# SYNTHESIS & MICROCONTACT MOLDING OF FUNCTIONALIZABLE HYDROGELS

by

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to my family

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# ABSTRACT


## SYNTHESIS & MICROCONTACT MOLDING OF FUNCTIONALIZABLE HYDROGELS

Hydrogels are crosslinked polymers that have large number of hydrophilic groups and capacity to hold water in their three dimensional network structure. These materials have a wide range of application in biomedical and materials science due to their controllable chemical and physical properties.

Dendron-polymer-dendron conjugate systems are novel polymeric materials to fabricate hydrogels. Multivalent dendron-polymer-dendron copolymers can be used to synthesize hydrogels that can be further functionalized with molecules and biomolecules of interest. In this project, synthesis of functionalizable hydrogels, characterizations, and post functionalization of hydrogels from dendron-polymer-dendron conjugates are reported. The resulting hydrogels are biodegradable and multivalent due to utilization of polyester dendrons, possess high swelling capacity due to presence of high molecular weight poly ethylene glycol. Since the hydrogel precursors have alkene and alkyne groups on their surfaces, crosslinking via photopolymerization and post functionalization by click chemistry is possible. Additionally, by micromolding methods, different surface patterns of functionalizable hydrogels can be realized. Successful covalent modification of these hydrogels via control of degree of functionalization was demonstrated by immobilization of fluorescent dyes and enzymes.

# ÖZET

## FONKSİYONEL HİDROJELLERİN SENTEZ VE MİKROKONTAK KALIPLANMASI

Hidrojeller çok sayıda hidrofilik gruba sahip ve bu yüzden yüksek sevide su tutma özelliği olan çarpraz bağlanmış polimerlerdir. Bu maddeler kontrol edilebilir kimyasal ve fiziksel özellikleri sayesinde, biomedikal uygulamalar ve malzeme bilimini de içeren geniş bir uygulama alanına sahiplerdir.

Dendron-polimer-dendron konjuge sistemleri, hidrojel yapımında kullanılan yeni plimer yapılarıdır. Çok dallı dendron-polimer-dendron kopolimerleri, istenilen moleküller ve biomoleküllerle ileri işlevselleştirilebilen hidrojellerin yapımında kullanılabilirler. Bu projede, dendron-polimer-dendron kopolimerlerinden sentezlenen aktif hidrojellerin karakterizasyonu ve ileri işlevselleştirilmesi anlatılmaktadır. Oluşan hidrojeller, kullanılan poliester dendron sayesinde çok dallı ve bioçözünürlüğü olan ve yapısındaki yüksek moleküler ağırlıktaki PEG sayesinde yüksek derecede su çekme özelliğine sahiptirler. Hidrojel ön maddesi üzerindeki alken ve alkin gruplarına sahip olduğundan foto polimerleşme ile çapraz bağlanıp “klik” kimyası ile ileri işlevselleştirilebilirler. Bununla beraber, mikro kalıplama yöntemleri yüzey üzerine farklı şekillerde kalıplanmış hidrojeller elde edilebilir. Hidrojellerin başarılı kovalent modifikasyonlarla kontrollü işlevselleştirilmeleri, floresan boya ve enzim bağlanmasıyla gösterildi.

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| | |
|---|---|
| Bis-MPA | 2,2-bis(hydroxymethyl)propionic acid |
| $CDCl_3$ | Deuterated chloroform |
| $CH_2Cl_2$ | Dichloromethane |
| $D_2O$ | Deuterated water |
| DCC | Dicyclohexylcarbodiimide |
| DMAP | N,N Dimethylaminopyridine |
| EtOAc | Ethyl Acetate |
| FT-IR | Fourier Transform Infrared |
| MeOH | Methanol |
| NMR | Nuclear Magnetic Resonance |
| PEG | Poly(ethylene glycol) |
| py | Pyridine |
| TEA | Triethylamine |
| TEG | Triethyleneglycol |
| THF | Tetrahydrofuran |
| FITC | Fluorescein isothiocyanate |

# 1. INTRODUCTION

## 1.1 Hydrogels

Hydrogels are polymers crosslinked via ionic interactions, chemical bonds, hydrogen bonds, hydrophilic interactions or physical bonds. These materials have large number of hydrophilic groups and capacity to hold fluid in their three dimensional network structure. Hydrogels absorb water and swell readily without dissolving (Figure 1.1) and shrink on drying.


Figure 1.1. Schematic representation of a swelling hydrogel

Hydrogels have a wide range of application area as biomedical applications including drug delivery, cell encapsulation and tissue repair, coating materials and devices. As a deep look for cell encapsulation application of hydrogels, scientists take advantage of the critical interactions between cells and extracellular matrix (ECM) to create bioactive materials capable of controlling cell function and tissue evolution (Figure 1.2). To determine the requirements of the microenvironment, they utilize hydrogels easily modified with respect to mechanical integrity, adhesive peptides, ECM molecules, degradability, and incorporation of drugs, to direct cellular differentiation through a variety of mechanisms [1-18].

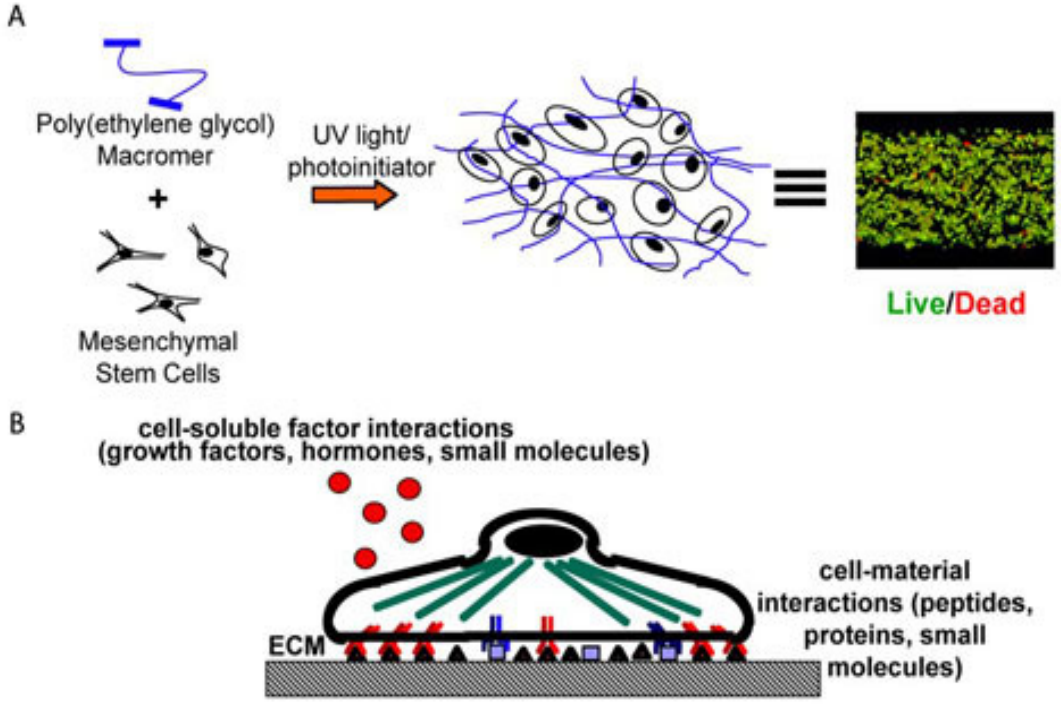



Figure 1.2. Hydrogels as a tool for cell encapsulation *(A)* and cell adhesion *(B)*

Solution of poly(ethylene glycol) macromolecules with vinyl functionalities is combined with stem cells and photopolymerized yielding viable encapsulated cells (Figure 1.2.a). Utilizing hydrogel microenvironments to direct encapsulated adult stem cell function in tissue engineering is an important area of interest. A thorough understanding of how material properties effect cell differentiation and tissue evolution is essential to tailor 'instructive materials' to direct cell function. Besides, hydrogel characteristics are altered to provide environment for cell-material interactions and soluble factor delivery to control cell differentiation and function (Figure 1.2.b) [19].

Hydrogels can be exploited to encapsulate and deliver cells and biomolecules therapeutically. Delivery characteristics can be controlled through alterations in hydrogel biophysical and biochemical structure and used as a powerful tool in tissue regeneration, wound healing, or control inflammation. Their biophysical and biochemical characteristics can be also altered to control angiogenic growth factor delivery.

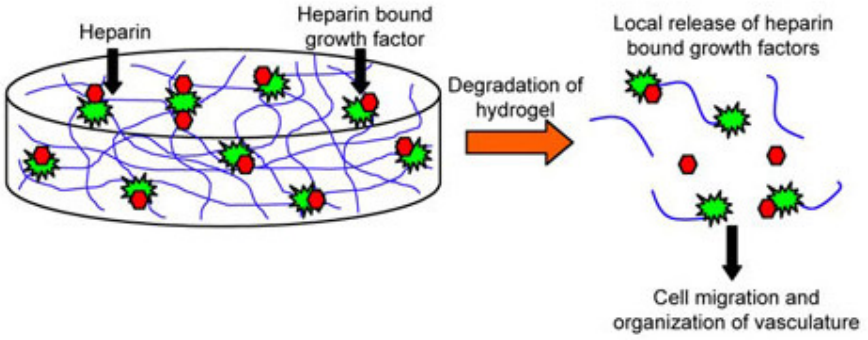



Figure 1.3. Hydrogel used to control angiogenic growth factor delivery

Hydrogels that designed to encapsulate cells and control the availability of therapeutic molecules such as growth factors, gene vectors, or small interfering RNA, will be useful for these therapeutic strategies. As an example, heparin functionalities which represent a blood thinner that prevents the formation of blood clots can be incorporated into hydrogels to control the release of angiogenic growth factors for treatment of chronic wounds (Figure 1.3.) [20].

### 1.2. Methods to Synthesize Hydrogels

Hydrogels can be represented according to their methods of synthesis as physically or chemically crosslinked. Physical hydrogels are formed by various reversible links [21]. These can be ionic interactions as in ionically crosslinked hydrogels and polyelectrolyte complexes and ionically crosslinked hydrogels are generally considered as biocompatible and well-tolerated. Ionic polymers can be crosslinked by the addition of di- or tri-valent counterions (Figure 1.4) [22].

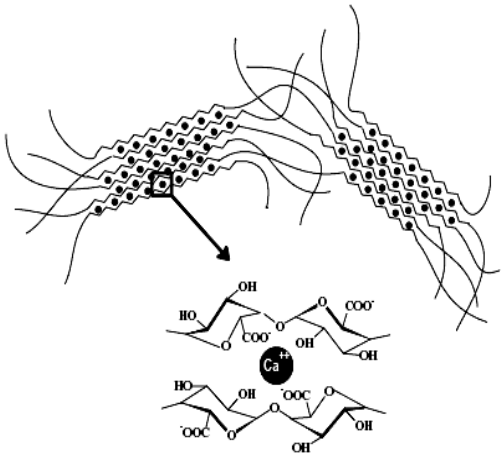



Figure 1.4. Gelation of sodium alginate by addition of $Ca^{+2}$ ions

Covalent crosslinking leads to formation of hydrogels with a permanent network structure, since irreversible chemical links are formed. This type of linkage allows absorption of water and/or bioactive compounds without dissolution and permits drug release by diffusion. Their non-permanent network is formed by reversible links.

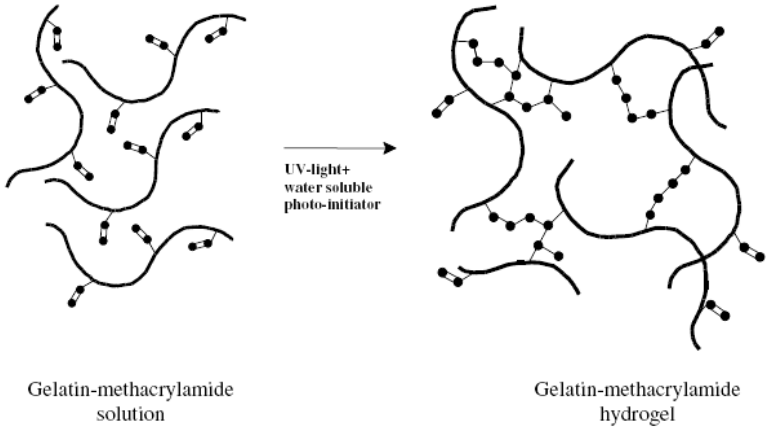



Figure 1.5. Chemical modification of gelatin with methacrylamide side groups

The methacrylamide side groups are introduced by reaction with methacrylic anhydride with the amino groups of lysine residues (Figure 1.5) [22]. The degree of modification, which can be varied by controlling the reaction conditions, determines to a large extent the mechanical properties of the resulting hydrogel. Photopolymerization, micheal addition reaction and click chemistry examples are some of the chemical crosslinking examples (Figure 1.6).

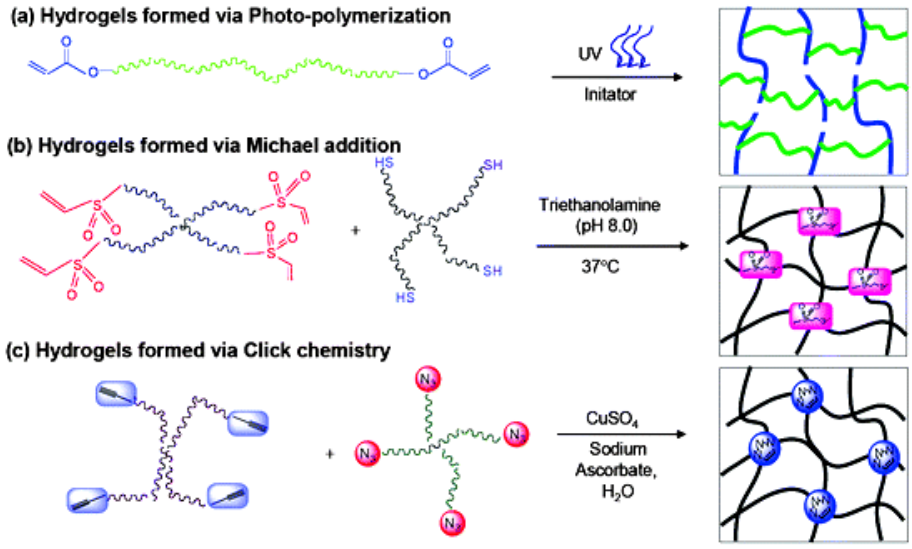



Figure 1.6. Hydrogel examples via chemical crosslinking methods [23]

### 1.3. Dendrimers

A synthetic, three-dimensional macromolecule formed using a nanoscale fabrication process. “Dendrimer” word comes from the Greek “dendra”, meaning “tree”. A dendrimer is built up from monomers, with new branches added in steps until a tree-like structure is created.

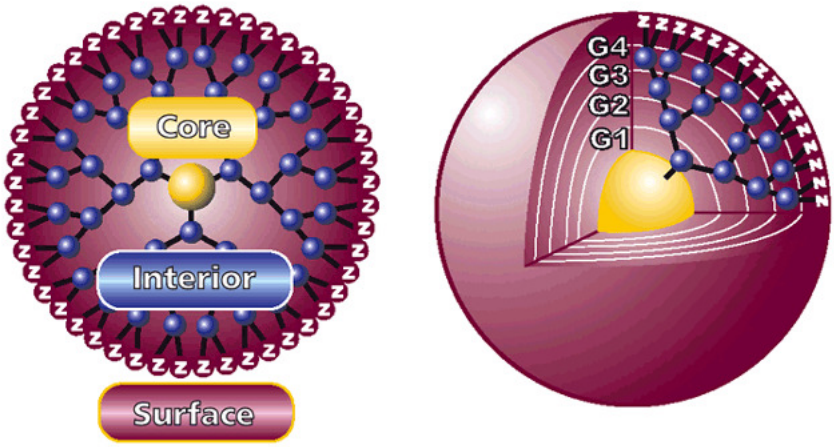



Figure 1.7. Dendrimer structure [24]

For a deeper look to dendrimer structure, the repeated units of the dendrimer between the core and surface that multiplies the number of surfaces each generation are the “branches”. The branches play a key roll in defining the chemical environment and void spaces of the interior of the dendrimer. The center of the dendrimer where the branches are attached is “core”. The core determines the initial degree of branching and contributes to the internal properties of the dendrimer and “dendron” is the dendrimer that radiates from fewer than all of its branch points at the core, often half a dendrimer. Finally, the number of synthesis cycles that the dendrimer has gone through is the “generation”; G1, G2, etc [24-25].

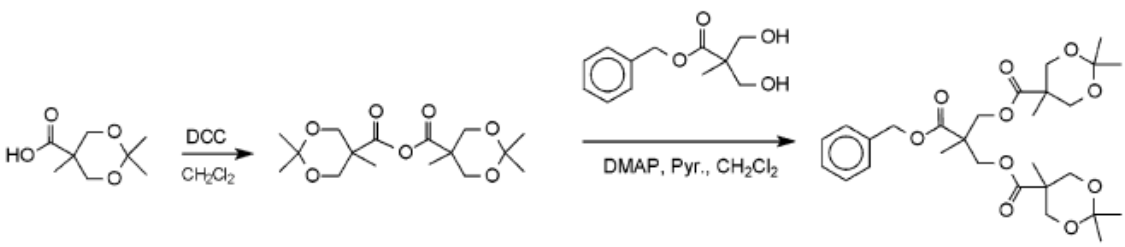



Figure 1.8. G2 benzyl ester dendron synthesized by coupling reaction

Dendrimers are very promising candidates as components of drug delivery systems. In contrast to conventional polymers, these molecules with their well-defined architecture possess a very low polydispersity or even a unique molecular weight, together with a highly regular branching pattern, and a strictly controlled multiplicity of reactive chain ends [26-30]. Therefore, it is possible to introduce or modify a specific number of functional groups on the periphery of a dendrimer, which can be used to alter its properties, such as its solubility or hydrodynamic volume, or can provide a specified number of readily accessed attachment points for drug loading. The design and synthesis of a polyester dendrimer scaffold based on 2,2-bis(hydroxymethyl)propionic acid reported in Figure 1.8. This scaffold is a promising candidate for drug delivery applications, as it shows very good biocompatibility and can be easily prepared [31].

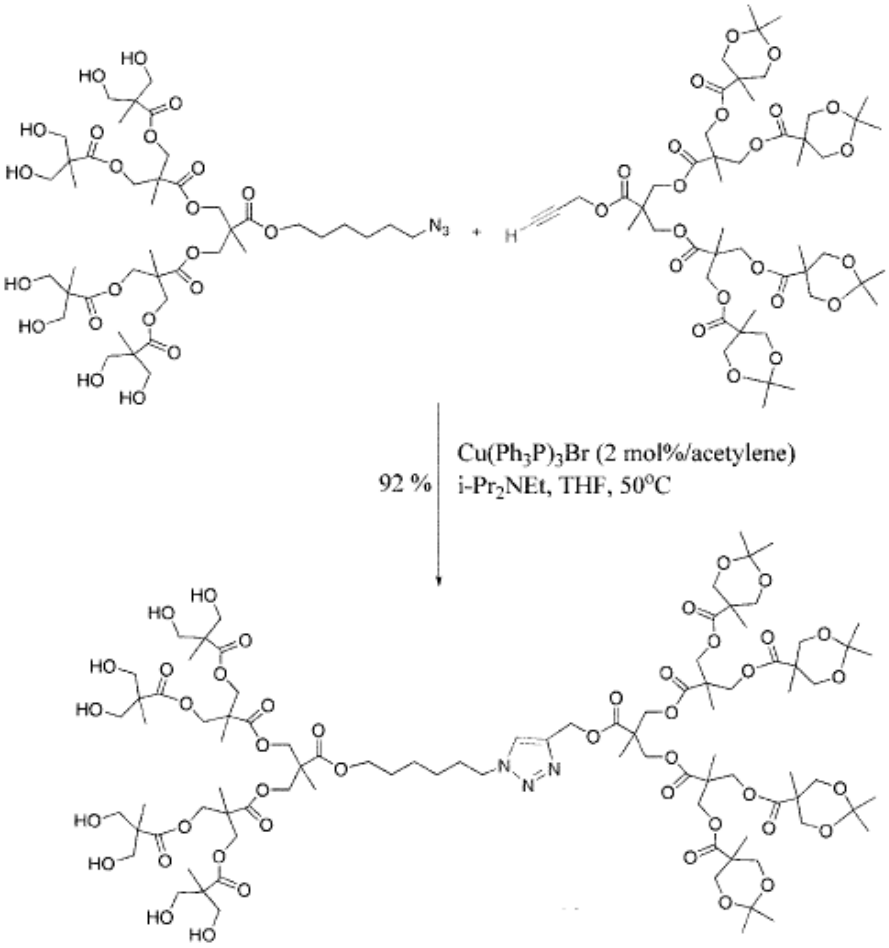



Figure 1.9. Dendrimer synthesized by Huisgen cycloadditon reaction

The same synthetic approach is selected that is based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) as a biocompatible building block and the resulting anhydride provides easy access to both the dendritic blocks containing either a single acetylene or azide group at the focal point. Coupling of the differentiated dendritic blocks containing a variety of chain end functional groups proceeded smoothly under the copper(I)- catalysis conditions using Click methodology (Figure 1.9) [31-37].

### 1.4. Dendritic Hydrogels

Dendritic macromolecules would be the ideal candidates or cross-linking agents [38, 39]. They have highly symmetrical, perfectly branched structures and multiple reactive sites placed in the interior and at the periphery of the macromolecules. The number of the junctions and their chemical composition can be easily controlled by the generation of the dendrimer. The fractal character of the dendritic interior provides unique possibilities for sizeselective encapsulation, manipulation and release of different substrates [40].

The synthesis of amphiphilic hydrogels with highly shape persistent cross-link junctions using PEG as linear blocks and surface-functionalized poly(benzyl ether) dendrimers is shown by Gitsov and Zhu (Figure 1.10). The synthetic strategy is based on the reaction of PEG with isocyanate or epoxy end groups as the hydrophilic component and hydrophobic dendritic poly(benzyl ethers) with amino groups at the periphery.

Dendrimers are highly branched polymers possessing three main structural zones consisting of a central core, internal branching layers, and peripheral groups [38]. Importantly, since these polymers possess narrow molecular weight distributions, unlike most polymers, one can correlate a specific biological response and physical property to an exact structure.

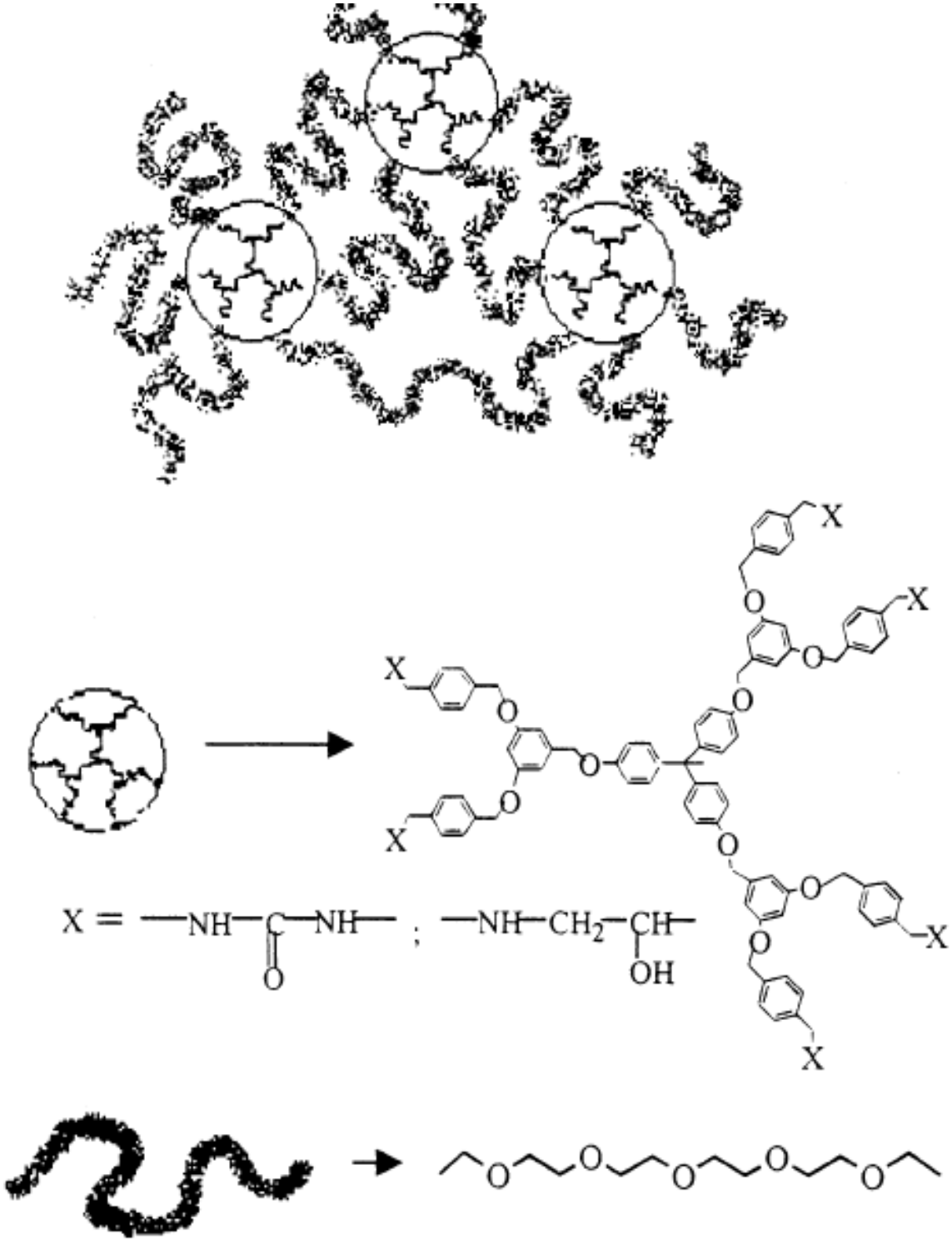



Figure 1.10. Schematic representation of a hydrogel constructed by linear and dendritic fragments

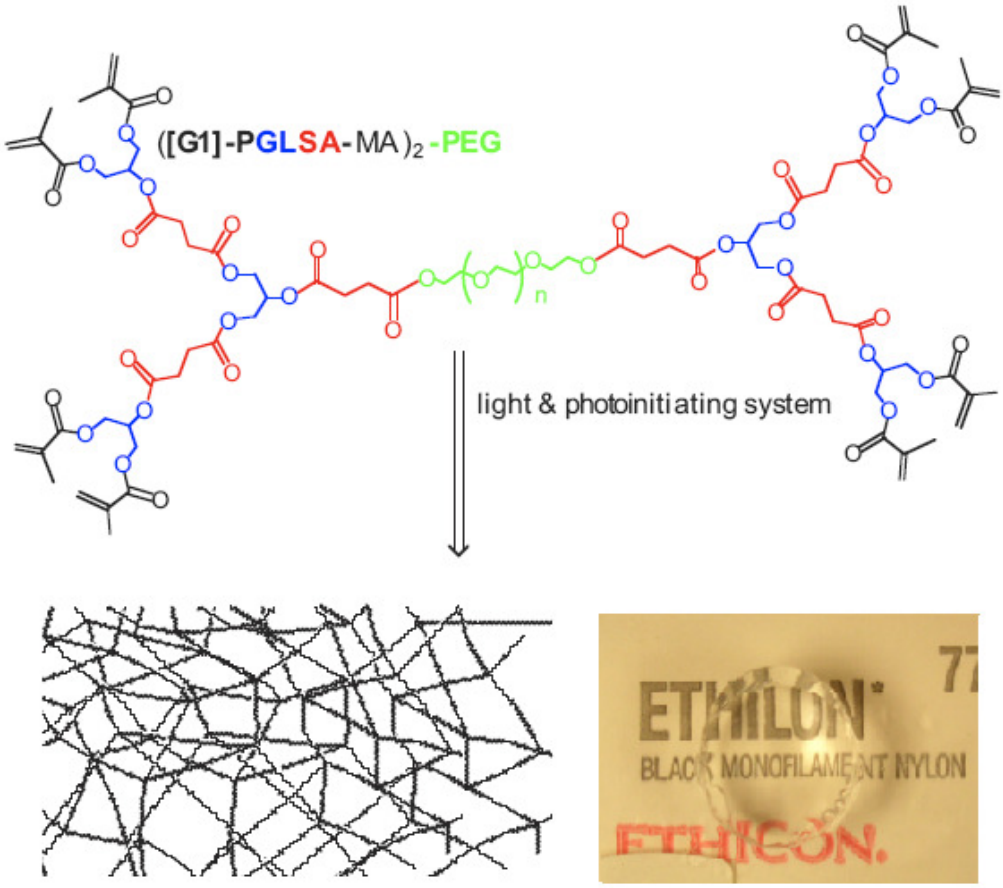



Figure 1.11. Scheme of the photocrosslinked hydrogel formation

The end groups of the dendritic macromolecule are modified to contain an acrylate or other free-radical polymerization group. Upon exposure to visible light the acrylated-modified dendritic macromolecule crosslinks to form a hydrogel. Photolysis of the solution using a 514 nm light initiates the free radical polymerization of the methacrylate moieties on the dendritic polymer (Figure 1.11). This initiating system has been previously used and shown to be non-toxic [41-44]. This photocrosslinking approach which delivers a liquid polymer to a site followed by solidification of the polymer via light to form a three-dimensional hydrogel network is an exciting modality being explored by many groups because of the advantages of photopolymerization including the ability to convert rapidly a liquid monomer or macromer to a gel at physiological temperatures with temporal and control during polymerization to form complex three-dimensional architectures with controlled mechanics [45].

### 1.5. Reactive Hydrogels

The ability to functionalize hydrogels is a useful tool for applications such as protein delivery in tissue engineering, guided cell growth and controlled drug delivery. Immobilization is generally accomplished either by physiabsorption or by covalent attachment of molecules of interest [46, 47].

