



1989

## Synthesis and Nucleophilic Reactions of Bifunctional Thiourea S,S,S-Trioxides

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Synthesis and Nucleophilic Reactions of  
Bifunctional Thiourea S,S,S-Trioxides

Dan Webb

A Thesis  
Submitted in Partial Fulfillment of the  
Requirements for  
Research Honors in Chemistry  
at  
Illinois Wesleyan University  
1989

# ABSTRACT:

The synthesis of bifunctional thioureas and the corresponding thiourea S,S,S-trioxides has been examined. Two methods for the synthesis of the bithioureas were employed. One involved the treatment of a diamine with silicon tetraisothiocyanate in benzene. The second involved treatment of the amine with ammonium thiocyanate in dilute acid. This latter synthesis was superior because of the ease of its use, the high yields obtained, and the purity of the products. Though this synthesis worked well for the preparation of phenylene-1,4-bis(thiourea), it yielded only bithiocyanate salts in the syntheses of aliphatic thioureas. The oxidation of the bithioureas was carried out using peracetic acid or hydrogen peroxide to give the corresponding thiourea S,S,S-trioxides.

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## INTRODUCTION:

### RATIONALE:

The synthesis and chemical reactions of monofunctional thiourea S,S,S-trioxides have previously been studied. Oxidation of parent thioureas with peracetic acid gives thiourea S,S,S-trioxides, which readily undergo nucleophilic displacement reactions with amino acids to give the corresponding guanidino acids.<sup>1-6</sup> The success of the oxidation of the parent monofunctional thiourea compounds to the corresponding S,S,S-trioxides suggests that oxidation of bifunctional thioureas to the corresponding bifunctional thiourea S,S,S-trioxides should also be possible. It has been shown that the monofunctional thiourea S,S,S-trioxides react with proteins at amino acid side chains. The bifunctional thiourea S,S,S-trioxides should undergo similar nucleophilic substitution reactions with amines and nucleophilic protein side chains. Thus it may be possible to prepare a new class of bifunctional protein crosslinking reagents which would be capable of cross-linking nucleophilic amino acid side chains such as lysine or cysteine.

**BACKGROUND:****Synthesis of Thioureas and Bifunctional Thioureas:**

Thioureas, 3, are often synthesized by the addition of primary or secondary amines or ammonia, 1, to alkyl or aryl isothiocyanates, 2, as shown in Figure 1.<sup>7-11</sup>

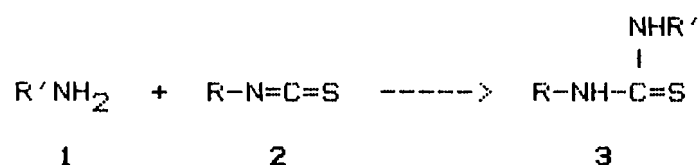


Figure 1

Phenylthiourea, 9, has been prepared by first making benzoyl isothiocyanate, 6, from ammonium thiocyanate, 4, and benzoyl chloride, 5, and then treating this product with aniline, 7, to form N-benzoyl-N'-phenylthiourea, 8. The final product is obtained by treatment with sodium hydroxide. This synthesis is shown in Figure 2.<sup>10</sup>



Primary amines, 2, have also been shown to react with carbon disulfide in the presence of diphenyl phosphite and pyridine to form symmetrically substituted thioureas, 13, as shown in Figure 4.<sup>7</sup>

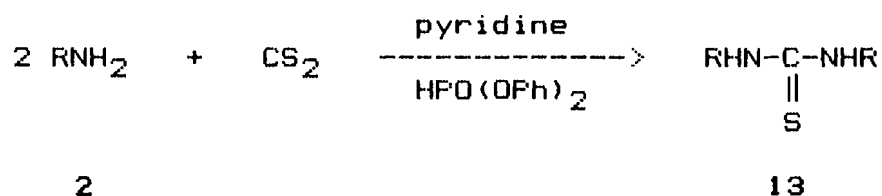


Figure 4

Zienty<sup>13</sup> and Thielke<sup>14</sup> have reported the synthesis of 1,3-dicyclohexyl-ethylenethiourea, 15, in 71% yield from N-formyl-N,N'-dicyclohexyl-ethylenediamine, 14, and elemental sulfur.

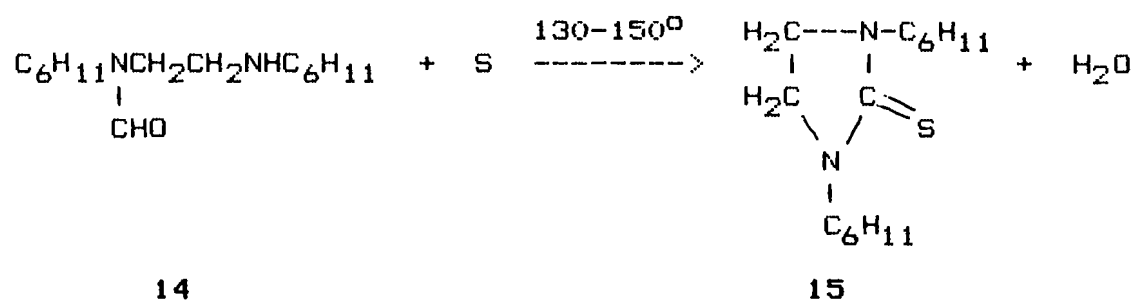


Figure 5



Many thiating reagents are also known that can be used to synthesize thioureas from the corresponding ureas. Thomsen and coworkers report the use of 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, Lawesson's reagent, 16, as a thiating reagent.<sup>15</sup> This method provides a general synthesis for the conversion of carbonyl groups to thiocarbonyl groups, as shown in Figure 6.<sup>15</sup>

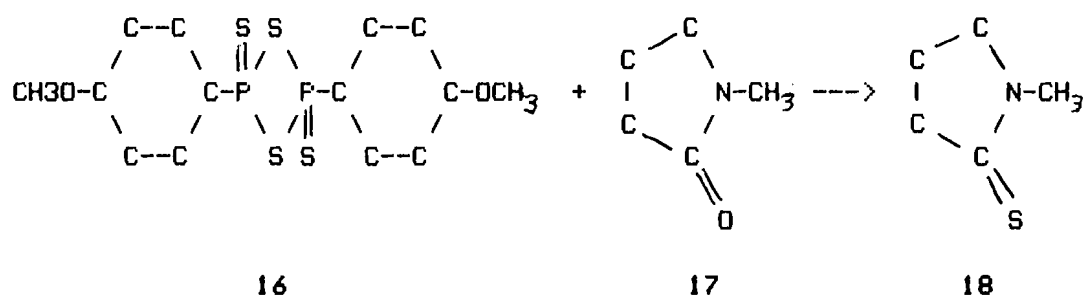
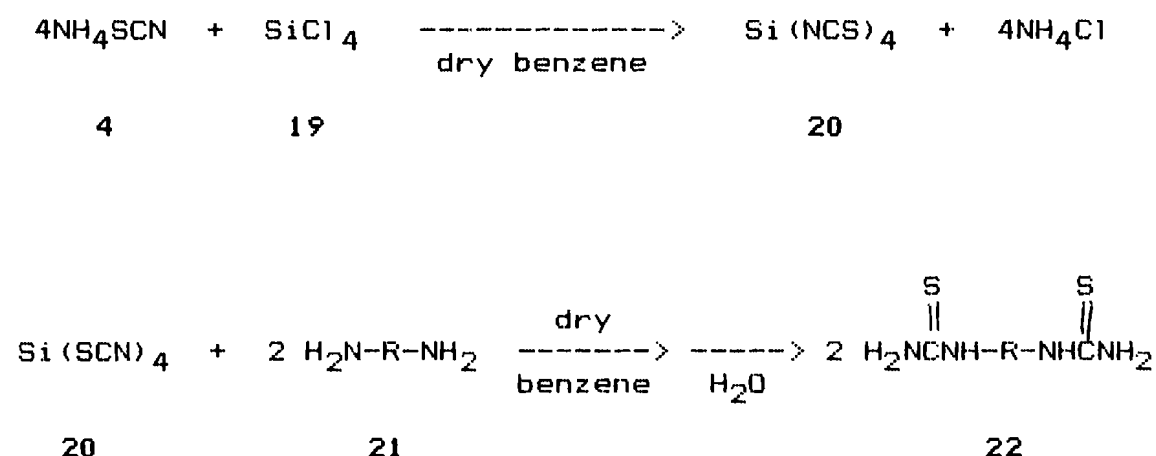


