ASPECTS OF THIONITRITES AND NITRIC OXIDE IN CHEMISTRY AND BIOLOGY

A Thesis Presented by

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ABSTRACT

This thesis is divided into three parts:

Part one is comprised of six chapters and provides a topical review of the main aspects of the chemistry and biology of nitric oxide and of thionitrites. The first chapter is a general introduction to the topic. The second chapter reviews the biology of nitric oxide. The third chapter provides a survey of some of the known chemistry of nitric oxide, with particular emphasis on those aspects which might be relevant in biological systems. The fourth chapter describes the biology of thionitrites in relation to NO. The fifth chapter describes in detail the chemistry of thionitrites, emphasising both those aspects which might be of biological importance and those which make the compounds of potential use in organic synthesis. The sixth chapter states the aims of the present project in the context of the current state of the field, as discussed in the preceding sections of the review.

Part two describes the work that has been carried out during this thesis and is divided into 6 chapters. The first three chapters describe the initial results of our studies aimed at finding novel potential uses of thionitrites in organic synthesis, focusing on the thermal or photochemically induced addition of thionitrites onto olefins. Chapter 1 describes the synthesis of unsaturated thionitrites. Chapter 2 reports on the intra-molecular cyclisation of thionitrites onto alkenes and its applications for the synthesis of episulfides and thiofuran compounds. Chapter 3 examines the inter-molecular version of this reaction, with particular emphasis on reactions involving trityl thionitrite, which was chosen as a model of a stable tertiary thionitrite. This thionitrite has been shown to add very efficiently to alkenes which contain an electron withdrawing or aromatic group and to conjugated dienes. The products obtained are the corresponding α-oximino sulfides. Chapters 4 and 5 describe the results obtained in studies towards achieving a better understanding of the reactivity of thionitrites in biological systems. Chapter 4 provides additional insight into the possible mechanism of NO release from bio-active thionitrites *in vivo*, by comparison of the biological activity of enantiomeric pairs of thionitrites in simple rat-artery models. On the other hand, the results discussed in Chapter 5 seem to indicate that a direct thionitrite-disulfide exchange reaction in solution systems which model physiological conditions does not take place. Finally, in Chapter 6, a series of overall conclusions have been drawn.

Part three provides a formal description of the experimental results and procedures employed throughout this work and is divided into 6 chapters. The first chapter describes the general procedures. The remaining five chapters describe the experimental details corresponding to the work described in Chapters 1 to 5 of the second part.

CONTENTS

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INDEX OF ABBREVIATIONS AND ACRONYMS

^{13}C NMR	Carbon Nuclear Magnetic Resonance (spectrum)
^{1}H NMR	Proton Nuclear Magnetic Resonance (spectrum)
AcOH	Acetic acid
addn	Addition (conditions)
APCI	Atmospheric Pressure Chemical Ionisation
aq.	Aqueous
Ar	Aromatic
Arg	Arginine
atm.	Atmosphere
b	Broad
BH_4	Tetrahydrobiopterin
Bn	Benzyl
Boc	*Tert*-butyloxycarbonyl
BtOH	1-Hydroxy benzotriazole
bp	Boiling point
Bu^t	*Tert*-butyl
Bu^tOK	Potassium *tert*-butoxide
CaM	Calmodulin
Cat.	Catalytic (amount)
cGMP	Cyclic guanosine 3', 5'-monophosphate
MeCN	Acetonitrile
COSY	Correlated Spectroscopy
Cys	Cysteine
CysNO	*S*-Nitrosocysteine
d	Doublet (^{1}H NMR)
Δ	Heating or thermolysis
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEPT	Distortion Enhancement by Polarisation Transfer
DIAD	Diisorpropyl azodicarboxylate

DMF	Dimethylformamide
DMPO	2, 2-Dimethylpyrrolidine *N*-oxide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
P. E.	Low boiling petroleum spirit (30/40 °C)
EA	Elemental analysis
EDRF	Endothelium Derived Relaxing Factor
EDT	Ethylenedithiol
EDTA	Ethylenediaminetetraacetate
EI	Electron Impact
equiv.	Molar equivalents
Et	Ethyl
ESR	Electron Spin Resonance
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EWD	Electron-withdrawing group
FAB	Fast Atom Bombardment
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
Fmoc	9-Fluorenylmethoxycarbonyl
GC	Gas Chrormatography
GC/MS	Coupled Gas Chrormatrography-Mass Spectrometry
Glu	Glutamic acid
Gly	Glycine
GSH	Glutathione
GSNO	*S*-Nitrosoglutathione
GSSG	Oxidised glutathione or glutathione disulfide
GTP	Guanosin 5'-triphosphate
h	Hour(s)
HSA	Human Serum Albumin
HCysNO	*S*-Nitrosohomocysteine

His	Histidine
HMQC	Heteronuclear Multiple Quantum Coherence
hν	Light
HPLC	High Performance Liquid Chromatography
HPLC/MS	Coupled HPLC-Mass Spectrometry
HRMS	High Resolution Mass (spectrum or spectroscopy)
IR	Infra-Red (spectrum or spectroscopy)
k	Rate constant
MeI	Methyl iodide
MeOH	Methanol
m	Multiplet (^{1}H NMR)
m	Meta
Me	Methyl
min	Minute(s)
mp	Melting point
MS	Mass (spectrum or spectroscopy)
NACysNO	*N*-Acetyl-*S*-nitrosocysteine
NAP	*N*-Acetylpenicillamine
NAP_2	*N*-Acetylpenicillamine disulfide
NADPH	Nicotinamide-adenine dinucleotide
Nle	Norleucine
NMM	*N*-Methylmorpholine
NOE	Nuclear Overhauser Effect
NOS	Nitric Oxide Synthase
o	Ortho
p	Para
Ph	Phenyl
ppm	Parts per million
pyBOP	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
q	Quartet (^{1}H NMR)
r. t.	Room temperature
RSNO	Thionitrite or *S*-nitrosothiol

s	Singlet (^{1}H NMR)
sat.	Saturated
sGC	Soluble Guanylate Cyclase
SNAP	*S*-Nitroso-*N*-acetylpenicillamine
SNOCAP	*S*-Nitrosocaptopril
SOD	Superoxide dismutase
SPPS	Solid Phase Peptide Synthesis
^{t}Bu	*Tert*-butyl
t	Triplet (^{1}H NMR)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Tr	Trityl or triphenylmethyl
UV	Ultra-violet (radiation or spectroscopy)
WHE	Wadsworth-Horner-Emmons

PART ONE: INTRODUCTION.

CHAPTER 1

GENERAL INTRODUCTION.

Some years ago, the free radical nitric oxide (NO) was simply regarded as a toxic molecule, one of the long list of environmental pollutants. However, major discoveries in the late 80s and early 90s provided ample evidence for its involvement in an increasing number of fundamental biological processes. As a result, NO is currently one of the most studied molecules of the biomedical sciences and this topic has been reviewed on a number of occasions from different points of view[1, 2]. Several books have also been edited on the subject[3]. Interest in this small gaseous molecule is increasing every year. In 1992 it was named "molecule of the year" by the journal Science[4] and only six years later, in 1998, Murad, Ignarro and Furchgott[5] were awarded a Nobel Prize for their seminal work in the field. This explosion of activity in the biological and medical fields has been accompanied by renewed interest from chemists in the basic chemistry of NO and some of its less-studied derivatives, in particular thionitrites (also called *S*-nitrosothiols).

The work described in this thesis is concerned first with preliminary studies of some new applications of thionitrites in organic synthesis and, secondly, with investigations into the mechanisms of several reactions of thionitrites which could be of biological relevance. It is the first thesis of this laboratory in the field. The purpose of this introductory survey is, therefore, to place these studies in context and hence to set a framework for future studies in the group. Given the vast amount of work which has been published in the field, it is not, by any means, intended to be exhaustive and many of the biological ideas will necessarily be presented in a simplified form.

Accordingly, the following review will cover, in the first instance, the essential aspects of the known biology and chemistry of NO. This will be followed by a description of the basic roles of thionitrites in biological systems in relation to NO. Finally, a more detailed account of the organic chemistry of thionitrites will be included.

CHAPTER 2

NITRIC OXIDE IN BIOLOGY.

2. 1. The nitric oxide story: an overview[1b, 1e].

The advent of the explosion of activity in the NO field can be traced back to 1987 when a cascade of discoveries showed that NO was synthesised by endothelial cells and that it had an essential biological role as a signalling molecule in the cardiovascular system. Shortly afterwards, the pathway for the biosynthesis of NO was elucidated and this gaseous molecule was also found to be synthesised by cells in the nervous and the immune systems. Within only one year, one of the enzymes involved in its biosynthesis (brain Nitric Oxide Synthase, bNOS) had been isolated and purified, and was being cloned. The other members of the family of the NOS were then isolated and cloned within a short period of time. Since then, many other biological and pathological roles of NO and many new aspects of its biosynthesis and modes of action have been discovered. Although many of the mechanistic details are still poorly understood and much remains to be investigated there are a few facts which are generally accepted and these are summarised below.

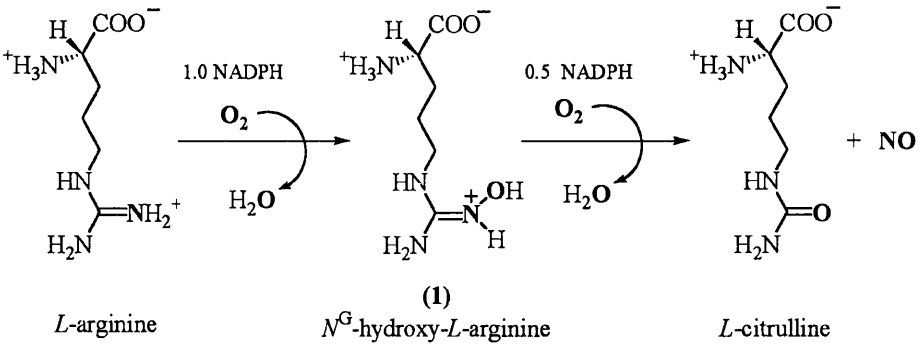
2. 2. NO biosynthesis, NO Synthases and the detection of NO[1,2].

It is now well established that in mammalian cells NO is synthesised from the semi-essential amino acid *L*-arginine and molecular O_2 with simultaneous production of *L*-citrulline. This involves a multi-step redox reaction (with transfer of a total of 5 electrons) as indicated in a simplified form in **Scheme 1**. The process is catalysed by one of the enzymes of a group of Nitric Oxide Synthases (NOS).

The first step, a two electron oxidation, involves the hydroxylation of one of *L*-arginine two equivalent guanidino nitrogen atoms to form N^G-hydroxy-*L*-arginine (**1**) as an enzyme-bound intermediate. The second step, an overall three electron oxidation, involves electron removal, oxygen insertion and carbon-nitrogen bond scission to form *L*-citrulline and the free radical NO. The same electron-donor, nicotinamide-adenine dinucleotide (NADPH **5**, later in **Figure 2**), is required for both steps. By means of isotopic labelling studies, Moncada and co-workers[6] showed that the source of oxygen in

both NO and citrulline is molecular oxygen and thus, NOS can be described as a dioxygenase.

They also showed that the source of nitrogen atom in NO is the guanidino nitrogen atom of arginine[7]. The oxygen atoms which are incorporated into NO and *L*-citrulline derive from distinct molecules of O_2.



Scheme 1. The biosynthesis of NO[7].

Two types of NOS have been characterised thus far. The first type is a constitutive enzyme, that is to say, it is permanently present and responds rapidly to activation. The second type of NOS is inducible and it is expressed in response to certain stimuli (usually associated with infection) such as cytokines. Another main difference between them is that the activity of the constitutive NOS is dependent upon the concentrations of Ca^{+2} and of calmodulin (CaM, a calcium binding protein) whereas the activity of the inducible enzyme is independent of both $Ca^{2+}$and calmodulin concentrations. It has been suggested that in the constitutive form of NOS only the Ca^{2+}-bound form of CaM can bind and activate the enzyme. In contrast, the inducible form of NOS already binds CaM strongly (so strongly that it is virtually impossible to obtain the enzyme without CaM) and is fully active at very low calcium concentrations, to such an extent that its activity *in vivo* can never be limited by this parameter.

Three isozymes of NO Synthase have been identified. Their structures, which all exhibit a certain degree of homology with the P-450 reductase, and their mechanisms of action are all very similar but small changes allow for different modes of regulation. The exact nature of the enzyme depends on the tissue from which it was obtained. Thus neuronal NOS (nNOS) is constitutive and is expressed by certain cells of the nervous system (neuronal cells and skeletal muscle cells). Endothelial NOS (eNOS) is also

constitutive and is expressed mainly in endothelial cells (cells lining the blood vessels) and in platelets (colourless cell fragments, containing granules, present in blood). It produces the endothelium derived relaxing factor (EDRF), which was an object of controversy for many years until, eventually, identified as NO independently by Moncada[7,8b] and by Ignarro[8a]. The third type of NOS, inducible NOS (iNOS) is expressed by the immune system (macrophages, hepatocytes, tumour cells) in response to stimuli released during infection.

All forms of the enzyme studied so far have been found to be dimeric. Each subunit of the dimer consists of two clearly differentiated domains: a reductase domain and an oxygenase domain (**Figure 1**).

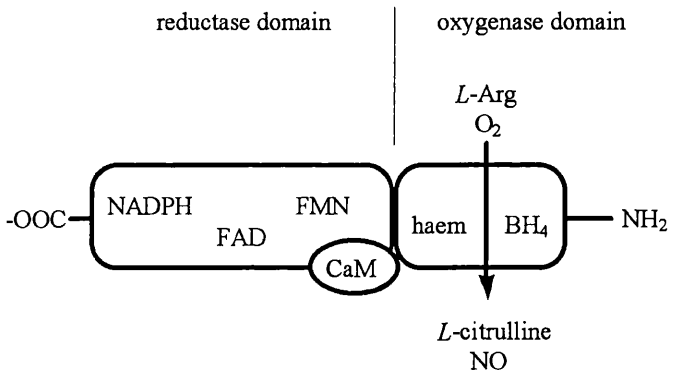


Figure 1. Domains and cofactors of NO-Synthase.

The reductase part contains one molecule each of two prosthetic groups (**Figure 2**): flavin-adenine dinucleotide (FAD, **2**) and flavin mononucleotide (FMN, **6**). It also contains a binding site for the cofactor NADPH (**5**). The oxygenase contains another prosthetic group (a haem, **4**) and two binding sites: one for the cofactor tetrahydrobiopterin (H_4B, **3**) and a second one for the substrate *L*-arginine. Linking the two domains is a sequence that forms the binding site for CaM. There is still much controversy with respect to what the full function of each of these groups is in NOS and how they interrelate. Reduced thiols have also been found to play an unspecified role in maintaining NOS activity.

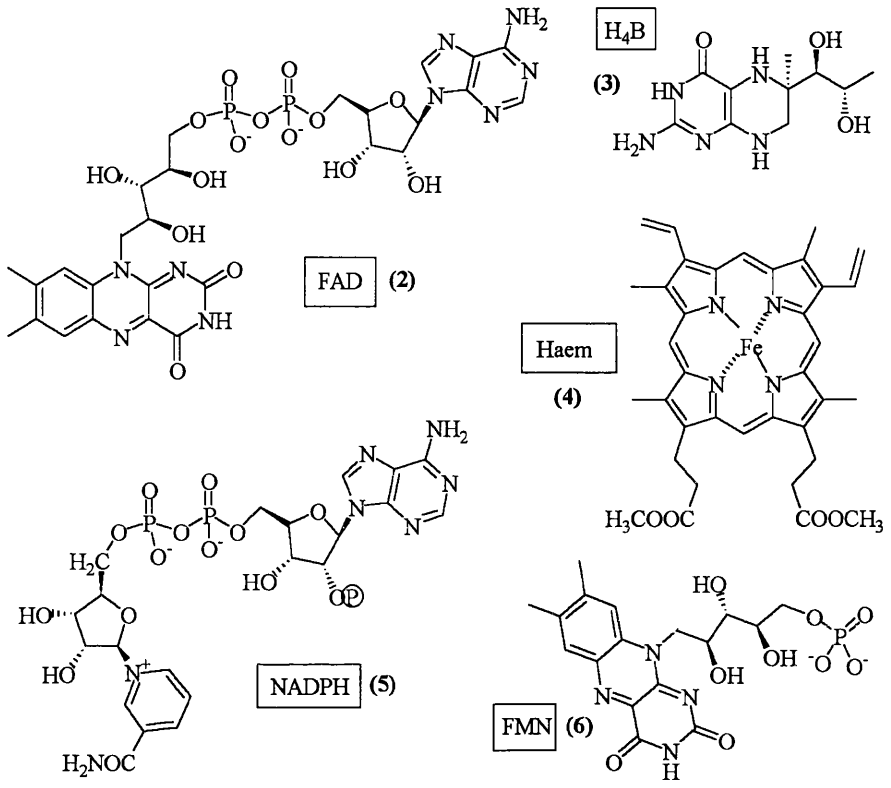
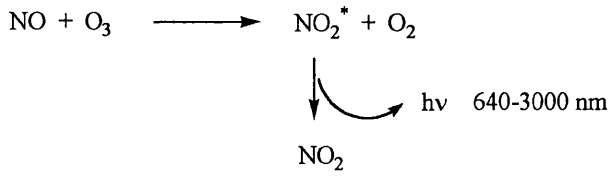


Figure 2. Cofactors used by NOS during NO biosynthesis.

Several techniques allow the monitoring of NO production in biological systems[3]. One of the most common is chemiluminescence. In order to use this technique, the biological mixture containing NO is purged with an inert gas such as N_2 to release NO into the gas phase. Oxidation of NO with ozone then produces nitrogen dioxide in an excited state. When this relaxes to the ground state, it releases light of a characteristic wavelength which can be quantified with a photomultiplier (**Scheme 2**). The reaction emits radiation in the region 640-3000 nm with a peak maximum at 1100 nm, but, for technical reasons, detection is better at 640-900 nm.

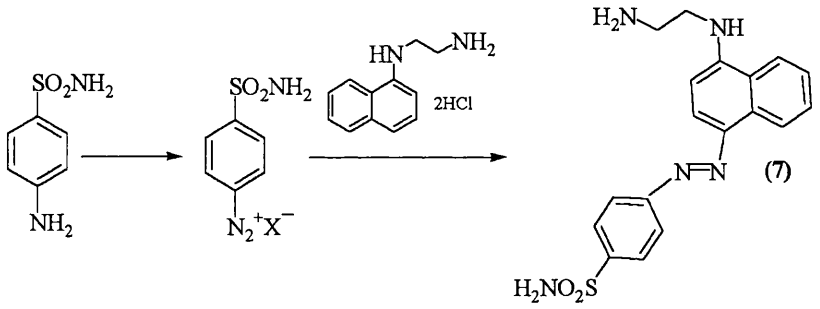


Scheme 2. Chemiluminescence assay for NO[3].

Another very extended technique involves an assay based on the Griess reaction (**Scheme 3**). Contrary to the chemiluminescence approach, this does not measure NO directly, but only after its conversion into nitrite and, therefore, it only allows to infer the

previous presence of NO. Solutions of biologically derived nitrite and nitrate are reduced to nitrite using a copper-coated cadmium column or the enzyme nitrate reductase. The resultant solution is then reacted with sulfanilamide in a solution of acidic *N*-(1-naphthyl)-ethylendiamine dihydrochloride to form the azo derivative **7**, which can be detected and quantified spectrophotometrically at 548 nm.

NO can also be detected by the use of spin-traps and ESR or by its reaction with oxyhaemoglobin (these techniques will be discussed later in more detail). Finally, a method of detection which has become very convenient in the last years is the electrochemical oxidation of NO using a NO selective microprobe electrode.



Scheme 3. Monitoring of NO production by the Griess reaction[3].

2. 3. The biological actions of NO[1-3].

The basic role of NO in the circulatory system, that of smooth muscle relaxation, was one of the first functions of NO to be identified[1a]. Smooth muscle is a type of tissue which surrounds hollow organs and tubes (mainly blood vessels) and its relaxation and contraction controls blood pressure. The process of relaxation has been known for many years to be triggered by a number of bio-molecules such as acetylcholine, bradikinin and histamine. However, before the discovery of the identity of EDRF as NO, it was thought that these molecules interacted directly with the muscle. Instead, it is now known that these compounds act on the endothelium and that this, in turn, produces a secondary messenger, now identified as NO, responsible for relaxation[9]. As well as the drugs mentioned, mere stress can trigger the process. Maintaining normal blood pressure requires vascular endothelial cells to constantly generate NO.

NO is involved in another important aspect of blood regulation. It is responsible for inhibition of platelets aggregation and adhesion (i.e. it reduces platelets affinity for

each other and for the blood vessel walls). In this way NO helps to regulate the process of blood clotting which is a natural defence mechanism of the body against injury and infection. This function of NO has implications in the treatment of arteriosclerosis, a process in which accumulation of blood clots in arteries can lead to fatal heart injury.

In the immune system NO mediates cytotoxicity and is used in the response of the body against foreign matter. It is synthesised by macrophages in high concentrations (around 1000 times higher than the basal production of NO in the cardiovascular or nervous systems). Macrophages are large phagocytic cells normally found in tissues that produce blood cells which ingest bacteria and cell debris (they evolve from monocytes which are the largest forms of leukocytes or white cells). NO generated by macrophages is able to kill or stop proliferation of target cells and this is achieved by different means: (i) NO can react with the iron-sulfur centers of several important macromolecules (impairing the cell's respiratory ability), (ii) it can also inhibit ribonucleotide reductase, necessary for DNA synthesis and this may be a way in which macrophages can inhibit the rapid growth of early tumour cells, (iii) finally, NO can react with other reactive species generated during infection as, for instance, superoxide. The substances generated by these processes are very destructive to DNA and lipid membranes, producing cell necrosis. Excessive expression of iNOS during acute infection may also have a contra-productive effect in causing severe vasodilatation. This condition is known as septic shock and can be fatal.

NO is present in both the central and peripheral nervous systems. However, the precise role of neuronal NO is not yet fully known. In the brain, it acts as a neurotransmitter and is believed to be involved in the processes of learning and memory building. It has also been suggested that over-production of NO could be responsible for brain damage during stroke and certain degenerative conditions such as senile dementia. NO also has a role in the peripheral nervous system, where it has been identified as a possible neurotransmitter for neurones known as non-adrenergic/non-cholinergenic. NO-sensitive neurones of this type are found in several peripheral tissues, including cardiovascular, uro-genital, respiratory and digestive systems.

2. 4. Non-enzymatic production of NO in humans.

Bacteria can synthesise NO from nitrite and nitrate *via* enzymatic pathways. Recently, a novel pathway for the production of nitric oxide in humans has been discovered which involves chemical reduction of inorganic nitrite and is non-enzymatic[10]. This process has been demonstrated to take place in the stomach, on the surface of the skin, in the ischemic heart and in infected nitrite-containing urine, that is, in places where strong and reducing conditions are expected to be found.

Two mechanisms have been proposed: (i) at low pH, nitrite is protonated to give nitrous acid which is in equilibria with N_2O_3. This, in turn, is in equilibrium with NO and NO_2. The direction in which these equilibria are driven depends on the ambient conditions and, under the conditions above the production of NO could be favoured (**Scheme 4**), (ii) in addition, when reducing compounds, such as ascorbate, are present, nitrous acid can be reduced directly to NO.

$$NO_2^- + H^+ \rightleftharpoons HNO_2 \xrightarrow{\text{ascorbate}} NO$$

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

$$N_2O_3 \rightleftharpoons NO + NO_2$$

Scheme 4. Chemical production of NO from nitrite in humans[10].

Human saliva contains both nitrate and nitrite. The former is taken up from the plasma by salivary glands and derives both from the diet and from the reaction of NO with oxyhaemoglobin (discussed later in more detail). The latter is obtained from dietary products and from bacterial reduction of nitrate in the oral cavity. Once nitrite reaches the stomach, the low pH present there favours its reduction to NO by the pathway discussed above. Thus, these processes seem to be part of a recycling cycle for NO produced enzymatically from *L*-arginine (**Figure 3**).

Most of the nitrate generated in the body is excreted in the urine. Nitrite is only excreted in very small quantities and, in practice, detection of nitrite in the urine is routinely used as a diagnosis of urinary tract infection since most of the bacteria involved in these situations may convert nitrate to nitrite by an enzymatic pathway. However, it has been pointed out that nitrite in infected urine can have a beneficial effect provided that the urine is made acidic. In this case nitrite can be converted to NO, by a similar

process to that occurring in stomach, and it can be used to destroy bacteria. This may provide an explanation of the beneficial effects of vitamin C, which is both acidic and reductive, in situations of urinary infection.

In a similar way, nitrate present in sweat is reduced to nitrite by bacteria and this is reduced to NO on the slightly acidic surface of the skin. This NO has been suggested to be involved in inhibition of pathogens as well as in regulation of skin blood flow. The production of NO by the skin may be enhanced by application of nitrite-containing saliva and this may explain why animals lick their wounds. The whole picture has been schematised below (**Figure 3**).

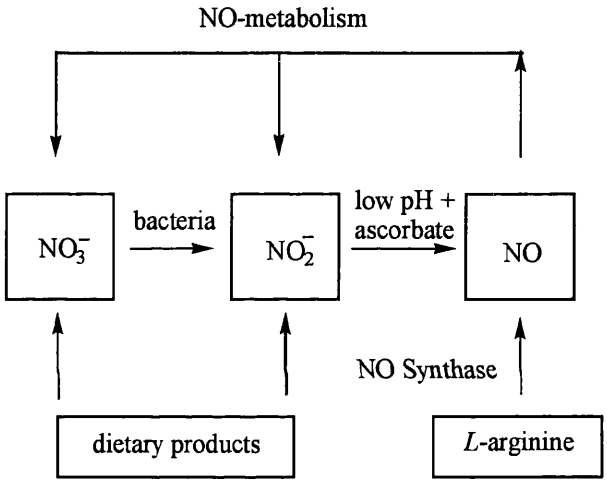


Figure 3. Enzymatic *vs* non-enzymatic production of NO in humans[10].

2. 5. Soluble Guanylate Cyclase: the NO receptor[1c,2b-c].

Interestingly, NO mediates most of its functions in the cardiovascular and nervous systems by stimulating the same enzyme, soluble Guanylate Cyclase (sGC) in the effector cells. In contrast to NOS, this enzyme is not so well defined and, at the time of this review, a crystal structure is still not available. Nevertheless, some details of its mode of action are known. NO exerts its activity by binding sGC through a haem prosthetic group. This binding activates the enzyme to catalyse the conversion of guanosine 5'-triphosphate (GTP) to cyclic guanosine 3',5'-monophosphate (cGMP). cGMP is an intracellular messenger that activates specific enzymes and ion channels producing a response to the formation of NO (**Figure 4**).

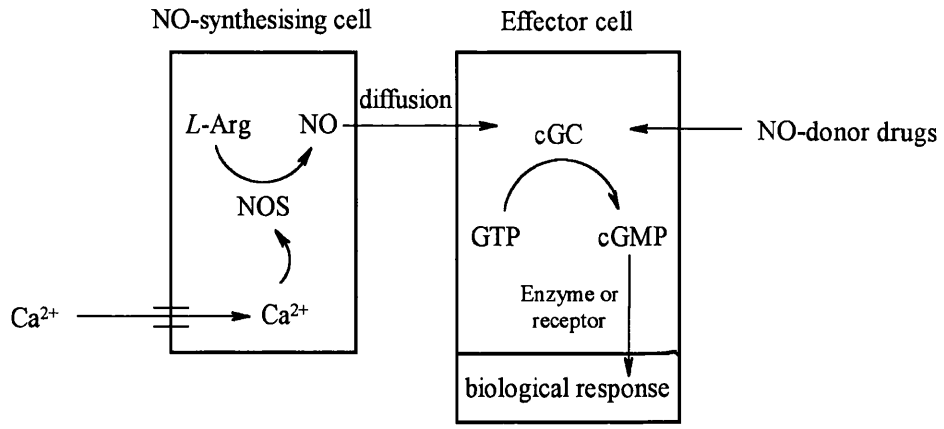


Figure 4. Biological actions of NO.

2. 6. The clinical aspects of NO.

Two of the most striking aspects of the physiology of NO are the large number of metabolic processes controlled by this simple molecule and the most extensive list of diseases that may be caused by either an increase or a decrease in its concentration. This has led scientists to search for specific activators and inhibitors of the enzymes involved in the physiology of NO (both Synthases and sGC) and also for specific exogenous donors of NO as therapeutic agents. These topics have been extensively reviewed in recent times [11,12,13 14].

CHAPTER 3

NITRIC OXIDE IN CHEMISTRY.

3. 1. Introduction[1c,2,15,16].

A basic knowledge of the chemistry of NO is a necessary prerequisite for the understanding of the chemistry of its derivatives, in particular the nitrosothiols (RSNO) studied in this thesis. In this section the most important properties and reactions of NO itself will be discussed. Those aspects of the chemistry of NO which are expected to be relevant in biological systems will also be emphasised.

3. 2. Physico-chemical properties and synthesis.

NO is a colourless gas at room temperature, with a solubility in water at 25 °C of $1.8x10^{-3}$ M, which is relatively low and comparable with those of molecular oxygen, molecular nitrogen and carbon dioxide, and which is unchanged within the pH range 2-13. It is slightly and almost equally soluble in other solvents[17]. NO is a neutral molecule and *in vivo* this allows easy diffusion across membranes. It contains an unpaired electron (i.e. it is a paramagnetic radical species) but is not ESR active in room temperature solutions or in the solid state unless bound to some radical trap (in fact, the ESR peak for NO exists but is highly broadened by spin delocalisation). In the laboratory NO can be synthesised by reduction of NO_2^- (with iodide or ascorbic acid) or NO_3^- (with copper). Commercially it is obtained by the catalytic oxidation of ammonia. For use in the laboratory it can also be conveniently purchased in cylinders.

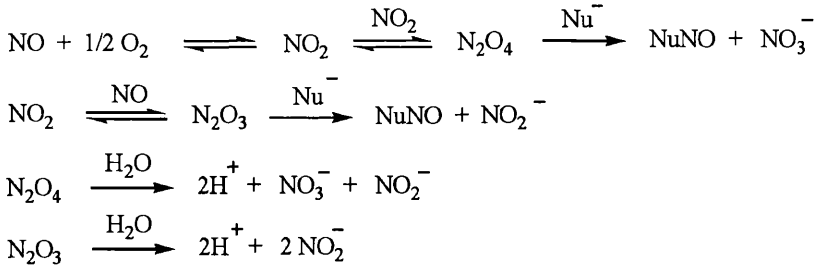
3. 3. NO as a radical.

NO is popularly regarded as being a highly reactive radical, but this does not in fact seem to be the case. For instance, it shows no tendency to dimerise or disproportionate. Furthermore, it is not "destructive" in the same way as the hydroxyl radical and its cytotoxicity cannot be simply a consequence of its radical nature. It is more likely that it is actually the products derived from the reaction of NO with other reactive oxygen intermediates which are responsible for its toxic actions. As a free radical, NO reacts easily with other free radicals or very reactive molecules such as molecular O_2,

superoxide radical anion ($O_2^{\cdot -}$), hydrogen peroxide and organic free radicals ($R_3C^{\cdot}$, $RO^{\cdot}$, $RS^{\cdot}$, etc.).

3. 3. 1. Reaction with O_2.

When NO reacts with O_2 either in solution or in the gas phase, NO_2 is formed (**Scheme 5**) probably through the intermediacy of a NO dimer (N_2O_2) . NO_2 can further react with NO to form N_2O_3 or with another molecule of NO_2 furnishing N_2O_4. Whilst in the gas phase and in organic solvents NO_2, N_2O_3 and N_2O_4 are in equilibrium, in aqueous solutions the two latter quickly hydrolyse. On hydrolysis, N_2O_3 yields nitrite as the sole product. N_2O_4 in water would give nitrite and nitrate in equimolecular amounts. However, it seems that in aerated aqueous solutions NO reacts to yield exclusively NO_2^- indicating that the reaction *via* N_2O_3 is favoured. The reaction of O_2 with NO in the gas phase or in saturated solutions, both in water and organic solvents, is very fast (in CCl_4 or H_2O, at 25 °C, the rate constant is in the order of 10^6 $M^{-2}s^{-1}$), giving characteristic brown fumes of NO_2. In contrast, in aqueous solutions containing physiological concentrations of O_2 (10^{-6} M) and NO (10^{-9}) this is a slow process. NO in these circumstances has a half-life of several hours, which would be long enough to allow for biological activity *in vivo*. Thus, the observed short physiological life of NO (from 6s to 30s) cannot be attributed to its reaction with O_2. *In vitro*, both N_2O_3 and N_2O_4 react with nucleophiles affording the corresponding nitrosated species. *In vivo* these reactions could be important in situations of oxidative stress in which NO is generated in large quantities.



Scheme 5. Reaction of NO with O_2 and subsequent processes.

3. 3. 2. Reaction with superoxide and subsequent reactions of peroxynitrite.

With superoxide radical anion ($O_2^{\cdot -}$) in aqueous solutions, NO gives peroxynitrite (^-OONO) or the corresponding conjugated acid (HOONO) depending on the pH with a rate constant of $5x10^9$ $M^{-2}s^{-1}$. Peroxynitrite anion is quite stable at alkaline pH but when

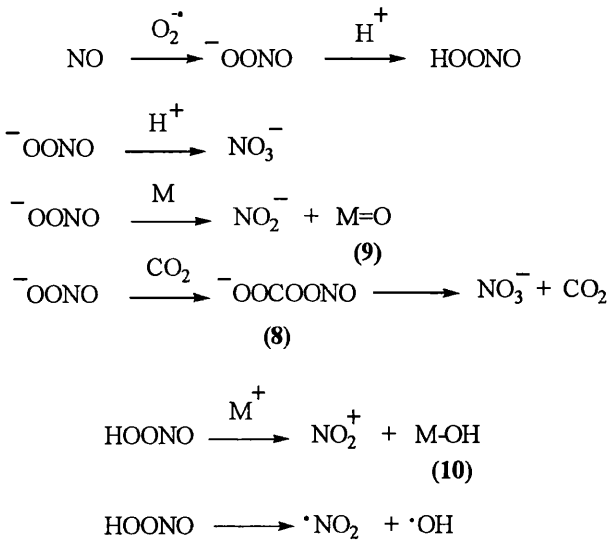
protonated at neutral or acidic pH (HOONO) it is highly unstable and may act as a strong oxidant or nitrating agent (**Scheme 6**). Indeed, peroxynitrite has been shown to react with virtually all classes of biomolecules *in vitro* and the process is believed to involve formation of NO_2 and hydroxyl radicals. Superoxide is generated in high concentrations by the body in situations of oxidative stress. In these conditions NO is also generated in large quantities and its reaction with superoxide is probably one of the ways by which NO effects its cytotoxicity. Furthermore, in contrast to the reaction of NO with O_2, its reaction with superoxide occurs at nearly the diffusion rate. Under normal physiological conditions ([NO]= 10^{-9} M and $[O_2^-]$= 10^{-7} M) the reaction would not be expected to contribute to the toxic properties of the system.

Interestingly, under acidic conditions peroxynitrite can also rearrange to inert nitrate and thus in this case NO would appear to act more as a superoxide scavenger than as a toxic radical. Hence, it can have either a deleterious or a beneficial effect depending on the conditions. The mechanisms and different pathways for the decomposition of HOONO in solution are still not understood and whether hydroxyl radicals and NO_2 radicals are formed at all as intermediates is under investigation. There is also evidence that peroxynitrite can act as a nitrosating agent as well as a nitrating species[18]. It has, for instance, been shown to nitrosate thiols under physiological conditions and this aspect will be addressed later.

Peroxynitrite anion reacts rapidly with carbon dioxide to yield the adduct nitrosoperoxycarbonate (**8**), which decomposes to NO_3^- and CO_2 in the absence of other reactive molecules. The rate constant of this reaction is large enough to make it the predominant route of peroxynitrite disappearance in normal biological fluids, where the total carbonate concentration is between 1mM and 25 mM.

It has been speculated than in the reactions of peroxynitrite *in vivo*, metals could play catalytic roles by forming intermediate oxene species (**9**, **Scheme 6**). In fact, peroxynitrite has been shown to be able to act as an electrophilic oxygen donor in its reaction with iodide (I^- + $^-$ONOO gives IO^- + NO_2^-). Peroxynitrite is also known to act as a nitrating agent in solution in the presence of transition metal ions such as Cu^{2+} or Fe^{3+}. It is clear that such a process, which is harmful to proteins, might contribute to the toxicity of this species *in vivo*. A mechanism has been proposed for this process in which

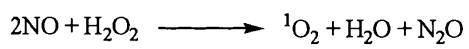
the O-O bond of peroxynitrous acid is polarised by the metal and undergoes heterolytic cleavage to give NO_2^+ and compound **10** as shown in **Scheme 6**.



Scheme 6. The formation of ^-ONOO from NO and $O_2^{\cdot -}$ and possible subsequent reactions

3. 3. 3. Reaction with hydrogen peroxide.

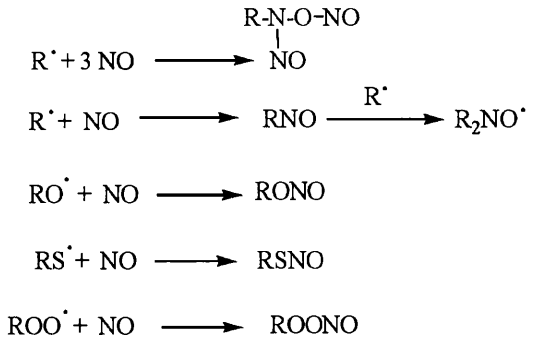
Reaction 1 may be an important process *in vivo* since the concentration of H_2O_2 in biological media is higher than that of superoxide and the product generated, singlet oxygen, is a highly reactive oxygen species which may contribute to cell injury.



Reaction 1. The reaction of NO with hydrogen peroxide.

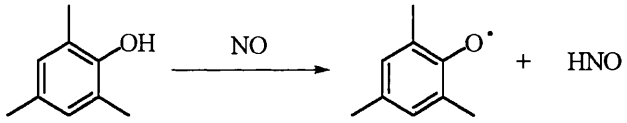
3. 3. 4. Radical coupling and hydrogen atom abstraction.

In solution, NO reacts with organic free radicals giving the corresponding nitrosated products (**Scheme 7**). This reaction may be relevant in biological systems and, in particular, in relation to the possible deactivation of enzymes which contain stable free radicals in the active site as, for example Ribonucleatide reductase. However, the radical reactivity of NO in water has almost not been studied and little is known about this possibility.



Scheme 7. Reactions of NO with organic free radicals.

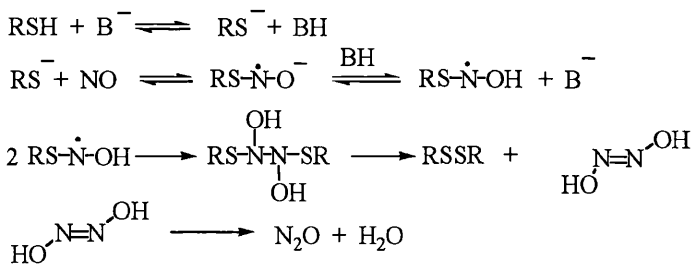
Although NO may abstract a hydrogen atom from neutral molecules, it should be appreciated that this reaction is much less efficient than in the case of the corresponding reaction with hydroxyl radicals. Some phenols have been shown to be transformed to phenoxy radicals by NO **(Reaction 2)**



Reaction 2. Hydrogen abstraction by NO.

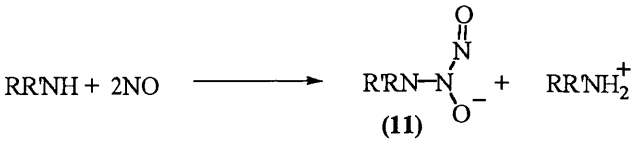
3. 4. NO as a nucleophile or electrophile.

NO itself has a very poor electrophilic or nucleophilic character. In general, NO is clearly not an electrophilic nitrosating agent, unless previously oxidised to NO^+ or another nitrogen oxide. In order for this to happen, aerobic conditions are needed. When oxygen is rigorously excluded, nitric oxide does not nitrosate nucleophiles such as S, N or O. An exception is found in the reaction with thiols at high pH[19]. In this case disulphides are obtained and the mechanism below has been proposed **(Scheme 8)**.



Scheme 8. Nitrosation of thiols by NO at alkaline pH[19].

In the absence of oxygen and in non-aqueous conditions, secondary amines react with excess NO yielding salts[20], the so called NONO-ates (**11**) which have been employed as NO sources (**Reaction 3**).



Reaction 3. Nitrosation of amines by NO in the absence of O_2.[20]

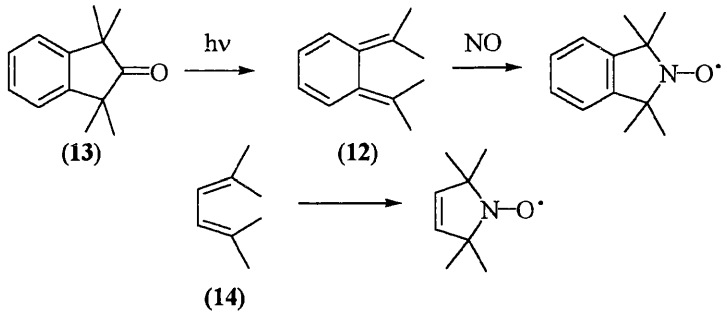
3. 5. Reaction with alkenes and dienes.

The reaction of NO with simple olefins in organic solvents has been the object of considerable controversy for some time[21] due to the complex mixture of products formed. The reaction is acknowledged to be radical in nature and initiated either by NO_2 present as an impurity or by a photochemical process[22]. It has been noted that pure NO does not add to alkenes unless trace amounts of NO_2 are first formed[23]. This can be present as an impurity from the oxidation of NO in the advent of traces of oxygen. It has also been proposed that at high pressures and when purified (oxygen free) NO was used, NO_2 could be formed simultaneous to N_2O by a known disproportionation reaction[24].

Even more discussion has been arisen in the reaction of NO with dienes, which was initially proposed to yield stable nitroxide radicals by a cycloaddition-type mechanism[25]. The first example (scheme 10) was shown with 5,6-diisopropylidenecyclohexan-1,3-diene (**12**), obtained by photolysis of 1,1,3,3,-tetramethylindan-2-one (**13**) and this was later extended to simpler dienes as shown. These molecules would be ideal to serve as NO spin-traps, to detect NO in solution by ESR spectroscopy. It is worth mentioning here that conventional nitrones and nitroso spin traps have proved unsuitable for trapping of NO. However, these studies had the limitation that they were conducted solely using

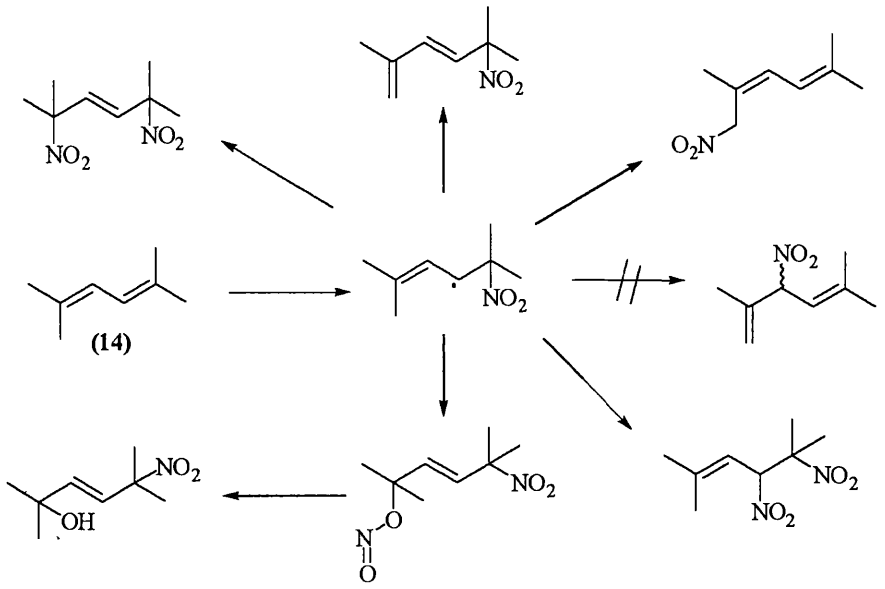
ESR data and that products were not isolated and characterised. Other authors, also basing their ideas on ESR data, suggested that for simple dienes a radical mechanism initiated by NO_2 would apply instead of the previously assumed ring-closure mechanism.[26]

In fact, Gabr and co-workers[27], some time after the publication of their first reports assuming a cheletropic mechanism for the reaction of NO with simple dienes[25], revised the structures assigned to the compounds detected by ESR and reassigned them as not being cyclic nitroxides of the type depicted in **Scheme 9**.



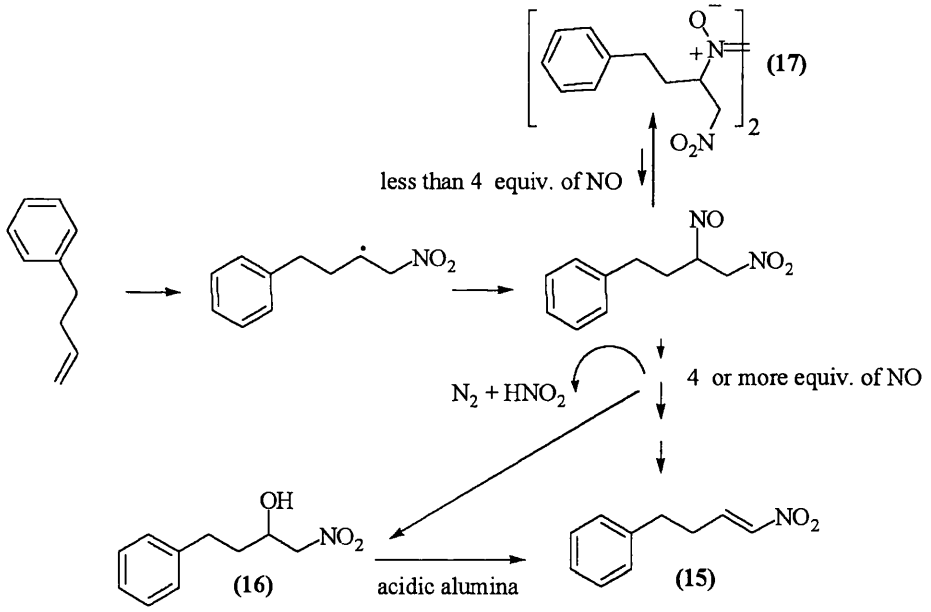
Scheme 9. Reactions of NO with some butadienes: initially proposed structures.[25]

In a detailed study, Kelly *et al.*[28] claimed to have resolved the conflicting data by isolating the adducts formed in the reaction of diene **14** with excess NO generated over 24 h. (**Scheme 10**).



Scheme 10. Reaction of excess NO with 2,4-dimethyl-2,3-hexadiene, products.[28]

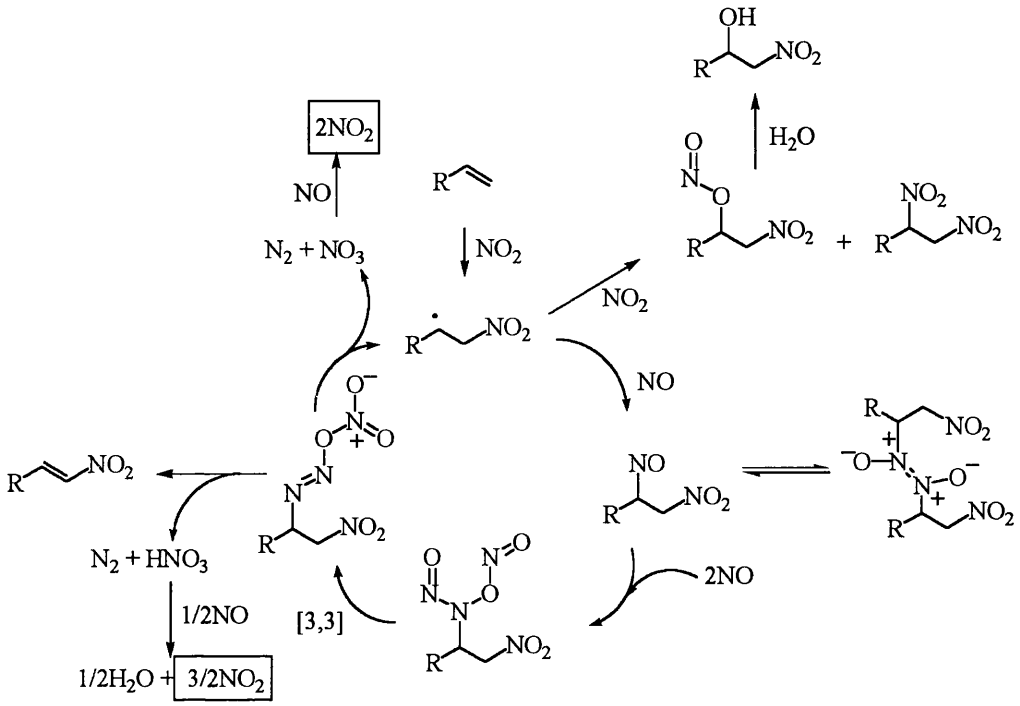
Since the structure of the majority of the products formed contained NO_2 groups, this posed a considerable problem: the oxygen source. It is inconceivable that only traces of nitrogen dioxide could be responsible. The answer to this problem was deduced mainly from previous investigations by the Mukaiyama group[29]. In Mukayiama's work, olefins with a terminal double bond or a double bond conjugated with an aromatic ring were successfully nitrated to nitro-olefins **15** on treatment with 1 atm of NO in DCM (**Scheme 11**). Nitro-alcohols **16** were also formed as by-products and they could also be converted to the final nitroalkenes in good yield by treatment with acidic alumina. Based on the analysis and quantification of solid and gaseous products generated in the reaction at different molar ratios of alkene to NO the following reaction pathway was proposed (**Scheme 11**).



Scheme 11. Reaction of NO with simple alkenes.[29]

Using only 1.1. molar equivalent of NO, nitroso-dimer **17** was isolated as the sole product in low yield. Four equivalents of NO were required for complete conversion of one equivalent of alkene, and one equivalent of N_2 and a total of one equivalent of nitrous and nitric acids were produced. If product **17** was treated with more NO, nitroalkene **15** and N_2 were again produced. Other authors had also isolated bis(nitrosonitroalkanes) as products in reactions of NO with alkenes and noted the stoichiometry and production of N_2 in the reactions of nitroso-dimers with excess

NO[20,21]. Kelly[28] assembled all those observations and proposed the following picture of all the possible pathways which could be taking place (**Scheme 12**)

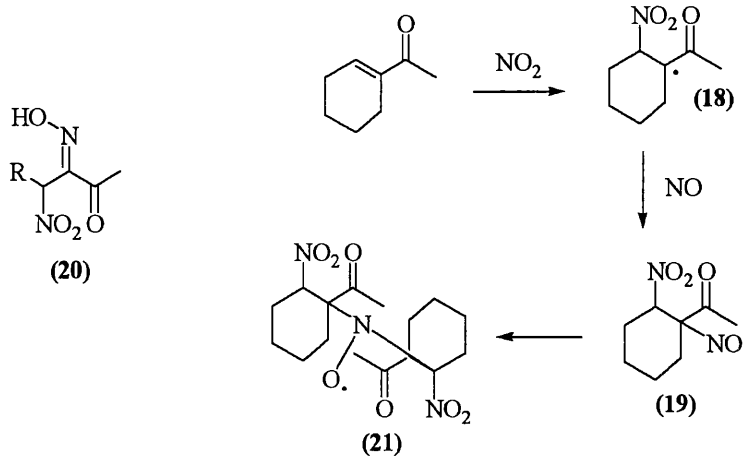


Scheme 12. Mechanism proposed for the reaction of NO with alkenes and dienes.[28]

It is worth noting that according to this mechanism, once the first steps are traversed, more NO_2 is produced than consumed and the reaction rate increases. This explains the isolation of so many products containing NO_2 and is in agreement with the stoichiometry of the other nitrogen-derivatives formed (N_2, nitrite and nitrate).

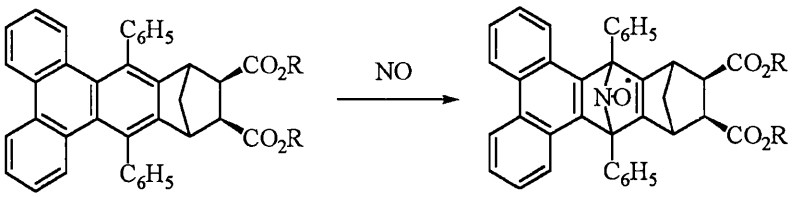
Park and Walton[23] also carried out an ESR study of the reactions of NO with alkenes containing electron-donor or electron-withdrawing groups both in the presence and absence of NO_2 (**Scheme 13**). They concluded that NO does not add to any type of simple alkene or diene in the absence of NO_2. If this gas is present, it adds onto alkenes generating β-nitro carbon-radicals **18** which subsequently react with NO to give α-nitroso-β-nitro compounds **19**. If an electron withdrawing group such as a carbonyl is present, these rearrange preferentially to the corresponding oximes **20**. In all other cases, such as tertiary nitroso-compounds which cannot tautomerise to the oximes or electron donating groups present on the double bond, the β-nitro-nitroso compounds trap other radicals to give various aminoxyl radicals such as **21.** Analogously, reaction of NO with a diene such as **14** gave ESR signals only in the presence of added NO_2 and the spectrum

was assigned as being a nitroxyl radical derived from the diene **14** with a structure analogous to that of **21**.



Scheme 13. Reaction of NO with several alkenes, ESR study.[23]

In contrast to simple dienes which appear to react with NO to give complex mixtures of products as discussed above, compounds of the quinodimethane family such as **12**, do seem to react with NO through a cheletropic mechanism. A recent review has been published on the topic[30] . The authors have extended the concept by introducing a fluorophoric system in the molecule which allows detection by fluorescence. They have also shown that these compounds can be loaded into cells, thus allowing for intracellular trapping of NO. An example is shown below.

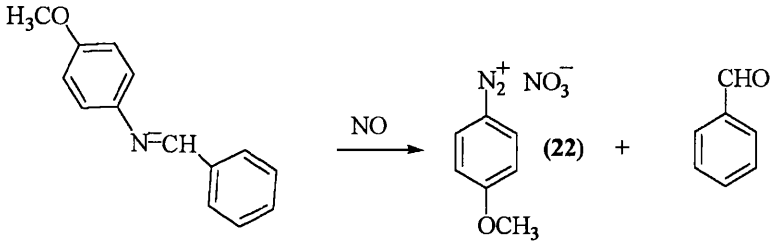


Reaction 4. Fluorescence cheletropic traps for NO.[30]

In summary, NO does not react with alkenes or simple dienes unless in the presence of traces of NO_2. In this case, the products formed in the presence of an excess of NO result from the addition of NO_2 and NO onto the double bond. The ESR signals detected correspond to acyclic aminoxyl radicals which are probably formed in small quantities. In contrast, the reactions of NO with quinodimethane-type alkenes do give

cyclic nitroxide radicals through a cheletropic mechanism, and these are sufficiently stable to be characterised by ESR and to be used as NO traps.

Finally, it is also interesting to mention a recent report[31] on the addition of NO onto the imine double bond of certain Schiff's bases. The authors reported that NO gas directly reacted with imines derived from *p*-methoxyaniline and that the product isolated in good yield was the diazonium nitrate **22**. (**Reaction 5**). The authors proposed a mechanism which involved addition of several NO molecules onto the N atom of the imine followed by rearrangement to give the final product together with N_2 in a similar fashion to that discussed above (**Scheme 12**) for alkenes.



Reaction 5. Reaction of NO with Schiff's bases.[31]

3. 6. Reactions with metals: co-ordination chemistry of NO[2b].

NO is an extremely powerful ligand, both for free and ligated metal ions. Nitrosyl complexes of transition metals (Ni, Mn, Ni, Pt, V, Mo, Cr, Fe, Ir, Ru, etc) have been known and used by both organic and inorganic chemists for many years and the topic has been reviewed several times[32]. In particular, there was a major expansion of interest in the 60s and 70s, due to their potential use as homogeneous catalysts. This is a very extensive issue and it is inappropriate to go into detail in the short space available here. Only those aspects of potential biological relevance will be summarised.

The electronic nature of the M-NO bond depends on the nature of the metal and the rest of ligands and, in general, it will be better described by one of three limiting forms: M^-NO^+, M^+NO^- or M–NO. The character of the bond can be conveniently monitored by IR. In general, low oxidation states of metals will give $M–NO^-$ and high oxidation states will give $M–NO^+$. In addition, NO can adopt a variety of co-ordination geometries.

The co-ordination chemistry of NO, when compared with those of O_2 and CO, is of great importance for the understanding of its biology. In particular, a knowledge of the reactions of NO with haem-iron is fundamental, since the receptor for NO *in vivo* is a haem-iron protein (sGC). Furthermore, its synthase is also a haem protein and, interestingly, in this case its activity is due to binding with O_2 and not with NO. Another vital iron metalloprotein, haemoglobin, also binds NO reversibly (**Scheme 14**). In contrast, the oxidised form of haemoglobin (oxyhaemoglobin) gives an irreversible reaction with NO transforming it into nitrate. Fortunately, the body has enzymes which allow the conversion of the final product, methaemoglobin (HbFe(III)), which does not bind O_2, back to active haemoglobin. Reaction with oxyHb may be one of the major routes of NO destruction *in vivo*. Some authors have suggested that reversible reaction with deoxyHb can act as a means of transporting NO in *vivo*. Incidentally, the reaction of NO with oxyHb is the basis of an analytical method for the determination of NO by differential spectrophotometry[33]. Cytochrome P450, another iron-haem enzyme, has also been found to bind NO.

$$\text{HbFe(II)} \overset{\text{NO}}{\rightleftharpoons} \text{HbFe(II)(NO)}$$

$$\text{HbFe(II)O}_2 \xrightarrow{\text{NO}} \text{HbFe(II)OONO} \longrightarrow \text{HbFe(III)} + \text{NO}_3^-$$

Scheme 14. Reactions of NO with the oxidised and reduced forms of haemoglobin.[2b]

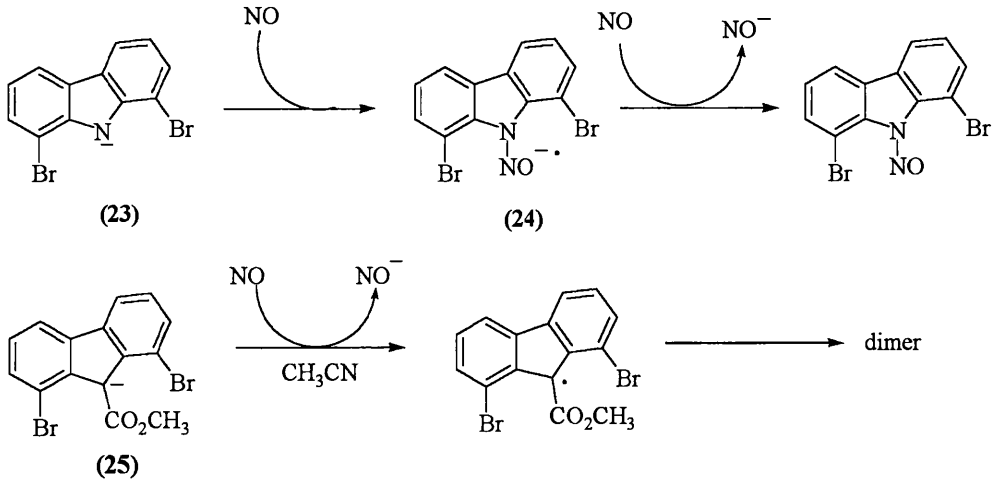
Cells contain a variety of other metalloproteins which could potentially receive NO as ligand. Not only are iron-metalloproteins of interest, but some reactions with zinc- and copper-containing proteins have also been reported. In addition, NO also reacts with non-haem iron. Whilst the reaction of NO with haem is essentially reversible, the binding with non-haem iron is of rather different character. There is a large amount of evidence[1b] that NO binds irreversibly to the iron atoms of the ferredoxin type iron-sulfur clusters in certain enzymes, leading to formation of iron-sulphur-nitrosyl complexes. This could be another of the mechanisms by which NO is cytotoxic.

A detailed account of the implications of the role of NO as a ligand for metals in biological systems has been published.[2b]

3. 7. Redox chemistry of NO.[3a]

In theory, NO can be oxidised to nitrosonium cation (NO^+) or reduced to the nitroxyl anion (NO^-) by single electron transfer reactions. Limited data is available on the values of the redox potentials for the couples NO^+/NO and NO/NO^- in solution. The former has only been determined in aprotic solvents: (+1.28, +1.34 and +1.48 V in acetonitrile, nitromethane and dichloromethane, respectively). The latter has a value of 0.25 V in water at pH 7.4. From these figures it looks rather easy to reduce NO to the nitroxyl anion, whilst oxidation to NO^+ would be more difficult. The first reaction is carried about by Fe(II) in solution or by Fe(II)-containing metalloenzymes, the second transformation requires relatively strong oxidants such as transition metals in high oxidation states. It has been pointed out that few biologically available reagents can provide the oxidising equivalents and, although H_2O_2 has been suggested as a possible candidate, this has not been proven[2b]. An NO reductase has been identified in eukaryotic systems, which reduces NO to N_2O, and this is thought to occur through the intermediacy of NO^-.

Very recently, an interesting study has appeared[34] in which the abilities of NO to act as an electrophile or as an electron acceptor in reactions with several anions have been compared (**Scheme 15**). A detailed thermodynamic analysis showed that NO reacts with amide anion **23** as an electrophile and this reaction is followed by an electron transfer from the better electron donor **24** to a second molecule of NO. A more explicit example of the role of NO as an electron acceptor is shown in its reaction with carbanion **25** (**Scheme 15**).



Scheme 15. NO as an electrophile vs NO as an electron-acceptor.[34]

3. 8. The chemistry of the redox activated forms of NO: NO^+ and NO^-.

In view of the large number of roles assigned to NO, it is difficult to understand how one single molecule could fulfil them all. It has been suggested that the other redox species associated with NO, the nitrosonium cation and the nitroxyl anion, can also function as biological mediators in an inter-related system[35]. This could also help to understand the problem of the conflicting roles of NO in some situations, for instance, its protective actions *vs.* its toxic behaviour. In some circumstances these species have been found to be generated *in vivo* together with neutral NO by SOD (Superoxide Dismutase). The product of NOS (NO^-, NO^+ or NO) seems to depend on the redox properties of the system under study. These species could also be generated from NO or from other nitrogen derivatives by redox processes.

As mentioned above, the NO^+ cation can be formed by oxidation of NO. This is true in organic solvents and many derivatives of NO^+, both ionic and covalent, but strongly polar, are known, which can be handled under anhydrous conditions. However, in aqueous solutions at neutral pH, NO^+ would have only a transient existence since it would immediately react with water to give nitrous acid by the **Reaction 6**.

$$NO^+ + H_2O \longrightarrow HNO_2 + H^+$$

Reaction 6. Hydrolysis of nitrosonium ion.

In strongly acid solutions the reverse process dominates and this reaction is used for the nitrosation of nucleophiles. At much lower acidity the actual nitrosating agent is believed to be $H_2NO_2^+$. Although the free nitrosonium cannot exist *in vivo*, there are several other species which can be considered as NO^+ carriers. For instance Fe^{3+} complexes with NO are better regarded as $Fe^{2+}NO^+$ and they readily release the nitrosonium ion which then nitrosates nucleophiles. Thionitrites, *vide infra,* can also be seen as donors of NO^+ in certain reactions. Other examples are: nitrosamines (R_2NNO), alkyl nitrites (RONO), N_2O_3 ($NO^+NO_2^-$) and N_2O_4 ($NO^+NO_3^-$).

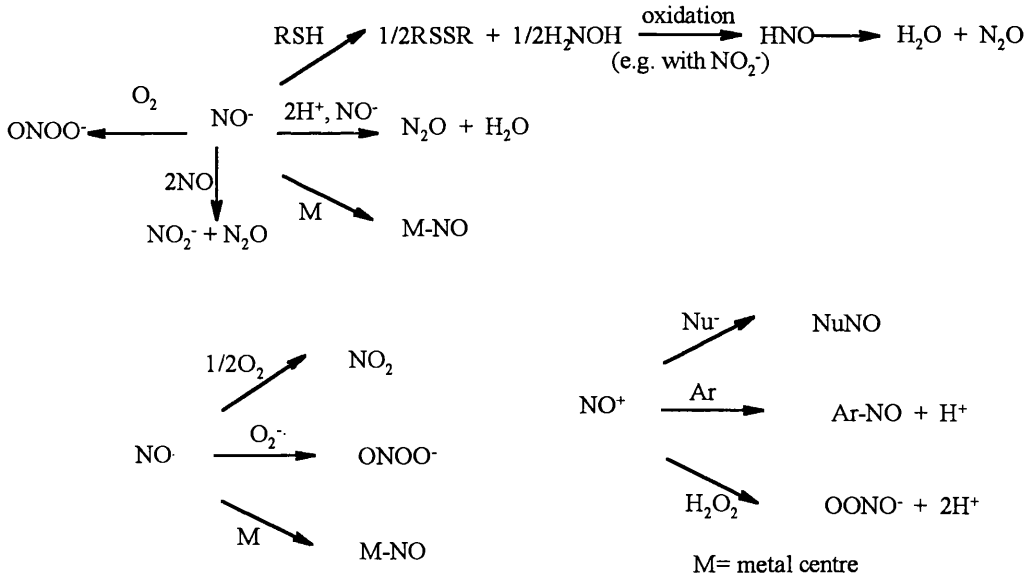
The chemistry of the nitroxyl anion NO^-, particularly in aqueous solution, has received considerably less attention. NO^- is involved in an acid-base equilibrium which in protic solvents is highly displaced towards the formation of HNO (hyponitrous acid) since this readily dimerises to give nitrous oxide (N_2O, **Reaction 7**). NO^- can also be

seen as formed from the ionization of HNO. This reaction is rarely seen in the laboratory since the dimerisation of HNO is so fast. However, dimerisation is a second order process and, therefore, its rate is very concentration dependent and would be slow at the concentrations of HNO which would occur in cells. The pK_a of HNO is 4.7 and hence ionisation will occur to give NO^- at physiological pH. HNO can be chemically obtained by decomposition of $HN_2O_3^-$ or $C_6H_5SO_2NHOH$[3a,16]. Certain metal complexes can be regarded as carriers of NO^-. For instance, $OsCl(NO)(CO)L_2$ can be protonated to give $OsCl(HNO)(CO)L_2$ and $[Co(NH_3)_5NO]^{2+}$ is a well-characterised example of a nitrosyl complex better regarded as a NO^- complex.

$$2NO^- + 2H^+ \rightleftharpoons 2HNO \longrightarrow N_2O + H_2O$$

Reaction 7. Nitroxyl acid-base equilibrium.

Some reactions of NO^- which could be of biological importance are, for instance, the reaction with Fe(III)haem giving Fe(II)NO[36] and the reversible addition to low molecular weight and protein thiols leading to sulphur oxidation with intermediate formation of RSNHOH[37]. In addition, NO^- is easily oxidised back to NO by superoxide dismutase. It can also react with two molecules of NO giving nitrite and nitrous oxide. The main reactions of NO^-, NO and NO^+ are collected in **Scheme 16**.



Scheme 16. Summary of the reactions of NO and its redox activated forms.

A detailed discussion of the implications of the chemistry of NO and its other redox activated forms in biology has been published.[35a]

CHAPTER 4

THIONITRITES IN BIOLOGY.

4. 1. Introduction.

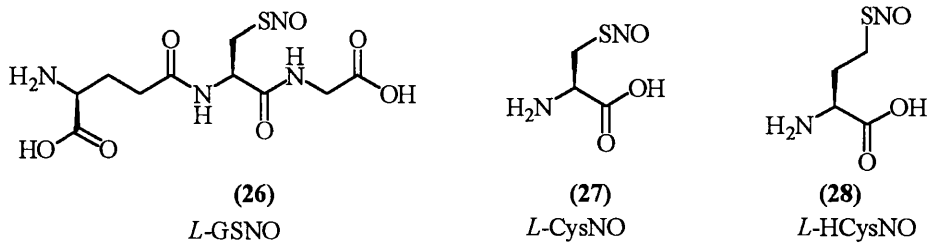
Thionitrites, also called *S*-nitrosothiols, are compounds of general formulae R-S-N=O where R can be an alkyl or an aryl group. They are the sulfur analogues of the much better known and studied alkyl nitrites of formulae RONO. In the last few years, thionitrites (RSNOs) have come into prominence as part of the 'nitric oxide story'. There are several ways in which thionitrites are related to NO, and all of them have important implications in the biomedical field. These will be discussed below.

4. 2. Thionitrites as endogeneous NO carriers *in vivo*.

Free NO is a fairly short-lived species in biological systems. This observation suggested that NO *in vivo* must be linked to a carrier molecule, forming a stable adduct. This would enable both the storage of NO and its transport from the site of formation to the site of action. Thionitrites are among the likely candidates for such role since they have been detected and quantified in living organisms. Other compounds, such as haem-containing proteins or iron-nitrosyl complexes of general structure $(RS)_2Fe(NO)_2$, both found *in vivo*, have also been proposed to play such a role.

Stamler and co-workers[38] determined that the concentration of free NO in blood or plasma is rather low (in the 10^{-9} M range). In contrast, thionitrites are present in concentrations of the order of 10^{-6} M. These circulate in blood primarily as *S*-nitrosoproteins (96 % in human plasma) with *S*-nitrosoalbumin being the most abundant species (82 % of the total content of *S*-nitrosoproteins). Other, less stable, low molecular weight thionitrites such as *S*-nitroso-*L*-glutathione (**26**, *L*-GSNO), *S*-nitroso-*L*-cysteine (**27**, *L*-CysNO), and *S*-nitroso-*L*-homocysteine (**28**, *L*-HCysNO), have also been detected and quantified *in vivo*[1d] (**Figure 5**). It has also been shown that, in biological fluids, RSNOs concentrations rise in states of inflammation and with the exogenous administering of NO gas. Levels fall with the administration of pharmacological inhibitors of nitric oxide synthases. As a result of these discoveries, the hypothesis has been drawn that stable long-lived thionitrites are formed in biological systems from NO and protein free thiols. These are subsequently delivered to the target site where they

react releasing NO, possibly through the formation of an intermediary less stable low molecular weight thionitrite.



(26) *L*-GSNO

(27) *L*-CysNO

(28) *L*-HCysNO

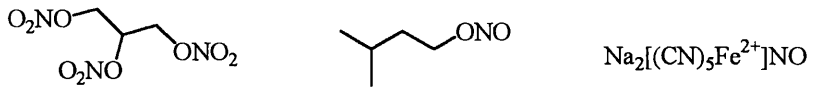
Figure 5. Structures of some of the most important biological thionitrites.

There is ample evidence that thiols react readily with nitrogen oxides in solution to form *S*-nitrosothiols and the mechanisms, as discussed in the following section, are well documented[39]. Nevertheless, the mechanism by which NO is delivered to and from thionitrites *in vivo* remains still undefined and is presently the subject of much research.

4. 3. Thionitrites in medicine: exogenous NO-releasing drugs.

As already mentioned, much research is currently devoted to obtaining drugs which allow the efficient administration of NO into the human body, as a treatment for disorders arising from its insufficient production *via* the normal pathways.

Some pharmacological NO-donors, namely organic nitrates such as **29**, organic nitrites such as **30** and sodium nitroprusside (**31, Figure 6**) have been used in medicine for a long time, even before the basic aspects of their biochemistry, involving NO, were recognised. There is evidence that *S*-nitrosothiols are in fact involved as active intermediates in the mechanisms of pharmacological action of these compounds. Ignarro and co-workers.[40] reported that several organic nitrites and nitrates, $NaNO_2$, sodium nitroprusside and NO were all able to activate the enzyme soluble guanylate cyclase, but this required the presence of thiols in the medium. They also observed that only some specific thiols were able to effectively act as co-factors. For example, glyceryl trinitrate was only active in the presence of cysteine among all the thiols tested. Furthermore, the activation of sGC by the pre-formed nitrosated thiols did not require the presence of added thiols. The process of activation was found to be pH dependent as expected if the thiolate forms of the thiols were involved in the trans-nitrosation reaction between the NO-donor studied and the added thiols.



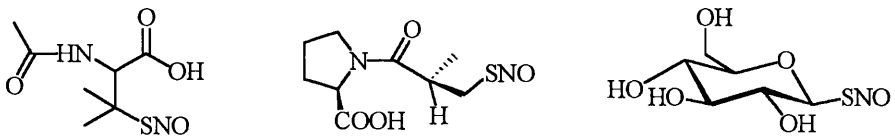
(29) glyceryl trinitrate

(30) isoamyl nitrite

(31) sodium nitroprusside

Figure 6. Some classical pharmacological NO-donors.

In recent years, many new classes of NO-donors have emerged and the topic has been reviewed several times[11, 12]. *S*-nitrosothiols are one such class of compounds and are receiving special attention given their possible role as 'natural NO-carriers' in biological systems. Many of them have been tested *in vivo* and found to have the same vasodilatation and platelet aggregation inhibiting properties as true NO gas[41]. The first demonstration that these compounds were pharmacologically active was the description of the antibacterial effects of CysNO[42]. Later, attention focused on the effects of thionitrites on the cardiovascular system. Studies have concentrated on the activity of CysNO (**27**), *N*-acetyl-*S*-nitrosocysteine (NACysNO), HCysNO (**28**), *S*-Nitroso-*N*-acetylpenicillamine (**32,** SNAP), *S*-nitrosocaptopril (**33**, SNOCAP), GSNO (**26**) and *S*-nitroso-*β*-*D*-glucose (**34**), (**Figure** 7).



(32) SNAP

(33) SNOCAP

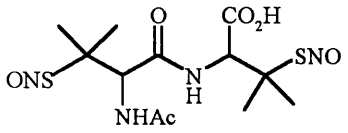
(34) *S*-nitroso-*β*-*D*-glucose

Figure 7. Structures of some thionitrite pharmacological NO-donors.

Other new thionitrites obtained by reacting commercially available thiols and nitrous acid have been pharmacollogically tested and found to be active. For instance, Kerr[41] obtained and tested the thionitrites derived from *β*-mercaptosuccinic acid, and thioglycerol. Stamler *et al.*[43] examined the reaction of several sulfhydryl containing proteins and studied their activity as vasodilators and platelet aggregation inhibitors. The proteins chosen were bovine serum albumin (abundant in plasma), tissue-type plasminogen activator (an endothelium derived enzyme) and cathepsin B (a lysosomal cysteine protease) and they were all found to possess EDRF-like effects.

In all these studies the thionitrites were prepared *in situ* before the biological tests and they were used in solution. Later, efforts have concentrated in trying to synthesise, isolate and fully characterise novel thionitrites before testing them *in vivo.*

Roberts[44] synthesised and characterised a series of thionitrites derived from penicillamine dipeptides. One of them (**35**) showed properties both in vitro and *in vivo* which were compatible with the liberation of free NO (e. g. decreasing of blood pressure in rats and rabbits and concomitant inhibition of platelet aggregation).



(35)

Wang[45] prepared and isolated three NO donors **36-38** based in SNAP by attaching this thionitrite to a sugar. The ability of these novel glyco-thionitrites to release NO in solution was examined and they were found to be more stable than SNAP (**Figure 8**).

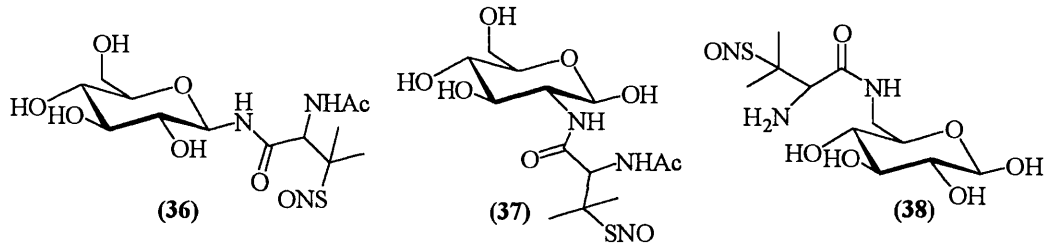


Figure 8. Structure of some glyco-thionitrites.[43]

Butler[46] has synthesised a series of thionitrites **39-43** derived from 1-thiosugars (glucose, galactose, xylose, maltose and lactose). Most of them were shown to be unstable either as solids or in solution. However, compound **39** was sufficiently stable to allow examination of its properties as a vasodilator using an isolated rat artery model. It also proved effective in human vascular smooth relaxation when delivered trans-dermally (**Figure 9**).

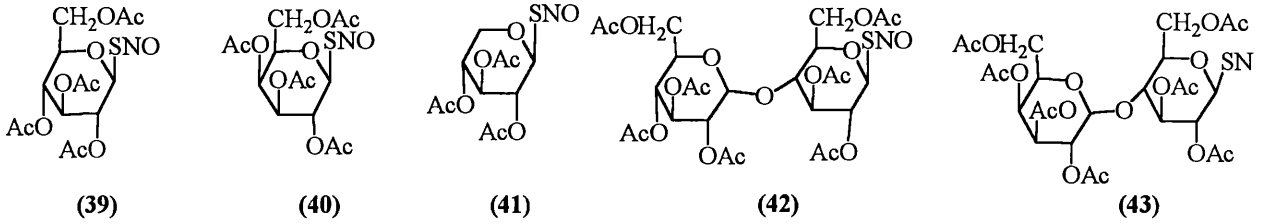
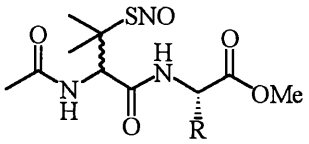


Figure 9. Sugar-derived *S*-nitrosothiols.[46]

Later, the same authors reported the synthesis of a series of SNAP-derived *S*-nitrosated dipeptides **44-53** (**Figure 10**) formed by linking SNAP to a selection of naturally occurring amino acid methyl esters[47]. All the compounds were found to be effective vasodilators in rat artery model analysis and some structure-activity relationships could be inferred.



R= H SNAP-gly **(44)**
R= Me SNAP-ala **(45)**
R= ^{i}Pr SNAP-val **(46)**
R= $CH_2{}^{i}Pr$ SNAP-leu **(47)**
R= CH_2Ph SNAP-phe **(48)**
R= CH_2CH_2SMe SNAP-met **(49)**
R= $CH(CH_2CH_3)CH_3$ SNAP-iso **(50)**
R= CH_2COOMe SNAP-asp **(51)**
R= CH_2CH_2COOMe SNAP-glu **(52)**
R= $CH_2CH_2CH_2$-(N) SNAP-pro **(53)**

Figure 10. Thionitrites derived from natural amino acids.[47]

4. 4. Thionitrites as intermediates in regulatory processes *in vivo*.

It has been shown that nitrosation of thiol residues in enzymes can modulate their activities[48]. This is both the case when thiols are part of the active site of the enzyme and when thiols are involved in hydrogen bonding or ionic interactions which determine the conformational preference of the protein section. Hence, in these cases, thionitrites would be acting as intermediates in processes such as the activation or deactivation of enzymes or receptors. Other similar regulatory processes with intermediate formation of thionitrites can be envisaged. This concept will be discussed in more detail at the end of the next section (5.7. Thiols, thionitrites, sulfides, disulfides and other sulfur compounds).

CHAPTER 5

THIONITRITES IN CHEMISTRY.

5. 1. General properties.

Thionitrites were firstly prepared almost a hundred years ago[49]. However, as already mentioned, since then, their chemistry has been much less developed than that of their oxygenated analogues, the alkyl nitrites. The main reason for this is their instability. In general all alkyl thionitrites are unstable to a certain extent. In particular, primary and secondary ones cannot be isolated in pure form and are difficult to handle. One important exception to this rule is the primary thionitrite *L*-GSNO (**26**)[50], which is indefinitely stable in the solid form if kept in the dark. Tertiary thionitrites are relatively stable and a few of them have been isolated and fully characterised. The most frequently cited in the chemical literature are *tert*-butylthionitrite (**54**)[51], tritylthionitrite (**55**)[52] and SNAP[53] (**32**), (**Figure 11**). Some aromatic thionitrites (**56-59**, **Figure 12**) have also been prepared and characterised in solution, but they were far too unstable (half-lives of the order of 2-7 min) to allow isolation[54].

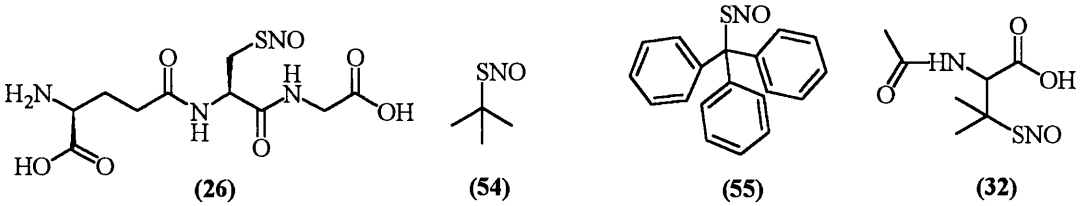


Figure 11. Alkyl thionitrites most frequently cited in the chemistry literature.

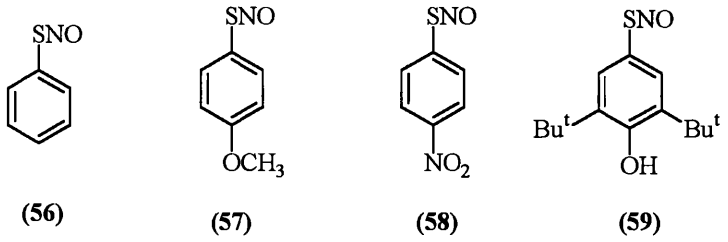


Figure 12. Some aromatic thionitrites that have been prepared but not isolated.[54]

The molecular structure of SNAP was obtained by X-ray crystallography[53] and the thionitrite is shown in **Figure 13**. The C-S bond is rather long and this could explain the

higher instability of thionitrites in comparison with alkyl nitrites, which have a shorter C-O bond distance. Other bonds and angles are as expected.

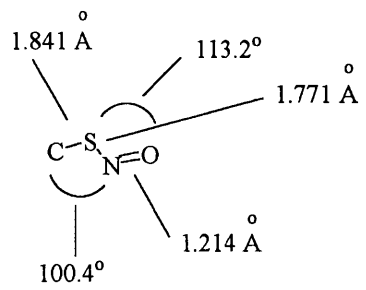


Figure 13. Structure of SNAP as seen by X-ray crystallography.

Thionitrites are strongly coloured compounds. Tertiary ones are green and primary and secondary ones are generally red. Aromatics give green-red solutions. They absorb in the UV-visible regions, with two maxima at around 350 nm and at around 550 nm, the first one being the most intense (ε= *ca.* 10^3). They can be solid, liquid or gas depending on their structure. For example, GSNO (**26**) is a pale pink solid, SNAP (**32**) and tritylthionitrite (**55**) are green crystalline solids and *tert*-butyl thionitrite (**54**) is a green oil. In the IR spectrum, the stretching frequency of the N=O bond around 1480-1530 cm^{-1} is diagnostic. In the ^1H and ^{13}C NMRs there is a significant downfield shift of the α proton and carbon signals with respect to those of the starting thiol upon nitrosation, which can be used to assess if nitrosation has taken place. Use of ^{15}N NMR has also been reported[55].

Two reviews containing the synthetic applications and known reactivity of thionitrites until the mid 80s were published by Oae[39c] in 1983, and by Williams[39a] in 1985. In 1993, Kim[56] published another survey covering several novel applications of thionitrites in organic chemistry. Some of the facts presented in those reviews and several other aspects of the chemistry and biochemistry of thionitrites will be discussed below.

5. 2. Reactivity of thionitrites: introduction and generalities.

In general, the reactions of thionitrites can be classified as belonging to one of three categories: (i) reactions involving homolytic fission of the S-N bond; (ii) reactions involving heterolytic cleavage of the S-N bond; and (iii) electron-transfer induced reactions.

(i) The reactions involving homolytic fission of the S-N bond result in release of NO with concomitant formation of intermediate thiyl radicals as schematised below (**Reaction 8**). In addition to having possible applicability in organic synthesis, these reactions are of pivotal importance for the understanding of the varied biological modes of action of thionitrites, which ultimately appear to be related to their ability to release NO. The thermal and photochemical decomposition reactions of RSNOs can be included in this category.

$$RSNO \longrightarrow RS^{\bullet} + NO^{\bullet}$$

Reaction 8. Homolytic cleavage of thionitrites.

(ii) The reactions involving heterolytic cleavage of the S-N bond may involve release or transfer of either NO^+ (**Reaction 9**) or, less frequently, NO^- (**Reaction 10**). Transnitrosation reactions between thionitrites and thiols or amines and formation of disulfides by nitrosation of proximal dithiols can also be included in this group.

$$RSNO \longrightarrow "RS^- + NO^+"$$

$$RSNO \longrightarrow "RS^+ + NO^-"$$

Reactions 9 and 10. Heterolytic cleavage of thionitrites.

Several authors have noted that the vasodilatatory properties of thionitrites are independent of the rate at which they release NO[42, 57]. That is, their stability towards homolytic fission does not correlate well with their biological activity in bioassays. As a consequence, it has been conjectured that heterolytic mechanisms of decomposition of RSNOs could also constitute significant pathways of their metabolism. In particular, transfer of NO^+ from thionitrites to thiols (with formation of a new thionitrite and a new thiol) is known to occur readily in solution[58]. The reaction has also been proved to take place under physiological conditions[59]. In addition, it has been found that transfer of NO^+ between thiols is much faster than release of NO from them (10s *vs.* several hours). The transnitrosation between thionitrites and amines is also known[39b]. In relation to this, there has been considerable concern for many years that nitrosation of secondary amines and amides by NO or some of its derivatives could lead to the formation of carcinogenic nitrosamines in the body.

The second possible form of heterolytic cleavage of the S-N bond, which would lead to formal release of NO^- or, more probably HNO, has been less studied. However, some examples have been reported[59] and they will be addressed in section 5.7.

(iii) Finally, thionitrites can participate in a third type of processes, namely electron-transfer reactions induced or catalysed by metals as, for example in the general **Reactions 11** and **12**. Clearly, many *in vivo* reactions take place via metal catalysis, with the metal being usually bound to a protein. Consequently, the study of the transformations of thionitrites in the presence of either free-metals or bound-metals (bound forming complexes or bound to proteins) is of great interest. The decomposition reactions of thionitrites catalysed by copper, mercury or iron salts would be included under this category.

Reactions 11 and 12. Electron-transfer processes of thionitrites.

In all of the types of transformations presented above, electron-transfer, whether inner or outer sphere is followed by cleavage of the thionitrite S-N bond. Nevertheless, transformations in which cleavage of the C-S bond takes place should also be considered. Although it is expected that they would have little biological significance, they may be interesting from the chemical point of view. In this context, the tendency of a thionitrite to give either S-N bond fission or C-S bond fission can be rationalised in terms of the relative weight of its two resonance forms depicted in **Figure 14**.

Figure 14. Thionitrite resonance forms.

Thus, the degree of double bond character of the S-N bond will determine the relative ease of its fission, with compounds in which form II is of predominant character being more stable towards S-N bond fission. Which of the two resonance forms contributes more to the electronic description of the substrate will be governed by the

nature of the R group attached to the sulfur atom. This particular aspect will be addressed later in more detail.

5. 3. Preparation of thionitrites.

5. 3. 1. General methods for the nitrosation of thiols[39b].

The preparation of thionitrites by nitrosation of thiols was first described in 1840[60], when a red colour was observed on addition of nitrous acid to thiol solutions. Since then, many other nitrosating agents have been developed for this transformation.

Both aromatic and aliphatic thionitrites have been prepared by treatment of thiols with NOX (X= Cl or Br), RONO, N_2O_4, N_2O_3, NO_2, HNO_2 or NO^+ salts. As expected, in most cases, the products were too unstable to be isolated. Oae[39c] suggested that the best method for effecting this transformation involved the use of N_2O_4 in $CHCl_3$ or CCl_4. Williams[39a] has advocated the use of the same reagent in an inert solvent such as $CHCl_3$, CCl_4, hexane, acetonitrile or ether, or the use of alkyl nitrites such as *tert*-butylnitrite in $CHCl_3$ as the best methods. In most of the studies currently carried out, nitrosation of thiols is achieved either by the use of sodium nitrite in acidified aqueous solutions or by the use of commercially available alkyl nitrites (*tert*-butylnitrite or isoamylnitrite) in organic solvents. Other reagents, which have little synthetic use, can also effect electrophilic nitrosation of thiols. These include ON-SCN, ON-$S^+C(NR_2)_2$, ONS^+Me_2, ON-$S_2O_3^-$. ON-SO_2R and ON-SR or metal nitrosyl complexes, among others.

From a kinetic point of view, the nitrosation of thiols has frequently been compared to the analogous reactions with alcohols or amines. Several conclusions can be drawn from these comparative studies: *viz.*, nitrosation of thiols is essentially, for all practical purposes irreversible, whilst nitrosation of alcohols is significantly reversible, nitrosation on sulfur is faster than on oxygen and on nitrogen and, when both sulfur and nitrogen are present in the same molecule, nitrosation occurs initially on the sulfur atom. However, *N*-nitrosated products can be recovered after more prolonged reaction times.

The nitrosation of thiols by nitrous acid (HNO_2) is an acid catalysed reaction which follows the rate equation r= k[HNO_2][RSH][H^+]. *k* values for many thiols have been determined[61] and indicate that the reaction rate tends towards the encounter-control limit. The true nitrosating species is believed to be $H_2NO_2^+$ or NO^+ formed from the

reaction of HNO_2 with acid (**Scheme 17**). The process is also catalysed by added nucleophiles (Cl^-, Br^-, SCN^-, thiourea) and, when these are present, the nitrosating species is assumed to be NOX, where X is the added nucleophile, formed from the reaction of HNO_2 with X. In this case the rate equation is better described as r= k_2[XNO][RSH], the same as when the nitrosation is carried out by using pure NOX as the nitrosating agent. Second order rate constants k_2 have also been determined for several thiols[61] . For very reactive substrates, the formation of XNO becomes the rate-determining step.

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \rightleftharpoons NO^+ + H_2O$$

$$NO^+ + RSH \longrightarrow RSNO + H^+$$

$$HNO_2 + H^+ + X^- \rightleftharpoons NOX + H_2O$$

$$NOX + RSH \longrightarrow RSNO + XH$$

Scheme 17. Nitrosation of thiols by nitrous acid, reactions involved.[61]

The nitrosation of thiols by alkyl nitrites has also been addressed from a mechanistic standpoint. Kinetic studies were carried out both in water[62] and in alcoholic solvents[63]. It was found that alkyl nitrites are efficient *S*-nitrosating agents in both solvents. By way of contrast, *N*-nitrosation in alcoholic solvents is only efficient when nucleophiles are added as catalysts. In mildly acidic water, alkyl nitrites are hydrolysed to give HNO_2 which, in turn, produces the effective reagent ($H_2NO_2^+$ or NO^+, **Scheme 18**). At basic pH, thiolate anions react directly with alkyl nitrites with direct transfer of NO^+. In alcoholic solvents, the mechanism is believed to involve some protonated form of the alkyl nitrite ($RO^+(H)NO$) and there is evidence that this is the true nitrosating agent and not free NO^+. If nucleophiles are added, the reaction is accelerated, again *via* the formation of NOX.

$$R'ONO \overset{H_2O}{\rightleftharpoons} R'OH + HNO_2$$

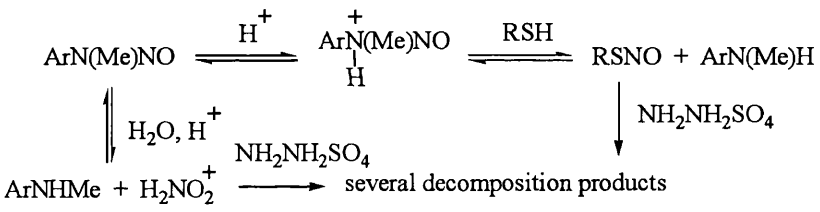
$$RSH \rightleftharpoons RS^- + H^+$$

$$R'ONO \overset{H^+}{\rightleftharpoons} R'\overset{+}{O}(H)NO$$

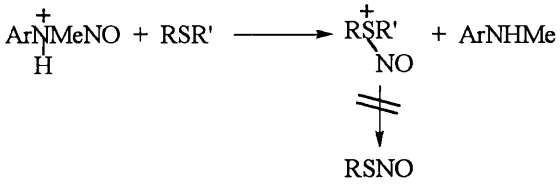
$$R'\overset{+}{O}(H)NO + RS^- \longrightarrow R'OH + RSNO$$

Scheme 18. Nitrosation of thiols by alkyl nitrites, reactions involved.[62, 63]

The nitrosation of thiols by nitrosamines has also been investigated by Williams and co-workers[64]. As a model, they analysed the kinetics of the reactions between *N*-methyl-nitrosoaniline and several thiols in aqueous acidic (H_2SO_4) solutions. In order to ensure irreversibility and to avoid complications arising from a competing reaction via $H_2NO_2^+$, hydrazine sulfate was added as a trap of the nitrite and thionitrite formed (**Scheme 19**). The conclusion drawn from this work was that direct nitrosation of thiols by nitrosamines (probably in their protonated forms) takes place but is very slow. Interestingly, it was also shown that substitution on the sulfur atom (for instance, *via* methylation) accelerates the reaction. This suggests that sulfides (RSR') would undergo nitrosation by nitrosamines quite readily. However, no leaving group would be available in this case to allow the formation of RSNO as the end product (**Reaction 13**). This issue was further explored and the ions derived from the nitrosation of sulfides by nitrous acid were found to be effective agents for the nitrosation of diverse substrates, effectively acting as catalysts in these processes.



Scheme 19. Nitrosation of thiols by nitrosamines.[64]



Reaction 13. Nitrosation of sulfides by nitrosamines.

5. 3. 2. Reactions of thiols with NO.

In the presence of oxygen, NO is able to nitrosate thiols and other nucleophiles. The intermediates involved in this process have been the object of much discussion. In a recent study[65], it was shown that the rate determining step of the nitrosation by oxygenated NO solutions was the same as that of the auto-oxidation of NO in solution.

This corresponds to the formation of N_2O_2, which is the precursor for NO_2 and N_2O_3, which, in turn, were formulated to be the only nitrosating species involved, in order to adjust the kinetic data obtained.

It is worth recalling here that the reaction of NO with low molecular weight thiols in the absence of oxygen, does not generally yield thionitrites but disulfides and that this is assumed to take place by the mechanism depicted in **Scheme 8**[19] in Chapter 3 (Nitric Oxide in Chemistry). Two other patterns for the reaction of NO with thiols under deaerated conditions were discovered later[66] and these will be discussed in section 5.7.

5. 3. 3. Other sources of thionitrites.

The preparation of thionitrites from thiols is the most common route. Nevertheless, thionitrites have also been obtained from disulfides although the reaction is of little use since a complex mixture of products is formed. This was first recognised by Oae[67], who described the oxidation of several unsymmetrical disulfides with excess N_2O_4 at 0 °C to give symmetrical disulfides, thiosulfonates and sulfonic acids as the main products. Thionitrites were detected as intermediates in these reactions (**Scheme 20**).

$$R'SSR \xrightarrow{N_2O_4} [RSNO + R'SNO] \longrightarrow RSSR + RSO_3H, R'SO_3H + RSSO_2R$$

Scheme 20. Formation of thionitrites from disulfides.[67]

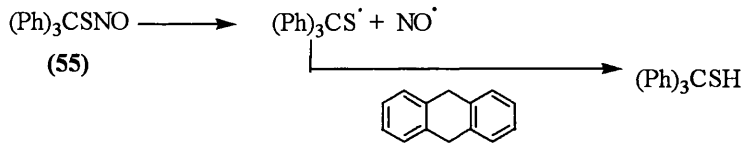
5. 4. Thermal decomposition of thionitrites.

It has been known for a long time that all thionitrites decompose at room temperature or upon heating to give nitric oxide and the corresponding disulfide (**Reaction 14**).

$$RSNO \xrightarrow{\Delta} RSSR + NO$$

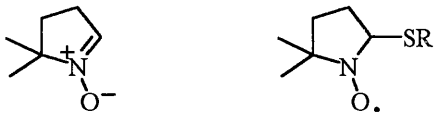
Reaction 14. Thermal decomposition of thionitrites.

The mechanism has been proved to involve homolytic fission of the S-N bond with formation of intermediate thiyl radicals as the first step. In 1968, Van Zwett *et. al.*[52] showed that thionitrite (**55**) decomposes upon heating to give tritylthiol in the presence of a radical scavenger such as 5, 10-dihydroanthracene and this was taken as a proof of the formation of intermediate thiyl radicals (**Reaction 15**).



Reaction 15. Scavenging of thiyl radicals formed during thermal decomposition of RSNOs.[52]

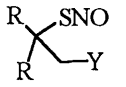
In 1978, Field[53] described the thermally induced polymerisation of methyl methacrylate in the presence of SNAP (**32**), concluding this was indicative of the homolysis of SNAP upon heating. Later, Josephy *et al.*[68] carried out a more rigorous investigation into the possible mechanism of the thermal decomposition of several thionitrites. In this work, thiyl radicals generated upon heating of thionitrite solutions were trapped with DMPO (**60a**) and the adducts formed (**60b**), (**Figure 15**) were shown to be ESR active and to exhibit the expected splitting.



(**60a**) (**60b**)

Figure 15.

In an attempt to rationalise the factors governing the thermal stability of thionitrites, Fontcave[69] synthesised a series of hydrosoluble thionitrites derived from cysteamines and mercaptoethanols of general formulae **61** and studied their thermally induced decomposition. The substrates were found to be increasingly stable with increasing substitution at the alpha carbon. It was also shown that the ability to release NO increased in the order: OH< $NHCOCH_3$<NH_3^+ for the substitution at the beta carbon. In addition, decomposition was highly dependent on the pH of the solutions, thionitrites becoming more stable at acidic pH. The authors explained these trends by making use of the argument based on the resonance forms of thionitrites, discussed earlier (**Figure 14**).

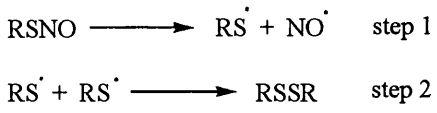


(**61**)

R= H, Me, Et
Y= $NH_3^+Cl^-$, OH, $NHCOCH_3$, H

According to this argument, form II would be expected to be stabilised in acidic conditions making thionitrites less labile towards NO release at low pH. The same resonance form would be destabilised by an R group with a positive charge on it, such as protonated amine, due to repulsion between two vicinal charges of the same sign. The higher stability of more substituted thionitrites was also ascribed to major stabilisation of

form II by the electron-releasing inductive effects of the alkyl groups at the alpha carbon. However, in a later study by Butler[70], it was conjectured that steric interactions in the dimerisation reaction of thiyl radicals leading to the product disulfide (step 2, **Scheme 21**) were likely to be the main factor responsible for the higher stability of more substituted thionitrites and that the degree of substitution at the alpha carbon was unlikely to contribute much to the strength of the S-N bond (affecting step 1).

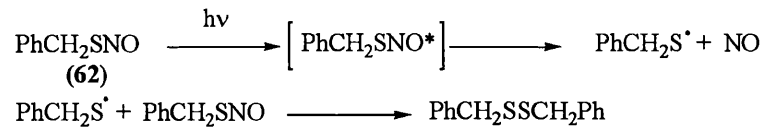


Scheme 21. Steps involved in the mechanism of thermal decomposition of RSNOs.[70]

The validity of this argument was proven both by means of *ab initio* calculations and experimental studies. The latter involved the use of differential scanning calorimetry and thermogravimetric analysis. Heats of reaction for the first step of the reaction were determined for two substrates, namely GSNO and SNAP, taken as models for primary and tertiary thionitrites respectively. The values for both models were found to be rather similar. Contrastingly, *ab initio* calculations indicated that tertiary disulfides such as di-(*tert*-butyl) disulfide formed in the second step are highly energetic (12.7 kJ/mol) whereas primary ones (such as diethyl disulfide) were considerably lower in energy (1.4 kJ/mol).

5. 5. Photochemistry of thionitrites.

