SYNTHESIS OF KEY INTERMEDIATES FOR BIOACTIVE COMPOUNDS

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UNDER THE SUPERVISION OF **DR. GOPAL SINGH BISHT**

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WAKNAGHAT

TABLE OF CONTENT

CERTIFICATE

This is to certify that the work titled **"Synthesis of key intermediates for bioactive compounds"** submitted by **"Nidhi and IshanTewari"** in partial fulfilment for the award of Bachelor of Pharmacy of Jaypee University of Information Technology; Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of his or any other degree or diploma.

Signature of Supervisor .....

Name of Supervisor Dr. Gopal Singh Bisht

Designation Senior Lecturer

Date 27/05/12

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Date: 27/05/012

SUMMARY

Bacterial resistance to conventional antibiotics is increasing at alarming rate so it is need to hour to search for new synthetic alternative with novel mode of action. Cationic antimicrobial peptides and peptidomimetics are one of such class. Cationic antimicrobial peptides are considered as suitable alternative to conventional antibiotics as they have nonspecific mode of action and multiple target.

Our project is to synthesise key intermediate synthesis for bioactive peptides and antibacterial peptidomimetics. These key intermediate includes synthesis of Fmoc & Boc protection of natural and unnatural amino acids, synthesis of hydrophobic bulk to be used in peptidomimetics synthesis. Boc protection of various compounds such as 6-amino caproic acid, 3-aminophenol, 4-amino benzoic acid, and Fmoc protection of tyrosine, leucine, valine, 3-aminophenol, 4-amino acid and 6-amino caproic acid was successfully completed. It is evident from literature that chalcone and its derivative possess potent biological activities. So it is decided to use chalcone derivative as hydrophobic bulk in antibacterial peptidomimetics synthesis. In the present work, we have synthesized some chalcone like molecule.We have also used micro wave to carry out organic synthesis wherever it is feasible as it provides better yield as compared to normal organic synthesis.

Our main purpose is to synthesise key intermediates for antibacterial peptidomimetics synthesis which are costly or not available commercially. Antibacterial peptidomimetics are one of the main classes of organic compounds to fight with bacterial resistance.

Ishan Tewari Nidhi

Signature of Students

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Date 27/05/12

LIST of FIGURE

NMR DATA FIGURE

LISTS of TABLES

List of Symbols And Acronyms Used

H-NMR	Proton Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
^0C	Degree Centigrade
R_f	Retention Factor
µM	Micro Molar
nM	Nano Molar
gm	Gram
mg	Milligram
µg	Microgram
ml	Millilitre
%	Percentage
Boc	Tert-butyloxycarbonyl
Fmoc	9-fluorenylmethoxycarbonyl
DMF	Dimethylformamide
DCM	Dichloromethane
HOBT	Hydroxybenzotriazole

Chapter-1

INTRODUCTION

1.1 INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds. Development of organic compounds has grown beyond traditional synthetic methods.[1] Research on various small organic molecule having various biological activities, such as anticancer, antibacterial, antiviral, peptidomimetics, etc. is going on around the world. These biologically active organic molecules are known as bioactive molecules. For synthesis of these bioactive compounds, various organic molecules are required known as key intermediates. Key intermediates are chemical substance produced during conversion of some reactant to a product. These can be recovered as a product if reaction is stopped at the point of generation of intermediate. In the present study, we are preparing key intermediates for peptidomimetics which includes synthesis of Fmoc and Boc protected natural and unnatural amino acids, and chalcone derivatives.[2]

A protecting group is introduced into a molecule by chemical modification of a functional group in order to obtain chemo selectivity in a subsequent chemical reaction. It plays an important role in multistep organic synthesis. 9-Fluorenylmethyloxycarbonyl (FMOC) group & tert-butyloxycarbonyl (BOC) group are commonly used in solid phase peptide synthesis[3]. The common method for introducing the FMOC group is by using Fluorenylmethyloxycarbonyl chloride (FMOC-Cl) & 9-Fluorenylmethyloxycarbonyl (FMOC-OSu). The advantage of Fmoc is that it is cleaved under very mild basic conditions (e.g. piperidine), but stable under acidic conditions. After base treatment, the nascent peptide is typically washed and then a mixture including an activated amino acid and coupling co-reagents is placed in contact with the nascent peptide to couple the next amino acid. After coupling, non-coupled reagents can be washed away and then the protecting group on the N-terminus of the nascent peptide can be removed, allowing additional amino acids or peptide material to be added to the nascent peptide in a similar fashion. The formation of Boc-protected amines and amino acids is conducted under either aqueous or anhydrous conditions. Protection of amino acid is done by reaction with a base and the anhydride Boc_2O. Active esters and other derivatives such as Boc-ONH_2 and Boc-N_3 can also be used. The Boc group is stable towards most nucleophiles and bases. *Tert*-butyl carbamates are cleaved under anhydrous acidic conditions with the production of *tert*-butyl cations.[4]

Chalcones are one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based food stuff, have been recently subjects of great interest for their interesting pharmacological activities. Chalcones are belonged to the flavonoids family. A vast number of naturally occurring chalcones are polyhydroxylated in the aryl rings. The radical

quenching properties of the phenolic groups present in many chalcones have raised interest in using the compounds or chalcone rich plant extracts as drugs or food preservatives .Chalcones have been reported to possess many useful properties, including anti-inflammatory, antimicrobial, antifungal, antioxidant, cytotoxic, antitumor and anticancer activities.[3]A number of chalcone derivatives, have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase , aldose reductase, epoxide hydrolase protein tyrosine kinase and quinonereductase. Chalcones having an α, β unsaturated carbonyl group are one of the important biocides and versatile synthons for various chemical transformations. Most of the chalcones are highly biologically active with a number of pharmacological and medicinal applications. Chalcones are also key precursors in the synthesis of many biologically important heterocycles such as benzothiazepine, pyrazolines, 1,4-diketones and flavones. In this study some chalcone like molecule was designed to use as hydrophobic moiety in peptidomimetics synthesis [5-6].

1.2. Objective of project:

1. Fmoc and Boc protection of natural and unnatural amino acid.
2. Microwave synthesis of amine and acids.
3. Synthesis of chalcone.

Chapter-2

LITRATURE REVIEW

2. LITRATURE REVIEW

Antimicrobial peptides (AMPs) are an essential part of innate immunity that evolved in most living organisms over 2.6 billion years to combat microbial challenge. These small cationic peptides are multifunctional as effectors of innate immunity on skin and mucosal surfaces and have demonstrated direct antimicrobial activity against various bacteria, viruses, fungi, and parasites. Innate immunity is necessarily rapid, cidal, redundant, and multifunctional. The antimicrobial function of innate immunity is mediated, in part, by small cationic peptides with potent antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, parasites, and some viruses. The principal mechanism of rapid killing of microbial pathogens is attributed to perturbation of the microbial cell membrane, but our understanding is incomplete and other mechanisms may also be operative [7].

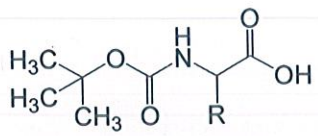
The driving force for the development of newer anti-infective is almost always the inevitable emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal agriculture (growth promoter in chickens, pigs, and feedlot cattle) usage. The pharmaceutical industry has continuously met this need by modifying existing antibiotics and developing newer antibiotics in a timely fashion. These successful efforts have produced the wide variety of currently available drug classes of antibiotics [beta lactams (penicillins, carbapenems, cepahalosporins), glycopeptides, macrolides, ketolides, aminoglycosides, fluoroquinolones, oxazolidinones, and others]. Similarly, there have been dramatic successes in developing effective anti-viral to kill important clinical viral pathogens (e.g., HIV, herpes viruses, and influenza). However, the rapid emergence of resistance is even a greater problem for life-threatening viral infections. The best example remains HIV, where the rapid emergence of resistance to single drugs posed daunting clinical problems. The only effective solution to this problem was to develop combination therapy involving several anti-viral with different mechanisms of inhibitory action. Currently, there are different approved drugs for anti-HIV therapy in use as components of combination therapy. They include nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, proteases, and viral entry blockers inhibitors [8].

One of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based food stuff, have been recently subjects of great interest for their interesting pharmacological activities). Chalcones belongs to the flavonoids family. A vast number of naturally occurring chalcones are polyhydroxylated in the aryl rings. The radical quenching properties of the phenolic groups present in many chalcones have raised interest in using the

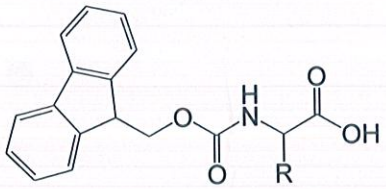
compounds or chalcone rich plant extracts as drugs or food preservatives. Chalcones have been reported to possess many useful properties, including anti-inflammatory, antimicrobial, antifungal, antioxidant, cytotoxic, antitumor and anticancer activities. A number of chalcone derivatives, have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase, aldose reductase, epoxide hydrolase protein tyrosine kinase and quinone reductase. Chalcones having an α, β unsaturated carbonyl group are one of the important biocides and versatile synthons for various chemical transformations. Most of the chalcones are highly biologically active with a number of pharmacological and medicinal applications. Chalcones have been used as anti AIDS agents, cytotoxic agents with anti-malarial, anti-angiogenic activity, anti-infective and anti-inflammatory and anti-tumor agents. Some chalcones were found to increase the level of the tumor suppressor protein p53 in various cancer cell lines by disrupting its complexes with the on co-protein MDM2. Chalcones are also key precursors in the synthesis of many biologically important heterocycles such as benzothiazepine, pyrazolines, 1,4-diketones and flavones . Some heterocyclic systems based on chalcone precursors are benzothiazepines, benzodiazepines, benzoxazepines, pyrimidines, pyrazoles, and oxazoles . Various substituted chalcones possess antioxidant, radical scavenging and, anticancer, anti-leishmanial, anti-mitotic anti-tumor and antibacterial properties, as well as P-glycoprotein mediated multidrug resistance[9].

2.1. PROTECTIONS OF AMINO AND SIDE-CHAIN FUNCTIONS

Chemical synthesis of peptides needs to protect the reactive functional groups, which are likely to interfere in peptide bond formation. These protecting groups must be easily removable without any damage to the assembled peptide. In peptide synthesis, there are two types of protections, which are employed: 1) the "temporary" protections of N-α-amino and carboxyl groups, and 2) the "permanent" protections of side chains in bifunctional amino acids. The temporary protective groups are selectively removed after the formation of each peptide bond without affecting the permanent protections of the side chain. Permanent protections are removed only ultimately after assembly of the peptide is completed. tert-Butyloxycarbonyl (Boc) (I) and the 9-fluorenylmethoxycarbonyl (Fmoc) group (II) are most commonly used to protect α-amino groups[10].



t-Butyloxycarbonyl group (Boc)



9-Fluorenylmethoxy-carbonyl group (Fmoc)

Figure 1: Amino function protecting groups

2.2. FMOC-CHEMISTRY

Incoming amino acids require protection of their amino group in order to obtain coupling to free amino attached to the resin. The 9-fluorenylmethoxycarbonyl (Fmoc) is used as temporary protection of α-amino group (figure 5). This group can be removed by organic bases like piperidine under mild conditions. This group is stable under acidic conditions, so acid labile groups could be used for protection of side chains of bifunctional amino acids. The electron withdrawing fluorine ring system of the 9-fluorenylmethyloxycarbonyl (Fmoc) group renders the lone hydrogen on the β -carbon very acidic and, therefore, susceptible to removal by weak bases. Following the abstraction of this acidic proton at the 9-position of fluorene ring system, β-elimination proceeds to give a highly reactive dibenzofulvene intermediate. Dibenzofulvene can be trapped by excess amine cleavage agents to form stable adducts [11].

