NOVEL DENDRON-POLYMER CONJUGATES FOR DRUG DELIVERY

by

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To my family

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LIST OF SYMBOLS/ ABBREVIATIONS

J	Coupling constant
υ	Frequency
ATRP	Atom transfer radical polymerization
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
D_2O	Deuterated water
DA	Diels-Alder
rDA	Retro Diels-Alder
DMAP	4-Dimethylaminopyridine
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOAc	Ethyl Acetate
FT-IR	Fourier Transform Infrared
G	Generation
GPC	Gel Permeation Chromotography
MeOH	Methanol
MHz	Mega hertz
MMA	Methylmetacrylate
NMR	Nuclear Magnetic Resonance
PEG	Poly(ethylene glycol)
PEGMEMA	Poly(ethylene glycol) monomethyl ether methacrylate
P*t*BA	tertiary Butylacrylate
SEC	Size-Exclusion Chromatography
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

ABSTRACT

NOVEL DENDRON-POLYMER CONJUGATES FOR DRUG DELIVERY

Multi-armed macromolecules have attracted increasing attention as polymeric scaffolds for biological applications. One of the significant ways to improve the properties of these architectures is the incorporation of dendrimers which are branched macromolecules with well-defined structures. Dendrimers provide the polymeric structure with multivalency and monodispersity especially important in bioconjugation area. First study in this thesis involves synthesis of dendron-polymer conjugates based on Diels-Alder/retro Diels-Alder strategy and drug conjugation to the periphery of these blobk copolymers. Biocompatible polyethylene glycol (PEG) is used as the polymer to provde water solubility to the hydrophobic molecules. Also, due to its anti-biofouling property, it reduces the non-specific interactions of the drug with the proteins in the body. End groups of PEG are functionalized with maleimide to undergo Diels-Alder cycloaddition with the biodegradable polyester dendrons having anthracene functionality at the core. Further functionalization of the periphery of dendron-polymer copolymers with alkyne groups yields a multifunctional dendritic polymer for bioconjugation via Huisgen type "click" reaction.

The second study involves the synthesis of multiarm star polymers based on core functionalized initiators via Atom Transfer Radical Polymerization (ATRP). Dendrons bearing maleimide groups in their core are furnished with initiators at end of their arms for polymerization of several acrylates and methacrylates. It is well known that maleimide group can readily react with thiols via Michael Addition reaction. Thiol containing tripeptide gluthathione is used to demonstrate the reactivity of the maleimide bearing dendritic polymers. Since the bioavailability of the conjugated biomolecule is remarkably affected by the density of the polymer chains around it, these dendritic macromolecules have great potential for protein delivery.

ÖZET

İLAÇ SALINIMI İÇİN YENİ DENDRON-POLİMER BİRLEŞİMLERİ

Çok kollu büyük moleküllerin biyolojik uygulamalarda polimerik yapılar olarak kullanılması çok ilgi çekicidir. Bu yapıların özelliklerinin geliştirilmesini sağlayan önemli yollardan biri yüksek derecede dallanmış ve iyi tanımlanmış dendrimerler ile birleştirilmeleridir. Bilhassa biyomoleküllerin çapraz bağlanması alanında çok onemli olan tek dağılımlı ve çok etkileşimli olma özellikleri polimerik yapılara dendrimerler ile kazandırılır. Bu tezde bahsedilen ilk çalışma, Diels-Alder/retro Diels-Alder stratejisine dayanan dendron polimer birleşimlerinin sentezlenmesi ve bu blok kopolimerlerin dış yüzeylerine ilaç bağlanmasını kapsar. Hidrofobik moleküllerin sudaki çözünürlüklerinin arttırılması için, polimer kısmı olarak biyouyumlu Polietilen glikol (PEG) kullanılmıştır. Ayrıca bu polimerler, vücutta ilaç ve proteinler arasında meydana gelebilecek spesifik olmayan etkileşimleri azaltır. Merkezinde antrasin fonksiyonu bulunduran biyobozunur poliester dendronlarıyla Diels-Alder halkasal katılım reaksiyonuna girebilmesi için PEG'in uç grupları maleimid ile fonksiyonelleştirildi. Sentezlenen dendron polimer kopolimerlerinin dış yüzeylerinin alkin gruplarıyla daha fazla fonsiyonelleştirilmesi, Huisgen cinsi klik reaksiyonu ile biyomoleküllerin çapraz bağlanması için çok fonksiyonlu gruplar taşıyan dendrimerli polimerlerin oluşumuyla sonuçlanır.

İkinci çalışma, Atom Transfer Serbest Radikal Polimerleşme (ATRP) yöntemi ile çok kollu star polimerlerin, merkezi fonksiyonelleştirilmiş başlatıcılar kullanılarak sentezlenmesini içerir. Merkezinde maleimid grubu bulunduran dendronların uçlarına, çeşitli akrilat ve metkrilatların polimerleştirilmesi için başlatıcılar takıldı. Maleimid grubunun tiollerle kolaylıkla Michael katılım reaksiyonuna girdikleri çok iyi bilinmektedir. Dendrimerli polimerlerde bulunan maleimid grubunun reaktif olduğunu göstermek için, tiol ihtiva eden üç aminoasitli bir peptid, glutatyon kullanılmıştır. Bağlanan biyomolekülün biyoyararlılığı çevresindeki polimer yoğunluğundan oldukça etkilendiği için, bu dendrimerli büyük moleküller protein salınımı için büyük potansiyele sahiptir.

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1.1. Dendrimers

Dendrites are regularly branched globular macromolecules with highly well defined monodisperse architecture. Its structure is mainly composed of three different units: a core, repeating units and surface groups (Figure 1.1). Symmetrically placed branching units around the core contribute to its uniform structure. The number of branching units from the core to the periphery refers to the generation of the dendrimer.

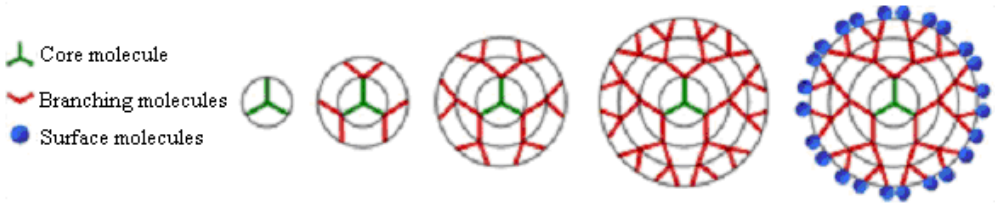

Figure 1.1. Schematic representation showing subunits of dendrimers

Branching units are the building blocks of the dendrimer and functional groups at the periphery are mostly chemically active to undergo further reactions. Dendron is a part of the dendrimer starting from a core and ending at the periphery. It does not contain the 'core' part. The starting point from which repeating units built the dendron is called as the focal point, which may also has chemically reactive groups (Figure 1.2).

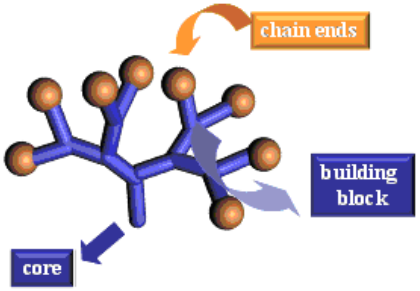

Figure 1.2. General structure of a dendron

Dendrons are synthesized by two approaches, one of which involves the growth of a dendron starting from the core. After the attachment of branching units, the surface groups are placed on the periphery. This is called as divergent synthesis. In the other method, convergent approach, firstly surface groups are attached onto the branching units and via a multivalent core, these small dendron pieces are combined. Synthesize dendrons are combined either via covalent bond forming reaction between their focal points or via non-covalent interactions (Figure 1.3) [1].

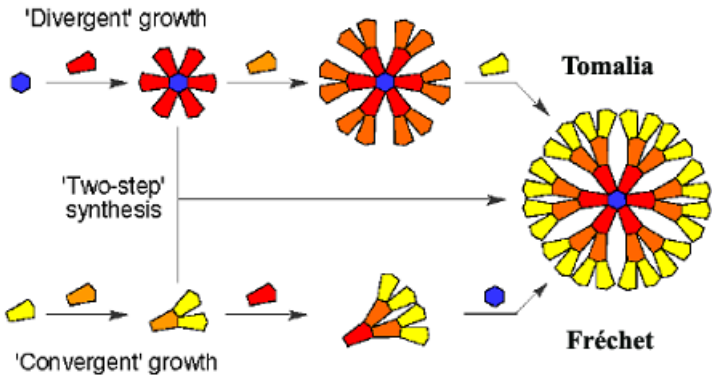

Figure 1.3. Synthesis of dendrimers via divergent and convergent method [1]

1.2. Click Chemistry

There are mainly 4 types of “Click” reactions which are ‘nucleophilic opening of highly strained rings’ such as epoxides, aziridines, cyclic sulfates, cyclic sulfamidates and aziridinium ions, ‘protecting group reactions’ such as acetals, ketals and their aza-analogs, most famous ones ‘cycloaddition reactions such as Diels-Alder [4+2] and Copper catalyzed Huisgen [3+2] or Huisgen 1,3 dipolar cycloadditon reactions [2].

1.2.1. Copper Catalyzed Huisgen [3+2] Cycloaddition

The most famous Click reaction is Huisgen [3+2] cycloaddition reaction. Azide-alkyne cycloaddition reaction is introduced by Rolf Huisgen in 1970 without using water and catalyst, azide and alkyne groups react at high temperature to produce a mixture of 1,4 and 1,5-disubstituted triazoles (Figure 1.4).

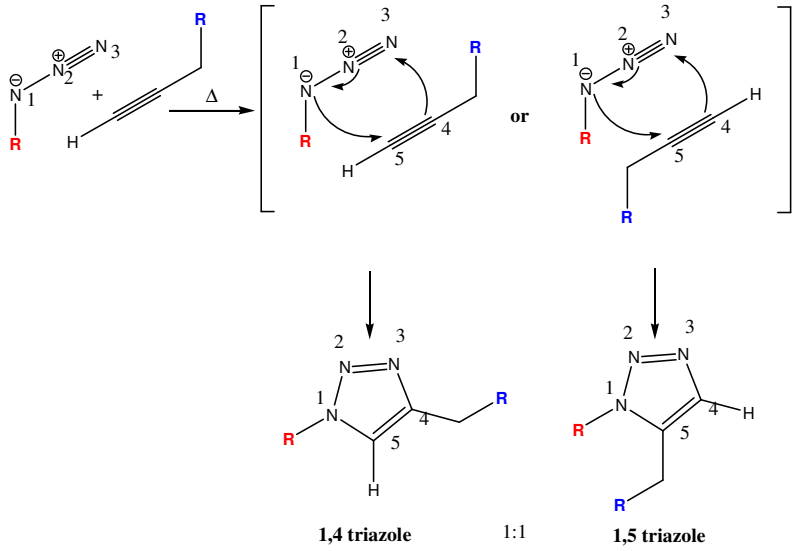

Figure 1.4. Click reaction [2]

In 2002 Sharpless and Fokin [3] introduced a Cu (I) catalyst in Huisgen reaction do that the catalyst directs the region-specific result of the reaction and results in only 1,4-disubstituted triazole (Figure 1.5). So far, it is called as copper (I)-catalyzed azide-alkyne reaction.

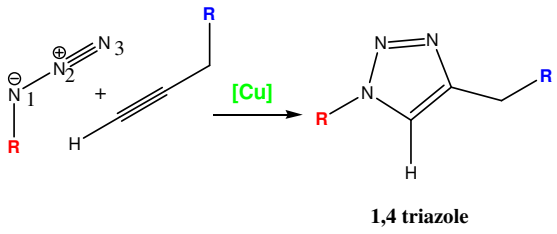

Figure 1.5. Copper catalyzed Huisgen reaction

Presence of Cu catalyst allows the synthesis of physiologically stable product in aqueous or organic solvents in high yields. The catalyst can be introduced Cu (I) salt (CuI or CuBr) or generated in situ by reduction of Cu (II) salts [4] and amine containing base such as 2,6-lutidine, triethylamine, pyridine and PMDETA. Commonly used Cu-systems are $CuSO_4$/ NaAsc and CuBr/PMDETA due to exclusive regiospecificity, mild reaction conditions, easy purification, high yield and functional group tolerance.

Click chemistry have been widely used in the synthesis of well-defined macromolecules such as dendrimer synthesis [5], dendronized polymers [6] and biological studies: building of fluorescent oligonucleotides for DNA sequencing [7], in situ assembly of acetylcholinesterase inhibitors [8] and synthesis of HIV-1 protease inhibitor [9].

1.3. Diels-Alder Reaction in Polymer Chemistry

Diels Alder reaction (DA) is a well known [4+2] cycloaddition reaction which involves the addition of an electron rich diene group to an electron poor dienophile. With the generation of new bonds by inter- or intramolecular interactions, DA reaction results in a six membered ring formation. The product obtained is referred as an adduct. Furan and anthracene derivatives have been widely explored as diene components in macromolecular construction. As the choice of dienophile, maleimides are mostly preferred due to their high reactivity and wide structural variability through the nature of the nitrogen substituents (Figure 1.6). Products of Diels Adler reactions can be obtained in high yiels without formation of offensive by-products [10].

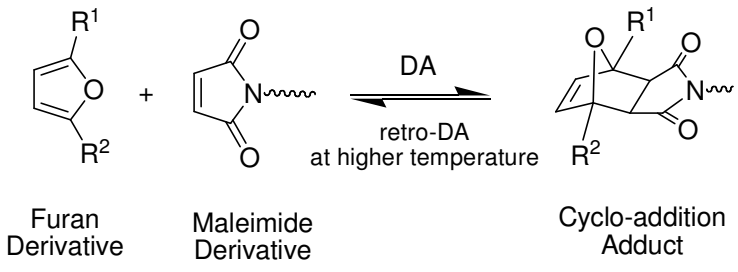

Figure 1.6. Representation of the DA and rDA reactions [10]

Diels Alder chemistry due to its effectiveness and uniqueness has become one of the outstanding and attractive tools to synthesize macromolecular structures such as dendrimers and polymers. In polymer chemistry, the DA reaction has been utilized to synthesize linear polymers as well as crosslinked structures. Linear polymers have been synthesized via successive DA cycloadditions involving multifunctional diene and dienophiles as the monomer, e.g. a di-furan derivative and a bismaleimide. For the crosslinked macromolecules, 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. For thermally reversible cross-linked polymers, the thermal sensitivity of the DA can be utilized to get their precursors back. This feature can be exploited in many applications, like the possibility of recycling or "mending" network-based materials.

1.3.1. Diels-Alder Reaction in Polymerization

DA "click" chemistry has been widely used for the synthesis of a diversity of polymers in the past. The DA reaction in polymer chemistry dates back to an early report about formation of oligomers of cyclopentadiene by Alder and coworkers in 1932. In 1961, Stille and co-workers successfully obtained high molecular weight polymers by Diels Alder chemistry [11]. In this study, they utilized the DA reaction between cyclopentadiene based dimers and bismaleimides. The study did not promise further developments due to the utilization of biscyclopentadienyl compounds that are highly prone to homopolymerize.

In 1990s, a variety of polymers such as polyimides, polyurethanes and acrylic copolymers have been synthesized utilizing DA reaction [12-14]. Gandini's group with their broader and long-standing interest in furan polymers has been engaged in different polymer applications of the Diels-Alder reaction involving furanic structures. In one of their more recent studies, they achieved linear macromolecular growth reversible DA polymerization by difurans (AA) reacting with bismaleimides (BB), which received much attention with respect to polymer synthesis and thermal reversion to the starting monomers (Figure 1.7) [15].

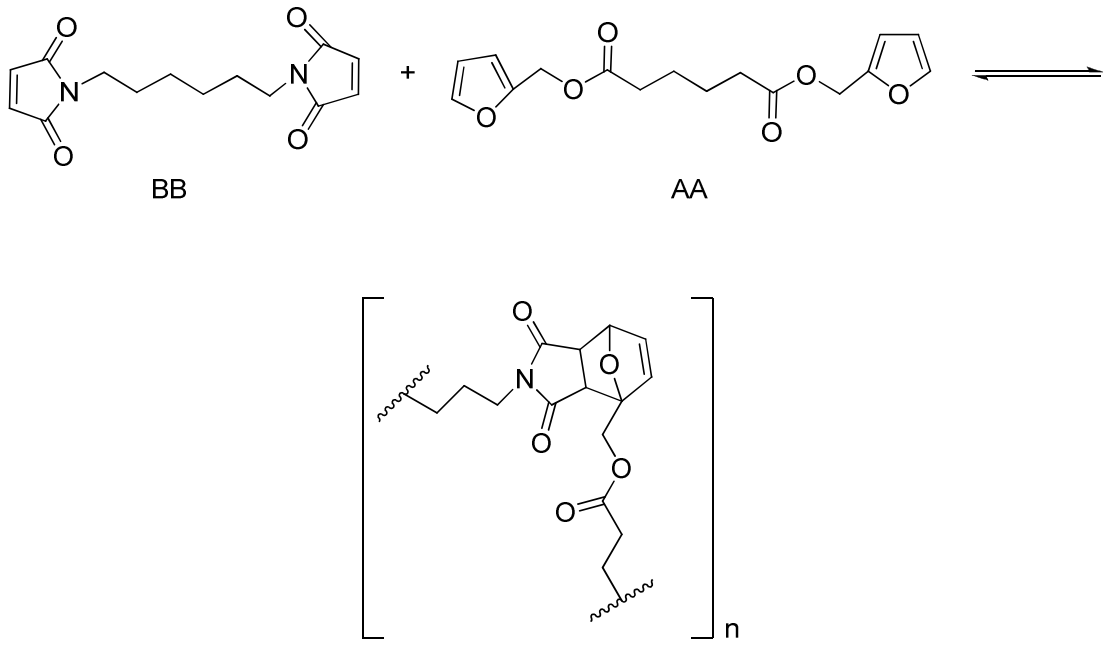

Figure 1.7. Reversible DA linear polymerization of a bismaleimide with a difuran comonomer [15]

Diels-Alder reaction has been widely used for bioimmobilization, peptide-peptide coupling, synthesis of diblock and graft copolymers and total synthesis due to its efficiency and reagent free conditions. In 2006, Howorka and co-workers prepared glass surfaces carrying a dense layer of Poly (ethylene glycol) (PEG) which is potential platforms for DNA oligonucleotide micro arrays [16]. The glass slides were first silanized by using 3-glycidoxypropyl trimethoxysilane (GPS). The epoxide groups of the GPS-silanized surface were hydrolyzed to diols which were subjected to oxidative cleavage resulted in the formation of aldehyde slides (Figure 1.8). To obtain a homogeneous thin layer of PEG, a solution of PEG diamine was applied onto the slides. PEG-grafted slides displaying terminal amino groups were incubated with succinimidyl 4-[p-maleimidophenyl] butyrate resulted in maleimide terminated surfaces which were then used to immobilize DNA oligonucleotides.

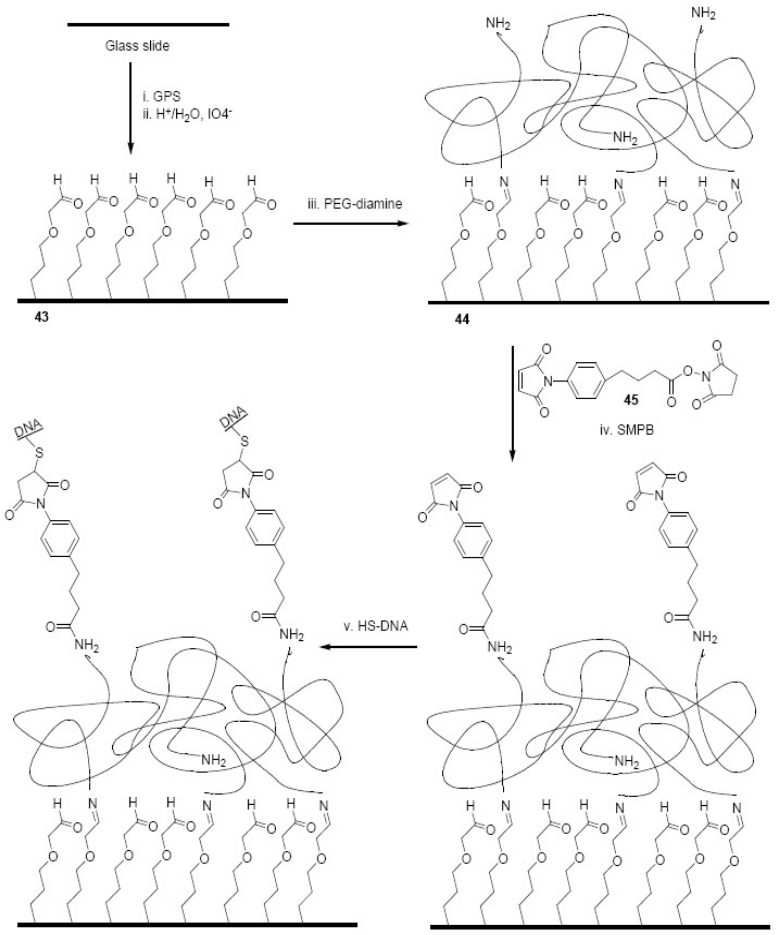

Figure 1.8. Preparation of PEG-grafted, maleimide terminated surfaces [16]

Tunca and co-workers synthesized graft copolymers via Diels-Alder reaction and copper catalyzed Huisgen reaction [17], (Figure 1.9) [18]. They have also utilized a combination of in situ click [3 + 2] and Diels-Alder [4 + 2] reactions to synthesize ABC triblock copolymers of PEG-PS-PMMA and PCL—PS-PMMA (Figure 1.10) [19].

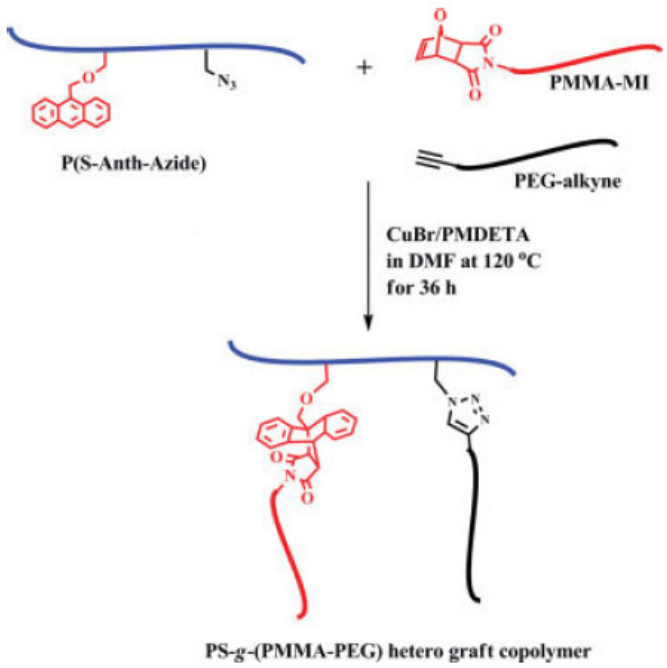

Figure 1.9. Synthesis of graft copolymer via double click reactions [18]

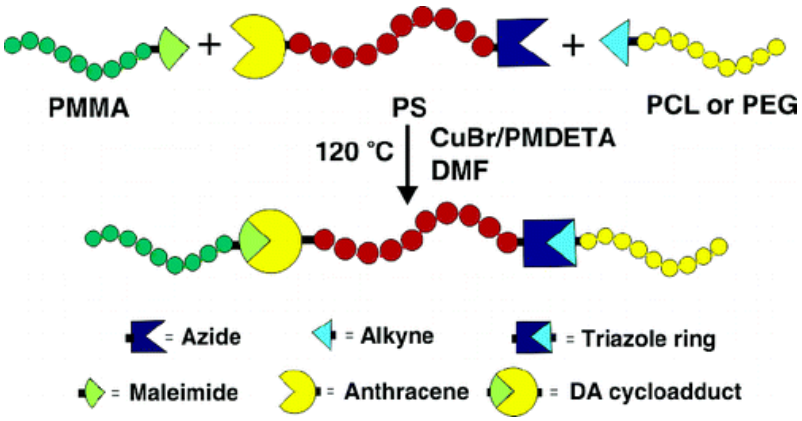

Figure 1.10. One-Pot synthesis of triblock copolymer [19]

1.3.1.1 Use of Diels-Alder Reaction in Synthesis of Reactive Polymers:

The maleimide group has been utilized as an efficient handle for conjugation chemistry. Polymers containig maleimide functionality have attracted considerable interest in biotechnology because it undergoes facile addition of furan and anthracene containing molecules via Diels Alder reaction. In 2004, Velonia and her coworkers prepared a series of R-functional maleimide polymethacrylates via copper-catalyzed living radical

polymerization (LRP) (Figure 1.11). The initiator containing a maleimide unit "protected" as a Diels-Alder adduct which then in the polymerization step has been reintroduced by retro-Diels-Alder reaction. These functional polymers have been successfully employed in conjugation of reduced glutathione bovine serum albumin (BSA) [20].

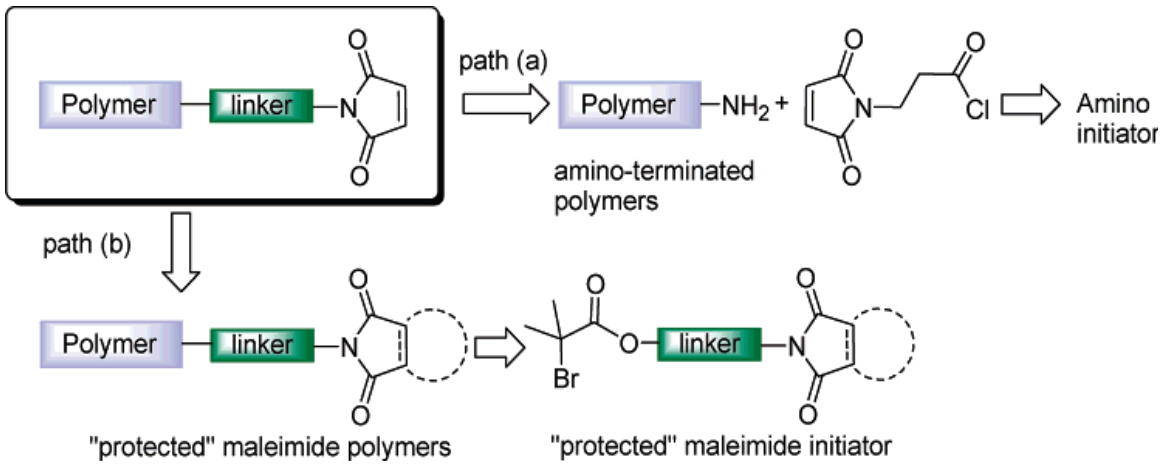

Figure 1.11. Retrosynthetic approaches to R-maleimide-functional macromolecules [20]

Maleimide group also enables the functionalization of polymers via Michael-type addition since it readily reacts with thiol containing molecules. Oftentimes the reaction goes to very high conversions without the need of additional reagents. In 2006 Bailey and Swager used furan masked maleimides to synthesize rhodamine modified poly(phenyleneethylenes) (PPE). Furan was removed quantitatively in solution under relatively mild thermal conditions via cycloreversion. The resulting unmasked polymer is functionalized with a compound containing a thiol or diene [21].

Recently, our research group has disclosed a novel strategy for the synthesis of reactive polymers bearing maleimide groups as side chain units. Polymers bearing protected maleimide groups as side chain substituents were prepared from a novel methacrylate based reactive monomer [22]. Protection of maleimide group before the polymerization prevents its incorporation into the polymer backbone. The reactive

maleimide units were then activated via rDA reaction to enable post-functionalization of the formed polymer with thiol containing molecules. (Figure 1.12).

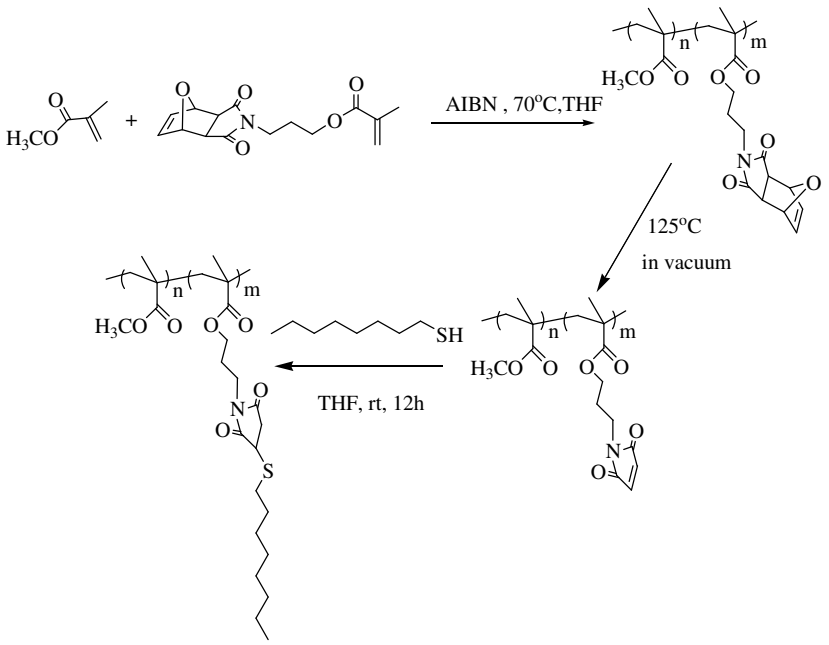

Figure 1.12. Synthesis of maleimide based reactive copolymer [22]

1.4. Dendronized Polymers

Dendronized polymers are defined as linear polymers with dendritic units [23]. In recent years, dendritic macromolecules have generated much attention. Incorparation of the dendrimers provides the polymers with special properties depending on the type and generation of dendron, attachment density along the backbone, functional groups at its periphery and degree of polymerization [24, 25]. Molecular architecture of dendronized polymers can be controlled with high precision in terms of size, stiffness and surface decoration.

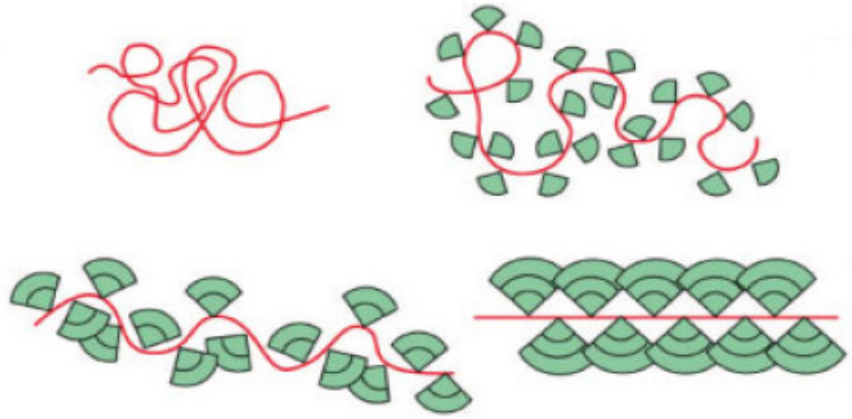

Figure 1.13. Different conformations of dendronized polymers [26]

Dendron polymer conjugates are rapidly becoming common macromolecular building blocks in both biopharmaceutical and materials research. Dendronized polymers have been investigated for different applications such as the synthesis of hierarchically structured materials, electronic and photonic applications, catalysis, drug delivery and applications in the biotechnology [27, 28].

Although a linear polymer mostly forms random-coil structures in solution or bulk, with the attachment of dendrimers, the backbone is fully extended as a consequence of sterical hinderence. Growing interest in these materials originates from the ability of end group funcionalities of dendrimers upon the adopted cylindrical, rodlike conformations.

There are three main pathways to synthesize the dendronized polymers in an efficient and preferably in a modular manner: namely, ‘macromonomer method’, ‘graft-to’ method, and the ‘graft from’ method. The macromonomer approach is based on the polymerization of a monomer that already bears a well defined dendron (Figure 1.14) [23]. This method ensures the presence of dendrons attached to all side chains in a homopolymer, meaning that maximum dendronization can be achieved. The disadvantage is the low degrees of polymerization due to steric hindrance generated by high generation dendrons. To overcome this problem, high monomer concentration should be used for the polymerization [29].

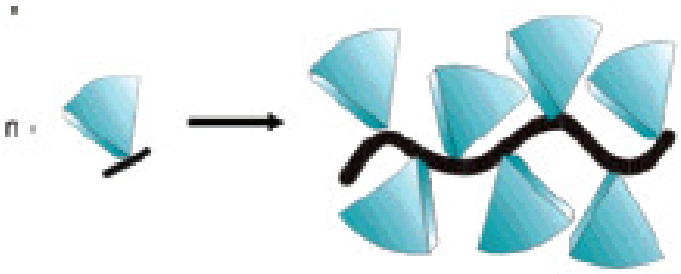

Figure 1.14. General scheme of macromonomer method [23]

The monomers were studied via several polymerization methods such as radical polymerization (Figure 1.15) [30, 31, 32], insertion polymerization [33], ring opening-metathesis polymerization (ROMP) [34, 35, 36], Suzuki polycondensation [37], Heck coupling [38] and Stille coupling [39].

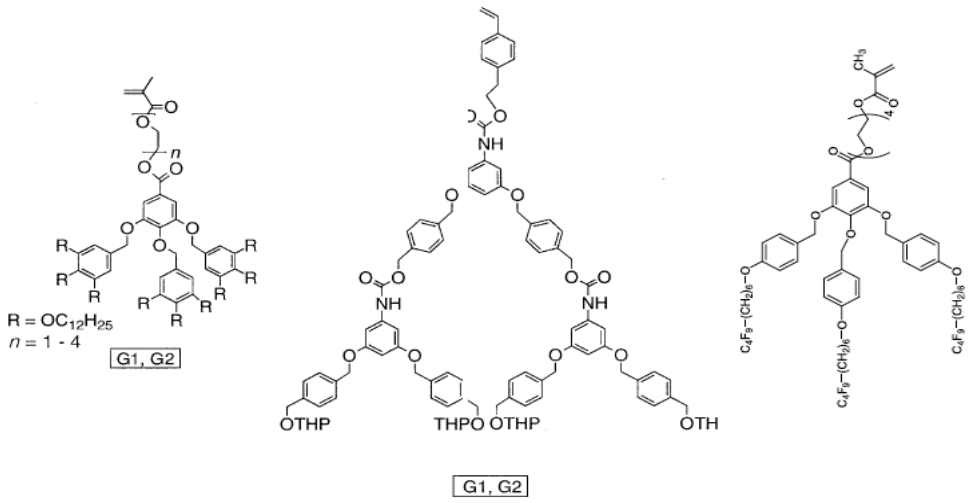

Figure 1.15. Structures of macromonomers used to obtain dendronized polymers by radical polymerization [30, 31, 32]

In both ‘graft from’ and ‘graft to’ approaches, the parent polymer chain is synthesized firstly and then dendron attachment are growth takes place. “Graft-from” approach involves a step wise growth of dendron from the polymer backbone after the attachment of G1 dendron onto polymer backbone (Figure 1.16) [23]. Dendron synthesis is

based on the divergent route on the polymer backbone. Although this approach seems to be challenging given the likelihood of defects in the structure, it is also utilized to gain maximum degree of dendronization. Figure 1.17 [40] and Figure 1.18 [41] shows examples of dendronized polymer by graft-from strategy.

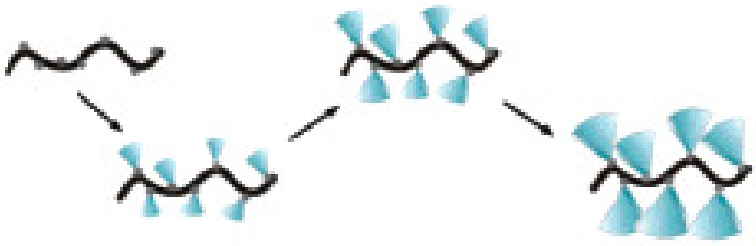

Figure 1.16. General scheme of graft-from method [23]

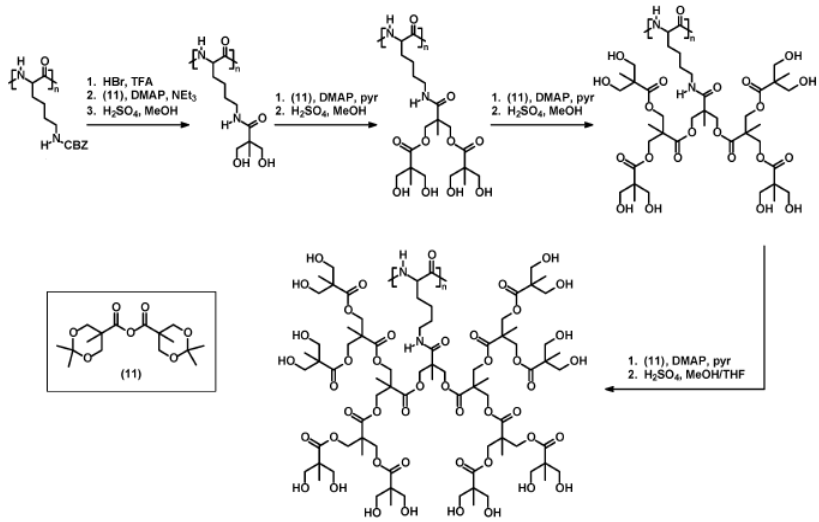

Figure 1.17. Synthesis of dendronized polymer by graft-from method [40]

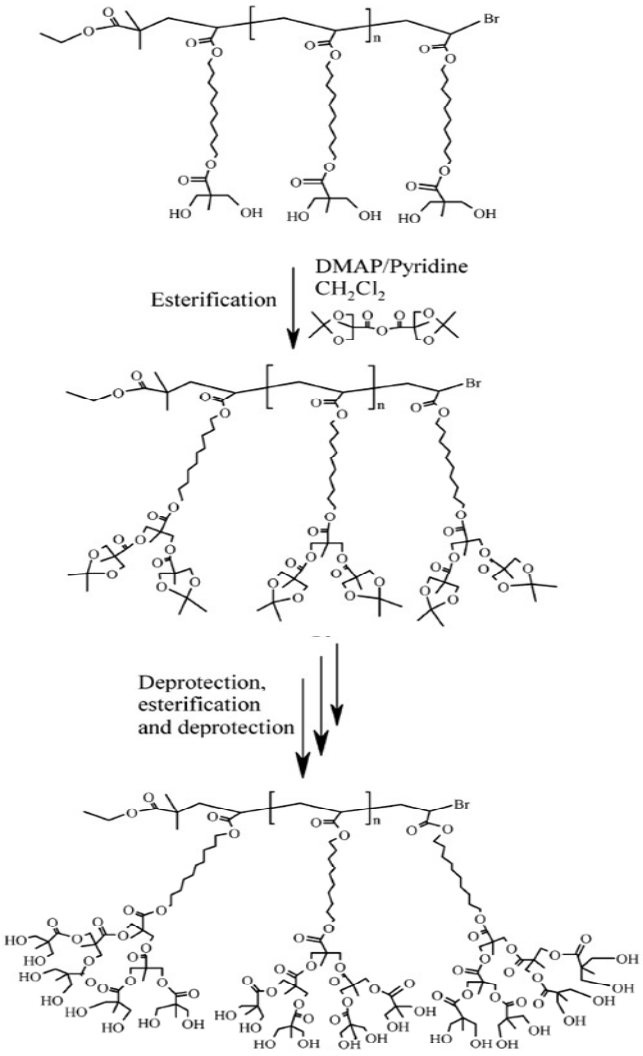

Figure 1.18. Synthesis of Polyester dendron based polymers by graft-from method [41]

On the other hand, in ‘graft-to’ approach, a preformed dendron is coupled to a polymer that contains pendant groups for attachment (Figure 1.19) [23]. Dendron sytnesis is based on the convergent route. This approach has the attractiveness of a modular ‘plug and play’ type interaction. Polymers with reactive side chains enables the generation of a family of polymers with the same backbone. According to the quantity of reactive groups at the backbone of the polymer, the appended side chain concentration can be adjusted as interested. In case of high generation de of the backbone is not achieved easily.

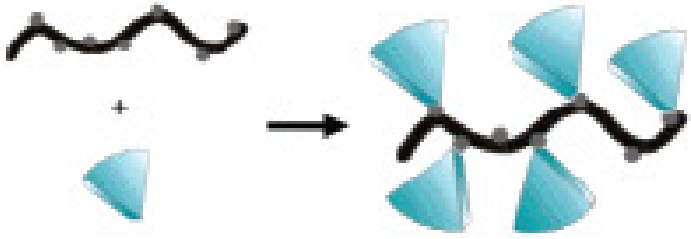

Figure 1.19. General scheme of graft-to method [23]

Perhaps the most widely used approach utilizes polymers containing N-hydroxysuccinimide based activated ester groups that can be easily functionalized with amine containing molecules for attachment through amide bond formation. Indeed, Hawker and coworkers evaluated the efficiency of polymers containing N-hydroxysuccinimide groups as side chains to obtain dendron grafted polymers by reacting them with dendrons containing amine groups at their focal points in Figure 1.20 [42]. Efficiency of such strategies depends upon efficient organic transformations that allow facile functionalization of the polymer backbone [43].

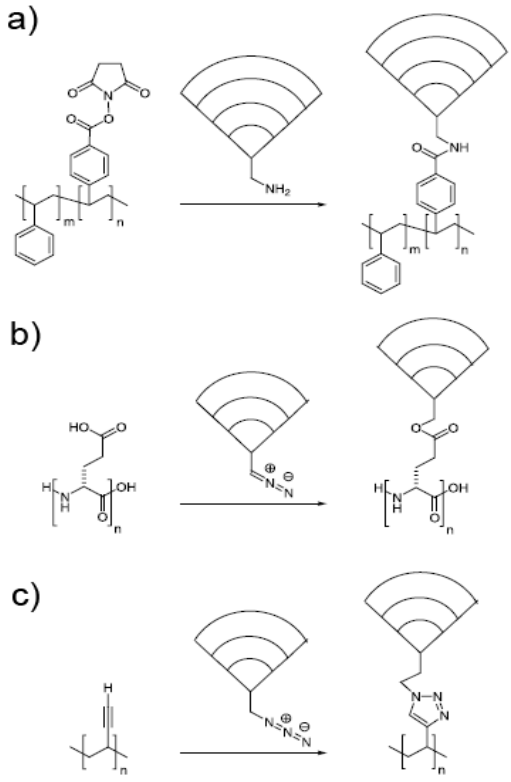

Figure 1.20. Examples of dendronized polymers provided by graft-to method a) amidation, b) esterification [44], c) copper catalyzed click reaction [45]

"Click reactions" have been utilized for achieving such efficient transformations due to their high reaction yields and mild reaction conditions. Recently, the copper catalyzed [3 + 2] Huisgen type cycloaddition reaction was utilized to obtain "dendronized polymer" with impressive effectiveness. In the same manner, the Diels-Alder cycloaddition reaction has also attracted considerable interest due to its "reagent free" reaction conditions. A recent study from our research group introduced a modular approach towards the synthesis of polymers dendron conjugates (Figure 1.21). 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 [46].