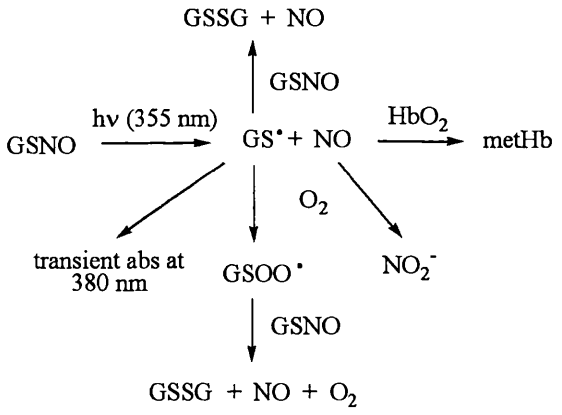
The first reports on the photochemical decomposition of thionitrites to give the same products as those obtained thermally, *viz.* NO and disulfides, date from the late 60s. Barrett and co-workers[71] studied the photochemically induced decomposition of benzyl thionitrite (**62**) and several other primary thionitrites in hexane solutions by irradiation at 365 nm. The mechanism was formulated to involve initial homolytic fission of the S-N bond to give intermediate free radicals, which could then react with another molecule of thionitrite to afford the final disulfides (**Scheme 22**). The simultaneous polymerisation of acrylonitrile and methyl methacrylate monomers and the decrease of the quantum yield of thionitrite disappearance in the presence of these additives were accepted as evidence for the formation of intermediate thiyl radicals.



Scheme 22. Photochemical decomposition of benzyl thionitrite.[71]

Twenty years later, the photochemical cleavage of SNAP was explored and also found to induce the polymerisation of methyl methacrylate monomers[53]. This effect was again considered to arise from the formation of thiyl radicals during the process.

More recently, a rather extensive analysis of the reaction of GSNO with visible or UV light (550 or 330 nm) in water or aqueous buffers has been reported[72]. The likely fate of all the radical species formed was investigated, as well as the effect of the presence or absence of several additives, such as molecular oxygen and different quenchers, on the final outcome of the reaction. The results are summarised in **Scheme 23**.



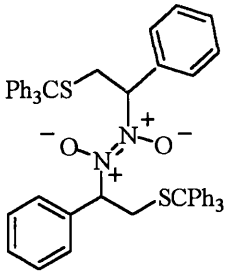
Scheme 23. Photochemical decomposition of GSNO.

Laser absorption spectroscopy studies determined that the mechanism does not involve simply homolysis followed by direct recombination of thiyl radicals, but rather reaction of thiyl radicals with another molecule of RSNO in its ground state. In the presence of a sufficient concentration of oxygen, thiyl radicals would react preferentially to give GSOO· which could, in turn, react with another molecule of GSNO to give more disulfide and NO. Thus, NO would be produced not only by direct homolysis but also *via* the reactions of GSNO with either GS· or GSOO·. In aerated aqueous solutions, NO was removed by conversion to nitrite or it reacted with oxyhaemoglobin to yield methaemoglobin and nitrate. Addition of *trans, trans*-2,4-hexadien-1-ol prevented the formation of GSOO· radicals in oxygen saturated solutions and also reduced the rate of

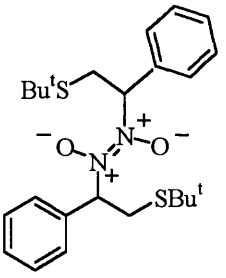
reaction between thiyl radicals and more GSNO, indicating that thiyl radicals react faster with dienes than with RSNOs or oxygen. Addition of reduced glutathione GSH or its anion had no effect on the observed kinetics and the measured amplitudes of absorption. Other authors[73] had noted that irradiation of GSNO either by visible or UV light enhanced its toxicity in cell cultures, thereby providing an indication for accelerated formation of NO.

Singh *et al.*[74] have investigated the mechanism of the photochemical decomposition of SNAP, GSNO and CysNO and compared it to the metal catalysed decomposition of the same substrates (*vide infra*). DMPO (**60a**) was used as a thiyl radical trap and the characteristic ESR spectra of the corresponding adducts (**60b**) were obtained. By way of contrast, metal catalysed decomposition of the same substrates in the dark did not generate ESR active compounds in the presence of added DMPO.

The photochemical decomposition of thionitrites either in organic or in aqueous solvents has not found general application in organic synthesis. Only one example can be found in the literature dating from 1979. Gowenlock and co-workers[75] used this reaction for the UV-induced synthesis of the α-alkylthio nitroso dimers **63** and **64**, derived respectively from the photochemical addition of trityl thionitrite (**55**) and *tert*-butylthionitrite (**54**) across the double bond of styrene.



(63)



(64)

Figure 16. UV-induced addition of thionitrites onto styrene.[75]

An analogous reaction with nitrosamines had been previously reported to yield either α-oximino amines **65** by direct addition onto alkenes, or oximes **66** and iminium ions **67** by addition followed by re-organisation and cleavage, depending on the conditions[76] (**Scheme 24**).

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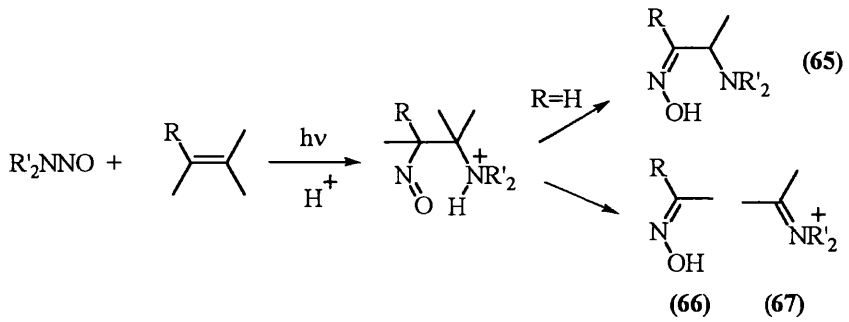


Scheme 24. Addition of nitrosamines across double bonds.[76]

5. 6. Metal-catalysed reactions of thionitrites.

The reactions of thionitrites in the presence of metal salts, especially of copper, have received considerable attention. In general, for the following discussion, they will be divided into three main groups, according to the nature of the products formed. The first group corresponds to reactions in which polysulfides, mainly trisulfides RS_3R and tetrasulfides RS_4R, are obtained as the major products. The work in this area has been carried out mainly by the group of Oae and co-workers and involves the use of organic solvents and copper salts. The second group includes decomposition of thionitrites in the presence of metals in aqueous solutions and gives only the disulfide RS_2R as the final product. This work is mainly by Williams and collaborators. Reactions of thionitrites with other metal ions, such as mercury or iron, to give various products will be discussed in a third section.

5. 6. 1. Polysulfides from thionitrites decomposition catalysed by copper salts.

During the late seventies, Oae[77] published a series of papers in which *tert*-butylthionitrite was used as a nitrosating agent in several organic transformations. In this context, they carried out studies into a modification of the known Sandmeyer reaction of diazonium salts to give halogenated aromatic compounds. They described the *in situ* formation of the diazonium salts from the corresponding anilines by mixing the aniline substrate, a copper salt (CuX_2, X= Cl, Br) and *tert*-butylthionitrite as the nitrosating agent, in place of nitrite or alkyl nitrites, previously used to achieve this transformation

(**Scheme 25**). Besides the expected halogenated aromatic compounds they isolated two products derived from the decomposition of the thionitrite. These were identified as the trisulfide **68**, obtained in high yield, and the tetrasulfide **69**, obtained as a minor product. They pursued this result further by treating several thionitrites with one equivalent of copper (II) halide obtaining similar results (**Scheme 25**, yields of polysulfides are based on the initial amount of sulfur). In addition, when the pure disulfide was exposed to the same conditions, it afforded a mixture of the trisulfide and the tetrasulfide, the latter being this time isolated as the major product (**Reaction 16**).

$$^{t}BuSNO + ArNH_2 + CuX_2 \xrightarrow[0\text{-}25\ ^{\circ}C,\ Ar]{MeCN} ArX + N_2 + (^{t}Bu)_2S_2 + (^{t}Bu)_2S_3$$

X= Cl, Br — 9 % — 88 % (**68**)

$$RSNO \xrightarrow[25\ ^{\circ}C]{CuCl_2\ 1\ equiv.} R_2S_2 + R_2S_3 + R_2S_4$$

(R= ^{t}Bu, C_5H_{11}, C_9H_{11}) — 9 % — 59-81 % (**68**) — 3-8 % (**69**)

Scheme 25. Modification of the Sandmeyer reaction using thionitrites.[77]

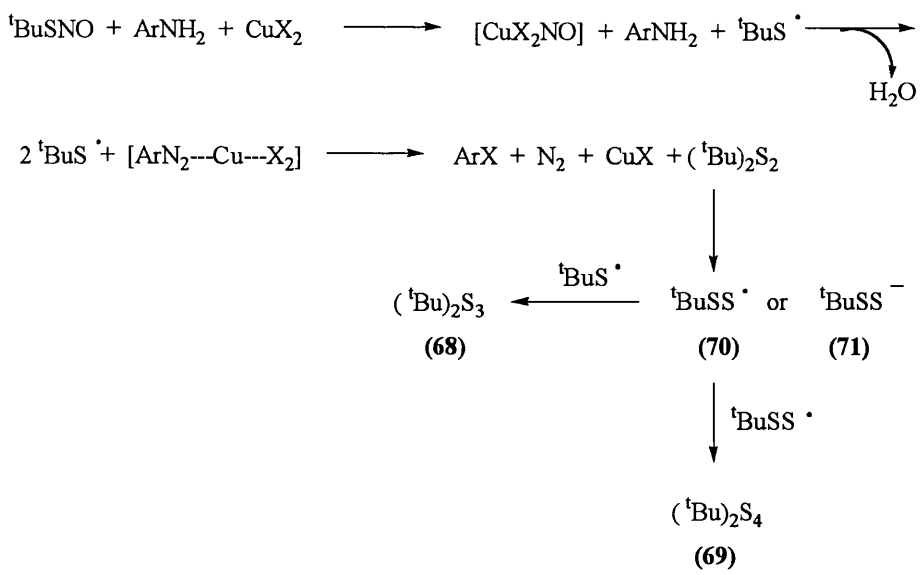
$$(^{t}Bu)_2S_2 \longrightarrow (^{t}Bu)_2S_3 + (^{t}Bu)_2S_4$$

25 % (**68**) — 65 % (**69**)

Reaction 16. Decomposition of disulfides in the presence of metal salts.[77]

In order to account for these results, the authors proposed the somewhat unusual mechanism depicted below (**Scheme 26**).

According to this mechanism, the reaction would be initiated by homolysis of the S-N bond to give an intermediate thiyl radical. This could then either dimerise or react with another molecule of RSNO to afford the disulfide. In turn, this would dissociate either by homolysis to give a perthiyl radical **70**, or heterolytic fission to give anion **71**. Subsequent reactions would lead to the formation of trisulfide or tetrasulfide as the final products.



Scheme 26. Transformations of thionitrites in the presence of copper halides.[77]

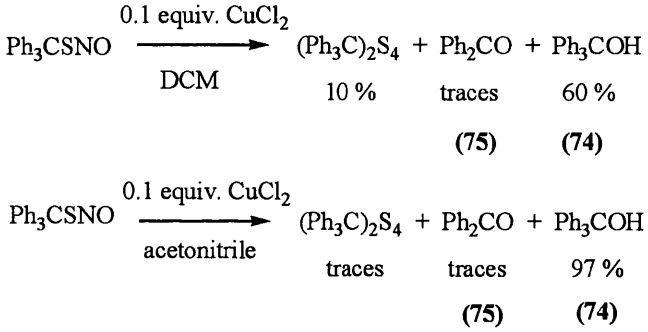
Recently, in our group[78], we explored the reactions of thionitrites with several metal salts. In agreement with previous observations, reaction of *tert*-butylthionitrite with an equimolecular amount of $CuCl_2$ in acetonitrile afforded the corresponding trisulfide in 93 % yield. In dichloromethane and using a catalytic amount of the copper salt (0.1 equiv.), the trisulfide was again isolated as the major product in 72 % yield. However, the use of dodecylthionitrite ($C_9H_{19}(CH_3)_2CSNO$) yielded mainly the disulfide under the same conditions. This last result is in contradiction to that obtained by Oae[77] with the very similar substrate *tert*-nonylthionitrite. The Japanese group reported isolation of the corresponding trisulfide in 77 % and no disulfide was detected.

Interestingly, reaction of pure adamantyl thionitrite **72** under the same conditions furnished the trisulfide in 33 % yield and traces of the disulfide (7 %). In contrast, when the sample of **72** employed was contaminated by traces of the corresponding thiol **73** (7 %) only the disulfide was obtained in 49 % after treatment with copper halides. The disulfide was also formed as the sole product upon exposure of a sample of the pure thiol **73** to identical conditions.

(72) (73)

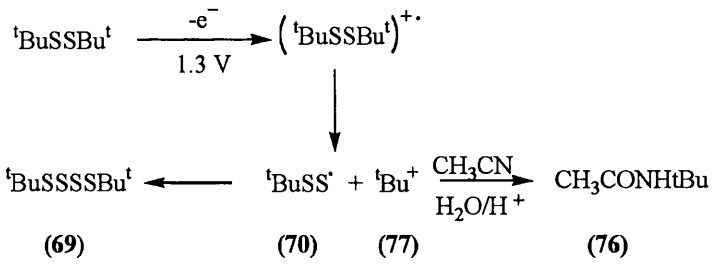
Figure 17. Adamantyl thiol and adamantyl thionitrite.[78]

When trityl thionitrite (**55**) was subjected to the same conditions (0.1 equiv. CuX_2 in DCM or acetonitrile), trityl alcohol (**74**) was identified as the major product together with traces of the tetrasulfide. Traces of benzophenone (**75**) were also detected in all of these experiments (**Scheme 27**). The mechanism must involve preferential cleavage of the C-S bond over fission of the S-N bond as the first step.



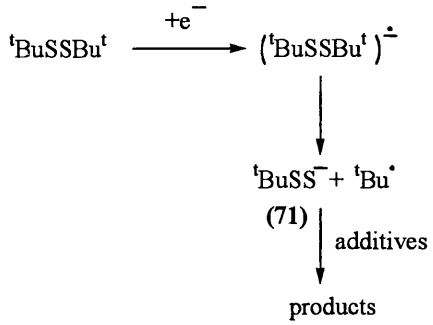
Scheme 27. Tritylthionitrite decomposition catalysed by copper.[78]

The most remarkable aspect of the transformations discussed above is the "growing sulfur chain effect". According to the mechanism proposed by Oae and co-workers[77], this would be ascribed to "spontaneous" fission of the C-S bond of an initially formed disulfide, to yield intermediates **70** or **71** . Such processes have been described to occur under electrochemical oxidative or reductive conditions. Thus, oxidation of *tert*-butyl disulfide in acetonitrile at a working potential of 1.3 V gave two main products, namely *tert*-butyltetrasulfide (**69**) (96 %) and *N-tert*-butylacetamide (**76**) (93 %)[79]. The authors explained the formation of **76** by the reaction of the *tert*-butyl cation (**77**) with the solvent, as in the Ritter reaction. Dimerisation of *tert*-butylperthiyl radicals **70** would afford the tetrasulfide (**69**), (**Scheme 28**).



Scheme 28. Electrochemical oxidation of disulfides.[79]

Conversely, under reductive conditions[80], *tert*-butyl disulfide was transformed into products whose formation could be rationalised in terms of a preferred C-S bond fission, yielding intermediate anion **71 (Scheme 29)**.



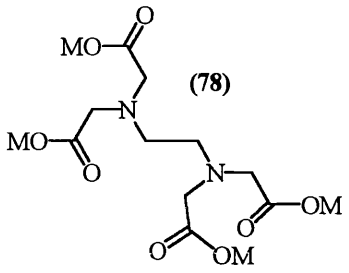
Scheme 29. Electrochemical reduction of disulfides.[80]

5. 6. 2. Disulfides from the decomposition of thionitrites catalysed by copper salts.

Decomposition of thionitrites in water or aqueous buffers in the presence of several metal ions (specially Cu^{2+}) has been the object of close scrutiny, specially since it was realised that these reactions might occur *in vivo*. Unfortunately, the majority of quantitative results reported before 1993 were found to be erratic and irreproducible. It was this time when Williams and co-workers[81] recognised that the presence of even trace amounts of copper salts as impurities in the distilled water employed in such studies, could catalyse the decomposition of thionitrites. A great deal of work has been carried out in this field after this discovery and a qualitative mechanistic picture has been established.

Several aspects of the reaction appear to be quite general. The role of Cu^{2+} in these reactions is catalytic and even concentration as low as 10^{-6} M can be sufficient to bring about the transformation. Thus, the presence of adventitious copper in distilled water

should not be neglected. In practise, the process can be completely inhibited if a chelating ligand for Cu^{2+} is added to the medium, and the addition of excess of Cu^{2+} over the concentration of the chelatant re-establishes decomposition. Ethylenediaminetetraacetate salts (**78**, EDTA) are the most frequently employed chelatants for such a purpose.



(78)

The initial products of decomposition are the disulfide and NO. The latter has been detected in unaerated solutions using a commercial NO-probe. In aerated aqueous solutions, NO is quantitatively transformed into nitrite. No significant levels of catalysis have been observed with other metal ions such as Mg^{2+}, Zn^{2+}, Ca^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+} or Fe^{3+}. However, ferrous ion has a similar effect to Cu^{2+} and Ag^{+} shows some catalysis but to a lesser extent. The decomposition is pH dependent, being enhanced at higher pHs and reaching a maximum for pH values around 7.

The first detailed kinetic study of this reaction, along with an investigation into structure-activity relathionships was carried out by Williams[82]. The authors followed spectrophotometrically the disappearance of the characteristic thionitrite absorptions in the 350 nm region of the spectrum. Reactions were monitored at 25 °C and pH 7.4, over a range of Cu^{2+} concentrations added in the form of hydrated $CuCl_2$. It was noted that within a particular range for each substrate the reaction showed first order behaviour with respect to the thionitrite concentration. Outside this range the kinetic pattern became more complicated. A relatively small intercept at added $[Cu^{2+}]= 0$ was always detected and was attributed to the decomposition brought about by adventitious copper. The processes were first order with respect to the concentration of Cu^{2+}. The rate equation $r= k[RSNO][Cu^{2+}] + k'$ was established. Values for k and k' were determined for a series of 18 thionitrites, some of which have been depicted in **Figure 18**, and some structure-reactivity relathionships could be inferred from them.

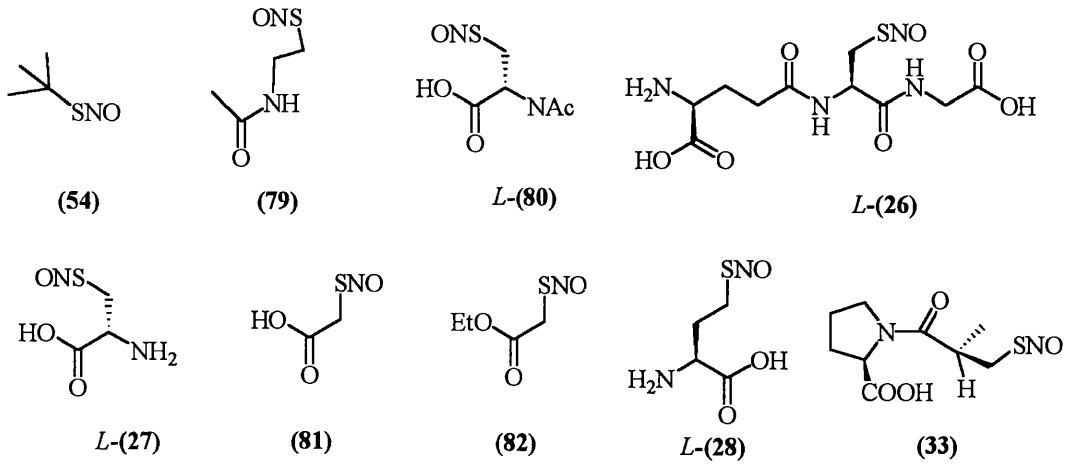


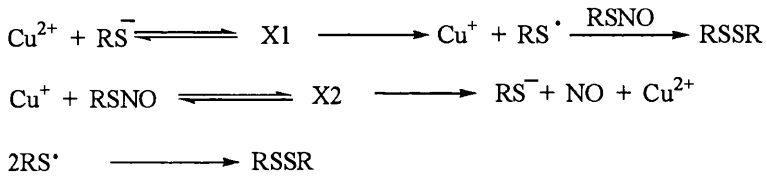
Figure 18. Structures of several of the thionitrites studied by Williams.[82]

The reaction was found to be almost non-existent for *tert*-butyl thionitrite (**54**), *S*-nitroso-*N*-acetylcysteamine (**79**), *S*-nitroso-*N*-acetylcysteine (**80**) and ethyl *S*-nitrosomercaptoacetate (**82**). Conversely, the decomposition of substrates containing free amines or carboxylates such as *S*-nitrosocysteine (**27**) or *S*-nitrosomercaptoacetic acid (**81**) was shown to be fast when these substituents were situated at a distance of 5 atoms from the nitroso-group nitrogen. Increasing this length by one additional methylene group, also reduced the rate of decomposition as was observed, for instance, for GSNO (**26**), *S*-nitrosohomocysteine (**28**) or *S*-nitrosocaptopryl (**33**). These results suggested that, for rapid reaction to occur, the copper needs to be bound to the substrate in a bidentate fashion (**Figure 19** below). The metal atom in this complex would be ligated through the N atom of the nitroso group and the N or O atom of the substituent, thus forming a six membered ring. Formation of a seven membered ring would be disfavoured. According to this premise, the authors found it difficult to explain why SNAP (**32**) should react at all under the conditions of study. For this particular case they evoked the implication of other factors such as the gem-dimethyl effect. On the same grounds, it was also found that decomposition was slowed when the potential chelating carboxylate or amino groups were protected.

In further studies, the reaction was found to be strongly inhibited in the presence of a chelating agent for Cu^+ (neocuproine) and consequently it was suggested that the reduced form of copper, Cu^+ could in fact be the true catalyst in this reaction[83]. Several facts were taken as confirming evidence for the involvement of Cu^+: *viz.* decomposition was effectively stopped in the presence of an excess of neocuprine, the characteristic

spectrum of the copper (I) adduct with this chelating agent which shows absorbance at 256 nm, was obtained and the decomposition of several thionitrites was accelerated by the addition of a Cu^+ salt instead of a Cu^{2+} one[84]. In addition, copper (I) chelation was shown to reduce the biological activity of thionitrites in washed human platelets[84c]. However, the effect of copper addition in the decomposition rates and biological activities of several thionitrites were found not to correlate. That is, there was a discrepancy between the effect of copper chelation on the chemical breakdown and on the biological actions of RSNOs. Thus, these results have to be interpreted with caution.

On the basis of all these observations the mechanism shown below was proposed (**Scheme 30**).



Scheme 30. Mechanism for the decomposition of thionitrites catalysed by copper in H_2O.[83]

According to this picture, Cu^{2+}would first be reduced to Cu^+. This, after acting as the catalyst in the decomposition of RSNOs, would be re-oxidised back to Cu^{2+} later in the reaction. It was first advanced that there is usually enough thiolate present in "pure" samples of thionitrite to bring about the reduction of Cu^{2+}. An alternative explanation was that thionitrites could partially be hydrolysed in the aqueous medium with or without the intervention of copper, to yield the necessary thiol. This last explanation was later confirmed during a study by the same authors[85]. Intermediate X1 above was proposed to be a structure based on $RSCu^+$ whereas X2 would be a bidentate complex of the thionitrite with Cu^+ (**Figure 19**).

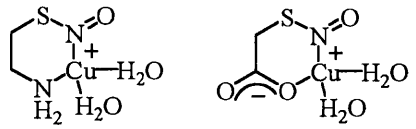


Figure 19. Structures proposed for intermediate X2.[83]

It has also been noted that the rate of the decomposition of stable thionitrites such as GSNO is dependent upon the presence of dissolved oxygen[83]. Furthermore, for these substrates a very long induction period is reproducibly observed. These effects have been

explained as related to the ability of oxygen to re-oxidise Cu^{+} to Cu^{2+}, thus preventing the decomposition of the thionitrite until all of the oxygen has been consumed. In agreement with this hypothesis, the induction period was completely suppressed if the reduced thiol (RSH) was added in catalytic quantities.

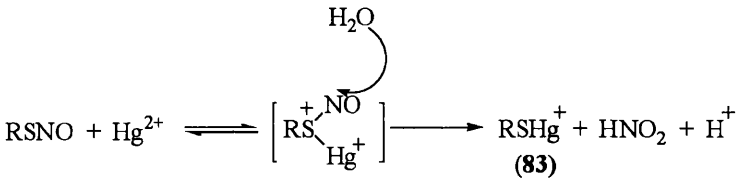
In order to establish the feasibility of these reactions in biological systems, Williams and co-workers[86] examined the decomposition of several thionitrites in the presence of three protein-bound copper sources: Gly-Gly-His-Cu^{2+}, $(His)_2Cu^{2+}$ and HSA-Cu^{2+}. Treatment of these with a thiol at pH 7.4 confirmed that the reduction of Cu^{2+} to Cu^{+} takes place under physiological conditions even when the metal atoms are bound to a protein. In addition, the three copper complexes were able to catalyse thionitrite decomposition. Nevertheless, the reactions in all cases were slower than when free copper ions were used. The decomposition of thionitrites was also examined in the presence of the glycoprotein ceruloplasmin (most of the total copper in plasma is bound to this protein) and the reaction was found to be first order with respect to the protein concentration.

Although the mechanism proposed by Williams and co-workers is now generally accepted and there is much experimental data in support of it, there are some features which are still not clear. One of these is the intermediate formation of thiyl radicals. For instance, Singh *et al.*[74] found that decomposition of thionitrites carried out in the presence of added or contaminating copper did not generate ESR adducts with DMPO used as a radical trap. Besides, the presence of DMPO had no effect on the yield of formation of the final disulfide as it would be expected if thiyl radicals were involved.

5. 6. 3. Decomposition of thionitrites in the presence of other metals.

5. 6. 3. 1. Reaction with mercury.

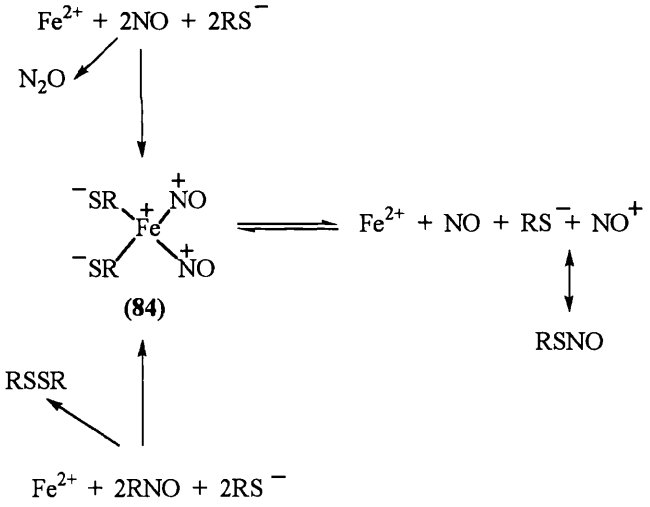
Hg^{2+} salts as stoichiometric reagents decompose thionitrites to their corresponding mercury complex (**83**) and nitrite, *via* the formal release of NO^{+}(**Reaction 17**)[87]. This reaction has been known for a long time and is the basis of an analytical test for thionitrites.[88]



Reaction 17. Reaction of thionitrites with mercury (II) salts.[87]

5. 6. 3. 2. Reaction with iron.

Studies of the formation and decomposition of thionitrites in the presence of Fe^{2+} salts and in the presence or absence of specific Fe^{2+} chelators, have determined that this ion catalyses both the formation of thionitrites from NO and thiols and the degradation of thionitrites in deoxygenated aqueous solutions[89] (**Scheme 31**). The conditions under which iron ions can preferentially catalyse the synthesis or the degradation of CysNO and GSNO in water at varied pHs have been investigated using optical and ESR spectroscopy. In all instances, formation of dinitrosyl iron complexes (**84**) could be detected by ESR.



Scheme 31. Catalysis of thionitrite formation and nitrosation by iron ions.[89]

In both instances the process of formation of **84** was found to be irreversible, the complex being in equilibrium with its constituents, Fe^{2+}, RS^- , NO and NO^+ (**Scheme 31**). The relative rates of the different reactions involved determined the ability of Fe^{2+} to catalyse either the accumulation of RSNO or its degradation to NO and disulfide. In turn, the relative values of these rates are governed by the relative concentrations of the different species implicated in this complicated system.

5. 6. 3. 3. Electrochemical studies of S-nitrosothiols.

Cyclic voltammetry of several thionitrites has been carried out using a Pt or Au electrode and a Ag/AgCl reference electrode (3M NaCl)[90] in water or acetonitrile at pH 7.4. It was found that the three substrates, SNAP (**32**), GSNO (**26**) and Glyco-SNAP (**36**) which were studied, exhibited only single reductions peaks at –0.97 V, –0.98 V and –0.91 V respectively, corresponding to the release of NO. The process was proved to be irreversible and to take place at a diffusion-controlled rate, giving disulfide as the final product. It was also noted that a linear relationship between the peak potential (ease of reduction) and the pK_a of the thiol existed. It was proposed that this relationship could be used to predict the reduction potential of other thionitrites. In order to account for the results observed, the mechanism below was proposed (**Scheme 32**):

$$RSNO \xrightarrow{e^-} RS^- + NO$$

$$RS^- + H^+ \rightleftharpoons RSH$$

$$RSNO + RS^- \longrightarrow RSSR + NO^-$$

$$2NO^- + 2H^+ \longrightarrow N_2O + H_2O$$

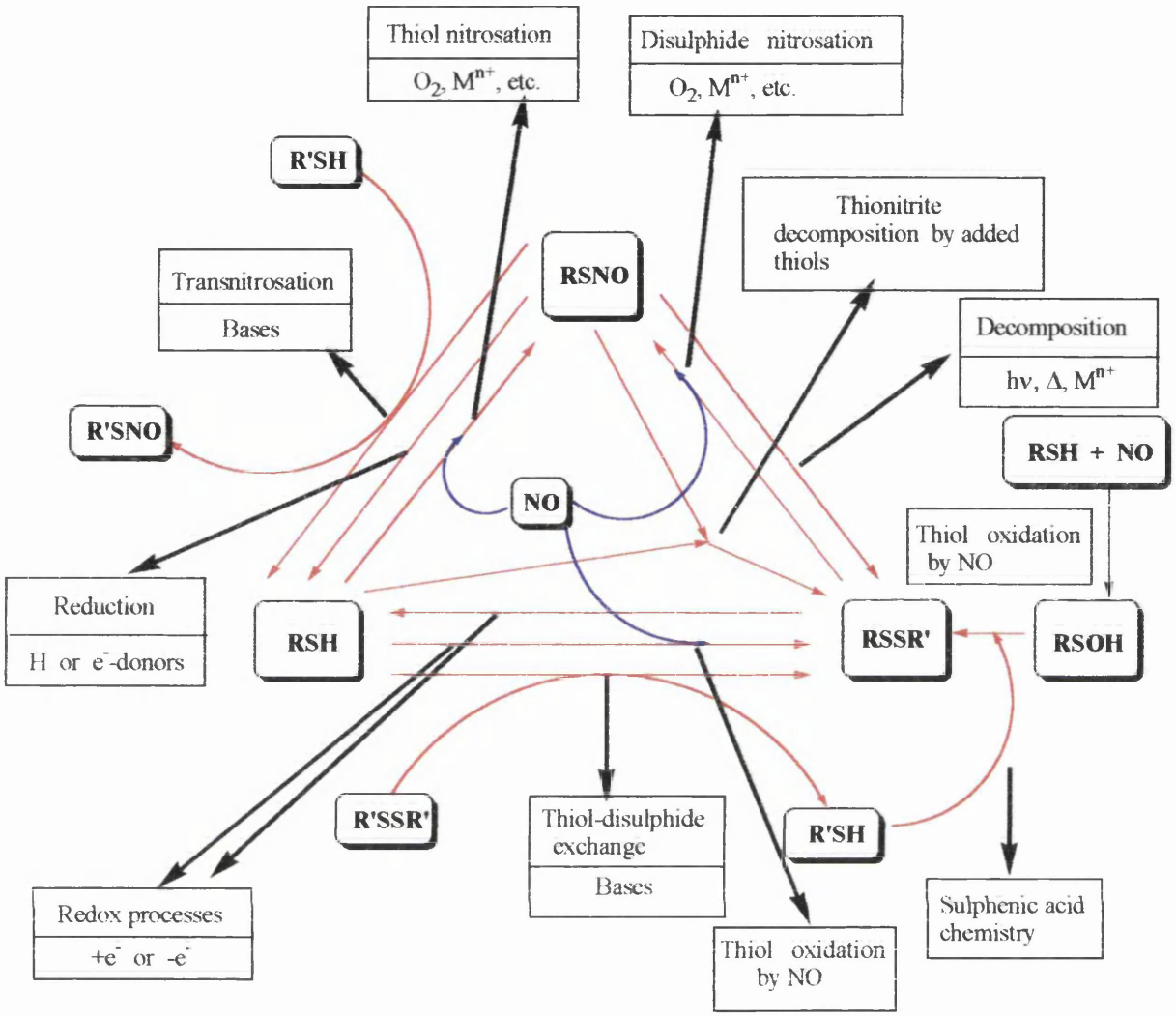
Scheme 32. Mechanism of the electrochemical decomposition of thionitrites.[90]

It is interesting to note that here the formation of disulfide is invoked to arise from the reaction of thiolate anion with thionitrite (*vide infra*, section 5.7) and does not involve formation of intermediate thiyl radicals. Their formation would imply that both an oxidation (of RS^- to $RS^{\cdot}$) and a reduction (of RSNO to RS^-) would have to take place in the same semi-cell, an event which is impossible.

5. 7. Thionitrites, thiols, sulfides, disulfides and other sulfur derivatives.

A large number of sulfur derivatives possess important biological activity. They range from thiols to sulfate esters, including compounds containing sulfur in oxidation states from –2 to +6[91]. As we have seen, nitrogen can also adopt a variety of oxidation numbers ranging from –3 for ammonia to + 5 for nitric acid or nitrates. All of these substances form an extremely complex interrelated system in biological systems to which metal salts have still to be added. In particular, some of the processes involving NO, nitrogen oxides, thiols, thionitrites and disulfides have been discussed or mentioned in the previous sections of this review: a) the nitrosation of thiols or disulfides by nitrogen

oxides, b) the oxidation of low molecular weight thiols by NO in non-aerated solutions giving rise to the formation of disulfides, c) the degradation of thionitrites to disulfides catalysed by heat, light or metal salts. Several other reactions involving thiols and disulfides are well-known. For instance thiols and disulfides are inter-converted by oxidation-reducing processes. They are also known to be involved in a fast exchange reaction which is catalysed by base[92]. In this section, some of the most relevant, among the other interactions between sulfur derivatives and NO derivatives which have received attention in the literature, will be discussed. Specifically, the last part of this survey will deal with three specific aspects *viz.*, 1. Interactions between thionitrites and thiols, namely transnitrosation and decomposition of thionitrites by added thiols. 2. The oxidation of thiols by unaerated NO to give higher oxidation states of sulfur (mainly sulfenic acids) or disulfides. 3. The nitrosylation and/or oxidation of protein thiols. A summary of the interactions covered in this review and how they relate to each other has been schematised in the next page (**Scheme 33**) and the substances which catalyse each of the processes have also been indicated.



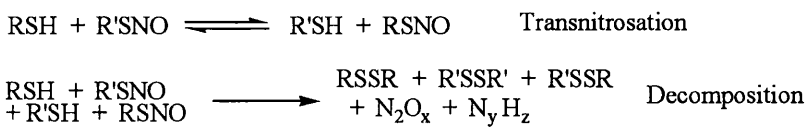
Scheme 33. Summary of the most important interactions between sulfur derivatives and NO.

5. 7. 1. thionitrites and thiols.

The reaction between a thiol and a thionitrite to give a mixture of disulfides has been known for some time[93]. However, a closer analysis revealed that in fact this transformation could be disconnected into two clearly differentiated processes. The primary products are those derived from the transfer of the NO^+ moiety between the thionitrite and the thiol, affording a thermodynamic mixture of the two corresponding thiols and two thionitrites (**Scheme 34**). The secondary process involves a complex cascade of reactions between the components of this mixture to yield, as the end products, a mixture of disulfides and several nitrogen derivatives of varied oxidation states.

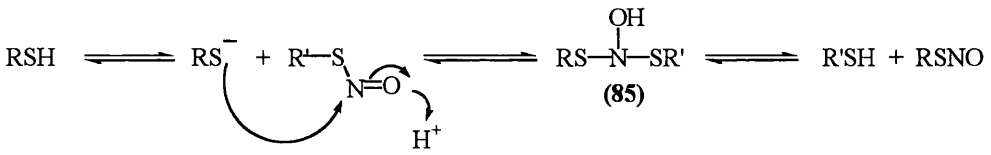
The transfer of the nitroso group between a thionitrite and a thiol has been shown to be an extremely fast process with the equilibrium being reached almost

instantaneously. This was first realised by monitoring the reaction either spectrophotometrically[94] or by HPLC[95] and detecting the formation of two new species (R'SH and RSNO) after a few seconds of mixing a thiol (RSH) and the thionitrite derived from a different thiol (R'SNO).



Scheme 34. Steps involved in the reaction between a thionitrite and a thiol.[94,95]

The first extensive investigation into the kinetics of this process was due to the group of Williams[96]. They studied the transnitrosation between a range of thionitrites and 2-hydroxyethanethiol, under conditions in which $[R'SH]_0 >> [RSNO]_0$ (in order to ensure irreversibility). The rate law $r = k_2[RSNO][R'S^-]$ was obtained and the values of k_2 were determined for several thionitrites. The reaction was found to be accelerated at higher pHs, as it would be expected if the thiolate form of the thiol was involved. It was also observed that transfer of the NO^+ group was faster from thionitrites containing an electron-withdrawing group, as would be expected if the nitroso group was acting as the electrophile. These observations were accounted for by the mechanism depicted below (**Scheme 35**) in which reaction occurs *via* attack of the thiolate anion as a nucleophile onto the nitrogen atom of the thionitrite nitroso group. According to this mechanism transnitrosation would proceed through intermediate **85** and no free NO^+ would be involved.

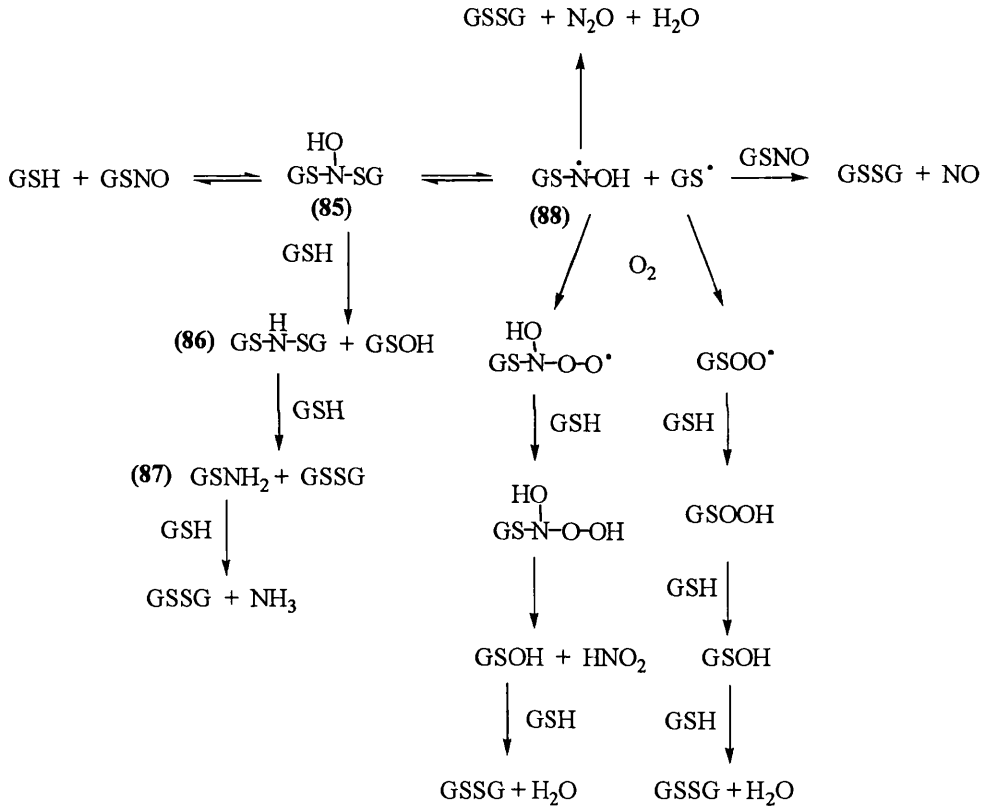


Scheme 35. Mechanism proposed for the transnitrosation between thionitrites and thiols.[96]

The second of the processes represented in **Scheme 34**, *viz.* the decomposition of thionitrites by added thiols, is much slower, although still faster than the spontaneous decomposition of the same thionitrites, and is characterised by an unexpectedly high degree of complexity. The reactions between several thiols and the thionitrites derived from them have been subjected to extensive analysis. In these particular situations,

transnitrosation becomes a degenerate process and therefore the system under study becomes simplified.

Thus, Singh *et al.*[97] have examined the decomposition of GSNO (**26**) in the presence of different concentrations of added GSH (between 1 and 10 fold excess). Disulfide (GSSG), nitrite, nitrous oxide and ammonia were characterised as the main products and the yields were determined for the reaction carried out under different conditions. In order to explain the stoichiometries observed, a complicated mechanistic picture was drawn which implicates different pathways for the oxygenated and non-oxygenated reactions (**Scheme 36**).



Scheme 36. Mechanisms proposed for the reaction between GSNO and GSH.[97]

It was suggested that both transnitrosation and formation of the disulfide proceed through the same intermediate **85**. From this intermediate several pathways are possible depending on the availability of O_2, GSH and GSNO. Reaction of **85** with two equivalents of GSH would lead to formation of disulfide and ammonia. Fission of intermediate **85** could lead to intermediate **88**, analogous to the intermediate invoked in the reaction between NO and thiols under deaerated conditions (**Scheme 9**). In the absence of oxygen, **88** would give rise to N_2O as the gaseous product and reaction of a

thiyl radical with another molecule of thionitrite would yield NO. In the presence of oxygen additional pathways have to be considered. Both intermediate **88** and thiyl radicals would react with O_2 to yield ultimately GSSG and nitrite as the end products.

A kinetic study of the reaction of CysNO with its corresponding thiol, in the presence of EDTA to scavenge metal ions has been carried out[98]. The rate equation $r = k_1[CysNO] + k_2[CysNO][CysSH]$ was determined. The first term accounts for the spontaneous decomposition of CysNO which is expected to be negligible in the case of GSNO but not for CysNO. Accordingly, k_1 was found to be independent of the pH whereas k_2 was dependent on this parameter. No product studies were undertaken for this compound.

In a recent investigation[99], the possible mechanism of the reaction between GSNO and GSH and, hence, by extension, between thionitrites and thiols in general, has been reconsidered. After a very comprehensive experimental study, the authors concluded that many of the observed products formed during the decomposition of GSNO by GSH may result from an initial generation of HNO by **Reaction 18**. This very reactive compound would arise from attack of thiolate onto the sulfur atom of thionitrite as opposed to the addition onto the N atom of the nitroso group which was invoked to explain transnitrosation.

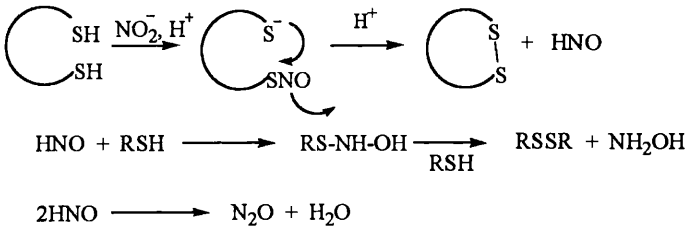
$$GSNO + GSH \longrightarrow GSSG + HNO$$

Reaction 18. Generation of HNO from a thionitrite-thiol reaction.[99]

Through a series of experiments with chemically generated HNO, the authors demonstrated possible pathways for the formation of N_2O, NH_3, NH_2OH, NO and NO_2^- (the nitrogen derivatives detected during decomposition of GSNO by GSH) by the reactions of HNO with itself, GSH, GSNO or other intermediates generated in the reactions of HNO with these two sulfur species. Mechanisms for these transformations were suggested.

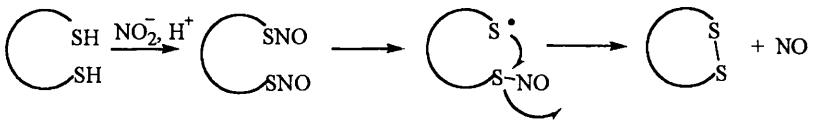
The intramolecular reaction between a thionitrite and a vicinal thiol has also been considered. Thus, Stamler *et al.*[59] showed that when substrates 1,3 and 1,4-dithiols were mono-nitrosylated with nitrous acid or by added GSNO under deaerated conditions, spontaneous formation of disulfide, hydroxylamine and nitrous oxide could be detected. The formation of the latter two products was taken to provide sufficient evidence for the

formation of HNO as an intermediate, which would be transformed to N_2O and NH_2OH by the reactions indicated below (**Scheme 37**; see also Scheme 8 and reaction 7 in Chapter 3. Nitric Oxide in Chemistry). This issue was additionally confirmed by the reduction of metmyoglobin (Fe(III)) by the products generated in the reaction (compare Scheme 8). Interestingly, when different quantities of a monothiol were incubated with a thionitrite under the same conditions, no hydroxylamine formation was detected, in agreement with the results of Singh *et al.*[97]. The mechanism below was proposed:



Scheme 37. Nitrosation of vicinal thiols and release of nitrous acid.[59]

The different possible reactions between dithiols were further examined as a model for dithiols in enzymes[100]. In addition to the decomposition of the mononitrosylated system, discussed above, the degradation of the dinitrosylated system was also studied for comparison purposes. Degradation of dinitrosylated dithiols gave NO as the only gaseous product together with the cyclic disulfide. Conversely, decomposition of the mononitrosylated dithiol yielded N_2O as the only detectable gaseous product, in a result which is partially in agreement with that of Stamler[59]. This product was proposed to arise from HNO formed as indicated in **Scheme 37**. Formation of NO from dinitrosylated substrates was assumed to take place *via* a different, homolytic pathway (**Scheme 38**)



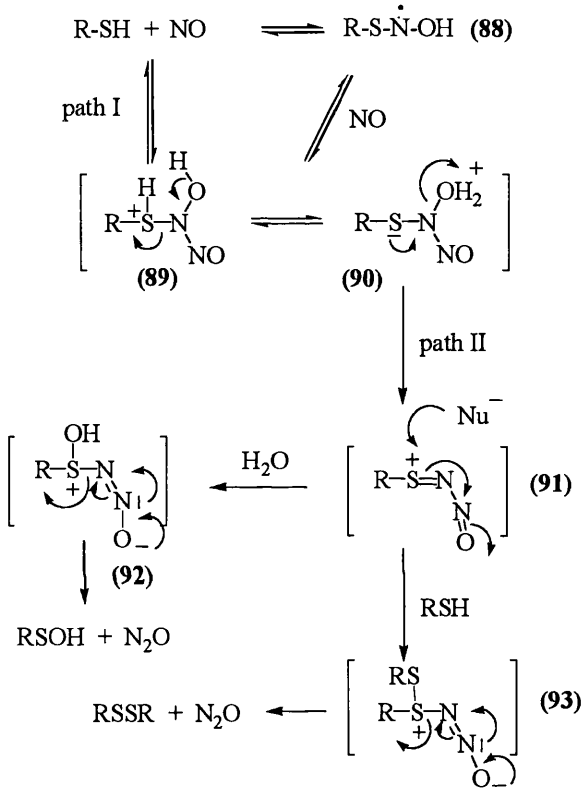
Scheme 38. Decomposition of dithionitrites.[100]

5. 7. 2. Thiols and NO.

The reaction between low molecular weight thiols and NO under deaerated conditions was discussed in Chapter 3. Nitric Oxide in Chemistry and was shown to give disulfides and N_2O as the end products. Two other patterns for the reaction of NO with thiols in deaerated conditions were discovered later[101] and are worth discussing here. The first one involves reaction of NO with high molecular weight thiols (such as human serum albumin, HSA) in deaerated aqueous solutions at pH 7.4. This reaction yielded the corresponding sulfenic acid instead of the expected disulfide and gave N_2O as the final gaseous product. Although the gaseous product formed was the same as that detected from the reaction of NO with a low molecular weight thiol, proposed to arise from the decomposition of HNO, the stoichiometry observed for both reactions differs: 1 mole of HSA was consumed for each mole of N_2O formed whereas 2 moles of low molecular weight thiol were consumed for each mole of N_2O formed under the same conditions. The second type of transformation of thiols by NO in the absence of oxygen corresponds to the oxidation of dithiols in water at pH 7.4. This should not be confused with the reactions discussed above which involve nitrosation and would derive from reaction with NO under aerated conditions or with a thionitrite (NO^+ donor to a thiol) under deaerated conditions. The reaction addressed here also yields a cyclic disulfide and N_2O in a 1: 1 stoichiometry and is analogous to the reaction with low molecular weigh thiols previously reported[19]. In order to explain the results for all the different types of thiols *via* a single mechanism the authors proposed that the transformations shown below (**Scheme 39**) would occur.

This mechanism could also serve to explain the formation of disulfides from low molecular weight thiols and differs from that proposed before[19] in several respects: instead of dimerisation of the initially formed intermediate radical **88**, which is an statistically unlikely event, the authors invoked reaction of **88** with another molecule of NO to yield intermediate **89-90**. This could either yield the starting materials back by a degenerate pathway I or could release H_2O furnishing intermediate **91** by pathway II. From here the mechanisms differ for the three types of thiols studied. In the case of low molecular weight thiols or dithiols, **91** would be attacked by another molecule of thiol in an intra or intermolecular fashion respectively, yielding the corresponding symmetrical or cyclic disulfide. In the case of high molecular weight thiols, attack onto **91** by a second

thiol molecule would be precluded for steric reasons. Instead, attack by a molecule of H_2O would be easier and would yield the sulfenic acid. Alternatively, sulfenic acid could be formed in all three cases and in the first two this would react with the remaining thiol yielding disulfides by a known reaction of sulfenic acids[102]. It is worth noting that according to this mechanism formation HNO as an intermediate does not need to be invoked since N_2O would be released directly from intermediates **92** or **93**.



Scheme 39. Different mechanisms proposed for the reaction of thiols with O_2-free NO.[101]

5. 7. 3. Nitrosylation/oxidation of protein thiols: NO signals.

In biological systems, NO and its derivatives have been found to interact with a large number of proteins. The modifications undergone by these proteins upon interaction with NO or its derivatives affect mainly two groups, namely iron-containing cofactors and thiol residues (mainly cysteines). A wide range of protein-types have been found to be either stimulated or inhibited by such processes, including several types of enzymes, receptors, transcription factors, membrane ion-channels and signalling proteins among others[103]. The modifications introduced into proteins after their synthesis are termed post-translational and many examples of these are known to be effected by other small gaseous molecules, specially O_2, CO and CO_2. Post-translational modifications of

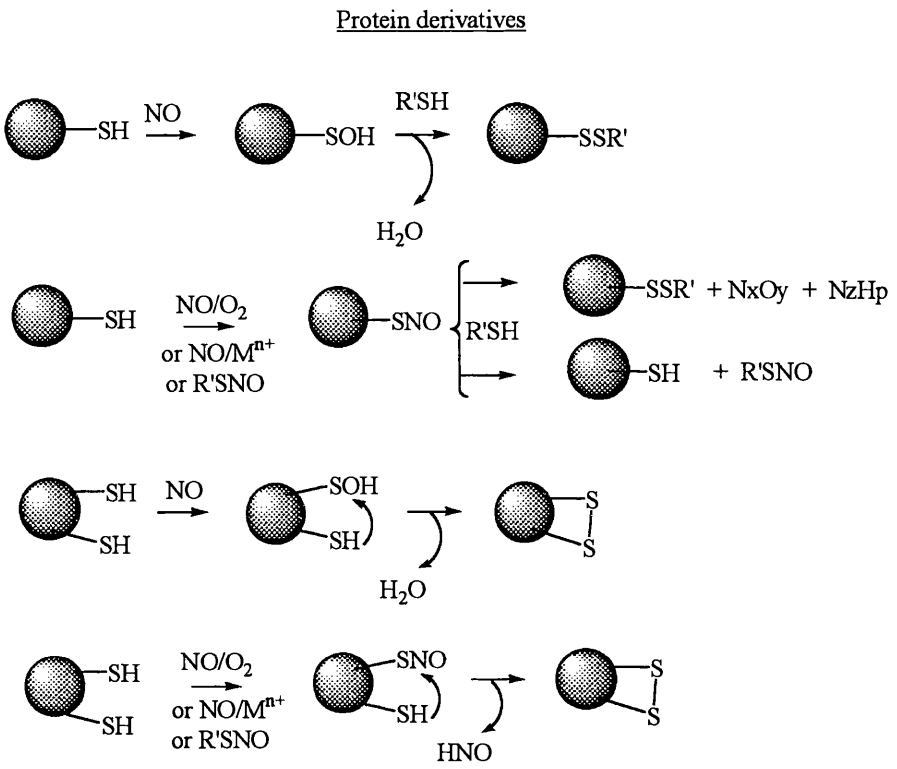
proteins regulate their activities and can be divided into two categories. The first one involves covalent or coordinative linkage of some species to a certain domain of the protein. The second one involves redox modification. For instance, oxygen covalently attaches to the haem group of haemoglobin or cytochrome P450. In a similar manner, activation of soluble Guanylate Cyclase (sGC), an intracellular signalling protein, involves reversible NO coordination to the haem-iron of the protein cofactor. These reactions trigger a cascade of molecular events inside the cells (signal transduction events) which result in a functional response at the cellular level *viz.*, activation or deactivation of certain processes.

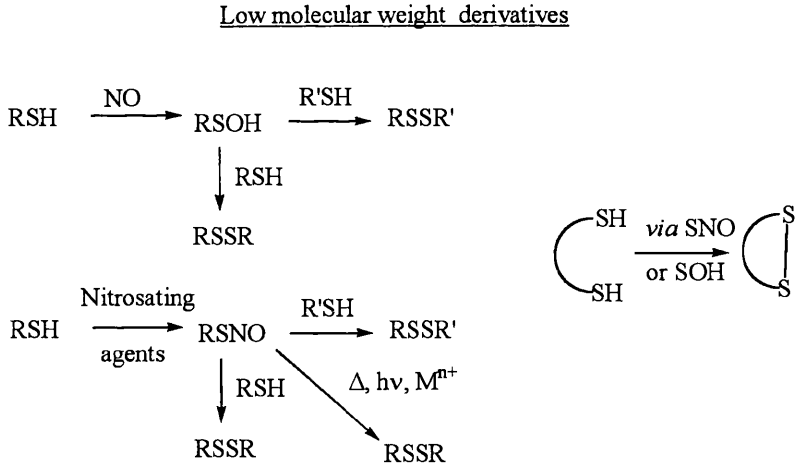
For the purposes of this review, we are more interested in the post-translational modifications introduced by NO into protein-thiol groups. NO-mediated regulation has been found to take place both *via* covalent addition of the NO moiety (nitrosylation) and by redox alterations of the thiol groups (mainly oxidation) by NO[104]. These processes can regulate protein function by several discrete mechanisms. Modifications of thiol groups which are distant from the active site bring about conformational changes, since hydrogen bonding and/or electrostatic interactions with the affected thiol group become altered. When the affected thiol is situated in the immediate vicinity of another thiol, nitrosylation or oxidation to a sulfenic acid may accelerate intramolecular disulfide-bond formation. Conversely, intermolecular reaction with another thiol results in formation of mixed disulfides. Finally, if the thiol group is situated at the receptor site of an enzyme, nitrosylation or oxidation may impair its recognition ability and result in inactivation. These events have been found to take place upon nitrosylation of protein thiols *in vitro* (both in solution and in cells) with a number of reagents. It has also been demonstrated that activation of Nitric Oxide Synthase, which regulates the endogenous production of NO, results in nitrosylation of certain protein thiols. However, the molecular mechanism for the *in vivo* *S*-nitrosylation of these molecules remains unknown. At least four possible mechanisms have been proposed: attack by a reactive NO species generated in a suitable molecular environtment, a somewhat controversial peroxynitrite-mediated *S*-nitrosylation, nitrosylation mediated by dinitrosyl-iron complexes and *S*-transnitrosation reactions with low molecular weight thiols such as GSNO. Similarly, exposure of protein thiols to NO under deaerated conditions *in vitro* has resulted in oxidation to the corresponding sulfenic acid. Nevertheless, although activation of endogenous NO

production has been shown to result in oxidation of certain protein thiols, the mechanism for this transfomation *in vivo* is again unknown.

It has been conjectured[105] that reversible post-translational modifications can be used in biological regulation whereas irreversible modifications cannot. The latter results in loss of function and plays a role in toxicity, but not in regulation. NO, GSNO and dinitrosyl-iron complexes, which are the major forms of NO-related activity *in vivo*, produce SNO, SOH and RSSR modifications of protein sulfur, all of which are reversible. Conversely, reactive oxygen species are apt to generate irreversible oxidations of sulfur to sulfinic and sulfonic acids. Thus, a picture has emerged of NO serving mainly as a regulator in signal transduction and of reactive oxygen species, sometimes in combination with NO, playing a larger role in toxicity.

In summary, it is worth insisting on the fact that the reactivity of sulfur derivatives varies dramatically when one moves from low molecular weight substrates to protein systems. Thus, the reactions which are expected to be relevant when studying GSNO or CysNO and their derivatives will be different to those which will become important when treating large proteins. For instance, protein nitrosothiols and sulfenic acids may be additionally stabilised by elements of protein structure or due to their size. Thus, certain protein sulfenic acids have been found to possess a high degree of stability in the absence of near thiols, whilst small sulfenic acids are prone to decomposition in solution by self-condensation or fast reaction with other thiols[106]. **Scheme 40** summarises the reactions which are considered to be of importance for protein sulfur compounds on the one hand and for low molecular weight sulfur derivatives on the other.





Scheme 40. Summary of the reactions of protein *vs* low molecular thiols and NO derivatives.

CHAPTER 6

CONCLUDING REMARKS AND AIMS.

In the past, most of the reactions of thionitrites found little application in organic synthesis. The main reason for this was their inherent instability. The development of modern techniques, which allow the manipulation of sensitive substrates, has re-opened the field of thionitrite chemistry and there is presently wide scope for new research. In this context, our first aim was to find new applications of the wide reactivity of thionitrites in modern organic methodology. We have concentrated in several reactions of thionitrites which are of radical nature.

The biochemistry of NO and thionitrites is still little understood and has turned out to be surprisingly complex. Hence, the drawing of conclusions from simple model studies requires extreme caution. Careful analysis of all the possible pathways implicated under a wide variety of conditions (i.e., presence or absence of oxygen, metal salts, contaminating thiols, etc.) have to be carried out before any conclusions can be inferred as to the possible mechanisms involved. Taking these issues into consideration, the second of our major aims was to study thionitrites from a biological standpoint, both by carrying out experiments in simple model systems, mimicking physiological conditions, and undertaking studies *in vitro* using life tissues.

PART TWO: RESULTS AND DISCUSSION.

CHAPTER 1

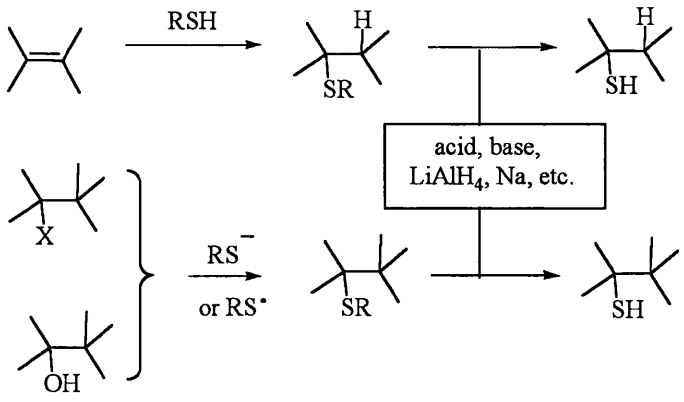
SYNTHESIS OF ALLYLIC TERTIARY THIONITRITES AND OF PRIMARY AND SECONDARY THIOLS FOR THE *IN SITU* PREPARATION OF PRIMARY AND SECONDARY THIONITRITES.

1. 1. Introduction.

As we have seen in the previous introductory section, both the thermal and photochemical decomposition of thionitrites appear to take place *via* cleavage of the S-N bond and formation of intermediate thiyl radicals. Given the prominent reactivity of thiyl radicals towards double bonds, it therefore seemed of interest to us to study the reactivity of alkene-containing thionitrites. Since tertiary alkyl thionitrites are more stable than primary, secondary or aromatic ones, our interest focused initially on the preparation of unsaturated tertiary alkyl thionitrites. Primary and secondary thionitrites are prone to rapid decomposition on attempted isolation. Nevertheless, they are sufficiently stable in solution if kept at low temperatures and, for some applications, they can be prepared *in situ* prior to their use. To this end, several primary and secondary unsaturated thiols were also prepared.

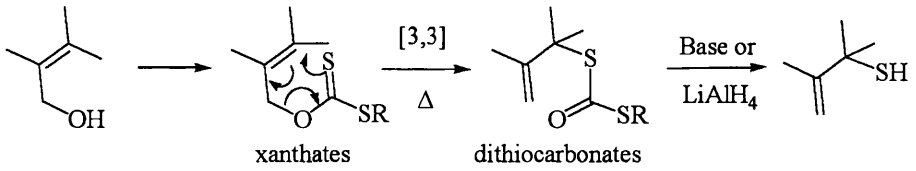
The most common route for the preparation of thionitrites involves direct nitrosation of thiols. Hence, the problem of thionitrite preparation can be reduced to the synthesis of the corresponding thiol. As already discussed, most previous workers in this area have synthesised thionitrites from commercially available thiols, either directly or after simple modifications, such as the attachment of other readily available moieties such as sugars or amino acids. However, this has limited the number of substrates which have been analysed. The preparation of alkene-containing thionitrites described herein, therefore, required longer synthetic sequences.

An inspection of the literature reveals that the synthesis of unsaturated thiols, many of which are considered to be unstable, has received little attention. In general, thiols can be prepared indirectly by the reactions of alkenes, alkyl halides or alcohols with sulfur containing-compounds such as thiols, thiol carboxylic acids, thiourea, xanthate salts, etc. Hydrolysis, reduction or some other form of bond cleavage of the resulting intermediates then furnishes the thiol (**Scheme 41**)[107].



Scheme 41. General methods for the synthesis of thiols[107].

For obvious reasons, the alkene routes are not suitable for accessing unsaturated thiols, unless the double bond is to be introduced in a later step, complicating the synthesis. On the other hand, most routes from alcohols or haloalkanes imply S_N2-type reactions and, therefore, become inefficient with hindered tertiary substrates. Other methods, useful for tertiary substrates, which are based on radical-type substitution processes, have been devised[108] but, again, these did not seem appropriate in our case, since the radicals involved would interfere with an already present double bond. A convenient route, which has been used to introduce a sulfur atom onto a quaternary carbon[109], is based on the thermal hetero-Claisen rearrangement of allylic xanthates to the corresponding dithiocarbonates (**Scheme 42**). Hydrolysis or reduction of these provides a regiospecific route to tertiary allylic thiols. The thiols thus obtained will contain a double bond on the allylic position in addition to any other double bond already present in the substrate.

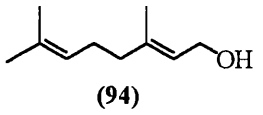


Scheme 42. Synthesis of tertiary allylic thiols by the hetero-Claisen rearrangement[109].

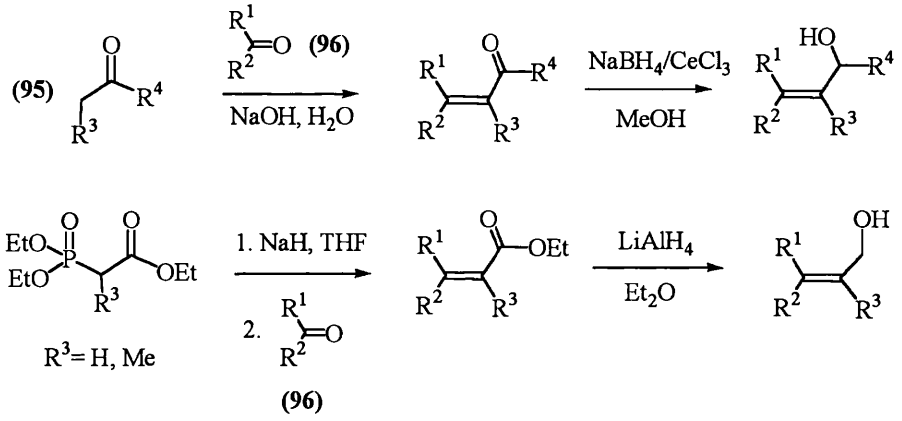
1. 2. Preparation of tertiary allylic thionitrites.

1. 2. 1. Synthesis of allyl alcohol precursors.

In order to apply this methodology, a series of allylic alcohols were first obtained. Geranyl alcohol (**94**) was commercially available. The rest of the alcohols used were prepared by $NaBH_4/CeCl_3$ reduction of α,β-unsaturated ketones or by $LiAlH_4$ reduction of α,β-unsaturated esters. The former can be obtained by aldol condensation of two suitably substituted ketones **95** and **96**. The latter were synthesised from a ketone (**96**) and triethyl phosphonoacetate or triethyl phosphonopropionate by the Wadsworth-Horner-Emmons (WHE) reaction. The two general routes have been illustrated below (**Scheme 43**).



(94)



Scheme 43. Routes chosen for the preparation of allylic alcohol precursors.

Thus, esters **97-102** were prepared from 4-*tert*-butylcyclohexanone, 5-hexen-2-one, 3-phenyl-2-propanone and cyclopentanone *via* the WHE approach. The corresponding allylic alcohols **103-108** were obtained after reduction with $LiAlH_4$. The results are summarised in **Table 1**. Alcohol **109** was obtained by $NaBH_4/CeCl_3$ induced reduction of isophorone (3,5,5-trimethyl-2-cyclohexen-1-one). Alcohol **110** was prepared in two steps from cyclopentanone *via* ketone **111** (**Scheme 44**).

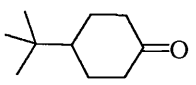
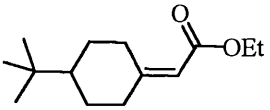
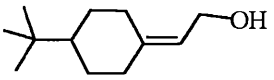
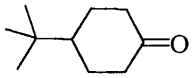
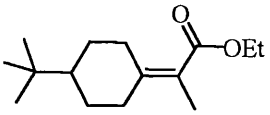
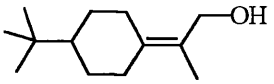
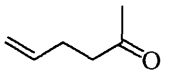
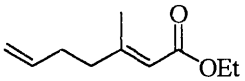
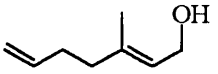
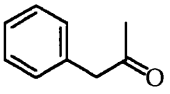
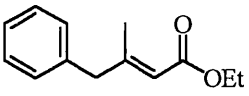
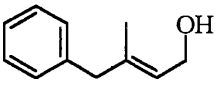
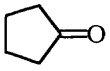
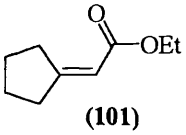
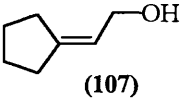
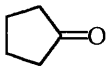
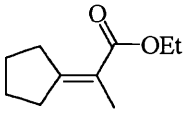
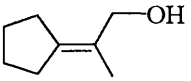
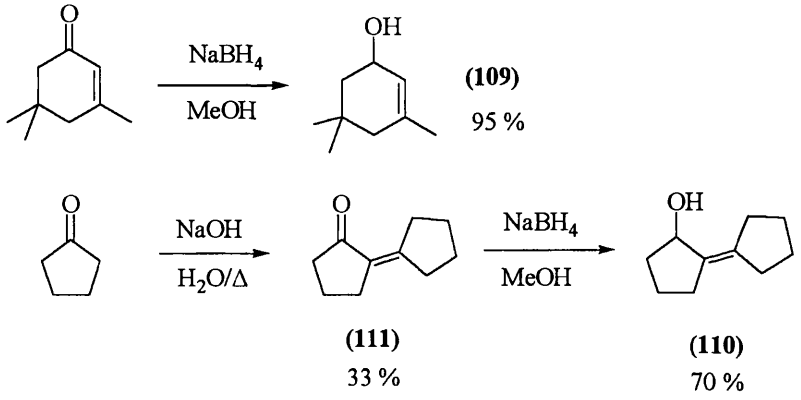
Starting ketone	Ester (yield)		Alcohol (yield)	
	 **(97)**	(99 %)	 **(103)**	(70-87 %)
	 **(98)**	(98 %)	 **(104)**	(71 %)
	 **(99)**	(86 %)	 **(105)**	(92 %)
	 **(100)**	(99 %)	 **(106)**	(84 %)
	 **(101)**	(99 %)	 **(107)**	(73 %)
	 **(102)**	(95 %)	 **(108)**	(50 %)

Table 1. Preparation of α, β-unsaturated esters and allylic alcohols.



Scheme 44. Preparation of allylic alcohols *via* reduction of α,β-unsaturated ketones.

1. 2. 2. Preparation of tertiary allylic dithiocarbonates.

Allylic alcohols **94** and **103-110** were next converted into their corresponding transposed tertiary allylic dithiocarbonates **112-122**. Formation of the intermediate xanthates was achieved by deprotonation of the alcohol with NaH or Bu^tOK in THF, followed by nucleophilic addition of the generated alkoxide anion onto CS_2 and, finally, trapping of the xanthate anion with MeI, following the usual one-pot literature procedure[109b]. Upon heating at reflux for 4-7 hours, the pre-formed xanthates quantitatively rearranged *in situ* to the corresponding transposed dithiocarbonates (see **Scheme 42** above). The structures of the tertiary dithiocarbonates prepared and the yields obtained by the two methods employed have been summarised in **Tables 2** and **3**. Assignment of the stereochemistry of thionitrites **112-115** will be discussed later in this chapter.

It has been reported that the rearrangement of certain allylic xanthates can occur during work-up[110] or on contact with silica[111] or alumina supports[112,113]. The isomerisation of tertiary xanthates seems to be extremely fast at room temperature and these cannot be isolated. Conversely, the rearrangement of primary xanthates, such as the ones we have prepared here, require high temperatures or acid catalysis. In agreement with these reports, we have observed that, on attempted purification by chromatography on silica gel, the xanthate derived from geraniol partially rearranged, yielding mixtures of xanthate and dithiocarbonate. Partial rearrangement was also observed upon standing at r.t., in $CDCl_3$ which may contain traces of acid. In the absence of acid and upon standing of the oil at room temperature, the process also took place, but was relatively slow (several days). Geranyl xanthate could be kept for several weeks at –20 °C, after neutral work-up of the reaction mixture. Complete isomerisation was achieved on heating at reflux in THF for 3-7 hours. For our purposes, xanthates were not isolated and rearranged *in situ* under the conditions described above.

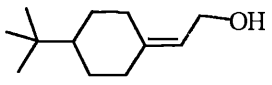
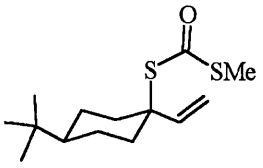
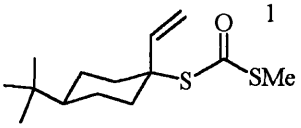
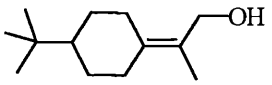
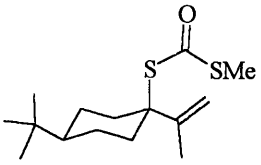
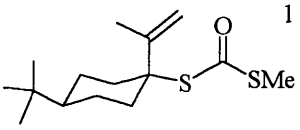
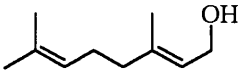
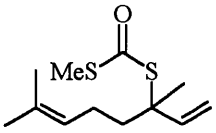
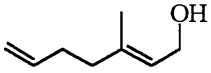
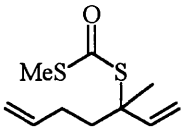
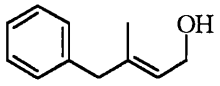
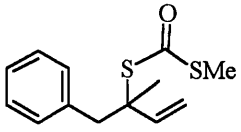
Alcohol	Tertiary dithiocarbonate	NaH (yield)	Bu^{t}OK (yield)
 (103)	 (112) 2 : 1  (113)	(43-55 %)	(65 %)
 (104)	 (114) 1.5 : 1  (115)	(39 %)	-
 (94)	 (116)	(53-63 %)	-
 (105)	 (117)	(37-55 %)	(66 %)
 (106)	 (118)	(47 %)	(57 %)

Table 2. Preparation of tertiary allylic thionitrites (I).

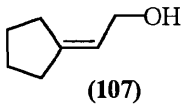
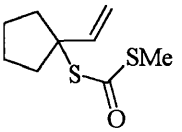
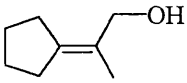
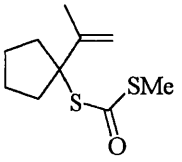
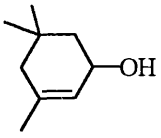
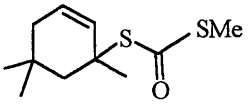
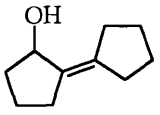
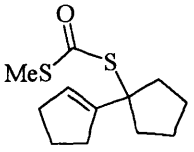
Alcohol	Tertiary dithiocarbonate	NaH (yield)	Bu^{t}OK (yield)
 (107)	 (119)	(26 %)	(76 %)
 (108)	 (120)	-	(52 %)
 (109)	 (121)	(*ca.* 35 %)	(70 %)
 (110)	 (122)	(20 %)	-

Table 3. Preparation of tertiary allylic thionitrites (II).

In our hands, Bu^{t}OK was found to give better results than NaH as the base. Isolated yields were higher and, in addition, when NaH was employed, by-products proved extremely difficult (sometimes impossible) to separate from the desired products by chromatography on silica. We also found that prolonged heating (more than 7 h) led to formation of small amounts of isomerised primary or secondary dithiocarbonates. In one occasion, an isomerised sulfide was obtained. In several instances, these products also appeared or their quantities increased during chromatography. Since longer purification was necessary when NaH was used as the base, isomerised products were isolated in relatively large quantities in those reactions (the yields have been collected in **Table 4**). In contrast, tertiary dithiocarbonates could be obtained pure after chromatography on silica gel when Bu^{t}OK was used, as long as prolonged heating was avoided.

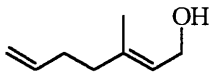
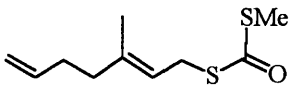
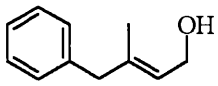
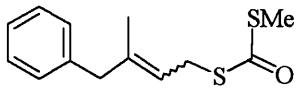
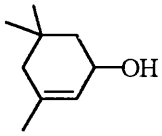
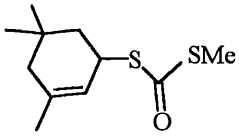
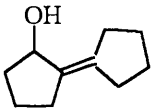
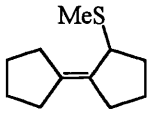
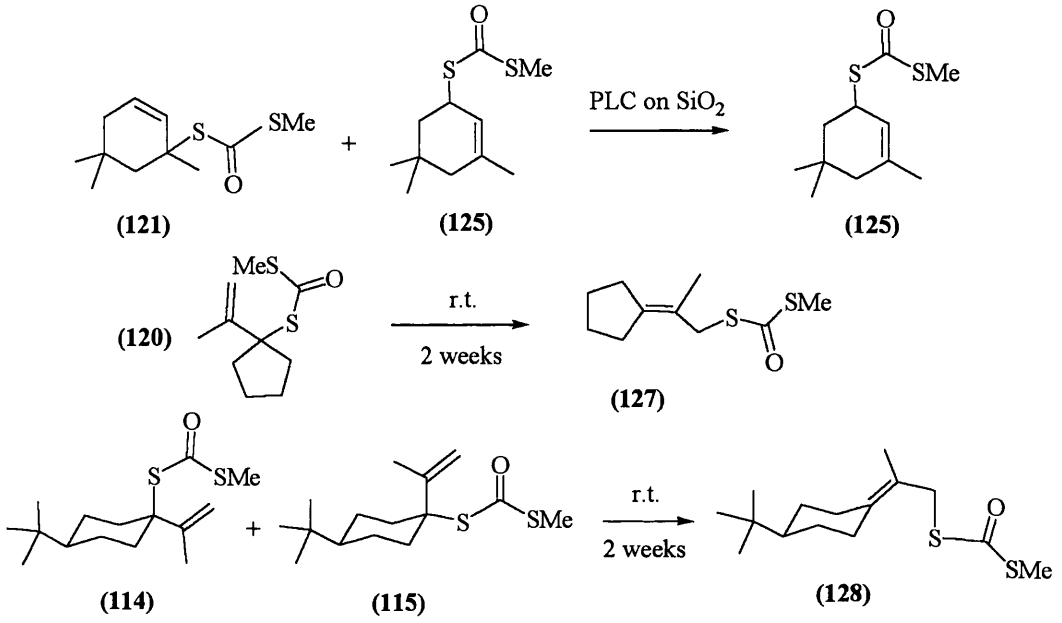
Alcohol	Rearranged by-products	Yield
 (105)	 (123)	(8-9 %)
 (106)	 (124)	(9-10 %)
 (109)	 (125)	(*ca.* 35 %)
 (110)	 (126)	(18 %)

Table 4. Isomerised by-products obtained during the NaH methodology.

Thus, we have detected two modes for further [1,3]-transposition of tertiary dithiocarbonates: *viz.* prolonged heating and lengthy chromatography on silica. Confirmation that the second mode of isomerisation was prevalent, was obtained from an attempt to separate dithiocarbonates **121** and **125** by preparative TLC on a silica plate (**Scheme 45**). This yielded isomerised secondary dithiocarbonate **125** as the only product after 3-4 hours of chromatography.

Furthermore, when tertiary dithiocarbonate **120** and the mixture of **114** and **115**, obtained by the NaH method, were left standing at room temperature under normal laboratory lighting for two weeks, rearranged primary dithiocarbonates **127** and **128** were formed quantitatively (**Scheme 45**). These results suggested that the isomerisations might be slow thermal or photochemical processes or Lewis acids catalysed conversions, depending upon the substrate and the conditions. In a comparative study, tertiary dithiocarbonate **118** was simultaneously exposed to three different conditions: heating at reflux in THF, standing under normal laboratory lighting (neat product) and reaction in a

silica gel suspension in THF. After 3 days, the crude of the first reaction showed the presence of both isomers of the dithiocarbonate in a proportion near to 1:1 (^{1}H NMR). Other by-products had also been formed. In the other two reactions, less than 10 % of the rearranged primary dithiocarbonate had formed after the same period of time.



Scheme 45. Observed isomerisations of tertiary allylic dithiocarbonates.

Formation of primary or secondary dithiocarbonates **123-125** and **127-128** can be rationalised in terms of a formal [1,3]-shift of the initially generated tertiary dithiocarbonates **114-115**, **117-118**, and **120-121**. Alternatively, they could arise from an oxygen to sulfur [1,3]-transposition of the allylic xanthate (**Figure 20**) *via* a four-membered transition state. Product **126** would arise from tertiary dithiocarbonate **122** *via* COS extrusion with concomitant double bond migration. The second type of process is well documented in the literature and a concerted mechanism *via* a six-membered transition state (retro-ene type process) has been proposed[114] (**Scheme 46**).

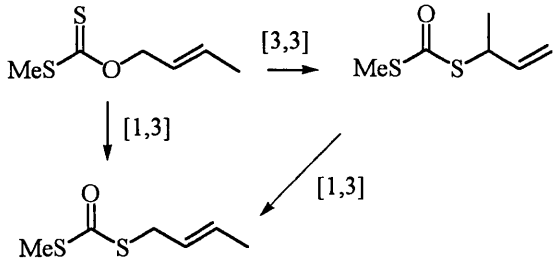
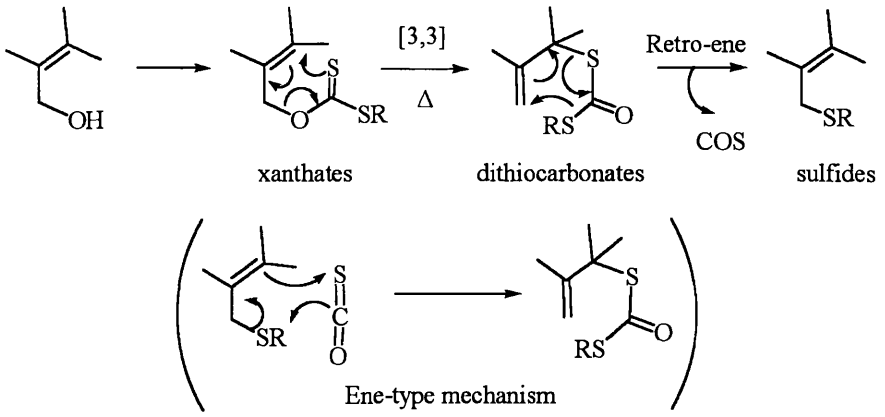
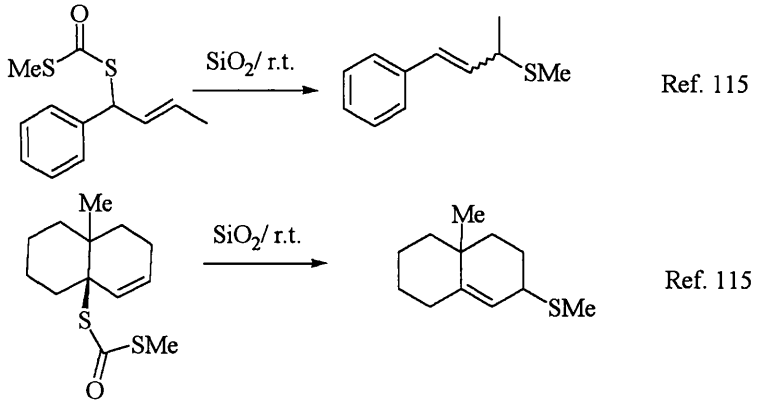


Figure 20. Possible isomerisation pathways for allylic thionitrites.



Scheme 46. Mechanism proposed for the formation of allylic sulfides from allylic xanthates[114].

High temperatures (200-300 °C) or prolonged heating are the conditions usually required to effect this transformation[115]. Some phenols and certain Lewis acids such as $AlCl_3$ have also been shown to act as catalysts[112, 116]. A smaller number of examples of the dithiocarbonate to sulfide isomerisation occurring at r.t., during chromatography on silica have also been reported[115]. These corresponded to substrates for which the alkene in the rearranged product was additionally stabilised by conjugation or hyperconjugation **(Scheme 47)**.

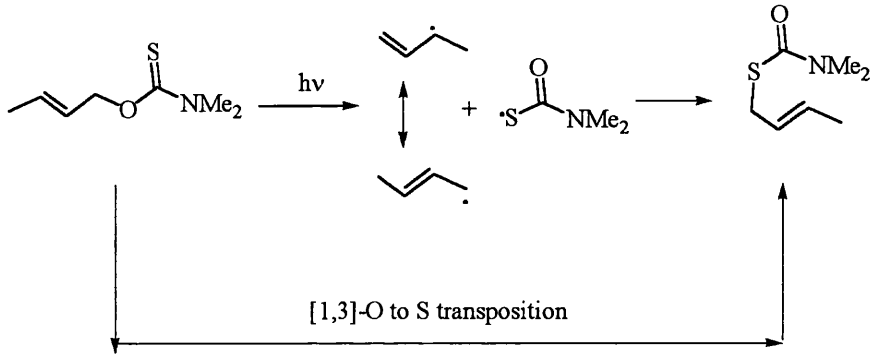


Scheme 47. Some facile reported dithiocarbonate to sulfide rearrangements[115].

The literature on the other type of rearrangement observed by us ([1,3]-shift) is relatively limited and, for the particular case of allylic dithiocarbonates or xanthates, no systematic studies have been undertaken. Incidentally, it has been pointed out that, in general, sulfur to sulfur allylic transpositions have found little application in organic synthesis[110]. Nevertheless, it is worth reviewing here some of the mechanisms which have been put forward in order to explain allylic shifts in sulfur containing systems since, as

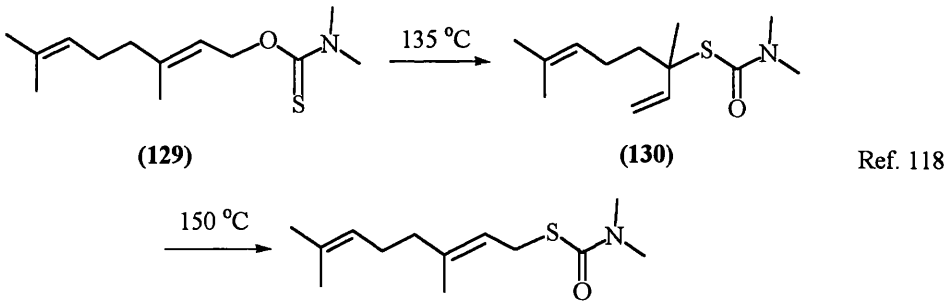
we will see in the following sections, these seem common to all of the allylic sulfur systems studied during this work, including dithiocarbonates, thiols and thionitrites.

For example, the [1,3]-oxygen to sulfur transposition of *O*-allyl thiocarbamates under photochemical conditions is believed to take place *via* homolytic cleavage of the C-O bond and radical recombination through the more nucleophilic sulfur atom to give the thermodynamically more stable alkene[117] **(Scheme 48)**.



Scheme 48. Radical isomerisation of *O*-allyl thiocarbonates[117].

Dialkylthiocarbamates have also been found to rearrange thermally. For instance, *O*-allyl thiocarbamate **129** rearranged initially to the corresponding *S*-allyl thiocarbamate **130** upon heating at 135 °C. When heating was continued at 150 °C, primary *S*-allyl thiocarbamate **131** started to form in small quantities[118] **(Scheme 49)**. The same product was detected in trace amounts when the reaction was carried out in the presence of certain transition metal salts[119].



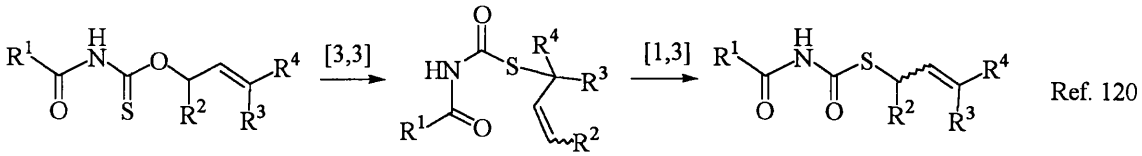
(129) **(130)** Ref. 118

(131)

Scheme 49. Thermally induced [1,3]-sigmatropic shift of an *O*-allylic thiocarbonate[118].

Upon heating, a series of *N*-acylmonothiocarbamates **132** were found to rearrange quantitatively to the corresponding *S*-substituted isomers **133**. After prolonged heating these were subsequently transformed into the corresponding [1,3]-shift products **134**

(**Scheme 50**)[120]. The authors demonstrated that the [1,3]-shift was always consecutive and never competitive with the [3,3]-shift, a result analogous to that observed by us. They pointed out that the driving force for the reaction would be the conversion of a terminal double bond to the thermodynamically more stable internal double bond.

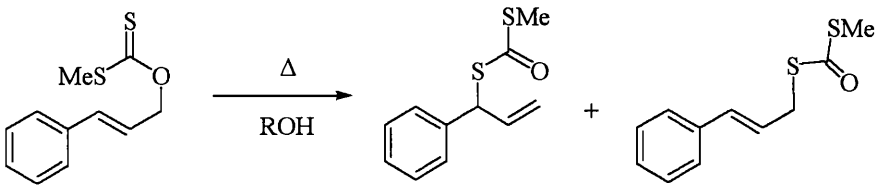


(132) **(133)** **(134)** Ref. 120

Scheme 50. [3,3] *vs* [1,3]-shifts of thiocarbamates[120].

In a more recent study[121], it was found that *N*-monoalkythiocarbamates undergo, in most cases, easier rearrangement to mixtures of carbamates upon heating at 120 °C-150 °C than their *N,N*-disubstituted counterparts. The authors suggested that, whereas a concerted [3,3]-sigmatropic rearrangement seems to predominate in the case of *N,N*-disubstituted thiocarbamates, two competitive paths could be followed in the case of the *N*-monosubstituted substrates: a concerted [3,3]-sigmatropic shift and a dissociative mechanism, either *via* a tight ion pair or *via* homolytic cleavage of the C-O bond. [1,3]-isomerised products would be formed from this intermediate.

The analogous reactions of xanthates have not been much studied. Nevertheless, in a recent report, the effect of solvent in the [3,3]-sigmatropic rearrangement of allylic xanthates to dithiocarbonates has been investigated[122]. It was concluded that the rearrangement could either occur *via* a concerted 6-membered transition state, or *via* an ionic transition state, depending on the solvent. Polar protic solvents favoured the second pathway and, in these, xanthate **135**, underwent rearrangement to mixtures of the two regioisomeric dithiocarbonates **136** and **137** (**Scheme 51**).

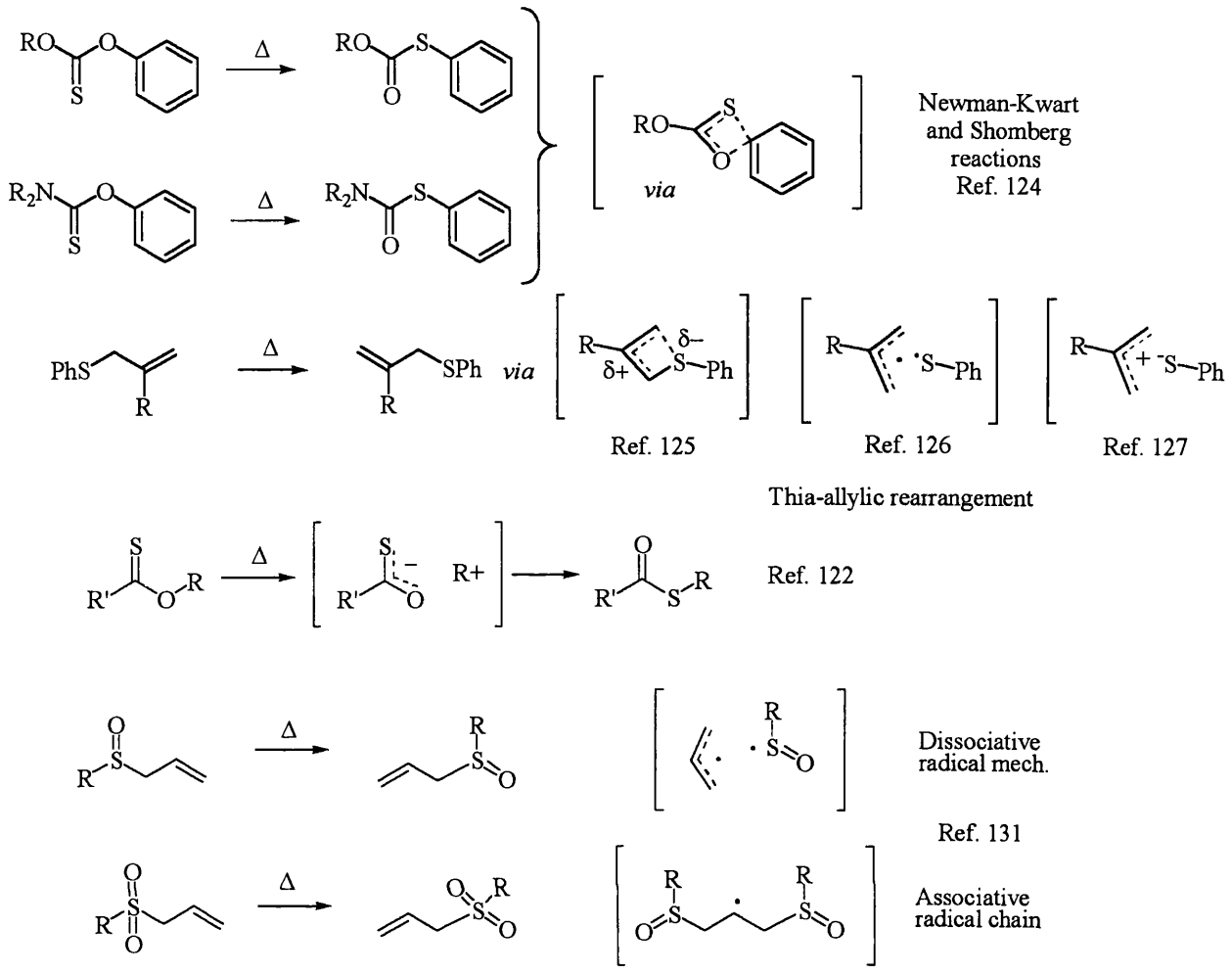


(135) **(136)** **(137)**

Scheme 51. Rearrangement of xanthates in polar protic solvents[122].

This and the rearrangement of the analogous xanthate containing a methyl group in the α-position of the double bond are virtually the only two examples of allylic xanthates affording mixtures of dithiocarbonates which can be found in the literature. On two occasions, mixtures were formed during pyrolysis of the xanthate at 150 °C[122].

Other classes of sulfur-containing systems have also been found to be susceptible to [1,3]-allylic migrations. With regard to the mechanisms of such reactions, some of them involve O to S and others S to S transpositions, but all of them have been explained by a reduced number of intermediates or transition states. For instance, *O*-aryl thiocarbonates and *O*-arylthiocarbamates rearrange upon pyrolysis (200-300 °C), affording their corresponding *S*-aryl isomers[123] (the Shömberg and Newman-Kwart reactions, **Scheme 52**). Allyl aryl sulfides can also yield [1,3]-rearranged products on heating[124] (thia-allylic rearrangement). Both processes are assumed to take place in a concerted fashion, *via* 4-membered transition states (**Scheme 52**). Under photochemical conditions or under thermal conditions for certain cyclic systems, a dissociative radical mechanism has been invoked for the thia-allylic rearrangement[125]. In the presence of acid, a dissociative ionic pathway has been proposed[126]. *O*-alkyl thionesters rearrange easily to thiolesters when the alkyl group can form relatively stable carbonium ions (e.g. benzyl) and the same effect is observed for xanthates. Conversely, rearrangement of ordinary primary or secondary xanthates requires heating above 200 °C. However, Lewis acids[127,128] and tertiary amines[129] have been found to play a catalytic role and the transformation can be effected at r.t. in the presence of these additives. The reaction in these cases is believed to occur *via* a "dissociation and return" pathway [122] (**Scheme 52**). Under some particular circumstances, allylic sulfoxides and sulfones have also been shown to give [1,3]-isomerised products thermally or photochemically [130]. For these substrates, both dissociative radical and associative radical chain mechanisms have been proposed.



Scheme 52. Summary of the mechanisms proposed for several [1,3]-sigmatropic shifts in sulfur systems.

In summary, [1,3]-allylic shifts in sulfur systems can take place either *via* concerted mechanisms involving 4-membered transition states, *via* dissociative or associative radical pathways, or *via* dissociative ionic pathways. The [1,3]-rearrangement observed by us could be rationalised in terms of any of the mechanisms illustrated above. A photochemical reaction is likely to involve a dissociative or associative radical pathway. An alternative thermal reaction is likely to occur by an ionic or radical dissociative mechanism. The reaction on silica is probably ionic. We have not undertaken additional studies in order to determine the possible mechanisms involved in each particular case. Nevertheless, it is worth pointing out the types of difficulty which may be encountered during the preparation of apparently simple tertiary allylic sulfur compounds and the conditions which might engender further reactivity.

1. 2. 3. Preparation of tertiary allylic thiols.

The classical methods for the preparation of thiols from dithiocarbonates imply either basic methanolysis (MeOH/NaOH) or reduction (Na or $LiAlH_4$)[107a]. Other, milder procedures, have been developed, for applications when the substrates contained sensitive functionalities or when the desired thiol was primary, in which case, oxidation to the disulfide is a fast process under strongly basic conditions[107b, c]. Since our own substrates seemed not to fall into either of these latter two categories, we initially chose the simpler methods for the preparation of tertiary thiols from dithiocarbonates **112-122** (**Tables 5** and **6**). Thiols **138** and **139** were derived from the mixture of dithiocarbonates **112-113** by basic methanolysis and could be separated after repeated chromatography. Analogously, thiols **141** and **142** were obtained from the mixture of dithiocarbonates **114-115** and were also separated at this stage. Thiol **144** was prepared following the same procedure. In all of these examples, unwanted allylic isomerised thiols (**140**, **143** and **145**) were formed only in trace amounts and, subsequent nitrosation afforded the desired tertiary thionitrites without problems, as discussed in the next section. However, the preparation of thiols **146**, which is structurally very similar to **144** and of thiols **148** and **155** gave disappointing results. The unwanted thiols of allylic isomerisation (**147**, **149** and **156**) were formed in substantial amounts, thus reducing the yield and complicating separation of the desired product. By TLC, it could be observed that both isomeric thiols were present from the beginning of the reaction, even at 0 °C.

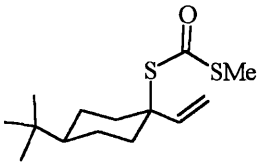
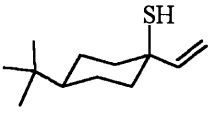
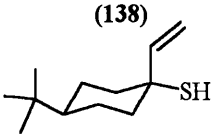
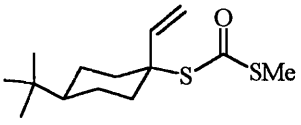
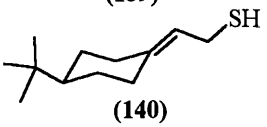
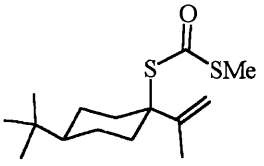
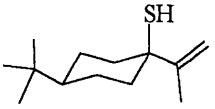
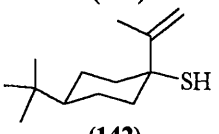
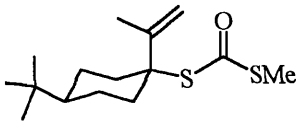
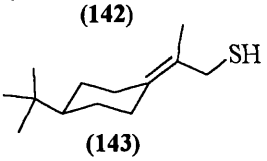
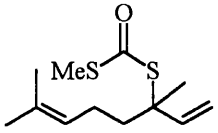
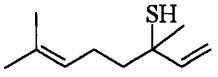
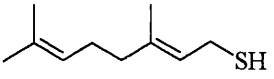
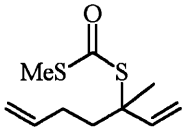
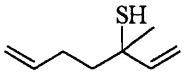
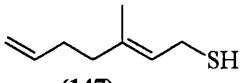
Dithiocarbonate	Thiol	MeOH/NaOH (yield)	$LiAlH_4$ (yield)	2-AP (yield)
 (112)	 (138)	(32-40 %)	-	-
	 (139)	(12 %)	-	-
 (113)	 (140)	(12 %)	-	-
 (114)	 (141)	(41 %)	-	-
	 (142)	(27 %)	-	-
 (115)	 (143)	(traces-9 %)	-	-
 (116)	 (144)	(56-63 %)	-	-
	 (145)	(5 %)	-	-
 (117)	 (146)	(10 %)	(10-16 %)	-
	 (147)	(30 %)	(20-30 %)	-

Table 5. Preparation of thiols from tertiary dithiocarbonates (I).

