DESIGN, SYNTHESIS AND CHARACTERIZATION OF THIOL REACTIVE DENDRONS FOR BIOCONJUGATION

by

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To My Dear Family

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ABSTRACT

DESIGN, SYNTHESIS AND CHARACTERIZATION OF THIOL REACTIVE DENDRONS FOR BIOCONJUGATION

Proteins and peptides are among the key bio-molecules which have gained considerable value as therapeutic agents in recent years. Proteins and peptides not only construct the structural material of bodily tissues, but also play an important role in various regulatory functions in the organism. The apparent size of a protein or a peptide is small, thus leading to easy filtration from the kidneys that results in a short circulation time in the body. Also peptide based drugs are prone to rapid degradation by various peptide cleaving enzymes found in the body. In order to improve the therapeutic value of proteins and peptides, novel protein-polymer conjugates can be designed that can shield them from unwanted enzymatic degradation, increase the solubility, and prolong the residence time of protein or peptide-based drugs. This thesis project focused on the synthesis of well-defined dendritic structures that can be used to synthesize multiarm polymers for protein-polymer conjugation. Three generations of dendrons containing a thiol reactive functional group, at their focal point were synthesized. Novel polyester dendrons that are biocompatible and biodegradable were synthesized and characterized for their purity and elemental composition via ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and FTIR spectroscopy. In addition to these, the effect of the number of generations on the rate of thiol addition reaction was investigated using UV-Vis spectroscopy.

ÖZET

BİYOBİRLİKTELİK İÇİN TİYOL REAKTİF DENDRONLARIN TASARIMI, SENTEZİ VE KARAKTERİZASYONU

Protein ve peptitler, son yıllarda tedavi ajanı olarak önemli değer kazanmış biyomoleküllerdir. Protein ve peptitler hem vücut dokularının yapısal malzemelerini yapar, hem de organizmaların çeşitli düzenleyici fonksiyonlarında önemli rol oynarlar. Protein ve peptitlerin boyutları küçüktür ve bu da vücuttaki dolaşım zamanlarının kısa olmasına neden olup, böbreklerden kolayca süzülmelerine yol açar. Peptit bazlı ilaçlar da vücuttaki enzimlerden ayrılan çeşitli peptitlerle hızla bozunma eğilimi gösterirler. Protein ve peptitlerin tedavi edici değerlerini geliştirmek için yeni protein-polimer birliktelikleri tasarlanabilir, böylece protein ve peptitlerin istenmeyen enzimatik bozunmalardan korununabilir, çözünürlüğü arttırılabilir ve protein ya da peptit bazlı ilaçların vücutta kalma süreleri uzatılabilir. Bu yüksek lisans çalışmasında, iyi tanımlanmış dendritik yapıların polimer olarak kullanıldığı protein-polimer birlikteliklerine odaklanıldı. Odak noktalarında tiyol reaktif fonksiyonel grup bulunduran üç nesil dendron sentezlendi. Biyouyumlu ve biyobozunur olan yani poliester dendronlar sentezlendi ve saflık elementel birleşimleri için ^{1}H-NMR, ^{13}C-NMR, yüksek çözünürlüklü kütle spektrometresi (HRMS) ve Fourier dönüşümlü kızılötesi spektroskopisi (FTIR) kullanılarak karakterize edildi. Bunlara ek olarak da nesil sayısının tiyol katkılı reaksiyonun hızına olan etkisi, UV-Vis spektroskopisi kullanarak incelendi.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS

J	Coupling constant
υ	Frequency

LIST OF ACRONYMS/ABBREVIATIONS

ATRP	Atom Transfer Radical Polymerization
Bis-MPA	2,2-bis(hydroxymethyl)propionic acid
CH_3COOH	Acetic acid
CH_2Cl_2	Dichloromethane
$CDCl_3$	Deuterated Chloroform
DCC	N,N'-Dicyclohexylcarbodiimide
DCU	N,N'-Dicyclohexylurea
DMAP	4-Dimethylaminopyridine
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et_3N	Triethylamine
EtOAc	Ethyl acetate
FTIR	Fourier Transform Infrared Spectroscopy
G_1	Generation 1 dendron
G_2	Generation 2 dendron
G_3	Generation 3 dendron
HCl	Hydrochloric acid
HPDS	Hydroxyethylpyridlydisulfide
HRMS	High Resolution Mass Spectrometry
H_2SO_4	Sulfuric Acid
MeOH	Methanol
NH_3	Ammonia
NHS	N-hydroxysuccinimidyl
NMR	Nuclear Magnetic Resonance
PAMAM	Poly(amido amine)
PDS	Pyridyl disulfide
PEG	Poly(ethylene glycol)
p-TsOH	p-Toluenesulfonic acid
RBF	Round Bottom Flask
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

1. INTRODUCTION

1.1. Proteins as Therapeutic Agents

In medicine and pharmacy, using peptides and proteins as drugs have been extensively explored because these compounds can destroy or regulate toxic or overproduced compounds in the body and imitate endogenous hormones, cytokines and antibodies. However, there are two major problems that needs to be addressed in order to widespread protein and peptide based therapeutics. The first major hurdle was the difficulty in producing sufficient quantities of the materials. Small peptides could be produced by chemical synthesis, but larger molecules could only be obtained by the extraction or purification of natural sources. Advancements in genetic engineering, this problem has enabled overcoming this problem to a large. The second major problem is to preserve the peptide and protein in its intact form during its delivery to the desired target. Because the digestive system destroys proteins, oral delivery of proteins is unavailable. Due to the rapid renal excretion and proteolytic digestion, injected proteins exhibit poor pharmacokinetics.

The use of proteins and peptides as therapeutics agents has increased in recent years because of (i) discovery of novel proteins and peptides, (ii) a better understanding of the mechanism of action in vivo, (iii) enhancements in expression or synthesis of proteins and peptides, and (iv) improvements in formulation technologies that enables delivery of polypeptides in vivo with desired pharmacokinetic and pharmacodynamic properties [1].

1.2. Peptide and Protein Pegylation

Proteins and peptides are ideal biomolecules for use as therapeutic agents because of their inherent specificity to various receptors in biological systems. Applications of naturally arising and recombinant proteins contain the fields of biotechnology, nanotechnology and medicine, and Covalent attachment of synthetic polymers to protein

has been demonstrated to significantly improve properties such as stability, biocompatibility and solubility. Moreover, the attachment of a polymer chain can be used to regulate protein activity [2].

In order to improve protein delivery, different approaches such as encapsulation of protein in insoluble matrices and liposomes, and immobilization onto polymer resins have been evaluated. However, to mask the protein surface from various proteolytic degradation in the body, conjugation to soluble polymers have demonstrated high levels of effectiveness. This protection of the protein achieved due to masking by a polymer, the so called "umbrella-like effect" (Figure 1.1), has been the most successful approach. Generally this involves the covalent coupling of a soluble poly(ethylene glycol) (PEG) with protein, a process which is known as pegylation .

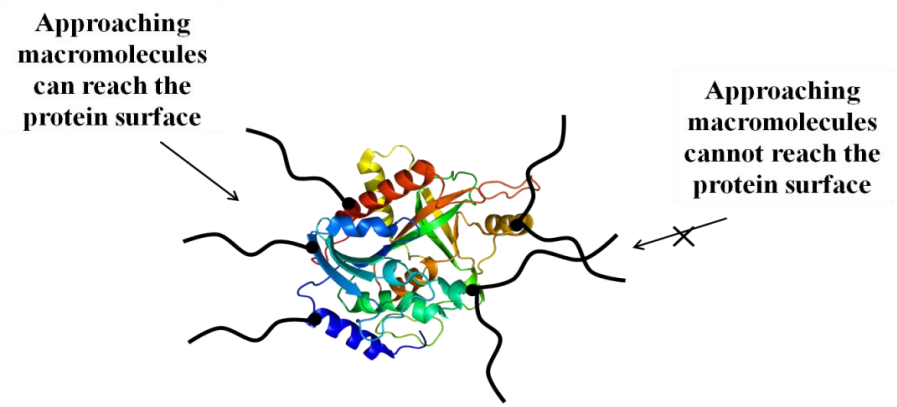


Figure 1.1. "Umbrella-like" effect masks larger protein surface. Figure was adopted from [4].

Pegylation supplies the real breakthrough in pharmaceutical properties of proteins and peptides. Polyethylene glycol is a polymer extensively used as a covalent modifier of biological macromolecules and particulates, and a carrier for low molecular weight drugs. PEG has a unique set of properties, containing absence of toxicity, immunogenicity and antigenicity; low, mass-dependent elimination via the kidney; high flexibility and high solubility in water and organic media [1,3,5]. Pegylation has several potential beneficial

effects involving increased bioavailability and plasma half-lives, declined immunogenicity, decreased proteolysis and enhanced solubility and stability. A significant function of the protein-polymer conjugates is also to rise the size of the protein, resulting in a reduction in the rate of clearance of the drug from the body through renal excretion (Figure 1.2) [6].

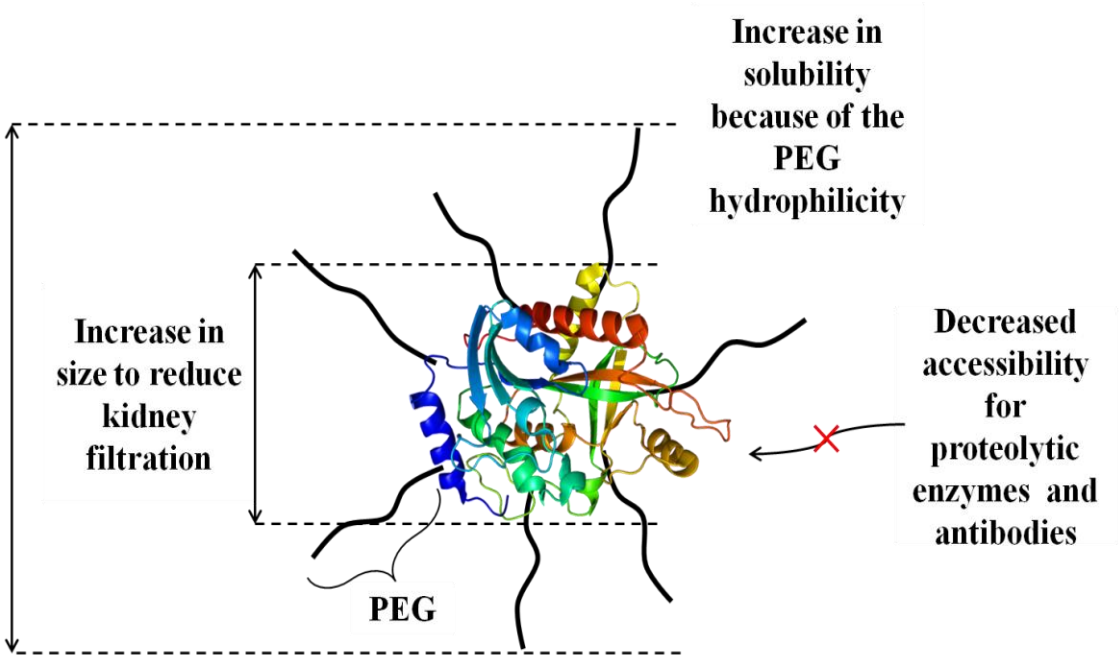


Figure 1.2. Main advantages of PEG-protein conjugates. Figure was adopted from [7].

1.3. Synthesis of Protein-Polymer Conjugates

The traditional way to prepare polymer bioconjugates is to modify polymers with protein-reactive end-groups, such as activated esters, that helps coupling between the polymer and the amino acid side-chains on the protein. As an alternative, nowadays, protein-reactive initiators have been used to synthesize polymers directly from proteins (Figure 1.3). These protein-reactive initiators used to yield polymers that contain a functional group at their chain-end which can be used for conjugation to the protein molecule. In different approach, the protein-reactive initiator can be first coupled to the protein to provide a biomolecule decorated with initiators for growing polymers. Polymerization from thus modified biomolecules leads to direct synthesis of polymer-protein conjugates (Figure 1.3) [2].

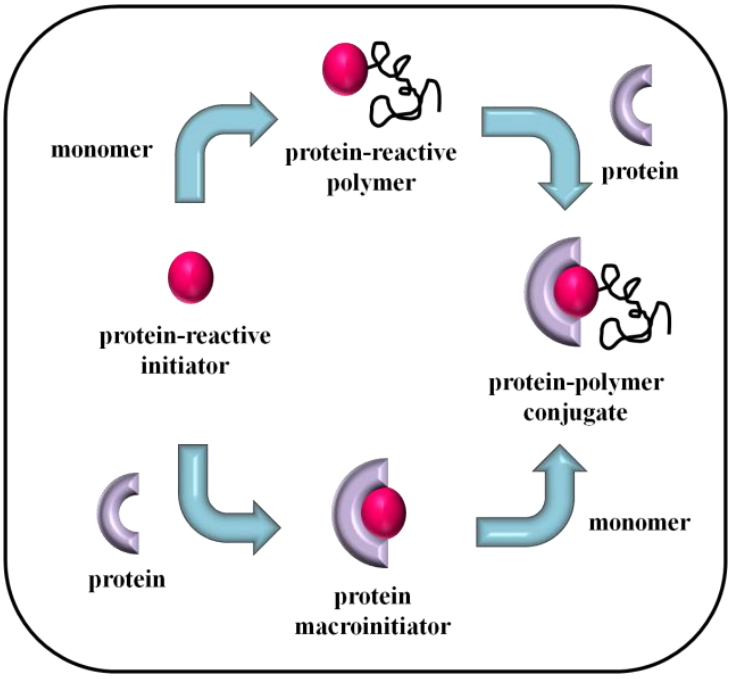


Figure 1.3. Routes to protein-polymer conjugates [2].

1.3.1. Post-Polymerization Modification Strategies

1.3.1.1. Amine-Reactive Polymers. The reactivity of the amine groups that are generally abundant in proteins, toward an extensive range of electrophiles, makes this an attractive method for bioconjugates formation. Amine-reactive polymers often contain activated esters such as N-hydroxysuccinimidyl (NHS) esters. Polymers with alternative end-groups containing mono- and dichlorotriazines, thioimidoesters, and aldehydes have also been synthesized for protein modification using the amine group on the proteins for comjugation (Figure 1.4).

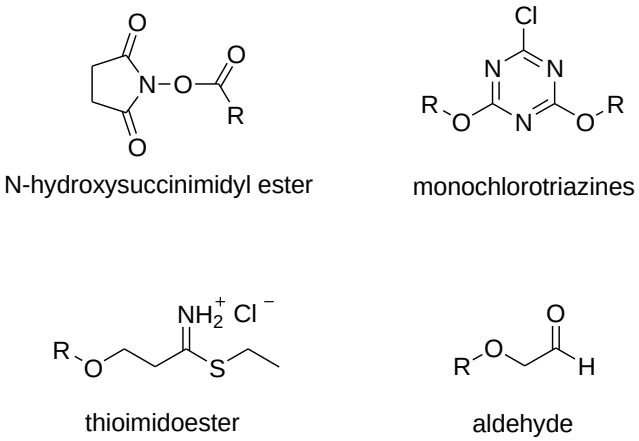
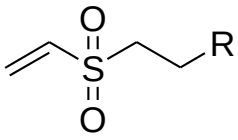


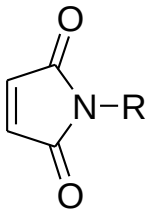
Figure 1.4. Amine-reactive end-groups.

The disadvantage of this route is that oftentimes a heterogeneous mixture of bioconjugates is obtained because of non-specific coupling with multiple amine group containing amino acid residues. This type of random and heterogeneous conjugation often causes a significant reduction in bioactivity. Therefore, alternative approaches to obtain well-defined biomolecule polymer conjugates are needed.

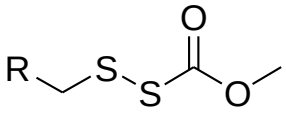
1.3.1.2. Thiol-Reactive Polymers. In order to create well-defined protein-polymer conjugates, modification of the sulfhydryl group of proteins can be utilized. Generally, the number of thiol-containing amino acid residues are less in number when compared to amine residues. Most of the cysteine groups that are found in proteins participate in disulfide bonds formation, hence free cysteine groups are present in low frequency, and therefore modification of this residue results in site-specific bioconjugates. The most popular thiol-reactive end-groups for protein-polymer conjugates that have been extensively investigated are vinyl sulfone, maleimide, and activated disulfide end-groups (Figure 1.5) [2].



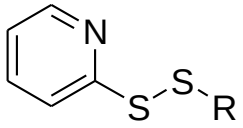
vinyl sulfone end group



maleimide end group



methoxy carbonyl activated disulfide group



pyridyl disulfide group

Figure 1.5. Thiol-reactive end-groups.

1.3.1.3. Polymers for Oxime Formation. In order to synthesize side-specific conjugates, oxime formation between a hydroxylamine group on a protein and a ketone or an aldehyde-functionalized polymer can be utilized (Figure 1.6). An aminooxy moiety can be incorporated into a protein using chemical modifications. Since either of the reacting functional groups used in the conjugation are not present in multiple numbers, this approach provides a desirable way to obtain well-defined protein-polymer conjugates in a chemoselective manner [2].

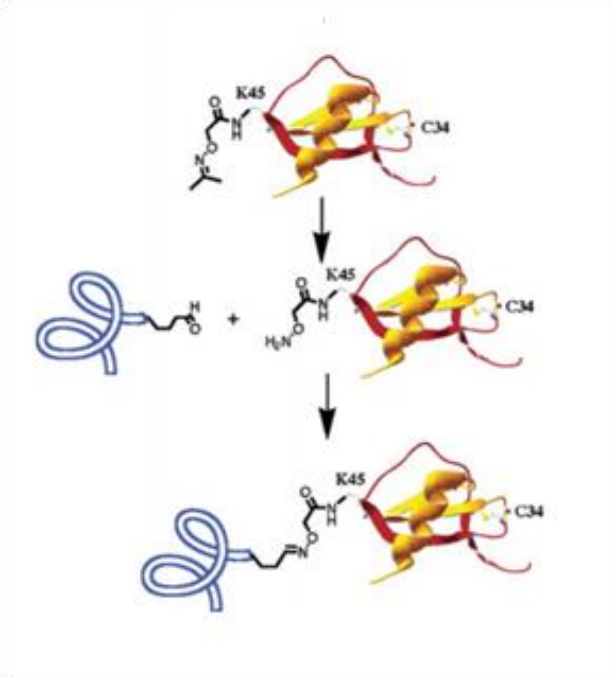


Figure 1.6. Protein-polymer conjugate via oxime formation [2].

1.3.1.4. Polymers for "Click" Conjugate Formation. The high yields and mild conditions of azide–alkyne 1,3-dipolar cycloaddition reactions makes them attractive candidates for preparation of bioconjugates (Figure 1.7). In order to synthesize conjugates in this route, the protein must be modified with an azide or alkyne moiety [2].

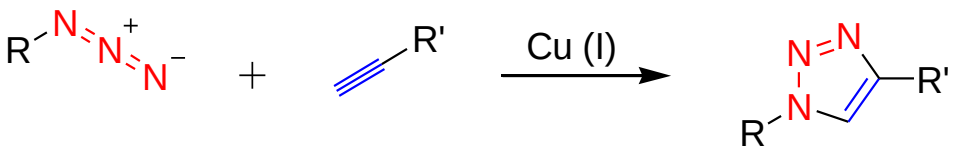


Figure 1.7. Bioconjugate formation via [3+2] cycloaddition.

1.4. Conjugation and Bioconjugation Reactions Using Thiols

Theoretically, there are mainly three approaches to synthesize polymer-protein conjugates. One of them is including the immobilization of the initiator and in this method,

the parent polymer chain is synthesized and then the biomolecule is attached. Another method for the synthesis of polymer-protein conjugate is the controlling agent onto the biomolecule. This method ensures the presence of biomolecules attached to the polymer. However, as a disadvantage, the biomolecules can generate steric hindrance. An alternative method for synthesizing polymer-protein conjugate is that functional polymers are post-modified with the desired biomolecules. In this approach, the biomolecule is coupled to a polymer that contains pendant groups for attachment (Figure 1.8) [8].

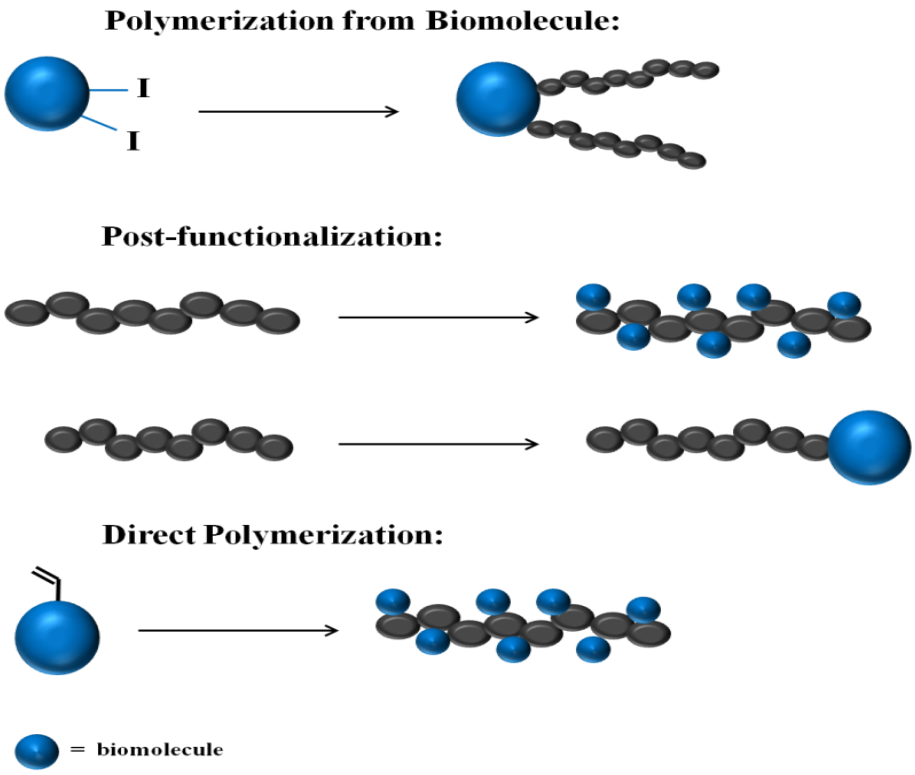


Figure 1.8. Potential pathways to bioconjugates.

In recent years, post-polymerization functionalization has been investigated as an attractive route to obtain protein-polymer bioconjugates Due to high conjugation efficiency, the Cu(I)-catalyzed alkyne-azide Huisgen 1,3-dipolar cycloaddition has been extensively investigated. However, the problems encountered with the removal of residual Cu(I) catalyst has shifted the attention from this reaction to metal free thiol-based conjugation reactions. The excellent nucleophilicity and the ability of thiols to participate in radical reactions make them a desirable choice. To date, thiol-halogen, thiol-parafluoro, thiol-ene, thiol-yne, thiol-vinylsulfone, thiol-maleimide, thiol-bisulfone and thiol-pyridyl disulfide reactions are the types of reactions that are commonly used to derivatize the thiol functional group (Figure 1.9).

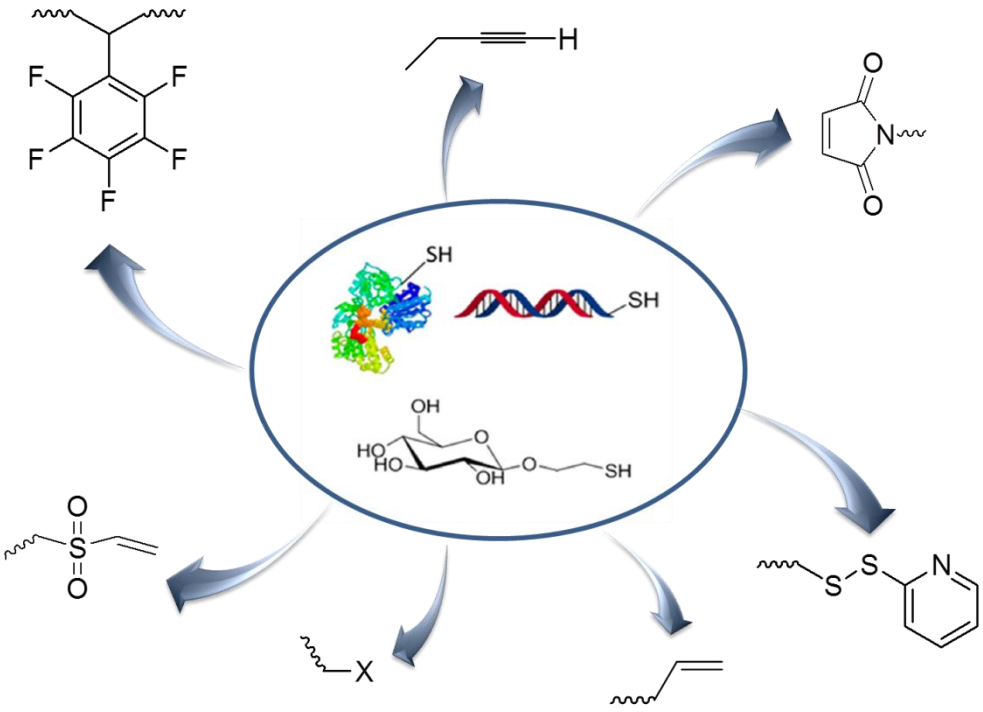


Figure 1.9. The types of thiol reactions. Figure was adapted from [8].

1.4.1. Thiol-Halogen Reactions

In order to synthesize glycopolymers, the simple nucleophilic substitution of halogenides and thiols are a robust way and this route has been discovered in the 1990s (Figure 1.10). Because halogenides can potentially act as chain transfer agents or interfere with initiators or catalysts, this approach is limited by the choice of polymerization techniques. Thiol-halogeno reactions need two-step process where a functional polymer is modified, followed by the nucleophilic substitution with thiols. In order to generate polymer-peptide conjugates, this pathway was also utilized, but it is significantly less commonly used than in the synthesis of glycopolymers [8].

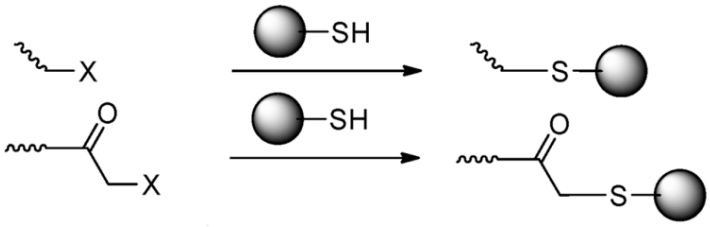


Figure 1.10. Thiol-halogen reactions.

