POLYMER-DENDRON BASED MICELLES AS DRUG DELIVERY SYSTEMS

by

Nuray Bilgen

B.S., Chemistry, Ondokuz Mayıs University, 2008

Submitted to the Institute for Graduate Studies in
Science and Engineering in partial fulfillment of
the requirements for the degree of
Master of Science

Graduate Program in Chemistry

Boğaziçi University

2012

Canım Annem, Babam ve Eşime,

ACKNOWLEDGMENTS

I would like to express my most sincere gratitude to my thesis supervisor Assoc. Prof. Rana Sanyal for her endless attention and scientific guidance throughout this study. I appreciate her support and useful comments throughout my laboratory work.

I wish to express my thanks to Prof. İlknur Doğan for her careful and constructive review of the final manuscript of my thesis.

I would extend my thanks to Assist. Prof. Ebru Türköz Acar for her careful and constructive review of the final manuscript of my thesis and for his helpful discussions regarding all my undergraduate programs.

I also thank my former and present labmates, Ece, Tuğçe, Nazlı, Betül, Pelin, Mehmet, Özlem, Sadık, Nergiz, Burcu and Merve for their enjoyable company and friendship.

I want to thank to Gamze Tanrıver for being my best friend and sharing all moments with me during lab work and outside.

I also want to thank Melike Eceoğlu and Özgül Gök for being with me all the times during my graduate studies and giving me support to bear with bad and good circumstances.

Finally, my deepest thanks go to my husband Emre, my mother, my father and my brother for their endless love, support and encouragement throughout these years.

This research has been supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (107T623).

ABSTRACT

POLYMER-DENDRON BASED MICELLES AS DRUG DELIVERY SYSTEMS

Polymeric micelles are remarkable nominees for drug delivery systems due to their excellent biocompatibility also they provide high loading capacity to hydrophobic inner core and increase drug solubility. In water, micelles can be formed via self-assembly and this micelle formation is connected to the solubility difference of the hydrophilic and hydrophobic part. Hydrophobic parts form the inner core of the micelle and hydrophilic part which surrounds the core form the sphere of micelle. In our study, polymer-dendron conjugates are prepared via Huisgen type ‘click’ reaction using polyester dendrons and PEG. Biocompatible PEG is used as the polymer to provide water solubility to hydrophobic drug molecules. The conjugation of Dendron to PEG followed by functionalization of the dendron periphery with hydrophobic moiety. This system provided the hydrophilic-hydrophobic balance necessary to form the polymeric micelles in aqueous media. Dendron surface was further decorated with drug molecules to yield the micellar drug delivery system.

ÖZET

İLAÇ SALIM SİSTEMLERİ OLARAK POLİMER-DENDRON YAPISINDAKİ MİSELLER

Polimerik misel yapılar, hidrofobik çekirdek kısmına istenilen hidrofobik molekülü yükleme kapasitesinin fazlalığı ve ilaç çözünürlüğünü arttırma ayrıca mükemmel biyouyumluluğu nedeniyle ilaç geliştirme sistemleri için dikkat çekici adaylardır. Amfifilik kopolimer yapısındaki miseller su içerisinde hidrofilik/hidrofobik bloklardaki çözünürlük farkına bağlı olarak herhangi bir dış etken olmaksızın oluşabilme özelliğine sahiptir. Hidrofobik kısım iç çekirdeği, hidrofilik kısımsa iç katmanı çevreleyen dış katmanı oluşturur. Bu tez çalışmasında, polimer-dendron konjugeleri poliester dendronlar ve PEG polimeri kullanılarak Huisgen tipi ‘klik’ reaksiyonu ile elde edilmiştir. Biyouyumlu PEG polimeri hidrofobik ilaç molekülünün su çözünürlüğünü arttırır. Sonraki basamakta dendron polimere bağlandıktan sonra ise dendron yüzeylerine hidrofobik fonksiyonel gruplar bağlanmıştır. Bu sistem ile sulu ortamda polimerik misel oluşumu için gerekli olan hidrofilik-hidrofobik denge sağlanır. Dendron yüzeyleri ilaç molekülleri ile bezenerek, ilaç salım sistemi potansiyeli olan misel yapılar elde edilmiştir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

Bis-MPA	2,2-bis(hydroxymethyl)propionic acid
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CMC	Critical micelle concentration
Comb	Combretastatin
CA-4	cis - combretastatin A-4
DCC	Dicyclohexylcarbodiimide
DDS	Drug delivery system
DLS	Dynamic light scattering
DMAP	N,N Dimethylaminopyridine
DMF	Dimethylformamide
EPR	Enhanced Permeability and Retention Effect
Et_2O	Diethyl ether
FT-IR	Fourier Transform Infrared
G	Generation
NMR	Nuclear Magnetic Resonance
PAMAM	Polyamidoamine
PEG	Poly(ethylene glycol)
PMDETA	N,N,N',N',N''-pentamethyldiethylenetriamine
PT	Polymer Therapeutics
py	Pyridine
STEM	Scanning transmission electron microscope
TEA	Triethylamine
TEM	Transmission electron microscopy
THF	Tetrahydrofuran

1. INTRODUCTION

1.1. Polymer Therapeutics (PT)

Polymer therapeutics (PT) has been one of the most alluring drug delivery techniques over past decades in term of notable potential in medicine, nanotechnology and therapeutic applications. PTs encompass polymeric drugs, polymer-drug conjugates, polymer-protein conjugates and polymeric micelles.

Ringsdorf first presented the purview of polymer-drug conjugates and opened an important approach to PT and also Duncan and coworkers are pioneers of PT [1].

Coupling low molecular weight anticancer drugs to macromolecules improve the therapeutic index of the drug. Clearly, polymer-drug conjugates improve drug solubility, reduce toxicity and undesired side effects, prolong drug residence in tumor through EPR effect and provide slow body clearance, increase blood circulation time, and overpass drug resistance.

Moreover, compared to small molecule, polymers have higher molecular weight, can carry multiple copies of drug leading to less frequent dose application and load different type of drugs on the same polymer. It is important to stress that choice of polymer is very important owing to fulfilling some fundamental requirements such as biocompatibility, stability under physical conditions, non-immunogenic, non-toxic and reduced side effects [2].

Novel polymeric architectures that are used in PT are graft, star shaped, branched, dendrimer and dendronized polymer [1] (Figure 1.1).

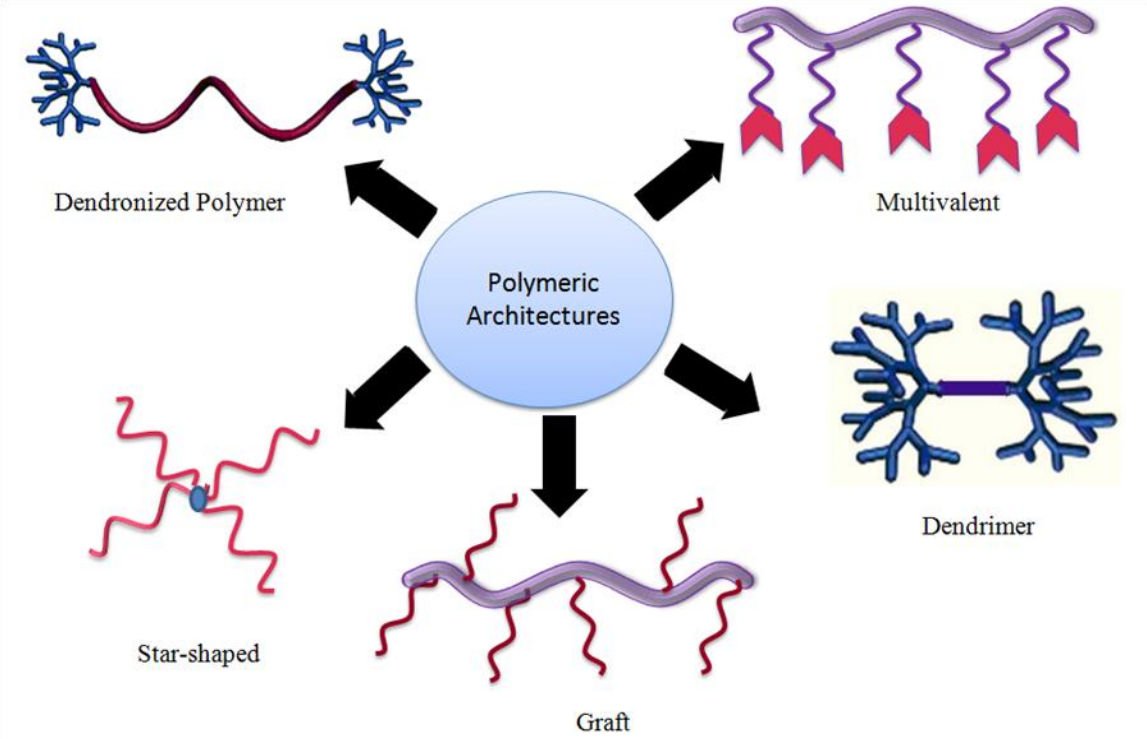

Figure 1.1. Schematic representation of novel polymeric architectures [1].

1.1.1. EPR Effect

In 1986, Maeda and Matsuma introduced a phenomenon, named 'Enhanced Permeability and Retention Effect' (EPR effect) for controlled drug delivery. Remarkable difference in tumor uptake between small molecule and biocompatible macromolecules, named SMANC that used in their study was observed. Macromolecule stayed in the body longer and accumulated more, also prolonged body clearance. Additionally, experiments after their collaboration with Prof. K. Ulbrich and Prof. R. Duncan supported previous results.

Tumor cells need oxygen and nutrients to grow and spread. The morphology of tumor cell differs from healthy cell in terms of the regulation of epitel cells; where there are holes between sicked cells, there is no deformation between non-tumor cells, so that accumulation of macromolecules like polymeric micelles easily accomponied for targetted drug delivery, the figure for EPR effect is given in Figure 1.2 [2].

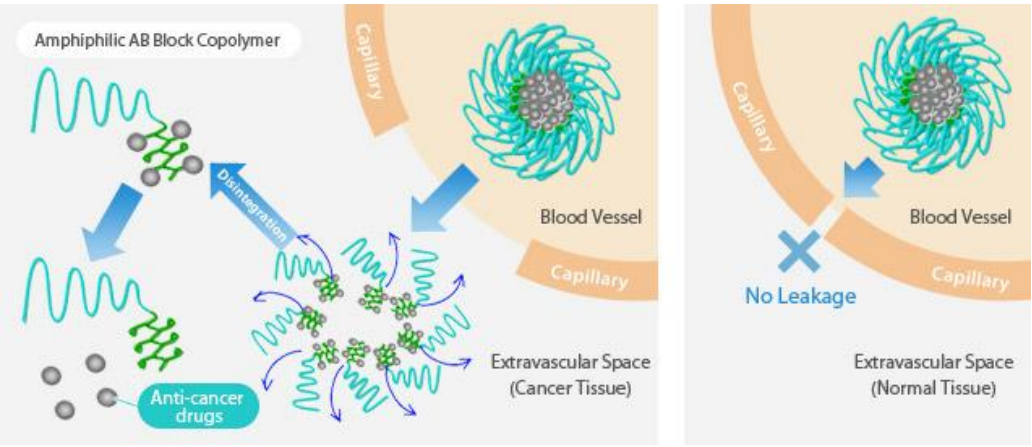

Figure 1.2. Schematic representation of EPR effect [3].

EPR effect offers significant benefits, namely prolonged half-life, stealth-character and non-immunogenicity, enhanced solubility, cellular uptake and stability, receptor-mediated drug targeting, patient compliance and quality of life [4, 5].

1.2. Dendrimers

Dendrimers are well-defined, monodisperse and highly branched macromolecular architectures. There are three different units in dendrimer structure: a core, repeating units and surface groups (Figure 1.3). Branching units are symmetrically placed around the core and contribute to its uniform structure. The generation of the dendrimer is referred to the number of branching units starting from the core to the periphery.

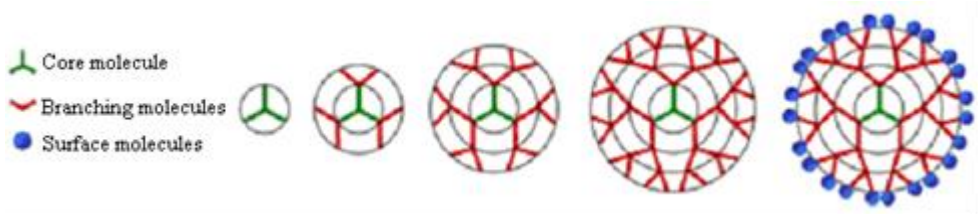

Figure 1.3. Schematic representation showing subunits of dendrimers.

Building blocks generate the branching points in the dendrimer structure. On the other hand, functional groups at the periphery are mostly chemically reactive to undergo further reactions, especially suitable for the conjugation of any biomolecule or drug of interest. The word “dendron” refers to one of the parts of a dendrimer starting from a core

and ending at the periphery. The starting point from which the repeating units built the dendron is called as the focal point, which may also be furnished with chemically reactive groups (Figure 1.4).

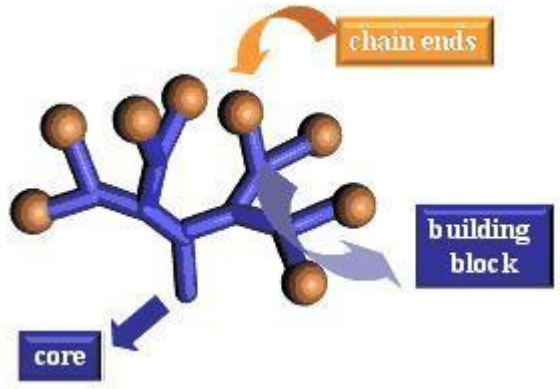

Figure 1.4. General structure of a Dendron.

Generally dendrimers are synthesized by two methods, one of which involves the growth of a dendron starting from the core and the other consists of the reverse process. With the attachment of branching units to each other, the surface groups are placed at the periphery. This is called as the divergent synthesis. In the other method, which is named as convergent approach, firstly surface groups are attached onto the branching units. Then, via a multivalent core, these small dendron pieces are combined to one another. Synthesized dendrons can be hold together either via covalent bonds which are results of reactions between their focal points or via non-covalent interactions (Figure 1.5) [6].

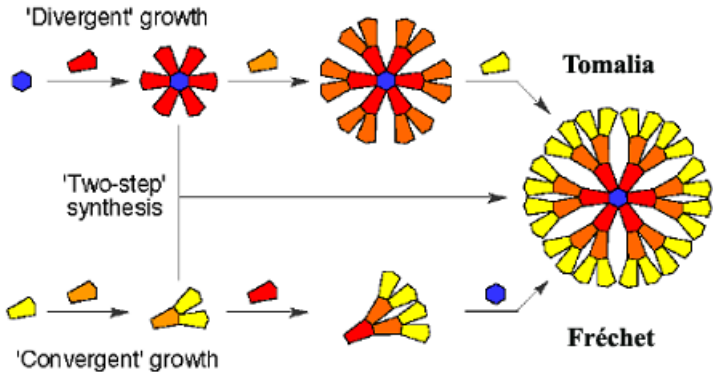

Figure 1.5. Synthesis of dendrimers via divergent and convergent method [6].

Unique properties of dendrites including high degree of branching, well-defined molecular weight and multivalency make them good nominees for drug delivery system.

For dendrimer synthesis, amines, polyesters, benzyls, and different types of backbones can be used. The promising architectur aliphatic polyester dendrimers based on 2,2-bis(hydroxymethyl) propionic acid can be used for the development of anticancer drug conjugation when they are attached to polymer scaffolds [7].

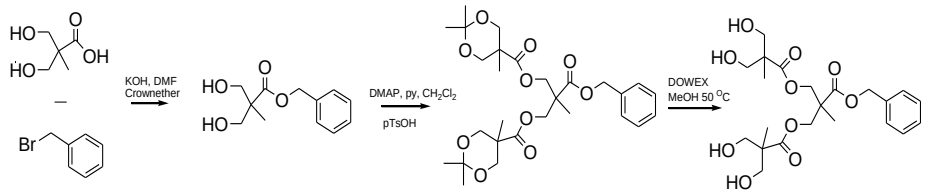

Figure 1.6. G2 benzyl ester dendron synthesized using coupling reaction.

It is very significant that well defined architectures and monodispersity of dendrimers supply low polydispersity beyond controlled multiplicity of reactive chain ends. By this way, the periphery of a dendrimer can be designed by coupling functional groups that simplifies the attachment of biomolecules, especially drug molecules. For the amplifying of molecular weight of a dendritic system, different types of polymers can be conjugated to the either core or periphery with the help of coupling or addition reactions.

1.3. 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 [8].

1.3.1. Copper Catalyzed Huisgen [3+2] Cycloaddition

In Huisgen [3+2] cycloaddition reaction, which is the most famous click reaction, azide-alkyne cycloaddition reaction is introduced by Rolf Huisgen in early 1960s 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 1.7).

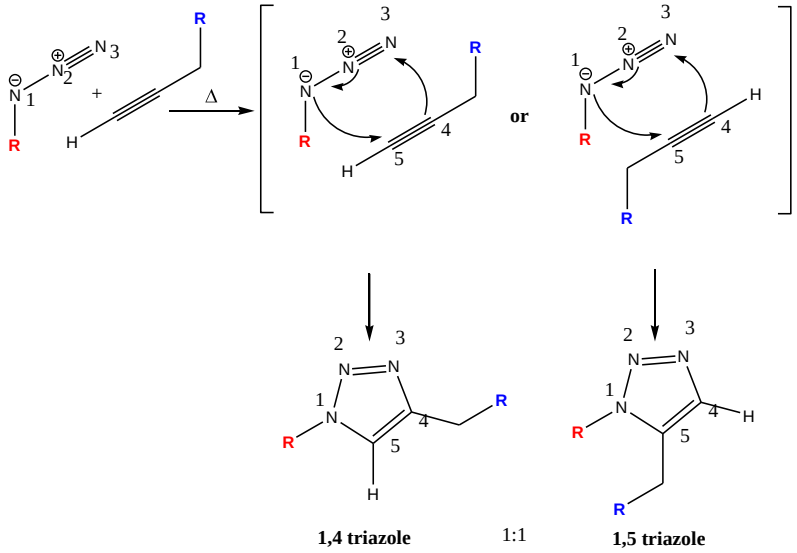

Figure 1.7. Click reaction [8].

In 2002, Sharpless and Fokin [9] introduced a Cu (I) catalyst in Huisgen reaction so that the catalyst directs the region-specific result of the reaction, which results in only 1,4-disubstituted triazole (Figure 1.8). So far, it is called as copper (I)-catalyzed azide-alkyne reaction.

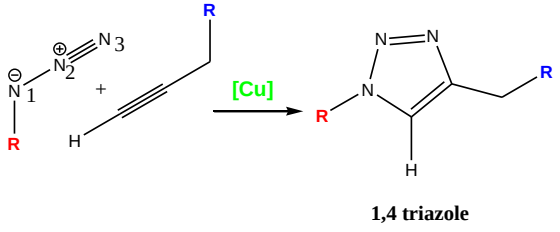

Figure 1.8. Copper catalyzed Huisgen reaction [9].

Presence of Cu catalyst allows the synthesis of a physiologically stable product in aqueous or organic solvents in high yields. The catalyst can be introduced as Cu (I) salt (CuI or CuBr) or generated in situ by reduction of Cu (II) salts [10] 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 their exclusive regiospecificity, mild reaction conditions, easy purification and high yield.

Click chemistry have been widely used in the synthesis of well-defined macromolecules such as dendrimer synthesis [11], dendronized polymers [12].

1.4. Dendron Polymer Conjugates Synthesis Using Copper Catalyzed Huisgen [3+2] Cycloaddition

Utilization of the azide/alkyne click reaction for the alteration of dendrimers has expanded significantly during the past two years. Because of the high yields gained by click chemistry, the application on multiple reaction sites can be easily derived.Thus, it can be applied on many new type dendron polymer conjugates as AB or AB_2 types and have been investigated up to now by different research groups.

Generally, for this kind of conjugate system PEG is preffered that is a hydrophilic polymer and used for many applications due to its reducing toxicity and better biodistribution characteristics. The advantage is gained from both rare properties of polymers and dendrimers via dendron-polymer conjugates.

In 2004, Frechet and Hawker have showed the synthesis of first generation of dendronized linear polymers as it can be seen in Figure 1.9 [13].

1.5. Polymer- Dendron- Drug Conjugates

In recent years, improving new polymeric materials to design innovative drug delivery systems has gained great interest because of the drawbacks of some potentially useful low molecular weight drug candidates. Lots of low molecular weight drugs suffer from low water solubility, bioavailabilty, as well as rapid elimination [14]. Attaching the

low MW drugs to high MW macromolecules is the solution for improving of water solubility and the circulation time of polymer-drug conjugates in the plasma [15].

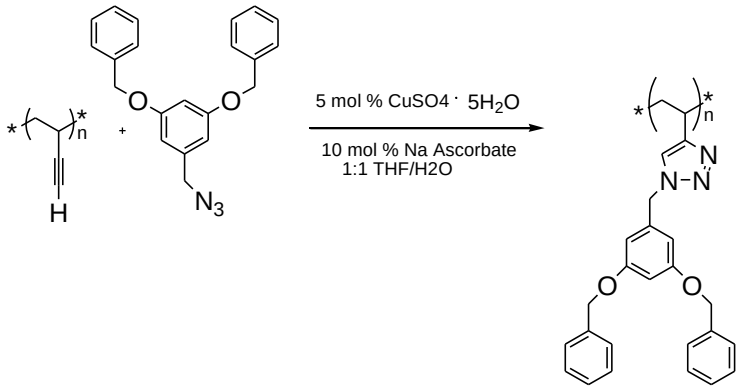

Figure 1.9. Syntetic route to first generation dendronized polymers [13].

Improving the circulation time of polymer-drug conjugate in the plasma is the reason for decreasing the rate of renal filtration that is related to molecular weight of the polymer-drug conjugate [16].

To embellish architectural ideality of the DDS agents and to combine more drug to high MW macromolecule, dendrimers' well defined and highly branched structures become very attractive. By way of attaching water soluble polymers to dendrimers through varying periphery and core of dendrimer, low MW drug molecule can be conjugated determinably.

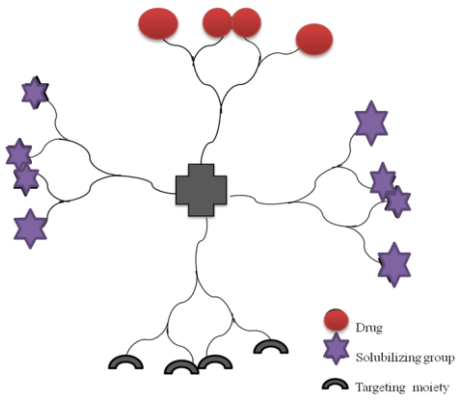

Figure 1.10. Schematic representation of a possible dendritic DDS.

Jean M. J. Frechet reported the polymer dendron conjugate synthesis in 2001, synthesized one, two, or four dendron bearing polymers. This work is the innovator of the area of dendron- polymer conjugated copolymer synthesis (Figure 1.11) [17].

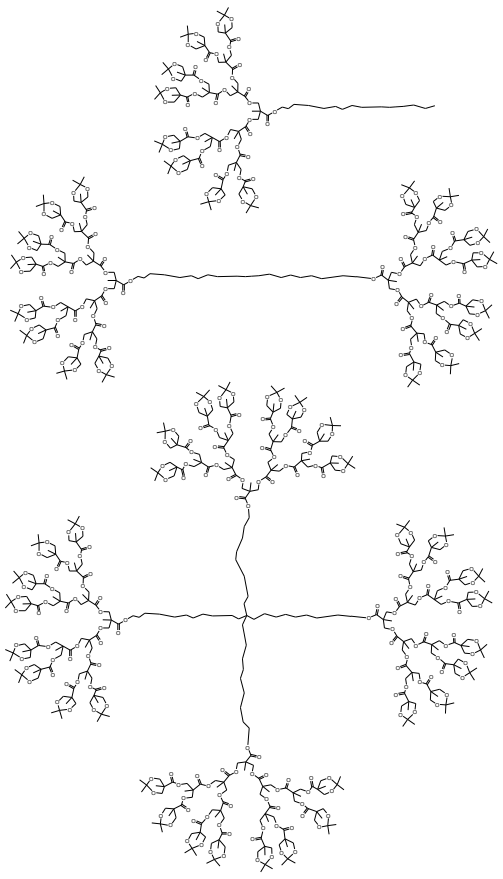

Figure 1.11. Synthesis of polymer dendron conjugate [17].

It is crucial for the polymeric scaffolds to have high water solubility that for bioconjugation and drug delivery applications. Poly(ethyleneglycol) (PEG) has been widely utilized to improve the water solubility and immunogenity. Bearing an antibiofouling property, it reduces the non-specific 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 introduced a new strategy for the synthesis of dendron-polymer conjugates that can be used as drug carriers. They attached a third generation polyester dendron to a PEG chain from the core of the dendron. Coupled hydrophobic drug

molecules to the periphery of the dendron are intended to release under acidic conditions due to the hydrolysis of ester groups in dendron structure (Figure 1.12) [18].

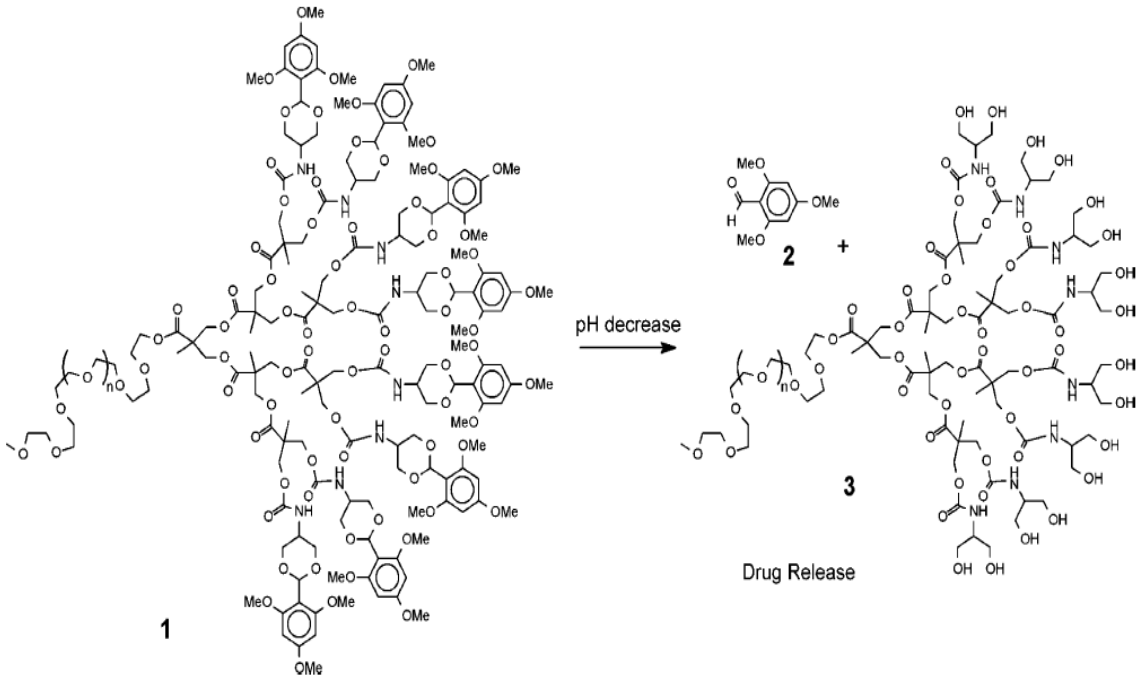

Figure 1.12. pH-responsive copolymers for controlled release of doxorubicin [18].

Ricardo Riguera published the synthesis of three generations of azido-terminated PEG-dendritic block copolymers in 2006. Monofunctional PEG reacted with a G1 dendron and grown up to G3. By means of copper (I)-catalyzed azide-alkyne [3+2] cycloaddition reaction the efficient surface functionalization of these dendritic polymers was achieved (Figure 1.13) [19].

