A PROJECT REPORT ON
"SYNTHESIS OF ANAGRELIDE IMPURITY"

INDUSTRIAL PROJECT REPORT CARRIED OUT AT

Anugraha Chemicals**, Bangalore**

IN PARTIAL FULLFILMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER OF SCIENCE IN CHEMISTRY

(ORGANIC CHEMISTRY)

SUBMITTED BY

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ACKNOWLEDGEMENT

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I take this opportunity to express my profound gratitude and deep regards to those people who have really been contributory in the successful completion of this internship.

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With love and affection, I wish to express my deep gratitude and thanks to my beloved **family, classmates and my friends** for their constant encouragement, patience, support and who are the foundation for all my success and achievements.

DECLARATION

I hereby declare that the matter embodied in this project report entitled **"SYNTHESIS OF ANAGRELIDE IMPURITY"** is the sole result of research work carried out by myself in Organic Synthesis R&D Laboratories, Bangalore, from Oct 2022 to Nov 2022 under the guidance of Dr. Suresh Babu, Anugraha Chemicals, Bangalore and Dr. Vishwanatha P, Dean and HOD Department of PG Studies in Chemistry SDM College, Autonomous Ujire.

I further declare that the work presented in the report is a genuine and bonafide work and it has not previously been formed the basis for the award of any degree, diploma fellowship or other similar title.

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CERTIFICATE

This is to certify that the work presented in the project dissertation entitled **"SYNTHESIS OF ANAGRELIDE IMPURITY"** is an authentic record of the original and independent work carried out by **Mr. RAKSHITH.R** under the guidance of **Dr. M. SURESH BABU** (Manager), R&D, Anugraha Chemicals, Bangalore), for the award of degree in Master of Science- Organic Chemistry from SDM College (Autonomous), Department of PG studies in Chemistry, Ujire, during the year 2022.

The matter embodied in this project work has not been submitted to any other institute or university for the award of any other degree or diploma.

Dr. Lokesh Ravilla

(Operations-Head)

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CHAPTER-1

Introduction to the Industry:

Anugraha Chemicals was established in 1991. It aspires to be one of the famed API and Intermediate Manufacturers of the world feeding to the Health Industry. Value creation through exploration and development of crucial technologies. Focusing on Innovation and creation of Intellectual Property. Their mission is to develop products and produce substantial value for the company in coming years.

Their crucial ideal is timely delivery, prompt responses to client queries, helping them to request process and support their ANDA/ Dossier on timely manner. They strive hard to give Quality API as per ICH guidelines and Quality Reg prospects.

Company offers services ranging from supporting the DMF (medicine Master train) and ANDA (shortened New Drug Application) throughout the lifecycle of the Product ranging from answering DL (Deficiency Letters) from Regulatory Agencies with sound specialized result. Form DMF in time supporting the ANDA holders for reaching request, bear a strong mate with sound knowledge on the nonsupervisory aspects and strength in Chemistry also logical Chemistry with a largely motivated platoon who execute the process efficiently on large scale, making sure that all of the operations are carried out in a way that meets the conditions and prospects of nonsupervisory agencies.

Installation is approved by US FDA, EDQM and ANSM for EU GMP compliance and is also audited & approved by several guests and large drugstore like Pfizer, GSK, Novartis, Sanofi Aventis, Proctor & Gamble etc for GMP compliance.

FACILITIES: ANUGRAHA is adequately equipped to facilitate quality and efficient research

Lab Experience.

I started my internship by visiting the R&D Lab knowing about the on-going synthesis of chemicals, the process involved in it and how it is carried on a research and development department. During the first week, I got the idea about the process, reactions and did a lot of literature survey about the chemicals used in the synthesis of organic compounds. Based on the structure of a molecule various journals, articles from different search engines like Reaxys & Scifinder were referred to gain more information about the molecular properties. The MSDS (Material Safety Data Sheet) provided by ANUGRAHA CHEMICALS was very helpful in order to carry out the reactions safely. The routine discussions with the senior officials helped me gain more information about the project.

Various sessions were called to discuss the safety issues in the company. Employees were asked to discuss the 'NEAR MISSES' that took place in the entire month. This kind of activity helped everyone to understand the importance of their personal as well as public safety and also helped everyone to work in a safe and sound environment.

CHAPTER-2

Environmental Health and Safety

Environmental protection, occupational health and safety at work are three major concerns of most of the leading companies all over the globe. EHS management has two general objectives: prevention of incidents or accidents that might result from abnormal operating conditions and reduction of adverse effects that result from normal operating conditions.

The Environmental Health and Safety (EHS) Department of Anugraha Chemicals is staffed by multidisciplinary professionals with responsibilities for ensuring a safe environment for employees. The EHS department of Anugrha Chemicals has following strict regulation to ensure the safety of employees and the trainees. Each employee and trainee has to undergo an EHS training program before entering the lab premises for the first time. Regular seminars on hazard management and safety are organized to assure employee understanding and compliance. Anugraha Chemicals ensures minimum release of chemicals to air water or land and protect and promote the health and safety of employees and visitors.

Personal Protective equipment:

Personal protective equipment (PPE) refers to protective clothing, helmets, goggles, or other garments or equipment designed to protect the wearer's body

from injury. The hazards addressed by protective equipment include physical, electrical, heat, chemicals, biohazards, and airborne particulate matter. Protective equipment may be worn for job-related occupational safety and health purposes. The purpose of personal protective equipment is to reduce employee exposure to hazards when engineering and administrative controls are not feasible or effective to reduce these risks to acceptable levels. PPE has the serious limitation that it does not eliminate the hazard at source and may result in employees being exposed to the hazard if the equipment fails.

Fig: Personal protective equipment (PPE)

Gloves

A glove is a garment covering the hand. Gloves have separate sheaths or openings for

each finger and the thumb; if there is an opening but no covering sheath for each finger they are called "fingerless gloves". Fingerless gloves with one large opening rather than individual openings for each finger are sometimes called gauntlets. Gloves which cover the entire hand or fist but do not have separate finger openings or sheaths are called mittens. Mittens are warmer than gloves made of the same material because fingers maintain their warmth better when they are in contact with each other. Reduced surface area reduces heat loss.

Apron

Lab aprons are synthetic rubber coated and are acid-resistant, alcohol-resistant and have a better oil-resistance than natural rubber.

- Aprons have an excellent abrasion and tear resistance.
- Lab Aprons are full cut and fitted with quality ties at neck and waist.

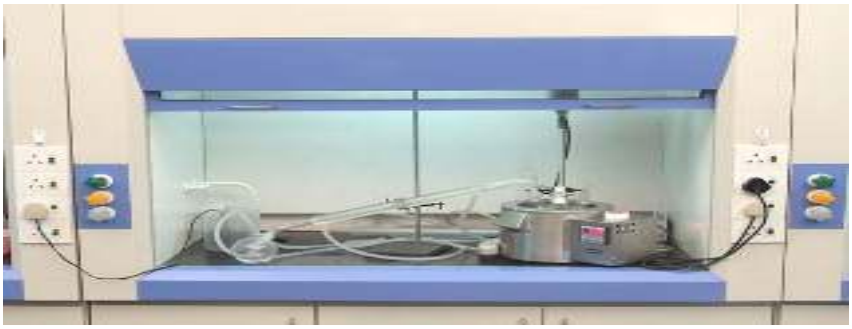
Safety Goggles

Molded of soft, clear, flexible vinyl that conforms to the face and nose, these goggles have 4 vents to eliminate fogging. With clear acetate lenses, the goggles can be worn over regular eye-glasses with complete comfort. 13mm (1/2") wide adjustable elastic headband.

Gas Mask

A gas mask is a device designed to protect the wearer from noxious vapors, dust, and other pollutants. Masks may be designed to carry their own internal supply of fresh air, or they may be outfitted with a filter to screen out harmful contaminants.

Fume Hood



A fume hood or fume cupboard is a type of local ventilation device that is designed to limit exposure to hazardous or toxic fumes, vapors or dusts. A fume hood is typically a large piece of equipment enclosing five sides of a work area, the bottom of which is most commonly located at a standing work height.

Two main types exist, ducted and re circulating (aka ductless). The principle is the same for both types: air is drawn in from the front (open) side of the cabinet, and either expelled outside the building or made safe through filtration and fed back into the room. Other related types of local ventilation devices include: clean benches, bio safety cabinets, glove boxes and snorkel exhausts. All these devices address the need to control airborne hazards or irritants that are typically generated or released within the local ventilation device. All local ventilation devices are designed to address one or more of three primary goals: to protect the user from inhaling toxic gases (fume hoods, bio safety cabinets, glove boxes);

- To protect the product or experiment (bio safety cabinets, glove boxes);
- To protect the environment (re circulating fume hoods, certain bio safety cabinets, and any other type when fitted with appropriate filters in the exhaust airstream). secondary functions of these devices may include explosion protection, spill containment, and other functions necessary to the work being done within the device.

CHAPTER - 3

Importance of Literature survey

Literature survey is a body of text that aims to review the critical points of current knowledge including substantive findings as well as theoretical and methodological contributions to a particular topic. Literature reviews are secondary sources, and as such, do not report any new or original experimental work.

A well-structured literature review is characterized by a logical flow of ideas; current and relevant references with consistent, appropriate referencing style; proper use of terminology; and an unbiased and comprehensive view of the previous research on the topic. Literature survey is the documentation of a comprehensive review of the published and unpublished work from secondary sources data in the areas of specific interest to the researcher. The library is a rich storage base for secondary data and researchers used to spend several weeks and sometimes months going through books, journals, newspapers, magazines, conference proceedings, doctoral dissertations, master's thesis, government publications and financial reports to find information on their research topic. With computerized databases now readily available and accessible the literature search is much speeder and easier and can be done without entering the portals of a library building.

The researcher could start the literature survey even as the information from the unstructured and structured interviews is being gathered. Reviewing the literature on the topic area at this time helps the researcher to focus further interviews more meaningfully on certain aspects found to be important is the published studies even if these had not surfaced during the earlier questioning. so the literature survey is important for gathering the secondary data for the research which might be proved very helpful in the research.

Databases for literature search include Reaxys (http://cn-www.reaxys.com), Scifinder (http://scifinder.cas.org), American Chemical Society

Journals(http://pubs.acs.org), Royal society of Chemistry (http://www.HYPERLINK "http://www.rsc.org)/"rscHYPERLINK "http://www.rsc.org)/".org),Science direct Journals (www.sciencedirect.com),WileyJournals (http://onlinelibrary.wiley.com). US and EU patent search etc. Various patent search engines are free patent online, patent lens (www.patentlens.net), espace.net (worldwide.espacenet.com), patinfo. nic, micro pat, india.bigpatents.org etc.