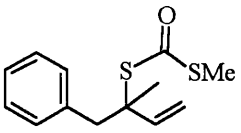
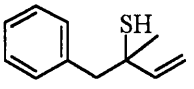
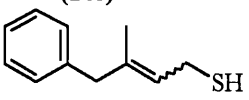
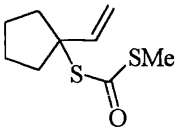
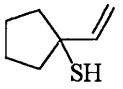
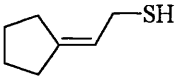
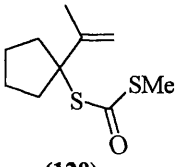
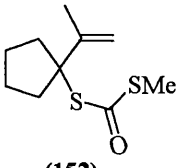
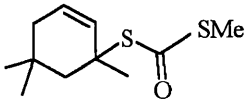
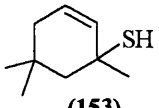
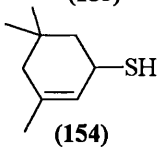
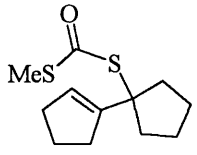
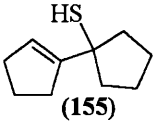
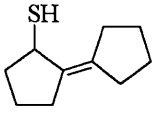
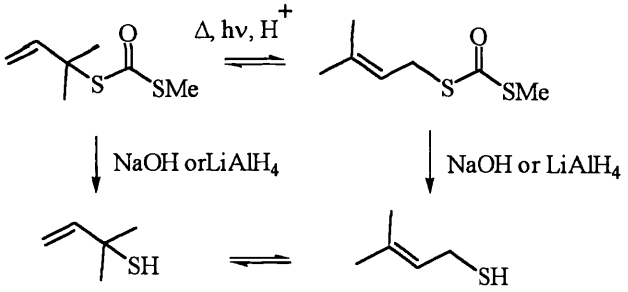
Dithiocarbonate	Thiol	MeOH/NaOH (yield)	$LiAlH_4$ (yield)	2-AP (yield)
 (118)	 (148)	(15 %)	(22 %)	(74 %)
	 (149)	(20 %)	(10 %)	(0 %)
 (119)	 (150)	-	-	(56 %)
	 (151)	-	-	(0 %)
 (120)	 (152)	-	-	(87 %)
 (121)	 (153)	-	(10 %)	(70-90 %)
	 (154)	-	(10 %)	(0 %)
 (122)	 (155)	(32 %)	-	-
	 (156)	(28 %)	-	-

Table 6. Preparation of thiols from tertiary dithiocarbonates (II). (AP= Aminopropanol)

A similar effect has been observed during the basic methanolysis of thiocarbamate **130**, which yielded only geranyl thiol **145**[118]. In this instance, the authors had solved their problem by using $LiAlH_4$ reduction as an alternative procedure. However, when we applied this methodology to our substrates, [1,3]-rearranged thiols were still obtained as major products and these were again detected by TLC from the start of the reaction. When the reaction mixtures were worked-up by addition of acid to pH 1-7, the unwanted isomerised thiol was almost always the only product. When the reactions were worked-up by addition of water following the reported method[118], mixtures were obtained in all cases. The proportion of the unwanted thiol was found to increase upon prolonged contact of the mixtures with silica gel.

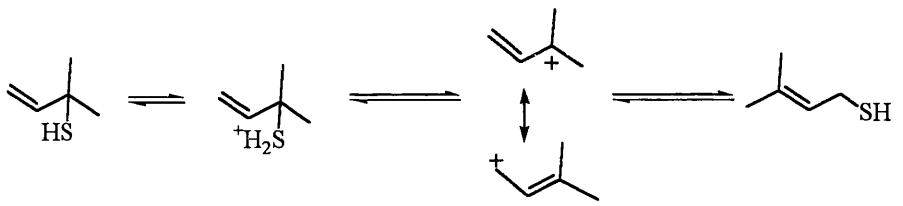
The formation of these [1,3]-rearranged thiols might be attributed to two competitive pathways: (i) isomerisation of the starting tertiary dithiocarbonates, followed by methanolysis, or (ii) rapid equilibration of the initially formed thiol, containing a terminal bond to the thermodynamically more stable thiol containing an internal double bond (**Scheme 53**).



Scheme 53. Possible pathways for the formation of primary and secondary allylic thiols from tertiary dithiocarbonates.

We have observed that the second pathway can take place both in neutral methanolic solutions and in the presence of added NaOH. Thus, stirring a sample of pure tertiary thiol **150** either in methanol or in methanol/NaOH led to complete isomerisation after 1 night. Nevertheless, this result does not preclude simultaneous reaction by the alternative pathway occurring during methanolysis, since as already mentioned before, ionic intermediates resulting from a C-S bond cleavage, would be favoured in methanolic solvents. Isomerisation of allylic thiols under acidic conditions (H^+ or SiO_2), or in solution in the absence of added acid, since the thiol already provides the acidic pH, would be rationalised in terms of formation of an intermediate stable allylic carbocation,

followed by sulfur attack at the less substituted site of the resulting conjugated system (**Scheme 54**). Analogous reactions of allylic alcohols or allyl esters are well known[110]. By way of contrast, isomerisation under strongly basic conditions, under which the thiol would be completely deprotonated, is more difficult to explain, and a concerted mechanism might be implicated. A concerted [1,3]-sigmatropic rearrangement would be allowed by the Woodward-Hoffmann rules. However, these processes are not very common and only a few examples have been reported in the literature, mostly involving allylic shifts with inversion of configuration of an alkyl or a silyl group[131].

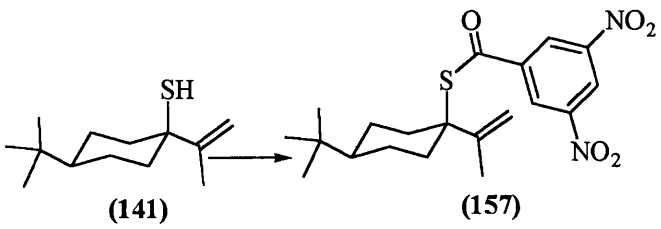


Scheme 54. Possible mechanisms proposed for the acid catalysed isomerisation of allylic thiols.

In practice, the use of a milder base such as 3-aminopropanol (AP, **Table 6**) has been found to be a better method for the conversion of dithiocarbonates to thiols in the case of sensitive substrates. When we applied this method, tertiary allylic thiols could be obtained free of their [1,3]-isomerised products and in higher yields to those recorded by the methods previously examined (**Tables 5** and **6**). It is interesting to mention that, in a few instances, rearranged thiols were also detected when this method was used, but only after very long reaction times (more than one day).

1. 2. 4. Determination of the stereochemistry of dithiocarbonates **112-115** and thiols **138**, **139**, **141** and **142**.

The stereochemistry of the cyclic thiol **141** was determined after preparation of a crystalline derivative **157**. Crystals suitable for X-ray analysis, were obtained by slow diffusion of P. E. through a solution of the compound in Et_2O. The three-dimensional structures of thiol **142** and of dithiocarbonates **114** and **115** could then be inferred from



(141) **(157)**

this result. On the other hand, the stereochemistry of thiol **138** could be deduced after crystallisation and X-ray analysis of one of the products obtained during the thermal decomposition of its thionitrite derivative (described later). This correlation enabled us to deduce the stereochemistry of the rest of the compounds of the series.

1. 2. 5. Preparation of tertiary thionitrites.

Many methods have been used for the preparation of thionitrites from thiols and these were reviewed in the introductory section. During previous studies in the group, it was found that nitrosation with $NaNO_2$, in acetic acid, at r.t., provided the best yields of tertiary thionitrites. Following this procedure, thionitrites **158-168** were obtained in moderate to good yields (**Figure 21**). All of them were green oils with red reflections and virtually all were sufficiently stable to allow purification by filtration through SiO_2. Only thionitrite **168** could not be obtained pure even after repeated filtrations and decomposed during manipulation affording complex mixtures.

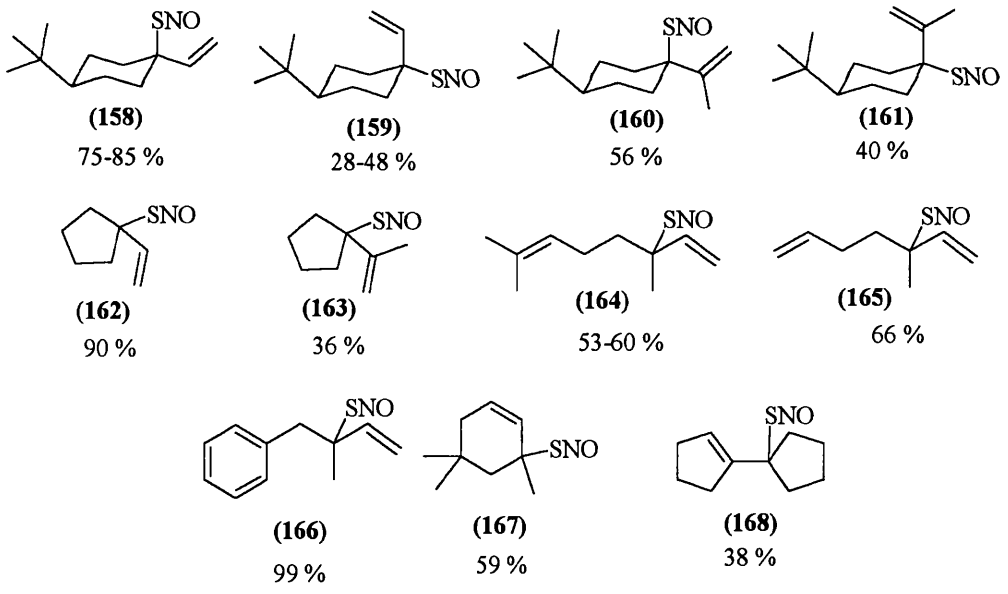
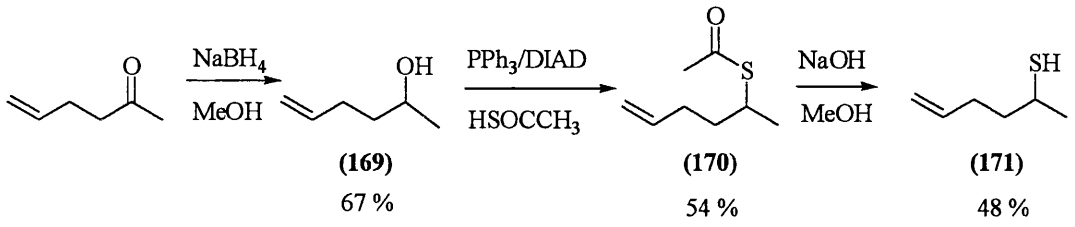


Figure 21. Tertiary allylic thionitrites obtained during this work (yields from the thiols).

1. 3. Preparation of primary and secondary thiols for the *in-situ* preparation of primary and secondary unsaturated thionitrites.

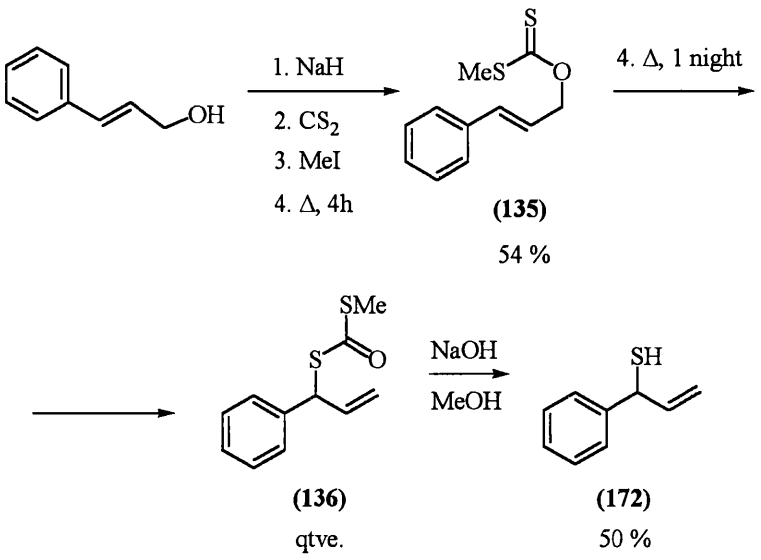
Secondary thiol **171** was synthesised in three steps from 5-hexen-2-one. Reduction of the ketone with $NaBH_4$ afforded alcohol **169**, which was then transformed into thioester **170** via a Mitsunobu-type reaction. Methanolysis of this intermediate in MeOH/NaOH, as described in the previous section, afforded thiol **171** (**Scheme 55**).



(169) 67 % **(170)** 54 % **(171)** 48 %

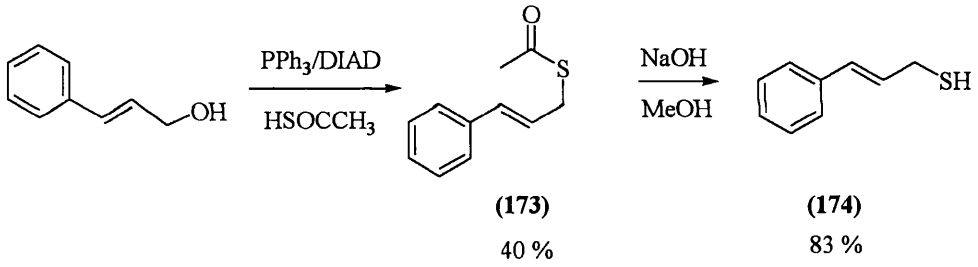
Scheme 55. Synthesis of thiol **171**.

In turn, secondary thiol **172** was synthesised in two steps from cinnamyl alcohol. Dithiocarbonate **136** was first obtained *via* a [3,3]-sigmatropic shift of cinammyl xanthate **135**, under the conditions previously described. Interestingly, heating at reflux for 4 hours and chromatography on SiO_2 furnished the unrearranged xanthate. The dithiocarbonate was only obtained after heating the xanthate in THF, at reflux, for a longer period (more than 1 night). This effect could well be related to the thermodynamically disfavoured rupture of the conjugated system which is necessary for the formation of **136** and the question has been noted before[133]. However, the authors of that study reported the spontaneous formation of both isomeric dithiocarbonates during purification, which we did not detect[132]. Thiol **172** was obtained free of isomerised thiol after basic methanolysis. It is worth noting that, in contrast to our own observations, this thiol had previously been found to rearrange readily under analogous conditions, yielding only cinnamyl thiol[118] (**Scheme 56**).



Scheme 56. Preparation of thiol **172.**

Finally, thiol **174** (cinnamyl thiol) was synthesised from cinnamyl alcohol by a procedure analogous to that employed for the synthesis of **171**, *via* the formation of intermediate thioester **173 (Scheme 57)**.



Scheme 57. Preparation of cinnamyl thiol **174**.

CHAPTER 2

THERMAL DECOMPOSITION OF ALKENE-CONTAINING THIONITRITES.

2. 1. Introduction.

The thermal decomposition of the prepared tertiary thionitrites was studied under an inert atmosphere of N_2, in DCM or benzene solutions, by heating at reflux or at 40 °C, respectively. The disappearance of the starting thionitrite could be conveniently monitored by noting the fading of the characteristic green colour. In all cases, the thionitrite had been completely consumed after *ca.* 7 h. After completion of the reaction, the solvent was evaporated and the resulting crude reaction mixtures separated by chromatography on silica gel.

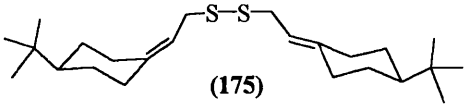
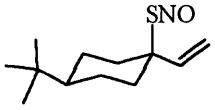
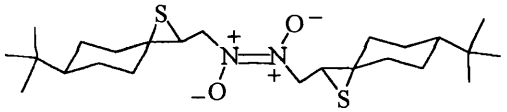
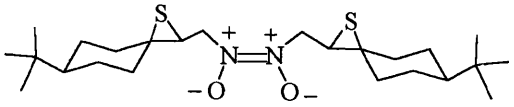
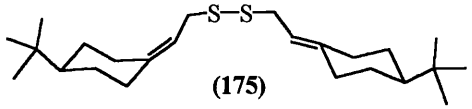
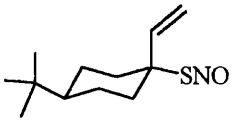
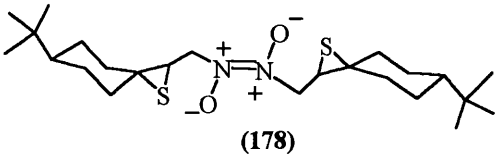
The primary and secondary thionitrites were prepared *in situ* immediately prior to the decomposition reaction, by mixing the thiol with a slight excess (1.1-1.2 equiv.) of *tert*-butyl nitrite, in benzene. The thionitrites formed almost instantly, as evidenced by the development of the characteristic intense red colour. After 10 min of standing at r.t., heating was started at 40 °C, until total disappearance of the thionitrite. To our surprise, this generally required very variable periods, ranging from 4 h to more than 1 day, depending on the experiment. On the basis of all literature precedents, we had expected that the decomposition of less substituted thionitrites would be faster than that of tertiary ones in the same solvents. However it should be pointed out that the tertiary thionitrites here used were pure, as ascertained by ^1H and ^{13}C NMR, whereas the solutions of primary and secondary thionitrites prepared *in situ* might well contain traces of unreacted thiol and *tert*-butyl nitrite, in addition to the *tert*-butyl alcohol generated as a result of thiol nitrosation. These substances might affect the thermal behaviour of thionitrites. Attempts to ensure complete nitrosation of thionitrite by addition of more equivalents of *tert*-butyl nitrite resulted in fast decomposition to complex mixtures, presumably by oxidative processes. *In situ* generation of thionitrites by other methods ($NaNO_2$, $NOBF_4$) led to irreproducible results, in terms of the products formed. It is also worthwhile noting that, as discussed in the introductory section, the higher stability of tertiary thionitrites over primary or secondary ones has been ascribed to steric hindrance in the disulfide formation step. In the case of decomposition of unsaturated thionitrites, both intra- and inter-molecular processes seem to be involved and, therefore, the overall picture is a

more complex one. Thus, comparison of the results obtained for tertiary substrates with those obtained for primary or secondary ones has to be made with some caution.

2. 2. Thermal decomposition of the 6-membered cyclic thionitrite series 158-161.

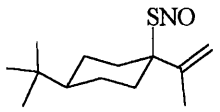
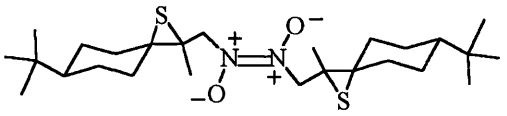
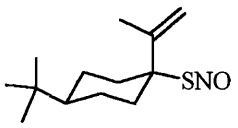
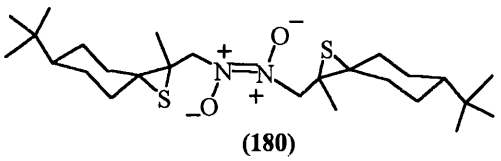
2. 2. 1. Product analysis.

The results obtained for the series of structurally very similar thionitrites **158-161** were approximately the same, with slight variation in product distribution depending on the solvent used and the substitution pattern of the substrate (**Tables 7** and **8**).

Thionitrite	Thermal decomposition products	Benzene (yield)	DCM (yield)
	 **(175)**	(16 %)	(18 %)
 **(158)**	 **(176)**	(30 %)[a]	(65 %)[a]
	 **(177)**	(9 %)[a]	-
	 **(175)**	(30 %)	(17 %)
 **(159)**	 **(178)**	(30 %)[a]	(65 %)[a]

(a) Only one isomer of the nitroso-dimer was formed.

Table 7. Thermal decomposition of thionitrites **158** and **159**.

Thionitrite	Thermal decomposition products	Benzene (yield)	DCM (yield)
 (160)	 (179)	(65 %)[a]	(73 %)[a]
 (161)	 (180)	(43 %)[a]	(72 %)[a]

(a) only one isomer was formed.

Table 8. Thermal decomposition of thionitrites **160** and **161**.

Two main products were separated after thermal decomposition of diastereomeric thionitrites **158** and **159** in DCM. These were identified as an allylic disulfide (**175**) and a highly symmetrical *N,N-trans*-nitroso-dimer containing a 2,3-episulfide ring (structures **176** and **178**, respectively from **158** and **159**). This conclusion was reached after analysis of their ^{1}H NMR, ^{13}C NMR, IR, MS (FAB) and HRMS spectra. In addition, correct elemental analyses were obtained for disulfide **175** and for the nitroso-dimer **176**. All of the signals on the ^{1}H NMR and ^{13}C NMR could be assigned by means of DEPT, COSY and HMQC experiments. Furthermore, the three dimensional structure of compound **176** was unequivocally assigned by X-ray analysis of a crystal obtained by slow diffusion of P.E. through a solution of the compound in DCM, at r.t. An analogous structure could be assumed for dimer **178**. As can be concluded from the yields quoted in the table, the *cis/trans* isomerism of the starting thionitrites has no effect on the final outcome of the reaction. This was not unexpected, since the *tert*-butyl group is situated too far in space from the reactive sulfur atom to produce any appreciable steric effect. It is also of interest that of the possible diastereisomers, only one is formed and this aspect will be discussed later in more detail.

Decomposition in benzene yielded the same products in slightly different proportions. In addition, when this solvent was used, a new product (**177**), considerably less polar than **176**, was obtained in small quantities from thionitrite **158**. The ^{1}H NMR spectrum of this compound was very similar to that of the *trans*-nitroso-dimer **176**,.with

the signal corresponding to the episulfide hydrogen and the hydrogens of the CHHN group shifted downfield between 6 and 8 ppm. Their ^{13}C NMR were considerably different, in particular the peak corresponding to the CHHN carbon which is shifted upfield 15 ppm. However, both spectra are compatible with the highly symmetrical 2,3-episulfide-nitroso-dimer pattern. Both products display the same IR spectrum and very similar MS. On the basis of these observations it seems plausible that **177** could have a structure analogous to that of **176**, but containing a thermodynamically less stable *cis*-N-N double bond. Alternative, compound **177** could be another diastereoisomer of the nitroso dimer. Unfortunately, correct elemental analysis or HRMS could not be attained for this product. Furthermore, crystals suitable for X-Ray analysis could not be obtained and therefore, this assignment remains speculative.

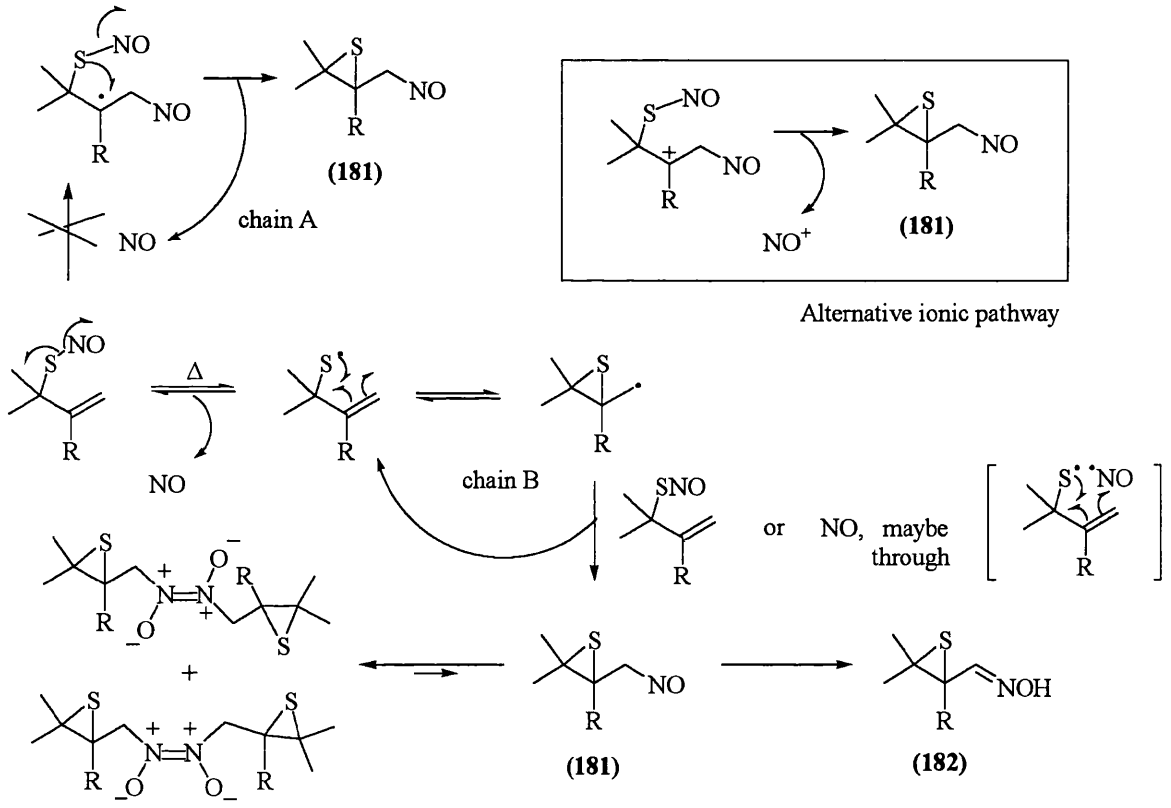
Thermal decomposition of thionitrites **160** and **161** afforded only one isolated compound in each case, both when the reaction was carried out in benzene and in DCM. The products were identified as the corresponding nitroso-dimers (structures **179** and **180**, respectively from **160** and **161**) on the basis of their spectra and by analogy with the products derived from thionitrites **158** and **159.**

For all of these four thionitrites, the reaction mixtures were less complex by TLC when experiments were carried out in DCM. In benzene, traces of other products of intermediate polarity between those of the disulfide and the corresponding nitroso-dimer were always detected but they could not be separated.

2. 2. 2. Mechanistic considerations.

In concordance with related literature precedents, discussed below, the rationale for the formation of the rearranged 2,3-episulfide nitroso-dimer products would implicate an initial homolytic fission of the thionitrite S-N bond, followed by a rapid, reversible, intramolecular 1,3-*exo* addition of the generated thiyl radical onto the allylic alkene (**Scheme 58**). Termination could then occur through reaction of the resulting primary alkyl radical with an NO molecule, possibly *via* a cage type-mechanism. Alternatively, the carbon-radical could react with another molecule of thionitrite in a radical-chain process (chain B). The possibility of a radical chain process initiated by the addition of NO to the double bond (chain A) can be ruled out, given the known lack of reactivity of NO towards alkenes, already discussed in the introductory section. The

product initially formed would be a primary 2,3-episulfide nitroso-monomer **181**. In solution, such compounds are known to be in equilibrium with their dimers, this equilibrium being very much displaced to the dimer in the case of aliphatic nitroso-compounds. Dimerisation can, in principle, occur to products with either a *cis* or a *trans* configuration around the N-N double bond and, under thermodynamic conditions, the *trans* isomer is generally the major product. Alternatively, **181** could irreversibly tautomerise to the corresponding oxime (**182**). The second course is favoured in polar protic solvents, but both pathways might be competitive, depending on the conditions and on the structure of the monomeric nitroso-compound. The first pathway appears to be favoured in the case of the primary nitroso-monomers, formed during the reactions here described.

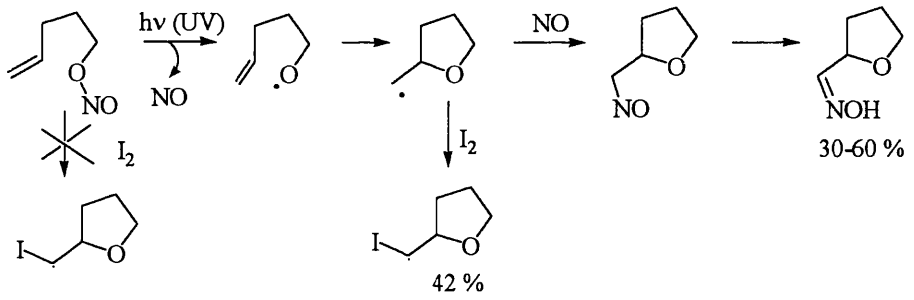


Scheme 58. Mechanism proposed for the formation of episulfide nitroso-dimers.

The 3-*exo* intramolecular cyclisation of thiyl radicals to give episulfides has not received much attention. A reaction analogous to the one here studied but starting from alkyl nitrites (ONO) to give α,β-epoxide oximes or nitroso-dimers has been reported to be brought about by irradiation with UV light[134a]. A mechanism analogous to the one depicted in Scheme 58 was proposed but was not demonstrated. It was also suggested

that a totally ionic mechanism *via* formation of NO^+ and RO^- would be unlikely. In order to rule out such a possibility in our case (**Scheme 58**, inset, above) we carried out the reaction in the presence of a catalytic amount of NO^+, using $NOBF_4$. The result was a complex mixture of products.

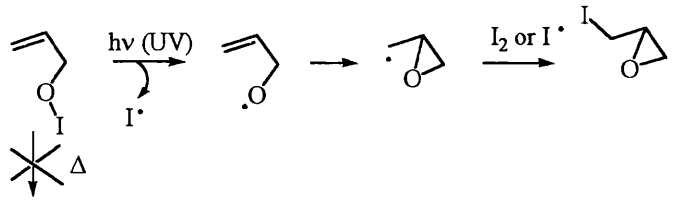
The analogous alkyl nitrites reaction to give a 5-membered ring has also been studied[134b, c]. Irradiation with UV light was found to generate alkoxy radicals which would efficiently add onto a double bond situated at a distance of 4 carbon atoms from the oxygen radical, followed by termination via NO trapping to give tetrahydrofurfural oximes (**Scheme 59**).



Scheme 59. Photochemical cyclisation of alkyl nitrites[134b,c].

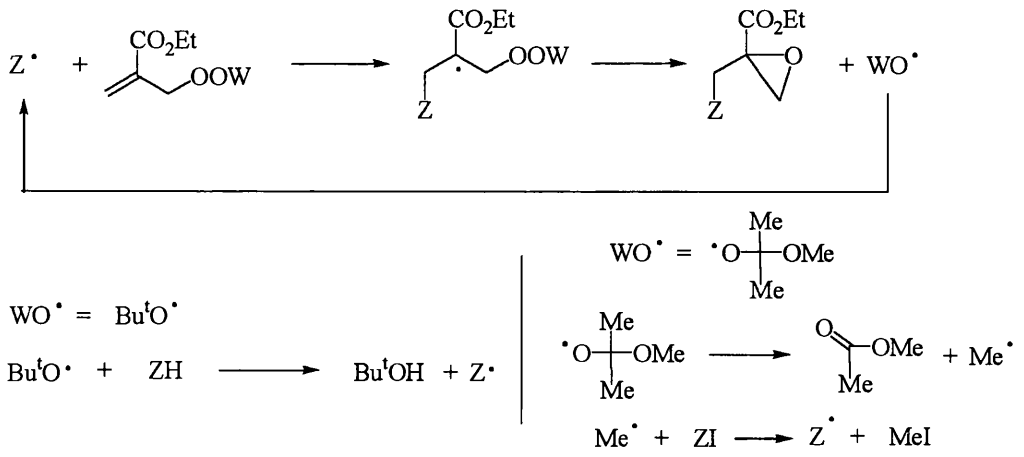
In that study, the intermediate carbon-radical formed could also be trapped by other radical traps such as I_2, Cl_2 or Br_2 (1-1.5 equiv.). The reaction leading to the oximes did not take place under thermal conditions (refluxing in benzene). This is in agreement with a stronger O-NO bond in comparison with the S-NO bond that is being broken in the reaction studied by us. In addition, iodine did not react with the alkyl nitrite upon heating in the dark. In comparison, when we treated thionitrite **158** with I_2 (0.2 to 1 equiv.), at r.t., the result was a complex mixture of products. This, again, is an indication of the higher reactivity of thionitrites, when compared with alkyl nitrites. Even if an α-iodosoepisulfide was formed from **158**, this did not seem to be stable in the conditions of our reaction. The authors also concluded that the process was not a chain reaction, given the low determined quantum yield. However, based again on the bond strength argument, it is possible that a radical chain could be more effective for thionitrites, since the S-N bond would be relatively easy to cleave. They did not study the possibility that the reaction was taking place in a "solvent cage".

In a related study, the photolysis (UV) of certain allylic alcohol hypoiodites was found to lead to the formation of α-iodosoepoxides[135a] (**Scheme 60**). A non-chain radical mechanism, involving trapping of the intermediate carbon-radical by iodine, was proposed. As in the reaction discussed above, no cyclisation was observed in the dark.



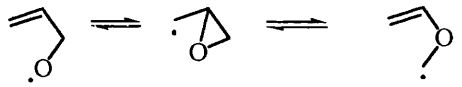
Scheme 60. Photochemical cyclisation of alkoxy hypoiodiotes[135a].

Maillard has studied the formation of epoxides via induced homolytic decomposition of unsaturated peroxyketals[135b] or alkyl 2-(1-*tert*-butylperoxyalkyl)propenoates[135c, d] (**Scheme 61**). The homolytic cleavage of the peroxide O-O bond is induced by the addition of an alkyl radical ($Z^{\cdot}$) to the unsaturation, followed by an SH_i reaction of the resulting carbon radical on the oxygen-oxygen bond. In order for the reaction to function as a chain, the alkoxy radical released in this process ($WO^{\cdot}$) must then regenerate the alkyl radical ($Z^{\cdot}$). This has been achieved by different pathways. For instance, $WO^{\cdot}$ was found to abstract a hydrogen atom from ZH, this being the solvent of the reaction[135b]. When the alkoxy radical was a 1-methoxy-1methylethoxy radical this was found to readily fragment giving a methyl radical ($R^{\cdot}$, R= Me) which, subsequently, could abstract an iodine atom from an alkyl iodide (ZI)[135c, d], regenerating $Z^{\cdot}$ for addition.



Scheme 61. Formation of epoxides from allyl peroxide derivatives[135b-d].

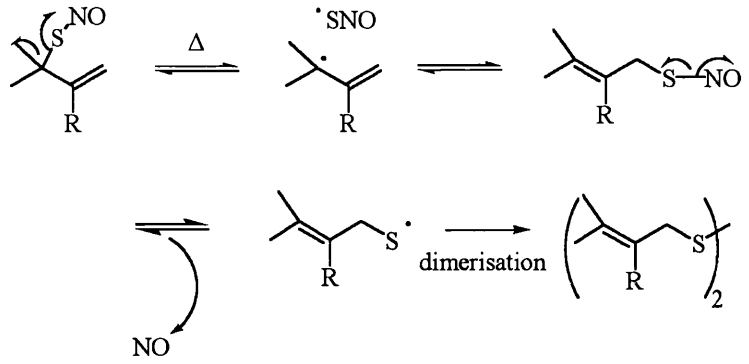
Another interesting point to note, when one compares the reactions *via* thiyl radicals with those *via* alkoxy radicals, is that in the latter, products resulting from competitive reactions of the alkoxy radicals are almost always detected. For example, in the reactions studied by Surzur[134c] (Scheme 59), products resulting from hydrogen abstraction by the alkoxy radical were also detected and, in the reaction in Scheme 60[135a], competitive fragmentation of the adjacent C-C bond was also detected. Substrates in which a carbon radical is situated alpha to an epoxide ring are known to give products resulting from different fragmentation processes[136] **(Scheme 62)**.



Scheme 62. Fragmentation of α, β-epoxy carbon radicals[136].

We did not observe any of the analogous processes in our sulfur systems. This is not surprising and is a reflection of the lower reactivity of thiyl radicals with respect to alkoxy radicals, which makes them more resistant towards hydrogen atom abstraction, and fragmentation processes. This also explains why an alternative mode of opening of the episulfide ring, generating a carbon centered radical, is unlikely.

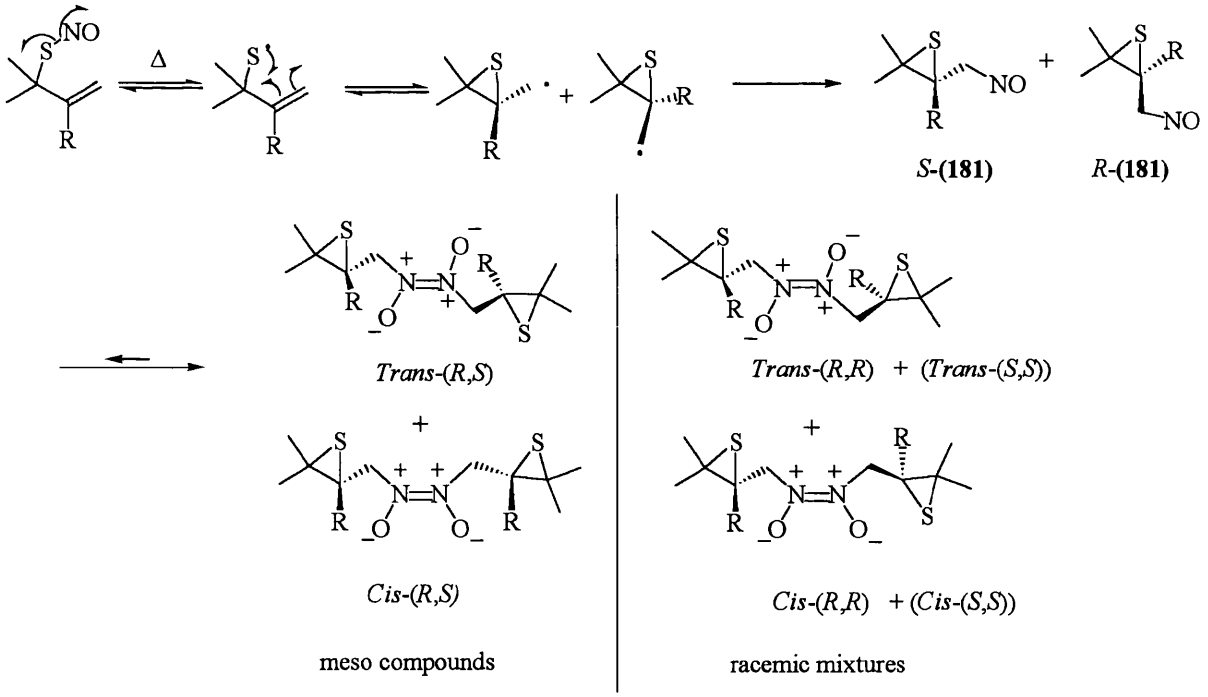
With regard to the formation of allylic disulfide **175** as a minor product, a likely mechanism would involve [1,3]-transposition of the thionitrite upon heating, probably through homolytic cleavage of the thionitrite C-S bond to give a relatively stable tertiary allyl radical. This could be followed by recombination to give the more stable alkene and then S-N bond fission to give a primary thiyl radical and facile dimerisation to the disulfide **(Scheme 63)**. A radical chain mechanism, similar to that mentioned in the previous chapter, could also be possible for the [1,3]-isomerisation step. An alternative ionic pathway with formation of an allyl carbocation and ONS^- seems unlikely, given the fact that the proportion of disulfide increased in a non-polar solvent such as benzene.



Scheme 63. Mechanism proposed for the formation of allylic disulfide **175**.

2. 2. 3. Stereochemical considerations.

The stereochemical outcome of nitroso-dimer formation reaction also deserves special consideration. In terms of the diastereoisomers formed, therefore, in addition to the *cis-trans* isomerism of the N-N double bond, one must consider the distinct diastereomers which can be formed as a result of the different possible combinations of the two enantiomeric nitroso-monomers ***S*** and ***R*-181 (Scheme 64)**.



Scheme 64. Stereochemical outcome of the nitroso-dimer formation.

In theory, a total of 6 possible diastereomeric nitroso-dimers could be formed: the two *trans R,R* and *S,S* enantiomers, their two *cis* counterparts, a *trans meso R,S* dimer and its *cis* analogue. In practice, the thermal decomposition of thionitrites **158-161** appears to yield only one of these diastereomers as the major product in all four cases, as

determined by NMR, where no appreciable splitting of the peaks is observed. In order to seek further confirmation of this aspect, nitroso-dimers **176**, **178** and **179**, **180** were analysed by GC-MS (EI). However, as it could have been foreseen, the dimers dissociated under the conditions of the GC chromatography or the MS (EI) spectrometry with the result that only fragments derived from the monomer could be detected. After decomposition of thionitrite **158** in benzene another diastereomer (**177**) was isolated. This was tentatively assigned a *cis* configuration, in view of the rather different polarity of this product with respect to that of **176** and of the large shift of the peak corresponding to the CHHN moiety on the ^{13}C NMR, which indicate that the environment around the N-N double bond is considerably different for both compounds. However, this assignment has not been unequivocally proven and, therefore, one of the other structures depicted in Scheme 64 could be more correct. The X-Ray analysis of a crystal of episulfide nitroso-dimer **176** unambiguously revealed that it possessed a *trans-R,S* absolute configuration. Given the very little variation in structure among the four thionitrites **158-161**, it is reasonable to assume the same configuration for the 4 nitroso-dimers obtained. All attempts to obtain crystals of **179**, appropriate for X-Ray diffraction analysis, were unproductive. As mentioned earlier, the preference for formation of a major diastereomer can probably be explained as molecular recognition on simple steric grounds.

2. 3. Decomposition of 5-membered cyclic thionitrites 162 and 163.

In contrast to the similarities observed between the thermal decomposition of the 6-membered cyclic thionitrite series, under the same conditions, thionitrites **162** and **163** gave rather different results. On heating in benzene, substrate **162** yielded an allylic disulfide (**183**) as the only isolable product (**Table 9**). In contrast, **163**, containing an additional methyl group at the α-position of the double bond, decomposed to give an inseparable mixture (2:1) of two diastereomeric episulfide nitroso-dimers (**184a-b**). These are presumably the two *trans*-isomers represented below, namely the *meso* compound and the dl mixture of the two *trans* enantiomers. In DCM, the results were virtually the same, as shown by TLC and ^{1}H NMR of the crude mixture, although the products were not isolated.

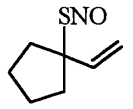
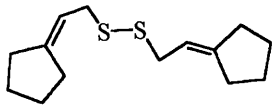
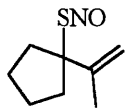
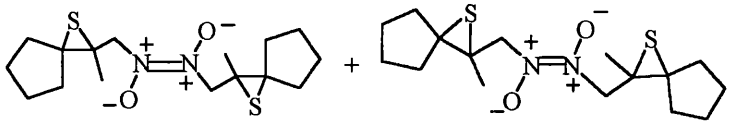
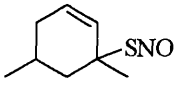
Thionitrite	Thermal decomposition products	(Yield)
 (162)	 (183)	(50 %)
 (163)	 (184a) + (184b)	(58 %)

Table 9. Thermal decomposition of thionitrites **162** and **163**.

It is interesting to point out that, as observed in the previous series of compounds, nitroso-dimer formation is favoured by substitution at the α-position of the alkene, whereas disulfide formation is favoured for vinylic substrates.

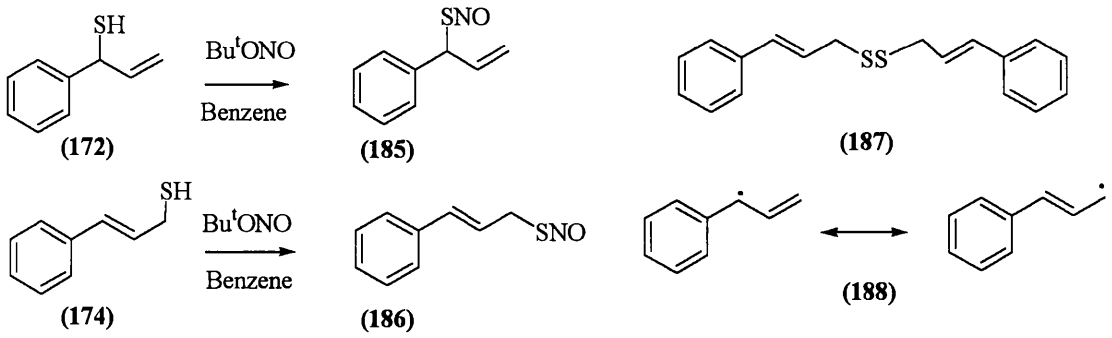
2. 4. Decomposition of cyclic thionitrite 167, containing an endocyclic double bond.


(167)

Thermal decomposition of thionitrite **167** in benzene or DCM yielded complex mixtures of mainly non-polar products, which could not be separated. It is hardly surprising that episulfide nitroso-dimers are not formed, since cyclisation followed by capture of NO would be a thermodynamically disfavoured process, involving formation of a strained system. In all of the products formed, the double bond has undergone an allylic shift, as detected in the ^{1}H and ^{13}C NMRs of the mixtures. However, these proved to be too complicated and the products were too close in polarity to allow separation even by preparative TLC.

2. 5. Decomposition of aromatic ring-containing thionitrites 166, 185 and 186.

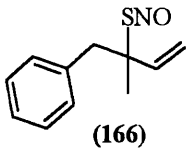
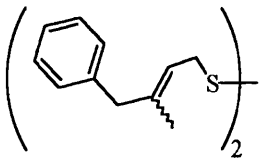
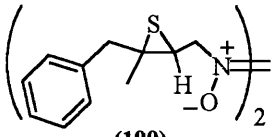
Thionitrites **185** and **186** were prepared *in situ* from thiols **172** and **174**, respectively, by the method described above (**Scheme 65**).



Scheme 65. *In situ* preparation of thionitrites **185** and **186**.

Upon heating, both thionitrites yielded the same major product, cinnamyl disulfide **187**, whose formation can be explained as the result of a preferred cleavage of the C-S bond over that of the S-N bond of the starting thionitrites. This is understandable considering that the resulting radical **188** (or the analogous carbocation) would be both allylic and benzylic and, therefore, highly stabilised by resonance with an extended conjugated system. Preferential recombination *via* the least substituted site, to give the more stable conjugated double bond, would then yield **187** in both cases and subsequent S-N bond cleavage would afford a primary thiyl radical which would readily dimerise to give the observed product. The *trans* alkene would expect to be formed under the thermodynamic conditions employed.

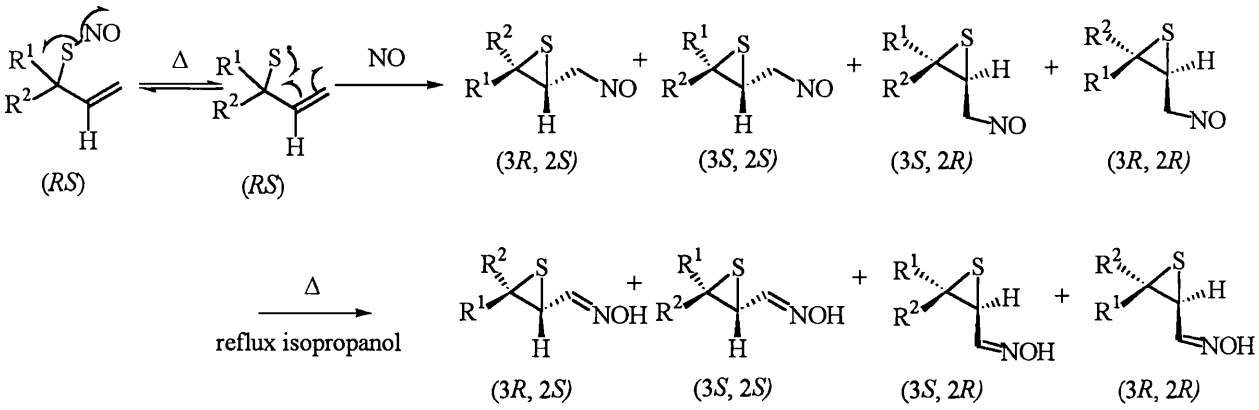
Some support for the above arguments comes from decomposition of thionitrite **166**, containing an aromatic ring which cannot achieve conjugation with the alkene. Reactions either in benzene or in DCM, gave rather different results (**Table 10**). Products were only isolated however from the reaction in benzene, but the crude mixtures were almost identical when the reaction was carried out in DCM, as seen by ^{1}H NMR and TCL.

Thionitrite	Thermal decomposition products	Benzene (yield)
 (166)	 (189)	(21 %)[a]
	 (190)	(52 %)[a]

(a) Mixtures of diastereomers were obtained.

Table 10. Thermal decomposition of thionitrite **166**.

The minor non-polar fraction corresponded to a diastereomeric mixture of disulfides **189**, arising from initial homolytic cleavage of the C-S bond with double bond migration. The major polar fraction was identified as a complex mixture of diastereomeric episulfide nitroso-dimers (**190**), analogous to those obtained previously from the cyclic systems. Given that thionitrite **166** is actually a racemic mixture and that an additional chiral centre is created upon cyclisation to give the episulfide ring, the mixture, as expected becomes more complex (**Scheme 66**). Combination of the four diastereomers depicted and *cis/trans* isomerism around the N-N double bond implies that a maximum of 32 diastereomers could be formed. Even assuming that the *cis* isomers will not form in appreciable amounts, and considering that some of the diastereomers would be formed in equal amounts as couples of enantiomers or *meso* compounds, we would still be left with 4 pairs of enantiomers and 2 *meso* compounds, that is, 6 possible sets of signals on the NMR.



(191a-d)

Scheme 66. Stereochemistry of episulfide nitroso-monomer formation for compounds with 2 chiral centres.

In order to facilitate interpretation of the spectra, the mixture of diastereomeric nitroso-dimers was converted into a mixture of oximes (**191a-c**) by heating at reflux in isopropanol for 1 night (**Scheme 66**). This reduced the number of possible compounds to 2 pairs of enantiomers which could, in turn, be *syn* or *anti* around the oxime C-N double bond, *i.e.* 4 possible sets of peaks. In addition, the spectra of these types of oximes were, as expected, simpler than those of the corresponding nitroso-dimers, which are complicated by the presence of diastereotopic protons (**Figure 22**, on the left). In agreement with this reasoning, after heating in isopropanol for 1 night, the polar fraction derived from the decomposition of thionitrite **166** was comprised of 4 compounds. By chromatography on SiO_2 this mixture was separated into two fractions containing two products each, one major and one minor. By means of bi-dimensional NOE experiments, the absolute stereochemistry around the episulfide ring was then assigned according to **Figure 23**.

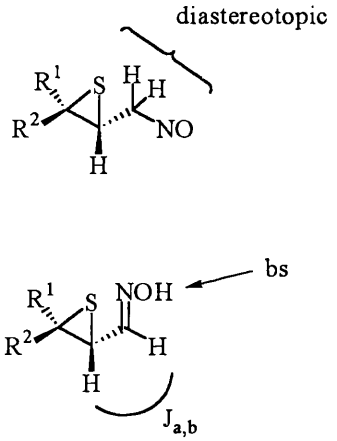


Figure 22. ^{1}H NMR of nitroso compounds *vs* that of oximes.

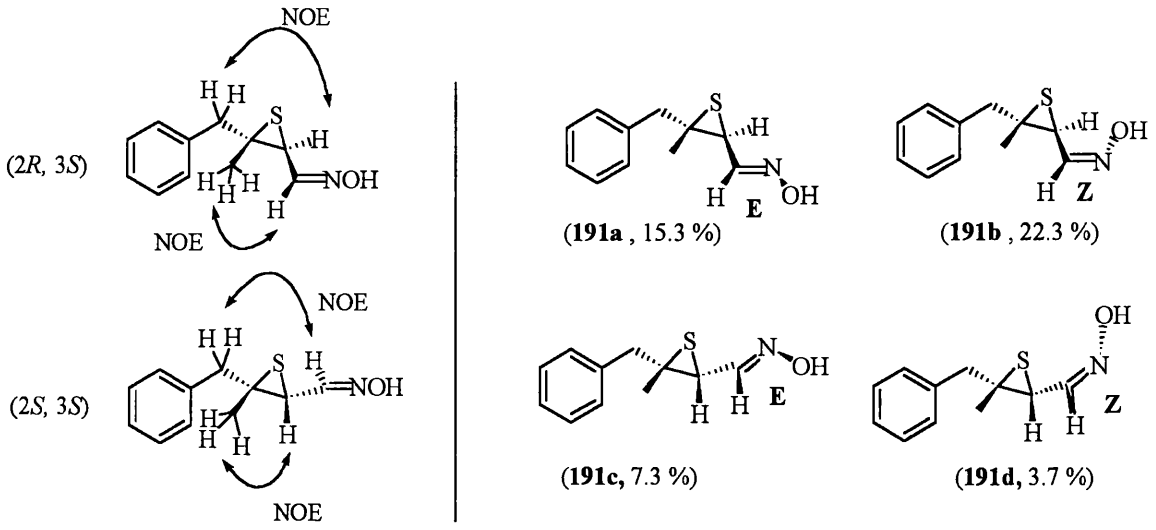
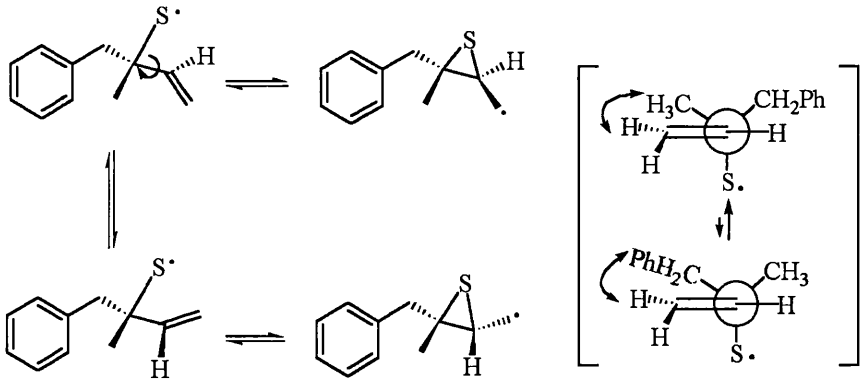


Figure 23. Assignment of stereochemistry of the oximes formed from decomposition of substrate **166**.

Thus, NOE experiments revealed that the two major oximes (**191a** and **b**) have the same relative stereochemistry of the episulfide ring (both are racemic mixtures). Therefore, they must have the opposite configuration of the oxime C-N double bond. In general, the signal for CHNOH on the ^{1}H NMR and that of CHNOH on the ^{13}C of oximes are shifted downfield for the *trans* isomer with respect to those of the *cis* counterpart. Thus **191a** must be the *trans* (*E*) oxime whereas **191b** would be the *cis (Z)* analog. Similarly, **191c** and **191d** both have the same relative configuration of the episulfide ring (as seen by NOE-exp.) and they must be the *trans* and *cis* isomers, respectively. This assignment is also in agreement with the fact that compounds **191a** and **191c** have very similar polarity and appear as a single spot on TLC, as do the pair of compounds **191b** and **191d**.

Hence, it appears that of the two possible conformations which can be adopted during the cyclisation step (**Scheme 67**), that in which the alkene and the bulky benzyl groups are farther away in space is favoured by a factor of approximately 4:1.

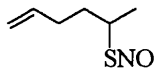


Scheme 67. Suggested explanation for the sterochemical outcome of the cyclisation of **166**.

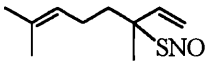
2. 6. Decomposition of linear thionitrites 164, 165 and 192.

2. 6. 1. Product studies.

In all the substrates analysed so far the double bond is placed in the allylic position with respect to the sulfur atom, so that 3-*exo* cyclisation takes place. In order to extend the scope of the reaction, the series of thionitrites **164**, **165** and **192** (depicted on the right), which now contain a double bond available for 1,5-*exo* cyclisation were prepared. The first two also contain, at the same time, an allylic double bond so that competitive reactions on both double bonds could, in principle, occur. The secondary thionitrite **192** was prepared *in situ* prior to heating as previously described and contains only one double bond.



(192)



(164)

After heating in benzene or DCM, thionitrite **164** (on the left) afforded very complex mixtures of both polar and non-polar products which could not be separated. In theory, these could be products formed as a result of the two competitive cyclisation processes which can take place. In principle, 1,5-*exo* cyclisation would be the kinetically favoured process. However, thiyl radical additions are known to be highly reversible and therefore under thermodynamic conditions mixtures could be possible. Furthermore, since the nitroso-compound formed as a result of a 1,5-*exo* cyclisation would be tertiary, it would not be capable of dimerising or tautomerising to give a more stable end product (nitroso-dimer or oxime). This could render this pathway slower and, maybe, reversible. In addition, the tertiary nitroso-compound formed could react with other radicals giving nitroxyl radicals of different types. The result of all these competitive pathways would be

a complicated mixture, in agreement with our observation. In order to simplify matters, thionitrite **165**, whose connectivity is analogous to **164** but contains no vinylic methyl groups on the remote double bond, was prepared. As expected, cyclisation of this thionitrite was much cleaner, with nitroso-dimers (**193**) formed as the main products (**Table 11**). The yields and structures of products were the same when the reaction was carried out in benzene or in DCM. Only traces of non-polar products were detected by TLC, which are presumably the result of a C-S homolytic fission, followed by migration of the allylic double bond, by analogy with the results obtained for the substrates previously studied. The mixture was still complex, since several diastereomers were formed. However, as before, spectra became much simplified by converting the mixture of nitroso-compounds into a mixture of oximes by refluxing in isopropanol for 1 night.

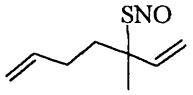
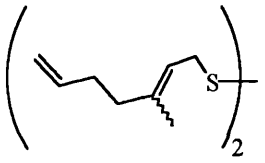
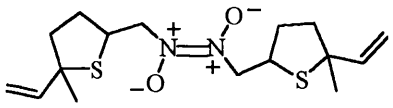
Thionitrite	Thermal decomposition products	benzene or DCM (yield)
 (165)		(traces)
	 (193)	(75 %)

Table 11. Thermal decomposition of thionitrite **165**.

The possible diastereomeric nitroso-monomers which could form, and their corresponding oximes have been depicted in **Figure 24**. In principle, 4 racemic pairs can be formed. Unfortunately, given that the substituents on the ring are now situated very far apart in space, NOE experiments did not allow the assignment of the relative stereochemistry around the five-membered ring in the products obtained. Both ^{1}H and ^{13}C NMR spectra revealed that the four possible oximes had been formed, and that they were present in a proportion 3.2:2:1.6:1.

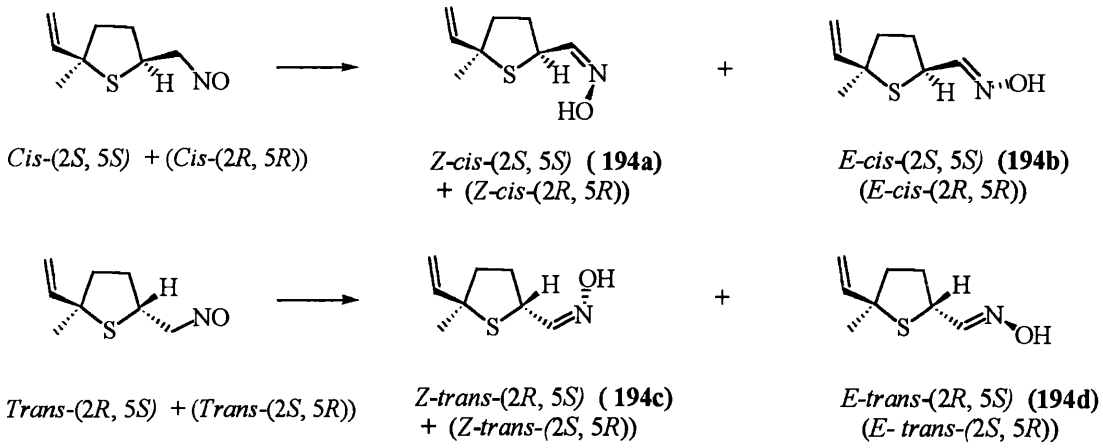


Figure 24. Stereochemical outcome of the 5-*exo* process.

To our surprise, the closely related thionitrite **192,** which was generated *in situ*, decomposed by a completely different pathway giving cyclic compounds **195** and **196** which could be separated by chromatography on SiO_2 (**Table 12**).

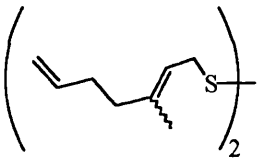
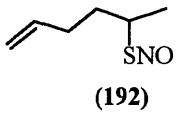
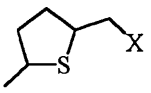
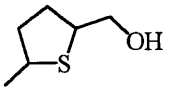
Thionitrite	Thermal decomposition products	Benzene or DCM (yield)
		(traces)
 **(192)**	 **(195)**	(*ca.* 19 %)
	 **(196)**	(19 %)

Table 12. Thermal decomposition of thionitrite **192**.

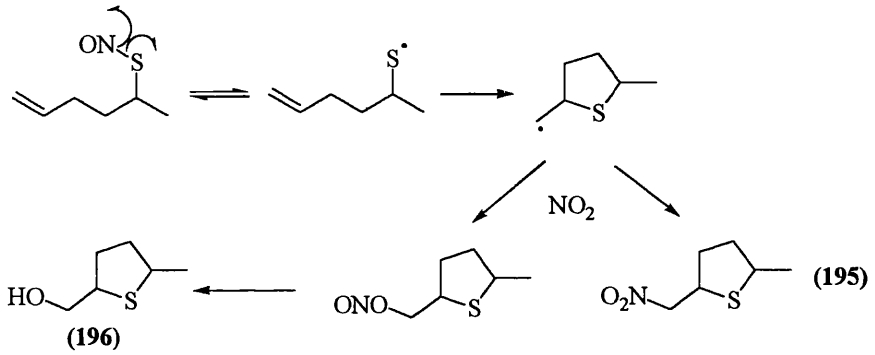
Alcohol **196** was actually separated as a 1:1 mixture of two diastereomers as depicted in **Figure 25**. Product **195** appears to be one single isomer of a cyclic compound with an unknown substituent X. Both ^{1}H and ^{13}C NMR and IR are compatible with X= NO_2. However, the MS results are not clear and, therefore, assignment at this time is only speculative.

(196a) **(196b)**

Cis-(2*S*, 5*S*) + (*Cis*-(2*R*, 5*R*)) *Trans*-(2*R*, 5*S*) + (*Trans*-(2*S*, 5*R*))

Figure 25. Alcohols **196**, stereochemistry.

The formation of both products (**195** and **196**) could be rationalised in terms of the trapping of NO_2 by the primary carbon-radical formed after cyclisation of the thiyl radical, either through N or through O (**Scheme 68**). However, the origin of NO_2 in the reaction mixture is not clear and further work is clearly required in order to clarify this point.



Scheme 68. Possible mechanism for the formation of products **195** and **196**.

2. 6. 2. Mechanistic considerations.

Some of the ideas discussed in section 2.2.2. are also applicable to this mode of reaction of unsaturated thionitrites (1,5-cyclisation), in particular, the comparison with the reaction illustrated in Scheme 59 (*viz.*, cyclisation of alkyl nitrites). There is one main difference between the reaction there discussed and the reaction of thionitrites: thiyl 5-*exo*-radical addition is a reversible process, whereas alkoxyl radical addition is irreversible. However, this does not seem to make much difference in practice and this might be attributed to the fact that the chain transfer or termination steps (reaction of the intermediate carbon-radical with another molecule of thionitrite or with NO) are very fast for the substrates here studied.

2. 7. Photochemical *vs.* thermally induced decomposition of unsaturated thionitrites.

The decomposition of thionitrite **158** also occurred upon irradiation with a tungsten lamp (500 W) for 4-5 hours in benzene. The same products as those obtained by the thermally induced decomposition were isolated in approximately the same yield. Since the photochemical reaction clearly occurs by a homolytic pathway, this result confirms the hypothesis that the radical mechanisms discussed above are likely to apply in the thermal decomposition as well.

CHAPTER 3

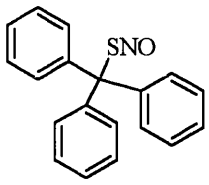
INTERMOLECULAR ADDITION OF THIONITRITES ONTO ALKENES.

3. 1. Introduction.

The results presented in the previous chapter demonstrated that the intramolecular addition of thionitrites across alkenes is an effective process which allows the simultaneous formation of two new heteroatom-carbon bonds (C-S and C-N or C=N). In continuation of this research, we then turned our attention to the study of the intermolecular version of such reactions. As mentioned in the introductory section, there was only one single paper in the literature of such a process by Gowenlock and co-workers[75], who reported the addition of tertiary trityl and *tert*-butyl thionitrites across the double bond of styrene in a reaction initiated by UV light. The authors also suggested that the reaction could be brought about thermally and that it would have synthetic utility, but the study was not pursued further. In view of the interesting results which we had obtained for the thermal intra-molecular reaction, we decided to investigate the intermolecular process in more detail, both under thermal and photochemical conditions.

3. 2. Preliminary studies.

3. 2. 1. Studies with the isolated thionitrite.



(55)

In the first instance, we set out to reproduce the literature results. For practical reasons, we concentrated our attention on the reactions with trityl thionitrite (**55**), which is a very stable, easy to manipulate, crystalline green solid. Gowenlock had used a UV mercury-lamp in order to initiate its decomposition[75]. However, since thionitrites absorb both in the UV and in the visible regions, we reasoned that a visible tungsten-lamp would produce a similar effect and its use would make the experimental procedure much simpler. In order to facilitate the identification all of the products formed during the reaction between trityl thionitrite and any chosen alkene, we first characterised the products which result from the thermal and photochemically induced decomposition of the isolated thionitrite in solution. Thus, heating a benzene solution of **55**, under N_2, at 65 °C for 5 h, yielded two products: the corresponding disulfide (**197**) and trityl alcohol (**74**) (**Table 13**). The same products were formed as a

result of irradiation with visible light for 1 night, along with traces of another product of intermediate polarity which was identified as benzophenone **75**.

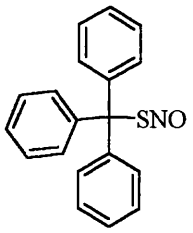
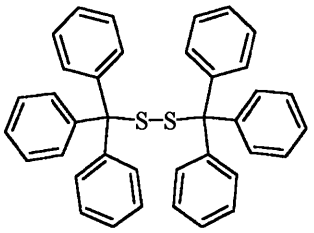
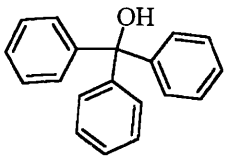
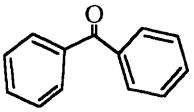
Thionitrite	Decomposition products	Δ (yield)	hν (yield)
 **(55)**	 **(197)**	(50 %)	(25 %)
	 **(74)**	(30 %)	(38 %)
	 **(75)**	(0 %)	(8 %)

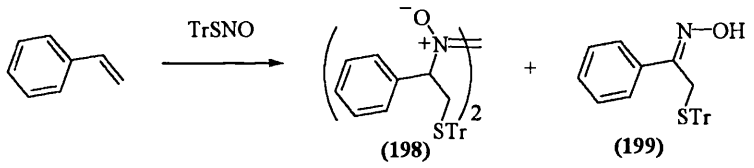
Table 13. Decomposition of trityl thionitrite.

It is clear that products **74** and **75** are the result of an initial fission of the C-S bond of the thionitrite to give the stable trityl radical or carbocation. However, the mechanism for their ultimate formation is not clear to us. Since reactions were carried out under N_2, it appears that the only possible sources of oxygen are the NO or SNO radicals or ONS^- anion generated upon cleavage of the S-N and C-S bonds of the substrate, or some derivative of those formed subsequently. It is worthwhile noticing that in all previous literature reports, the decomposition of this particular thionitrite has always been assumed to yield the corresponding disulfide, by analogy with what occurs for most other thionitrites studied. As we have seen in the previous chapter and we see here again, the general assumption that thionitrites yield disulfides upon decomposition is not always true, in practice.

3. 2. 2. Reactions with alkenes.

Once it was clear what products could be expected to form simultaneously with the addition product of **55** onto a given alkene, a benzene solution of the thionitrite, prepared according to the literature[52] and containing an excess of styrene, was irradiated with a 500 Watt Tungsten-lamp, at r. t. under the conditions indicated below (**Table 14**, 2nd entry) until it became colourless (1h). As expected, the mixture formed contained the products described above, along with styrene and the desired addition product, isolated in the form of a mixture of diastereomeric nitroso-dimers **198** and oxime **199**. The stereochemistry of oxime in the latter product is as drawn, as determined by X-Ray Crystallography (see appendix 1).

We found that, in order to achieve comparable yields under our conditions to those reported using a UV-lamp (1st entry on **Table 14**), 6 equivalents of styrene were required instead of the 3 used in the paper by Gowenlock, at a slightly lower concentration of thionitrite (4th entry). The same transformation could be brought about thermally by heating the thionitrite-alkene mixture in benzene at 65 °C for 1-2 h and yields were somehow lower. Again, the reaction worked better at lower concentrations (0.02 *vs.* 0.06, last two entries).

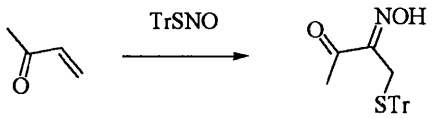


Styrene equiv.	Thionitrite conc. (M)	Initiation	197 (yield)	74 (yield)	75 (yield)	Addition products	(Yield)
3	0.03	UV	-	-	-	**198**	(60 %)[a]
3	0.03	Vis	c	c	c	**198-199**	(*ca.* 25 %)[b]
6	0.03	Vis	c	c	c	**198-199**	(40 %)
6	0.02	Vis	(0 %)	(12 %)	(5 %)	**198-199**	(48 %)
6	0.02	Δ	(2 %)	(34 %)	(0 %)	**198-199**	(38 %)
6	0.06	Δ	(0 %)	(0 %)	(59 %)	**198-199**	(27 %)

(a) Literature result in an unspecified solvent. (b) Calculated from ^{1}H NMR of the crude mixture. (c) Not separated. **Table 14.** Addition of trityl thionitrite onto styrene.

The effects of concentration and of the method employed for initiation were also analysed for the addition of **55** onto methyl vinyl ketone (**Table 15**). In this case the addition product was the ketone **200**, isolated in all cases as a single isomer whose stereochemistry was not assigned.

A concentration of 0.02 M seemed optimum for the photochemical reaction whereas a concentration of 0.04 M reduced the yield. Results were better at this concentration (0.04 M) when the reaction was carried out thermally, although the yields were lower than those of the photochemical process. Subsequently, these conditions were applied in the reactions of **55** with other alkenes.



(200)

Ketone equiv.	Thionitrite conc. (M)	Initiation	197 (yield)	74 (yield)	75 (yield)	Addition products	(Yield)
6	0.02	Vis	(7 %)	(26 %)	(2 %)	**200**	(57 %)[b]
6	0.04	Vis	a	a	a	**200**	(40 %)[b]
6	0.02	Δ	(28 %)	(31 %)	(0 %)	**200**	(29 %)[b]
6	0.04	Δ	(20 %)	(33 %)	(0 %)	**200**	(37 %)[b]
6	0.06	Δ	(28 %)	(31 %)	(0 %)	**200**	(28 %)[b]

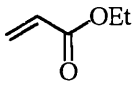
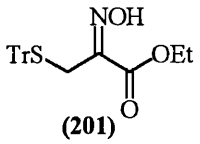
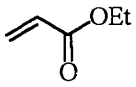
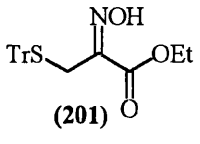
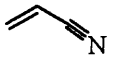
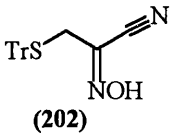
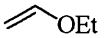
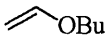
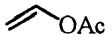
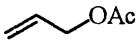
(a) Not separated. (b) Only one isomer of the oxime was formed.

Table 15. Addition of trityl thionitrite onto methyl vinyl ketone.

3. 3. Substrate scope: initial studies.

A systematic study was then undertaken in order to assess the substrate scope of the thermally induced addition reaction which was elected because of the simplicity of the experimental *setup* involved. In all cases we used trityl thionitrite and the optimised conditions previously found, *viz.* thionitrite concentration= 0.04 M in benzene, 6 equiv. of the alkene. The results are summarised in **Table 16**. Overall, it was found that addition took place for substrates containing either electron-withdrawing or neutral substituents which can conjugate with the double bond, whereas substrates bearing electron-donating groups conjugated with the double bond gave no addition products.

Similarly, addition did not take place onto non-conjugated alkenes such as cyclohexene, or allyl acetate.

Alkene	Initiation	Addition products	(Yield)
	Δ	 **(201)**	(5-10 %)[a]
	Vis	 **(201)**	(5-10 %)[a]
	Δ	 **(202)**	(30 %)[b]
	Δ	No addn.	-
	Δ	No addn.	-
	Δ	No addn.	-
	Δ	No addn.	-
	Δ	No addn.	-

(a) A single isomer of the oxime was formed. (b) The two isomers of the oxime where formed in a 1:1 proportion.

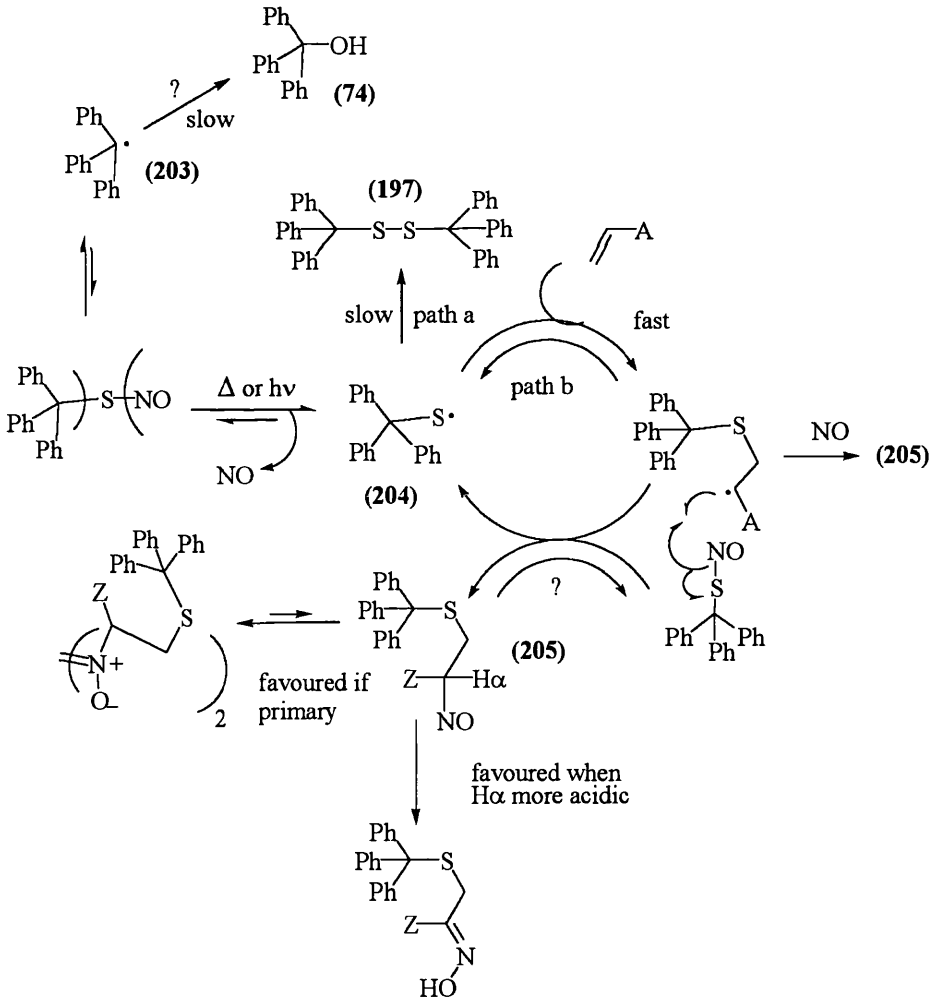
Table 16. Initial results of substrate studies.

It is interesting to compare this reaction with the addition of thiols to olefins, which is the simpler reaction involving addition of thiyl radicals. In particular, the results obtained from the reactions with ethyl acrylate were unexpected, since thiols are known to add to this substrate very effectively. The very different reactivity observed for methyl vinyl ketone (**Table 15**) or acrylonitrile in comparison with ethyl acrylate was also surprising, since thiols also add to all of them effectively[137]. It is known that non-activated alkenes, such as cyclohexene and allyl acetate react with alkane thiols such as dodecathiol four to ten times slower than do activated alkenes[138]. Thus, the lack of addition products for these substrates was not totally unexpected. In contrast, the overall

reactivity of n-butyl vinyl ether towards dodecanethiol is about two times higher than that of methyl acrylate[138], a result in contradiction with ours. Addition of alkane thiols onto alkenes conjugated with an alkene, an aromatic ring or an electron-withdrawing substituent is known to occur in high yields, in agreement with our results. These somehow contradictory observations led us to analyse the possible individual steps of the mechanism in order to be able to introduce further elements of optimisation at different stages of the reaction.

3. 4. The possible reaction mechanism and further optimisation.

By analogy with the mechanism proposed for the intra-molecular addition of thionitrites onto alkenes, the inter-molecular process may occur *via* the reactions illustrated in **Scheme 69**.



Scheme 69. Possible mechanism for the intra-molecular addition of thionitrites onto alkenes.

According to this mechanism, decomposition of trityl thionitrite could be initiated by two competitive processes: fission of the C-S bond or cleavage of the S-N bond. The resulting trityl radical **203** would eventually give rise to product **74** (and to a lesser extent to **75**). On the other hand, the thiyl radical **204** could dimerise giving **197**, by pathway a, or could be incorporated into an alternative pathway b, which after several steps would afford the observed addition products. Formation of by-products **197** and **74** appears to be a rather slow process, since decomposition of trityl thionitrite in the absence of an alkene or in the presence of a non-activated alkene requires around 5-10 times longer than addition onto reactive alkenes. Once thiyl radicals are incorporated into pathway b, 3 consecutive steps would lead to the formation of the observed addition products: (i) reversible thiyl radical addition onto the double bond, (ii) carbon radical adduct reaction with NO or RSNO and (iii) dimerisation or tautomerisation of the resulting nitroso-monomer. The overall rate of addition (relative to the rates of formation of by-products **197** and **74**) would thus depend on the relative rates of these three steps and their degree of reversibility. In order to simplify matters, it is better to discuss the aspects likely to affect each of the three steps separately.

3. 4. 1. Thiyl radical addition onto alkenes.

This is a reversible process[139, 140, 141]. Thus, thiyl radicals disappear in the presence of alkenes with the same rate they are consumed in their absence, indicating that the addition is reversible and that decay is due to dimerisation processes only. The addition is made irreversible by rapid, irreversible and selective quenching of the resulting carbon radical-adducts. This has been typically achieved by reaction with oxygen[141,142], reduction[140, 141] as in the trapping of a hydrogen atom from a thiol, addition onto another double bond, generally in an intra-molecular fashion[143], or reaction with a diselenide or ditelluride[144]. The analogous reaction with a disulfide appears to be less effective[144]. There are also examples in which a final irreversible step of this type can be preceded by another rapid equilibration process, for instance a skeletal rearrangement. Two typical examples are the opening of α-cyclopropanes or α-epoxides[145, 146].

The position of the equilibrium of thiyl radical addition onto an alkene is determined by the stability and character of the resulting radical adduct. Radicals can be stabilised by resonance, for instance with another alkene, an aromatic ring or a carbonyl group. On the other hand, radicals such as that resulting from addition onto cyclohexene

will be unstable and the loss of thiyl radical giving the alkene back will be a fast process. The same effect will be observed for the radical resulting of addition onto a vinyl-ether type compound. An effective overall process will be one for which the irreversible step following addition is faster than the loss of thiyl radical from the intermediate carbon-radical.

In general, two dominant factors influence the rate of radical addition reactions: polar and enthalpy effects. In addition, two other factors may also influence the outcome of the reactions: steric and solvent effects[147a]. Enthalpy effects are directly related to the stability of the product formed in the process, discussed above (**Figure 26**). In fact, this refers to free-energy values but, in order to simplify, entropic effects can be neglected. The term polar effect is used to describe the influence on the activation energy of any charge transfer which may occur in proceeding from reactants to the transition state. In **Figure 26**, two extreme cases in which polar effects act favourably or disfavourably have been represented. In this respect, thiyl radicals have been found to be moderately electrophilic in character. This means that a polar resonance structure such as $RS^{-}\ {}^{+}CR_2C{\cdot}XR$, predominates in the transition state. However, it has been demonstrated that for moderately electrophilic radicals both enthalpic effects and polar effects affect the rate of the reaction and, in fact, the latter predominate in most cases when varying the alkene.

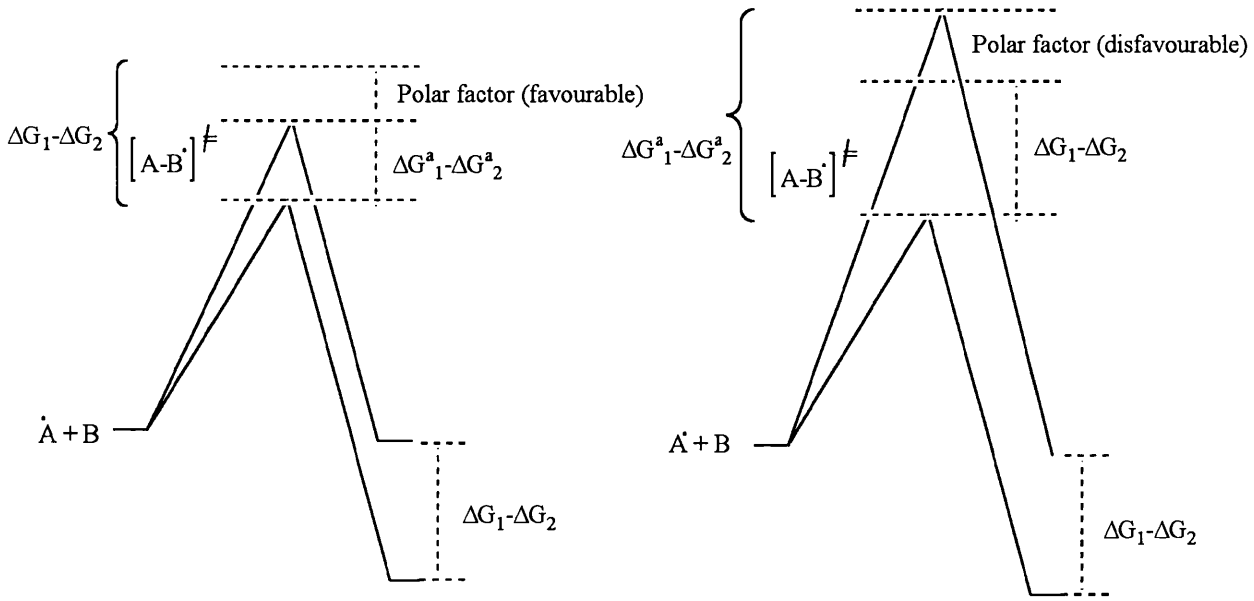
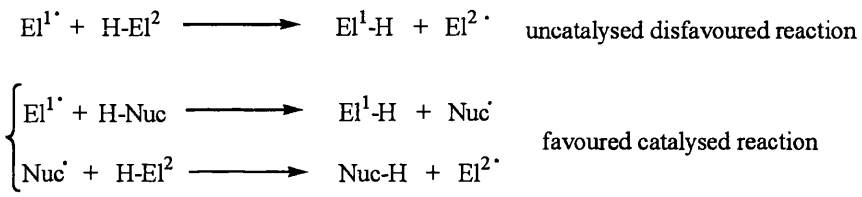


Figure 26. Schematic representation of the influence of enthalpy and polar effects in radical additions.

Negative polar effects can, in certain cases, be overcome. For instance, Roberts[147b] has elegantly resolved the problem of disfavouring polar effects in hydrogen abstraction reactions by the application of the concept of polarity-reversal catalysis. The principle underlying polarity-reversal catalysis in hydrogen-atom transfer is generalised in **Scheme 70**, where El· and Nuc· represent an electrophilic and a nucleophilic radical, respectively. The lack of stabilising charge-transfer in the transition state for the direct abstraction (1st equation) can be overcome by including a *hydridic* catalyst H-Nuc, when a single step process is replaced by a cycle of two hydrogen-atom transfer reactions with favourable polar effects. Similarly, a slow hydrogen abstraction from a nucleophilic hydrogen donor from a nucleophilic radical can be promoted by a protic catalyst H-El.



Scheme 70. Polarity-reversal catalysis of hydrogen abstraction[147b].

Polar and enthalpy effects due to variation in the structure of the thiyl radical can also be qualitatively rationalised, but this is not applicable in our case, since the thiyl radical used is always the same. These factors have to be considered when different thionitrites are compared. Nevertheless, it is worth mentioning that aryl thiyl radicals are generally less reactive than their alkyl counterparts. This is rationalised in terms of an enthalpy effect, i.e. aryl thiyl radicals are more stable than alkyl ones. But when comparing the reactivity of, for instance, several substituted aryl thiyl radicals, polar effects became important.

Thus, the rates of addition of several *para*-substituted aryl thio radicals (*p*-XC_6H_4S·) to *iso*-butyl vinyl ether, vinyl acetate or acrylonitrile were determined[147] and found to decrease in the order: X= NO_2 < Cl < H < CH_3 < OCH_3 and this was ascribed to predominant polar effects, i.e. stabilisation of the $XC_6H_4S^-$ form in the transition state. However, if one considers the reactivity of a given thiyl radical (for example X= OCH_3) towards the three different olefins, this decreases in the order: acrylonitrile > *iso*-butyl vinyl ether > vinyl acetate. And this is better explained in terms of an enthalpy (resonance) effect, since, if a polar effect was predominant, we would expect the

opposite order for addition, i.e. XArSCHCH^{+}OR would be more stable than XArSCHCH^{+}CN in the transition state. In another study[148149], naphthalenylthio radicals were found to add to several vinyl derivatives with the same order: styrene >> methyl methacrylate > acrylonitrile > *iso*-butyl vinyl ether > vinyl acetate. Hence, again, enthalpy (resonance) effects predominate over polar (stabilisation of charge-transfer forms) effects when comparing the reactivities of several alkenes. Similarly, the rates of addition of a given *p*-substituted aryl thiyl radical towards several monosubstituted alkenes was found to decrease in the order: ethyl vinyl sulfide > phenyl vinyl sulfide >> ethyl vinyl ether ~vinyl sulfoxide ~ vinyl sulfone. This correlates better with the resonance effects than with the polar effects. When comparing the reactivities towards styrenes containing different substituents[150], it was found that a small polar effect applied, and hence for *p*-substituted styrenes of general formulae *p*-XArCH=CH_2 reactivity decreased in the order: X= Cl > H > Me > OCH_3, although the variation in the rate constant values was very small. Dienes are, in general, very reactive towards thiyl radicals with the rate constants being the same order of magnitude as those of styrenes or vinyl sulfides and around 2 orders of magnitude more reactive than vinyl carbonyl derivatives. This is attributable to resonance effects[151]. When comparing substituted dienes, polar effects again predominate.

In summary, the first step, thiyl radical addition onto an alkene is governed predominantly by enthalpy effects and, in accordance with this, additions are fast onto olefins containing groups which can stabilise the resulting carbon-radical by resonance (dienes, styrenes, vinyl sulfides and carbonyl derivatives). Similarly, the rate is decreased if the thiyl radical is stabilised by resonance. Additions are slower onto olefins which do not contain resonance-stabilising groups. Among these, reaction is faster when the double bond is more of an electron-donor due to polar effects. Similarly, when comparing substrates in a particular family, polar effects again predominate. If one collects the data reported in the references given above, the following order of reactivity can be deduced for a given thiyl radical: styrene ~ diene ~ vinyl sulfide >> acrylonitrile > acrylate ~ vinyl ether ~ vinyl sulfoxide ~ vinyl sulfone > vinyl acetate >> cyclohexene.

So far, only the rate of addition has been considered. However, as mentioned above, the overall result of the reaction will be determined both by the rates of the first

equilibrium and of the subsequent irreversible reaction and situations can be found in which either the first or the second is the rate-determining step.

Thiyl radicals have usually been generated from thiols or disulfides and, thus, most examples found in the literature deal with these. For instance, 2-butenes isomerise quickly from their *cis* to their *trans* isomers in the presence of small quantities of thiol[152] or disulfide, because the radical adducts formed upon addition cannot be quenched fast enough. These are examples in which chain propagation is the rate-determining step and, therefore, the addition remains reversible. In a similar manner, addition of thiyl radicals generated from disulfides affords products which are derived from alternative reactions of the intermediate carbon-radical adduct due to the fact that propagation of a chain by reaction with another molecule of disulfide or termination by reaction with another thiyl radical are not efficient processes[144] and, therefore, the addition remains reversible. Conversely, if a large excess of a thiol is used or if the thiol is chosen so that hydrogen atom abstraction from the S-H bond by the carbon radical is facile, the product is that derived from addition of the thiol, and here the rate-determining step is the addition and this becomes essentially irreversible. If the rates of hydrogen atom abstraction of several alkyl radicals from a particular thiol are compared, again both enthalpy and polar effects become apparent[138]. Thus, hydrogen atom abstraction by the benzyl radical obtained after thiyl radical addition onto styrene is very slow. This is explained by the high stability of the carbon-radical. In contrast, hydrogen abstraction by a cyclohexyl or cyclopentyl radical is comparatively much faster, due to the higher reactivity of the corresponding carbon-radical. In the styrene case, the rate determining step of the overall thiol-addition process is the chain propagation but, because the addition is very fast and essentially irreversible, in a competitive reaction the overall process would be favoured for styrene in comparison with cyclopentene. In the cyclopentene case, the chain propagation is relatively fast (when compared to the styrene reaction) but, both steps, addition and hydrogen abstraction have similar rate constants and the addition still remains reversible as proved by the fact that a fast isomerisation process can still take place before hydrogen abstraction. Thus, although the addition of a thiol onto styrene is slower, formation of disulfide by-products will be diminished due to the low degree of reversibility of the addition. By way of comparison, addition of a thiol onto cyclopentene is faster, but more reversible. In order to solve this problem, one can accelerate the

hydrogen abstraction step by adding a large excess of thiol, thus making the addition appear less reversible. The reaction of phenylthiol with cyclooctene is an example in which addition is the rate determining step. Whereas the addition of thiyl radicals to methyl acrylate is faster than to methyl vinyl ether, additions of thiols are twice as fast for the second case. This can be ascribed to a higher rate of hydrogen atom abstraction in the vinyl ether case and this would be explained by a polar effect, since the corresponding carbon radical is more nucleophilic. Taking all of these factors into consideration, additions of thiols have been carried out effectively on all types of alkenes (electron-rich, electron-poor and non-activated) by adequate tuning of the reaction conditions.

In comparison to the reactions discussed in the preceding paragraph, irreversible quenching by trapping of oxygen is very fast in all cases and there is very little variation depending on the structure of the alkene. This, in contrast to the reaction with a thiol, is not a chain reaction if the thiyl radicals are generated from disulfides. In our particular case, the second step would be either a reaction of the intermediate carbon-radical adduct with a molecule of thionitrite in a chain process as in the thiol-addition reaction or, alternatively trapping by NO in a non-chain process.

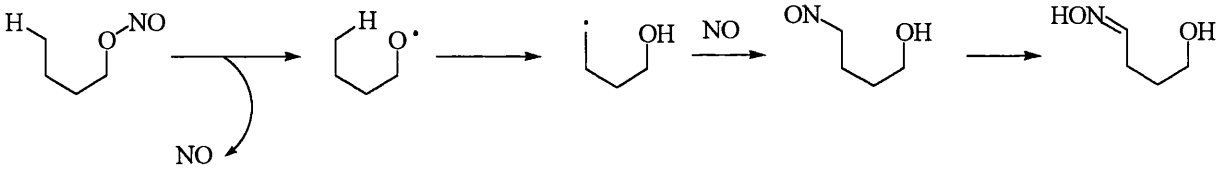
3. 4. 2. The second and third steps: chain transfer with thionitrite *vs* NO trapping and oxime formation.

Let us first consider the non-chain reaction. NO is known to be a very good radical trap for all types of carbon-radicals. Thus, there are examples in the literature in which NO has trapped electron-poor[153], electron-rich[154] and electronically neutral carbon-radicals[159]. In many of these examples, the carbon radical has been generated in the presence of a large excess of NO. In this respect, the reaction of a thiyl radical with an alkene in the presence of a large excess of NO would, in theory, lead to the same products as those formed during the addition of thionitrites onto olefins. Under these conditions, the precedent addition equilibrium would become essentially irreversible in a similar way to those reactions when a large excess of oxygen is added to the reaction mixture. However, there is an inherent difficulty to such an approach in the case of NO. Whereas O_2 reacts selectively with carbon radicals and not with thiyl radicals, NO reacts with both. Hence, in the presence of an excess of NO, thiyl radicals would be

competitively consumed by formation of thionitrites and this would diminish the rate of addition. Indeed, when we reacted trityl thionitrite with cyclohexene under a NO atmosphere, the decomposition of the thionitrite, as monitored by the fading of the green colour, was not complete after several days and no addition product had formed.

During the thermal decomposition of trityl thionitrite in the presence of alkenes, NO is expected to be formed in much lower concentrations than those in some of the experiments reported in the references above. Trapping of NO by an alkyl radical is an irreversible process under our conditions. Under UV irradiation or heating at very high temperatures, nitroso-monomers are known to revert to the alkyl radicals and NO[155]. Although irreversible, the process is likely to be relatively slow given the low concentration of NO present at each particular moment. It seems reasonable to assume that reaction of the carbon-radical with another molecule of RSNO, present in much higher concentrations, would be preferred. Nevertheless, there are examples in the literature in which trapping of NO in a non-chain process is preferred over a radical chain pathway by attack of a carbon centered radical with a second molecule of RONO. One could argue that this might be ascribed to the fact that O-NO bond cleavage is relatively difficult.

The classical example of such a situation is the Barton nitrite photolysis reaction. The mechanism of this transformation has been studied in great detail and is believed to involve the steps depicted in **Scheme 71**[156].

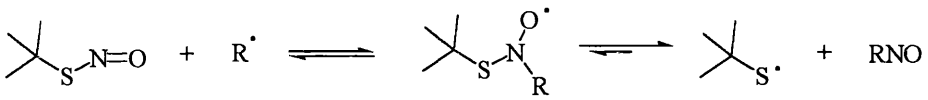


Scheme 71. Accepted mechanism of the Barton nitrite photolysis[156].

This has been proved to be a non-chain, "non-cage" process. If the addition of thionitrites was a non-chain process equivalent to this, there would be one main difference between the two reactions. The process leading to the formation of the carbon-radical in the Barton reaction is irreversible, whilst the process leading to the carbon radical in thionitrite additions is the reversible addition of a thiyl radical. In the former procedure, the carbon radical formed can only react with NO, fragment, or add

intra-molecularly onto an appropriately sited double bond. Intermolecular addition has also been effected, but required the use of a very large excess of the olefin (~ 80 equiv.)[157]. Both processes have been found to occur and this implies that NO trapping is not always the predominant process. In the absence of any other possible courses for reaction, NO trapping is responsible for the structure of the final product. In our case, the carbon-radical has another alternative pathway to follow which involves loss of thiyl radical. This is likely to become more important for non-stabilised alkyl radicals and this effect could explain the lack of addition product formation in the reactions of trityl thionitrite with electron-rich or non-activated alkenes (*vide infra*).

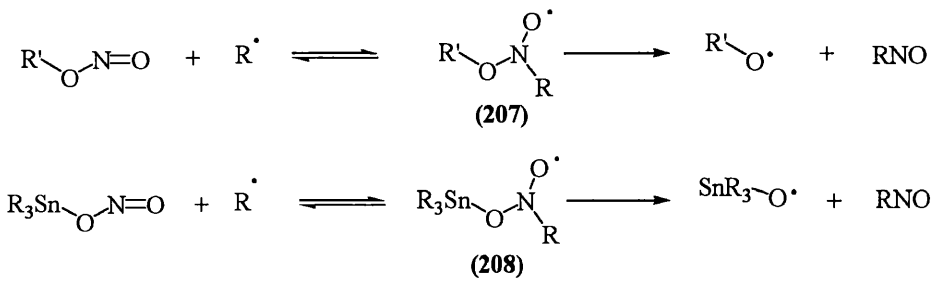
Let us now consider a more likely chain-process. We can envisage two possible modes of reaction: the first one, analogous to the reaction with a thiol, would involve NO abstraction in a more or less concerted process; the second one, would involve addition of the carbon-radical onto the N=O bond of a thionitrite molecule, with generation of an intermediate aminoxyl radical (**206**), (**Scheme 72**). This could then dissociate to give the nitroso monomer and a thiyl radical or revert to the initial carbon radical. Since nitroso-compounds have been used as traps for thiyl radicals[158], recombination of a thiyl radical with RNO to give again intermediate **206** is also feasible, unless RNO disappears quickly through other pathways and thus, the overall second step could, in principle, be reversible.



(**206**)

Scheme 72. Proposed pathway of nitroso-monomer formation.

An analogous type of process was invoked by Murphy [159] to explain the formation of oximes from the reaction of carbon-radicals with either Bu_3SnONO or RONO (**Scheme 73**). The authors suggested that this was a reasonable assumption given the excellent trapping properties of nitroso compounds towards alkyl radicals. Incidentally, it is worth noticing that here an alternative non-chain process by NO trapping is not possible.



Scheme 73. Carbon radical additions onto the N=O of nitrites[159].

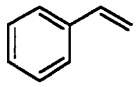
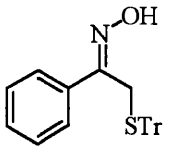
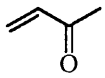
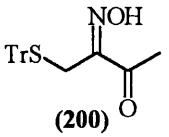
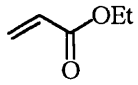
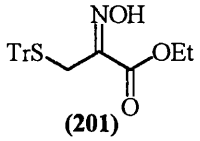
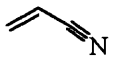
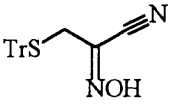
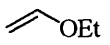
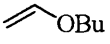
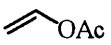
Nevertheless, there are two main differences between the processes illustrated in Schemes 72 and 73. First, the carbon-radicals generated in the work of Murphy[159] are formed irreversibly. By way of contrast, their formation in our work is the result of a reversible thiyl radical addition. Secondly, formation of RNO in Scheme 73 is accompanied by formation of an alkoxyl radical whereas a thiyl radical is formed in our work. Recombination of the alkoxyl radical with the nitroso compound to give back intermediates **207** or **208** would be feasible, since alkyl nitrites have been proved to act as spin-traps of alkoxy radicals[160]. Nevertheless, since alkoxy radicals are more reactive than thiyl radicals, the former might disappear before recombination *via* fragmentation or hydrogen abstraction pathways, rendering the overall process irreversible. Conversely, thiyl radicals do not tend to fragment or abstract hydrogen. They could either combine with the nitroso monomer, reverting to intermediate **206**, or react with the alkene, a process which is also reversible!!. Hence, in our case, both the first and the second steps in the overall addition process (Scheme 69, *vide supra*) might be reversible.

Irreversibility in the second step might be ensured by different means. Following the reasoning applied for the optimisation of thiol addition onto alkenes, it might be possible to favour the forward reaction by changing the structure of the thionitrite so as to form a more stable thiyl radical or by increasing its concentration. Given that thiyl radicals are involved in several equilibria, complications could arise from the first approach. However, in order to test the second possibility, we performed the reaction with slow addition of the alkene onto a solution of trityl thionitrite. We expected that, under these conditions, the chain transfer and hence the overall process would be favoured. In practice, no substantial effect was observed. Another way of influencing the irreversibility of the chain transfer step is by ensuring that the initial nitroso product (RNO) is rapidly and irreversibly converted into the corresponding oxime or nitroso-

dimer. Tautomerisation of a nitroso monomer to the oxime is known to be accelerated by polar solvents[161]. Dimerisation of a nitroso-dimer can be reversible and is favoured for primary monomers. It is more difficult to control other factors which may affect the dimerisation[161]. On the other hand, formation of oximes would be favoured by a more acidic Hα proton. This might explain the difference in reactivity between methyl vinyl ketone and ethyl acrylate. In this respect, we reasoned that carrying out the reaction in isopropanol might accelerate the last step of the overall process, leading to the formation of the desired addition products. In practice, for solubility reasons, these reactions had to be conducted in a 2:1 mixture of benzene and isopropanol.

To our delight, under these conditions, yields of products resulting from thermal induced addition increased in all cases for substrates containing a resonance stabilising group on the double bond (**Table 17**). However, for those substrates which had not given addition products under the previously employed conditions, the result did not vary.

So, where do the main differences of behaviour between the two types of substrates reside?

