FUNCTIONAL HYDROGELS
VIA
CLICK CHEMISTRY

by
Hüseyin Altın
B.S. in Chemistry, Boğaziçi University, 2007

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
2009

FUNCTIONAL HYDROGELS
VIA
CLICK CHEMISTRY

APPROVED BY:

Assist. Prof. Amitav Sanyal ……...
(Thesis Supervisor)

Assist. Prof. Rana Sanyal ……...

Prof. Gürkan Hızal ……

DATE OF APPROVAL: …….

... to My Mother

ACKNOWLEDGEMENTS

I would like to express my most sincere appreciation to my thesis supervisor Asst. Prof. Amitav SANYAL for his endless attention and scientific supervision throughout this study. I appreciate his great support and useful comments during my laboratory work. I would extend my thanks Asst. Prof. Rana SANYAL for all the helpful discussions regarding my thesis work and for her help during my masters program.

I wish to express my thanks to Prof. Dr. Gürkan HIZAL for his careful and constructive review of the final manuscript and for serving in my committee.

I would like to extend my thanks to Ayla TÜRKEKUL, Burcu SELEN ÇAĞLAYAN and Bilge GEDİK ULUOCAK for their patience and help running of NMR and SEM imaging experiments supporting my laboratory work.

I would also thank Cem ÖZTÜRK, Fırat ÖZDEMİR, Murat TONGA, Özgül GÖK, Ali Hikmet KARAYEL, İrem KOSİF, İdil İpek YILMAZ, Serap YAPAR, Gülen YEŞİLBAĞ, Duygu ÇITAK and Nergiz CENGİZ for their enjoyable company and friendship. I would like to thank to all my friends and all the members of the faculty in the Chemistry Department.

Finally my sincere thanks go to my love, Dilek and my family for their endless love, support and encouragement throughout my life.

This work was partially supported by Bogazici University Research Fund BAP 07H502, 07M105, 09S107 and NOVARTIS and The Scientific and Technological Research Council of Turkey (TÜBİTAK Project No: 108T898).

ABSTRACT

FUNCTIONAL HYDROGELS VIA CLICK CHEMISTRY

Hydrogels are three dimensionally crosslinked water soluble polymer networks which can absorb high quantity of water. In recent years, there has been a remarkable increase in the number of articles related to hydrogels due to their adjustable chemical and physical properties. As a consequence of the unique properties, hydrogels are widely used and waiting to be used in medical applications such as controlled drug release, cell growth media and tissue engineering, wound healing biological adhesives. For such applications attachment of desired molecules to the hydrogels are extremely important.

Here, syntheses of functionalizable hydrogels by using dendron-polymer-dendron conjugates are successfully done and characterizations of molecules and post functionalization of hydrogels are reported. In this study, multivalency and mono dispersity properties of polyester dendrons are combined with the biocompatible, water soluble, polyethyleneglycol polymers by using [3+2] Huisgen type copper catalyzed cycloaddition chemistry to synthesize dendron –polymer conjugates. These copolymers are used to synthesize Functional hydrogels again by [3+2] Huisgen type “Click” reaction. Synthesized functional hydrogels are characterized with SEM and ATR FTIR and their high swelling properties are investigated. They are further functionalized covalently with a third “Click” reaction.

ÖZET

KLİK KİMYASI YÖNTEMİYLE FONKSİYONEL HİDROJELLER

Hidrojeller, suda çözünebilen polimerlerin üç boyutta çağraz bağlarla ağ yapmasıyla oluşan ve yüksek miktarda su emebilen yapılardır. Son yıllarda hidrojellerle alakalı makale sayısında gözle görüülür birartış yaşanmaktadır, bu da hidrojellerin ayarlanabilen kimyasal ve fiziksel özelliklerinden kaynaklanmaktadır. Bu eşsiz özelliklerin sonucu olarak, hidrojeller tıbbi uygulamalarda çokça kullanılmakta ve kullanılmayı beklemektedirler, örneğin kontrollü ilaç salınımı, hücre büyüme ortamı, doku mühendisliği, yara iyileştirici biyolojik yapışkan olarak. Bu uygulamalar için istenilen moleküllerin hidrojele bağlanması oldukça önemlidir

Burada, dendrimer-polymer-dendrimer bileşimleri kullanılarak fonksiyonel hidrojeller üretimi başarıyla yapılmış ve moleküllerin ve sonradan fonksiyonel hale getirilmiş hidrojellerin nitelikleri rapor edilmiştir. Bu çalışmada dendron –polymer bileşimleri üretmek için çok yapışma değerlikli ve tek dağlımlı özellikteki poliester dendronlar suda çözünebilen ve canlı dokularla uyum gösterebilen poli etilen glikol polymerleriyle [3+2] Huisgen tipi bakır katalizörlü dairesel ekleme kimyası kullanılarak sentezlenmiştir. Fonksiyonel hidrojel üretimi için bu kopolimerlerin [3+2] Huisgen tipi klik reaksiyonu ile yapılmıştırÜretilen fonksiyonel hidrojellerin karakterizasyonu SEM ve ATR FTIR ile yapılmış ve su yüksek su emme özellikleri ölçülmüştür. Bu hidrojellerin ileriki aşamadaki fonksiyonel hale getirilmesi yine [3+2] Huisgen tipi “klik” reaksiyonuyla yapılmıştır.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iv
ABSTRACT v
ÖZET vi
LIST OF FIGURES ix
LIST OF TABLES xiv
LIST OF ABBREVIATIONS xv
1. INTRODUCTION 1
1.1. Hydrogels 1
1.2. Azide Alkyne Huisgen Cycloaddition Click Reaction 2
1.3. Synthesis of Hydrogels by Using "Click" Reaction 3
1.4. Functional Hydrogels 8
1.5. Dendrimers and Dendrons 9
1.6. Dendron-Polymer Based Functional Copolymers 10
1.7. Dendron-Polymer Based Hydrogels 13
2. AIM OF THE STUDY 15
3. RESULTS AND DISCUSSION 16
3.1. Synthesis of Functional Hydrogels 16
3.2. Characterization of Functional Hydrogels 19
3.3. Functionalization of Hydrogels 22
3.4. Swelling Properties of Hydrogels 24
3.5. Temperature Effect on Swelling Properties of Hydrogels 26
3.6. Morphological Studies 27
4. EXPERIMENTAL 31
4.1. General Methods and Materials 31
4.2. Synthesis of Dendrons 31
4.2.1. Synthesis of 2nd Generation Alkyne Functionalized Dendron (1) 31
4.2.2. Synthesis of 3rd Generation Alkyne Functionalized Dendron (2) 32
4.3. Synthesis of PEG bis-azides (3 and 4) 33
4.4. Synthesis of [G2]4OH[PEG2K] Copolymer (5) 33
4.5. Synthesis of [G2]4OH[PEG6K] Copolymer (6) 34

4.6. Synthesis of [G3]8OH[PEG6K] Copolymer (7) 35
4.7. Functionalization of the [G2]4OH[PEG2K] Copolymer (8) 36
4.8. Functionalization of the [G2]4OH[PEG6K] Copolymer (9) 38
4.9. Functionalization of the [G3]8OH[PEG6K] Copolymer (10) 39
4.10. General Synthesis of Hydrogels via [3+2] Huisgen "Click" Chemistry 40
4.11. Functionalization of Hydrogels 40
4.11.1. Functionalization with 4-Azido-N-ethyl-1,8-naphthalimide 41
4.11.2. Functionalization with Bodipy azide 41
4.11.3. Functionalization with Biotin Azide 42
4.12. Measurements 42
4.12.1. Scanning Electron Microscopy Analysis of the Hydrogel Samples 42
4.12.2. Physical Property Analysis (Water Uptake) 43
4.12.3. Temperature Dependence of Swelling Ratio 43
5. CONCLUSIONS 44
APPENDIX: SPECTROSCOPY DATA 45
6. REFERENCES 68

LIST OF FIGURES

Figure 1.1. Effect of hydrogels in wound healing 1

Figure 1.2. Hydrogels can be generated by crosslinking wide range of monomers 2

Figure 1.3. Click reaction between an azide and a terminal alkyne by heat 3

Figure 1.4. Click reaction between an azide and a terminal alkyne with Cu catalyst 3

Figure 1.5. Synthesis of PEG based hydrogel formed by click reaction 4

Figure 1.6. Crosslinked PVA via [3+2] Huisgen Click 5

Figure 1.7. Synthesis of P(NIPAAm-co-HEMA)-based hydrogels 6

Figure 1.8. Hydrogel structure of polysaccharide 7

Figure 1.9. Peptide linked click hydrogels 7

Figure 1.10. NTA containing hydrogel synthesis and Histidine tagged protein structure 8

Figure 1.11. Dendrimer Structure 9

Figure 1.12. Generations of dendrons 9

Figure 1.13. Dendrimer synthesis by click reaction 10

Figure 1.14. Fréchet reported the PEG based dendron polymer conjugates with one, two and four functional telechelic PEGs. 11

Figure 1.15. Schematic representation of dendron polymer dendron conjugates 12

Figure 1.16. PEG-dendron block copolymers for targeted drug delivery systems. 13

Figure 1.17. Formation of hydrogel from PGLBA-PEG macromolecule, ... 14

Figure 2.1. General Scheme for the synthesis of functionalized hydrogels 15

Figure 3.1. Synthesis and functionalization of G2 and G3 block copolymer 17

Figure 3.2. Hydrogel formation 18

Figure 3.3. Picture of transparent hydrogel 18

Figure 3.4. FTIR spectroscopies of (a) G3 dendron **2** (b) PEG-6K bis-azide **4** (c) PEG dendron copolymer **7** (d) PEG-dendron alkynyl functionalized copolymer **10** (e) Functional 6KG3 (1:4) Hydrogel 19

Figure 3.5. ^{1}H NMR spectrum of [G2]4OH[PEG6K] **6** and [G2]4OR[PEG6K] **9** 21

Figure 3.6. ^{1}H NMR spectrum of [G3]8OH[PEG6K] **7** and [G3]8OR[PEG6K] **10** 21

Figure 3.7. Azides for hydrogel functionalization 22

Figure 3.8. Fluorescence microscope images of functionalized hydrogels (a) with 4-Azido-N-ethyl-1,8-naphthalimide (**12**) (b) control of a without catalyst (c) with biotin azide (**14**)-streptavidin (d) control of c without catalyst (e) with bodipy azide (**16**) (f) control of e without catalyst 23

Figure 3.9. Relative fluorescence values of functionalized hydrogels with streptavidin and their controls that contain descending amount of functional groups. 24

Figure 3.10. Water uptake comparison of 2KG2 (1:3) and 6KG2 (1:3) hydrogels 25

Figure 3.11. Water uptake comparison of 6KG2 (1:3) (1:2) and (1:1) hydrogels 26

Figure 3.12. Water uptake comparison of 6KG3 (1:4), (1:3), (1:2), (1:1) hydrogels 26