The ACS Journals searches used in this report are -

- Journal of Organic Chemistry
- Organic Letters
- Journal of American Chemical Society
- Journal of Medicinal Chemistry

The RSC Journals searches used in this report are -

- Chemical Communications
- Organic and Bimolecular Chemistry

  The Wiley journals searches used in this report are -

- Angewandte Chemie International Edition

The Science Direct Journals searches used in this report are -

- Tetrahedron
- Tetrahedron: Asymmetry

CHAPTER-4

Instruments and techniques

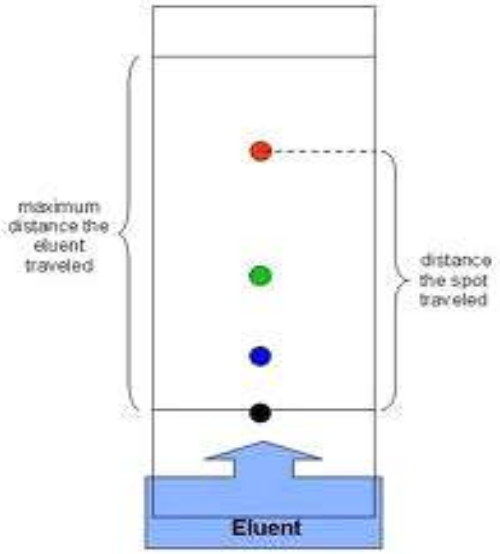
In this work these parameters played an important role they are:

- Thin layer chromatography
- Column Chromatography
- High performance chromatography
- Gas chromatography
- Vacuum Distillation
- Rotary Evaporator
- IR
- NMR

Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.



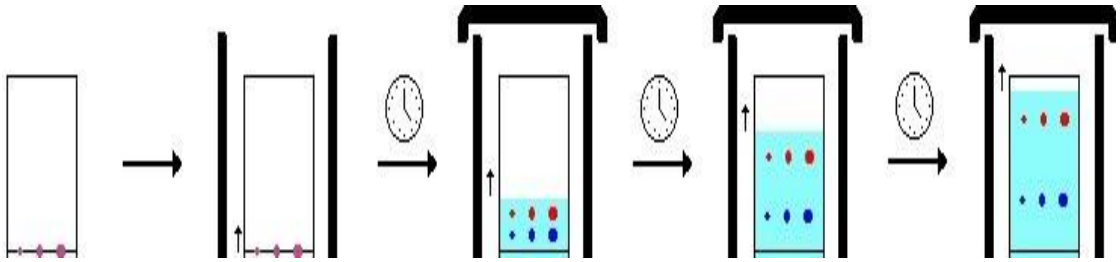
To run a TLC, the following procedure is carried out:

A small spot of solution containing the sample is applied to a plate, about 1.5 cm from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber.

The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent

moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches no higher than the top of the filter paper in the chamber, the plate should be removed (continuation of the elution will give a misleading result) and dried.

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_F value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column.



Thin layer chromatography can be used to:

- Monitor the progress of a reaction
- Identify compounds present in a given substance
- Determine the purity of a substance.

Visualization

- Ultraviolet: Look at the plate under the light first when working with compounds with conjugated double bond systems.
- Iodine: Shake with powdered I2. You can then heat the plate to remove the iodine stain, and use a liquid TLC stain as usual.
- Anisaldehyde (Use for carbonyl groups)
- Ceric Ammonium Molybdate (Use for hydroxy groups)
- Ninhydrin (Use for amines)
- Phosphomolybdic Acid (General useful)
- Potassium Permanganate (General useful)

Circle the spots on the TLC plate to have a permanent record how far the compound travelled on the plate. Also draw a sketch of the developed plate in your lab notebook.

Column Chromatography

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. The latter prevents cross-contamination and stationary phase degradation due to recycling.

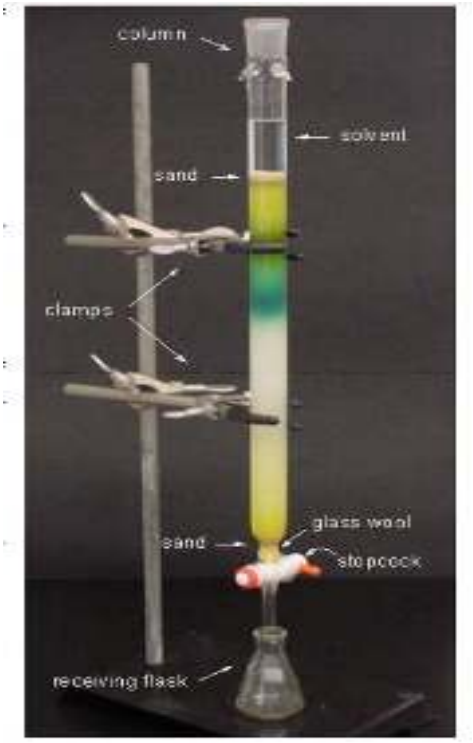
The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom. Two methods are generally used to prepare a column; the dry method, and the wet method.

In this work wet method is used where, a slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipetted on top of the stationary phase.

Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put on

top of the column.

The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, e.g.by analytical chromatography, UV absorption, or fluorescence. Color compounds (or fluorescent compounds with the aid of an UV lamp) can be seen through the glass wall as moving band.



The Adsorbent

Silica gel (SiO_2) and alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. These adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label: "Silica gel 60 mesh" or "Silica gel 230-400 mesh" is examples. This number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. If there are more holes per unit area, those holes are smaller, thus allowing only smaller silica particles go through the sieve. The relationship is: the larger the mesh size, the smaller the adsorbent particles.

Adsorbent particle size affects how the solvent flows through the column. Smaller particles (higher mesh values) are used for flash chromatography; larger particles (lower mesh values) are used for gravity chromatography. For example, 70–230 mesh silica gel is used for gravity columns and 230–400 mesh for flash columns. Results were less than acceptable when large 60-200 mesh material was used, but remarkably improved when a 200-400 mesh material was in the column. Equally important: particle sizes less than 40 microns offered no significant improvement in resolution in this system.

The Solvent

The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique.

Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound.

Column Chromatography Procedure

Packing a (silica gel) column:

- Use a piece of wire to add a plug of cotton to the bottom of the column. There should be just enough cotton that the sand and silica will not fall out of the column.
- Clamp the column to a ring stand and add enough sand to fill the curved portion of the column.
- Place a pinch clamp on the tubing, then fill the column 1/4 to 1/3 full with the initial eluent.
- Prepare slurry of silica in the initial eluent by pouring dry silica into a beaker of eluent. (Add a volume of silica gel, such as 20 mL, to approximately double the volume of eluent, 40 mL) **CAUTION**: Keep the dry silica in your hood and be careful not to inhale the lightweight substance.
- Quickly but carefully pour the slurry into the column. Stir and pour immediately to maximize the amount of silica that goes into the column instead of remaining behind in the beaker.

- Use a Pasteur pipette to rinse any silica that is sticking to the sides of the column. Allow the silica to settle while eluent continues to drip into the flask.

Loading a sample onto the column

Once the silica has settled, using a long Pasteur pipette, carefully add your sample to the column. Use ~ 1 mL of eluent to rinse your container and pipette. Repeat this two or three time to completely transfer your sample onto the silica gel.

Eluting the sample

Once you have rinsed your sample onto the silica, carefully add eluent to the top of the column. Add more eluent as necessary. The eluent collected prior to the elution of sample can be recycled. The composition of the eluent can be changed as the column progresses. If the eluent composition is to be changed, always start with least polar solvent/mixture and change to the more polar solvent/mixture.

[Fair, J. D.; Kormos, C.M.J.Chromatography.A **2008**, *1211*(1-2), 49-54. (doi:10.1016/j.chroma.2008.09.085)

High performance liquid chromatography:

The components of a basic high-performance liquid chromatography [HPLC] system

shown in the simple diagram in Figure E.

A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically milliliters per minute. An injector [sample manager or autosampler] is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column (most compounds have no colour, so we cannot see them with our eyes). The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the eluate containing that purified compound for further study. This is called preparative chromatography [discussed in the section on HPLC Scale].

Note that high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the conduit for the mobile phase, sample, and separated compound bands.

Operation:

A simple way to understand how we achieve the separation of the compounds contained in a sample is to view the diagram in Figure G.

Mobile phase enters the column from the left, passes through the particle bed, and exits at the right. Flow direction is represented by green arrows. First, consider the top image; it represents the column at time zero [the moment of injection], when the sample enters the column and begins to form a band. The sample shown here, a mixture of yellow, red, and blue dyes, appears at the inlet of the column as a single black band. [In reality, this sample could be anything that can be dissolved in a solvent; typically the compounds would be colorless and the column wall opaque, so we would need a detector to see the separated compounds as they elute.]

After a few minutes [lower image], during which mobile phase flows continuously and steadily past the packing material particles, we can see that the individual dyes have moved in separate bands at different speeds. This is because there is a competition between the mobile phase and the stationary phase for attracting each of the dyes or analytes. Notice that the yellow dye band moves the fastest and is about to exit the column. The yellow dye likes [is attracted to] the mobile phase more than the other dyes. Therefore, it moves at a *faster* speed, closer to that of the mobile phase. The blue dye band likes the packing material more than the mobile phase. Its stronger attraction to the particles causes it to move significantly *slower*. In other words, it is the most retained compound in this sample mixture. The red dye band has an intermediate attraction for the mobile phase and therefore moves at an *intermediate* speed through the column. Since each dye band moves at different speed, we are able to separate it chromatographically.

4.4. Gas chromatography:

It is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.[1][2]

In gas chromatography, the *mobile phase* (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. Helium remains the most commonly used carrier gas in about 90% of instruments although hydrogen is preferred for improved separations.[3] The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (an homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a *gas chromatograph* (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness. chromatography, such as HPLC, TLC), but has several notable differences. First, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a the gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness. Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences.

4.5 Rotary evaporator

Distillation is a method of separating mixtures based on differences in their volatilities in a boiling liquid mixture. Distillation is a unit operation, or a physical separation process, and not a chemical reaction.

Commercially, distillation has a number of applications. It is used to separate crude oil into more fractions for specific uses such as transport, power generation and heating. Water is distilled to remove impurities, such as salt from seawater. Air is distilled to separate its components—notably oxygen, nitrogen, and argon—for industrial use. Distillation of fermented solutions has been used since ancient times to produce distilled beverages with higher alcohol content. The premises where distillation is carried out, especially distillation of alcohol are known as a distillery.