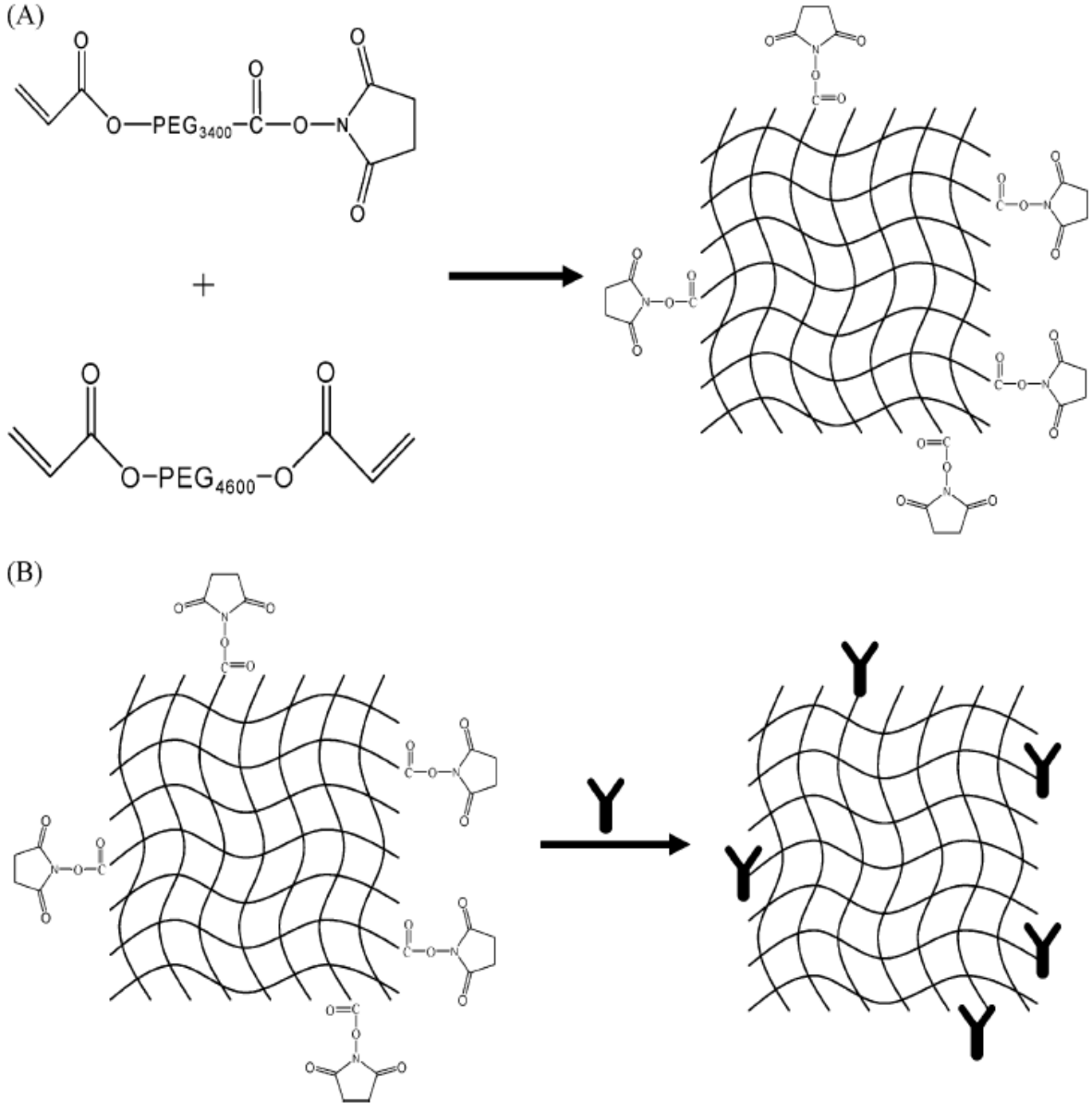



Figure 1.12. (A) Schematic representation of photopolymerization of PEG4600 diacrylate and *N*-hydroxysuccinimide-PEG-acrylate, (B) conjugation an antibody to the surface of these hydrogels

As Charles Y. Cheung and Kristi S. Anseth reported, poly(ethylene glycol diacrylate) was polymerized with *N*-hydroxysuccinimide-PEG-acrylate by UV-initiated photopolymerization. Thereafter, mouse anti-human IgG was conjugated to these hydrogels (Figure 1.12). Developing systems with higher degrees of grafting proteins or any other biomolecules on hydrogel surfaces allows long-term graft survival [48].

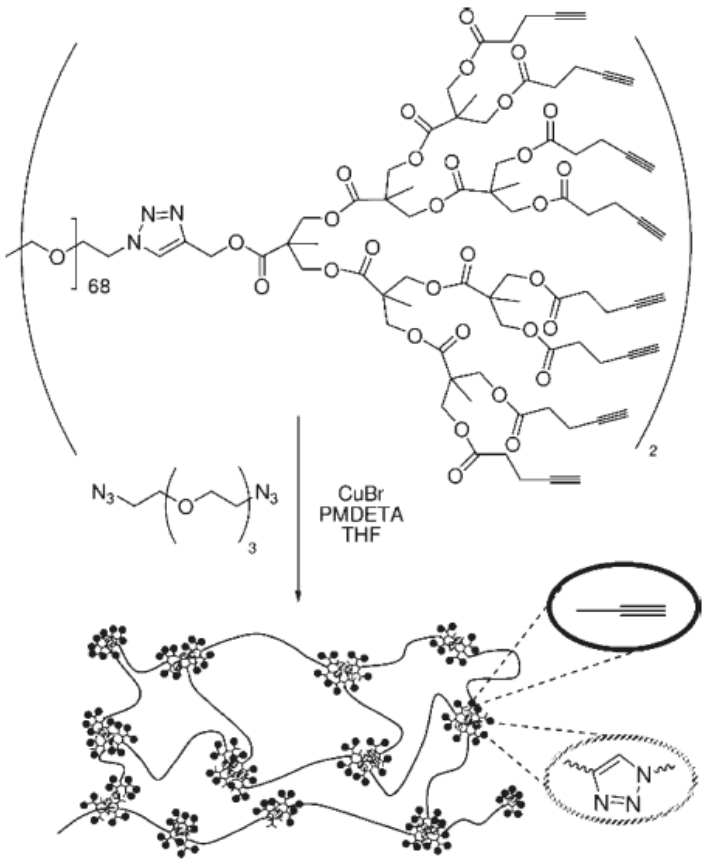



Figure 1.13. Hydrogel Formation via Cross-Linking of Dendron-Polymer Conjugates

Another functionalizable hydrogel model is shown by Huseyin Altın via Huisgen-type copper-catalyzed click reaction between polyester dendrons and linear PEG polymers to access dendron-polymerdendron conjugates necessary for the hydrogel formation [56, 57]. Functionalization of the dendron periphery with alkyne groups affords hydrogel precursors and some of these alkyne groups are cross-linked using a bisazide. Residual alkynes of the resulting hydrogel allow covalent functionalization of the hydrogel via “click” reaction. [46]

### 1.6. Patterning of Hydrogels

Tissue engineering requires a matrix to guide cell growth and differentiation within a three-dimensional system and cells are needed to be guided in chemically patterned volumes inside a hydrogel [49, 50]. For control of cell growth in a spatially directed manner and better mimic the complex cell-adhesive architecture of the central nervous system, there should be more sophisticated 3D-modified volumes such as chemical channels, subsurface tunnels, and networks.

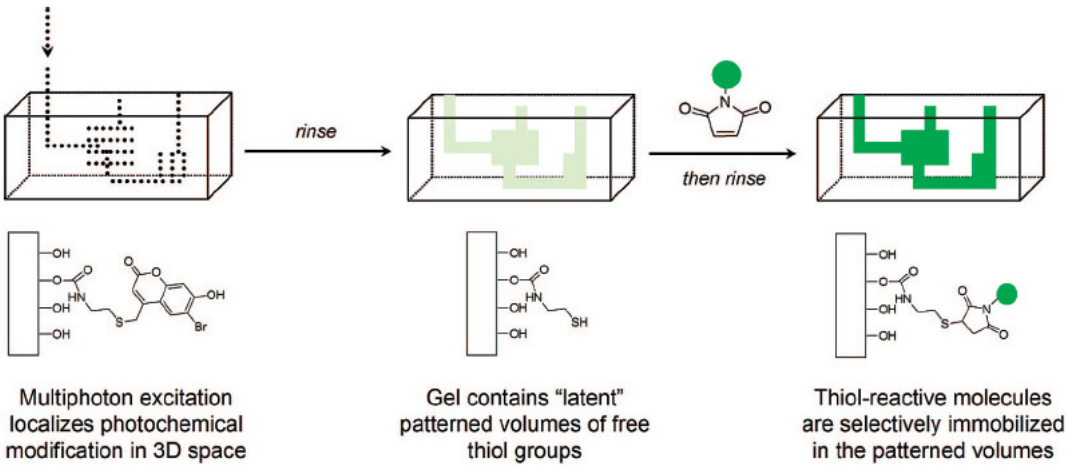



Figure 1.14. Schematic representation chemical patterning in hydrogels

Therefore, 3D chemically defined patterns used for photoinitiated polymerization. The resulting gel contains free thiol groups witch can go further functionlaization meaning thiol reactive groups can be immobilized in the patterned volumes (Figure 1.14) [51]. The resulting patterned hydrogels have applications in tissue engineering, where they can be used to control cell behavior, alter gene expression [52], regulate the structure of the resulting tissue [53], and affect the mechanical properties of the developing tissue [54, 55].

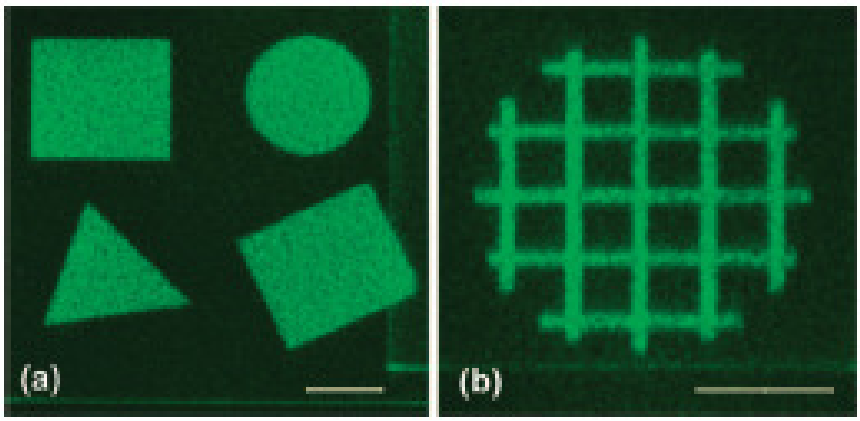



Figure 1.15. Confocal micrographs of fluorescent hydrogels in geometric shapes *(a)* and shape of a grid *(b)* [51]

Fluorescent pictures of hydrogels were created by photochemical immobilization of fluorescently labeled maleimide molecule on hydrogel sample having scale bars of 50 $\mu$m (Figure 1.15).

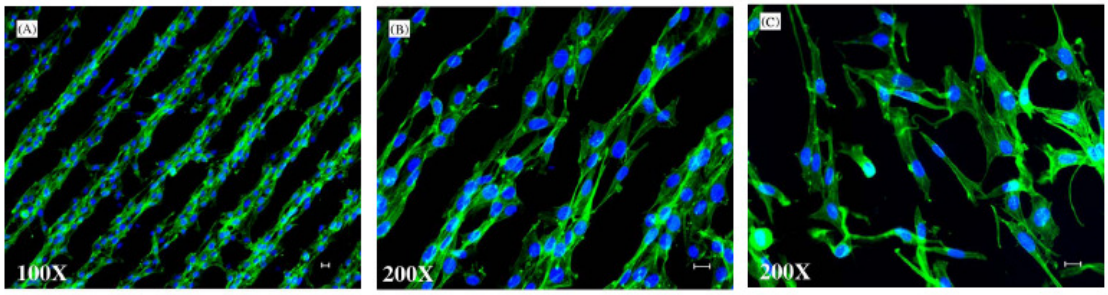



Figure 1.16. Fluorescent photomicrograph of cells plated on patterned hydrogels (Size bar 20 $\mu$m)

The surfaces of HA (hyaluronic acid) and HA–collagen hydrogels were patterned by casting on Teflon dishes that contained patterns. Immediately after crosslinking, the pattern on the hydrogel was complementary to the pattern of the Teflon dish. Fluorescence microscopy showed that the cells were aligned with the pattern when plated on the patterned surface after hydration (Figure 1.16) [55].

# 2. AIM OF THE STUDY

Multivalency in dendrons is a promising property to have reactive crosslinked materials. In our study dendrons are combined with polyethylene glycol molecules to have Dendron-polymer-dendron conjugates by Huisgen click reaction. The surface of these conjugates functionalized with methacrylate and alkyne groups. In the presence of different ratios of methacrylates, UV curing is used to form crosslinked materials which still have unreacted alkyne groups on the surface. These unreacted alkyne groups are used for further functionalization of the obtained hydrogels.

Characterization methods like IR spectroscopy and fluorescent microscopy, the presence of triple bonds in the hydrogels is proved. Thereafter, with microcontact printing methods, we show the shape and size control of the hydrogels. Overall, we achieve the synthesis of hydrogels that have high water absorbance, biocompatibility and biodegradability, have controlled shape and size as candidates for medical applications.

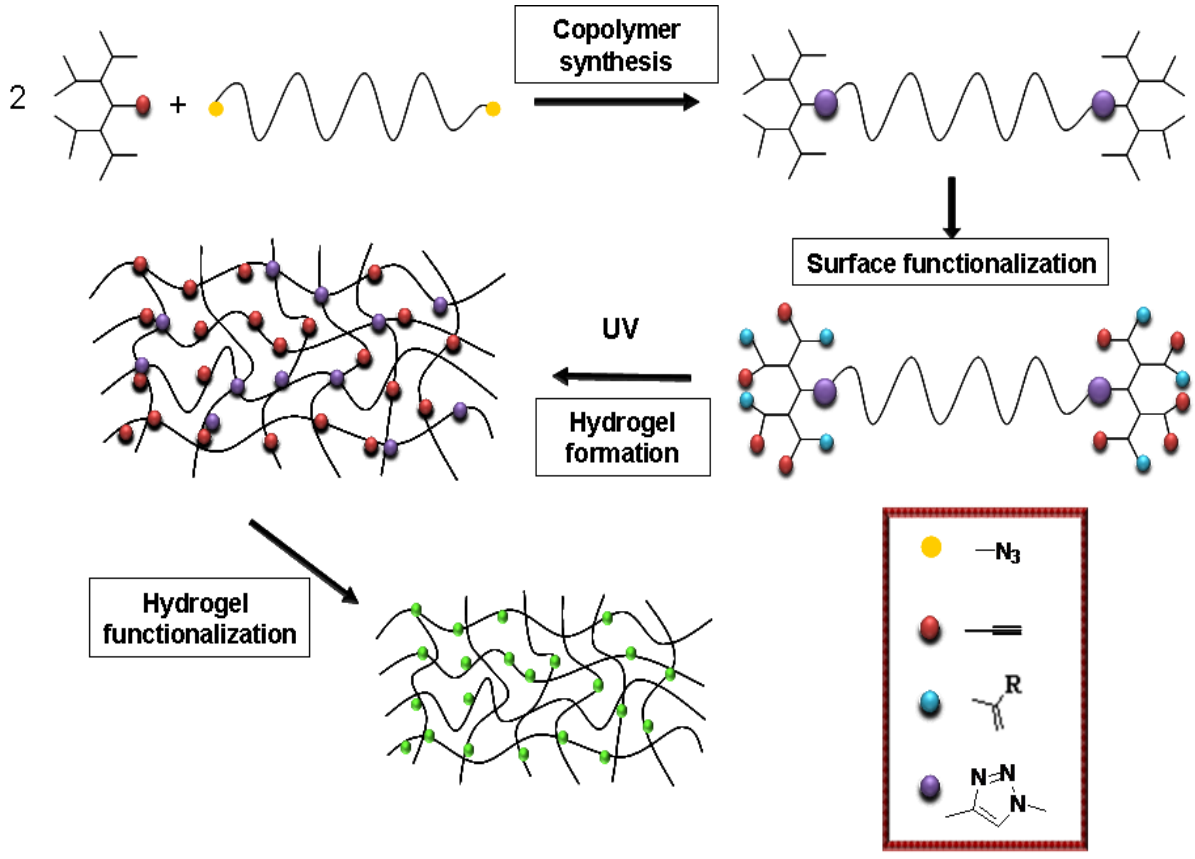



Figure 2.1. General representation of the synthesis of functional hydrogels

# 3. RESULTS AND DISCUSSION

## 3.1. Synthesis of Functional Hydrogels

Dendron-polymer-dendron copolymer hydrogel precusers are synthesized by combining biodegradable polyester dendrons and biocompatible and hydrophilic linear polyethylene glycol molecules via Huisgen cycloaddition reaction. The surface groups of the dendrons are functionalized with methacrylic anhydride and pentynoic anhydride to obtain alkyne and alkene functionalities at the periphery. Finally, hydrogels are synthesized via UV irradiatiob and further functionalized by again Huisgen cycloaddition reaction.

Synthesis of ABA type triblock copolymers that consist of G3 polyester dendrons and PEG molecules and their functionalization are shown in Figure 3.2. As A block of this copolymer, polyester dendrons were chosen. Synthesis of these second and third generation denrons was performed according to literature. B block of the copolymer were linear PEG diazides, were synthesized according to literature. The copolymers were named according to their dendron generation, surface functionality and length of the PEG group ie. [G3]8OH[PEG6K] represents the third generation dendrons are third generation having eight alcohol groups on the surface with the middle block PEG MW=6000.

The copolymers were synthesized via the “click” reaction of G2 and G3 polyester dendrons that bear alkyne functionality at their core and PEG diazide molecules with 2K, 4K and 6K molecular weight in the presence of CuBr and PMDETA and THF as solvent. Functionalization of the terminal alcohol groups was performed by acylation reaction with pentynoic anhydride and methacrylic anhydride in different ratios in the presence of pyridine and DMAP yielding molecules **3**, **4**, **5**, **7**, **8**, **9**, **10**, **12**, **14**. The methacrylate groups of the copolymers were utilized for the crosslinking by UV irradiation forming desired hydrogels.

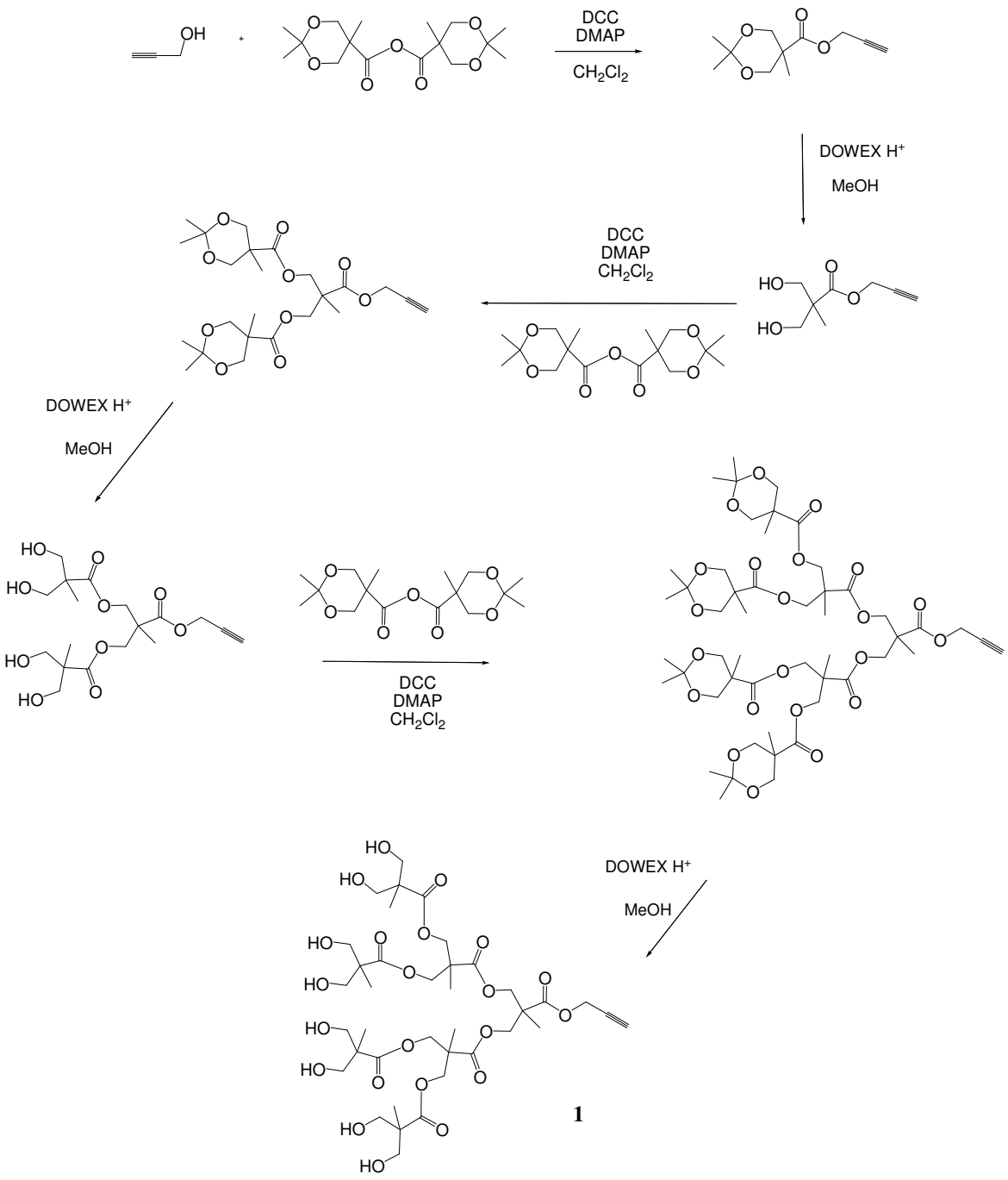



Figure 3.1. Synthesis of G3 polyester dendron

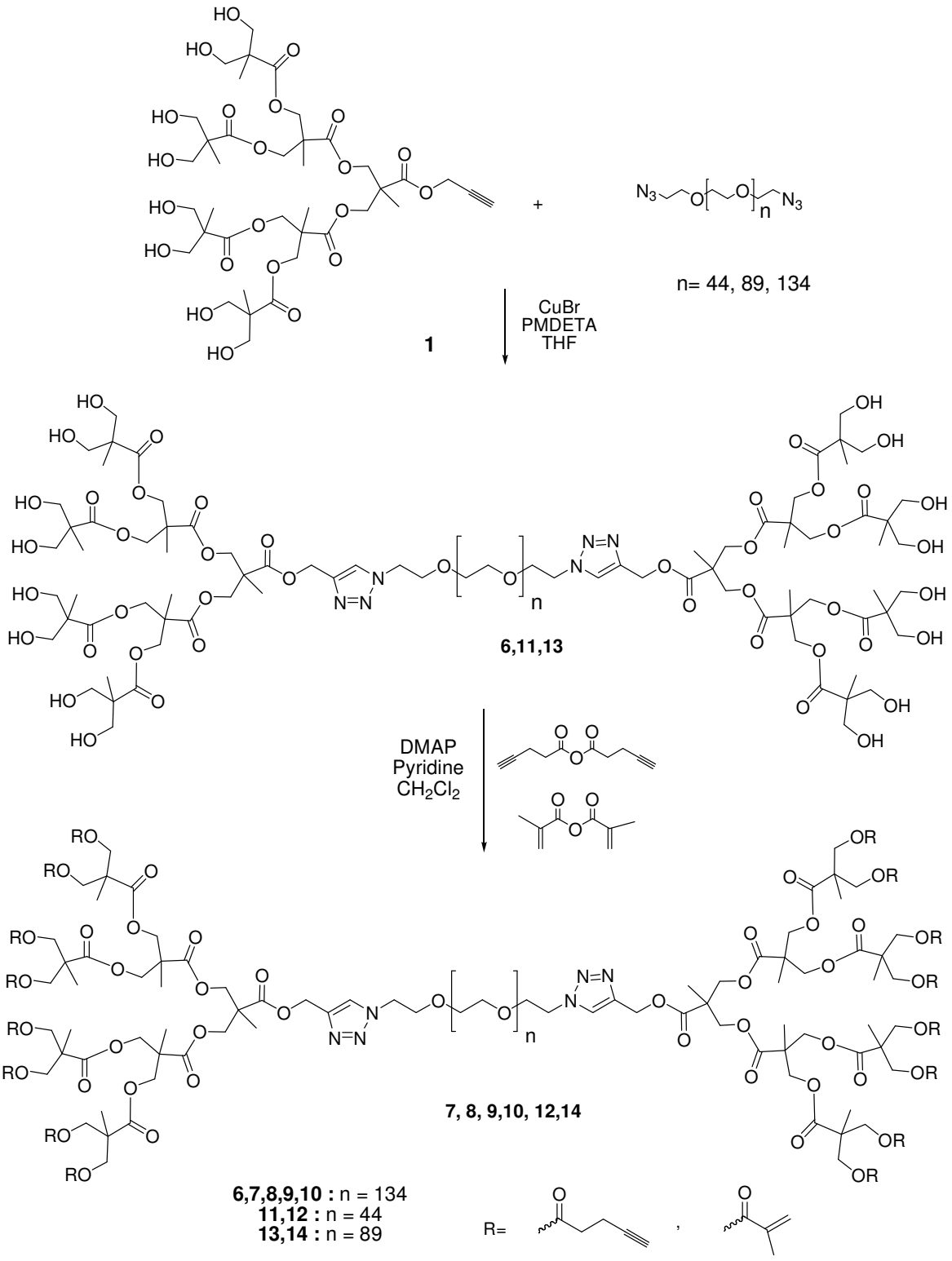



Figure 3.2. Synthesis and functionalization of G3 block copolymers

Alkene functionalized copolymers were dissolved copolymers in water and EtOH mixture and crosslinked in the presence of a photoinitiator under UV for 5 minutes to obtain hydrogels. To improve the solubility of the copolymers EtOH was used as a cosolvent. Depending on the ratio of alkene to alkyne groups at the periphery, hydrogels with different alkyne amounts were obtained.

