Synthesis and Application of Novel N-halamine Antimicrobial Materials

by

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Abstract

Several novel antimicrobial N-halamines have been synthesized and were incorporated into water-soluble latex paint. These paints were expected to render good biocidal capability and the antimicrobial activity to be long-lasting. The physical properties of these paints have been examined. These paints were coated onto different materials. Chlorine loading was monitored by a potassium iodide/thiosulfate procedure. A chlorine stability study was completed under different conditions: lab light and UVA light. The rechargeability of these paints has also been studied. The biocidal test result was also obtained.

The second part of the research was designed to study the chlorine activities if bonded to different kinds of nitrogen atoms in 5,5-dimethylhydantoin. Selective chlorination was done, first, by blocking the each nitrogen atom in turn with a methyl group. Thus, 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethylhydantoin were the examined compounds. The solubility of these two compounds was tested. Research has also been done to study the chlorine stabilities and biocidal efficacies of these two compounds. During the UVA stability test, an interesting hydrogen atom migration reaction was observed. Further experiments were used to propose several mechanisms for this kind of reaction.

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Chapter 1

Introduction

1.1 Chronology of antimicrobial techniques

In the past several thousand years, numerous epidemics have happened. Records show that the well-known black death that happened during 1347-1353 killed at least one-third of the European population. The large number of deaths was caused by *Yersinia pestis*, which was carried by human blood.[1,2] The same microbe, *Yersinia pestis*, is also responsible for the Plague of Justinian in the year 542.[2] Current data has indicated a total European population loss of 50% to 60% between 542 and 700.[2] The majority of deaths during the influenza pandemic of 1918-1919 was caused by the influenza virus, and the population of the victims was more than 40 million worldwide.[2,3] Influenza spread was seasonal around the world, leading to the deaths of between 250,000 and 500,000 people each year.[3] In the last decade, several deadly and easily infectious viruses have already claimed hundreds of people's lives across the world. Among them, SARS, H5N1 (which is known as Bird flu), and H1N1 (which is known as Swine flu) are the culprits for a great number of deaths globally.[4,5]

The use of microorganisms to produce biological weapons is well documented. Attempts to use biological warfare agents date back to ancient times.[6,8] Scythian archers made infectious arrows by dipping them in decomposing bodies with manure around 400 BC. Romans used dead animals to contaminate their enemy's water sources in 300 BC. Hannibal

won a naval victory over King Eumenes II of Pergamon by firing earthen vessels full of venomous snakes into the enemy ships in 190 BC. During the battle of Tortona in 1100s, dead and decomposing human bodies were used to poison enemy's wells. Plague-infected corpses were used during the battle of Kaffa in the 1300s, and it resulted in an epidemic within the enemy forces.

During World War I, Germany developed several kinds of biological weapons, including wheat fungus.[7] They spread plague in St. Petersburg, Russia, and infected mules with glanders in Mesopotamia. During the Sino-Japanese War (1937-1945) and World War II, Unit 731 of the Imperial Japanese Army conducted human experimentation on thousands of victims, mostly Chinese, Russian, and American prisoners. In military campaigns, the Japanese army used biological weapons on Chinese soldiers and civilians. The massive biological attack caused a plethora of deaths within 10 years.[7,8]

The infectious diseases and biological weapons mentioned above result from the presence of pathogenic microbial agents, and they are collectively called microorganisms. Microorganisms are very tiny, unicellular organisms that are found everywhere on the earth. Although some microorganisms are good ones for human beings and animals, others can be detrimental and cause diseases. The harmful organisms mostly include pathogenic bacteria, tuberculosis, anthrax, and fungi. They cause various diseases such as malaria, plague, sleeping sickness, and toxoplasmosis. These microorganisms can also spoil food, contaminate water, ruin clothes, rot wood, foul water pipes and ships, interfere with equipment, and produce odors in carpets and diapers, or on living organisms.[9]

Effective methods have to be developed to kill the infectious microorganisms.[10] Archaeological evidences indicate that human beings have used biocides as preservatives for food and water since the earliest civilizations. The Egyptians, Chinese, and Persians practiced food preservation, drinking water sanitation, and antisepsis for wounds and injuries.

In the late 1800s, John Tyndall showed that spores could be killed by repetitive heating. Nicholas Appert suggested a way to preserve food by sealing vegetables and fruit in glass jars and heating them.[10] In 1857, Pasteur developed the process known as "pasteurization" to control microorganisms.[11] In 1886, Semmel Weiss proved that physicians could prevent puerperal fever by washing their hands after performing autopsies and prior to assisting in childbirth. Pasteur, Koch and Wolff Hugel developed scientific procedures for both dry heating and steam sterilization in the late 1800s.[10,11]

Chemicals such as alcohol (wine), copper and silver were considered to be some of the earliest disinfectants.[10-12] Mercuric chloride was used as an antiseptic and a wood preservative by Arab physicians. Coal, tar and wood tar were used as disinfectants in the distilling industries and in the preservation of ship timber. Chlorine and formaldehyde were discovered to be effective disinfectants in 1700. Since this idea was first adopted in 1843, chlorine has been used for water treatment up to this day. Copper sulfate, zinc chloride, sodium permanganate, acids, alkalis, sulfurs and alcohols were also excellent disinfectants of the time.[10,12]

The present need for new, effective and specific biocides is much more urgent than ever before. People of the present live relatively longer times than their grandparents did 60 years ago. Many factors have contributed to this change, including medical innovations and

nutritional improvements. The wide range application of sanitation and disinfection has also played a very important role in attaining longevity. Infection control in the hospital mainly depends upon the use of a variety of specialized antimicrobial agents. In addition to the cleaning and disinfectant solutions used in general housekeeping, there are various formulations especially used for demanding tasks, such as operating room protection and sterilization or high-level disinfection of delicate and heat-sensitive medical devices. The appropriate and careful use of these products is a major issue for infection control.[13]

A dramatic increase in the population of elderly people is expected in the very near future. With the occurrence of antibiotic resistance, we face increased pressure on the ability to deal with harmful microbes. One solution to this dilemma is to maintain sanitation and use disinfection products. Sanitation is an important and helpful way to prevent contact with microbes, but it could not last for a long time. Therefore, disinfection of surfaces is essential in the controlling of microorganisms. To be effective, a disinfectant must stay attached to the surface for a designated amount of time. A disinfectant cannot kill organisms if it is not in contact with the surface.[14]

Biocides have a variety of environmental applications. The largest single use of antimicrobial agents is for the purification of drinking water. The disinfection of drinking water can eliminate disease-producing contaminants and unpleasant taste or odor, and it has substantially reduced the instances, and therefore deaths, of cholera, hepatitis, and amoebic dysentery. In areas where water is not adequately disinfected, outbreaks of these diseases still occur. At present, the most common method to disinfect drinking water is the use of free

chlorine and chloramines (a mixture of chlorine and ammonia). Roughly 90 percent of the drinking water in the United States is disinfected with these compounds, and only one percent is disinfected through the use of ozone. These antimicrobial agents are used to provide more than 200 million people with disinfected drinking water.[15,16]

The development of antimicrobial surfaces using antimicrobial coating or impregnated surfaces is promising for hospitals and public facilities. Most of them are aiding in the protection of surfaces against environmental spoilage. Antimicrobial paint is such an antimicrobial material capable of inhibiting the proliferation of bacteria and other harmful microorganisms. An antimicrobial paint film can be produced by applying a liquid paint composition to a work surface, wall, ceiling or floor. This polymer film embedded with antimicrobial substance can kill bacteria or prevent their growth on the surface.[17-22]

The cause of food-borne illness is widely recognized to be infection by pathogenic microorganisms. Continuing incidents of food poisoning demonstrate the limited effect of some food sanitation practices and the importance of antimicrobials usage in the maintenance of our food supply. Deaths and illness due to food-borne organisms are preventable with appropriate precautions including hand washing with an efficacious product and disinfection of surfaces that come in contact with contaminated food. Organisms, typically responsible for food poisoning, are spread by the fecal-oral route. Contamination may occur via direct contact to a person's hands or from consumption of food from an infected surface.

During this century, considerable efforts have been focused on better techniques for studying and isolating microorganisms. These studies have furthered the development of a

larger amount of more effective antimicrobials which could be used to solve specific problems. Scientists today are also studying antimicrobial efficacy, its metabolic pathways in the human body and environmental toxicity in order to develop even better biocides. The development of antibiotics offers a powerful tool against bacterial infections and has saved the lives of millions of people. However, researchers are also beset by the bacteria-resistance problem that results from the widespread and sometimes inappropriate use of antibiotics. In addition to identifying new biocides, scientists are improving their knowledge of the application of these materials. The right biocide for a specific application can solve specific problems and benefit people. The understanding of the structure and metabolic processes of bacteria is the fundamental basis for the improvement of biocides.[23,24]

1.2 The structure of bacteria

Bacteria are single-celled organisms such as that pictured in Figure 1.1. From outside inward, bacteria are surrounded by capsule, cell wall, membrane, and cytoplasm. There are ribosomes, a nucleoid, and plasmids floating in cytoplasm, and there is no membrane-bounded nucleus or other membrane-bounded organelles. Flagella or pili are sometimes grown on the bacteria surface. Two groups of bacteria have a general rounded shape: the Gram-negative and the Gram-positive. They have a general rod-like shape and stain as red color or blue, the bacilli.[9, 25, 26] The bacteria chosen for our study are *Staphylococcus aureus* (Figure1.2) and *Escherichia coli* (Figure1.3). *Staphylococcus aureus* are commonly notated as *S. aureus*, have a round shape, and are Gram-positive. Meanwhile, *Escherichia coli* are commonly notated as *E. coli*, have a rod shape, and are Gram-negative.

Because *E. coli* have an outer membrane layer, they are Gram-negative and thus can survive some antimicrobials such as quaternary ammonium salts. The Gram-negative organisms have thicker cell walls compared to the Gram-positive organisms (Figure 1.4).

Bacterial food poisoning may occur in two ways: either by the direct presence of bacteria in consumed food, or by toxins produced by bacteria that remain in the food.[27] Bacterial organisms responsible for direct infection include *Salmonella spp., shigella spp., Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Botulism,* and *Vibrio spp.* The organisms that produce toxins are *Staphylococcus aureus*, *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Clostridium botulinum* and *Vibrio cholera.*

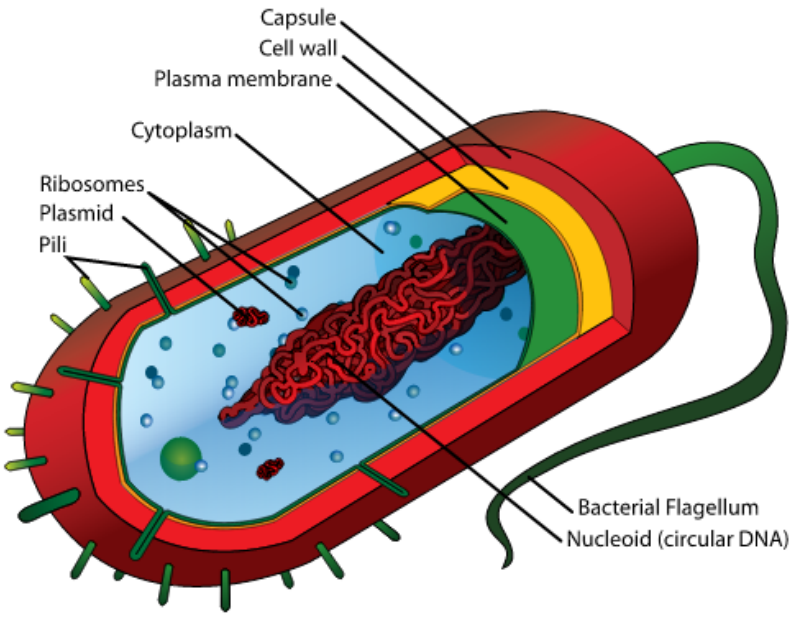


Figure 1.1 Bacteria

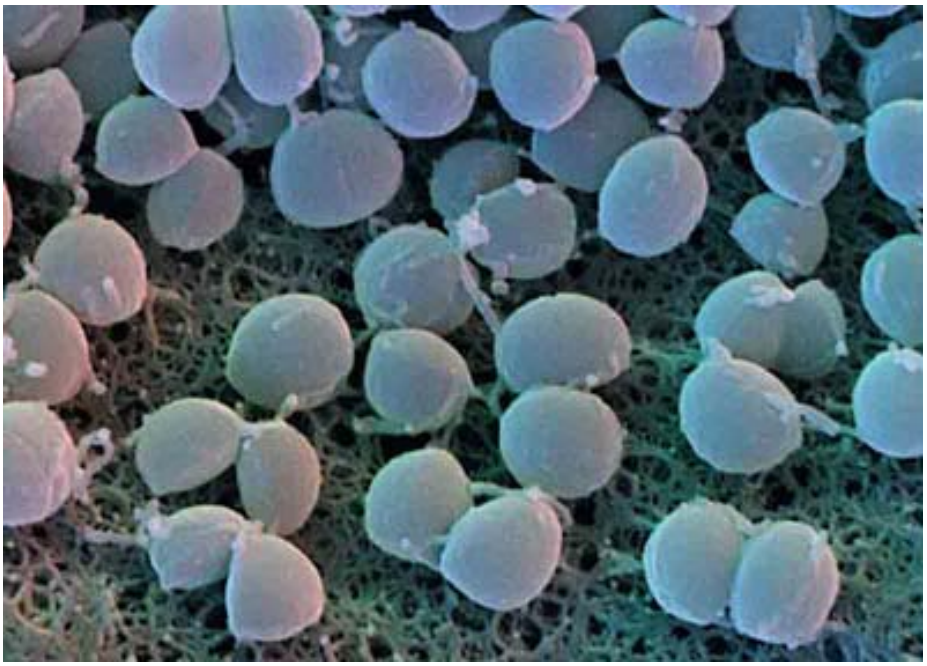


Figure 1.2. *Staphylococcus aureus*

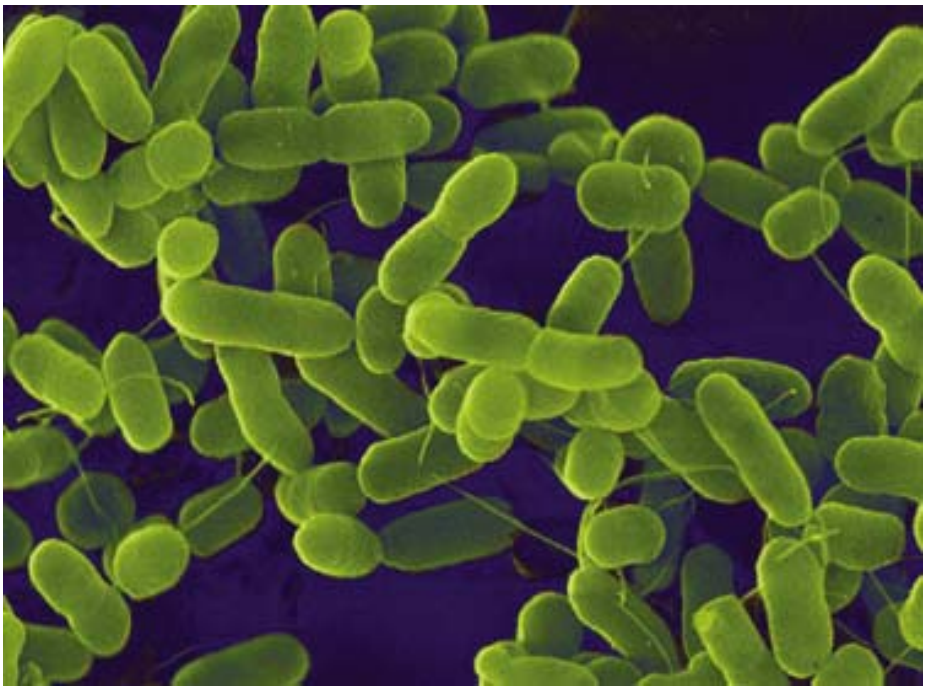


Figure 1.3. *Escherichia coli*

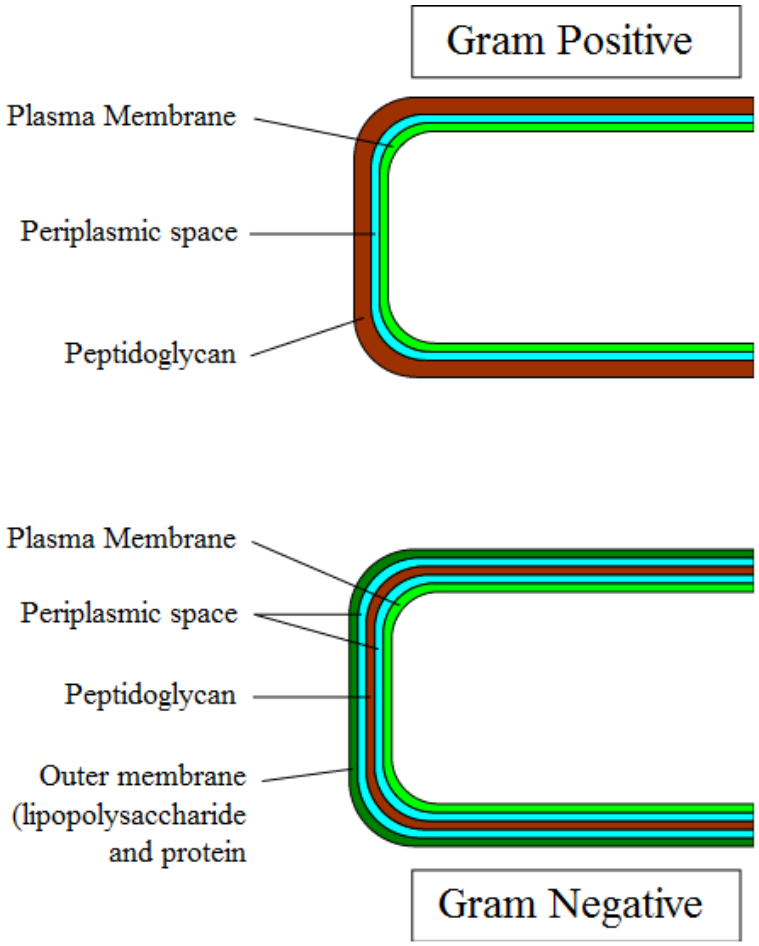


Figure 1.4. Structures of Gram-positive bacteria and Gram-negative bacteria

Biocides are represented by a wide range of chemical agents. Their actions on the bacterial cells are multifarious. Understanding the mechanism of action of biocides is important in optimizing their application and combating the resistance. A common mechanism is the damaging to the integrity of cell membranes. There are several stages of interactions between biocide and bacteria cell. The first phase is the initial binding and accumulation of biocides on the cell surface, followed by the structural alteration of the cell outer layer. The third stage would be the penetration of the biocides into the cytoplasmic membrane. The critical site on the membrane is damaged by the chemical ingredient in the biocide and eventually this causes the cell to leak. The reactions between biocide and the

cytoplasmic constituents may occur, but the rupture of the cell membrane is probably the main cause for bacterial cell death.[27-29]

1.3 Antimicrobial Agents

Chemical biocides not only play a key role in the preservation of products such as foods, beverages, cosmetics, and pharmaceutical formulations but also provide protection against spoilage and degradation in a wide range of industrial and environmental applications. The systematic study of their mechanisms of action renders a direction to their future design and development, providing insight into new agents, resistance mechanisms, and toxicological problems, and offers guidance on their correct usage.

Any substance that acts against microbes (such as a disinfectant, sanitizer or sterilant) is an antimicrobial biocide. There are many names for biocides that prevent the growth of microorganisms, such as algaecide, antifoulant, bactericide, bacteriostat, disinfectant, fungicide, fungistat, mildewcide, preservative, sanitizer, slimicide, sterilant, and virucide.[30]

Biocides usually bring rapid effective population reductions to a system. There are various biocides, some of which have a wide range of killing effect on many kinds of bacteria. Generally, they are divided into two groups: oxidizing agents and non-oxidizing agents. Chlorine, bromine, hypochlorite, peroxide and ozone are examples of oxidizing agents. Non-oxidizing agents include aldehydes, alcohols, chlorinated phenolics, heavy metals, organo-sulfur compounds, quaternary ammonium compounds, and organic acids.[31]

Below is an introduction to the typical biocides:

1. Chlorine. Chlorine is the most widely used industrial biocide today. It has been used for

disinfection in water supplies and for the removal of tastes and odors from water for several hundred years. The amount of chlorine required by a water system is determined by chlorine demand, contact time, pH and the temperature of the water, the volume of water and the amount of chlorine lost during aeration. When chlorine gas enters a water supply, it will be hydrolyzed to form hydrochlorous acid which is the active ingredient for biocidal activity.[32]

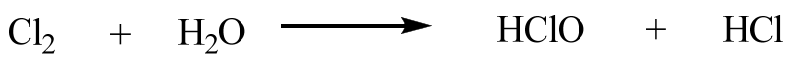


Figure 1.5. The hydrolysis of chlorine gas in water

Hydrochlorous acid is responsible for the oxidation reactions in the cytoplasm of microorganisms after diffusion through the cell walls. Chlorine disturbs the production of ATP, an essential compound for the respiration of microorganisms. The bacteria in the water will die as a consequence of lack of energy.[32, 33] However, chlorine can form potentially hazardous byproducts. It has been replaced in certain parts of the world.

2. Peroxides. Hydrogen peroxide has been used as a wound cleaner, surface cleaner, surface disinfectant, and especially in sterilization applications (in liquid or gas forms). It is environmentally friendly and rapidly degrades into water and oxygen, leaving no harmful residues. It also has a broad spectrum of uses in killing bacteria, fungi, and spores. However, concentrated hydrogen peroxide solutions are both corrosive and carcinogenic.[34-36]

3. Ozone. Ozone is naturally unstable but is a powerful oxidizing agent. As a biocide, ozone acts in a similar way as does chlorine. It disturbs the formation of ATP and causes difficulty in cell respiration, resulting in bacterial death from loss of life-sustaining energy.[36, 37] However, ozone is an atmospheric pollutant, is toxic to human beings, and is known to

degrade almost all polymers and oxidize metals upon contact.[38-40]

4. Organo-sulfur compounds. Organo-sulfur compounds act as biocides by inhibiting cell proliferation. A variety of organo-sulfur compounds can function in different pH ranges. Normally energy is transferred in bacterial cells when the iron oxidation state changes from Fe^{3+} to Fe^{2+}. Organo-sulfur compounds remove the Fe^{3+} by complexion as an iron salt. The transfer of energy in the cells is thus stopped and immediate cell death follows.[41]

5. Quaternary Ammonium Salts (Quats). Quaternary ammonium salts (Figure 1.6) are surface-active chemicals that consist generally of one nitrogen atom, surrounded by substituents on four bonding sites of the nitrogen atom. These compounds are mostly effective against bacteria in alkaline pH ranges. They are positively charged and will bond to the negatively charged sites on the bacterial cell wall. These electrostatic bonds will cause the bacteria die of cell wall stresses. They also cause normal flow of life-sustaining compounds through the cell wall to stop by decreasing its permeability. Use of quaternary ammonium salts is limited, because they are not effective against Gram-negative bacteria and spores. Also, some bacteria such as *Staphylococci* and *Lactobacilli* become resistant to compounds in this class. Quaternary ammonium compounds require a lengthy alkyl group to achieve a biocidal function.[42-44]

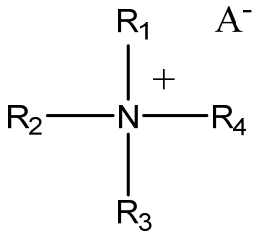


Figure 1.6. Structure of Quats

6. Alcohols. Alcohols are widely used in medical, food service, and consumer products. However, they need to reach a significant concentration (usually 15-70 %) in order to be effective. The short chain alcohols, such as ethanol, isopropanol, and n-propanol, are popular for their "near-instant" antimicrobial activity. Alcohols generally inactivate spores and viruses by acting on fatty outer coats such as that of a mycobacterium. Alcohols are popular in hand cleansers and hand sanitizers due to their speedy action. Although ethanol, isopropanol, and n-propanol show excellent immediate biocidal activity, they evaporate rapidly and lack of persistent antimicrobial activity. They also lead to problems due to dry skin after frequent usage.