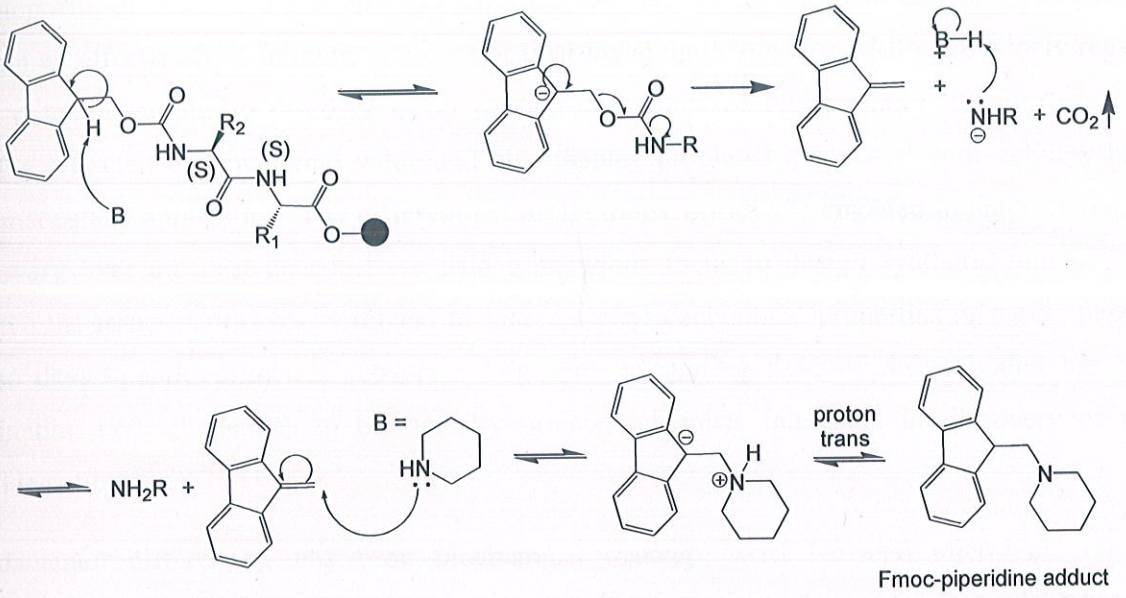


Figure 2. Mechanism of Fmoc Deprotection

2.3. BOC-CHEMISTRY

In the Boc-chemistry, *tert*-butyloxycarbonyl (Boc) group (I) is used for temporary protection of α-amino group. This group can be readily removed by treating with tri-fluoroacetic acid (figure 5). When Boc group (I) is to be used for temporary protection of the N-α-amino group, the side chains of bifunctional amino acids are first protected by benzyl based derivatives such as benzyl esters, ethers etc. These benzyl based protecting groups are removed by much stronger acids like hydrofluoric acid and trifluoromethane-sulphonic acid. The protections of amino functions with Boc group (I) are used both in classical solution as well as solid phase peptide synthesis procedure. The Boc group can be added to the amine under aqueous conditions using di-*tert*-butyl dicarbonate in the presence of a base such as sodium bicarbonate. The Boc group is stable towards most nucleophiles and bases.*tert*-Butyl carbamates are cleaved under anhydrous acidic conditions with the production of *tert*-butyl cations [12].

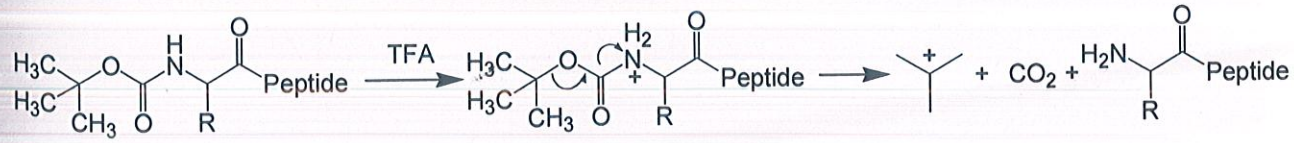


Figure 3. Mechanism of Boc removal

Conventional antibiotic resistance is increasing at a rate that far exceeds the pace of new development of drugs. Natural antimicrobial peptides have been identified as major candidates to sustain an effective host immune response against invading pathogens. AMPs are widely regarded as a potential targets to discover novel peptide antibiotics of future. Early literature on native AMP's reflected the medicinal value and also display problems associated with delivery hinder pharmaceutical application. The different research groups worldwide engaged in antibacterial drug discovery over the past decade have paid tremendous focus to design synthetic mimics which resolve the issue of drawbacks related to inherent physicochemical properties of native peptides. These days "peptidomimetics" covers a large and expanding field of research that has offers fascinating new challenges to biologically oriented chemists interested in discovery of novel peptide antibiotics [13].

Fundamental differences, based on biochemical makeup, exist between microbial cells and mammalian host cells responsible for the selectivity of antimicrobial peptides. Amphipathic nature of essentially all biomembranes is because of the elementary component phospholipids. From these perspectives, it follows that the net charge of a biomembrane is based largely upon its phospholipid stoichiometry. Besides, significant distinctions include membrane composition and architecture, energetics such as transmembrane potential and polarization, cationic property contributes most because of electostaticintraction between positively charged AMP's and anionic surface charge of microbial membrane. Minor factor, hydrophobic interactions between the hydrophobic regions of the antimicrobial peptides and the zwitterionic phospholipids (electrically neutral) surface of the bacterial membranes, also responsible for the cellular assotiation. Moreover, cholesterol, a major constituent of mammalian cellular membranes, can reduce the activity of AMPs by stabilizing the lipid bilayer or by directly interacting and neutralizing them [14].

Following are some BOC and FMOC protected structures synthesised by Lucia Raffaella Lampariello, Daniela Piras, Manuela Rodriquez, and Maurizio Taddei. Using these intermediates, they synthesised hydroxamic dipeptides [15].

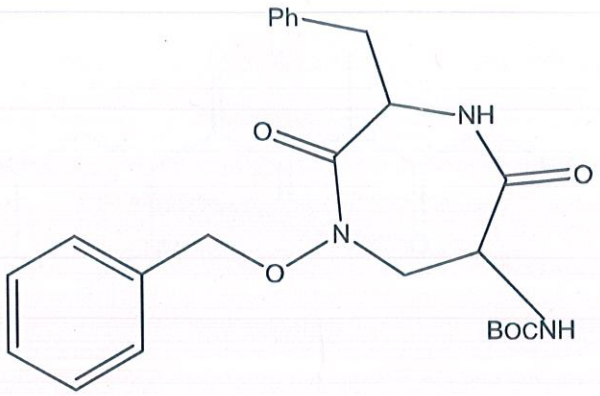


Figure. 4

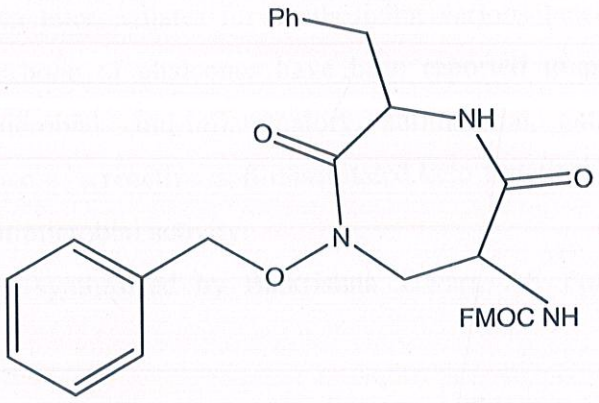


Figure. 5

Following are BOC and FMOC protected amine structures synthesised by Eric Biron, Jayanta Chatterjee, and Horst Kessler. Using these intermediates, they synthesised 1,3-oxazole, thiazole, and imidazole-containing peptides on solid phase from dipeptides [16].

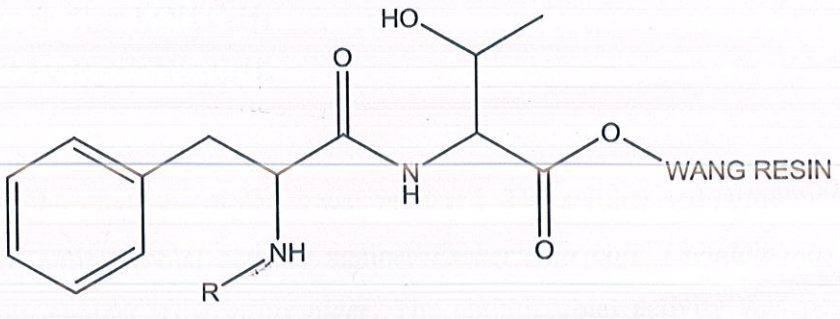
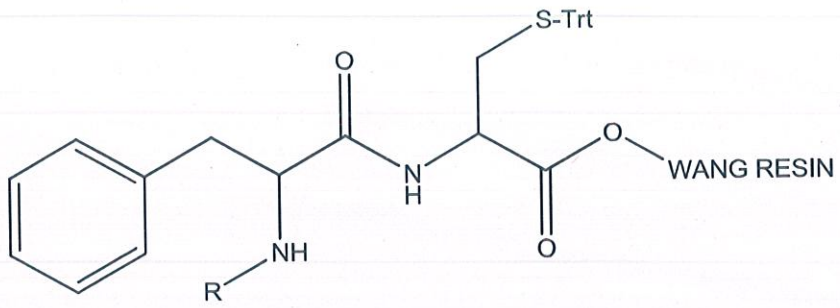


Figure:-6



R= FMOC

Figure. 7

2.4. Chalcones

Chalcones are well known intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial, anti-inflammatory, antimalarial, antileishmanial, antioxidant, antitubercular. The presence of a reactive α, β-unsatutated keto function in chalcones was found to be responsible for their antimicrobial activity.

Few chalcone derivatives synthesised by Balkrishna Tiwari, AS Pratapwar, AR Tapas, SR Butleand BS Vatkar are:

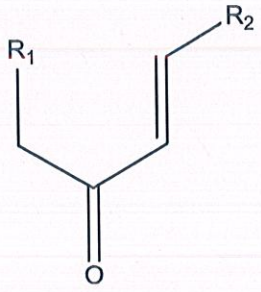


Figure. 8

1) R_1= 4F C_6H_4, R_2= 3-OH C_6H_4
2) R_1= 4F C_6H_4, R_2= 3-$NO_2C_6H_4$

So a series of similar chalcone moieties was prepared. The synthesised compounds were screened for their in vitro antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger*. The antimicrobial activity was performed by filter paper disc plate method at concentration 100 µg/mL [7].