Figure 6

Thomsen and coworkers also lists many other thiating agents, such as H<sub>2</sub>S, H<sub>2</sub>S/HCl, H<sub>2</sub>S<sub>2</sub>/HCl, (Et<sub>2</sub>Al)<sub>2</sub>S, (EtAlS)<sub>n</sub>, SiS<sub>2</sub>, B<sub>2</sub>S<sub>3</sub>, PCl<sub>5</sub>/Al<sub>2</sub>S<sub>3</sub>/Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>S/H<sub>2</sub>SO<sub>4</sub>, P<sub>2</sub>S<sub>5</sub>, P<sub>2</sub>S<sub>5</sub>/pyridine, P<sub>2</sub>S<sub>5</sub>/NEt<sub>3</sub>, P<sub>2</sub>S<sub>5</sub>/NaHCO<sub>3</sub>, RPS(OR')<sub>2</sub>, PSCl<sub>x</sub>(NMe<sub>2</sub>)<sub>3-x</sub> (x=0 to 3), and SCNCOOEt,<sup>15</sup> but state that 16 has the advantage of giving reproducible results in high yields and can be obtained in one step from commercially available starting materials.

Neville and McGee have reported that excellent yields (97-100%) of N-mono- and N,N'-di-substituted thioureas can be obtained by the reaction of silicon tetraisothiocyanate, 20, with primary amines.<sup>8</sup> In this synthesis,  $\text{Si}(\text{NCS})_4$  is first made from treatment of  $\text{SiCl}_4$ , 19, with  $\text{NH}_4\text{SCN}$ , 4. The amine or diamine is then treated with  $\text{Si}(\text{NCS})_4$  to give the desired thiourea product. These reactions are shown in Figure 7.



where  $\text{R} = -\text{C}_6\text{H}_4-$ ,  $-\text{CH}_2\text{CH}_2-$ ,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$ , etc.

Figure 7

Wolfe, Loo, and Arnold<sup>16</sup> also report a synthesis of the bifunctional thiourea, 1,4-phenylenebis(thiourea), 24, which involves direct reaction of 1,4-phenylenediamine, 23, with 4 under acidic conditions. They obtained the product in 96 % yield.

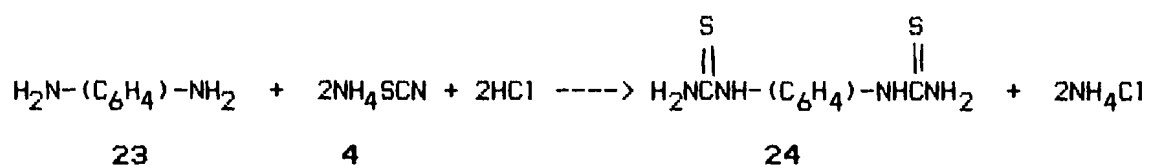


Figure 8

### Synthesis of Thiourea S,S,S-Trioxides:

The syntheses of many monofunctional thiourea S,S,S-trioxides have been reported, most using either hydrogen peroxide or peracetic acid as the oxidant.<sup>1,2,17-26</sup> Walter<sup>17,18</sup> has prepared thiourea S,S,S-trioxides, 25, by oxidation of thioureas, 3, with 4-5 equivalents of peracetic acid. The solvent was either chloroform or a mixture of chloroform, methanol, and ethanol, and the temperature was maintained at  $-10^\circ\text{C}$ . The reaction is shown below in Figure 9.

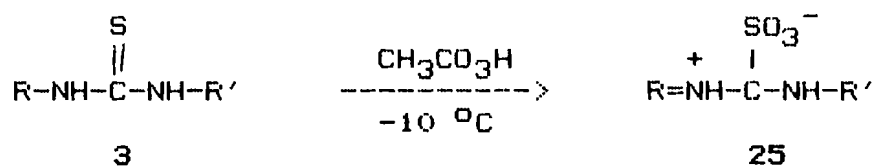


Figure 9

The S,S,S-trioxide, 27, of the 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) salt of N-cyano-N'-methylthiourea, 26, has been prepared by oxidation of the parent thiourea compound

with 35% hydrogen peroxide in water at 0 °C, using Na<sub>2</sub>WO<sub>4</sub>•H<sub>2</sub>O as a catalyst, as shown in Figure 10.<sup>19</sup>

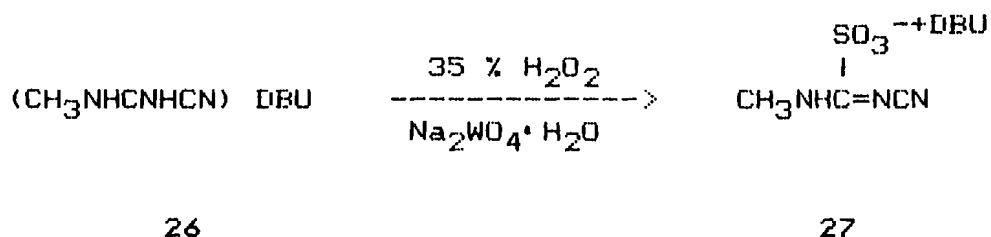


Figure 10

Oxidation of ethylene thiourea, 13, with hydrogen peroxide in CCl<sub>4</sub> at 0 °C has also been reported, by Marshall and Singh, to form the S,S,S-trioxide, 28, according to Figure 11.<sup>20</sup>

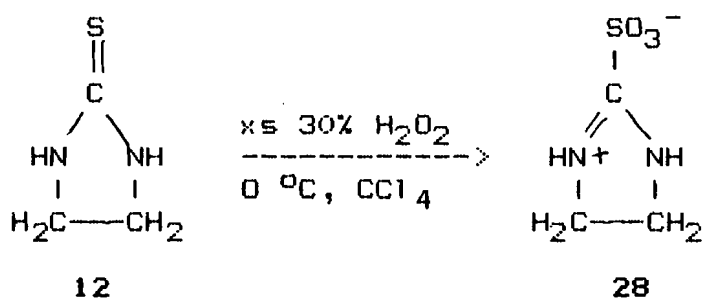


Figure 11

A typical procedure has been given by Miller<sup>2</sup> and Bischoff,<sup>1,2</sup> which involves treatment of a freshly prepared peracetic acid solution with a solution of the thiourea.

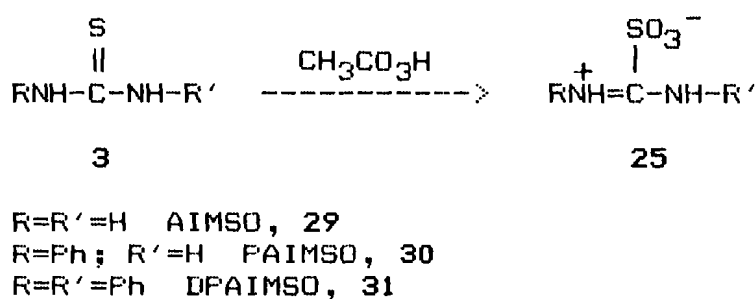


Figure 12

They found that some of the factors which affect the synthesis included the reaction solvent, reaction temperature, rate of addition of the thiourea solution, and quantity of the oxidant. They were able to use methanol as a solvent for reaction, for example, in the synthesis of aminoiminomethanesulfonic acid (AIMSO), 29 and N-phenylaminoiminomethanesulfonic acid (FAIMSO), 30, but found significant decomposition to the bisanilinium salt, 32, when using methanol as the solvent in the synthesis of N,N'-diphenylaminoiminomethanesulfonic acid (DFAIMSO), 31.

