ORTHOGONALLY FUNCTIONALIZABLE MALEIMIDE BASED STYRENIC POLYMERS

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ABSTRACT

ORTHOGONALLY FUNCTIONALIZABLE MALEIMIDE BASED STYRENIC POLYMERS

Polymers containing maleimide groups on their side chains have been synthesized by utilization of a novel styrenic monomer containing a masked maleimide unit. Diels-Alder reaction between furan and maleimide was adapted for the protection of the reactive maleimide double bond in the monomer prior to polymerization. AIBN initiated free radical polymerization was utilized for synthesis of copolymers containing masked maleimide groups. No unmasking of the maleimide group was evidenced under the polymerization conditions. The maleimide groups in the side chain of the polymers were unmasked into their reactive form by utilization of retro Diels-Alder reaction. This cycloreversion was monitored by thermo gravimetric analysis (TGA), ^{1}H and ^{13}C NMR spectroscopy.

Orthogonally multifunctional styrene based copolymers were obtained by copolymerization of the maleimide based monomer both with N-hydroxysuccinimide containing styrene monomer and alkene containing styrene monomer. Further functionalization studies were performed with two different thiols by using both Michael addition and thiol-ene click chemistry.

Crosslinking studies for maleimide based styrenic copolymers were also performed as an extension of the project. Furfuryl amine was introduced to the polymer and the solution was spin coated on to the silicon surfaces. By heating the surface under vacuum, first Diels-Alder reaction between maleimide and furfuryl amine occured, then, due to the Michael addition of amines to free maleimides, crosslinked network was obtained. Remaining unreacted maleimides were functionalized both with bodipy bromide as a control and bodipy thiol. Fluorescence microscopy was used to investigate the degree of immobilization.

ÖZET

ORTOGONAL OLARAK İŞLEVSELLEŞTİRİLEBİLEN MALEİMİD KÖKENLİ STİRENLİ POLİMERLER

Yan zincirlerinde maleimid grubu içeren polimerler korunmuş maleimid grubu içeren yeni stirenli monomer kullanılarak sentezlendi. Polimerizasyondan önce monomerdeki reaktif maleimidin çift bağını korumak için furan ve maleimid arasında oluşan Diels-Alder tepkimesi kullanıldı. Korunmuş maleimid grupları içeren kopolimerlerin sentezi için AIBN başlatıcılı serbest radikal polimerizasyonu kullanıldı. Bu polimerizasyon koşulları altında hiç korunmamış maleimid grubu kalmadığı gösterilmiştir. Retro Diels-Alder tepkimesi sayesinde polimerlerin yan zincirlerinde bulunan maleimid grupları açılarak reaktif hallerine dönüştürüldü. Bu siklo-dönüşüm, termo gravimetrik analiz (TGA), ^{1}H ve ^{13}C NMR spektroskopileri kullanılarak izlenmiştir.

Hem alken içeren hem de N-hidroksi-süksinimid içeren stiren monomerinin maleimid kökenli monomer ile kopolimerizasyonu sonucunda ortogonal çok fonksiyonlu stiren kökenli kopolimerler elde edildi. Michael katılması ve tiol-en klik kimyası kullanılarak iki değişik tiol varlığında sonraki işlevselleştirme çalışmaları gerçekleştirildi.

Projenin devamı olarak maleimid kökenli stiren kopolimeri için çapraz bağlanma çalışmaları yapıldı. Furfuril amine polimerle biraraya getirildi ve slikon yüzey bu çözelti ile spin kaplama yöntemi kullanılarak kaplandı.

Yüzeyin vakum altında ısıtılmasıyla once maleimid ve furfural amin arasında Diels-Alder tepkimesi gerçekleştirildi, daha sonra aminlerin serbest maleimidlere Michael katılımı ile çapraz bağlı ağlar oluşturuldu. Geriye kalan bağlanmamış maleimidler hem kontrol olarak kullandığımız bodipi bromür hem de bodipi tiol ile işlevselleştirildi. Bağlanmanın derecesi floresan mikroskopu ile incelendi.

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LIST OF SYMBOLS / ABBREVIATIONS

ATR	Atom transfer coupling
ATRP	Atom transfer radical polymerization
BSA	Bovine serum albumin
DA	Diels-Alder
DNA	Deoxyribonucleicacid
NMR	Nuclear Magnetic Resonance
PEG	Poly(ethyleneglycol)
RAFT	Reversible addition–fragmentation chain transfer
rDA	Retro Diels-Alder
THF	Tetrahydrofuran
UV	Ultraviolet
AIBN	2,2’-azobisisobutyronitrile
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
DMF	Dimethylformamide
DMPA	2,2-dimethoxy-2-phenylacetophenone
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl Acetate
FT-IR	Fourier Transform Infrared
GPC	Gel Permeation Chromotography
HPLC	High Performance Liquid Chromatography
MeOH	Methanol
NHS	N-hydroxysuccinimide
SEC	Size Exclusion Chromotography
TEA	Triethylamine
TGA	Thermogravimetric Analysis

1. INTRODUCTION

1.1. Reactive Polymers

Recent years has witnessed an increase in design and synthesis of novel reactive polymers amenable to efficient post-functionalization. This is due to an increased application of such polymers in areas as materials science, and biomedical applications. These advancements are possible to an increased availability of a variety of very efficient chemical transformations [1-2].

Post-polymerization modification leading to the functionalization of the polymers is based on the polymerization of monomers having different functional groups. Chemoselectivity and orthogonality concepts are taking into account while conducting the reactions in order to modify the side chain of polymers [3].

In recent years multifunctionalization of polymers using various ‘orthogonal’ reactive functional groups have taken place (Figure 1.1). Weck and coworkers have pushed the limits by demonstrating orthogonal functionalization of a terpolymer using covalent synthetic strategies via combination of three different transformations such as maleimide thiol coupling, 1,3-dipolar cycloaddition and hydrazone formation. These transformations offering site-specific functionalizations, result in orthogonal multifunctional polymers [4].

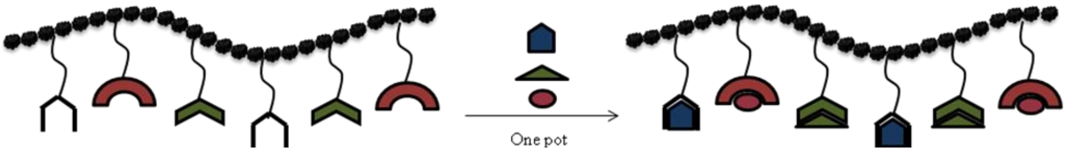


Figure 1.1. Schematic representation of the orthogonal multifunctionalization of copolymers

They copolymerized three different monomers to obtain the random terpolymer (Figure 1.2). For the following stepwise and orthogonal functionalization, they used benzenethiol, phenylacetylene, and benzhydrazine in order to functionalize maleimide, azide and ketone functionalities respectively.

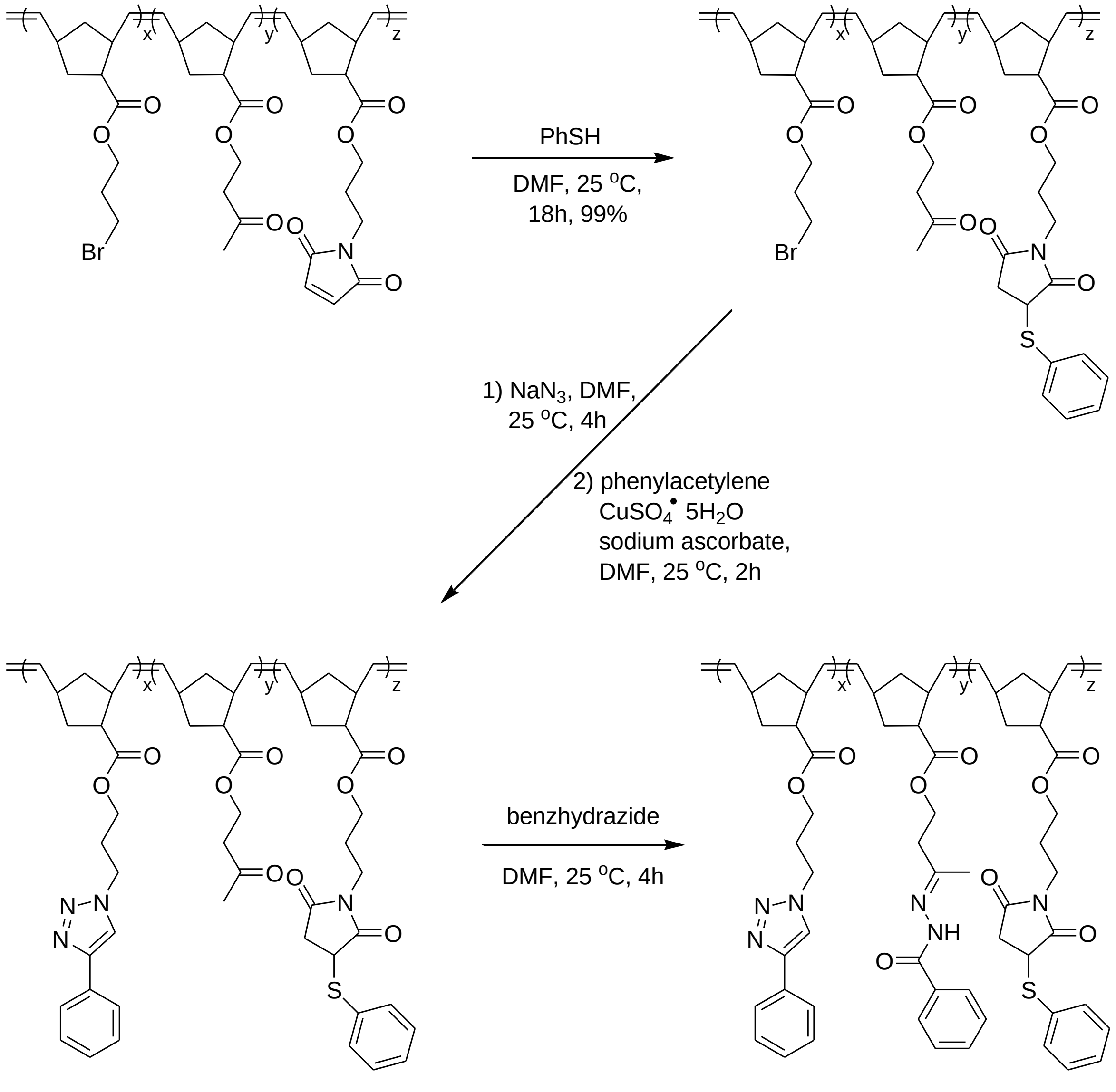


Figure 1.2. Synthesis and covalent multifunctionalization of terpolymer

Click chemistry based on the copper (I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes has been used mostly in the preparation of dendrimers, block copolymers, coupling of telechelic polymers and also in the area of polymer functionalization. An early example developed by Hawker and coworkers includes the one-pot combinations of click chemistry and other highly efficient chemical transformations, such as esterifications and amidation, for the multifunctionalization of a wide range of linear random terpolymers using simultaneous or cascade approaches [5]. They utilized the cascade strategy using a 9:1 random copolymer of styrene and N-acryloxy succinimide. To a solution of this copolymer in THF were added propargylamine and methyl 4-(azidomethyl)benzoate in one pot synthesis. They proved the orthogonal, complete cascade functionalization with the NMR spectrum indicating the complete conversion of active ester group into amide linkage and acetylene group into triazole ring (Figure 1.3).

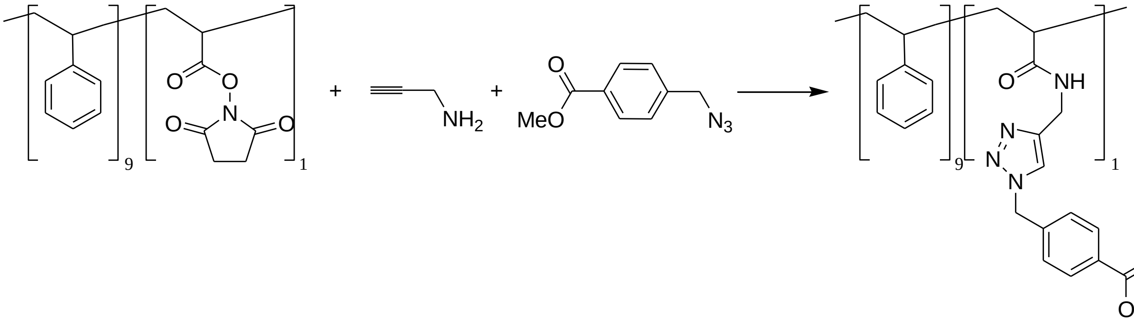


Figure 1.3. Orthogonal functionalization of random copolymer via click chemistry

For immobilization of biomolecules, a much sought after functionalization is via thiols as cystein residues can be engineered at specific sites of biomolecules. Cystein residues can react with maleimides, orthopyridyl disulfide units, vinyl sulfones and iodoacetamides. Reactivity of PEG-vinylsulfone towards thiols is very slow under basic conditions but getting faster when the pH increases. PEG-iodoacetamides may also form a stable thioether linkage but reacts slowly with thiols. The reaction of orthopyridyl disulfide-PEG units specifically with sulfhydryl groups under both acidic and basic conditions leads to a stable disulfide bond formation with proteins. On the other hand, since maleimides have high reactivity to thiols even under acidic conditions, the thioether linkage between the PEG-maleimide and protein is very stable [6].

Thiol reactive polymers based on the pyridyl-disulfide group have been recently reported as end functionalization or side chains [7-8]. Thayumanavan and coworkers have shown the synthesis of polymers by copolymerizing the two monomers containing pyridyl-disulfide and N-hydroxy succinimide units as reactive functional groups that are complementary to thiols and amines respectively (Figure 1.4). The substituents were incorporated on to the polymer either in one pot (independent of the order of addition) or by a sequential addition strategy.

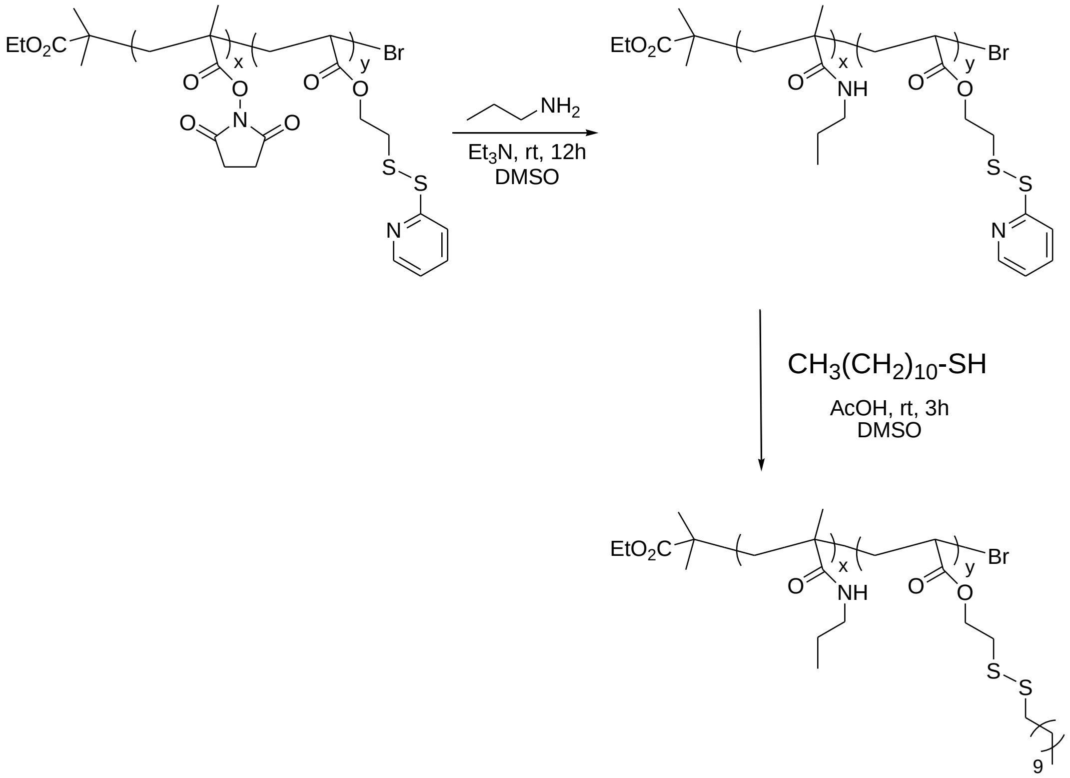


Figure 1.4. Functionalization of random copolymer

As an alternative, efficient thiol attachment can be done to the maleimide functional group. Often the reaction does not require any additional reagents and does not generate any byproducts. While the maleimide group have been extensively exploited in biomolecular immobilization on various metallic and glass surfaces [9-11], reports of polymeric surfaces modified by maleimides are rare.

Recent work by Howorka and coworkers highlight the potential such platforms hold towards DNA oligonucleotide microarrays [12], (Figure 1.5). First of all, the researchers prepared glass slides carrying a dense layer of maleimide poly(ethylene glycol) (PEG) which were then silanized with glycidoxypropyl trimethoxysilane. The epoxide groups of the GPS-silanized surface were hydrolyzed to diols in order to achieve an aldehyde functionality via oxidation of diols. Poly(ethylene glycol)-diamine was grafted onto the surface to obtain homogeneous thin layer of PEG. PEG-grafted slides bearing terminal amino groups were functionalized with succinimidyl 4-[p-maleimidophenyl] butyrate resulted in maleimide terminated surfaces for further immobilization of thiolated DNA oligonucleotides.

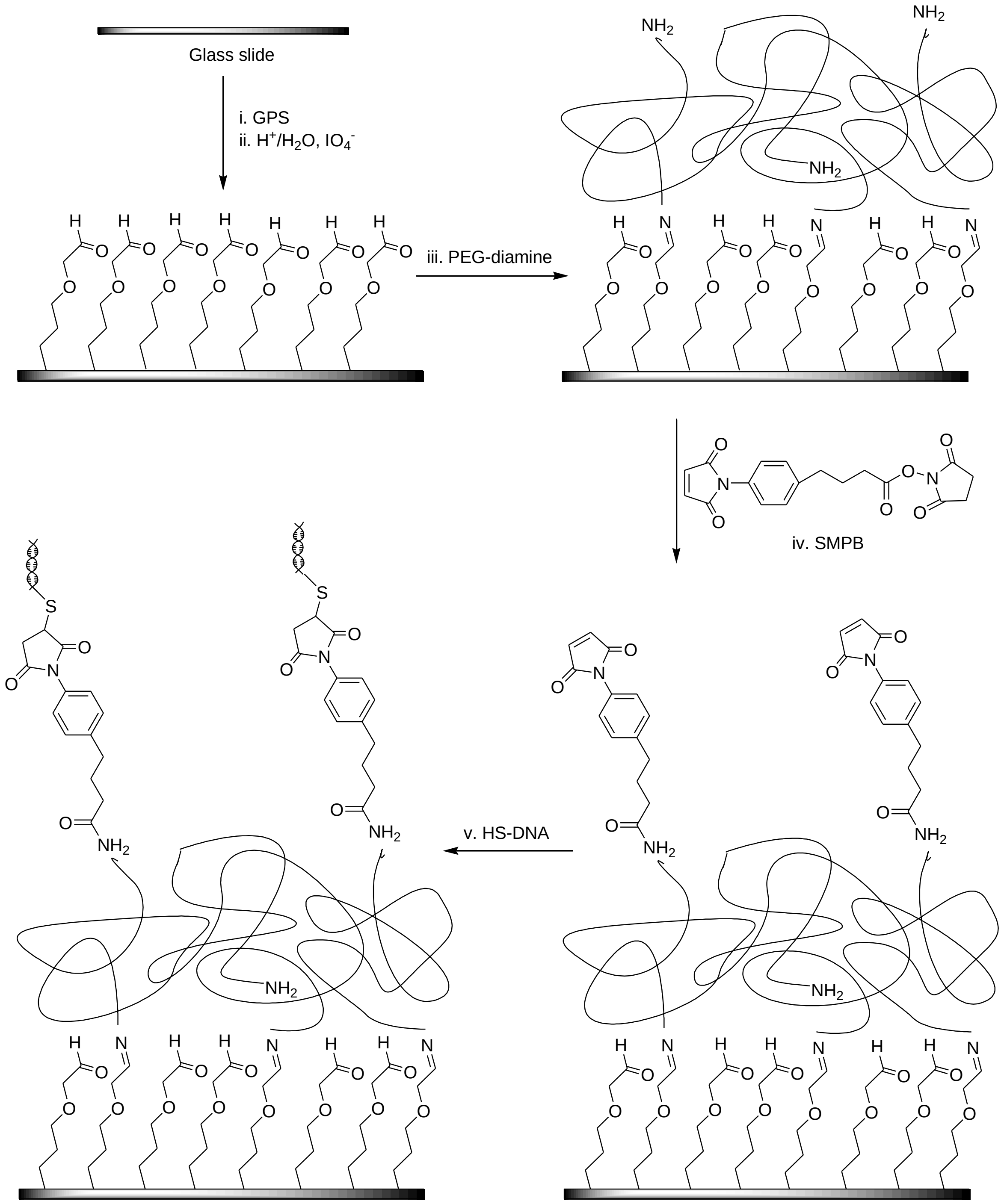


Figure 1.5. Schematic representation of the procedure for preparing PEG-grafted, maleimide terminated surfaces for the immobilization of thiolated DNA oligonucleotides

Very recently, remarkable advances have been made in this area by utilization of protected maleimide based initiators to obtain polymers with maleimides as their end groups using a Diels-Alder based maleimide protection-deprotection strategy [13-14].

Haddleton and co-workers reported two retrosynthetic approaches to obtain maleimide end functionalized macromolecules to target cysteine residues on the proteins. They improved following two independent approaches to introduce the maleimide moiety into the polymers (a) a post functionalization of a preformed primary amine-terminated polymer and (b) the use of a "protected" maleimide initiator for the polymerization step followed by deprotection to give the expected maleimide terminated polymers (Figure 1.6). These α-functional methacrylate polymers have been succesfully employed in coupling reactions with a tripeptide and a model protein Bovine Serum Albumin (BSA).

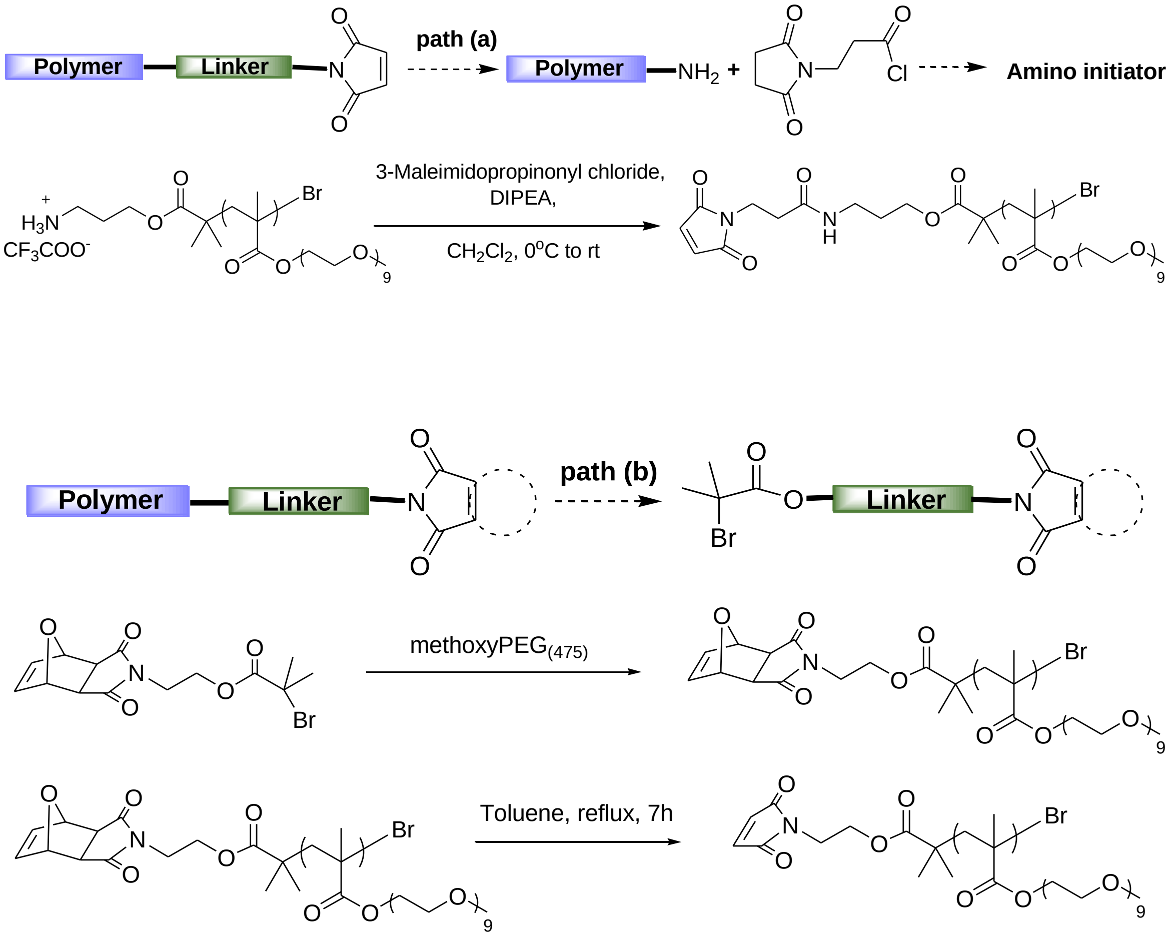


Figure 1.6. Synthesis of maleimide terminated polymer

In a recent study, Maynard et al. reported the synthesis of telechelic polystyrene containing maleimides at both ends for side selective conjugation to free cysteines of proteins using ATRP and atom transfer coupling reaction (ATR). An ATRP initiator with protected maleimide functionality was prepared and used to polymerize styrene. Semitelechelic styrene polymers were dimerized through ATR coupling to obtain bis-functionalized polymer. Resulting telechelic polymer was conjugated to *N*-acetyl-L-cysteine methyl ester after heating to activate maleimide groups (Figure 1.7).

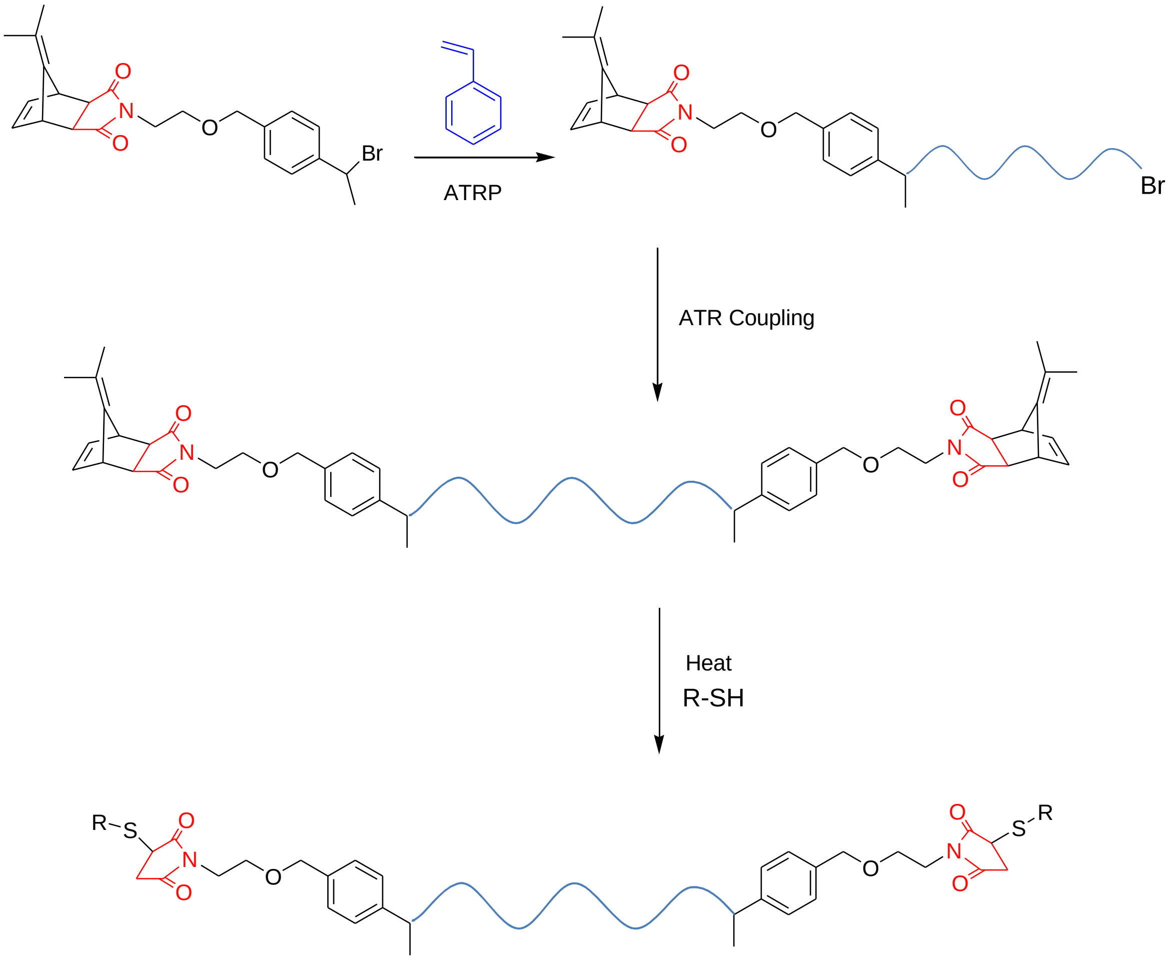


Figure 1.7. Synthesis of maleimide terminated telechelic polystyrene by ATR coupling

Apart from potential applications in biotechnology, since maleimide is an excellent dienophile participating in [4+2] Diels-Alder reaction in a reversible manner under mild conditions, maleimide containing polymers have attracted considerable interest from material science where cycloaddition chemistry has been widely explored [15-16].

1.2. Diels Alder Reaction in Polymer Chemistry

The Diels-Alder (DA) reaction is one of the most widely used and powerful synthetic methods when simplicity and efficiency of the reaction is taking into account. This chemistry based on the [4+2] cycloaddition of a conjugated, electron-rich “diene” functional group to an electron-poor “dienophile” unit [17-18]. In this cycloaddition reaction, addition of a dienophile to a conjugated diene results in a cyclic product referred as an adduct with the formation of new σ bonds, which are energetically more stable than the π bonds [19] (Figure 1.8).

The Diels- Alder reaction can be carried out under mild conditions in high yields without the formation of byproducts. Sharpless and coworkers proposed that the Diels-Alder reaction is included in click chemistry concept because of the high efficiency and modular nature of this reaction [20].

Furan and anthracene derivatives have been widely explored as diene components in macromolecular construction whereas maleimide due to their high reactivity and wide structural variability through the nature of the nitrogen substituents, are preferred as the choice of dienophile (Figure 1.8). One of the most relevant and attractive aspects of the DA reaction is the thermoreversibility, which implies that the adducts can be readily converted to the starting material, known as retro Diels-Alder (rDA) reaction [21-22].

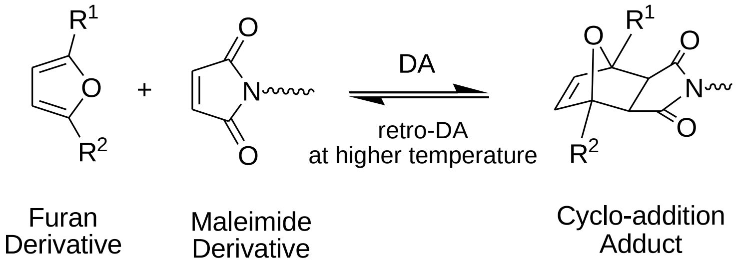


Figure 1.8. Representation of the DA and rDA reactions

The thermoreversible nature of the Diels-Alder reaction makes it an attractive candidate for self-healing polymeric materials [23-32]. Unfortunately, the free radical reactive double bond of the maleimide does not allow the use of a monomer containing this functional group. Hence, the maleimide functionalized polymers are usually obtained by post functionalization of the parent copolymers by a maleimide containing small molecule.

