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Preparation and Photochemical Degradation Studies
of Tertiary Benzyl N-Nitrosocarbamates

Thesis

Submitted to

The college of Arts and Sciences

UNIVERSITY OF DAYTON

In Partial Fulfillment of the Requirements for

The Degree

Master of Science in Chemistry

by

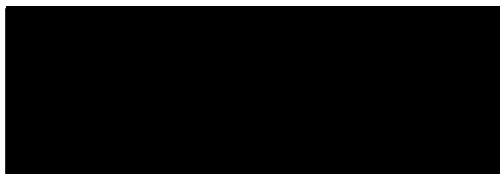
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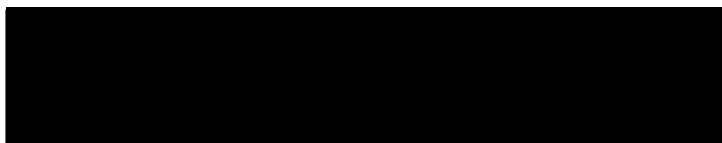
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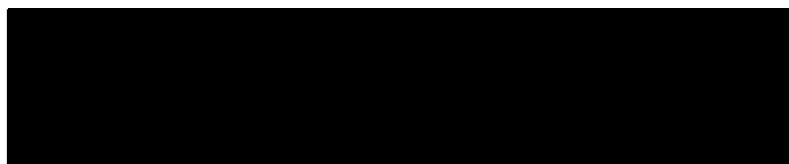
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ABSTRACT

PREPARATION AND PHOTOCHEMICAL DEGRADATION STUDIES OF TERTIARY BENZYL N-NITROSOCARBAMATES

Satya Nagaraju Addaganti Venkata

University of Dayton

Advisor: Dr. Vladimir A. Benin

I am reporting the synthesis, and photochemical studies on some tertiary benzyl N-nitrosocarbamates with 2-(methylthio)ethyl or thiobis(2-aminoethyl) functionality, as a part of long-term goal to design and prepare novel photolabile structures that could be used as substances for controlled release of DNA alkylating and/or crosslinking agents. The synthesis was accomplished by reaction of benzyl chloroformates with the corresponding amines, resulting in the preparation of carbamates, which were subsequently nitrosated to yield the target structures. Photolytic studies were conducted at 250 and 350 nm, and the products of decomposition were isolated and characterized, providing an insight into the mechanism of photochemical degradation.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank sincerely my research advisor, Dr. Vladimir Benin for his continuous support through out my masters program. He showed me different ways to approach a research problem and the need to be persistent to accomplish any goal. He has provided me with the freedom to learn on my own and I was always comfortable expressing my ideas to him. I would like to thank him for his patience and understanding which helped me in good preparation of my thesis. I would like to thank my parents for providing me an opportunity to study abroad. I would also like to thank the University of Dayton, Department of Chemistry for providing me excellent opportunity to start my career in the field of research and development. Finally and equally important, I would like to thank all faculty and staff for their support and help during these years. I also would like to thank my friends for all their support and help.

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LIST OF ABBREVIATIONS

NMR.....Nuclear magnetic resonance

CDCl₃.....Deuterated chloroform

CHAPTER 1

INTRODUCTION

N-nitroso compounds (NOCs) have been implicated as important factors in the etiology of several cancers including gastric, esophageal, intestinal, bladder and to some extent, kidney.¹ NOCs have been known for almost 40 years ago to be present in food treated with sodium nitrite, which made fish meal hepatotoxic to animals through formation of N-nitrosodimethylamine (NDMA).² Since then, N-nitroso compounds have been shown in animal experiments to be the most broadly acting and the most potent group of carcinogens. Over three hundred nitrosamines have been tested for carcinogenicity and 90% of these compounds show activity.² Careful dose response studies have shown that many of these compounds have a high degree of potency, and N-nitrosodimethylamine has been found to be carcinogenic in more than 20 species of animals.²

Humans are exposed to N-nitroso compounds (NOCs) from diet, tobacco smoke and other environmental sources, as well as from endogenous synthesis, which contributes to 45 – 75% of total exposure.³ Various NOCs have been found to be carcinogenic in multiple organs in at least 40 animal species including higher primates.³ Despite continuing concern that NOCs may be

causally related to gastrointestinal cancer, the epidemiological literature has failed so far to support this link with any degree of conviction.⁴

All humans excrete the non-carcinogenic amino acid derivative N-nitrosoproline in their urine.⁵ This finding and other studies clearly demonstrate that N-nitroso compounds are formed within the human body.⁶ Over the past twenty year period a great deal of effort has been expended in an attempt to establish an epidemiological connection between human cancer and endogenous N-nitroso compound formation and exogenous exposure to preformed N-nitroso compounds.

While highly definitive conclusions are difficult to make, the evidence connecting nitrosamines and more reactive N-nitroso compounds to human cancer can be summarized as follows: tobacco and tobacco smoke contain significant concentrations of nitrosamines. Two of the more carcinogenic are N-nitrosornicotine (NNN) and 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK), both derived from the nitrosation of nicotine.⁷ Careful dose response studies have shown NNK to be a powerful lung carcinogen in three species of rodents, despite the mode of administration.⁷ Molecular fragments from NNK and NNN have been found in the hemoglobin and DNA of humans exposed to high concentrations of the nitrosamines.⁸ To date, the nitrosamines derived from the nicotine alkaloids are the only significant carcinogenic agents found in the smokeless tobacco.⁸ On the other hand, because of the many carcinogenic agents in tobacco, a precise epidemiological connection between tobacco related cancers and nitrosamines is difficult to make.

There is also good evidence linking N-nitroso compounds to gastric cancer in areas where there is a high dietary nitrate and nitrite intake. The collaborative work of Correa and Tannenbaum is notable in this regard.⁹ This evidence, coupled with the fact that nitrosamines are extremely potent carcinogens in a wide variety of animals, regardless of species, suggests that these nitroso compounds are also human carcinogens.¹⁰ Every type of animal was susceptible to carcinogenesis by N-nitroso compounds; and although there is no direct evidence to associate them with human cancer, it is believed that they are probably carcinogenic to humans as well.¹¹ Figure 1 gives few examples of structures whose carcinogenicity is well documented.^{1,12-14}

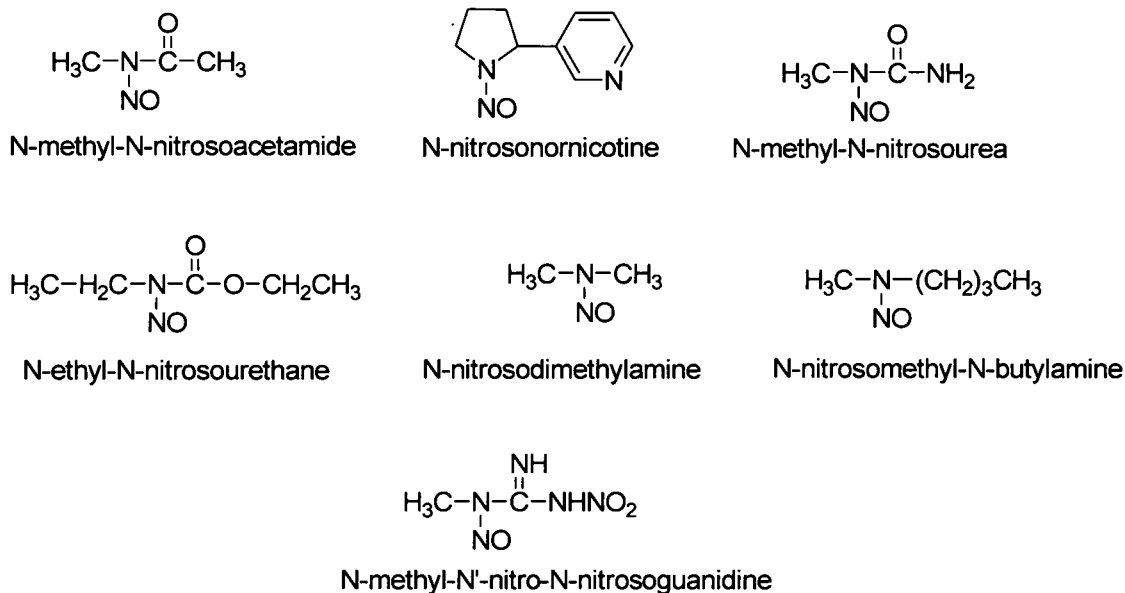


Figure 1: Typical examples of N-nitroso compounds

N-nitroso compounds can be placed in two broad categories (Figure 2). Of these the nitrosamines, being amides of nitrous acid, are the most stable and

are formally derived from the reaction of the secondary amine with nitrous acid. The second class of N-nitroso compounds (N-nitrosoamide type) are those substances which have a carbonyl group attached to the nitrogen bearing the NO group. Members of this class include the reactive N-nitrosoamides, N-nitrosocarbamates, and N-nitrosoureas. The instability of the compounds of the N-nitrosoamide type is due to the joining of two very electron-deficient functional groups (NNO and CO).

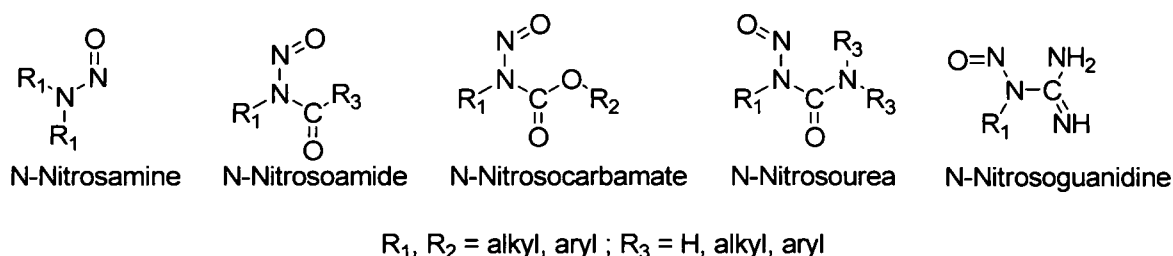
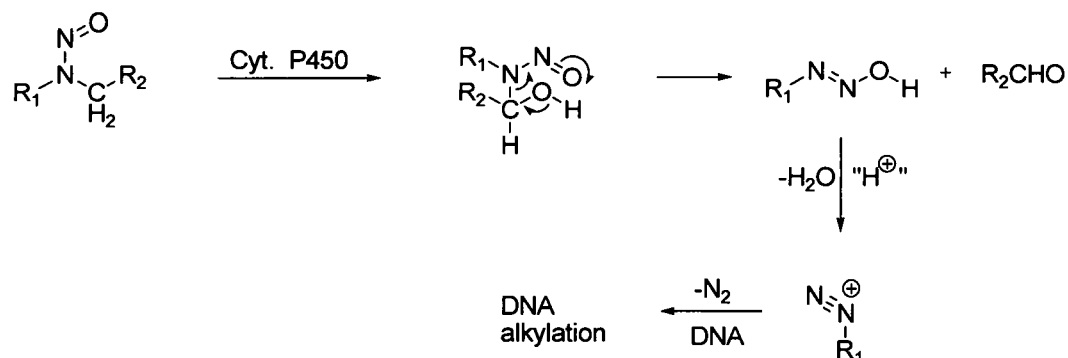


Figure 2: General classification of N-nitroso compounds

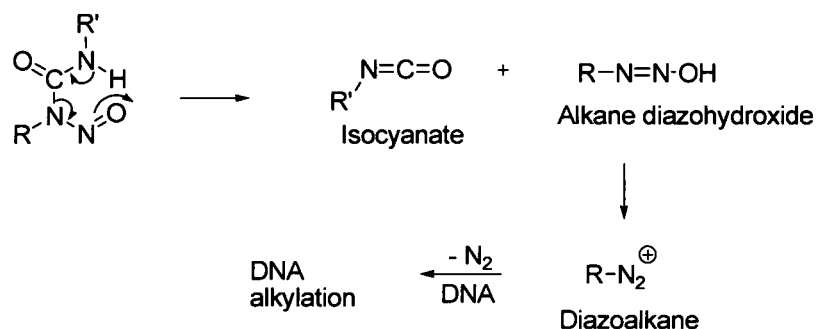
In general, N-nitrosamines such as N-nitrosodialkylamines need to be metabolized *in vivo* by cytochrome P450 to elicit their mutagenic or carcinogenic properties.² While the mode of carcinogenic biochemical activation is not known for all nitrosamines, many of them are activated through the process of α -hydroxylation (Scheme 1). The α -hydroxy nitrosamines resulting from this process are chemically unstable and decompose readily to diazonium ions, which are aggressive alkylating agents. In biological systems, the result is the alkylation of DNA, RNA, and protein. The induced DNA alkylation finally results in carcinogenesis.²

Scheme 1



By contrast, N-nitrosoamides such as N-nitroso-N-alkylureas, known as direct mutagens (require no biochemical activation), do not need metabolic conversion and readily undergo heterolysis to give alkylating species *via* the unstable diazotic acid (alkanediazohydroxide) (Scheme 2).¹⁵ Regardless of what their true nature might be (alkanediazohydroxides, alkyldiazonium ions, carbonium ion, or related ion pair or triplets), these alkylating species are believed to initiate the carcinogenic process by attacking critical nucleophilic sites of macromolecular constituents of target tissues (DNA, RNA, proteins).¹⁶

Scheme 2



While the deleterious effects of many N-nitroso compounds have been the focal point of research and practical work over the last several decades, several new types of compounds are receiving clinical attention and efforts continue to be directed at developing new efficacious antitumor drugs from N-nitrosoureas. Two N-nitrosoureas (Figure 3), BCNU (bischloroethyl N-nitrosourea) and CCNU (1-chloroethyl-3-cyclohexyl-1-nitrosourea), have been used as anticancer agents for a number of years. Despite their structural similarity to the carcinogenic nitrosoureas, these chloroethyl derivatives show great promise as effective anti-tumor agents.¹⁷⁻²⁰

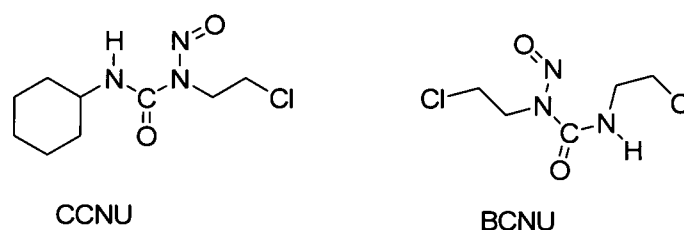
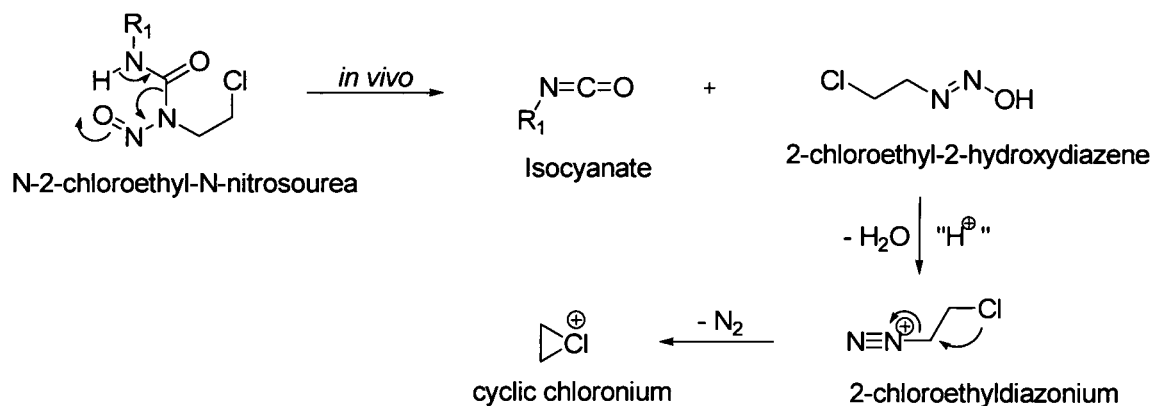


Figure 3: Few examples of N-nitroso compounds that are used as anti-tumor agents

It is believed that the anti-cancer activity of N-2-chloroethyl-N-nitrosoureas is due to the generation, *in vivo*, of 2-chloroethyldiazonium cation, which further decomposes to generate a cyclic chloronium cation (Scheme 3). The latter reacts with both protein and DNA to form an alkylated products, and a second alkylation event on alkylated base can lead to DNA cross-links owing to the presence of the chloro leaving group.^{21,22} DNA interstrand cross-links prevent separation of the DNA double strands during the replication process, which

eventually inhibits DNA synthesis, resulting in a cytotoxic effect.²³ The process of decomposition *in vivo* also leads to the generation of an isocyanate, which serves as a carbamoylating agent for proteins²⁴, by inhibiting cellular glutathione reductase activity by up to 90% at pharmacological doses. GR is susceptible to attack from exogenous electrophiles, particularly carbamoylation from alkyl isocyanates, rendering the enzyme unable to catalyze the reduction of oxidized glutathione. Evidence implicates inhibition of GR as a cause of the pulmonary toxicity often seen in high-dose BCNU-treated animals and human cancer patients.^{25,26,27}

Scheme 3



In contrast to N-nitrosoureas, N-nitrosocarbamates should decompose without the production of an isocyanate, but the process of decomposition seems to be one with higher activation energy. As a consequence, so far, they have, as a class, been much less explored as potential anticancer agents.³⁹