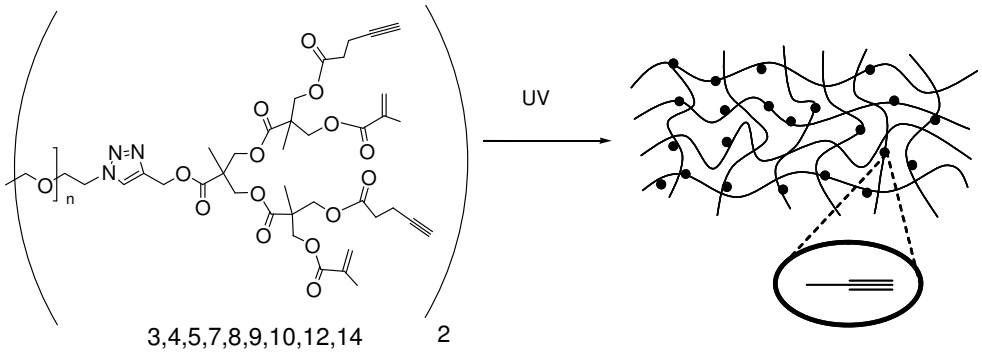



Figure 3.3. Schematic representation of hydrogel formation

### 3.2. Characterization of Copolymers and Functional Hydrogels

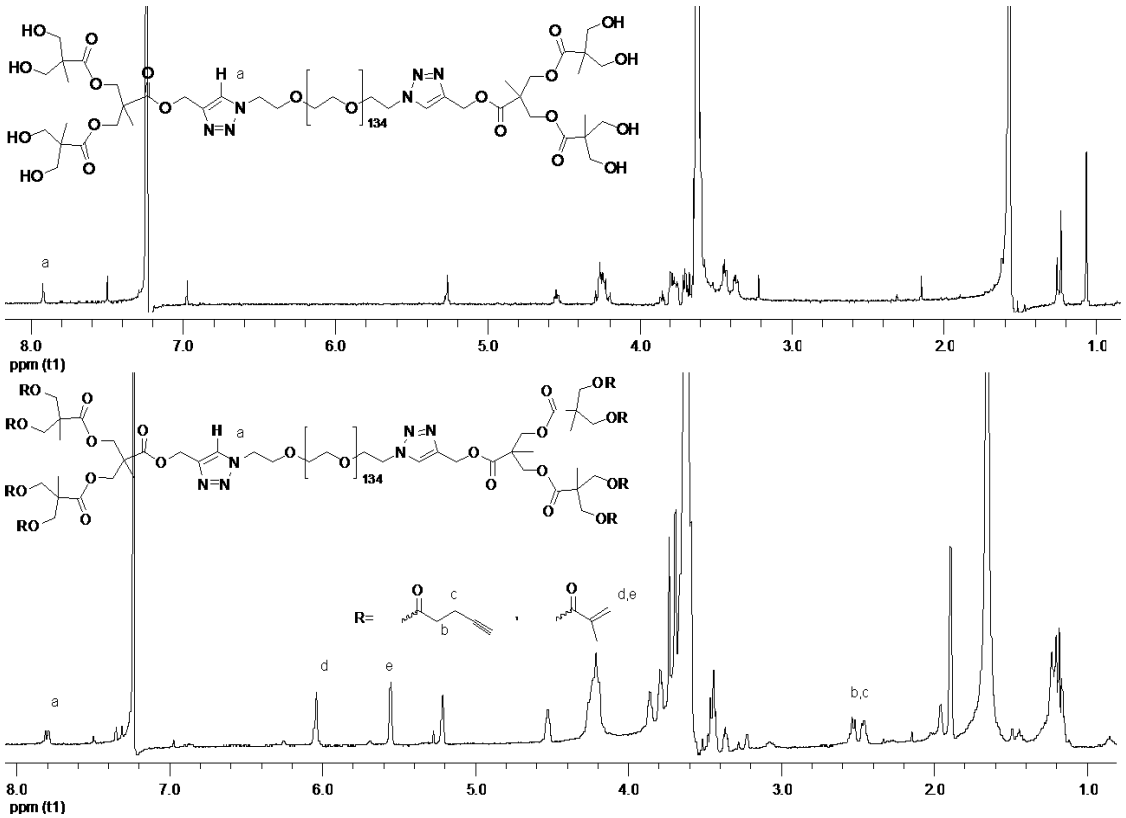



Figure 3.4. $^{1}$H NMR spectrum of [G2]4OH[PEG6K] **2** and [1:1] [G2]4OR[PEG6K] **3**

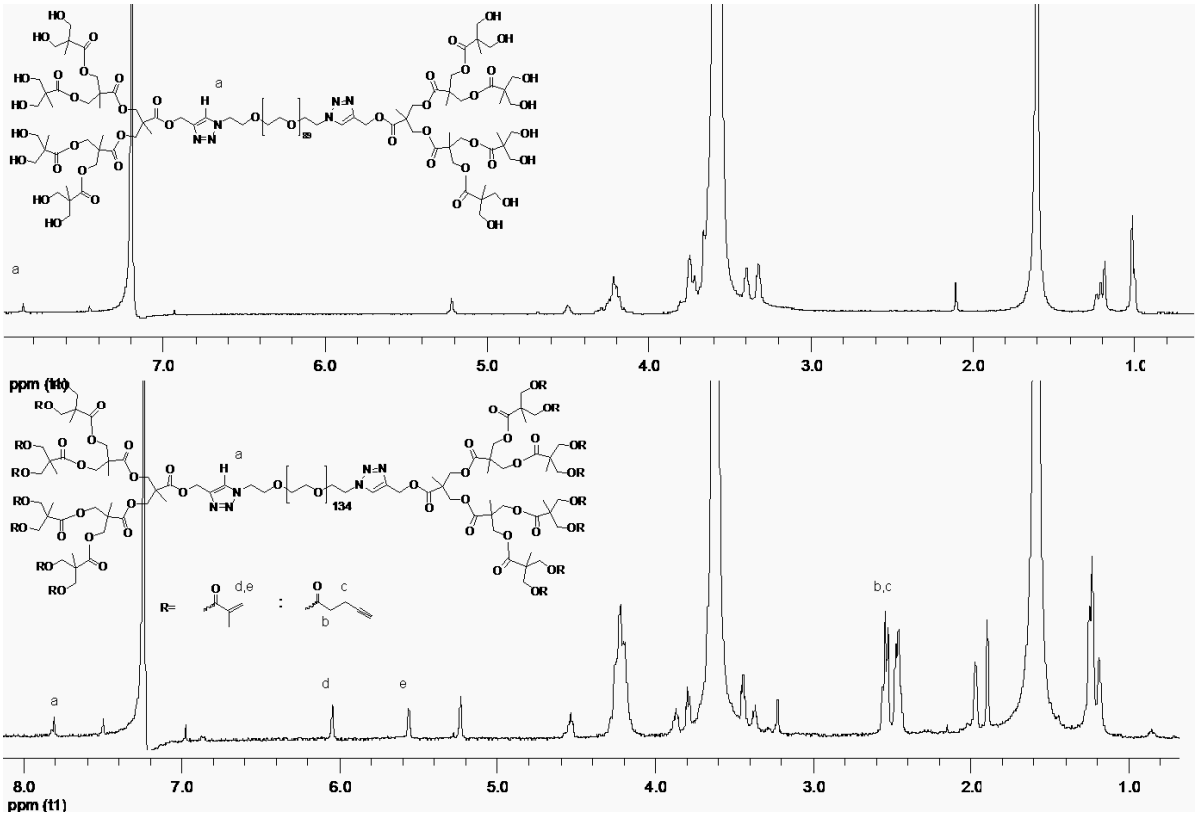



Figure 3.5. $^{1}$H NMR spectrum of [G3]8OH[PEG6K] **6** and [1:1] [G3]8OR[PEG6K] **8**

In $^{1}$H NMR spectrum copolymers G26K $_{(1:1)}$ and G36K $_{(1:1)}$ have additional peaks coming from alkene and alkyne functionalities. The hydrogen of the triazole ring that is formed after cycloaddition reaction was observed as a peak at around 7.8 ppm and signed as “a”. The two hydrogens of the alkene carbon in functionalized copolymers were observed at around 6.1 ppm and 5.6 ppm and signed as “d” and “e”. Finally the peak due to the four hydrogens next to the alkyne group was observed at around 2.5 ppm and signed as “b” and “c” (Figure 3.4, Figure 3.5).

IR spectra were also taken for characterization of the copolymers and hydrogels to show some specific peaks of these molecules.

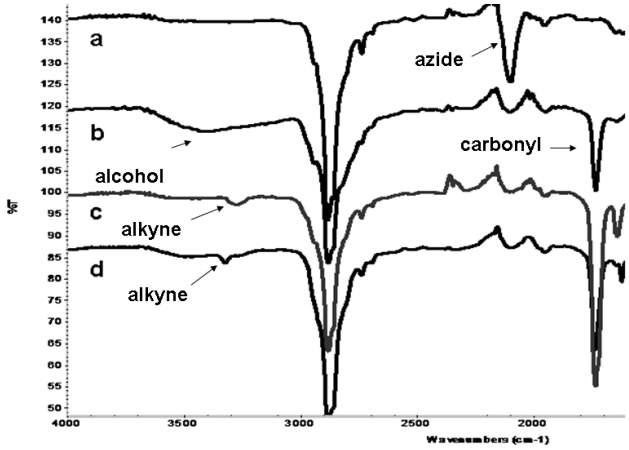



*Figure* 3.6. FTIR spectroscopies of *(a)* PEG6K bisazide, *(b)* PEG-dendron copolymer, *(c)* functionalized copolymer, *(d)* functional hydrogel

IR spectroscopy of PEG6K-bisazide has the specific stretching value at around 2100 $cm^{-1}$ doe to azide functionality (Figure 3.6 a). Subsequently, in IR spectroscopy of the G3 dendron PEG6K combination, disappearance of the peak due to azide group and formation of a new peak of carbonyl groups at around 1730 $cm^{-1}$ and a broad peak at 3440 $cm^{-1}$ due to alcohol groups of dendrons were observed (Figure 3.6 b). Thereafter, functionalization of the copolymer was performed and stretching due to alcohol groups of the dendrons in copolymer structure was exchanged with a new peak of alkyne functionality at 3280 $cm^{-1}$ (Figure 3.6 c). In order to check the availability of these alkyne groups after crosslinking step, we need to see the same stretching and it was observed at again around 3280 $cm^{-1}$ (Figure 3.6 d).

### 3.3. Swelling Properties of Hydrogels

Hydrogels are examined to understand their swelling properties. Formation of hydrogels from constructs 6KG2 and 6KG3 having PEG molecular weight of 6000 were discussed according to different ratios of double and triple bonds; 2:1, 1:1, 1:2 and 1:1, 1:2, 1:3, respectively and water uptake measurements were attempted as explained above. As anticipated, a decrease in triple bond ratio resulted in an increase in the water uptake of the hydrogel. Swelling behaviour of G26K constructed hydrogels are compared as a function of time and the equilibrium hydration levels are described in Figure 3.7. The same data also collected and a diagram representation was given for 6KG3 constructed hydrogels in Figure 3.8. The hydrogels containing higher number of alkene functionality than alkyne

showed higher degree of swelling for 6KG2(2:1), where for 6KG3 the same equivalent of alkene and triplalkyne containing hydrogel 6KG3(2:1) gave the highest swelling among all. Besides, as the PEG length increases water absorption degree increases so, the hydrogel that is of PEG6K absorbs more water than PEG4K and it absorbs more than PEG2K. (Figure 3.9)

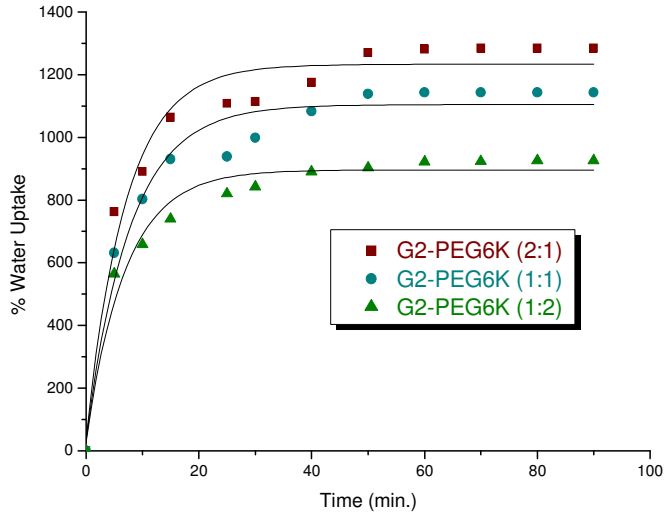



Figure 3.7. Water Uptake Comparison of **6KG2$_{(2:1)}$;6KG2$_{(1:1)}$;6KG2$_{(1:2)}$** Hydrogels.

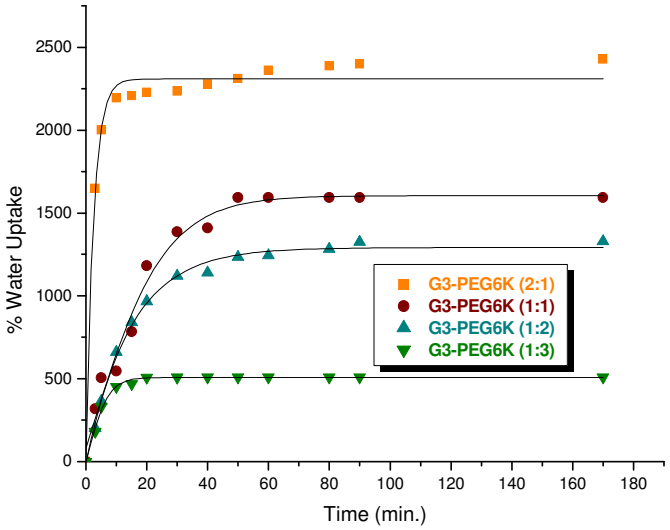



Figure 3.8. Water Uptake Comparison of **6KG3$_{(2:1)}$; 6KG3$_{(1:1)}$; 6KG3$_{(1:2)}$; 6KG3$_{(1:3)}$**Hydrogels

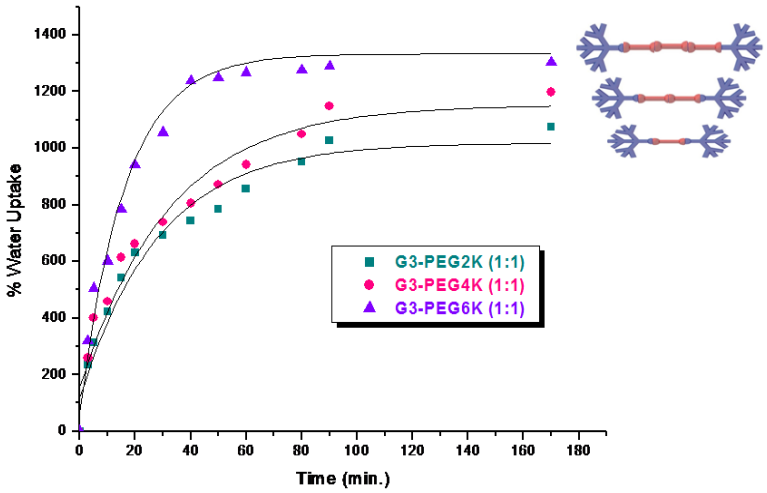



Figure 3.9. Water Uptake Comparison of **2KG3$_{(1:1)}$; 4KG3 $_{(1:1)}$; 6KG3$_{(1:1)}$**Hydrogels.

The macromolecules **3**, **4**, **5**, **7**, **8**, **9**, **10**, **12**, **14** that are combinations of dendrons and PEG have different alkene to alkyne ratio and different PEG lengths. (Table 3.1)

Table 3.1. Properties of hydrogels with variations in dendron-polymer structure, alkene to alkyne ratio and polymer length.

| Item | HYDROGEL | PEG | DENDRON | Double bond:Triple bond ratio | Water uptake % | Available functional groups |
|---|---|---|---|---|---|---|
| 1 | PEG6KG2 (2:1) | 6000 | G2 | 2 : 1 | 1286 | 2 |
| 2 | PEG6KG2 (1:1) | 6000 | G2 | 1 : 1 | 1144 | 4 |
| 3 | PEG6KG2 (1:2) | 6000 | G2 | 1 : 2 | 926 | 6 |
| 4 | PEG6KG3 (2:1) | 6000 | G3 | 2 : 1 | 2428 | 6 |
| 5 | PEG6KG3 (1:1) | 6000 | G3 | 1 : 1 | 1593 | 8 |
| 6 | PEG6KG3 (1:2) | 6000 | G3 | 1 : 2 | 1329 | 10 |
| 7 | PEG6KG3 (1:3) | 6000 | G3 | 1 : 3 | 510 | 12 |
| 8 | PEG2KG3 (1:1) | 2000 | G3 | 1 : 1 | 1075 | 8 |
| 9 | PEG4KG3 (1:1) | 4000 | G3 | 1 : 1 | 1197 | 8 |

The hydrogels labeled according to their dendron generation first, PEG length second and finally double bond to triple bond ratio. As alkene ratio on the copolymer perifery decreases, alkyne ratio increases, meaning that in the resulting hydrogel number of available functional group increases.

### 3.4. Morphological Studies

SEM micrographs of dry hydrogels are clearly observed 100 μm and 10 μm scales (Figure 3.10-11-12-13). As the crosslinker ratio increases, the smaller pores on the hydrogel surface can be easily observed referring that the G36K $_{(1:3)}$ hydrogel is the one that has smallest pore size (Figure 3.13)

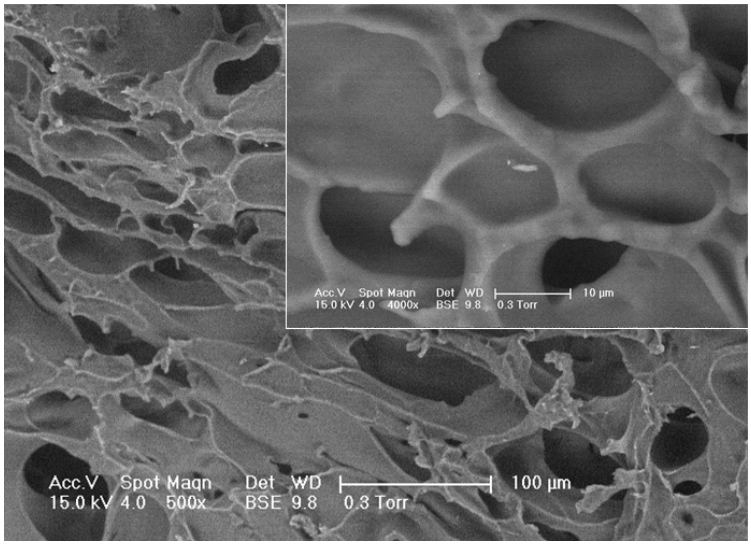



Figure 3.10. SEM images of dry hydrogel G36K $_{(2:1)}$

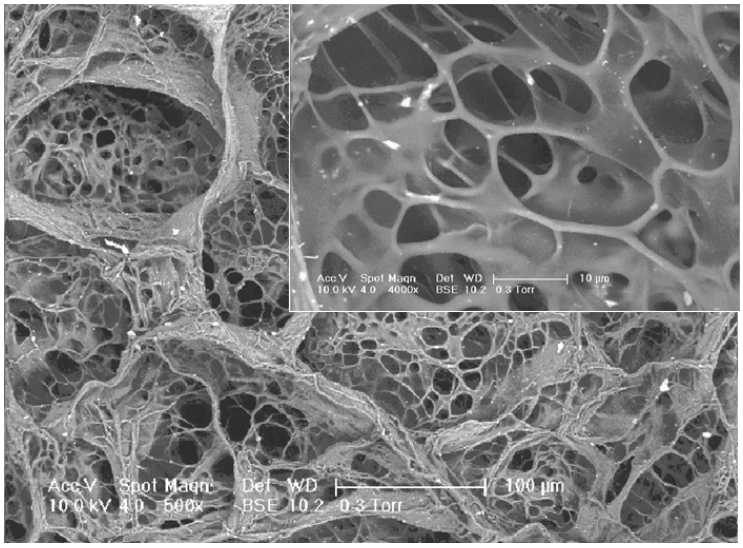



Figure 3.11. SEM images of dry hydrogel G36K $_{(1:1)}$

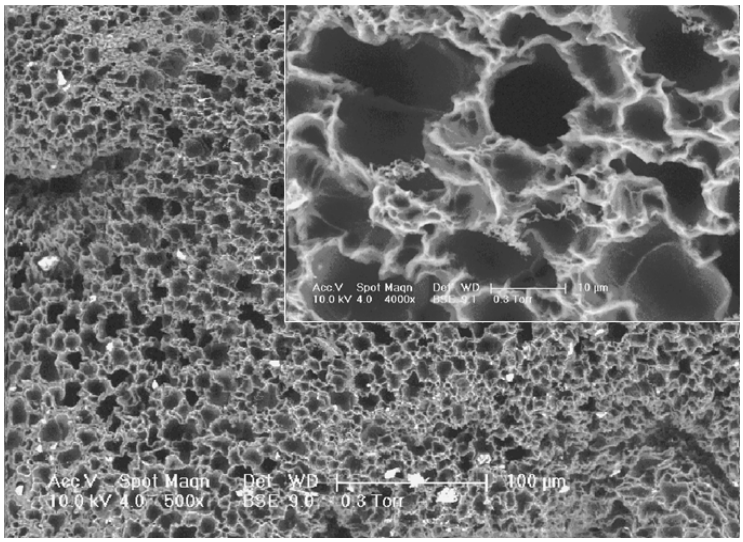



Figure 3.12. SEM images of dry hydrogel of G36K $_{(1:2)}$

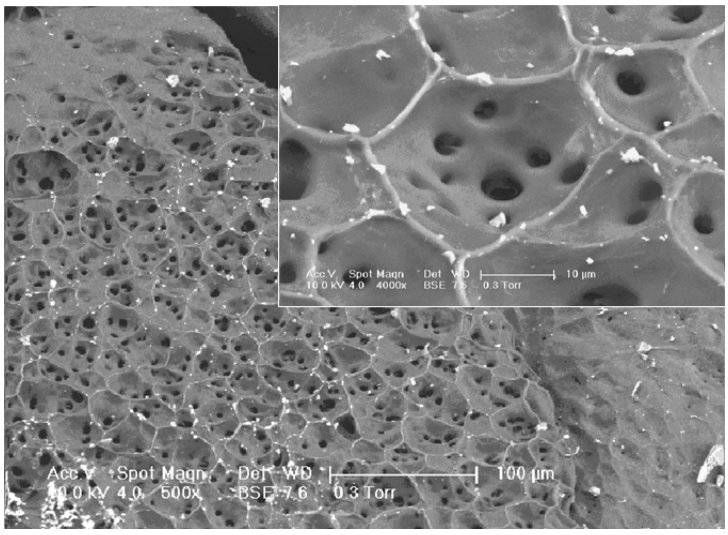



Figure 3.13. SEM images of dry hydrogel of G36K $_{(1:3)}$

### 3.5. Functionalization of Hydrogels

To further demonstrate the presence and reactivity of alkyne groups, the hydrogels were reacted with azide containing molecules: bodipy azide and biotin azide in a [3+2) Huisgen cycloaddition.

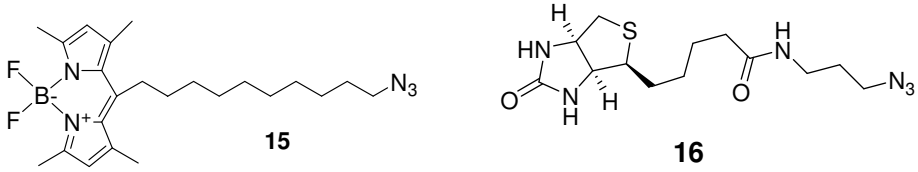



Figure 3.14. Azides that are used for hydrogel functionalization

Bodipy azide **15** has an emission at green shifted wavelengths and it is used for visualization under fluorescence microscope. The second azide: biotin azide was attached for immobilization as an example of protein attachment.

Two identical hydrogels were treated with desired azide, one with and the other without the $CuSO_4$ – sodium ascorbate and catalyst system to observe the physical absorption. The functionalized hydrogels became fluorescent and were observed under fluorescence microscope. Bodipy dye immobilized hydrogel were observed under fluorescence microscope in 1 ms fluorescent light (Figure 3.15).

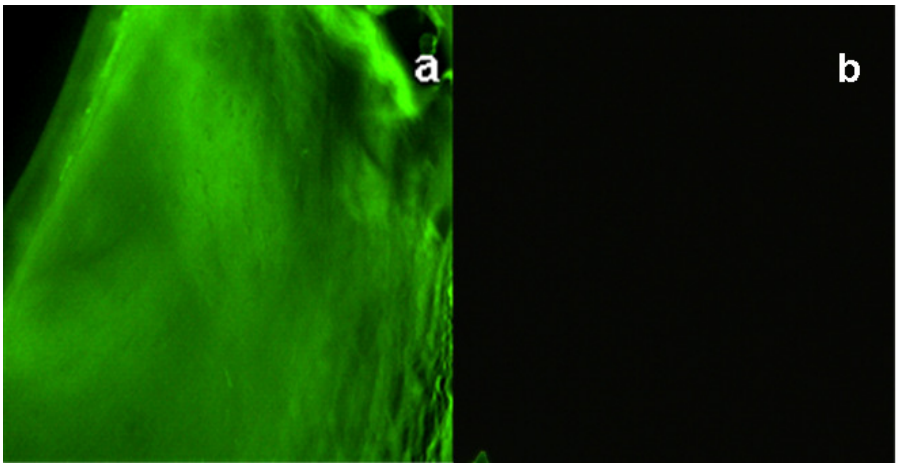



Figure 3.15. Fluorescence microscope images of functionalized hydrogels (*a*) with biotin azide (*b*) control without catalyst

Since we had many hydrogels that have different numbers of functional groups, fluorescent intensities altered according to change of available alkyne groups of hydrogels (Figure 3.16, Figure 3.18).

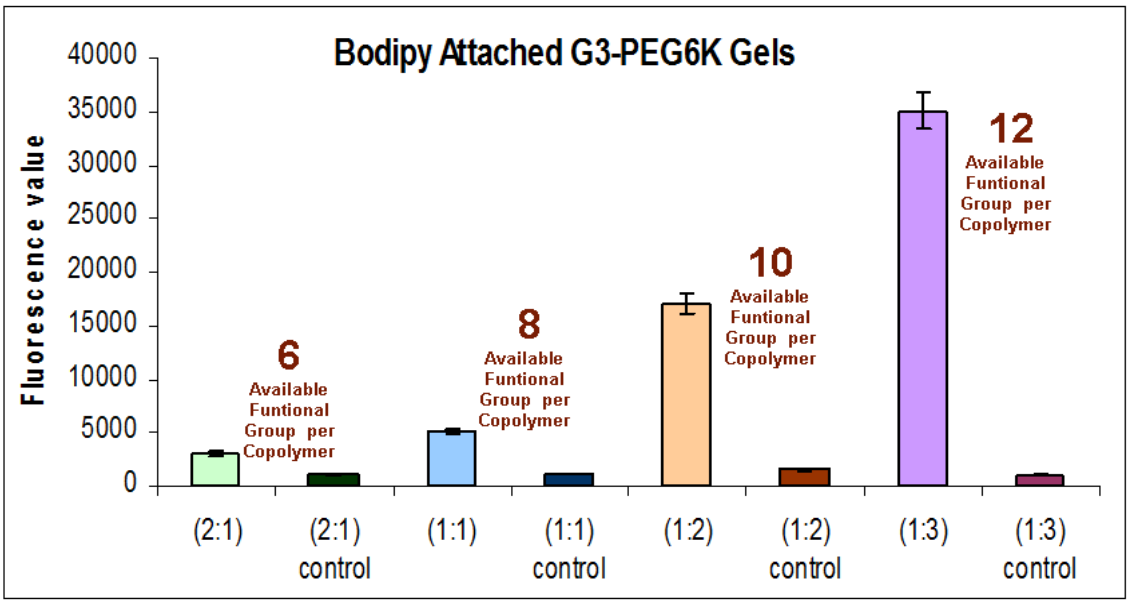



Figure 3.16. Relative fluorescence intensities of functionalized hydrogels with bodipy and their controls that contain descending amount of functional groups. (Under 1 ms fluorescence light)

Hydrogels from alkene – alkyne functionalized G3-PEG6K copolymers that have different number of alkyne groups were reacted with bodipy azide. Figure 3.9 is a summery of the results that shows the fluorescence intensity of the bodipy functionalized hydrogels increases as the number of the available alkyne groups in the hydrogels increases. The most fluorescent one is the hydrogel of $G36K_{(1:3)}$ which has the largest number of available alkyne groups among the all hydrogels.

To demonstrate the enzyme immobilization, two identical hydrogels were reacted with biotin azide **16** one in the presence of $CuSO_4$ – sodium ascorbate catalyst and one in the absence of the catalyste. The both hydrogels were incubated with FITC labeled streptavidin under 50 ms fluorescent light. (Figure 3.17)

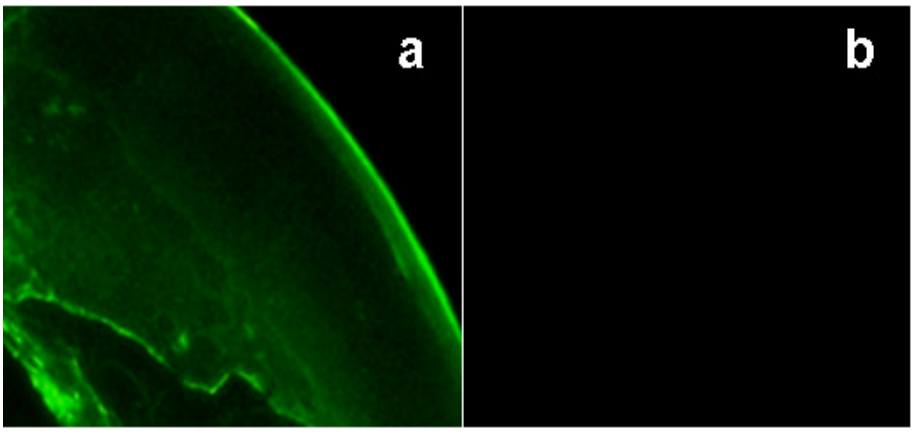



Figure 3.17. Fluorescence microscope images of functionalized hydrogels (*a*) with biotin azide (*b*) control without catalyst

The hydrogel that is reacted with biotin azide in the presence of $CuSO_4$ – sodium ascorbate catalyst system and immersed into FITC labeled streptavidin shows a fluorescent image under fluorescence microscope. The hydrogel reacted without the catalyst system shows that there is almost no physical absorption of the biotin and streptavidin moleules (Figure 3.17).

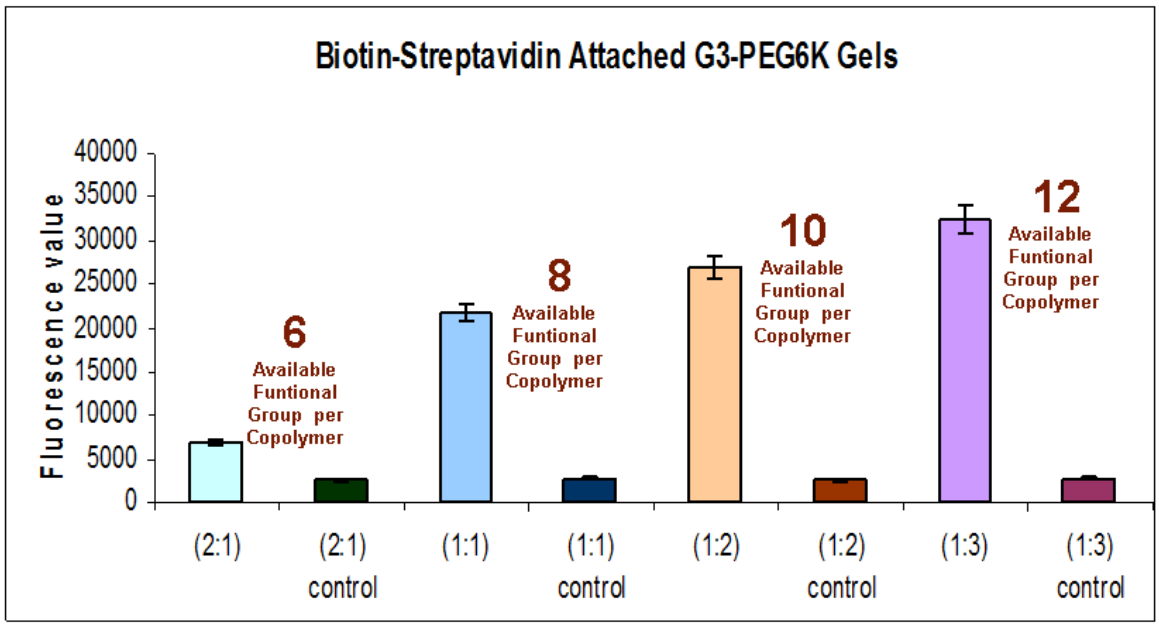



Figure 3.18. Relative fluorescence intensities of functionalized hydrogels with streptavidin and their controls that contain descending amount of functional groups. (Under 50 ms fluorescence light)

The same trend with bodipy attached hydrogels was observed for fluorescence intensities of the biotin functionalized and streptavidin attached hydrogels. As the number of the available alkyne groups in the hydrogel increases, the fluorescence intensity of the hyrogel increases. The most fluorescent one is the hydrogel of $G36K_{(1:3)}$ which has the largest number of available alkyne groups among the all hydrogels.