Diels-Alder reaction have attracted attention of the researchers generally in two main application areas: (i) synthesis of polymers where the pendant groups cross-link through successive DA coupling reactions and (ii) synthesis of polymers involving multifunctional diene and dienophiles as the monomer. Both types of polymer can revert to their precursors through the rDA reaction.

1.2.1. Cross Linking via Diels-Alder Reaction

DA reaction have been utilized to induce the cross-linking to the polymer structure by taking the advantage of the inter-macro-molecular couplings with a difunctional complementary reagent such as furan copolymer plus bismaleimides or polymer bearing maleimide moieties plus difurans. The thermal sensitivity of the DA reaction makes it an attractive candidate for thermally reversible cross-linking. The first study of thermally reversible networks from linear polymers bearing furan as a diene and maleimide as a dienophile has reported by Saegusa and coworkers in 1990 [33]. In later studies of the same system, Chujo and co-workers introduced the maleimide and furan functionalities to the side chain of the polymer [28] (Figure 1.9).

Canary and Stevens revealed the synthesis of thermally reversible cross-linking of polystyrene [34]. Poly[styrene-co-(maleic anhydride)] esterified with furfuryl alcohol was thermally crosslinked with maleimidomethylated polystyrene which was synthesized by the tin (IV) chloride catalyzed Friedel-Crafts reaction between polystyrene and N-chloromethylmaleimide. Reversal of crosslinking via rDA occurred rapidly at 150°C but at this temperature, polymers posed stability problems due to the furfuryl groups (Figure 1.10).

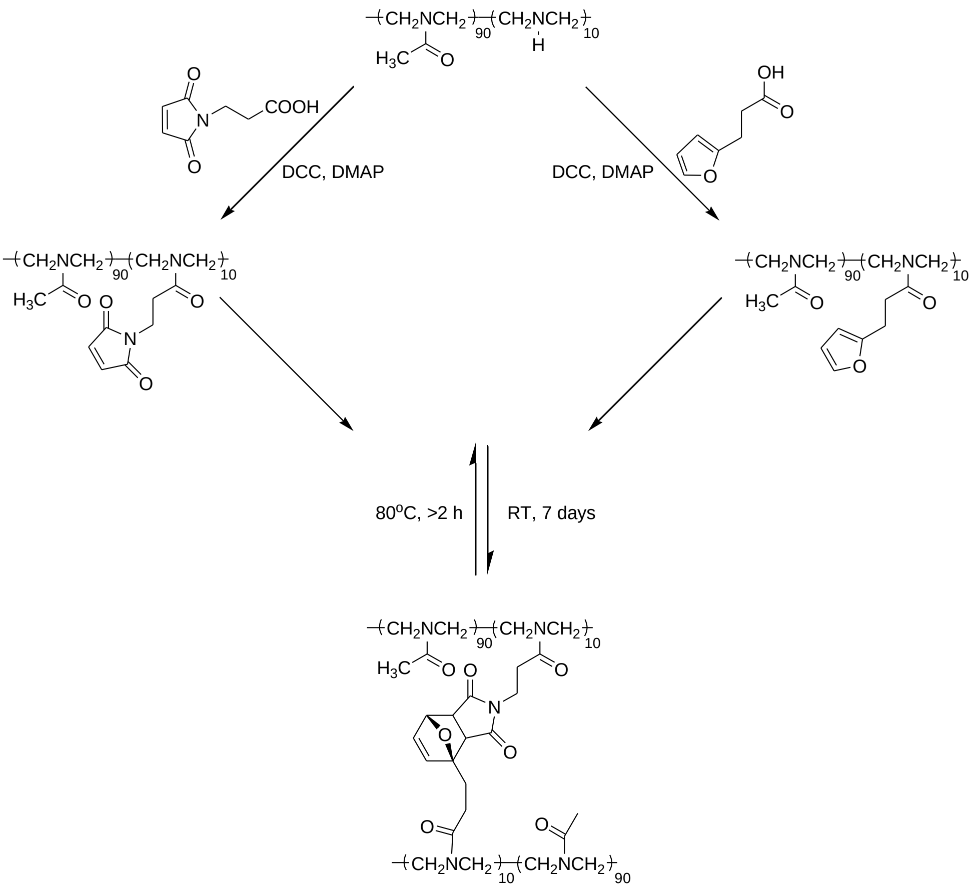


Figure 1.9. Cross-linking via Diels–Alder cycloaddition reaction between pendant furan and maleimide moieties of thermally reversible polymer

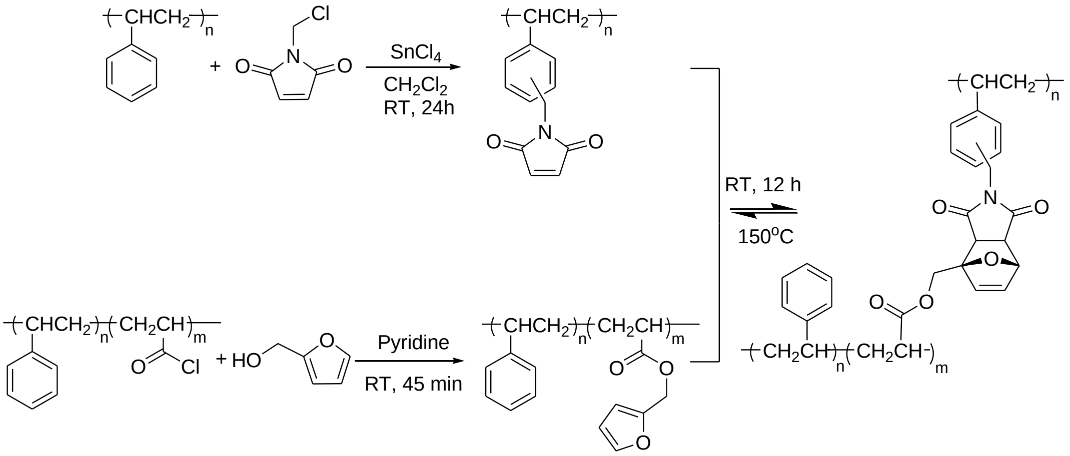


Figure 1.10. Reversible crosslinking of polystyrene

Gandini and coworkers also noticed the similar problem that they easily constructed the crosslinked network but the resultant gels did not undergo the rDA reaction. To overcome this issue, they used excess furan trap while performing the retro Diels-Alder reaction between the furan containing styrene copolymer and N,N-methylenediphenylbismaleimide (MDPBM). The use of an excess furan reacting with the released MDPBM, successfully induced the rDA and prevented the side reactions related to the furfuryl moieties [26] (Figure 1.11).

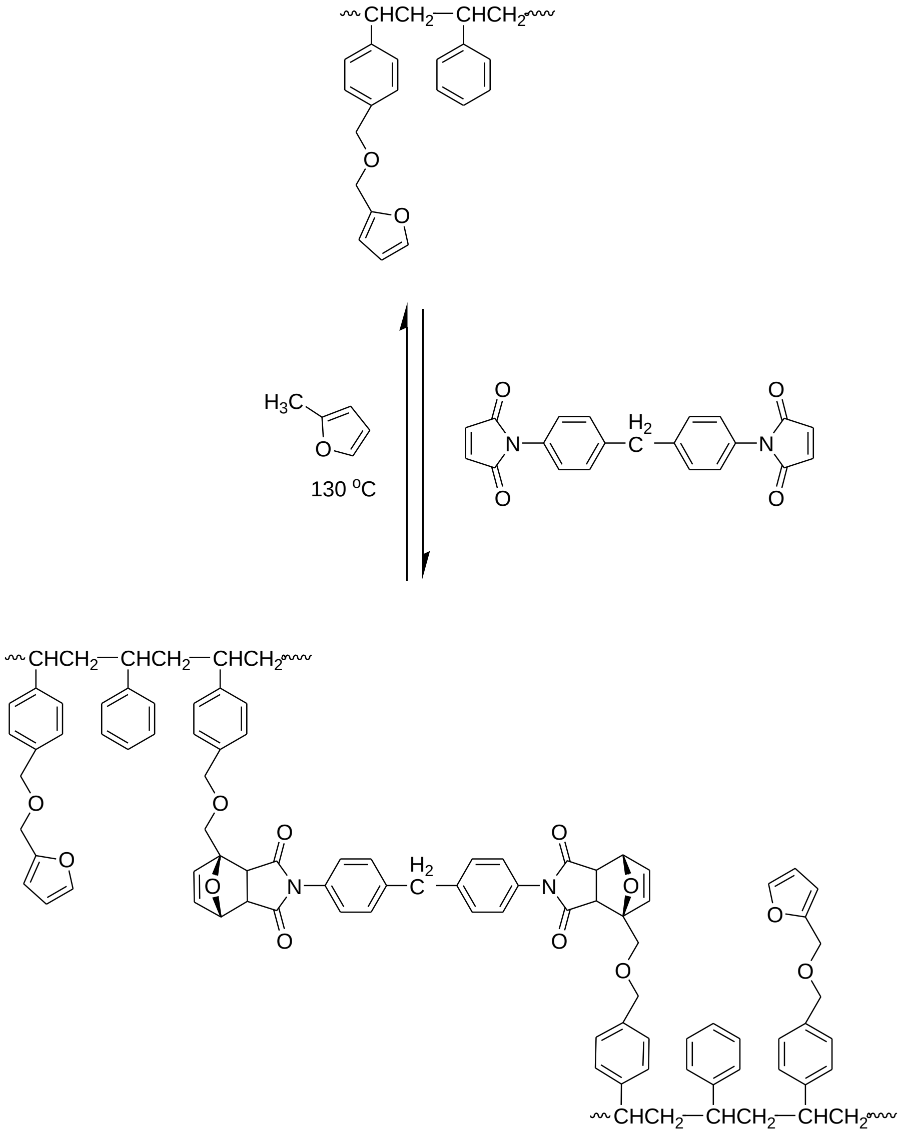


Figure 1.11. Use of bismaleimides as crosslinkers

1.2.2. Polymerization via Diels-Alder Reaction

DA “click” chemistry is a suitable method for the synthesis of polymers; it has been widely used for the synthesis of a diversity of polymers in the past. Utilization of DA reaction in polymerization dates back to an early report about formation of oligomers of cyclopentadiene by Alder and coworkers in 1932. In 1961, Stille and co-workers accomplished the DA reaction between cyclopentadiene based dimers and bismaleimides in order to produce high molecular weight polymers [35]. The study did not promise further developments due to the utilization of biscyclopentadienyl compounds that are highly prone to homopolymerize.

In 1990s, DA polymerization has given rise to the synthesis of variety of polymers such as polyimides, polyurethanes and acrylic copolymers [36-37]. In the early nineties, series of papers including DA polymerization between difuran derivative and bismaleimide have been published. Gandini’s group described the synthesis of polyadducts from the AA & BB type monomers containing difuran and aromatic bismaleimides via Diels-Alder type polycondensation reaction [38] (Figure 1.12).

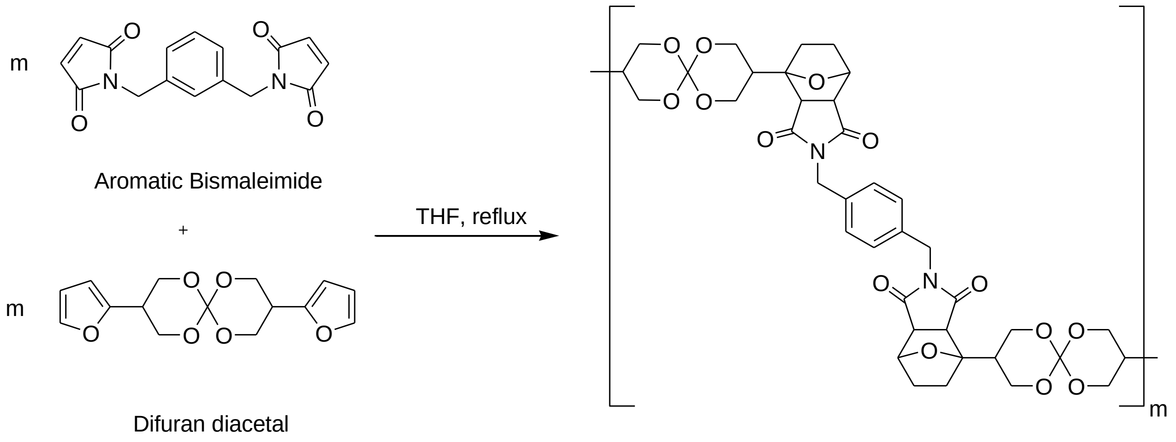


Figure 1.12. Diels-Alder polycondensation of difunctional monomers

McElhanon and Wheeler [39] demonstrated the synthesis of benzyl aryl ether dendron and dendrimers based on thermally reversible furan-maleimide Diels-Alder reactions. The study revealed that after 1 h of heating, % 40 of dendrons had dissociated to maleimide and furan dendron. On the other hand, full restoration of the original structure was observed after cooling to 65 °C for couple of days (Figure 1.13). This work was the first example of a covalent thermally reversible dendrimer design exploiting the DA/rDA approach.

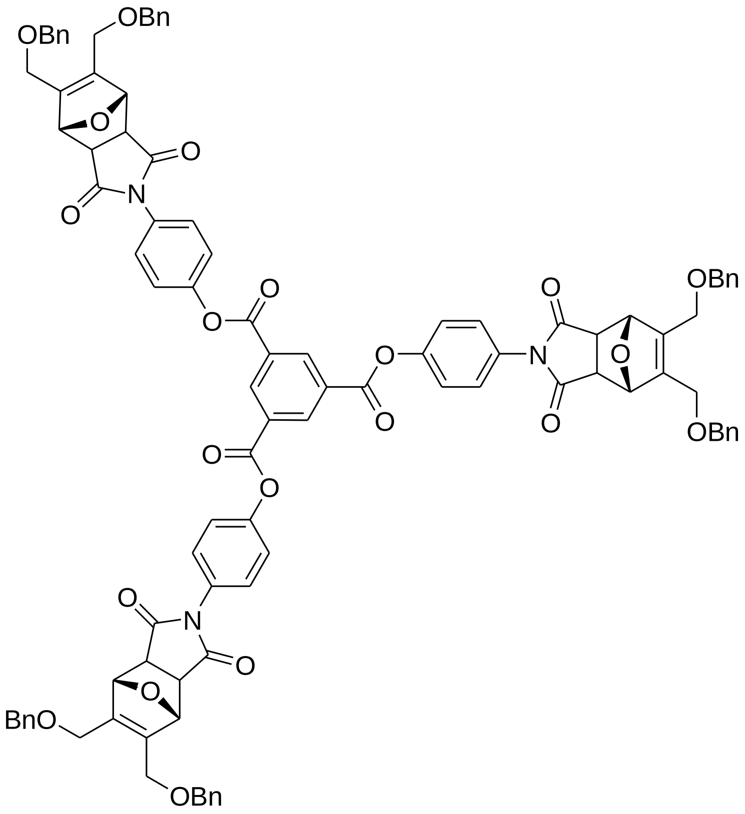


Figure 1.13. Synthesis of first generation dendrimer via DA

<u>1.2.2.1. Synthesis of Reactive Polymers via Diels-Alder Reaction:</u> Polymers containing functional groups can be utilized to attach desired molecules using very efficient and clean chemical transformations like thermoreversible Diels-Alder reaction. Polymers containing dendrons as side chains, often referred to as “dendronized polymers” are rapidly becoming common macromolecular building blocks in both biopharmaceutical and materials research. A recent study from our research group introduced a modular approach towards the synthesis of polymers dendron conjugates.(Figure 1.14) In this study Diels-Alder cycloaddition between the anthracene containing polymer and latent reactive dendrons containing masked maleimide groups at the core leads to quantitative functionalization of the polymer chains to afford dendronized polymers [40].

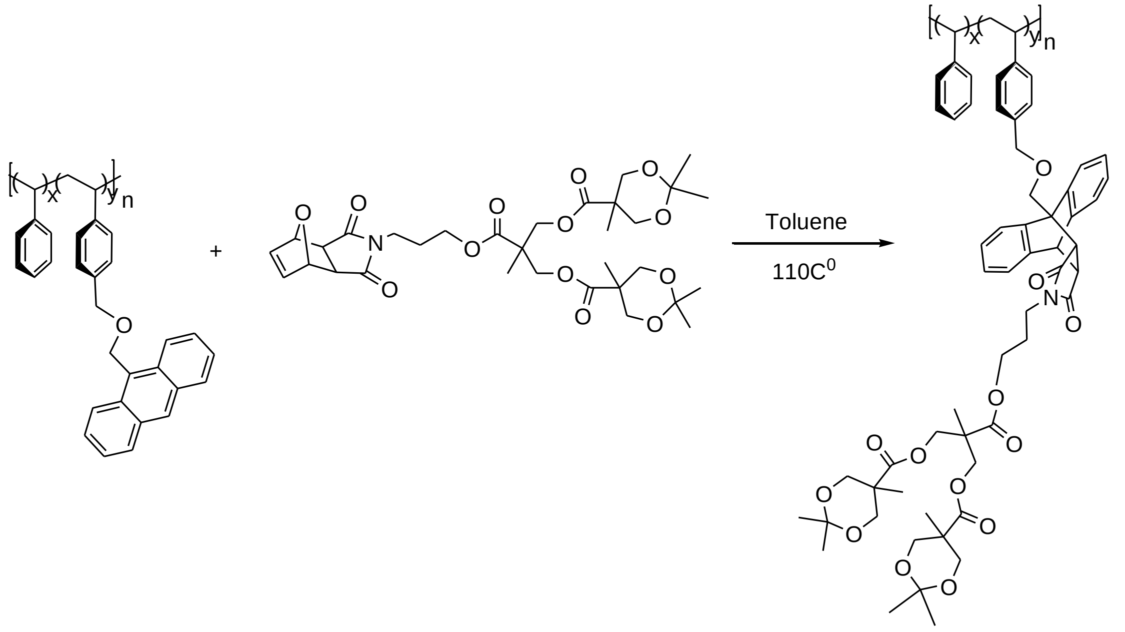


Figure 1.14. Synthesis of dendron-polymer conjugate

Recently, our research group has shown the synthesis of novel methacrylate based reactive polymers containing furan-protected maleimide groups as side chain units. The protected maleimide units were then activated via rDA reaction to enable post-functionalization of the resulting polymer with thiol containing molecules [41] (Figure 1.15).

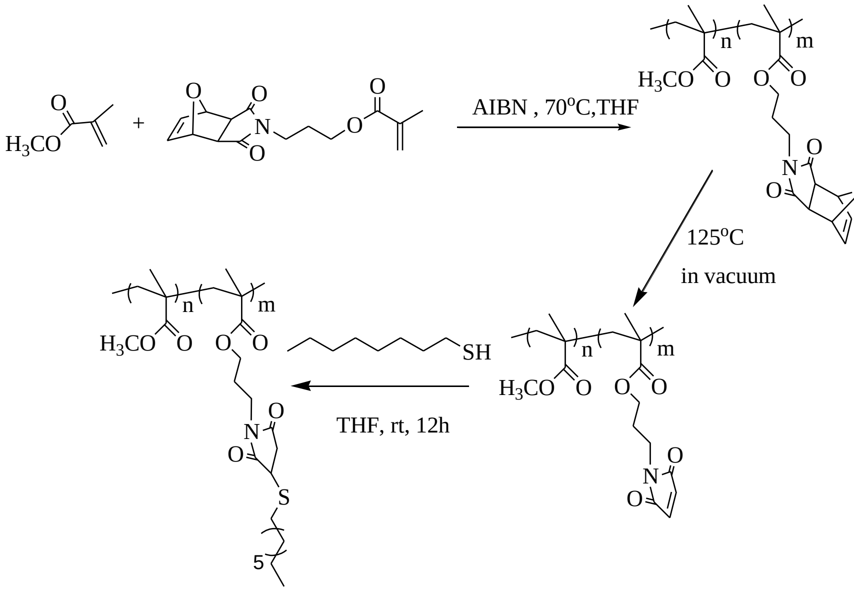


Figure 1.15. Synthesis of maleimide based reactive copolymer

1.3. Thiol-ene Click Chemistry

Click chemistry, specifically the copper(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes, is highly useful technique in polymer science due to its regiospecific conversion, compatibility with many of the functional groups and efficient postpolymerization applicability [42-43]. However, there are remarkable limitations of this strategy, such as the necessity of a metal catalyst, the lack of ability to control the reaction photochemically and performing reactions without using any solvent [44]. To meet the need of efficient, robust and orthogonal click reactions, thiol-ene click chemistry has been discovered. This chemistry is mainly based on the addition of thiols to nonactivated alkenes via thermally or UV initiated free-radical step-growth mechanism.

The basic kinetics of the photochemically initiated thiol-ene click reaction was demonstrated in Figure 1.16. There are three steps as in free-radical polymerization mechanism: initiation, propagation and termination. First of all, a thiyl radical is formed under UV light using photoinitiator. Then this thiyl radical is introduced to the carbon of an ene functionality which is included in propagation 1. At the second propagation step, the carbon-centered radical subsequently abstract the hydrogen of the thiol group resulting in the ‘clicked’ product and a thiyl radical which is then terminated via radical-radical coupling [45].

Thiol-ene chemistry has easy access to a wide variety of thiol and alkene starting materials such as commercially available thiols including cysteine containing peptides, alkyl halides, alcohols, RAFT reagents and also alkenes that can be readily converted to thiols. In addition to wide availability and associated stability of starting materials, the solvent free reaction condition of this chemistry prevents the formation of harmful by products. Furthermore, purification of the resulting product is based on the elimination of any excess mercaptans by utilizing simple precipitation techniques [46].

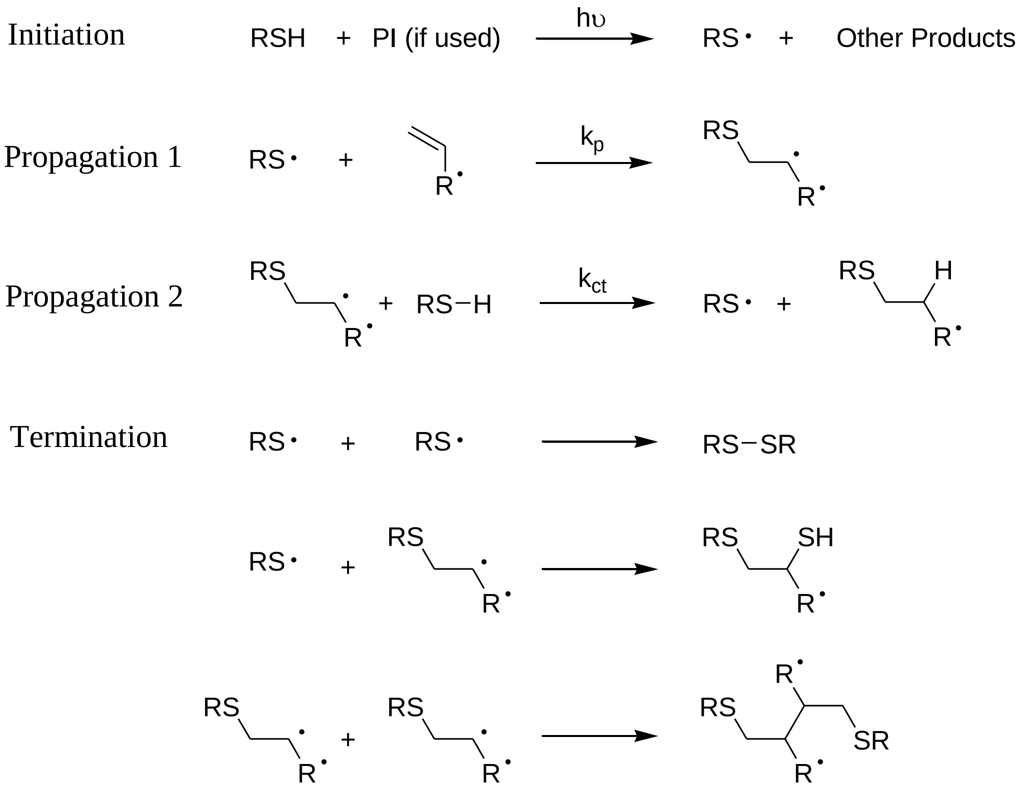


Figure 1.16. General thiol-ene photopolymerization process

The thiol-ene chemistry has recently attracted significant attention in the materials arena and this rapid as well as high yield synthetic strategy has prompted the researchers to utilize this reaction while developing new, functional, thiolated macromolecules [45].

Hawker and co-workers have highlighted its application in the synthesis of dendritic macromolecules. They demonstrated the efficient synthesis of poly(thio-ether) dendrimers by conducting the solvent free thiol-ene reactions for not only construction of the dendritic backbone but also functionalization of the chain ends [44] (Figure 1.17).

In 2007, Schlaad and co-workers [47] utilized thiol-ene chemistry to modify side chains of poly[2-(3-butenyl)-2-oxazoline] photochemically with five different thiols having diverse functionality. These thio-click reactions were performed under an inert atmosphere by irradiation with UV light for 24 h.

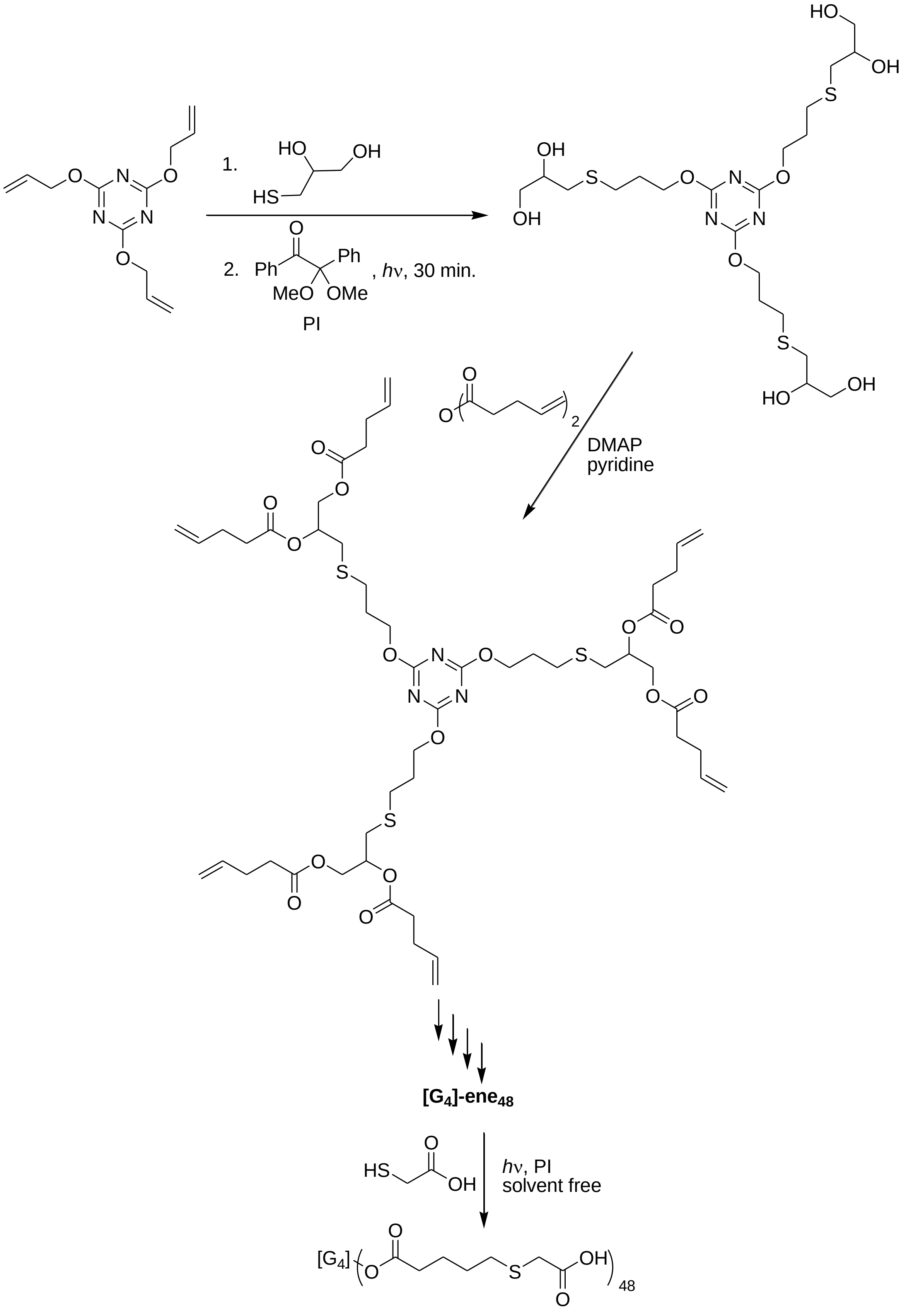


Figure 1.17. Synthesis of functionalized dendritic macromolecule via thiol-ene reaction

Thiol-ene click chemistry can also be performed with thermal initiation. David and Kornfield reported the design of the polymer structure where thiols can be coupled to the polybutadiene bearing pendant vinyl groups under thermal conditions within 2-6 hours, using a thermal radical initiator [48].

In order to examine the efficiency and orthogonality of both photochemically and thermally initiated thiol-ene click coupling reactions, Hawker and coworkers reported various click reactions through the mercaptan functionalization of a series of alkene-functional polymers [46]. Figure 1.18 represents the two of the copolymer that would undergo thiol-ene coupling.

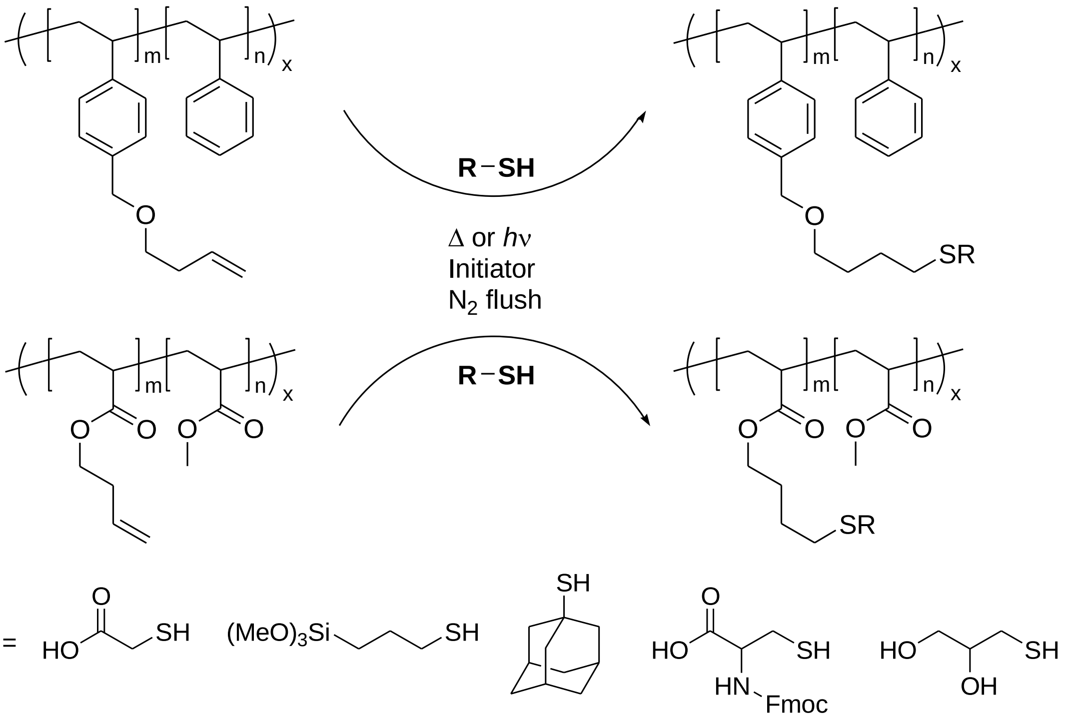


Figure 1.18. Functionalization of styrenic and methacrylic copolymers with different thiols

As an extension of this work, they also proved the orthogonality of the thiol-ene reaction with the the copper-catalyzed azide/alkyne cycloaddition chemistry. They synthesized an asymmetric telechelic polymer bearing an alkene at one chain end and an azide at the other chain end. Thioglycolic acid and propargyl alcohol were incorporated on to the polymer in a stepwise fashion independently of the order of addition (Figure 1.19).