In recent years considerable amount of effort in drug design has been focused on the development of drugs that are supposed to release their active species in the desired locality and/or conditions, and also on identifying structures with photosensitive groups that would cleave upon irradiation with near UV or visible light, yielding active intermediates for biological applications.²⁸ First reported by Barltrop and Schofield in 1962²⁹, photolabile protecting groups have found numerous applications in biology in the past decade.^{30,31} The protecting groups (also known as “caging” groups) can render a bioactive compound inert until they are removed by photolysis, thus releasing the compound rapidly. Some examples (Figure 4) of commonly used photolabile caging groups include o-nitrobenzyl³², desyl³³ and 2-methoxy-5-nitrophenyl (MNP).³⁴ However, the commonly employed 2-nitrobenzyl photosensitive protecting group³⁵ has found limited use in compounds destined for biochemical systems, due to the release of a toxic by-product: 2-nitrobenzaldehyde.³⁶

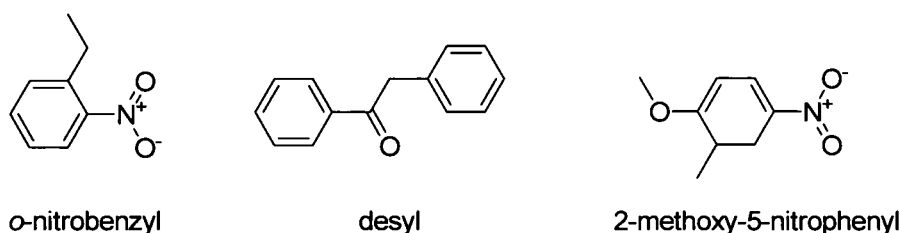
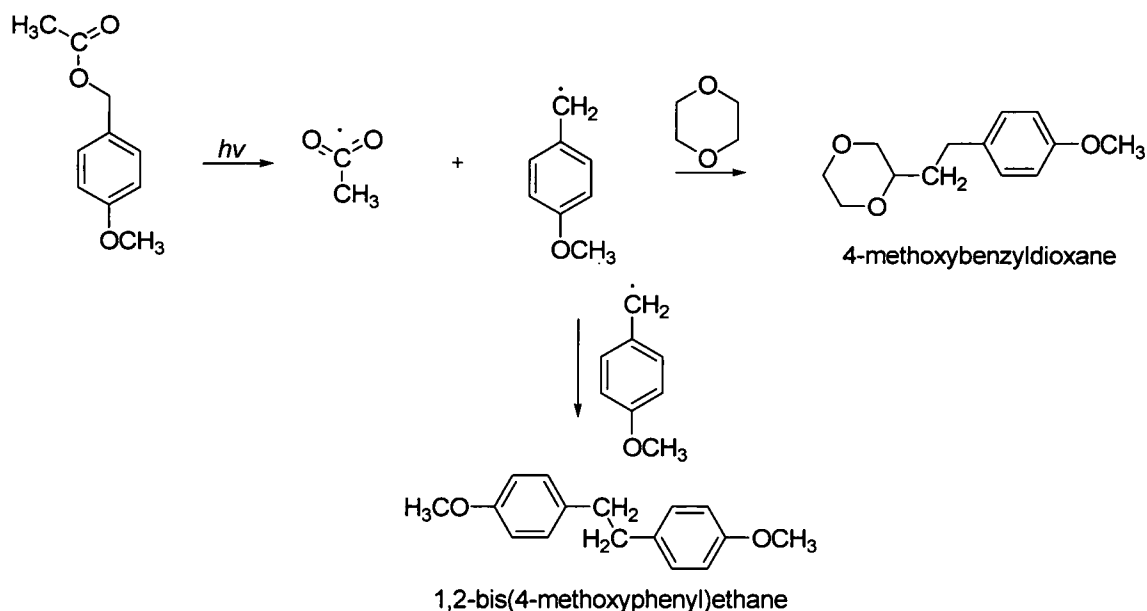


Figure 4: Examples of commonly used photolabile caging groups

Zimmerman first demonstrated the efficient photosolvolysis of benzyl acetate in 50% aqueous dioxane. He found that benzyl acetates undergo a

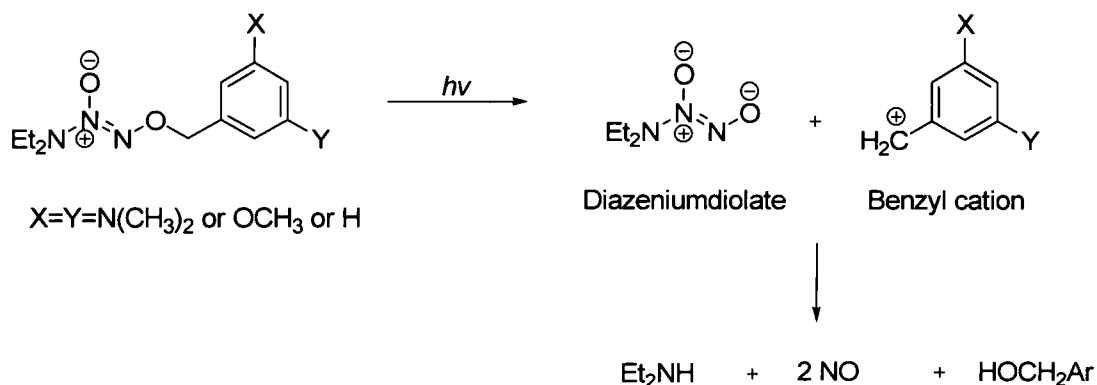
photolysis reaction leading to radical-derived products 4,4'-dimethoxybibenzyl and 4-methoxybenzylidioxane (Scheme 4).³⁷

Scheme 4



Based on the studies done by Zimmerman, recently Toscano *et al.* have begun an investigation of photosensitive protecting groups for diazeniumdiolates $[N(O)=NO]^-$. They have examined the use of the substituted benzylic derivatives in the photochemical cleavage to yield diazeniumdiolates. Their studies showed that the pattern of substitution in the benzyl group greatly influenced the products of photodecomposition.³⁸ It was found that compounds having benzene rings with π -donor groups at the 3- and 5-positions tend to decompose *via* heterolytic, rather than homolytic bond cleavage, and generate resonance stabilized (in the excited state) benzylic carbocations (Scheme 5).³⁸

Scheme 5



Based on the above mentioned studies by Toscano *et al*, and also on the observed alkylating activity of ethyl-N-2-chloroethyl-N-nitrosocarbamate⁴⁰, which is structurally similar to the clinically used N-2-chloroethyl-N-nitrosourea anticancer drugs, we proposed that certain N-nitrosocarbamates could be developed into a new class of anticancer agents, capable of releasing the active substance in controlled conditions, photolytically, without generating harmful by-products such as isocyanates.

The objective of this study was to design, synthesize and study a series of tertiary benzyl N-nitrosocarbamate structures containing 2-(methylthio)ethyl (**1**), N-2-(dimethylamino)amino (**2**) or thiobis(2-aminoethyl) (**3**) functionalities respectively (Figure 5). They represent classes of potentially photolabile structures, which could lead to the development of a novel methodology for controlled release of species with ability to act as efficient DNA alkylating agents and/or interstrand cross-linkers, such as cyclic ammonium or sulfonium cations.

The latter are similar to the intermediates in the metabolism/decomposition of N-2-chloroethyl-N-nitrosoureas^{41,42} or sulfur and nitrogen mustards.⁴³ The effect of substitution in the benzyl group on the rate and mechanism of photochemical and thermal degradation was also examined.

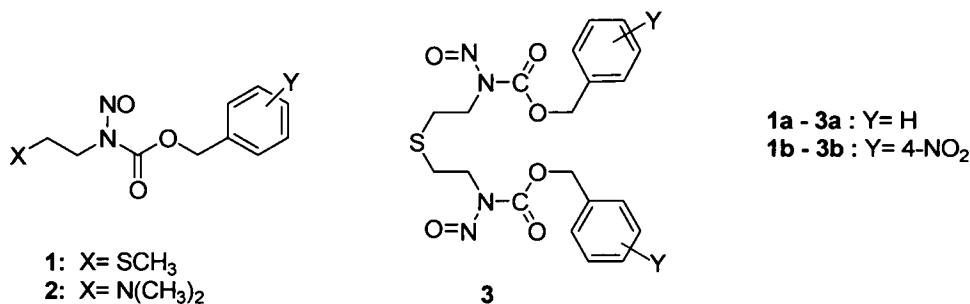
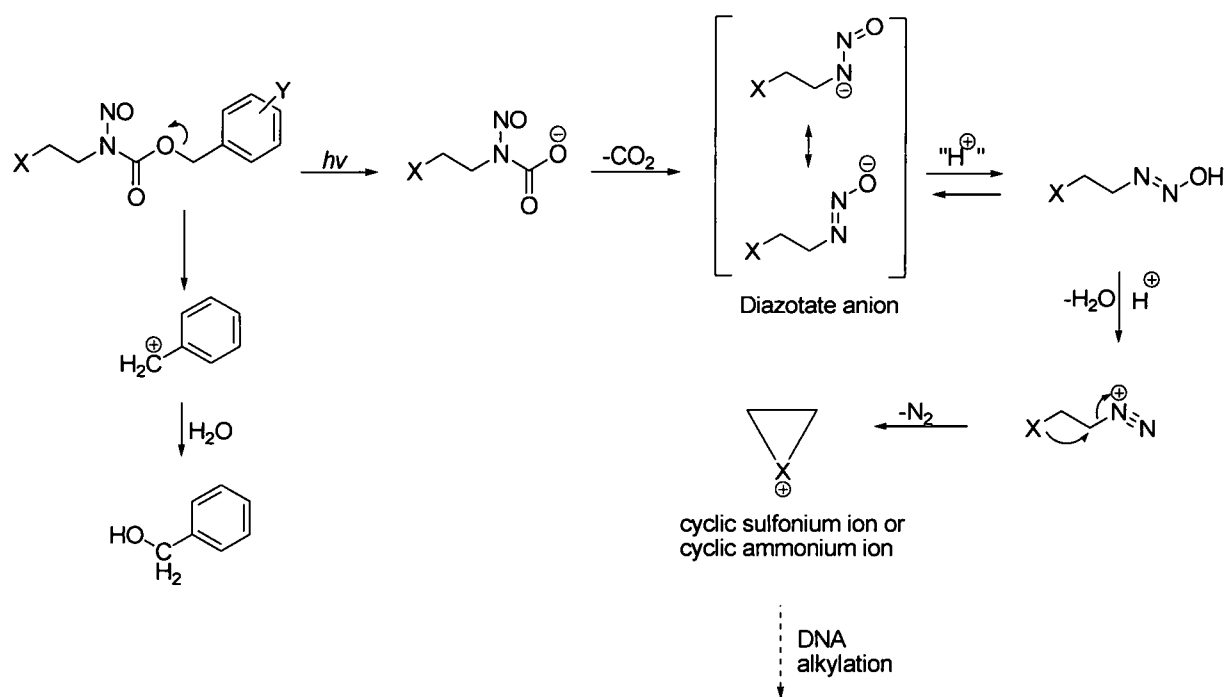


Figure 5: Target structures containing 2-(methylthio)ethyl (**1**), N-2-(dimethylamino)amino (**2**) or thiobis(2-aminoethyl) (**3**) functionalities.

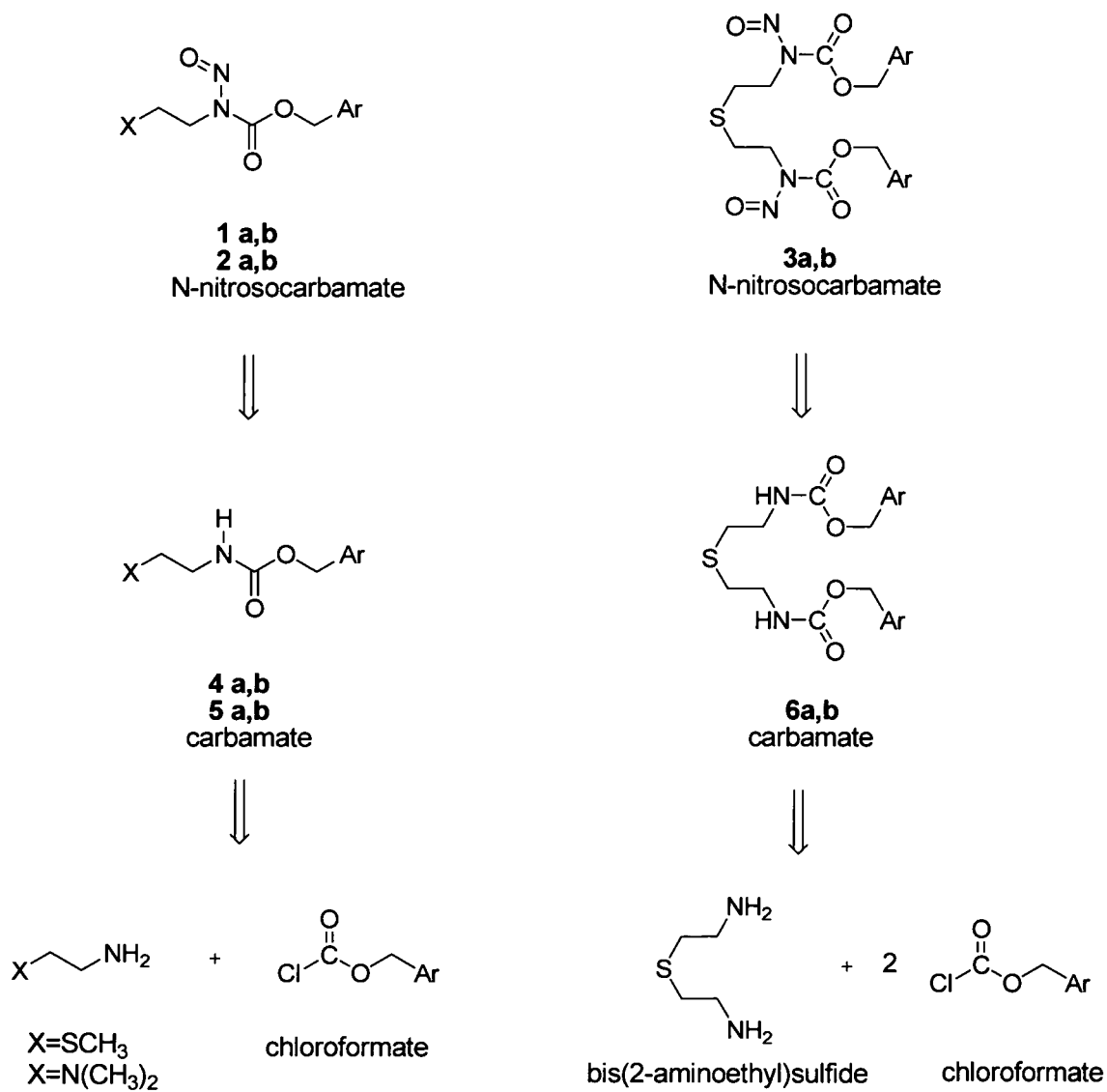
The target structures, upon heterolytic mode of cleavage of the benzylic C – O bond, would be expected to yield the corresponding benzylic carbocation and a carbamate anion. The benzylic carbocation is expected in its turn to react with a nucleophile in the medium (water in aqueous medium) and generate a neutral and relatively harmless by-product (Scheme 6). The carbamate anion is unstable and expected to decompose readily to generate a diazotate anion. Protonation of the latter and loss of water would lead to generation of a diazonium cation, which loses nitrogen to yield a cyclic ammonium or sulfonium cation.⁴⁴⁻⁴⁷ The latter are expected to readily alkylate DNA nucleophilic sites.

Scheme 6



The retrosynthetic pathway (Scheme 7) of the target compounds is given below.

Scheme 7

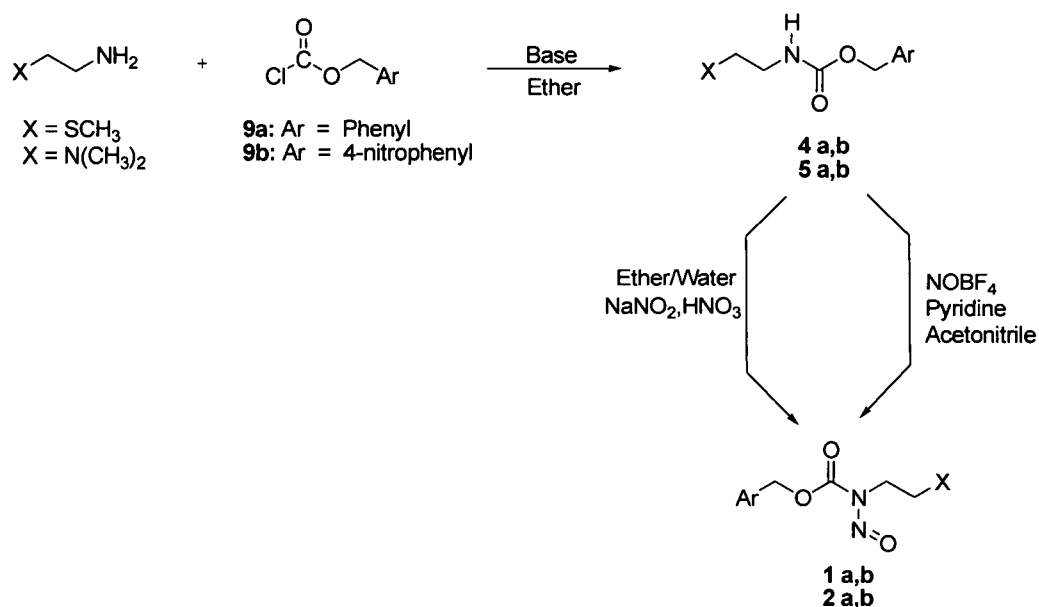


CHAPTER 2

RESULTS AND DISCUSSION

I. PREPARATION OF BENZYL N-NITROSOCARBAMATES: The preparation of these structures was conducted in accordance with the synthetic sequence reflected in Scheme 8. Reaction of the benzyl chloroformate (commercially available) with 2-methylthio)ethylamine or 2-(dimethylamino)ethylamine generated the corresponding carbamate, which was then nitrosated in anhydrous or aqueous conditions, to yield the target structure.