7. Aldehydes. Formaldehyde and glutaraldehyde (Figure 1.7) are broad spectrum of biocides well known for the killing of bacterial spores. Formaldehyde has been used for hundreds of years as an embalming agent. It is effective in killing water-borne bacteria, and its vapor has been applied to the sterilization of inanimate surfaces such as chicken houses and equipment. However, formaldehyde is a potent carcinogen, so there are restrictions on its useage. The most commonly used "cold sterilant" is glutaraldehyde, which is used to sterilize surgical instruments as an alternative to heat sterilization in autoclaves. Unfortunately, many people become allergic to it after contact.[32]

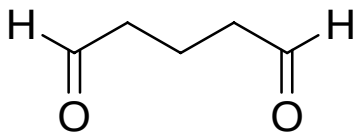
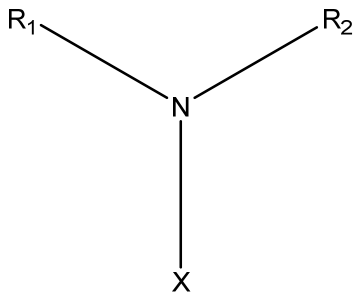


Figure 1.7. Structure of Glutaraldehyde

1.4 N-halamine

Biocidal polymers, such as N-halamine antimicrobial polymers, have received much attention in recent years. N-halamines are particularly efficacious, and they are safe to humans and the environment. They also have long-term stability, regenerability, and effectiveness toward a broad spectrum of microorganisms. There is little chance for organisms to develop a resistance because a halogen is the active oxidant.[42,43]

N-halamines, as a class of biocides, is defined as compounds containing one or more nitrogen-halogen covalent bond(s). The halogen can be chlorine or bromine which can be released and acts like the free halogen in aqueous media. The relatively stable N-Cl bond leads to their long-lasting germicidal properties.[44] And only a very small amount of "free halogen" can be found in water due to their low hydrolyses constants. Toxic byproduct are not significantly produced. The general structure of N-halamines is shown in Figure 1.8.



R_1, R_2,=H, Cl, Br, inorganic group, organic group
X= Cl, Br

Figure 1.8. Structure of N-halamines

The structure of the N-halamine can have organic groups (alkyl group, carbonyl group), inorganic groups (phosphate, sulfate), hydrogen, or halogens to replace the R_1 and R_2. If the substituent is an inorganic group or hydrogen, the resulting compoundd is referred to as an

inorganic N-halamine (Figure 1.9). Organic N-halamines are more complicated than inorganic N-halamines, with substituents R_1 and R_2 being organic groups. Amines, amides, and imides are in this category (Figure 1.10).[45-47]

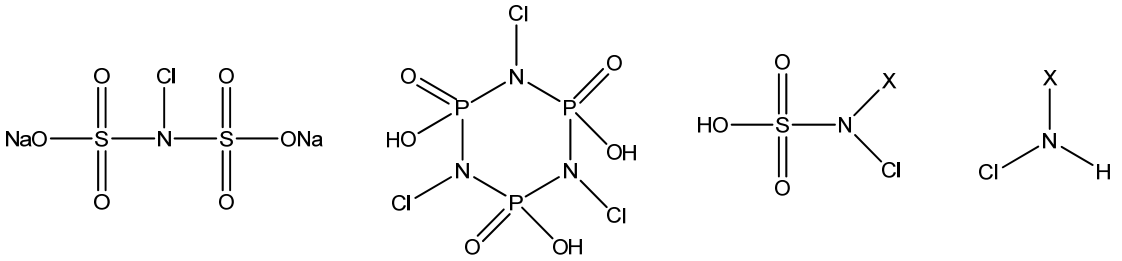


Figure 1.9. Structure of inorganic N-halamines.

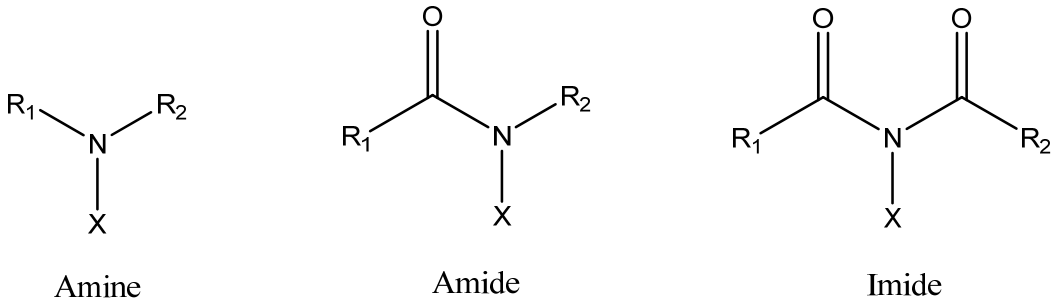


Figure 1.10. Organic N-halamines

The structure of the N-halamines determines the order of the inactivation of bacteria. The killing rate of bacteria by N-halamine follows the sequence imide>amide>amine.[46] However, the trend of N-halamine stability is inversely related to biocidal capability.[48] N-halamines with cyclic structures are more stable and applicable due to the absence of an α-hydrogen. This means that the α-hydrogen dehydrohalogenation (Figure 1.11) is impossible. Cyclic N-halamines are very efficient, broad-spectrum biocides that can last for a relatively long time.

They are stable in aqueous solutions because there is a low concentration of "free chlorine" released into water. They will not react significantly with organic compounds to produce toxic $CHCl_3$.[49]

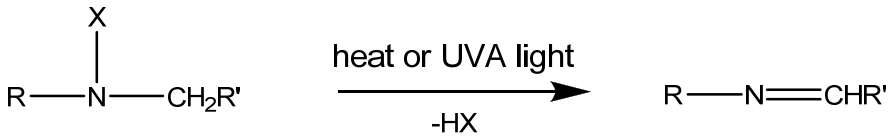
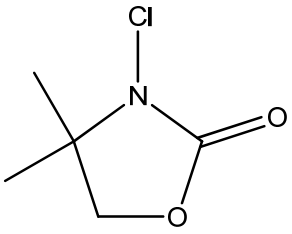
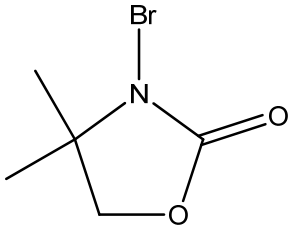


Figure 1.11. Dehydrohalogenation of α-hydrogen.

Kovacic and co-workers proposed the idea of N-halamine compounds in 1968, and several compounds were synthesized in the following years. Kaminski and co-workers were the first to demonstrate the antimicrobial behavior of halogenated oxazolidinone compounds (Figure 1.12) in 1976.[49, 50]



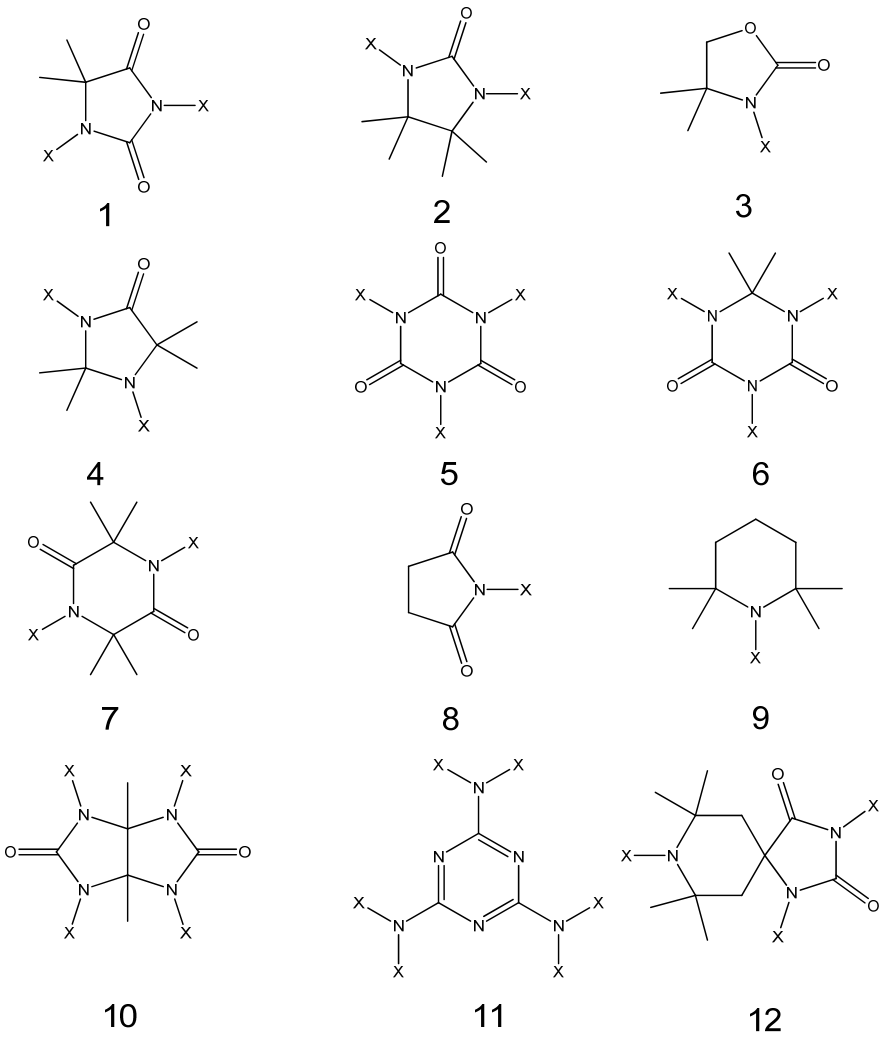
3-Chloro-4,4-dimethyl-2-oxazolidinone



3-Bromo-4,4-dimethyl-2-oxazolidinone

Figure 1.12. Structures of 3-chloro-4,4-dimethyl-2-oxazolidinone and 3-bromo-4,4-dimethyl-2-oxazolidinone

The synthesis and application of novel N-halamine biocides have been greatly advanced in the past several decades. Numerous novel N-halamine compounds have been synthesized by the Worley research group at Auburn University since the 1980s. The structures of some typical N-halamine compounds are shown in Figure 1.13.[51-54]



For X=H, 1: 5,5-dimethylhydantoin; 2: 4,4,5,5-tetramethyl-1,3-imidazolidin-2-one; 3: 4,4-dimethyl-2-oxazolidinone; 5: 2,4,6-trihydroxy-1,3,5-triazine (cyanuric acid); 6: 6,6-dimethyl-1,3,5-triazine-2,4-dione; 7: 3,3,6,6-tetramethyl-2,5-piperazine-dione; 8: 2,5-pyrrolidinedione (succinimide); 9: 2,2,6,6-tetramethylpiperidine; 10: 3a,6a-dimethyltetrahydroimidazo[4,5]imidazole-2,5(1H,3H)-dione; 11:1,3,5-triazine-2,4,6-triamine (melamine); 12: 7,7,9,9-tetramethyl-1,3,6-triazospiro[4,5]decane-2,4-dione.

Figure 1.13. Structures of cyclic organic N-halamines (X= H, Cl, Br)

Organic N-halamines are expected to render excellent biocidal capability. The unique property of N-halamines is that they can be repeatedly charged by simply reacting them with sodium hypochlorite solution or household bleach after many applications. Water disinfection was the primary research area in Worley's group during the period 1980 to 2000. Recent research has been focused on the application of the cyclic N-halamines to water treatment and

surface protection.

1.5 Biocidal materials

Biocidal polymeric materials have been developed to have antimicrobial applications in numerous areas, and one of them is in water treatment. There are three major approaches to make polymeric biocides. The first strategy would be the polymerization of biocidal monomers by themselves or with other monomers to generate biocidal homopolymers or heteropolymers. The second one is to graft or coat N-halamine precursor monomers onto polymer backbones or to modify the polymer units to form N-halamine derivatives. For example, biocidal moieties can graft onto a commercial polymeric resin by modifying its surface. The third one is to add N-halamine monomers or polymers to the host polymers just before polymer processing or fiber extrusion. Precautions need to be taken in making N-halamine polymer derivatives. The addition of too many biocidal moieties may change the properties of some of these polymers, and it might lead to problems with leaching.

N-halamine compounds can be designed to generate N-halamine monomers during the first step, followed by monomer polymerization with the assistance of polymerization initiators. The polymerization method frequently used in Worley's group is to use monomers having unsaturated bonds in the molecules (such as vinyl group).[55-61] Usually 2,2'-azobisisobutyronitrile (AIBN, Figure 1.14) and peroxide compounds such as potassium persulfate and hydrogen peroxide are used to initiate the polymerizing reaction.

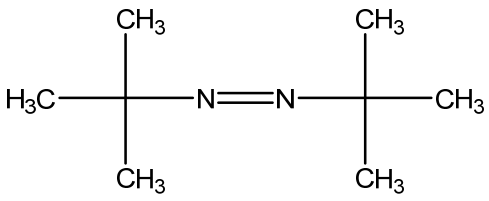
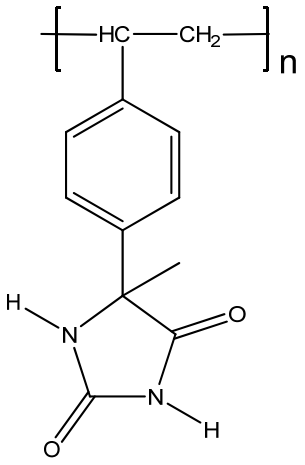
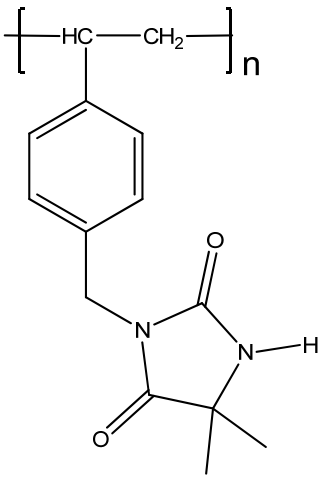


Figure 1.14. Structure of AIBN

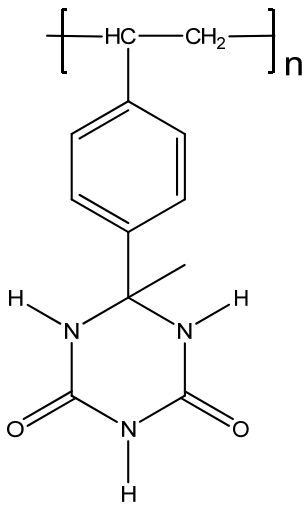
Several N-halamine polymers have already been synthesized by following this free radical initiation technique. Former group members in Worley's group made great contributions to the synthesis of N-halamine polymers. The structures of the prepared polymers representatives are shown in Figure 1.15. The main characteristic of these structures is that all monomers have the cyclic N-halamine moiety. Schemes 1.1 and 1.2 illustrate typical polymerization using N-halamine monomers. The N-halamine polymers are insoluble in water and exist as beads at ambient temperature. They have been shown to inactivate numerous species of bacteria, fungi, and even viruses within a short contact time. Once the oxidative chlorine (or bromine) has been exhausted, the polymers can be recharged upon exposure to household bleach or sodium hypochlorite solution. Poly I and Poly II are among the best biocidal polymers for water treatment. The best means of producing Poly I and Poly II (Figure 1.15), however, is by functionalizing polystyrene or chloromethylated polystyrene beads.[56-61]



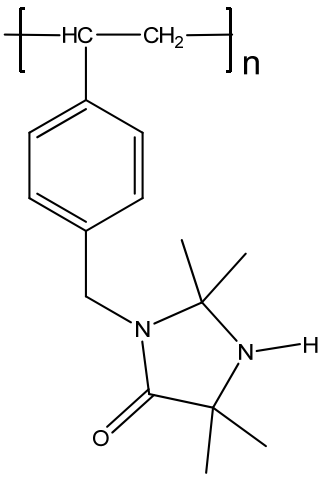
Poly I



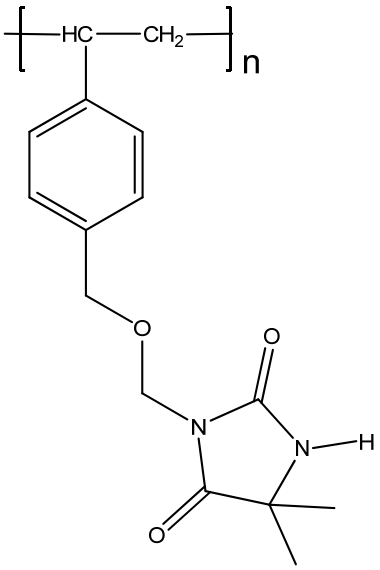
Poly II



Poly-CTD



PI

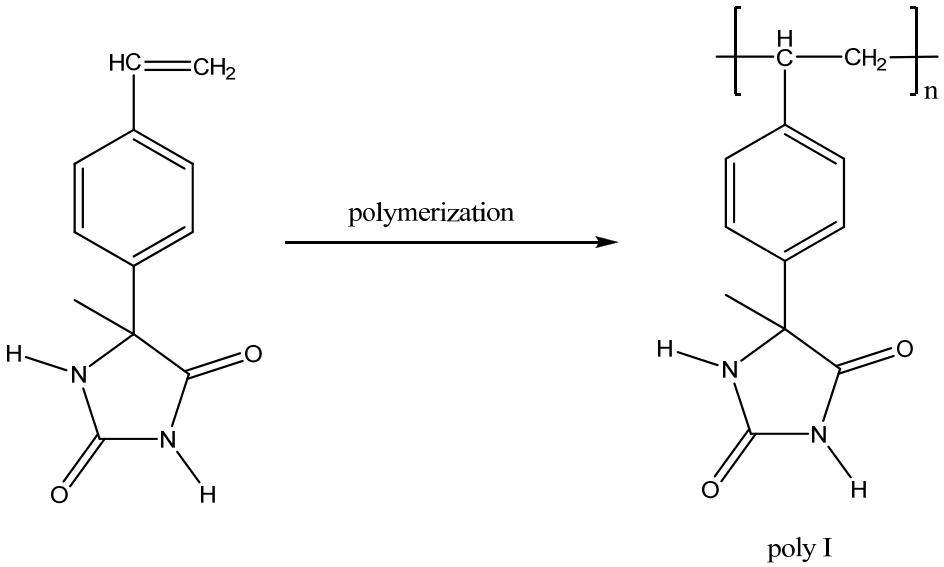


PHMH

X=Cl, Br, H

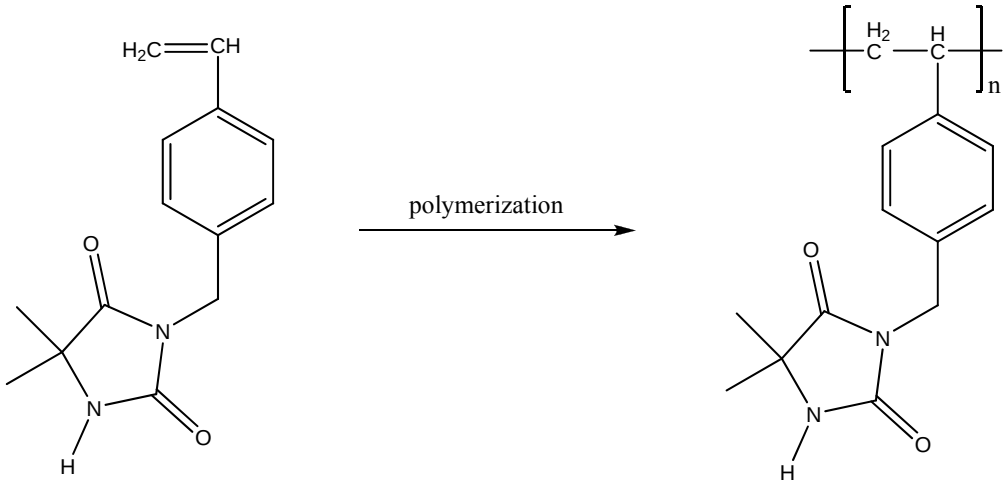
Poly[1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin] (Poly I)
Poly[1-chloro-3-(4'-vinylbenzyl)-5,5-dimethylhydantoin] (PVBDMH, Poly II)
Poly[1,3,5-trichloro-6-(4'-vinylphenyl)-1,3,5-triazine-2,4-dione] (Poly-CTD)
Polystyrene imidazolidinone (PI)
Polystyrene hydroxymethylhydantoin (PHMH)

Figure 1.15. Structures of polystyrene N-halamine polymers



poly I

Scheme 1.1. The formation of poly I



poly II

Scheme 1.2. The formation of poly II

N-halamine precursors can also bond to substrates via different tethering groups such as siloxanes, hydroxyl groups, and epoxides. Figure 1.16, 1.17 and 1.18 show several attaching approaches of 5,5-dimethylhydantoin onto cellulose which has OH groups on its surface.[62-65]

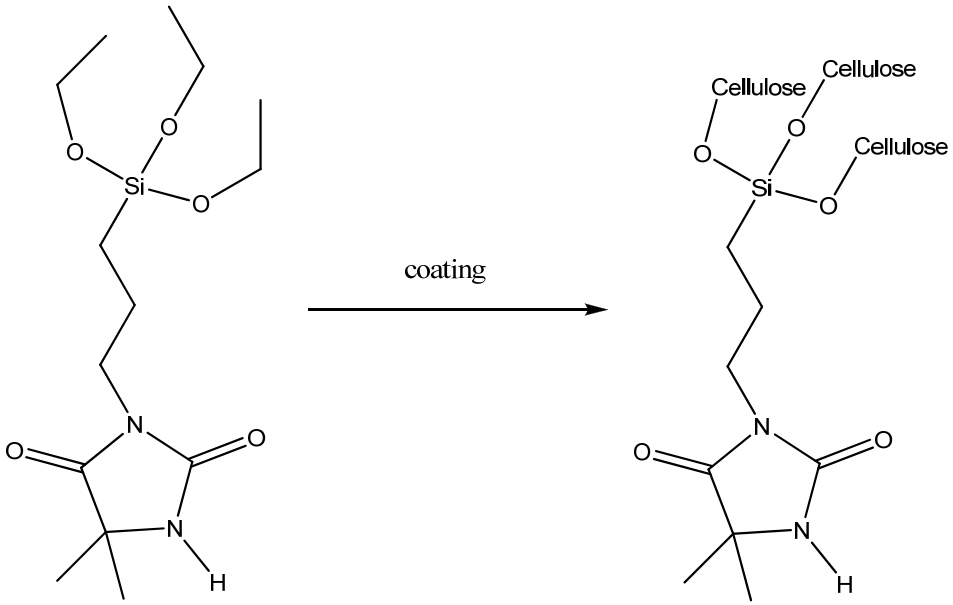


Figure 1.16. The attachment of hydantoin siloxane onto cellulose

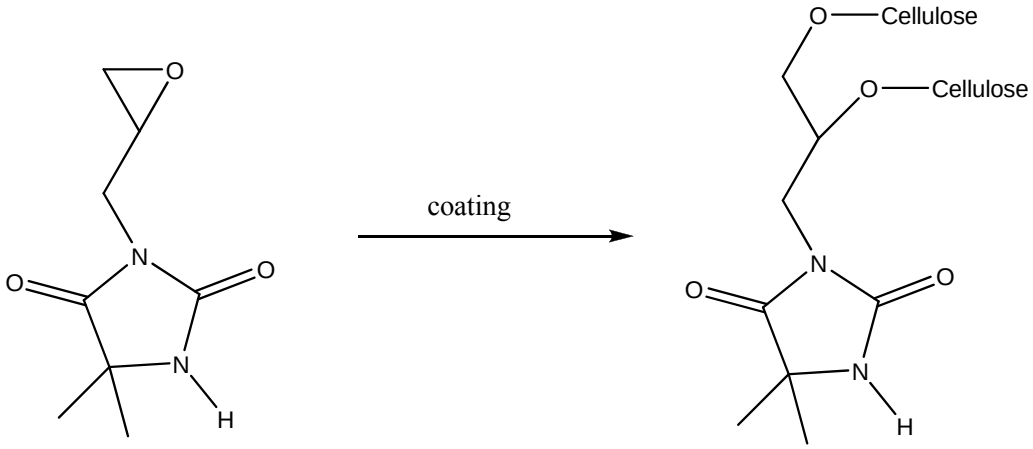


Figure 1.17. The attachment of hydantoin epoxide onto cellulose

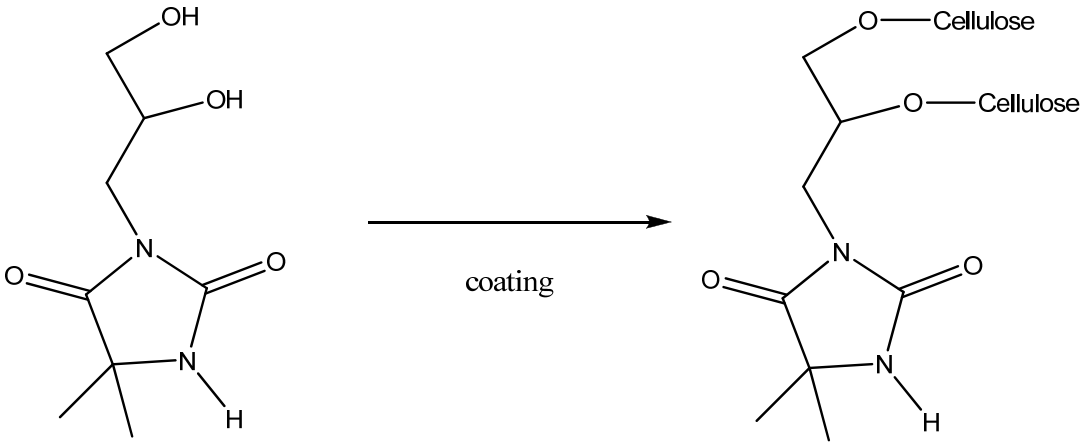
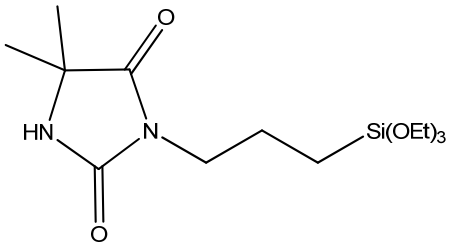
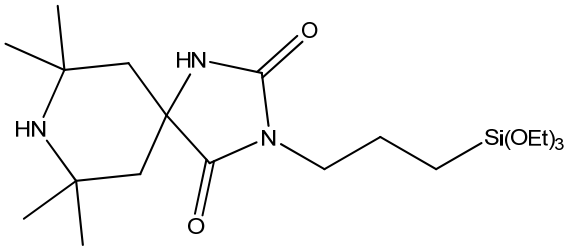


Figure 1.18. The attachment of hydantoin diol onto cellulose

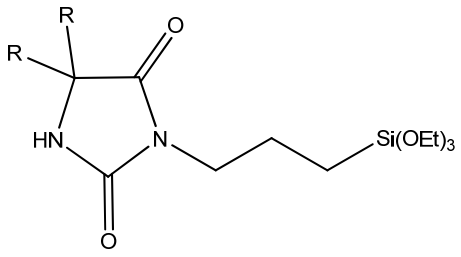
Alkoxy silane (siloxane) is an excellent adhesive for bonding various organic groups onto substrates. A large number of N-halamine precursors have been coated onto substrates by using this coupling agent (Figure 1.16). Synthesis of siloxanes was carried out by the reaction of 5,5-dialkylhydantoin salt and 3-chloropropyltriethoxysilane. Both 5,5-dimethyl-3-(3'-triethoxysilylpropyl)hydantoin (BA-1) and 3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro[4,5]decane-2,4-dione (Figure 1.19) are being employed in industrial settings. Cellulose, polyester, sand, silica gel, and paint can be coated with N-chlorohydantoinyl siloxanes, and these substrates show excellent antimicrobial capabilities.