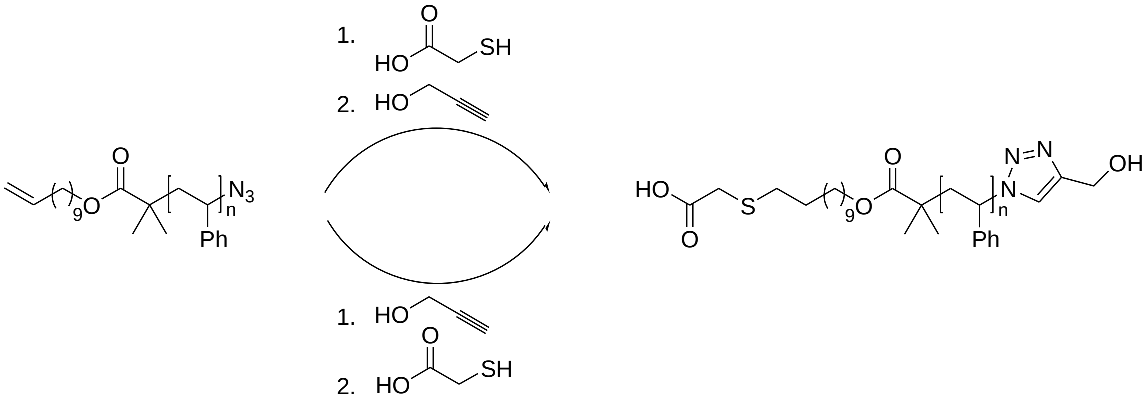


Figure 1.19. Orthogonal stepwise functionalization with both thiol-ene and CUAAC click reactions

2. AIM OF THE PROJECT

The methodology discussed in this project utilizes a Diels-Alder/retro Diels-Alder (rDA) reaction based strategy for the synthesis of maleimide side chain containing styrene polymers. A Diels-Alder reaction with furan protects the reactive maleimide group in the monomer prior to the polymerization, while a retro Diels-Alder reaction after the polymerization deprotects the maleimide group to its reactive form. Synthesis of novel masked maleimide monomers and their copolymerization with styrene via AIBN free radical polymerization, subsequent activation via the retro Diels-Alder reactions to afford novel reactive polymers is demonstrated.

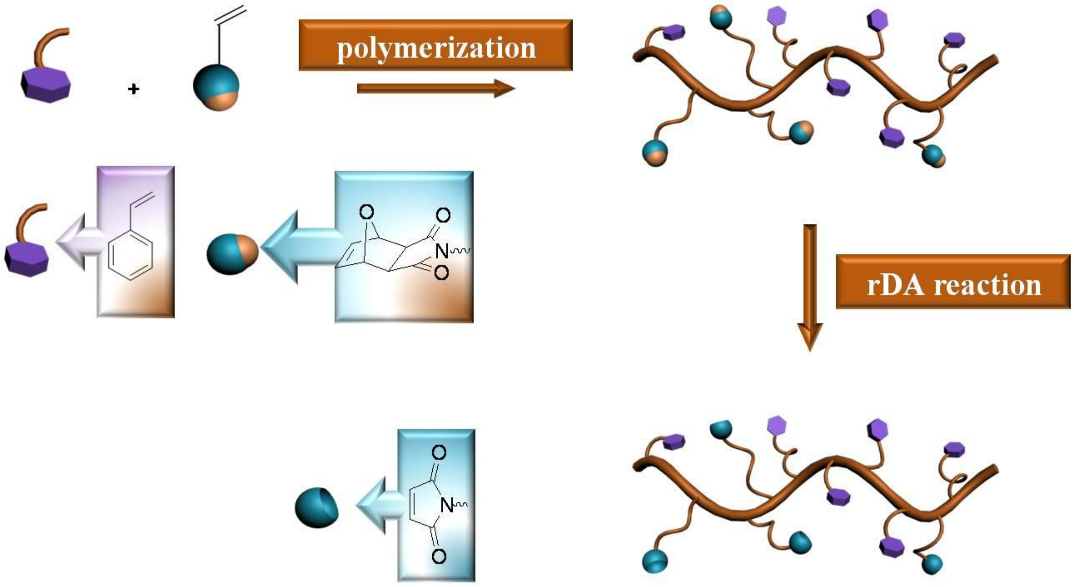


Figure 2.1. Design of the polymer structure

As an extension of this research, synthesis of copolymers containing two orthogonal reactive groups that would allow multifunctionalization in a controlled fashion is undertaken. The maleimide based monomer can be copolymerized with other reactive monomers such as amine reactive N-hydroxy succinimide based monomer and thiol-reactive alkene based monomer. While the former copolymer allows orthogonal one-pot functionalization with amine containing and thiol containing molecules, the later copolymer represents a novel class of macromolecules that allow efficient functionalization by two different thiols. Thiols were incorporated on the polymer via Michael addition and thiol-ene click chemistry respectively.

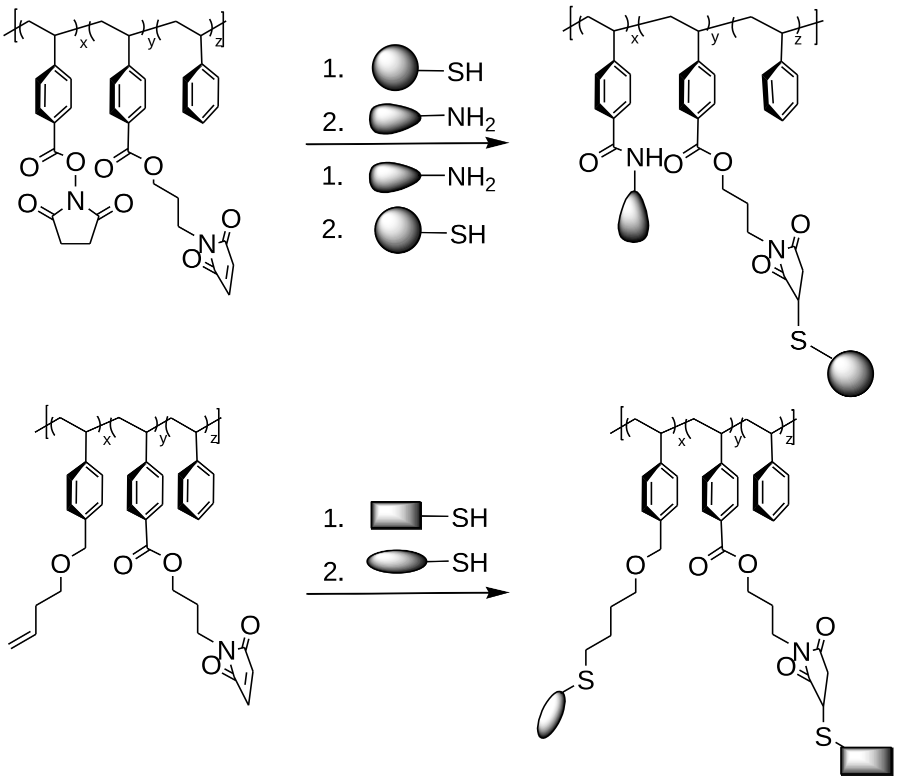


Figure 2.2. The synthesis of orthogonally functionalizable thiol and amine reactive polymers, and thiol-thiol reactive polymers

Finally, crosslinking studies for maleimide based styrenic copolymers were performed. Solution of the activated polymer with furfuryl amine in DMSO was prepared and spin coated on to the silicon surfaces. Surfaces were then heated to 60 ^{o}C in order to provide the Diels-Alder reaction between maleimide and furfuryl amine, as well as the crosslinked network due to the Michael addition of amines to free maleimides. Remaining unreacted maleimides were functionalized both with bodipy bromide as a control and bodipy thiol. Fluorescence microscopy was used to investigate the degree of immobilization.

3. RESULTS AND DISCUSSION

3.1. Latent-Reactive Maleimide Based Monomers

Latent reactive monomer **1** and monomer **2** was synthesized according to the procedure that is written in the experimental part. Monomer **1** was synthesized via the reaction of cycloadduct **1** with chloromethylstyrene in presence of K_2CO_3 and THF (Figure 3.1). Monomer **2**, containing the protected maleimide Diels-Alder adduct, was synthesized by the reaction of alcohol **2** with 4-vinyl benzoic acid (Figure 3.3). The alcohol **2** was obtained by reacting the Diels-Alder adduct of furan and maleimide with 3-amino-1-propanol according to previously reported literature [49]. Purity of the monomers was proved with the NMR (^{1}H and ^{13}C) spectra (Figure 3.2)

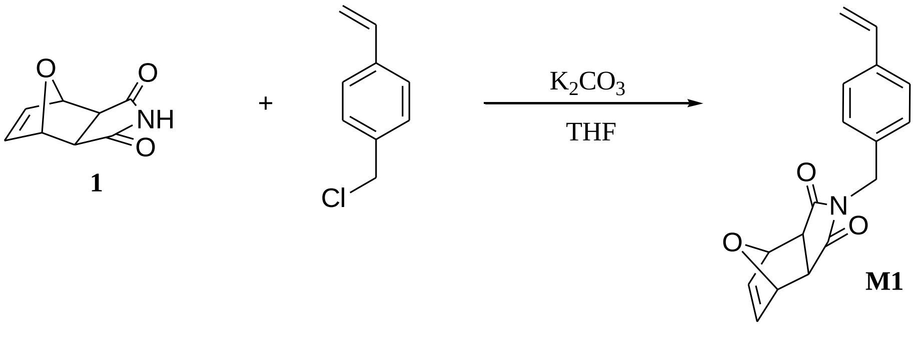


Figure 3.1. Synthesis of Styrene based Latent-Reactive Monomer **M1**

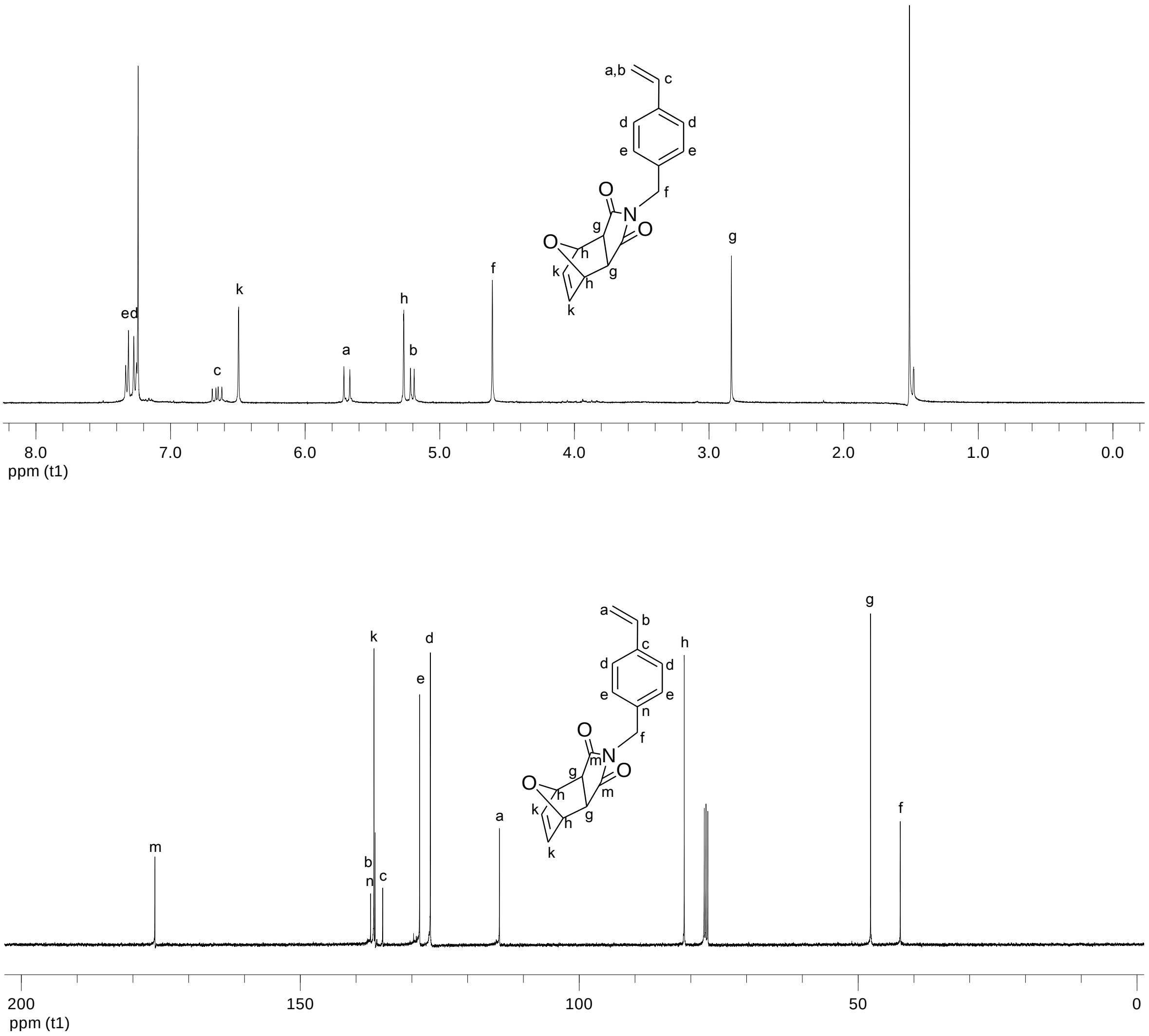


Figure 3.2. ^{1}H NMR and ^{13}C NMR spectrum of monomer M1

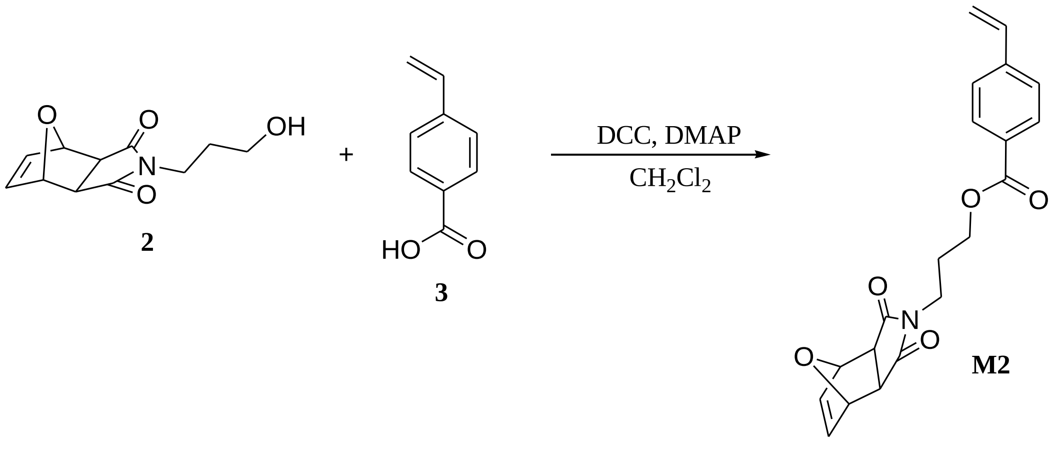


Figure 3.3. Synthesis of styrene based latent-reactive monomer M2

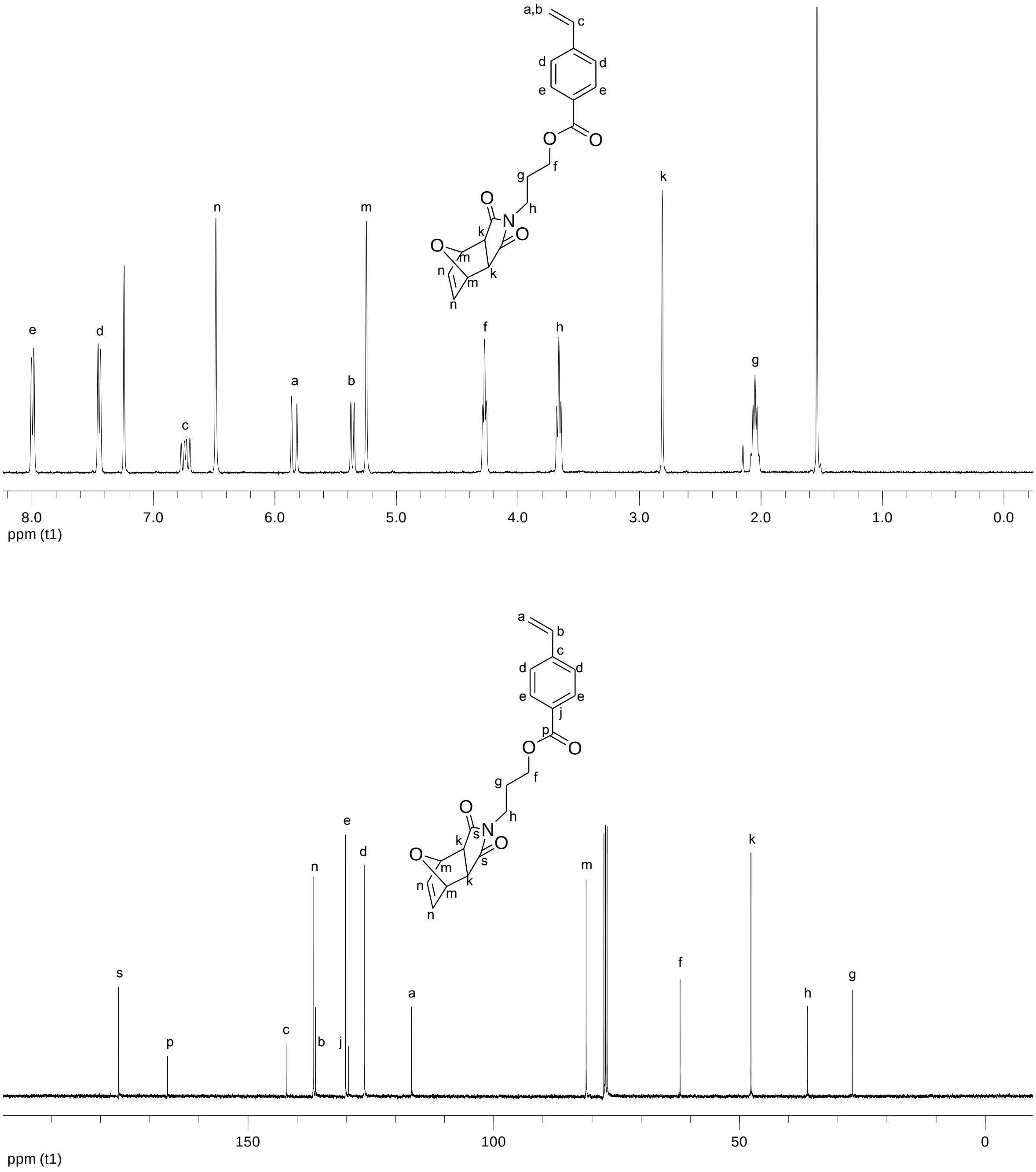


Figure 3.4. ^{1}H NMR and ^{13}C NMR spectrum of monomer M2

There are two types of vinylic protons in the ^{1}H NMR spectra of the monomers and each of them was readily distinguishable. In the ^{1}H NMR spectrum of the monomer **1**, the resonances of the vinylic protons on the styrenic part appear at 5.20, 5.70 and 6.65 ppm whereas the vinylic protons on the bicyclic oxanorbornene moiety appear at 6.49 ppm. Besides, the resonances of the carbon atoms of the styrene bond appear at 114.3 and 136.5 ppm whereas the carbon atoms of the oxanorbornene part appear at 136.8 ppm. When monomer **2** was taking into account, the vinylic protons which belong to styrene appear at 5.36, 5.84 and 6.74 ppm. On the other hand, the vinylic protons of the bicyclic oxanorbornene moiety appear at 6.48 ppm. Additionally, the carbon resonances of the styrene were observed at 116.7 and 136.3 whereas oxanorbornene moiety appeared at 136.7 ppm. In order to prove the accuracy of the monomer structures, elemental analysis was also performed and the data were displayed in Table 3.1.

Table 3.1. Results of elemental analysis

Monomers	**Nitrogen (%)**		**Carbon (%)**		**Hydrogen (%)**		**Sulfur (%)**	
	Theor.	Calc.	Theor.	Calc.	Theor.	Calc.	Theor.	Calc.
Monomer 1	4.979	4.762	72.58	72.299	5.374	5.53	0	0
Monomer 2	3.964	3.855	67.979	67.831	5.419	5.506	0	0

3.2. Maleimide Based Reactive Copolymers: Characterization and Functionalization

3.2.1. General Polymerization Procedure

AIBN initiated free radical polymerization was utilized for the synthesis of copolymers with different ratio of maleimides. Monomer **1** and monomer **2** were copolymerized with styrene in presence of THF in order to obtain latent reactive polymers **P-M1-1** and **P-M2-1** (Figure 3.5). After the polymerization of the monomers, resonances of the vinylic protons which belong to styrene disappeared whereas the peak coming from the bicyclic oxanorbornene remained unchanged.

Polymerizations were performed by changing the feed ratio, the reaction time and the dilution. It was observed that dilute reaction conditions results in lower molecular weight and lower conversions. Among the different solvents surveyed for polymerizations, lower polydispersities and higher conversions were obtained upon using THF as a solvent. Table 3.1 shows a summary of different polymerization conditions that were investigated and the results obtained. The composition of the copolymers could be easily determined from the integration of the ^{1}H NMR spectra (Figure 3.6). The ratio of area under the peak at 2.81 and 5.24 ppm corresponds to the bridge protons and the bridgehead protons of the protected monomers respectively, and the part of the area under the peak at around 6.5-8.0 ppm belongs to the protons coming from the styrene monomer.

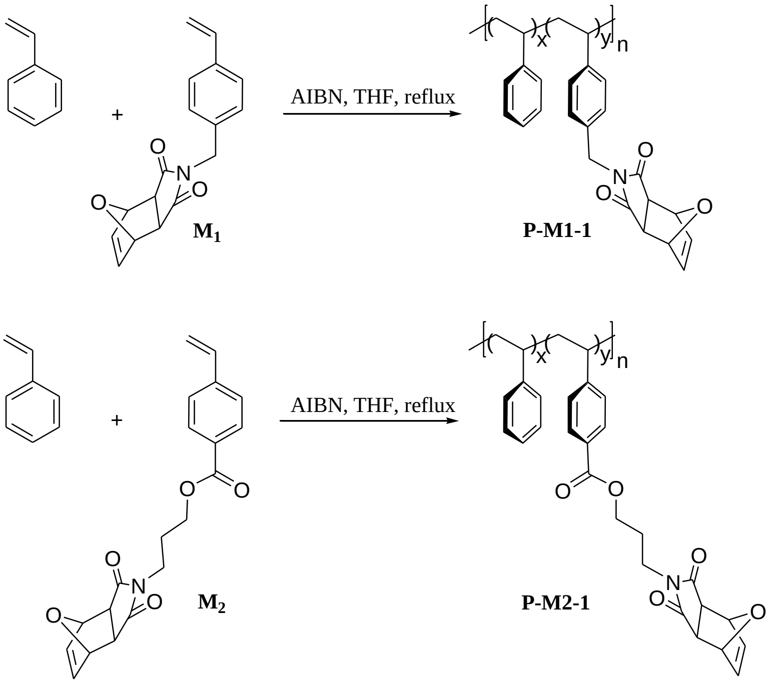


Figure 3.5. Synthesis of the latent-reactive polymer

Table 3.2. Synthesis of random copolymers containing latent reactive monomer 2[a]

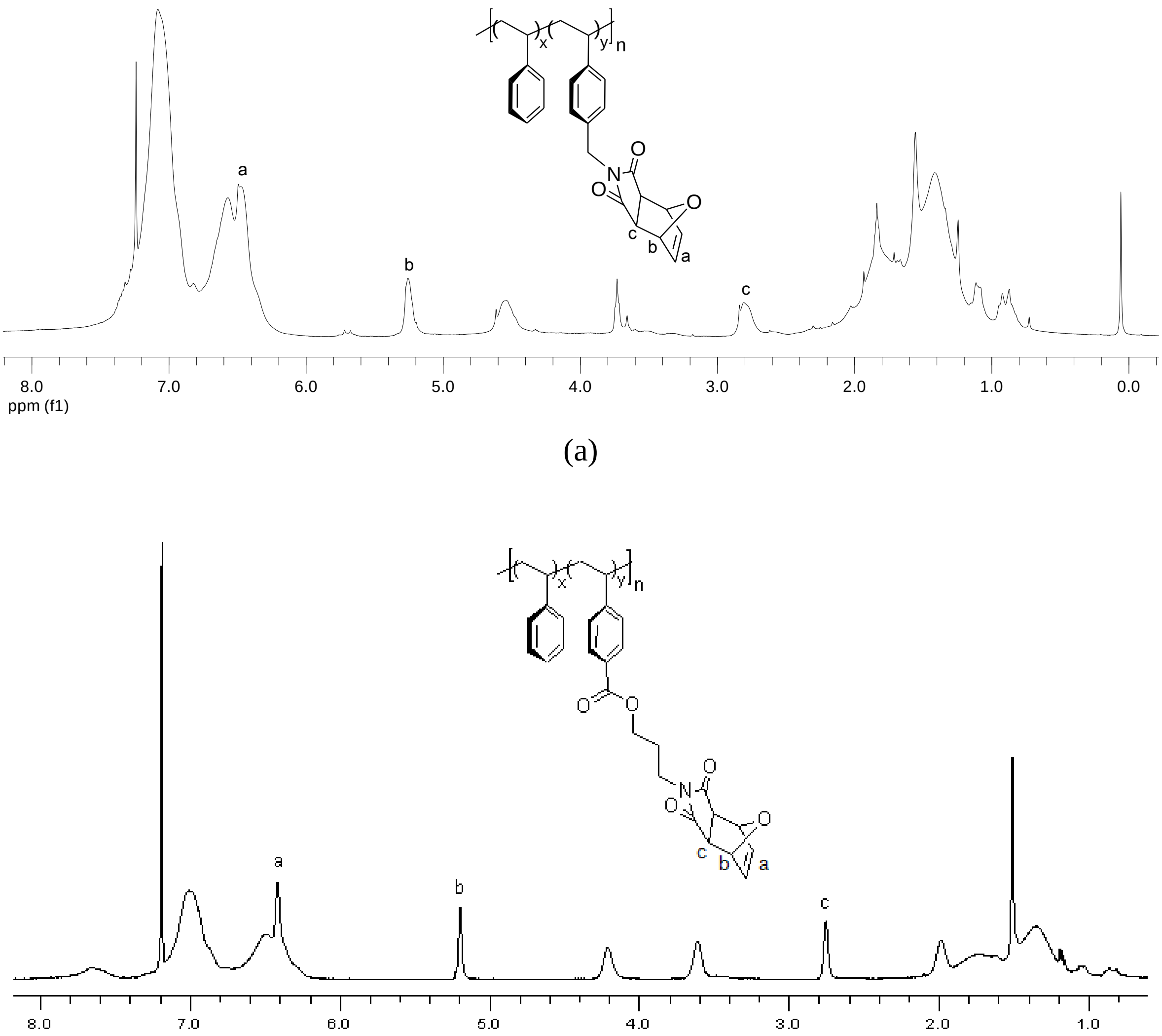
Polymer	Solvent	$F_{theoretical}$[b] (%)	$F_{calculated}$ (%)	% yield	M_n[c]	M_w/M_n[c]
P1	THF	50.0	33.0	33	6563	1.74
P2	THF	33.3	25.0	39	5406	1.51
P3	THF	20.0	20.2	41	5600	1.64
P4	THF	11.1	16.6	38	5214	1.49
P5	DMF	11.1	-[d]	42	8330	3.16
P6	Acetonitrile	11.1	-[d]	10	3900	1.6

[a] Initiator, AIBN; temperature, 65 °C; time, 12 h; monomer/solvent, 1/130.

[b] Feed ratio Styrene : Monomer **2**.

[c] Estimated by SEC eluted with THF, using polystyrene calibrations.

[d] NMR spectra were not taken due to bad results.



(a)

(b)

Figure 3.6. 1H NMR spectrum of copolymers (a) **P-M1-1** and (b) **P-M2-1**

3.2.2. Activation of the Polymer

The maleimide groups in the side chain of the polymers were unmasked into their reactive form by utilization of retro Diels-Alder reaction. The polymers were heated overnight at 110 ^{o}C in toluene. This resulted in complete cycloreversion of the furan-maleimide adducts to afford polymers containing maleimide side chain for further functionalization (Figure 3.7). ^{1}H-NMR analysis proved that the cycloreversion was almost quantitative (Figure 3.8). Appearance of a new peak at 6.63 ppm, accompanied by disappearance of peaks at 5.24, 2.81 and 6.47 ppm corresponding to the oxabicyclic moiety confirmed successful cycloreversion. Additionally, GPC analysis revealed that no undesirable degradation took place during the cycloreversion (Figure 3.9).

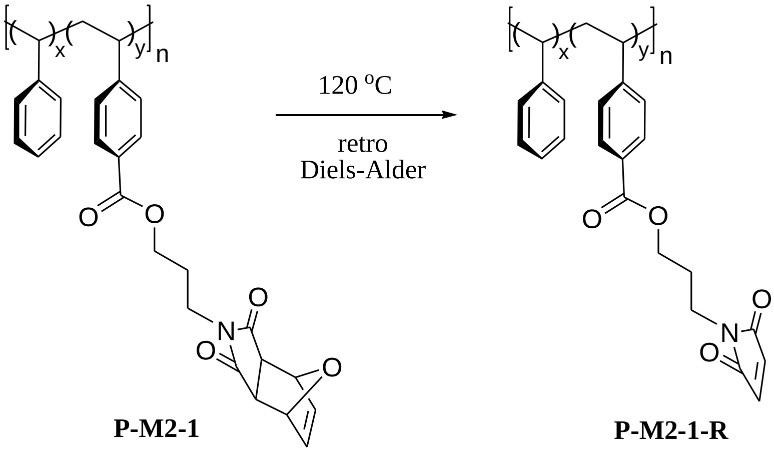


Figure 3.7. Synthesis of the Reactive Polymer **P-M2-1-R** via rDA Reaction

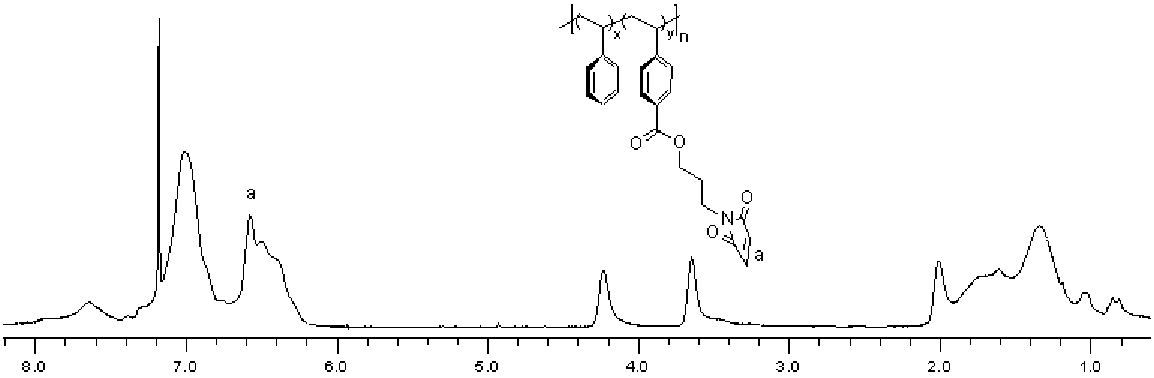


Figure 3.8. ^{1}H NMR spectrum of reactive polymer **P-M2-1-R**

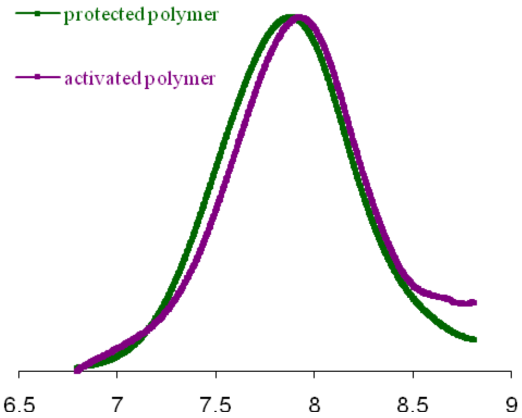


Figure 3.9. GPC graph of protected and activated polymer

Thermogravimetric analysis (TGA) was used to determine the thermal stability of the copolymers. It also confirms that the weight loss of furan increases proportionally with the increase of maleimide percentage in polymers. TGA of the polymers **P (1–4)** showed a weight loss starting at 115 °C (Figure 3.10). A consistent increase in weight loss of the polymers was observed upon increasing the amount of furan based monomer. According to the TGA analysis the observed weight losses were 11.4%, 8.9%, 7.6% and 6.6% for polymers **P1**, **P2**, **P3** and **P4**, where the expected weight losses were 14.8%, 12.1%, 8.5%, 5.7% respectively. Similar observations have been reported before and the discrepancies have been attributed to the broad range of local environments around the cycloadducts [50].