Scheme 8

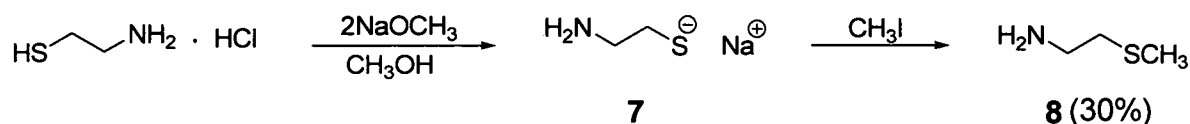


The presence of a sulfide group in carbamates (**4a,b** and **5a,b**) is not expected to pose problems in the nitrosation step. There are no known examples of S-nitrosation of sulfides that yield stable S-nitroso products⁴⁸, but an S- to N-nitroso rearrangement has been suggested to occur both inter- and intramolecularly in some reactions, such as N-nitrosation of dimethylsulfide⁴⁹ or the de-amination of methionine and S-methyl cysteine.⁵⁰ DFT calculations could not find an energy minimum for S-nitroso derivatives of the carbamate, but a structure was located that corresponds to the intermediate for S- to N-nitroso rearrangement.

A. Preparation of 2-(methylthio)ethylamine (8): It was prepared according to a literature procedure⁵¹, from the hydrochloric salt of 2-aminoethanethiol (Scheme 9). The hydrochloric salt of 2-aminoethanethiol was first neutralized and deprotonated with sodium methoxide, to yield sodium-2-aminoethanethiolate (**7**). The sulfide center (S⁻) of the 2-aminoethanethiolate

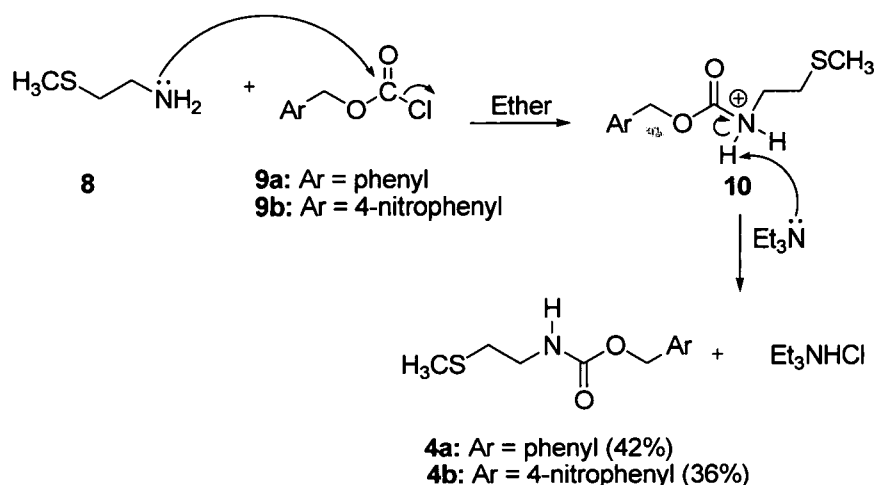
anion, being relatively more nucleophilic than the amino (NH₂) group, undergoes preferential nucleophilic substitution reaction with iodomethane to yield 2-(methylthio)ethylamine (**8**).

Scheme 9



B. Preparation of benzyl N-2-(methylthio)ethylcarbamate (4a) and of 4-nitrobenzyl N-2-(methylthio)ethylcarbamate (4b): The synthesis of these carbamates was achieved by the reaction of 2-(methylthio)ethylamine (**7**) with the corresponding commercially available chloroformates, in presence of equivalent amount of triethylamine. The amino group serves as a nucleophile and attacks the carbonyl carbon of the chloroformate (**9a,b**), resulting in elimination of a chloride ion, the better leaving group, to yield the intermediate structure **10**, which is then deprotonated by triethylamine to form the corresponding carbamate (**4a,b**) (Scheme 10).

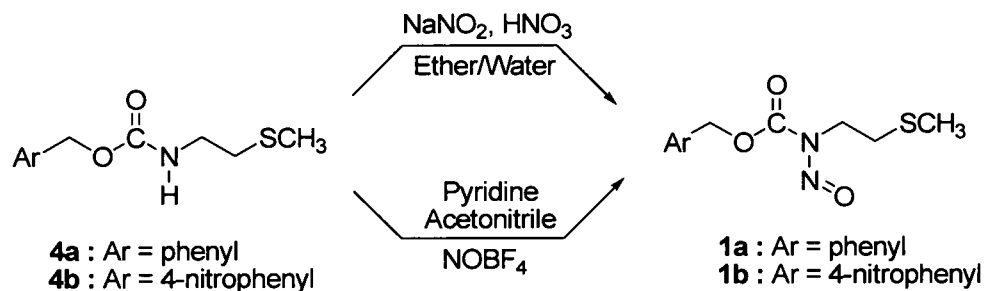
Scheme 10



C. Preparation of benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (1a) and of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (1b):

Nitrosation reactions were done as outlined in Scheme 11. The reactions were carried out utilizing two different methods.

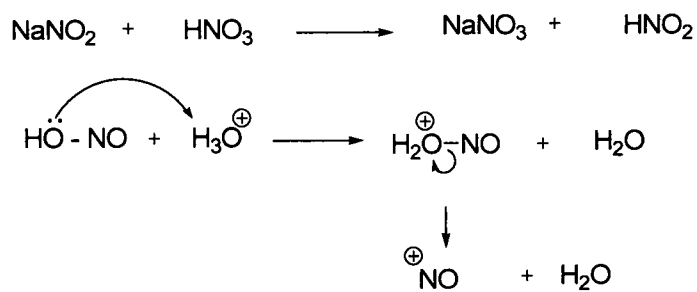
Scheme 11

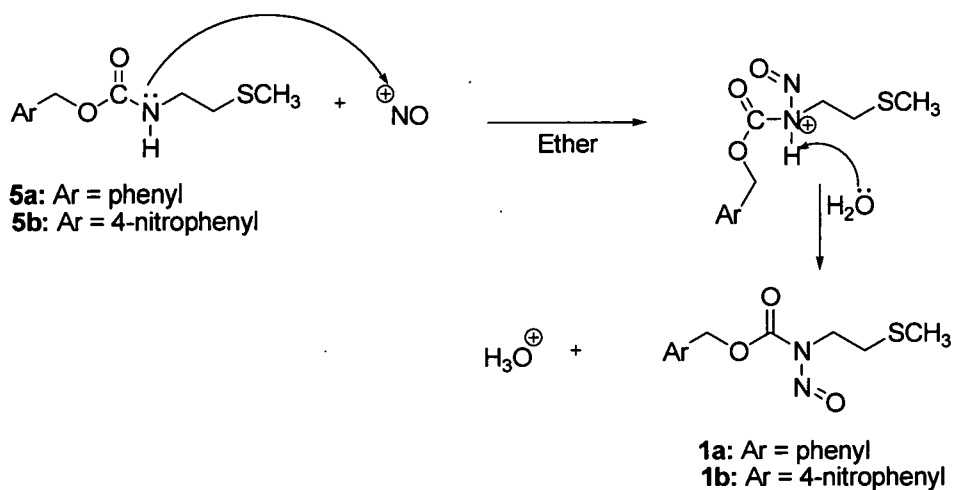


Compound	Method	Yield (%)
1a	Aqueous	20
	Anhydrous	43
1b	Aqueous	Not done
	Anhydrous	40

Method 1 (Aqueous method)⁵²: This method involves a two phase (water-ether) system, with the nitrosating agent ^+NO generated in the aqueous layer, upon reaction of NaNO_2 with HNO_3 (Scheme 12). The nitrosating agent then attaches to nitrogen in the carbamate, followed by deprotonation by water to yield the target nitroso carbamate (**1a,b**). This method gave the desired product, but the yield was low.

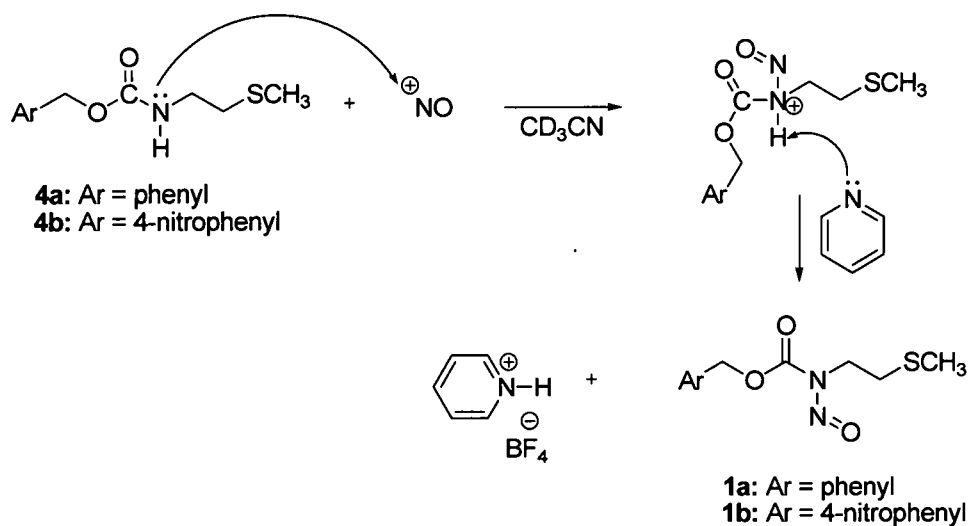
Scheme 12





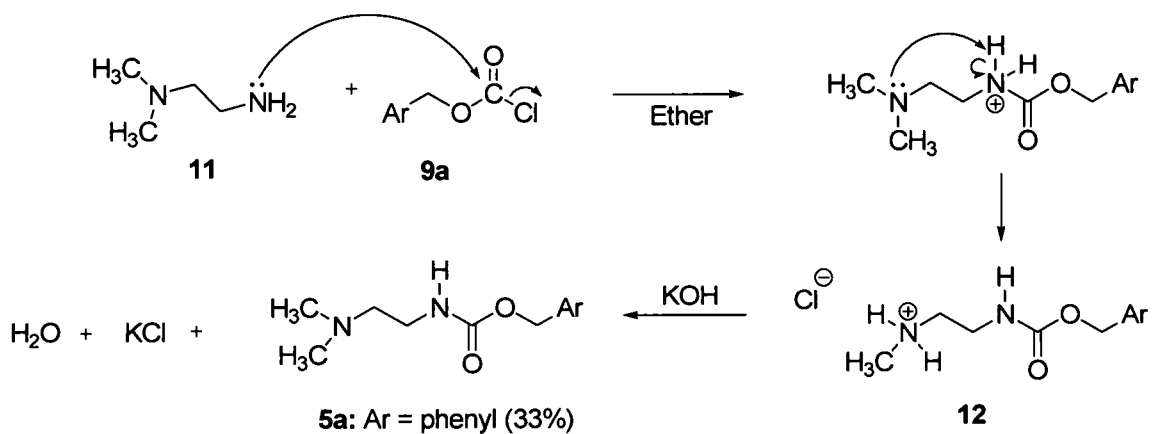
Method 2 (Anhydrous method)⁵³: Solid nitrosonium tetrafluoroborate (NOBF_4) is used as the nitrosating agent. The reaction is conducted in the presence of anhydrous pyridine, in anhydrous acetonitrile, at -20°C . The nitrosating agent ^+NO attaches to the nitrogen center of the carbamate, followed by deprotonation by pyridine to yield the corresponding nitrosocarbamate (**1a,b**) (Scheme 13). Better yields were obtained by this method.

Scheme 13



D. Preparation of benzyl 2-(N,N-dimethylamino)ethylcarbamate (5a) and 4-nitrobenzyl 2-(N,N-dimethylamino)ethylcarbamate (5b): The synthesis of these carbamates was accomplished by the reaction of N,N-dimethylethylenediamine with the corresponding benzyl chloroformates (Scheme 13). N,N-Dimethylethylenediamine (**11**) attacks the carbonyl carbon of the chloroformate (**9a**), leading to elimination of chloride ion, the better leaving group, followed by deprotonation by the nitrogen of dimethylamino group to yield the corresponding carbamate as the salt **12**. The salt can be neutralized by equimolar aqueous solution of potassium hydroxide to yield the corresponding carbamate (**5a**).

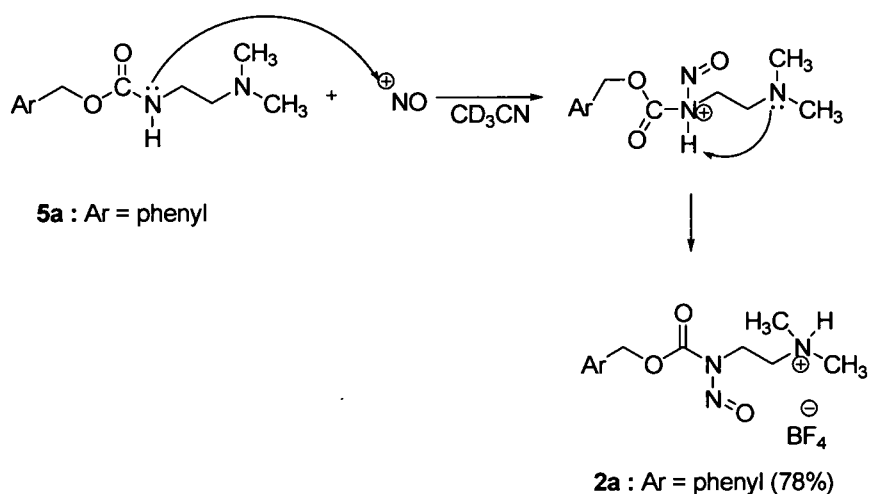
Scheme 14



E. Preparation of benzyl 2-(N,N-dimethylamino)ethyl-N-nitrosocarbamate (2a):

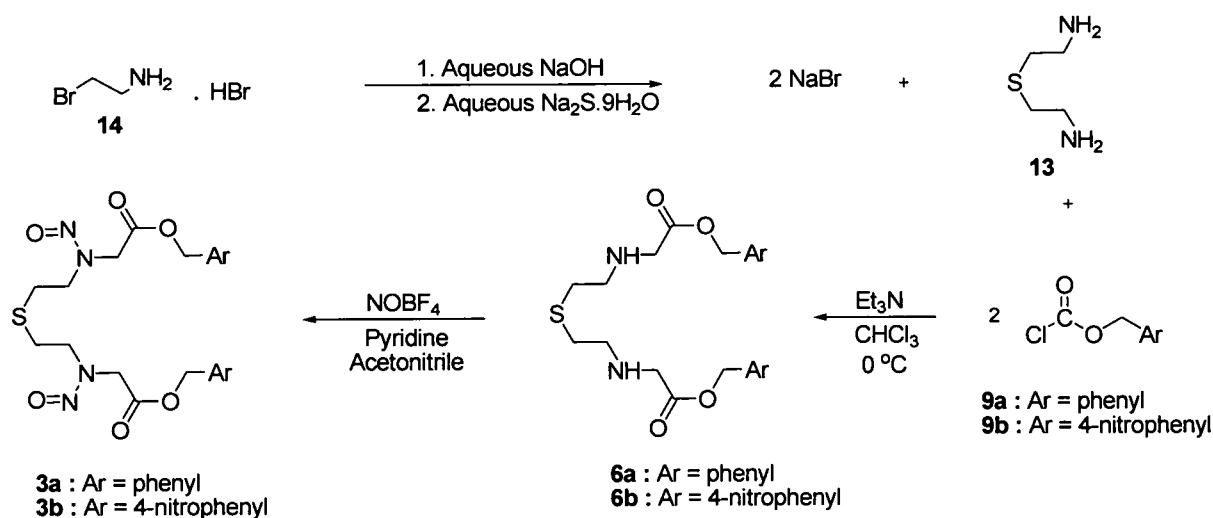
Anhydrous method⁵³: Attachment of ^+NO is followed by deprotonation by the nitrogen of the dimethylamino group, to yield the corresponding nitrosocarbamate as salt (**2a**) (Scheme 15).

Scheme 15



II. PREPARATION OF BENZYL BIS(N-NITROSOCARBAMATES): The preparation of these structures (**3a,b**) was conducted in accordance with the synthetic sequence reflected in Scheme 16. Reaction of the chloroformates (**9a,b**) bis(2-aminoethyl)sulfide (**13**) generated the corresponding carbamates (**6a,b**), which were subsequently nitrosated in anhydrous condition, to yield the target structures (**3a,b**). Bis(2-aminoethyl)sulfide was prepared according to a literature procedure⁵⁴ (Scheme 17), from the commercially available hydrobromide salt of 2-bromoethylamine (**14**).