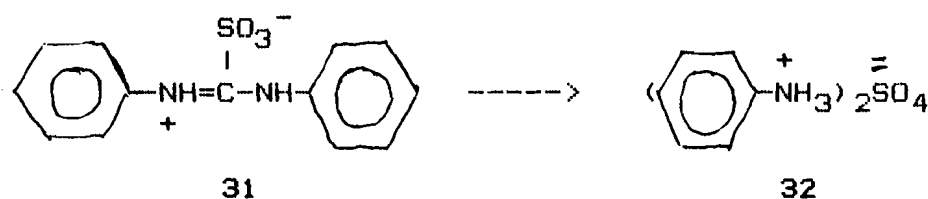


Figure 13

They also found that the optimum temperature for the preparation of AIMS0 was between 10 and 20 °C, but that for the preparation of FAIMS0 was between 0 and 10 °C. During preparation of AIMS0, if the temperature fell below 10 °C, aminoiminomethanesulfinic acid (AIMS), 34, formed instead, as shown in Figure 14.

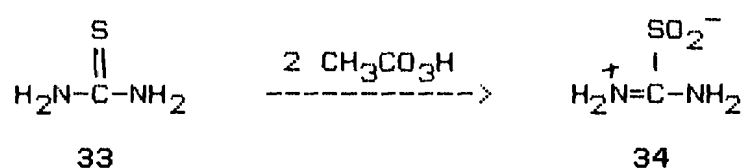


Figure 14

Improved yields of AIMS0 were seen when the thiourea was added slowly and the temperature was controlled carefully. Also, it was found that if too much (an excess of more than 10 %) oxidant was used in the preparation of DPAIMS0, decomposition occurred to 32, formed presumably via a diphenylformamidinium intermediate.

When Kharkanis and Field attempted to oxidize N,N'-disubstituted thioureas with 5 equivalents of hydrogen peroxide for 1 hour at 0 to 5 °C and also for 7 hours at 25°C, they obtained only the formamidinium bisulfate salt, 36.<sup>21</sup> This is shown in figure 15.

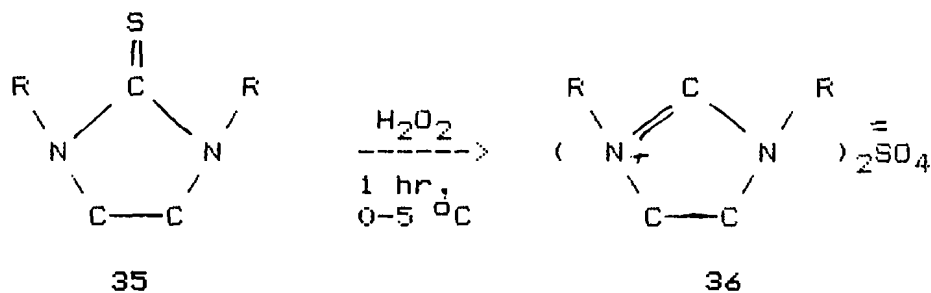


Figure 15

Other attempts at forming the trioxides of thiourea compounds have also failed. Walter and Reuss obtained only the trisubstituted formamidine, 38, in their oxidation of 3,3-dibenzyl-1-(2-chlorophenyl)thiourea, 37, with peracetic acid below 10 °C.<sup>22</sup> This reaction is shown in Figure 16.

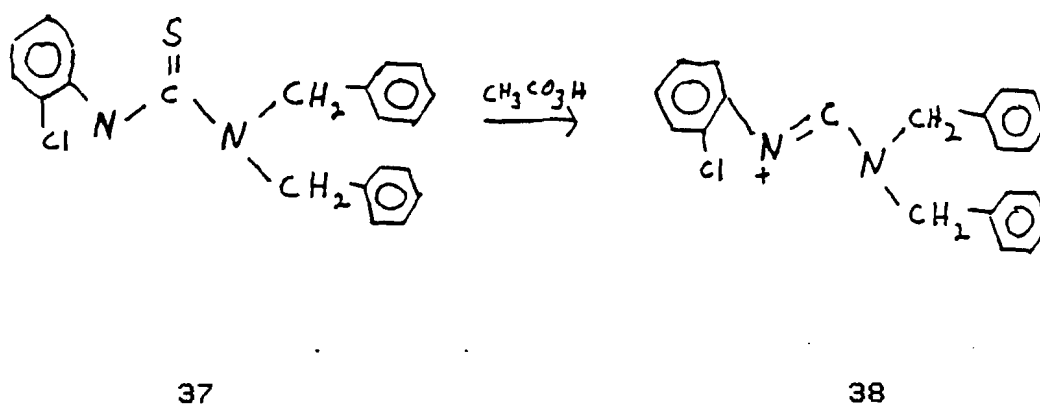


Figure 16

Shibasaki, Koizumi, and Matsumura obtained only N,N'-diphenylformamidine when they attempted to oxidize





nucleophilic attack of the amine on the AIMS $\text{O}$ .<sup>28</sup> The reaction is shown below in Figure 18.

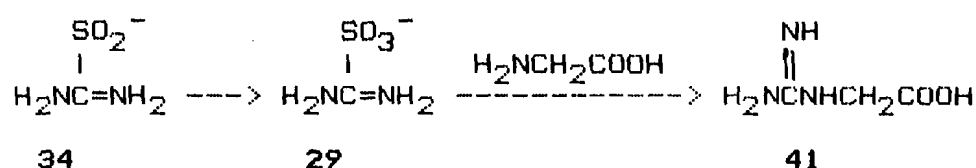


Figure 18

Similar reactions have also been reported.<sup>19,29,30</sup> Alhede and Gelting report the preparation of guanidinium iodides, 43, from N-N'-dimethylaminoiminomethanesulfonic acid, 42, according to figure 19.<sup>29</sup>

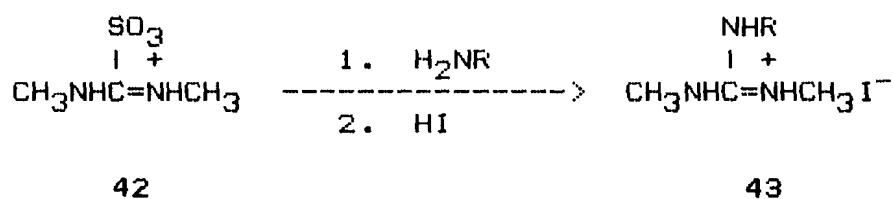


Figure 19

Teraji reported the preparation of cimetidine, 46, a drug used to treat ulcers, in 32% yield according to Figure 20.<sup>19</sup>

