DEVELOPMENT OF HETEROCYCLIC CARBOXYL ACTIVATING GROUPS AND THEIR APPLICATIONS IN PEPTIDE SYNTHESIS

Thesis submitted to the University of Calicut
in partial fulfilment of the requirements
for the award of the Degree of
DOCTOR OF PHILOSOPHY
in Chemistry under the Faculty of Science

By
M.P. RAJAN, M.Phil

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALICUT

MAY 2003

DECLARATION

I hereby declare that the thesis entitled **"Development of Heterocyclic Carboxyl Activating Groups and Their Applications in Peptide Synthesis"** is an authentic record of the research work carried out by me under the supervision and guidance of **Dr. E. Purushothaman**, Professor, Department of Chemistry, University of Calicut. No part of this thesis has been presented for any other degree or diploma earlier.

University of Calicut,
May 2003.

M.P. RAJAN

Dr. E. PURUSHOTHAMAN
Professor

CERTIFICATE

This is to certify that the thesis entitled **"Development of Heterocyclic Carboxyl Activating Groups and Their Applications in Peptide Synthesis"** is an authentic record of the research work carried out by **Mr. M.P. Rajan** under my supervision and guidance during the period October 1995 to May 2003 in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** under the Faculty of Science of the Calicut University. The work presented in this thesis has not been submitted for any other degree or diploma earlier. It is also certified that Mr. M.P. Rajan has fulfilled the course requirements and passed degree of Master of Philosophy in Chemistry of this University.

University of Calicut
May 2003.

Dr. E. PURUSHOTHAMAN
(Supervising Teacher)

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CONTENTS

ABBREVIATIONS

Abbreviations and nomenclature used for the amino acids and peptides are in agreement with the recommendations of the IUPAC-IUB commission on Biochemical Nomenclature, *Biochem. J.*, 219, 345 (1984); *Eur. J. Biochem.*, 138, 9 (1984). In addition, the following abbreviations were used.

AcOH	-	Acetic acid
Boc	-	Butyloxycarbonyl
bp	-	Boiling point
Bz_2O_2	-	Benzoyl peroxide
Bz	-	Benzoyl
Bzl	-	Benzyl
C	-	Celsius
CD	-	Circular dichroism
CLPSER	-	Cross-linked polystyrene polyethylene glycol acrylate resin
DCC	-	Dicyclohexylcarbodiimide
DCM	-	Dichloromethane
DCU	-	Dicyclohexylurea
DIEA	-	N,N'-Diisopropylethylamine
DMA	-	Dimethylacetamide
DMF	-	Dimethylformamide
DMSO	-	Dimethylsulfoxide
DPT	-	4,6-Dimethylpyrimidine-3-thione
DTT	-	5,6-Diphenyl-1,2,4-triazine-2-thione
EtOH	-	Ethanol
Fmoc	-	9-Flurenylmethyloxycarbonyl
g	-	Gram
HBTU	-	2-(1H-Benzotriazol-1-yl) 1,1,3,3-tetramethyluronium hexafluorophosphate
h	-	Hour
HMB	-	4-Hydroxymethylbenzamidomethyl

HOBt	-	1-Hydroxybenzotriazole
HPLC	-	High performance liquid chromatography
IR	-	Infrared
MALDI	-	Matrix assisted laser desorption ionization
Me	-	Methyl
MeOH	-	Methanol
mg	-	Milligram
mL	-	Millilitre
min	-	Minute
mp	-	Melting point
NBS	-	N-Bromosuccinimide
N-BzDPT	-	N-Benzoyl-4,6-dimethylpyrimidine-2-thione
N-AcDPT	-	N-Acetyl-4,6-dimethylpyrimidine-2-thione
N-PrDPT	-	N-Propionyl-4,6-dimethylpyrimidine-2-thione
N-PaDPT	-	N-Phenylacetyl-4,6-dimethylpyrimidine-2-thione
N-BzDTT	-	N-Benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione
N-AcDTT	-	N-Acetyl-5,6-diphenyl-1,2,4-triazine-3-thione
N-PrDTT	-	N-Propionyl-5,6-diphenyl-1,2,4-triazine-3-thione
N-PaDTT	-	N-Phenylacetyl-5,6-diphenyl-1,2,4-triazine-3-thione
NMP	-	N-Methylpyrolidone
NMR	-	Nuclear magnetic resonance
OD	-	Optical density
Ph	-	Phenyl
SPPS	-	Solid phase peptide synthesis
TEA	-	Triethylamine
TFA	-	Trifluoroacetic acid
THF	-	Tetrahydrofuran
TLC	-	Thin layer chromatography
UV	-	Ultra Violet

INTRODUCTION AND OBJECTIVES

M.P. Rajan “Development of heterocyclic carboxyl activating groups and their applications in peptide synthesis” Thesis.Department of Chemistry, University of Calicut, 2003

INTRODUCTION AND OBJECTIVES

Chapter 1

INTRODUCTION AND OBJECTIVES

The growth of Organic Chemistry, from the very beginning, has kept pace with the growth of Organic Synthesis. The essence of practical Chemistry is synthesis - reproducible synthesis of strictly characterised systems. Therefore, synthesis, is a vital and most exciting part of Organic Chemistry.

Organic chemistry, during the last century, has been deeply oriented with a trend towards overspecialisation within the discipline. During these days Heterocyclic Chemistry, has undoubtedly led to many exciting developments in the field of synthesis. Outstanding methods for obtaining functionalised organic compounds of varied interest can be formed by employing a heterocyclic system either as a precursor or a carrier for formation.[1] Chemical investigators look at heterocyclic systems with great enthusiasm towards their potential synthetic utility. Nowadays, heterocyclic chemistry has made tremendous contributions towards pharmaceuticals, plasticizers, pesticides, herbicides, dyes and for many other potential applications. Recently, the use of heterocyclic compounds has been extended to the field of peptide synthesis also.

Synthesis of a peptide with a well defined sequence of amino acid residues is fairly a complex process. The major problem associated with peptide synthesis lies in the formation of these amides or peptide bonds that couple amino acids. The synthesis of naturally occurring peptides and their structural analogues for biological

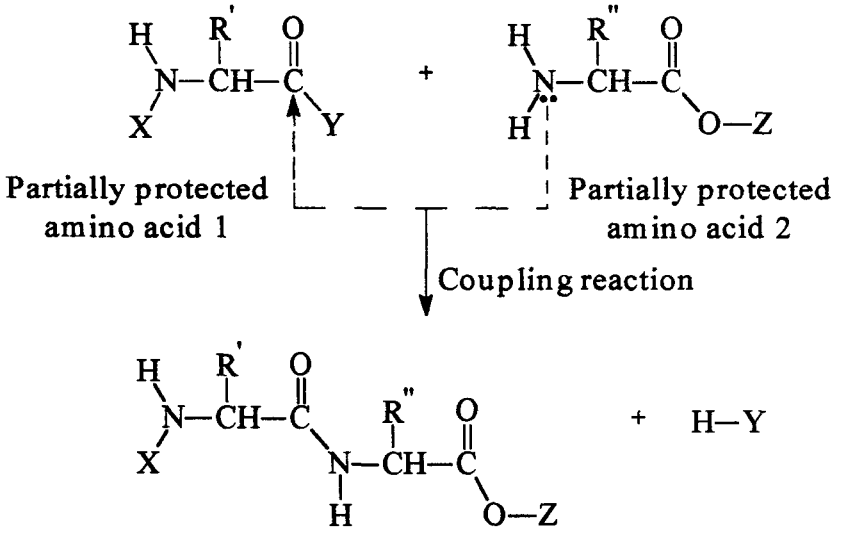
and pharmacological investigations necessitates the availability of efficient and modified peptide synthesis strategies. In the present work, it is envisaged to design novel carboxyl activating groups to be effectively applied in the synthesis of peptides.

Peptide chemistry in association with molecular biology has made great advances in the last two decades and still continue to be a prominent area of active research. Recent advances in biotechnology has made great contributions in understanding the life process of living organisms and health science. These help in developing new synthetic vaccines that can compete with bacterial and viral infections, enzyme mechanism and also in understanding the action of neuro-peptides[2-4]. Using synthetic peptides, studies on mechanism of hormone action, and of enzyme-substrate, antigen-antibody and protein-DNA interaction have been made possible Peptides and their derivatives with antibacterial, antiendotoxic, antibiotic-potentiating or antifungal properties are being developed for the use as a novel class of antimicrobial agents and as the basis for making transgenic disease-resistant plants and animals.[5]

Synthetic and structural studies of the peptides are essential for the development of new methods for the preparation of peptides and also for the action of proteins in living systems. Numerous investigations are underway to develop new strategies to synthesize medium to large peptides with high purity. A number of approaches for the rapid and stepwise synthesis of many peptides have been reported[6-8]. Synthesis of biologically active peptides for clinical purposes requires a

strategy to enable a side product free synthesis of every intermediate and allows analytical procedures for control of reactions and purity of sequential peptides. It is impossible to carry out peptide synthesis as a mere routine because of undesired side reactions during synthesis. Hence it is absolutely necessary to recognize and to understand such undesired reactions and to know how to tackle them.

Amino acids contain several different reactive groups: a carboxyl group, an amino group and often another functional group on the side chain. In the usual protocol, a number of operations like protection, activation, coupling and deprotection are necessary for the incorporation of each amino acid residue in the peptide chain. Thus the formation of even short and medium sized peptide chain is a laborious procedure which involves considerable time and effort. To prevent unwanted combinations in the peptide synthesis, during the amide bond formation between aminoacid molecules, one of these groups must be protected. Anyhow, peptide bond formation from carboxyl function poses problems. Therefore, spontaneous formation of amide bond requires activation of one of these functional groups. Activation of amino component is not an easy task. Hence carboxyl component has to be activated and this activated carboxyl group leads to coupling (Scheme **1**) by nucleophilic attack of the amino group. Further additions to the peptide chain must be proceeded by this method of protecting and deprotecting the various amino acid groups.



Scheme 1
Synthesis of a peptide

The amino protecting group[9-12] functions in such a way that it decreases the basicity and nucleophilicity of the nitrogen atom, preventing protonation and acylation. It also makes the α-carboxyl group free from its zwitterion for subsequent activation. In a similar manner, the amino group of the amino acid to be acylated, should also be freed from the zwitterion state by a proper carboxyl protecting group in order to recover the nucleophilic character necessary for the reaction. In the case of polyfunctional amino acids, used in peptide synthesis, their side chain functions must also be selectively blocked to prevent undesirable side reactions.

The terminology "activation of carboxyl component" means the augmentation of the electrophilic character of the carboxyl carbon atom. In the carboxyl group, the already low electron density of this carbon is further decreased by the electron

withdrawing inductive effect of the activating substituent. The resulting electrophilic centre of an amino acid easily promotes the attack of the nucleophilic amino group of another molecule to form a peptide bond.

The activation of carboxyl group by converting to azides,[13] acid halides,[14] mixed anhydrides,[15-18] and active esters achieve high reactivity of carboxyl moiety towards nucleophilic attack. At the same time, the rigorous conditions such as the use of base, temperature etc. make the synthesis much more troublesome. Hence activation of carboxyl group with a mild precursor is a real problem and to overcome this task a number of activating groups have been reported in recent years.

The principles of organic photochemical reactions are also being effectively exploited in designing photochemically removable and activating groups for synthetic purposes. Photochemical methods usually provide facile, mild and specific means of activation of functional groups under different conditions. These photochemical activation approaches have been offered effective synthetic methods for peptides[19,20], macrolides[21,22] and carbohydrates[23,24].

The classical approach to peptide synthesis has yielded impressive successes, in recent years, in the preparation of several biologically active peptides. But these procedures, however, are not ideally suited to the synthesis of long chain polypeptides due to technical difficulties of purification and solubility, as the number of amino acid residues increases. Solid phase peptide synthesis introduced by R.B. Merrifield in 1963 overcomes some of these difficulties. In this method the first amino acid of the chain is attached to a solid polymer by a covalent bond, then the

succeeding amino acids one at a time in a stepwise manner were added until the desired sequence has been achieved, and finally the peptide formed is removed from the solid support. Here the growing peptide chain is firmly attached to a completely insoluble solid support so that it can be conveniently filtered and washed from reagents and by-products. This approach simplifies the manipulations and shorten the time required for the synthesis of peptides and also provides a route to the synthesis of high molecular weight polypeptides which are impossible by conventional methods.

Conventionally, activation of functional group is achieved by converting the acids to halides, azides, mixed anhydrides and active esters. Of course, in these methods high reactivity of carboxyl function towards nucleophilic attack is achieved, but rigorous conditions such as use of base, temperature etc. are required. Moreover, the electron withdrawing groups like halogen which enhances the electrophilicity of carboxyl carbon, increases the possibility of racemization. Recently heterocyclic thiols have been developed as excellent carboxyl activating groups.

Thus, the main objective here, is the systematic study on 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine as carboxyl activating groups. The aminolysis and esterification of N-acyl derivatives of 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine with various amines, alcohols and selective aminolysis with amino alcohols are carried out in order to verify the suitability of 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-

mercapto-4,6-dimethylpyrimidine as effective candidates for carboxyl group activation.

After providing a brief review on different carboxyl activating groups and their applications in chapter 2, the thesis deals with the activation of carboxyl groups using 3-mercapto-5,6-diphenyl-1,2,4-triazine in chapter 3. Here, apart from the synthesis and characterisation of 3-mercapto-5,6-diphenyl-1,2,4-triazine, the preparation of different N-acyl derivatives of the mercaptotriazine and their characterisation using different analytical and spectral techniques are also included. The aminolysis, esterification and selective aminolysis are the other parts of the study included in this chapter. Spectrophotometric monitoring of aminolysis and esterification reactions using different acyl derivatives are also discussed in chapter3.

Chapter 4 deals with the carboxyl activation studies using 2-mercapto-4,6-dimethylpyrimidine. The effective utilization of 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrmidine as carboxyl activating groups in peptide synthesis is the subject matter of chapter 5. Chapter 6 sums up the observations of the investigation and the conclusions that are arrived at. The references are given at the end of each chapter.

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DEVELOPMENTS AND APPLICATIONS OF DIFFERENT CARBOXYL ACTIVATING GROUPS

M.P. Rajan “Development of heterocyclic carboxyl activating groups and their applications in peptide synthesis” Thesis.Department of Chemistry, University of Calicut, 2003

Chapter 2

DEVELOPMENTS AND APPLICATIONS OF DIFFERENT CARBOXYL ACTIVATING GROUPS

2.1. Introduction

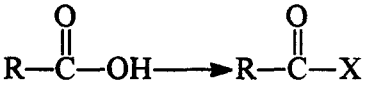
More than a century ago, the first synthetic peptide was reported by Curtius[1] and later by Emil Fischer. According to Hofmeister & Fischer the structure of proteins is best represented by chains of amino-acids linked to each other through amide bonds. This made peptide synthesis more pragmatic and synthesis of small, well defined peptides became indispensable for the study of the specificity of proteolytic enzymes. Thus with the discovery of biologically active peptides, the objectives of synthesis underwent a dramatic change.

In later years, formation of the peptide bond through acid azides and acid chlorides became well established but a general approach for the peptide synthesis was not available. The synthesis of oxytoxin by du Vigneaud and his associates[2], in 1953, gave the first significant accomplishment to the development of peptide synthesis. Later, adaptation of mixed anhydrides[3-5] for peptide bond formation, the introduction of active esters[6-8] and the discovery of coupling agents[9,10] followed each other in rapid succession to rejuvenate the peptide synthesis. Remarkable developments have been made during the last five decades and search for improvements in peptide synthesis is still continuing. Now there is a rich choice of procedures that make the synthesis of peptides, of any kind, possible.

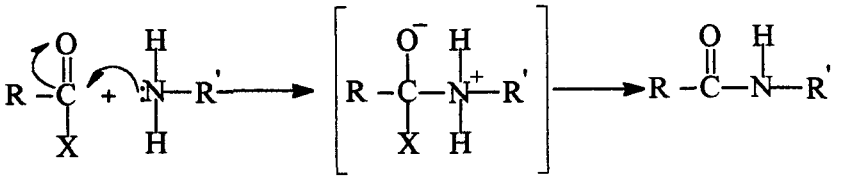
2.2. Carboxyl group activation

The basis of the peptide synthesis is the formation of amide bonds that couple amino acids. Two amino acid molecules unite together through an amide linkage ie. by a peptide bond to yield a dipeptide with the simultaneous elimination of water molecule, which is an example of common condensation reaction. This reaction has an equilibrium that favours reactants rather than products. To make the reaction thermodynamically more favourable, the carboxyl group must be chemically activated. So far no practical method is available for the activation of the amino component, therefore the carboxyl component has to be converted to a reactive form.

The activation of a carboxyl group mainly depends on the nature of the electron attracting substituent which diminishes the electron density on the carboxyl carbon. The electrophilic character of the carboxyl carbon has to be enhanced for the activation of the carboxyl group and hence the hydroxyl group must be replaced by an electron withdrawing substituent (X). Thus the facile nucleophilic attack of the amino group at room temperature is possible through the activation strategy. The course of activation and coupling of amino acids are as given in Scheme **1**.



Activation



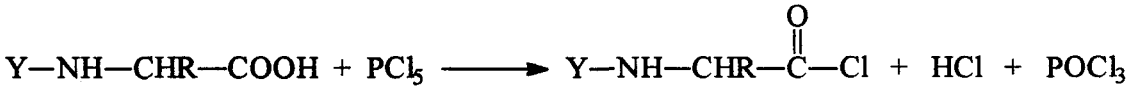
Coupling

Scheme 1

The different activation approaches commonly used are discussed here.

2.2.1. Acid chloride

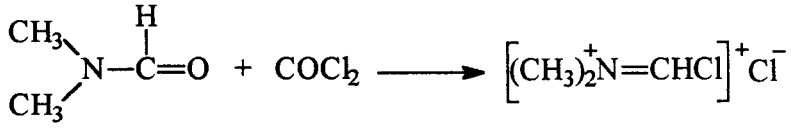
The acid chloride activation was first reported in 1903 by E. Fischer. Here, the carboxyl group of the amino acid **1** (partially blocked by the amino-protecting group Y) is converted to the chloride **2** by the treatment of phosphorous pentachloride, phosphorous trichloride or thionyl chloride[11].



1 **2**

As chlorinating agents Heslinga and Arenes proposed α-chlorovinyl ether and α,α'-dichlorodiethyl ether but Rieche and co-workers[12] suggested the more accessible dichloromethylether. Zoaral and Arnold[13] used another chlorinating agent, dimethyl

chloromethylene ammonium chloride **4** which could be readily prepared from dimethylformamide **3** in presence of phosgene.



3 **4**

However, the acid chloride method of activation is not without shortcomings. Obviously, the reactivity of acid chlorides is more than what is actually needed in peptide synthesis. Brenner defined this phenomenon as "overactivation". This creates highly reactive intermediates during the course of peptide synthesis. Such highly reactive intermediates can affect side chain functions, as in the case of asparagine in which carbonamide group **5** is converted to a nitrile **6** in the presence of chlorinating agents.

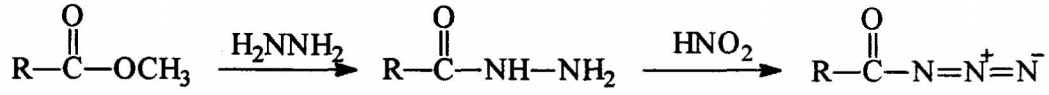
5 **6**

Therefore it is clear that not only the coupling reaction, but the process of activation also has to be carried out under mild conditions.

2.2.2. Acid azide

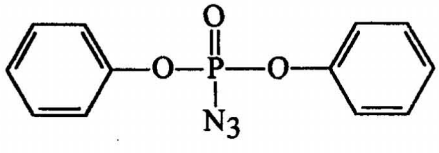
Acid azide was developed by Curtius[14] as a contemporary method to acid chloride method and is still widely used in peptide synthesis. Its unusual acceptance

is mainly due to the resistance of azide activated peptide derivatives to racemization. The azide method was, regarded as the only approach which allows retention of chiral purity during the coupling of peptides. Another universal advantage of azide method is the hydrazinolysis procedure in which the carboxyl group protected in the form of an alkyl ester **7**, is changed to an acid hydrazide **8**, which is then transformed to reactive azide **9**.



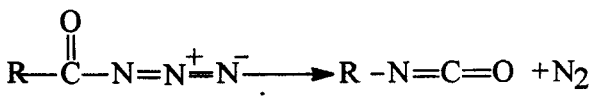
7 **8** **9**

Curtius method has been modified[15] usefully by replacing sodium nitrite with alkyl nitrite. Later, a very effective method[16] has been reported using diphenyl phosphorazidate (**10**) which could convert to azide.



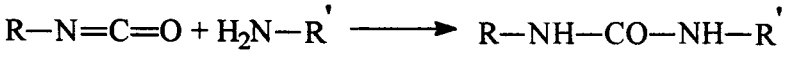
10

An important drawback of the azide method is Curtius rearrangement by which the azide gets converted to more reactive isocyanate **11**.



9 **11**

The isocyanate thus formed reacts with amine to form urea derivative **12** which resembles the desired peptide and it can be separated only with difficulty.



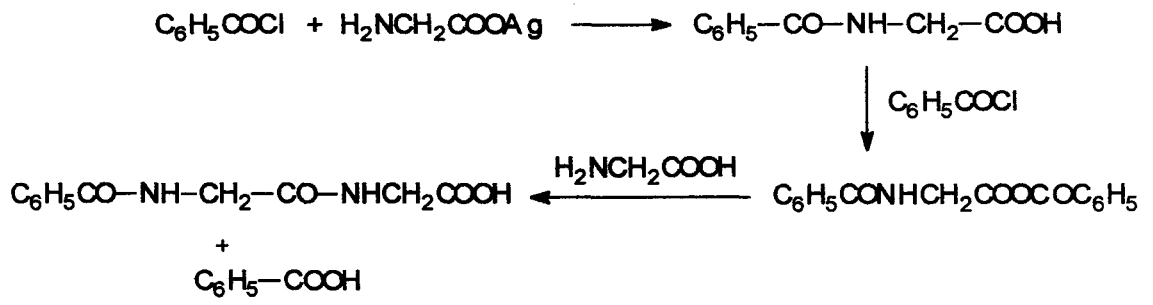
11 **12**

To reduce the extent of Curtius rearrangement, azide preparation and coupling is carried out at low temperatures and at high concentration of reactants. Another inherent problem associated with azide method is the slow peptide bond formation, which takes several days to complete when it is carried out at low temperature. In spite of all these shortcomings, azide coupling is one of the good methods in peptide synthesis.

2.2.3. Acid anhydride

In search for new acylating agents, one of the most efficient methods developed again by Curtius was using acid anhydrides. According to him, he could isolate a dipeptide in an unexpected side reaction, i.e., during the preparation of hippuric acid. The reaction product reacted with unconsumed acid chloride to form a

mixed anhydride[17] which was attacked by glycine and produced the unexpected dipeptide derivative benzoylglycinylglycine (Scheme 3).

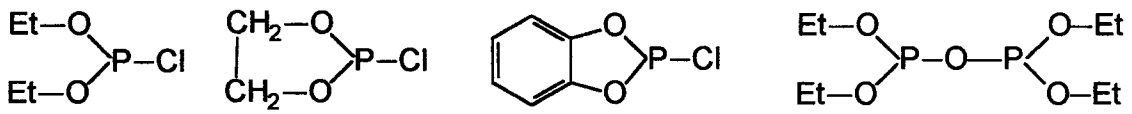


Scheme 3

The most common difficulty associated with the mixed anhydrides was the formation of undesired acylation product, which was evidenced from a number of acylations carried out using symmetrical and unsymmetrical anhydrides. Symmetrical anhydrides were used to tackle this problem. Nonapeptides have been efficiently synthesised using symmetrical anhydrides without isolating and identifying the intermediates.[18] The symmetrical anhydrides of *t*-butoxycarboxyl amino acids were used conveniently to synthesise peptides on polymeric carrier.[19] Easy regeneration of the excess anhydride is an advantage here. Using symmetrical anhydride a linear dipeptide, corresponding to antamonide, has been synthesised in very high yield.

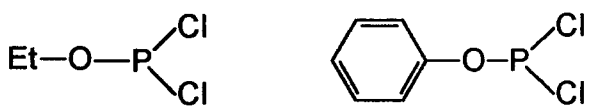
Anderson and co-workers[20] introduced mixed anhydrides of protected amino acids with derivatives of phosphorous acid. Such effective reagents are the chlorides

of diethylphosphite (**13**), ethylenechlorophosphite (**14**), *o*-phenylenechlorophosphite (**15**) and tetraethylpyrophosphite (**16**).



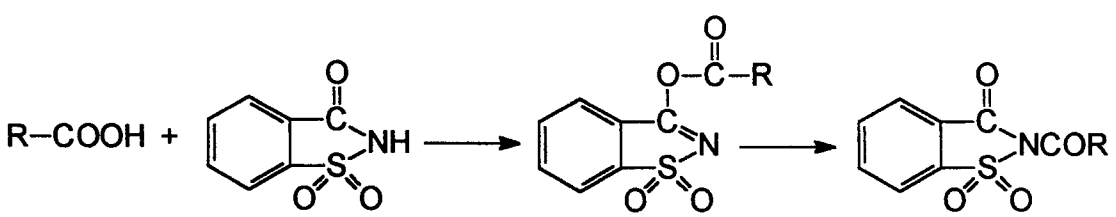
13 **14** **15** **16**

Ethyldichlorophosphite (**17**)[21] and phenyldichlorophosphite (**18**)[22] are new addititions in this class.



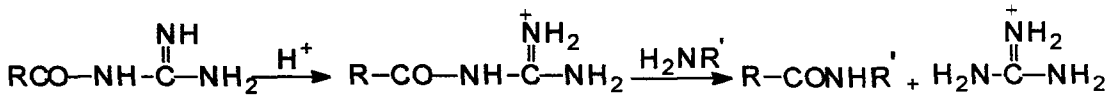
17 **18**

A novel application of saccharin (**19**)[23] is its use in the synthesis of mixed anhydrides with acylamino acids.



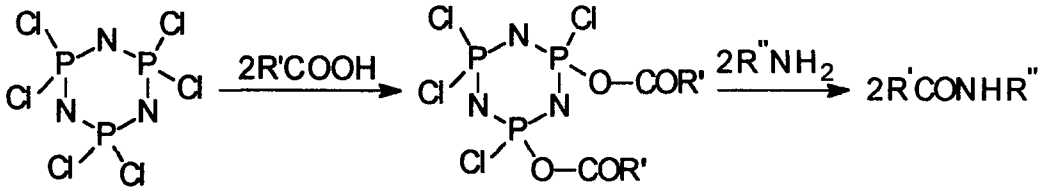
19 **20** **21**

The use of acyl guanidine for the synthesis of peptide is gaining importance.[24] In this case, acyl guanidine (**22**) reacts fairly effectively with amines, forming amides **23**.



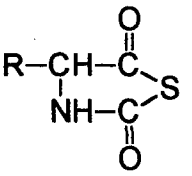
22 **23**

A very significant application of mixed anhydride in peptide synthesis is the use of hexachlorocyclotriphosphatriazine (**24**), which can react with two molecules of acylamino acid to form an unusual mixed anhydride **25**.



24 **25**

The application of internal anhydrides like N-carboxy anhydrides which are readily prepared from amino acids by treatment with phosgene[25] proved to be very effective in peptide synthesis. For amino acids like histidine and glycine, it has been observed[26,27] that thio analogues of N-carboxy anhydrides (thiazolidine-2,5-diones) (**26**) are very useful.



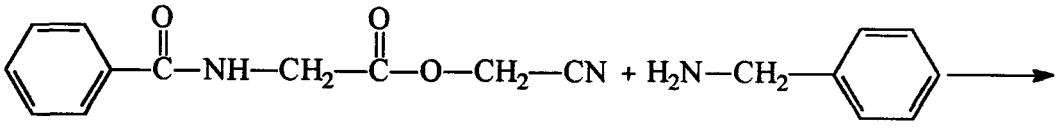
26

The N-carboxy anhydride method has been used to synthesis peptide fragments consisting 4-8 amino acid residues.

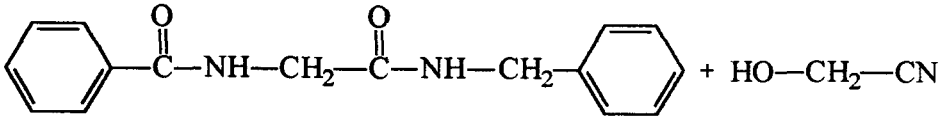
2.2.4. Active esters

Carboxyl group activation can also be achieved effectively by the conversion of protected amino acids to their reactive esters.[28] Only if a single electrophilic center is present in the acylating agent, unequivocal coupling is achieved. Since there are two electrophilic groups in mixed anhydrides, the requirement of an ideal acylating agent cannot be satisfied. The requirement of unequivocal coupling is fulfilled in acid chlorides and also in acid azides but the activated intermediates are prone to side reactions such as racemization in acid chlorides and Curtius rearrangement in acid chlorides and acid azides respectively. The added advantage in esters is that of the leaving group. In active ester method, the reaction is fairly fast and depends on the nature of the leaving group. The contributions made by Curtius and Fischer show that esters of acylated amino acids and peptides represent 'energy-rich' intermediates whose chemical and optical purity can be tested before hand and they show parallel to the natural amino acids in protein biosynthesis.[29]

Simple esters like methyl or ethyl esters undergo aminolysis, but amides form very slowly. The reaction rate can be increased at elevated temperature or by the use of excess amine. Schwyzer and his co-workers[30,31] investigated the aminolysis of a series of modified methyl esters, each carrying a different electron withdrawing substituent on the methyl group. Thus the reactivity of the cyanomethyl ester (**27**) tested with benzylamine (**28**) as the nucleophile, was found to be fairly sufficient for application in peptide synthesis:

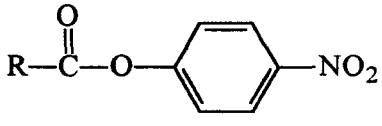


27 **28**



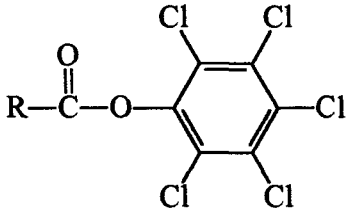
29 **30**

Among active aryl esters, *o*, *m* & *p*-nitrophenyl esters[32] have been studied in detail and out of them para derivatives **31** were selected as most effective[33] in peptide synthesis. At the same time, Farrington and coworkers proposed the application of *p*-nitrophenyl thioesters, and found them to be potent acylating agents.



31

In subsequent years, a number of aryl esters having electron withdrawing substituents have been introduced. Out of these, the pentachlorophenyl esters (**32**)[34,35] drawn much attention due to its high reactivity, but the steric hindrance of the bulky activating groups poses problems. Anyhow, replacement of chlorine by fluorine[36] produced highly potent active ester with less steric hindrance, which can retain its reactivity in the matrix of peptidyl polymers. A number of peptides including oxytocin, corticotropin and antamocide have been synthesised using active ester **33**.



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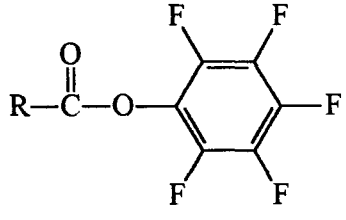
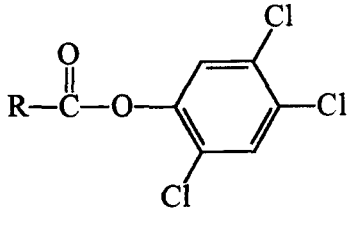


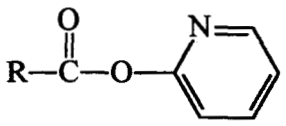
33

Later, the extensive study made by Pless and coworkers[37] showed that 2,4,5-trichlorophenyl esters (**34**) were excellent in reactivity owing to its less steric hindrance than pentachlorophenyl esters.



34

Esters of 2-hydroxypyridine (**35**) and 2-mercaptopyrimidine[38] (**36**) were recognised as highly reactive and the desired peptides were obtained in good yield.



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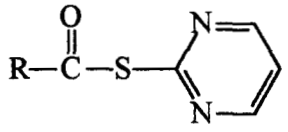
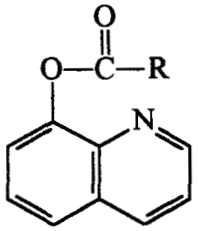


36

Activated esters whose reactivity could be explained by intramolecular base catalysis have drawn much attention. Esters derived from 8-hydroxyquinoline (**37**)[39] were considered as the most important ones. Other prominent esters were based on 2-mercaptopyrimidine (**38**) and 1-hydroxypiperidine (**39**).[40-42]



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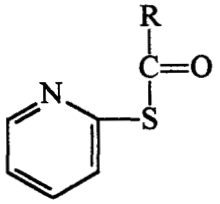

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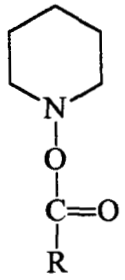
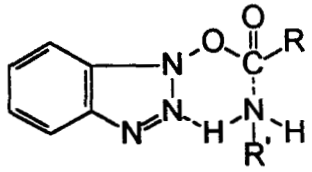


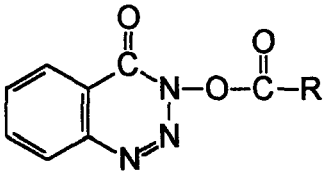
39

Esters of 1-hydroxybenzotriazole (HOBT) **40** were used as very good acylating agent and their reactivity is attributed to anchimeric assistance.[43]

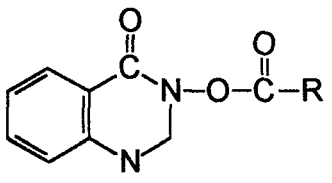


40

It should also be mentioned that the O-acyl derivatives of 3-hydroxy-3,4-dihydrobenzotriazine-4-one (**41**) and 3-hydroxy-3,4-dihydroquinazoline-4-one (**42**) were applied for the catalysis[44, 45] of otherwise moderately reactive aryl esters.

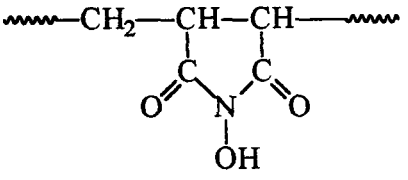


41



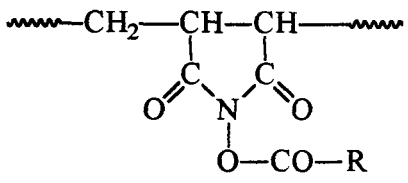
42

Later, Bodansky observed the effectiveness of tetrazole[46] as catalyst to accelerate the rate of aminolysis (in dimethylformamide in the presence of a base) with active esters. The copolymerisation of ethylene with maleic anhydride followed by the treatment with hydroxylamine yielded a polymer **43** resembling to N-hydroxysuccinimide. Activated esters obtained from polymers containing N-hydroxyimide groups were highly promising in the hands of peptide investigators.



43

To produce an activated polymeric ester **44**, N-protected amino acid was added to the above polymers with the aid of carbodiimide.



44

Polymeric activated esters are convenient because they obviate the need for the procedure involving the removal of excess activated ester and the ester component. Moreover, they exhibit good acylating activity similar to that of the esters obtained from N-hydroxysuccinimide.

2.3. Photochemical Methods of Activation

Advanced techniques of organic photochemical reactions are being advantageously exploited in designing removable photosensitive protecting groups and also, recently, in achieving photochemical means of activation of functional groups. These photochemical activation methods are now being properly utilized in the synthetic strategies of different classes of biopolymers and other complex natural products.[47]

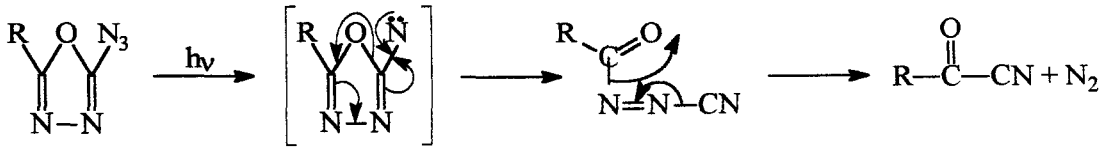
The basic advantage of the photochemical reactions is that it provides convenient methods for activation of carbonyl, hydroxyl and carboxyl functions under mild neutral conditions. In the photochemical activation methodology, the functional group is derivatised with a light sensitive chromophore, which can serve as a latent activator of the functional group.[48] Here the functional group is converted to an active form, on irradiation with light of suitable wave length, and the light

sensitive chromophores are removed. The active species possessing enhanced reactivity at the functional group towards the desired reaction permits synthesis under mild neutral conditions. These activation methods provide effective synthetic approaches under mild neutral photochemical conditions for peptides[49], macrolides[50,51] and carbohydrates[52,53].

In peptide synthesis, photochemical reactions have wide application in which photolabile groups are used as protecting as well as activating groups and also as handles of polymeric support.[54-57] The present review, comprises the different photochemical activation approaches covering the action and advantages of different photolabile carboxyl activating groups employed in various synthetic methodologies.

2.3.1. 5-Azido-1,3,4-oxadiazoles

Confalone and Woodward[58] put forward a promising approach to synthesise peptides on the basis of photochemistry of 2-substituted-5-azido-1,3,4-oxadiazole (**45**). Here, in the heterocyclic system[59] the carboxyl function of a suitably protected amino acid was incorporated by a five step reaction starting from the corresponding acid hydrazide. On irradiation, these 1,3,4-oxadiazoles afforded, the activated acyl cyanide **48** corresponding to the carboxylic acid, with the extrusion of two molecules of nitrogen.

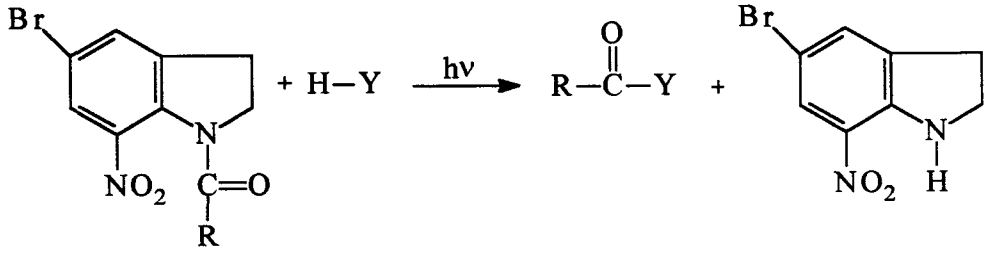


45 **46** **47** **48**

When this 2-substituted 5-azido-1,3,4-oxadiazole was irradiated in the presence of nucleophiles, the reactive acyl cyanide with the nucleophile was resulted which shows that on irradiation in the presence of alcohols and amines corresponding esters and amides are being produced accordingly.

2.3.2. Nitroindolines

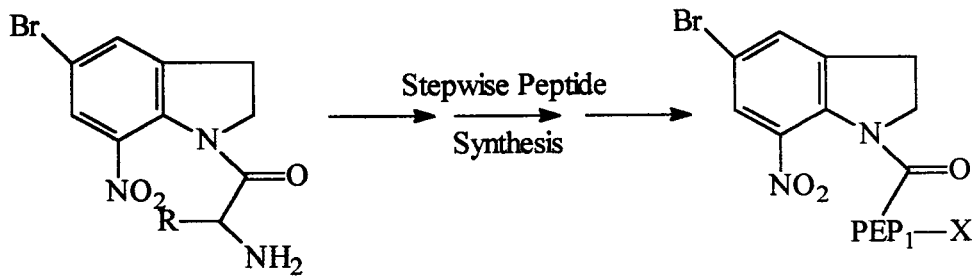
The photochemically generated activated carboxyl group was introduced by Amit, Dass and Patchornik[60] with a view to formulating racemisation-free coupling of peptide segments. Unusual photochemical properties of 5-bromo-7-nitroindolyl (Bni) group[61] was the basis of this condensation. It was found that N-acyl derivatives of 5-bromo-7-nitroindoline (**49**) and aromatic or aliphatic acids undergo efficient photosolvolysis to yield acids, esters or amides depending upon the nucleophiles present during irradiation.[62] Irradiation of **49** at 420 nm or below, activates the acyl function towards the nucleophilic attack. In the presence of water, this results in photolysis of the amide bond with quantitative formation of a free carboxylic acid and 5-bromo-7-nitroindoline (Bni) (**51**).



49 **50** **51**

Carboxylic acid derivatives were produced by a unique photoacylation reaction of compound **49**, on irradiation in the presence of other nucleophiles,. Thus it is clear that Bni group can be used to protect the carboxyl function as well as to activate it upon irradiation towards the attack of nucleophiles.

The above reaction was successfully utilized by Amit *et al.* for the photochemically activated coupling of peptide segments in which Bni group was used to protect the carboxyl function in addition to the activation on irradiation. The Bni group initially may be used to block the c-terminus during the stepwise synthesis of peptide segment **53** and finally to couple this segment photochemically to a second segment as shown below.



52 **53**

$$\xrightarrow[h\nu(420\ nm)]{H_2N\text{—}PEP_1\text{—}Y} X\text{—}PEP_1\text{—}\overset{\overset{O}{\|}}{C}\text{—}NH\text{—}PEP_2\text{-}Y + \mathbf{51}$$

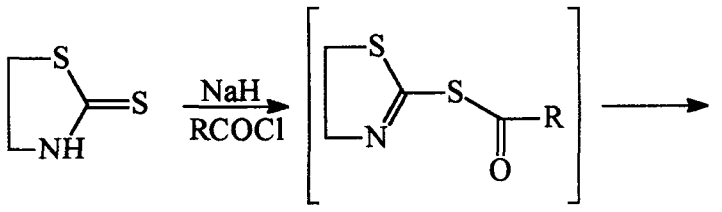
54

PEP_1 & PEP_2	:	Different peptide segments
X, Y	:	Protecting groups

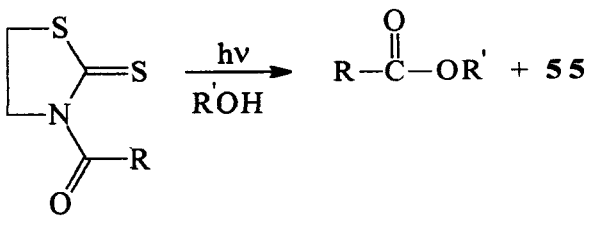
The Bni function is a unique example of a group which can be used for both protection and activation of the terminal carboxylic function of peptide segments. Moreover, the change in the mode of protection to activation requires no additional chemical manipulations. The optical purity of the products obtained from the photochemically activated coupling was also found to be very high.

2.3.3. Thionothiazolidines

Burton and White[63] had established the photochemical activation of carboxyl group through N-acyl derivative of 2-thionothiazolidine (**55**). Thus, photolysis of the N-acyl derivatives of **55** in the presence of ethanol produced the corresponding ethyl esters. The N-acyl derivatives were prepared by the treatment of the sodium salt of **55** in THF with the corresponding acyl chloride in benzene. The kinetic product[64,65] in this reaction was the S-acyl derivative **56** which actually undergoes a facile S → N thermal rearrangement to yield the N-acylated product **57**.