In 2011, Lisa M. Kaminskas and her group were sythesized G5- PEGylated polylysine dendrimer with covalently attached doxorubicin and doxorubicin loaded PEGylated liposome. They compared pharmocokinetic, antitumor activity and toxicity profile of dendrimer and liposome-associated doxorubicin. In terms of pharmacokinetic properties and antitumor activity dendrimer associated doxorubicin showed similar performance to liposamal doxorubicin, however, it exhibited lower systemic toxicity [20].

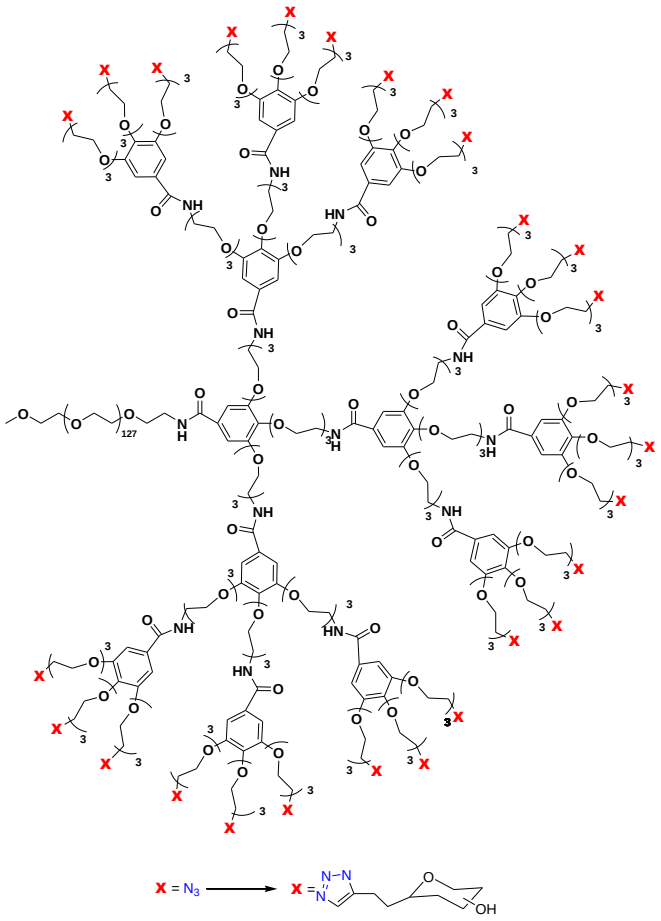

Figure 1.13. Synthesis of azido-terminated PEG-dendritic block copolymers [19].

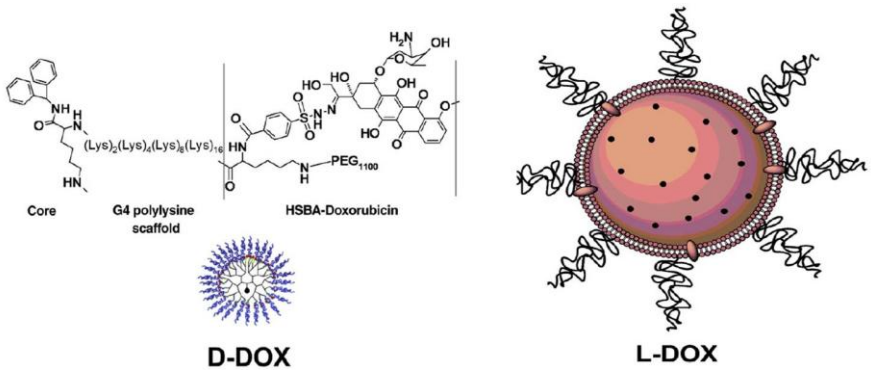

Figure 1.14. Schematic representation of dendrimer and liposomal doxorubicin [20].

1.6. Polymer-Dendron Based Micelles

Today, chemotherapy is used to treat cancer still have some primary problems like lack of selectivity of anticancer drugs against tumor cells. For this reason, not only tumor cells but also cells of gastrointestinal tract and bone marrow are influenced by high cytotoxic effects of these drugs. This disadvantage lead up to a narrow therapeutic index of many anticancer drugs [21]. On the other hand, treatment of different type tumor cells needs high dosage of drug and this means high toxicity to healthy cells. So for the adequate treatment, different vehicles are neccesary to get rid of the difficulties of high dosage anticancer drugs treatment and to decrease toxicity.

Disadvantages of drug molecules is because of the small molecular weight for that their combination to highly branched dendronized polymers will be reason for eliminating all side effects.Normally, dendronized polymers have thousands of MW when self-assemble in aqueous media will have millions of MW. Polymeric micelles are emerging as ideal vehicles for drug delivery [22]. By these nanocarriers, solubility and longevity of drugs can be increased, and controlled release by environmental sensitive or external stimuli can be accompanied. Also via enhanced permeability and retention effect, accumulation of drugs in solid tumors can be achieved [23].

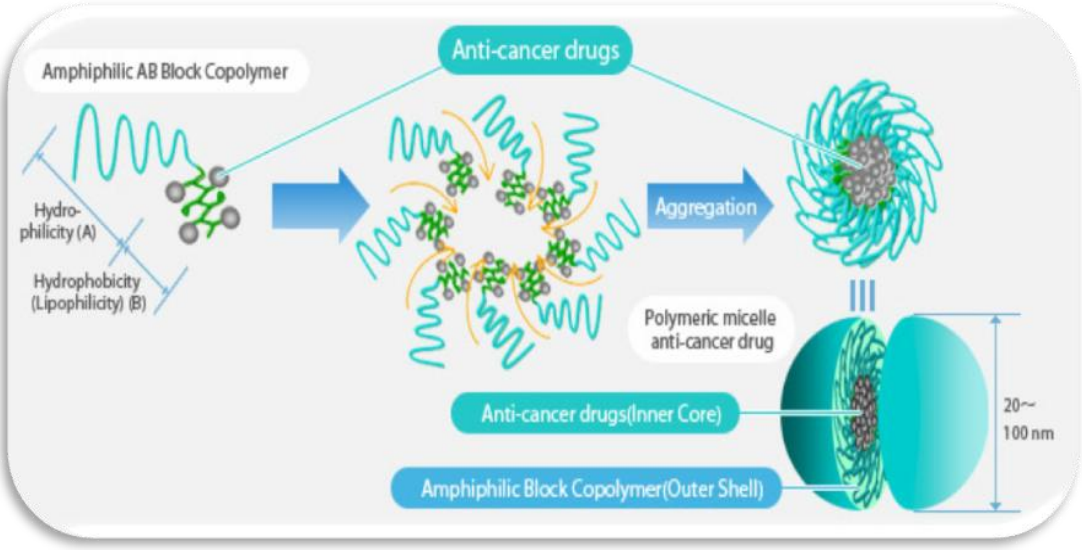

Figure 1.15. A typical structure of a polymer-dendron-drug based micelle [3].

The typical structure of a polymer-dendron based micelle in water is given in Figure 1.19, the structure of polymeric micelles involves a core and a shell; hydrophobic part forms the inner core which encapsulates poorly water-soluble drug and hydrophilic part forms the shell around the core which is usually polyethylene type polymer that protects the drug from aqueous environment and provides polymeric micelle a site specific passive targeting drug delivery [24].

The critical micelle concentration, the minumum concentration of conjugated polymers tends to form micelle in aqueous medium.This term include thermodynamic stability of the polymeric micelle and the measurement was performed using flourescence method with generally pyrene as a probe. Pyrene which is unsoluble in water will preferentially partitions into a hydrophobic micellar core from water to be soluble. Upon changing the polarity of its microenvironment, pyrene undergoes a red shift (long excited-state lifetime) in the excitation spectrum. Fluorescence peak excimer-to-monomer intensity ratio (I_E/I_M) were plotted against the logarithm of polymer concentrations to determine CMC as the onset of micellization. Amphiphilic polymers generally have lowered CMC values, usually 10^{-6} M (Figure 1.16) [25-32].

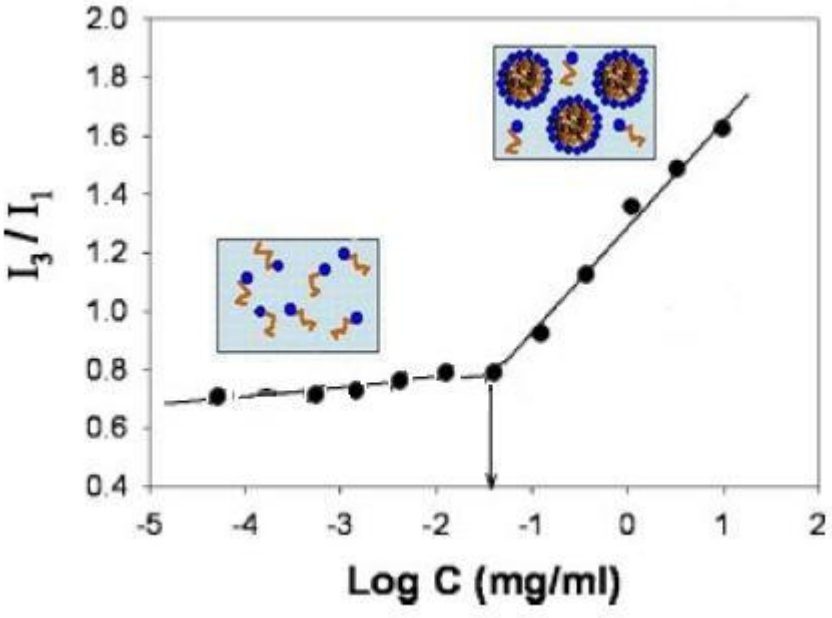

Figure 1.16. Represantation of CMC.

For targetting the tumor, pharmaceutical drug carriers in plasma should possess properties like (i) biodegradability, (ii) small particle size, (iii) high loading capacity, (iv) prolonged circulation and accumulation in right pathological site in the body [33].

In the early 1990s, Kataoka's group presented polymeric micelles as drug delivery systems, by development of doxorubicin-conjugated block copolymer micelle [34]. Polymeric micelles have also been examined for the delivery of many chemoterapeutics in preclinical and clinical studies. Liposomes, lipid based drug delivery systems, nanoparticles are also reported for solid-tumor targetting agents but they are the victim of acquired drug resistance and poor targeting. Polymeric micelles are the only reported drug delivery systems that establish multi drug resistance using different approaches like passive targeting, pH-sensitive and thermosensetive drug delivery systems [35].

In 2004, R. Gillies and J.M.J. Frechet have synthesized polyester dendron, and hydrophobic groups were attached to the dendrimer periphery by highly acid-sensitive cyclic acetals with the goal of developing a pH-responsive micelle system. By this way, stable micelles were formed in aqueous solution at neutral pH. However to disintegrate the micelle into unimers, mildly acidic pH was required, following the loss of the hydrophobic groups upon acetal hydrolysis [35].

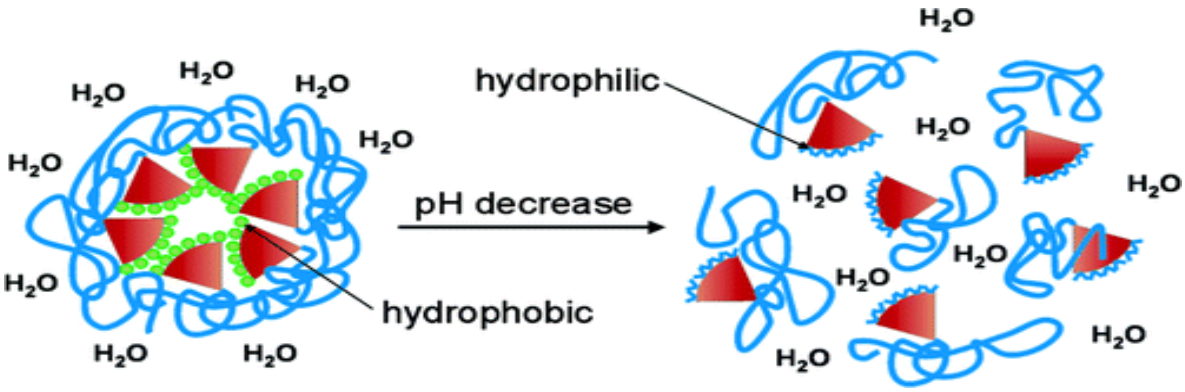

Figure 1.17. Schematic for drug release from a pH-sensitive micelle [35].

Kim and coworkers have synthesized the amphiphilic dendron-PEG conjugate and showed the micellar characteristics in water.They were synthesized different generations of urethane-amide based dendrons by a convergent route and dendrons were attached to

MeO-PEG(M_n=2000). The mean diameter of these micellar systems was investigated with dynamic light scattering methodology and were found between 90-170 nm range and it is dependent on generation of the dendron [37].

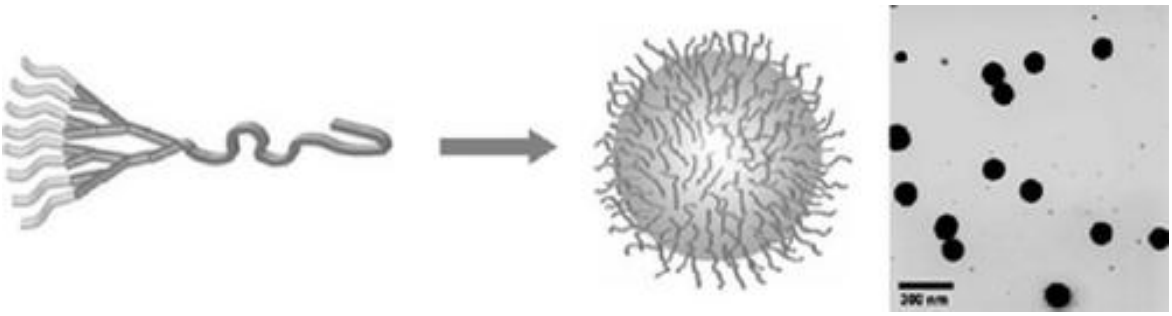

Figure 1.18. Schematic representation and TEM image of PEG-3GOc micelles [37].

Hammond and coworkers have designed and synthesized an amphiphilic dendritic copolymer based on poly (n-dodecyl-L-glutamate) as a hydrophobic block and a hydrophilic polyester dendron block modified with PEG.

In aqueous solution, hydrobic and hydrophilic block formed successively the micelles' inner core and exterior shell. The critical micelle concentration (CMC) measurements were performed by using the flourescence method and was found to be 10^{-8} M approximately [38].

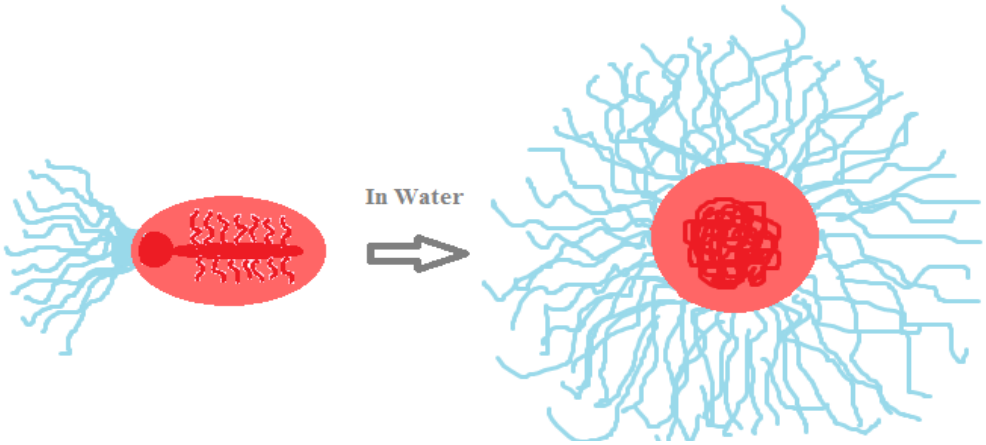

Figure 1.19. Self assembly of dendron-polymer based micelles in water [38].

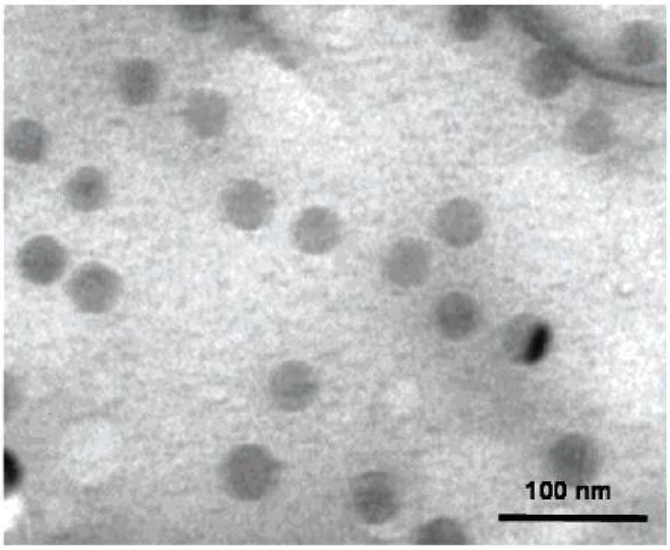

Figure 1.20. TEM image of comb-dendritic micelles [38].

In 2008, a new class of linear-dendron-like poly(ε-caprolactone)-b-poly(ethylene oxide) (PCL-b-PEO) copolymers was synthesized by using controlled ring-opening polymerization (ROP) of ε-caprolactone (CL) followed by a click conjugation with azide-terminated PEO (PEO-N_3).

DLS and TEM analyses demonstrated that the biodegradable micelles and vesicles with different sizes (less than 100 nm) self-assembled from these Dm-PCL-b-PEO copolymers in aqueous solution. Both the PEO composition and the linear-dendron-like architecture of copolymers controlled the morphology and the average size of nanoparticles.

This provides a functional strategy not only for the synthesis of biodegradable and amphiphilic block copolymers with linear-dendron-like architecture but also for fabricating biocompatible nanoparticles with suitable size for controlled drug release [39].

In 2010, Jiandong and coworkers were synthesized PLGA-PEG-PLGA block copolymer containing pH sensitive group N-Boc Histidine as an end group. In water, this copolymer formed the micelle PLGA rich core and PEG rich shell above CMC and they were used Doxorubicin as a model anticancer drug. In vitro drug relase from micelles of N-

Boc Histidine capped copolymer showed difference between pH=6.2 and pH=7.4 due to the pH sensitivity of N-Boc Histidine [40].

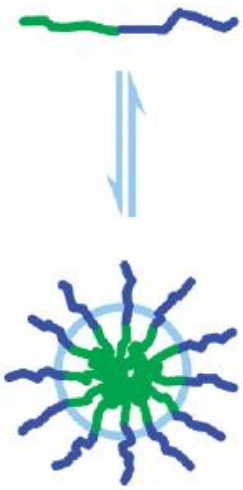

Figure 1.21. Traditional Star-like Micelles Self-Assembled fromAmphiphilic Diblock Copolymer [39].

In 2010, Jiandong and coworkers were synthesized PLGA-PEG-PLGA block copolymer containing pH sensitive group N-Boc Histidine as an end group. In water, this copolymer formed the micelle PLGA rich core and PEG rich shell above CMC and they were used Doxorubicin as a model anticancer drug. In vitro drug relase from micelles of N-Boc Histidine capped copolymer showed difference between pH=6.2 and pH=7.4 due to the pH sensitivity of N-Boc Histidine [40].

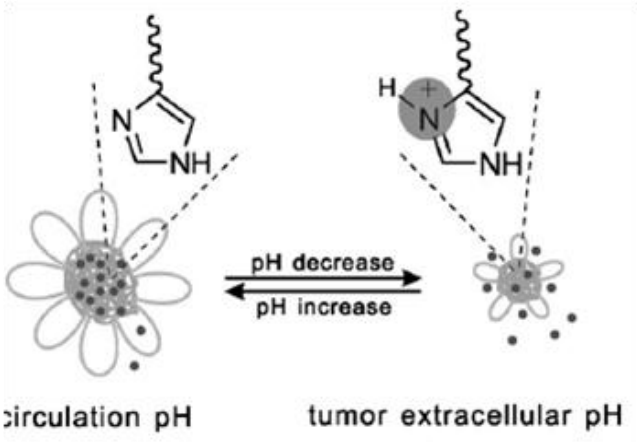

Figure 1.22. Schematic presentation the micelles composed of amphiphilic polymers with end capped by N-Bochistidine [40].

In Lei Zhou's group, folate functionalized biodegradable amphiphilic dendrimer-like star polymer with a well defined poly(l-lactide) (PLLA) star polymer core and six hydrophilic polyester dendrons based on 2,2-bis(hydroxy methyl propionic acide was synthesized by using ring opening polymerization for drug delivery (Figure 1.29) [41].

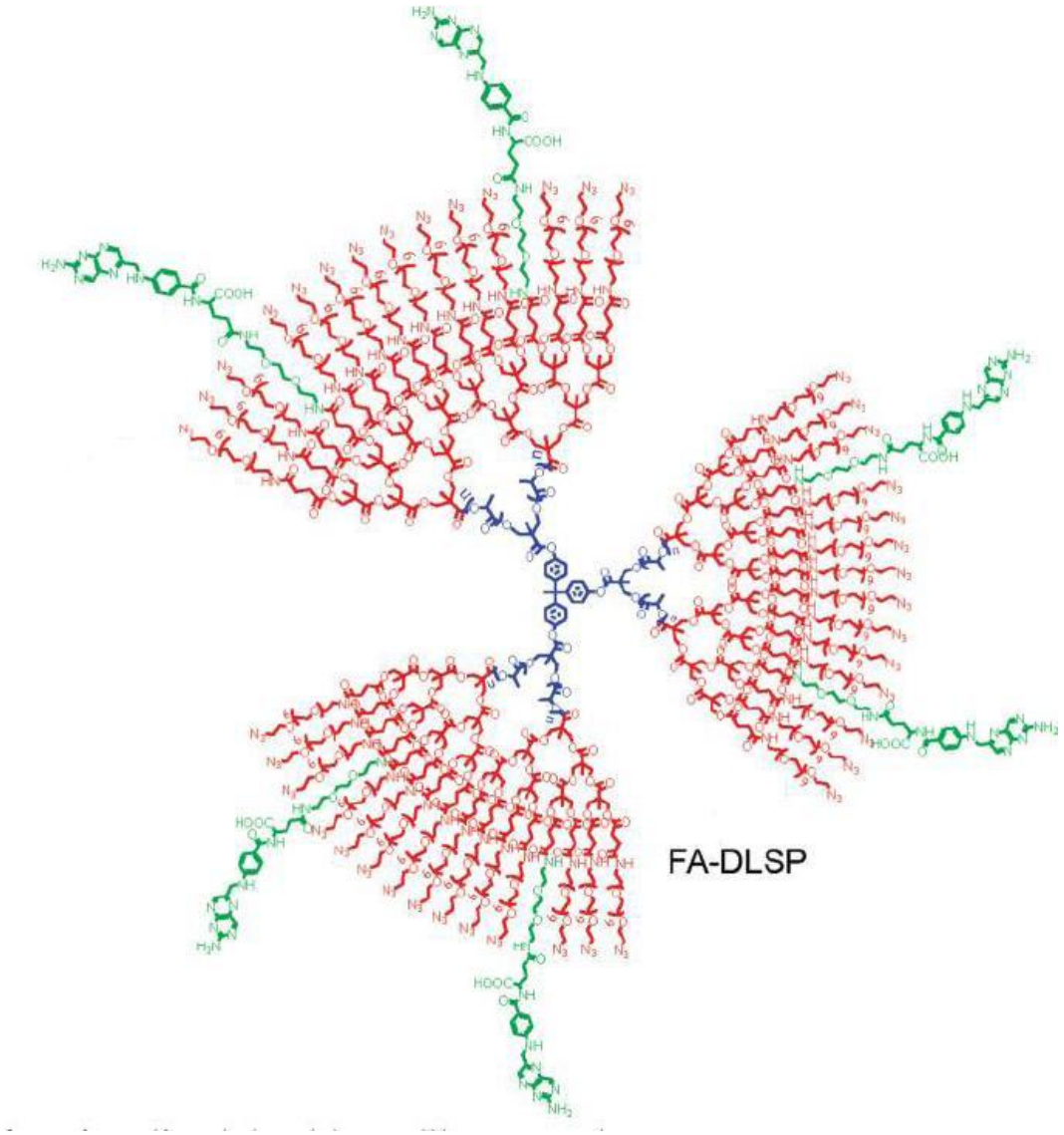

Figure 1.23. Synthesis of Folate-Functionalized Dendrimer-Like Star Polymer [41].

This macromolecule formed unimolecular micelles in aqueous medium and its unimolecular micelles' particle size determined ~15nm by dynamic light scattering and TEM (Figure 1.30). Hydrophobic anticancer drug doxorubicin was encapsulated by utilizing from this molecule's hydrophobic core. The in vitro drug release study revealed that the release of DOX at pH 5.3 was much faster than that at pH 7.4.

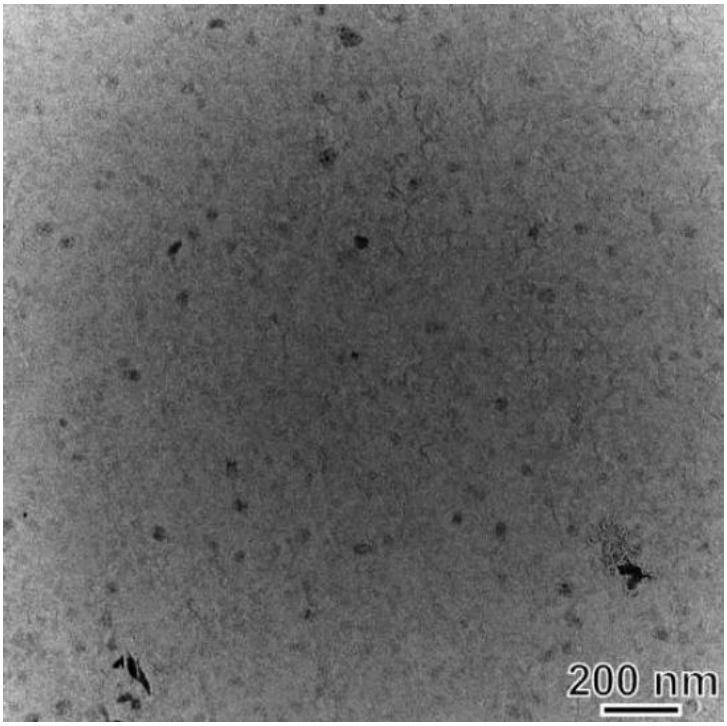

Figure 1.24. TEM micrograph for Folate-Functionalized Dendrimer-Like Star Polymer [41].

2. AIM OF THE STUDY

2.1. Micelle Formation From Functionalized Dendron-Polymer Conjugate and Drug Conjugated Dendron-Polymer Conjugates

Micelles have attractive potential for carrying hydrophobic molecules , thus they are remarkable nominees for drug delivery systems. Design of delivery system should include both hydrophobic and hydrophilic blocks in order obtain micellar structures. Using polymer-dendron conjugates is considerably attractive to get micellar structure because this conjugation brings multivalency, well defined architectural properties, in addition to biocompatible and biodegradable facilities to the system.

In this study, biodegradable polyester dendrons were used and gave reaction with functional biocompatible polyethylene glycol polymers chain by using Huisgen type click reaction. Because of the surface properties of polyester dendrons, alkyne functionality was brought to the system. This system composed of both hyrophilic and hydrophobic blocks can be described an amphiphilic design. Hence, the conjugates tend to form micellar structures in water via self-assembly. In this thesis covers studies of getting micellar structures and the results were obtained as expected.

After the synthesis of alkyne functionalized dendron-polymer conjugate, their end group is ready to one more click reaction to couple azide carrying drug molecule. This click reaction is applied on the generation 1, 2 and 3 polyester dendron conjugated PEG5K copolymers and COMB functionalized with azide molecules were synthesized. Micelle formation from these drug conjuagetes was performed via self-assembly in water.