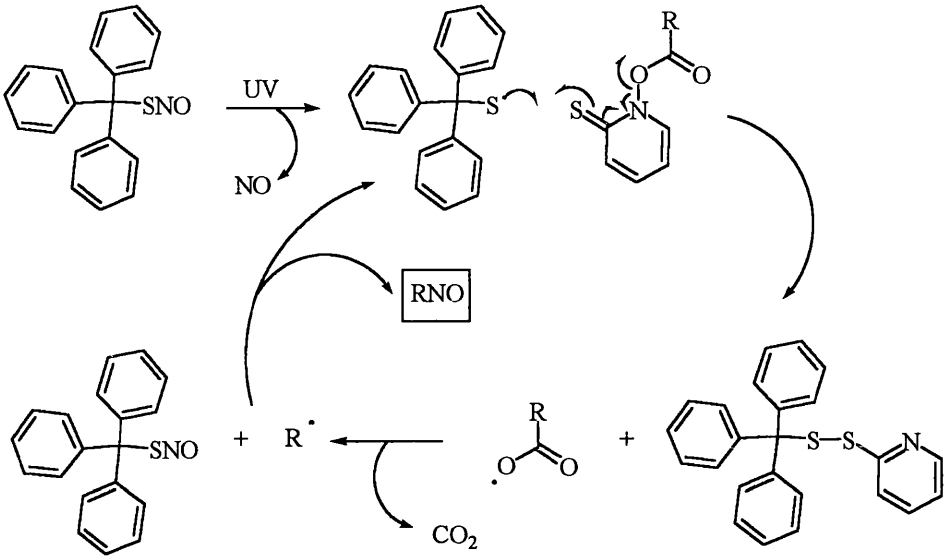
Alkene	Initiation	Addition products	(Yield)
	Δ	 **(199)**	(72 %)[a]
	Δ	 **(200)**	(55 %)[a]
	Δ	 **(201)**	(23 %)[a]
	Δ	 **(202)**	(35 %)[b]
	Δ	No addn.	-
	Δ	No addn.	-
	Δ	No addn.	-
	Δ	No addn.	-

(a). Only one isomer of the oxime was formed. (b) The two isomers of the oxime were formed in a 1:1 proportion.

Table 17. Additon of trityl thionitrite onto alkenes in benzene:isopropanol.

Trapping of NO from an alkyl nitrite (RONO) has been found to be effective for both stabilised and non-stabilised radicals in the reaction discussed above[159]. Furthermore, trapping of NO from a thionitrite has also been found to be effective in a radical chain process for non-stabilised carbon-radicals in a reaction reported by Motherwell and co-workers[162]. The authors studied the decarboxylative nitrosation of esters 2-mercaptopyridine *N*-oxide in the presence of trityl thionitrite. The reaction was

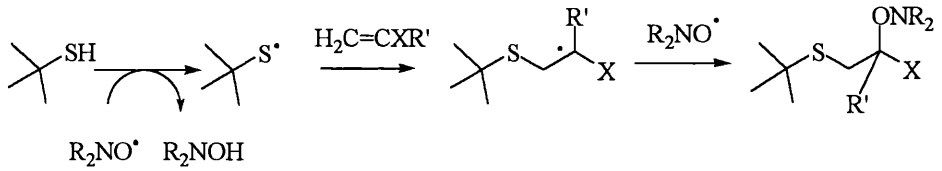
initiated by homolytic cleavage of the thionitrite S-N bond (UV light) and the following radical-chain mechanism was proposed (**Scheme 74**):



Scheme 74. Decarboxylative nitrosation with thionitrites[162].

Again, the major difference with the reaction herein studied is related to the issue of reversibility. First, the carbon radical generated in Scheme 74 is formed irreversibly, due to simultaneous formation of a stable, neutral gas molecule (CO_2). In addition, the chain transfer step, even if one assumes it taking place *via* formation of an intermediate such as **206**, would also become irreversible, since it is followed by rapid and eventually irreversible reaction of the thiyl radical with another molecule of the hydroxamic ester. Hence, in this case, thiyl radicals are consumed irreversible during the chain-reaction, whereas in our case, all the steps in which these may be involved are reversible and the only alternative pathway by which they might be consumed would be dimerisation to give the unwanted disulfide.

Similar results to those obtained by us, where reported by Heiland[163] in the trapping of carbon-radicals generated by thiyl radical addition onto an alkene with an aminoxyl radical trap (**Scheme 75**). This is a non-chain process, in which the aminoxyl trap is present in excess (> 2 equiv.) from the outset of the reaction and is used both to trap the carbon-radical adduct generated upon thiyl addition and to generate thiyl radicals from thiols.

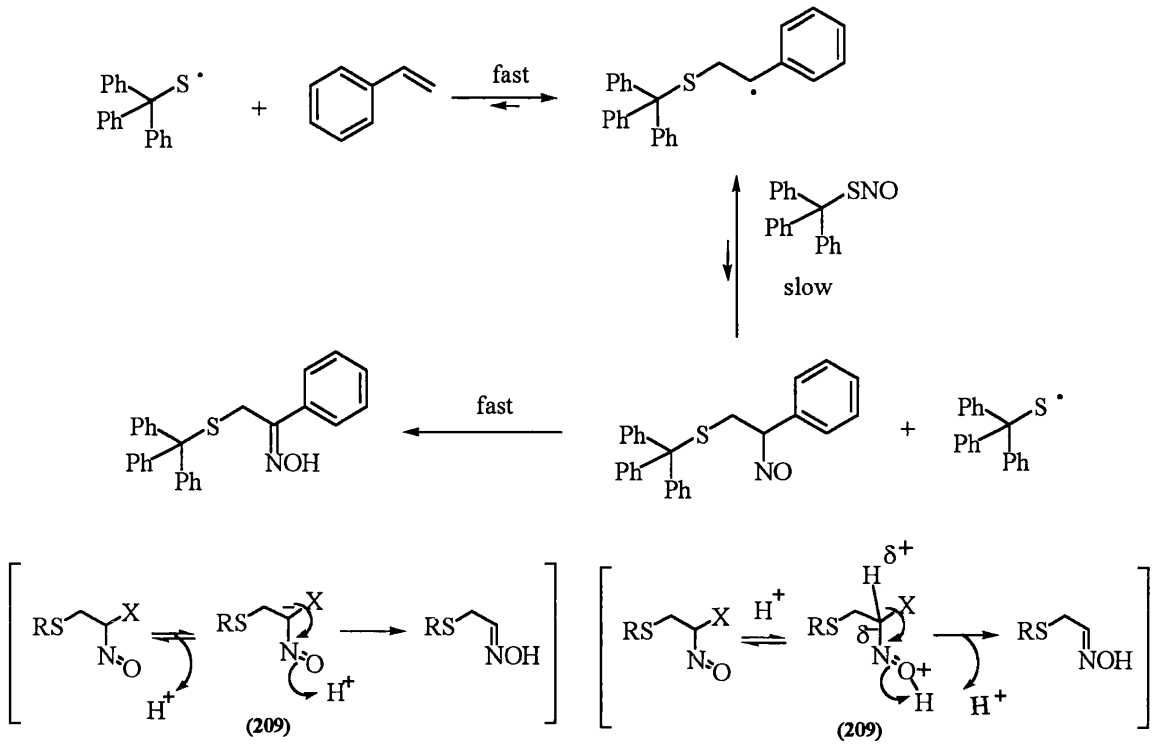


X	yield of addition	yield of disulfide
CN	44	56
$COOCH_3$	54	46
OAc	26	66

Scheme 75. Trapping of carbon-radicals generated by thiyl radical additions[163].

As in our reaction, the yield of addition is higher for acrylonitrile and acrylate than for vinyl acetate with the yield of disulfide formation increasing in the inverse direction. However, the yield of addition is better for acrylate than for acrylonitrile whereas we observe the opposite effect. In the reaction illustrated in Scheme 75, the only step likely to be reversible is the addition of the thiyl radical. Following the arguments developed above for the thiol addition reaction, addition will be faster and less reversible for acrylonitrile (or acrylate) than for vinyl acetate, due to the resonance effect. For the same reasons, trapping will be faster for the substrate containing the acetate. Thus, for acrylonitrile, trapping will be the rate determining step and the overall process will have little reversibility. The overall result is a higher yield of addition. In contrast, in the acetate case, the addition will be slower and more reversible and trapping will be fast. Although the overall forward process is likely to be fast, if trapping is not faster than the back reaction in the previous equilibrium, the overall process will still be reversible, giving time for more disulfide to be formed.

Analogous arguments can be put forward to explain the results in the addition of thionitrites to alkenes, but this has to be done with more caution due to the higher degree of complication involved in our reaction with the presence of several competitive equilibria. Thus, for a substrate such as styrene the addition will be expected to be fast and little reversible (**Scheme 76**). In turn, the chain transfer would be slow and more reversible, since it would revert to a stable carbon-radical. The last step, tautomerisation, is always irreversible. All of the equilibria can then be displaced to favour product formation.

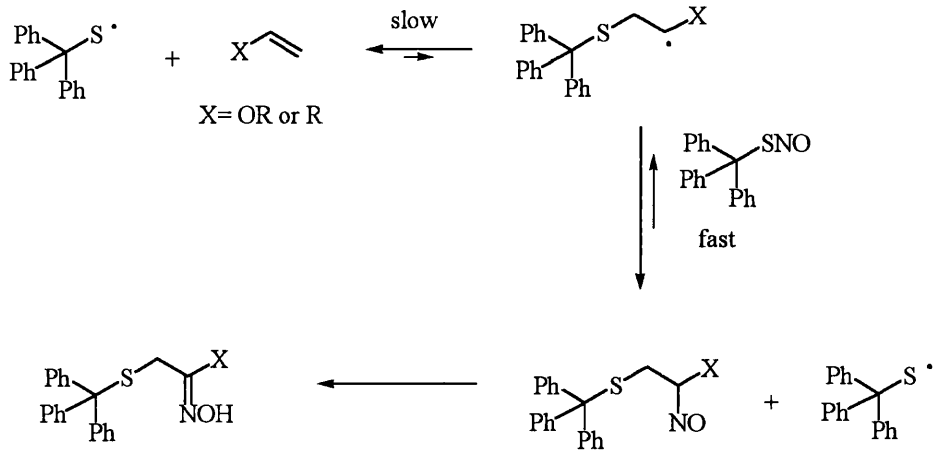


Scheme 76. Mechanism proposed for the addition of trityl thionitrite onto an activated alkene.

Thus, when the tautomerisation rate is increased by using a polar solvent, yields should increase, as observed. However, even if tautomerisation was relatively slow, the overall direction in which both of the preceding equilibria are displaced implicates that the species in higher concentration in the equilibrium would be the carbon-radical and not the thiyl radical and thus dimerisation would still be a minor process, in agreement with our observations.

Conversely, for a substrate such as an alkyl vinyl ether or cyclohexene (**Scheme 77**), the addition is expected to be slow and highly reversible. The chain transfer step would be relatively fast and less reversible. In this case, both equilibria would be directed in the direction of thiyl radical formation, favouring dimerisation to the disulfide. In addition, tautomerisation would be disfavoured, since a species such as **209**, where X is an electron-donating group or an alkyl group would not be very stable. It would appear, then, that even under induced tautomerisation by isopropanol, the last step is far too slow, for these substrates, to make the second equilibrium and, in turn, the first one, leading to the wanted addition product, totally irreversible. Hence, for these substrates,

dimerisation to the disulfide becomes a highly competitive process and, in practice, it seems to be the most predominant one.



Scheme 77. Mechanism proposed for non-activated alkenes.

We have only considered formation of an oxime as the last step. Formation of a nitroso-dimer is also possible although it might be reversible. Factors affecting both reactions may be different. In all of the successful examples of intra-molecular reaction discussed in the previous chapter, dimerisation seems to predominate. This is in agreement with the fact that the nitroso-monomers formed are primary. Dimerisation for secondary alkyl-nitroso compounds is also known, but might be slower for the substrates which we have studied. In addition, the intra-molecular process could involve reaction in a solvent-cage and more work would be needed in order to ascertain the differences in the mechanism for the intra- and inter-molecular processes.

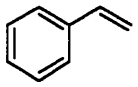
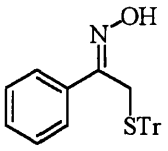
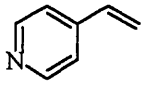
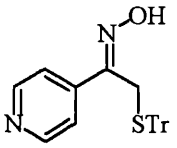
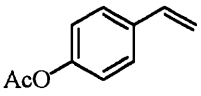
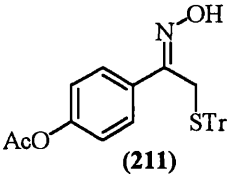
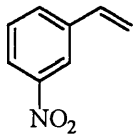
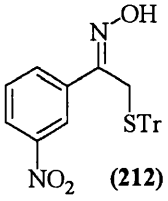
3. 5. Extending the applicability of the reaction.

Once optimised conditions were established, studies were initiated towards determining the general applicability of the reaction by assessing the effects of structural variation within families of alkene substrates.

3. 5. 1. Styrene derivatives.

Introduction of a *para* electron-withdrawing group in the aromatic ring (pyridine) resulted in an increase of the yield of the addition product (**Table 18**). Conversely, introduction of the electron-donating acetoxy group in the same position, led to a decrease in the yield. This is in disagreement with the effects observed during thiyl

radical addition. It is in agreement with the hypothesis that the acidity of the Hα proton in the initially formed nitroso-monomer is a key factor. Following this rationale, we expected that introduction of a nitro group on the *meta* position would not have much effect on the yield. However, although, qualitatively, this seemed to be the case (TLC), the resulting addition product proved to be very unstable and decomposed during chromatography giving only a 10 % isolated yield. In all cases only one isomer of the corresponding oxime was formed.

Alkene	Initiation	Addition products	(Yield)
	Δ	 **(199)**	(72 %)[a]
	Δ	 **(210)**	(87 %)[a]
	Δ	 **(211)**	(23 %)[a]
	Δ	 **(212)**	(10 %)[a]

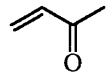
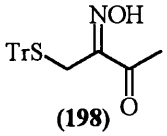
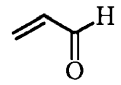
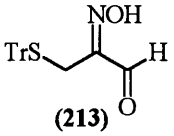
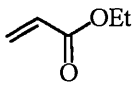
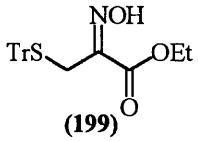
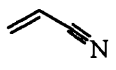
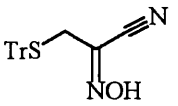
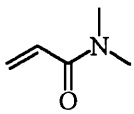
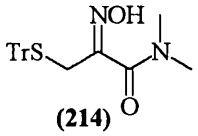
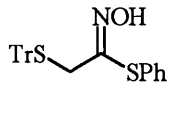
(a) Only one isomer of the oxime was formed.

Table 18. Trityl thionitrite addition onto styrene derivatives.

3. 5. 2. Carboxylic acid derivatives and related substrates.

As can be seen from the results summarised in **Table 19**, the yields of isolated addition products seem to correlate both with the acidity of the H_α proton in the initially formed nitroso-monomer and with the polar effects in the addition step. These two effects are now acting in opposite directions, i.e. the ability of carboxylic acid derivatives

to stabilise an intermediate such as RSCHC(−)X will vary with X exactly in the opposite direction to their ability to stabilise a polar charge-transfer form such as RSCH(+)X in the transition state.

Alkene	initiation	addition products	(yield)
	Δ	 **(198)**	(55 %)[a]
	Δ	 **(213)**	(42 %)[a]
	Δ	 **(199)**	(23 %)[a]
	Δ	 **(200)**	(40 %)[b]
	Δ	 **(214)**	(42 %)[a]
	Δ	 **(215)**	(29 %)[b]

(a) Only one isomer of the oxime was formed. (b) The two isomers of the oxime were formed in a 1:1 proportion

Table 19. Trityl thionitrite addition onto carboxylic acid derivatives.

Yields obtained from the additions onto acrylonitrile, ethyl acrylate or vinyl sulfide are between 15 and 20 percent lower than those obtained for methyl vinyl ketone and this correlates well with a value of the pKa of around 25 for former and a value around 20 for the later. We could have expected to obtain a similar yield for the reactions with methyl vinyl ketone and acrolein. Nevertheless, the product obtained from the reaction with the latter proved to be unstable and decomposed slowly even if kept in the freezer

and this could explain the difference. Based only on acidity effects, acrylamide would be expected to react less efficiently. However, the opposite result was obtained and this might be ascribed to a predominant polar effect in the addition step. The different reactivity of the ester and the nitrile, which have a very similar pK_a, can be ascribed to the different rates of addition of tritylthiyl radical onto both substrates.

3. 5. 3. Addition onto conjugated dienes.

We anticipated that reactions onto conjugated dienes would work in a similar fashion to styrene-type substrates, given the resemblance in electronic character between a benzyl and an allyl group. In effect, this proved to be the case, as demonstrated by the results summarised in **Table 20**. As expected, only 1,4-addition products were isolated.

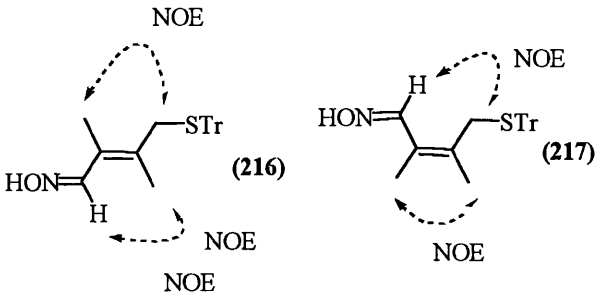


Figure 27. Observed NOE effects for **216**.

Reactions with substituted dienes provided an additional element of interest, since several stereoisomers and regioisomers could be formed. For instance, the reaction with 2,4-dimethyl-1,3-butadiene afforded mostly isomer **216**, as determined by means of NOE experiments (see **Figure 27**, above). In a similar manner, ^{1}H and ^{13}C NMR and NOE experiments revealed that the reaction onto 1-acetoxy-1,3-butadine had resulted in formation of only one regioisomer, that in which the tritylthiyl radical had added to the least substituted position of the diene, due to a predominant steric effect. In addition only one stereoisomer around the C=C double bond can be detected (structure **218a**, in **Figure 28**), since the NOE effect illustrated, between the CH_2S and the =CH groups, could be observed. The stereochemistry around the C=N double bond (oxime) has not been determined, but, as in most of the examples presented before, only one isomer was formed. It is interesting to note that here the NO is transferred onto a carbon

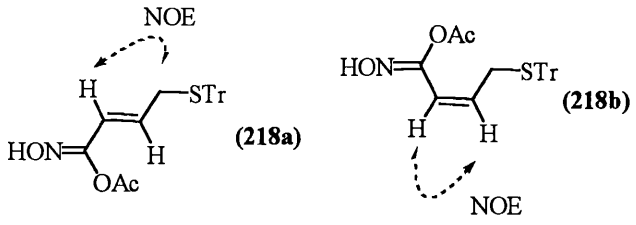


Figure 28. Observed NOE effects for **218**.

which is next to an electron-donating group. Hence, it appears that the negative acidity effect is overcome by more favourable electronic effects.

From isoprene, two major regioisomers were formed, both of which involve attack of the thiyl radical through the less substituted end of the diene (**Figure 29**). Two diastereomers of the first regioisomer were isolated and three of the second regioisomer. The stereochemistry around the double bond for 4 of them could be determined by NOE experiments.

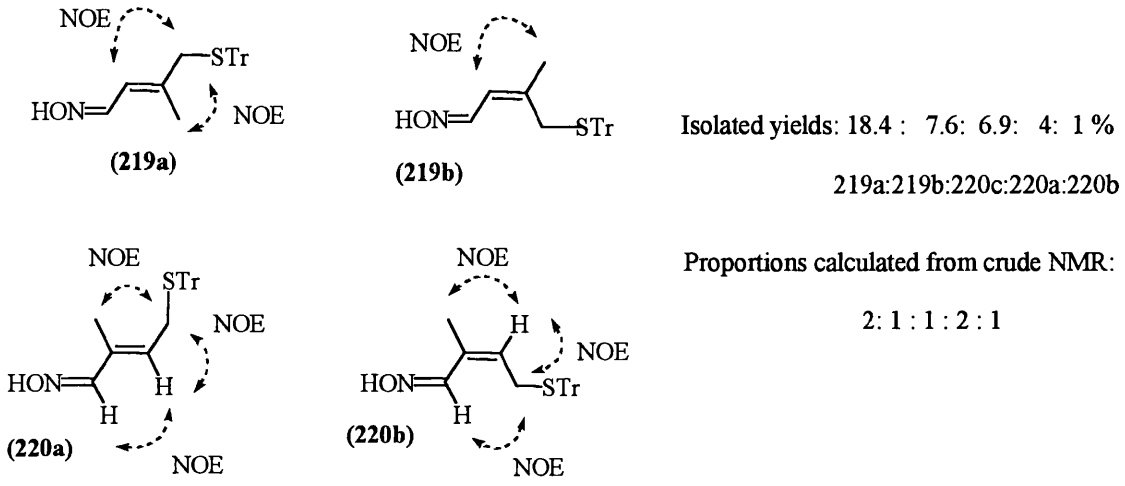
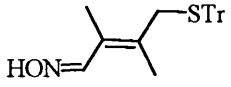
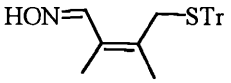
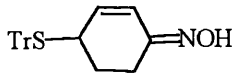
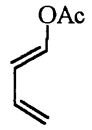
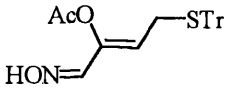
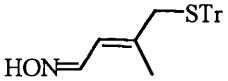
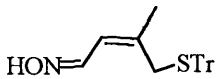
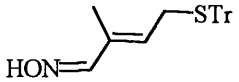
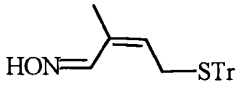
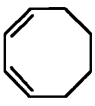
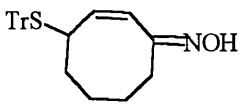


Figure 29. Observed NOE effects for the isoprene addition products.

As expected, the major products are the all-*trans* isomers (**219a** and **220a**).

Diene	Initiation	Addition products	(Yield)
	Δ	 **(216)**	(75 %)[a]
		 **(217)**	traces[a]
	Δ	 **(221)**	(42 %)[a]
	Δ	 **(218a)**	(10 %)[a]
	Δ	 **(219a)**	(18.4 %)[b]
		 **(219b)**	(7.9 %)
		 **(219c)**	(6.6 %)
		 **(220a)**	(4 %)
		 **(220b)**	(1 %)
	Δ	 **(222)**	(17 %)[a]

(a) Only one isomer of the oxime was formed. (b) These are isolated yields, the spectrum of the crude gives a proportion 2: 1 : 1 : 2 : 1.

Table 20. Trityl thionitrite addition onto dienes.

3. 5. 4. Other substrates.

The reaction of tritylthionitrite was also studied with a large number of other substrates, without the formation of addition products (**Figure 30**). The lack of reactivity towards trityl thionitrite can be explained using different arguments, depending on the substrate. For substrates containing either a double bond which is conjugated with an electron-donating group, or which is not activated, the arguments exposed in the previous sections apply. For alkenes with a higher degree of substitution, steric effects might predominate. The reaction was also attempted with dienes such **223**, **224**, **225** and **226** for which reaction with thiols are known to result in addition of thiyl radical, followed by 5-*exo* cyclisation onto the other double bond and H atom capture to give products of general structure **227**[143]. In our system, no cyclised products of any type were detected. Non-cyclised products have been found to predominate in similar reactions depending on the conditions and on the structure of the substrate[143]. Clearly, more work is needed in order to clarify this point.

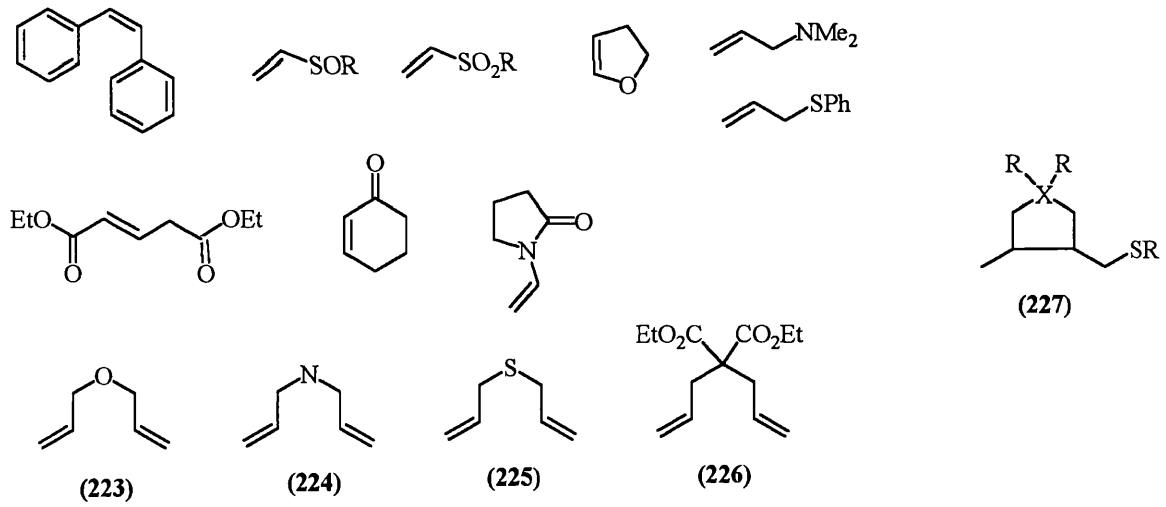


Figure 30. Other substrates examined.

3. 6. A case study: the reaction of trityl thionitrite with *N,N*-diallylacrylamide.

The addition of thiyl radicals onto *N,N*-diallyacrylamide (**228**) is known to give cyclised products which result form a predominant initial attack onto the conjugated double bond (**229**), in agreement with the arguments developed before for thiyl radical additions. When we studied the thermal decomposition of trityl thionitrite in the presence

of this substrate, we did obtain a small yield of a cyclised product derived from addition of the trityl thiyl radical onto the conjugated double bond (**230**, **Figure 31**, 7.5 %), both in the presence and in the absence of isopropanol. However, to our surprise, this product had not trapped NO but instead it had undergone hydrogen atom abstraction. This is an additional indication of how ineffective NO trapping by non-stabilised radicals is under the conditions employed. Again, more work would be required in order to clarify this issue.

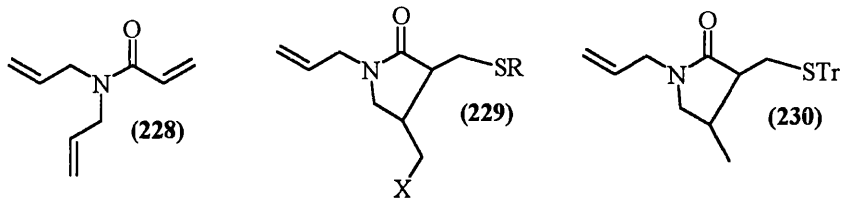


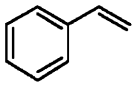
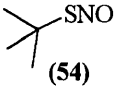
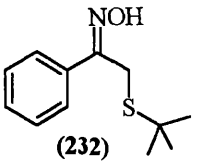
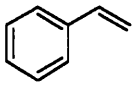
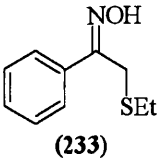
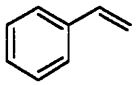
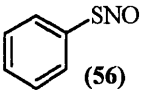
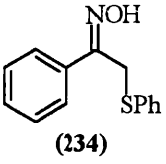
Figure 31. Addition onto allyl acrylamide.

3. 7. Extension of the limits of the reaction to other thionitrites.

In parallel with our investigations into the intra-molecular addition of thionitrites onto alkenes (chapter 2 of this part), the next step in this study involved extending the scope of the reaction so as to be useful for other thionitrites, including primary and secondary ones.

To this end, thionitrites were prepared *in situ* following the procedure described in the previous chapter. Once the thionitrite had formed, the reaction mixture was diluted with isopropanol and the alkene (6 equiv.) was added to a final concentration equivalent to that used above, in the reactions involving trityl thionitrite. Heating was then effected at 65 °C, until total bleaching of the characteristic colours (red for primary thionitrites, green for tertiary ones and red-brown for aromatic ones).

The first examples studied gave very encouraging results (**Table 21**). Thus, addition of pre-formed *tert*-butyl thionitrite (**54**), ethyl thionitrite (**231**) and phenyl thionitrite (**56**) onto styrene yielded addition products **232**, **233** and **234**. The low yield in the case of ethylthionitrite was attributed to the high volatility of the product under the conditions employed.

Alkene	Thionitrite	Init.	Addition products	(Yield)
	 (54)	Δ	 (232)	(38 %)[a]
	EtSNO (231)	Δ	 (233)	(15 %)[a]
	 (56)	Δ	 (234)	(36 %)[a]

(a) Only one isomer of the oxime was formed.

Table 21. Addition of *in situ* generated thionitrites onto styrene.

By way of contrast, when the reactions of pre-formed thionitrites **62**, **237**, and **57** with methyl vinyl ketone were attempted under the same conditions (**Table 22**), unexpected products **235**, **236** and **238** were formed. These would arise from addition of the thiyl radical followed by reduction of the resulting carbon radical.

Since isopropanol has been shown to be able to effect the reduction of certain carbon centered radicals, the reaction was attempted in the absence of this co-solvent, but the result did not vary. We then reasoned that the difference in reactivity might be related to the differences in structure, since both the alkene and the thionitrites were different to those employed in Table 21. In order to test this concept, the reactions of phenylthionitrite with styrene and with methyl vinyl ketone were performed at the same time and using the same batch of *tert*-butyl thionitrite under the same conditions. To our surprise, this time both reactions gave the corresponding reduced products. Subsequent attempts to reproduce the results in Table 21 were unsuccesiful and lack of time precluded any further investigations into this issue.

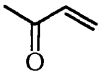
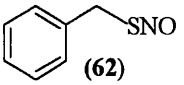
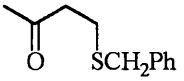
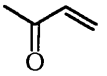
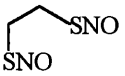
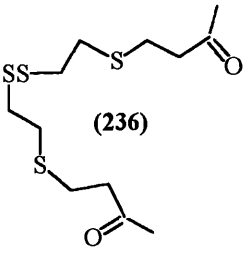
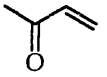
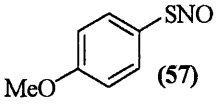
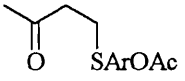
Alkene	Thionitrite	Init.	Addition products	(yield)
	 (62)	Δ	 (235)	(16 %)
	 (237)	Δ	 (236)	(29 %)
	 (57)	Δ	 (238)	(51 %)

Table 22. Addition of *in situ* prepared thionitrites onto methyl vinyl ketone.

CHAPTER 4

SYNTHESIS AND BIOLOGICAL EVALUATION OF ENANTIOPURE THIONITRITES.

4. 1. Introduction.

As discussed in the introductory section, the mechanisms of release of NO from thionitrites in solution are well-established. The process can be catalysed by heat, light, or certain metal salts. By way of contrast, there are strong indications that in biological systems the release of NO from thionitrites is a cell-mediated process and, therefore, requires stereospecific recognition by a receptor site[57,164]. In order to investigate this hypothesis further we have accordingly prepared three pairs of enantiomeric thionitrites. The corresponding *L*-isomers possess well characterised biological activity. Hence, we set out to investigate if, under the same conditions, the *D*-isomers also showed similar activity. We have measured the dilatatory properties of these substrates using simple isolated aorta-ring models.

4. 2. Substrate preparation.

4. 2. 1. Generalities.

The compounds chosen for study were the two naturally occurring thionitrites *S*-nitrosoglutathione (**26**) and *S*-nitrosocysteine (**27**) and the exogenous NO-donor *S*-nitroso-*N*-acetylpenicillamine (**32**) (**Figure 32**).

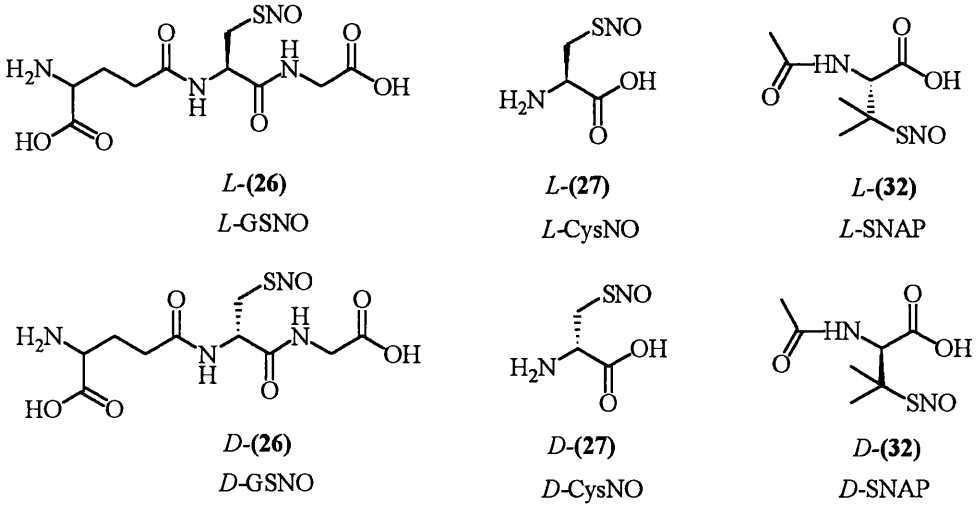
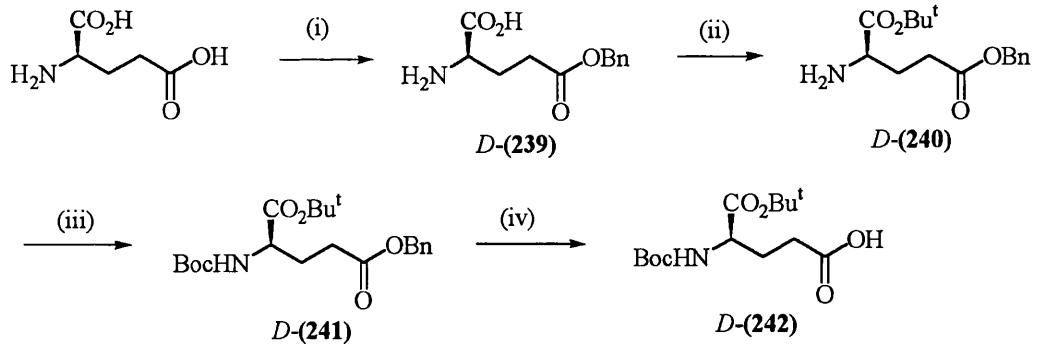


Figure 32. Bio-active thionitrites studied.

Both enantiomers of the corresponding thiols *L*- and *D*-cysteine are commercially available and, therefore, obtaining both enantiomers of **27** required only nitrosation. Both enantiomers of penicillamine are also commercially available. Preparation of both enantiomers of **32** involved introduction of an acetyl group onto the amide nitrogen, followed by nitrosation. These operations were performed following literature procedures, as indicated in the experimental section. By way of contrast, only the natural (*L*) isomer of glutathione is available and, accordingly, the *D*-isomer had to be prepared. For comparative purposes, this was undertaken both by using a solid-phase peptide synthesis approach (SPPS) and in solution. The details will be discussed below. Once the two enantiomers of glutathione (GSH) were in our hands, nitrosation following literature procedures gave *L*- and *D*- **26**.

4. 2. 2. Solid-phase synthesis of *D*-glutathione

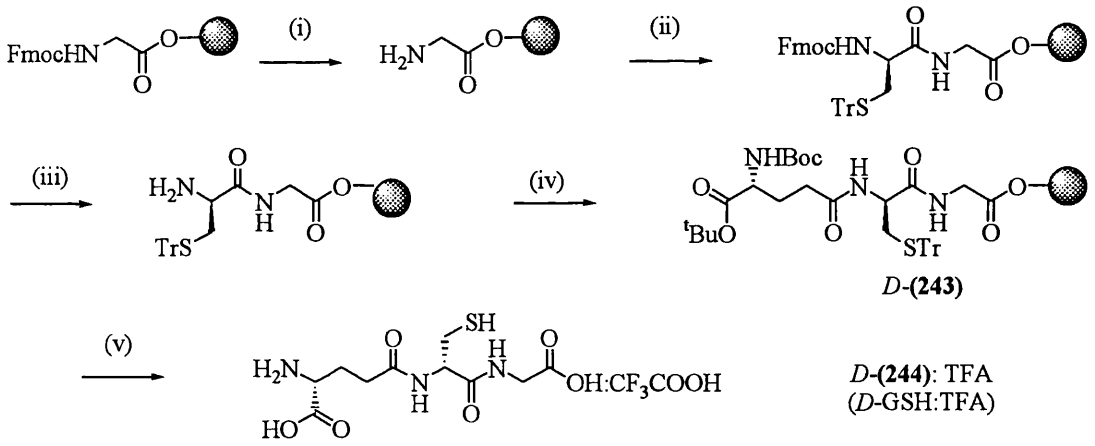
Several syntheses of *L*-GSH using solution phase chemistry have been previously reported and, in several of these, the oxidised tripeptide (GSSG) was obtained after deprotection[165]. To the best of our knowledge, the SPPS approach has not been previously employed for the preparation of either enantiomer of glutathione. We chose to apply the Fmoc strategy for SPPS and the following protecting groups for the side chains: the sulfur of cysteine was protected as the trityl (Tr) thioether, the α-carboxylic acid group of glutamic acid was protected as its *tert*-butyl ester (^{t}Bu) and the *N*-terminal group was protected as the *tert*-butyloxycarbonyl (Boc) derivative. The resin was linked to the first amino-acid through the TFA-labile 4-hydroxymethylphenoxyacetic acid ester. By choosing this type of resin and this protecting group strategy, we expected to be able to carry out the cleavage of the peptide from the resin and the deprotection of all functional groups in one single operation, by using acidic conditions. The suitably protected glutamic acid derivative (*D*-**242**) was not commercially available and was assembled in three steps following literature methods **(Scheme 78)**.



(i) $PhCH_2OH$, Na_2SO_4, HBF_4>Et_2O, r. t., 24 h, 94 % (ii) $(CH_3)_2CH{=}CH_2$, H_2SO_4, dioxane, r. t., 5 h, not isolated (iii) di-*tert*-butyl dicarbonate, Et_3N, H_2O/dioxane, r. t., 1 night., 50 % two steps. (iv) H_2, Pd/C 5 %, EtOH, 16 h, 50 %

Scheme 78. Preparation of the protected glutamic acid derivative.

Our approach to the solid-phase synthesis of *D*-GSH is outlined in **Scheme 79.**



(i) piperidine/DMF 20 % v/v, r. t., 30 min (ii) Fmoc-*D*-Cys(STr)-OH, pyBOP, NMM, DMF, r. t., 3 h (iii) piperidine/DMF 20 % v/v, r. t., 30 min (iv) Boc-*D*-Glu-O^{t}Bu, pyBOP, NMM, DMF, r. t., 3 h (v) TFA/CH_2Cl_2 95 % v/v, EDT, 3 h, prep-HPLC. Overall yield 20 %.

Scheme 79. Solid-phase synthesis of *D*-glutathione.

The first amino acid of the sequence (Gly) was initially protected (Fmoc) and linked to the polystyrene/polydimethylacrylamide resin. Fmoc deprotections were carried out with 20 % piperidine in *N,N*-dimethylformamide (DMF). The efficiency of the deprotections was qualitatively checked by the Ninhydrin (Kaiser[166]) test. The peptide chain was elongated by successive coupling of Fmoc-*D*-Cys(STr)-OH and Boc-*D*-Glu-O^{t}Bu. Coupling reactions were performed in DMF using pyBOP$^{\rightarrow}$ as the coupling reagent in the presence of *N*-methylmorpholine (NMM). A ratio 2:2:2:1 of added amino

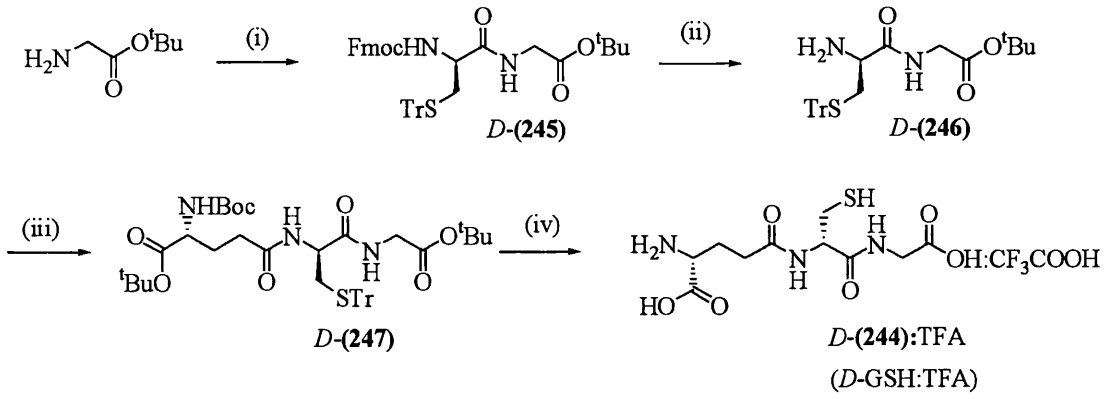
acid: pyBOP: NMM: linked peptide was employed in order to ensure quantitative couplings. Completion of the coupling was qualitatively checked by the Ninhydrin test.

The cleavage of the peptidyl-resin (*D*-**243**) and simultaneous deprotection of all functional groups was achieved by exposure to TFA in DCM (95 %) with ethanedithiol (EDT) as scavenger. The crude product was purified by repeated reverse-phase-HPLC. The pure tripeptide was isolated as its TFA salt, in an overall 20 % yield, and was identical by analytical HPLC to a commercial sample of *L*-GSH. The TFA salt of *D*-GSH thus obtained and that derived from *L*-GSH, by reaction with one equivalent of TFA, showed equal but opposite specific optical rotations. Both salts were nitrosated to give *L* and *D*-**26** as described in the experimental section.

It is worth mentioning that the nitrosation of the glutathione isomers appeared to be extremely sensitive to the presence of trace impurities in the sample of GSH used and that **26** decomposed *in situ* after a few minutes, unless a very pure sample of the thiol was used. This involved purification of the sample of the *D*-thiol by prep-HPLC several times (2 or 3, depending on the batch). We reasoned that the thiol might be obtained purer by a solution-phase approach, given that it should be possible to separate most impurities by chromatography on SiO_2 prior to the cleavage step. Contrastingly, impurities might accumulate through the different steps during the SPPS approach, leading to the necessity of extensive and time-consuming purification by HPLC after cleavage.

4. 2. 3. Solution-synthesis of *D*-glutathione.

The same protecting group strategy as that described for the SPPS approach was chosen. The general approach is outlined in **Scheme 80**. The terminal carboxylic acid was protected as its *tert*-butyl ester. All operations were carried as before but in DCM, instead of DMF. All intermediates were isolated and purified by chromatography on SiO_2. After final deprotection, the crude tripeptide was purified by prep-HPLC (once). Overall, this approach proved to be more convenient than the SPPS approach, discussed above, since it required less HPLC work. It also allowed us to obtain larger quantities of the pure tripeptide at a much lower cost, given the generally high cost of the resins employed for SPPS.



(i) Fmoc-*D*-Cys(STr)-OH, pyBOP, NMM, DCM, r. t., 3 h, 93 % (ii) piperidine/DCM 20 % v/v, r. t., 30 min, 73 % (iii) Boc-*D*-Glu-O^{t}Bu, pyBOP, NMM, DCM, r. t., 3 h, 90 % (iv) TFA/CH_2Cl_2 95 % v/v, EDT or Et_3SiH, 3 h, prep-HPLC, 35 %.

Scheme 80. Solution phase-synthesis of *D*-glutathione.

4. 3. Biological tests.

4. 3. 1. Generalities.

Enantiopure thionitrites employed in biological studies were freshly prepared *in-situ* for every experiment, by dissolving the corresponding enantiomer of the thiol in water, in the presence of 1 equivalent of $NaNO_2$ and acidifying the solution to pH 2 with HCl. The solutions turned immediately green or red and were used on the day in order to avoid thermal or photochemical decomposition. During experimentation they were kept in the dark at 0 °C.

Dissected rat thoracic aortas were cleaned of connective tissue and cut into rings (3-4 mm wide). Aortic rings were suspended in 25 mL organ baths containing Krebs-bicarbonate buffer, maintained at 37 °C and gassed with 95 % O_2/ 5 % CO_2 (**Figure 33**).

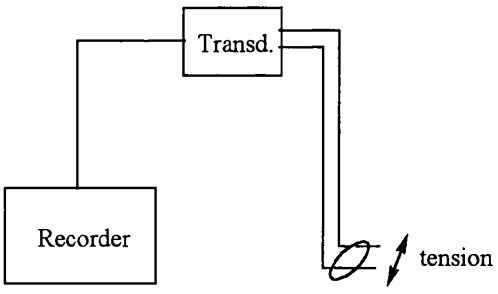


Figure 33.

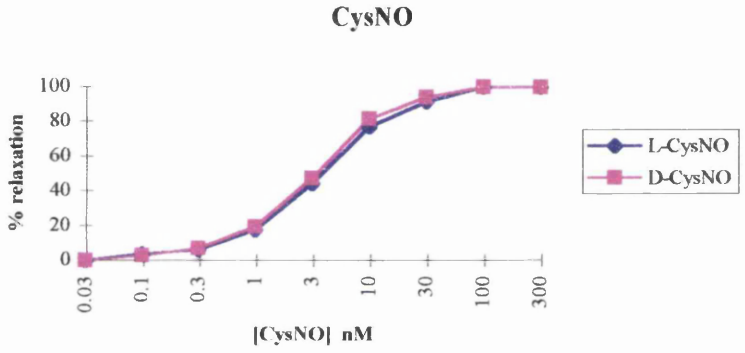
A resting tension of 1.0 g was applied to each tissue and changes in isometric tension were measured using a force displacement transducer, connected to a chart recorder. The tissues were allowed to equilibrate for 60 min prior to experimentation and, then, a concentration of phenylephedrine (PE), producing a sub-maximal (70-85 % of the maximum) contraction, was added. Once the response had

stabilised, the vasodilator acetylcholine was added to asses the integrity of the endothelium.

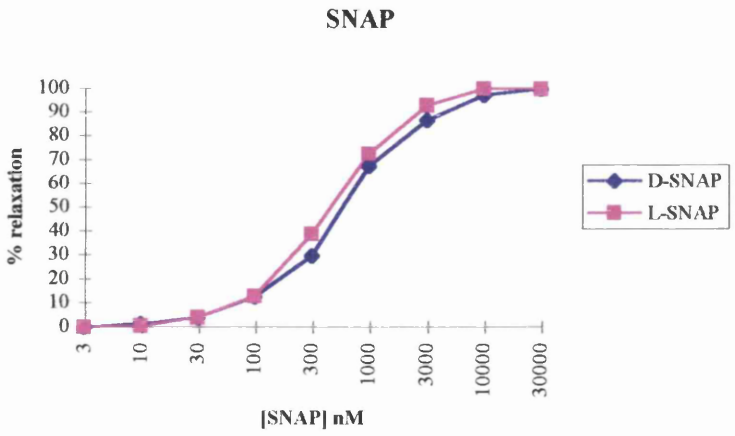
After discarding the damaged tissues, and once a stable response to PE was obtained, concentration-response curves to *D*- and *L*-CysNO (0.03-300 nM), SNAP (0.01-30 μM) and GSNO (0.01-10 μM) were obtained.

4. 3. 2. Bio-assay results.

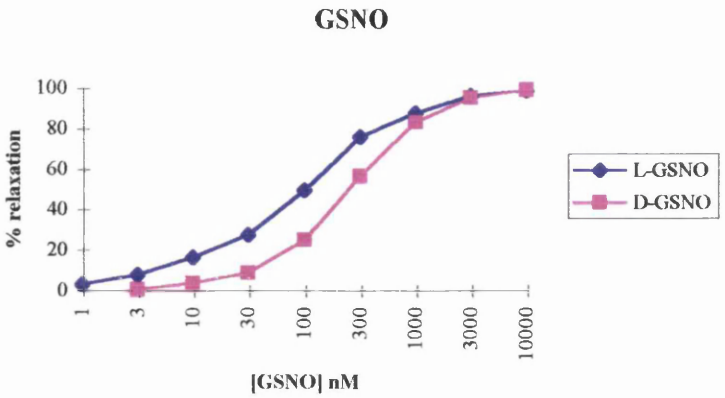
The results obtained for each of the three couples of enantiomers are graphically represented below (Graphs 1, 2 and 3). Relaxations are expressed as a percentage reversal of the PE-induced tone. Doses are expressed in concentrations in nM units.



Graph 1. Relaxation of rat aorta rings by CysNO enantiomers



Graph 2. Relaxation of rat aorta rings by SNAP enantiomers.



Graph 3. Relaxation of rat aorta rings by GSNO enantiomers.

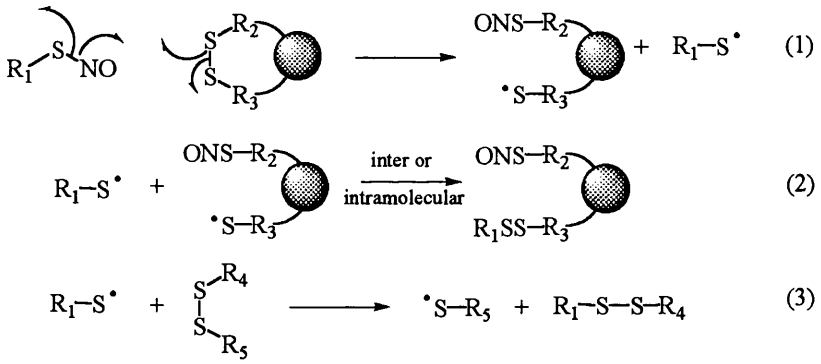
The results obtained were rather interesting. Both enantiomers of CysNO and SNAP displayed identical activity. This was not unexpected. CysNO is very labile and releases NO in solution very quickly, even at r.t. In agreement, it can be seen from the plots above that the concentration needed to achieve the same activity is around 100 times smaller for CysNO than it is for SNAP. Thus, it is reasonable to assume that decomposition of this thionitrite *in vivo* might be virtually spontaneous. On the other hand, SNAP is not an endogenous thionitrite, so the observation that both enantiomers are recognised equally by cells is not unreasonable. In contrast, GSNO is one of the most important endogenous thionitrites, believed to be the principal form of NO transport in the human body. Accordingly, cells seem to recognise the natural (*L*) isomer better than the (*D*) isomer and activity is between 2 or 3 times higher for the natural enantiomer. This result is an indication that the release of NO from this particular thionitrite in biological systems might well be cell-mediated, with the involvement of either an enzyme or a receptor.

CHAPTER 5

A STUDY OF THE DISULFIDE-THIONITRITE EXCHANGE REACTION.

5. 1. Introduction.

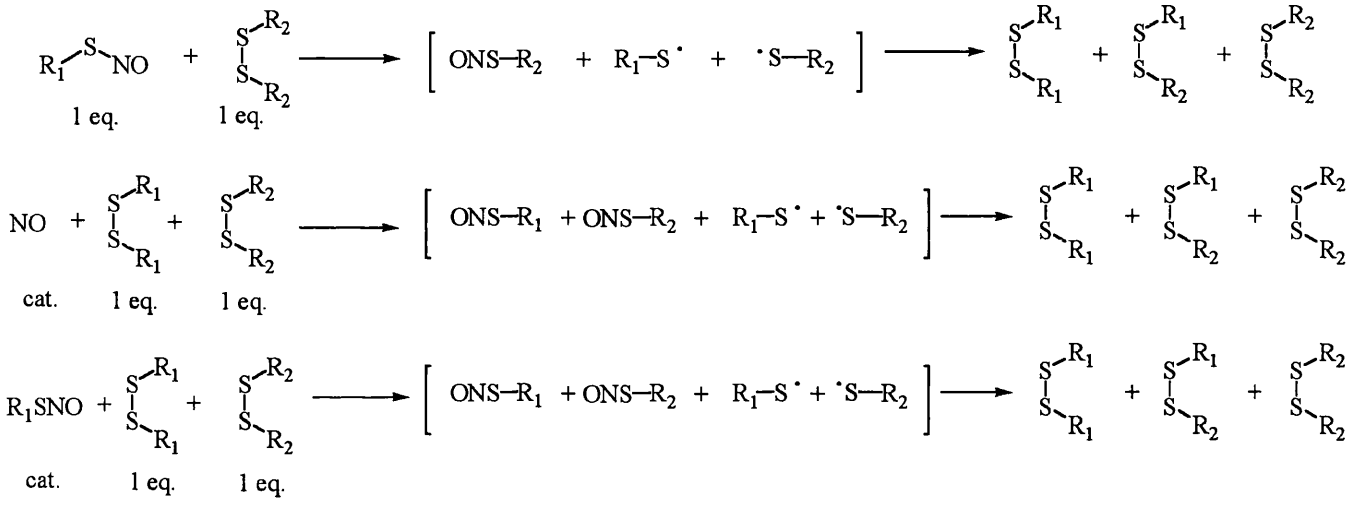
As we have discussed in the introductory section, in biological systems thionitrites form part of a complex interrelated system which includes NO, thiols, disulfides and other sulfur derivatives. Many of the reactions between the varied elements of this system have been studied in relative detail in recent years. In this context, Girard and Potier[167], in 1993, published a hypothesis article entitled "NO, thiols and disulfides". They proposed that NO *in vivo* could be involved in either a series of complex radical chain or oxido-reductive processes, which altogether could constitute the elementary steps of more complex signal transduction or regulatory cascades. Some of the radical reactions discussed in their paper are illustrated below (**Scheme 81**).



Scheme 81. Examples of reactions of thionitrites involving reversible thiol-disulfide exchanges.

According to this hypothesis, NO can participate in the formation of disulfide linkages from two thiol residues, via their corresponding thionitrites, either by inter- or intra-molecular reactions. These processes are known to take place and have been reviewed in the previous sections. Similarly, it was suggested that NO or thionitrites could be responsible for the cleavage of disulfide linkages in biological systems. For instance, a thionitrite could react by a radical mechanism with a disulfide with formation of a new thionitrite and a new disulfide in the same molecule (equations (1) and (2) in Scheme 81) or a thionitrite and a disulfide in a different molecule (equation (3)).

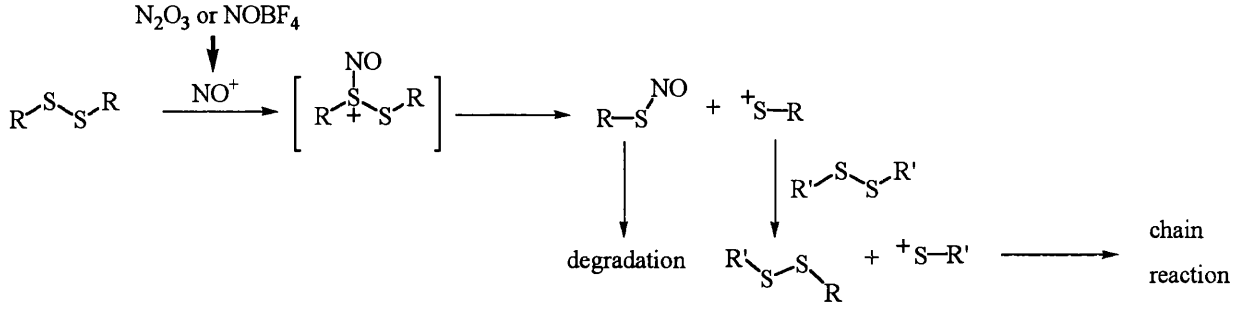
Analogously, NO could cleave a disulfide with formation of a thionitrite and a thiyl radical which could be engaged in further reactivity. It therefore seemed of interest to examine the possibility of a thionitrite-disulfide exchange process or NO- or thionitrite-catalysed disulfide-disulfide exchange processes operating in solution under conditions which mimic physiological ones. We reasoned that in simple model systems three types of processes could be envisaged (**Scheme 82**).



Scheme 82. Possible disulfide linkage cleavage processes involving NO or thionitrites.

Whilst the interactions between biological thiols and NO or NO-carriers such as thionitrites have received much attention, the reactions between these two species and substrates containing disulfide linkages have virtually not been analysed. Only very recently, during the writing of the present thesis, a related study was published[168] which reported that scrambling of disulfides, i. e. disulfide-disulfide exchange, could be catatysed by NO, but only in the presence of air. This suggested the participation of NO^+ or a higher oxidation state oxide of nitrogen, generated from the reaction of NO with O_2. In fact, this is not a new reaction since the formation of unsymmetrical disulfides from symmetrical ones in the presence of stoichiometric N_2O_4 had been reported, and is mentioned in the introductory section of this thesis[67]. The interesting observation was that only catalytic (0.005 equiv.) amounts of NO and O_2 were needed and, therefore, the reaction might well be of relevance in biological systems. However, all the reactions studied involved non-biological disulfides and were carried out in acetonitrile, conditions which are very far from those in biological systems. The reaction could also be brought

about by a catalytic amount of $NOBF_4$. In agreement with this observation, an ionic mechanism involving nitrosation of one of the disulfide sulfur atoms followed by cleavage with simultaneous formation of a thionitrite and a sulfenium cation was proposed (**Scheme 83**).



Scheme 83. Mechanism proposed for the scrambling of disulfides catalysed by NO/O_2.[168]

5. 2. Generalities of our work and choice of substrates.

For our own work, we had chosen to study the reactions of disulfides with thionitrites (1st and 3rd reactions in Scheme 82) under conditions which mimicked biological ones. To this end, we chose as substrates a series of water-soluble, biologically-active, disulfides and thionitrites or some simple derivatives of these, obtained in order to aid solubility or reaction monitoring (**Figure 34**). Most reactions were carried out in aerated phosphate buffer water solutions, at physiological temperature (37 °C) and pH (7.4). These conditions were only modified when studies towards the understanding of the possible reaction mechanism were undertaken. The rate of disappearance of the thionitrites was qualitatively monitored by analytical reverse-phase HPLC, with UV detection set at λ= 215 nm (amide bond) and the composition, in terms of product structure, of the final disulfide mixtures was determined by HPLC-MS. The yields of the final sulfur-containing products were determined from the ^1H NMR of the mixtures after freeze-drying the solvent. Reactions carried out in the absence of added metal salts were undertaken in the presence of a catalytic amount (0.2 equiv.) of the metal chelator EDTA, in order to ensure complexation of adventitious concentrations of metal ions. The effect of adding known concentrations of copper (II), from a $CuCl_2$>H_2O stock solution of pH 7.0, was also analysed and these reactions were performed in the absence of added EDTA.

Concentrations of thionitrite and disulfide substrates were kept constant at a value of 10^{-2} M when these acted as stoichiometric reagents.

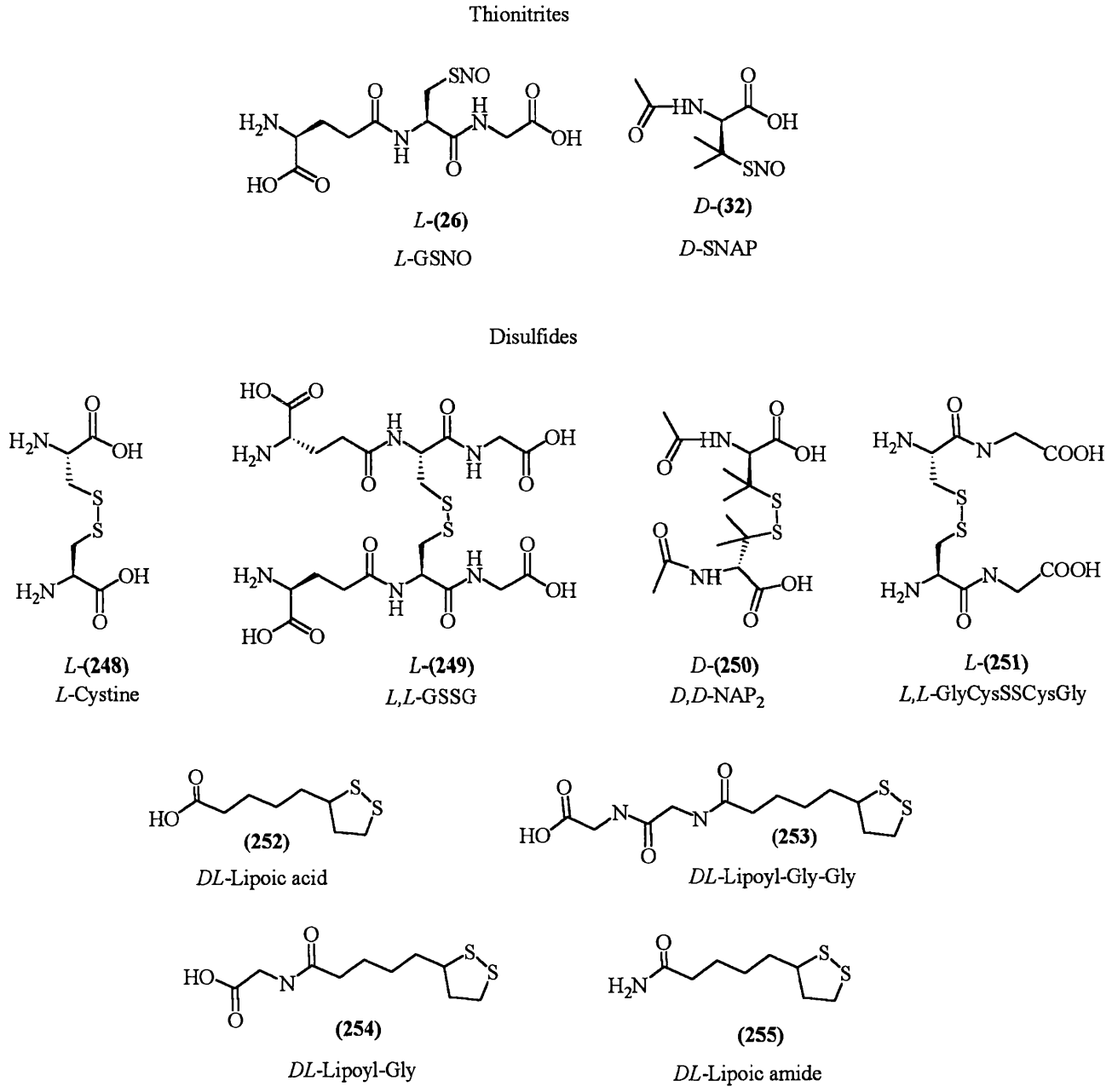
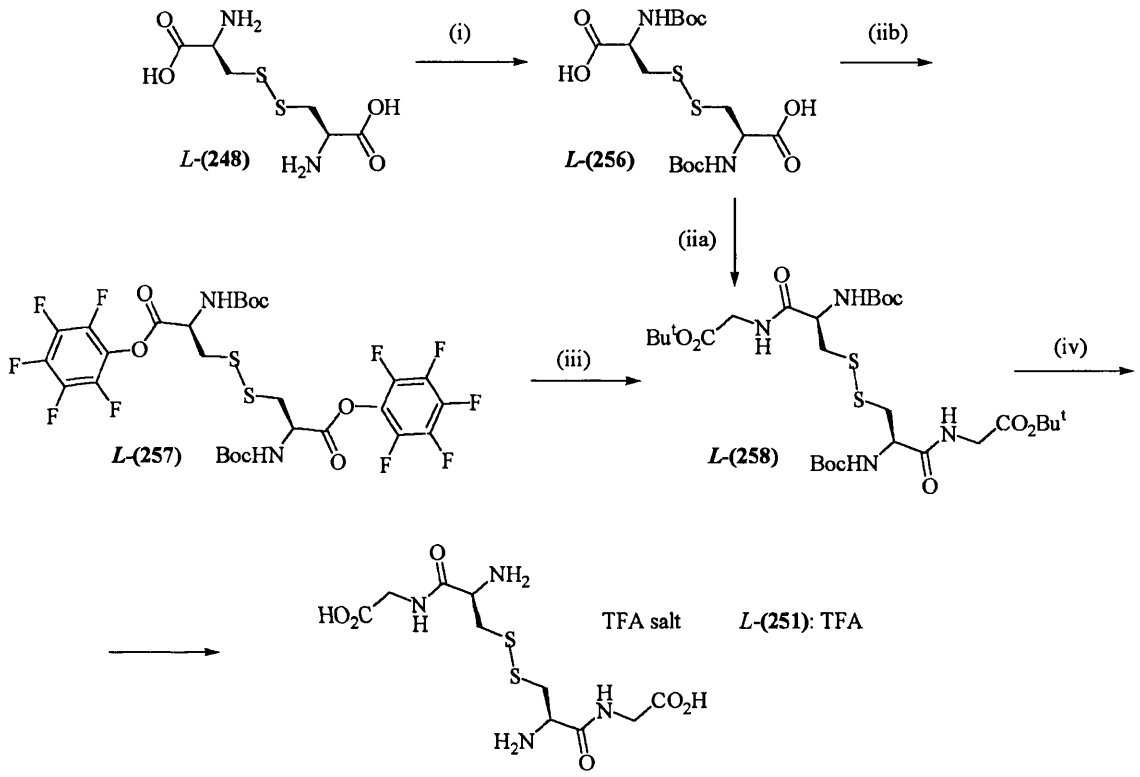


Figure 34. Substrates chosen for our disulfide-thionitrite exchange studies.

5. 3. Substrate preparation.

Thionitrites *L*-**26** and *D*-**32** were prepared by nitrosation of the corresponding thiols with $NaNO_2$, following literature procedures, as described in Chapter 4 of the experimental section. Disulfides *L*-**248** and *L*-**249** were purchased. The disulfide from*N*-acetyl-*D*-penicillamine (*D*-**250**) was obtained by oxidation of the corresponding thiol

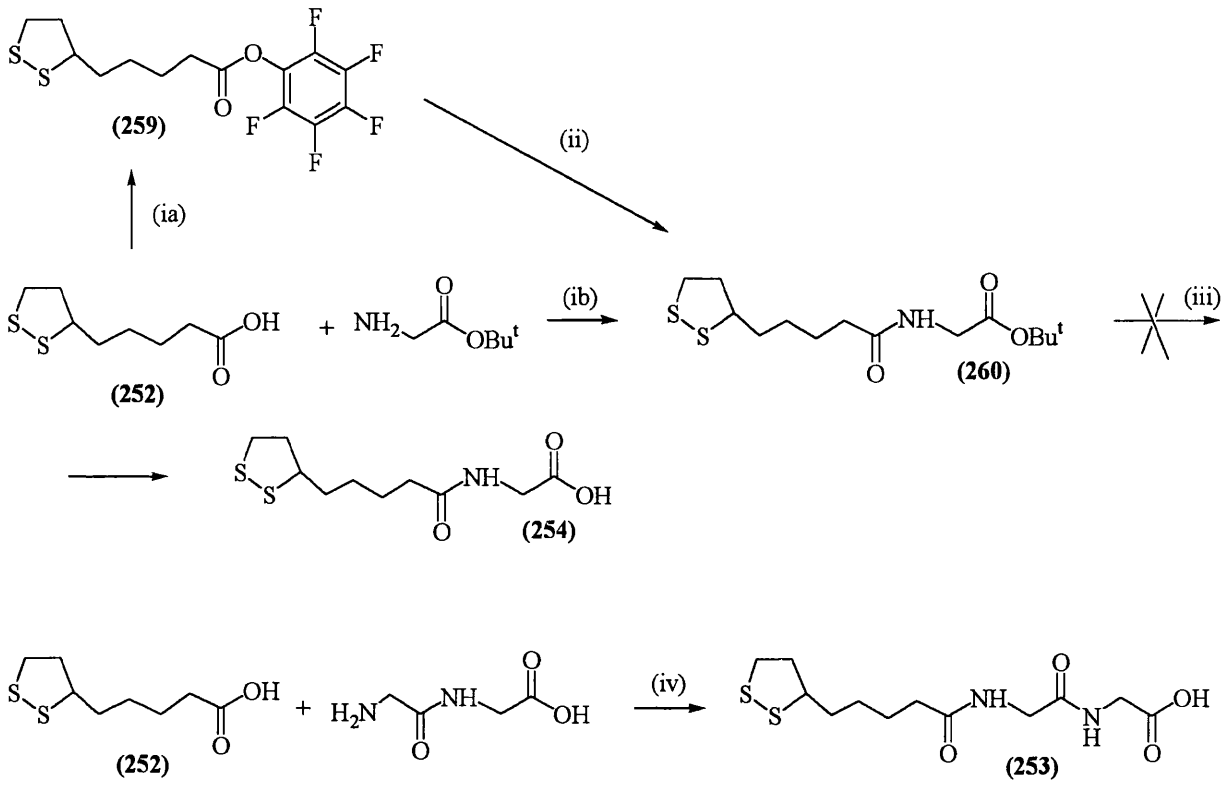
with I_2. Protected disulfide *L*-**258** was obtained in two or three steps from cystine and *tert*-butyl glycinate (**Scheme 84**), *via* pyBOP$^{\rightarrow}$ or pentafluorophenyl activation, respectively. However, the final product (*L*-**250**), obtained after cleavage of the *tert*-butyl ester protecting groups, did not have the desired degree of purity and, accordingly was not used for our subsequent exchange studies.



(i) NaOH 1M, di-*tert*-butyl dicarbonate, r. t., 1 night, 34 %. (iia) *tert*-butyl glycinate, pyBOP, NMM, DCM, r. t., 3 h, 54 %. (iib) pentafluorophenol, DCC, EtOAc/THF, r. t., 2 h, 80 %. (iii) *tert*-butyl glycinate, r. t., 2 h, 68 %. (iv) TFA/DCM 95 % v/v, EDT or $HSiEt_3$ as scavengers, r. t., 3 h, yield< 45 %.

Scheme 84. Preparation of cystyl derivative *L*-**251**.

Lipoic acid (**252**) and lipoic amide (**255**) are commercially available. Derivative **254** of lipoic acid could be obtained protected (**260**) without much difficulty (**Scheme 85**) *via* the pyBOP or pentafluorophenyl activation approaches. Nevertheless, acid deprotection led in all cases to formation of polymeric material. In contrast, derivative **253** was easily obtained as the unprotected material from lipoic acid and glycylglycine, following the literature procedure. The analogous procedure from unprotected glycine and lipoic acid to yield **253** was reported to fail and also led to the formation of polymeric material.



(ia) pentafluorophenol, DCC, EtOAc/THF, r.t., 2 h, 75 %. (ib) pyBOP, NMM, DCM, r.t., 3 h, 17 %. (ii) *tert*-butyl glycinate, DCM, r.t., 2 h 68 %. (iii) TFA/DCM 95 % v/v, EDT or Et_3SiH as scavengers, polymeric material. (iv) isobutyl chloroformate, THF, Et_3N, 0 °C, 10 min, followed by NaOH 1M, r.t., 15 min, 45 %.

Scheme 85. Preparation of lipoic acid derivatives **253** and **254.**

5. 4. Initial studies: exchange processes between SNAP and GSSG.

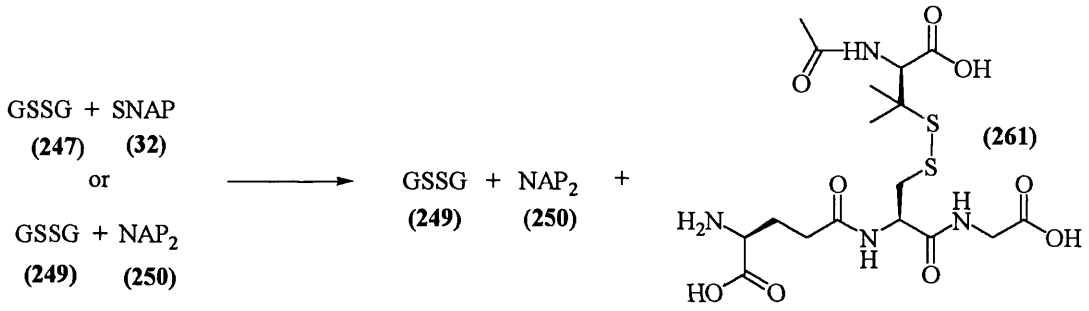
5. 4. 1. Preliminary results.

The configurations of the products are as indicated in Figure 34 above and, for simplicity, they will not be indicated in the discussion below.

Initially, we analysed the reaction between SNAP and GSSG under a variety of conditions. In order to ensure that under the conditions of our experiments GSSG and NAP_2 , formed from the decomposition of SNAP, could not exchange in the absence of the thionitrite, we carried out a series of blank experiments. To this end, equal amounts of GSSG and NAP_2 were mixed in a phosphate buffer at pH 7.4 in the presence of either catalytic amounts of EDTA or $CuCl_2$ and the mixtures stirred at 37 °C. After two days, no "scrambling" of the initial disulfides was observed **(Table 23)**.

In the next preliminary experiments it was found that, under physiological conditions (pH 7.4 and 37 °C), SNAP had decomposed after three days in the presence

of one equivalent of GSSG and catalytic EDTA to give a mixture of GSSG, NAP_2 and the mixed disulfide **261** in a proportion *ca.* 2:1:1.3. Conversely, when 0.2 equiv. or 1 equiv. of Cu^{2+} were added in the form of $CuCl_2$, no exchange had taken place after the same period of time, and the final mixture only contained GSSG and NAP_2. In view of these results we then decided to monitor the disappearance of the starting thionitrite in order to be able to compare the reaction times for the two processes. We determined that, under physiological conditions and in the presence of EDTA, SNAP decomposition in the presence of stoichiometric GSSG, required *ca.* 77 h. Furthermore, both GSSG and the mixed disulfide appeared simultaneously as the thionitrite was consumed. Contrastingly, decomposition in the reactions containing Cu^{2+} was complete in less than 7 h and did not give exchange products (**Table 23**).



GSSG	NAP_2	SNAP	additive[a]	atm.	time	GSSG	NAP_2	261
1 equiv.	1 equiv.	-	EDTA (0.2 equiv.)	air	48 h	50 %[b]	50 %	0 %
1 equiv.	1 equiv.		Cu^{2+}(0.2 equiv.)	air	48 h	50 %	50 %	0 %
1 equiv.	1 equiv.		Cu^{2+}(1 equiv.)	air	48 h	50 %	50 %	0 %
1 equiv.	-	1 equiv.	EDTA (0.2 equiv.)	air	77 h	53 %	20.5 %	26.5
1 equiv.	-	1 equiv.	Cu^{2+}(0.2 equiv.)	air	< 7 h	66.7 %	33.3 %	0 %
1 equiv.	-	1 equiv.	Cu^{2+}(1 equiv.)	air	< 7 h	66.7 %	33.3 %	0 %
1 equiv.	-	1 equiv.	EDTA (0.2 equiv.)	N_2	< 6 days	53 %	20.5 %	26.5

(a) All reactions were carried out at pH 7.4 and 37 °C in aqueous phosphate buffer solutions. (b) Molar proportions.

Table 23. SNAP-GSSG exchange reactions: results of preliminary experiments.

For comparison purposes and in order to rule out a reaction with the participation of O_2, we carried out the reaction between SNAP and GSSG, under physiological conditions, in the presence of catalytic EDTA and under an inert atmosphere of N_2. This gave the same composition of the final mixture to that obtained when air was present. In addition, after 13 h, the relative areas of the peaks corresponding to GSSG, **261**, SNAP and NAP_2 on the respective HPLC traces were of the same order, which is indicative that the reaction rates are similar for both processes.

5. 4. 2. Reactions at different copper concentrations.

In order to determine if exchange would occur at lower copper concentrations, of the order of those which can be found in biological systems, we carried out the reaction between SNAP and GSSG under physiological conditions and in the presence of a range of increasingly lower Cu^{2+} concentrations, keeping the concentrations of the thionitrite and the disulfide constant (10^{-2} M) (**Table 24**). Even at copper concentrations as low as 10^{-8} M, the thionitrite had been completely consumed after a period between 3 and 7 h and no exchange was observed in any of the experiments.

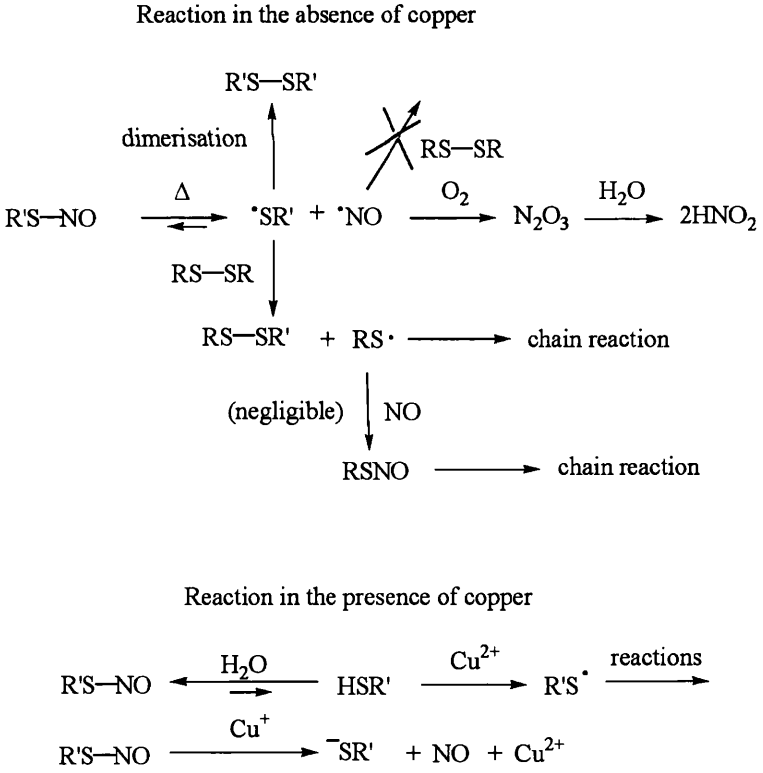
GSSG	SNAP	$[Cu^{2+}]$ [a] (M)	GSSG	NAP_2	260
1 equiv.	1 equiv.	0.1	66.7 %.	33.3 %	0 %.
1 equiv.	1 equiv.	0.01	66.7 %.	33.3 %	0 %.
1 equiv.	1 equiv.	10^{-3}	66.7 %.	33.3 %	0 %.
1 equiv.	1 equiv.	10^{-4}	66.7 %.	33.3 %	0 %.
1 equiv.	1 equiv.	10^{-5}	66.7 %.	33.3 %	0 %
1 equiv.	1 equiv.	10^{-6}	66.7 %.	33.3 %	0 %
1 equiv.	1 equiv.	10^{-7}	66.7 %.	33.3 %	0 %.
1 equiv.	1 equiv.	10^{-8}	66.7 %.	33.3 %	0 %.

(a) Added as $CuCl_2$.

Table 24. SNAP-GSSG exchange reactions at different copper concentrations.

5. 4. 3. The possible mechanism of the reaction.

We initially envisaged that the mechanism of the exchange reaction could involve the steps illustrated in **Scheme 86**. On the basis of the results reported[168], we ruled out the possibility of a reaction between NO and the disulfide, leading to the cleavage of the S-S bond. In addition, in the presence of O_2, NO is likely to be quickly transformed into N_2O_3 and, in contrast to the literature study[168], this species would, in our system, disappear from the medium by reaction with the solvent (water) at a faster rate than by reaction with the disulfide. For the same reason, the extent of the reactions of NO with thiyl radicals to give back the initial thionitrite or the thionitrite derived from the other thiyl radicals generated during the reaction, are likely to be negligible. On the other hand, the reaction between thiyl radicals and disulfides is known to occur in non-polar solvents[169] and it seemed reasonable to suppose that the process might also take place in water.



Scheme 86. Initially proposed mechanisms for the formation of mixed disulfides.

However, there are several effects which were difficult to explain on the basis of this mechanism. The decomposition of thionitrites in water, catalysed by copper, has also been proposed to occur *via* the formation of intermediate thiyl radicals, as was discussed

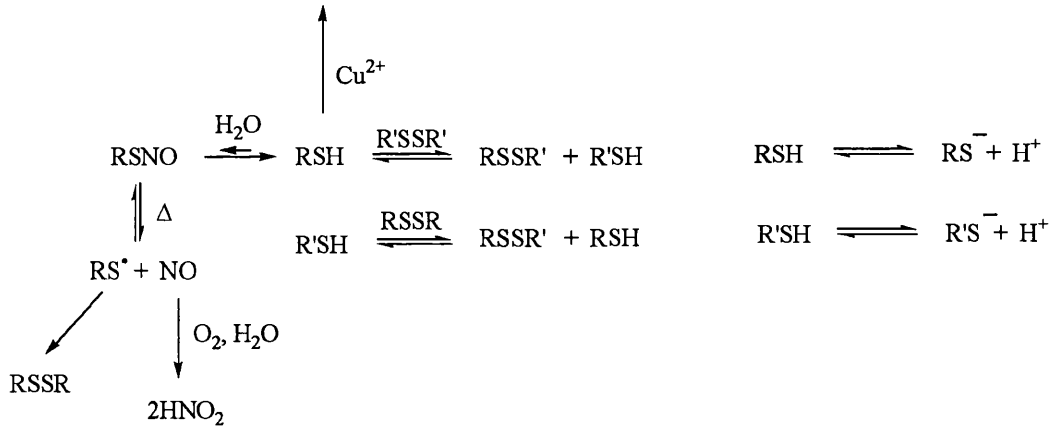
in the introductory section (see also **Scheme 86**, above). Thus, if the decomposition of thionitrites catalysed by metal salts is faster than under thermal conditions, one can assume that the rate at which thiyl radicals are generated in the first case will also be faster, since subsequent reactions of these with the starting thionitrite or other species can be expected to have the same rate constants in both systems. Therefore, we would expect that the scrambling of disulfides would also take place in the presence of copper and that it would even be more favourable. The effect which we observe is exactly the opposite. In addition one would expect that thiyl radicals would react in a chain process and, therefore, the overall process would be relatively fast. Furthermore, the reaction between thiyl radicals and disulfide has been found to take place only in the presence of O_2[169], whereas our exchanges also occur under an inert atmosphere of N_2.

It appears that the species responsible for the scrambling disappears too fast in the copper catalysed reaction. This species could, in principle, be the thionitrite itself or the derived thiol. In subsequent experiments we determined that the exchange reaction in the absence of copper is highly dependent on the pH of the medium and that for pHs equal or above 7, exchange is always observed, whereas for pHs below that value no exchange occurs (**Table 25**). In addition, as was expected, we found that the decomposition of SNAP under acidic pH is slightly slower than the reaction[92] at basic pH The strong pH dependence of the exchange reaction is in clear agreement with a reaction which is affected by the position of the thiol acid-base equilibrium. The thiol-disulfide exchange is a well known reaction which is catalysed by traces of base, and which takes place by attack of the thiolate anion onto one of the disulfide sulfurs. Consequently, the rate of this reaction is highly dependent on the pH. For instance, it was determined that the reaction of penicillamine with cystine was around 500 times faster at pH 8.05 and 100 times at pH 6.93 than it was at pH 5.07 [170]. In addition, determined rate constants for the reaction of penicillamine with several disulfides are of the order of 10^{-1}-10^{2}, and this value would be in agreement with a quite slow equilibration reaction (several days), specially considering that the thiol in our system would be generated by hydrolysis of the thionitrites in very small concentrations[86]. In the copper reactions, the thiol generated would rapidly be oxidised by copper(II) and would not have time to react with the disulfide.

GSSG	SNAP	pH	time	SNAP	GSSG	NAP_2	261
1 equiv.	1 equiv.	1.68	88 h	~5 %	66.6 %	~28 %	0 %.
			120 h	0 %	66.6 %.	33.3 %	0 %.
1 equiv.	1 equiv.	3.77	88 h	~6 %	66.6 %	~29 %	0 %.
			120 h	0 %	66.6 %	33.3 %	0 %.
1 equiv.	1 equiv.	5.80	88 h	~6 %	66.6 %	~29 %	0 %.
			120 h	0 %	66.6 %	33.3 %	0 %
1.2 equiv.	1 equiv.	7.00	96 h	0 %	56 %	16 %	27 %
1.2 equiv.	1 equiv.	8.00	96 h	0 %	62 %	19 %	19 %.
0.8 equiv.	1 equiv.	11.00	88 h	0 %	47 %	29 %	23 %

Table 25. SNAP-GSSG exchange reactions at different copper concentrations.

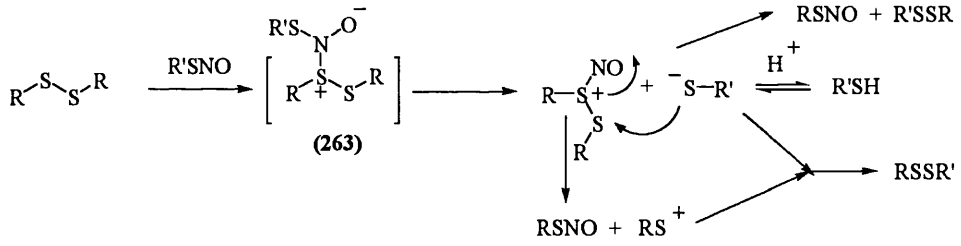
With respect to the proportions obtained of the three disulfides, it is difficult to predict how these would change with the pH, since the series of equilibria depicted below would be involved and we do not have enough information which would allow to predict the position of each of them (**Scheme 87**).



Scheme 87. Mechanism proposed for the exchange between thionitrites and disulfides in water.

Reactions at acidic pH were also carried out in the presence of added thiol (0.2 equiv. of NAP) in order to ensure that the lack of exchange was due to the protonation of the thiol at this pH and not to a less favoured hydrolysis of the thionitrite at acidic pH. As expected, no appreciable exchange was produced. In addition, reaction of equimolecular amounts of GSSG and NAP_2, under physiological conditions in the presence of EDTA and a catalytic amount of SNAP resulted in no appreciable exchange. After 5 days, less than a 10 % of exchange could be detected, probably attributable to catalysis by the small quantity of thiol generated in the medium from the hydrolysis of SNAP. Thus, it appears that reaction 3 in Scheme 82 does not occur either under our conditions.

One could argue that an alternative ionic mechanism could also be possible (**Scheme 88**). This would involve nucleophilic attack of the disulfide onto the NO group of the thionitrite in an analogous manner to that proposed for the reaction of disulfides with NO^+. Intermediate **263** could then collapse giving a thiolate and a cation such as the one formed in that reaction, which would cleave giving another thionitrite and a sulfonium cation which would rapidly combine with the thiolate. If this reaction is sufficiently slow, it could also be that in the presence of copper, the thionitrite disappears too quickly. However, the pH dependence is more difficult to explain by this mechanism.

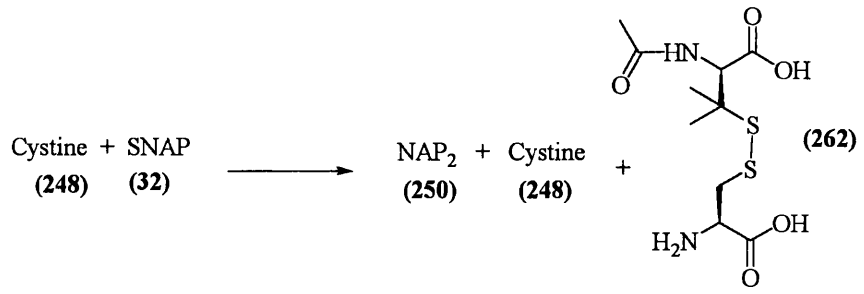


Scheme 88. Alternative mechanism for the thionitrite-disulfide exchange.

5. 5. Exchange studies with other substrates.

During the reactions between SNAP (**32**) and cystine (**248**), mixed disulfide **262** was formed both in the presence and in the absence of copper (**Table 26**). In the presence of catalytic copper only a 10.5 molar proportion of the mixed disulfide was detected, whereas in the absence of added copper this increased by four fold. Thus, as in the reaction between SNAP and GSSG, the degree of exchange increases when copper

is not present. The fact that now a certain amount of exchange can still take place even when copper is present might be due to a higher rate of the reaction between the thiol from SNAP and the disulfide cystine, in comparison to the analogous reaction with GSSG.

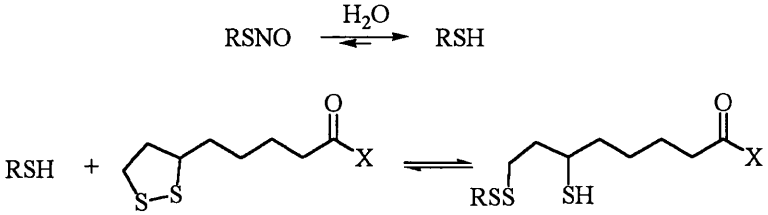


Cystine	SNAP	additive[a]	time	Cystine	NAP_2	262
1 equiv.	1 equiv.	EDTA (0.2 equiv.)	88 h	46 %[b]	13 %	41 %
1 equiv.	1 equiv.	Cu^{2+}(10^{-8} M)	68 h	61.5 %	28 %	10.5 %

(a) Reactions were carried out at pH 7.4 and 37 °C in aqueous phosphate buffer solutions. (b) Molar proportions.

Table 26. SNAP-Cystine exchange reactions.

Reactions between SNAP and cyclic disulfides **253** and **255,** under physiological pH and temperature, did not give exchange products, neither in the presence nor in the absence of copper. Only the starting cyclic disulfide and the disulfide derived from SNAP decomposition were detected after 5 days. This was not totally unexpected if the reaction occured *via* a thiol-disulfide exchange mechanism. Cleavage of the ring disulfide can easily be followed by intra-molecular attack of the resulting thiol, thereby reverting to the initial product (**Scheme 89**)

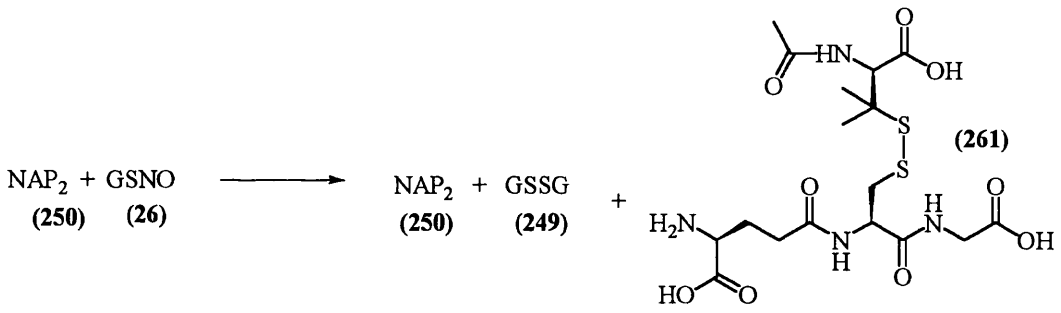


Scheme 89. Exchange between thionitrites and cyclic disulfides.

Reactions involving GSNO (**26**) as the thionitrite gave rather different results to those obtained with SNAP. Exchange did not take place between GSNO and NAP_2 (**250**) even after a few days, neither in the presence nor in the absence of added copper.

Monitoring the disappearance of the thionitrite (GSNO) by HPLC revealed that this decomposed much more slowly than SNAP under the same conditions (**Table 27**).

For instance, in the presence of 10^{-8} M copper only 10 % of the disulfide from GSNO had been formed after 2 h and 16 % after 68 h Similarly, in the absence of added copper, after 96 h only 19 % of the thionitrite disulfide had been formed. Thus, GSNO is much more stable than SNAP under the conditions here studied and this observation is in agreement with all previous reports[82]. It is reasonable to suggest that GSNO might also be more resistant to hydrolysis than SNAP. Unfortunately, there are not available data in the literature which permits a comparison of the tendency to hydrolysis of the two thionitrites to be made[85].



NAP_2	GSNO	additive[a]	time	GSNO	GSSG	NAP_2	261
1 equiv.	1 equiv.	EDTA (0.2 equiv.)	3 h	50 %	0 %[b]	50 %	0 %
		EDTA (0.2 equiv.)	96 h	31 %	19 %[b]	50 %	0 %
1 equiv.	1 equiv.	Cu^{2+}(10^{-8} M)	2 h	40 %	10 %	50 %	0 %
		Cu^{2+}(10^{-8} M)	68 h	34 %	16 %	50 %	0 %

(a) Reactions were carried out at pH 7.4 and 37 °C in aqueous phosphate buffer solutions. (b) Molar proportions.

Table 27. GSNO-NAP_2 exchange reactions.

GSNO (**26**) did not exchange with cystine (**248**) in the presence of 10^{-8} M copper (II). In the absence of copper ions exchange appeared to take place. However, the ^1H NMR of the resulting mixture is too complex to allow for a determination of the relative proportions of products. With the cyclic disulfides **253** and **255** no exchange with GSNO was observed under any conditions, similarly to what had occurred with SNAP.

CHAPTER 6

CONCLUSIONS.

The first part of the foregoing study has clearly demonstrated some novel potential uses of thionitrites in synthetic organic methodology.

The results presented in the second chapter have shown that the thermally induced reactivity of unsaturated thionitrites is rich and varied and that it offers wide scope for research into further applications. The difficulties inherent in the synthesis of stable unsaturated tertiary thionitrites, discussed in the first chapter, restrict the applicability of this chemistry in these particular instances. However, the fact that the thionitrites used can be generated *in situ*, without the need for isolation, was expected to expand the scope of this chemistry to primary and secondary substrates, which are more easily accessible. The products formed in some preliminary experiments reflect the simple fact that, when thionitrites are generated *in situ*, the resulting mixtures are more complex and predictions become difficult. It is expected that results in the desired direction could be achieved by a careful optimisation of the reaction conditions and, clearly, further work is needed in this direction.

In general, products of the thermal decomposition of unsaturated thionitrites have been formed by pathways which implicate both formal cleavage of the thionitrite S-N bond and of the C-S bond. The latter type of processes has been observed when at least two structural elements of the substrate render the resulting carbon radical stable. We have seen examples in which this is achieved by formation of tertiary allylic and benzylic-allylic carbon radicals. Doubly benzylic thionitrites were also shown to be susceptible to C-S bond fission. Additionally, some preliminary experiments showed that the thermal reactions carried out in this study can also be brought about by photochemical activation, through the simple use of visible light.

The thermal decomposition of unsaturated thionitrites has allowed the preparation of episulfide and thiofuran type compounds in which two new heteroatom-carbon bonds have been formed, namely a carbon-sulfur and a carbon-nitrogen bond.

The results presented in the third chapter have demonstrated that the thermal addition of trityl thionitrite, chosen as a model of stable tertiary thionitrites in general, onto alkenes, can be extended to the inter-molecular mode if appropriate substituents are

introduced on the double bond. This thionitrite has been proven to add very efficiently to vinylic alkenes containing electron donating, aromatic or olefin conjugated groups on the β-position, both under thermal and photochemical conditions. The products obtained are the corresponding α-oximino sulfides. Other olefin substrates have worked inefficiently and, in a first approximation, it has been possible to rationalise some of the factors likely to affect the overall process in terms of a mechanism involving reversible thiyl radical addition as the first step, followed by reversible reaction of the resulting carbon radical with another molecule of thionitrite in a radical chain process. Under certain conditions, a last irreversible step can lead to the tautomeric oxime of the initially formed nitroso monomer. Underlying enthalpy (resonance) effects seem to play a predominant role in determining the relative rates of the three steps. Polar and steric effects appear also to be manifest in some particular cases, although to a lesser extent. At this point, our understanding of the reaction mechanism still remains mostly speculative and much more research is evidently needed in order to solve this addition reaction. Clearly, we are still at an elementary level of providing a new radical tool for the generation of more complex frameworks, but some obvious transformations of the products resulting from the thermally or photochemically induced addition of trityl thionitrite onto alkenes can be envisaged. For instance, mild acidic treatment would lead to trityl group cleavage from the resulting sulfide with exposure of the underlying thiol function. Treatment of the oxime under appropriate conditions would allow access to other nitrogen functional groups such as amines or amides. One can also envisage incorporation of the thionitrite addition reaction into a cascade of other radical processes, thus allowing access to even more complicated structures.

The studies described in Chapters four and five are of a very different character and were aimed at achieving a better understanding of the reactivity of thionitrites in much more complicated biological systems. The results discussed in chapter four have provided additional insight into the possible mechanism of NO release from bio-active thionitrites *in vivo*. Comparison of the biological activity of enantiomeric pairs of thionitrites in simple rat-artery models has given interesting results. Both enantiomers of CysNO and SNAP displayed identical activity. In contrast, both enantiomers of GSNO, one of the most important endogenous thionitrites, believed to be the principal form of NO transport in the human body, clearly displayed different potencies. Thus, endothelial

cells seemed to recognise the natural (*L*) isomer better than the (*D*) isomer and activity was about 2 or 3 times higher for the natural enantiomer. This result is an indication that the release of NO from this particular thionitrite in biological systems might well be cell-mediated, with the involvement of either an enzyme or a receptor and has opened the door to new research in the biomedical field aimed at identifying such a macromolecule.

The results discussed in the fifth chapter seem to indicate that a direct thionitrite-disulfide exchange reaction in solution systems which model physiological conditions does not take place. Under certain conditions and for some particular substrates, the thionitrite can became hydrolysed and the resulting thiol can initiate a thiol-disulfide exchange reaction. The overall result is a formation of a mixture of symmetrical and unsymmetrical disulfides from stoichiometric quantities of a thionitrite and a disulfide.

PART THREE: EXPERIMENTAL SECTION.

CHAPTER 1

GENERAL PROCEDURES

^{1}H NMR spectra were recorded either at 400 MHz on a Varian VXR-400 or a Bruker AMX400 instrument, or at 300 MHz on a Bruker AMX-300 instrument or at 500 MHz on a Brucker Avance-500 instrument. ^{13}C NMR spectra were recorded at 125.8, 100.6 MHz or 75.4 on the instruments above. Residual protic solvent was taken as internal standard. Spectra run in D_2O were referenced to trimethylsilyl-d_4-propionic acid used as an external standard. Chemical shifts are given in ppm and coupling constants in Hz. The abbreviations used to indicate multiplicity are: s= singlet, d= doublet, t= triplet, q= quartet, dd= double doublet, dt= double triplet, b= broad, m= multiplet and ddd= double double doublet. Infrared spectra were recorded as thin films on sodium chloride plates, as KBr discs or in solution ($CHCl_3$ or CCl_4), as indicated in each case, on a Perkin-Elmer FT-IR 1605 instrument. In some cases, only assigned peaks have been quoted. Mass spectra were recorded under electron impact, atmospheric pressure chemical ionisation or fast atom bombardment conditions. They were recorded by the staff of the Mass Spectroscopy Service at the School of Pharmacy of the University of London or by the staff of the Mass Spectroscopy service of UCL. Melting points were taken on a Reichert hot stage instrument and are uncorrected. Optical rotations were taken with a 'POLAAR 2000' instrument by Optical Activity Ltd.

P.E. refers to light petroleum (b.p. 30-40 °C). Ether refers to diethyl ether, which when used as a reaction solvent was distilled under nitrogen from sodium benzophenone-ketyl, as was tetrahydrofuran. Benzene and toluene were distilled from molten sodium. When used as reaction solvent, dichloromethane was distilled from phosphorus pentoxide or calcium hydride. Methanol was distilled from magnesium turnings and stored over 4 □ molecular sieves. Ethanol refers to absolute ethanol (>99.7 %) and was used as received. Dimethyl formamide was peptide synthesis grade and was used as received. All other reagents and solvents were purified following the usual procedures.

Analytical thin layer chromatography was performed on pre-coated aluminium backed plates (Merck Kieselgel 60 F_{254}) and visualised either with ultraviolet light (254 nm), potassium permanganate solution [2.0 g K_2CO_3 in H_2O (125 mL) + 3.5 g KMO_4 in H_2O (125 mL) + 5 % sol. NaOH (5 mL)], anisaldehyde solution [6 g anisaldehyde + 250

mL EtOH + conc. H_2SO_4 (2.5 mL)], solution of molybdate (IV) [conc. H_2SO_4 (250 mL), ammonium molybdate tetrahydrate, H_2O (250 mL)], solution of palladium [$PdCl_2$ 0.5 % in H_2O + few drops of conc. HCl] or ninhydrin solution [ninhydrin 0.3 % in ^nBuOH + 3 % AcOH, 10 min 125 °C]. Preparative thin layer chromatography was performed on pre-coated glass backed plates (Merck Kieselgel 60 F_{254}) and visualised with ultraviolet light (254 nm). Preparative column chromatography was performed at low positive pressure on Merck Kieselgel 60. Analytical HPLC was carried out on a Shimadzu LC-10 AS instrument with a 25 cm x 0.5 cm reverse phase Hichrom KR100-5C18-4179 column, connected to a UV-vis SPD-6A detector set at 215 nm and a CR6A recorder. Preparative HPLC was performed on a Gilson 306 instrument with a 22 cm x 250 cm hyperprep pep100A C18 8μ column (from Alltech), connected to a UV-vis Gilson 118 detector set at 215 nm, a FC204 fraction collector and a 231 XL sampling injector. Solvents used were HPLC grade and degassed prior to use. HPLC-MS was carried out on a HP-1100 instrument connected to a Quattro LC Mass Spectrometer from Micromass. The GC-MS was carried out by Dr. Carmen Escolano at Kingston University on a Perkin Elmer 8600 instrument equipped with a capillary column Perkin Elmer 12QC/BP1 0.25. Heating was started at 75 °C for 3 min and increased to 275 °C in 20 min, then kept at this temperature for 7 min. X-Ray chrystallography was carried out by Dr. Derek Tocher at UCL and Dr. Alexandra Slawin at Laughborough University.