Some compounds have very high boiling points. To boil such compounds, it is often better to lower the pressure at which such compounds are boiled instead of increasing the temperature. Once the pressure is lowered to the vapor pressure of the compound (at the given temperature), boiling and the rest of the distillation process can commence. This technique is referred to as vacuum distillation and it is commonly found in the laboratory in the form of the rotary evaporator.

Vacuum distillation is a method of distillation whereby the pressure above the liquid mixture to be distilled is reduced to less than its vapor pressure (usually less than atmospheric pressure) causing evaporation of the most volatile liquid(s) (those with the lowest boiling points). This distillation method works on the principle that boiling occurs when the vapor pressure of a liquid exceeds the ambient pressure. Vacuum distillation is used with or without heating the solution.

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) is an effect whereby magnetic nuclei in a magnetic field absorb and re-emit electromagnetic (EM) energy. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and other factors. This allows the observation of specific quantum mechanical magnetic properties of an atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals and non-crystalline materials through NMR spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field. It is this feature that is exploited in imaging techniques; if a sample is placed in a non-uniform magnetic field then the resonance frequencies of the sample's nuclei depend on where in the field they are located. Since the resolution of the imaging technique depends on the magnitude of magnetic field gradient, many efforts are made to

develop increased field strength, often using

superconductors. The effectiveness of NMR can also be improved using hyper polarization, and/or using two-dimensional, three-dimensional and higher-dimensional multi-frequency techniques.

The principle of NMR usually involves two sequential steps:

- The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field H_0.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field (H_0) and the nuclei of observation.

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to either the chemical shift, Zeeman Effect or the Knight Shift effect or a combination of both, on the resonant frequencies of the nuclei present in the sample.

Liquid chromatography–Mass spectrometry

Liquid chromatography–mass spectrometry is a chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and selectivity. Generally its application is oriented towards the general detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture). Preparative LC-MS system can be used for fast and mass directed purification of natural-products extracts and new molecular entities important to food, pharmaceutical, agrochemical and other industries.

Mass spectrometry

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

Typical MS procedure:

- A sample is loaded onto the MS instrument and undergoes vaporization.
- The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions).
- The ions are separated according to their mass-to-charge ratio in an analyzer by

electromagnetic fields.

- The ions are detected, usually by a quantitative method.
- The ion signal is processed into mass spectra.

MS instruments consist of three modules:

- An ion source, which can convert gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase).
- A mass analyzer, which sorts the ions by their masses by applying electromagnetic fields.
- A detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

Different mass analyzers that can be used in LC/MS:

- Single quadrapole
- Triple quadrapole
- Ion trap
- Time of flight (TOF)
- Quadrapole-time of flight (Q-TOF).

CHAPTER-5

Chemicals and their material safety data sheet

Chemicals required:

- O-CHLORO BENZALDEHYDE
- CONCENTRATED NITRIC ACID
- CONCENTRATED SULFURIC ACID
- DM WATER
- ETHYL ACETATE
- N HEXANE
- METHANOL
- CHLOROFORM
- DICHLOROMETHANE
- SODIUM HYDROXIDE
- SILICA GEL
- ACETONE
- TOLUENE
- SODIUM BOROHYDRATE
- DICHLORO METHANE (DCM)
- TRIETHYL AMINE
- ISOPROPANOL HYDROCHLORIDE (IPA HCL)
- THIONYL CHLORIDE
- HYDROCHLORIC ACID
- STANNUS CHLORIDE

Material safety data sheet of compounds (MSDS):

1. O-Chloro benzaldehyde:

- Physical state: liquid
- Melting point: **:** 64-69^{0}C
- Potential acute health effects: Causes severe skin burns and eye damage.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting and Diarrhea. May cause central nervous system effects.
- Inhalation: It can irritate the nose and throat causing coughing and shortness of breath.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation - Do not breath vapors or spray mist, remove from exposure lie down move to fresh air, obtain medical attention.

    Ingestion - clear mouth with water, get medical attention

2. Concentrated Nitric acid:

- Physical state: Liquid
- Color: Light Yellow
- Boiling point**:** 83^{0}C
- Potential acute health effects: Causes burns of eyes, skin and mucous membranes.
- Ingestion: May cause burns of tongue and stomach, headache, nausea, vomiting and it may be fetal.
- Inhalation: It can burn sensation, dry nose and throat, cough, chest pain and difficulty in breathing.

- Precautions:
    - Skin contact - wash off immediately with soap and plenty of water.

      while removing all contaminated clothes and shoes obtain medical attention.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation - Do not breath fumes /gas/vapors, remove from exposure lie down move to fresh air, Obtain medical attention.
    - Ingestion - Clear mouth with water, get medical attention.
    - Use in outdoors or well-ventilated area
    - Keep only in original container.

3. **Concentrated sulfuric acid***:*
    - Physical state: Liquid
    - Color: Colorless
    - Boiling point**:** 37^0C
    - Potential acute health effects: Causes skin (pain, redness and burns) and eye damage, it may lead to blindness.
    - Ingestion: May cause headache, nausea, vomiting and it can burn holes in stomach.
    - Inhalation: It can irritate the nose and throat causing coughing and shortness of breath.
    - Precautions:
        - Skin contact - wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes. Seek medical

          Attention/advise immediately.
        - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.

- Inhalation - Do not breath vapors/gas/fumes, remove from exposure lie down move to fresh air, Obtain medical attention.
- Ingestion - Clear mouth with water, get medical attention.
- Use in outdoors or well-ventilated area
- Keep only in original container.

4. DM Water (H2O):

- Physical state: Liquid
- Boiling point**:** 100 ^{0}C
- Potential acute health effects: Eye(irritant), skin (irritant), prolonged and for repeated contact may cause irritation and for dermatitis.
- Ingestion While this material is not reported, or expected to be specifically toxic, ingestion may cause other toxins to be introduced (carried) into the body. Do not drink!
- Inhalation: Inhalation of liquid or vapor can cause shortness of breath, and in extreme deluge circumstances, drowning may occur
- Advice to physician: No specific antidote. Treat symptomatically and supportively.

5. Ethyl acetate:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:** 77.1^{0}C
- Potential acute health effects: Cause Eye irritation, Skin irritation, prolonged and for repeated contact may cause irritation for dermatitis.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting and

  diarrhea. May cause central nervous system effects.
- Inhalation: It can irritate the nose and throat causing coughing and shortness of breath.
- Precaution:

- Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
- Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
- Inhalation - Do not breath vapors or spray mist, remove from exposure lie down move to fresh air, obtain medical attention.
- Ingestion - clear mouth with water, get medical attention.

6. N hexene:

- Color: Colorless
- Boiling point**:** 69^0C
- Potential acute health effects: Cause Eye irritation, Skin irritation, prolonged and for repeated contact may cause irritation for dermatitis.
- Ingestion: May cause peripheral nerves, resulting in weakness or numbness of lower limbs, may cause central nerve system depression.
- Inhalation: It can cause respiratory irritation, dizziness, nausea, or unconsciousness occurs.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation - Do not breath vapors or spray mist, remove from exposure lie down move to fresh air, obtain medical attention.
  - Ingestion - clear mouth with water, do not induce vomiting. get medical attention.

7. Methanol:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:** 64.7^0C
- Potential acute health effects: in case of skin contact (irritant), of eye contact(irritant), prolonged and for repeated contact may cause irritation and for dermatitis.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting and

  diarrhea. May cause central nervous system effects.
- Inhalation: It can irritate the lungs causing coughing and /or shortness of breath.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation – Move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
  - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice

8. Chloroform:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:** 61.2^0C
- Potential acute health effects: Cause Eye irritation, Skin irritation, prolonged and for repeated contact may cause irritation for dermatitis.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting

and diarrhea. May cause central nervous system effects.

- Inhalation: It can irritate the nose and throat causing coughing and shortness of breath.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation - Do not breath vapors or spray mist, remove from exposure lie down move to fresh air, obtain medical attention.

Ingestion - clear mouth with water, get medical attention;

9. Dichloromethane:

- Physical State: liquid
- Melting point**:** ^{0}C
- Potential acute health effects:
- Ingestion:
- Inhalation:
- Precaution: Skin contact;

10. Sodium hydroxide:

- Physical state: Solid
- Color: White
- Melting point**:** 400^{0}C
- Potential acute health effects: in case of skin contact (irritant), of eye contact(irritant) leads to corneal damage or blindness and also it is skin itching.
- Ingestion: May cause fetal, pain in upper abdomen, blood in feces, vomiting and nausea.
- Inhalation: It can irritate the lungs causing coughing and /or shortness

of breath.

- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
    - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

11. Silica gel:

- Physical state: Solid
- Color: White
- Melting point**:** 1710 ^{0}C
- Potential acute health effects: Eye (irritant), skin (irritant), prolonged and for repeated contact may cause irritation and for dermatitis.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting and Diarrhea. May cause central nervous system effects.
- Inhalation: May cause respiratory track irritation, may cause narcotic effects.
- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation - Do not breath vapors or spray mist, remove from exposure lie down move to fresh air, obtain medical attention.

- Ingestion - clear mouth with water, get medical attention.

12. Acetone:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:** 56^0C
- Potential acute health effects: Cause Eye irritation, Skin irritation.
- Ingestion: May cause gastrointestinal track irritation with abdominal pain, nausea, vomiting, diarrhea.
- Inhalation: It can irritate the nose and throat causing coughing and shortness of breath.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
  - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

13. Toluene:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:** 111^0C
- Potential acute health effects: Cause Eye irritation, Skin irritation.
- Ingestion: May cause CNS effects (headache, dizziness, ataxia, drowsiness, hallucinations, tremor, seizures and coma), vomiting and nausea.
- Inhalation: It can irritate the nose and throat, dizziness, feeling of

being drunk, confusion and anxiety.

- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
    - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

14. Sodium borohydrate:

- Physical state: Solid
- Color: White
- Melting point**:** 400^0C
- Potential acute health effects: in case of skin contact (irritant), of eye contact(irritant) leads to corneal damage or blindness and also it is skin itching.
- Ingestion: May cause fetal, pain in upper abdomen, blood in feces, vomiting and nausea.
- Inhalation: It can irritate the lungs causing coughing and /or shortness of breath.
- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.

- Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

15. Tri ethyl amine:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:**89.28 ^{0}C
- Potential acute health effects: in case of skin contact (irritant), of eye contact(irritant), prolonged and for repeated contact may cause irritation and for dermatitis.
- Ingestion: May cause gastro intestinal irritation with nausea vomiting and
  Diarrhea.
- Inhalation: It can irritate the lungs causing coughing and /or shortness of breath.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
  - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.
  -

16. Thionyl chloride:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:**76^{0}C
- Potential acute health effects: It causes sever skin burns and eye

damage.