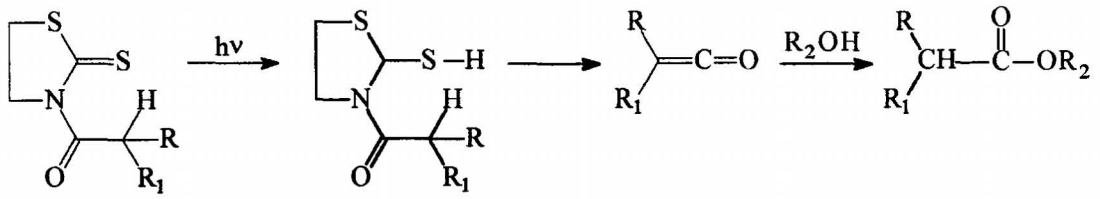
55 **56**



57 **58**

The acylated 2-thionothiazolidines, which are quite stable towards acidic and basic reagents, on irradiation with a 450 W mercury lamp, undergo cleavages of the acyl substituent and the parent 2-thionothiazolidines **55** was recovered in quantitative yield. But irradiation in ethanol produced the corresponding ethyl ester, with an efficiency which is markedly dependent on the nature of the R substituent.[66] Aryl derivatives afford good yield, whereas aliphatic systems give only poor yields.

The mechanism (scheme **4**) involving abstraction of α-hydrogen by the sulphur atom was postulated for this photochemical activation process[67]. This was analogous to the mechanistic pathway for the photolysis of *o*-phenylethylthiobenzoates investigated by Burton and coworkers[68] and this activation methodology, has now been extended to the synthesis of a number of amides and peptides[69,70].

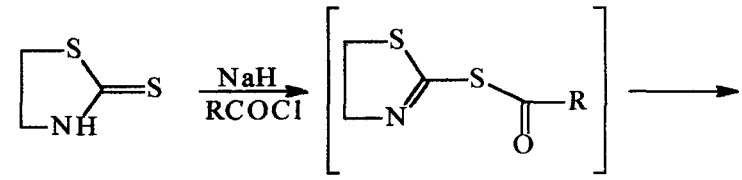


59 **60** **61** **62**

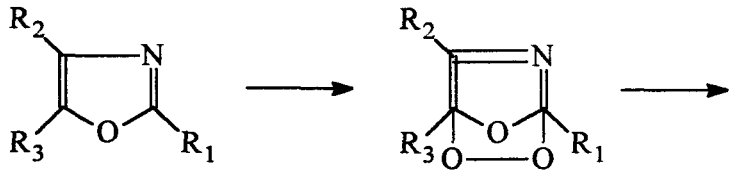
Scheme 4.

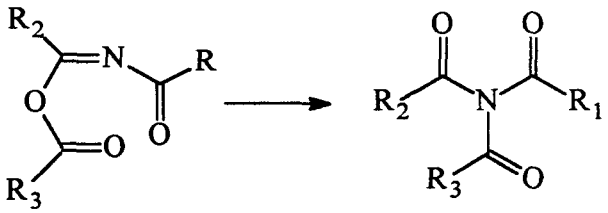
2.3.4. Activation *via* oxazole-triamide rearrangement

Wassermann and coworkers[71] observed that oxazole system may serve as a protecting and activating group for the carboxyl function. They formulated this from the observations that the conversion of oxazoles **63** to triamides **66** by the action of singlet oxygen, effectively transforms each of the carbon atoms in the oxazole ring to a carboxylate derivative (Scheme 5).



63 **64**

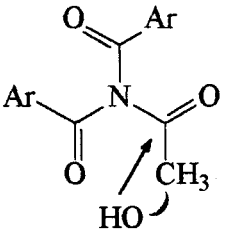




65 **66**

Scheme 5

Thus, with triamides formed from 2-alkyl-4,5-diphenyloxazoles, on photooxygenation undergo selective nucleophilic attack at the acyl carbonyl group derived from the carbon at 2-position of the oxazole ring, where the steric hindrance is the least as shown in **67**.



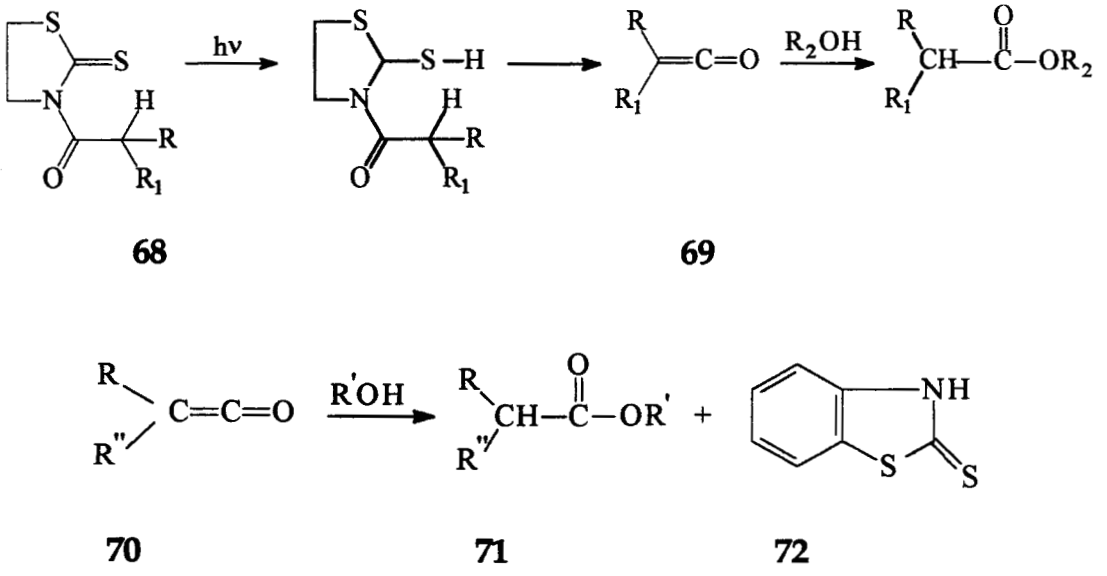
67

The oxazole group considered to be an excellent carboxyl protecting group since it is relatively unreactive towards hydrolysis in both acids and bases, is stable towards many types of oxidation and reduction procedures. It is also capable of yielding the activated carboxyl group under conditions of oxidation which do not affect most functional groups. Wassermann *et al.* exploited this selective reaction for the synthesis of macrocyclic lactones and peptides. In an N-acylated amino acid, the carboxyl group was protected by conversion to an oxazole derivative which on photooxygenation regenerated the carboxyl group in the activated triamide form of the peptide coupling. An intramolecular acylation process was used in the synthesis of lactones by the oxazole-triamide rearrangement.

2.3.5. Benzothiazoline-2-thione and benzoxazoline-2-thione

In this approach, carboxylic acids were derivatised[72] with benzothiazoline-2-thione. The acyl derivatives, thus obtained on irradiation with alcohols under neutral

photochemical conditions to form esters. As shown in Scheme 3, α-hydrogen abstraction by sulphur has been proposed[73] as the mechanism. It was also shown that 3-benzoyl benzothiazoline-2-thione, even on prolonged irradiation, with UV-visible light using different alcohols did not afford any benzoate. The failure of 3-benzoyl benzothiazoline-2-thione to undergo any photolytic cleavage with alcohols was attributed to the absence of α-hydrogen in the acyl functions (Scheme 6).



68 **69**

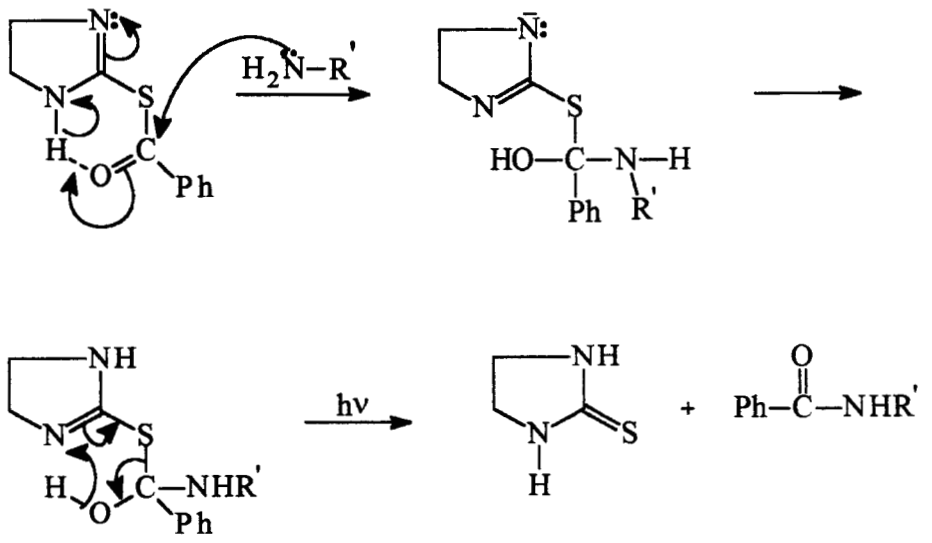
70 **71** **72**

Scheme 6. Photochemical activation of 3-acylbenzothiazoline-2-thione

2.3.6. 2-Mercaptoimidazol-2-ene

It has been reported[74] the photochemical activation of carboxyl function *via* 2-mercaptoimidazol-2-ene. On irradiation, the carboxyl group activated by 2-mercaptoimidazol-2-ene undergoes photolytic cleavage with light into an active form and the light sensitive chromophore has been removed.

Even without possessing α-hydrogen atom, the S-benzoyl-2-mercaptoimidazol-2-ene underwent facile aminolysis under photochemical conditions. In this case, the nucleophile can attack the slightly polarised carboxyl carbon, since its polarisation is enhanced by the intramolecular hydrogen bond with imidazole NH. The suggested mechanistic pathway is represented in Scheme 7.



Scheme 7

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ACTIVATION OF CARBOXYL GROUPS THROUGH 3-MERCAPT0-5, 6-DIPHENYL-1,2,4-TRIAZINE

M.P. Rajan “Development of heterocyclic carboxyl activating groups and their applications in peptide synthesis” Thesis.Department of Chemistry, University of Calicut, 2003

ACTIVATION OF CARBOXYL GROUPS THROUGH 3-MERCAPTO-5, 6-DIPHENYL-1,2,4-TRIAZINE

Chapter 3

ACTIVATION OF CARBOXYL GROUPS THROUGH 3-MERCAPTO-5, 6-DIPHENYL-1,2,4-TRIAZINE

3.1. Introduction

Research activities in peptides are growing at an unassuming pace, since synthetic peptides have emerged as a powerful tool for studying peptide or protein structures and their complexities. Recently biomedical research including molecular biology,[1,2] immunology,[3-5] pharmacology,[6,7] enzymology[8-10] and neurobiology[11,12] have established their importance. Synthesis of peptides, chemically confirms the structures of naturally occurring peptides and enable them to obtain in bulkier quantities for further investigation and in the preparation of artificial vaccines and new potent drugs. The goal of peptide synthesis principally lies with the formtion of amide bonds in which the union of amino and carboxyl groups is the key step. Activation and coupling of amino acids often results in overall loss of free energy which is achieved either by catalysts or by the use of activated functional groups. Till now, no practical approach is reported for the activation of amino group.

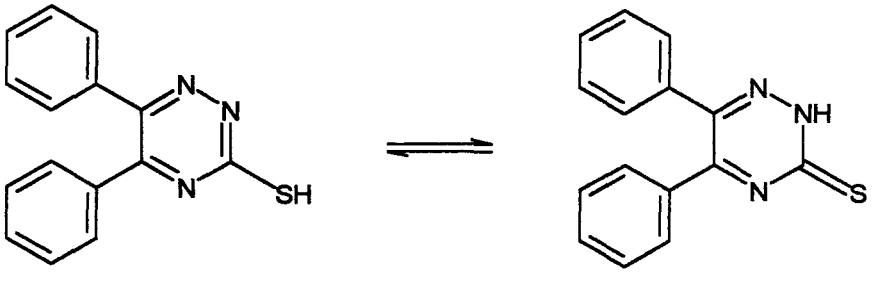
Carboxyl group activation which eventually leads to the peptide synthesis has the history of more than a century old and benzoyl glycine was the first activated synthetic derivative achieved. Afterwards the peptide bond formation by converting to azides and chlorides got established. Two decades later, in 1953 peptide synthesis got acceleration when oxytoxin[13] was synthesised by du Vigneaud and co-workers.

This was followed in rapid succession by the introduction of mixed anhydrides,[14-17] active esters[18-20] as carboxyl activating groups and also the discovery of coupling reagents[21,22] which gave an unprecedented impeteus to the development of peptide synthesis. Thus, it would be desirable to use amino acid derivatives with strong electron withdrawing groups making the carboxyl cation more prone to nucleophilic attack and thereby achieving high reaction rates at ambient temperature. Though, azides and acid chlorides were used to meet with this task, the over activation by halides and the possible loss of chiral integrity at the α-carbon asymmetric centre make them as weak choices for carboxyl group activation. However, conventional activating groups such as mixed anhydrides and active esters make the carboxyl moiety for better reactivity towards nucleophilic attack. Generally thiol esters are more reactive than esters towards nucleophilic substitution reaction. The relatively high reactivity is attributed to the better leaving group ability of the mercaptide than that of alkoxide. The facile cleavage of the acyl sulphur bond is considered as the basis for the important biological transformations, the best proclaimed example being coenzyme A, which is found in biological system functioning as an acylating agent.

Nowadays, a number of heterocyclic systems, having a thiol function have been proved to be effective in carboxyl group activation.[23,24] A number of new methods have been developed for the preparation of thiol esters. Active role of thiol esters in biochemical processes and their reactivity with various nucleophiles have led investigators to choose them as attractive synthetic intermediates in organic synthesis. Carboxyl group activated heterocyclic compounds possess enhanced

reactivity, which promotes synthesis under mild conditions. This has been found to provide effective synthetic methods for peptides,[25,26] macrolides[27,28] and carbohydrates.[29,30] In recent years there has been tremendous advance in the chemistry of heterocyclic compounds.[31] Most of them were found to be biologically active and are being used as herbicides,[32-36] cross linking agents in polymers,[37] dyes and in pharmaceuticals.[38-40]

Among the heterocyclic compounds triazines have drawn much attention as plant growth regulators,[41-46] disinfectants[47] and bleaching agents.[48] The facile formation of triazine thiols from easily available precursors prompted to exploit the utility of trazine as carboxyl activating group in organic synthesis. Thus the present chapter deals with the study of 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) which can easily be tautomerised to thione function **2'** to serve as a novel carboxyl activating group under mild conditions.



2 **2'**

The investigation concentrates mainly to:

i) the synthesis of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) and its characterisation using different analytical and spectral methods.

ii) derivatisation of compound **2** with different aliphatic and aromatic acids to yield N-acyl derivatives and characterisation of the derivatised compounds.

iii) aminolysis and esterification of N-acyl-5,6-diphenyl-1,2,4-triazine-3-thione with various aliphatic and aromatic amines, alcohols and selective aminolysis using amino alcohols and characterisation of the product formed to demonstrate the suitability of N-acyl-5,6-diphenyl-1,2,4-triazine-3-thione as mild carboxyl activating moiety.

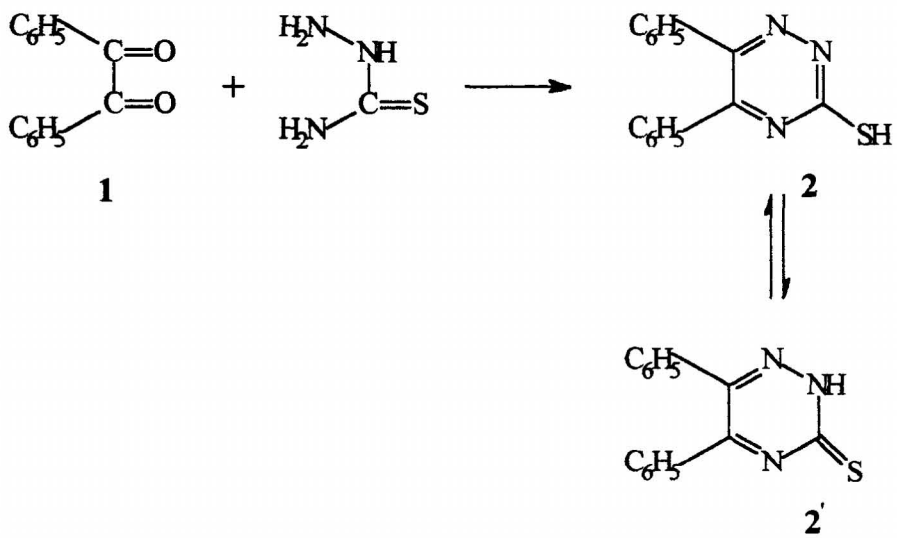
iv) spectrophotometric monitoring studies of aminolysis and esterification reactions using N-acyl-5,6-diphenyl-1,2,4-triazine-3-thione in order to give strong support to carboxyl activation.

3.2. Results and Discussions

3.2.1. Synthesis of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2)

1,2,4-triazine thiol derivatives are usually synthesised by condensing thiosemicarbazide with 1,2-dicarbonyl compounds.[49] Thus, synthesis of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was carried out by refluxing equimolar alcoholic solution of benzil and aqueous thiosemicarbazide. Ammonium acetate was then added and the mixture refluxed for 10 h. The slightly orange red colour developed initially became deep orange red after 1 h The formation of new product was

evidenced by tlc. After the completion of the reaction (10 h), the mixture was cooled and the crystals separated were filtered and recrystallised from ethanol to afford orange red crystals of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) in 95% yield. UV-visible spectrum in chloroform (**Fig. 3.1**) gave absorption band λ_{max} 321 nm at an absorbance value of 3.48. IR (KBr) spectrum (Fig **3.2**) of the compound **2** showed characteristic SH stretching frequency at 2360 (w) cm^{-1} and C=S stretching frequency at 1128 (s) cm^{-1}. It is clear from the above signals that two tautomeric structures are possible for the triazine thiol as **2** & **2'**. ^1H NMR ($DMSOd_6$) spectrum (Fig. **3.3**) gave two characteristic signals. The multiplet, at δ 7.5 - 7.2 ppm (10H,*m*) and a singlet for NH proton at δ 5.2 (1H,*s*).The mass spectrum (Fig. **3.4**) gave the molecular ion peak at m/z 265. The base peak occurs at m/z 178 for fragment $Ph—C\equiv C—Ph^{\dagger}$. The other prominent peaks at m/z 89, 76 and 51 could also be explained.



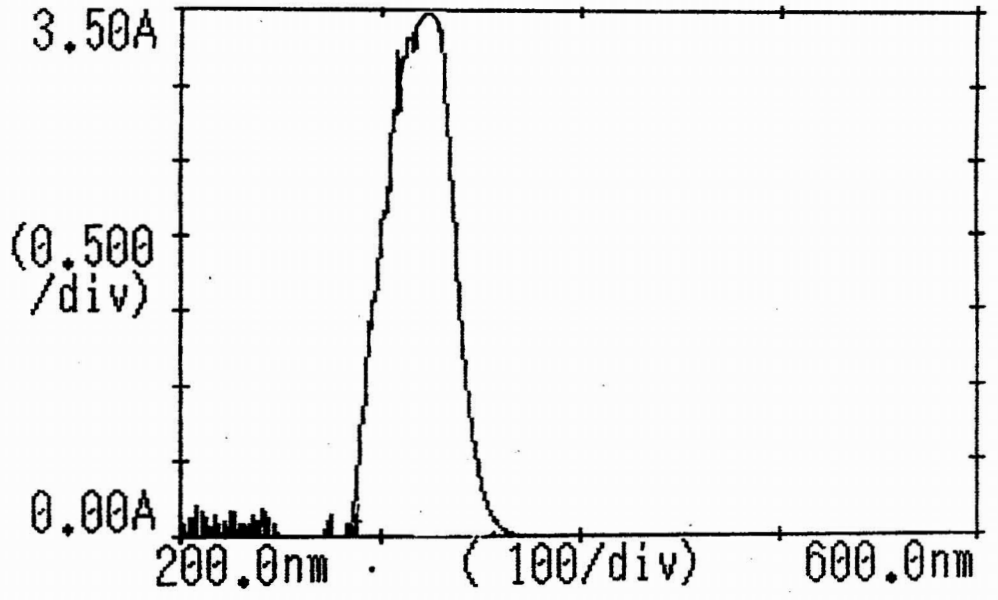


Fig. 3.1. UV-visible spectrum of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2) in chloroform

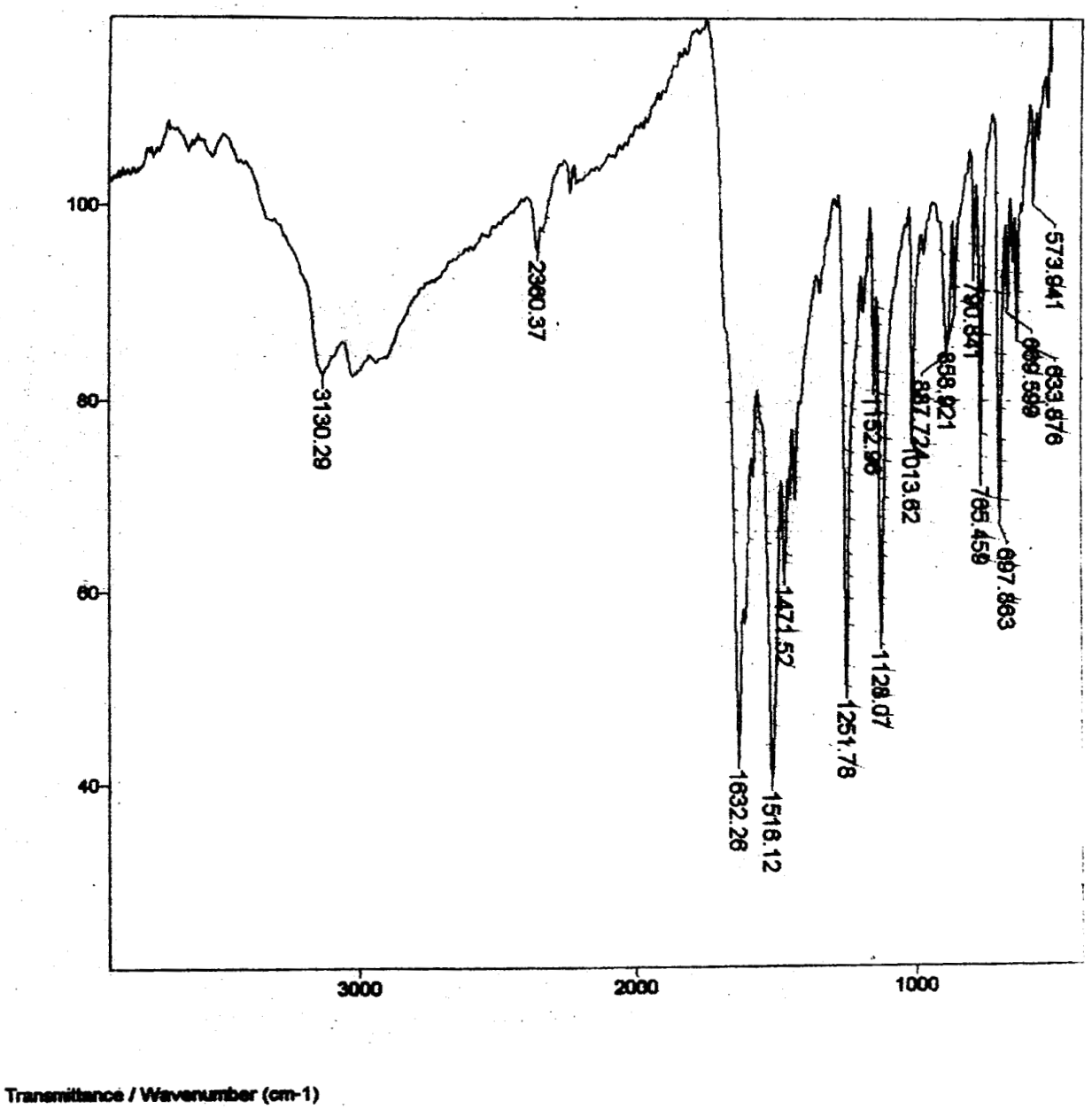


Fig. 3.2. IR (KBr) spectrum of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2)

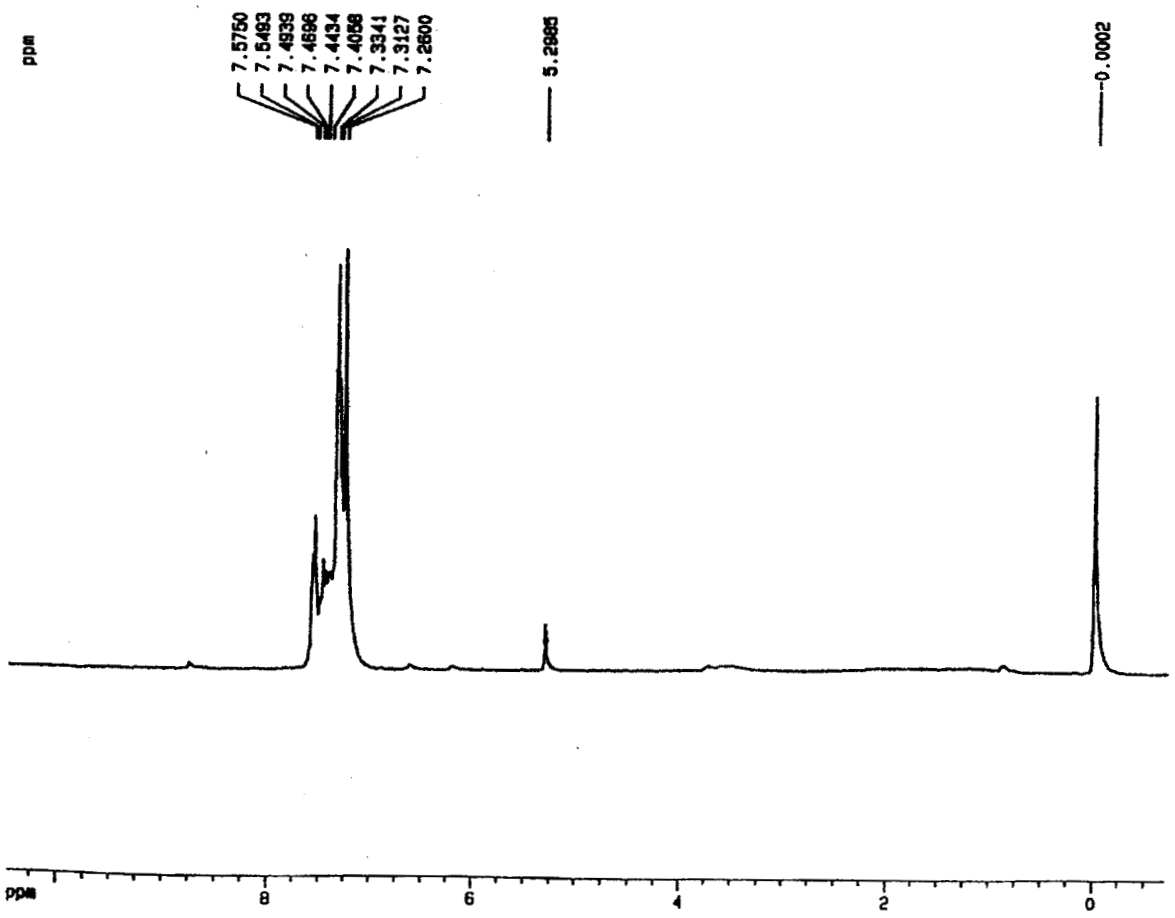


Fig. 3.3. ^{1}HNMR spectrum of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2)

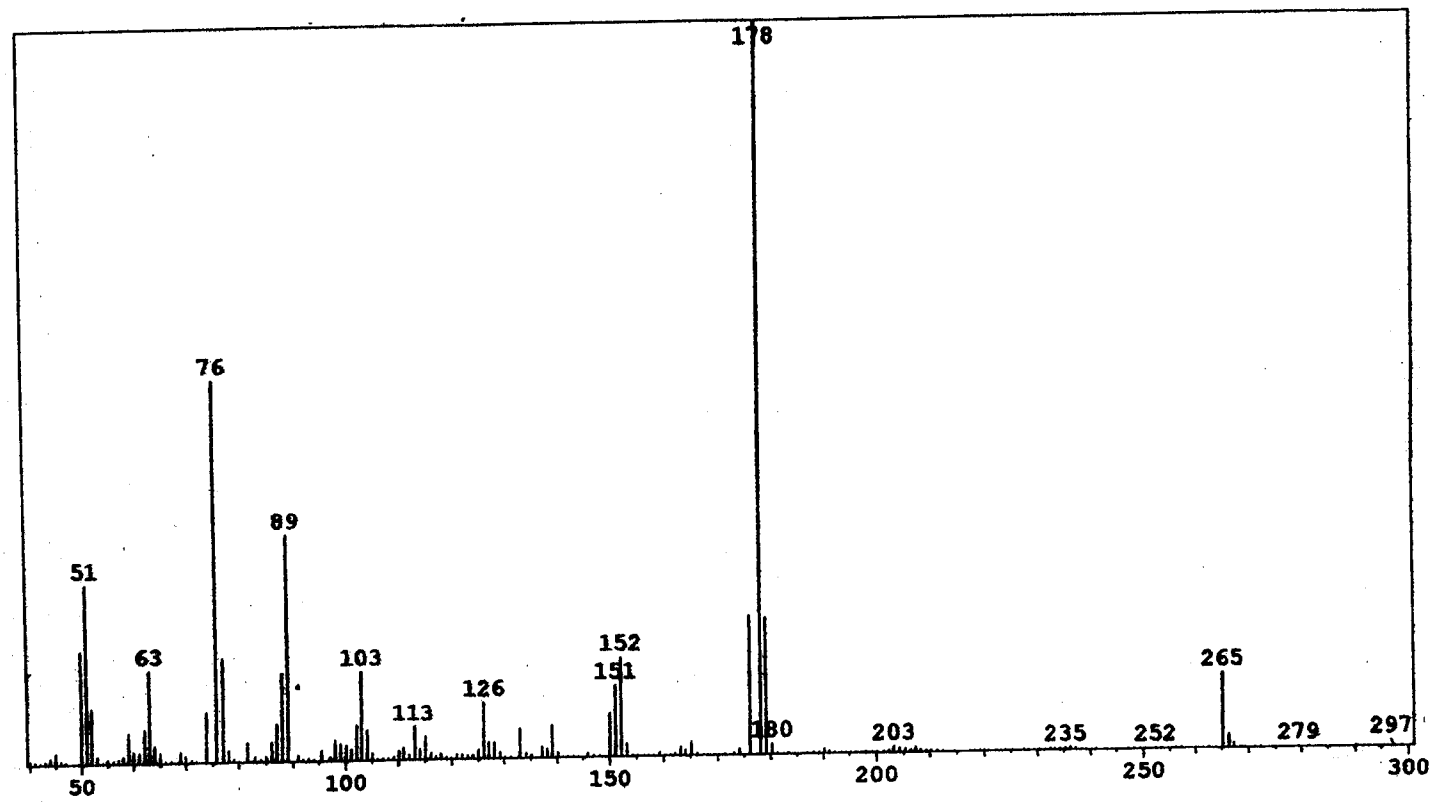


Fig. 3.4. Mass spectrum of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2)

3.2.2. N- Benzoyl -5,6-diphenyl-1,2,4-triazine-3-thione (3')

Schotten - Baumann method

The benzoylation of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was carried out by Schotten Baumann reaction. In this method 0.001 molar solution of (**1**) in 5% NaOH was shaken vigorously with 0.5 mL benzoyl chloride till the odour of benzoyl chloride disappeared. Benzoylation proceeds smoothly and the sparingly soluble benzoyl derivative separated as a solid. The solid benzoyl derivative formed was recrystallised from ethanol to get pale yellow crystals with mp 125°C in 85% yield, which was characterised as N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**3'**) by UV, IR, NMR and mass spectra. UV-visible absorption spectrum in chloroform (Fig. **3.5**) showed λ_{max} at an absorbance of 1.70. IR (KBr) spectrum (Fig. **3.6**) exhibits C=O stretching at 1707 cm^{-1} with the absence of SH stretching frequency. Stretching frequency at 1189 cm^{-1} may be due to the presence of C=S grouping. ^{1}HNMR spectrum (Fig. **3.7**) showed multiplets at 7.9 - 7.2 ppm (15H,*m*) indicates the presence of three phenyl groups. ^{13}CNMR spectrum (Fig. **3.8**) showed clearly the different types of carbon atoms present in the compound **3'**. Carbonyl group is indicated by the signal at 166. Mass spectrum (Fig. **3.9**) gave m^+ ion peak at m/z 369 The base peak found at m/z 178 and the peak at m/z 105 can be accounted as $Ph—C{\equiv}C—\overline{Ph}\,]^{+}$ and $C_6H_5—\overline{CO}\,]^{+}$ respectively.

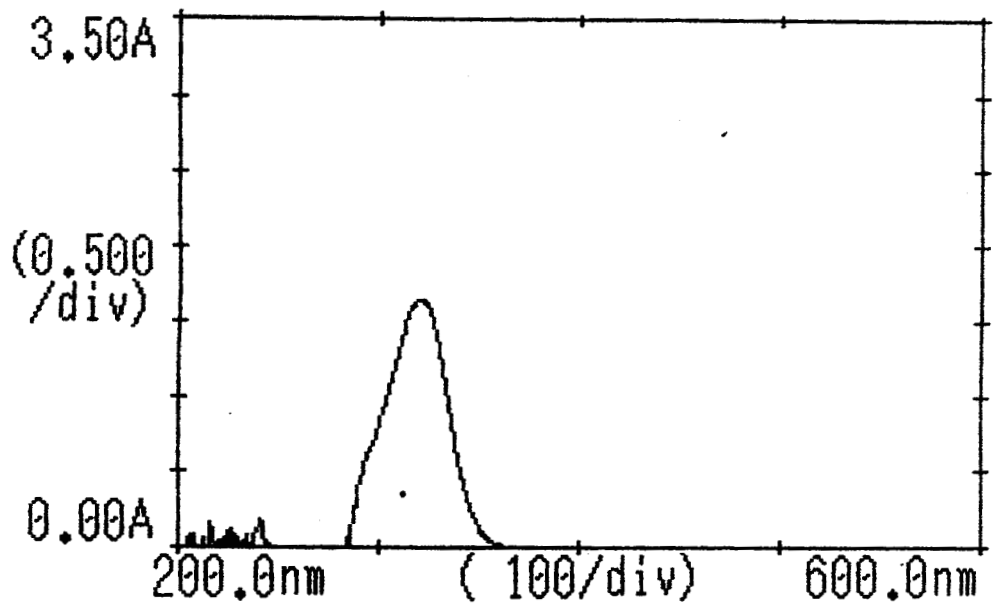


Fig. 3.5. UV-visible spectrum of N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (3') in chloroform

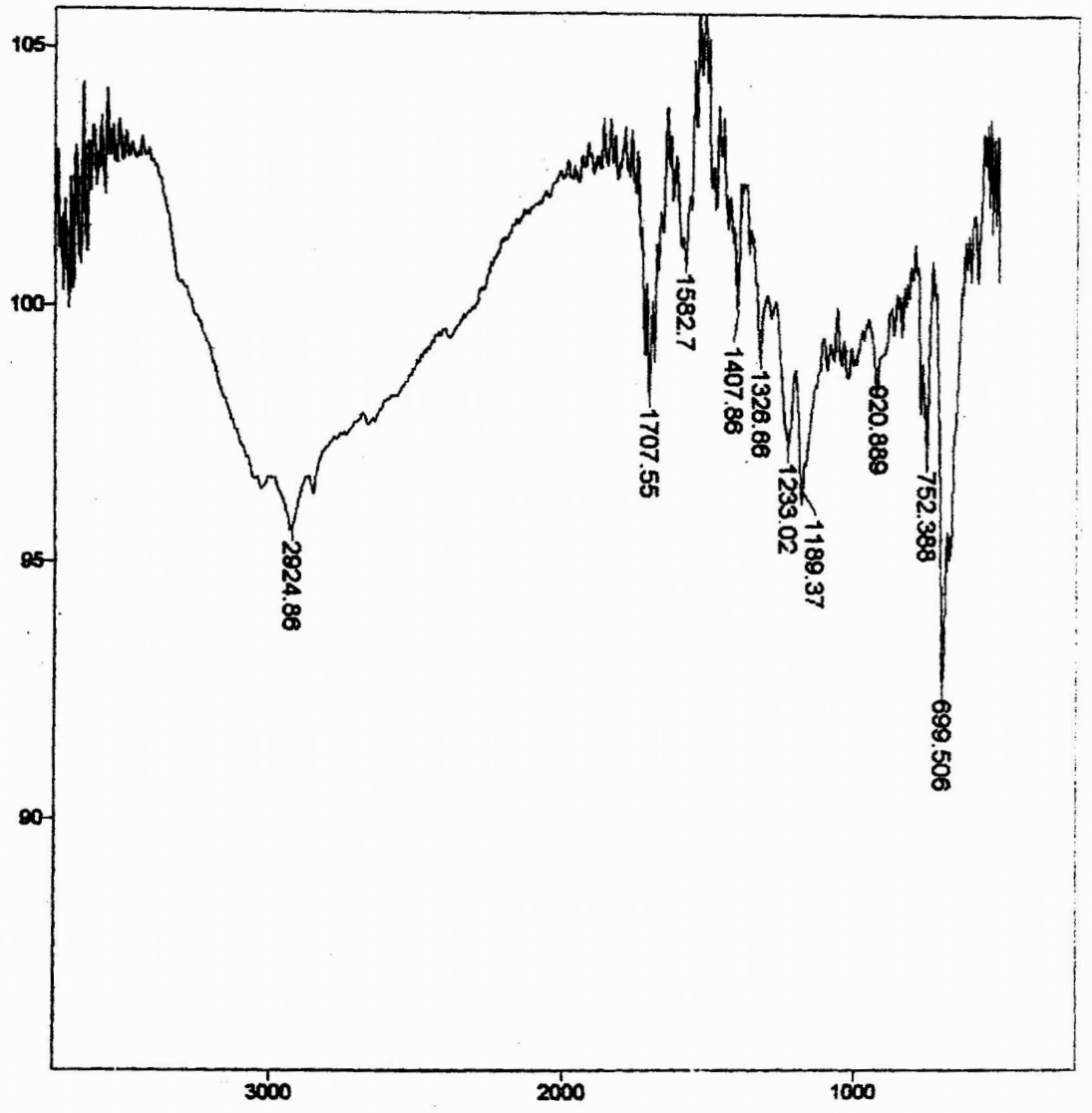


Fig. 3.6. IR (KBr) spectrum of N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (3')

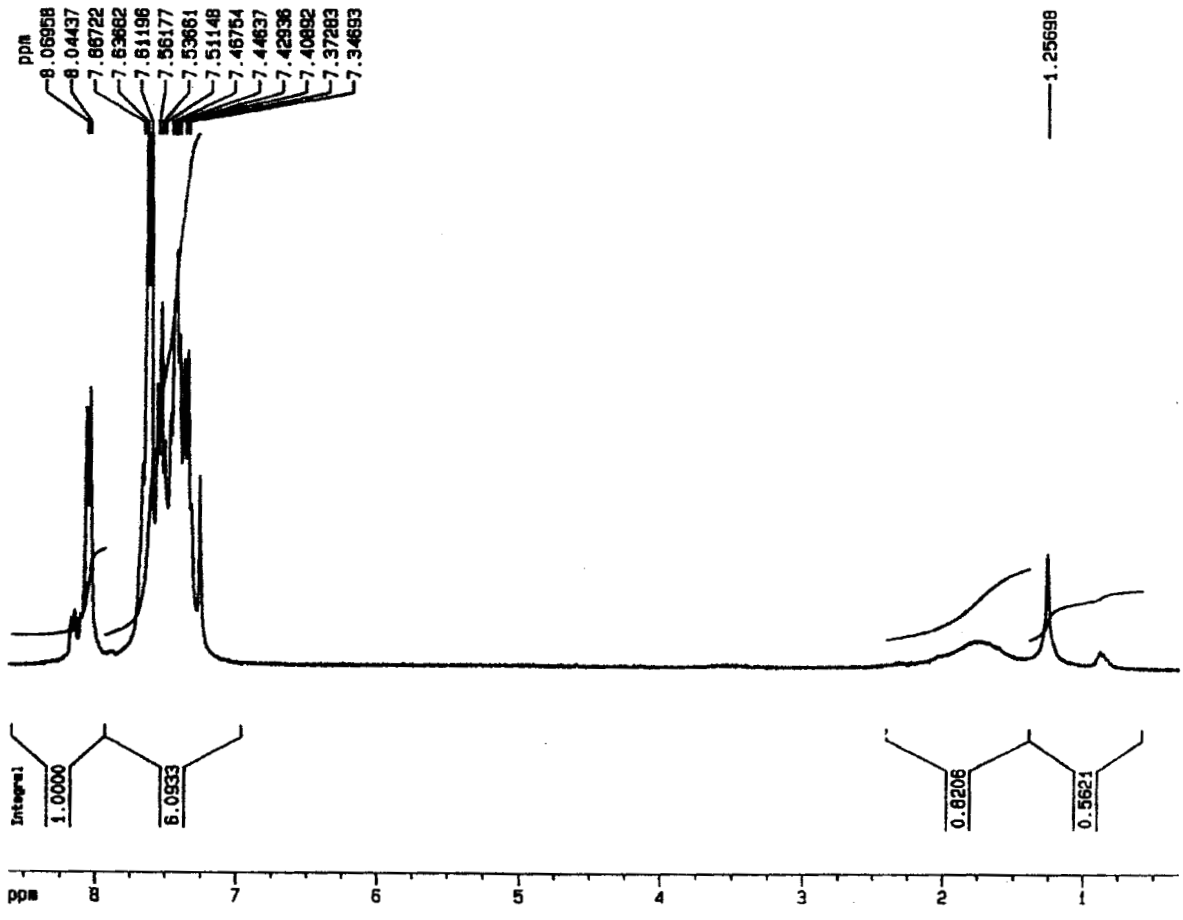


Fig. 3.7. ^{1}HNMR spectrum of N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (3')