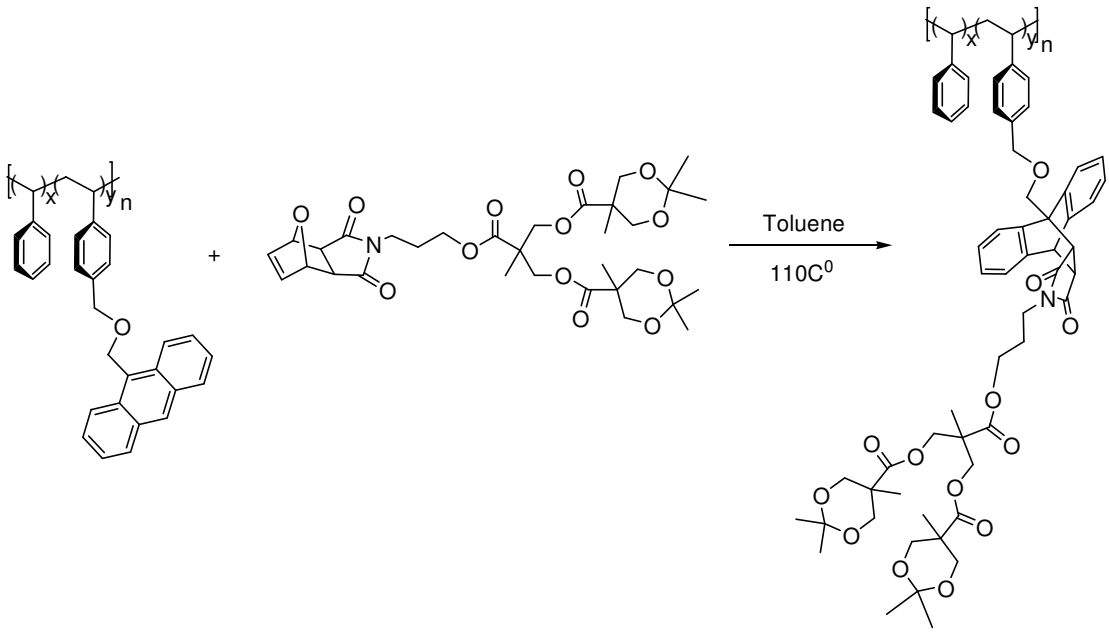

Figure 1.21. Synthesis of dendronized polymer via Diels Alder reaction [46]

1.4.1. Dendron-Polymer Based Functional Copolymers

Being attached to the dendrimers, polymers became very branched compact architectures with the dendrons mostly looking towards the outside of this structure. The reactive end groups at the periphery of dendrons are available for further functionalization a variety of small molecules of interest. The ability to conjugate especially therapeutic agents and proteins makes dendronized polymers very attractive polymeric scaffolds for bioapplications.

Apart from the attachment of dendrons to the pendant groups of the linear polymer, dendronized polymers can also be achieved by the addition of dendrons to the ends of the linear polymer. These types of block copolymers are mostly synthesized by 'graft-to' approach which includes the preparation of polymer and dendron parts separately. For the synthesis of this type of di or triblock copolymers, the polymer used should have reactive end groups to interact with the focal point of the dendron to be attached. Also the polymerization starts from the focal point of the dendron. Here, besides the functional groups on the surface of the dendron, the free end of the polymer can also be attached to a small molecule such as drugs and proteins or it is coupled to another dendron to yield a triblock dendritic polymer (Figure 1.22).

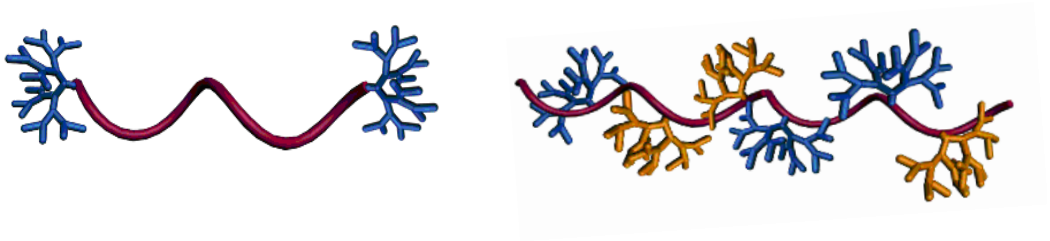

Figure 1.22. General scheme of end and side chain functionalized dendritic polymers

In 2001, Jean M. J. Frechet reported the polymer dendron conjugate synthesis. One, two, or four dendron bearing polymers are synthesized and this work is one of the pioneers in dendron- polymer conjugated copolymers (Figure 1.23) [47].

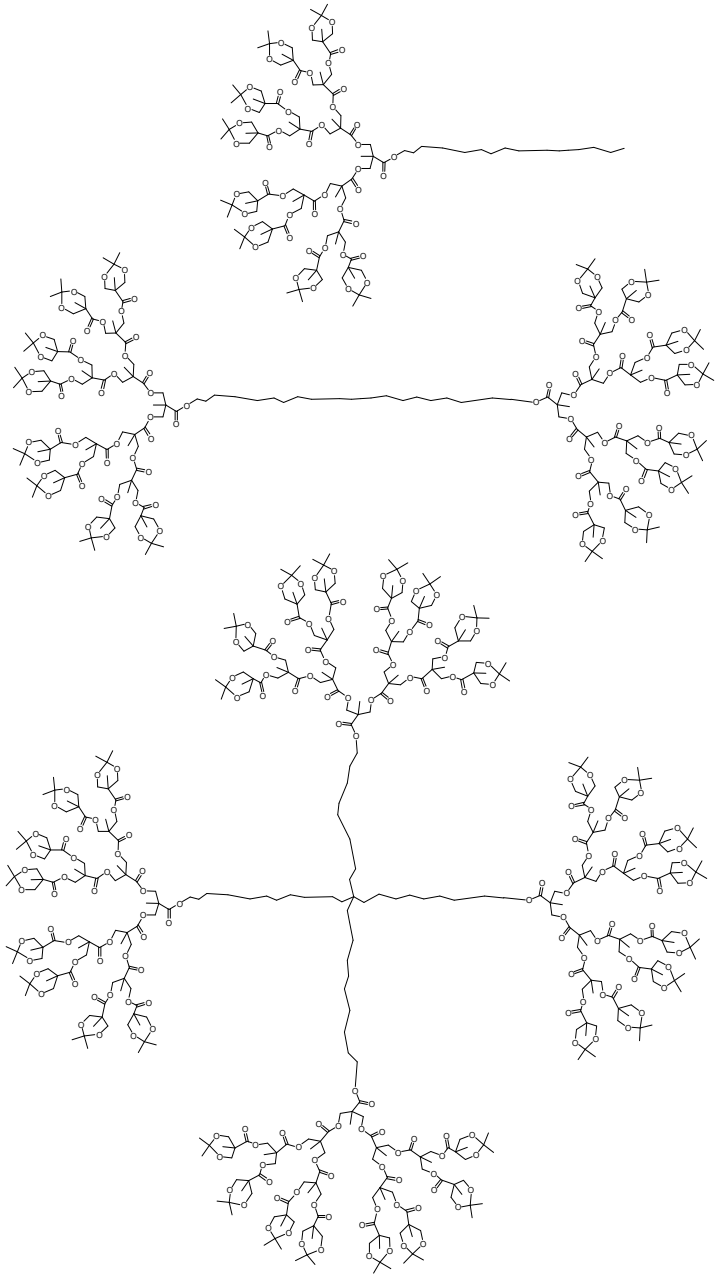

Figure 1.23. Synthesis of polymer-dendron conjugate [47]

It is crucial for the polymeric scaffolds that are aimed to be used in bioconjugation and drug delivery that they should have high water solubility. Poly(ethyleneglycol) (PEG) has been widely utilized to improve the water solubility and immunogenity. Bearing an antibiofouling property, it reduces the nonspecific interactions towards the proteins. It is used for many applications, due to its property of lowering toxicity and giving better biodistribution, to any molecule, polymer, or surface to which it is covalently bonded

In 2005, Gillies and Fre´chet published a paper revealing a new approach for the synthesis of dendron-polymer conjugates as drug carriers. They used a third generation polyester dendron and attached it to a PEG chain. Conjugated hydrophobic drug molecule to the end groups of the dendron are aimed to release under acidic conditions due to the hydrolysis of the ester groups in dendron structure (Figure 1.24) [48].

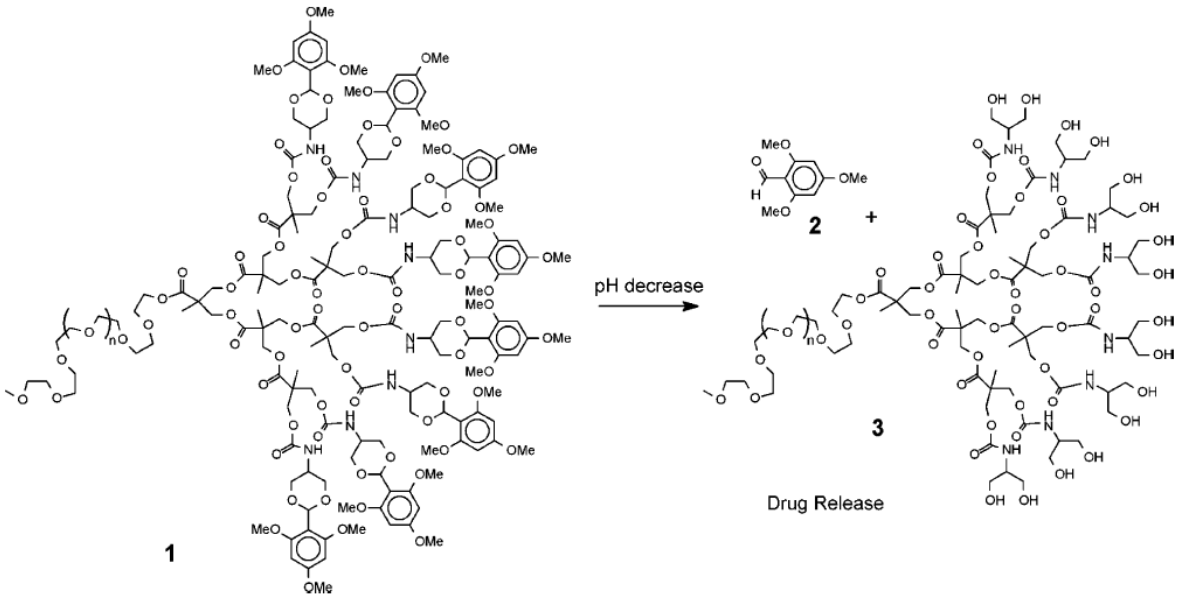

Figure 1.24. pH-responsive copolymers for controlled release of doxorubicin [48]

Ricardo Riguera published the synthesis of three generations of azido-terminated PEG-dendritic block copolymers. Mono functional PEG reacted with a G1 dendron and grown up to G3. The efficient surface functionalization of these dendritic polymers by means of copper (I)-catalyzed azide-alkyne [3+2] cycloaddition reaction (Figure 1.25) [49].

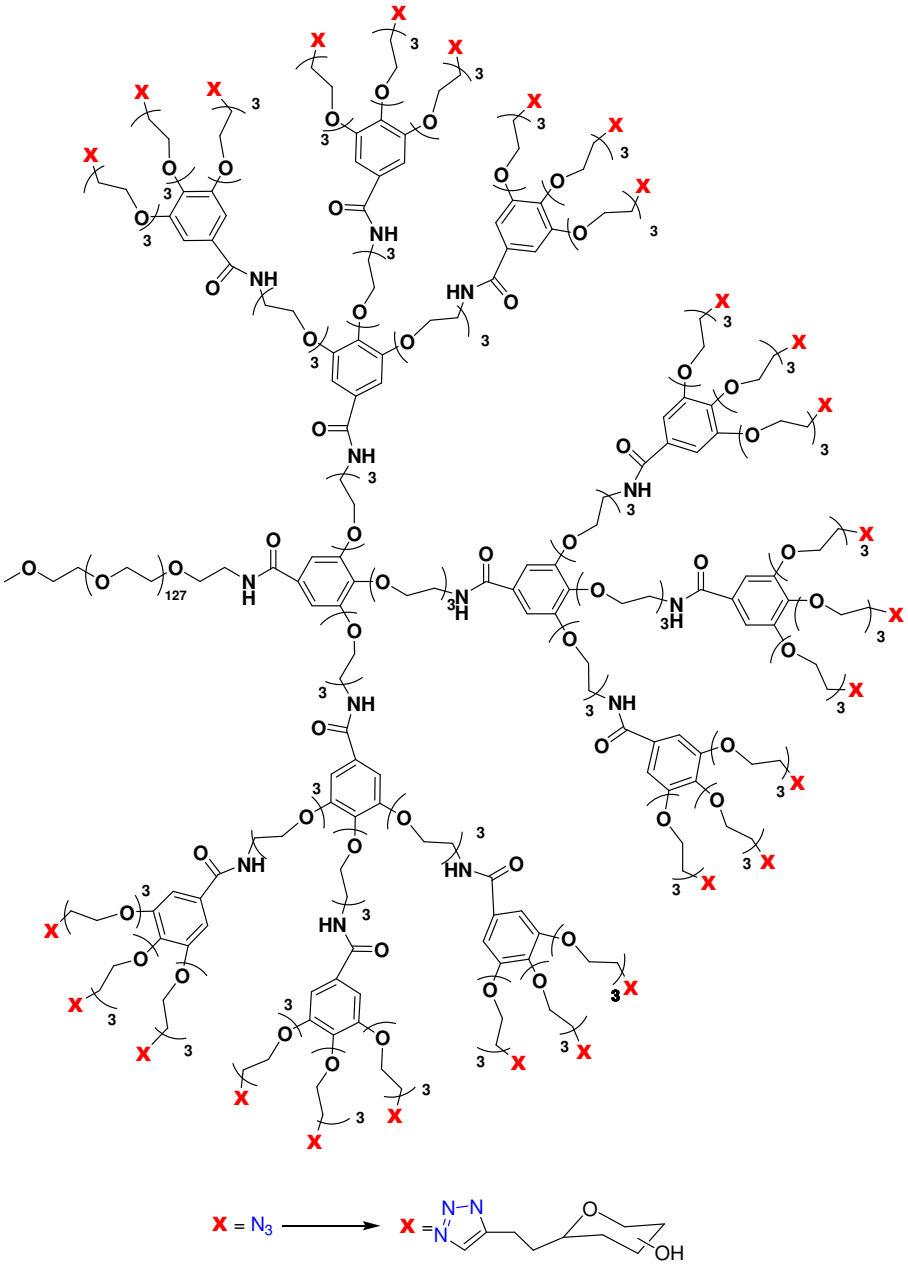

Figure 1.25. Synthesis of azido-terminated PEG-dendritic block copolymers [49]

1.5.Multiarm Star Polymers

Multiarm star polymers are three-dimensional compact macromolecules with many linear arms extending from the core (Figure 1.26) [50]. Linear arms of similar molecular weight and narrow molecular weight distribution placed around a central core, giving the star shape to the macromolecule. Star polymers have gained great attention as multi-armed macromolecules due to their outstanding bulk and solution properties. By using a multifunctional initiator core, the number of arms in the final star like scaffold can be pre-determined as interested and further functionalization of these groups permit additional modifications or cross-linking which are useable in both biotechnology and material sciences.

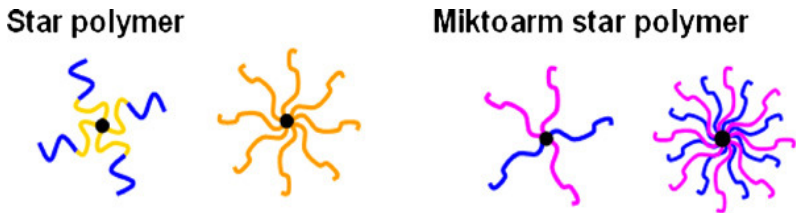

Figure 1.26. Categories of star polymers [50]

Until recently, living anionic [51] and cationic [52] polymerizations were the most widely used methods to obtain well defined multiarm star polymers. Recent advances in various living free radical polymerization techniques such as nitroxide mediated polymerizations (NMP) [53] and atom transfer radical polymerizations (ATRP) [54] have enriched the toolbox to access multiarm star polymers due to the wider functional group tolerance and less-stringent experimental conditions of these polymerizaion techniques.

There are two commonly used approaches to synthesize multiarm star polymers. For the *'arm-first'* method, end-reactive living polymers are synthesized first and then either quenched via a multifunctional coupling agent to form the core part or they bind to each other by difunctional small vinyl compounds or monomers. The other is the *'core first'* procedure which includes the synthesis of star polymer with the living polymerization initiated by a multifunctional core. Here, the resultant star polymer has arms with the same number of initiators in the structure of the core, which leads to more organized arms around the core.

Wiltshire and Qiao, in 2006, investigated the synthesis of core cross-linked star polymers (CCS) by 'arm first' method. ATRP of nondegradable monomers were combined with ROP of degradable lactone-based monomers. Hydrolysis degredable part was also studied to remove the labile component (Figure 1.27) [55].

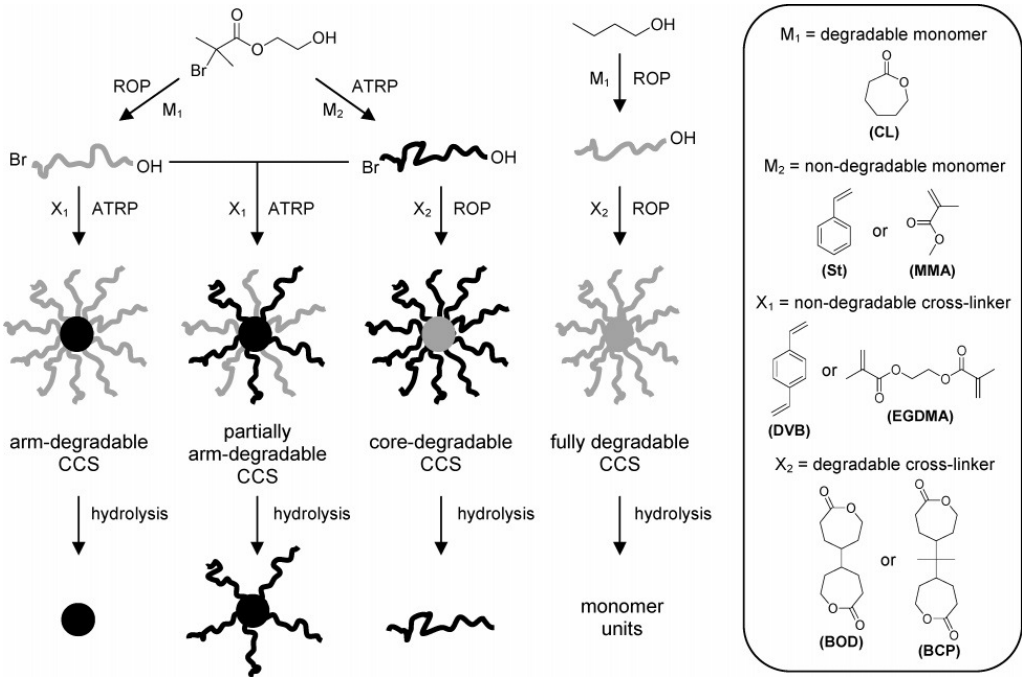

Figure 1.27. Generalized schematic of core cross-linked star polymer formation [55]

Liu et al. Presented a study involving the synthesis of mixed-arm star polymers ATRP and RAFT using the "core first" method. Here, Poly styrene was attached to a multifunctional a hyperbranched polyglycerol (HPG) core (Figure 1.28) [56].

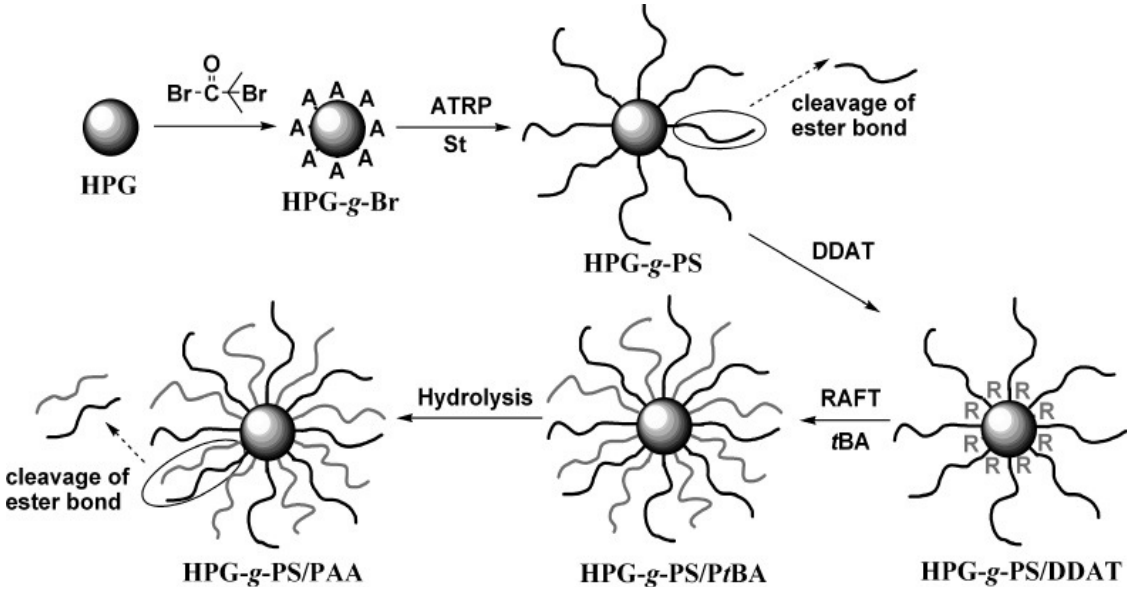

Figure 1.28. Synthetic procedure of HPG-*g*-PS/PtBA(PAA) mixed-arm star polymers and their cleavage by Sodium Hydroxide [56]

Furthermore, water soluble multiarm macromolecular scaffolds are attractive candidates for polymer conjugated drug delivery. Attachment of water soluble polyethylene glycol chains to drug molecules has been studied extensively both as dendritic structures and as linear scaffolds to increase bioavailability. Interestingly, the polymer drug conjugates with multiarm structures possessed longer circulation time compared to the linear polymers of the same molecular weight. [57, 58]

Schubert and his coworkers synthesized a new amphiphilic star-shaped architectures with dense hydrophilic shells by a combination of ROP and ATRP, leading to the formation of well-defined 4-, 6-, 8-, and 12-arm star PCL*p*PEGMAs. The presence of PEG makes the whole system a promising candidate for drug delivey (Figure 1.29) [59].

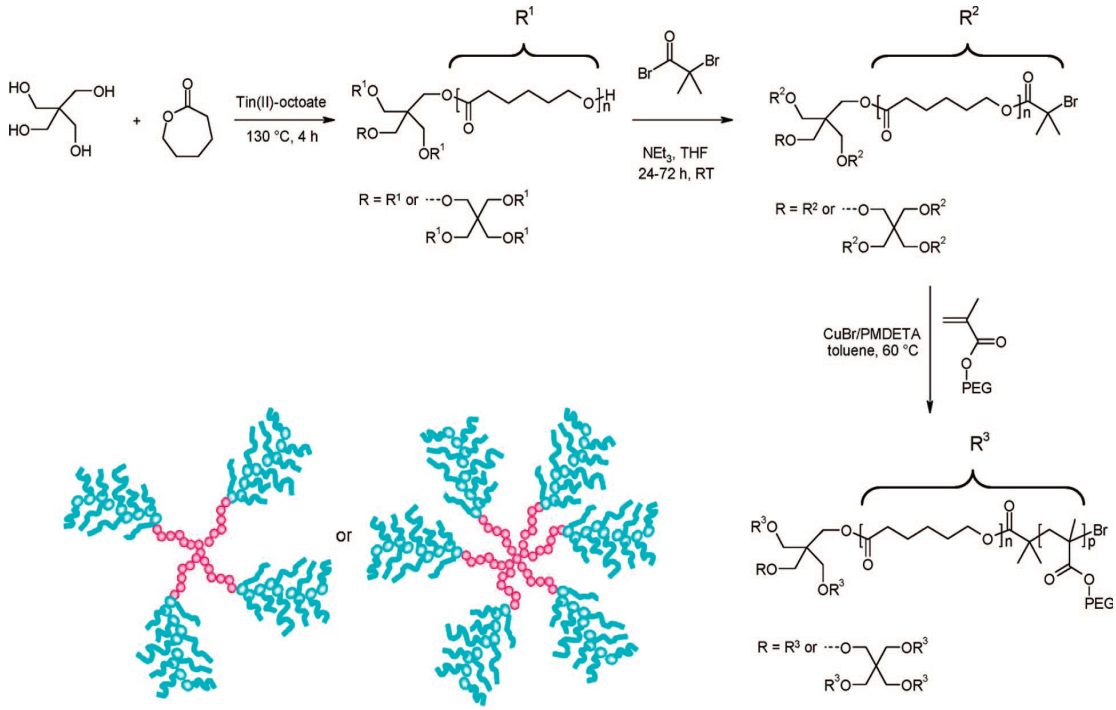

Figure 1.29. Star-Shaped Polymers using PEGylation [59]

Synthesis of multiarm star polymers bearing functional group at their core has been predominantly limited to the 'core-first' approach. By using a multifunctional initiator core, the number of arms in the final star like scaffold can be controlled. An alernative approach to obtain multiarm star polymers appended with molecules of interest at their core relies on conjugation of the desired molecule onto the reactive core of the multiarm star polymer. The efficiency of this conjugation would rely upon the effectiveness of the conjugation chemistry used for attachment to the reactive core.

In 2006, published a paper revealing the synthesis of core-functionalized star polymers with high star yield and narrow MWD using the MM method. Here, two kinds of functionalities, pyrene groups and hydroxyl groups, were introduced into the star core with high encapsulation efficiency through the use of functional ATRP initiators (Figure 1.30) [60].

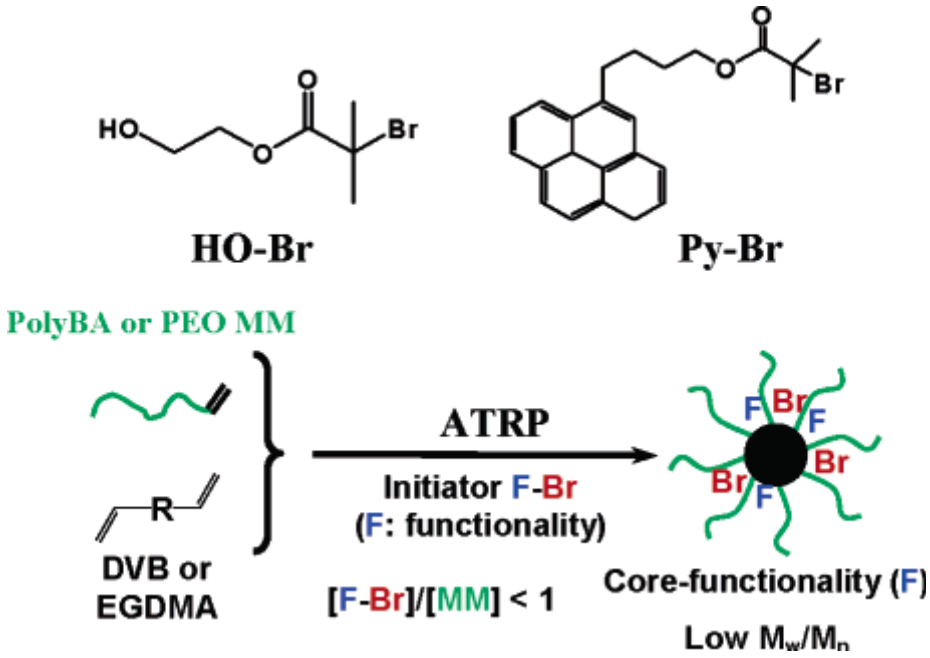

Figure 1.30. Synthesis of core-functionalized star polymers [60]

'Click' reactions have emerged as a desirable methodology for synthesis of polymeric materials such as the block copolymers, the complex macromolecular structures and post polymerization transformations due to their high efficiency under mild reaction conditions. In the literature, there are a lot of studies that examines Cu(I)-catalyzed [3 + 2] Huisgen and [4 + 2] Diels-Alder reactions to synthesize multiarm star polymers.

Also Tunca and coworkers are considerably interested in click reactions to get multimeric star polymers. One of their works is mainly based on the Diels Alder reaction between anthracene and maleimide groups. The study includes two types of multiarm star block copolymers: (PS)m-polyDVB-(PMMA)n and (PS)m-polyDVB-(PtBA)k prepared by ''arm-first'' methodology (Figure 1.31) [61].

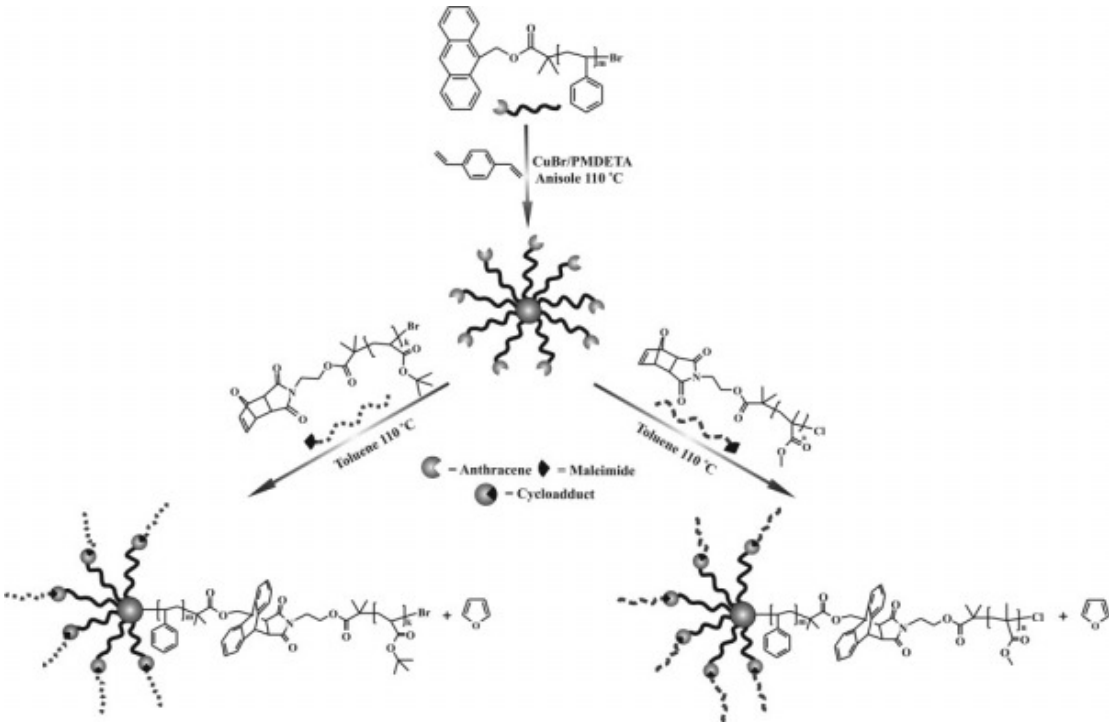

Figure 1.31. Synthesis of multiarm star polymers based on Diels Alder reaction [61]

2. AIM OF THE STUDY

2.1. Dendron-Polymer Conjugates for Drug Delivery

Polyethylene glycol (PEG) improves the biocompatibility, hydrophilicity and increased circulation time of the resultant drug conjugate in the body. On the other side, its limited loading capacity can be increased by the incorparation of dendrimers into the system. So dendrimeric polymer scaffolds being multivalent and monodisperse are highly desirable. More functional groups provided by dendrimers leads to an increase in the amount of drug molecules loaded into the water soluble polymeric supports. Hence the pharmacokinetic properties of drug are expected to be improved. The aim of this study is to illustrate a new methodology towards ABA type triblock copolymers that act as soluble supports for drug delivery. Telechelic PEG polymers with maleimide end groups were synthesized and reacted with dendrons containing anthracene fuctionality at their focal point via Diels-Alder cycloaddition reaction to obtain multivalent dendron polymer-

dendron conjugates. Thereafter the periphery of the dendrons are functionalized by alkyne groups which then reacts with an azide containing antiretroviral drug, AZT via Huisgen type 'click' reaction. (Figure 2.1).

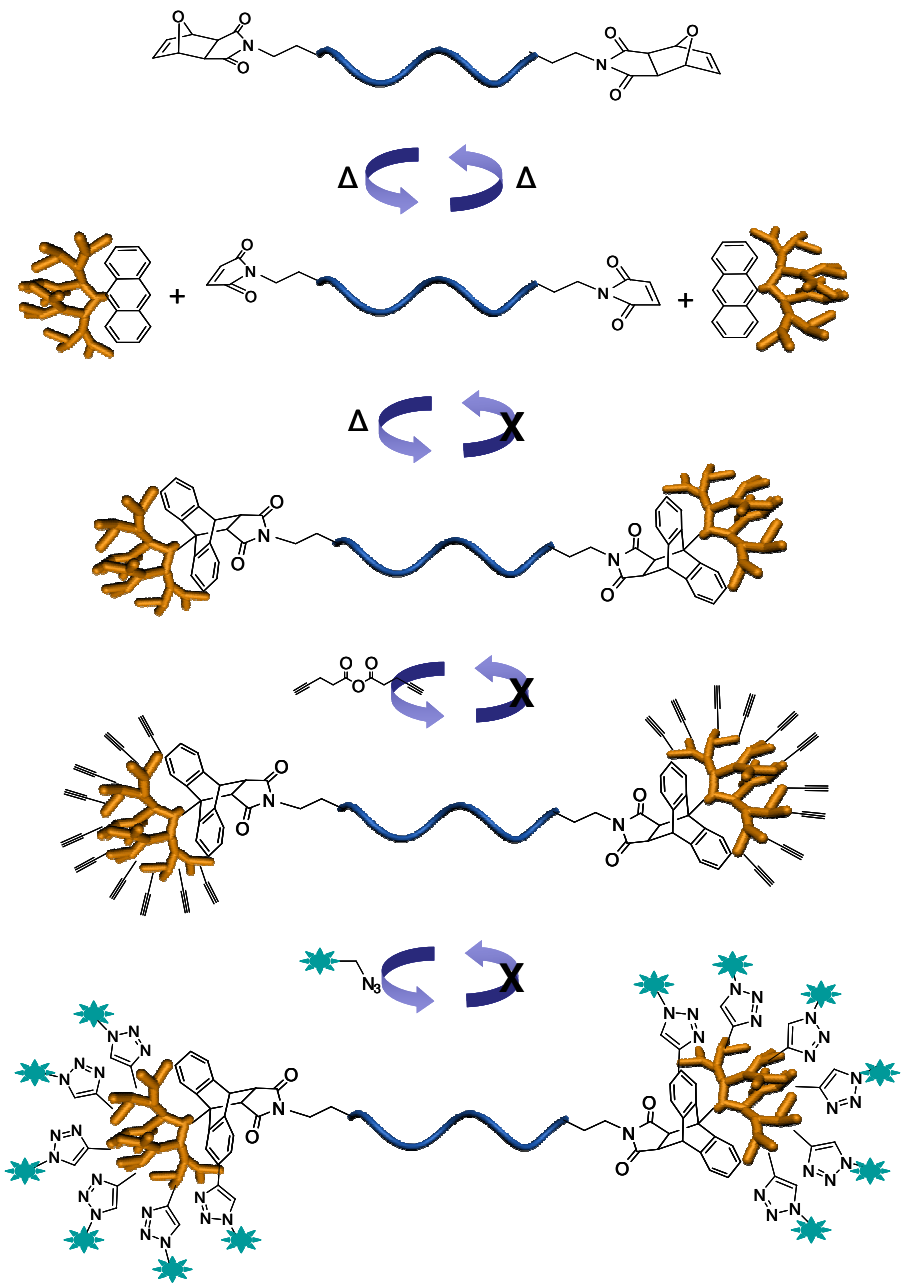

Figure 2.1. Synthetic approach for drug conjugated dendron-polymer copolymers

2.2. Thiol Reactive Multiarm Star Polymers

Multi-armed star polymers possess interesting solution properties that make them attractive candidates for polymer conjugated drug delivery. In this study, a Diels-Alder/retro Diels-Alder (rDA) reaction based strategy is utilized to synthesize multiarm polymers containing a thiol-reactive maleimide functional group at the focal point. Incorporation of dendritic initiators provide more compact three dimentional structure and the resultant multiarm star polymers possessed longer circulation time compared to the linear polymers of the same molecular weight. Maleimide functionality comes from masked maleimide based dendritic initiators with which polymerization from the core with various methacrylate and acrylate monomers is successfully carried out. Afterwards, retro Diels-Alder reaction is examined to deprotect the maleimide unit at the focal point, whose reactivity is then verified by the conjugation of a thiol containing tripeptide, glutathione via Michael addition under reagent free conditions (Figure 2.2).

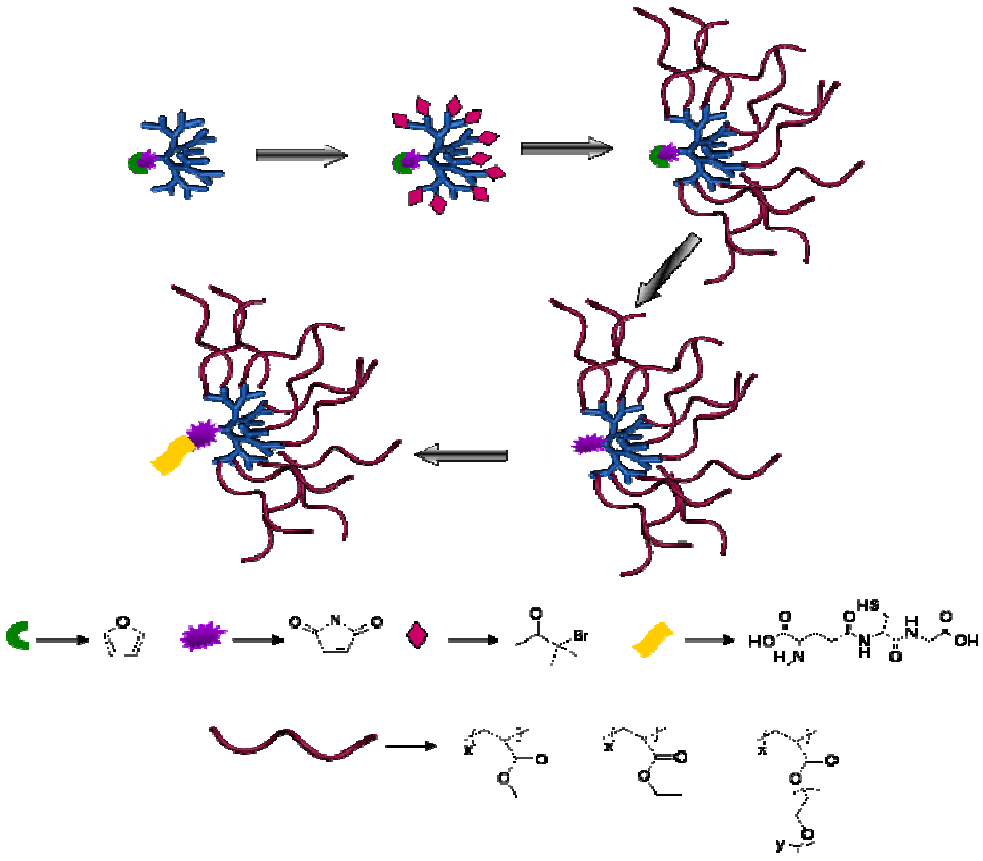

Figure 2.2. Synthetic approach for core reactive multiarm star polymers.

3. RESULTS AND DISCUSSION

3.1. Dendron-Polymer Conjugates

Biocompatible hydrophilic linear PEG polymers are end-functionalized by maleimide groups and reacted with biodegradable polyester dendrons bearing anthracene functionality at the core via Diels Alder 'click' chemistry. Further functionalization of the periphery of the dendrons with alkyne groups facilitates the attachment of a drug molecule (AZT, antiretroviral drug) having an azide group via Huisgen type 'click' reaction. Synthesis of dendron-polymer-dendron ABA triblock copolymers via Diels Alder reaction, their functionalization with alkyne groups and drug attachment to the periphery are shown in Figure 3.1.

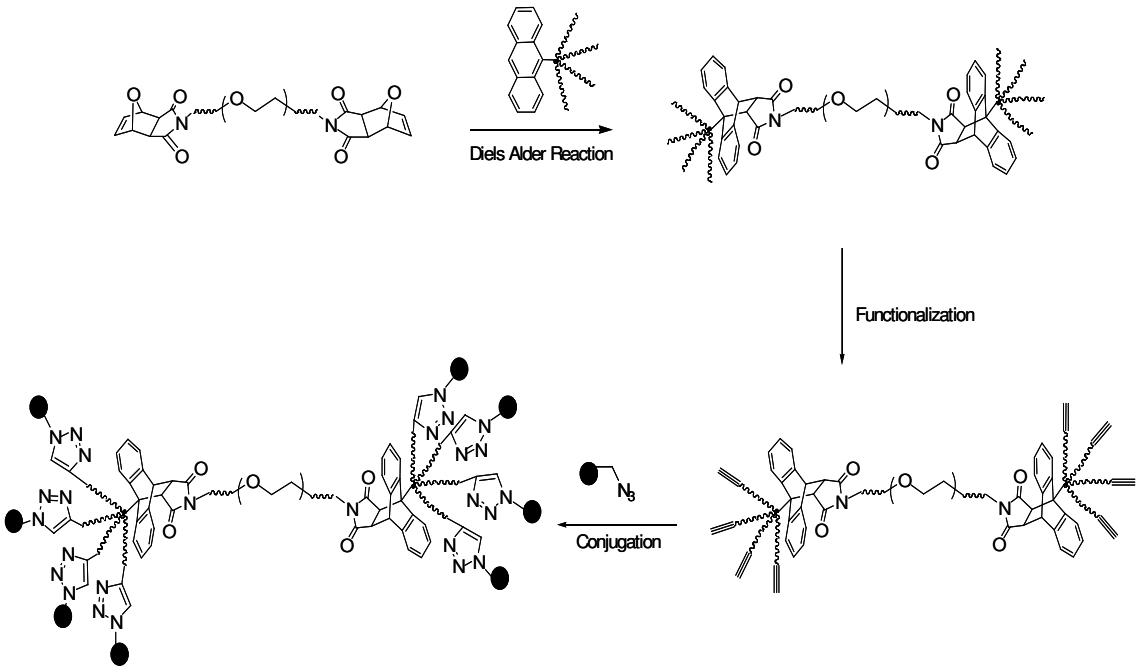

Figure 3.1. The synthesis of drug conjugated dendron polymer copolymers

Dendron-polymer conjugates are designed as diblock (AB) and triblock (ABA) copolymers. The polymer part involves PEG (Poly (ethylene) glycol) as its homo and hetero telechelic structures. Length of these PEG polymers that are explored in this study is 2K. For the dendron part, anthracene core bearing polyester dendrons of three generations are synthesized.

Since the synthesized multifunctional polymeric scaffold is aimed to be used as a carrier for an antiretroviral drug, the copolymer to which drug is attached should be water soluble. Due to its hydrophilic structure and biocompatibility, Poly (ethylene glycol) (PEG) is preferred. Its presence makes the whole system water soluble. Moreover, incorporation of PEG prevents bioadhesion due to its antibiofouling property. This protein resistivity comes from the steric repulsion between hydrated PEG chains and proteins.