BA-1



TTDD Siloxane



BA-1 derivatives

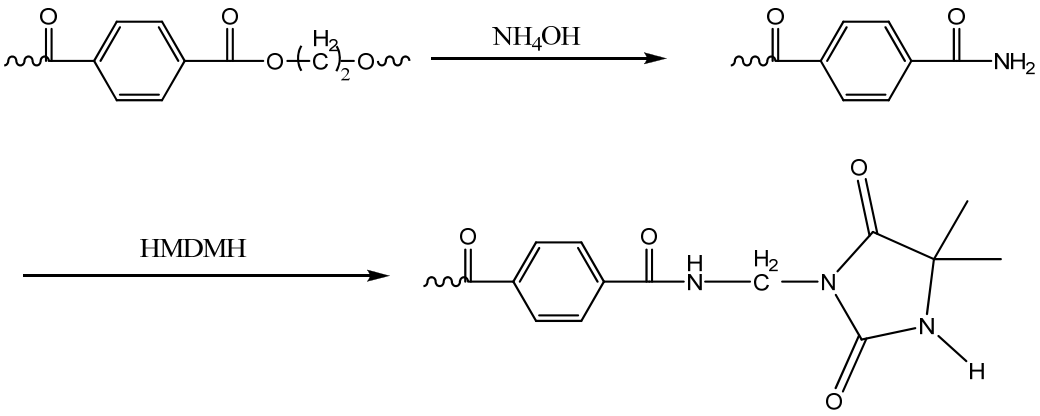
Figure 1.19. Structures of N-halamine siloxanes

Hydantoinylepoxide derivatives are frequently used as surface coupling agents in

Worley's group. They can be synthesized by reacting the appropriate hydantoin salt with epichlorohydrin. This new kind of N-halamine compound can be coated onto cellulose (Figure 1.17). After being treated with household bleach or sodium hypochlorite solution, the cellulose becomes an antimicrobial material. Biocidal tests show that it is extremely effective in killing both Gram-positive and Gram-negative bacteria requiring short contact times. Hydantoin expoxide is water soluble and shows great promise as a commercial biocidal material.

N-halamines with diol groups can also be coated onto cellulose to produce a biocidal material. However, this coating procedure requires a cross-linking agent 1,2,3,4-butanetetracarboxylic acid (BTCA) (Figure 1.18). The cellulose containing hydantoin diol shows effective biocidal activity against both Gram-positive and Gram-negative bacteria.

Biocidal materials can also be made by grafting techniques. These polymers include, but are not limited to, polyamide, polyester, and polypropylene. After being modified chemically, the hydantoin derivatives can form covalent bonds with the polymer backbone (Figure 1.20). These polymers have been proved to render very good biocidal capability.[66,67]

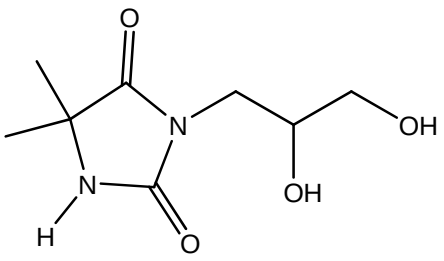


HMDMH: 3-hydroxymethyl-5,5-dimethylhydantoin

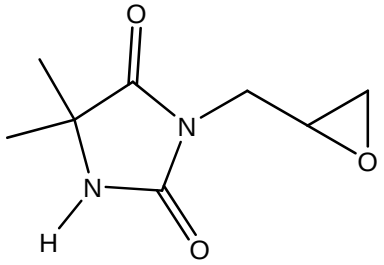
Figure 1.20. Hydantoin derivative grafting onto polyester.

1.6 Research projects

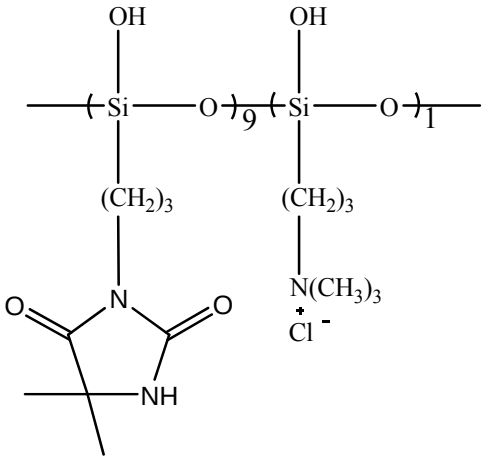
Two major research projects have been undertaken in this work. The first project was to develop long-lasting and effective antimicrobial paint. A commercial water-based latex paint was modified with hydantoin derivatives and is expected to provide potent antimicrobial activities against bacteria without changing the original properties. This paint was applied onto transparencies, wood chips and glass, and then chlorinated. Chlorine stability, rechargeability and biocidal efficacies were evaluated. Figure 1.21 shows the structures of the hydantoin derivatives that were used in this project.



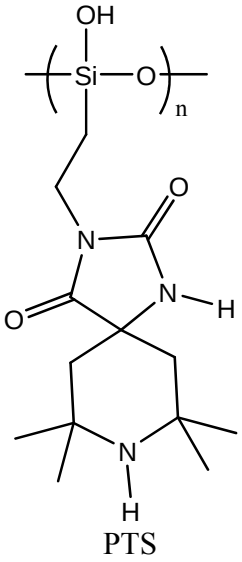
HD



HE



PHQS



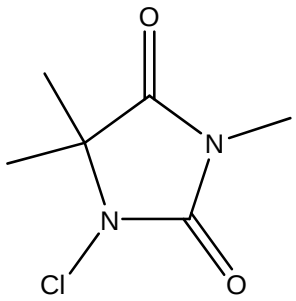
PTS

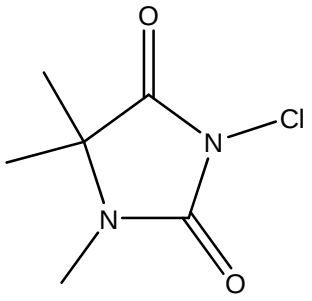
HD: 3-(2,3-dihydroxypropyl)-5,5-dimethylhydantoin; HE: 3-glycidyl-5,5-dimethyl- hydantoin; PHQS: poly[3-(5,5-dimethylhydantoinylpropyl)siloxane-co-trimethylammonium-propylsiloxane chloride; PTS: poly[3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-tri- azaspiro[4.5]decane-2,4-dione]

Figure 1.21. The structures of hydantoin derivatives

The aim in the second project was to compare the chlorine activity between two chlorinated compounds: 3-chloro-1,5,5-trimethylhydantoin and 1-chloro-3,5,5-trimethyl-hydantoin (Figure 1.22). The location of the N-Cl bond in these two compounds resulted in different chlorine activity. Solubility, stability, and biocidal activity were tested. During a UVA irradiation stability test, hydrogen migration was encountered. The mechanism of

hydrogen migration is reported in Chapter 5.





1-Chloro-3,5,5-trimethylhydantoin 3-Chloro-1,5,5-trimethylhydantoin

Figure 1.22. Structures of 3-chloro-1,5,5-trimethylhydantoin and 1-chloro-3,5,5-trimethylhydantoin

Chapter 2

Novel N-halamines incorporated latex paint (I)

2.1. Materials and instruments

All chemicals used in this research were purchased from Fisher Scientific (Fair Lawn, NJ) or Aldrich Chemicals (Milwaukee, WI) and used as received without further purification unless otherwise noted. Commercial latex paint, Olympic Premium Interior Latex Paint, was bought from local Lowe's store. Chlorination was accomplished with household bleach (Clorox ®, Inc., Oakland, CA). NMR spectra of the synthesized compounds were recorded by a Bruker 400 MHz spectrometer. An Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA, Type A, 315-400 nm) was used for UVA degradation studies.

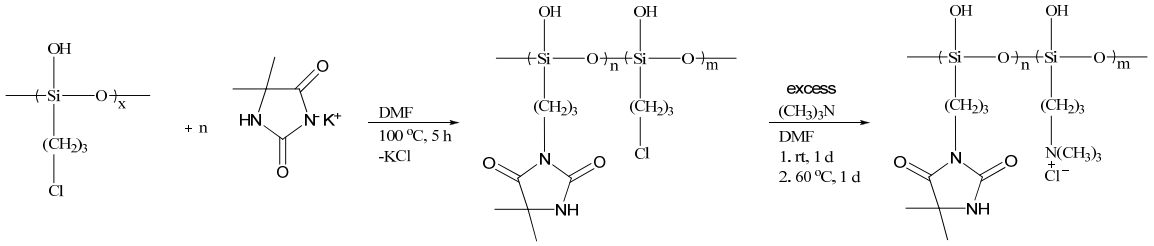
2.2. Synthesis of poly[3-(5,5-dimethylhydantoinylpropyl)siloxane-co-trimethyl-ammoniumpropylsiloxane] chloride (PHQS, 9:1)[68, 69]

25.35 g (0.1 mol) of 3-chloropropyltriethoxysilane was dissolved in 100 mL of 0.1 N HCl and stirred at room temperature for 2 h. Water was removed by evaporation. The white solution containing poly(3-chloropropylsiloxane) (PCPS), was dried in a vacuum oven overnight.

The hydantoin salt was synthesized by the following procedure: 11.89 g (0.09 mol) of 5,5-dimethylhydantoin and 5.74 g (0.09 mol) of potassium hydroxide were dissolved in 100 mL of ethanol. After refluxing at 95 ^{o}C for 10 min, the solid chemicals were completely dissolved. When the solution cooled to room temperature, the solvent was removed. Finally,

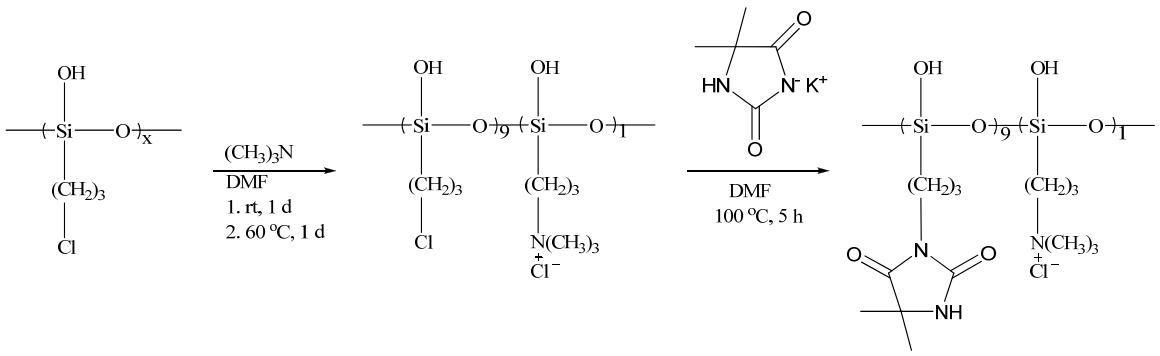
the product was dried in a vacuum oven for 2 days.

The newly synthesized poly(3-chloropropylsiloxane) and the hydantoin salt were mixed into 150 mL of anhydrous DMF. After 5 hour of mixing under stirring at 100 °C, KCl was precipitated on the bottom of the flask. The inorganic salt was removed by filtration when the mixture cooled down to room temperature. Next, 8 mL of trimethylamine (0.1 mol, 40 % solution in water by wt) were added, and the reaction mixture was stirred at room temperature for 24 h and then at 60 °C for another 24 h. After removing most of the DMF by evaporation, the reaction residue was washed with $CHCl_3$ twice to remove traces of DMF. A white solid product was produced and the yield was 83.7%. Scheme 2.1 summarizes the procedure used this synthesis.

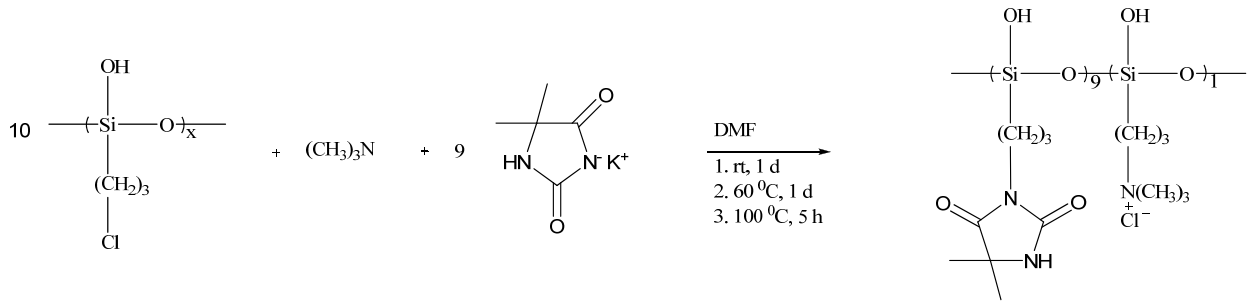


Scheme 2.1. Preparation of PHQS by a two-step process.

Previously PHQS was synthesized by a different two-step reaction and a one-step reaction in our group.[70] The two-step reaction is illustrated in Scheme 2.2. PCPS was reacted with trimethylamine first and then with the hydantoin salt. The molar ratio of the hydantoin salt and trimethylamine was 9:1. The one-step reaction is summarized in Scheme 2.3, where all ingredients (PCPS, hydantoin salt, and trimethylamine) were mixed at the same time in a molar ratio of 10: 9: 1. PHQS (1:1) and PHQS (5:1) were also synthesized by following the same procedure.



Scheme 2.2. Preparation of PHQS by a second two-step process



Scheme 2.3. Preparation of PHQS by a one-step process

^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.50 (20H), 1.20-1.24 (54H), 1.50-1.67 (20H), 3.01-3.30 (29H).

2.3. Preparation of biocidal paint coatings

A former group member has demonstrated that the solubility of PHQS in water is 15%.[69] 0.15 g of PHQS and 1 g of distilled water were mixed together. When the solid was dissolved, 14 g of commercial latex paint was added into the beaker with stirring for 2 min. The resulting formulation was immediately spread onto the surfaces of 10 pieces of wood and air dried for 16 h at ambient temperature. Paint samples with higher PHQS concentrations were also prepared, which were more viscous and hard to coat onto wood due to the strong bonding between

PHQS molecules and latex paint. The paint sample with 2% of PHQS could be coated onto wood perfectly with a reasonable chlorine loading (test documented in the next section). Therefore, all of the paint samples that were used in the following steps contained 2% of PHQS (9:1).

2.4. Chlorination and analytical titration

The chlorination was done by a modified procedure: 200 mL of 10% aqueous solution of commercial bleach (pH=7) was sprayed onto the wood chips which were coated with 1% of PHQS, repeating the spray procedure 4 times. The total chlorination process lasted for 1 h. After rinsing thoroughly with tap water and then distilled water, the painted wood chips were air dried at ambient temperature overnight and then at 45°C for 1 h. Rechlorination also followed the same procedure.

Determination of the chlorine loading was carried out by a modified iodometric/ thiosulfate titration method. In a 125 mL conical flask, 0.25 g of potassium iodide was dissolved in 10 mL of 0.1 N acetic acid and 90 mL of absolute ethanol. Each piece of painted wood chip was cut into small pieces and added to the flask. Standardized 0.00375 M sodium thiosulfate solution was slowly added to the flask until reaching the endpoint (from yellow to colorless), and the solution remained colorless for 1 min. The amount of sodium thiosulfate solution consumed was recorded. The same procedure was usually repeated 3 times, and the average titration volume was used for calculations.

The surface concentration of the bound oxidative chlorine was calculated according to the following equation:

$$Cl^{+}\left(atom/cm^{2}\right)=\frac{6.02\times10^{23}\times N\times V}{2\times A} \quad (2.1)$$

Where N is the normality (equiv/L), and V is the volume (L) of the sodium thiosulfate solution, and A is the surface area in cm^2 of the wood chips.

2.5. Stability test of the halogenated N-halamine paint

Stability of the chlorinated paint coating was measured using UV light (Type A, 315-400 nm) and laboratory light. The paint samples were kept in a vacuum desiccator while being exposed to the lab light. After a specific time of exposure to UVA irradiation or lab light, the paint samples were titrated immediately, and the same procedure was applied to rechlorinated samples. Paint samples containing PHQS (1:1, 5:1) were also prepared. However, the chlorine loading were not as high as that containing PHQS(9:1) (data is not shown).

Table 2.1. The stability and rechargeability of chlopaint samples under lab light

Time (week)	Cl^+ atom/cm^2	
	Under lab light	Rechlorination
0	1.71×10^{17}	
1	1.68×10^{17}	1.58×10^{17}
2	1.65×10^{17}	1.61×10^{17}
4	1.60×10^{17}	1.67×10^{17}
6	1.51×10^{17}	1.51×10^{17}
8	1.45×10^{16}	1.53×10^{16}
10	1.41×10^{16}	1.69×10^{16}
12	1.37×10^{17}	1.66×10^{17}

Table 2.2. The stability and rechargeability of paint samples under UVA light

Time (h)	Cl^+ atom/cm^2	
	Under UVA light	Rechlorination
0	1.68×10^{17}	
0.25	1.66×10^{17}	1.68×10^{17}
0.5	1.52×10^{17}	1.71×10^{17}
1	1.44×10^{17}	1.63×10^{17}
2	1.33×10^{17}	1.57×10^{17}
4	1.20×10^{16}	1.65×10^{16}
6	1.05×10^{16}	1.73×10^{16}
8	0.98×10^{17}	1.71×10^{17}

According to Table 2.1, this new biocidal paint coating showed excellent stability under lab light, retaining 80% of its chlorine after 12 weeks. The rechlorination result showed that the paint coating could be easily recharged to the original chlorine level.

As shown by the data in Table 2.2, the N-Cl bonds of this paint coating were relatively stable, with 58% active chlorine remaining after exposure to UVA light for 8 h. After rechlorination, all of the oxidative chlorine was regained. Some of the data even showed levels a little higher than the initial readings, possibly because the paint coating was more porous and hydantoin moieties in the inner coating were adding chlorine atoms.

2.6. Biocidal efficacy test

Both chlorinated and unchlorinated original latex paint samples and N-halamine paint samples were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895). A "sandwich test" was employed to evaluate the efficacies of the samples. For a typical test, 25 μL of the bacterial suspension was placed in the center of a sample (2.54 cm × 2.54 cm) in a sterile Petri dish and covered with a second identical size of sample. After contact times of 30, 60, and 120 min, the samples were placed in sterile conical centrifuge tubes containing 5.0 mL of 0.02 N sodium thiosulfate (sterile) to quench any oxidative chlorine and stirred for 2 min. The samples were then removed, and the quenched solution was diluted with 100 mM phosphate buffer, pH 7. Then 25μL diluted solution was placed on a Trypticase soy agar plate. The plates were incubated at 37^{o}C for 24 h, and then the bacterial colonies were recorded for further analysis.

The biocidal test on wood samples showed unexpected results (data not shown). The chlorinated latex paint with PHQS did not show an improved biocidal effect compared to controls not containing the polymer. There might be two reasons for the test results. The first reason is that the latex paint had some ingredients that could kill bacteria. The second one, which might be the main reason, is that the wood chips could hold the bacteria inside the pores. Thus most of the bacteria could remain in the wood chips after the quenching step. In order to carry out the biocidal efficacy of this antimicrobial paint, other paint coating carriers needed to be considered. Transparency sheet is one of the best choices (coating and chlorination procedures were the same). The biocidal test procedure was the same as above. The samples involved in the following test were coated on PET film transparency. The

chlorinated antimicrobial paint sample used in this biocidal test had 1.16×10^{17} atom/cm^2 Cl^+.

Table 2.3. Biocidal paint efficacy against the microorganism: *E. coli O157:H7*

Sample	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
Unchlorinated latex paint sample	0	9.20×10^4	0
	30	2.08×10^4	0.01
	60	1.74×10^4	0.08
	120	1.68×10^4	0.09
Chlorinated latex paint sample	0	9.20×10^4	0
	30	2.14×104	0.01
	60	1.94×10^4	0.08
	120	1.14×10^4	0.13
Unchlorinated PHQS N-halamine latex paint sample	0	9.20×10^4	0
	30	2.88×10^4	0.02
	60	2.41×10^4	0.07
	120	2.28×10^4	0.10
Chlorinated PHQS N-halamine latex paint sample	0	9.20×10^4	0
	30	1.41×10^4	0.81
	60	0	4.97

	120	0	4.97

Table 2.4. Biocidal paint efficacy against the microorganism: *S. aureus*

Sample	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
Unchlorinated latex paint sample	0	9.60×10^4	0
	15	3.15×10^4	0.48
	30	2.88×10^4	0.52
	60	3.08×10^4	0.49
Chlorinated latex paint sample	0	9.60×10^4	0
	15	3.22×10^4	0.63
	30	2.55×10^4	0.58
	60	1.94×10^4	0.69
Unchlorinated PHQS N-halamine latex paint sample	0	9.60×10^4	0
	15	6.10×10^4	0.19
	30	4.75×10^4	0.36
	60	2.15×10^4	0.54
Chlorinated PHQS N-halamine latex paint sample	0	9.60×10^4	0
	15	1.18×10^2	2.49
	30	0	4.98

	60	0	4.98

The biocidal results shown in Tables 2.3 and 2.4 suggest that this new paint containing PHQS has a strong antimicrobial activity after the chlorination. It could inactivate both Gram-positive and Gram-negative bacteria within 30 min of contact.

No significant reduction of bacteria population was detected in the three control samples. This indicates that the original latex paint has little antimicrobial capability. Thus, only the latex paint containing PHQS N-halamine can have the antibacterial function. From the biocidal result using transparency material, we conclude that the failure of previous biocidal testing using wood chips as paint carrier probably resulted from the wood chips absorbing most of the bacteria solution.

2.7. Conclusion

A water-soluble latex paint containing a N-halamine copolymer has been prepared. This new paint shows a strong biocidal efficacy against *S. aureus* and *E. coli* after being coated onto transparency films. The bacteria, *S. aureus* and *E. coli*, could be inactivated within 60 min of contact with log reductions of 4.98 and 4.97, respectively. The antimicrobial activity was long-lasting and could be easily monitored by a potassium iodine/starch test, and if the activity was accidentally lost, it could be regenerated by another halogenation treatment. Based on the advantages of this new paint, it is highly promising as a commercially viable antimicrobial material.