## 3.6. Microcontact Molding of Hydrogels

### 3.6.1. PDMS Molding

Hydrogels are patterned by using PDMS mold. Onto a silicon wafer that was first functionalized with 3(trimethoxysilyl)propylacrylate, a PDMS mold having channels in micron size was put. The gel precursor which is prepared according to the same procedure with the bulk hydrogels was dropped from the edge of mold and let it move through the channels of mold and cured under UV light for 5 minutes. Finally the mold was removed and the hydrogel modified surface was washed with solvents (Figure 3.19). Optical microscopy image of the hydrogel (Figure 3.21, a), the fluorescent microscopy images of bodipy azide and biotin azide functionalized hydrogels (Figure 3.21, b, c) and the SEM image of the patterned hydrogel show the well shaped patterns (Figure 3.20).

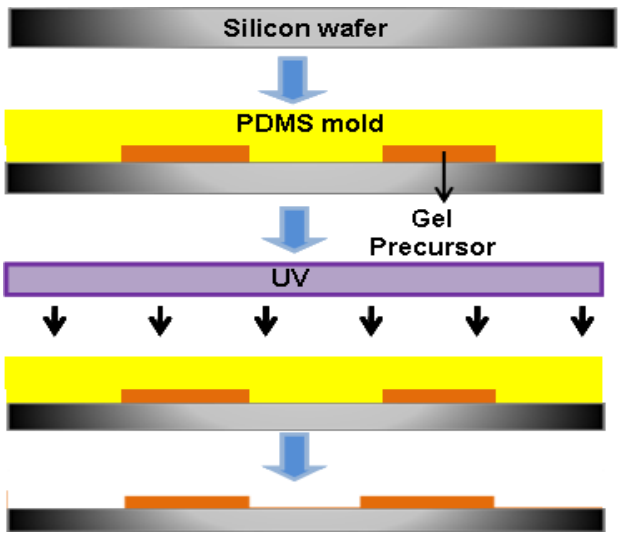



Figure 3.19. Schematic representation of PDMS molding on hydrogels

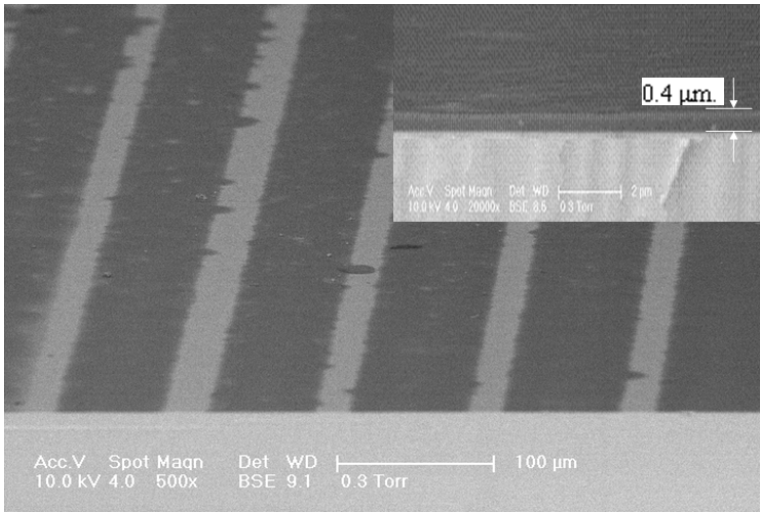



Figure 3.20. SEM images of patterned. hydrogel G36K $_{(1:1)}$ by PDMS molding

SEM image of surface modified with hydrogel shows well defined patterns having 60 µm width and 0.4 µ thickness.

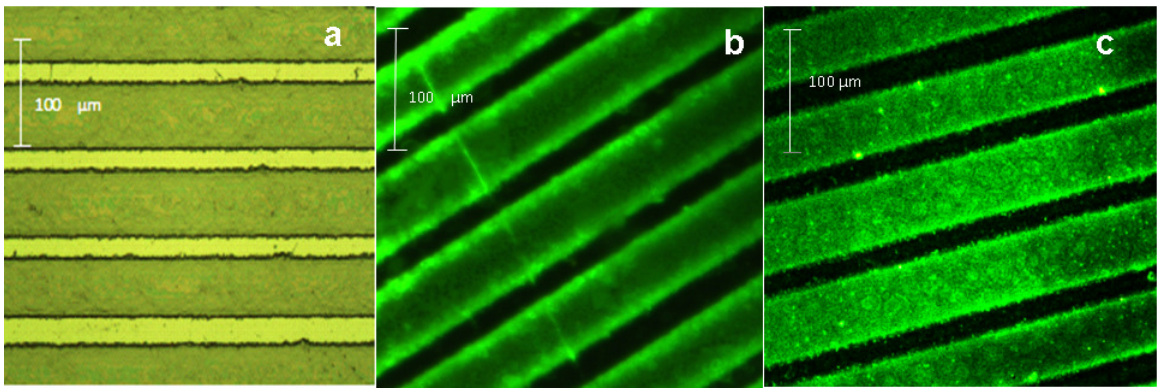



Figure 3.21. The microscope images of hydrogels *(a)* patterned with PDMS mold *(b)* patterned and functionalized with bodipy azide; *(c)* with biotin azide.

In Figure 3.21, the images of patterned hydrogels can be observed. The "a" is the optical microscope image of silicon surface modified with hydrogel from *[2:1] [G3]8OR[PEG6K]* precursor. The second one, "b" is the fluorescence microscope image of the patterned haydrogel from same precursor that is functionalized with bodipy azide after patterning process, with 1 ms fluorescence light. Finally, "c" is the fluorescence microscope image of the patterned haydrogel from same precursor that is functionalized

with biotin azide and immersed into FITC labeled streptavidine molecule, under 50 ms fluorescence light.

### 3.6.2. Spin Coating and Patterning via UV Mask

To the silicon wafer that was functionalized with 2% 3(trimethoxysilyl)propylacrylate, the gel precursor that contains the photoinitiator was dropped. The surface was spin coated in 2000 rpm speed for 10 seconds. To avoid the solvent loss before crosslinking, the coated surface was immediately put on a UV mask. The system was cured under UV (475 nm) for 5 minutes. The mask removed and the surface washed with EtOH and THF several times to remove the parts that were not crossliked.

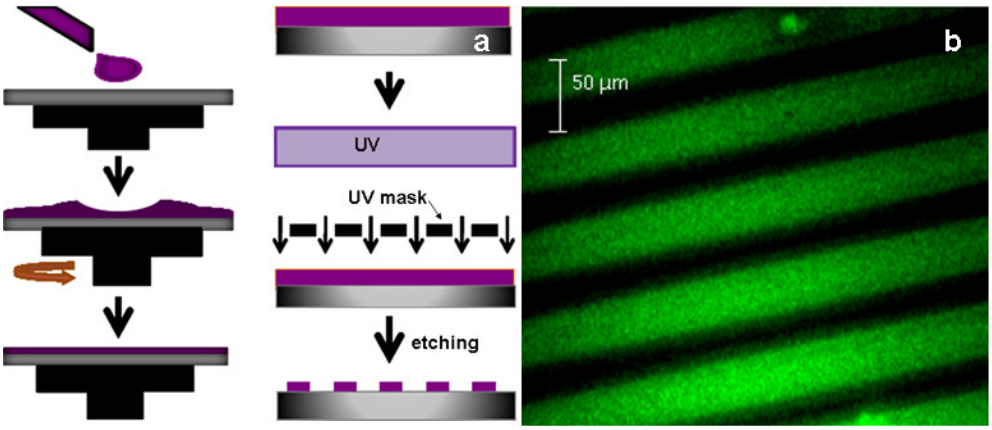



Figure 3.22. *(a)* Representative scheme of spin coating and UV-mask system, *(b)* fluorescence microscope images of patterned hyrogels from precursor by this system

Although PDMS molding is a successful method for patterning, the edges of the patterns are not sharp enough, so another method is used to have better shapes. First, onto a silicon wafer previously prepared hydrogel precursor was dropped and let it spin with 2000 rpm speed for ten seconds. Thereafter, a chromium based UV mask was put onto this coated surface and let the precursor crosslinked under UV light for five minutes following the etching process. The fluorescence microscope image of the surface modified with hydrogel from *[2:1] [G3]8OR[PEG6K]* precursor after functionalization with bodipy azide shows the nice patterns (Figure 3.22). The SEM image of the surface that is modified with

this method shows that the patterns are well defined and the edges are sharper refering better shape control (Figure 3.23).

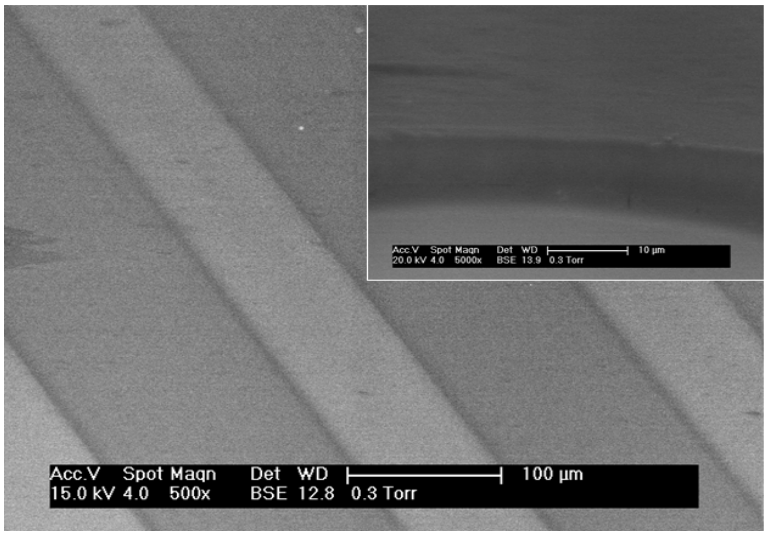



Figure 3.23. SEM images of patterned. hydrogel G36K $_{(1:1)}$ by “spin coating and UV mask system”

# 4. EXPERIMENTAL

## 4.1. General Methods and Materials

2,2-Bis(hydroxymethyl)propionic acid (BMPA), Dowex X50WX2, propargyl alcohol, and 4-pentynoic acid were purchased from Alfa Aesar. All poly(ethylene glycol) were obtained from Fluka. All solvents were purchased from Merck and used as obtained without further purification unless otherwise noted. Azide-functionalized PEGs were synthesized accordingto literature procedures. [58]

The monomer and copolymer characterizations involved $^{1}$H NMR spectroscopy (Varian 400 MHz) and Fourier transform infrared (ATR-FTIR) spectroscopy (Thermo Fisher Scientific Inc. Nicolet 380).. Elemental analyses were obtained from Thermo Electron SpA FlashEA 1112 elemental analyzer (CHNS separation column, PTFE; 2 m; 6 _ 5 mm). The dry and wet surfaces of the hydrogels were observed with an ESEM-FEG/ EDAX Philips XL-30 (Philips, Eindhoven, The Netherlands) instrument using an accelerating voltage of 10kV. Functionalized hydrogels were visualized with Zeiss Observer. Z1 inverted fluorescent microscope.

## 4.2. Preparation of Dendron-Polymer-Dendron ABA Triblock Copolymer Systems

### 4.2.1. Synthesis of compound [G2]4OH[PEG6K] 2

PEG-6K-diazide (250 mg, 0.040 mmol) and propargyl [G2]4[OH] (50 mg, 0.103 mmol) were dissolved in dry THF (4 mL). In a separate flask were dissolved CuBr (0.6 mg, 0.004 mmol) and N,N,N',N",N"-Pentamethyldiethylenetriamine (PMDETA, 0,69 µL, 0.004 mmol) in dry THF (1 mL) purging with $N_2$. The mixture transferred onto azide-propargyl alcohol solution and stirred at 30 $^{o}$C for 24 h. After reaction is completed, the solution is diluted with THF (5 mL) and column with aluminium oxide ($Al_2O_3$, 4 cm in small pipette) to remove copper. The solvent was evaporated under *vacuo* and the desired product was precipitated with Et2O after dissolving in THF (3 mL) , filtered and dried under *vacuo* yielding compound (260 mg, 86% ) as a yellowish white solid. $^{1}$H NMR ($CDCl_3$, δ, ppm) 7.78 (s, 2H), 5.16 (s, 4H), 4.45 (t, 4H, *J* = 5.0 Hz), 4.24 (d, 4H, *J* = 11.0 Hz ), 4.17 (d, 4H, *J* = 11.0 Hz ), 3.78 (t, 4H, *J* = 5.0 Hz), 3.72-3.36 (m, 180H), 2.57 (s, 8H) 1.19 (s, 6H),

0.96 (s, 12H). FTIR ($cm^{-1}$): 3408, 2881, 1733. $C_{126}H_{236}N_6O_{64}$ Calcd: C, 52.97; H, 8.25 N, 2.94. Found: C, 53.01; H, 8.51 N, 3.00.

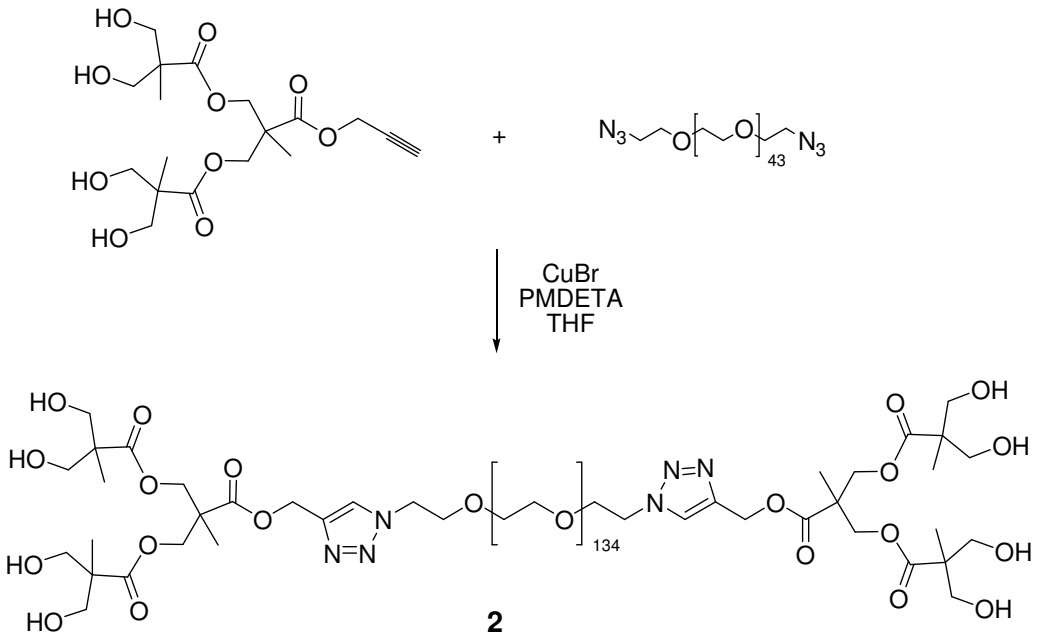



Figure 4.1. Schematic synthesis of compound [G2]4OH[PEG6K] **2**

### 4.2.2. Synthesis of compound [1:1] [G2]4OR[PEG6K] 3

[G2]4OH[PEG6K] (60 mg, 0.008 mmol), pyridine (0.2 mL) and DMAP (1.0 mg, 0.008 mmol) dissolved in dry $CH_2Cl_2$ (5 mL) in a round bottom flask. To the stirring reaction miwture was added methacrylic anhydride (4.78 µL, 0.032 mmol) and continued stirring for 24 h at 40 $^oC$ under $N_2$. Then, 4-pentynoic anhydride (6.25 mg, 0.032 mmol) was added to the reaction mixture and continued stirring for another 24h at 30 $^oC$ under $N_2$. Crude product was purified by precipitation in diethyl ether to give a yellowish solid (56 mg, 77%). $^1H$ NMR ($CDCl_3$, δ, ppm) 7.79 (s, 2H), 6.04 (s, 4H), 5.56 (s, 4H), 5.22 (s, 4H), 4.53 (s, 6H), 4.21 (s, 16H), 3.86 (t, 10H, *J* = 4,7 Hz), 3.79 (s, 8H), 3.69 (s, 8H), 3.68-3.59 (m, 540H), 2.68 (t, 4H, *J* = 6.59 Hz), 2.47 (t, 4H, *J* = 6.2 Hz), 1.96 (s, 2H), 1.89 (s, 8H), 1.23 (s, 6H), 1.20-1.17 (m, 12H). FTIR ($cm^{-1}$): 3317, 2882, 1738. $C_{344}H_{632}N_6O_{163}$ Calcd: C, 54.23; H, 8.79 N, 1.54. Found: C, 54.49; H, 8.98 N, 1.66.

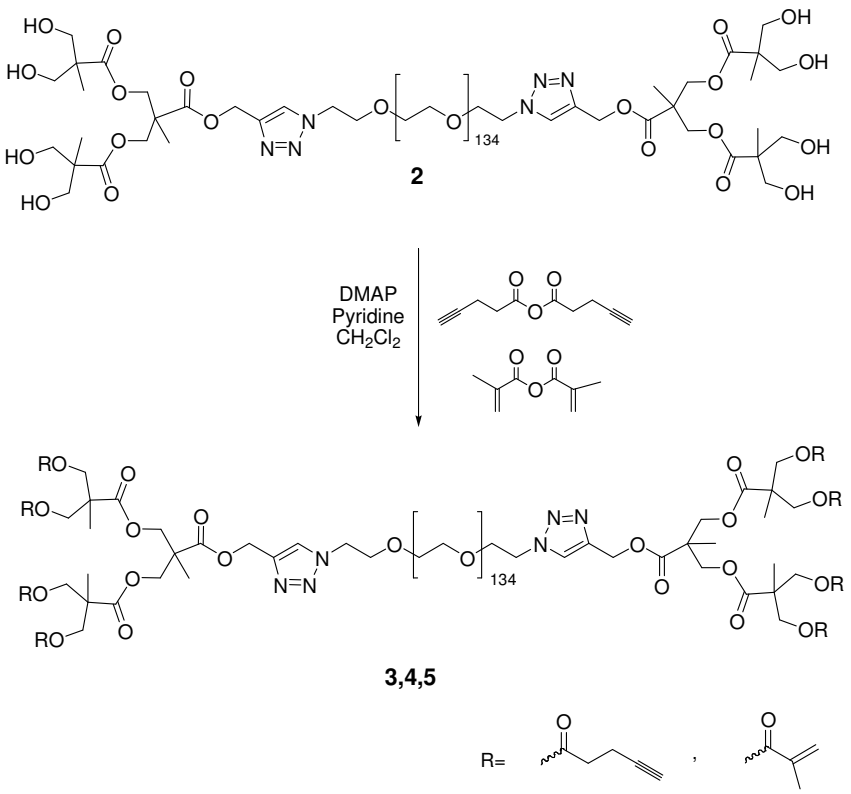



Figure 4.2. Schematic synthesis of compounds [G2]4OR[PEG6K] **3**, **4**, **5**

### 4.2.3. Synthesis of compound [1:2] [G2]4OR[PEG6K] 4

Synthesized with the same procedure as compound *(3)* by using *[G2]4OH[PEG6K]* (60 mg, 0.008 mml), pyridine (0.2 mL) and DMAP (1.0 mg, 0.008 mmol), dry $CH_2Cl_2$ (5 mL), methacrylic anhydride (6.53 µL, 0.024 mmol), 4-pentynoic anhydride (4.68mg, 0.040 mmol) yielding product as a yellowish viscous liquid (65 mg, 91%). $^1$H NMR ($CDCl_3$, δ, ppm) 7.78 (s, 2H), 6.06 (s, 2H), 5.56 (s, 2H), 5.29 (s, 6H), 4.52 (s, 6H), 4.24 (s, 16H), 3.8 (t, 10H, *J* = 4,75 Hz), 3.77 (s, 8H), 3.69 (s, 8H), 3.66-3.58 (m, 540H), 2.54 (t, 8H, *J* = 5.79 Hz), 2.46 (t, 8H, *J* = 4.66 Hz), 1.99 (s, 2H), 1.86 (s, 8H), 1.2 (s, 6H), 1.19-1.13 (m, 12H). FTIR ($cm^{-1}$): 3288, 2880, 1738. $C_{345}H_{632}N_6O_{163}$ Calcd: C, 56.97; H, 9.25 N, 1.64. Found: C, 56.45; H, 9.38 N, 1.35.

### 4.2.4. Synthesis of compound [2:1] [G2]4OR[PEG6K] 5

Synthesized with the same procedure as compound *(3)* by using *[G2]4OH[PEG6K]* (100 mg, 0.015 mmol), pyridine (0.3 mL) and DMAP (1.4 mg, 0.011 mmol), dry $CH_2Cl_2$ (8 mL), methacrylic anhydride (10.88 µL, 0.073 mmol), 4-pentynoic anhydride (7.8 mg,

0.044 mmol) yielding product as a yellowish viscous liquid (80 mg, 67%). $^{1}$H NMR ($CDCl_3$, δ, ppm) 7.81 (s, 2H), 6.05 (s, 6H), 5.56 (s, 6H), 5.23 (s, 3H), 4.50 (s, 6H), 4.23 (s, 16H), 3.88 (t, 10H, *J* = 4,72 Hz), 3.79 (s, 8H), 3.68 (s, 8H), 3.65-3.58 (m, 540H), 2.54 (t, 3H, *J* = 6.42 Hz), 2.46 (t, 3H, *J* = 5.9 Hz), 1.97 (s, 2H), 1.88 (s, 8H), 1.26 (s, 6H), 1.22-1.16 (m, 12H). FTIR ($cm^{-1}$): 3288, 2881, 1738. $C_{343}H_{632}N_6O_{163}$ Calcd: C, 53.97; H, 9.25 N, 1.94. Found: C, 53.88; H, 9.11 N, 1.36.

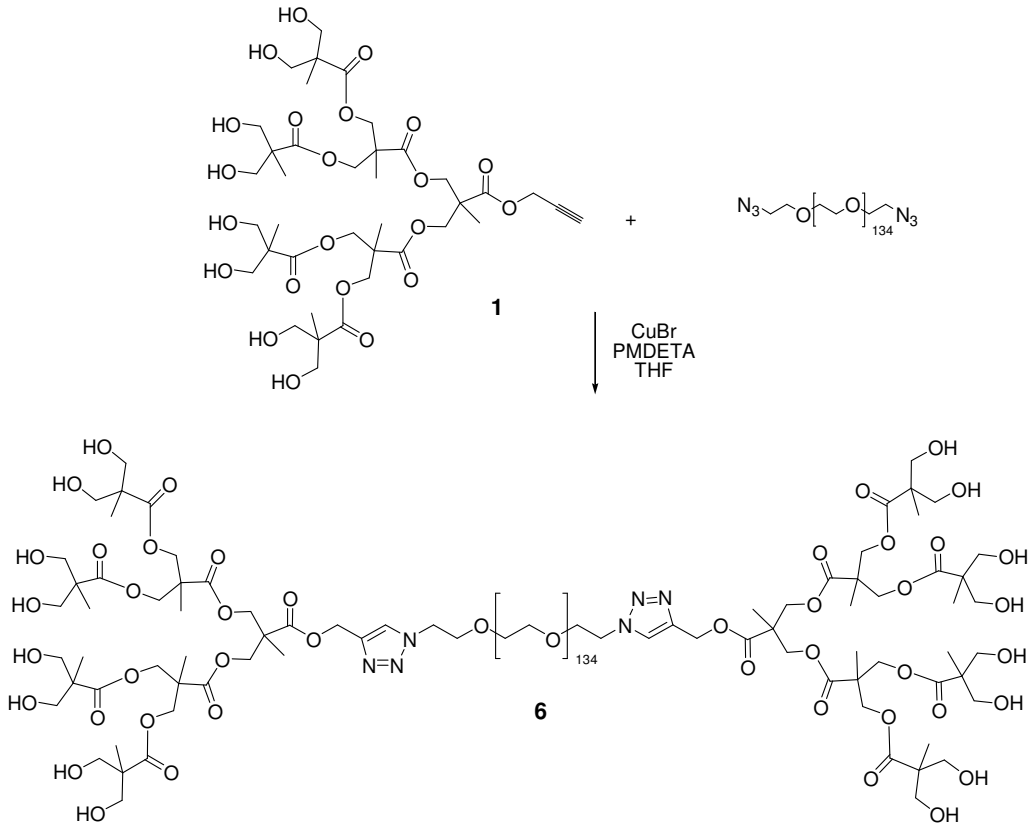



Figure 4.3. Schematic synthesis of compound [G3]8OH[PEG6K] **6**

### 4.2.5. Synthesis of compound [G3]8OH[PEG6K] 6

PEG-6K-Diazide (750 mg, 0.12 mmol) and propargyl [G3]8[OH] (250 mg, 0.29 mmol) were dissolved in dry THF (8 mL). In a separate flask were dissolved CuBr (1.8 mg, 0.013 mmol) and N,N,N',N",N" Pentamethyldiethylenetriamine (PMDETA, 2.61 µL, 0.013 mmol) in dry THF (2 mL) and purged with $N_2$. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 30 $^{o}$C for 24 h. The solution is diluted

with THF (10 ml) and column with aluminium oxide ($Al_2O_3$, 4 cm in small pipette) to remove copper. The solvent was evaporated under *vacuo* and the desired product was precipitated with diethyl ether after dissolving in THF (3 mL) , filtered and dried under *vacuo* yielding compound (850 mg, 85%) as a white solid. $^1$H NMR ($CDCl_3$, δ, ppm) 7.91 (s, 2H), 5.27 (s, 4H), 4.54 (t, 4H, *J* = 4 Hz), 4.28-4.19 (m, 24H), 3.85 (t, 4H, *J* = 4 Hz), 3.80-3.42 (m, 540H), 1.25-1.17 (m, 24H), 1.06 (s, 18H). FTIR (cm$^{-1}$): 3444, 2882, 1732. $C_{348}H_{664}N_6O_{179}$ Calcd: C, 50.97; H, 8.25 N, 3.04. Found: C, 50.67; H, 8.54 N, 3.29.

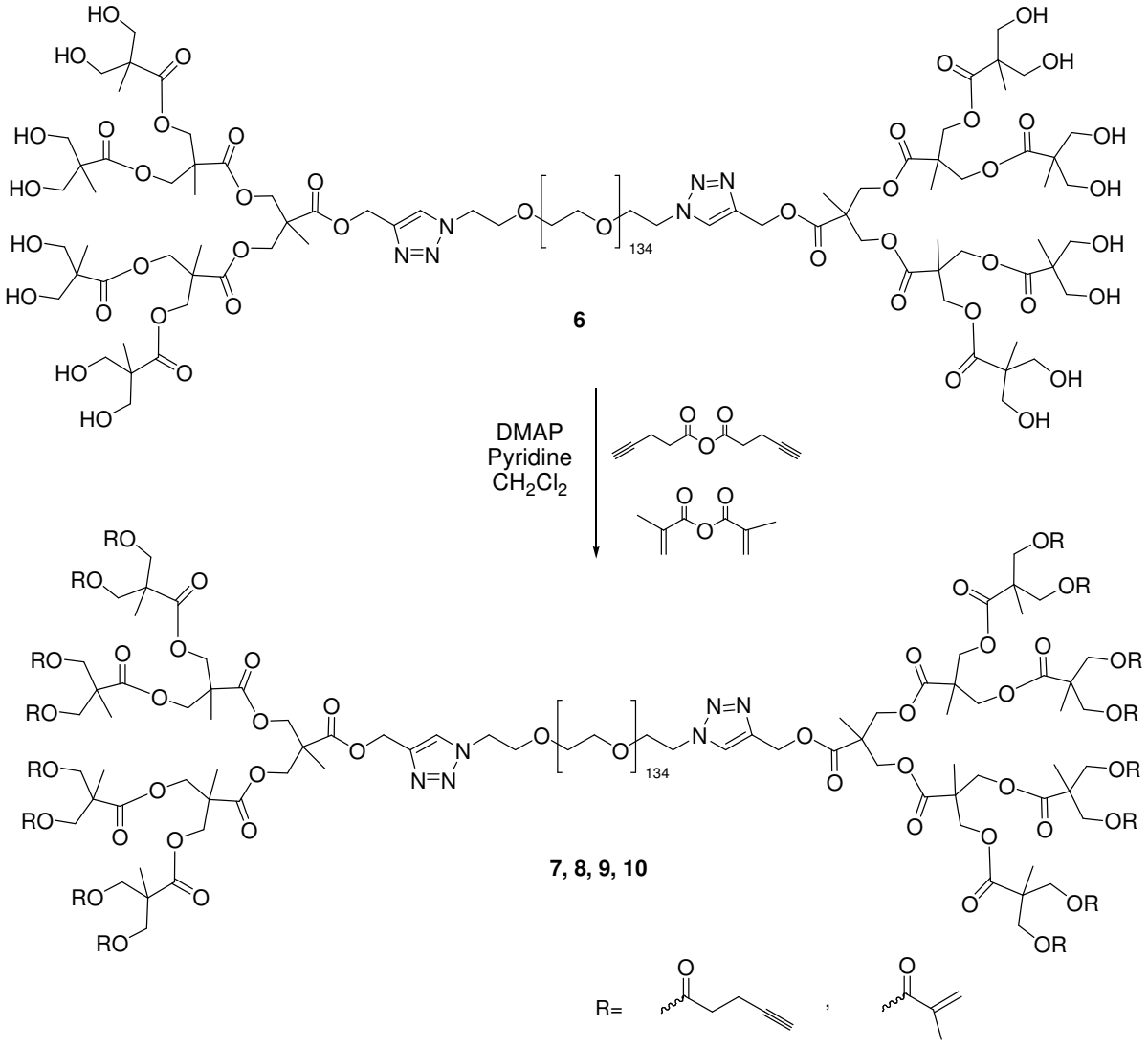



Figure 4.4. Schematic synthesis of compounds [G3]8OR[PEG6K] **7**, **8**, **9**, **10**

### 4.2.6. Synthesis of compound [2:1] [G3]8OR[PEG6K] 7

[G3]8OH[PEG6K] (80 mg, 0.01 mmol), pyridine (0.2 mL) and DMAP (1.2 mg, 0.012 mmol) were dissolved in dry $CH_2Cl_2$ (5 mL) in a 25 mL round bottom flask. To the stirring reaction mixture was added methacrylic anhydride (15.3 µL, 0.1 mmol) and continued stirring for 24 h at 40 $^oC$ under $N_2$. Then 4-pentynoic anhydride (11 mg, 0.06 mmol) added to the reaction and continued stirring overnight at room temperature under $N_2$. Crude was purified by precipitation in diethyl ether to give 80 mg of a yellowish solid. (75 % yield). $^1H$ NMR ($CDCl_3$, δ, ppm) 7.80 (s, 2H), 6.04 (s, 10H), 5.58 (s, 10H), 5.22 (s, 5H), 4.51 (t, 4H, *J* = 5.2 Hz), 4.23-4.19 (m, 56H), 3.88 (t, 2H, *J* = 4.9 Hz), 3.80-3.42 (m, 540H), 2.54 (t, 10H, *J*=6.89 Hz), 2.45 (t, 10H, *J*=6.81 Hz), 1.97 (s, 12H), 1.9 (s, 18H), 1.62 (s, 40H), 1.24 (s, 24H), 1.20 (s, 6H). FTIR ($cm^{-1}$): 3280, 2869, 1735. $C_{418}H_{728}N_6O_{194}$ Calcd: C, 55.97; H, 8.25 N, 3.04. Found: C, 56.07; H, 8.26 N, 3.29.