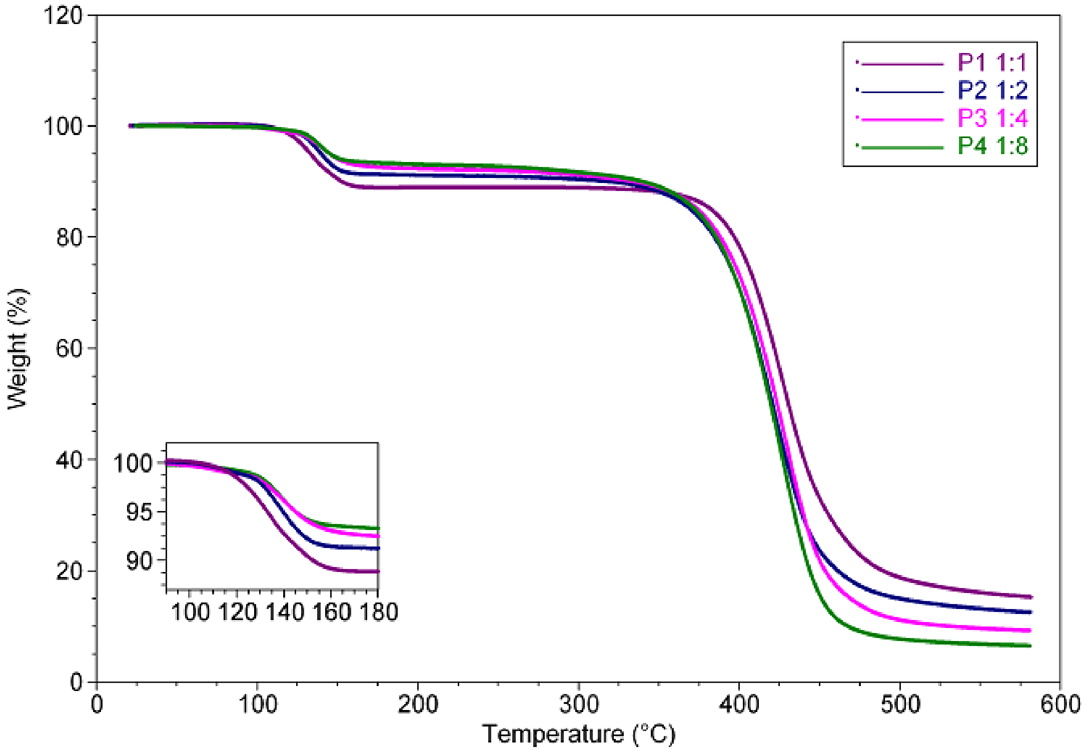


Figure 3.10. Thermogravimetric analysis of polymers with varying ratios of maleimide

3.3. Orthogonally Reactive Polymers: Characterization and Functionalization

3.3.1. Copolymers Containing Succinimide and Alkene Side Chains

To demonstrate the versatility of the maleimide based monomers, copolymerization with other styrene based reactive monomers, successful activation via thermal cycloreversion and subsequent efficient 'orthogonal' functionalization was explored. Styrene based N-hydroxysuccinimide and alkene appended monomers were synthesized according to previously reported literature. Monomer **3** was synthesized from the esterification of 4-vinyl benzoic acid with N-hydroxysuccinimide according to the previous reports [51]. Synthesis of monomer **4** was based on the chloride displacement from 4-vinylbenzyl chloride, using 3-buten-1-ol [52].

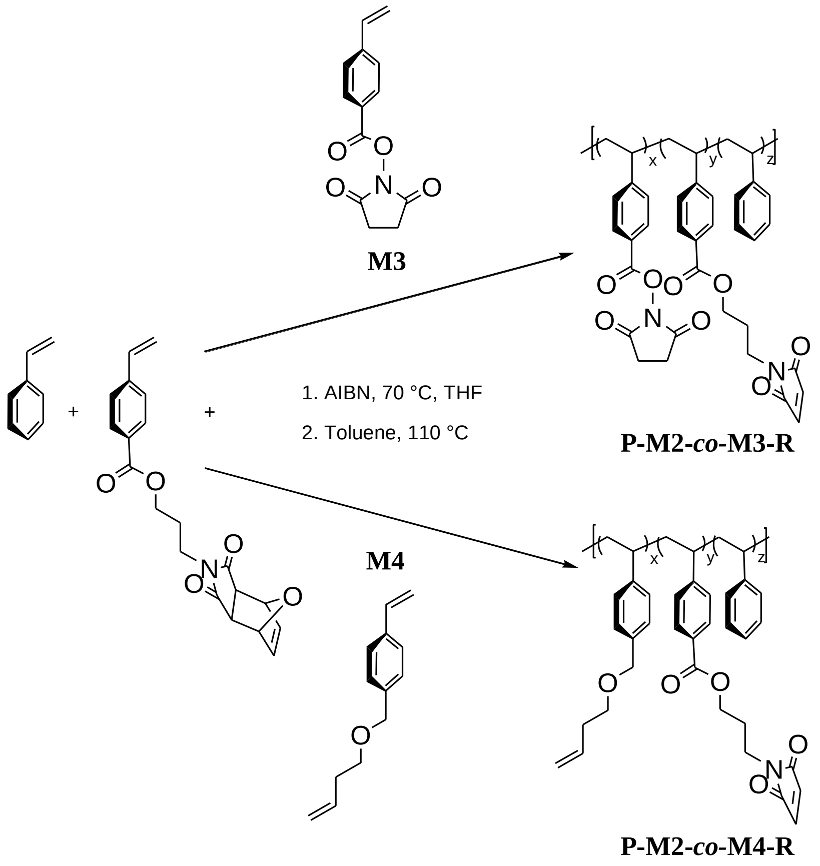
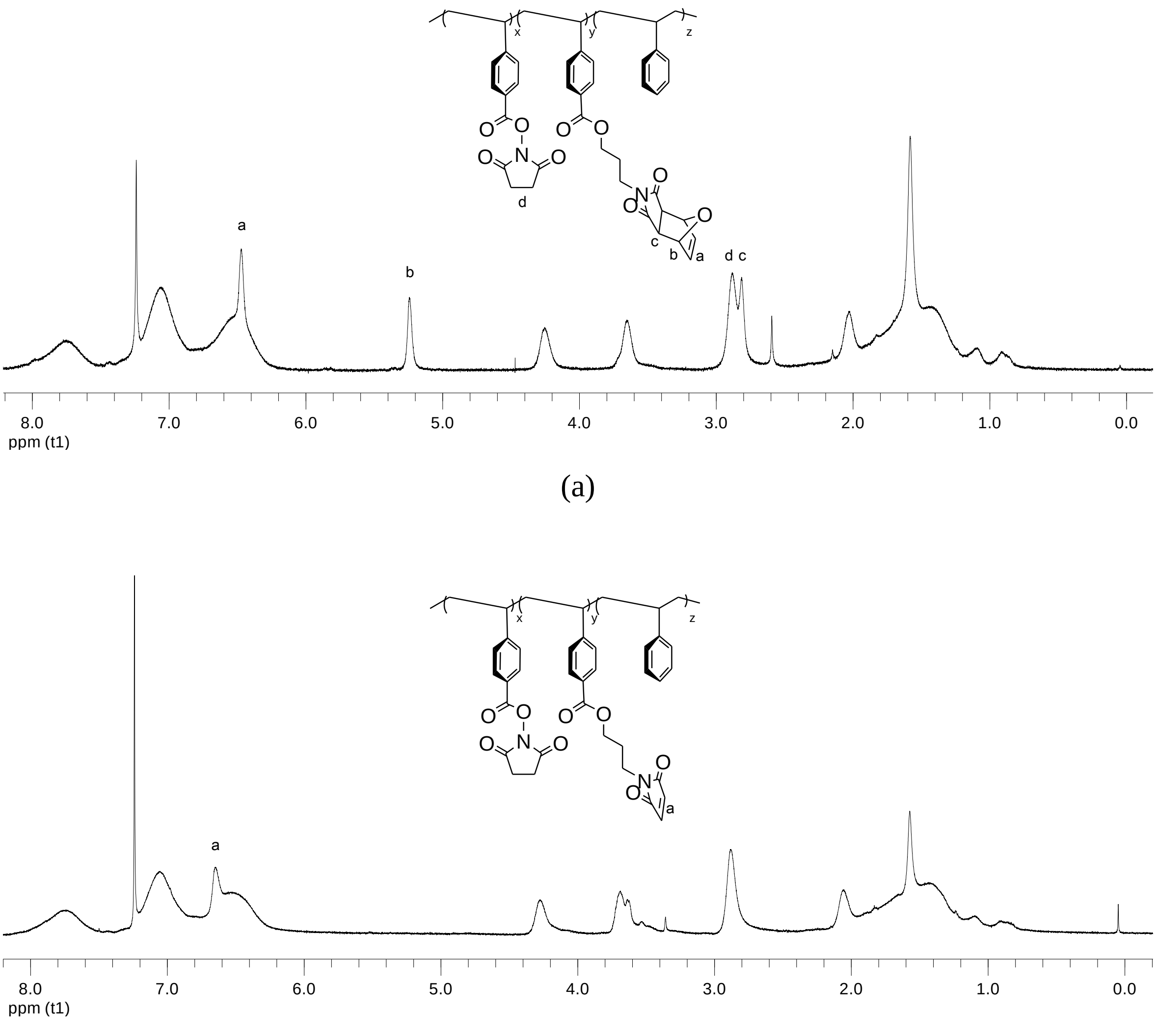


Figure 3.11. Synthesis of the orthogonally thiol-amine reactive polymer **P-M2-*co*-M3-R** and thiol-thiol reactive polymer **P-M2-*co*-M4-R**



(a)

(b)

Figure 3.12. ^{1}H NMR spectra of thiol-amine reactive polymer before (a) and after (b) rDA

Succinimide based monomer **M3** was copolymerized with the maleimide containing monomer **M2** resulting in the polymer **P-M2-*co*-M3**. Upon subjecting copolymer **P-M2-*co*-M3** to the rDA reaction, **P-M2-*co*-M3-R** which accommodates the amine reactive succinimide group and thiol reactive maleimide moiety along the same polymer backbone is obtained (Figure 3.11). Copolymer **P-M3-*co*-St** was synthesized to serve as a reference polymer in order to analyze the TGA results of the copolymers.

By using the same approach, maleimide containing monomer **M2** was copolymerized with the monomer **4** which has an alkene functionality resulting in the polymer **P-M2-*co*-**

M4. Retro Diels-Alder reaction took place for further functionalization of the polymer **P-M2-*co*-M4-R** (Figure 3.11).

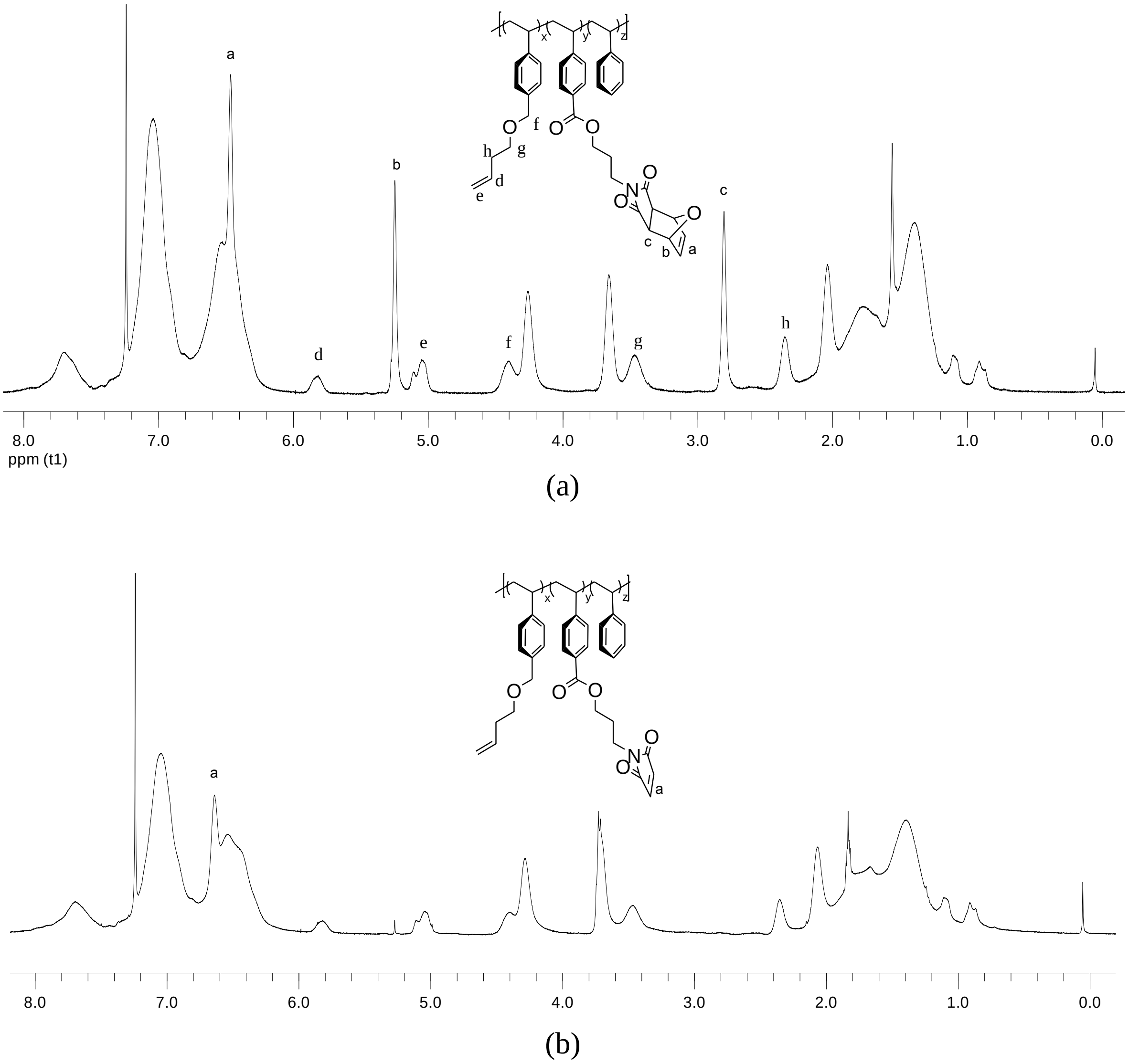


Figure 3.13. ^{1}H NMR spectra of thiol-thiol reactive polymer before (a) and after (b) rDA

^{1}H-NMR spectrum of the copolymer **P-M2-*co*-M3-R** confirms that oxabicyclic moiety was effectively converted to the reactive maleimide functional group whereas the succinimide moiety undamaged during the cycloreversion step (Figure 3.12). Relative stoichiometry of both the reactive groups in the parent polymer can be determined by integration of the proton resonances of the maleimide double bond (6.65 ppm) and succinimide unit (2.88 ppm).

When copolymer **P-M2-*co*-M4-R** was taking into account, the effectivity of retro Diels-Alder attracted attention as the same with **P-M2-*co*-M3-R.** Since copolymer has been synthesized from the monomer which has an alkene functionality, side chain of the polymer contains stoichiometric amount of double bonds which were easily detected from the H^1-NMR spectrum at 5.83 and 5.04 ppm (Figure 3.13).

Table 3.3. Synthesis of random copolymers containing succinimide and alkene side chains [a]

Polymer	$F_{\text{theoretical}}$[b] (%)	$F_{\text{calculated}}$ (%)	% yield	M_n[c]	M_w/M_n[c]
P-M2-*co*-M3	16.6	20.0	52	3822	1.54
P-M2-*co*-M4	11.1	10.5	54	5460	1.62

[a] Initiator, AIBN; temperature, 65 °C; time, 12 h; solvent, THF; monomer/solvent, 1/325 (for P-M2-*co*-M3), 1/225 (for P-M2-*co*-M4).

[b] Feed ratio Styrene : Monomer **2** : Monomer **3**.

[c] Estimated by SEC eluted with THF, using polystyrene calibrations.

Table 3.3 displays a summary of polymerization conditions of random copolymers containing succinimide and alkene side chains. The composition of the copolymers could be easily determined from the integration of the ^{1}H NMR spectra belongs to these copolymers.

3.3.2. Orthogonal Functionalization of the Succinimide Based Copolymer: P-M2-*co*-M3-R

Selective stepwise functionalization of the maleimide-succinimide containing copolymer was investigated by reacting it first with 11-undecenethiol in order to functionalize the maleimides on the side chain. Since succinimides are reactive towards amines, 3-amino-1-propanol was used as a nucleophile for further functionalization of the copolymer (Figure 3.14). Disappearance of the peak due to maleimide’s double bond in the ^{1}H NMR spectra and presence of expected new peaks at 5.8 and 4.95 ppm due to attachment of the 11-undecenethiol moiety implied successful Michael addition (Figure

3.15-a). Furthermore, thiol addition step has no effect on the succinimide unit since there was no change at 2.88 ppm which belongs to the proton resonance of succinimide. Further reaction of this polymer with amine resulting in the disappearance of the peak belongs to NHS confirms that the amidation was successfully done (Figure 3.15-b). Apart from the stepwise functionalization, one pot synthesis was also utilized in order to prove the orthogonality of the functionalization. As expected, same product was synthesized according to the ^{1}H-NMR spectroscopy.

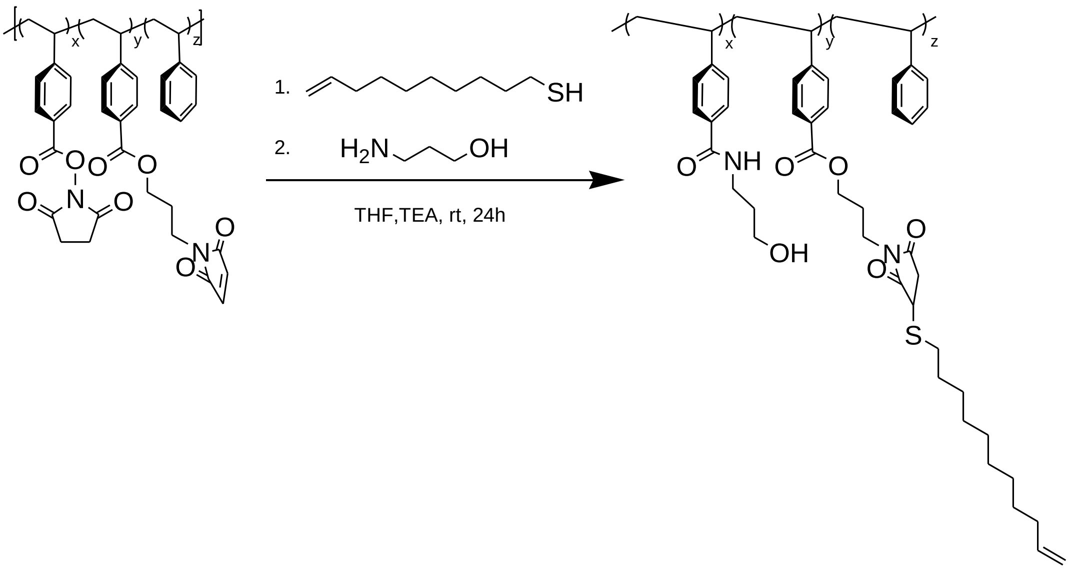
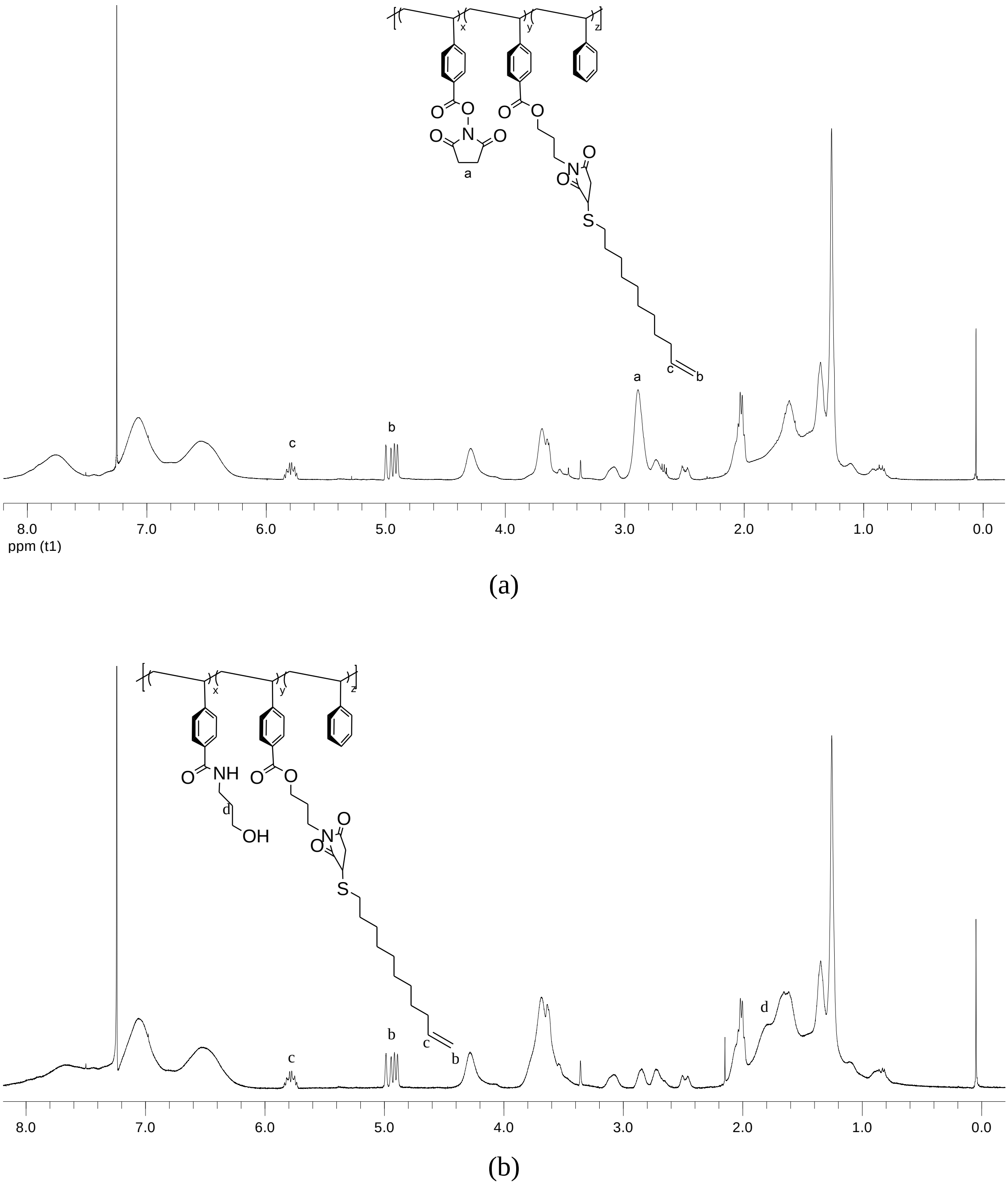


Figure 3.14. Functionalization of the thiol-amine reactive polymer **P-M2-*co*-M3-F1-F2**

TGA analysis was also performed for this copolymer. Unlike the maleimide based polymers (P1, P2, P3, P4), an additional weight loss was observed around 250 ^{o}C. In order to investigate the origin of this weight loss, copolymerization of styrene and succinimide monomer **M3** was carried out to obtain **P-M3-*co*-St**. As anticipated, during TGA weight loss was observed in the region of 250 ^{o}C, probably due to degradation of the succinimide moiety. Since there is no maleimide in the copolymer, no weight loss was detected in the region of 120 ^{o}C due to lack of any furan adducts in the side chains (Figure 3.16).



(a)

(b)

Figure 3.15. ^{1}H-NMR spectrum of thiol (a) and amine (b) functionalized **P-M2-*co*-M3-F**

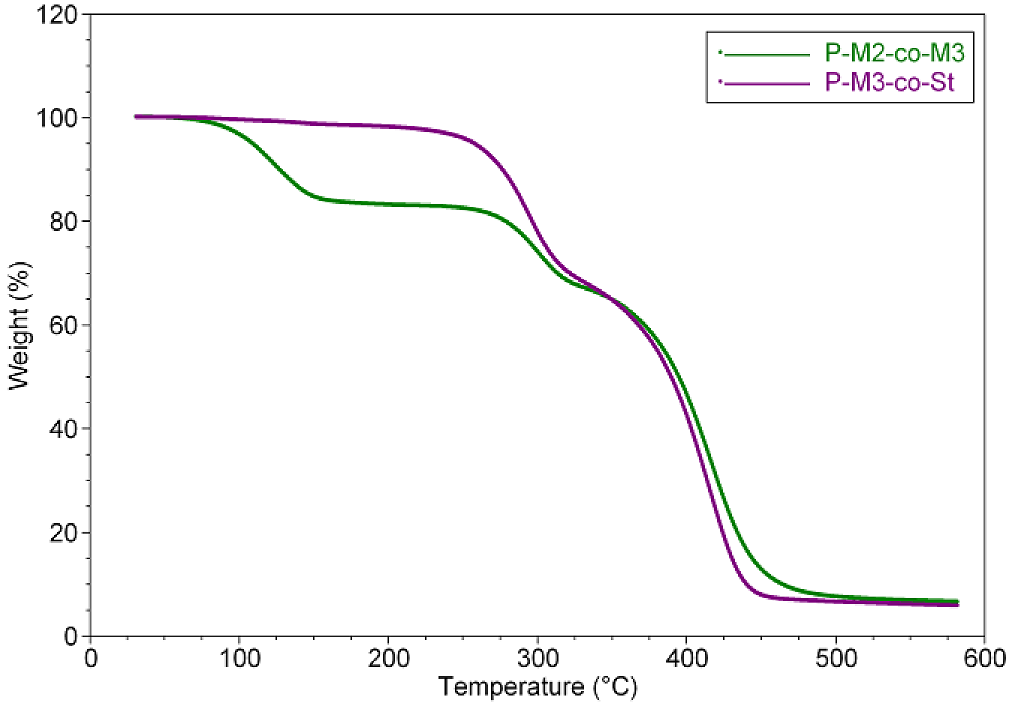


Figure 3.16. Thermogravimetric analysis of succinimide based styrenic copolymers

3.3.3. Functionalization of the Alkene Based Copolymer: P-M2-*co*-M4-R

Stepwise functionalization was performed for the maleimide-alkene functional copolymer. Two different thiols were added to the copolymer by using different reaction conditions. Hexane thiol was added via Michael-type conjugate addition in order to functionalize the maleimide units, whereas UV-initiated thiol-ene click chemistry was utilized for functionalization of double bonds of the monomer **4** by using ferrocenyl-hexane thiol and the thioglycerol in presence of DMPA (2,2-dimethoxy-2-phenylacetophenone) as a photoinitiator (Figure 3.17).

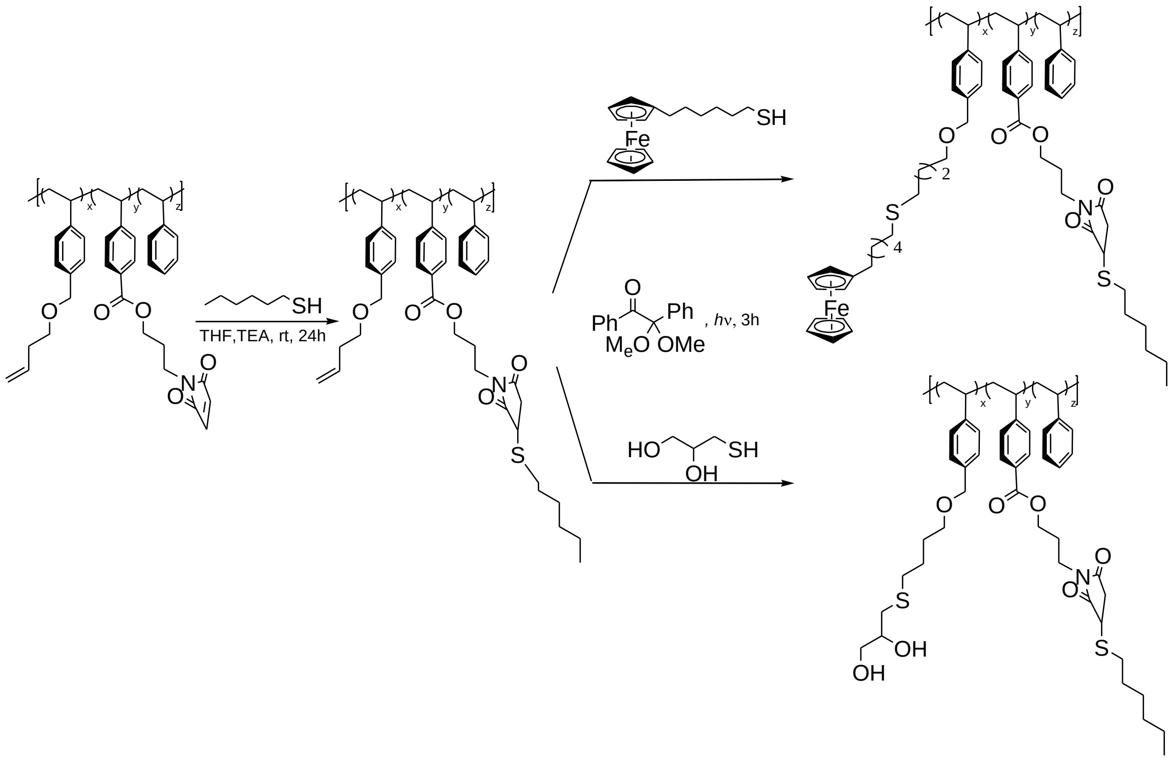
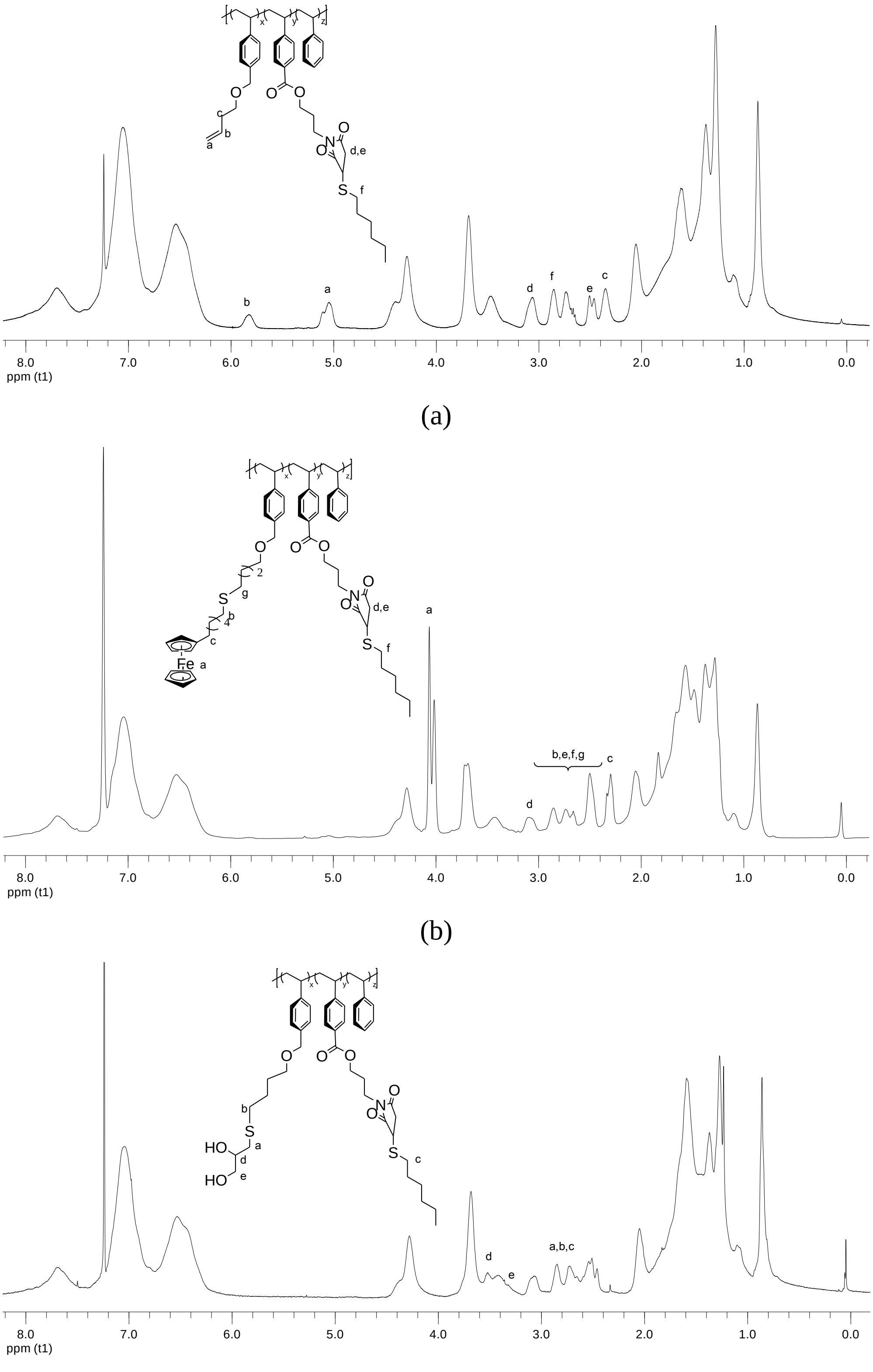


Figure 3.17. Functionalization of the thiol-thiol reactive polymer **P-M2-*co*-M4-R**

Disappearance of the peak due to maleimide double bond in the ^{1}H NMR spectra and presence of expected new peaks due to attachment of the hexanethiol implied successful Michael addition. Furthermore, the disappearance of the peaks at 5.83 and 5.04 ppm corresponding to the double bond of the monomer **4** along with appearance of expected peaks have become an evidence for the successful addition of ferrocenyl-hexane thiol and the thioglycerol via thiol-ene click reaction (Figure 3.18).