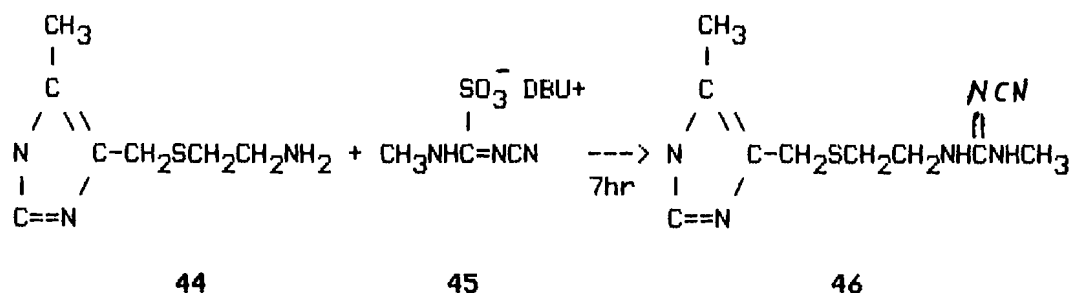
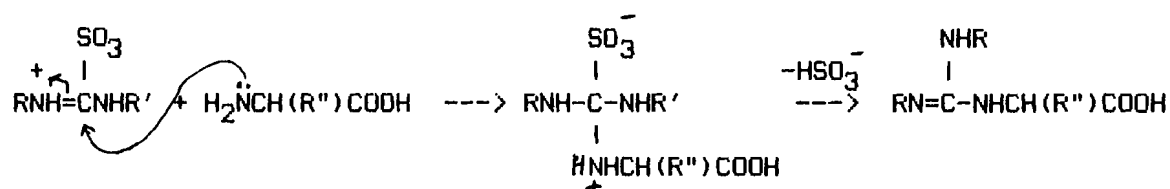


Figure 20

Claiborne reports the reaction of the 2-S,S,S-trioxide of thioriboflavin with an amino acid side chain of p-hydroxybenzoate hydroxylase.<sup>30</sup> DiIanni and coworkers describe the inactivation of *Escherichia coli* glutamine synthetase by thiourea trioxide.<sup>31</sup>

Maryanoff<sup>3-6</sup> and coworkers have reported the synthesis of guanidines from the reaction of amines with thiourea trioxides. Independent work by Miller<sup>2</sup> and Bischoff<sup>1</sup> has shown that guanidino acids can be prepared by the reaction of amino acids with thiourea S,S,S-trioxides. The following mechanism has been suggested.<sup>1</sup>



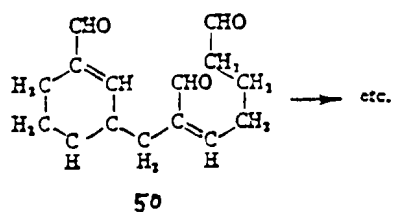
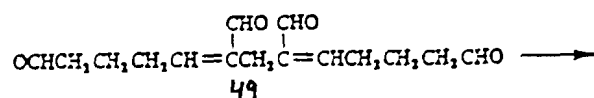
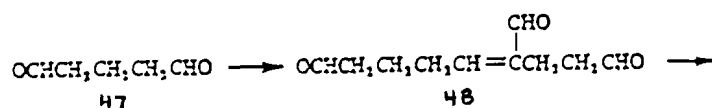
Scheme 1

### Protein Crosslinking Reactions:

There has been a great deal of interest in the cross-linking reactions of proteins.<sup>32-42</sup> These reactions have been used to determine intramolecular distances, to stabilize proteins and membranes, and to label molecules.

A good review of reactions of proteins with bifunctional crosslinking reagents has been given by Wold.<sup>32</sup> The use of glutaraldehyde to stabilize proteins for electron microscopic investigation has been reported.<sup>33</sup> It is thought that the reagent partially polymerizes to smaller oligomers that may react with protein amino acid groups. This is illustrated in Figure 21.

## 1. Polymerization (aldol condensations)



## 2. Cross-linking reactions

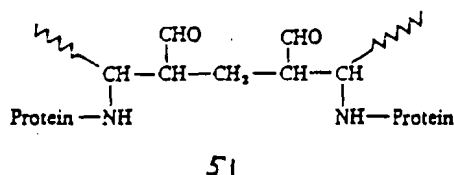


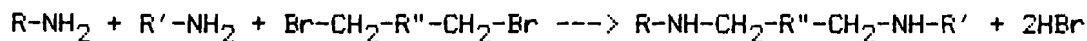
Figure 21

It has also been suggested that sulfhydryl and amino groups attack the aldehyde carbons directly, forming a covalent bond and the alkylol group.<sup>32</sup>

Bifunctional alkyl and aryl halides are also very useful as protein crosslinking reagents. These reagents primarily react with sulfhydryl groups, sulfides, imidazole, and amino groups, with the reaction at SH favored at neutral to slightly alkaline pH and the reaction with amino groups

favored at higher pH.<sup>32</sup> A typical reaction is shown below  
 in figure 22.

17



52

53

Figure 22

Typical bifunctional alkyl and aryl halide reagents are  
 shown below in Figure 23.<sup>32,33</sup>

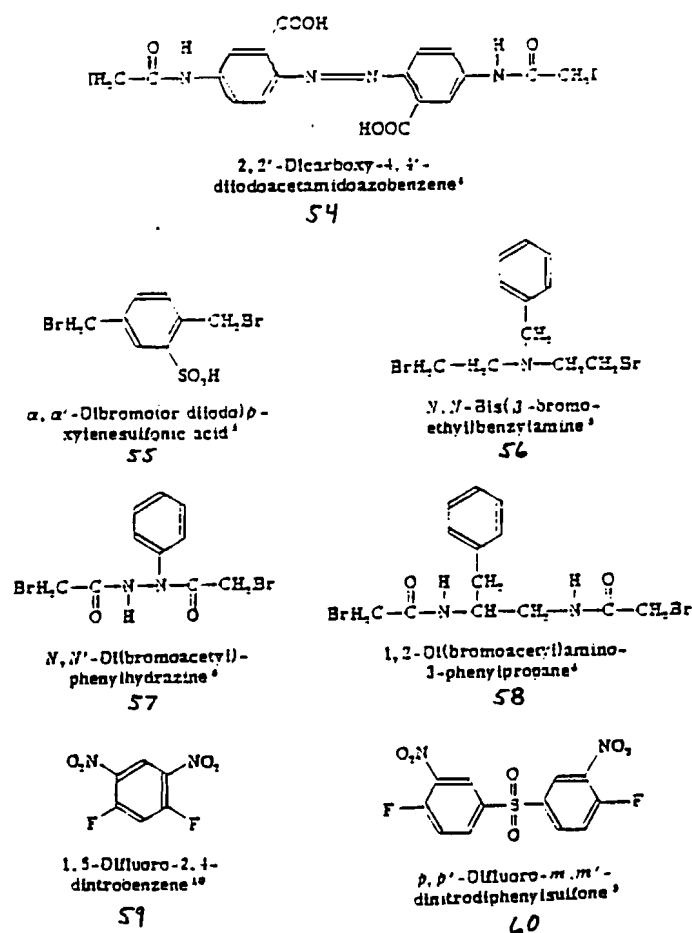


Figure 23

Wold has studied the reaction of  
 p,p'-difluoro-m,m'-dinitro-diphenylsulfone with bovine serum  
 albumin to produce synthetic tertiary bonds and has found

this product to be resistant to the conditions of chemical hydrolysis of the peptide.<sup>34</sup> Hiremath and Day have studied the reaction of  $\alpha,\alpha'$ -Dibromo (or diiodo)  $p$ -phenyloxylsulfonic acid with lysozyme.<sup>35</sup> Marfey and coworkers have studied the three dimensional structure of bovine pancreatic ribonuclease A by cross-linking it with 1,5-difluoro-2,4-dinitrobenzene to determine positions of closely spaced groups.<sup>36,37</sup> Berg and coworkers have used this reagent to increase the strength of the membrane of human erythrocytes.<sup>38</sup>

Many bifunctional maleimide derivatives are also known,<sup>32,33</sup> some of which are shown in Figure 24.