Chapter-3

Material & Methods

3. Material& Methods

3.1. Materials

Amino acids L-Tyrosine, L-Leucine, L-valine, 6-aminocaproic acid, 3-aminophenol, para aminobenzoic acid, were purchased from Loba Chemie. L-Phenylalanine and L-aspartic Acid from Spectrochem and Merck respectively. Solvents such as methanol, ethanol, Ethyl acetate, Actone, hexane, Dimethylformamide (DMF) and Dichloromethane (DCM) were obtained from Merck. Sodium hydroxide, acetic acid, potassium hydrogen sulphate, sodium bicarbonate were purchased from Fischer Scientific. Fmoc is purchased from Spectrochem. Other chemicals used were dioxane and BOC anhydride, benzaldehyde, 3-nitro benzaldehyde, 3-nitroaniline, acetophenone were obtain form Loba Chemie. Zinc chloride, Stannous chloride, etc.

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3.2. Experimental procedure

3.2.1. Synthesis of Fmoc-L-tyrosine

In the aq. solution of L-tyrosine (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added drop wise Fmoc-su (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.2. Synthesis of Fmoc-L-Valine

In the aq. solution of L-valine (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added dropwise Fmoc-su (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.3. Synthesis of Fmoc-L-leucine

In the aq. solution of L-leucine (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added drop wise Fmoc-su (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.4. Synthesis of Fmoc-L-phenylalanine

In the aq. solution of L-phenylalanine (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added drop wise Fmoc-su (1 eq.) as a solution in 1, 4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.5. Synthesis of Fmoc-L-aspartic acid

In the aq. solution of L-aspartic acid (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added drop wise Fmoc-su (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethylacetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.6. Synthesis of Fmoc- 6-aminocaproic acid

In the aq. solution of 6-aminocaproic acid (1eq.), sodium bicarbonate (2eq) was added with stirring and the resulting solution is cooled to 0 °C. Then, added dropwise Fmoc-su (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at 0°C for 5-6 hours and then allowed to

warm at room temperature. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.7. Synthesis of Boc-3-aminophenol (using sodium bicarbonate)

In the aq. solution of 3-amino phenol (1eq.), sodium bicarbonate (2eq) was added with stirring. Then, added drop wise Boc (*tert*-Butyloxycarbonyl anhydride) (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at room temperature for 5-6 hours. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.8. Synthesis of Boc-3-aminophenol (using acetic acid)

In the aq. solution of 3-aminophenol (1eq.), acetic acid (2eq) was added with stirring. Then, added drop wise Boc (*tert*-Butyloxycarbonyl anhydride) (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at room temperature for 5-6 hour. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.9. Synthesis of Boc– 4-aminobenzoic acid

In the aq. solution of 4-amino benzoic acid (1eq.), sodium bicarbonate (2eq) was added with stirring. Then, added drop wise Boc (*tert*-Butyloxycarbonyl anhydride) (1 eq.) as a solution in 1,4-dioxane. The resulting mixture was stirred at room temperature for 5-6 hours. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.10. Synthesis of Boc-6 aminocaproic acid

In the aq. solution of 3-amino phenol (1eq.), sodium bicarbonate (2eq) was added with stirring. Then added drop wise Boc-anhydride (1 eq.) as a solution in 1, 4-dioxane. The resulting mixture was stirred at room temperature for 5-6 hours. TLC was used to check the progress of reaction. For work up, water was then added and mixed with ethyl acetate. The organic (ethyl acetate) layer was separated and aqueous layer was acidified to a pH of 3-4 by using 10% HCl. After that it was extracted with ethyl acetate and separated. Rota evaporator was used to evaporate ethyl acetate layer leaving the product behind. Product formation was confirmed by compliance with standard product.

3.2.11. Synthesis of N-benzoyl phenylalanine

10 grams of glycone was added into 100 ml of 10% sodium hydroxide. Then 18 ml of benzoyl chloride was added to it. Then the solution was shaken vigorously. The solution was cooled by adding some ice to it. Then acidification was done by using concentrated HCl and pH was adjusted in range of 3 to 4. Precipitates were filtered and dried. Then, dried precipitates were dried and were dissolved in carbon tetrachloride and heated for 10 minutes. Resultant precipitates were filtered and dried. Dried filtrate was again dissolved in hot water for recrystellization. Crystals of hippuric acid were filtered and dried.

3.2.12. Reaction of Nicotinic acid with cyclohexylamine

Reaction of nicotinic Acid with cyclohexylamine was tried in microwave. Reaction started with 0.5 grams nicotinic acid and 0.72 grams and was tried at different power levels i.e. power level 1 for 9 minutes; power level 2 for 6 minutes; power level 5 for 8 minutes. But the reaction was not successful.

3.2.13. Reaction of Nicotinic acid with hippuric acid

Reaction of Nicotinic acid with hippuric acid was tried in microwave. Reaction started with 0.5 grams of nicotinic acid and 0.9 grams of hippuric acid and was tried at different power levels i.e. heated at power Level 2 for 5 minutes and at power level 6 for 7 minutes. The reaction was not successful.

3.2.14. Amide coupling Reaction

Reaction of cyclohexyl amine (1 eq.) with benzoic acid (1 eq.) was carried out in microwave. Mixture of both reactants was heated at power 2 for 10 minutes in microwave.

3.2.15. Synthesis of chalcone

Chalcones can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of sodium hydroxide as a catalyst.An efficient synthesis of chalcones was carried out based on the reaction between acetophenone and 3 nitro benzaldehyde using sodium hydroxide and ethanol. The above mixture was allowed to stirred for 4-5 hr.the reaction was monitored using TLC. Once the reaction is completed, HCl was added to get the precipitate which was then filtered and dried to obtain the product.

3.2.16. Selective reduction of chalcone (NI/001/32) with $Zn/NH_4Cl/C_2H_5OH/H_2O$

Chalcone (0.5mmol) in ethanol (50mL) was added to water solution (8 mL) containing 1.07 g (20 mmol) ammonium chloride at room temperature and stirred vigorously with 0.195 g (3 mmol) zinc powder added in three equal portions at intervals of 15min. Stirring was continued for 15 min. The progress of the reaction was monitored by TLC using petroleum ether : ethyl acetate (90:10; v/v). No reaction occurred.

3.2.17. Reduction of chalcone (NI/001/32) nitro group to amine group using stannous chloride

A solution of nitro compound and stannous chloride dehydrate (eq) in methanol (3ml/mmo)l of nitro) was refluxed until the TLC analysis indicate all the staring material had reacted. The methanol was evaporated rota evaporater. The residue was adjusted to pH 8 with saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3t). The combined organic extracts were dried over Na_2SO_4 and evaporated in to vacuo to obtain crude product.

3.2.18. Purification of Amine compound (NI/003/39)

Product (NI/003/39) 0.005gm was taken and 2ml of di-ethyl ether and dil. HCl was added to a mixture at a room temperature .Then aq. and organic layer was seprated. Aq. Layer contains chalcone. After that aq. layer was taken and NaOH was added till alkali pH. Then organic layer was collected and TLC was checked to confirm whether the reaction has completed. Product was taken and solvent was removed from using rotavapour to obtain the purified product.

3.2.19. Reaction of benzaldehyde and 3-nitroaniline Using methods (reflux)

3 nitro aniline (0.005) gm and benzaldehyde(0.0038) was taken and ethanol(2)ml was added in to mixture. 2-3 drop of HCl added in to mixture and was refluxed for 4-5 hr at 50c. At different time of interval were checked for the using TLC. After that product were taken and solvent was removed from the using rotavapour to obtain product.

3.2.20. Reduction of compound (NI/004/47) nitro group to amine group using tin chloride

A solution of nitro compound and stannous chloride dihydrate (eq) in methanol (3ml/mmol) of nitro) was refluxed until the TLC analysis indicate all the staring material had reacted. The methanol was evaporated rota evaporater. The residue was adjusted to PH 8 with saturated NaHCO3 solution and then extracted with ethyl acetate (3t). The combined organic extracts were dried over Na2SO4 and evaporated in to vacuo to afforded crude product.

3.2.21. Reaction of 3-nitroaniline and 3-nitrobenzaldehyde (Schiff base)

1.51gm (10mmol) of 3-nitroaniline was taken and added in to water(5ml). And other side1.38 gm (10mmol) 3-nitro benzaldehyde was added in to ethanol, both reactant mixed with each other and stirred for 3-4 hr. At different time interval were checked for the product using TLC. No reaction occurred.

Chapter-4

Results and Discussion

4. Results and Discussion

4.1. Synthesis of Fmoc protected amino acid

4.1.1. F-moc L-tyrosine

Synthesis of f-moc protected L-tyrosine is a single step procedure. Synthesized F-moc L-tyrosine compounds were confirmed by using TLC (Hexane 2 : 1 Ethyl acetate) and was confirmed by compliance with standard TLC. Percentage yield of f-moc tyrosine is 73%.Rf value is 0.4. White to light yellow crystal powder obtained.

Reaction:

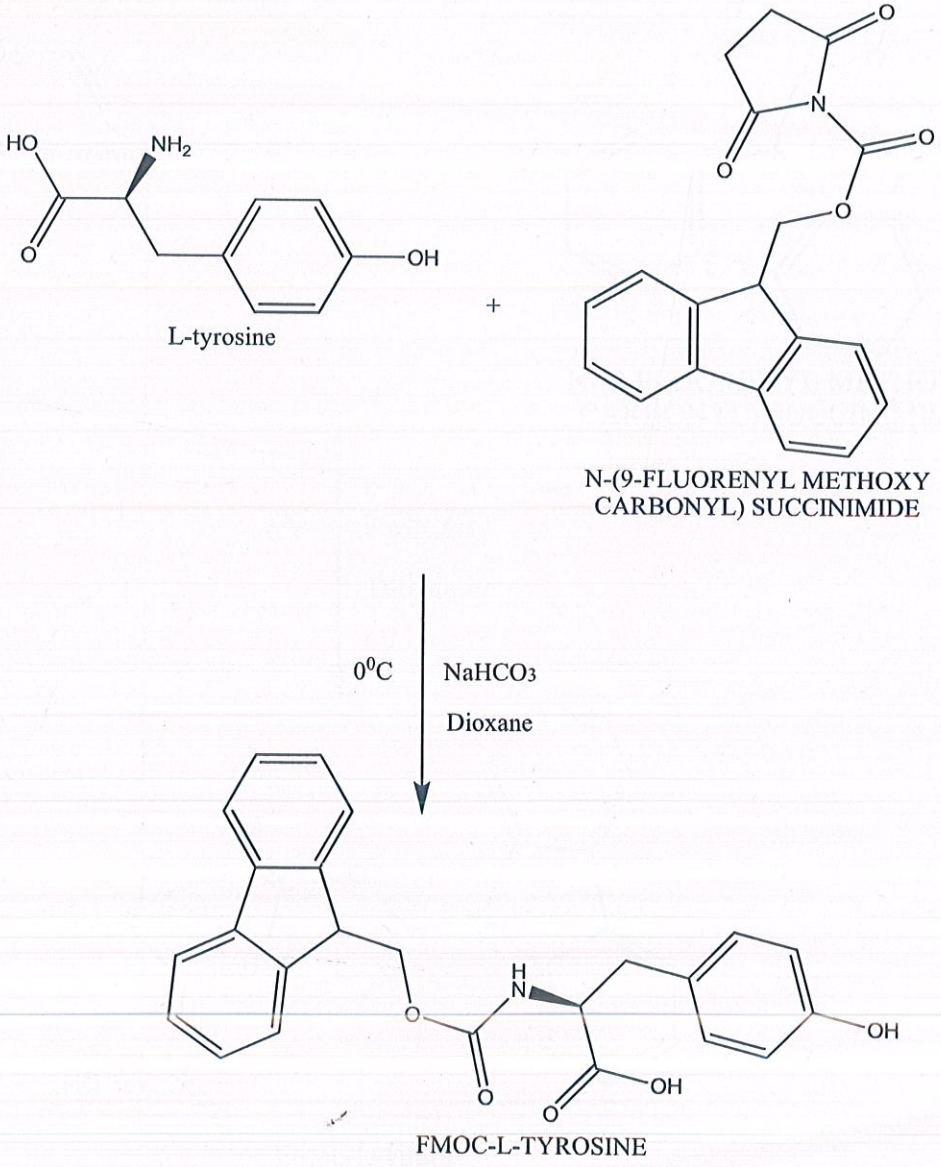
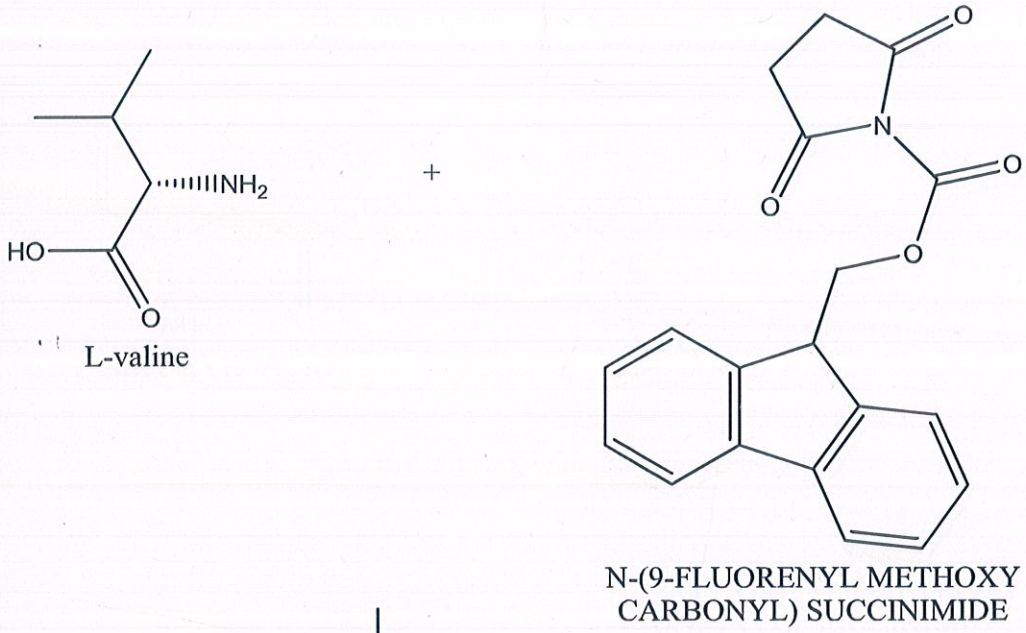


Figure. 9

4.1.2. Synthesis of F-moc valine

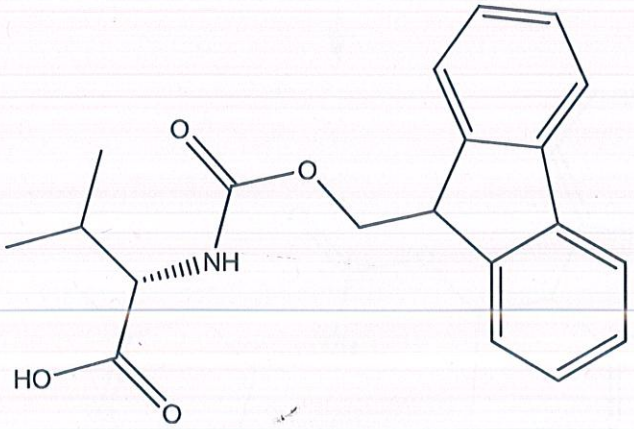
Synthesis of f-moc protected L-aspartic acid is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of f-moc aspartic acid is 97.2%, R_f value is 0.5.

Reaction:



L-valine + N-(9-FLUORENYL METHOXY CARBONYL) SUCCINIMIDE

0^0C | $NaHCO_3$ | Dioxane



Fmoc-L-valine

Figure. 10

4.1.3. Synthesis of F-moc aspartic acid

Synthesis of fmoc protected L-aspartic acid is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of f-moc aspartic acid is 38%, R_f value is 0.4.

Reaction:

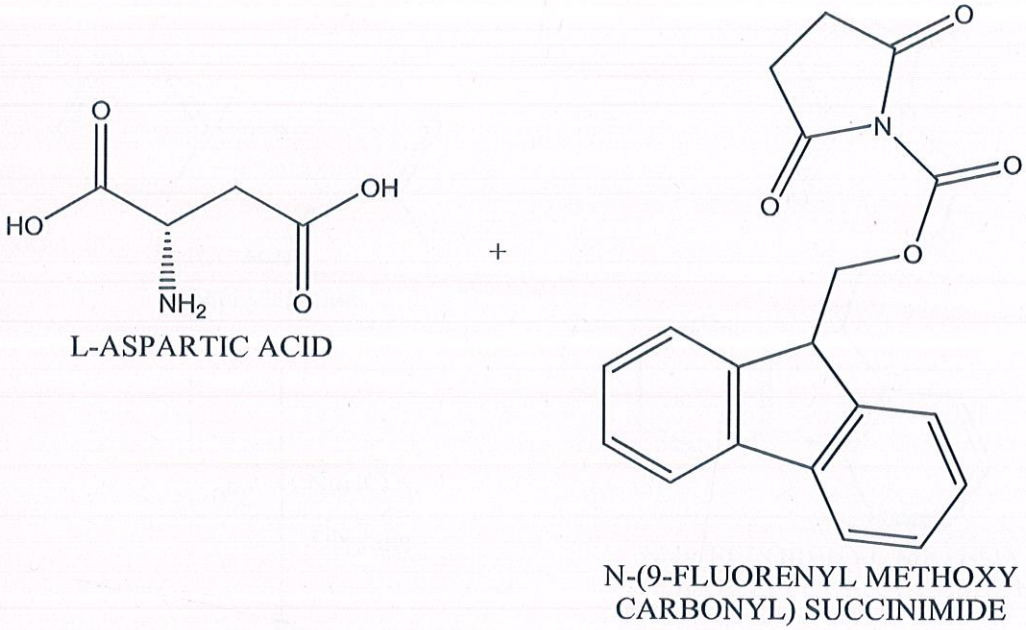


Figure. 11

4.1.3. Synthesis of FMOC- phenylalaline

Synthesis of f-moc protected L-phenylalaline is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of f-mocphenylanaline is 93%, R_f value is 0.5.

Reaction:-

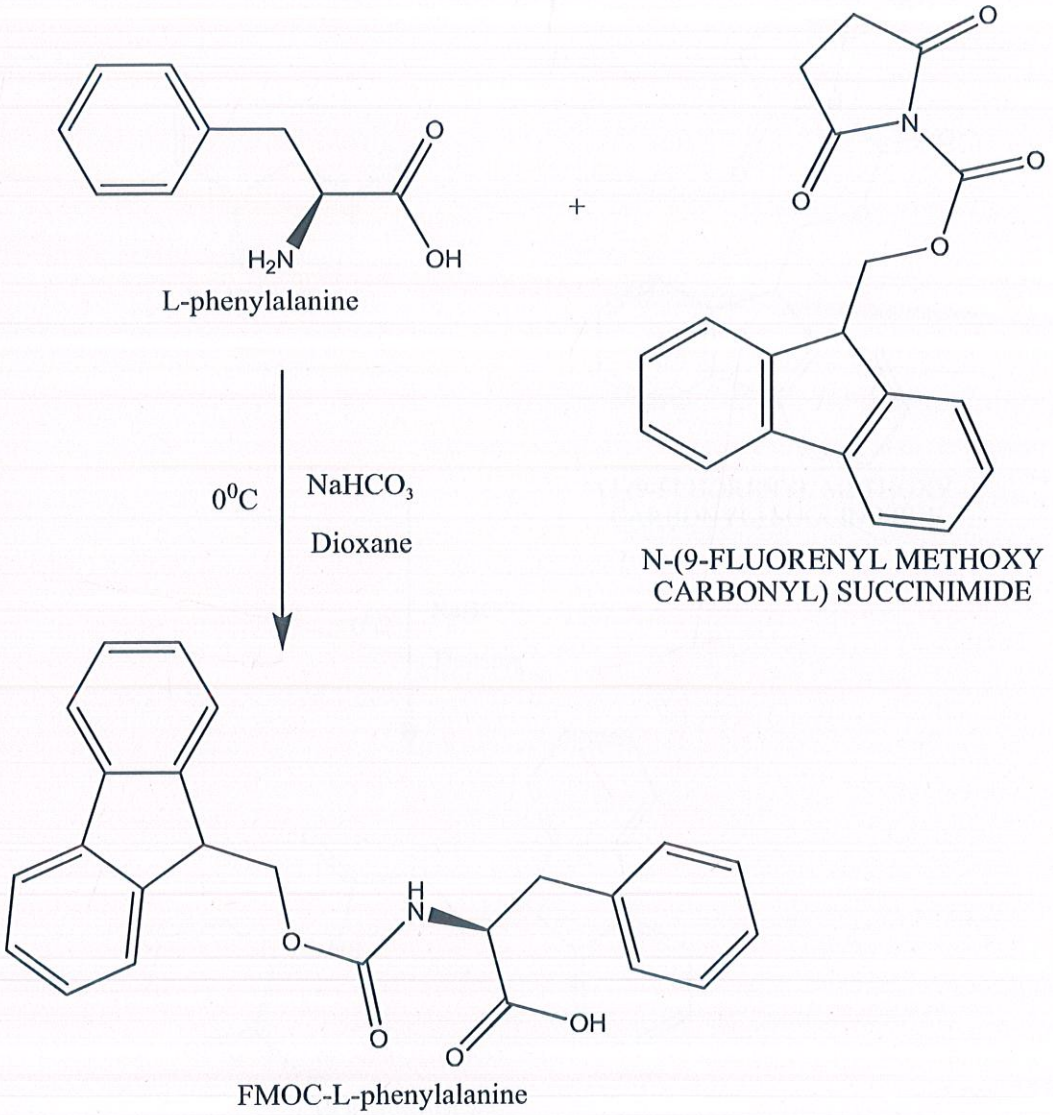


Figure. 12

4.1.4. Synthesis of FMOC-L-leucine

Synthesis of f-moc protected L-leucine is a single step procedure.Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of f-moc leucine is 63.43%, R_f value is 0.6.