(a)

(b)

(c)

Figure 3.18. NMR spectrum of thiol- thiol functionalized polymer **P-M2-*co*-M4-R**. (a) Functionalization with hexanethiol, (b) Functionalization with ferrocenyl-hexane thiol, (c) Functionalization with thioglycerol

3.4. Crosslinking Studies

In addition to synthesis of side chain functionalizable reactive polymers, crosslinking studies for maleimide based styrenic copolymers were also performed (Figure 3.20). First of all, polymer having unmasked maleimides was dissolved in DMSO and furfuryl amine was introduced into this solution for further Diels-Alder cycloaddition reaction. This sample was then spin coated on to the silicon surfaces and heated to 60 ^{o}C under vacuum. The starting polymer containing 1:4 ratios of maleimide and styrene first undergoes Diels-Alder reaction with required amount of furfuryl amine in order to functionalize the one forth of the maleimides. The pendant amines then reacted with the free maleimides via Michael addition resulting in the formation of the crosslinked network.

To demonstrate the functionality of the remaining unreacted maleimide groups present on the surface, bodipy thiol was introduced (Figure 3.19). The surface was incubated with a solution of BodipyC10SH in THF at room temperature overnight. Since maleimides have high reactivity towards thiols, BodipyC10SH was easily incorporated onto the surface and after several washing with THF, fluorescence was detected with fluorescence microscopy. As a control, the same surface was incubated with a solution of BodipyC10Br and no fluorescence was observed as expected.

(a) (b)

Figure 3.19. Fluorescence microscope images of crosslinked polymer with a) BodipyBr, b)Bodipy SH

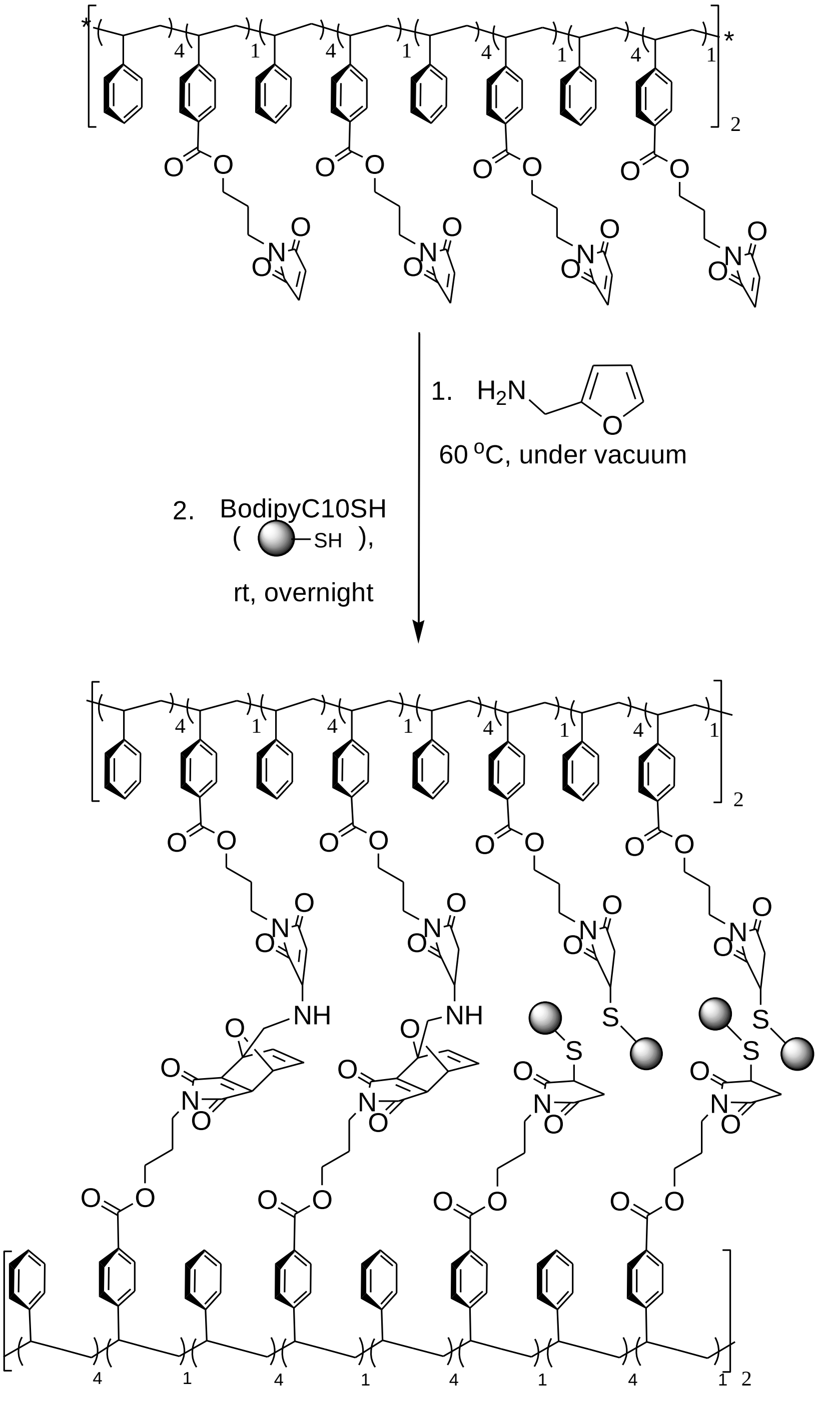


Figure 3.20. Structure of crosslinked and functionalized polymer

4. EXPERIMENTAL

4.1. Materials and Methods

All chemicals were used as received from manufacturer (Merck, Aldrich, Alfa Aesar, Riedel de Haen). Dry solvents (CH_2Cl_2, THF, toluene) was obtained from ScimatCo Purification System, other dry solvents were dried of molecular sieves. Styrene was obtained from commercial resources and was filtered from aluminum oxide prior to use. Column chromatography was performed using silicagel-60 (43-60 nm). Thin layer chromatography was performed using silica gel plates (Kiesel gel 60 F254, 0.2mm, Merck). Plates were viewed under 254nm UV lamp otherwise plates are developed either by $KMnO_4$ stain. Infrared spectroscopy was carried out on Thermo Scientific Nicolet 380 FT-IR spectrophotometer. ^1H NMR (operating at 400 MHz) were recorded on Varian Mercury-MX in $CDCl_3$ as solvent at the Advanced Technologies Research and Development Center at Boğaziçi University. Thermogravimetric analysis (TGA) was done on TA instrument. The molecular weights were estimated by gel permeation chromatography (GPC) with polystyrene as a standard and with refractive index detector, and the sample was eluted with dry THF.

4.2. Maleimide Based Styrenic Polymers

4.2.1. Synthesis of Furan-Maleimide Monomer (M1)

To a solution of the furan-maleimide cyclic adduct **1** (1.00 g, 6.06 mmol) and vinylbenzyl chloride (4.27 mL, 30.30 mmol) in acetone (20 mL) was added K_2CO_3 (1.674 g, 12.12 mmol). The solution was refluxed under a nitrogen atmosphere for 16 h at 65 ^oC. To the reaction mixture was added dichloromethane (50 mL) and the mixture was washed with H_2O (3 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give a white residue that was purified by flash chromatography on SiO_2 (EtOAc: Hexane: CH_2Cl_2) affording 500 mg (29.4 % yield) of **M1** as a white solid. ^1H NMR ($CDCl_3$, ppm): 7.31 (d, 2H, *J*=8.2 Hz, CH=CH), 7.27 (d, 2H, *J*=8.5 Hz, CH=CH), 6.65 (dd, 1H, *J*=10.8, 6.8 Hz, CH= CH_2), 6.49 (s, 2H, CH=CH), 5.69 (d, 1H, *J*=17.6 Hz,

CH_2=CH), 5.27 (s, 2H, CH bridgehead protons), 5.20 (d, 1H, *J*=11.2 Hz, CH_2=CH), 4.61 (s, 2H, NCH_2), 2.83 (s, 2H, CH-CH, bridge protons); ^{13}C NMR ($CDCl_3$) 176.0, 137.4, 136.8, 136.6, 135.2, 128.6, 126.7, 114.3, 81.2, 77.2, 76.9, 47.7, 42.4.

4.2.2. Synthesis of Furan-Maleimide Monomer (M2)

To a solution of the alcohol **2** (1.13 g, 5.06 mmol) and 4-vinylbenzoic acid **3** (0.50 g, 3.37 mmol) in CH_2Cl_2 (10 mL) was added EDCI (0.71 g, 3.71 mmol) and DMAP (0.041 g, 0.337 mmol). The solution was heated under a nitrogen atmosphere for 20 h. To the reaction mixture was added dichloromethane (50 mL) and the mixture was washed with saturated $NaHCO_3$ (1x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give a light yellow residue that was purified by flash chromatography on SiO_2 (EtOAc: Hexane) affording 1.00 g (83.3 % yield) of **M2** as a white solid. ^{1}H NMR ($CDCl_3$, ppm): 7.99 (d, 2H, *J*=7.52 Hz, CH=CH), 7.45 (d, 2H, *J*=7.64 Hz, CH-CH), 6.74 (dd, 1H, *J*=6.06, 11.1 Hz, CH=CH_2), 6.48 (s, 2H, CH=CH), 5.84 (d, 1H, *J*=17.5 Hz, CH_2=CH), 5.36 (d, 1H, *J*=10.8 Hz, CH_2=CH), 5.25 (s, 2H, CH bridgehead protons), 4.27 (t, 2H, *J*=5.9 Hz, OCH_2), 3.66 (t, 2H, *J*=6.90 Hz, NCH_2), 2.81 (s, 2H, CH-CH, bridge protons), 2.05 (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$) 176.3, 166.4, 142.2, 136.7, 130.2, 126.3, 116.7, 81.2, 62.1, 47.6, 36.1, 27.0.

4.2.3. General Polymerization Procedure

In a typical experiment, to a solution of styrene (0.29 mL, 2.49 mmol) and monomer **M2** (0.11 g, 0.311 mmol) in dry THF (15 mL), was added 2, 2'-azobisisobutyronitrile (AIBN, 15.3 mg, 0.09 mmol). The mixture was degassed and then heated to reflux. At the end of the reaction, THF was evaporated under *vacuo*. The polymer was dissolved in minimum amount of dichloromethane and added to a cold methanol to precipitate the polymer as a solid (29 % yield). ^{1}H NMR for polymer **P-M2-1** ($CDCl_3$, ppm): 8.0-6.2 (br, 9H, aromatic of PS), 6.47 (s, 2H, CH=CH), 5.25 (s, 2H, CH bridgehead protons), 4.26 (br s, 2H, OCH_2), 3.66 (br s, 2H, NCH_2), 2.81 (s, 2H, CH-CH bridge protons), 2.03 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.82 (m, CH_2 along polymer backbone).

4.2.4. General Procedure for retro Diels-Alder Reaction

Polymer **P-M2-1** (20 mg) was dissolved in dry toluene and heated at 110 ^{o}C, overnight to obtain **P-M2-1-R**. Quantitative conversion of the oxabicyclic moiety to the maleimide functional group was observed from the ^{1}H-NMR spectroscopy. ^{1}H NMR for polymer **P-M2-1-R** ($CDCl_3$, ppm): 8.0-6.2 (br, 9H, aromatic of PS), 6.64 (s, 2H, CH=CH), 4.29 (br s, 2H, OCH_2), 3.70 (br s, 2H, NCH_2), 2.07 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.82 (m, CH_2 along polymer backbone).

4.3. Orthogonally Functionalizable Copolymers

4.3.1. Synthesis of thiol and amine reactive polymer P-M2-co-M3

To a solution of monomer 2 (0.2 g, 0.57 mmol), monomer 3 (0.14g, 0.57 mmol) and styrene (0.26 ml, 2.28 mmol) in dry THF (15 ml), was added 2,2'-azobisisobutyronitrile (AIBN, 18.6 mg, 0.113 mmol). The mixture was degassed and then heated to reflux for 12h. At the end of the reaction, THF was evaporated under *vacuo*. The polymer was dissolved in minimum amount of dichloromethane and added to cold methanol to precipitate the polymer as a solid (52% yield). Mn: 3822, Mw/Mn: 1.54. ^{1}H NMR ($CDCl_3$, ppm): 8.20-6.20 (br, 13H, aromatic of PS), 6.47 (s, 2H, CH=CH), 5.24 (s, 2H, CH bridgehead protons), 4.25 (br, 2H, OCH_2), 3.65 (br s, 2H, NCH_2), 2.88 (s, 4H, CH_2-CH_2 of NHS), 2.81 (s, 2H, CH-CH bridge protons), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.82 (m, CH_2 along polymer backbone).

4.3.2. Synthesis of thiol - thiol reactive polymer P-M2-co-M4

To a solution of monomer 2 (0.1 g, 0.28 mmol), monomer 4 (0.053g, 0.28 mmol) and styrene (0.23 ml, 1.98 mmol) in dry THF (6 ml), was added 2,2'-azobisisobutyronitrile (AIBN, 13.8 mg, 0.034 mmol). The mixture was degassed and then heated to reflux for 12h. At the end of the reaction, THF was evaporated under *vacuo*. The polymer was dissolved in minimum amount of dichloromethane and added to cold methanol to precipitate the polymer as a solid (54% yield). Mn: 5460, Mw/Mn: 1.62. ^{1}H NMR ($CDCl_3$, ppm): 7.85-6.2 (br, 13H, aromatic of PS), 6.47 (s, 2H, CH=CH), 5.83 (br, 1H, $CH=CH_2$), 5.25 (s, 2H, CH bridgehead protons), 5.04 (br, 2H, $CH=CH_2$), 4.41 (br, 2H, St-CH_2-O), 4.26 (br, 2H, OCH_2), 3.65 (br, 2H, NCH_2), 3.46 (br, 2H, St-CH_2-O-CH_2), 2.81 (s, 2H, bridge protons), 2.35 (s, 2H, O-CH_2-CH_2-$CH=CH_2$), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.80 (m, CH_2 along polymer backbone).

4.3.3. Retro Diels-Alder reaction of P-M2-co-M3

P-M2-*co*-M3 (0.05 g, 0.013 mmol) was dissolved in dry toluene and heated at 110°C for 12 hours. NMR analysis proved quantitative conversion of the oxabicyclic moiety to the maleimide functional group. NMR for polymer **P-M2-*co*-M3-R**: ^{1}H NMR ($CDCl_3$, ppm): 8.20-6.20 (br, 13H, aromatic of PS), 6.65 (s, 2H, CH=CH), 4.25 (br, 2H, OCH_2), 3.65 (br s, 2H, NCH_2), 2.88 (s, 4H, CH_2-CH_2 of NHS), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.82 (m, CH_2 along polymer backbone).

4.3.4. Retro Diels-Alder reaction of P-M2-co-M4

P-M2-*co*-M4 (0.05 g, 0.009 mmol) was dissolved in dry toluene and heated at 110°C for 12 hours. NMR analysis proved quantitative conversion of the oxabicyclic moiety to the maleimide functional group. NMR for polymer **P-M2-*co*-M4-R**: ^{1}H NMR ($CDCl_3$, ppm): 7.85-6.20 (br, 13H, aromatic of PS), 6.65 (s, 2H, CH=CH), 5.83 (br, 1H, $CH=CH_2$), 5.04 (br, 2H, $CH=CH_2$), 4.41 (br, 2H, St-CH_2-O), 4.26 (br, 2H, OCH_2), 3.65 (br, 2H, NCH_2), 3.46 (br, 2H, St-CH_2-O-CH_2), 2.35 (s, 2H, O-CH_2-CH_2-$CH=CH_2$), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.80 (m, CH_2 along polymer backbone).

4.4. Functionalization Studies for Reactive Copolymers

4.4.1. Functionalization of P-M2-*co*-M3-R

<u>4.4.1.1. Addition of thiol to polymer</u>: **P-M2-*co*-M3-R** (0.03 g, 0.008 mmol) and 11-undecene thiol (0.03 g, 0.164 mmol) were dissolved in 3 ml of THF and TEA (4.5 µl, 0.03 mmol) was added to the solution. The mixture was stirred at room temperature for 24h. After evaporation of THF, the solid residue was dissolved in minimum amount of dichloromethane and precipitated in methanol affording to yellowish polymer **P-M2-*co*-**

M3-F1. ^{1}H NMR ($CDCl_3$, ppm): 8.2-6.2 (br, 13H, aromatic of PS), 5.8 (m, 1H, $CH=CH_2$ of thiol), 4.95 (dd, 2H, $CH=CH_2$ of thiol), 4.25 (br, 2H, OCH_2), 3.65 (br s, 2H, NCH_2), 3.01 (b, 1H, CH_2-CH-S), 2.88 (s, 4H, CH_2-CH_2 of NHS), 2.84 (b, 1H, CH-S-CH_2), 2.74 (b, 2H, CH_2-S), 2.49 (b, 1H, CH_2-CH-S), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.82 (m, CH_2 of thiol and CH_2 along polymer backbone).

4.4.1.2. Addition of amine to polymer: **P-M2-*co*-M3-F1** (0.015 g, 4.1*e^{-3} mmol) and 3-amino-1-propanol (5µl, 0.065 mmol) were dissolved in 3 ml of THF and TEA (2.5 µl, 0.018 mmol) was added to the solution. The mixture was heated to reflux for 12h. After evaporation of THF, the solid residue was dissolved in minimum amount of dichloromethane and precipitated in methanol affording to yellowish polymer **P-M2-*co*-M3-F2**. ^{1}H NMR ($CDCl_3$, ppm): 8.2-6.2 (br, 13H, aromatic of PS), 5.8 (m, 1H, $CH=CH_2$ of thiol), 4.95 (dd, 2H, $CH=CH_2$ of thiol), 4.25 (br, 2H, OCH_2), 3.65 (br s, 2H, NCH_2), 3.01 (b, 1H, CH_2-CH-S), 2.84 (b, 1H, CH-S-CH_2), 2.74 (b, 2H, CH_2-S), 2.49 (b, 1H, CH_2-CH-S), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 1.81 (b m, 2H, N- CH_2-CH_2-O), 2.1–0.82 (m, CH_2 of thiol and CH_2 along polymer backbone).

4.4.2. Functionalization of P-M2-*co*-M4-R

4.4.2.1. Synthesis of **P-M2-co-M4-F1**: **P-M2-*co*-M4-R** (0.03 g, 0.0054 mmol) and hexanethiol (23 µl, 0.164 mmol) were dissolved in 3 ml of THF and TEA (6.8 µl, 0.049 mmol) was added to the solution. The mixture was stirred at room temperature for 24h. After evaporation of THF, the solid residue was dissolved in minimum amount of dichloromethane and precipitated in methanol affording to polymer **P-M2-*co*-M4-F1**. ^{1}H NMR ($CDCl_3$, ppm): 7.85-6.20 (br, 13H, aromatic of PS), 5.83 (br, 1H, $CH=CH_2$), 5.04 (br, 2H, $CH=CH_2$), 4.41 (br, 2H, St-CH_2-O), 4.26 (br, 2H, OCH_2), 3.65 (br, 2H, NCH_2), 3.46 (br, 2H, St-CH_2-O-CH_2), 3.06 (br, 1H, CH_2-CH-S), 2.85 (br, 1H, CH-S-CH_2), 2.74 (br, 2H, CH_2-S), 2.47 (br, 1H, CH_2-CH-S), 2.35 (s, 2H, O-CH_2-CH_2-$CH=CH_2$), 2.04 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.80 (m, CH_2 along polymer backbone).

4.4.2.2. Synthesis of **P-M2-co-M4-F2**: **P-M2-*co*-M4-R** (0.01 g, 0.0018 mmol), ferrocenyl-hexane thiol (8.16 mg, 0.027 mmol) and DMPA (0.7 mg, 0.0027 mmol) were dissolved in the minimum amount of the THF required to solubilize all reactants. The mixture was purged with nitrogen for 5 min. and then was exposed to UV light at 365nm for 3 hours. The resulting polymer was then purified by precipitating it in methanol. ^{1}H NMR of **P-M2-*co*-M4-F2** ($CDCl_3$, ppm): 7.85-6.2 (br, 13H, aromatic of PS), 4.36 (br, 2H, St-CH_2-O), 4.28 (br, 2H, OCH_2), 3.70 (br, 2H, NCH_2), 3.42 (br, 2H, St-CH_2-O-CH_2), 3.09 (br, 1H, CH_2-CH-S), 2.85 (br, 1H, CH-S-CH_2), 2.70 (br, 2H, CH_2-S), 2.50 (br, 1H, CH_2-CH-S), 2.29 (s, 2H, Fer-CH_2), 2.05 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.80 (m, CH_2 along polymer backbone).

4.4.2.3. Synthesis of **P-M2-co-M4-F3**: **P-M2-*co*-M4-R** (0.005 g, 8.9*e^{-4} mmol), thioglycerol (2.34 µl, 0.027 mmol) and DMPA (0.46 mg, 0.0018 mmol) were dissolved in the minimum amount of the THF required to solubilize all reactants. The mixture was purged with nitrogen for 5 min. and then was exposed to UV light at 365nm for 3 hours. The resulting polymer was then purified by precipitating it in methanol. ^{1}H NMR of **P-M2-*co*-M4-F3** ($CDCl_3$, ppm): 7.85-6.2 (br, 13H, aromatic of PS), 4.36 (br, 2H, St-CH_2-O), 4.28 (br, 2H, OCH_2), 3.68 (br, 2H, NCH_2), 3.52 (br, 1H, CH-OH), 3.42 (br, 2H, St-CH_2-O-CH_2), 3.33 (br, 1H, CH-OH), 3.06 (br, 1H, CH_2-CH-S), 2.85 (br, 1H, CH-S-CH_2), 2.70 (br, 2H, CH_2-S), 2.50 (br, 1H, CH_2-CH-S), 2.05 (m, 2H, $NCH_2CH_2CH_2O$), 2.1–0.80 (m, CH_2 along polymer backbone).

4.5. Crosslinking Studies and Functionalization

4.5.1. Synthesis of crosslinked polymer and immobilization on to the surface

The 1:4 ratio of **P-M2-1-R** (0.015 g, 2.7*e^{-3} mmol) and furfuryl amine (0.5 µl, 5.35*e^{-3} mmol) were dissolved in 0.5 ml of DMSO. The solution was then spin coated on to the silicon surfaces. In order to obtain the crosslinked polymer, surface was heated to 60 ^{o}C under vacuum for 1 hour.

4.5.2. Functionalization with fluorescent dye

The solution of BodipyC10SH in THF with a concentration of 1mg/1ml was prepared. The crosslinked surface was incubated with this solution at room temperature, overnight. Surface was washed several times with THF and fluorescent images were taken.

As a control the same surface was incubated with a solution of BodipyC10Br and again fluorescent images were taken after several washing.

5. CONCLUSIONS

Two novel styrenic monomers containing a protected maleimide unit was synthesized and polymerized via AIBN catalyzed free radical polymerization. Polymers containing different ratios of maleimides were converted to their reactive forms by retro Diels-Alder reaction for further functionalization.

As an extension, polymers bearing both maleimide and succinimide units in their side chains, which are reactive towards thiols and amines respectively, were successfully synthesized to achieve orthogonal functionalization both in a single step and a stepwise manner. An additional copolymer was synthesized via using alkene based and maleimide based monomers which were then functionalized with two different thiols by using both Michael addition and thiol-ene click chemistry.

Finally, surface crosslinking was achieved by utilizing the Michael addition. Maleimide based styrenic polymer reacted with the furfuryl amine via Diels-Alder reaction followed by the Michael addition of amines to the unreacted maleimides resulted in the formation of crosslinked polymer on silicon surface. To demonstrate the reactivity of surface due to the remaining free maleimides, fluorescent dye was immobilized onto the surface and fluorescence microscopy images were taken. As expected, thiol functional dye was reacted with maleimide and showed fluorescence, however, bromo functional dye was washed out and no fluorescence was observed.

APPENDIX

^{1}H NMR, ^{13}C NMR and IR data for the synthesized compounds are given.