Elemental analysis was carried out by the staff at the Microanalysis Service of University College London.

CHAPTER 2

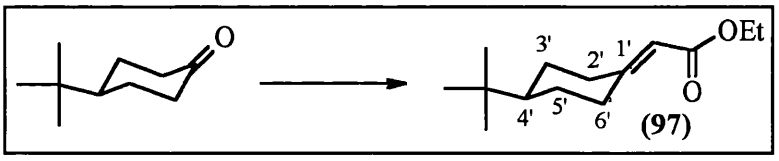
SYNTHESIS OF ALLYLIC TERTIARY THIONITRITES AND OF PRIMARY AND SECONDARY THIOLS FOR THE *IN SITU* PREPARATION OF PRIMARY AND SECONDARY THIONITRITES.

2. 1. Preparation of allylic alcohols.

2. 1. 1. From α,β-unsaturated esters.

2. 1. 1. 1. Synthesis of α,β-unsaturated esters, general procedure.

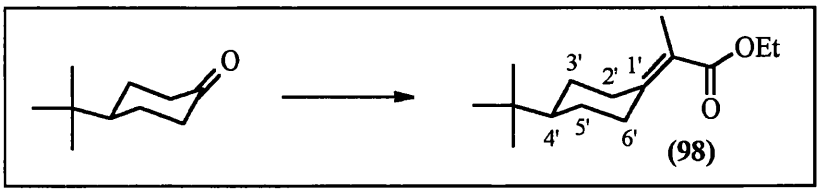
In a typical run, triethylphosphonoacetate (23.7 g, 21 mL, 100 mmol) or triethylphosphonopropionate (23.8 g, 21.4 mL, 100 mmol) was added dropwise, at 0 °C and under N_2, to a suspension of sodium hydride (60 % in oil, 4.2 g, 100 mmol) in 100 mL of anhydrous THF. After stirring at the same temperature for 30 min, the corresponding ketone (33 mmol) was added slowly, dissolved in 30 mL of dry THF. When addition was complete, the temperature was allowed to rise to room temperature and the mixture stirred for 2 h. The reaction was quenched by careful addition of aq. saturated NH_4Cl and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and the solvent eliminated at reduced pressure to yield a crude oil which was purified, in each particular case, as indicated in parenthesis below. All esters were isolated as colourless oils.



(97)

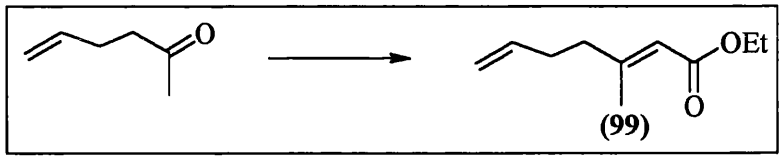
Ethyl 2-(4-tert-butylcyclohexylidene)ethanoate (97). (filtration through SiO_2, P.E./Et_2O 95/5; 99 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.57 (1H, s, C<u>H</u>=), 4.13 (2H, q, J= 7.4, C<u>H</u>$_2$O), 3.87 (1H, dd, J= 13.8, 2.2, $C_{2'(6')}$<u>H</u>H), 2.30 (1H, dd, J= 13.5, 2.0 $C_{2'(6')}$H<u>H</u>), 2.15 (1H, bt, J= 14.0, 3.2, $C_{2'(6')}$H<u>H</u>), 1.87 (2H, bd, J= 13.0, 4.4, 2 x $C_{3'(5')}$<u>H</u>H), 1.82 (1H, td, J= 13.5, 4.2, $C_{2'(6')}$H<u>H</u>), 1.26 (3H, t, J= 7.4, C<u>H</u>$_3$CH$_2$O), 1.24-1.18 (3H, m, 2 x $C_{3'(5')}$H<u>H</u> + $C_{4'}$<u>H</u>), 0.85 (9H, s, (<u>C</u>H$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 166.81 (<u>C</u>=O), 163.47 (<u>C</u>=), 112.65 (<u>C</u>H=), 59.41 (<u>C</u>H$_2$O), 47.73 ($\underline{C}_{4'}$H), 37.83 ($\underline{C}_{2'(6')}$H$_2$), 32.39 ((CH$_3$)$_3$<u>C</u>), 29.50, 29.16 and 28.41 (ring <u>C</u>H$_2$), 27.51 (<u>C</u>H$_3$)$_3$C), 14.29

($\underline{C}H_3CH_2O$). **IR** (neat): ν_{max}/cm^{-1} 2937, 2845, 1719 (C=O), 1651 (C=C), 1445, 1250, 1165, 1132, 1033, 912. **MS** (FAB): m/z 225 ($(M+H)^+$, 29), 179 ($(M-OEt)^+$, 14), 154 (100), 136 (86). **HRMS** (FAB): found 225.1855, $C_{14}H_{25}O_2$ $(M+H)^+$ requires 225.1855.



(98)

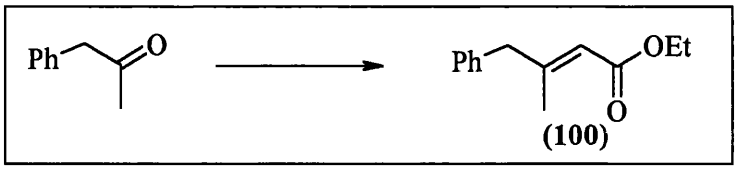
Ethyl 2-(4-tert-butylcyclohexylidene)propanoate (98). (filtration through SiO_2, P.E./Et_2O 95/5; 98 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.16 (2H, q, J= 7.0, $C\underline{H}_2O$), 3.06 (1H, dm, J= 12.6, $C_{2'(6')}\underline{H}H$), 2.66 (1H, dm, J= 13.4, $C_{2'(6')}H\underline{H}$), 1.83 (3H, s, $C\underline{H}_3C$=), 1.90-1.75 (2H, m, 2 x $C_{2'(6')}H\underline{H}$), 1.27 (3H, t, J= 7.0, $C\underline{H}_3CH_2O$), 1.25-1.10 (5H, m, 2 x $C_{3'(5')}\underline{H}_2$ + $C_{4'}\underline{H}$), 0.82 (9H, s, $(C\underline{H}_3)_3C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 170.60 ($\underline{C}$=O), 147.57 and 119.47 ($\underline{C}$=), 60.08 ($\underline{C}H_2O$), 47.93 ($\underline{C}_{4'}H$), 32.42 ($(CH_3)_3\underline{C}$), 31.94, 30.94, 29.68 and 28.68 (ring $\underline{C}H_2$), 27.54 ($(\underline{C}H_3)_3C$), 15.13 and 14.27 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2928, 2868, 1714 (C=O), 1637 (C=C), 1457, 1367, 1283, 1241, 1175. **MS** (FAB): m/z 239 ($(M+H)^+$, 81), 238 ($M^{+\cdot}$, 19), 237 ($(M-H)^+$, 100), 223 ($(M-Me)^+$, 3), 211 (4), 209 ($(M-Et)^+$, 15), 207 (21), 193 ($(M-OEt)^+$, 7), 165 (2), 149 (4), 137 (6), 115 (10), 74 (4). **HRMS** (FAB): found: 239.2025, $C_{15}H_{27}O_2$ $(M+H)^+$ requires 239.2011.



(99)

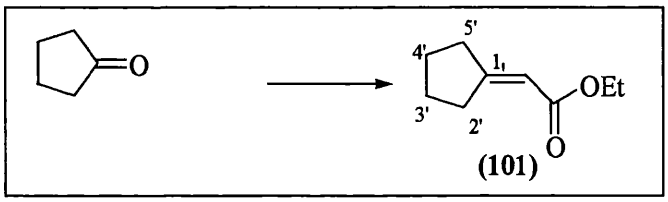
Ethyl (E)-3-methyl-2,6-heptadienoate (99). (chromatography on SiO_2, P.E./Et_2O 98/2; 86 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, m, $C\underline{H}$=CH_2), 5.67 (1H, s, =$C\underline{H}CO_2Et$), 5.04 (1H, d, J= 8.4, $C\underline{H}H$=C), 4.99 (1H, d, J= 20.6, $CH\underline{H}$=C), 4.13 (2H, q, J= 7.2, $C\underline{H}_2O$), 2.23 (4H, m, $C\underline{H}_2C\underline{H}_2$), 2.17 (3H, s, =$CC\underline{H}_3$), 1.28 (3H, t, J= 7.2, $C\underline{H}_3CH_2O$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 166.74 ($\underline{C}$=O), 159.00 ($\underline{C}$=), 137.23, 115.84 and 115.25 ($\underline{C}$(H)=), 59.45 ($\underline{C}H_2O$), 40.12 and 31.49 ($\underline{C}H_2$), 18.69 and 14.29 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2980, 2938, 1717 (C=O), 1649 (C=C), 1445, 1368, 1275, 1223, 1149, 1045, 914, 864. **MS** (FAB): m/z 169 ($(M+H)^+$, 89), 168 ($M^{+\cdot}$, 6),

123 ($(M-OEt)^+$, 53), 95 ($(M-CO_2Et)^+$, 72). **HRMS** (FAB): found 269.1240, $C_{10}H_{17}O_2$ $(M+H)^+$ requires 269.1229.



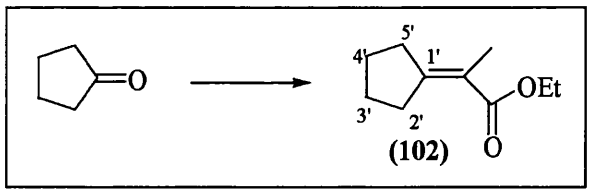
(100)

Ethyl (E)-3-methyl-4-phenyl-2-butenoate (100). (chromatography on SiO_2, P.E./Et_2O 98/2; 99 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.35-7.17 (5H, m, ArH), 5.66 (1H, s, =CH), 4.12 (2H, q, J= 7.2, CH_2O), 3.41 (2H, s, CH_2Ph), 2.95 (3H, s, $=CCH_3$), 1.25 (3H, t, J= 7.2, CH_3CH_2O). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 166.71 (C=O), 158.27, 137.72, 129.14, 128.53, 126.66 and 126.62 ($C_{Ar}(H)$ + C(H)=), 62.45 (CH_2O), 48.65 and 47.06 ($PhCH_2$ + $=CCH_3$), 14.30 (CH_3CH_2O). **IR** (neat): ν_{max}/cm^{-1} 2999, 2918, 1720 (C=O), 1650 (C=C), 1497, 1457, 1396, 1369, 1287, 1275, 1213, 1145, 1044. **MS** (FAB): m/z 205 ($(M+H)^+$, 90), 159 ($(M-OEt)^+$, 55), 131 ($(M-CO_2Et)^+$, 20). **HRMS** (FAB): found 205.1220, $C_{13}H_{16}O_2$ $(M+H)^+$ requires 205.1229.



(101)

Ethyl 2-cyclopentylidenethanoate (101)[171]. (chromatography on SiO_2, P.E./Et_2O 95/5; 99 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, m, J= 2.0, CH=), 4.14 (2H, q, J= 7.08, CH_2O), 2.77 (2H, td, J= 7.32, 1.12, $C_{2'(5')}H_2$), 2.43 (2H, t, J= 7.24, $C_{2'(5')}H_2$), 1.8-1.6 (4H, m, $C_{3'}H_2C_{4'}H_2$), 1.28 (3H, t, J= 7.04, CH_3CH_2O). **^{13}C NMR** ($CDCl_3$, 400 MHz): δ 169.04 (C=O), 166.91 (C=), 111.65 (CH=), 59.38 (CH_2O), 35.92 and 32.59 ($C_{2'(5')}H_2$), 26.40 and 25.46 ($C_{3'(4')}H_2$), 14.36 (CH_3).

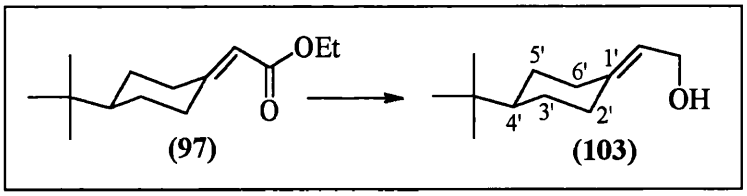


(102)

Ethyl 2-cyclopentylidenepropanoate (102). (chromatography on SiO_2, P.E./Et_2O 98/2; 95 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.17 (2H, q, J= 7.16, $C\underline{H}_2O$), 2.71 (2H, m, $C_{2'(5')}\underline{H}_2$), 2.35 (2H, m, $C_{2'(5')}\underline{H}_2$), 1.84 (3H, m, $C\underline{H}_3C$=), 1.75-1.60 (4H, $C_{3'}\underline{H}_2C_{4'}\underline{H}_2$), 1.29 (3H, t, J= 7.16, $C\underline{H}_3CH_2O$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 168.39 ($\underline{C}$=O), 160.19 and 118.52 ($\underline{C}$=), 59.73 ($\underline{C}H_2O$), 34.17 and 34.06 ($\underline{C}_{2'(5')}H_2$), 27.13 and 25.50 ($\underline{C}_{3'(4')}H_2$), 15.91 and 14.33 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2957, 2871, 1707 (C=O), 1642 (C=C), 1275 (C–O), 1191, 1097, 1061, 1027, 772. **MS** (FAB): m/z 169 ($(M+H)^+$, 59), 138 ($(M-HEt)^{+\cdot}$, 63), 123 ($(M-OEt)^+$, 89), 110 (100). **HRMS** (FAB): found 169.1229, $C_{10}H_{17}O_2$ $(M+H)^+$ requires 169.1220.

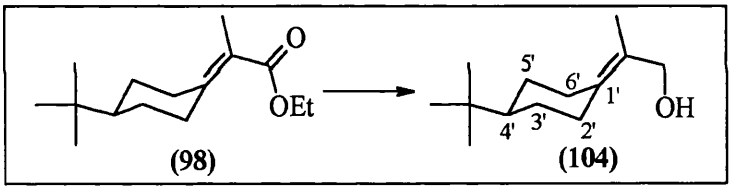
2. 1. 1. 2. Reduction of α,β-unsaturated esters to allylic alcohols, general procedure.

The corresponding ester (16.2 mmol), dissolved in 50 mL of dry Et_2O, was added dropwise over 30-45 min, at 0 °C and under N_2, to a suspension of $LiAlH_4$ (1 g, 32.4 mmol) in 65 mL of dry Et_2O. After addition was complete, the resulting mixture was stirred for 1 h at 0 °C and, then, for another hour at r. t. The reaction was quenched by very slow addition of cold H_2O until all of the excess $LiAlH_4$ had been destroyed, a white precipitate being formed. The crude mixture was filtered through Celite®, dried over $MgSO_4$ and the solvent removed at reduced pressure. The corresponding allylic alcohols were obtained as colourless oils, with the exception of **104** which was a white solid. In the majority of the cases the product was of sufficient purity to allow the next step to be carried out without additional purification. When additional purification was needed, the conditions of chromatography have been indicated in parenthesis below.



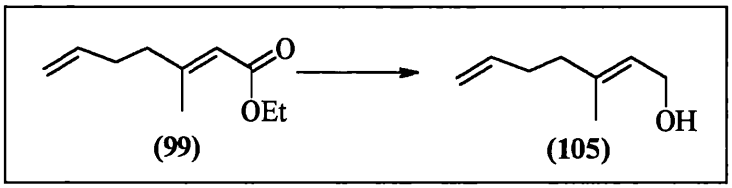
(97) (103)

2-(4-Tert-butylcyclohexylidene)-1-ethanol (103). (70-87 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.31 (1H, t, J= 7.2, C<u>H</u>=), 4.10 (2H, d, J= 7.2, C<u>H</u>$_2$OH), 2.66 (1H, bd, J= 13.7, $C_{2'(6')}$<u>H</u>H), 2.22 (1H, bd, J= 13.4, $C_{2'(6')}$<u>H</u>H), 2.03 (1H, bt, J= 12.9, $C_{2'(6')}$<u>H</u>H), 1.83 (2H, m, $C_{2'(6')}$H<u>H</u> + $C_{3'(5')}$H<u>H</u>), 1.73 (1H, dt, J= 13.0, 4.2, $C_{3'(5')}$H<u>H</u>), 1.46 (1H, bs, O<u>H</u>), 1.4-0.8 (3H, m, 2 x $C_{3'(5')}$H<u>H</u> + $C_{4'}$<u>H</u>), 0.81 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 144.29 (<u>C</u>=), 119.87 (<u>C</u>H=), 58.55 (C<u>H</u>$_2$OH), 48.22 (<u>C</u>$_{4'}$H), 36.85 (<u>C</u>$_{2'(6')}$H$_2$), 32.40 ((CH$_3$)$_3$<u>C</u>), 28.95, 28.58 and 28.46 (ring <u>C</u>H$_2$), 27.55 ((<u>C</u>H$_3$)$_3$C). **IR** (neat): ν_{max}/cm^{-1} 3356 (b, OH), 2980, 1667 (C=C). **MS** (FAB): m/z 205 ($(M+Na)^+$, 5), 181 ($(M-H)^+$, 53), 165 ($(M-OH)^+$, 100), 137 (38). **HRMS** (FAB): found 181.1584, $C_{12}H_{22}O$ $(M-H)^+$ requires 181.1592.



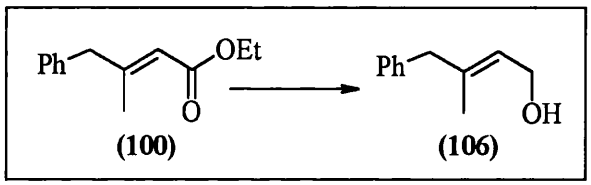
(98) (104)

2-(4-Tert-butylcyclohexylidene)-1-propanol (104). (white solid after chromatography on silica gel, DCM; 71 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.13 and 4.9 (1H each, 2 x d, J= 11.2, C<u>H</u>HOH), 2.75 (1H, dm, J= 13.4, $C_{2'(6')}$<u>H</u>H), 2.65 (1H, dm, J= 13.6, $C_{2'(6')}$<u>H</u>H), 1.73 (3H, s, C<u>H</u>$_3$C=), 1.90-1.65 (4H, m, 2 x $C_{2'(6')}$H<u>H</u> + 2 x $C_{3'(5')}$<u>H</u>H), 1.20-0.95 (2H, m, 2 x $C_{3'(5')}$H<u>H</u>), 0.92 (1H, bt, J= 12.3, $C_{4'}$<u>H</u>), 0.81 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 137.66 and 123.89 (<u>C</u>=), 63.38 (<u>C</u>H$_2$OH), 48.27 (<u>C</u>$_{4'}$H), 32.40 ((CH$_3$)$_3$<u>C</u>), 30.53, 29.88, 29.01 and 28.35 (ring <u>C</u>H$_2$), 27.56 ((<u>C</u>H$_3$)$_3$C), 16.24 (<u>C</u>H$_3$). **IR** (CCl_4): ν_{max}/cm^{-1} 3340 (b, OH), 2960, 2363, 1445, 1366, 1278, 1240, 1174, 1102, 1004. **MS** (FAB): m/z 195 ($(M-H)^+$, 2), 179 ($(M-OH)^+$, 48), 154 (38), 137 (87), 123 (10), 109 (84), 105 (5). **HRMS** (FAB): found 195.1758, $C_{13}H_{23}O$ $(M-H)^+$ requires 195.1749.



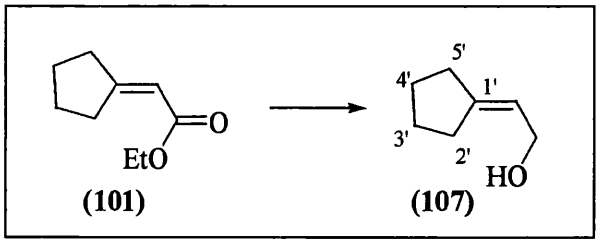
(99) (105)

(E)-3-Methyl-2,6-heptadien-1-ol (105). (92 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, m, C<u>H</u>=CH_2), 5.39 (1H, m, =C<u>H</u>CH_2OH), 4.99 (1H, d, J= 17.2, C<u>H</u>H=), 4.93 (1H, d, J= 10.4, CH<u>H</u>=), 4.12 (2H, d, J= 7.2, C<u>H_2</u>OH), 2.20-2.05 (4H, m, C<u>H_2</u>C<u>H_2</u>), 1.65 (3H, s, C<u>H_3</u>). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 139.23, 138.26 and 123.63 (<u>C</u>(H)), 114.62 (=<u>C</u>H_2), 59.36 (<u>C</u>H_2OH), 38.80 and 31.94 (<u>C</u>H_2), 16.23 (<u>C</u>H_3). **IR** (neat): ν_{max}/cm^{-1} 3395 (b, OH), 2952, 2825, 1675 (C=C), 1456, 1438, 1364, 1284, 1170. **MS** (FAB): m/z 125 ($(M-H)^+$, 32), 109 ($(M-OH)^+$, 100), 95 (45), 81 (33), 67 (31). **HRMS** (FAB): found 125.0956, $C_8H_{13}O$ $(M-H)^+$ requires 125.0966.

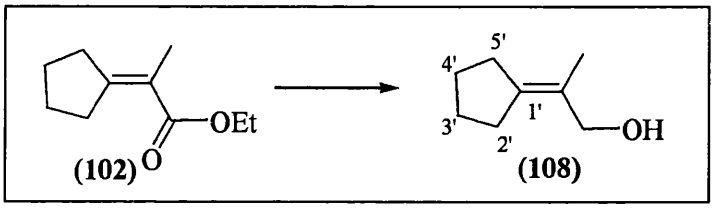


(100) (106)

(E)-3-Methyl-4-phenyl-2-buten-1-ol (106). (84 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.28-7.13 (5H, m, Ar<u>H</u>), 5.47 (1H, m, C<u>H</u>=), 4.16 (2H, d, J= 6.8, C<u>H_2</u>OH), 3.30 (2H, s, C<u>H_2</u>Ph), 1.60 (3H, s, C<u>H_3</u>). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 139.38, 138.96, 128.92, 128.31, 126.17 and 125.22 (<u>C</u>$_{Ar}$(H) + <u>C</u>(H)=), 59.43 (<u>C</u>H_2OH), 46.01 (<u>C</u>H_2Ph), 16.13 (<u>C</u>H_3). **IR** (neat): ν_{max}/cm^{-1} 3351 (b, OH), 2922, 1649 (C=C), 1448, 1381, 1222, 1148, 1004, 738, 699. **MS** (FAB): m/z 185 ($(M+Na)^+$, 15), 162 ($M^{+\cdot}$, 15), 145 ($(M-OH)^+$, 100). **HRMS** (FAB): found 145.1017, $C_{11}H_{13}$ $(M-OH)^+$ requires 145.1021.



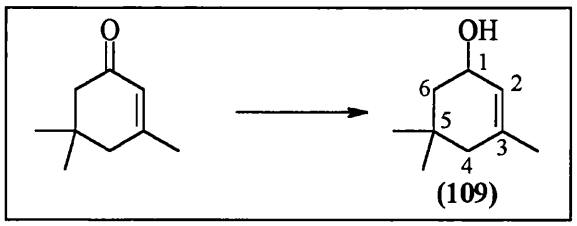
***2-Pentylidene-1-ethanol (107)*[172].** (73 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.38 (1H, m, C<u>H</u>=), 3.98 (2H, dm, J= 6.8, C<u>H$_2$</u>OH), 2.20 (4H, m, 2 x $C_{2'(5')}$<u>H_2</u>), 1.55 (4H, m, $C_{3'}$<u>H_2</u>$C_{4'}$<u>H_2</u>).



2-Cyclopentylidene-1-propanol (108). (chromatography on SiO_2, CH_2Cl_2; 50 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.08 (2H, bs, C<u>H$_2$</u>OH), 2.26 (2H, m, $C_{2'(5')}$<u>H_2</u>), 2.2 (2H, m, $C_{2'(5')}$<u>H_2</u>), 1.70 (3H, s, C<u>H$_3$</u>), 1.65-1.60 (4H, m, $C_{3'}$<u>H_2</u>$C_{4'}$<u>H_2</u>). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 140.85 and 124.4 (<u>C</u>=), 64.9 (<u>C</u>H$_2$OH), 30.9 and 29.8 (<u>$C_{2'(5')}$</u>H_2), 26.9 and 26.2 (<u>$C_{3'(4')}$</u>H_2), 16.74 (<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 3340 (b, OH), 2952, 2868, 1661 (C=C), 1435, 1378, 1011. **MS** (EI): m/z 126 ($M^{+\cdot}$, 10), 108 ($(M-H_2O)^{+\cdot}$, 52), 93 ($M-H_2O-Me)^+$, 100), 77 (45), 67 (69), 55 (54). **HRMS** (EI): found 126.1041, $C_8H_{14}O$ ($M^{+\cdot}$) requires 126.1045.

<u>2. 1. 2. Preparation of allylic alcohols by reduction of α,β-unsaturated ketones.</u>

2. 1. 2. 1. Preparation of 3,5,5-trimethyl-2-cyclohexen-1-ol.

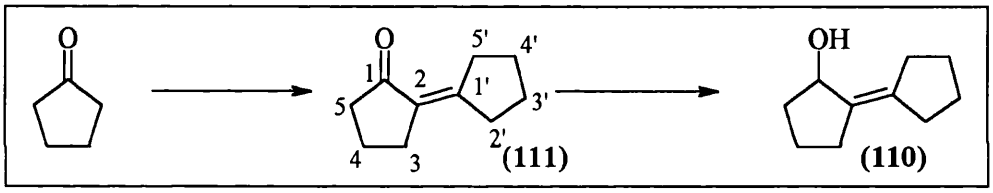


Commercially available 3,5,5-trimethyl-2-cyclohexen-1-one (10 g, 72 mmol) and $CeCl_3.7H_2O$ (27 g, 72 mmol) were dissolved in 200 mL of MeOH and, at 0 °C, $NaBH_4$ (2.8 g, 72 mmol) was added slowly. The mixture was allowed to warm to r. t. and stirred

until all the starting ketone had been consumed. After quenching with H_2O and extracting with Et_2O (twice), the organic layer was dried over $MgSO_4$ and the solvent evaporated under vacuum, affording a crude oil, which was purified by filtration through silica gel (DCM, 9.68 g, 95 %).

3,5,5-Trimethyl-2-cyclohexen-1-ol (109)[173]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.42 (1H, m, $C_2\underline{H}$=), 4.23 (1H, m, $C_1\underline{H}OH$), 1.87 (1H, d, J= 17.4, $C_4\underline{H}H$), 1.77 (1H, dd, J= 12.2, 5.6, $C_6\underline{H}H$), 1.68 (3H, s, $C\underline{H}_3$), 1.61 (1H, d, J= 17.4, $C_4H\underline{H}$), 1.22 (1H, dd, J= 12.2, 9.2, $C_6H\underline{H}$), 0.99 (3H, s, $C\underline{H}_3$), (3H, s, $C\underline{H}_3$) . **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 135.73 ($\underline{C}$=), 123.71 ($\underline{C}$H=), 66.65 ($\underline{C}$HOH), 45.09 and 44.08 ($\underline{C}_{4(6)}H_2$), 31.25, 31.02 and 26.12 ($\underline{C}H_3$), 23.50 ($\underline{C}_5$). **IR** (neat): ν_{max}/cm^{-1} 3351 (b, OH), 2904, 1639 (C=C), 1375, 1250, 1003, 895.

2. 1. 2. 2. Preparation of 2-cyclopentylidene 1-cyclopentanol.



Cyclopentanone (20 g, 21 mL, 0.24 mol) was dissolved in H_2O (50 mL) and NaOH (2.85 g, 0.07 mol) was added in one portion. The resulting solution was stirred at r. t. for one day, becoming yellow/orange after the first few minutes. The reaction was quenched with AcOH and extracted with toluene. The organic layer was separated and washed once with saturated aq. $NaHCO_3$ and once with brine, dried over $MgSO_4$ and the solvent evaporated under vacuum. The crude oil was distilled at low pressure (0.3 mm Hg, T= 115 °C) giving compound **111** as a colourless oil in 33 % yield. Reduction of 10 g (6.6 mmol) of **111** was carried out with $NaBH_4$ (252 mg, 6.6 mmol), in the presence of $CeCl_3 \cdot 7H_2O$ (2.5 g, 6.6 mmol), in MeOH, under the conditions described above (*2. 1. 2. 1*). Chromatography on silica gel (P.E./Et_2O 95/5), afforded alcohol **110** in 70 % yield (7.1 g).

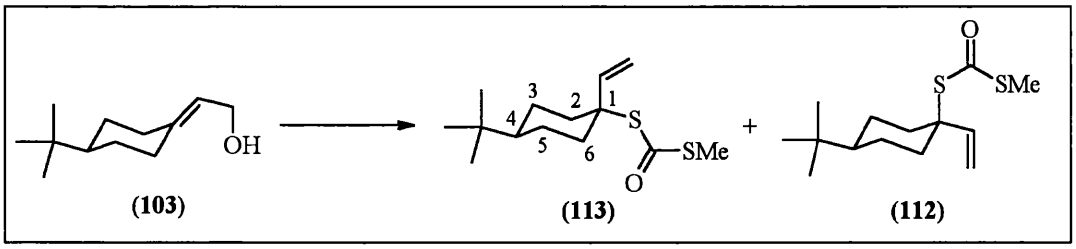
2-Cyclopentylidene 1-cyclopentanone (111)[174]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 2.78 (2H, m), 2.54 (2H, m), 2.32 (4H, m), 1.92 (2H, m), 1.75 (4H, m). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 207.78 (C=O), 159.34 and 129.5 (=$\underline{C}_{2(1')}$), 39.78, 34.28, 32.53, 29.50, 26.92, 25.21 and 20.06 (rings $\underline{C}H_2$s).

2-Cyclopentylidene 1-cyclopentanol (110)[175]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.58 (1H, $C_1\underline{H}OH$), 2.36 (2H, m), 2.15 (1H, m), 2.10 (1H, m) and 1.86-1.66 (10H, m). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 139.04 and 135.0 (=$\underline{C}_{2(1')}$), 73.75 ($\underline{C}_1HOH$), 36.71, 31.63, 30.63, 29.94, 27.04, 26.31 and 23.29 (rings $\underline{C}H_2$s).

2. 2. Synthesis of tertiary allylic dithiocarbonates.

2. 2. 1. Using NaH as the base, general procedure.

In a typical reaction, the corresponding allylic alcohol (20.6 mmol) was added slowly, at 0° C and under N_2, to a suspension of NaH (60 % in mineral oil, 1.23 g, 30.8 mmol) in 75 mL of anhydrous THF. The mixture was stirred at r. t. for a period between 30 min and 1.5 h (turning pale yellow). CS_2 (3.7 mL, 62.0 mmol) was then added and stirring continued for an additional period of 1 to 1.5 h (solution turned orange or brown). Finally, MeI (7.7 mL, 123 mmol) was added and the mixture heated at reflux for a period of at least 5 or 6 h (formation of yellow precipitate was observed). The reaction was quenched with saturated aq. NH_4Cl solution (added carefully) and extracted with Et_2O. The organic layer was washed with aqueous NH_4Cl (once), followed by aq. $NaHCO_3$ (once) and brine (once) and dried over $MgSO_4$. After evaporation of the solvent, the brown oil obtained was purified by repeated chromatography on silica gel (usually two or three times), using the solvent system specified in each case in parenthesis. All dithiocarbonates were isolated as yellow oils.



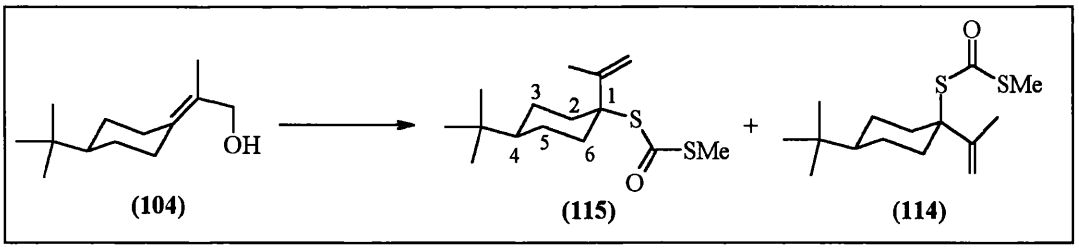
(103) (113) (112)

P.E., followed by P.E./Et_2O 98/2; 43-55 % of the mixture of the two diastereomers *cis:trans* 2:1 was obtained. The products were not separated. Only traces of the two diastereomers could be obtained separately and independently characterised. They were used as the mixture in the next step.

Cis S-methyl S-(4-tert-butyl-1-vinylcyclohexyl) dithiocarbonate (112). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.16 (1H, dd, J= 17.2, 10.7, C<u>H</u>=), 5.16 (1H, dd, J= 17.3, 0.88, CH<u>H</u>=), 5.05 (1H, dd, J= 10.7, 0.92, CH<u>H</u>=), 2.33 (3H, s, SC<u>H</u>$_3$), 2.22 (2H, bd, J= 13.7, 2 x $C_{2(6)}$H<u>H</u>), 1.72 (2H, bt, J= 13.5, 2 x $C_{2(6)}$H<u>H</u>), 1.65 (4H, m, 2 x $C_{3(5)}$<u>H</u>$_2$), 1.02 (1H, m, C_4<u>H</u>), 0.87 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 189.51 (<u>C</u>=O), 139.18 (<u>C</u>H=), 117.05 (<u>C</u>H$_2$=), 57.63 (<u>C</u>S), 47.42 (<u>C</u>$_4$H), 36.10 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.34 ((CH$_3$)$_3$<u>C</u>), 27.41 ((<u>C</u>H$_3$)$_3$C), 23.92 (2 x <u>C</u>$_{3(5)}$H$_2$), 12.78 (S<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 2948, 1718 (C=O), 1643 (C=C), 1361. **MS** (FAB): m/z 273 ((M+H)$^+$, 24), 165 ((M–SCOSMe)$^+$, 89).

Trans S-methyl S-(4-tert-butyl-1-vinylcyclohexyl) dithiocarbonate (113). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.90 (1H, dd, J= 17.6, 10.7, C<u>H</u>=), 5.33 (1H, d, J= 17.6, CH<u>H</u>=), 5.31 (1H, d, 10.7, CH<u>H</u>=), 2.45 (3H, s, SC<u>H</u>$_3$), 2.42 (2H, bd, J= 12.6, 2 x $C_{2(6)}$H<u>H</u>), 1.90 (2H, bt, J= 12.6, 2 x $C_{2(6)}$H<u>H</u>), 1.65 (2H, bd, J= 11.7, 2 x $C_{3(5)}$H<u>H</u>), 1.2 (2H, qd, J= 11.5, 3.3, 2 x $C_{3(5)}$H<u>H</u>), 0.95 (1H, tt, J= 12.0, 2.7, C_4<u>H</u>), 0.82 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.0 (<u>C</u>=O), 143.53 (<u>C</u>H=), 112.12 (<u>C</u>H$_2$=), 59.08 (<u>C</u>S), 47.64 (<u>C</u>$_4$H), 35.46 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.37 ((CH$_3$)$_3$<u>C</u>), 27.44 ((<u>C</u>H$_3$)$_3$C), 22.96 (2 x <u>C</u>$_{3(5)}$H$_2$), 12.82 (S<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 2968, 2921, 2862, 1714 (C=O), 1637 (C=C), 1062.

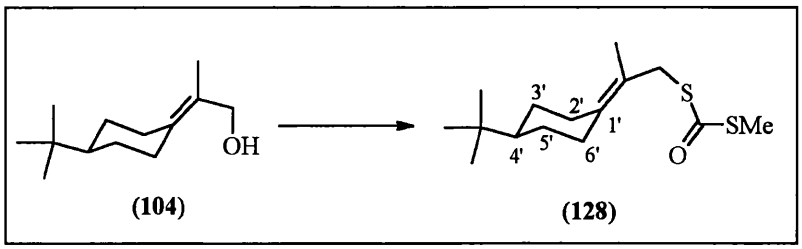
IR (neat, of the mixture): ν_{max}/cm^{-1} 2948, 2864, 1720 (C=O), 1640 (C=C). **MS** (FAB, of the mixture): m/z 273 ((M+H)$^+$, 22), 245 (7), 211 (4), 165 ((M-SCOSMe)$^+$, 74), 123 (34), 117 (8), 109 (100). **HRMS** (FAB, of the mixture): found 273.1360, $C_{14}H_{25}S_2O$ (M+H)$^+$ requires 273.1347.



(104) (115) (114)

P.E., followed by P.E./Et_2O 98/2; 39 % of the mixture of the two diastereomers *cis:trans* 1.5:1 was obtained. The products were not separated and are analysed as the mixture.

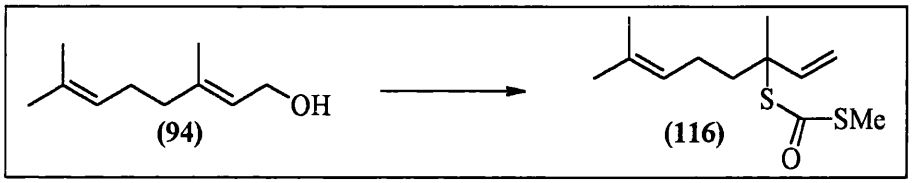
Cis/trans S-methyl S-[(4-tert-butyl-1-(2-propenyl)cyclohexyl)] dithiocarbonate (114/115). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.20 (1H, s, C<u>H</u>H= minor isomer), 5.18 (1H, m, C<u>H</u>H= minor), 5.05 (1H, s, CH<u>H</u>= major), 4.94 (1H, m, CH<u>H</u>= major), 2.57 (2H, bd, J= 12.1, 2 x $C_{2(6)}$<u>H</u>H), 2.44 (2H, bd, J= 12.1, 2 x $C_{2(6)}$<u>H</u>H), 2.33 (6H, s, 2 x S<u>C</u>H_3), 1.98 (2H, m, 2 x $C_{2(6)}$H<u>H</u>), 1.88 (3H, d, J= 0.8, (<u>C</u>H_3)C= major), 1.82 (3H, s, (<u>C</u>H_3)C= minor), 1.65 (2H, m, 2 x $C_{2(6)}$H<u>H</u>), 1.70-1.20 (10H, m, 4 x $C_{3(5)}$<u>H</u>$_2$ + 2 x C_4<u>H</u>), 0.87 (9H, s, (C<u>H</u>$_3$)$_3$C major), 0.81 (9H, s, (C<u>H</u>$_3$)$_3$C minor). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.2 (<u>C</u>=O), 148.21 and 141.13 (<u>C</u>=), 116.88 and 111.81 (<u>C</u>H_2=), 62.30 and 61.69 (<u>C</u>S), 47.61 and 47.52 (<u>C</u>$_4$H), 35.38 and 34.83 (4 x $C_{2(6)}$<u>H</u>$_2$), 32.38 (2 x (CH_3)$_3$<u>C</u>), 27.62 and 27.51 ((<u>C</u>H_3)$_3$C), 24.08 and 23.42 (4 x $C_{3(5)}$<u>H</u>$_2$), 19.89 and 19.80 (=C<u>C</u>H_3), 14.11 and 12.86 (S<u>C</u>H_3). **IR** (neat): ν_{max}/cm^{-1} 2948, 2865, 1713 (C=O), 1641 (C=C), 1445, 1367, 900, 847. **MS** (FAB): m/z 287 ($(M+H)^+$, 11), 271 ($(M-Me)^+$, 2), 211 ($(M-COSMe)^+$, 14), 179 ($(M-SCOSMe)^+$, 87), 123 (72), 109 (59), 95 (32), 81 (29), 69 (26), 57 (100), 41 (37), 29 (14). **HRMS** (FAB): found 287.1520, $C_{15}H_{27}S_2O$ $(M+H)^+$ requires 287.1503.



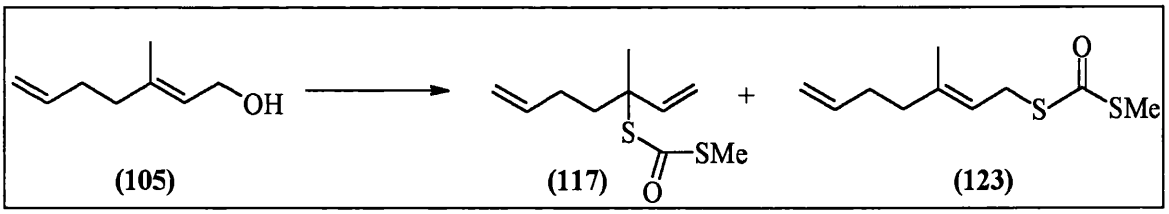
(104) (128)

If the crude reaction mixture from alcohol **104** was chromatographed after standing at r. t. for 2 weeks, only one rearranged product (**128**) was separated in 34 % yield.

***S*-Methyl *S*-[2-(4-*tert*-butyl-1-cyclohexylidene)propyl] dithiocarbonate (128).** ^{1} **H NMR** ($CDCl_3$, 400 MHz): δ 3.76 (2H, s, $C\underline{H}_2S$), 2.67 (2H, m, 2 x $C_{2'(6')}\underline{H}H$), 2.41 (3H, s, $C\underline{H}_3S$), 1.85 (2H, m, 2 x $C_{2'(6')}H\underline{H}$), 1.75 (2H, m, 2 x $C_{3'(5')}H\underline{H}$), 1.69 (3H, s, $C\underline{H}_3$), 1.15 (1H, m, $C_{4'}\underline{H}$), 0.95 (2H, bq, 2 x $C_{3'(5')}H\underline{H}$), 0.83 (9H, s, $(C\underline{H}_3)C$). ^{13} **C NMR** ($CDCl_3$, 100.6 MHz): δ 190.33 ($\underline{C}$=O), 139.04 and 118.11 ($\underline{C}$=), 48.07 ($C_{4'}\underline{H}$), 34.39 ($C\underline{H}_2S$), 32.33 ($(CH_3)_3\underline{C}$), 30.51, 30.39, 28.72 and 28.32 ($\underline{C}H_2$), 27.52 ($(\underline{C}H_3)_3C$), 17.52 and 13.00 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2954, 2862, 1739 (C=O), 1647 (C=C), 1444, 1366, 1310, 1236, 1183, 1039, 970, 870.



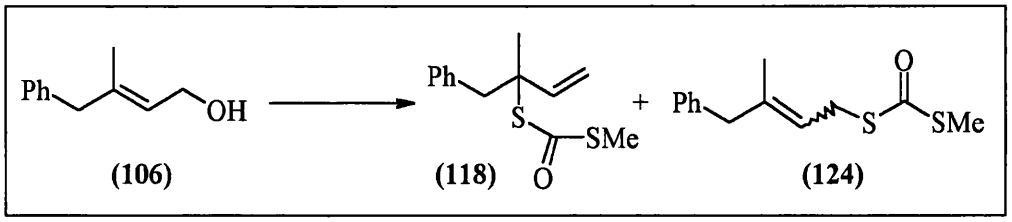
***S*-Methyl *S*-[3-(3,7-dimethyl-1,6-octadienyl)] dithiocarbonate (116)[176].** (P.E.; 53-63 %). ^{1}**H NMR** ($CDCl_3$, 400 MHz): δ 6.06 (1H, dd, J= 17.4, 11.0, $C\underline{H}=CH_2$), 5.21 (1H, d, J= 17.4, $CH\underline{H}$=), 5.17 (1H, d, J= 11.0, $CH\underline{H}$=), 5.09 (1H, m, $(Me)_2C=C\underline{H}$), 2.36 (3H, s, $SC\underline{H}_3$), 2.4-1.8 (4H, m, $C\underline{H}_2C\underline{H}_2$), 1.69 (3H, s, $C\underline{H}_3$), 1.62 (3H, s, $C\underline{H}_3$), 1.60 (3H, s, $C\underline{H}_3$). ^{13}**C NMR** ($CDCl_3$, 100.6 MHz): δ 188.51 ($\underline{C}$=O), 141.53 ($\underline{C}$=), 132.20 and 123.36 ($\underline{C}$H=), 114.18 (=$\underline{C}H_2$), 57.60 ($\underline{C}$S), 40.19 (=$C\underline{C}H_2$), 23.26 ($\underline{C}H_2$), 25.65, 23.62 and 17.60 ($\underline{C}H_3$), 12.81 ($S\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2890, 1710 (C=O), 1643 (C=C), 850. **MS** (FAB): m/z 245 ($(M+H)^+$, 15), 244 ($M^{+\cdot}$, 80), 137 ($(M-SCOSMe)^+$, 100). **HRMS** (FAB): found 245.1046, $C_{12}H_{21}S_2O$ $(M+H)^+$ requires 245.1034.



P.E., followed by P.E./Et_2O 99/1. Two fractions were isolated in order of increasing polarity.

(E) S-Methyl S-[1-(3-methyl-2,6-heptadienyl)] dithiocarbonate (123). (8-9 %, slightly impure). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.85-5.70 (1H, m, C<u>H</u>=CH_2), 5.33 (1H, bt, J= 7.8, =C<u>H</u>CH_2S), 5.01 (1H, bd, J= 17.2, C<u>H</u>H=), 4.95 (1H, bd, J= 9.2, CH<u>H</u>=), 4.03 (2H, d, J= 7.9, $C\underline{H}_2$S), 2.56 (3H, s, $SC\underline{H}_3$), 2.2-2.1 (4H, m, $C\underline{H}_2C\underline{H}_2$), 1.72 (3H, s, $C\underline{H}_3$). **IR** (neat) ν_{max}/cm^{-1}: 2918, 1698 (C=O), 1639 (C=C), 1418, 1380, 1218, 1066, 961, 911. **MS** (EI): m/z 217 ($(M+H)^+$, 8), 185 (32), 141 ($(M-COSMe)^+$, 17), 109 ($(M-SCOSMe)^+$, 77), 66 (98), 90 (42), 80 (35).

S-Methyl S-[3-(3-methyl-1,6-heptadienyl)] dithiocarbonate (117). (37-55 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.04 (1H, dd, J= 17.7, 10.5, =C<u>H</u>CS), 5.79 (1H, m, CH_2=C<u>H</u>CH_2), 5.20 (1H, d, J= 17.7, C<u>H</u>H=CHCS), 5.17 (1H, d, J= 10.5, CH<u>H</u>CHCS), 5.03 (1H, dd, J= 17.1, 1.7, C<u>H</u>H=$CHCH_2$), 4.96 (1H, dd, J= 10.12, 1.2, CH<u>H</u>=$CHCH_2$), 2.37 (3H, s, $SC\underline{H}_3$), 2.4-1.8 (4H, s, $C\underline{H}_2C\underline{H}_2$), 1.61 (3H, s, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.50 (<u>C</u>=O), 141.38 and 137.79 (<u>C</u>H=), 114.90 and 114.40 ($\underline{C}H_2$=), 57.43 (<u>C</u>S), 39.34 and 28.87 ($\underline{C}H_2$), 23.68 ($\underline{C}H_3$), 12.83 ($S\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2965, 2928, 2856, 1717 (C=O), 1642 (C=C), 1457, 1411, 1373, 994, 915, 854. **MS** (FAB): m/z 217 ($(M+H)^+$, 5), 215 ($(M-H)^+$, 65), 201 ($(M-Me)^+$, 35), 109 ($(M-SCOSMe)^+$, 74). **HRMS** (FAB): found 217.0719, $C_{10}H_{17}S_2O$ $(M+H)^+$ requires 217.0721.



P.E., followed by P.E./Et_2O 99/1. Two fractions were separated in order of increasing polarity.

(ZE)-S-Methyl S-[1-(3-methyl-4-phenyl-1-butenyl)] dithiocarbonate (124). (9-10 %; slightly impure; proportions of *Z:E* isomers varied with the experiment). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.32-7.15 (5H, m, ArH), 5.70 and 5.65 (1H in overall, 2 x t, J= 8.4, =CHCH$_2$S), 4.03 and 3.95 (2H in overall, 2 x d, J= 8.4, CH$_2$S), 3.42 and 3.32 (2H in overall, 2 x s, CH$_2$Ph), 2.77 (3H in overall, s, SCH$_3$), 1.68 and 1.60 (3H in overall, 2 x s, CH$_3$). **IR** (neat, of the mixture): ν_{max}/cm^{-1} 2926, 1712 (C=O), 1641 (C=C), 1440, 1379, 680. **MS** (EI, of the mixture): m/z 254 ($(M+2H)^+$, 41), 204 ($(M-HSMe)^{+\cdot}$, 3), 176 ($(M-HCOSMe)^{+\cdot}$, 26), 145 ($(M-SCOSMe)^+$, 129 (38), 108 (36), 91 (100).

S-Methyl S-[3-(3-methyl-4-phenyl-1-butenyl)] dithiocarbonate (118). (47 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.30-7.15 (5H, m, ArH), 6.16 (1H, dd, J= 17.2, 10.8, CH=), 5.15 (1H, d, J= 10.8, CHH=), 5.12 (1H, d, J= 17.2, CHH=), 3.24 (1H, d, J= 13.6, CHHPh), 3.15 (1H, d, J= 13.6, CHHPh), 2.39 (3H, s, SCH$_3$), 1.53 (3H, s, CH$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.30 (C=O), 130.92, 128.84, 128.32, 127.81, 126.78, 126.20, 119.95 and 114.59 (C(H)$_{Ar}$+ CH(H)=), 57.77 (CS), 46.16 (CH$_2$Ph), 28.75 and 23.34 (CH$_3$ and SCH$_3$). **IR** (neat): ν_{max}/cm^{-1} 3028, 2928, 1716 (C=O), 1643 (C=C), 1453, 1076, 853. **MS** (FAB): m/z 253 ($(M+H)^+$, 13), 225 (16), 154 (37), 145 ($(M-SCOSMe)^+$, 100). **HRMS** (FAB): found 253.0703, $C_{13}H_{16}S_2O$ $(M+H)^+$ requires 253.0721;.

GC/MS (EI) of the mixture:

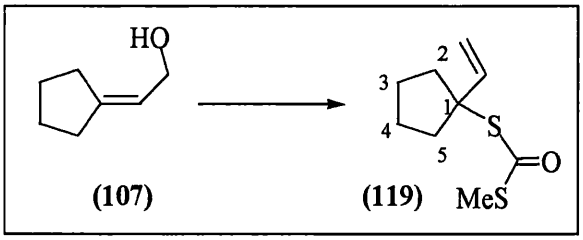
3.18 min (5 %): MS m/z 73 (10), 61 ($(COS+H)^+$, 12), 43 (100).

17.75 and 17.9 min (14 %): MS m/z 144 ($(M-HSCOSMe)^{+\cdot}$, 54), 129 (85), 117 (21), 105 (13), 91 (100), 77 (15), 65 (43), 51 (23), 41 (37).

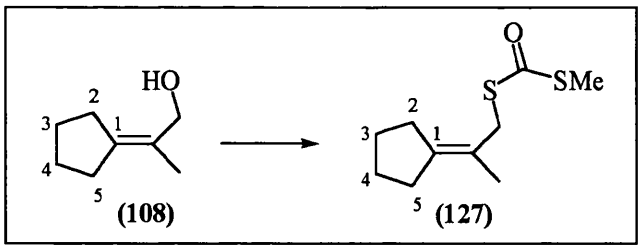
22.94 min (46 %): MS m/z 91 ($PhCH_2^+$, 100)

23.6 min (19.7 %): MS m/z 144 (48), 129 (100), 115 (23), 91 (87), 77 (23).

23.32 and 23.67 (same peaks as above on the MS).

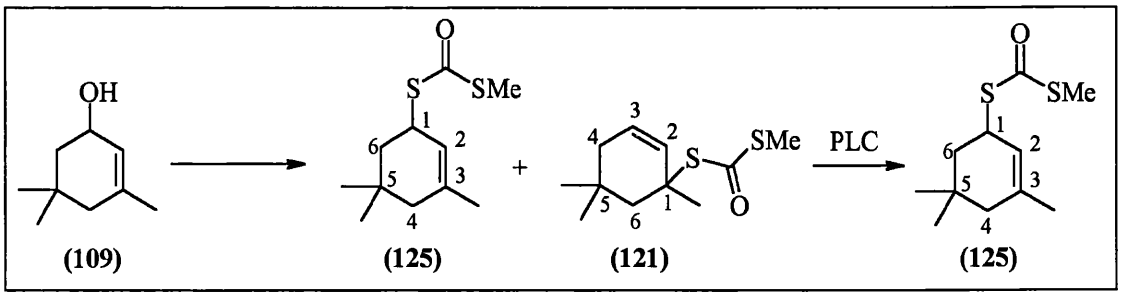


***S*-Methyl *S*-(1-vinylcyclopentyl) dithiocarbonate (119).** (P.E.; 26 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.15 (1H, dd, J= 17.2, 10.4, C<u>H</u>=), 5.20 (1H, d, J= 17.2, CH<u>H</u>=), 5.08 (1H, d, J= 10.4, CH<u>H</u>=), 2.36 (3H, s, SC<u>H</u>$_3$), 2.04-2.00 (4H, m, 2 x $C_{2(5)}\underline{H}_2$), 1.79-1.75 (4H, m, 2 x $C_{3(4)}\underline{H}_2$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.90 (<u>C</u>=O), 141.3 (<u>C</u>H=), 113.2 (=<u>C</u>H$_2$), 62.79 (<u>C</u>S), 37.7 (2 x $\underline{C}_{2(5)}H_2$), 23.3 (2 x $\underline{C}_{3(4)}H_2$), 12.77 (S<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 2950, 2865, 1720 (C=O), 1649 (C=C), 1280, 856, 750. **MS** (FAB): m/z 203 ($(M+H)^+$, 33), 154 ($(M-HSMe)^{+\cdot}$, 100), 127 ($(M-COSMe)^+$, 45). **MS** (EI): 95 ($(M-SCOSMe)^+$, 100). **HRMS** (FAB): found 203.0550, required for $C_9H_{15}S_2O$ $(M+H)^+$ 203.0564.



***S*-Methyl *S*-(2-cyclopentylidenepropyl) dithiocarbonate (127).** (27 %; obtained when the reaction mixture from alcohol **106** was left standing at r. t. for 2 weeks prior to chromatography). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.69 (2H, bs, C<u>H</u>$_2$S), 2.39 (3H, s, SC<u>H</u>$_3$), 2.24 (2H, m, $C_{2(5)}\underline{H}_2$), 2.15 (2H, m, $C_{2(5)}\underline{H}_2$), 1.62 (7H, m, $C_3\underline{H}_2C_4\underline{H}_2$ + =CC<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 190.14 (<u>C</u>=O), 142.81 and 119.17 (<u>C</u>=), 35.82 (<u>C</u>H$_2$S), 31.18, 31.06, 26.81 and 26.55 (ring <u>C</u>H$_2$), 18.38 (<u>C</u>H$_3$), 13.00 (S<u>C</u>H$_3$). **IR** (neat) ν_{max}/cm^{-1} 2952, 2866, 1738 (C=O), 1646 (C=C), 1230, 870. **MS** (EI): m/z 201 ($(M-Me)^+$, 8), 183

((M–SH)$^+$, 7), 141 ((M–COSMe)$^+$, 5), 109 ((M–SCOSMe)$^+$, 100), 93 (9), 77 (15), 67 (57).



P.E., 75 % of the mixture of the two diastereomers in varied proportions up to 1:1. The products were not separated. **121** was later obtained pure by another method, allowing full characterisation. When separation of a fraction of the mixture was attempted by preparative TLC, **125** was the only product obtained.

***S*-Methyl *S*-(1,5,5-trimethyl-2-cyclohexen-1-yl) dithiocarbonate (121).** **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.78 (1H, dt, J= 10.0, 2.0, =C_2H), 5.72 (1H, dt, J= 10.0, 3.6, =C_3H), 2.12 (1H, d, J= 14.5, C_6HH), 1.84 (1H, bd, J= 16.4, C_4HH), 1.73 (1H, bd, J= 16.4, C_4HH), 1.58 (1H, d, J= 14.5, C_6HH), 1.45 (3H, s, CH_3), 0.98 (3H, s, CH_3), 0.90 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 189.03 (C=O), 129.59 and 127.52 ($C_{2(3)}$H=), 53.85 (C_1S), 48.21 (C_6H_2), 38.28 (C_4H_2), 30.37 (C_5), 30.17 and 29.57 (3 x CH_3), 12.76 (SCH_3). **IR** (neat): ν_{max}/cm^{-1} 2953, 2888, 1720 (C=O), 1641 (C=C), 1458, 1367, 1146, 1052, 857. **MS** (FAB): m/z 155 ((M–COSMe)$^+$, 9), 123 ((M–SCOSMe)$^+$, 11). **HRMS** (FAB): found 231.0894, $C_{11}H_{19}S_2O$ $(M+H)^+$ requires 231.0877.

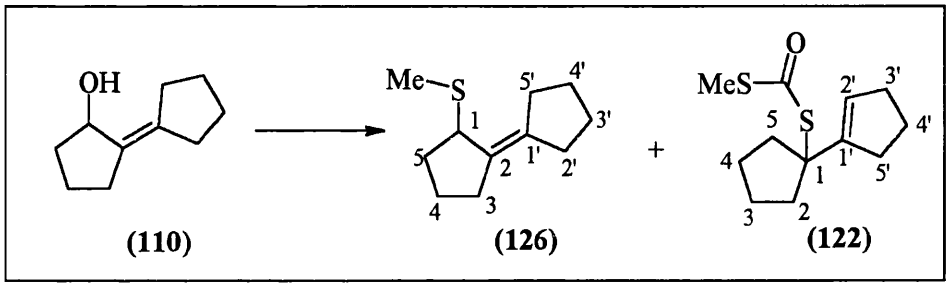
***S*-Methyl *S*-[1-(3,5,5-trimethyl-2-cyclohexenyl)] dithiocarbonate (125).** **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.36 (1H, m, C_2H=), 4.36 (1H, m, C_1HS), 2.40 (3H, s, CH_3S), 1.84 (1H, d, J= 13.9, C_4HH), 1.82 (1H, dd, J= 11.4, 6.1, C_6HH), 1.64 (1H, d, J= 13.9, C_4HH), 1.65 (3H, s, CH_3), 1.40 (1H, dd, J= 11.4, 9.8, C_6HH), 0.95 (3H, s, CH_3), 0.92 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 189.88 (C=O), 137.90 (=C_3), 119.66 (C_2H=), 43.49 and 42.21 ($C_{4(6)}H_2$), 41.46 (C_1H), 31.41 (CH_3), 30.75 (C_5), 25.89 and 23.85 (CH_3), 12.89 (SCH_3). **IR** (neat): ν_{max}/cm^{-1} 2951, 2907, 1734 (C=O), 1642 (C=C), 1456, 1374, 1309.

GC/MS (EI) of the mixture:

12.44 min (8.7 %): MS m/z 170 ($(M–COS)^{+\cdot}$, 3), 123 ($(M–SCOSMe)^{+}$, 100), 81 (52)

18.56 min (15.8 %): MS m/z 123 (100), 81 (32)

19.2 min (65.8 %): MS m/z 230 ($M^{+\cdot}$, 2), 155 ($(M–Me)^{+}$, 1), 123 ($(M–SCOSMe)^{+}$, 100), 107 (32), 81 (20).



P.E. was used as the eluant. Two fractions were isolated in order of increasing polarity.

***S*-Methyl *S*-[1-(1-cyclopentylidenecyclopentyl)] dithiocarbonate (122).** (20 %, slightly impure) **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.52 (1H, bd, C_2-H=), 2.42 (3H,,s, CH_3S), 2.3-1.2 (14H, several multiplets, rings CH_2s). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 184.90 (C=O), 128.64 and 126.19 (=$C_{1'(2')}$(H)), 49.61 (C_1S), 36.55, 31.96, 31.62, 31.26, 30.39, 27.02, 24.03, 22.68 and 26.53 (ring CH_2), 14.14 (SCH_3). **IR** (neat): ν_{max}/cm^{-1} 2979, 2870, 1719 (C=O), 1638 (C=C), 1445, 1310, 1239, 1139, 1041, 967, 857. **MS** (FAB): m/z 241 ($(M–H)^{+}$, 5), 181 (7), 165 (7), 154 (14), 135 (100).

2-Cyclopentylidene-1-cyclopenty methyl sulfide (126). (18 %, only partially separated from **122**). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.7 (1H, bd, C_1-H), 2.5 (1H, m), 2.3-2.1 (5H, m), 2.05 (3H, s, SCH_3), 1.9 (3H, m), 1.8-1.6 (5H, m). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 138.19 and 131.63 (=$C_{1'(2)}$), 49.51 (C_1HS), 34.09, 33,49, 31.58, 31.25, 30.06, 26.97, 26.45, 23.40 and 22.30 (ring CH_2), 14.74 (SCH_3). **IR** (neat): ν_{max}/cm^{-1}: 2925, 2868, 1459, 1381, 1262, 1138. **MS** (FAB): m/z 181 ($(M–H)^{+}$, 10), 135 ($(M–SCOSMe)^{+}$, 100).

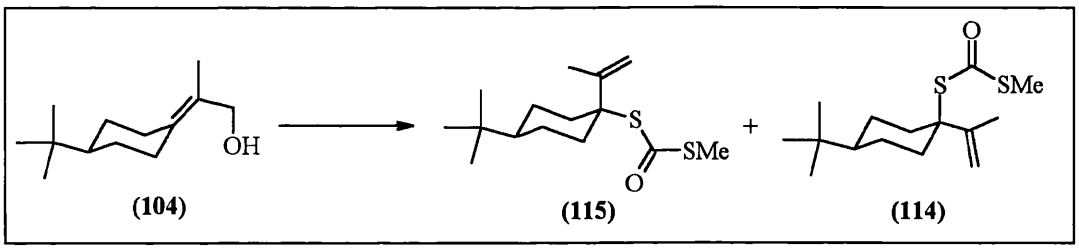
GC/MS (EI) of the mixture:

11.6 and 11.7 min (5 % each): MS m/z 134 $(M–HSCOSMe)^{+\cdot}$, 95), 92 (62), 91 (100).

16.8 and 16.9 min (38 % and 43 %): MS m/z 182 ($(M^{+\cdot}$, 3), 135 $(M–SCOSMe)^{+}$, 45), 134 (100), 92 (32), 91 (33).

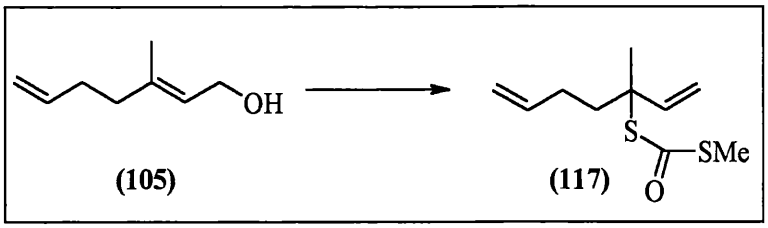
2. 2. 2. Using Bu^tOK as the base, general procedure.

In a typical run, the alcohol (20.6 mmol), dissolved in 20 mL of dry THF was added dropwise, under N_2 and at 0 °C, to a solution of Bu^tOK (30.9 mmol) in THF (100 mL). The mixture was allowed to warm to r. t. and stirred for 1 h. CS_2 (61.8 mmol) was then added and the reaction heated at reflux for one additional hour (becoming brown or orange). Next, MeI (123 mmol) was added, and the mixture kept at reflux for 4 h. The reaction was then quenched by addition of aq. saturated NH_4Cl solution and extracted with Et_2O. The organic layer was washed with aq. NH_4Cl (once), followed by aq. $NaHCO_3$ (once) and brine (twice), dried over $MgSO_4$ and filtered off. After evaporating the solvent, the crude residues were purified by chromatography on silica gel. Repeated chromatography was not needed by this method since the crude mixtures were less complex.



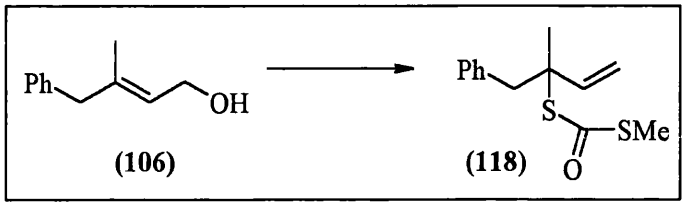
(104) (115) (114)

Cis/trans S-methyl S-[4-tert-butyl-1-(2-propenyl)cyclohexyl] dithiocarbonates (114/115). (P.E., followed by P.E./Et_2O 98/2; 65 % of the pure mixture of the two diastereomers, not separated). The spectra of the products were identical with those already recorded by the mixture **114/115** obtained by method 2.2.1.



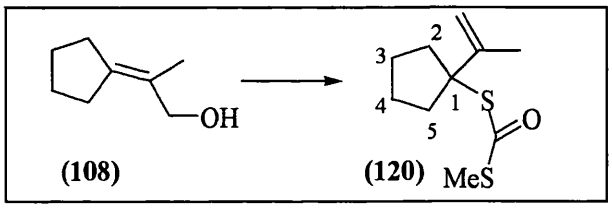
(105) (117)

***S*-methyl *S*-[3-(3-methyl-1,6-heptadienyl)] dithiocarbonate (117).** (P.E., followed by P.E./Et_2O 99/1; 66 %). The product was obtained as a pure single isomer and displayed the same spectral data as **117** obtained by method 2.2.1.



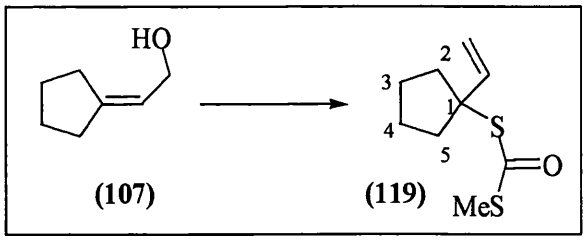
(106) (118)

***S*-Methyl *S*-[3-(3-methyl-4-phenyl-1-butenyl)] dithiocarbonate (118).** (P.E. followed by P.E./Et_2O 99/1; 57 %). The product was obtained as a single isomer and displayed the same spectra as **118** obtained by method 2.2.1.



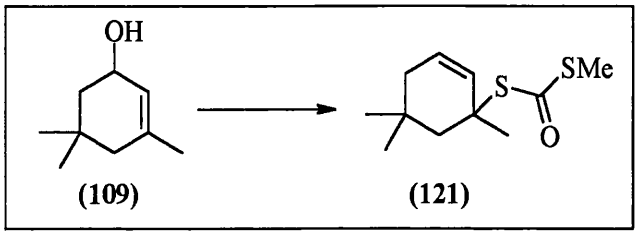
(108) (120)

***S*-Methyl *S*-(2-propenylcyclopentyl) dithiocarbonate (120).** (P.E.; 52 %, pure single isomer). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.05 (1H, bs, =C<u>H</u>H), 4.91 (1H, bs, =CH<u>H</u>), 2.31 (SC<u>H</u>$_3$), 2.25 (2H, m, 2 x $C_{2(5)}\underline{H}_2$), 1.95 (2H, m, 2 x $C_{2(5)}\underline{H}_2$), 1.9-1.7 (4H, m, $C_3\underline{H}_2C_4\underline{H}_2$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 188.69 (<u>C</u>=O), 145.76 (=<u>C</u>), 112.57 (=<u>C</u>H$_2$), 68.09 (<u>C</u>S), 37.13 (2 x $\underline{C}_{2(5)}H_2$), 23.06 (2 x $\underline{C}_{3(4)}H_2$), 12.68 (<u>C</u>H$_3$). **IR** (CCl_4): ν_{max}/cm^{-1} 2958, 2871, 1717 (C=O), 1654, (C=C), 1448, 1374, 1374, 1309, 1210, 1178, 1065, 963, 897, 852. **MS** (FAB): m/z 217 ($(M+H)^+$, 30), 201 ($(M-Me)^+$, 8), 168 ($(M-HSMe)^{+\cdot}$, 1), m/z 141 ($(M-COSMe)^+$, 109 ($(M-SCOSMe)^+$, 100). **HRMS** (FAB): found 217.0710, $C_{10}H_{17}S_2O$ $(M+H)^+$, requires 217.0710.



(107) (119)

***S*-Methyl *S*-(1-vinylcyclopentyl) dithiocarbonate (119).** (P.E.; 76 %). The product displayed the same spectral data as those obtained for **119** obtained by method 2.2.1.



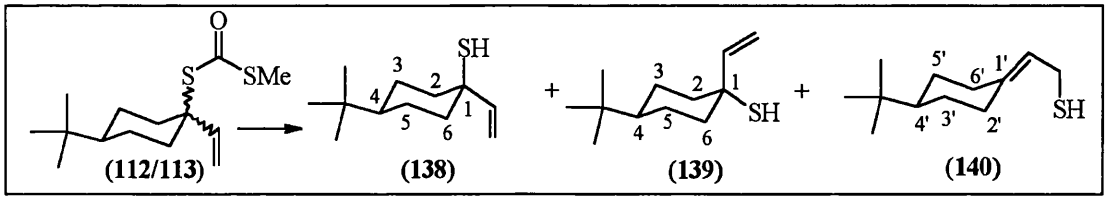
(109) (121)

***S*-Methyl *S*-(1,5,5-trimethyl-2-cyclohexenyl) dithiocarbonate (121).**(P. E.; 70 %). The product was obtained as a single isomer and gave the same spectra as **121** obtained by method 2.2.1.

2. 3. Preparation of tertiary allylic thiols.

2. 3. 1. By basic methanolysis, general procedure.

Typically, NaOH (681 mg, 17 mmol) was added in one portion to a solution of the corresponding dithiocarbonate (8.5 mmol) in 20 mL of MeOH and the mixture stirred at r. t. for 2 h. This was followed by acidification with aq. HCl 2N to pH 7 and extraction with Et_2O. The organic layer was further washed with brine (once) and the aqueous layer extracted with two further portions of Et_2O. The combined organic layers were dried over $MgSO_4$ and the solvent evaporated under *vacuo*. The crude reaction mixture was purified by chromatography on silica gel (in several cases more than one separation by chromatography were needed), using P.E. as the solvent in all cases. The thiols were isolated as colourless oils, except when indicated otherwise.



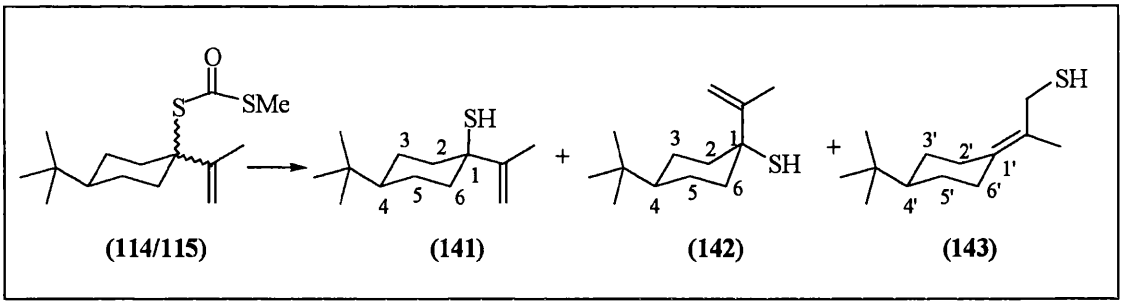
(112/113) (138) (139) (140)

Two or three columns were required. Two fractions were separated in order of increasing polarity. The second one was formed by two products (**139** and **140**)

***Cis* 4-*tert*-butyl-1-vinyl-1-cyclohexanethiol (138).** (32-40 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.08 (1H, dd, J= 17.4, 10.6, C<u>H</u>=), 5.06 (1H, d, J= 17.4, C<u>H</u>H=), 4.92 (1H, d, 10.6, CH<u>H</u>=), 1.86 (2H, bd, J= 10.7, 2 x $C_{3(5)}$<u>H</u>H), 1.70 (2H, bd, J= 10.7, 2 x $C_{3(5)}$H<u>H</u>), 1.60-1.45 (4H, m, 2 x $C_{2(6)}$H<u>H</u> + 2 x $C_{2(6)}$H<u>H</u>), 0.81 (1H, m, C_4<u>H</u>), 0.89 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 148.15 (<u>C</u>H=), 109.32 (<u>C</u>H$_2$=), 49.57 (<u>C</u>SH), 47.90 (<u>C</u>$_4$H), 38.71 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.40 ((CH$_3$)$_3$<u>C</u>), 27.40 ((<u>C</u>H$_3$)$_3$C), 23.02 (2 x <u>C</u>$_{3(5)}$H$_2$). **MS** (FAB): m/z 199 ((M+H)$^+$, 22), 197 ((M–H)$^+$, 35), 165 ((M–SH)$^+$, 100). **HRMS** (FAB): found 199.1510, $C_{12}H_{23}S$ (M+H)$^+$ requires 199.1520.

***Trans* 4-*tert*-butyl-1-vinyl-1-cyclohexanethiol (139).** (24-25 %, impurified by inseparable compound **140** in a proportion 1:1). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, dd, J= 16.8, 8.3, C<u>H</u>=), 5.18 (1H, d, J= 16.8, C<u>H</u>H=), 5.07 (1H, d, 8.3, CH<u>H</u>=), 2.15 (2H, bd, J= 14.0, 2 x $C_{2(6)}$<u>H</u>H), 1.72 (2H, bt, 2 x $C_{2(6)}$H<u>H</u>), 1.52 (2H, bd, J= 14.1, $C_{3(5)}$<u>H</u>H) 1.2-0.9 (3H, m, 2 x $C_{3(5)}$H<u>H</u> + C_4<u>H</u>), 0.78 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 141.83 (<u>C</u>H=), 119.71 (<u>C</u>H$_2$=), 47.33 (<u>C</u>SH), 40.77 (<u>C</u>$_4$H), 36.64 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.25 ((CH$_3$)$_3$<u>C</u>), 27.44 ((<u>C</u>H$_3$)$_3$C), 24.61 (2 x <u>C</u>$_{3(5)}$H$_2$).

***2-(4-Tert*-butylcyclohexylidene)ethen-1-thiol (140).** (mixture with **139**, the product was later obtained pure by another method, allowing full characterisation). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.25 (1H, t, J= 7.8, C<u>H</u>=), 3.13 (2H, t, J= 7.8, C<u>H</u>$_2$SH), 2.63 (1H, m, $C_{2'(6')}$<u>H</u>H), 2.30 (1H, m, $C_{2'(6')}$<u>H</u>H), 1.90 (2H, m, 2 x $C_{2'(6')}$H<u>H</u>), 1.65 (1H, m, $C_{4'}$<u>H</u>), 1.3-0.8 (4H, m, 2 x $C_{3'(5')}$<u>H</u>$_2$), 0.82 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 143.18 (<u>C</u>=), 116.54 (<u>C</u>H=), 53.41 (<u>C</u>H$_2$SH), 48.3 (<u>C</u>$_4$H) , 36.05 (<u>C</u>$_{2'(6')}$H$_2$), 32.40 ((CH$_3$)$_3$<u>C</u>), 30.45, 29.67 and 28.6 (<u>C</u>H$_2$), 27.56 ((<u>C</u>H$_3$)$_3$C). **MS** (FAB): m/z 199 (M+H, 37), 197 ((M–H)$^+$, 98), 165 ((M–SH)$^+$, 80), 141 (17), 109 (100), 123 (37). **HRMS** (FAB): found 199.1510, $C_{12}H_{23}S$ (M+H)$^+$ requires 199.1520.



(114/115) (141) (142) (143)

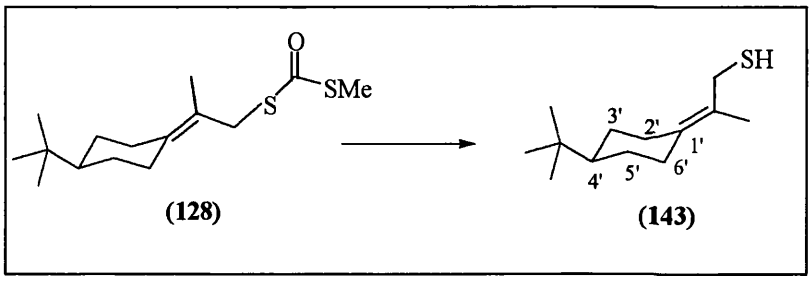
Two fractions were isoltated in order of increasing polarity. The second one was formed by two products (**142** and **143**).

***Cis* 4-*tert*-butyl-2-propenyl-1-cyclohexanethiol (141).** (white solid; 41 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.87 (1H, bs, C<u>H</u>H=), 4.74 (1H, bs, CH<u>H</u>=), 2.03 (2H, bd, J= 10.7, 2 x $C_{2(6)}$<u>H</u>H), 1.71 (2H, bt, 2 x $C_{2(6)}$H<u>H</u>), 1.94 (3H, s, (C<u>H</u>$_3$)C=), 1.65-1.54 (4H, m, 2 x $C_{3(5)}$<u>H</u>$_2$), 0.90 (1H, m, C_4<u>H</u>), 0.89 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 152.96 (<u>C</u>=), 108.45 (<u>C</u>H$_2$=), 51.77 (<u>C</u>SH), 47.85 (<u>C</u>$_4$H), 38.19 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.41 ((CH$_3$)$_3$<u>C</u>), 27.55 ((<u>C</u>H$_3$)$_3$C), 23.38 (2 x <u>C</u>$_{3(5)}$H$_2$), 19.56 (<u>C</u>H$_3$). **MS** (FAB): m/z 211 ($(M-H)^+$, 30), 179 ($(M-SH)^+$, 100), 149 (7), 123 (77), 109 (47), 95 (19), 69 (22), 57 (93), 41 (27). **HRMS**: found 211.1503, $C_{13}H_{23}S$ $(M-H)^+$ requires 211.1520.

***Trans* 4-*tert*-butyl-2-propenyl-1-cyclohexanethiol (142).** (27 %, contains traces to 2:1 of **143**). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.97 (1H, s, C<u>H</u>H=), 4.93 (1H, s, CH<u>H</u>=) 2.40 (2H, bd, J= 12.6, 2 x $C_{2(6)}$<u>H</u>H), 1.71 (2H, bt, J= 12.0, 2 x $C_{2(6)}$H<u>H</u>), 1.87 (3H, s, (C<u>H</u>$_3$)C=), 1.87-1.70 (2H, m, 2 x $C_{3(5)}$<u>H</u>H), (3H, m, 2 x $C_{3(5)}$H<u>H</u>+ C_4<u>H</u>), 0.81 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 142.30 (<u>C</u>=), 112.03 (<u>C</u>H$_2$=), 52.50 (<u>C</u>SH), 47.41 (<u>C</u>$_4$H), 39.78 (2 x <u>C</u>$_{2(6)}$H$_2$), 32.42 ((CH$_3$)$_3$<u>C</u>), 27.53 ((<u>C</u>H$_3$)$_3$C), 24.62 (2 x <u>C</u>$_{3(5)}$H$_2$), 19.66 (<u>C</u>H$_3$). **MS** (FAB, of the mixture): m/z 211 ($(M-H)^+$, 26), 179 ($(M-SH)^+$, 100), 123 (35), 109 (37), 95 (12), 69 (22), 57 (84), 41 (14). **MS** (EI, of the mixture): m/z 211 ($(M-H)^+$, 10), 178 ($(M-SH_2)^{+\cdot}$, 60), 123 (85), 79 (52). **HRMS** (FAB): found 211.1503, $C_{13}H_{23}S$ $(M-H)^+$ requires 211.1520.

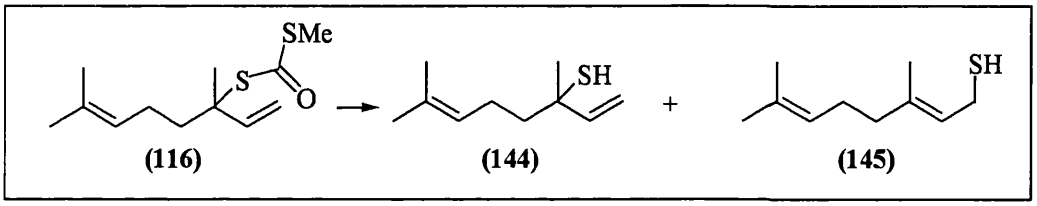
2-(4-Tert-butylcyclohexylidene)propane-1-thiol (143). (impure, the product was later obtained by another method, allowing full assignment of spectra) **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.19 (2H, d, J= 8.0, C<u>H</u>$_2$SH), 2.67 (2H, bd, J= 15.0, 2 x $C_{2'(6')}$<u>H</u>H), 1.86 (2H, m, 2 x $C_{2'(6')}$H<u>H</u>), 1.75 (2H, bd, 2 x $C_{3'(5')}$<u>H</u>H), 1.38 (1H, t, J= 8.0, S<u>H</u>), 1.18 (1H,

m, $C_{4'}\underline{H}$), 1.0 (2H, bq, 2 x $C_{3'(5')}H\underline{H}$), 0.86 (s, 9H, $(C\underline{H}_3)_3C$). **MS** (EI): m/z 212 ($M^{+\cdot}$, 5), 179 ($(M-SH)^+$, 45), 123 (85), 109 (75), 95 (83), 69 (50).



(128) **(143)**

2-(4-Tert-butylcyclohexylidene)propane-1-thiol (143). (qtve.). The product displayed the same ^{1}H NMR spectrum as **143** obtained as above.

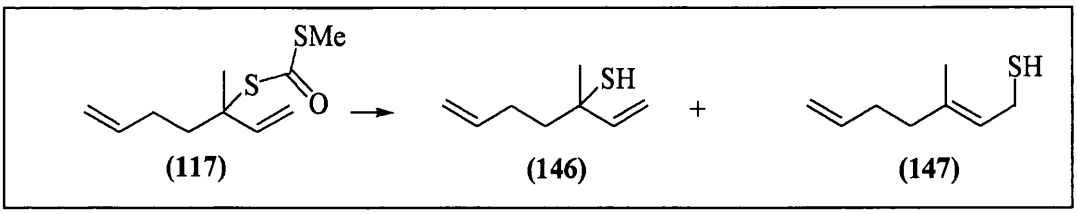


(116) **(144)** **(145)**

Two products were separated in order of increasing polarity.

3,7,-Dimethyl-1,6-octadien-3-thiol (144)[177,178]. (56-63 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.97 (1H, ddd, J= 17.2, 10.5, 0.8, =C$\underline{H}$CS), 5.10 (1H, d, J= 17.2, C$\underline{H}$H=), 5.10 (1H, m, $(Me)_2C$=C$\underline{H}$), 4.98 (1H, dd, J= 10.5, 0.8, CH$\underline{H}$=), 2.2-2.0 and 1.8-1.7 (4H, m, $C\underline{H}_2C\underline{H}_2$), 1.70 (3H, s, $C\underline{H}_3$), 1.62 (3H, s, $C\underline{H}_3$), 1.50 (3H, s, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 146.23 ($\underline{C}$=), 132.10 and 124.20 ($\underline{C}$H=), 111.15 (CH_2=), 48.23 ($\underline{C}$SH), 44.34 ($\underline{C}H_2CS$), 28.52 ($\underline{C}H_3CS$), 26.15, 24.12 and 17.5 (2 x $\underline{C}H_3$ + $\underline{C}H_2$). **IR** (neat): ν_{max}/cm^{-1} 2967, 2922, 2907, 1633.8 (C=C), 1449, 1352, 915. **MS** (FAB): m/z 171 ($(M+H)^+$, 12), 169 ($(M-H)^+$, 21), 137 ($(M-SH)^+$, 47). **HRMS** (FAB): found 169.1045, $C_{10}H_{17}S$ $(M-H)^+$ requires 169.1051.

(E)-3,7-Dimethyl-2,6-octadien-1-thiol (145)[179].(5 %, impure). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.36 (1H, t, J= 7.32, =C$\underline{H}CH_2S$), 5.08 (1H, t, J= 7.32, $(CH_3)_2C$=C$\underline{H}$), 3.17 (2H, t, J= 7.32, C$\underline{H}_2$SH), 2.1-2.0 (4H, m, $C\underline{H}_2C\underline{H}_2$), 1.69 (3H, s, $C\underline{H}_3$), 1.67 (3H, s, $C\underline{H}_3$), 1.61 (3H, s, $C\underline{H}_3$), 0.95 (1H, t, J= 7.32, S$\underline{H}$).

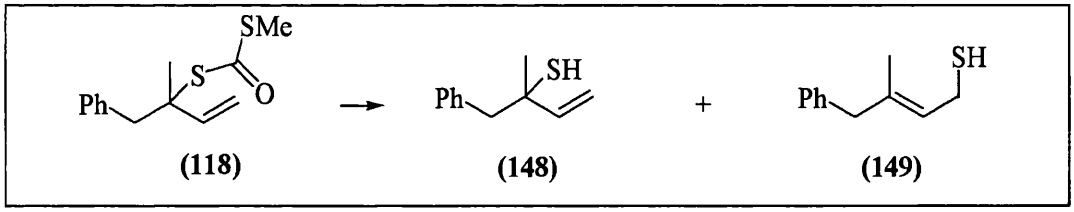


(117) (146) (147)

Two products were separated in order of increasing polarity.

3-Methyl-1,6-heptadien-3-thiol (146). (10 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.96 (1H, dd, J= 17.2, 10.6, =C<u>H</u>CS), 5.79 (1H, m, CH_2=C<u>H</u>), 5.10 (1H, dd, J= 17.2, 0.76, C<u>H</u>H=), 5.03 (1H, dd, J= 17.1, 1.68, CH<u>H</u>=), 4.98 (1H, dd, J= 10.6, 0.7, C<u>H</u>H=), 4.95 (1H, dt, J= 10.6, 1.3, CH<u>H</u>=), 2.15-2.05 (2H, m, C<u>H</u>$_2$), 1.8-1.7 (2H, m, C<u>H</u>$_2$), 1.97 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 145.74 and 138.14 (<u>C</u>H=), 114.48 and 111.35 (<u>C</u>H_2=), 48.14 (<u>C</u>S), 43.32, 29.53 and 28.52 (2 x <u>C</u>H_2 + <u>C</u>H_3). **MS** (FAB): m/z 143 ($(M+H)^+$, 21), 142 ($M^{+\cdot}$, 6), 141 ($(M-H)^+$, 25), 109 ($(M-SH)^+$, 67). **HRMS** (FAB): found 143.0815, $C_8H_{15}S$ $(M+H)^+$ requires 143.0894.

<u>(E)</u>-3-Methyl-2,6-heptadien-1-thiol (147). (30 %, impure). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.79 (1H, m, C<u>H</u>=CH_2), 5.35 (1H, dt, J= 7.3, 1.2, =C<u>H</u>CH_2S), 5.01 (1H, dq, J= 15.4, 1.72, C<u>H</u>H=), 4.95 (1H, dd, J= 10.38, 0.68, CH<u>H</u>=), 3.17 (2H, t, J= 7.32, C<u>H</u>$_2$S), 2.20-2.10 (4H, m, C<u>H</u>$_2$C<u>H</u>$_2$), 1.66 (3H, s, C<u>H</u>$_3$), 1.40 (1H, t, J= 7.32, S<u>H</u>). **MS** (FAB): m/z 143 ($(M+H)^+$, 19), 142 ($M^{+\cdot}$, 5), 141 ($(M-H)^+$, 35), 109 ($(M-SH)^+$, 100).



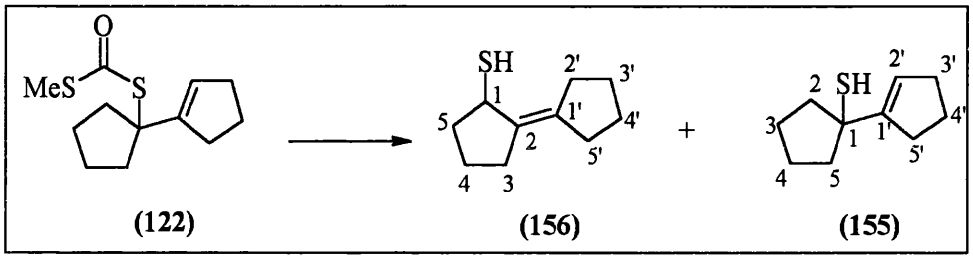
(118) (148) (149)

Two products were separated in order of increasing polarity.

3-Methyl-4-phenyl-1-buten-3-thiol (148). (15 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.4-7.2 (5H, m, Ar<u>H</u>), 6.07 (1H, dd, J= 17.2, 10.6, C<u>H</u>=), 5.07 (1H, d, J= 17.2, C<u>H</u>H=), 5.01 (1H, d, J= 10.6, CH<u>H</u>=), 2.98 (2H, s, C<u>H</u>$_2$Ph), 1.48 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 145.35, 136.93, 130.76, 127.69 and 126.63 (<u>C</u>$_{Ar}$(H) + <u>C</u>=) and 111.60 (<u>C</u>H=), 50.47 (<u>C</u>H_2Ph), 48.56 (<u>C</u>SH), 28.18 (<u>C</u>H_3). **MS** (FAB): m/z 179

($(M+H)^+$, 5), 178 ($M^{+\cdot}$, 5), 177 ($(M-H)^+$, 13), 145 ($(M-SH)^+$, 100). **HRMS**: found 177.0725, $C_{11}H_{13}S$ $(M-H)^+$ requires 177.0738.

(ZE)-3-Methyl-4-phenyl-2-buten-1-thiol (149). (20 %, impure; proportions of *Z:E* varied for different experiments). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.3-7.1 (5H, m, ArH), 5.44 and 5.42 (1H in overall, 2 x t, J= 8.2, CH=), 3.28 (2H in overall, s, CH_2Ph), 3.17 (2H in overall, t, J= 8.2, CH_2SH), 1.68 (3H, s, CH_3), 0.85 (1H, t, J= 8.0, SH). **MS** (FAB): m/z 354 ($(2M-2H)^+$, 5), 178 ($(M+H)^+$, 7), 145 ($(M-SH)^+$, 100), 91 (25).



(122) **(156)** **(155)**

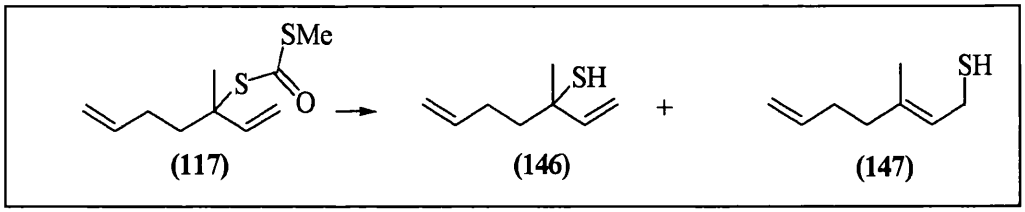
Two products were separated in order of increasing polarity.

Cyclic (155). (32 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.49 (1H, quintuplet, J= 1.8, $C_{2'}$H=), 2.50-2.44 (2H, m), 2.36-2.30 (2H, m), 2.00-1.84 (8H, m), 1.72-1.66 (2H, m). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 150.12 and 122.67 (=$C_{1'(2')}$(H)), 54.24 (C_1SH), 41.04, 32.48, 32.35, 23.62, 23.48, 23.13.

Cyclic (156). (28 %, impure). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.9 (1H, m, C_1HSH).

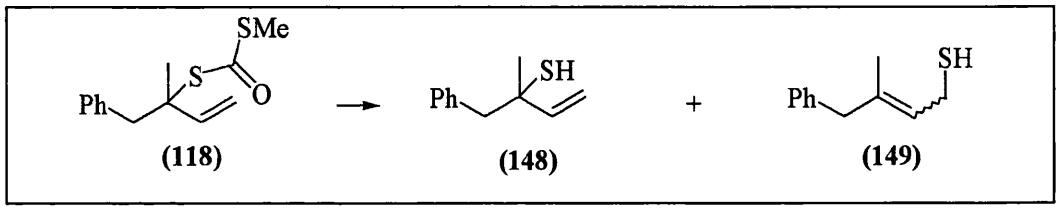
2. 3. 2. By reduction with $LiAlH_4$, general procedure.

The corresponding dithiocarbonate (1.74 mmol), in 5 mL of anhydrous Et_2O was added dropwise, at 0° C and under N_2, to a suspension of $LiAlH_4$ (188 mg, 6.08 mmol) in 15 mL of Et_2O. The mixture was allowed to warm to r. t. and stirred for a period between 3 and 4 h. Quenching was accomplished by careful addition of H_2O at 0° C. The resulting solution was filtered through Celite® and dried over $MgSO_4$. The crude product, obtained after elimination of the solvent, was purified as in method 2.3.1. Thiols were isolated as colourless oils.



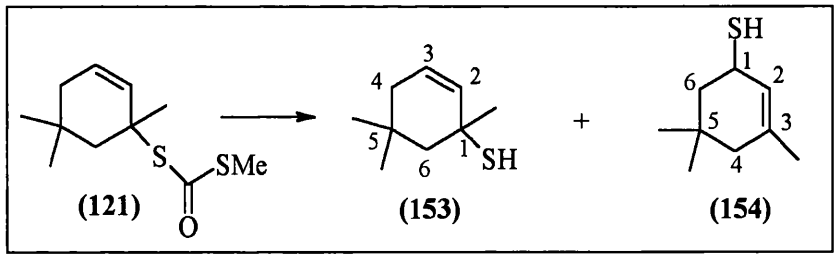
3-Methyl-1,6-heptadien-3-thiol (146). (10-16 %). The product recorded the same spectra as that obtained by method 2.3.1.

(E)-3-Methyl-2,6-heptadien-1-thiol (147). (20-30 %, impure). The product recorded the same spectra as that obtained by method 2.3.1.



3-Methyl-4-phenyl-1-buten-3-thiol (148). (22 %). The product recorded the same spectra as that obtained by method 2.3.1.

(ZE)-3-Methyl-4-phenyl-1-buten-1-thiol (149). (10 %, impure). The spectra of the product were the same as those obtained by method 2.3.1.



Overall 20 % yield for two steps (from the alcohol) of an the inseparable mixture of **153** and **154** in a proportion 1:1 was obtained. Product **153** was later obtained pure by another method, allowing full characterisation.

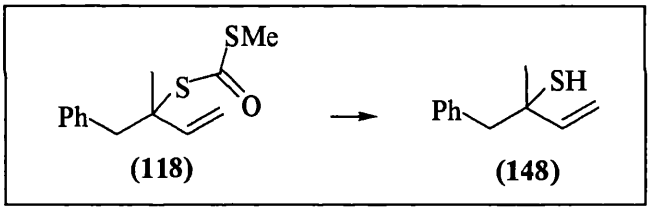
1,5,5,-Trimethyl-2-cyclohexen-1-thiol (153). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.10 (1H, bd, J= 10.3, =C_2H), 5.84 (1H, dt, J= 10.8, 4.4, =C_3H), 1.91 (1H, d, J= 14.1, C_6HH), 1.79 (2H, m, C_4H_2), 1.72 (1H, d, J= 14.1, C_6HH), 1.48 (3H, s, CH_3), 1.08 (3H, s, CH_3), 0.97 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 134. 76 and 123.56 (C=), 52.07 (CSH), 43.60, 38.08, 35.02, 30.46, 30.33, 29.00. **MS** (FAB): m/z 155 ($(M-H)^+$,

6), 123 ((M–SH)$^+$, 100). **HRMS** (FAB): found 155.0885, $C_9H_{15}S$ (M–H)$^+$ requires 155.0894.

3,5,5,-Trimethyl-2-cyclohexen-1-thiol (154). **^{1}H NMR** ($CDCl_3$, 400 MHz, in the mixture with **153**): δ 5.40 (1H, bs, =C_2H), 3.49 (1H, m, C_1HSH). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 134.29 and 124.26 (C=), 47.59 (CHSH), 43.41, 34.59, 31.34, 31.25, 25.06, 23.69.

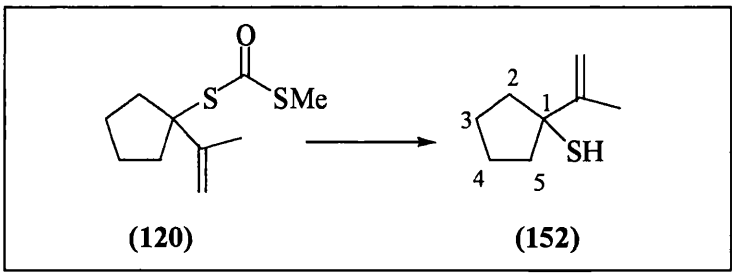
2. 3. 3. By aminolysis with 3-aminopropanol, general procedure.

In a typical run, the dithiocarbonate (3.96 mmol) was mixed with 3-aminopropanol (7.94 mmol) at r. t., and the mixture stirred for 4-5 h. The product was isolated by extraction with pentane. After drying over $MgSO_4$ and evaporation of the solvent at reduced pressure, the product was obtained as an oil. In some cases this oil was already of sufficient purity, whilst in others (as indicated in parenthesis below) an additional purification by chromatography (P.E. as the solvent) was necessary.



(118) **(148)**

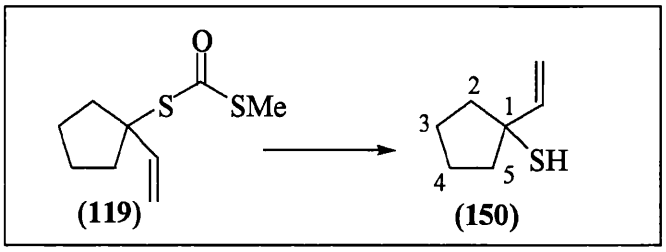
3-Methyl-4-phenyl-1-buten-3-thiol (148). (74 %). The product was obtained as a pure single isomer and gave the same spectral data as those obtained by methods 2.3.1 and 2.3.2.



(120) **(152)**

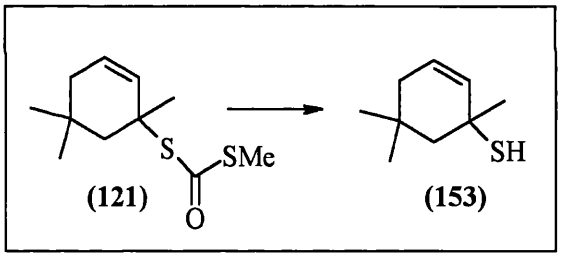
1-(2-Propenyl)-1-cyclopentanethiol (152). (87 %, pure single isomer). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.84 (1H, s, CHH=), 4.70 (1H, t, J= 1.2, CHH=), 1.93 (3H, s, CH_3), 2.00-1.85 (4H, m, 2 x $C_{2(5)}H_2$), 1.80-1.70 (4H, m, $C_3H_2C_4H_2$). **^{13}C NMR** ($CDCl_3$,

100.6 MHz): δ 151.03 (C=), 108.70 (CH_2=), 59.0 (CSH), 40.05 (2 x $C_{2(5)}H_2$), 22.65 (CH_3), 20.12 (2 x $C_{3(4)}H_2$). **MS** (FAB): m/z 141 ($(M-H)^+$, 20), 109 ($(M-SH)^+$, 100). **HRMS** (FAB): found 141.0731, $C_8H_{13}S$ $(M-H)^+$ requires 141.0738.



(119) **(150)**

1-Vinyl-1-cyclopentanethiol (150). (56 %; pure single isomer). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.07 (1H, dd, J= 17.2, 10.4, =CH), 5.11 (1H, d, J= 17.2, =CHH), 4.92 (1H, d, J= 10.5, =CHH), 2.0-1.6 (8H, m, 2 x CH_2CH_2). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 145.28 (CH=), 110.70 (CH_2=), 54.95 (CSH), 41.15 and 23.38 (4 x CH_2). **MS** (FAB): m/z 127 ($(M-H)^+$, 40), 95 ($(M-SH)^+$, 100). **MS** (EI): m/z 127 ($(M-H)^+$, 45), 95 (100). **HRMS** (FAB): found 127.0581, $C_7H_{11}S$ $(M-H)^+$ requires 127.0575.



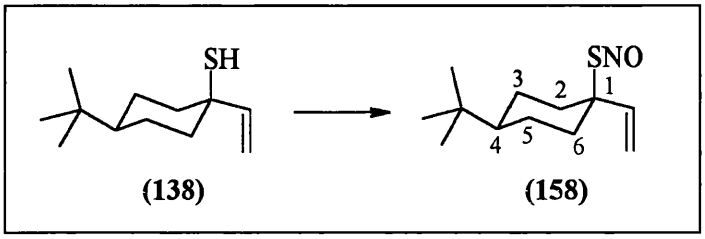
(121) **(153)**

1,5,5,-Trimethyl-2-cyclohexen-1-thiol (153). The product was obtained as a pure single isomer after chromatography on silica gel (70-90 %). The spectral data were identical with those already obtained for the product prepared by reduction with $LiAlH_4$.

2. 4. Synthesis of tertiary allylic thionitrites, general procedure.