- Ingestion: May cause gastro intestinal irritation with nausea and vomiting.
- Inhalation: It can irritate the lungs causing coughing and /or shortness of breath.
- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
    - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
    - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
    - Ingestion - clear mouth with water. feel unwell, do not induce vomit, seek medical attention/advice.

17. Hydrochloric acid:

- Physical state: Liquid
- Color: Colorless
- Boiling point**:**50.5 ^{0}C
- Potential acute health effects: It cause sever skin burns and eye damage.
- Ingestion: May cause burns to lips, oral cavity, upper airway, esophagus and digestive track.
- Inhalation: It causes damage to mucous membranes in noses, throat, lungs and bronchial system.
- Precaution:
    - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.

- Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
- Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
- Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

18. Stannus chloride:

- Physical state: Solid
- Color: White
- Melting point**:**247 ^{0}C
- Potential acute health effects: It may cause skin irritation and sever eye damage.
- Ingestion: May cause burns to the gastric/intestinal mucosa, possible esophageal perforation.
- Inhalation: It cause irritation of nasal mucous membranes, respiratory difficulties, coughing.
- Precaution:
  - Skin contact - Wash off immediately with soap and plenty of water. While removing all contaminated clothes and shoes obtain medical attention/advise.
  - Eye contact – Rinse exposed eye gently using water for 15 minutes. seek medical advice/attention.
  - Inhalation – move to fresh air, comfortable for breathing. If feel unwell seek medical advice.
  - Ingestion - clear mouth with water. feel unwell, seek medical attention/advice.

CHAPTER-6

ABOUT ANAGRELIDE

Introduction:

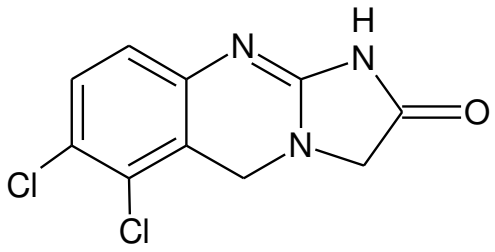
Anagrelide is a heterocyclic aromatic organic compound with the chemical formula $C_{10}H_7Cl_2N_3O$. It is a white color hygroscopic solid with a strong odor. It is a drug used for the treatment of essential thrombocytosis or over production of blood platelets. It is also has been used in the treatment of chronic myeloid leukemia.

Anagrelide is a platelet-reducing agent used to lower dangerously elevated platelet levels (that is to treat thrombocythemia) in patients with myeloproliferative neoplasms. It is an oral imidazo quinazoline that was first approved for use in the US in 1997. It appears to carry a better response rate than other thrombocythemia treatments (that is busulfan, hydroxyurea) and may be better tolerated.

Anagrelide decreases platelet counts by suppressing transcription factors necessary for the synthesis and maturation of platelet-producing cells. The drug itself appears to have a relatively short residence time in the body necessitating twice or four times daily dosing. However, given that the pharmacological effect of anagrelide therapy is reliant on a gradual suppression of platelet-producing cells, it may take 7 to 14 days for its administration to be reflected in reduced platelet counts - for this reason any changes to anagrelide doses should not exceed 0.5 mg/day in any one week.

Evidence from animal studies suggests anagrelide may impair female fertility. Female patients of reproductive age should be advised of the potential for adverse effects on fertility prior to initiating therapy.

Structure:



Chemical Formula: $C_{10}H_7CL_2N_3O$

Weight:

Average: 256.008

Monoisotopic: 254.9966

Melting Point: 280^0C

Vapor Pressure: 6.8X10-10 mm Hg at 25 °

Applications:

- An antidepressant;
- Medicine to improve blood flow;
- A blood thinner (such as warfarin or Coumadin) or other medicine to treat or prevent blood clots;
- NSAIDs (nonsteroidal anti-inflammatory drugs)--aspirin, ibuprofen (Advil, Motrin), naproxen (Aleve), celecoxib, diclofenac, indomethacin, meloxicam, and others.
- Anagrelide may impair female fertility. Female patients of reproductive age should be advised of the potential for adverse effects on fertility prior to initiating therapy.

Side Effects:

Common side effects are headache, diarrhea, unusual weakness/fatigue, hair loss, nausea.

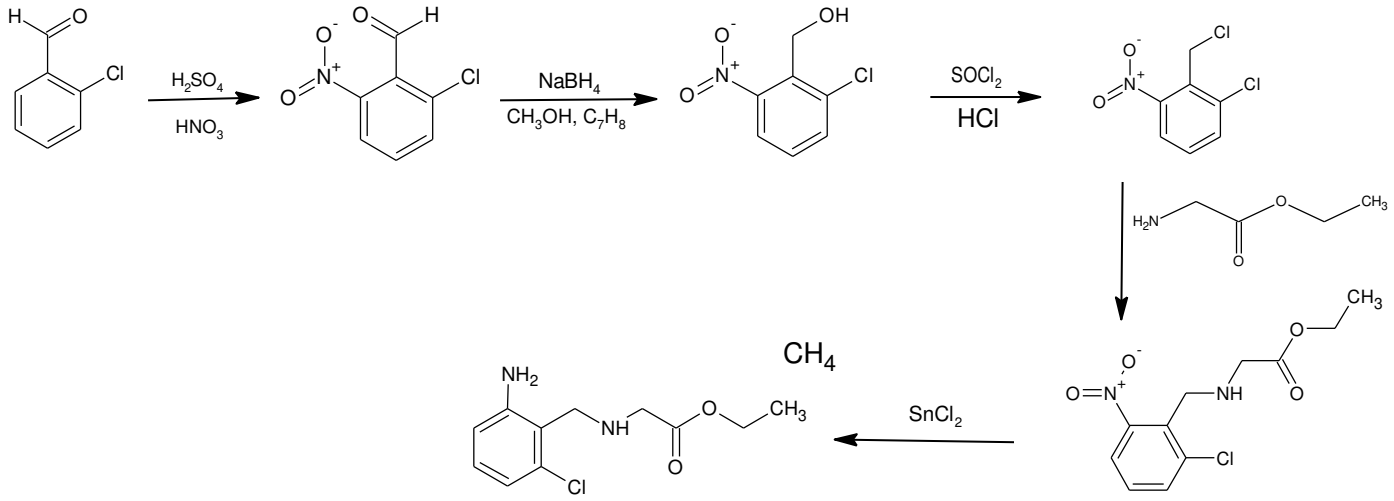
The same MRC trial mentioned above also analyzed the effects of anagrelide on bone marrow fibrosis, a common feature in patients with myelofibrosis. The use of anagrelide was associated with a rapid increase in the degree of reticulin deposition (the mechanism by which fibrosis occurs), when compared to those in whom hydroxyurea was used. Patients with myeloproliferative conditions are known to have a very slow and somewhat variable course of marrow fibrosis increase. This trend may be accelerated by anagrelide. This increase in fibrosis appeared to be linked to a drop in hemoglobin as it progressed. Stopping anagrelide (and switching patients to hydroxyurea) appeared to reverse the degree of marrow fibrosis. Thus, patients on anagrelide may need to be monitored on a periodic basis for marrow reticulin scores, especially if anemia develops, or becomes more pronounced if present initially.

Less common side effects include: congestive heart failure, myocardial infarction, cardiomyopathy, cardiomegaly, complete heart block, atrial fibrillation, cerebrovascular accident, pericarditis, pulmonary infiltrates, pulmonary fibrosis, pulmonary hypertension, pancreatitis, gastric/duodenal ulceration, renal impairment/failure and seizure.

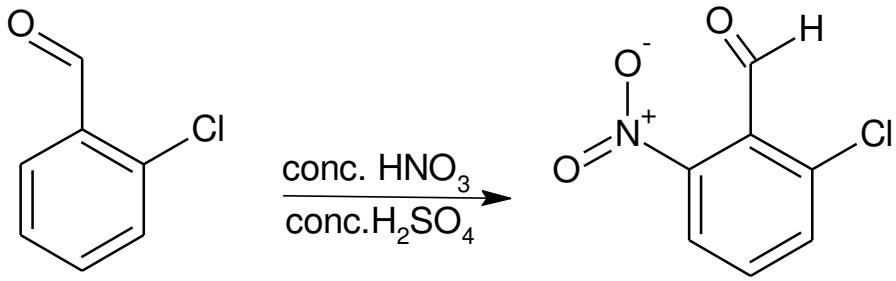
Due to these issues, anagrelide should not generally be considered for first line therapy for Essentia.

Chapter 7

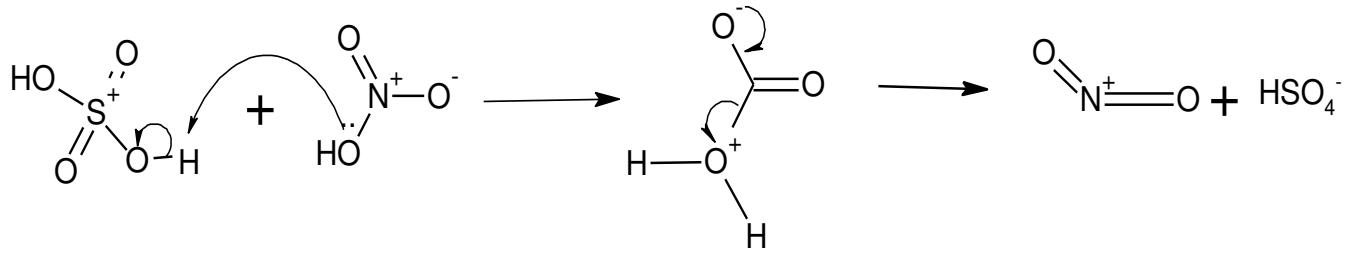
Scheme:

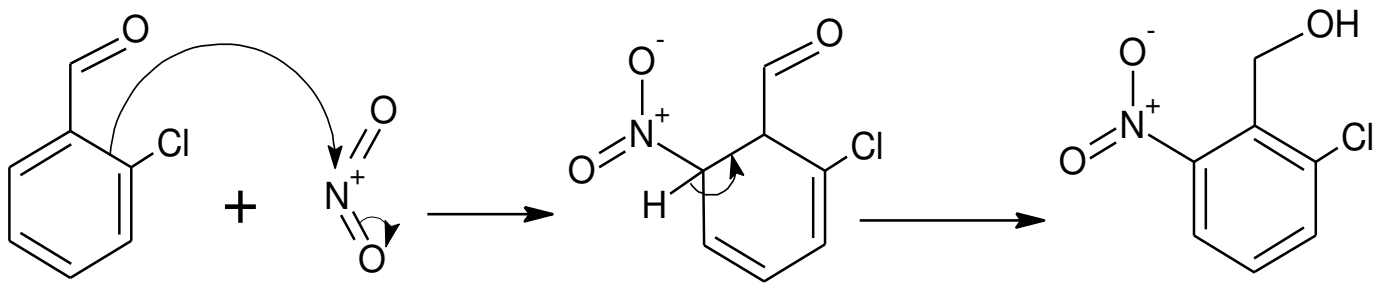


Synthesis of 2-chloro 6-nitro benzaldehyde



Mechanism:





Raw Materials:

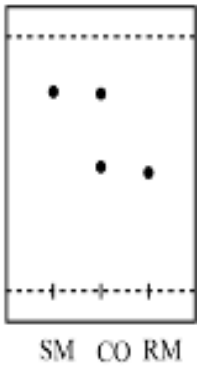
Sl.No.	Chemicals used	Qty	Mol. Wt.	Moles	Molar ratio
1	2 chloro Benzaldehyde	20.0 g	140	0.0142	1 eq
2	Conc. Nitric Acid	12.4 mL	63	0.019	1.461eq
3	Conc. Sulfuric Acid	147.2 mL	98	0.150	11.538
4	DM Water	500 mL	18.02	.....	
5	Ethyl Acetate	200 mL	88.1	.....	