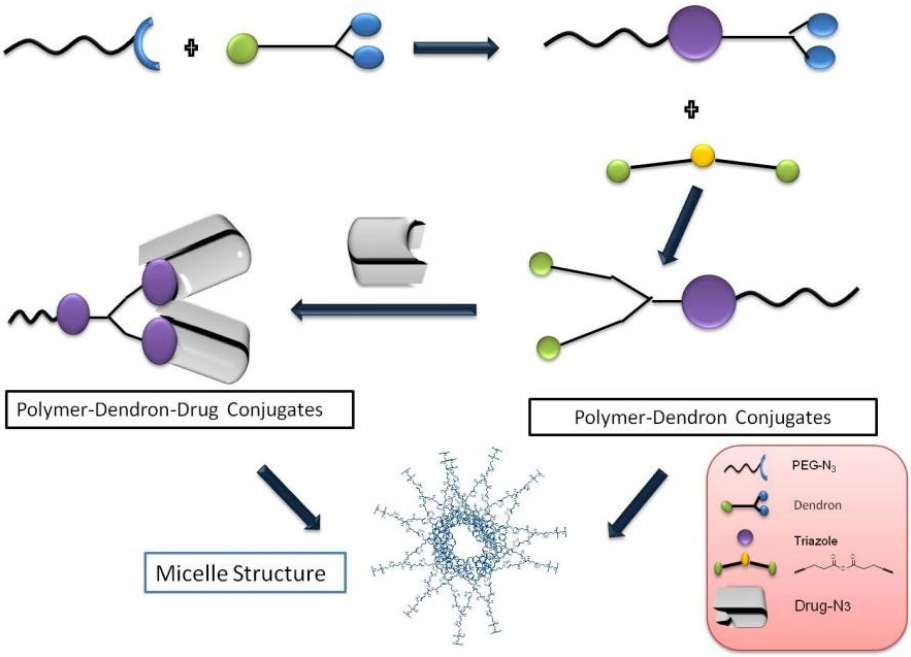

Figure 2.1. General scheme for micellar structures of functionalized dendronized polymer and their drug conjugates

3. RESULTS AND DISCUSSION

The project consists of three parts:

(i) Synthesis of dendronized polymers and their functionalization

(ii) Conjugation of drug molecules on dendronized polymers

(iii) Micelle formation of dendronized drug conjugated polymers

3.1. Dendronized Polymers

Because linear PEG polymers have low loading capacity, PEG alone can carry maximum two drug molecules on its periphery. Making dendronized polymers bearing branching unit combats with this drawback. Hence, azide functionalized polymer and then alkyne functionalized dendronized polymers are synthesized as demonstrated in Figure 3.1. Moreover, functionalization of dendronized polymers bearing alkyne or azide groups facilitates the attachment of drug molecules via the Huisgen type ‘click’ reaction.

3.1.1. Preperation of Dendronized Polymers

To obtain polymer-dendron conjugates ,biodegredable polyester dendrons and biocompatible, water soluble PEG polymer were conjugated via Huisgen type [3+2] cycloaddition reaction in the presence of Cu(I)Br. Three generations of the polyester dendron, G1 ,G2 and G3 were used in this study. The molecular weight of PEG that was used in this study was 5000 Da. Firstly, Mesylated PEG synthesized and then azide ended PEG 3 was obtained (Figure 3.1).

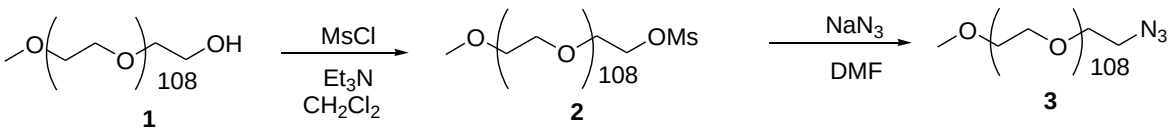

Figure 3.1. Synthesis of azide ended PEG.

After the synthesis of polyester dendrons 4, 5 and 6 which were synthesized according to literature, biodegradable polyester dendrons and biocompatible hydrophilic linear PEG polymers were combined via Huisgen type "click" chemistry to obtain polymer-dendron conjugates. [G1]2OH[PEG5K] molecule bears two alcohol groups and the number of functional hydroxy groups increase with increasing generation. The desired copolymers [G1]2OH[PEG5K] (7), [G2]4OH[PEG5K] (8), and [G3]8OH[PEG5K] (9) were obtained by the reaction of alkyne core functionalized G1 (4), G2 (5) and G3 (6) polyester dendrons and azide ended PEG (3) in the presence of Cu(I)Br and PMDETA in THF (Figure 3.2).

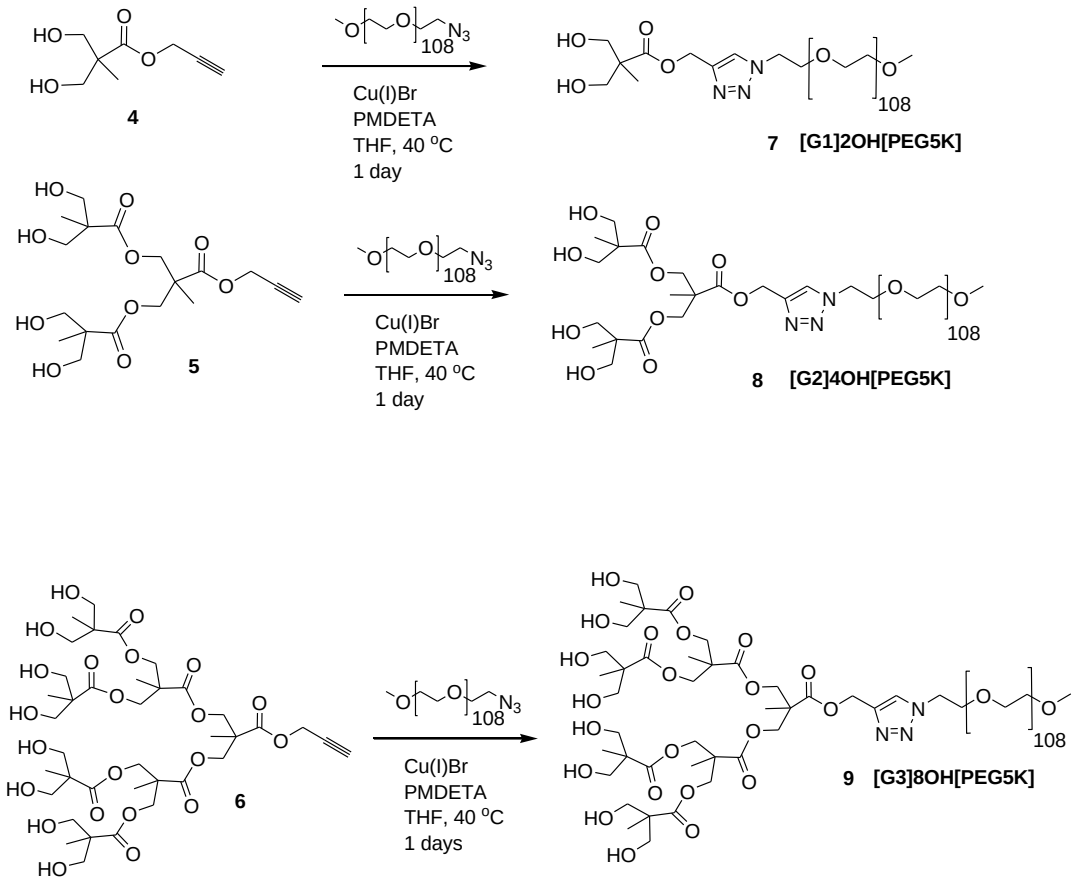

Figure 3.2. Synthesis of dendronized polymers.

Functionalization of the surface alcohol groups thus obtained copolymers were realized via the acylation reaction with pentynoic anhydride (11) in the presence of pyridine yielding copolymers (13), (14), and (15). Alkyne groups on the surface of the dendrons were now ready for the second ‘click” reaction to couple drug molecules (Figure 3.4).

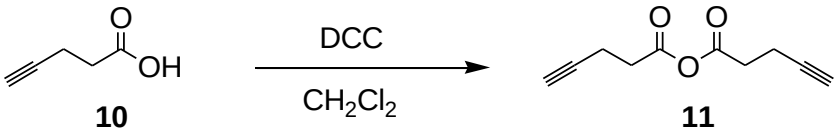

Figure 3.3. Synthesis of 4-pentynoic anhydride.

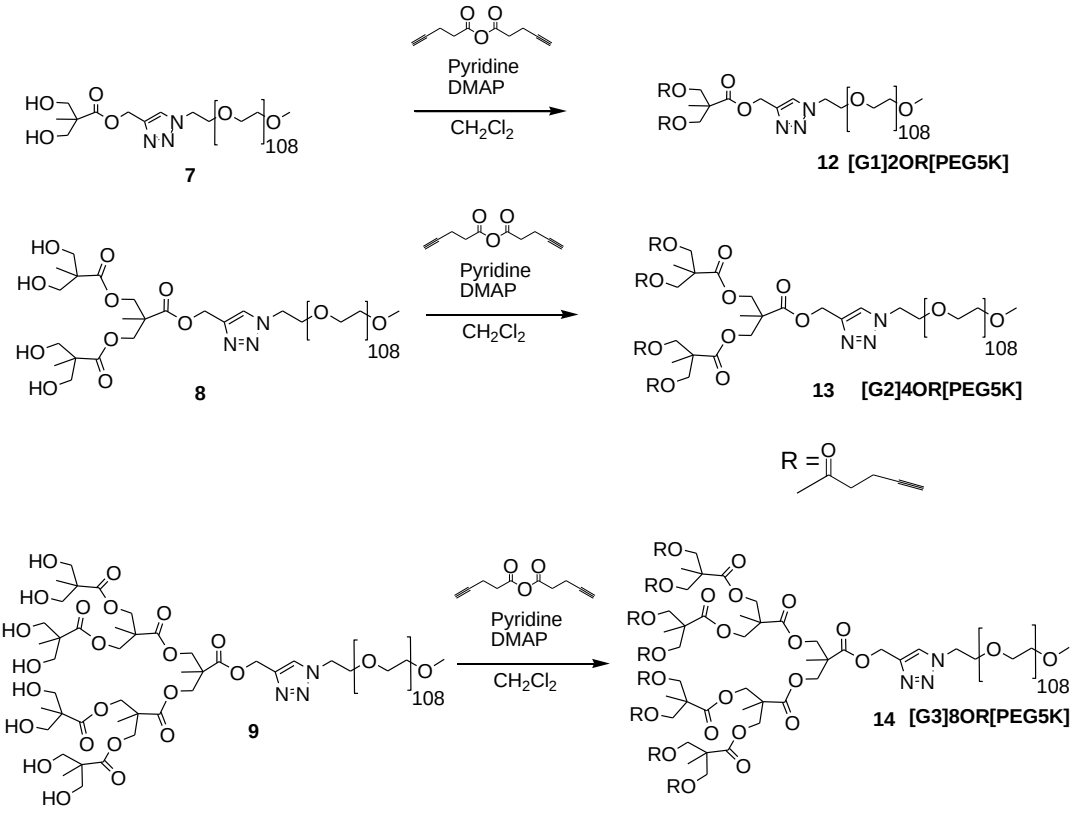

Figure 3.4. Synthesis of alkyne ended dendronized polymers

All synthesized products were determined by FT-IR, which the functional group alterations can be traced easily.

To detect and compare functional groups on the molecules, IR spectra were taken (Figure 3.5). The FT-IR of azide ended PEG5K (3) showed a sharp peak at 2106 cm^{-1}**(a)** due to the C-N bond stretching. After the conjugation with G2 dendron, this peak disappeared and the characteristic broad peak between 3100 - 3500 cm^{-1} which representing the hydroxy groups on the surface of dendrons and also, the carbonyl stretch which belong to the carbonyl esters of dendron units at 1734 cm^{-1} were observed. Copolymer (13) (c) did not contain any alcohol or azide groups, but the alkyne stretching at 3276 cm^{-1} was detectable which formed due to the reaction of the hydroxy terminated copolymer (8) with 4-pentynoic anhydride.

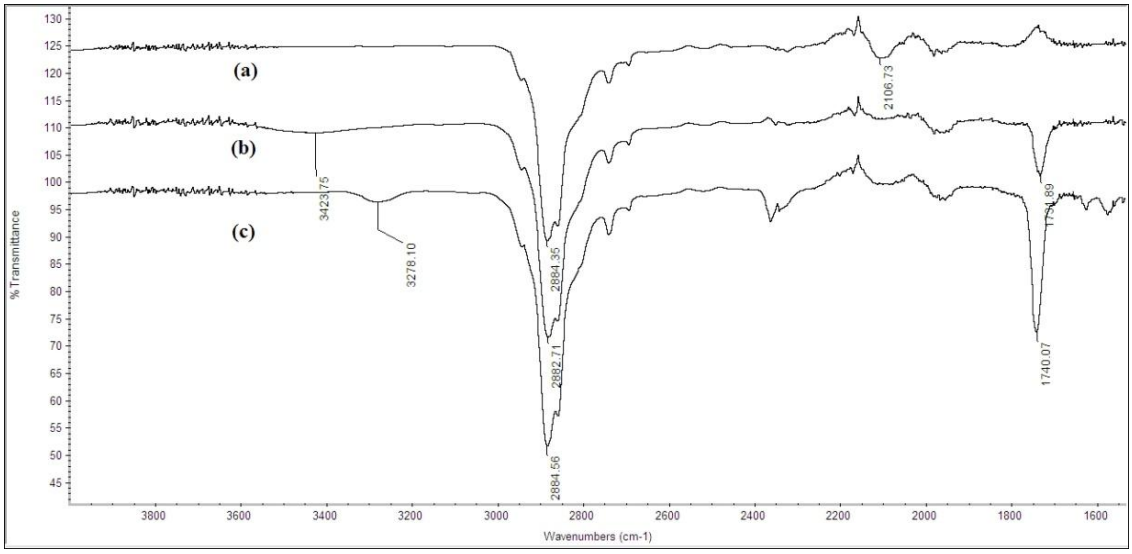

Figure 3.5. FT-IR results of (a) [PEG5K]-N_3 (3), (b) [G2]4OH[PEG5K] (8), (c) [G2]4OR[PEG5K] (13).

The same changes also detected for G3 conjugated polymers on FT-IR measurement. In the click reaction azide group of the polymers and the core alkyne proton forms a triazole ring so that azide functional group disappears at 2100 cm^{-1} and detected a peak at 3435 cm^{-1} for hydroxy groups and at 1731 cm^{-1} for carbonyl groups of dendron units appearance support the synthesis of 9. After the functionalization of –OH groups the peak at 3282 cm^{-1} was seen and broad peak between 3100 - 3500 cm^{-1} disappeared.

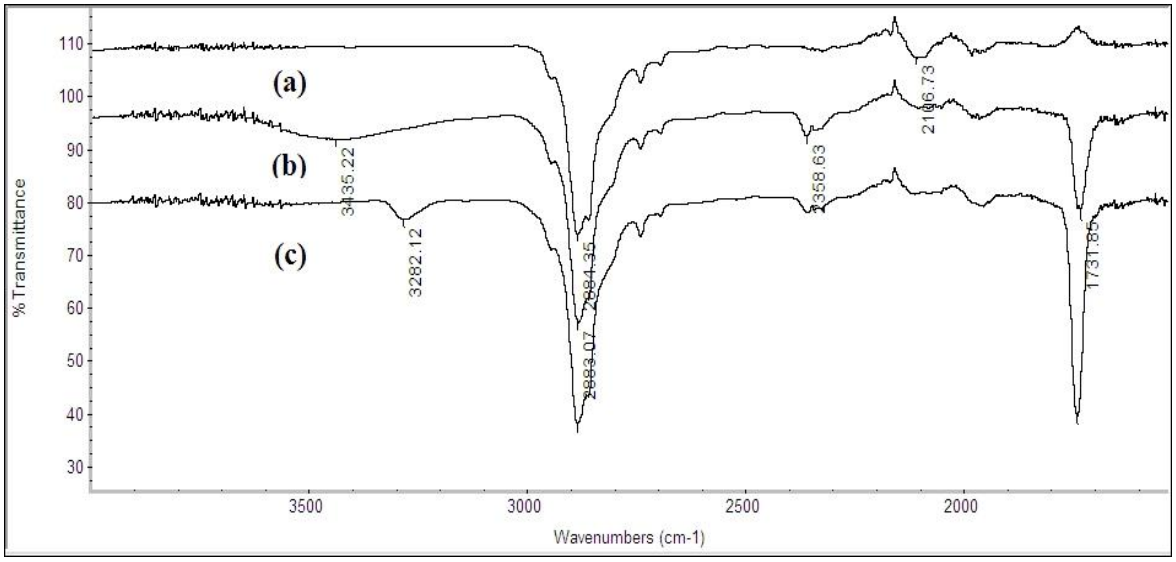

Figure 3.6. FT-IR results of (a) [PEG5K]-N_3 (3), (b) [G3]8OH[PEG5K] (9), (c) [G3]8OR[PEG5K] (14).

The synthesis of the conjugates and their functionalization were also monitored via ^{1}H- NMR to approve the FT-IR data.

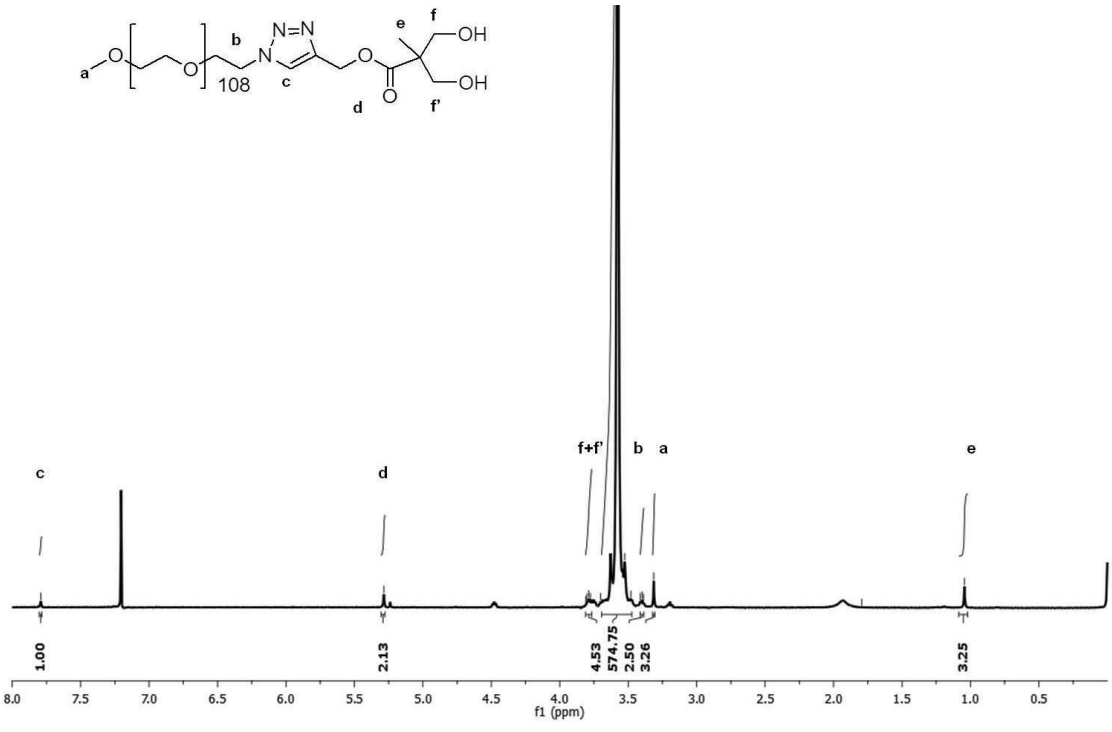

Figure 3.7. ^{1}H NMR of [G1]2OH[PEG5K] (7).

From the ^{1}H NMRs of G1, G2 and G3 dendron conjugated polymers in Figure 3.7, Figure 3.8 and Figure 3.9, respectively, the H assignments can be seen easily. After the Huisgen type click reaction between the G1 G2, G3 dendrons and azide ended PEG 5K 1,4-triazole formation occurs between the alkyne core of dendrons and N_3 ends of PEG polymers. This triazole hydrogen can be defined from ^{1}H- NMR of dendronized polymers as a singlet at 7.79 ppm. A singlet at 5.2 ppm is present that belongs to the $–CH_2$ near –O-OC of dendron and again two singlets come at 1.04 ppm and 3.31 ppm which, succesively, belong to the the methyl Hs of dendron and the terminal alkane H of polymer.

The ^{1}H NMR of (Figure 3.8) (Figure 3.9) also displays the products which are the result of the click reaction of G2 and G3 dendrons with azide ended PEG. They give the same peaks for triazole and other expected H's with different H integrations due to the change of generation number.

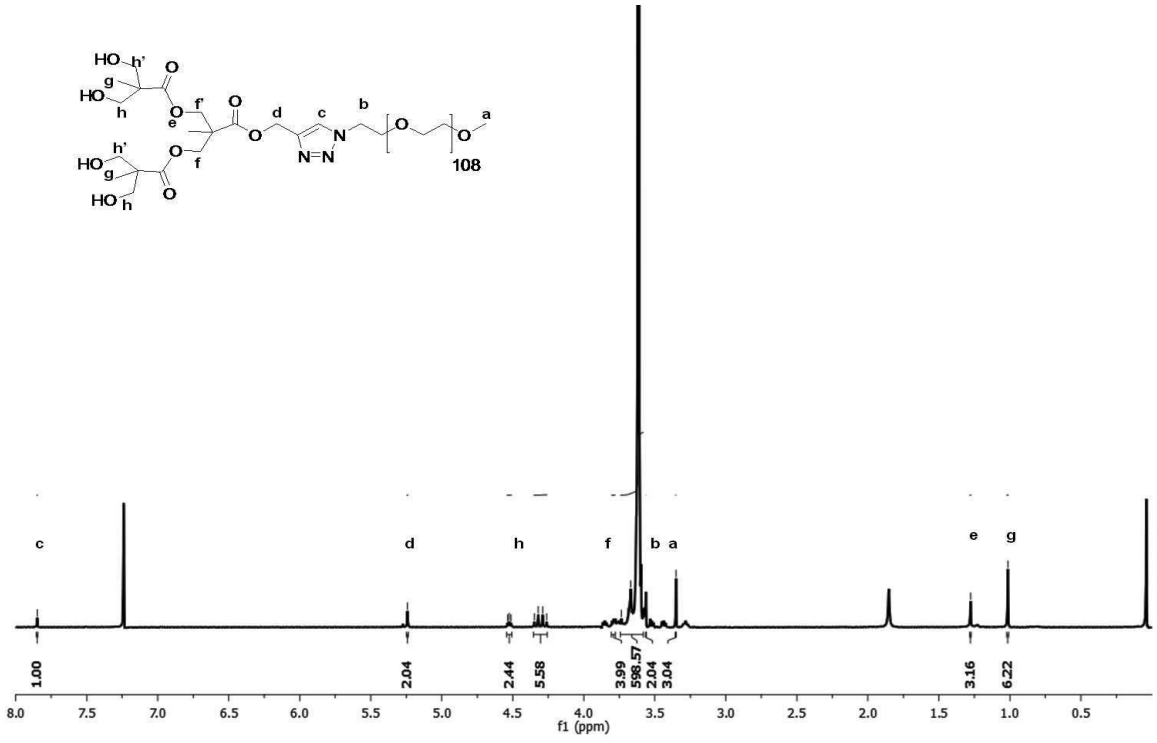

Figure 3.8. ^{1}H NMR of [G2]4OH[PEG5K] (8).

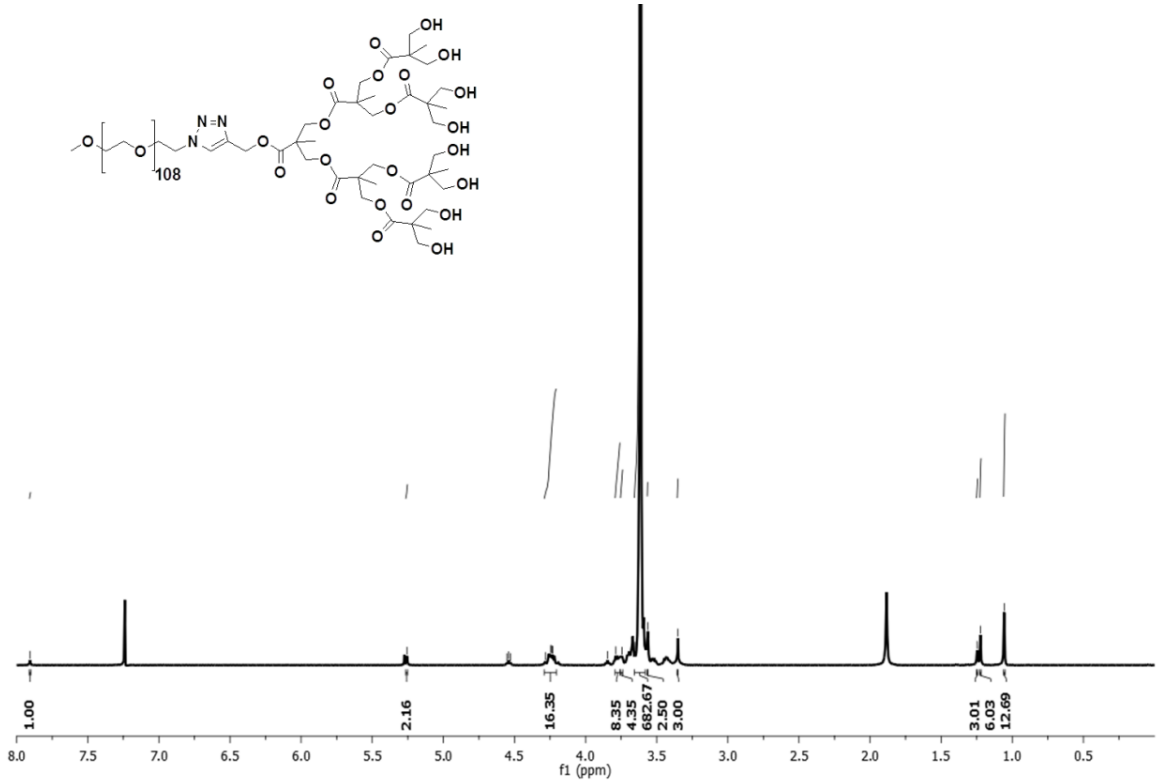

Figure 3.9. ^{1}H NMR of [G3]8OH[PEG5K] (9).

Alkyne functionalized dendronized polymers which are product of the acylation reaction can also be characterized due to the new proton peaks on NMR spectrum. In Figure 3.10, proton NMR of 12 new proton peaks at 2.55 and 2.47 ppm as triplet are seen due to two neighbour $-CH_2$ groups and a singlet at 1.98 for terminal alkyne proton of pentynoic acid.

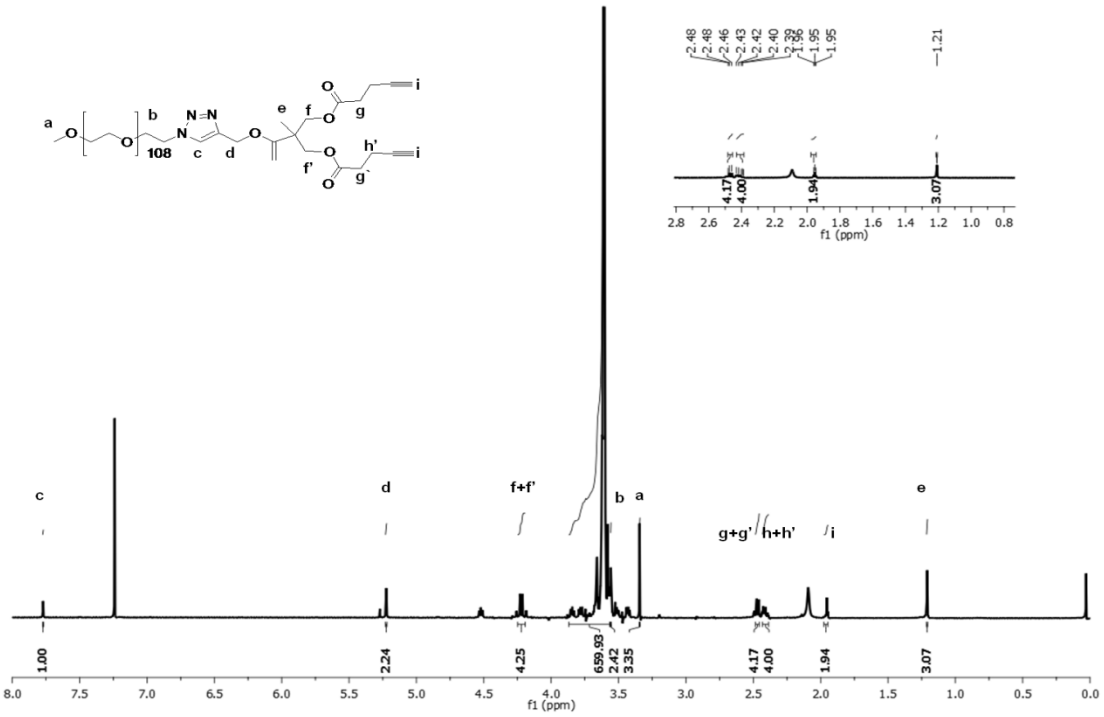

Figure 3.10. ^{1}H NMR of [G1]2OR[PEG5K] (12).