1.4.2. Thiol-Parafluoro Reactions

With using a wide range of techniques, pentafluorostyrenes can be polymerized. The advantages of this pathway (Figure 1.11) are not only easy access to several polymer architectures but also easy monitoring of reactions with thiols using ^{19}F-NMR spectroscopy. Compared to the thiol-halogen reaction, this route seems to be faster, and full conversion was achieved. However, this efficient reaction has yet to be tested using peptides or proteins [8].

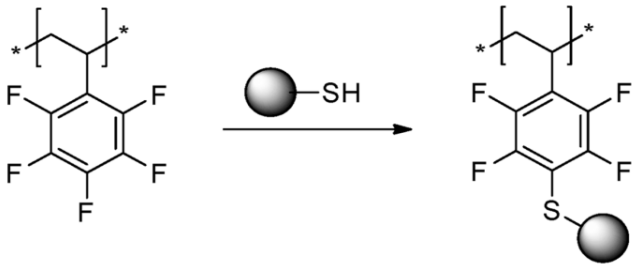


Figure 1.11. Thiol-parafluoro reactions.

1.4.3. Thiol-ene Reactions

Thiol-based reactions are probably controlled by thiol-ene chemistry, especially in the synthesis of glycopolymers. The reaction between thiols and pendant double bonds is the most common approach in creating glycopolymers with postfunctionalization (Figure 1.12). With using a radical source, thiol-ene reactions are commonly initiated; however, depending on the structure of the vinyl functionality Michael addition reactions or enzymatic synthesis can offer an alternative approach. Conversely, most reports on bioconjugation focus on the radical pathway [8].

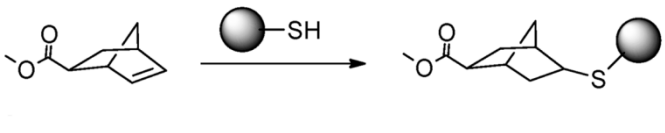


Figure 1.12. Thiol-ene reactions.

1.4.4. Thiol-yne Reactions

The easy access of the reactive polymer is the advantage of the thiol-yne reactions (Figure 1.13). In response to the thiol-ene reactions, that require the synthesis of polymers with vinyl pendant groups either by post-functionalization or by careful adjustment of the reactivity of the two vinyl groups, the thiol-yne pathway can benefit from monomers, that can be easily polymerized in a controlled manner [8].

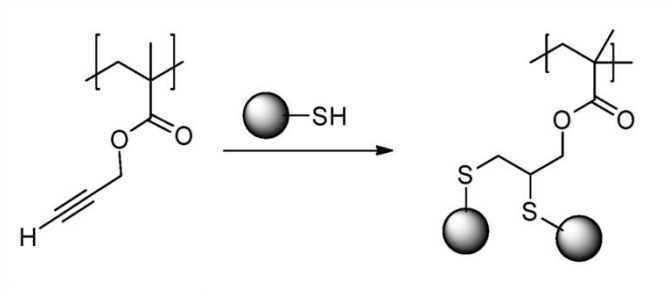


Figure 1.13. Thiol-yne reactions.

1.4.5. Thiol-Vinylsulfone Reactions

The reaction between thiols and vinylsulfone groups does not require any additional catalyst (Figure 1.14). Simple stirring in a preferably slightly alkaline aqueous solution or in an organic solvent can result in full conversion in less than a day. Polymers with pendant vinyl sulfone groups can be prepared for instance by ring-opening polymerization resulting in polymers which can be reacted with peptides. The thiol-vinyl sulfone approach is particularly attractive when polymers are utilized that have been generated using the RAFT process [8].

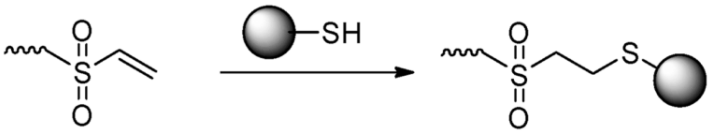


Figure 1.14. Thiol-vinylsulfone reactions.

1.4.6. Thiol-Maleimide Reactions

For conjugating polymers to peptides or proteins, the thiol-maleimide reactions are the most popular thiol-based route (Figure 1.15). Any heat or catalyst are not required for the nucleophilic addition of thiols to maleimide, and simple stirring of the two reactants at room temperature often adequate to achieve complete conversion. Protecting the maleimide functionality prior to radical polymerization is the only limitation [8].

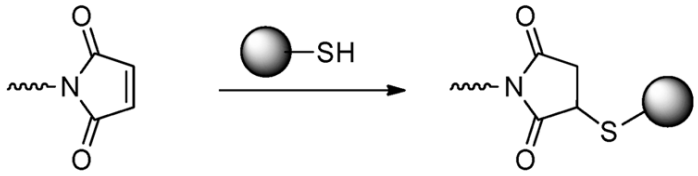


Figure 1.15. Thiol-maleimide reactions.

1.4.7. Thiol-Pyridyl Disulfide Reactions

The fast reaction between thiols and pyridyl disulfides (PDS) is a very popular approach to polymer-peptide conjugates (Figure 1.16). The pyridyl disulfide undergoes a fast exchange with free thiol groups resulting in mixed disulfides. With the exchange reactions, the yellow pyridine-2-thione that allows easy monitoring of the rate of the reaction is formed. The linkage formed between the polymer and biomolecule is based on a disulfide bridge, that can be cleaved again in the reductive cell environment allowing the release of the biomolecule. Polymers with PDS groups can be directly synthesized, having either a pendant group or a single reactive entity at the end of the polymer chain. The bioconjugation with peptides or proteins is usually carried out at ambient temperature without the addition of any catalyst [8].

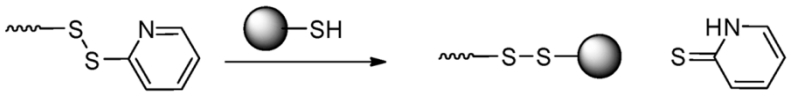


Figure 1.16. Thiol-pyridyl disulfide reactions.

1.4.8. Thiol-Thiosulfonates Reactions

The use of thiosulfonates, that can be easily accessed by nucleophilic substitution of alkyl halides is an emerging tool for bioconjugation (Figure 1.17). For example, PEG modified with a terminal thiosulfonate was reacted at ambient temperature for one hour resulting in the formation of a disulfide bridge [8].

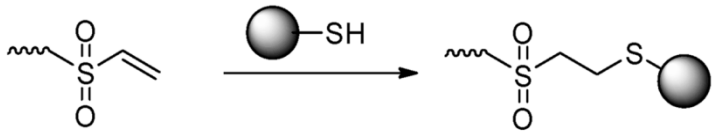


Figure 1.17. Thiol-thiosulfonates reactions.

1.5. Using of Disulfide and Pyridyl Disulfide Groups

Cleavable bonds, such as disulfides, are of interest for designing biomacromolecules because they can be easily degraded in the cytoplasm of cells in the presence of reduced glutathione, offering an pathway to the release of proteins/siRNA, imaging agents, drugs, or the synthesis of biodegradable polymers. There are two types of disulfides which are symmetric and asymmetric. Asymmetric disulfides are essentially formed using thiol-exchange chemistry with an electrophilic (activated) sulfur reagent (Figure 1.18), while a symmetric disulfide is formed by oxidation of a thiol with an oxidizing agent, such as iodine, oxygen, or $FeCl_3$.

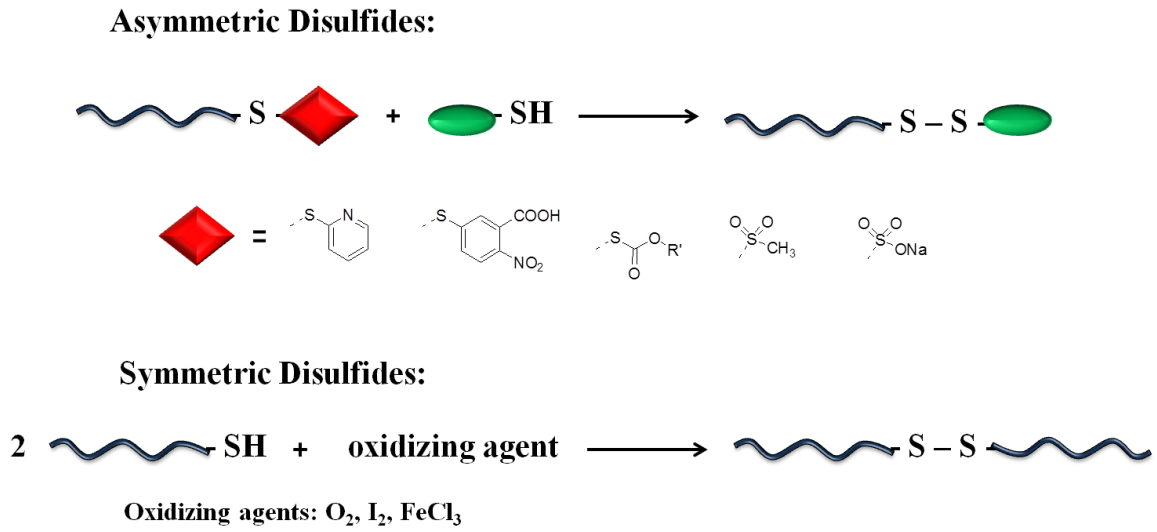


Figure 1.18. Asymmetric and symmetric disulfides.

The components which have pyridyl disulfide group are novel and glutathione reactive. The pyridyl disulfide bond can be reacted with a thiol-containing molecules via disulfide-exchange chemistry, targeting ligand or other functional compounds, forming an asymmetric disulfide bond to such molecules, and releasing the by product, thiopyridone (Figure 1.19). This reaction can be carried out in organic or in aqueous media at room temperature to high yields, and is unaffected by the presence of functional groups such as amines, carboxylic acids, or hydroxyl groups [9, 10].

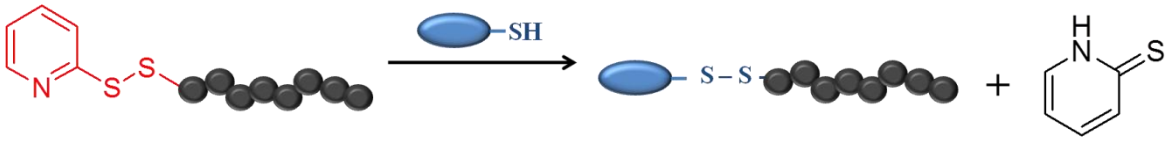


Figure 1.19. Forming a disulfide bond and thiopyridone as a byproduct [10].

Pyridyl disulfide group has an effective group towards the disulfide exchange reaction with thiols under mild conditions. Due to the leaving character of thiopyridone, pyridyl disulfide group demonstrates increased reactivity towards the attack of free thiol-tethered derivatives. The formation of reversible disulfide linkages via thiol-disulfide

exchange reaction make the pyridyl disulfide group a favorable functional group in biological applications for the preparation of the cleavable conjugates of biomolecules such as proteins and thiol-activated oligonucleotides [11].

1.6. DENDRIMERS AND DENDRONS

Polymers that consist entirely of branched repeat units have two basic types which are dendrimers and hyperbranched polymers. Hyperbranched polymers exhibit an irregular architecture with incompletely reacted branch points throughout the structure because they are usually product of a noniterative polymerization procedure (Figure 1.9). Dendrimers, on the other hand, are regularly branched, highly ordered and globular macromolecules synthesized by a stepwise organic reactions. The structure of dendrimers consists of three different architectural regions: (i) a focal moiety or core, (ii) layers of branched repeat units emanating from the core, and (iii) end groups on the outer layer of repeat units (Figure 1.20). The variations between dendrimers and hyperbranched polymers are the structural perfection of dendrimers causing an exact number of concentric layers of branching points, or generations [12].

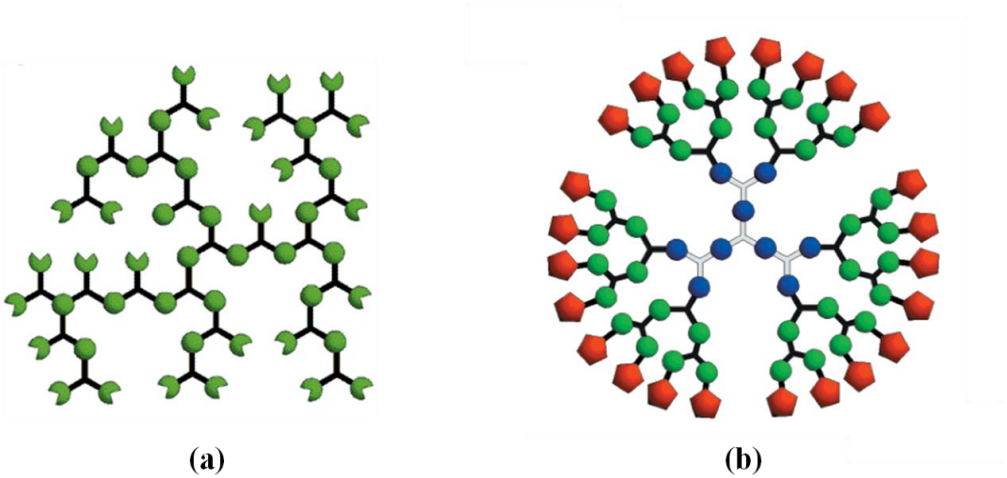


Figure 1.20. Schematic representation of hyperbranched polymer (a) and dendrimer (b).

The first paper about dendrimers was published in 1985 by Tomalia *et al.* and since then many others with several various repeating units have been published. Dendrimers are monodisperse which makes them highly attractive building blocks for applications such as formation of enzyme mimicking cavities [13], targeted drug delivery [14], etc.

1.6.1. Structure of Dendrimers

A dendrimer has three main parts which are a core, branching repeat units and surface groups that are often referred to as peripheral groups (Figure 1.21). Diversity of the dendrimers are characterized by the core via number of functional groups on it. Focal point of a dendron can has reactive groups that can be used to add repeating units and thus grow the dendrimers.

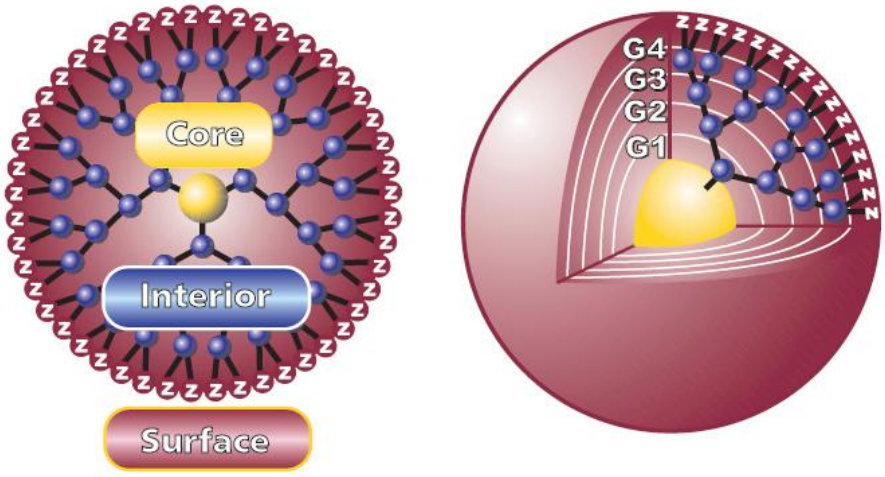


Figure 1.21. Schematic representation showing subunits of dendrimers [15].

Dendron is a part of the dendrimer, and it starts from the core and ends at the periphery. A dendron does not possess a core but instead contains a focal point. The focal point is the fragment from which repeating units that built the dendron originates (Figure 1.22).

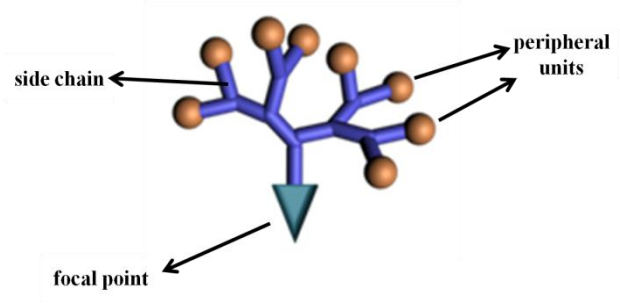


Figure 1.22. General structure of a dendron.

Each branching point at a dendrimer or a dendron is named as a new generation. As the number of branching points increases, the generation of the dendrimers or dendrons also increases (Figure 1.23).

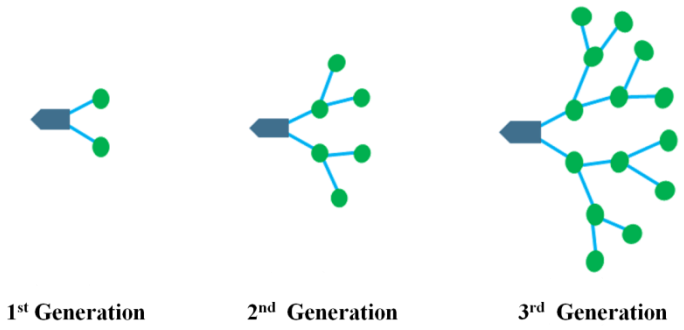


Figure 1.23. Illustration of generation of the dendrons.

1.6.2. Historical Development

The first publication in the area of synthesis of dendritic macromolecules was published by Vögtle *et al.* in 1978 [16]. The products were defined as "cascade molecules" by Vögtle and prepared via stepwise organic synthesis methodology. In 1985, Tomalia and coworkers introduced the word "dendrimer" to the literature. They synthesized the poly (amidoamine) dendrimer that is abbreviated as PAMAM (Figure 1.24). The same year, Newkome and his coworkers published the synthesis of molecules called "arborols". With arborols, three branching units at each generation can be obtained [17]. With these pioneering publications, dendrimers have gained great attention and a large number of papers have been published related to dendrimers and their different applications.

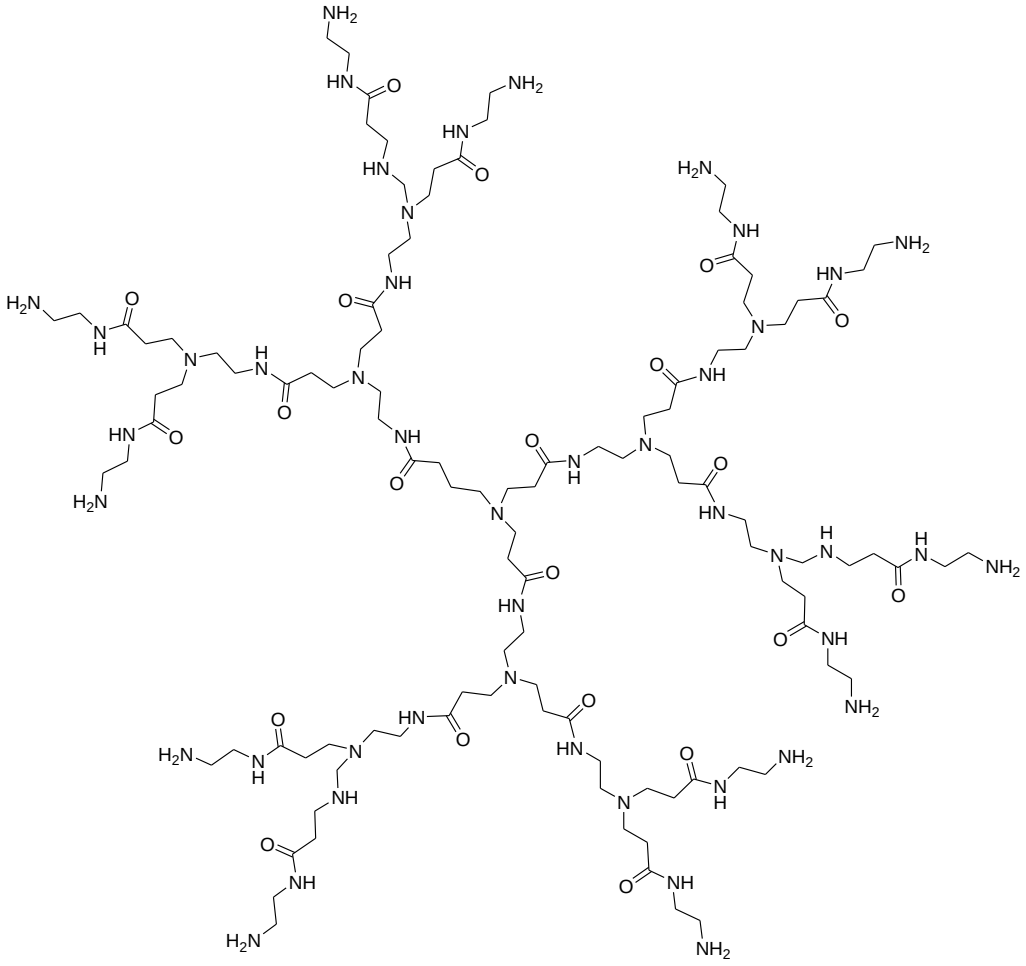


Figure 1.24. Second generation PAMAM dendrimer.

1.6.3. Synthesis Methods

Dendrimers can be synthesized using either a “divergent” or a “convergent” methods. In the “divergent” method, synthesis of the dendrimers start from the core by attachment of branching units and finally installation the surface groups at the periphery. In convergent method, on the other hand, dendrimers start with the attachment of surface groups onto the branching units and further coupling of these small dendrons to the multivalent core molecule results in formation of the desired dendrimer. These two methods of synthesis will be explained in detail in the following parts.

1.6.3.1. Divergent Method.

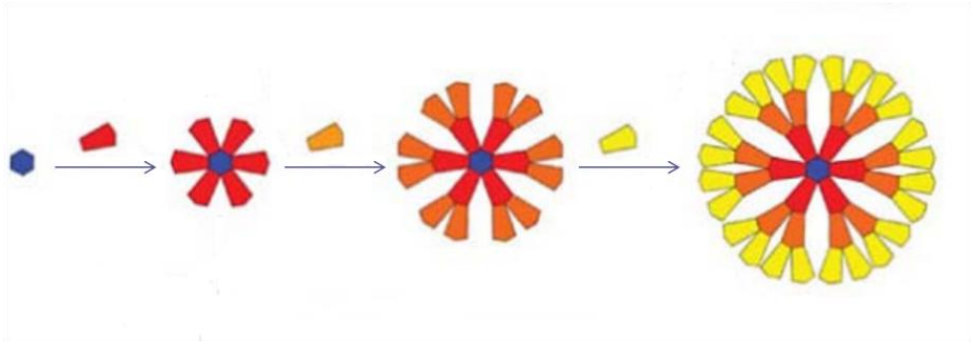


Figure 1.25. Schematic representation of divergent method [18].

In divergent method, the synthesis of dendrimer starts with a core molecule, that should have a multifunctional molecule to provide the branched structure. With the addition of repeating units to the core molecule, the growth of dendrimer continues. Thus, the final shape of the dendrimer is formed. According to the usage of the dendrimers, monomers that have one active and two or more inactive sites are chosen. After the attachment of the repeating unit to the core molecule, the inactive sites on the monomer are activated and reacting the newly formed compound with the same monomer in order to synthesize the dendrimer with higher generation (Figure 1.14). For example, PAMAM (Figure 1.13), dendrimers are usually synthesized via divergent method [19].

Because the molecular weight of the dendrimer doubles with the production of each generation, divergent synthesis is a desirable method for large scale dendrimer synthesis. In order to drive the coupling reactions to completion, this brings about the necessity of using a large excess of monomer. Unreacted monomers can be removed by conventional separation techniques such as distillation, chromatography, crystallization etc.

In addition to the advantages, the divergent method has a major disadvantages that is the loss of structural control upon reaching the higher generations. This situation arises from the rising number of coupling reactions that needs to be accomplished as the dendrimer generation increases. For instance, in order to convert a second generation

PAMAM dendrimer into a third generation PAMAM dendrimer, 24 coupling reactions must be completed on the same molecule. Complete conversion for such high number of reactions per molecule is very difficult and therefore it leads to polydispersity in the formed product. Furthermore, dendrimers synthesized possess a lot of structural defects; that are often very difficult to detect and quantify.

1.7. Core Functionalized Dendrons

Dendrimers are monodispered, highly branched, globular structure without defects have made them attractive building blocks in macromolecular chemistry. The ability to selectively append different functional groups onto the surface renders them multifunctional as opposed to being simply multivalent, while the well-defined multivalent construct of the dendrimer surface itself makes this class of macromolecules unique. Recent developments have focused on both the efficient core and surface functionalization of dendrimers and the synthesis of dendrimers with orthogonal reactive groups.

Hawker *et al*. utilized the copper catalyzed [3 + 2] Huisgen-type cycloaddition reaction between two different core functionalized dendrons. One of them has an alkyne group at its core and the other dendron has an azide unit at its focal point. With using these two dendrons, orthogonally functionalizable surface block dendrimers were obtained (Figure 1.26) [20].

Sanyal *et al*. utilized Diels-Alder based synthetic strategies to obtain unsymmetrical dendrimers. For this aim, they used two different dendrons. One of them has a maleimide group at its core and the other dendron has a furan unit at its focal point (Figure 1.27) [21].

As another example for Dials-Alder “click” chemistiry, Sanyal and his coworkers used the anthracene and furan groups to obtain dendron-polymer conjugate. For this aim, anthracene containing polyester dendron and furan-protected maleimide-containing PEG polymers were used (Figure 1.28) [22].