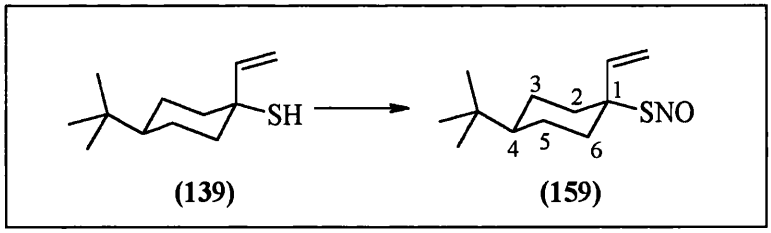
The corresponding thiol (0.98 mmol) was dissolved in *ca.* 6 mL of glacial AcOH at r. t. Then, $NaNO_2$ (148 mg, 2.11 mmol), dissolved in 1mL of H_2O, was added in one portion to the thiol solution and a green colour, characteristic of the thionitrite, developed instantly. The mixture was stirred at r. t. for 1 h, in the dark, by covering the flask and the fume-hood with Al foil, in order to prevent photochemical decomposition. The reaction was then partitioned between H_2O and DCM and the organic layer separated and washed twice with H_2O. After drying and evaporating the solvent at

reduced pressure, the residual AcOH was separated by azeotropic distillation (several times) with cyclohexane, in the dark. The resulting crude product was purified by filtration through silica, using P.E. as the eluant in all cases. All thionitrites were isolated as green/red oils. The instability of the nitrosothiols prevented, in most cases, high ressolution mass measurements.



(138) **(158)**

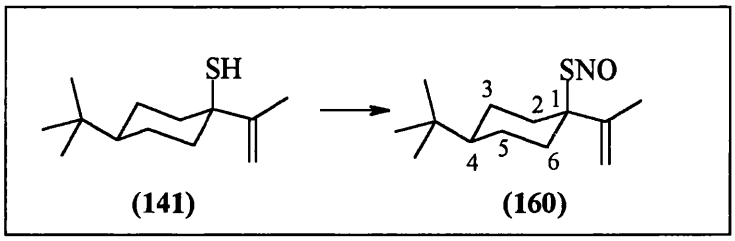
***Cis* 4-*tert*-butyl-1-vinyl-1-cyclohexanethioniotrite (158).** (85-75 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.38 (1H, dd, J= 17.6, 10.8, C<u>H</u>=), 5.29 (1H, d, J= 17.6, C<u>H</u>H=), 5.15 (1H, d, 10.8, CH<u>H</u>=), 2.60 (2H, bd, J= 14.0, 2 x $C_{2(6)}$<u>H</u>H), 1.99 (2H, bt, J= 14.1, 2 x $C_{2(6)}$H<u>H</u>), 1.86 (2H, bd, 2 x $C_{3(5)}$<u>H</u>H), 1.33-1.25 (3H, m, 2 x $C_{3(5)}$H<u>H</u> + C_4<u>H</u>), 0.83 (9H, s, ($C\underline{H}_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 144.18 (<u>C</u>H=), 112.56 (<u>C</u>H$_2$=), 59.43 (<u>C</u>SNO), 47.33 (<u>C</u>$_4$H), 36.00 (2 x <u>C</u>$_{2(6)}$H$_2$), 27.41 ((CH$_3$)$_3$<u>C</u>), 27.25 ((<u>C</u>H$_3$)$_3$C), 23.02 (2 x <u>C</u>$_{3(5)}$H$_2$). **IR** (neat): ν_{max}/cm^{-1} 1632 (C=C), 1507 (N=O). **MS** (FAB): m/z 229 ($(M+2H)^+$, 8), 197 ($(M-NO)^+$, 100), 165 ($(M-SNO)^+$, 53). **UV** ($CHCl_3$): λ_{max} 597 (ε 10.7), 350 (ε 0.7 x 10^3), 295 (ε 0.6 x 10^3), 250 (ε > 2 x 10^3).



(139) **(159)**

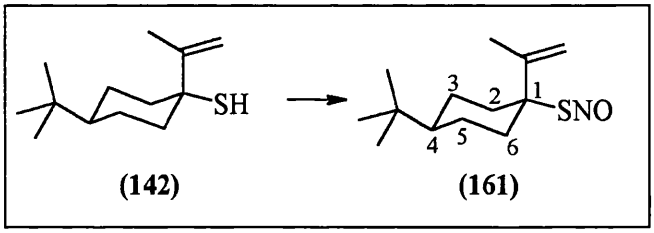
***Trans* 4-*tert*-butyl-1-vinyl-1-cyclohexanethionitrite (159).** (28-48 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.15 (1H, dd, J= 17.2, 10.4, C<u>H</u>=), 5.46 (1H, d, J= 17.2, C<u>H</u>H=), 5.43 (1H, d, 10.4, CH<u>H</u>=), 2.65 (2H, bd, J= 14.4, 2 x $C_{2(6)}$<u>H</u>H), 2.32 (2H, bt, 2 x $C_{2(6)}$H<u>H</u>), 1.81 (2H, bd, J= 14.2, 2 x $C_{3(5)}$<u>H</u>H), 1.29 (2H, bq, J= 13.2, 2 x $C_{3(5)}$H<u>H</u>), 1.19 (1H, tt, J= 11.9, 3.4, C_4<u>H</u>), 0.88 (9H, s, ($C\underline{H}_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 139.22 (<u>C</u>=), 117.82 (<u>C</u>H$_2$=), 60.37 (<u>C</u>SNO), 47.44 (<u>C</u>$_4$H), 36.77 (2 x <u>C</u>$_{2(6)}$H$_2$), 29.57 ((CH$_3$)$_3$<u>C</u>), 27.53 ((<u>C</u>H$_3$)$_3$C), 23.95 (2 x <u>C</u>$_{3(5)}$H$_2$). **IR** (neat): ν_{max}/cm^{-1} 2931, 2868, 1505

(N=O), 1369, 1134. **MS** (FAB): m/z 228 ($(M+H)^+$, 10), 197 ($(M-NO)^+$, 49), 165 ($(M-SNO)^+$, 73).



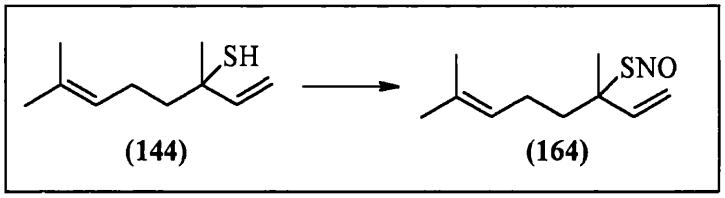
(141) **(160)**

***Cis* 4-*tert*-butyl-1-(2-propenyl)-1-cyclohexanethioniotrite (160).** (56 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.26 (1H, s, =CHH), 5.04 (1H, bs, =CHH), 2.82 (2H, bd, J= 14.2, 2 x $C_{2(6)}$HH), 2.03 (2H, bdt, J= 14.2, 2.5, 2 x $C_{2(6)}$HH), 1.87 (3H, s, (CH_3)C=), 1.81 (2H, bd, J= 13.2, 2 x $C_{3(5)}$HH), 1.45 (2H, bq, J= 14.0, 2 x $C_{3(5)}$HH), 1.31 (1H, m, C_4H), 0.86 (9H, s, $(CH_3)_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 149.13 (C=), 112.25 (=CH), 63.71 (CSNO), 47.92, 35.77 (2 x $C_{2(6)}H_2$), 32.44 ($(CH_3)_3C$), 27.52 ($(CH_3)_3C$), 23.50 (2 x $C_{3(5)}H_2$), 19.85 (CH_3). **IR** (neat): ν_{max}/cm^{-1} 2931, 2868, 1507 (N=O), 1369, 1134. **MS** (FAB): m/z 241 ($M^{\cdot+}$, 1), 212 (15), 211 ($(M-NO)^+$, 100), 179 ($(M-SNO)^+$, 70), 149 (13), 123 (40), 109 (77), 95 (12), 81 (27), 69 (31), 57 (74).



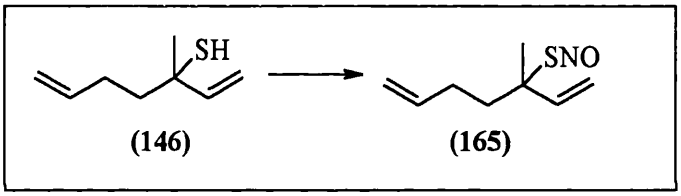
(142) **(161)**

***Trans* 4-*tert*-butyl-1-(2-propenyl)-1-cyclohexanthioniotrite (161).** (40 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.38 (1H, s, CHH=), 5.25 (1H, m, CHH=), 2.90 (2H, bd, J= 14.2, 2 x $C_{2(6)}$HH), 2.27 (2H, tm, J= 14.0, 2 x $C_{2(6)}$HH), 1.86 (3H, s, C(CH_3)=), 1.81 (2H, bd, J= 13.2, 2 x $C_{3(5)}$HH), 1.45 (2H, bq, J= 14.0, 2 x $C_{3(5)}H_2$), 1.29 (1H, m, C_4H), 0.87 (9H, s, $(CH_3)_3$C). **IR** (neat): ν_{max}/cm^{-1} 2931, 2868, 1507 (N=O), 1369, 1134. **MS** (FAB): m/z 241 ($M^{\cdot+}$, 2), 212 (15), 211 ($(M-NO)^+$, 100), 179 ($(M-SNO)^+$, 63), 154 (50), 136 (54), 123 (23), 109 (68), 95 (15), 81 (24), 69 (42), 57 (82).



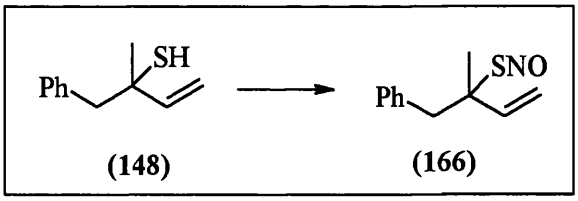
(144) (164)

3,7-Dimethyl-1,6-octadien-3-thionitrite (164). (53-60 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.30 (1H, dd, J= 17.2, 10.8, =CHCSNO), 5.34 (1H, d, J= 17.2, CHH=), 5.27 (1H, dd, J= 10.8, CHH=), 5.11 (1H, m, $(Me)_2C=CH$), 1.84-1.7 (4H, m, CH_2CH_2), 1.98 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.57 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 141.54 (C=), 132.28 and 123.12 (CH=), 114.81 ($=CH_2$), 60.19 (CSNO), 40.97 (CH_2), 25.49, 24.37, 23.20 and 17.48 (2 x CH_2 + 2 x CH_3). **IR** (neat): ν_{max}/cm^{-1} 2980, 2970, 1631 (C=C), 1501 (N=O). **MS** (FAB): m/z 201 ($(M+2H)^+$, 12), 169 ($(M-NO)^+$, 100), 137 ($(M-SNO)^+$, 68).



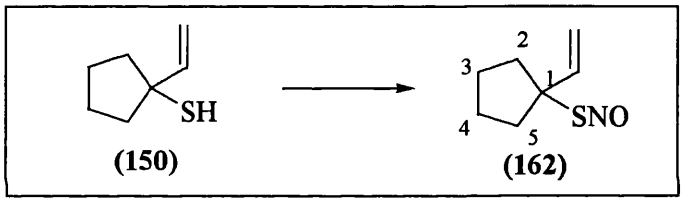
(146) (165)

3-Methyl-1,6-heptadien-3-thionitrite (165). (66 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.80 (1H, m, $CH_2=CHCH_2$), 6.34 (1H, d, J= 17.4, CHH=CHCS), 6.29 (1H, dd, J= 17.3, 10.6, =CHCS), 5.28 (1H, d, J= 10.7, $CHH=CHCH_2$), 5.04 (1H, dd, J= 17.4, 1.64, CHH=CHCS), 4.97 (1H, dd, J= 8.9, 1.2 $CHH=CHCH_2$), 2.4-2.2 (4H, m, CH_2CH_2), 1.97 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 141.48 and 137.61 (CH=), 115.20 and 115.14 ($CH_2=$), 60.00 (CSNO), 40.27 (CH_2CSNO), 28.87 and 24.56 (CH_2 and CH_3). **IR** (neat): ν_{max}/cm^{-1} 2978, 2928, 1642 (C=C), 1502 (N=O), 1412, 1374. **MS** (FAB): m/z 194 ($(M+Na)^+$, 50), 171 (M^+, 5), 141 ($(M-NO)^+$, 100), 109 ($(M-SNO)^+$, 89).



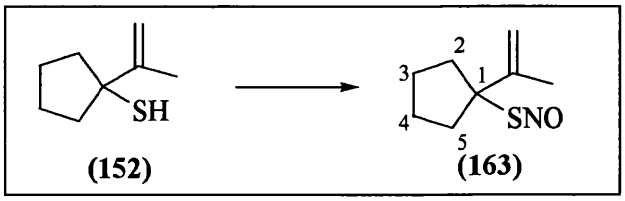
(148) (166)

3-Methyl-4-phenyl-1-buten-3-thionitrite (166). (99 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.32 (3H, m, Ar<u>H</u>), 7.20 (2H, m, Ar<u>H</u>), 6.43 (1H, dd, J= 17.3, 10.6, C<u>H</u>=), 5.31 (1H, d, J= 17.12, CH<u>H</u>=), 5.30 (1H, d, J= 10.7, CH<u>H</u>=), 5.54 (2H, d, J= 3.6, C<u>H</u>$_2$Ph), 1.95 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 141.30, 135.78, 130.99, 127.97, 126.97 and 115.37 (4 x <u>C</u>$_{Ar}$(H) + 2 x <u>C</u>H(H)=), 59.45 (<u>C</u>SNO), 47.29 (<u>C</u>H$_2$Ph), 24.29 (<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 1638 (C=C), 1497 (N=O). **MS** (FAB): m/z 207 (M^{+}, 2), 177 ($(M–NO)^{+}$, 17), 145 ($(M–SNO)^{+}$, 100). **UV** ($CHCl_3$): λ_{max} 600 (ε 18), 340 (ε 0.2 x 10^3), 260 (ε > 0.4 x 10^3).



(150) (162)

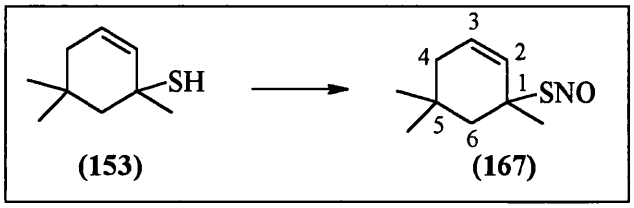
1-Vinyl-1-cyclopentenethionitrite (162). (90 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.34 (1H, dd, J= 17.2, 10.4, C<u>H</u>=), 5.31 (1H, d, J= 17.2, CH<u>H</u>=), 5.19 (1H, d, J= 10.4, CH<u>H</u>=), 2.33-2.29 (4H, m, 2 x C$_{2(5)}$<u>H</u>$_2$), 1.83-1.79 (4H, m, C$_3$<u>H</u>$_2$C$_4$<u>H</u>$_2$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 141.34 (<u>C</u>H=), 114.07 (<u>C</u>H$_2$=), 66.5 (<u>C</u>SNO), 37.8 and 23.5 (4 x <u>C</u>H$_2$). **IR** (neat): ν_{max}/cm^{-1} 2961, 2873, 1503 (N=O). **MS** (FAB): 317 ($(2M–H)^{+}$, 10), 159 ($(M+2H)^{+}$, 37), 127 ($(M–NO)^{+}$, 100).



(152) (163)

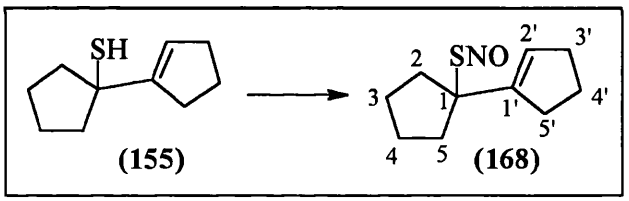
1-(2-Propenyl)-1-cyclopentenethionitrite (163). (36 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.24 (1H, bs, CH<u>H</u>=), 5.01 (1H, t, J= 1.32, C<u>H</u>H=), 2.6 (2H, m, C$_{2(5)}$<u>H</u>$_2$), 2.4 (2H, m, C$_{2(5)}$<u>H</u>$_2$), 2.00-1.80 (4H, m, C$_3$<u>H</u>$_2$C$_4$<u>H</u>$_2$), 1.89 (3H, d, J= 1.4, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 146.5 (<u>C</u>=), 112.7 (<u>C</u>H$_2$=), 55.0 (<u>C</u>SNO), 37.3 (2 x <u>C</u>$_{2(5)}$H$_2$),

23.1 and 20.7 (2 x $\underline{C}_{3(4)}H_2$ + $\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 3093, 2961, 2887, 2364, 1638 (C=C), 1501 (N=O), 1448, 1376, 899. **MS** (FAB): m/z 141 ($(M-NO)^+$, 33), 109 ($(M-SNO)^+$, 100).



(153) **(167)**

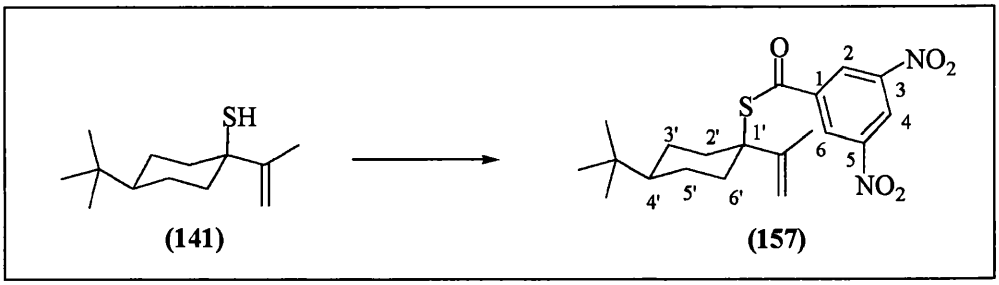
1,5,5-Trimethyl-2-cyclohexen-1-thionitrite (167). (59 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.03 (1H, d, J= 9.8 , $=C_2\underline{H}$), 5.90 (1H, m, $=C_4\underline{H}$), 2.41 (1H, d, J= 14.6, $C_6\underline{H}H$), 2.08 (1H, d, J= 14.6, $C_6H\underline{H}$), 2.02 (3H, s, $C\underline{H}_3CSNO$), 1.98 (2H, m, $C_4\underline{H}_2$), 1.07 (3H, s, $C\underline{H}_3$), 0.93 (3H, s, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 129.37 and 128.35 ($\underline{C}H=$), 49.17 ($\underline{C}SNO$), 38.40, 31.05, 30.98, 30.83, 30.22, 28.86. **IR** (neat): ν_{max}/cm^{-1} 1638 (C=C), 1499 (N=O). **MS** (FAB): m/z 187 ($(M+2H)^+$, 1), 155 ($(M-NO)^+$, 3), 123 ($(M-SNO)^+$, 100).



(155) **(168)**

1-(1-Cyclopentenyl)-1-cyclopentyl thionitrite (168). (*ca.* 38 %, impure). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.82 (1H, m, $C_{2'}\underline{H}=$), 2.55-2.35 (4H, m), 1.90-1.80 (4H, m), 1.40-1.20 (2H, m).

2. 5. Synthesis of a crystalline derivative of thiol 141 and X-Ray analysis for the determination of its stereochemistry.



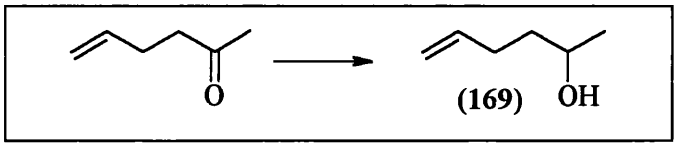
(141) (157)

Thiol **141** (40 mg, 0.24 mmol) was dissolved in dry DCM (10 mL) and Et_3N (31 μL, 0.29 mmol) and DMAP (cat.) were added at r. t. and under N_2. 3,5-Dinitrobenzoyl chloride (87 mg, 0.48 mmol) was then added, the solution turning red and then yellow and a precipitate forming. The reaction was quenched by addition of aq. NH_4Cl solution, washed with aq. $NaHCO_3$ and with H_2O, dried over $MgSO_4$ and the solvent eliminated at reduced pressure. The remaining brown solid was chromatographed on SiO_2 (P.E./Et_2O 80/20) giving the product as a white solid (31 mg, 32 %). This was recrystallised from Et_2O-PE in the form long transparent needles suitable for X-Ray analysis.

S-(4'-Tert-butyl-1-vinylcyclohexyl)-3, 5-dinitro-1-thiobenzoate (157). **mp** (Et_2O/P.E.): 181-182 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 9.17 (1H, s, $C_4\underline{H}$), 9.05 (2H, s, 2 x $C_{2(6)}\underline{H}$), 5.19 (1H, s, =C$\underline{H}$H), 5.02 (1H, s, =CH$\underline{H}$), 2.60 (2H, bd, J= 12.6, 2 x $C_{2'(6')}\underline{H}$H), 1.88 (3H, s, C$\underline{H_3}$), 1.74 (2H, bt, J= 12.7, 2 x $C_{2'(6')}$H$\underline{H}$), 1.65 (2H, bt, J= 14.0, 2 x $C_{3'(4')}\underline{H}$H), 1.42 (2H, bq, J= 14.6, 2 x $C_{3'(5')}$H$\underline{H}$), 1.03 (1H, t, J= 12.2, $C_{4'}\underline{H}$), 0.85 (9H, s, $(CH_3)_3C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 187.05 ($\underline{C}$=O), 148.89, 147.04, 141.23, 127.08, 122.15, 113.36 (4 x C_{Ar}(H) + 2 x =$\underline{C}(H_2)$), 62.44 ($\underline{C}$S), 47.83 ($\underline{C}_{4'}$H), 35.31 ($\underline{C}H_2$), 32.66 ($\underline{C}(CH_3)_3$), 27.74 (($\underline{C}H_3)_3C$), 23.93, 22.65 and 20.28 (CH_2), 14.35 ($\underline{C}H_3$). **MS** (FAB)*:* m/z 406 ($M^{+\cdot}$, 5), 289 (30), 277 (62). **HRMS** (FAB)*:* Found 406.1540, $C_{20}H_{26}N_2SO_5$ ($M^{+\cdot}$) requires 406. 1562. **X-ray** (see apendix).

2. 6. Synthesis of 5-hexen-2-thiol (171).

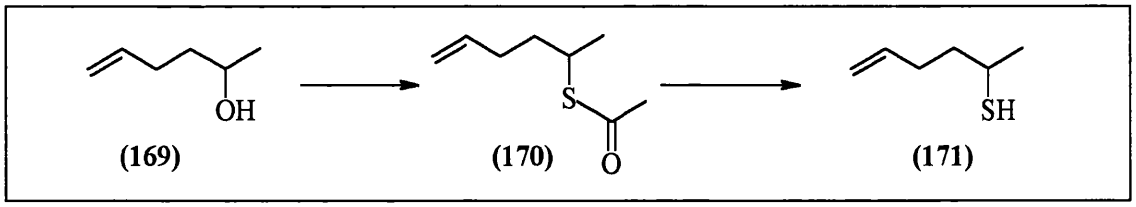
2. 6. 1. Preparation of 5-hexen-2-ol (**169**).



Alcohol **169** was obtained by reduction of commercially available 5-hexen-2-one (10 g, 100 mmol) with $NaBH_4$ (4 g, 100 mmol) in MeOH (50 mL), at 0 °C, for 2 hours as described in section *1.1.2.1.* of this Chapter. After quenching with aq. NH_4Cl solution and extracting with Et_2O, the organic layer was washed with two more portions of H_2O and the solvent evaporated under vacuum. The product was isolated as a colourless oil, after chromatography on silica gel (DCM, 6.67 g, 67 %).

5-Hexen-2-ol (169)[180]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.85 (1H, m, =C<u>H</u>), 5.05 (1H, dd, J= 18.0, 1.4, C<u>H</u>H=), 4.98 (1H, dd, J= 8.0, 1.0, CH<u>H</u>=), 3.84 (1H, m, C<u>H</u>OH), 2.2-2.1 (2H, m, C<u>H</u>$_2$), 1.6-1.5 (2H, m, C<u>H</u>$_2$), 1.36 (1H, d, J= 4.8, O<u>H</u>), 0.84 (3H, d, J= 6.2, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 138.46 (<u>C</u>H=), 114.76 (<u>C</u>H$_2$=), 67.71 (<u>C</u>HOH), 38.24 and 30.16 (<u>C</u>H$_2$), 23.47 (<u>C</u>H$_3$).

2. 6. 2. Synthesis of thiol **171**.



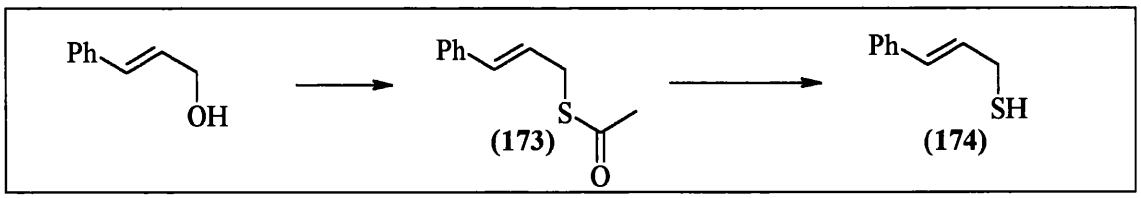
Triphenylphosphine (5.3 g, 20.3 mmol) and diisopropylazadicarboxylate (4 mL, 20.3 mmol) were mixed in THF (50 mL), under N_2 and at 0 °C, and the resulting suspension stirred at this temperature for 30 min. Alcohol **169** (923 mg, 9.23 mmol) was then added, followed by thioacetic acid (1.4 mL, 20.3 mmol). The reaction was stirred at 0 °C for 45 min and then allowed to warm to r. t. and stirred for 3 hours. The solvent was evaporated and the resulting precipitate re-dissolved in benzene and chilled. After filtration the solvent was evaporated and the crude oil re-dissolved in EtOAc. The solid

impurities were filtered and the solvent evaporated again. The crude reaction mixture was purified by chromatography on silica gel (P.E./Et_2O 99/1), giving thioester **170** as a yellow oil, in 54 % yield (787 mg). Methanolysis of 1 g of the thioester was carried out as described above (2. 3. 1) for other dithiocarbonates, using NaOH, in MeOH, at r. t., for 2 h. Thiol **171** was obtained as a yellow oil in 48 % yield (352 mg), after chromatography on silica gel (P.E.).

S-(5-Hexen-2-yl) thioacetate (170). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, m, C<u>H</u>=), 5.03 (1H, dq, J= 16.8, 1.7, CH<u>H</u>=), 4.97 (1H, d, J= 10.2, CH<u>H</u>=), 3.57 (1H, m, C<u>H</u>SCOCH$_3$), 2.2-2.1 (2H, m, C<u>H</u>$_2$), 1.7-1.6 (2H, m, C<u>H</u>$_2$), 1.31 (3H, d, J= 6.96, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 195.86 (<u>C</u>=O), 137.66 (<u>C</u>H=), 115.04 (=<u>C</u>H$_2$), 38.98 (<u>C</u>S), 35.57 and 31.13, (<u>C</u>H$_2$), 21.23 (<u>C</u>H$_3$). **IR** (neat): ν_{max}/cm^{-1} 2932, 2845, 1692 (C=O). **MS** (FAB): m/z 149 ($(M+H)^+$, 16), 133 ($(M-Me)^+$, 7).

5-Hexen-2-thiol (171). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.80 (1H, m, =C<u>H</u>), 5.05 (1H, dq, J= 17.1, 1.04, C<u>H</u>H=), 4.98 (1H, dd, J= 10.1, 0.8, CH<u>H</u>=), 2.80 (1H, m, C<u>H</u>S), 2.2-2.1 (2H, m, C<u>H</u>$_2$), 1.8-1.3 (3H, m, C<u>H</u>$_2$ and S<u>H</u>), 1.31 (3H, d, J= 6.7, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 137.87 (<u>C</u>H=), 115.01 (<u>C</u>H$_2$=), 45.89 (<u>C</u>SH), 35.25 and 31.18 (<u>C</u>H$_2$), 20.62 (<u>C</u>H$_3$). **MS** (FAB): m/z 115 ($(M-H)^+$, 18), 82 ($(M-SH)^+$, 47). **HRMS** (FAB): found 115. 0572, $C_6H_{12}S$ $(M-H)^+$ requires 115.0581.

2. 7. Preparation of 3-phenyl-2-propen-1-thiol (174).



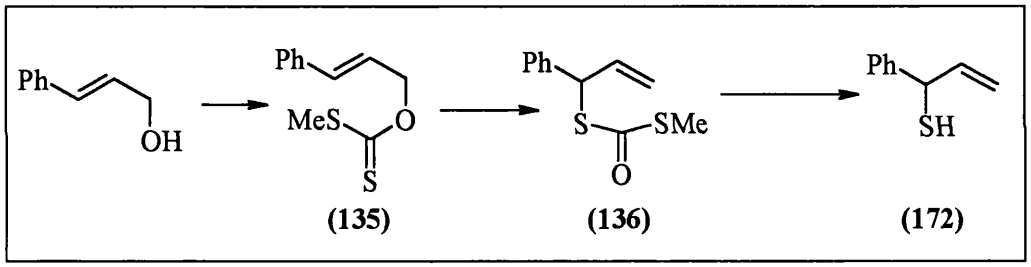
The procedure described above for the obtention of thioester **170** and thiol **171** was followed, starting from 2 g. of the alcohol. Products were purified as indicated in parenthesis below.

S-(3-Phenyl-2-propen-1-yl) thioacetate (173). (chromatography on SiO_2, P.E./Et_2O 99/1; 40 %, yellow oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.36-7.20 (5H, m, Ar<u>H</u>), 6.57 (1H, d, J= 15.72, =C<u>H</u>Ph), 6.17 (1H, dt, J= 15.7, 7.4, =C<u>H</u>CH$_2$), 3.71 (2H,

dd, J= 7.5, 1.1, C$\underline{H}_2$S), 2.37 (3H, s, C$\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 204.10 ($\underline{C}$=O), 136.53, 133.08, 128.53, 127.68, 126.34 and 124.37 (4 x $\underline{C}_{Ar}$(H) + 2 x =$\underline{C}$H), 31.77 and 30.53 ($\underline{C}H_2S$ + $\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2921, 2856, 1687 (C=O), 1132, 961. **MS** (FAB): m/z 192 ($M^{+\cdot}$, 39), 117 ($(M-SCOMe)^+$, 100).

3-Phenyl-2-propen-1-thiol (cinnamyl thiol, 174)[181]. (chromatography on SiO_2, P.E.; 83 %, colourless oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.20- 7.40 (5H, m, Ar$\underline{H}$), 6.50 (1H, d, J= 15.7, =C$\underline{H}$Ph), 6.33 (1H, dt, J= 15.7, 6.2, PhCH=C$\underline{H}$), 3.36 (2H, t, J= 7.4, C$\underline{H}_2$SH), 1.54 (1H, t, J= 7.4, S$\underline{H}$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 136.63, 130.77, 128.71, 128.58, 127.60, 127.55, 126.42 and 126.33 (4 x $\underline{C}_{Ar}$(H) + 2 x $\underline{C}$H=), 27.31 ($\underline{C}H_2$SH). **MS** (FAB): m/z 149 ($(M-H)^+$, 15), 117 ($(M-SH)^+$, 100), 115 (18), 105 (17). **HRMS** (FAB): found 149.0423, C_9H_9S $(M-H)^+$ requires 149.0425.

2. 8. Preparation of 3-phenyl-1-propen-3-thiol (172).



(135) (136) (172)

Dithiocarbonate **136** was prepared by [3,3] rearrangement of the corresponding xanthate (**135**), derived from cinammyl alcohol, following the general procedure given in section 2.2.1. The xanthate could, in practice, be isolated after reflux for 4 hours. Additional heating for 1 night provided the dithiocarbonate. Thiol **172** was obtained by methanolysis of the dithiocarbonate, using NaOH in MeOH, following the general procedure described above (2. 3. 1). The products were purified as indicated in parenthesis below.

$\underline{S}$-Methyl $\underline{O}$-(3-phenyl-2-propenyl) xanthate (135). (chromatography on SiO_2, P.E.; yellow oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.4-7.3 (5H, m, Ar$\underline{H}$), 6.49 (1H, d, J= 15.7, =C$\underline{H}$), 6.31 (1H, dt, J= 15.7, 7.2, =C$\underline{H}$C$\underline{H}_2$O), 4.14 (2H, dd, J= 7.2, 1.4, C$\underline{H}_2$O), 2.78 (3H, s, C$\underline{H}_3$S). **^{13}C NMR** ($CDCl_3$, 400 MHz): δ 220.5 ($\underline{C}$=S), 136.62 and 131.17

(=C), 128.56, 127.69, 127.67 and 126.47 (C(H)$_{Ar}$), **MS** (FAB): m/z 225 ((M+H)$^+$, 3), 133 ((M–SSMe)$^+$, 29), 117 ((M–OCSSMe)$^+$, 100), 105 (46).

S-Methyl S-[3-(3-phenyl-2-propenyl)] dithiocarbonate (136). (chromatography on SiO_2, P.E.; 54 % from the alcohol, yellow oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.38-7.28 (5H, m, ArH), 6.12 (1H, m, =CH), 5.43 (1H, dt, J= 7.0, 1.2, CHSCOCH$_3$), 5.27 (1H, dt, J= 17.3, 0.96, CHH=), 5.23 (1H, dt, J= 10.2, 1.16, CHH=), 2.42 (3H, s, CH$_3$S). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 188.54 (C=O), 139.00, 136.30, 128.76, 128.09, 127.78 and 117.48 (4 x C$_{Ar}$(H) + 2 x CH(H)=), 51.96 (CHS), 13.11 (CH$_3$S). **IR** (neat): ν_{max}/cm^{-1} 3020, 2910, 1736 (C=O), 1638 (C=C), 864. **MS** (FAB): m/z 225 ((M–H)$^+$, 1), 179 ((M–SMe)$^+$, 43), 150 ((M–COSMe)$^+$, 2), 149 (22), 118 (10), 117 (100). **HRMS** (FAB): found 225.0420, $C_{11}H_{12}S_2O$ (M+H)$^+$ requires 225.0408.

1-Phenyl-2-propen-1-thiol (172)[181]. (chromatography on SiO_2, P.E.; 50 %, colourless oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.40-7.25 (5H, m, ArH), 6.20 (1H, m, CH=), 5.23 (1H, dt, J= 16.8, 1.2, CHH=), 5.12 (1H, dd, J= 10.0, 0.8, CHH=), 4.75 (1H, t, J= 6.2, CHS), 2.05 (1H, t, J= 6.2, SH). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 142.33, 140.31, 128.72, 127.48, 127.30, 114.96, 46.55 (CHSH). **MS** (FAB): m/z 149 ((M–H)$^+$, 7), 117 (M–SH)$^+$, 100). **HRMS** (FAB): found 149.0423, C_9H_9S (M–H)$^+$ requires 149.0425.

CHAPTER 3

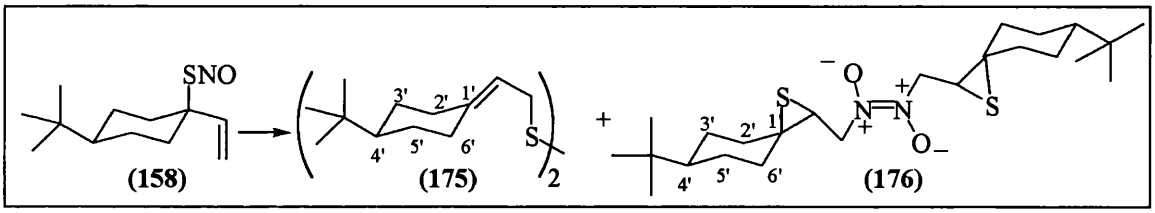
THERMAL DECOMPOSITION OF ALKENE-CONTAINING THIONITRITES.

3. 1. Tertiary allylic thionitrites.

3. 1. 1. Thermal decomposition in DCM.

In a general procedure, the thionitrite (1 mmol) was dissolved in 20 mL of dry DCM, under N_2, and the solution heated at reflux for a period of time between 7 and 24 h (until the control by TLC indicated total consumption of starting material and the solution had lost its green colour completely). The final solutions were pale yellow in all of the cases. The solvent was evaporated under vacuum and the crude reaction mixture separated by chromatography on silica gel, using a gradient of polarity (see details for each particular case). In some occasions, one or several of the separated fractions needed additional purification by chromatography or by preparative TLC.

*3. 1. 1. 1. Thermal decomposition of thionitrite **158**.*



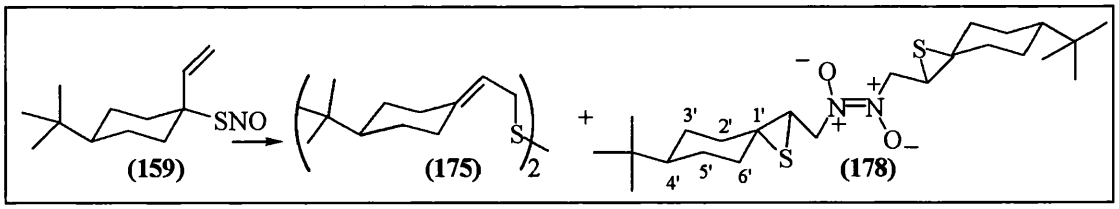
Purification was carried out by chromatography on silica, starting from P.E. and increasing the polarity until P.E/Et_2O 70/30. Two fractions were separated in order of increasing polarity:

Bis-[2-(4-tert-butyl-cyclohexylidene)-1-ethyl] disulfide (175). (P.E.; 18 %, colourless oil). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.22 (1H, t, J= 7.9, C<u>H</u>=), 3.40 (2H, m, C<u>H</u>$_2$S), 2.71 (1H, bd, J= 13.2, $C_{2'(6')}$<u>H</u>H), 2.27 (1H, bd, J= 13.0, $C_{2'(6')}$<u>H</u>H), 2.05 (1H, bt, 14.0, $C_{2'(6')}$H<u>H</u>), 1.88 (2H, bt, J= 14.0, $C_{2'(6')}$H<u>H</u> + $C_{3'(5')}$<u>H</u>H), 1.80 (1H, bd, J= 14.0, $C_{3'(5')}$H<u>H</u>), 1.25-1.00 (3H, m, $C_{3'(5')}$<u>H</u>$_2$ + $C_{4'}$<u>H</u>), 0.86 (9H, s, (C<u>H</u>$_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 145.2 (<u>C</u>=), 115.6 (<u>C</u>H=), 48.4 (<u>C</u>$_{4'}$H), 37.0 and 36.7 (ring <u>C</u>H$_2$ + <u>C</u>H$_2$S), 32.5 ((CH$_3$)$_3$<u>C</u>), 29.1, 28.8 and 28.7 (<u>C</u>H$_2$), 27.7 ((<u>C</u>H$_3$)$_3$C).

IR (CCl_4): ν_{max}/cm^{-1} 2944, 2867, 1661 (C=C), 1479, 1444, 1353. **MS** (FAB): m/z 231 (M'=(M–$C_{12}H_{22}$+H)$^+$, 16), 197 ((M'–SH)$^+$, 39), 165 ((M'–SSH)$^+$, 84). **MS** (APCI): m/z 396 ((M+2H)$^+$, 12), 395 ((M+H)$^+$, 43), 231 (34), 197 (19), 166 (14), 165 (100). **HRMS** (FAB): found 395.2806, $C_{24}H_{43}S_2$ (M+H)$^+$ requires 395.2797; found 230.1163, $C_{12}H_{22}S_2$ (M+H–$C_{12}H_{21}$)$^+$ requires 230.1144. **EA**: found C 73.04, H 10.89, S 16.15 %, $C_{24}H_{42}S_2$ requires C 73.02, H 10.73, S 16.24 %.

Trans nitroso-dimer 176. (P.E./Et_2O 75/25; 65 %; white solid). **mp** (Et_2O/P.E.): 118-119.5 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.76 (1H, dd, J= 14.6, 5.8, C<u>H</u>HN), 4.41 (1H, dd, J= 14.6, 8.7, CH<u>H</u>N), 3.32 (1H, dd, J= 8.3, 5.7, SC<u>H</u>CH_2N), 2.24 (1H, dt, J= 12.9, 3.9, $C_{2'(6')}$<u>H</u>H), 2.1-1.8 (3H, m, $C_{2'(6')}$<u>H</u>H + 2 x $C_{2'(6')}$H<u>H</u>), 1.60 (1H, bd, J= 13.5, $C_{3'(5')}$<u>H</u>H), 1.4-1.25 (3H, m, $C_{3'(5')}$<u>H</u>H + 2 x $C_{3'(5')}$H<u>H</u>), 1.25 (1H, m, C_4<u>H</u>), 0.89 (9H, s, ($C\underline{H}_3)_3C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 59.58 (<u>C</u>H_2N), 56.11 (episulfide <u>C</u>S), 47.56 (<u>C</u>$_4$H), 40.10 (episulfide <u>C</u>HS), 39.38 ($\underline{C}_{2'(6')}H_2$), 33.05 ($\underline{C}_{2'(6')}H_2$), 32.58 ($(CH_3)_3\underline{C}$), 27.54 ($(\underline{C}H_3)_3C$), 26.78 and 26.71 ($\underline{C}_{3'(5)}H_2$). **IR** ($CCl_4$): ν_{max}/cm^{-1} 2957, 2867, 1366, 1120, 908. **MS** (FAB): m/z 455 ((M+H)$^+$, 5), 259 (12), 197 (98), 165 (32), 107 (31). **HRMS** (FAB): found 455.2760, $(C_{12}H_{21}SNO)_2H$ (M+H)$^+$ requires 455.2766. **EA**: found C 63.51, H 9.28, N 6.08, S 14.47 %; requires C 63.39, H 9.32, N 6.16, S 14.10 %. A sample of this compound was recristalised from DCM/P.E. and the X-ray of a single crystal was obtained (see Appendix)

*3. 1. 1. 2. Thermal decomposition of thionitrite **159**.*

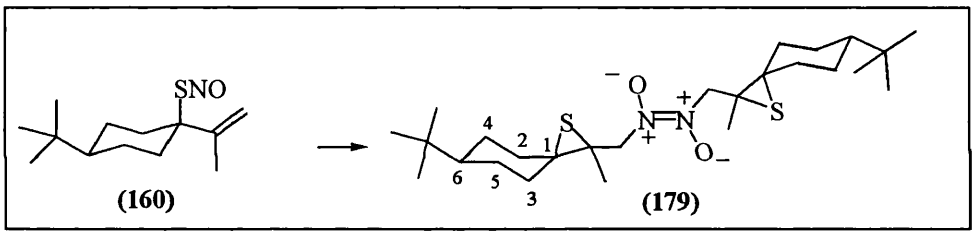


The crude was purified as indicated in the previous reaction. Two fractions were separated in order of increasing polarity.

Bis-[2-(4-tert-butylcyclohexylidene)-1-ethyl] disulfide (175). (P.E.; 17 %; colourless oil). This compound displayed the same spectral data as compound **175** from the decomposition of thionitrite **159**.

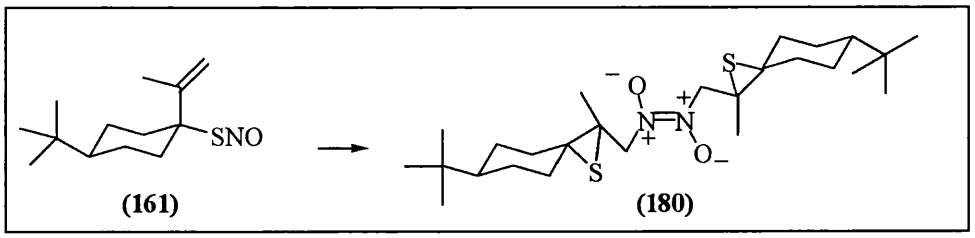
Nitroso dimer 178. (P.E./Et_2O 75/25; 65 %; white solid). **mp** (Et_2O/P.E.): 198.8-199.5 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.55 (2H, d, J= 6.8, C$\underline{H}_2$N), 3.31 (1H, t, J= 6.8, SC$\underline{H}$CH$_2$N), 2.22 (2H, m, 2 x $C_{2'(6')}\underline{H}H$), 1.8 (2H, m, 2 x $C_{2'(6')}H\underline{H}$), 1.74 (1H, m, $C_{3'(5')}\underline{H}H$), 1.47 (1H, m, $C_{3'(5')}\underline{H}H$), 1.27 (1H, m, $C_{3'(5')}H\underline{H}$), 1.14 (1H, m, $C_4\underline{H}$), 1.04 (1H, m, $C_{3'(5')}H\underline{H}$), 0.85 (9H, s, (C$\underline{H}_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 60.0 (C$\underline{H}_2$N), 52.7 (episulfide $\underline{C}$S), 46.83 ($\underline{C}_4$H), 41.33 ($\underline{C}_{2'(6')}H_2$), 40.66 (episulfide $\underline{C}$HS), 33.58 ($\underline{C}_{2'(6')}H_2$), 32.37 ((CH$_3$)$_3\underline{C}$), 28.57 and 28.46 ($\underline{C}_{3'(5')}H_2$), 27.60 (($\underline{C}H_3$)$_3$C). **IR** ($CCl_4$): ν_{max}/cm^{-1} 2947 , 2863, 1638, 1448, 1394, 1365, 1239, 1194, 1036, 986. **MS** (FAB): m/z 455 ((M+H)$^+$, 21), 391 (18), 362 (16), 289 (22), 259 (100), 243 (17), 197 (98), 165 (32), 107 (31). **HRMS** (FAB): found 455.2750, $(C_{12}H_{21}SNO)_2H$ (M+H)$^+$ requires 455.2760.

*3. 1. 1. 3. Thermal decomposition of thionitrite **160.***



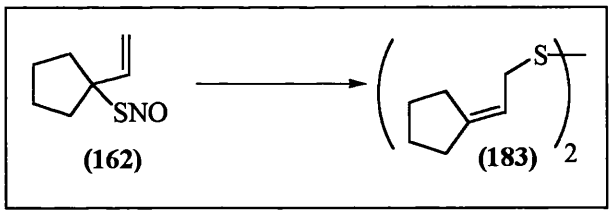
***Trans* nitroso-dimer 179** (chromatography on SiO_2, P.E./Et_2O 75/35; 73 %; white solid). **mp**: 145-148 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.06 (1H, d, J= 14.5, C$\underline{H}$HN), 4.28 (1H, d, J= 14.5, CH$\underline{H}$N), 2.20-1.80 (3H, m, 2 x $C_{2'(6')}\underline{H}H$ + $C_{2'(6')}H\underline{H}$), 1.75-1.70 (1H, m, $C_{2'(6')}H\underline{H}$), 1.70 (3H, s, C$\underline{H}_3$), 1.40-1.02 (5H, m, 2 x $C_{3'(5')}\underline{H}_2$ + $C_4\underline{H}$), 0.90 (9H, d, J= 1.0, (C$\underline{H}_3$)$_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 66.99 (C$\underline{H}_2$N), 61.62 (episulfide $\underline{C}$S), 49.14 (episulfide $\underline{C}$S), 47.14 ($\underline{C}_4$H), 34.64 ($\underline{C}_{2'(6')}H_2$), 34.30 ($\underline{C}_{2'(6')}H_2$), 32.42 ((CH$_3$)$_3\underline{C}$), 27.54 (($\underline{C}H_3$)$_3$C), 26.85 and 26.66 ($\underline{C}_{3'(5')}H_2$), 21.75 ($\underline{C}H_3$). **IR** ($CCl_4$): ν_{max}/cm^{-1} 2947 , 2846, 1693, 1551, 1454, 1362, 1260, 1200, 1178. **MS** (FAB): m/z 483 ((M+H)$^+$, 7), 473 (1) 211 (100). **HRMS** (FAB): found 483.3060, $(C_{13}H_{23}SNO)_2H$ (M+H)$^+$ requires 483.3079.

*3. 1. 1. 4. Thermal decomposition of thionitrite **161.***



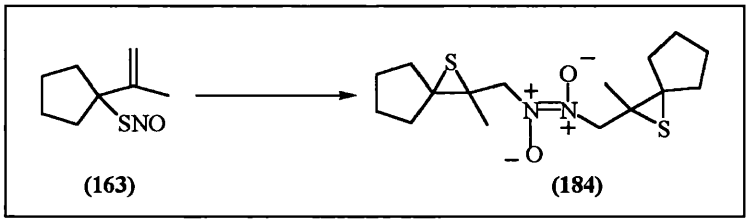
Trans* nitroso-dimer *180. (chromatography on SiO_2, P.E./Et_2O 75/25; 72 %, white solid). **mp**: 136-138 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.75 (1H, d, J= 15.0, CHHN), 4.63 (1H, d, J= 15.0, CHHN), 2.20-1.95 (4H, m, 3 x $C_{2'(6')}$HH), 1.75 (2H, m, 2x $C_{3'(5')}$HH), 1.79 (3H, s, CH_3), 1.3-1.15 (3H, m, 2 x $C_{3'(5')}$HH + $C_{4'}$H), 0.87 (9H, s, $(CH_3)_3C$). **^{13}C NMR** ($CDCl_3$, 400 MHz): δ 63.39 (CH_2N), 57.7 (episulfide CS), 49.86 (episulfide CS), 47.32 ($C_{4'}H$), 36.13 (ring CH_2), 32.36 ($(CH_3)_3C$), 27.59 ($(CH_3)_3C$), 27.54 and 27.50 (ring CH_2), 22.10 (CH_3). **IR** (CCl_4): ν_{max}/cm^{-1} 2952 , 2865, 1454, 1363, 1217, 1187, 1033. **MS** (FAB): m/z 483 ($(M+H)^+$, 6.9), 473 (4), 451. (5), 289 (17), 211 (100). **HRMS** (FAB): found 483.3060, $(C_{13}H_{23}SNO)_2H$ $(M+H)^+$ requires 483.3079.

*3. 1. 1. 5. Thermal decomposition of thionitrite **162.***



Bis [1-(2-cyclopentyliden)ethyl] disulfide (183). (chromatography on SiO_2, P.E.; major product by ^{1}H NMR of the crude and TLC, but only isolated after reaction in benzene, allowing full characterisation). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.36 (1H, tq, J= 7.9, 2.2, =CH), 3.41 (2H, d, J= 7.9, CH_2S), 2.3 (4H, m, 2 x CH_2), 1.70-1.55 (4H, m, CH_2CH_2). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 149.5 (=C), 115.3 (=CH), 39.5, 34.1, 29.1 and 26.5 (5 x CH_2). **MS** (FAB): m/z 254 ($M^{+\cdot}$, 32), 221 ($(M-SH)^+$, 8), 161 (38), 127 (100). **HRMS** (FAB): found 254.1154, $C_{14}H_{22}S_2$ ($M^{+\cdot}$) requires 254.1163.

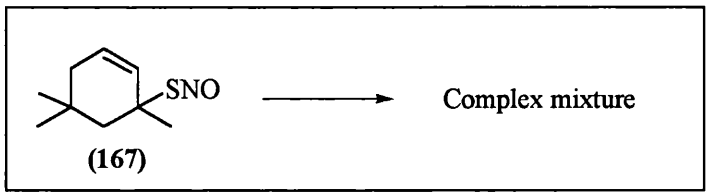
*3. 1. 1. 6. Thermal decomposition of thionitrite **163**.*



(163) (184)

Nitroso-dimer 184. (Chromatography on SiO_2, P.E/ Et_2O 80/20 to 70/30; mixture of two diastereomers in proportion 2:1 by ^1H NMR of the crude mixture, not isolated; isolated only later after reaction in benzene, allowing full characterisation). **mp**: 119-121 °C (Et_2O/P.E.). **^1H NMR** ($CDCl_3$, 400 MHz): δ 5.20 and 5.16 (2H overall, 2 x d, J= 15.2, C<u>H</u>HN), 4.14 and 14.11 (1H overall, d, J= 15.1, CH<u>H</u>N), 2.32 (2H, m, 2 x $C_{2'(5')}$<u>H</u>H), 1.87 (6H, m, 2 x $C_{2'(5')}$H<u>H</u> and 2 x $C_{3'(4')}$<u>H_2</u>), 1.66 and 1.67 (3H overall, s, <u>CH_3</u>). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 65.79 and 65.73 (<u>CH_2</u>N), 65.47 and 65.30 (episulfide <u>C</u>S), 46.81 (2 x episulfide <u>C</u>S), 35.81 and 35.77 (4 x <u>$C_{2(5)}$</u>H_2), 25.54, 25.16, 25.09 (4 x ring <u>$C_{3(4)}$</u>H_2), 23.24 and 23.14 (<u>CH_3</u>). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3002, 2967, 2887, 1444, 1362, 1217, 1186. **MS** (FAB): m/z 365 $((M+Na)^+$, 2), 344 $((M+2H)^+$, 47), 343 $((M+H)^+$, 3), 312 $((M-S)^+$, 33), 286 (100), 141 $((M/2-NO)^+$, 100), 109 $((M/2-NO-S)^+$, 73). **HRMS** (FAB): found 344.1592, $C_{16}H_{28}S_2N_2O_2$ $(M+2H)^+$ requires 344.1580.

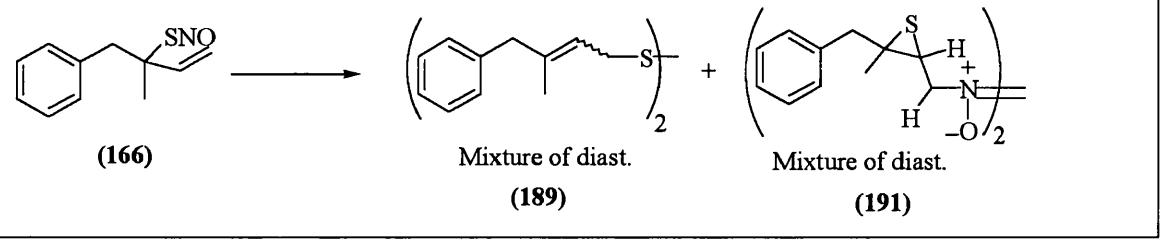
*3. 1. 1. 7. Thermal decomposition of thionitrite **167**.*



(167)

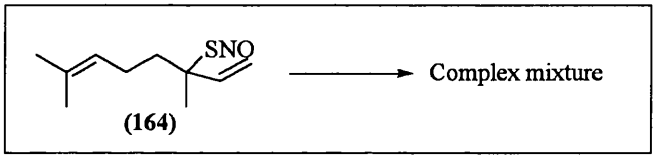
A complex mixture of non-polar products (P.E.) was obtained and its components could not be separated.

*3. 1. 1. 8. Thermal decomposition of thionitrite **166.***



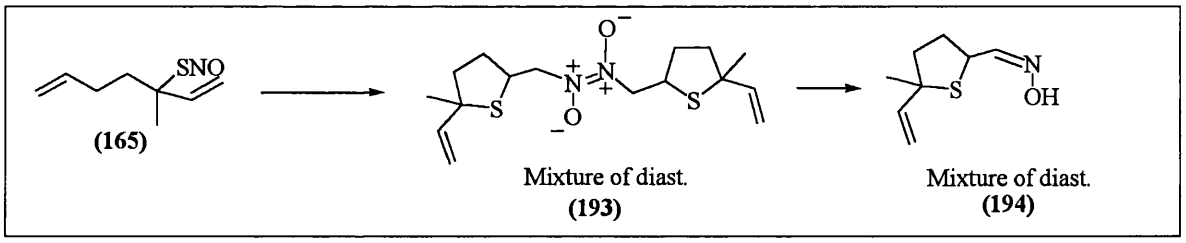
By TLC and ^{1}H NMR of the crude mixture, the composition is practically the same as that when the reaction was carried out in benzene. Products where only separated in that case and characterisation is shown below.

*3. 1. 1. 9. Thermal decomposition of thionitrite **164.***



The result was a complex mixture of compounds of all polarities which could not be separated.

*3. 1. 1. 10. Thermal decomposition of thionitrite **165.***



Chromatography on silica gel (P. E./Et_2O 80/20 to 70/30). A mixture of diastereomeric nitroso-dimers was isolated. This was heated at reflux in isopropanol for 1 night, affording a mixture of oximes.

Nitroso-dimers 193 (75 %, mixture of diastereomers). **^{1}H NMR** ($CDCl_3$, 400 MHz)**:** δ 5.8 (2H, m, 2 x =C<u>H</u>), 4.7 (2H, m, 2 x C<u>H</u>HN), 4.5 (4H, m, 2 x =C<u>H</u>$_2$), 4.4 (2H, m, 2 x CH<u>H</u>N), 3.2 (1H, m, C<u>H</u>S), 2.1 (4H, m, 2 x ring C<u>H</u>$_2$), 1.9 (2H, m, 2 x ring C<u>H</u>H), 1.78 (2H, m, 2 x ring CH<u>H</u>), 1.60 (6H, s, 2 x C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6

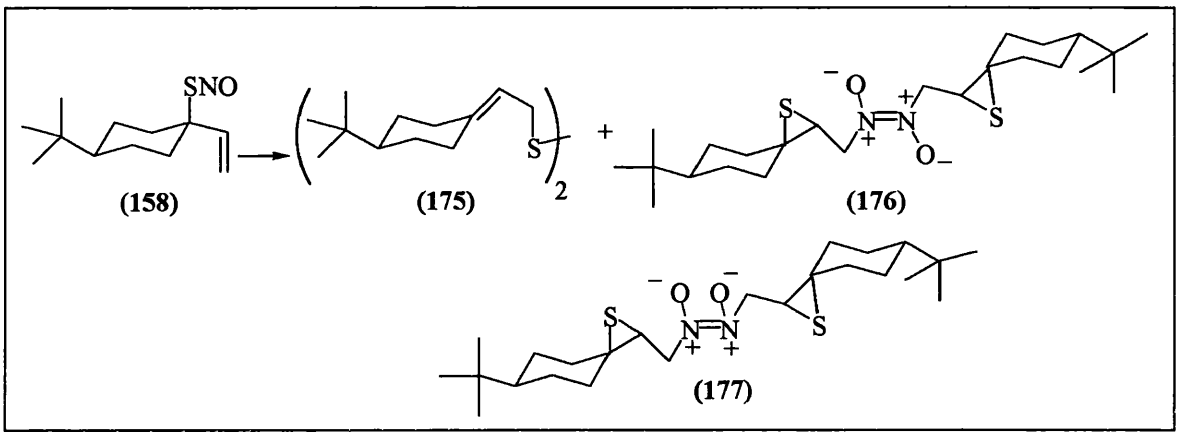
MHz): δ 137.45, 137.32, 115.41, 115.17, 65.85, 60.12, 60.10, 59.96, 50.34, 42.42, 41.37, 40.36, 35.57, 32.18, 31.67, 27.05, 20.57, 20.55, 15.27. **IR** (CCl_4): ν_{max}/cm^{-1} 2928, 2855, 1641, 1448, 1380, 1350, 1261, 1225, 1098, 1003. **MS** (FAB): m/z 342 ($M^{+\cdot}$, 10), 191 (10), 141 ($(M/2-NO)^+$, 25), 107 ($(M/2-NO-SH_2)^+$, 44). **MS** (EI): m/z 278 ($(M-S_2)^+$, 3), 256 (60), 160 (55).

5-Methyl-5-vinyl-2-hydroximinomethyltetrahydrothiophene (194). (yellow oil, mixture of four diastereomers in proportion *ca.* 3.2: 2: 1.6: 1) **^{1}H NMR** ($CDCl_3$, 300 MHz)**:** δ 7.98 (3.2H, d, J= 10.0, C<u>H</u>=NOH), 7.96 (1.6H, d, J= 10.0, C<u>H</u>=NOH), 7.28 (2H + 1H, m, 2 x C<u>H</u>=NOH), 6.46 (2H + 1H, d, J= 10.0, 2 x ring C_2<u>H</u>), 5.86 (3.2H + 1.6H, d, J= 10.0, 2 x ring C_2<u>H</u>), 4.72 (7.8H, m, 4 x =C<u>H</u>), 4.96 (15.6H, m, 4 x =C<u>H_2</u>), 2.15 (15.6H, m, 4 x ring C<u>H_2CH_2</u>), 1.84 (3H, s, C<u>H_3</u>), 1.81 (6H, s, C<u>H_3</u>), 1.80 (4.8H, s, C<u>H_3</u>), 1.76 (9.6H, s, C<u>H_3</u>). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 150.30, 150.03, 149.33 and 148.98 (4 x <u>C</u>HNOH), 147.77, 147.51, 138.73, 138.02, 137.96, 137.78, 138.67, 119.34 and 118.52 (<u>C</u>H), 115.91, 115.81, 115.55 and 115.50 (4 x =<u>CH_2</u>), 39.94, 39.67, 32.75, 32.71, 32.51, 32.44, 32.13 and 32.08 (8 x ring <u>CH_2</u>), 24.80, 24.53, 17.59 and 17.56 (4 x <u>CH_3</u>). **IR** (neat): ν_{max}/cm^{-1} 3259 (OH), 3078, 2923, 2853, 1642, 1449, 913. **MS** (FAB): m/z 141 ($(M/2-NO)^+$, 100).

<u>3. 1. 2. Reactions in benzene.</u>

Typically, the thionitrite was dissolved in 20 mL/mmol of dry benzene, under N_2, and the solution heated at 40 °C for a period of 7 to 24 h. From this point, the procedure described for the reactions in DCM was followed.

*3. 1. 2. 1. Thermal decomposition of thionitrite **158.***



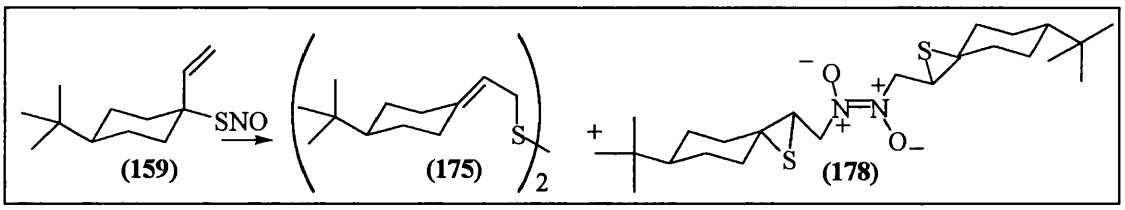
Purification by chromatography on silica, starting from P.E. and increasing the polarity until P.E./Et_2O 85/15. Three fractions were separated. The same purification method was used in the examples below.

Bis-[2-(4-tert-butylcyclohexylidene)-1-ethyl] disulfide (175). (P.E.; 16 %). It displayed the same spectral data as shown before.

Nitroso-dimer 177. (P.E./Et_2O 98/2, 9 %, white solid). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.84 (1H, dd, J= 14.8, 5.4, C<u>H</u>HN), 4.47 (1H, dd, J= 14.8, 9.2, CH<u>H</u>N), 3.32 (1H, dd, J= 9.2, 5.4, SC<u>H</u>CH_2N), 2.27 (1H, dt, J= 13.0, 4.0, $C_{2'(6')}$<u>H</u>H), 2.10-1.87 (3H, m, $C_{2'(6')}$<u>H</u>H + 2 x $C_{2'(6')}$H<u>H</u>), 1.4-1.25 (5H, m, 2 x $C_{3'(5')}$<u>H_2</u> + C_4<u>H</u>), 0.89 (9H, s, (C<u>H_3</u>$)_3$C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 75.49 (<u>C</u>H_2N), 56.25 (episulfide <u>C</u>S), 47.5 (<u>C_4</u>H), 40.16 (ring <u>C</u>H_2), 32.73 (($CH_3)_3$<u>C</u>), 27.59 ((<u>C</u>$H_3)_3$C), 27.42, 26.78 and 26.65 (ring <u>C</u>H_2). **IR** (CCl_4) ν_{max}/cm^{-1}: 2959, 2868, 1556, 1367, 1162. **MS** (FAB): m/z 454 ($(M-H)^+$, 0.75), 259 (4.0), 197 (56), 165 (96), 107 (100).

<u>Trans</u> nitroso dimer 176. (P.E./Et_2O 75/25; 30 %). See spectra above.

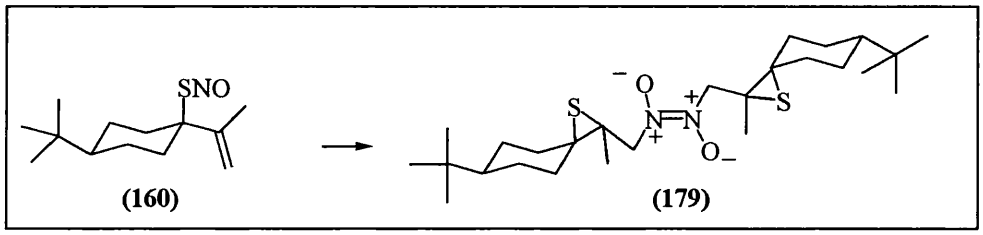
*3. 1. 2. 2. Thermal decomposition of thionitrite **159**.*



Bis-[2-(4-tert-butylcyclohexylidene)-1-ethyl] disulfide (175). (P.E.; 30 %). See spectra above.

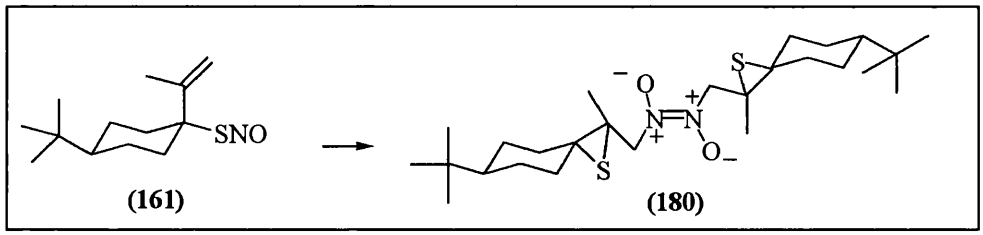
Nitroso-dimer 178. (P.E./Et_2O; 30 %). See spectra above.

*3. 1. 2. 3. Thermal decomposition of thinoitrite **160**.*



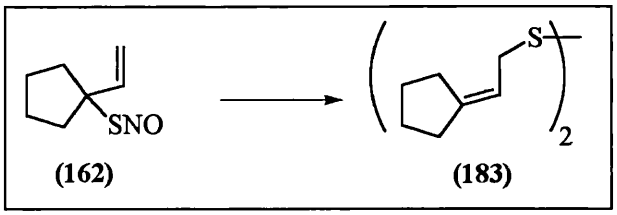
Nitroso-dimer 179. (P.E./Et_2O 75/25; 43 %). See spectra above.

*3. 1. 2. 4. Thermal decomposition of thionitrite **161**.*



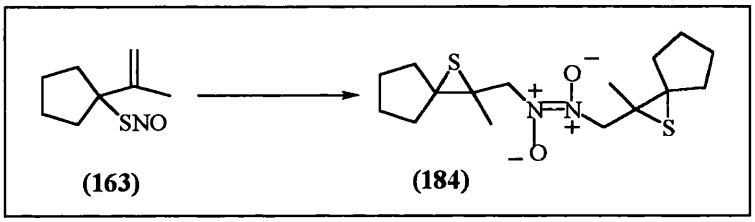
Nitroso-dimer 180. (P.E./Et_2O 75/25; 65 %). See spectra above.

*3. 1. 2. 5. Thermal decomposition of thionitrite **162**.*



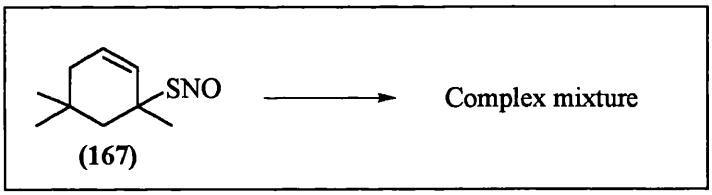
Bis [1-(2-cyclopentyliden)ethyl] disulfide (183). (P. E., 50 %). See spectra above.

*3. 1. 2. 6. Thermal decomposition of thionitrite **163**.*



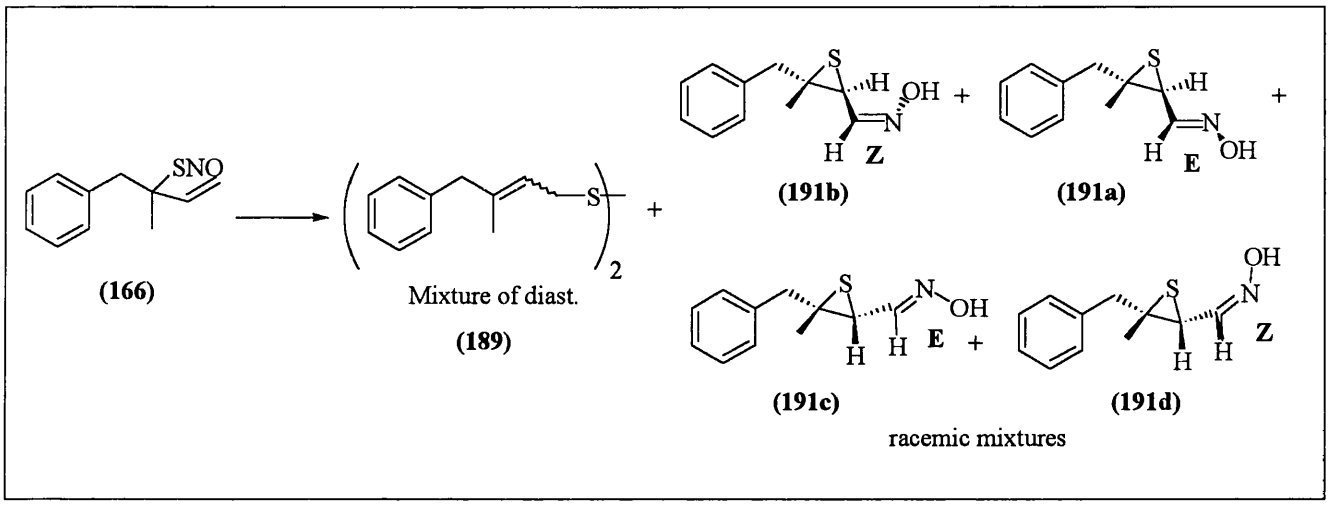
Nitroso-dimers 184. (chromatography on SiO_2, P.E./Et_2O 75/25; 58 %; mixture of two diastereomers proportion 2:1; white solid). See spectra above.

*3. 1. 2. 7. Thermal decomposition of thionitrite **167**.*



A complex mixture of non-polar products (P.E.) was obtained and its components could not be separated.

3. 1. 2. 8. Thermal decomposition of thionitrite 166.



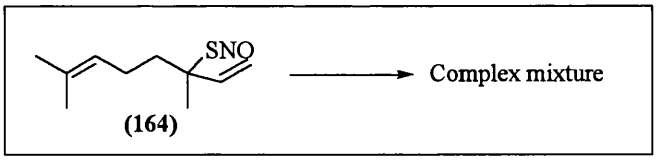
Two fractions were isolated by column chromatography on silica gel (P.E. to P.E/ Et_2O 70/30). The least polar corresponded to a mixture of diastereomers of disulfide **189**. The polar fraction corresponded to a complex mixture of diastereomeric nitroso-dimers (overall yield 52 %). In order to facilitate NMR analysis this mixture was transformed into a mixture of the corresponding oximes (**191**), by heating at reflux in isopropanol for one night. After evaporation of the solvent, the residue was additionally separated by column chromatography, affording two polar fractions. Each of the these contained two isomeric oximes.

(ZE) Bis-[3-methyl-4-phenyl-2-butenyl] disulfides 189. (approximately 2:1 of the *Z* and *E* isomers; not assigned; colourless oil; 21 %). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ for the mixture 7.2-7.3 (total of 5H, m, ArH), 5.35 and 5.31 (total of 1H, 2 x m, =CH), 3.44 and 3.36 (total of 2H, 2 x m, CH_2S), 3.36 and 3.24 (total of 2H, 2 x m, CH_2Ph), 1.57 and 1.55 (total of 3H, 2 x s, CH_3). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 150.1, 140.3 and 140.1 (C), 129.35, 129.06, 128.82, 128.72 and 126.58 (CH), 126.5 (C), 121.59 (CH), 46.51 (minor $PhCH_2$), 38.30, 37.82 and 37.83 (CH_2), 23.81(minor CH_3), 16.62 (major CH_3). **IR** (neat): ν_{max}/cm^{-1} 3025, 2972, 2918, 1657, 1601, 1493, 1450, 1207, 1080, 733, 699. **MS** (FAB): m/z 355 ($(M+H)^+$, 19), 281 (24), 221 (31), 219 (29), 211 ($(M/2 + SH_2)^+$, 52), 177 ($(M/2)^+$, 100). **HRMS** (FAB): found 355.1565, $C_{22}H_{27}S_2$ $(M+H)^+$ requires 355.1554.

(2,3)-Episulfide-4-phenyl-1-butane oximes (191a,c). (mixture 2:1, thick oil, 23 %). ***(±)-oxime 191a*** [2-(*R,S*)-3-(*S,R*) 15.3 %]. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.44 (1H, bs, NOH), 7.99 (1H, d, J= 10.4, CH=NOH), 7.25-7.10 (5H, m, ArH), 5.93 (1H, dd, J= 10.4, 1.1, CHS), 3.39 (2H, s, CH_2), 1.69 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 149.36 (CH=NOH), 147.06 (CS), 138.84 (C_{Ar}), 129.40, 128.98 and 126.93 ($C_{Ar}H$), 119.83 (CHS), 46.84 (CH_2), 17.37 (CH_3). **MS** (FAB): m/z 207 (M^+, 8), 191 ($(M-O)^+$, 6), 176 ($(M-NOH)^+$, 100), 158 (22), 147 (20), 143 ($(M-SNO)^+$, 32). ***(±)-oxime 191c*** [2-(*S,R*)-3-(*S,R*), 7.6 %]. **^{1}H NMR** ($CDCl_3$, 100 MHz): δ 8.44 (1H, bs, NOH), 8.15 (1H, d, J= 10.2, CH=NOH), 7.25-7.10 (5H, m, ArH), 6.01 (1H, d, J= 10.2, CHS), 3.51 (2H, s, CH_2), 1.70 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 149.15 (CH=NOH), 146.53 (CS), 138.84 (C_{Ar}), 129.40, 128.97 and 126.93 ($C_{Ar}H$), 120.23 (CHS), 38.99 (CH_2), 24.42 (CH_3). **IR** (neat, of the mixture): ν_{max}/cm^{-1} 3260 (b, OH), 3062, 3024, 2925, 1647, 1601, 1487, 1448, 1380, 1342, 1282, 1706, 969, 870, 742. **MS** (FAB, of the mixture): m/z 176 ($(M–NOH)^+$, 100). **HRMS** (FAB, of the mixture): found 207.0718, $C_{11}H_{13}SNO$ (M^+) requires 207.0733.

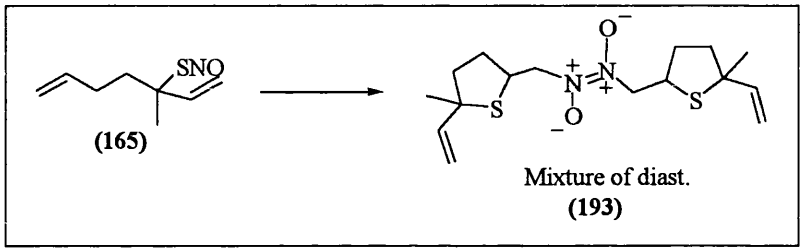
(2,3)-Episulfide-4-phenyl-1-butane oximes (191b,d). (mixture 6:1, white solid, 26 %). ***(±)-oxime 191b*** [2-(*R,S*)-3-(*S,R*), 22.3 %]. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.5 (1H, bs, NOH), 7.30 (1H, d, J= 9.8, CH=NOH), 7.25-7.15 (5H, m, ArH), 6.55 (1H, dd, J= 9.8, 0.9, CHS), 3.38 (2H, s, CH_2), 1.73 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 149.02 (CH=NOH), 147.03 (CS), 138.70 (C_{Ar}), 129.41, 128.99 and 126.97 ($C_{Ar}H$), 114.72 (CHS), 47.12 (CH_2), 17.21 (CH_3). ***(±)-oxime 191d*** [2-(*S,R*)-3-(*S,R*), 3.7 %]. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.5 (1H, bs, NOH), 7.52 (1H, d, J= 9.8, CH=NOH), 7.25-7.15 (5H, m, ArH), 6.62 (1H, dd, J= 9.8, 0.9, CHS), 3.52 (2H, s, CH_2), 1.74 (3H, s, CH_3). **IR** ($CHCl_3$, of the mixture): ν_{max}/cm^{-1} 3217 (b, OH), 3069, 3024, 2917, 1645, 1450, 902. **MS** (FAB, of the mixture): m/z 176 ($(M–NOH)^+$, 100). **HRMS** (FAB of the mixture): found 207.0718, $C_{11}H_{13}SNO$ (M^+) requires 207.0733.

*3. 1. 2. 9. Thermal decomposition of thionitrite **164**.*



The result was a complex mixture of compounds of all polarities, which could not be separated.

*3. 1. 2. 10. Thermal decomposition of thionitrite **165**.*



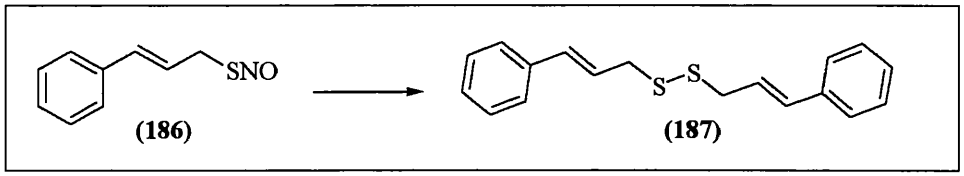
Nitroso-dimers 193 (SiO_2; P.E./Et_2O 80/20; 75 %, mixture of diastereomers). See spectra above.

3. 2. Thermal decompositon of unsaturated primary and secondary thionitrites.

3. 2. 1. *In-situ* preparation of unstable thionitrites, general procedure.

Primary and secondary thionitrites were prepared in solution by dissolving the corresponding thiol in benzene, under N_2, and adding 1.1-1.2 equivalents of *tert*-butylnitrite. The solutions turned immediatelly red. After 10-15 min, heating was started at 40 °C, until total disappearance of the colour (generally from 4 h to 1-2 days). The solvent was evaporated at low pressure and the crude reaction mixture separated by chromatography on silica gel.

3. 2. 2. Thermal decomposition of thionitrite **186**.

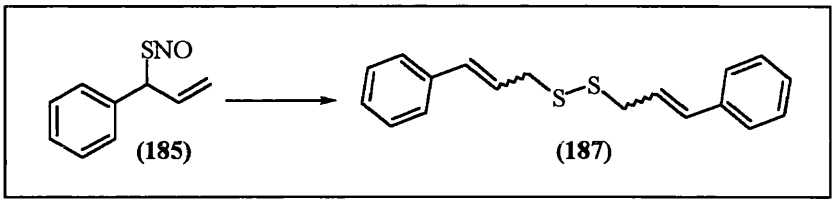


(186) (187)

Chromatography on SiO_2 (P. E.)

Bis [3-phenyl-2-propen-1-yl] disulfides. (50 %, mixture of diastereomers of which the all-*trans* (**187**) was the major one in all cases; white solid). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.38-7.25 (5H, m, ArH), 6.48 (1H, d, J= 15.8, =CH), 6.20 (1H, dt, J= 15.8, 7.6, =$CHCH_2S$), 3.51 (2H, dd, J= 7.6, 1.0, CH_2S). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 136.59, 133.57, 128.58, 127.68, 126.36 and 124.46, 42.45 (CH_2S). **IR** ($CHCl_3$, of the mixture): ν_{max}/cm^{-1} 3027, 2923, 1635, 1599, 1492, 1449, 1412, 1209, 962, 749, 695. **MS** (FAB): m/z 299 ($(M+H)^+$, 3), 298 ($M^{+\cdot}$, 3), 149 ($(M/2)^+$, 11), 117 ($(M/2-S)^+$, 100). **HRMS** (FAB): found 298.0850, $C_{18}H_{18}S_2$ ($M^{+\cdot}$) requires 298.0864.

3. 2. 3. Thermal decomposition of thionitrite **185**.

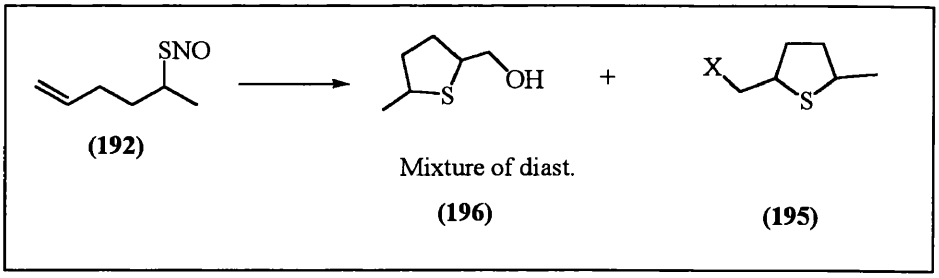


(185) (187)

Chromatography on SiO_2 (P.E.)

Bis [3-phenyl-2-propen-1-yl] disulfides (187). (chromatography on SiO_2 (P.E.); 50 %; mixture of diastereomers of which the all-*trans* (**187**) was the major one, in all cases; white solid). See spectra above.

3. 2. 4. Thermal decomposition of thionitrite **192**.



Two fractions were isolated in order of increasing polarity:

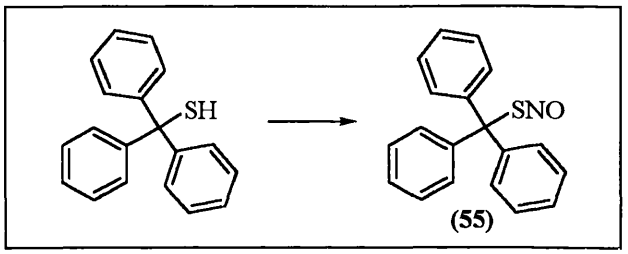
Tetrahydrothiophene 195. (P. E. 80/20; 19 %). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 4.35 (2H, d, J= 11.4, $C\underline{H}_2X$), 4.07 (1H, m, $C_2\underline{H}$), 3.47 (1H, m, $C_5\underline{H}$), 2.3-2.0 (2H, m, ring $C\underline{H}_2$), 1.7-1.6 (2H, m, ring $C\underline{H}_2$), 1.27 (3H, d, J= 11.4, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 80.46 ($\underline{C}H_2X$), 45.60 ($\underline{C}H$), 44.67 ($\underline{C}H$), 38.77 ($\underline{C}H_2$), 34.18 ($\underline{C}H_2$), 22.43 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 2959, 2929, 2863, 1554, 1446, 1377. **MS** (EI): m/z 191 ((cycle + N_2O_3)$^{+\cdot}$, 25), 149 (54), 115 ((cycle)$^+$, 55), 81 (43), 57 (53), 43 (32).

2-Hydroxymethyl-4-methyltetrahydrothiophene (196). (P. E. 80/20; 19 %). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 3.90 (1H, bs), 3.5-3.3 (3H, m), 3.45 (1H, m, $C\underline{H}S$), 2.89 (1H, dd, J= 15.4, 1.8), 2.69 (1H, m, $C\underline{H}S$), 2.67 (1H, dm, J= 15.4), 2.1-1.3 (8H, m, 2 x ring $C\underline{H}_2C\underline{H}_2$), 1.26 and 1.13 (3H each, d, J= 6.8, 2 x $C\underline{H}_3$).**^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 66.27 ($\underline{C}H_2$), 62.41 ($\underline{C}H$), 52.47 ($\underline{C}H$), 44.75 ($\underline{C}H$), 38.74 ($\underline{C}H_2$), 37.47 ($\underline{C}H$), 36.91 ($\underline{C}H_2$), 32.76 ($\underline{C}H_2$), 32.69 ($\underline{C}H_2$), 31.14 ($\underline{C}H_2$), 23.20 ($\underline{C}H_3$), 21.88 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 3367 (OH), 2926, 1654, 1034. **MS** (FAB): m/z 133 ((M+H)$^+$, 100), 115 ((M–HO)$^+$, 47).

CHAPTER 4

INTERMOLECULAR ADDITION OF THIONITRITES ONTO ALKENES.

4. 1. Preparation of trityl thionitrite (55).

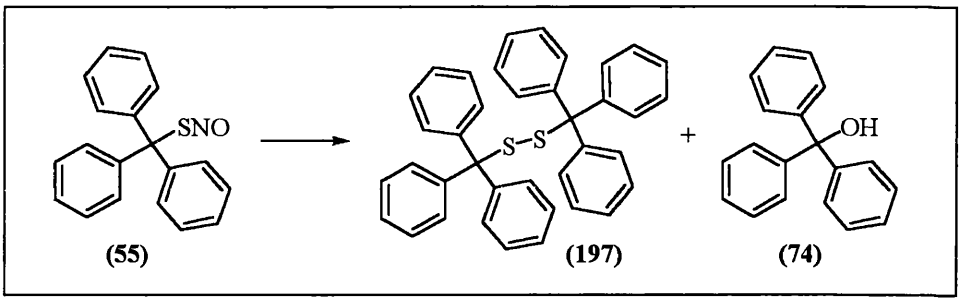


(55)

Triphenylmethyl mercaptan (trityl thiol, 1.7 g, 42 mmol) was dissolved in benzene (24 mL) and sodium nitrite (3.5 g, 50.4 mmol) was added, dissolved in H_2O (10 mL). The resulting mixture was cooled to 5 °C and sulfuric acid (15 %, cat.) was added dropwise. The reaction was stirred for 15 min in the dark (covering the flask with Al foil). It was diluted with additional benzene and washed several with several portions of H_2O. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude thionitrite was re-crystallised from $CHCl_3$/EtOH, in the dark, affording the product in the form of bright green prisms (9.45 g, 73 %).

Trityl thionitrite (55). **mp** ($CHCl_3$/EtOH): 100 °C decomp., lit[182]: 99 °C (decomp.). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.30 (9H, m), 7.14 (6H, m). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 143.60 (3 x $\underline{C}_{Ar}$), 129.91 and 128.17 (6 x $\underline{C}_{Ar}$H each), 127.61 (3 x $\underline{C}_{Ar}$), 76.0 (<u>C</u>SNO). **MS** (EI): m/z 276 ($(M–NO+H)^+$, 0.6), 275 ($(M–NO)^+$, 1.1), 274 (3), 244 ($(M–SNO+H)^+$, 38), 243 (Tr^+, 100), 242 (79), 241 (80), 215 (24), 166 (Ph_2CH^+, 37), 165 (100). **MS** (FAB): m/z 275 ($(M–NO)^+$, 20), 244 (24), 243 (Tr^+, 100). 165 (67). **UV** ($CHCl_3$): λ_{max} 240, 352, 602.

4. 2. Thermal decomposition of trityl thionitrire.

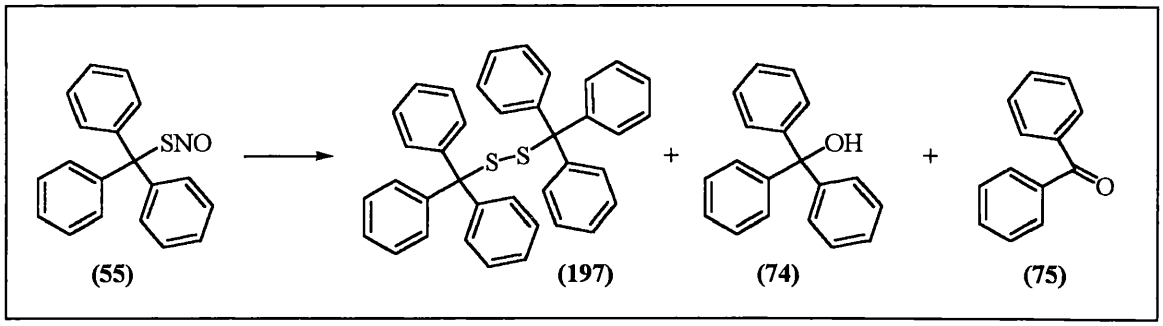


Trityl thionitrite (**55**) was dissolved in dry benzene under N_2. The solution was heated at 65 °C for 5 h, until it became yellow. The solvent was evaporated under reduced pressure and the crude reaction mixture separated by chromatography on silica gel (P.E./Et_2O 80/20), affording two products in order of increasing polarity:

Trityl disulfide (197). (50 %; white solid)[183] **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.40-7.19 (9H, m), 7.24 (6H, s). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 143.46 (3 x $\underline{C}_{Ar}$), 130.25 and 127.81 (6 x $\underline{C}_{Ar}$H each), 127.95 (3 x $\underline{C}_{Ar}$), 72.54 ($\underline{C}$S). **IR** (CH_2Cl_2): ν_{max}/cm^{-1} 3077, 3025, 1924, 1897, 1810, 1595, 1492, 1446, 1184, 1077, 1031, 667. **MS** (EI): m/z 486 ($(M-S_2)^{+\cdot}$, 2), 409 (5), 244 ($(M-SNO+H)^+$, 30), 243 (Tr^+, 100), 241 (79), 166 (Ph_2CH^+, 94), 165 (100).

Trityl alcohol (74). (30 %; white solid)[184] **mp** (DCM/P.E.): 165 °C. lit[184]: 166 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.30 (15H, m, Ar$\underline{H}$), 2.81 (1H, s, O$\underline{H}$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 146.82, 127.92, 127.90, 127.26, 81.99. **IR** (CH_2Cl_2): ν_{max}/cm^{-1} 3590 (OH), 3027, 1958, 1906, 1816, 1598, 1491, 14447, 13332, 1160, 1013, 894. **MS** (FAB): m/z 260 ($M^{+\cdot}$, 13), 244 (21), 243 (Tr^+, 100), 183 ($(M-C_6H_5)^+$, 24), 165 (32), 154 (41), 136 (35).

4. 3. Photochemical decomposition of trityl thionitrite.



Trityl thionitrite (**55**) was dissolved in dry benzene under N_2. The resulting solution was irradiated with visible light (500 Watt tungsten lamp) at r. t. until total disappearace of the green colour was observed (1 night). The solvent was then removed under *vacuo* and the remaining residue was separated by chromatography on silica gel (P.E./Et_2O 80/20) , affording three products in order of increasing polarity:

Trityl disulfide (197).(25 %; white solid). This product displayed the same spectral data as the product obtained from the thermal decomposition of trityl thionitrite.

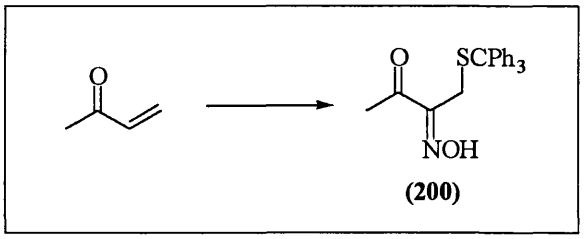
Benzophenone (75). (8 %; white solid). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.81 (2H, m), 7.53 (3H, m). **IR** (CH_2Cl_2): ν_{max}/cm^{-1} 3067, 1658, 1597, 1445, 1314, 1273, 944, 919. **MS** (FAB): m/z 183 ($(M+H)^+$, 67), 105 ($(M+H–Ph)^+$, 69).

Trityl alcohol (74). (38 %; white solid). This product displayed the same spectral data as the product obtained from the thermal decomposition of trityl thionitrite.

4. 4. Addition of trityl thionitrite onto alkenes under optimised thermal conditions, general procedure.

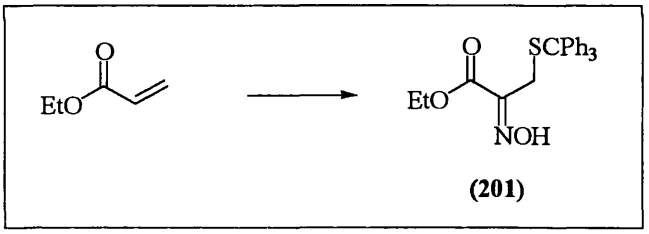
Trityl thionitrite (**55**, 200 mg, 0.65 mmol) was dissolved in 10 mL of isopropanol and 5 mL of benzene. The solution was bubbled though with nitrogen for 5-10 min and the alkene (6 equivalents) was added. The green mixture was then heated at 65 °C until total disappearence of the green colour (usually between 30 min and 1 h). The solvent was evaporated at reduced pressure and the residue chromatographed (solvent system indicated between brackets for each case). Only the addition products are indicated. In all cases, the unreacted excess of the alkene and products derived from the thermal decomposition of trityl thionitrite were also separated but have not been quantified.

4. 4. 1. Derivatives of carboxylic acids and related.



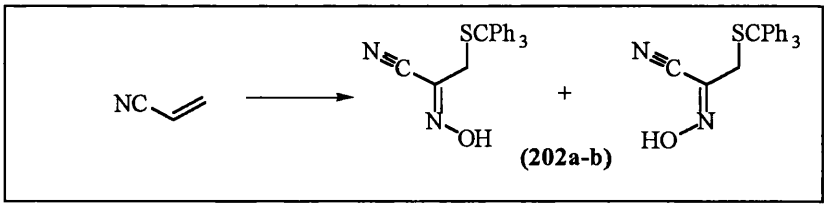
(200)

2-Hydroxyimino-1-tritylthio-3-butanone (200). (P.E./Et_2O 98:2 to 70:30; 56 %; white solid; single isomer). **mp** (P.E./Et_2O): 84-85 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.27 (1H, bs, NO<u>H</u>), 7.25-7.06 (15H, m, Ar<u>H</u>), 3.03 (2H, s, C<u>H</u>$_2$S), 2.12 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 195.42 (<u>C</u>=O), 156.46 (<u>C</u>=N), 144.64 (3 x <u>C</u>$_{Ar}$), 130.11 and 128.35 (6 x <u>C</u>$_{Ar}$H each), 127.20 (3 x <u>C</u>$_{Ar}$H), 67.77 (<u>C</u>S), 25.64 (<u>C</u>H$_3$), 22.92 (<u>C</u>H$_2$S). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3292 (bs, OH), 3046, 2923, 2861, 1681 (C=O), 1594 (C=N), 1486, 1440, 1368, 1163, 1086, 998. **MS** (FAB): m/z 398 ($(M+Na)^+$, 100). **HRMS** (FAB): found 398.1191, $C_{23}H_{21}SNO_2$ $(M+Na)^+$ requires 398.1175.



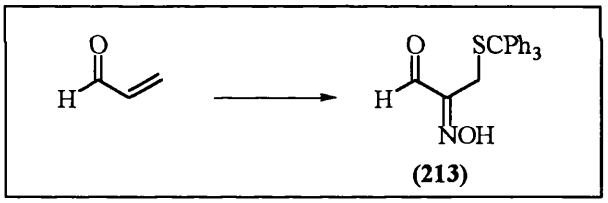
(201)

Ethyl 2-Hydroxyimino-1-tritylthiopropanoate (201). (P.E./Et_2O 98:2 to 70:30; 25 %; white soli; single isomer). **mp** (Et_2O/P.E): 120-121 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.99 (1H, bs, NO<u>H</u>), 7.38-7.14 (15H, m, Ar<u>H</u>), 4.18 (2H, q, J= 9.5, $CH_3C\underline{H}_2O$), 3.20 (2H, s, C<u>H</u>$_2$S), 1.28 (3H, t, J= 9.5, C<u>H</u>$_2$O). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 162.37 (<u>C</u>=O), 149.00 (<u>C</u>=NOH), 144.18 (3 x <u>C</u>$_{Ar}$), 128.64 and 126.93 (6 x <u>C</u>$_{Ar}$H each), 125.80 (3 x <u>C</u>$_{Ar}$H), 66.36 (<u>C</u>S), 61.04 (<u>C</u>H$_2$O), 24.68 (<u>C</u>H$_2$S), 13.05 (<u>C</u>H$_3$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3400 (b, OH), 1715 (C=O), 1575 (C=N). **MS** (FAB): m/z 428 ($(M+Na)^+$, 17), 328 (5), 244 ($(Tr+H)^{+\cdot}$, 90), 243 (Tr^+, 92), 165 (100), 154 (27). **HRMS** (FAB): found 428.1296, $C_{24}H_{23}SNO_3Na$ $(M+Na)^+$ requires 428.1304.



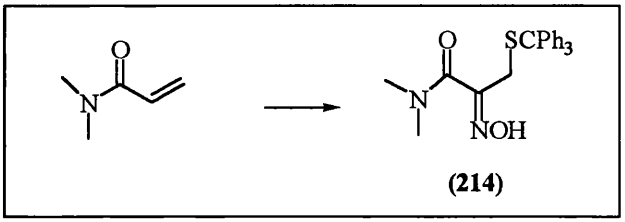
(202a-b)

(ZE)-2-Hydroxyimino-1-tritylthioethylnitrile (202a-b). (P.E./Et_2O 98:2 to 70:30; mixture 1:1 of the two isomeric oximes, not separated; 41 %; white solid; highly hygroscopic). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.41-7.20 (30H, m, ArH), 3.19 (2H, s, CH_2S), 3.14 (2H, s, CH_2S). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 143.71 and 143.66 (3 x C_{Ar} each), 136.72 and 130.61 (C=NOH), 129.54, 129.52, 129.20 and 128.20 (6 x $C_{Ar}H$ each), 127.16 and 127.14 (3 x $C_{Ar}H$ each), 113.81 and 109.32 (CN), 67.91 and 66.10 (CS), 32.73 and 27.36 (CH_2S). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3305 (b, OH), 3059, 3028, 2915, 2864, 2210 (w, CN), 1595 (C=N), 1487, 1441, 1261, 1024. **MS** (FAB): m/z 381 ($(M+Na)^+$, 15/50), 243 (Tr^+, 100), 165 (25). **HRMS** (FAB): found 381.1038, $C_{22}H_{18}SN_2ONa$ $(M+Na)^+$ requires 381.1050.



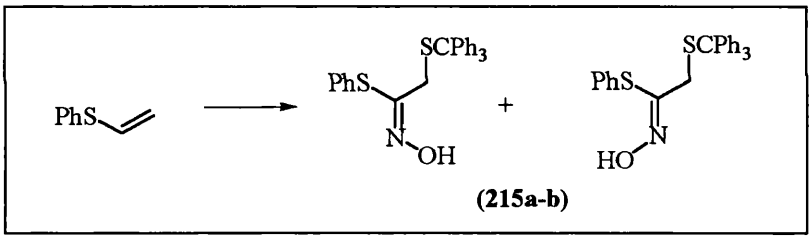
(213)

2-Hydroxyimino-3-tritylthio-1-butanal (213). (P.E./Et_2O 95:5 to 70:30; 42 %; white-yellow, highly hygroscopyc solid; single isomer). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 9.70 (1H, bs, NOH), 9.25 (1H, s, CHO), 7.34-7.15 (15H, m, ArH), 3.07 (2H, s, CH_2S). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 189.03 (C=O), 156.75 (C=N), 144.04 (3 x C_{Ar}), 129.62 and 127.95 (6 x $C_{Ar}H$ each), 126.83 (3 x $C_{Ar}H$), 67.36 (CS), 21.79 (CH_2S). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3450 (b, OH), 3017, 1708 (C=O), 1418, 1358, 1220, 1083. **MS** (FAB): m/z 384 ($(M+Na)^+$, 7), 243 (Tr^+, 100). **HRMS** (FAB): found 384.1034, $C_{22}H_{19}NO_3SNa$ $(M+Na)^+$ requires 384.1050.



(214)

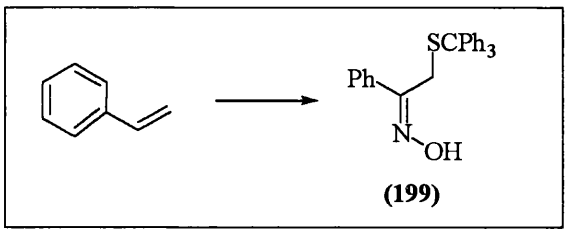
2-Hydroxyimino-N,N-dimethyl-3-tritylthiopropanamide (214). (DCM/MeOH 99:1; second chromatography with Et_2O; 42 %; white solid; single isomer). **mp** (Et_2O/P.E.): 184-186 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.10 (1H, bs, NOH), 7.43-7.18 (15H, m, ArH), 3.30 (2H, s, CH_2S), 2.93 and 2.87 (3H each, 2 x s, 2 x CH_3). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 165.20 (C=O), 151.64 (C=N), 144.68 (3 x C_{Ar}), 130.02 and 128.39 (6 x $C_{Ar}H$ each), 127.26 (3 x $C_{Ar}H$), 67.89 (CS), 39.30 and 35.86 (CH_3), 26.55 (CH_2S). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3345 (b, OH), 3009, 2939, 1636 (C=O), 1496, 1442, 1410, 966. **MS** (FAB): m/z 427 ($(M+Na)^+$, 3), 387 ($(M-OH)^+$, 1), 243 (Tr^+, 100). **HRMS** (FAB): found 427.1456, $C_{24}H_{24}N_2O_2SNa$ $(M+Na)^+$ requires 427.1473.