Also ^{1}H NMR analysis of shows that [G2]4OR[PEG5K] (13) and [G3]8OR[PEG5K] (14) alkyne derivatives were successfully achieved (Figure 3.11) and (Figure 3.12). Appearance of new peaks at 2.45 ppm and 2.52 ppm that belong to the alkyne functional group and at 1.95 ppm from alkyne proton proves the formation of alkyne ended dendronized polymers.

3.1.2. Drug Conjugation to the Functionalized Dendronized Polymers

Conjugation of drug molecules to macromolecules provides many unprecedented advantages such as; increase in accumulation in the tumor via EPR effect, so, increase in the amount of drug into the tumor ,decrease in drug resistance and, reduction of clearance rate. Moreover, dendronized polymer bounded drug molecule is stable in plasma due to the conjugation. The drug can release from the macromolecule to exhibit the anti-angiogenic effect via pH difference, enzymatically and so on. In this way, the effect of the polymer-bound drug to normal cells is diminished by decreasing the potential side effects, in other words, decrease in toxicity. For these reasons, in the study, these benefits were utilized to

improve therapeutic effects of drug molecules alone. Furthermore, drug molecules in the study were attached on dendronized polymer covalently.

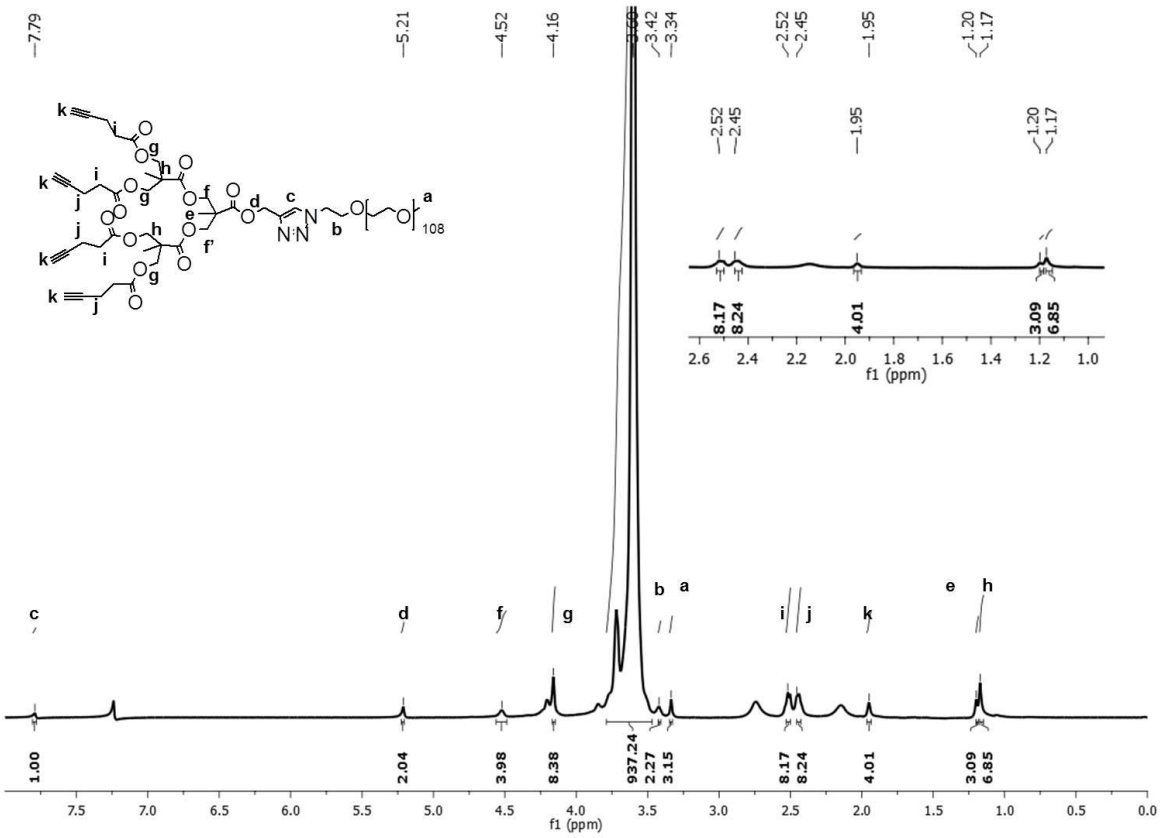

Figure 3.11. ^{1}H NMR of [G2]4OR[PEG5K] (13).

PEG is a biocompatible polymer but dendronized polymer –drug conjugates that are used in the study both biocompatible and biodegradable because ester groups in dendronized polymer-drug conjugates make them susceptible to hydrolysis in aqueous media and so, become biodegradable.

This gave a remarkable opportunity in terms of clearance rate and drug release profile. By taking all these pivotal advantages, conjugation of CA-4 bearing azide groups on functionalized dendronized polymer bearing alkyne unit is achieved via Huisgen type ‘click’ reaction.

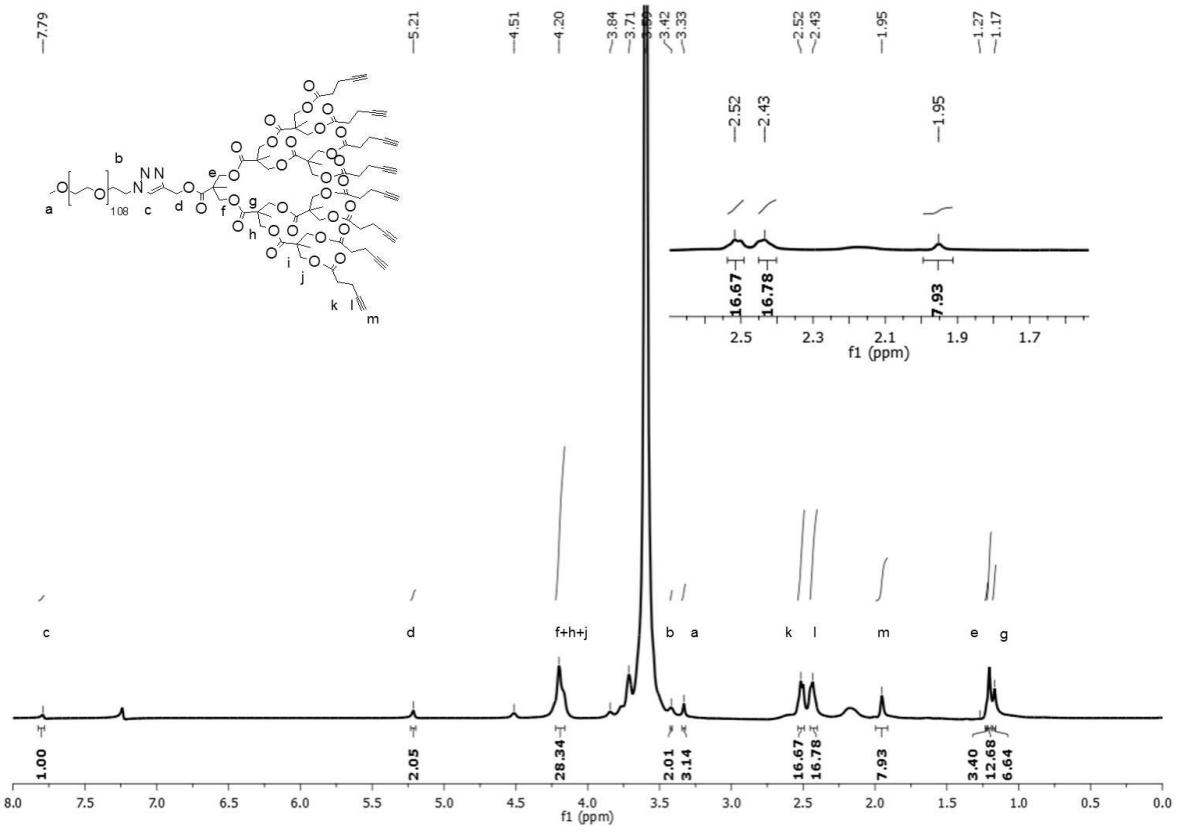

Figure 3.12. ^{1}H NMR of [G3]8OR[PEG5K] (14).

The general scheme for drug combination to dendronized polymers is given in Figure 3.13. Conjugation efficiency for these reactions with 12, 13 are 100% and for 14 are 75%, respectively. The reason for not having 100% conjugation for 17 may be the steric hinderence effect of drug molecule, it may not give cycloaddition reaction for each arms of dendrons.

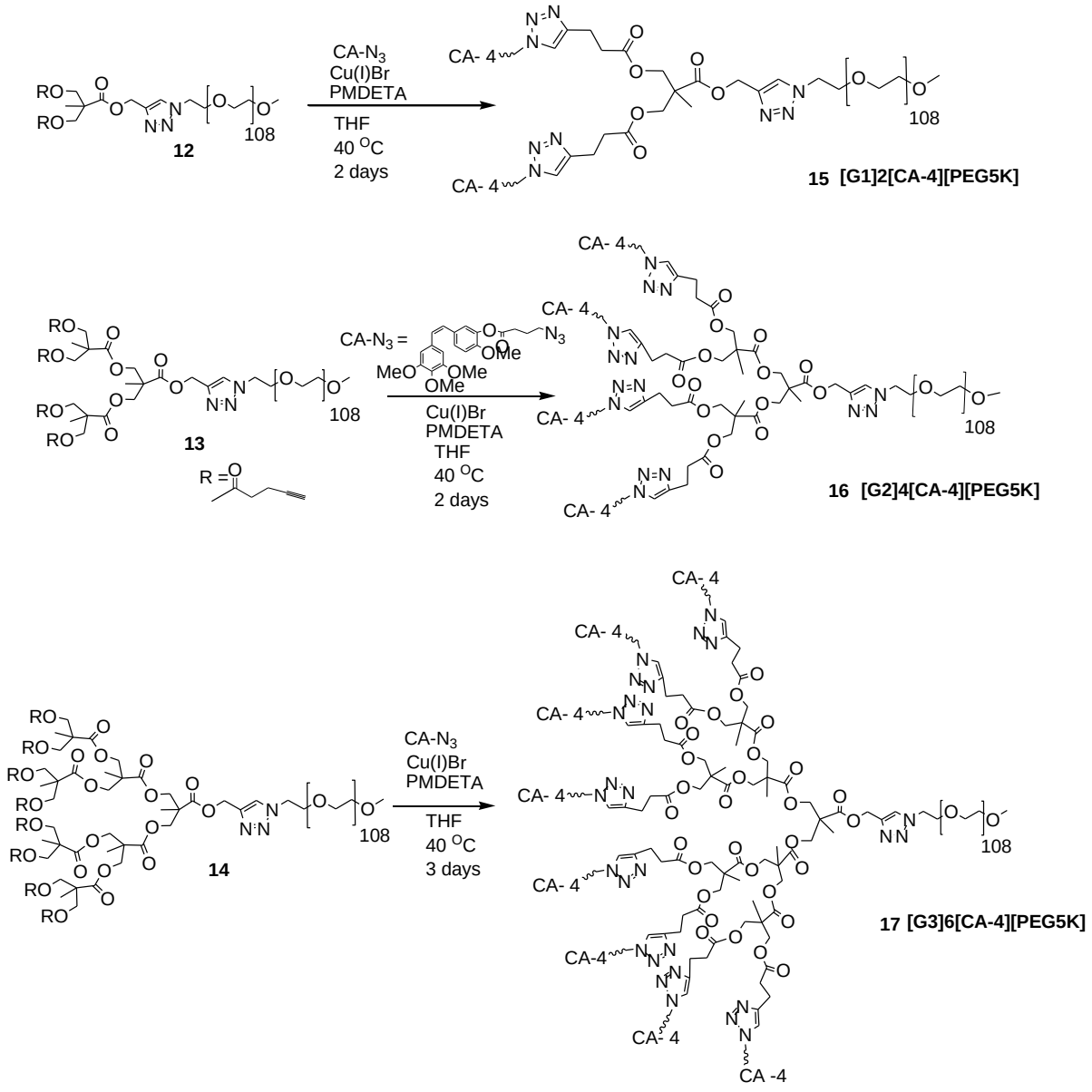

Figure 3.13. Genereal scheme for drug conjugation to dendronized polymers.

Whether click reaction worked successfully or not was monitored by ^{1}H-NMR. Figure 3.14, 3.15 and 3.16 exhibits results of conjugation. After conjugation, peak at 2.45 ppm and 2.52 ppm that belong to the alkyne functional group are shifted to down field (between 4.00 – 4.50 ppm) and integral results showing 100% conjugation with drug only 75% for (17), which proves that the click reaction has worked successfully for (15) and (16). New triazole proton formation was expected and the peak gave at 7.41 ppm and proton integration shows that 2 drugs molecule are combined to (12). One of the specific peaks of drug molecule comes at 7.09 ppm shown with letter ‘o’ at Figure 3.14. Specific

peaks belonging to drug molecule (peaks o, m, n, r, p, l, j, k) are evidence for the conjugation of drug molecule on dendronized polymer. From ^{1}H NMR of product (16) and **(**17) in Figure 3.15 and Figure 3.16.

The same proton peaks from drug molecules are also present. When integration of triazole 1 (between alkyne core of dendron and PEG azido) and triazole 2 (between alkyne functionalized dendron arm and Comb azido) gets into account 1 to 4 and 1 to 6 ratio is observed and this means drug conjugation is finished for 16 is not finished for 17.

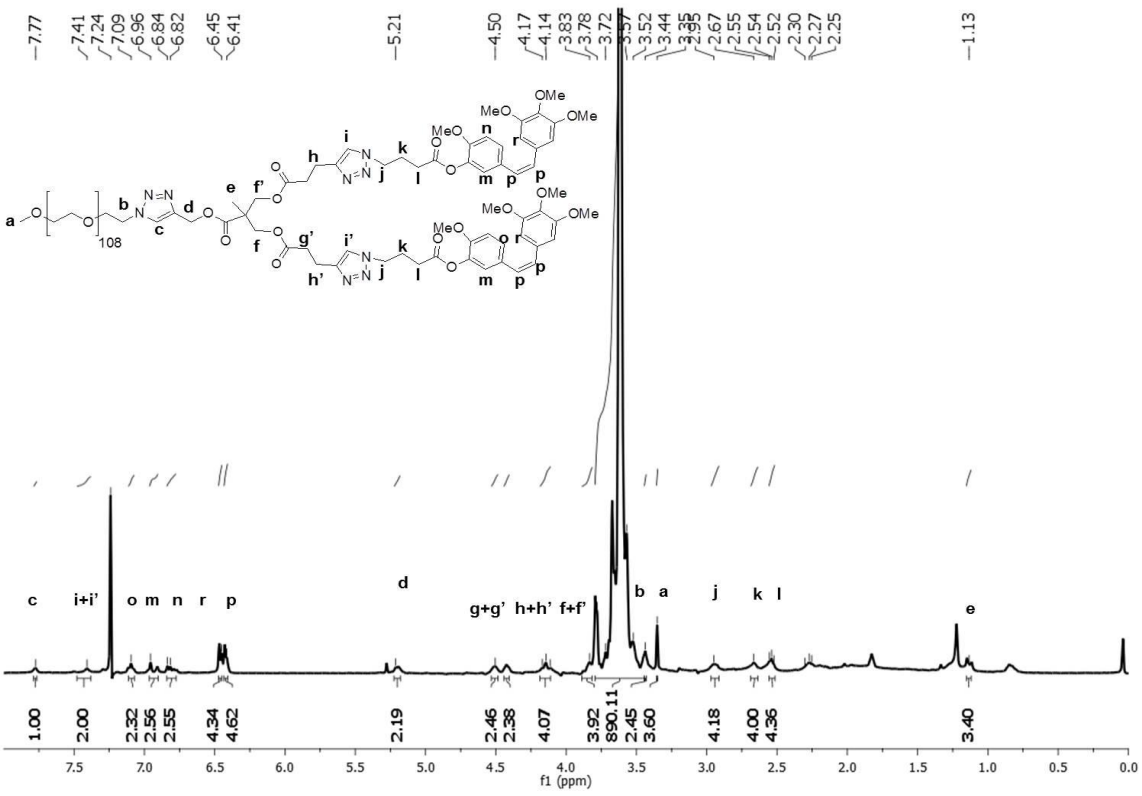

Figure 3.14. ^{1}H NMR of [G1]2[CA-4][PEG5K] (15).

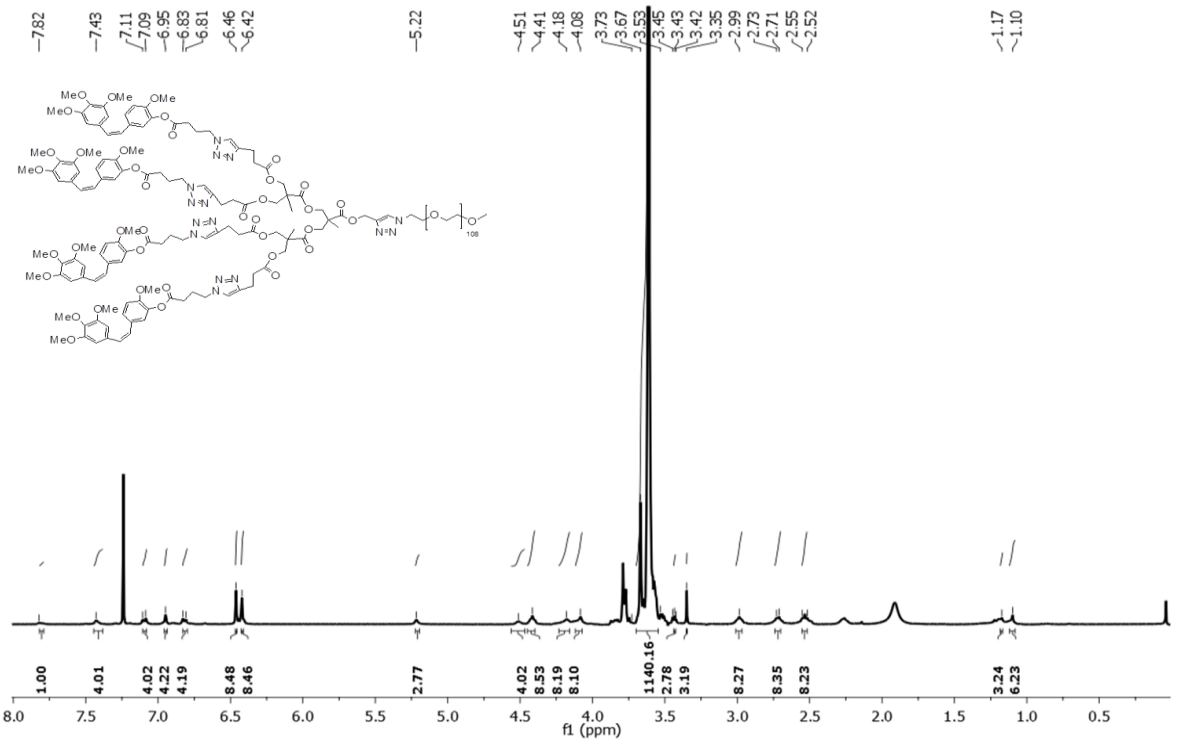

Figure 3.15. ^{1}H NMR of [G2]4[CA-4][PEG5K] (16).

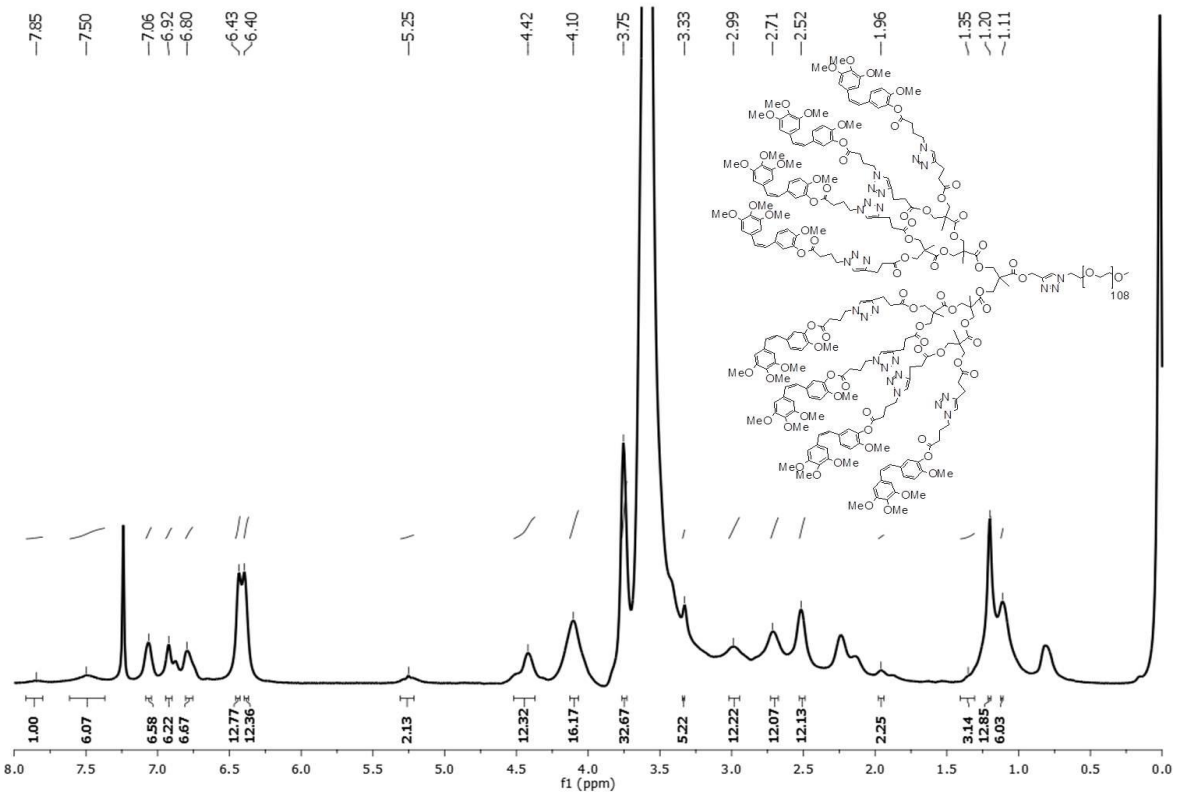

Figure 3.16. ^{1}H NMR of [G3]6[CA-4][PEG5K] (17).

3.1.3. Micelle Formation from Dendronized Polymers and Drug Conjugated Polymers

Polymeric micelles are nanoscale shell-core structures constructed from amphiphilic block copolymer in aqueous solution. The hydrophobic core may serve as a nanoreservoir for hydrophobic drugs, while the hydrophilic shell allows the stabilization of micelles in aqueous media. Alkyne ended and drug conjugated dendronized polymers tend to form micellar structures in aqueos media due to the hydrophilic-hydrophobic composition.

Polymeric micelles have presented special interests because these carriers have some advantages, including easiness of preparation, small and uniform particle size (10–100 nm), high stability, high drug-loading capacity, biodegradability, controllable drug release profiles, long systemic circulation time, and enhanced accumulation in tumor via the enhanced permeability and retention (EPR) effect.

3.1.3.1. Fluorescence Experiments. Fluorescence measurement is run to scan alkyne ended and drug conjugated dendronized polymers' micelle formation and pyrene is used as a probe because normally pyrene is not soluble in water and it will be soluble in the hydrophobic core, then it will give characteristic fluorescence peaks when dissolve.

1.8 x 10^{-4} M of pyrene stock solution was prepared and 10 μL of pyrene-acetone solution was added into vials for each sample. In order to get rid of acetone solution, all vials waited in decicator at high vacuum at least 3 h. Different concentrations of conjugates (10^{-4} to 10^{-10} M) were prepared in distilled water. After the preparation of each sample, 3 ml of these conjugate solutions were added into each vial respectively and the solutions were sonicated for 1 hour at room temperature. The final pyrene concentration was $6x10^{-7}$M. After keeping the each sample for 24 h, micelle formations of conjugates were characterized by Fluorescence spectroscopy.

Fluorescence data were collected by excitation experiments at 300-360 nm range using 5 nm width. Pyrene has characteristic peaks at 334 and 338 nm wavelenght; therefore, micelle formation in these data was explained by the shift of the wavelenghts and the critical micelle concentrations can be determined from the log (concentration) vs

I_{338}/I_{334} graphs. The excitation data for product (14) is given in Figure 3.17. From the excitation graph of conjugate 14 it is obvious that the pyrene intensity increases as the concentration increases.

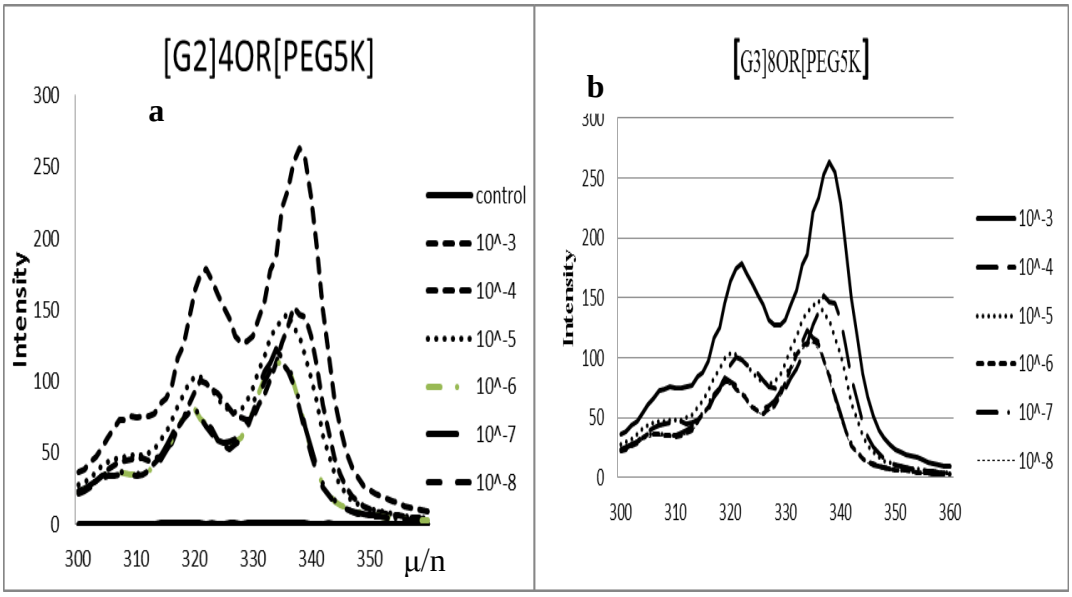

Figure 3.17. Excitation graph of (a) [G2]4OR[PEG5K] (13) and (b) [G3]8OR[PEG5K] (14).