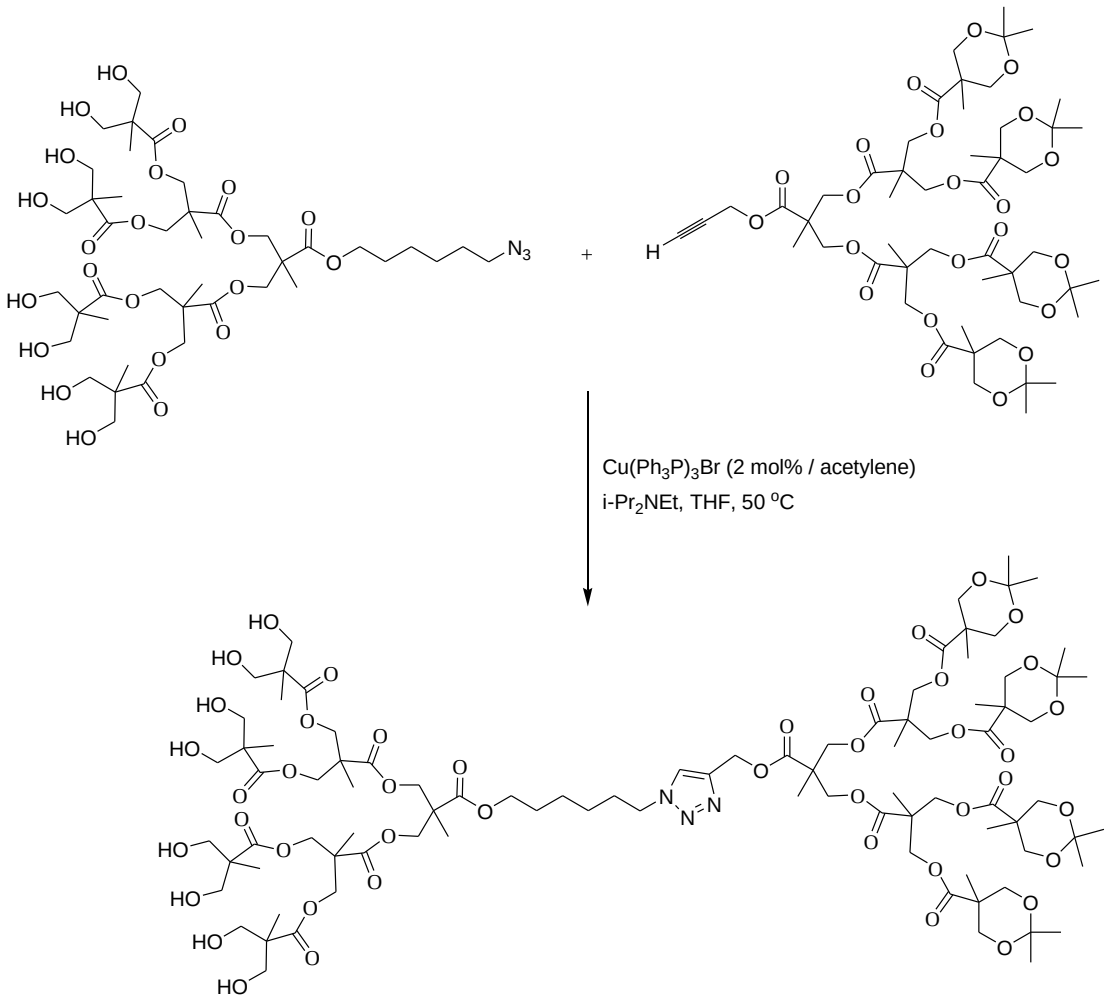


Figure 1.26. Synthesis of unsymmetrical dendrimer using click chemistry.

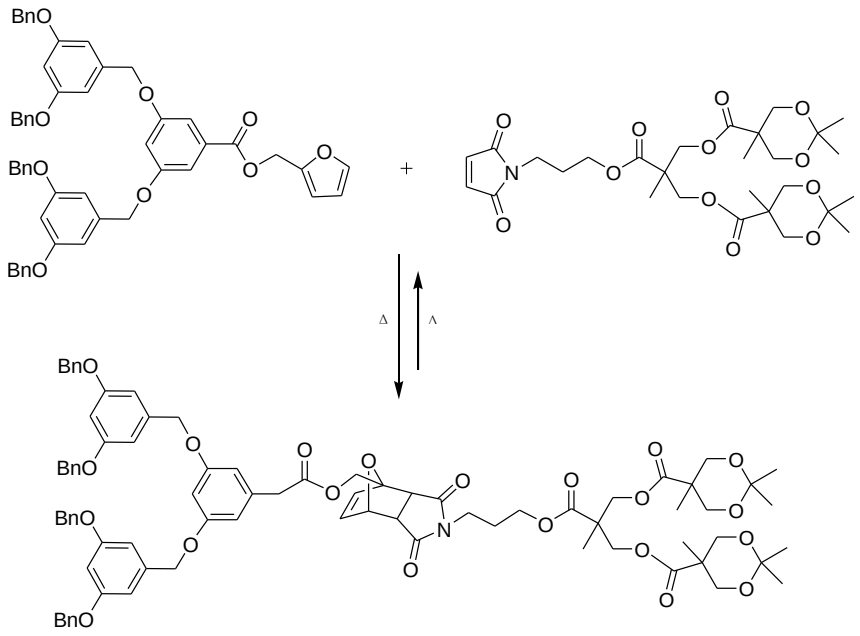


Figure 1.27. Synthesis of unsymmetrical dendrimer using Diels-Alder reaction.

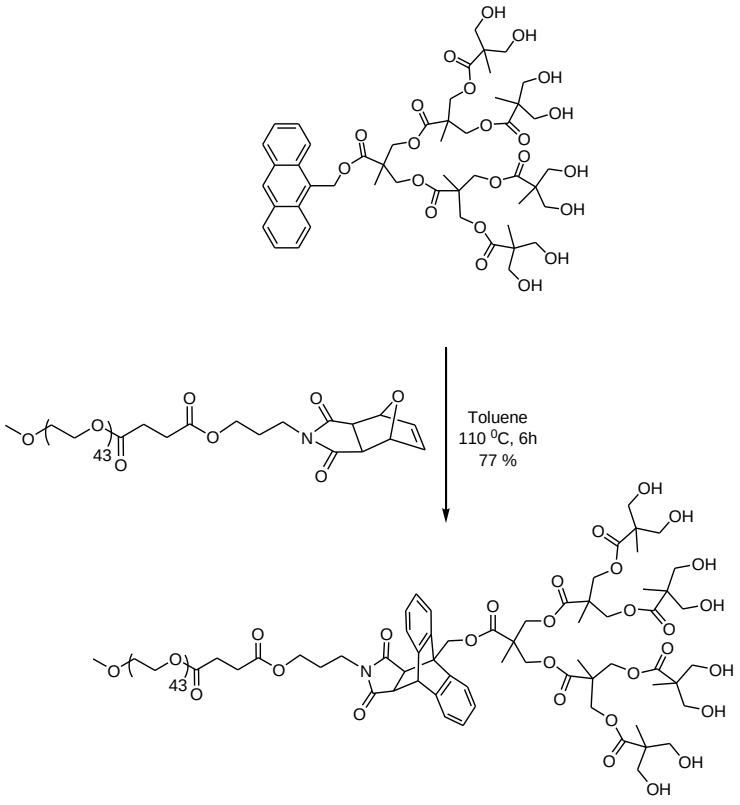


Figure 1.28. Synthesis of dendron–polymer conjugate via the Diels–Alder reaction.

As an alternative strategy for the usage of core functionalized dendrons, thiol-ene click chemistry can be an example. Malkoch *et al.* utilized thiol and double bond containing polyester dendrons to synthesize an unsymmetrical dendrimer (Figure 1.29) [23].

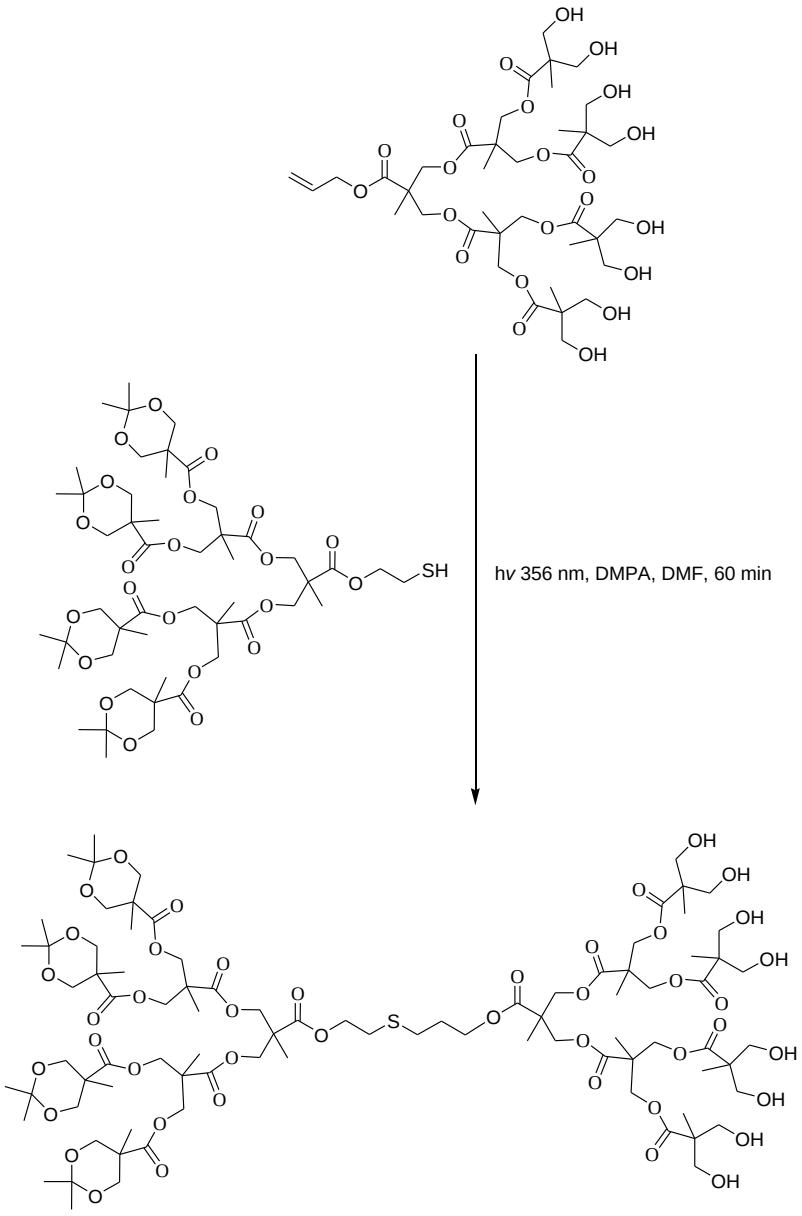


Figure 1. 29. Synthesis of an asymmetrical dendrimer via thiol-ene click chemistry.

2. AIM OF THE STUDY

The main aim of this thesis to design novel dendritic molecules that can be used to obtain multiarm polymers containing a thiol reactive core that will be cleavable. To achieve the synthesis of such multiarm polymers, dendrons containing a thiol-reactive focal point needs to be synthesized. These dendrons will be appended with initiators for polymerization to obtain multiarm polymers. The focal point of thus obtained multi-arm polymers will contain a thiol-reactive group that can be cleaved when desired.

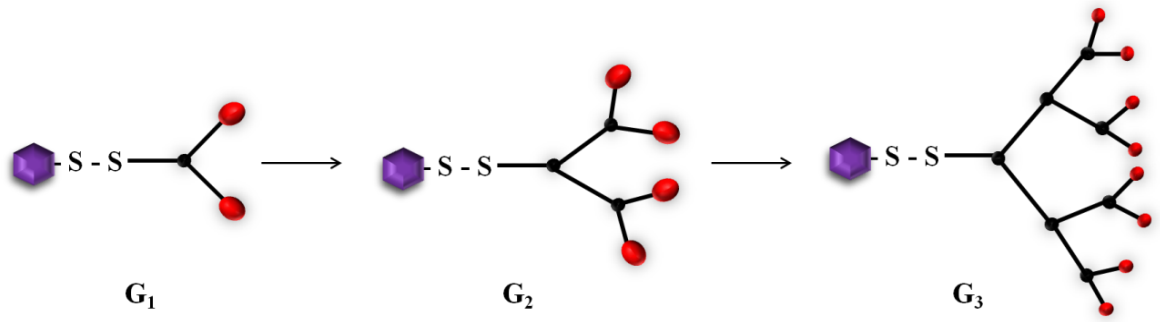


Figure 2.1. Schematic representation of 1st, 2nd and 3rd generation dendrons.

In particular, this thesis focuses on the synthesis of novel dendrons that contain the thiol-reactive functional group at their focal point. Three generations of polyester dendrons that are biocompatible and biodegradable will be synthesized and characterized. After synthesizing three generations of polyester dendrons, the effect of the number of generations on the rate of disulfide exchange reactions at the focal point is explored. For this aim, these dendrons which have pyridyl disulfide

group (PDS) at their core are reacted with thiol containing molecules via disulfide exchange reaction. During this reaction, thiopyridone is liberated as a byproduct and this release of thiopyridone can be monitoring with UV spectrometry.

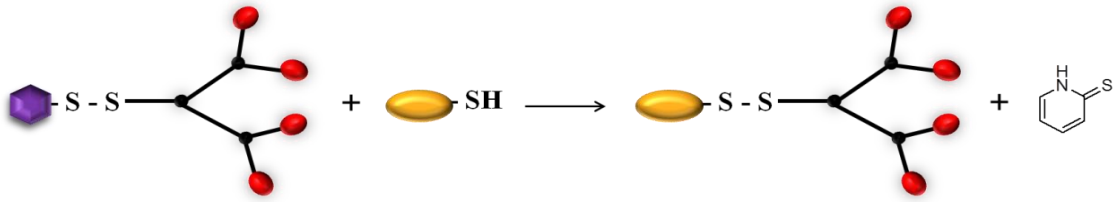


Figure 2.2. Schematic representation of disulfide exchange reaction.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Pyridyl Disulfide Based Polymers

In order to synthesize the desired dendrons, first hydroxyethylpyridlydisulfide (HPDS) was synthesized according to literature procedures [24]. For the synthesis of HPDS as a core molecule in the divergent synthesis of polyester dendron, aldrithiol-2 was reacted with mercaptoethanol in methanol in the presence of acetic acid (Figure 3.1). The product was obtained in high purity as evident from its ^{1}H NMR (Figure 3.2) and FTIR spectra (Figure A.15).

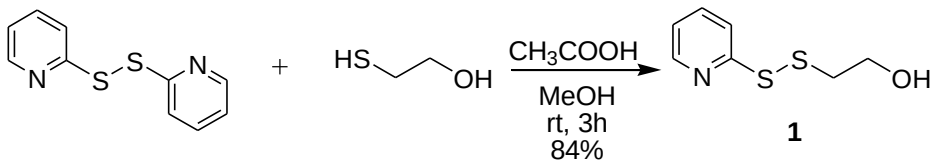


Figure 3.1. Synthesis of HPDS molecule.

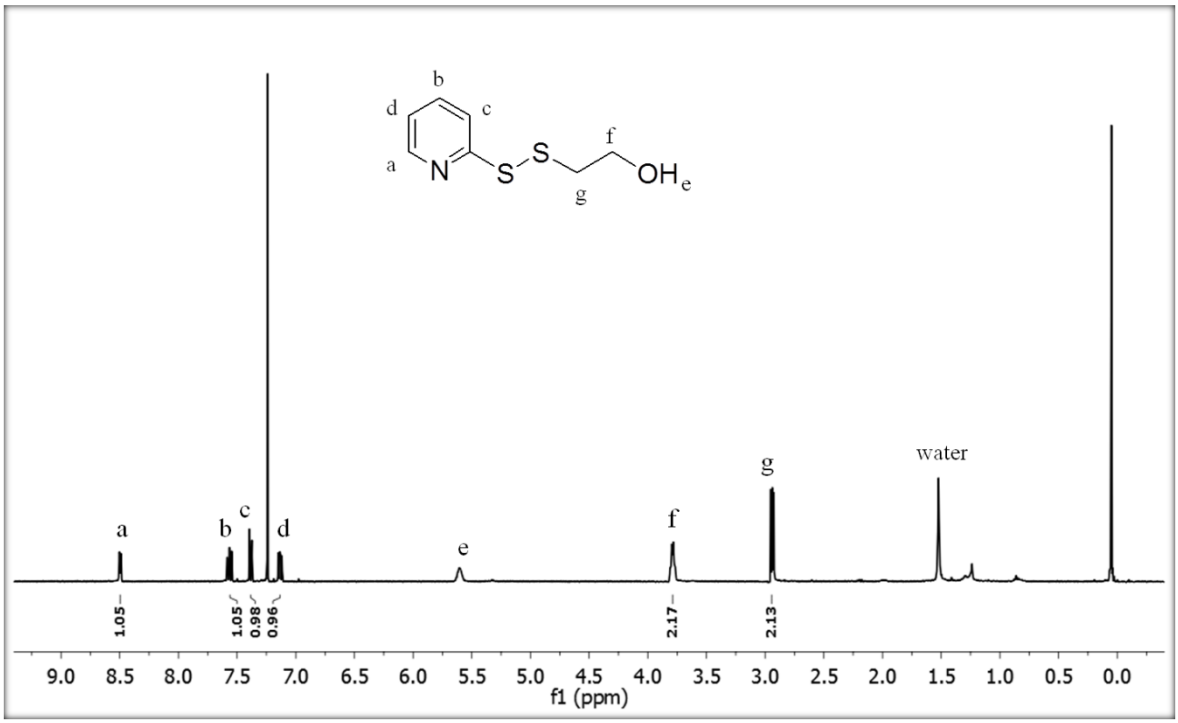


Figure 3.2. ^{1}H NMR spectrum of HPDS molecule (**1**).

According to the ^{1}H NMR, peaks between 8.50 – 7.12 ppm correspond to aromatic protons, while the proton resonances around 3.80 and 2.94 ppm belong to the two different CH_2 groups on the HDPS molecule.

3.2. Synthesis of the 1st Generation Dendrons

In order to synthesize the 1st generation pyridyl disulfide functionalized protected dendron, **1**, acetonide-protected 2,2-bis(hydroxymethyl)propionic acid was prepared according to literature [25]. This molecule was reacted with HPDS in anhydrous CH_2Cl_2 and in the presence of EDCI and DMAP (Figure 3.3). For the synthesis of the 1st generation pyridyl disulfide functionalized dihydroxyl group containing dendron (**5**), compaund **4** was reacted in THF using 1M HCl (Figure 3.3). The product was obtained in high purity as evident from its ^{1}H NMR (Figure 3.4), ^{13}C NMR (Figure A.4 and Figure A.6), FTIR spectra (Figure A.16 and Figure A.18) and HRMS (Figure A.22 and Figure A.24).

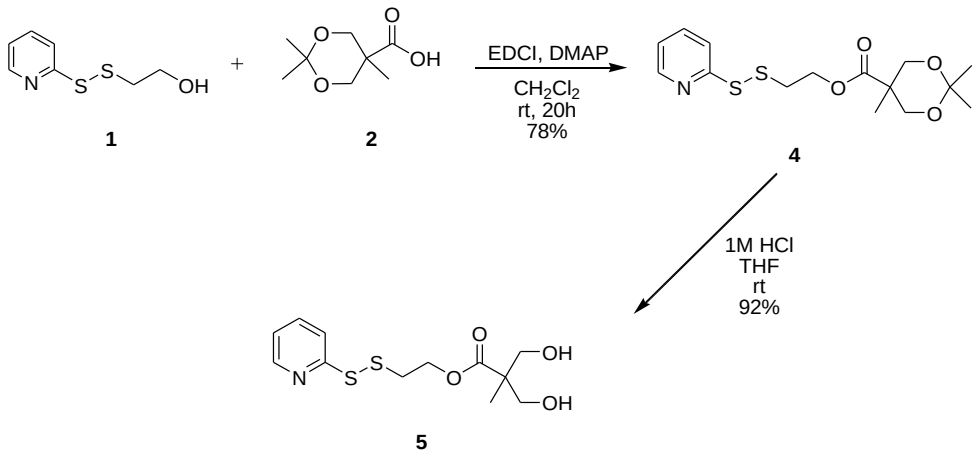


Figure 3.3. Synthesis of 1st generation dendrons.

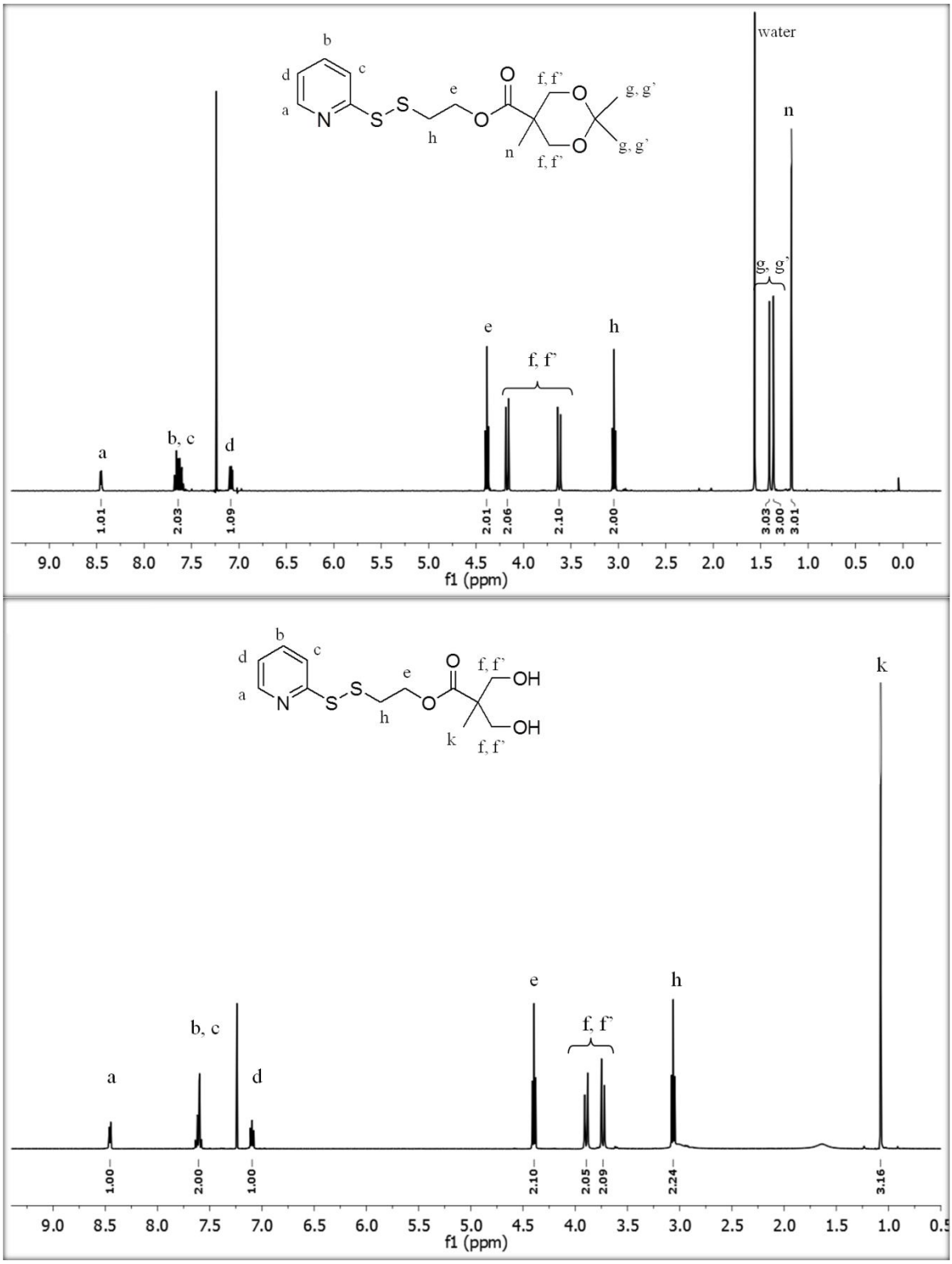


Figure 3.4. ^{1}H NMR spectra of 1st generation dendrons (**4** and **5**).

The proton assignments from the ^{1}H NMRs of dendrons **4** and **5** can be seen in Figure 3.4. For both of these dendrons, the proton resonances between 8.50 – 7.00 ppm belong to the aromatic protons, peaks around 4.5 and 3.0 ppm correspond to two different CH_2 groups on the core, and the peaks around 4.0 and 3.5 ppm belong to CH_2 groups of the fragment belonging to the bis-methyl propanoic acid. The singlet proton resonances at 1.41 and 1.37 ppm for dendron **4** belong to CH_3 groups of the acetal protecting group. After deprotection in acidic media, due to removal of the dimethylacetal group, these peaks disappears in the ^{1}H NMR spectrum of dendron **5** .

3.3. Synthesis of the 2nd Generation Dendrons

In order to synthesize 2nd generation pyridyl disulfide functionalized protected dendron, **6**, first acetonide-protected 2,2-bis(hydroxymethyl)propionic anhydride (**3**) was synthesized according to literature procedure [26]. The dihydroxy group containing first generation dendron **5** was reacted with the anyhdride **3** in anhydrous CH_2Cl_2 in the presence of DMAP and pyridine (Figure 3.5). The product was obtained in high purity as evident from its ^{1}H NMR (Figure 3.6 and Figure 3.7), ^{13}C NMR (Figure A.8 and Figure A. 10), FTIR spectra (Figure A.18 and Figure A.19) and HRMS (Figure A.24 and Figure A. 25).

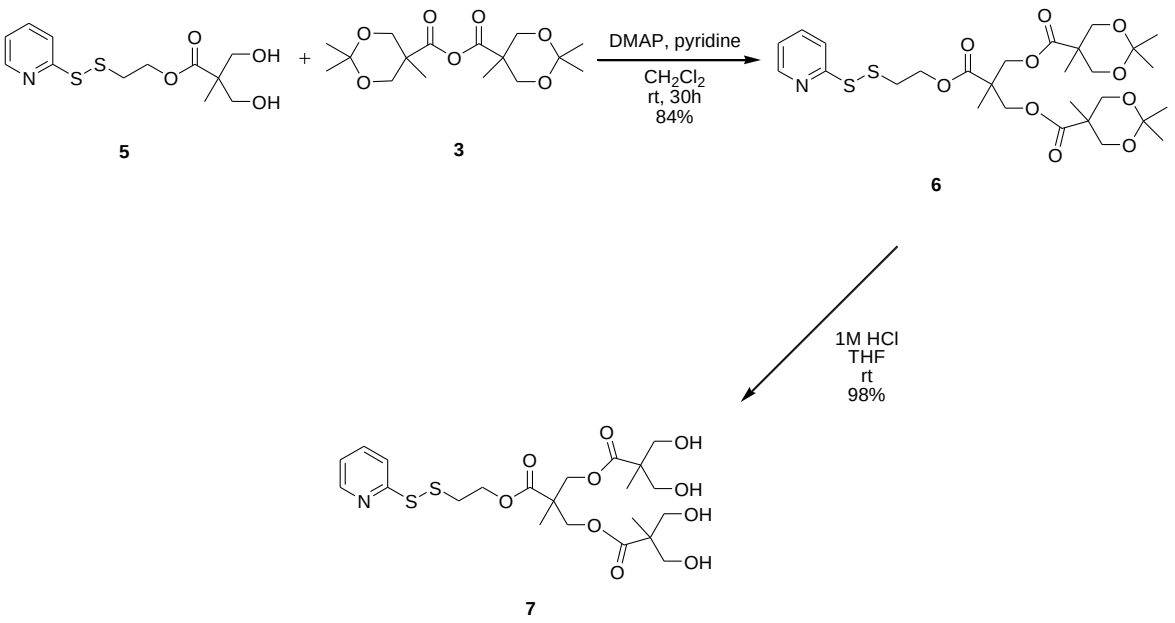


Figure 3.5. Synthesis of 2nd generation dendrons.