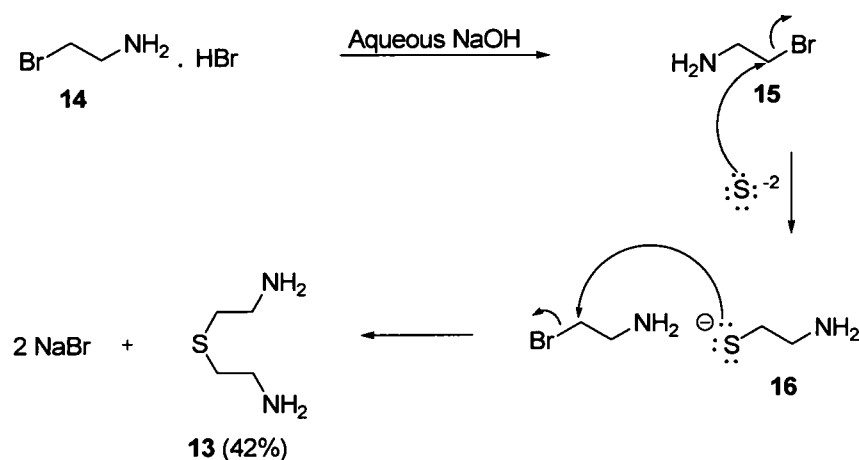
Scheme 16



A. Preparation of bis(2-aminoethyl)sulfide (13**):** Bis(2-aminoethyl)sulfide was prepared according to the literature procedure⁵⁴, starting from the hydrobromide salt of 2-bromoethylamine (**14**). The hydrobromide salt of 2-bromoethylamine was neutralized with aqueous sodium hydroxide to yield 2-

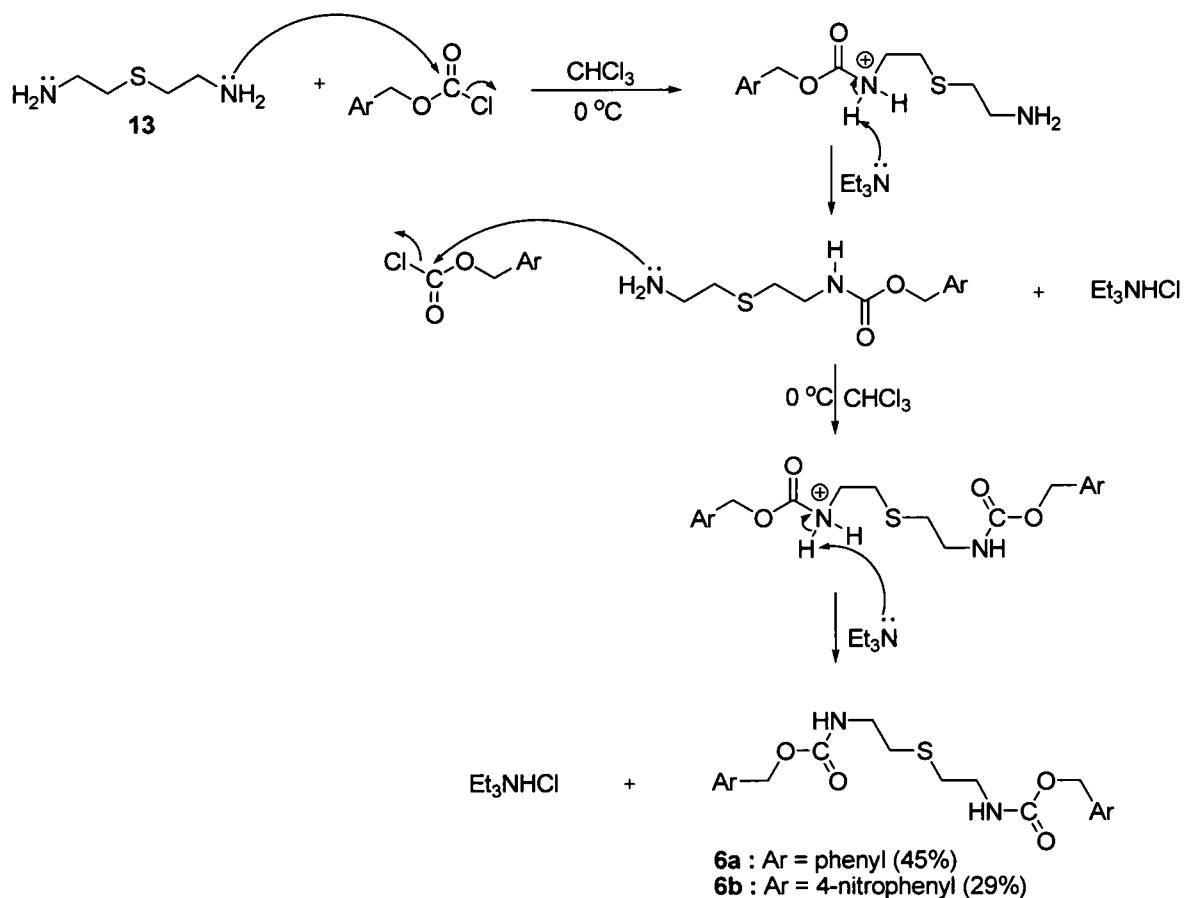
bromoethylamine **15**. The sulfide ion, present in the aqueous solution of sodium sulfide, reacts with 2-bromoethanamine to yield 2-aminoethanethiolate (**16**). The latter attacks another molecule of 2-bromoethanamine to yield bis(2-aminoethyl)sulfide (**13**) and sodium bromide (Scheme 17).

Scheme 17



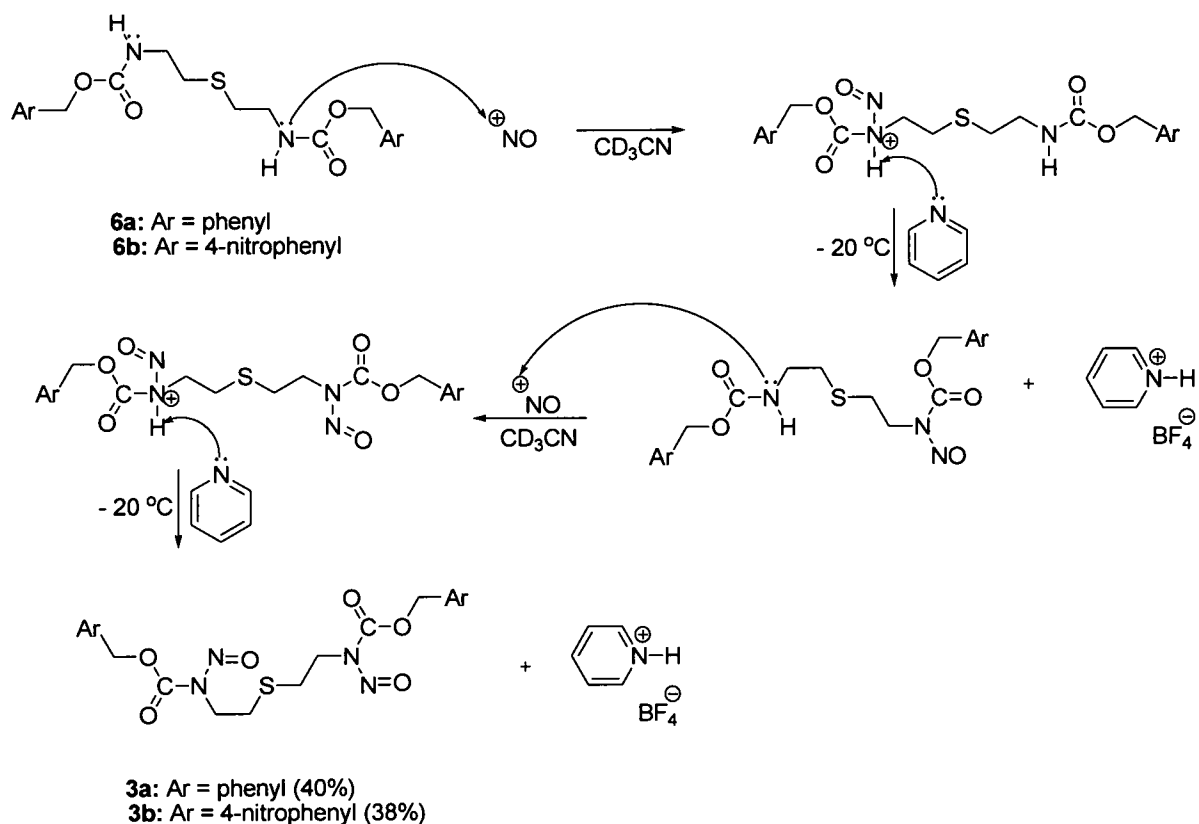
B. Preparation of bis[2-(benzyloxycarbonylamino)ethyl]sulfide (6a) and bis[2-(4-nitrobenzyloxycarbonylamino)ethyl]sulfide (6b): The synthetic procedure used for the preparation of these structures is similar to the protocol outlined in Scheme 10. The carbamates were generated by adding the corresponding chloroformates (**9a,b**) to a solution of bis(2-aminoethyl)sulfide (**13**) in chloroform, at 0 °C under nitrogen. The process involves a sequence of two substitution – deprotonation cycles (Scheme 18).

Scheme 18



C. Preparation of bis[2-(benzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide (3a) and bis[2-(4-nitrobenzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide (3b): Nitrosation reactions were done using the methodology outlined in Scheme 9. Solid nitrosonium tetrafluoroborate (NOBF_4) was used as the nitrosating agent in a stirred mixture of the corresponding carbamate (**6a,b**) and pyridine, in anhydrous acetonitrile, at $-20\text{ }^\circ\text{C}$ (Scheme 19).

Scheme 19



III. PHOTOCHEMICAL STUDIES

A. Photochemical studies of benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate: The photochemical studies were done on a mg scale, using quartz NMR tubes. Irradiations were performed using a Rayonet photochemical reactor and the results were monitored by using a 300MHz Bruker FT-NMR. The pattern of photolytic degradation at 250 nm is showed in Figure 6. Studies at 350nm were also done and found that the degradation follows a similar pattern

and yield the same products. We observed that the rate of degradation was faster at 250 nm when compared to degradation at 350 nm.

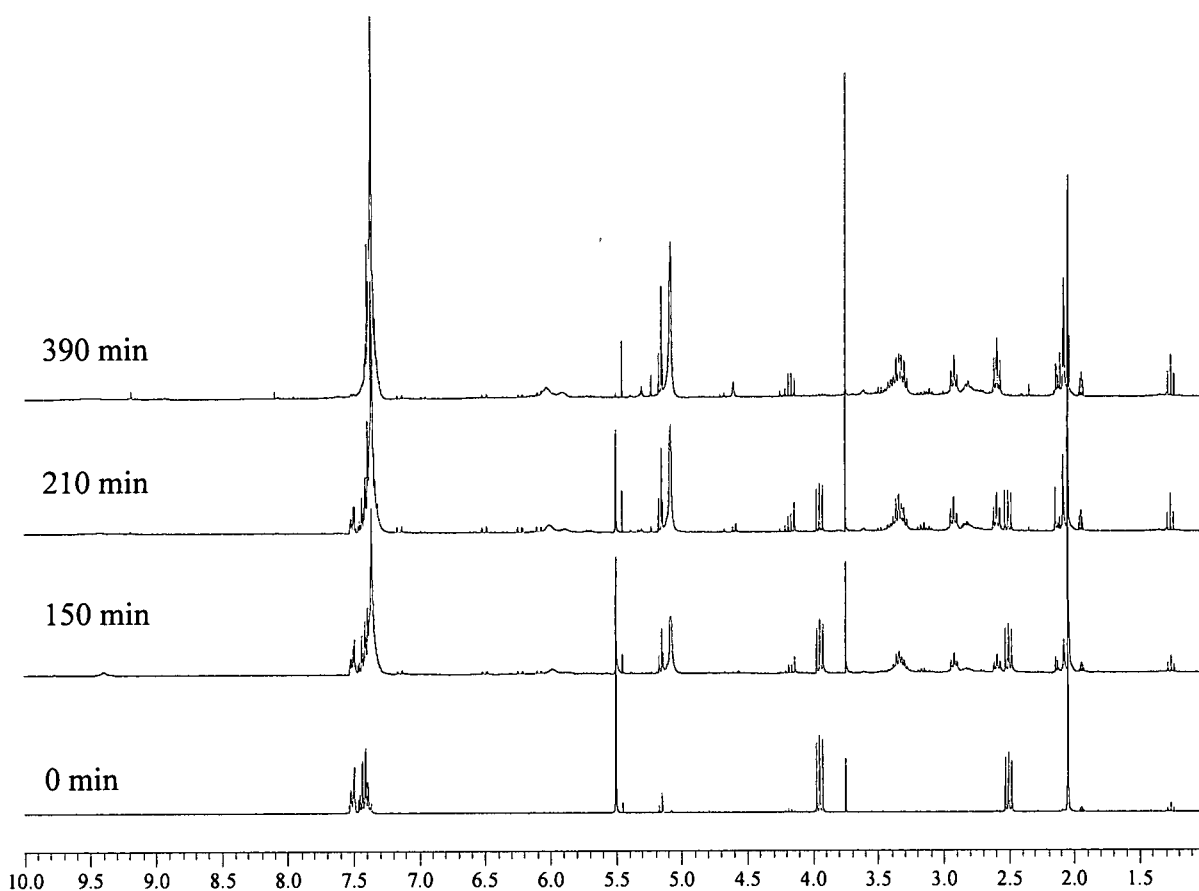


Figure 6: Stacked plot of ^1H NMR spectra of the reaction mixture from photolytic degradation of benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate upon irradiation at 250 nm.

The mixture obtained by photolysis was separated by column chromatography, yielding three fractions.

FRACTION 1: The fastest moving spot on TLC, colorless oil.

The peaks in ^1H NMR spectrum at δ 2.57 and 3.28 ppm with integral intensities 1.9 and 2.0 suggest the presence of two adjacent $-\text{CH}_2$ groups and the presence of singlet at δ 5.05 ppm with an integral intensity of 2.0 indicates the presence of a benzylic $-\text{CH}_2$ group. The singlet at δ 2.07 ppm, with an integral intensity of 3.0, most likely belongs to the methyl group attached to sulfur. The peaks at δ 7.28 – 7.38 ppm are assigned to the aromatic protons. All the peaks in ^1H NMR spectrum are consistent with the peaks in ^{13}C NMR spectrum. The appearance of a broad peak at δ 5.76 ppm showed that the irradiation caused denitrosation of the starting structure leading to formation of an $-\text{NH}$ group i.e. formation of benzyl N-2-(methylthio)ethylcarbamate.

FRACTION 2: The second fastest moving spot on TLC, yellow oil. The NMR spectra of this fraction were not clear. It seems like the fraction was a mixture of products. Further analysis of this fraction was not possible as the quantity was very small ($< 5\text{mg}$).

FRACTION 3: The slowest moving spot on TLC, white solid, major degradation product. The ^1H NMR (Figure 7) and ^{13}C NMR spectra (Figure 8) are shown below.

Figure 7 compares the ^1H NMR spectrum of the Fraction 3 with that of the starting N-nitrosocarbamate. The presence of peaks at δ 2.90 and 3.31 ppm with integral intensities 1.9 and 2.0 respectively suggest the presence of two adjacent

–CH₂ groups in the unknown product. The singlet at δ 5.06 ppm with an integral intensity 2.0 suggest the presence of benzylic –CH₂ group. The appearance of a broad peak in ¹H NMR spectrum at δ 5.86 ppm with an integral intensity 0.7 suggest that the irradiation caused denitrosation of the starting structure, leading to formation of an -NH group. The peak at δ 7.30 – 7.37 ppm with an integral intensity 5.7 are assigned to the aromatic protons.

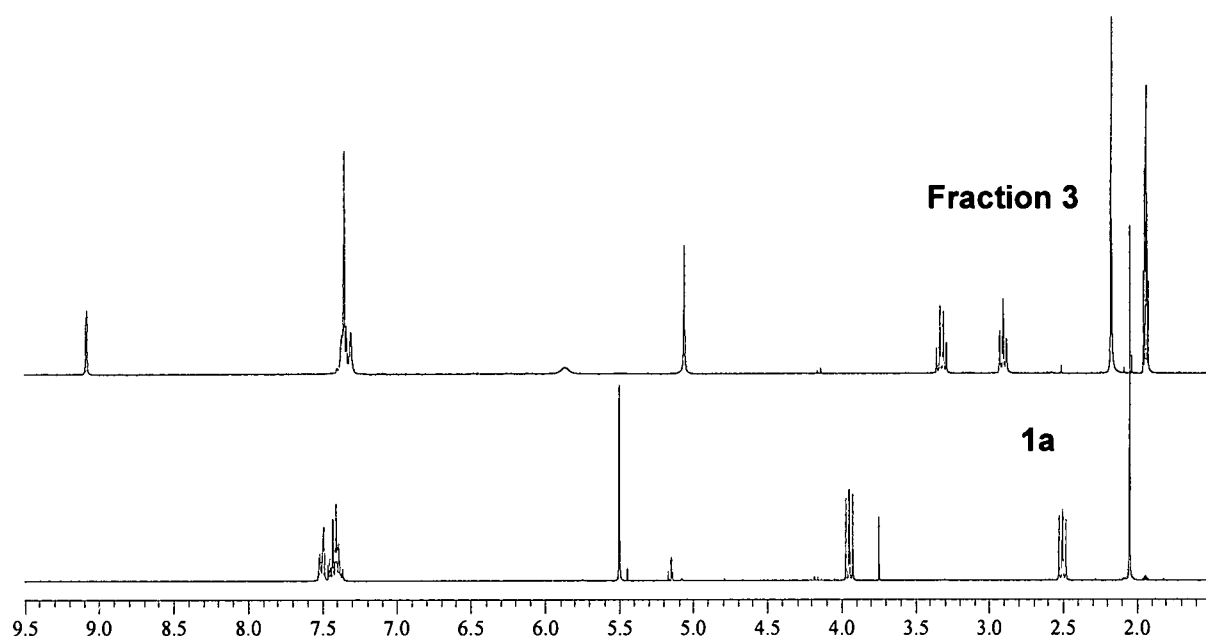


Figure 7: ¹H NMR Spectrum of Fraction 3 compared to starting structure **1a**

Based on the pattern of peaks in the ¹H NMR spectrum, we suggest that the photolytic degradation product contains two adjacent –CH₂ groups, an -NH group, and a benzyl group. Noticeable is the disappearance of the peak for the methyl group attached to sulfur and appearance of a new singlet at δ 9.08 ppm with an integral intensity of 0.91. By comparing integral intensities of all other

peaks, we suggest that the peak at δ 9.08 ppm corresponds to a single proton, part of yet unknown substructure. Some possibilities are groups such as, $-\text{HC}=\text{O}$ or $-\text{HC}=\text{N}-$.