### 4.2.7. Synthesis of compound [1:1] [G3]8OR[PEG6K] 8

Synthesized using the same procedure using [G3]8OH[PEG6K] (100 mg, 0.012 mmol), pyridine (0.2 mL) and DMAP (1.2 mg, 0.012 mmol), dry $CH_2Cl_2$ (8 mL), methacrylic anhydride (16 µL, 0.100 mmol), 4-pentynoic anhydride (18.3 mg, 0.092 mmol). Yielding product as a yellowish viscous liquid (93 mg, 81 %). $^1H$ NMR ($CDCl_3$, δ, ppm) 7.81 (s, 2H), 6.07 (s, 4H), 5.56 (s, 4H), 5.23 (s, 4H), 4.54 (t, 4H, *J* = 5 Hz), 4.25-4.18 (m, 56H), 3.85 (t, 2H, *J* = 5 Hz), 3.80-3.42 (m, 540H), 2.55 (t, 16H, *J*=6.7 Hz), 2.45 (t, 16H, *J*=6.4 Hz), 1.98 (s, 12H), 1.92 (s, 18H), 1.6 (s, 40H), 1.24 (s, 24H), 1.22 (s, 6H). FTIR ($cm^{-1}$): 3280, 2879, 1737. $C_{420}H_{728}N_6O_{194}$ Calcd: C, 53.97; H, 8.25 N, 1.94. Found: C, 53.63; H, 8.28 N, 1.81.

### 4.2.8. Synthesis of compound [1:2] [G3]8OR[PEG6K] 9

Synthesized using [G3]8OH[PEG6K] (100 mg, 0.012 mmol), pyridine (0.2 mL) and DMAP (1.2 mg, 0.012 mmol), dry $CH_2Cl_2$ (8 mL), methacrylic anhydride (11.5 µL, 0.072 mmol), 4-pentynoic anhydride (22.85 mg, 0.116 mmol). Yielding product as a yellowish viscous liquid (95 mg, 82 %). $^1H$ NMR ($CDCl_3$, δ, ppm) 7.82 (s, 2H), 6.08 (s, 3H), 5.58 (s, 3H), 5.23 (s, 7H), 4.54 (t, 8H, *J* = 5 Hz), 4.25-4.18 (m, 56H), 3.85 (t, 2H, *J* = 5.12 Hz), 3.80-3.42 (m, 540H), 2.58 (t, 20H, *J*=6.1 Hz), 2.44 (t, 20H, *J*=6.5 Hz), 1.98 (s, 14H), 1.92

(s, 20 H), 1.6 (s, 40H), 1.24 (s, 24H), 1.22 (s, 6H). FTIR ($cm^{-1}$): 3261, 2877, 1734. $C_{422}H_{728}N_6O_{194}$ Calcd: C, 52.97; H, 8.25 N, 1.94. Found: C, 52.84; H, 8.26 N, 2.02.

### 4.2.9. Synthesis of compound [1:3] [G3]8OR[PEG6K] 10

Synthesized using [G3]8OH[PEG6K] (100 mg, 0.012 mmol), pyridine (0.2mL) and DMAP (1,2 mg, 0.012 mmol), dry $CH_2Cl_2$ (8 mL), methacrylic anhydride (7.68 µL, 0.051 mmol), 4-pentynoic anhydride (27.4 mg, 0.154 mmol). Yielding product as a yellowish viscous liquid (98 mg, 83 %). $^1$H NMR ($CDCl_3$, δ, ppm) 7.80 (s, 2H), 6.06 (s, 2H), 5.60 (s, 2H), 5.23 (s, 4H), 4.54 (t, 4H, *J* = 5 Hz), 4.25-4.18 (m, 56H), 3.85 (t, 2H, *J* = 5.1 Hz), 3.80-3.42 (m, 540H), 2.59 (t, 24H, *J*=6.8 Hz), 2.43 (t, 24H, *J*=6.7 Hz), 1.98 (s, 18H), 1.92 (s, 14 H), 1.6 (s, 40H), 1.24 (s, 24H), 1.22 (s, 6H). FTIR ($cm^{-1}$): 3280, 2880, 1736, 1658. $C_{424}H_{728}N_6O_{194}$ Calcd: C, 56.97; H, 8.85 N, 2.04. Found: C, 56.87; H, 9.06 N, 2.21.

### 4.2.10. Synthesis of compound [G3]8OH[PEG2K] 11

PEG-2K-Diazide (105 mg, 0.05 mmol) and propargyl [G3]8[OH] (95 mg, 0.11 mmol) were dissolved in dry THF (4 mL). In a separate flask were dissolved CuBr (1.5 mg, 0.0108 mmol) and N,N,N',N'',N'' Pentamethyldiethylenetriamine (PMDETA, 2.5 µL, 0.0125 mmol) in dry THF (1 mL) and purged with $N_2$. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 30 $^o$C for 24 h. The solution is diluted with THF (10 ml) and column with aluminium oxide ($Al_2O_3$, 4 cm in small pipette) to remove copper. The solvent was evaporated under *vacuo* and the desired product was precipitated with diethyl ether after dissolving in THF (3 mL) , filtered and dried under *vacuo* yielding a brownish viscous compound (145 mg, 73 %) $^1$H NMR ($CDCl_3$, δ, ppm) 7.87 (s, 2H), 5.22 (s, 4H), 4.5 (t, 4H, *J*=3.97), 4.21-4.18 (m, 28H), 3.74 (d, 14 H), 3.67 (d, 18H), 3.63 (s, 16H), 3.6-3.55 (m, 140H), 3.42 (s, 10H), 2.1 (s, 8H), 1.97 (s, 4H), 1.2-1.18 (m, 18H), 1.01 (s, 20H). FTIR ($cm^{-1}$): 3390, 2878, 1731. $C_{170}H_{308}N_6O_{90}$ Calcd: C, 49.67; H, 8.16 N, 3.98. Found: C, 49.81; H, 7.90 N, 4.02.

### 4.2.11. Synthesis of compound [1:1] [G3]8OR[PEG2K] 12

[G3]8OH[PEG2K] (85 mg, 0.029 mmol), pyridine (0.2 mL) and DMAP (1.0 mg, 0.008 mmol) dissolved in dry $CH_2Cl_2$ (5 mL) in a round bottom flask. To the stirring reaction

micture was added methacrylic anhydride (26.9 µL, 0.183 mmol) and continued stirring for 24 h at 40 $^{o}$C under $N_2$. Then, 4-pentynoic anhydride (32.1 mg, 0.183 mmol) was added to the reaction mixture and continued stirring for another 24h at 30 $^{o}$C under $N_2$. Crude product was purified by precipitation in diethyl ether to give a yellowish solid (95 mg, 94 %). $^1$H NMR ($CDCl_3$, δ, ppm) 7.85 (s, 2H), 6.05 (s, 4H), 5.56 (s, 4H), 5.23 (s, 4H), 4.53 (s, 4H), 4.28-4.20 (m, 36H), 3.86 (t, 4H, *J*=4.67 Hz), 3.81-3.78 (m, 150H), 3.44 (t, 4H, *J*=4.82 Hz), 3.37 (t, 4H, *J*=5.03), 3.22 (s, 4H), 2.54 (t, 4H, *J*=6.42 Hz), 2.47 (t, 4H, *J*=5.95 Hz), 1.96 (s, 8H), 1.9 (s, 20H), 1.27 (s, 10H), 1.24 (s, 24H), 1.19 (s, 18H). FTIR (cm$^{-1}$): 3264, 2877, 1737, 1673. $C_{242}H_{372}N_6O_{105}$ Calcd: C, 53.08; H, 8.16 N, 2.44. Found: C, 53.43; H, 7.84 N, 2.12.

### 4.2.12. Synthesis of compound [G3]8OH[PEG4K] 13

PEG-4K-Diazide (200 mg, 0.049 mmol) and propargyl [G3]8[OH] (100 mg, 0.115 mmol) were dissolved in dry THF (6 mL). In a separate flask were dissolved CuBr (1.4 mg, 0.009 mmol) and N,N,N',N",N" Pentamethyldiethylenetriamine (PMDETA, 1.31 µL, 0.009 mmol) in dry THF (1 mL) and purged with $N_2$. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at 30 $^{o}$C for 24 h. The solution is diluted with THF (10 ml) and column with aluminium oxide ($Al_2O_3$, 4 cm in small pipette) to remove copper. The solvent was evaporated under *vacuo* and the desired product was precipitated with diethyl ether after dissolving in THF (3 mL) , filtered and dried under *vacuo* yielding a brownish viscous compound (268 mg, 89%) $^1$H NMR ($CDCl_3$, δ, ppm) 7.9 (s, 2H), 5.26 (s, 4H), 4.54 (t, 8H, *J*=4.13 Hz), 4.29-4.20 (m, 42H), 3.85 (t, 8H, *J*=5 Hz), 3.79-3.76 (m, 34H), 3.7 (s, 22H), 3.64-3.6 (m, 360H), 3.5 (s, 12H), 3.44 (t, 18H, *J*=5.07 Hz), 3.38 (t, 18H, *J*=5.18 Hz), 2.38 (s, 10H), 2.3 (s, 10H), 2.15 (s, 6H), 1.23 (s, 68H), 1.06 (s, 46H). FTIR (cm$^{-1}$): 3408, 2883, 1735. $C_{258}H_{484}N_6O_{134}$ Calcd: C, 52.97; H, 8.25 N, 1.34. Found: C, 53.02; H, 8.54 N, 1.09.

### 4.2.13. Synthesis of compound [1:1] [G3]8OR[PEG4K] 14

Synthesized by the same procedure as compound (11) via using [G3]8OH[PEG4K] (225 mg, 0.038 mmol), pyridine (0.3mL) and DMAP (5.0 mg, 0.04 mmol), dry $CH_2Cl_2$ (7 mL), methacrylic anhydride (46,1 µL, 0.311 mmol), 4-pentanoic anhydride (48.3 mg, 0.311

mmol) yielding product as a white solid (215 mg, 86 %). $^1$H NMR ($CDCl_3$, δ, ppm) 7.8 (s, 2H), 6.04 (s, 8H), 5.56 (s, 8H), 5.23 (s, 4H), 4.53 (t, 4H, *J*=5 Hz), 4.28-4.19 (m, 60H), 3.86 (s, 4H), 3.79 (s, 3H), 3.74-4.54 (m, 360H), 3.44 (s, 4H), 3.22 (d, 8H), 2.54 (t, 12H, *J*=6.65 Hz), 2.46 (t, 12H, *J*=6.3 Hz), 1.96 (s, 10H), 1.89 (s, 24H), 1.27-1.17 (m, 48H). FTIR ($cm^{-1}$): 3276, 2883, 1764, 1644. $C_{330}H_{548}N_6O_{149}$ Calcd: C, 54.97; H, 8.25 N, 1.94. Found: C, 55.32; H, 8.34 N, 1.69.

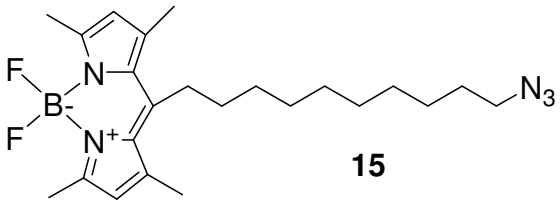
### 4.3. General Synthesis of Hydrogels via Photocrosslinking Chemistry

Formation of hydrogel photocrosslinking was achieved according to literature. Poly(ethyleneglycol)-bis-oktaacetylene **7** (20 mg, 2.52 μmol), was dissolved in 1:1 EtOH:$H_2O$ mixture (0.05 mL). To another vial was added 2-2-Dimethoxy-2-phenylacetophenone (99 %) (3 mg, 0.012 mmol) and was dissolved in N-vinyl prylollidone (20 μL). This solution was mixed with gel precursor under ultrasound to give a clear solution. The final mixture was cured under UV light (475 nm) for 5 minutes. The crosslinked material was washed with MeOH and the vial was broken to let the hyrogel out. To remove the solvents and unreacted chemicals, hyrogels were washed with MeOH for 2 hours and finally was with deionized water. The hydrogel sample was quickly frozen and further freeze-dried under *vacuo* until the solvent removed, yielding **6KG3$_{(1:1)}$** (14.2 mg, 84% gel content).

### 4.4. Functionalisation of Hydrogels

#### 4.4.1 Functionalization with BODIPY azide

Two identical cylindrically shaped hydrogels were synthesized (each about 20 mg) and placed into two different vials. Bodipy azide (0.25 mg, 0.00058 mmol), (Figure 4.5) in THF (2.5 mL) was added to each of the vials. PMDETA (1.20 μL, 0.0058 mmol) and CuBr (1.00 mg, 0.007 mmol) were added to vial #1 for copper catalyzed cycloaddition. Vial#2 was left without catalyst addition to control the nonspecific adsorptions of the hydrogels. The reactions were stirred at room temperature for 12 h. After the reaction was completed hydrogels were washed with EtOH to remove any trapped dye molecules and then washed with an aqueous EDTA solution (5%, pH ~7-8) to extract the trapped Cu(I)Br and finally washed with deionized water.

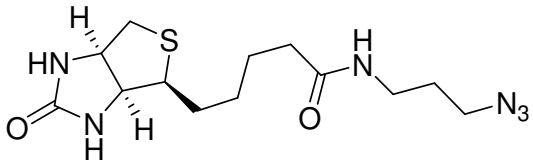



**15**

Figure 4.5. Chemical structure of BODIPY azide

### 4.4.2 Functionalization with Biotin azide

Similarly, two identical cylindrically shaped hydrogels were synthesized (20 mg) and placed into two different vials and reacted with biotin azide (1 mg, 3.3 μmol), (Figure 4.6) in the presence of $CuSO_4$( 1mg, 0.0063 mmole) and sodium ascorbate (1 mg, 0,005 mmole) for 12h. As a control the same hydrogel is treated with biotin azide in the absence of $CuSO_4$ and sodium ascorbate. After the reaction completed at 30 $^oC$, the gel was first transferred to a pH ~7-8 EDTA water solution (5%) to extract the trapped $CuSO_4$ and finally washed with pure deionized water. Thereafter, hydrogels are incubated with FITC labeled streptavidin (0.1 mg/ml of PBS buffer, pH 7.4) for 15 minutes. After incubation, hydrogels are kept in water for another 15 minutes, washed with water several times.




**16**

Figure 4.6. Chemical structure of Biotin azide

## 4.5. Microcontact Molding of Hydrogels

### 4.5.1. PDMS Molding

Poly(ethyleneglycol)-bis-oktaacetylene **7** (20 mg, 2.52 mol), was dissolved in 1:1 EtOH:$H_2O$ mixture (0.05 mL). To another vial was added 2-2-Dimethoxy-2-phenylacetophenone (99 %) (3 mg, 0.012 mmol) and was dissolved in N-vinyl prylollidone (20 μL). This solution was mixed with gel precursor to give a clear solution. A silicon wafer functionalized by immersing the surface into 2% 3(trimethoxysilyl)propylacrylate

solution in ethanol/water (95/5) for 2 hours then by heating at 110 °C for 1 hour. Onto this silicon wafer a PDMS mold which has 60 to 30 micron size -polymer to channel- was put. The gel precursor was dropped from the edge of mold and let it move through the channels of mold. This system cured under UV (475 nm) for 5 minutes and waited for 12h. Finally the mold was removed and the hydrogel modified surface was washed with EtOH anf THF several times.

### 4.5.2. Spin Coating and Patterning via UV mask

A silicon wafer was functionalized with 2% 3(trimethoxysilyl)propylacrylate as in the PDMS molding method and the gel precursor that contains the photoinitiator was dropped. The surface was spin coated in 2000 rpm speed for 10 seconds. To avoid the solvent loss before crosslinking, the coated surface was immediately put on a UV mask. The system was cured under UV (475 nm) for 5 minutes. The mask removed and the surface washed with EtOH and THF several times to remove the polymer that was not crossliked on the silicon wafer surface.

## 4.6. Measurements

### 4.6.1. Scanning Electron Microscopy (SEM) Analysis

The hydrogel samples first swelled with distilled water and were frozen to be ready for lyophilization. The ones that are patterned on a silicon wafer were dried with $N_2$ gas. The dry hydrogels studied by a scanning microscope.

### 4.6.2. Phisical Property Analysis

The weight of the hydrogel dried under *vacuum* is recorded and water uptake taste is performed by recording the new weight of the gel in water in every 5 minutes. Measurements done until the equilibrium hydration degree was reached. Swelling ratio (W) was calculated by the Equation (1) in which $M_f$ is the weight in equilibrium and $M_i$ is the initial weight of the sample.

$$W = (M_f - M_i) / M_i \times 100 \qquad \text{Equation (1)}$$

# 5. CONCLUSIONS

Synthesis of functionalizable hydrogels by click chemistry is done by combining G2 and G3 polyester dendrons with PEG. Attachment of active groups like alkene and alkyne to the surface of these copolymers gives the property of multifunctionality. Photo initiated polymerization gives the hydrogels that are further functionalizable by click chemistry.

Characterizations of the molecules are reported and the claimed properties of hydrogels are proved by different methodologies.

Synthesized hydrogels are great candidates for biomedical applications, electronics and material science due to their physical and chemical properties.

# APPENDIX A: SPECTROSCOPY DATA

$^{1}$H NMR and FTIR spectra of the synthesized compounds are given below. Needed regions of NMR data were extended.