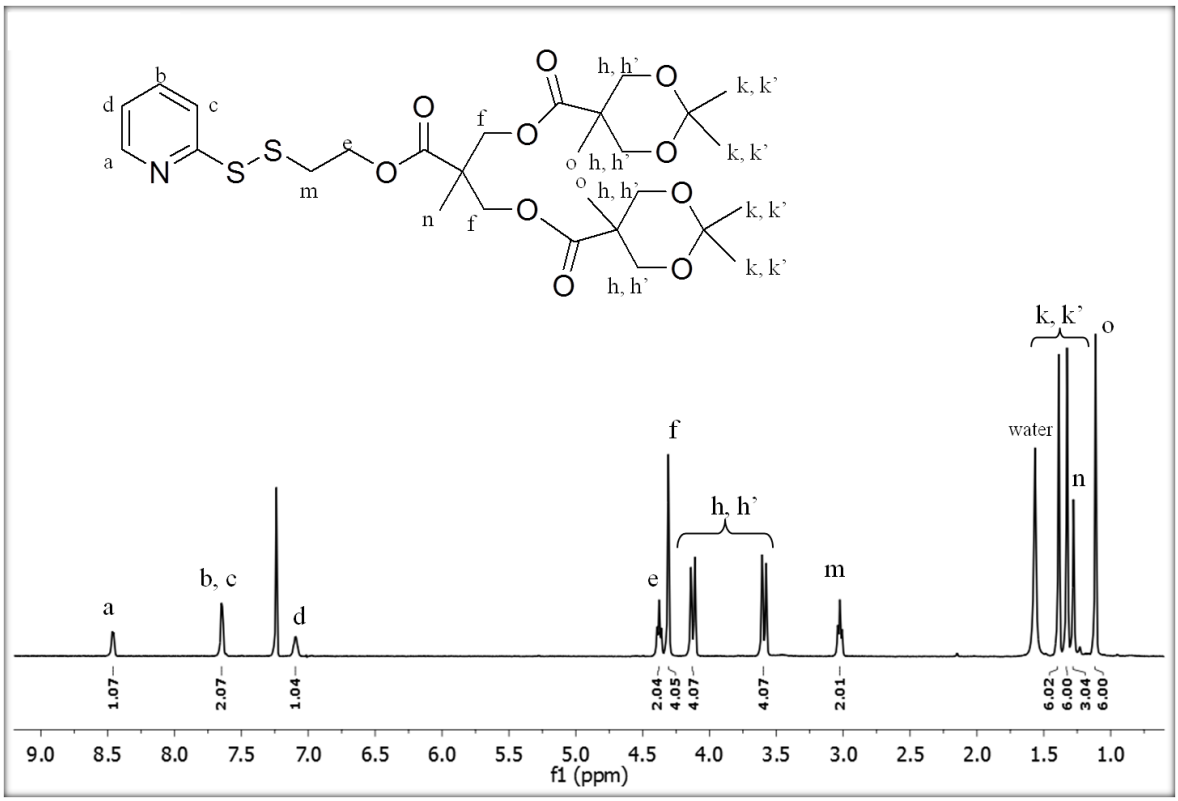


Figure 3.6. ^{1}H NMR spectrum of 2nd generation dendron **6**

The proton assignment from the ^{1}H NMR of dendron **6** can be seen in Figure 3.6. For that dendron, the proton resonances between 8.50 – 7.00 ppm belong to the aromatic protons, peaks around 4.38 and 3.0 ppm correspond to two different CH_2 groups on the core, the singlet proton resonance at 4.31 ppm belongs to the ester protons and the peaks around 4.12 and 3.60 ppm correspond to CH_2 groups of the fragment belonging to the bis-metyl propanoic acid. The singlet proton resonances at 1.39, 1.33, 1.28 and 1.11 ppm for dendron **6** belong to CH_3 groups of the acetal protecting groups.

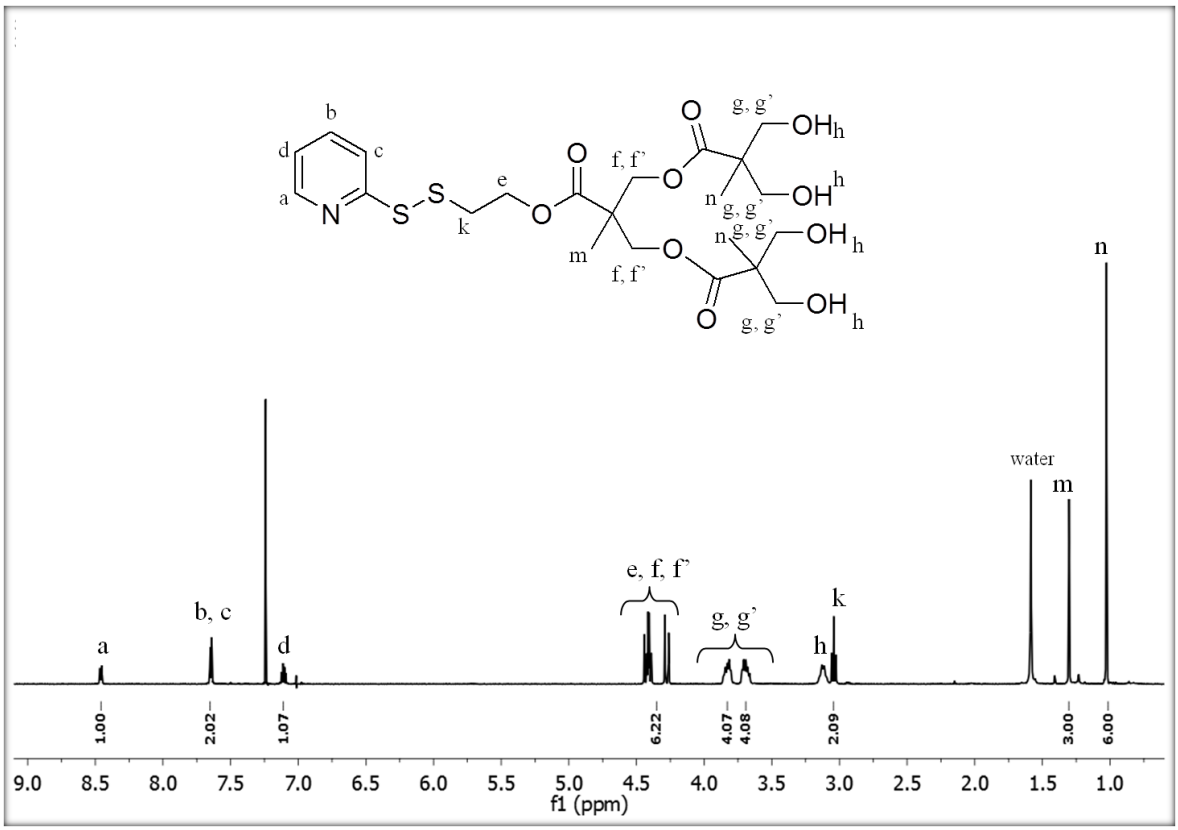


Figure 3.7. ^{1}H NMR spectrum of 2nd generation dendron **7**

After deprotection of dendron **6** in acidic media, the proton assignment from the ^{1}H NMR of dendron **7** can be seen in Figure 3.7. For that dendron, the proton resonances between 8.50 – 7.00 ppm belong to the aromatic protons, peaks around 4.50 and 3.0 ppm correspond to two different CH_2 groups on the core, the proton resonance at 4.50 ppm belongs to the ester protons and the peaks between 3.80 and 3.65 ppm correspond to CH_2 groups of the fragment belonging to the bis-metyl propanoic acid. The singlet proton

resonances at 1.30 and 1.03 ppm for dendron **6** belong to CH_3 groups of the acetal protecting groups. According to the ^1H NMR of dendron **7**, it can be seen the removal of the dimethylacetal groups.

3.4. Synthesis of the 3rd Generation Dendrons

In order to synthesize the 3rd generation pyridyl disulfide functionalized protected dendron, (**5**), first the acetonide-protected 2,2-bis(hydroxymethyl)propionic anhydride was reacted with tetra-hydroxy group containing dendron, **4**, in CH_2Cl_2, in the presence of DMAP and pyridine (Figure 3.8) and this dendron was deprotected, in MeOH, in the presence of 18M H_2SO_4. The third generation dendron **8** and **9** was obtained in high purity as evident from its ^1H NMR (Figure 3.9 and Figure 3.10), ^{13}C NMR (Figure A.12 and Figure A.14), FTIR spectra (Figure A.20 and Figure A.21), HRMS (Figure A.26).

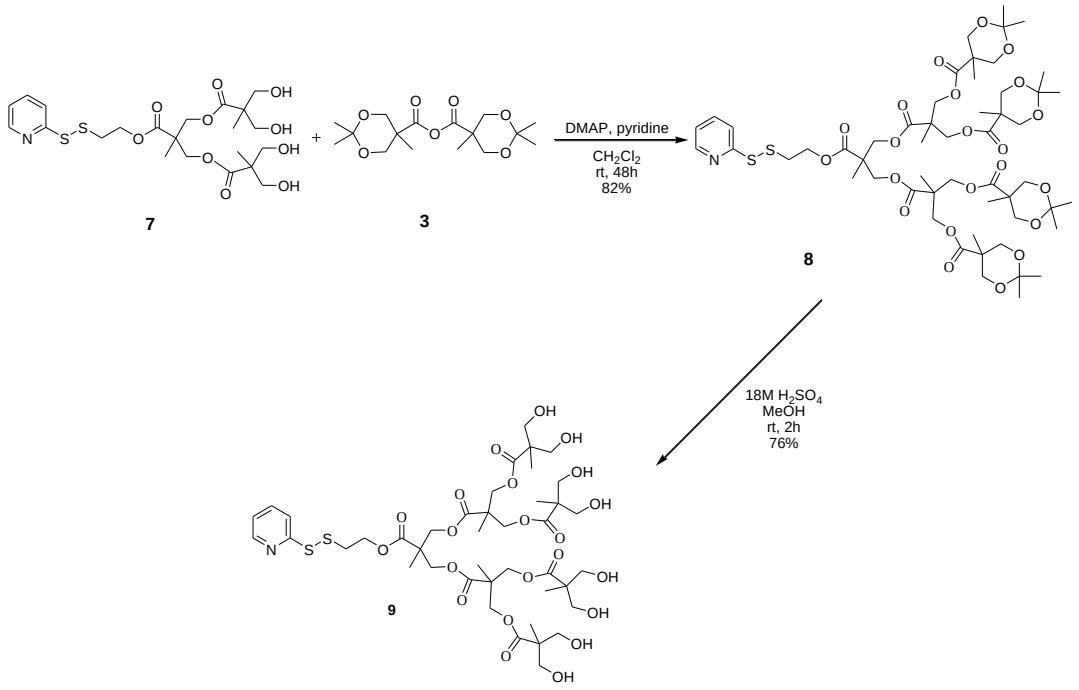


Figure 3.8. Synthesis of 3rd generation dendrons.

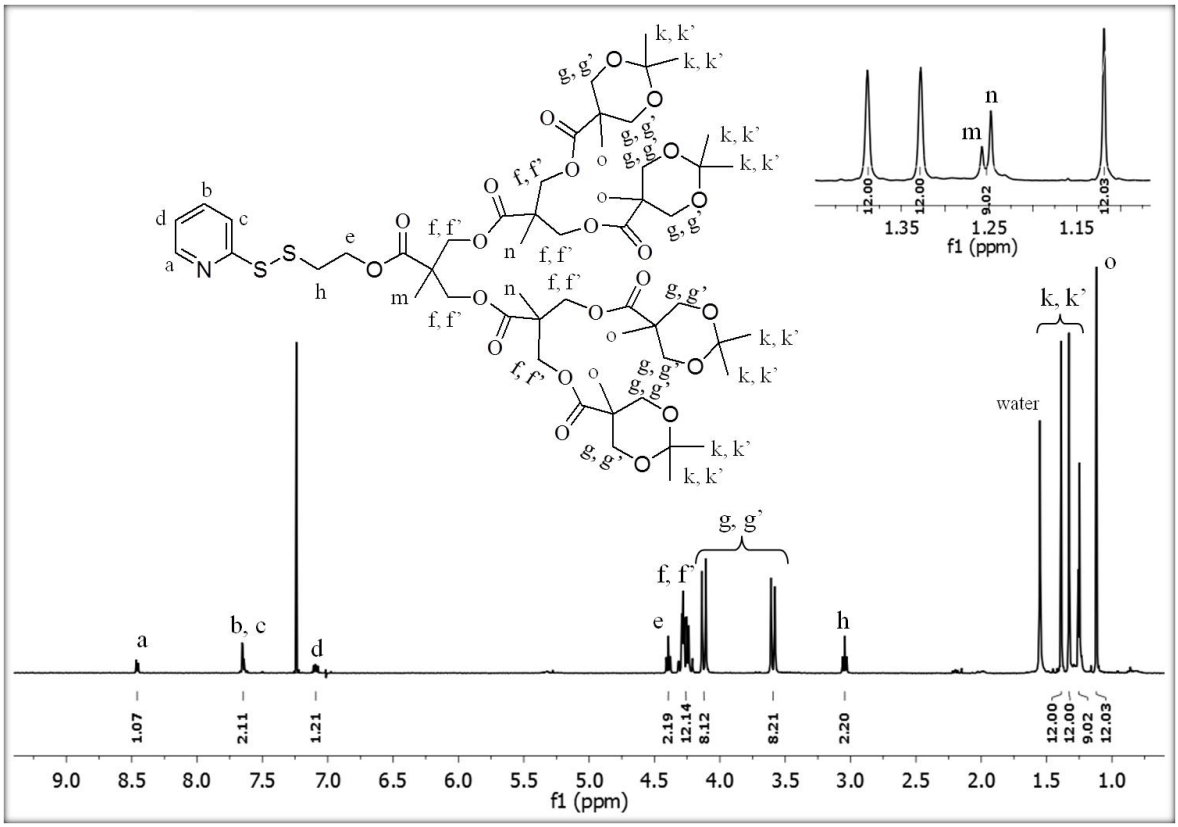


Figure 3.9. ^{1}H NMR spectrum of 3rd generation dendron **8**

The proton assignment from the ^{1}H NMR of dendron **8** can be seen in Figure 3.8. For that dendron, the proton resonances between 8.50 – 7.00 ppm belong to the aromatic protons, peaks around 4.39 and 3.04 ppm correspond to two different CH_2 groups on the core, the proton resonance around 4.25 ppm belongs to the ester protons and the peaks between 4.13 and 3.60 ppm correspond to CH_2 groups of the fragment belonging to the bis-metyl propanoic acid. The singlet proton resonances at 1.39, 1.33, 1.26, 1.25 and 1.12 ppm for dendron **8** belong to CH_3 groups of the acetal protecting groups.

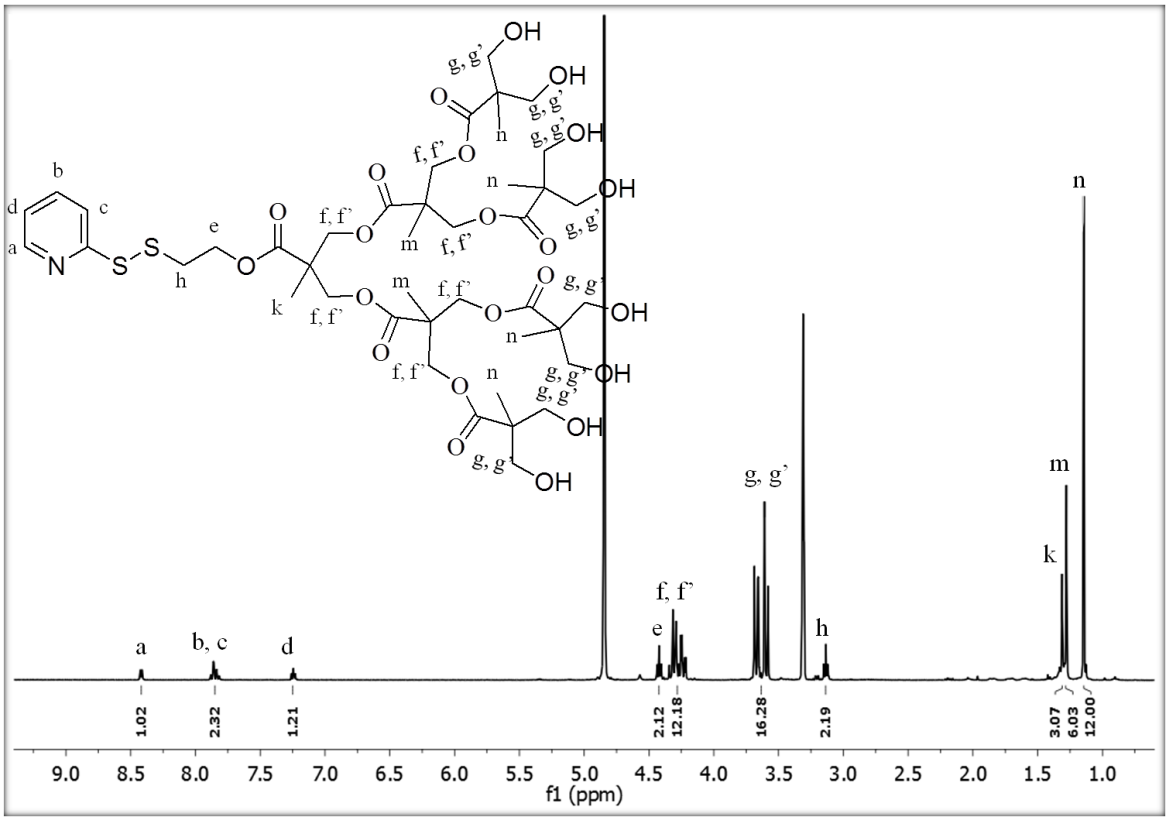


Figure 3.10. ^{1}H NMR spectrum of 3rd generation dendron **9**

After deprotection of dendron **8** in acidic media, the proton assignment from the ^{1}H NMR of dendron **9** can be seen in Figure 3.9. For that dendron, the proton resonances between 8.50 – 7.00 ppm belong to the aromatic protons, peaks around 4.42 and 3.50 ppm correspond to two different CH_2 groups on the core, the proton resonance at 4.25 ppm belongs to the ester protons and the peaks between 3.69 and 3.58 ppm correspond to CH_2 groups of the fragment belonging to the bis-metyl propanoic acid. The singlet proton resonances at 1.31, 1.28 and 1.14 ppm for dendron **6** belong to CH_3 groups of the acetal protecting groups. According to the ^{1}H NMR of dendron **7**, it can be seen the removal of the dimethylacetal groups.

3.5. Disulfide Exchange Reaction of G_3 Dendron via Free Thiol Containing Molecule

In order to perform the conjugation studies between the dendron containing thiol-reactive functional group at its core and free thiol containing molecule, the third generation polyester dendron were reacted with thioglycerol via disulfide exchange reaction (Figure 3.11). During this reaction, thiopyridone was liberated as a byproduct and the release of thiopyridone was monitoried with UV spectrometry (Figure 3.12).

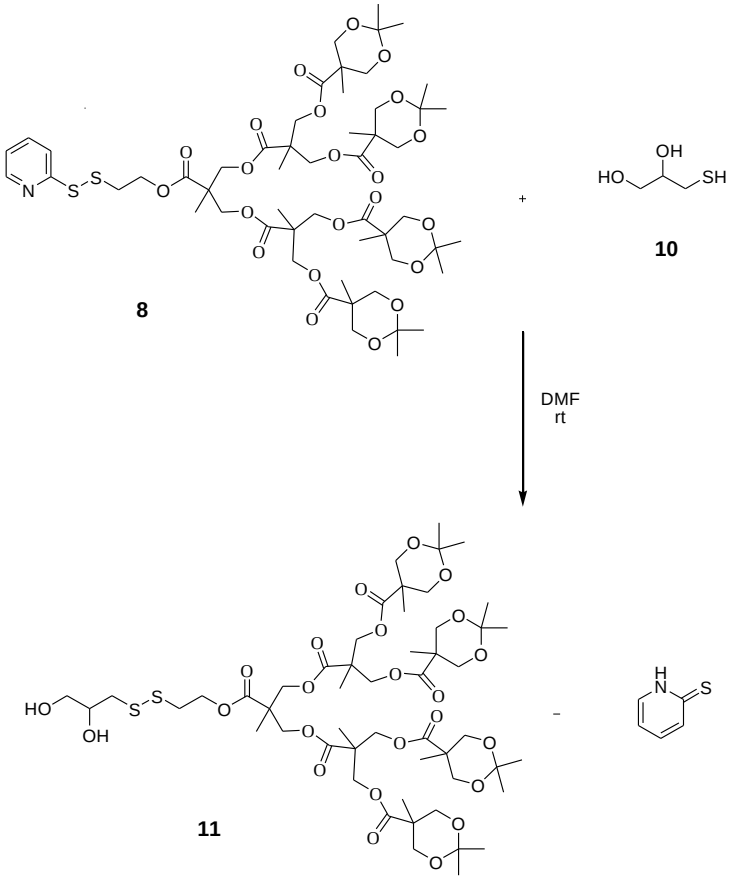


Figure 3.11. Disulfide exchange reaction

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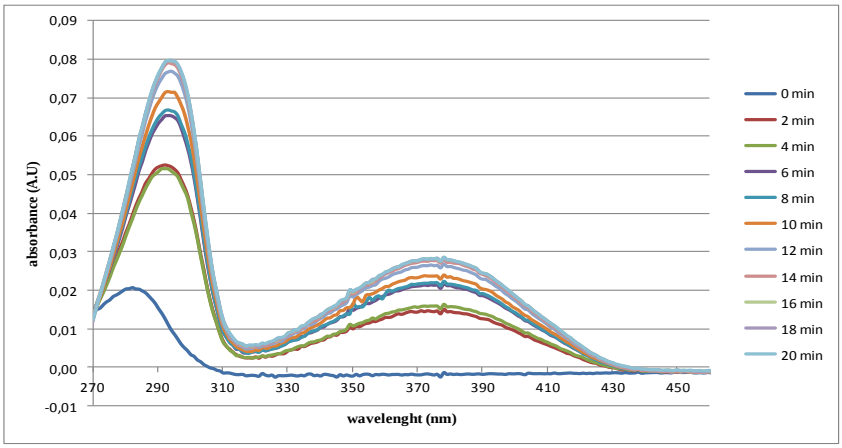


Figure 3.12. Thiopyridone release profile of G_3 dendron monitored at 375 nm

According to the UV spectrometry data, at time 0, the third generation dendron has not any absorbance at 375 nm because the thiopyridone molecule was intact. After thioglycerol was added to the reaction media, thiopyridone starts to liberate by giving an increasing absorbance at its characteristic wavelength, 375 nm, with increasing time.

4. EXPERIMENTAL

4.1. General Method and Materials

Aldrithiol-2, 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), 7M NH_3 in methanol and 4-dimethylaminopyridine (DMAP) were purchased from Aldrich. p-Toluenesulfonic acid (p-TsOH), 2,2-dimethoxypropane, N,N'-Dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) were purchased from Alfa Aesar. 2-mercaptoethanol, triethylamine and pyridine were purchased from Merck. All solvents were purchased from Merck and used as obtained without further purification unless otherwise noted. The dendrons characterizations involved ^{1}H NMR and ^{13}C NMR spectroscopy (Varian 400 MHz), Fourier transform infrared (ATR-FT-IR) spectroscopy (Thermo Fisher Scientific Inc. Nicolet 380) and HRMS (Agilent, 1200/6210).

4.2. Synthesis of Polyester Dendron

4.2.1. Synthesis of HPDS

Aldrithiol-2 (5.0 g, 23.0 mmol) was dissolved in 15 mL methanol. Glacial acetic mercaptoethanol (0.81 mL, 11.5 mmol) in 3 mL methanol was added drop-wise to the mixture at room temperature. The clear solution was stirred for 3 h at room temperature under N_2. After reaction was completed, all volatiles were evaporated and the residue was

extracted with water until the TLC shows only two spots. The crude product obtained as a yellow oil was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane as the eluent. The pure product was dried under *vacuo* yielding compound **1** as a yellow oil (1.81 g, 84% yield) (Figure 4.1) [23]. ^{1}H NMR ($CDCl_3$, δ, ppm) 8.50 – 8.49 (s, 1H), 7.58 – 7.54 (s, 1H), 7.40 – 7.37 (s, 1H), 7.15 – 7.12 (s, 1H, aromatic H meta to nitrogen), 5.60 (s, 1H, OH), 3.81 – 3.77 (m, 2H, SCH_2CH_2OH), 2.94 (t, 2H, J = 4.0 Hz, SCH_2CH_2).

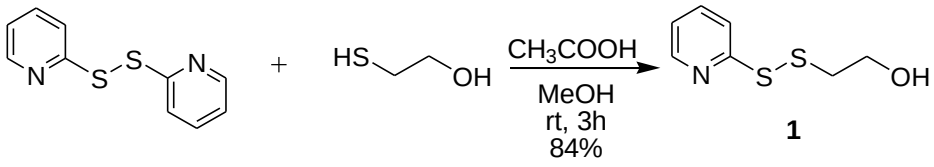


Figure 4.1. Synthesis of HPDS molecule.

4.2.2. Synthesis of Acetonide-Protected 2,2-bis(hydroxymethyl)propionic Acid

Bis-MPA (5.0 g, 37.0 mmol) and a catalytic amount of p-toluenesulfonic acid (p-TsOH) were added to a 100 mL round bottom flask (RBF) under N_2. 20 mL dry acetone that was dried over molecular sieves was added to the mixture. 2,2-dimethoxypropane (6.9 mL, 56.0 mmol) was added to the clear solution. The mixture was stirred for 3 h at room temperature under N_2. At the end of the reaction, to neutralize the excess p-TsOH, 0.3 mL Et_3N (triethylamine) was added to the round bottom flask and acetone was evaporated. The white residue was diluted to 25 mL with CH_2Cl_2 and extracted with water (2 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4 and all volatiles were evaporated. The white crude product was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane (60/40, v/v) as eluent. The pure product was dried under *vacuo* yielding as a white solid (5.03 g, 78% yield) (Figure 4.2) [25]. ^{1}H NMR ($CDCl_3$, δ, ppm) 4.16 (d, 2H, J = 12.0 Hz, OCH_2), 3.68 (d, 2H, J = 12.0 Hz, OCH_2), 1.44 (s, 3H, $C(CH_3)_2$), 1.40 (s, 3H, $C(CH_3)_2$).

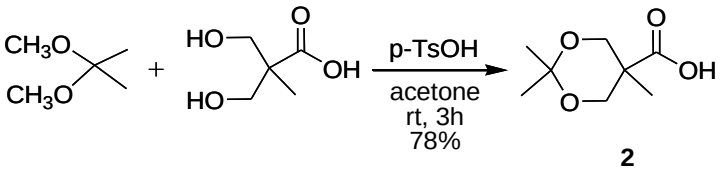


Figure 4.2. Synthesis of acetonide-protected 2,2-bis(hydroxymethyl)propionic acid.