In the ^{13}C NMR spectrum (Figure 8), we noticed the peaks corresponding to two $-\text{CH}_2$ groups at δ 30.6 and 41.0 ppm, peak at δ 70.1 ppm that corresponds to benzyl CH_2 group, peaks at δ 126.0 - 136.9 ppm corresponding to aromatic group (i.e. benzene ring) and a peak at δ 156.0 ppm that corresponds to a carbonyl carbon. We also observed the disappearance of the peak for the methyl group attached to sulfur ($\text{S}-\text{CH}_3$) at δ 15.3 ppm and appearance of an additional peak at δ 145.3 ppm.

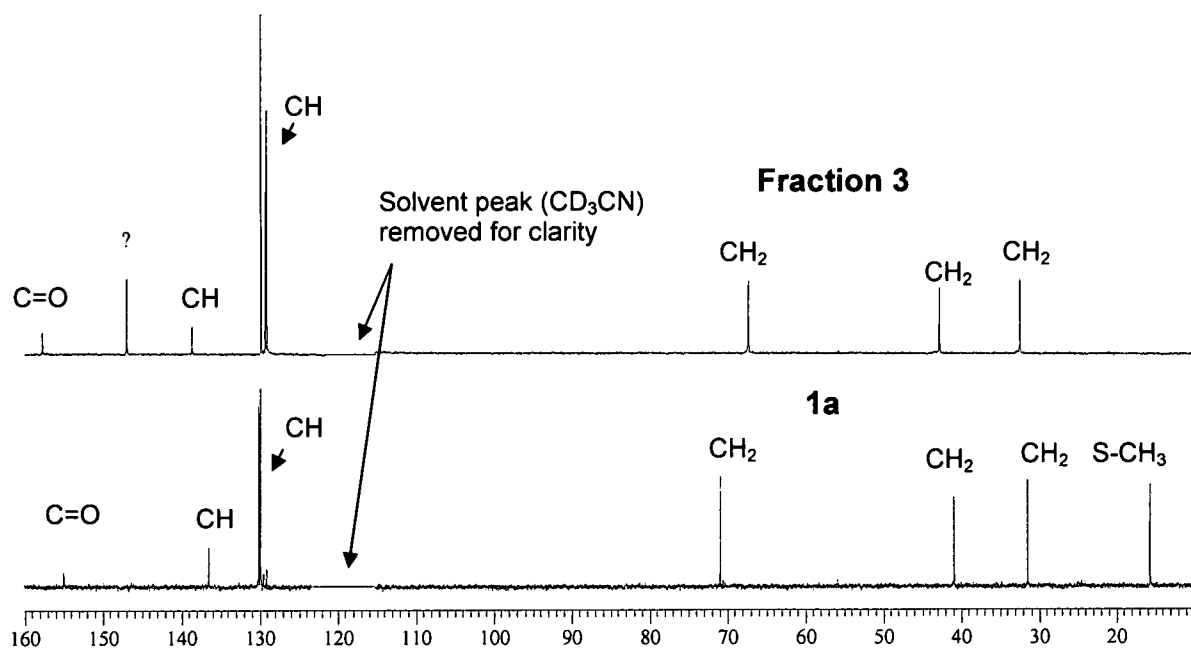


Figure 8: ^{13}C NMR spectrum of Fraction 3 compared to benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate, (**1a**).

Based on the above NMR data, we propose a structure for the decomposition product as shown in Figure 9. The HRMS data gave a molecular weight for the unknown fraction of 277.0755 a.u. The molecular weight of the known portion is 178.09. The unknown part therefore has approximate mass of 99 a.u.

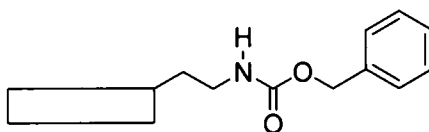
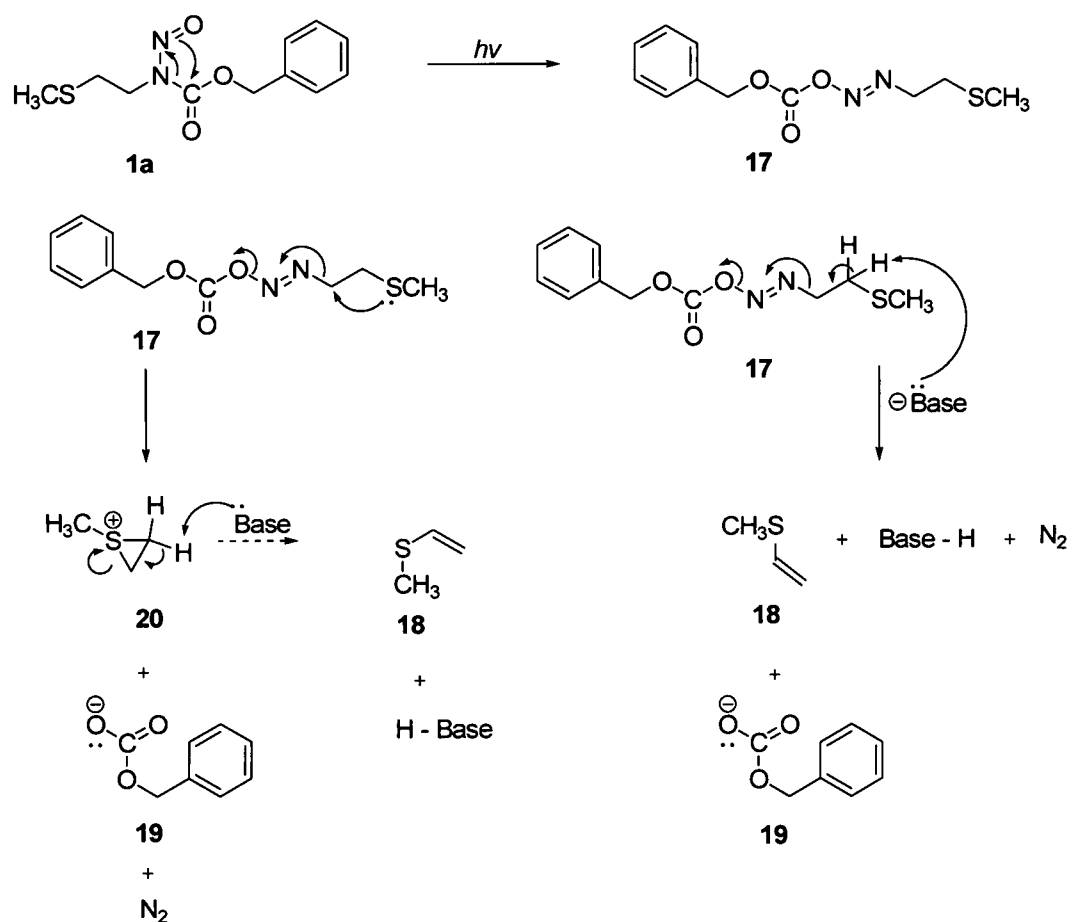


Figure 9: Suggested structure for the decomposition product in fraction 1

Although we could not isolate it as a fraction, the pattern of peaks in the ^1H NMR spectrum of the crude photolysis mixture at δ 6.00 - 6.60 ppm (Figure 6) suggest the presence of one more decomposition product with a monosubstituted C=C bond. We propose the formation of methyl vinyl sulfide (**18**). Two mechanisms for generation of the latter can be proposed, as shown in Scheme 20. **Mechanism 1:** Conversion of benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate to benzyloxycarbonyl diazotate (**17**), which could occur as a photochemically allowed concerted four-electron transfer process. Rearrangement of the latter, followed by fragmentation yield 1-methylthiiranium cation (**20**), which upon deprotonation by the base would yield methyl vinyl sulfide (**18**), nitrogen and benzyl carbonate anion (**19**). **Mechanism 2:** Deprotonation of benzyloxycarbonyl diazotate (**17**), followed by β -elimination would yield methyl vinyl sulfide (**18**).

Scheme 20



Overall, based on NMR spectral analysis, we have identified several decomposition products, which are listed in Figure 10.

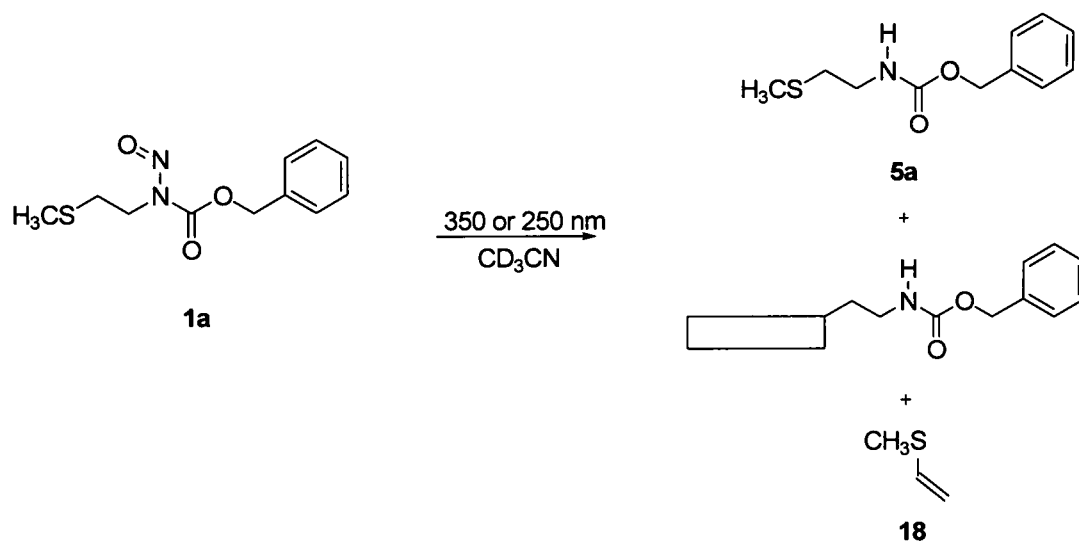


Figure 10: List of possible photolytic degradation products of benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (**1a**).

B. Photolytic Studies of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (1b): The photochemical studies were done on an NMR scale, using quartz NMR tubes. Irradiations were performed using a Rayonet photochemical reactor and monitoring of the results was done by NMR measurements, using 300 MHz Bruker FT-NMR. NMR spectra of the photolytic degradation mixture at 250 nm are shown in Figure 11. Studies at 350 nm were also done and found that the degradation follows a similar pattern and yield the same products. We observed that the rate of degradation was faster at 250 nm compared to degradation at 350 nm.

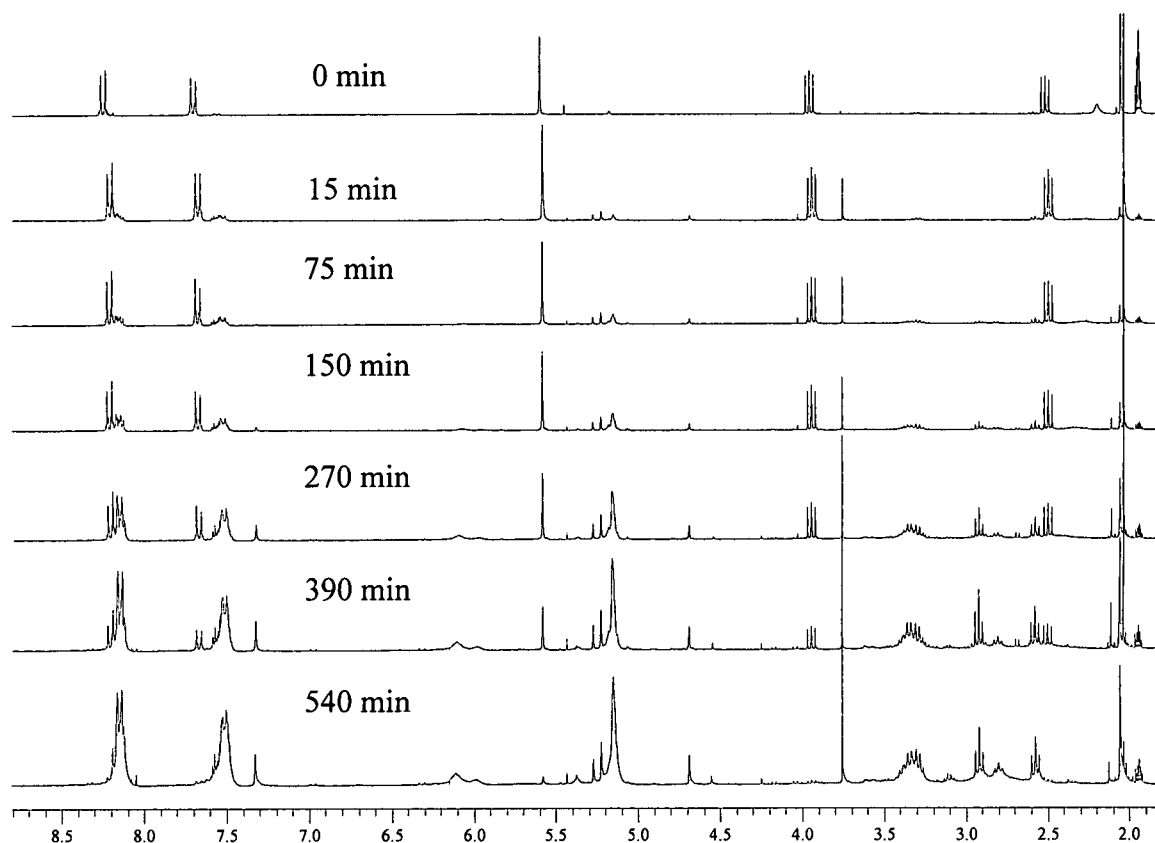


Figure 11: Stacked plot of ^1H NMR spectra of the reaction mixture from photolytic degradation of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate upon irradiation at 250 nm.

The compounds obtained by photolysis were separated by column chromatography. We isolated three major fractions from the chromatographic separation. The reported yields are probably lower as the reactions were run on a small scale and also because of some difficulties with the separation of the recognized products from one another and from minor by-products. All three major fractions were subjected to NMR analysis.

FRACTION 1: Fastest moving spot on TLC. White solid. Mp 76 – 82 °C. The ^1H NMR and ^{13}C NMR spectra are given below (Figure 12).

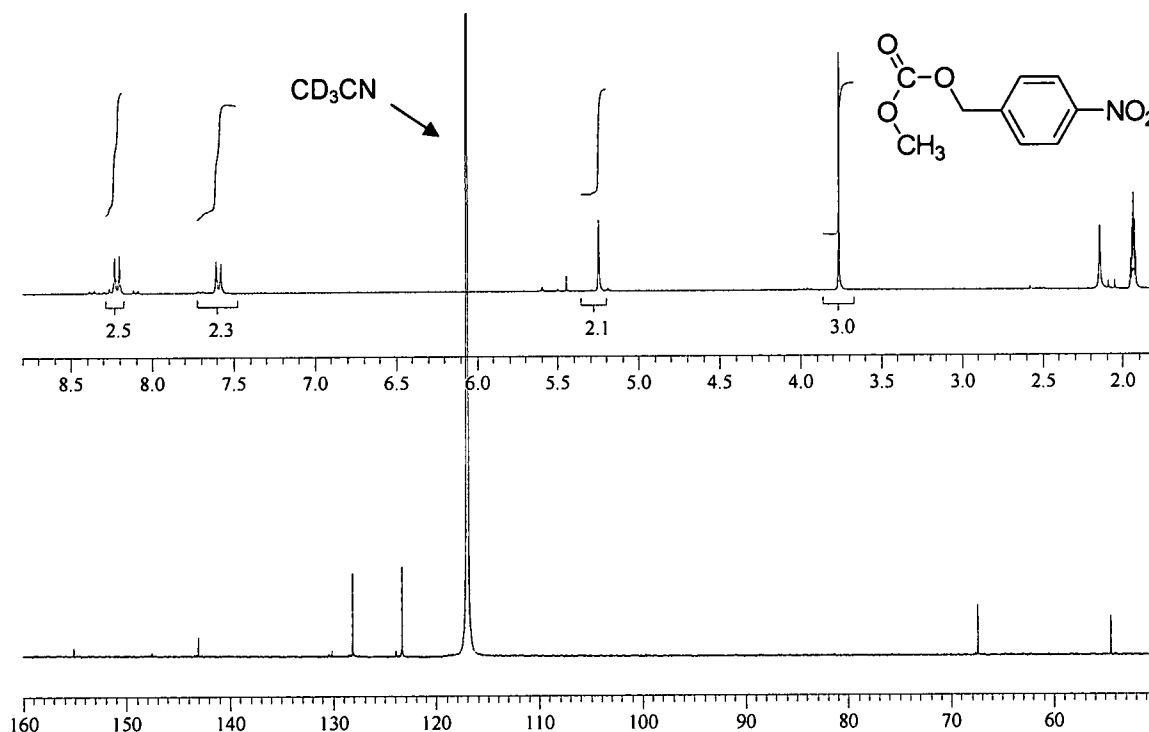
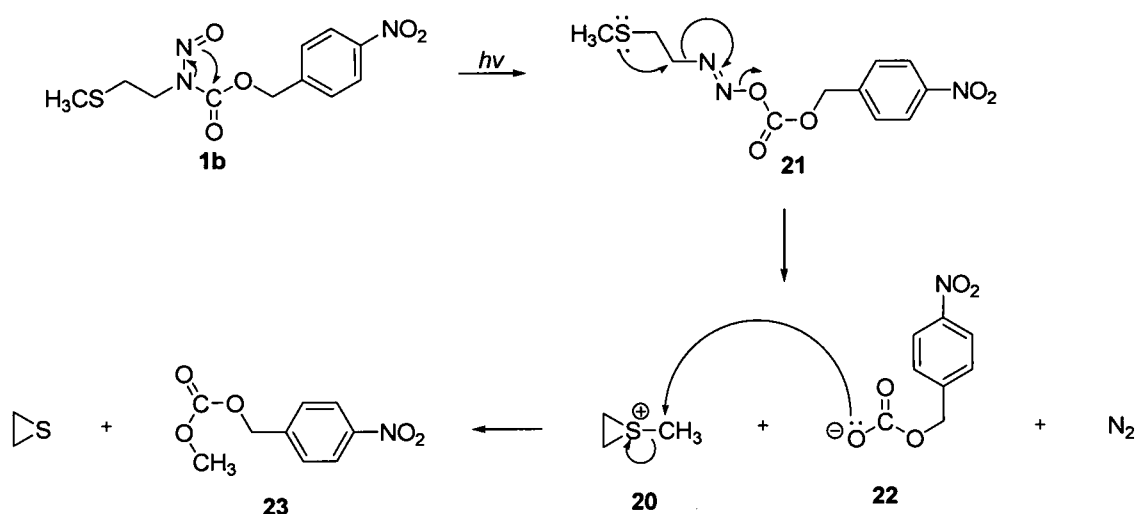


Figure 12: ^1H - and ^{13}C -NMR spectra of methyl 4-nitrobenzyl carbonate

In the ^1H NMR spectrum, we observed two doublets at δ 7.58 and 8.22 ppm with integral intensities 2.1 and 2.5 respectively in the aromatic region, which most likely belong to the protons of the 4-nitrobenzyl group. A singlet with an integral intensity of 3.0 at δ 3.76 ppm corresponds probably to a $-\text{CH}_3$ group attached to O-center. A singlet at δ 5.24 ppm, with an integral intensity of 2.1, can be assigned to a benzylic $-\text{CH}_2$ group.