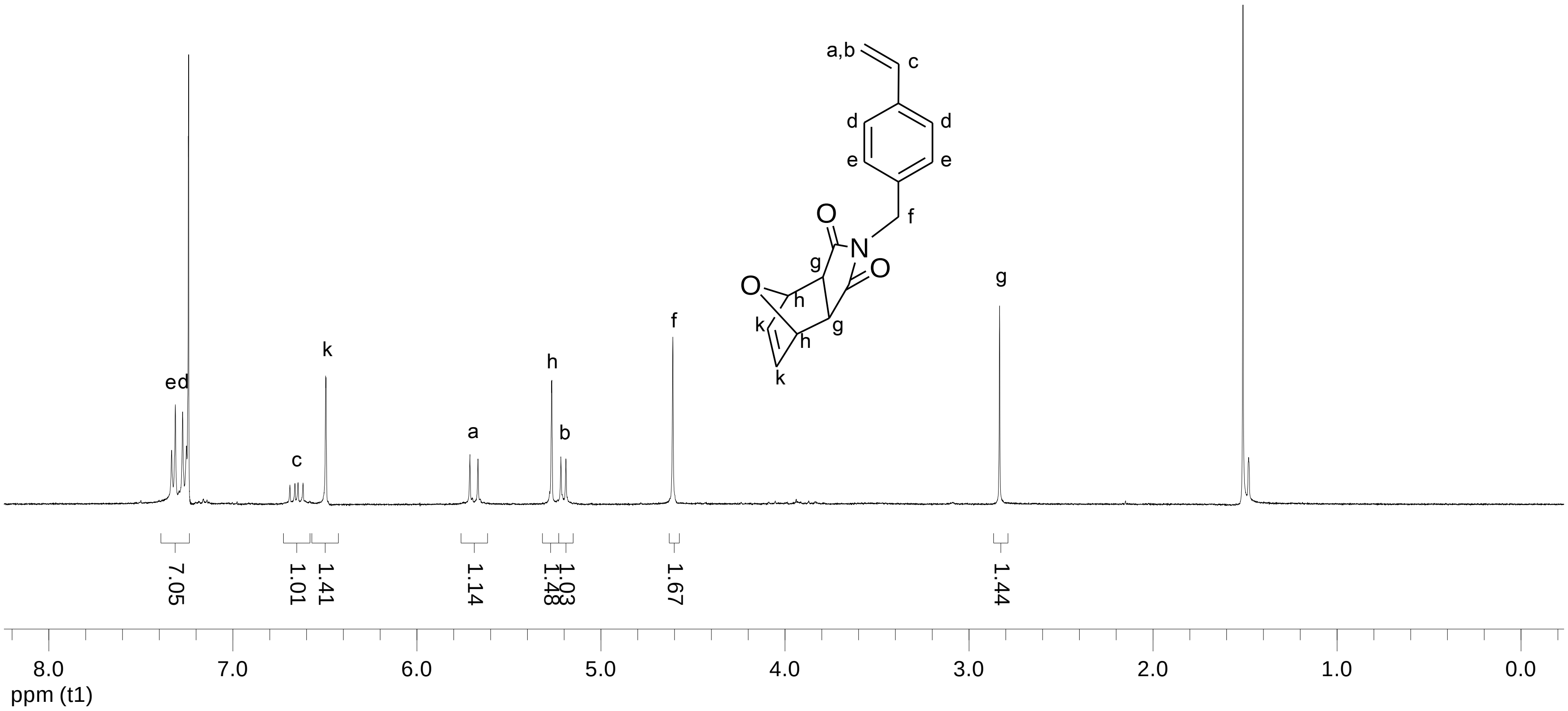


Figure A.1. ^{1}H-NMR spectrum of monomer 1

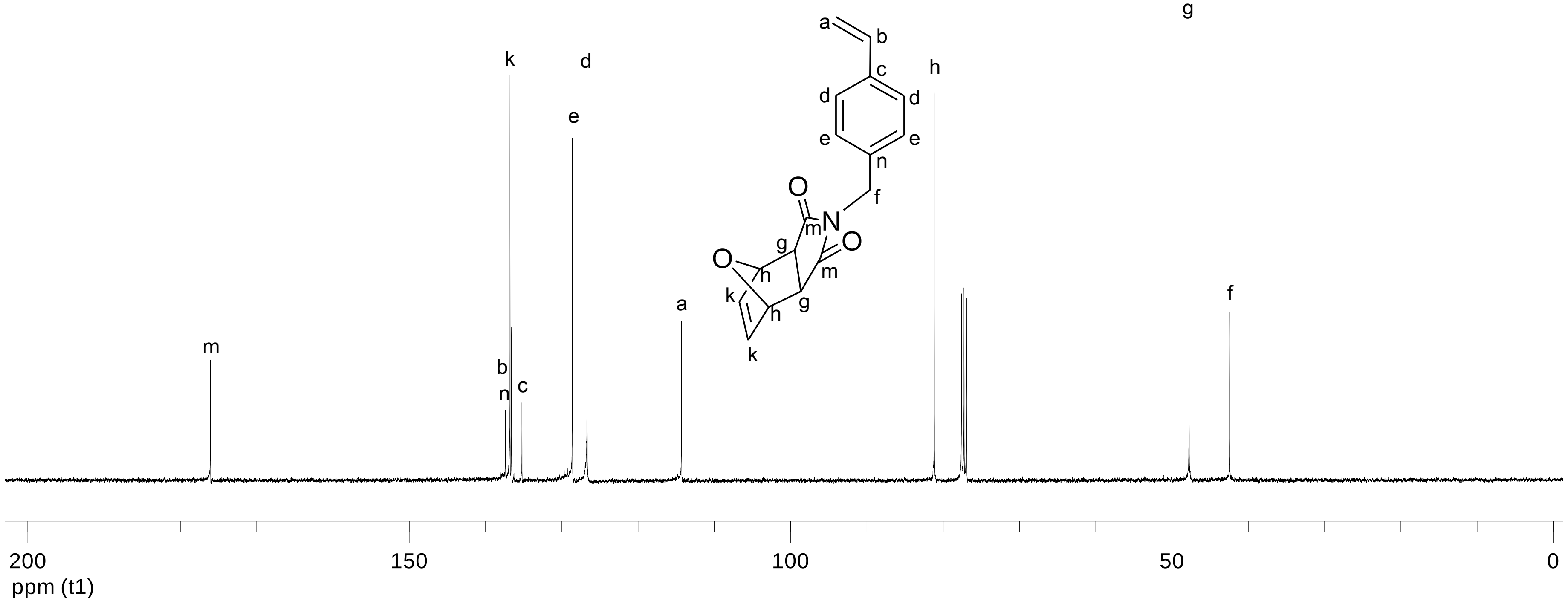


Figure A.2. ^{13}C-NMR spectrum of monomer 1

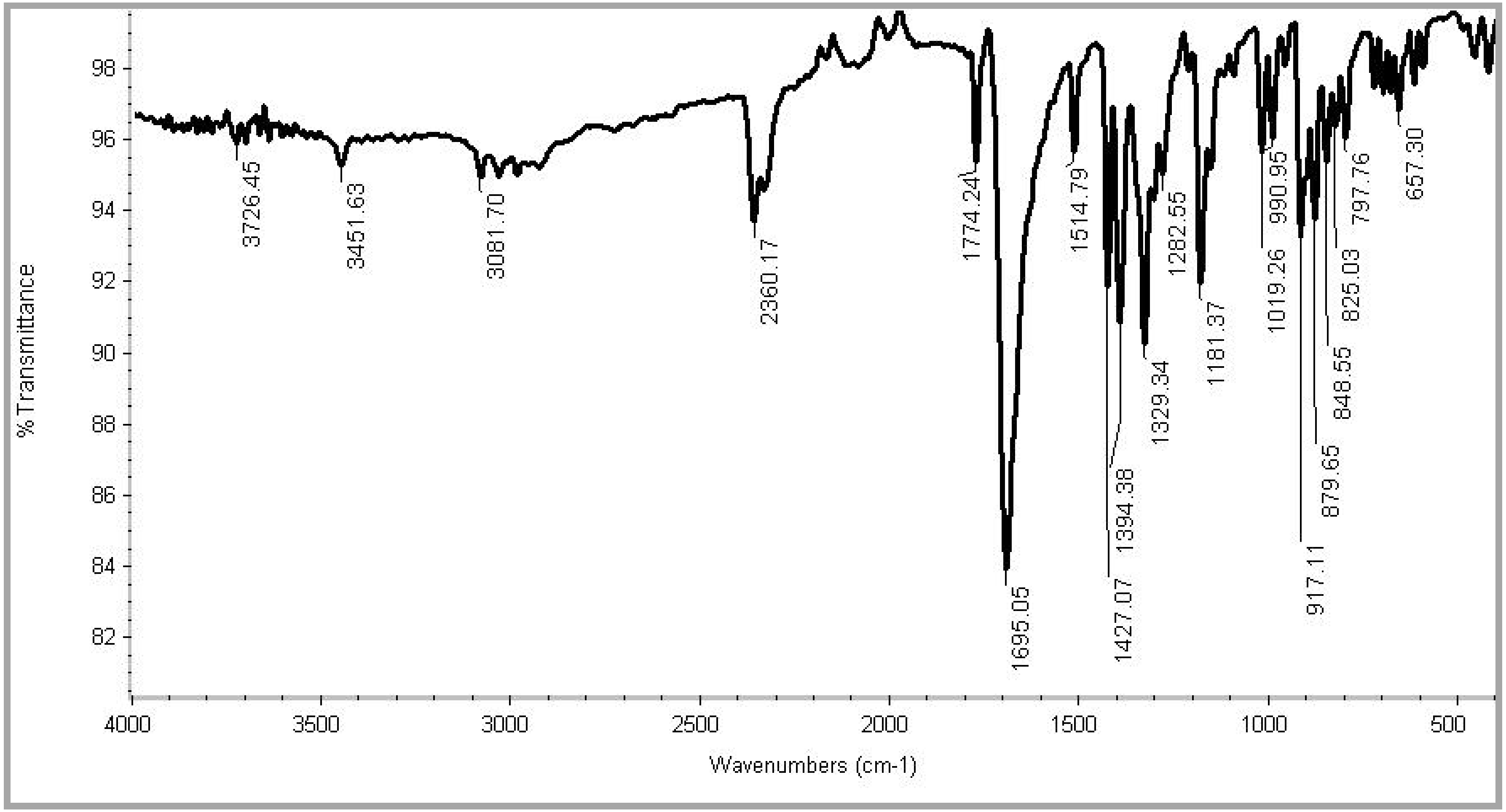


Figure A.3. IR spectrum of monomer 1

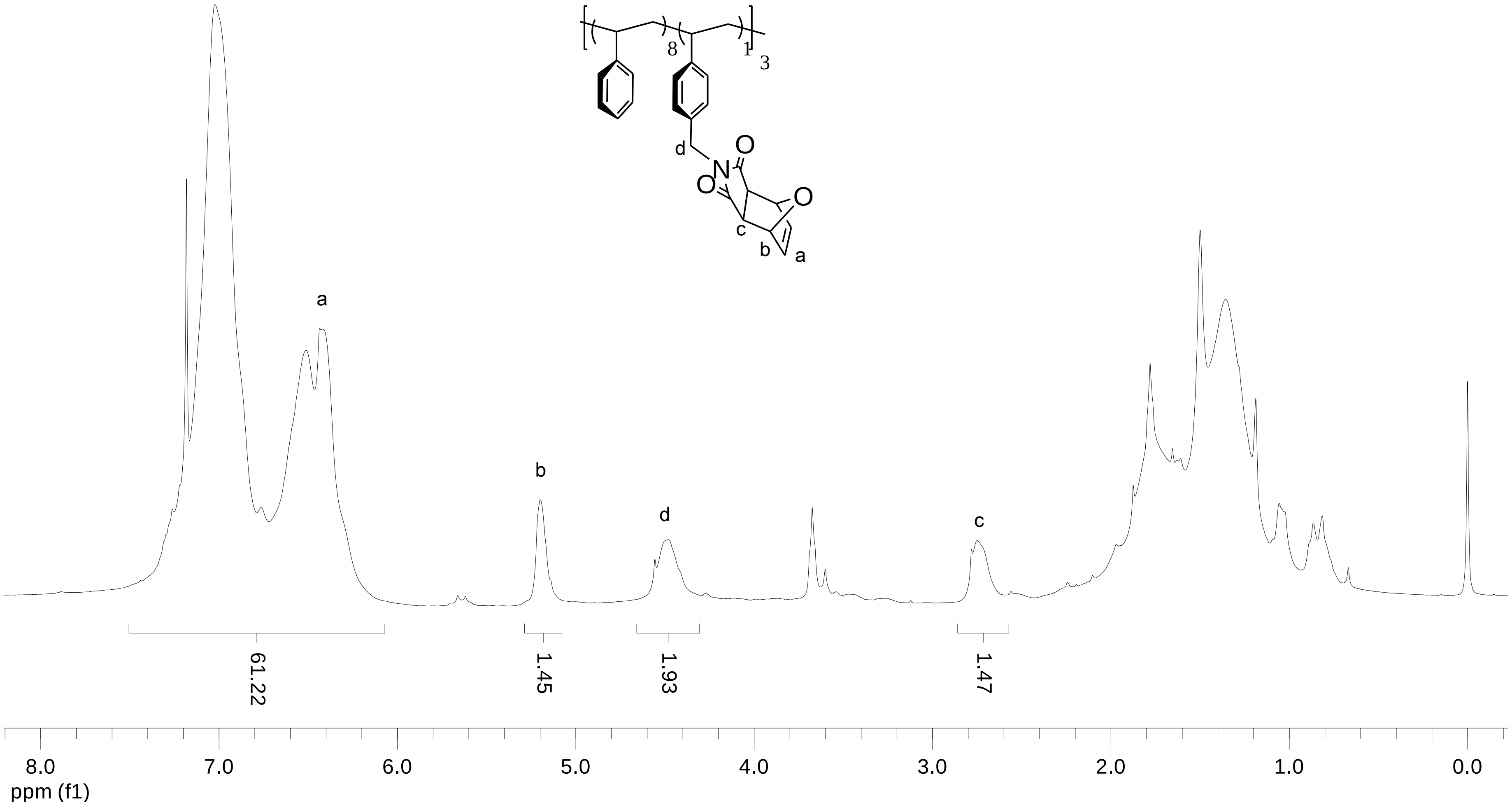


Figure A.4. ^{1}H-NMR spectrum of 1:8 polymer from monomer 1

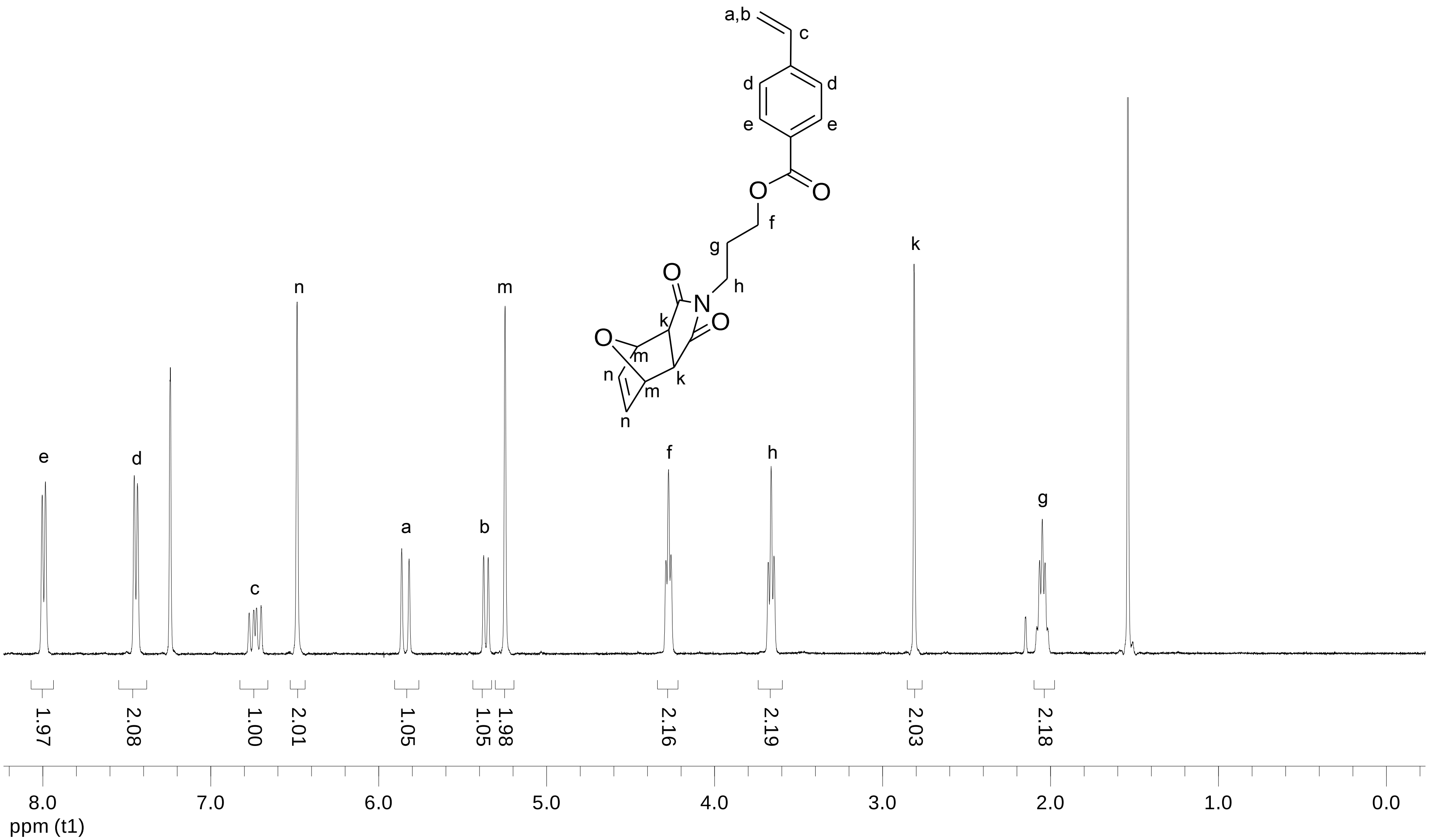


Figure A.5. ^{1}H-NMR spectrum of monomer 2

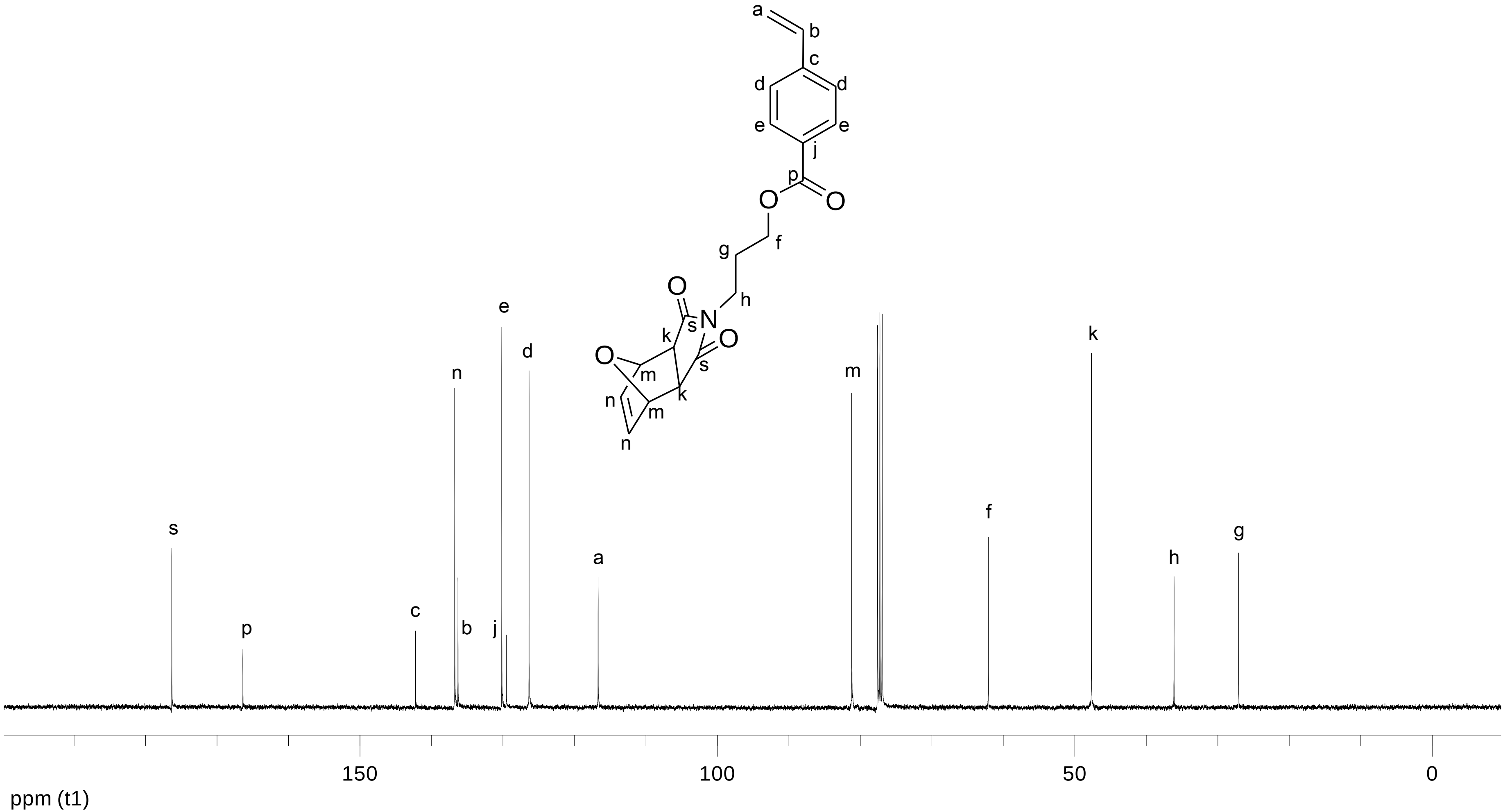


Figure A.6. ^{13}C-NMR spectrum of monomer 2

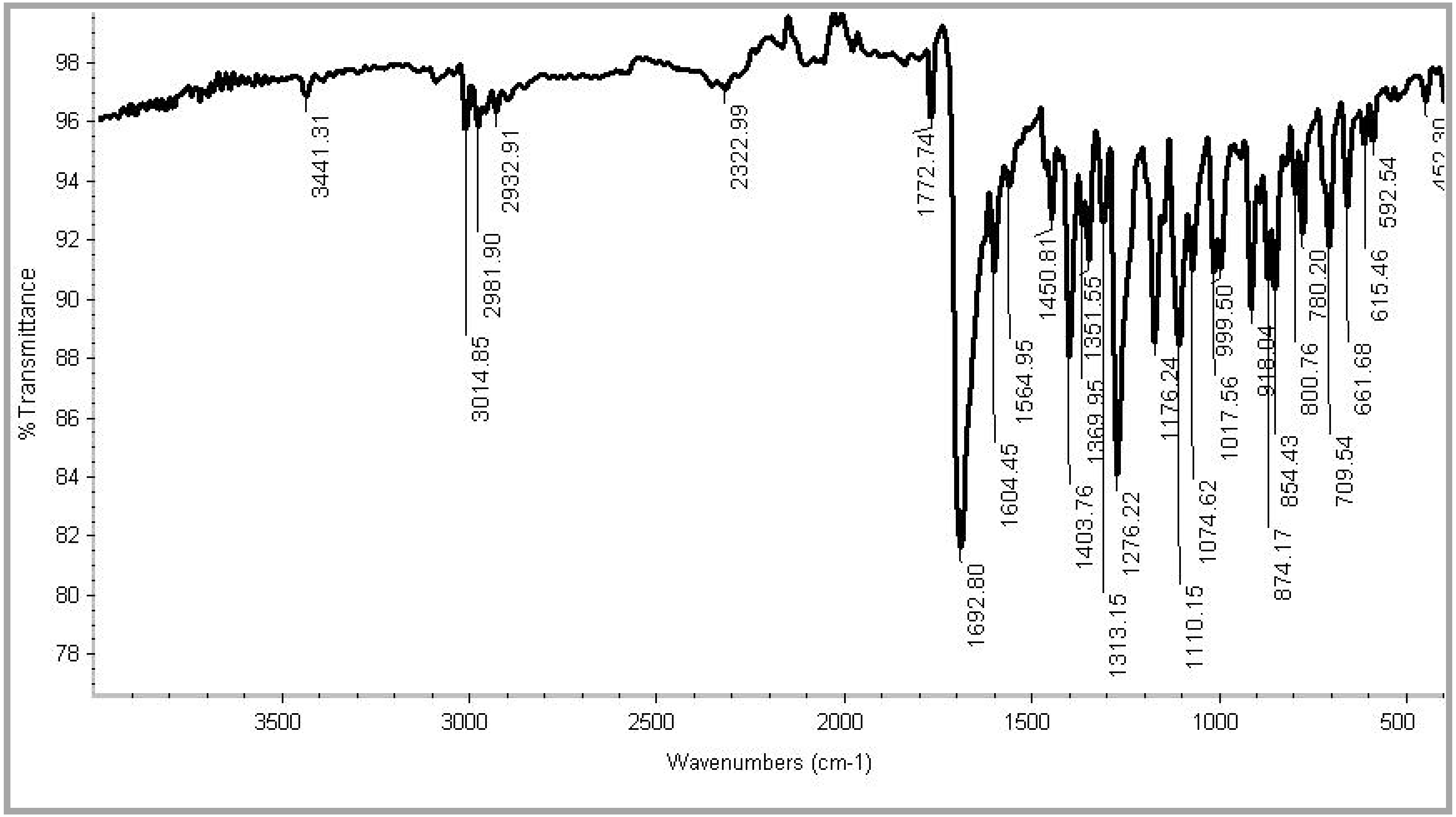


Figure A.7. IR spectrum of monomer 2

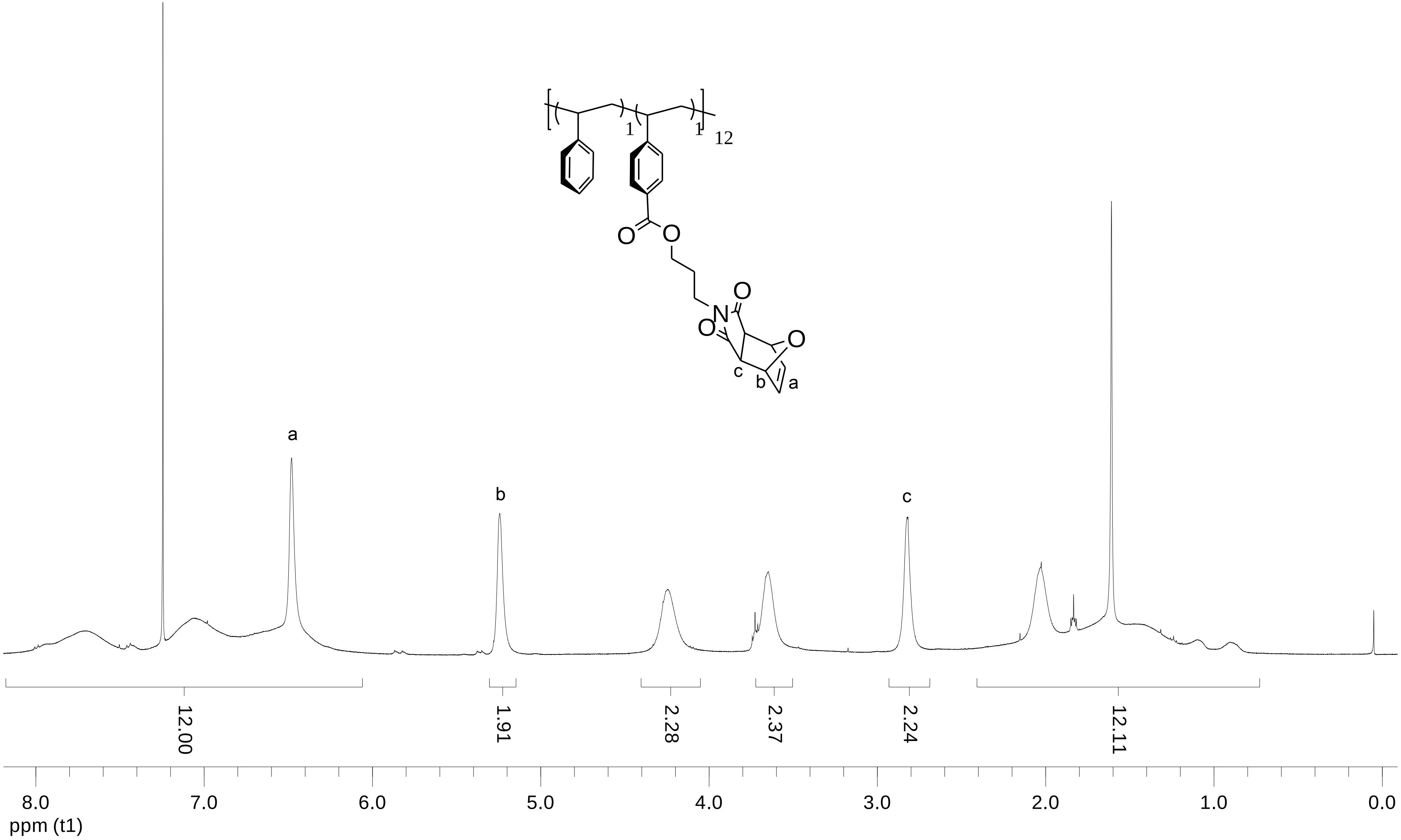


Figure A.8. ^{1}H-NMR spectrum of 1:1 polymer

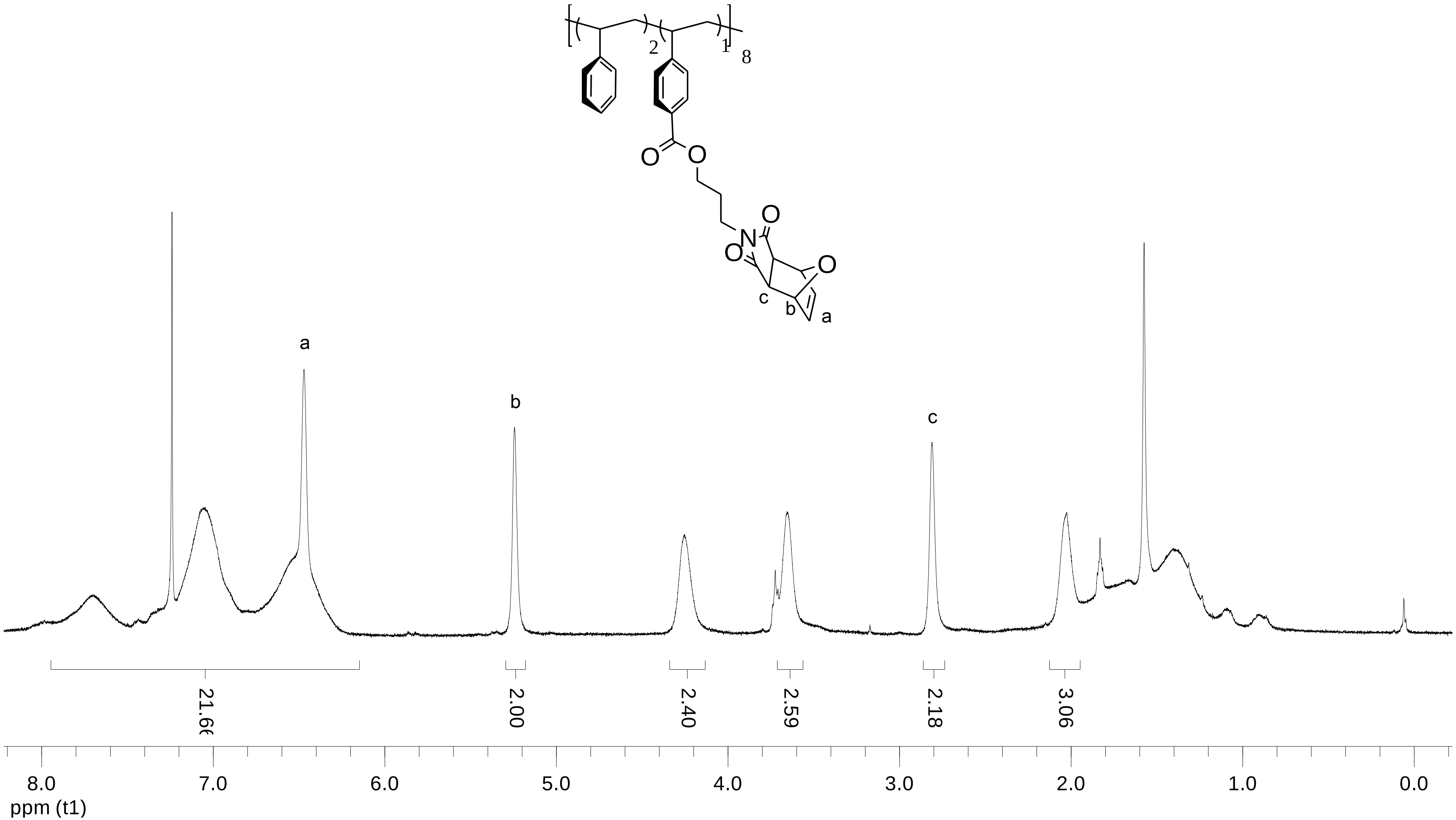


Figure A.9. ^{1}H-NMR spectrum of 1:2 polymer

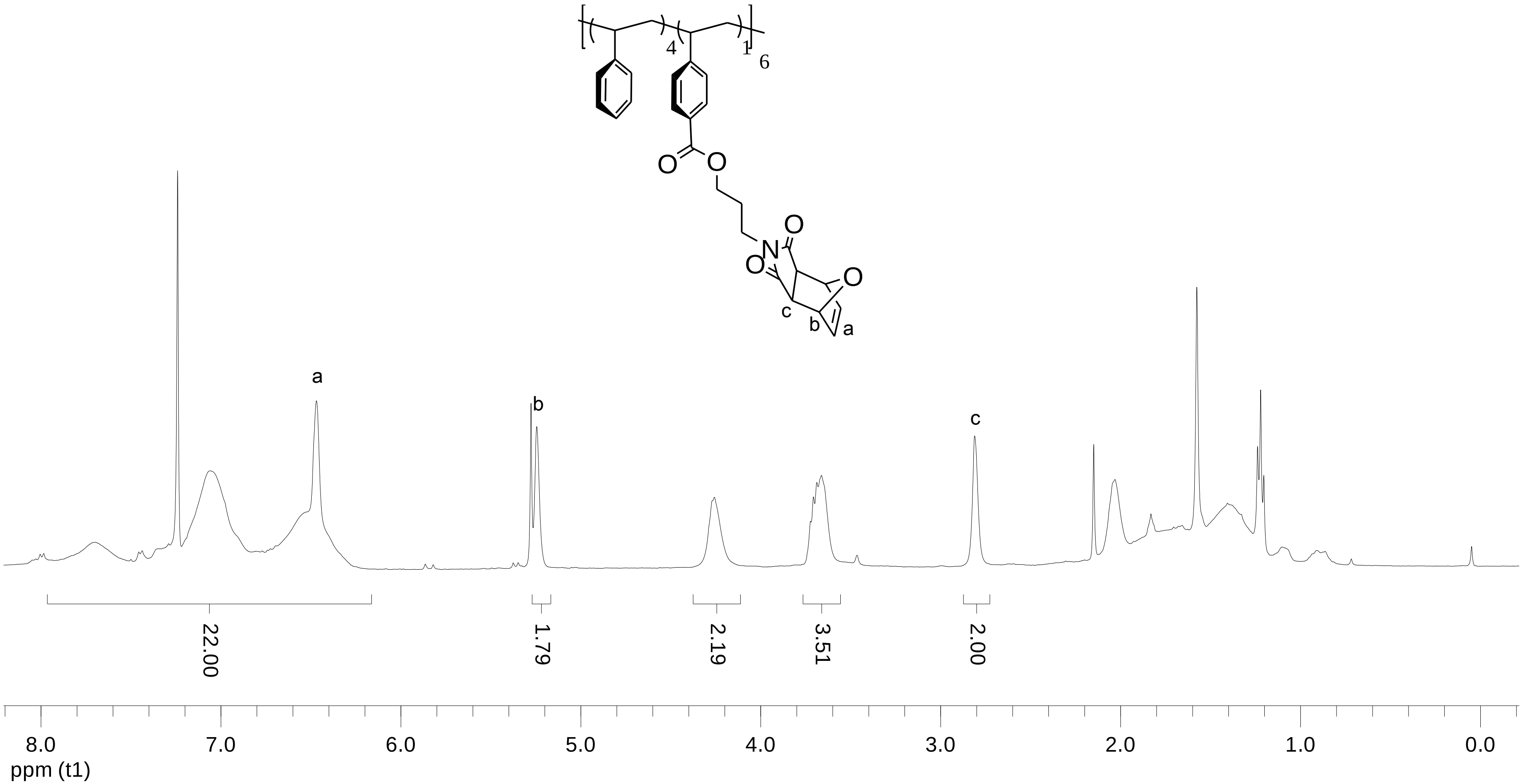


Figure A.10. ^{1}H-NMR spectrum of 1:4 polymer

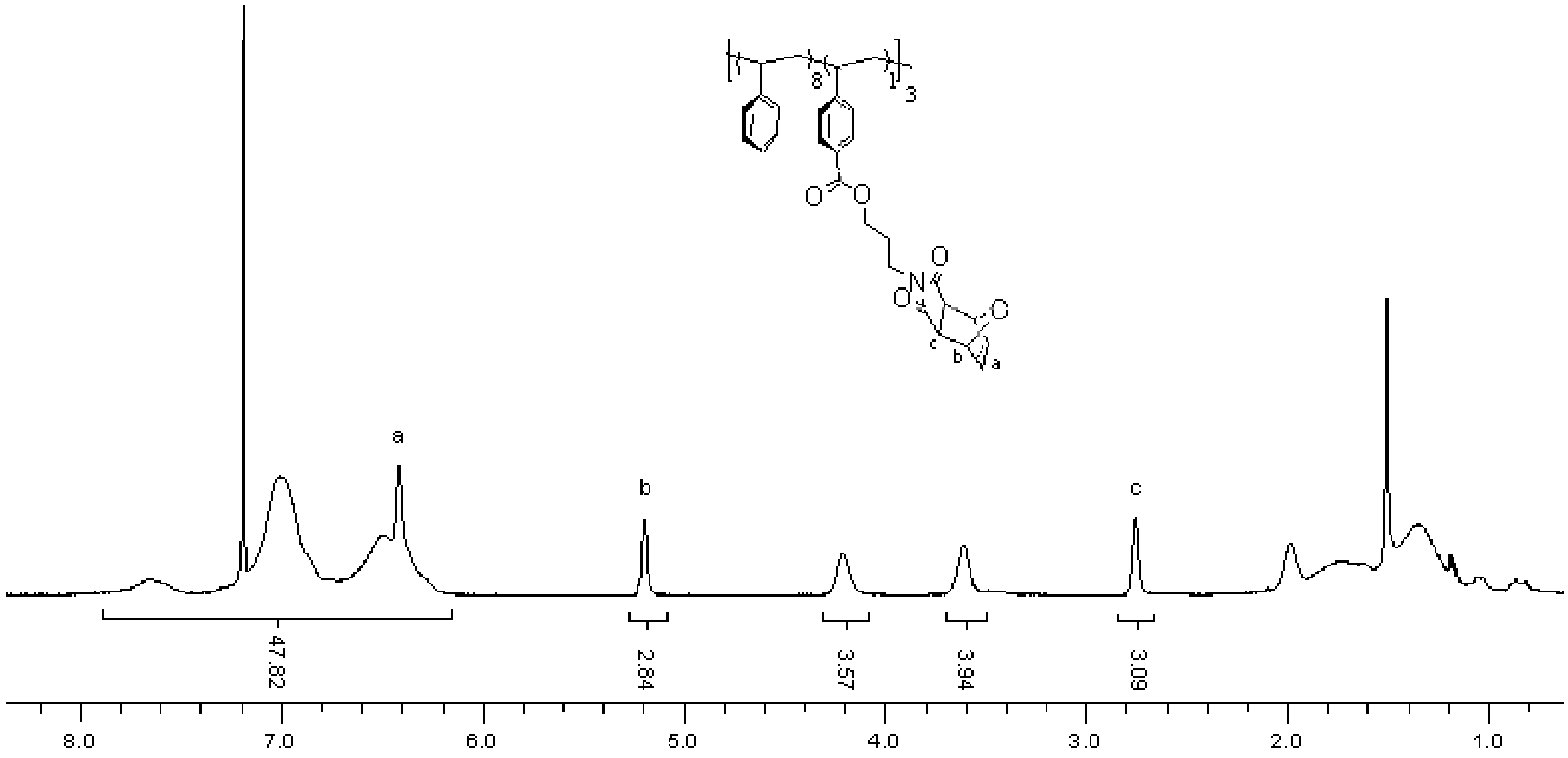