4.2.3. Acetonide-Protected 2,2-bis(hydroxymethyl)propionic Anhydride

Compound **2** (2.7 g, 15.0 mmol) was dissolved in 35 mL CH_2Cl_2 under N_2. A solution of DCC (1.55 g, 7.5 mmol) in 15 mL CH_2Cl_2 was added to the clear solution under N_2. The mixture was stirred for 20 h at room temperature. After reaction was completed DCU was removed via filtration and CH_2Cl_2 was evaporated. The residue was washed with cold ethyl acetate (EtOAc) and filtrated again to get rid of DCU. EtOAc was evaporated and the pure product was dried under *vacuo* (2.65 g, 98% yield) (Figure 4.39) [26].

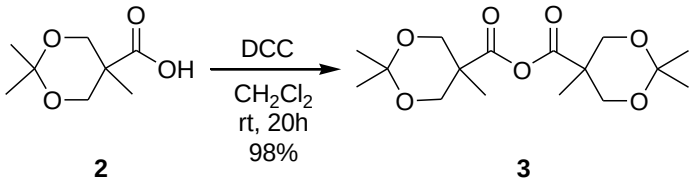


Figure 4.3. Synthesis of acetonide-protected 2,2-bis(hydroxymethyl)propionic anhydride.

4.2.4. Synthesis of 1st Generation Pyridyl Disulfide Functionalized Protected Dendron

Compound **2** (0.505 g, 2.9 mmol), EDCI (0.612 g, 3.19 mmol) and DMAP (0.106 g, 0.87 mmol) were added to a 25 mL round bottom flask (RBF). In order to dissolve these

reagents, 10 mL CH_2Cl_2 was also added to the RBF. A solution of HPDS (1.63 g, 8.7 mmol), compound **1**, in 4 mL CH_2Cl_2 was added to the clear mixture. After stirring for 20 h at room temperature, reaction mixture was diluted to 30 mL with CH_2Cl_2 and extracted with 7% $NaHCO_3$ solution (3 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4. The crude product was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was dried under *vacuo* yielding (0.78 g, 78% yield) as a yellow oil (Figure 4.4). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.45 (d, 1H, *J* = 4), 7.68 – 7.58 (m, 2H), 7.09 (d, 1H, *J* = 16), 4.39 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2), 4.17 (d, 2H, *J* = 12.0 Hz, OCH_2), 3.62 (d, 2H, *J* = 12.0 Hz, OCH_2), 3.05 (t, 2H, *J* = 6.4 Hz, SCH_2), 1.41 (s, 3H, $C(CH_3)_2$), 1.37 (s, 3H, $C(CH_3)_2$), 1.18 (s, 3H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 174.0, 159.6, 149.8, 137.1, 120.9, 119.8, 98.1, 66.0, 62.5, 41.9, 37.3, 24.7, 22.5, 18.6. HRMS m/z: $[M + H]^+$ Calcd for $C_{15}H_{21}NO_4S_2H$ 344.0990 ; Found 344.0994; $[M + Na]^+$ Calcd for $C_{15}H_{21}NO_4S_2Na$ 366.0810; Found 366.0815. FTIR (cm^{-1}): 2990.3, 1723.8.

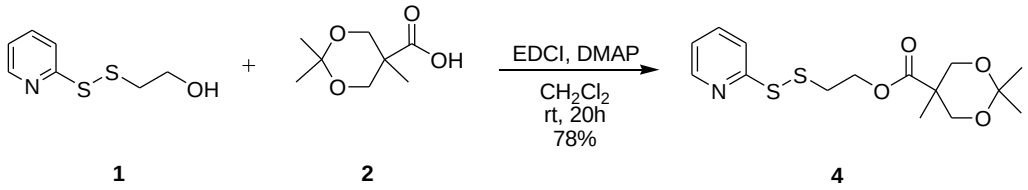


Figure 4.4. Synthesis of dendron **4**.

4.2.5. Synthesis of 1st Generation Pyridyl Disulfide Functionalized Deprotected Dendron

Compound **4** (2.08 g, 6.05 mmol) was dissolved in 20 mL THF and then 20 mL 1M HCl was also added to the mixture. The resulting mixture was stirred at room temperature

until the consumption of compound **4** was observed via TLC. At the end of the reaction, THF was evaporated and the residue was diluted to 50 mL with CH_2Cl_2. The crude was extracted with 10% Na_2CO_3 solution (2 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and all CH_2Cl_2 was evaporated. The crude product was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was dried under *vacuo* yielding as a yellow oil (1.74 g, 92% yield) (Figure 4.5). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.46 – 8.44 (m, 1H), 7.64 – 7.58 (m, 2H), 7.11 – 7.08 (m, 1H), 4.40 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2), 3.90 (d, 2H, *J* = 11.2 Hz, OCH_2), 3.73 (d, 2H, *J* = 11.2 Hz, OCH_2), 3.06 (t, 2H, *J* = 6.4, SCH_2), 1.08 (s, 3H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 175.5, 159.0, 149.8, 137.1, 121.1, 120.2, 68.4, 62.4, 49.4, 37.7, 17.1. HRMS m/z: $[M + H]^+$ Calcd for $C_{12}H_{17}NO_4S_2H$ 304.0677; Found 304.0657; $[M + 2H]^+$ Calcd for $C_{12}H_{17}NO_4S_2H_2$ 305.0755; Found 305.0688. FTIR (cm^{-1}): 3357.0, 2940.1, 1721.8.

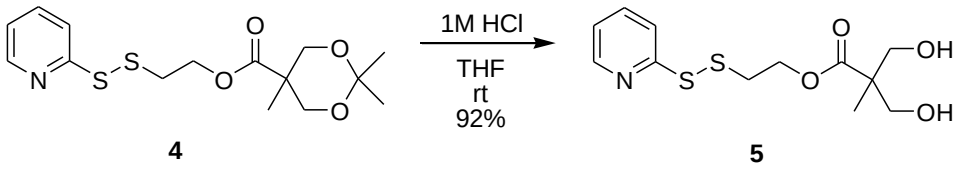
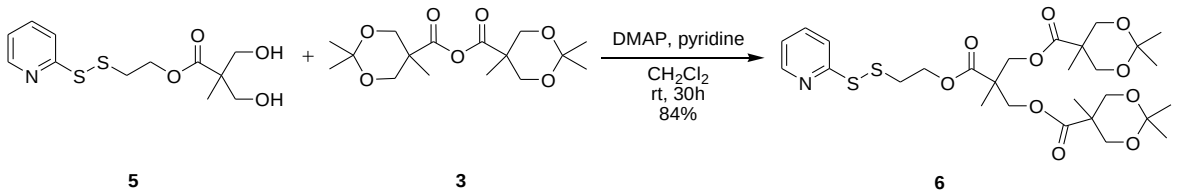


Figure 4.5. Synthesis of dendron **5**.

4.2.6. Synthesis of 2nd Generation Pyridyl Disulfide Functionalized Protected Dendron

Compound **3** (4.63 g, 14.0 mmol) and DMAP (0.46 g, 3.79 mmol) were dissolved in 70 mL CH_2Cl_2 in a 250 mL round bottom flask. A solution of compound 5 (1.44 g, 4.74 mmol) in 30 mL CH_2Cl_2 was added also the round bottom flask. Pyridine (2.26 mL, 28.0 mmol) was added to the clear mixture. After stirring 30 h at room temperature, to get rid of the excess anhydride, 2.26 mL (same amount with pyridine) water was added to the reaction mixture. The reaction crude was extracted with 1M $NaHSO_4$ solution (3 x 40 mL),

then 10% Na_2CO_3 solution (3 x 40 mL) and with brine solution (40 mL). After extraction, the organic layer was dried over anhydrous Na_2SO_4 and all CH_2Cl_2 were evaporated. The crude product was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was dried under *vacuo* yielding as a yellow oil (2.44 g, 84% yield) (Figure 4.6). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.47 – 8.46 (d, 1H), 7.65 – 7.64 (m, 2H), 7.11 – 7.08 (m, 1H), 4.38 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2), 4.31 (s, 4H, OCH_2), 4.12 (d, 4H, *J* = 12.0 Hz, OCH_2), 3.59 (d, 4H, *J* = 12.0 Hz, OCH_2), 3.02 (t, 2H, *J* = 6.4 Hz, SCH_2), 1.39 (s, 6H, $C(CH_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$), 1.28 (s, 3H, $C(CH_3)$), 1.11 (s, 6H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 173.5, 172.3, 159.4, 149.8, 137.1, 119.9, 120.0, 98.1, 66.0, 65.3, 62.8, 46.8, 42.0, 36.9, 25.2, 22.0, 18.5, 17.7. HRMS m/z: $[M + H]^+$ Calcd for $C_{28}H_{41}NO_{10}S_2H$ 616.2250; Found 616.2213; $[M + 2H]^+$ Calcd for $C_{12}H_{17}NO_4S_2H_2$ 617.2328; Found 617.2245; $[M + 3H]^+$ Calcd for $C_{28}H_{41}NO_{10}S_2H_3$ 618.2407; Found 618.2210. FTIR (cm^{-1}): 2989.8, 1732.5.



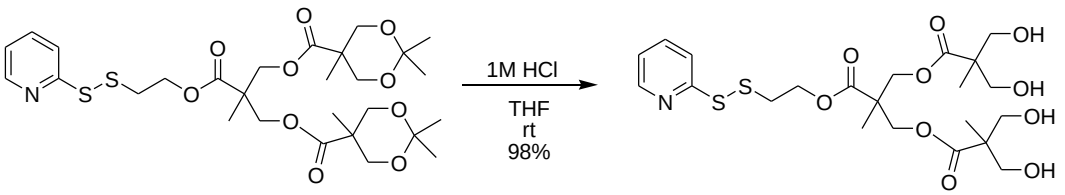
5 3 6

Figure 4.6. Synthesis of dendron **6**.

4.2.7. Synthesis of 2nd Generation Pyridyl Disulfide Functionalized Deprotected Dendron

Compound **6** (1.33 g, 2.16 mmol) was dissolved in 30 mL THF and then 30 mL 1M HCl was also added to the mixture. The resulting mixture was stirred at room temperature until the consumption of compound **6** was observed via TLC. At the end of the reaction,

THF was evaporated and the residue was diluted to 50 mL with EtOAc. The cure was extracted with 5% $NaHCO_3$ solution (2 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and all EtOAc were evaporated. The pure product was dried under *vacuo* yielding as a yellow oil (1.13 g, 98% yield) (Figure 4.7). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.47 – 8.45 (m, 1H), 7.65 – 7.64 (m, 2H), 7.12 – 7.09 (m, 1H), 4.44 – 4.26 (m, 6H, SCH_2CH_2 and OCH_2), 3.86 – 3.80 (m, 4H, OCH_2), 3.72 – 3.66 (m, 4H, OCH_2), 3.15 – 3.10 (m, 4H, OH), 3.04 (t, 2H, J = 6.4 Hz, SCH_2), 1.30 (s, 3H, $C(CH_3)$), 1.03 (s, 6H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 175.1, 172.7, 159.2, 149.8, 137.2, 121.1, 120.2, 67.8, 64.8, 63.0, 49.7, 46.4, 36.9, 18.1, 17.1. HRMS m/z: $[M + H]^+$ Calcd for $C_{22}H_{33}NO_{10}S_2H$ 536.1624; Found 536.1594; $[M + Na]^+$ Calcd for $C_{22}H_{33}NO_{10}S_2Na$ 558.1444; Found 558.1404; FTIR (cm^{-1}): 3370.1, 2937.6, 1725.0.



6 **7**

Figure 4.7. Synthesis of dendron **7**.

4.2.8. Synthesis of 3rd Generation Pyridyl Disulfide Functionalized Protected Dendron

Compound **3** (2.69 g, 7.46 mmol) and DMAP (0.182 g, 1.49 mmol) were dissolved in 40 mL CH_2Cl_2 in a 100 mL round bottom flask. A solution of compound **7** (0.50 g, 0.93 mmol) in 10 mL CH_2Cl_2 was added also the round bottom flask. Pyridine (0.75 mL, 9.33 mmol) was added to the clear mixture. After stirring 48 h at room temperature, to get rid of the excess anhydride, 0.75 mL (same amount with pyridine) water was added to the reaction mixture. The reaction crude was extracted with 1M $NaHSO_4$ solution (3 x 20 mL), then 10% Na_2CO_3 solution (3 x 20 mL) and with brine solution (20 mL). After extraction,

the organic layer was dried over anhydrous Na_2SO_4 and all CH_2Cl_2 were evaporated. The crude product was then purified by column chromatograph using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was dried under vacuo yielding as a yellowish solid (0.71 g, 82% yield) (Figure 4.8). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.47 – 8.45 (m, 1H), 7.66 – 7.64 (m, 2H), 7.11 – 7.08 (m, 1H), 4.39 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2), 4.32 – 4.21 (m, 12H, OCH_2), 4.13 (d, 8H, *J* = 12.0 Hz, OCH_2), 3.60 (d, 8H, *J* = 12.0 Hz, OCH_2), 3.04 (t, 2H, *J* = 6.4 Hz, SCH_2), 1.39 (s, 12H, $C(CH_3)_2$), 1.33 (s, 12H, $C(CH_3)_2$), 1.26 (s, 3H, $C(CH_3)$), 1.25 (s, 6H, $C(CH_3)$), 1.12 (s, 12H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 173.5, 171.85, 171.83, 159.3, 149.8, 137.1, 121.0, 120.0, 98.1, 66.96, 66.91, 64.9, 63.0, 46.9, 46.7, 42.0, 36.9, 25.2, 22.0, 18.5, 17.7, 17.6. HRMS m/z: $[M + H]^+$ Calcd for $C_{54}H_{81}NO_{22}S_2H$ 1160.4770; Found 1160.4756; $[M + 2H]^+$ Calcd for $C_{54}H_{81}NO_{22}S_2H_2$ 1161.4848; Found 116.4887; $[M + 3H]^+$ Calcd for $C_{54}H_{81}NO_{22}S_2H_3$ 1162.4926; Found 1162.4783. FTIR (cm^{-1}): 2990.2, 1731.6.

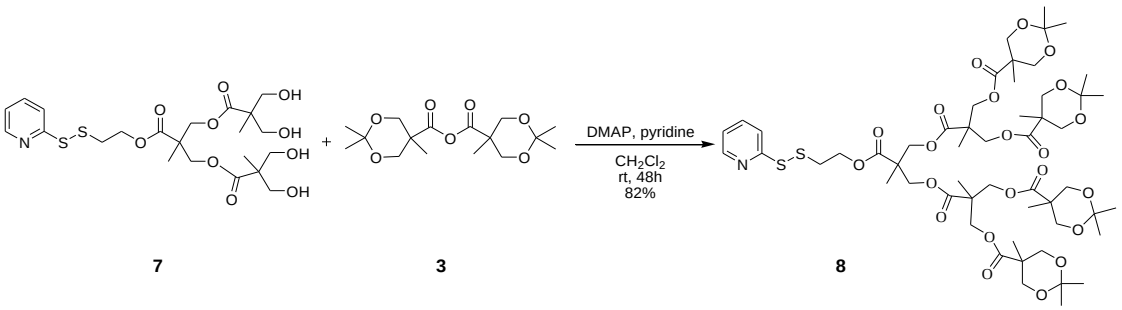


Figure 4.8. Synthesis of dendron **8**.

4.2.9. Synthesis of 3rd Generation Pyridyl Disulfide Functionalized Deprotected Dendron

Compound **7** (0.100 g, 0.086 mmol) was dissolved in 65 mL MeOH and then 18M H_2SO_4 (0.32 mL, 5.82 mmol) was added to the mixture. After stirring for 2 h at room temperature, in order to neutralize the reaction mixture, 7M NH_3 in methanol was added until pH is 7. The salt, $(NH_4)_2SO_4$, was filtered with filter paper and MeOH was evaporated. To get rid of the remaining salt, the residue was washed with the minimum

amount of water and the pure product was obtained as a white solid (0.086 g, 62%) (Figure 4.9). ^{1}H NMR (CD_3OD, δ, ppm) 8.43 – 8.41 (m, 1H), 7.86 – 7.84 (m, 2H), 7.26 – 7.23 (m, 1H), 4.42 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2), 4.34 – 4.22 (m, 12H, OCH_2), 3.69 – 3.58 (m, 16H, OCH_2), 3.14 (t, 2H, *J* = 6.4 Hz, SCH_2), 1.31 (s, 3H, $C(CH_3)$), 1.28 (s, 6H, $C(CH_3)$), 1.14 (s, 12H, $C(CH_3)$). ^{13}C NMR (CD_3OD, δ, ppm) 174.5, 172.3, 172.2, 159.5, 149.1, 137.9, 121.2, 120.0, 65.8, 64.8, 64.4, 62.8, 50.4, 46.5, 36.9, 33.3, 16.8, 16.6, 15.9. FTIR (cm^{-1}): 3283.1, 2938.7, 1729.7.

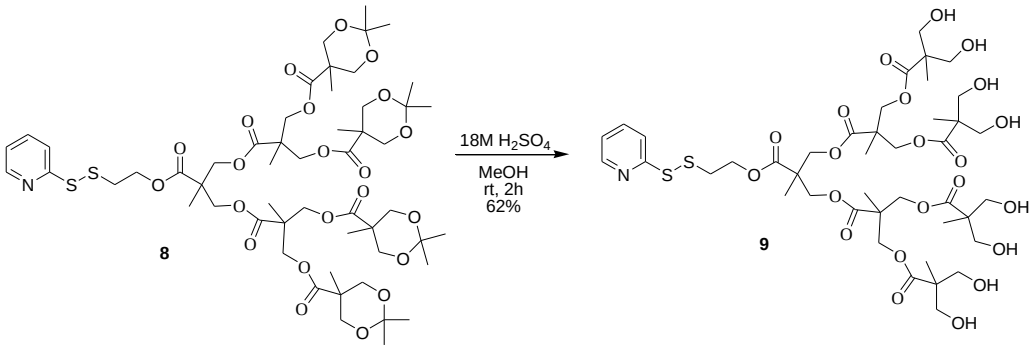


Figure 4.9. Synthesis of dendron **9.**

4.2.10. Disulfide Exchange Reaction of G_3 Dendron via Free Thiol Containing Molecule

Compound **8** (0.016 mg, 1.40 x 10^{-5} mmol) was dissolved in 3 mL DMF and UV data was monitored for time 0. After collecting data, thioglycerol (1.20 x 10^{-3} mL, 1.40 x 10^{-5} mmol) was added to the mixture. After stirring, at room temperature, the release of thiopyridone was monitored via UV spectrometry for every 2 minutes.

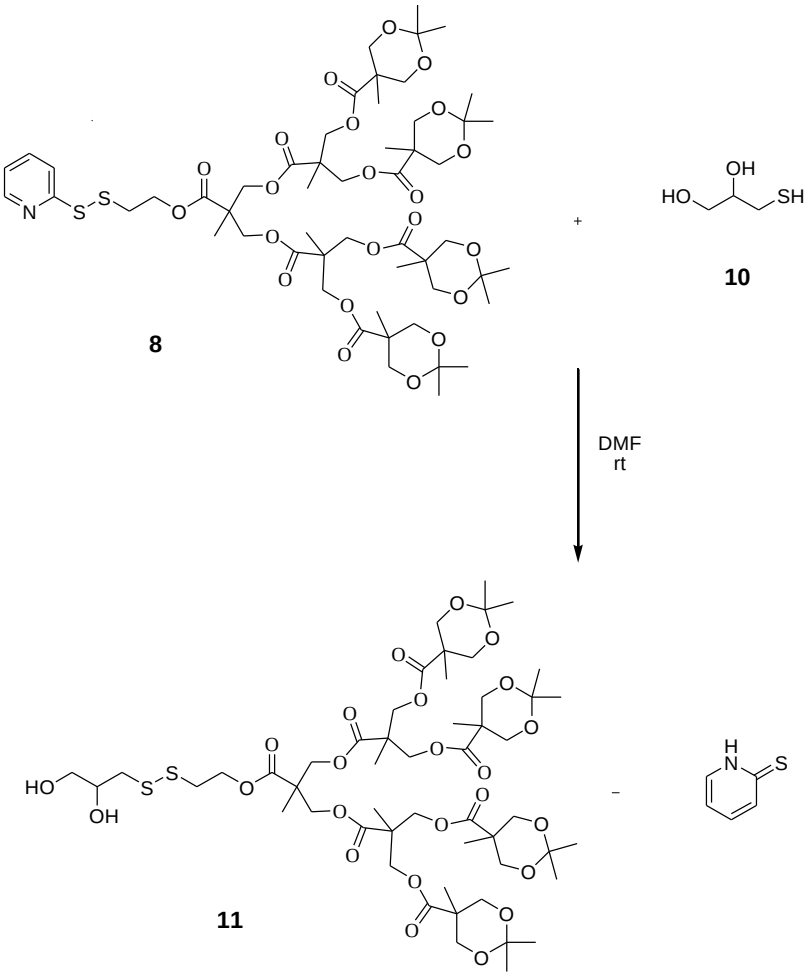


Figure 4.10. Disulfide exchange reaction.

5. CONCLUSION

In the study, three generations novel polyester dendrons containing thiol-reactive functional group at their focal point were synthesized using divergent growth strategy. These biocompatible and biodegradable dendrons which have pyridyl disulfide group (PDS) at their core was characterized via ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and FTIR spectroscopy. These novel dendrons containing thiol-reactive functional group at their core were reacted with thiol containing molecules via disulfide exchange reaction. During this reaction, thiopyridone was liberated as a byproduct and this release of thiopyridone was monitoried with UV spectrometry.

APPENDIX A: SPECTROSCOPY DATA

^{1}H NMR, ^{13}C NMR, FT-IR, and HRMS spectra of the synthesized products are included.

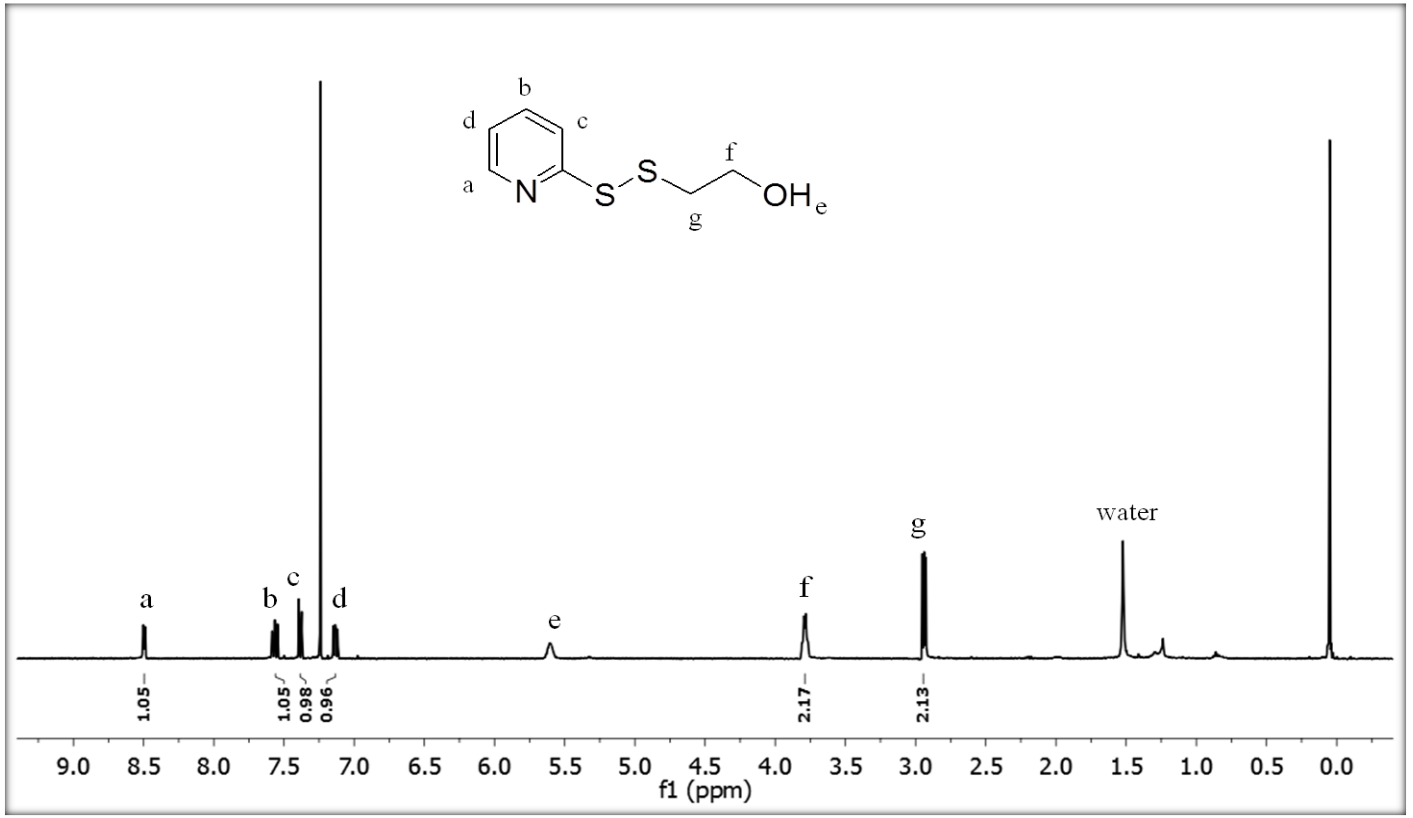


Figure A.1. ^{1}H NMR spectrum of product **1**.

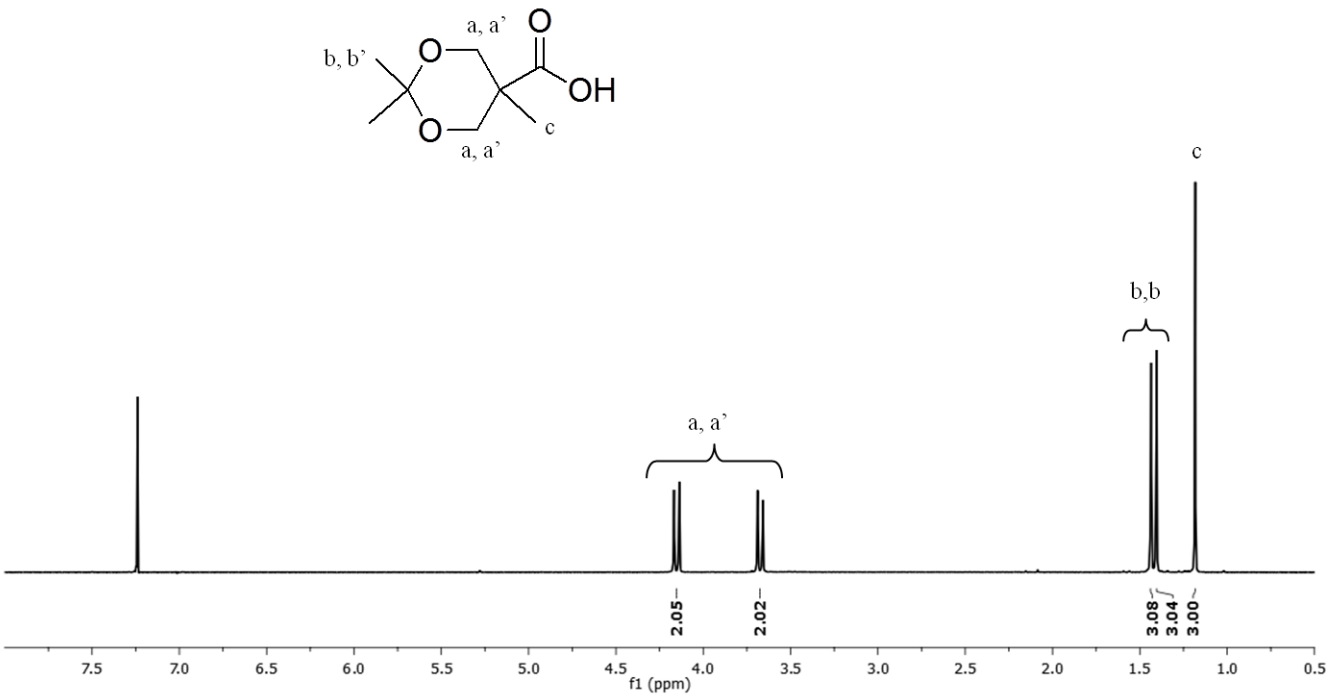


Figure A.2. ^{1}H NMR spectrum of product **2**.