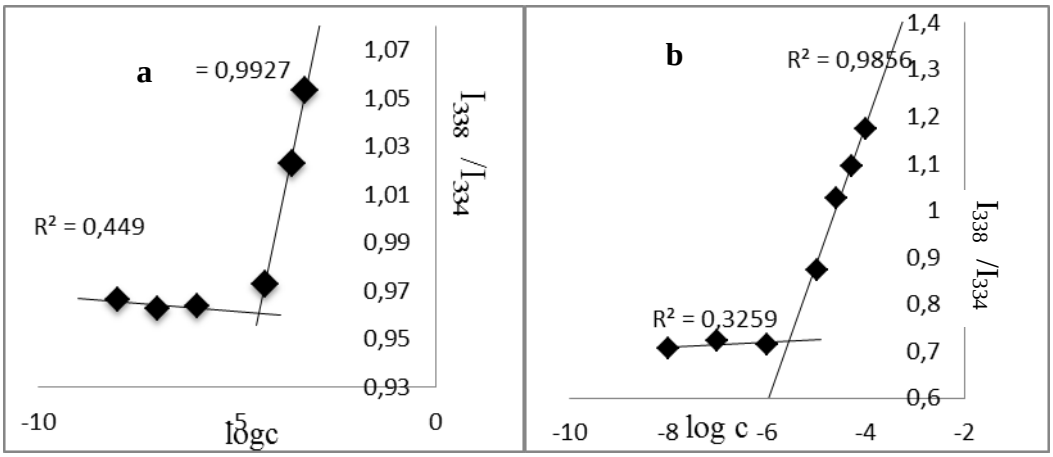

Figure 3.18. Log C vs I_{338}/I_{334} graph of (a) [G2]4OR[PEG5K] (13) and (b) [G3]8OR[PEG5K] (14).

Table 3.1. CMC values for polymer-dendron conjugates.

Dendron-Polymer Conjugate	CMC (M)
13	$3.6x10^{-5}$
14	$3.5x10^{-6}$
16	$3.9x10^{-6}$
17	$1.5x10^{-6}$

According to the Table 3.1, it is obvious that there is a trend between the conjugates related to the generations of dendron. The highest CMC value was seen for generation three dendron with PEG 5K was expected.

For drug conjugates micellar structure tend to be formed at lower concentration than copolymers. This is the result of increased hydrophobic length in copolymers.

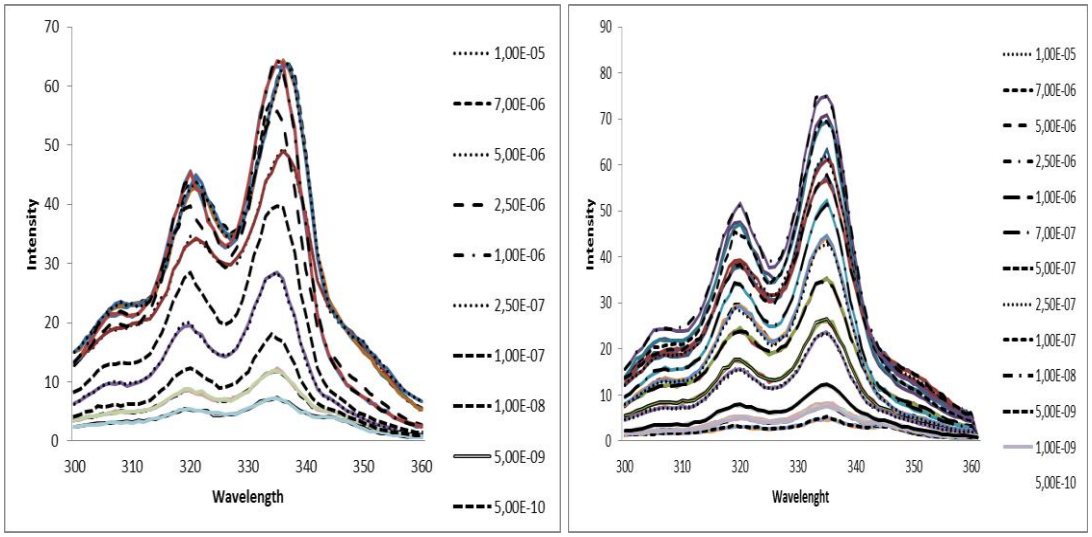

Figure 3.19. Excitation graph of ; (a) [G2]4[CA-4][PEG5K] (16),
(b) [G3]6[CA-4][PEG5K] (17).

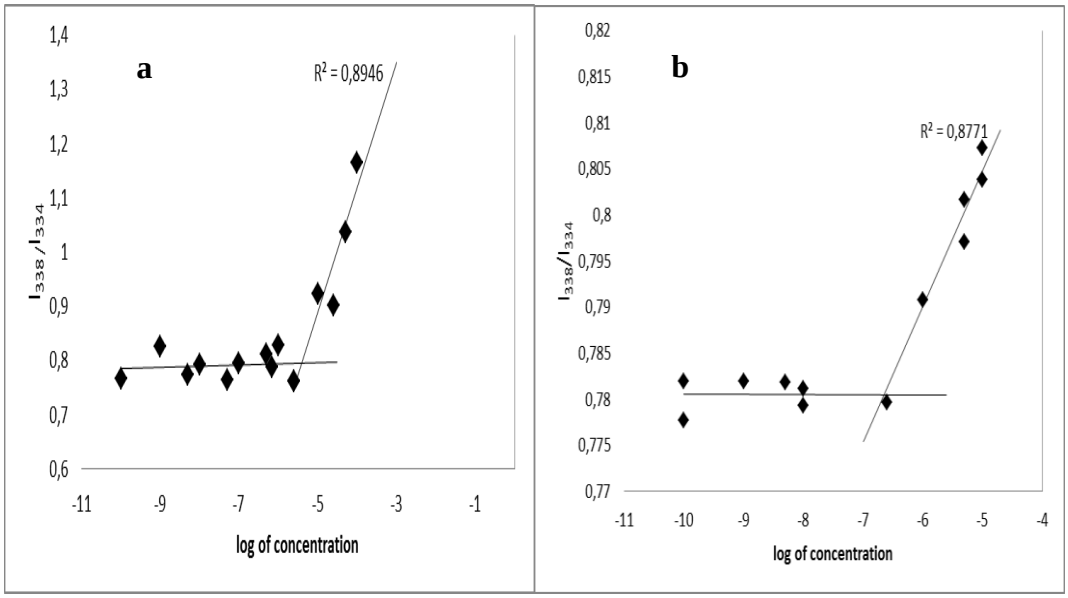

Figure 3.20. CMC graphs of copolymers; (a) [G2]4[CA-4][PEG5K] (16), (b) [G3]6[CA-4][PEG5K] (17).

For product (15) no shift for pyrene peaks were observered, means micelle formation was not seen, so other measurements did not performed using this product.

3.1.3.2. Dynamic Light Scattering (DLS) Measurements. In order to determine the effective diameter and the size distributions of micelle structures of dendronized polymers and drug conjugated dendronized polymers after finding the desired CMC, dynamic light scattering (DLS) experiments were performed above these concentrations. Solutions were prepared using distilled H_2O at desired concentrations and the analysis of particle size of these micelles were done using dynamic light scattering (DLS) at 25 ^oC and 90^o angle.

Since volume vs diameter graphs tells the dispersity of micellar structures at the same diameter, from these graphs micellar structures dispersion can be concluded that in (a) and (b) unimodular micelle formation, where as in (c) bimodular micelle formations are seen.

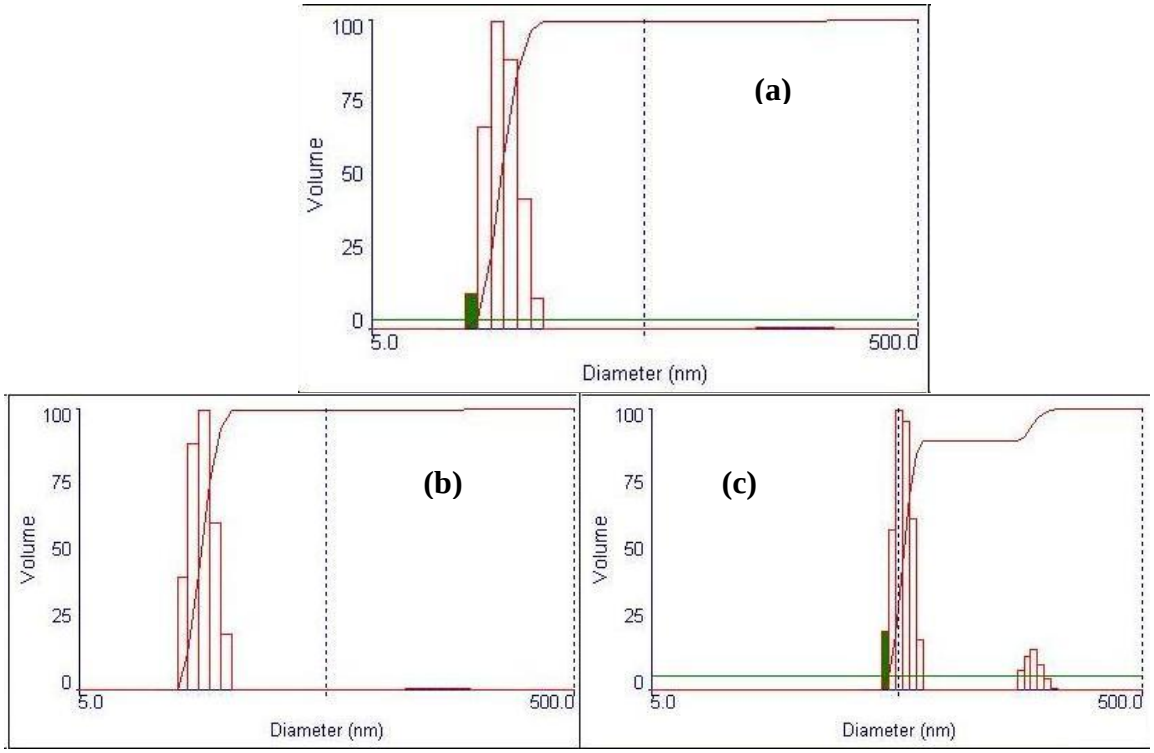

Figure 3.21. Volume vs diameter graph of a) [G3]8OR[PEG5K] (14), b) [G2]4[CA-4][PEG5K] , and c) [G3]6[CA-4][PEG5K] (17).

Table 3.2. Effective diameters of samples [G2]4OR[PEG5K] (13) and [G3]8OR[PEG5K] (14).

Product	Con. (M)	Effective diameter nm	Product	Conc. (M)	Effective diameter nm
13	10^{-4}	195	14	10^{-4}	69

The smallest micelle diameter was found in DLS as 69 nm for sample (14) and the biggest one is for (13). Actually, the smallest micelle formation were expected to be formed (13) according to its hydrophobic/hydrophilic ratio but this did not observed.

Introducing hydrophobic drug molecule to the system the tendency of micelle formation of copolymer increased. The diameter of non-coagulated [G3]6[CA-4][PEG5K] (17) product found to be around 70 nm and micellar structures is unimolecular.

Table 3.3. Effective diameters of samples [G2]4[CA-4][PEG5K] and [G3]6[CA-4][PEG5K] (17).

Product	Concentration (M)	Effective Diameter (nm)	Concentration (M)	Effective Diameter (nm)
16	10^{-4}	16	10^{-5}	21
17	10^{-5}	72	10^{-6}	65

4.EXPERIMENTAL

4.1. General Methods and Materials

2,2-Bis(hydroxymethyl) propionic acid (BMPA), Dowex X50WX2, Propargyl Alcohol, 4-pentynoic acid were purchased from Alfa Aesar. All polyethylene glycols were obtained from Fluka. All solvents were purchased from Merck and used as obtained without further purification unless otherwise noted. Azide functionalized PEG were synthesized according to literature procedures. The monomer and copolymer characterizations involved ^{1}H NMR spectroscopy (Varian 400 MHz) and Fourier transform infrared (ATR-FT-IR) spectroscopy (Thermo Fisher Scientific Inc. Nicolet 380). Micelle formations were characterized using Fluorescence spectroscopy (Cary Eclipse) , Dynamic Light Scattering 90 Plus Particle Size Analyzer instrument (Brookhaven Instruments Cooperation).

4.2. Synthesis of Dendrons and Dendron-Polymer-Dendron Conjugates

Alkyne functionalized dendrons G1ol, G2ol and G3ol were synthesized according to literature procedures. Via deprotection reaction G1ol, G2ol and G3ol were obtained from G1trp, G2trp and G3trp.

4.2.1. Synthesis of Generation1 Polyester Dendron

In order to obtain first generation polyester dendron, firstly G1-Acid was synthesized. Bis-MPA 5.00g (37.27 mmol), p-TsOH 0.35g (1.83 mmol), and 2,2-dimethoxypropane 6,8 mL (55.30 mmol) in 20 mL of dry acetone was stirred for 3 h at room temperature. The organic phase was diluted with 50 mL of CH_2Cl_2, extracted with 2x30 mL of distilled water and dried with Na_2SO_4, finally the solvent was evaporated in order to obtain the white product, which is G1 Acid, in 81% yield. In order to obtain first generation polyester dendron, G1 Anhydride was synthesized by the reaction of 9.37 g (53 mmol) of G1 Acid and 5.54 g (27 mmol) of DCC (Dicyclohexylcarbodiimide) in 40 mL of dry CH_2Cl_2. The reaction was stirred at room temperature under N_2 gas for 4h. The precipitated white product, which is DCU, was filtered off in a sintered glass and again

washed with cold EtOAc in order to get rid of the left DCU. After the second filtration of DCC byproduct and evaporation of the solvent 8.70 g of G1-Anhydride was obtained in. 99% yield.

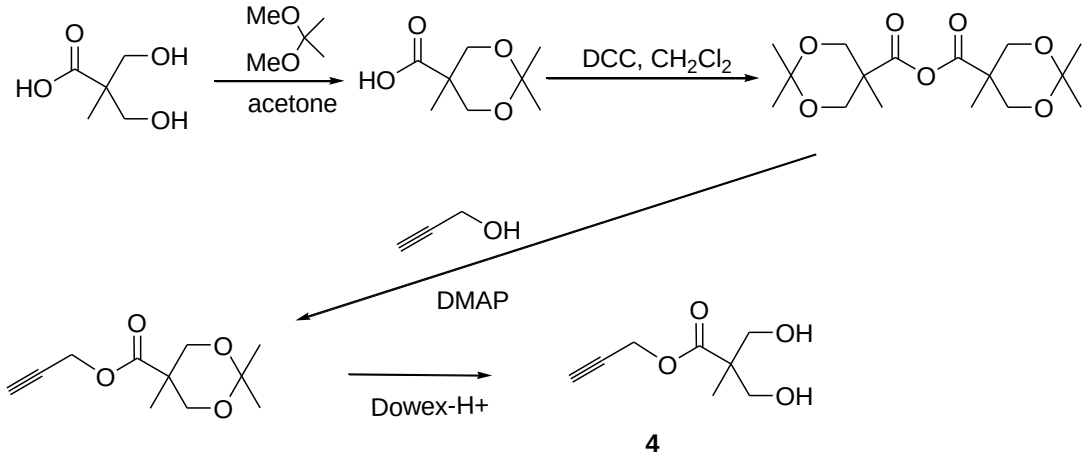

Figure 4.1. Synthesis of G1 Dendron

For the synthesis of the G1 dendron, G1 Anhydride (7.45 g, 23 mmol) reacted with propargyl alcohol (0.89 ml, 15 mmol), DMAP (1.84 g, 15 mmol) and pyridine (1.81 mL, 23 mmol) in 25 mL of dry CH_2Cl_2 at room temperature under N_2 gas. The reaction mixture was stirred at room temperature overnight, and 1.81 mL of distilled water was added into the reaction mixture after 20 hr. The organic phase was diluted with 50 mL of CH_2Cl_2 and extraction was done with 3x50 mL of $NaHSO_4$ (1M), 3x50 mL of Na_2CO_3 (%10) and 2x50 mL of brine solution. Finally the organic phase was dried using Na_2SO_4 and evaporated to obtain protected G1 dendron (3.2 gr). In order to deprotect this dendron, DOWEX (1 gr) was used in 40 mL of methanol at 40^oC under N_2 gas; therefore, protected first generation dendron was converted into first generation alcohol in 91% yield. The scheme of this reaction can be seen in Figure 4.1.

4.2.2. Synthesis of Generation 2 Dendron

Dowex X50WX2 (1.00g) was placed in a 100 mL Erlenmeyer flask and washed methanol (5 x 30 mL). Subsequently, G2-Alkyne (6.00 g, 0.028 mmol), Dowex X50WX2 (1.00 g) and methanol (60 mL) were added to a 100 mL rb flask and stirred for 24 h.

Dowex was removed via filtration and the filtrate was dried under *vacuo* yielding G2-ol (5.30 g, 99%) as a white solid. ^{1}H NMR (CD_3OD, δ, ppm) 4.75 (s, 2H), 4.48 (d, 2H, *J* = 11.2 Hz), 4.31 (d, 2H, *J* = 11.2 Hz), 3.9 (dd, 4H, *J* = 10.8, 8.0 Hz), 3.60 (dd, 4H, *J* = 10.8, 8.0 Hz), 2.49 (s, 1H), 1.34 (s, 3H), 1.05 (s, 6H). FT-IR (cm^1): 3260, 1718.

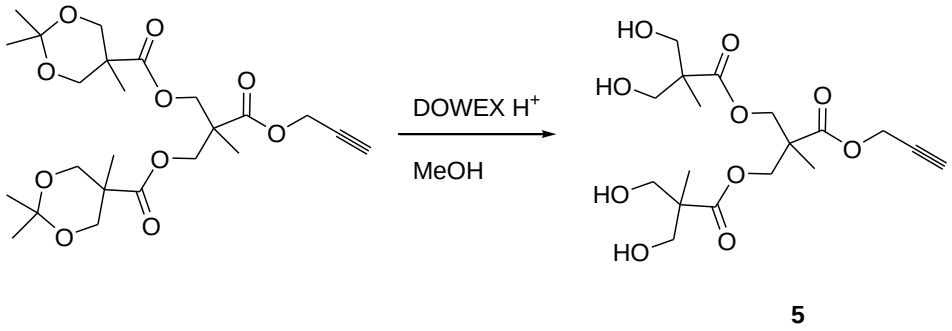

Figure 4.2. Synthesis of Generation 2 dendron

4.2.3. Synthesis of Generation 3 Dendron

Dowex X50WX2 (1.00g) was placed in a 100 mL Erlenmeyer flask and washed methanol (5 x 30 mL). To the G3-Alkyne (3 g, 2.92 mmol), Dowex X50WX2 (2 tips) and methanol (60 mL) was added to a 100 mL rb flask and stirred for 24 h at 40 ^{o}C. After the reaction was completed Dowex was removed via filtration and the filtrate was dried under *vacuo* yielding G3-ol (2.80 g, 96%) as a white solid. ^{1}H NMR (CD_3OD, δ, ppm) 4.76 (s, 2H), 4.38-4.24 (m, 12H), 3.82 (d, 8H, *J* = 10.3 Hz), 3.73 (d, 8H, *J* = 10.1 Hz), 2.43 (s, 1H), 1.23 (s, 3H), 1.16 (s, 6H), 1.14 (s, 12H). FT-IR (cm^{-1}): 3292, 1728.

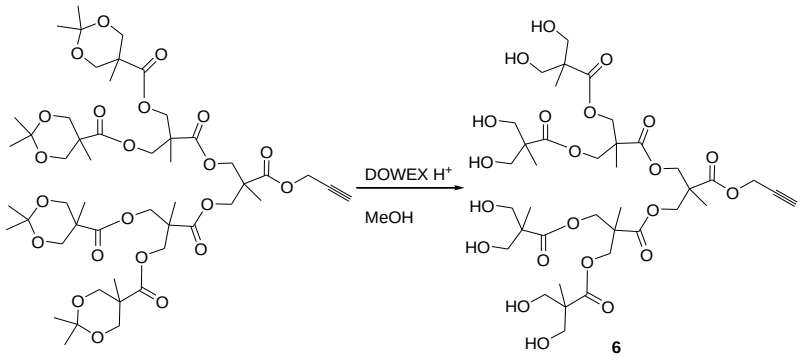

Figure 4.3. Synthesis of Generation 3 Dendron.

4.2.4. Synthesis of Compound [G1]2OH[PEG5K]

PEG-5K-monoazide (500 mg 0.1 mmol) and propargyl [G1]2[OH] (70 mg, 0.41mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (3.3 mg, 0.023 mmol) and N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 4.2 μL, 0.25 mmol) in dry THF (3 mL) and purged with N_2. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 40^o C for 24 h. The solvent was then evaporated, and the crude product was dissolved in THF (50 mL) the solution was filtered through Al_2O_3 column to remove copper salts. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O, filtered, and dried in *vacuo*, yielding compound [G1]2OH[PEG5K] (420 mg, 81%) as a yellowish-white solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.79(s, 1H), 5.29 (s, 2H), 3.81-3.78 (m, 4H), 3.40-3.39 (m, 2H) , 3.31 (s, 3H), 1.04 (s, 3H).

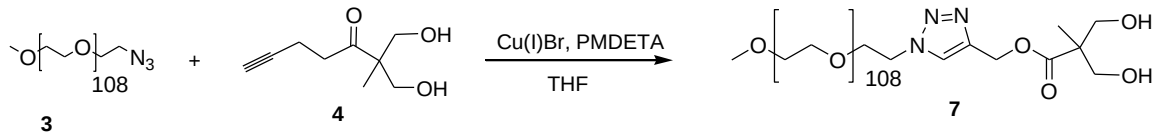

Figure 4.4. Synthesis of [G1]2OH[PEG5K].

4.2.5. Synthesis of Compound [G2]4OH[PEG5K]

PEG-5K-monoazide (400 mg 0.08 mmol) and propargyl [G2]4[OH] (64. 3 mg, 0.016mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (1,15 mg, 0.008 mmol) and N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 1.66 μL, 0.25 mmol) in dry THF (2 mL) and purged with N_2. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 40^o C for 24 h. The solvent was then evaporated, and the crude product was dissolved in THF (50 mL) the solution was filtered through Al_2O_3 column to remove copper salts.. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O, filtered, and dried in *vacuo*, yielding compound [G2]4OH[PEG5K]. (320 mg, 75%) as a yellowish-white solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.85 (s, 1H), 5.24 (s, 2H), 4.53-4.26 (m, 8H), 3.74-3.67 (m, 4H), 3.45-3.38 (m, 2H), 3.35 (s, 3H), 1.28 (s, 3H), 1.01 (s, 6H).

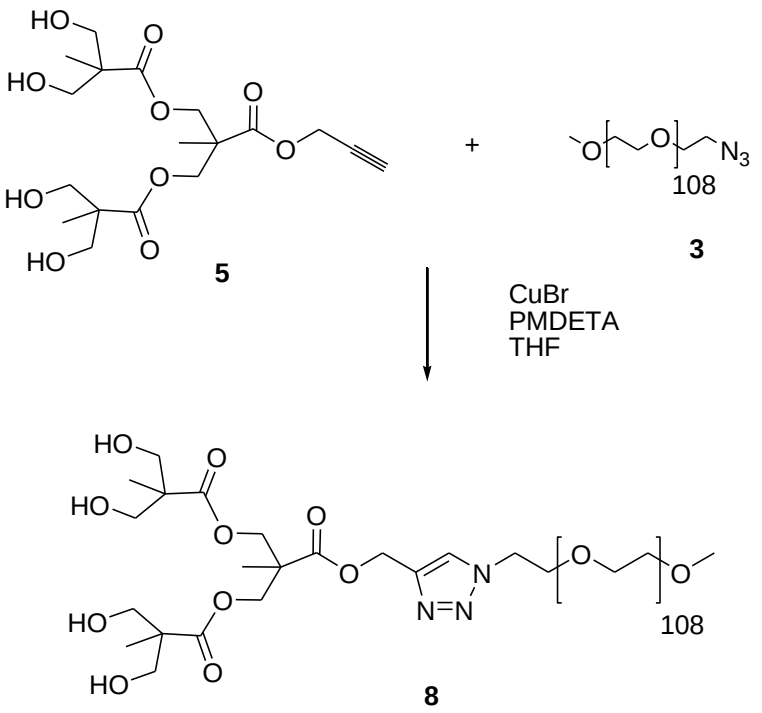

Figure 4.5. Synthesis of [G2]4OH[PEG5K].

4.2.6. Synthesis of Compound [G3]8OH[PEG5K]

PEG-5K-monoazide (400 mg, 0.079 mmol), propargyl [G3]8[OH] (138 mg 0.158 mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (1.14 mg 0.008), and PMDETA (1.66 μL) in dry THF (2 mL) and purged with N_2. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 40^o C for 24 h. The solvent was then evaporated, and the crude product was dissolved in THF (50 mL) the solution was filtered through Al_2O_3 column to remove copper salts. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O filtered, and dried in *vacuo*, yielding compound [G3]8OH[PEG5K] (350 mg, 77%) as a yellowishwhite solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.91 (s, 1H), 5.26 (s, 2H), 4.25-3.74 (m, 28H), 3.56 (s, 2H), 3.35 (s, 3H), 1.25 (s, 3H), 1.22(s, 6H), 1.06(s, 12H).

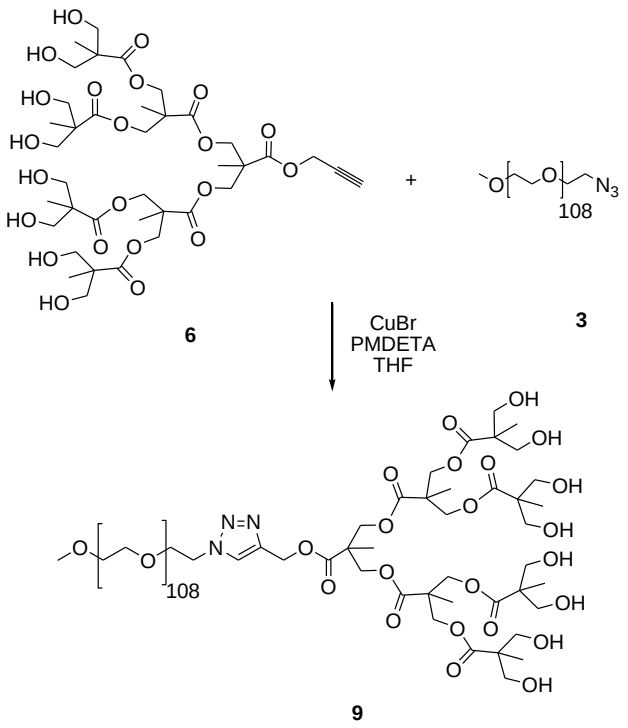

Figure 4.6. Synthesis of [G3]8OH[PEG5K].

4.2.7. Synthesis of [G1]2OR[PEG5K]

[G1]2OH[PEG5K] (420 mg, 0.08 mmol), pyridine (66 μL), and DMAP (4 mg, 0.0212 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL roundbottom flask. To the stirring reaction mixture was added 4-pentynoic acid anhydride (57.6 mg, 0.317 mmol) and continued stirring for 24 h at room temperature under N_2. Pyridine:water solution (1:1) was added to the reaction mixture and stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (30mL) and then extracted with 1M $NaHSO_4$ (3 *30 mL), 10% Na_2CO_3 (3 *30 mL), and then with brine (1*30 mL). Combined organic layers were dried over anhydrous Na_2SO_4, and the residue was concentrated in *vacuo*. The crude product was purified by precipitation in cold diethylether to give as a yellowish-brown viscous liquid (380mg, 87%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.77 (s, 1H), 5.22 (s, 2H), 4.23-4.21 (s, 4H), 3.58 (s, 2H), 3.34 (s, 3H), 2.48-2.39 (m, 8), 1.95 (s, 2H), 1.21 (s, 3H).

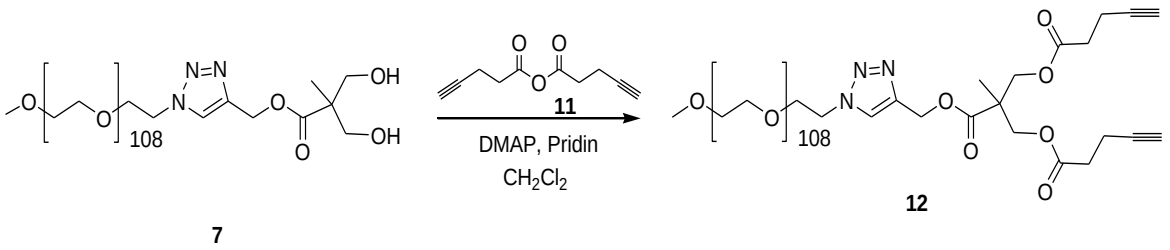

Figure 4.7. Synthesis of [G1]2OR[PEG5K].