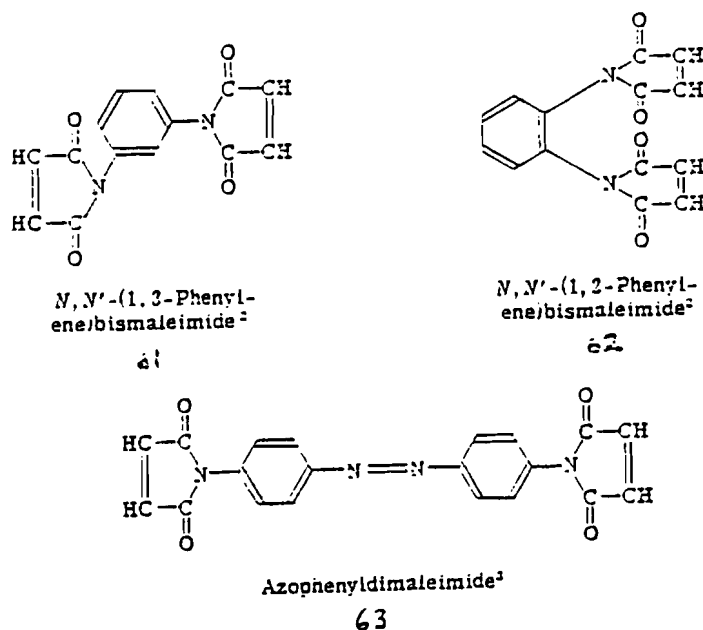


Figure 24

These reagents are specific for sulfhydryl groups.<sup>32,39</sup> They form thioether linkages and crosslink proteins as shown in Figure 25.<sup>39</sup>

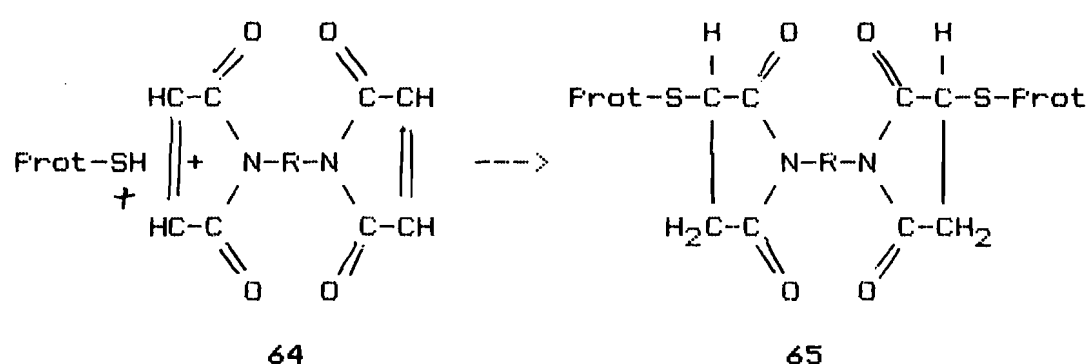


Figure 25

Moore and Ward have used N,N'-(1,3-phenylene)-bis-maleimide to crosslink bovine plasma albumin.<sup>39</sup> They studied the increased resistance of reduced wool to alkali, oxidation, and reduction by replacing the disulfide cross-links with bis-maleimide cross-links.<sup>39</sup> Bis(N-maleimidomethyl) ether, another bifunctional maleimide reagent, has been used to cross-link hemoglobin.<sup>32</sup>

The many bifunctional isocyanates are also known to react as crosslinking reagents, primarily reacting with amino groups in the proteins to form substituted ureas, as shown in Figure 26.<sup>32</sup>

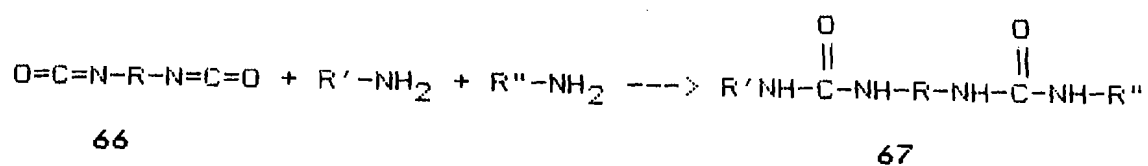


Figure 26

Some of these isocyanate reagents are shown in Figure 27.

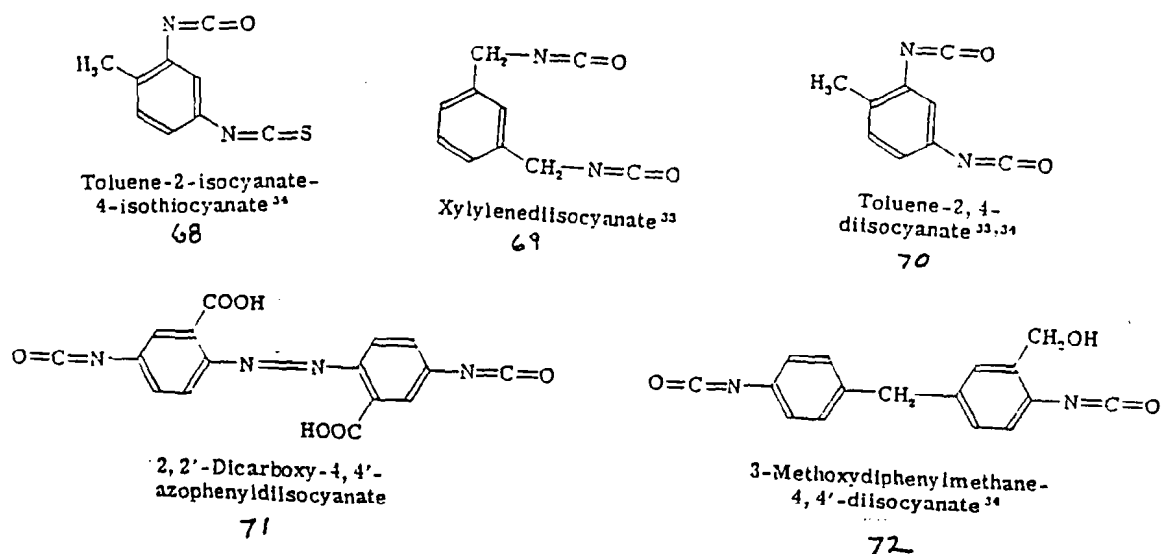


Figure 27

These reagents have been used to label antibody molecules by attaching electron-dense proteins to the antibodies.<sup>40</sup>

Schink and Singer have linked bovine serum albumin with bovine gamma-globulin and ferritin with rabbit

gamma-globulin.<sup>40</sup> Compound 72 has also been used to study the interresidue distances of the amino acids of

myoglobin.<sup>32</sup>



Another type of bifunctional crosslinking reagent is the diimidoester, which reacts with amino groups to give amidines as shown in Figure 28.<sup>32,41</sup>

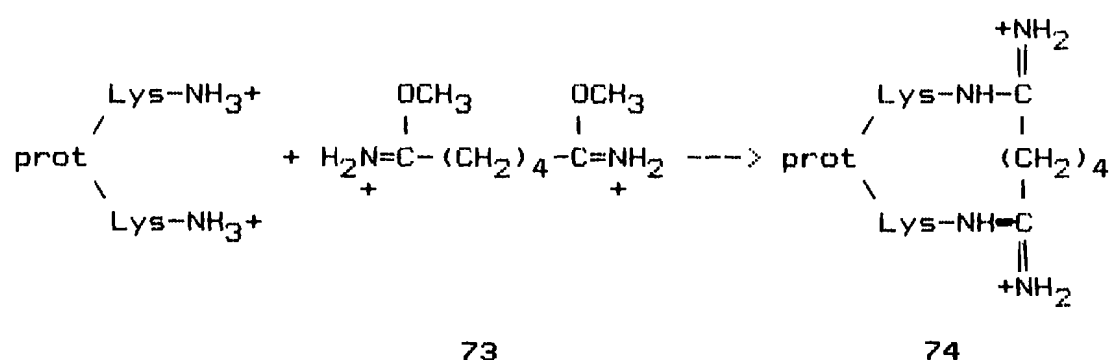


Figure 28

Dimethyladipimide, 73, has been used to study interresidue distances in bovine pancreatic ribonuclease A.<sup>41</sup> The reaction of imidoesters with insulin in similar reactions has been described by Hunter and Ludwig, who also suggest the use of diimidoesters as protein crosslinking reagents.<sup>42</sup>