Figure 3.13. Temperature dependence of water uptake 27

Figure 3.14. SEM images of wet hydrogel 6KG2 (1:1.5) 28

Figure 3.15. SEM images of dry hydrogel 6KG2 (1:1.5) 28

Figure 3.16. SEM images of dry hydrogel 6KG2 (1:3) 29

Figure 3.17. SEM images of dry hydrogel 6KG3 (1:1) 29

Figure 3.18. SEM images of dry hydrogel 6KG3 (1:1) 30

Figure 3.19. SEM images of dry hydrogel 6KG3 (1:3) 30

Figure 4.1. Synthesis of 2nd Generation of Dendron (**1**) 31

Figure 4.2. Synthesis of 2nd Generation of Dendron (2) 32

Figure 4.3. Synthesis of PEG bis-azides (**3** and **4**) 33

Figure 4.4. Synthesis of [G2]4OH[PEG2K] Copolymer (**5**) 34

Figure 4.5. Synthesis of [G2]4OH[PEG6K] Copolymer (**6**) 35

Figure 4.6. Synthesis of [G3]8OH[PEG6K] Copolymer (**7**) 36

Figure 4.7. Synthesis of [G2]4OR[PEG2K] Copolymer (**8**) 37

Figure 4.8. Synthesis of [G2]4OR[PEG6K] Copolymer (**9**) 38

Figure 4.9. Synthesis of G3]8OR[PEG6K] Copolymer (**10**) 39

Figure 4.10. Structure of 4-Azido-N-ethyl-1,8-naphthalimide (**12**) 41

Figure 4.11. Structure of Bodipy azide (**16**) 41

Figure A.1. ^{1}H NMR spectrum of 2nd generation polyester dendron **1** 46

Figure A.2. ATR FTIR spectrum of 2nd generation polyester dendron **1** 47

Figure A.3. ^{1}H NMR spectrum of 3rd generation polyester dendron **2** 48

Figure A.4. ATR FTIR spectrum of 3rd generation polyester dendron **2** 49

Figure A.5. ^{1}H NMR spectrum of PEG 2K bis azide **3** 50

Figure A.6. ATR FTIR spectrum of PEG 2K bis azide **3** 51

Figure A.7. ^{1}H NMR spectrum of PEG 6K bis azide 4 52

Figure A.8. ATR FTIR spectrum of PEG 6K bis azide **4** 53

Figure A.9. ^{1}H NMR spectrum of [*G2]4OH[PEG2K]* **5** 54

Figure A.10. ATR FTIR spectrum of [*G2]4OH[PEG2K]* **5** 55

Figure A.11. ^{1}H NMR spectrum of *[G2]4OH[PEG6K]* **6** 56

Figure A.12. ATR FTIR spectrum of *[G2]4OH[PEG6K]* **6** 57

Figure A.13. ^{1}H NMR spectrum of *[G3]8OH[PEG6K]* **7** 58

Figure A.14. ATR FTIR spectrum of *[G3]8OH[PEG6K]* **7** 59

Figure A.15. ^{1}H NMR spectrum of *[G2]4OR[PEG2K]* **8** 60

Figure A.16. ^{1}H NMR spectrum of *[G2]4OR[PEG2K]* **8** 61

Figure A.17. ^{1}H NMR spectrum of *[G2]4OR[PEG6K]* **9** 62

Figure A.18. ATR FTIR spectrum of *[G2]4OR[PEG6K]* **9** 63

Figure A.19. ^{1}H NMR spectrum of *[G3]8OR[PEG6K]* **10** 64

Figure A.20. ATR FTIR spectrum of *[G3]8OR[PEG6K]* **10** 65

Figure A.21. ^{1}H NMR spectrum of Bodipy azide **16**..... 66

Figure A.22. ATR FTIR spectrum of Bodipy azide **16** 67

LIST OF TABLES

Table 3.1. Properties of hydrogels with different variations in dendron polymer and linker structure and quantity..... 20

LIST OF ABBREVIATIONS

bFGF	Basic Fibroblast Growth Factor
Bis-MPA	2,2-bis(hydroxymethyl)propionic acid
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
D_2O	Deuterated water
DCC	Dicyclohexylcarbodiimide
DMAP	N,N Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl Acetate
FITC	Fluorescein Isothiocyanate
FT-IR	Fourier Transform Infrared
HEMA	(Hydroxyethyl)methacrylate
MeOH	Methanol
NMR	Nuclear Magnetic Resonance
NTA	Nitrilotriacetic acid
P(…)	Poly(….)
PAMAM	Polyamidoamine
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PEMA	Poly(ethyl methacrylate)
PLLA	Poly-L-lactide
PNIPAAm	Poly(N-isopropyl acrylamide)
PVA	Poly(vinyl alcohol)
Py	Pyridine
TEA	Triethylamine
TEG	Triethyleneglycol

1. INTRODUCTION

1.1. Hydrogels

Recent years have witnessed remarkable advances in the area of synthesis of well defined hydrogels. Tremendous increase in the number of published articles in that area also shows the great importance of hydrogels. Interest in developing novel synthetic approaches in this area of research is fueled by the hydrogel based emerging technologies from off-the-shelf consumer products.

Hydrogels are three dimensionally crosslinked water soluble polymer networks which can absorb high quantity of water. Chemical and physical properties of hydrogels can be adjusted by wide range of chemicals. By changing the structure of monomer or monomers, one can set the properties of hydrogels such as swelling ratio, crosslink density, crosslinking method, biodegradability, biocompatibility, hydrophilicity, porosity, transparency and functionality.

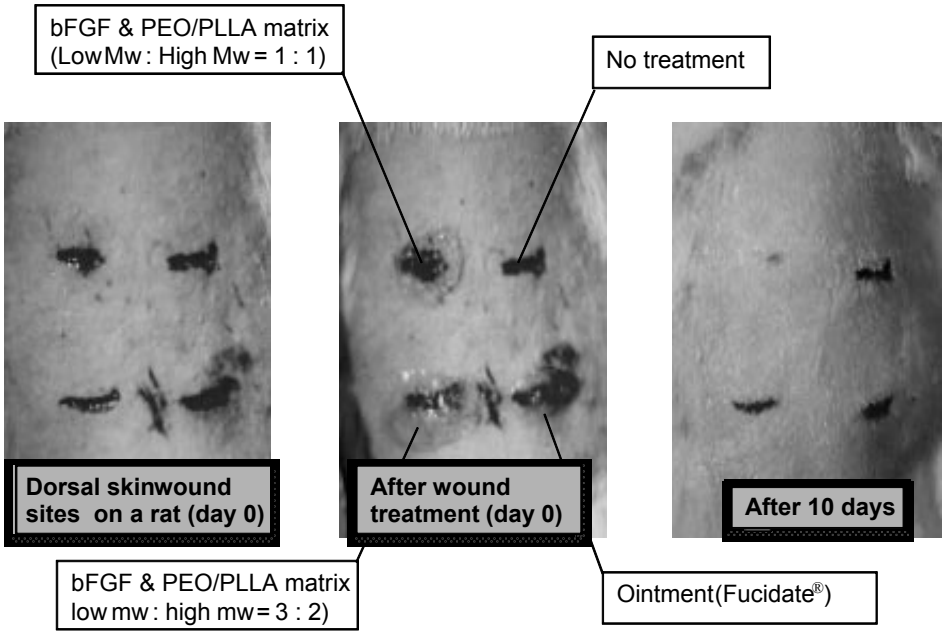


Figure 1.1. Effect of hydrogels in wound healing

As a consequence of this adjustable features and modifications, today's hydrogels are widely used in medical applications for example controlled drug release, cell growth and artificial tissue engineering, and wound healing biological adhesives [1-24].

Bae showed in 2000 the effect of hydrogels on wound healing (Figure 1.1) compared to traditional methods [25]. According to his experiments wounds which were covered bFGF film showed complete recovery after 10 days while the control wound sites were only partially healed.

Advances in this area have seen developments in new crosslinking methodologies and synthesis of designer hydrogels that can be precisely functionalized. (Figure 1.2) There are chemical and physical crosslinking methods to form a hydrogel ranging from crosslinking by radical polymerization and crosslinking using enzymes, to crosslinking by ionic interactions and crosslinking by hydrogen bonding [26]. Here we will discuss the formation of hydrogels by using "Click" reaction which is a chemical cross linking method for hydrogels.

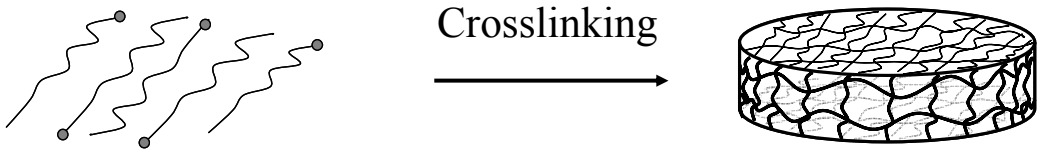


Figure 1.2. Hydrogels can be generated by crosslinking wide range of monomers

1.2. Azide Alkyne Huisgen Cycloaddition Click Reaction

In 1970s, Rolf Huisgen introduced alkyne azide cycloaddition reaction of molecules with increasing temperature without using a catalyst [27]. Unfortunately, this reaction did not produce a single product but formed a mixture of 1,4 and 1,5 heterocyclic products (Figure 1.3).

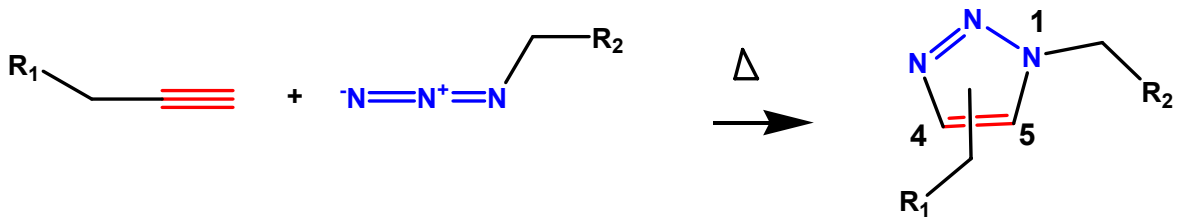


Figure 1.3. Click reaction between an azide and a terminal alkyne by heat

In 2001, K. Barry Sharpless and Morten Meldal improved this reaction by using Cu[I] catalyst and achieved the reaction regioselectively, the synthesis of a single 1,4 product at room temperature (Figure 1.4) [28] . Due to the simplicity in the conditions and reagents of reaction as easy as clicking two pieces together it is called as “click” chemistry. Click reaction allows the synthesis of regiospecific, physiologically stable heterocycles with high chemical yield in aqueous or organic solvents.

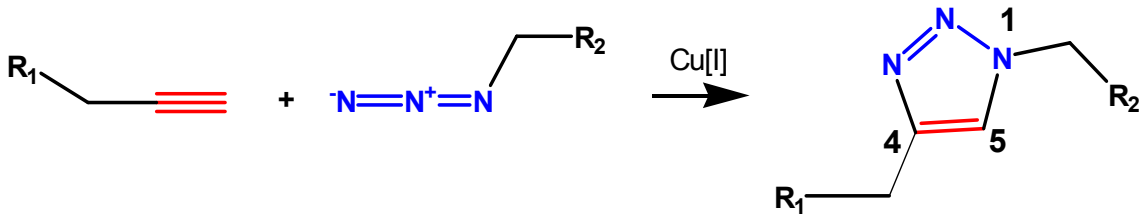


Figure 1.4. Click reaction between an azide and a terminal alkyne with Cu catalyst

1.3. Synthesis of Hydrogels by Using “Click” Reaction

Synthesis of hydrogels by using [3+2] Huisgen type “click” reaction has been explored by many researchers. Such hydrogels were reported by Hawker and coworkers in 2006 to synthesize well defined hydrogels using Huisgen type crosslinking of telechelic PEGs with multivalent azide based crosslinkers (Figure 1.5) [29]. They used different molecular weights of PEGs to show change in physical properties of hydrogels. As the chain length of PEGs used in hydrogel increased, degree of swelling is also increased. Hydrogels formed by photocrosslinking instead of cycloadditon had lower degree of swelling showing that the click reaction increased the physical properties hydrogels. Also some of the unreacted azide groups of the crosslinker in this reaction could be available for further functionalization.