Procedure:

Three necked 250 ml round bottom flask was fixed to Overhead stirrer.

Added conc. nitric acid (12.4ml) to the round bottom flask (RB) and charged 2-chloro Benzaldehyde (20.0g) at room temperature stirred for 10 minutes and the clear solution was obtained. Then charged with conc. sulfuric acid (147.2ml) drop wise at 0-5^{0}C temperature. It was stirred for 1 hour. Reaction mixture appeared to be white precipitate in the round bottom flask which indicates the completion of reaction. Then finally removed it from the ice bath. Completion of reaction was confirmed by TLC.

TLC Method *TLC Sample Preparation*: 1mL of the reaction mixture was taken in a vial, starting material dissolved in ethyl acetate. Above samples spotted on TLC plate. TLC was run in ethyl acetate. The starting material was consumed completely in reaction mixture.



SM- starting material

CO- co spot (starting material + reaction mass)

RM-reaction mixture

Mobile phase**:** Ethyl acetate and Methanol in the ratio 9:1

Work up:

Quenched the reaction mass in ice cold demineralized water (500 ml).

Stirred it for 10 mins.

Extracted the reaction mass with ethyl acetate (200 ml).Then again extracted with (100 ml) ethyl acetate

Washed the ethyl acetate layer with demineralized water (200 ml).

Dried ethyl acetate layer over anhydrous sodium sulphate and filtered it.

Concentrated the ethyl acetate layer over vacuum rotavapor to get solid.

The reaction was observed through TLC. (If two spots occurred then it is Refluxed with cyclohexane in round bottom flask (RB) and concentrated over rotavapor using cyclohexane layer).

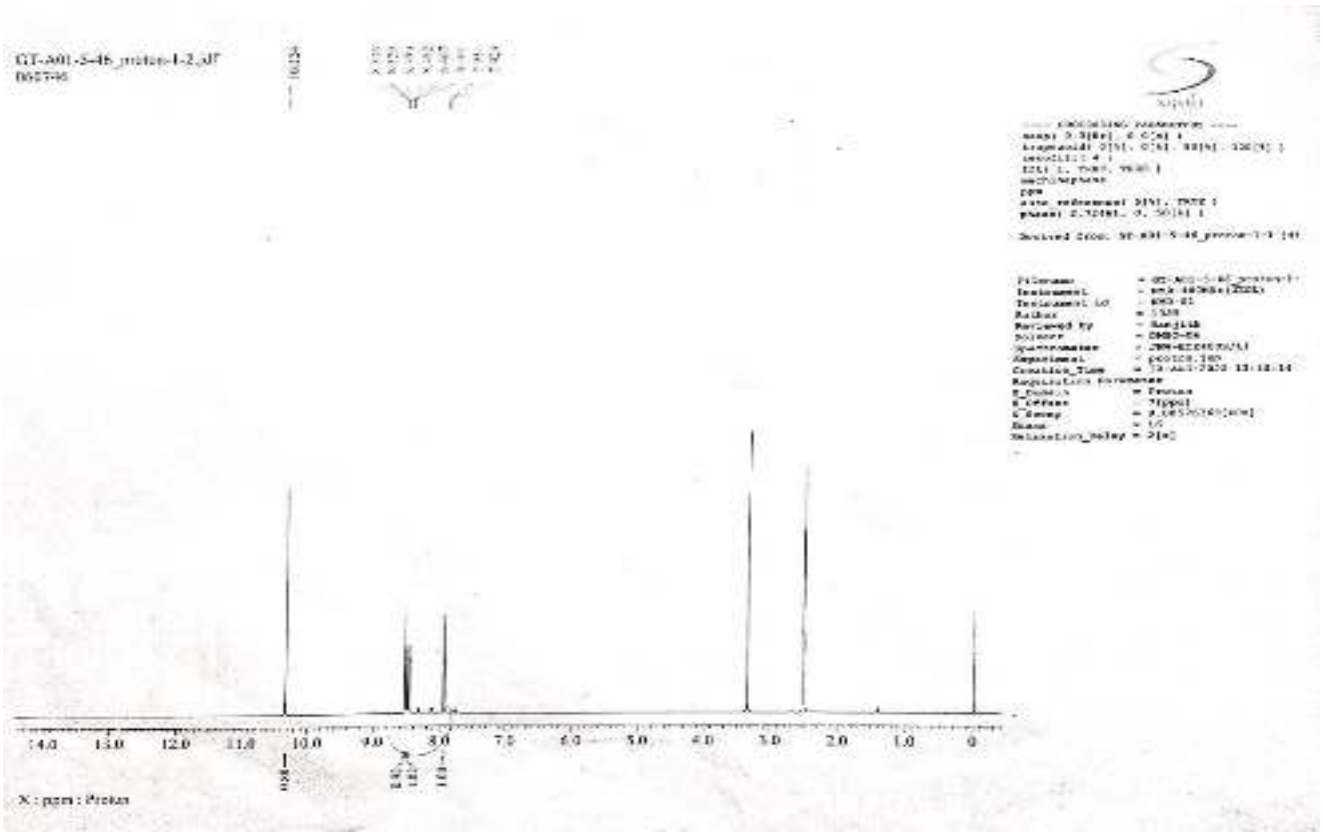
Then dried the product and weighed it. Completion of reaction was confirmed by TLC.

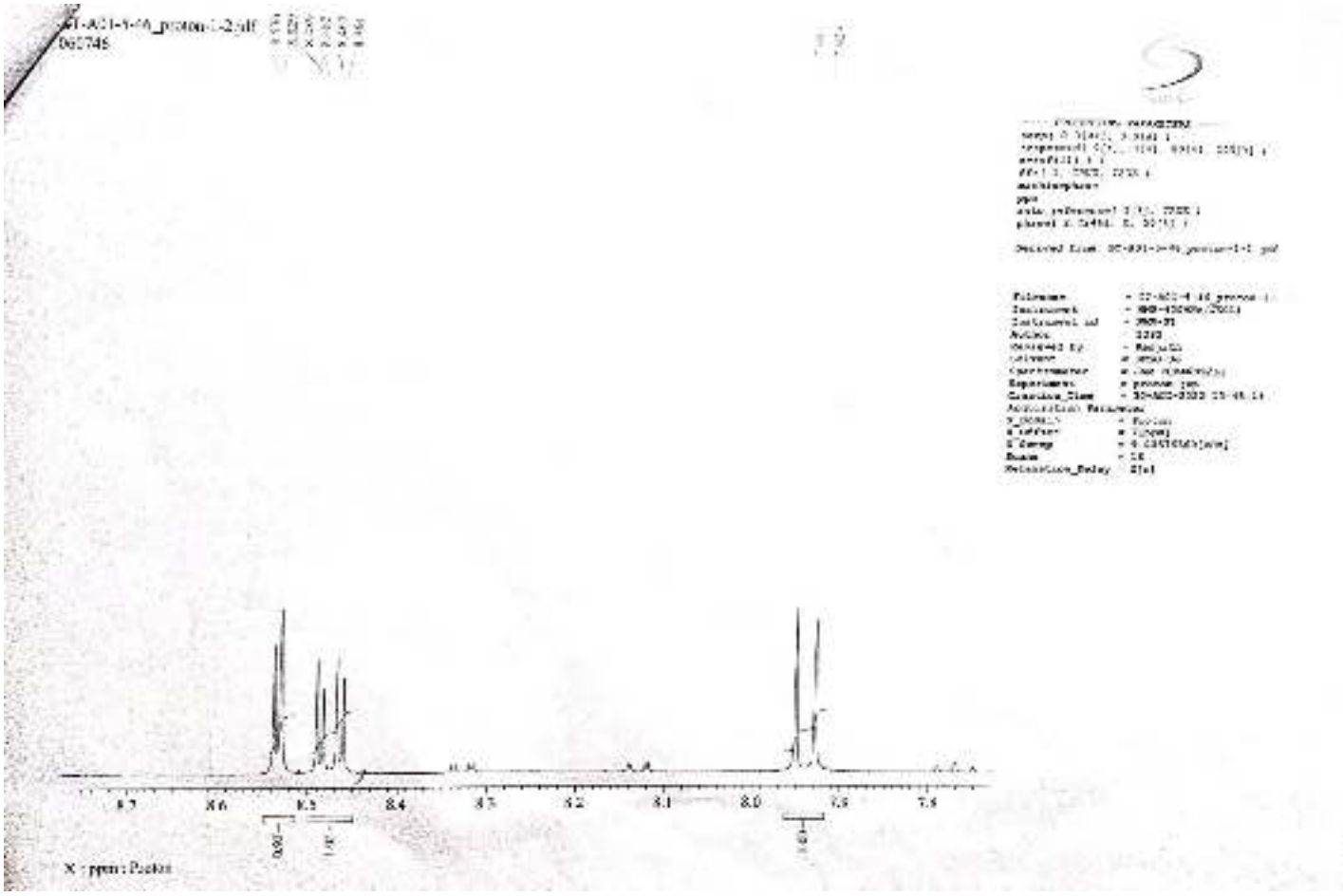
Result: The purity was good because TLC showed single spot. Theoretical M.P was 83°C to 84°C. Observed melting point was 82°C to 86°C.