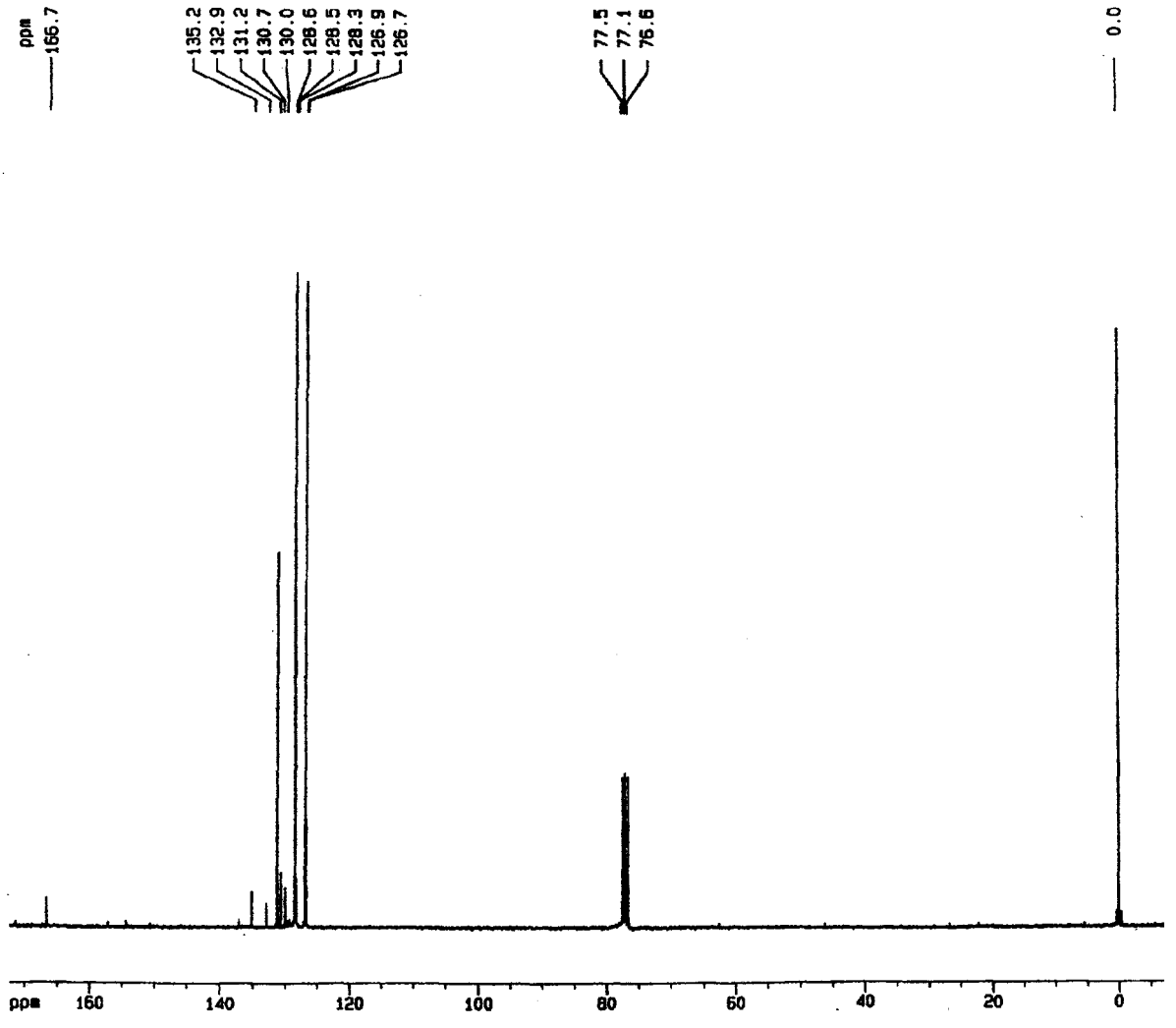


Fig. 3.8. ^{13}CNMR spectrum of N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (3')

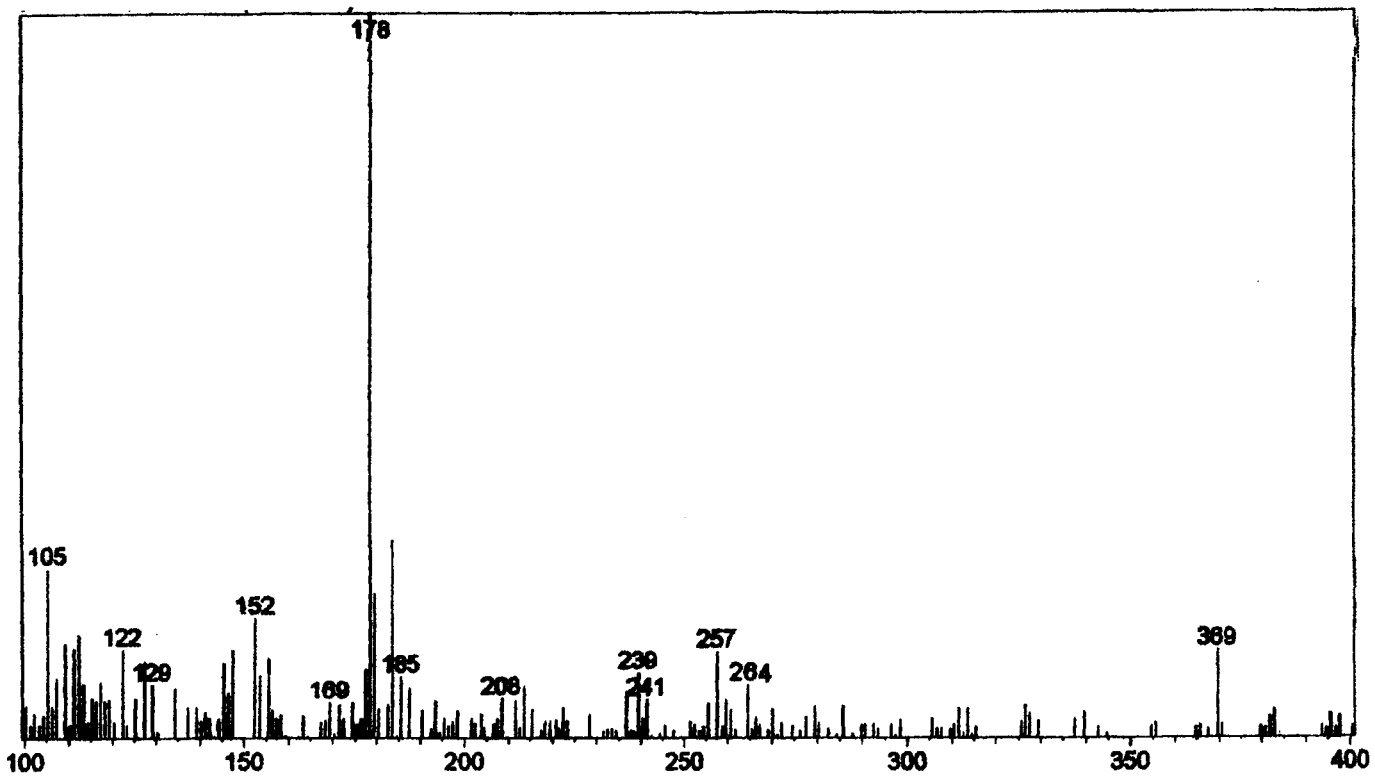
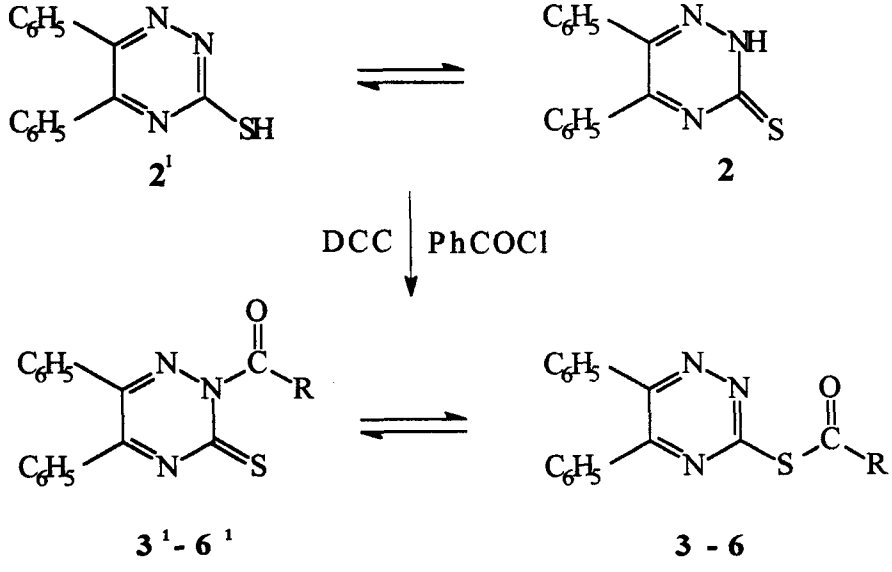


Fig. 3.9. Mass spectrum of N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (3')

DCC Coupling method

Benzoylation of (**2**) was also done by DCC coupling method.[50] Here an equimolar solution of benzoic acid and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) in THF & CH_2Cl_2 mixture (1:4), an equivalent amount of DCC in CH_2Cl_2 was added with constant stirring for 1 hour in an ice bath. DCU precipitated was filtered off. The concentrated filtrate was separated using silica gel column. The product separated was recrystallised from ethanol to afford pale yellow crystals with mp125°C in 90% yield. Mixed melting point with the compound obtained by Schotten-Baumann reaction of **2** did not show any depression and it was homogeneous to tlc.

Both S-acyl (**3**) and N-acyl (**3'**) derivatives are reasonably possible.



2' **2** **3'-6'** **3 - 6**

3, 3': R = C_6H_5-
4, 4': R = CH_3-
5, 5': R = CH_3-CH_2-
6, 6': R = C_6H_5- CH_2-

Scheme 3.1

The formation of the N-benzoyl derivative **3'** from S-benzoyl-5,6-diphenyl-1,2,4-triazine (**3**) can be explained as an S → N thermal rearrangment of the kinetic product **3** to the thermodynamically more stable N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**3'**). Similar explanations were offered to the rearranged products observed in the case of acyl derivatives of 2-thionothiazolidenes.[51,52]

The facile formation of N-benzoyl-5,6-diphenyl-1,2,4-triazine (**3'**) from 3-mercapto-5,6-diphenyl-1,2,4-triazine-3-thione (**2**) prompted to prepare other acyl derivatives. Thus, N-acetyl-5,6-diphenyl-1,2,4-triazine-3-thione (**4'**), N-propionyl-5,6-diphenyl-1,2,4-triazine-3-thione (**5'**) and N-phenylacetyl-5,6-diphenyl-1,2,4-triazine-3-thione (**6'**) were prepared (Scheme **3.1**) in good yield from 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). The analytical and spectral data (Fig. **3.10–3.13**) of the acyl derivatives are presented in Table **3.1**.

Table 3.1. Characterisation data of N-acyl-5,6-diphenyl-1,2,4-triazine-3-thiones

Acyl derivatives	mp (°C)	Yield (%)	Spectral data		
			UV ($CHCl_3$, nm)	IR (KBr, cm^{-1})	^{1}HNMR (DMSOd_6, δ)
N-Benzoyl-5,6-diphenyl-1,2,4-triazine [N-BzDTT (**3'**)]	125	90	321	1189, C=S 1407, C=S 1705,C=O	7.2 - 7.9 (15H,*m*)
N-Acetyl-5,6-diphenyl-1,2,4-triazine [N-AcDTT (**4'**)]	180	80	307	1171, C=S 1480, C=S 1684,C=O	2.1 (3H,*s*) 7.3 - 7.7 (10H,*m*)
N-Propionyl-5,6-diphenyl-1,2,4-triazine [N-PrDTT (**5'**)]	162	80	305	1128, C=S 1450, C=S 1700,C=O	1.9 (3H,*s*) 3.2 (2H,*m*) 7.2 - 7.6 (10H,*m*)
N-Phenylacetyl-5,6-diphenyl-1,2,4-traizine [N-PaDTT (**6'**)]	191	85	315	1127, C=S 1471, C=S 1686,C=O	3.3 (2H,*s*) 7.2 - 7.7 (15H,*m*)

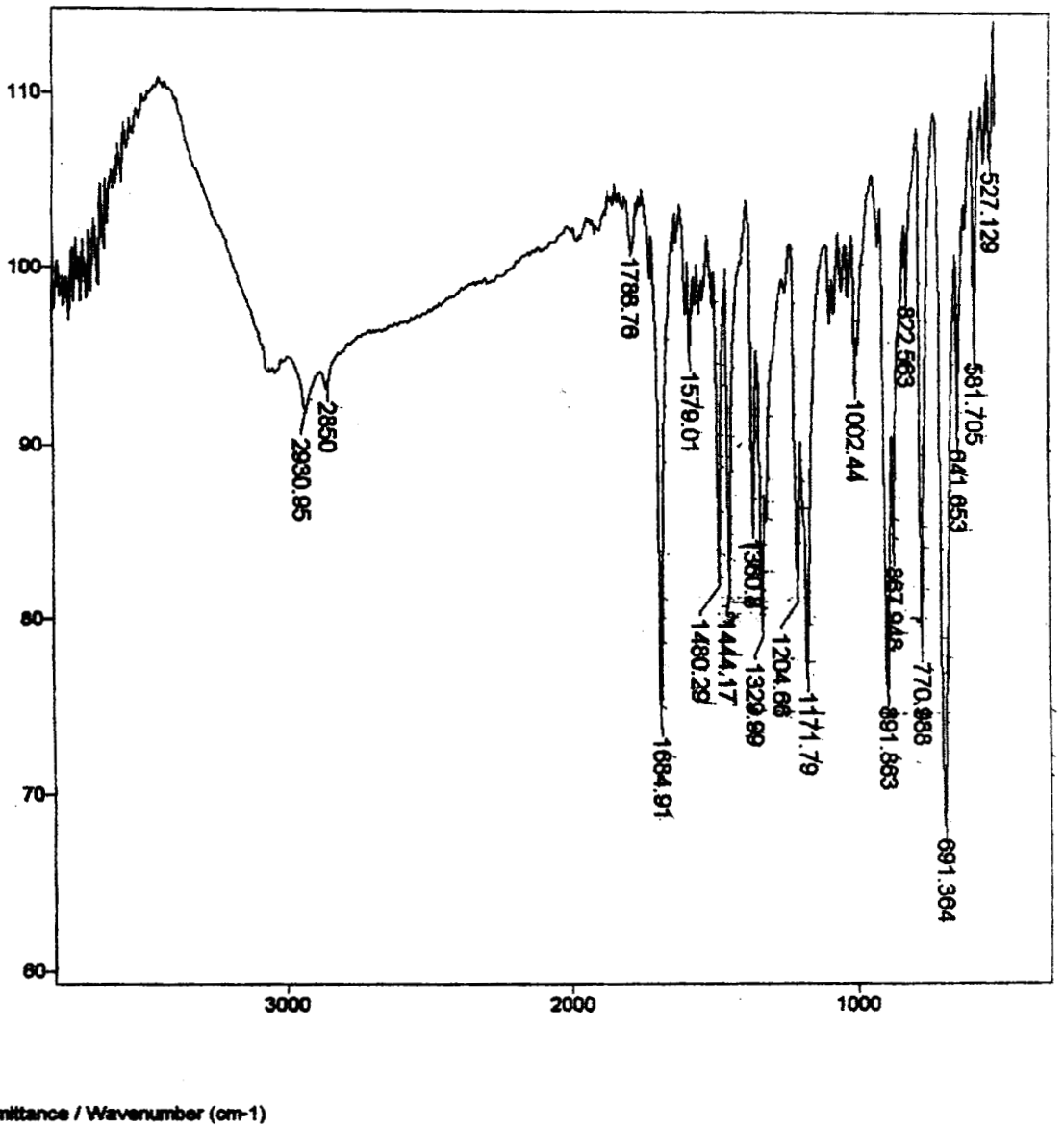


Fig. 3.10. IR (KBr) spectrum of N-acetyl-5,6-diphenyl-1,2,4-triazine-3-thione (4')

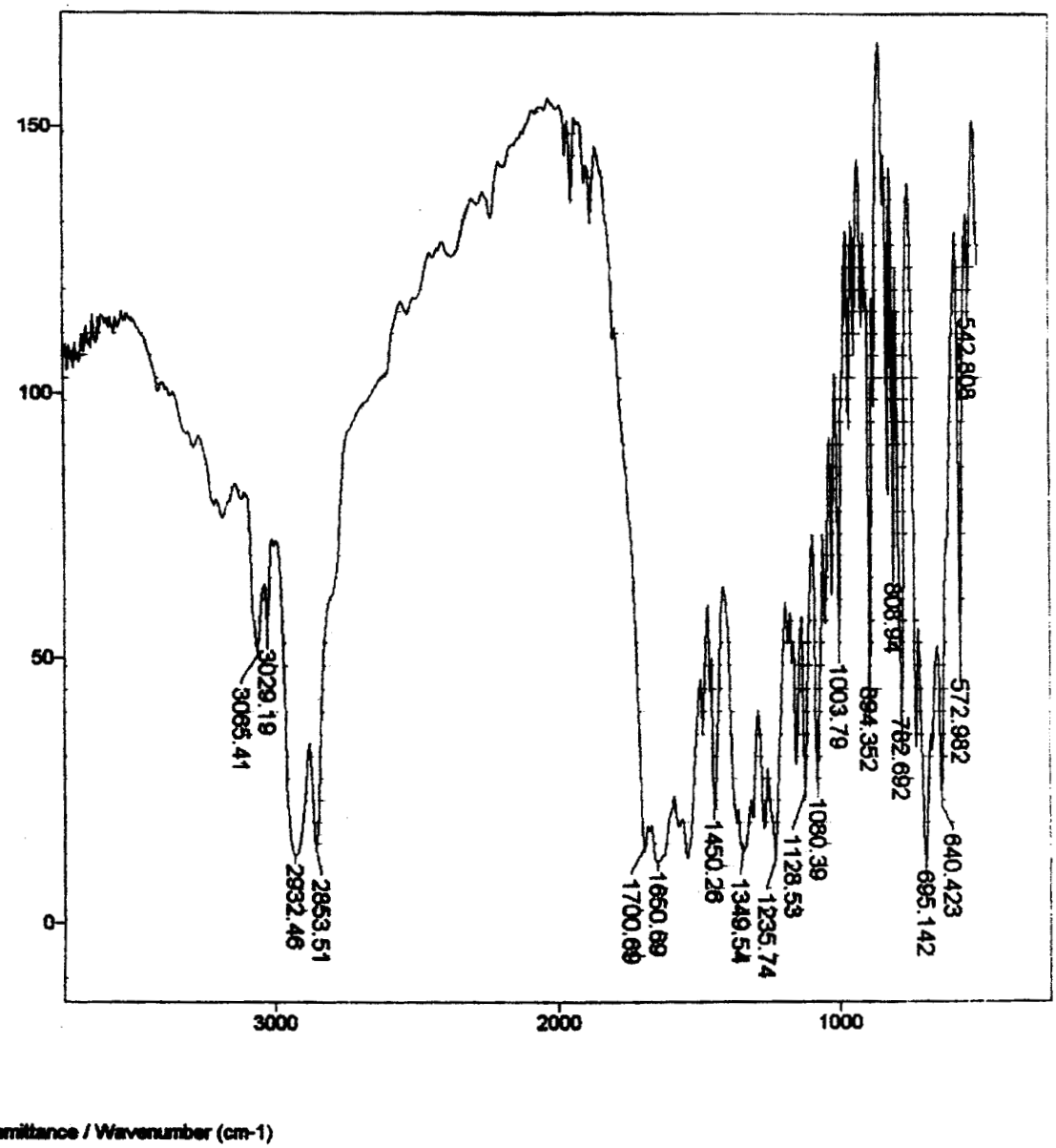


Fig. 3.11. IR (KBr) spectrum of N-propionyl-5,6-diphenyl-1,2,4-triazine-3-thione (5')

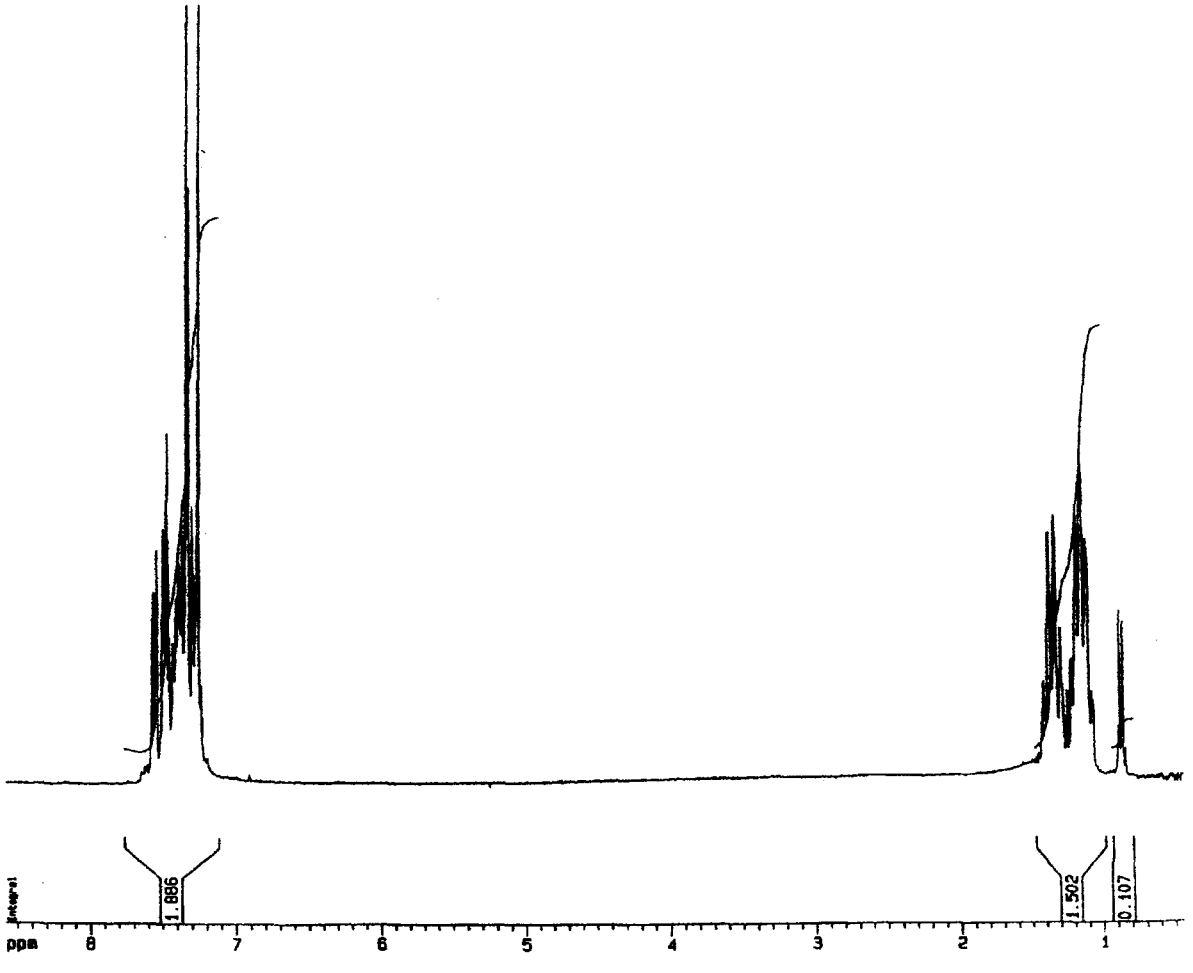


Fig. 3.12. ^{1}HNMR spectrum of N-propionyl-5,6-diphenyl-1,2,4-triazine-3-thione (5')

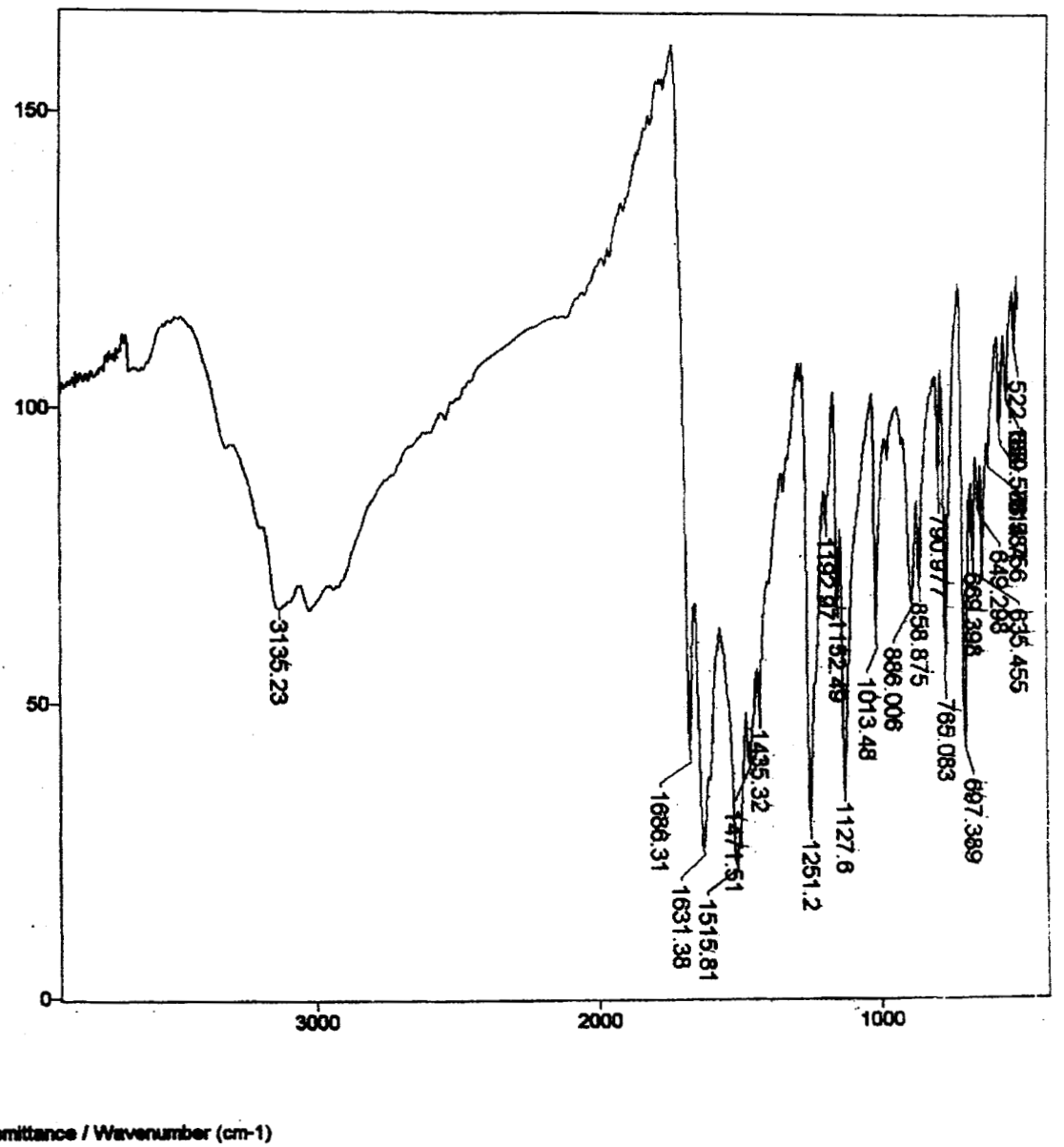


Fig. 3.13.

IR (KBr) spectrum of N-phenylacetyl-5,6-diphenyl-1,2,4-triazine-3-thione (6')

3.2.2.1. Acylation of amines (8) using N-BzDTT (3'): Formation of amides (9)

The suitability of DTT (**2**) as carboxyl activating group was established by studying acylation reaction with simple amines. Thus, a chloroform solution of N-BzDTT (**3'**) was treated with an equimolar amount of freshly distilled amines or amino alcohols. Benzoylation reactions were very effective and rapid. The simultaneous regeneration of pale orange DTT (**2**) was observed by tlc and spectrophotometrically.

Initially, aminolysis reaction was carried out by treating a chloroform solution of the N-BzDTT (**3'**) with an equimolar amount of freshly distilled aniline (**8a**). The benzoylation was almost complete in 5 minutes. Regeneration of 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was observed by the colour change of the reaction mixture to pale red orange. The reaction mixture was concentrated by evaporation and separated by column chromatography (neutral alumina). The first fraction obtained was identified as benzanilide (**9a**) in 90% with mp 162°C (lit.[58] mp 163°C). Presence of benzanilide was further evidenced by tlc. Mixed melting point with an authentic sample did not show any depression. The regenerated 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also in quantitative yield.

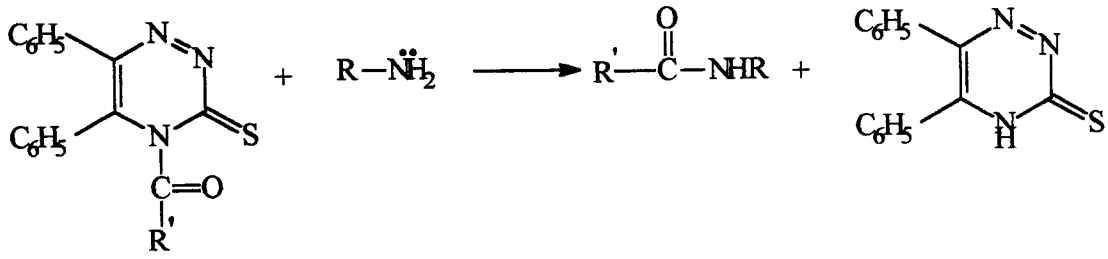
In order to establish the general nature of aminolysis the reaction was carried out with different amines (Scheme **3.2**). All the reactions were carried out at room temperature. In all the cases, 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also

regenerated in almost quantitative yield. The details of the reactions are given in Table 3.2.

Table 3.2. Acylation of amines/alcohols using N-BzDTT (3')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Time of reaction (min.)	Yield (%)
Aniline (**8a**)	Benzanilide (**9a**)	162 (163)[58]	7	90
2-Methyl aniline (**8b**)	N-Benzoyl-2-methyl aniline (**9b**)	141 (144)[58]	5	85
4-Methyl aniline (**8d**)	N-Benzoyl-4-methyl aniline (**9d**)	154 (158)[60]	5	90
4-Chloraniline (**8e**)	N-Benzoyl-4-chloraniline (**9e**)	190 (193)[60]	10	65
4-Methoxy aniline (**8f**)	N-Benzoyl-4-methoxy aniline (**9f**)	150 (154)[60]	6	75
Benzylamine (**8g**)	N-Benzoyl benzamide (**9g**)	106 (106)[60]	8	70
Ethylamine (**8j**)	N-Ethyl benzamide (**9j**)	248	5	75
Methyl alcohol (**10a**)	Methyl benzoate (**11a**)	bp 196 (199)[60]	20	52*
Ethyl alcohol (**10b**)	Ethyl benzoate (**11b**)	bp 211 (212)[60]	20	47*
Benzyl alcohol (**10f**)	Benzyl benzoate (**11f**)	bp 302 (314)[59]	20	40*
Ethanol amine (**8m**)	N-(2-hydroxyethyl) benzamide (**9m**)	161 (163)[60]	10	75
Diethanol amine (**8n**)	N,N-bis(2-hydroxyethyl) benzamide (**9n**)	150 (153)[60]	10	65
p-Amino phenol (**8p**)	N-(4-hydroxyphenyl) benzamide (**9p**)	228 (234)[60]	12	70

*Yield Calculated based on the amount of DTT regenerated.



8a-p **9a-p** **2'**

3':R' = C_6H_5–	**8**	**9**
4':R' = CH_3–	**8**	**12**
5':R' = CH_3–CH_2–	**8**	**15**
6':R' = C_6H_5–CH_2–	**8**	**18**

R: **a**: C_6H_5–
b: *o*-$C_6H_4(CH_3)$–
c: *m*-$C_6H_4(CH_3)$–
d: *p*-$C_6H_4(CH_3)$–
e: *p*-$C_6H_4(Cl)$–
f: *p*-$C_6H_4(OCH_3)$–
g: C_6H_5–CH_2–
h: N-$C_6H_5(C_2H_5)$–
i: CH_3–
j: CH_3–CH_2–
k: CH_3–CH_2–CH_2–
l: CH_3–CH_2–CH_2–CH_2–
m: HO–CH_2–CH_2–
n: (HO–CH_2–CH_2–)$_2$
o: HO–CH_2–CH_2–CH_2–CH_2–
p: *p*-$C_6H_4(OH)$–

Scheme 3.2

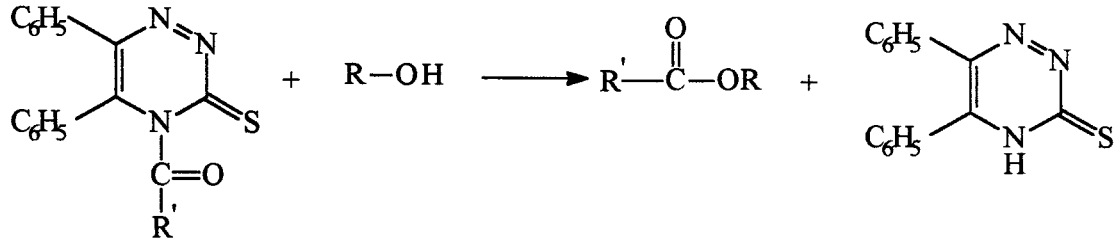
3.2.2.2. Acylation of alcohols (10) using N-BzDTT (3'): formation of esters (11)

Formation of ester from carboxylic acid and alcohol is basically a reversible reaction and hence isolation of the desired product requires complicated purification procedures.[53] Acyl halide or anhydride method, therefore, is applied to make the process essentially irreversible. Transesterification in the presence of acidic catalysts such as *p*-toluene sulphonic acid[54] is an another pathway for the synthesis of esters.

The smooth benzoylation of amines using N-BzDTT (**3'**) under room temperature conditions prompted to extend this nucleophilic reaction with other weak nucleophiles such as alcohols. Thus, when **3'** (20 mmol) in chloroform was added to an equimolar quantity of ethyl alcohol (**10b**), even after stirring for a long time, no evidence for the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was noticed. Any how, when the reaction mixture was heated to about 70 °C for 5 minutes, the solution turned pale red orange with a clear fruity smell of the ester. Column chromatographic separation yielded ethyl benzoate (**11b**) bp 211°C (lit.[60] bp 212°C) and 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was regenerated in almost quantitative yield. Further, the very slow regeneration of **2** was also monitored spectrophotometrically.

To generalise the esterification, the reaction was tried with other alcohols (Scheme **3.3**). The reaction conditions and analytical details are presented in Table

3.2. In all the reactions tried, the nature and course of reactions were similar to that of ethyl alcohol.



10a-f **11a-f** **2'**

3':R' = C_6H_5–	**10**	**11**
4':R' = CH_3–	**10**	**13**
5':R' = CH_3–CH_2–	**10**	**16**
6':R' = C_6H_5–CH_2–	**10**	**19**

R: **a:** CH_3–
b: CH_3–CH_2–
c: CH_3–CH_2–CH_2–
d: CH_3–CH_2–CH_2–CH_2–
e: CH_3–CH_2–CH_2–CH_2–
f: C_6H_5–CH_2–

Scheme 3.3

The above observations clearly demonstrate that though alcohols can be used as nucleophiles, they are weak in nucleophilic character to attack the already activated acyl group present in N-BzDTT (**3'**). It was also observed that N-BzDTT does not react with water.

The above aminolysis and esterification reactions clearly show that the reactivity of N-BzDTT (**3'**) is sufficient enough to couple directly with the amino group under room temperature conditions and alcoholic group requires higher temperaure due to the weak nucleophilic nature of alcohols.

3.2.2.3. Reactions of N-BzDTT (3') with amino alcohols (8): Selective aminolysis

The facile aminolysis with amines under room temperature conditions and the sluggish nature of esterification using alcohols prompted to carry out the selective aminolysis using amino alcohols (Scheme **3.2**). In the case of amino alcohols, amino nucleophiles were selectively reacted and the respective amides were obtained in good yield.

Thus, a chloroform solution of N-BzDTT (**3'**) when treated with an equimlar solution of ethanolamine (**8m**) in chloroform, a pale yellow solid product was found to be precipitated. The reaction mixture was further stirred for 5 minutes. TLC and spectrophotometric investigation showed the formation of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). The solid product obtained was separated using neutral alumina column. One fraction separated in 75% yield was identified as N-(2-hydroxyethyl)benzamide (**9m**) mp 161°C (lit.[61] mp 163 °C)..

The selective aminolysis was also repeated using diethanolamine (**8n**), and *p*-aminophenol (**8p**) to obtain the corresponding amides N,N-bis (2-hydroxyethyl benzamide (**9n**) with mp 150°C (lit.[61] mp 153 °C) in 65% yield and N-(4-hydroxyphenyl)benzamide (**9p**) mp 228°C (lit.[61] mp 234 °C) in 70% yield (table**3.2**).

Here also DTT (**2**) was regenerated which was identified by the colour change as well as spectrophotometrically.

In all the cases of aminolysis and esterification reactions, the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was observed. This was evidenced by monitoring the reactions spectrophotometrically.

3.2.3. Spectrophotometric monitoring of aminolysis and esterification using N-BzDTT (3')

The electronic transitions of the thiocarbonyl group have been investigated in detail.[55-57] The carbonyl and thiocarbonyl chromophores exhibit qualitatively similar bonding properties since both possess similar nuclear frame work. Anyhow, the C=S bond is weaker than the C=O bond (Ca. 430 kJ mol^{-1} *vs.* Ca. 635 kJ mol^{-1}) and the excited electronic states are found at lower energies in the sulphur containing species. Basically, two prominent electronic transitions ($n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$) are observed in thiocarbonyl chromophores. The $n \rightarrow \pi^*$ transition of C=S chromophore is found at longer wave lengths than the corresponding carbonyl compounds. This is understandable since the ionization potential of the sulphur lone pair is smaller than that of the oxygen lone pair. These differences in the energy and spacing of electronic states lead to differences in the absorption spectra of the compounds. These electronic absorption spectral characteristics of thiocarbonyl chomophores facilitated to study the rates of aminolysis and esterification reactions using N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**2'**). Further, in order to monitor the aminolysis and esterification spectrophotometrically, actually, the rate of regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) from N-benzoyl-5,6-

diphenyl-1,2,4-triazine-3-thione (**3'**) during the course of aminolysis/esterification reactions (Fig. **3.14–3.18**) was followed since 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) and N-benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**3'**) give distinct peaks differing in absorbance in their UV-visible spectra.

Thus, the following part of the investigation is devoted to study the UV-visible absorption spectra of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) and N-benoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**3'**) during aminolysis and esterification reactions and to correlate these with the extent of the reactions. These experimentations were carried out by observing the intensity of the absorption bands at specific wave lengths at regular intervals. Since the aminolysis reaction taking places are very fast in nature, only a qualitative correlation are made here.