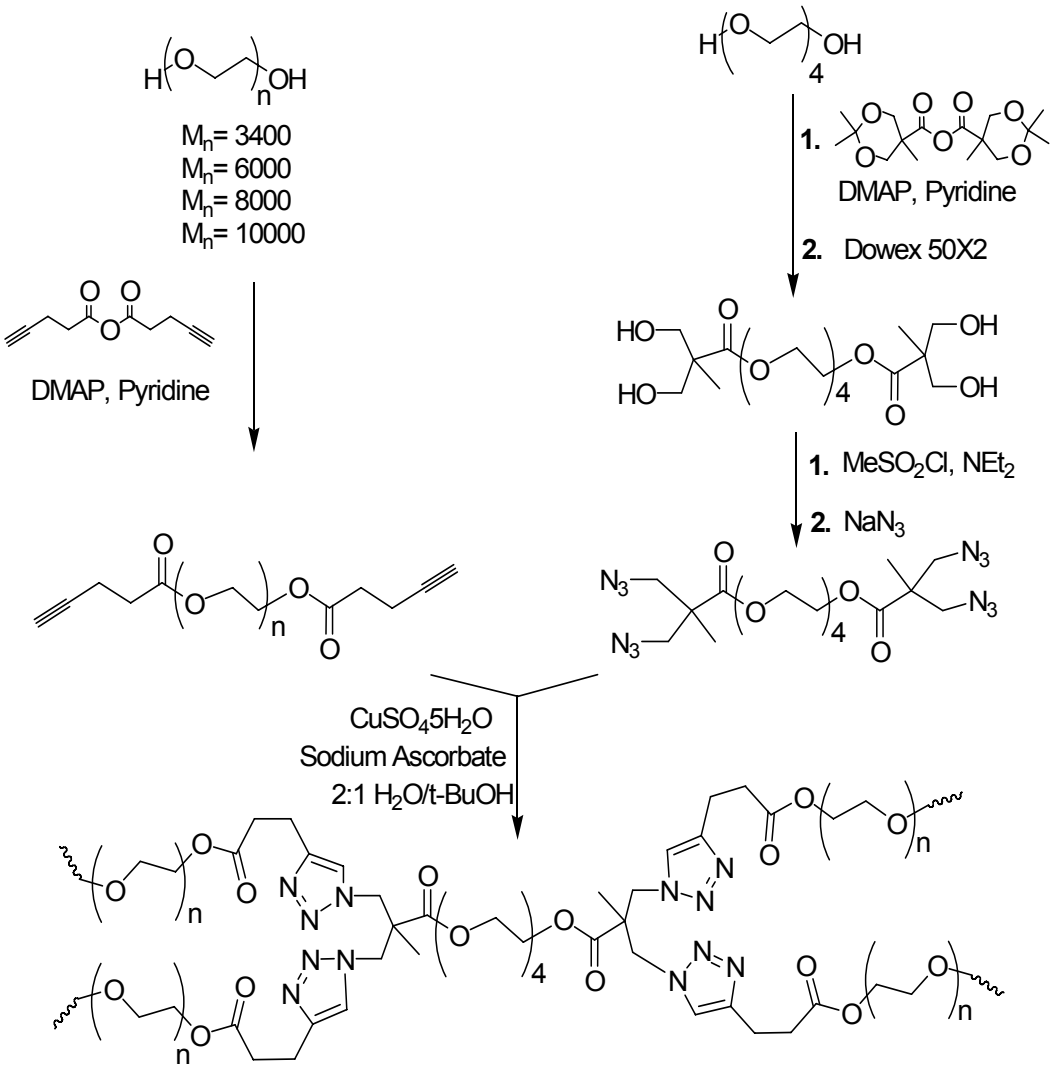


Figure 1.5. Synthesis of PEG based hydrogel formed by click reaction

Hilborn reported the polyvinyl alcohol based hydrogels crosslinked with click reaction (Figure 1.6) [30]. Two polymers one having alkynyl and the other one having azide pendant functionalities on the backbone were crosslinked via two methods. In the first method, telechelic PEG-diazide was used as a crosslinker for the PVA multiply functionalized with alkyne. In the second, hydrogels were obtained by the reaction between two PVA components multiply functionalized with azide and alkyne groups. Hydrogels obtained from two poly functionalized PVA components were characterized by higher elastic moduli compared to hydrogels prepared with the bifunctional crosslinker.

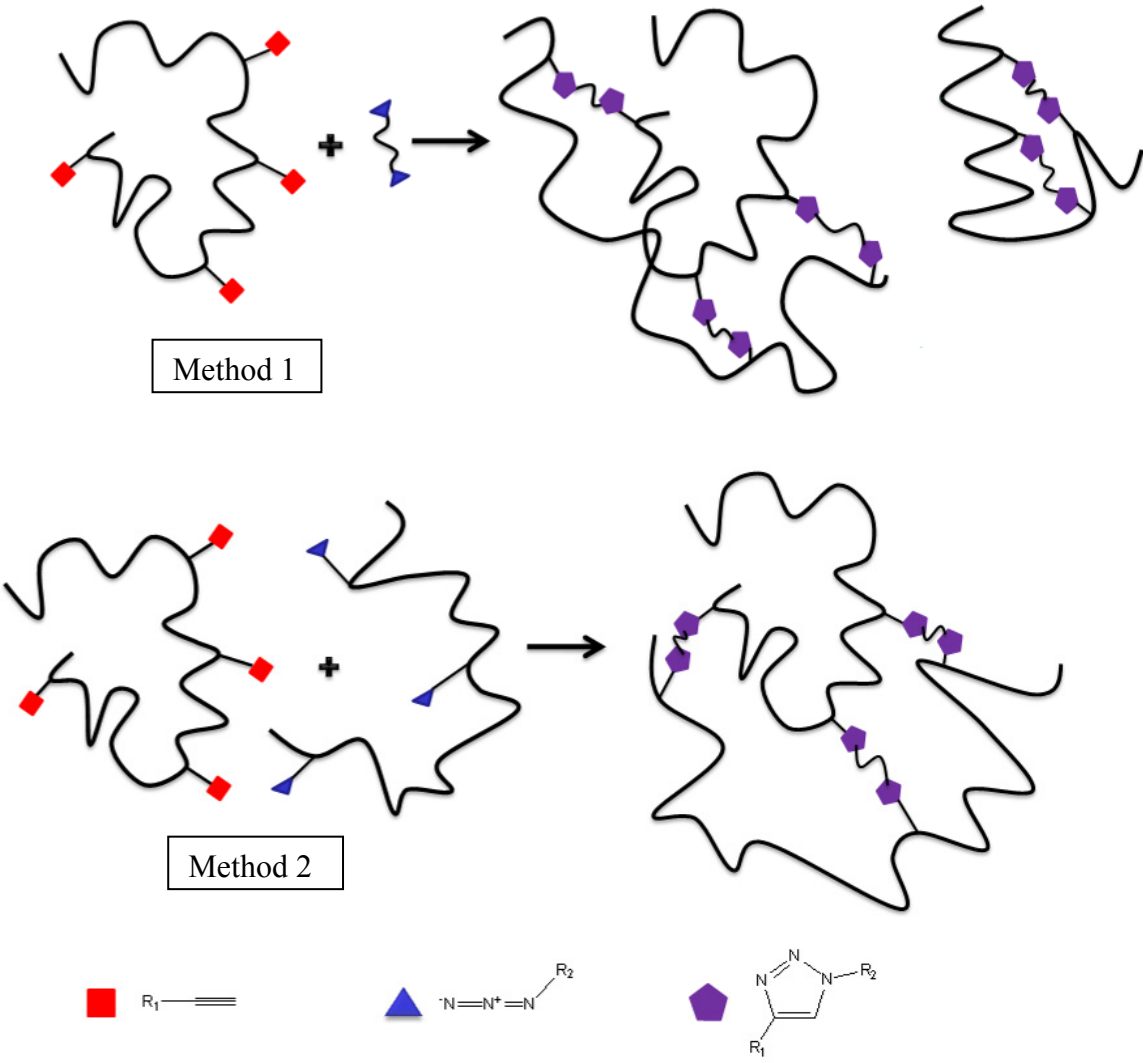


Figure 1.6. Crosslinked PVA via [3+2] Huisgen Click

Syntheses of thermoresposive hydrogels are also possible by random polymerization of N-Isopropylacrylamide (NIPAAm) and hydroxylethyl methacrylate (HEMA) monomers (Figure 1.7) [31]. Compared with the conventional PNIPAAm hydrogel, the P(NIPAAm-co-HEMA)-based hydrogels has a faster shrinking rate. For instance, all the P(NIPAAm-co-HEMA)-based hydrogels lose at least 80% water within 5 min. However, only 40% water is lost for conventional PNIPAAm hydrogel within the same time frame.