Reaction:

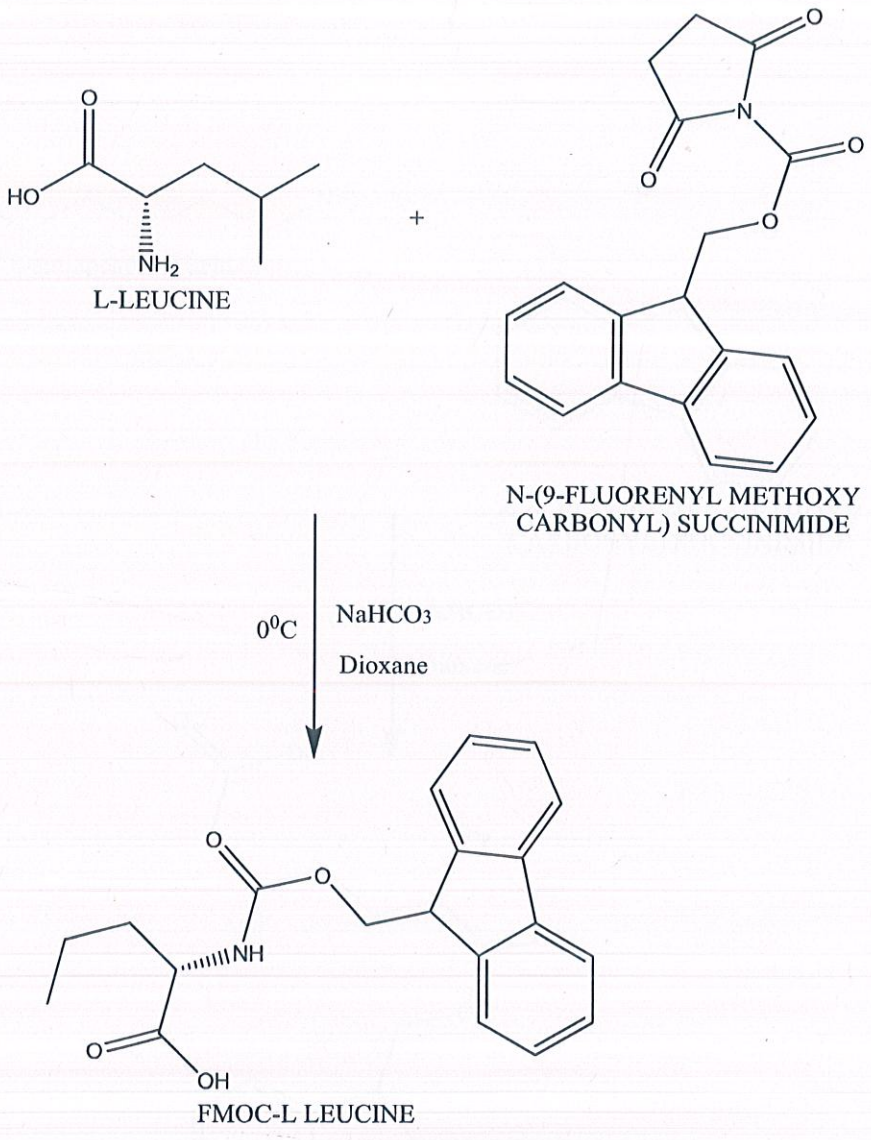


Figure. 13

4.1.5. Synthesis of FMOC -6-amino caproic acid

Synthesis of FMOC protected 6- amino caproic acid is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of f-moc 6-amino caproic acid is 87%, R_f value is 0.7.

Reaction:

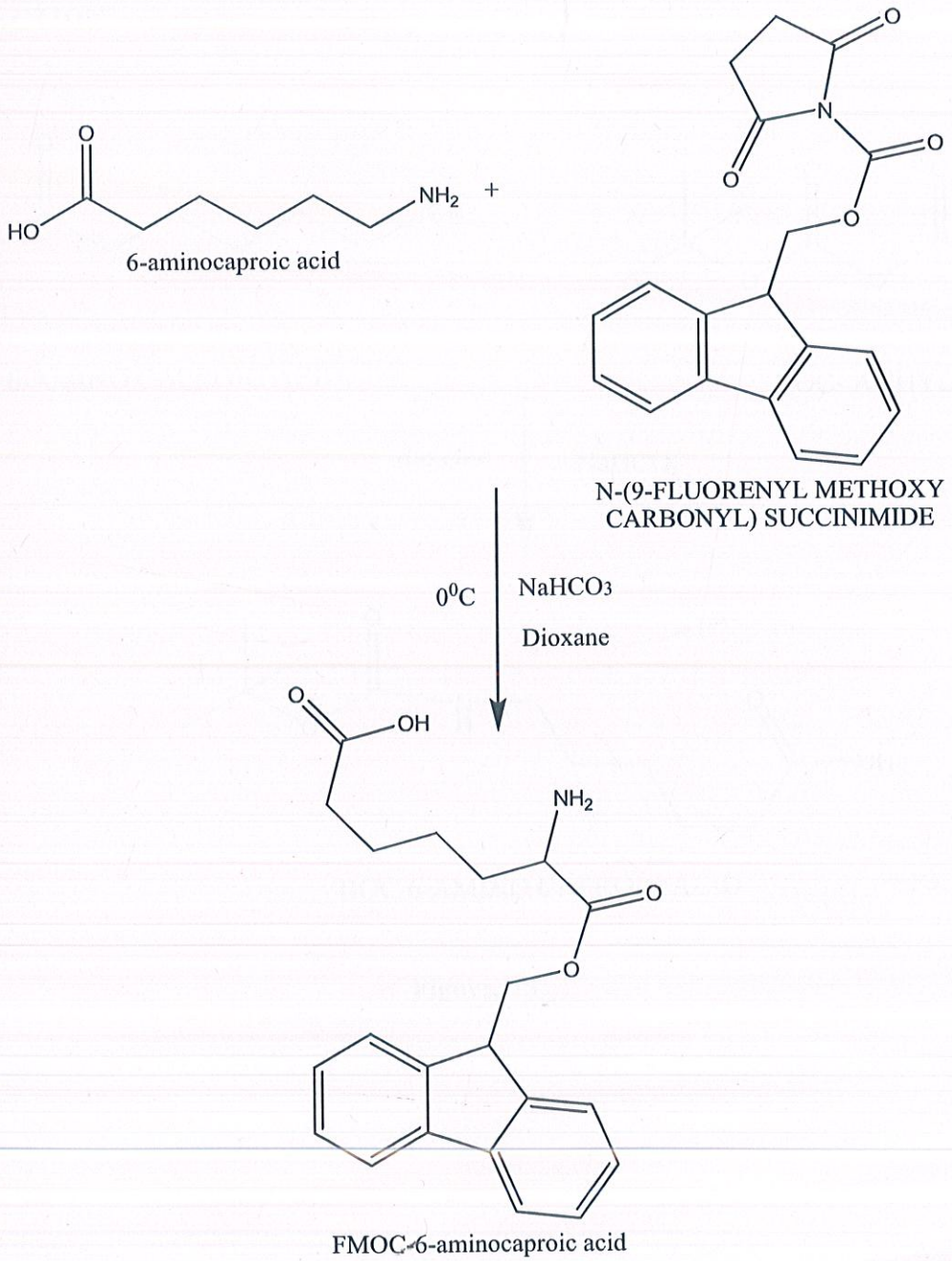


Figure. 14

4.2. Synthesis of BOC protected amino acids

4.2.1. Synthesis of BOC 6-amino caproic acid

Synthesis of BOC protected 6-aminocaproic acid is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of Boc 6-aminocaproic acid is 71%, R_f value is 0.5.

REACTION:

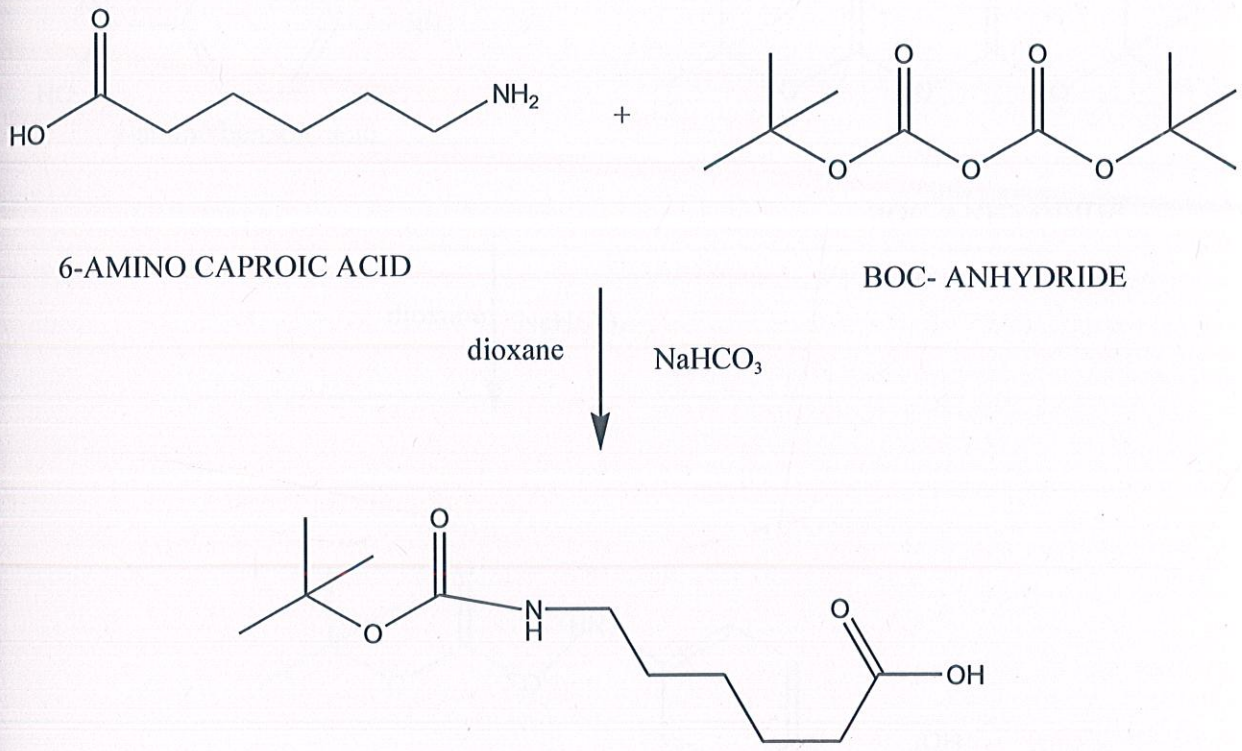


Figure. 15

4.2.3. Synthesis of Boc 4-amino benzoic acid

Synthesis of Boc protected 4-amino benzoic acid is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of Boc 4-amino benzoic acid is 43.4%. R_f value is 0.6.