4.2.8. Synthesis of Compound [G2]4OR[PEG5K]

[G2]4OH[PEG5K] (287 mg, 0.053 mmol), pyridine (43 µL), and DMAP (2.6 mg, 0.0212 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL roundbottom flask. To the stirring reaction mixture was added 4-pentynoic acid anhydride (57mg, 0.317 mmol) and continued stirring for 24 h at room temperature under N_2. Pyridine:water solution (1:1) was added to the reaction mixture and stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (30mL) and then extracted with 1M $NaHSO_4$ (3 *30 mL), 10% Na_2CO_3 (3 *30 mL), and then with brine (1*30 mL). Combined organic layers were dried over anhydrous Na_2SO_4, and the residue was concentrated in *vacuo*. The crude product was purified by precipitation in cold diethylether to give as a yellowish-brown viscous liquid (290 mg, 95%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.79 (s, 1H), 5.21 (s, 2H), 4.52 (s, 4H), 4.16 (m, 8H), 3.42 (s ,2H), 3.34 (s, 3H), 2.52-2.45 (m, 16), 1.95 (s, 4H), 1.20 (s, 3H), 1.17 (s, 6H).

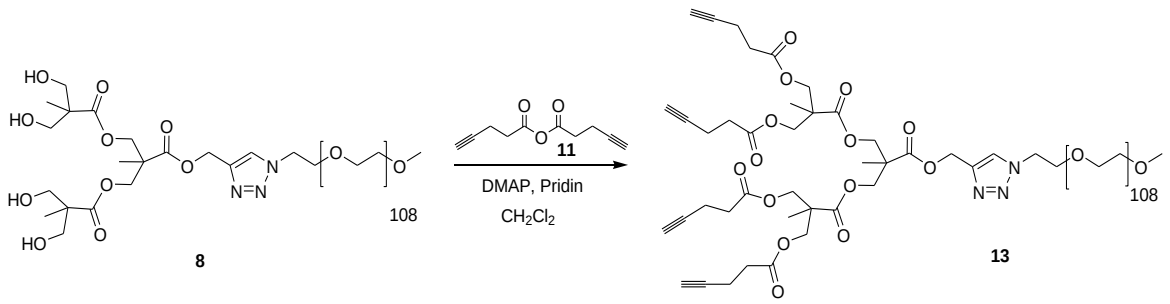

Figure 4.8. Synthesis of [G2]4OR[PEG5K].

4.2.9. Synthesis of Compound [G3]8OR[PEG5K]

Synthesized via the same procedure as compound [G2]4OR[PEG5K] using [G3]8OH[PEG5K] (150 mg, 0.026 mmol) pyridine (21 μL), DMAP (1.27 mg, 0.0104 mmol), and 4-pentynoic acid anhydride (55 mg, 0.305 mmol) to give as a yellowish-white solid (150 mg, 87 %). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.79 (s, 1H), 5.21 (s, 2H), 4.20 (m, 28H), 3.42 (s, 2H), 3.33 (s, 3H), 2.52-2.43 (m, 32H) 1.95 (s, 8H), 1.27 (s, 3H), 1.20 (s, 12), 1.17 (s, 6H).

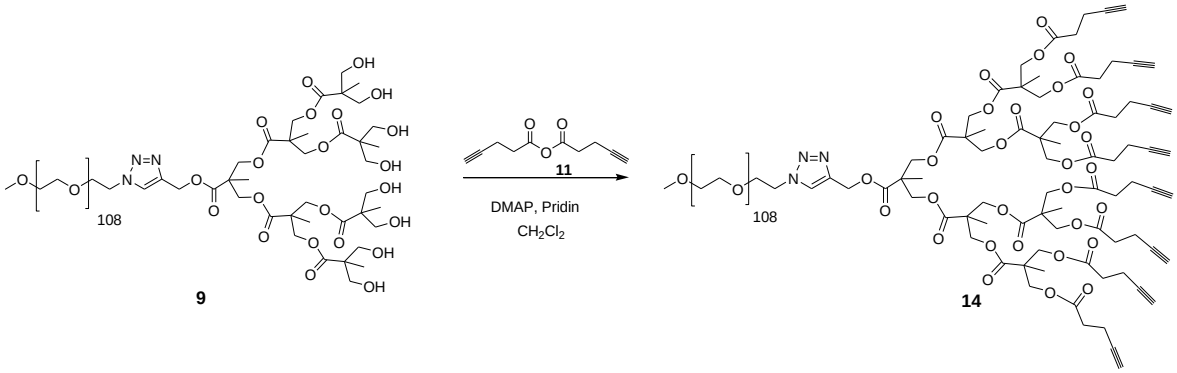

Figure 4.9. Synthesis of [G3]8OR[PEG5K].

4.2.10. Synthesis of Compound [PEG5K]-[G1]-[2Drug]

[G1]2OR[PEG5K] (60mg, 0.011 mmol) and C-N_3 (14.3mg, 0.033mmol) were dissolved in dry THF (2 mL). In a separate flask were dissolved CuBr (0.32 mg, 0.002mmol) and PMDETA (1 μL) in dry THF (2 mL) and purged with N_2. The mixture was then transferred onto C- N_3-[G1]2OR[PEG5K] solution and stirred at 40^o C for 2days. The solvent was then evaporated, and the crude product was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (10 mL) to remove copper salts. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O, filtered, and dried in *vacuo*, yielding compound of [PEG5K]-[G1]-Drug (60mg, 85%) as a yellowish-white solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.77 (s, 1H), 7.41 (s, 2H), 7.11-7.09 (d, 2H, *J* = 8 Hz), 6.96 (s, 2H), 6.84-6.82 (d, 2H, *J*= 8 Hz), 6.45 (s, 4H), 6.41 (s, 4H), 5.21 (s, 2H), 4.50-4.14 (m, 8H), 3.83-3.72 (m , 4H), 3.44 (s, 2H), 3.35(s, 3H), 2.95 (s, 4H), 2.67 (s, 4H), 2.55-2.52 (m, 4H), 1.13 (s, 3H).

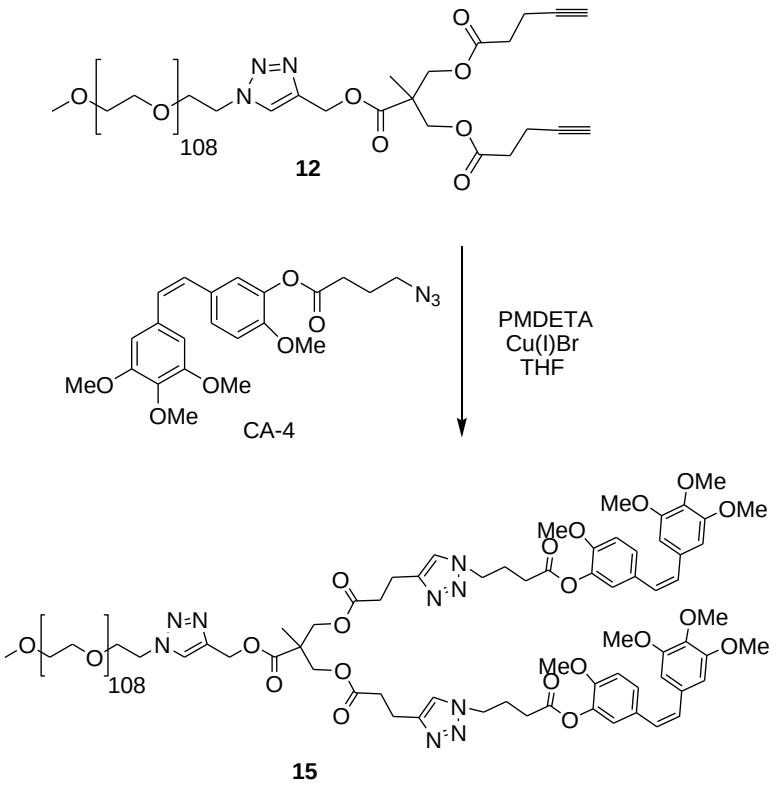

Figure 4.10. Synthesis of [PEG5K]-[G1]-[2Drug].

4.2.11. Synthesis of Compound [PEG5K]-[G2]-[4Drug]

[G2]4OR[PEG5K] (86.5mg, 0.015mmol) and C-N_3 (30mg,0.07mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (0.5 mg, 0.003mmol) and PMDETA (1.5 μL) in dry THF (2 mL) and purged with N_2. The mixture was then transferred onto C- N_3-[G2]4OR[PEG5K] solution and stirred at 40^o C for 2days. The solvent was then evaporated, and the crude product was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (10 mL) to remove copper salts. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O, filtered, and dried in *vacuo*, yielding compound of [PEG5K]-[G2]-Drug (100mg, 89%) as a yellowish-white solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.82 (s, 1H), 7.43 (s, 4H), 7.11-7.09 (d, 4H, *J* = 8 Hz), 6.95 (s, 4H), 6.83-6.81 (d, 4H, *J*= 8 Hz), 6.46 (d, 8H, *J* = 8 Hz), 6.42(d, 8 H, *J* = 8 Hz), 5.22(s, 2H), 4.51 (s, 4H), 4.41 (s, 8H), 4.18 (s, 8H), 4.08 (s, 8 H), 3.45-3.42 (m, 2 H), 3.35 (s, 3H), 2.99 (s, 8 H), 2.73-2.71 (m, 8H), 2.55-2.52 (m, 8H), 1.17 (s, 3), 1.10 (s, 6H).

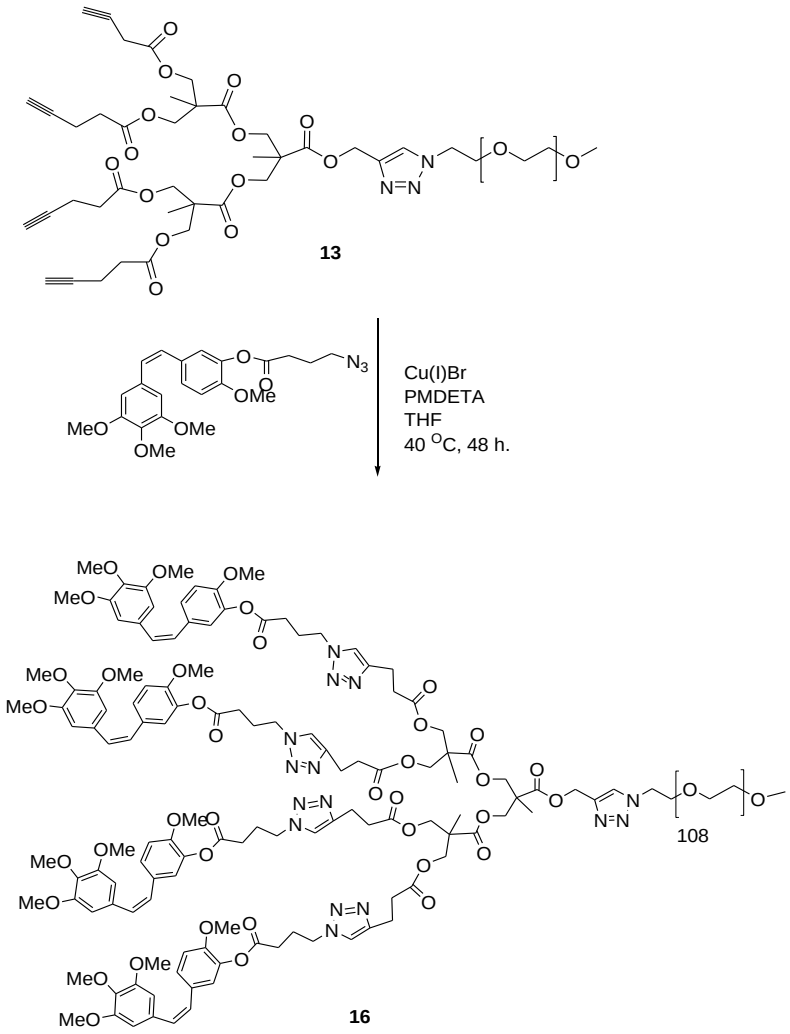

Figure 4.11. Synthesis of [PEG5K]-[G2]-[4Drug].

4.2.12. Synthesis of Compound [PEG5K]-[G3]-[6Drug]

[G3]8OR[PEG5K] (32mg ,0.0048mmol) and C-N_3 (20mg, 0.048mmol) were dissolved in dry THF(4 mL). In a separate flask were dissolved CuBr (0.14mg, 0.00096mmol), and PMDETA (1μL) and purged with N_2. The mixture was then transferred onto C- N_3-[G3]8OR[PEG5K] solution and stirred at 40^o C for 3 days. The solvent was then evaporated, and the crude product was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (10 mL) to remove copper salts. The solvent was concentrated in *vacuo*, and the desired product was precipitated with Et_2O, filtered, and dried in *vacuo*, yielding compound [PEG5K]-[G3]-[8Drug] (40 mg, 83 %) as a yellowishwhite solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.85 (s, 1H), 7.50 (s, 6H), 7.06 (s, 6H) 6.92 (s, 6H), 6.80 (s, 6H), 6.43 (s,

12H), 6.40(s, 12 H), 5.25(s, 2H), 4.42-4.10 (m, 40H), 3.75 (s, 32H) , 3.33 (s, 5H), 2.99 (s, 12 H), 2.71(s, 12H), 2.52(s, 12H), 1.96 (s , 2H), 1.35 (s, 12H), 1.20 (s, 6H), 1.11 (s, 3H).

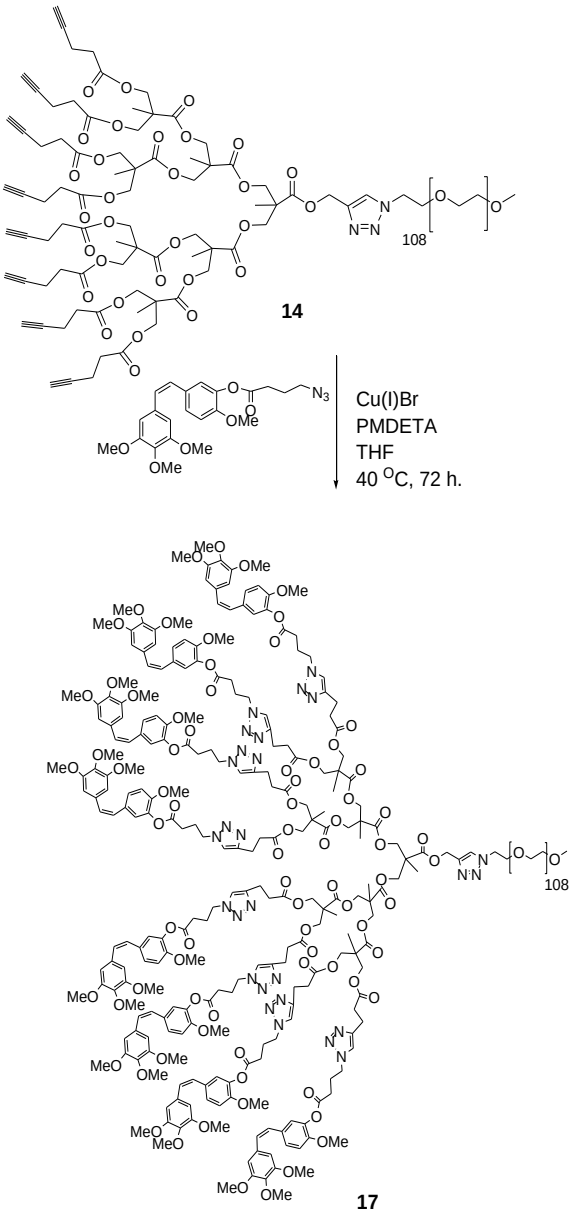

Figure 4.12. Synthesis of [PEG5K]-[G3]-[8Drug].

5. CONCLUSION

Synthesis of azide ended PEG and dendronized polymers, and conjugation of drug molecules on functionalized dendronized polymers were achieved successfully.Dendrons which conjugated to azide ended PEG bearing alkyne functionality enhance the drug loading capacity of PEG polymer. CA-4 containing azide functionality was reacted with alkyne groups of the copolymer via Huisgen type ‘click’ chemistry. It is also expected from this conjugation that the drug will show higher water solubility and improved pharmacokinetic properties.

Furthermore, micellar structures were obtained in aqueous media due to the hydrophobic-hydrophilic interactions of alkyne functionalized copolymers and drug conjugates. DLS and fluorescence experiments were performed for micellar systems. As expected, the diameters of micelles obtained from the drug conjugates were found to be smaller than the micelles obtained from the alkyne appended parent triblock copolymer.

APPENDIX

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

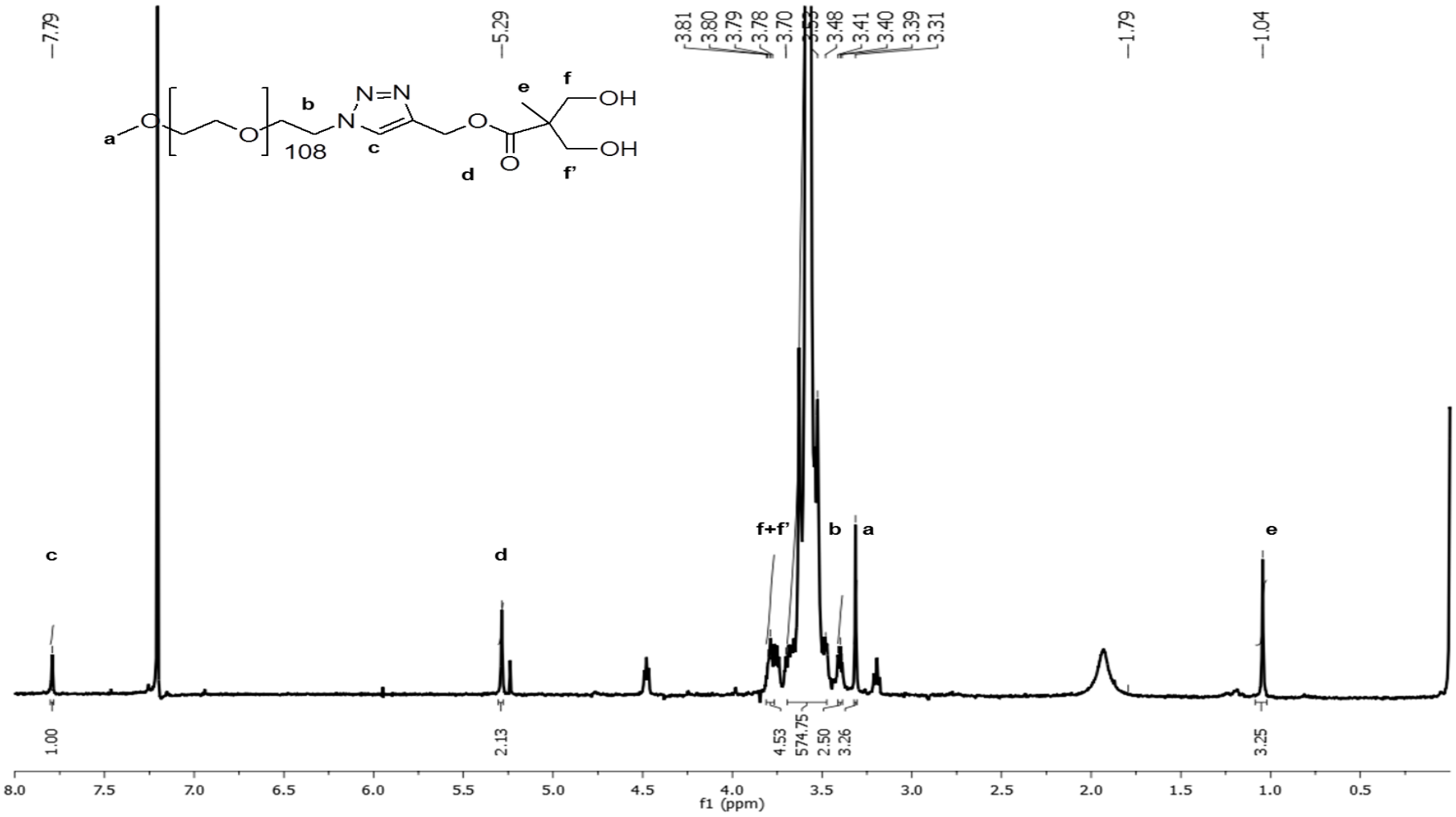

Figure A.1. ^{1}H NMR spectrum of [G1]2OH[PEG5K] (7).

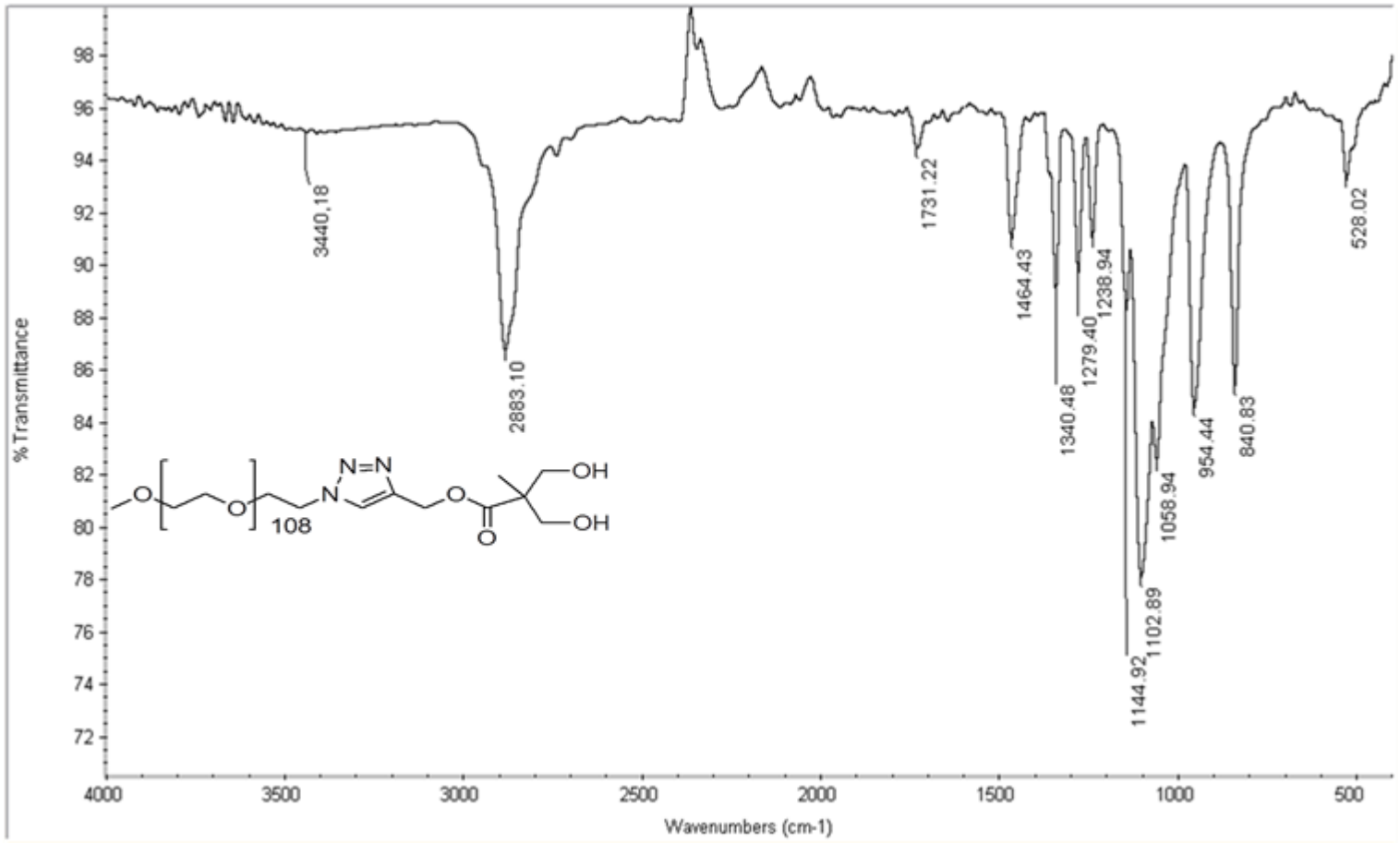

Figure A.2. IR spectrum of [G1]2OH[PEG5K] (7).

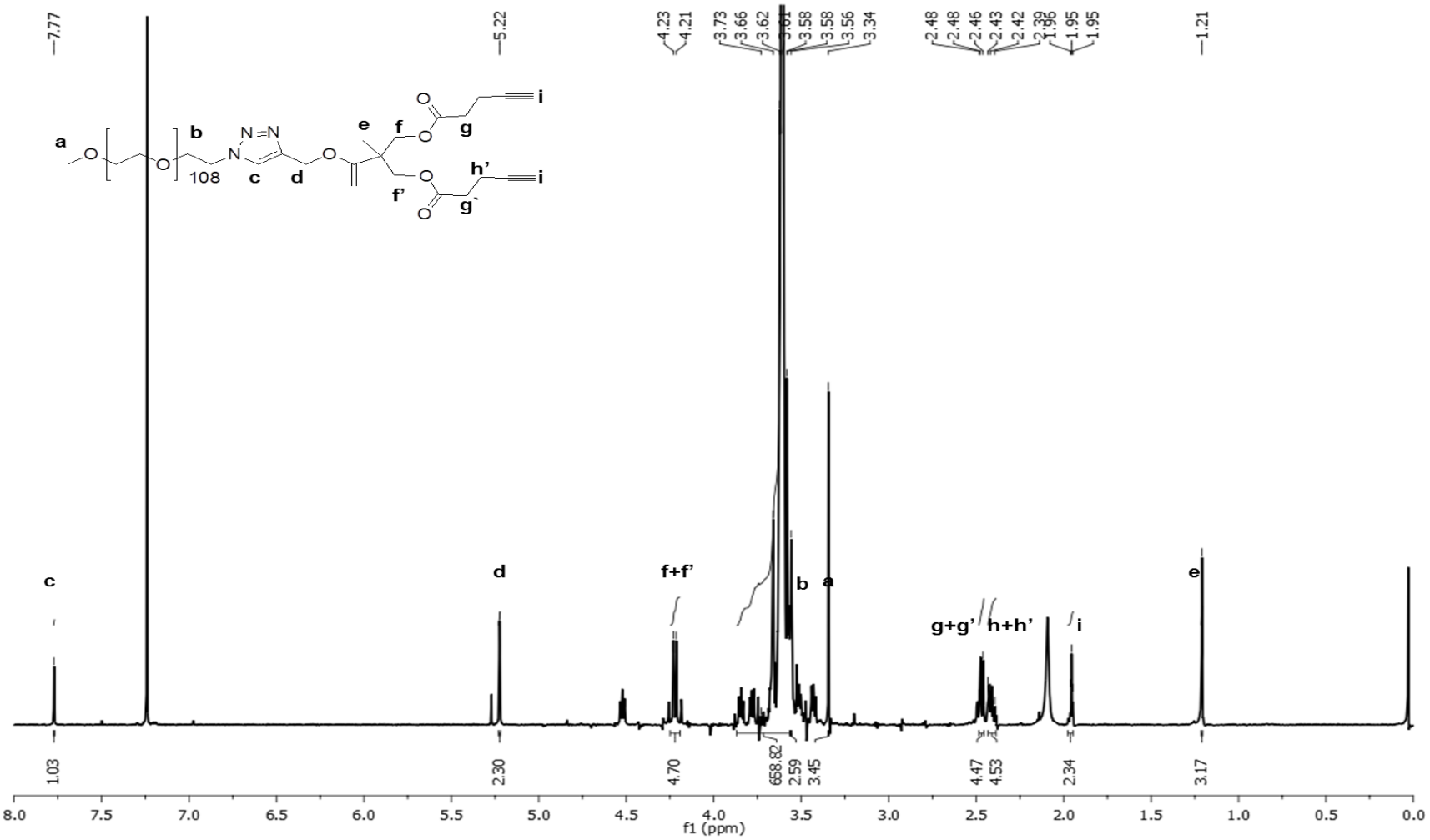

Figure A.3. ^{1}H NMR spectrum of [G1]2OR[PEG5K] (12).

