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The Reactions of N-Phenylaminoiminomethanesulfonic Acid (PAIMSO) with Meldrum's Acid

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**The Reactions of
N-Phenylaminoiminomethanesulfonic Acid
(PAIMSO) with Meldrum's Acid**

Milana-Minja Maletic

**An Honors Paper Submitted in Partial Fulfillment of
Research Honors and Chemistry 499
at Illinois Wesleyan University**

April, 1991

Abstract

Reactions of N-phenylaminoiminomethanesulfonic acid (PAIMSO), a stable trioxide of N-phenylthiourea, with Meldrum's acid, a cyclic diester, were carried out under various conditions. The desired product should be N-phenylamidino acetic acid which could cyclize to give a β -lactam. The reactions were carried out in aqueous solution as well as in different organic solvents. In aqueous solution, the main product was N-phenylurea, obtained through a nucleophilic displacement reaction between water and PAIMSO. In pyridine, two or three equivalents of PAIMSO may undergo condensation. No traces of an addition product were observed. This was also true for the reactions carried out in organic solvents under acidic conditions.

APPROVAL PAGE


Honors Paper

The Reactions of
N-Phenylaminoiminomethanesulfonic Acid
(PAIMSO) with Meldrum's Acid

Presented by

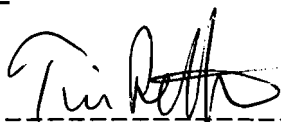
Milana-Minja Maletic

Project Advisor:

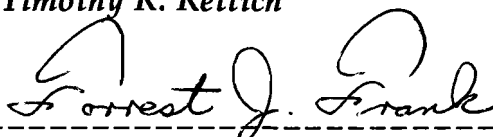


Judith J. Bischoff

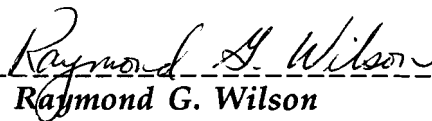
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Timothy R. Rettich



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Raymond G. Wilson

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Chapter I

INTRODUCTION

1.1 Rationale

There is a great deal of evidence that S-oxides of thioureas are intermediates in some biological reactions of the unsubstituted as well as the substituted thioureas.¹⁻⁴ The S,S,S-trioxides of thioureas are susceptible to nucleophilic attack by amino acids to form stable guanidino acids^{5,6} and by primary amines to form guanidines.⁷ Thus it is proposed that thiourea S,S,S-trioxides (4) are also susceptible to nucleophilic attack by enolates of diethyl malonate or 2,2-dimethyl-1,3-dioxan-4,6-dione (Meldrum's acid) (11). It is further postulated that products from these reactions would be the N-substituted amidino acetic acids (28). These compounds are of interest because some homologous amidino carboxylic acids have been shown to have antifungal activity. It is also proposed that intramolecular ring closure of amidino acetic acid could be effected to form a β -lactam ring, a functional group present in penicillin (23) and cephalosporin (24) antibiotics, which is partially responsible for the biological activity of these classes of antibiotics.

1.2 Background

1.2.1 Occurrence of Oxidized Thioureas

It has been reported in several articles¹⁻³ that the metabolism of biologically active thioureas involves oxidation at sulfur. This oxidized intermediate can then undergo nucleophilic attack followed by elimination of the oxidized sulfur moiety, or it can, alternatively, undergo elimination or

reduction reactions.⁴ *In vivo*, oxidation of thioureas is believed to be catalyzed by FADMO (FAD-containing monooxygenase).^{4,8,9} Oxidation of thioureas with FADMO *in vitro* is a sequential reaction in which the thiourea-S,S-dioxide is the main product.⁴ However, further oxidation of this compound to S,S,S-trioxide does not seem to be catalyzed by FADMO (Figure 1).

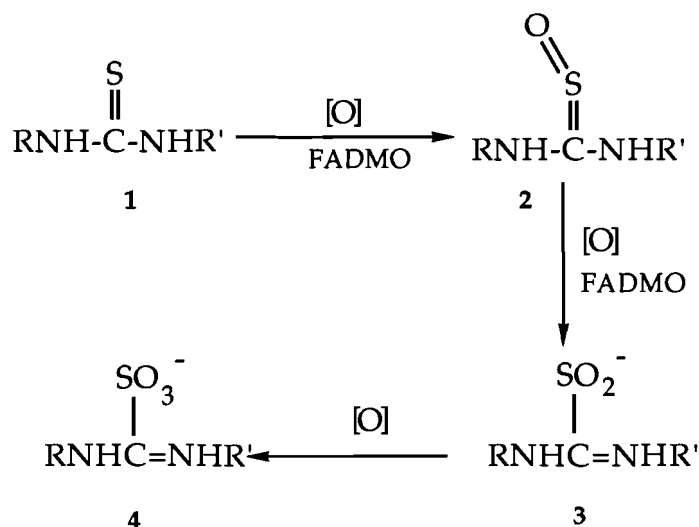


Figure 1

Many synthetic methods are known for the preparation of thiourea-S,S,S-trioxides. The most common preparation is oxidation of thioureas with a strong oxidizing agent with, or without a catalyst.⁴ For instance, Walter, whose decade-long research in the area of oxidation of thioureas and thioamides resulted in the publication of over 30 articles,⁵ prepared both the dioxides (3) and trioxides (4) of thioureas using various methods. He suggested a method for oxidation of N-phenylthiourea (5) to the corresponding S,S,S-trioxide (6) using an excess of freshly prepared peracetic acid in CHCl_3 , or a combination of CHCl_3 and ethanol.¹⁰ This oxidation is shown in Figure 2.

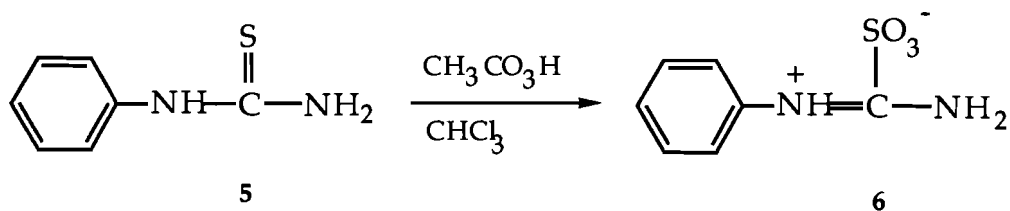


Figure 2

Bischoff also reported the oxidation of thiourea and mono- and disubstituted phenylthioureas to the corresponding trioxides with peracetic acid in methanol (10-20 °C) with yields ranging from 43 to 83%.⁴

An alternative oxidant used in the preparation of the oxides of thioureas is hydrogen peroxide. This oxidant is mostly used for the various preparations of the dioxide derivatives. Walter, for instance, prepared dioxides using 30% H_2O_2 in pyridine or ethanol at temperatures close to 10 °C.⁴ Several other procedures for the preparation of various dioxides using 30% H_2O_2 were reported. These procedures involved various solvents (e.g. water, carbon tetrachloride) at temperatures close to 0 °C.⁴ Maryanoff and coworkers carried out the oxidation of phenylthiourea to N-phenylaminoiminomethanesulfonic acid [PAIMSO (6)] by H_2O_2 as a slurry in water at low temperature to give 80% yield. To effect rapid oxidation they used a sodium molybdate catalyst.⁵ This reaction is shown in Figure 3.

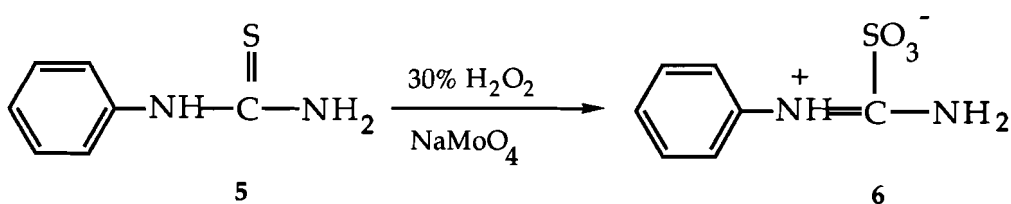


Figure 3

Thiourea trioxides can also be prepared by direct oxidation of the corresponding dioxides.⁴ The oxidation, in some cases, occurs spontaneously at slightly elevated temperatures (39 °C).

It is also possible to prepare trioxides of thioureas by nucleophilic displacement. One of these reactions involves nucleophilic displacement of the thiomethyl group of substituted S-methylisothiuronium iodide salts (**4a**) with Ag_2SO_3 at room temperature. The yields for this reaction were poor, ranging from 13-75%.⁴ In another nucleophilic displacement reaction, the thiomethyl group of 1-methylthio-1-methyliminomethanesulfonate (**4b**) can be replaced with a primary amine nucleophile.⁴

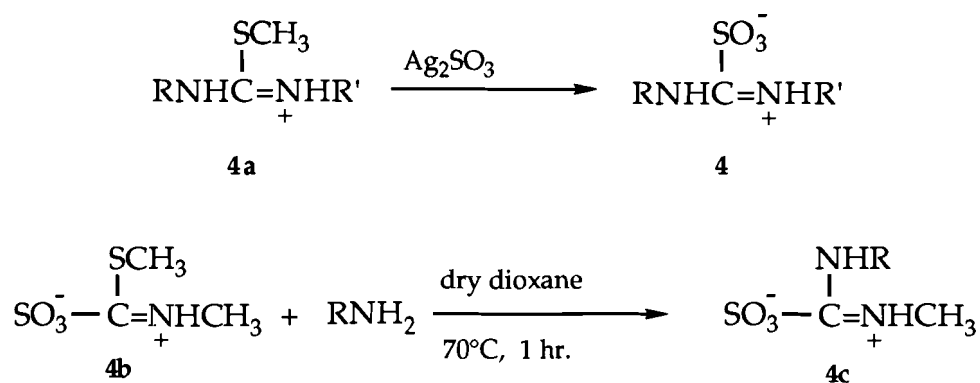


Figure 4 (a,b)

1.2.2 Properties of Thiourea S-Oxides

The three S-oxide derivatives of thiourea: sulfenic, sulfinic, and sulfonic acids differ in their thermal as well as their chemical stability. Monoxides, or sulfenic acids (**2**), are thermally very labile.⁵ In most cases, depending on the N-substituent, these species are transient and can only be detected by chromatographic methods. Bulky N,N'-substituted thiourea monoxides have been isolated at low temperatures,¹¹ but they immediately decompose to the starting thiourea and urea when heated to 40 °C. Formation of the corresponding thiourea (**1**) and urea (**7**) was also observed upon treatment of sulfenic acids with acid or base. In some cases traces of

formamidine were formed. The decomposition of sulfenic acids in acid or base is shown in Figure 4:

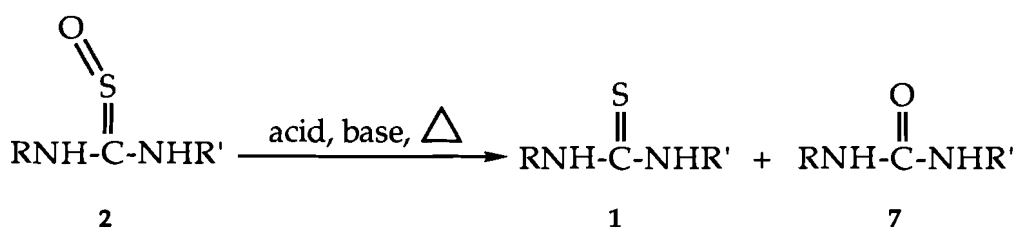


Figure 5

The S,S-dioxides, or sulfinic acids (3), are thermally more stable than the monosubstituted derivatives. They can be isolated at temperatures slightly below room temperature.⁵ Sulfenic acids are also unstable to acid or base treatment and decompose to the corresponding ureas. Furthermore, upon treatment with acid, the SO₂⁻ group may be eliminated to form a formamidine.

The S,S,S-trioxides, or sulfonic acids (4), are much more thermally stable than the other two oxide derivatives of thioureas. They are very stable in the presence of acid, but at various alkaline pH's form the corresponding urea.¹²

1.2.3 Nucleophilic Displacement Reactions of Sulfonic Acids

Since thiourea-S,S,S-trioxides have been shown to be much more stable than mono- or dioxides of thioureas (12), they are more suitable reactants for the nucleophilic displacement reactions under acidic or basic conditions. The most important nucleophilic displacement reaction of activated thioureas is guanylation (Figure 5). This reaction has been studied in several laboratories:⁵⁻⁷

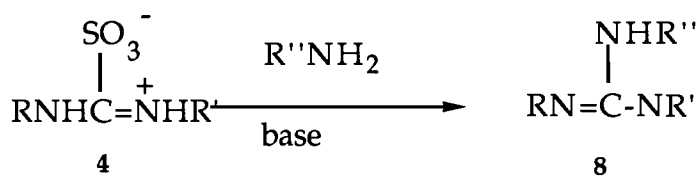


Figure 6

The guanylation reaction is particularly interesting because it provides a simple preparative method for guanidino acids⁴ and guanidines^{5,7} under mild conditions, without evolution of unpleasant or toxic gases. Earlier guanylation methods involved reaction of ammonia or different amine derivatives with cyanamides, carbodiimides, chloroformamidines, or dichloroisocyanides. All of these starting materials are either corrosive, toxic, or moisture sensitive.¹³

In the procedure developed by Maryanoff and coworkers,⁵ synthesis of guanidines (8) from thiourea-S,S,S-trioxides using amine nucleophiles was carried out at room temperature in acetonitrile solutions. After a simple work-up, the desired guanidine was obtained in 80-95% yield. Kim and coworkers⁷ also devised a synthesis of guanidines from thiourea-S,S,S-trioxides. The prepared monosubstituted guanidines from equimolar amounts of a primary amine and aminoiminomethanesulfonic acid (AIMSO), the unsubstituted thiourea-S,S,S-trioxide. These syntheses were carried out in absolute methanol, at room temperature, with yields of guanidines ranging from 50-80%.