Chapter 3

Novel N-halamines incorporated latex paint (II)

3.1. Materials and instruments

All chemicals used in this research were purchased from Fisher Scientific (Fair Lawn, NJ) or Aldrich Chemicals (Milwaukee, WI) and used as they were received with no further purification unless otherwise noted. Commercial latex paint, Olympic Premium Interior Latex Paint, was bought from local Lowe's store. Chlorination was achieved with household bleach (Clorox ®, Inc., Oakland, CA). NMR spectra of the synthesized compounds were recorded by a Bruker 400 MHz spectrometer. An Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA, Type A, 315-400 nm) was used for UVA degradation studies.

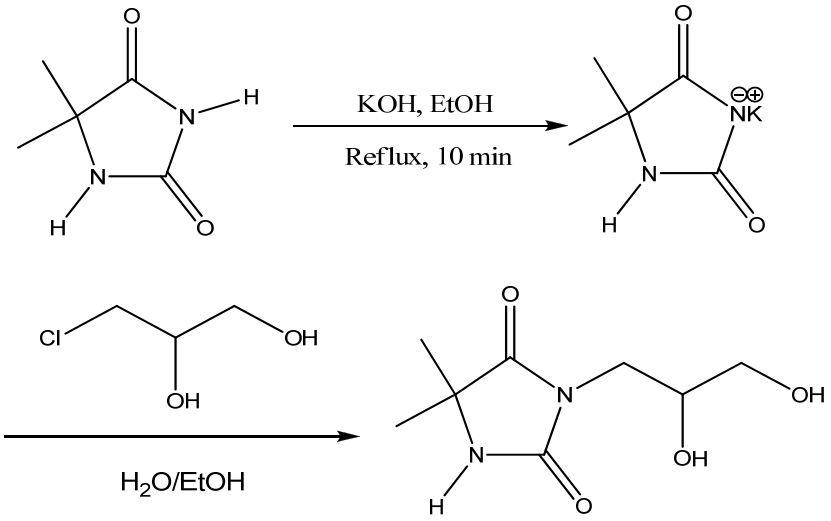
3.2.1. Properties and applications of hydantoin diol (HD) and hydantoin epoxide (HE)

3-(2,3-Dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione (hereinafter hydantoin diol, or HD) and 3-glycidyl-5,5-dimethylhydantoin (hereinafter hydantoin epoxide, or HE) are good N-halamine precursors. Both of them are water-soluble and can be coated onto cotton fabrics. The only difference is that HE can be directly coated onto cotton fabrics while HD

needs the assistance of the cross-linking agent 1,2,3,4-butanetetracarboxylic acid (BTCA). Former group members have already demonstrated that these two chlorine coated cellulose samples have excellent antimicrobial properties in inactivating *Staphylococcus aureus* and *Escherichia coli*. The coated samples endured water washing and were stable upon UVA irradiation. My objective was to incorporate the novel N-halamine compounds into commercial available latex paint to create antimicrobial materials. The latex paint, HD and HE are all water soluble. Thus, HD (or HE) and latex paint can be mixed together to develop an antimicrobial paint.

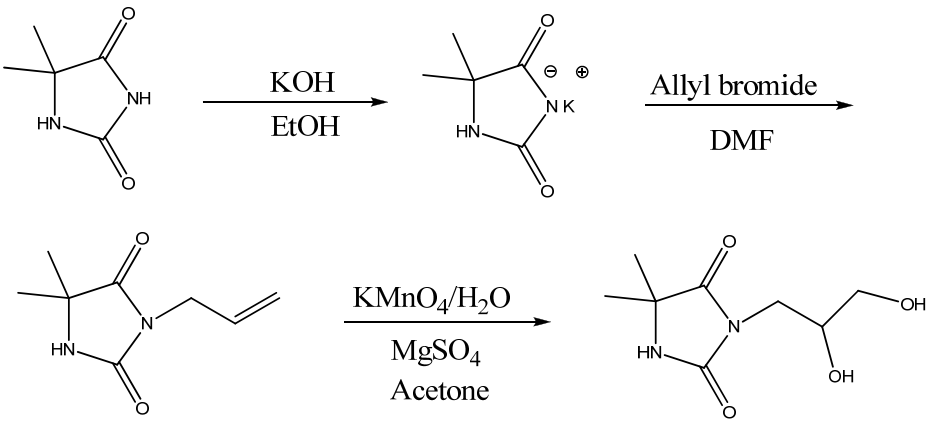
3.2.2. Synthesis of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione (HD)[69]

Scheme 3.1 shows the synthesis procedure. 6.6 g (0.05 mol) of 5,5-dimethylhydantoin and 3.3g (0.05 mol) of potassium hydroxide were mixed in 150 mL of ethanol and refluxed for 10 min. Then, an equimolar of 3-chloropropanediol in 50 mL of water was added and stirred for 16 h at ambient temperature. After the reaction was complete, ethanol and water were removed, and 50 mL of acetone were added to the flask. Potassium chloride was produced as a side product that was removed by filtration. Transparent, viscous oil was obtained after the acetone solvent was evaporated. The experimental yield was 91.3%. The desired product was stored in sealed vials for subsequent experiments.



Scheme 3.1. Synthesis of HD through procedure I

An alternative method was also applied for the synthesis of HD (Scheme 3.2).[69] 0.01 mol $KMnO_4$ was dissolved in 40 mL of distilled water, and the $KMnO_4$ solution was then added dropwise into 40 mL of acetone in a 250 mL flask containing 0.01 mol 3-allyl-5,5-dimethylimidazolidine-2,4-dione and 0.02 mol $MgSO_4$. The whole mixture was continuously stirred for 5-10 min, and filtered thereafter. Acetone and water were removed under reduced pressure. $MgSO_4$ was removed by adding acetone and then filtering. After removing acetone by evaporation, a yellowish sticky product was formed.



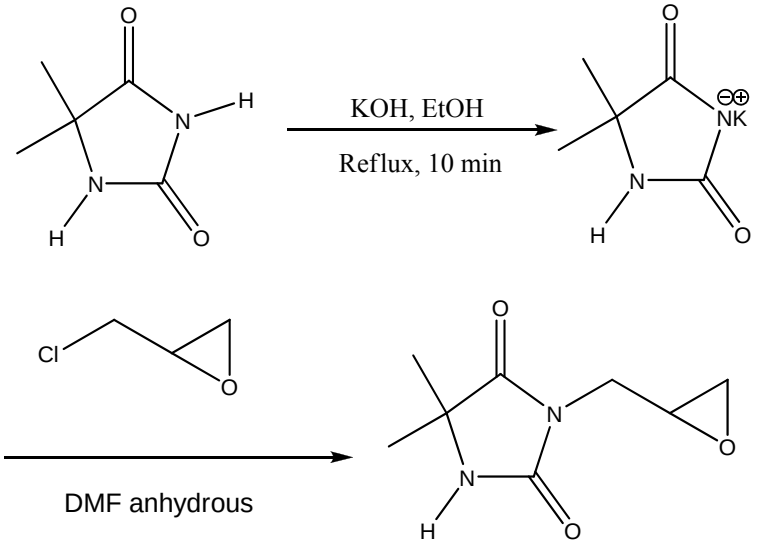
Scheme 3.2. Synthesis of HD through procedure II

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (s, 6H), 3.29 (t, 4H), 3.70 (s, 1H); ^{13}C NMR (DMSO-d_6)

δ 25.1, 41.9, 58.1, 64.6, 68.7, 156.2, 178.1.

3.2.3. Synthesis of 3-glycidyl-5,5-dimethylhydantoin (HE)

6.6 g (0.05 mol) of 5,5-dimethylhydantoin and 3.24 g of KOH (0.05 mol) were mixed in 100 mL of EtOH and refluxed for 10 min. A solid potassium salt was obtained after removal of solvent under reduced pressure and dried under vacuum at 60°C for 1 day. Then, the dry salt and 4.76 g of epichlorohydrin (0.05 mol) (in 100 mL of DMF) were mixed and stirred for 10 h at ambient temperature with nitrogen gas protection. After the solution cooled, solid KCl was removed by filtration, and then DMF was removed at reduced pressure to yield a white solid.[70] ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 6H), 3 (t, 4H), 3.70 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.77, 23.51, 31.23, 61.78, 157.3, 171.2.



Scheme 3.3. Synthesis of HE

3.2.4. Incorporation of HD and HE into latex paint

0.5 g of HD (or HE) was dissolved in 2 g of distilled water. Then 7.5 g of commercial latex paint were added into the beaker with constant stirring for 2 min. The resulting formulation was

immediately spread onto the surfaces of transparency slides. The paint coated transparency slides were dried in air at ambient temperature for 16 h.

According to the instruction of the latex paint, 5.77 g of paint was required for each transparency slide. For the purpose of comparison, 5 g, 7 g, 9 g, 10 g and 15 g of paint were used, respectively.

Table 3.1. Latex paint coating test

The amount of paint that is used for coating (g)	5	7	9	10	15
The amount of liquid paint that adheres onto each slide (g)	2.93	3.40	3.37	3.42	3.39

Data in Table 3.1 showed that the maximum paint that bonded onto the transparency was around 3.4 g. This amount of coating can be achieved by using 7 g of latex paint.

In order to get the best latex paint coating, the dry weight of the latex paint also needed to be measured. As the data shown in Table 3.2, the dry weight of the latex paint was around 50% of the applied paint weight.

Table 3.2. Latex paint ingredient test

Sample	Paint weight (g)	The percentage of

	Original	After drying	the dry ingredient
Control	3.40	1.68	49.6%
Paint-HD (5%)	3.36	1.76	52.4%
Paint-HE (5%)	3.14	1.62	51.6%

3.2.5. Chlorination and analytical titration

The painted transparency slides were cut into pieces of 25 cm^2. The small slices were immersed into a 10% solution of commercial bleach at pH 7 for 1 h. After rinsing thoroughly with tap water and distilled water, the painted slides were air dried at ambient temperature overnight and then at 45°C for 1 h.

The bound oxidative chlorine was quantified using the modified iodometric/thiosulfate titration method described in Chapter 2. The surface concentration of the bound oxidative chlorine was calculated according to equation 2.1:

$$Cl^{+}\left(atom/cm^{2}\right)=\frac{6.02\times10^{23}\times N\times V}{2\times A} \qquad (2.1)$$

where N is the normality (equiv/L), V is the volume (L) of the sodium thiosulfate solution, and A is the slide surface area in cm^2.

3.2.6. Chlorine loading test with different N-halamine concentration

Using the same procedures for coating biocidal paint film onto transparency slides, different amounts of HD (or HE) were used to test the maximum chlorine loading on the latex paint. Four latex painted slides were prepared with four HD concentrations: 7% (0.28 g diol), 11% (0.47 g), 17% (0.78 g) and 21% (1 g). Slides were chlorinated in 10% aqueous solution of

commercial bleach (pH 7) for 1 h. The biocidal painted films were washed first with the tap water and then distilled water. They were dried at ambient temperature overnight. Any free chlorine remaining on the painted films was removed by heating in a oven (45 ^{o}C) for 1 h. All titrations were usually conducted in triplicate, and the average value was used for calculations.

Table 3.3. Chlorine loading test with different HD concentrations

HD concentration	0	7%	11%	17%	21%
Cl^{+} (atom/cm^2)	1.6×10^{16}	1.9×10^{16}	2.1×10^{16}	2.1×10^{16}	2.5×10^{16}

Table 3.4. Chlorine loading test with different HE concentrations

HE concentration	0	11%	21%
Cl^{+} (atom/cm^2)	1.6×10^{16}	2.1×10^{16}	2.2×10^{16}

The titration results clearly shows that the chlorine loading on the HD and HE painted films was not increasing as dramatically as the concentrations of the hydantoin derivatives were increasing, and the paint incorporated with HE did not show higher chlorine loading. This indicates that both of HD and HE did not interact with latex paint, and most of the materials were washed off before the titration. Even though the value of chlorine atom/cm^2 of the treated paint was a little higher than that of the pure latex paint, the expected bacterial killing power was not good.

3.3.1 Latex paint incorporated with Poly TTDD siloxane polymer

TTDD (7,7,9,9-tetramethyl-1,3,8-triazaspiro[4,5]-decane-2,4-dione), a new N-halamine

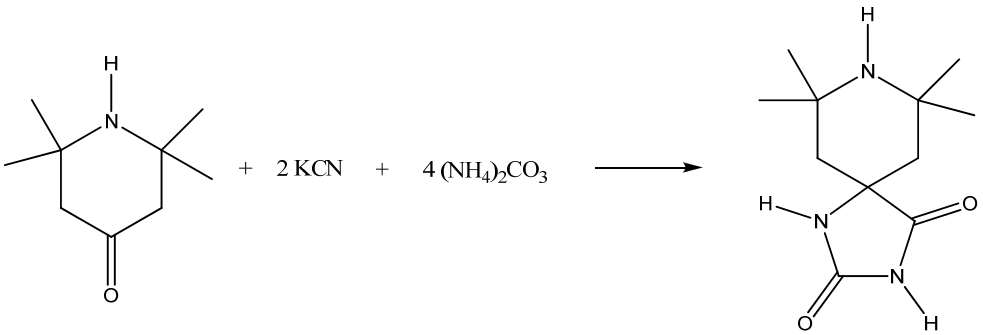
precursor, has been shown to be promising in antimicrobial applications. It can be easily bonded to the surfaces of silica gel particles and cellulose with the assistance of the siloxane tethering group. After being treated with hypochlorite solution, these materials showed excellent biocidal properties. Much work has been done to incorporate TTDD derivatives with paint to form antimicrobial paint.

3.3.2. Synthesis of 7,7,9,9-tetramethyl-1,3,8-triazaspiro[4,5]-decane-2,4-dione (TTDD)[72]

In a 500 mL flask, 15.6g (0.1 mol) of 2,2,4,4-tetramethyl-4-piperidone, 13.5g (0.2mol) of potassium cyanide and 43.2g (0.4mol) of ammonium carbonate were mixed in 200 mL of water/ethanol (1:1) solution. The molar ratio of these three reactants is 1:2:4. These chemicals were stirred at ambient temperature for 3 days. After the reaction finished, a white solid was obtained using filtration. 500 mL of hot water were used to purify the product. The final product was dried in the oven, and the yield was 94.8%.

An alternative method to synthesize the desired compound is as follows: in a Parr high-pressure reactor, 15.6g (0.1 mol) of 2,2,4,4-tetramethyl-4-piperidone, 13.5g (0.2 mol) of potassium cyanide, 43.2g of ammonium carbonate, 100 mL of ethanol and 100 mL of water were mixed. The reaction proceeded on for 12h with stir mixing at 85 ^{o}C. After the reactor cooled to room temperature, the product was filtered. Then 500 mL of hot water was slowly added into the funnel to remove all the impurities. The final product was dried in the vacuum oven for 1 day at 50℃. The yield was 97.1 %.[69]

^{1}H NMR TTDDK^{+} (D_2O, 400 MHz) δ 1.27 (s, 6H), 1.31 (s, 6H), 2.10 (s, 4H); ^{13}C NMR (D_2O, 400 MHz) δ 27.23, 33.78, 42.58, 48.65, 64.32, 157.90, 176.49.



Scheme 3.4. Synthesis of TTDD

3.3.3. Synthesis of poly [3-(3-triethoxysilylpropyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro-[4.5]-decane-2,4-dione] (PTS)[72]

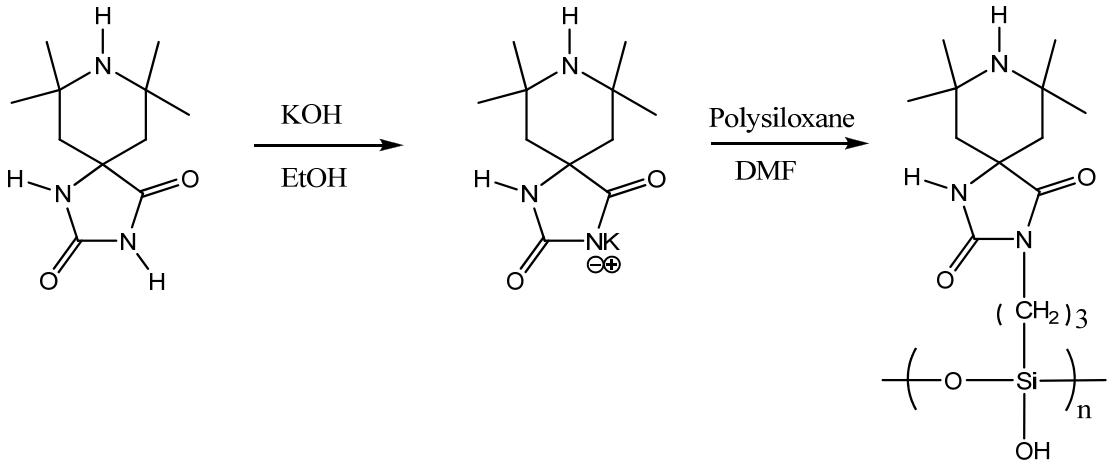
The potassium salt of TTDD was prepared by the following steps: 11.25 g (0.05mol) of TTDD and 3.24 g (0.05 mol) of 88.7% potassium hydroxide were added into 100 mL of ethanol and refluxed at 95 ^{o}C for 10 min. After removal of the ethanol and water produced during salt formation, the TTDD salt was dried in the vacuum oven at 45 °C for 1 day. This product was obtained in near quantitative yield.

The siloxane polymer was obtained through the following procedure: 12.67 g (0.05 mol) of 95% 3-chloropropyltriethoxysilane and 100 mL of 0.1M HCl were mixed together in a 250 mL flask and stirred at ambient temperature for 2h. After removal of the water and newly formed ethanol, the crude product was washed with distilled water and dried in the vacuum oven at ambient temperature for 1 day.

Then 100 mL of anhydrous DMF was mixed with the potassium salt and stirred at 60 ^{o}C until maximum dissolution occurred. At this stage, the siloxane polymer was added into the mixture. The temperature was increased to 100^{o}C and continuously stirred for 8 h. After cooling down to ambient temperature, the inorganic salt, which was potassium chloride, was

removed by filtration. Most of the DMF could be removed by reduced pressure evaporation. A known amount of chloroform was used to wash the reaction residue from trace amounts of DMF yeilding a white solid product (the yield was 82.7%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.54 (t, 2H), 1.08 (s, 6H), 1.14 (s, 6H), 1.40-1.59 (t, 6H), 3.69 (s, 4H), 8.40 (s, 1H).



Scheme 3.5. Synthesis of PTS

3.3.4. Solubility test of PTS

The solubility of this new polymer is a major concern since quantitative analysis are needed. In order to perfectly mix the unhalogenated PTS into latex paint, this polymer must be water soluble. A great deal of effort has been devoted to enhance the water solubility of PTS. Increasing the acidity seems to be the easiest and best way to achieve the goal. Solubility measurement of PTS was summarized in Table 3.5.

Table 3.5. Solubility test of PTS in water under different pH condition

pH value	Solubility of PTS

7	None
5	<1%
3	5%
1	8%

* The value of the solubility in Table 3.6 was not very accurate.

Data in Table 3.6 showed that when the pH value decreased, the solubility increased because of protonation of the amine N. According to our experience, a 5% solubility is acceptable for further chlorination analysis.

3.3.5. Preparation of biocidal latex paint film incorporating PTS.

0.1 g of PTS and 2 g of distilled water (adjusted to pH 3 with HCl) were mixed together with constant stirring until all of the solid was dissolved into the water. Then, 10 g of commercial latex paint were added to the same beaker, and stirred for 2 min. The resulting formulation was immediately spread onto the surfaces of 8 pieces of wood, and the paint coated wood was dried in air at ambient temperature for 16 h.

3.3.6. Chlorination and analytical titration

The chlorination process was performed using the same procedure as for PHQS paint coatings: briefly, 200 mL of 10% aqueous solution of commercial bleach (pH 7) was sprayed onto the wood chips for 4 times. The total chlorination time lasted for 1 h. After rinsing thoroughly with tap water and then distilled water, the painted wood chips were air dried at ambient temperature overnight and then at 45°C for 1 h.

The chlorine loading was quantified using the same titration method mentioned in

Chapter 2. The titration results (data not shown) indicated that the chlorine loading was 2.07×10^{17} atom/cm^2 which was higher than any of the 1% N-halamine paints that were tested before. However, the latex paint was destroyed after the pH 3 water was added. This paint could easily come off the wood (or transparency). Thus, latex paint with PTS could not be used as biocidal material even though it has a higher chlorine loading capacity.

3.4. Conclusion

Several compounds have been successfully coated onto cotton and rendered very good for biocidal activity. We synthesized these compounds and mixed them with commercial latex paint to determine if they exhibit biocidal activity. To our surprise, none of them could kill bacteria. The reason for failing to kill bacteria is that these compounds could not be incorporated into latex paint. According to our experience, we suggest that the candidate compound for incorporation into commercial latex paint should exhibit the following features: (1) be water-soluble under mild conditions (e.g. room temperature, pH=7). The N-halamine copolymer discussed in Chapter 2 fulfills this stipulation; (2) the N-halamine must bond to the polymer particles in the latex paint.

Chapter 4

Chlorine activity comparison between 3-chloro-1,5,5-trimethylhydantoin and 1-chloro-3,5,5-trimethylhydantoin

4.1. Materials

All chemicals applied in the research were purchased from Fisher Scientific (Fair Lawn, NJ) or Aldrich Chemicals (Milwaukee, WI) and used as they were received without further purification unless otherwise noted. The Trypticase soy agar was from Difco Laboratories, Detroit, MI. Chlorination was accomplished with household bleach (Clorox, Inc., Oakland, CA). Bacteria *S. aureus* ATCC 6538 and *E. coli* O157:H7 ATCC 43895 (American Type Culture Collection, Rockville, MD) were selected for the compound efficacy testing.

4.2. Instruments

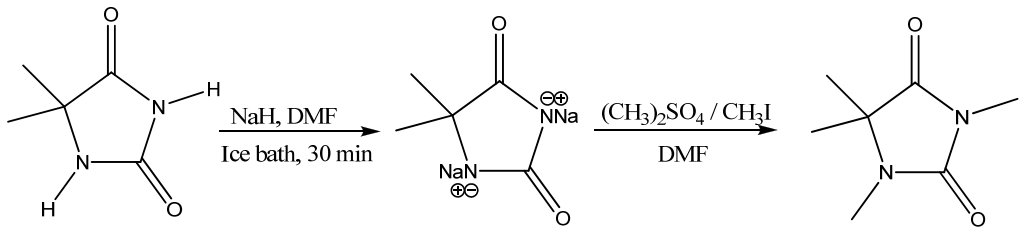
NMR spectra of the synthesized compounds were recorded by a Bruker 400 MHz spectrometer. FTIR spectra were obtained with a Nicolet 6700 FT-IR spectrometer. An

Accelerated Weathering Tester (The Q-panel Company, Cleveland, OH, USA, Type A, 315-400 nm) was used for UVA degradation studies.

4.3.1. Synthesis of 1,3,5,5-tetramethylhydantoin

1,3,5,5-Tetramethylhydantoin was designed as a prototypical compound to test the reaction conditions and identify the position in the molecule of the two newly added methyl groups by means of ^{1}HNMR. First, 3.3 g (0.025 mol) of 5,5-dimethylhydantoin were dissolved in 100 mL of anhydrous DMF. The solution was kept on ice for 5 min under nitrogen gas protection. Secondly, 2.0 g (0.05 mol) of sodium hydride (60% in mineral oil) were slowly added into the solution. After most of the hydrogen had evolved from the solution, mixing was continued for an extra 30 min until the solution became clear. Then, 3.2 g (0.025 mol) of dimethyl sulfate (or 0.05 mol potassium iodide) were added into the solution and mixed at room temperature for 5 h. After the complete removal of the DMF, 100 mL of acetone were used to precipitate the inorganic salt. When acetone was vaporized, 100 mL of ethyl acetate were added to extract the product from the mixture. Finally, a pure white solid appeared after vaporizing the solvent. The overall yield was 90.7% (85.9% for CH_3I).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 6H), 2.81 (s, 3H), 2.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 21.99, 24.31, 60.19, 62.05, 155.42, 178.41.



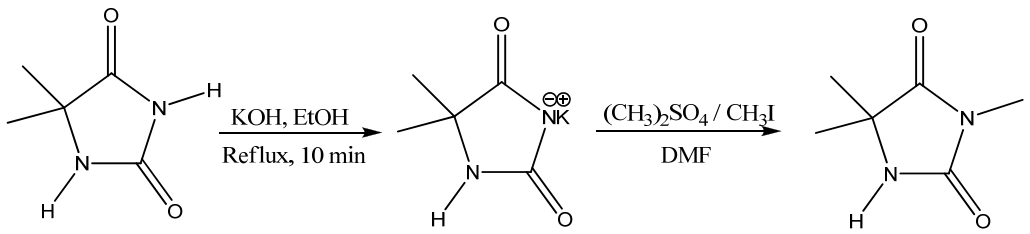
Scheme 4.1. Preparation of 1,3,5,5-tetramehtylhydantoin by a two-step process.