For triblock copolymer synthesis, PEG, as the interior part, is end-functionalized by furan protected maleimide groups. Firstly, end groups of PEG are converted to carboxylic acid from alcohol so that via EDCI coupling, PEG can react with an alcohol bearing a protected maleimide group. The resultant polymer is called as bis-maleimide PEG. Instead of PEG diol, if monomethoxy PEG is used, then it ends up with furan masked maleimide group at one end, which is called as mono-maleimide PEG. Figure 3.2 shows the details of this synthesis.

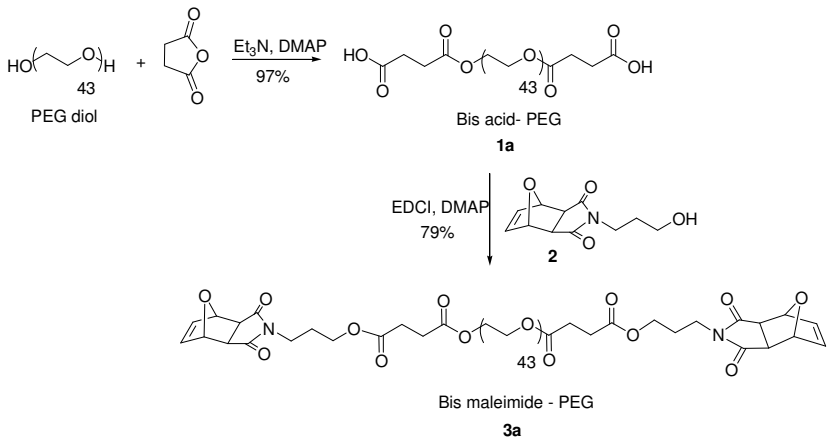

Figure 3.2. Synthesis of Bis-maleimide PEG

Peaks at 6.48 and 5.23 ppm corresponding to the oxabicyclic moiety observed in ^{1}H NMR analysis proved the presence of the maleimide group in the structure of bis-maleimide PEG (Figure 3.3, peaks h&g respectively).

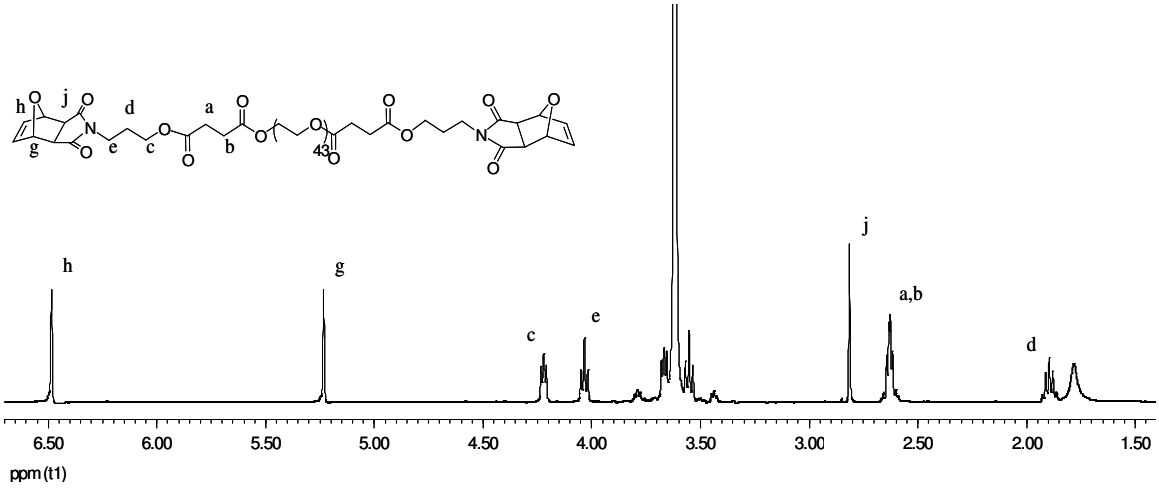

Figure 3.3. ^{1}H NMR spectrum of Bis-maleimide PEG

A block of the copolymers is choosen as an aliphatic polyester dendron with anthracene group at the core. Ester groups in dendron structure makes its hydrolysis easier in aqeuous media so the A block of the copolymers become biodegredable. Dendrons are synthesized by the convergent method, meaning that the growth of the repeating units of the dendron starts from the anthracene core towards the periphery. The repeating unit (4) is synthesized and converted to its anhydride form (5) according to literature protocols [62,63]. Its reaction with 9-Anthracene methanol ends up with 1st generation dendron bearing anthracene group at its core. The acetonide groups of this G1 dendron is achieved via treatment with DOWEX, H^{+} which yiels free alcohol groups to continue the syhthesis of 2nd and 3rd generation dendrons in the same manner (Figure 3.4).

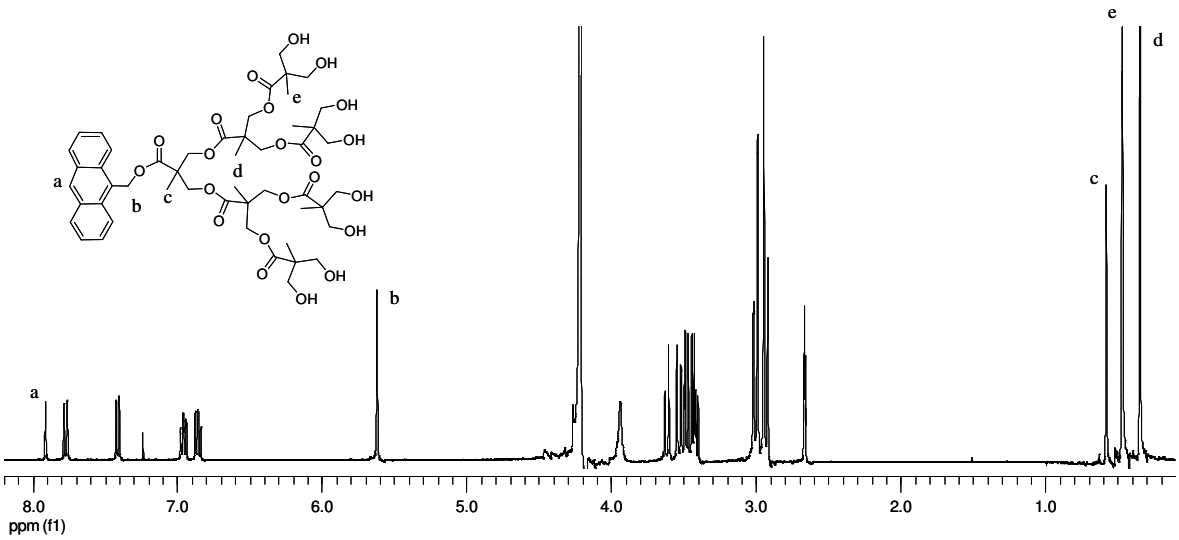

Figure 3.4. ^{1}H NMR spectrum of G3-Anthracene Dendron

3.1.1. Diels-Alder Reaction of Dendrons and Polymers

Diblock and triblock copolymers are synthesized by Diels Alder reaction for which maleimide group at the end of PEG polymer acts as a diene and the dienophile is the polyester dendron with an anthracene moiety at the core. Diels Alder reaction takes place under reagent free conditions with a high yield. Since maleimide group in the structure of bis-maleimide PEG is protected by furan, it should be deprotected to be activated for further reactions. Activation of maleimide and its reaction with anthracene are done in one step due to the reversibility of Diels Alder reaction between furan and maleimide at elevated temperatures. So, during the synthesis of copolymer (110 ^{o}C), maleimide that is unmasked via retro Diels-Alder reaction, irreversibly reacts with anthracene group. These dendrionized polymers are named according to the dendron generations used: i.e. G2-PEG represents a diblock copolymer with G2 dendron and G2-PEG-G2 refers to its triblock form.

Diels Alder reaction between furan masked maleimide and anthracene groups results in disappearance of peaks at 6.48 and 5.23 ppm belonging to furan moiety. Peak at 7.91 ppm coming from the hydrogen at the first position of anthracene is disappeared and also

other characteristic aromatic proton peaks of anthracene betweeen 7.3 and 8.4 ppm completely disappeared. After cycloaddition reaction, proton resonances from the anthracene unit at 5.56-5.35 ppm (m, 2H, $CH_2OC=O$) and 4.72 (s, 1H, CH bridgehead protons) are shifted to downfield. (Figure 3.5, peak f&g, respectively).

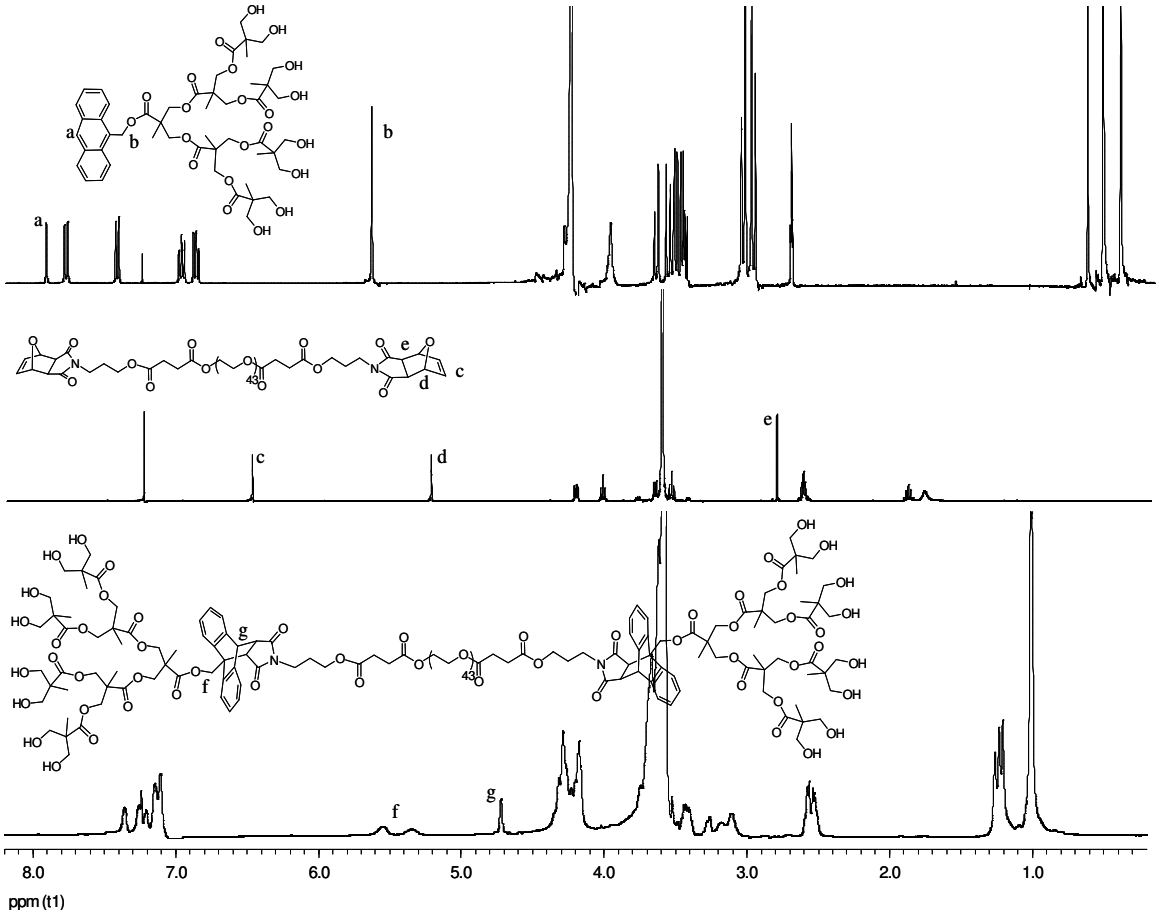

Figure 3.5. ^{1}H NMR spectra of 3a, 9c and P6

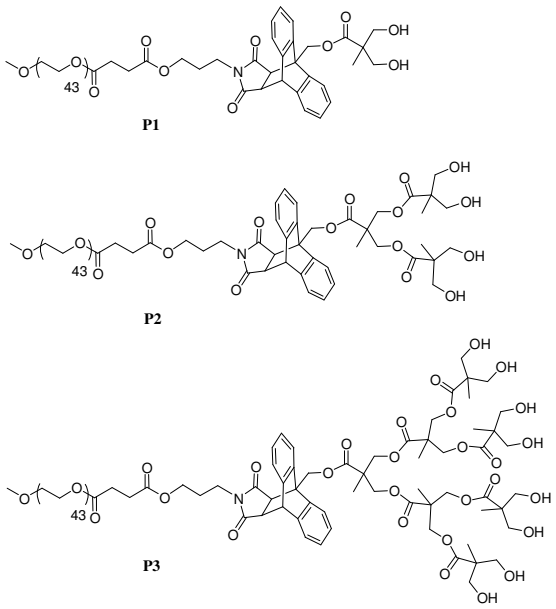

Figure 3.6. Synthesized diblock copolymers

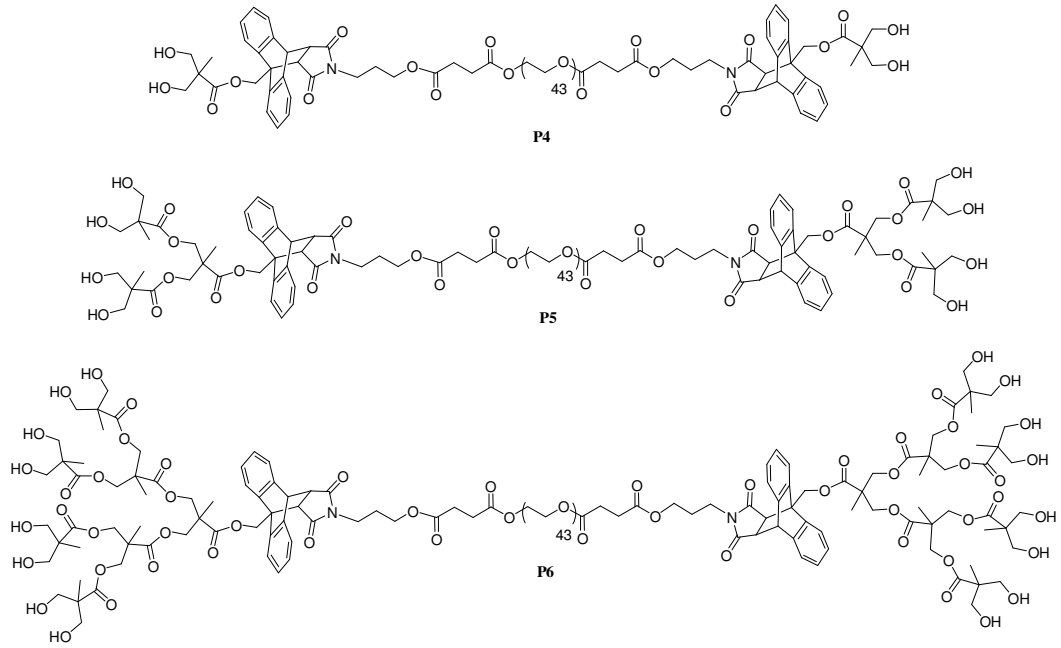

Figure 3.7. Synthesized triblock copolymers

Diels Alder reaction is monitored by GPC traces of Dendron-polymer copolymers and their starting materials. It is evident from the GPC traces that molecular weight of dendronized polymers increases upon attachment of higher generation dendrons (Figure 3.8).

Table 3.1 shows a summary of characterization data of Diels Alder products that are synthesized and the results obtained. In all cycloaddition reactions, mono-maleimide PEG: Anthracene dendron 1:1.05 and Bis-maleimide PEG: Anthracene dendron 1:2.10 ratios have been used. It has been observed that the cycloaddition reaction is fast enough that it completed within 6 hours. Polydispersity values increases as the generation of the dendron increases, which may be due to the increase in the branching going from G1 dendron to G3 dendron.

Table 3.1. Synthesis and Characterization of Dendron-Polymer Copolymers

Entry	Polymer[a]	Time (h)	Temp. ($^{\circ}$C)	Yield (%)	GPC[b]		$M_{n,\,theo}$
					M_n (g/mol)	M_w/M_n	
1	PEG-G1	6	110	84	3160	1.15	2560
2	PEG-G2	6	110	79	3440	1.15	2800
3	PEG-G3	6	110	77	3600	1.30	3260
4	G1-PEG-G1	6	110	96	3660	1.18	3120
5	G2-PEG-G2	6	110	83	4490	1.16	3590
6	G3-PEG-G3	6	110	84	4980	1.27	4520

[a] $[MI]_0$:[Dendron] = 1:1.05 (entries 1-3), 1:2.10 (entries 4-6). [b] Calibration with linear PS as standard. GPC samples are prepared in THF.

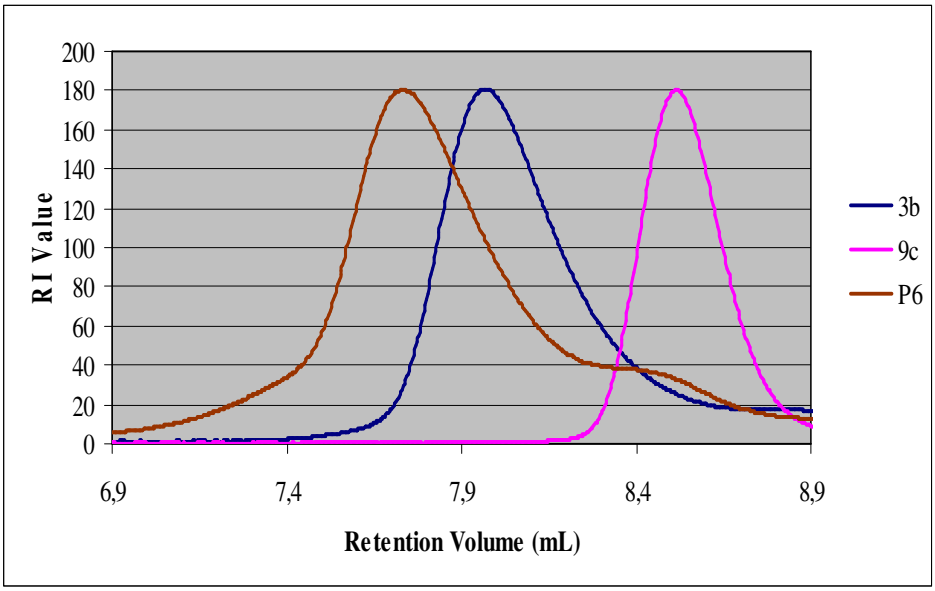

Figure 3.8. GPC traces of 3b, 9c and P6

Additon of dendrons to the ends of PEG during the DA reaction was monitored by UV spectroscopy where disappearance of characteristic finger like anthracene peaks at around 300-400 nm verifies the quantitative cycloadduct formation (Figure 3.9).

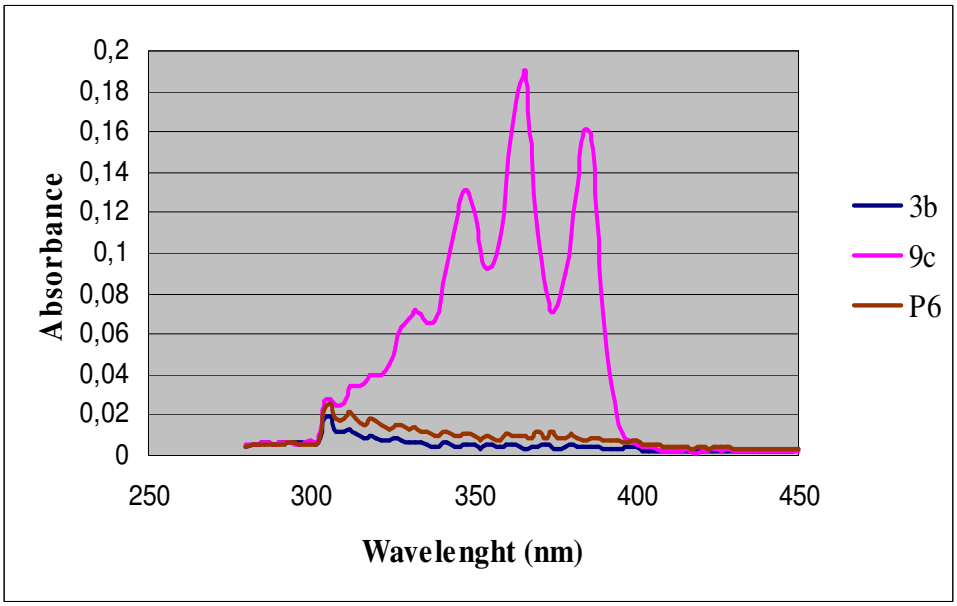

Figure 3.9. UV Visible absorption properties of 3a, 9c and P6

3.1.2. Activation of G2-PEG-G2

Functionalization of the alcohol groups at the periphery of the triblock copolymers were obtained via the acylation reaction with pentynoic anhydride in the presence of pyridine. This reaction yields alkyne functionalized ABA block copolymer (**P7**). Alkyne groups on the surface of the dendrons were now ready for the Huisgen type 'click" reaction to conjugate molecules of interest. ^{1}H NMR analysis shows that the triple bond addition was successfully achieved. Appearance of a new peak at 1.89 ppm (peak c, Figure 3.10.) proves the presence of triple bonds on the copolymer.

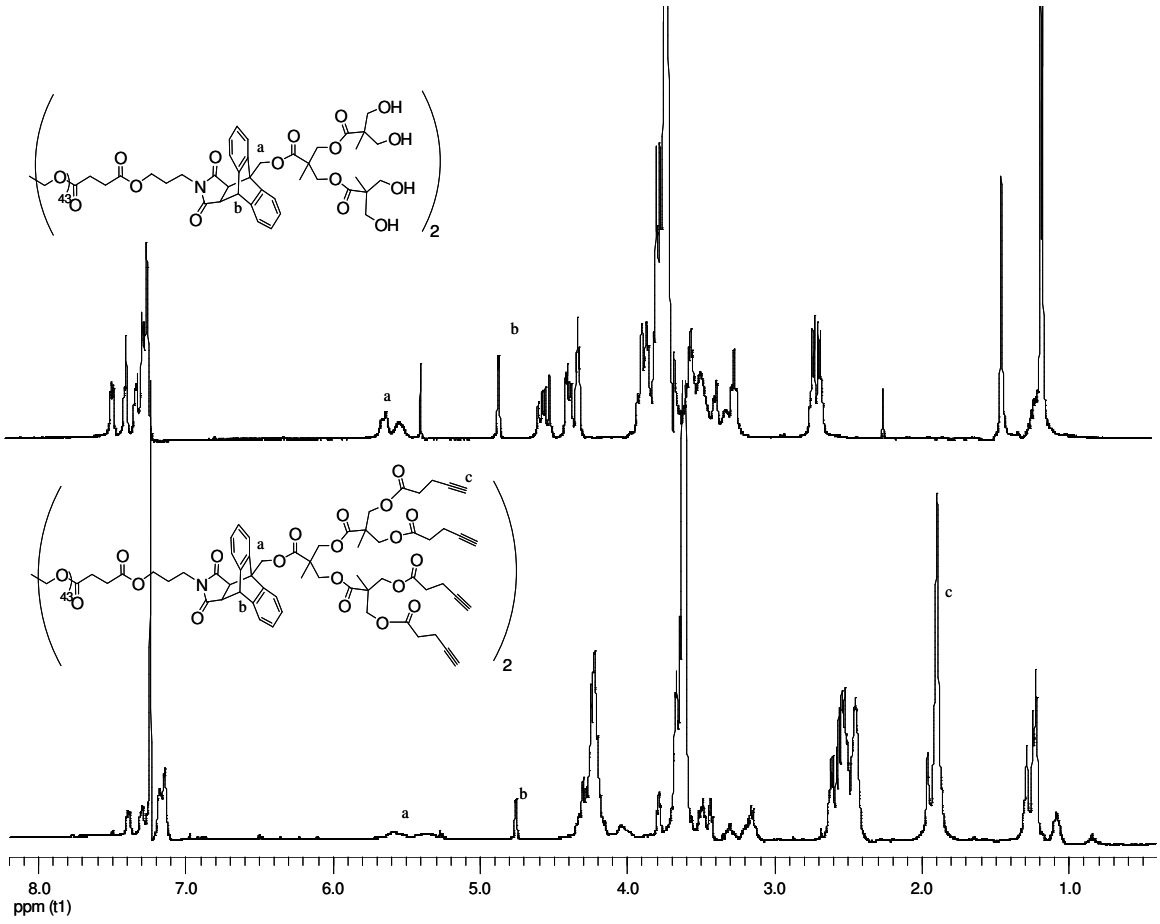

Figure 3.10. ^{1}H NMR spectra of Polymer 5 (a) and Polymer 7(b)

3.1.3. Conjugation with AZT

Conjugation of the drug molecule to the multivalent dendritic polymer support is achieved via Huisgen type 'click' reaction **(P8)**. AZT is antiretroviral drug having an azide functionality by means of which it is conjugated to the dendrionized polymers with alkyne functionality at the periphery. The attachment of drug molecules is monitored by ^{1}H NMR analysis (in DMSO). Appearance of a new peak at 8.37 ppm (peak a, Figure 3.11.) proves the presence of triazol ring on the copolymer. Also disappearance of peak belonging to terminal hydrogen of alkyne group at 1.89 ppm (peak, d, Figure 3.11.) indicates complete conjugation. Purification of the resultant drug-conjugated copolymer was not possible since by precipitating the polymer it is not possible to remove the excess unbound free drug could not be removed. Attempts to obtain pure polymer drug conjugates using methods such as membrane dialysis will be targeted.

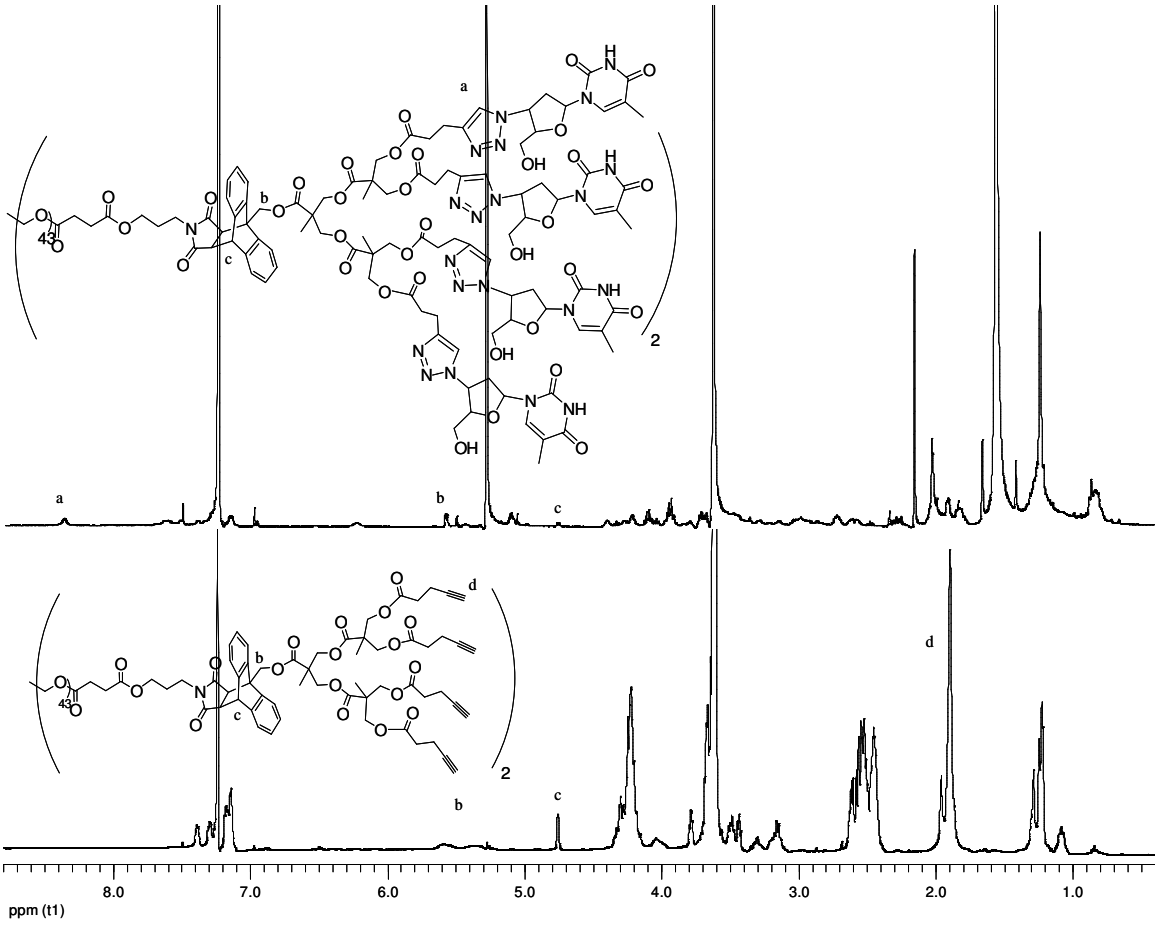

Figure 3.11. ^{1}H NMR spectra of polymer **P8** (a) and polymer **P7** (b)

3.2. Thiol Reactive Multiarm Star Polymers

The synthesis of multiarm star polymers bearing reactive maleimide core was synthesized using the Atom Transfer Radical Polymerization (ATRP) method. Post fuctionalization of these dendritic polymers was achieved by retro Diels-Alder reaction and then the activity of maleimide moiety at the core is verified by Michael Addition reaction with a thiol containing tripeptide, glutathione. Synthesis of multiarm star polymers via ATRP, activation of maleimide group and glutathione attachment to the core is shown in Figure 3.12.

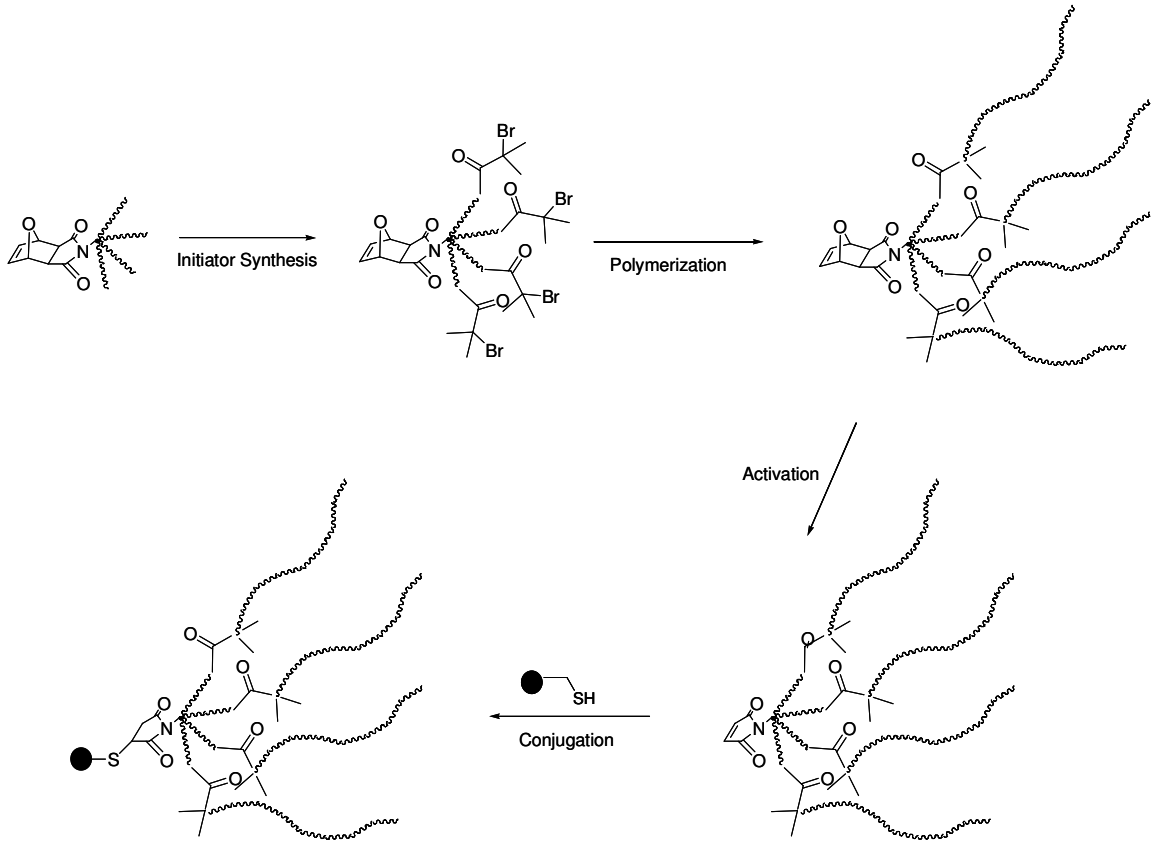

Figure 3.12. General scheme for the synthesis of multiarm star polymers

As the multiarm initiator for the polymerization, biodegradable polyester dendron with maleimide functionality at the core was chosen and three different generations of dendrons were synthesized according to the literature procedures [64]. The alcohol groups

at the periphery of the dendrons were reacted with a brominated compound so halogen groups needed to initiate the polymerizations were gained succssesfully.

To prevent the interference of double bond of maleimide group from participating into the polymerization reactions, starting from the dendron synthesis it is protected by a furan moiety. Acetal protected polyester dendrons **10-12** were prepared using a divergent growth synthetic strategy starting with a furan protected N-hydroxy propyl maleimide. The acetal protecting groups at the periphery of these dendrons were removed using the acidic resin DOWEX 50W-X2 to furnish the polyols. Subsequently, the hydroxyl groups at the periphery of the dendrons were esterified by 2-bromoisobutyryl bromide in the presence of triethyl amine to yield the multiarm initiators **13-15** in good yields (Figure 3.13).

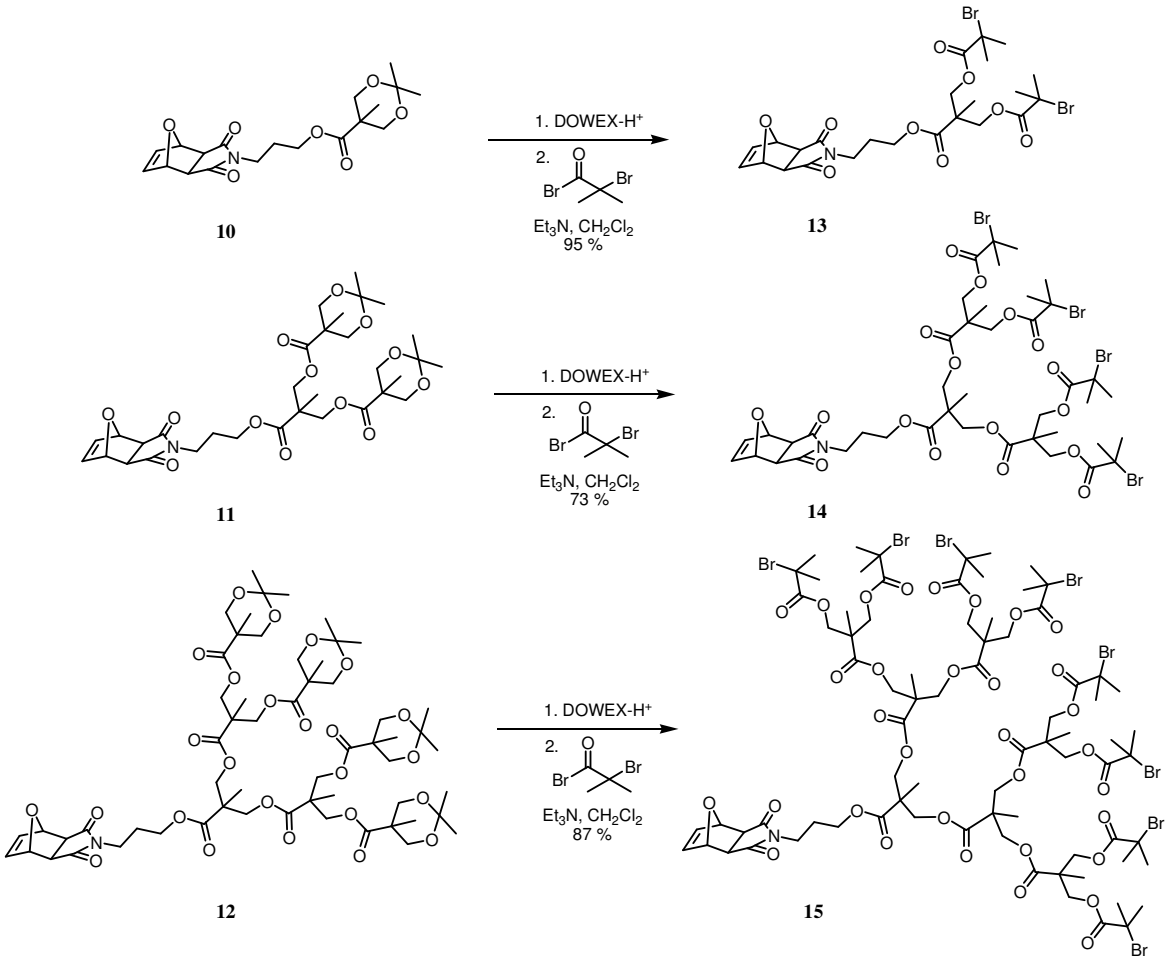

Figure 3.13. Synthesis of Maleimide based Initiators

The dendritic multiarm initiators were obtained in pure form after column chromatography. All dendritic initiators were characterized with ^{1}H NMR, ^{13}C NMR and elemental analysis for their structural assignments and purity. For example, the presence of the bicyclic unit composed of the furan protected maleimide unit was evident from the proton resonances at 2.83, 5.23 and 6.48 ppm for the second generation dendritic initiator **14** (Figure 3.14). The carbon NMR provides further support of the structure due to the presence of four different carbonyl peaks as expected. It is very important to obtain the multiarm initiators in very high purity in order to synthesize polymers with monomodal molecular weight distribution.

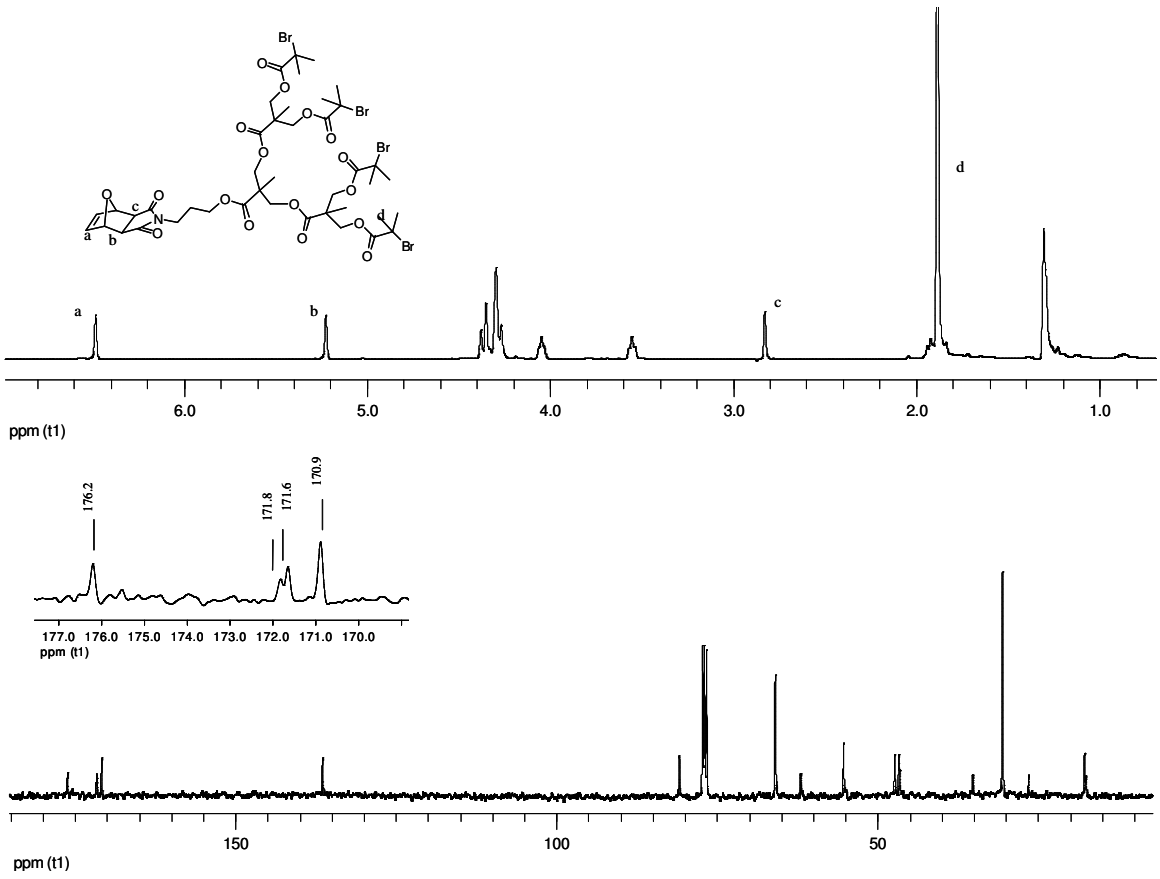

Figure 3.14. ^{1}H NMR and ^{13}C NMR spectra of G2-Initiator **(14)**

Especially for biological applications, it is important to utilize polymers with narrow polydisperties and known architectures. To have control over molecular weight distribution, a living free radical polymerization (ATRP) is chosen for obtaining desired polymers. As a controlled living polymerization method, ATRP is initiated by an alkyl halide (R-X) and catalyzed by a transition metal complex. In this study, dendritic initiators were used along with CuBr/PMDETA complex as the catalyst system. Reaction time and solvent ratio were adjusted for obtaining polymers with narrow polydispersities.

Initially, polymerizations with different monomers were investigated using the first two generations of the multiarm dendritic initiators containing furan protected maleimide unit at their core. Organosoluble monomers methyl methacrylate, tert-butyl acrylate and water soluble monomer polyethyleneglycol monomethylether methacrylate were utilized to obtain two and four arm polymers with masked maleimide cores (Figure 3.15). The third generation dendritic initiator is used for the polymerization of PEGMEMA monomer. Polymerization temperatures were kept at or below 70 ^{o}C to prevent any unmasking of the maleimide units at the core of the initiators. Up to 70 ^{o}C, maleimide group preserves its protected form as observed by ^{1}H NMR analysis of the obtained polymers. Reactions were terminated at low conversions in order to obtain polymers with well-defined arms and low polydispersity. A good correlation was observed between the molecular weights detected by size exclusion chromatography for the PMMA and PtBA polymers using PS calibrations as well as TD-GPC.