(215a-b)

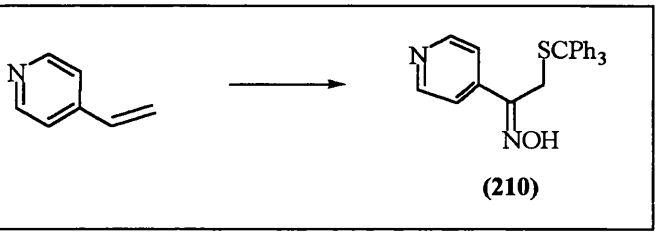
(ZE)-1-Phenylthio-2-tritylthio-1-ethane oxime (215a-b). (P.E./Et_2O 98:2 to 70:30; mixture 1:1 of the two isomeric oximes; 29 %; white-yellow highly hygroscopyc solid). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.93 (2H, m, 2 x =CNOH), 7.40-7.12 (20H, m, ArH), 3.10 (2H, s, CH_2S), 2.63 (2H, s, CH_2S). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 156.42 and 152.80 (C=NOH), 144.57 and 144.52 (Tr C_{Ar}), 136.54 and 134.66 (Ph C_{Ar}), 130.36, 130.05, 129.99, 129.75, 129.63, 129.62, 129.36, 128.37, 128.31, 127.19 and 127.02 ($C_{Ar}H$), 67.87 and 67.26 (CS), 34.43 and 29.95 (CH_2S). **IR** ($CHCl_3$):ν_{max}/cm^{-1} 3279 (OH), 3056, 3024, 2921, 2850, 1595 (C=N), 1478, 1442, 1381, 1182, 1085, 969, 912. **MS** (FAB): m/z 464 ($(M+Na)^+$, 17/10), 364 ($(M-Ph)^+$, 40/10), 243 (Tr^+, 100), 165 (62), 154 (17). **HRMS** (FAB): found 464.1119, $C_{27}H_{23}NOS_2Na$ $(M+Na)^+$ requires 464.1109.

4. 4. 2. Styrene-type compounds.



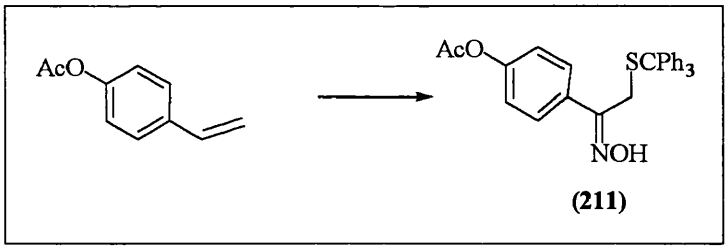
(199)

(Z)-1-Phenyl-2-tritylthio-1-ethane oxime (199). (P.E./Et_2O 98:2 to 70:30; 72 %; white solid). **mp** (DCM/P.E.): 145-147 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.93 (1H, bs, NO<u>H</u>), 7.65 (7H, dm, Ar<u>H</u>), 7.64-7.42 (13H, m, Ar<u>H</u>), 3.55 (2H, s, $C\underline{H}_2S$). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 155.10 (<u>C</u>=NOH), 144.65 (3 x $\underline{C}_{Ar}$), 134.71 ($\underline{C}_{Ar}$), 130.10, 129.86, 128.80, 128.40, 127.22, 126.72 ($\underline{C}_{Ar}H$), 70.30 (<u>C</u>S), 26.80 ($\underline{C}H_2S$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3313 (b, OH), 3056, 2923, 1682, 1635, 1595 (C=N), 1486, 1445. **MS** (FAB): m/z 432 ($(M+Na)^+$, 8/50), 410 ($(M+H)^+$, 17/50), 378 ($(M-NOH)^+$, 8/50), 234 (Tr^+, 100). **HRMS** (FAB): found 410.1579, $C_{27}H_{24}SNO$ $(M+H)^+$ requires 410.1597. **X-ray:** see data in appendix.



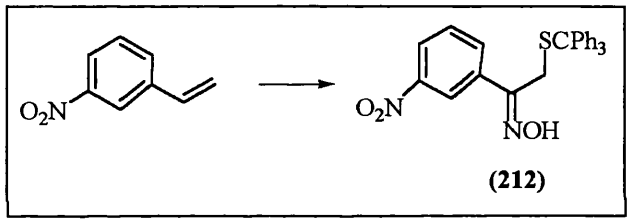
(210)

2-(4'-Pyridyl)-1-tritythio-2-ethane oxime (210). (P.E./Et_2O 80:20, followed by re-crystallisation from $CHCl_3$/P.E.; 87 %; crystalline solid; single isomer). **mp** (MeOH/P.E): 206 °C decomp. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.71 (1H, bs, O<u>H</u>), 8.54 (2H, d, J= 5.0 Hz, pyr $\underline{C}_{orto}H$), 7.52 (d, 6H, Ar<u>H</u>), 7.34-7.24 (13H, m, Ar<u>H</u>), 3.42 (2H, s, $C\underline{H}_2S$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 153.14 (<u>C</u>=NOH), 150.21 (pyr <u>C</u>H), 144.50 ($\underline{C}_{Ar}$), 130.02, 128.46, 127.34 and 120.82 ($\underline{C}_{Ar}H$), 68.10 (<u>C</u>S), 25.94 ($\underline{C}H_2S$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3379 (b, OH), 3010, 2929, 2853, 1731, 1598 (C=N), 1443, 1249, 950, 830. **MS** (FAB): m/z 411 ($(M+H)^+$, 100). **HRMS** (FAB): found 411.1531, $C_{26}H_{23}SN_2O$ $(M+H)^+$ requires 411.1519. **EA**: Found C 76.08, H 5.45, N 6.77, S 8.21 %, calculated for $C_{26}H_{22}SN_2O$ C 76.07, H 5.41, N 6.82, S 7.81 %.



(211)

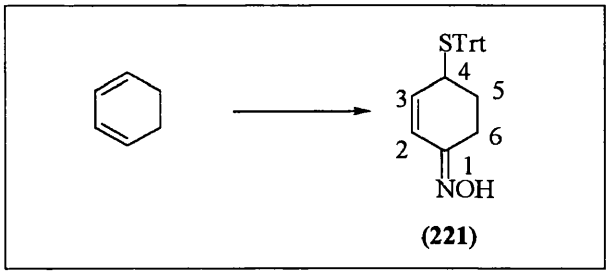
1-(4'-Acetoxy-1-phenyl)-2-tritylthio-1-ethane oxime (211). (P.E./Et_2O 80:20; 22 %; white hygroscopyc solid; single isomer; unstable). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.3-6.8 (19H, m, ArH), 3.21 (2H, s, CH_2S), 2.20 (3H, s, CH_3CO). **^{13}C NMR** ($CDCl_3$, 400 MHz): δ 169.69 (C=O), 154.21 (C=NOH), 66.33 (CS), 26.93 (CH_2S), 21.59 (CH_3). **IR** (CH_3): ν_{max}/cm^{-1} 3580 (OH), 3055, 3002, 1753 (C=O), 1593 (C=N), 1502, 1441, 1365, 1205 (C-O), 1159, 1008, 916. **MS** (FAB): m/z 243 (Tr^+, 100). Instability of the product precluded HRMS measurements.



(212)

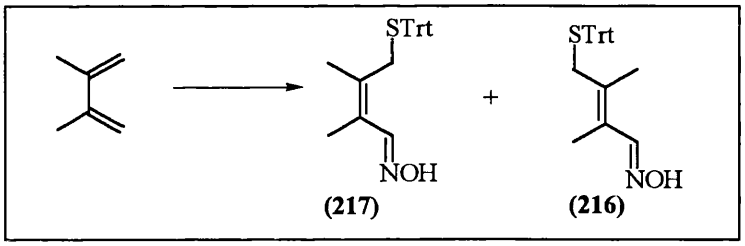
1-(3-Nitrophenyl)-2-tritylthio-1-ethan oxime (212). (P.E./Et_2O 97:3 to 80:20; 10 %; white hygroscopyl solid; single isomer). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.17 (2H, m, nitro-ring $C_{Ar}H$), 8.10 (1H, d, J= 5.4, nitro-ring $C_{Ar}H$), 7.52 (1H, d, J= 5.4, nitro-ring $C_{Ar}H$), 7.43-7.17 (15H, m, trityl ArH), 3.33 (2H, s, CH_2S). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 153.33 (C=N), 148.76 (nitro-ring C_{Ar}), 144.38 (trityl C_{Ar}), 136.51 (nitro-ring C_{Ar}), 132.38 (nitro-ring $C_{Ar}H$), 129.97, 128.53 (trityl 6 x $C_{Ar}H$ each), 127.38 (trityl 3 x $C_{Ar}H$), 124.39 and 121.76 (nitro-ring $C_{Ar}H$), 68.06 (CS), 26.58 (CH_2S). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3565, 3299, 3055, 3017, 1533 (C=N), 1487, 1441, 1350, 1213. **MS** (FAB): m/z 477 ($(M+Na)^+$, 2), 243 (Tr^+, 100). **HRMS** (FAB): found 477.1249, $C_{27}H_{22}N_2O_3SNa$ $(M+Na)^+$ requires 477.1275.

4. 4. 3. Dienes.



(221)

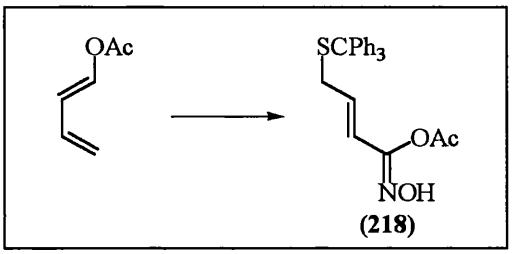
4-Tritylthio-2-cyclohexen-1-oxime (221). (P.E./Et_2O 98:2 to 70:30; 42 %; white solid; single isomer). **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 5.96 (1H, d, J= 8.1, =C_2H), 5.88 (1H, dd, J= 8.1, 3.9, =C_3H), 3.06 (1H, m, CHS), 2.58 (1H, m, ring CHH), 2.49 (1H, m, ring CHH), 1.53 (2H, m, ring CH_2). **^{13}C NMR** ($CDCl_3$, 125.8 MHz): δ 156.09 (C=N), 145.13 (3 x C_{Ar}), 136.42 and 124.79 (=$C_{2(3)}$H), 129.98 and 128.37 (6 x C_{Ar}H each), 127.16 (3 x C_{Ar}H), 68.38 (CS), 40.96 (CHS), 27.13 and 19.73 (ring $C_{5(6)}H_2$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3191, 3056, 2928, 2850, 1594 (C=N), 1489, 1440, 1382, 1266, 999, 802, 1741. **MS** (FAB): m/z 408 ($(M+Na)^+$, 17/10), 386 ($(M+H)^+$, 57/10), 307 ($(M-Ph)^+$, 25), 244 ($(Tr+1)^+$, 100), 243 (Tr^+, 45), 242 ($(Trt-1)^+$, 45), 176 (34), 165 (72), 154 (52). **HRMS** (FAB): found 386.1579, $C_{25}H_{24}SNO$ $(M+H)^+$ requires 386.1568



(217) **(216)**

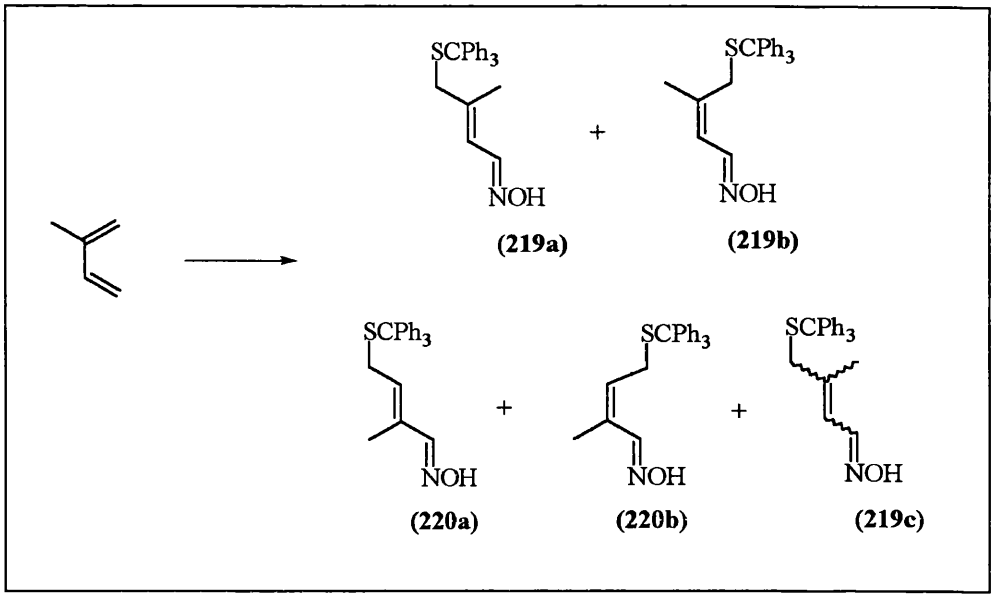
(2,3-Z) and (2,3-E) 2,3-Dimethyl-4-tritylthio-2-buten-1-oxime (217, 216). (P.E./Et_2O 98:2 to 70:30; 75 % of a mixture of 216 with traces of 217, not separated; white solid; stereochemistry of the oxime bond unknown). **mp** (P.E./Et_2O): 123-124 °C. ***Major product (216, (2,3-E)).*** **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.07 (1H, s, CHNOH), 7.41-7.38 (6H, m, ArH), 7.25-7.16 (9H, m, ArH), 2.77 (2H, s, CH_2S), 1.76 (3H, s, CH_3), 1.67 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 150.30 (C=N), 144.59 (3 x C_{Ar}), 136.34 (C=), 129.50, 127.95 and 126.71 (15 x C_{Ar}H), 127.04 (C=), 66.90 (CS), 37.32 (CH_2S), 18.24 and 13.17 (CH_3). ***Minor product (217, (2,3-Z)).*** **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.94 (1H, s, CHNOH), 2.85 (2H, s, CH_2S), 1.78 (3H, s, CH_3), 1.76 (3H, s,

$C\underline{H}_3$). **IR** (neat, of the mixture): ν_{max}/cm^{-1} 3285 (b, OH), 3059, 2925, 2874, 1685 (C=C), 1594 (C=N), 1487, 1441, 1421, 1365, 1162, 1086, 994, 740. **MS** (FAB, of the mixture): m/z 378 ($(M+H)^+$, 15/50), 243 (Tr^+, 100), 165 (22), 154 (14). **HRMS** (FAB): found 378.0953, $C_{25}H_{16}SNO$ $(M+H)^+$ requires 378.0947.



(218)

(2,3-E)-1-Acetoxy-4-tritylthio-2-buten-1-oxime (218). (P.E./Et_2O 80:20 to 50:50 10 %; white hygroscopic solid; single isomer). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.80 (1H, bs, NO$\underline{H}$), 7.46-7.24 (15H, m, Ar$\underline{H}$), 6.59 (1H, dt, J= 15.0, 7.6, =C$\underline{H}CH_2S$), 5.59 (1H, d, J= 15.0, C$\underline{H}$=C), 2.90 (2H, dd, J= 7.2, 1.2, C$\underline{H}_2$S), 2.37 (3H, s, C$\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 168.75 ($\underline{C}$=O), 143.25 (3 x $\underline{C}_{Ar}$), 142.22 ($\underline{C}$=NOH), 128.54, 127.03 and 125.90 (15 x $\underline{C}_{Ar}H$), 119.08 (=$\underline{C}$H), 66.44 ($\underline{C}$S), 32.47 ($\underline{C}H_2S$), 17.37 ($\underline{C}H_3$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3352, 3009, 2925, 2849, 1784, 1708, 1647, 1487, 1441, 1357, 1213. **MS** (FAB): m/z 440 ($(M+Na)^+$, 98), 340 (100). **HRMS** (FAB): found 440.1278, $C_{26}H_{23}SNO_3Na$ $(M+Na)^+$ requires 440.1296.



Three main fractions were separated, each of them containing several diastereomers. The first fraction corresponds to regioisomer **220** and the second and third fractions to regioisomer **219**.

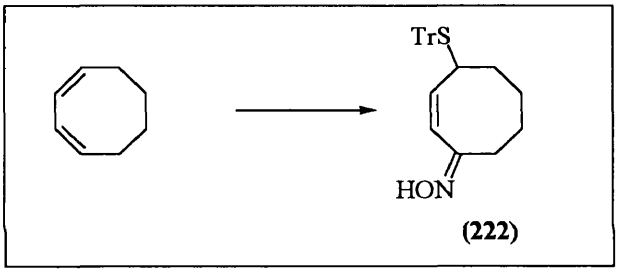
2-Methyl-4-tritylthio-2-buten-1-oximes 220a and 220b (P. E./Et_2O, 80/20; 5 %; formed by two diastereomers in proportions 4:1). ***Oxime 220a*** (***(2,3-E)***, 4 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.61 (1H, b, O<u>H</u>), 7.53 (1H, s, C<u>H</u>CNOH), 7.4 (15H, m, Ar<u>H</u>), 5.47 (1H, t, J= 7.7, =C<u>H</u>), 2.85 (2H, d, J= 7.7, C<u>H</u>$_2$S), 1.72 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 154.52 (<u>C</u>=NOH), 144.94 (3 x <u>C</u>$_{Ar}$), 133.85 (=<u>C</u>), 132.53 (=<u>C</u>H), 129.96 and 128.39 (6 x <u>C</u>$_{Ar}$H each), 127.27 (3 x <u>C</u>$_{Ar}$H), 67.56 (<u>C</u>S), 30.33 (<u>C</u>H$_2$S), 11.73 (<u>C</u>H$_3$). ***Oxime 220b*** (***(2,3-Z)***, 1 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.86 (1H, s, C<u>H</u>CNOH), 7.61 (1H, b, O<u>H</u>), 7.5 (15H, m, Ar<u>H</u>), 5.57 (1H, t, J= 7.7, =C<u>H</u>), 2.81 (2H, d, J= 7.7, C<u>H</u>$_2$S), 1.61 (3H, s, C<u>H</u>$_3$).

3-Methyl-4-tritylthio-2-buten-1-oximes 219a and 219b (P. E./Et_2O, 80/20; 23 %; formed by two diastereomers in proportions 4:1). ***Oxime 219a*** (***(2,3-E)***, 18.4 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.7 (bs, OH), 7.86 (1H, d, J= 10.2, C<u>H</u>CNOH), 7.5 (15H, m, Ar<u>H</u>), 5.76 (1H, d, J= 10.2, =C<u>H</u>), 2.79 (2H, s, C<u>H</u>$_2$S), 1.68 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 148.51 (<u>C</u>=NOH), 144.55 (3 x <u>C</u>$_{Ar}$), 142.04 (=<u>C</u>), 130.03 and 128.45 (6 x <u>C</u>$_{Ar}$H each), 127.19 (3 x <u>C</u>$_{Ar}$H), 112.31 (=<u>C</u>H), 67.67 (<u>C</u>S), 41.88 (<u>C</u>H$_2$S), 17.14 (<u>C</u>H$_3$). ***Oxime 219b*** (***(2,3)-Z***, 4.6 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.62 (1H, d,

J= 10.2, CHCNOH), 7.5 (15H, m, ArH), 5.81 (1H, d, J= 10.2, =CH), 2.57 (2H, s, CH_2S), 1.77 (3H, s, CH_3).

3-Methyl-4-tritylthio-2-buten-1-oximes 219b and 219c (P. E./Et_2O, 80/20; 10 %; formed by two diastereomers in proportions 2:1). ***Oxime 219b*** (3.3 %). See spectra above. *Oxime 219c.* (unknown stereochemistry, 6.6 %). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.6-7.5 (16H, m, ArH + CHCNOH), 6.37 (1H, d, J= 10.2, =CH), 2.81 (2H, s, CH_2S), 1.75 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 147.26 (C=NOH), 144.95 (3 x C_{Ar}), 130.00 and 128.34 (6 x $C_{Ar}H$ each), 129.39 (=C), 127.19 (3 x $C_{Ar}H$), 127.66 (=CH), 66.29 (CS), 41.88 (CH_2S), 15.68 (CH_3).

MS (FAB, of the mixture): m/z 374 ($(M+H)^+$, 28), 307 (67), 289 (64), 259 (100). **HRMS** (FAB, of the mixture): found 374.1593, $C_{24}H_{24}SNO$ $(M+H)^+$ requires 374. 1579.

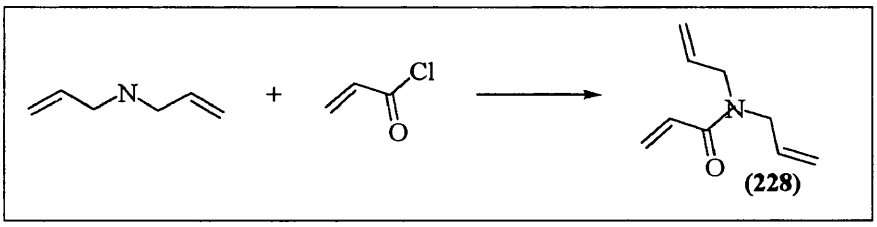


(222)

4-Tritylthio-2-cyclooctene-1-oxime (221). (P.E./Et_2O 98:2 to 70:30; 17 %; white solid; single isomer; highly hygroscopic). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.4-7.2 (15H, m, ArH), 5.80 (1H, d, J= 12.80, $=C_2H$), 5.62 (1H, dd, J= 12.8, 7.5, $=C_3H$), 3.4 (1H, m, CHS), 2.0-1.0 (8H, m, 4 x ring CH_2). **^{13}C NMR** ($CDCl_3$, 125.8 MHz): δ 159.0 (C=NOH), 145.39 (3 x C_{Ar}), 137.75 ($=C_{2(3)}H$), 130.12 and 128.32 (6 x $C_{Ar}H$ each), 127.86 ($=C_{2(3)}H$), 127.15 (3 x $C_{Ar}H$), 68.67 (CS), 43.68 (CHS), 35.19, 24.55 and 23.58 (4 x ring CH_2). **MS** (FAB): m/z 436 ($(M+Na)^+$, 35/50), 244 (Tr^+, 100). **HRMS** (FAB): found 436.1711, $C_{27}H_{27}NOSNa$ $(M+Na)^+$ requires 436.1740.

4. 4. 4. Addition onto *N,N*-diallylacrylamide.

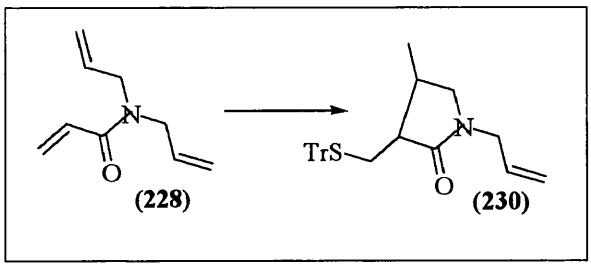
4. 4. 4. 1. Synthesis of N,N-diallylacrylamide.



Following the literature procedure[185], acryloyl chloride (4.1 mL, 50 mmol) in 10 mL of dry benzene was added slowly over a solution of diallylamine (6.17 mL, 50 mmol) and Et_3N (6.9 mL, 50 mmol) in 20 mL of dry benzene, at °C and under N_2. A yellow precipitate formed immediately. This suspension was stirred at r. t. for 1 h and then it was partitioned between Et_2O and 2N HCl. The organic layer was washed several times with 2N HCl followed by brine and dried over $MgSO_4$. The solvent was removed under vacuum affording diallylacrylamide, which did not require any further purification.

N,N-Diallylacrylamide (228)[185]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.40 (1H, dd, J= 16.7, 10.3, CH_2=C<u>H</u>), 6.26 (1H, dd, J= 16.7, 2.0, C<u>H</u>H=), 5.71 (2H, m, 2 x C<u>H</u>=CH_2), 5.57 (1H, dd, J= 10.2, 2.0, CH<u>H</u>=), 5.07 (4H, m, 2 x $C\underline{H}_2$), 3.93 (2H, m, $C\underline{H}_2$=), 3.81 (2H, bs, $C\underline{H}_2$=). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 165.89 (<u>C</u>=O), 132.52 and 127.83 (=<u>C</u>H), 127.76 (=<u>C</u>H_2), 127.30 (=<u>C</u>H), 116.99 and 116.24 (=<u>C</u>H_2), 48.62 and 47.93 (<u>C</u>H_2C=).

4. 4. 4. 2. Addition of tritylthionitrite onto N,N-diallylacrylaminde.

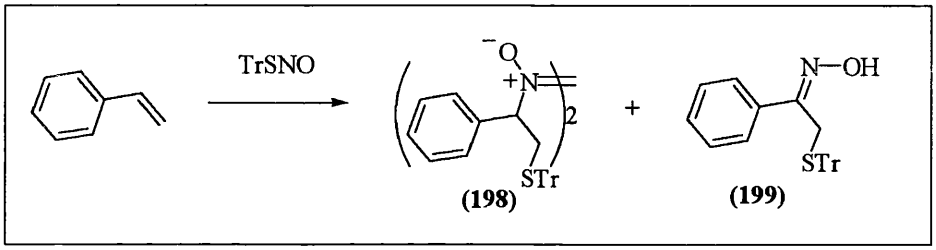


N-Allyl-4-methyl-5-tritylthiomethylpyrrolidone (230). (7.5 %, white hygroscopic solid). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.57 (1H, m, C<u>H</u>=), 5.09 (2H, m, $C\underline{H}_2$=), 3.75 (2H, J= 6.05 =CHC<u>H</u>$_2$), 3.17 (1H, dd, J= 9.6, 8.1), 2.67 (1H, J= 9.9, 7.4), 2.49 (1H, dd,

J= 11.9, 4.0), 2.31 (1H, dd, J= 11.9, 7.4), 2.00 (2H, m, $C\underline{H}_2S$), 0.91 (3H, d, J= 6.2, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 174.50 ($\underline{C}$=O), 145.08 (3 x $\underline{C}_{Ar}$), 132.77 ($\underline{C}$H=), 130.09 (6 x $\underline{C}H_{Ar}$), 128.29 (6 x $\underline{C}H_{Ar}$), 127.04 (3 x $\underline{C}H_{Ar}$), 118.30 (=$\underline{C}H_2$), 67.21 ($\underline{C}$S), 52.64 ($\underline{C}H_2$), 49.67 ($\underline{C}$H), 45.67 ($\underline{C}H_2$), 32.58 ($\underline{C}H_2$), 32.51 ($\underline{C}$H), 19.39 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 3081, 2982, 1649, 1443, 1276, 1225, 993, 922, 746, 699, 382. **MS** (FAB): m/z 243 (Tr^+, 100).

4. 5. Addition of trityl thionitrite onto alkenes under photochemical conditions.

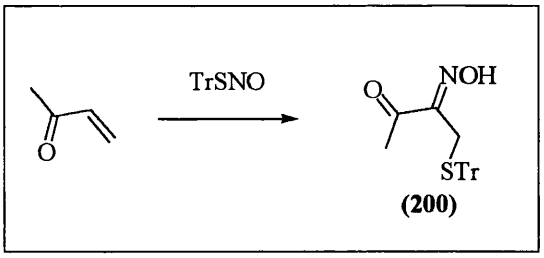
4. 5. 1. Additions onto styrene.



Styrene equiv.	thionitrite conc. (M)	addition products	yield
3	0.03	**198-199**	*ca.* 25 %
6	0.03	**198-199**	40 %
6	0.02	**198-199**	48 %

Trityl thionitrite (concentrations indicated in the table) was dissolved in 15 mL of benzene. The solution was bubbled though with nitrogen for 5-10 min and the alkene (6 equivalents) was then added. The resulting green mixture was irradiated with a tungsten lamp (500 Watts) until total disappearence of the green colour (usually between 30 min and 1 h) was observed. The solvent was evaporated at reduced pressure and the residue chromatographed (solvent system indicated in parenthesis for each case). Only the addition products are indicated. Mixtures of **198** and **199** were converted into oxime **199** by heating in isopropanol at reflux for one night. In all cases, the unreacted excess of the alkene and products derived from the photochemical decomposition of trityl thionitrite were also separated but have not been quantified. See spectra above.Only one isomer of the oxime was obtained in all cases.

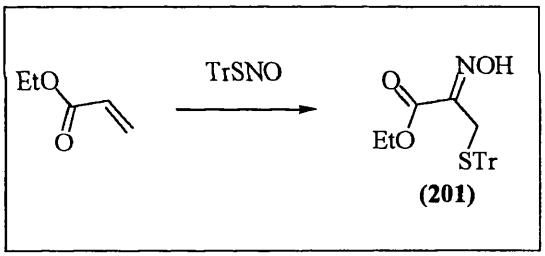
4. 5. 2. Additions onto methyl vinyl ketone and ethyl acrylate.



(200)

thionitrite conc. (M)	addition products	yield
0.02	**200**	57 %
0.04	**200**	40 %

The procedure described in section 4. 5. 1 was followed, using methyl vinyl ketone as the alkene. The product obtained displayed the same spectral data as that obtained by the thermally induced reaction, described before. Only one isomer of the oxime was obtained in all cases.



(201)

Following the same procedure, oxime **201** was obtained from ethyl acrylate, using a concentration of 0.04 M of trityl thionitrite (5-10 % yield). The product displayed the same spectral data as those obtained from the thermally induced reaction. See above.

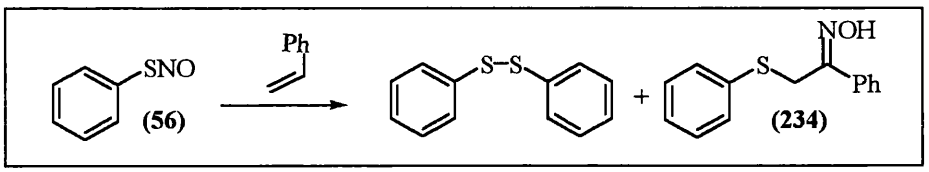
4. 6. Addition of other thionitrites onto alkenes under thermal conditions.

4. 6. 1. *In-situ* generation of thionitrites and addition onto alkenes, general procedure.

The corresponding thiol (2 mmol, 1 equiv.) and *tert*-butyl nitrite (238μL, 2.2 mmol, 1.1 equiv.) were mixed in benzene (5 mL), at r. t. and under N_2. The thionitrite formed almost instantaneously, giving the characteristic colour (green for tertiary thionitrites and red for primary or secondary ones). The solution was left standing at r. t. for 10 min and an excess of alkene was then added (6 equiv.). The mixture was diluted with previously degassed isopropanol (10 mL) and the reaction heated at 60-65 °C until

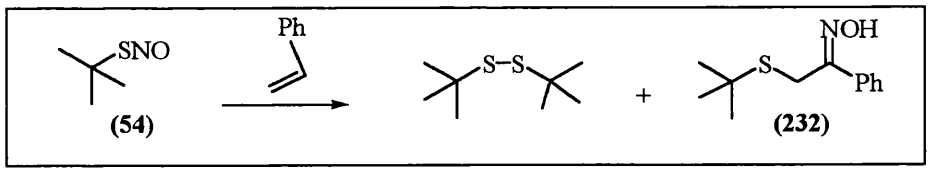
total disappearance of the colour (between 30 min and several days). The solvent was then removed under vacuum and the remaining residue separated by chromatography on silica gel (P.E./Et_2O 98:2 to 70:30 in all cases). Products are presented in order of increasing polarity.

4. 6. 1. 1. Additions onto styrene.



Diphenyl disulfide. (11 %; white solid). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.50 (4H, d, J= 7.5, 4 x $C_{orto}\underline{H}$), 7.24 (2H, t, J= 7.3, 2 x $C_{para}\underline{H}$), 7.32 (4H, dd, J= 7.8, 7.6, 4 x $C_{meta}\underline{H}$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 166.99 (2 x $\underline{C}$), 129.06, 127.46, 127.13 (10 x $\underline{C}H$). **MS** (FAB): m/z 218 ($M^{+\cdot}$, 70), 147 (95), 136 (100). **HRMS** (FAB): found 218.0230, $C_{12}H_{10}S_2$ ($M^{+\cdot}$) requires 218.0225.

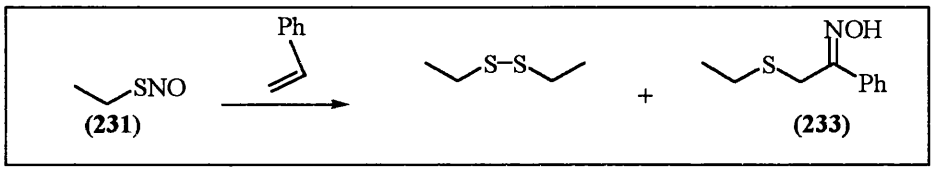
1-Phenyl-2-phenylthio-1-ethane oxime (234). (36 %; white solid). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.65 (1H, bs, NO$\underline{H}$), 7.56 (2H, m, Ar$\underline{H}$), 7.39 (5H, m, ArH), 7.24 (3H, m, Ar$\underline{H}$), 4.22 (2H, s, $C\underline{H}_2S$). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 155.22 ($\underline{C}$=NOH), 135.02 and 134.36 ($\underline{C}_{Ar}$), 130.96, 129.53, 128.84, 128.52, 126.98 and 126.42 ($\underline{C}_{Ar}H$), 28.40 ($\underline{C}H_2S$). **MS** (FAB): m/z 244 ($(M+H)^+$, 100), 213 ($(M-NO)^+$, 15), 228 (7), 185 (15), 135 (24), 123 (40). **HRMS** (FAB): found 244.0799, $C_{14}H_{14}NOS$ $(M+H)^+$ requires 244.0796.



Di-tert-butyl disulfide. (8 %, colourless oil). **^{1}H NMR** ($CDCl_3$, 75.4 MHz): δ 1.28 (18H, s). **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 46.08 ($\underline{C}$), 30.52 (6x $\underline{C}H_3$).

2-Tert-butylthio-1-phenyl-1-ethane oxime (232). (38 %; white solid). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.21 (1H, s, NO$\underline{H}$), 7.65 (2H, m, Ar$\underline{H}$), 7.36 (3H, m, Ar$\underline{H}$), 3.86

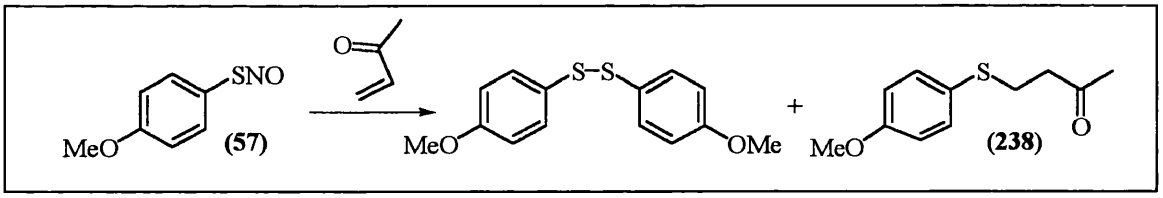
(2H, s, C<u>H</u>$_2$S), 1.37 (18H, s, (C<u>H</u>$_3$)C). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 155.70 (<u>C</u>=NOH), 129.51 (<u>C</u>$_{Ar}$), 129.42, 128.50 and 126.43 (<u>C</u>$_{Ar}$H), 43.46 (<u>C</u>), 30.49 ((<u>C</u>H$_3$)C). **IR** ($CHCl_3$): ν_{max} 3314 (OH), 2962, 2923, 2861, 1635, 1559 (C=N), 1449, 1364, 1309, 1153, 1052, 934 **MS** (FAB): m/z 224 ($(M+H)^+$, 62), 207 ($(M-O)^+$, 22), 193 ($(M-NO)^+$, 21), 168 (100). **HRMS** (FAB): found 224.1100, $C_{12}H_{18}S$ $(M+H)^+$ requires 224.1109.



Diethyl disulfide. (10 %, colourless oil).

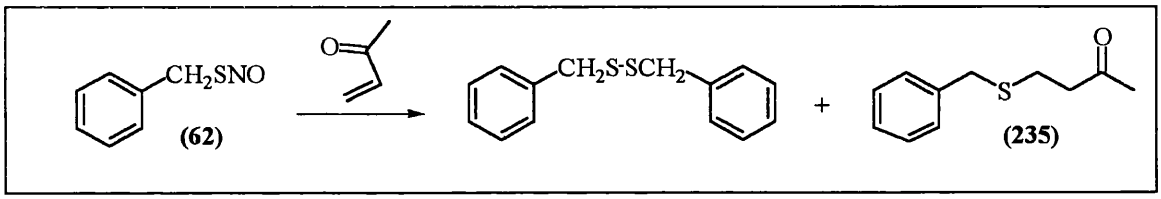
2-Ethylthio-1-phenyl-1-ethane oxime (233). (15 %; colourless oil). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.67 (2H, m, ArH), 7.37 (3H, m , ArH), 3.84 (2H, s, C<u>H</u>$_2$S), 2.58 (2H, q, J= 7.4, C<u>H</u>$_2$CH$_3$), 1.25 (3H, t, J= 7.4, C<u>H</u>$_3$CH$_2$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 156.55 (<u>C</u>=NOH), 134.97 (<u>C</u>$_{Ar}$), 129.99, 129.02, 126.85 (<u>C</u>$_{Ar}$H), 27.05 and 24.79 (C<u>H</u>$_2$S), 15.08 (C<u>H</u>$_3$). **IR** (neat): ν_{max}/cm^{-1} 3283 (OH), 3056, 2970, 2928, 2877, 1635, 1558 (C=N), 1497, 1444, 1230, 942. **MS** (FAB): m/z 196 ($(M+H)^+$, 100), 135 (90). **HRMS** (FAB): found 196.0791, $C_{10}H_{14}SNO$ $(M+H)^+$ requires 196.0796.

4. 6. 1. 2. Additions onto methyl vinyl ketone.



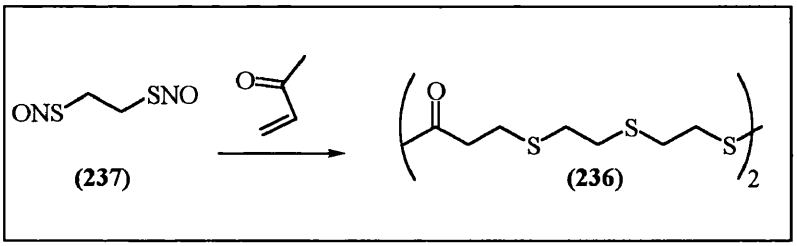
Bis-(4-methoxyphenyl) disulfide. (33 %; yellow oil). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.32 (4H, d, J= 7.5, 4 x C$_{orto}$<u>H</u>), 6.74 (4H, d, J= 7.5, 4 x C$_{para}$<u>H</u>), 3.67 (6H, d, 2 x C<u>H</u>$_3$OMe). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 160.38 (2 x <u>C</u>), 133.06 (4 x <u>C</u>H), 128.80 (2 x <u>C</u>), 115.11 (4 x <u>C</u>H). **IR** (neat): ν_{max}/cm^{-1} 3008, 2939, 2900, 2830, 1590, 1488, 1457, 1285, 1247, 1110, 1098, 1028, 818. **MS** (FAB): m/z 278 ($M^{+\cdot}$, 100). **HRMS** (FAB): found 278.0423, $C_{14}H_{14}O_2S_2$ ($M^{+\cdot}$) requires 278.0435.

4-(4-Methoxyphenylthio)-2-butanone (238). (51 %, yellow oil) **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.51 and 6.02 (4H, 2 x d, J= 15.4, 4 x $C_{orto}\underline{H}$), 3.70 (3H, d, $C\underline{H}_3OMe$), 2.93 and 2.61 (2H each, 2 x t, J= 7.3, $C\underline{H}_2C\underline{H}_2$), 2.08 (3H, s, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 207.66 ($\underline{C}$=O), 159.55 ($\underline{C}_{Ar}$), 135.34 (2 x $\underline{C}_{Ar}H$), 125.92 ($\underline{C}_{Ar}$), 115.04 (2 x $\underline{C}_{Ar}H$), 55.73 ($\underline{C}H_3O$), 43.69 ($\underline{C}H_2$), 30.48 ($\underline{C}H_2$), 30.01 ($\underline{C}H_3$). **IR** (neat): ν_{max}/cm^{-1} 3002, 2947, 2838, 1707 (C=O), 1590, 1559, 1496, 1357, 1285, 1239, 1177, 1099, 1028, 826. **MS** (FAB): m/z 210 ($M^{+\cdot}$, 100). **HRMS** (FAB): found 210.0709, $C_{11}H_{14}O_2S$ ($M^{+\cdot}$) requires 210.0715.



Dibenzyl disulfide. (34 %; colourless oil). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.38 (5H, m, Ar$\underline{H}$), 3.69 (4H, s, 2 x $C\underline{H}_2S$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 137.90 (2 x $\underline{C}$), 129.96 and 129.02 (2 x $\underline{C}$H each), 127.50 (2 x $\underline{C}$H). **MS** (FAB): m/z 245 ($(M-H)^+$, 37), 213 (M/2, 100). **HRMS** (FAB): found 278.0423, $C_{14}H_{14}O_2S_2$ ($M^{+\cdot}$) requires 278.0435.

4-Benzylthio-2-butanone (235).(16 %; colourless oil) **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 7.22(5H, m, Ar$\underline{H}$), 3.57 (2H, s, $C\underline{H}_2Ph$), 2.56 (4H, s, $C\underline{H}_2C\underline{H}_2$), 2.03 (3H, s, $C\underline{H}_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 207.27 ($\underline{C}$=O), 138.66 ($\underline{C}_{Ar}$), 129.23 and 128.96 (2 x $\underline{C}_{Ar}H$ each), 127.48 ($\underline{C}H_{Ar}$), 43.73 ($\underline{C}H_2$), 37.11 ($\underline{C}H_2Ph$), 30.43 ($\underline{C}H_3$), 25.60 ($\underline{C}H_2$). **MS** (FAB): m/z 195 ($(M+H)^+$, 100).



(237) (236)

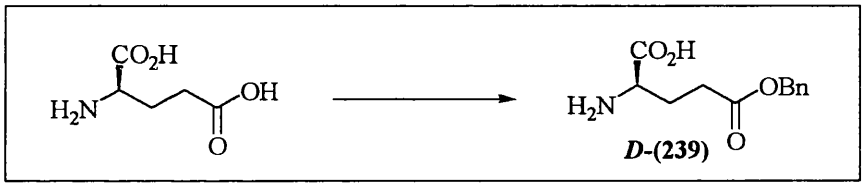
Product 236.(29 %). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 2.94 (4H, m), 2.80 (4H, m), 2.74 (4H, m), 2.16 (3H, s, C<u>H</u>$_3$). **^{13}C NMR** ($CDCl_3$, 75.4 MHz): δ 207.05 (<u>C</u>=O), 44.02, 38.78 and 32.22 (<u>C</u>H$_2$), 30.56 (<u>C</u>H$_3$). **IR** (CCl_3): ν_{max}/cm^{-1} 3010, 1714 (C=O), 1416, 1364, 1159. **MS** (FAB): m/z 327 ($(M+H)^+$, 4), 255 ($(M-CH_2CH_2COMe)^+$, 10), 195 ($(255-SCH_2CH_2)^+$, 8), 163 ($(195-S)^+$, 98), 131 ($(163-S)^+$, 100), 103 ($(131-CH_2CH_2)^+$, 49), 71 ($(CH_3COCH_2CH_2)^+$, 5), 43 ($(CH_3CO)^+$, 27).

CHAPTER 5

SYNTHESIS AND BIOLOGICAL EVALUATION OF ENANTIOPURE THIONITRITES.

5. 1. Preparation of *α-tert*-butyl N^{α}-(*tert*-butyloxycarbonyl) *D*-monoglutamate for the synthesis of *D*-glutathione.

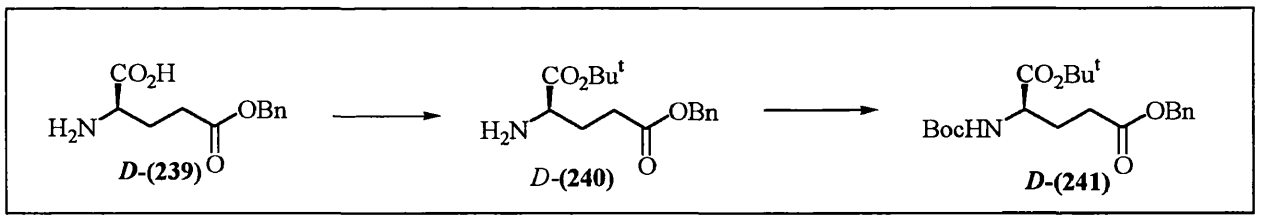
5. 1. 1. *γ*-Benzylation of *D*-glutamic acid.



Following the described procedure[186], a mixture of *D*-glutamic acid (10 g, 68 mmol) and anhydrous sodium sulfate (10 g, 68 mmol) was suspended in benzyl alcohol (125 mL) and tetrafluoroboric acid etherate (54 %, 18.5 mL) was added under N_2 by means of a xyringe. The suspension was stirred at r. t. for 24 h and then it was diluted with THF (500 mL) and filtered with the aid of activated charcoal (over a pad of Celite®). The clear filtrate was treated with 20.5 mL of Et_3N and a white solid precipitated. The solvent was eliminated under vacuum, furnishing a white slurry which was triturated with EtOAc (500 mL), collected by filtration, washed with EtOAc and dried under vacuum. The product was obtained as a white amorphous solid in 94 % yield (15.20 g).

γ-Benzyl D-monoglutamate (D-Glu(OBn)-OH, D-239). **mp** (EtOAc): 160-162 °C, lit.[187] (EtOAc): 161-162 °C. **^{1}H NMR** (D_2O + D_2SO_4; 400 MHz)[188]: δ 7.0-6.9 (5H, m, ArH), 4.67 (2H, s, CH_2Ph), 3.64 (1H, t, J= 6.6, $C_\alpha H$), 2.18 (2H, t, J= 7.8, CH_2COOBn), 1.77 (2H, m, CH_2C_α). **^{13}C NMR** (D_2O + D_2SO_4, 75.4 MHz): δ 174.47 and 171.77 (C=O), 139.82 (C_{Ar}), 129.58, 128.83 and 128.43 (CH_{Ar}), 64.99 (CH_2Ph), 52.95 ($C_\alpha H$), 30.24 and 25.40 (CH_2). **IR** (CCl_4): ν_{max}/cm^{-1} 3464-3401 (b, OH and NH_2), 3020, 2921, 2585, 1722 (C=O), 1577, 1513, 1409, 1174. **MS** (FAB): m/z 260 ($(M+Na)^+$, 17), 238 ($(M+H)^+$, 15), 154 (90), 136 (87), 102 (84), 91 (Bn^+, 100), 77 (Ph^+, 44). **HRMS** (FAB): found 238.1079, $C_{12}H_{16}NO_4$ $(M+H)^+$ requires 238.1060.

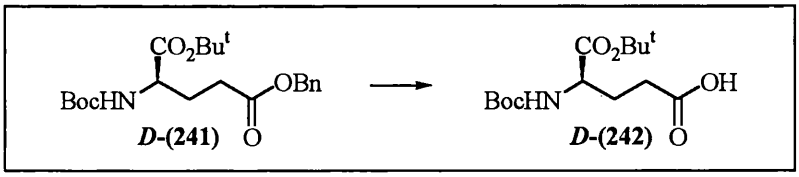
5. 1. 2. Preparation of fully protected *D*-glutamic acid.



Following the described procedure[189], γ-benzyl *D*-monoglutamate (*D*-**239**, 5 g, 21 mmol) was dissolved in a mixture of dioxane (50 mL) and concentrated sulfuric acid (5 mL). The solution was placed in a 100 mL pressure bottle and cooled to –78 °C. Isobutylene (35 mL) was added and the mixture allowed to warm to r. t. and stirred for 5 h. The solution was poured carefully onto a cold, stirred mixture of Et_3N/H_2O (30:50 mL). To this dilute, cold mixture, di-*tert*-butyl dicarbonate (4.3 g, 20 mmol) was added and the mixture stirred at r. t. for 16 h. The dioxane/H_2O was removed under *vacuo* to give an oily residue, which was suspended in EtOAc and made acidic (pH 2) with aq. $KHSO_4$ solution. The EtOAc extract was washed with H_2O (50 mL) and brine (25 mL) and dried over $MgSO_4$. After evaporation of the solvent, the oily residue was purified by column chromatography, furnishing the pure product as a yellow oil, in 55 % yield (4. 56 g).

γ-Benzyl α-tert-butyl N^{α}-(tert-butyloxycarbonyl)-D-diglutamate (Boc-D-Glu(OBn)-OBu^t, D-241). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.40-7.30 (5H, m, ArH), 5.14 (2H, s, CH_2Ph), 5.10 (1H, m, NH), 4.25 (1H, m, $C_{\alpha}H$), 2.52-2.38 (2H, m, CH_2COOBn), 2.23-2.15 (1H, m, $CHHC_{\alpha}$), 2.01-1.92 (1H, m, $CHHC_{\alpha}$), 1.46 (9H, s, $(CH_3)_3C$), 1.44 (9H, s, $(CH_3)_3C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 172.62 and 171.26 (CC=O), 155.31 (NHC=O), 135.76 (C_{Ar}), 128.51 and 128.16 (CH_{Ar}), 82.14 and 74.72 (CO), 66.38 (CH_2Ph), 53.30 ($C_{\alpha}H$), 30.28 (CH_2), 28.25 and 28.03 ($(CH_3)_3C$), 27.93 (CH_2). **IR** (neat): ν_{max}/cm^{-1} 3375 (NH), 2970, 1735 (C=O), 1732 (C=O), 1502, 1454, 1367, 1254, 1157, 1052. **MS** (FAB): m/z 416 ($(M+Na)^+$, 25), 394 ($(M+H)^+$, 18), 338 ($(M+H-C_4H_8)^+$, 18), 282 ($(M+H-2 \times C_4H_8)^+$, 100), 238 ($(M+H^+-2 \times C_4H_8-CO_2)^+$, 67), 192 (20), 154 (50), 136 (24), 91 (Bn^+, 100), 57 ($Bu^{t\,+}$, 69). **HRMS** (FAB): found 394.2245, $C_{21}H_{32}NO_6$ $(M+H)^+$ requires 394.2230.

5. 1. 3. γ-Benzyl group removal.



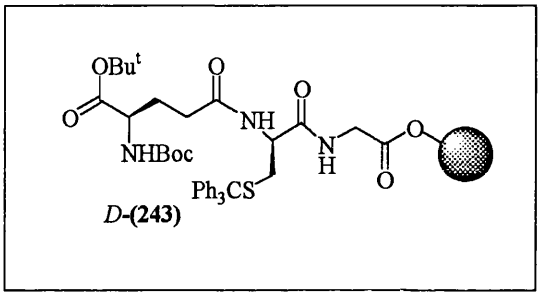
Following the literature procedure[189], γ-benzyl *a-tert*-butyl N^{α}-(*tert*-butyloxycarbonyl)-*D*-diglutamate (***D*-241**, 13.48 g, 33.8 mmol) and 5 % palladium on carbon (1.02 g) were mixed in 100 mL of absolute ethanol and stirred under a positive pressure of H_2 for 16 h. The catalyst was then filtered and the filtrate evaporated to give an oil which was triturated with Et_2O and re-crystallised from P.E./Et_2O, providing the product as a white amorphous solid in 50 % yield (5.14 g).

α-Tert-butyl N^{α}-(tert-butyloxycarbonyl)-D-monoglutamate (Boc-D-Glu-OBu^t, D-242). **mp** (P.E/Et_2O): 103-106 °C, lit[189] (P.E/Et_2O): 102-105 °C. $[\alpha]_D^{25}$ = +30.4 (*c* 1, MeOH), lit[189] $[\alpha]_D^{25}$ = –26.5 (*L* isomer, *c* 1, MeOH). **^{1}H NMR** (DMSO-d_6, 400 MHz): δ 7.12 (1H, d, J= 7.8, NH), 4.06 (1H, m, $C_{\alpha}H$), 2.25 (2H, m, CH_2), 1.85 (1H, m, CHH), 1.70 (1H, m, CHH), 1.63 (9H, s, $(CH_3)_3C$), 1.62 (9H, s, $(CH_3)_3C$). **^{13}C NMR** (DMSO-d_6, 100.6 MHz): δ 173.86 and 171.63 (CC=O), 155.65 (NHC=O), 80.43 and 78.18 (CO), 53.65 ($C_{\alpha}H$), 30.13 (CH_2), 28.26 and 27.72 ($(CH_3)_3C$), 26.02 (CH_2). **MS** (FAB): m/z 326 ($(M+Na)^+$, 32), 304 ($(M+H)^+$, 12), 270 (13), 248 ($(M+H-C_4H_8)^+$, 19), 204 ($(M+H-CO_2-C_4H_8)^+$, 10), 148 ($(M+H-2 \times C_4H_8-CO_2)^+$, 43), 130 (22), 84 (11), 91 (Bn^+, 100), 57 ($Bu^{t\,+}$, 74). **HRMS** (FAB): found 326.1580, $C_{14}H_{25}O_2NNa$ $(M+Na)^+$ requires 326.1570.

5. 2. Preparation of *D*-glutathione (*D*-GSH).

5. 2. 1. Solid phase-approach.

5. 2. 1. 1. Solid phase synthesis of resin-bound Boc-γ-D-Glu(OBu^t)-D-Cys(STr)-Gly (243).



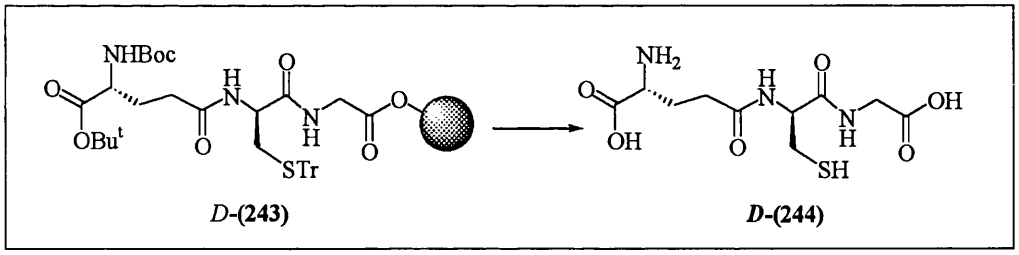
D-(243)

The title compound was synthesised on a solid phase support using the Fmoc (9-fluorenylmethoxycarbonyl) protecting group strategy. The resin (NovaSyn PA500®) and the protected cysteine residue (Fmoc-*D*-Cys(STr)-OH) were purchased from Nova Biochem. The suitably protected *D*-glutamic acid residue (Boc-*D*-Glu-OBu^t) was synthesised from *D*-Glutamic acid following described procedures as indicated in the previous section. The solid support employed was formed by a polydimethylacrylamide-polystyrene composite, functionalised with the first amino acid in its Fmoc protected form (Fmoc-Gly, 0.59 mmol/g), linked to the polymer in the form of its 4-hydroxymethylphenoxyacetic acid ester (Fmoc-Gly-[OCH_2PhCH_2CO]-Nle-$NHCH_2CH_2NH$-polymer).

10.4 g (weight equivalent to 6.5 mmol of amino-acid) of Fmoc-Gly-Nova Syn PA 500 resin were suspended in DCM (300 mL) for one night prior to the synthesis. The swollen resin was then filtered and washed several times with DMF. This was followed by suspension in a mixture of piperidine/DMF (20 % v/v, 1 L), in order to achieve the deprotection of the terminal Fmoc group. This mixture was shaken at r. t. at regular intervals of 5-10 min, for a total of 30 min The by-products of the deprotection were separated by filtration and the resin was washed several times with DMF (a total of 1 L was used). The degree of deprotection was qualitatively analysed by the Ninhydrin (Kaiser)[190] test. The second amino-acid (Fmoc-*D*-Cys(STr)-OH) was then coupled to the free amino group of glycine. Activation was achieved by derivatisation of the Cys

residue in the form of its benzyloxytriazole ester (BtOH) by mixing 15.2 g (26 mmol, 4 equiv.) of Fmoc-*D*-Cys(STr)-OH with 13.4 g (26 mmol, 4 equiv.) of pyBOP and 2.78 (26 mmol, 4 equiv.) of *N*-methylmorpholine (NMM) in 85 mL of DMF. After stirring this mixture at r. t. for 10 min, the pre-formed activated ester was added to a suspension of the resin in 300 mL of dry DMF. The mixture was left standing at r. t. with occasional swirling for 3 h. After filtration and washing with a total of 1 L of DMF, Kaiser test gave negative (colourless beads) indicating coupling had been completed. Deprotection of the terminal Fmoc group was carried out as before with piperidine/DMF 20 % v/v for 30 min Coupling of the last amino-acid (Boc-*D*-Glu-OBu^t was carried out as previously by pre-forming the activated ester of the glutamic acid residue from 4.2 g (13 mmol, 2 equiv.) of Boc-*D*-Glu-OBu^t)-OH, 6.7 g (13 mmol, 2 equiv.) of pyBOP and 1.36 (13 mmol, 2 equiv.) of NMM in 85 mL of DMF. The pre-formed activated ester was then poured onto a suspension of the resin in 300 mL of DMF and these were allowed to react at r. t. for 3 h with occasional swirling. The resin was filtered and washed successively with a total of 1 L of DMF, 0.5 L of freshly distilled AcOH, 1 L of DCM and 1 L of Et_2O. The shrunk resin was then dried overnight in a desiccator at low pressure. The final weight of dry resin was 9.36 g.

5. 2. 1. 2. Cleavage from the resin and deprotection.



D-(243) *D*-(244)

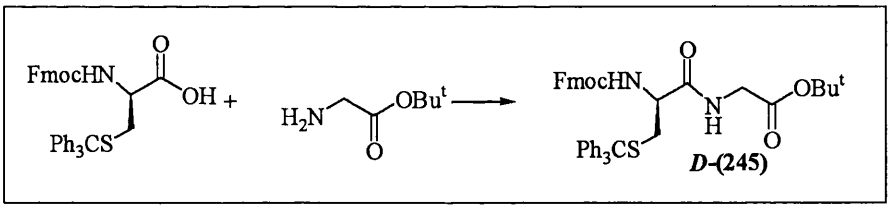
1 g of the dried resine was suspended in 20 mL of TFA/DCM 95 % v/v and 1 mL of EDT was added as scavenger. The mixture was allowed to react at r. t. with occassional shaking for 3 h and then the resin was separated by filtration and washed several times with neat TFA. The solvent was evaporated at low pressure and the residue was partitioned between Et_2O and H_2O. The aqueous layer was separated, washed several times with Et_2O and filtered in order to separate solid impurities. The clear solution was lyophilized giving a white solid. This was additionally purified by preparative reverse-phase HPLC furnishing 60 mg (overall 20 %) of the tri-peptide in the

form of its TFA salt as a white hygroscopic solid.

D-Glutathione TFA salt (D-GSH·TFA, D-γ-Glu-D-Cys-Gly-OH·TFA, D-244:TFA). $[\alpha]_D^{25} = +8.0$ (*c* 0.5, H_2O), a prepared sample of the TFA salt of the *L*-isomer recorded $[\alpha]_D^{25} = -8.5$ (*c* 0.5, H_2O). **^{1}H NMR** (D_2O, 400 MHz): δ 4.37 (1H, t, J= 6.2, Cys $C_\alpha\underline{H}$), 3.85 (1H, t, 6.2, Glu $C_\alpha\underline{H}$) 3.83 (2H, d, J= 1.58, Gly $C_\alpha\underline{H_2}$), 2.92 (2H, dd, J= 5.9, 2.6, $C\underline{H_2}S$), 2.61 (2H, m, Glu $C\underline{H_2}$), 2.25 (2H, m, Glu $C\underline{H_2}$). **^{13}C NMR** (D_2O, 100.6 MHz): δ 174.95, 173.44, 172.99 and 172.81 ($\underline{C}$=O), 55.94 (Cys $\underline{C}_\alpha$H) 52.93 (Glu $\underline{C}_\alpha$H), 41.44 (Gly $\underline{C}_\alpha H_2$), 31.23 (Glu $\underline{C}H_2$), 25.85 ($\underline{C}H_2S$), 25.67 (Glu $\underline{C}H_2$). **MS**[191] (FAB): m/z 308 ($(M+H)^+$, 100), 273 ($(M-SH_2)^{+\cdot}$, 12), 223 (20), 207 (50), 131 (31), 115 (100). **HRMS** (FAB): found 308.0910, $C_{10}H_{17}SN_3O_6$ $(M+H)^+$ requires 308.0916. **HPLC** (Hypersil PEP 100 C18; 0.1 % TFA/H_2O): retention time 7.9 min, 92-95 % pure, contains ca. 3-5 % of the disulfide (identical to a sample of commercial *L*-GSH).

5. 2. 2. Solution phase approach.

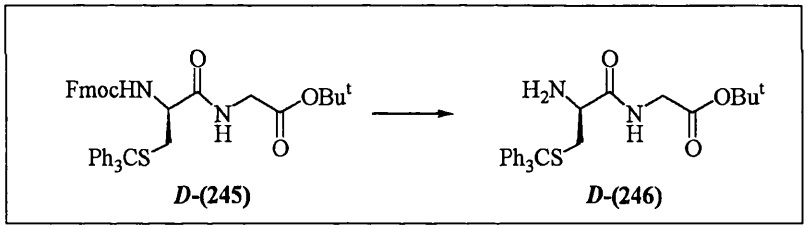
5. 2. 2. 1. Glycine-cysteine coupling.



PyBOP (650 mg, 1.25 mmol) and NMM (137 μL, 1.25 mmol) were dissolved in DCM (2 mL) and the solution added in one portion over a solution of Fmoc-*D*-Cys(STr)-OH (293 mg, 0.5 mmol) in DCM (2 mL). After stirring at r. t. for 10 min the resulting mixture was poured onto a solution of Gly-O^{t}Bu hydrochloride (84 mg, 0.5 mmol) in 10 mL of DCM. The mixture was stirred at r. t. for 3 h and then washed with H_2O (3 times), dried over $MgSO_4$ and the solvent evaporated off. The residue was filtered through SiO_2 (EtOAc/P.E. 5/5) giving the protected dipeptide as a white amorphous solid (324 mg, 93 %).

Tert-butyl [N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-S-trityl-D-cysteinyl]glycinate (Fmoc-D-Cys(STr)-Gly-OH, D-245). **mp** (Et_2O/P.E.): 90-91 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.72 (2H, t, J= 7.7, ArH), 7.55 (2H, d, J= 7.6, ArH), 7.39 (6 H, d, J= 7.1, ArH), 7.36 (2H, q, J= 7.3, ArH), 7.25 (9H, m, ArH), 7.19 (2H, q, J= 7.2, ArH), 6.31 (1H, bs, NH), 4.97 (1H, bd, J= 6.8, NH), 4.36 (2H, d, J= 7.1, Fmoc CH_2O), 4.18 (1H, t, J= 6.7, Fmoc CH), 3.80 (3H, m, Cys $C_{\alpha}H$ and Gly $C_{\alpha}H_2$), 2.64 (2H, m, CH_2S), 1.42 (9H, s, $(CH_3)C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 169.94 and 168.25 (C=O), 144.29, 143.65 and 141.26 (C_{Ar}), 129.54, 128.06, 127.69, 127.05, 126.89, 125.03 and 119.95 (CH_{Ar}), 82.36 (CO), 67.31 (CH_2O), 47.05 (Fmoc CH), 53.92 (Cys $C_{\alpha}H$), 42.07 (Gly $C_{\alpha}H_2$), 33.74 (CH_2S), 27.98 ($(CH_3)_3C$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3418 (NH), 3019, 1730 (C=O), 1669 (C=O), 1498, 1239. **MS** (FAB): 699 ($(M+H)^+$, 30/50), 456 (14/50), 400 (57/50), 243 (Tr^+, 100). **E. A.**: found C 73.52 %, H 6.04 %, N 4.02 %, S 5.03 %, calculated C 73.90 %, H 6.06 %, N 4.01 %, S 4.59 %.

5. 2. 2. 2. Fmoc-deprotection.



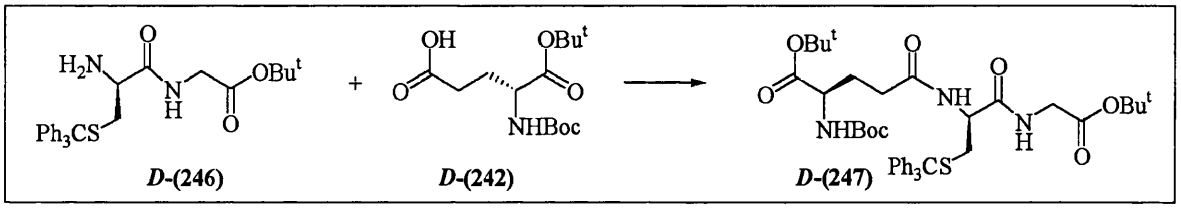
D-(245) *D*-(246)

The protected dipeptide ***D*-245** (282 mg, 0.4 mmol) was dissolved in piperidine/DCM 20 % v/v and the solution stirred at r. t. for 15 min The reaction mixture was then washed with H_2O (twice), and dried over $MgSO_4$. After filtration, the solvent was evaporated off and the crude product purified by chromatography on SiO_2 (EtOAc/P. E. 30/70). The free amine (*D*-**246**) was obtained as an amorphous solid in 73 % yield (141 mg).

Tert-butyl (S-trityl-D-cysteinyl)glycinate (D-Cys(STr)-Gly-OBu^t, D-246). **mp** (Et_2O/P.E.): 84-85 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.43 (1H, t, J= 4.8, Gly NH), 7.28-7.19 (15H, m, ArH), 3.87 (1H, dd, J= 18.3, 5.6, Gly $C_{\alpha}HH$), 3.73 (1H, dd, J= 18.3, 5.6, Gly $C_{\alpha}HH$), 2.98 (1H, dd, J= 8.8, 3.7, Cys $C_{\alpha}H$), 2.74 (1H, dd, J= 12.8, 3.7, CHHS)), 2.53 (1H, dd, J= 12.8, 8.8, CHHS), 1.42 (9H, s, $(CH_3)_3C$).

^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 173.05 and 168.85 (C=O), 144.54 (C_{Ar}), 129.56, 127.94 and 126.77 (CH_{Ar}), 82.11 (CO), 66.98 (CS), 53.92 (Cys $C_\alpha H$), 41.66 (Gly $C_\alpha H_2$), 37.25 (CH_2S), 27.98 ($(CH_3)C$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3382 (NH), 3013, 1776 (C=O), 1710 (C=O), 1518, 1441, 1370. **MS** (FAB): m/z 477 ($(M+H)^+$, 18), 243 (Tr^+, 100). **HRMS** (FAB): found 477.2198, $C_{28}H_{33}N_2O_3S$ $(M+H)^+$ requires 477.2212. **E. A.** : found C 70.21 %, H 7.03 %, N 5.84 %, S 6.81 %, calculated C 70.56 %, H 6.77 %, N 5.88 %, S 6.73 %.

5. 2. 2. 3. D-Cysteinyl-glycinate coupling with protected D-glutamic acid.



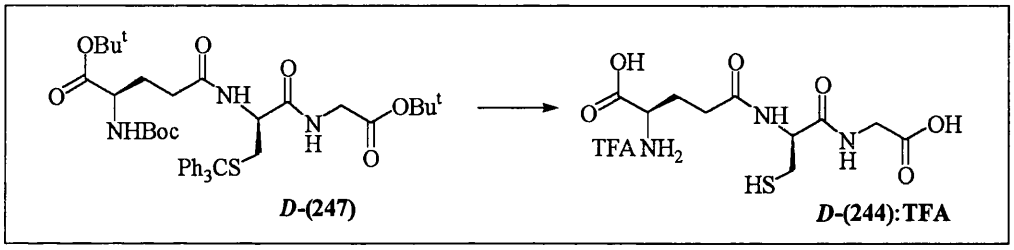
D-(246) *D*-(242) *D*-(247)

A solution of pyBOP (325 mg, 0.62 mmol) and NMM (68 μL, 0.62 mmol) in 5 mL of DCM was poured onto a solution of Boc-*D*-Glu-OBu^t (***D*-242**, 76 mg, 0.25 mmol) in 5 mL of DCM at r. t. After 10 min, the resulting mixture was poured onto a solution of the protected dipeptide ***D*-246** (120 mg, 0.25 mmol) in 10 mL of DCM. The mixture was stirred at r. t. for 3 h and then washed with H_2O (x 3) and extracted with DCM. After drying over $MgSO_4$, the solvent was evaporated under vacuum and the residue chromatographed (SiO_2, P.E./EtOAc 85/15) furnishing fully protected *D*-GSH (***D*-247**) as a white solid (172 mg, 90 %).

Tert-butyl [N$^\alpha$-(tert-butyloxycarbonyl)-α-tert-butyl-D-γ-glutamyl]-(S-trityl-D-cisteinyl)glycinate (Boc-D-γ-Glu(OBu^t)-D-Cys(STr)-GlyOBu^t, D-247). **mp** (Et_2O/P.E.): 79-80 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.51 (6H, d, J= 11.7, ArH), 7.39-7.31 (9H, m, ArH), 6.99 (1H, bt, J= 5.4, Gly NH), 6.31 (1H, bd, J= 7.0, Cys NH), 5.33 (1H, bd, J= 8.0, Glu NH), 4.23 (1H, m, Cys $C_\alpha H$), 4.18 (1H, m, Glu $C_\alpha H$), 3.95 (1H, dd, J= 10.7, 5.4, Gly $C_\alpha HH$), 3.85 (1H, dd, J= 10.7, 5.4, Gly $C_\alpha HH$), 2.86 (1H, dd, J= 13.3, 7.6, CHHS), 2.68 (1H, dd, J= 13.3, 5.3, CHHS), 2.28 (2H, m, Glu CH_2), 1.79 (2H, m, Glu CH_2), 1.52 (18H, s, 2 x $(CH_3)_3C$), 1.50 (9H, s, $(CH_3)C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 172.25, 171.39, 170.00 and 168.37 (C=O), 144.40 (C_{Ar}), 129.56, 128.05

and 126.85 ($\underline{C}H_{Ar}$), 82.18 and 79.95 ($\underline{C}O$), 67.21 ($\underline{C}S$), 60.89 ($\underline{C}O$), 53.50 and 52.11 (Cys and Glu $\underline{C}_{\alpha}H$), 42.11 (Gly $\underline{C}_{\alpha}H$), 32.83 (Glu $\underline{C}H_2$), 31.98 ($\underline{C}H_2S$), 29.11 (Glu $\underline{C}H_2$), 28.31, 27.99 and 27.93 (($\underline{C}H_3)_3C$). **IR** ($CHCl_3$): ν_{max}/cm^{-1} 3423 (NH), 3013, 2978, 1730 (C=O), 1700 (C=O), 1678 (C=O), 1500, 1445, 1368, 1157. **MS** (FAB): m/z 762 ($(M+H)^+$, 60/50), 626 (25/50), 520 (65/50), 243 (Tr^+, 100). **HRMS** (FAB): found 762.3778, $C_{42}H_{56}O_8SN_3$ $(M+H)^+$ requires 762.3788.

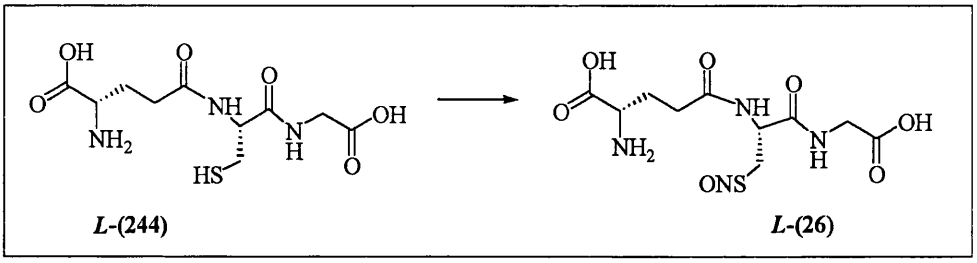
5. 2. 2. 4. Deprotection.



Protected *D*-glutathione (***D*-247,** 1.1 g, 1.57 mmol) was dissolved in TFA/H_2O (97.5 /2.5 % v/v) and triethylsilane (2.5 %) was added as scavenger. The solution was stirred at r. t. for 3 h. The solvent was then evaporated and distilled H_2O was added. The solid impurities were separated by filtration and the aqueous phase freeze-dried. The crude product *D*-GSH (TFA salt) was additionally purified by preparative reverse phase HPLC (same conditions as previously), purity 90 %, contains 8 % of the disulfide). *D*-glutathione was obtained in the form of its TFA salt as a white hygroscopic solid and it displayed the same spectral data as the product obtained by solid-phase synthesis the same optical rotation value (237 mg, 35 %).

5. 3. Nitrosation of glutathione enantiomers.

5. 3. 1. Preparation of *S*-nitroso-*L*-glutathione (*L*-GSNO).



5. 3. 1. 1. Using HCl as the acid.

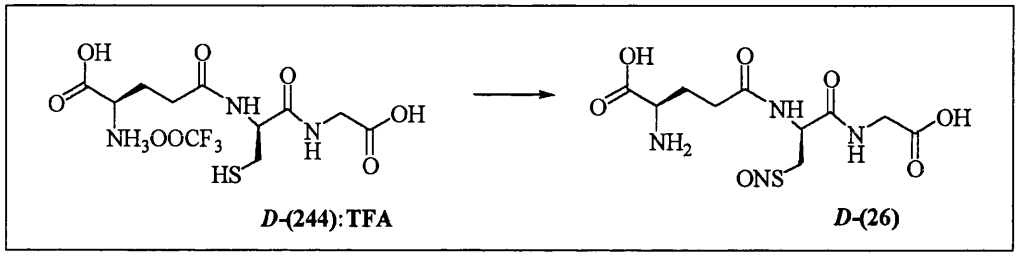
Commercial *L*-GSH (***L*-244**) (35 mg, 0.11 mmol) was suspended in 0.1 mL of distilled H_2O and HCl 0.1 N (1 equiv., 0.1 mL) was added with a micro-syringe. The solid immediately became soluble. The mixture was cooled to 5 °C and 9 mg (1 equiv.) of $NaNO_2$ was added in one portion, the solution immediately turning deep red. Under a flow of N_2, the solution was stirred at the same temperature for 1 h , a pink precipitate forming after the first 15 min. This was filtered and washed successively with cold H_2O (0.1 mL), cold acetone (0.1 mL) and cold Et_2O (0.1 mL). The product (pink solid) was dried in a desiccator for 2 days and kept in a cold dry place and covered with aluminium foil in order to prevent thermal and photochemical decomposition (37 mg, 39 %).

***S*-Nitroso-*L*-glutathione (*L*-GSNO, *L*-γ-Glu-*L*-Cys(SNO)-Gly, *L*-26).** $[\alpha]_D^{25}$: + 42 (*c* 1, H_2O), lit[50]: + 47 (*c* 1.31, H_2O). **^{1}H NMR** (D_2O, 300 MHz): δ 4.50 (1H, t, J= 7.0, Cys $C_\alpha\underline{H}$), 3.93 (1H, m, C<u>H</u>HSNO), 3.79 (1H, m, CH<u>H</u>SNO), 3.75 (2H, s, Gly $C_\alpha\underline{H}_2$), 3.64 (1H, t, J= 6.3, Glu $C_\alpha\underline{H}$), 2.27 (2H, t, J= 7.6, Glu $C\underline{H}_2$), 1.95 (2H, m, Glu $C\underline{H}_2$). **^{13}C NMR** (D_2O, 75.4 MHz): δ 176.59, 175.36 and 173.75 (<u>C</u>=O), 55.57 ($\underline{C}_\alpha H$), 54.46 ($\underline{C}_\alpha H_2$), 43.40 ($\underline{C}_\alpha H$), 32.97, 32.98 and 27.81 ($\underline{C}H_2$). **MS** (FAB): m/z 337 ($(M+H)^+$, 25). **HRMS** (FAB): found 337.0828, $C_{10}H_{17}N_4SO_7$ $(M+H)^+$ requires 337.0818. **HPLC** (Hypersyl C18, 0.1 % TFA/H_2O): 14.6 min, 97 % pure, contains 1 % of the disulfide (0.1 % TFA, MeOH/H_2O gradient from 1% to 35 % in 50 min: retention time 9.5 min). **UV** (H_2O): λ_{max} 335 (ε 7.68 x 10^2 $mol^{-1}cm^{-1}$), 545 (ε 10.22 $mol^{-1}cm^{-1}$), lit[24]: 335 (ε 9.22 x 10^2), 545 (ε 15.9).

5. 3. 1. 2. Using TFA as the acid.

The reaction was carried out as above but using 9 μL (1 equiv.) of TFA instead of HCl and 0.2 mL of H_2O as the solvent. The product was obtained as a pink solid in 30 % yield and displayed the same spectral data and HPLC trace as the product obtained by the method described above. By HPLC the product was found to be pure 95 %, containing *ca.* 3 % of the disulfide.

5. 3. 2. Preparation of *S*-nitroso-*D*-glutathione (*D*-GSNO).



The synthesis was carried out as above but without addition of TFA since the starting tripeptide was obtained in the form of its TFA salt and contains exactly one equivalent of the necessary acid. The product displayed the same spectral data and HPLC trace as the *L*-enantiomer and optical rotation of opposite sign.

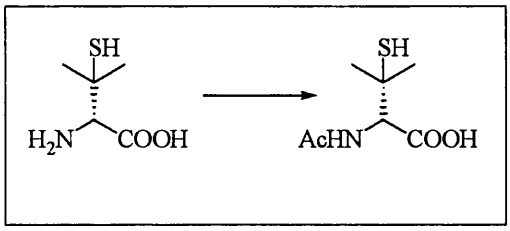
***S*-Nitroso-*D*-glutathione (*D*-GSNO, *D*-γ-Glu-*D*-Cys(SNO)-Gly-OH), *D*-26).** $[\alpha]_D^{25}$: −35 (*c* 1, H_2O). **HPLC** (against a sample of *L*-GSNO, 0.1 % TFA/H_2O): retention time 14.6 min; purity: 85 %; contains *ca.* 10 % of the disulfide.

5. 3. 3. *In situ* preparation of *S*-nitrosoglutathione isomers.

D and *L*-GSNO used in biological studies were freshly prepared *in-situ* every day by dissolving the corresponding enantiomer of the thiol (***D*-GSH·TFA** or ***L*-GSH**) in H_2O, in the presence of 1 equivalent of $NaNO_2$, and acidifying the solution to pH 2 with TFA. The solutions turned immediately red and, between experiments, they were kept in ice and in the dark, in order to avoid thermal or photochemical decomposition. UV absorption of the two solutions were measured for comparison and solutions diluted until the same reading was obtained for the two enantiomers, in order to compensate for presence of different quantities of disulfide in the samples of the two thiols. The differences were never larger than 5 %.

5. 4. Preparation of the *D* and *L* isomers of *S*-nitrosopenicillamine.

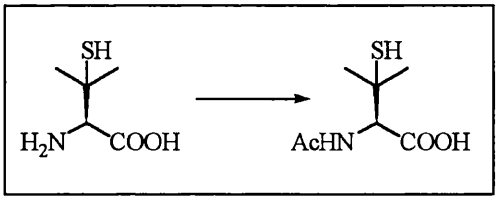
5. 4. 1. *N*-Acetylation of *D*-penicillamine.



Following the literature procedure[192] *D*-penicillamine (500 mg, 2.6 mmol) was suspended in 0.7 mL of distilled H_2O and $(Ac)_2O$ (530.86 mg, 490 μL, 5.2 mmol) was added at r. t. The resulting suspension was stirred at r. t. for 3 h. The white precipitate was collected by filtration and washed with cold H_2O. The product was recrystallised from boiling H_2O and isolated in the form of transparent prisms (34 %). It displayed the same ^{1}H NMR as a sample of commercial *D*-NAP.

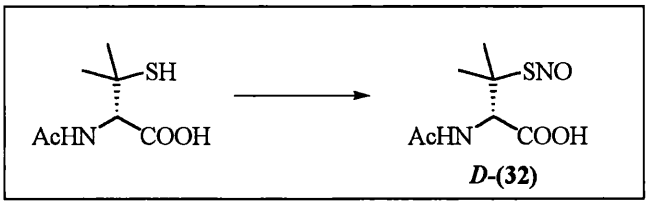
***N*-Acetyl-*D*-penicillamine (*D*-NAP).** **mp** (H_2O): 195 ^{0}C decomp.; commercial sample mp.: 185-190 °C, decomp. $[\alpha]_D^{25}$: + 7.7 (*c* 0.7, EtOH/H_2O 1:1); commercial *D*-NAP $[\alpha]_D^{25}$: + 10.1 (*c* 1, EtOH/H_2O 1:1). **^{1}H NMR** (D_2O, 300 MHz): δ 4.30 (1H, s, $C_\alpha\underline{H}$), 1.91 (3H, s, $COC\underline{H}_3$), 1.32 (3H, s, $C\underline{H}_3$), 1.26 (3H, s, $C\underline{H}_3$). **HPLC** (Hypersyl C18, 0. 1% TFA, MeOH/H_2O gradient from 1% to 35 % in 50 min): retention time 19 min, one single peak.

5. 4. 2. Acetylation of *L*-penicillamine.



Following the procedure described for the *D*-isomer, *L*-acetylpenicillamine was obtained as transparents prisms in 31 % yield and displayed the same ^{1}H NMR and HPLC trace as the the *D* isomer. ***N*-Acetyl-*L*-penicillamine (*L*-NAP).** **mp** (H_2O): 195 ^{0}C decomp. $[\alpha]_D^{25}$: –7.7 (*c* 0.7, EtOH/H_2O 1:1). **^{1}H NMR** (D_2O, 300 MHz): δ 4.30 (1H, s, $C_\alpha\underline{H}$), 1.91 (3H, s, $COC\underline{H}_3$), 1.32 (3H, s, $C\underline{H}_3$), 1.26 (3H, s, $C\underline{H}_3$).

5. 4. 3. Preparation of *S*-nitroso-*N*-acetyl-*D*-penicillamine.



D-(32)

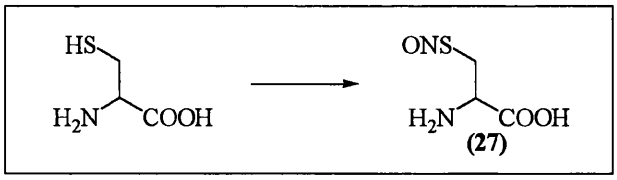
Following the literature procedure[193], *N*-acetyl-*D*-penicillamine (1 g, 5.23 mmol) was dissolved in 8 mL of 1M HCl and 12 mL of MeOH, at r. t. A solution of $NaNO_2$ (732 mg) in 12 mL of H_2O was then added slowly, over a period of 25 minutes. Once the addition was finished, the mixture was stirred for 15 min and a green crystalline solid started to precipitate. This was filtered, washed with cold H_2O, and dried in a desiccator overnight. The filtrate solution was freeze-dried, the resulting solid washed with 10 mL of cold H_2O and dried as before. The overall yield of the two crops was 44 %.

***S*-Nitroso-*N*-Acetyl-*D*-penicillamine (*D*-SNAP, *D*-32).** **mp** (H_2O/MeOH): 152-154 °C, lit[193]: 153-154 °C. $[\alpha]_D^{25}$: –48 (*c* 1, EtOH/H_2O 1:1). **^{1}H NMR** (D_2O, 300 MHz)**:** δ 5.23 (1H, s, $C_\alpha\underline{H}$), 2.03 (6H, s, 2 x $C\underline{H}_3$), 2.02 (3H, s, $COC\underline{H}_3$). **IR** (KBr): ν_{max} /cm^{-1}: 3345 (NH), 2978, 2931, 1941, 1715 (C=O), 1606, 1543, 1504, 1301, 1223, 1114. **MS** (FAB): m/z 221 ($(M+H)^+$, 100), 191 ($(M+H-NO)^{\cdot+}$, 69), 190 ($(M-NO)^+$, 63), 176 (59). **HRMS** (FAB): found 221.0605, $C_{17}H_{13}O_4N_2S$ $(M+H)^+$ requires 221.0596. **HPLC** (Hypersyl C18, TFA 0.1 %, MeOH/H_2O gradient from 1% to 35 % in 50 min): retention time 38 min, one single peak. **UV** (H_2O): λ_{max} /nm 340 (ε 9.05 x 10^2 $M^{-1}cm^{-1}$), 595 (ε 8.41 $M^{-1}cm^{-1}$).

6. 4. 4. *In-situ* preparation of *D* and *L* enantiomers of *S*-nitrosopenicillamine.

D and *L*-SNAP employed in biological studies were freshly prepared *in-situ* for every experiment, by dissolving the corresponding enantiomer of the acetylated thiol (***D*** or ***L-NAP***) in H_2O, in the presence of 1 equivalent of $NaNO_2$ and acidifying the solution to pH 2 with HCl. The solutions turned immediately green and were used straightaway, in order to avoid thermal or photochemical decomposition.

5. 5. *In situ* preparation of *D* and *L* enantiomers of *S*-nitrosocysteine.



(27)

As described above, these were nitrosated *in-situ* prior to the biological tests, by dissolving 1 equiv. of the free thiol (both enantiomers were commercially available) and 1 equiv. of $NaNO_2$ in an aqueous solution of pH 2. The solution turned red after a few seconds and was used immediately after, in order to avoid spontaneous decomposition.

5. 6. Biological evaluation of the stereospecificity of nitric oxide-release from bioactive thionitrites.

5. 6. 1. General method.

Male Sprague-Dawley rats (approximate weight 250 g, from Charles Rives) were stunned and killed by cervical dislocation. The thoracic aorta was dissected out, cleaned of connective tissue and cut into rings (3-4 mm wide). Aortic rings were suspended in 25 mL organ baths containing Krebs-bicarbonate buffer (composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^{-1} 128, HCO_3^- 25, HPO_4^- 1.2, SO_4^- 1.2, *D*-glucose 11), maintained at 37 °C and gassed with 95 % O_2/ 5 % CO_2. A resting tension of 1.0 g was applied to each tissue and changes in isometric tension measured using a force displacement transducer (FT03) connected to a Rikadenki chart recorder. The tissues were allowed to equilibrate for 60 min prior to experimentation. During this time the tissues were washed with buffer three times, and the tension re-adjusted to baseline.

Tissues were primed with KCl (48 mM) before a concentration (0.1 μM) of phenylephedrine (PE) producing a sub-maximal (70-85 % of the maximum) contraction was added. Once the response had stabilised (*ca.* 5 min), acetylcholine (1 μM) was added to asses the integrity of the endothelium. If the contractions to PE were not maintained or relaxations (> 60 %) of the PE induced tone to acetylcholine were not observed, the tissues were discarded.

Tissues were washed over 30 min after which a cumulative concentration-response curve to PE was constructed. The tissues were then washed over 60 min to restore basal tone before contracting to approximately 80 % of the maximum PE-induced response. Once a stable response to PE was obtained, concentration-response curves to *D*- and *L* CysNO (0.03-300 nM), SNAP (0.01-30 μM) and GSNO (concentrations) were obtained.

5. 6. 2. Data analysis.

Relaxations are expressed as a percentage reversal of the PE-induced tone. Doses are expressed in concentrations either in nM or μM units. Responses were calculated as means from at least 4 separate experiments. A t-test was used to asses the significance of differences between dose-response curves. A P value <0.05 was taken to indicate a statistically significant difference. Statistical analysis was performed using Prism software.

5. 6. 3. Results obtained for *D* and *L*- CysNO.

[*L*-CysNO]	% relaxation	for	n= 8	tissues	from	n= 2	animals		means
0.03	0	0	0	0	0	0	0	0	0
0.1	1	0	4	3	7	5	6	3	3.625
0.3	3	0	4	7	13	7	10	7	6.375
1	13	5	17	12	20	17	30	28	17.75
3	31	23	33	43	51	52	70	52	44.375
10	75	53	80	81	71	83	90	86	77.375
30	94	77	100	95	86	95	95	90	91.5
100	100	100	100	100	100	100	100	100	100
300	100	100	100	100	100	100	100	100	100

[*D*-CysNO]	% relaxation for n= 8 tissues from n= 2 animals								means
0.03	0	0	0	0	0	0	0	0	0
0.1	0	0	4	0	4	3	7	6	3
0.3	3	0	6	3	6	4	18	16	7
1	7	4	20	12	7	21	51	35	19.625
3	50	17	55	55	24	55	74	50	47.5
10	87	73	88	90	54	85	88	88	81.625
30	96	93	94	95	87	97	96	96	94.25
100	100	100	100	100	100	100	100	100	100
300	100	100	100	100	100	100	100	100	100

5. 6. 4. Results obtained for *D*- and *L*-SNAP.

[L-SNAP]	% relaxation (n= 9 tissues from n= 2 animals)									mea
3	0	0	0	0	0	0	0	0	0	0
10	0	2	2	0	0	0	0	2	0	0.66
30	1	8	2	4	4	8	3	3	4	4.11
100	12	29	4	18	12	14	6	5	15	12.7
300	34	67	25	55	38	36	38	27	31	39
1000	81	92	59	91	69	60	72	63	66	72.5
3000	94	100	87	100	88	94	91	85	97	92.8
10000	100	100	100	100	100	100	100	100	100	100
30000	100	100	100	100	100	100	100	100	100	100

[D-SNAP]	% relaxation (n= 9 tissues from n= 2 animals)									mea
3	0	0	0	0	0	0	0	0	0	0
10	0	0	2	2	8	0	0	0	0	1.33
30	1	0	5	5	14	2	3	3	3	4
100	11	10	17	18	27	4	11	5	10	12.5
300	15	27	43	38	56	14	30	23	22	29.7
1000	62	71	90	70	78	38	72	64	63	67.5
3000	85	94	95	87	84	64	90	92	91	86.8
10000	98	100	100	100	95	88	99	96	100	97.3
30000	100	100	100	100	100	100	100	100	100	100

5. 6. 5. Results obtained for *D*- and *L*-GSNO.

[L-GSNO]	% relaxation (n= 18 tissues from n= 6 animals)								
1	0	0	3	7	3	0	0	5	4
3	0	7	12	10	6	5	5	4	0
10	4	4	13	16	14	10	14	13	12
30	5	11	16	25	18	16	23	16	28
100	25	25	34	62	39	37	32	46	76
300	81	80	79	82	79	83	89	82	97
1000	99	94	91	98	92	97	99	97	100
3000	100	100	100	100	100	100	100	100	100
10000	100	100	100	100	100	100	100	100	100

(table continued below)

									means
0	0	4	17	9	3	5	0	4	3.556
0	0	7	44	16	4	14	4	11	8.2778
0	0	13	71	42	21	30	8	20	16.944
0	0	16	88	79	67	44	18	33	27.944
4	3	29	98	93	90	75	60	70	49.889
12	15	51	100	98	99	88	79	83	76.5
31	34	76	100	100	100	96	93	91	88.222
72	76	94	100	100	100	100	100	100	96.778
96	95	100	100	100	100	100	100	100	99.5

[D-GSNO]	% relaxation (n= 18 tissues from n= 6 animals)								
3	0	0	0	0	0	0	3	5	4
10	0	0	0	4	3	0	5	11	8
30	0	0	0	9	5	3	12	18	15
100	10	5	12	24	15	10	29	28	28
300	55	23	46	68	38	30	73	80	77
1000	89	78	94	88	85	66	93	98	96
3000	99	97	100	97	97	87	100	100	100
10000	100	100	100	100	100	100	100	100	100

(table continued below)

									means
0	0	0	0	3	0	0	3	0	1
0	0	0	4	7	4	16	11	3	4.222
0	0	0	10	28	12	27	21	6	9.2222
0	3	6	57	79	38	56	33	28	25.611
19	12	25	83	96	69	82	70	82	57.111
49	32	73	98	100	94	93	87	96	83.833
81	77	92	100	100	100	100	100	100	95.944
95	99	100	100	100	100	100	100	100	99.667

CHAPTER 6

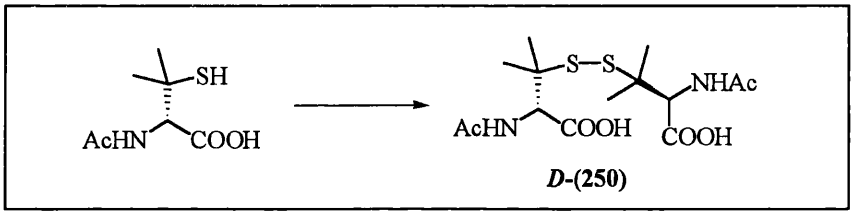
A STUDY OF THE THIONITRITE–DISULFIDE EXCHANGE REACTION.

6. 1. Preparation of thionitrites.

S-nitroso-*L*-glutathione and *S*-nitroso-*N*-acetyl-*D*-penicillamine, used for the exchange studies described below, were prepared as solids, as described in Chapter 5 of the experimental.

6. 2. Preparation of disulfides.

6. 2. 1. Synthesis of *N*-acetyl-*D*-penicillamine disulfide.



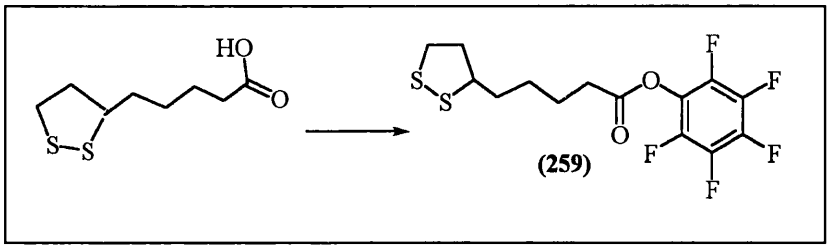
***D*-(250)**

Commercial *N*-acetyl-*D*-penicillamine (500 mg, 2.6 mmol) was dissolved in 6 mL of H_2O and 6 mL of MeOH, at r. t. An iodine solution (663 mg, 2.6 mmol in 5 mL of MeOH) was added slowly until permanent brown coloration developed, evidence of a slight excess of I_2. Methanol was then evaporated and the H_2O was eliminated by freeze-drying. The resulting yellow crude solid was crystallised from MeOH/P.E. furnishing the disulfide as a white solid in quantitative yield.

(D,D)-Bis-(2-acetamido-1,1-dimethyl-2-carboxyethyl)disulfide (N-Acetyl-D-penicillamine disulfide, D-NAP$_2$, D-250). **mp** (MeOH/P.E.): 210-212 °C decomp., lit[194] (aq. EtOH): 207 °C, lit[195]: 128-131 °C. **^{1}H NMR** (D_2O, 300 MHz): δ 4.12 (1H, s, $C_\alpha\underline{H}$), 1.88 (3H, s, $C\underline{H_3}CO$), 1.27 (3H, s, $C\underline{H_3}$), 1.22 (3H, s, $C\underline{H_3}$). **^{13}C NMR** (D_2O, 75.4 MHz): δ 187.5 and 175.0 ($\underline{C}$=O), 50.76 ($\underline{C}_\alpha$H), 26.14, 23.50 and 21.93 ($\underline{C}H_3$). **MS** (m/z, FAB): m/z 403 ($(M+Na)^+$, 100), 381 ($(M+H)^+$, 30). **HRMS** (FAB): found 403. 0987, $C_{14}H_{24}N_2O_6S_2Na$ $(M+Na)^+$ requires 403.0974. **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 43 min, one single peak.

6. 2. 2. Attempted synthesis of lipoylglycine.

6. 2. 2. 1. Preparation of the pentafluorophenyl ester of lipoic acid.

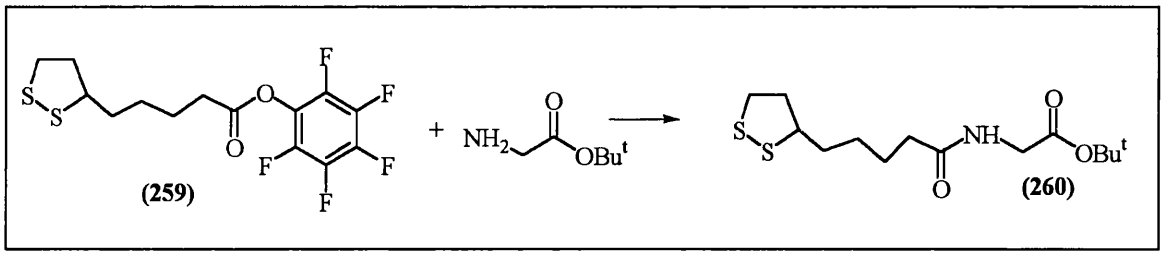


(259)

Pentafluorophenol (0.9 g, 5.33 mmol) and lipoic acid (1g, 4.85 mmol) were mixed in THF/EtOAc (12 mL/20 mL), at 0 °C. DCC (1.0 g, 4.85 mmol) was then added and the resulting solution stirred at 0 ^{0}C for 30 min and then at r. t. for 2 h. The precipitate formed was removed by filtration and the solvent evaporated. Hexane was added and the precipitate formed separated by filtration. The remaining solution was extracted with DCM and washed with $NaHCO_3$ and H_2O. After drying with $MgSO_4$ the solvent was evaporated at reduced pressure, giving the product as a yellow thick oil.

Pentaflurophenyl 5-(1,2-dithiolan-3-yl)pentanoate (pentafluorophenyl lipoate, 259). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.59 (1H, tt, J= 14.3, 6.48, C<u>H</u>SS), 3.18 (2H, m, C<u>H$_2$</u>SS), 2.69 (2H, t, J= 7.32, C<u>H$_2$</u>CO), 2.48 (1H, m, ring CH<u>H</u>), 2.0-1.5 (7H, m, ring CH<u>H</u> + 3 x chain C<u>H$_2$</u>). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 169.32 (<u>C</u>=O), 142.43-136.12 (m, <u>C</u>F_{Ar}), 56.12, 40.16, 38.49, 34.47, 34.36, 33.07, 28.43, 24.44. **IR** (neat) ν_{max}/cm^{-1}: 2926, 1786 (C=O), 1516, 1100, 999. **MS** (FAB): 372 ($M^{+\cdot}$, 100), 339 ((M–$^{\cdot}$SH)$^+$, 7), 307 (M–$^{\cdot}$SSH)$^+$, 5), 189 (32), 161 (28), 154 (17), 136 (18). **HRMS** (FAB): found 372.0355, $C_{14}H_{13}S_2F_5O_2$ ($M^{+\cdot}$) requires 372.0289.

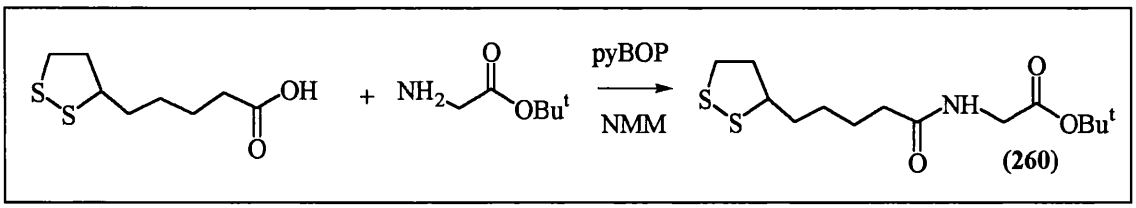
6. 2. 2. 2. Coupling of lipoic acid with tert-butyl glycinate; method a: via the pentafluorophenyl ester.



The pentafluorophenyl ester of lipoic acid (**259**, 1g, 2.68 mmol) was added (in 5 mL of DCM) over a solution of Gly-OBu^t (224 mg, 234 μL, 1.71 mmol) in 15 mL of DCM at r. t. The resulting mixture was stirred at r. t. for 2 h. The reaction was worked up by diluting in more DCM and washing with sat. $NaHCO_3$ solution and H_2O. The organic layer was dried over $MgSO_4$ and filtered off. The residual solid was dissolved in EtOAc/hexane and chromatographed on SiO_2 using EtOAc/hexane 4:5 as eluant. The product was obtained as a thick oil in 68 % yield.