- Theoretical yield: 15.32 g
- Experimental yield: 13.28 g
- Percentage of yield: 86.68

ANALYTICAL DATA:

NMR SPECTROSCOPY



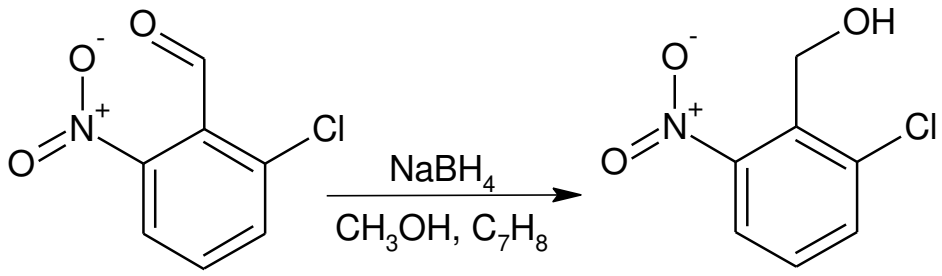


J-Coupling Analysis Report

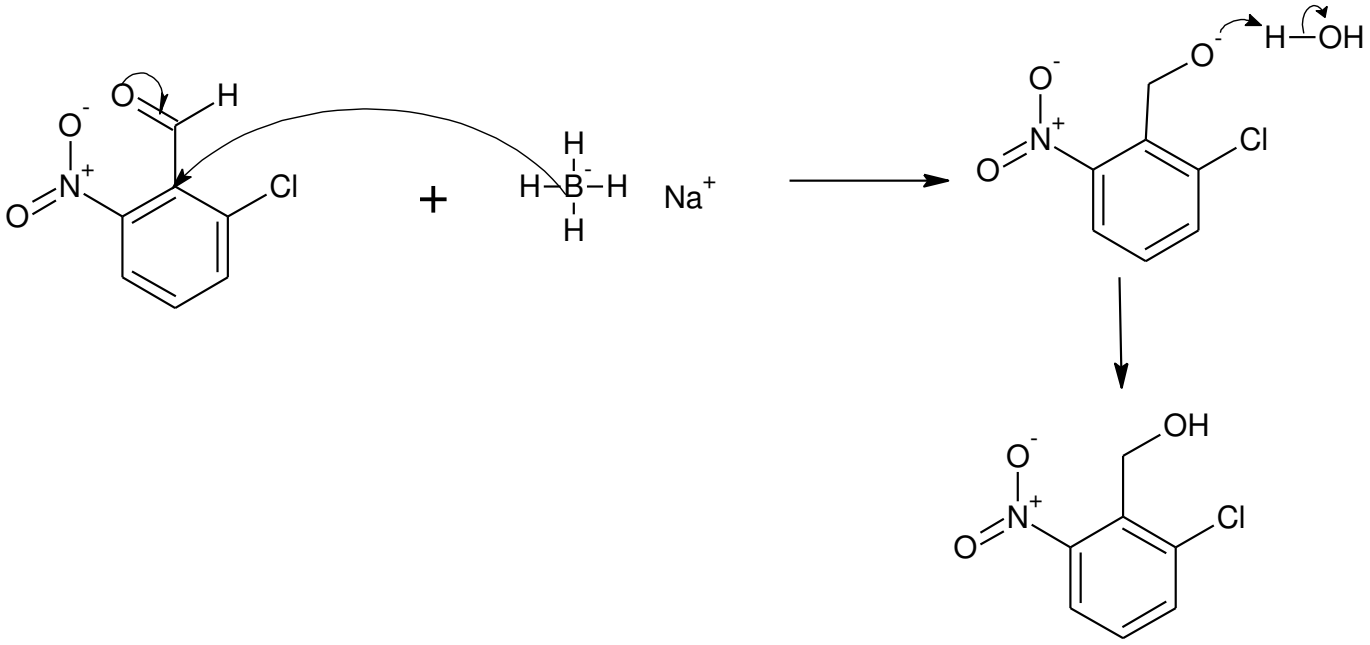
Path = \\backup\D\NMR-400 NEW\Raw Data-400\Aug-2022\GT-A01-5-66_proton-1-2.jdf

Position	Integral	Pattern	J
10.32[ppm]	1	s	
8.53[ppm]	1	d	J1 = 2.7[Hz]
8.48[ppm]	1	dd	J1 = 8.7[Hz], J2 = 2.8[Hz]
7.94[ppm]	1	d	J1 = 8.8[Hz]

Synthesis of 2 -chloro 6-nitrobenzyl alcohol



Mechanism:



Raw materials:

Sl.No	Chemicals used	Qty	Mol. wt.	Moles	Mole ratio
1	Ortho chloro 6-nitro benzaldehyde	13.28g	185.01g/mol	0.07177	1 eq
2	Sodium borohydride	3.30g	37.83g/mol	0.05733	1.1eq
3	Toluene	67.65 ml	92.14g/mol	0.1151	……
4	Methanol	3.30 ml	32.04g/mol	…….	……..

Procedure:

Three necked 250 ml round bottom flask (RB) was fixed to overhead stirrer.

Then charged Ortho chloro 6-nitro benzaldehyde (13.28g) and added toluene (67.65 ml), with maintained temperature of 0-5 ^{0}C with stirring.

Charged with Methanol stirred it for 10 minutes.

Then charged sodium borohydride (3.30g) drop wise up to 20 minutes.

After getting clear solution, Completion of reaction was confirmed by TLC.

TLC Method

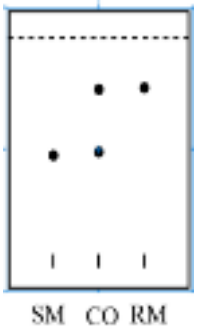
TLC Sample Preparation: 1mL of the reaction mixture was taken in a vial, starting material dissolved in ethyl acetate. Above samples spotted on TLC plate. TLC was run in ethyl acetate. The starting material was consumed completely in reaction mixture.

SM- starting material

CO- co spot (starting material + reaction mass)

RM-reaction mixture

Mobile phase**:** Ethyl acetate and Methanol in the ratio 9:1



Work up:

Added Demineralized water (30 ml) to that reaction mass.

Stirred for 15 minutes at room temperature. (RT)

Extracted toluene layer and aqueous layer using separating funnel. Then removed the aqueous layer.

Washed toluene layer 3 times using DM water (25 ml).

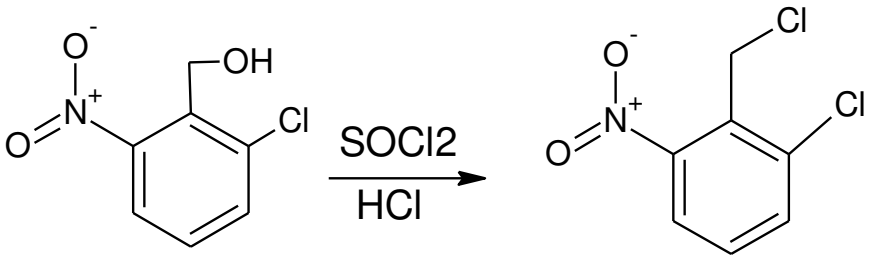
Dried toluene layer over Anhydrous Sodium Sulphate and filtered it.

Concentrated toluene layer using rotavapor pump. Completion of reaction was confirmed by TLC and weighed the product.

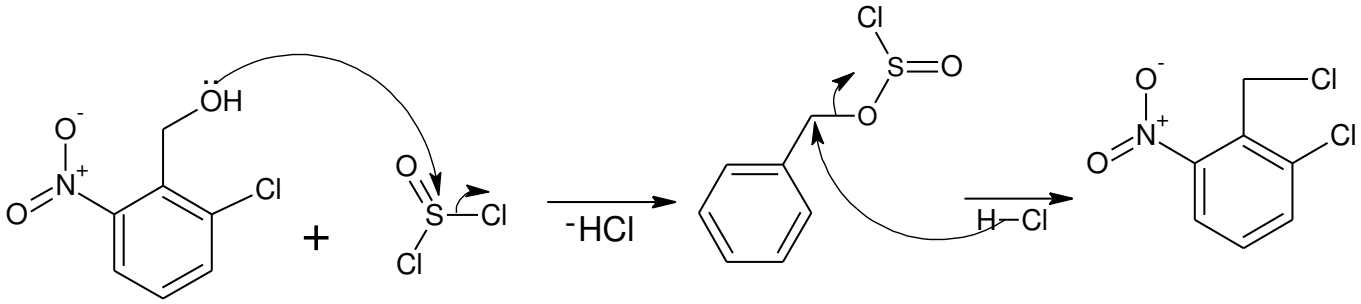
Result: The purity was good because TLC showed single spot. Theoretical M.P was 135 ^{0}C. Observed M.P was 132^{0}C.

- The theoretical yield: 13.06 g
- The experimental yield: 12.9 g
- Percentage yield: 98.77

Synthesis of 2-chloro 6-nitrobenzyl chloride



Mechanism:



Raw materials:

Sl.No	Chemicals used	Qty	Mol. wt.	Moles	Mole ratio
1	Ortho chloro 6-nitro benzyl alcohol	12.9g	187	0.0745	1 eq
2	Tri Ethyl Amine	4.455g	101.19	0.0440	1.7 eq
3	Thionyl Chloride	5.8ml	118.97	0.0245	2.3 eq
4	Toluene	56.30ml	92.14	…..	……

Procedure:

- Three necked 250ml round bottom flask (RB) was fixed to overhead stirrer
- Charged the Ortho chloro 6-nitro benzyl alcohol (12.9g) added with toluene (56.30ml) just to dissolve product and added tri ethyl amine (TEA), about 4.455g. Maintained temperature 0-5^{0}C.
- Stirred it until solution temperature becomes stable for above condition.
- Charged thionyl chloride ($SOCl_2$) slowly for about 15 mins.
- Stirred it for 2 hours in water bath at 50-60^{0}C. Completion of reaction was confirmed by TLC

TLC method

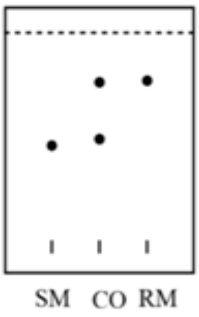
TLC Sample Preparation: 1mL of the reaction mixture was taken in a vial, starting material dissolved in ethyl acetate. Above samples spotted on TLC plate. TLC was run in ethyl acetate. The starting material was consumed completely in reaction mixture.

SM- starting material

CO- co spot (starting material + reaction mass)

RM-reaction mixture

Mobile phase**:** Ethyl acetate and Methanol in the ratio 9:1



Work up:

Product was washed with demineralized water (DM) for three time, each time with (60 ml).

Separated the toluene layer and aqueous layer, removed the aqueous layer.

Dried the toluene layer with anhydrous sodium sulphate solution.