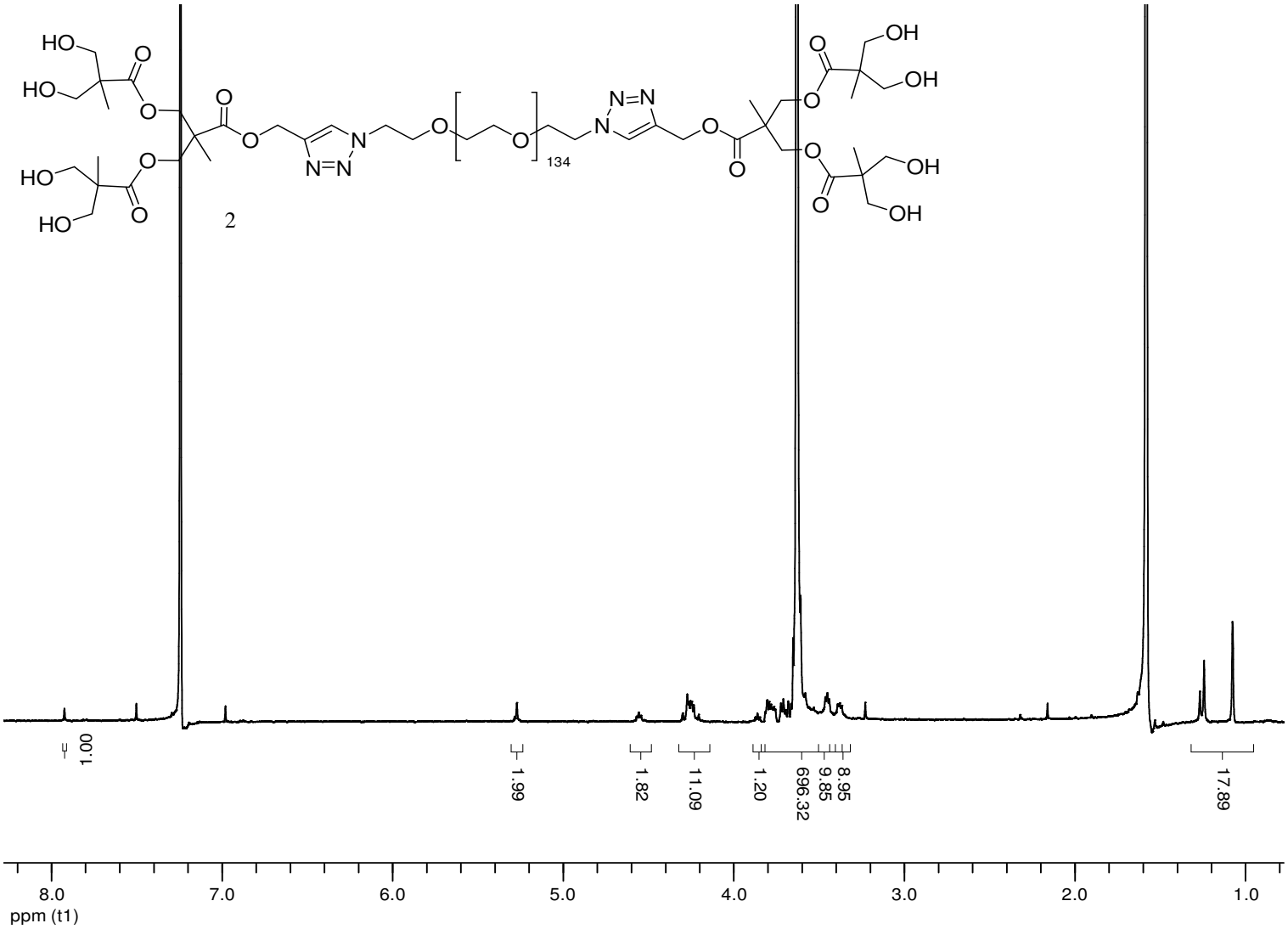



Figure A.1. $^{1}$H NMR spectrum of *[G2]4OH[PEG6K]* **2**

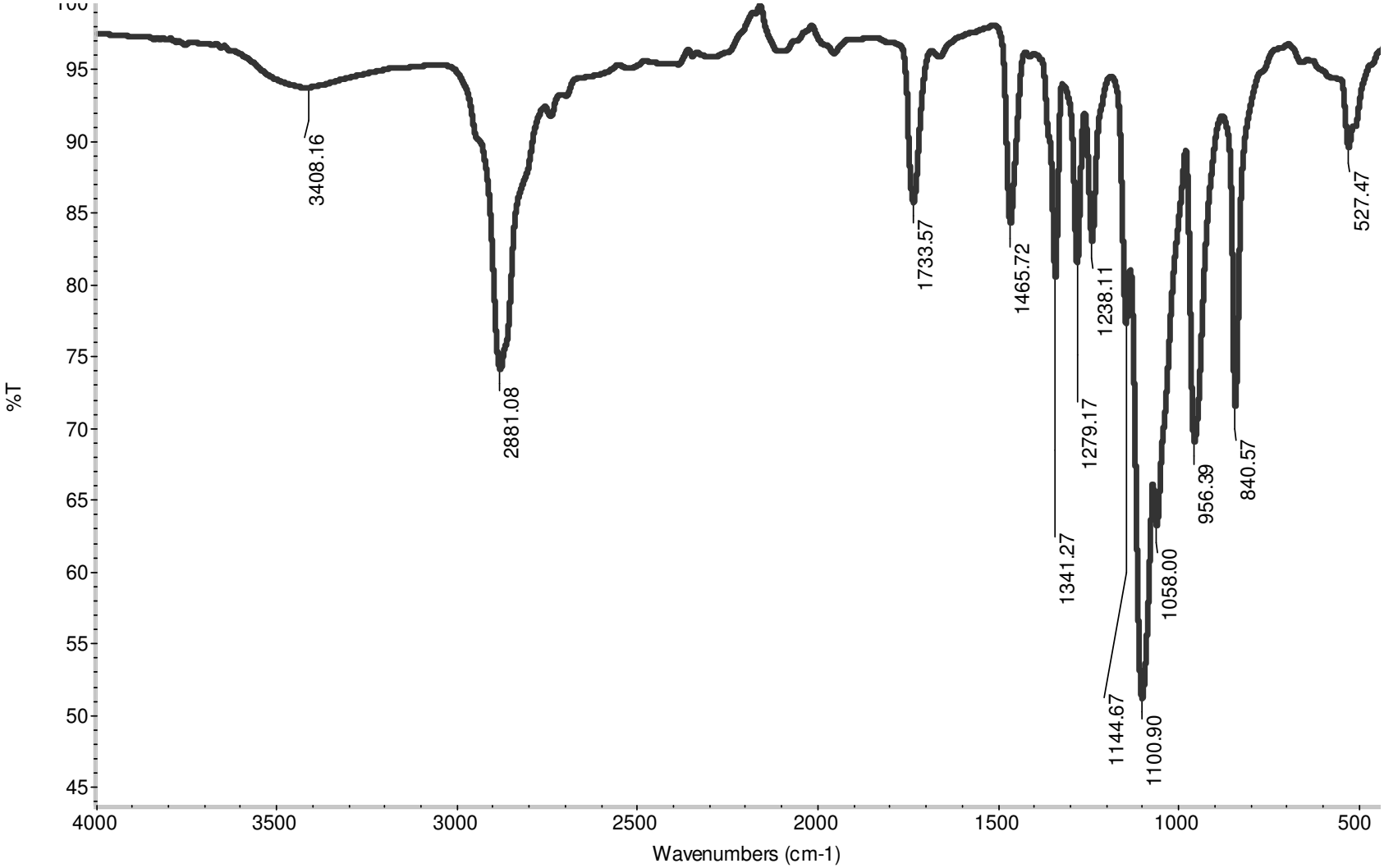



Figure A.2. IR spectrum of *[G2]4OH[PEG6K]* **2**

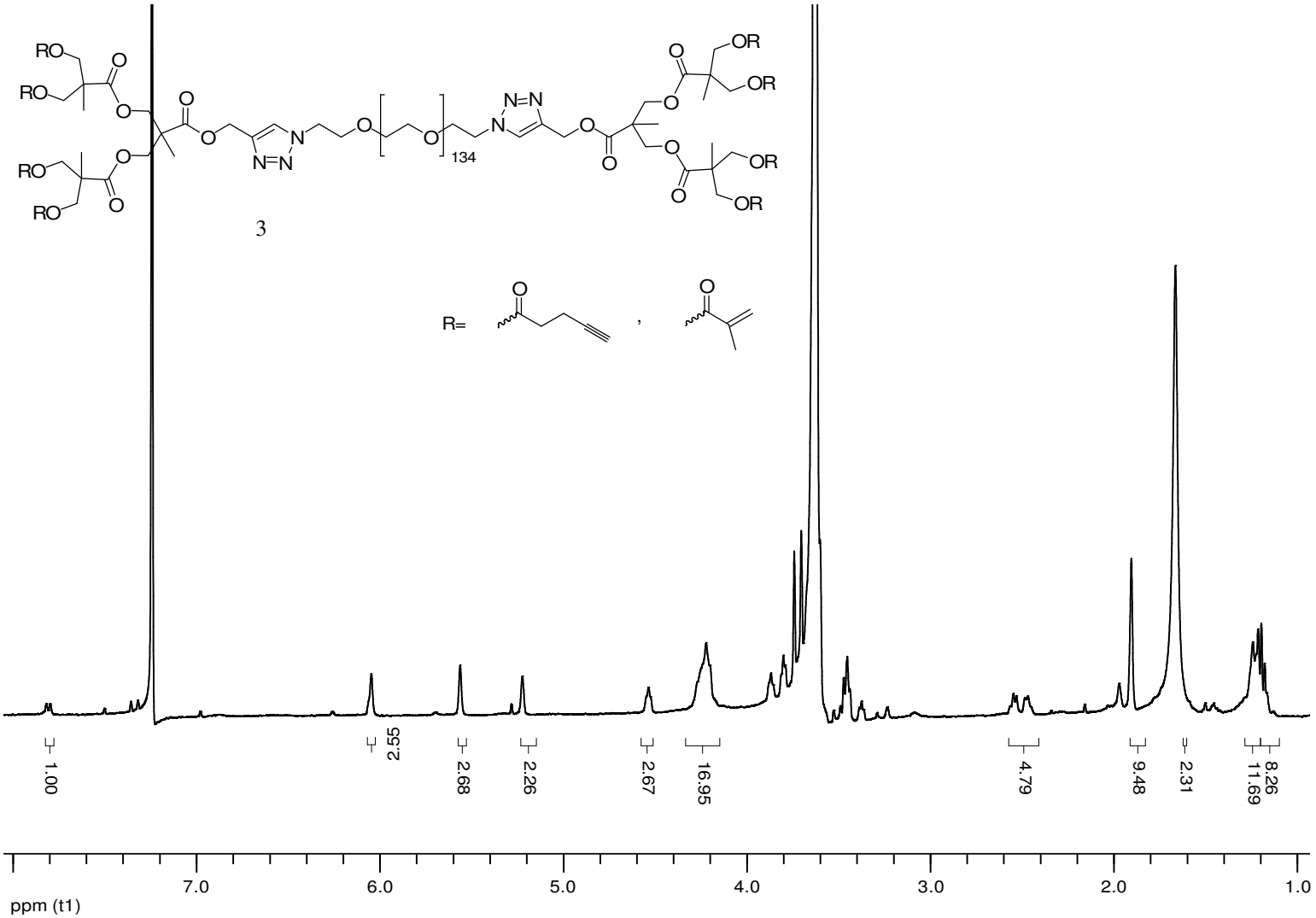



Figure A.3. $^{1}$H NMR spectrum of *[1:1] [G2]4OR[PEG6K]* **3**

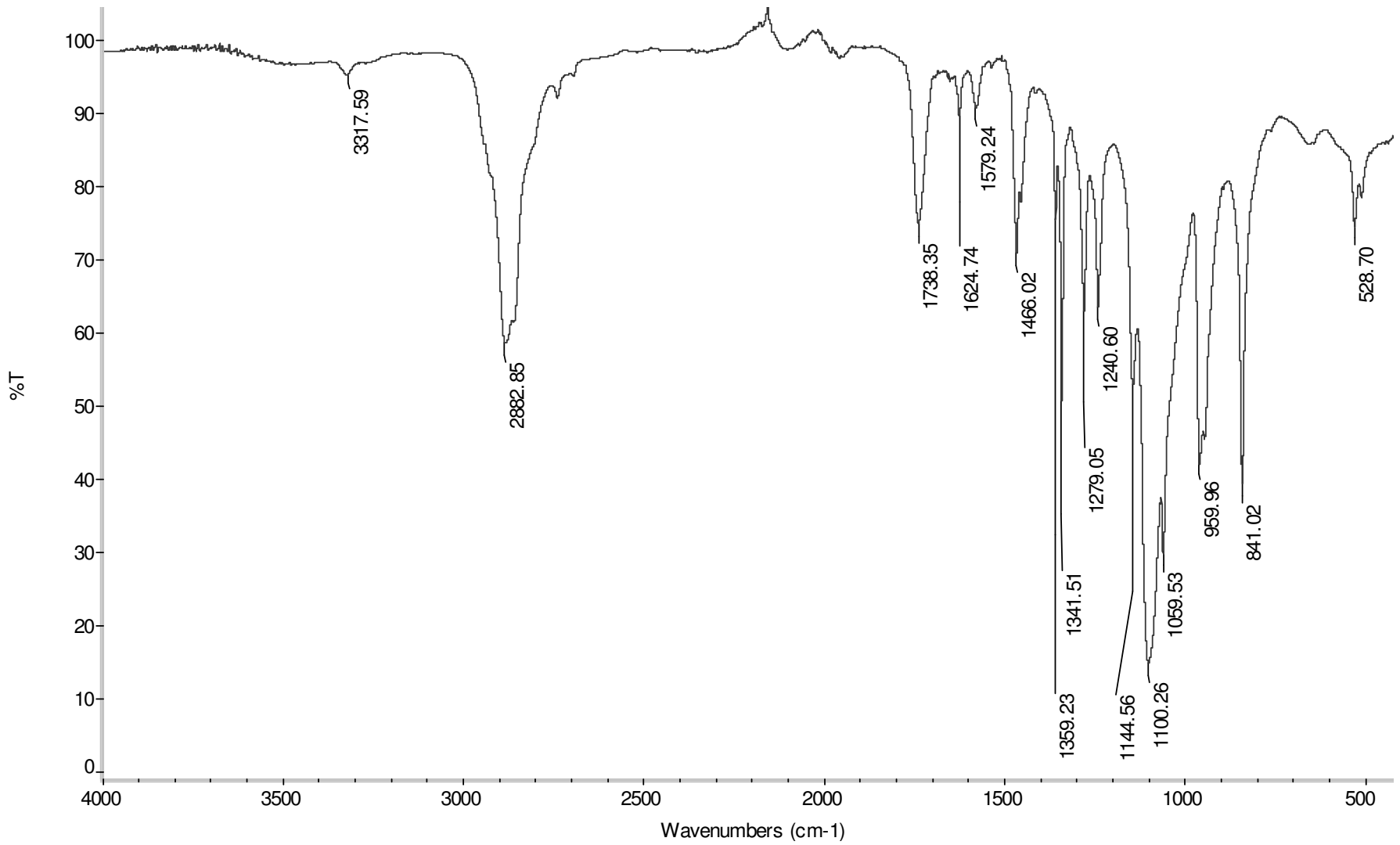



Figure A.4. IR spectrum of *[1:1] [G2]4OR[PEG6K]* **3**

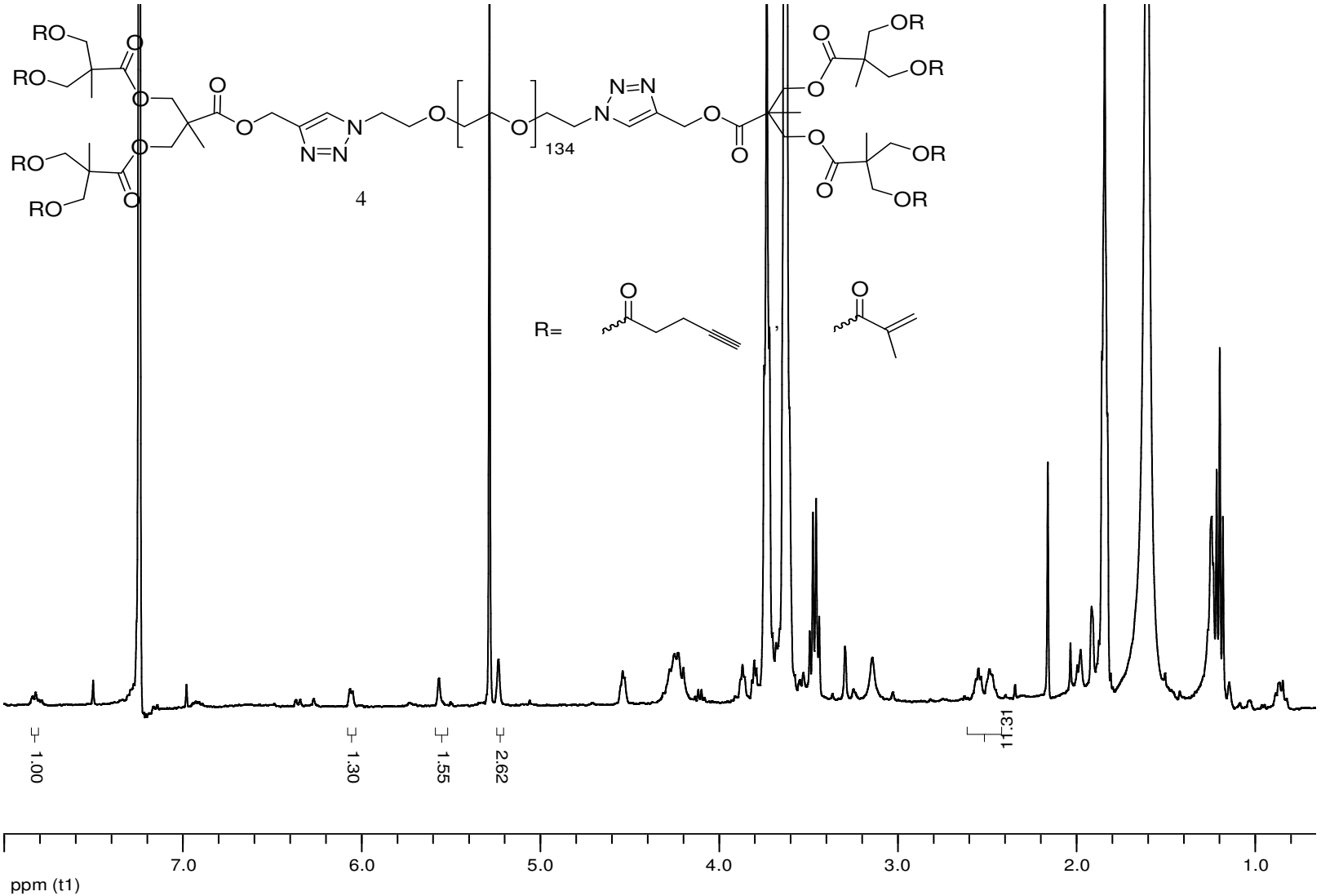



Figure A.5. $^{1}$H NMR spectrum of *[1:2] [G2]4OR[PEG6K]* **4**

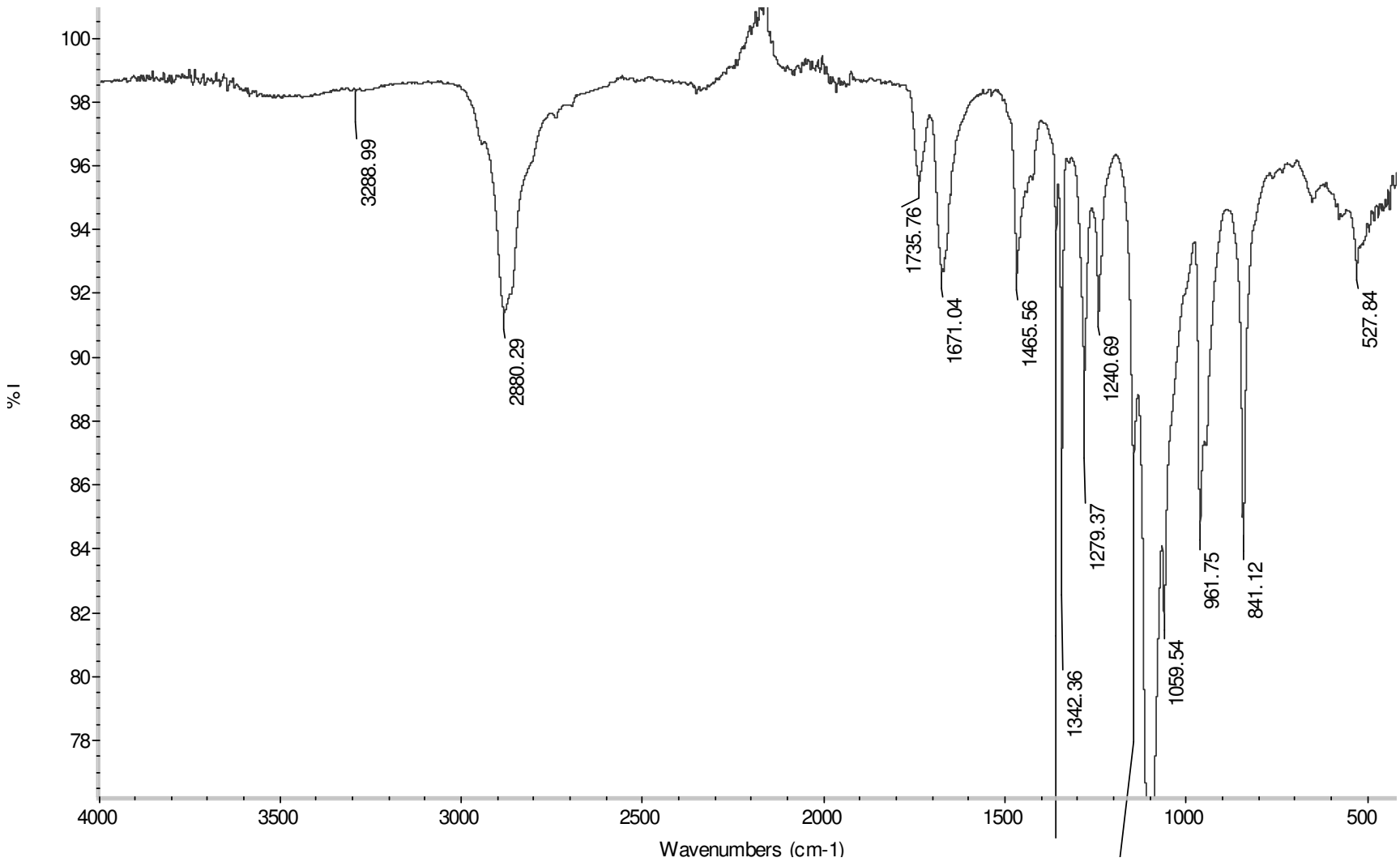



Figure A.6. IR spectrum of *[1:2] [G2]4OR[PEG6K]* **4**

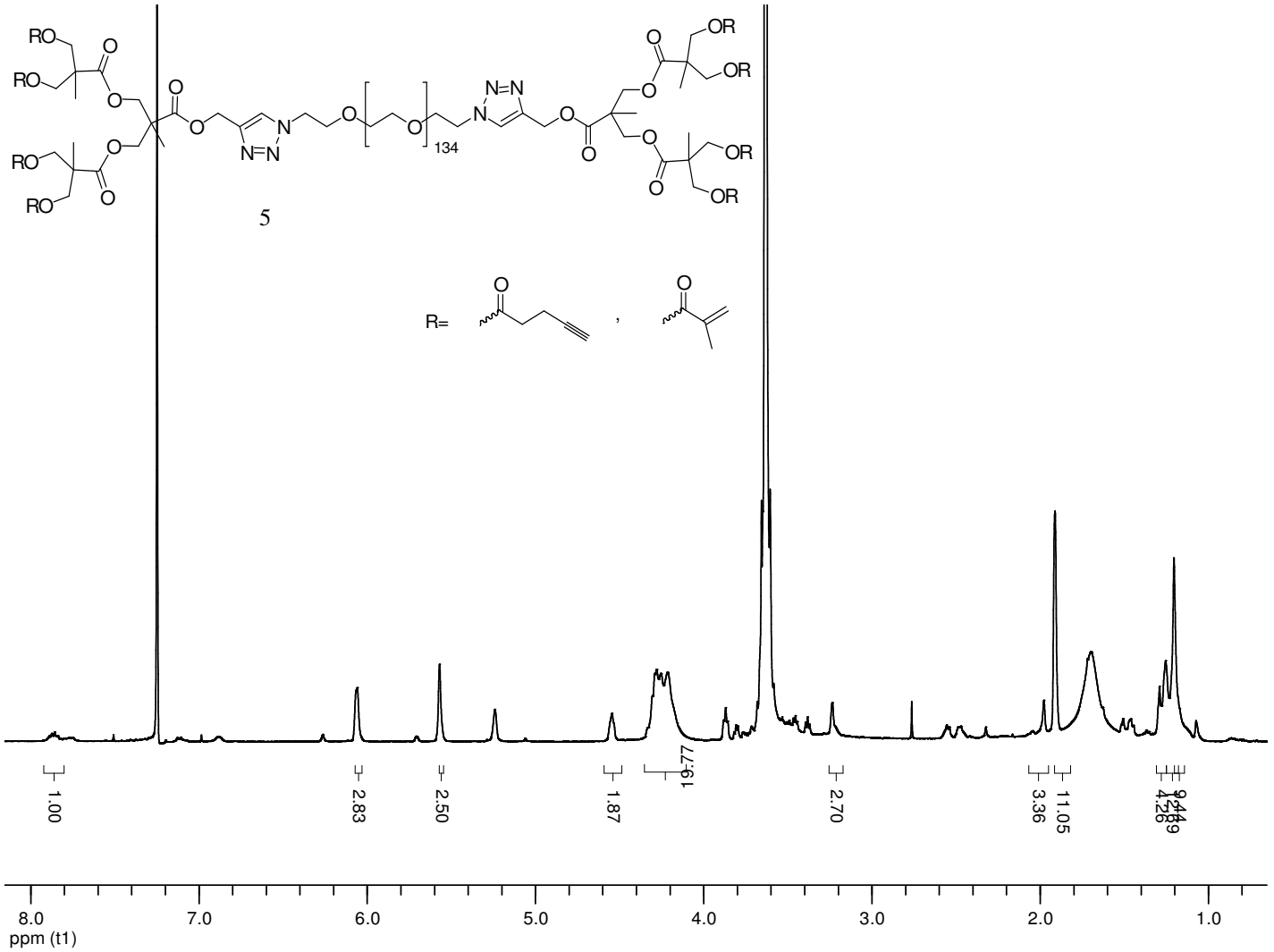



Figure A.7. $^{1}$H NMR spectrum of *[2:1] [G2]4OR[PEG6K]* **5**

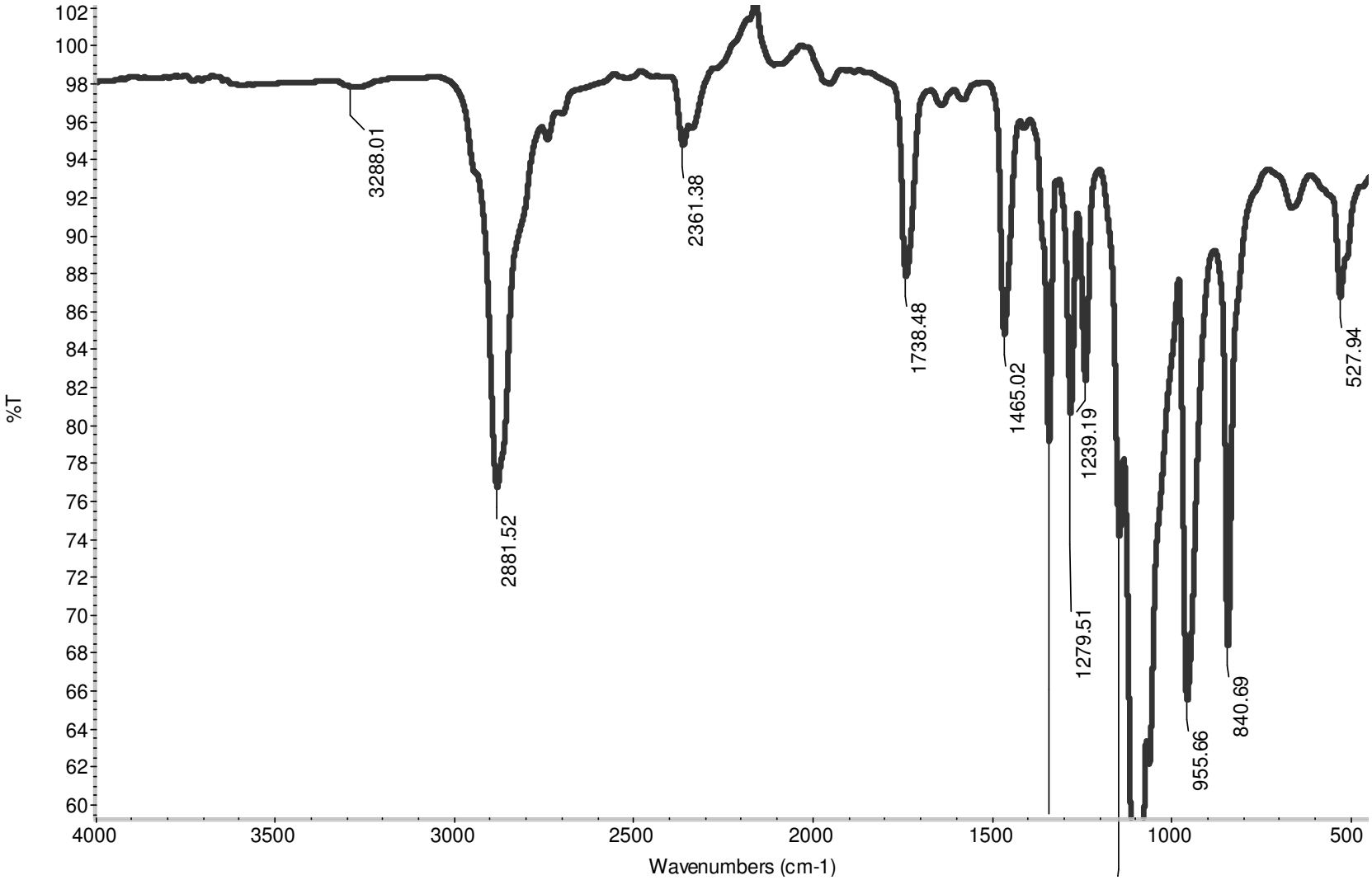



Figure A.8. IR spectrum of *[2:1] [G2]4OR[PEG6K]* **5**

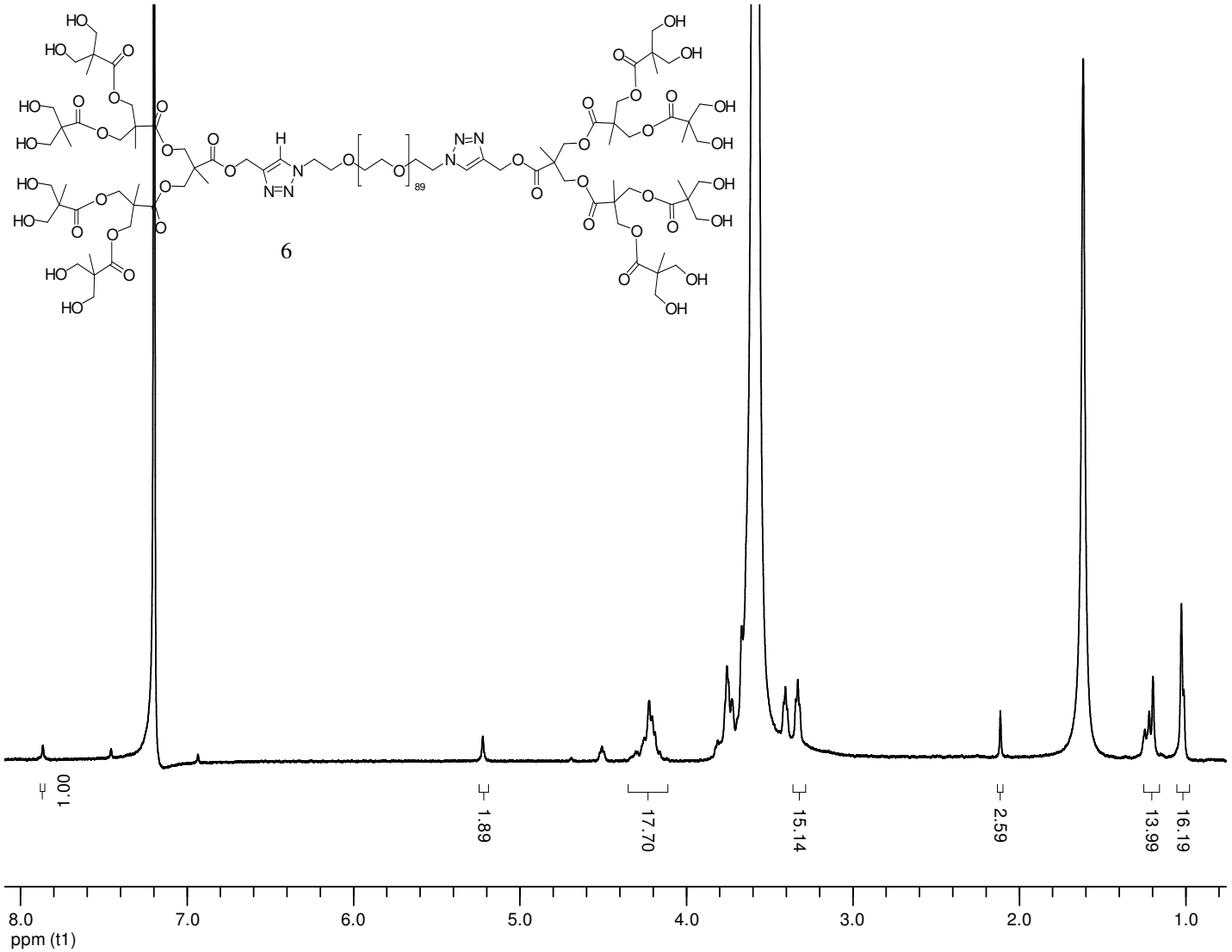



Figure A.9. $^{1}$H NMR spectrum of *[G3]8OH[PEG6K]* **6**