### Objective

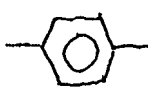
The goals of this research were multifold. The following investigations were carried out:

- (1) synthesis and characterization of 1,2-ethylene bis(thiourea), 75, 1,4-phenylenebis(thiourea), 24, 1,3- propylenebis(thiourea), 76, and 1,4-butylenebis(thiourea), 77, and
- (2) oxidation of these compounds with peracetic acid or hydrogen peroxide and
- (3) isolation and characterization of the resulting bifunctional thiourea S,S,S-trioxides; ethylene-1,2-bis(thiourea S,S,S-trioxide), 78, and phenylene-1,4-bis(thiourea S,S,S-trioxide), 79.

RESULTS AND DISCUSSION:

The properties and yields of several bifunctional thioureas are given in Table 1.

Table 1  
Yields and Properties of Bisthioureas<sup>a</sup>

Bisthiourea	m.p., °C	lit. m.p., °C	IR bands, cm <sup>-1</sup>	% Yield
-CH <sub>2</sub> CH <sub>2</sub> -	199-200	202	2970, 2040, 1473, 1024, 812, 468	45
	217-218	218	3140, 1604, 1530, 1058, 780, 510	93
-CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -	1. >400 2. 51-79	--- ---	3100-2780sb, 1970wb, 1710mb, 1370sb 3200-2780sb, 2030s, 1390s	--- ---
-CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -	1. 350d 2. sub.350	--- ---	3080-2780sb, 2030s, 1720mb, 1375sb 3100-2740sb, 1980wb, 1720mb, 1360sb	--- ---

(a) Using procedure of Wolfe, Loo, and Arnold.<sup>16</sup>

The synthesis of bifunctional thioureas was attempted using both the procedure of Neville and McGee<sup>8</sup> and that of Wolfe, Loo, and Arnold.<sup>16</sup> The latter procedure seemed to be superior because of the ease of its use, the high yields obtained, and the purity of the products.

There were numerous difficulties with the procedure of Neville and McGee,<sup>8</sup> the primary one being the preparation of pure silicon tetraisothiocyanate, 21. First, the preparation of 21 has to be run under anhydrous conditions due to the decomposition of the product in the presence of water. It was found that the white silicon tetraisothiocyanate decomposed in air immediately after isolation to form a yellow solid: mp partially melted at 140 °C [lit.<sup>8</sup> 144]. This supports the findings of Neville and McGee,<sup>8</sup> who reported that the silicon tetraisothiocyanate readily hydrolyzed on contact with water (in the air or in solution) to form gelatinous silica and thiocyanic acid according to Figure 26.

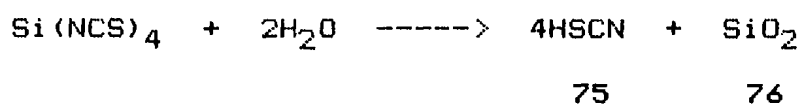


Figure 29

Thiocyanic acid, 75, is not very stable, however, and slowly breaks down to form a yellow solid,<sup>44</sup> which has been identified as a polymer of the free acid.<sup>45</sup>

When 21 was used immediately after its isolation to prepare phenylene-1,4-bis(thiourea), a light-yellow solid was obtained. This solid was shown to be impure by thin layer chromatography and infrared spectroscopy. TLC results for this reaction are given in Table 2.

Table 2: TLC Results of Phenylene-1,4-Bisthiourea Synthesis<sup>a</sup>

Solvent System	R <sub>f1</sub>	R <sub>f2</sub>
methylene chloride	0.00	0.00
benzene	0.00	0.00
iso-propanol	0.25	0.34
i-Pr-OH/benzene (50:50)	0.50	0.57
n-butanol	0.43	0.54
ethanol/methanol (50:50)	0.55	0.61

(a) By method of Neville and McGee.<sup>8</sup>

The IR indicated the presence of some starting material, with the some 1,4-phenylenediamine bands at 1500, 820, and 500 cm<sup>-1</sup>.

Phenylene-1,4-bis(thiourea) is insoluble in most solvents (water, benzene, acetone, methanol, ethanol, isopropanol, chloroform, and methylene chloride), and thus

cannot be purified by recrystallization. It is soluble, however, in DMSO.

Thin layer chromatography gave inconclusive results. When the crude phenylene-1,4-bis(thiourea) was analyzed by TLC using benzene and methylene chloride, the sample did not move from the origin. In isopropanol, n-butanol, isopropanol/benzene (50:50), and ethanol/methanol (50:50), very little separation of the compounds, presumably the bithiourea and the starting 1,4-phenylenediamine (determined by spotting against 1,4-phenylenediamine), was observed.

When the procedure of Neville and McGee<sup>8</sup> was used to synthesize ethylene-1,2-bis(thiourea), only a gummy residue formed. No purification or analysis of this residue was performed.

The method of Wolfe, Loo, and Arnold<sup>16</sup> gave better results. Phenylene-1,4-bis(thiourea) was obtained in 93% yield. The product was easy to isolate because the starting materials, 1,4-phenylenediamine and ammonium thiocyanate, are soluble in aqueous acid, while the bithiourea is insoluble. Thus the product precipitates readily out of solution as it forms, and it is easily recovered.

When the procedure of Wolfe, Loo, and Arnold<sup>16</sup> was used for the preparation of ethylene-1,2-bis(thiourea), the product did not precipitate from solution. A product did crystallize, however, upon evaporation of some of the water. Recrystallization using ethanol/water gave a product which

melted at 201-202 °C [lit.<sup>8</sup> 202 °C], suggesting that a pure product was obtained. Infrared spectroscopy showed absorption bands at 3080 (N-H stretch), 2970 (N-H stretch), 2040 (thiocyanate), and 1023 (C=S)  $\text{cm}^{-1}$ .

Attempts were made to prepare propylene-1,3-bis(thiourea) and butylene-1,4-bis(thiourea) by analagous reactions. However, the solid product obtained after addition of acetone was shown by IR spectroscopy to be the inorganic salt ammonium chloride, as compared with an authentic sample. Ammonium chloride was formed from excess ammonium thiocyanate and concentrated hydrochloric acid. When the remaining filtrate was evaporated, a solid was obtained which gave bands in the IR spectra that appeared to be due to the presence of an organic compound, but the strongest peaks in the spectrum corresponded to ammonium thiocyanate, as compared to an authentic sample. The melting points and IR bands for these salts are given in Table 3. Apparently, little, if any, of the starting material reacted to form the bithiourea.

Table 3: Inorganic salts

Salt	m.p., °C <sup>43</sup>	IR bands, cm <sup>-1</sup>
NH <sub>4</sub> SCN	subl. 340	3120-2780sb, 2030s, 1385s
NH <sub>4</sub> Cl	149.6	3100-2980, 1970wb, 1720wb, 1390s

The organic products obtained were probably the diisothiocyanate salts of the corresponding diamines. These ionic compounds would be expected to have high solubility in water and would show the characteristic thiocyanate ion IR band at 2060 cm<sup>-1</sup>. These same properties were seen for the products from the reactions of the aliphatic diamines. The equation shown in Figure 30 is the reaction which has occurred with ethylenediamine.