REACTION:

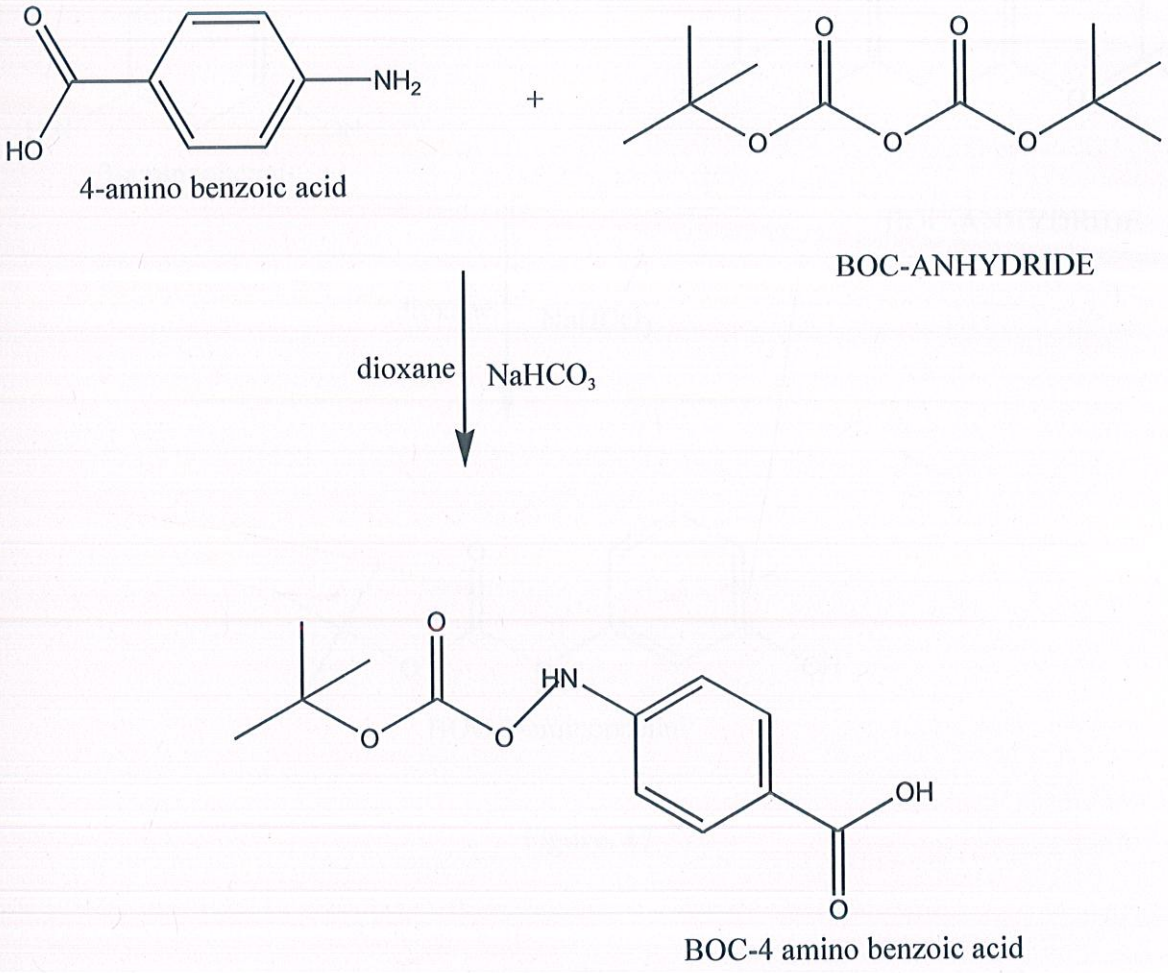


Figure. 16

4.2.4 Synthesis of Boc -3-aminophenol by method 1(using sodium bicarbonate)

Synthesis of Boc protected 3-aminophenol is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of Boc 3-aminophenol is 61.34%, R_f value is 0.6.

REACTION:

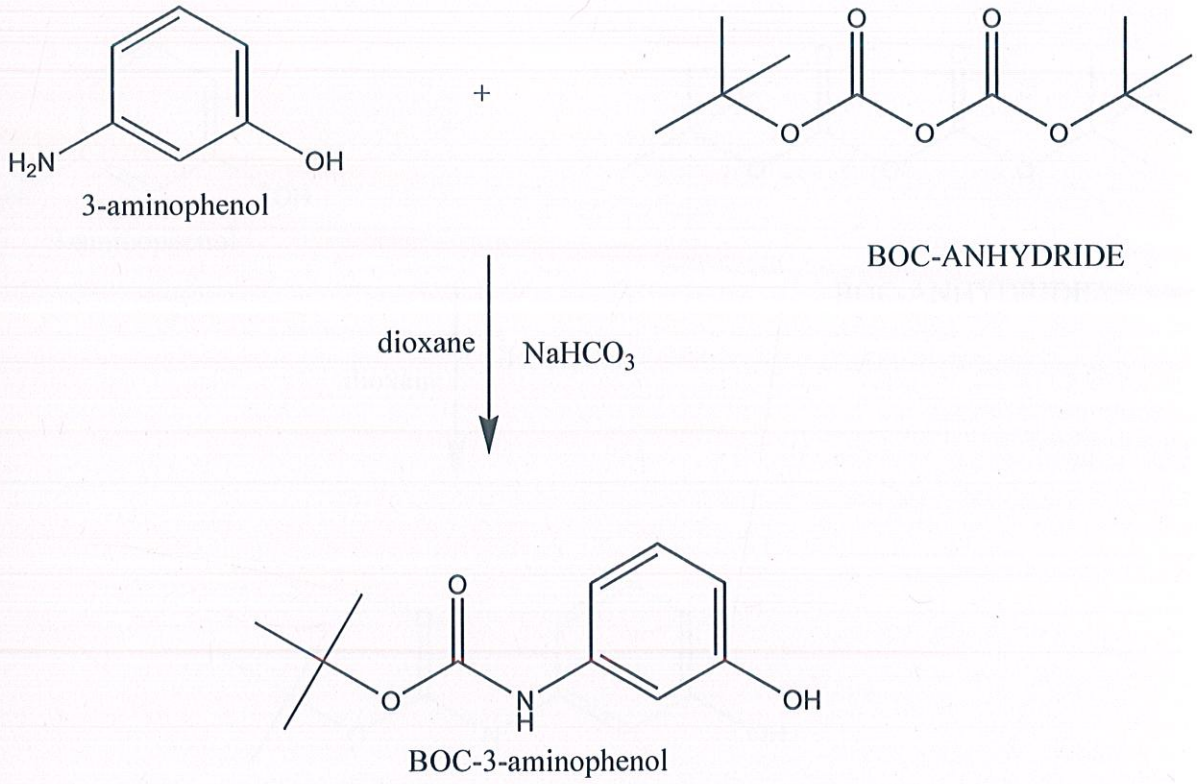


Figure. 17

4.2.5 Synthesis of Boc 3-aminophenol by method 2 (using acetic acid)

Synthesis of Boc protected 3-aminophenol is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of Boc 3-aminophenol is 70%, R_f value is 0.5.

Reaction:

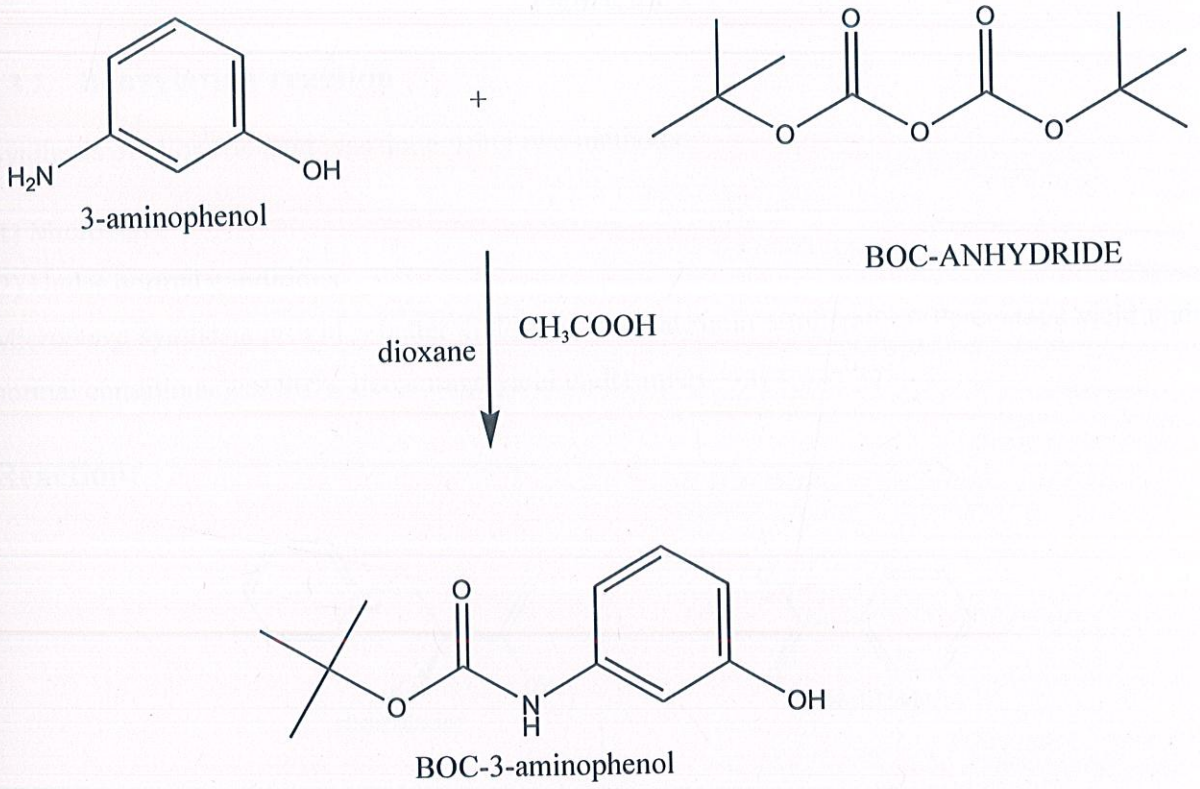


Figure. 17

4.3. Synthesis using microwave oven

4.3.1. Synthesis of hippuric acid

A synthesis of hippuric acid involves the acylation of glycine with benzoyl chloride

Synthesis of Hippuric acid was done using two methods:

1) Microwave
2) Room temprature.

Microwave synthesis give best yield than done at room temperature.Percentage yield in microwave was 61.34% . Percentage yield under normal conditions was 41.46%

REACTION:

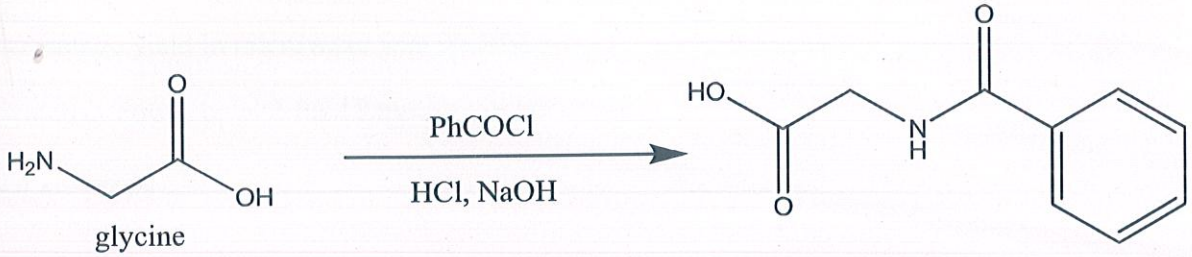


Figure. 18

4.3.2. Benzylation reaction

Synthesis of Hippuric acid was done using two methods:

1) Microwave

2) Under normal conditions

Microwave synthesis provides better yield than done at room temperature. Percentage yield under normal conditions was 90%. Percentage yield under micro wave was 96%.