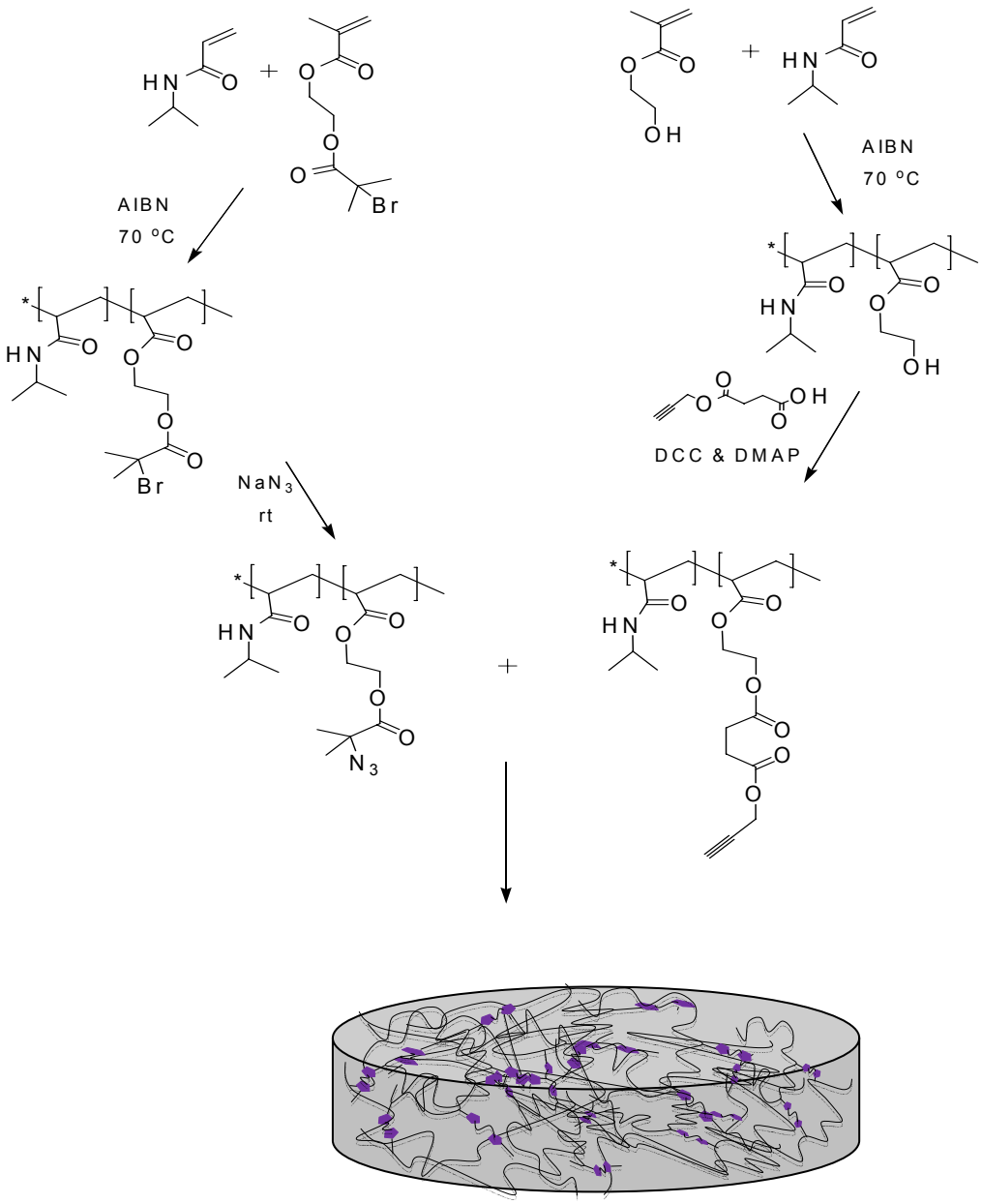


Figure 1.7. Synthesis of P(NIPAAm-co-HEMA)-based hydrogels

Crescenzi and coworkers reported a novel hydrogel crosslinked with copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction [32]. This hydrogel was composed of two polysaccharides with azide and alkyne containing side chains (Figure 1.8). The resulting gel could be utilized for controlled drug release or as cell growth media.

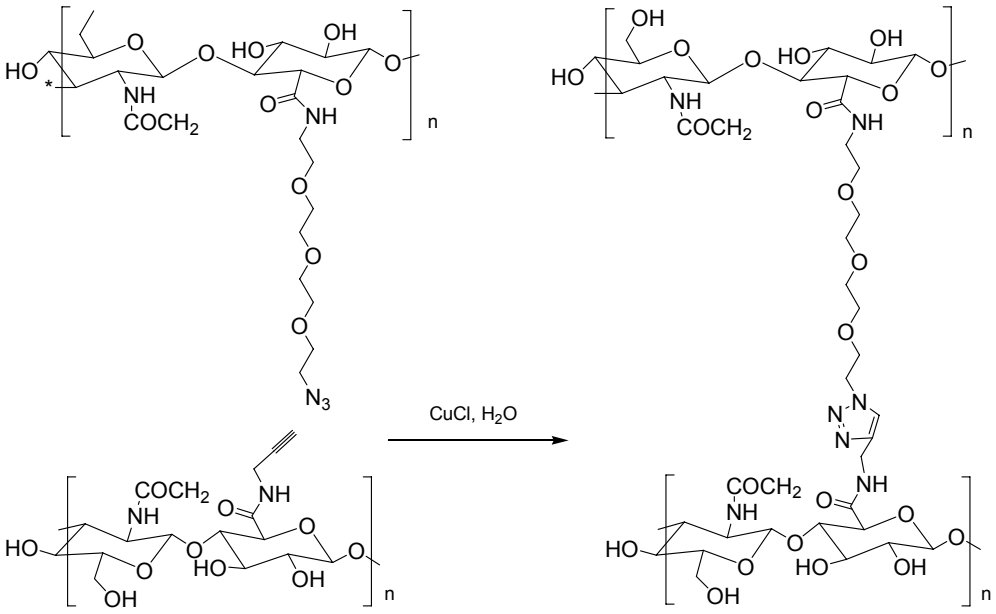


Figure 1.8. Hydrogel structure of polysaccharide

Cell growth in a hydrogel formed by click reaction is reported by Yang et. al. in 2008 (Figure 1.9) [33]. Tetra-hydroxy terminated 4-arm PEG was functionalized with acetylene and was reacted with peptide diazide and PEG diazide to produce hydrogels via a copper mediated 1,3-cycloaddition generating a triazole linkage as the networking forming reaction. The researchers observed that by attaching, Arg-Gly-Asp (RGD) containing (2.7–5.4 mM) peptides to the hydrogel, significantly improved cell attachment and greater cell proliferation rate when compared to the hydrogels without any peptides.

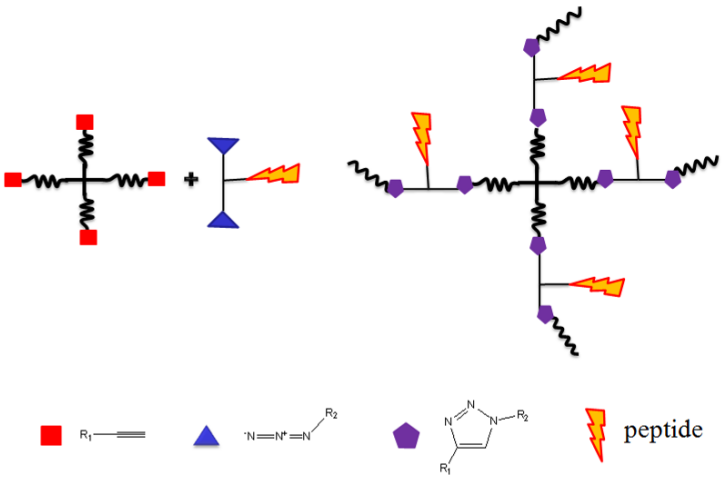


Figure 1.9. Peptide linked click hydrogels

This functionalization to obtain desired properties will be discussed in the next section.

1.4. Functional Hydrogels

Traditional approaches for incorporating entities such as small molecules, peptides and large biomacromolecules like enzymes or growth factors into hydrogel matrix have relied upon encapsulation and physiabsorption [34, 35]. Post functionalization of hydrogels has been evaluated as an attractive alternative in recent years. This approach relies on the presence of reactive functional groups in the hydrogel matrix that can undergo efficient functionalization under mild reaction conditions. Advent of "Click" reactions has dramatically influenced post polymerization functional group transformations due to their near quantitative conversions and mild reaction conditions.

Syntheses of functional hydrogels by using different molecules are also possible. For example, in 2005 Ober et.al. reported the synthesis of functional hydrogel surfaces using the advantage of the strong affinity of histidine (His) residues for metal-ion-nitrilotriacetic acid (NTA) complexes (Figure 1.10) [36]. In this study, they used HEMA and NTA copolymer to form hydrogel because HEMA hydrogels have good mechanical properties. NTA molecules can form a complex easily with metals. Histidine tagged peptides are bounded to hydrogel surface. This type of hydrogels can be used for biosensor and biochip applications.

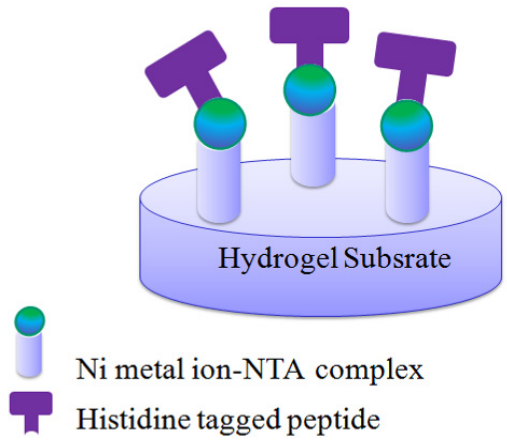


Figure 1.10. NTA containing hydrogel synthesis and Histidine tagged protein structure

1.5. Dendrimers and Dendrons

Dendrimers are monodisperse globular macromolecules that have two or more geometrically symmetric branching units around a core [37]. When we look closer into the segments, the central part is the core and the outer part is the periphery and each branching unit building the structure from the core to the periphery is the indication of generation. In Figure 1.11 three generations of a dendrimer are shown, namely first generation (G1), second generation (G2) and third one is (G3).

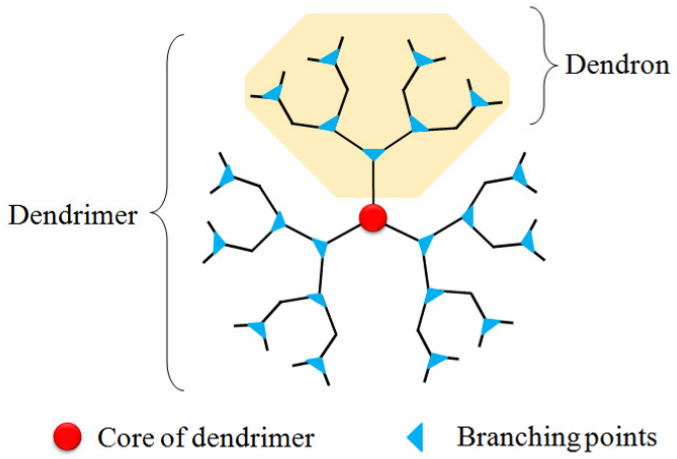


Figure 1.11. Dendrimer Structure

Part of a dendrimer starting from a core and ending at the periphery is called as a dendron (Figure 1.12). Classifications of the generations of a dendron are exactly in the same as the dendrimers. There are two methods to synthesize dendrons, one starting from the core and growing through the periphery, the divergent method and the other one start from the periphery and building towards the core, the convergent method.

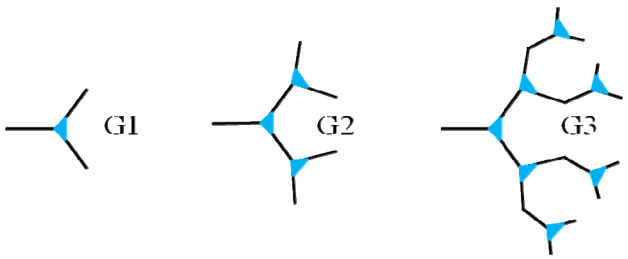


Figure 1.12. Generations of dendrons

Click reaction can be used to combine different kinds of dendrons efficiently. Hawker and coworkers reported a dendrimer prepared by click chemistry (Figure 1.13) [38]. They showed the two different dendrons can form a dendrimer by utilizing click reaction with high yield. Produced dendrimer can be orthogonally functionalized and used.