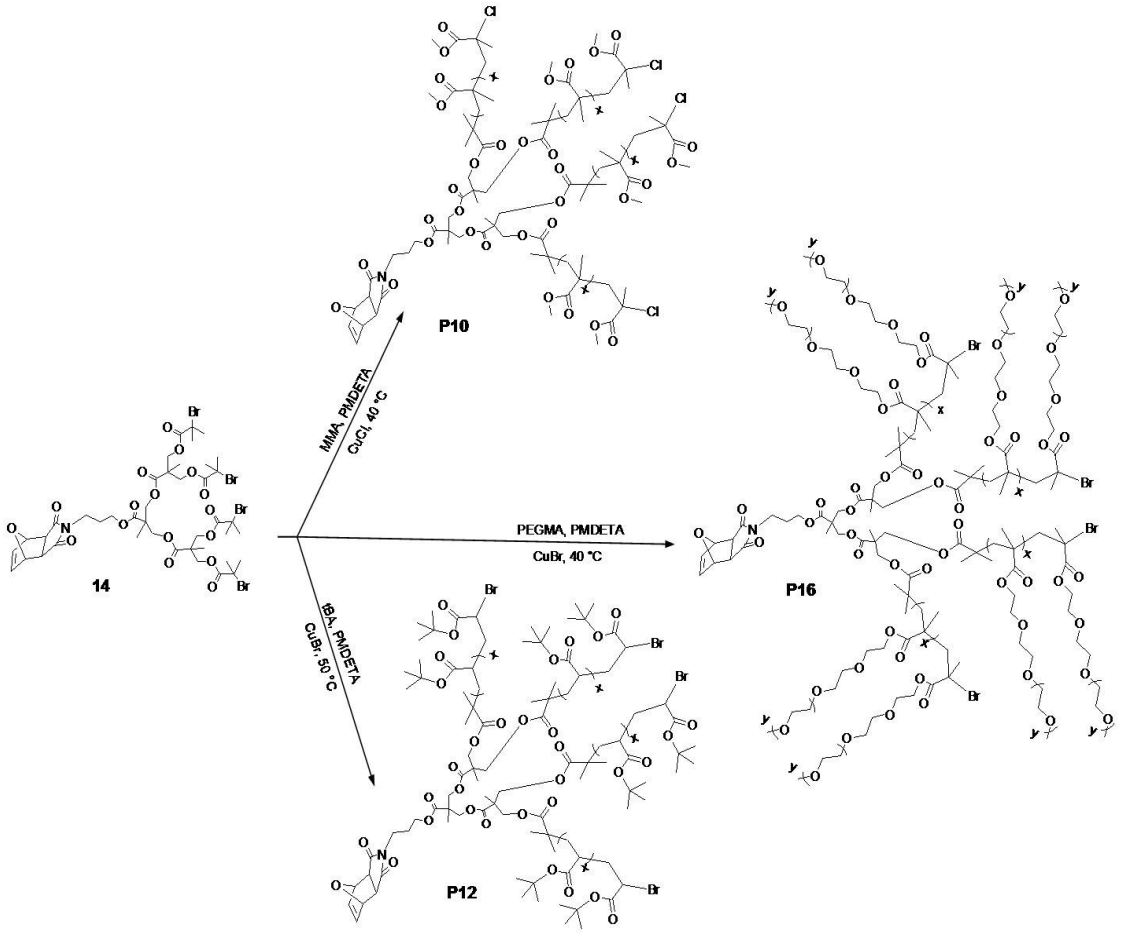

Figure 3.15. Synthesis of Protected-Core Polymers

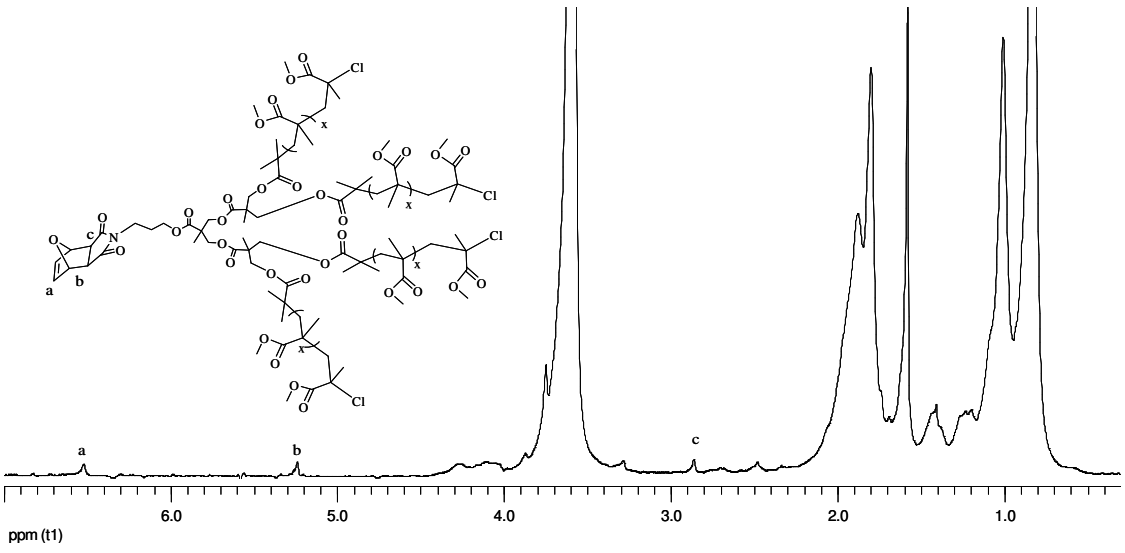

Figure 3.16. ^{1}H NMR spectrum of Polymer **10**

Since biological reactions takes place in aqueous media, the polymeric scaffolds aimed to be used for drug delivery, bioconjugation, etc. should have higher water solublity, which is mostly achieved by incorporation of PEG. In this study, water soluble multiarm star polymers with a reactive maleimide core were obtained by using polyethylene glycol monomethylether methacrylate (PEGMA) as the monomer. Polymers with low polydispersity and good conversions were obtained at 70 ^{o}C.

All polymers synthesized were characterized also with size exclusion chromatography. It is evident from the GPC results that as the generation of the dendritic initiator increases, the average molecular weights of the corresponding polymers synthesized at the same conditions are also increased (Figure 3.17). The PEG based polymers were not analyzed using the triple detection system due to their known affinity for the utilized size exclusion chromatography columns. The polymerization conditions and the results of ^{1}H NMR and TD-SEC analysis are given in Table 1.

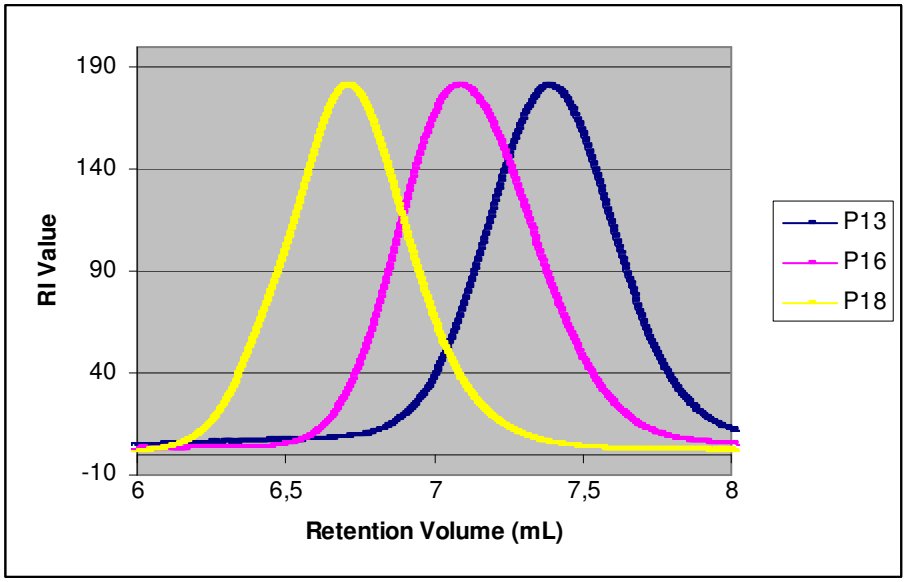

Figure 3.17. GPC traces of P13, 16 and 18

Table 3.2. Synthesis and Characterization of Maleimide-Core Polymers

Entry	Polymer[a]	$[M]_0/[I]_0$	[I]	Time (min)	Temp. (^oC)	Conv. (%)	GPC[b]		TD-GPC[c]		$M_{n,theo}$	$M_{n,NMR}$
							M_n (g/mol)	M_w/M_n	M_n (g/mol)	M_w (g/mol)		
9	G1-PMMA	200	**G1**	90	40	21	6190	1.17	6650	8280	4920	5950
10	G2-PMMA	400	**G2**	180	40	27	12880	1.13	16930	19650	12000	15240
11	G1-PtBA	200	**G1**	120	50	22	5520	1.12	6260	7545	6280	6790
12	G2-PtBA	400	**G2**	180	50	19	9350	1.09	11860	15430	10900	11580
13	G1-PEGMA	200	**G1**	120	40	29	17820	1.13			37060	31640
14	G1-PEGMA	200	**G1**	120	70	51	30950	1.17			64690	59610
15	G2-PEGMA	400	**G2**	60	40	15	21510	1.11			42270	33060
16	G2-PEGMA	400	**G2**	120	40	34	36280	1.18			94330	77400
17	G2-PEGMA	400	**G2**	120	70	53	51790	1.22			146390	259270
18	G3-PEGMA	800	**G3**	240	40	14	32470	1.12			80420	92380
19	G3-PEGMA	800	**G3**	240	70	42	77490	1.22			241250	243130

[a] $[I]_0$:[CuCl]:[PMDETA] = 1:2:2 (entries 9-10), $[I]_0$:[CuBr]:[PMDETA] = 1:2:2 (entries 11-12), 1:2.4 (entries 13-19). [b] Calibration with linear PMMA (entries 9 and 10), PS (entries 11-19) as standards. [c] GPC in THF with triple detector system, d*n*/d*c* = 0.076mL/g (entries 1 and 2), 0.049 mL/g (entries 3 and 4).

3.2.1. Activation of the Polymer

The polymers containing the masked maleimide group were converted to their reactive form upon subjecting them to the retro Diels-Alder reaction. Polymer **15** was heated under vacuum at 100 ^{o}C for 5h. Quantitative unmasking of the maleimide group was evident from disappearance of proton resonances at 2.83, 5.23 and 6.48 ppm due to the bicyclic core, and appearance of a new proton resonance at 6.73 ppm corresponding to the maleimide proton (Figure 3.18).

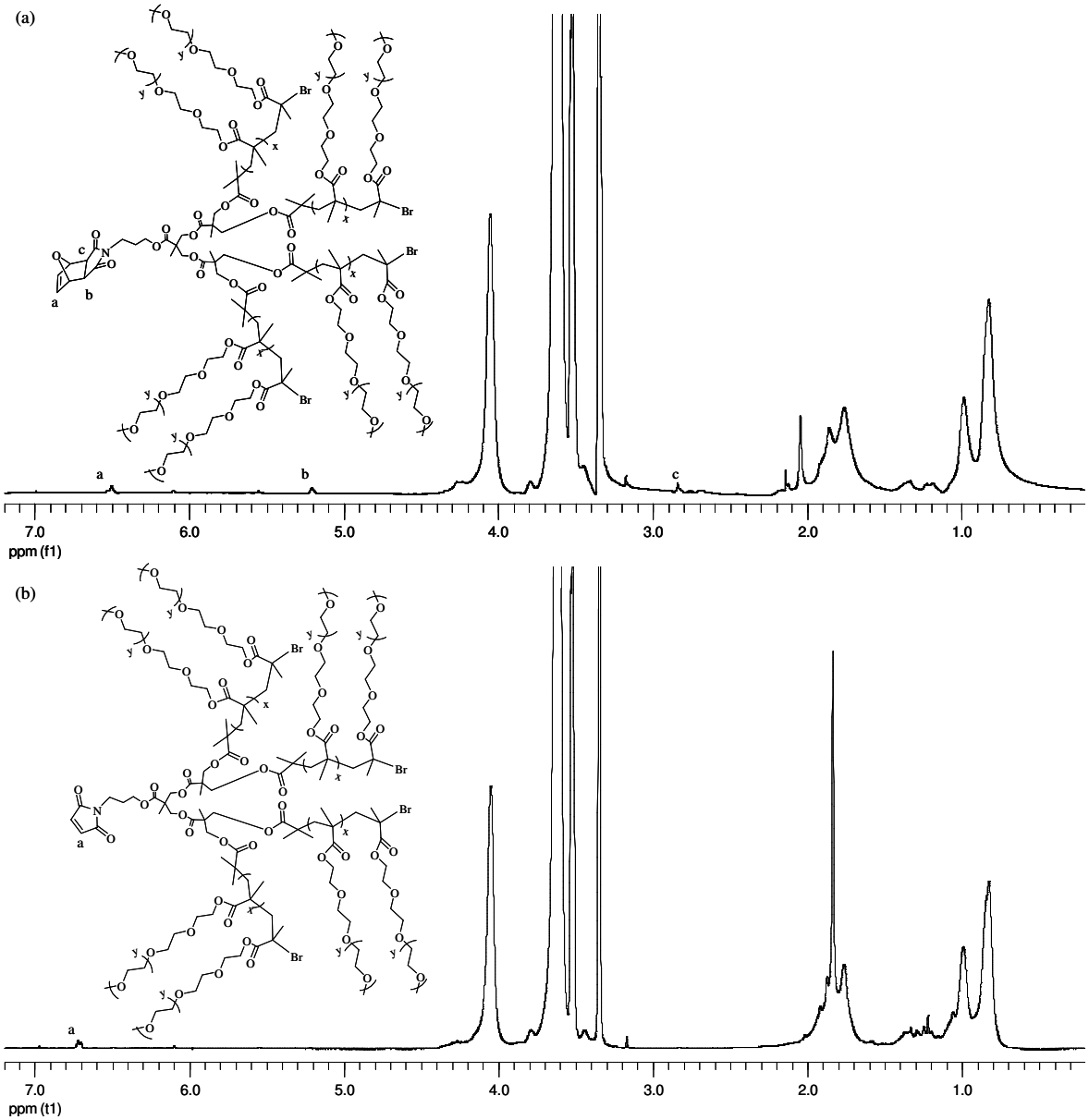

Figure 3.18. ^{1}H NMR spectra of Polymer **15** (a) and Polymer **20** (b) (in $CDCl_3$)

3.2.2. Functionalization with Glutathione

The next step was to evaluate the efficiency of conjugation of thiol containing molecules to the maleimide core of the water soluble multiarm polymer **20**. Especially for conjugation of biomolecules, reactivity of maleimide group towards the thiolinated molecules is considered as an advantage, because cystein residues in the structure of proteins and enzymes contain thiol functionality. For this purpose a cysteine containing tripeptide glutathione was selected.

The conjugate addition was carried out by treatment of the polymer with glutathione in water at room temperature. Polymer **21** was obtained in pure form after thoroughly dialysis in water to eliminate any residual unbound glutathione. Complete conjugation was observed due to complete disappearance of the maleimide resonance at 7.03 ppm and appearance of new proton signals at 2.17 and 2.56 ppm belonging to the glutathione moiety (Figure 3.19).

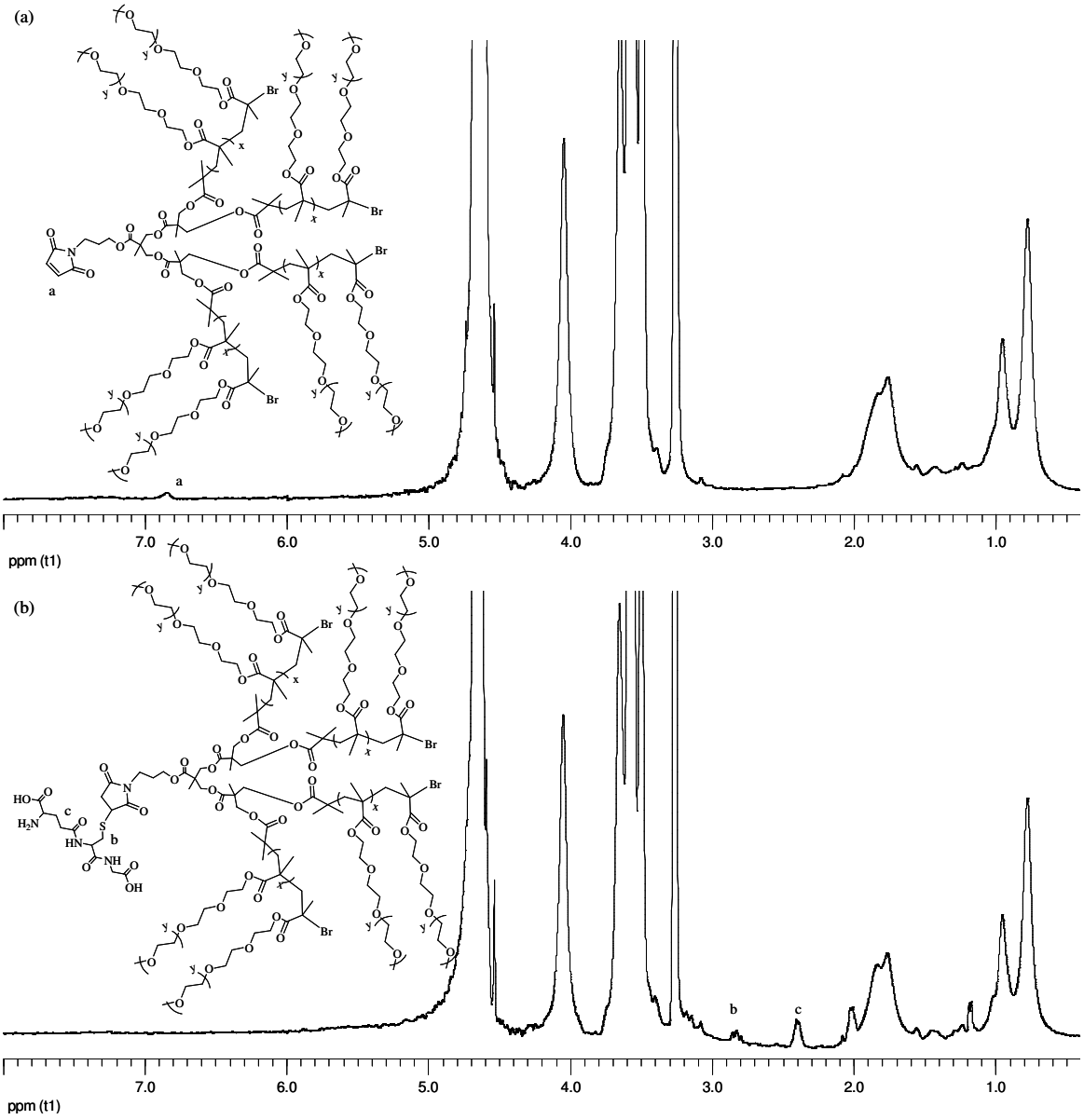

Figure 3.19. ^{1}H NMR spectra of Polymer **20** (a) and Polymer **21** (b) (in D_2O)

4. EXPERIMENTAL

4.1. Materials and Methods

All reagents were obtained from commercial sources (Merck, Aldrich and Alfa Aesar) and were used as received unless otherwise stated. Dry solvents (CH_2Cl_2, THF, toluene) was obtained from ScimatCo Purification System, other dry solvents were dried over molecular sieves. 4, 5, 10, 11 and 12 were prepared according to literature procedures [62, 64] Poly (ethylene) glycol diol (2K) and monomethoxy Poly (ethylene) glycol (2K) were dried under vacuum after azeotropic distillation with toluene. Methyl methacrylate (MMA, 99%, Aldrich), Poly(ethylene glycol) methyl ether metacrylate (Mw = 300) (PEGMA, 99%, Aldrich) and *tert*-butylacrylate (*t*BA, 99%, Aldrich) were passed through basic alumina column to remove inhibitor and then distilled over CaH_2 in vacuum prior to use. *N, N, N', N'', N''*-pentamethyldiethylenetriamine (PMDETA, Aldrich) was distilled over NaOH prior to use. The dendron and polymer characterizations involved ^1H solution NMR spectroscopy (Varian 400 MHz and and Bruker 260 MHz), FlashEA^R 1112 Series Elemental Analyzer (CHNS Separation Column, PTFE; 2m; 6x5mm), UV-Vis spectroscopy and Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer 1600 Series). The molecular weights were estimated by gel permeation chromatography (GPC) analysis using a Viscotek GPCmax VE-2001 analysis system. PLgel (length/ID 300 mm 3 7.5 mm, 5 lm particle size) Mixed-C column was caliberated with polystyrene standards, using refractive index detector. THF was used as elutent at a flow rate of 1 mL/min at 30 ^oC.

4.1.1. Instrumentation

The conventional size exclusion chromatography (SEC) measurements were carried out with an Agilent instrument (Model 1100) consisting of a pump, refractive index, and UV detectors. Four Waters Styragel columns (HR 5E, HR 4E, HR 3 and HR 2), (4.6 mm internal diameter, 300 mm length, packed with 5 μm particles) were used in series. The

effective molecular weight ranges were 2000–4,000,000, 50–100,000, 500–30,000, and 500–20,000, respectively. THF was used as eluent at a flow rate of 0.3 mL/min at 30 ^{o}C. Toluene was used as an internal standard. The apparent molecular weights and polydispersities were determined with a calibration based on linear PS or PMMA standards using PL Caliber Software from Polymer Laboratories. The second SEC system with a Agilent 1200 model pump, four Waters Styragel columns (guard, HR 5E, HR 4, HR 3, and HR 2), and a Viscotek TDA 302 triple detector (RI, dual laser light scattering (LS) (λ=670 nm, 90^{o} and 7^{o}) and a differential pressure viscometer), (TD-SEC) was conducted to measure the absolute molecular weights in THF with a flow rate of 0.5 mL/min at 35 ^{o}C. All three detectors were calibrated with a PS standard having narrow molecular weight distribution (Mn=115,000 g/mol, Mw/Mn =1.02, [g] = 0.519 dL/g at 35 ^{o}C in THF, dn/dc = 0.185 mL/g) provided by Viscotek company. Data analyses were performed with Omni-Sec version 4.5 software from Viscotek Company.

4.2. Dendron-Polymer Conjugates for Drug Delivery

4.2.1. Synthesis of Bis-Maleimide PEG

To a solution of dried PEG (2 g, 1 mmol) dissolved in THF (5 mL) was added triethylamine (0.42 mL, 2.99 mmol) at 0 ^{o}C. In a separate flask were dissolved succinic unhydride (0.34 g, 3.42 mmol) and DMAP (0.049 g, 0.40 mmol) in THF (5 mL) and transferred dropwise onto the PEG solution at 0 ^{o}C over 30 min. The clear solution was stirred for 20 h at room temperature under N_2. All volatiles were removed under *vacuo* and the product was precipitated in cold diethyl ether, filtered and dried under vacuum. The precipitation procedure was repeated twice to give pure bis-acid PEG as white solid **1a** (2.13 g, 97%). Then, polymer **1a** (1.50 g, 0.68 mmol) was dried under vacuum after azeotropic distillation with toluene. To a solution of 1a dissolved in CH_2Cl_2 (15 mL), were added the furan protected maleimide group containing alcohol (2) (0.90 g, 4.10 mmol), DMAP (0.016 g, 0.14 mmol) and EDCI (0.29 g, 1.49 mmol). The solution was stirred for 20 h at room temperature under N_2. To the reaction mixture was added CH_2Cl_2 (30 mL) and the mixture was washed with saturated $NaHCO_3$ (2 x 30 mL). The combined organic

layers were dried over anhydrous Na_2SO_4 and all volatiles were evaporated. After precipitation twice with cold diethyl ether, filtered and dried under vacuum to give pure bis-maleimide PEG as a white solid **3a** (1.40g, 79%). ^{1}H NMR ($CDCl_3$, δ, ppm) 6.48 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.22 (t, 2H, *J* = 4.8 Hz, OCH_2), 4.03 (t, 2H, *J* = 6.0 Hz, NCH_2), 3.64-3.62 (m, 2H, OCH_2CH_2 of PEG), 2.82 (s, 2H, CH-CH bridge protons), 2.65 (t, 2H, *J*= 5.2 Hz, CH_2C=O), 2.61 (t, 2H, *J*= 5.2 Hz CH_2C=O), 1.90 (tt, 2H, *J*= 6.0, 4.8 Hz, $NCH_2CH_2CH_2O$).

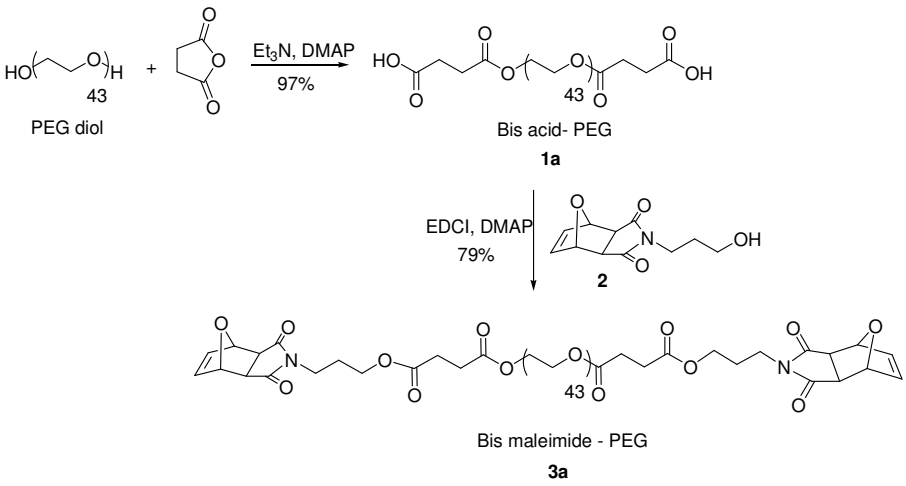

Figure 4.1. Bis-Maleimide PEG Synthesis

4.2.2. Synthesis of Mono-Maleimide PEG

To a solution of dried monomethoxy PEG (2 g, 1 mmol) dissolved in THF (5 mL) was added triethylamine (0.21 mL, 1.50 mmol) at 0 ^oC. In a separate flask were dissolved succinic unhydride (0.17 mg, 1.70 mmol) and DMAP (0.024 mg, 0.20 mmol) in THF (5 mL) and transferred dropwise onto the PEG solution at 0 ^oC over 30 min. The clear solution was stirred for 20 h at room temperature under N_2. All volatiles were removed under *vacuo* and the product was precipitated in cold diethyl ether, filtered and dried under vacuum. The precipitation procedure was repeated twice to give pure mono-acid PEG as white solid **1b** (1.95 g, 93%). Then, polymer **1b** (1.50 g, 0.71 mmol) was dried under vacuum after azeotropic distillation with toluene. To a solution of 1b dissolved in CH_2Cl_2

(10 mL), were added the furan protected maleimide group containing alcohol (2) (0.49 g, 2.14 mmol), DMAP (0.09 g, 0.07 mmol) and EDCI (0.15 g, 0.78 mmol). The solution was stirred for 20 h at room temperature under N_2. To the reaction mixture was added CH_2Cl_2 (20 mL) and the mixture was washed with saturated $NaHCO_3$ (2 x 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and all volatiles were evaporated. After precipitation twice with cold diethyl ether, filtered and dried u nder vacuum to give pure mono-maleimide PEG as a white solid **3b** (1.22 g, 75%). ^{1}H NMR ($CDCl_3$, δ, ppm) 6.48 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.22 (t, 2H, *J* = 4.8 Hz, OCH_2), 4.03 (t, 2H, *J* = 6.4 Hz, NCH_2), 3.82-3.50 (m, 4H, OCH_2CH_2 of PEG), 3.35 (s, 3H, OCH_3 of PEG), 2.82 (s, 2H, CH-CH bridge protons), 2.64 (t, 2H, *J*= 5.2 Hz, CH_2C=O), 2.61 (t, 2H, *J*= 5.2 Hz, CH_2C=O), 1.89 (tt, 2H, *J*= 6.0, 4.8 Hz, $NCH_2CH_2CH_2O$).

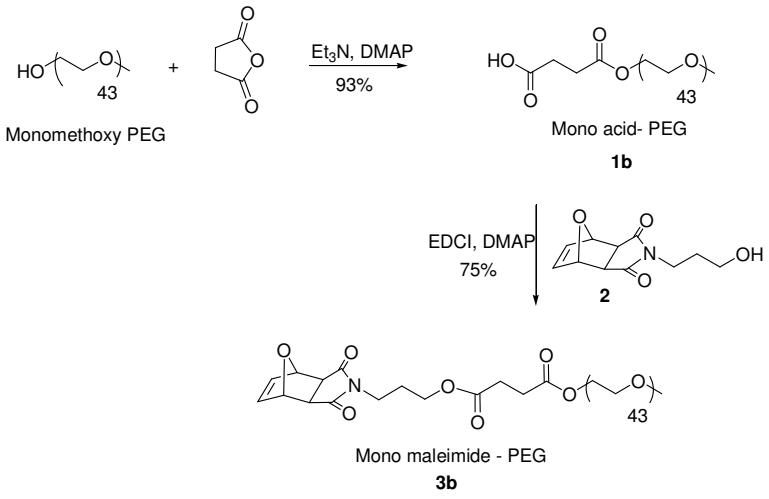

Figure 4.2. Mono-Maleimide PEG Synthesis

4.2.3. Synthesis of Anthracene Functionalized Poly (ester) Dendrons

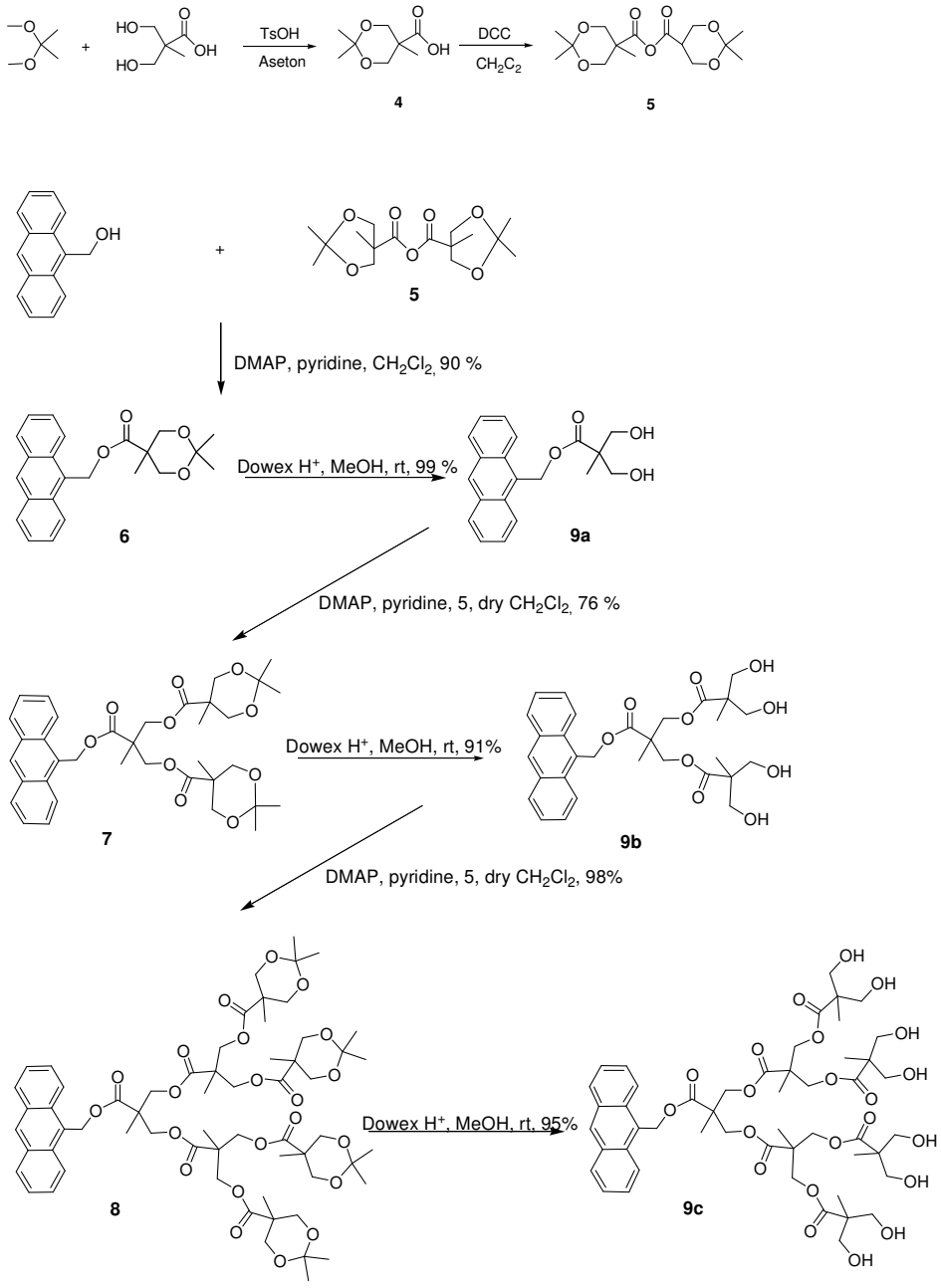

Figure 4.3. Divergent synthesis of anthracene functionalized poly (ester) dendron

4.2.3.1. Synthesis of 1st Generation Anthracene Functionalized Dendron

Compounds **4** and **5** were synthesized according to the previously reported literature procedures [62, 63]. To a solution of 5 (4.25 g, 12.86 mmol) in CH_2Cl_2 (40 mL), 9-anthracene methanol (1.79 g, 8.58 mmol), DMAP (0.42 g, 3.43 mmol) and pyridine (2.50 mL) was added. The mixture was stirred at room temperature for 20 hours followed by quenching of excess anhydride with (1:1) mixture of pyridine and water (8.20 mL) for 5 h. Reaction mixture was extracted with 1 M $NaHSO_4$ (3 x 60 mL), 10 % Na_2CO_3 (3 x 60 mL) and then with brine (1 x 60 mL) combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. The product was obtained as a pure yellow solid **6** (5.70 g, 90 %).

Compound **6** (2.00 g, 5.30 mmol) was dissolved in MeOH (30 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of 5 was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_3$. The product was obtained by filtration to give **9a** as a yellow solid (1.78 g, 99%). ^1H NMR ($CDCl_3$, δ, ppm) 8.51 (s, 1H, ArH), 8.30 (d, 2H, *J*= 9.2 Hz, ArH), 8.03 (d, 2H, *J*= 9.2 Hz, ArH), 7.57 (dd, 2H, *J*= 7.6, 6.4 Hz, ArH), 7.49 (dd, 2H, *J*= 7.6, 6.4 Hz, ArH), 6.21 (s, 2H, CH_2-Ar), 3.85 (d, 2H, *J*= 10.8 Hz, CH_2 ester protons), 3.66 (d, 2H, *J*= 11.2 Hz, CH_2 ester protons), 2.68 (s, 2H, OH), 0.97 (s, 3H, $C(CH_3)$). Anal. calcd for G1-Antracene Dendron [$C_{20}H_{20}O_4$]: C, 74.06; H, 6.21; O, 19.73. Found: C, 74.25; H, 6.44; O, 19.31.

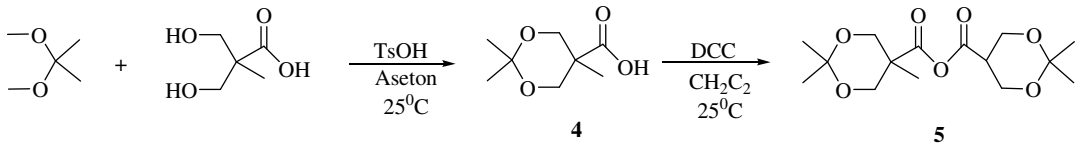

Figure 4.4. G1-Anhydride Synthesis

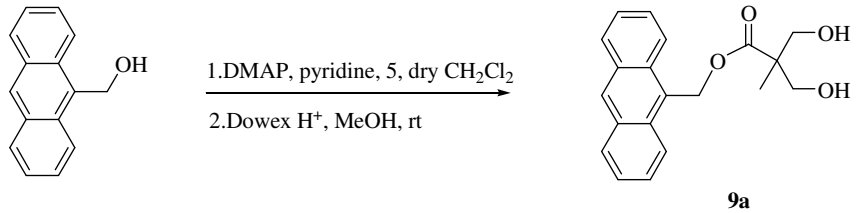

Figure 4.5. G1-Anthracene Dendron Synthesis

4.2.3.2. Synthesis of 2nd Generation Anthracene Functionalized Dendron

Compound **9a** (0.32 g, 0.99 mmol) was added to a solution of DMAP (0.06 g, 0.49 mmol), pyridine (0.72 mL) and compound 5 (1.14 g, 3.45 mmol) in CH_2Cl_2 (10 mL). The mixture was then stirred at room temperature for 20 h followed by quenching of excess anhydride with (1:1) mixture of pyridine and water (2.40 mL) for 5 h. Reaction mixture was diluted with CH_2Cl_2 (30 mL) and extracted with 1 M $NaHSO_4$ (3 x 40 mL), 10 % Na_2CO_3 (3 x 40 mL) and then with brine (1 x 40 mL) combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. Crude product was purified by column chromatography to give **7** as a yellow solid (0.48 g, 76 %). Compound **7** (0.48 g, 0.76 mmol) was dissolved in MeOH (18 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of 6 was observed via TLC. The resin was then filtered off and washed with MeOH. Crude product was purified by column chromatography to give **9b** as a yellow solid (0.38 g, 91 %). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.51 (s, 1H, ArH), 8.32 (d, 2H, *J*= 8.8 Hz, ArH), 8.02 (d, 2H, *J*= 8.8 Hz, ArH), 7.57 (dd, 2H, *J*= 8.8, 7.6 Hz, ArH), 7.49 (dd, 2H, *J*= 8.8, 7.6 Hz, ArH), 6.23 (s, 2H, CH_2-Ar), 4.34 (d, 2H, *J*= 11.2 Hz, CH_2 ester protons), 4.22 (d, 2H, *J*= 11.2 Hz, CH_2 ester protons), 3.70-3.51 (m, 8H, CH_2 ester protons), 2.90-2.86 (m, 4H, OH), 1.25 (s, 3H, $C(CH_3)$), 0.80 (s, 6H, $C(CH_3)$). Anal. calcd for G2-Antracene Dendron [$C_{30}H_{36}O_{10}$]: C, 63.44; H, 6.61; O, 29.95. Found: C, 63.52; H, 6.82; O, 29.66.

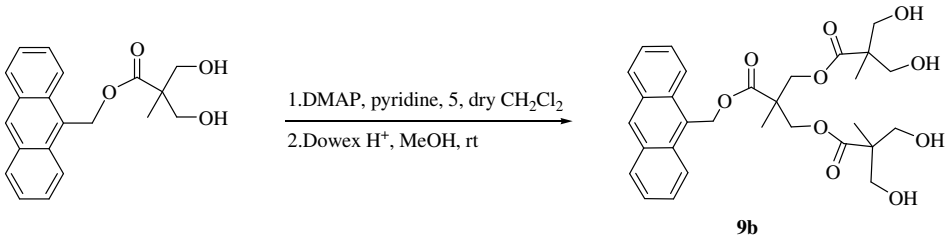

Figure 4.6. G2-Anthracene Dendron Synthesis

4.2.3.3. Synthesis of 3rd Generation Anthracene Functionalized Dendron

Compound **9b** (0.40 g, 0.72 mmol) was added to a solution of DMAP (0.19 g, 1.55 mmol), pyridine (2.70 mL) and compound 5 (1.90 g, 5.75 mmol) in CH_2Cl_2 (18 mL). The mixture was then stirred at room temperature for 20 h. Excess anhydride was quenched with (1:1) mixture of pyridine and water (9 mL) for 5 h. Reaction mixture was diluted with CH_2Cl_2 (20 mL) and then extracted with 1 M $NaHSO_4$ (3 x 40 mL), 10 % Na_2CO_3 (3 x 40 mL) and then with brine (1 x 40 mL) combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. The product was obtained as a pure light yellow solid **8** (0.83 g, 98 %). Compound **8** (0.83 g, 0.71 mmol) was dissolved in MeOH (20 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of 7 was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_3$. The product was obtained by filtration to give **9c** as a yellow solid (0.68 g, 95 %). ^{1}H NMR (CD_3COD, δ, ppm) 7.91 (s, 1H, ArH), 7.78 (d, 2H, *J*= 8.8 Hz, ArH), 7.41 (d, 2H, *J*= 8.8 Hz, ArH), 6.96 (dd, 2H, *J*= 8.0, 6.8 Hz, ArH), 6.86 (dd, 2H, *J*= 8.0, 6.8 Hz, ArH), 5.62 (s, 2H, CH_2-Ar), 3.52 (m, 16H, CH_2 ester protons), 2.97 (m, 20H, CH_2 ester protons and OH), 0.58 (s, 3H, $C(CH_3)$), 0.47 (s, 12H, $C(CH_3)$), 0.35 (s, 6H, $C(CH_3)$). Anal. calcd for G3-Antracene Dendron [$C_{50}H_{68}O_{22}$]: C, 57.64; H, 6.80; O, 35.56. Found: C, 57.65; H, 6.55; O, 35.80.

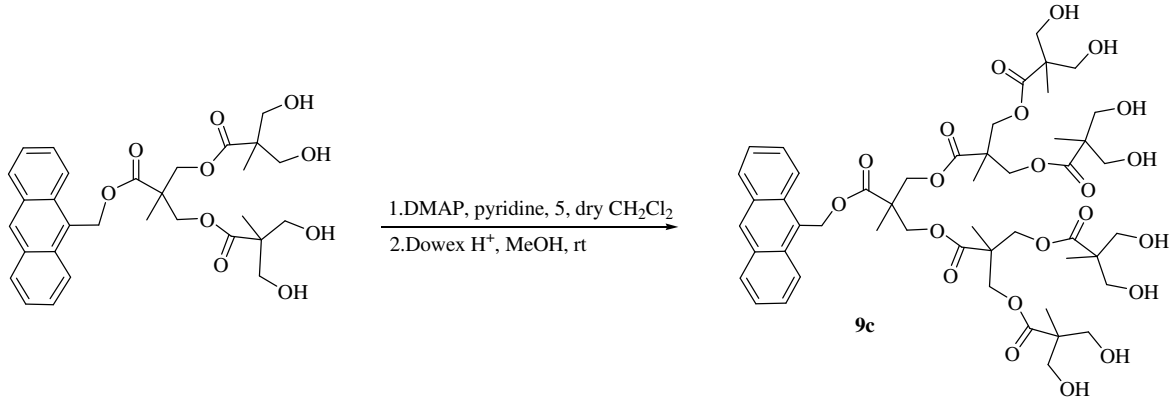

Figure 4.7. G3-Anthracene Dendron Synthesis

4.2.4. General Procedure for Diblock Copolymer Synthesis

Mono-maleimide PEG **(3b)** (0.20 g, 0.09 mmol) and G3-anthracene dendron **(9b)** (0.092 g, 0.09 mmol) were dissolved in Toluene (4 mL) and the mixture was heated under Nitrogen at 110 ^{o}C for 6 hours. Then all volatiles were removed by *vacuo* and after precipitation twice with cold diethyl ether, the product was filtered and dried under vacuum to give **P3** as a light yellow solid (0.22 g, 77 %).