Reaction:

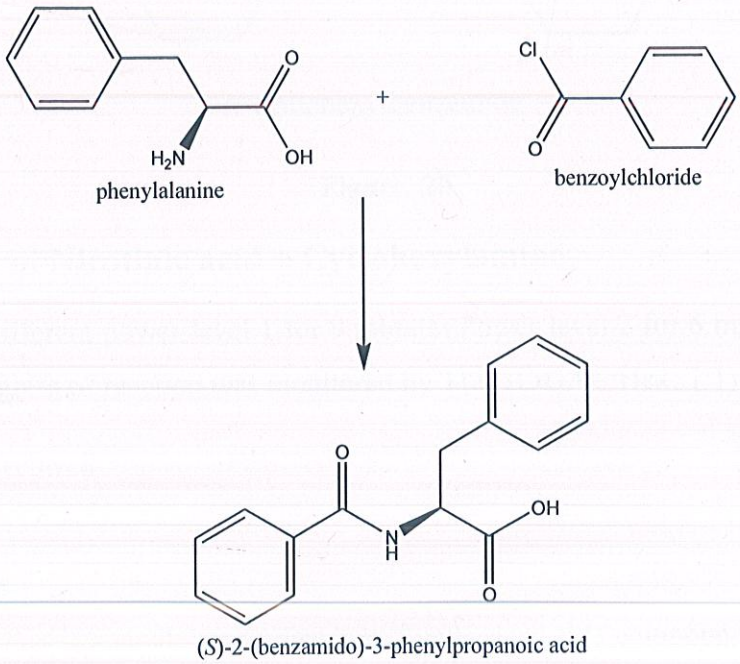


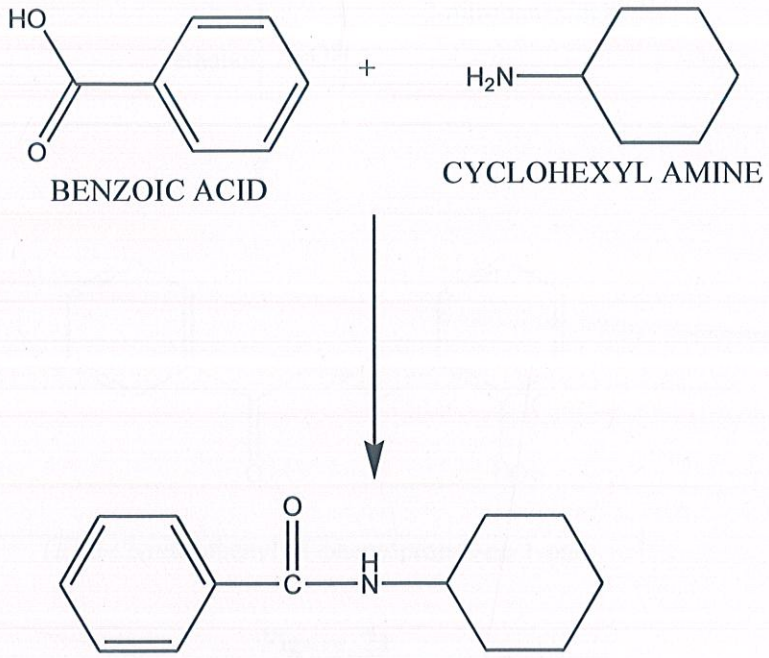
Figure. 19

4.3.3. Amide Coupling-

Percentage yield in microwave was 96.30%

Heated at Power level 2 for 10 minutes in microwave.

REACTION:-



N-cyclohexylbenzamide

Figure. 20

4.3.3. Reaction of Nicotinic acid + Cyclohexylamine

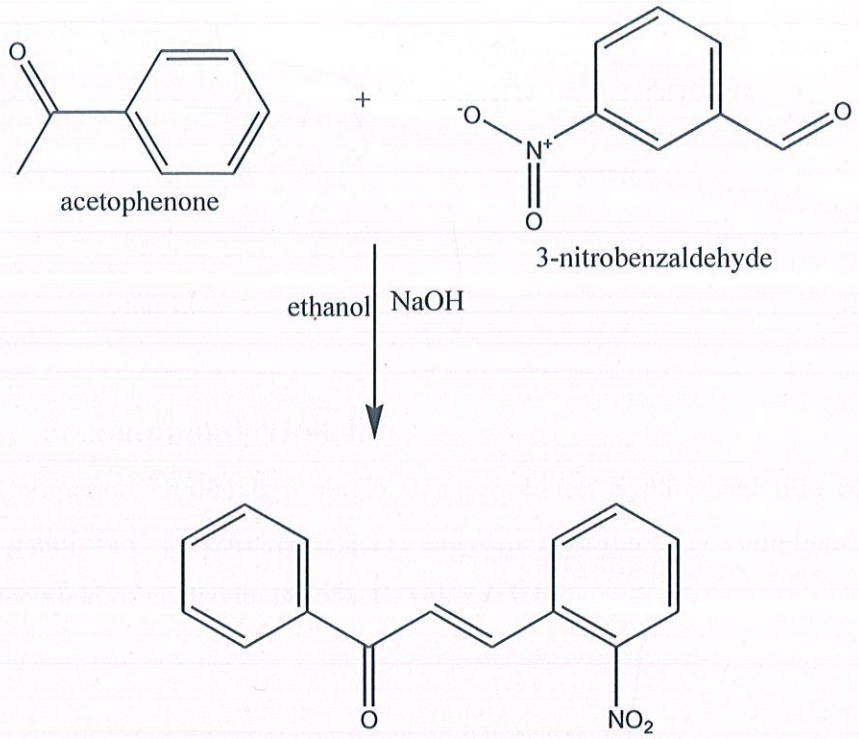
In microwave at different power level 1 for 9 minutes,Power level 2 for 6 minutes, Power level 5 for 8 minutes. Progress of reaction was monitored by TLC (EtOAC:Hex::1:1)

Reaction did not occurred.

4.4. Chalcone

Synthesis of chalcone is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of chalcone is 85%, R_f value is 0.7.

Reaction:-



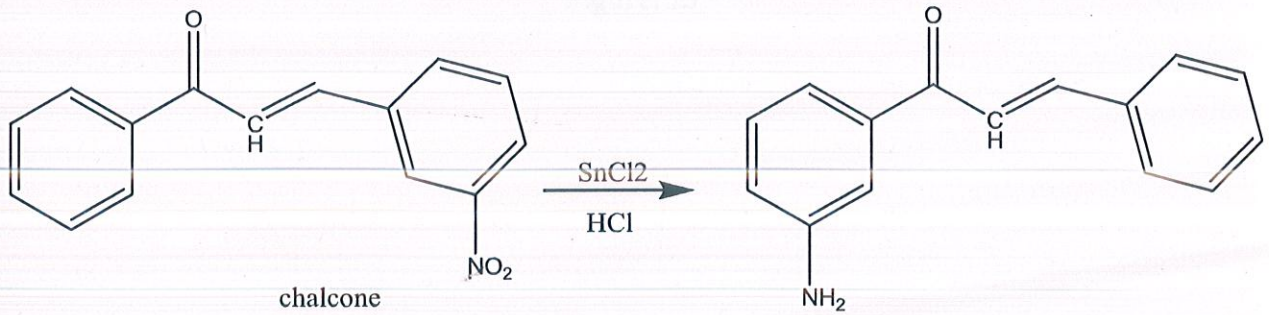
(*E*)-3-(2-nitrophenyl)-1-phenylprop-2-en-1-one

Figure. 21

4.5. Synthesis of compound NI/001

Synthesis of Compound NI/001 is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of chalcone is 90%. R_f value is 0.4.

Reaction:-



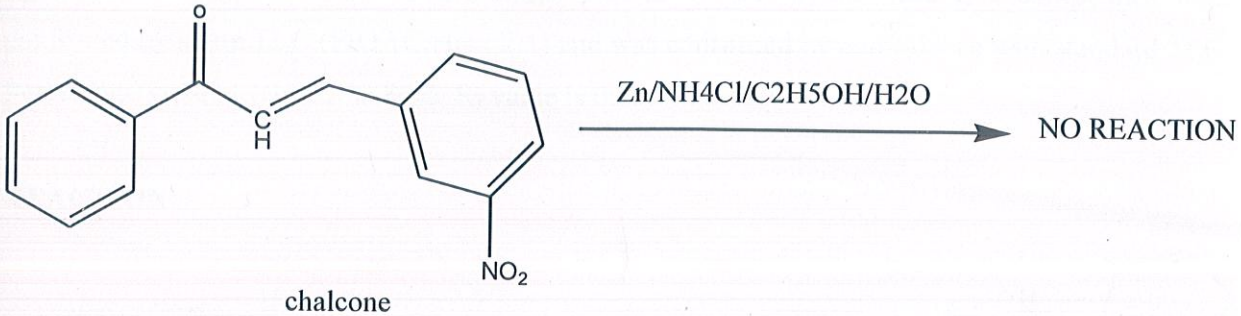
chalcone

(*E*)-1-(3-aminophenyl)-3-phenylprop-2-en-1-one

NI/001

Figure. 22

Reaction:

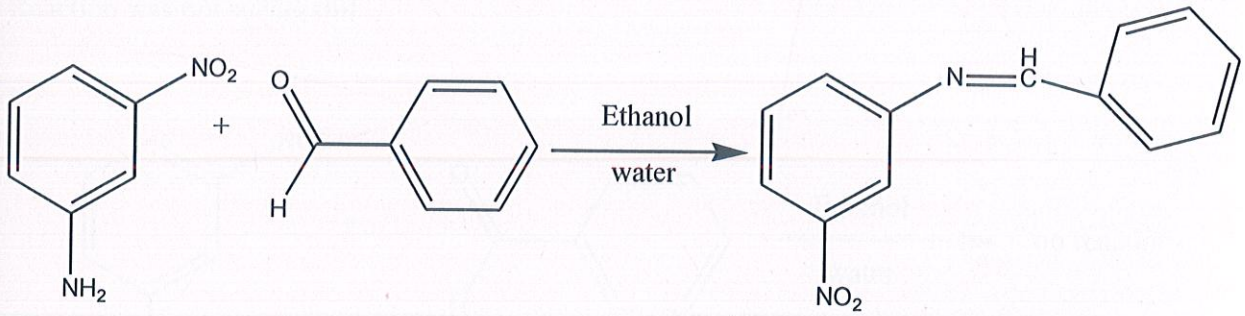


chalcone

4.6. Synthesis of compound NI/004:

Synthesis of Compound NI/004 is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of chalcone is 75%, R_f value is 0.6

Reaction:-



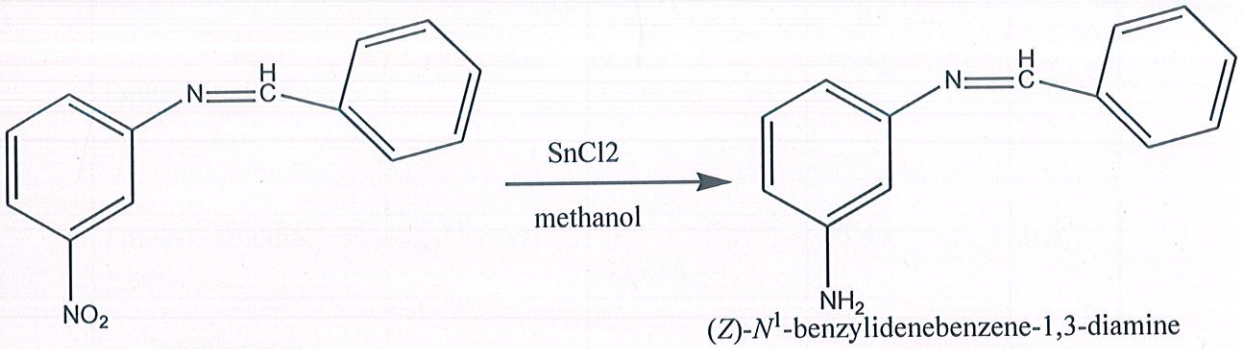
3-nitro aniline benzaldehyde (*Z*)-*N*-benzylidene-3-nitrobenzenamine NI/004/047

Figure. 23

4.7. Synthesis of NI/005

Synthesis of Compound NI/005 is a single step procedure. Synthesized title compounds were confirmed by using TLC (EtOAC:Hex::2:1) and was confirmed by compliance with standard TLC. Percentage yield of NI/005 is 86%, R_f value is 0.5.

