **11**) were tested antibacterial and antifungal activity, some of them demonstrated mild to moderate antimicrobial activity against most of the test organism.

- Therefore this methodology could be utilized to synthesize the biologically important iodo-aniline and iodo-acetanilide derivatives. This method will be attractive to both organic and medicinal chemistry.