In the ^{13}C NMR spectrum, we noticed the peak at δ 56.1 ppm corresponding to the carbon of $-\text{OCH}_3$ group, and peak at δ 69.1 ppm that corresponds to benzyl CH_2 group, peaks at δ 125.0, 128.6, 144.7, 149.1 ppm corresponding to aromatic group (i.e. 4-nitrobenzene ring) and a peak at δ 156.7 ppm that corresponds to a carbonyl carbon.

Scheme 21



Based on the above NMR spectral analysis and literature reference⁵⁵ we suggest that Fraction 1 is methyl 4-nitrobenzyl carbonate (23). The proposed mechanism for the formation of the latter is shown in Scheme 21. Conversion of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate to 4-nitrobenzyloxycarbonyl diazotate (21), which could occur as a photochemically allowed concerted four-electron transfer process, is followed by fragmentation to yield N_2 , 4-nitrobenzyl carbonate anion (22) and 1-methylthiiranium cation (20). The 4-

nitrobenzyl carbonate anion is subsequently methylated by the 1-methylthiiranium cation to yield thiirane and methyl 4-nitrobenzyl carbonate (**23**).

FRACTION 2: The second fastest moving spot on TLC. Off-white solid. Mp 146 - 152 °C. The ^1H NMR and ^{13}C NMR spectra are shown in Figure 13.

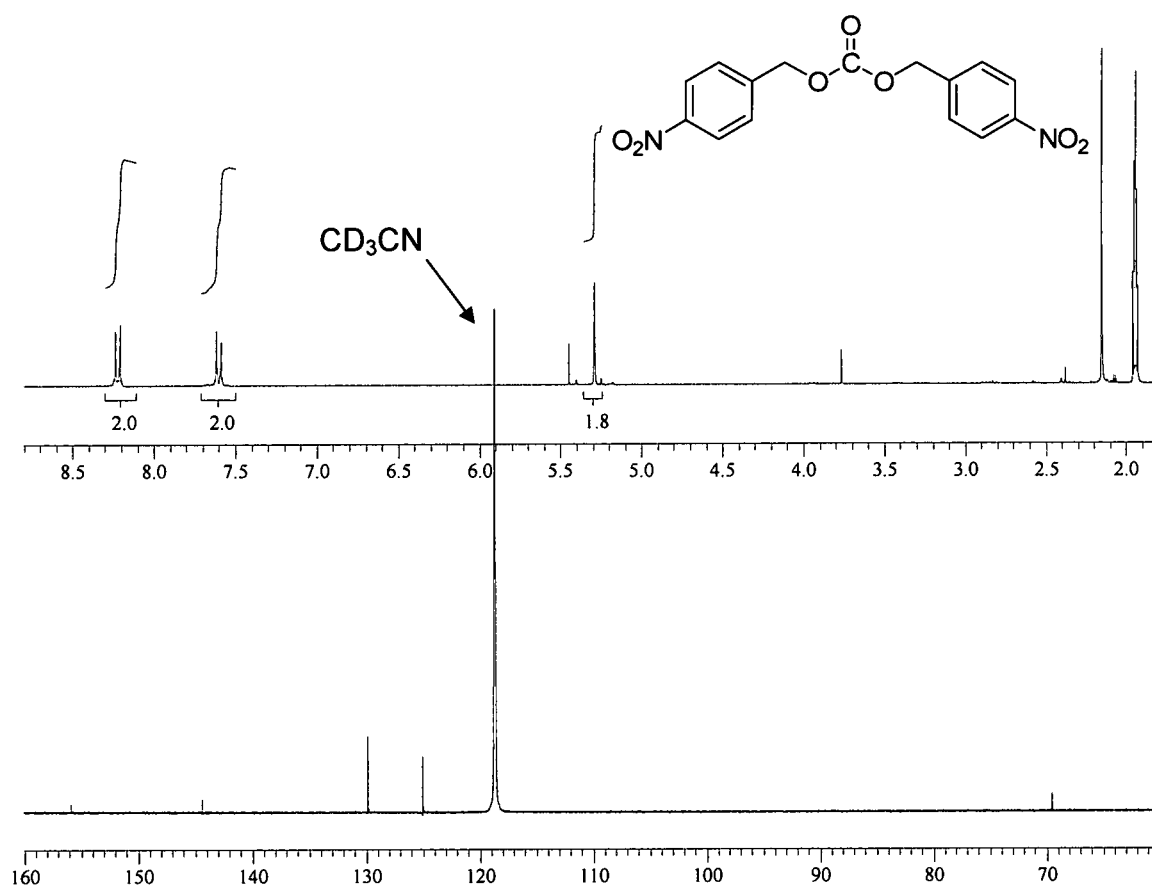


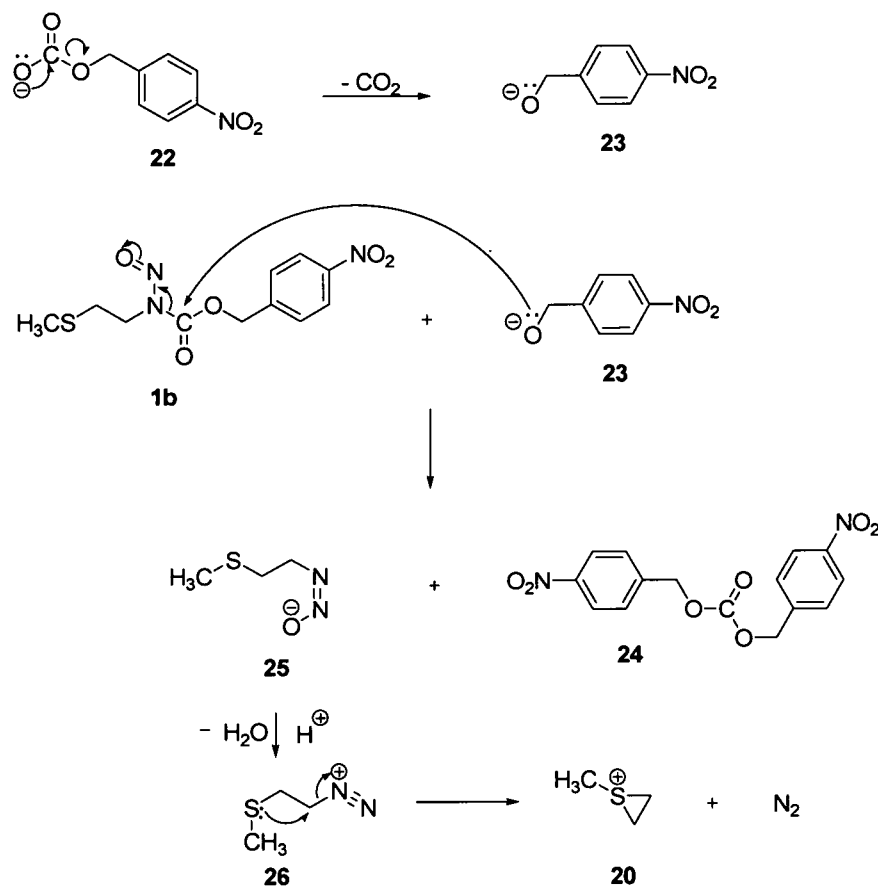
Figure 13: ^1H and ^{13}C NMR spectra of 4-nitrobenzyl carbonate

^1H NMR spectrum shows two doublets at δ 7.58 and 8.22 ppm with integral intensities 2.0 and 2.0 respectively, which we assign to the protons of the

4-nitrobenzyl group. A singlet at δ 5.28 ppm with an integral intensity of 2.0, is assigned to the benzylic $-\text{CH}_2$ group. The peaks in ^{13}C NMR spectrum are consistent with the peaks in ^1H NMR spectrum. We noticed the peak at δ 69.5 ppm that corresponds to the benzyl CH_2 group, peaks at δ 125.0, 128.6, 144.7, 149.1 ppm corresponding to aromatic group (i.e. 4-nitrobenzene ring) and a peak at δ 155.8 ppm that corresponds to a carbonyl carbon.

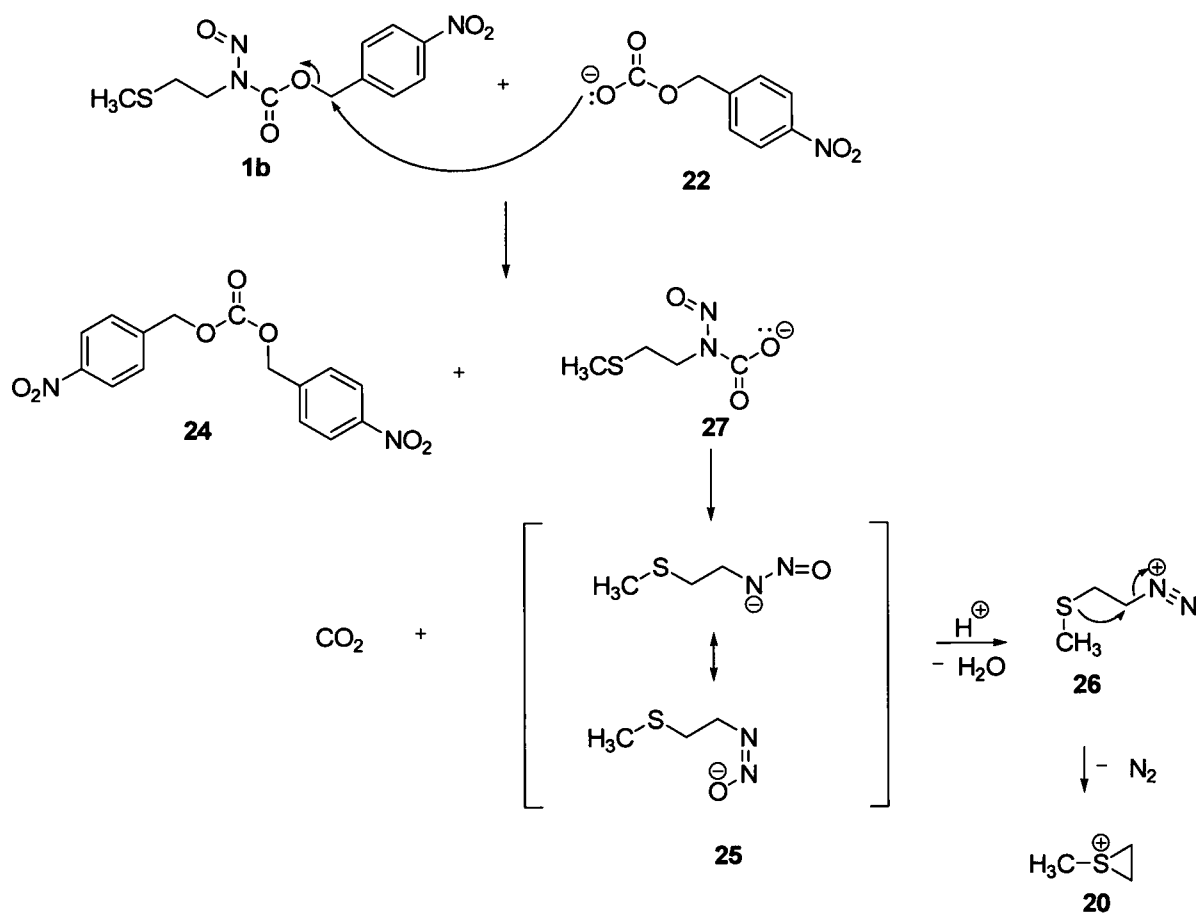
Based on the above NMR spectral analysis and literature reference⁵⁶ we suggest that Fraction 2 is 4-nitrobenzyl carbonate (**24**). The suggested mechanism for the formation of the latter is shown in Scheme 22.

Scheme 22



Decarboxylation of 4-nitrobenzyl carbonate anion (**22**) formed as shown in Scheme 21, would yield 4-nitrobenzyl oxide anion (**23**). The anion acts as a nucleophile and attacks the carbonyl carbon of **1b** to yield 4-nitrobenzyl carbonate (**24**) and the diazotate anion (**25**). Protonation of the latter and loss of water would lead to generation of 2-(methylthio)ethyl diazonium cation (**26**), which, upon loss of nitrogen, would yield the 1-methylthiranium cation (**20**).

Scheme 23



Alternatively, the 4-nitrobenzyl carbonate anion (**22**), formed as shown in Scheme 21, acts as a nucleophile and attacks the benzylic carbon of **1b** to yield 4-nitrobenzyl carbonate (**24**) and a 2-(methylthio)ethyl-N-nitrosocarbamate anion (**27**). Decarboxylation of the latter yield the diazotate anion **25**, whose fragmentation would lead again to the formation the 1-methylthiranium cation (**20**) (Scheme 23).

FRACTION 3: The slowest moving spot on TLC. White solid. Mp 57 - 60 °C.

The ^1H NMR and ^{13}C NMR spectra are shown in Figure 14.

The peaks in ^1H NMR spectrum at δ 2.58 and 3.35 ppm with integral intensities 2.0 and 1.9 suggest the presence of two adjacent $-\text{CH}_2$ groups and the presence of singlet at δ 5.24 ppm with an integral intensity of 2.0 indicates the presence of a benzylic $-\text{CH}_2$ group. The singlet at δ 2.10 ppm, with an integral intensity of 3.0, most likely belongs to the methyl group attached to sulfur. The appearance of a broad peak at δ 5.91 ppm showed that the irradiation caused denitrosation of the starting structure leading to formation of an $-\text{NH}$ group i.e. formation of 4-nitrobenzyl N-2-(methylthio)ethylcarbamate (**2b**). All the peaks in ^1H NMR spectrum are consistent with the peaks in ^{13}C NMR spectrum.

In the ^{13}C NMR spectrum, we noticed the peaks corresponding to two $-\text{CH}_2$ groups at δ 34.7 and 41.0 ppm, the peak at δ 65.9 ppm corresponds to benzyl CH_2 group, peaks at δ 124.9, 129.2, 146.4, 148.8 ppm corresponding to

aromatic group (i.e. 4-nitrobenzene ring), a peak at δ 157.3 ppm that corresponds to a carbonyl carbon and a peak at δ 15.5 ppm assigned to the carbon of the methyl group attached to sulfur (S-CH₃).