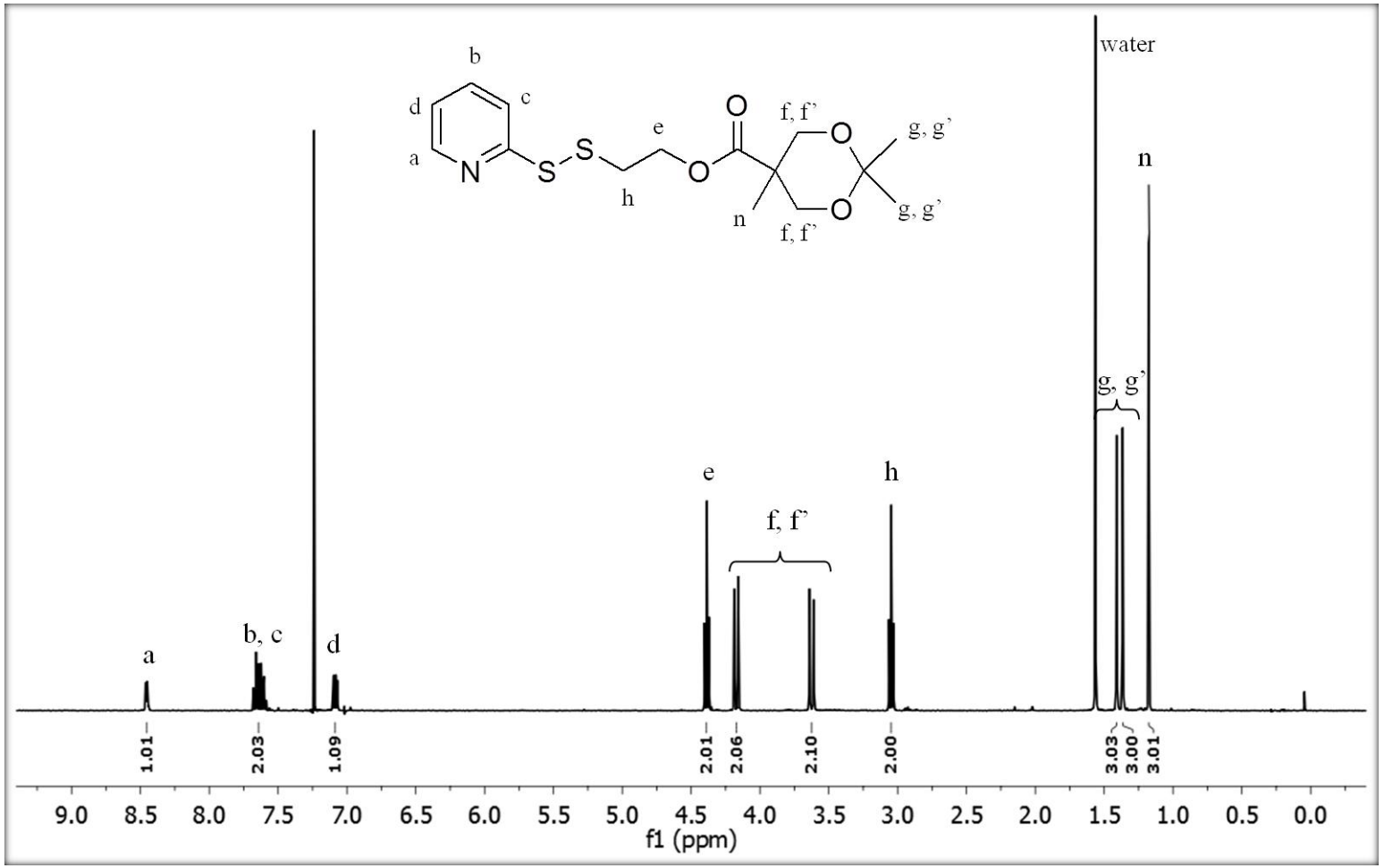


Figure A.3. ^{1}H NMR spectrum of product **4**.

Figure A.4. ^{13}C NMR spectrum of product **4**.

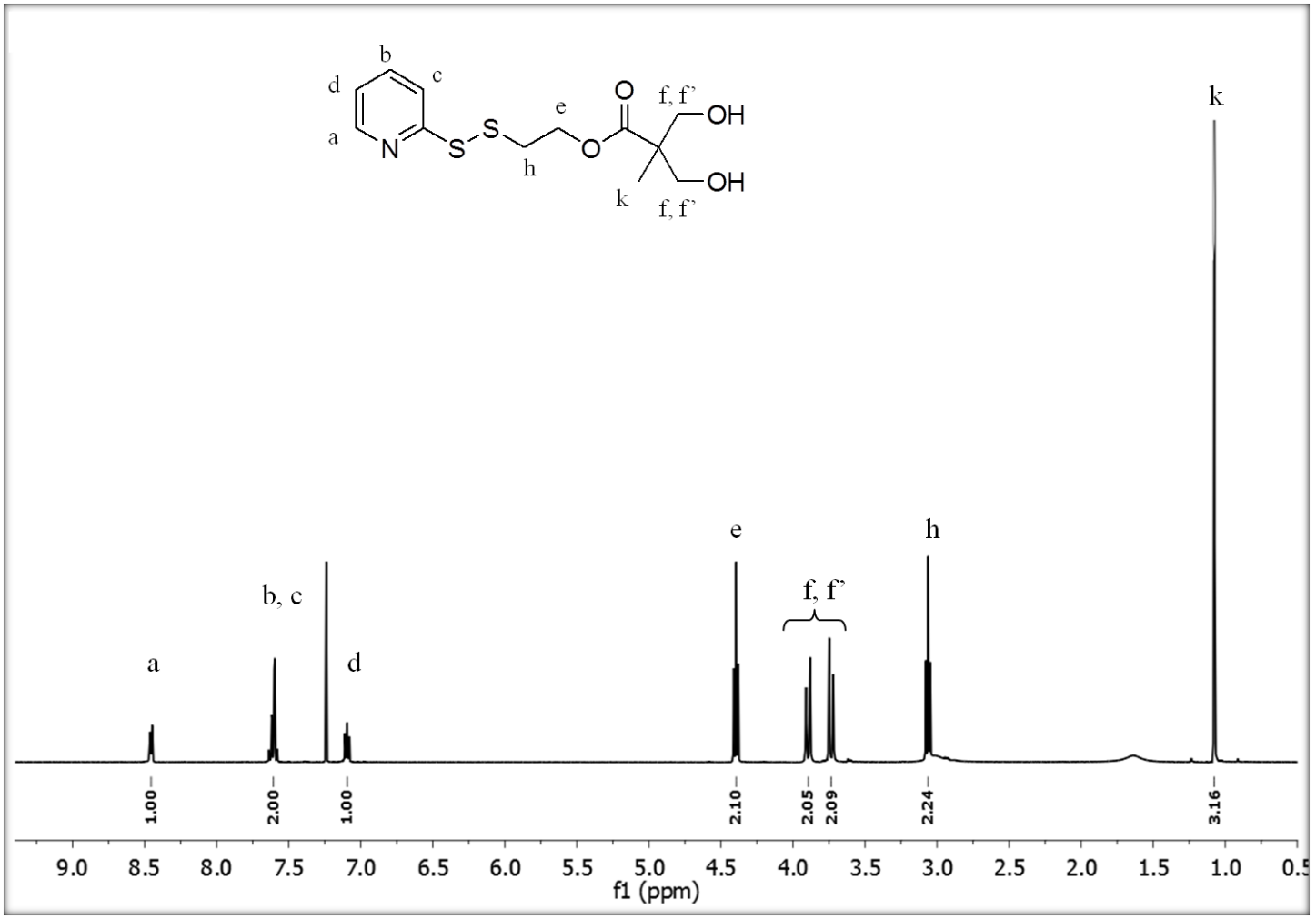


Figure A.5. ^{1}H NMR spectrum of product **5**.

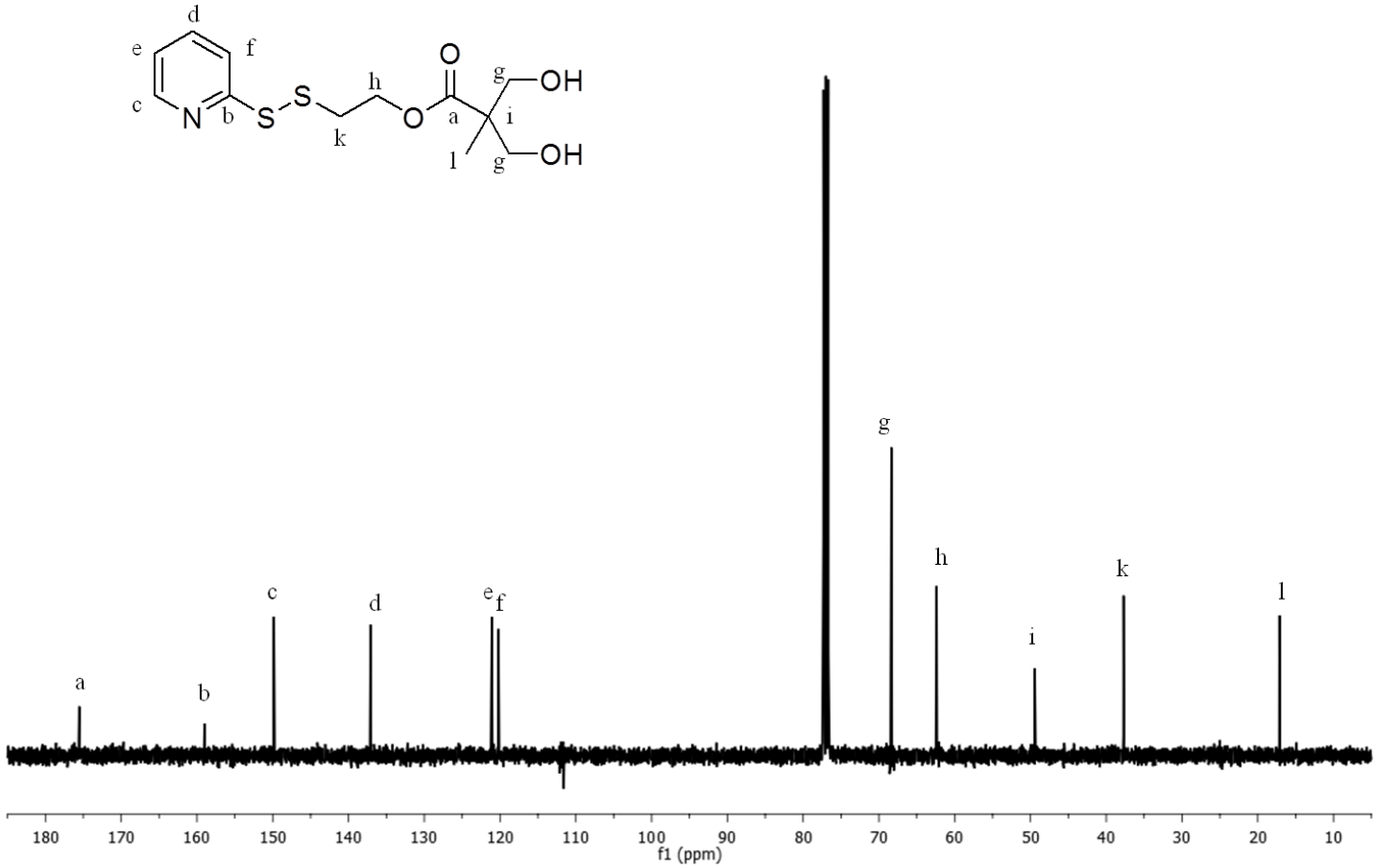


Figure A.6. ^{13}C NMR spectrum of product **5**.

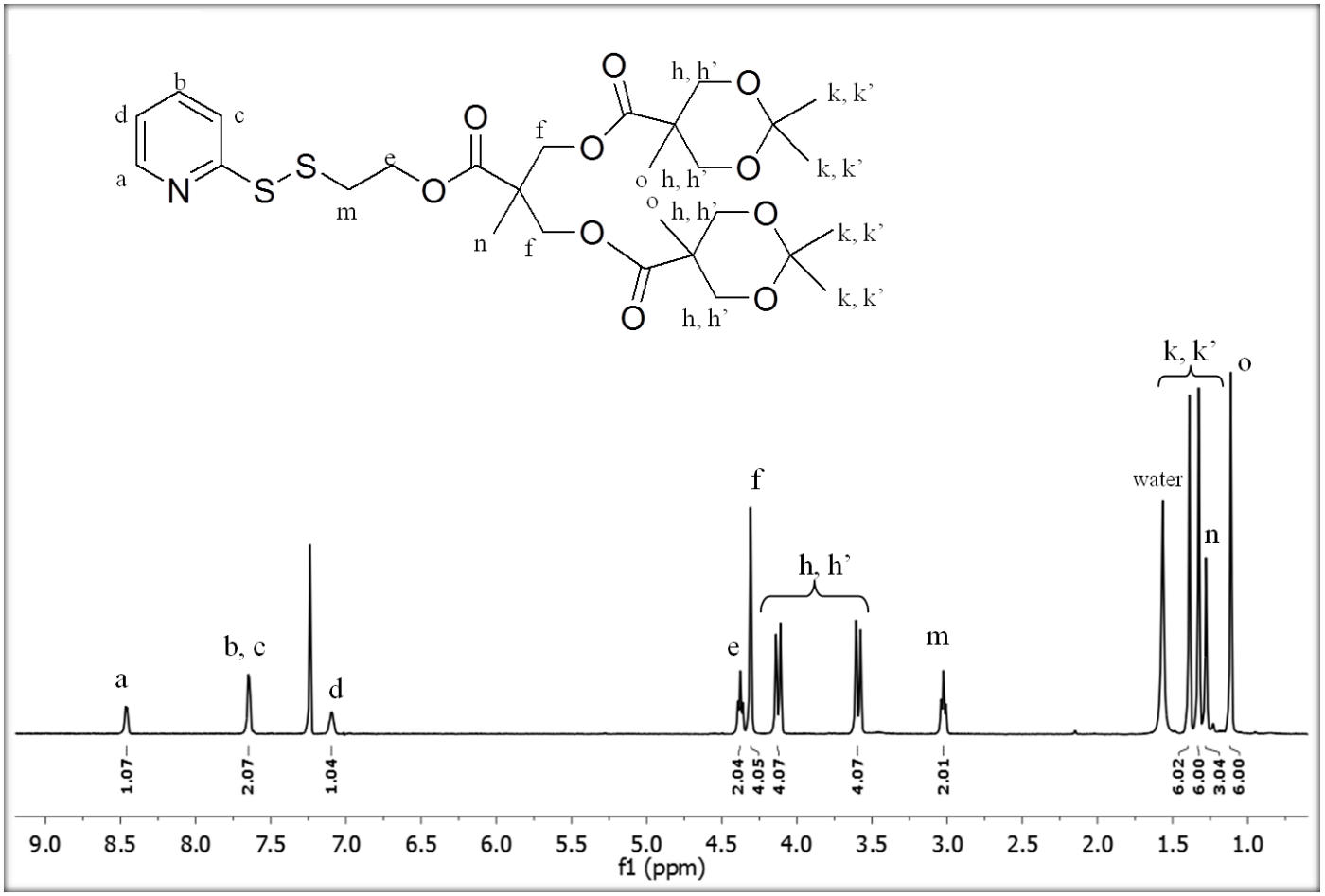


Figure A.7. ^{1}H NMR spectrum of product **6**.

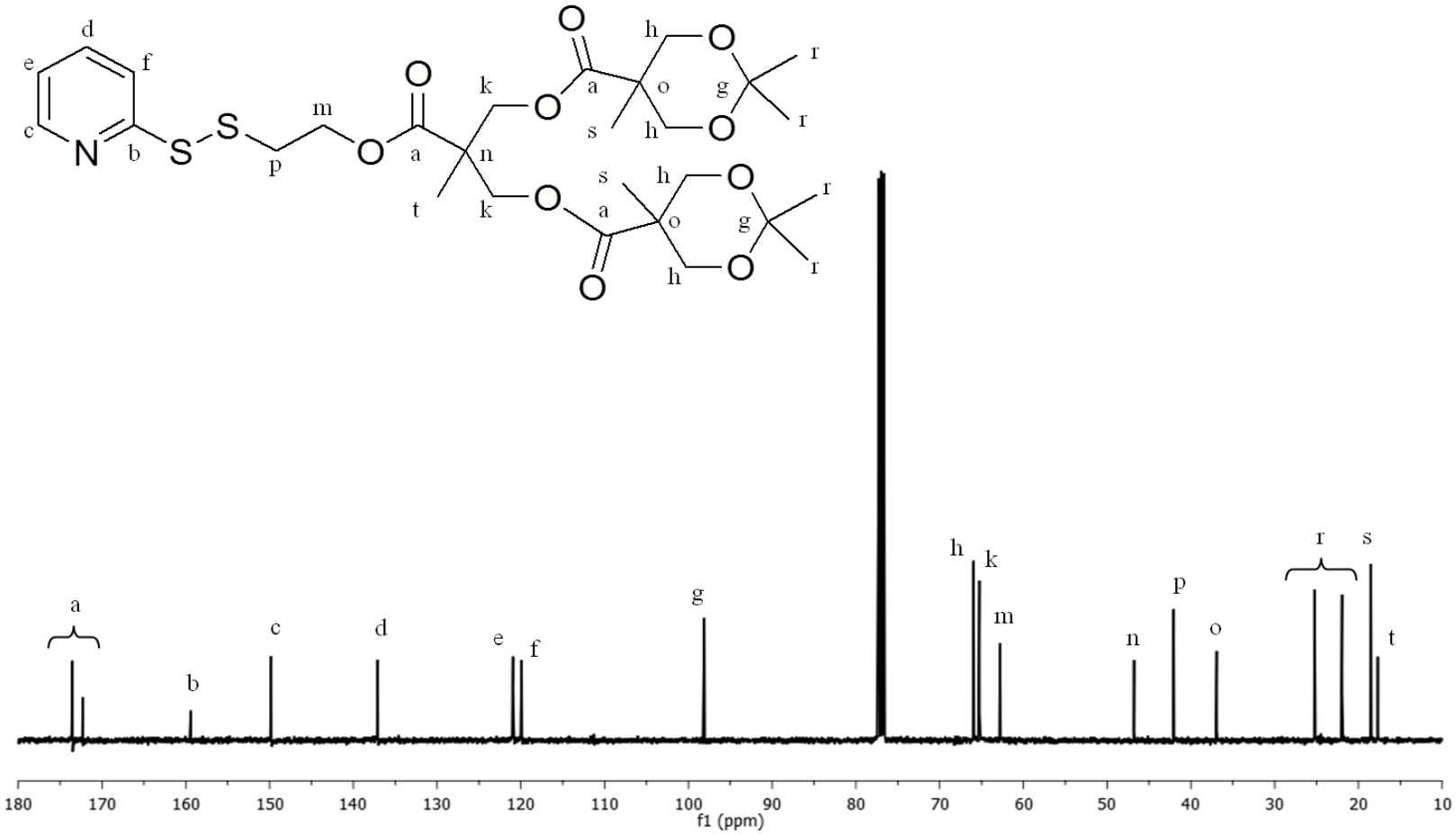


Figure A.8. ^{13}C NMR spectrum of product **6**.

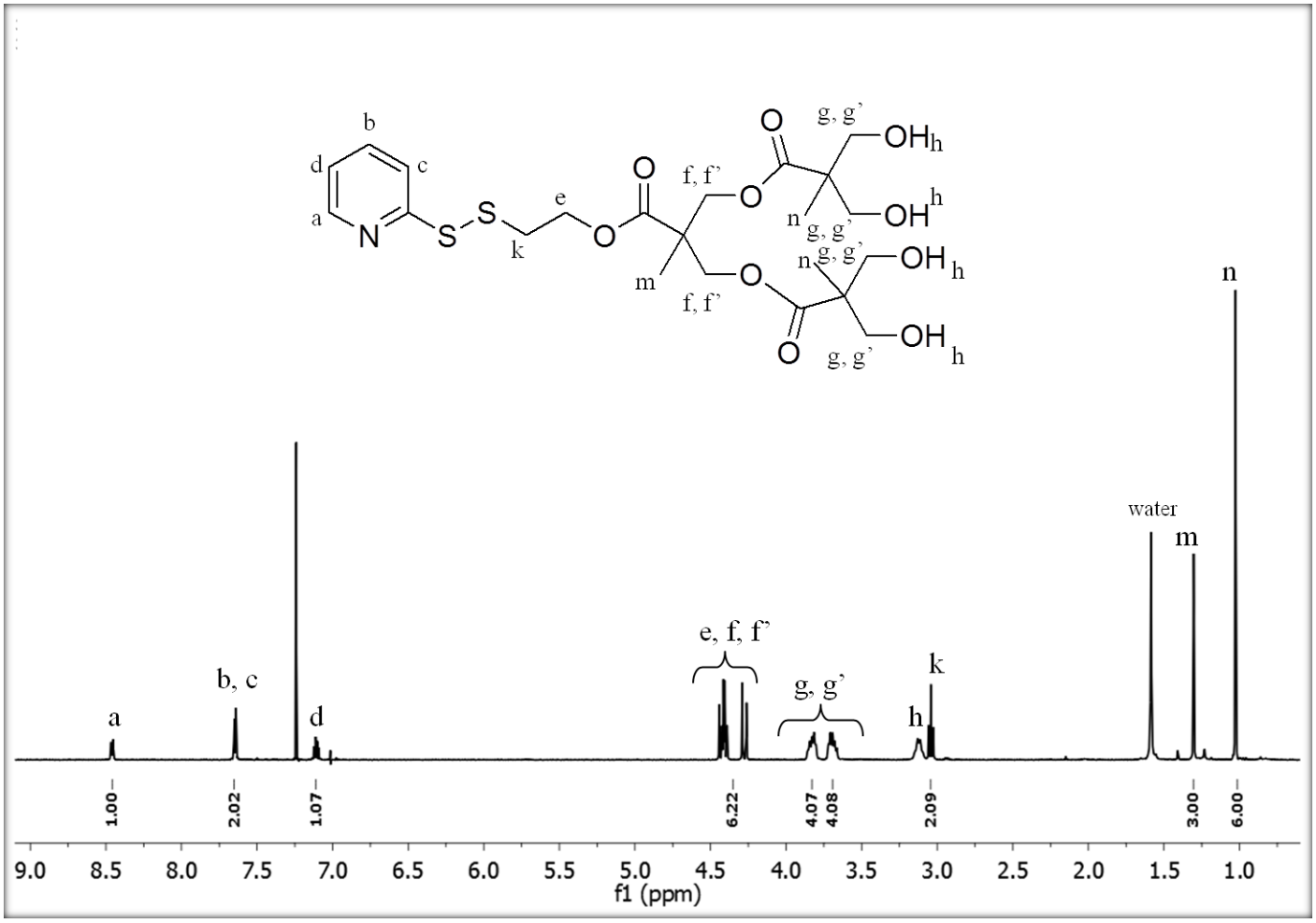


Figure A.9. ^{1}H NMR spectrum of product **7**.

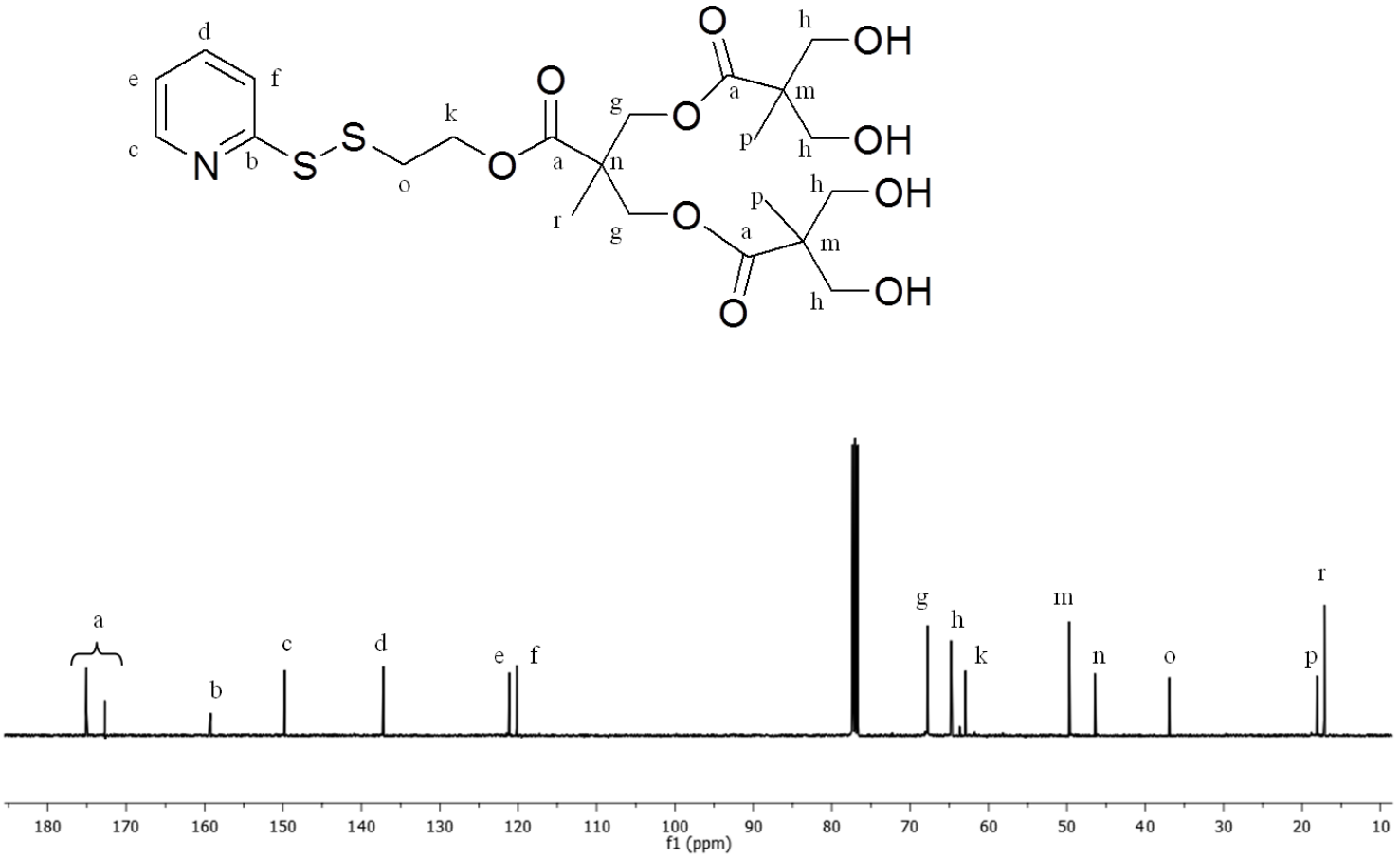


Figure A.10. ^{13}C NMR spectrum of product **7**.