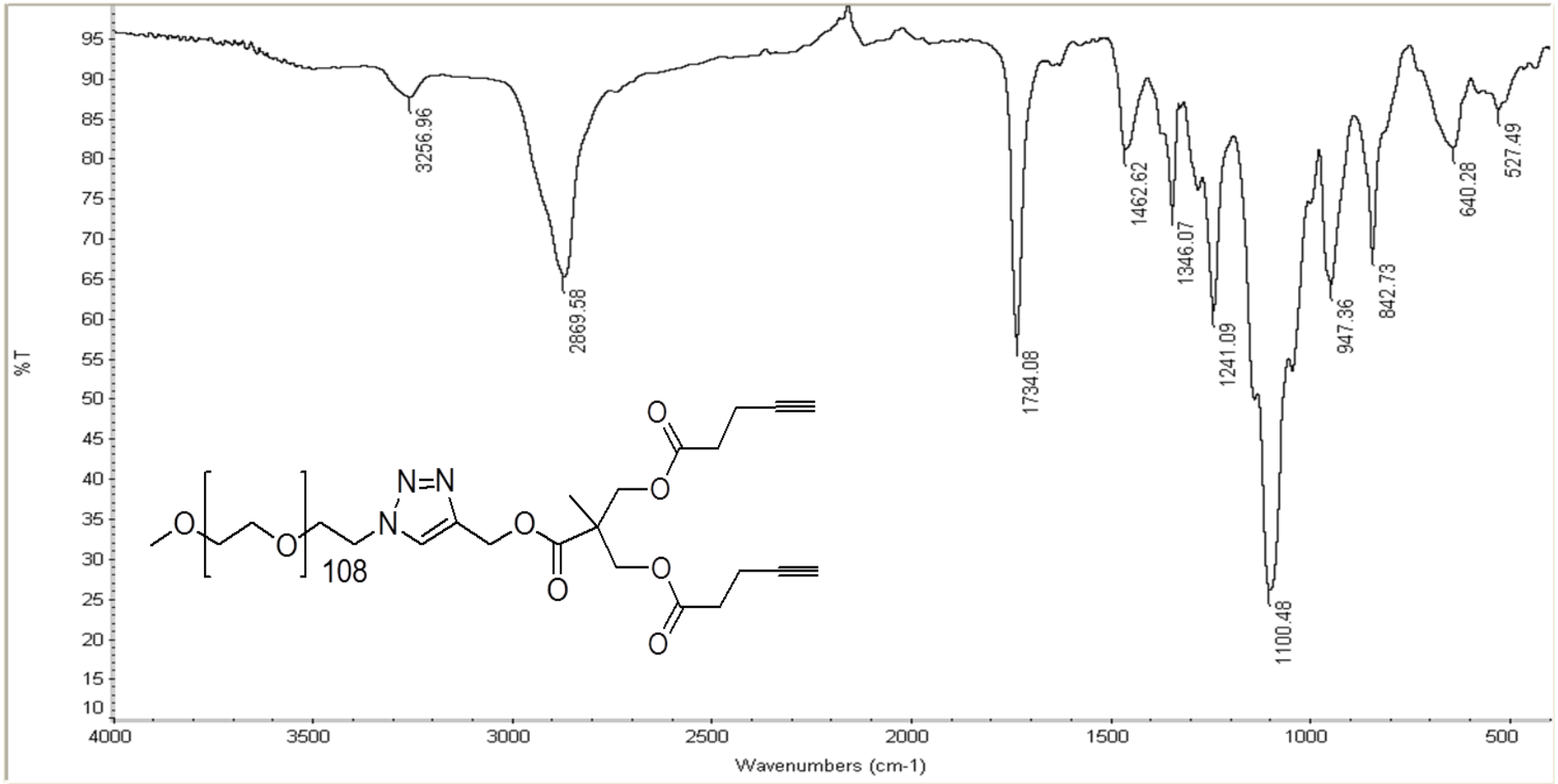

Figure A.4. IR spectrum of [G1]2OR[PEG5K] (12).

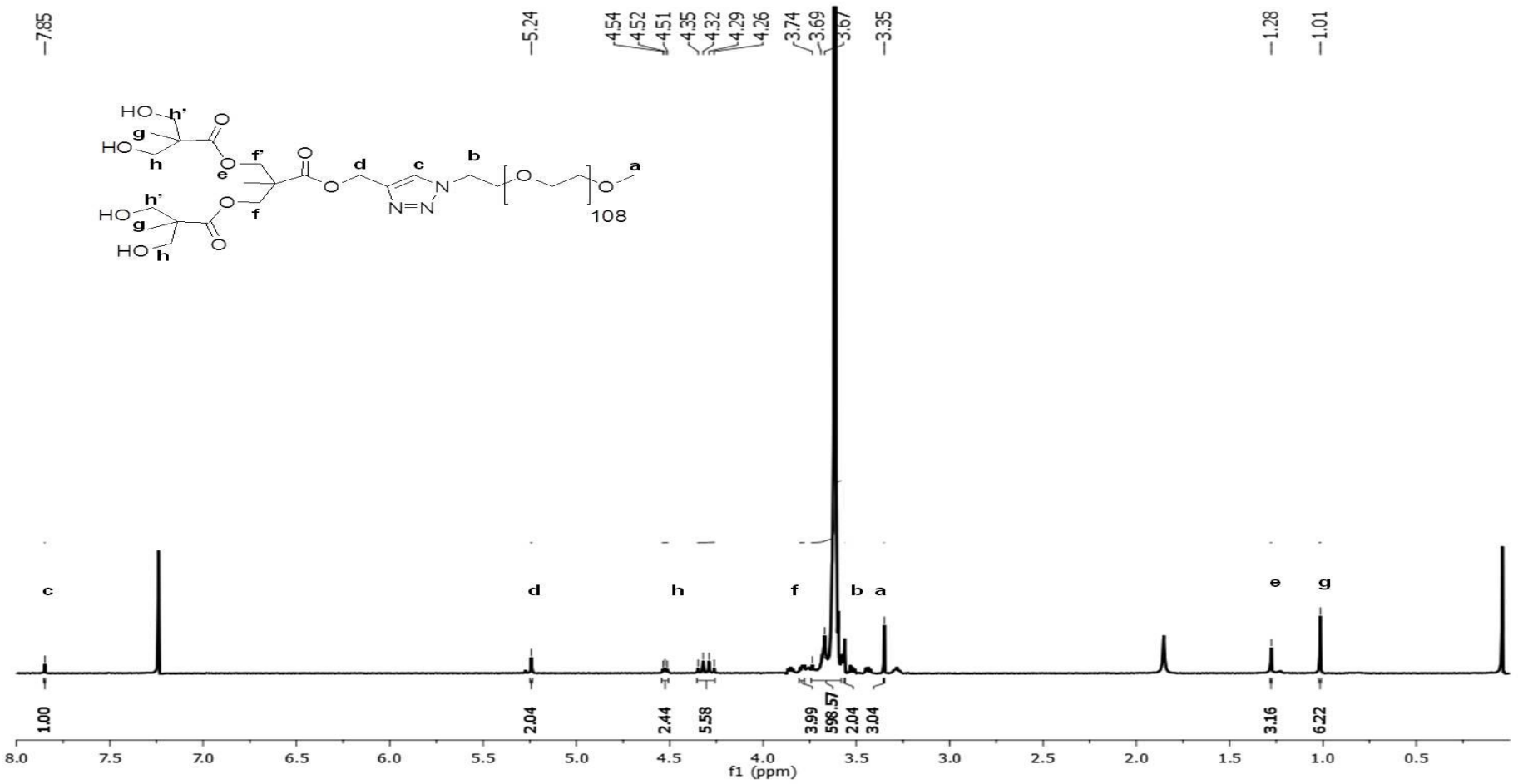

Figure A.5. ^{1}H NMR spectrum of [G2]4OH[PEG5K] (8).

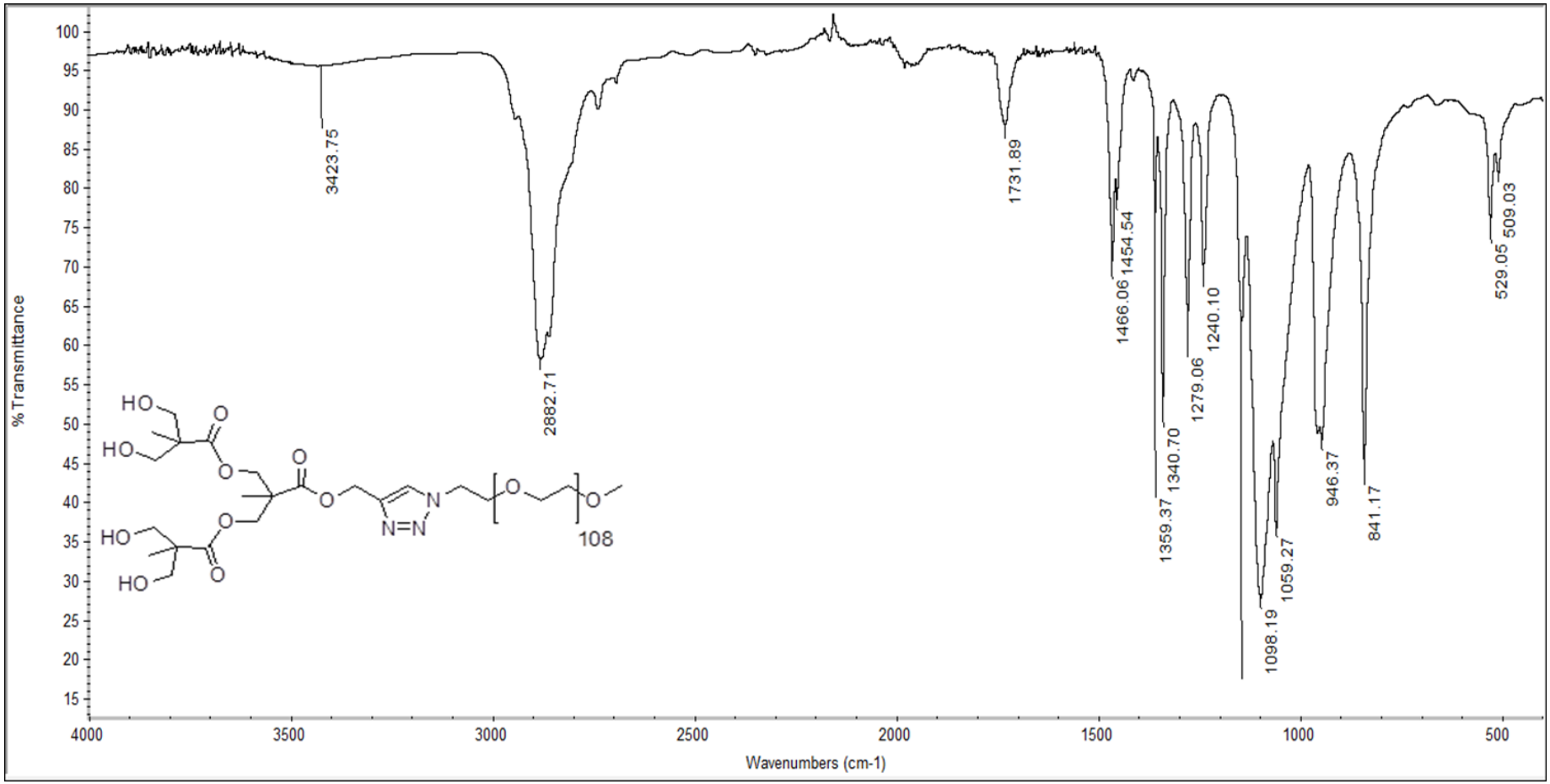

Figure A.6. IR spectrum of [G2]4OH[PEG5K] (8).

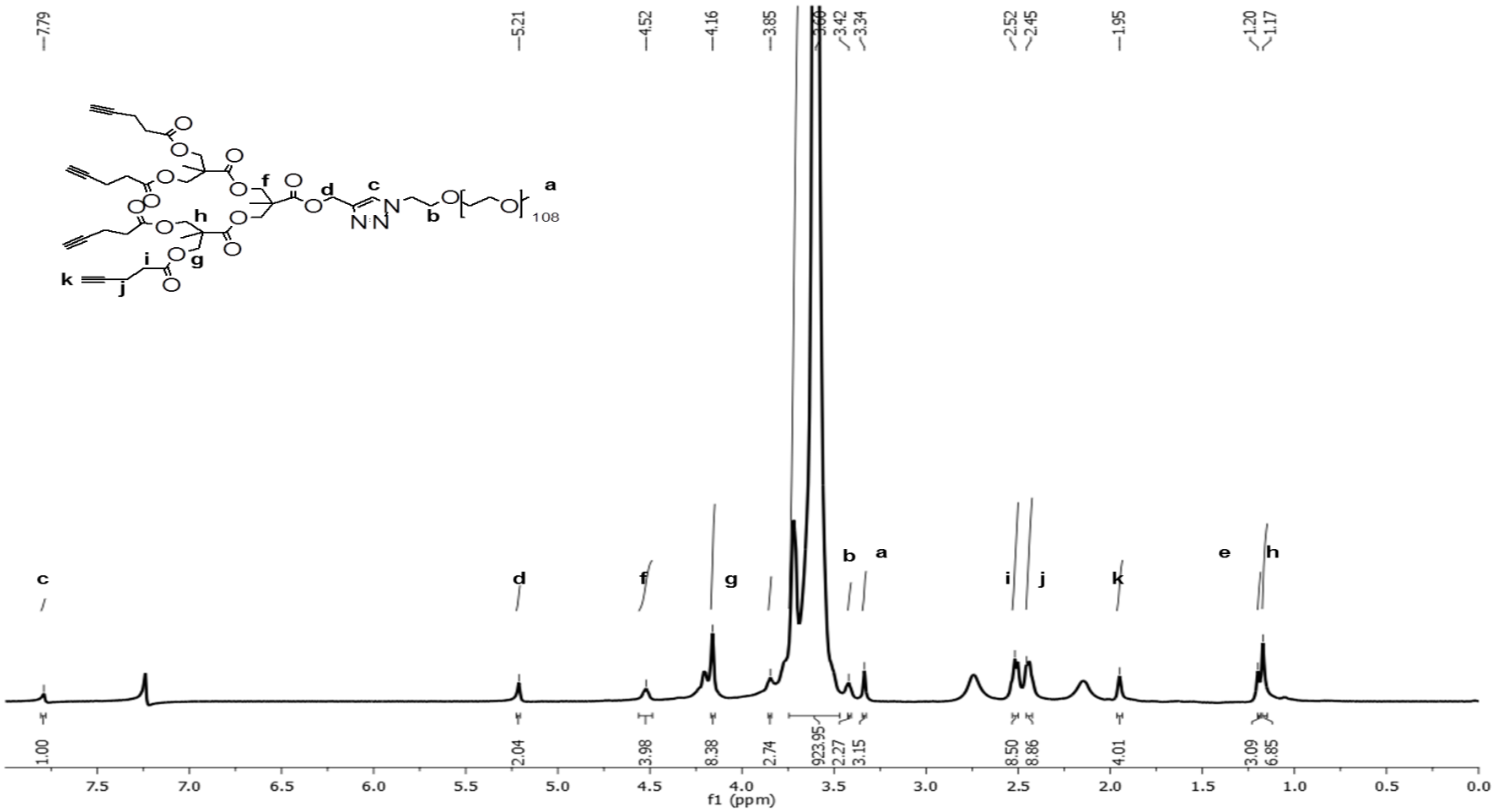

Figure A.7. ^{1}H NMR spectrum of [G2]4OR[PEG5K] (13).

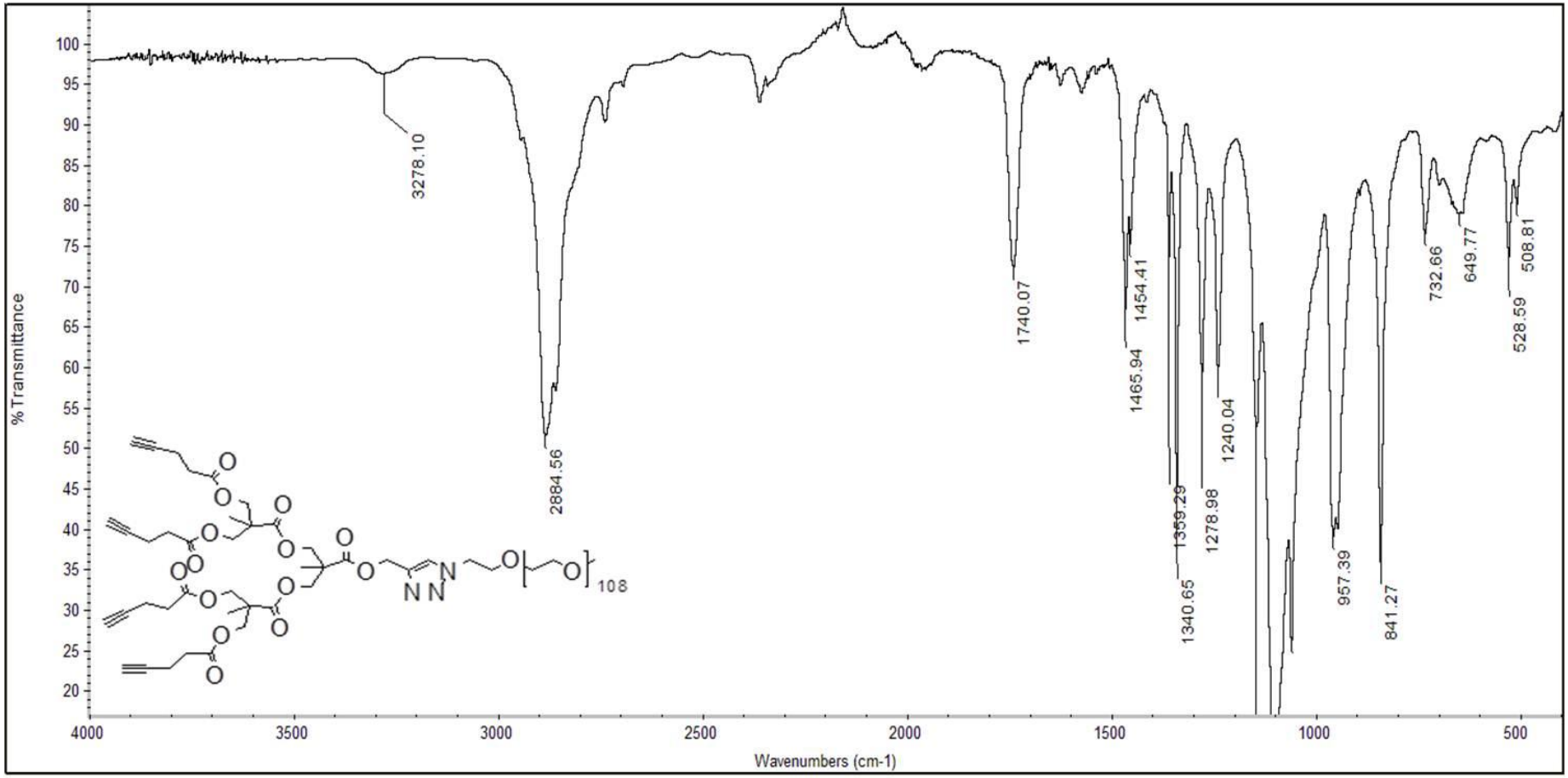

Figure A.8. IR spectrum of [G2]4OR[PEG5K] (13).

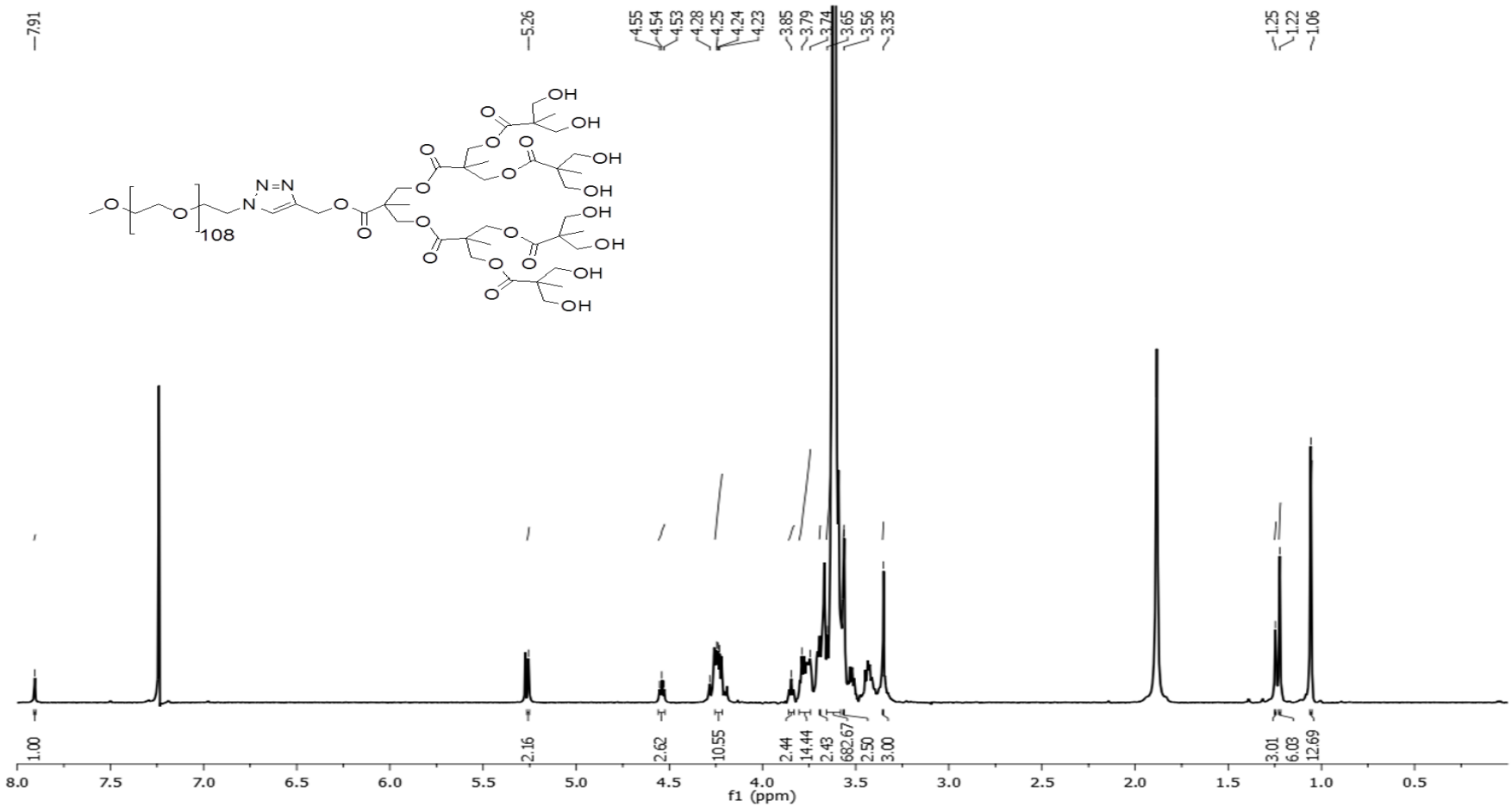

Figure A.9. ^{1}H NMR spectrum of [G3]8OH[PEG5K] (9).

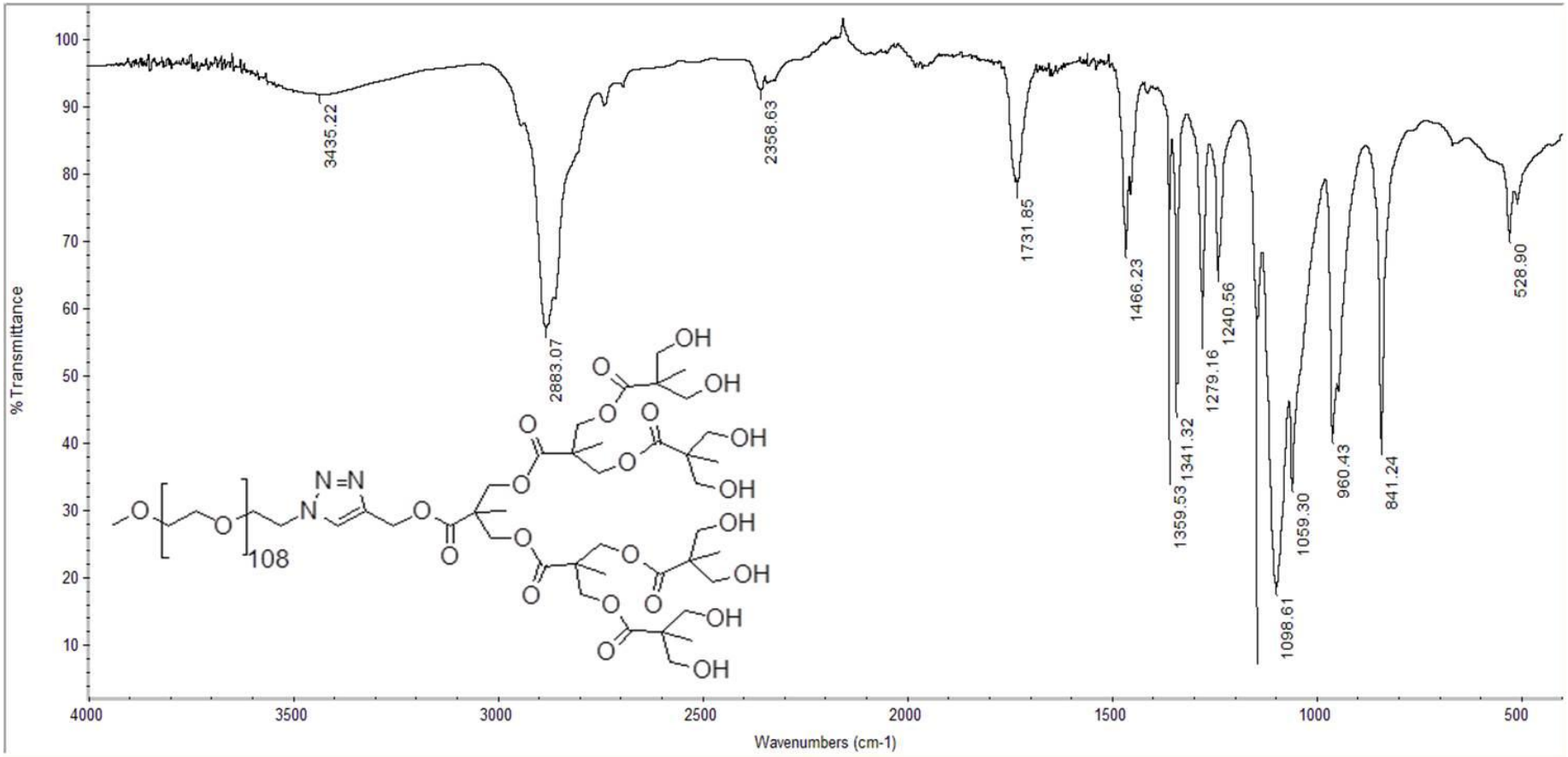

Figure A.10. IR spectrum of [G3]8OH[PEG5K] (9).