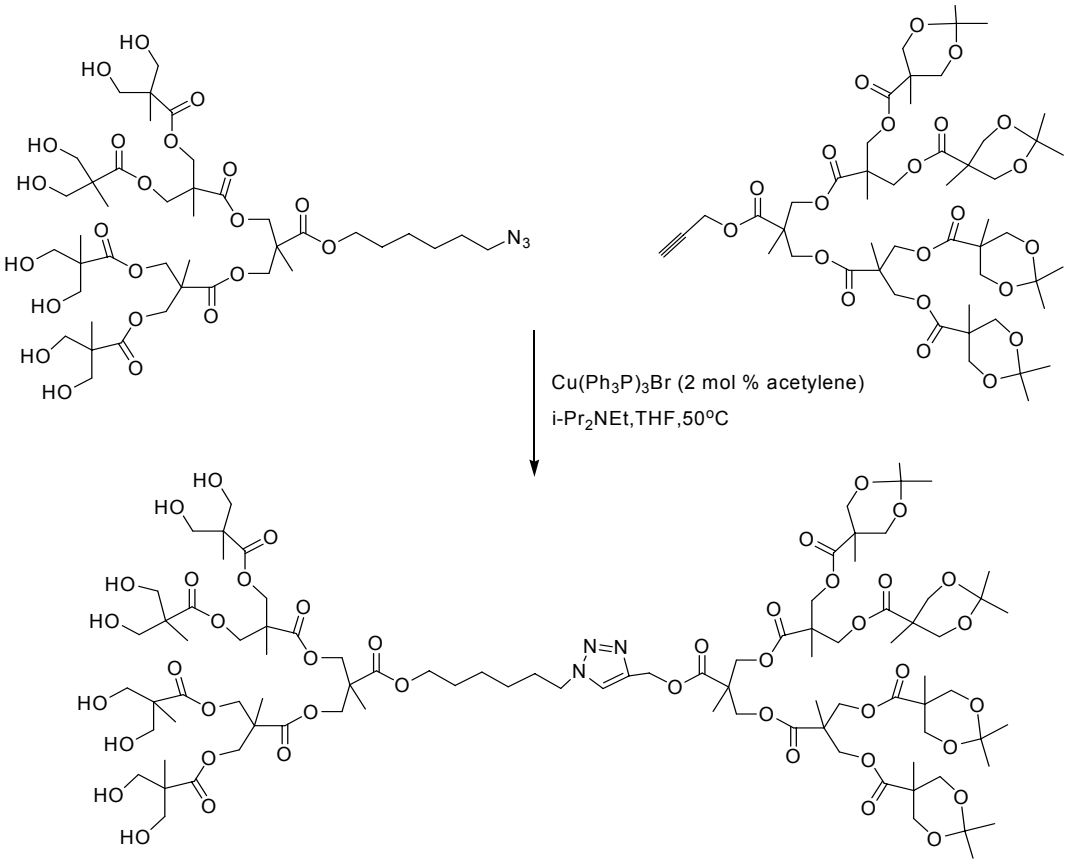


Figure 1.13. Dendrimer synthesis by click reaction

1.6. Dendron-Polymer Based Functional Copolymers

Triblock copolymers are receiving attention due to their special properties. By attaching a telechelic polymer with a dendron one can to utilize the advantages of both polymers and dendrons. Commonly, poly(ethyleneglycol) (PEG), a hydrophilic polymer, is used as the middle polymer section due to its properties that make it suitable for medical applications. In drug delivery applications, PEG is used to decrease the immunogenicity against protein drugs, passive targeting for cancer chemotherapy agents and to increase water solubility and bioavailability in general [39].

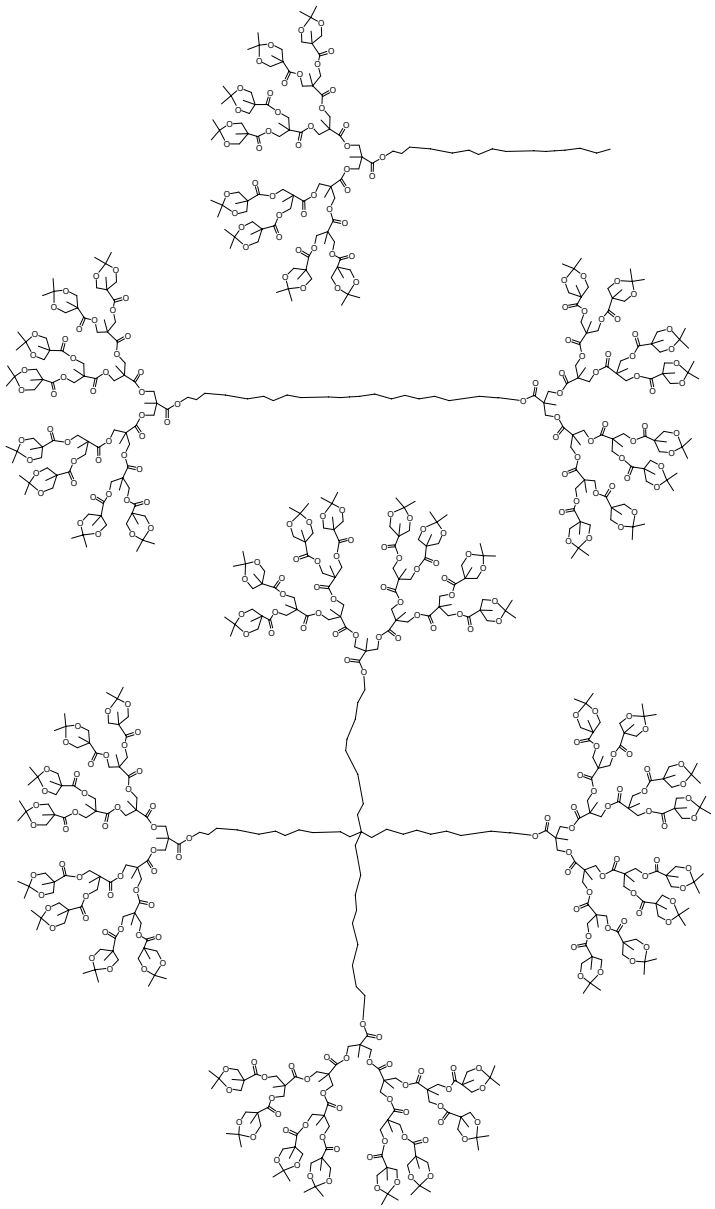


Figure 1.14. Fréchet reported the PEG based dendron polymer conjugates with one, two and four functional telechelic PEGs.

In 2001, Frechet reported the polymer dendron conjugate synthesis with multiarm PEG as the center and polyester dendron as the end groups (Figure 1.14) [40]. One, two or four dendron bearing copolymers were synthesized pioneering the water soluble dendron-polymer field. These dendron-polymer hybrids have interesting properties due to the intrinsic differences between the PEGylated core and dendritic endgroups of the of the copolymer, leading to form a amphiphilic molecule and can be used as surface modifiers,[41] “stimulusresponsive” materials,[42] and drug carriers,[43] for instance.

Namazi and coworkers synthesized dendron polymer dendron conjugates for smart drug delivery (Figure 1.15) [44, 45]. Surface of the dendrons were functionalized with carboxylic acids for increased water solubility and further partial functionalization. They used two different procedures to determine availability of functional groups. First one is benzyl alcohol.second procedure is the entrapment of Rose Bengal, a kind of fluorescent salt based on physical interactions also known as complexation method. İn both cases, the loading or binding capacity of linear–dendritic macromolecules depends on their generation; as the generation increases, binding capacity increases.

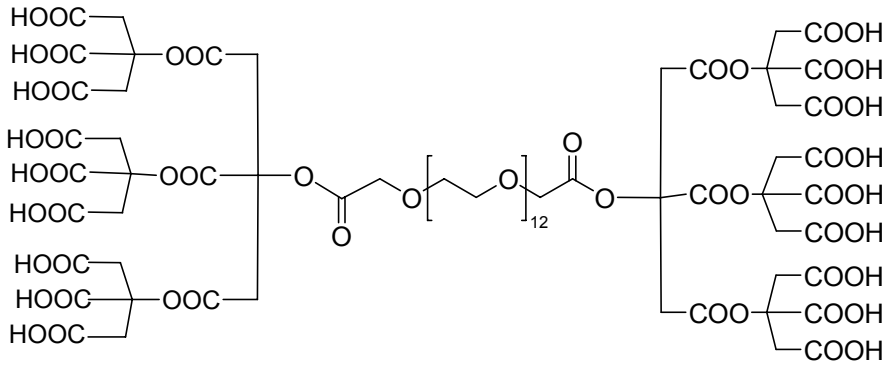


Figure 1.15. Schematic representation of dendron polymer dendron conjugates

Riguera published the synthesis of PEG-dendron block copolymers which had surface functionalities reactive for click reaction [46]. Mono functional PEGs were reacted with a G1 dendron and further grown up to G3 divergently (Figure 1.16). Functional surface groups of the dendrons contained azide functionality and they were reacted with alkyne containing unprotected carbohydrates via copper (I)-catalyzed [3+2] Huisgen cycloaddition in excellent yields. The reaction proceeded at room temperature, under aqueous conditions, and required only catalytic amounts of Cu. The resulting PEGylated glycodendrimers demonstrated an increased capacity to aggregate lectins with increasing dendron generation. These block copolymers represent a novel glycoconjugate structural design benefiting from the beneficial properties of PEG, that are envisioned as potential tools for the study of carbohydrate receptor interactions and as attractive building blocks for the production of active targeted drug delivery systems.

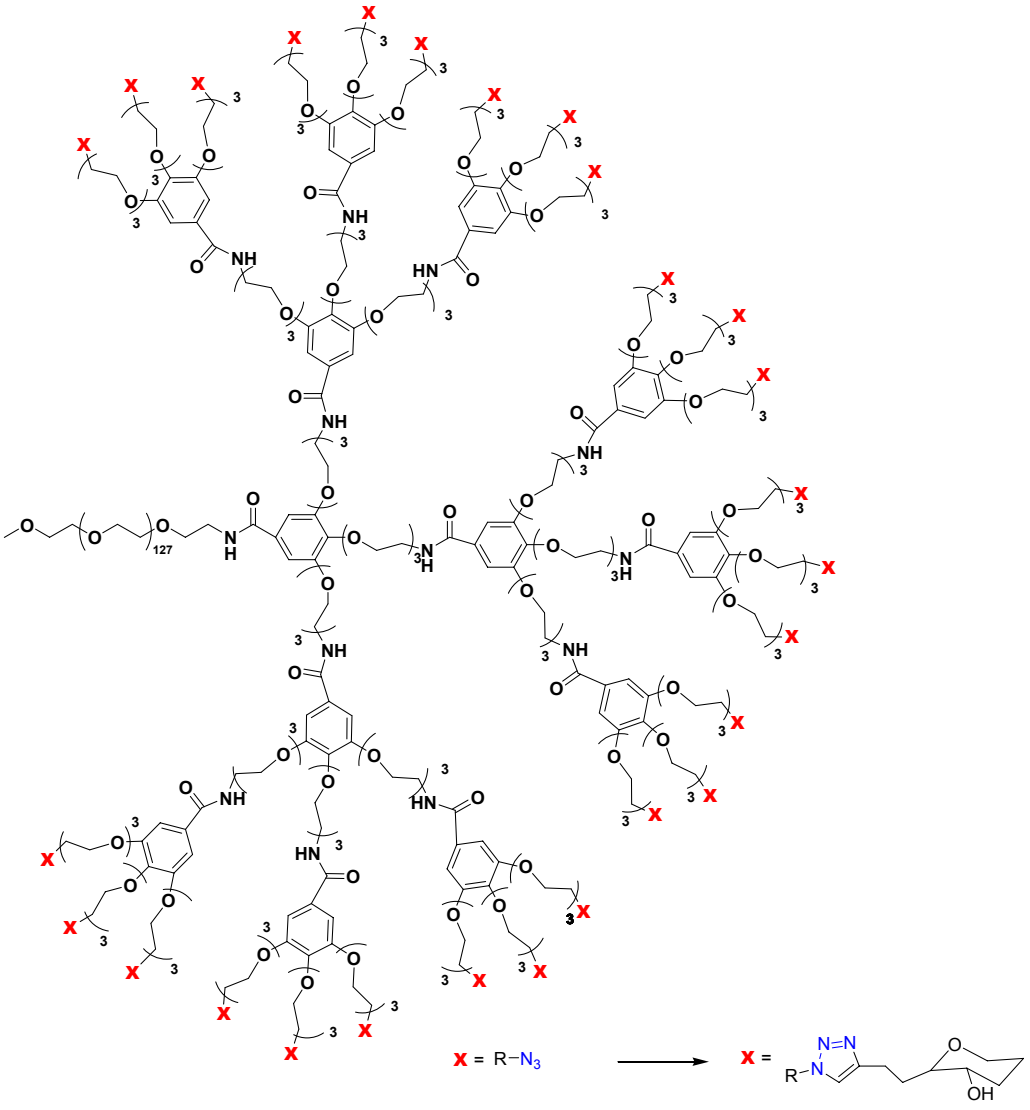


Figure 1.16. PEG-dendron block copolymers for targeted drug delivery systems.