^{1}H NMR data for G1-PEG (P1) ($CDCl_3$, δ, ppm) 7.39-7.29(m, 4H, ArH), 7.19-7.16 (m, 4H, ArH), 5.54-5.42 (m, 2H, $CH_2OC=O$), 4.77 (s, 1H, CH bridgehead proton), 4.22 (t, 2H, *J* = 4.4 Hz, OCH_2), 3.90-3.83 (m, 4H, CH_2 ester protons), 3.79 (t, 2H, *J* = 4.8 Hz, NCH_2), 3.70-3.58 (m, 4H, OCH_2CH_2 of PEG), 3.49-3.42 (m, 2H, $NCH_2CH_2CH_2O$), 3.35 (s, 3H, OCH_3 of PEG), 3.28 (bs, 1H, CH-CH bridge proton), 3.18 (t, 1H, *J*= 6.8 Hz, CH-CH bridge proton), 2.97 (bs, 2H, OH), 2.64-2.55 (m, 4H, $O=C-CH_2CH_2-C=O$), 1.12 (s, 3H, $C(CH_3)$).

^{1}H NMR data for G2-PEG (P2) ($CDCl_3$, δ, ppm) 7.34-7.10 (m, 8H, ArH), 5.50-5.40 (m, 2H, $CH_2OC=O$), 4.71 (s, 1H, CH bridgehead proton), 4.44-4.36 (m, 2H, OCH_2), 4.25-4.16 (m, 4H, CH_2 ester protons), 3.96-3.90 (m, 2H, NCH_2), 3.74-3.39 (m, 18H, OCH_2CH_2 of PEG, CH_2 ester protons, $NCH_2CH_2CH_2O$ and OH), 3.30 (s, 3H, OCH_3 of PEG), 3.24-3.11 (m, 2H, CH-CH bridge protons), 2.57-2.52 (m, 4H, $O=C-CH_2CH_2-C=O$), 1.12 (d, 6H, *J*= 6.0 Hz, $C(CH_3)$), 1.02 (d, 3H, *J*= 4.8 Hz, $C(CH_3)$).

^{1}H NMR data for G3-PEG (P3) (CDCl3, δ, ppm) 7.41-7.14 (m, 8H, ArH), 5.62-5.39 (m, 2H, $CH_2OC=O$), 4.76 (d, 1H, *J*= 3.2 Hz, CH bridgehead proton), 4.40-4.20 (m, 16H, OCH_2, NCH_2 and CH_2 ester protons), 3.80-3.49 (m, 28H, OCH_2CH_2 of PEG, CH_2 ester protons and OH), 3.35 (s, 3H, OCH_3 of PEG),3.45-3.42 (m, 2H, $NCH_2CH_2CH_2O$), 3.17-3.13 (m, 2H, CH-CH bridge protons), 2.63-2.54 (m, 4H, $O=C-CH_2CH_2-C=O$), 1.31-1.26 (m, 9H, $C(CH_3)$), 1.05 (d, 12H, *J*= 5.2 Hz, $C(CH_3)$).

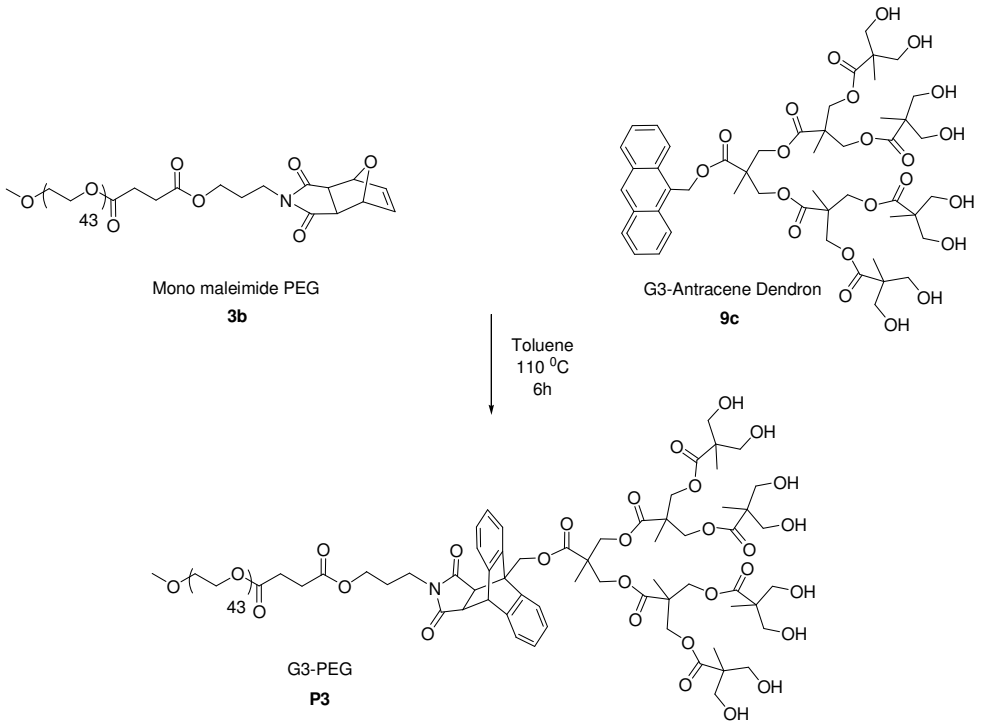

Figure 4.8. Diels-Alder reaction to synthesize G3-PEG **(P3)**

4.2.5. General Procedure for Triblock Copolymer Synthesis

Bis-maleimide PEG **(3a)** (0.20 g, 0.08 mmol) and G3-anthracene dendron **(9b)** (0.16 g, 0.15 mmol) were dissolved in Toluene (5 mL) and the mixture was heated under Nitrogen at 110 ^{o}C for 6 hours. Then all volatiles were removed by *vacuo* and after precipitation twice with cold diethyl ether, the product was filtered and dried under vacuum to give **P6** as a light yellow solid (0.29 g, 84 %).

^{1}H NMR data for G1-PEG-G1 (P4) ($CDCl_3$, δ, ppm) 7.38-7.18 (m, 8H, ArH), 5.54-5.40 (m, 2H, CH_2OC=O), 4.76 (s, 1H, CH bridgehead proton), 4.22 (bs, 2H, OCH_2), 3.90-3.79 (m, 6H, CH_2 ester protons and NCH_2), 3.70-3.16 (m, 8 H, CH-CH bridge protons, OCH_2CH_2 of PEG, $NCH_2CH_2CH_2O$ and OH,), 2.62-2.57 (m, 8H, O=C-CH_2CH_2-C=O), 1.12 (s, 6H, $C(CH_3)$).

^{1}H NMR data for G2-PEG-G2 (P5) ($CDCl_3$, δ, ppm) 7.48-7.25 (m, 8H, ArH), 5.64-5.50 (m, 2H, CH_2OC=O), 4.84 (d, 1H, *J*= 2.8 Hz, CH bridgehead proton), 4.58-4.50 (m, 2H, OCH_2), 4.39-4.30 (m, 4H, CH_2 ester protons), 3.90-3.81 (m, 2H, NCH_2), 3.79-3.60 (m, 16H, OCH_2CH_2 of PEG, CH_2 ester protons, $NCH_2CH_2CH_2O$ and OH), 3.56-3.51 (m, 1H, CH-CH bridge protons,), 3.39-3.23 (m, 1H, CH-CH bridge protons,), 2.71-2.66 (m, 4H, O=C-CH_2CH_2-C=O), 1.42 (s, 3H, $C(CH_3)$), 1.15 (d, 6H, *J*= 5.6 Hz, $C(CH_3)$).

^{1}H NMR data for P3-PEG-G3 (P6) ($CDCl_3$, δ, ppm) 7.36-7.10 (m, 8H,), 5.56-5.35 (m, 2H, CH_2OC=O), 4.72 (s, 1H, CH bridgehead protons), 4.32-4.18 (m, 16H, OCH_2, NCH_2 and CH_2 ester protons), 3.75-3.52 (m, 26H, OCH_2CH_2 of PEG, CH_2 ester protons and OH), 3.44-3.40 (m, 4H, $NCH_2CH_2CH_2O$), 3.28-3.10 (m, 2H, CH-CH bridge protons), 2.58-2.52 (m, 4H, O=C-CH_2CH_2-C=O), 1.26 (s, 3H, $C(CH_3)$), 1.22 (d, 6H, *J*= 10.4 Hz, $C(CH_3)$), 1.01 (d, 12H, *J*= 2.8 Hz, $C(CH_3)$).

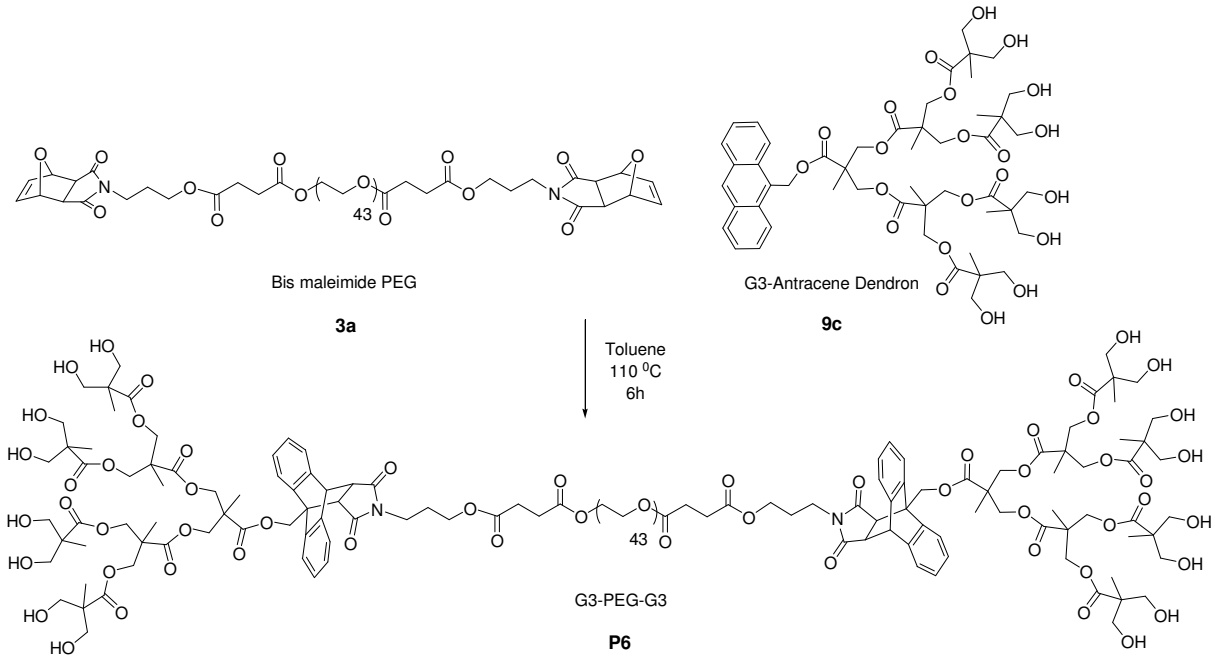

Figure 4.9. Diels-Alder reaction to synthesize G3-PEG-G3 (**P6**)

4.2.6. Functionlization of G2-PEG-G2

G2-PEG-G2 **(P5)** (0.30 g, 0.08 mmol), pyridine (0.27 mL) and DMAP (0.016 g, 0.13 mmol) dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL round bottom flask. To the stirring reaction mixture was added 5-pentynoic acid anhydride (0.18 g, 1.01 mmol) and continued stirring for 20 h at room temperature under N_2. Pyridine:water solution (0.6 mL, 1:1) was added to the reaction mixture and stirred at room temperature for 5 h. Reaction mixture was diluted with CH_2Cl_2 (20 mL) and then extracted with 1 M $NaHSO_4$ (3 x 20 mL), 10% Na_2CO_3 (3 x 20 mL) and then with brine (1 x 20 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and the residue was concentrated in *vacuo*. Crude product was purified by precipitation in diethyl ether to give **P7** as a white solid (0.21 g, 60 % yield). $M_{n,theo}$= 4219, $M_{n,GPC}$= 4703, M_w/M_n= 1.15, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.40-7.14 (m, 8H, ArH), 5.66-5.27 (m, 2H, CH_2OC=O), 4.76 (d, 1H, *J*= 1 Hz, CH bridgehead proton), 4.35-4.11 (m, 6H, OCH_2 and CH_2 ester protons), 3.79 (m, 2H, *J*= 4.8 Hz, NCH_2), 3.72-3.42 (m, 12H, OCH_2CH_2 of PEG, CH_2 ester protons and $NCH_2CH_2CH_2O$), 3.38-3.26 (m, 1H, CH-CH bridge protons,), 3.21-3.10 (m, 1H, CH-CH bridge protons,), 2.62-2.46 (m, 20H, O=C-CH_2CH_2-C=O and CH_2CH2-CCH of), 1.89 (bs, 4H, CCH), 1.29-1.22 (m, 9H, C(CH_3)).

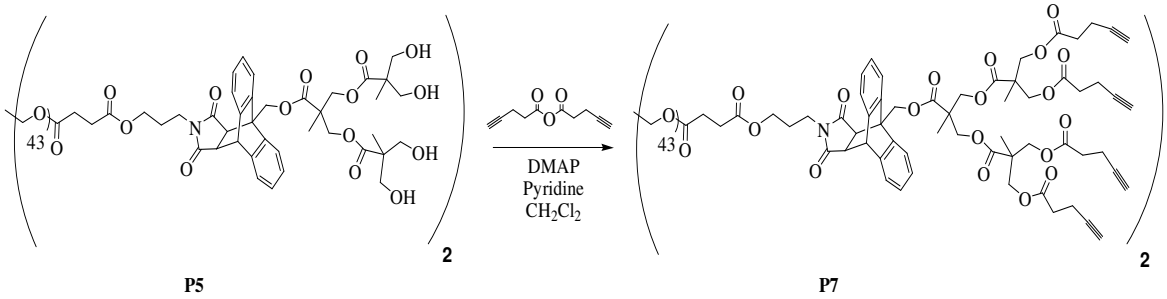

Figure 4.10. Synthesis of **P7**

4.2.7. Conjugation with AZT

CuBr (0.8 mg, 0.006 mmol) and PMDETA (1.2 μL, 0.006 mmol) were dissolved in dry THF (2 mL). In a separate flask were dissolved AZT (0.02 g, 0.085 mmol) and triple bond functionalized triblock copolymer (P7) (0.03 g, 0.007 mmol) in dry THF (2 mL) and purged with N_2. This mixture was then transferred onto the former solution and stirred at 35 ^{o}C for 24 h. The solvent was then evaporated and the crude product was dissolved in CH_2Cl_2 (20 mL) and extracted with H_2O (5 mL) to remove copper salts. The solvent was concentrated under *vacuo* and the desired product was precipitated with Et_2O, filtered and dried under *vacuo* yielding Polymer 8 (0.02 g, 45%) as a white solid. $M_{n,theo}$= 6355, $M_{n,GPC}$= 7253, M_w/M_n= 1.32, relative to PS.

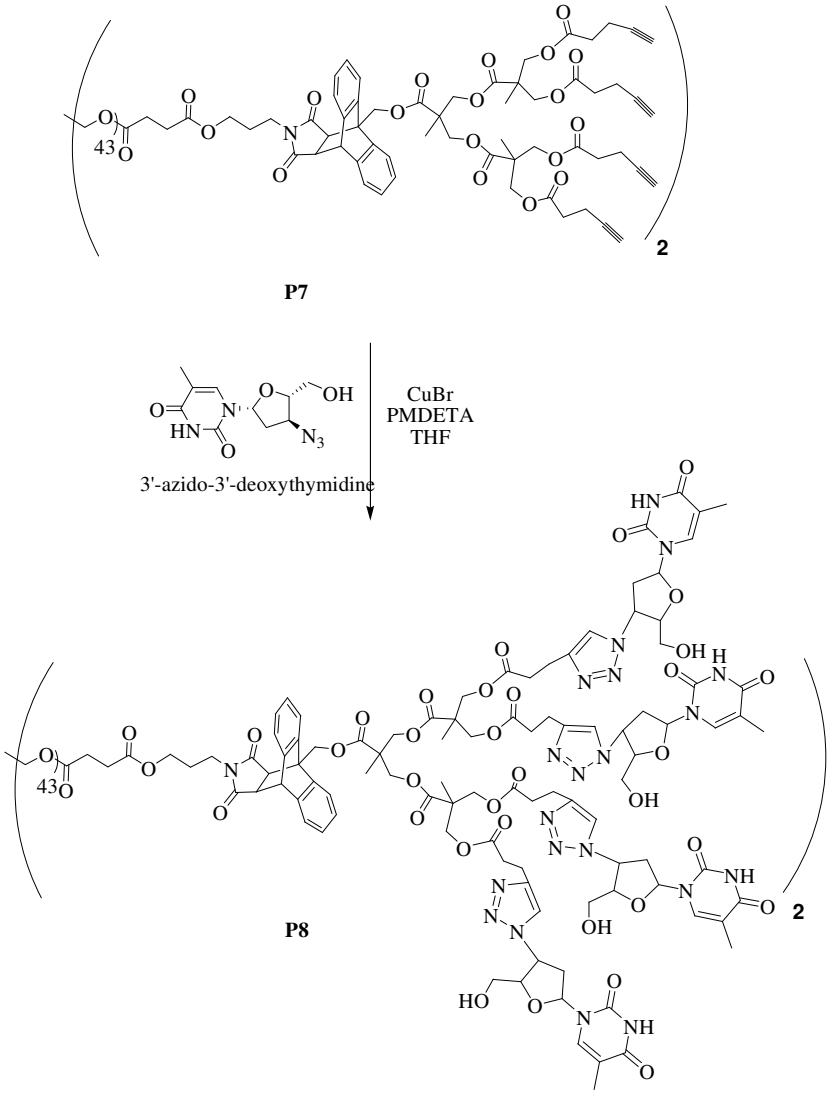

Figure 4.11. Synthesis of **P8**

4.3. Thiol Reactive Multiarm Star Polymers

4.3.1. Synthesis of G1-Maleimide Initiator

Compound **10** (1.0 g, 2.6 mmol) was dissolved in MeOH (15 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at ambient temperature until the consumption of 10 was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_2$. The product was obtained by filtration to give a white solid (0.89 g, 99% yield). The solid (1.00 g, 3.01 mmol) was then added to a solution of triethylamine (1.47 mL, 10.55 mmol) and DMAP (0.11 g, 0.90 mmol) in THF (15 mL) under N_2. The mixture was cooled to 0 °C in an ice bath. On the other side, 2-bromo-isobutyryl bromide (1.12 mL, 9.04 mmol) was diluted in THF (60 mL) and added into the former mixture dropwise (30 min). The obtained white suspension was stirred for 3 h at 0 °C, then warmed to room temperature and stirred for 22 hours. The ammonium salt formed was filtered off and the residue was concentrated in *vacuo*. The product was obtained as a pure white solid **13** (1.85 g, 95 %). ^{1}H NMR ($CDCl_3$, δ, ppm) 6.49 (s, 2H, CH=CH), 5.24 (s, 2H, CH bridgehead protons), 4.41 (d, 2H, *J*= 5.5 Hz, CH_2 ester protons), 4.32 (d, 2H, *J*= 5.5 Hz, CH_2 ester protons), 4.08 (t, 2H, *J*= 6.2 Hz, OCH_2), 3.56 (t, 2H, *J*= 6.8 Hz, NCH_2), 2.83 (s, 2H, CH-CH bridge protons), 1.96-1.90 (m, 14H, $NCH_2CH_2CH_2O$, $CBr(CH_3)$), 1.35 (s, 3H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 176.1, 172.1, 170.9, 136.5, 80.9, 66.2, 62.0, 55.4, 47.4, 46.7, 35.4, 30.6, 26.5, 17.8. Anal. calcd for G1-Maleimide Initiator [$C_{24}H_{31}Br_2NO_9$]: C, 44.78; H, 4.96; N, 2.18. Found: C, 44.52; H, 4.97; N, 2.06

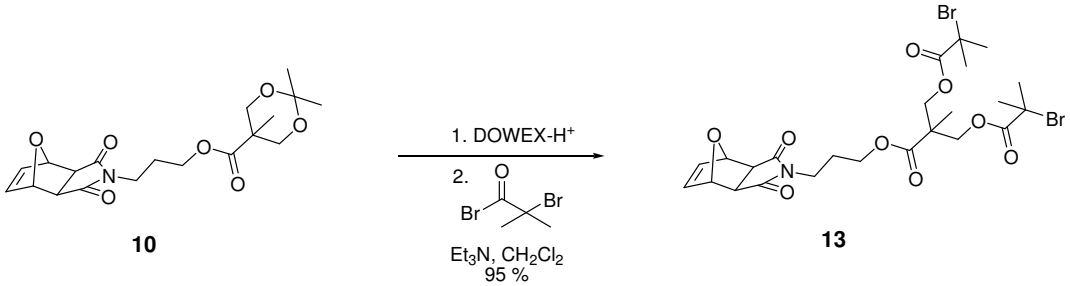

Figure 4.12. G1-Maleimide Initiator Synthesis

4.3.2. Synthesis of G2-Maleimide Initiator

Compound **11** (1.00 g, 1.52 mmol) was dissolved in MeOH (20 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at ambient temperature until the consumption of 11 was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_2$. The product was obtained by filtration to give a white solid (0.85 g, 98% yield). The solid (1.00 g, 1.75 mmol) was then added to a solution of triethylamine (1.7 mL, 12.25 mmol) and DMAP (0.13 g, 1.05 mmol) in THF (20 mL) under N_2. The mixture was cooled to 0 °C in an ice bath. On the other side, 2-bromo-isobutyryl bromide (1.3 mL, 10.50 mmol) was diluted in THF (80 mL) and added into the former mixture dropwise (30 min). The obtained white suspension was stirred for 3 h at 0 °C, then warmed to room temperature and stirred for 22 hours. The ammonium salt formed was filtered off and the residue was concentrated in *vacuo*. The product was obtained as a pure white solid **14** (1.49 g, 73 %). ^{1}H NMR ($CDCl_3$, δ, ppm) 6.48 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.38-4.27 (m, 12H, CH_2 ester protons), 4.05 (t, 2H, *J*= 6.2 Hz, OCH_2), 3.55 (t, 2H, *J*= 6.2 Hz, NCH_2), 2.83 (s, 2H, CH-CH bridge protons), 1.94-1.84 (m, 26H, $NCH_2CH_2CH_2O$, $CBr(CH_3)$), 1.31 (s, 9H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 176.2, 171.8, 171.6, 170.9, 136.5, 80.9, 66.0, 62.1, 55.4, 47.4, 46.8, 46.6, 35.2, 26.6, 17.8, 17.6**.** Anal. calcd for G2-Maleimide Initiator [$C_{42}H_{57}Br_4NO_{17}$]: C, 41.48; H, 5.17; N, 1.15. Found: C, 41.31; H, 4.87; N, 1.06.

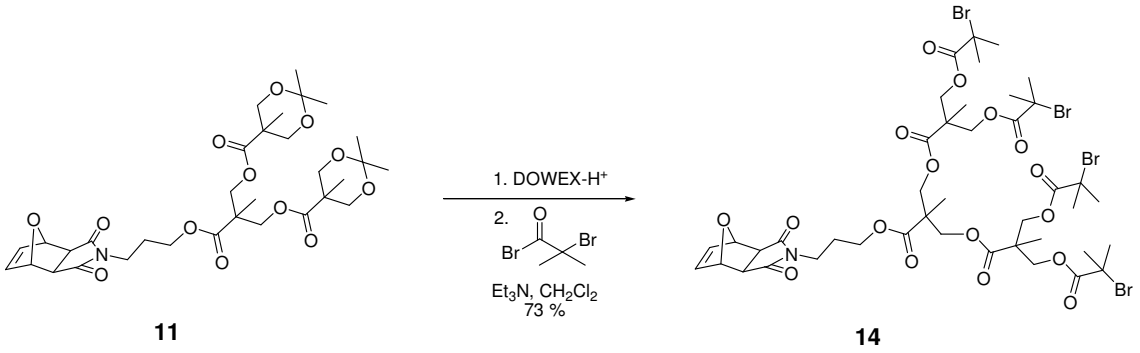

Figure 4.13. G2-Maleimide Initiator Synthesis

4.3.3. Synthesis of G3-Maleimide Initiator

Compound **12** (0.50 g, 0.42 mmol) was dissolved in MeOH (10 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at ambient temperature until the consumption of 12 was observed via TLC. The resin was then filtered off and washed with MeOH. Crude product was purified by column chromatography to give a white solid (0.41 g, 95% yield). The solid (40 mg, 0.039 mmol) was then added to a solution of triethylamine (75 µL, 0.54 mmol) and DMAP (6 mg, 0.046 mmol) in THF (5 mL) under N_2. The mixture was cooled to 0 °C in an ice bath. On the other side, 2-bromo-isobutyryl bromide (57 µL, 0.46 mmol) was diluted in THF (25 mL) and added into the former mixture dropwise (30 min). The obtained white suspension was stirred for 3 h at 0 °C, then warmed to room temperature and stirred for 22 hours. The ammonium salt formed was filtered off and the residue was concentrated in *vacuo*. The product was obtained as a pure light yellow solid **15** (75 mg, 87 %). ^{1}H NMR ($CDCl_3$, δ, ppm) 6.50 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.39-4.24 (m, 28H, CH_2 ester protons), 4.04 (t, 2H, *J*= 5.2 Hz, OCH_2), 3.56 (t, 2H, *J*= 6.2 Hz, NCH_2), 2.84 (s, 2H, CH-CH bridge protons), 1.94-1.89 (m, 50H, $NCH_2CH_2CH_2O$, $CBr(CH_3)$), 1.54 (s, 12H, $C(CH_3)$), 1.31 (s, 6H, $C(CH_3)$), 1.26 (s, 3H, $C(CH_3)$). ^{13}C NMR ($CDCl_3$, δ, ppm) 176.1, 171.6, 171.4, 170.8, 136.5, 81.0, 66.3, 66.0, 65.7, 62.1, 55.4, 47.4, 46.8, 35.2, 30.6, 26.6, 17.9, 17.6. Anal. calcd for G3-Maleimide Initiator [$C_{78}H_{109}Br_8NO_{33}$]: C, 40.78; H, 5.12; N, 0.61. Found: C, 40.79; H, 4.90; N, 0.47.

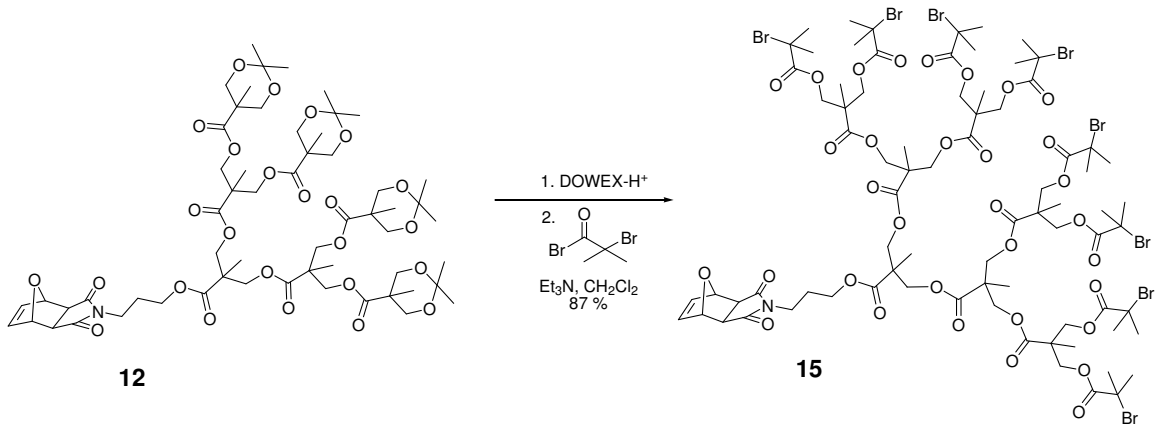

Figure 4.14. G3-Maleimide Initiator Synthesis

4.3.4. Synthesis of furan protected maleimide end-functionalized G1-PMMA (P9)

G1-PMMA was prepared by ATRP of MMA. In a 25 mL of Schlenk tube, MMA (3.00 mL, 28 mmol), PMDETA (0.058 mL, 0.280 mmol), CuCl (0.028 g, 0.280 mmol), toluene (3 mL), and **G1-Initiator (13)** (0.089 g, 0.140 mmol) were added, and the reaction mixture was degassed by three freeze-pump–thaw (FPT) cycles, and left in argon. The tube was then placed in a thermostated oil bath at 40 °C for 1.5 h. The polymerization mixture was diluted with THF, passed through a neutral alumina column to remove the catalyst, and precipitated in hexane. The polymer was dried for 24 h in a vacuum oven at 40°C. $[M]_0/[I]_0$ = 200; $[I]_0$:$[CuCl]_0$:$[PMDETA]_0$=1:2:2; conversion = 21%. $M_{n,theo}$= 4920, $M_{n,NMR}$= 5950, $M_{n,GPC}$= 6190, M_w/M_n= 1.17, relative to linear PMMA. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.51 (s, 2H, CH=CH), 5.24 (s, 2H, CH bridgehead protons), 4.33-4.08 (m , 6H, OCH_2 and CH_2 ester protons), 3.58 (br s, 5H, OCH_3 and NCH_2), 2.85 (s, 2H, CH-CH bridge protons), 1.87–0.82 (m, 22H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone)

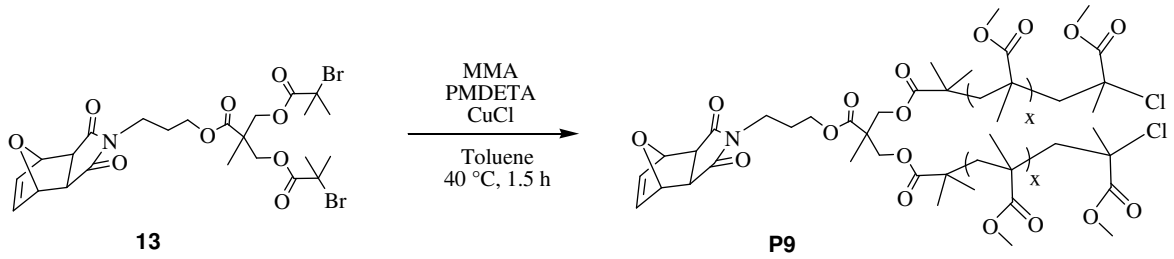

Figure 4.15. G1-PMMA Synthesis

4.3.5. Synthesis of furan protected maleimide end-functionalized G2-PMMA (P10)

G2-PMMA was prepared by ATRP of MMA. In a 25 mL of Schlenk tube, MMA (3.00 mL, 28 mmol), PMDETA (0.058 mL, 0.280 mmol), CuCl (0.028 g, 0.280 mmol), toluene (3 mL), and **G2-Initiator (14)** (0.082 g, 0.070 mmol) were added, and the reaction mixture was degassed by three FPT cycles, and left in argon. The tube was then placed in a thermostated oil bath at 40 °C for 3 h. The polymerization mixture was diluted with THF, passed through a neutral alumina column to remove the catalyst, and precipitated in

hexane. The polymer was dried for 24 h in a vacuum oven at 25 °C. $[M]_0/[I]_0$ = 400; $[I]_0$:$[CuCl]_0$:$[PMDETA]_0$=1:4:4; conversion = 27%. $M_{n,theo}$= 12000, $M_{n,NMR}$= 15240, $M_{n,GPC}$= 12880, M_w/M_n= 1.13, relative to linear PMMA. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.52 (s, 2H, CH=CH), 5.24 (s, 2H, CH bridgehead protons), 4.26-4.06 (m , 14H, OCH_2 and CH_2 ester protons), 3.58 (br s, 5H, OCH_3 and NCH_2), 2.86 (s, 2H, CH-CH bridge protons), 1.87–0.83 (m, 40H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

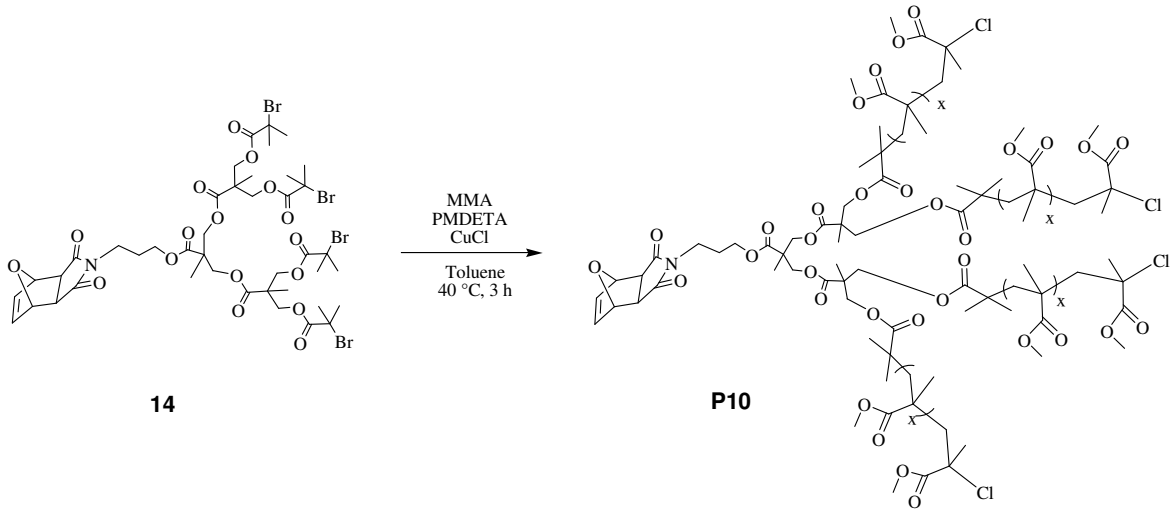

Figure 4.16. G2-PMMA Synthesis

4.3.6. Synthesis of furan protected maleimide end-functionalized G1-P*t*BA (P11)

G1-P*t*BA was prepared by ATRP of *t*BA. In a 25 mL of Schlenk tube, *t*BA (5.0 mL, 34.0 mmol), PMDETA (0.071 mL, 0.340 mmol), CuBr (0.048 g, 0.34 mmol), ethylene carbonate (0.86 g) and the initiator **G1-Initiator (13)** (0.108 g, 0.17 mmol) were added, and the reaction mixture was degassed by three FPT cycles, and left in argon. The tube was then placed in a thermostated oil bath at 50 °C for 2 h. The polymerization mixture was diluted with THF, passed through a basic alumina column to remove the catalyst. The excess of THF was evaporated under reduced pressure and the mixture was precipitated into cold methanol/water (80/20; v/v). After decantation, the polymer was dissolved in CH_2Cl_2, extracted with water and the water phase was again extracted with CH_2Cl_2, and combined organic phase was dried over Na_2SO_4. Finally, the organic phase was evaporated to give G1-P*t*BA. The polymer was dried for 24 h in a vacuum oven at 40 °C. $[M]_0/[I]_0$=

200; $[I]_0$:[CuBr]:[PMDETA]=1:2:2; conversion = 22 %. $M_{n,theo}$= 6280, $M_{n,NMR}$= 6790, $M_{n,GPC}$= 5520, M_w/M_n= 1.12, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.49 (s, 2H, CH=CH), 5.22 (s, 2H, CH bridgehead protons), 4.16-4.04 (m , 6H, OCH_2 and CH_2 ester protons), 3.54 (br s, 2H, NCH_2), 2.83 (s, 2H, CH-CH bridge protons), 2.21 (br s, 1H, CH along polymer backbone),1.81–1.09 (m, 28H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, $OC(CH_3)_3$ and CH_2 along polymer backbone).

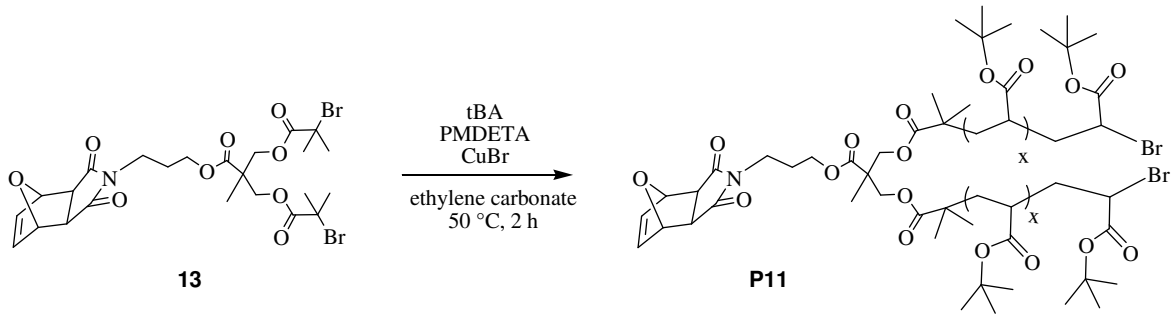

Figure 4.17. G1-P*t*BA Synthesis

4.3.7. Synthesis of furan protected maleimide end-functionalized G2-P*t*BA (P12)

G2-P*t*BA was prepared by ATRP of *t*BA. In a 25 mL of Schlenk tube, *t*BA (5.0 mL, 34.0 mmol), PMDETA (0.071 mL, 0.340 mmol), CuBr (0.048 g, 0.34 mmol), ethylene carbonate (0.86 g) and the initiator **G2-Initiator (14)** (0.10 g, 0.085 mmol) were added, and the reaction mixture was degassed by three FPT cycles, and left in argon. The tube was then placed in a thermostated oil bath at 50 °C for 3 h. The polymerization mixture was diluted with THF, passed through a basic alumina column to remove the catalyst. The excess of THF was evaporated under reduced pressure and the mixture was precipitated into cold methanol/water (80/20; v/v). After decantation, the polymer was dissolved in CH_2Cl_2, extracted with water and the water phase was again extracted with CH_2Cl_2, and combined organic phase was dried over Na_2SO_4. Finally, the organic phase was evaporated to give G2-P*t*BA. The polymer was dried for 24 h in a vacuum oven at 40 °C. $[M]_0/[I]_0$= 200; $[I]_0$:[CuBr]:[PMDETA]=1:4:4; conversion = 19 %. $M_{n,theo}$= 10900, $M_{n,NMR}$= 11580, $M_{n,GPC}$= 9350, M_w/M_n= 1.09, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.49 (s, 2H, CH=CH), 5.22 (s, 2H, CH bridgehead protons), 4.23-4.01 (m , 14H, OCH_2 and CH_2 ester protons), 3.54 (br s, 2H, NCH_2), 2.84 (s, 2H, CH-CH bridge protons), 2.22 (br s, 1H, CH

along polymer backbone),1.81–1.08 (m, 46H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, $OC(CH_3)_3$ and CH_2 along polymer backbone).