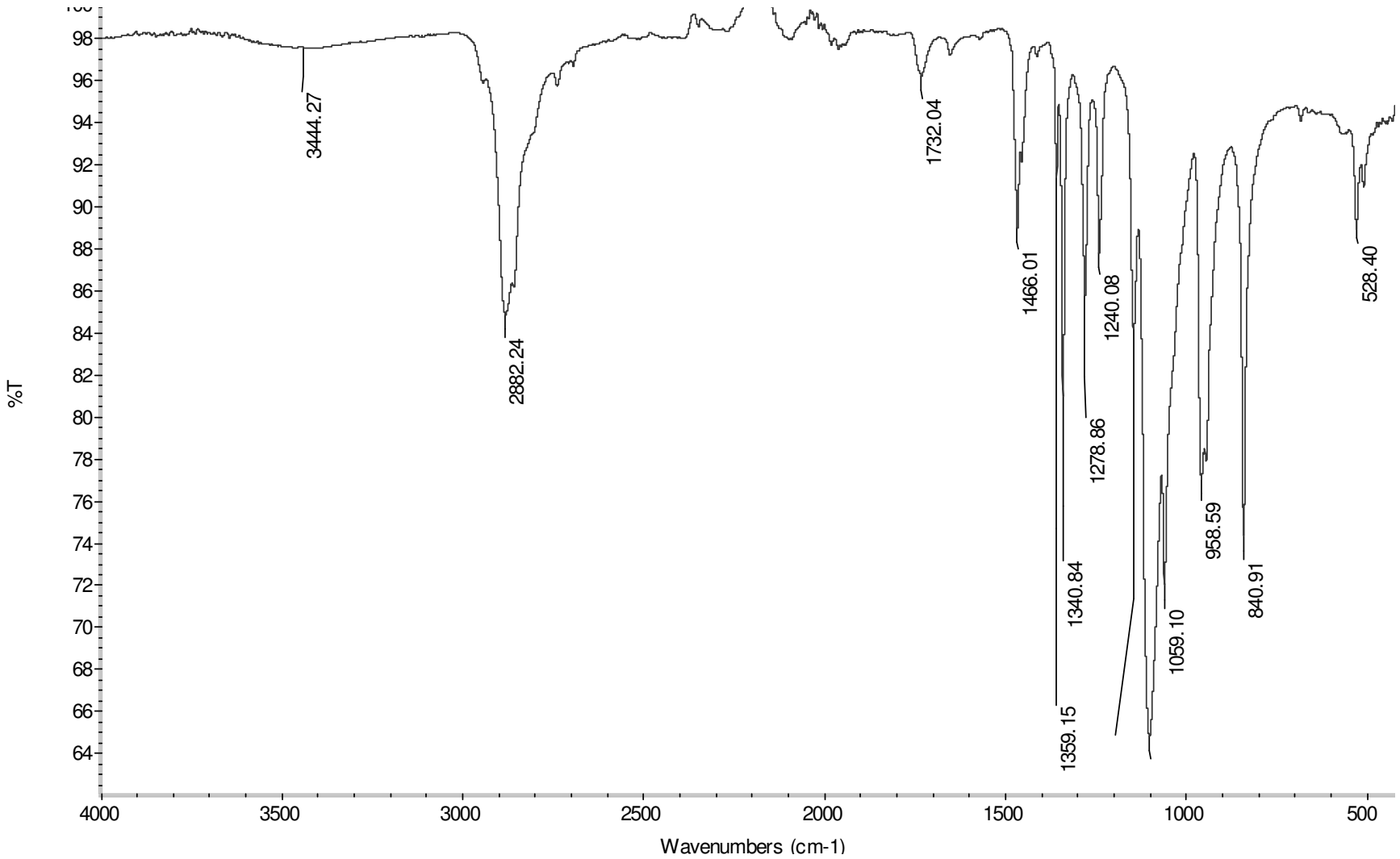



Figure A.10. IR spectrum of *[G3]8OH[PEG6K]* **6**

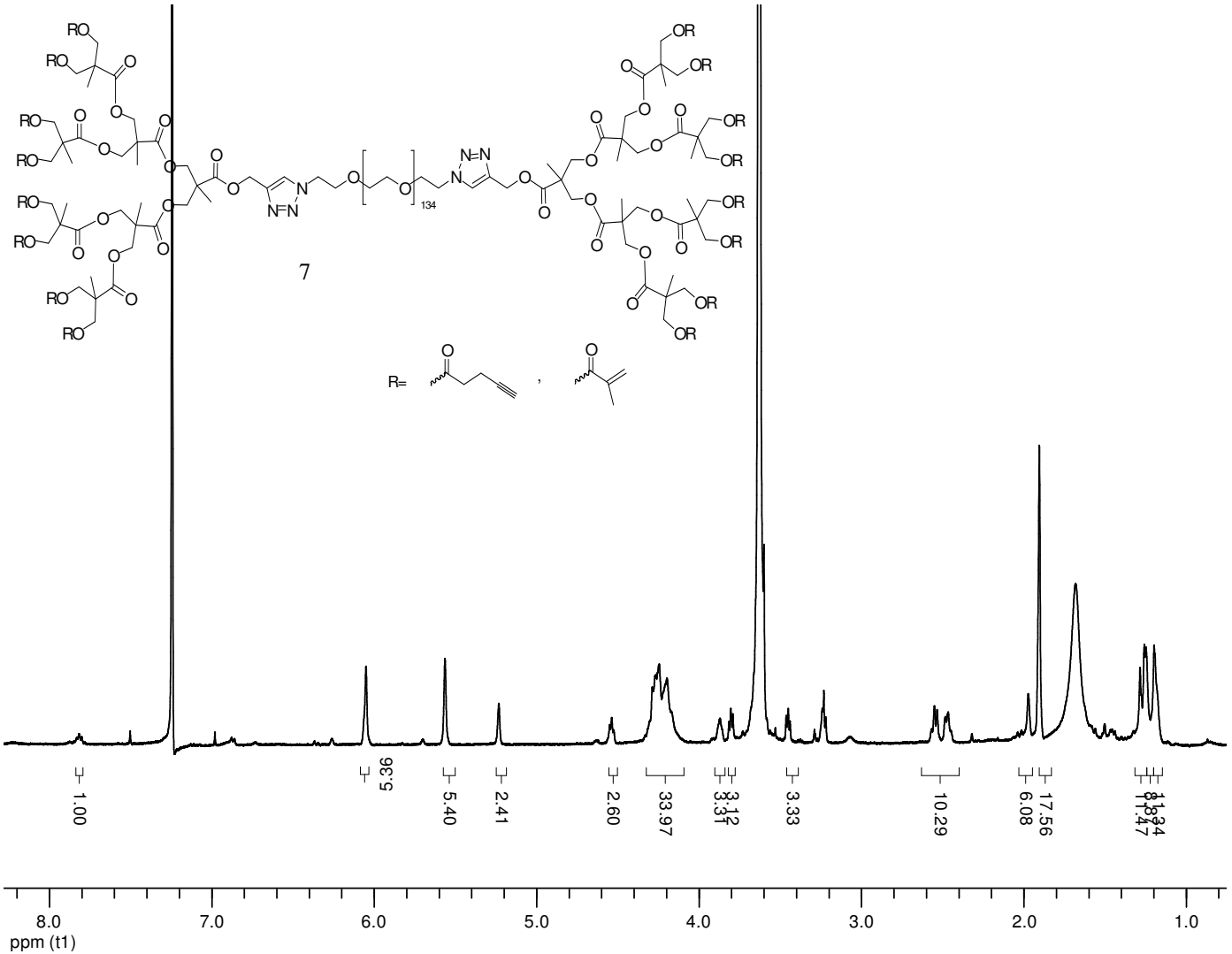



Figure A.11. $^{1}$H NMR spectrum of *[2:1] [G3]8OR[PEG6K]* **7**

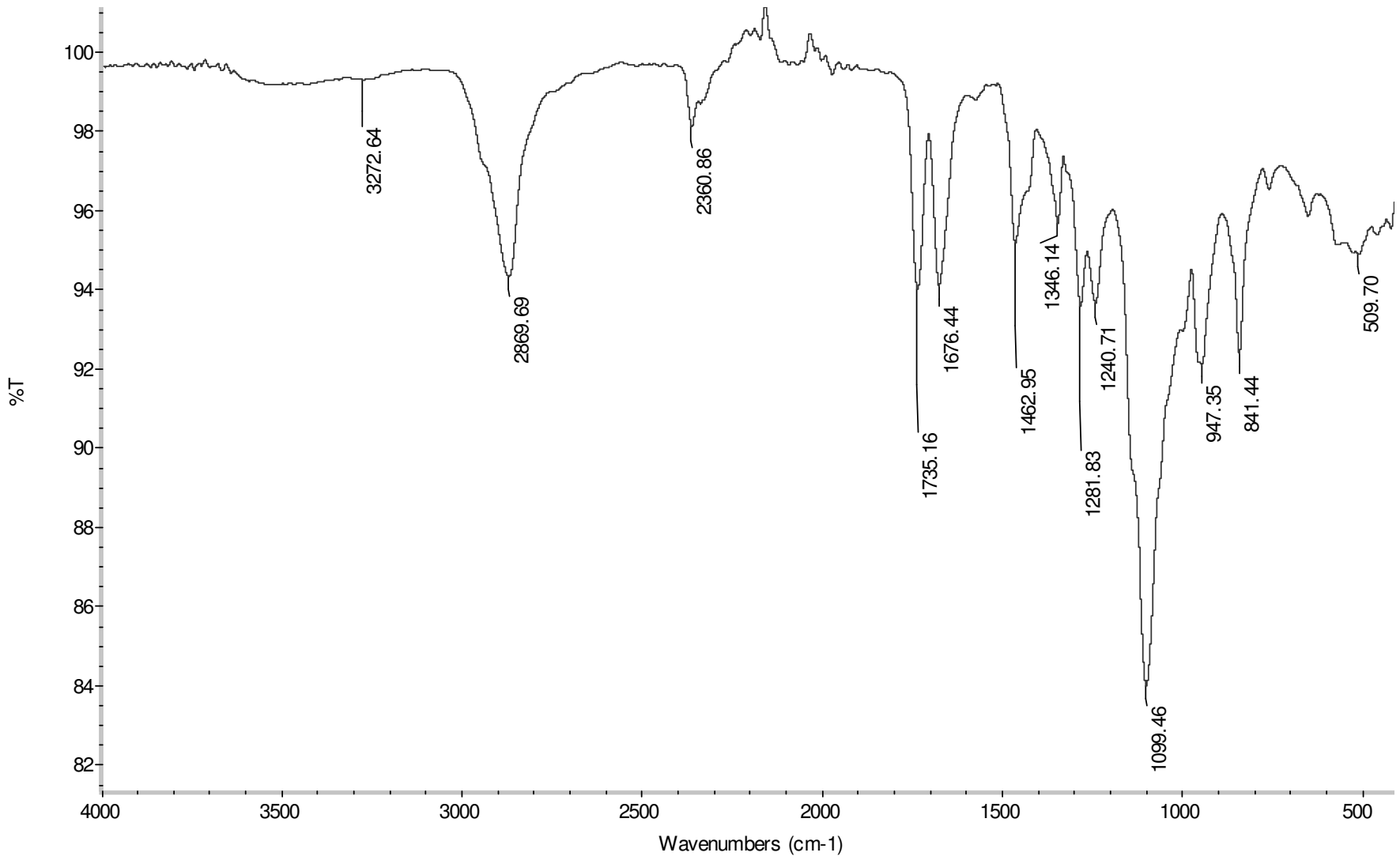



Figure A.12. IR spectrum of *[2:1] [G3]8OR[PEG6K]* **7**

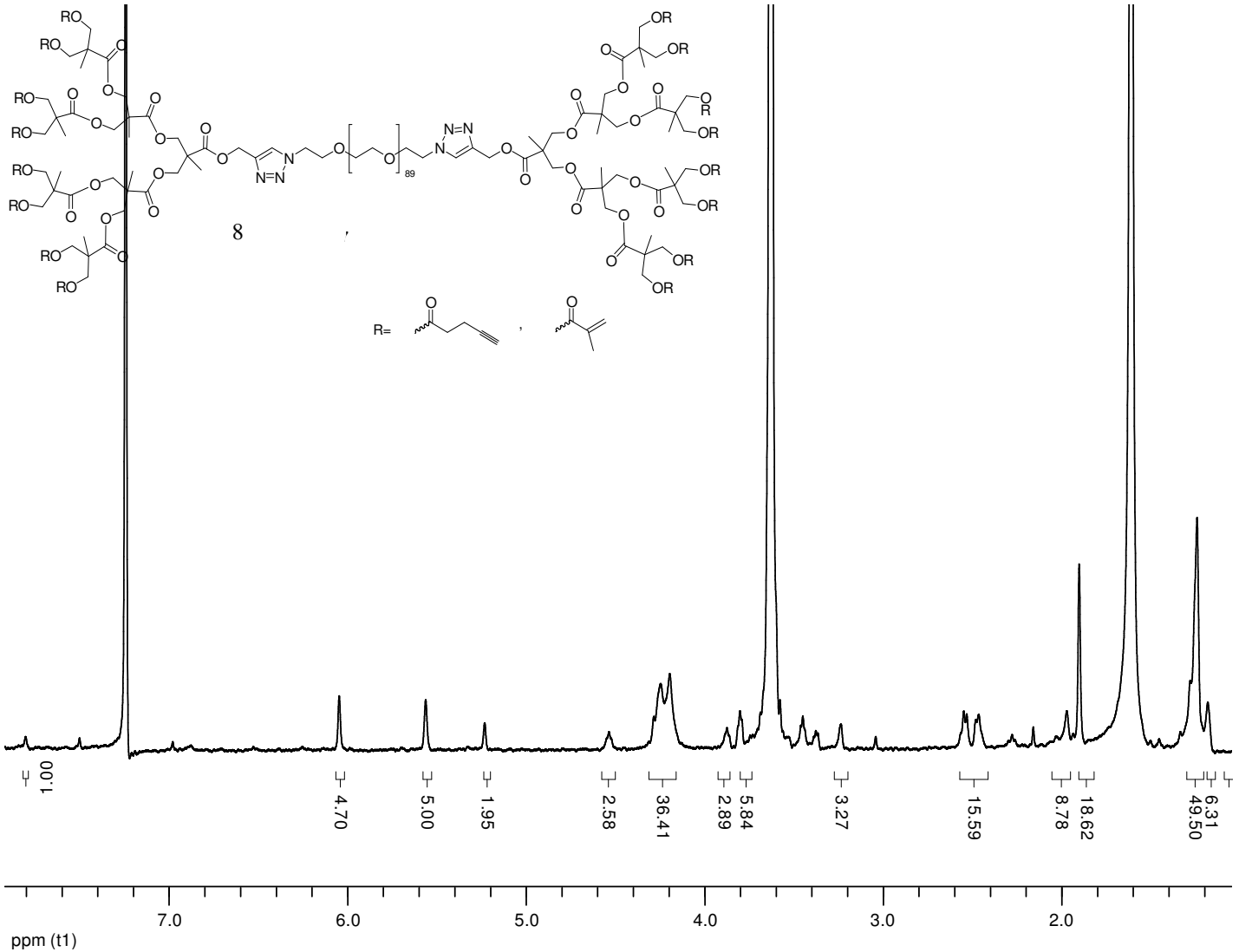



Figure A.13. $^{1}$H NMR spectrum of *[1:1] [G3]8OR[PEG6K]* **8**

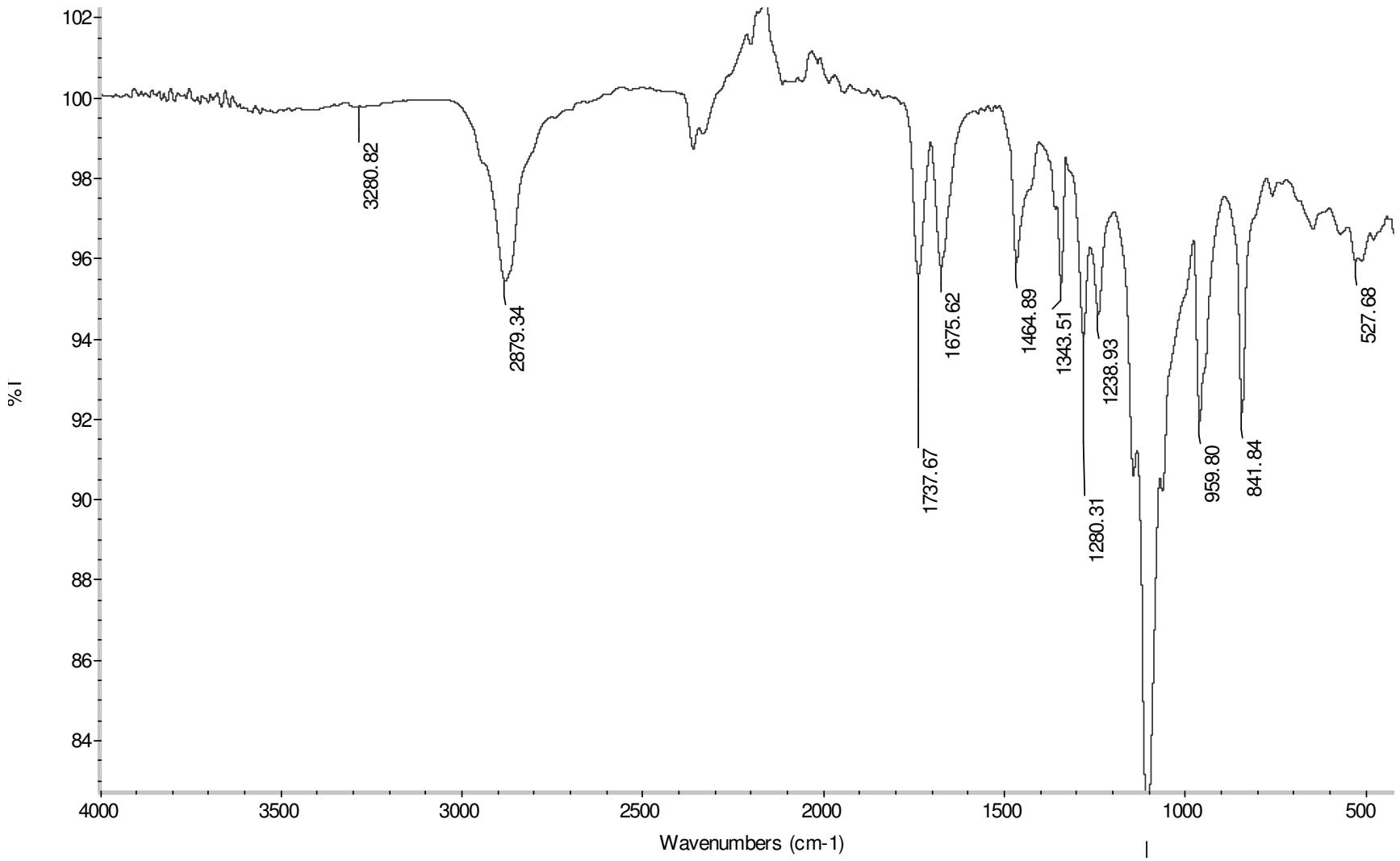



Figure A.14. IR spectrum of *[1:1] [G3]8OR[PEG6K]* **8**

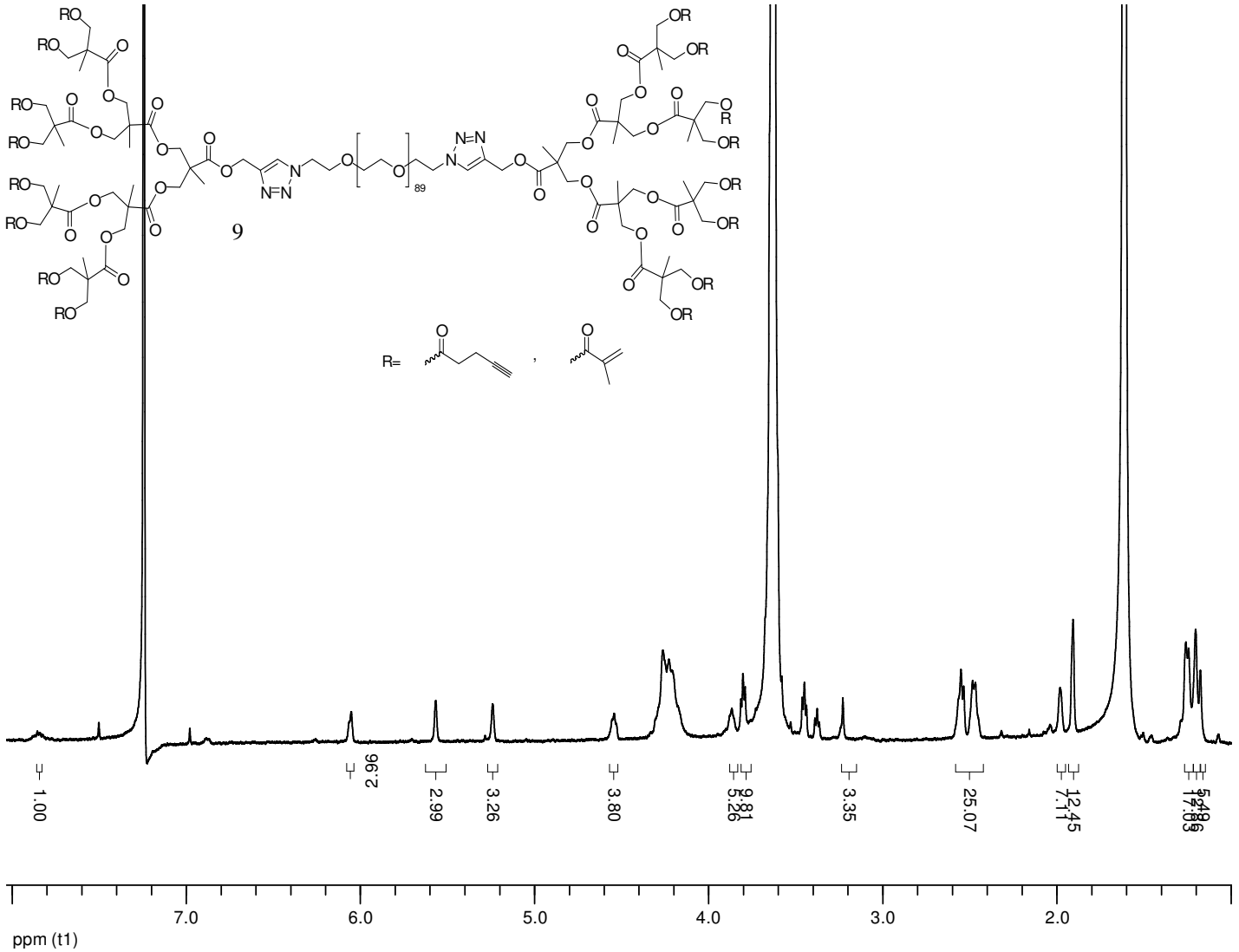



Figure A.15. $^{1}$H NMR spectrum of *[1:2] [G3]4OR[PEG6K]* **9**

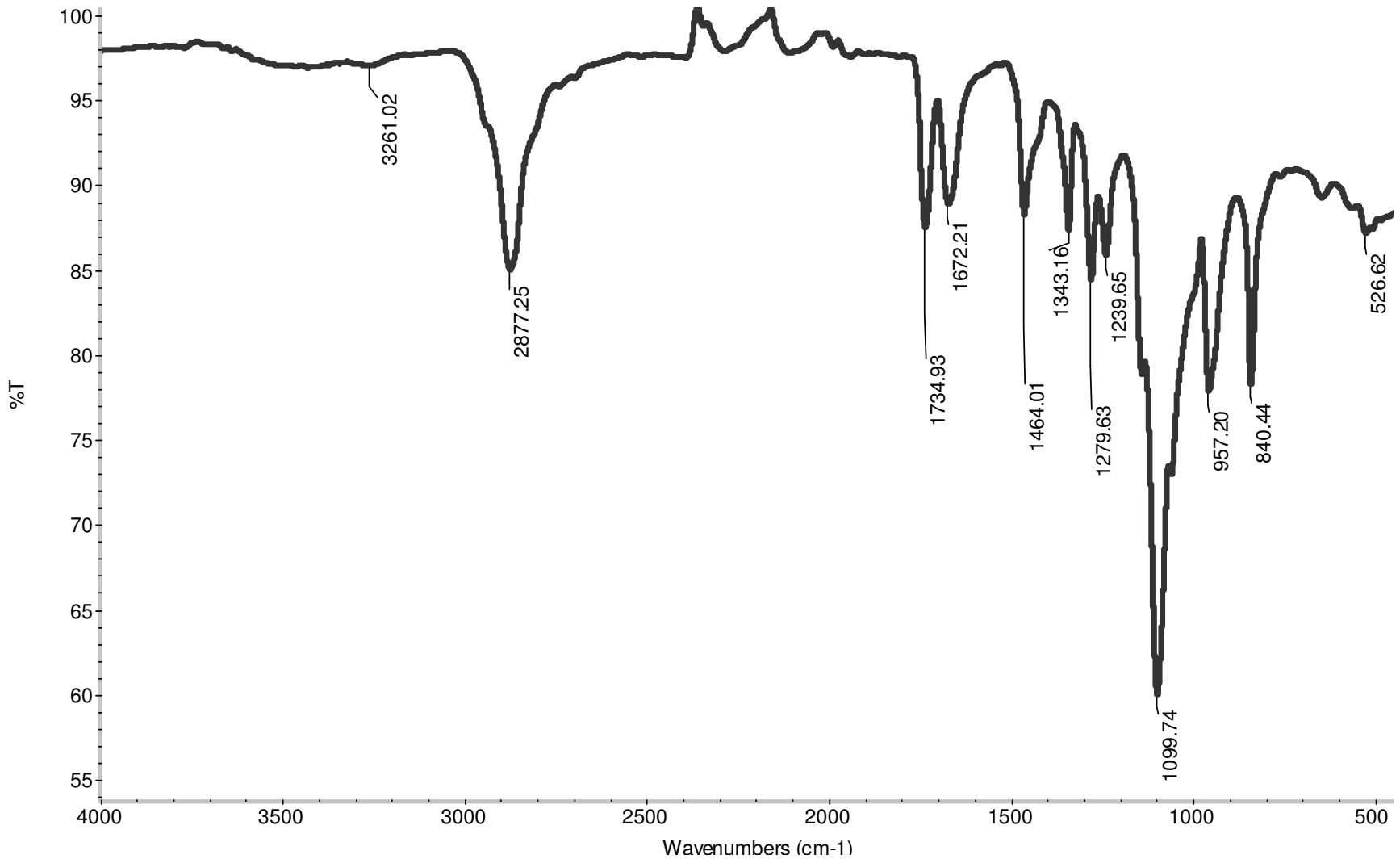



Figure A.16. IR spectrum of *[1:2] [G3]4OR[PEG6K]* **9**

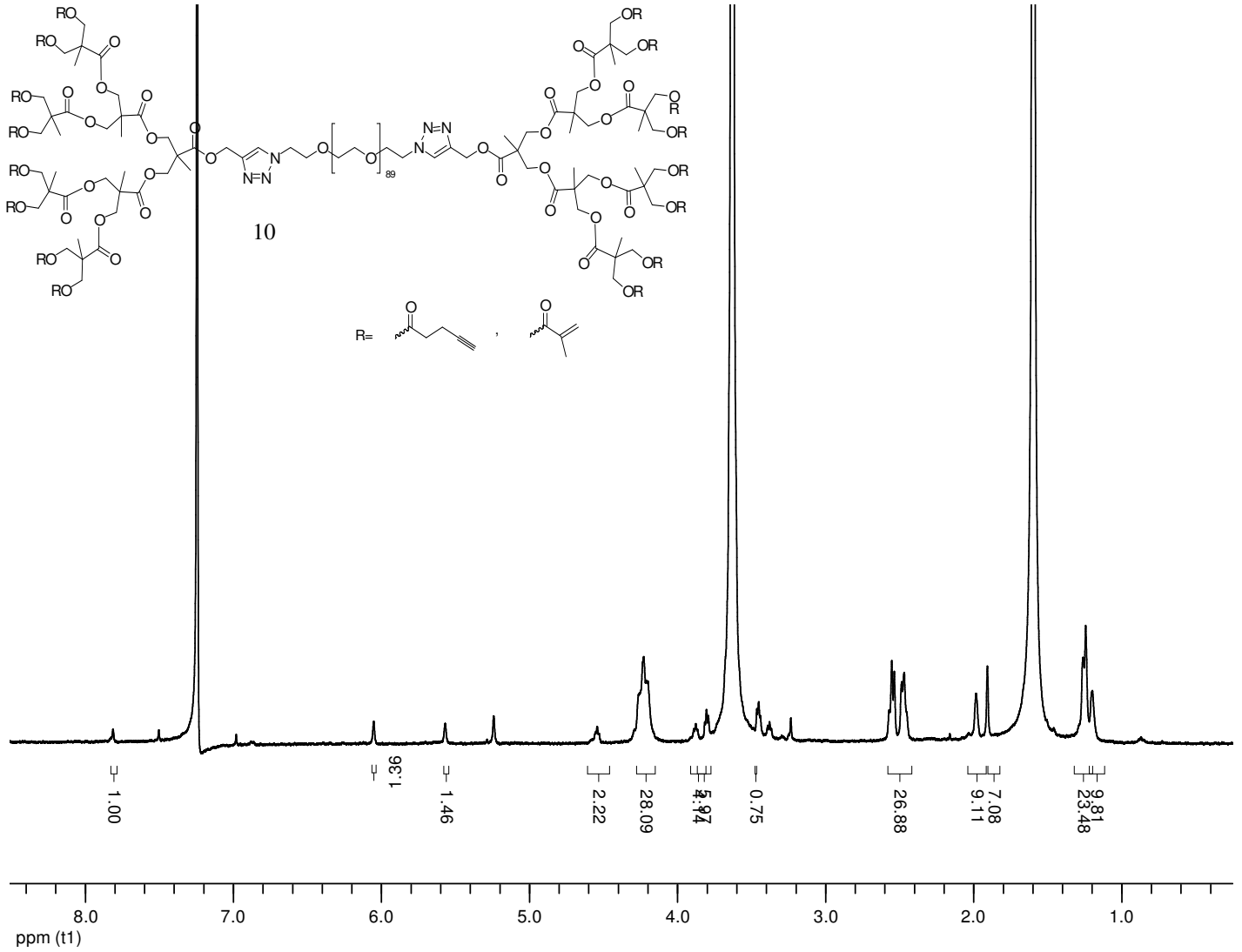



Figure A.17. $^{1}$H NMR spectrum of *[1:3] [G3]8OR[PEG6K]* **10**

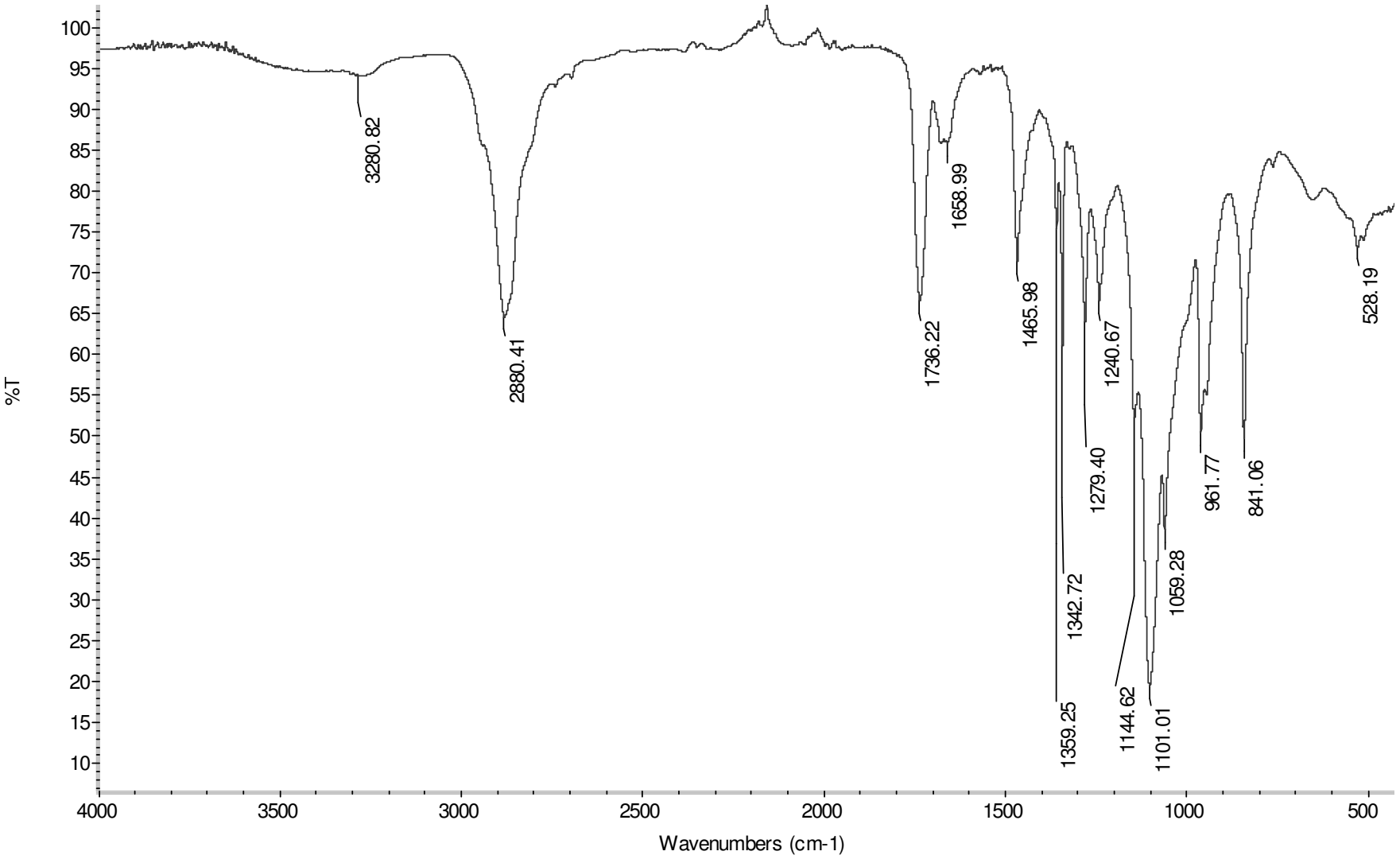