Bischoff and Miller⁶, on the other hand, studied the formation of guanidino acids (9). They observed that by reacting thiourea-S,S,S-trioxides with various amino acids in 1 M K₂CO₃ solution, guanidino acids could be prepared in 45-80% yields. In their studies, guanylation occurred in minutes or days depending on the N-substituents on AIMSO. The reaction for the preparation of guanidino acids is shown in Figure 6.

The mechanism for guanylation proposed by Bischoff⁴ involves nucleophilic attack of the amine on the sp^2 hybridized carbon of the thiourea- S,S,S -trioxide, resulting in the formation of a tetrahedral intermediate. The sulfite group is then eliminated from this unstable intermediate and the sp^2 hybridized carbon atom is regenerated. Guanylation needs to be carried out under alkaline conditions since base acts to generate the free amine from the amino acid.

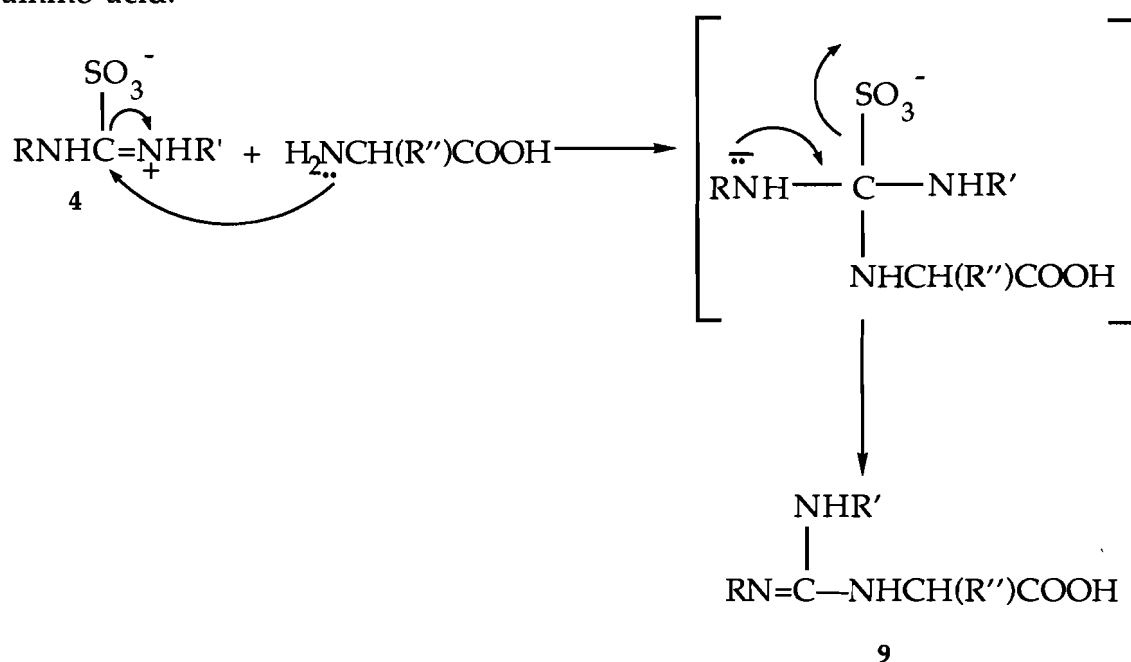


Figure 7

Maryanoff and coworkers⁶ also studied the mechanism of guanylation. They used both chromatographic and spectroscopic methods in their experiments. When they followed the reactions with tlc, they observed an intermediate in the reaction mixture. This observation lead them to propose two mechanisms: addition/elimination and elimination/addition. In the first mechanism, the intermediate is formed by addition of a nucleophile to the sp^2 hybridized carbon, followed by elimination of the sulfite group to form a guanidine (Figure 6). In the second mechanism, the sulfite group is

first eliminated from the same carbon to generate a carbodiimide intermediate (**10 a**). The carbodiimide may further tautomerize to form cyanamide or undergo nucleophilic attack by the amine nucleophile to form a guanidine. This mechanism is shown in Figure 7.

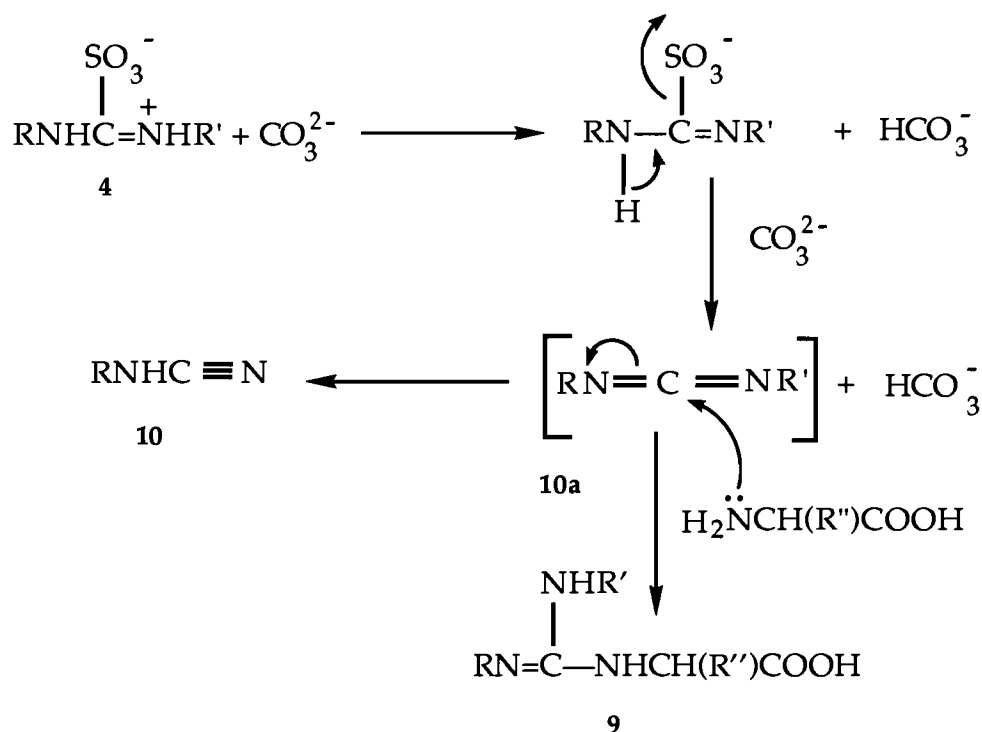


Figure 8

The intermediate, when studied by IR spectroscopy, did not show absorptions characteristic of carbodiimide (**10 a**) or cyanamide (**10**) molecules (1900 and 2300 cm^{-1}).⁶ The elimination/addition mechanism proposed by Maryanoff and coworkers was ruled out. Guanylation, therefore, probably occurs through the addition/elimination mechanism, as suggested by Bischoff.⁴

1.2.4 Nucleophilic Displacement Reactions of β -Dicarbonyls

It is well known that diethyl malonate is an excellent nucleophile because it readily loses a proton under basic conditions ($\text{pK}_a = 13$)¹⁴ to form an enolate anion. The enolate ion can be used to convert an alkyl halide to an α -substituted acetic acid. This is the malonic ester synthesis, one of the classic and best known carbonyl alkylation reactions.¹⁴ This acidity is not unique to malonic ester. The α -hydrogen atoms in all β -dicarbonyls are very acidic because they are “flanked by two carbonyl groups.”¹⁴ When one of the α -hydrogens is lost, the filled p-orbital overlaps with the neighboring carbonyl-group p-orbitals and the α -carbon acquires sp^2 character. This p-orbital overlap further allows for the negative charge on the α -carbon to be delocalized and the enolate ion is stabilized by resonance. Diesters are more acidic than diketones, since they can delocalize the negative charge on the enolate ion over a larger number of atoms. The p-orbitals of the two additional oxygen atoms in diesters participate in the delocalization of charge.

Meldrum's acid (**11**), 2,2-dimethyl-1,3-dioxan-4,6-dione, is a cyclic diester with strongly acidic properties. It is comparable in strength to acetic acid ($\text{pK}_a = 4.76$)¹⁵ and some 10 pK units more acidic than acyclic diesters (e.g. diethyl malonate). The explanation for this facile proton loss is in the rigidity of the cyclic structure of the diester. As it is the case with acyclic diesters, the enolate ion of cyclic esters is stabilized by resonance. In addition to this, the α -hydrogens are easily accessible to the base (not sterically hindered) since they are located above the plane of the ring and their removal occurs much more readily. The resonance stabilization of the enolate of Meldrum's acid is shown in Figure 8.

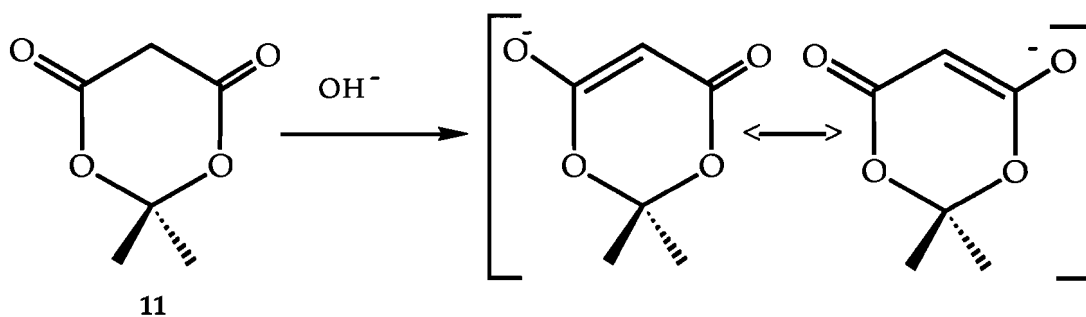


Figure 9

Meldrum's acid has been extensively used in synthesis as an excellent nucleophile. A review by McNab presents many possibilities for the synthetic uses of this cyclic malonate.¹⁵ In one of the reactions, Smith carried out a malonic ester-type synthesis with Meldrum's acid to effect substitution of CH_2COOH on the 4-position of the quinoline ring (12). This synthetic method used highly acidic enols.¹⁶ Under such acidic conditions, the N atom in the quinoline molecule carries a positive charge because it is protonated and thus serves as an "electron sink". The mechanism for the reaction is shown below (Figure 9). It is evident that this reaction would not be possible if the N-containing ring were not activated by protonation.

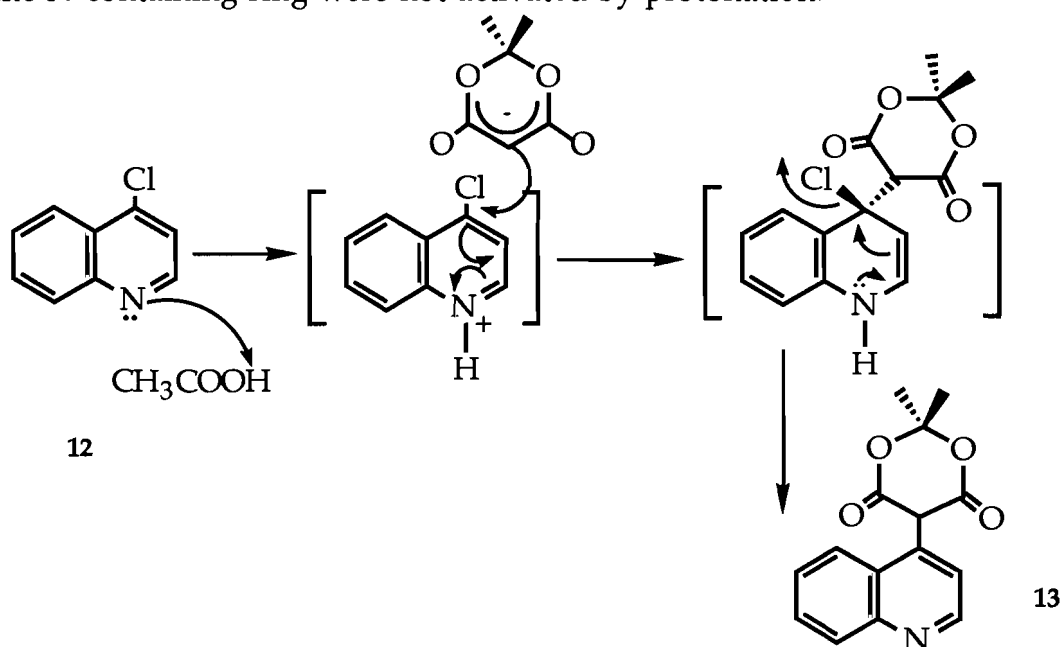


Figure 10

Similar substitution reactions were carried out using pyridine.¹⁷ The reactions were carried out under basic conditions, with the ring nitrogen activated (positively charged) by methylation. No reaction occurred in the systems with unmethylated pyridine derivatives, showing that the activated nitrogen atom was essential for the substitution to occur. The mechanism for this reaction is the same as the one shown in Figure 9, with the electrophilic site on 4-chloropyridine instead of 4-chloroquinoline.