Filtered sodium sulphate using cotton.

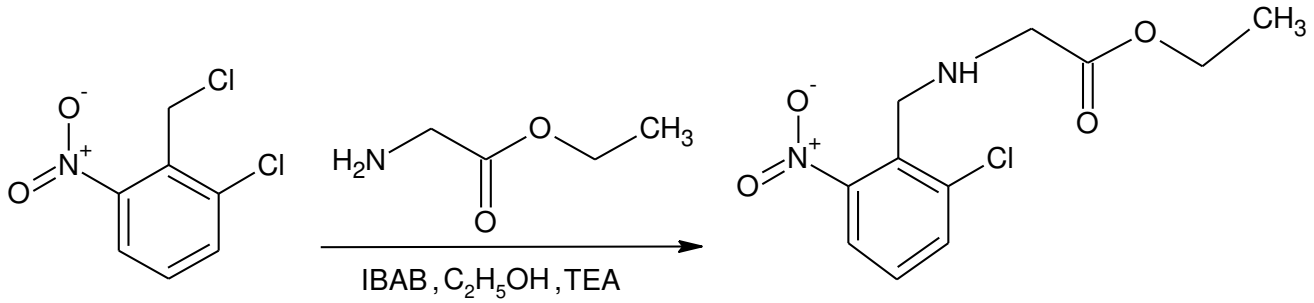
Concentrated the toluene layer over rotatory vapor vacuum pump.

Dried the product. Checked the TLC and weighed the product.

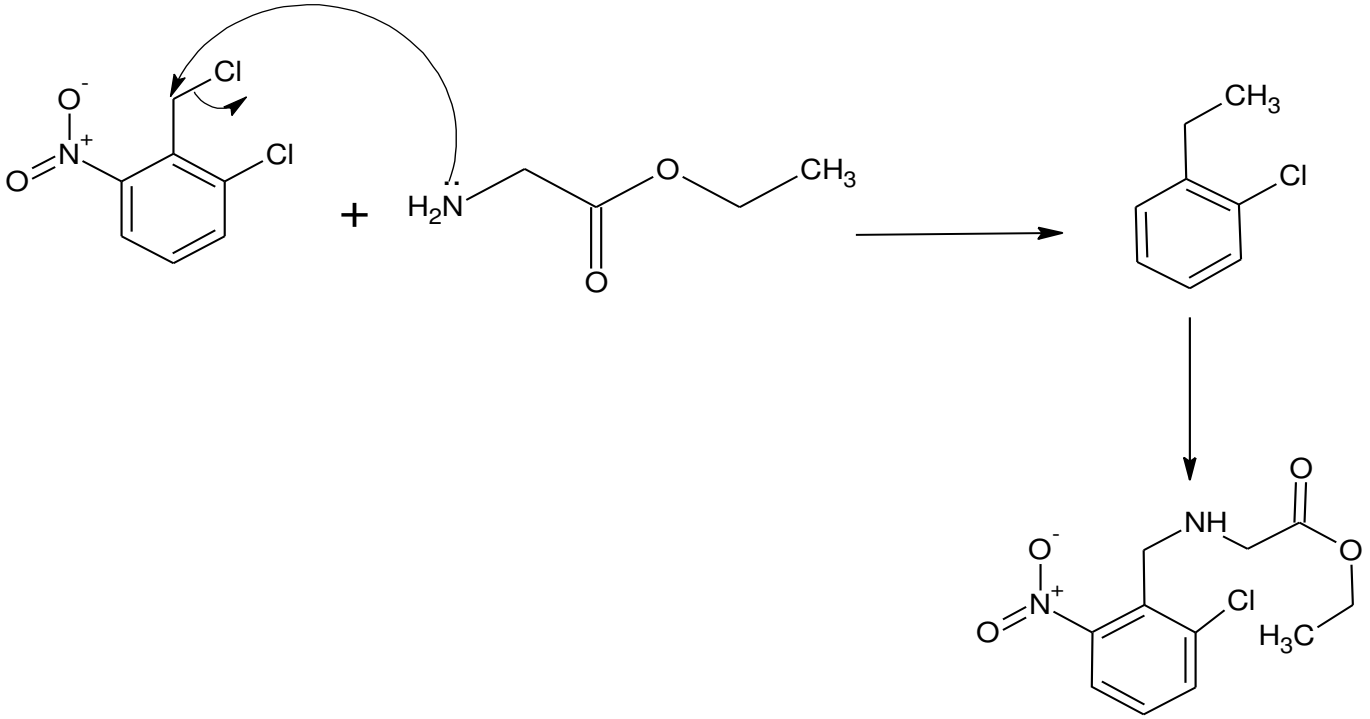
Result: The purity was good because TLC showed single spot. Theoretical M.P was 137.9-141^{0}C. Observed M.P was 130^{0}C.

- The theoretical yield: 11.76 g
- The experimental yield: 10.1g
- Percentage yield: 85.88

Synthesis of 2-chloro 6-nitrobenzylethyl glycine



Mechanism:



Raw materials:

Sl.No	Chemicals used	Qty	Mol. Wt.	Moles	Mole ratio
1	Ortho chloro 6-nitro benzyl chloride	10.1 g	205g/mol	0.049	0.1eq
2	Ethyl glycine	25g	139.58g/mol	0.1791	2eq
3	IBAB	0.2g	……	----	---
4	Ethanol	10ml	46.07g/mol	…..	.....
5	Tri Ethyl amine	22.94g	101.19g/mol	0.2267	2.5eq

Procedure:

Three necked 250 ml round bottom flask (RB) was fixed to Overhead Stirrer. Then charged Ortho chloro 6-nitro benzyl chloride (10.1g) ethyl glycine, IBAB catalytic quantity (0.2g) and tri ethyl amine (22.94 g). kept it for stirring in water bath at 50 ^{0}C about 9 hours. Completion of reaction was confirmed by TLC

TLC Method

TLC Sample Preparation: 1mL of the reaction mixture was taken in a vial, starting material dissolved in ethyl acetate. Above samples spotted on TLC plate. TLC was run in ethyl acetate. The starting material was consumed completely in reaction mixture.

SM- starting material.

CO- co spot (starting material + reaction mass)

RM-reaction mixture.

Mobile phase**:** Ethyl acetate and Methanol in the ratio 9:1

Work up:

Transferred reaction mixture (RM) to single neck round bottom (RB) flask and concentrated the ortho chloro 6-nitro benzyl chloride solution over rotatory vapour vacuum pump

Charged demineralized water (60 ml), stirred for half an hour.

Filtered the aqueous layer, charged solid product to beaker, and added dichloromethane (100 ml) and then stirred.

Dried the reaction mixture (RM) over anhydrous sodium sulphate and filtered through cotton.

Took round bottom flask (RB) and charged the above solution, then added Charcoal.

Stirred for 15 minutes at room temperature and refluxed it for 30 minutes, then filtered through high flow bed.

To the filtrate added n-hexane (10 ml). and concentrated the above reaction mixture (RM).

Charged 20 ml n-hexane, cooled it to temperature about 0-5 ^{0}C.

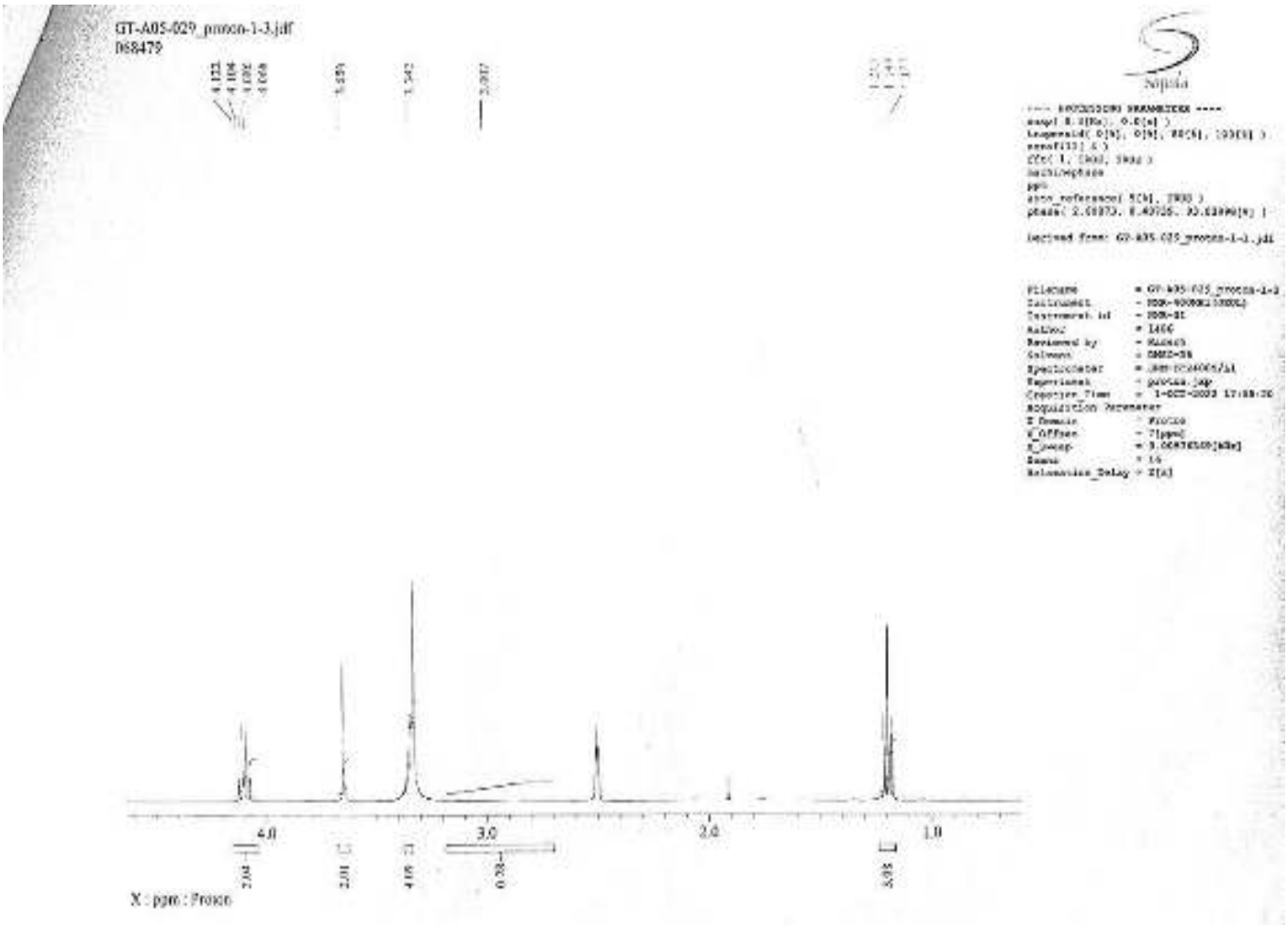
Filtered the precipitate. Dried the product and weighed it.