4.3.2. Synthesis of 3,5,5-trimethylhydantoin

5,5-Dimethylhydantoin (13.21 g, 0.1 mol) and potassium hydroxide (6.28 g, 0.1 mol) were mixed in a flask with 100 mL ethanol, and stirred at 95 °C for 10 min. After cooling to room temperature, the solvent was removed through evaporation. A pure white hydantoin salt was left in the flask and dried in the vacuum oven at 45 °C for one day. At this stage, 100 mL of anhydrous DMF were added to dissolve the hydantoin salt, and 6.4 g (0.05 mol) dimethyl sulfate thereafter. The reaction proceeded for 2 h at 95 °C under nitrogen gas protection. After the mixture cooled to room temperature, the inorganic salt, potassium sulfate, was eliminated by filtration, and DMF was removed by reduced pressure evaporation. The synthesized compound was dried in the oven for 1 day and weighed 12.87 g. The yield was 91.8% for the white powder.

An alternative synthesis of 3,5,5-Trimethylhydantoin is stated below: the starting step is the same as the above. The difference is the chemical used after the first drying step. 5,5-dimethylhydantoin (13.21 g) and potassium hydroxide (6.28 g) were mixed in a flask with 100 mL ethanol, and stirred at 95 °C for 10 min. After drying, the hydantoin salt was dissolved in 100 mL of anhydrous DMF, followed by the addition of 14.2 g (0.1 mol) of iodomethane. The reaction continued for 5 h with constant stirring at room temperature. The newly formed potassium salt was removed by filtration, and the final product was obtained by evaporating all of the DMF. The white solid weighed 12.61 g, and the calculated yield was 90.1%.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 6H), 3.04 (s, 3H), 5.82 (s, 1H); ^{13}C NMR($CDCl_3$, 400 MHz) δ 21.42, 24.80, 59.20, 154.07, 175.43.



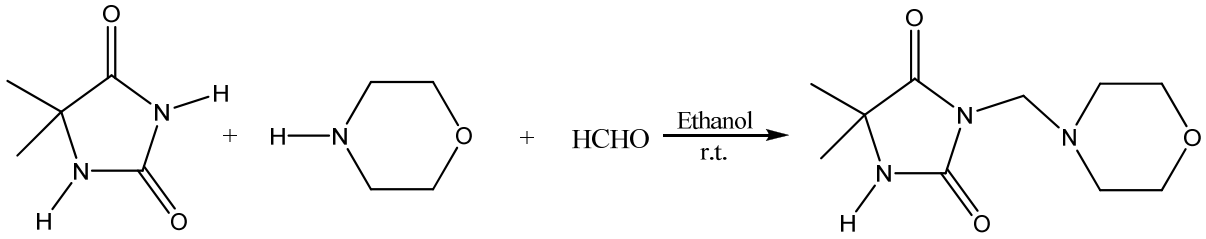
Scheme 4.2. Preparation of 3,5,5-trimehtylhydantoin by a two-step process.

4.3.3. Synthesis of 1,5,5-trimethylhydatoin

Synthesis of 1,5,5-Trimethylhydatoin involved three steps.

a. Synthesis of 3-morpholinomethyl-5,5-dimethylhydantoin

13.21 g (0.1 mol) of 5,5-Dimethylhydantoin, 8.8 g (0.1 mol) of morpholine and 8 g (0.1 mol) of 40% (wt) formaldehyde were mixed in a flask containing 100 mL of ethanol. The reaction took place at ambient temperature with constant stirring. The white product precipitate started to appear after 2 h of mixing, and the reaction was complete in 5 h. After the removal of ethanol, the white product was dried at ambient temperature for 2 days. The yield was 98.1%. This white solid easily decomposes at temperatures higher than 50°C. Therefore, the whole procedure was carried out at room temperature.

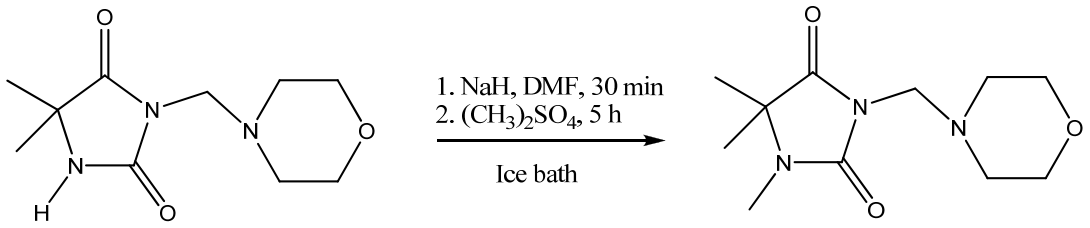


Scheme 4.3. Preparation of 1,5,5-trimethylhydantoin, first step.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.30 (s, 6H), 2.44 (t, 4H), 3.52 (t, 4H), 4.22 (s, 2H), 8.32 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 25.36, 58.23, 59.72, 66.47, 156.32, 179.01.

b. Synthesis of 3-morpholinomethyl-1,5,5-trimethylhydantoin

11.35 g (0.05 mol) of 3-morpholinomethyl-5,5-dimethylhydantoin were dissolved in 100 mL of anhydrous DMF. The solution was kept in ice for 5 min under nitrogen gas protection. Then, 2 g (0.05 mol) of sodium hydride (60% in mineral oil) were slowly added into the solution. After most of the hydrogen gas had evolved from the solution, it was stirred for 30 more min until becoming clear. At this point, 3.2 g (0.025 mol) of dimethyl sulfate were added and continuously stirred for 5 h at room temperature. After removal of DMF, 100 mL of acetone were added to precipitate the inorganic salt. The unwanted acetone was evaporated, and 100 mL of ethyl acetate were used to extract the product from the mixture. A pure white solid was obtained after vaporizing the solvent. The yield was 90.7%. For drying of product, an equimolar quantity of iodomethane was used, instead of dimethyl sulfate. The yield of the product was 85.7%.



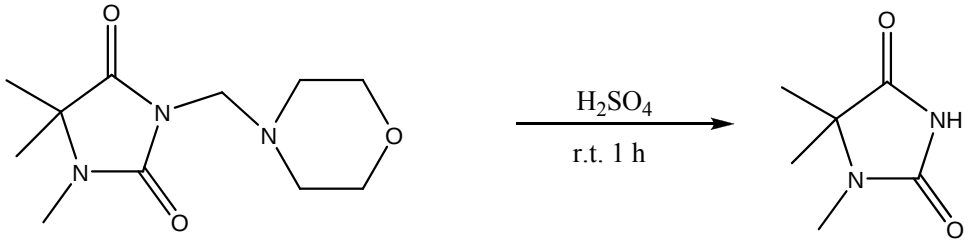
Scheme 4.4. Preparation of 1,5,5-trimethylhydantoin, second step.

^{1}H NMR (DMSO- d_6, 400 MHz) δ 1.27 (s, 6H), 2.41 (t, 4H), 2.71 (s, 3H), 3.43 (t, 4H), 4.14 (s, 2H)

c. Synthesis of 1,5,5-trimethylhydantoin

Synthesis of 1,5,5-trimethylhydantoin was conducted first by mixing 6 g (0.025 mol) of 3-morpholinomethyl-1,5,5-trimethylhydantoin with 50 g of 10% sulfuric acid and stirring at 60 °C for 1 h. Then 50 mL of ethyl acetate were used to extract the organic product. The white

powder was obtained after removing the aqueous layer and evaporating the solvent. The product yield was 59.5%.



Scheme 4.5. Preparation of 1,5,5-trimethylhydantoin, third step.

^{1}H NMR (DMSO- d_6, 400 MHz) δ 1.27 (s, 6H), 2.73 (s, 3H), 10.76 (s, 1H); ^{13}C NMR (DMSO- d_6, 400 MHz) δ 21.99, 24.31, 62.05, 155.42, 178.41.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 6H), 2.88 (s, 3H), 8.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 21.96, 24.24, 62.63, 155.07, 177.41

4.4. Chlorination of 3,5,5-trimethylhydantoin and 1,5,5-trimethylhydantoin.

The chlorination can be achieved by two independent methods:

Protocol 1.

0.05 mol of 3,5,5-trimethylhydantoin (or 1,5,5-trimethylhydantoin) was mixed with 150 mL of 50% chlorox solution (pH=7) and stirred for 1 h. Most of the chlorinated product separated and remained on the top of the solution. Chloroform was used to extract the product. After removing the top aqueous layer, dried magnesium sulfate (1.5 g) was added into the organic solution to remove the residual water. The chloroform was then transferred to a new vial and brought to dryness by evaporation. The white product was dried in the oven at 45 °C for 1 h and stored in the refrigerator for future use.

Protocol 2.

3,5,5-Trimethylhydantoin (or 1,5,5-trimethylhydantoin) and trichloroisocyanuric acid (TCCA, molar ratio, 1:3) were dissolved in acetone with vigorous stirring for 30 min at room temperature. When the acetone was evaporated, hexane was added to the mixture. The insoluble solids were filtered off, and the residue was dried through evaporation. The chlorinated hydantoin derivative was obtained as a white powder.

Yields of chlorination of 3,5,5-trimethylhydantoin from protocols 1 and 2 was 85.3% and 80.2%, respectively. The ^{1}HNMR parameters for the functional groups are: ^{1}HNMR ($CDCl_3$, 400 MHz) δ 1.48 (s, 6H), 3.10 (s, 3H); ^{13}CNMR ($CDCl_3$, 400 MHz) δ 22.20, 25.67, 66.00, 154.77, 174.39. The IR spectra of 3,5,5-trimethylhydantoin and 1-chloro-3,5,5-trimethyl-hydantoin are shown in Figure 4.1.

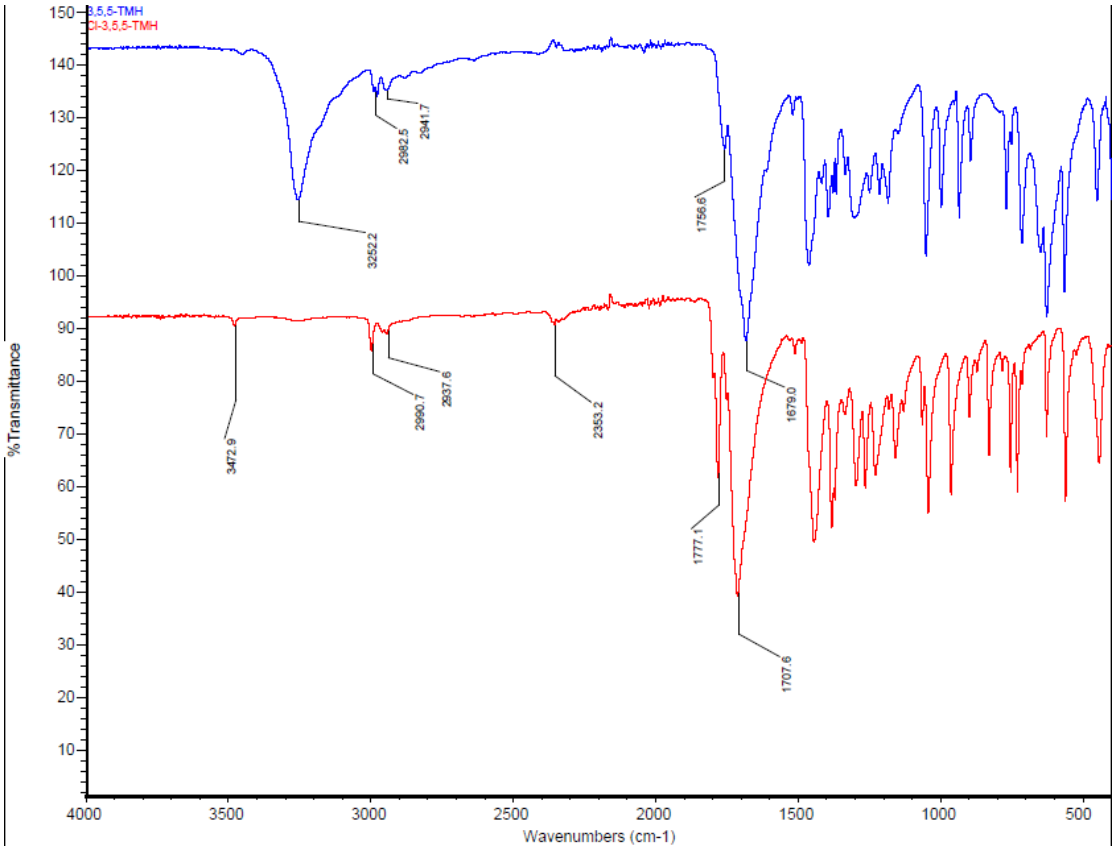


Figure 4.1. IR spectra of 3,5,5-trimethylhydantoin (upper line) and

1-chloro-3,5,5-trimethylhydantoin (lower line).

For the chlorination of 1,5,5-trimethylhydantoin, the yield was 80.2% from protocol 1 and 72.9% from protocol 2. The ^{1}HNMR parameters for the functional groups are: ^{1}HNMR ($CDCl_3$, 400Hz) δ 1.47 (s, 6H), 2.95 (s, 3H); ^{13}CNMR ($CDCl_3$, 400Hz) δ 22.33, 25.31, 62.80, 151.16, 172.24. The IR spectra of 1,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin are shown in Figure 4.2.

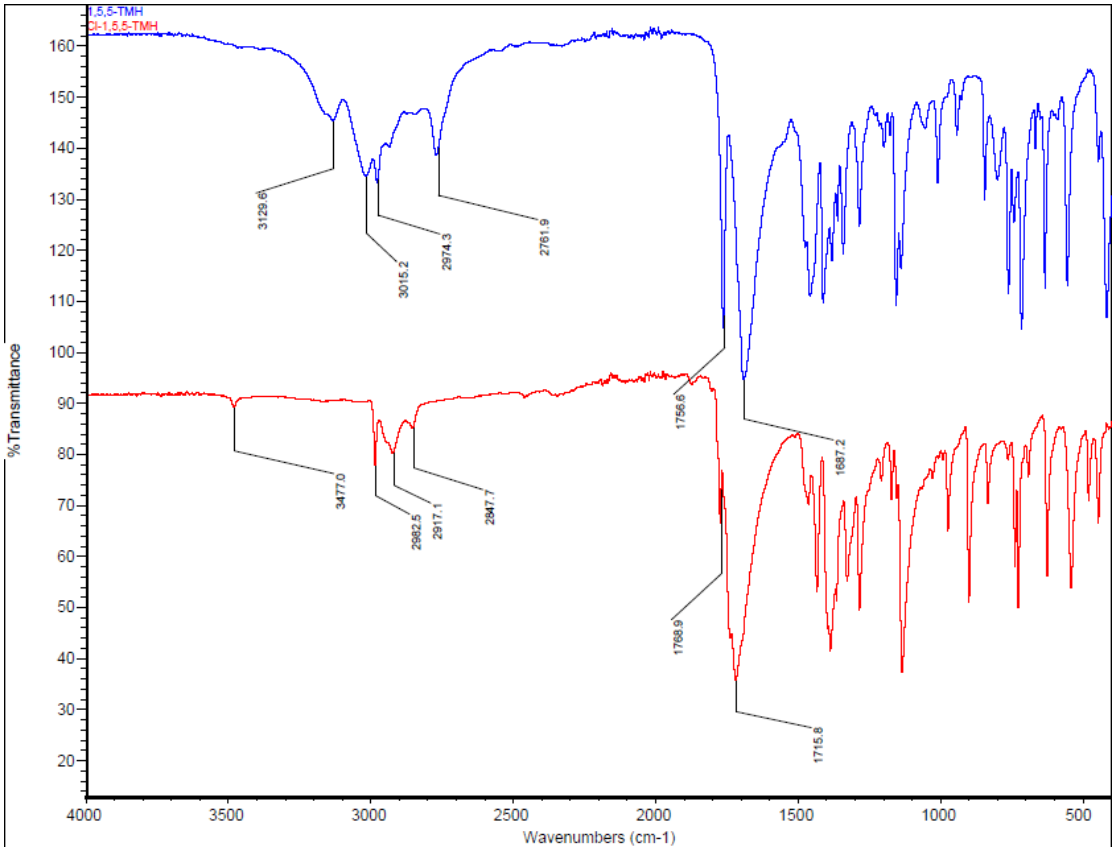


Figure 4.2. IR spectra of 1,5,5-trimethylhydantoin (upper line) and 3-chloro-1,5,5-trimethylhydantoin (lower line).

4.5. Solubility test of chlorinated hydantoin derivatives

Organic and water solubility tests were performed on the newly synthesized compounds of 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl- hydantoin. Both compounds

showed excellent solubility in organic solvents such as chloroform. The water solubility of these two compounds was conducted to test their antimicrobial ability. Some of the N-Cl bonds may break when dissolved in water. If this is the case, there will be two different forms of chlorine in the solution, free chlorine and bonded chlorine. The normal titration method used in this lab can only test the total amount of chlorine. An assumption is applied for the water solubility test: there will be no free chlorine in the solution. Then, we could use the normal titration method to determine the amount of the chlorine in the two chlorinated hydantoin derivatives.

The experiment was performed using the following procedure: 0.3 g of 1-chloro-3,5,5-trimethylhydantoin and distilled water (10 g, 15 g or 20 g) were mixed with continuous stirring for 20 min. There were insoluble compounds staying on the bottom of the vial, and they were removed by filtration. By removing the insoluble compounds, the maximum solubility has been achieved. For each condition, the titration was done 4 times, and the average value was used for the evaluation. The solubility test of the 3-chloro-1,5,5-trimethylhydantoin followed the same procedure as that of 1-chloro-3,5,5-trimethylhydantoin.

The normal iodometric/thiosulfate titration method was employed for determining the solubility of the two chlorinated hydantoin compounds. For a certain amount of chlorinated hydantoin compound to be tested, 0.25 g potassium iodide, 50 mL of distilled water, 10 drops of 4 N acetic acid and 10 drops of 0.5% starch solution were added for the titration. The bound chlorine was calculated by the following equation:

$$S = \frac{0.0375 \times V \times 176.45}{2 \times W} \times 1000 \qquad (4.1)$$

where S is the solubility (g/L) of the compound, V is the volume (L) of the original sodium thiosulfate solution, and W is the amount (g) of chlorinated compound. The results are listed in the Table 4.1.

Table 4.1. The solubility of chlorinated compounds in water

Compound	Solubility (g/L)			
	0.3 g / 10 g	0.3 g / 15 g	0.3 g / 20 g	Average value
1-Chloro-3,5,5-trimethylhydantoin	9.62	9.71	9.67	9.66
3-Chloro-1,5,5-trimethylhydantoin	1.73	1.70	1.71	1.71

0.3 g / 10 g, 0.3 g / 15 g and 0.3 g / 20 g means 0.3 g of compound and 10, 15, or 20 g of distilled water were used during the experiments.

To our surprise, the solubility of 1-chloro-3,5,5-trimethylhydantoin was almost 6 times higher than that of 3-chloro-1,5,5-trimethylhydantoin (9.66/1.71 ≈ 6). Theoretically, 3-chloro-1,5,5-trimethylhydantoin should be much more polar than 1-chloro-3,5,5-trimethylhydantoin. Further energy calculation is needed to explain this unusual result.

4.6. Stability test of the aqueous solutions of 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin

From Table 4.1, we know that 1-chloro-3,5,5-trimethylhydantoin is more soluble than

3-chloro-1,5,5-trimethylhydantoin. The next question is which compound is more stable? To answer this question, we need to determine the concentration (Cl^+) of the compounds under room temperature at different lengths of storage time in darkness. The container was closed with a cover on it. The normal titration method was used to determine the amount of the remaining oxidative chlorine. The starting concentration was the same for these two compounds. Samples were titrated in triplicate. The average value was used for the analysis.

The equation used to calculate the concentration of Cl^+ is:

$$\text{The concentration of } Cl^+ \text{ (ppm)} = \frac{0.0375 \times V \times 35.45}{2 \times W} \times 1000 \qquad (4.2)$$

where V is the volume (L) of the original sodium thiosulfate solution, and W is the amount (g) of chlorinated compound. The collected data is summarized in Table 4.2.

Table 4.2. The stability of chlorinated compounds in water

Time (week)	The concentration of Cl^+ (ppm)	
	1-Chloro-3,5,5-trimethylhydantoin	3-Chloro-1,5,5-trimethylhydantoin
0	300	300
1	298	285
2	292	272
3	292	259
4	286	246
5	278	239
6	275	232

7	271	226
8	263	219
9	260	212
10	260	207
12	255	196
14	251	187

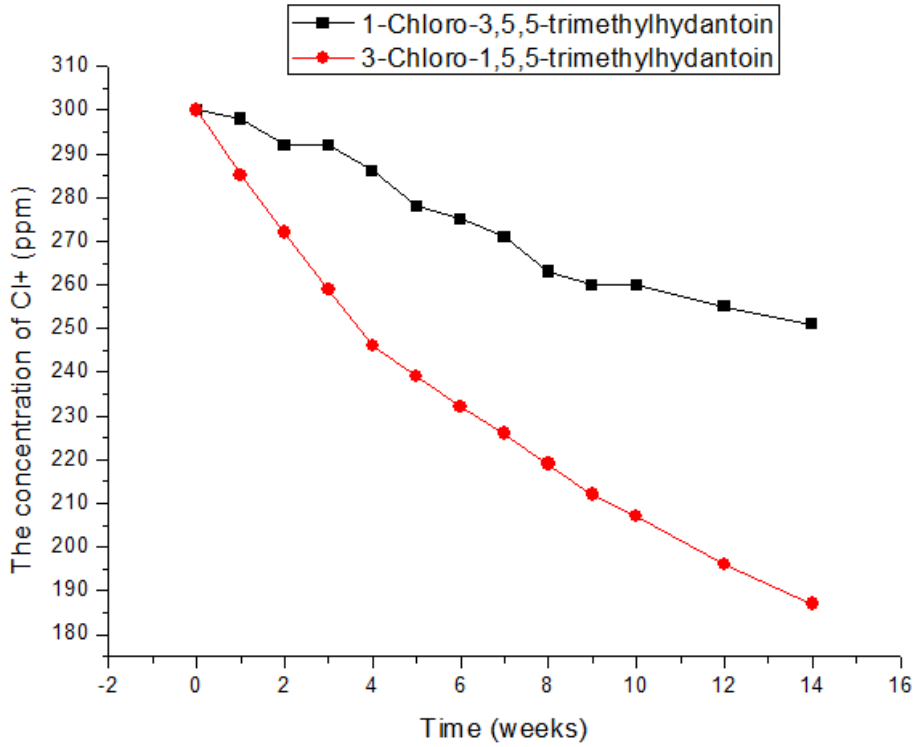


Figure 4.3. Different Cl^+ loss rates of the two chlorinated compounds in water.

Figure 4.3 shows the rates of Cl^+ loss in 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin solution. The Cl^+ loss for 1-chloro-3,5,5-trimethyl-hydantoin is much slower than that for 3-chloro-1,5,5-trimethylhydantoin. The result indicates that the chlorine on the amide position is much more tightly bonded than that on the imide position. This conclusion was further confirmed by the experiments described in the following

section.

4.7. Stability test of 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin under solid state conditions

The biocidal efficacy was related to two factors: the loading of Cl^+ and the types of N-Cl bonds. The N-Cl bond might not be stable and gradually transforms into an N-H bond at room temperature. In order to test whether this is the case, an experiment was designed by storing the two solid compounds in darkness at room temperature. These two compounds were kept in vials with covers on them. The normal iodometric/thiosulfate titration method was employed in determining the stability of the oxidative chlorine. For a certain amount of compound to be tested, 0.25 g potassium iodide, 50 mL of distilled water, 10 drops of 4 N acetic acid and 10 drops of 0.5% starch solution were added for the titration. The equation used to calculate the concentration of Cl^+ is:

$$\text{wt \% of } Cl^+ = \frac{0.0375 \times V \times 35.45}{2000 \times W} \times 100 \qquad (4.3)$$

where V is the volume (L) of the original sodium thiosulfate solution, and W is the amount (g) of chlorinated compound. The collected data is summarized in Table 4.3.