Figure A.18. IR spectrum of *[1:3] [G3]8OR[PEG6K]* **10**

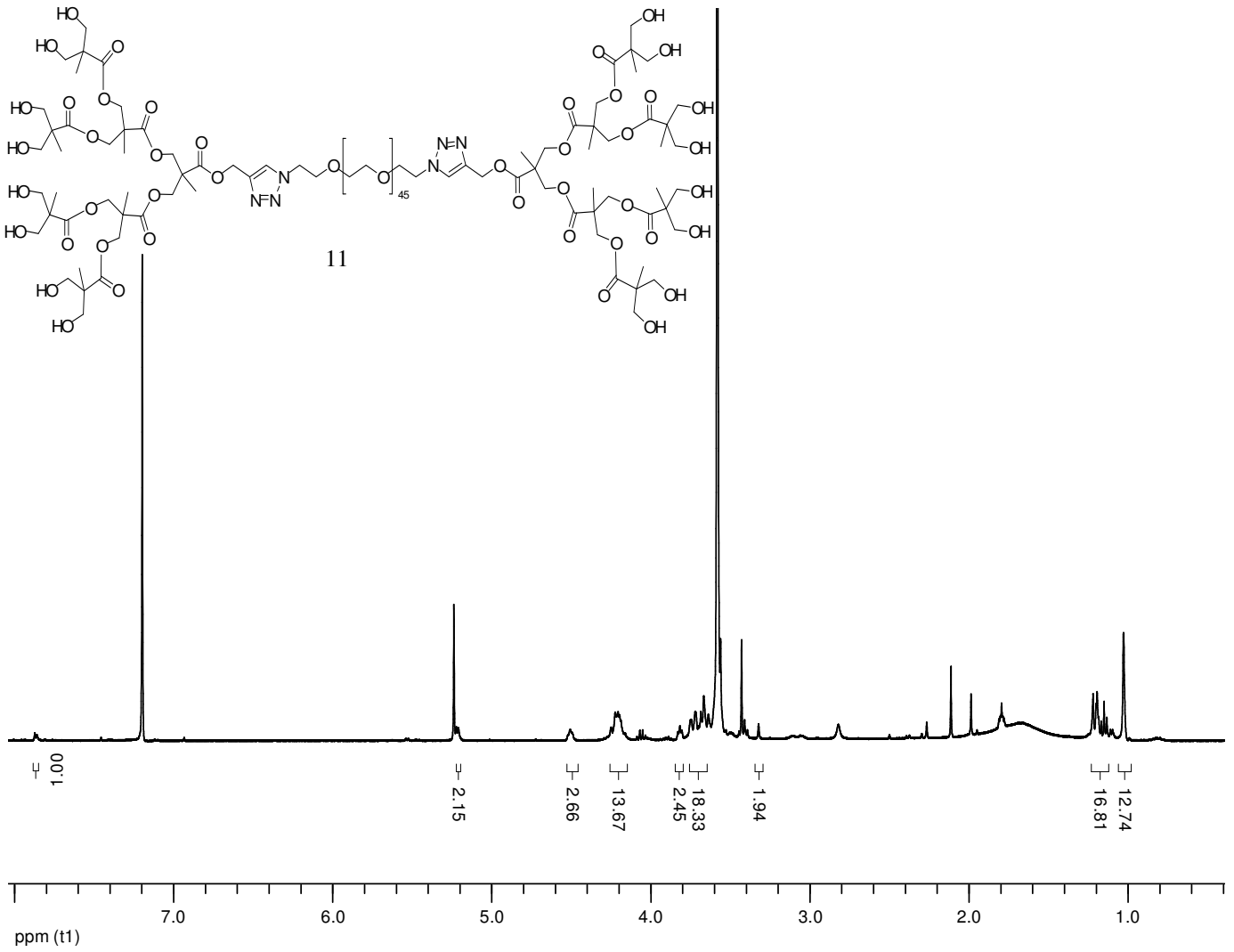



Figure A.19. $^{1}$H NMR spectrum of *[G3]8OH[PEG2K]* **11**

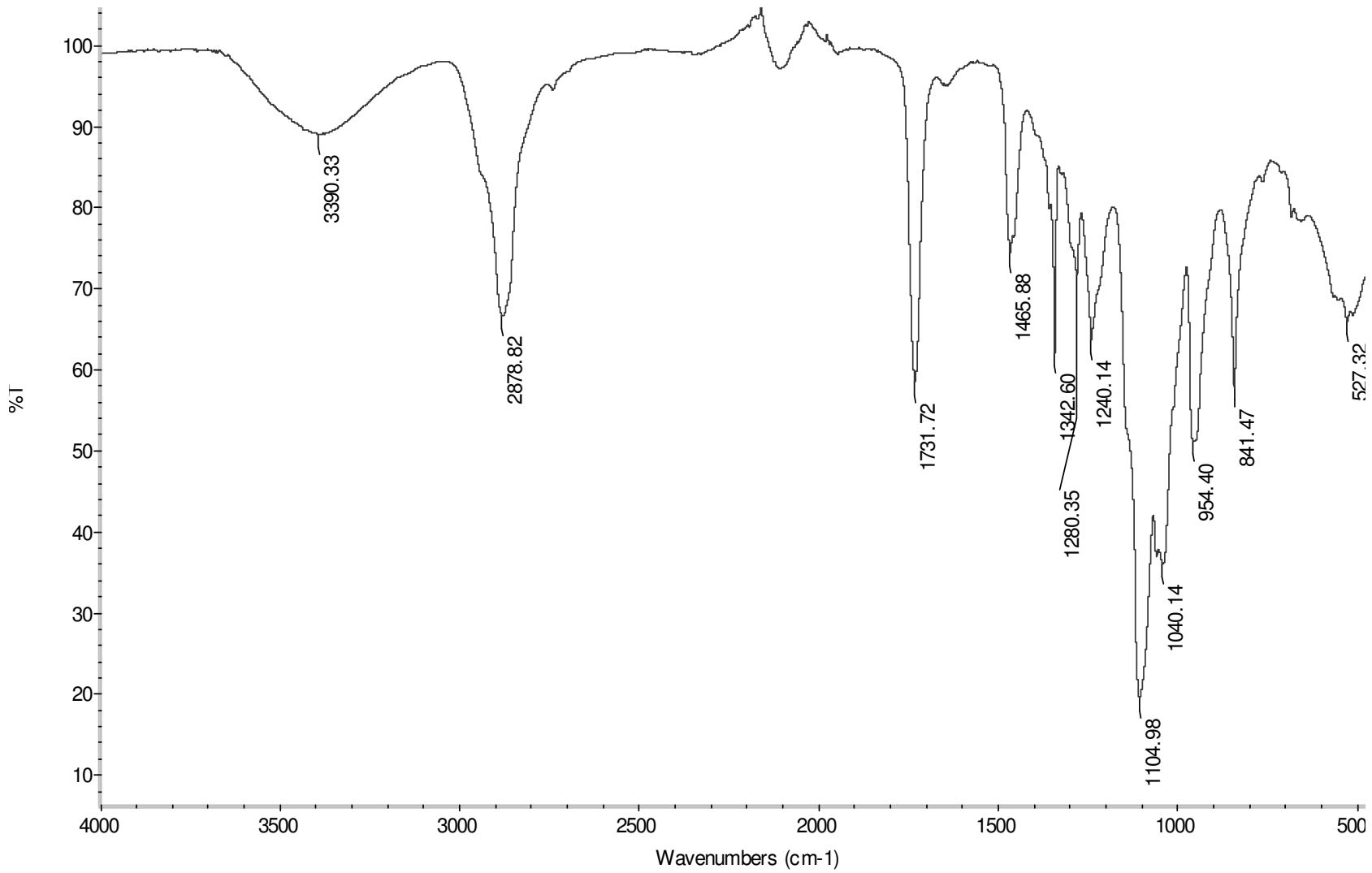



Figure A.20. IR spectrum of *[G3]8OH[PEG2K]* **11**

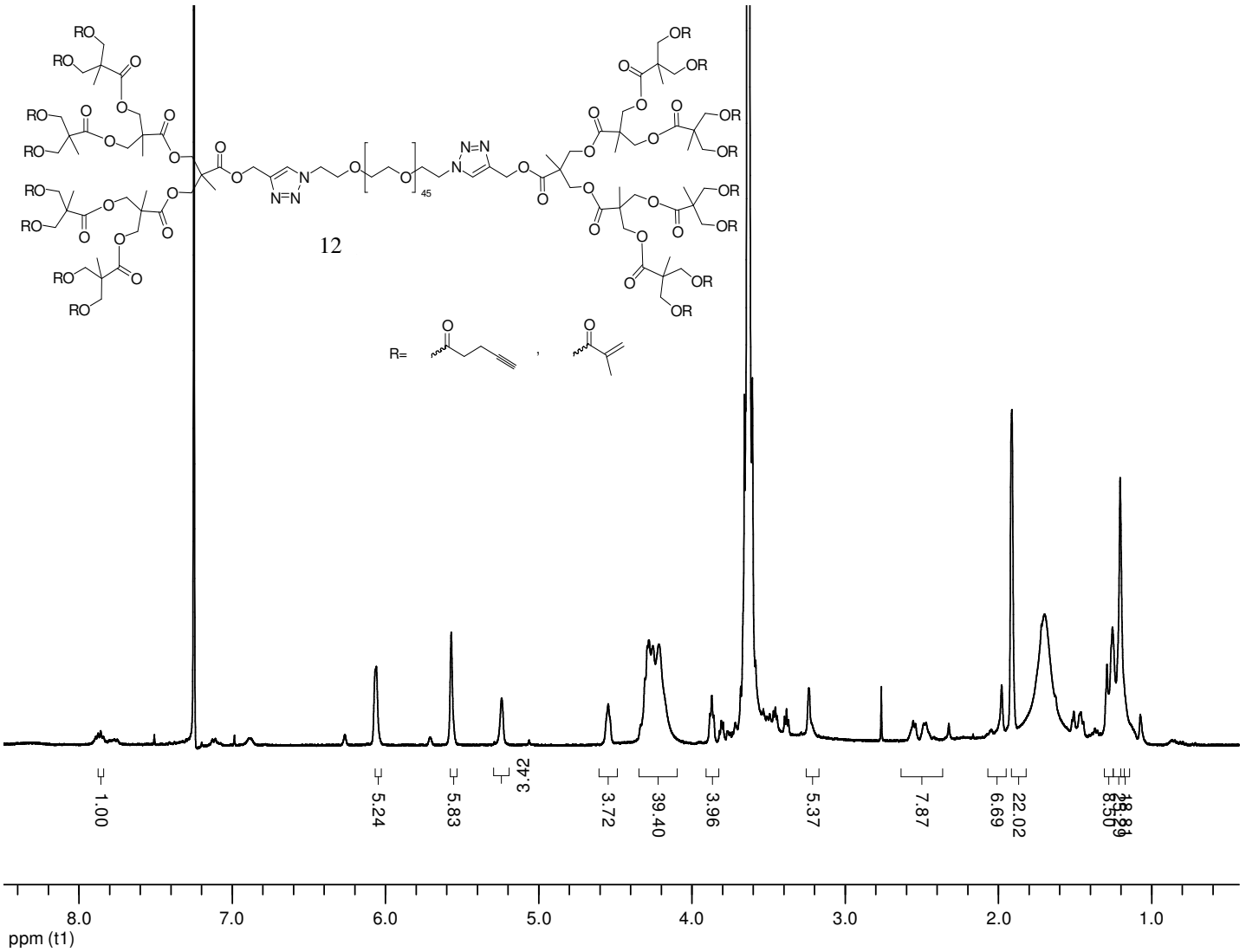



Figure A.21. $^{1}$H NMR spectrum of *[1:1] [G3]8OR[PEG2K]* **12**

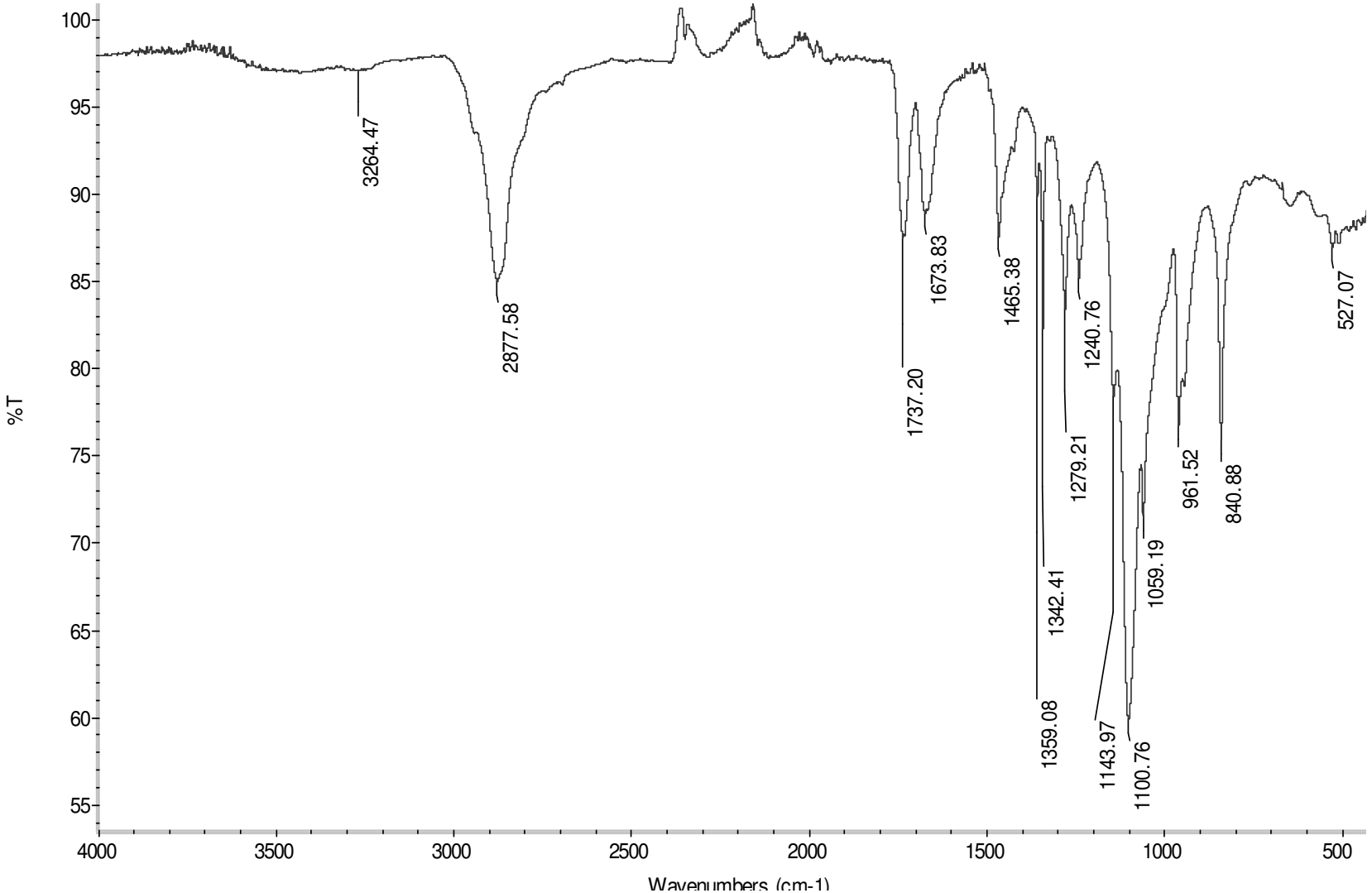



Figure A.22. IR spectrum of *[1:1] [G3]8OR[PEG2K]* **12**

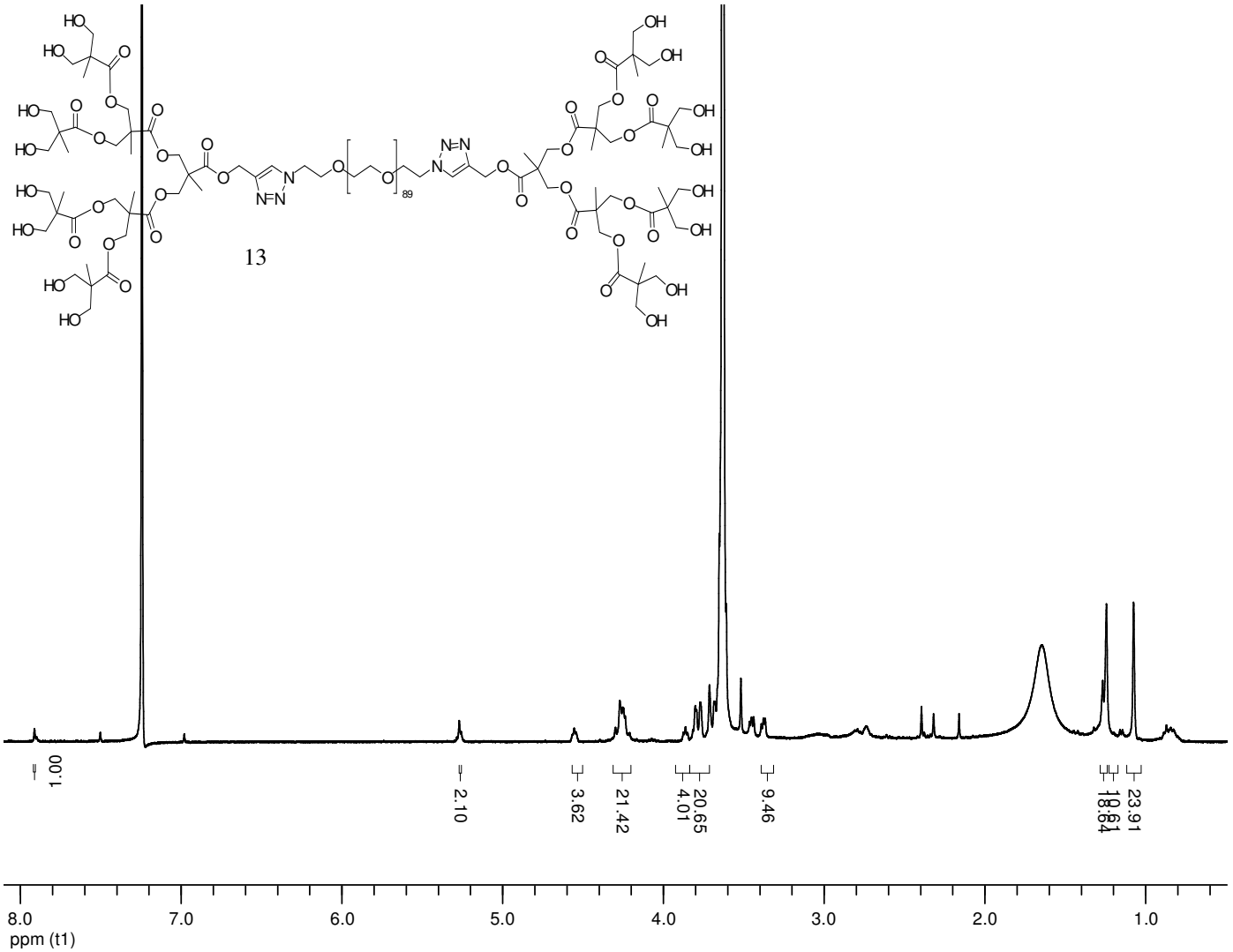



Figure A.23. $^{1}$H NMR spectrum of *[G3]8OH[PEG4K]* **13**

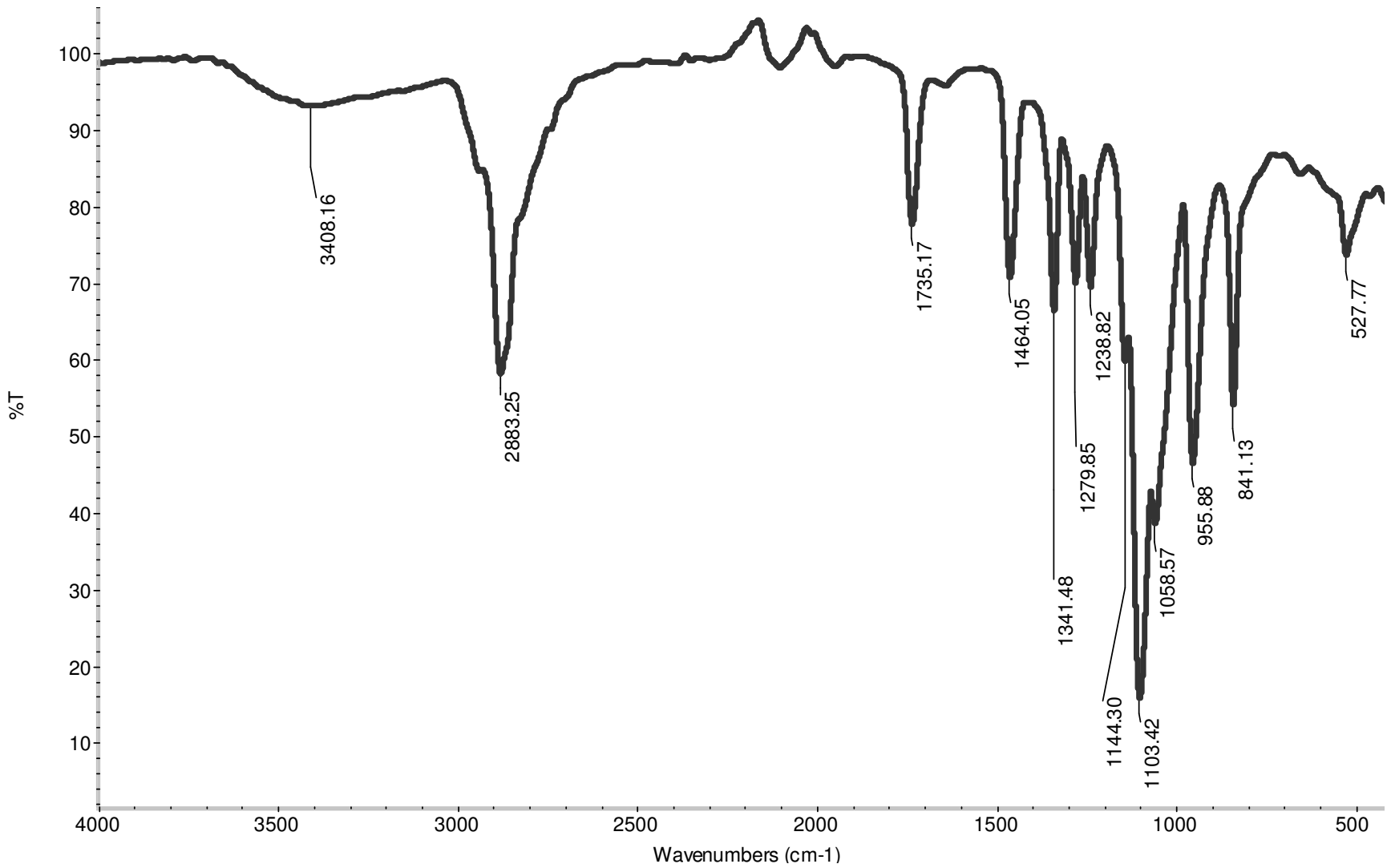



Figure A.24. IR spectrum of *[G3]8OH[PEG4K]* **13**

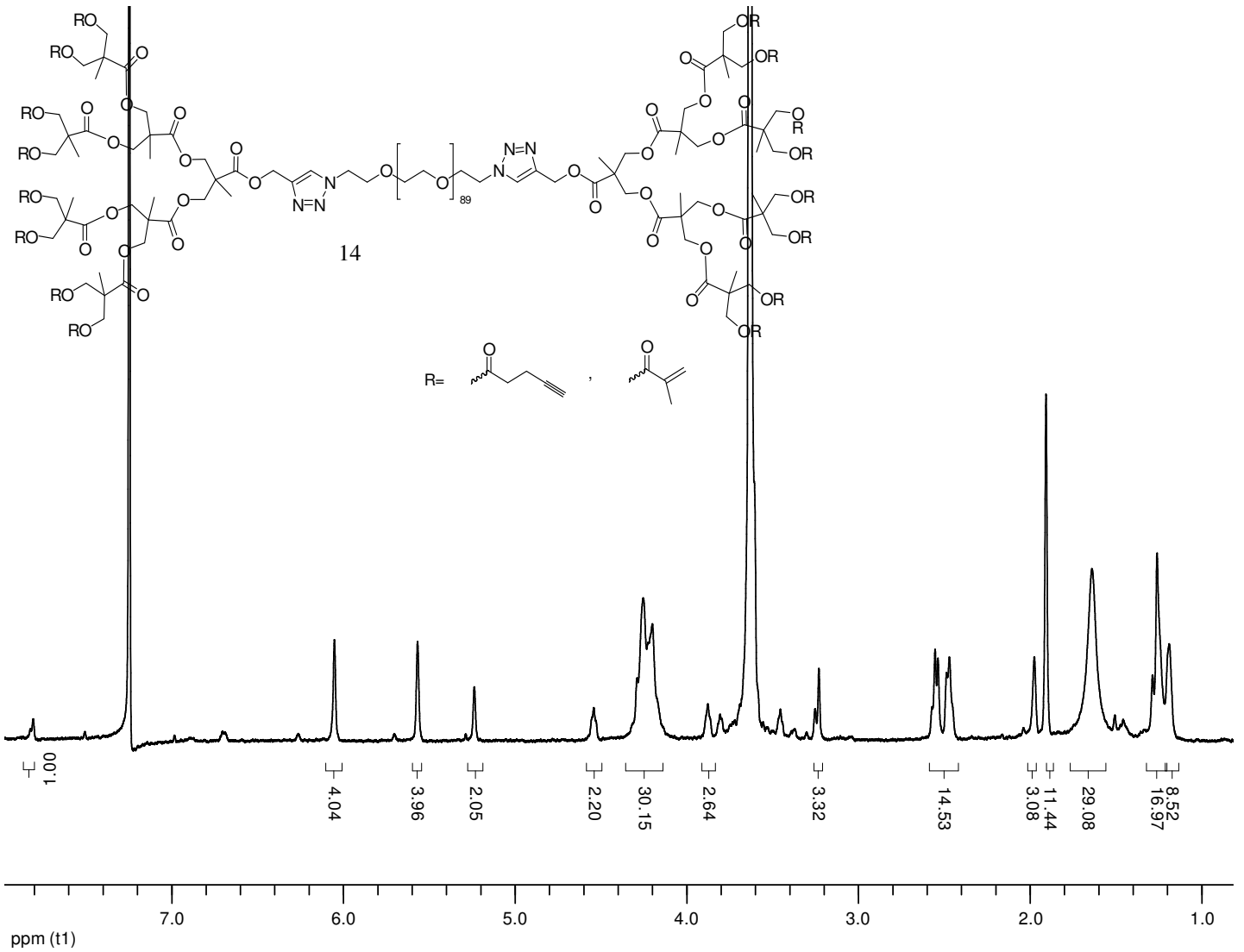



Figure A.25. $^{1}$H NMR spectrum of *[1:1] [G3]8OR[PEG4K]* **14**

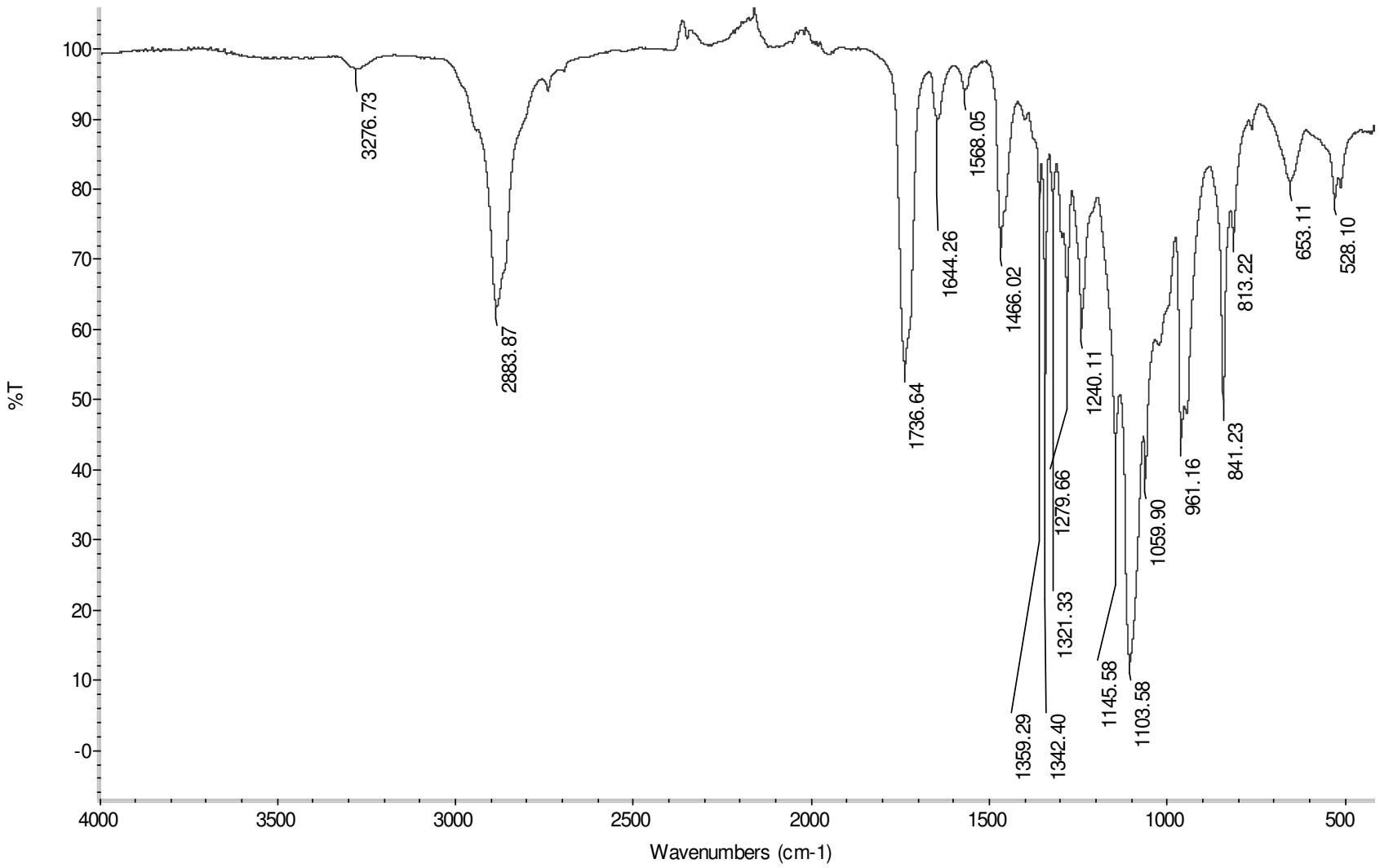



Figure A.26. IR spectrum of *[1:1] [G3]8OR[PEG4K]* **14**

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