Figure A.11. ^{1}H-NMR spectrum of 1:8 polymer

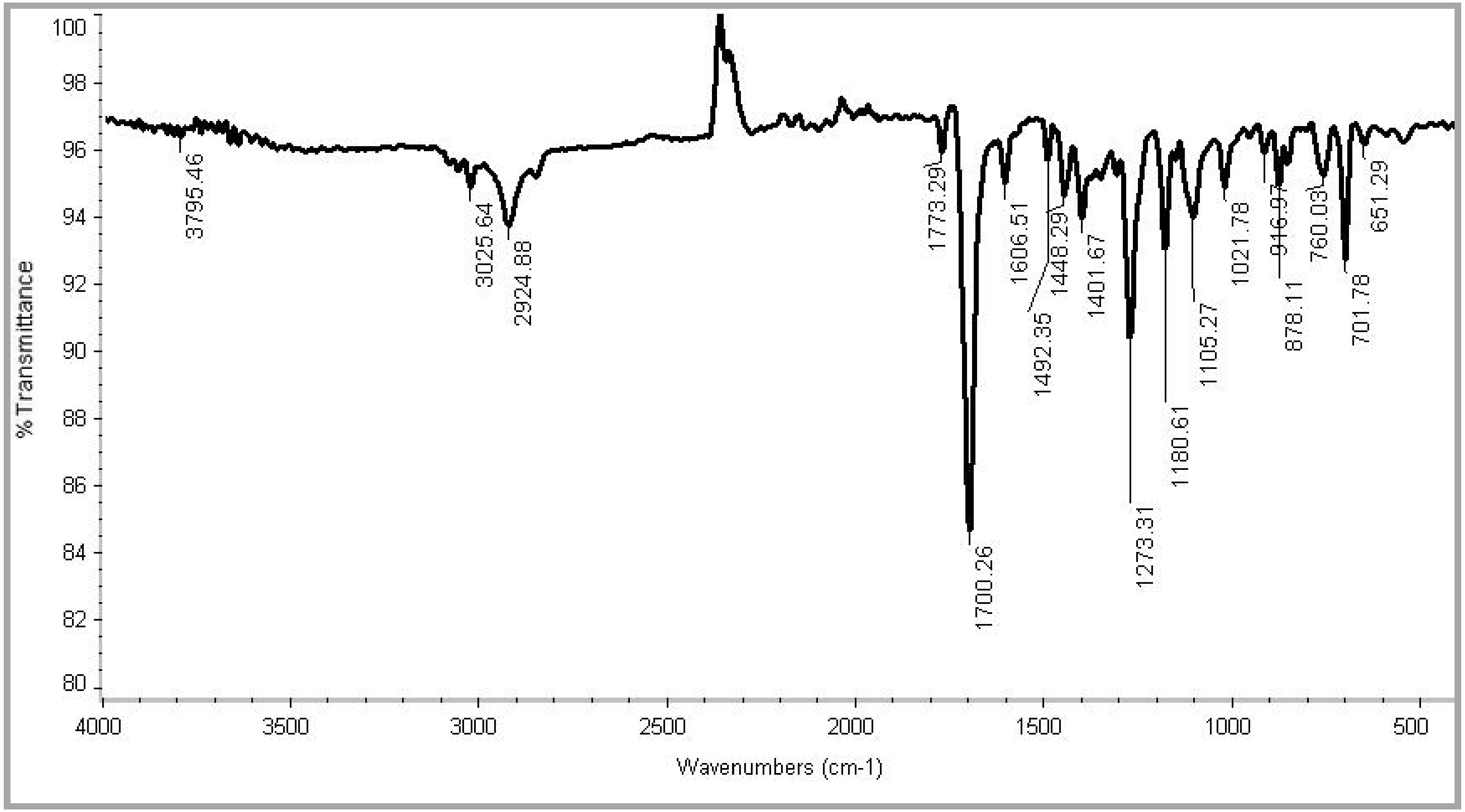


Figure A.12. IR spectrum of 1:4 polymer

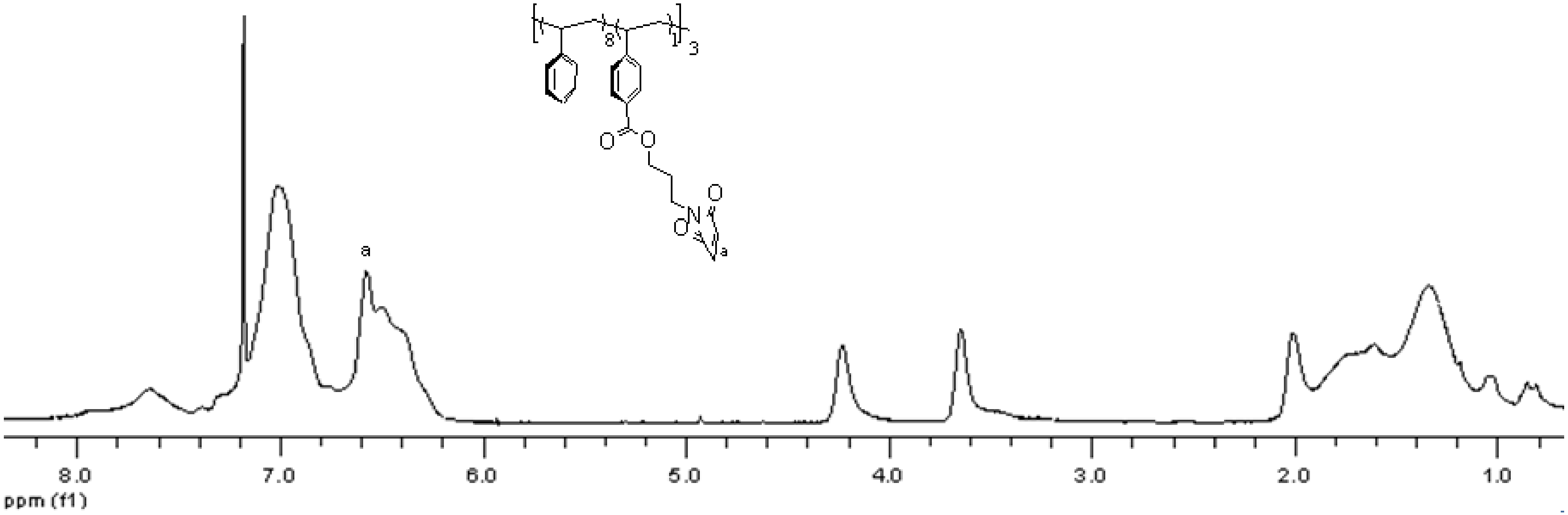


Figure A.13. ^{1}H-NMR spectrum of activated polymer

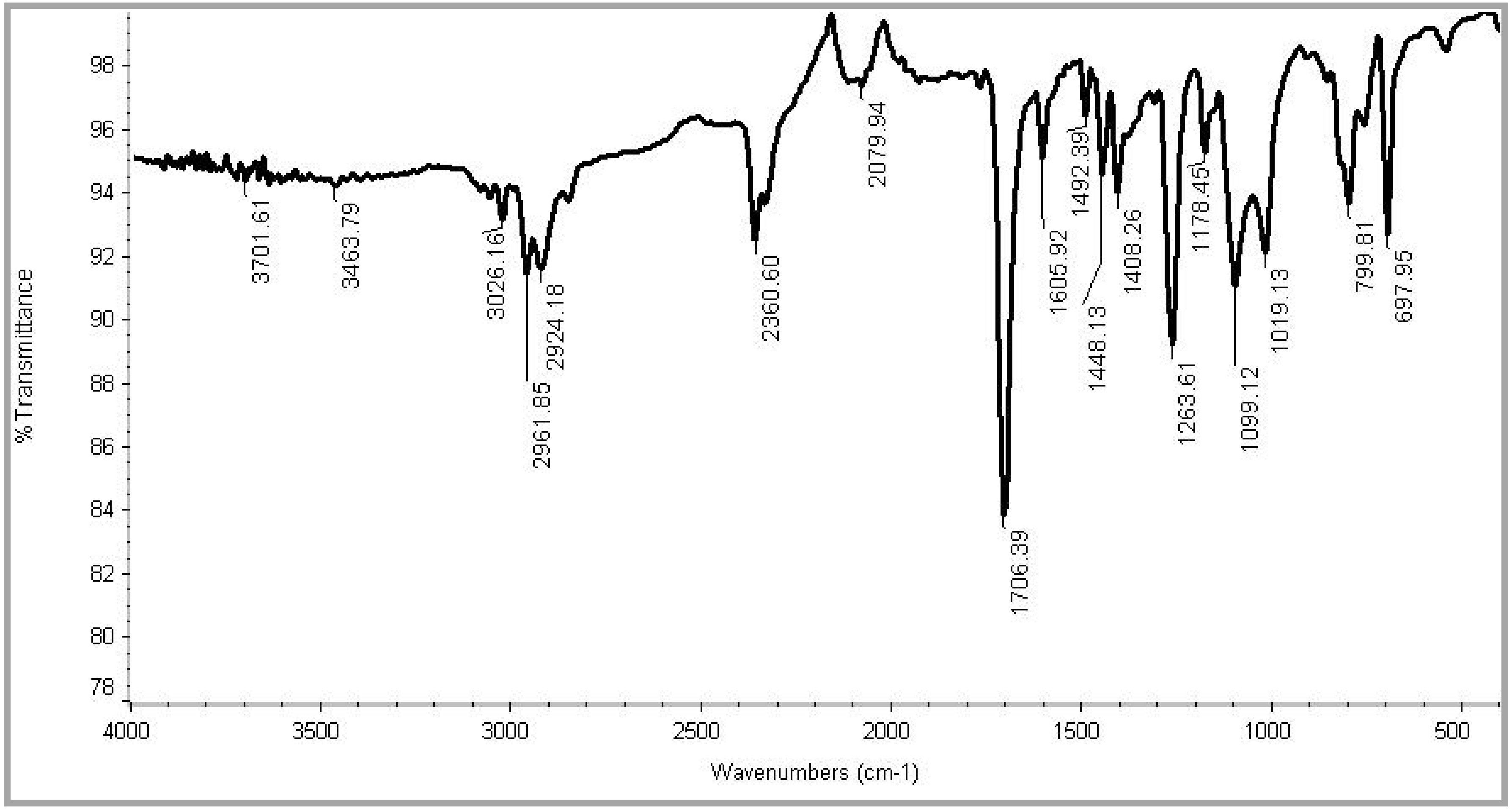


Figure A.14. IR spectrum of activated 1:4 polymer

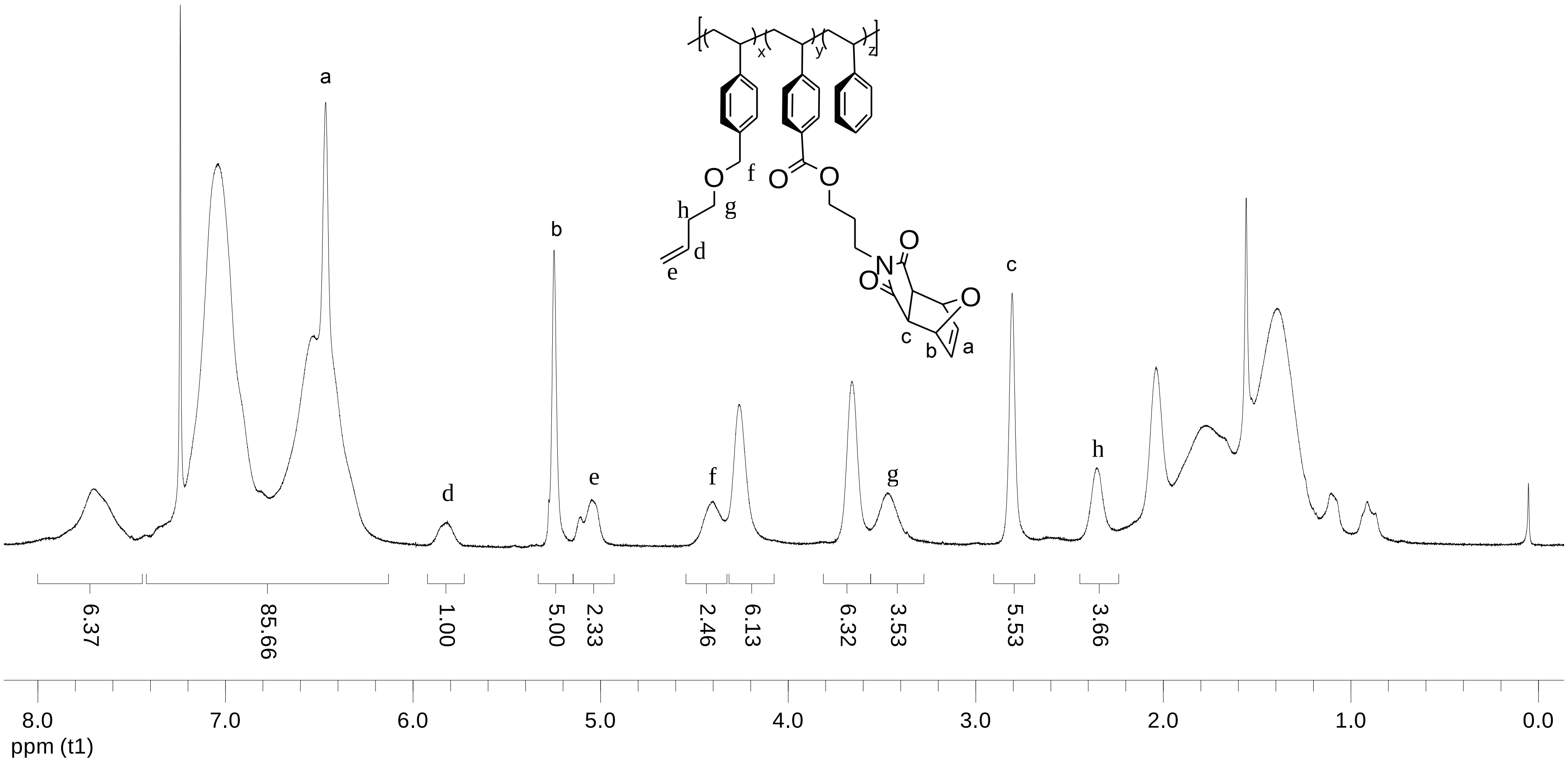


Figure A.15. ^{1}H-NMR spectrum of the alkene-maleimide-styrene copolymer

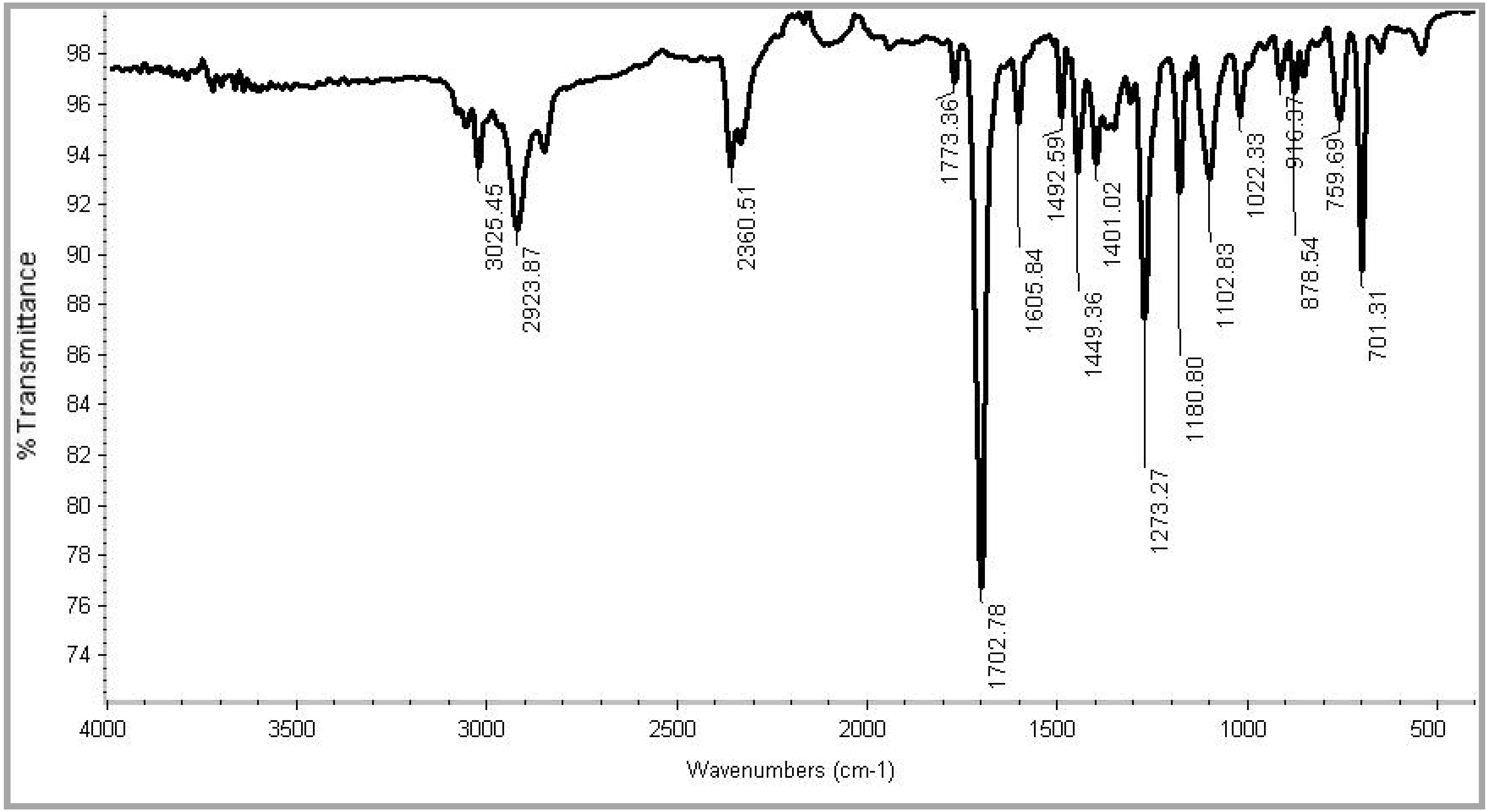


Figure A.16. IR spectrum of the alkene-maleimide-styrene copolymer

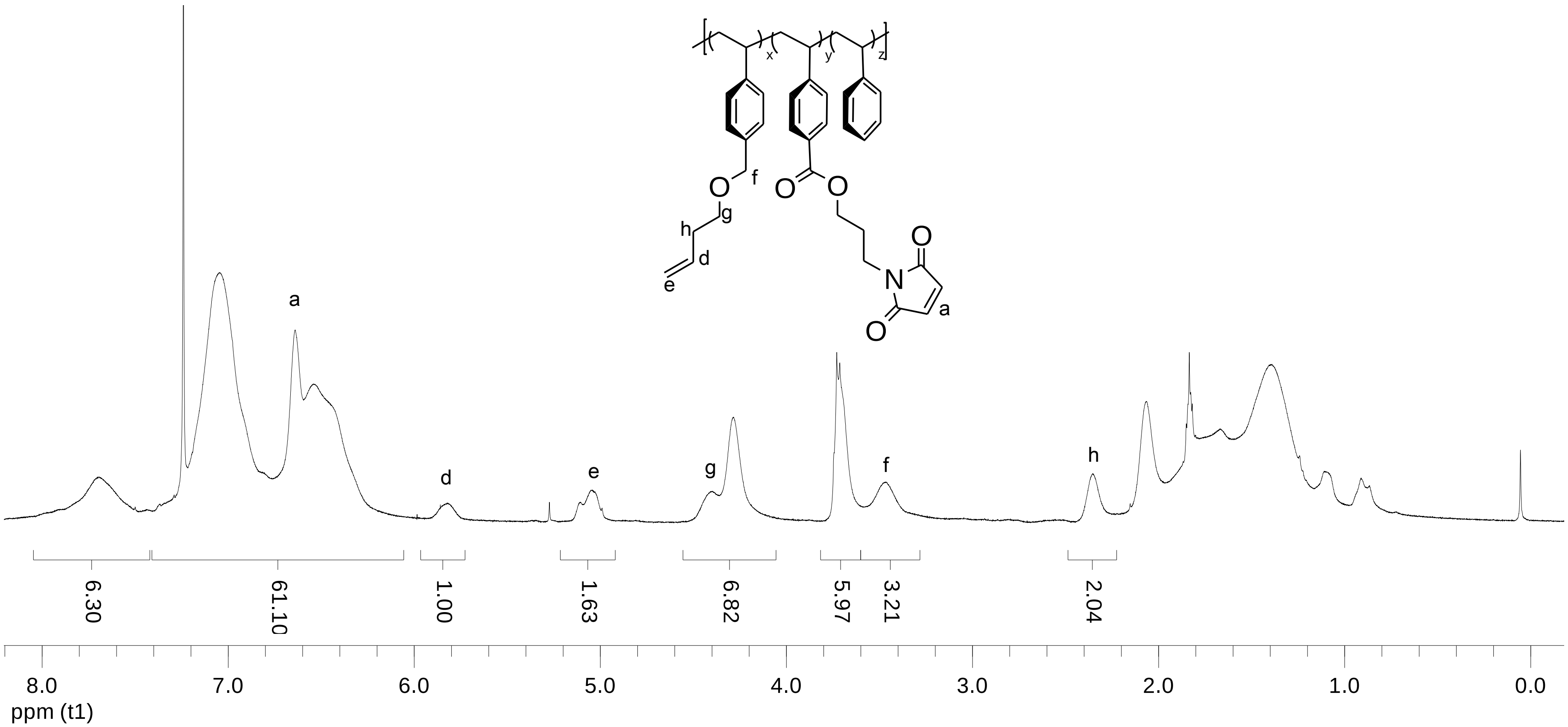


Figure A.17. ^{1}H-NMR spectrum of the activated alkene-maleimide-styrene copolymer

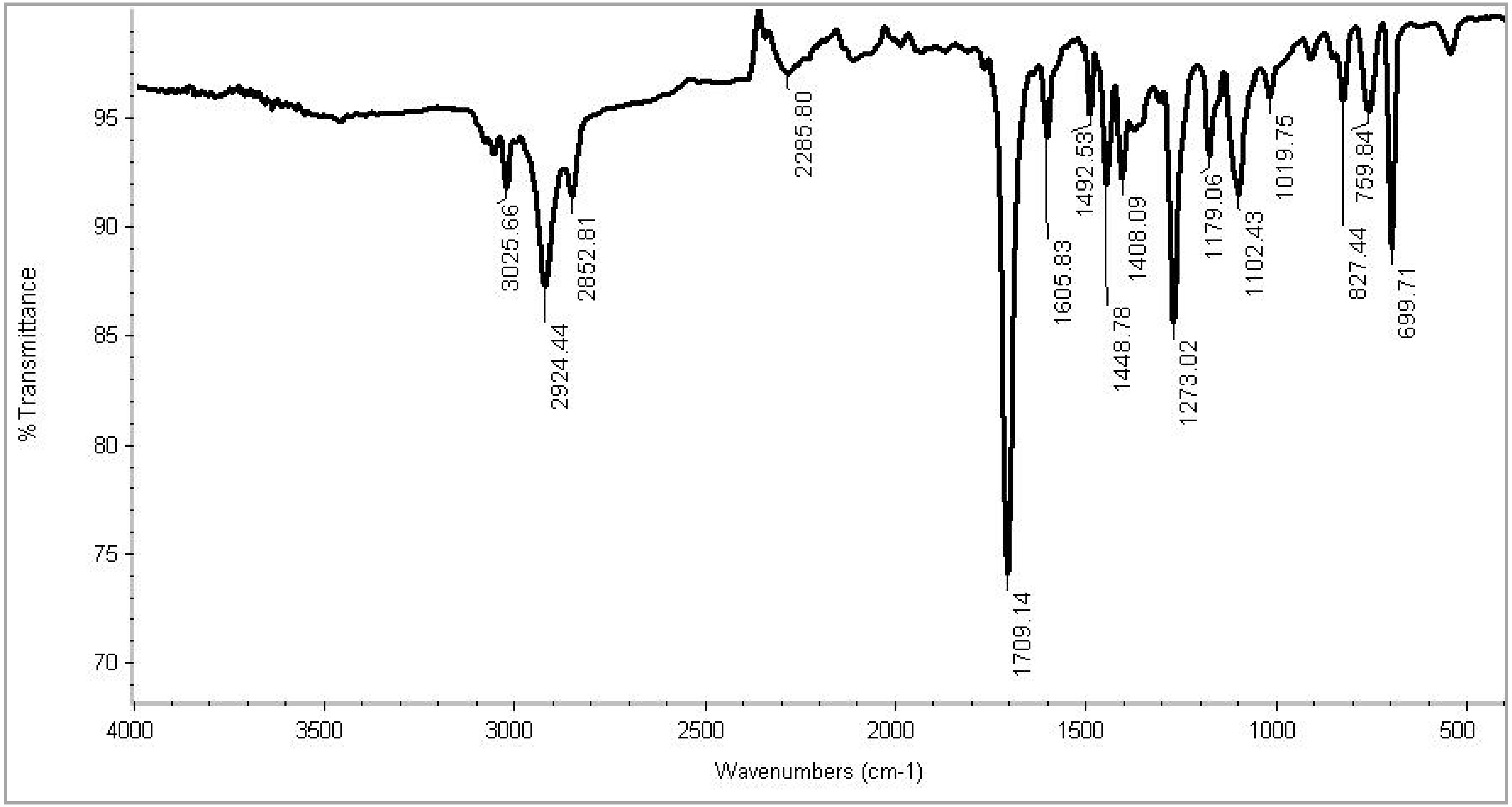


Figure A.18. IR spectrum of the activated alkene-maleimide-styrene copolymer

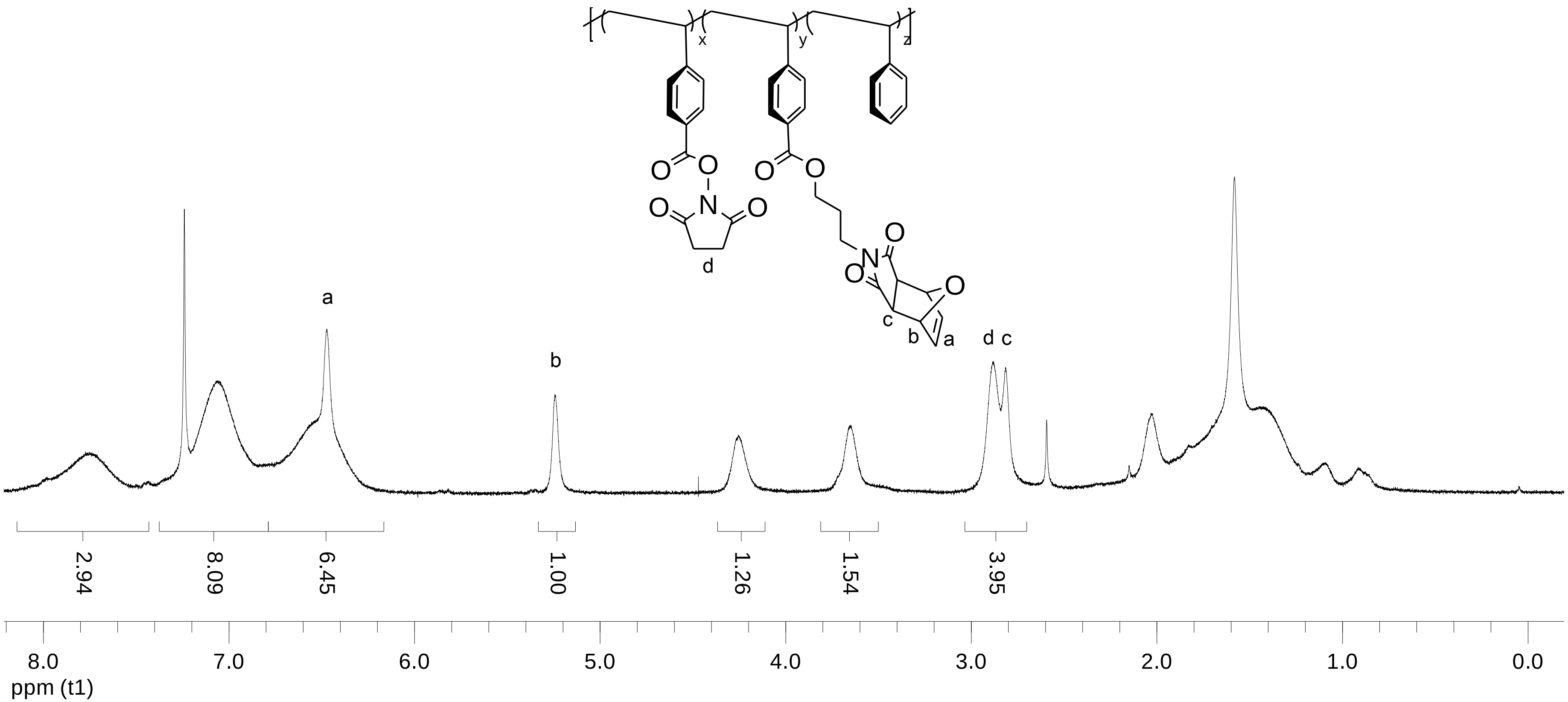


Figure A.19. ^{1}H-NMR spectrum of the N-hydroxysuccinimide-maleimide-styrene copolymer