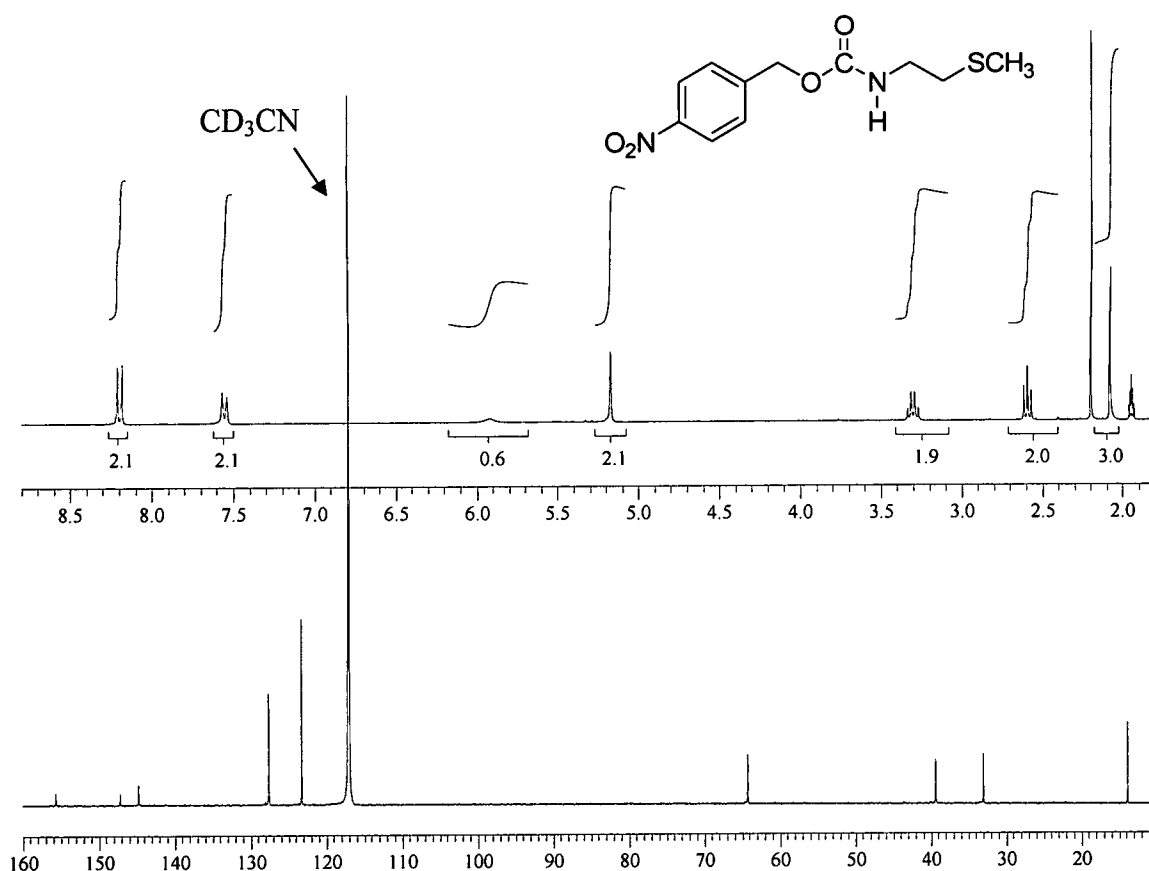


Figure 14: ¹H and ¹³C NMR spectra of 4-nitrobenzyl N-2-(methylthio)ethylcarbamate.

In this case too, we could not isolate the fraction with peaks in the ¹H NMR spectrum of the crude mixture at δ 6.35 – 7.00 ppm. Similarly, we assign them to methyl vinyl sulfide (**18**).

Overall, based on NMR spectral analysis and literature references we have identified several possible products from photolytic degradation of **1b**. Figure 15 outlines those products. Their percent yields at 250 nm and 350 nm are shown in Table 1.

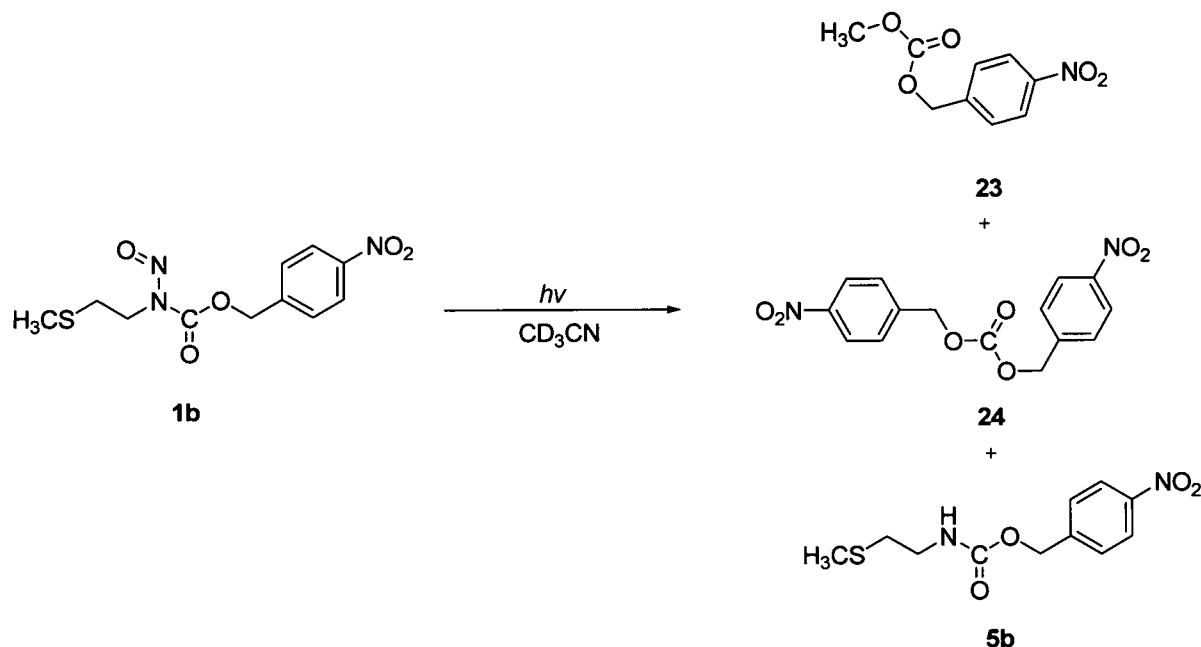


Figure 15: List of products from photolytic degradation of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate.

Table 1: Photolytic decomposition products of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate and their percent yields at 250 nm and 350 nm

Product	% Yield	
	250 nm	350 nm
Methyl 4-nitrobenzyl carbonate	17	18
4-Nitrobenzyl carbonate	21	14
4-Nitrobenzyl 2-(methylthio)ethylcarbonate	25	20

IV. CONCLUSIONS FROM THE PHOTOLYSIS EXPERIMENTS:

We have synthesized several target structures and examined the photodecomposition of structures **1a** and **1b**. The photolytic data on **1a** (partially) and **1b**, and the suggested mechanisms for the formation of photodecomposition products, provide substantial indirect support for our original hypothesis, namely the formation of 1-methylthiiranium cation upon irradiation. Based on this we conclude that the studied structures hold considerable promise as potential photolabile alkylating agents.

CHAPTER 3

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra of intermediate and target compounds were recorded at 300 MHz and 75 MHz respectively and referenced to the solvent (CDCl_3 : 7.27 ppm and 77.0 ppm; acetonitrile- d_3 : 1.93 ppm and 118.6 ppm). Photochemical studies were conducted in a Rayonet photochemical reactor, using quartz NMR tubes. Elemental analysis was provided by Atlantic Microlab, Norcross, GA. HRMS data was provided by the Mass Spectrometry and Proteomics facility at the Ohio State University. Nitrosonium tetrafluoroborate was purchased from the Lancaster chemical company. 2-Aminoethanethiol hydrochloride and 4-nitrobenzyl chloroformate were purchased from Acros Organics.

2-(Methylthio)ethylamine (8): A solution of cysteamine hydrochloride (5.02 g, 44.01 mmol) in methanol (10 mL) was added very slowly to sodium methoxide (4.75 g, 88.02 mmol, 16.48 mL (30% weight)) for about 30 min, under nitrogen. The mixture was kept stirring for 10 min in an ice bath. Iodomethane (6.24 g, 44.01 mmol, 2.73 mL) was added dropwise over a period of one hour. The mixture was stirred at room temperature for 24 hrs, kept in the freezer for 15

min, and vacuum filtered. The filtrate was distilled at 85 °C (30 mm Hg), using a Vigreux column to obtain the product as a light yellow liquid. Yield: 1.18 g (30%).

^1H NMR (CDCl_3) δ 1.75 (broad s, 2H), 2.06 (s, 3H), 2.56 (t, J = 6.4 Hz, 2H), 2.85 (t, J = 6.3 Hz, 2H).

Benzyl N-2-(methylthio)ethylcarbamate (4a): A solution of 2-(methylthio)ethylamine (2.90 g, 31.83 mmol) in ether (20 mL) was stirred for 5 min and then methylene chloride (5 mL) was added. Triethylamine (3.22 g, 31.83 mmol, 4.43 mL) was added and the solution stirred for 10 min at 0 °C under nitrogen. Benzyl chloroformate (5.43 g, 31.83 mmol, 4.54 mL) in ether (10 mL) was added dropwise to the mixture under nitrogen for about 30 min. The mixture was stirred for 24 hrs, vacuum filtered and the solvent evaporated under reduced pressure. Based on thin layer chromatography, the residue was separated by passing through a short silica gel filter (hexane : methylene chloride = 1 : 1 and then pure methylene chloride) to obtain the product as a colorless oily liquid. Yield : 2.81 g (42%).

^1H NMR (CDCl_3) δ 2.14 (s, 3H) , 2.62 (t, J = 6.3 Hz, 2H) , 3.38 (m, 2H), 5.15(s, 2H), 5.28 (broad s, 1H) 7.30 - 7.38 (m, 5H). ^{13}C NMR (CDCl_3) δ 15.3, 30.4, 39.4, 69.8, 128.5, 128.7, 128.9, 134.4, 153.7; HRMS (FAB^+) m/z Calcd. for $\text{C}_{11}\text{H}_{15}\text{NO}_2\text{S}$ [$\text{M}+\text{Na}$] $^+$ 248.0721, found 248.0721.

Benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (1a): AQUEOUS METHOD: A mixture of sodium nitrite (1.37 g, 19.92 mmol) in water (4 mL) was added to a mixture of benzyl N-2-(methylthio)ethylcarbamate (0.47 g, 2.22 mmol)

in ether (4 mL). Without stirring or cooling, nitric acid (2.66 mL, 35%) was added directly to the lower layer over one hour. Then 8 mL of water and 8 mL of ether were added, the organic layer was separated, dried (MgSO_4) and the solvent removed under reduced pressure to yield the corresponding benzyl N-2-(methylthio)ethyl N-nitrosocarbamate. Further purification was done by passing the mixture through a short silica gel filter (hexane : methylene chloride = 1 : 1). The solvent was evaporated to obtain the product as a light yellow liquid. Yield: 0.53 g (20%).

^1H NMR (CDCl_3) δ 2.10 (s, 3H), 2.50 (t, J = 6.3 Hz, 2H) , 3.95 (m, 2H), 5.52 (s, 2H), 7.38 - 7.47 (m, 5H).

ANHYDROUS METHOD: Solid nitrosonium tetrafluoroborate (NOBF_4) (0.82 g, 7.09 mmol) was added in one portion to a stirred mixture of Benzyl N-2-(methylthio)ethylcarbamate (1.00 g, 4.73 mmol) and anhydrous pyridine (0.56 g, 7.09 mmol, 0.57 mL) in anhydrous acetonitrile (10 mL) at $-20\text{ }^\circ\text{C}$. The mixture was stirred at $0\text{ }^\circ\text{C}$ under nitrogen for 2 hours and the progress of the reaction was observed by thin layer chromatography (hexane : methylene chloride = 1 : 1). The product exhibited a rapidly moving yellow spot. The solvent was removed under reduced pressure and the product was purified by passing the residue through a short silica gel filter (hexane : methylene chloride = 1 : 1). The solvent was removed under reduced pressure to obtain the product as a light yellow liquid. Yield : 0.49 g (43%).

^1H NMR (CDCl_3) δ 2.10 (s, 3H) , 2.46 (t, J = 6.3 Hz, 2H) , 3.95 (m, 2H), 5.52 (s, 2H), 7.35 - 7.41 (m, 5H). ^{13}C NMR (CDCl_3) δ 15.3, 30.4, 39.4, 69.8, 128.5,

128.7, 128.9, 134.4, 153.7; HRMS (FAB⁺) m/z Calcd. for C₁₁H₁₄N₂O₃S [M+Na]⁺ 277.0623, found 277.0628.

4-Nitrobenzyl N-2-(methylthio)ethylcarbamate (4b): A mixture of 2-(methylthio)ethylamine (0.20 g, 2.19 mmol) in ether (20 mL) was stirred for 5 min, followed by addition of methylene chloride (5 mL). Triethylamine (0.22 g, 2.19 mmol, 0.30 mL) was added and the mixture stirred for 10 min at 0 °C under nitrogen. A solution of 4-nitrobenzyl chloroformate (0.47 g, 2.19 mmol) in ether (10 mL) was added dropwise under nitrogen for about 30 min. The mixture was stirred for 24 hrs, filtered under vacuum and the solvent evaporated under reduced pressure. The 4-nitrobenzyl N-2-(methylthio)ethylcarbamate was separated using a short silica gel filter (hexane : ethyl acetate = 2 : 1). Solvent was removed under reduced pressure to obtain the product as a light yellow solid. Yield: 0.21 g (36%). Mp 57 – 60 °C.

¹H NMR (CDCl₃) δ 2.12 (s, 3H) , 2.68 (t, J = 6.3 Hz, 2H) , 3.42 – 3.44 (t, J = 6.3 Hz, 2H), 5.18 (s, 2H), 5.21 (broad s, 1H), 7.53 (d, J = 8.7 Hz, 2H), 8.24 (d, J = 8.7 Hz, 2H). ¹³C NMR (CD₃CN) δ 15.5, 34.7, 41.0, 65.9, 124.9, 129.2, 146.4, 148.8, 157.3; Anal. Calcd. for C₁₁H₁₄N₂O₄S: C, 48.88; H, 5.22; N, 10.36. Found: C, 49.07; H, 5.31; N, 10.19.

4-Nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (1b): Solid nitrosonium tetrafluoroborate (NOBF₄) (0.14 g, 1.25 mmol) was added in one portion to a stirred mixture of 4-nitrobenzyl N-2-(methylthio)ethylcarbamate (0.17 g, 0.62 mmol) and anhydrous pyridine (0.09 g, 1.25 mmol, 0.10 mL) in anhydrous acetonitrile (10 mL) at –20 °C. The mixture was stirred at 0 °C under nitrogen for

2 hours and the progress of the reaction was observed by thin layer chromatography (hexane : ethyl acetate = 1 : 2). The product exhibited a rapidly moving yellow spot. The solvent was removed under reduced pressure and the residue was purified by passing through a short silica gel filter (hexane : ethyl acetate = 1 : 2). The solvent was removed under reduced pressure to give the product as light yellow liquid. Yield: 0.07 g (38%).

^1H NMR (CD_3CN) δ 2.11 (s, 3H) , 2.51 (t, J = 6.3 Hz, 2H) , 3.95 (t, J = 6.3 Hz, 2H), 5.61 (s, 2H), 7.67 (d, J = 8.7 Hz, 2H) , 8.29 (d, J = 8.7 Hz, 2H). ^{13}C NMR (CD_3CN) δ 15.6, 31.3, 40.9, 69.3, 125.0, 130.1, 143.8, 149.3, 154.8; Anal. Calcd. for $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_5\text{S}$: C, 48.88; H, 5.22; N, 10.36. Found: C, 49.07; H, 5.31; N, 10.19.

Benzyl 2-(N,N-dimethylamino)ethylcarbamate (5a): A mixture of 2-(N,N-dimethylethylenediamine (2.0 g, 22.70 mmol, 2.4 mL) in benzene (10 mL) was stirred for 5 min at 0 °C under nitrogen. A solution of benzyl chloroformate (3.86 g, 22.70 mmol, 3.22 mL) in benzene (10 mL) was added dropwise under nitrogen for about 30 min. The mixture was stirred for 24 hrs. The product, clumpy white precipitate, obtained after decanting the solvent, was treated with aqueous solution of potassium hydroxide, followed by extraction with ether (4 x 25 mL). The combined ether extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the product as light yellow oil. Yield: 1.63 g (33%).

^1H NMR (CDCl_3) δ 2.20 (s, 6H) , 2.37 (t, J = 6.3 Hz, 2H) , 3.24 (q, J = 6.3 Hz, 2H), 5.08 (s, 2H), 5.54 (broad s, 1H), 7.28 - 7.36 (m, 5H).

Benzyl 2-(N,N-dimethylamino)ethyl-N-nitrosocarbamate (2a): Solid nitrosonium tetrafluoroborate (NOBF₄) (0.26 g, 2.25 mmol) was added in one portion to a stirred mixture of benzyl 2-(N,N-dimethylamino)ethylcarbamate (0.50 g, 2.25 mmol) and anhydrous acetonitrile (15 mL) at -20 °C. The mixture was stirred at 0 °C under nitrogen for 4 hours. The solvent was then distilled out at room temperature to obtain the product as a yellowish white solid salt. Yield: 0.60 g (78%).

¹H NMR (CD₃CN) δ 2.85 (s, 7H), 3.12 (q, J = 6.4 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 5.53 (s, 2H), 7.38 – 7.45 (m, 5H).

Bis(2-aminoethyl)sulfide (13): An aqueous solution (10 mL) of sodium hydroxide (1.95 g, 48.80 mmol) was added dropwise to a stirred aqueous solution (10 mL) of 2-bromoethylamine hydrobromide (10.00 g, 48.80 mmol). An aqueous solution (10 mL) of sodium sulfide (5.85 g, 24.40 mmol) was then added to above solution for about 30 min. The mixture was stirred at room temperature for 24 hrs. Most of the solvent was removed under reduced pressure and the white precipitate (NaBr) was removed by vacuum filtration. The filtrate was made strongly basic with aqueous potassium hydroxide and the yellow oil, which formed, was extracted with chloroform (4 x 25 mL). The combined chloroform extracts were dried (Na₂SO₄) and the solvent removed under reduced pressure, to obtain the product as brown oily liquid. Yield: 1.24 g (42%).

¹H NMR (CD₃CN) δ 1.21 (broad s, 4H), 2.43 (t, J = 6.5 Hz, 4H), 2.69 (t, J = 6.3 Hz, 4H).

Bis[2-(benzyloxycarbonylamino)ethyl]sulfide (6a): A solution of bis(2-amino ethyl)sulfide (0.50 g, 4.16 mmol) in chloroform (20 mL) was stirred for 5 min. Then triethylamine (0.84 g, 8.33 mmol, 1.16 mL) was added at once. The solution was stirred for 10 min at 0 °C and a solution of benzyl chloroformate (1.42 g, 8.33 mmol, 1.18 mL) in chloroform (10 mL) was added dropwise under nitrogen for about 30 min. The mixture was stirred for 24 hrs, vacuum filtered, and the solvent removed under reduced pressure. The residue was recrystallized from toluene to obtain the product as a white solid. Yield: 0.73 g (45%). Mp 91 – 95 °C.