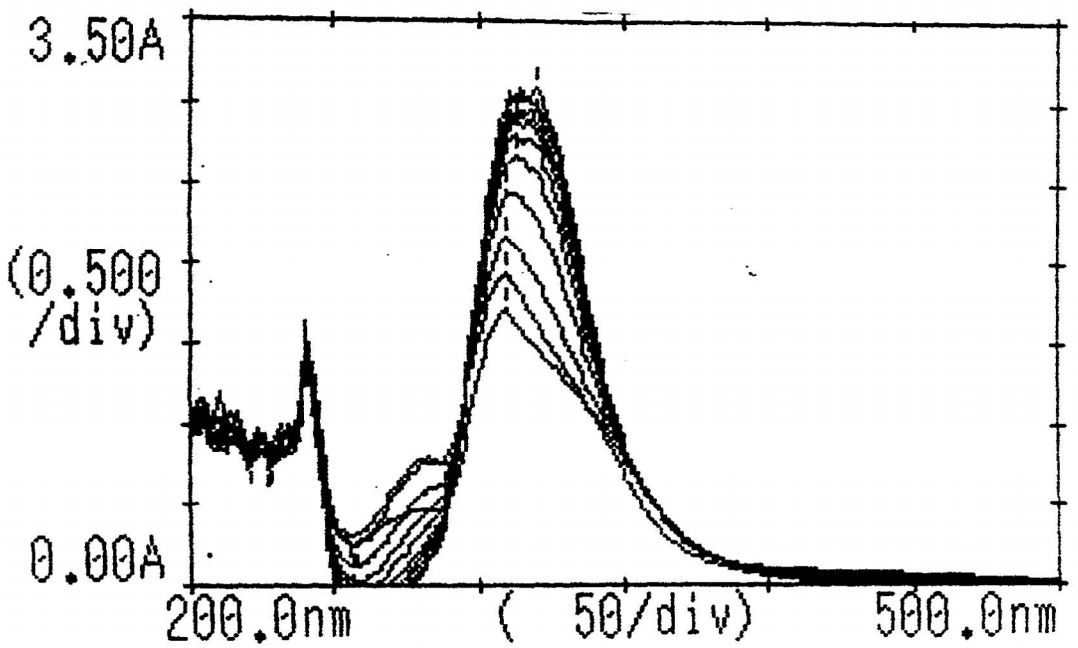


Fig. 3.14. UV-Visible spectrum – Reaction of aniline with N-BzDTT (3')

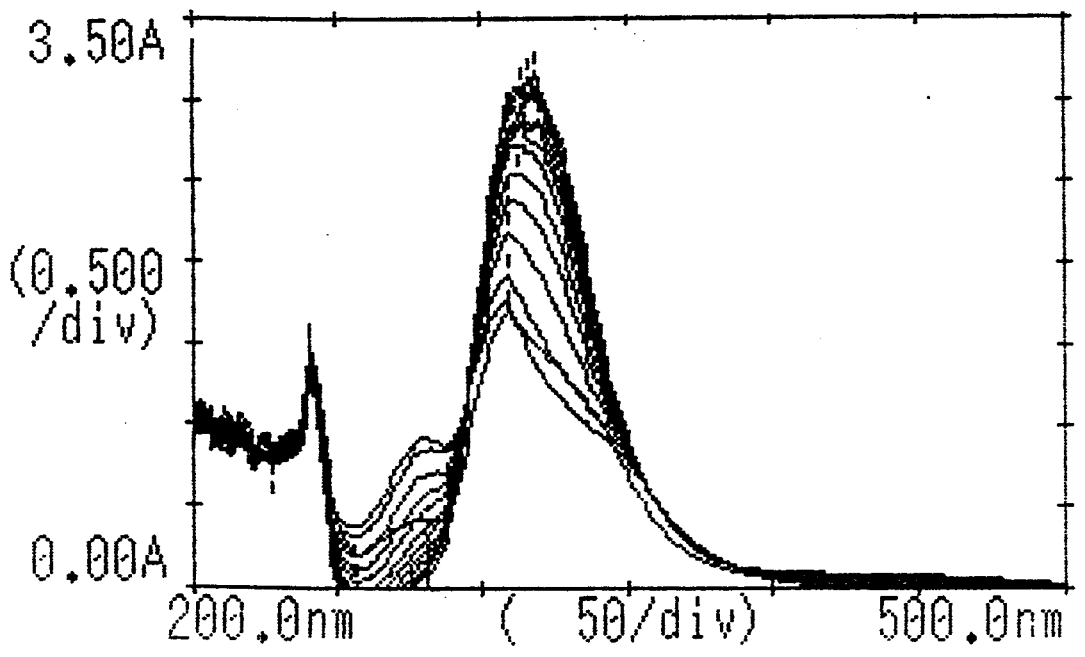


Fig. 3.15. UV-Visible spectrum – Reaction of *o*-toluidine with N-BzDTT (3')

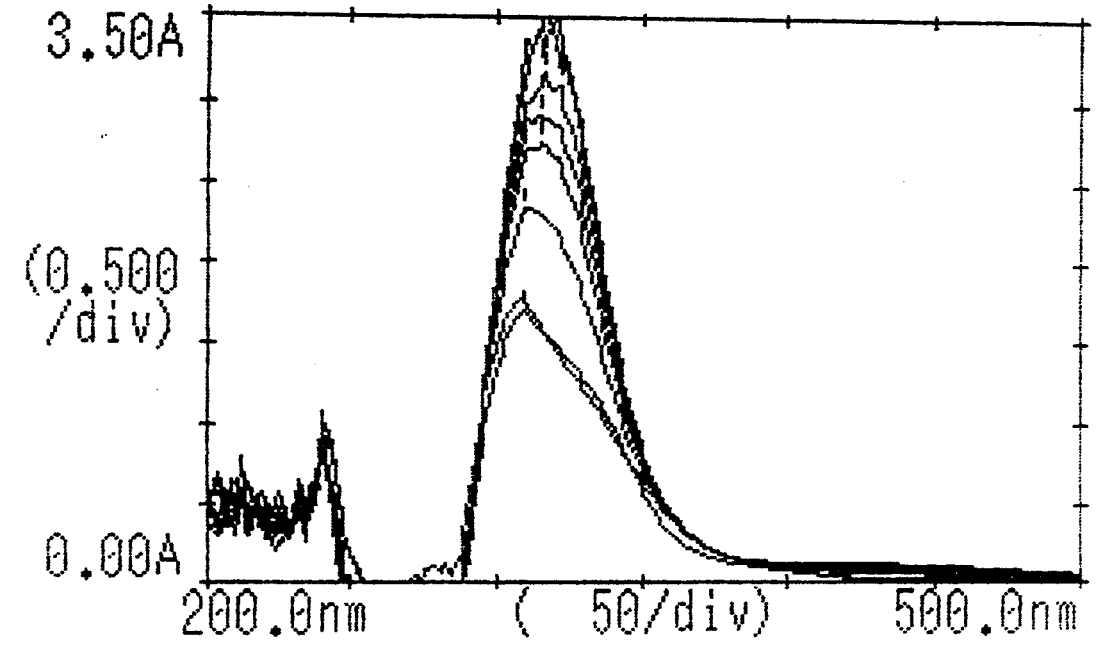


Fig. 3.16. UV-Visible spectrum – Reaction of *n*-propylamine with N-BzDTT (3')

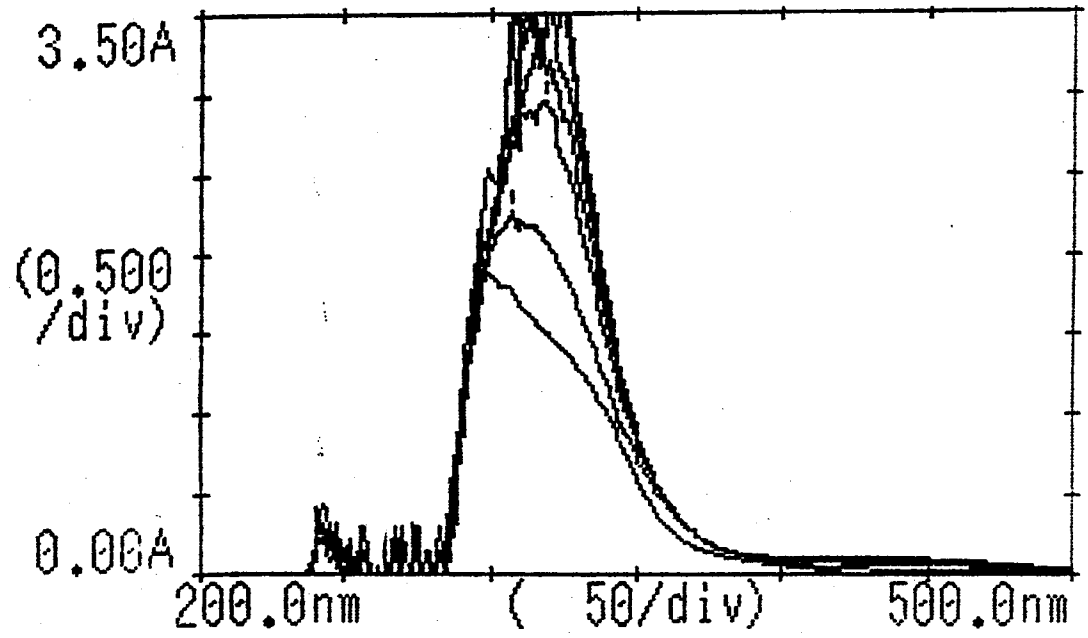


Fig. 3.17. UV-Visible spectrum – Reaction of *n*-butylamine with N-BzDTT (3')

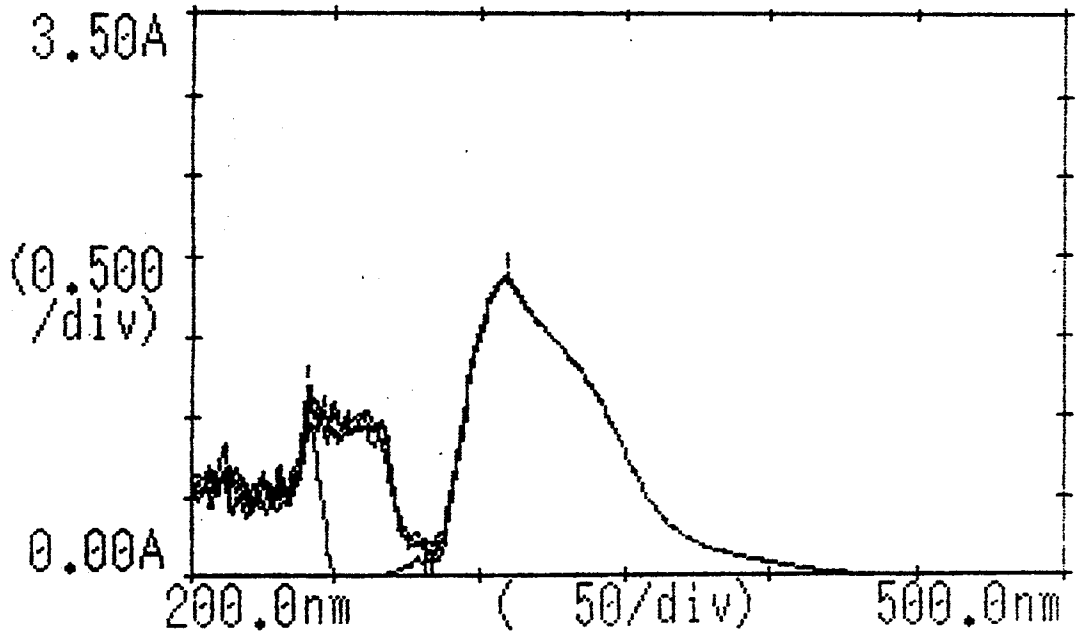


Fig. 3.18.UV-Visible spectrum – Reaction of benzyl alcohol and N-BzDTT (3')

3.2.4. N-Acetyl-5,6-diphenyl-1,2,4- triazine- 3-thione

3.2.4.1. Acylation of amines (8) using N-AcDTT (4'): Formation of amides (12)

The facile acylation of primary amines using N-BzDTT (**3'**) was also extended to N-AcDTT (4'). Thus, when N-AcDTT (**4'**) in chloroform and freshly distilled aniline (**8a**) in chloroform were mixed in equimolar proportion (5 mml) for 10 minutes, a pale red orange colour was gradually appeared. The reaction was followed by tlc and spectrophotometrically. When the reaction was over, the mixture was concentrated and separated by column chromatography. Recrystallisation from alcohol afforded a white solid which was identified as acetanilide (**12a**) mp 112°C (lit.[58]mp 114°C) in 85% yield. 2-Mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also separated as another product.

The aminolysis reaction was then carried out with other primary amines **(Scheme 3.2)**. The products formed and the characterisation data are presented in Table **3.3**.

Table 3.3. Acylation of amines/alcohols using N-AcDTT (4')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Time of reaction (min.)	Yield (%)
Aniline (**8a**)	Acetanilide (**12a**)	112 (114)[58]	8	85
2-Methyl aniline (**8b**)	N-(*o*-Tolyl) acetamide (**12b**)	111 (110)[58]	6	88
3-Methyl aniline (**8c**)	N-(*m*-Tolyl) acetamide (**12c**)	62 (65.5)[58]	6	80
4-Methyl aniline (**8d**)	N-(*p*-Tolyl) acetamide (**12d**)	156 (154)[59]	6	90
Benzylamine (**8g**)	N-Benzylacetamide (**12g**)	59 (61)[58]	10	70
Ethanol (**10b**)	Ethyl acetate (**13b**)	bp 75 (77)[59]	20	48*
1-Propanol (**10c**)	*n*-Propyl acetate (**13c**)	bp 90 (101)[58]	22	42*
1-Butanol (**10d**)	*n*-Butyl acetate (**13d**)	bp 121 (126.5)[58]	25	39*
Ethanol amine (**8m**)	N-(2-Hydroxyethyl) acetamide (**12m**)	161 (166)[59]	12	75
Diethanol amine (**8n**)	N,N-Bis (2-hydroxyethyl) acetamide (**12n**)	152 (153)[58]	15	65
4-Aminophenol (**8p**)	N-(4-Hydroxyphenyl) acetamide (**12p**)	180 (184)[59]	20	70

* Yield calculated based on the amount of DTT regenerated.

3.2.4.2. Esterification of N-AcDTT (4') with alcohols (10): formation of esters (13)

The ease of acylation of different amines under room temperature conditions prompted to extend the reaction to other weak nucleophilic reagents like alcohols using N-AcDTT (**4'**). Thus, when equimolar quantity of N-AcDTT (**4'**) in chloroform

was treated with ethyl alcohol **(10b)**, even after stirring for about 1 hour, no sign of regeneration of 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was observed. Anyhow, the reaction mixture when heated to 70 °C, the solution became pale red orange with a fruity smell of the ester. Column chromatogrphic separation of the mixture yielded ethyl acetate (**13b**) bp 75 °C (lit.[59]bp77 °C) and DTT (**2'**). The reaction was then tried using other alcohols (Scheme **3.3**), the details of which are given in Table **3.3**.

3.2.4.3. Reaction of N-AcDTT (4') with aminoalcohols (8): Formation of hydroxy amides (14)

Now it is clear that N-AcDTT (**4'**) is very reactive towards amines whereas the reactivity towards alcohols and phenols is very slow. Hence if amino alcohols or phenols are used, selective aminolysis is possible. Based on this generalisation, when an equimolar (2 mmol) proportion of N-AcDTT (**4'**) and ethanolamine (**8m**) in chloroform stirred for 10 minutes, the red orange colour was found to be gradually developed. The product separated using neutral alumina column, identified as N-(2-hydroxyethylacetamide (**14m**) from mp 165°C (lit.[59]166°C) m.mp. and homogeneous tlc. This selective aminolysis was further established by extending to other aminoalcohols and phenols. The details of the reaction carried out and the products formed are part of the Table **3.3**. In all the above reactions, DTT (**2**) was also obtained in good yield. Spectrophotometric monitoring in all cases of aminolysis/esterification again confirmed the regeneration of the original thiol **2** (Fig. **3.19 - 3.23**).

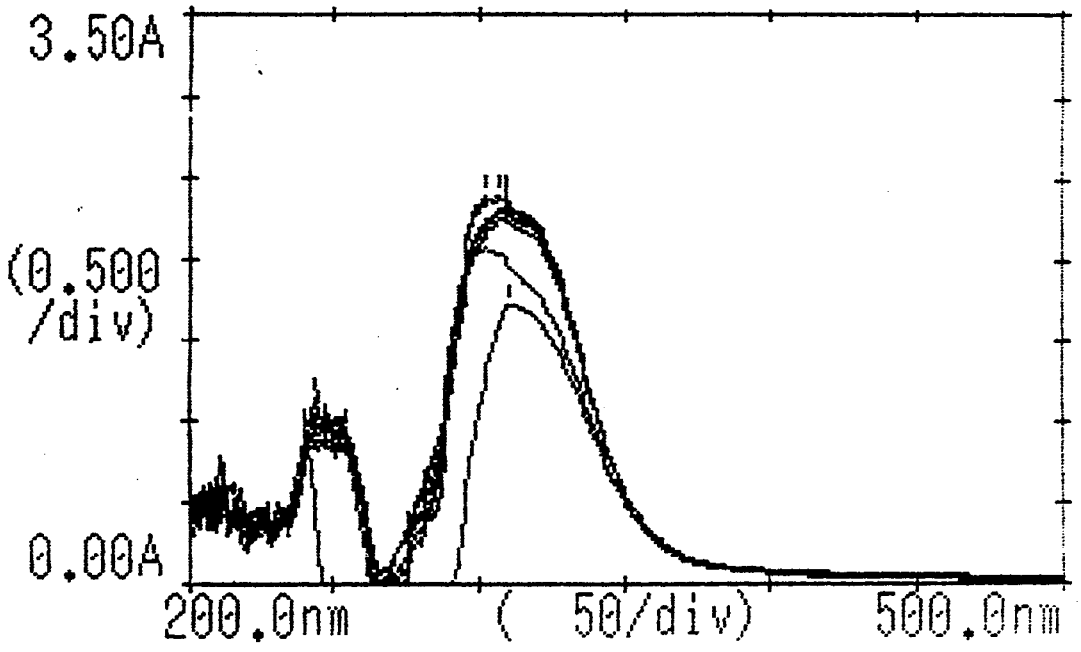


Fig. 3.19. UV-Visible spectrum – Reaction of N-AcDTT with aniline

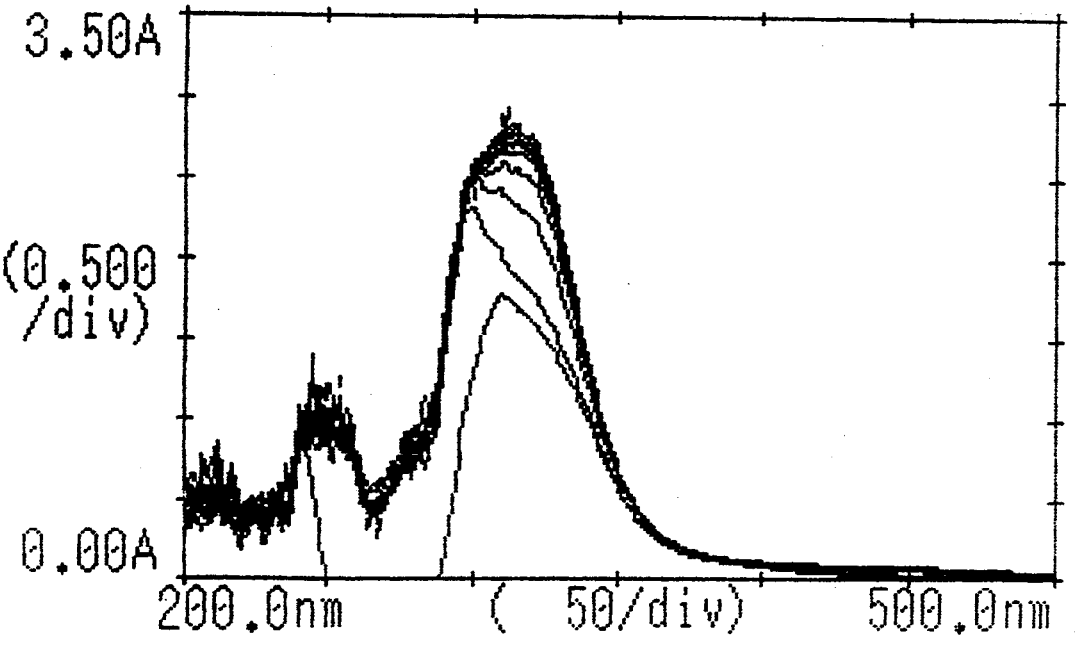


Fig. 3.20. UV-Visible spectrum – Reaction of N-AcDTT with *o*-toluidine

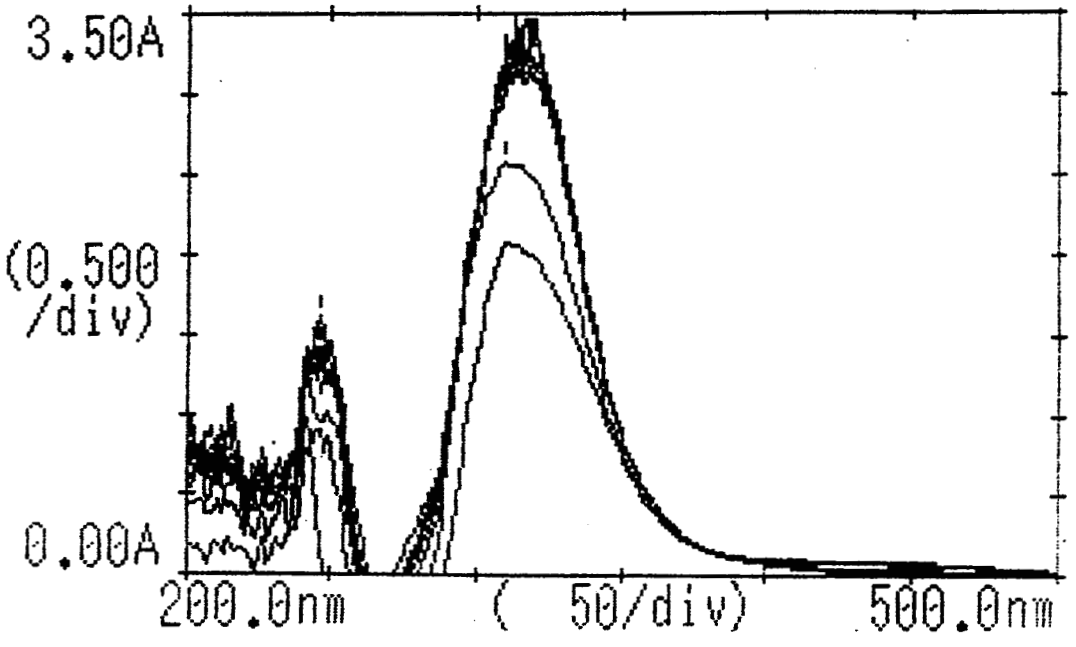


Fig. 3.21. UV-Visible spectrum – Reaction of N-AcDTT with *n*-propylamine

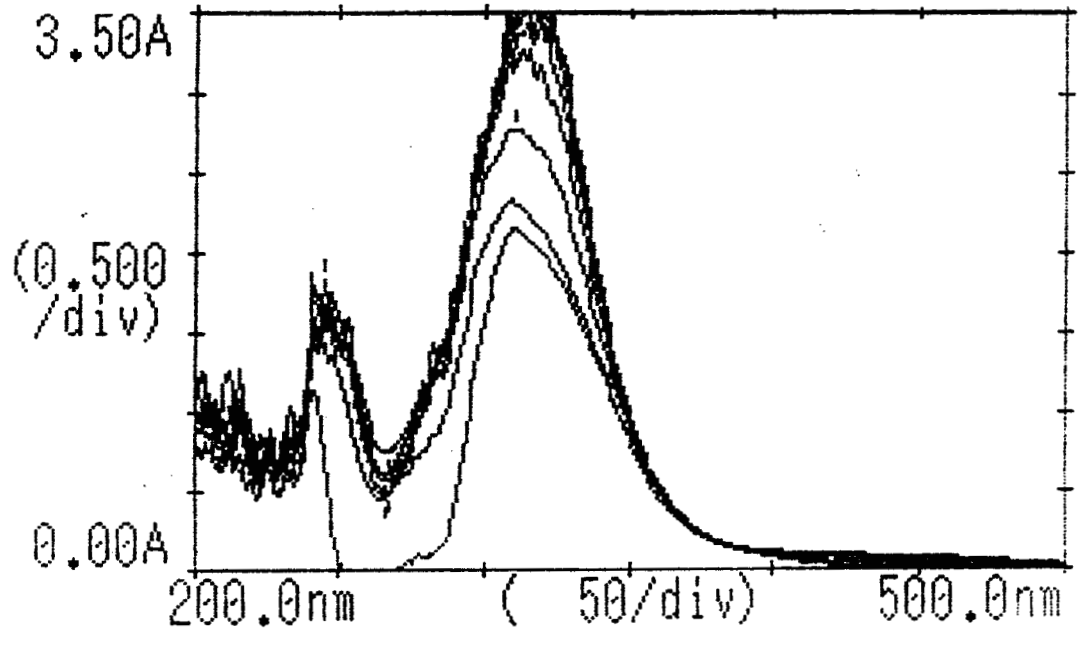


Fig. 3.22. UV-Visible spectrum – Reaction of N-AcDTT with *n*-butylamine

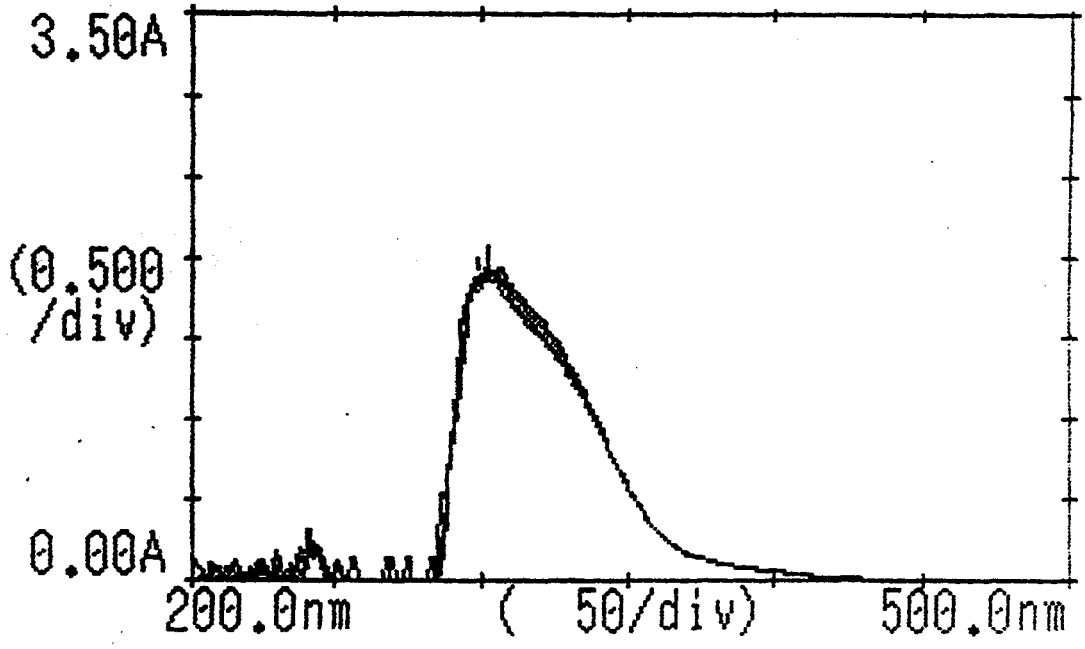


Fig. 3.23. UV visible spectrum – Reaction of N-AcDTT with benzyl alcohol

3.2.5. N-Propionyl-5,6-diphenyl-1,2,4-triazine-3-thione

3.2.5.1.Aminolysis of N-PrDTT (5') with amines (8): Formation of amides (15)

In order to prove the suitability of 3-mercapto-5,6-dipheyl-1,2,4-triazine as an efficient carboxyl activating reagent, N-PrDTT (**5'**) was treated with different amines. Thus when an equimolar (4 mml) solution of N-PrDTT (**5'**) was mixed with a freshly distilled solution of aniline (**8a**), orange red colour was developed immediately. The intensity of the orange red colouration was gradually increased which indicated the regeneration of the 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). This was also evidenced by tlc and spectrophotometric monitoring. The mixture was then separated using neutral alumina column. The compound eluted first was recrystallized from alcohol and characterised as N-phenyl propionamide (**15a**) which

melts at 104°C (lit.[58] mp 105°C). Similar reactions were carried out using other amines (Scheme **3.2**), the details and the characterisation data are presented in Table **3.4**.

Table 3.4. Acylation of amines/alcohols using N-PrDTT (5')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Time of reaction (min.)	Yield (%)
Aniline (**8a**)	N-Phenyl propionamide (**15a**)	104(105)[58]	12	72
4-Methyl aniline (**8d**)	N-(*p*-Tolyl) propionamide (**15d**)	122(126)[59]	10	70
Ethyl amine (**8j**)	N-Ethyl propionamide (**15j**)	146(149)[58]	8	68
n-Propyl amine (**8k**)	N-(*n*-Propyl) propionamide (**15k**)	156(154)[58]	8	70
n-Butyl amine (**8l**)	N-(*n*-Butyl) propionamide (**15l**)	174(177)[58]	10	65
Methanol (**10a**)	Methyl propionate (**16a**)	bp 75(80)[59]	18	44*
1-Propanol (**10c**)	*n*-Propyl propionate (**16c**)	bp 106(122)[58]	20	40*
1-Butanol (**10d**)	*n*-Butyl propionate (**16d**)	bp 133(145)[58]	20	38*
Benzyl alcohol (**10f**)	Benzyl propionate (**16f**)	bp 209(220)[58]	25	35*
Ethanolamine (**8m**)	N-(2-Hydroxy ethyl) propionamide (**17m**)	143(145)[58]	15	75
Diethanol amine (**8n**)	N,N-Bis(2-hydroxy ethyl) propionamide (**17n**)	175(179)[58]	15	65
4-Amino phenol (**8p**)	N-(4-Hydroxy phenyl) propionamide (**17p**)	152(156)[59]	20	60

*Yield calculated based on the amount of DTT regenerated.

3.2.5.2. Esterfication reaction of N-PrDTT (5') with alcohols (10)

As observed in the case of **(3.2.2.2)** & **(3.2.4.2)** the estrification reactions of N-PrDTT (**5'**) were found to the sluggish with different alcohols like methanol (**10a**), 1-propanol (**10c**), 1-butanol (**10d**) and benzyl alcohol (**10f**) **(scheme 3.3).**. Here, the formation of the ester was noticed for about 25 minutes at 70°C, in addition to the isolation of DTT (**2'**). Table **3.4** provides the details of the reaction conditions and analytical data of the products formed.

3.2.5.3. Reactions of N-PrDTT (5') with amino alcohols & phenols (8)

Analogous to N-BzDTT and N-AcDTT, N-PrDTT (**5'**) has also been proved to be an activated N-acyl derivative from the aminolysis reactions with various amines. Moreover, very poor reactivity was observed with alcohols at room temperatures, which prompted to undertake the selective aminolysis with amino alcohols.

Thus, when an equimolar proportion of N-PrDTT (**5'**) in chloroform and ethanolamine (**8m**) in chloroform was stirred about 20 minutes, the orange red colour was gradually developed. The mixture was then subjected to column chromatographic separation using neutral alumina colum. The separated product was recrystallysed from alcohol to afford crystals of N-(2-hydroxy ethyl) propionamide (**17m**) in 75% yield. Mp 143°C (lit.[58] mp 145°C). The same selective aminolysis was further established by extending the reaction using different amino alcohols and phenols such as diethanolamine (**8n**), 4-aminophenol (**8p**) to get respective hydroxy substituted amides, N,N-bis (2-hydroxyethyl) propionamide (**17n**), N-(4-hydroxyphenyl) propionamide(**17p**). The characterisation data are

presented in Table **3.4**. The formation of DTT (**2'**) in all cases was evidenced by tlc and spectrophotometrically (Fig. **3.24 - 3.27**).

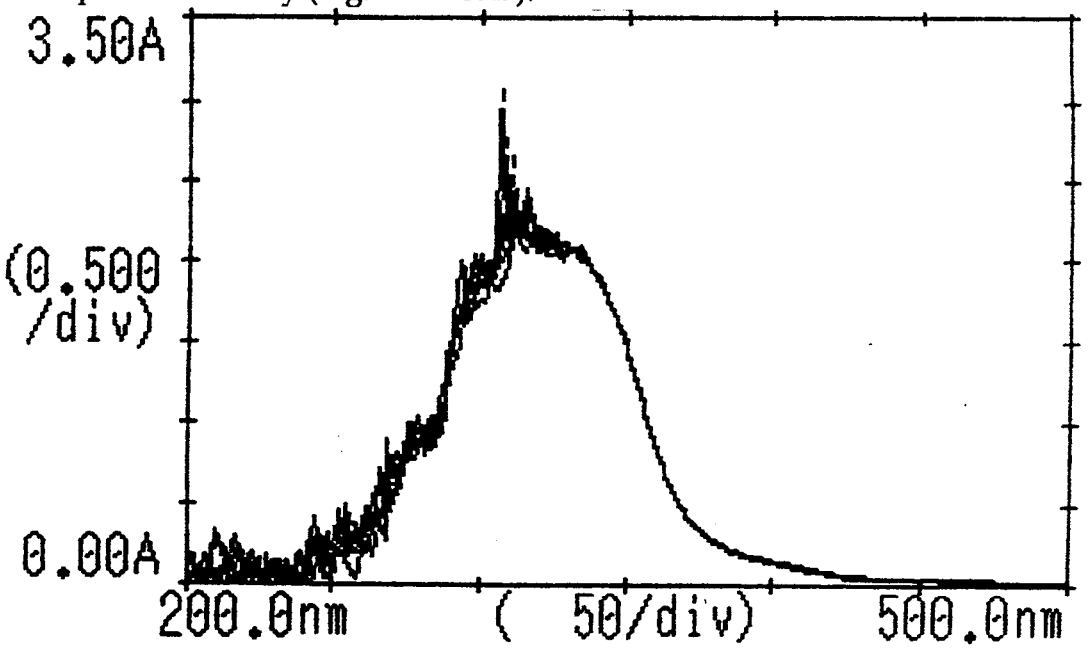


Fig. 3.24. UV visible spectrum – Reaction of N-PrDTT (5') with aniline

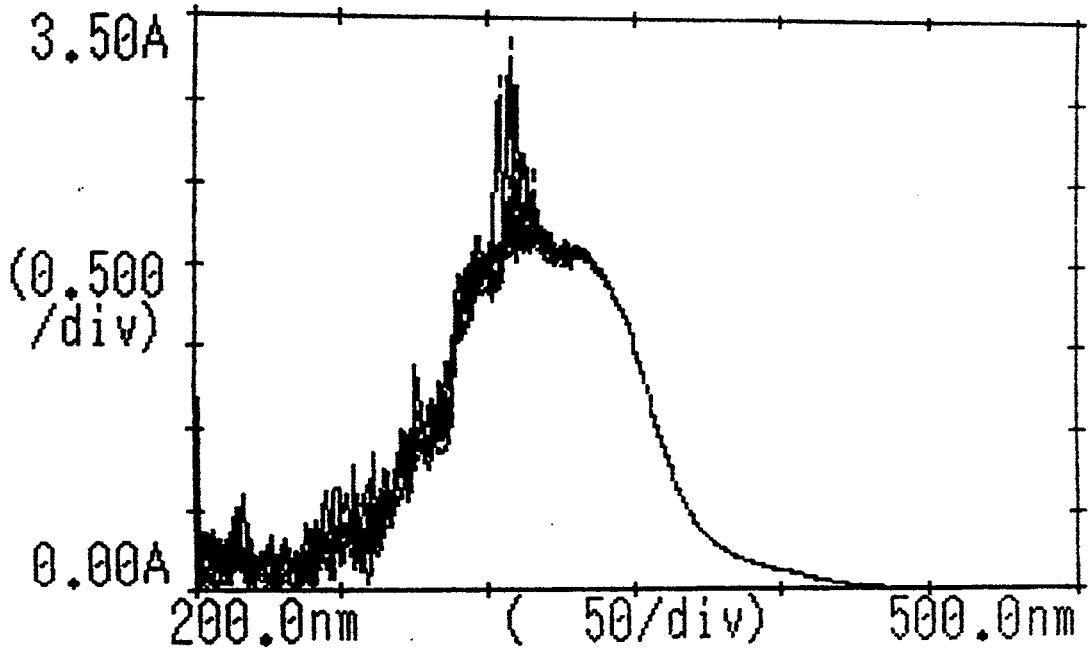


Fig. 3.25. UV visible spectrum – Reaction of N-PrDTT (5') with *o*-toluidine

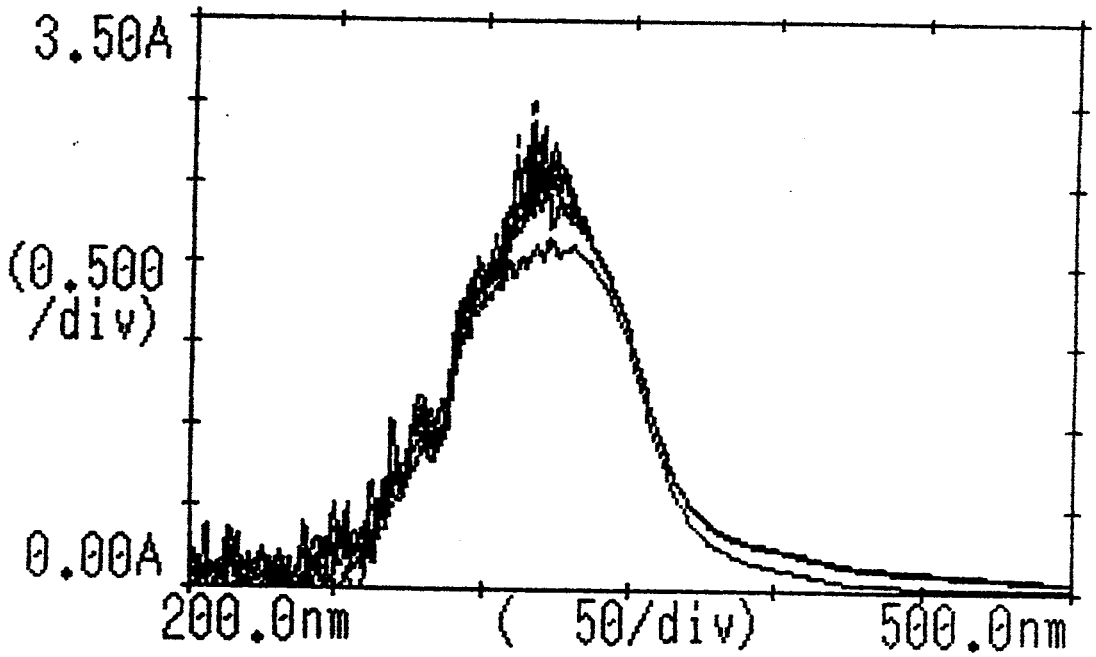


Fig. 3.26. UV visible spectrum – Reaction of N-PrDTT (5') with *n*-propylamine

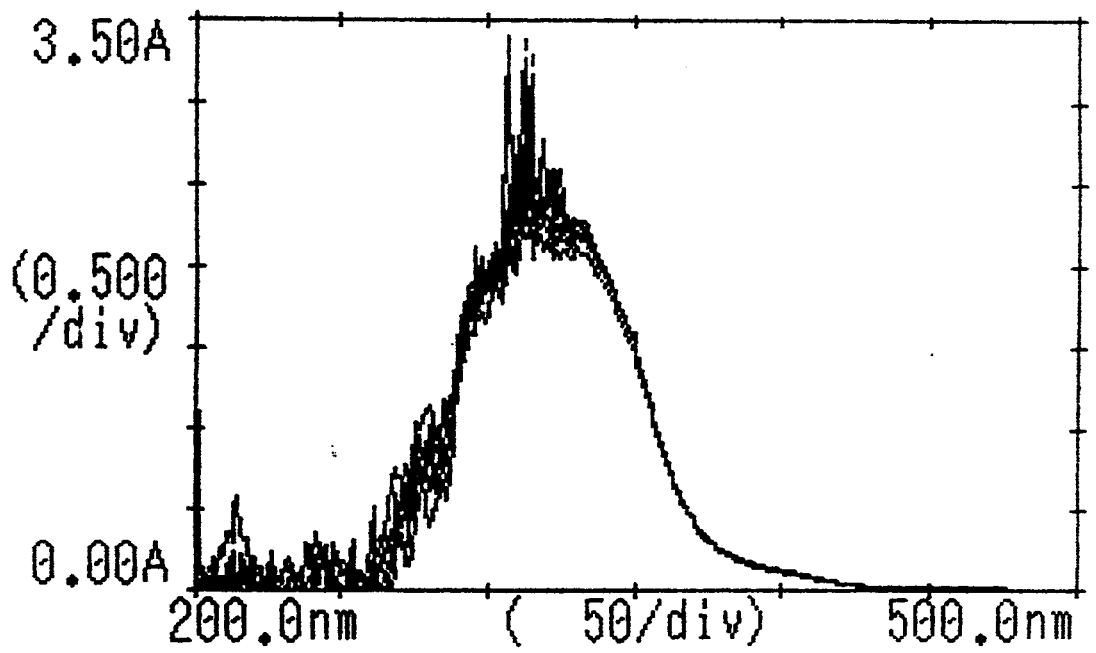


Fig. 3.27. UV visible spectrum – Reaction of N-PrDTT (5') with *n*-butylamine

3.2.6. N-Phenylacetyl-5,6-diphenyl-1,2,4-triazine-3-thione(6')

3.2.6.1. Aminolysis of N-PaDTT (6') with amines (8): formation of amides (18)

N-PaDTT (**6'**) has also been found to be an activated carboxyl derivative by conducting various aminolysis reactions. Here, when a chloroform solution of N-PaDTT (**6'**) was treated with a solution of freshly distilled aniline (**8a**), immediate colour change was noticed and within 5 minutes and the orange red colour developed became intense. The mixture was stirred for another 10 minutes and seperated by column chromatographic method using neutral alumina column. The first fraction of the product was identified as phenyl acetyl amino benzene (**18a**): yield 95% , mp 116 °C (lit[58] mp 118 °C). 3-Mercapto-5,6-diphenyl-1,2,4-triazine (2) was isolated as another compound.

The behaviour of N-PaDTT **(6')** was also studied using other amines (Scheme **3.2**). The products formed in the different reactions were separated by column chromatographic method and characterised. Characterisation data are given in table **3.5**. In all the above cases, DTT (**2'**) was isolated in almost quantitative yield.

3.2.6.2. Esterification reaction of N-PaDTT (6') with alcohols: formation of esters (19)

When a solution of N-PaDTT **(6')** in chloroform (40 mL) was treated with ethyl alcohol (**10b**), there was no indication for the regeneration of DTT (**2'**) even after stirring for about 2 hours at room temperature. Anyhow, when the mixture was heated to about 70°C, for 5 minutes fruity smell of the ester was noticed with a

simultaneous orange red colour indicating the regeneration of DTT (**2'**). Column chromatographic separation afforded ethyl phenyl acetate (**19b**) with bp 220°C. (lit[59]. bp 227°C) and the regeneration of DTT (**2'**). The other esterification reactions carried out using N-PaDTT (**6'**) and alcohols are presented in table **3.5** In all the above cases, DTT (**2'**) has been isolated.

Table 3.5. Acylation of amines/alcohols using N-PaDTT (6')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) °C	Time of reaction (min.)	Yield %
Aniline (**8a**)	Phenyl acetyl amino benzene (**18a**)	116 (118)[58]	8	85
2-Methylaniline (**8b**)	Phenyl acetyl amino (2-methyl) benzene (**18b**)	156 (159)[59]	7	70
4-Methylaniline (**8d**)	Phenyl acetyl amino (4-methyl) benzene (**18d**)	135 (136)[59]	7	73
Benzyl amine (**8g**)	N-Benzoyl-2-phenyl ethanamide (**18g**)	118 (122)[59]	8	70
Ethyl amine (**8j**)	Phenyl acetyl aminoethane (**18j**)	169 (174)[58]	8	82
Methanol (**10a**)	Methyl phenyl acetate (**19a**)	bp 209 (215)[59]	15	55*
Ethanol (**10b**)	Ethyl phenyl acetate (**19b**)	bp 220 (227)[59]	15	52*
1-Pentanol (**10e**)	*n*-Pentyl phenyl acetate (**19e**)	bp 260 (265)[59]	20	45*
Benzyl alcohol (**10f**)	Benzyl phenyl acetate (**19f**)	bp 305 (317)[59]	18	43*
Ethanolamine (**8m**)	N-(2-Hydroxyethyl) phenyl acetamide (**20m**)	150 (157)[58]	10	78
Diethanolamine (**8n**)	N,N-Bis-(2-hydroxyethyl) benzamide (**20n**)	130 (136)[58]	12	72
4-Aminophenol (**8p**)	N-(2-Hydroxyphenyl) benzamide (**20p**)	180 (190)[58]	15	70

*Yield calculated based on the regenerated amount of DTT.

3.2.6.3. Reaction of N-PaDTT (6') with aminoalcohols & phenols (20)

Due to the better nucleophilicity of amines than alcohols, it could be seen that the esterification of N-PaDTT **(6')** with alcohols are very sluggish in nature. This led to carry out selective aminolysis reaction using amino alcohols. Thus, when an equimolar solution of N-PaDTT **(6')** in chloroform was stirred with ethanolamine **(8m)** in chloroform for 15 minutes, the pale orange red colour was gradually developed. The mixture was separated using neutral alumina column and the products obtained were recrystallised from alcohol and identified as N-(2-hydroxyethyl) phenyl acetamide (**20m**) mp 150°C (lit.[58] mp 152°C) in 80% yield. Simultaneously 3-mercapto-5,6-diphenyl-1,2,4-triazine **(2')** was also isolated almost quantitatively.

To generalise the above selective aminolysis reaction, the reaction was repeated with a few amino alcohols and phenols (Scheme **3.2**). Hence, diethanolamine (**8m**), 4-aminophenol (**8p**) gave N,N-bis (2-hydroxy ethyl) benzamide (**20m**), N-(2-hydroxyphenyl) phenyl benzamide (**20p**) respectively. Characterisation data has been provided in Table **3.5**. In all the above cases DTT (**2'**) was regenerated. Regeneration of DTT (**2'**) was again evidenced by spectrophotometric monitoring studies (Fig. **3.28 - 3.31**).

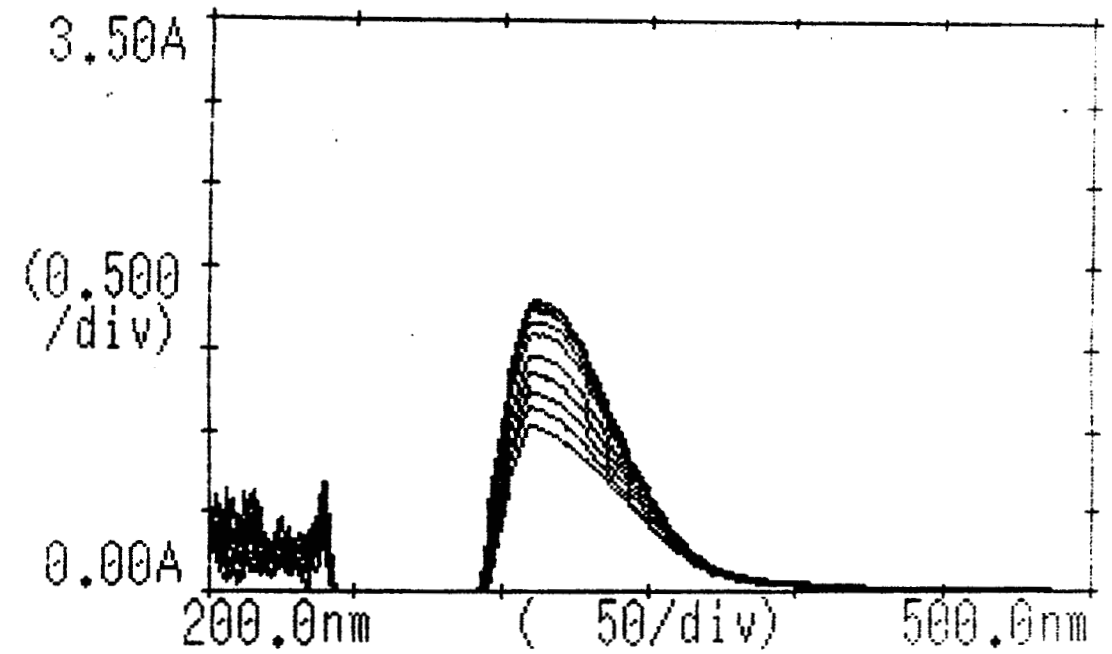


Fig. 3.28. UV-visible spectrum – Reaction of N-PaDTT (6') with aniline.