Table 4.3. The stability of chlorinated compounds in the solid state

Time (week)	Cl^+ % remaining in the solid	
	1-chloro-3,5,5-trimethylhydantoin	3-chloro-1,5,5-trimethylhydantoin
Theoretical	20.09	20.09
0	20.01	19.99
1	19.97	19.80
2	19.97	19.68
3	19.90	19.49
4	20.03	18.90
5	19.91	18.61
6	20.00	18.19
7	19.98	17.67
8	19.98	17.00
9	20.01	16.67

10	19.88	15.91
12	19.78	15.03
14	19.65	14.09
16	19.51	13.17
18	19.39	12.09
20	19.11	11.28

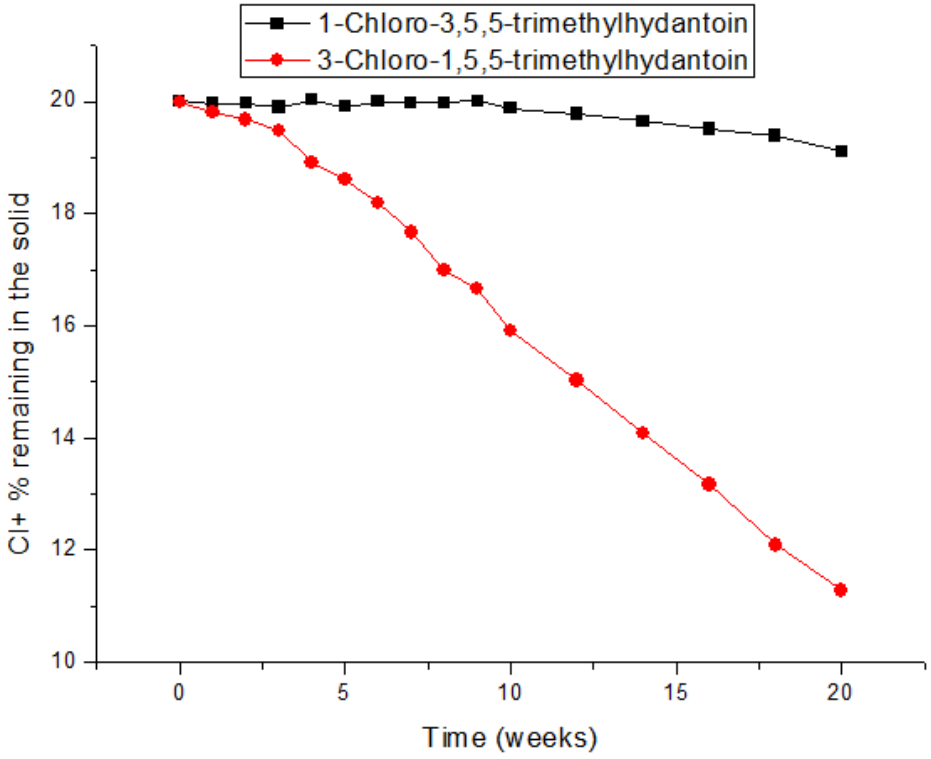


Figure 4.4. Different Cl^+ loss rates of the two chlorinated compounds (solid state) at room temperature.

From Figure 4.4 we conclude that the loss of Cl^+ is very slow in the solid form of 1-chloro-3,5,5-trimethylhydantoin (amide) during the test period of 20 weeks. Compared to 1-chloro-3,5,5-trimethylhydantoin (imide), the loss of Cl^+ in 3-chloro-1,5,5-trimethyl-

hydantoin is very fast. This data proved that the chlorine on 1-chloro- 3,5,5-trimethylhydantoin was much more stable than that on 3-chloro-1,5,5-trimethylhydantoin. 1-Chloro-3,5,5-trimethylhydantoin was extremely stable in the first 10 weeks. The chlorine started to be lost slowly after that. The chlorine loss rate for 3-chloro-1,5,5-trimethylhydantoin was relatively faster than for 1-chloro-3,5,5- trimethylhydantoin.

4.8. UVA stability test of 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin

The stability of antimicrobial functional groups of two chlorinated hydantoin derivatives was investigated under UVA irradiation. The test time spans 96 hours under UVA irradiation, which will cause the N-Cl bond to break and eventually a N-H bond will be formed. This reaction can be measured by the normal titration method (mentioned in 4.7) to determine how much chlorine is still attached to the compound. The collected data is summarized in Table 4.4.

Table 4.4. The stability of solid chlorinated compounds under UVA irradiation

Time (h)	Cl^+ % remaining in the compound	
	1-chloro-3,5,5-trimethylhydantoin	3-chloro-1,5,5-trimethylhydantoin
Theoretical	20.09	20.09
0	19.91	19.73

1	19.53	18.84
2	19.18	17.21
3	18.78	15.43
4	18.03	15.05
5	17.76	13.06
6	16.92	11.27
7	16.16	10.14
8	15.97	7.59
10	15.09	4.56
12	14.06	2.00
14	12.91	1.09
16	12.04	0.57
18	11.73	0.14
20	10.87	0
22	10.29	0
24	9.69	0
48	5.51	0
72	3.98	0
96	0.02	0

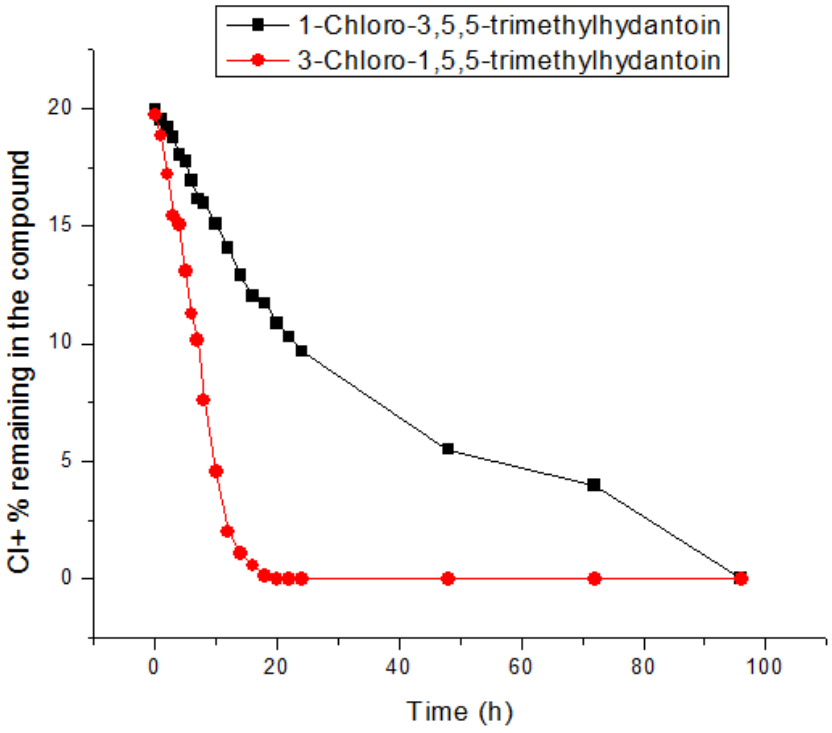


Figure 4.5. Different Cl^+ loss rates of the two chlorinated compounds under UVA irradiation.

The data shown in Figure 4.5 demonstrated that the amount of chlorine in these two compounds was reduced at different rates by the UVA light treatment. Chlorine in 1-chloro-3,5,5-trimethylhydantoin (amide) decreased more slowly than that in 3-chloro-1,5,5-trimethylhydantoin (imide). There was no oxidative chlorine left in 3-chloro-1,5,5-trimethylhydantoin after exposure to UVA light for 20 h. In contrast, a substantial amount of Cl^+ reminded in 1-chloro-3,5,5-trimethylhydantoin after 20 hour UVA irradiation. Prolonged exposure to UVA irradiation also resulted in the loss of the Cl^+ in 1-chloro-3,5,5-trimethylhydantoin (96 hours in this case).

The UVA stability difference between these two chemicals can be explained as follows: for the 5,5-dimethylhydantoin, there are two carbonyl groups adjacent to the N atom that is at the

third position (the imide N atom), and there is only one carbonyl group and an alkyl group adjacent to the N atom that is located at the first position (the amide N atom). The electron density at the amide N atom is much higher than that of the imide N atom. Thus the N-Cl bond located at the first position is much more stable than that of the N-Cl located at the third position.

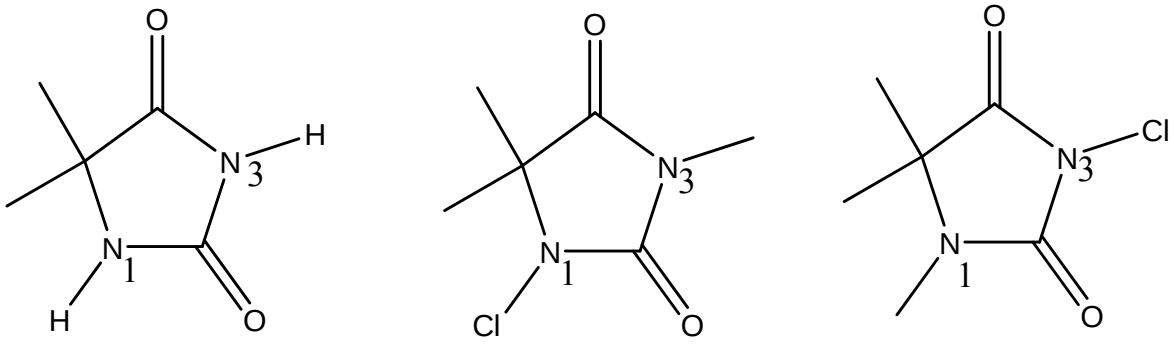


Figure 4.6. The structure of 5,5-dimethylhydantoin, 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethylhydantoin (from left to right)

We observed that these two compounds underwent different transformations under UVA light. 1-chloro-3,5,5-trimethylhydantoin gradually melts under UVA light and eventually becomes a gel-like substance after 20 h irradiation, while 3-chloro-1,5,5-trimethylhydantoin still remains a solid. After 20 h exposure to the UVA light, these two chemicals were placed in NMR tubes to test the structural change by NMR methods. The NMR spectra showed that extra signals besides the expected ones. This indicates that a certain amount of the compounds has decomposed to other derivatives during the UVA irradiation. The discussions on the structural change will be presented in Chapter 5.

4.9. Biocidal efficacy testing

The newly synthesized compounds were challenged with Gram-positive *Staphylococcus*

aureus ATCC 6538 and Gram-negative *Escherichia coli* O157:H7 ATCC 43895. A modified AATCC 100-199 method was used to analyze the antimicrobial efficacy. The unchlorinated control was included for the comparison. Concentration dependent assays were performed with a range of concentrations for the chlorinated compounds. First, the solution was injected into a sterile test tube and mixed with bacterial solution. The bacteria were in full contact with the chlorinated compound and stirred for 5 s. Samples were removed at the intervals after mixing 1.0, 5.0, and 10.0 min for bacterial population analysis. Each time, 1 mL of inoculated solution was transferred to a new tube and quenched with 1.0 mL of sterile 0.02 N sodium thiosulfate solution to remove all oxidative chlorine and vigorously stirred for 2 min. Serial dilutions of the quenched solutions were made using pH 7, 100 mM phosphate buffer. Each dilution of the culture was placed on a Trypticase soy agar plate for colony development. The plates were incubated at 37 ^{o}C for 24 h, and the colonies in the appropriate dilution were recorded for further analysis.

Table 4.5. Biocidal test of control compounds against the microorganism:

E. coli O157:H7

Sample (10 ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
3,5,5-Trimethylhydantoin	0	2.40×10^{8}	0
	1	2.27×10^{7}	1.03
	5	2.19×10^{7}	1.04
	10	2.24×10^{7}	1.03
1,5,5-Trimethylhydantoin	0	2.40×10^{8}	0
	1	1.81×10^{7}	1.12
	5	1.84×10^{7}	1.12
	10	1.36×10^{7}	1.25

Table 4.6. Biocidal test of control compounds for the microorganism: *S. aureus*

Sample (10 ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
3,5,5-Trimethylhydantoin	0	3.07×10^{8}	0
	1	1.54×10^{8}	0.29
	5	1.65×10^{8}	0.27
	10	1.30×10^{8}	0.35
1,5,5-Trimethylhydantoin	0	3.07×10^{8}	0
	1	1.76×10^{8}	0.24
	5	1.49×10^{8}	0.31
	10	1.28×10^{8}	0.38

Table 4.7. Biocidal test of 1-chloro-3,5,5-trimethylhydantoin solution against the microorganism: *E. coli O157:H7*

Sample concentration (ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
10	0	3.73×10^{8}	0
	1	2.67×10^{5}	3.15
	5	1.60×10^{2}	6.39
	10	0	8.57
5	0	3.73×10^{8}	0
	1	2.08×10^{8}	0.25
	5	8.53×10^{2}	5.64
	10	1.87×10^{2}	6.30
1	0	3.73×10^{8}	0
	1	1.92×10^{8}	0.29
	5	1.55×10^{8}	0.38
	10	8.53×10^{7}	0.64
0.5	0	3.73×10^{8}	0
	1	2.37×10^{8}	0.20
	5	1.79×10^{8}	0.32
	10	1.09×10^{8}	0.53

Table 4.8. Biocidal test of 3-chloro-1,5,5-trimethylhydantoin solution against the microorganism: *E. coli O157:H7*

Sample concentration(ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
10	0	3.73×10^{8}	0
	1	2.45×10^{5}	3.18
	5	0	8.57
	10	0	8.57
5	0	3.73×10^{8}	0
	1	2.03×10^{8}	0.27
	5	5.60×10^{2}	5.82
	10	4.80×10^{2}	5.89
1	0	3.73×10^{8}	0
	1	3.47×10^{8}	0.03
	5	2.48×10^{8}	0.18
	10	1.07×10^{8}	0.54
0.5	0	3.73×10^{8}	0
	1	3.68×10^{8}	0.01
	5	3.23×10^{8}	0.06
	10	1.65×10^{8}	0.35

Table 4.9. Biocidal test of 1-chloro-3,5,5-trimethylhydantoin solution against the microorganism: *S. aureus*

Sample concentration (ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
10	0	8.20×10^{7}	0
	1	0	7.91
	5	0	7.91
	10	0	7.91
5	0	8.20×10^{7}	0
	1	3.47×10^{7}	1.03
	5	1.12×10^{3}	4.87
	10	0	7.91
1	0	8.20×10^{7}	0
	1	4.27×10^{7}	0.28
	5	4.00×10^{7}	0.31
	10	3.20×10^{7}	0.41
0.5	0	8.20×10^{7}	0
	1	6.93×10^{7}	0.07
	5	6.40×10^{7}	0.11

	10	4.27×10^{7}	0.28

Table 4.10. Biocidal test of 3-chloro-1,5,5-trimethylhydantoin solution against the microorganism: *S. aureus*

Sample concentration (ppm)	Contact time (min)	Total bacterial concentration (CFU)	Log reduction
10	0	8.20×10^{7}	0
	1	0	7.91
	5	0	7.91
	10	0	7.91
5	0	8.20×10^{7}	0
	1	0	7.91
	5	0	7.91
	10	0	7.91
1	0	8.20×10^{7}	0
	1	1.60×10^{2}	5.71
	5	5.30×10^{1}	6.19
	10	5.30×10^{1}	6.19
0.5	0	8.20×10^{7}	0
	1	4.80×10^{7}	0.23
	5	3.47×10^{7}	0.37

	10	2.03×10^{7}	0.60

The control experiments were designed to determine if the unchlorinated compounds have any biocidal effect. The data are listed in Tables 4.5 and 4.6. As expected, the unchlorinated compounds 3,5,5-trimethylhydnatoin and 1,5,5-trimethylhydantoin have no biocidal capability. Tables 4.7 and 4.8 shows the biocidal effect against bacteria *E. coli O157:H7* using 1-chloro-3,5,5-trimethylhydantoin or 3-chloro-1,5,5-trimethylhydantoin treated samples. The data indicate that compound 1-Chloro-3,5,5-trimethylhydantoin could kill all of the bacteria within 10 min at 10 ppm concentration, while 3-chloro-1,5,5-trimethylhydantoin (10 ppm) could inactivate all the bacteria within 5 min. Therefore, at the concentration level of 10 ppm, compound 3-chloro-1,5,5-trimethylhydantoin shows the faster transfer of Cl^{+} to the bacterial cells. A significant bacteria reduction was also observed for both treatments at the concentration of 5 ppm. The lower concentration (less than 1 ppm) had no effect in killing bacteria. Tables 4.9 and 4.10 provide the biocidal effect against bacteria *S. aureus* using 1-chloro-3,5,5-trimethylhydantoin or 3-chloro-1,5,5-trimethylhydantoin treated samples. At higher concentration (10 ppm), both compounds could kill all of the bacteria within 1 min. At the concentration of 5 ppm, 3-chloro-1,5,5-trimethylhydantoin could kill all of the bacteria within 1 min, while 1-chloro-3,5,5-trimethylhydantoin required 10 min. At the lower concentration (1 ppm), 3-chloro-1,5,5-trimethylhydantoin performed the dramatic killing effect in 5 min, while 1-chloro-3,5,5-trimethylhydantoin did not kill the bacteria. The Gram-negative and Gram-positive biocidal data clearly demonstrates that the imide N bond

chlorine is much more active than that of amide N.

4.10. Conclusion

Two novel hydantoin derivatives, 3,5,5-trimethylhydantoin and 1,5,5-trimethylhydantoin, have been synthesized. After being chlorinated, each compound has one N-Cl group. The three comprehensive stability tests demonstrate that the N-Cl function in 3,5,5-trimethylhydantoin is much more stable than that in 1,5,5-trimethylhydantoin. The most plausible reason is that the electron density on the imide N is lower than that on the amide N. The lower electron density resulted in the easier breakage of the N-Cl bond in 1,5,5-trimethylhydantoin. The biocidal data supports this hypothesis.

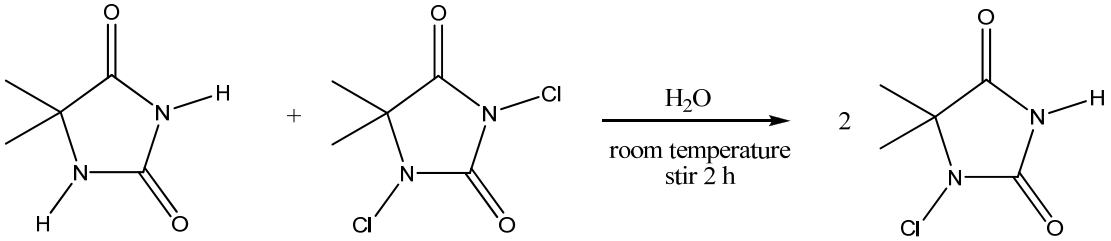
Chapter 5

Hydrogen atom transfer reaction (HATR) generated by N-centered radicals

5.1. Synthesis of 1-chloro-5,5-dimethylhydantoin

3.94 g (0.02 mol) of 1,3-dichloro-5,5-dimethylhydantoin and 2.64 g of (0.02 mol) 5,5-dimethylhydantoin were mixed in 150 mL of water. The mixture was stirred for 2 h at room temperature and then filtered. The white solid was brought to dryness at ambient temperature; the yield was 95.1%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.50 (s, 6 H), 8.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 22.0, 67.3, 154.0, 174.9

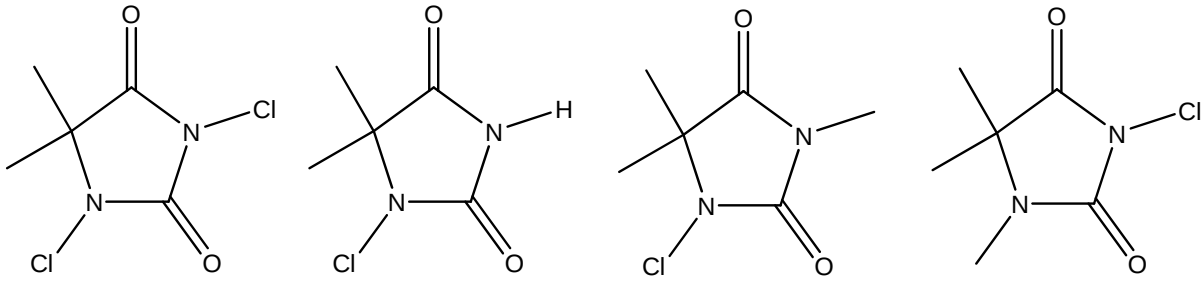


Scheme 5.1. Synthesis of 1-chloro-5,5-dimethylhydantoin

5.2. HATR test under UVA light

The compounds used in this test were 1,3-dichloro-5,5-dimethylhydantoin (**1**), 1-chloro-5,5-dimethylhydantoin (**2**), 1-chloro-3,5,5-trimethylhydantoin (**3**) and 3-chloro-1,5,5-trimethylhydantoin (**4**). Each compound was exposed to UVA light for a different time

period. The new products were characterized by NMR. Experiments were performed with solid compounds (Test **A)** or with the solution of each compound (dissolved in $CDCl_3$) (Test B).



1 **2** **3** **4**

Figure 5.1. The structure of the compounds involved in the HATR test

5.3. Chlorine determination

Titration is a good tool to analyze the amount of oxidative chlorine. The normal iodometric/thiosulfate titration method was employed in determining the stability of the oxidative chlorine. For a certain amount of chlorinated hydantoin compound to be tested through titration, 0.25 g potassium iodide, 50 mL of distilled water, 10 drops of 4 N acetic acid and 10 drops of 0.5% starch solution were added. The equation used to calculate the wt % of Cl^+ is:

$$\text{wt \% of } Cl^+ = \frac{0.0375 \times V \times 35.45}{2000 \times W} \times 100 \qquad (4.3)$$

where V is the volume (L) of the original sodium thiosulfate solution, and W is the amount (g) of compound. The results are listed in Table 5.1.

Table 5.1. The amount of chlorine loss under UVA light exposure

Time (d)	Oxidative chlorine loading (Cl^+%)			
	Compound			
	1	**2**	**3**	**4**
0	36.01	21.82	20.09	20.09
1	33.85	20.91	9.69	0
6	24.63	20.41	0	0

Data in Table 5.1 clearly shows that the stability sequence of the N-Cl bond is: **2**>**3**>**4**. According to the UVA stability test of compound **1**, the imide chlorine was lost within 15 days of UVA irradiation, while the amide chlorine could last more than 55 days of UVA exposure. The stability difference of **3** and **4** was reasoned in Chapter 4. The explanation for the rapid loss of the chlorine in **3** and **4** is that the additional alkyl group drastically decreases the stability of the N-Cl bond.