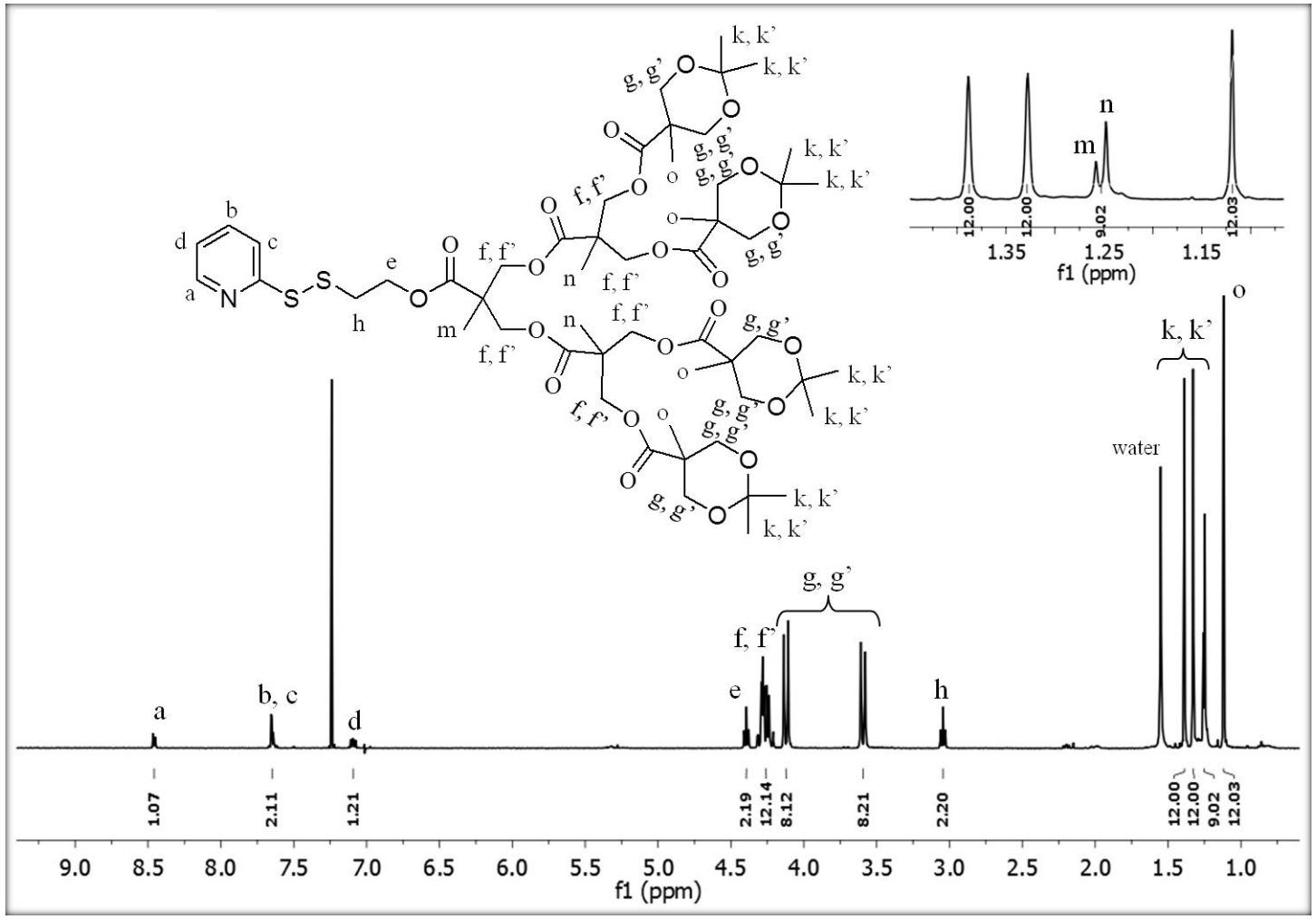


Figure A.11. ^{1}H NMR spectrum of product **8**.

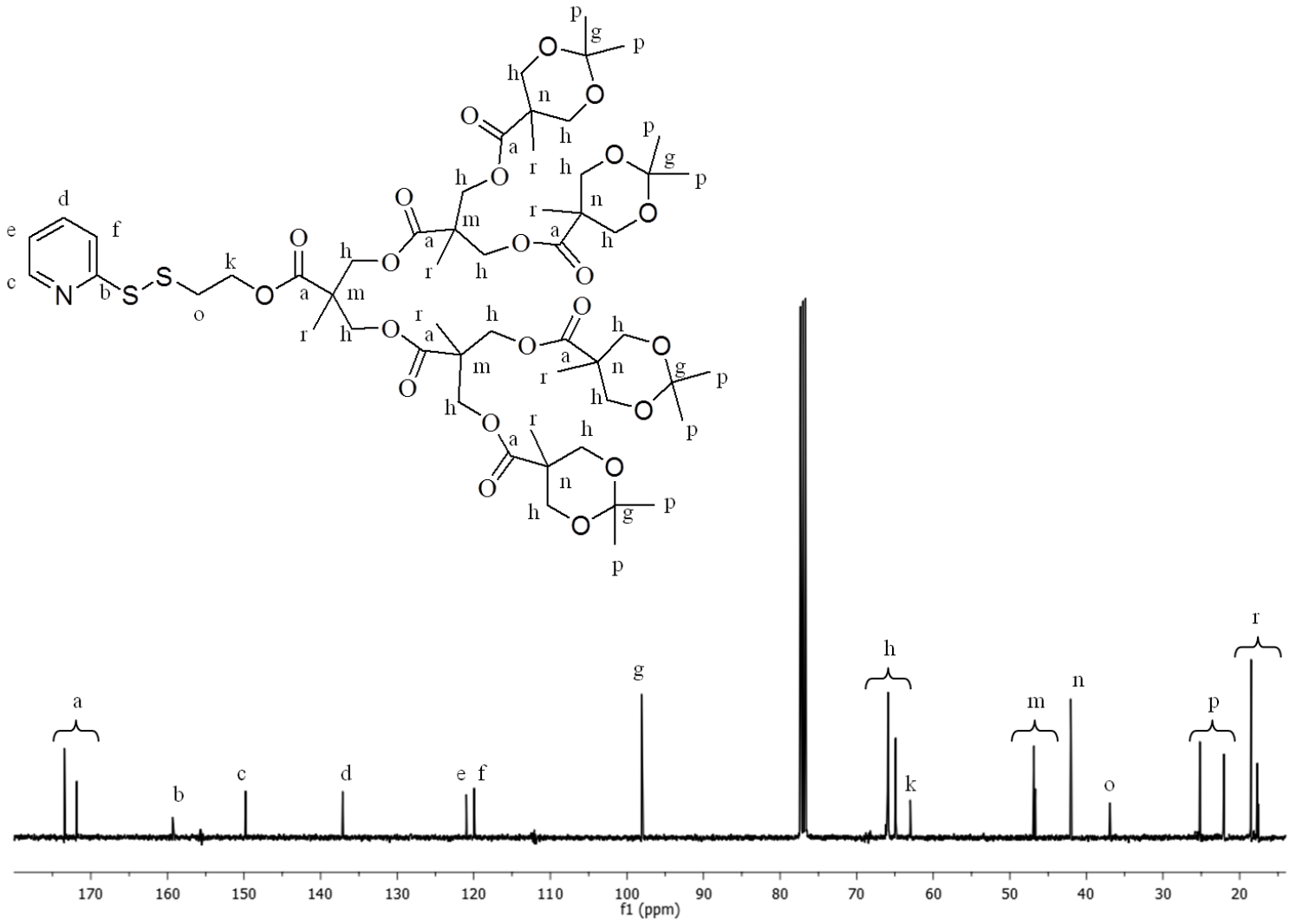


Figure A.12. ^{13}C NMR spectrum of product **8**.

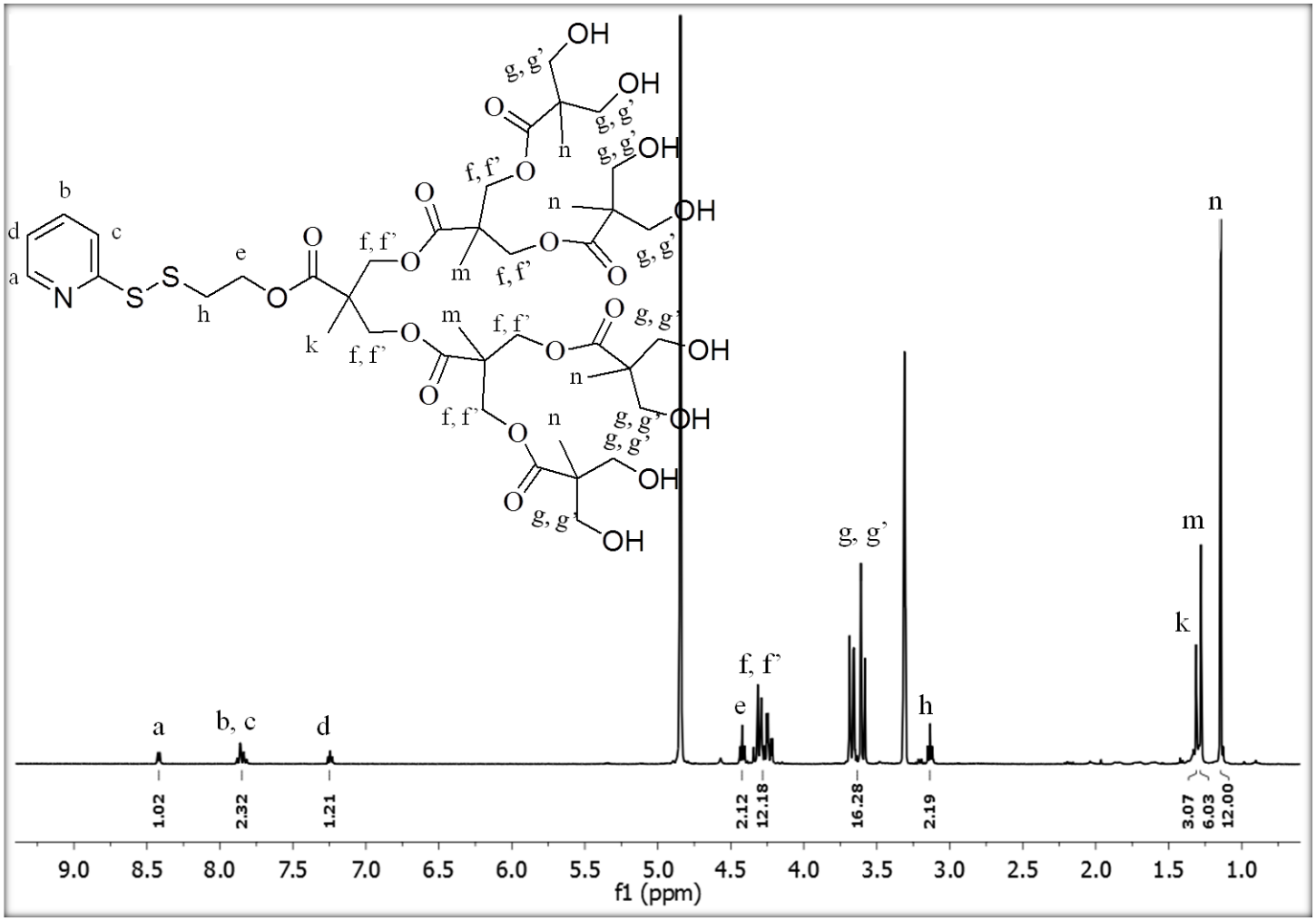


Figure A.13. ^{1}H NMR spectrum of product **9**.

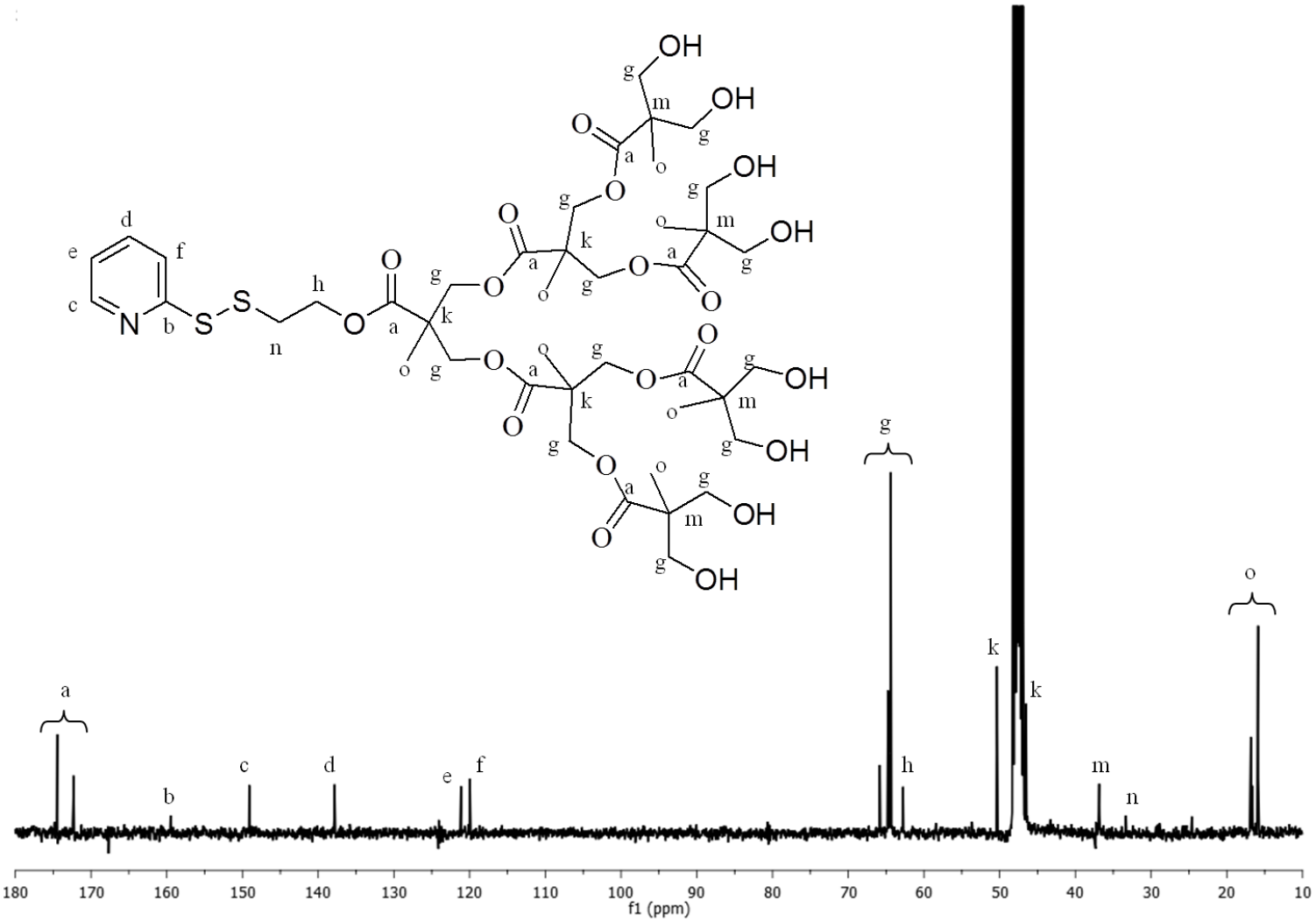


Figure A.14. ^{13}C NMR spectrum of product **9**.

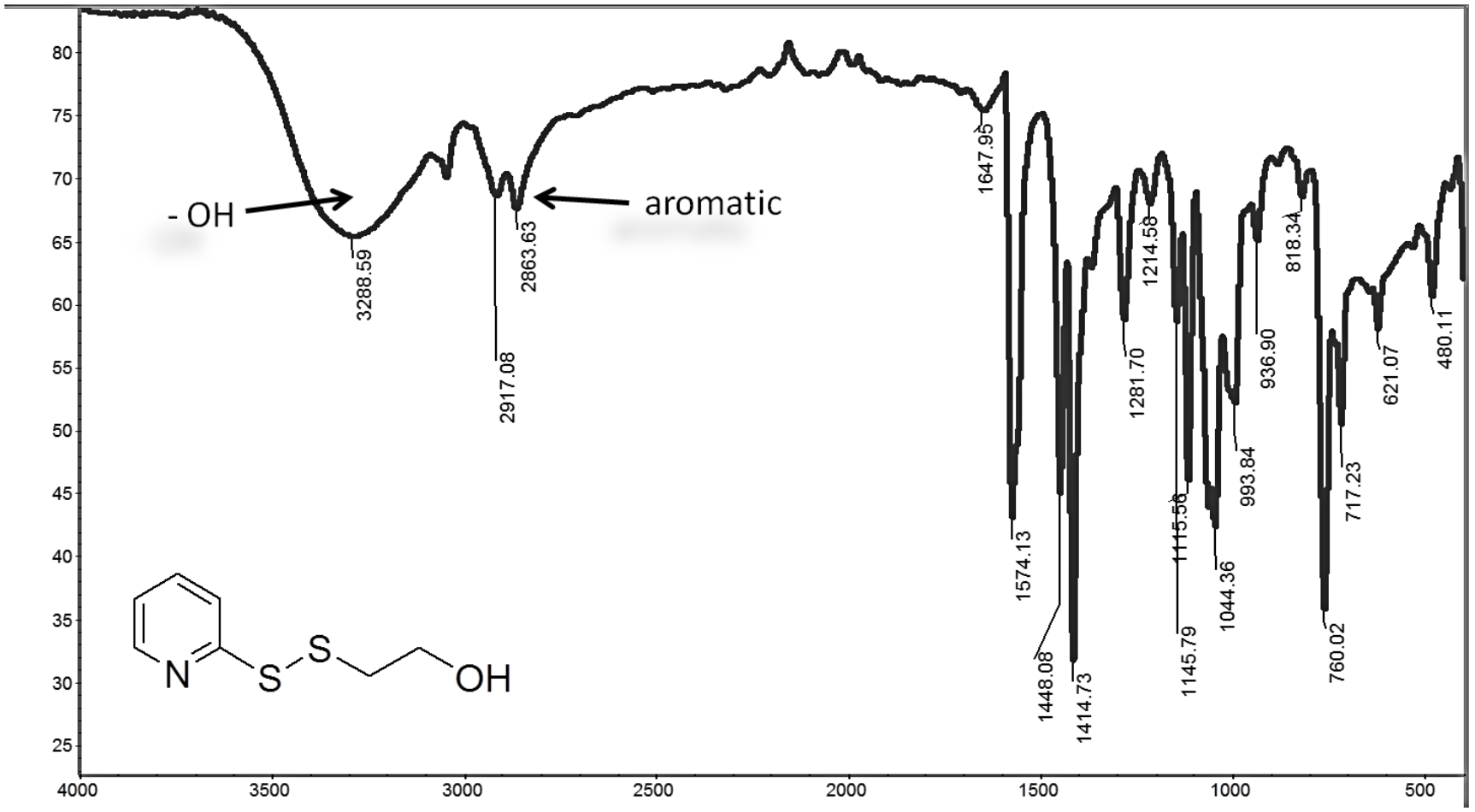


Figure A.15. FT-IR spectrum of product **1**.

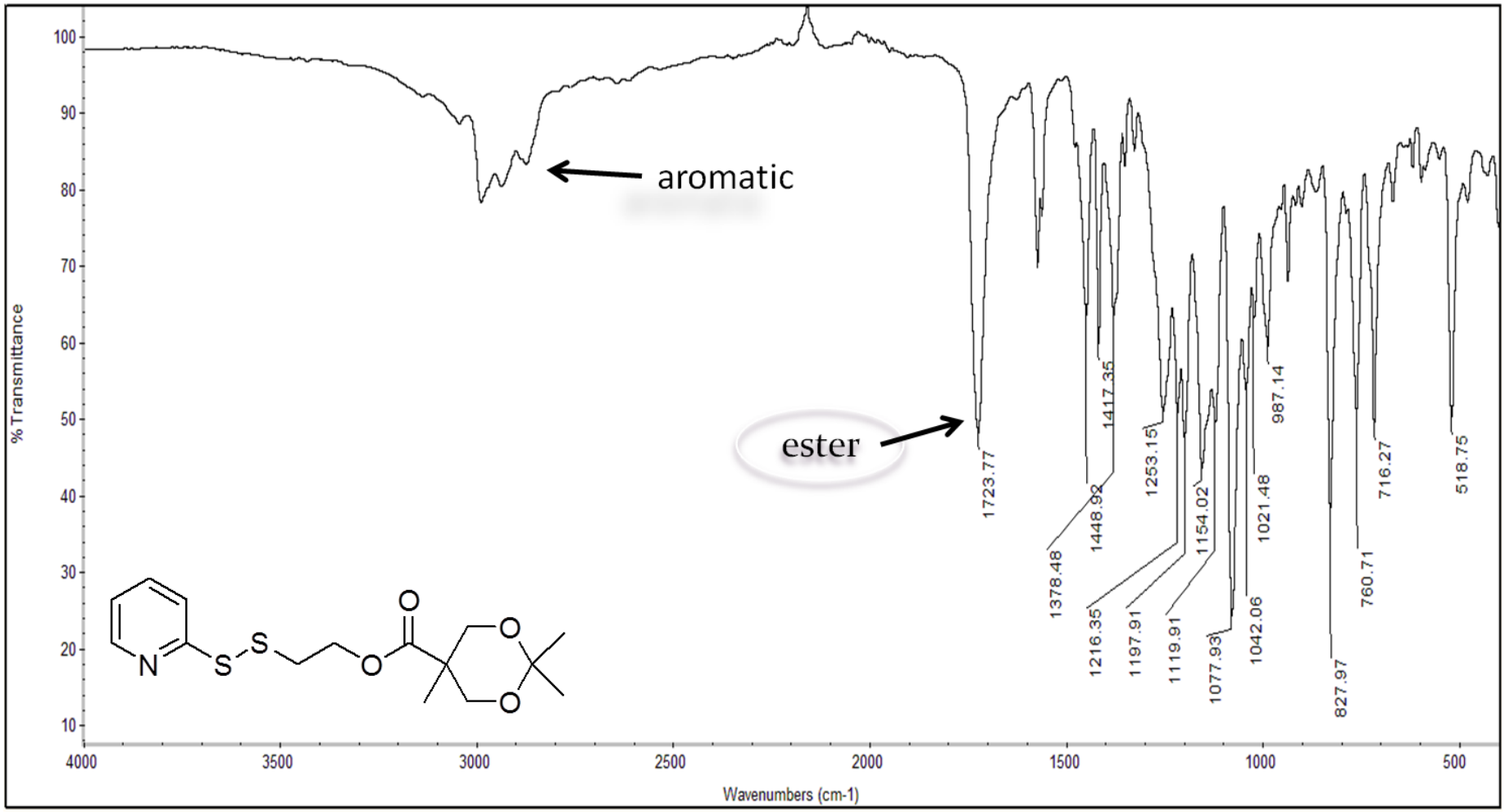


Figure A.16. FT-IR spectrum of product **4**.

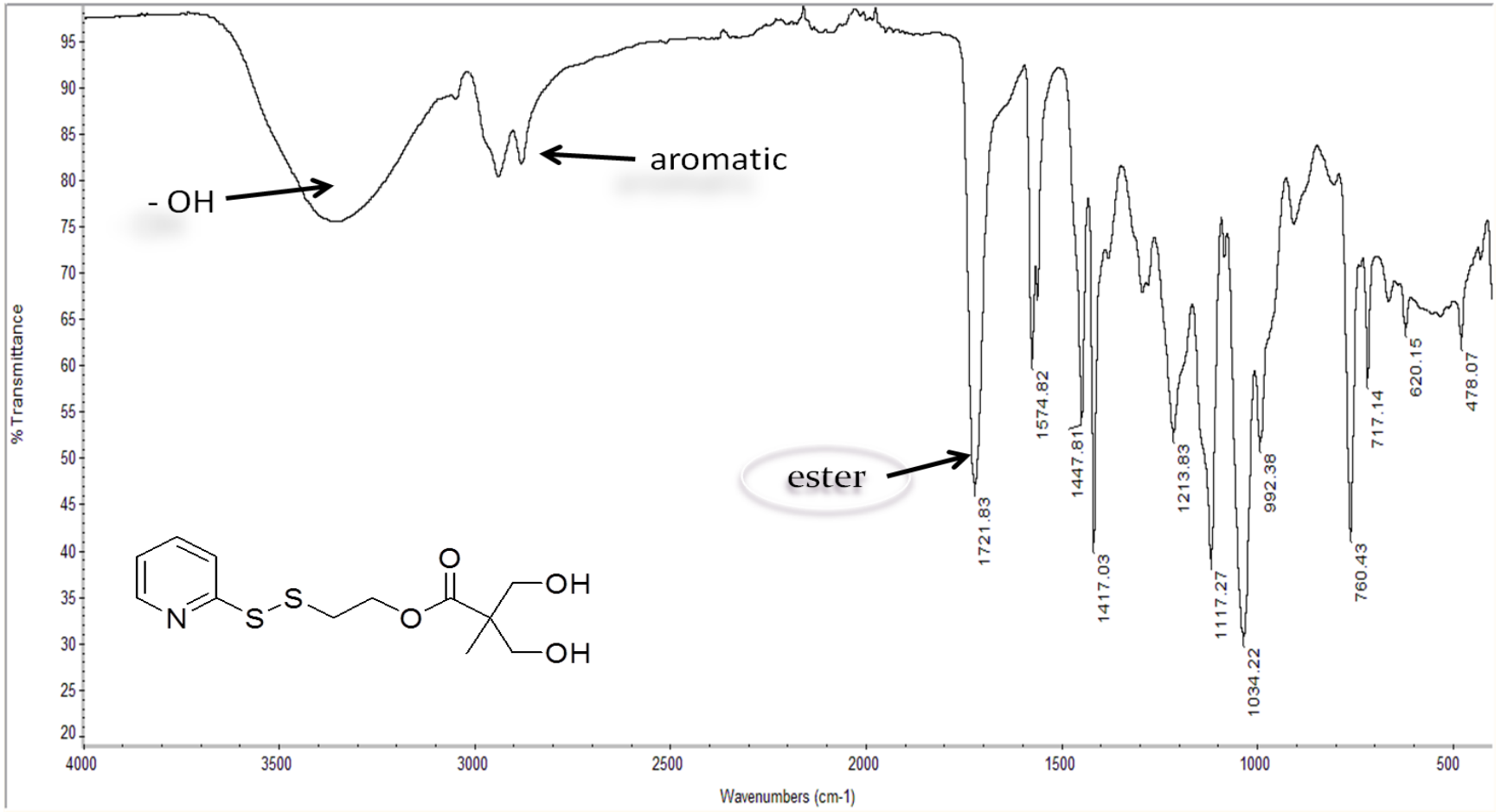


Figure A.17. FT-IR spectrum of product **5**.