Since the trioxides of thioureas are zwitterionic compounds (have a protonated N atom) that contain an sp^2 hybridized carbon atom susceptible to nucleophilic attack and are stable under acidic conditions, their reaction with Meldrum's acid in an acidic solution may proceed by a mechanism similar to that shown in Figure 9, yielding the desired amidino acetic acid as the main product. Furthermore, the reactions of quinolines under acidic conditions, described previously, were carried out at temperatures slightly above room temperature (55° C). At this temperature both Meldrum's acid and thiourea-S,S,S-trioxides are stable.

Another interesting reaction is the condensation of Meldrum's acid with Mannich bases ($RCH_2NR'_2$). Smith¹⁸ prepared a lactone (15) in high yield when treating Meldrum's acid with 1-[(dimethylamino)-methyl]-2-naphthol (14). This reaction was also carried out under slightly elevated temperatures (55 °C) in 1,2-dimethoxyethane/acetic anhydride mixtures and is shown in Figure 10.

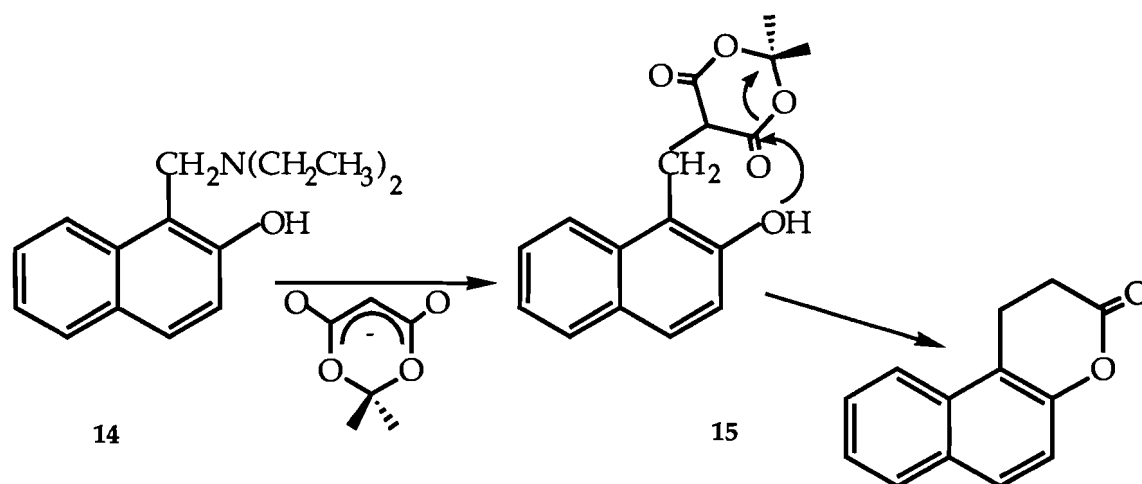


Figure 11

A similar ring closure to form a β -lactam may be effected in the amidinoacetic acids formed from thiourea-*S,S,S*-trioxides.

1.2.5 Amidino Carboxylic Acids

Some functionalized amidines are known to have biological activity. For instance, the *N*-substituted amidinoformic acid (22) is a very unique and interesting moiety biologically.¹⁹ This moiety has been identified as a part of kasugamycin (Figure 11), an antibiotic used in prevention of rice blasts and *Pseudomonas* infection in humans.

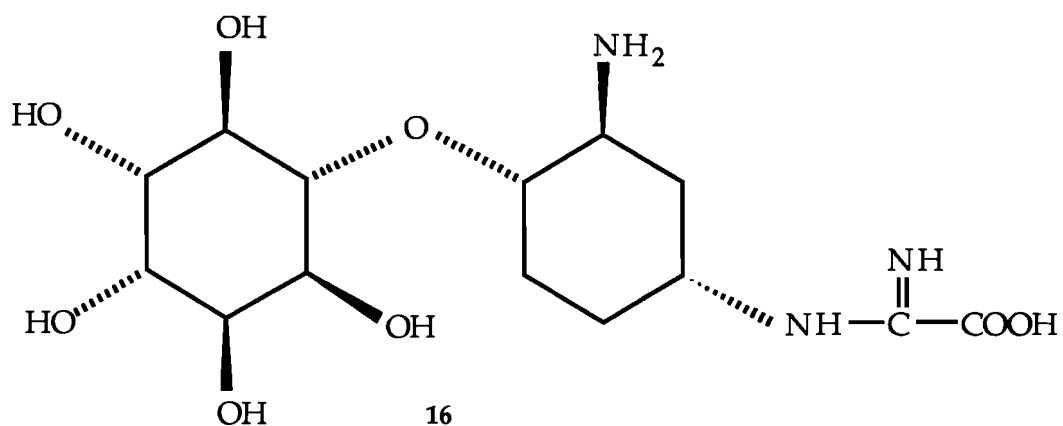


Figure 12

Another functionalized amidine that is known to have antibiotic properties is *cis*- β -acrylamidine (17). This compound has been reported as a cytotoxic agent²⁰ and was isolated from the fermentation broth of an unidentified actinomycete. This compound was inactive *in vitro* against a number of different microorganisms, but has been shown to have antifungal activity. The reduction of this compound to amidinopropionic acid (18) can be accomplished easily and is shown in Figure 12.²¹

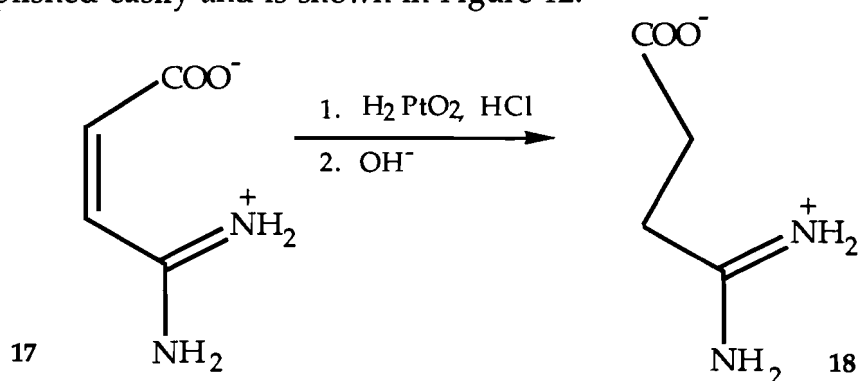


Figure 13

The two amidino carboxylic acids shown above are both very pertinent to this study since they differ structurally from amidino acetic acid only in the length of the carboxylic acid chain. All of the early methods for the preparation of these amidino acetic acids (28) afforded very low yields.¹⁹ However, the method for synthesis of various N-substituted and unsubstituted amidinoformic acids (22) developed by Nii and coworkers,¹⁹ required mild conditions and afforded excellent yields (92-95%). Their syntheses involved selective nucleophilic substitution with amino groups on the carbon of the "cyano group equivalent" of cyanoformates after it had been activated. A cyanoformate (19) was first converted into thioamide (20) either by addition of hydrogen sulfide or by treatment of benzyl alcohol-HCl followed by phosphorus pentasulfide. This thioamide was activated by alkylation with triethyloxonium tetrafluoroborate to afford thiooxamimidate

(21). Further addition of an amine in methylene chloride at room temperature afforded the corresponding N-substituted or N-unsubstituted amidinoformic acid esters. Hydrolysis of this compound gave the free acid. This mechanism is shown in Figure 13.

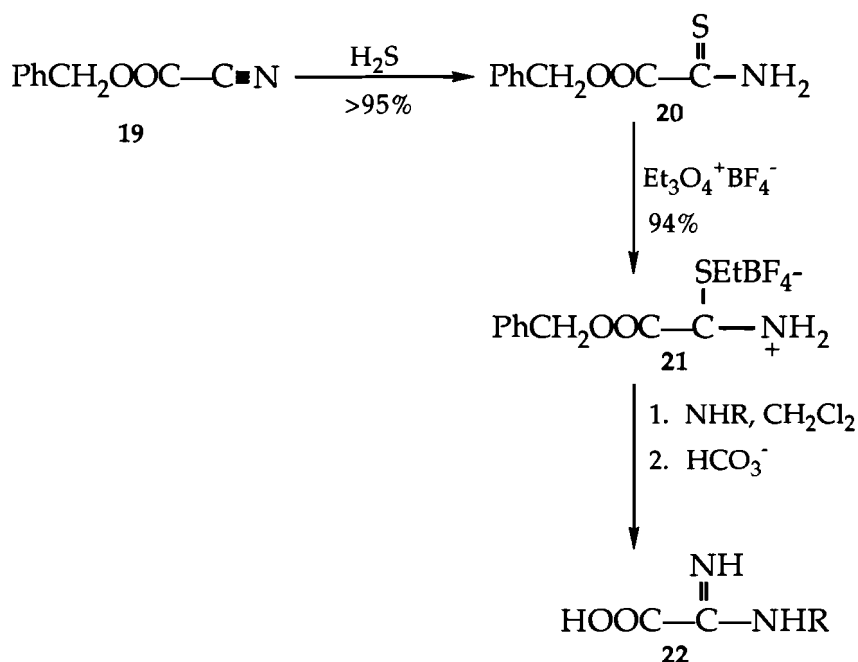


Figure 14

1.2.6. β -Lactam Antibiotics

Penicillin (23) and cephalosporin (24) antibiotics are characterized by the β -lactam ring. The general structure of both of these antibiotics is shown in Figure 14. Penicillin contains a 4-membered β -lactam ring fused to a 5-membered thiazolidine ring, where the R group is variable. Cephalosporins also contain the β -lactam ring fused to a 6-membered S-containing ring. In a cephalosporin molecule both R and R' groups are variable. The X group can be either H or -OCH₃ group.²²

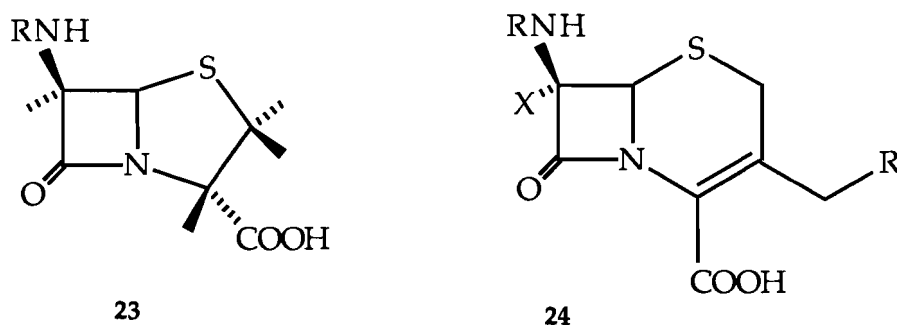


Figure 15

Since its discovery, penicillin has been known to inhibit the growth of bacteria. However, the mechanism of action of penicillin was not understood till later. Joshua Lederberg discovered that penicillin interfered with the synthesis of the bacterial cell wall.²² He came to this conclusion by observing that rod-shaped cells of *E. coli* growing in a hypertonic medium (a medium that has a very high osmotic pressure) changed into spherical cells when penicillin was added to the culture. These newly formed spherical bacteria had a fragile cytoplasmic membrane, but lacked most of the outer cell wall of their parents. When the osmotic pressure inside the cell was much greater than that outside the cell, these bacteria lacking a cell wall would burst. Bacterial cell wall consists of alternating units of two amino sugars: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The NAM units are linked to peptide chains, which are, in turn, cross-linked. In *Staphylococcus aureus* bacteria, crosslinking occurs when the amino group of a terminal glycine chain is inserted into the bond containing 2 D-alanine units of another chain to form a new bond (Figure 15).

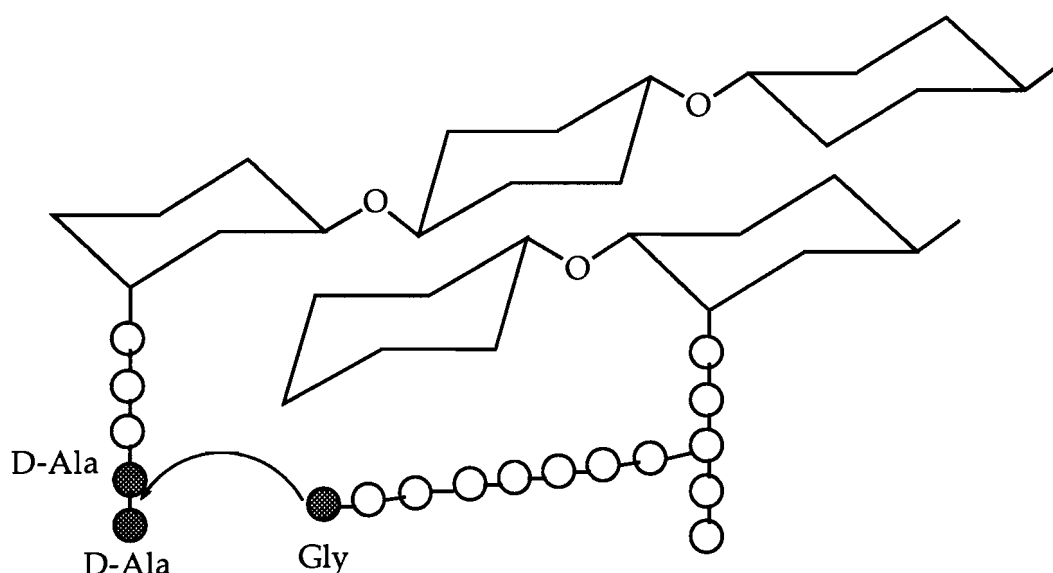


Figure 16

The enzyme that catalyzes this insertion in growing bacteria, transpeptidase, is attacked by penicillin and cephalosporin antibiotics. β -Lactam antibiotics also attack carboxypeptidase which is an enzyme that removes terminal D-alanine units by hydrolysis, but does not result in cross-linking.