5.4. NMR data analysis

All of the NMR experiments were performed using $CDCl_3$ as a solvent. Compound **3** completed the loss reaction in 6 days, while **4** finished within 1 day. The result of test **B** was as follows:

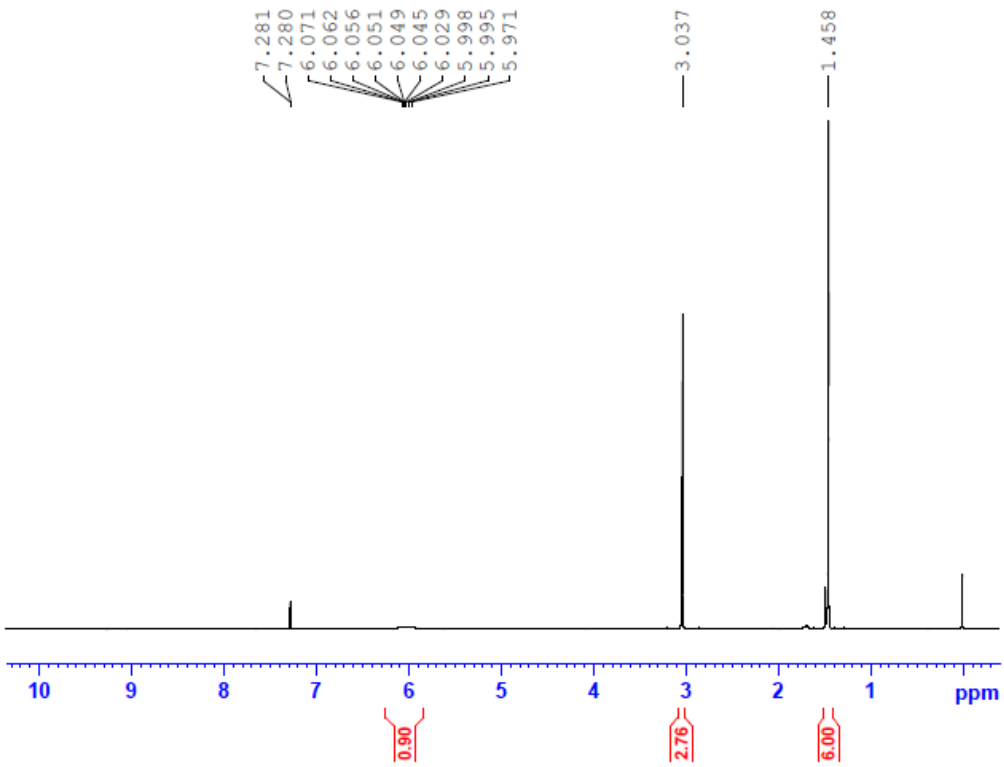


Figure 5.2. [1]HNMR spectrum of compound 3,5,5-trimethylhydantoin

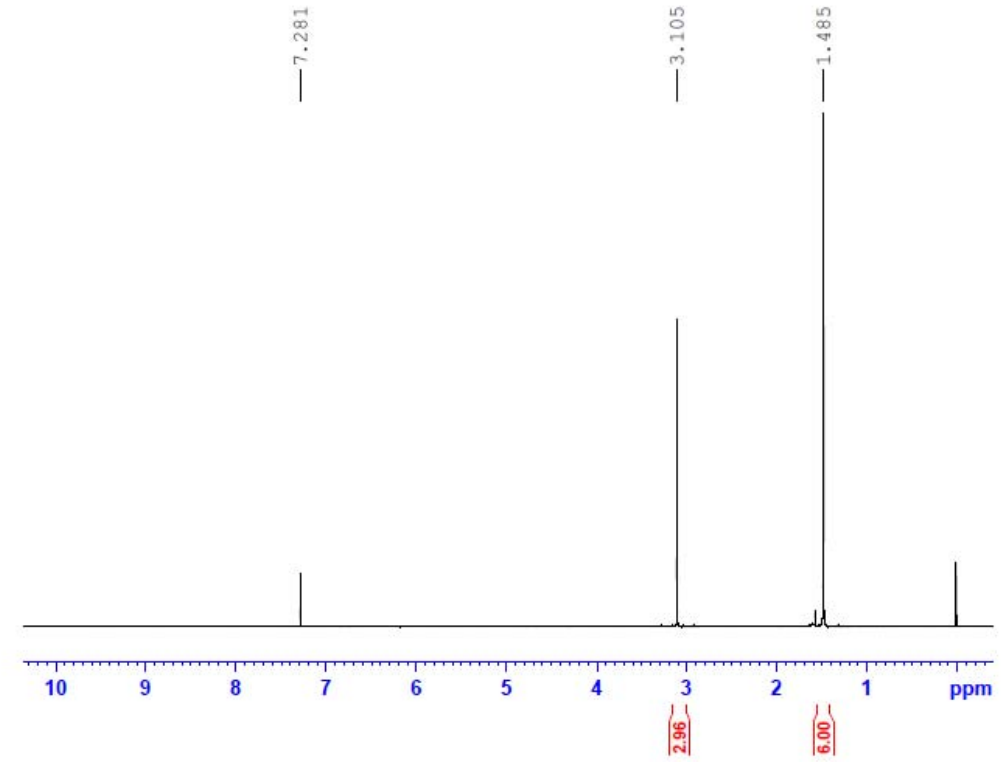


Figure 5.3. [1]HNMR spectrum of compound 3

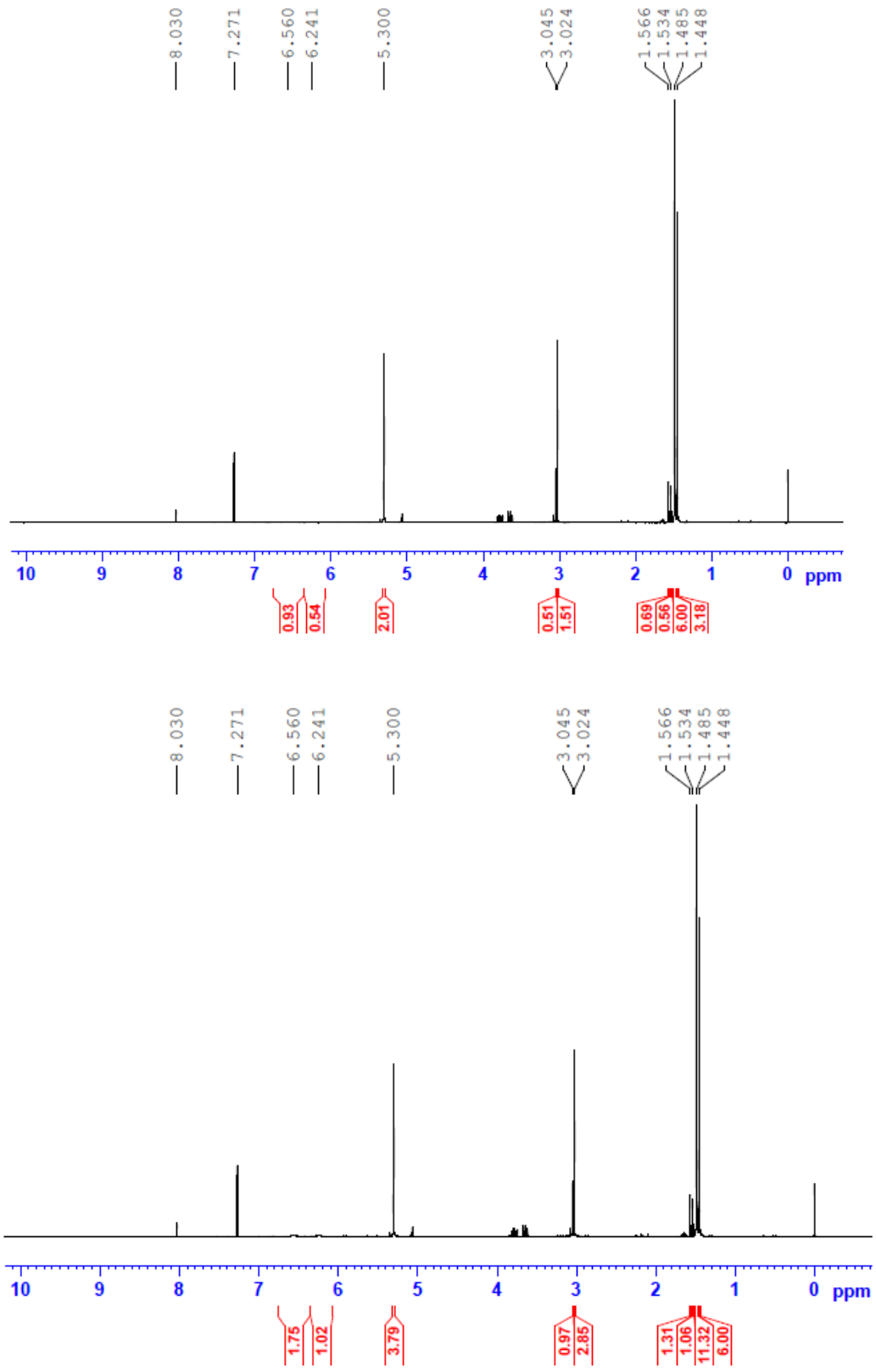


Figure 5.4. [1]HNMR spectrum of compound 3 after 6 days UVA irradiation

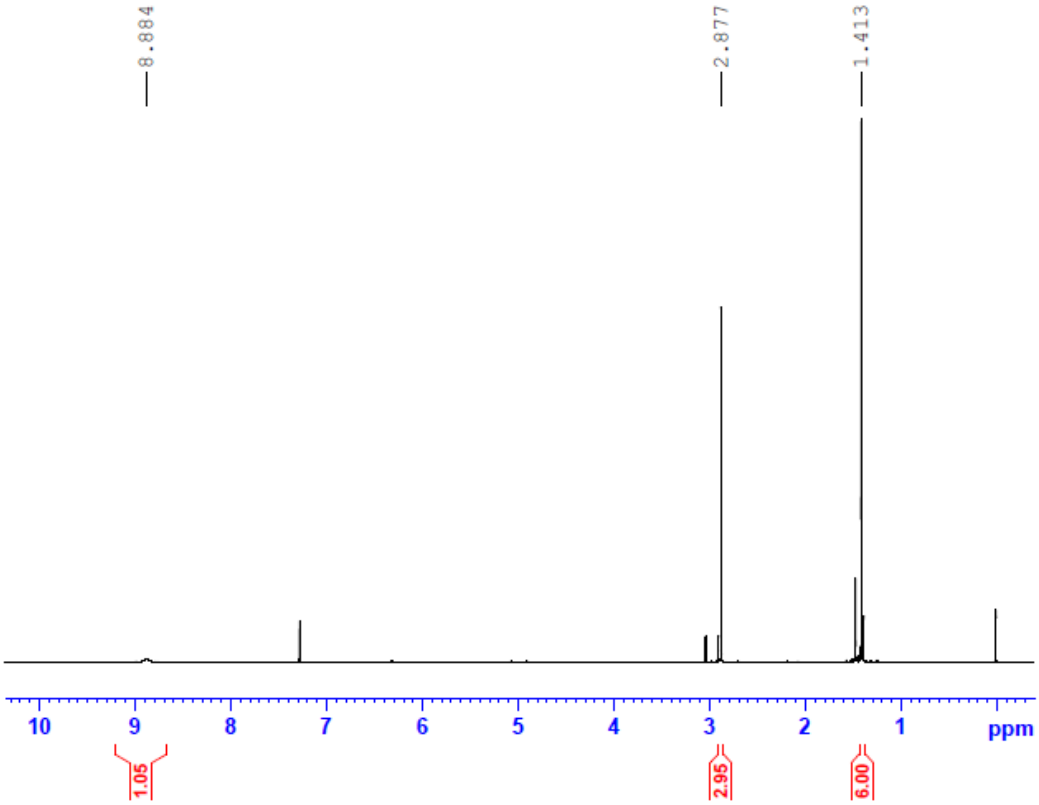


Figure 5.5. [1]HNMR spectrum of 1,5,5-trimethylhydantoin

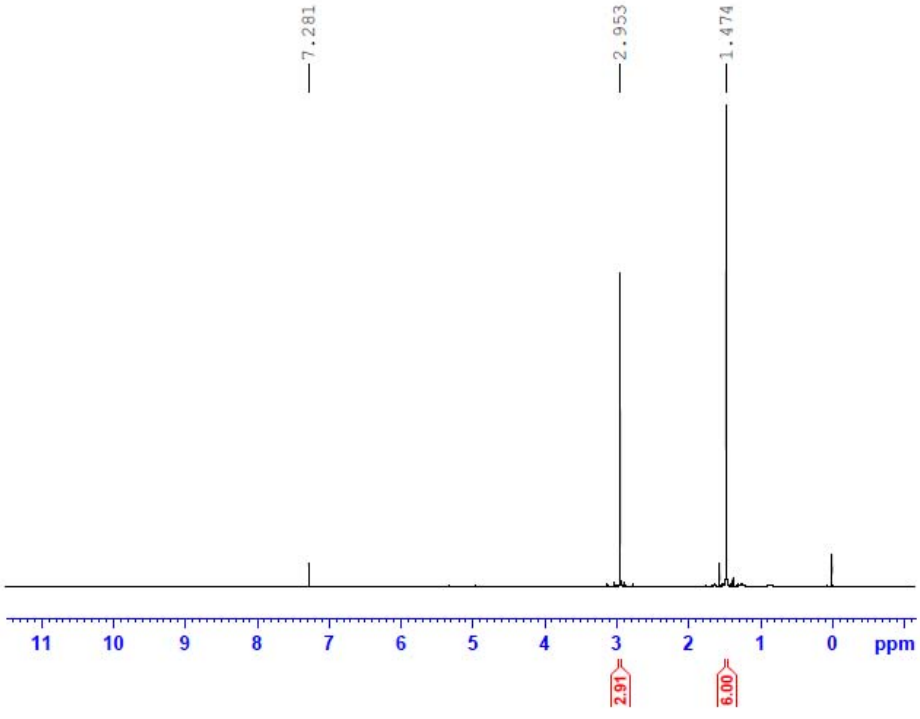


Figure 5.6. [1]HNMR spectrum of compound 4

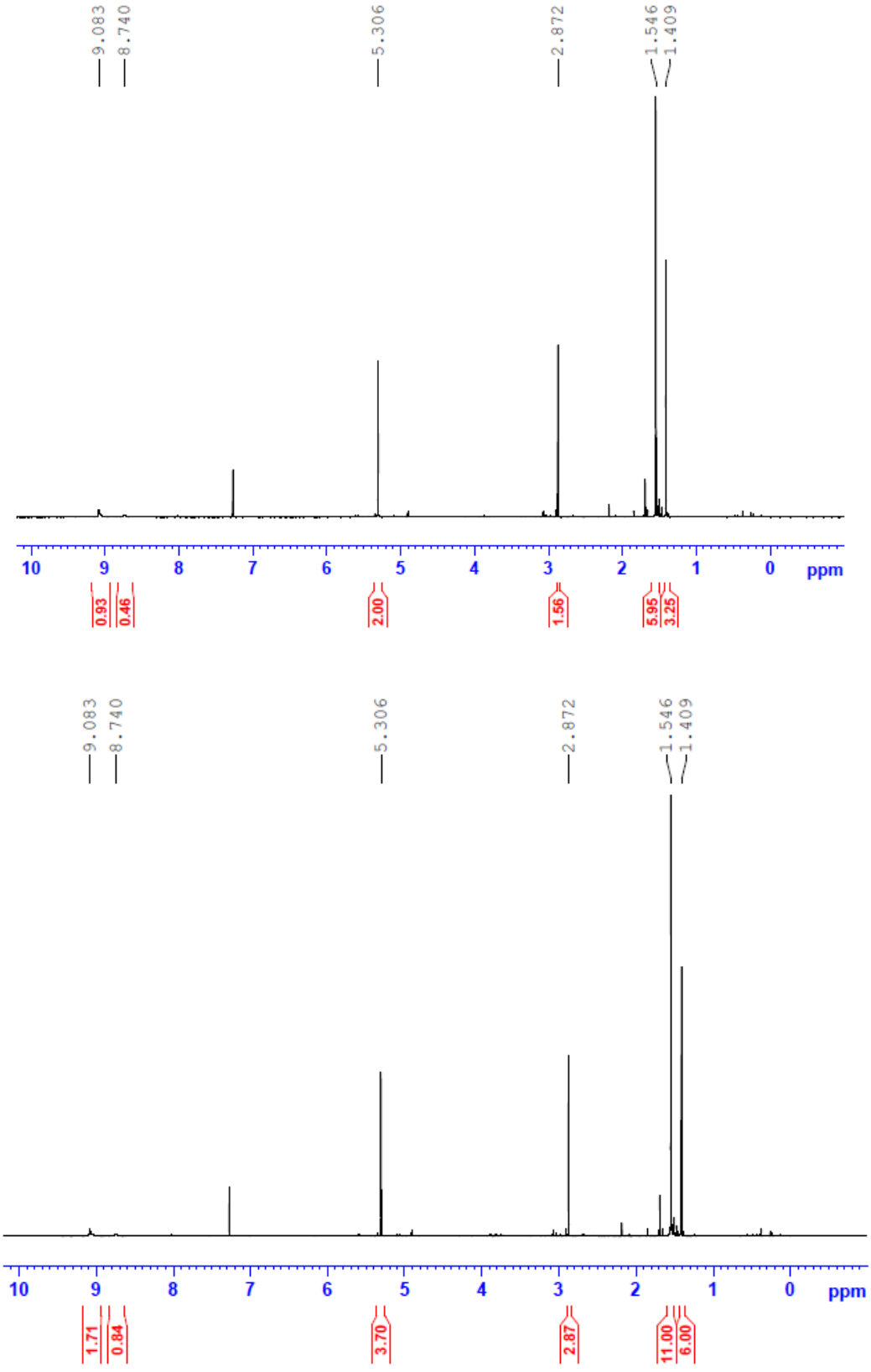
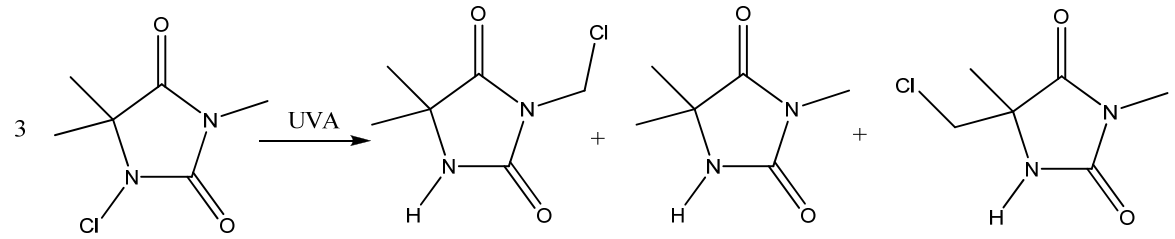


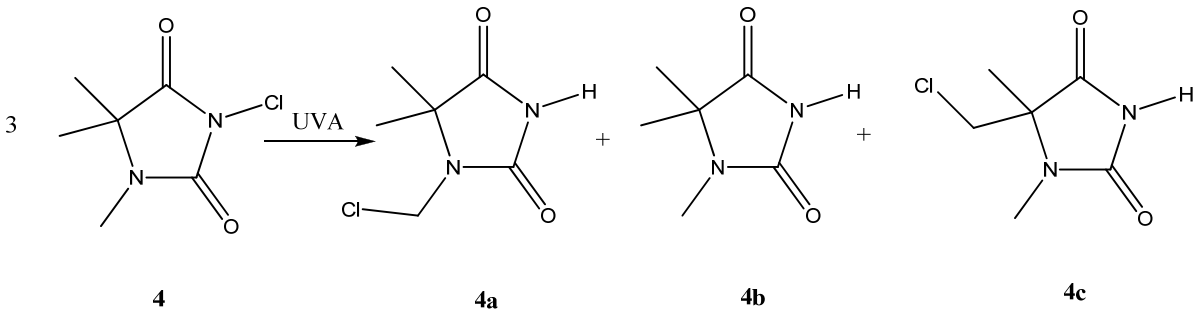
Figure 5.7. [1]HNMR spectrum of compound 4 after 1 day UVA irradiation

5.5. Experimental explanation

Theoretically, there are three possible decomposition products after UVA exposure for compound **3** and **4**. Their structures are shown below:



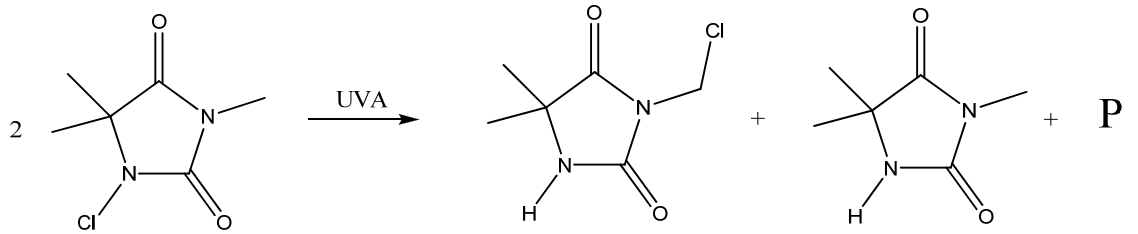
Scheme 5.2. The possible reaction products of compound **3** under UVA light



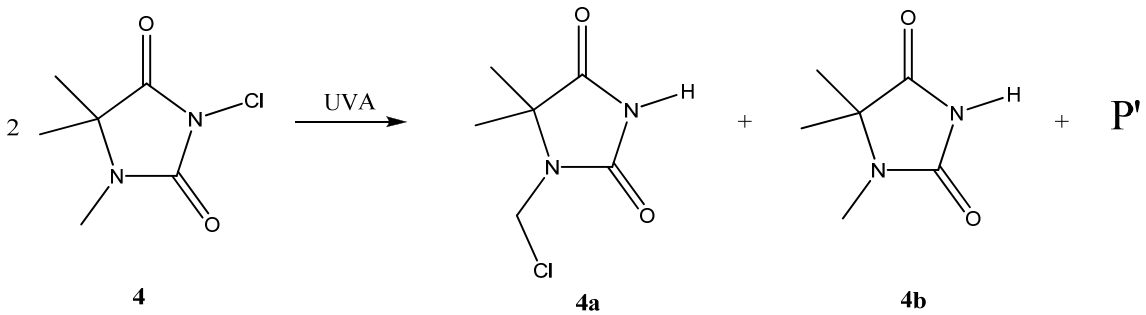
Scheme 5.3. The possible reaction products of compound **4** under UVA light

For **3**, the signals with δ 1.45 (s, 6 H), 3.02 (s, 3H), 6.24 (s, 1H) are very similar to those of 3,5,5-trimethylhydantoin (**3b**). The signals with δ 1.49 (s, 6H), 5.30 (s, 2H), 6.56 (s, 1H) indicate another compound generated during the UVA irradiation. This new compound should have two methyl groups, one methylene group and one N-H. The NMR data of the product is consistent with compound **3a**. For **4**, the signals with δ 1.41 (s, 6 H), 2.87 (s, 3H), 8.78 (s, 1H) are very similar to those of 1,5,5-trimethylhydantoin (**4b**). The signals with δ 1.54 (s, 6H),

5.31 (s, 2H), 9.08 (s, 1H) imply that another compound has two methyl groups, one methylene group and one N-H. The NMR data of the product is consistent with compound **4a**. It clearly shows that both compound **3** and **4** can generate two major products during UVA irradiation. One is the original unchlorinated compound, and the other is the migration product. Compounds **3c** and **4c** should have two methyl groups exhibiting different resonances (the chemical shift should be around 1.5 for one and 3.0 for the other) and one N-H (the value of δ should be higher than 6). However, the NMR spectrum does not provide any evidence that compounds **3c** and **4c** are generated. The NMR spectra of compounds **1** and **2** shows that there are no products other than the dechlorination product and migration product after 7 days UVA irradiation. The data above indicate that a methyl hydrogen at the 5th position (Figure 4.6) is reluctant to transfer as compared to hydrogen atoms of the single methyl hydrogen.



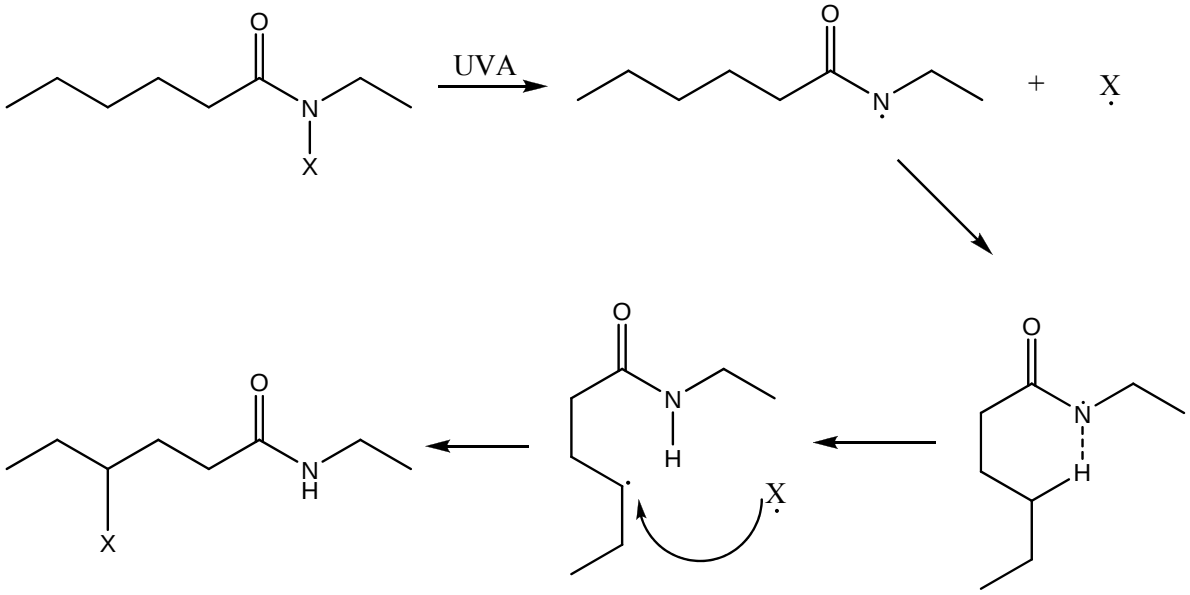
Scheme 5.4. The reaction products of compound 3 under UVA irradiation



Scheme 5.5. The reaction products of compound 4 under UVA irradiation

* Where P and P' are unknown products

These transformations involve a process called a hydrogen transfer reaction (Scheme 5.6). In general, the mechanism of [1, 5] (or [1, 4]) HATR can be considered as follows: after being activated, the N-X bond breaks and generates two radicals, an amidyl (or imidyl) and a halogen radical. Then, the N-centered radical twists to form a 5 or 6 membered ring which could be considered as the transition intermediate. The hydrogen atom to be transferred is very close to the amidyl radical in the transition intermediate. After hydrogen atom transfer, a new radical is generated. This new radical is attacked by the halogen radical with the formation of the migration product.[73, 74]

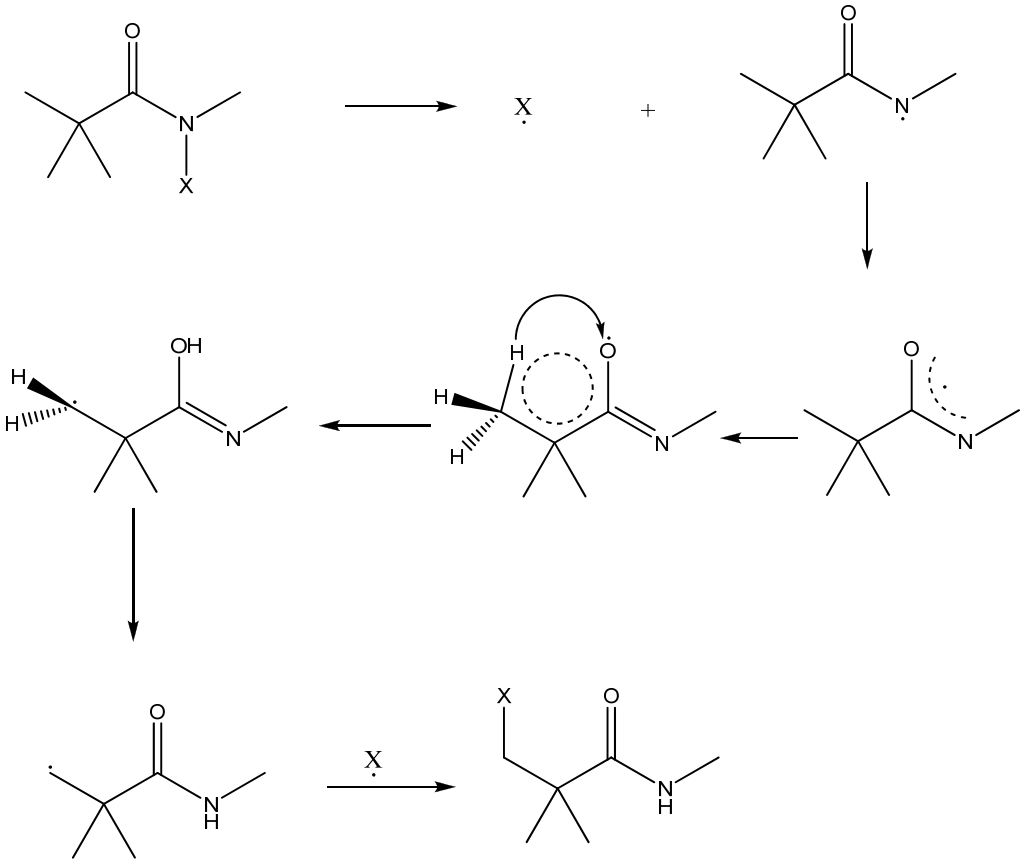


Scheme 5.6. The proposed mechanism of intramolecular HATR.