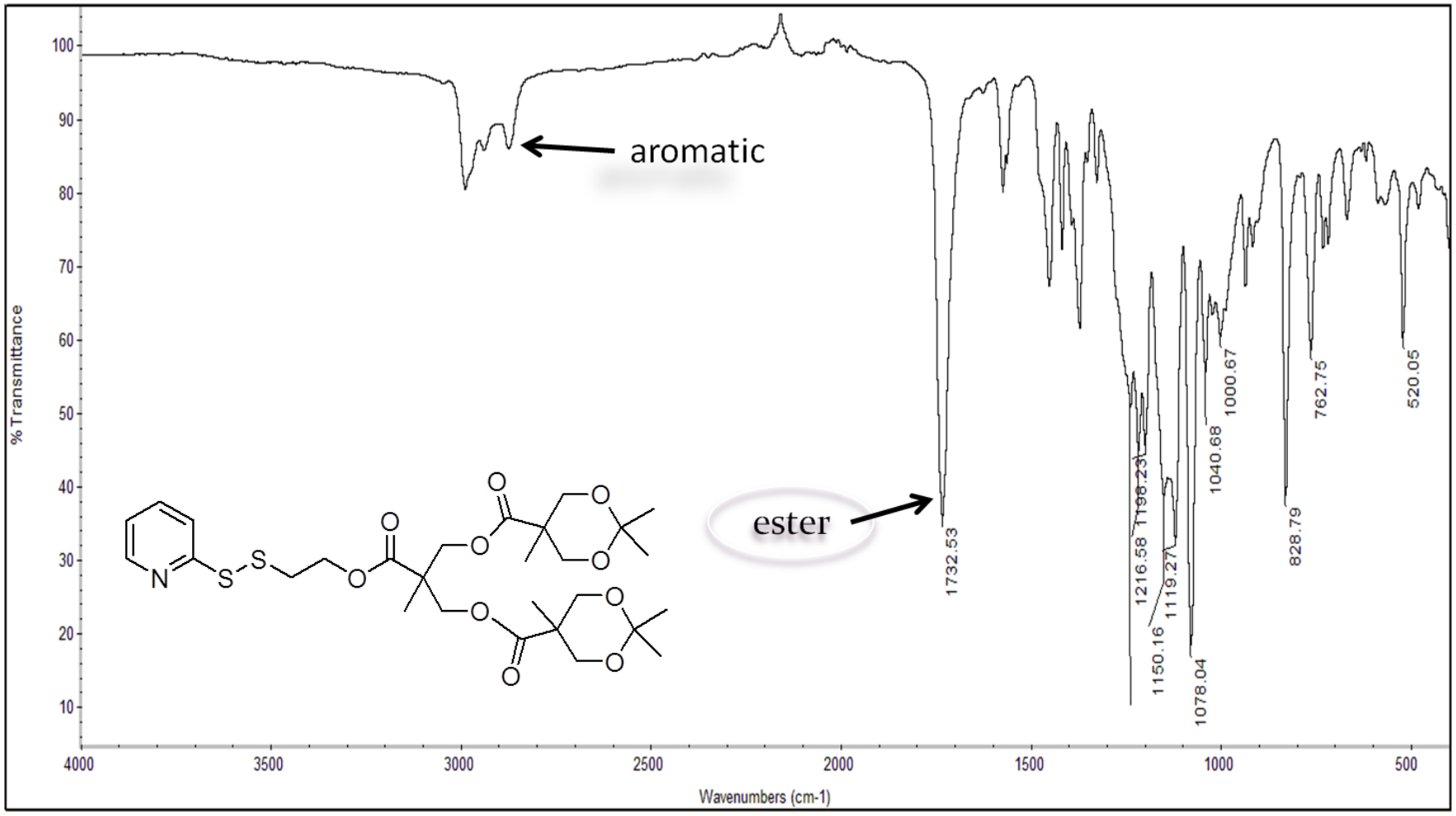


Figure A.18. FT-IR spectrum of product **6**.

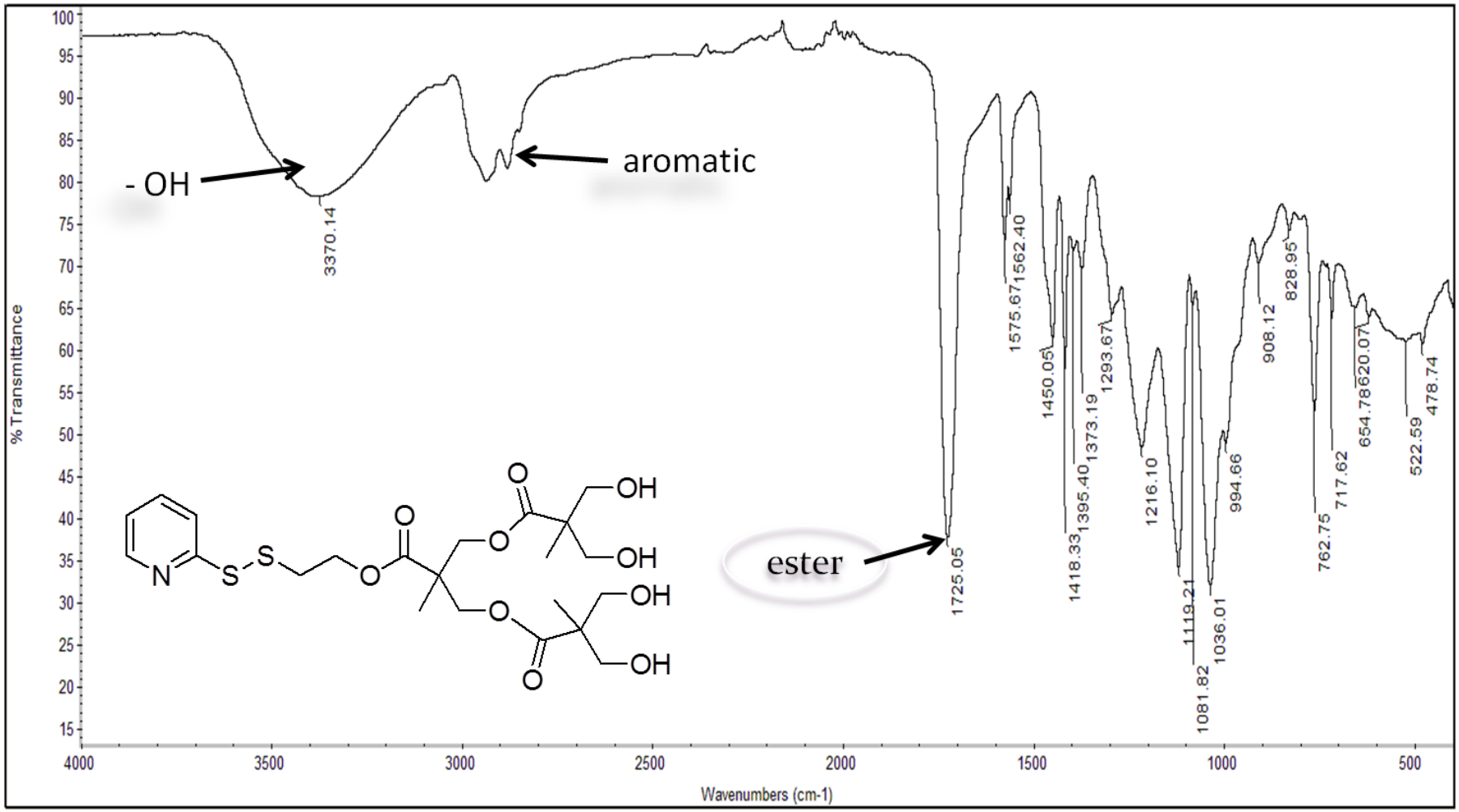


Figure A.19. FT-IR spectrum of product **6**.

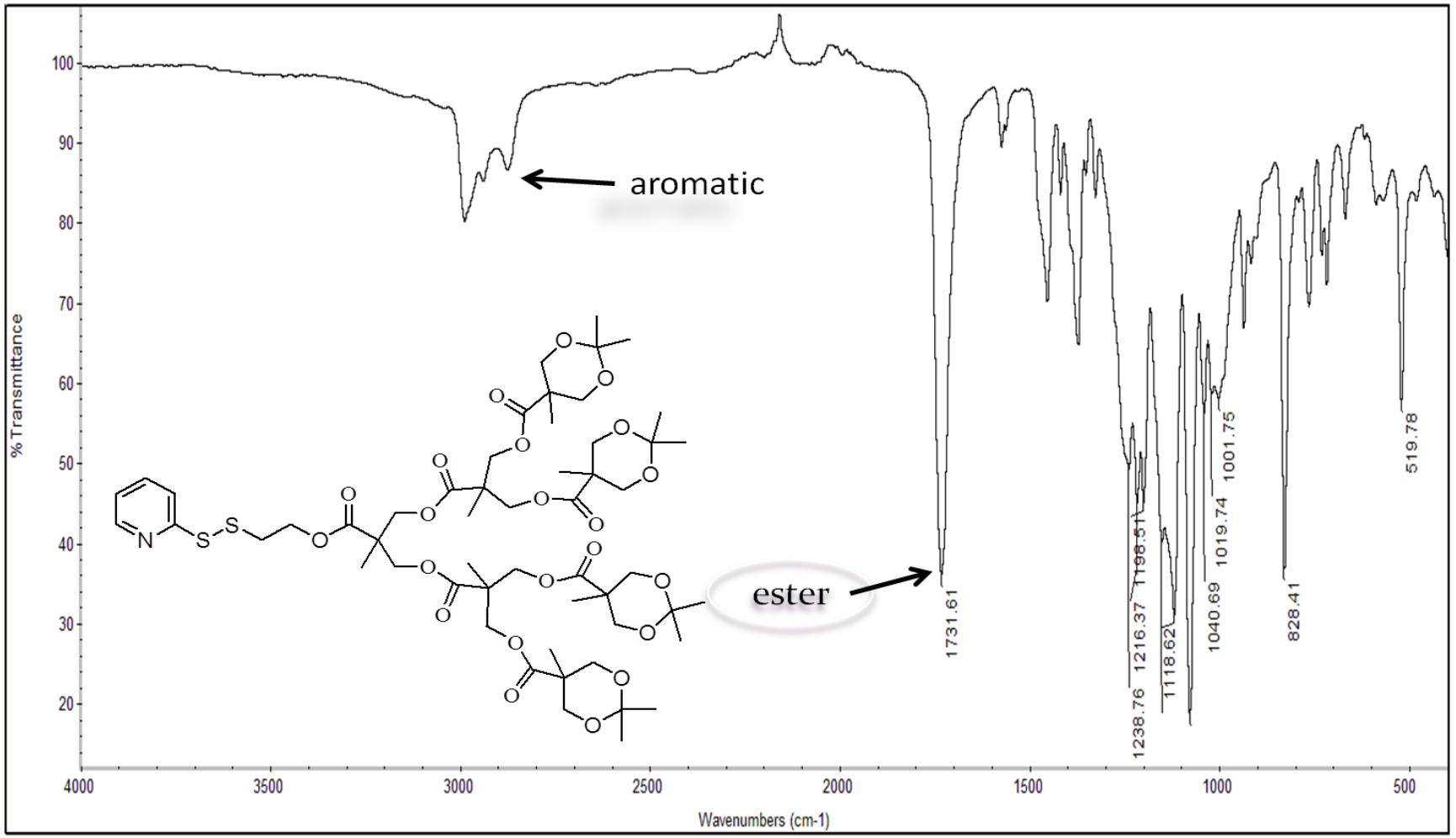


Figure A.20. FT-IR spectrum of product **6**.

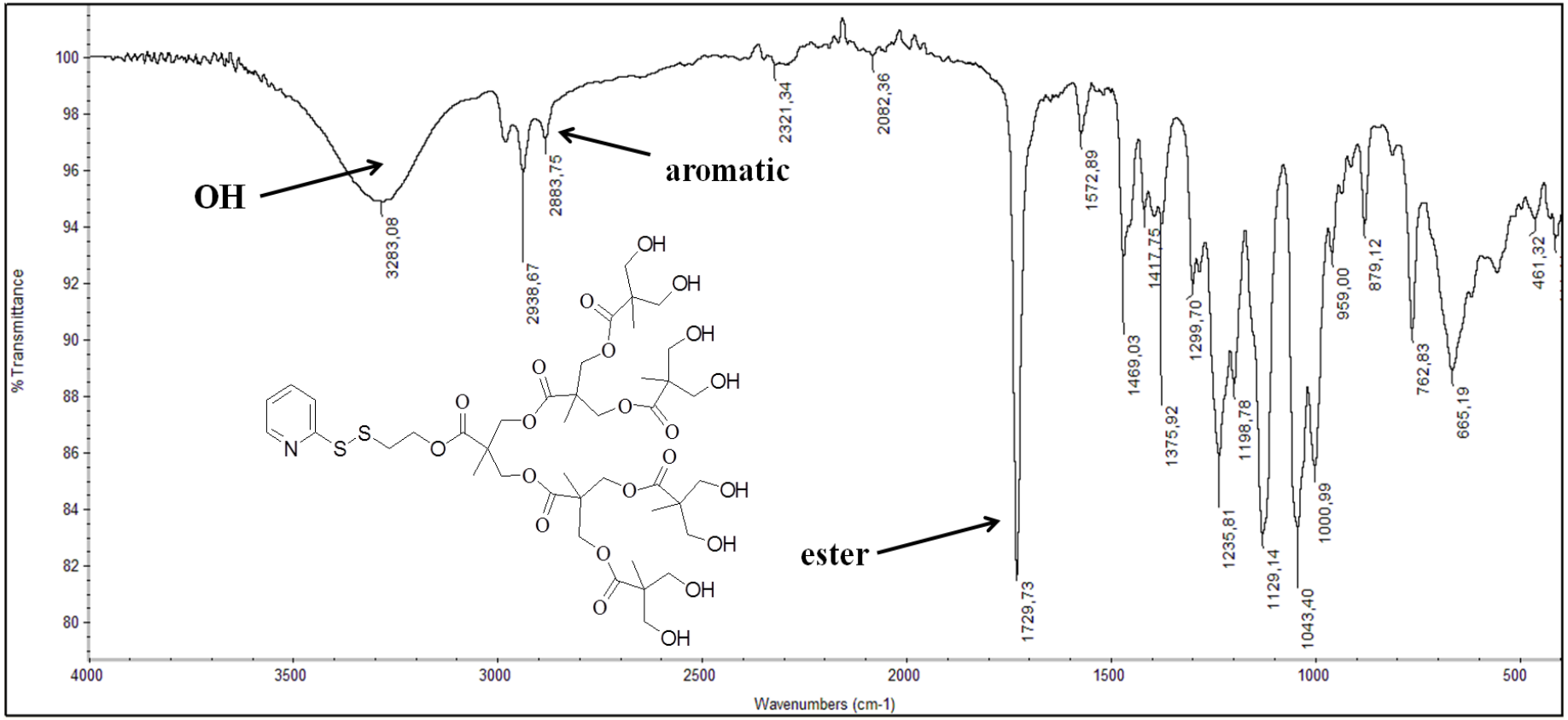


Figure A.21. FT-IR spectrum of product **9**.

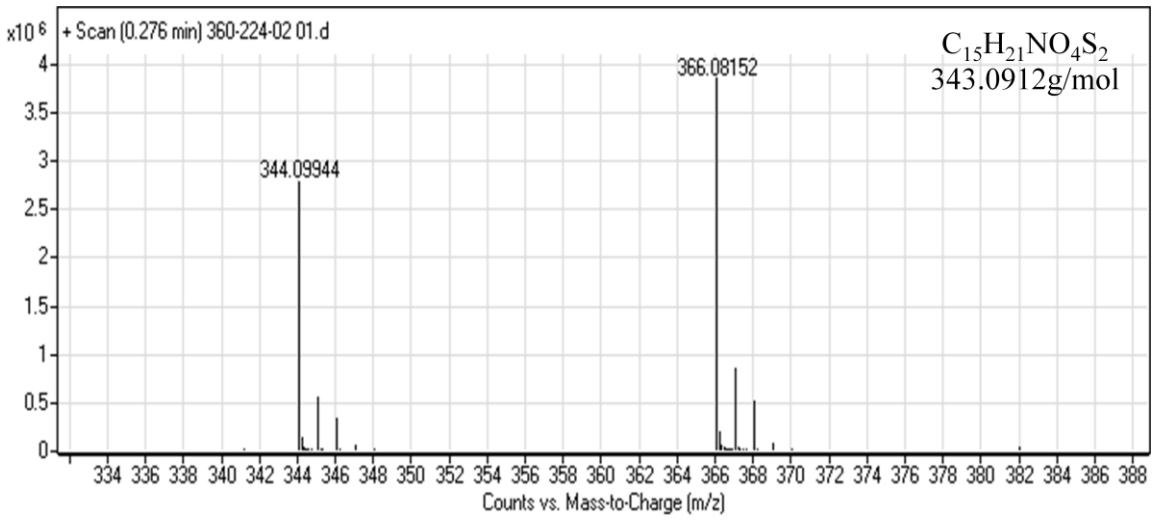


Figure A.22. HRMS spectra of product **4**.

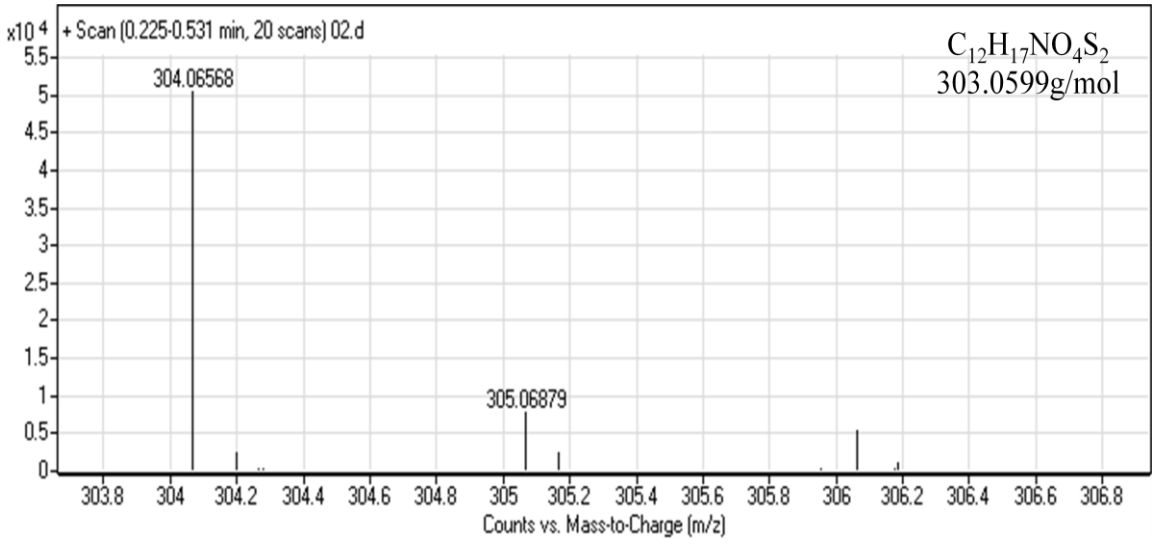


Figure A.23. HRMS spectra of product **5**.

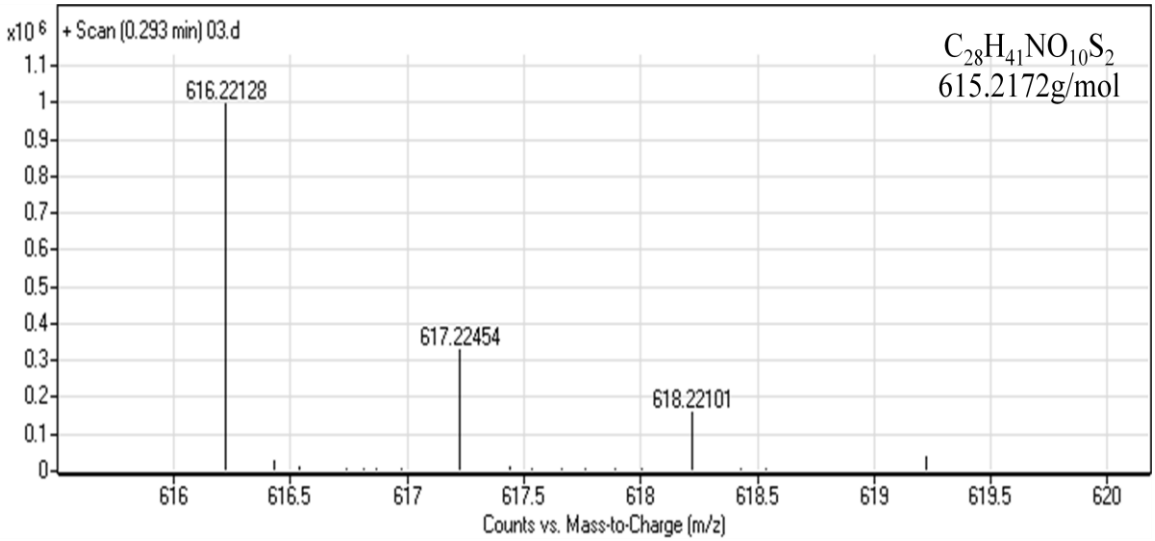


Figure A.24. HRMS spectra of product **6**.

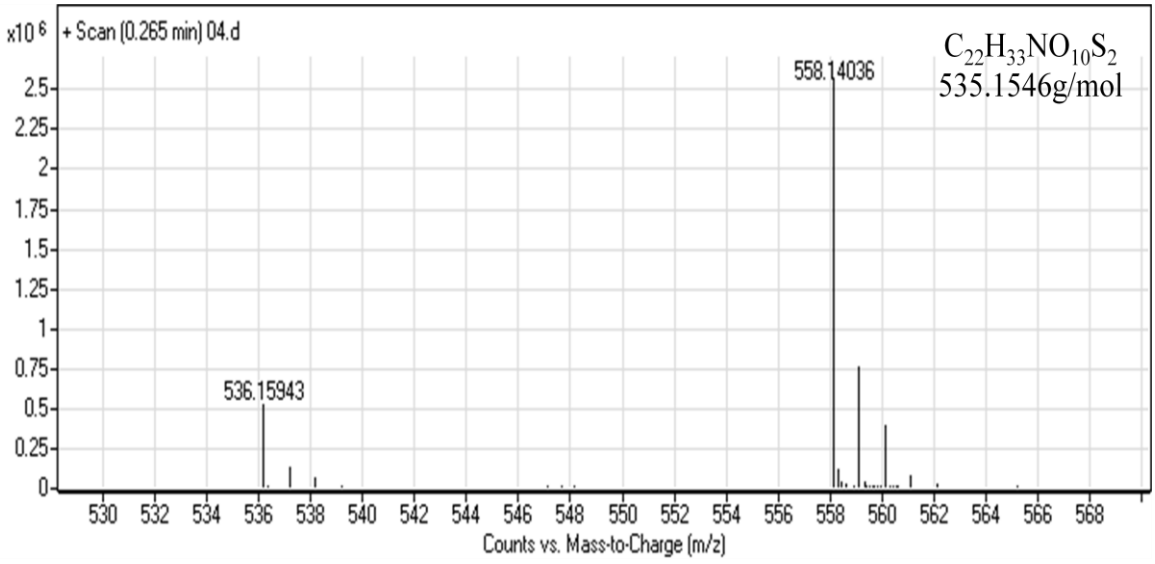


Figure A.25. HRMS spectra of product **7**.

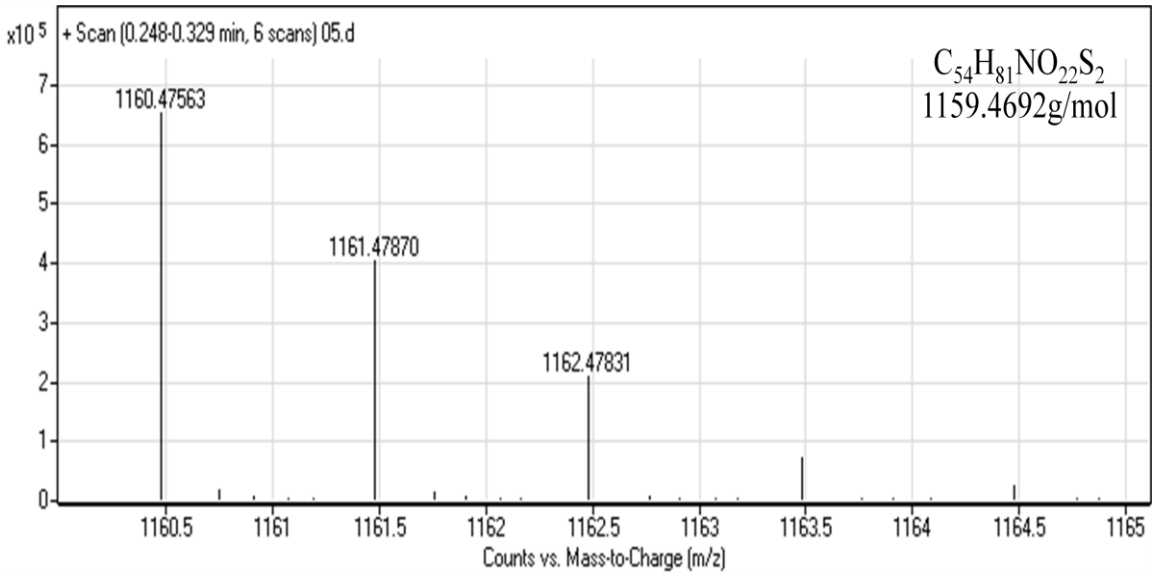


Figure A.26. HRMS spectra of product **8**.

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