The mechanism of penicillin action was proposed by Waxman and Strominger.²³ Penicillin, as well as cephalosporin, serves as a substrate analog to peptidoglycan transpeptidase. Both of these antibiotics are structurally similar to the acyl D-alanyl-D-alanine terminals (25) of the peptide chains in bacterial cell walls (Figure 16).

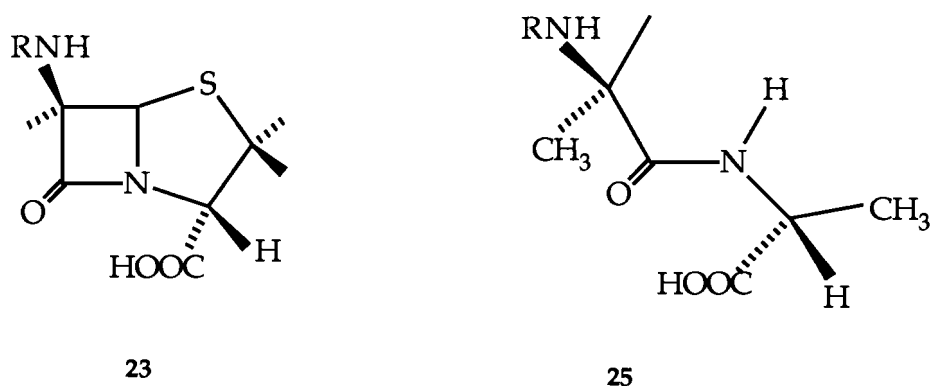


Figure 17

This structural similarity is most evident when the free carboxyl groups and the terminal asymmetrical centers are aligned, resulting in similar positioning of the highly reactive CO-NH β -lactam bond and the peptide bond cleaved during transpeptidase (as well as CPase) reaction. It has been further postulated that transpeptidase reacts with the peptide substrate to form an acyl-enzyme intermediate. In this step D-alanine is eliminated. The subsequent reaction with the free amino group in another peptide chain would lead to the cross-link formation. However, when penicillin (or cephalosporin) covalently bind to the transpeptidase (or CPase) active site, the active CO-NH β -lactam bond is cleaved and an inactive penicilloyl-enzyme compound (26) is formed. This compound permanently inhibits the action of transpeptidase and the formation of the bacterial cell wall²³ (Figure 17).

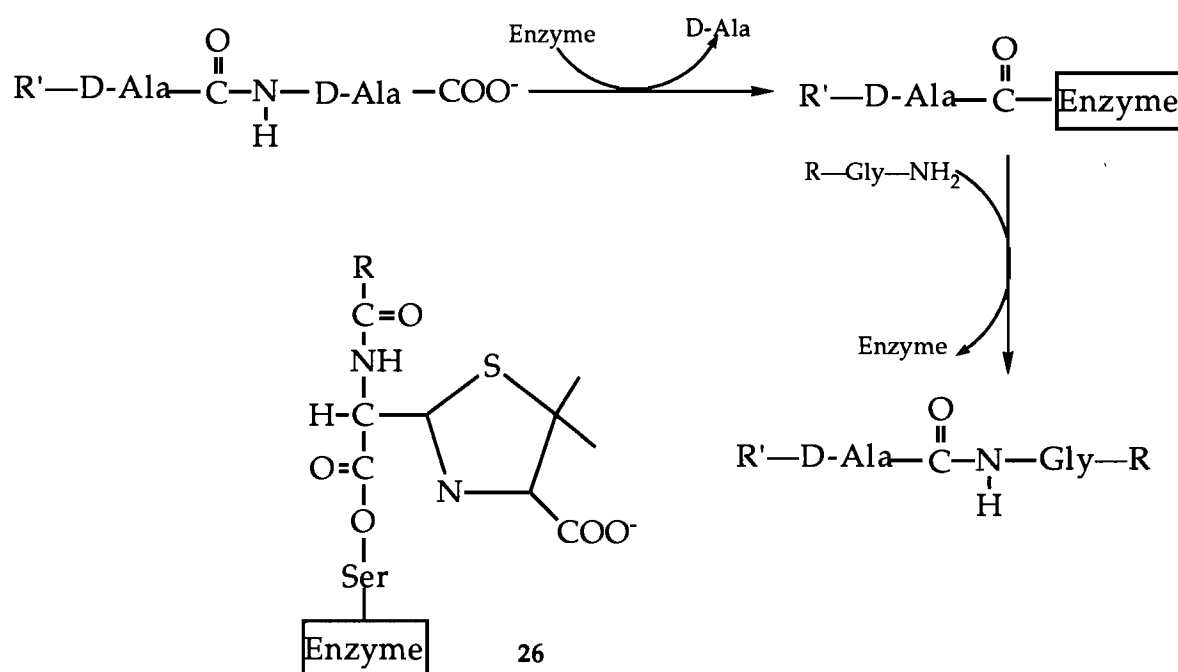
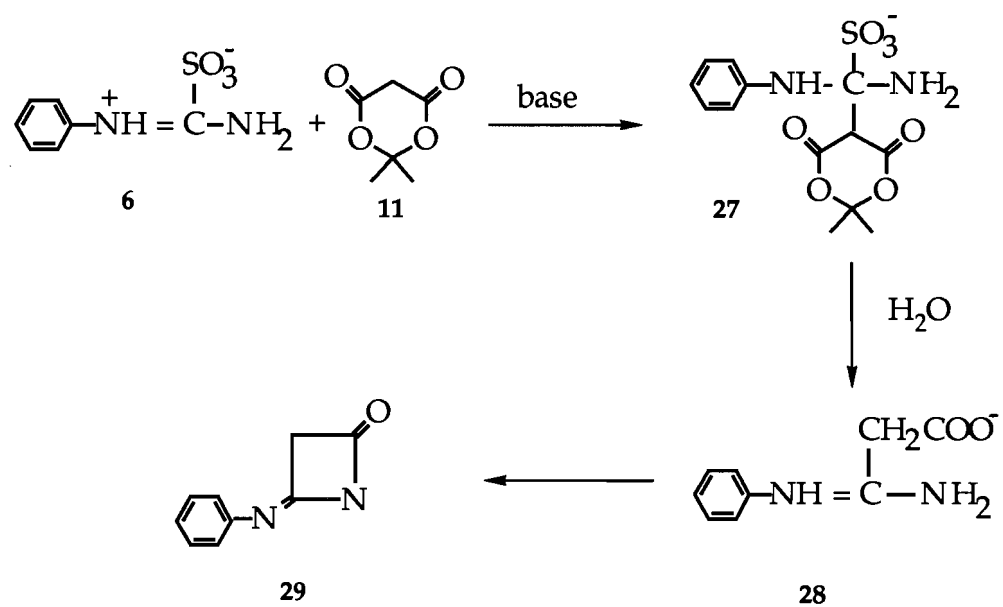


Figure 18

Since the early work on various penicillin and cephalosporin antibiotics, some bacteria have developed their own protective mechanism against these antibiotics. They can synthesize an enzyme called β -lactamase that catalyzes the dissociation of the penicilloyl-enzyme compound. This discovery initiated new research in the field of β -lactam antibiotics. For instance, other β -lactams have been developed that do not contain penicillin or cephalosporin ring systems. Some of these β -lactams are highly resistant to bacterial β -lactamases. Different synthetic as well as semisynthetic methods have been used for the preparation of β -lactams.²² As bacteria continue to adjust to the β -lactam antibiotics, research in this field has become increasingly important.

1.3 Goals of This Project

Our goal was to develop a simple method for β -lactam synthesis using N-phenylaminoimino-methanesulfonic acid, PAIMSO, as a starting material. By reaction of PAIMSO with Meldrum's acid, a CH_2COOH group could be introduced onto the amidine carbon. Once the amidino acetic acid (**28**) is formed, ring closure may occur through another nucleophilic addition reaction to form a lactam (**29**). Depending on the substituents on thiourea, other functionalities may also be introduced onto the ring (Figure 18).

**Figure 19**

This synthesis may provide an entry into a new class of antibiotics.

Chapter II

RESULTS AND DISCUSSION

Table 1 summarizes the physical constants of the reactants used for the preparation of PAIMSO as well as for the nucleophilic displacement reactions of PAIMSO and Meldrum's acid in various solvents.

Table 1: Physical Constants of Reactants

	M.W. (g/mol)	M.P. (°C)	IR Spectrum
Phenylurea	136.17	147	Appendix 1 - 3
1-phenyl-2-thiourea	152.23	154	Appendix 1 - 5
PAIMSO	200.22	166.5-167	Appendix 1 - 1,2
Meldrum's acid	144.14	94-96	Appendix 1 - 4

2.1. Oxidation of N-Phenylthiourea

In Table 2, the percent yields and melting points for the four preparations of PAIMSO are given. The first trial was discarded because the product that was prepared was not PAIMSO due to a procedural error.

Table 2: Synthesis of PAIMSO

Reaction	Mass of PTU* used (g)	Mass of PAIMSO prepared (g)	% Yield	M.P. (°C)	Comments
1	5.00	0.427	6.4	253-255	discarded
2	5.024	2.779	42.1	155-156	Appendix 1-1
3	5.004	2.292	34.7	156-156.5	-
4	5.012	2.895	43.9	160-162.5	Appendix 1-2

*PTU = 1-phenyl-2-thiourea

The addition of oxidant, peracetic acid, to the solution of 1-phenyl-2-thiourea in methanol, is the most important step of this synthesis. This addition needs to be carried out at temperature 0-10 °C. Slightly higher or lower temperatures during this oxidation step will result in lower yields of PAIMSO.⁴ Although the side products formed above 10 °C are not known, it has been suggested that PAIMSO undergoes decomposition to yield aniline and formamidine.⁴ When the solution was left sitting overnight, in order for oxidation to occur, the reaction mixture changed from colorless to brown. This development of color was probably due to a small amount of decomposition of PAIMSO to aniline.⁴ Furthermore, when the excess solvent was being removed from the reaction mixture *in vacuo*, the temperature was maintained under 50 °C. At a higher temperature, PAIMSO underwent decomposition (Figure 4).

In the first preparation of PAIMSO the reaction mixture was heated to 65 °C, resulting in decomposition of PAIMSO. Although no experimental evidence exists in support of this decomposition of PAIMSO to aniline and formamidine, it has been shown that N, N'-diphenylaminoiminomethane-sulfonic acid (DPAIMSO) undergoes acid or base catalyzed decomposition.⁴ The product of this decomposition in protic solvents (*e.g.* methanol) is bisanilinium sulfate $[(\text{PhNH}_3^+)_2\text{SO}_4^{2-}]$ formed through a formamidinium sulfate intermediate ($\text{PhN}^+\text{H}=\text{CHNHPH}$).

PAIMSO was identified by the melting point and IR spectroscopy. In the last three preparations of PAIMSO, the yields obtained ranged from 34.7% to 43.9% and the melting points were: 155-156, 156-156.5, and 160-162 °C, respectively. It is important to note that the melting point range was very small (1-2 °C) and that each sample of PAIMSO decomposed with bubbling at the melting point (as described in the literature).⁴ The literature melting

point is slightly higher than the melting points obtained in this experiment: 166.5-167 °C.⁴ Another source gave a melting point of PAIMSO that was even higher: 171-172 °C.¹¹ Despite these differences from the literature values in the MP of PAIMSO, the IR spectra obtained for all of the preparations of PAIMSO were identical and agreed with the literature spectra (spectra for preparations 2 and 4 are included in the Appendix 1 - Figures 1 and 2). The characteristic bands for S=O symmetric and asymmetric stretches are present at 1065 and 1245 cm⁻¹ (lit. 1060 and 1253 cm⁻¹, respectively).^{4,26} The two out-of-plane bending bands characteristic of the monosubstituted benzene ring were also present at 755 and 790 cm⁻¹.²⁶

2.2. Nucleophilic Displacement Reactions

The R_f values obtained from the tlc analyses of the different reaction mixtures are presented in Table 3.

**Table 3: Thin Layer Chromatography;
R_f Values of the Components of Different Reaction Mixtures**

Standard		Aqueous Solution		Pyridine Solution		Acidic Solution
Compound	R _f value	Spot	R _f value	Control	R _f value	R _f value
PTU*	0.70	1	0.74	-	0.66	0.21
PU [†]	0.45	2	0.42	0.54	0.54	-
MA**	0.08	3	0	0.09	0.10	-
PAIMSO	0	-	-	0.04	0.02	-

*PTU = Phenylthiourea

[†]PU = Phenylurea

**MA = Meldrum's acid

2.2.1 Reactions of PAIMSO and Meldrum's Acid in Aqueous Solution

The summary of the nucleophilic displacement reactions of PAIMSO with Meldrum's acid is presented in Table 4. The reactions were carried out in 1:1:1 molar proportions of the reactants, as shown in Table 4.