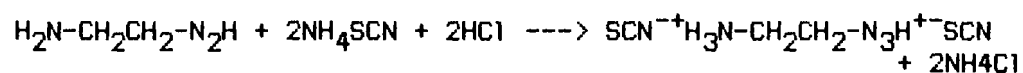


Figure 30



The structure of this salt was verified qualitatively by the addition of ferric chloride to an aqueous solution of the solid to give the characteristic deep red color of  $\text{Fe}(\text{SCN})^{2+}$ .



Figure 31

All products of the reactions involving aliphatic diamines gave positive  $\text{Fe}^{3+}$  tests, suggesting that these products were the bisisothiocyanate salts.

The product obtained from the ethylenediamine was indeed the thiocyanate salt, which had a melting point of 200-201 °C. This melting point is the same as that reported by Neville and McGee<sup>8</sup> for their preparation of 1,2-ethylenebis(thiourea). It is possible that Neville and McGee did not obtain ethylene bis(thiourea) but rather the dithiocyanate salt. No IR results were reported for their product. Thus they did not rule out the possibility of formation of this thiocyanate salt. They reported that their compound was analyzed correctly for nitrogen, but because the salt and the bithiourea are isomeric, their nitrogen analysis would not rule out formation of the salt. The percent composition of carbon, hydrogen, nitrogen, and sulfur for each compound is given in Table 5.

Table 4: Elemental Percentages

Compound	% C	% H	% N	% S
ethylene-1,2-bis(thiourea)	27	6	31	36
ethylenedi-ammonium thiocyanate	27	6	31	36

The fact that the melting point obtained for the ethylenediammonium thiocyanate salt is the same as that reported by Neville and McGee<sup>8</sup> suggests that they did not obtain the bithiourea but instead obtained the thiocyanate salt.

Thiocyanate ion was analyzed titrimetrically via the Volhard method.<sup>46</sup> If the solid were the dithiocyanate salt of ethylenediamine, then it would be expected to be 69.9 % thiocyanate by mass. Titrimetric analysis showed that the solid was 65.2 % thiocyanate. Since there is only a 7% error between these two values, this analysis suggests that the product was most likely the thiocyanate salt of ethylenediamine, rather than the bithiourea.

A possible explanation for why the aliphatic amines formed salts rather than undergoing nucleophilic addition reactions, as in the case of phenylene diamine, can be

rationalized on the basis of the dissociation constants of the aliphatic diamines and the aromatic diamine. The  $pK_{a1}$  of ethylenediamine is 6.85, while that of 1,4-phenylenediamine is 2.67,<sup>47</sup> thus ethylenediamine is more basic than the phenylene diamine. Therefore ethylene diamine is protonated much more easily than the phenylene diamine. Upon protonation, the amino group is no longer available as a nucleophile, and therefore this group cannot undergo nucleophilic reactions.

The preparation of the phenylene-1,4-bis(thiourea-S,S,S-trioxide) seems to have been successful. The synthesis resulted in a good yield (68%) of a light-yellow solid. Melting point and IR results for this oxidation product and other known thiourea S-oxides are given in Table 5. The major IR spectra bands (1680, 1248, 1057, 622  $\text{cm}^{-1}$ ) appeared at nearly the same frequencies as those for N-phenyl-thiourea-S,S,S-trioxide<sup>1,2</sup> (1666, 1253, 1060, 639  $\text{cm}^{-1}$ ) and were bands that do not occur in the spectra of the thiourea dioxide. The product did not melt below 400 °C. Isolation of this compound was different than that of the N-phenyl-thiourea trioxide, though, as described by Bischoff.<sup>1</sup> Whereas the N-phenyl-thiourea-S,S,S-trioxide was soluble in the methanolic solution,<sup>1</sup> the bifunctional compound was insoluble; therefore, it could be collected by suction filtration of the reaction mixture. Evaporation of the solvent from the filtrate gave no identifiable product.

Table 5: Oxidation of Thioureas and Bisthioureas

Thiourea	Oxidant	IR Bands, cm <sup>-1</sup>	m.p., °C <sup>a</sup>	lit. m.p., °C <sup>a,b</sup>	% Yield
ethylene bisthiourea	CH <sub>3</sub> CO <sub>3</sub> H	1: 1220w, 1080s 1630w <sup>b</sup>	>400	---	9.4
		2: 1030 <sup>c</sup>	335-338	---	6.7
	H <sub>2</sub> O <sub>2</sub>	1213w, 1070s 1630w	>400	---	---
phenylene bisthiourea	CH <sub>3</sub> CO <sub>3</sub> H	1: 1680, 1248, 1057, 622 <sup>b</sup>	>400	---	68
		2: 1510, 1245 <sup>c</sup>	>400	---	0.9
thiourea (dioxide) <sup>d</sup>	commer- cially available	1052, 1012	---	126	---
thiourea (trioxide) <sup>e</sup>	CH <sub>3</sub> CO <sub>3</sub> H	1220s, 1050s 1690s	131	112- 115	51-83
N-phenyl thiourea (trioxide) <sup>f</sup>	CH <sub>3</sub> CO <sub>3</sub> H	1666, 1253, 1060, 639	166.5 -167	171- 172	43-76

(a) All compounds decompose at their melting point.

(b) Solid from reaction mixture filtration.

(c) Solid from evaporation of solvent from filtrate.

(d) Dioxide of thiourea, AIMS.<sup>1,2</sup>(e) Trioxide of thiourea, AIMS.<sup>1,2</sup>(f) Trioxide of N-phenylthiourea, PAIMS.<sup>1,2</sup>

### SUGGESTIONS FOR FUTURE WORK:

Investigation of the reaction according to Neville and McGee<sup>8</sup> should be continued. Running the bithiourea synthesis reaction with a little excess  $\text{Si}(\text{NCS})_4$  (10-25%) would allow complete reaction of the diamine. The excess  $\text{Si}(\text{NCS})_4$  could then be destroyed with water to give thiocyanic acid and silica gel, both of which could be removed from soluble products by filtration.

The preparation of aliphatic thioureas should be performed using a modification of the synthesis of Wolfe, Loo, and Arnold.<sup>16</sup> The reaction should be carried out under neutral conditions. The aliphatic amines and the ammonium thiocyanate are soluble in water, and the reaction should proceed as expected, with the unprotonated amino groups attacking the thiocyanate groups to form the bithioureas.

Once the bithioureas have been obtained, attempts should be made to prepare the S,S,S-trioxides. If these oxidations succeed, a study of the reactions of the bifunctional thiourea-S,S,S- trioxides with amino acids should be investigated. These model studies could lead to a study of the protein crosslinking abilities of these bifunctional thiourea S,S,S-trioxides.

## EXPERIMENTAL PROCEDURE:

### Determination of Physical and Spectroscopic Properties:

Melting points were determined using a "Mel-Temp" capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 398 infrared spectrophotometer. All samples were solid and were analyzed in a KBr pellet. Intensities of the reported bands are abbreviated as s, m, w, and b to indicate strong, medium, weak, or broad absorbances, respectively.

### Analysis of reaction mixtures and products:

Thin layer chromatography was employed to analyze reaction mixtures, using Kodak 131B1 Silica Gel plates with fluorescent indicator. Ultraviolet light was used to visualize the spots.

### Commercially Available Starting Materials:

Silicon tetrachloride and all starting diamines (ethylenediamine, 1,3-diaminopropane, 1,4-diaminobutane, 1,4-phenylenediamine) were obtained from Aldrich Chemical Company. Only the ethylenediamine was purified by distillation before use. Baker's Analyzed ammonium thiocyanate was used. Hydrogen peroxide (30%) and acetic anhydride were obtained from Fisher Scientific.