1.7. Dendron-Polymer Based Hydrogels

Dendron-polymer-dendron based triblock copolymers have been also utilized for the synthesis of hydrogels. Polymerizable groups such as acrylate double bonds at the end of the hydrophobic blocks allow photocrosslinking of the structure to provide chemically crosslinked hydrogels. Grinstaff and coworkers reported the synthesis of biodegradable photocrosslinkable hybrid dendritic-linear triblock copolymers and applied them to seal corneal lacerations [47].

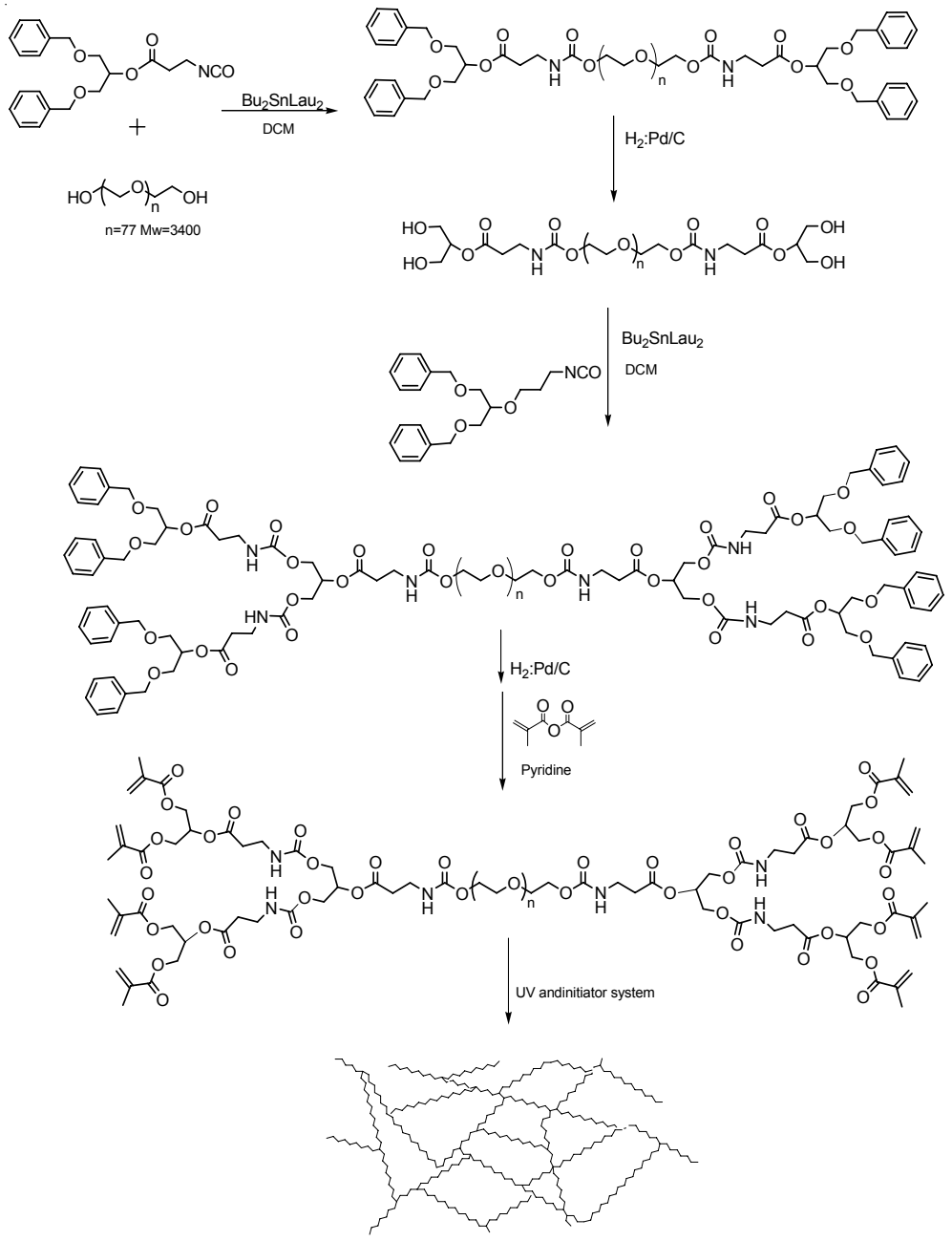


Figure 1.17. Formation of hydrogel from PGLBA-PEG macromolecule.

In these works, dendritic macromolecules composed of natural metabolites (i.e., succinic acid, glycerol, and *β*-alanine) connected to a nonimmunogenic poly(ethylene glycol) linear polymer by ester and carbamate functionalities in an ABA-type architecture (Figure 1.17).

2. AIM OF THE STUDY

We envisioned that the multivalent nature of the dendrons may allow utilization of some of the reactive end groups for crosslinking to afford a hydrogel, whereas the residual reactive groups can allow covalent post functionalization of the hydrogels with molecules of interest. In our design, we utilized the Huisgen-type click reaction to access dendron-polymer-dendron conjugates necessary for the hydrogel formulation. Thereafter, the dendron surfaces were functionalized with alkyne groups. Some of these alkyne groups at the periphery of the dendrons were utilized for cross linking via Huisgen-type click reaction with a small bisazide containing molecule to afford the hydrogel. The residual alkyne groups remain available for efficient post functionalization of the hydrogel using the click chemistry. Well defined functional Hydrogels successfully synthesized and by using different characterization methods such as IR, fluorescent microscopy, we achieve to prove that these residual unreacted alkyne groups are available for click reaction High water swelling, biocompatible and biodegradable hydrogel have a great potential for medical applications.

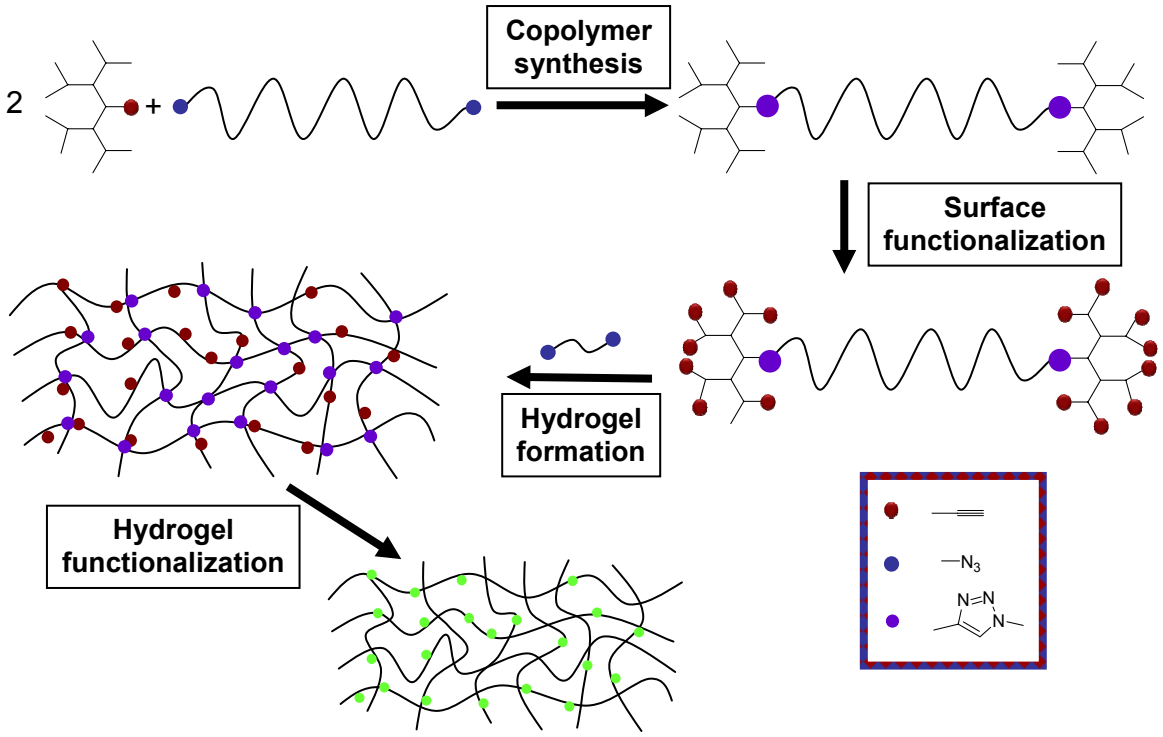


Figure 2.1. General Scheme for the synthesis of functionalized hydrogels

3. RESULTS AND DISCUSSION

3.1. Synthesis of Functional Hydrogels

Biodegradable polyester dendrons and biocompatible hydrophilic linear PEG polymers were combined via Huisgen type "click" chemistry to obtain dendron-polymer-dendron conjugates. Functionalization of the dendron periphery of the dendrons with alkyne groups affords reactive hydrogel precursors. While some of these alkyne groups are crosslinked using a bisazide to fabricate the hydrogel (second Huisgen type "click" reaction), the residual alkynes allow efficient covalent functionalization of the hydrogel matrix with molecules of interest via the third consecutive "click" reaction (Figure 3.1).

Synthesis of two different G2 dendron-polymer–dendron ABA triblock copolymers via Huisgen "Click" reaction and their functionalization with alkyne groups is shown in Figure 3.1. Aliphatic polyester dendrons were chosen as the A block of the copolymer. For the synthesis of the alkyne core bearing protected polyester dendrons literature procedure was followed (See SI). Deprotection of the acetonide groups of these G2 and G3 dendrons were achieved via treatment with DOWEX, H^+ yielding second generation dendron **1** and third generation **2**, respectively. Azide containing telechelic PEG polymers **3** and **4** were synthesized according to literature protocols. These triblock copolymers are named according to the dendron generations and length of PEG used: i.e. [G2]4OH[PEG2K] represents two G2 dendrimers with alcohol as the surface functionality bearing four alcohol groups as the A block and PEG polymer with MW=2000 as the middle (B) block. Two different PEG lengths, 2K and 6K, were explored for this study. The desired copolymers [G2]4OH[PEG2K] (**5**) and [G2]4OH[PEG6K] (**6**) were obtained via the reaction of alkyne core functionalized G2 polyester dendron **1** and bisazido PEG **3** or **4** in the presence of CuBr and PMDETA in THF. Functionalization of the surface alcohol groups thus obtained triblock copolymers were realized via the acylation reaction with pentynoic anhydride in the presence of pyridine yielding copolymers **8** and **9**. Similar procedures were applied for the G3 dendron **2** (Figure 3.1) yielding [G3]8OH[PEG6K] (**7**) and alkyne functionalized ABA block copolymer (**10**). Alkyne groups on the surface of the dendrons were now ready for the second 'click" reaction forming the hydrogels.