Table 4: Nucleophilic Displacement in Aqueous Solution

Reaction	Mass of PAIMSO used (g)	Mass of Meldrum's acid used(g)	Mass of K ₂ CO ₃ used (g)	Mass of product formed(g)	M.P. of the product (°C)
1 (1:1:1)	0.400	0.285	0.276	0.180 (66% for PU)*	136-140 Mixture MP with PU: 136-147
2 (1:1:1)	2.003	1.429	1.379	1.218 (89% for PU)	125-137

*PU = phenylurea

Several problems occurred when this malonic ester-type synthesis was carried out with Meldrum's acid in an aqueous solution. First, PAIMSO was only soluble in water in the presence of one equivalent of base (such as K₂CO₃). Second, water was found to be a better nucleophile than was Meldrum's acid under these basic conditions. As a result, the main product of this reaction was N-phenylurea which was obtained in 89% yield (in Reaction 3, Table 3) and identified by the melting point and IR.

Both reactions of PAIMSO with Meldrum's acid in aqueous solution yielded a mixture of products, with the main product being N-phenylurea (66-89% yield). The melting point range for phenylurea was different in each reaction (136 - 140 °C and 125 - 137 °C), but in both cases it was only a few degrees lower than the melting point of N-phenylurea (147 °C)²⁵, indicating an impure product. The IR spectra of N-phenylurea and the product from

this reaction, however, are almost identical (Appendix 1 - 3 and 6, 7). Some of the more prominent peaks shared by both spectra are: 3300, 1655, 1600, 1565, 760, and 710 cm^{-1} .

A possible mechanism for the formation of N-phenylurea in these reactions was the same nucleophilic addition/elimination mechanism proposed for the malonic ester-type synthesis of phenylamidinoacetic acid using Meldrum's acid and PAIMSO. This reaction would proceed through a tetrahedral intermediate with the nucleophile being water, followed by elimination of the sulfite (Figure 6). This proposed mechanism is shown in Figure 20; however, there is no experimental evidence to support this mechanism.

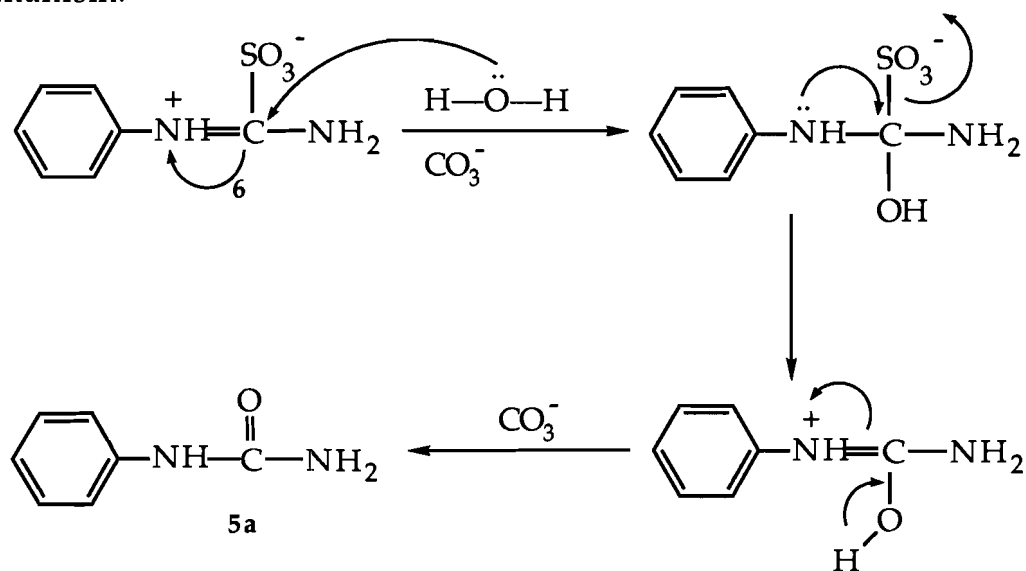


Figure 20

A small amount of a byproduct was detected in the reaction mixture by tlc. Several attempts were made to isolate this product, however these methods were unsuccessful. For instance, when a separation was carried out using thin layer chromatography, two spots were observed (Table 3). On the thick layer chromatography plate, however, many more bands were observed. One of the reasons for this may be the larger quantity that is used in thick

layer chromatographic separations. The reaction mixture was probably composed of a large number of byproducts that were present in very small quantities and undetectable by thin layer chromatography, but when applied on the thick plate, they were observable. There were, however, only two well defined regions on the thick layer chromatographic plate. When these two bands were separated and analyzed, the band with the smaller R_f was found to contain N-phenylurea. The melting point of this isolated material had a wide value range and was approximately 10 °C lower than the melting point of N-phenylurea (Table 5). The IR spectrum (Appendix 1 - Figure 8), however, did not differ significantly from the IR of phenylurea or the IR of the mixture before the separation was carried out (Appendix 1 - 6, 7).

The band with the higher R_f gave a mixture of unknown identity. The melting point was quite broad (106 - 121 °C), indicating a mixture of many compounds. The IR spectrum of this sample (App. 1 - 8) was also very similar to the spectra of N-phenylurea and the unpurified mixture.

**Table 5: Thick Layer Chromatography;
Separation of the Aqueous Reaction Mixture**

	Top Layer	Bottom Layer
% Recovery	56%	22%
M.P. (°C)	106-121	134-138
IR - Characteristic peaks (cm⁻¹)	1450(w), 1395(w), 1410(w), 1370(w) (Appendix 1 - 8)	1455(w), 1365 (m) (Appendix 1 - 9)

Column chromatography of the reaction mixture on alumina, was used to obtain five fractions which were analyzed by IR. The spectra for fractions 2 and 4 are shown in the Appendix (Figures 11 and 12). When these

two samples were analyzed by tlc, sample 2 contained the byproduct of the reaction that had the highest R_f value (in the tlc analysis), but sample 4 did not. The IR spectra of these two fractions were quite similar to each other and to the spectrum of N-phenylurea. Both of these spectra show that there was no trace of a β -lactam (the usual C=O stretching frequency of a 4-membered lactam is $\sim 1745\text{cm}^{-1}$)²⁶ or a carboxylic acid (typical O-H stretching frequency is between $3000\text{-}2500\text{ cm}^{-1}$ and very broad)²⁶. Finally the strong C=O stretching absorption frequency at 1660 cm^{-1} seen in both spectra is very characteristic of ureas.²⁶ This peak, together with the 1600 cm^{-1} N-H stretch (characteristic for amides)²⁶ is strong evidence that both of the spectra contain very large amounts of N-phenylurea. The two spectra were also compared to the spectrum of Meldrum's acid (Appendix 1 - Figure 4), but no similarities were found.

2.2.2 Reactions in Organic Solvents - Pyridine

Since the nucleophilic addition reaction of Meldrum's acid was shown to be unsuccessful under aqueous conditions, another set of reaction conditions were used. Corey has shown that Meldrum's acid readily condenses with an aldehyde in pyridine to form an alkene (11a).²⁴ The reaction is shown in Figure 21. In this reaction pyridine is used both as a solvent and as a base necessary to generate the enolate ion of Meldrum's acid. The reactions occurred under mild conditions ($50\text{ }^{\circ}\text{C}$) and afforded excellent yields of the alkene products (80-85%).²⁴

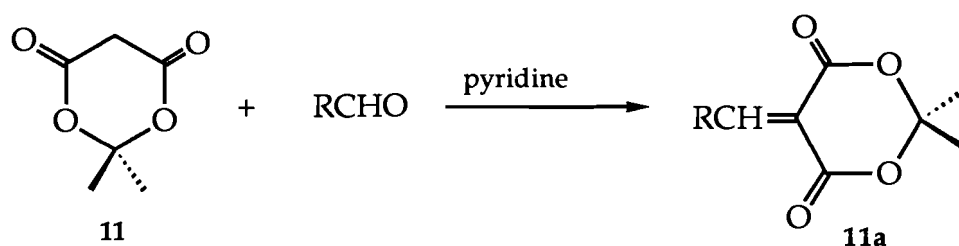


Figure 21

Similar results were expected to be obtained when Meldrum's acid was reacted with PAIMSO in pyridine. Pyridine (pKa for pyridinium ion is 5.35)²⁵ was used to generate the enolate of Meldrum's acid and as the solvent for the reaction. PAIMSO, was not readily soluble in pyridine. It dissolved only after vigorous stirring and heating. When following the reaction using tlc, four spots were observed shortly after all of the reactants were completely dissolved in the reaction mixture. The reaction mixture also changed from colorless to brown. No precipitate was formed when the reaction was poured into water.

The control reaction (PAIMSO in pyridine), when analyzed by tlc had only three distinguishable components which all corresponded to the tlc spots observed in the reaction mixture that included Meldrum's acid (Table 3). The additional spot present in this reaction mixture had the largest R_f value for the eluant used (1:1 ethyl acetate : ether) and could not, therefore, be Meldrum's acid (R_f value for Meldrum's acid is 0.08 - Table 3).

When the reaction of PAIMSO and Meldrum's acid was heated, it proceeded, at first, with the change in color and the formation of an additional component in the mixture (observed by tlc). When the reaction mixture was poured into an ice/water mixture no precipitate formed. However, after two days some precipitate formed in the solution. The amount of precipitate was just sufficient to obtain a melting point and an IR spectrum. This compound was found to have a melting point of 235-239 °C

with decomposition. The IR spectrum included bands at 3280, 1590, 1530, 1280, 720, 680 cm^{-1} and is shown in the Appendix (12). It can be seen that there is no peak with carbonyl stretching frequency ($1650 - 1760 \text{ cm}^{-1}$)²⁶ in the spectrum. However, all of the target compounds of this synthesis (phenylamidinoacetic acid, or the β -lactam) include a $\text{C}=\text{O}$ bond. The lack of this peak indicates that the precipitate was probably a decomposition or a condensation product of either PAIMSO or Meldrum's acid, possibly due to the alkaline conditions that were applied, that does not include a carbonyl group. Formation of the possible condensation products of PAIMSO is shown in Figure 22. It is well known that PAIMSO undergoes nucleophilic attack by an amine to form a guanidine (Figure 6, p. 6). In pyridine, the free amine group of PAIMSO may have served as a good nucleophile. The condensation products formed from two equivalents of PAIMSO (30) and three equivalents of PAIMSO (31, 32) are both shown in Figure 22.

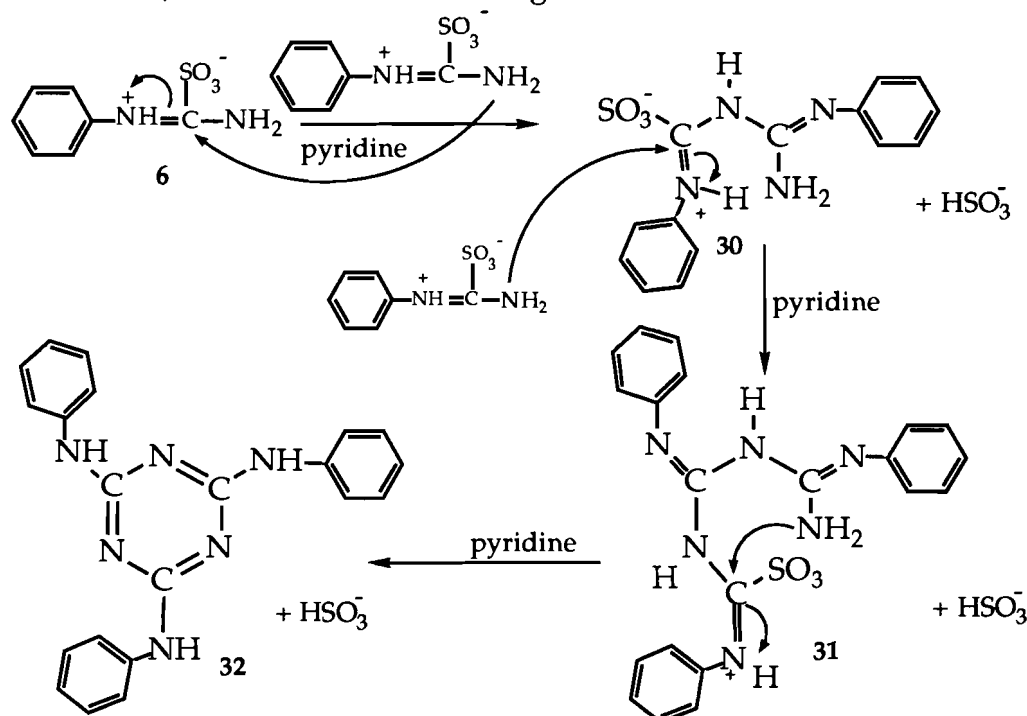


Figure 22

Since both the IR and the melting point of the product formed from this reaction indicated that the product was a mixture, this condensation reaction yielding a mixture of products is a likely side reaction.

A possible reason that the addition of Meldrum's acid to PAIMSO in pyridine was unsuccessful is in the operation of the reaction. When carrying out a "malonic-ester type synthesis," it is very important to follow a certain order of addition of the reactants to the reaction mixture. The usual way of carrying out the reaction is to first dissolve the β -diketone or a diester in a basic solution to generate the enolate ion and then slowly add the other reactant to this reaction mixture. However, the order of addition of the reactants to the reaction mixture described here was not observed in this experiment. Instead, both PAIMSO and Meldrum's acid were dissolved in pyridine at the same time. Under such alkaline conditions, in pyridine, PAIMSO was probably deprotonated. This deprotonated PAIMSO is shown in Figure 23. From this figure it is obvious that PAIMSO cannot undergo nucleophilic attack, since it does not have an activated N atom.