¹H NMR (CD₃CN) δ 2.61 (t, J = 6.3 Hz, 4H) , 3.25 (q, J = 6.3 Hz, 4H), 5.04 (s, 4H), 5.81 (broad s, 2H), 7.28 - 7.36 (m, 10H). ¹³C NMR (CD₃CN) δ 32.5, 41.6, 67.2, 129.0, 129.2, 129.8, 138.7, 157.7; Anal. Calcd. for C₂₀H₂₄N₂O₄S: C, 61.83; H, 6.23; N, 7.21. Found: C, 61.73; H, 6.25; N, 7.33.

Bis[2-(4-nitrobenzyloxycarbonylamino)ethyl]sulfide (6b): A solution of bis(2-aminoethyl)sulfide (0.20 g, 1.66 mmol) in chloroform (10 mL) was stirred for 5 min. Triethylamine (0.33 g, 3.33 mmol, 0.46 mL) was added at once. The mixture was stirred at 0 °C for 10 min and then a solution of 4-nitrobenzyl chloroformate (0.71 g, 3.33 mmol) in chloroform (10 mL) was added dropwise under nitrogen for about 30 min. The mixture was stirred for 24 hrs, vacuum filtered, and the solvent removed under reduced pressure. The compound was purified by passing through a short silica gel filter (ethylacetate : hexane = 2 : 1). The product was recrystallized from toluene as a light yellow solid. Yield: 0.23 g (29%). Mp 108 – 111 °C.

^1H NMR (CD_3CN) δ 2.63 (t, J = 6.3 Hz, 4H), 3.29 (m, 4H), 5.16 (s, 4H), 5.94 (broad s, 2H), 7.54 (d, J = 8.7 Hz, 4H), 8.17 (d, J = 8.7 Hz, 4H). ^{13}C NMR (CD_3CN) δ 32.6, 41.6, 65.9, 124.9, 129.2, 146.6, 148.8, 157.4; Anal. Calcd. for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_8\text{S}$: C, 50.20; H, 4.63; N, 11.71. Found: C, 49.62; H, 4.73; N, 11.38.

Bis[2-(benzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide (3a): Solid nitrosonium tetrafluoroborate (NOBF_4) (0.44 g, 3.84 mmol) was added in one portion to a stirred mixture of bis[2-(benzyloxycarbonylamino)ethyl]sulfide (0.50 g, 1.28 mmol) and anhydrous pyridine (0.30 g, 3.84 mmol, 0.31 mL) in anhydrous acetonitrile (10 mL) at -20°C . The mixture was stirred at 0°C under nitrogen for 4 hours and the progress of the reaction was observed by thin layer chromatography (hexane : ethyl acetate = 2 : 1). The product exhibited a rapidly moving yellow spot. The solvent was removed under reduced pressure and the product was purified by passing the residue through a short silica gel filter (hexane : ethyl acetate = 2 : 1). The solvent was removed under reduced pressure to give the product as a light yellow liquid. Yield: 0.23 g (40%).

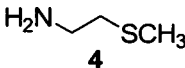
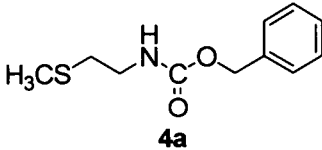
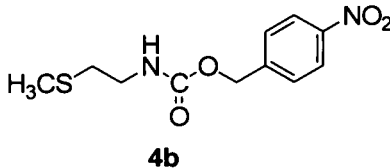
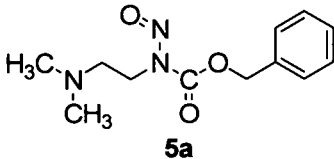
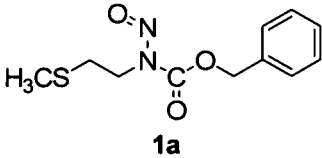
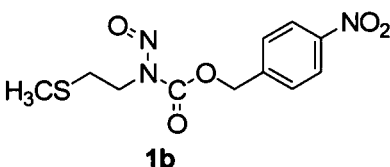
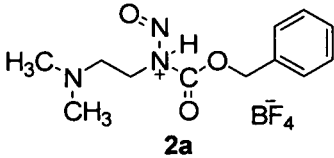
^1H NMR (CD_3CN) δ 2.48 (t, J = 6.3 Hz, 4H), 3.85 (t, J = 6.3 Hz, 4H), 5.47 (s, 2H), 7.38 - 7.47 (m, 10H). ^{13}C NMR (CD_3CN) δ 28.8, 40.9, 70.9, 129.9, 130.0, 130.1, 136.4, 154.8; Anal. Calcd. for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_6\text{S}$: C, 53.80; H, 4.97; N, 12.55. Found: C, 53.94; H, 5.08; N, 12.42.

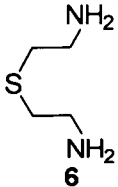
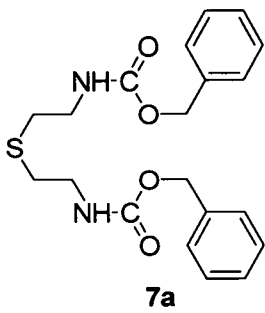
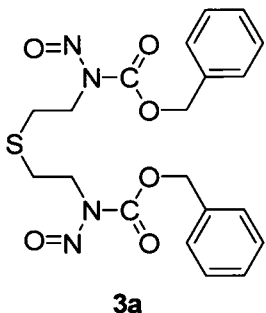
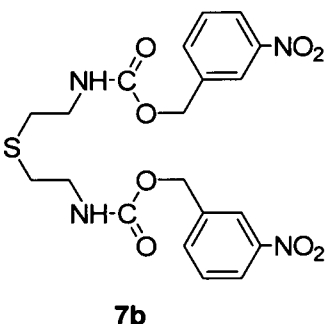
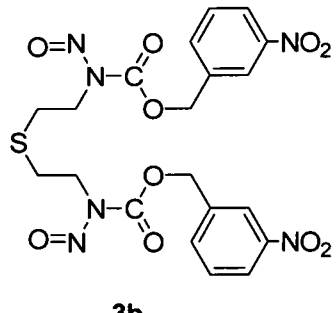
Bis[2-(4-nitrobenzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide (3b): Solid nitrosonium tetrafluoroborate (NOBF_4) (0.14 g, 1.23 mmol) was added in one portion to a stirred mixture Bis[2-(4-nitrobenzyloxycarbonyl amino) ethyl] sulfide (0.20 g, 0.41 mmol) and anhydrous pyridine (0.09 g, 1.23 mmol, 0.10 mL)

in anhydrous acetonitrile (10 mL) at $-20\text{ }^{\circ}\text{C}$. The mixture was stirred at $0\text{ }^{\circ}\text{C}$ under nitrogen for 4 hours and the progress of the reaction was observed by thin layer chromatography (hexane : ethyl acetate = 2 : 1). The product exhibited a rapidly moving yellow spot. The solvent was removed under reduced pressure and the product was purified by passing the residue through a short silica gel filter (hexane : ethyl acetate = 1 : 2). The solvent was removed under reduced pressure to obtain the product as a light yellow liquid. Yield: 0.08 g (38%).

^1H NMR (CD_3CN) δ 2.51 (t, $J = 6.3\text{ Hz}$, 4H) , 3.88 (t, $J = 6.3\text{ Hz}$, 4H) , 5.57 (s, 4H), 7.65 (d, $J = 8.7\text{ Hz}$, 4H), 8.20 (d, $J = 8.7\text{ Hz}$, 4H). ^{13}C NMR (CD_3CN) δ 28.9, 40.9, 69.3, 125.0, 130.0, 143.7, 149.2, 154.7; HRMS (FAB^+) m/z Calcd. for $\text{C}_{20}\text{H}_{20}\text{N}_6\text{O}_{10}\text{S}$ $[\text{M}+\text{Na}]^+$ 559.0859, found 559.0856.

Table 2: Synthesized structures and their yields

Structure	Yield (%)
 <p>4</p>	30
 <p>4a</p>	42
 <p>4b</p>	36
 <p>5a</p>	33
 <p>1a</p>	<p>Aqueous: 20</p> <p>Anhydrous: 43</p>
 <p>1b</p>	<p>Aqueous: Not done</p> <p>Anhydrous: 43</p>
 <p>2a</p>	<p>Aqueous: Not done</p> <p>Anhydrous: 78</p>

Structure	Yield (%)
 <p>6</p>	42
 <p>7a</p>	45
 <p>3a</p>	40
 <p>7b</p>	29
 <p>3b</p>	38

Photochemical studies of benzyl N-2-(methylthio)ethyl N-nitrosocarbamate:

Benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (0.4 g, 1.66 mmol) (**1a**) was dissolved in acetonitrile- d_3 (0.75 mL) and transferred into a quartz NMR tube. The NMR tube was irradiated at 250 nm in a Rayonet photochemical reactor. The effect of irradiation on the compound was monitored at 15, 30, 60, 90, 150, 210, 390 min time intervals by ^1H NMR (16 scans) measurements. Irradiation continued until the entire sample was decomposed. It took nearly six and half hours for the complete decomposition to occur. Then the sample was separated into different fractions using column chromatography (silica gel, hexane : ethyl acetate = 1 : 1). We observed three separate fractions. The first fraction was colorless oil (15 mg). The second fraction was a light yellow liquid (<5 mg). The third fraction, white solid, was purified by recrystallization from acetonitrile and it is the major degradation product (50 mg).

Similar kind of studies were conducted on benzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (0.20 g, 0.83 mmol), by irradiating the sample at 350 nm. We irradiated the sample for three hours and product decomposition was monitored at 15, 30, 60, 90, 150, 180 min time intervals by ^1H NMR (16 scans) measurements. The product mixture was separated by column chromatography (silica gel, hexane : ethyl acetate = 1 : 1). We observed three separate fractions. The first fraction was colorless oil (10 mg). The second fraction was a light yellow liquid (< 2 mg). The third fraction, white solid, was purified from acetonitrile and it is the major degradation product (30 mg). The white solid from

the third fraction was purified by recrystallization from acetonitrile. All the products were subjected to NMR spectral analysis.

Before photolysis: ^1H NMR (CDCl_3) δ 2.10 (s, 3H) , 2.46 (t, J = 6.3 Hz, 2H) , 3.95 (m, 2H), 5.52 (s, 2H), 7.35 - 7.41 (m, 5H). ^{13}C NMR (CDCl_3) δ 15.3, 30.4, 39.4, 69.8, 128.5, 128.7, 128.9, 134.4, 153.7

Fraction 1: ^1H NMR (CD_3CN) δ 2.08 (s), 2.57 (t, J = 6.6 Hz), 3.30 (t, J = 6.3 Hz), 5.05 (s), 5.76 (broad s, 1H), 7.28 - 7.41 (m, 5H).

Fraction 3: ^1H NMR (CD_3CN) δ 2.17 (s), 2.92 (t, J = 6.7 Hz), 3.31 (t, J = 6.5 Hz), 5.06 (s), 5.86 (broad s, 1H), 7.30 - 7.37 (m, 5H), 9.08 (s). ^{13}C NMR (CD_3CN) δ 30.6, 41.0, 65.6, 127.3, 127.5, 128.1, 136.9, 145.3, 156.0.

Photochemical studies of 4-nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate: 4-Nitrobenzyl N-2-(methylthio)ethyl-N-nitrosocarbamate (**1b**) (0.14 g, 0.46 mmol) was dissolved in acetonitrile- d_3 (0.75 mL) and transferred into a quartz NMR tube. The NMR tube was irradiated at 250 nm in Rayonet photochemical reactor. The effect of irradiation on the compound was monitored at 15, 30, 75, 90, 150, 210, 270, 390, 450, 540 min time intervals by ^1H NMR measurements (16 scans). Irradiation continued until the entire sample was decomposed. It took nearly nine hours for the complete decomposition to occur. Then the sample was separated into different fractions using column chromatography (silica gel, hexane : ethyl acetate = 2 : 1). We observed three major fractions from the separation. The first fraction was a white solid (25 mg).

The second fraction was a off- white solid (30 mg). The third fraction was a white solid (30 mg).

Similar kind of studies were conducted on 4-nitrobenzyl N-2-(methylthio)ethyl-N-Nitrosocarbamate (0.10 g. 0.33 mmol), by irradiating the sample at 350 nm. The effect of irradiation on the compound was monitored at 30, 90, 150, 240, 360, 480 min time intervals by ^1H NMR measurements (16 scans). Irradiation continued until the entire sample was decomposed. It took nearly eight hours for the complete decomposition to occur. We irradiated the sample for 8 hrs and product decomposition was monitored by ^1H NMR measurements. The product mixture was separated by column chromatography (silica gel, hexane : ethyl acetate = 2 : 1), we got three different products based on the TLC studies. The first product was a white solid (20 mg). The second product was a off-white solid (10 mg). The third product was a white solid (20 mg). All the decomposition products were subjected to NMR spectral analysis.

NMR data of photochemical degradation products at 250 nm:

Before photolysis: ^1H NMR (CD_3CN) δ 2.11 (s, 3H) , 2.51 (t, J = 6.3 Hz, 2H) , 3.98 (t, J = 6.3 Hz, 2H), 5.61 (s, 2H), 7.67 (d, J = 8.7 Hz, 2H), 8.29 (d, J = 8.7 Hz, 2H). ^{13}C NMR (CD_3CN) δ 15.6 (SCH_3), 31.3 (CH_2), 40.9 (CH_2), 69.3 (CH_2), 125.0 (CH), 130.1 (CH), 143.8 (CH), 149.3 (CH), 154.8 ($\text{C}=\text{O}$).

Fraction 1: ^1H NMR (CD_3CN) δ 2.14 (s, 3H) , 5.24 (s, 2H), 7.60 (d, J = 8.7 Hz, 2H), 8.23 (d, J = 8.7 Hz, 2H). ^{13}C NMR (CD_3CN) δ 56.1 (OCH_3), 69.1(CH_2), 125.0 (CH), 128.6 (CH), 144.7 (CH), 149.1 (CH), 156.7 ($\text{C}=\text{O}$).

Fraction 2: ^1H NMR (CD_3CN) δ 5.28 (s, 2H), 7.61 (d, $J = 8.7$ Hz, 2H), 8.23 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 69.5 (CH_2), 125.0 (CH), 129.8 (CH), 144.6 (CH), 149.2 (CH), 155.8 (C=O).

Fraction 3: ^1H NMR (CD_3CN) δ 2.19 (s, 3H), 2.58 (t, $J = 6.3$ Hz, 2H), 3.28 (q, $J = 6.3$ Hz, 2H), 5.17 (s, 2H), 5.91 (broad s, 1H), 7.56 (d, $J = 8.7$ Hz, 2H), 8.21 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 15.5 (SCH_3), 34.7 (CH_2), 41.0 (CH_2), 65.9 (CH_2), 124.9 (CH), 129.2 (CH), 146.4 (CH), 148.8 (CH), 157.3 (C=O).

NMR data of photochemical degradation products at 350 nm:

Before photolysis: ^1H NMR (CD_3CN) δ 2.11 (s, 3H), 2.51 (t, $J = 6.3$ Hz, 2H), 3.97 (t, $J = 6.3$ Hz, 2H), 5.61 (s, 2H), 7.67 (d, $J = 8.7$ Hz, 2H), 8.29 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 15.6, 31.3, 40.9, 69.3, 125.0, 130.1, 143.8, 149.3, 154.8.

Fraction 1: ^1H NMR (CD_3CN) δ 2.15 (s, 3H), 5.24 (s, 2H), 7.60 (d, $J = 8.7$ Hz, 2H), 8.22 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 56.0 (OCH_3), 69.0 (CH_2), 125.0 (CH), 129.2 (CH), 144.6 (CH), 149.1 (CH), 156.7 (C=O).

Fraction 2: ^1H NMR (CD_3CN) δ 5.28 (s, 2H), 7.61 (d, $J = 8.7$ Hz, 2H), 8.23 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 69.4, 124.6, 128.2, 144.3, 146.3, 151.6.

Fraction 3: ^1H NMR (CD_3CN) δ 2.21 (s, 3H), 2.91 (t, $J = 6.3$ Hz, 2H), 3.35 (q, $J = 6.3$ Hz, 2H), 5.18 (s, 2H), 6.02 (broad s, 1H), 7.55 (d, $J = 8.7$ Hz, 2H), 8.18 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (CD_3CN) δ 15.6 (SCH_3), 32.4 (CH_2), 42.8 (CH_2), 66.0 (CH_2), 124.8 (CH), 128.4 (CH), 146.5 (CH), 149.0 (CH), 157.4 (C=O).

CHAPTER 4

FUTURE WORK

1. Photochemical degradation studies of bis[2-(N-benzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide and bis[2-(N-4-nitrobenzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide.
2. Preparation and photochemical degradation studies of 3,4,5-trimethoxybenzyl [(2-methylthio)ethyl]-N-nitrosocarbamate and bis[(N-3,4,5-trimethoxybenzyloxycarbonyl-N-nitrosoamino)ethyl]sulfide.
3. Preparation and photochemical degradation of benzyl 2-(N,N-dimethylamino)ethyl-N-nitrosocarbamate, 4-nitrobenzyl-2-(N,N-dimethylamino)-ethyl-N-nitrosocarbamate, and 3,4,5-trimethoxybenzyl 2-(N,N-dimethylamino)-ethyl-N-nitrosocarbamate.

CHAPTER 5

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