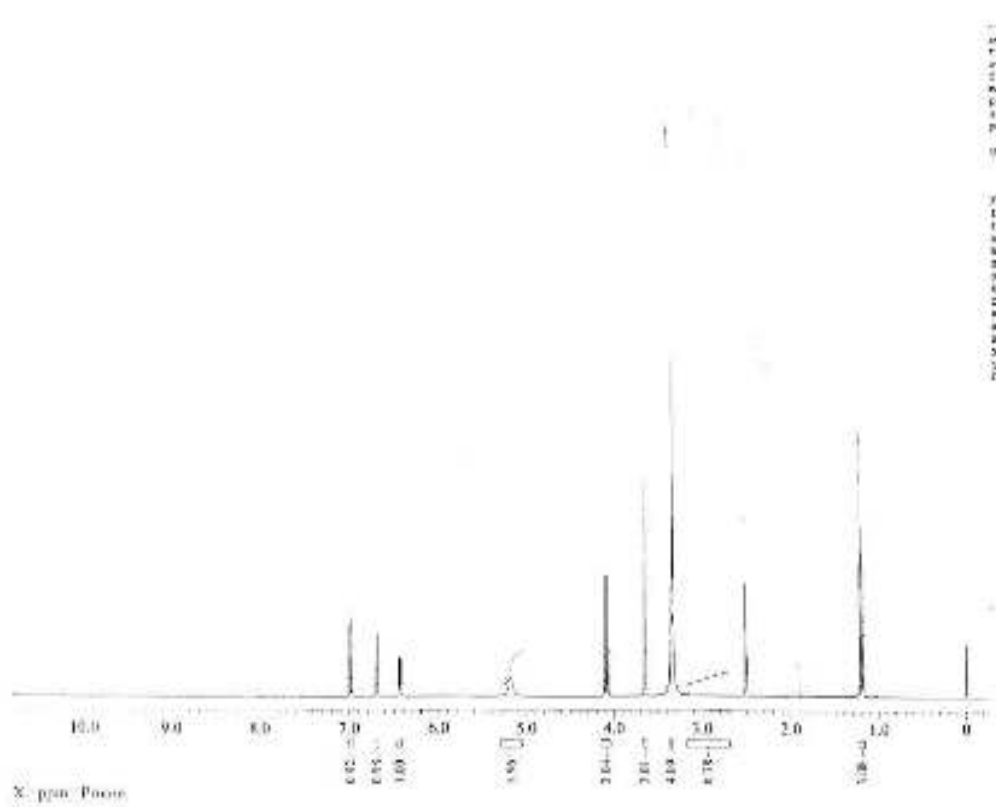
Result: The purity was good because TLC showed single spot. Theoretical M.P was 137.9 - 141^{0} C. Observed M.P was 130^{0}C.

- The theoretical yield: 7.62 g
- The experimental yield: 7.11 g
- Percentage yield: 93.30

Analytical data

NMR Spectroscopy:

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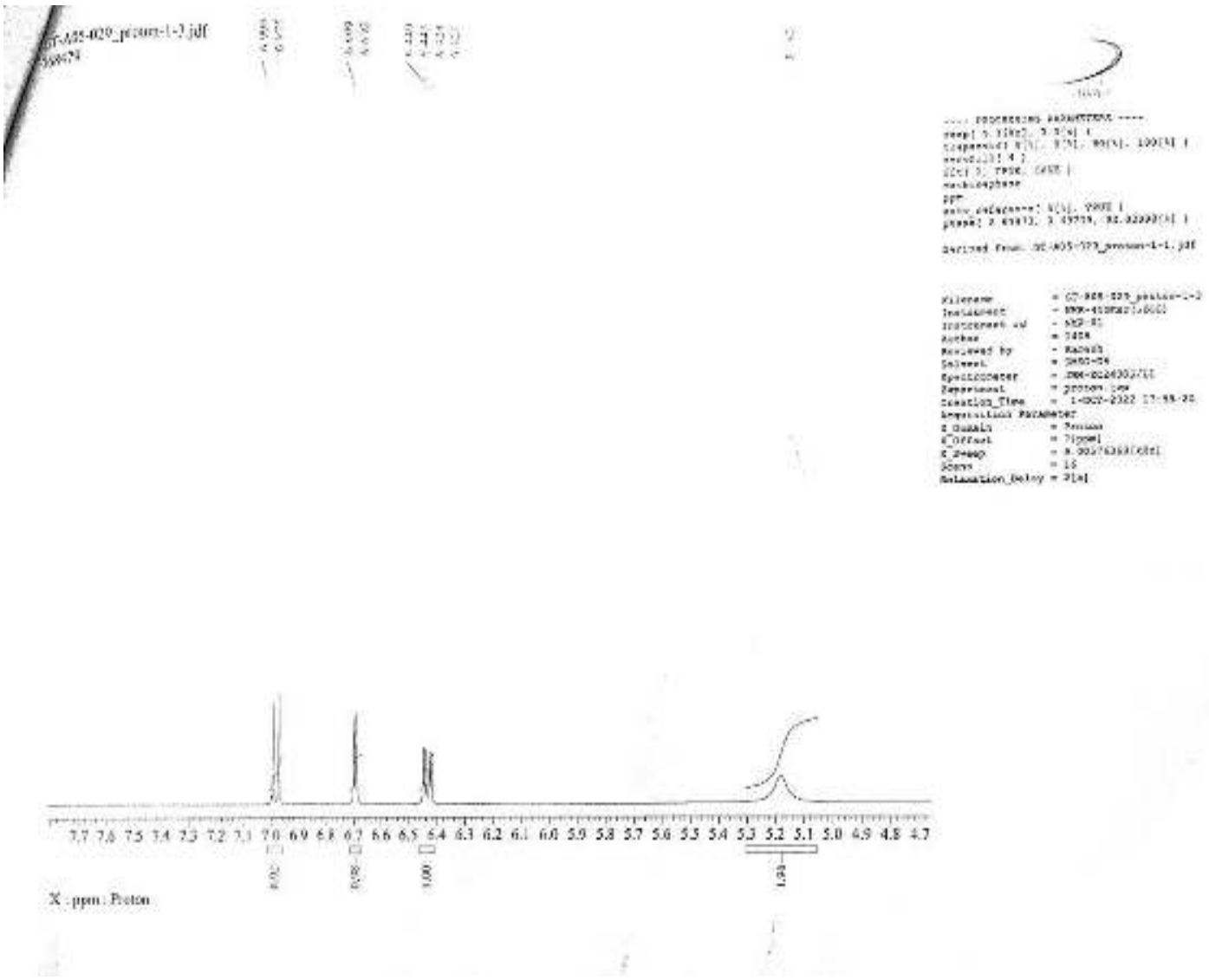
0



J-Coupling Analysis Report

Path = \\backup\D\NMR-400 NEW\Raw Data-400\Sep-2022\GT-A05-029_proton-1-3.jdf

Position	Integral	Pattern	J
6.99[ppm]	1	d	J1 = 8.5[Hz]
6.70[ppm]	1	d	J1 = 2.7[Hz]
6.44[ppm]	1	dd	J1 = 8.5[Hz], J2 = 2.7[Hz]
5.18[ppm]	2	s	
4.09[ppm]	2	q	J1 = 7.1[Hz]
3.65[ppm]	2	s	
3.34[ppm]	4	s	
3.01[ppm]	1	s	
1.19[ppm]	3	t	J1 = 7.1[Hz]

CHAPTER-8

CONCLUSION

During this project I have learned the lab techniques such as literature search, planning of reaction, execution of reaction, Isolation procedure such as column chromatography and characterization of the organic compounds using ^{1}HNMR and IR. Along with the lab technique learned and practiced EHS (Environmental Health and safety) policy. Safe handling of Chemicals, personal and work place safety, disposal of used chemicals, also learned to work in lab as a team.

The present work involved the synthesis of Anagrelide intermediate via Gabriel synthesis. The reaction of aldehyde on nitration, reduction and chlorination gives the subsequent compounds. The Chloro compound on Gabriel synthesis obtained amino compound which on condensation with ethyl bromoacetate and finally on reduction of nitro group with stannous chloride gives the final intermediate and we got good yield.

CHAPTER-9

Result and Discussion:

Compounds	Theoretical M. P	Observed M. P	Theoretical yield	Observed yield	Percentage yield
2-chloro 6-nitrobenzaldehyde	83°C- 84°C	84°C-86°C	15.32 g	13.28 g	86.68
2-chloro 6-nitrobenzyl alcohol	135^0C-137^0C	132^0C-134^0C	13.06 g	12.9 g	98.77
2-chloro 6-nitrobenzyl chloride	137.9^0C-141^0C	136^0C-140.3^0C	11.76 g	10.1 g	85.88
2-chloro 6-nitrobenzyl ethyl glycine	137.9^0C -141^0C	131.4^0C-139.1^0C	7.62 g	7.11 g	93.30
6-amino 2-chloro glycine ethyl ester	137.9^0C-141^0C	130^0C-135.5^0C	11.36 g	10.70 g	94.19

The observed yield of (2-chloro 6-nitrobenzaldehyde) was 13.28 g which is almost closer to theoretical yield i.e., 15.32 g ,thus the percentage yield obtained was 86.68. The compound melting point was 84^0C-86^0C. The observed yield of the (2-chloro 6-nitrobenzyl alcohol) was 12.9 g which was almost closer to theoretical yield i.e.,13.06 g, thus the percentage yield obtained was 98.77. The compound melting point was 132^0C-134^0C. The observed yield of (2-chloro 6-nitrobenzyl chloride) was 10.1 g which was almost closer to theoretical yield i.e.,11.76 g, thus the percentage yield obtained was 85.88. The compound melting point was 136^0C-140.3^0C. The observed yield of (2-chloro 6-nitrobenzyl ethyl glycine) was 7.11 g which was almost closer to theoretical yield i.e.,7.62 g, thus the percentage yield obtained was 93.30. The compound melting point was 131.4^0C-139.1^0C. The observed yield of (6-amino 2-chloro glycine ethyl ester) was 10.70 g which was almost closer to theoretical yield i.e.,11.36 g, thus the percentage yield obtained was 94.19. The compound melting point was 130^0C-135.5^0C.

References:

1. Vogel's textbook of Practical Organic Chemistry,

2. Heterocyclic Chemistry II edition by TLG lich