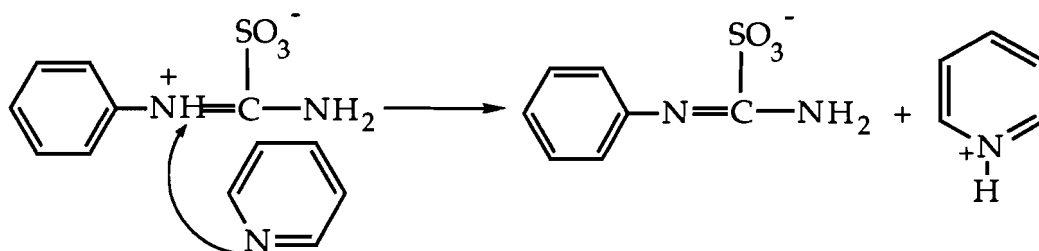


Figure 23

2.2.3 Reaction of PAIMSO and Meldrum's Acid Under Acidic Conditions

Since the attempts to effect nucleophilic addition of Meldrum's acid on PAIMSO under alkaline conditions were unsuccessful, the reaction was attempted under acidic conditions. It has been reported by Maryanoff⁵ that

PAIMSO is very stable in acidic solution. Some procedures that used an enol form of Meldrum's acid as a nucleophile were described previously (Figure 9). These reactions carried out by Scoville and Smith¹⁶ involved the use of an activated N atom that served as an "electron sink." Thiourea-S,S,S-trioxides also have the activated nitrogen (N with a positive charge) that could act as an electron acceptor and their nucleophilic addition reactions (*e.g.* guanylation) may proceed through a similar mechanism. This mechanism is illustrated in Figure 9 in the introduction.

This reaction was carried out in methanol and 1,2-dimethoxyethane. The enol was generated in the presence of acetic anhydride, as it had been previously done by Scoville and Smith.¹⁶ However, even when the reaction was allowed to run for several days with vigorous stirring and heating (~50 °C), no solid precipitated when the reaction mixture was poured into an ice/water mixture. Since no change was observed by tlc when the reactants were stirred for a long period of time, no reaction occurred under these conditions.

2.3 Conclusions

Nucleophilic addition of Meldrum's acid on PAIMSO can not be effected under the experimental conditions described in this paper. In aqueous solutions, water is a better nucleophile than the enolate ion of Meldrum's acid and it undergoes nucleophilic addition on PAIMSO to form N-phenylurea in good yields (66-89%). In pyridine, nucleophilic addition of Meldrum's acid on PAIMSO cannot be effected under the conditions of this experiment. A possible side reaction is the condensation of two or three equivalents of PAIMSO. Finally, there is no evidence that enol of Meldrum's acid, in an acidic solution, accomplishes nucleophilic attack on PAIMSO in the solvents such as methanol or 1,2-dimethoxyethane.

2.4 Suggestions for Further Study

Nucleophilic addition may possibly be effected under acidic conditions, but in a different solvent. Acetic acid has been used to generate the enol of Meldrum's acid, as well as the solvent for the reaction in different nucleophilic addition and substitution reactions.¹⁶ If PAIMSO is soluble in acetic acid, nucleophilic addition of PAIMSO may be effected under these conditions.

Other nucleophiles may also undergo nucleophilic attack on PAIMSO. For instance, ethyl cyanoacetate also has an acidic hydrogen and forms an enolate under basic conditions. The proposed reaction scheme is shown in Figure 20.

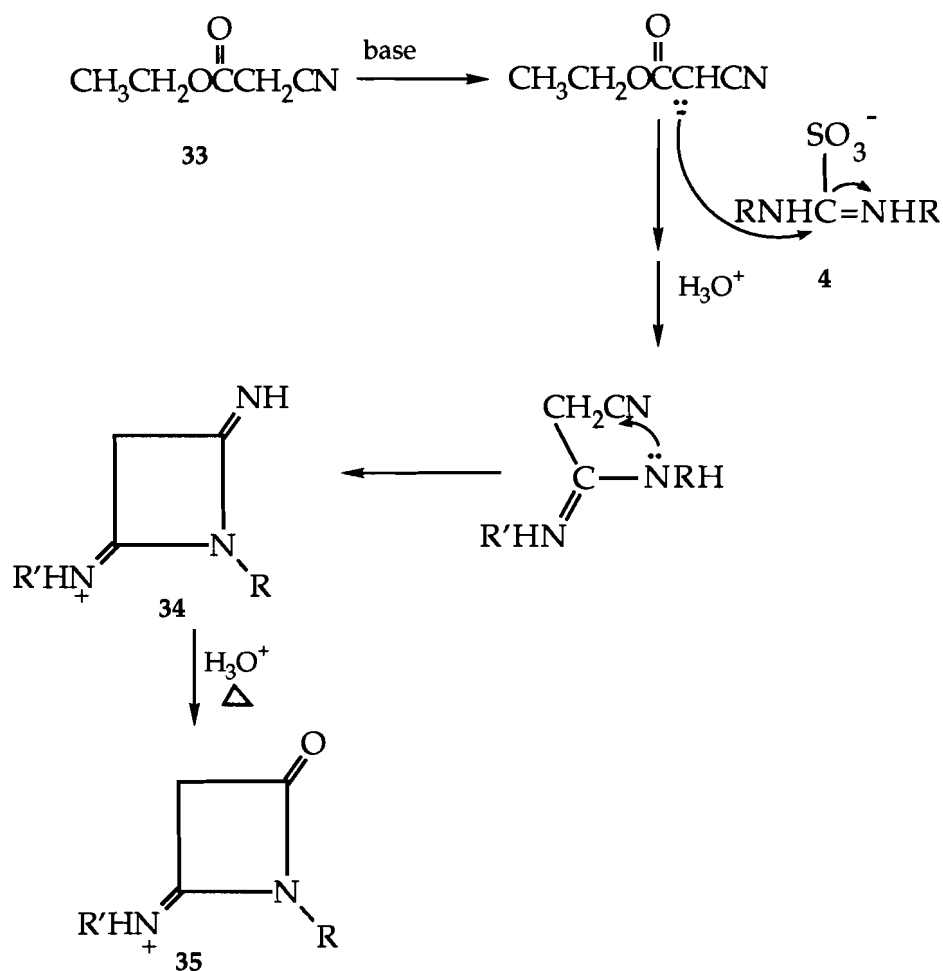


Figure 24

Chapter III

EXPERIMENTAL

3.1 General Information

The melting points were determined using a "Mel-Temp" capillary melting point apparatus and are uncorrected.

Infrared spectra were recorded on a Perkin Elmer model 398 infrared spectrophotometer. All of the samples analyzed were solids and were analyzed using KBr pellets.

Reaction mixtures were analyzed by thin layer chromatography on either Kodak Silica Gel plates with a fluorescent indicator or Alumina plates. The solvent systems used were determined for each reaction. Spots were visualized using FCNP solution⁴ [10% NaOH : 10% $K_3Fe(CN)_6$: 10% $Na_2Fe(CN)_5NO \cdot 2H_2O$: H_2O (v/v: 1:1:1:3)], ultraviolet lamp at 254 nm, or a sand/iodine chamber. When the dry plates were sprayed with the FCNP solution, different components of the reaction appeared in different colors allowing some components of the reaction mixtures to be identified. The colored spots from the FCNP solution were not permanent. The plates were examined under the UV light before being sprayed with the FCNP solution.

Thick layer chromatography was used to separate products from the reaction of PAIMSO with Meldrum's acid in an aqueous solution. It was carried out on 1.0 mm Kodak F 254 silica gel plates using ether as eluant. The bands were desorbed with methanol which was evaporated overnight from an open beaker.

Column chromatography on a 10-inch long, 1-inch wide alumina column was carried out for the same separation. The eluants used were: petroleum ether, toluene, and methanol.

The starting materials, 1-phenyl-2-thiourea (97%) and Meldrum's acid (98%) were obtained from Aldrich and used without further purification. Meldrum's acid was kept refrigerated and in a sealed bottle to prevent decomposition. All solvents were used without purification.

3.2 Preparation of N-Phenylaminoiminomethanesulfonic Acid (PAIMSO)

Peracetic acid used for the oxidation of 1-phenyl-2-thiourea was prepared by slow addition of 12.5 mL of acetic anhydride to 10.1 mL of 30% hydrogen peroxide cooled in an ice bath. A drop of concentrated sulfuric acid was added to the reaction mixture causing a violent reaction. After reaction subsided another 28 mL of acetic anhydride were added to the mixture in small portions. The mixture was gradually allowed to warm to room temperature and left to stand for at least one day.

In a separate Erlenmeyer flask, 5 g of N-phenylthiourea were dissolved in 350 mL of methanol. This solution was cooled to 0-10 °C in an ice bath. Peracetic acid was placed in a 1-L beaker and cooled in an ice bath also. The phenylthiourea solution was added in small portions to the peracetic acid at such a rate to maintain the temperature at around 10°C. At this temperature greatest yields were obtained. After the addition was complete, the mixture was allowed to stand at room temperature for one day. The solution changed from colorless to brown. Any crystals that formed at this point were removed by gravity filtration and discarded. The filtrate was concentrated on a rotary evaporator (40 °C) until precipitate formed. The solid was separated by suction filtration and then it was washed with several portions of cold methanol to yield 2.4-2.8 g of PAIMSO (35-43%): MP 155-157 °C (lit. 166.5 - 167)⁴ with bubbling; IR: 1070(s), 1230(s), 1267(s), 1680(s) cm⁻¹. PAIMSO is stable for several months if kept refrigerated.

3.3 Nucleophilic Displacement Reactions

3.3.1 Reaction of PAIMSO and Meldrum's Acid in Aqueous Medium

In a 25 mL Erlenmeyer flask 0.276 g (2.0 mmol) of K_2CO_3 and 0.288 g (2.0 mmol) Meldrum's acid were dissolved in 5 mL of water. With frequent swirling, 0.400 g PAIMSO (2.0 mmol) were added to the solution in small spatulafuls, over 45 minutes. The reaction was followed using tlc. The plates were developed in 1:1 mixture of ether and ethyl acetate. After the addition of PAIMSO was complete, the reaction mixture was allowed to stand at room temperature for a day. The white crystals (0.180 g - 0.244 g) that precipitated from the mixture were separated by suction filtration and dried overnight in air. The isolated crystals were shown to be a mixture when analyzed by tlc (1:1 ether:ethyl acetate) and had the following characteristics: MP 136-140 °C; IR: 1655(s), 1600(s), 1565(s), 710(m), 760(m) cm^{-1} . The mixture was separated using column or thick layer chromatography. These chromatography methods were described previously. Yield for N-phenylurea: 65-90%.

3.3.2 Reaction of PAIMSO and Meldrum's Acid in Pyridine

In a 25 mL Erlenmeyer flask 0.285 g of Meldrum's acid (2.0 mmol) were dissolved and 0.400 g PAIMSO (2.0 mmol) were added to the solution with vigorous stirring. The addition of PAIMSO was *not* carried out slowly, over an extended period of time. The reaction mixture was stirred overnight at room temperature (in the second trial it was stirred overnight at 50 °C) and it changed from a colorless solution to a golden yellow solution. The reaction was followed by tlc. Four spots were observed (Table 4). In the first trial, when the reaction mixture was poured over ice water, no precipitate formed. However, in the second trial (at 50 °C), the reaction mixture was allowed to react for several days. When the mixture was poured into ice water, no

precipitate formed immediately. After one day, needle-like crystals formed in the solution. The precipitate was collected by gravity filtration and washed with cold water. The product (0.0082 g) was analyzed using MP and IR spectroscopy: MP 235 - 239 °C, IR 3280, 1590, 1530, 1280, 720, 680 cm^{-1} .

A control reaction was also run with 0.100 g of PAIMSO in 5 mL of pyridine both at room temperature and at 50 °C. This reaction mixture was also followed using tlc (95% ethanol). In this reaction, three different components were detected by tlc. All of these spots had R_f values corresponding to the ones detected in the reaction mixture with Meldrum's acid. The component with the highest R_f value from the reaction mixture was not observed in the control.

3.3.3. Reaction of PAIMSO and Meldrum's Acid under Acidic Conditions

In a 25 mL Erlenmeyer flask, 0.360 g Meldrum's acid (2.8 mmol) and 0.521 g PAIMSO (2.8 mmol) were dissolved in 5 mL of methanol. To this mixture, 5 mL acetic anhydride were added with stirring. The reaction mixture was stirred overnight at room temperature. When the solution was poured over ice-water, no precipitate formed. The reaction was followed by tlc, but only one spot was observed on the tlc plate. The reaction mixture was then made alkaline using NH_4OH ; however, no solid precipitated. The mixture was also concentrated *in vacuo* at 65 °C, leaving a thin film of brown oil.

The same procedure was repeated in 1,2-dimethoxyethane. In addition, the reactions in both solvents were carried out at 50 °C. All of the preparations yielded the same results. Under all of the conditions applied, no reaction took place between Meldrum's acid and PAIMSO.