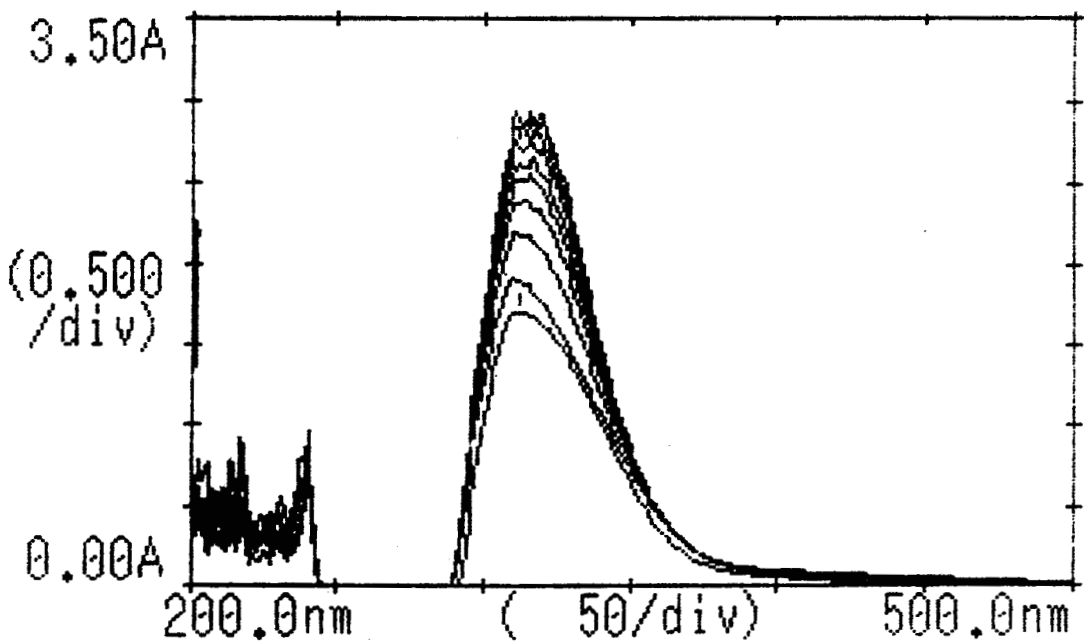


Fig. 3.29. UV-visible spectrum – Reaction of N-PaDTT (6') with *o*-toluidine.

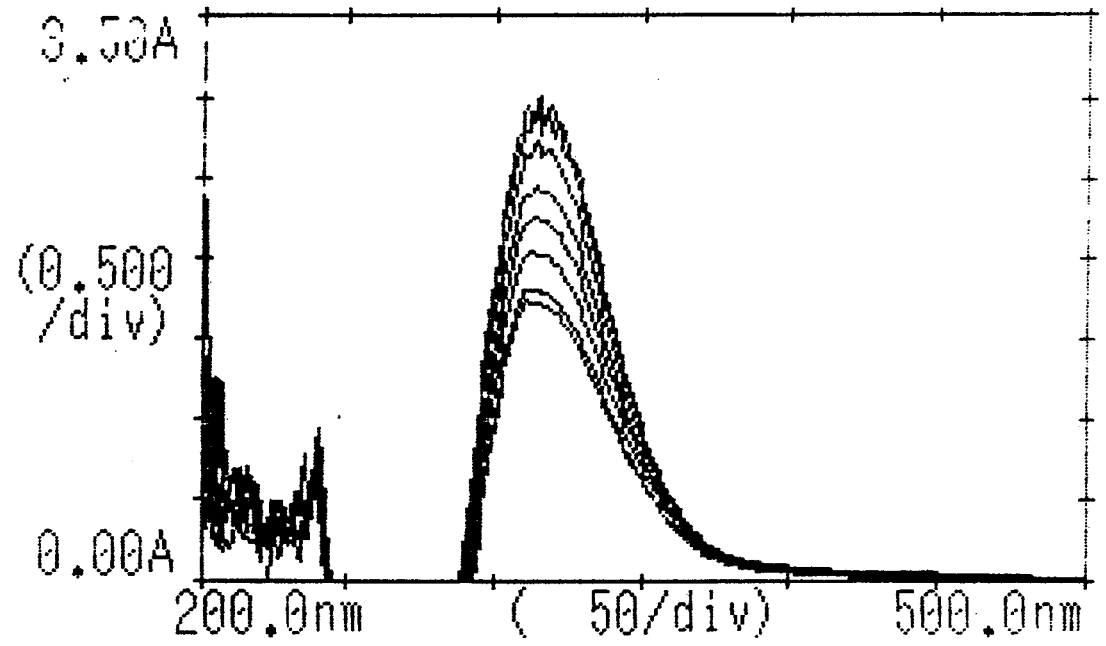


Fig. 3.30. UV-visible spectrum – Reaction of N-PaDTT (6') with *n*-propylamine.

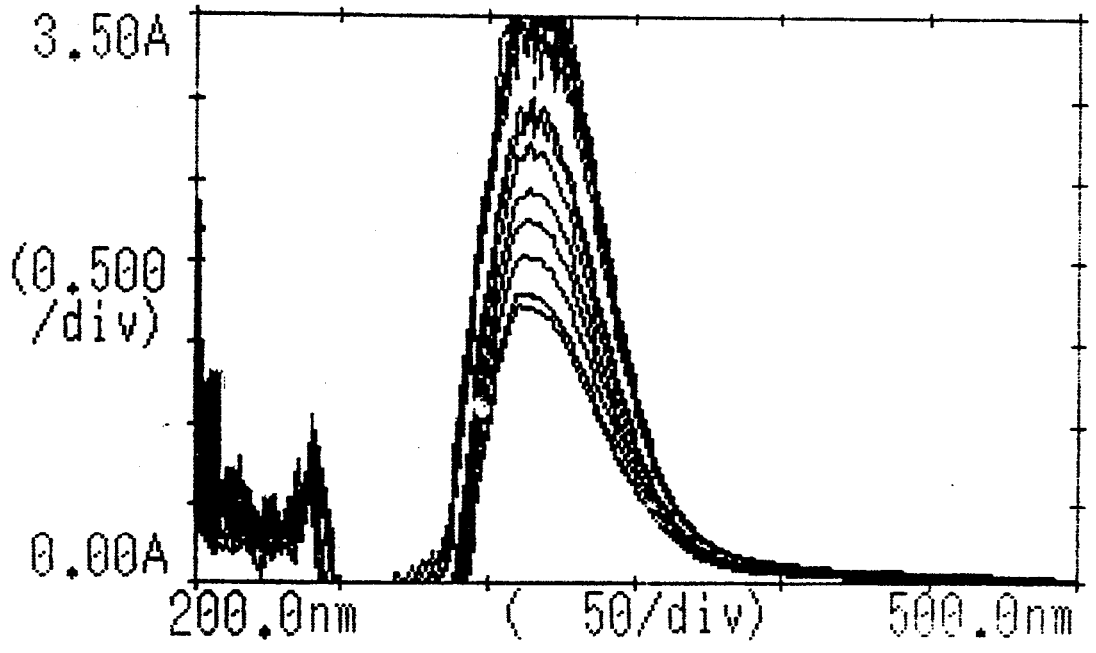


Fig. 3.31. UV-visible spectrum – Reaction of N-PaDTT (6') with *n*-butylamine.

3.2.7. Spectrophotometric monitoring of aminolysis and esterification using N-acyl-5,6-diphenyl-1,2,4-triazine-3-thione with amines, amino alcohols and alcohols/phenols

When a dilute solution of aniline (0.1 mmol) was mixed with an equimolar solution of N-BzDTT **(3')** in chloroform and the course of aminolysis reaction was followed by spectrophotometric scannings at every 30 secs, it was found that the initial absorbance value at 1.7 gradually increased and after 5 minutes it remained constant Fig. **3.14** It is obvious from the relative absorbance values that, as the reaction proceeds, more and more DTT **(2')** is regenerated so that absorbance value gradually increased. After 5 minutes, absorbance value remained unchanged. Initial sudden jump in the absorbance shows that the major portion of the aminolysis would have completed with in 2-4 minutes. The gradual increase in the absorbance after the above period is a clear indication of the slow process. The clear demonstration of the aminolysis reaction as a spectrophotometric tool paved the way to extend the reaction using different aliphatic and aromatic primary amines and also selective aminolysis with amino alcohols. Thus, the spectrophotometric scannings were repeated using *o*-toluidine (Fig. **3.15**), *n*-propylamine (Fig. **3.16**), *n*-butylamine (Fig. **3.17**) and benzyl alcohol (Fig. **3.18**). In all spectral scannings the regeneration of DTT **(2')** is in confirmity with the nucleophilicity of NH_2 group or OH group present.

From the experimental observations, it is very much obvious that the esterification rections of N-BzDTT **(3')** is sluggish at room temperature conditions.

As already evidenced, the weak nucleophilic nature of the alcohols and phenols were also established by the spectrophotometric studies by carrying out esterification reactions using N-BzDTT **(3')** with different alcohols like ethanol, benzyl alcohol, m-nitrophenol and m-cresol. Thus, when a chloroform solution of benzyl alcohol (0.1 mmol) was added to an equimolar solution of N-BzDTT **(3')** followed by scanning the UV-Visible spectrum (Fig. **3.18**) at definite time intervals of 30 secs, only a very slow increase in the absorption of the characteristic peak at 321 nm was observed. This shows that the regeneration of DTT **(2')** ie. rate of esterification is very slow.

3.2.7.1. Electronic effects on aminolysis and esterification

Fundamentally, electronic effects like inductive/mesomeric effects and the nucleophilicity of the amines or alcoholic group play an important role in the rate of different reactions. Therefore, in addition to the investigation on the extent of aminolysis/esterification of individual reactions, a comparative study on different amines, amino-alcohols and alcohols/phenols in order to make a correlation with the electronic arrangement of nucleophiles was also carried out. Effort is made here only to get a qualitative idea on the electronic effects operating and not for a quantitative treatment on the various kinetic parameters involved in it.

Aliphatic and aromatic amines like ethylamine, *n*-propylamine, *n*-butylamine, aniline, *o*-toluidine, *p*-chloraniline, *o*-ethylaniline, polyfunctional compounds like ethylenediamine, ethanolamine, diethanolamine, 4-aminophenol and hydroxy

compounds like benzyl alcohol, *n*-butylalcohol, *m*-nitrophenol and *m*-cresol were used to study the rate of benzoylation/esterification using N-BzDTT **(3')**.

The relative absorbance in aminolysis using various amines and amino alcohols has been plotted against time in seconds (Fig. **3.14-3.31**) so that the extent of the reaction could easily be studied and compared. From the figure, it is observed that aliphatic amines showed better reactivity than aromatic amines towards benzoylation. This may be due to the electron releasing effect of the alkyl groups in aliphatic amines and consequent increase in nucleophilicity on nitrogen centre of the amino group so that the extent of aminolysis is comparatively higher than that of aromatic amines. Amongst aliphatic amines, *n*-butyl amine showed the highest rate than that of *n*-propyl amine and ethyl amine, which is undoubtedly in agreement with the influence of electron releasing inductive effect of alkyl groups. Aniline showed the least reactivity towards benzoylation. Here electron withdrawing effect of the phenyl group would have influenced the reactivity at the nitrogen centre of the amino group. In the case of *m*-toluidine, a better reactivity than aniline has been observed. This may be due to the positive inductive effect of methyl group at the *meta* position which in turn makes the reaction faster than aniline.

Further, *o*-ethylaniline showed more reactivity than *m*-toluidine. This could be due to the presence of electron donating ethyl group at the *o*-position of the benzene ring. In aliphatic polyfunctional compounds like ethylenediamine, ethanolamine and diethanolamine an increase in the reaction trend was observed. This again may be due the increase in availability of electrons at the nitrogen atom of the amino group. An exceptional behaviour was observed in the case of *p*-

chloroaniline where a very high reactivity was noticed. This anomalous behaviour could not be explained on the basis of electronic effect.

It has already been discussed that esterification of N-BzDTT **(3')** using alcohols and phenols are sluggish in nature, probably due to the less nucleophilicity of the hydroxy group than amino group. Therefore, a thorough and systematic comparative spectrophotometric investigation using different alcohols/phenols could not be conducted. However, the behaviour of benzyl alcohol towards the reactivity with N-BzDTT **(3')** was compared with that of *m*-nitrophenol.

The relative case of aminolsyis of benzoyl derivative with alphatic, aromatic and hydroxyamines and its very poor response in esterification with alcohols and phenols at room temperature have been discussed earlier. Spectrophotometric monitoring has been found to very successful in the case of benzoyl derivative of 3-mercapto-5,6-diphenyl-1,2,4-triazine (2) and hence the investigation was extended to other acyl derivatives like N-AcDTT **(4')**, N-PrDTT **(5')** and N-PaDTT **(6').**

It is obvious from the spectrophotometric investigations that aminolysis are comparably effective but esterification reactions are very weak as in the case of N-BzDTT **(3')**. However, the rate of aminolysis/esterification reactions are found to be in the order, N-BzDTT > N-PaDTT > N-AcDTT > N-PrDTT. This is in full agreement with the electronic effects of phenyl, phenyl acetyl, methyl and ethyl groups attached to carbonyl group in the respective acyl triazine deriatives. The leaving group ability of phenyl group is the highest among other groups due to its electron withdrawing nature than the phenyl acetyl group. The positive field effect

of ethyl group is better than that of the methyl group attached to carbonyl group and this is in tune with the observations form spectrophotometric experiments. In short, 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) could be used as a carboxyl activating group in peptide synthesis, in which the peptide linkage will be effectively formed with ease.

3.3. Experimental

3.3.1. Materials and Methods

The solvents were of reagent grade and further purified according to literature procedure. Melting points were determined in open capillaries on a Toshniwal capillary melting point apparatus and are uncorrected. Thin layer chromatography was carried out by using precoated silica gel plates. In column chromatography (100 cm x 2 cm) neutral alumina and silica gel were used as adsorbants and the solvent systems used were petroleum ether - ethyl acetate (4:1), chloroform, methanol and water.

UV-visible spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Monotoring studies were carried out spectrophotometrically by conducting the reactions in the cuvet of spectrophotometer. IR spectra were recorded on Shimadzu IR-470 spectrophotometer using KBr pellets. ^{1}HNMR and ^{13}CNMR and mass spectroscopic measurements were recorded elsewhere.

3.3.2. Synthesis 3-mercapto-5,6-diphenyl-1,2,4-triazine (2)

3.3.2.1. Benzoin

A solution of pure benzaldehyde (25 g, 0.235 mol) and rectified spirit (32.5 mL) was gently boiled with a 10% solution of sodium cyanide for half an hour. Cooled the contents in ice bath, filtered and washed with cold water, drained well and dried. The crude benzoin was then recrystallised from hot rectified spirit to yield pale yellow crystals. Yield. 22.5 g (90%), mp 137 °C.

3.3.2.2. Benzil

Benzoin (10 g) was treated with conc. HNO_3 (50 mL) and heated in water bath for 1.5 hours with occasional shaking. Poured into cold water (200 mL) with vigorous shaking until the oil crystallised completely as a yellow solid. Washed with water, filtered and recrystallised from ethanol to afford pale yellow shining crystals of benzil. Yield 8.1g (86 %), mp 95 °C.

3.3.2. 3. 3-Mercapto-5,6-diphenyl-1,2,4-triazine (2)

A solution of benzil (4.2 g, 0.01 mol) in ethanol (60 mL) was refluxed and to the refluxing solution an equimolar solution of thiosemicarbazide (1.82 g, 0.02 mol) in water (12 mL) was added. To the reaction mixture, ammonium acetate crystals were added till the solution became turbid. The orange red colour appeared initially became deep red orange after 1 h. The mixture was refluxed for another 10 h. The orange red colour appeared initially became deep red orange after 1 h. After 10 h, the reaction mixture was cooled in ice and the crystals separated were filtered.

Recrystlalised from alcohol to afford yellow crystals of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**), Yield 2.52 g(95%) , mp 205 °C.

3.3.3. N-BzDTT (3')

The benzoylation of 3-Mercapto-5,6-diphenyl-1,2,4-triazine **(2)** was carried out by Schotten Baumann method. 10 millimolar solution of **2** (2.65 g) was prepared in 10% NaOH. To this solution, benzoyl chloride (0.25 mL) was added in drops with thorough shaking. The sparingly soluble benzoyl derivative was separated as pale yellow solid. Filtered, washed with cold water and then recrystallised from ethanol to get pale yellow crystals of N-BzDTT **(3')** which was characterised by IR,NMR and Mass spectra. Yield 2.77 g (75%). mp 125°C.

Benzoyalation by DCC coupling method

DCC coupling method was also adopted to prepare N-BzDTT **(3')**. In a typical procedure a solution of benzoic acid (1.22 g, 10 mmol) and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**,2.65 g) in a 1:4 mixture of THF and CH_2Cl_2 (10 mL), was treated with DCC (10 mmol) in methylene chloride (5 mL) with constant stirring at < 5 °C for about 1 h. The precipitated DCU was filtered off. The filtrate was concentrated and separated by colum chromatography using silica gel column. Recrystallisation from alcohol afforded yellow crystals of N-BzDTT **(3')**. Yield 3.32 g (90%), mp 125°C. Mixed melting point with the sample prepared by the Schotten-Baumann method did not show any depression.

3.3.3.1. Aminolysis of N-BzDTT (3') Formation of amides (9)

To a solution of N-BzDTT (**3'**, 0.74 g, 2 mmol) in chloroform (10 mL), freshly distilled aniline (**8a,** 0.19 mL, 2 mmol) was added with constant stirring at room temperature. The orange red colour appeared immediately deepened after 5 minutes. The reaction was followed by tlc and also spectrophotometrically. The mixture was concentrated and then separated by column chromatography (neutral alumina). The first fraction separated was evaporated to dryness. White crystalline solid obtained was recrystallized from alcohol to afford benzanilide **(9a),** yield 3.35g, (90%),. mp 162°C (lit.[58] mp 163°C). The other fraction separated was isolated and identified as 3-mercapto-5,6-diphenyl-1,2,4-trazine (**2**) almost in quantitative yield.

Similar aminolysis reactions were repeated, with 2-methyl aniline (**8b**), 4-methylaniline (**8d**), 4-chloraniline (**8e**), 4-methoxyaniline (**8f**), benzylamine (**8g**), ethylamine (**8j**), so that respective amides N-benzoyl-2-methylaniline (**9b**), N-benzoyl-4-methylaniline (**9d**), N-benzoyl-4-chloraniline (**9e**), N-benzoyl-4-methoxyaniline (**9f**), N-benzoyl benzamide (**9g**), N-ethyl benzamide (**9j**) were formed. In all the above cases, 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was regenerated in almost quantitative yield. The characterisation of the products formed were already given in Table **3.2.** Sudden regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also clearly followed spectrophotometrically.

3.3.3.2. Reaction of N-BzDTT (3') with alcohols: Formation of esters (11)

N-BzDTT (**3'**, 1.48 g, 4 m mol) was dissolved in chloroform (20 mL) and treated with ethanol (25 mL). The mixture was heated to about 70 °C, for 5 minutes, the solution became deep red orange with a conspecuous odour of the ester. Then the reaction mixture was concentrated and separated using a neutral alumina column to afford ethyl benzoate (**11b**), yield 47% bp 211°C (lit.60 bp 212°C). Even though 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was regenerated, it was in very small quantity. The slow regeneration of **2** was also monitored spectrophotometrically.

Esterification reaction was generalised by extending the reaction using other alcohols like methyl alcohol (**10a**), benzyl alcohol (**10f**). The products obtained were methyl benzoate (**11a**) and benzyl benzoate (**11f**) respectively. In both cases, nature and course of reaction were similar to that of ethyl alcohol. Characterisation data of the products formed are already presented in Table **3.2**.

3.3.3.3. Selective aminolysis of N-BzDTT (3') with amino alcohols (8)

N-BzDTT (**3'**, 0.74 g; 2 mmol) was dissolved in chloroform (10 mL). Ethanolamine (**8m**, 0.12 g, 2 mmol) was then added to it. Stirred thoroughly for about 5 minutes. Intense red orange colour was developed indicating the sudden regeneration of DTT (**2**). The reaction was monitored by tlc and spectrophotometrically. The products formed were separated using neutral alumina column. The first fraction separated was concentrated and recrystallised from

alcohol to afford N-(2-hydroxy ethyl) benzamide (**9m**), yield .248g (75%), mp 161°C (lit[61]. mp 163°C).

Similar reaction was carried out using diethanolamine to get the respective amide, N,N-bis (2-hydroxyethyl) benzamide. Characterisation data are already given in Table **3.2.**

3.3.4. Synthesis of N-AcDTT (4')

DTT (**2**, 1.4 g, 4 mmol) and acetic acid (0.24 mL, 4 mmol) were dissolved in a mixture of THF and CH_2Cl_2 (10 mL) in 1:4 ratio and stirred in an ice bath. The precipitated DCU was filtered off and the filtrate was separated using silica gel column. The product obtained was pale red orange crystalline solid which was identified as N-AcDTT (**4'**). Yield 0.98 g.(80%), mp 180°C.

3.3.4.1. Aminolysis of N-AcDTT (4'): Formation of amides (12)

N-AcDTT (**4'**, 0.614 g, 2 mmol) in chloroform (10 mL) was treated with freshly distilled aniline (**8a**, 0.2 mL, 2 mmol) dissolved in chloroform at room temperature and stirred well for 15 minutes. A pale red orange colour was gradually developed. The product formed was evidenced by tlc. The reaction mixture was separated by alumina column and recrystallised from alcohol. The products separated were identified as acetanilide (**12a**) yield 0.228 g (85%), mp 112°C (lit.[58] mp 114 °C) and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**).

The aminolysis was also extended to other amines like 2-methyl aniline (**8b**), 3-methyl aniline (**8c**), 4-methyl aniline (**8d**), benzyl amine (**8g**). Corresponding

amides N-(*o*-tolyl) acetamide (**12b**), N-(*m*-tolyl) acetamide (**12c**), N-(*p*-tolyl) acetamide (**12d**), N-benzyl acetamide (**12g**) were formed. Characterisations data have been given in table **3.3**. The aminolysis reactions were also followed spectrosphotometrically.

3.3.4.2. Esterification of N-AcDTT (4'): formation of esters (13)

N-AcDTT (**4'**, 0.614 g, 2 mmol) in chloroform (10 mL) was treated with 10 mL of ethanol (**10b**). Stirred the solution for 1 hour. But there was no indication of regeneration of 3-mercapto-5,6-diphenyl-1,24-triazine (**2**). When the reaction mixture was heated to about 70°C, the red orange colour was developed immediately in addition to the fruity smell of the ester. The mixture was then separated by alumina column and the product obtained was identified by tlc and bp of ethyl acetate (**13b**) bp 75 °C (lit[59].bp 77 °C) 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also regenerated, not in quantitative yield.

The esterification reaction was then carried out with other alcohols like 1-propanol (**10c**), 1-buthanol (**10d**) to get the respective esters n-propyl acetate (**13c**), n-butyl acetate (**13d**). Simultaneously 3-mercapto-5,6-dipheyl-1,2,4-triazine (**2**) crystals were also obtained.

3.3.4.3. Reaction of N-AcDTT (4') with aminoalcohols (8): Formation of hydroxy amides (14)

A chloroform solution (10 mL) of N-AcDTT (**4'**, 0.614g, 2 mml) was reacted with ethanolamine (**8m**, 0.12g, 2 mmol). Shaken up the reaction mixture well for about 5 minutes. Intense red orange colour developed indicated the regeneration of

DTT (**2**). The product thus afforded was separated using neutral alumina column so that the first fraction obtained was characterised as N-(2-hydroxy ethyl) acetamide (**9m**). mp 165°C (lit.[59] mp 166°C) in 75% yield. (0.15 g). The reaction was monitored by tlc as well as spectrophotometrically indicating the regeneration of DTT (**2**) almost in quantitative yield.

To generalise the selective aminolysis reaction, the above procedure was repeated with diethanolamine (**8n**) & 4-aminophenol (**8p**). The respective amides obtained were N,N-bis (2-hydroxy ethyl) acetamide (**14n**), & N-(4-hydroxy phenyl) acetanilide (**14p**). 3-Mercapto-5,6-diphenyl-1,2,4-triazine (**2**) in all the above cases was separated almost in quantitative yield. Characterisation data of all the above products have been produced in Table **3.3**.

3.3.5. Synthesis of N-PrDTT (5')

An equimolar solution of DTT (**2**, 2.65 g, 10 mmol) and propionic acid (0.74 g, 10 mmol) in 10 mL of THF and methylene chloride mixture (1:4) was treated with an equal proportion of DCC (2.06 g, 10 mmol) in methylene chloride (3 mL). Stirred by keeping in an ice bath for 1 hour. The precipitated DCU was filtered off. Using silica gel column, the mixture obtained was separated. The formed product was evidenced by tlc and recrystallised from alcohol. The yellow crystalline compound was characterised as **N-PrDTT (5')** , yield 2.5 g (80%), mp 162 °C .

3.3.5.1. Aminolysis of N-PrDTT (5') with amines (8): formation of amides (15)

N-PrDTT (**5'**, 0.642 g, 2 mmol) in chloroform (10 mL) was treated with freshly distilled aniline (**8a**, 0.2 mL, 2 mmol). Gradually a pale red orange colour was developed. The reaction mixture was stirred for about 10 minutes. Intensity of the colour of the reaction mixture was increased and after 10 minutes it became steady, which showed the complete regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) from the reaction mixture. This was again confirmed by following tlc as well as spectrophotometrically. The mixture was separated by neutral alumina column. The compound eluted first was recrystallised from alcohol and characterised as N-phenyl propionamide (**15a**) yield 2.1 g (72%), mp 104°C (lit.[58] mp 105°C).

With a view to generalising this acylation reaction, the above aminolysis reaction was repeated with 4-methyl aniline (**8d**), ethyl amine (**8j**), *n*-propyl amine (**8k**) and *n*-butyl amine (**8l**) so that respective amides, N-(*p*-tolyl) propionamide (**15d**), N-ethyl propionamide (**15j**), N-(*n*-propyl) propionamide (**15k**), N-(*n*-butyl) propionamide (**15l**) were afforded. The characterisation data are presented in Table **3.4** .There was simultaneous separation of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**).

3.3.5.2. Esterification reaction of N-PrDTT (5') using alcohols and phenols (10) : formation of esters (16)

Esterification N-PrDTT (**5'**) was also studied and found to be poor when compared to aminolysis reactions. Thus when a solution of N-PrDTT (**5'**, 1.44 g, 20 mmol) in chloroform (40 mL) was treated with methyl alcohol (**10a**, 20 mL), even

after stirring for about 2 hours, no regeneration of 3-mercapto-5,6-diphenyl 1,2,4-triazine occurred. But when the reaction mixture was heated to about 70 °C, fruity smell of the ester was noticed, with simultaneous development of red orange colour indicating the regeneration of 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). When the products were separated using neutral alumina column yielded methyl propionate (**16a**) which was also evidenced by tlc. The course of esterification was also monitored spectrophotometrically; yield 44%, bp 75°C (lit.[59] bp80 °C).

This esterification reaction was also extended to other alcohols like 1-propanol (**10c**), 1-butanol (**10d**) and benzyl alcohol (**10f**). In all cases respective esters *n*-propyl propionate (**16c**), *n*-butyl propionate (**16d**) and benzyl propionate (**16f**) were isolated. Characterisation data are shown in Table **3.4.**

3.3.5.3. Reactions of N-PrDTT (5') with aminoalcohols and phenols (8): selective aminolysis

An equimolar proportion of N-PrDTT (**5'**, 0.74, 10 mmol) in chloroform (20 mL) and ethanolamine (**8m**, 10 mmol) was stirred for about 20 minutes, the red orange colour was gradually developed. The mixture thus obtained when subjected to column chromatographic separation (neutral alumina), different products were separated. The first fraction recrystallised from alcohol afforded yellow crystals of N-(2-hydroxymethyl propionamide **(17m)** yield 75%, mp 143°C (lit.[58] mp 145°C). The regenerated 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was almost in quantitative yield.

The selective aminolysis was then extended to diethanolamine (**8n**) & 4-aminophenol (**8p**) to obtain respective hydroxyamides, N,N-bis (2-hydroxy ethyl) propionamide (**17n**), N-(4-hydroxy phenyl) propionamide (**17p**). Characterisation data of the products obtained are produced in Table **3.4**. In all of the above cases regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was in good yield.

3.3.6. Synthesis of N-PaDTT (6')

An equimolar solution of phenyl acetic acid (2.68 g; 20 mmol) and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**, 5.3 g, 20 mmol) in 40 mL THF and CH_2Cl_2 mixture (1:4) were treated with an equivalent quantity of DCC (4.12 g; 20 m mol) in 7 mL CH_2Cl_2 with constant stirring in an ice bath. The reaction mixture was stirred for 1 hour in the ice bath. Precipitated DCU was fillered off and the concentrated reaction mixture was separated using silica gel column. Product separated recrystallised from alcohol to afford dark green amorphous mass of N-PaDTT (**6'**) yield 5.72 g (85%), mp 191°C.

3.3.6.1. Aminolysis of N-PaDTT (6') with amines (8): formation of amides (18)

N-PaDTT (**6'**, 0.268 g; 2 mmol) in chloroform (10 mL) was mixed up with freshly distilled aniline (**8a**, 0.2 mL, 2 mmol). The reaction mixture was stirred for 5 minutes under the room temperature conditions. During the course of reaction, the dark green colour changed to red orange indicating the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). The products were separated using alumina column. The first fraction was identified as phenyl acetyl aminobenzene (**18a**) yield 0.31 g

(85%), mp 117°C (lit.[58] mp 118°C). 3-Mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also separated as biproduct in almost quantitative yield.

To verify the generality of the above reaction, aminolysis was extended to other amines like 2-methylaniline (**8b**), 4-methylaniline (**8d**), benzyl amine (**8g**), ethyl amine (**8j**). The respective amides phenyl acetyl amino (2-methyl) benzene (**18b**), phenyl acetyl amino(4-methyl)benzene (**18d**), N-Benzoyl-2-phenylethanamide (**18g**), phenyl acetylamino ethane (**18j**), were obtained in 70-80% yield. The reactions were also followed by tlc and spectrophotometrically. Using neutral alumina column all the products were seperated and characterised. The characterisation data are given in Table **3.5.** In all aminolysis reactions 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was isolated almost in quantitative yield.

3.3.6.2. Reaction of N-PaDTT (6') with alcohols & penols (10) : formation of esters (20)

Ethyl alcohol (**10b**, 20 mL) was treated with a solution of N-PaDTT (**6'**, 1.34 g, 10 mmol) in chloroform (40 mL). Even after stirring throughly for about 2 hours at room temperature, there was no indication for the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**). But, when the reaction mixture heated to about 70°C, dark green colour was gradually changed to red orange. Simultaneously the pleasant fruity odour of the ester formed was also noticed. The colour change to red orange was the indication of the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-trazine (**2**). Column chromatographic separation (neutral alumina) afforded ethyl phenyl acetate (**19b**) which was also evidenced by tlc.

The esterification reaction was then repeated with other alcohols like methanol (**10a**), 1-pentanol (**10e**), benzyl alcohol (**10f**). The corresponding esters obtained were methyl phenyl acetate(**19a**), *n*-pentyl phenyl acetate(**19e**), benzyl phenyl acetate(**19f**). 3-Mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was isolated in all the above cases as a biproduct. The course of reactions were evidenced by tlc and spectrophotometric measurements. Characterisation data have been produced in Table **3.5**.

3.3.6.3. Selective aminolysis reaction of N-PaDTT (6') with amino alcohols and phenols (20)

A chloroform solution (20 mL) of N-PaDTT (**6'**, 1.34g, 10 mmol) was stirred with ethanolamine (**8m**, 0.6 mL, 10 mmol). The dark green coloured solution, thus obtained, was gradually changed to a pale red orange solution indicating the regeneration of DTT (**2'**). The mixture, after 15 minutes stirring was then separated using neutral alumina column. The first fraction obtained was recrystallized from alcohol and identified as N-(2- hydroxyethyl) phenyl acetamide (**20m**) yield 1.4 g (80%), mp 150 °C (Lit.[58] mp 152°C),. Simultaneously 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) was also regenerated almost in quantitative yield.

Selective aminolysis was then generalised by extending to diethanolamine (**8m**) and 4-aminophenol (**8p**) so that N,N-bis(2-hydroxyethyl) benzamide (**20n**) and N-(2-hydroxyphenyl) benzamide (**20p**) were obtained in addition with the regeneration of 2-mercapto-5,6-diphenyl-1,2,4-triazine (**2**) (Table **3.5**).

3.3.7. UV-Visible absorption spectra of N-BzDTT (3'), N-AcDTT (4'), N-PrDTT (5') and N-PaDTT (6') during aminolysis and esterification

To carry out the spectrophotometric investigation on aminolysis of **N-BzDTT (3')**, a very dilute solution (0.1 mmol) was prepared in chloroform and taken in the cuvet of Schimadzu UV-160A spectrophotometer. The initial absorbance value was noted. Then a 0.01 mmol solution of ethylamine in chloroform was transferred to the cuvet. Stirred well with the aid of a capillary tube and the absorbance value measured immediately. Then the solution was stirred again and absorbance values were measured. The procedure was repeated at an interval of 30 secs.

The same procedure was repeated for the spectrophotometric monitoring of aminolysis/esterification of N-AcDTT **(4')**, N-PrDTT **(5')** and N-PaDTT **(6')** with different amines and alcohols at regular time intervals of 30 secs (Fig.**3.14-3.31**).

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ACTIVATION OF CARBOXYL GROUP THROUGH 2-MERCAPTO- 4,6-DIMETHYLPYRIMIDINE

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ACTIVATION OF CARBOXYL GROUP THROUGH 2-MERCAPTO- 4,6-DIMETHYLPYRIMIDINE

Chapter 4

ACTIVATION OF CARB OXYL GROUP THROUGH 2-MERCAPTO-4,6-DIMETHYLPYRIMIDINE

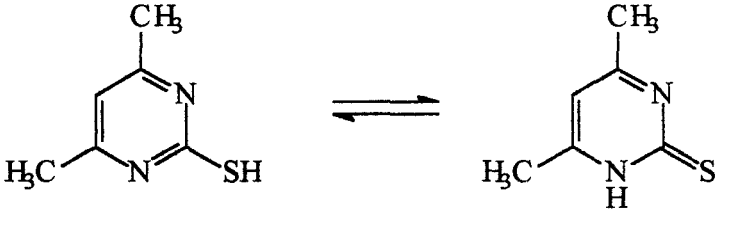
4.1. Introduction

A number of heterocyclic systems having a thiol function have been proved to be effective in carboxyl group activation. Actually thiol esters are more reactive than esters towards nucleophilic substitution.[1] A number of new methods have been developed for the preparation of thiol esters[2-4]. Active role of thiol esters in biochemical processes and their reactivity with various nucleophiles have led investigators to choose them as attractive synthetic intermediates in organic synthesis.[5-7] Their high reactivity may be due to the better leaving group ability of the thiide group than that of the alkoxide.

This led to investigate carboxyl group activation *via* heterocyclic thiols, especially using triazine and diazine thiols. In the preceding chapter, it has been shown that 3-mercapto-5,6-diphenyl-1,2,4-triazine acts as a potent carboxyl activating group. In order to find out another choice of carboxyl activating group, the studies on 2-mercapto-5,6-dimethylpyrimidine have been carried out in detail. Hence the work presented in this chapter is the investigation on the effectiveness of 2-mercapto-4,6-dimethylpyrimidine as carboxyl activating group.

In order to establish the carboxyl activating nature of 2-mercapto-4,6-dimethylpyrimidine **(1)**, as in the case of 3-mercapto-5,6-diphenyl-1,2,4-triazine

(Chapter 3) it has been converted to various acyl derivatives by suitable acylation reactions so that S-acyl derivatives which easily tautomerise to the more stable N-acyl derivatives are formed. These derivatised compounds were found to be very efficient in acylation when subjected to aminolysis and esterification reactions. During the course of these reactions, the amides are isolated in very good yield in addition to the formation of equivalent amount of original thiol. The UV-visible spectra of the pyrimidines and other monocyclic azines have been theoretically interpreted by Green and Tong[29]. Any how, the spectra of 2- and 4-mercaptopyrimidines, and those of the available S- and N-methyl derivatives, indicate[30,31] that these compounds exist largely in the thiol form in the neutral aqueous solution. The efficiency of these acyl derivatives in aminolysis and esterification processes were measured spectrophotometrically by scanning the UV-visible spectrum of the compound regenerated during the course of the reaction, in addition to the isolation of amides or esters, in very high yield.



1 **1'**

Thus the present chapter mainly deals with

i) the synthesis of 2-mercapto-4,6-dimethylpyrimidine (**1**) followed by analytical and spectral characterisations.

ii) the derivatisation of **1** to afford different acyl derivatives **2** using aliphatic and aromatic acids like benzoic acid, acetic acid, proprionic acid and phenylacetic acid and characterisation of these acyl derivatives using analytical and spectral methods.

iii) the investigation of compound **1** as carboxyl activating group, by carrying out aminolysis and esterification reactions on the above N-acyl derivatives using different amines and alcohols.

iv) the selective aminolysis reactions using amino alcohols and phenols.

v) the study of the electronic excitation behaviour of 2-mercapto-4,6-dimethylpyrimidine (**1**) spectrophotometrically which eventually leads to follow the rate of amide formation and esterification reactions.

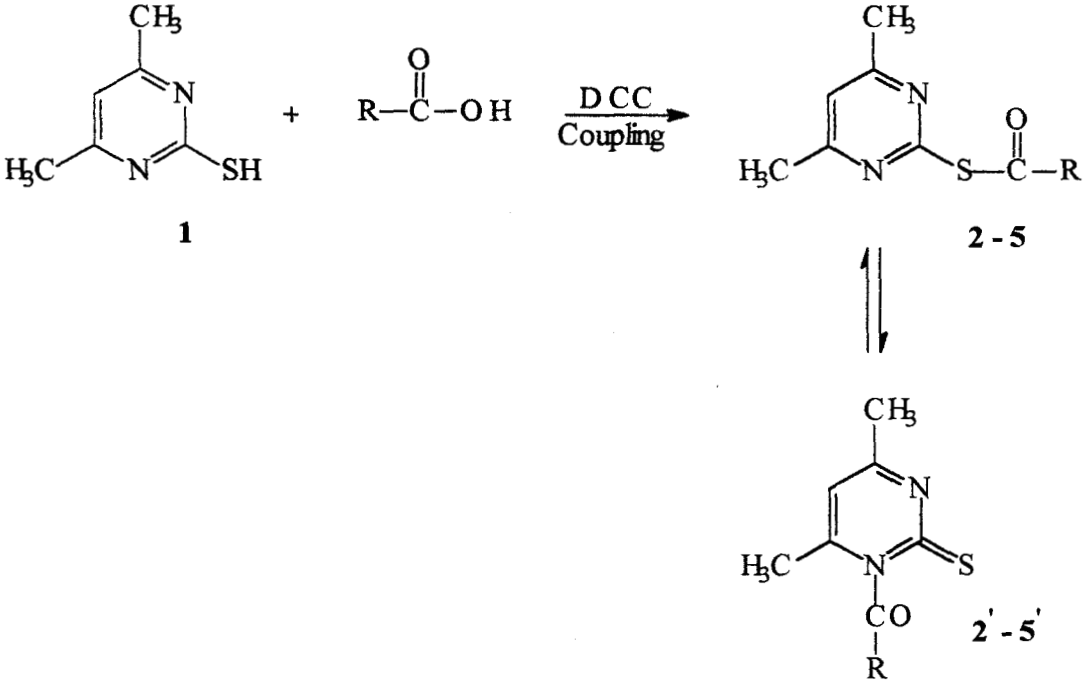
4.2. Results and discussions

4.2.1. Synthesis of 2-mercapto-4,6-dimethylpyrimidine (1)

To an aqueous solution of 2-mercapto-4,6-dimethylpyrimidine hydrochloride[12] sodium carbonate solution was added in drops with constant stirring. An yellow solid formed at the neutralisation point (pH 7.8) was separated and then recrystallised from ethanol to afford shining yellow crystals of 2-mercapto-4,6-dimethylpyrimidine (**1**) in 75% yield, mp 206°C (lit.[14] mp 209-210°C).

4.2.2. Synthesis of N-acyl-4,6-dimethylpyrimidine-2-thiones (2')

N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**) was synthesised by the DCC coupling method[13] as well as using benzoyl chloride applying the general procedure of acylation in the presence of triethylamine as base. Here in DCC coupling method an equimolar proportion of thiol **1** and benzoic acid in THF and methylene chloride (1:4) was stirred with DCC in methylene chloride in an ice-bath with constant stirring for about 1 hour. The precipitated DCU was then filtered off. The mixture thus obtained was separated by neutral alumina column. The product obtained (evidenced by tlc) was recrystallised from benzene to afford pale yellow crystals with m.p. 68°C in 85% yield which was characterised as N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**) by different analytical and spectral methods. The other N-acyl derivatives such as N-acetyl-4,6-dimethylpyrimidine-2-thione (**3'**), N-PrDPT (**4'**) and N-PaDPT (**5'**) were also obtained from acetic acid, propionic acid, phenyl acetic acid respectively (Scheme **4.1**).



2, 2′:R = C_6H_5–
3, 3′:R = CH_3–
4, 4′:R = CH_3–CH_2–
5, 5′:R = C_6H_5–CH_2–

Scheme 4.1

The details of different N-acyl derivatives prepared and their spectral (Fig. **4.1-4.6)** and other characterisation data are presented in Table **4.1**. ^{13}CNMR spectrum (Fig. **4.6)** clearly indicated the different types of carbon atoms present in compound **2′**.