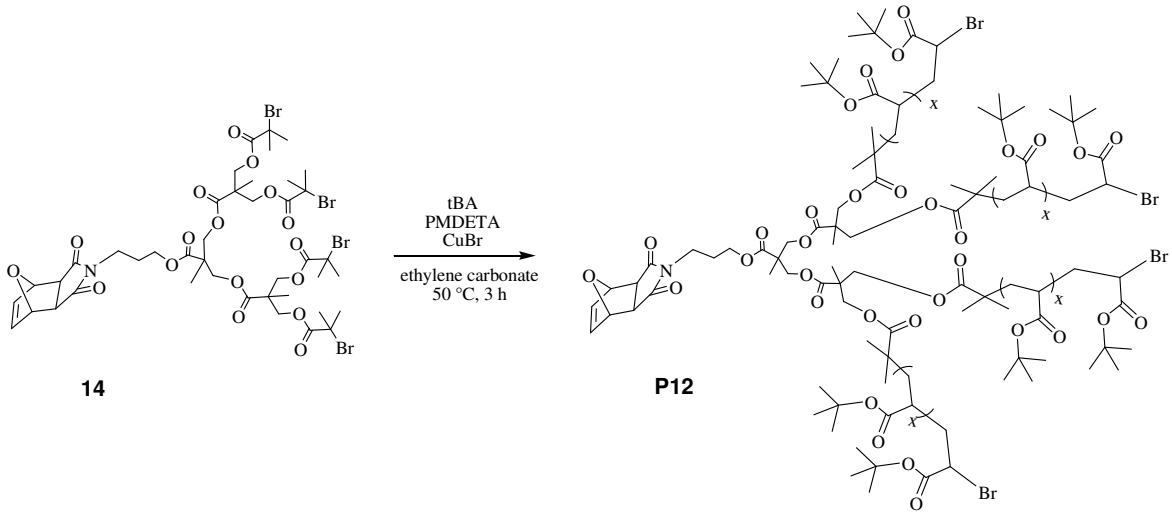

Figure 4.18. G2-P*t*BA Synthesis

4.3.8. Synthesis of furan protected maleimide end-functionalized G1-PEGMEMA (P13)

G1-PEGMEMA was prepared by ATRP of PEGMEMA. Furan protected G1-initiator (**13)** (20 mg, 0.031 mmol) dissolved in minimum amount of degassed anisole was introduced into a flask containing CuBr (9.0 mg, 0.063 mmol), degassed PMDETA (21.8 mg, 0.125 mmol) and degassed PEGMA (1.79 mL, 6.28 mmol) dissolved in degassed anisole (8.95 ml) under stirring. The flask was then placed in a thermostated oil bath at 40 °C for 120 min. After polymerization the reaction mixture was passed through a neutral alumina column to remove the catalyst and precipitated in diethyl ether twice. G1-PEGMEMA was obtained as a white solid. $[M]_0/[I]_0$= 200; $[I]_0$:[CuBr]:[PMDETA]=1:2:4; conversion = 29 %. $M_{n,theo}$=37060, $M_{n,NMR}$= 31640, $M_{n,GPC}$= 17820, M_w/M_n= 1.13, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.51 (s, 2H, CH=CH), 5.22 (s, 2H, CH bridgehead protons), 4.29-4.21 (m , 6H, OCH_2 and CH_2 ester protons), 4.06 (br s, 2H, OCH_2 of PEGMEMA), 3.80-3.41 (m, 2H, OCH_2 of PEGMEMA), 3.56 (br s, 5H, OCH_3 and NCH_2), 2.84 (s, 2H, CH-CH bridge protons), 1.92–0.84 (m, 22H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

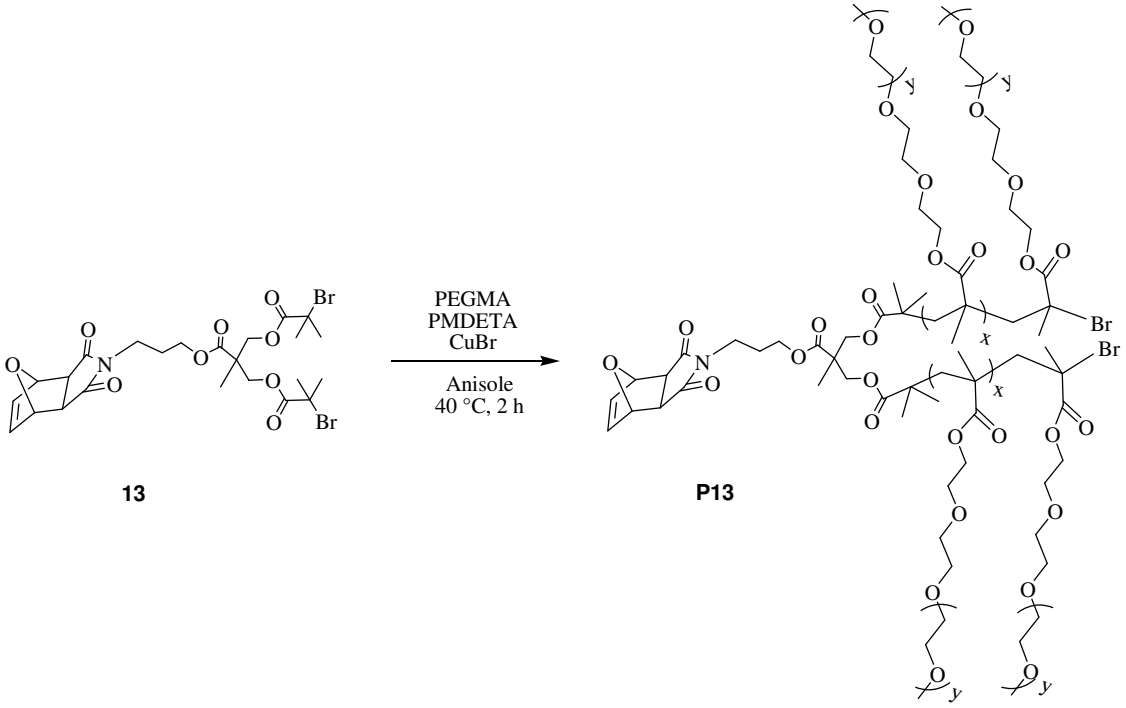

Figure 4.19. G1-PEGMEMA Synthesis

4.3.9. Synthesis of furan protected maleimide end-functionalized G2-PEGMEMA (P16)

G2-PEGMEMA was prepared by ATRP of PEGMEMA. Furan protected G2-initiator **(14)** (20.0 mg, 0.017 mmol) dissolved in minimum amount of degassed anisole was introduced into a flask containing CuBr (9.8 mg, 0.068 mmol), degassed PMDETA (23.7 mg, 0.137 mmol) and degassed PEGMA (1.96 mL, 6.85 mmol) dissolved in degassed anisole (9.80 mL) under stirring. The flask was then placed in a thermostated oil bath at 40 °C for 60 min. After polymerization the reaction mixture was passed through a neutral alumina column to remove the catalyst and precipitated in diethyl ether twice. G2-PEGMA was obtained as a white solid. $[M]_0/[I]_0$= 400; $[I]_0$:[CuBr]:[PMDETA]=1:4:8; conversion = 15 %. $M_{n,theo}$= 42270, $M_{n,NMR}$= 33060, $M_{n,GPC}$= 21510, M_w/M_n= 1.11, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.49 (s, 2H, CH=CH), 5.19 (s, 2H, CH bridgehead protons), 4.25-4.19 (m , 14H, OCH_2 and CH_2 ester protons), 4.03 (br s, 2H, OCH_2 of PEGMEMA), 3.78-3.44 (m, 2H, OCH_2 of PEGMEMA), 3.34 (br s, 5H, OCH_3 and NCH_2), 2.82 (s, 2H, CH-CH bridge protons), 1.95–0.81 (m, 40H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

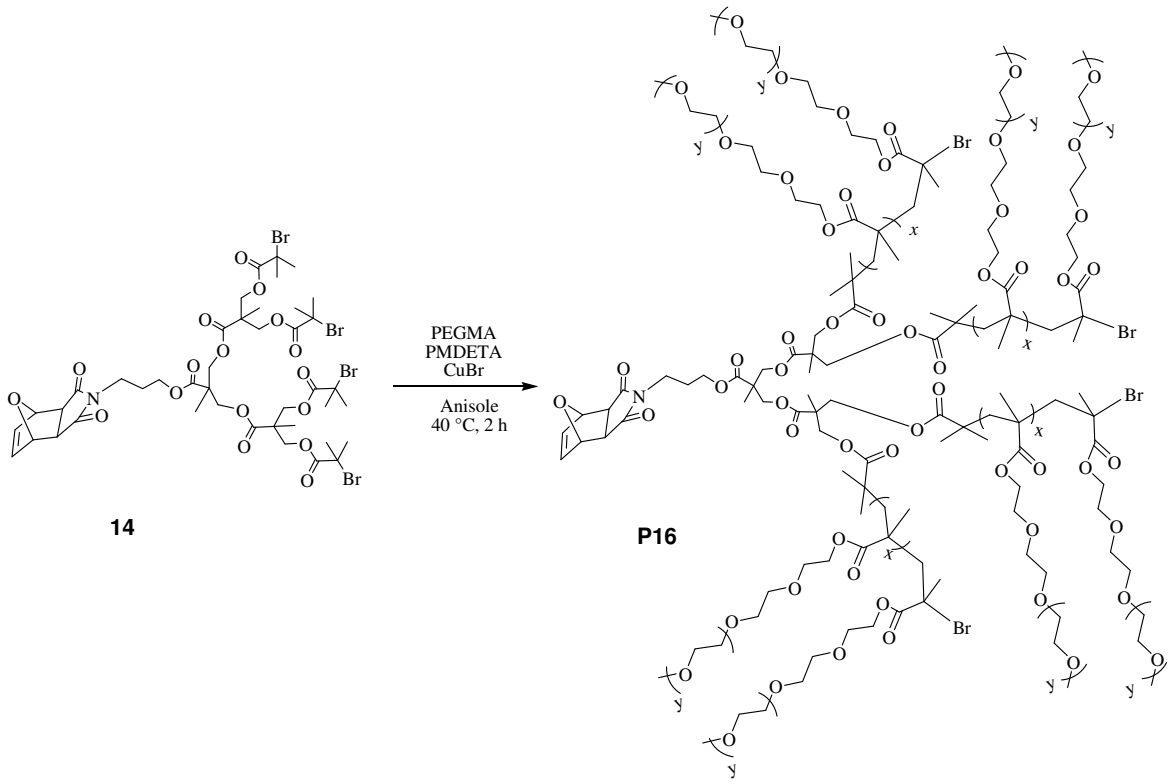

Figure 4.20. G2-PEGMEMA Synthesis

4.3.10. Synthesis of furan protected maleimide end-functionalized G3-PEGMEMA (P18)

G3-PEGMEMA was prepared by ATRP of PEGMEMA. Furan protected G3-initiator **(15)** (20.0 mg, 0.009 mmol) dissolved in a minimum amount of degassed anisole was introduced into a flask containing CuBr (10.3 mg, 0.072 mmol), degassed PMDETA (30.0 mg, 0.144 mmol) and degassed PEGMA (2.05 mL, 7.18 mmol) dissolved in degassed anisole (10.25 ml) under stirring. The flask was then placed in a thermostated oil bath at 40 °C for 240 min. After polymerization the reaction mixture was passed through a neutral alumina column to remove the catalyst and precipitated in diethyl ether twice. G3-PEGMA was obtained as a white solid. $[M]_0/[I]_0$= 800; $[I]_0$:[CuBr]:[PMDETA]=1:8:16; conversion = 14 %. $M_{n,theo}$= 80420, $M_{n,NMR}$= 92380, $M_{n,GPC}$= 32470, M_w/M_n= 1.12, relative to PS. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.46 (s, 2H, CH=CH), 5.13 (s, 2H, CH bridgehead

protons), 4.27-4.15 (m , 30H, OCH_2 and CH_2 ester protons), 4.02 (br s, 2H, OCH_2 of PEGMEMA), 3.76-3.38 (m, 2H, OCH_2 of PEGMEMA), 3.31 (br s, 5H, OCH_3 and NCH_2), 2.79 (s, 2H, CH-CH bridge protons), 1.98–0.80 (m, 76H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

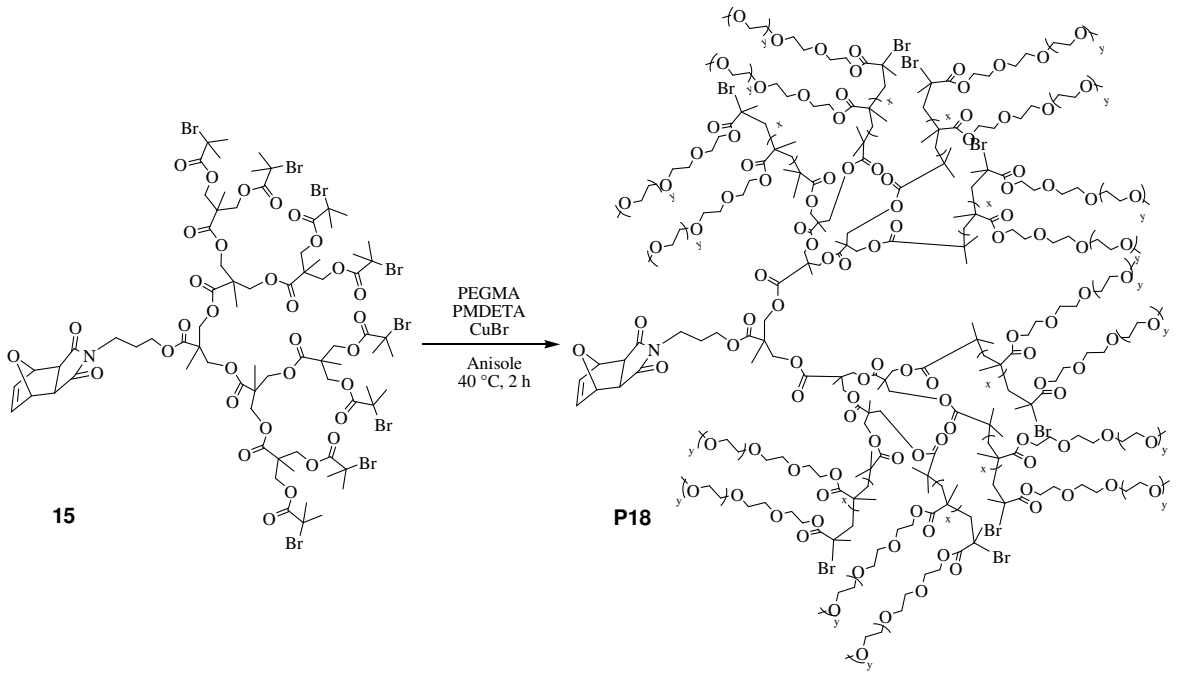

Figure 4.21. G3-PEGMEMA Synthesis

4.3.11. Activation of P15 by cyclorevesion reaction (P20)

P15 (25.0 mg, 1.16 μmol) was heated at 100 °C for 5 hours under high vacuum. NMR analysis proved quantitative conversion of the oxabicyclic moiety to the maleimide functional group. ^1H NMR (D_2O, δ, ppm) 7.03 (s, 2H, CH=CH), 4.21 (br s, 16H, OCH_2, CH_2 ester protons and OCH_2 of PEGMEMA), 3.90-3.66 (m, 2H, OCH_2 of PEGMEMA), 3.42 (br s, 5H, OCH_3 and NCH_2), 1.92–0.93 (m, 40H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone). ^1H NMR ($CDCl_3$, δ, ppm) 6.73 (s, 2H, CH=CH), 4.41-4.05 (m, 16H, OCH_2, CH_2 ester protons and OCH_2 of PEGMEMA), 3.80-3.44 (m, 2H, OCH_2 of PEGMEMA), 3.17 (br s, 5H, OCH_3 and NCH_2), 2.02–0.83 (m, 40H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

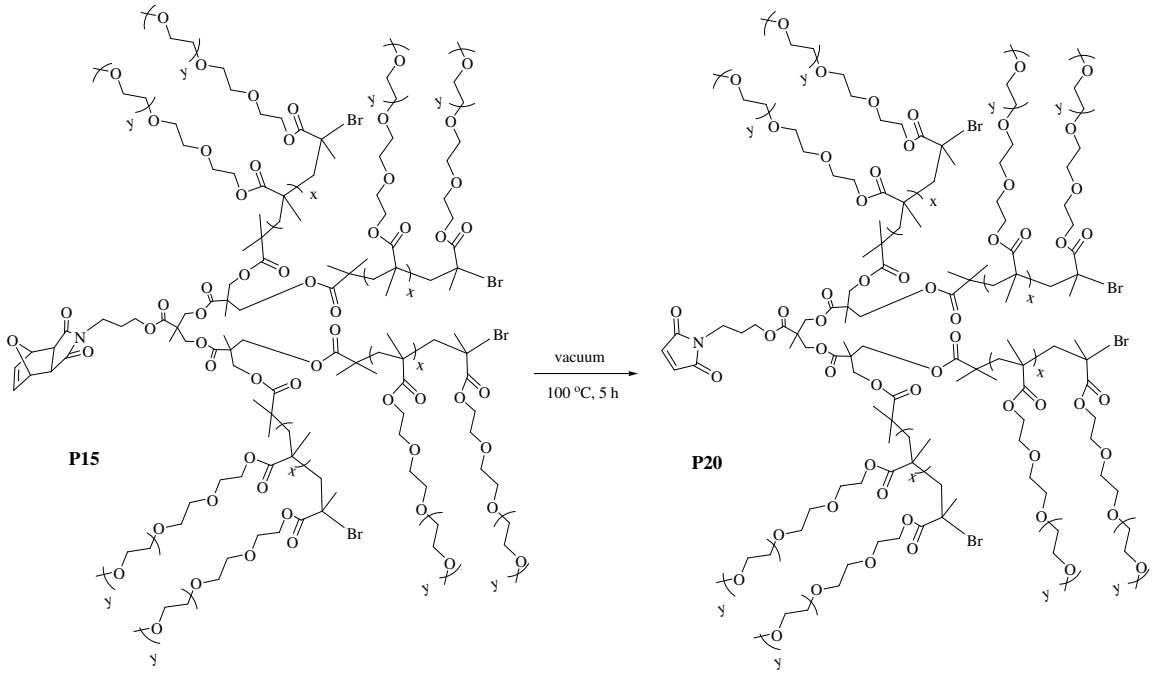

Figure 4.22. Activation of G2-PEGMEMA

4.3.12. Functionalization with glutathione (P21)

P20 (10 mg, 0.50 μmol) was dissolved in D_2O (0.5 mL) and degassed for 30 minutes. Glutathione was added (0.2 mL from 10 mg/mL stock solution in D_2O) and reacted at room temperature for 24 hours. ^{1}H NMR (D_2O, δ, ppm) 4.21 (br s, 16H, OCH_2, CH_2 ester protons and OCH_2 of PEGMEMA), 3.92-3.66 (m, 2H, OCH_2 of PEGMEMA), 3.42 (br s, 5H, OCH_3 and NCH_2), 2.56 (br s, 2H, SCH_2 of glutathione), 2.17 (br s, 4H, $COCH_2CH_2$ of glutathione), 1.98–0.92 (m, 40H, $NCH_2CH_2CH_2O$, $C(CH_3)$, $CBr(CH_3)$, CH_2 and CH_3 along polymer backbone).

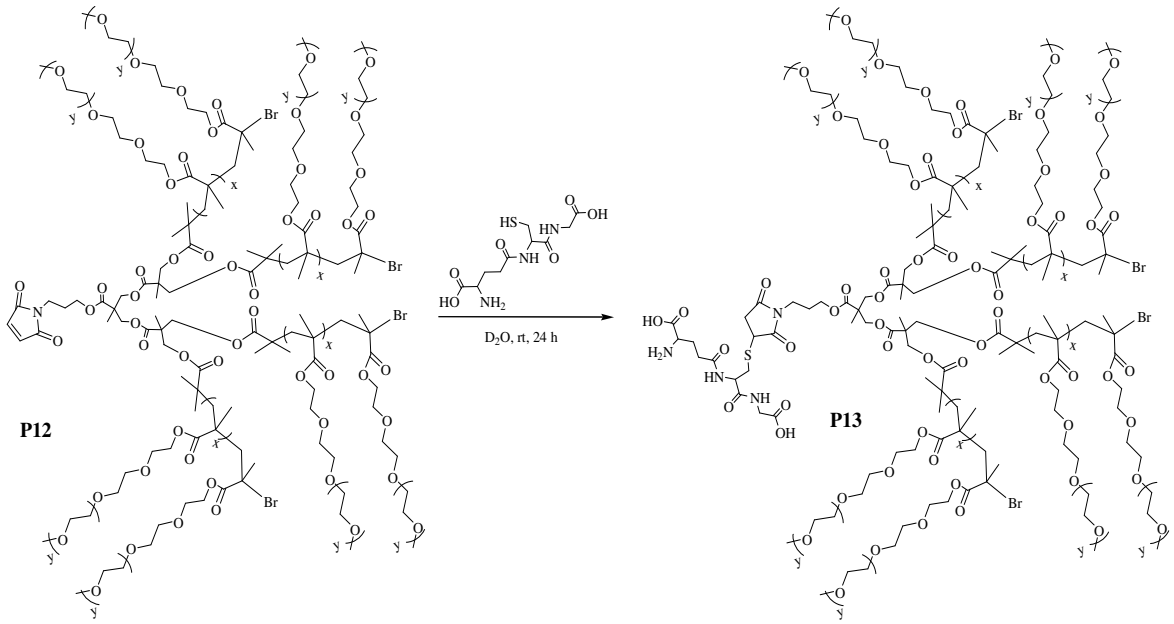

Figure 4.23. Functionalization of G2-PEGMEMA with glutathione

5. CONCLUSIONS

In the first study, dendron-polymer diblock and triblock copolymers were synthesized using a novel Diels-Alder/retro Diels-Alder strategy. Furan masked maleimide containing homo/hetero telechelic PEG polymers were synthesized as interior block which provides the resultant copolymer with water solubility, anti-biofouling property and biocompatibility. As the exterior part, three generations of anthracene functionalized biodegradable polyester dendrons were synthesized. During the Diels Alder cycloaddition reaction at high temperature, furan group is released to unmask the maleimide group that reacts irreversibly with the anthracene moiety at the focal point of the dendron. The reaction ends up with dendron-polymer conjugates as well-defined and multivalent polymeric scaffolds which have a considerable potential to be used as carriers for drug delivery applications. Functionalization of the periphery of the copolymers with alkyne groups was achieved by acylation reaction and an antiretroviral drug AZT, containing azide functionality was reacted with alkyne groups of the copolymer via Huisgen type 'click' chemistry. This construct improves the drug loading capacity of PEG polymer. It is also expected that the drug will show higher water solubility and improved pharmacokinetic properties. As a future work, release of the drug molecule will be investigated.

In the second study, multiarm star polymers containing thiol-reactive maleimide groups at their focal point have been synthesized via atom transfer radical polymerization (ATRP) of various methacrylates and acrylates (MMA, *t*BA and PEGMEMA). Multiarm structure is obtained by utilizing three generations of maleimide functionalized dendritic initiators. The maleimide group at the core was obtained quantitatively in its native form by heating the polymer via retro Diels-Alder reaction. Post polymerization functionalization of the activated maleimide group was successfully achieved by using a thiol containing tripeptide, glutathione via Michael addition reaction.

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APPENDIX

^{1}H and ^{13}C NMR spectra of the synthesized products are included.