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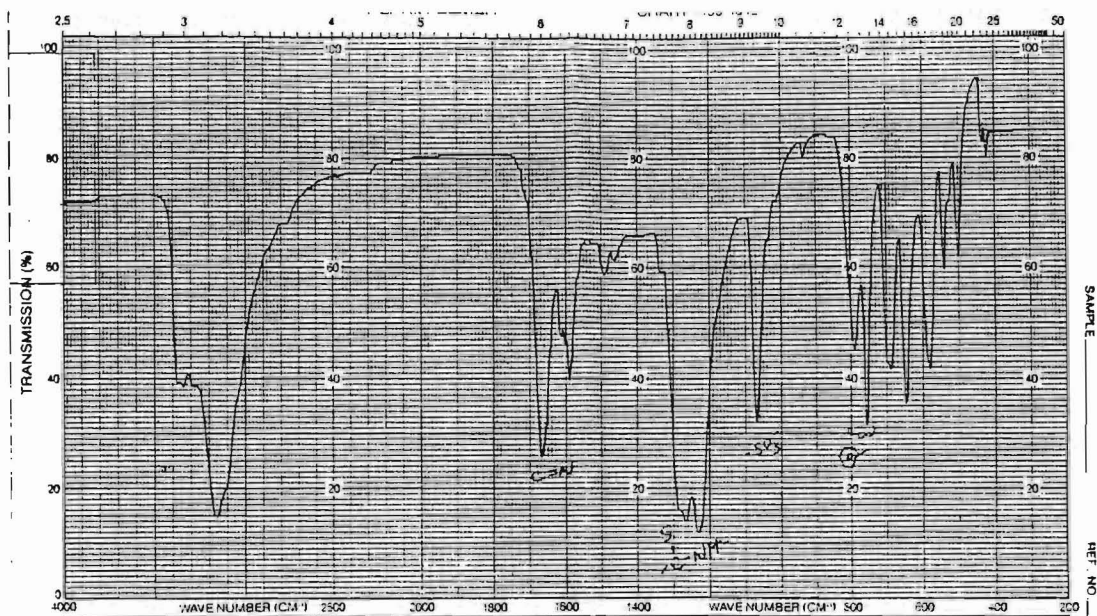
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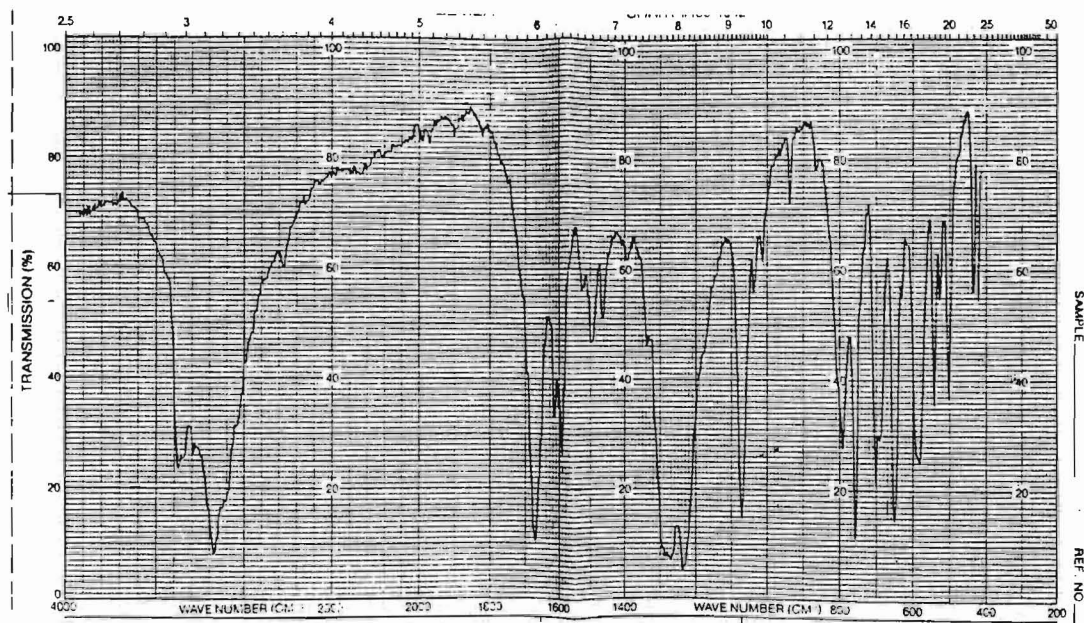
Appendix 1

Infrared Spectra

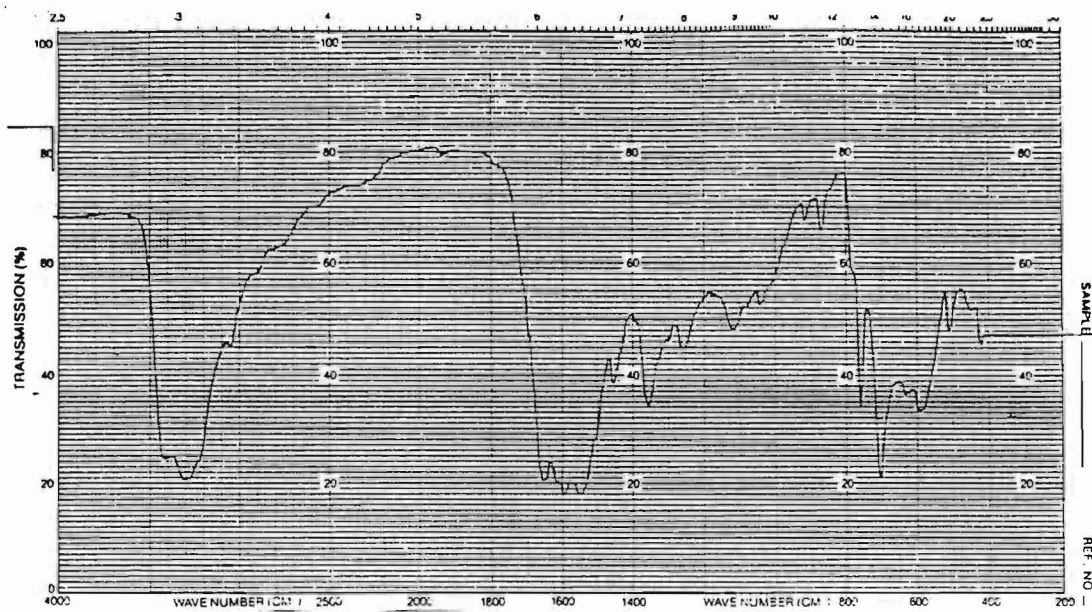
1. PAIMSO: preparation 2
2. PAIMSO: preparation 4
3. Phenylurea
4. Meldrum's acid
5. 1-Phenyl-2-thiourea
6. Product from the nucleophilic displacement reaction in aqueous solution (Trial 1)
7. Product from the nucleophilic displacement reaction in aqueous solution (Trial 2)
8. Top layer from the thick layer chromatographic separation of the product from nucleophilic displacement reaction in aqueous solution
9. Bottom layer (same as above)
10. Column chromatography: separation of the same reaction, Fraction 2
11. Column chromatography: Fraction 4
12. Product from the reaction in pyridine