Table 4.1. Characterisation Data of N-Acyl-4,6-dimethylpyrimidine-2-thiones

Acyl derivative	Mp (°C)	Yield (%)	Spectral data		
			UV ($CHCl_3$, nm)	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, δ)
N-Benzoyl-4,6-dimethylpyrimidine-2-thione [N-BzDPT **(2')**]	68	85	301 358	1236, C=S 1436, C=S 1705, C=O	2.37(6H,*m*) 6.7(1H,*s*) 7.2-7.9(4H,*m*)
N-Acetyl-4,6-dimethylpyrimidine-2-thione [N-AcDPT **(3')**]	210	80	303 362	1236, C=S 1439, C=S 1709, C=O	2.35(9H,*m*) 6.5(1H,*s*)
N-Propionyl-4,6-dimethylpyrimidine-2-thione [N-PrDPT **(4')**]	187	72	302 360	1191, C=S 1367, C=S, 1728, C=O	2.35(9H,*m*) 3.7(2H,*m*) 6.5(1H,*s*)
N-Phenylacetyl-4,6-dimethylpyrimidine-2-thione [N-PaDPT **(5')**]	110	85	307 355	1243, C=S 1436, C=S 1712, C=O	2.2(6H,*m*) 3.4(2H,*m*) 6.6(1H,*s*)

Many experimental and theoretical studies have been reported[14-21] that pyridines and pyrimidines substituted with an -SH or -OH group at 2- or 4-positions are fundamentally tautomeric systems. In addition, the formation of the N-acyl derivatives from the tautomeric S-acyl-4,6-dimethylpyrimidine-2-thiones can reasonably be explained as an S→N thermal rearrangement of the kinetic product to the more stable N-acyl-4,6-dimethylpyrimidine-2-thione by close analogy with the rearranged products observed in the case of acyl derivatives of 2-thionothiazolidines.[22]

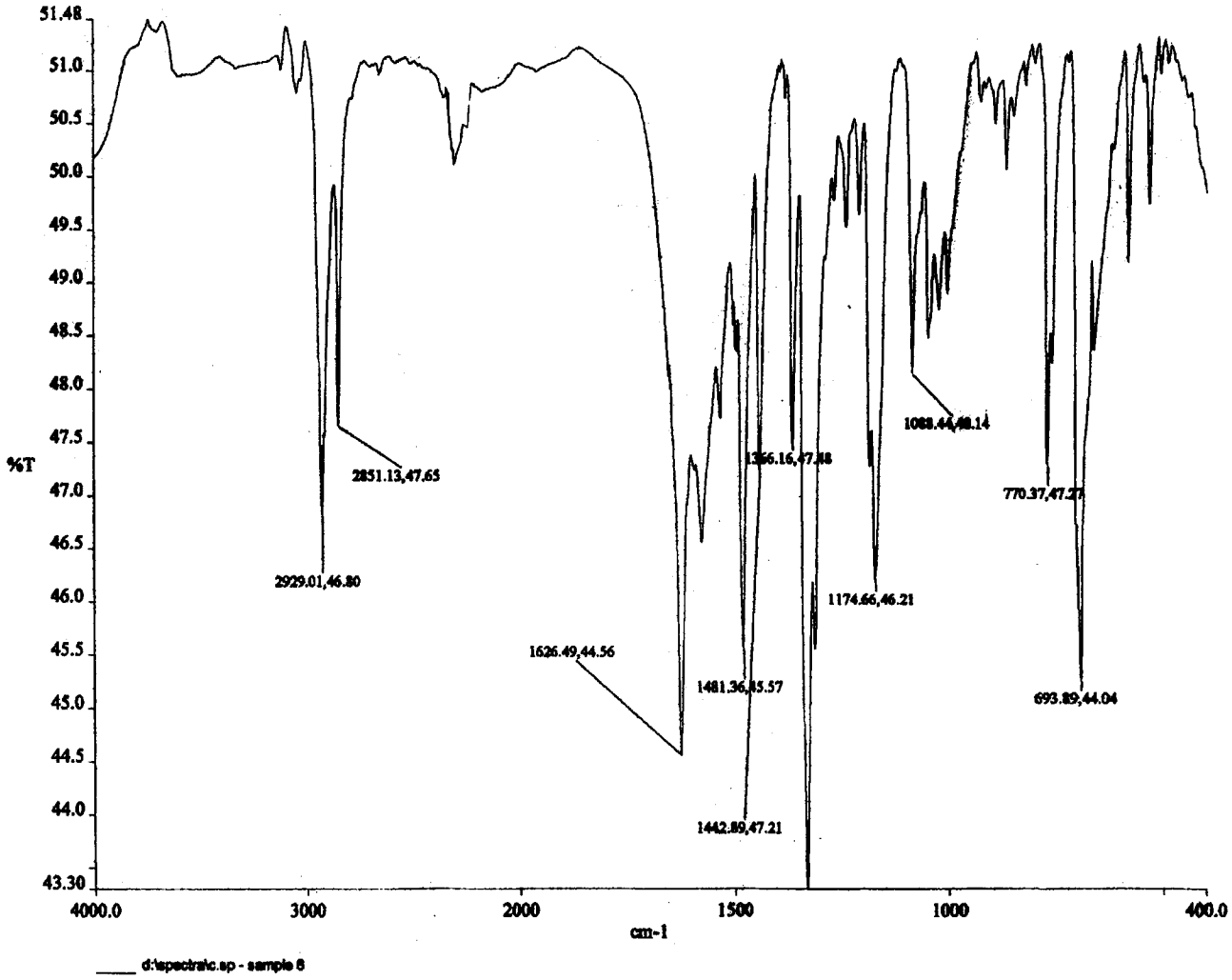


Fig. 4.1. IR (KBr) Spectrum of 2-Mercapto-4,6-dimethylpyrimidine (1)

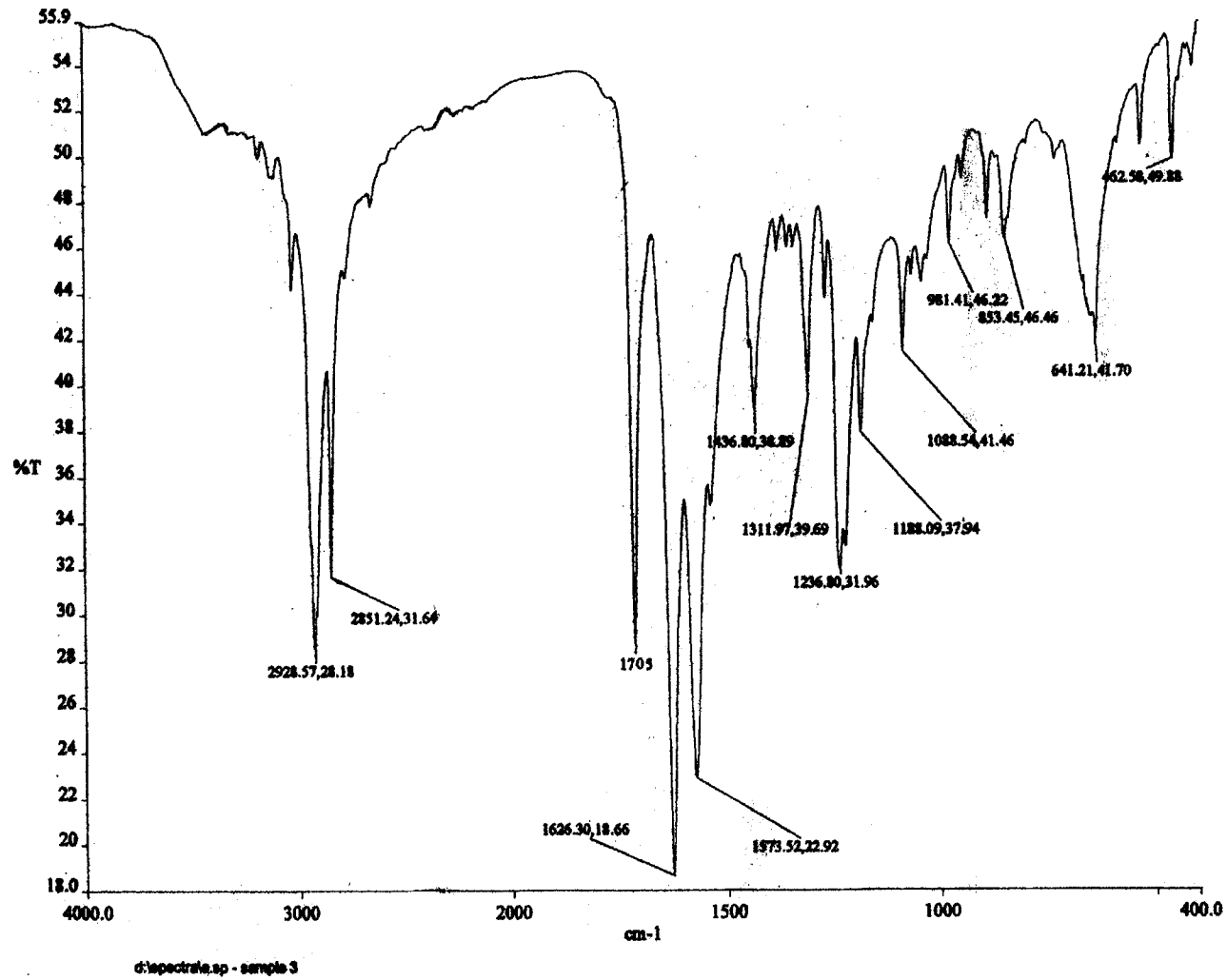


Fig. 4.2. IR (KBr) Spectrum of N-BzDPT (2')

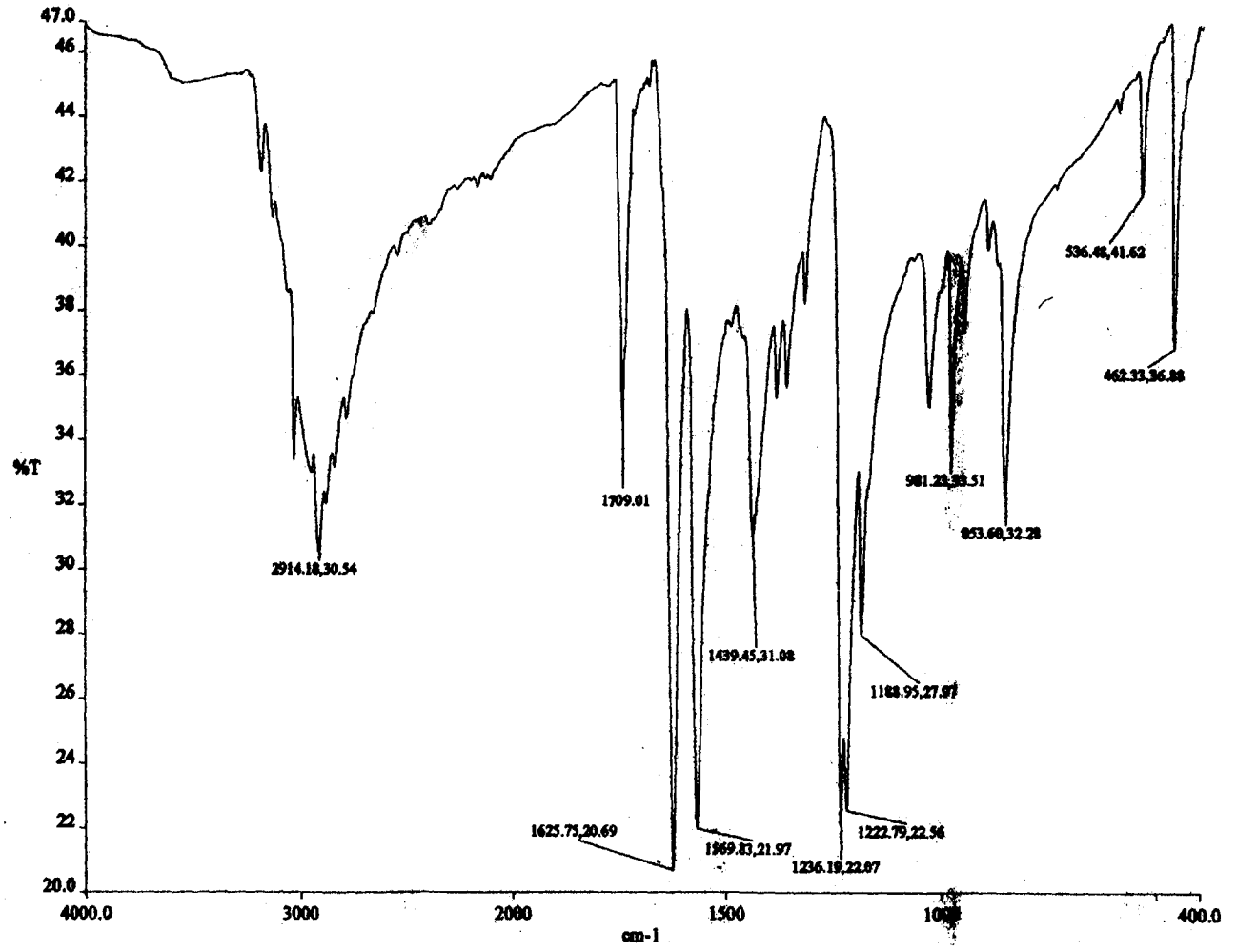


Fig. 4.3. IR (KBr) Spectrum of N-AcDPT (3')

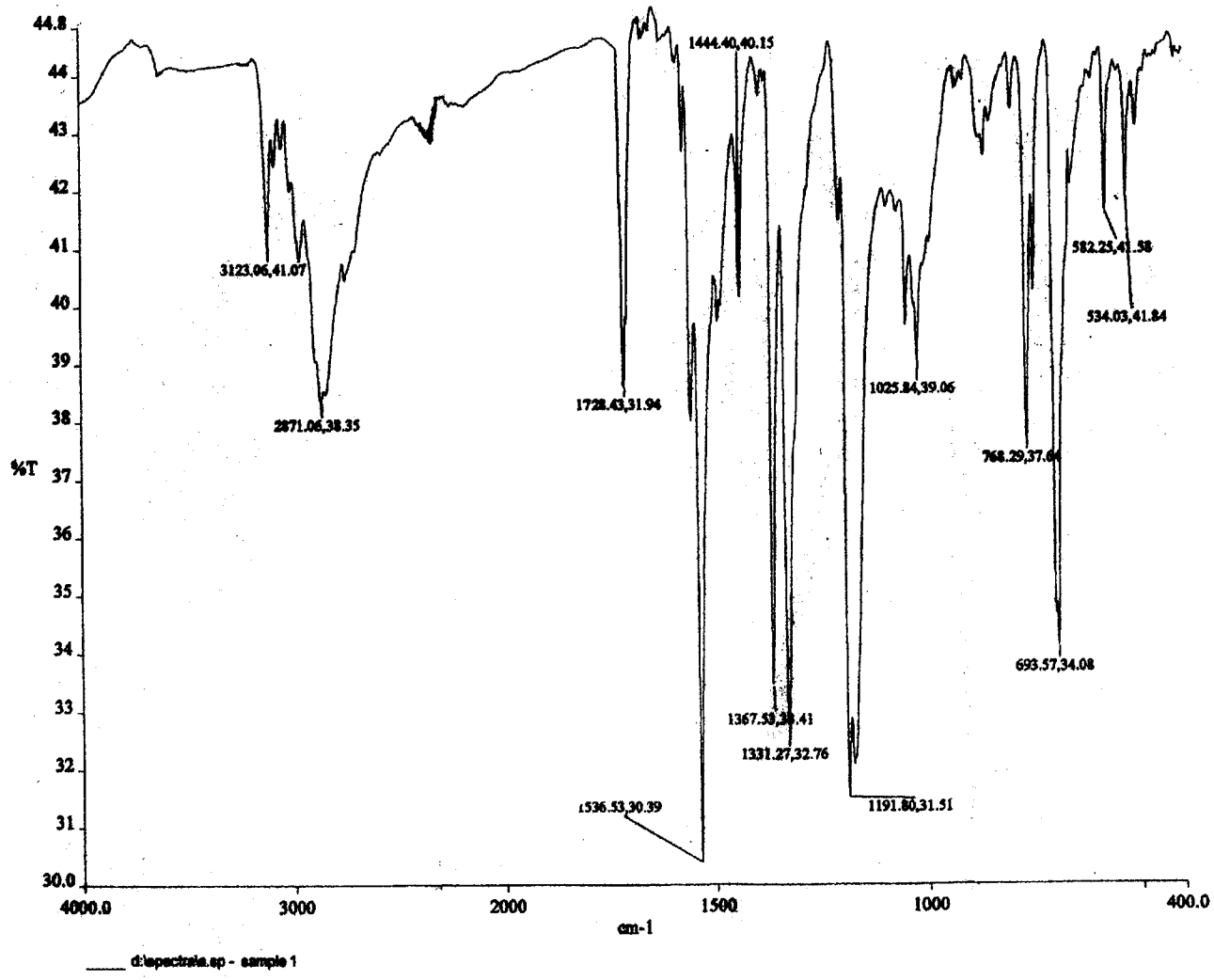


Fig. 4.4. IR (KBr) Spectrum of N-PrDPT (4')

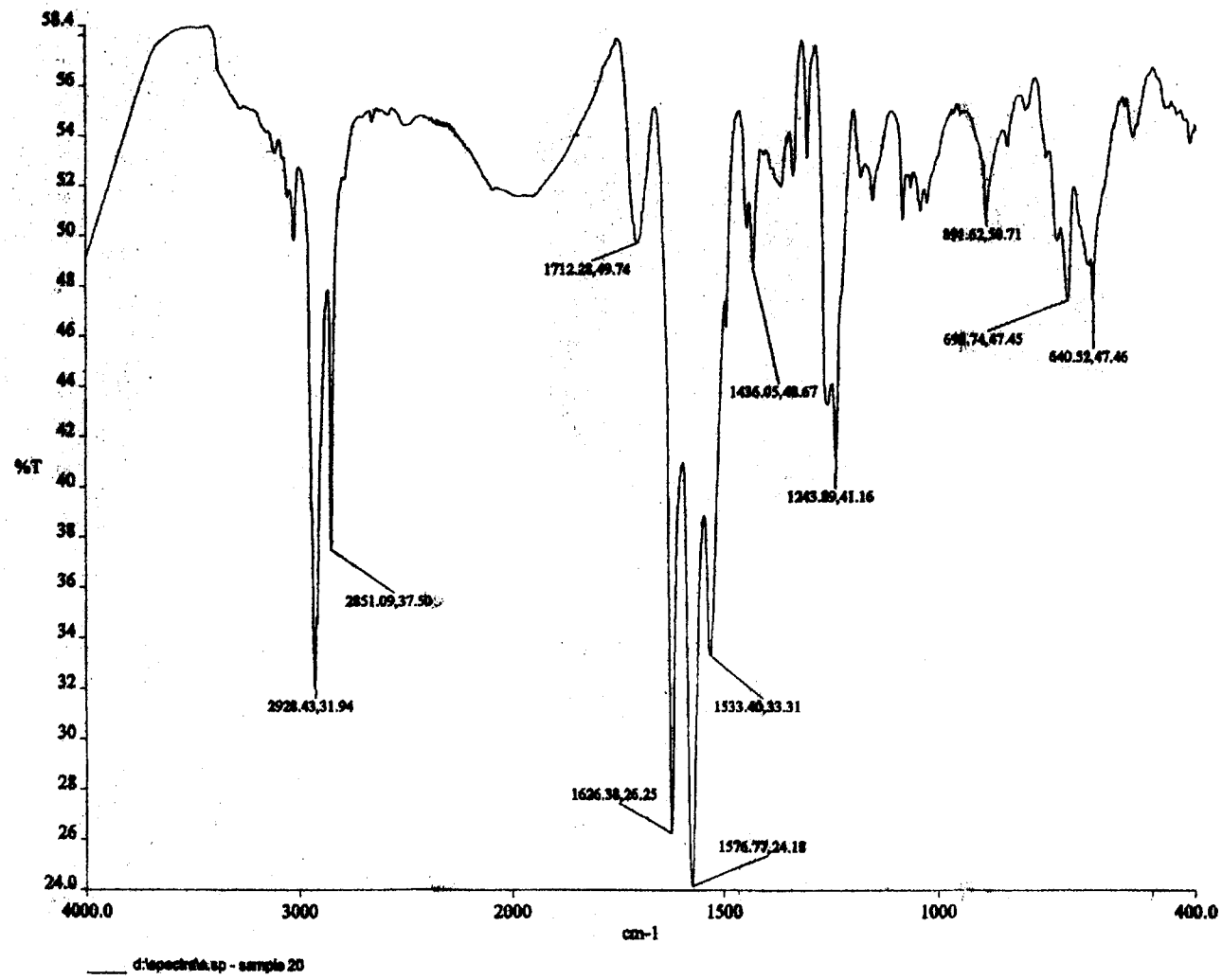


Fig. 4.5 IR (KBr) Spectrum of N-PaDPT (5')

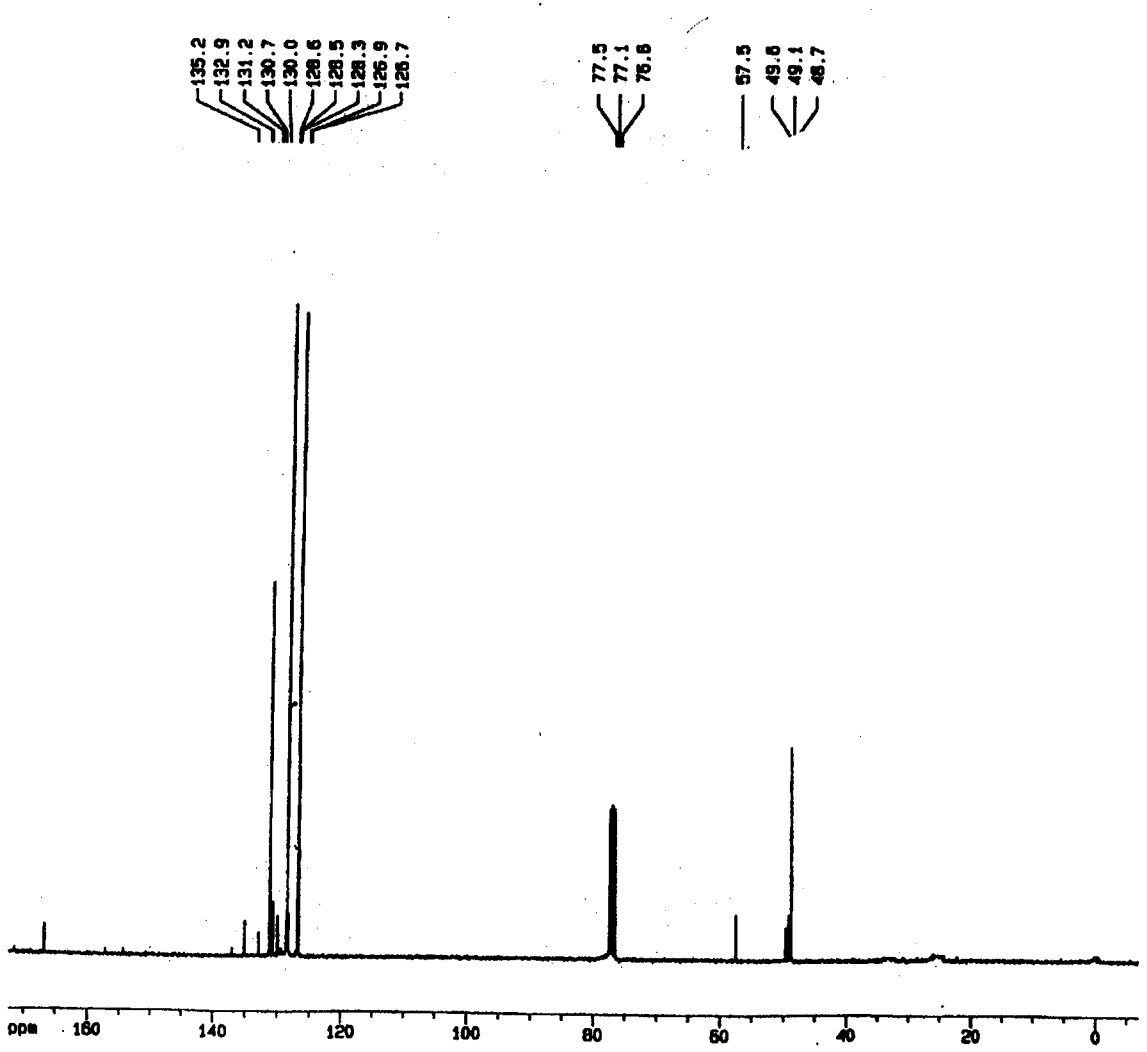


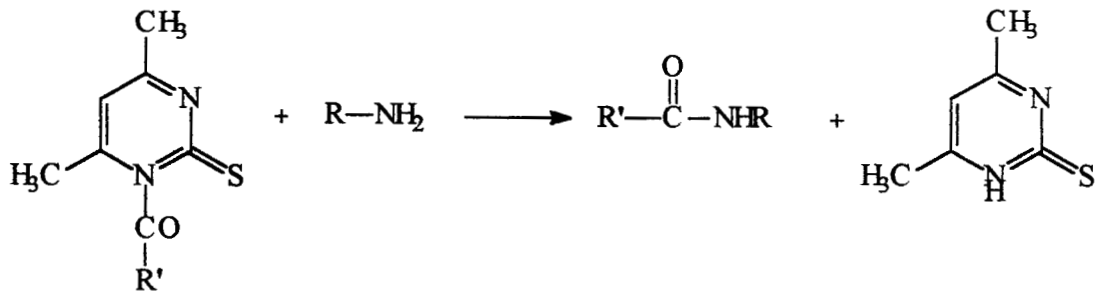
Fig. 4.6 ^{13}CNMR Spectrum of N-BzDPT (2')

4.2.2.1. Aminolysis of N-acyl-4,6-dimethylpyrimidines using amines (8): Formation of amides.

The suitability of 4,6-dimethylpyrimidine-2-thione (**1**) as a potent carboxyl activating group was established by carrying out the acyl group transferring efficiency of N-acyl-4,6-dimethyl-pyrimidine-2-thiones using aromatic and aliphatic amines. During the course of these aminolysis reactions respective amides were formed and were characterised. Moreover, the amide formation reactions were also monitored spectrophotometrically.

Thus, initially, a chlorofrm solution of N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**) was treated with an equimolecular amount of aniline (**8a**). The benzoylation rection was found to be very effective, at the same time, an yellow colour of 4,6-dimethylpyrimidine-2-thione (**1**) was observed, which was evidenced by tlc. The amide formed was separated by column chromatography, recrystallised from alcohol and the product obtained was identified as benzanilide **(9a)** by homogenous tlc, mp 159°C (lit.[24]mp 163°C) and mixed melting point, yield 85%. The aminolysis reaction was also followed spectrophotometrically by scannings the UV-visible spectra.

To generalise, the aminolysis reaction of N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2**) the reaction was repeated with other amines. The details are given in table **4.2.**



2′ **8a – p** **9a – p** **1**

2′:R′ = C_6H_5–	**8**	**9**
3′:R′ = CH_3–	**8**	**12**
4′:R′ = CH_3–CH_2–	**8**	**14**
5′: R′ = C_6H_5–CH_2–	**8**	**16**

R: **a**: C_6H_5–
b: *o*–$C_6H_4(CH_3)$–
c: *m*–$C_6H_4(CH_3)$–
d: *p*–$C_6H_4(CH_3)$–
e: *o*-$C_6H_4(OH)$–
f: *p*-$C_6H_4(OH)$–
g: *p*–$C_6H_4(Cl)$–
h: *p*-$C_6H_4(NO_2)$ –
i: *p*–$C_6H_4(OCH_3)$–
j: C_6H_5–CH_2–
k: $C_{10}H_7$–
l: CH_3–
m: CH_3–CH_2–
n: CH_3–CH_2–CH_2–
o: HO–CH_2–CH_2–
p: (HO–CH_2–CH_2–)$_2$

Scheme 4.2

In order to establish the carboxyl activated nature of N-acyl dimethylpyrimidine-2-thione, the aminolysis reactions were further carried out using other acylpyrimidine derivatives like N-AcDPT (**3'**), N-PrDPT (**4'**), N-PaDPT (**5'**)

with similar set of amines. In all the cases, the respective amides formed were separated by column chromatography. The amides obtained were in almost quantitative yield except in the case of N-PrDPT (**4'**). The formed amides were evidenced by tlc and characterised by analytical methods. The characterisation data of the amides are presnted in Tables **4.2, 4.3, 4.4** and **4.5** respectively.

Table 4.2. Benzoylation of amines (8) / alcohols (10) using N-BzDPT (2')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Reaction time (min.)	Yield (%)
Aniline (**8a**)	Benzanilide (**9a**)	159(163)[24]	10	85
2-Methyl aniline (**8b**)	N-Benzoyl-2-methyl aniline (**9b**)	143(144)[24]	8	90
3-Methyl aniline (**8c**)	N-Benzoyl-3-methyl aniline (**9c**)	123(125)[14]	9	82
4-Methyl aniline (**8d**)	N-Benzoyl-4-methyl aniline (**9d**)	160(158)[14]	8	87
1-Naphthyl amine (**8k**)	N-(1-Naphthyl) benzamide (**9k**)	159(161)[14]	12	70
2-Chloraniline (**8g**)	N-Benzoyl-2-chloraniline (**9g**)	95(99)[14]	15	55
4-Nitraniline (**8h**)	N-Benzoyl-4-nitraniline (**9h**)	189(199)[14]	15	50
Ethanolamine (**8o**)	N-(2-Hydroxyethyl) benzamide (**9o**)	156(163)[27]	12	77
Diethanol amine (**8p**)	N,N-bis(2-hydroxyethyl) benzamide (**9p**)	147(153)[27]	12	75
4-Amino phenol (**8f**)	N-(4-hydroxyphenyl) benzamide (**9f**)	231(234)[27]	15	65
Benzyl alcohol (**10a**)	Benzyl benzoate (**11a**)	bp 304(314)[14]	25	54*
Methyl alcohol (**10b**)	Methyl benzoate (**11b**)	bp 193(199)[14]	22	50*
Ethyl alcohol (**10c**)	Ethyl benzoate(**11c**)	bp 204(212)[14]	23	48*

* Yield Calculated based on the amount of DPT regenerated.

Table 4.3. Acylation of amines (8) / alcohols (10) using N-AcDPT (3')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Reaction time (min.)	Yield (%)
Aniline (**8a**)	Acetanilide (**12a**)	110(114)[24]	12	82
2-Methyl aniline (**8b**)	N-(*o*-Tolyl)acetamide (**12b**)	108(110)[24]	9	85
3-Methyl aniline (**8c**)	N-(*m*-Tolyl)acetamide (**12c**)	63(65.5)[24]	9	83
4-Methyl aniline (**8d**)	N-(*p*-Tolyl)acetamide (**12d**)	153(154)[25]	7	83
Ethyl amine (**8m**)	N-Ethylacetamide (**12m**)	190(205)[24]	7	77
n-Propyl amine (**8n**)	*n*-Propylacetamide (**12n**)	bp 211(215)[24]	8	74
Benzyl amine (**8j**)	N-Acetyl benzamide (**12j**)	56(61)[24]	10	78
4-Methoxylaniline (**8i**)	N-(4-Methoxyphenyl) acetamide (**12i**)	79(81)[24]	12	75
Ethanol amine (**8o**)	N-(2-Hydroxyethyl) acetamide (**12o**)	162(166)[25]	14	72
Diethanol amine (**8p**)	N,N-Bis(2-hydroxyethyl) acetamide (**12p**)	146(153)[24]	15	59
2-Aminophenol (**8e**)	N-(2-Hydroxyphenyl) acetamide (**12e**)	205(209)[24]	14	68
4-Aminophenol (**8f**)	N-(4-Hydroxyphenyl) acetamide (**12f**)	174(170)[24]	14	70
Benzyl alcohol (**10a**)	Benzyl acetate (**13a**)	bp 201(215.5)[24]	23	50*
n-Propyl alcohol (**10d**)	*n*-Propyl acetate (**13d**)	bp 92(101)[24]	22	45*
n-Butyl alcohol (**10e**)	*n*-Butyl acetate (**13e**)	bp 118(126.5)[24]	22	45*

* Yield calculated based on the amount of DPT regenerated.

Table 4.4. Acylation of amines (8) / alcohols (10) using N-PrDPT (4')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Reaction time (min.)	Yield (%)
Aniline (**8a**)	N-Phenyl propionamide (**14a**)	102(105)[24]	14	75
2-Methyl aniline (**8b**)	N-(*o*-Tolyl) propionamide (**14b**)	[illegible]	12	68
4-Methylaniline (**8d**)	N-(*p*-Tolyl) propionamide (**14d**)	124(126)[25]	12	70
Ethyl amine (**8m**)	N-Ethyl propionamide (**14m**)	147(149)[24]	11	77
n-Propylamine (**8n**)	N-(*n*-Propyl) propanolamide (**14n**)	152(154)[25]	11	75
Ethanol amine (**8o**)	N-(2-Hydroxyethyl) propionamide (**14o**)	141(145)[25]	15	60
Diethanol amine (**8p**)	N,N-bis(2-Hydroxyethyl) propionamide (**14p**)	172(179)[25]	16	58
4-Aminophenol (**8f**)	N-(4-Hydroxy phenyl) propionamide (**14f**)	149(156)[24]	18	50
Benzyl alcohol (**10a**)	Benzyl propionate (**15a**)	bp 210(220)[25]	25	46*
n-Propyl alcohol (**10d**)	Propyl propionate (**15d**)	bp 116(122)[25]	23	48*
n-Butyl alcohol (**10e**)	Butyl propionate (**15e**)	bp 134(145)[25]	23	46*

* Yield calculated based on the amount of DPT regenerated.

Table 4.5. Acylation of amines (8) / alcohols (10) using N-PaDPT (5')

Amines/ alcohols/ amino alcohols used	Amides / esters formed	mp/bp (lit. mp/bp) (°C)	Reaction time (min.)	Yield (%)
Aniline (**8a**)	Phenyl acetyl amino benzene (**16a**)	115(118)[24]	11	86
2-Methyl aniline (**8b**)	Phenyl acetyl amino (2-methyl) benzene (**16b**)	160(159)[25]	8	86
4-Methyl aniline (**8d**)	Phenyl acetylamino (4-methyl) benzene (**16d**)	138(136)[25]	10	90
Benzyl amine (**8j**)	N-Benzyl-2-phenyl ethanamide (**16j**)	120(122)[25]	10	90
Ethanol amine (**8o**)	N-(2-Hydroxyethyl) phenylacetamide (**16o**)	148(152)[25]	12	85
Diethanol amine (**8p**)	N,N-Bis(2-hydroxyethyl) phenylacetamide (**16p**)	127(136)[24]	15	78
4-Amino phenol (**8f**)	N-(4-Hydroxy phenyl) phenoxy acetamide (**16f**)	185(190)[24]	16	68
Benzyl alcohol (**10a**)	Benzyl phenyl acetate (**17a**)	bp 300(317)[25]	20	63
Methyl alcohol (**10b**)	Methyl phenyl acetate (**17b**)	bp 207(218)[25]	18	55*
Ethyl alcohol (**10c**)	Ethyl phenyl acetate (**17c**)	bp 217(227)[25]	18	53*
Isobutyl alcohol (**10f**)	Isobutyl phenyl acetate (**17f**)	bp 235(247)[24]	20	47*
n-Pentyl alcohol (**10g**)	*n*-Pentyl phenyl acetate (**17g**)	bp 256(265)[25]	20	50*

* Yield calculated based on the amount of DPT regenerated.

Further, it has been observed that amide formation with various acyl derivatives of 2-mercapto-4,6-dimethylpyrimidine (**1**) were effective and aminolysis reactions occurred irrespective of the nature of the acyl group present on 4,6-

dimethylpyrimidine-2-thione. Another specific observation noticed was that all the reactions took place at room temperature conditions and the original thione regenerated was almost in quantitative yield. In all the cases, the time taken for amide formation was around 10-15 minutes except with N-propionyl 4,6-dimethyl-pyrmidine-2-thione (**4'**).

Spectrophotometric monitoring studies of the acyl transfer reactions were repeated using acyl derivatives **2', 3', 4'** and **5'** so as to verify the regeneration of original thiol during the amide formation reactions, under room temperature conditions. In all the cases 0.1 millimolar solutions of acyl derivatives and respective amines in chloroform solutions were used. The UV-visible scannings, which show the regeneration of 2-mercapto-4,6-dimethylpyrimidine (**1**) were followed in every 30 seconds. In all the reactions, pale yellow colour was developed within 2-3 minutes showing the regeneration of thiol with simultneous formation of respective amides with the exception of N-propionyl-4,6-dimethylpyrimidine-2-thione (**4'**) in which the reaction was very slow. Some of the typical scanning results are presented in Fig. **4.7 - 4.15**

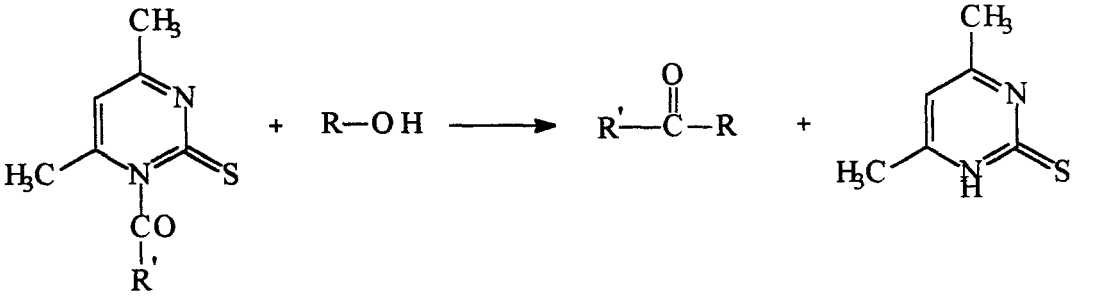
From the scanning results, slight differences have been observed in the rate of amide formation as, N-BzDPT (**2'**) > N-PaDPT (**5'**) > N-AcDPT (**3'**) > N-PrDPT (**4'**). This order of reactivity is in full agreement with the field effects of benzoyl, phenyl acetyl, acetyl and propionyl groups. Among the phenyl and phenyl acetyl groups, the latter is having a better electron releasing group and therefore acyl group cleavage is most facilated in benzoyl derivative. The same trend in rate of reactivity

in terms of isolated yields of amides has also been observed. In brief, all the N-acyl derivatives could be used as activated carboxyl components, benzoyl derivative being the best among them.

4.2.2.2. Esterification reactions of N-acyl-4,6-dimethylpyrimidine-2-thiones with alcohols: Formation of esters

Analogous to the aminolysis of N-acyl-4,6-dimethylpyrimidine-2-thiones with strong nucleophiles like amines, which resulted in the formation of amides, esterification reactions at room temperature were tried with weak nucleophiles like alcohols. Thus, when a 4 millimolar solution of N-BzDPT (**2'**) was dissolved in benzyl alcohol (**10a**) at room temperature, even after prolonged stirring for about 15 minutes, no characteristic colour change was observed. But when heated to 60-70 °C for 5 minutes, the mixture became pale yellow indicating the regeneration of 4,6-dimethylpyrimidine-2-thione (**1**) with a fruity smell. The formation of the products were also evidenced by tlc. Column chromatographic separation of the mixture afforded 4,6-dimethylpyrimidine-2-thione (**1**) and benzyl benzoate (**11a**) with bp 304 °C (lit[14]. bp 314°C)

Similar esterification was carried out using methanol (**10b**) and ethanol (**10c**) using the same procedure and the respective esters, methyl benzoate (**11b**) and ethyl benzoate (**11c**) were isolated (Scheme **4.4**).



2′ **10a-g** **11a-g** **1′**

2′:R′ = C_6H_5-	**10**	**11**
3′:R′ = CH_3-	**10**	**13**
4′:R′ = CH_3-CH_2-	**10**	**15**
5′:R′ = C_6H_5-CH_2-	**10**	**17**

R: **b**: C_6H_5- CH_2-
b: CH_3-
c: CH_3-CH_2-
d: CH_3-CH_2- CH_2-
e: CH_3-CH_2- CH_2-CH_2-
f: CH_3-$CH(CH_3)$- CH_2-
g: CH_3-CH_2- CH_2-CH_2- CH_2-

Scheme 4.4

In order to generalise the esterification reactions the other acyl pyrimidine derivatives, N-AcDPT (**3'**), N-PrDPT (**4'**) and N-PaDPT (**5'**) were also subjected to esterification using different alcohols.

The failure in esterification reactions at room temperature also prompted to carry out spectrophotometric monitoring similar to that of aminolysis. It has been clearly found that the esterification reactions using N-acyl-4,6-dimethylpyrimidine-2-thiones (Fig. **4.9, 4.12 & 4.15**) were sluggish as in the case of N-acyl-5,6-diphenyl-

1,2,4-triazine-3-thiones (Chapter **3.2.2.2**) which points to the weak nucleophilic nature of alcohol/phenols.

4.2.2.3. Selective aminolysis using N-acyl-4,6- dimethylpyrimidine-2-thione with amino alcohols and phenols: Formation of hydroxy amides

The success achieved in aminolysis reactions of N-acyl-4,6-dimethyl pyrimidine-2-thiones, prompted to extend the selective aminolysis reactions with aminoalcohols and phenols. Here the reactions were tuned to isolate the hydroxyamides and also to study the UV-visible spectra of the selective aminolysis reactions.

In a typical selective aminolysis procedure, an equimolecular proportion of N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**) and ethanolamine (**8o**) in chloroform were stirred thoroughly for 10 minutes at room temperature. Gradually a pale yellow colour was developed indicating the regeneration of 2-mercapto-4,6-dimethylpyrimidine (**1**) which was confirmed by tlc. Working up the reaction mixture by evaporation followed by column chromatographic purification gave a solid, which was recrystallised from alcohol. Thin layer chromatographic identification and melting point confirmed that the product is N-(2-hydroxymethyl) benzamide (**9o**), yield 77%, mp 156°C (lit[27]. mp 163°C).

The reaction was then repeated, with diethanolamine (**8p**) and 4-aminophenol (**8f**) so that respective hydroxy amides, N,N-bis(2-hydroxyethyl) benzamide (**9p**) and

N-(4-hydroxyphenyl)benzamide (**9f**) were isolated. The characterisation data of the products formed are presented in Table **4.2**.

The carboxyl activation efficiency of 4,6-dimethylpyrimidine-2-thione (**1**) towards selective aminolysis reactions were again studied using N-AcDPT (**3'**), N-PrDPT (**4'**) and N-PaDPT (**5'**), so that respective hydroxyamides were afforded without much difference in the nature of the reactivity. Anyhow, the selective aminolysis reaction with N-propionyl-4,6-dimethylpyrimidine-2-thione (**4'**) was weak compared to other acyl derivatives. Characterisation details have been presented in tables **4.3, 4.4** & **4.5**. In all the cases, 2-mercapto-4,6-dimethylpyrimidine (**1**) was regenerated in very good yield.

Similar to aminolysis reactions using N-acyl-4,6-dimethylpyrimidine-2-thione, selective aminolysis was also followed by spectrophotometric measurements. Here, again, the experiments were conducted using 0.1 mmolar solutions of respective acyl derivative and each amino alcohol and the scannings were done in every 30 secs at room temperature till the completion of the reaction. The increase in the absorbance clearly indicated the regeneration of 2-mercpto-4,6-dimethylpyrimidine (**1**) during the selective aminolysis reactions. In all these experiments, the spectrophotometric measurements were fully in agreement with the rate of observed yield of hydroxy amides.