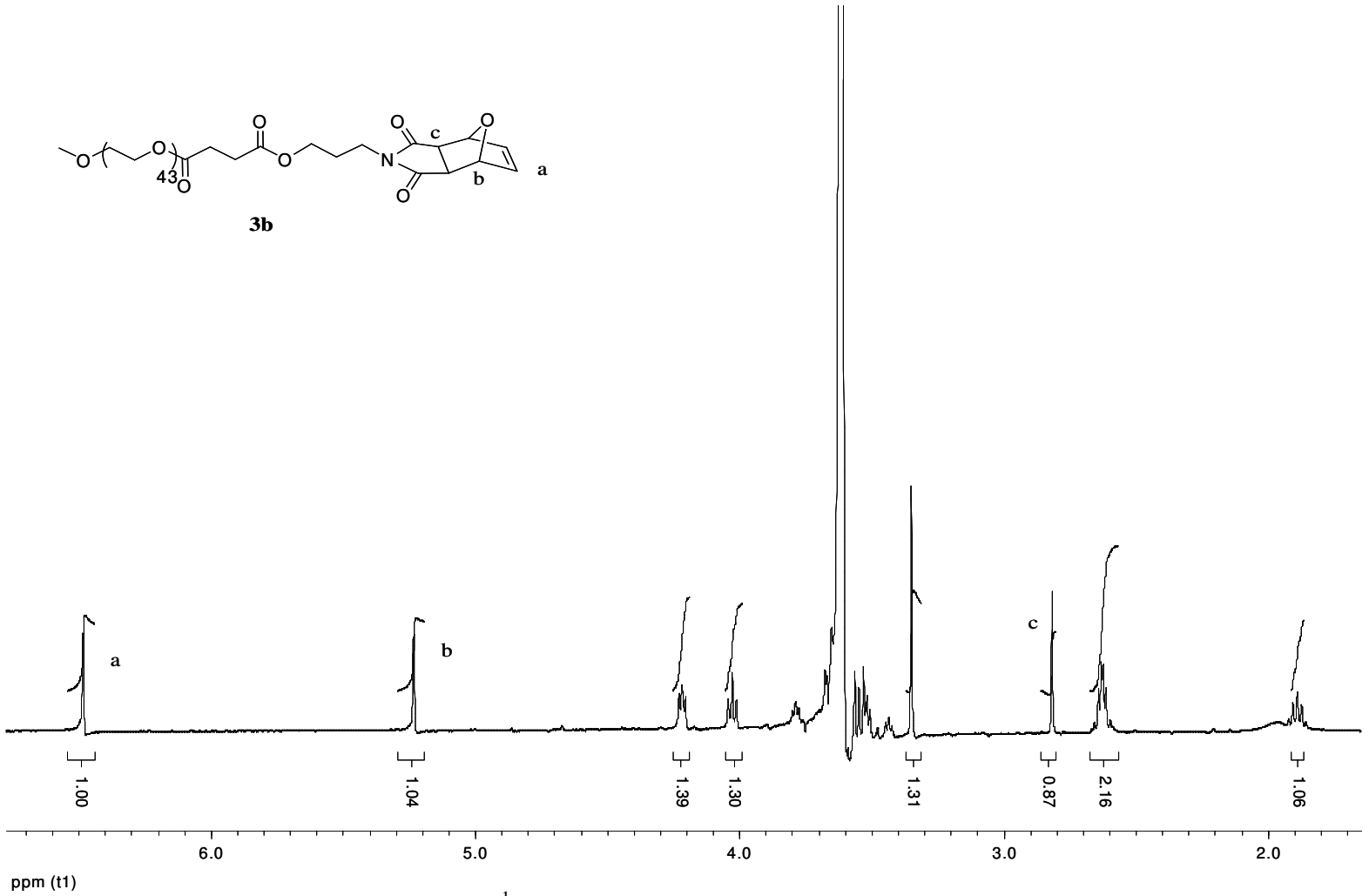

Figure A.1. ^{1}H NMR spectrum of Mono-maleimide PEG

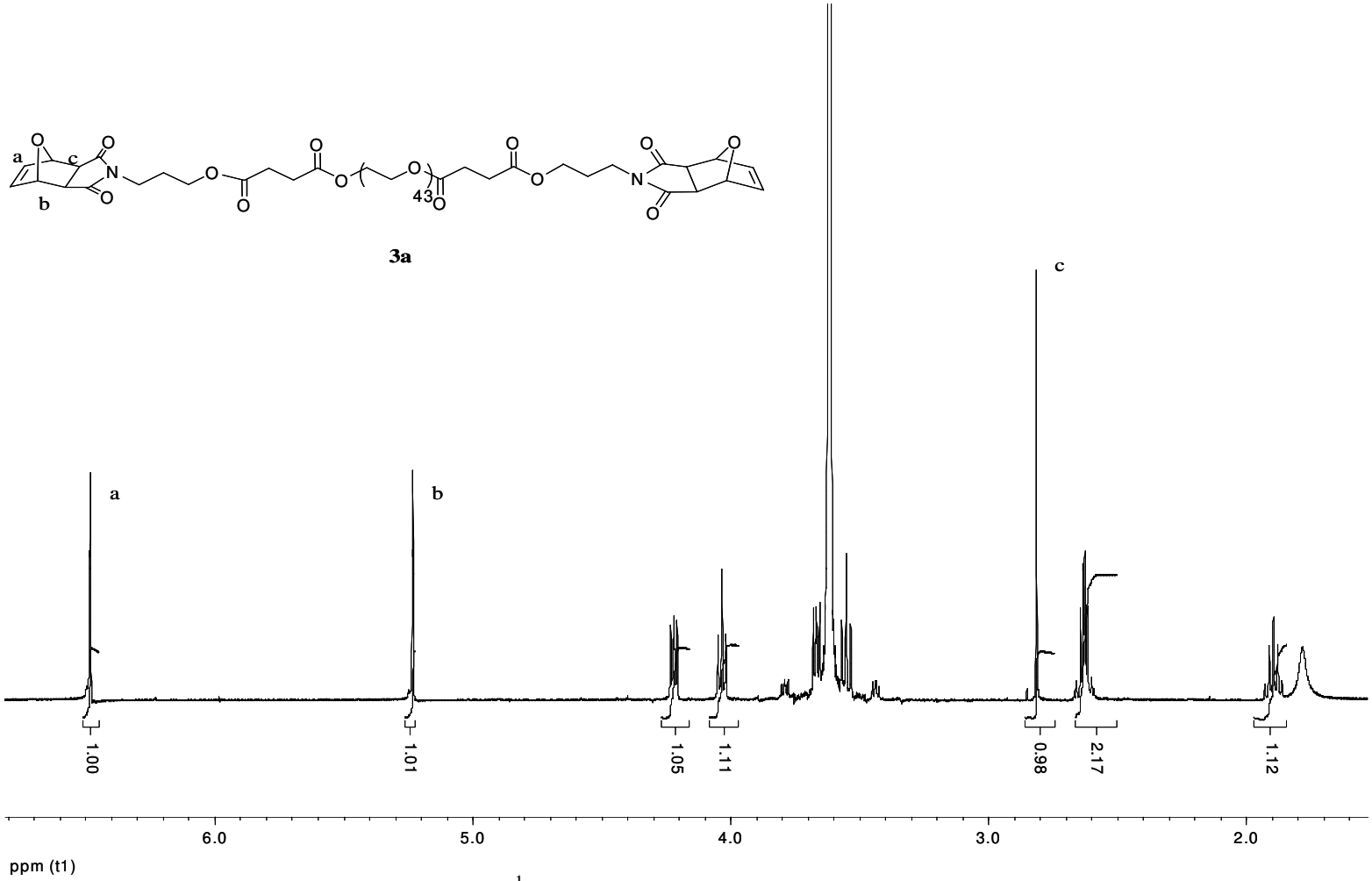

Figure A.2. ^{1}H NMR spectrum of Bis-maleimide PEG

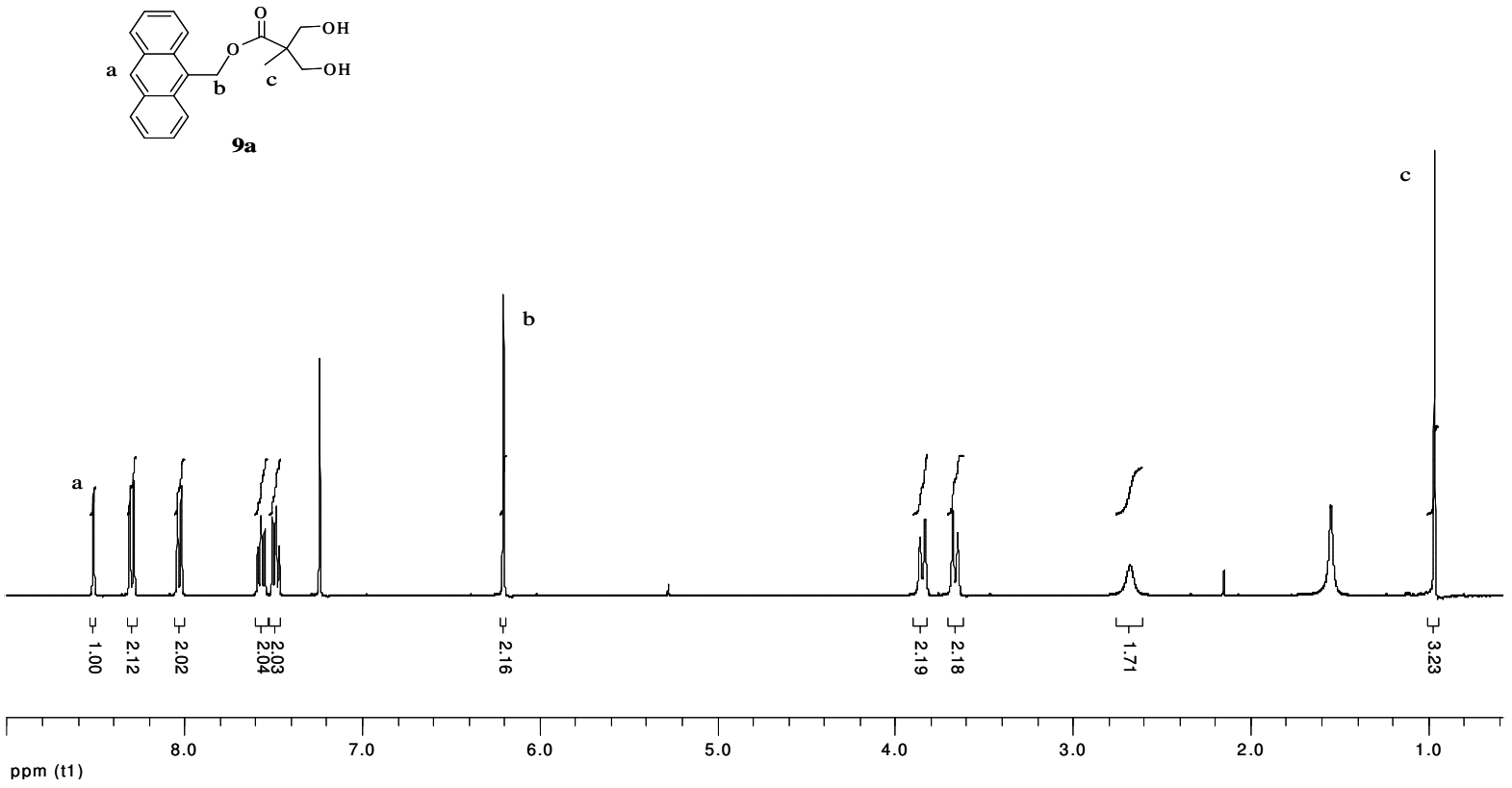

Figure A.3. ^{1}H NMR spectrum of 1st generation anthracene dendron

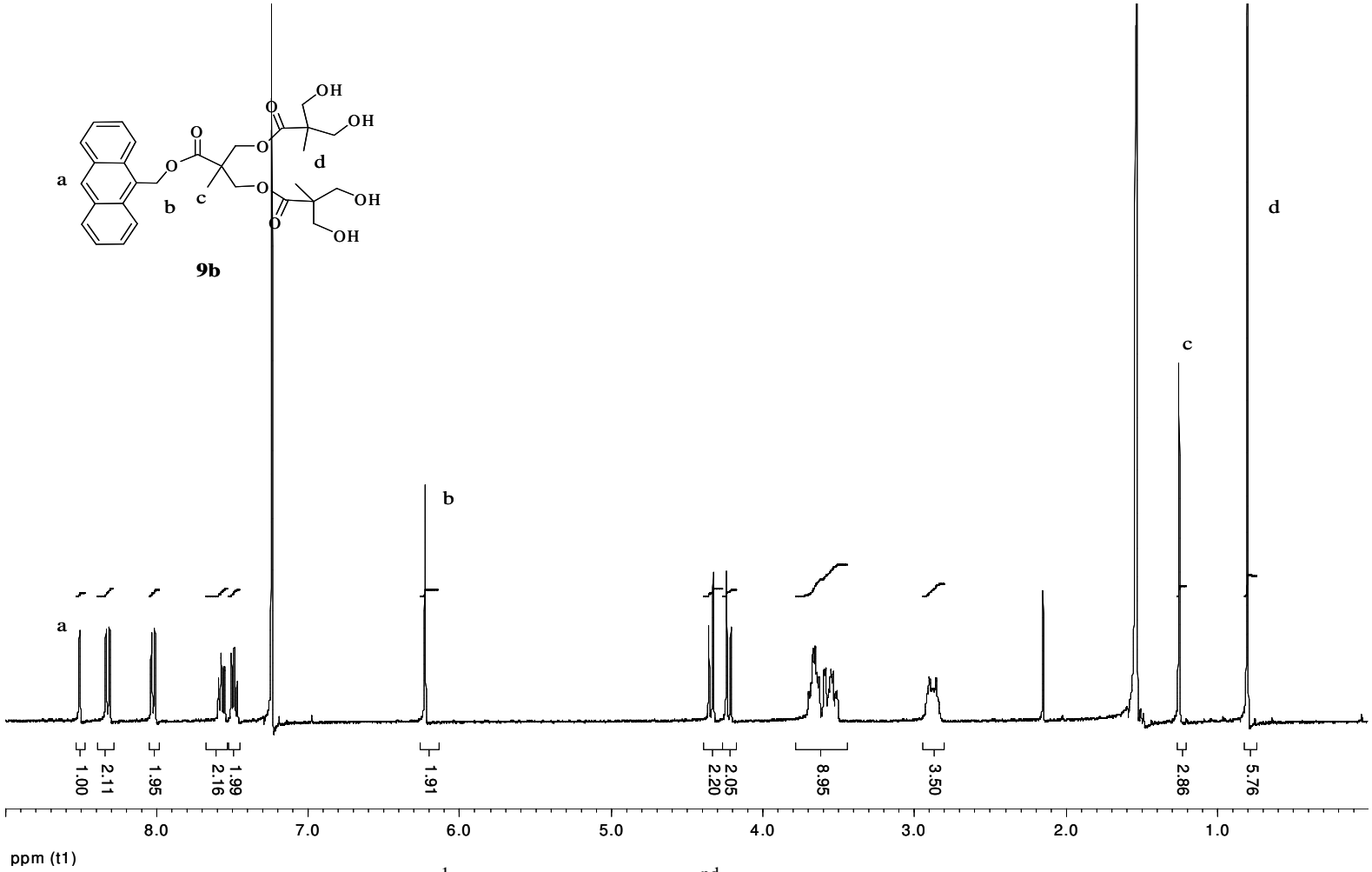

Figure A.4. ^{1}H NMR spectrum of 2nd generation anthracene dendron

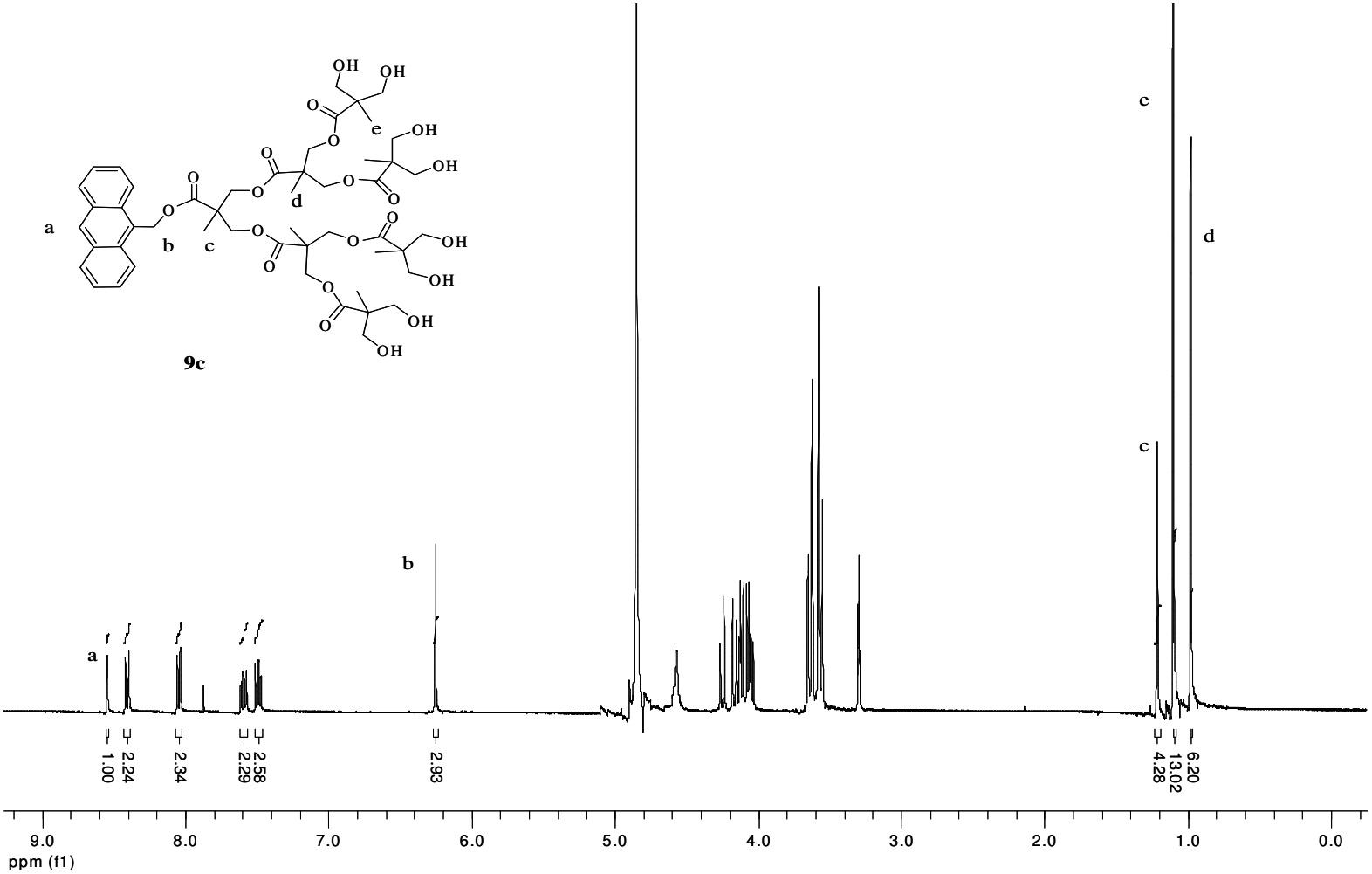

Figure A.5. ^{1}H NMR spectrum of 3rd generation anthracene dendron

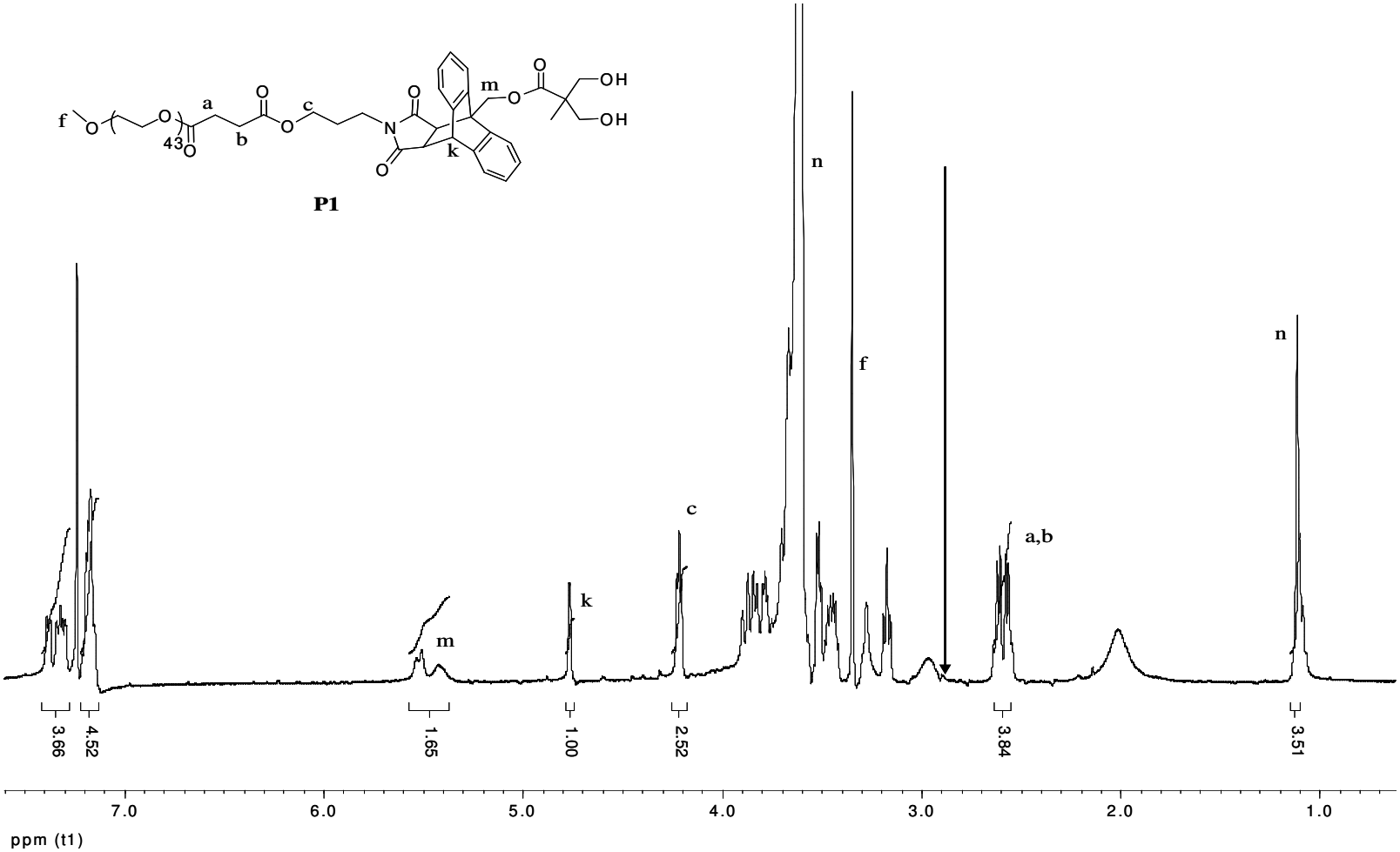

Figure A.6. ^{1}H NMR spectrum of **P1**

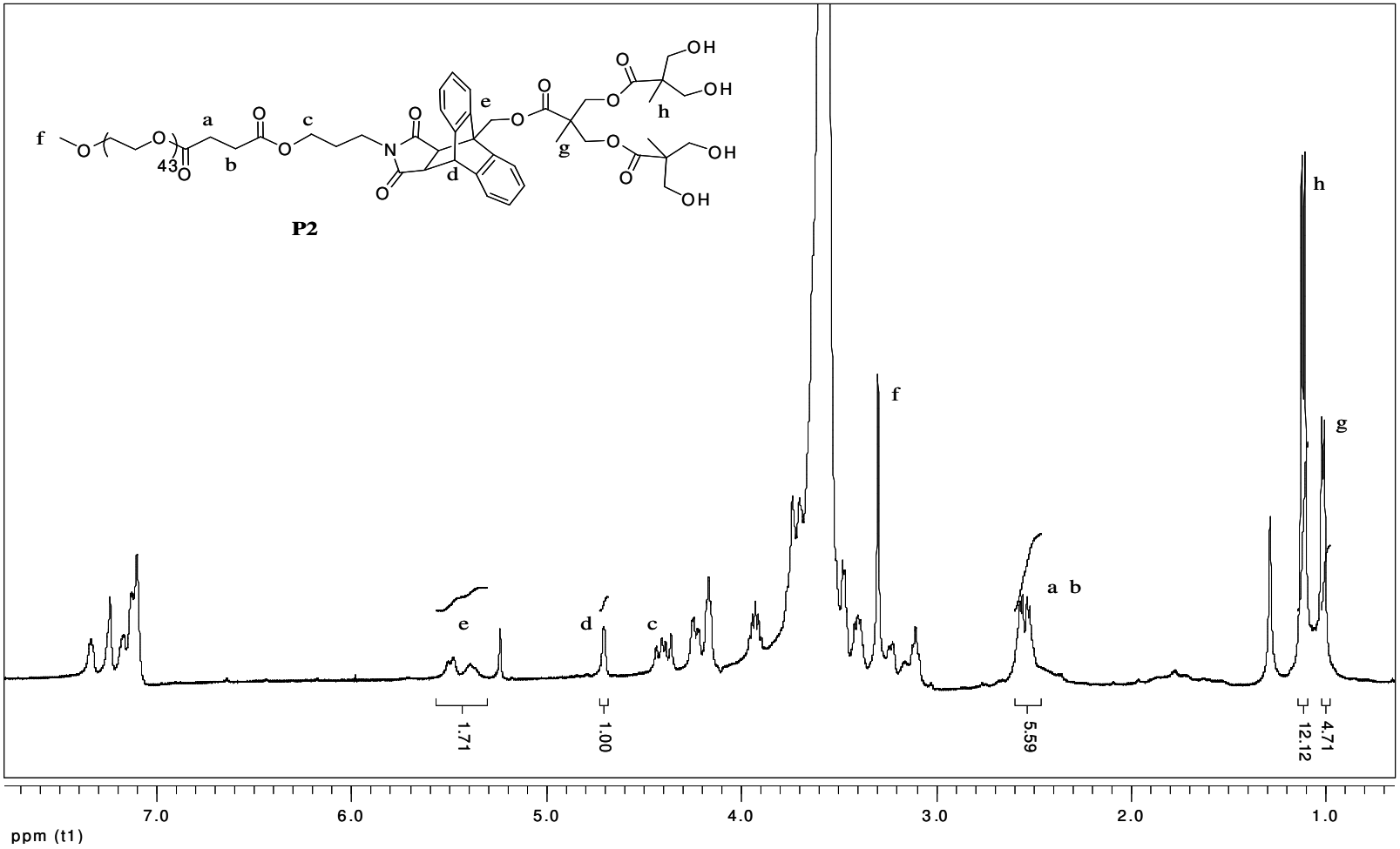

Figure A.7. ^{1}H NMR spectrum of **P2**

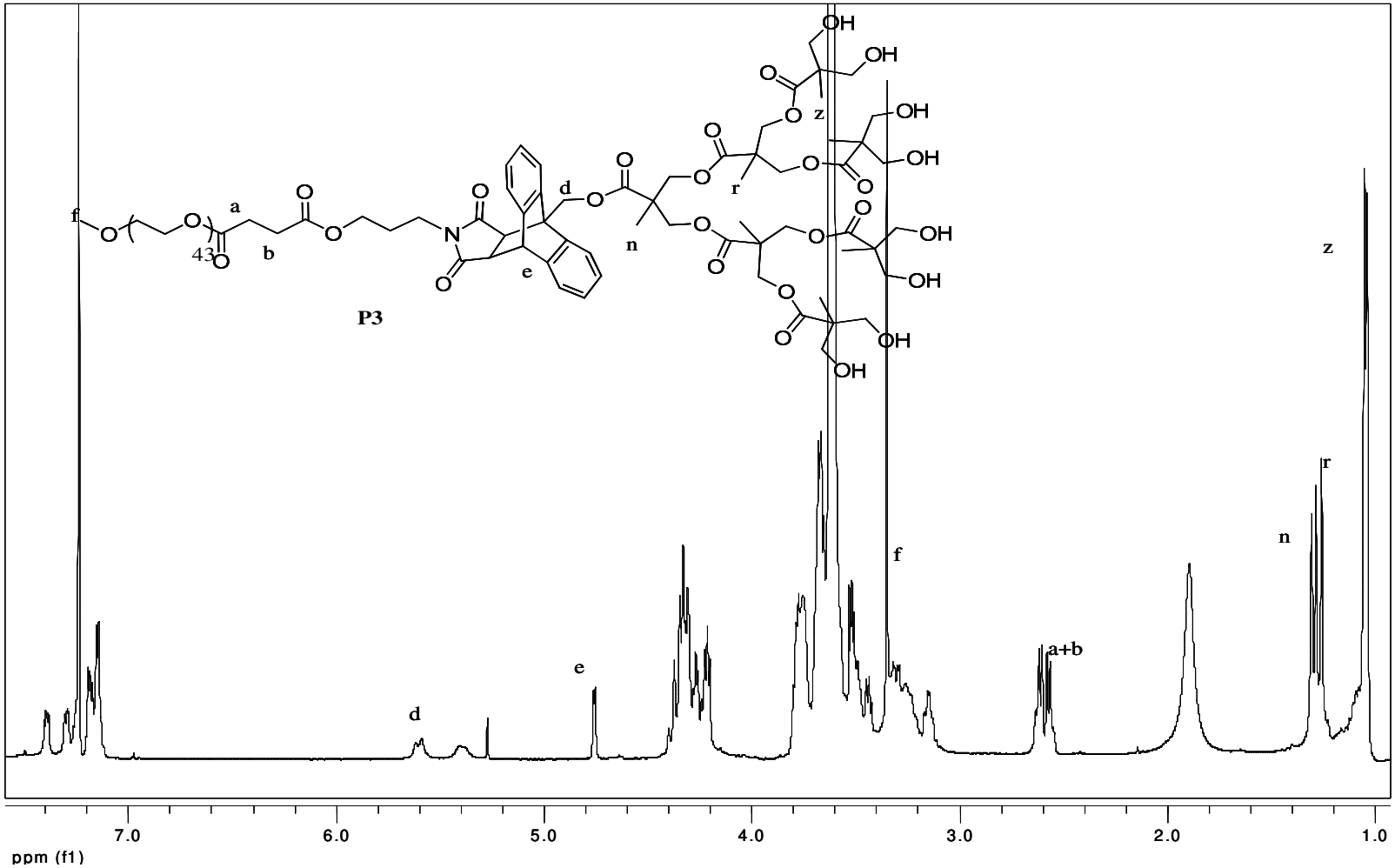

Figure A.8. ^{1}H NMR spectrum of **P3**

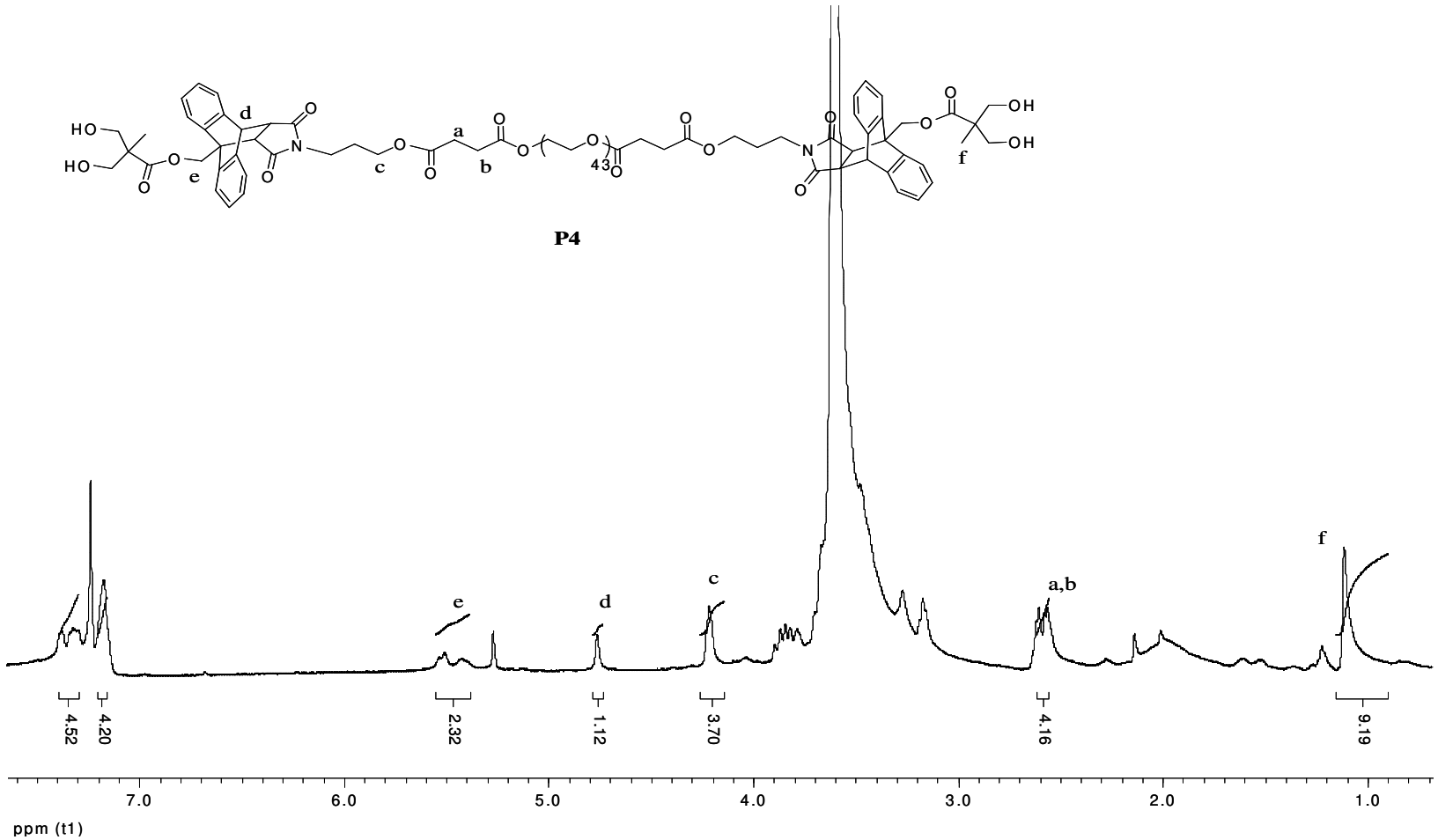

Figure A.9. ^{1}H NMR spectrum of **P4**

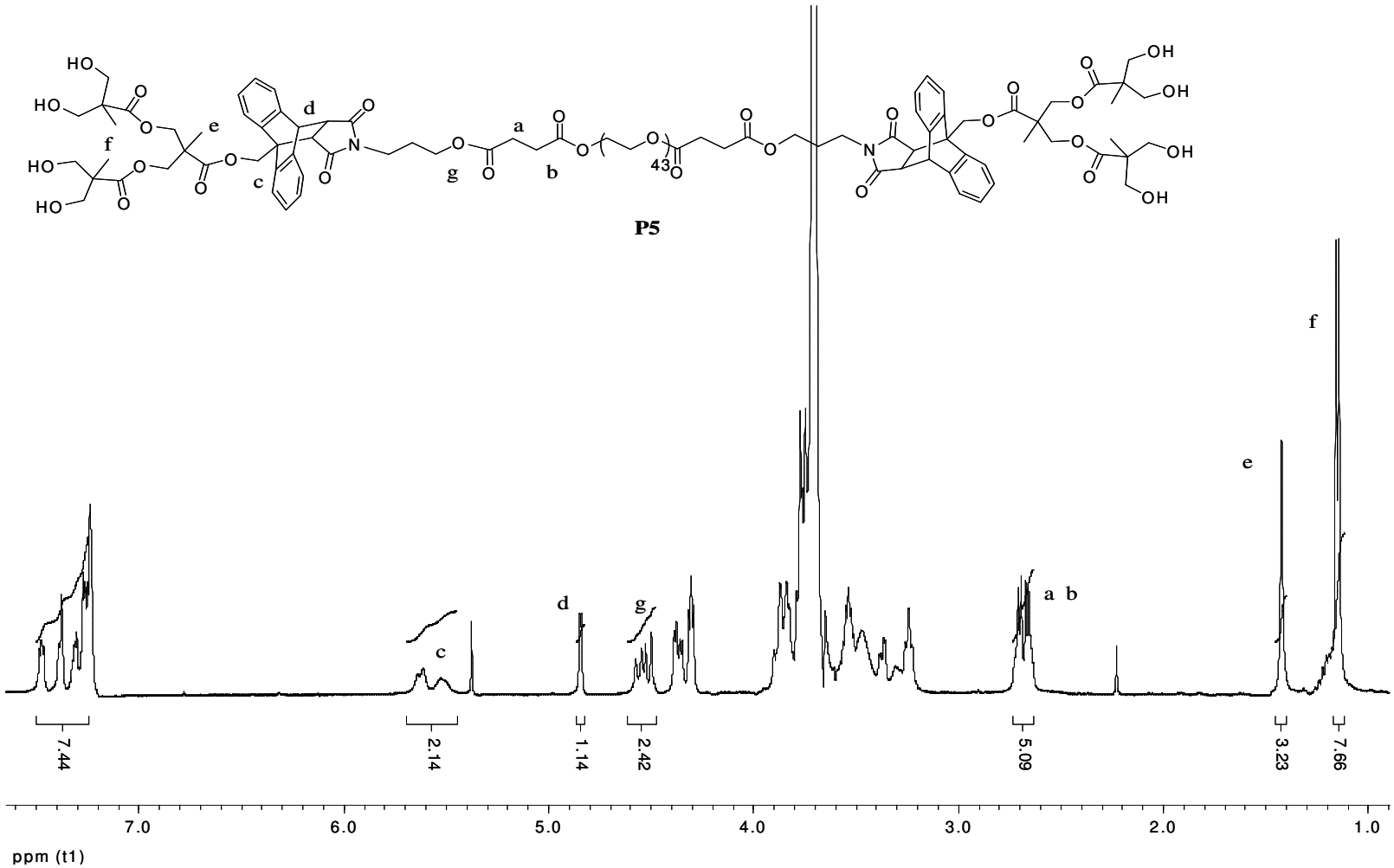

Figure A.10. ^{1}H NMR spectrum of **P5**

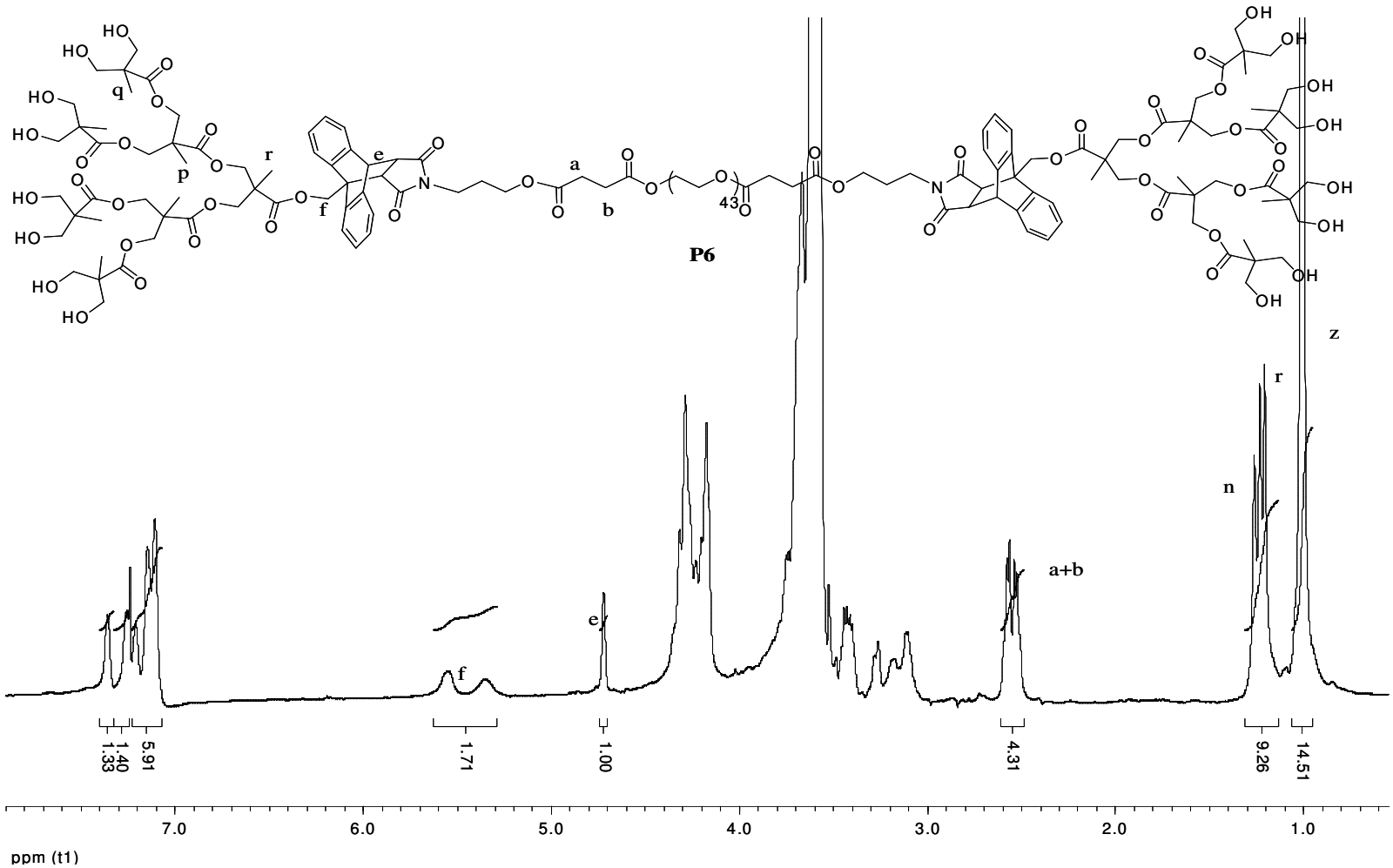

Figure A.11. ^{1}H NMR spectrum of **P6**

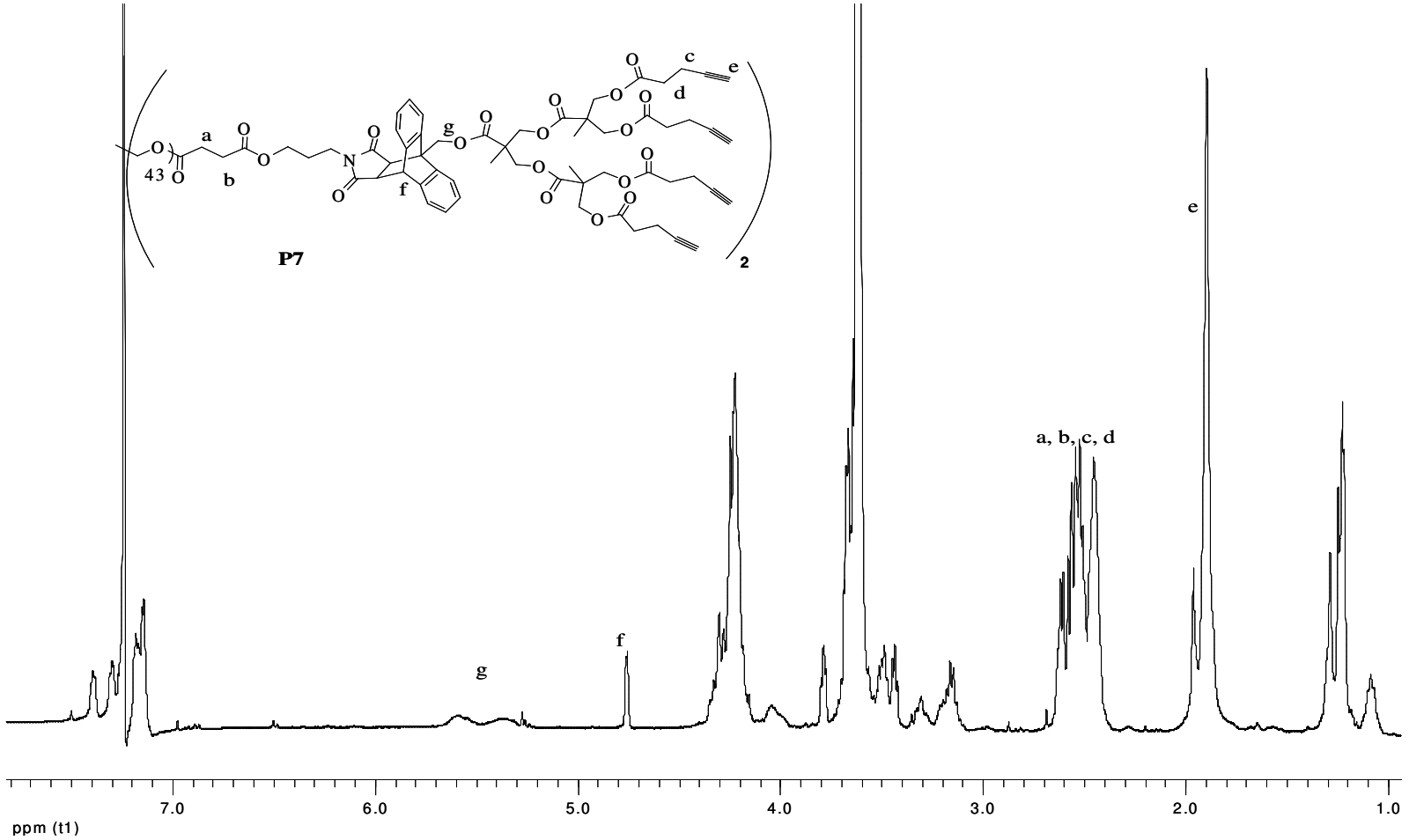

Figure A.12. ^{1}H NMR spectrum of **P7**

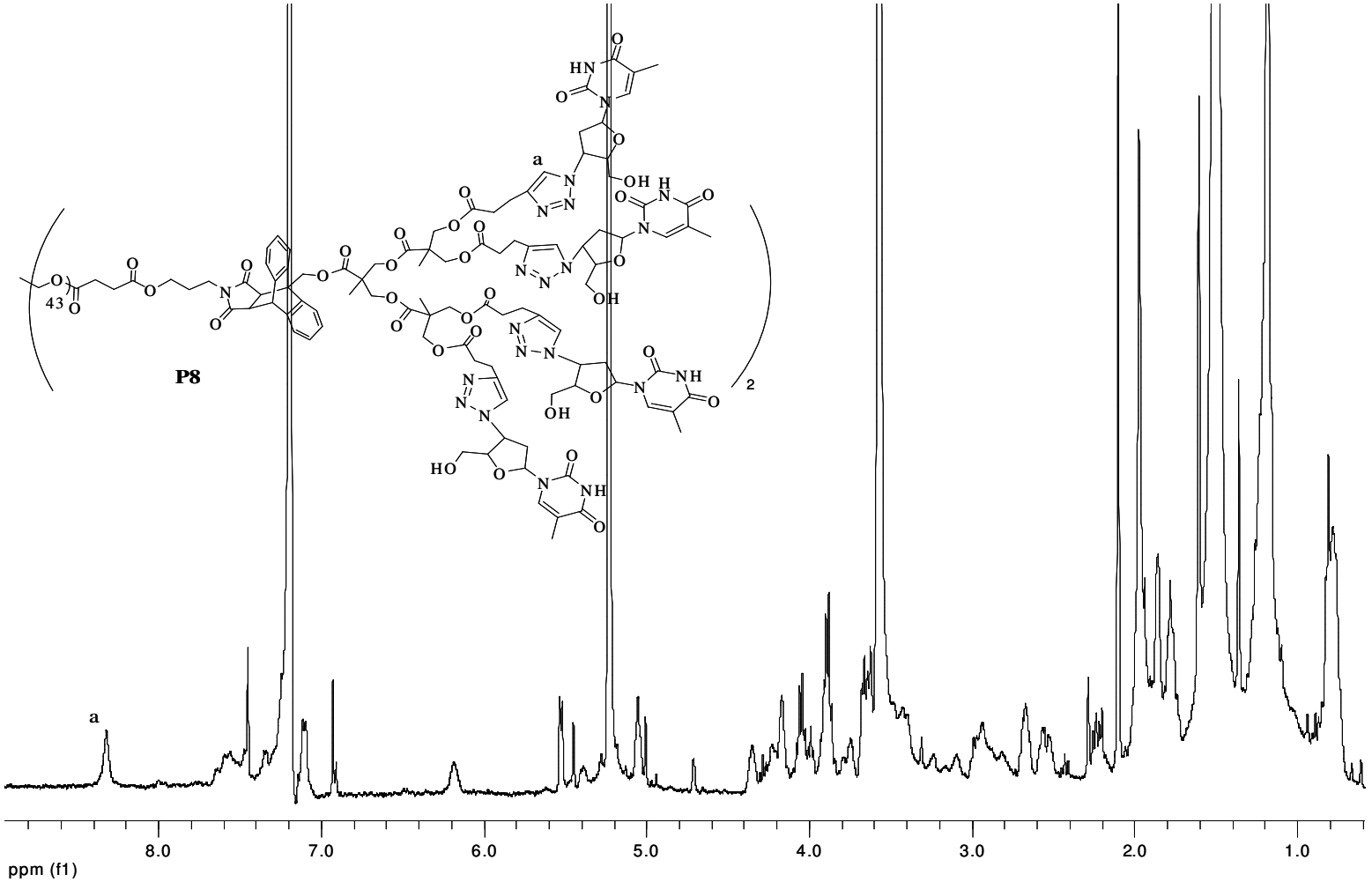

Figure A.13. ^{1}H NMR spectrum of **P8** (in DMSO)