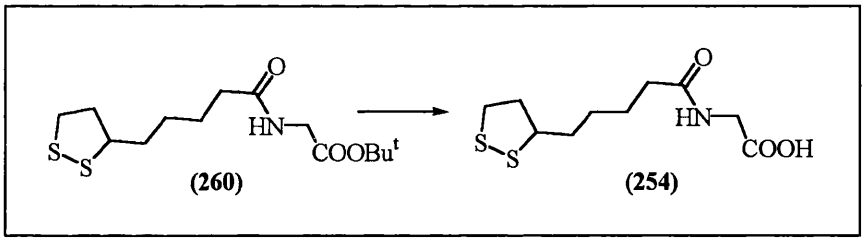
Tert-butyl N^{α}-[5-(1,2-dithiolan-3-yl)pentanoyl]glycinate (tert-butyl lipoylglycinate, 260). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.05 (1H, bs, NH), 3.90 (2H, d, J= 5.0, $C_{\alpha}H_2$), 3.54 (quint., 1H, J= 6.5, CHSS), 3.15 (2H, m, CH_2SS), 2.42 (1H, m, ring CHH), 2.22 (2H, t, J= 7.5, CH_2CO), 1.88 (1H, m, ring CHH), 1.80-1.60 (6H, m, 3 x chain CH_2), 1.64 (9H, s, $(CH_3)_3C$). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 172.75 and 169.11 (C=O), 82.23 (CO), 56.26 (Gly $C_{\alpha}H_2$), 41.90, 40.18, 38.38, 36.00, 34.52, 28.75, 27.95, 25.15. **IR** (neat)ν_{max}/cm-1: 3313 (NH), 3085, 2921, 2856, 1741 (C=O), 1654 (C=O), 1540, 1365, 1224, 1157, 1037, 944. **MS** (FAB): 452 $((M+Cs)^+$, 100), 319 $((M+Na)^+$, 18), 296 ($M^{+\cdot}$, 22), 264 $((M-S)^{+\cdot}$, 82), 225 (22), 189 (72). **HRMS** (FAB): found 452.0330, $C_{14}H_{25}S_2O_3NCs$ $(M+Cs)^+$ requires 452.0310.

6. 2. 2. 3. Coupling of lipoic acid to tert-butyl glycinate; method b: via pyBOP activation.



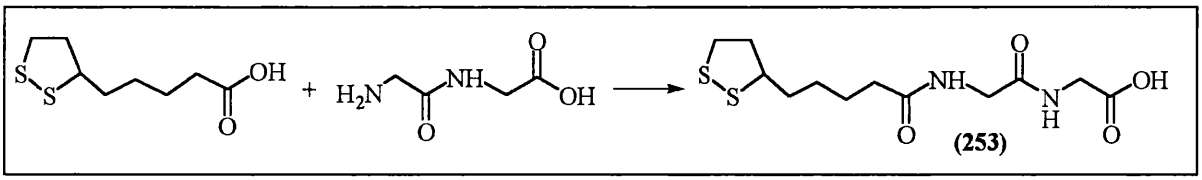
Lipoic acid (412 mg, 2 mmol) was mixed with pyBOP (1.3 g, 5 mmole) and NMM (274 μL, 5 mmol) in anhydrous DCM (15 mL). After 10 min of stirring at r. t. the mixture was poured over a solution of *tert*-butyl glycinate hydrochloride (168 mg, 2 mmol) in DCM (10 mL). The reaction was stirred at r. t. for 3 h and it was diluted with more DCM and washed with H_2O. After drying over $MgSO_4$ and evaporation of the solvent in *vacuo* the residue was chromatographed on silica gel (P.E./Et_2O 6/4). The product (**260**) was obtained as a yellow oil in 17 % yield (109 mg) and displayed the same spectral data as the product obtained by method a.

6. 2. 2. 4. Attempted deprotection to lipoylglycine.



Protected *tert*-butyl lipoylglycinate (**260**) was dissolved in TFA/H_2O 95 %/2.5 % and EDT or triethylsilane (2.5 %) were added as a scavenger. The mixture was stirred at r. t. for 3 h and the solvent evaporated under vaccum. The product polymerised when purification was attempted on several occasions. Formation of **254** could not be detected by ^1H NMR of the crude mixture after evaporation of the solvent .

6. 2. 3. Preparation of lipoylglycylglycine.



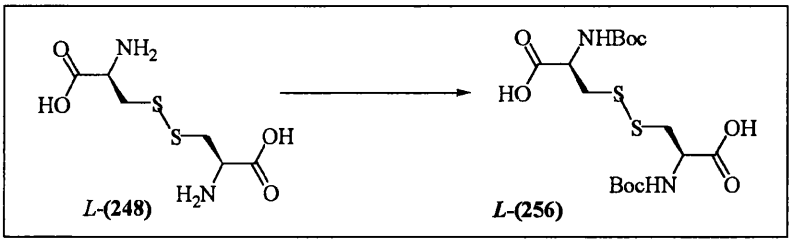
(253)

Following the described procedure[196], lipoic acid (350 mg, 1.69 mmol) and Et_3N (240 μL, 1.69 mmol) were mixed in THF (15 mL) at 0 ^{0}C and isobutyl chloroformate (225 μL, 1.69 mmol) was added slowly dissolved in THF (2 mL). The resulting mixture was stirred at 0 ^{0}C for 10 min. This solution, which contained the pre-formed mixed lipoyl isobutyl carbonate was added over a solution of glycylglycine (231 mg, 1.69 mmol) in NaOH 1M and the resulting mixture stirred at r. t. for 15 min. The reaction was acidified by addition of HCl 1 M and extracted with $CHCl_3$. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated in *vacuo*. The resulting solid residue was crystallised from a minimum volume of hot ethanol and the product was obtained as a yellow solid (45 %).

N^{α}-[5-(1,2-dithiolan-3-yl)pentanoyl]glycylglycine (lipoylglycylglycine, 253). **mp** (EtOH): 137-139 °C, lit[196] (EtOH): 138-139 °C. **^{1}H NMR** (CD_3OD, 300 MHz)**:** δ 3.88 (2H, s, Gly $C_{\alpha}\underline{H}_2$), 3.34 (2H, s, Gly $C_{\alpha}\underline{H}_2$), 3.19 (2H, s, 2 x N$\underline{H}$), 3.60 (1H, m, C$\underline{H}$SS), 3.05 (2H, m, $C\underline{H}_2$SS), 2.44 (1H, m, ring C$\underline{H}$H), 2.24 (2H, t, J= 7.3, $C\underline{H}_2$CO), 1.88 (1H, m, ring CH$\underline{H}$), 1.82 (4H, m, chain $C\underline{H}_2C\underline{H}_2$), 1.44 (2H, m, chain $C\underline{H}_2$). **^{13}C NMR** (CD_3OD, 100.6): δ 176.52, 172.88 and 172.14 ($\underline{C}$=O), 57.51 ($\underline{C}$HSS), 43.32 and 41.72 (Gly $\underline{C}_{\alpha}H_2$), 41.29, 39.35, 36.63, 35.76, 29.90 and 26.44 (CH_2). **IR** (KBr): ν_{max}/cm^{-1} 3298 (NH), 3079, 2924, 2853, 2658, 2534, 1738 (C=O), 1652, 1606, 1551, 1395, 1356, 1223. **MS** (FAB): m/z 343 ($(M+Na)^+$, 48), 321 ($(M+H)^+$, 45), 320 ($M^{\cdot+}$, 35), 289 (34), 262 (50), 246 (25), 206 (37), 189 (100), 176 (43)**. HRMS** (FAB): found 321.0956, $C_{12}H_{21}N_2O_4S_2$ $(M+H)^+$ requires 321.0943. **HPLC** (Hypersyl C18, TFA 0.1 % gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 52 min, one single peak.

6. 2. 4. Synthesis of *L,L*-cystyldiglycine derivative

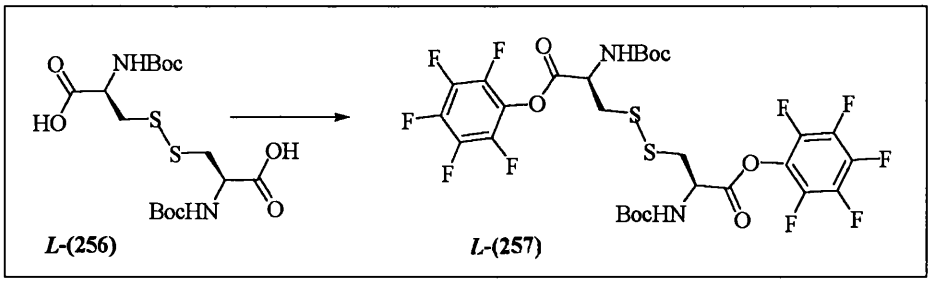
6. 2. 4. 1. Bis-Boc-protection of L-cystine.



By a modification of the literature procedure[197] *L*-cystine (2.4 g, 10 mmol) was dissolved in 22 mL of 1M NaOH and *tert*-butyldicarbonate (4.4 g, 20 mmol) was added in portions over a period of 2 h. The resulting clear solution was stirred at r. t. for 1 night. The milky solution was then acidified to pH 1 by addition of aq. $KHSO_4$ and extracted with EtOAc. The organic layer was separated and dried over $MgSO_4$ and the solvent was evaporated under *vaccuo* furnishing a white solid (1.47 g, 34 %).

N,N-Bis-(tert-butyloxycarbonyl)-L-cystine (L-256). **mp** (EtOAc): 136-138 °C, lit.[197] 137-139 °C. **^{1}H NMR** (D_2O + NaOD, 400 MHz): δ 3.86 (1H, m, $C_{\alpha}H$), 2.86 (1H, m, CHHS), 2.57 (1H, m, CHHS), 1.09 (9H, s, $(CH_3)_3C$). **IR** (CCl_4) ν_{max}/cm^{-1}: 3367 (NH), 3112, 3008, 2769, 1753 (C=O), 1728 (C=O), 1685, 1517, 1241, 1168. **MS** (FAB): m/z 463 ($(M+Na)^+$, 45), 441 ($(M+H)^+$, 5), 407 ($M-SH)^+$, 4), 329 (17), 285 (32), 57 (100, $^tBu^+$).

6. 2. 4. 2. Preparation of the dipentafluorophenyl ester of N,N-bis-Boc-L-cystine.

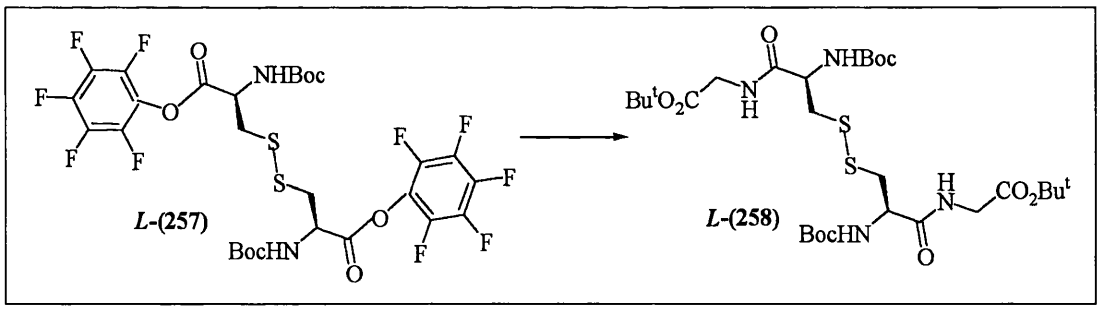


Following the literature procedure[197], pentafluorophenol (1.2 g, 3.6 mmol) and bis-Boc-cystine (***L*-256**, 790 mg, 1.8 mmol) were mixed in THF/EtOAc (12 mL/20 mL) at 0 °C. DCC (1.4 g, 3.6 mmol) was then added and the resulting solution stirred at 0 °C for 30 min and then at r. t. for 2 h. The precipitate formed was removed by filtration and the

solvent evaporated. Hexane was added and the precipitated separated by filtration, the remaining solution was extracted with DCM and washed with $NaHCO_3$ and H_2O. After drying with $MgSO_4$ the solvent was evaporated, giving the product as a white solid. This was crystallised from DCM/hexane (1.1 g, 80 %).

Dipentafluorophenyl N,N-bis-(tert-butyloxycarbonyl)dicystinate (L-257)[197]. **mp** (DCM/hexane): 180-181 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz): δ 7.40 (1H, d, J= 7.64, NH), 4.15 (1H, m, C_αH), 2.00 (2H, d, J= 1.8, CH_2S), 0.91 (9H, s, $(CH_3)_3C$). **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 5.41 (1H, m), 4.96 (1H, m), 3.35 (2H, m), 1.48 (9H, s). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 167.20 and 154.85 (C=O), 139.74, 139.64 and 124.36 (CF_{Ar}), 81.11 (CO), 53.04 (Cys C_αH), 40.55 (CH_2S), 28.22 ($(CH_3)C$). **IR** (neat) ν_{max}/cm^{-1}: 3379 (NH), 2921, 2856, 1807 (C=O), 1774 (C=O), 1687, 1512, 1175, 995. **MS** (FAB): m/z 795 ($(M+Na)^+$, 3), 773 (M^+, 1), 661 (10), 617 (100), 573 (25), 405 (10), 361 (12). **HRMS** (FAB): found 795.0869, $(C_{14}H_{13}NO_4SF_5)_2Na$ $(M+Na)^+$ requires 795.0889.

6. 2. 4. 3. Coupling of cystine with glycine-tert-butyl ester; method a: via the pentafluorophenyl ester.

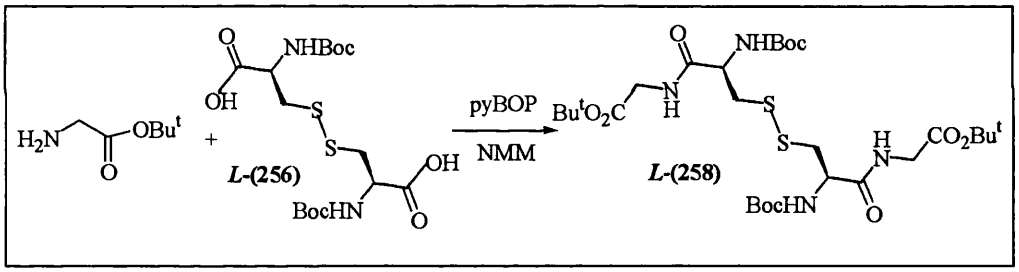


Tert-butyl ester glycinate (224 mg, 234 μL, 1.7 mmol) was dissolved in 15 mL of DCM and the pentafluorophenyl ester *L*-**257** (600 mg, 0.77 mmol) was then added at r. t. The mixture was stirred at r. t. for 2 h and then extracted with more DCM, washed with aq. sat. $NaHCO_3$ (once) and with H_2O (once). The organic layer was dried and filtered off. After evaporation of the solvent at reduced pressure, the remaining residue (foam) was chromatographed (SiO_2, hexane/EtOAc 5/5), giving the product as a white amorphous solid (620 mg, 68 %).

Di-tert-butyl [N,N-bis-(tert-butyloxycarbonyl)cystinyl]diglycinate[198] (L-258).

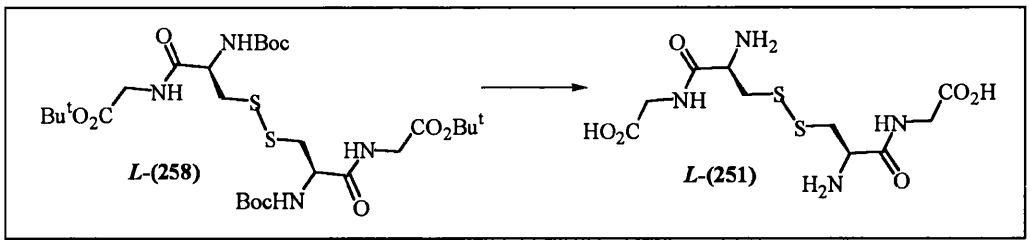
mp: 127-129 ^{0}C, lit[198] 131 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.09 (2H, m, 2 x Gly NH), 5.54 (2H, d, J= 9.5, 2 x Cys NH), 4.95 (2H, m, 2 x Cys C_αH), 4.10 (2H, dd, J= 17.8, 6.5, 2 x Gly C_αHH), 3.78 (2H, dd, J= 17.8, 5.2, 2 x Gly C_aHH), 3.10 (2H, dd, J= 14.6, 3.8, 2 x CHHS), 2.94 (2H, dd, J= 14.6, 10.7, 2 x CHHS), 1.45 (18H, s, 2 x (CH_3)C), 1.44 (18H, s, 2 x (CH_3)C). **^{13}C NMR** ($CDCl_3$, 100.6 MHz): δ 170.69 and 168.31 (C=O), 155.87 (NH(C=O)OC), 81.87 and 80.21 (CO), 54.53 (Cys C_αH) and 47.07 (Gly $C_\alpha H_2$), 41.55 (CH_2S), 28.35 and 28.01 ((CH_3)C). **IR** (neat): ν_{max}/cm^{-1} 3335 (NH), 2965, 2921, 2856, 1725 (C=O), 1687 (C=O), 1664, 1512, 1147. **MS** (FAB): m/z 667 ($(M+H)^+$, 100), 611 (32), 567 (76), 555 (12), 511(35). **HRMS** (FAB): found 799.2023, $(C_{14}H_{25}O_2N_2S)_2Cs$ $(M+Cs)^+$ requires 799.1998.

6. 2. 4. 4. Coupling of cystine and tert-butylglycinate; method b: via pyBOP activation.



Bis-Boc cystine ***L*-256** (442 mg, 0.5 mmol) was dissolved in DCM (10 mL) and NMM (508 μL, 2.5 mmol) and pyBOP® (2.6 g, 2.5 mmol) were added at r. t. The mixture was stirred at r. t. for 10 min and then poured onto a solution of glycine *tert*-butyl ester hydrochloride (336 mg, 1 mmol) in DCM (5 mL). The resulting mixture was stirred at r. t. for 3 h, and then it was diluted with DCM, washed with H_2O and the organic solvent evaporated at reduced pressure, giving a yellow paste. This was chromatographed on silica gel (P.E./EtOAc 1:1), furnishing the product as a white amorphous solid (376 mg, 54 %). This displayed the same mp and spectral data as the product obtained by method 6. 2. 4. 3.

6. 2. 4. 5. Preparation of deprotected bis-glycylcystine .



The protected peptide ***L*-258** (600 mg, 0.9 mmol) was dissolved in 20 mL of TFA/DCM (95 %/5 %) and a few drops of EDT or triethylsilane were added as scavenger. The solution was stirred at r. t. for 3 h and then the solvent evaporated under reduced pressure, giving a yellow oily residue. Et_2O was added and a white solid precipitated. This was collected by filtration and washed with cold Et_2O (417 mg, of the impure TFA salt). The product proved to be impure by HPLC and unsuitable for ulterior studies.

Cystinyldiglycine TFA salt (L-251). **^{1}H NMR** (D_2O, 400 MHz): δ 4.26 (2H, dd, J= 7.7, 5.1, 2 x Cys $C_\alpha\underline{H}$) 3.91 (4H, s, 2 x Gly $C_\alpha\underline{H}_2$), 3.24 (2H, dd, J= 15.1, 5.0, 2 x C$\underline{H}$HS), 3.17 (2H, s, N$\underline{H}$), 3.04 (2H, dd, J= 15.1, 7.7, 2 x CH$\underline{H}$S). **HPLC** (Hypersyl C18, 0.1 % TFA, MeOH/H_2O gradient from 1% to 35 % in 50 min) : retention time around 4 min, purity: < 45 %.

6. 3. Thionitrite-disulfide exchange experiments.

In all the reactions described below, the same quantities of reagents were used. In all cases 0.027 mmoles of each stoichiometric reagent were employed: *D*-SNAP (*D*-**32**, 6 mg), *L*-GSNO (*L*-**26**, 9.2 mg), *D*-NAP_2 (*D*-**250**, 10.4 mg), *DL*-lipoylglycylglycine (**253**, 8.6 mg), *DL*-lipamide (**255**, 6 mg), cystine (**248**, 6.5 mg), *L*-GSSG (*L*-**249**, 16.6 mg). The additives EDTA, copper(II) and thiol (NAP) were added in catalytic amounts (indicated for each case).

6. 3. 1. Disulfide-disulfide exchange: blank experiments.

In order to ensure that a disulfide-disulfide exchange reaction did not take place under the conditions of our thionitrite-disulfide exchange experiments, a series of blank reactions were carried out. These involved mixing one equivalent each (0.027 mmol) of two disulfides, in 2.5 mL of phosphate buffer of pH 7.4 (final concentration 10^{-2} M). The mixtures were stirred at 37 °C in the presence of either 0. 2 equivalents of the metal complexant EDTA or 0.2 equivalents of $CuCl_2 \cdot H_2O$. After 2 days, water was eliminated by freeze-driyng and the remaining solid mixture was analysed by ^1H NMR (D_2O). In all the experiments carried out (GSSG+Cystine, GSSS+NAP_2 and Cystine+NAP_2), both with EDTA and in the presence of added copper, only the peaks corresponding to the starting disulfides were observed. Peaks corresponding to a mixed disulfide were not detected in any case.

6. 3. 2. Thionitrite-disulfide exchange reactions: general method.

One equivalent (0.027 mmol) of one of the disulfides prepared above (*DL*-lipoylglycylglycine (**253**) or *N*-acetyl-*D*-penicillamine disulfide (*D*-**250**) or of the commercially available disulfides *L*-glutathione disulfide (*L*-**249**), *DL*-lipamide (**255**) or *L*-cystine (*L*-**248**), was mixed with one equivalent (0.027) of *S*-nitroso-*L*-glutathione (*L*-**26**) or *N*-acetyl-*S*-nitroso-*D*-penicillamine (*D*-**32**), prepared as in section 4, in 2.5 mL of phosphate buffer of the indicated pH (final concentration of disulfide and thionitrite= 10^{-2} M). In a few cases and in order to aid solubility the reagents were mixed in 2 mL of phosphate buffer and 0.5 of a co-solvent (EtOH or DMF). The reactions were carried out either under an atmosphere of air or under a possitive pressure of N_2 (provided by a baloon), as indicated for each experiment. All reactions were carried out at 37 °C (± 2 °C). In the case of the reactions carried out in the absence of metal salts, EDTA (0.2 equiv.) was added in order to ensure complexation of adventitious copper which may be present in the distilled H_2O used. When reactions were carried out in the presence of added copper, this was introduced in the form of hydrated $CuCl_2$ to the final concentration indicated in each case. Experiments were carried out in the dark in order to minimise photochemical decomposition. The evolution of the exchange-reactions was monitored by reverse-phase HPLC (Hypersyl C18, 0.1 % TFA, MeOH/H_2O gradient from 2 % to 35 % in 50 min, detection at λ 215 nm (amide bond)). When the reactions

were completed (complete disappearance of the peak corresponding to the thionitrite) or when no detectable changes in the composition of the mixture were observed between several consecutive HPLC-injections, the reactions were stopped and the solvent eliminated by freeze-drying. The solid residue was analysed by ^{1}H NMR (D_2O) and/or by HPLC-MS. The yields of final products were calculated by integration of the best resolved ^{1}H NMR peaks and the identity of the products confirmed from the mass spectrum obtained for each HPLC peak.

6. 3. 3. SNAP-GSSG exchange experiments.

6. 3. 3. 1. Under physiological conditions (pH 7.4, 37 °C, aerated solutions).

The reaction was carried out in the presence of 0. 2 equiv. of EDTA. and in an aerated solution. After 77h a mixture of GSSG, NAP_2 and the mixed disulfide was obtained in a molar proportion 53: 20.5: 26.5 %.

GSSG (249). **^{1}H NMR** (D_2O, 400 MHz): δ 4.65 (2H, m overlapped with the water peak, 2 x Cys $C_\alpha\underline{H}$), 3.83 (4H, s, 2 x Gly $C_\alpha\underline{H}_2$), 3.68 (2H, t, J= 5.6, 2 x Glu $C_\alpha\underline{H}$), 3.14 (2H, dd, J= 14.4, 4.4, 2 x C$\underline{H}$HS), 2.84 (2H, dd, J= 14.4, 9.0, 2 x CH$\underline{H}$S), 2.39 (4H, m, 2 x C$\underline{H}_2$), 2.03 (4H, m, 2 x C$\underline{H}_2$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 9.8 min

NAP_2 (250). **^{1}H NMR** (D_2O, 300 MHz): δ 4.12 (1H, s, $C_\alpha\underline{H}$), 1.88 (3H, s, C$\underline{H}_3$CO), 1.27 (3H, s, C$\underline{H}_3$), 1.22 (3H, s, C$\underline{H}_3$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 43 min

Mixed disulfide 261. **^{1}H NMR** (D_2O, 400 MHz, only the best resolved peaks are given): δ 4.52 (1H ,m, Cys $C_\alpha\underline{H}$), 4.04 (1H, NAP Gly C_αH), 2.99 (1H, dd, J= 14.4, 4.4, Cys C$\underline{H}$HS), 1.21 (3H, s, C$\underline{H}_3$), 1.17 (3H, s, C$\underline{H}_3$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 24 min

HPLC/MS (of the mixture,): 9.59 min (GSSG, $M^{+\cdot}$ = 612), 17.37 (GSNAP, $(M+H)^+$ = 497), 23.12 min (NAP_2, $(M+H)^+$ = 381).

6. 3. 3. 2. Study at different pHs.

The reactions were carried out as above, but variying the pH between 1 and 12. The solutions in all cases were aerated. Proportions of the products were obtained by integration of the ^{1}H NMR peaks (see above).

GSSG	SNAP	pH	time	SNAP	GSSG	NAP_2	261
1 equiv.	1 equiv.	1.68	88 h	~5 %	66.6 %	~28 %	0 %
			120 h	0 %	66.6 %	33.3 %	0 %
1 equiv.	1 equiv.	3.77	88 h	~6 %	66.6 %	~29 %	0 %
			120 h	0 %	66.6 %	33.3 %	0 %
1 equiv.	1 equiv.	5.80	88 h	~6 %	66.6 %	~29 %	0 %
			120 h	0 %	66.6 %	33.3 %	0 %
1.2 equiv.	1 equiv.	7.00	96 h	0 %	56 %	16 %	27 %
1.2 equiv.	1 equiv.	8.00	96 h	0 %	62 %	19 %	19 %
0.8 equiv.	1 equiv.	11.00	88 h	0 %	47 %	29 %	23 %

6. 3. 3. 3. Study at different copper concentrations.

The reaction was carried out at pH 7.4, in the presence of variying concentrations of $CuCl_2$, in aerated solutions. Concentrations were successively increased by ten-fold from 10^{-8} M to 0.1 M. Mixed disulfide was not obtained in any case and reactions were finished after 3 to 7 h, as determined by HPLC.

6. 3. 3. 4. In the presence of added thiol.

Reactions were carried out at pH 3.77 or 5.80, in the presence of 0. 2 equiv. of added thiol (NAP) and 0.2 equiv. of EDTA, in aerated solutions. No mixed disulfide was detected after 5 days.

6. 3. 3. 5. GSSG-NAP_2 in the presence of catalytic SNAP.

The reaction was carried out at pH 7.4, in the presence of 0.2 equiv. of SNAP and 0.2 equiv. of EDTA. After 5 days, less than *ca.* 10 % exchange was detected by HPLC.

6. 3. 3. 6. Reaction under N_2.

The reaction was carried out at pH 3.77 or 5.80, in the presence of 0.2 equiv. of EDTA, and under an atmosphere of N_2. In order to ensure total absence of O_2, the solutions were submitted to several freeze-pump-thaw cycles and saturated with N_2 prior to starting the heating at 37 °C. Additional possitive pressure of N_2 was achieved by attaching a baloon connected through a needle to the reaction vessel. No mixed disulfide **261** was detected by HPLC.

6. 3. 4. GSNO-NAP_2 exchange.

6. 3. 4. 1. Under physiological conditions.

The reaction between GSNO and NAP_2 was carried out in the conditions described above for SNAP and GSSG. The thionitrite decomposed much more slowly in this case. The results, as determined by HPLC (molar concentrations are approximate) are summarised below. Exchange product **261** was not detected.

NAP_2	GSNO	additive	time	GSNO	GSSG	NAP_2	261
1 equiv.	1 equiv.	EDTA (0.2 equiv.)	3 h	50 %	0 %	50 %	0 %
		EDTA (0.2 equiv.)	96 h	31 h	19 %	50 %	0 %

6. 3. 4. 2. In the presence of copper.

The reaction was carried out as described above in the presence of 10^{-8} M $CuCl_2$. No exchange product was formed and the results, as determined by HPLC (molar concentrations are approximate) are summarised below.

NAP_2	GSNO	additive[a]	time	GSNO	GSSG	NAP_2	261
1 equiv.	1 equiv.	$Cu^{2+}(10^{-8}$ M)	2 h	40 %	10 %	50 %	0 %
		$Cu^{2+}(10^{-8}$ M)	68 h	34 %	16 %	50 %	0 %

6. 3. 5. SNAP-cystine exchange.

6. 3. 5. 1. Under physiological conditions.

The reaction was carried out in the presence of 0. 2 equiv. of EDTA. and in an aerated solution. After 88 h a mixture of cystine, NAP_2 and the mixed disulfide **261** was obtained in a molar proportion 46: 13: 41 %.

Cystine (248). **^{1}H NMR** (D_2O, 400 MHz): δ 3.99 (2H, m, 2 x Cys $C_\alpha\underline{H}$), 3.52 (2H, dd, J= 13.5, 4.5, 2 x $C\underline{H}HS$), 2.84 (2H, dd, J= 13.5, 7.5, 2 x $CH\underline{H}S$), 2.39 (4H, m, 2 x $C\underline{H}_2$), 2.03 (4H, m, 2 x $C\underline{H}_2$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 9.8 min

NAP_2 (250). **^{1}H NMR** (D_2O, 300 MHz): δ 4.12 (1H, s, $C_\alpha\underline{H}$), 1.88 (3H, s, $C\underline{H}_3CO$), 1.27 (3H, s, $C\underline{H}_3$), 1.22 (3H, s, $C\underline{H}_3$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 43 min

Mixed disulfide 262. **^{1}H NMR** (D_2O, 400 MHz, only the best resolved peaks are given): δ 4.16 (1H, NAP Gly $C_\alpha H$), 1.31 (3H, s, $C\underline{H}_3$), 1.27 (3H, s, $C\underline{H}_3$). **HPLC** (Hypersyl C18, 0.1 % TFA gradient MeOH/H_2O from 1% to 35 % in 50 min): retention time 25 min

HPLC/MS (of the mixture,): 12.78 (CysNAP, M+2H = 242), 31.45 min (NAP_2, M+H = 381).

6. 3. 5. 2. In the presence of copper

The reaction was carried out as described above in the presence of 10^{-8} M $CuCl_2$. Exchange product **262** was formed after 68 h in a 10.5 molar percentage. Cystine and the disulfide derived from SNAP were present in 61.5 and 28 % molar concentrations, respectively, as approximately determined by HPLC.

6. 3. 6. GSNO-cystine exchange.

6. 3. 6. 1. *Under physiological conditions.*

The reaction was carried out in the presence of 0. 2 equiv. of EDTA. and in an aerated solution. After 5 days a mixture of cystine, NAP_2 and exchange product was formed, but the ^1H NMR was too complicated to allow differenciation of the peaks. Cysteine is not detectable under UV and, therefore, analysis of the mixture by HPLC did not solve the problem.

6. 3. 6. 2. In the presence of copper.

The reaction was carried out as described above in the presence of 10^{-8} M $CuCl_2$. Exchange product was not formed after 5 days, as determined by ^1H NMR of the mixture.

6. 3. 7. SNAP or GSNO exchange with cylclic disulfides: lipamide and lipoylglycylglycine.

6. 3. 7. 1. Under physiological conditions.

Reactions between SNAP or GSNO and cyclic disulfides **253** and **255,** under physiological pH and temperature, did not give exchange products, neither in the presence nor in the absence of copper. Only the starting cyclic disulfide and the disulfide derived from SNAP decomposition were detected after 5 days. In the case of GSNO, most of the thionitrite was still present after 5 days.

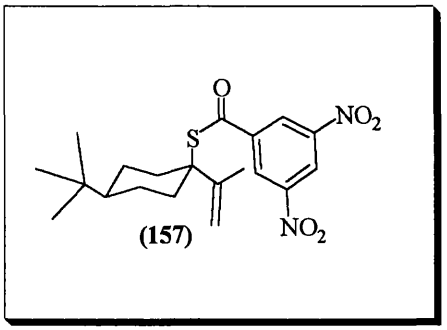
6. 3. 7. 2. In the presence of copper.

Reactions between SNAP or GSNO and cyclic disulfides **253** and **255,** under physiological pH and temperature, in the presence of 10^{-8} M $CuCl_2$ did not give exchange products, neither in the presence nor in the absence of copper. Only the starting cyclic disulfide and the disulfide derived from SNAP decomposition were detected after 5 days. In the case of GSNO, most of the thionitrite was still present after 5 days.

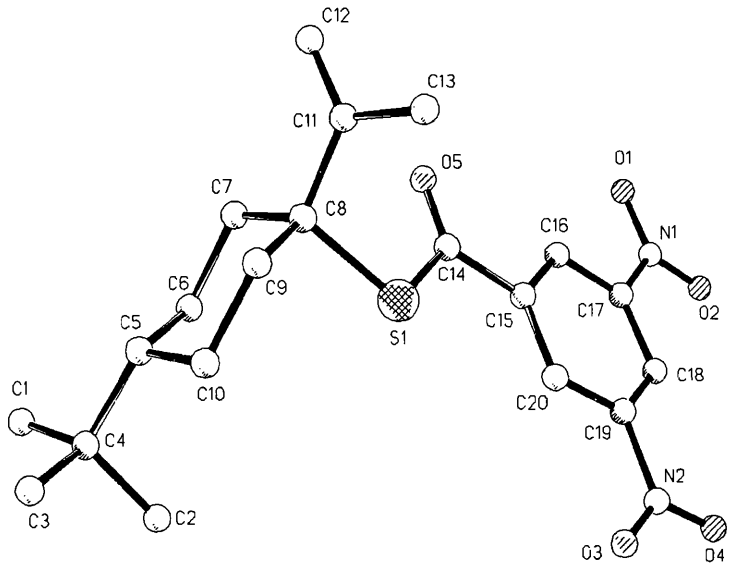
APPENDICES.

APPENDIX 1: X-RAY CRYSTALLOGRAPHIC DATA.

X-Ray Structure determination for 157.



(157)



Crystal Data:

Empirical formula	$C_{20}\ H_{26}\ N_2\ O_5\ S$
Formula weight	406.49
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system	MONOCLINIC
Space group	P2(1)/n
Unit cell dimensions	a = 6.5080(10) A alpha = 90 deg. b = 17.881(4) A beta = 96.62(3) deg. c = 18.102(4) A gamma = 90 deg.
Volume	2092.5(7) A^3
Z	4
Density (calculated)	1.290 Mg/m^3
Absorption coefficient	0.187 mm^-1
F(000)	864
Crystal size	0.74 x 0.72 x 0.64 mm

Data Collection:

Theta range for data collection	2.54 to 25.07 deg.
Index ranges	0<=h<=7, 0<=k<=21, -21<=l<=21
Reflections collected	4031
Independent reflections	3685 [R(int) = 0.0282]

Solution and refinement:

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3681 / 0 / 254
Goodness-of-fit on F^2	1.021
Final R indices [I>2sigma(I)]	R1 = 0.0458, wR2 = 0.1186
R indices (all data)	R1 = 0.0582, wR2 = 0.1321
Extinction coefficient	0.029(2)
Largest diff. peak and hole	0.213 and -0.214 e.A^-3

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **257**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
S(1)	1344(1)	8924(1)	8122(1)	50(1)
N(1)	8913(3)	11277(1)	8567(1)	65(1)
N(2)	3149(4)	11069(1)	10080(1)	71(1)
O(1)	9712(3)	11053(1)	036(1)	87(1)
O(2)	9607(3)	11781(1)	8977(1)	98(1)
O(3)	1628(4)	10726(1)	10210(1)	93(1)
O(4)	3878(4)	11592(2)	10446(1)	113(1)
O(5)	4645(2)	9150(1)	7437(1)	64(1)
C(1)	1889(5)	5697(2)	9048(2)	94(1)
C(2)	998(5)	6818(2)	9754(2)	85(1)
C(3)	-1778(5)	6038(2)	9101(2)	88(1)
C(4)	389(4)	6353(1)	9055(1)	60(1)
C(5)	390(3)	6823(1)	8338(1)	48(1)
C(6)	2500(3)	7159(1)	8229(1)	52(1)
C(7)	2448(3)	7572(1)	7495(1)	49(1)
C(8)	834(3)	8197(1)	7385(1)	43(1)
C(9)	-1275(3)	7871(1)	7528(1)	51(1)
C(10)	-1230(3)	7444(1)	8256(1)	53(1)
C(11)	637(3)	8565(1)	6622(1)	54(1)
C(12)	1635(5)	8311(2)	6082(2)	84(1)
C(13)	-801(4)	9211(2)	6484(2)	77(1)
C(14)	3660(3)	9337(1)	7932(1)	47(1)
C(15)	4400(3)	9980(1)	8437(1)	45(1)
C(16)	6220(3)	10329(1)	8290(1)	49(1)
C(17)	6966(3)	10921(1)	8734(1)	51(1)
C(18)	5995(4)	11181(1)	9317(1)	58(1)
C(19)	4216(3)	10820(1)	9451(1)	54(1)
C(20)	3386(3)	10224(1)	9025(1)	49(1)

Bond lengths [A] and angles [deg] for **257**.

S(1)-C(14)	1.748(2)
S(1)-C(8)	1.865(2)
N(1)-O(1)	1.212(3)
N(1)-O(2)	1.220(3)
N(1)-C(17)	1.481(3)
N(2)-O(3)	1.210(3)
N(2)-O(4)	1.211(3)
N(2)-C(19)	1.470(3)
O(5)-C(14)	1.207(2)
C(1)-C(4)	1.527(4)
C(2)-C(4)	1.527(4)
C(3)-C(4)	1.529(4)
C(4)-C(5)	1.547(3)
C(5)-C(10)	1.526(3)
C(5)-C(6)	1.533(3)
C(6)-C(7)	1.517(3)
C(7)-C(8)	1.531(3)
C(8)-C(11)	1.522(3)
C(8)-C(9)	1.541(3)
C(9)-C(10)	1.521(3)
C(11)-C(12)	1.316(3)
C(11)-C(13)	1.489(3)
C(14)-C(15)	1.513(3)
C(15)-C(20)	1.386(3)
C(15)-C(16)	1.391(3)
C(16)-C(17)	1.383(3)
C(17)-C(18)	1.372(3)
C(18)-C(19)	1.373(3)
C(19)-C(20)	1.386(3)
C(14)-S(1)-C(8)	103.90(9)
O(1)-N(1)-O(2)	124.4(2)
O(1)-N(1)-C(17)	118.3(2)
O(2)-N(1)-C(17)	117.3(2)
O(3)-N(2)-O(4)	124.3(2)
O(3)-N(2)-C(19)	117.9(2)
O(4)-N(2)-C(19)	117.9(2)
C(2)-C(4)-C(1)	108.7(2)
C(2)-C(4)-C(3)	108.0(2)
C(1)-C(4)-C(3)	108.2(2)
C(2)-C(4)-C(5)	111.9(2)
C(1)-C(4)-C(5)	110.3(2)
C(3)-C(4)-C(5)	109.6(2)
C(10)-C(5)-C(6)	108.7(2)
C(10)-C(5)-C(4)	114.2(2)
C(6)-C(5)-C(4)	114.1(2)
C(7)-C(6)-C(5)	112.0(2)
C(6)-C(7)-C(8)	114.5(2)
C(11)-C(8)-C(7)	114.8(2)
C(11)-C(8)-C(9)	109.5(2)
C(7)-C(8)-C(9)	108.3(2)
C(11)-C(8)-S(1)	109.70(14)
C(7)-C(8)-S(1)	110.64(14)
C(9)-C(8)-S(1)	103.38(13)
C(10)-C(9)-C(8)	114.0(2)
C(9)-C(10)-C(5)	113.2(2)
C(12)-C(11)-C(13)	119.9(2)
C(12)-C(11)-C(8)	121.8(2)
C(13)-C(11)-C(8)	118.2(2)
O(5)-C(14)-C(15)	120.0(2)
O(5)-C(14)-S(1)	124.9(2)
C(15)-C(14)-S(1)	115.07(14)
C(20)-C(15)-C(16)	119.8(2)
C(20)-C(15)-C(14)	123.9(2)
C(16)-C(15)-C(14)	116.3(2)
C(17)-C(16)-C(15)	118.8(2)
C(18)-C(17)-C(16)	123.0(2)
C(18)-C(17)-N(1)	119.4(2)
C(16)-C(17)-N(1)	117.6(2)
C(19)-C(18)-C(17)	116.6(2)
C(18)-C(19)-C(20)	123.1(2)
C(18)-C(19)-N(2)	118.5(2)
C(20)-C(19)-N(2)	118.4(2)
C(19)-C(20)-C(15)	118.6(2)

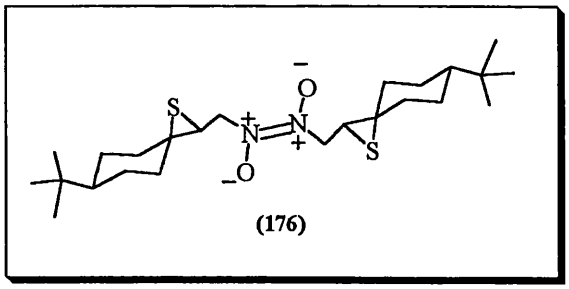
Anisotropic displacement parameters (A^2 x 10^3) for **257**.
The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a*^2 U11 + + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
S(1)	45(1)	49(1)	60(1)	-12(1)	19(1)	-8(1)
N(1)	57(1)	54(1)	82(1)	15(1)	-9(1)	-14(1)
N(2)	77(1)	75(1)	61(1)	-15(1)	3(1)	9(1)
O(1)	69(1)	83(1)	112(2)	-2(1)	25(1)	-25(1)
O(2)	94(1)	84(1)	112(2)	-11(1)	-4(1)	-47(1)
O(3)	101(2)	104(2)	81(1)	-20(1)	37(1)	-8(1)
O(4)	111(2)	121(2)	105(2)	-66(2)	14(1)	-8(2)
O(5)	55(1)	67(1)	77(1)	-19(1)	29(1)	-14(1)
C(1)	118(3)	65(2)	102(2)	25(2)	26(2)	13(2)
C(2)	98(2)	95(2)	60(2)	1(2)	3(1)	-5(2)
C(3)	96(2)	88(2)	82(2)	20(2)	16(2)	-30(2)
C(4)	68(1)	54(1)	60(1)	4(1)	10(1)	-5(1)
C(5)	45(1)	44(1)	56(1)	-7(1)	9(1)	-5(1)
C(6)	40(1)	51(1)	67(1)	0(1)	9(1)	2(1)
C(7)	37(1)	49(1)	64(1)	-5(1)	16(1)	3(1)
C(8)	36(1)	43(1)	52(1)	-7(1)	9(1)	-1(1)
C(9)	35(1)	49(1)	71(1)	0(1)	7(1)	-3(1)
C(10)	37(1)	54(1)	71(1)	-1(1)	16(1)	-5(1)
C(11)	47(1)	59(1)	57(1)	0(1)	9(1)	-1(1)
C(12)	93(2)	103(2)	61(2)	13(2)	24(1)	24(2)
C(13)	82(2)	75(2)	74(2)	15(1)	10(1)	14(1)
C(14)	41(1)	45(1)	56(1)	-4(1)	9(1)	-2(1)
C(15)	43(1)	41(1)	52(1)	2(1)	3(1)	-2(1)
C(16)	46(1)	45(1)	57(1)	5(1)	4(1)	-2(1)
C(17)	47(1)	42(1)	62(1)	9(1)	-5(1)	-5(1)
C(18)	64(1)	46(1)	62(1)	-2(1)	-12(1)	-1(1)
C(19)	58(1)	53(1)	49(1)	-2(1)	-1(1)	10(1)
C(20)	49(1)	46(1)	52(1)	2(1)	2(1)	3(1)

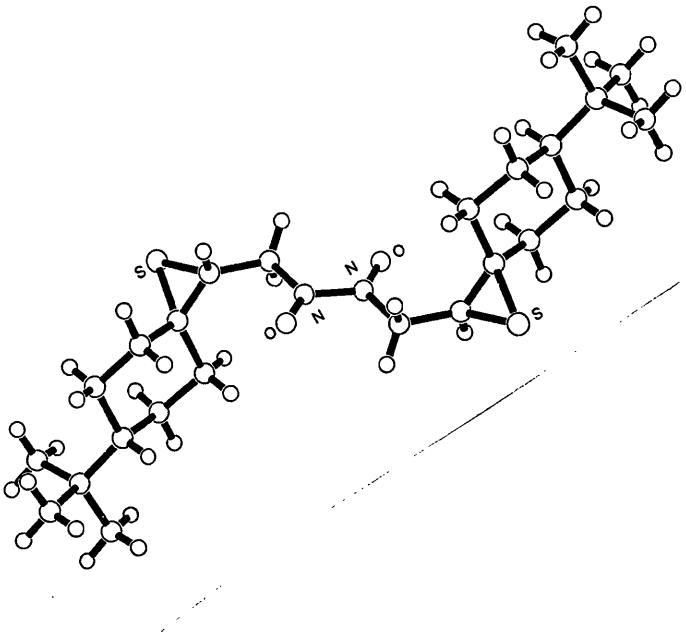
Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **257**.

	x	y	z	U(eq)
H(1A)	1512(5)	5381(2)	8625(2)	80
H(1B)	3234(5)	5906(2)	9016(2)	80
H(1C)	1914(5)	5407(2)	9495(2)	80
H(2A)	948(5)	6526(2)	10197(2)	80
H(2B)	2385(5)	6992(2)	9732(2)	80
H(2C)	84(5)	7238(2)	9761(2)	80
H(3A)	-2196(5)	5735(2)	8672(2)	80
H(3B)	-1806(5)	5744(2)	9543(2)	80
H(3C)	-2708(5)	6453(2)	9112(2)	80
H(5A)	55(3)	6489(1)	7927(1)	80
H(6A)	3483(3)	6758(1)	8239(1)	80
H(6B)	2936(3)	7492(1)	8633(1)	80
H(7A)	2220(3)	7221(1)	7092(1)	80
H(7B)	3769(3)	7803(1)	7469(1)	80
H(9A)	-1783(3)	7553(1)	7121(1)	80
H(9B)	-2218(3)	8283(1)	7538(1)	80
H(10A)	-1006(3)	7783(1)	8669(1)	80
H(10B)	-2546(3)	7207(1)	8274(1)	80
H(12A)	2523(5)	7884(2)	6173(2)	80
H(12B)	1512(5)	8553(2)	5605(2)	80
H(13A)	-772(4)	9399(2)	5989(2)	80
H(13B)	-2175(4)	9046(2)	6545(2)	80
H(13C)	-399(4)	9600(2)	6837(2)	80
H(16A)	6944(3)	10159(1)	7888(1)	80
H(18A)	6552(4)	11589(1)	9622(1)	80
H(20A)	2136(3)	9987(1)	9138(1)	80

X-Ray Structure determination for 176.



(176)



Crystal Data:

Empirical formula	$C_{24}H_{42}N_2O_2S_2$
Formula weight	454.73
Crystal Color, Habit	clear, needle
Temperature	293(0) K
Wavelength	1.54178 A
Crystal system	MONOCLINIC
Lattice type	Primitive
Space group	P2(1)/a
Omega Scan Peak Width at Half-height	0.44 deg.
Unit cell dimensions	a = 11.32 (2) A b = 6.13 (1) A beta = 104.99(6) deg. c = 19.082(7) A gamma = 90 deg.
Volume	1278(2) A^3
Z	2
Density (calculated)	1.182 Mg/m^3
Absorption coefficient	0.2047 mm^-1

F(000)	496
Crystal size	0.03 x 0.04 x 0.12 mm

Data Collection:

Diffractometer	Rigaku AFC7S
Theta range for data collection	20.6 to 31.1 deg.
Reflections collected	2237
Independent reflections	2121 [R(int) = 0.220]
Corrections	Lorentz-polarization Absorption (trans.factors: 0.3794-1.0000) Decay (3.83 % decline)

Solution and refinement:

Refinement method	Full-matrix least-squares on F^2
p-factor	0.0010
Anomalous dispersion	All non-hydrogen atoms
No Observations (I.2.00σ(I))	329
No Variables	62
Reflection/Parameter Ratio	5.31
Goodness-of-fit on F^2	4.59
Max Shift/Error in Final Cycle	3.36
Residuals R; Rw	0.128; 0.088
Largest diff. peak and hole	0.89 and -0.60 e.A^-3

Table 1. Atomic coordinates and B_{iso}/B_{eq}

atom	x	y	z	B_{eq}
S(2)	0.3844(8)	-0.013(2)	0.1543(4)	5.2(3)
O(1)	0.611(2)	-0.311(5)	0.0269(9)	4.3(6)
N(1)	0.515(2)	-0.396(5)	0.018(1)	3.2(6)
C(1)	0.409(2)	-0.318(7)	0.049(1)	3.6(8)
C(2)	0.459(2)	-0.111(7)	0.089(1)	3.4(8)
C(3)	0.539(2)	-0.083(6)	0.155(1)	2.2(7)
C(4)	0.634(3)	0.075(7)	0.170(1)	4.9(9)
C(5)	0.692(2)	0.136(7)	0.247(1)	3.8(8)
C(6)	0.746(2)	-0.067(7)	0.290(1)	3.0(7)
C(7)	0.643(2)	-0.231(8)	0.280(1)	5.0(9)
C(8)	0.589(2)	-0.295(7)	0.203(1)	2.9(7)
C(9)	0.820(3)	-0.009(8)	0.365(1)	3.9(7)
C(10)	0.737(2)	0.086(8)	0.412(1)	5.3(9)
C(11)	0.929(3)	0.133(7)	0.369(1)	5.5(9)
C(12)	0.871(3)	-0.221(8)	0.403(1)	6(1)
H(1a)	0.3328	-0.3118	0.0104	4.3844
H(1b)	0.3974	-0.4191	0.0839	4.3844
H(2)	0.4647	-0.0039	0.0502	4.2432
H(4b)	0.5952	0.1951	0.1426	5.9423
H(4a)	0.6924	0.0125	0.1491	5.9423
H(5b)	0.6344	0.2032	0.2670	4.6203
H(5a)	0.7551	0.2319	0.2437	4.6203
H(6)	0.8030	-0.1326	0.2678	3.7081
H(7b)	0.5803	-0.1648	0.2981	5.9975

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B_{eq}
H(7a)	0.6730	-0.3561	0.3086	5.9975
H(8b)	0.6479	-0.3664	0.1819	3.5932
H(8a)	0.5224	-0.3941	0.1995	3.5932
H(10a)	0.7837	0.1155	0.4596	6.3928
H(10b)	0.6998	0.2147	0.3894	6.3928
H(10c)	0.6726	-0.0162	0.4147	6.3928
H(11a)	0.9716	0.1599	0.4183	6.5665
H(11b)	0.9827	0.0702	0.3442	6.5665
H(11c)	0.8993	0.2694	0.3467	6.5665
H(12a)	0.8037	-0.3144	0.4013	7.2641
H(12b)	0.9232	-0.2856	0.3768	7.2641
H(12c)	0.9157	-0.1994	0.4520	7.2641

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

Table 2. Bond Lengths($\AA$)

atom	atom	distance	atom	atom	distance
S(2)	C(2)	1.78(3)	S(2)	C(3)	1.80(3)
O(1)	N(1)	1.18(3)	N(1)	N(1)	1.44(7)
N(1)	C(1)	1.55(4)	C(1)	C(2)	1.51(5)
C(2)	C(3)	1.36(3)	C(3)	C(4)	1.42(5)
C(3)	C(8)	1.61(5)	C(4)	C(5)	1.49(3)
C(5)	C(6)	1.53(6)	C(6)	C(7)	1.52(5)
C(6)	C(9)	1.51(4)	C(7)	C(8)	1.50(3)
C(9)	C(10)	1.57(5)	C(9)	C(11)	1.49(5)
C(9)	C(12)	1.53(7)			

Table 3. Bond Lengths($\AA$)

atom	atom	distance	atom	atom	distance
C(1)	H(1a)	0.97	C(1)	H(1b)	0.95
C(2)	H(2)	1.01	C(4)	H(4b)	0.94
C(4)	H(4a)	0.93	C(5)	H(5b)	0.93
C(5)	H(5a)	0.93	C(6)	H(6)	0.95
C(7)	H(7b)	0.95	C(7)	H(7a)	0.95
C(8)	H(8b)	0.96	C(8)	H(8a)	0.96
C(10)	H(10a)	0.95	C(10)	H(10b)	0.94
C(10)	H(10c)	0.97	C(11)	H(11a)	0.96
C(11)	H(11b)	0.94	C(11)	H(11c)	0.96
C(12)	H(12a)	0.94	C(12)	H(12b)	0.96
C(12)	H(12c)	0.95			

Table 4. Bond Angles(°)

atom	atom	atom	angle	atom	atom	atom	angle
C(2)	S(2)	C(3)	45(1)	O(1)	N(1)	N(1)	124(4)
O(1)	N(1)	C(1)	126(4)	N(1)	N(1)	C(1)	109(4)
N(1)	C(1)	C(2)	103(3)	S(2)	C(2)	C(1)	117(3)
S(2)	C(2)	C(3)	68(2)	C(1)	C(2)	C(3)	130(4)
S(2)	C(3)	C(2)	67(2)	S(2)	C(3)	C(4)	122(3)
S(2)	C(3)	C(8)	114(3)	C(2)	C(3)	C(4)	124(3)
C(2)	C(3)	C(8)	118(4)	C(4)	C(3)	C(8)	107(3)
C(3)	C(4)	C(5)	119(3)	C(4)	C(5)	C(6)	110(4)
C(5)	C(6)	C(7)	106(3)	C(5)	C(6)	C(9)	111(4)
C(7)	C(6)	C(9)	120(3)	C(6)	C(7)	C(8)	114(3)
C(3)	C(8)	C(7)	110(4)	C(6)	C(9)	C(10)	111(3)
C(6)	C(9)	C(11)	115(3)	C(6)	C(9)	C(12)	107(4)
C(10)	C(9)	C(11)	112(4)	C(10)	C(9)	C(12)	105(3)
C(11)	C(9)	C(12)	106(3)				

Table 5. Bond Angles(°)

atom	atom	atom	angle	atom	atom	atom	angle
N(1)	C(1)	H(1a)	110.5	N(1)	C(1)	H(1b)	109.6
C(2)	C(1)	H(1a)	119.0	C(2)	C(1)	H(1b)	106.6
H(1a)	C(1)	H(1b)	107.6	S(2)	C(2)	H(2)	115.4
C(1)	C(2)	H(2)	105.2	C(3)	C(2)	H(2)	116.1
C(3)	C(4)	H(4b)	101.2	C(3)	C(4)	H(4a)	102.3
C(5)	C(4)	H(4b)	112.0	C(5)	C(4)	H(4a)	110.1
H(4b)	C(4)	H(4a)	111.5	C(4)	C(5)	H(5b)	109.6
C(4)	C(5)	H(5a)	103.8	C(6)	C(5)	H(5b)	111.2
C(6)	C(5)	H(5a)	110.1	H(5b)	C(5)	H(5a)	112.2
C(5)	C(6)	H(6)	109.0	C(7)	C(6)	H(6)	104.9
C(9)	C(6)	H(6)	104.7	C(6)	C(7)	H(7b)	107.2
C(6)	C(7)	H(7a)	108.0	C(8)	C(7)	H(7b)	108.7
C(8)	C(7)	H(7a)	110.1	H(7b)	C(7)	H(7a)	109.2
C(3)	C(8)	H(8b)	108.5	C(3)	C(8)	H(8a)	108.6
C(7)	C(8)	H(8b)	112.3	C(7)	C(8)	H(8a)	109.6
H(8b)	C(8)	H(8a)	107.9	C(9)	C(10)	H(10a)	110.0
C(9)	C(10)	H(10b)	108.6	C(9)	C(10)	H(10c)	111.4
H(10a)	C(10)	H(10b)	110.4	H(10a)	C(10)	H(10c)	107.8
H(10b)	C(10)	H(10c)	108.7	C(9)	C(11)	H(11a)	109.9
C(9)	C(11)	H(11b)	112.1	C(9)	C(11)	H(11c)	107.6
H(11a)	C(11)	H(11b)	109.4	H(11a)	C(11)	H(11c)	107.9
H(11b)	C(11)	H(11c)	109.9	C(9)	C(12)	H(12a)	107.5
C(9)	C(12)	H(12b)	107.6	C(9)	C(12)	H(12c)	112.6
H(12a)	C(12)	H(12b)	109.4	H(12a)	C(12)	H(12c)	110.7

Table 5. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
H(12b)	C(12)	H(12c)	108.9				

Table 6. Non-bonded Contacts out to 3.60 $\AA$

atom	atom	distance	ADC	atom	atom	distance	ADC
S(2)	O(1)	3.57(2)	44504	O(1)	C(2)	3.36(5)	65503
O(1)	C(1)	3.38(4)	54504				

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

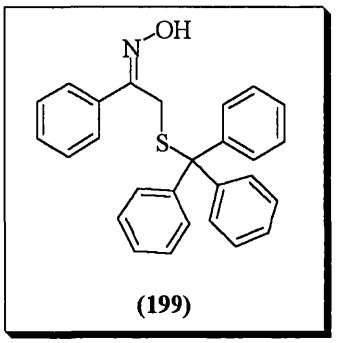
For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

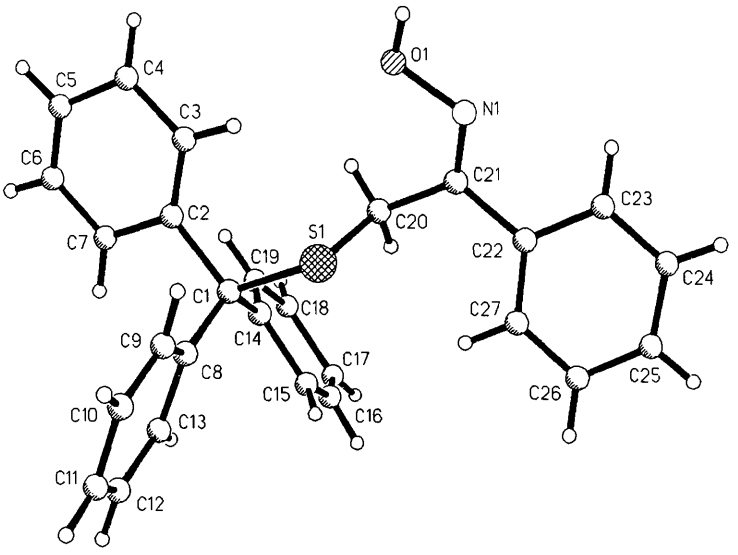
Symmetry Operators:

(1)	X,	Y,	Z	(2)	1/2-X,	1/2+Y,	-Z
(3)	-X,	-Y,	-Z	(4)	1/2+X,	1/2-Y,	Z

X-Ray Structure deteremination for 199



(199)



Crystal data

Identification code	str659
Empirical formula	C21.60 H18.40 N0.80 O0.80 S0.80
Formula weight	327.62
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system	TRICLINIC
Space group	P-1
Unit cell dimensions	a = 9.350(2) A alpha = 83.24(3) deg. b = 17.044(3) A beta = 75.79(3) deg. c = 17.123(3) A gamma = 74.68(3) deg.
Volume	2547.2(8) A^3
Z	4
Density (calculated)	1.068 Mg/m^3
Absorption coefficient	0.143 mm^-1
F(000)	864

Crystal size	0.73 x 0.48 x 0.42 mm

Data collection

Theta range for data collection	2.69 to 22.55 deg.
Index ranges	0<=h<=10, -17<=k<=18, -17<=l<=18
Reflections collected	7111
Independent reflections	6623 [R(int) = 0.0719]

Solution and refinement.

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6623 / 0 / 541
Goodness-of-fit on F^2	1.037
Final R indices [I>2sigma(I)]	R1 = 0.2124, wR2 = 0.4889
R indices (all data)	R1 = 0.2696, wR2 = 0.5340
Largest diff. peak and hole	1.078 and -1.269 e.A^-3

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for 1. U(eq) is define as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
S(1)	2017(3)	8209(2)	6823(2)	75(1)
O(1)	-98(8)	9113(5)	5434(6)	95(3)
N(1)	1351(10)	9286(6)	5097(7)	82(3)
C(1)	1815(13)	7227(7)	7410(7)	64(4)
C(2)	147(16)	7122(12)	7513(9)	108(6)
C(3)	-950(17)	7625(16)	7183(11)	155(9)
C(4)	-2414(21)	7379(22)	7332(15)	233(17)
C(5)	-2605(27)	6698(23)	7874(16)	223(16)
C(6)	-1544(28)	6257(22)	8171(15)	219(16)
C(7)	-118(23)	6429(13)	8032(12)	147(8)
C(8)	1987(14)	7345(9)	8205(9)	73(4)
C(9)	1205(18)	8047(10)	8646(10)	96(5)
C(10)	1334(23)	8212(11)	9365(11)	115(6)
C(11)	2308(24)	7661(14)	9785(11)	116(6)
C(12)	3085(19)	6936(13)	9390(11)	100(5)
C(13)	2967(14)	6777(9)	8654(10)	91(5)
C(14)	2926(16)	6520(7)	6923(7)	72(4)
C(15)	4532(16)	6534(8)	6850(8)	70(4)
C(16)	5498(35)	6022(15)	6457(13)	154(11)
C(17)	5528(29)	5458(18)	6053(16)	193(18)
C(18)	3842(38)	5413(8)	6122(8)	181(14)
C(19)	2751(21)	5943(7)	6537(8)	86(5)
C(20)	2283(12)	7987(7)	5804(8)	84(5)
C(21)	2457(12)	8732(7)	5246(8)	73(4)
C(22)	4027(12)	8870(7)	4935(7)	67(3)
C(23)	4301(15)	9356(7)	4201(8)	82(4)
C(24)	5789(15)	9492(9)	3931(10)	99(5)
C(25)	6893(17)	9119(12)	4346(12)	121(6)
C(26)	6635(14)	8648(8)	5047(10)	84(4)
C(27)	5222(12)	8507(8)	5297(8)	79(4)
S(51)	2993(3)	6771(2)	1853(2)	73(1)

O(51)	3855(8)	5140(5)	880(6)	86(3)
N(51)	5343(9)	5294(6)	689(7)	66(3)
C(51)	2345(10)	7252(7)	2796(7)	70(4)
C(52)	1266(12)	6840(7)	3533(8)	55(3)
C(53)	649(13)	7143(9)	4213(8)	73(4)
C(54)	-280(15)	6775(11)	4834(9)	105(6)
C(55)	-573(16)	6061(11)	4674(11)	98(6)
C(56)	23(17)	5748(10)	3946(13)	110(6)
C(57)	1012(12)	6088(8)	3304(10)	93(5)
C(58)	3761(12)	7313(7)	3111(9)	73(4)
C(59)	4242(14)	6909(8)	3776(9)	87(4)
C(60)	5607(16)	6904(9)	3965(10)	92(4)
C(61)	6559(18)	7338(11)	3447(13)	122(6)
C(62)	6114(16)	7746(11)	2801(11)	108(5)
C(63)	4761(13)	7764(8)	2607(9)	91(5)
C(64)	1342(12)	8121(7)	2639(7)	63(3)
C(65)	289(14)	8196(9)	2161(9)	87(5)
C(66)	-628(14)	8961(10)	1992(10)	93(5)
C(67)	-542(15)	9627(9)	2307(9)	84(4)
C(68)	442(17)	9572(9)	2755(12)	104(5)
C(69)	1380(14)	8824(9)	2971(8)	77(4)
C(70)	4391(12)	5832(7)	2061(7)	69(4)
C(71)	5505(12)	5628(7)	1260(8)	62(3)
C(72)	7045(15)	5847(10)	1175(9)	90(5)
C(73)	7681(20)	5861(17)	1717(13)	169(10)
C(74)	9110(22)	6063(18)	1637(17)	188(12)
C(75)	9819(24)	6213(17)	866(18)	182(12)
C(76)	9249(39)	6195(32)	339(18)	406(41)
C(77)	7766(31)	5938(31)	450(14)	400(38)

Bond lengths [A] and angles [deg] for 1.

S(1)-C(20)	1.77(2)	S(51)-C(70)	1.839(11)
S(1)-C(1)	1.878(10)	O(51)-N(51)	1.436(10)
O(1)-N(1)	1.429(10)	N(51)-C(71)	1.245(14)
N(1)-C(21)	1.258(14)	C(51)-C(64)	1.56(2)
C(1)-C(8)	1.45(2)	C(51)-C(58)	1.58(2)
C(1)-C(14)	1.54(2)	C(51)-C(52)	1.626(14)
C(1)-C(2)	1.58(2)	C(52)-C(53)	1.27(2)
C(2)-C(3)	1.35(2)	C(52)-C(57)	1.48(2)
C(2)-C(7)	1.43(2)	C(53)-C(54)	1.40(2)
C(3)-C(4)	1.49(2)	C(54)-C(55)	1.39(2)
C(4)-C(5)	1.42(4)	C(55)-C(56)	1.34(2)
C(5)-C(6)	1.25(4)	C(56)-C(57)	1.43(2)
C(6)-C(7)	1.40(2)	C(58)-C(59)	1.37(2)
C(8)-C(9)	1.42(2)	C(58)-C(63)	1.43(2)
C(8)-C(13)	1.44(2)	C(59)-C(60)	1.39(2)
C(9)-C(10)	1.34(2)	C(60)-C(61)	1.39(2)
C(10)-C(11)	1.39(2)	C(61)-C(62)	1.33(2)
C(11)-C(12)	1.41(2)	C(62)-C(63)	1.38(2)
C(12)-C(13)	1.36(2)	C(64)-C(65)	1.40(2)
C(14)-C(19)	1.31(2)	C(64)-C(69)	1.40(2)
C(14)-C(15)	1.48(2)	C(65)-C(66)	1.40(2)
C(15)-C(16)	1.21(2)	C(66)-C(67)	1.34(2)
C(16)-C(17)	1.24(4)	C(67)-C(68)	1.31(2)
C(17)-C(18)	1.57(3)	C(68)-C(69)	1.41(2)
C(18)-C(19)	1.29(2)	C(70)-C(71)	1.52(2)
C(20)-C(21)	1.516(14)	C(71)-C(72)	1.55(2)
C(21)-C(22)	1.504(14)	C(72)-C(73)	1.23(2)
C(22)-C(27)	1.38(2)	C(72)-C(77)	1.27(2)
C(22)-C(23)	1.43(2)	C(73)-C(74)	1.44(2)
C(23)-C(24)	1.43(2)	C(74)-C(75)	1.35(3)
C(24)-C(25)	1.37(2)	C(75)-C(76)	1.16(3)
C(25)-C(26)	1.37(2)	C(76)-C(77)	1.52(3)
C(26)-C(27)	1.36(2)	C(20)-S(1)-C(1)	104.2(5)
S(51)-C(51)	1.793(14)	C(21)-N(1)-O(1)	113.9(8)

C(8)-C(1)-C(14)	119.6(11)	C(27)-C(22)-C(21)	123.0(10)
C(8)-C(1)-C(2)	107.1(9)	C(23)-C(22)-C(21)	117.9(10)
C(14)-C(1)-C(2)	107.9(11)	C(24)-C(23)-C(22)	116.7(13)
C(8)-C(1)-S(1)	103.8(8)	C(25)-C(24)-C(23)	119.6(13)
C(14)-C(1)-S(1)	108.1(7)	C(26)-C(25)-C(24)	123.6(13)
C(2)-C(1)-S(1)	110.1(9)	C(25)-C(26)-C(27)	116.5(13)
C(3)-C(2)-C(7)	121.9(14)	C(26)-C(27)-C(22)	124.0(11)
C(3)-C(2)-C(1)	125.8(13)	C(51)-S(51)-C(70)	104.0(5)
C(7)-C(2)-C(1)	112.3(14)	C(71)-N(51)-O(51)	109.2(9)
C(2)-C(3)-C(4)	116(2)	C(64)-C(51)-C(58)	110.5(10)
C(5)-C(4)-C(3)	118(2)	C(64)-C(51)-C(52)	104.5(8)
C(6)-C(5)-C(4)	122(2)	C(58)-C(51)-C(52)	107.1(9)
C(5)-C(6)-C(7)	123(3)	C(64)-C(51)-S(51)	107.3(8)
C(6)-C(7)-C(2)	119(2)	C(58)-C(51)-S(51)	109.4(8)
C(9)-C(8)-C(13)	110.6(14)	C(52)-C(51)-S(51)	118.0(9)
C(9)-C(8)-C(1)	124.2(12)	C(53)-C(52)-C(57)	123.4(11)
C(13)-C(8)-C(1)	125(2)	C(53)-C(52)-C(51)	124.6(11)
C(10)-C(9)-C(8)	128(2)	C(57)-C(52)-C(51)	111.9(11)
C(9)-C(10)-C(11)	121(2)	C(52)-C(53)-C(54)	123(2)
C(10)-C(11)-C(12)	114(2)	C(55)-C(54)-C(53)	117(2)
C(13)-C(12)-C(11)	125(2)	C(56)-C(55)-C(54)	120.2(14)
C(12)-C(13)-C(8)	122(2)	C(55)-C(56)-C(57)	124(2)
C(19)-C(14)-C(15)	114.4(12)	C(56)-C(57)-C(52)	111(2)
C(19)-C(14)-C(1)	133.9(14)	C(59)-C(58)-C(63)	115.2(11)
C(15)-C(14)-C(1)	111.6(12)	C(59)-C(58)-C(51)	127.3(10)
C(16)-C(15)-C(14)	117(2)	C(63)-C(58)-C(51)	117.1(11)
C(15)-C(16)-C(17)	136(3)	C(58)-C(59)-C(60)	124.6(13)
C(16)-C(17)-C(18)	108(2)	C(61)-C(60)-C(59)	118(2)
C(19)-C(18)-C(17)	119(2)	C(62)-C(61)-C(60)	118.7(14)
C(18)-C(19)-C(14)	125(2)	C(61)-C(62)-C(63)	124.1(14)
C(21)-C(20)-S(1)	110.7(10)	C(62)-C(63)-C(58)	119.3(14)
N(1)-C(21)-C(22)	117.8(10)	C(65)-C(64)-C(69)	117.7(12)
N(1)-C(21)-C(20)	123.6(10)	C(65)-C(64)-C(51)	117.9(12)
C(22)-C(21)-C(20)	118.2(10)	C(69)-C(64)-C(51)	124.4(11)
C(27)-C(22)-C(23)	119.0(10)	C(64)-C(65)-C(66)	120.4(14)

C(67)-C(66)-C(65)	120.3(14)	C(73)-C(72)-C(77)	118(2)
C(68)-C(67)-C(66)	120.3(13)	C(73)-C(72)-C(71)	127.1(14)
C(67)-C(68)-C(69)	123(2)	C(77)-C(72)-C(71)	114(2)
C(64)-C(69)-C(68)	118.1(14)	C(72)-C(73)-C(74)	127(2)
C(71)-C(70)-S(51)	105.9(7)	C(75)-C(74)-C(73)	114(2)
N(51)-C(71)-C(70)	128.3(10)	C(76)-C(75)-C(74)	120(2)
N(51)-C(71)-C(72)	117.6(11)	C(75)-C(76)-C(77)	124(3)
C(70)-C(71)-C(72)	114.2(12)	C(72)-C(77)-C(76)	116(2)

Anisotropic displacement parameters (A^2 x 10^3) for 1. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
S(1)	57(2)	64(2)	98(3)	54(2)	-21(2)	-24(2)
O(1)	32(4)	103(6)	145(8)	85(6)	-34(5)	-34(4)
N(1)	33(5)	92(7)	118(9)	65(6)	-24(5)	-37(5)
C(1)	65(8)	83(9)	51(7)	36(6)	-9(6)	-55(7)
C(2)	53(8)	184(17)	95(10)	57(11)	-15(8)	-74(10)
C(3)	48(9)	298(28)	133(15)	75(16)	-27(9)	-95(14)
C(4)	58(11)	488(52)	167(21)	95(27)	-48(12)	-115(21)
C(5)	84(16)	422(47)	173(22)	115(26)	-14(15)	-152(24)
C(6)	121(19)	386(44)	201(25)	132(26)	-66(18)	-183(27)
C(7)	120(15)	188(19)	171(18)	82(15)	-60(13)	-116(15)
C(8)	50(7)	72(9)	94(11)	41(8)	-11(7)	-33(7)
C(9)	103(12)	97(12)	73(10)	55(9)	-21(9)	-23(10)
C(10)	151(17)	100(12)	82(12)	38(10)	-30(11)	-24(11)
C(11)	136(16)	152(17)	73(11)	56(12)	-23(11)	-87(14)
C(12)	92(12)	126(15)	82(12)	54(11)	-23(10)	-51(11)
C(13)	53(8)	97(10)	102(12)	64(9)	-15(7)	-17(7)
C(14)	102(11)	42(7)	46(7)	18(6)	11(7)	-9(7)
C(15)	74(9)	41(7)	79(9)	23(6)	-5(7)	-9(7)
C(16)	218(28)	93(16)	84(15)	30(11)	-17(16)	43(18)
C(17)	137(19)	162(26)	137(23)	95(19)	69(17)	73(19)
C(18)	470(44)	25(8)	33(8)	-16(7)	-2(15)	-79(15)
C(19)	175(16)	29(6)	60(8)	-16(6)	-13(9)	-42(8)
C(20)	33(6)	70(8)	129(11)	77(8)	-10(6)	-23(5)
C(21)	38(6)	75(8)	106(10)	52(7)	-23(6)	-30(6)
C(22)	38(6)	79(8)	81(8)	25(7)	-4(6)	-29(6)
C(23)	65(8)	64(8)	110(11)	41(7)	-11(7)	-31(7)
C(24)	55(9)	88(10)	144(13)	39(9)	7(9)	-47(8)
C(25)	42(9)	143(15)	177(18)	17(14)	-12(10)	-45(9)
C(26)	47(7)	83(9)	128(12)	24(9)	-31(8)	-30(7)
C(27)	32(6)	94(9)	100(10)	53(8)	-17(6)	-20(6)
S(51)	47(2)	79(2)	84(2)	47(2)	-20(2)	-21(2)

O(51)	39(4)	107(7)	125(7)	25(6)	-17(4)	-53(5)
N(51)	30(5)	94(8)	82(7)	12(6)	-10(5)	-40(5)
C(51)	19(5)	85(8)	102(10)	61(7)	-16(6)	-32(5)
C(52)	39(6)	60(7)	64(8)	34(6)	-20(6)	-16(5)
C(53)	41(7)	96(10)	70(9)	30(8)	0(6)	-23(6)
C(54)	55(8)	126(14)	93(11)	55(10)	8(7)	1(9)
C(55)	53(9)	112(14)	116(14)	70(11)	-12(9)	-35(9)
C(56)	64(9)	88(11)	173(18)	77(12)	-26(11)	-48(8)
C(57)	30(6)	76(9)	161(14)	54(9)	-12(7)	-31(6)
C(58)	34(6)	59(7)	125(11)	21(7)	-20(7)	-22(6)
C(59)	48(7)	90(10)	123(12)	44(9)	-33(8)	-29(7)
C(60)	62(9)	88(10)	130(13)	15(9)	-46(9)	-14(8)
C(61)	57(10)	123(14)	189(19)	34(14)	-58(11)	-18(9)
C(62)	50(8)	126(13)	151(15)	28(12)	-11(9)	-54(9)
C(63)	41(7)	108(11)	128(12)	54(9)	-22(7)	-49(7)
C(64)	39(6)	65(8)	78(8)	29(7)	3(6)	-30(6)
C(65)	53(7)	89(10)	131(12)	57(9)	-45(8)	-41(7)
C(66)	49(8)	86(11)	135(13)	49(10)	-34(8)	-13(8)
C(67)	54(8)	60(9)	133(13)	47(8)	-25(8)	-25(7)
C(68)	67(9)	57(9)	187(17)	21(10)	-22(11)	-29(8)
C(69)	49(7)	80(10)	95(10)	22(8)	-11(7)	-21(7)
C(70)	44(6)	64(7)	81(9)	38(6)	-6(6)	-11(6)
C(71)	36(6)	63(8)	86(9)	24(7)	-11(6)	-24(6)
C(72)	46(8)	149(13)	92(10)	41(9)	-22(8)	-65(8)
C(73)	77(12)	317(32)	143(17)	57(18)	-30(12)	-121(17)
C(74)	84(14)	329(36)	192(23)	59(23)	-70(15)	-119(19)
C(75)	111(16)	311(33)	194(25)	32(22)	-59(17)	-168(20)
C(76)	279(41)	951(123)	140(23)	-24(38)	19(23)	-479(66)
C(77)	228(30)	1035(115)	91(16)	-9(31)	-8(16)	-453(56)

Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for 1.

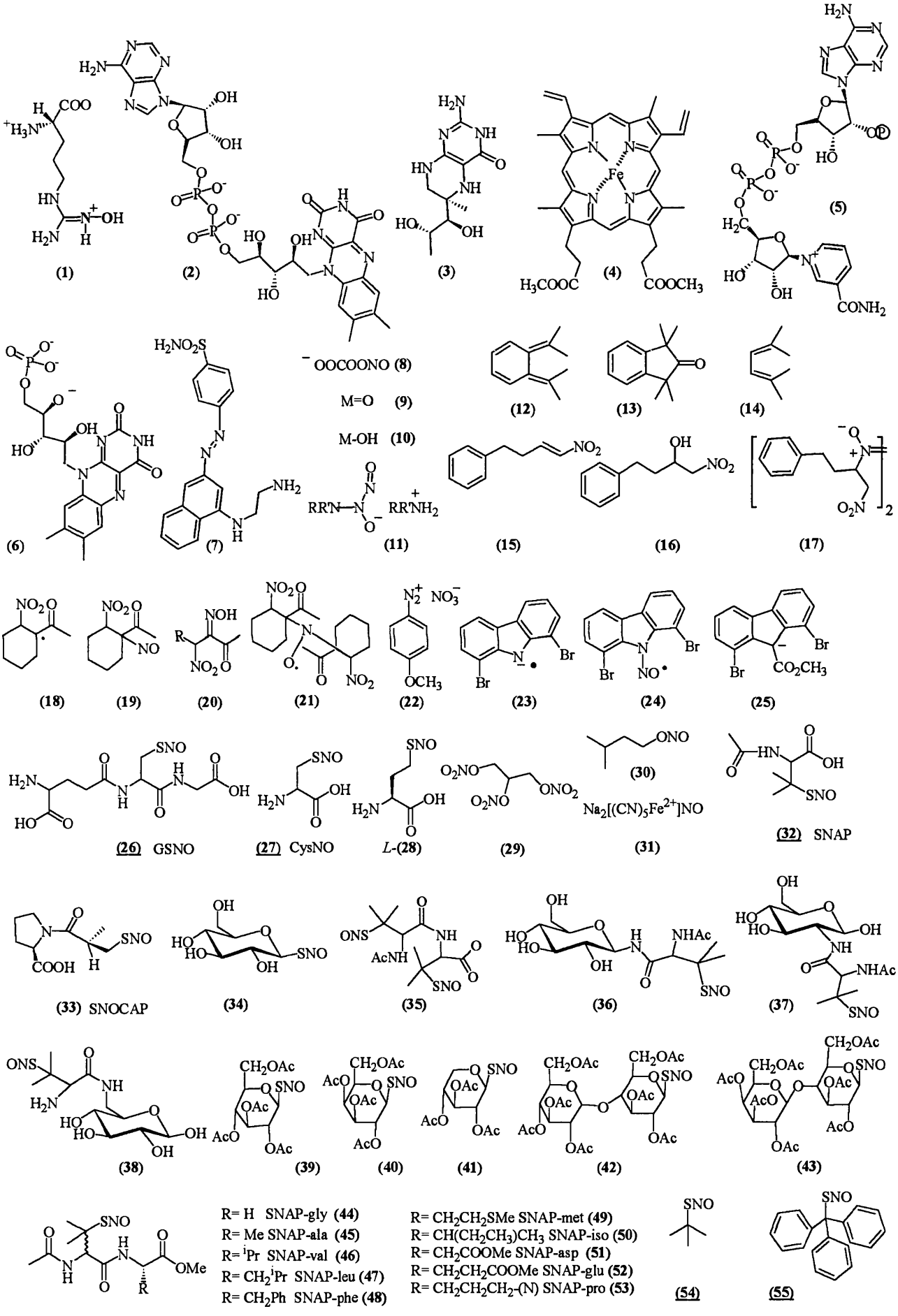
	x	y	z	U(eq)
H(1A)	-793(8)	9501(5)	5300(6)	80
H(3A)	-724(17)	8080(16)	6832(11)	80
H(4A)	-3214(21)	7675(22)	7073(15)	80
H(5A)	-3571(27)	6561(23)	7991(16)	80
H(6A)	-1725(28)	5779(22)	8494(15)	80
H(7A)	690(23)	6088(13)	8261(12)	80
H(9A)	500(18)	8447(10)	8393(10)	80
H(10A)	726(23)	8708(11)	9607(11)	80
H(11A)	2452(24)	7766(14)	10296(11)	80
H(12A)	3752(19)	6531(13)	9662(11)	80
H(13A)	3553(14)	6274(9)	8418(10)	80
H(15A)	4755(16)	6944(8)	7107(8)	80
H(17A)	6409(29)	5093(18)	5757(16)	80
H(18A)	3745(38)	4881(8)	6343(8)	80
H(18B)	3694(38)	5468(8)	5581(8)	80
H(19A)	1720(21)	5920(7)	6578(8)	80
H(20A)	1427(12)	7811(7)	5739(8)	80
H(20B)	3181(12)	7555(7)	5659(8)	80
H(23A)	3527(15)	9570(7)	3900(8)	80
H(24A)	6015(15)	9858(9)	3473(10)	80
H(25A)	7918(17)	9156(12)	4110(12)	80
H(26A)	7400(14)	8457(8)	5356(10)	80
H(27A)	5047(12)	8128(8)	5750(8)	80
H(51A)	3761(8)	4908(5)	490(6)	80
H(53A)	834(13)	7653(9)	4292(8)	80
H(54A)	-700(15)	7005(11)	5351(9)	80
H(55A)	-1206(16)	5790(11)	5085(11)	80
H(56A)	-244(17)	5262(10)	3865(13)	80
H(57A)	1451(12)	5862(8)	2786(10)	80
H(59A)	3578(14)	6618(8)	4142(9)	80
H(60A)	5904(16)	6593(9)	4429(10)	80

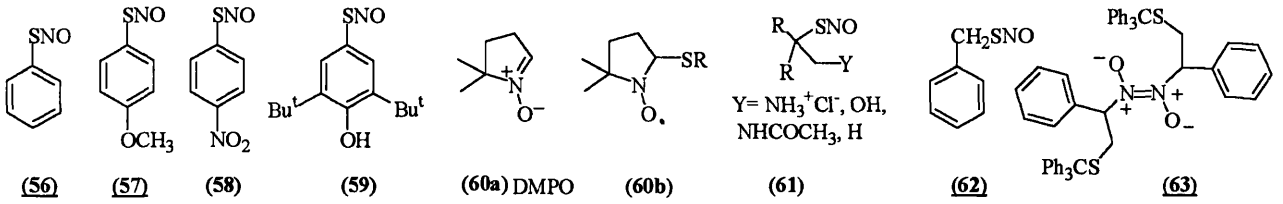
H(61A)	7482(18)	7377(11)	3574(13)	80
H(62A)	6790(16)	8032(11)	2439(11)	80
H(63A)	4488(13)	8068(8)	2134(9)	80
H(65A)	215(14)	7717(9)	1944(9)	80
H(66A)	-1342(14)	9011(10)	1659(10)	80
H(67A)	-1178(15)	10150(9)	2191(9)	80
H(68A)	482(17)	10055(9)	2979(12)	80
H(69A)	2072(14)	8797(9)	3310(8)	80
H(70A)	3903(12)	5395(7)	2259(7)	80
H(70B)	4905(12)	5915(7)	2454(7)	80
H(73A)	7166(20)	5720(17)	2252(13)	80
H(74A)	9490(22)	6145(18)	2086(17)	80
H(75A)	10847(24)	6265(17)	759(18)	80
H(76A)	9701(39)	6383(32)	-191(18)	80
H(77A)	7419(31)	5793(31)	16(14)	80

APPENDIX 2: STRUCTURE INDEX.

STRUCTURE INDEX

(The numbers of those compounds which have been prepared during this work are underlined)





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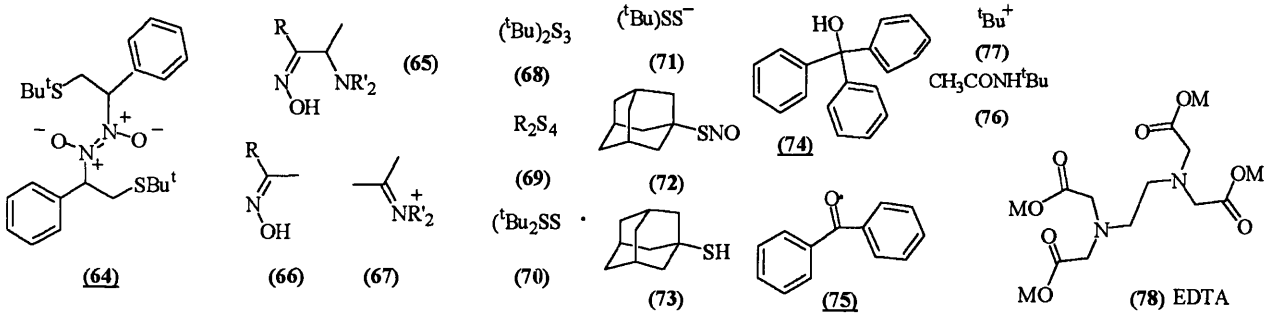

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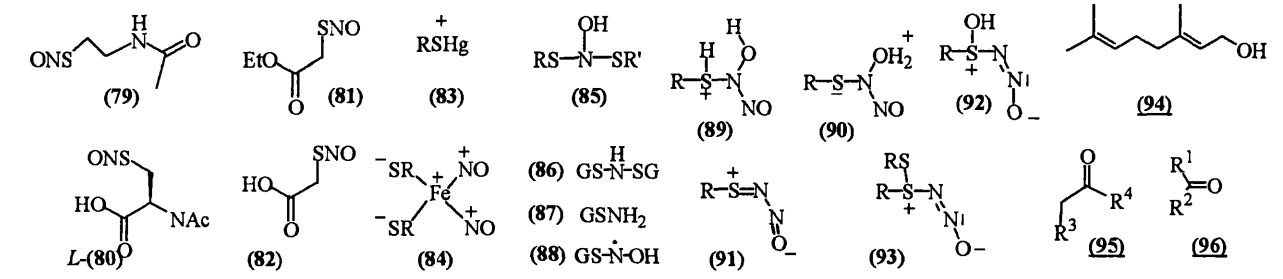

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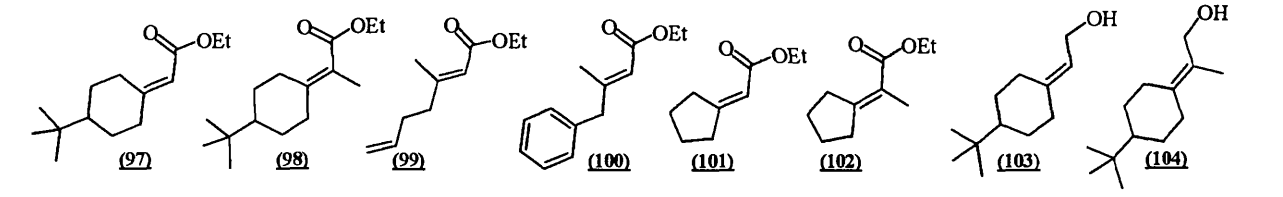

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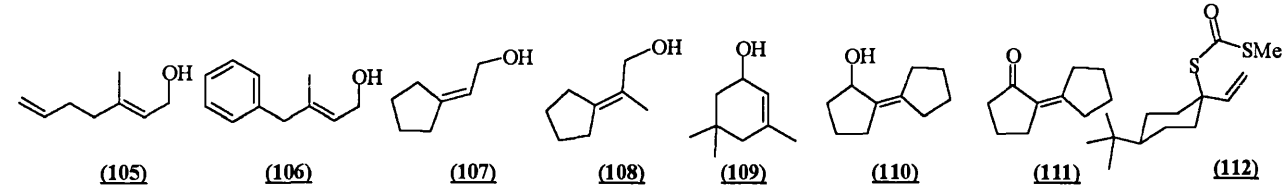

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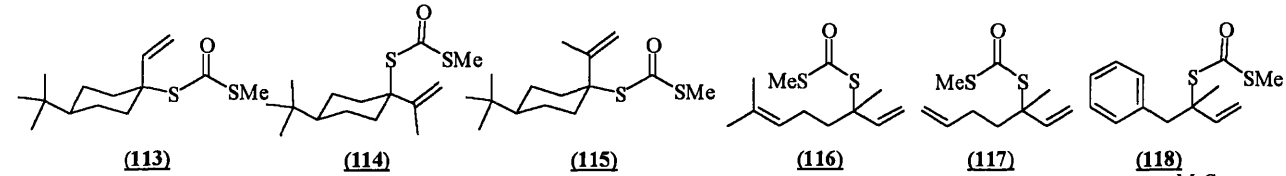

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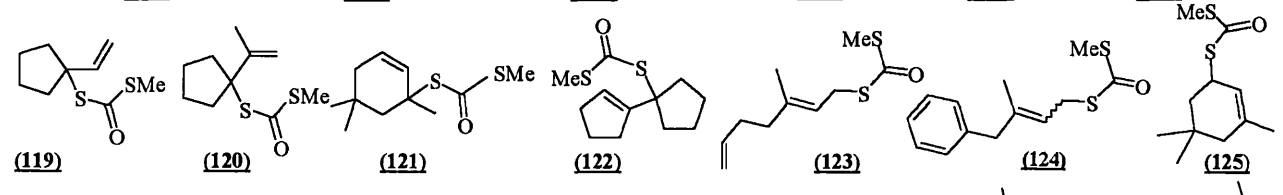

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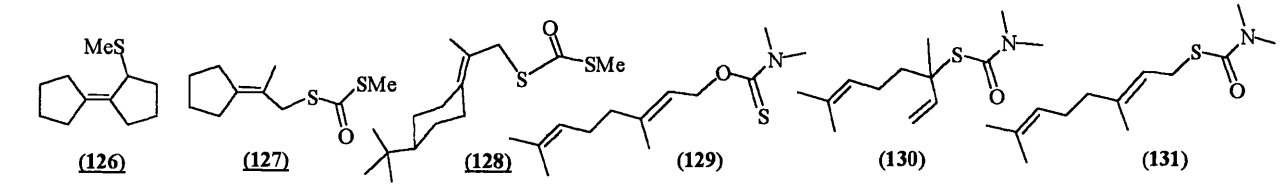

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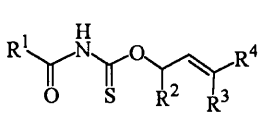

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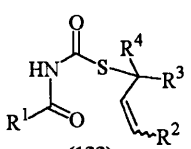

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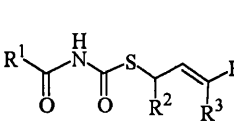

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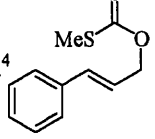

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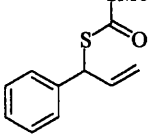

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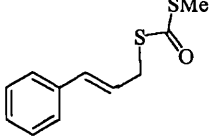

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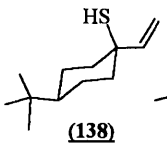

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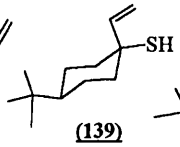

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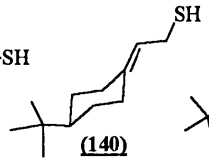

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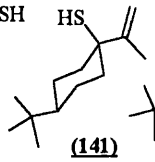

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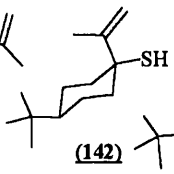

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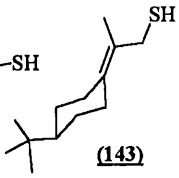

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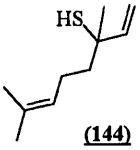

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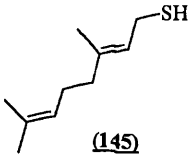

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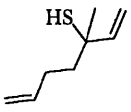

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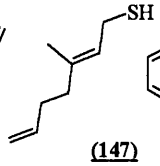

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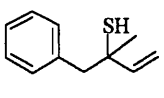

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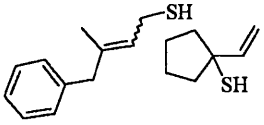

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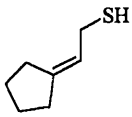

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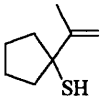

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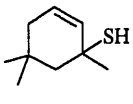

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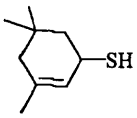

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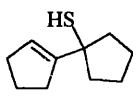

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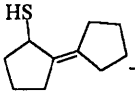

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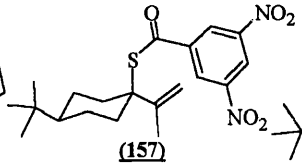

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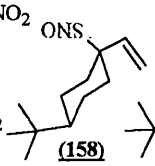

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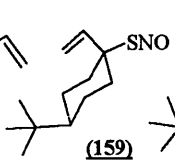

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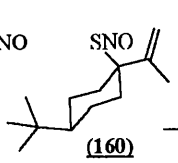

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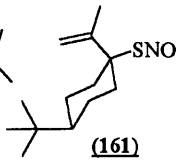

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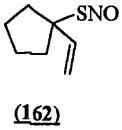

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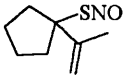

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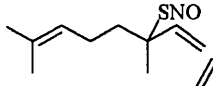

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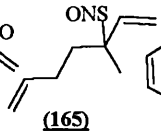

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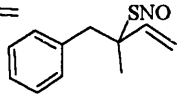

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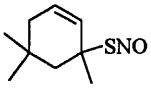

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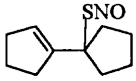

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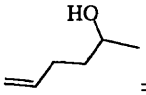

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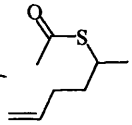

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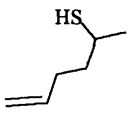

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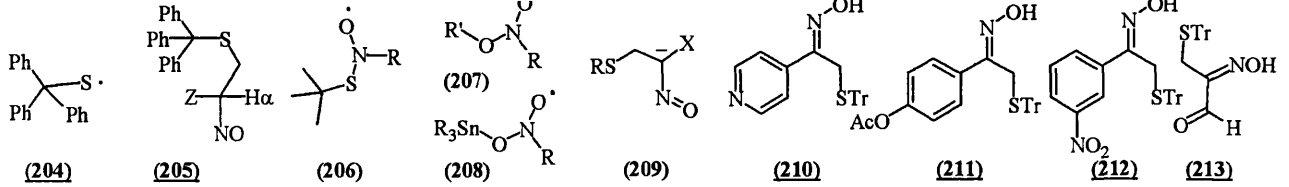

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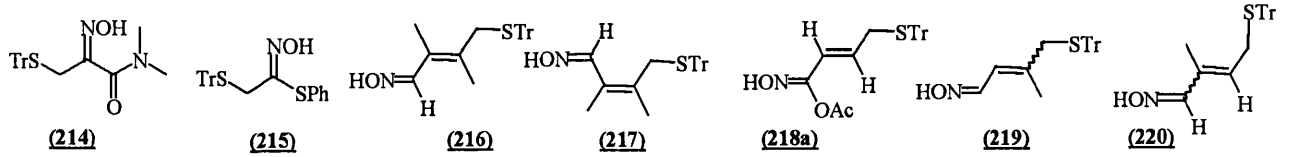

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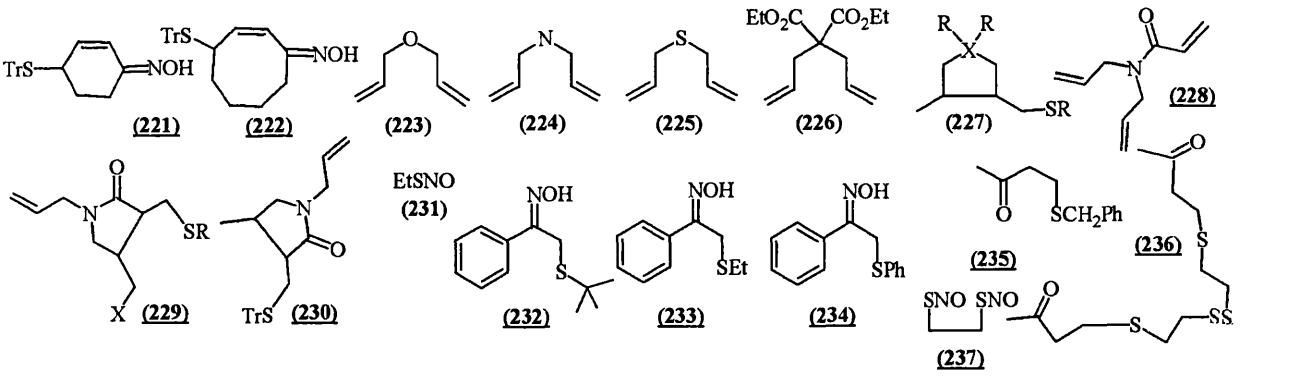

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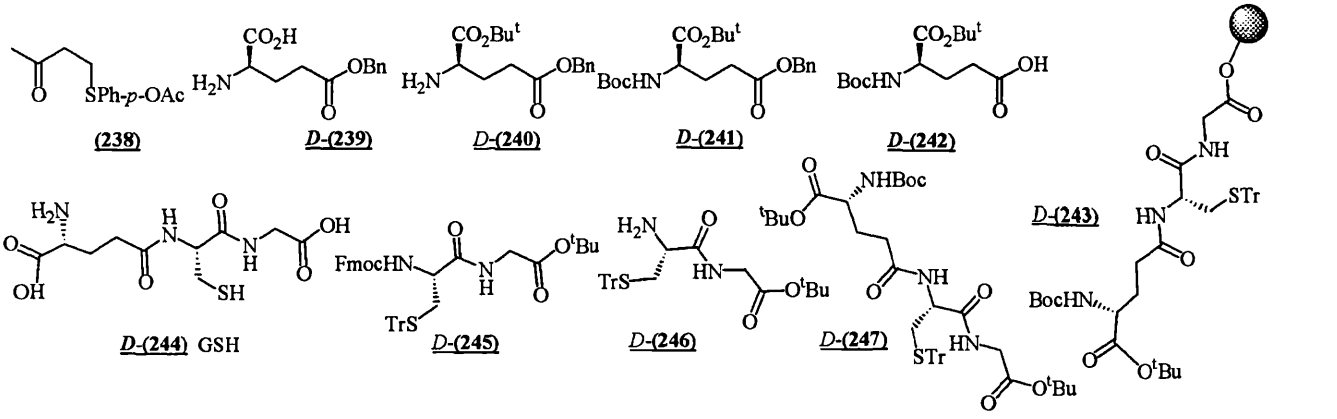

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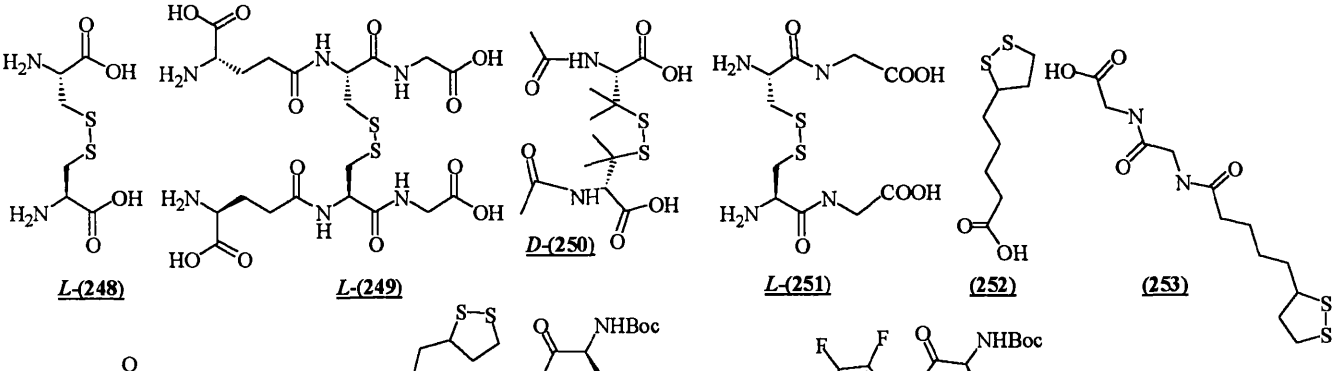

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