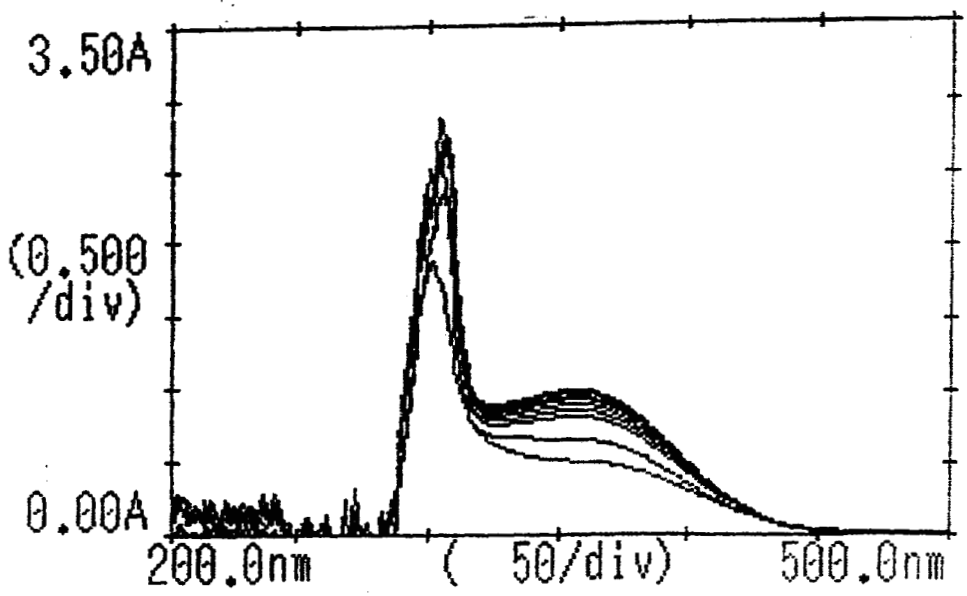


Fig. 4.7. UV-Visible spectrum: Reaction of N-AcDPT (3') with aniline

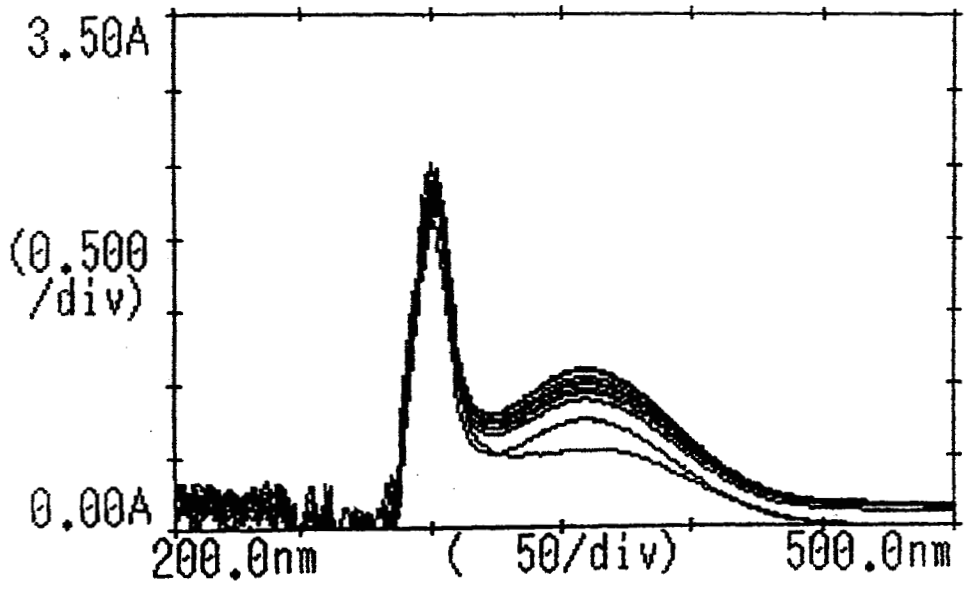


Fig. 4.8. UV-Visible spectrum: Reaction of N-AcDPT (3') with *o*-toluidine

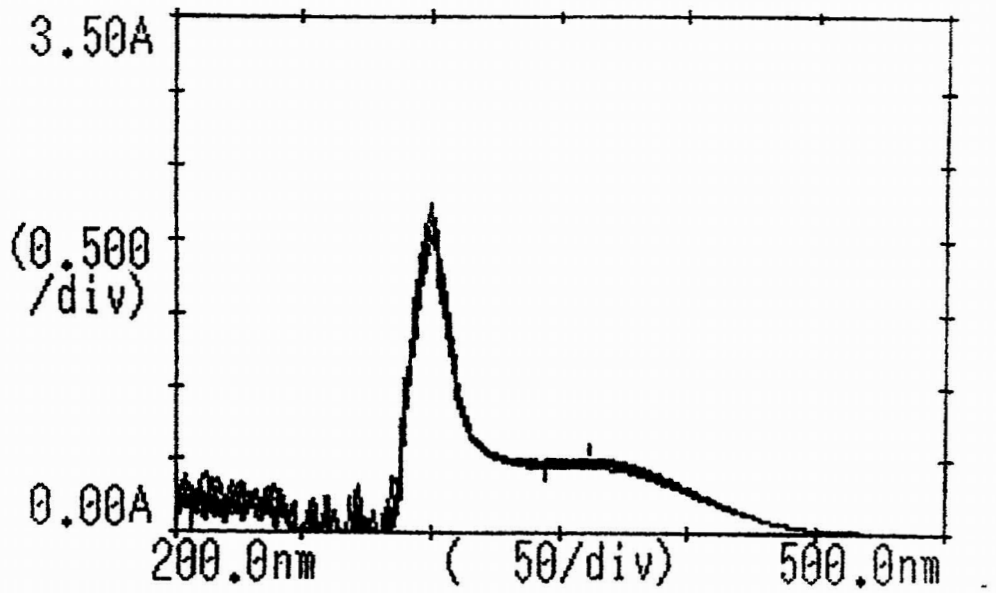


Fig. 4.9. UV-Visible spectrum: Reaction of N-AcDPT (3') with benzylalcohol

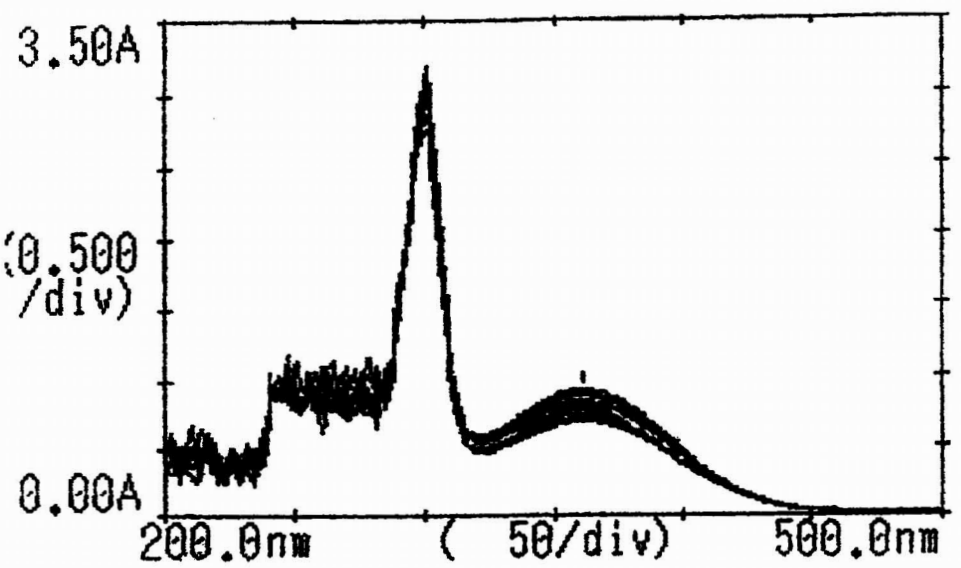


Fig. 4.10. UV-Visible spectrum: Reaction of N-PrDPT (4') with aniline

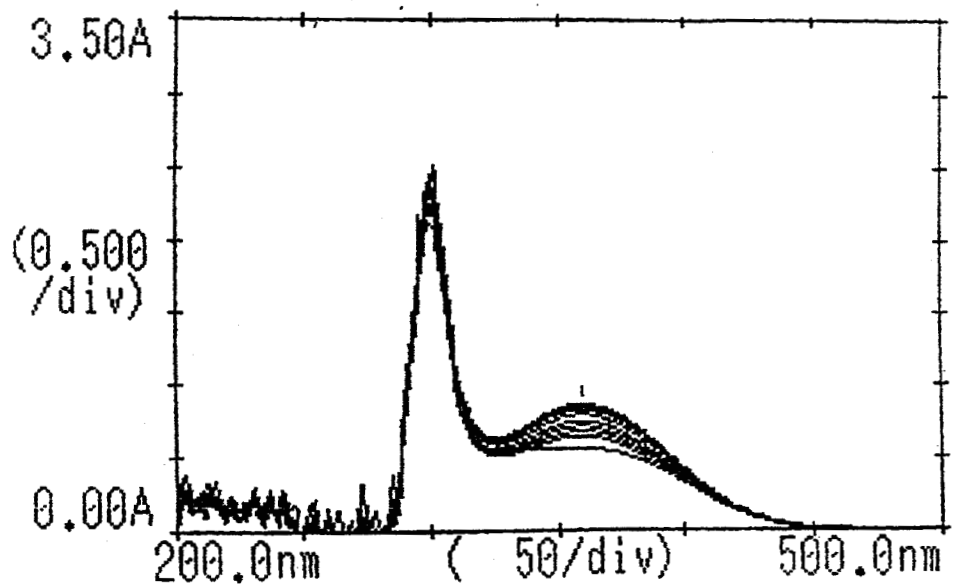


Fig. 4.11. UV-Visible spectrum: Reaction of N-PrDPT (4') with *o*-toluidine

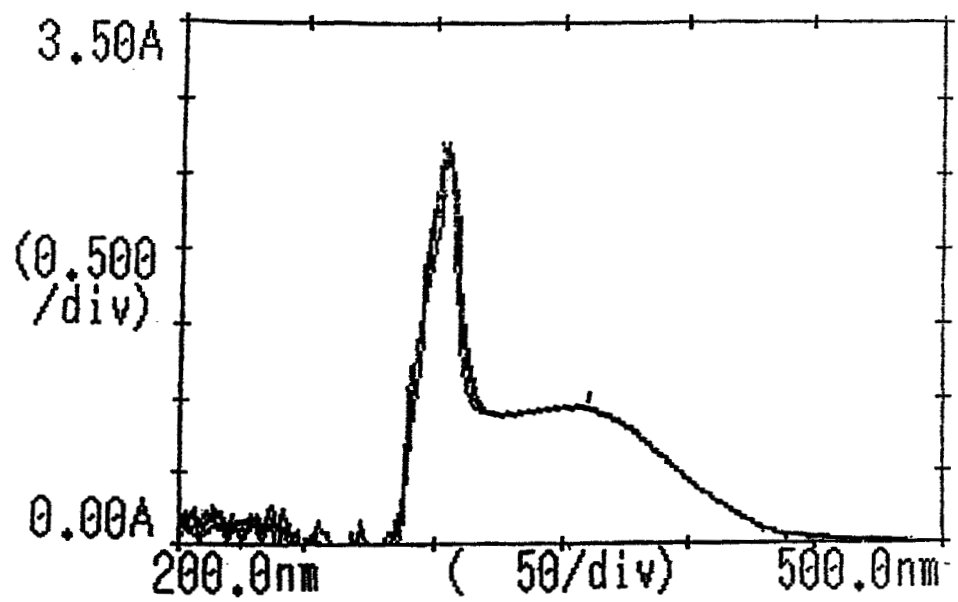


Fig. 4.12. UV-Visible spectrum: Reaction of N-PrDPT (4') with benzylalcohol

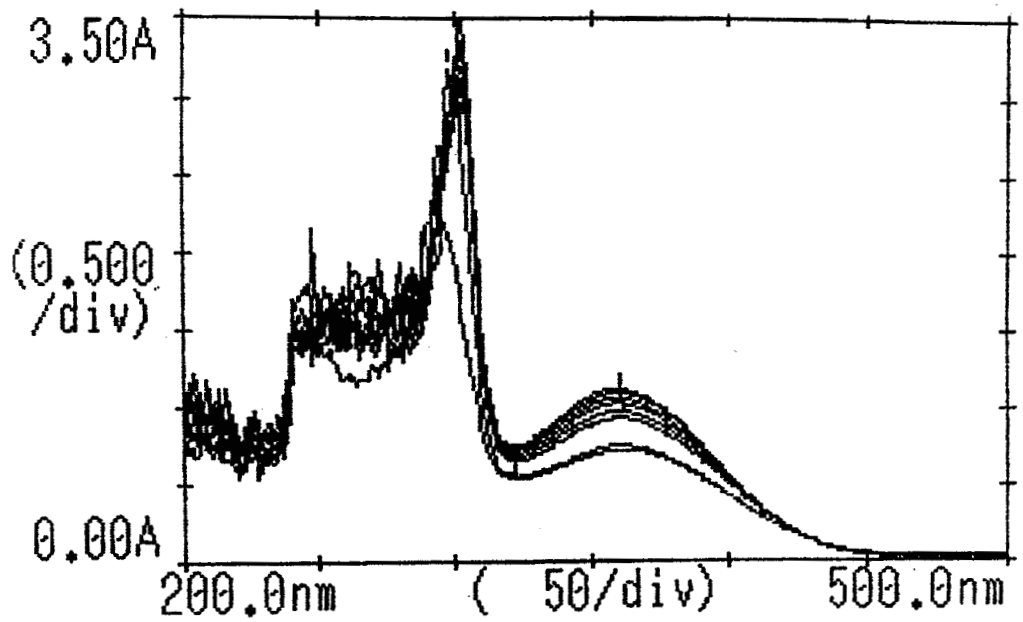


Fig. 4.13. UV-Visible spectrum: Reaction of N-PaDPT (5') with *o*-toluidine

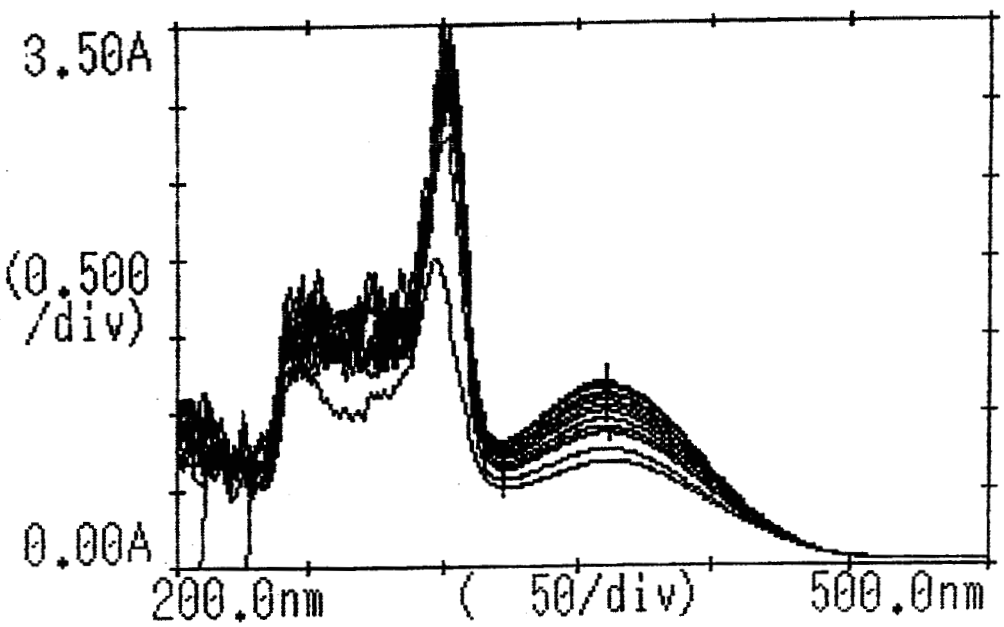


Fig. 4.14. UV-Visible spectrum: Reaction of N-PaDPT (5') with aniline

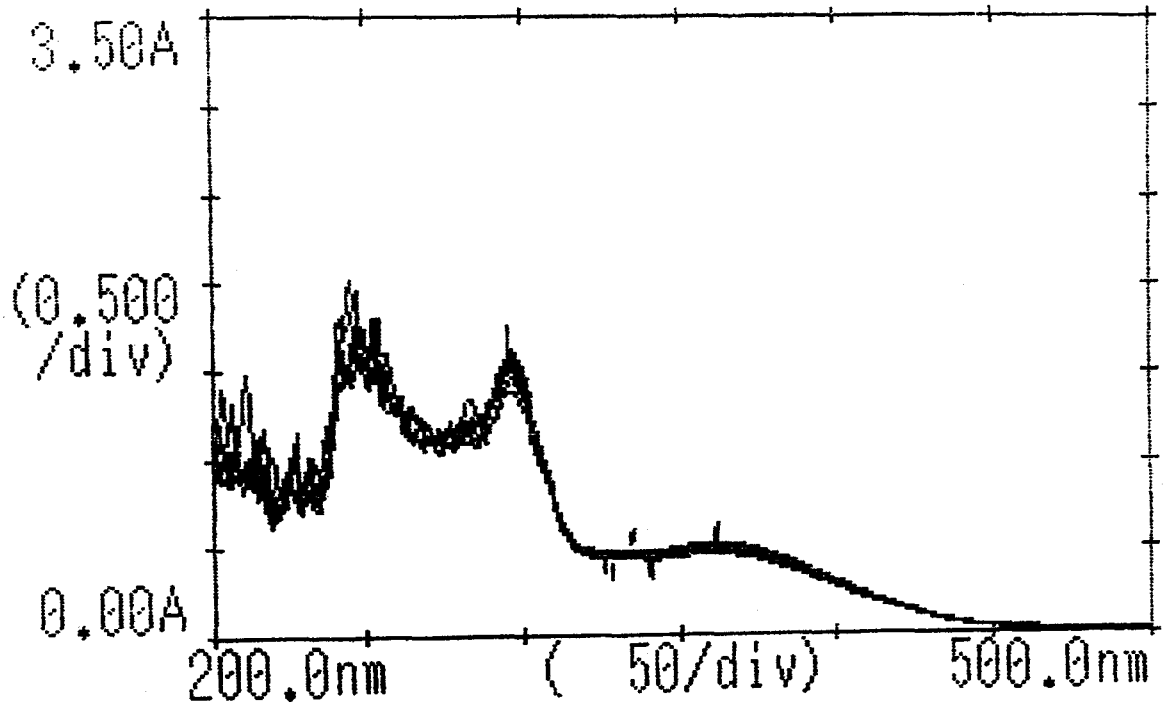


Fig. 4.15. UV-Visible spectrum: Reaction of N-PaDPT (5') with benzyl alcohol

4.3. Experimental

4.3.1. Synthesis of 2-mercapto-4,6-dimethylpyrimidine hydrochloride

In a typical procedure, con. hydrochloric acid (75 mL) was added to a suspension of finely powdered thiourea (38 g, 0.5 mol) in acetylacetone (60 g, 0.6 mol) and ethanol (1.25 lit.) and the mixture was then refluxed for 2 hours. Yellow needle shaped crystals of 2-mercapto-4,6-dimethylpyrimidine hydrochloride afforded on cooling was separated by filtration. Recrystallisatin form alcohol gave yellow crystals of 2-mercapto-4,6-dimethylpyrimidine hydrochloride: yield, 80%, mp 240 °C.

4.3.2. 2-Mercapto-4,6-dimethylpyrimidine (1)

2-Mercapto-4,6-dimethylpyrimidine hydrochloride was dissolved in water and to this solution, a saturated aqueous solution of sodium carbonate was added in drops with constant stirring. At the neutral point (pH 7-8), yellow crystals were formed. It was then separated by filtration and dried. The product thus obtained was recrystallised to afford yellow crystals of 2-mercapto-4,6-dimethylpyrmidine (**1**): : yield 70%, mp 206°C (lit[14]. mp 209-210°C).

4.3.3. N-acyl-4,6-dimethylpyrimidine-2-thiones (2'), (3'), (4') & (5')

N-Benzoyl-4,6-dimethylpyrimidine (**2'**) was prepared first by DCC coupling method[24] and also using the general procedure of acylation with benzoyl chloride in presence of triethylamine as base.[13]

By applying the usual DCC coupling method, 2-mercapto-4,6-dimethylpyrimidine (**1**, 3.5 g, 25 mmol) and benzoic acid (3.05 g, 25 mmol) were dissolved in 80 mL THF and methylene dichloride (1:4) and this was then added to a solution of DCC (5.2 g, 25 mmol) in 14 mL methylene dichloride in drops with constant stirring in an ice bath. Stirring continued for 1 h and the DCU precipitated was filtered off. The filtrate thus obtained was separated by neutral alumina column using benzene-ethylacetate (3:1) mixture as solvent. The product obtained was recrystalised from benzene to afford yellow crystals of N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**) which was identified by different spectra: yield 85%, mp 72°C.

The DCC coupling method was also used to prepare other N-acyl derivatives using acetic acid (1.5 g, 25 mmol), propionic acid (1.8 g, 25 mmol) and phenylacetic acid (2.8 g, 25 mmol) and equimolar proportions of DCC (5.2 g, 25 mmol). The respective acyl derivatives, N-AcDPT (**3'**), N-PrDPT (**4'**) and N-PaDPT (**5'**) were afforded. They were isolated, purified by column chromatography and characterised. Characterisation data have already been given in Table **4.1**.

4.3.4. Aminolysis of N-acyl-4,6-dimethylpyrimidine-2-thiones

N-Benzoyl-4,6-dimethylpyrimidine-2-thione (**2'**, 1 g, 4 mmol) dissolved in chloroform (15 mL) was mixed with freshly distilled aniline (**8a**, 0.38 g, 4 mmol). The reaction mixture was agitated well. A pale yellow colouration was developed in the solution within 3-4 minutes indicating the regeneration of thiol **1** which was also confirmed by tlc. From the reaction mixture, the solvent was evaporated off and the residue thus obtained was extracted with water, since DPT (**1'**) is highly soluble in water. The white crystalline product was separated by filtration, purified by chromatography (neutral alumina), then recrystallised from alcohol to get crystals of benzanilide (**9a**, yield 85%, mp 159°C (lit[24]. mp 163°C).

The spectrophotometric monitoring of aminolysis was carried out using 0.1 millimolar chloroform solution of **2'** with equimolar amount of aniline **8** and the UV-visible spectrum was taken at every 30 secs. Absorbance values were gradually increased at room temperature conditions and became steady after **3** minutes.

Simultaneously the colour of the reaction mixture became yellow indicating the regeneration of 2-mercapto-4,6-dimethylpyrimidine (**1**).

Now the entire procedure of aminolysis was repeated using other amines 2-methylaniline (**8b**), 3-methylaniline (**8c**), 4-methylaniline (**8d**), 1-naphthylamine (**8k**), 2-chloraniline (**8g**) and 4-nitraniline (**8h**), so that respective amides N-benzoyl-2-methylaniline (**9b**), N-benzoyl-3-methylaniline (**9c**), N-benzoyl-4-methylaniline (**9d**), 1-naphthylbenzamide (**9k**), N-benzoyl-2-chloraniline (**9g**) and N-benzoyl-4-nitraniline (**9h**) were obtained. Characterisation details have already been given in table **4.2**.

The aminolysis reaction to isolate amides and spectrophotometric monitoring were repeated using other acyl derivatives N-AcDPT (**3'**), N-PrDPT (**4'**) and N-PaDPT (**5'**). These experiments were conducted at room temperature. Characterisation data and spectral scanning results have already given in Table **4**, and fig. **4.7-4.15** respectively. In all the cases, 2-mercapto-4,6-dimethylplyrimidine (**1**) has been isolated in almost quantitative yield.

4.3.5. *Reactions of N-acyl-4,6-dimethylpyrimidine-2-thiones with alcohols: Formation of esters*

N-Benzoyl-4,6-dimethylpyrimidine-2-thione(**2**, 2.5g, 10mmol) was dissolved in 40 mL benzyl alcohol (**10a**) and stirred well. There was no indication of the regeneration of **1** even after prolonged stirring. Then the reaction mixture was heated on a water bath to 60-70°C for 5 minutes. A fruity smell with a simultaneous colour

change to pale yellow indicating the regeneration of **1**was developed. The product formed was evidenced by tlc. Column chromatographic separation of the mixture using neutral alumina afforded benzyl benzoate (**11a**) bp 304°C (lit[14]. bp 314°C) and **1** in very good yield. The reaction was also followed by UV-visible spectral measurements using 0.1 millimolar solutions of **2'** and benzyl alcohol (**10a**). Absorbance values were noted at regular interval of 30 secs. The increase in the absorbance values were negligibly small.

The esterification reaction was repeated using other alcohols like methyl alcohol (**10b**) and ethyl alcohol (**10c**) and other acyl derivatives under similar conditions. In all the cases esterification occurred only when heated and the respective esters, methyl benzoate (**11b**) and ethyl benzoate (**11c**) formed were separated by chromatographic method. The details have already been given in Table **4.2.**

4.3.6. Selective aminolysis of acyl-derivatives with aminoalcohols and phenols: Formation of hydroxy amides

A chloroform solution (15 mL) of N-benzoyl-4,6-dimethylpyrimidine-2-thione (**2**, 1g, 4 mmol) were treated with ethanolamine (**8o**, 0.24 g, 4 mmol) and stirred well for 10 minutes at room temperature. Indicating the regeneration of 4,6-dimethyl-pyrimidine-2-thione (**1**), an yellow colour was developed within 2-3 minutes. This was also evidenced by tlc. The excess chloroform was evaporated off. The residue thus obtained was extracted with water and filtered. The solid product thus obtained

was purified by neutral alumina column and recrystallised from benzene to afford N-hydroxyethyl benzamide (**9o**). Yield 80%, mp 156°C, (lit[27]. mp163°C)

Similar procedure was repeated using diethanolamine (**8p**) and 4-aminophenol (**8f**) with the same proportion so that N,N-bis(hydroxyethyl)benzamide (**9p**) and 4-(hydroxyphenyl)benzamide (**9f**) were yielded (table **4.2**). The similar sets of selective aminolysis using ethanolamine, diethanolamine, 2-aminophenol and 4-aminophenol were also carried out with N-AcDMPT (**3'**), N-PrDMPT (**4'**) and N-PaDMPT (**5'**) derivatives.

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PEPTIDE SYNTHESIS USING 3-MERCAPTO-5,6-DIPHENYL-1,2,4-TRIAZINE & 2-MERCAPTO-4,6-DIMETHYLPYRIMIDINE

M.P. Rajan “Development of heterocyclic carboxyl activating groups and their applications in peptide synthesis” Thesis.Department of Chemistry, University of Calicut, 2003

PEPTIDE SYNTHESIS USING 3-MERCAPTO-5,6-DIPHENYL-1,2,4-TRIAZINE & 2-MERCAPTO-4,6-DIMETHYLPYRIMIDINE

Chapter 5

PEPTIDE SYNTHESIS USING 3-MERCAPTO-5,6-DIPHENYL-1,2,4-TRIAZINE & 2-MERCAPTO-4,6-DIMETHYLPYRIMIDINE

5.1. Introduction

There has been a constant upsurge to develop new and improved strategies in peptide synthesis since the turn of 20th century. Peptides are important in biological processes from the most humble bacteria to the most complex mammal is beyond doubt, having roles as diverse as neurotransmission and antibiotic activity; in addition to their functions as hormones and toxins. This wide spectrum of activity makes them as a class of compounds of significant interest in areas from medicinal chemistry to molecular biology. The early studies were encouraged by the emerging formulations of protein structure.[1] Drastic developments in biotechnology of new proteins and in pharmaceutical chemistry led to great demands in synthetic peptides. Later, the realisation that other biologically important molecules also had simpler amino acid sequences stimulated the investigation on peptide synthesis.

In recent years, the classical approach of peptide synthesis has yielded impressive successes in the synthesis of several biologically active peptides.[2-6] Pharmacological activity of several peptides helped in investigating the structure activity relationship which led to improved methods in peptide synthesis.

Chemical assembly to form peptides is achieved either by the "solution phase" or by the "solid phase" method. Though, solution phase peptide synthesis is very much effective in the synthesis of shorter peptides, long chain peptides become ineffective mainly because of their low solubility. Peptide synthesis in solution phase has now much advanced by introducing new protecting groups, activating groups, coupling reagents and additives, the latter often being used to reduce the risk of racemisation. Still solution phase peptide synthesis suffers from some important set backs such as

(i) Synthesis involves slow, tedious and laborious processes. Thus to obtain a peptide of high purity, the constituent amino acids are coupled together in a stepwise manner beginning at the C-terminal of the peptide. After each completed amino acid addition, the intermediate peptide is separated from any remaining reactants before its characterisation, which leads to lengthy synthetic procedures.

(ii) Difficulties in both the purification and coupling steps due to the increasing insolubility of the growing peptide chains.

(iii) Considerable reduction in overall yield which results due to the large number of steps, e.g. chromatography, crystallisation, transfers etc. required during the synthesis.

In spite of all these difficulties, solution phase peptide synthesis still continues even today.

Peptide chemists realised the need to facilitate long peptides and in 1962 Merrifield introduced[7-9] the most versatile technique which had a revolutionary effect on peptide synthesis - the solid phase peptide synthesis (SPPS). Merrifield opined that by attaching the growing peptide chain to an insoluble polymeric support, excess reagents and byproducts from the synthetic cycles could be removed by simple washing and filtrations. It could also overcome the lengthy intermediate purification steps of classical peptide synthesis. Merrified's approach, coupled with the improved purification techniques like chromatography and HPLC, revolutionarised peptide synthesis.[10,11] The important features of solid phase peptide synthesis are:

(i) A peptide is synthesised while its C-terminus is covalently attached to an inosluble polymeric support. Thus byproducts or constituent amino acid present in excess are enabled to be separated from the growing peptide.

(ii) The reactions of the polymer supported peptide are driven to completion by using excess of reactants and reagents.

(iii) The growing peptide is retained by the polymer in a single reaction vessel throughout the synthesis so that no mechanical loss occur.

(iv) The final peptide is detached from its polymer support by a single cleavage step at the end of the synthesis. This final cleavage step should not degrade the assembled peptide.

(v) The operations involved, such as washing and filtration are simple and rapid. With the success in the standardisation of these steps involved in peptide synthesis

the whole process can be automated[12,13] which is one of the most attractive features of the SPPS strategy.

(vi) The spent resin can be recycled.

In spite of these advantages, Merrified's solid phase method, is not without limitations and have been reviewed in detail.[14-17] The major disadvantages are:

(i) Non-compatibility of resin and growing peptide chain.

(ii) Lack of stability of peptide resin linkage under conditions of synthesis.

(iii) The non-equivalence of functional groups attached to the polymer support.

(iv) Formation of error peptides due to truncated and failure sequences.

(v) Peptide conformation changes in macroscopic environments inside the polymer matrix and also due to peptide resin linkage.

However, systematic and well defined strategies of protection, activation, coupling, and removal of protecting groups enable SPPS a great success.

In this context it will be appropriate to know about the different steps involved in solid phase peptide synthesis, which is given as in Scheme **5.1**.

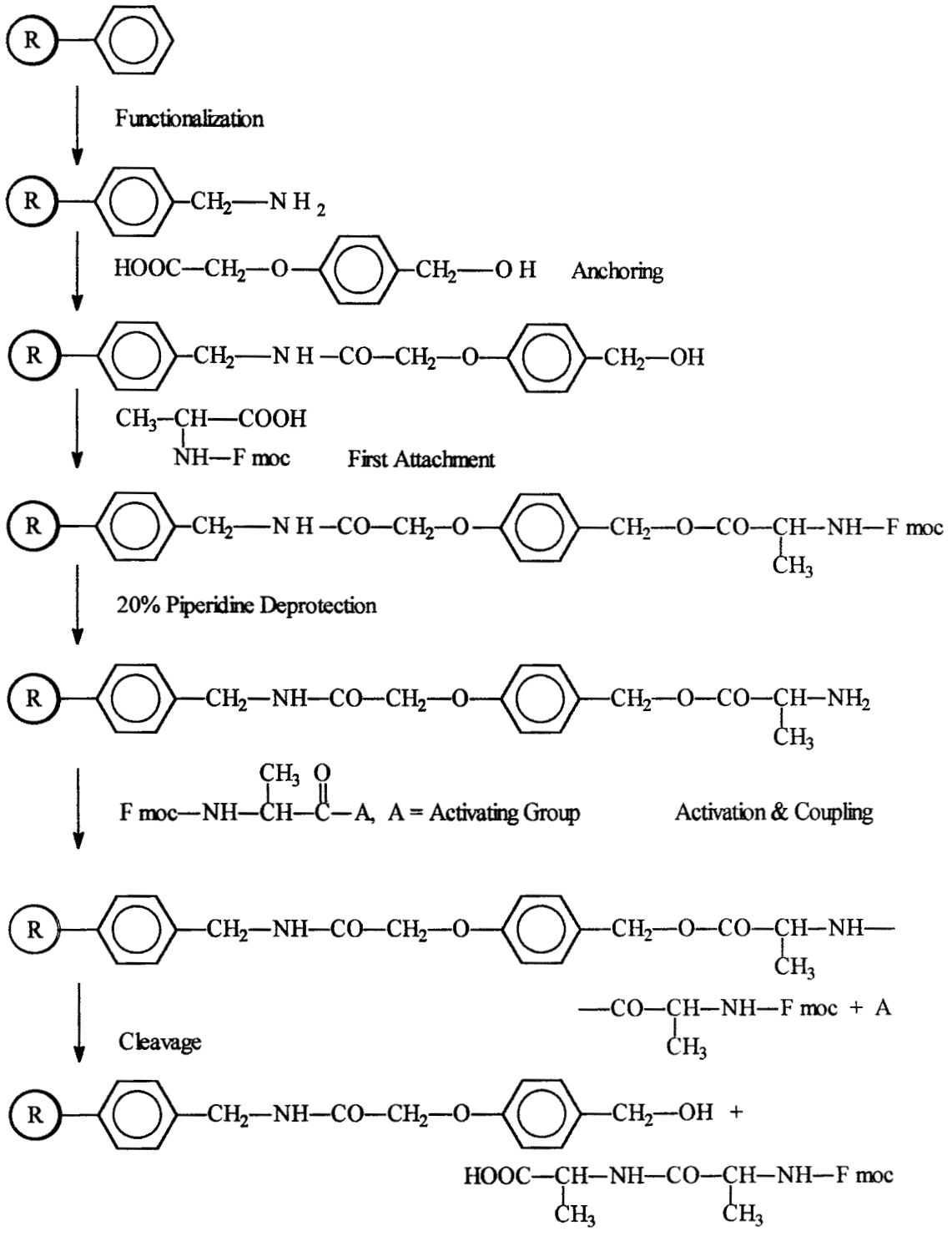


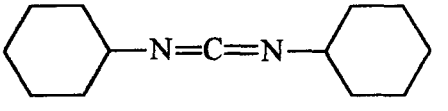
Fig. 5.1. Solid Phase Peptide Synthesis applying carboxyl activating group

From the different steps detailed above, it can be seen that the core part of the rapid and quantitative synthesis of peptides, principally, depend on activation of

carboxyl function of the amino acid and then coupling with the other amino acid. A large number of activating and coupling reagents have been reported in the literature. Thus, an overview of the important carboxyl activating groups, which enabled SPPS strategy very simple, is given here.

In SPPS, the deciding factors of the efficiency of coupling reactions include the nature of acylating agent, protected as well as activated amino acid species and the solvation of the resin-bound growing peptide chain. Subsequent amino acids must be added to the growing peptide-resin in a highly activated form to ensure their rapid and quantitative coupling. So it is only appropriate to introduce some of the important coupling reagents used in the solid phase peptide synthesis.

DCC mediated coupling has been widely used for many years in peptide synthesis. The basic drawback in the use of DCC (**1**) was the formation of insoluble DCU during acylation. Diisopropyl carbodiimide (DIP CDI, **2**) and t-butylethyl carbodiimide (**3**) are the other carbodiimides used in SPPS.[18,19]



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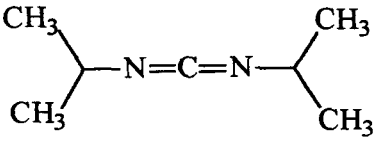

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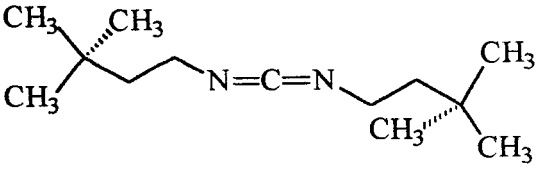

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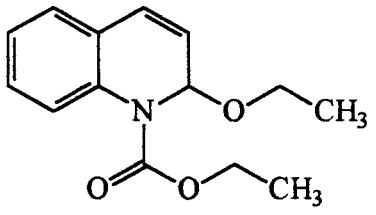
N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, **4**) has also been used for the coupling of successive amino acids in SPPS.[20]



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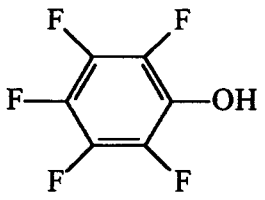
Pentafluorophenyl (pfp, **5**) and 3-hydroxy-2,3-dihydro-4-oxo-benztriazine (**6**) were also found to be promising coupling reagents.[21,22]



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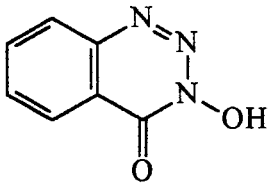

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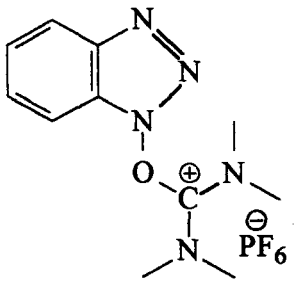
The synthesis of hydrophobic peptides are difficult because of internal aggregation of constituent amino acids by association between protected chain with the synthetic support matrix. These problems can be minimised by the use of 2-(1H-benzotriazol-1-yl) - 1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU, **7**) or 2-(1H-benzotriazol-1-yl) - 1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU, **8**) during the coupling process.[23-25] The coupling reaction proceeds rapidly with low level of racemisation. HOBt (**9**) can act as a catalyst for thio coupling reaction and the reaction can be activated by a base like DIEA (**10**).



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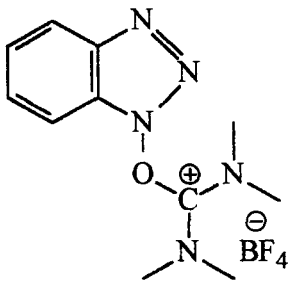

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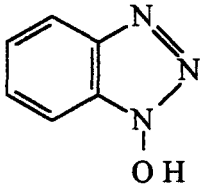

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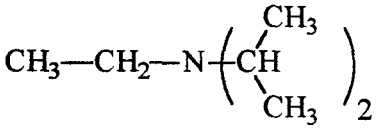

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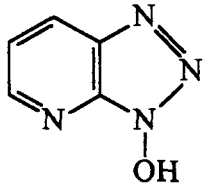
7-Aza-1-hydroxybenzotriazol (HOAt, **11**) and its uronium salt O-(7-azabenzotriazol-1-yl) - N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, **12**) in the presence of an activating base DIEA were used as activating agent for acylation reaction.[26]



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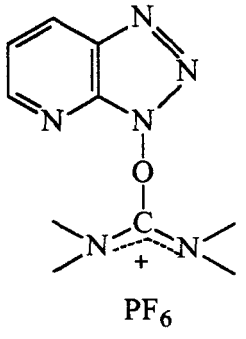

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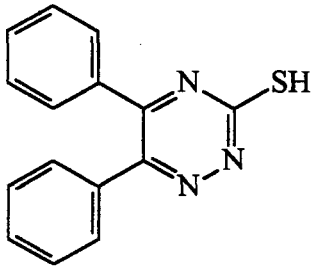
The use of the above heterocyclic reagents as activating agent in SPPS and ample proof that 3-mercapto-5,6-diphenyl-1,2,4-triazine (**13**) and 2-mercapto-4,6-dimethylpyrimidine (**14**) as carboxyl activating groups from the facile aminolysis and esterification reactions stimulated to apply the capability of these groups in peptide synthesis, where the F moc aminoacids are easily converted to **15** and **16.**



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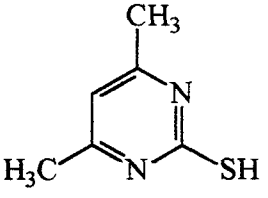

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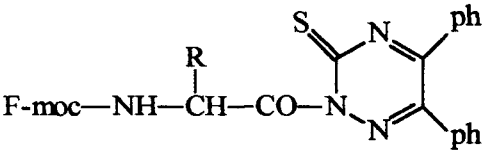

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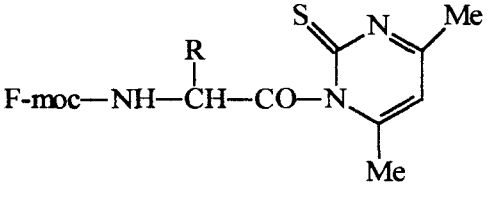


16

Thus the present chapter deals with:

i) synthesis of monomer bisacrylamidopropyl polyethyleneglycol (Acr_2PEG).

ii) synthesis of polymeric support - crosslinked polystyrene-polyethylene glycol acrylate resin (CLPSER).

iii) functionalisation of resin-aminomethylation.

iv) aminocapacity determination of the resin by picric acid method.

v) anchor attachment to CLPSER.

vi) C-terminal Fmoc-amino acid incorporation.

vii) ninhydrin test for coupling reactions using compound **13** & **14** as carboxyl activating groups.

viii) time-dependent Fmoc-amino acid coupling using thiols **13** and **14**.

ix) Synthesis of model peptide G-A using thiols 13 and 14.

x) synthesis of peptide, acyl carrier protein (ACP) fragment (65-74) using **13**.

xi) synthesis of peptide G-A-A-A using **14**.