However, the compound we used has a methyl group on a ring instead of the long-chain alkyl group, and the 5 membered hydantoin ring is rigid. It is impossible for the methyl group to twist in order to form a 5 or 6 membered ring when the N-Cl becomes a N radical. Thus the

result of our observation cannot be simply explained by this mechanism.

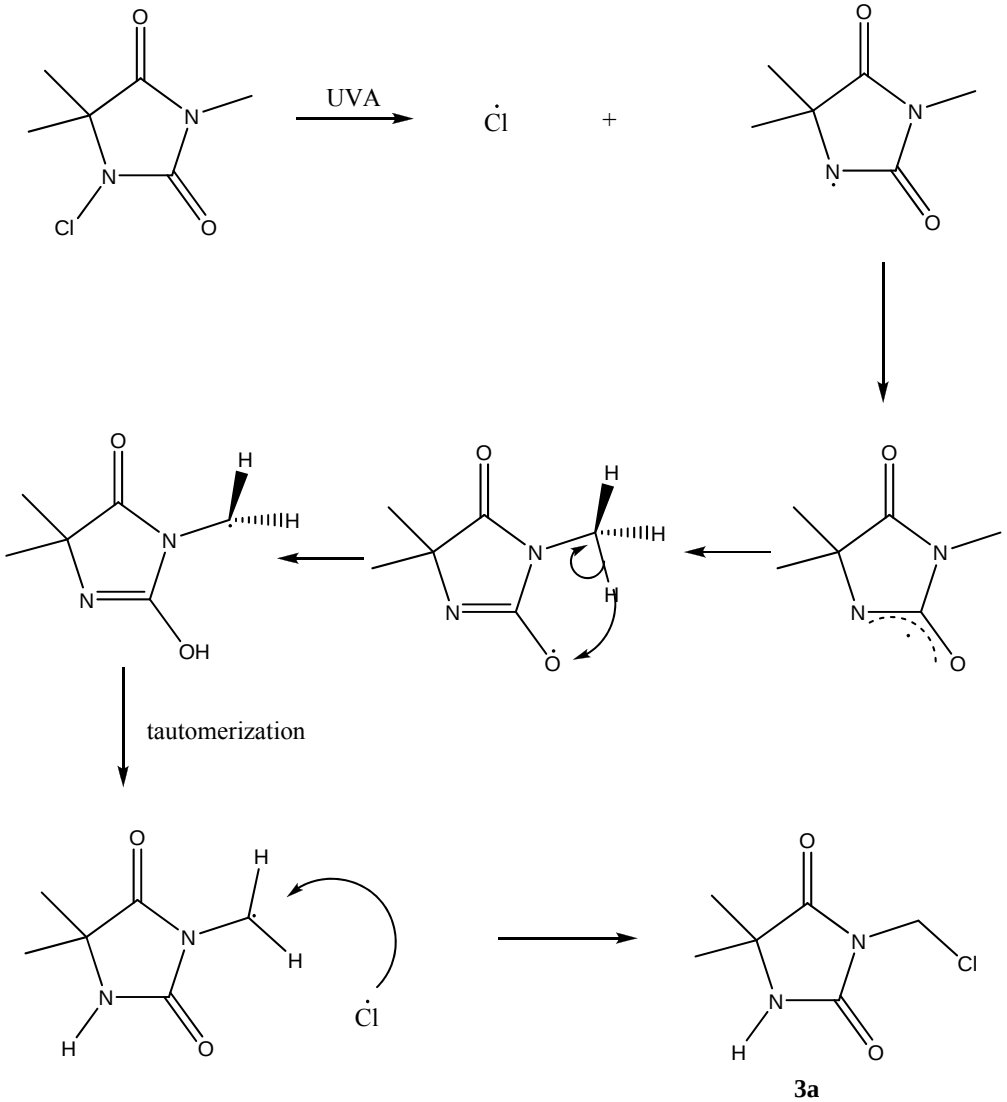
We propose a new mechanism for the migration reaction with sterically hindered reactants (Scheme 5.7). The first step would be the N-Cl bond breakage and the formation of Cl and amidyl radicals. After formation, the amidyl radical delocalizes electron density on the oxygen atom. Then, a hydrogen atom on the adjacent methyl group moves to this oxygen radical and generates a methylene radical. Finally, the chlorine radical attacks the methylene radical to form a stable product.



Scheme 5.7. The proposed mechanism of intramolecular HATR.

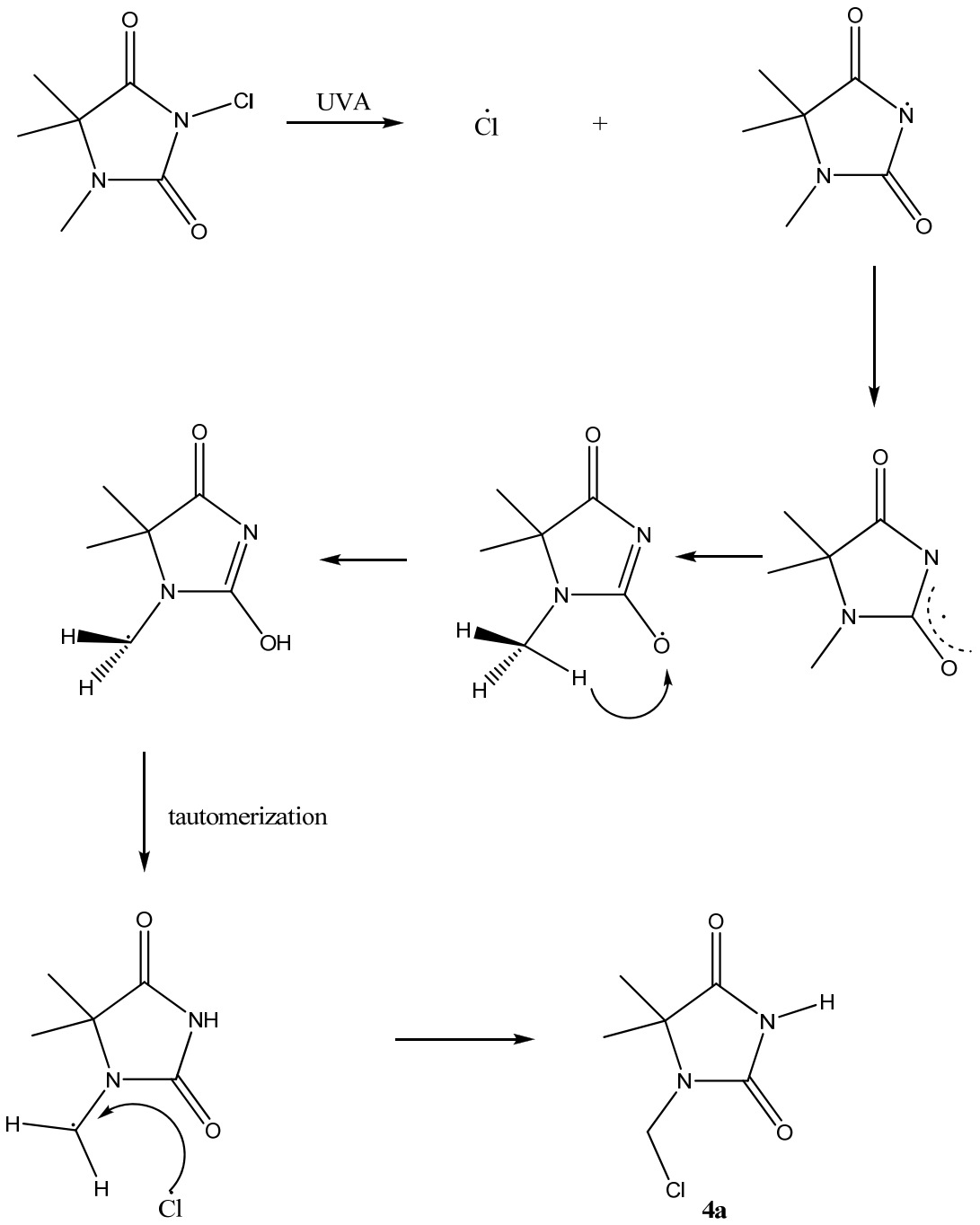
The formation mechanism of **3a** and **4a** can be proposed in Scheme 5.8 and 5.9. It mainly follows the proposed mechanism described in Scheme 5.7. The interesting feature in these products is that there are two carbonyl groups in the hydantoin ring. To be emphasized is that

only the one at the 2nd position is involved in the migration. The explanation for this phenomenon is: after the amidyl (or imidyl) radical is formed, the single electron will be delocalized. The carbonyl group on the 2nd position has two nitrogen atoms on both sides while the one on the 4th position only has one nitrogen atom as its neighbor. Thus, the electron density on the carbonyl group at the 2nd position is lower than that on the carbonyl group at the 4th position. The single electron prefers to be shared by the carbonyl group with lower electron density. The chlorine radical can only exit for a short time. After it is generated, it might be trapped by the carbonyl group that does not involve in the transition state.



3a

Scheme 5.8. The proposed mechanism of **3a** formation in Test A.



Scheme 5.9. The proposed mechanism of **4a** formation in Test A.

As the new radical forms through migration, a hydrogen atom might be generated from the decomposition products, or the walls of the container to form compounds **3b** and **4b**.

The solution radiation reaction has a different pattern in products ratio (Table 5.2). The

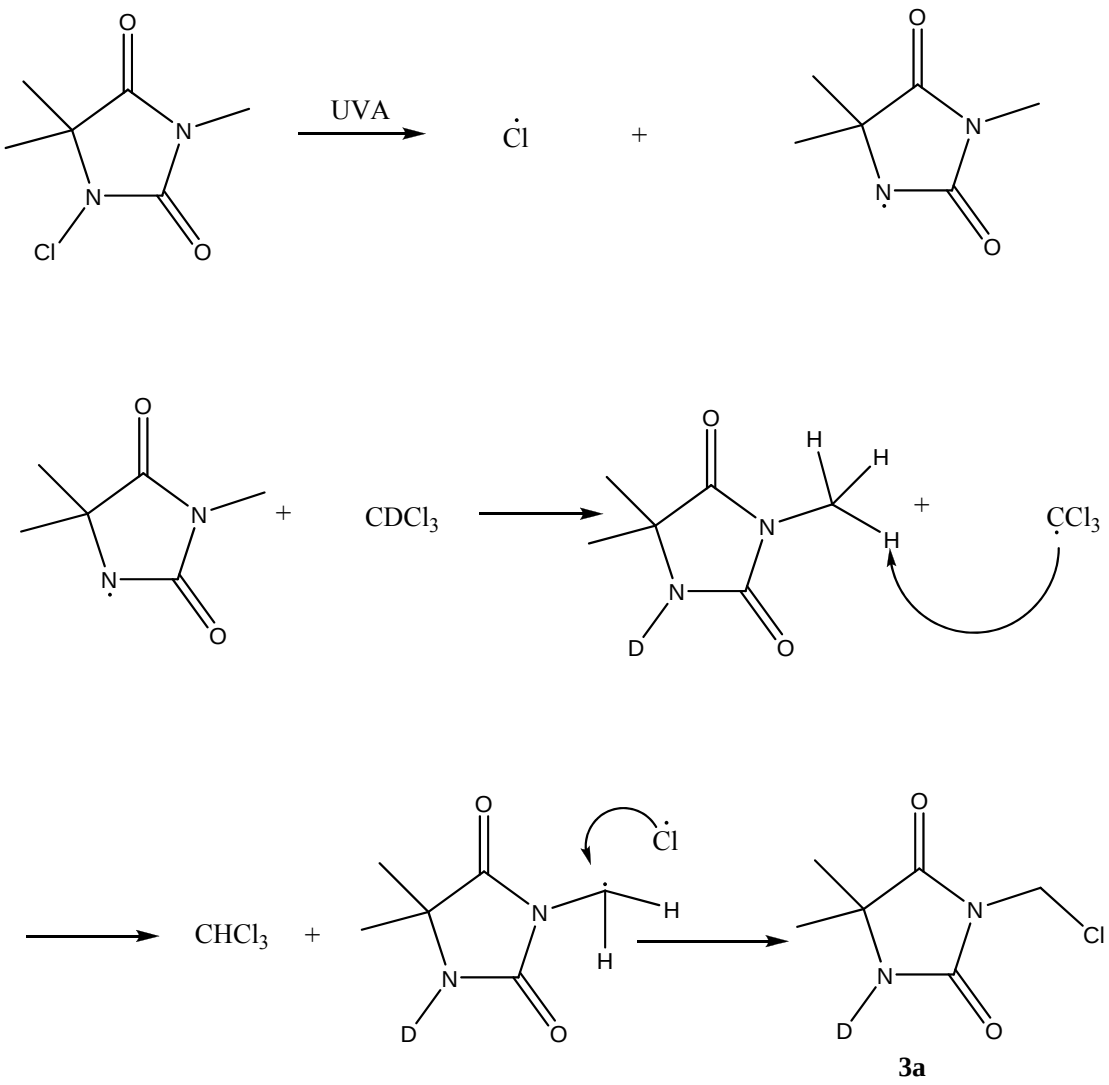
solvent $CDCl_3$ obviously affects the ratio of the final products. The explanation would be that the products of Test A are formed from the intramolecular hydrogen migration while the intermolecular hydrogen transfer is the case during Test B.

Table 5.2. Final product ratio under UVA light exposure

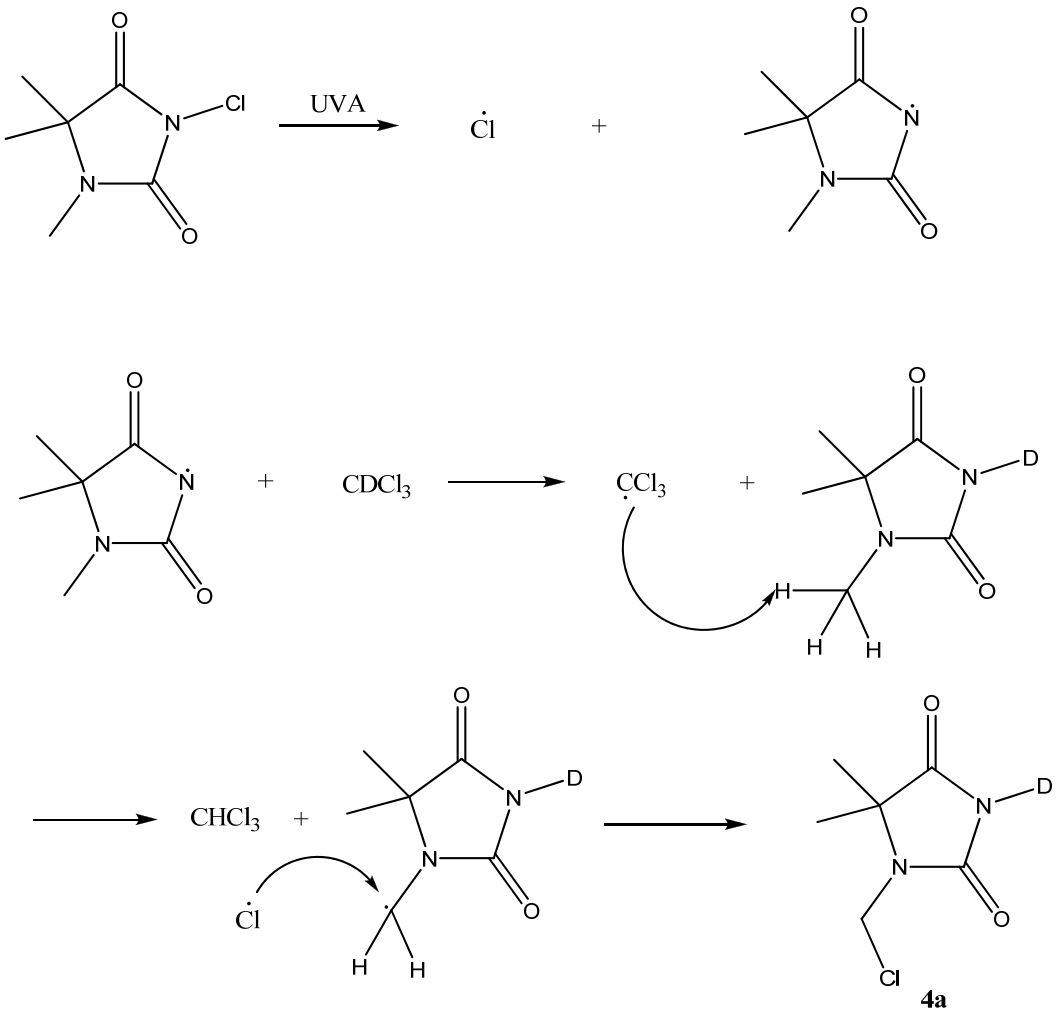
	Product ratio	
	3a : 3b	**4a : 4b**
Test **A**	10:1	1.5:1
Test **B**	11:6	11:6

A reasonable reaction mechanism for the product shift from **3a** to **3b** or **4a** to **4b** in Test B is shown in Scheme 5.11 and 5.12. A new radical is involved in this intermolecular migration. The first step is the N-Cl bond breaking. The amidyl (or imidyl) radical abstracts a hydrogen (or deuterium) from the solvent (chloroform or $CDCl_3$). CCl_3 radical and **3b** (or **4b**) are formed. Then, this new radical prefers to attack the methyl group that bonds to the nitrogen atom rather than the one that bonds to the carbon atom. This steps leads to the formation of chloroform and a methylene radical which can react with chlorine radical and generate **3a** (or **4a**). However, the mechanism of the formation of compounds **3b** and **4b** is still unknown.

The oxygen that exists in the $CDCl_3$ might also affect the mechanism. However, there is no evidence that can show the corresponding products. It might consume some of the decomposition products as the process continues. After a short reaction time, there should be no oxygen remaining in the solvent.



Scheme 5.10. The proposed mechanism of **3a** formation in Test B.



Scheme 5.11. The proposed mechanism of **4a** formation in Test B

Other alternative explanations for the data in Table 5.2 are mentioned below.

In Test A, the rate-determining step is the breakage of the N-Cl bond in the formation of **3**. The amidyl radical quickly becomes delocalized between the nitrogen atom and the adjacent carbonyl group and the oxygen atom in the carbonyl group eventually becomes oxygen radical. This oxygen radical then abstracts a hydrogen atom from the adjacent methyl group. The chlorine radical then attacks this methylene radical and ends the reaction. Due to the high migration rate and the low chlorine radical formation rate, the side reaction barely proceeds. Thus, the main product is **3a**. There is only a small amount of **3b** formed.

The rate-determining step is the radical migration step in the formation of **4**. The N-Cl can easily break into imidyl radical and chlorine radical. As the imidyl radical accumulates, a considerable amount of **4b** can be generated. So the ratio of **4b** in the product is much higher.

In Test B, CCl_3 radical plays the key role during the intermolecular migration. The ratio of **3a** : **3b** and the ratio of **4a** : **4b** are almost the same. This means CCl_3 radical speeds up the side reaction for **3** and the radical migrating step for **4**. The rate-limiting step of these two reactions here is the N-Cl bond breaking step. Due to the stronger bond energy of the N-Cl in compound 3, the overall reaction time of **3** is much longer than that of **4**.

5.6. Conclusion

The hydrogen migration phenomenon was observed during the UVA stability test in Chapter 4. Additional experiments were designed for the study of the mechanisms. Two different mechanisms were proposed, and they are intramolecular and intermolecular migration. Further experiments and possibly calculations are needed to prove the mechanisms. These two mechanisms can also be used for the explanation of why the N-Cl bond in 3,5,5-trimethylhydantoin is much more stable than that in 1,5,5-trimethylhydantoin in Chapter 4.

Chapter 6

Conclusions and recommendations

A great amount of N-halamines have been synthesized in Worley's group. They have been shown to provide excellent antimicrobial capability after being coated onto cotton fabric or grafted onto some polymers. Are these compounds able to render great biocidal efficacy after being incorporated into latex paint? Can this antimicrobial ability last for a long time? PHQS, HD, HE, and PTS have been tested as described in Chapter 2 and 3. Latex paint is a commonly water-soluble paint. The N-halamines that are to be tested should share the same physical property. PHQS, HD and HE are water-soluble. PTS can dissolve in water after being treated with acid. PHQS incorporated latex paint showed a strong biocidal efficacy against *S. aureus* and *E. coli* after being coated onto transparency film. The oxidative chlorine was very stable. This means that the antimicrobial activity was long-lasting, and it could be recharged by simple rechlorination. Based on the advantages of the new paints, they seem highly promising as commercial antimicrobial material. Paints that contain HD or HE were tested in Chapter 3. These water-soluble N-halamine precursors could generate homogeneous paints with latex paint. However, there was no strong bonding between them and the paint so that these compounds could easily be washed away from the paint. Thus these paints had no useful antimicrobial capabilities. PTS can dissolve in water only if being treated with acid first. However, original latex paint will interact with acid. Thus, PTS incorporated latex paint

could not render useful antimicrobial ability.

According to our experience, we suggest that the candidate compound for incorporation into commercial latex paint should have the following features: (1) water-soluble under mild conditions (e.g. room temperature, pH=7); (2) bond to the polymer particles in the latex paint. Other compounds that are water-soluble and contain siloxane groups might be the next ones that should be tested.

The hydantoin ring has two N-H bonds which can form N-Cl after chlorination. However, the biocidal activities of these two N-Cl groups might not be the same. Experiments in Chapter 4 and 5 provided evidence to demonstrate this difference. The compounds involved were 1-chloro-3,5,5-trimethylhydantoin and 3-chloro-1,5,5-trimethyl-hydantoin. Theoretically, the electron density on the imide N is lower than that on the amide N. This indicates that the N-Cl bond in 3-chloro-1,5,5-trimethylhydantoin is weaker than that in 1-chloro-3,5,5-trimethylhydantoin. Three different stability tests were done. Biocidal efficacy tests were also examined. All of the experiments indicate that the N-Cl bond in 1-chloro-3,5,5-trimethylhydantoin is much more stable than that in 3-chloro-1,5,5-trimethylhydantoin.

The UVA stability test in Chapter 4 indicates that hydrogen migration occurred during the test. Experiments thereof were designed for the study of the mechanisms. The additional methyl group decreased the stability of the N-Cl compared to 1-chloro-5,5-dimethylhydantoin and 1,3-dichloro-5,5-dimethylhydantoin. Chlorine atoms preferred to bond to the newly formed methyl group. Two different mechanisms were proposed based on

different reaction conditions. Hydrogen migration occurring in the solid state compounds might proceed though an intramolecular mechanism. The reaction that occurred in the chloroform solvent involved intermolecular hydrogen transfer. Theoretical calculations are needed to prove the mechanisms. These two mechanisms also provide strong evidence to demonstrate that the N-Cl bond in 1-chloro-3,5,5-trimethylhydantoin is much more stable than that in 3-chloro-1,5,5-trimethylhydantoin.

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