Most solvents (benzene, methanol, acetone, iso-propanol, carbon tetrachloride, hexanes, toluene, and methylene chloride) were obtained from Fisher Scientific. Dimethyl sulfoxide was obtained from Aldrich Chemical Company. Ethanol (95%) was obtained from U.S. Industrial Chemicals.

Other compounds used include thiourea, sulfuric acid, ammonium chloride, ferric nitrate, and ferric chloride which were obtained from Fisher Scientific. Hydrochloric acid was obtained from VWR Scientific, potassium bromide from Mallinckrodt Chemical Works, and silver nitrate from Spectrum Chemical Manufacturing Corporation.

#### Preparation of Compounds:

Silicon tetraisothiocyanate (21). The procedure of Neville and McGee<sup>8</sup> was used to prepare  $\text{Si}(\text{NCS})_4$  in nearly quantitative yield: m.p. approx.  $140^\circ\text{C}$  [lit.<sup>8</sup>  $144^\circ\text{C}$ ].

Phenylene-1,4-bis(thiourea) (24). The white  $\text{Si}(\text{NCS})_4$  was used without further purification in an attempt to synthesize phenylene-1,4-bis(thiourea) according to the procedure of Neville and McGee.<sup>8</sup> An impure yellow solid was obtained in 10% yield: mp above  $230^\circ\text{C}$  [lit.<sup>8</sup>  $218^\circ\text{C}$ ]; IR: 2800sb, 2020s, 1500m, 820w,  $500\text{ cm}^{-1}$ . TLC showed presence of impurity.

Phenylene-1,4-bis(thiourea) was also synthesized according to the procedure of Wolfe, Loo and Arnold.<sup>16</sup> The yellow solid that was obtained was recrystallized from DMSO/water to give pure

product in 52% yield: m.p. 217-218 °C [lit.<sup>8</sup> 218 °C]; IR 3320m, 3240s, 3140s, 1604s, 1530s, 1058m, 780m, 510m  $\text{cm}^{-1}$ ; [lit. IR<sup>16</sup> 3330, 3260, 3170, (NH and  $\text{NH}_2$ ) 1070 (C=S)  $\text{cm}^{-1}$ ].

Ethylene-1,2-bis(thiourea) (77). An attempt to synthesize this product by the method of Neville and McGee<sup>8</sup> gave only a gummy residue. Using the procedure of Wolfe, Loo, and Arnold,<sup>16</sup> afforded 45% yield of the dithiocyanate salt of ethylenediamine: m.p. 201-202 °C; IR 2980s, 2040s, 1475s, 1025s, 810m, 465m  $\text{cm}^{-1}$ .

Propylene-1,3-bis(thiourea) (78). Attempts to prepare this compound by the procedure of Wolfe, Loo, and Arnold<sup>16</sup> yielded only inorganic salts. Evaporation of solvent resulted in precipitation of ammonium thiocyanate: mp partial at 360 °C [lit.<sup>43</sup> 149.6 °C]; IR 3100sb, 2020s, 1385s  $\text{cm}^{-1}$  [authentic 3100-2800sb, 2020s, 1390s  $\text{cm}^{-1}$ ]. Addition of acetone resulted in precipitation of ammonium chloride: mp sublimes at 350 °C [lit.<sup>43</sup> subl. at 340 °C]; IR 3100-2740sb, 1980w, 1720m, 1360s  $\text{cm}^{-1}$  [authentic 3100sb, 1980w, 1720m, 1390s <sup>$\text{cm}^{-1}$</sup> ].

Butylene-1,4-bis(thiourea) (79). Attempts at preparing this compound by the procedure of Wolfe, Loo, and Arnold<sup>16</sup> yielded only inorganic salts. Evaporation of solvent resulted in precipitation of ammonium thiocyanate: mp did not melt below 400 °C [lit.<sup>43</sup> 149.6 °C]. Addition of acetone resulted in



precipitation of ammonium chloride: IR 3100-2800sb, 1970w, 1710m, 1380s  $\text{cm}^{-1}$  [authentic 3100sb, 1980w, 1720m, 1390s].<sup>cm<sup>-1</sup></sup>

Peracetic acid (80). Peracetic acid was prepared according to the procedure of Bischoff.<sup>1</sup>

Ethylene-1,2-bis(thiourea-S,S,S-trioxide) (81). This compound was synthesized by a method analogous to that used by Bischoff for the preparation of AIMS0 (thiourea trioxide).<sup>1</sup> Equimolar amounts of ethylene-1,2-bisthiourea (5.757 g, 0.03229 mole) and peracetic acid were reacted in approximately 1 liter of methanol (adding the bisthiourea in 900 ml methanol slowly to the peracetic acid), keeping the temperature between 10 and 20 °C using an ice bath. Upon formation of a solid, the reaction vessel was removed from the bath, and the reaction was allowed to come to room temperature. The mixture was allowed to sit overnight at room temperature. The solid that formed was removed by suction filtration to obtain 0.833 g (9.4% yield) of a white solid: m.p. turned brown at about 300 °C but did not melt up to 400 °C; IR: 2950sb, 1630w, 1535m, 1345m, 1080sb, 615s  $\text{cm}^{-1}$ .

Methanol was evaporated in vacuo from the filtrate until crystals formed. These were collected by suction filtration and rinsed with cold methanol to afford 0.591 grams of a white solid (6.7% yield): mp 335-338 °C with bubbling; IR 2900sb, 2050m, 1600s, 1500s, 1030s, 820m  $\text{cm}^{-1}$ .

Compound 81 was also prepared using 30%  $\text{H}_2\text{O}_2$  as the oxidant, but no quantitative results were recorded: mp. turned dark

brown around 300 °C but did not melt up to 400 °C; IR: 2900sb, 1630w, 1535m, 1340m, 1070sb, 610s  $\text{cm}^{-1}$ .

Phenylene-1,4-bis(thiourea-S,S,S-trioxide) (82). This compound was synthesized using a procedure analogous to that used by Bischoff<sup>1</sup> for the synthesis of FAIMSO. Freshly prepared peracetic acid (6 equivalents) was treated with a mixture of 5.569 grams of phenylene-1,4-bisthiourea in 900 ml methanol. The solid bisthiourea was insoluble in the methanol but formed a somewhat soluble product when added to the oxidant. The reaction was allowed to stand overnight at room temperature. The solid that formed was collected by suction filtration. A brown solid, 5.392 grams (68% yield, if the S,S,S-trioxide) was obtained: m.p. did not melt up to 400 °C; IR: 3150sb, 1680s, 1248s, 1057s, 622m  $\text{cm}^{-1}$ .

The methanol was evaporated from the filtrate, and the resulting dark brown/black solid was recovered in 0.9% yield: m.p. >400 °C; IR: 3200mb, 1670m, 1600m, 1510m, 1250m, 1055m, 620w  $\text{cm}^{-1}$ .

#### Quantitative determination of thiocyanate:

Thiocyanate ion was determined by the Vollhard method.<sup>46</sup> A 20.00 ml aliquot of 0.103 Molar  $\text{AgNO}_3$  was added to 0.102 grams of the salt. White  $\text{AgSCN}$  precipitated. The excess  $\text{Ag}^+$  was back-titrated with 0.125 Molar thiocyanate solution, using  $\text{Fe}^{3+}$  as an indicator of the endpoint. This required 6.72 ml of the

standard thiocyanate solution. The percent of thiocyanate in the sample was found to be 69.9 (theoretical for  $\text{SCN}^- + \text{NH}_3\text{CH}_2\text{CH}_2\text{NH}_3^+ - \text{SCN}$  is 65.2 percent).

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