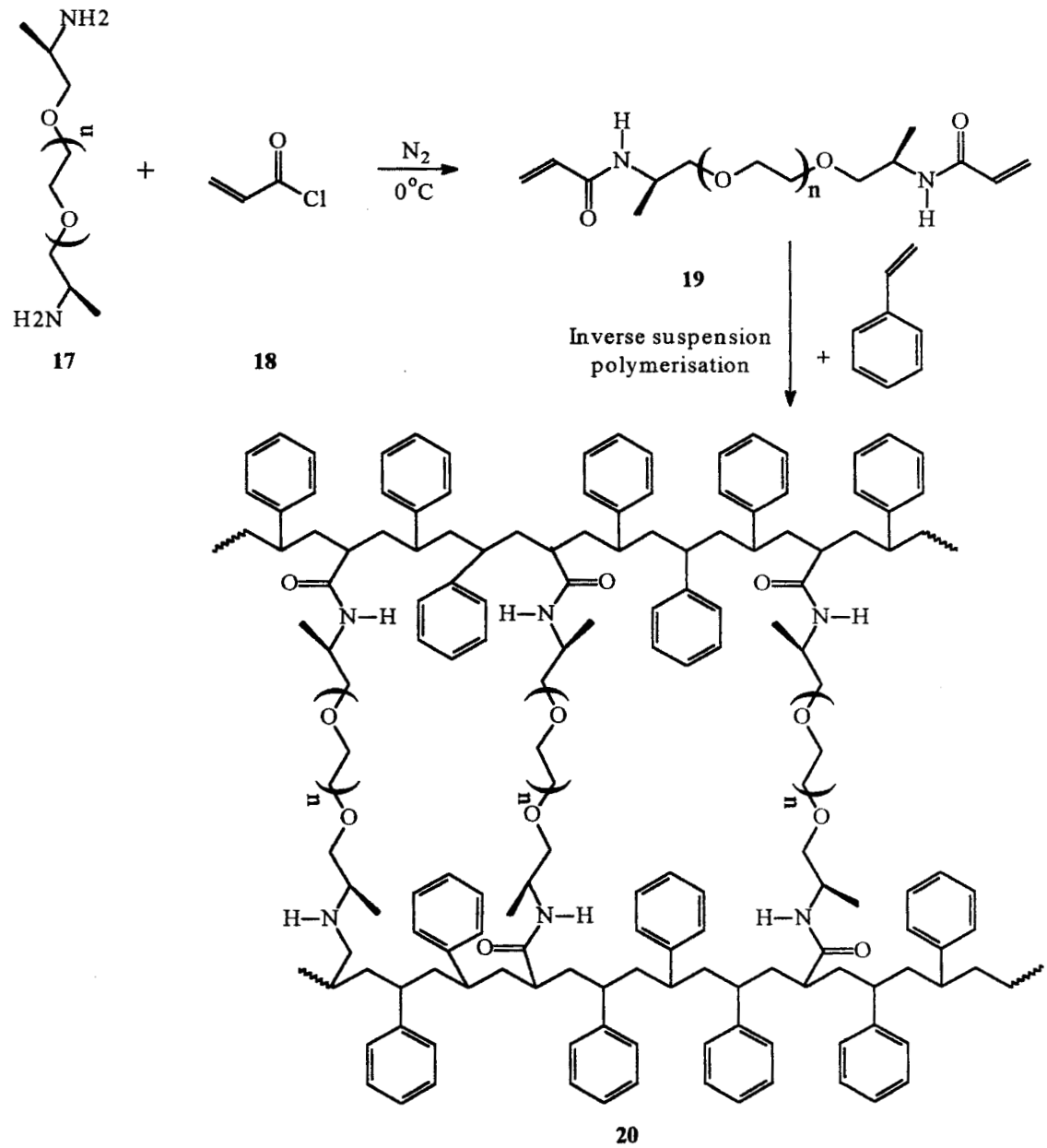
xii) characterisation of the synthesised peptides ACP.

5.2. Results and Discussion

5.2.1. Synthesis of Acr_2PEG crosslinked polystyrene (CLPSER, 20)

In SPPS, the first and foremost important part is the synthesis of polymeric support, that is resin. In the present work, the resin used was crosslinked polystyrene-ethyleneglycol acrylate (CLPSER, **20**) and it also synthesised by an established procedure[27] from the monomer - cross linker, O,O'-bis(2-acrylamido propyl)polyethylene $glycol_{1900}$ (Acr_2PEG, **19**). Acr_2PEG, in turn, was synthesised initially by treating acryloyl chloride (**18**) with O,O'-bis(2-aminopropyl) polyethylene $glycol_{1900}$ [$(NH_2)_2PEG$, **17**] in the presence of diisopropylethylamine (Scheme **5.1**). The copolymer was then prepared by polymerisation of Acr_2PEG_{1900} and styrene (Scheme **5.1**) using sorbitan monolaurate as the suspension stabilizer, and a mixture of ammonium peroxodisulfate and benzoyl peroxide as the radical initiators. The

resin afforded, though it was reported earlier, was characterised using gel-phase ^{13}C NMR (Fig. 5.1) and infrared (KBr) spectroscopic techniques.



Scheme 5.1

Synthesis of CLPSER Support (20) from O,O'bis(2-acrylamidopropyl)polyethylene glycol$_{1900}$

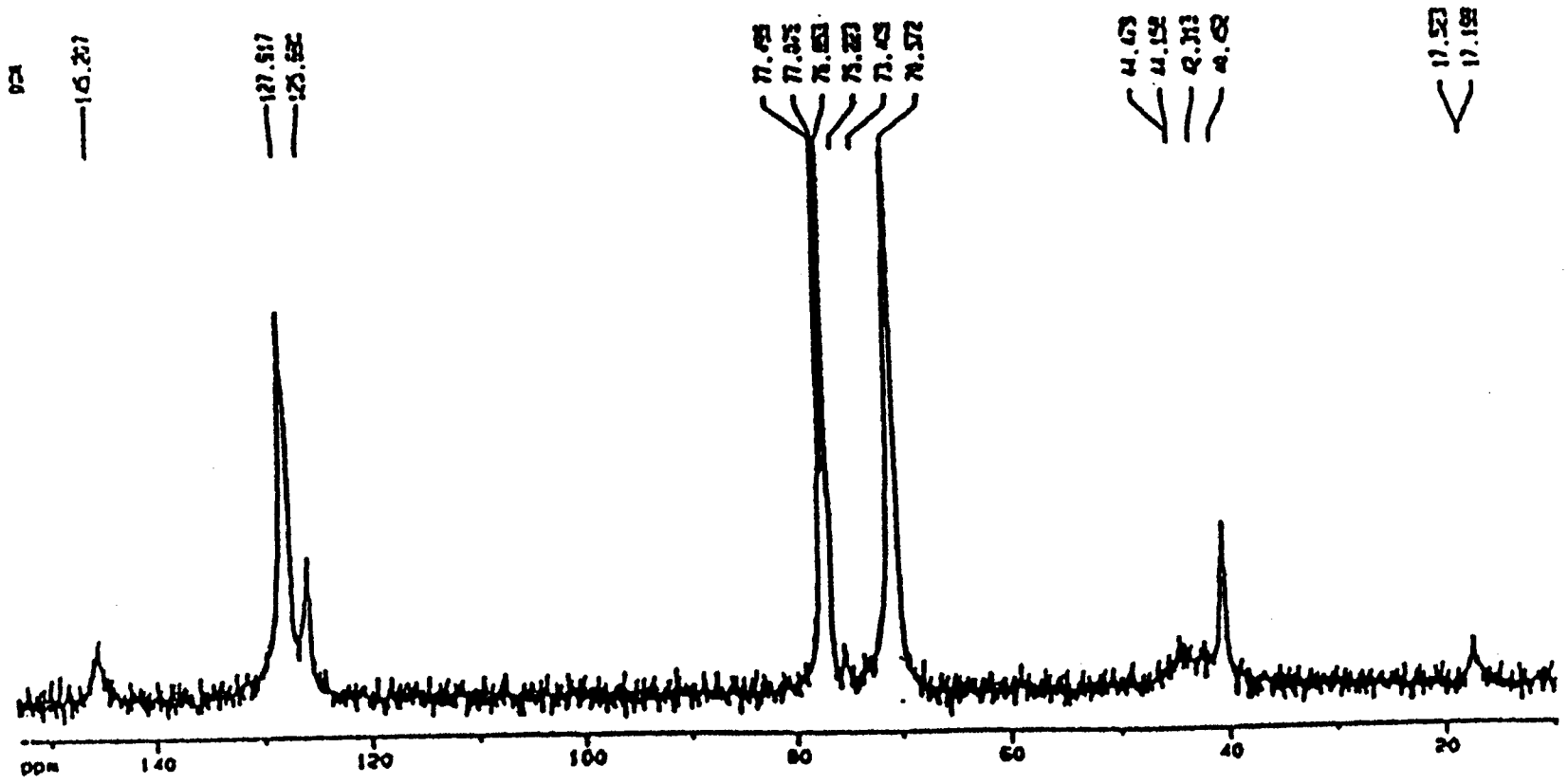


Fig. 5.1. Gel-phase 13C NMR spectrum of CLPSER in CDCl3

5.2.2. Functionalisation of CLPSER resin (20) - Aminomethylation

CLPSER-resin was functionalised using aminomethylation method. The amino capacity of the aminomethyl CLPSER resin was then determined by picric acid method.[28]

The pre-swollen CLPSER-resin in DMF was refluxed with potassium phthalmide and the resin thus obtained was subjected to hydrazinolysis. The extent of functinalisation was estimated by picric acid method.

5.2.3. Anchor attachment to CLPSER (20)

The covalent attachment of the growing peptide chain to the polymeric support has been found to be the critical problem of efficient peptide synthesis in SPPS.[29] A chemical linkage of peptide to the support using a suitable 'handle' unit allows the whole synthesis under well defined mild conditions. These linkers are bifunctional spacers that incorporate on some end having features of a smoothly cleavable protecting group and at the other end allow coupling to a previously functionalised support. These linkages are easily formed, stable to repeated cycles of acylation and deprotection and also easily cleaved at the end of the synthesis without damage to newly formed peptide bond. These handles also help to cleave the polypeptides as free acids.

Here, 4-hydroxymethyl phenoxyacetic acid (HMPA) was used as the anchoring unit for the peptide synthesis. The pre-swollen aminomethyl CLPSER

resin was treated with equimolar mixture of HMPA, HOBt, HBTU and DIEA so as to form anchored CLPSER-HMPA resin.

5.2.4. C-Terminal Fmoc amino acid incorporation

The CLPSER-HMPA resin was incorporated with a C-terminal F-moc amino acid, Fmoc-Glycine, in the presence of MSNT and N-methylimidazole. The percentage incorporation of amino acid was estimated by measuring the optical density (OD). The OD measured was 0.8984. Thus the amino capacity of the resin after the C-terminal attachment was obtained as 0.1198.

5.2.5. Synthesis of G-A using 3-mercapto-5,6-diphenyl-1,2,4-triazine(13)

The Fmoc alanine (3.5 mmol excess), DCC (3.5 mmol excess) and thiol **13** (3.5 mmol excess) were dissolved in minimum NMP, N-methylpyrrolidone in a small R.B. flask and kept for 1 h. Filtered off the precipitated DCU in a Gisin tube and the filtrate (activated amino acid) was used for coupling.

The C-terminal glycine incorporated CLPSER-HMPA resin swollen in DMF was subjected to Fmoc deprotection using 20% piperidine in DMF. Then the above activated amino acid filtrate was added to the swollen resin so as to start coupling. The extend of coupling was followed by ninhydrin test.[30] Finally the dipeptide fragment G-A was cleaved from the resin using the mixture TFA, water, thioanisol, ethanedithiol and phenol. The filtrate thus obtained was treated with ice cold diethylether and the precipitated dipeptide G-A was identified by homogeneous tlc

in the methanol: chloroform (1:7) solvent system. A single spot showed the peptide to be homogeneous.

5.2.6. Synthesis of G-A using 2-mercapto-4,6-dimethylpyrimidine (14)

Similar to the above procedure, G-A was synthesised by substituting compound **14** as carboxyl activating group instead of **13**. The dipeptide gave a homogeneous tlc with that of the G-A synthesised using compound 13 as carboxyl activating group. The homogeneity was checked by tlc using methanol: chloroform (1:9) as solvent system. A single spot indicates the homogeneity of the peptide.

5.2.7. Time dependent amino acid coupling using thiol 13

Resin on which first attachment has been carried out was subjected to deprotection. To this reaction mixture, triazine thiol instead of the usual coupling agent HOBt, Fmoc alanine, HBTU and DIEA was added all in 3.5 mmol excess in DMF medium. At regular interval of time, a small portion of the resin was withdrawn, washed and kept overnight in vacuum desiccator. In the next day, each sample of the withdrawn resin was weighed and subjected to deprotection using 3 mL of 20% piperidine. The details are given in Table **5.1**.

Table 5.1. Time dependence of the amino acid coupling using CLPSER Resin, F-moc alanine and thiol (13)

Sl. No.	Time (min)	Wt. of resin (mg)	Absorption	Amino capacity
1	5	7.5	0.6586	0.0554
2	10	4.8	0.610	0.0847
3	15	3.7	0.533	0.0960
4	20	7.8	1.259	0.1075
5	25	5.8	1.053	0.1210
6	30	7.5	1.368	0.1216
7	35	6.3	1.154	0.1222
8	45	4.6	0.846	0.1227
9	55	6.6	1.233	0.1245

$$\text{Amino capacity} = \frac{\text{OD} \times 0.11 \times 10}{1.65 \times \text{wt. of resin}}$$

From table **5.1** it can be seen that amino capacity gradually increases and after 25 minutes it remained almost constant which clearly demonstrates the efficiency of thiol **13** as carboxyl activating group in peptide synthesis. It could be seen that the amino acid coupling was moderately fast at the initial stage of the procedure and then proceeds slowly.

5.2.8. Time dependent amino acid coupling using thiol 14.

Following the above methodology, time dependence of amino acid coupling using thiol **14** has been carried out and found to be successful. Here CLPSER -

HMPA resin (52 mg) was used. The details of the OD measurements and amino capacity are shown in Table **5.2.**

Table 5.2. Time dependence of the amino acid coupling using CLPSER Resin, F-moc alanine and thiol (14)

Sl. No.	Time (min)	Wt. of resin (mg)	OD measurement	Amine capacity
1	5	4.0	0.107	0.01783
2	10	5.4	0.223	0.02760
3	15	5.9	0.359	0.04056
4	20	6.3	0.443	0.04687
5	25	5.6	0.423	0.05035
6	30	6.2	0.854	0.09184
7	40	4.2	0.639	0.1015
8	50	5.7	0.872	0.1020
9.	60	5.1	0.784	0.1026

From the calculated values of amino capacity during the amino acid coupling in the presence of thiol **(14)** as carboxyl activating group again proved its suitability in carboxyl group activation. Unlike thiol **13**, started rather slowly and the coupling took about 40 minutes for completion. This shows that thiol **14** is also an attractive carboxyl activating group, but less effective than thiol **13**.

Anyhow, it is very much clear that thiols **13** as well as **14** are promising carboxyl activating reagents.

5.2.9. Synthesis of (65-74) fragment of Acyl Carrier Protein (ACP)* on CLPSER using thiol 13.

The effectiveness of the new carboxyl activating group **13** was further established by synthesising ACP on CLPSER (**20**). 4-Hydroxymethylphenoxyacetic acid (HMA) handle was attached to the aminomethyl resin using HBTU, HOBt and DIEA. C-Terminal Fmoc-glycine was then attached to 0.01 mmol of CLPSER-HMPA resin by an ester bond using MSNT in presence of N-methyl imidazole. The extent of attachment was measured from the UV absorbance of the adduct of dibenzofulvene and piperidine formed by the treatment of accurately weighed Fmoc-amino acid attached resin with 20% piperidine in DMF. After the cleavage of the Fmoc-protection with 20% piperidine in DMF, the remaining Fmoc acids - Aspargine, Isoleucine, Tyrosine, Aspartic acid, Isoleucine, Alanine, Alanine, Glutamine and Valine - were coupled in succession using 3 equivalents of HBTU, thiol **13** and DIEA. The amino acid and coupling reagents required were calculated, weighed and dissolved in a definite volume of DMF. This solution was uniformly distributed in the resin and the coupling reaction was continued for 30 minutes. At the end, the peptide formed was cleaved from the resin using TFA in presence of scavengers. The peptide obtained was an off white powder. The HPLC profile of peptide (Fig. **5.2**) obtained from the CLPSER showed a sharp single major peak corresponding to the target peptide which was confirmed by MALDI-TOF MS (Fig. **5.3**). Maldi TOF MS: m/z $[(M+H)^+]$, 1085 & 1101.

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* Val-Gln-Ala-Ile-Asp-Tyr-Ile-Asn-Gly

Peak at 1085 & 1101 are sodiated and potassiated peaks respectively. The smaller peaks, ie. peaks before 1085 at 1068, 1052, 1030 etc. are fragment ion peaks formed due to high laser power. Spectrum shows that the sample is pure without any deleted or truncated sequence or impurity. Further the purity of the sample is in agreement with HPLC time course analysis. There is only one major peak indicating the presence of ACP without impurities.

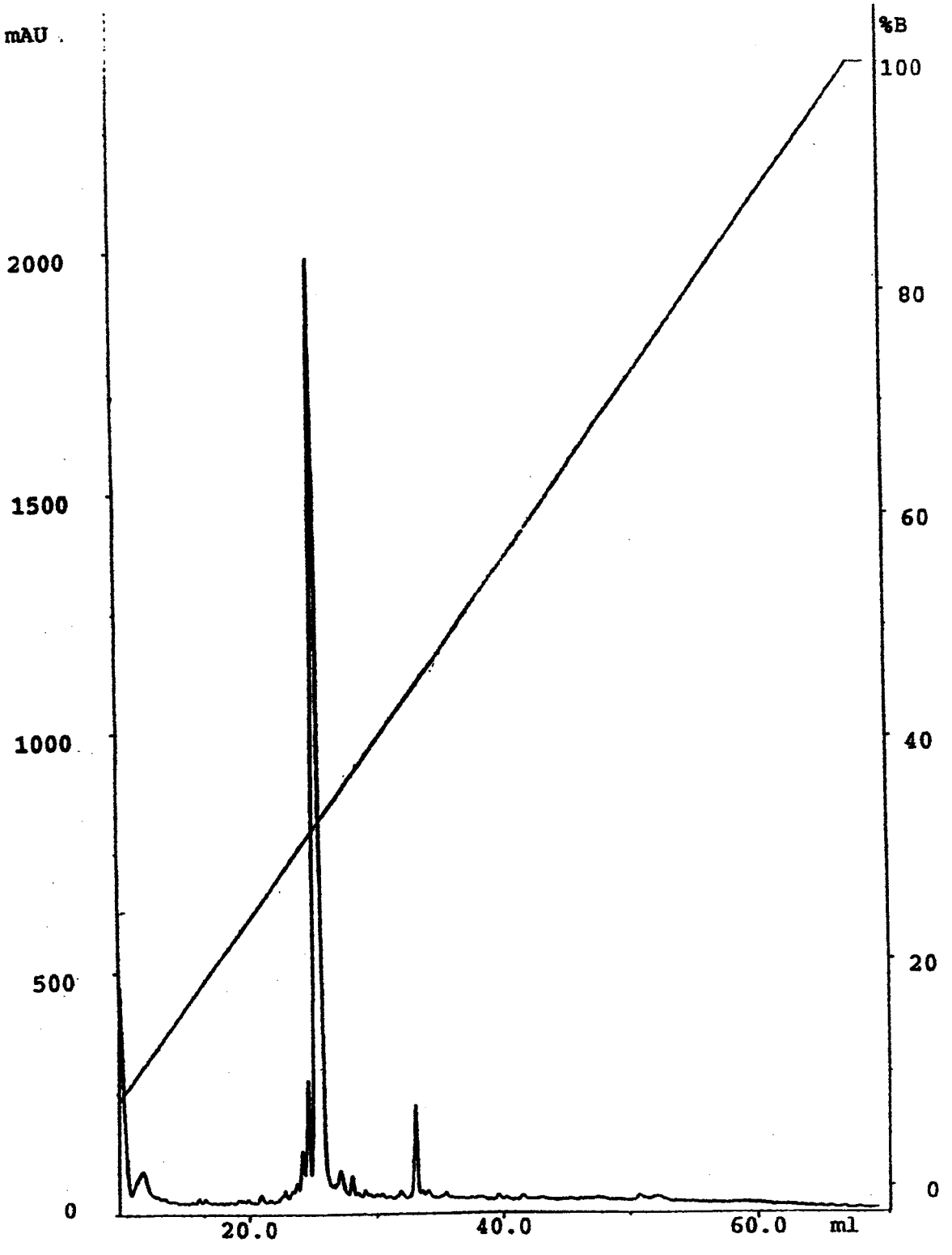

Fig. 5.2. HPLC time course analysis of peptide (ACP)

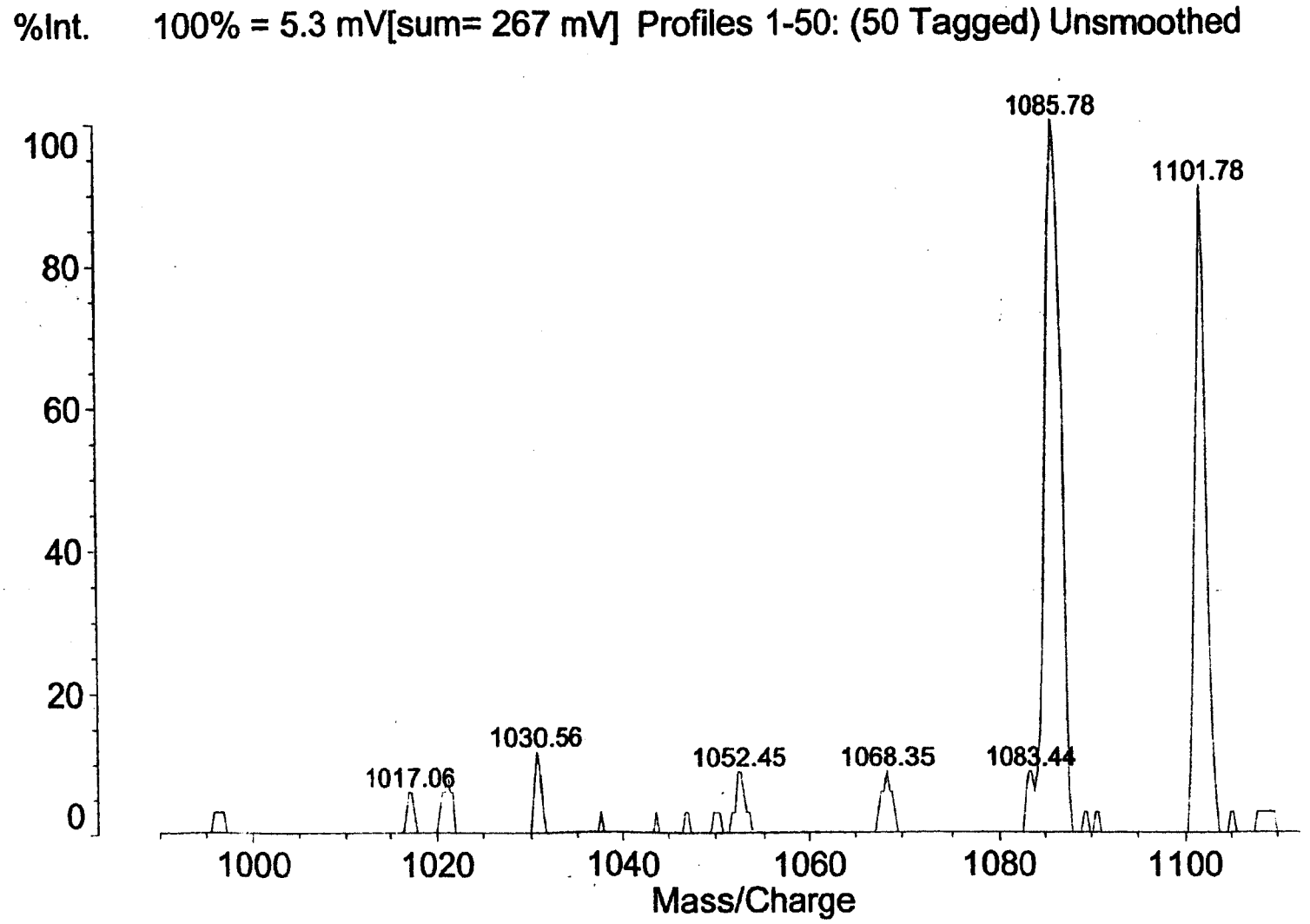

Fig. 5.3. MALDI-TOF MS of ACP

5.2.10. Synthesis of tetrapeptide G-A-A-A on CLPSER using thiol 14

The tetrapeptide G-A-A-A on CLPSER was synthesised using the above protocol. Here F-moc amino acids - glycine & alanine - and thiol **14** were used. The time for the completion of coupling was found to be 45 minutes. The peptide thus obtained was an off white powder. The purity was checked by tlc using 1-butanol: acetic acid: water (4:1:1) as the solvent system. A single spot was obtained indicating the homogeneity of the peptide.

From the synthesis of above peptides, it has been clearly established that thiols **13** and **14** are potent carboxyl activating reagents.

5.3. Experimental

5.3.1. General

4-Hydroxymethylphenoxyacetic acid (HMPA), 1-hydroxybenzotriazole (HOBt), Fmoc-amino acids, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluranium hexafluorophosphate (HBTU), dicyclohexyl carboiimide (DCC), 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) wer purchased from Novabiochem Ltd. (UK). N-Methylimidazole, styrene and sorbitan monolaurate were purchased from Aldrich (Sigma-Aldrich Corp., USA). Thioanisol, ethanedithiol, piperidine and diisopropylethylamine (DIEA) were purchased from Sigma. O,O'-bis(2-aminopropyl)polyethyleneglycol$_{1900}$ [$(NH_2)_2$PEG], N-(3-bromopropyl) phthalimide and N,N,N',N'-tetramethylethylenediamine (TEMED)

were purchased from Fluka, Sigma-Aldrich Copr., USA. All solvents used were of HPLC grade purchased from E. Merck, India, BDH (India) and SISCO Chemicals (Bombay). ^{13}C NMR measurements were recorded on a varian Unity 400 operating at 100 MHz. HPLC was performed on a Pharmacia Akta Purifier instrument using C_{18} Sephasil Peptide reverse-phase semipreparative column. Amino acid analysis was carried out on an LKB 4151 Alpha plus instrument. Mass spectra of peptides were performed in a Kratos PC Kompact MALDI-TOF MS spectrometer.

5.3.2. Synthesis of bisacrylamidopropyl polyethyleneglycol$_{1900}$ (Acr_2PEG, 19)

A mixture of bisaminopropylpolyethylene glycol [$(NH_2)_2PEG$, 10 g] and diisopropylethylamine (1.6 mL) in DCM (12 mL) was stirred at 0 °C under nitrogen atmosphere and acryloyl chloride (**18**, 0.8 mL, 1 eqn.) was added in drops. Then stirring was continued for 1 h at 0 °C. The salt formed was filtered and washed with DCM (5 x 25 mL). The combined filtrate and washings were concentrated to dryness. Hydroquinone stabilized ether (25 mL) was added to the residue and the product was allowed to crystallize for 5 h at 0 °C under mechanical stirring. The crude product was filtered off, washed with stabilized ether (5 x 25 mL) and dried, yield 7.5 g (75%). The cross linker was dissolved in DCM, the solution was filtered, the filtrate was concentrated and Acr_2PEG was again recrystallized from peroxide free unstabilized ether. Acr_2PEG (**19**) as obtained as a white powder, dried under vacuum and stored at -20 °C. Yield: 7.5g (75%).

5.3.3. Synthesis of Cross-linked polystyrene polyethylene glycol acrylate resin (CLPSER)

Synthesis of CLPSER (**20**) was carried out by crosslinking Acr_2PEG (**19**) with styrene. Thus, styrene made free from the inhibitors by washing with 1% NaOH solution followed by distilled water (2 x 50 mL) and dried over anhydrous sodium sulphate. The polymerization reaction was carried out in 1 L double-walled cylindrical flask with a three bladed propellar stirrer, a fixed counter blade, an inert gas inlet tube, a thermometer and a condenser. The copolymer was synthesised in a 1:3 ratio of crosslinker to styrene using the following procedure.

Heptane (120 mL) and CCl_4 (90 mL) were added to the polymerization vessel and stirred at 70 °C at 1500 rpm. Acr_2PEG (**19**, 2.64 g) was dissolved in DMF (30 mL), mixed thoroughly with styrene (0.429 mL), benzoyl peroxide (0.2 g), ammonium peroxidisulphate (0.2 g) and sorbitan monolaurate (0.25 g in 1.25 mL DMF) and added to the reaction vessel. The reaction mixture was stirred at 1500 rpm and the temperature was increased to 80 °C. After 2 min, TEMED (1 mL) was added and polymerization was allowed to proceed under nitrogen atmosphere for 3 h. The copolymer obtained was washed thoroughly with water (5 x 50 mL), ethanol (5 x 50 mL), benzene (5 x 50 mL), toluene (50 mL) and methanol (5 x 50 mL) and then soxhleted with toluene, methanol and acetone. The polymer was dried in vacuum over P_2O_5 to afford dry polymer - CLPSER resin (**20**): Yield 2.7 g.

5.3.4. Functionalisation of CLPSER resin (20) - Aminomethylation

CLPSER (**20**, 0.12 mmol/g, 1 g) was made to swell in DMF for 1 h. Excess DMF was removed, potassium phthalimide (0.22 g, 1.2 mmol) dissolved in DMF (1 mL) was added to the resin and the mixture was stirred at 120 °C for 12 h. The resin was filtered and washed with DMF (5 x 50 mL), DCM (3 x 50 mL), THF (3 x 50 mL) and ether (3 x 50 mL). It was dried under vacuum. The dried resin was swollen in distilled ethanol (20 mL) for 1 h, hydrazine hydrate (0.02 mL) was then added and the reaction mixture was refluxed at 80 °C for 8 h. The resin was collected by filtration washed with hot ethanol (5 x 50 mL) methanol (3 x 50 mL) and ether (3 x 50 mL), and dried under vacuum. The amino capacity of the resin was determined by Picric acid method.[18]

5.3.5. Estimation of amino group by picric acid method

Functionalised CLPSER (**20**, 7 mg) was taken in a sintered funnel and swelled in DCM for 30 minutes and treated with reagent I and reagent II as follows:

Reagent I : Picric acid (0.1 M) in CH_2Cl_2

Reagent II : Triethylamine 5% in CH_2Cl_2

(i) The swelled resin treated with Reagent I (2 x 3 mL x 5 min).

(ii) Washed with CH_2Cl_2 (5 x 3 mL x 1 min)

(iii) The picrate was eluted with Reagent II (2 x 2.5 mL x 2 min) and saved the elute.

(iv) Made up the eluted solution to 15 mL in ethanol.

(v) 0.5 µl of the above solution made upto 5 mL in ethanol to give a suitable absorbance.

Absorbance at 358 nm of the picrate solution was noted and the free amino group was estimated using the equation

$$\frac{OD \times 5 \times 15 \times 1000 \times 10^{-5}}{0.145 \times 0.5 \times \text{wt. of the resin}}$$

(vi) Amino capacity of the resin was estimated as 0.1212.

5.3.6. Anchor attachment to CLPSER (20)

The resin was dried in a lyophilizer. The aminomethyl CLPSER resin (225 mg, 0.1212 mmol/g) was made to swell in DMF in a peptide synthesizer for 1 h. Excess DMF was drained off. Then a mixture of HMPA (1.74 mg, 0.35 mmol), HBTU (36.2 mg, 0.35 mmol), HOBt (12.9 mg, 0.35 mmol) in DMF (1.5 mL) was added. DIEA (16.3 µl, 0.35 mmol) in DMF (0.5 mL) was added to the reaction mixture and the mixture was kept for 1 h with occasional stirring. After 1 h the reaction mixture was drained off and washed with DMF (4 x 25 mL), methanol (4 x 25 mL) and ether (4 x 25 mL) and dried over night under vacuum. The resin was cooled at -70°C for 2 h and subjected to lyophilisation.

5.3.7. C-Terminal Fmoc amino acid incorporation

The dried CLPSER-HMPA resin (200 mg, 0.1212 mmol/g) was swollen in dry DCM (100 mL). After 1 h, excess DCM was removed and a C-terminal F-moc amino acid, Fmoc-Glycine (0.5 mml), MSNT (0.5 mmol) and N-methylimidazole (0.45 mmol) mixture in dry DCM (5 mL) was added. Nitrogen gas as a continuous stream was bubbled through the reaction mixture. After 1 h, the resin was washed with dry DCM (5 x 10 mL), ethanol (5 x 10 mL) and ether (5 x 10 mL) and dried under vacuum. The dried resin (5 mg) was mixed with 2 mL 20% piperidine in DMF for half an hour. The percentage incorporation of amino acid was estimated by measuring the optical density (OD) of the above solution containing dibenzotoluene-piperidine adducts at 290 nm. The OD measured was 0.8984. Thus amino capacity of the resin after the C-terminal attachment per g was

$$= \frac{0.8984 \times 0.11 \times 10}{1.65 \times 57} = 0.1198$$

5.3.8. Synthesis of dipeptide G-A using 3-mercapto-5,6-diphenyl 1,2,4-triazine (13)

Fmoc alanine (3.5 mmol excess), DCC (3.5 mmol excess) and thiol **13.** (3.5 mmol excess) were dissolved in minimum N-methylpyrrolidone in a small R.B. flask. Kept for 1 h. Filtered off the precipitated DCU in a Gisin tube and the filtrate (activated amino acid) used for coupling.

Then C-terminal glycine incorporated CLPSER-HMPA resin in a silanized 15 mL glass peptide synthesizer made to swell for 1 h. To this swollen resin 10 mL 20% piperidine in DMF (10 mL) was added so that Fmoc deprotection was carried out washing DMF. Then the above activated amino acid filtrate was added to the swollen resin. The extent of coupling was monitored by ninhydrin test.[20] Absence of bluish violet colouration indicates the completion of coupling (40 min). Again 20% piperidine in DMF (10 mL) was added for 30 min more for deprotection and again ninhydrin test was carried out.

The dipeptide fragment G-A was cleaved from the resin using the following procedure. Dipeptidyl resin was suspended in a mixture of TFA (3 mL), water (150 mL), thioanisol (150 μL), ethanedithiol (150 mL) and phenol (200 mL) and the mixture was kept at room temperature for 6 h. Then the suspension was filtered, washed with TFA (1 mL), DCM and the filtrate was concentrated (10 mL) under reduced pressure at 40°C until the TFA reweers moved from the filtrate. To the filtrate ice cold diethylether (10 mL) was added, the precipitated dipeptide G-A was washed with ice cold ether (6 x 10 mL) to remove the scavangers and discs. The precipitated dipeptide G-A was identified by tlc.

5.3.9. Synthesis of G-A using 2-mercapto-4,6-dimethyl pyrimidine(14)

The above procedure was repeated using thiol **14** as the carboxyl activating group instead of thiol **13**. The dipeptide formed was also identified by homogeneous tlc.

5.3.10. Time-dependent amino acid coupling using thiol 13.

Resin after first attachment (61 mg) was swelled in DMF for 1 h. Then it was subjected to deprotection for 30 min. by washing with DMF (10 mL x 7). Tested for deprotection using ninhydrin. Then reaction mixture thiol (**13**, 3.5 mmol excess), Fmoc alanine (3.5 mmol excess), HBTU (3.5 mmol excess) and DIEA (3.5 mmol excess) was added in DMF and it was mixed properly to get uniform distribution. At definite time intervals, a small amount of resin was withdrawn for 1 h. The withdrawn resin was washed with DMF (3 mL x 5), methanol (2 mL x 5). Dried overnight in vacuum desiccator. In the next day, weight of the resin was taken. Then it was subjected to deprotection using 3 mL 20% piperidine. 1 mL of this solution was used for OD measurement. Time interval, weight of the resin and reflection OD are shown in table **5.1.**

5.3.11. Time dependent amino acid coupling using thiol 14

The above procedure was repeated using 52 mg of thiol (**14**) instead of thiol **(13)**. Time, wt. of resin, OD measurement and amino capacity determination are shown in table **5.2.**

5.3.12. Synthesis of acyl carrier protein (ACP) on CLPSER (20) using thiol 13

CLPSER-HMPA-Gly-Fmoc (100 g, 0.01 mmol Gly) was used for the preparation of the ACP fragment (65-74). The resin was taken in a manual peptide synthesiser and swelled in DMF for 1 h. Fmoc protection was removed by 20% piperidine in DMF (10 mL) for 20 min and washed the resin with DMF (6 x 20 mL).

Coupling reactions were carried out in a minimum volume of DMF as solvent. The respective F-moc amino acid (3.5 mmol excess) was added to the swollen resin. HBTU (36 mg, 0.1 mmol), thiol (**13**, 30 mg, 0.1 mmol) and DIEA (17.7 μL, 0.1 mmol) were added to it and the solution was mixed thoroughly. The reaction mixture was then kept for 40 min with occasional shaking. The solution was filtered and washed with DMF (6 x 10 mL). The coupling and deprotection steps were monitored by ninhydrin test. The synthetic operations are as follows:

i) Wash with DMF (6 x 1 min)

ii) Fmoc-deprotection with 20% piperidine in DMF.

iii) Wash with DMF (6 x 1 min)

iv) Acylation was carried out with 3.5 mmol excess of Fmoc-amino acid, HBTU, thiol **13** and DIEA relative to the amino capacity of the C-terminal amino acid.

v) Wash with DMF (6 x 1 min).

After incorporating the remaining amino acids, F-moc protection of N-terminal amino acid was removed with 20% piperidine in DMF (1 x 10 mL x 20 min). The resin was washed with DMF (6 x 10 mL), NMP (5 x 10 mL), DCM (5 x 10 mL), MeOH (5 x 10 mL) and ether (5 x 10 mL), and dried under vacuum.

The peptide was cleaved from the peptidyl resin by suspending in TFA (2.85 mL), ethanedithiol (75 μL) and water (75 μL) and the mixture was kept at room temperature. After 6 h, the suspension was filtered, washed with TFA (1 mL), DCM (10 mL) and the filtrate was concentrated under reduced pressure at 40 °C until the

TFA was removed from the filtrate. Then the peptide was precipitated by the addition of ice cold ether. The peptide was washed thoroughly with ether to remove the scavangers added and dried. The peptide was further purified by dissolving in aqueous 2% acetic acid and passed through a Sephadex G-10 column (50 cm X 1 cm). The clutching fractions containing the peptide were collected and lyophilized.

5.3.13.Synthesis of G-A-A-A using thiol 14

The tetrapeptide G-A-A-A was synthesised by substituting thiol **14** as carboxyl activating group instead of **13** and monotored similar procedure applied for the synthesis of ACP.

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SUMMARY AND OUTLOOK

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SUMMARY AND OUTLOOK

Chapter 6

SUMMARY AND OUTLOOK

The basic criteria for the synthesis of peptides are embedded with strategies that enable the amino acid coupling easy under mild conditions and to attain maximum purity for the sequential peptides obtained. Peptide chemistry, nowadays, seeks advanced techniques to synthesise peptides with well defined sequential arrangement of amino acids to mimic naturally occurring biologically active peptides.

In the present work, the main thrust is to find out transient carboxyl activating groups which could be effectively applied in peptide synthesis. Thus, two suitable and potent carboxyl activating heterocyclic thiol compounds, 3-mercpato-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine are introduced here. The efficiency of these easily accessible heterocyclic systems as versatile carboxyl activating groups have been subjected to investigations using their acyl derivatives, by the chemical conversion of respective amides or esters, in addition to spectrophotometric monitoring, exploiting the UV-visible absorption spectra.

Thus, for the above purpose, these mercapto heterocyclic systems were derivatised to their acyl derivatives so that the respective S-acyl derivatives formed initially were easily tautomerised to the thermodynamically more stable N-acyl derivatives. With a view to proving their acyl transferring efficiency, these N-acyl derivetives were then made to react with aliphatic, aromatic and hydroxyamines

under room temperature conditions. In addition, those acyl derivatives were subjected to esterification reactions with different alcohols and phenols. Moreover, the above reactions were studied spectrophotometrically by scanning the UV-visible spectrum of the compounds regenerated during the course of reactions. Finally the application of these activating carboxyl group in peptide synthesis employing the SPPS was also carried out

The results obtained in these investigations are summarised as follows:

(i) 3-Mercapto-5,6-diphenyl-1,2,4-triazine and 2-Mercapto-4,6-dimethyl pyrimidine were found to be excellent carboxyl activating groups and were synthesised and characterised using established procedures. Anyhow, the former thiol has been proved to be more effective in carboxyl group activation than the latter.

(ii) The above thiol systems were subjected to acylation reactions and the derivatised products were characterised as N-acyl-5,6-diphenyl-1,2,4-triazine-3-thione and N-acyl-4,6-dimethylpyrimidine-2-thione using different analytical and spectral techniques.

(iii) The suitability of carboxyl activation of the above acyl derivatives were established by aminolysis reactions using various amines and amino alcohols and esterification reactions using alcohols and phenols. But esterification rections were found to be very sluggish and those observations were in close

agreement with the theoretical predictions. The products formed during aminolysis and esterification reactions were amides and esters respectively.

Further, aminolysis reactions in particular, were found to be so quick under room temperature conditions and the respective amides were formed in almost very good yield. Regeneration of original thiols in quantitative yield is a significant feature of the reactions because these reactions can be clearly monitored, chemically and spectrophotometrically, in addition to purification and recycling.

(iv) The relative ease of aminolysis using N-AcDTT and AcDPT with aliphatic, aromatic and hydroxylamines has been monitored, during the course of these reactions spectrophotometrically, exploiting UV-visible absorption spectra and the results obtained were found to be in agreement with the chemical conversions. These aminolysis reactions clearly prove that the N-acyl derivatives of both thiol systems are well carboxyl activated species, which indicates that both heterocyclic thiols are excellent carboxyl activating functions. On the contrary, by the following esterification reactions chemically as well as spectrophotometrically, it has been observed that alcohols and phenols show very poor response in esterification reactions indicating their weak nucleophilicity. From the spectrophotometric monitoring studies, during the course of aminolysis reactions conspicuously indicate that the reactions have a profound influence on the field effect of

amines and the rate of aminolysis is directly proportional to the nucleophilicity on nitrogen centre.

(v) Carboxyl activating nature of 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine, were further confirmed by solid phase peptide synthesis. Here, CLPSER resin was applied as polymeric support and synthesised smaller peptides and ACP by substituting these thiols by replacing the usual carboxyl activationg reagents. The peptides synthesised were tested for their purity by HPLC and characterized.

Thus, from different chemical and spectrophotometric methods, it has been clearly showed that 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine are novel candidates for carboxyl group activation. These systems offer effective synthetic strategies, specifically in peptide synthesis and in general organic synthesis. Moreover, it is possible to illustrate the fundamentals of reaction mechanisms in organic chemistry. The aminolysis and esterification carried out here are not as a tool for the preparation of simple amides and esters, but to demonstrate the suitability of the selective mercapto heterocyclic systems in carboxyl group activation.

The spectrophotometric monitoring study clearly traces the regeneration of the original thiol in quantitative yield. Regenerated thiol could be recycled so that the entire experimental operations are done cost effectively. The characteristic colour change that is orange-red and yellow indicating the regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine respectively

observed during the reactions makes the chemical and spectroscopic investigations very simple. Good shelf life of the activated carboxyl derivatives is one of the added attractions.

Since peptides synthesised, using SPPS strategy on CLPSER polymeric support carrying thiols as carboxyl activating groups in very high purity, peptides of sequential array could also be synthesised by replacing 3-mercapto-5,6-diphenyl-1,2,4-triazine and 2-mercapto-4,6-dimethylpyrimidine instead of the usual carboxyl activating groups in SPPS. These carboxyl activating functions are easy to prepare and handle unlike conventional reagents. Moreover, the success achieved in the research work on the heterocyclic thiols as new choice of carboxyl activating groups can be extended in different perspectives. Different mercaptotriazine and diazine derivatives could be synthesised by applying similar synthetic procedures and those mercapto derivatives can be subjected to carboxyl activated measures so that still better and new carboxyl activating groups might be emerged in future.