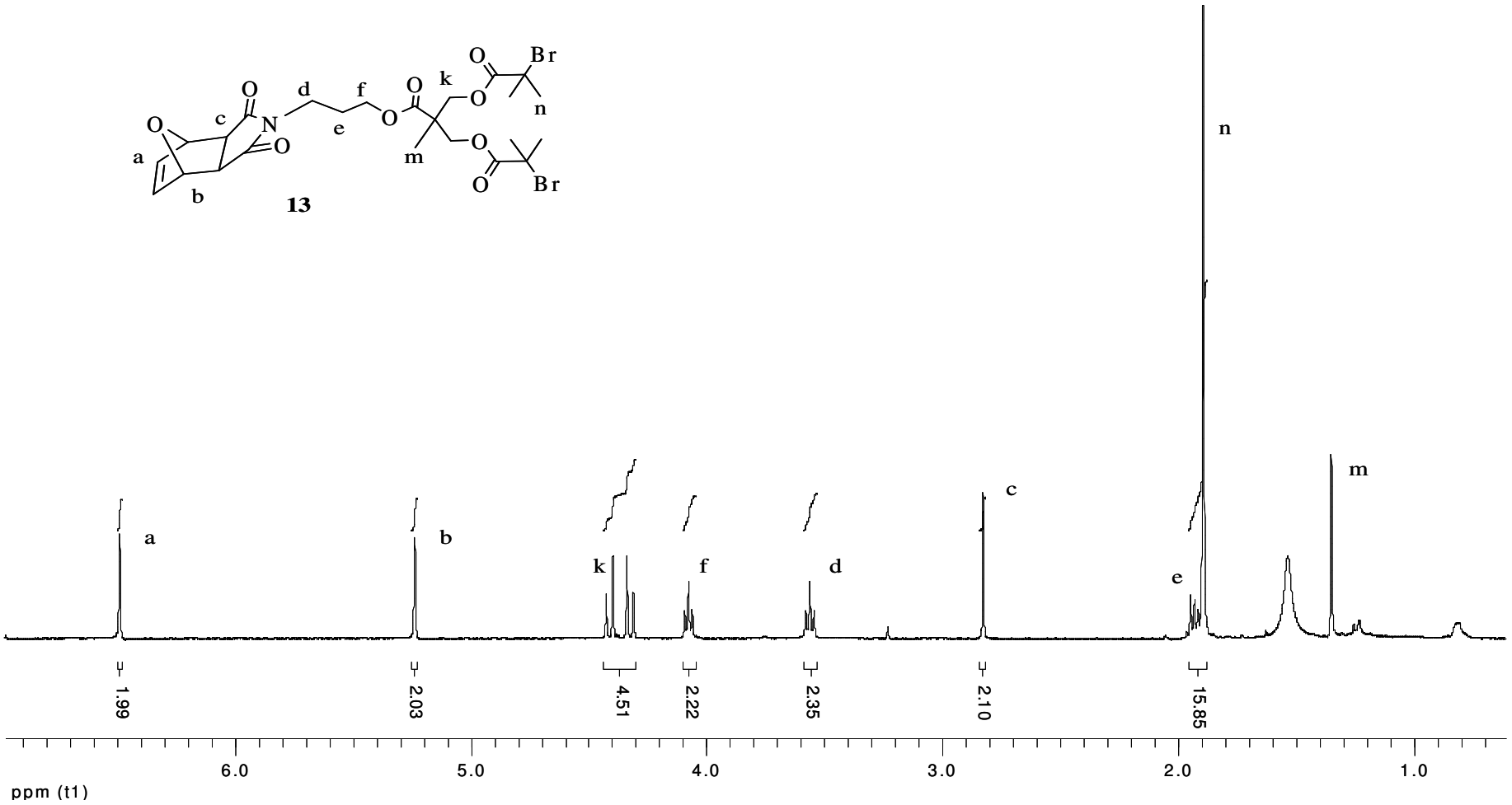

Figure A.14. ^{1}H NMR spectrum of 1st generation dendritic initiator

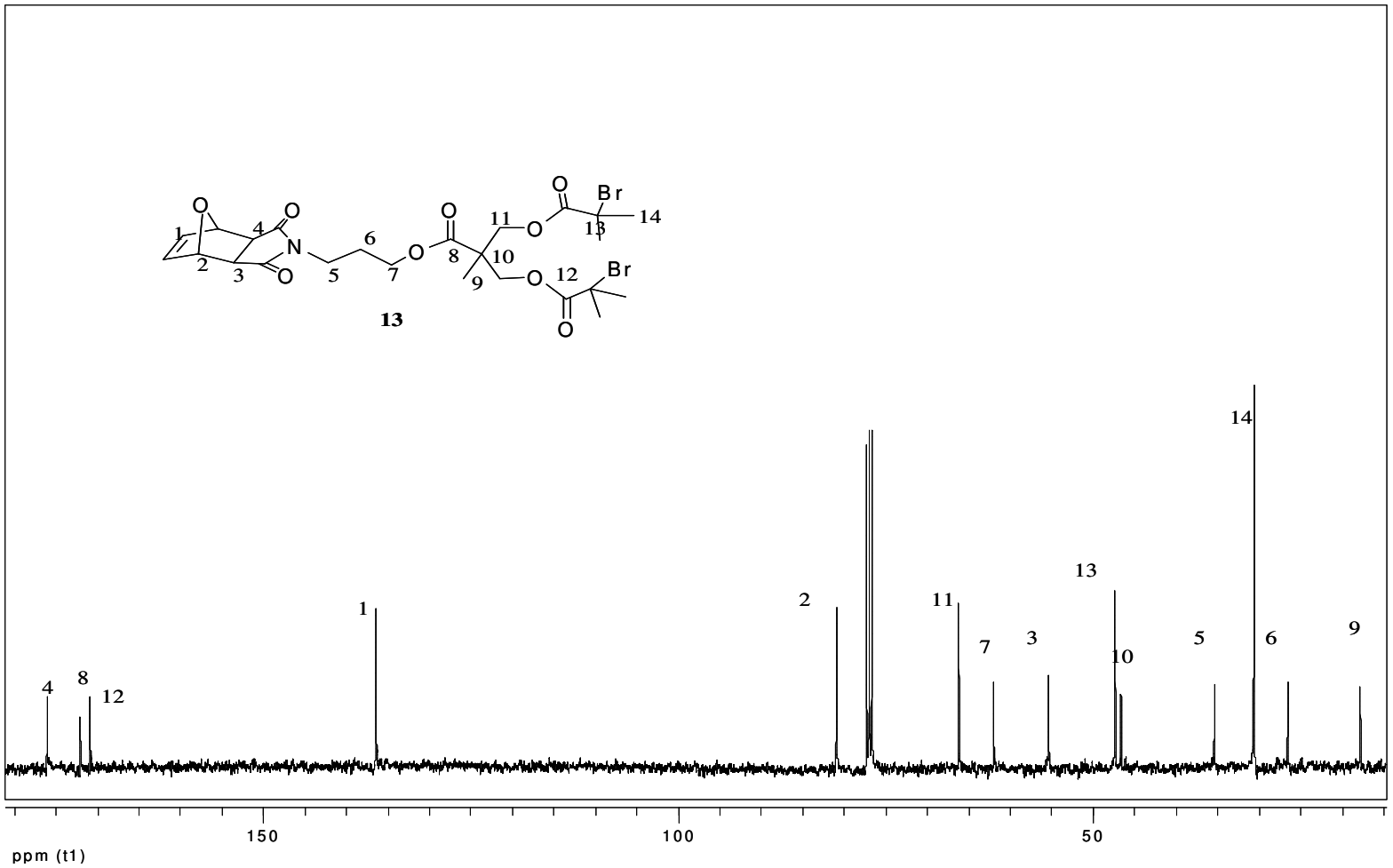

Figure A.15. ^{13}C NMR spectrum of 1st generation dendritic initiator

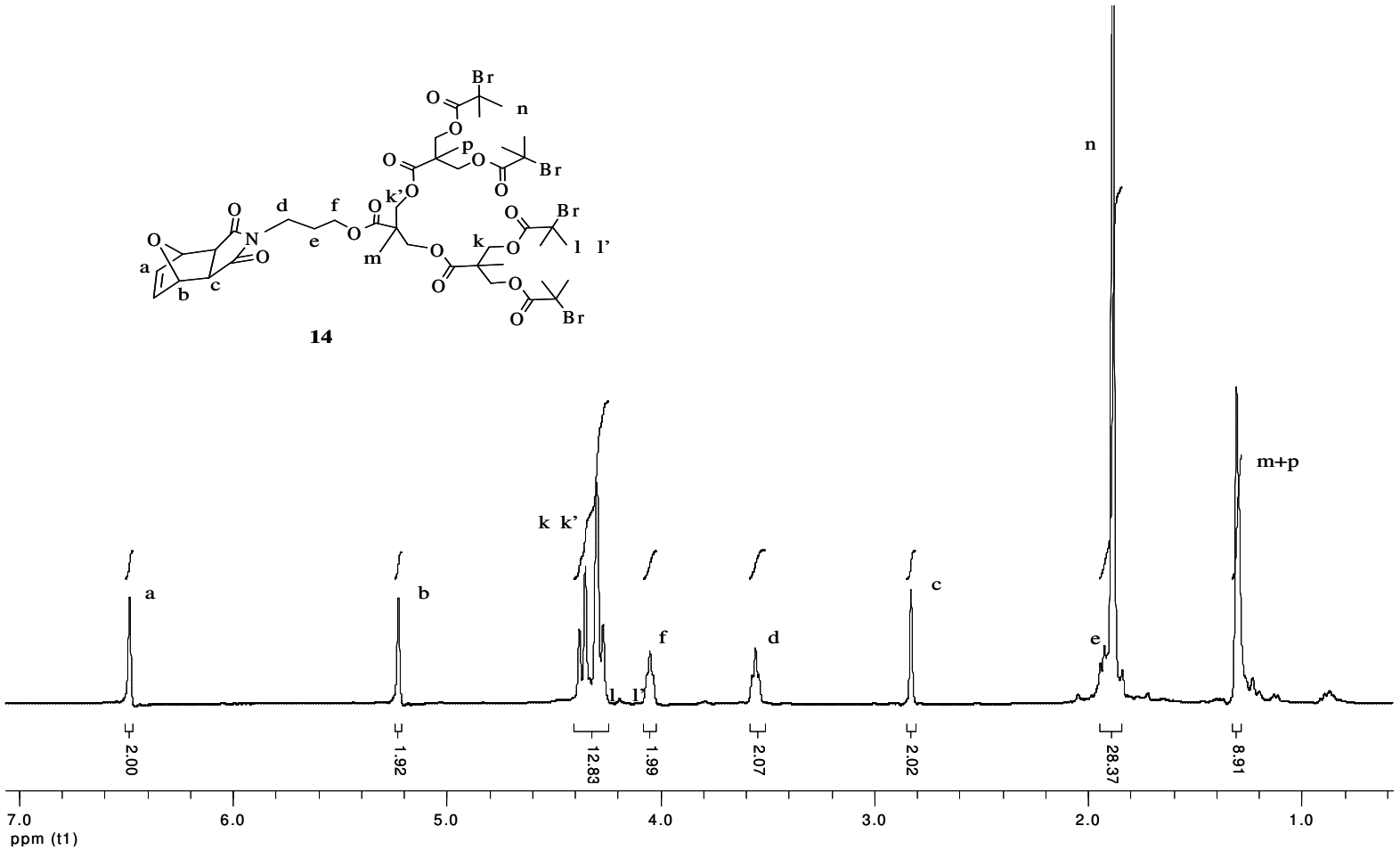

Figure A.16. ^{1}H NMR spectrum of 2nd generation dendritic initiator

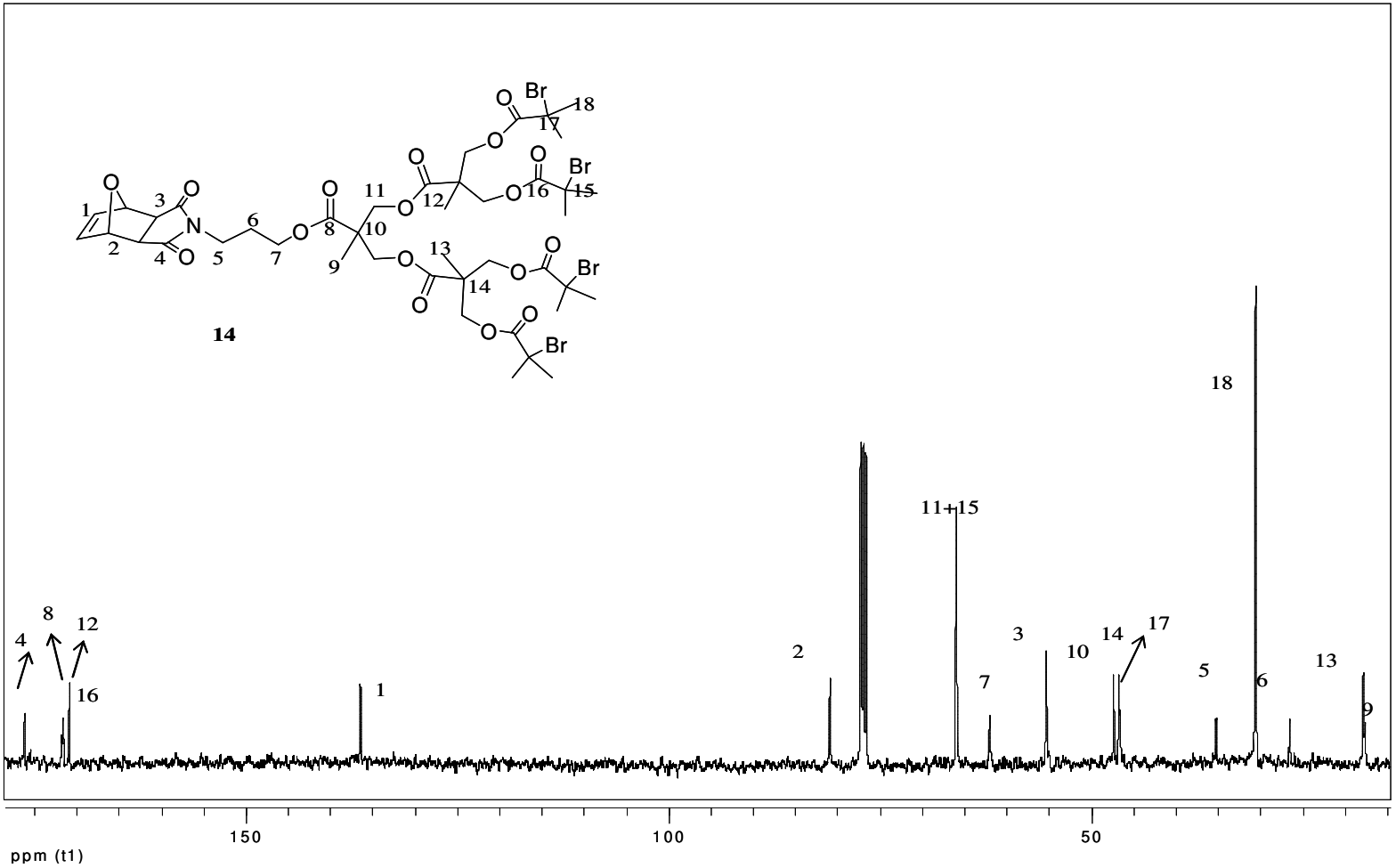

Figure A.17. ^{13}C NMR spectrum of 2nd generation dendritic initiator

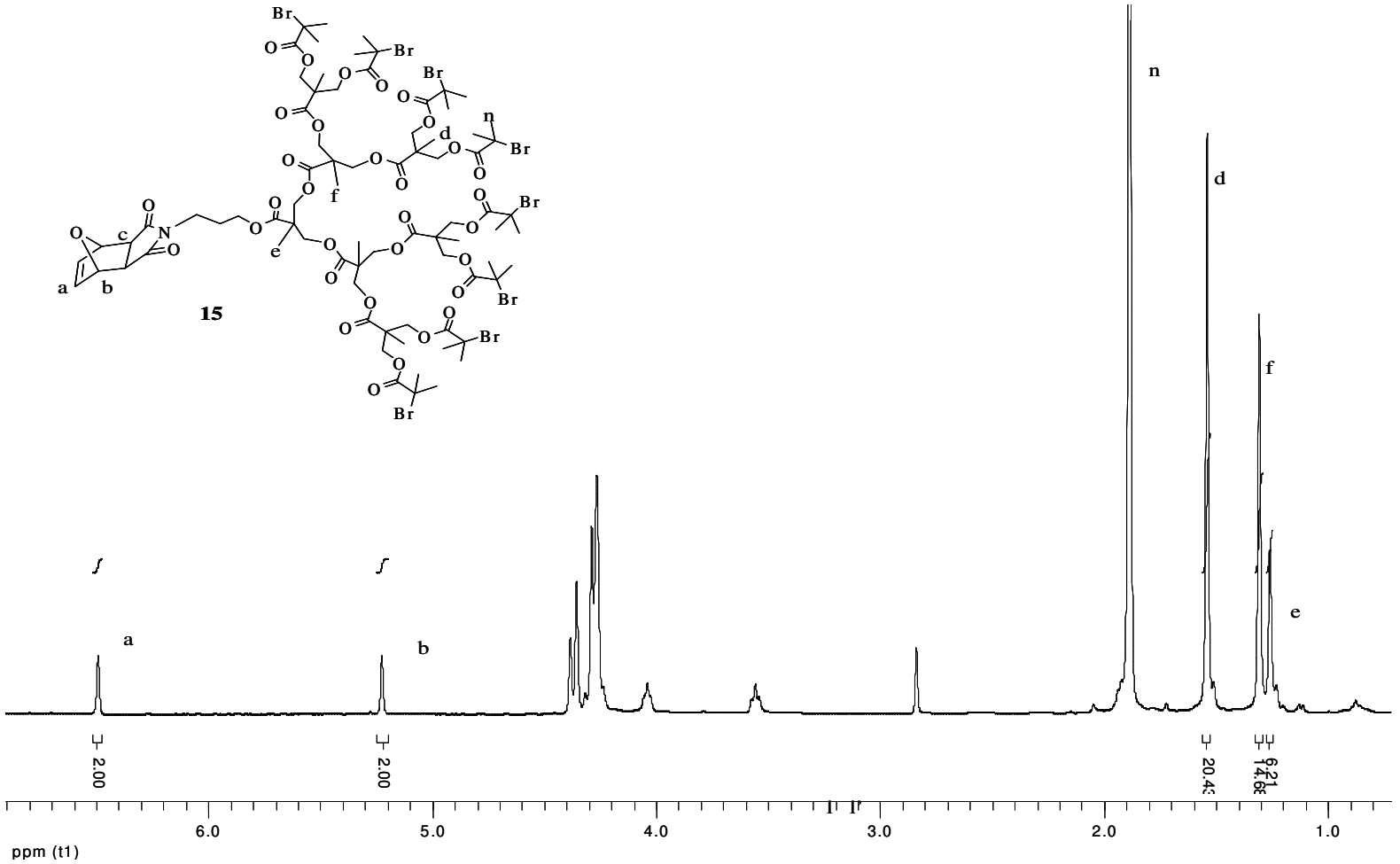

Figure A.18. ^{1}H NMR spectrum of 3rd generation dendritic initiator

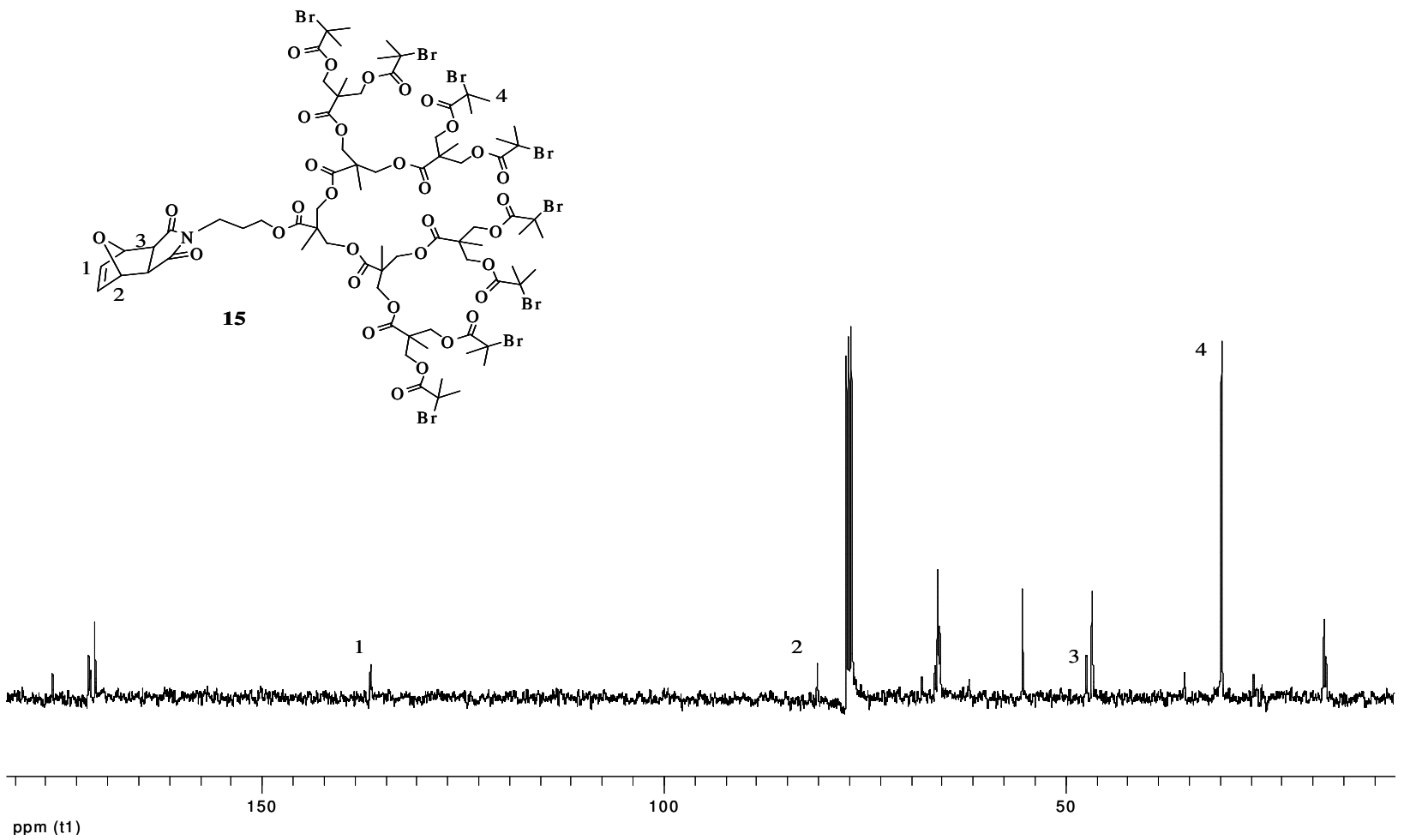

Figure A.19. ^{13}C NMR spectrum of 3rd generation dendritic initiator

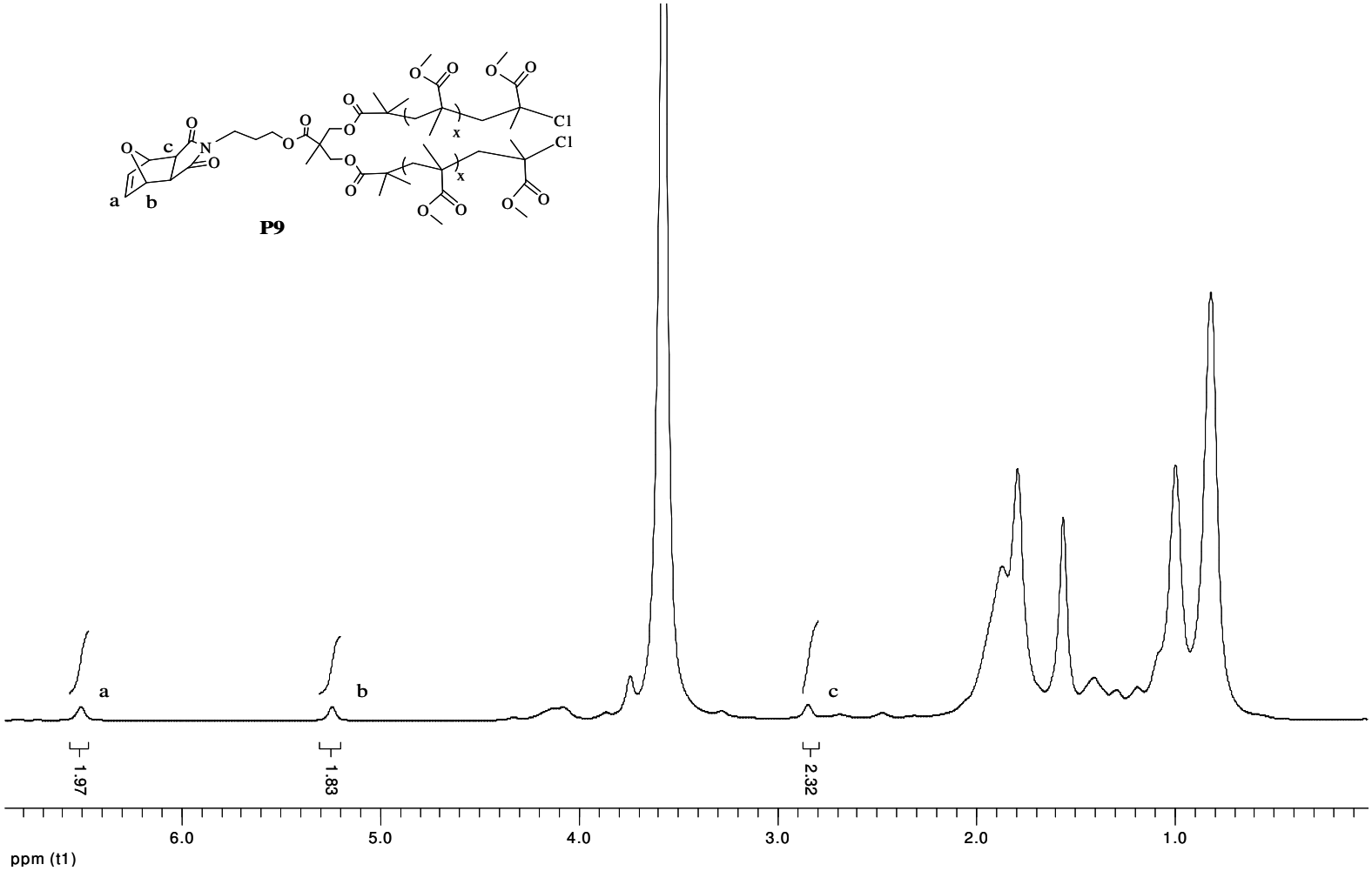

Figure A.20. ^{1}H NMR spectrum of **P9**

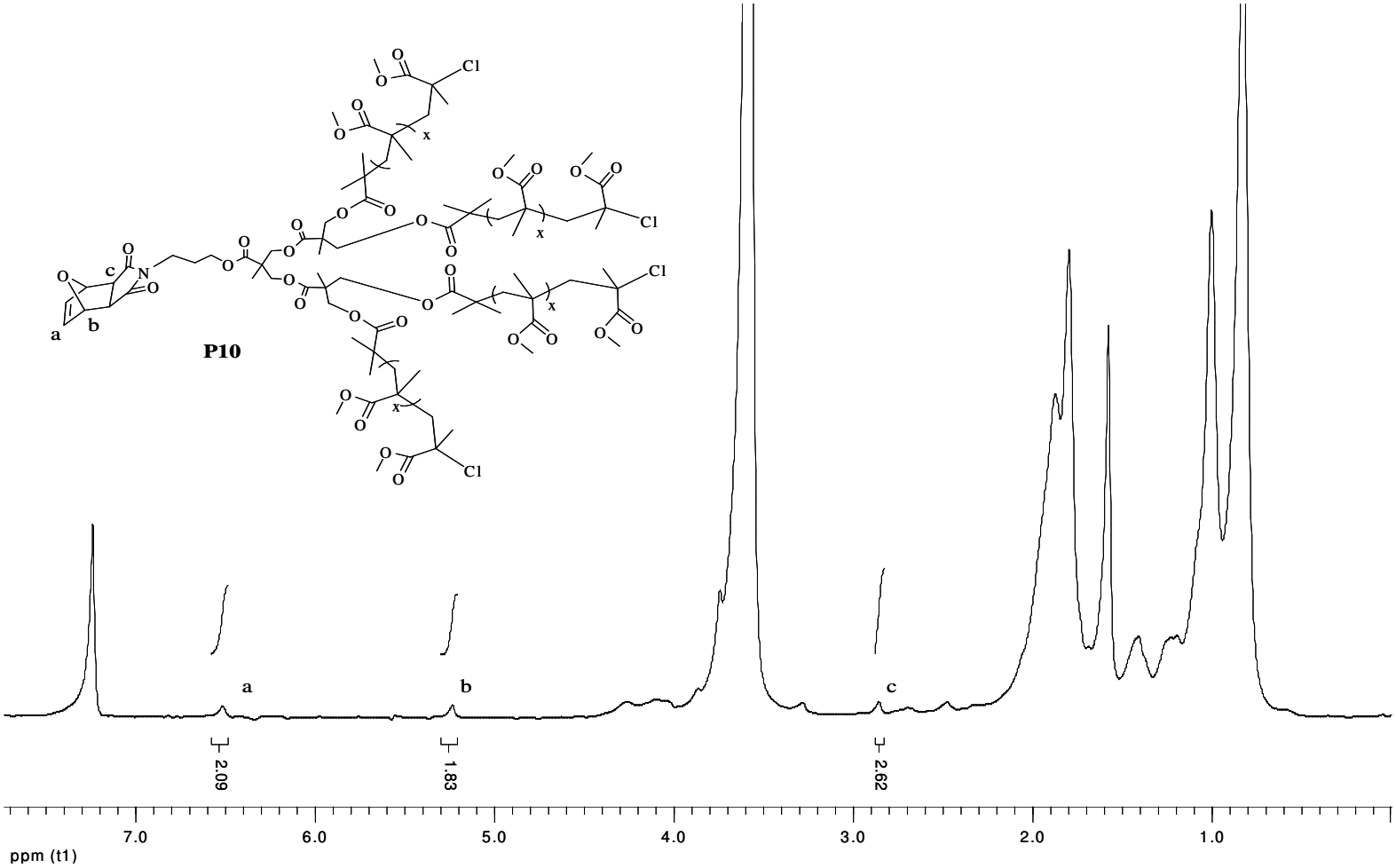

Figure A.21. ^{1}H NMR spectrum of **P10**

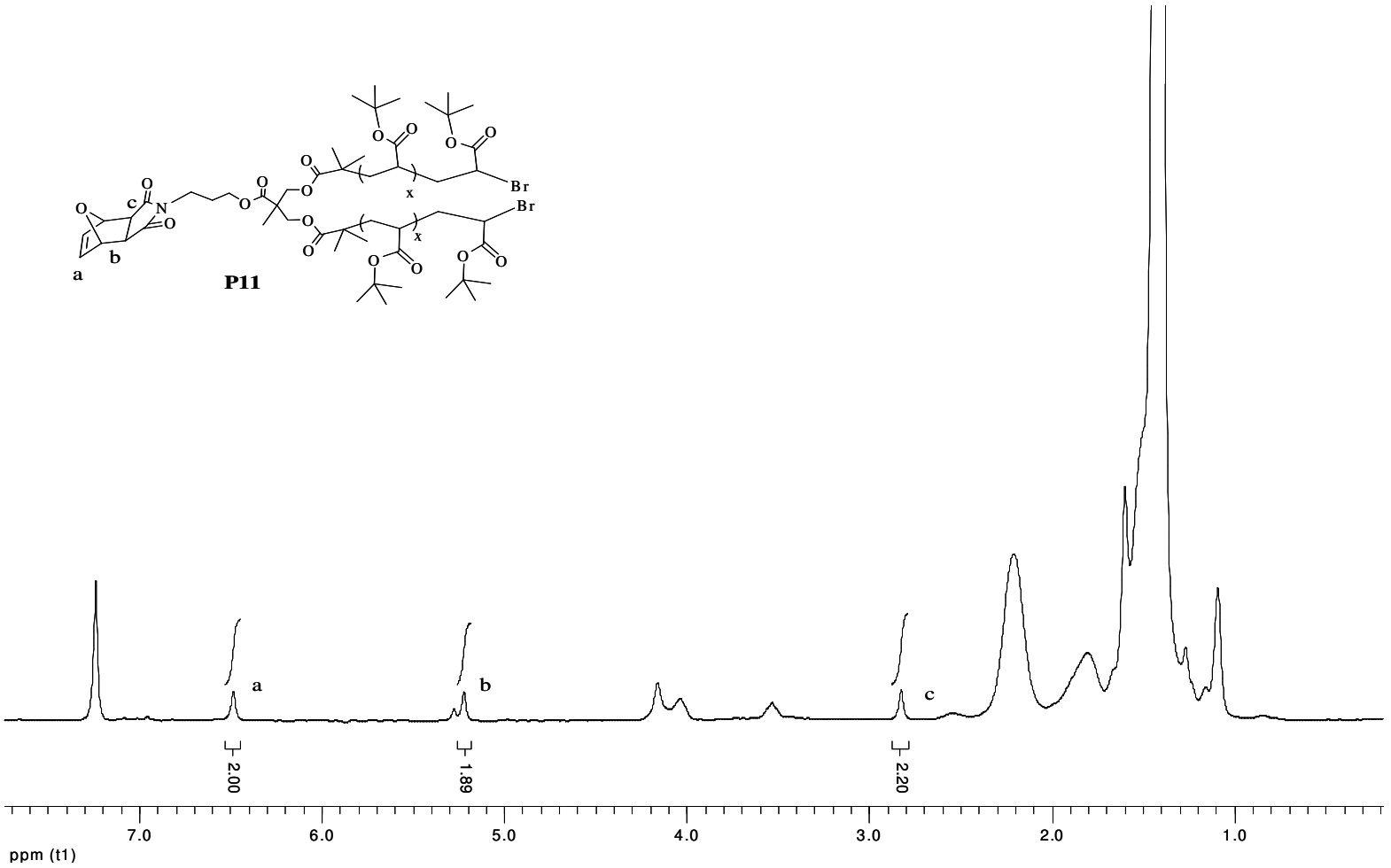

Figure A.22. ^{1}H NMR spectrum of **P11**

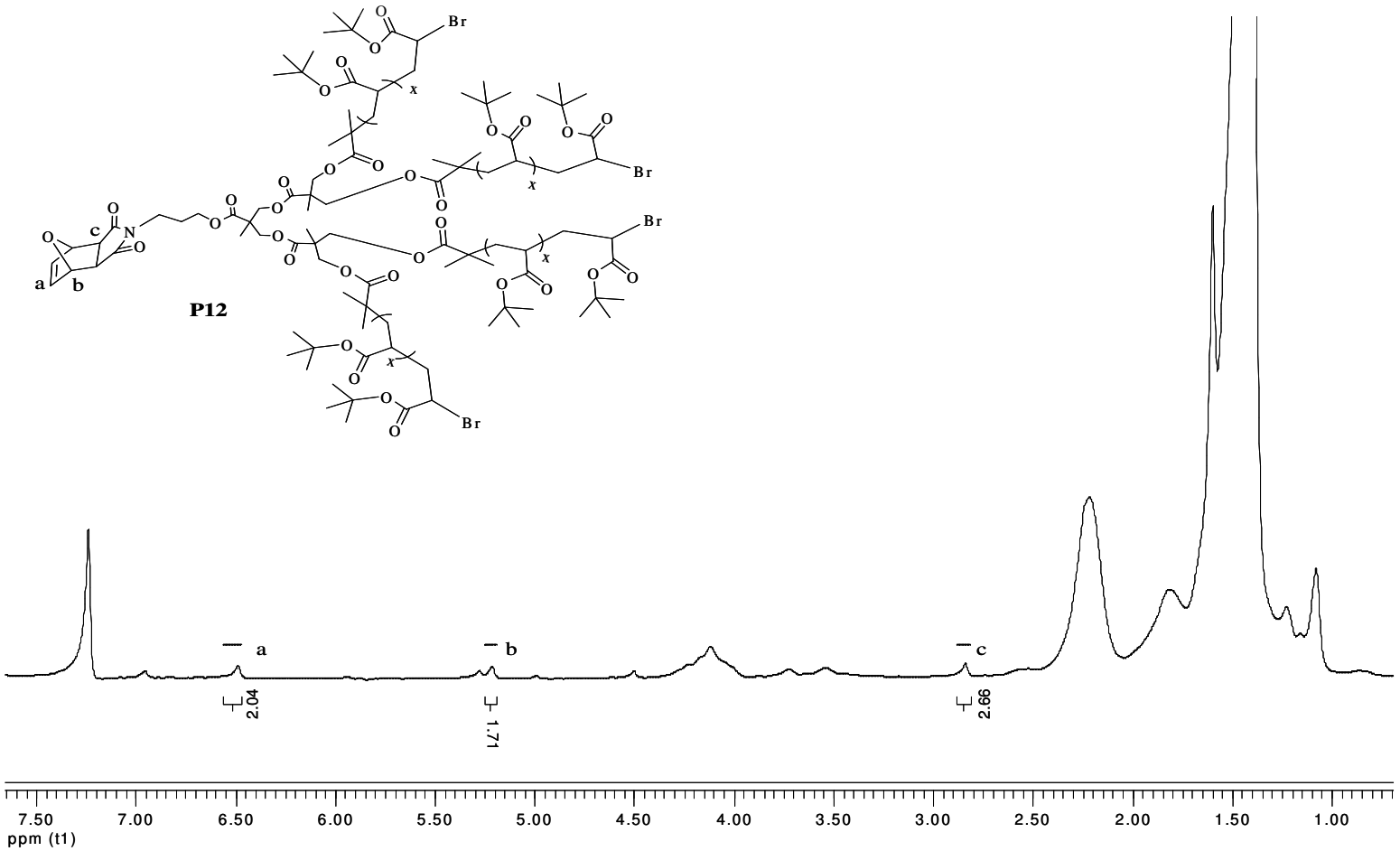

Figure A.23. ^{1}H NMR spectrum of **P12**

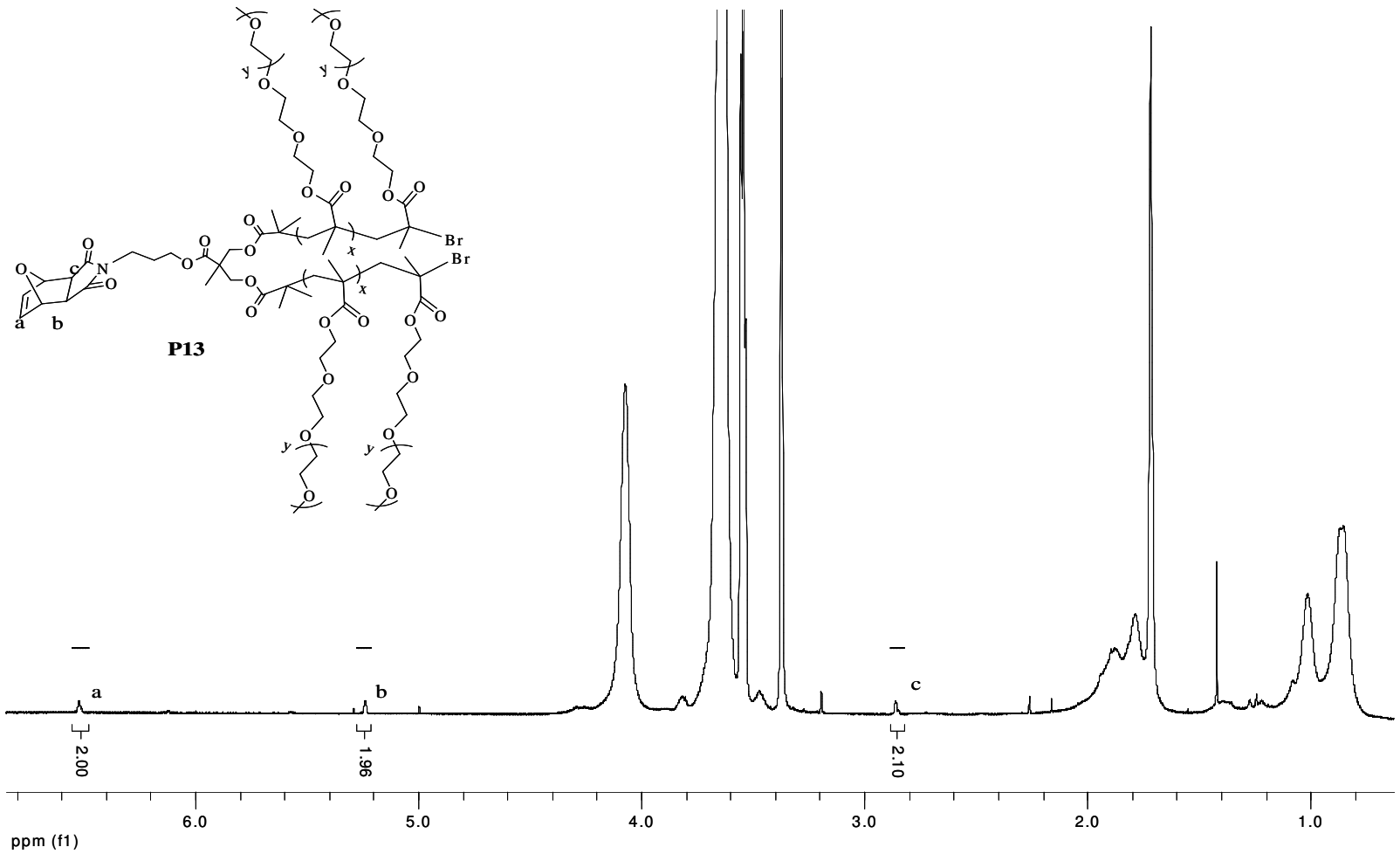

Figure A.24. ^{1}H NMR spectrum of **P13**

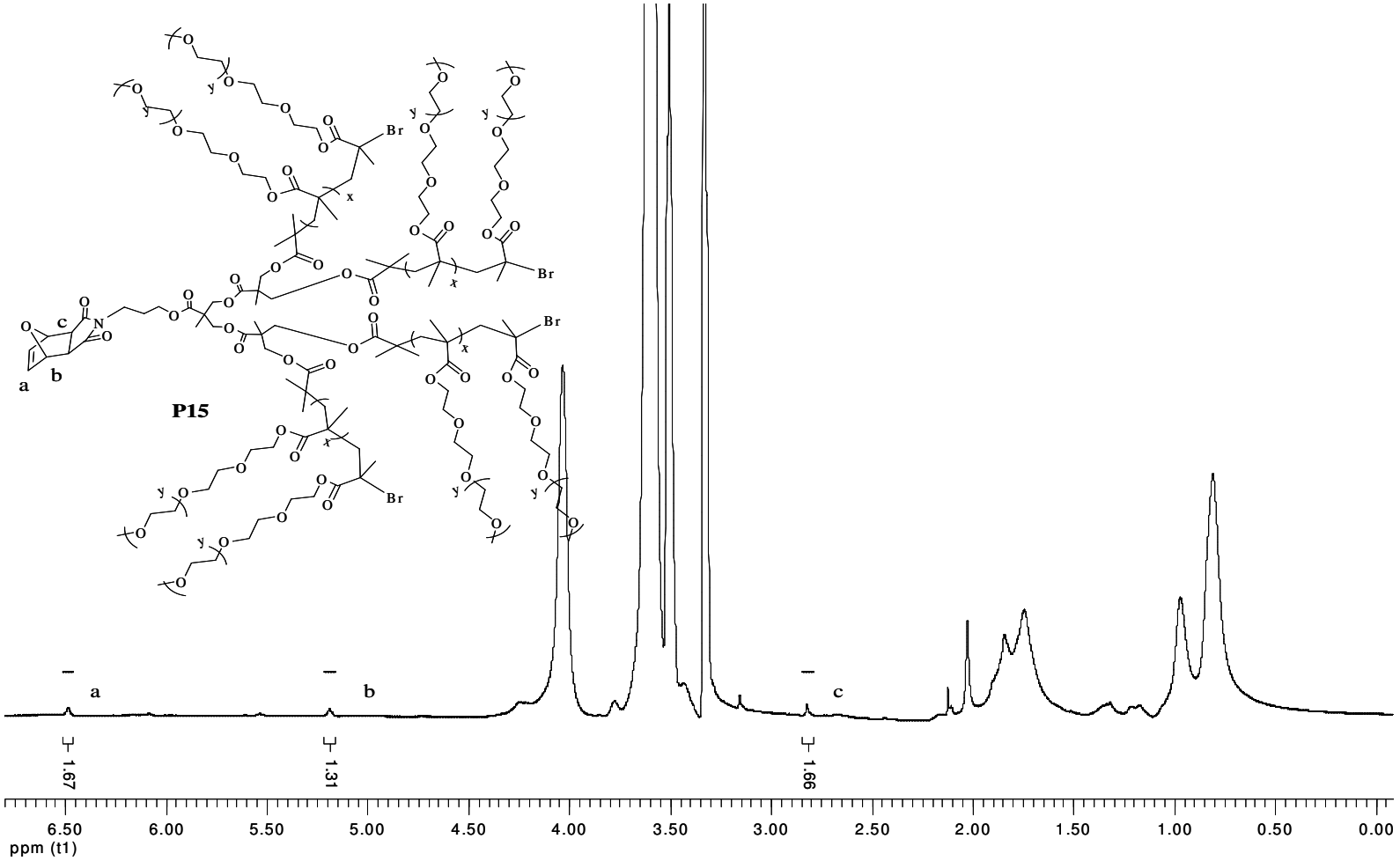

Figure A.25. ^{1}H NMR spectrum of **P15**

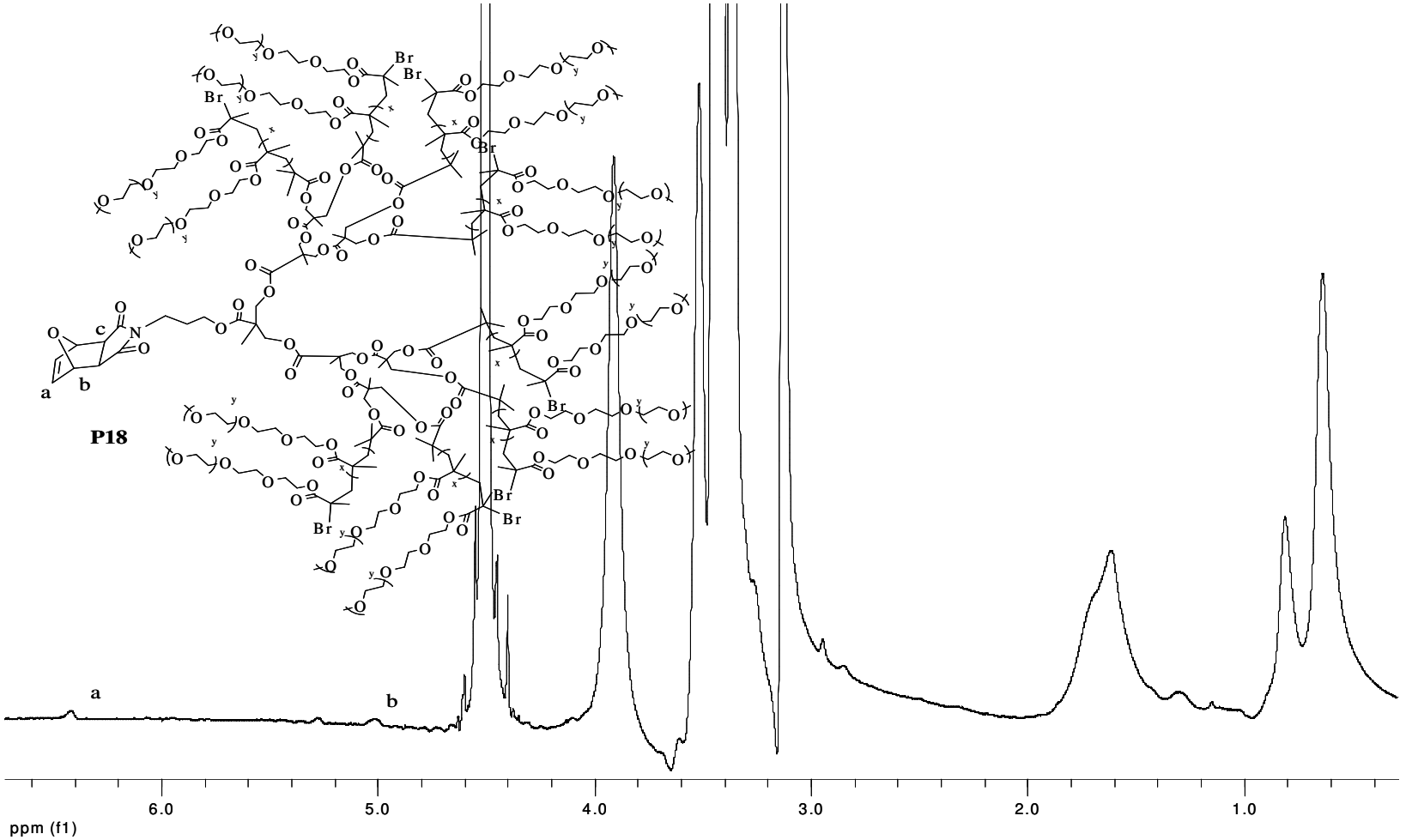

Figure A.26. ^{1}H NMR spectrum of **P18**

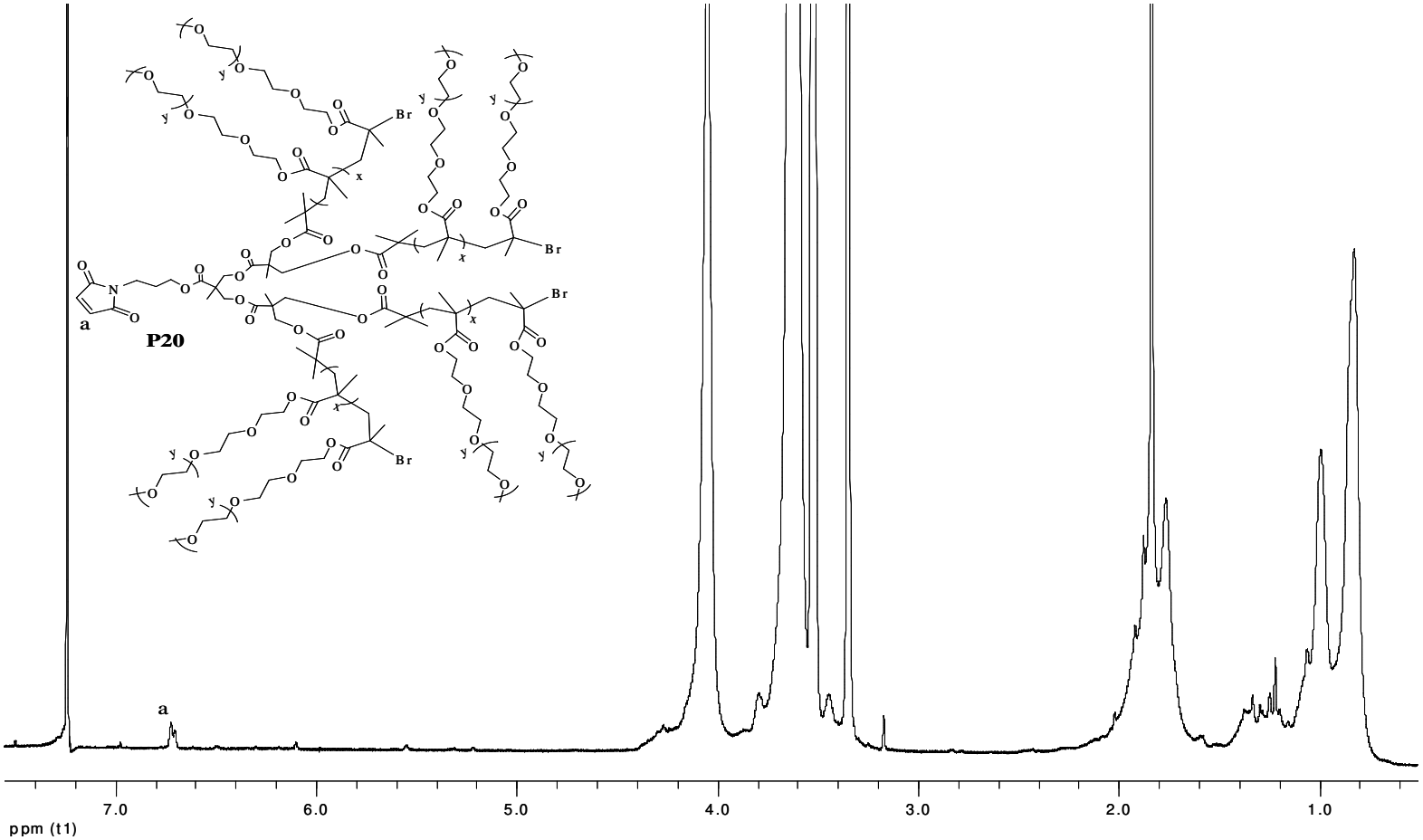

Figure A.27. ^{1}H NMR spectrum of **P20** (in $CDCl_3$)

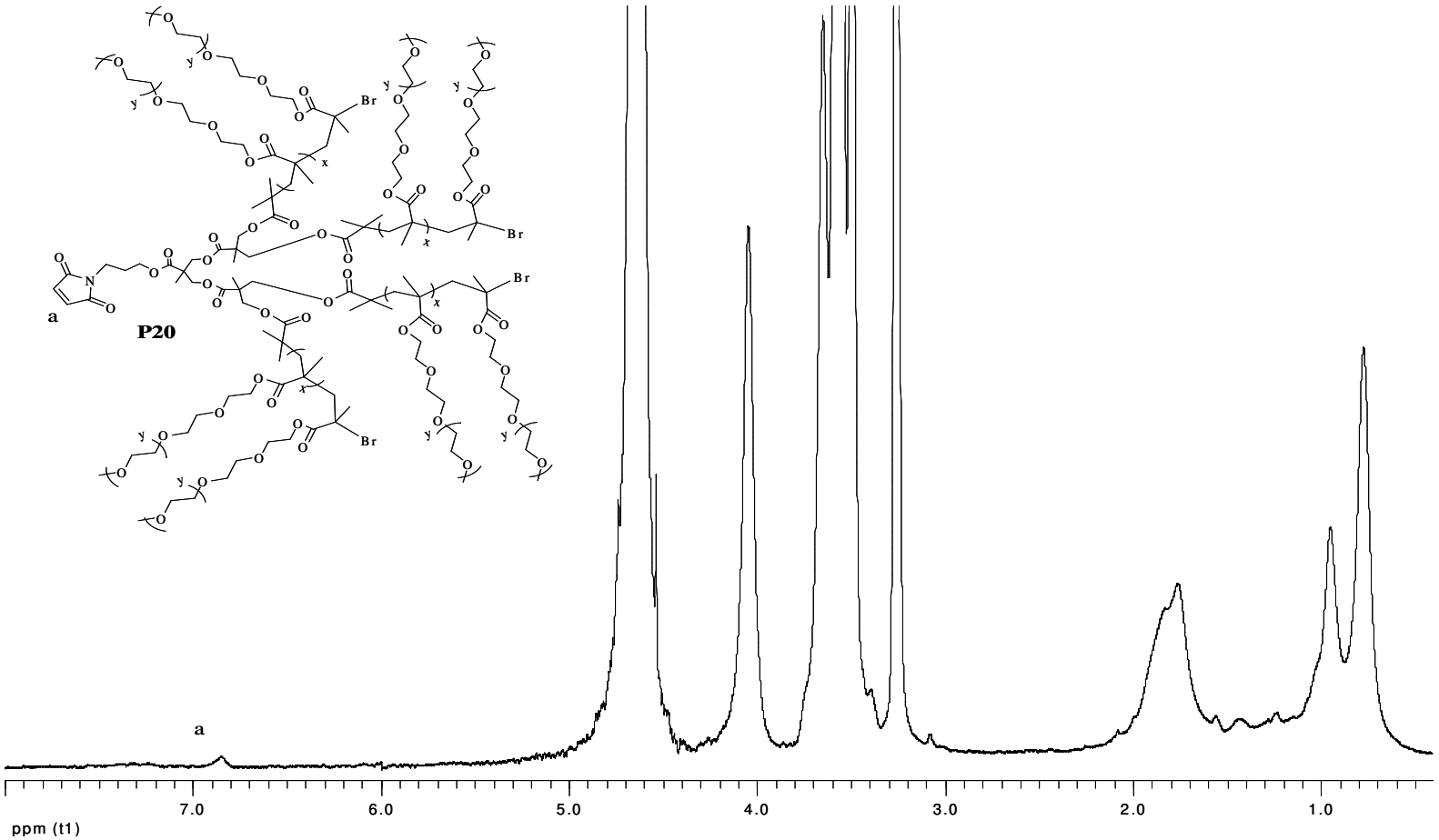

Figure A.28. ^{1}H NMR spectrum of **P20** (in D_2O)

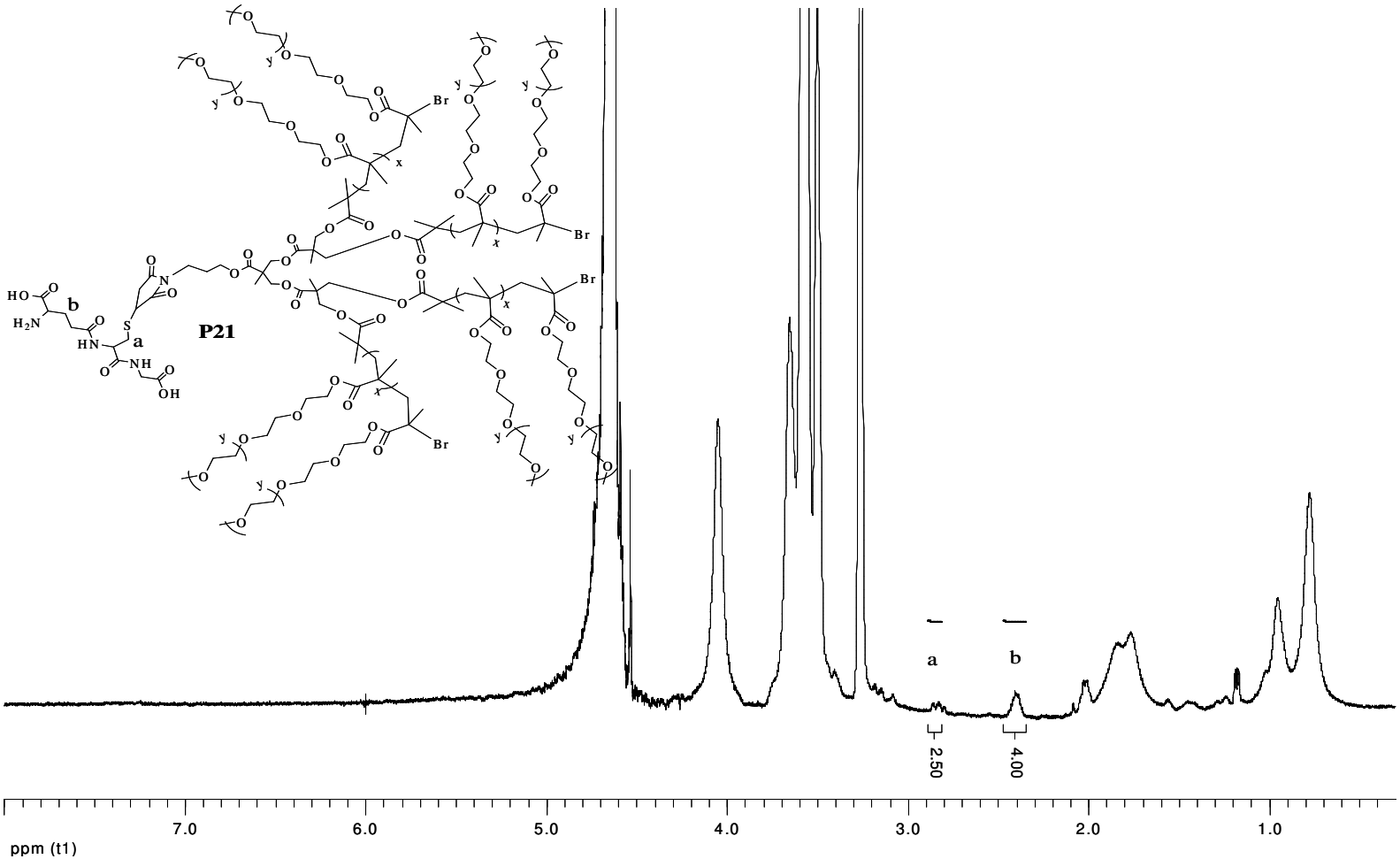

Figure A.29. ^{1}H NMR spectrum of **P21** (in D_2O)

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