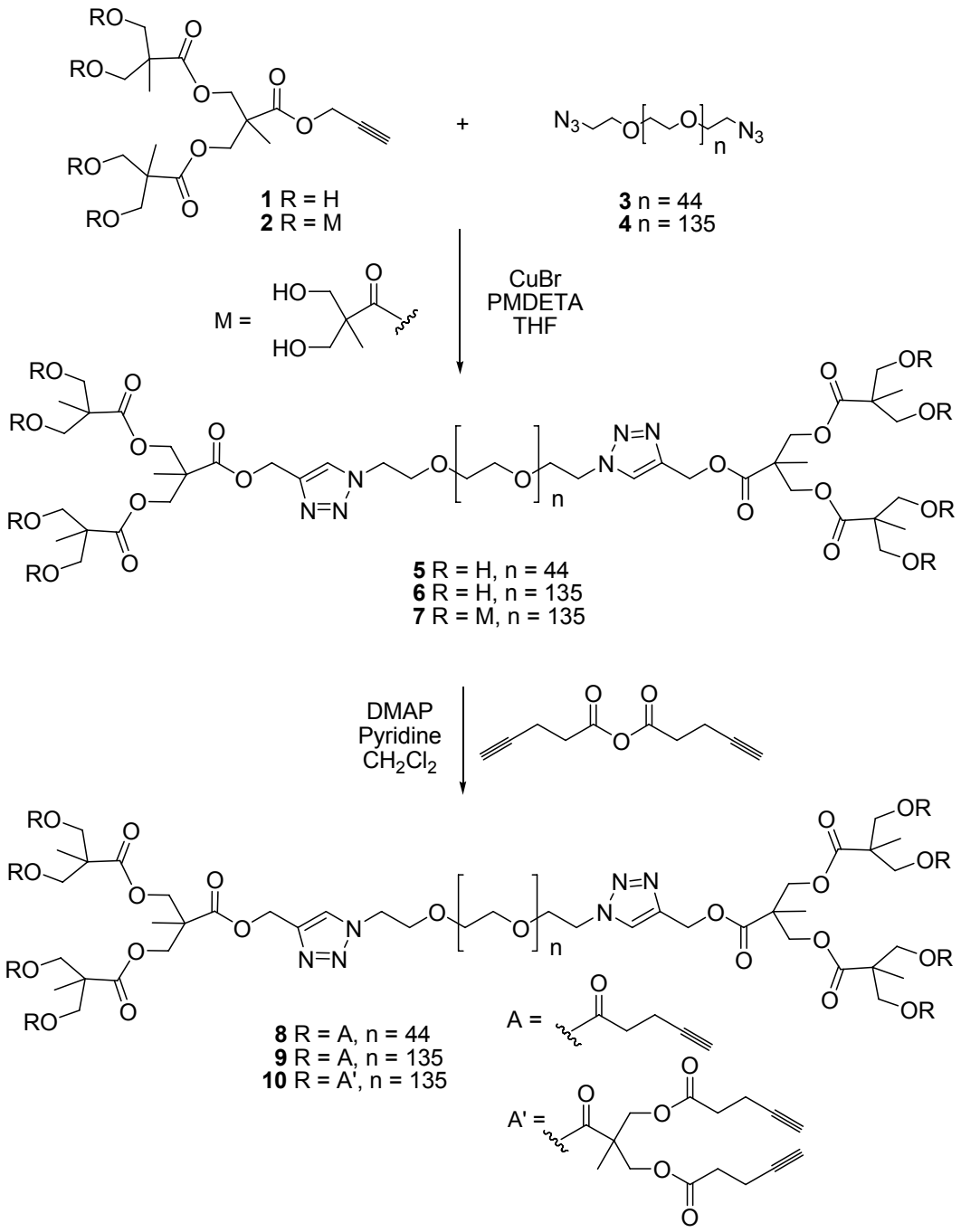


Figure 3.1. Synthesis and functionalization of G2 and G3 block copolymer

Well-defined hydrogels were prepared by the Huisgen "click" reaction between alkyne functionalized dendron-PEG-dendron triblock copolymers **8**, **9**, **10** and tetraethylene glycol bisazide (**11**) (Figure 3.2). Although dendron-polymer conjugates **5**, **6** and **7** have good water solubility, after the functionalization of the surface groups, the alkyne bearing shorter PEG chain conjugate **8** no longer bear the same property. Therefore, during the crosslinking of the dendron-polymer conjugate **8** in water, organic solvents such

as ethanol and THF were added to improve the solubility of the copolymer. Utilization of only ethanol during the gelation procedure was also successful and led to clear gels (Figure 3.3). In order to improve the water solubility of the dendron-polymer conjugate **8**, longer PEG chain (MW = 6000) was employed with G2 dendron and the resulting conjugate **9** was gelled in water without any solubility problems. On the other hand, hydrogel formation with the conjugate **10** in water as the solvent was not successful. One can envision that as the number of end groups increase with increasing generation, the hydrophobicity of the conjugate will increase rendering the overall copolymer less soluble in water. Thus, conjugate **10** was gelled in H_2O with the addition of THF and/or ETOH. The synthesized gels were subsequently placed in distilled water for 2 days to remove unreacted monomer with periodic replacement of the water used for leaching the organic solvents used for gelation.

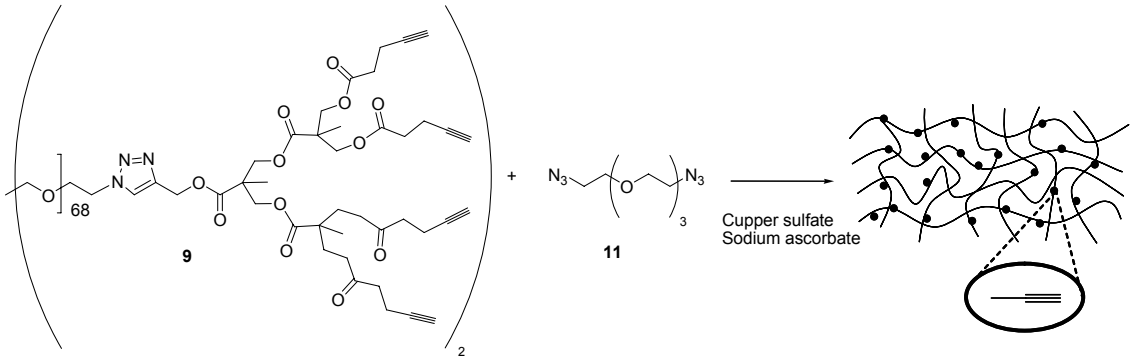


Figure 3.2. Hydrogel formation



Figure 3.3. Picture of transparent hydrogel

3.2. Characterization of Functional Hydrogels

For full characterization of the hydrogel precursors and for establishing the presence of residual alkyne functionality on the hydrogels, infrared spectra were taken. Figure 2 shows the IR spectra of starting components, namely the G3 dendron (a) and the bisazido PEG 6K (b), the copolymer (c) and the alkyne functionalized copolymer (d) and finally the 6KG3 (1:4) hydrogel. The IR spectra of the G3 dendron and the PEG diazide are given for comparison. The azide strech can be seen clearly at 2099 cm^{-1} for bisazido PEG (b) and is not present for the copolymers as expected. The alcohol surface groups in copolymer [G3]8OH[PEG6K] (**7**) (3435 cm^{-1}) are converted to alkyne groups in [G3]8OR[PEG6K] (**8**). The C-H strech of the alkyne functionality is observed at 3279 cm^{-1} for the copolymer [G3]8OR[PEG6K] (**8**) and this peak is noticeable at 3249 cm^{-1} for the hydrogel 6KG3 (1:4) with eight remaining alkynes, theoretically, demonstrating the presence of unreacted triple bonds.

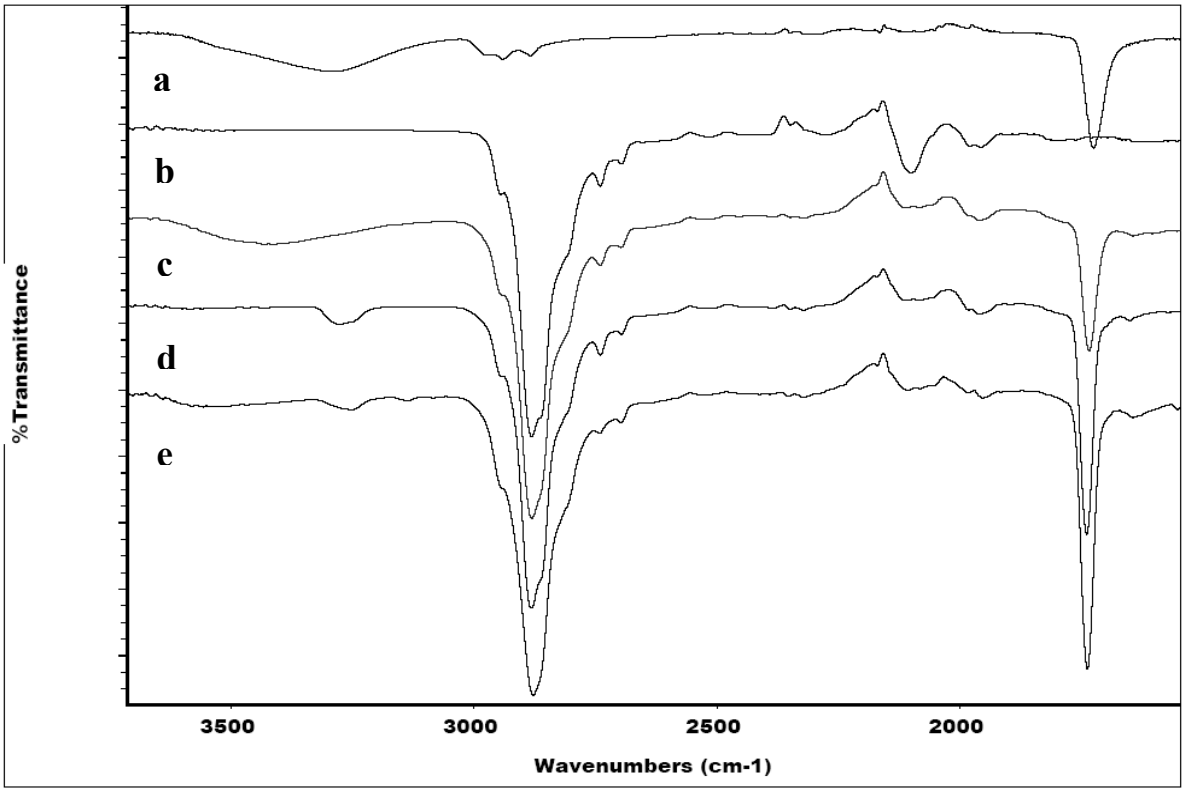


Figure 3.4. FTIR spectroscopies of products (a) G3 dendron 2 (b) PEG-6K bis-azide 4 (c) PEG dendron copolymer 7 (d) PEG-dendron alkynyl functionalized copolymer 10 (e) Functional 6KG3 (1:4) Hydrogel

The PEG-dendron macromolecules **8**, **9** and **10** were gelled with different ratios of tetraethylene glycol bisazide (**11**) resulting in a variety of hydrogels with different number of available functional groups (Table 3.1).