REACTION:



NI/004/047

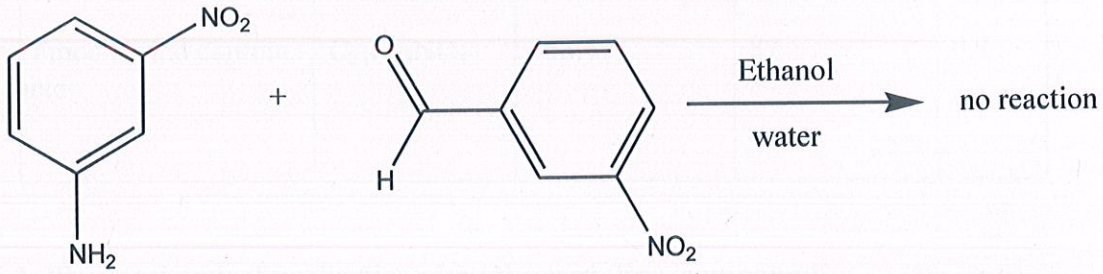
(*Z*)-N^1-benzylidenebenzene-1,3-diamine

NI/005/48

Figure. 24

4.8. Reaction of 3-nitrobenzaldehyde and 3-nitro aniline

Reaction was not successful



3-nitro aniline

3-nitro benzaldehyde

Table 1. Physicochemical properties of synthesized Fmoc compound

S.NO.	Compund Name	Mol. Formula	Mol wt.	%	R_f value
1	Fmoc-L-tyrosine	$C_{24}H_{21}NO_5$	403.14	73	0.4
2	Fmoc-L-valine	$C_{20}H_{21}NO_4$	339.39	97.22	0.5
3	Fmoc-L-leucine	$C_{21}H_{23}NO_4$	353.18	63.43	0.6
4	Fmoc-L- phenylene	$C_{24}H_{21}NO_4$	387.18	93	0.5
5	Fmoc-L-aspartic acid	$C_{19}H_{17}NO_6$	355.34	38	0.4
6	Fmoc-amino caproic acid	$C_{21}H_{23}NO_4$	353.41	87	0.7

Table 2. Physicochemical properties of synthesized Boc compound

S.No.	Compound Name	Mol.Formula	Mol.Wt	%Yield	Rf Value
1	BOC-3 aminophenol	$C_{11}H_{15}NO_3$	209.42	61.34	0.7
2	BOC-6-aminocaproic acid	$C_{15}H_{24}N_2O_6$	231.32	71	0.5
3	BOC-PABA	$C_{12}H_{15}NO_4$	237	43.4	0.6

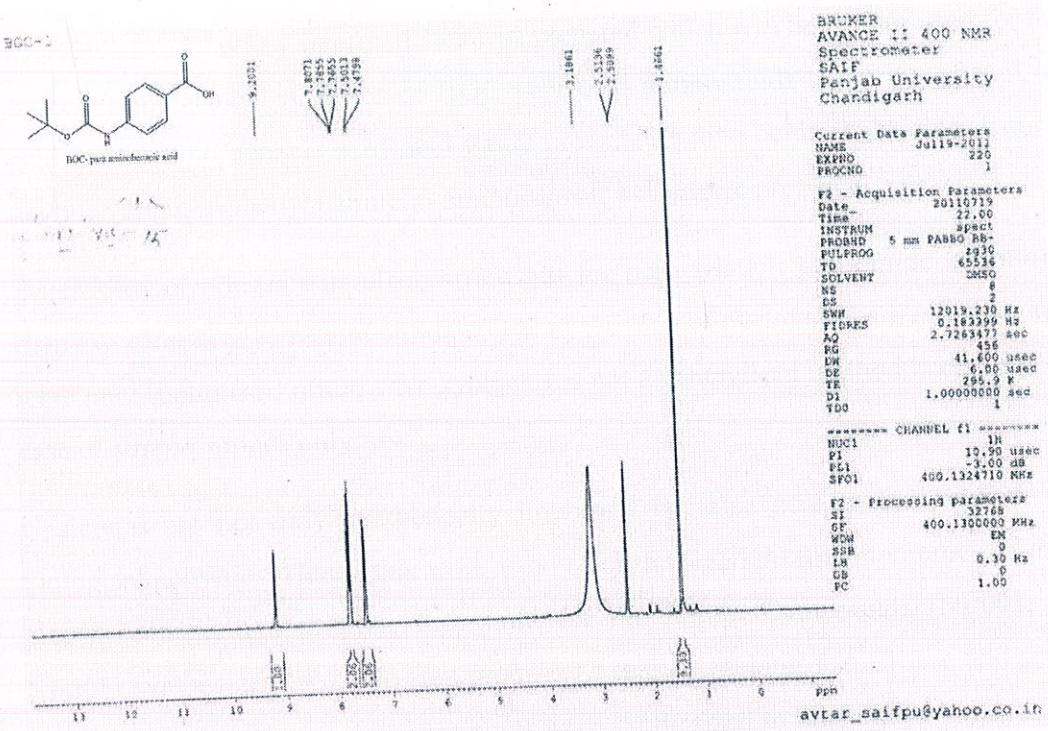
Table 3. Physicochemical properties of synthesized Benzylated, Glycine and Phenylalanine:

S.NO	Compound name	Mol. formula	Mol. wt	% yield	Rf value
1	Hippuric acid	$C_9H_9NO_3$	189.17	41.46	0.4
2	3-phenyl propanoic acid	$C_9H_{10}O_2$	150.17	90	0.6

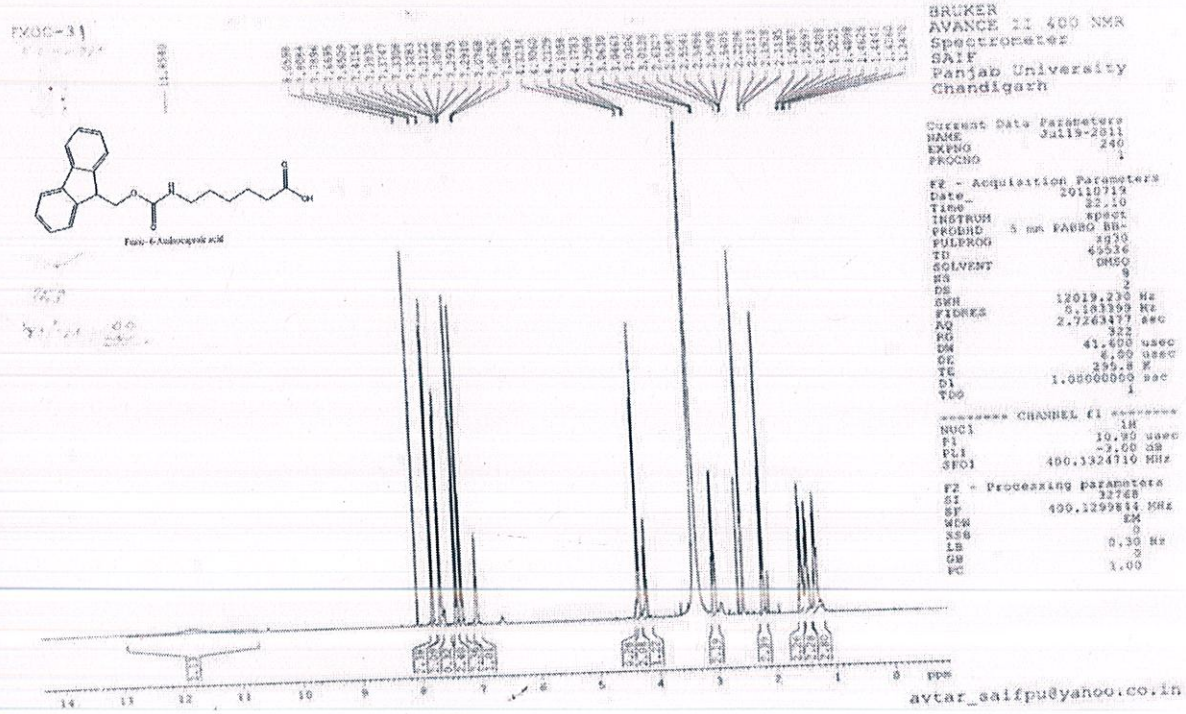
Table 4. Physicochemical properties of synthesized chalcone:

S.NO	Compound code & name	Mol.formula	Mol.wt	%yield	Rf value
1	Chalcone (NI/001)	$C_{15}H_{12}NO_2$	238	90	0.4
2	NI/003	$C_{15}H_{12}NH_2$	208	85	0.6
3	NI/004	$C_{13}H_{10}N_2O_2$	226	75	0.6
4	NI/005	$C_{13}H_{10}N_2H_2$	196	86	0.5

NMR of Boc-PABA



NMR of 6- aminocaproic acid



CONCLUSION

A series of various key intermediates was prepared which will be further used for the preparation of bioactive molecules. A series of BOC protected amino acids (3-amino phenol, 6-amino caproic acid, para amino benzoic acid) and Fmoc protected amino acids (L-leucine, L-tyrosine, L-aspartic acid, L-valine, L-phenylalanine, 6-aminocaproic acid) were prepared.

Microwave synthesis was also carried out for benzoylation and amide coupling. Two reactions (benzoylation of glycine and amide coupling reaction of cyclohexyl amine) were successful but rest were not. That means microwave synthesis is not a generalised method because it was successful in case of simple amines only.

Chalcones are not only synthetically important but also possesses a wide range of promising biological activities. Hence this work is an effort to highlight the importance of the chalcone in the present context and promise they hold for the future. Compounds (NI/001, NI/003, /NI/004, NI/005) synthesized by a two step process 1st step by making Schiff base and second reduction of nitro group to amine. All the compounds were synthesized in fairly good yields and were confirmed by using NMR and TLC by compliance with standard compound.

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BIO-DATA

1. Ms. Nidhi

   CGPA- 6.8 (equivalent to 74%)

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   Industrial Training- successfully completed 1 month training at Ipca Laboratories, Dehradun, Uttarakhand

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