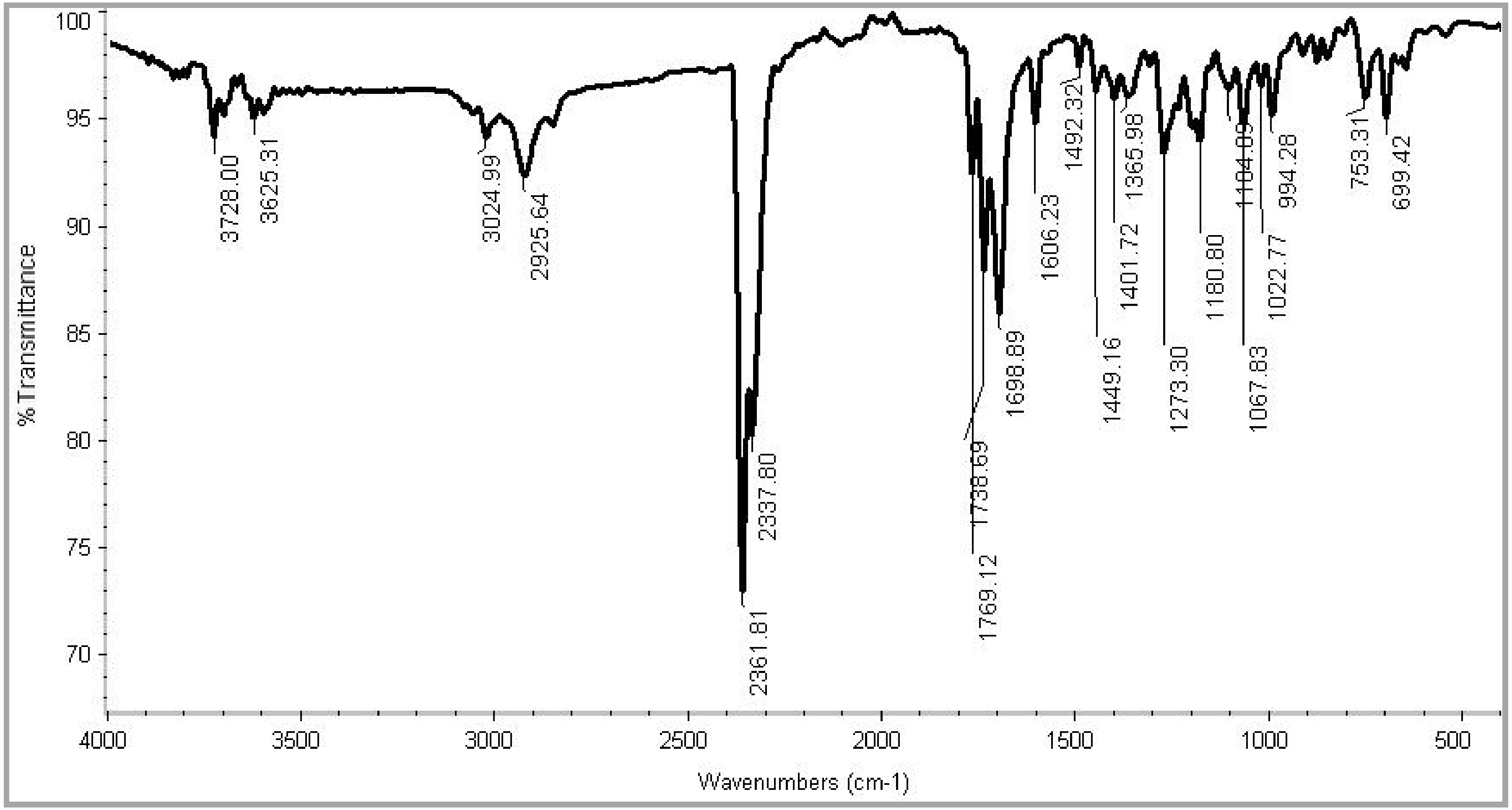


Figure A.20. IR spectrum of the N-hydroxysuccinimide-maleimide-styrene copolymer

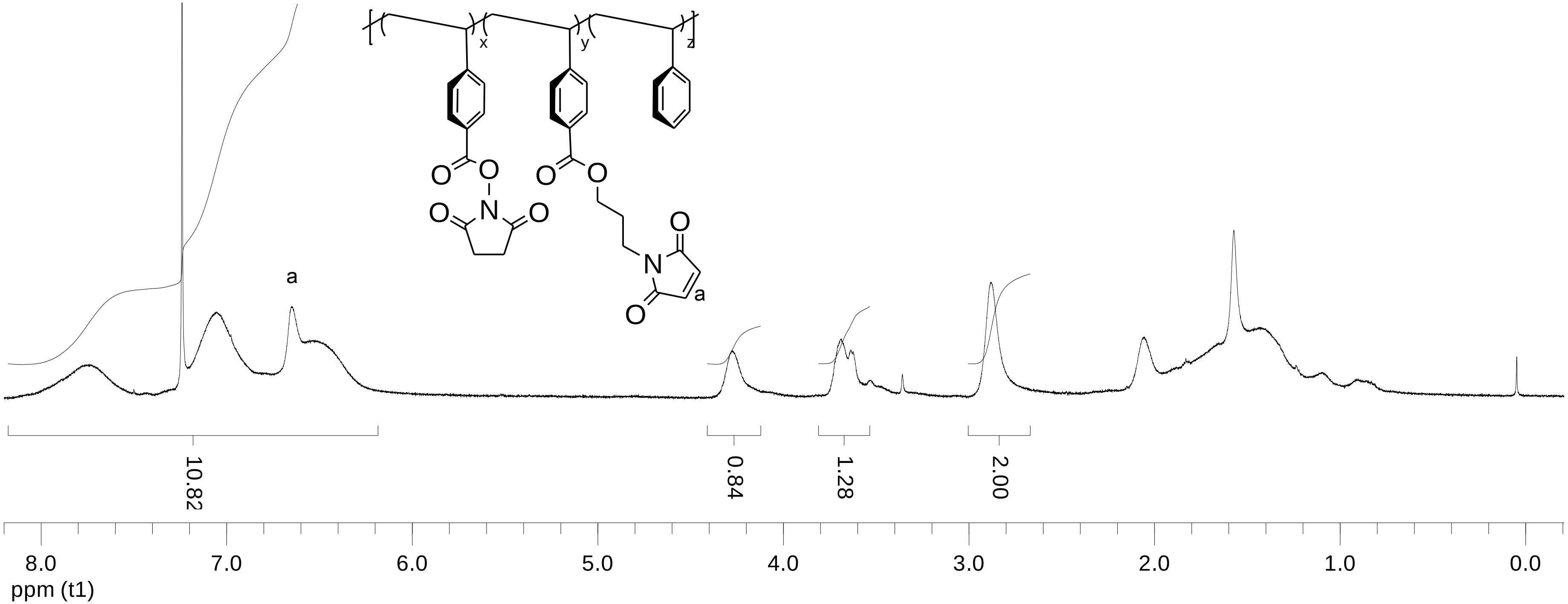


Figure A.21. ^{1}H-NMR spectrum of the activated N-hydroxysuccinimide-maleimide-styrene copolymer

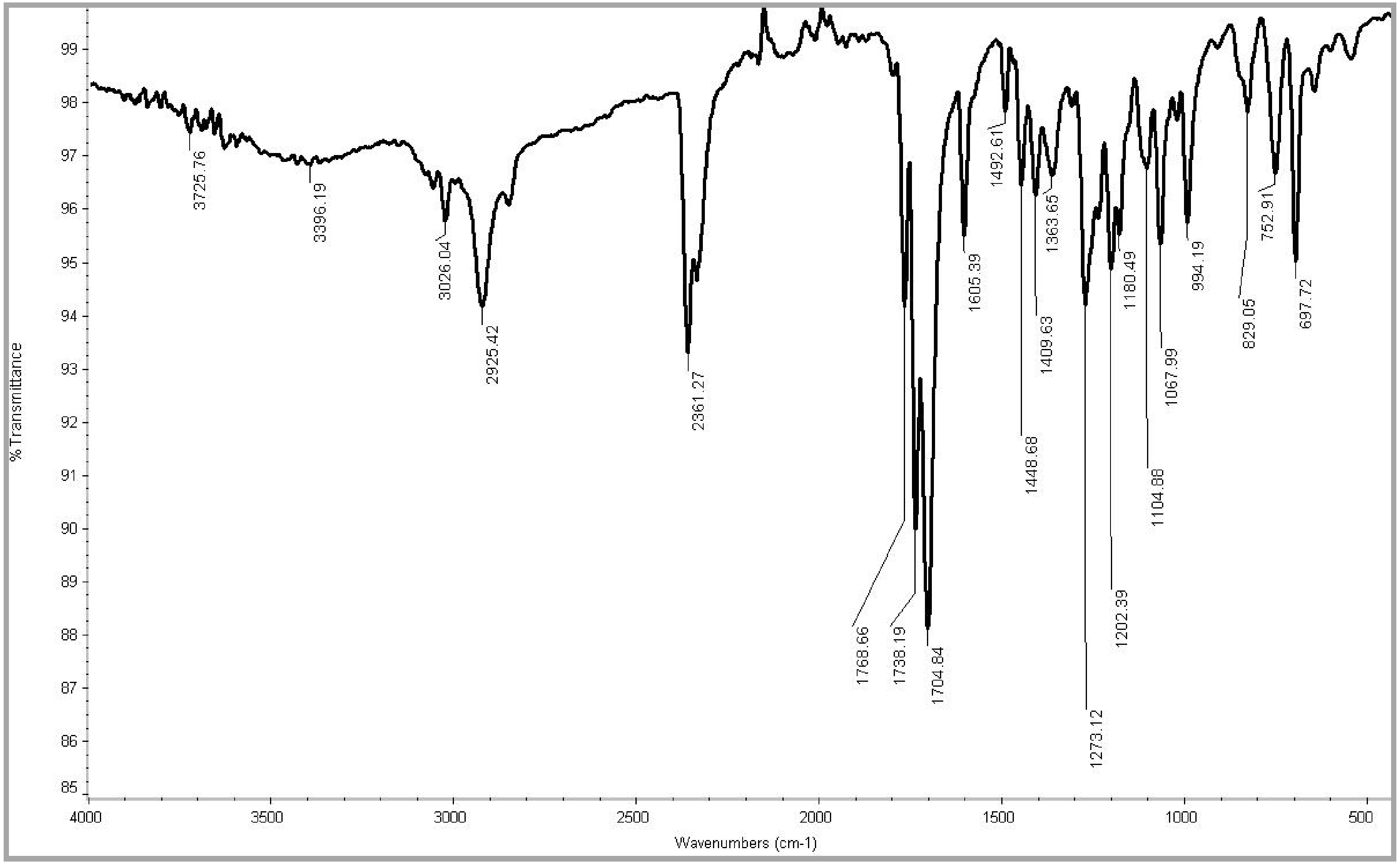


Figure A.22. IR spectrum of the activated N-hydroxysuccinimide-maleimide-styrene copolymer

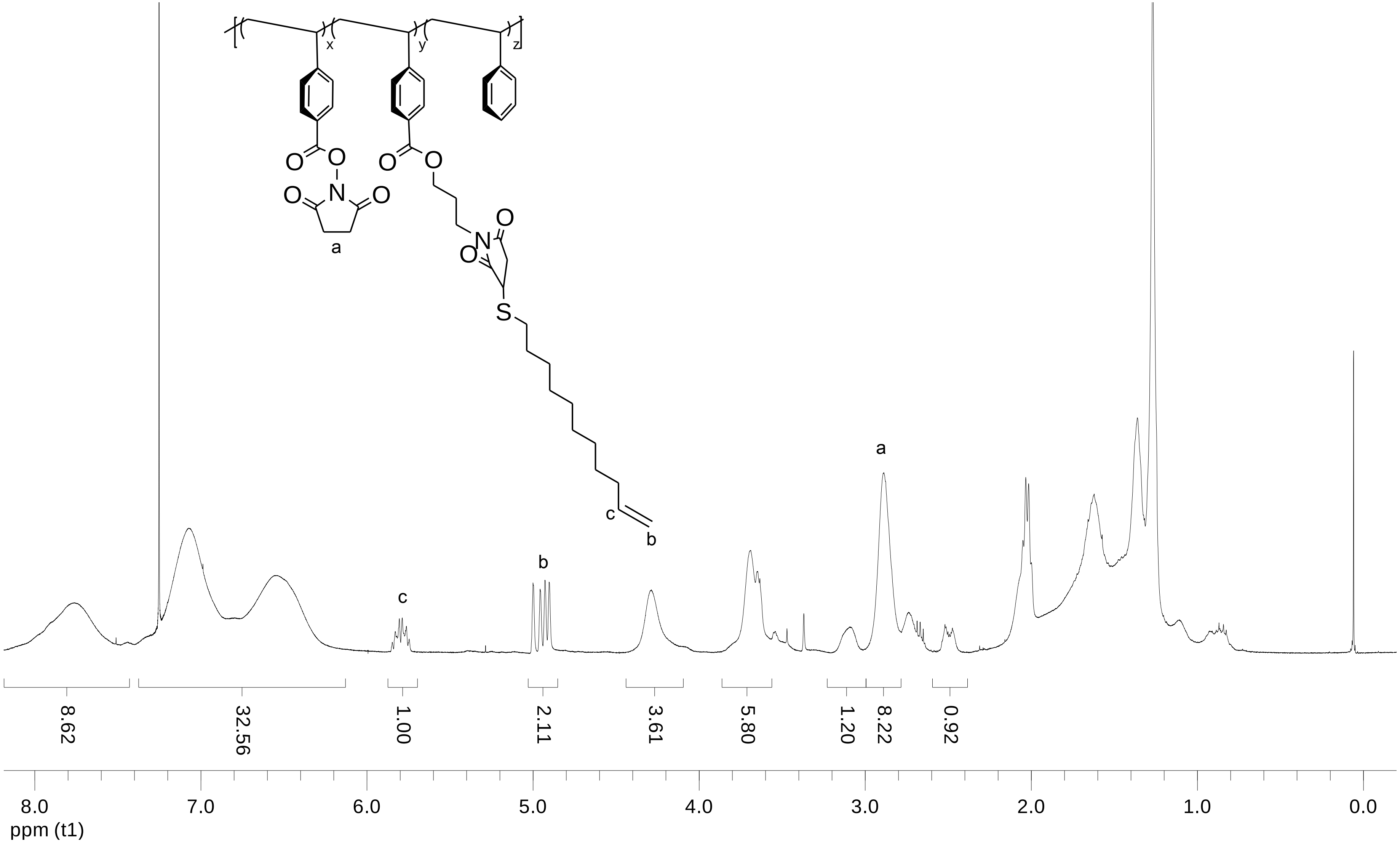


Figure A.23. ^{1}H-NMR spectrum of thiol functionalized N-hydroxysuccinimide-maleimide-styrene copolymer

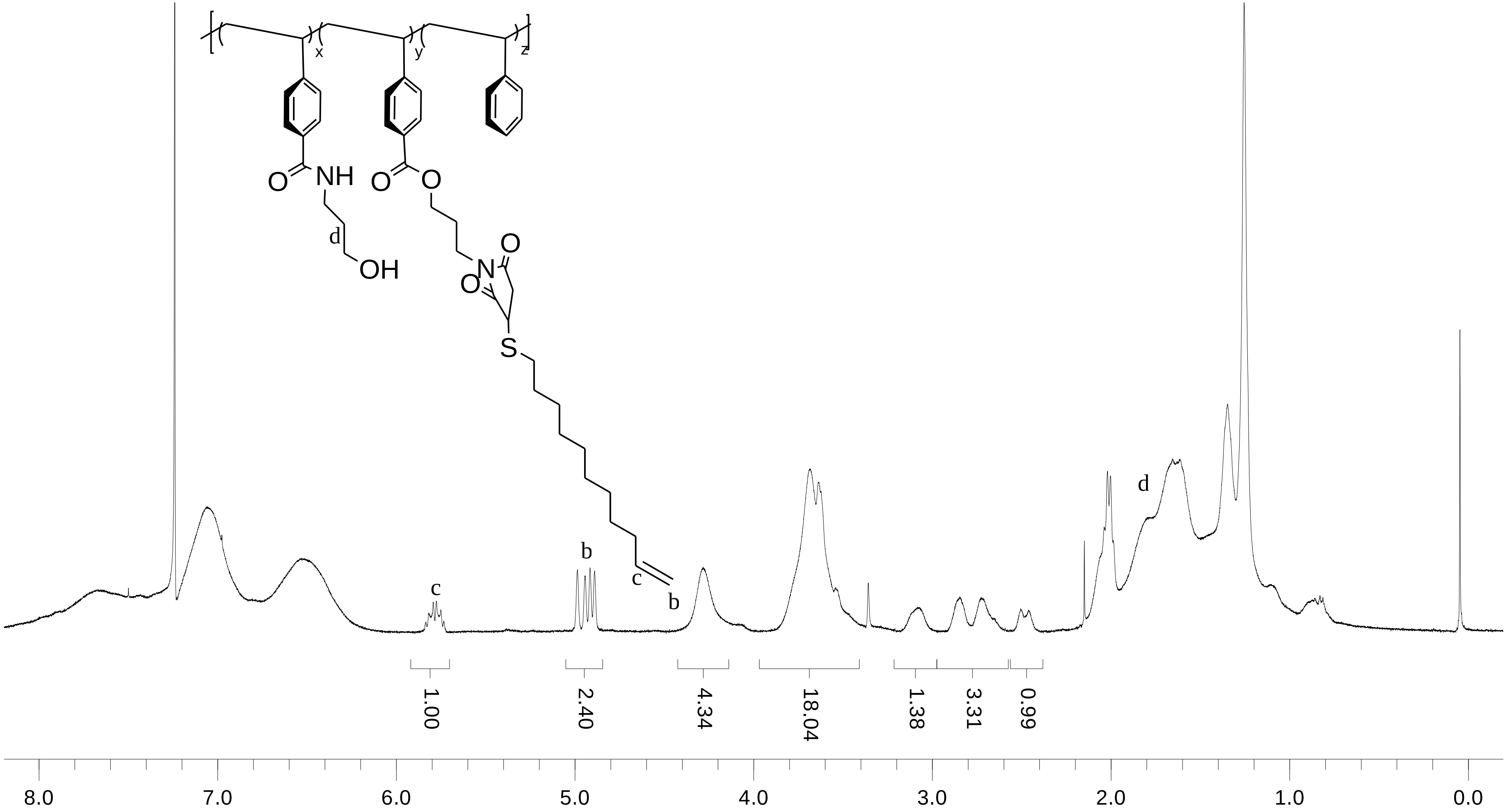


Figure A.24. ^{1}H-NMR spectrum of amine and thiol functionalized N-hydroxysuccinimide-maleimide-styrene copolymer

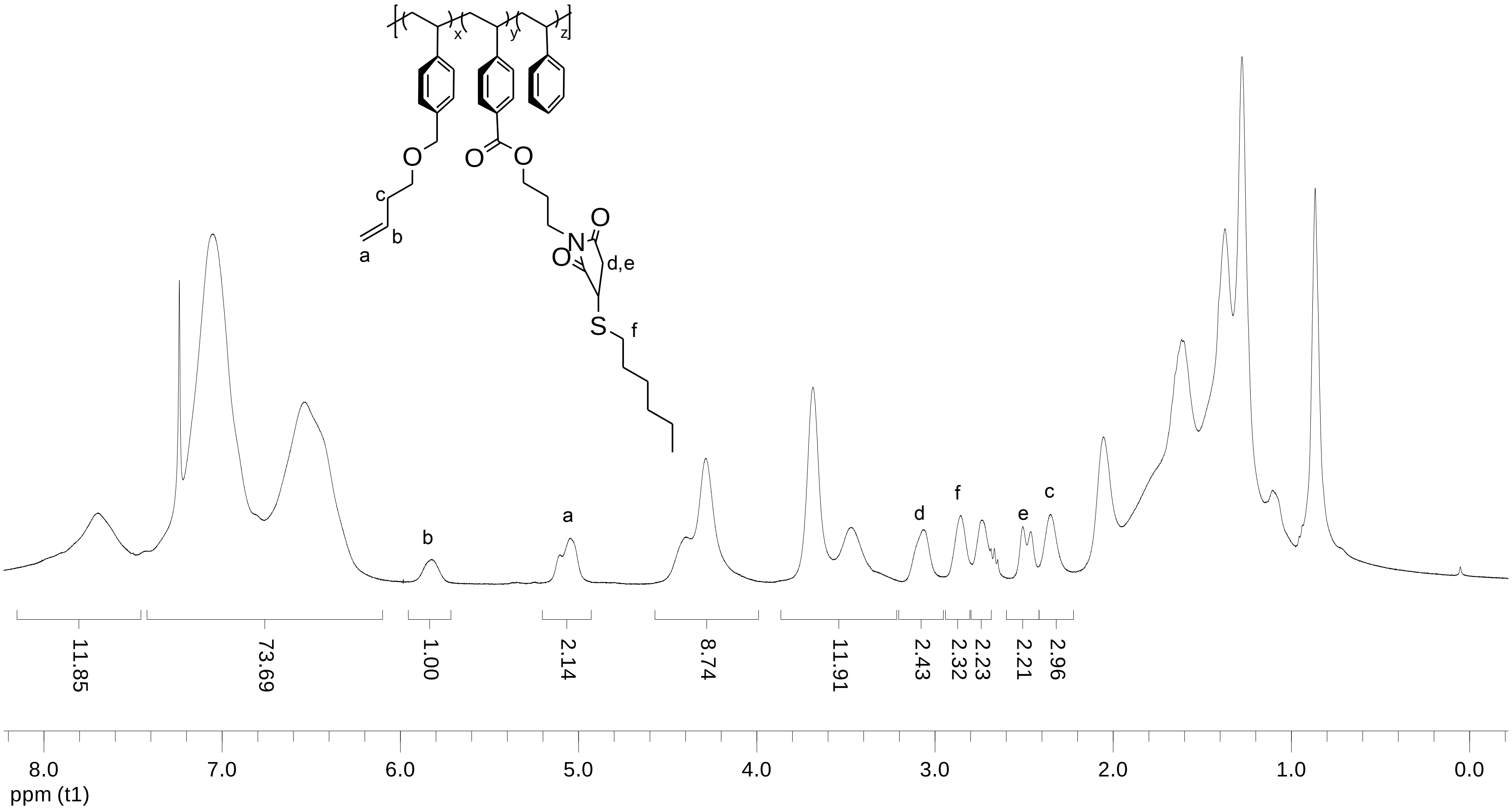


Figure A.25. ^{1}H-NMR spectrum of the hexanethiol functionalized alkene-maleimide-styrene copolymer

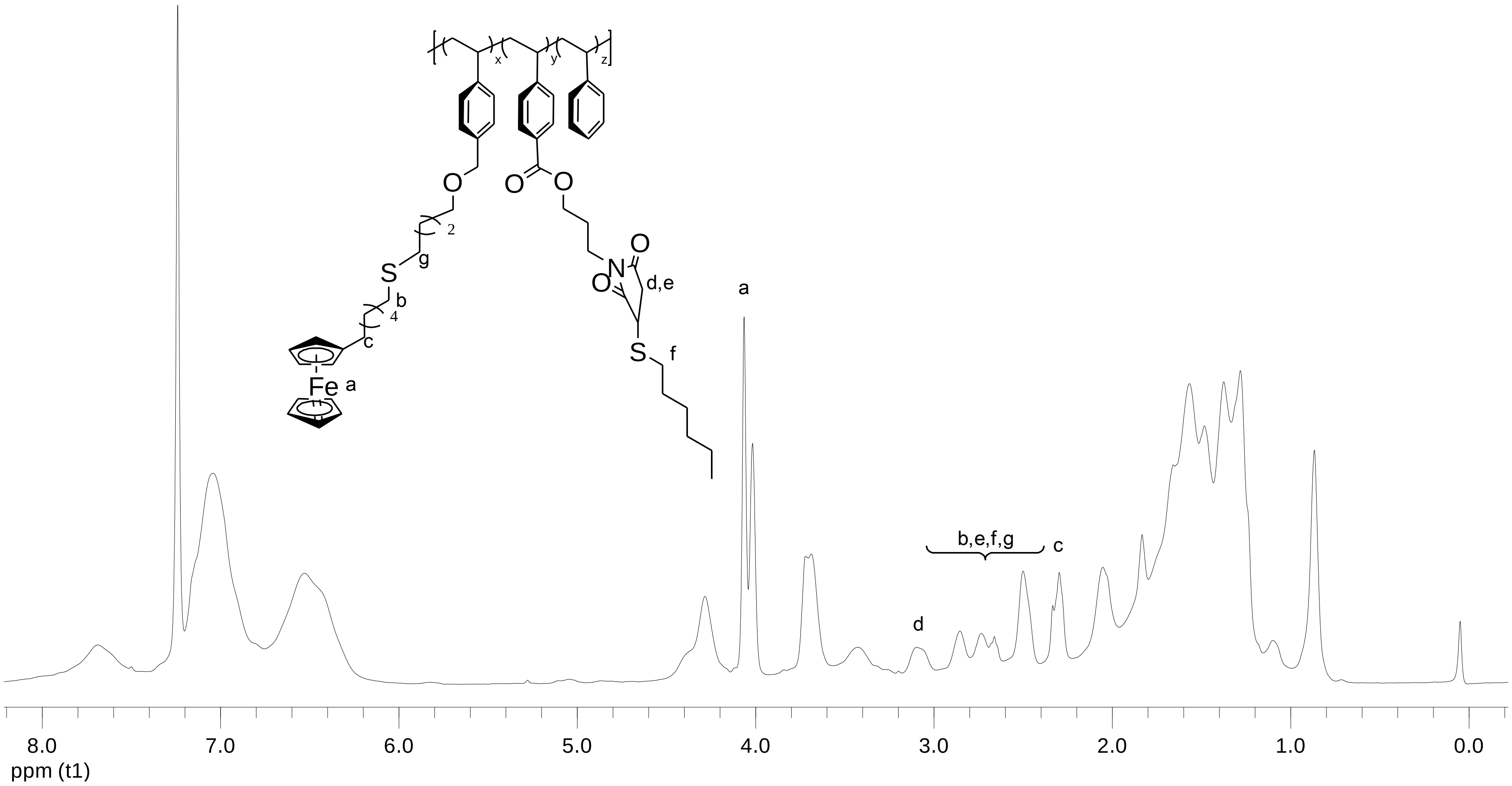


Figure A.26. ^{1}H-NMR spectrum of the ferrocenyl(hexanethiol) functionalized alkene-maleimide-styrene copolymer

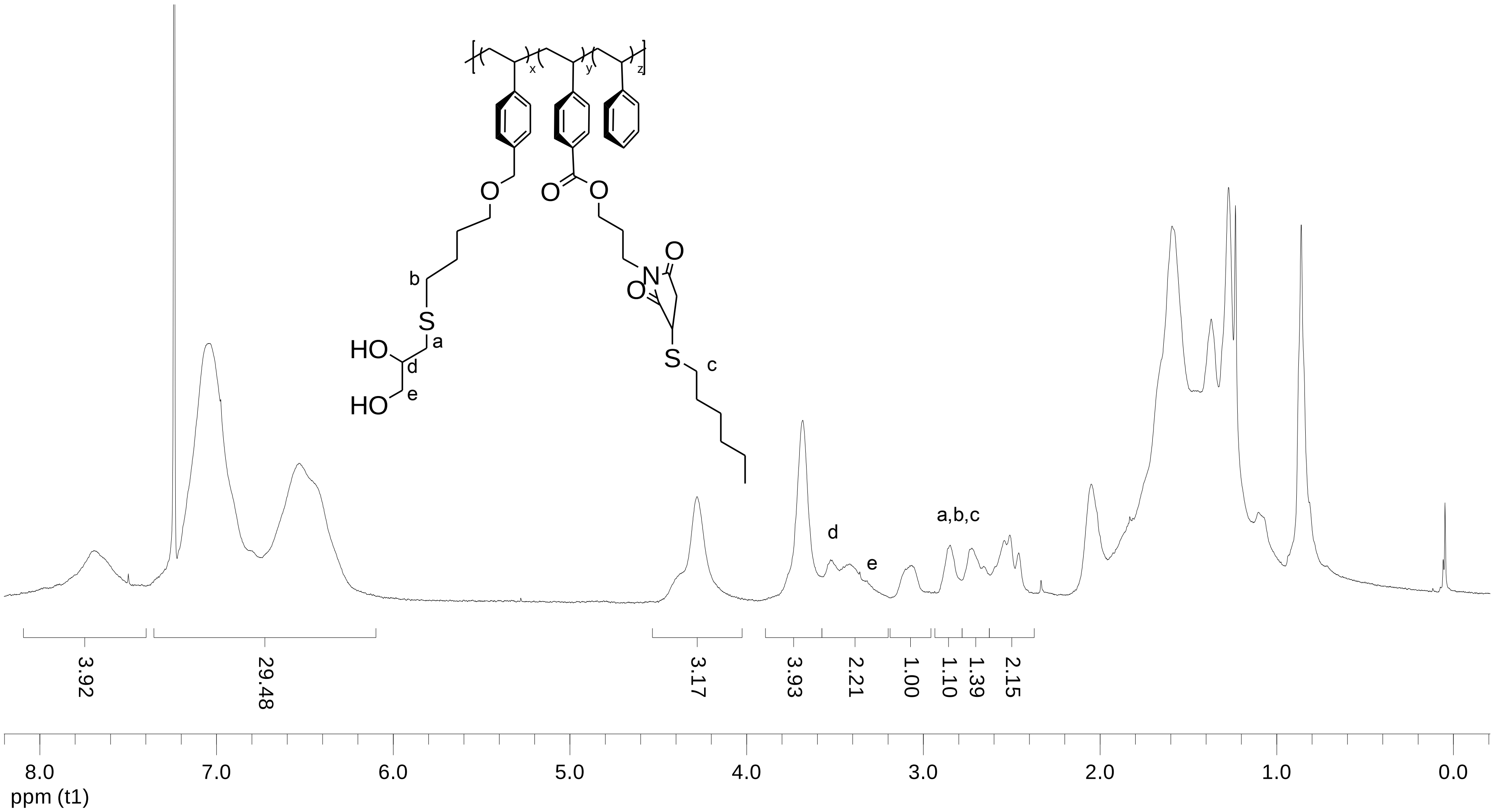


Figure A.27. ^{1}H-NMR spectrum of the thioglycerol functionalized alkene-maleimide-styrene copolymer

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