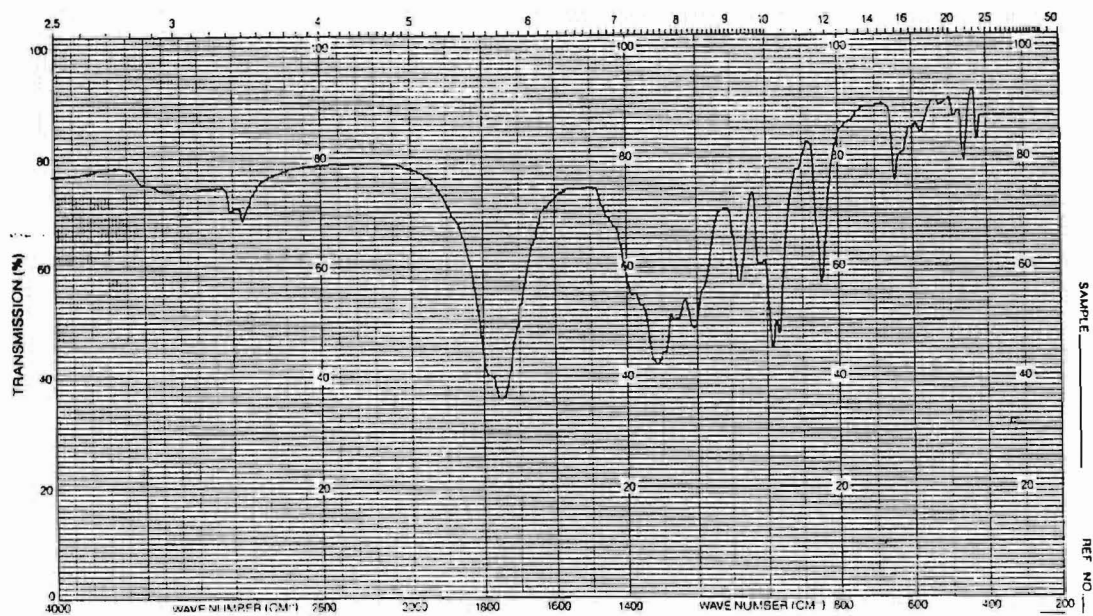
1. PAIMSO: preparation 2



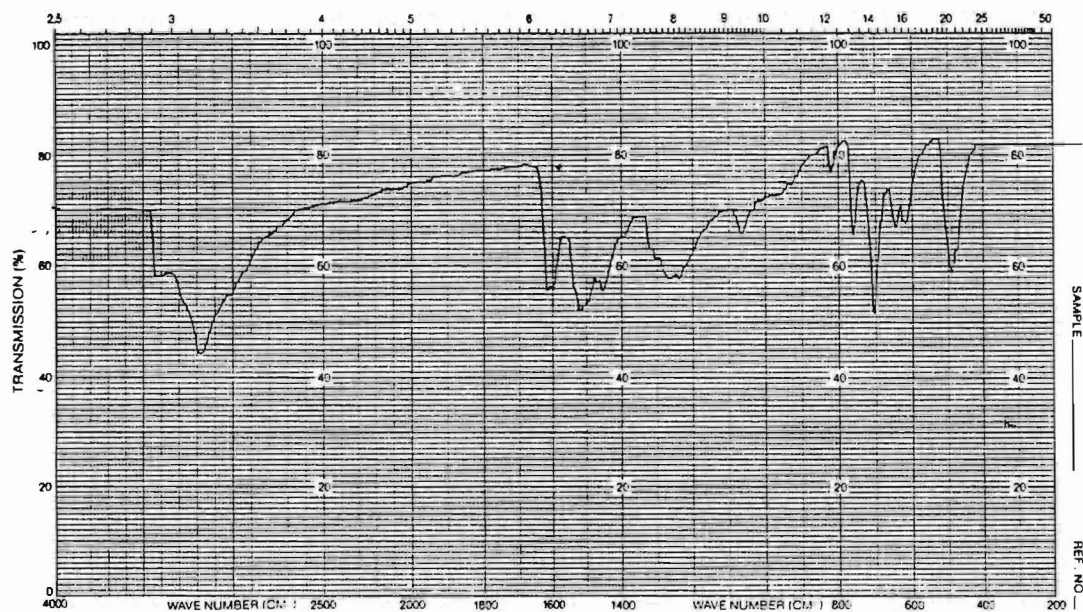
2. PAIMSO: preparation 4



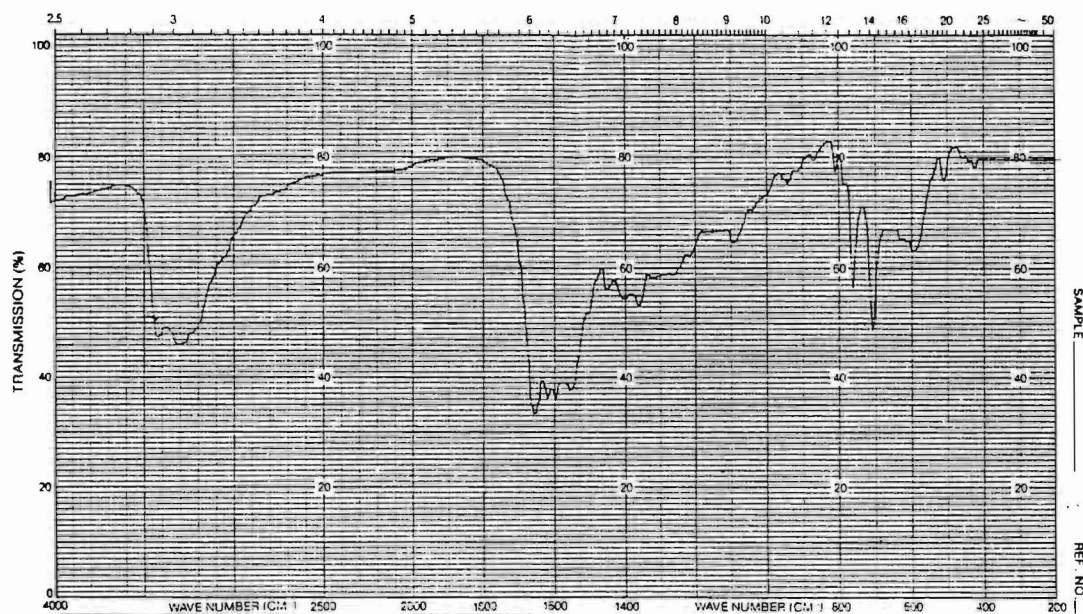
3. Phenylurea



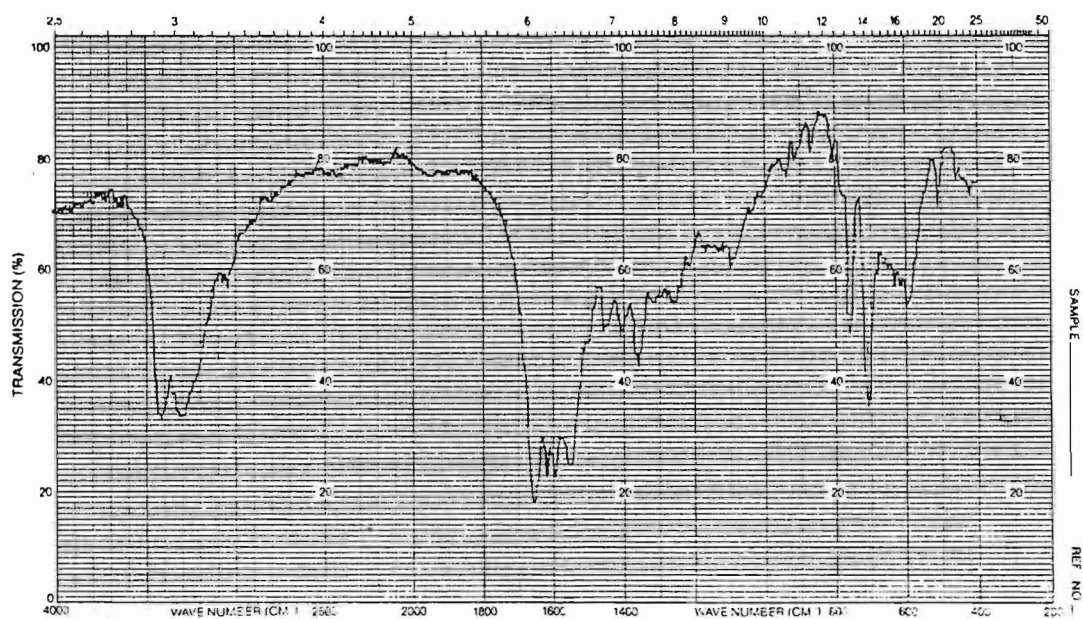
4. Meldrum's acid



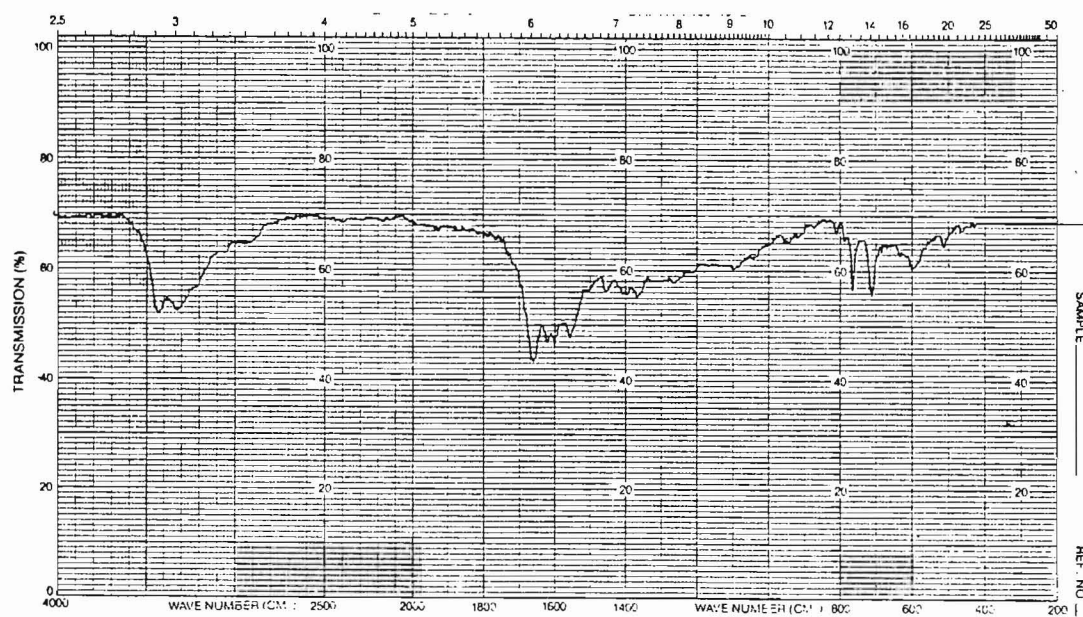
5. 1-Phenyl-2-thiourea



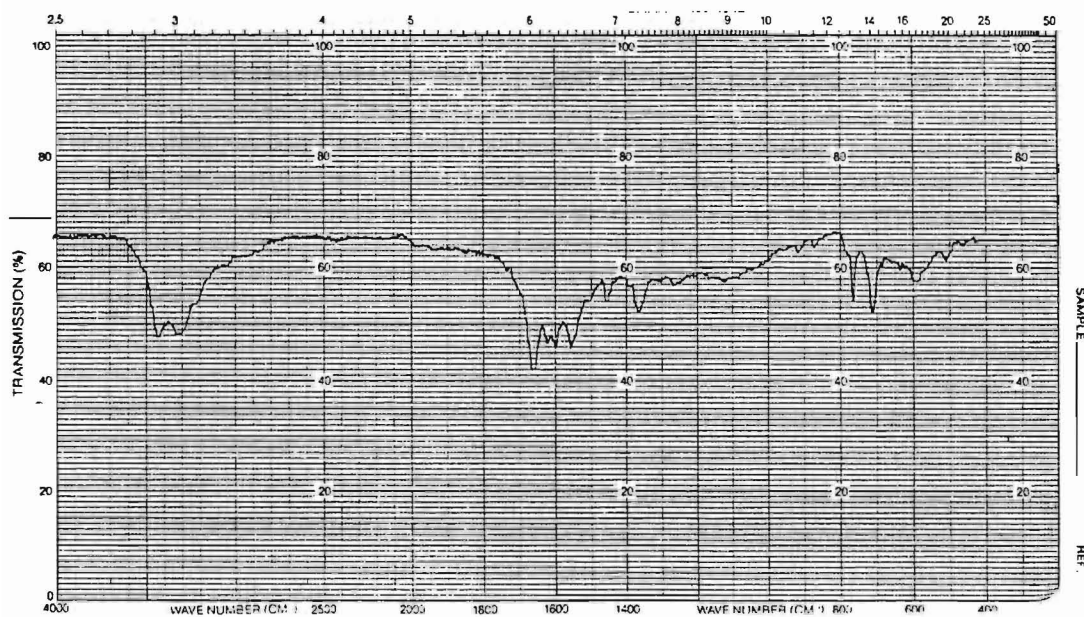
6. Product from the nucleophilic displacement reaction in aqueous solution (trial 1)



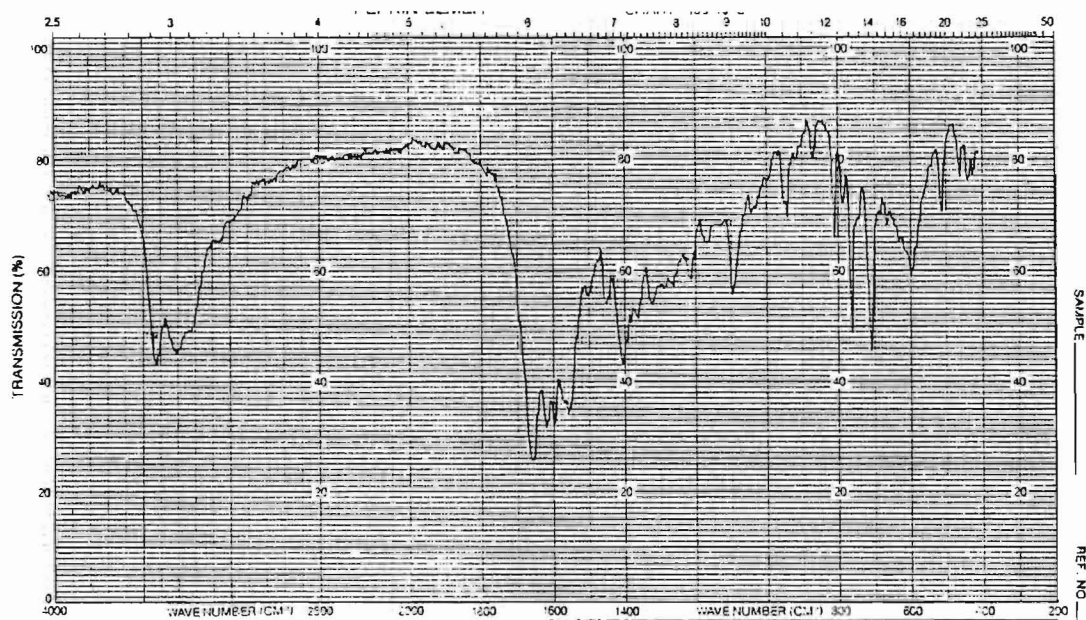
7. Product from the nucleophilic displacement reaction in aqueous solution (trial 2)



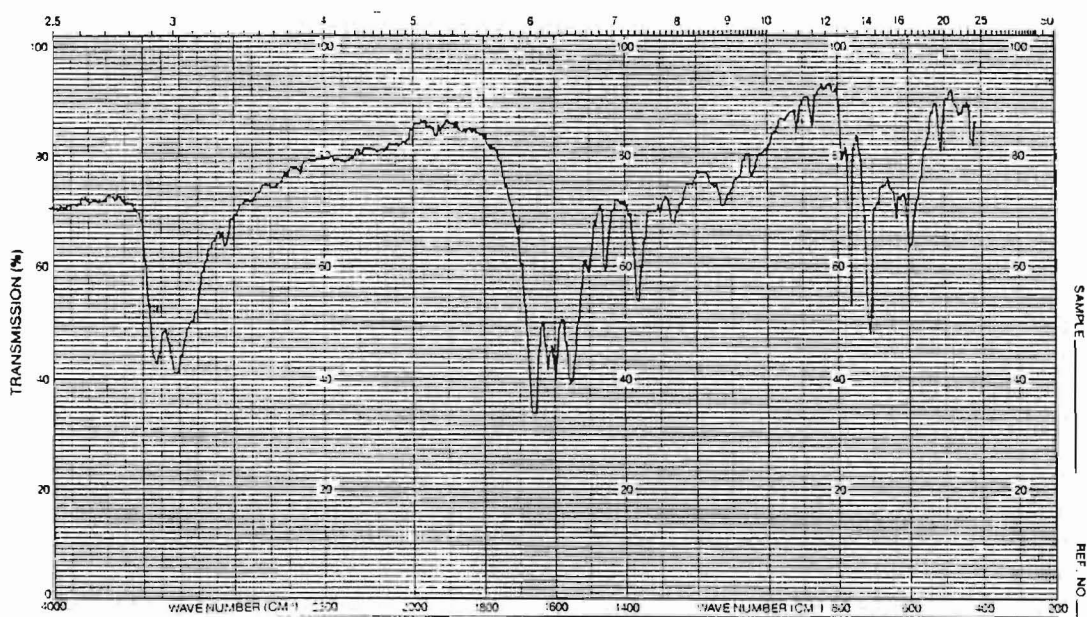
8. Top layer from the thick layer chromatographic separation of the product from nucleophilic displacement reaction in aqueous solution



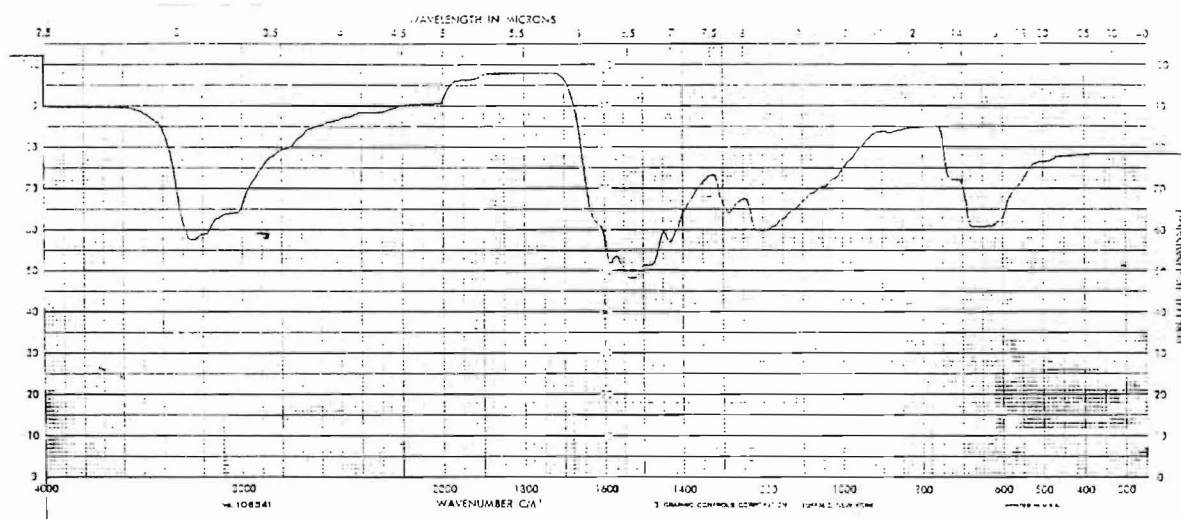
9. Bottom layer (same as above)



10. Column chromatography: separation of the same reaction, Fraction 2



11. Column chromatography: Fraction 4



12. Product from the reaction in pyridine