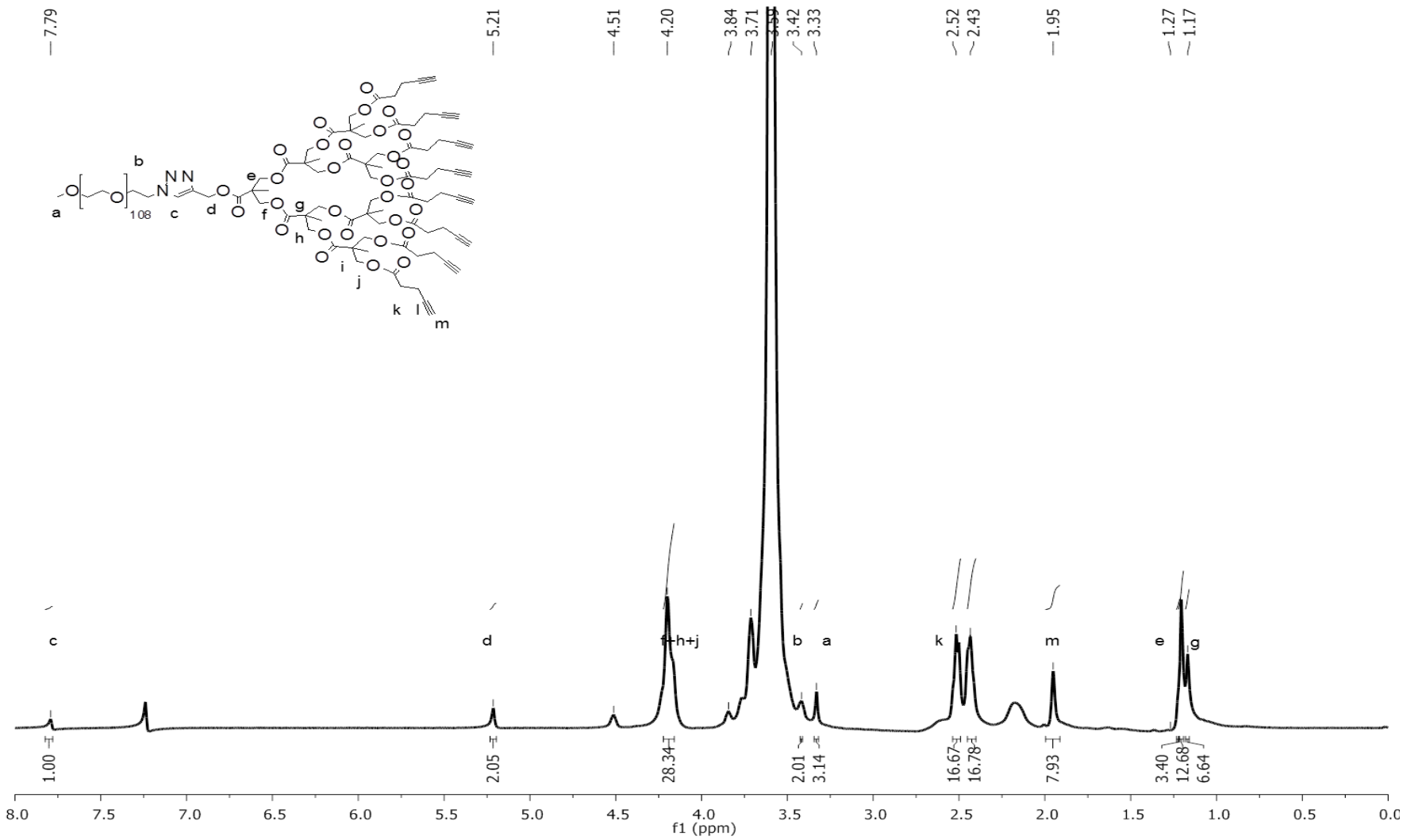

Figure A.11. ^{1}H NMR spectrum of [G3]8OR[PEG5K] (14).

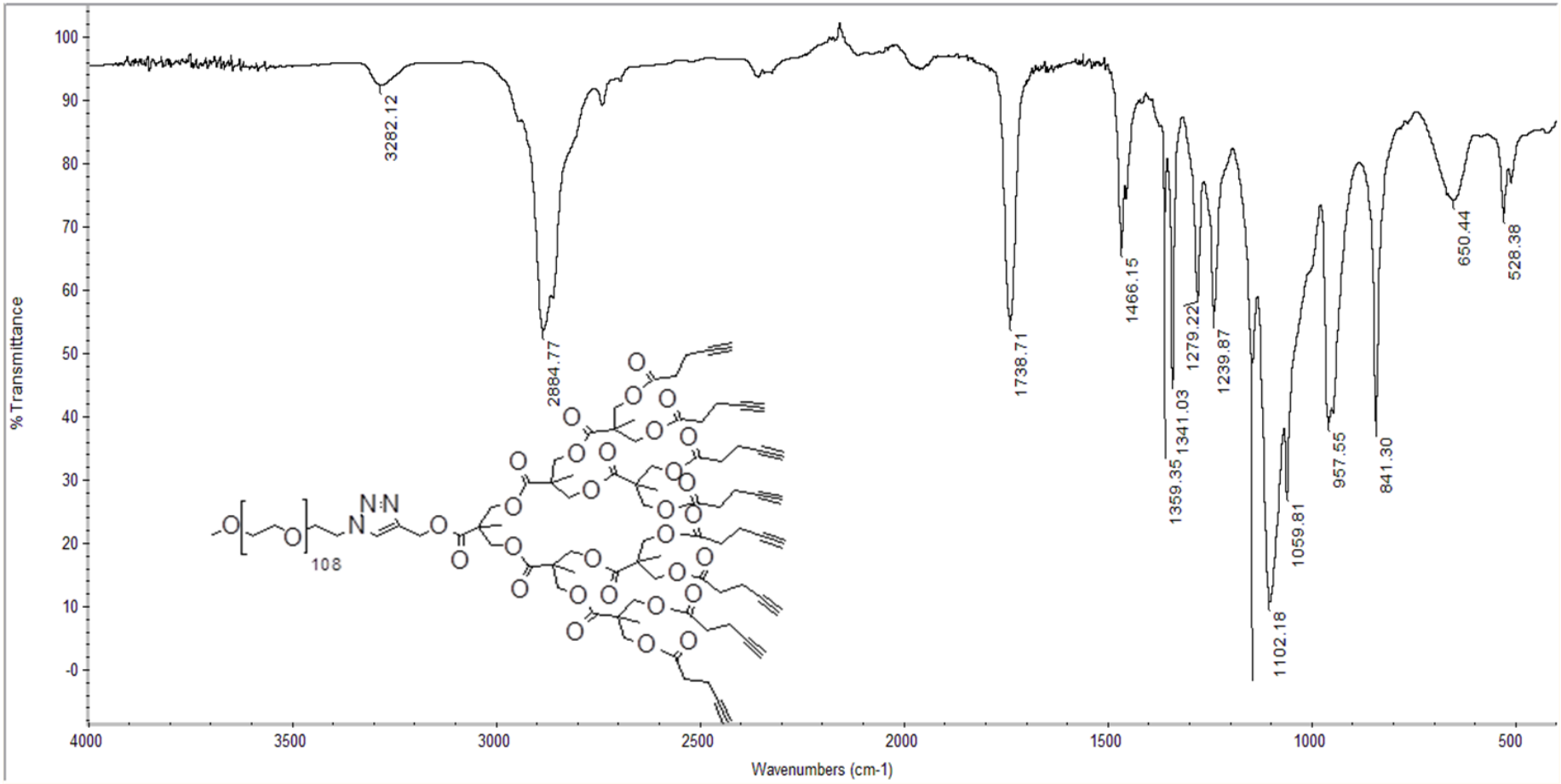

Figure A.12. IR spectrum of [G3]8OR[PEG5K] (14).

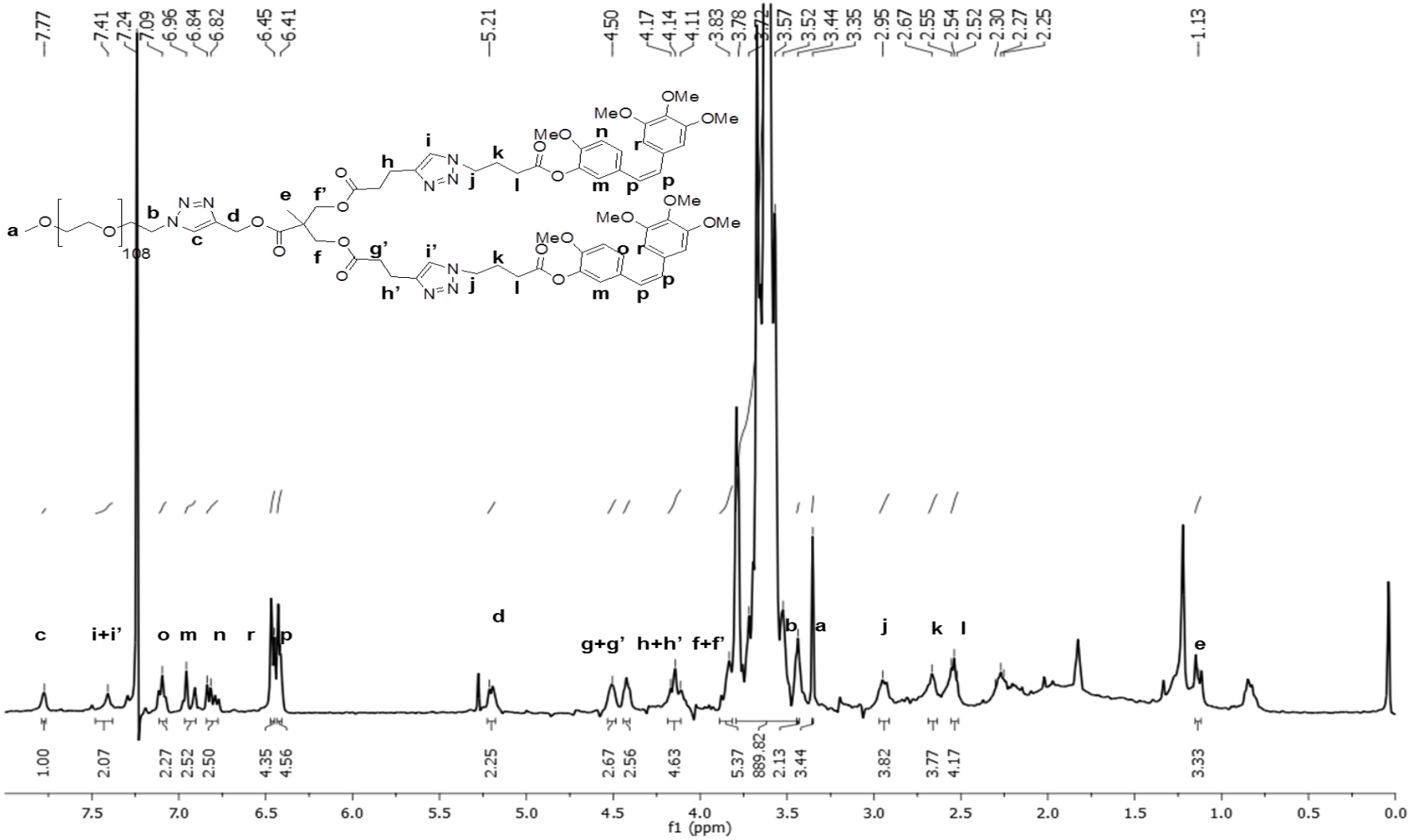

Figure A.13. ^{1}H NMR of [G1]2[CA-4][PEG5K] (15).

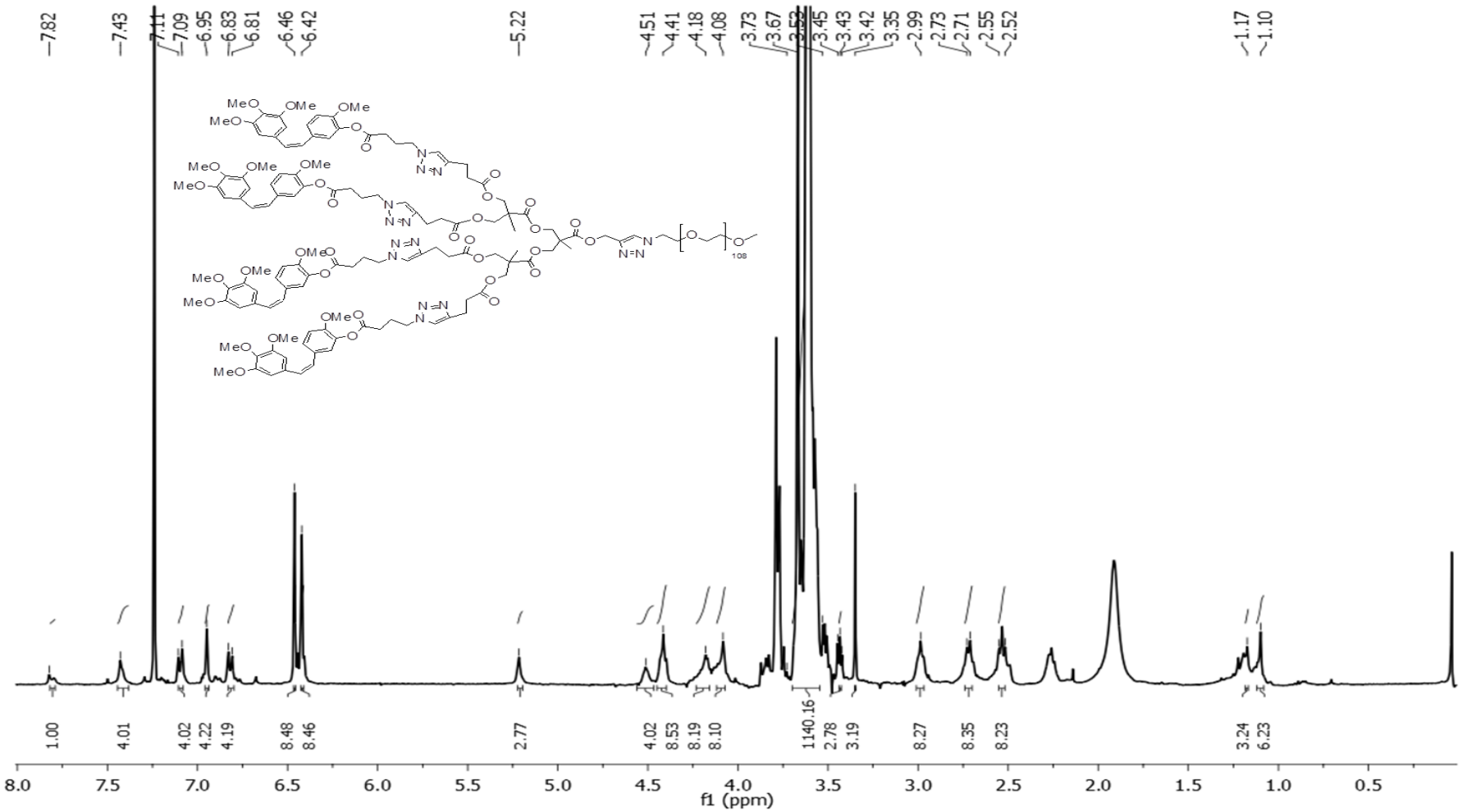

Figure A.14. ^{1}H NMR of [G2]4[CA-4][PEG5K] (16).

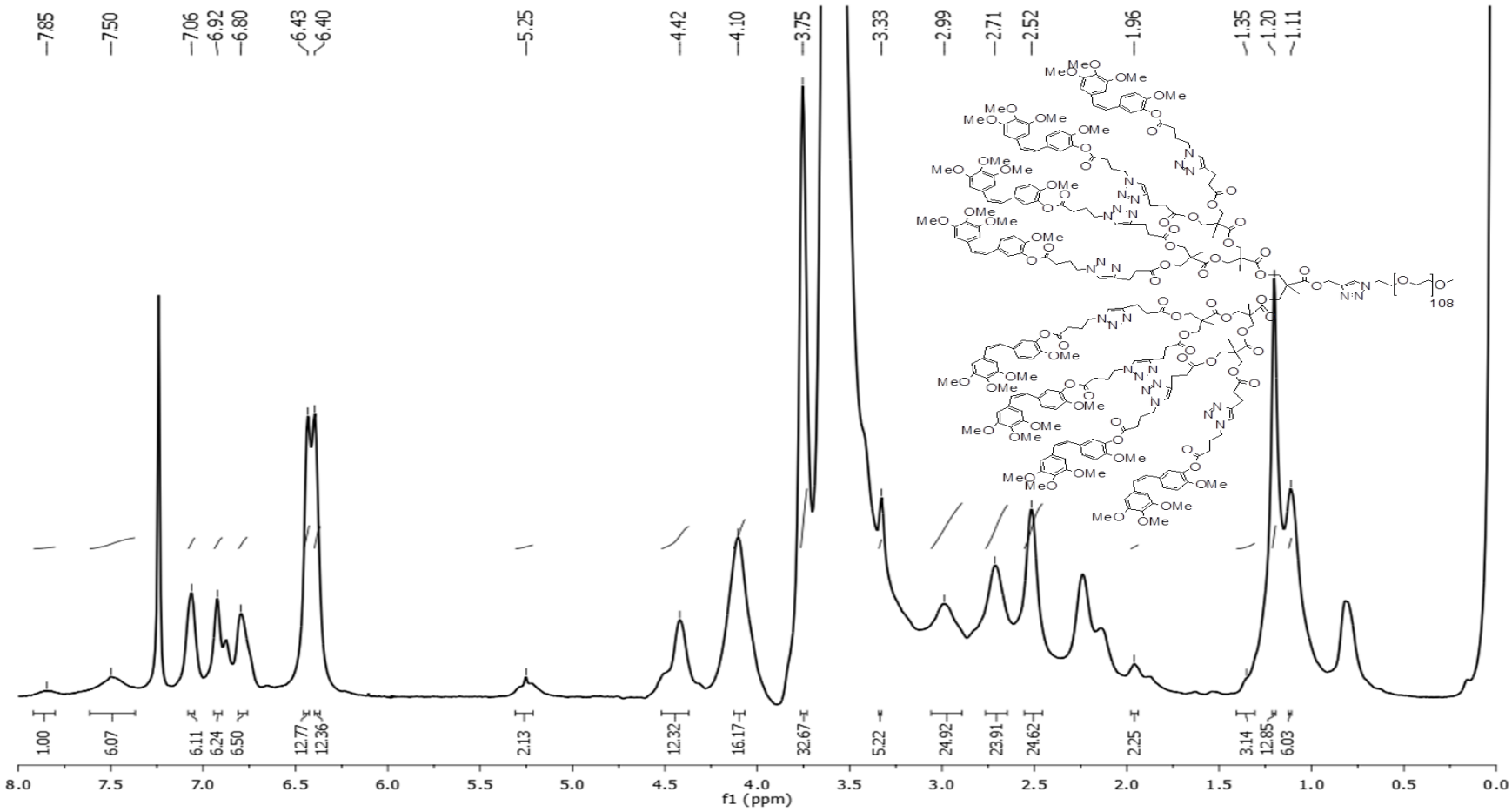

Figure A.15. ^{1}H NMR of [G3]6[CA-4][PEG5K] (17).

REFERENCES

1. Liu, S., R. Maheshwari, and K. L. Kiick, "Polymer-Based Therapeutics", *Macromolecules*, Vol. 42, No. 1, pp. 3-13, 2009.
2. Duncan, R., "The Dawning Era of Polymer Therapeutics", *Nature- Drug Discovery*, Volume 2, No. 5, pp. 347-360, 2003.
3. Kayaku, N., "Polymeric micelle Anticancer Drug", 2009, http://www.nipponkayaku.co.jp/english/rd/institute, acccessed at January 2012.
4. Iyer, A. K., G. Khaled, J. Fang, and H. Maeda, "Exploiting the Enhanced Permeability and Retention Effect for Tumor Targeting", *Drug Discovery Today*, Vol. 11, No. 17/18, pp. 812-818, 2006.
5. Maeda, H., "Polymeric Drugs for Efficient Tumor-Targeted Drug Delivery Based on EPR Effect", *European Journal of Pharmaceutics and Biopharmaceutics,* Vol. 71, No. 3, pp. 409–419, 2009.
6. Matthews, O. W., N. S. Andrew, and J. F. Stoddart, "Dendrimers Branching out from Curiosities into New Technologies", *Progress in Polymer Science,* Vol. 23, No. 1, pp. 1-56, 1998.
7. Medina, S. H., and E. H. El-Sayed, "Dendrimers as Carriers for Delivery of Chemotherapeutic Agents", *Chemical Reviews,* Vol.109, pp. 3141-3157, 2009.
8. Kolb, H., M. Finn, and B. Sharpless, "Click Chemistry", *Angewandte Chemie International Edition,* Vol. 40, pp. 2004-2021, 2001.
9. Rostovtsev, V. V., L. G. Green, V. V. Fokin, and K. B. Sharpless, "A stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes", *Angewandte Chemie International Edition,* Vol. 41, pp. 2596-2599, 2002.
10. Feldman, A. K., B. Colasson, and V. V. Fokin, "One-Pot Synthesis of 1,4-Disubstitued 1,2,3-Triazoles from In-situ Generated", *Organic Letters*, Vol. 6, No. 22, pp. 3897-3899, 2004.
11. Lee, J. W., J. H. Kim, S. C. Han, W. S. Shin, and S. H. Jin, "Synthesis of Simetrical and Unsymetrical PAMAM Dendrimers by Fusion Azide and Alkyne Functionalized PAMAM Dendrons", *Bioconjugate Chemistry,* Vol. 18, No. 2, pp. 579-584, 2007.

12. Polaske, N. W., D. V. McGrath, and J. R. McElhanon, "Thermally Reversible Dendronized Linear AB Step-Polymers via Click Chemistry", *Macromolecules,* Vol. 44, No. 9, pp. 3203–3210, 2011.
13. Helms B. , J. L. Mynar, C. J. Hawker, and J. M. J. Frechet, "Dendronized Linear Polymers via Click Chemistry" , *Journal of the American Chemical Society*, Vol. 126, pp. 15020-15021, 2004.
14. Maeda H., J. Wu., T. Sawa, Y. Matsumura, and K. Hori, 'Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics: a Review', *Journal of Controlled Release*, Vol. 65, pp. 271-284, 2000.
15. Lee C. C., J. A. Mackay, J. M. J. Frechet, and F. C. Szoka, "Designing Dendrimers for Biological Applications", *Nature Biotechnology*, Vol. 23, No. 12, pp. 1517-1527, 2005.
16. Gillies, E. R., and J. M. J. Frechet, "Designing Macromolecules for Therapeutic Applications: Polyester Dendrimer – Poly(ethylene oxide) "Bow-tie" Hybrids with Tunable Molecular Weight and Architecture", *Journal of the American Chemical Society*, Vol. 124, pp. 14137-14146, 2002.
17. Ihre, H., O. L. P. D. Jesus, and J. M. J. Frechet, "Polyester Dendritic Systems for Drug Delivery Applications: Desing, Synthesis and Characterization", *Bioconjugate Chemistry*, Vol. 123, No. 5908-5917, 2001.
18. Gillies, E. R. and J. M. J. Frechet, "Dendrimer and Dendritic Polymers in Drug Delivery", *Drug Discovery Today*, Vol. 10, No. 1, 2005.
19. Megia, E. F., J. Correa, and R. Riguera, "Clickable PEG-Dendritic Block Copolymers", *Biomacromolecules,Vol.* 7, No. 11, pp. 3104-3111, 2006.
20. Kaminskas, M. L., J.H.C. Porter, "A comparison of Changes to Doxorubicin Pharmacokinetics, Antitumor Activity, and Toxicity Mediated by PEGylated Dendrimer and PEGylated Liposome Drug Delivery Systems", *Nanomedicine*, Vol. 8, No. 1, pp. 103-111, 2012.
21. Yokoyama, M., "Polymeric Micelles Drug Carries for Tumor Targeting", *Polymeric Drug Delivery*, Vol. 3, pp. 27-36, 2006.
22. Ganta, S., H. Devalapally, A. Shahiwala, and M. Amiji, "A review of Stimuli–Responsive Nanocarriers for Drug and Gene Delivery", *Journal of Controlled Release*, Vol. 126, pp. 187–204, 2008.

23. Maeda, H., G. Y. Bharate, and J. Daruwalla, "Tumor Selective Delivery of Macromolecular Drugs via The EPR effect: Background and Future Prospects", *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 71, pp. 409 –419, 2009.
24. Kayaku, N., "Polymeric Micelle Anticancer Drug", 2009, http://www.nipponkayaku.co.jp/english/rd/institute, accessed at January 2012.
25. Kataoka, K., A. Harada, and Y. Nagasaki, "Block Copolymer Micelles for Drug Delivery: Design, Characterization and Biological Significance", *Advanced Drug Delivery Reviews*, Vol. 47, pp. 113-131, 2001.
26. Klok, H. A., "Advanced Drug Delivery Devices via Self Assembly of Amphiphilic Block Copolymers", *Advanced Drug Delivery Re*views, Vol. 53, pp. 95-108, 2001.
27. Kwon, G. S., "Poly (ethylene oxide)-Block-Poly(amino acid) Micelles for Drug Delivery", *Advanced Drug Delivery Re*vievs, Vol. 54, pp. 169-190, 2002.
28. Hubbell, J. A., "Enhancing drug function", *Science*, Vol. 300, pp. 595-596, 2002.
29. Anger, P., P. Bharadwaj, and L. Novotny, "Enhancement and Quenching of Single-Molecule Fluorescence", *Review Letters*, Vol. 96, pp. 113002-113006, 2006.
30. Winnik, F. M., "Flourescence Studies of Aqueos Solutions of Poly(N-isopropylacrylamide) below and above Their LCST", *Macromolecules*, Vol. 23, pp. 233–242, 1990.
31. Farinha, J. P. S., S. Picarra, K. Miesel, and J. M. G. Martinho, "Flourescence study of the Coil-Globule Transition of a PEO Chain in Toluen", *Journal of Physical Chemistry*, Vol. 105, pp. 10536–10545, 2001.
32. Picarra, S., P. Relogio, C. A. M. Afonso, J.M.G. Martinho, and J. P. S. Farinha, "Coil-Globule Transition of Poly(Dimethylacrylamide): Flourescence and Light Scattering Study", *Macromolecules*, Vol. 36, pp. 8119–8129, 2003.
33. Torchilin, V. P., "Cell Penetrating Peptide-Modified Pharmaceutical Nanocarriers for Intracellular Drug and Gene Delivery", *Biopolymers*, Vol. 90, pp. 604–610, 2008.
34. Yokoyama, M., T. Okano, Y. Sakurai, S. Suwa, and K. Kataoka, "Introduction of Cisplatin into Polymeric micelle", *Journal of Controlled Release*, Vol. 39, pp. 351–6, 1996.

35. Gref, R., Y. Minamitake, M. T. Percocchia, V. Trubetskoy, V. P. Torchillin, and R. Langer, "Biodegradable Long-Circulating Polymeric Nanospheres", *Science,* Vol. 263 pp. 1600–1603, 1994.
36. Gillies, E. R., and J. M. J. Frechet, "Stimuli-Responsive Supramolecular Assemblies of Linear-Dendritic Copolymers", *Journal of the American Chemical Society*, 126, 11936–11943, 2004.
37. Kim, S. and K. Park, "Synthesis and Micellar Characteristics of Dendron-PEG Conjugates", *Langmuir*, Vol. 21 No. 10 pp. 4334–4339, 2005.
38. Tian, L., and P. T. Hammond, "Comb-Dendritic Block Copolymers as Tree-Shaped Amphiphiles for Nanoparticle Self-Assembly", *Chemistry of Materials,* Vol. *18, No. 17* pp. 3976–3984, 2006.
39. Hua, C., S. M. Peng, and C. M. Dong, "Synthesis and Characterization of Linear-Dendron- Like Poly(€-Caprolactone)-b-poly(ethylene oxide) Copolymers via the Combination of Ring-Opening Polymerization and Click Chemistry", *Macromolecules,* Vol. 41, No. 18, pp. 6686–6695, 2008
40. Chang, G., C. Li, W. Lu, J. Ding, "N-Boc-Histidine-Capped PLGA-PEG-PLGA as a Smart Polymer for Drug Delivery Sensitive to Tumor Extracellular pH", *Macromolecular Bioscience*, Vol. 10, No.10, pp. 1248–1256, 2010
41. Cao, W., J. Zhou, A. Mann, Y. Wang, and L. Zhu, "Folate-Functionalized Unimolecular Micelles Based on a Degradable Amphiphilic Dendrimer-Like Star Polymer for Cancer Cell-Targeted Drug Delivery", *Biomacromolecules*, Vol. 12, pp. 2697–2707, 2011