Item	HYDROGEL	PEG	DENDRON	Alkyne : diazide mol ratio	Water uptake %	Available functional groups
1	**2KG2 (1:3)**	**2000**	**G2**	**1 : 3**	**804**	**2**
2	**6KG2 (1:3)**	**6000**	**G2**	**1 : 3**	**1182**	**2**
3	**6KG2 (1:2)**	**6000**	**G2**	**1 : 2**	**1009**	**4**
4	**6KG2 (1:1.5)**	**6000**	**G2**	**1 : 1.5**	**954**	**5**
5	**6KG2 (1:1)**	**6000**	**G2**	**1 : 1**	**898**	**6**
6	**6KG3 (1:4)**	**6000**	**G3**	**1 : 4**	**2015**	**8**
7	**6KG3 (1:3)**	**6000**	**G3**	**1 : 3**	**1286**	**10**
8	**6KG3 (1:2)**	**6000**	**G3**	**1 : 2**	**1036**	**12**
9	**6KG3 (1:1)**	**6000**	**G3**	**1 : 1**	**938**	**14**

Table 3.1. Properties of hydrogels with different variations in dendron polymer and linker structure and quantity.

The hydrogels were labeled according to the molecular weight of the PEG and "A" block generation of the dendron, i.e. second generation dendron - PEG 2K = 2KG2. The different alkyne-diazide ratios ranging from 1:1 to 1:4 used during gelation are shown in Table 3.1. 2KG2 and 6KG2 dendron-PEG copolymers have a total of eight alkynes on the periphery. Since tetraethylene glycol bisazide (**11**) has two azide groups, three equivalents of the azide would react with six of the peripheral alkynes, leaving two alkynes for further functionalization (Table 3.1, items 1 and 2). Two equivalents of the diazide **11** would result in four available alkynes (Table 3.1, item 3) and decreasing amounts (1.5 and 1 eqv) of the diazide **11** would leave five and six available alkynes, respectively (Table 3.1, items 4 and 5). 6KG3 dendron-PEG copolymers on the other hand have a total of sixteen peripheral alkynes.

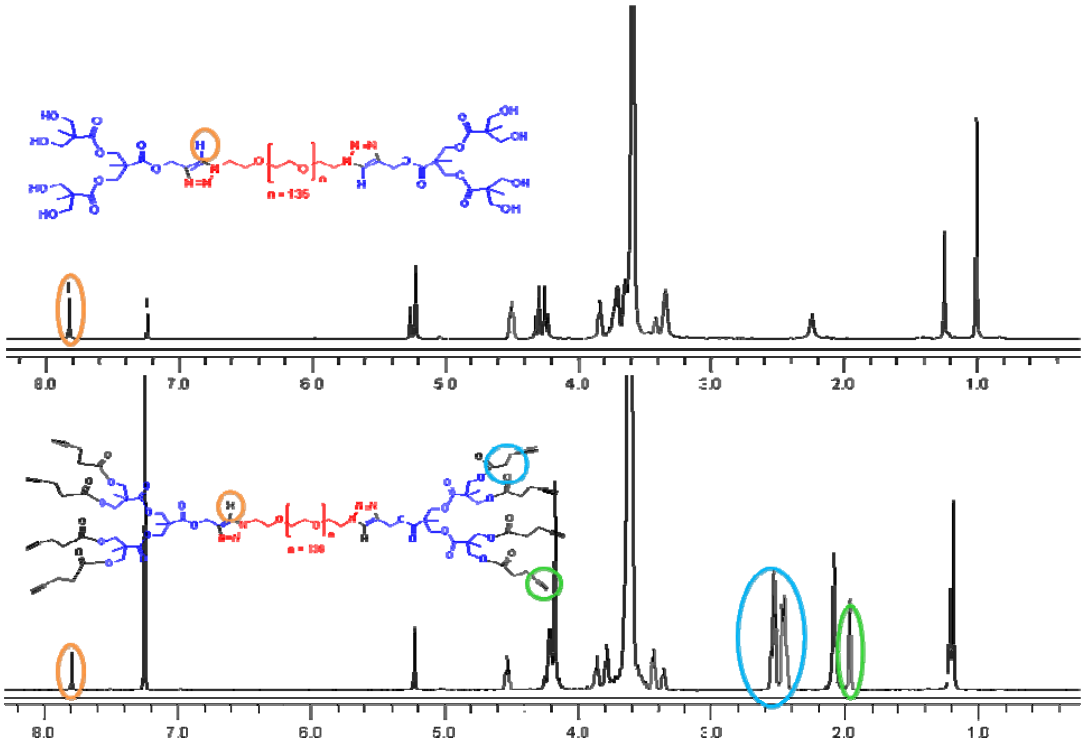


Figure 3.5. ^{1}H NMR spectrum of [G2]4OH[PEG6K] **6** and [G2]4OR[PEG6K] **9**

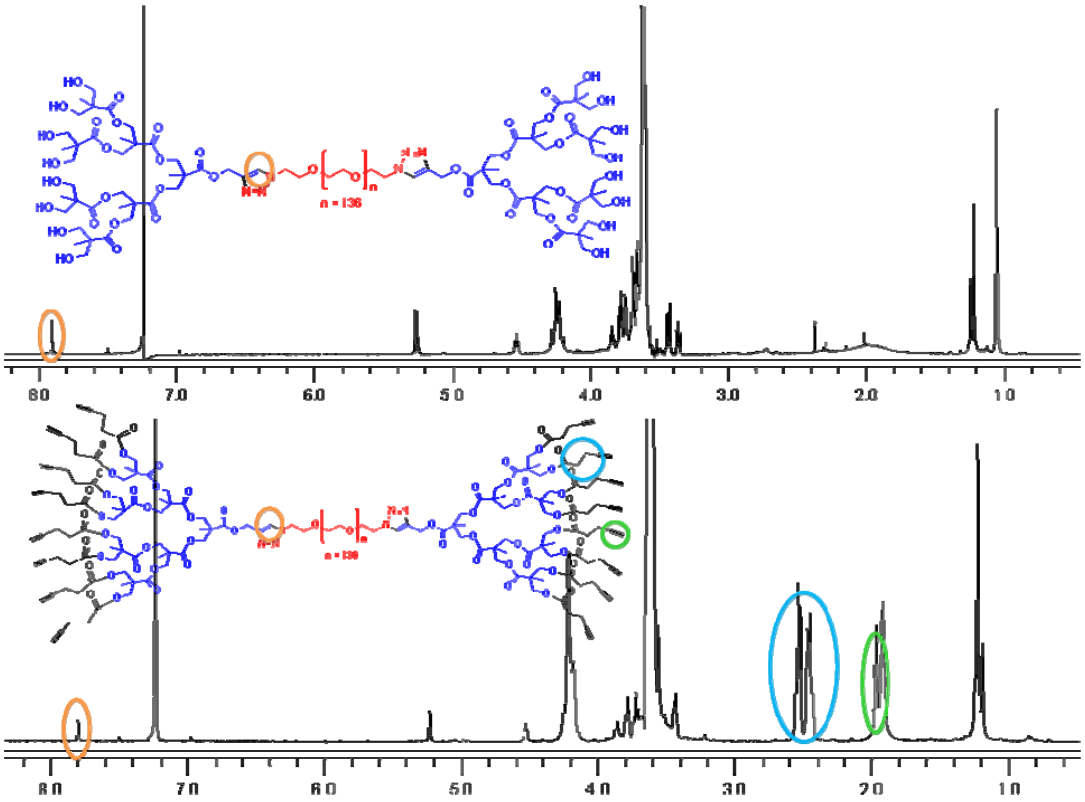


Figure 3.6. ^{1}H NMR spectrum of [G3]8OH[PEG6K] **7** and [G3]8OR[PEG6K] **10**

Four equivalents of the diazide **11** would leave eight alkynes for functionalization (Table 3.1, item 6), and decreasing equivalents of the diazide **11** would result in increasing available alkynes in the hydrogel (Table 3.1, items 7, 8 and 9).

If we compare the ^{1}H NMR spectrum of copolymers **6** and **9** there are remarkable differences. ^{1}H NMR spectrum of copolymer **9** contains additional peaks coming from reacted pentynoic anhydride functionalization of copolymer **6** (Figure 3.5). The same difference in the ^{1}H NMR spectrum of copolymers **7** and **10** are also observed (Figure 3.6).

3.3. Functionalization of Hydrogels

To demonstrate the presence and reactivity of remaining alkyne groups, the hydrogels were reacted with azide bearing molecules in a [3+2] Huisgen cycloaddition. The choice of azides were as follows: The first dye, 4-Azido-*N*-ethyl-1,8-naphthalimide (**12**), shows no fluorescence but has click-activated fluorogenic properties showing an emission maximum between 400 – 500 nm. The second dye, bodipy azide **16** has an emission at red-shifted wavelengths. The third and last azide, biotin azide (**14**) is attached for immobilization of streptavidin as an example of protein attachment on the hydrogels.

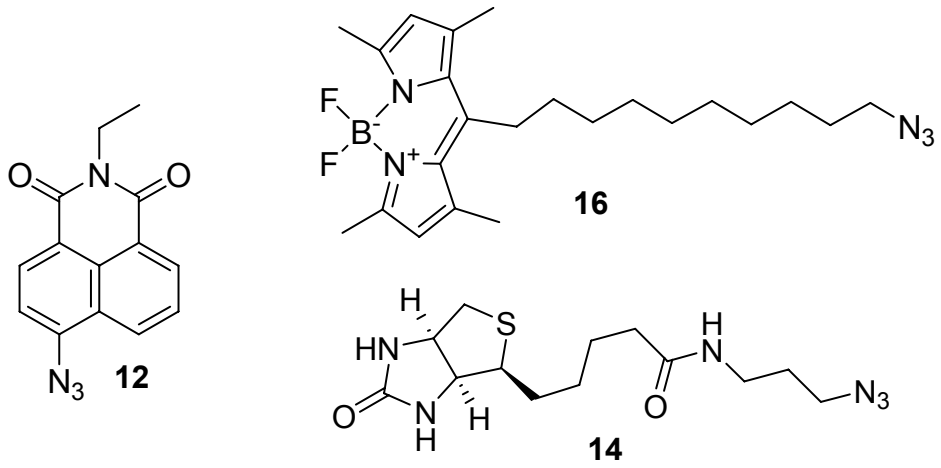


Figure 3.7. Azides for hydrogel functionalization

The functionalized hydrogels were visualized under the fluorescent microscope (Figure 3.7). The cylindrical hydrogel was divided into two parts and as one part was treated with the desired azide and the catalyst system, the other part was treated with only

the azide for adsorption control. The catalyst system for the naphthalimide dye **12** and the biotin azide (**16**) was $CuSO_4$-sodium ascorbate, whereas the bodipy azide required CuBr-PMDETA for solubility reasons. The fluorescence microscope images of the catalyst bearing reactions produced colored images (Figure 3.8 a-f).

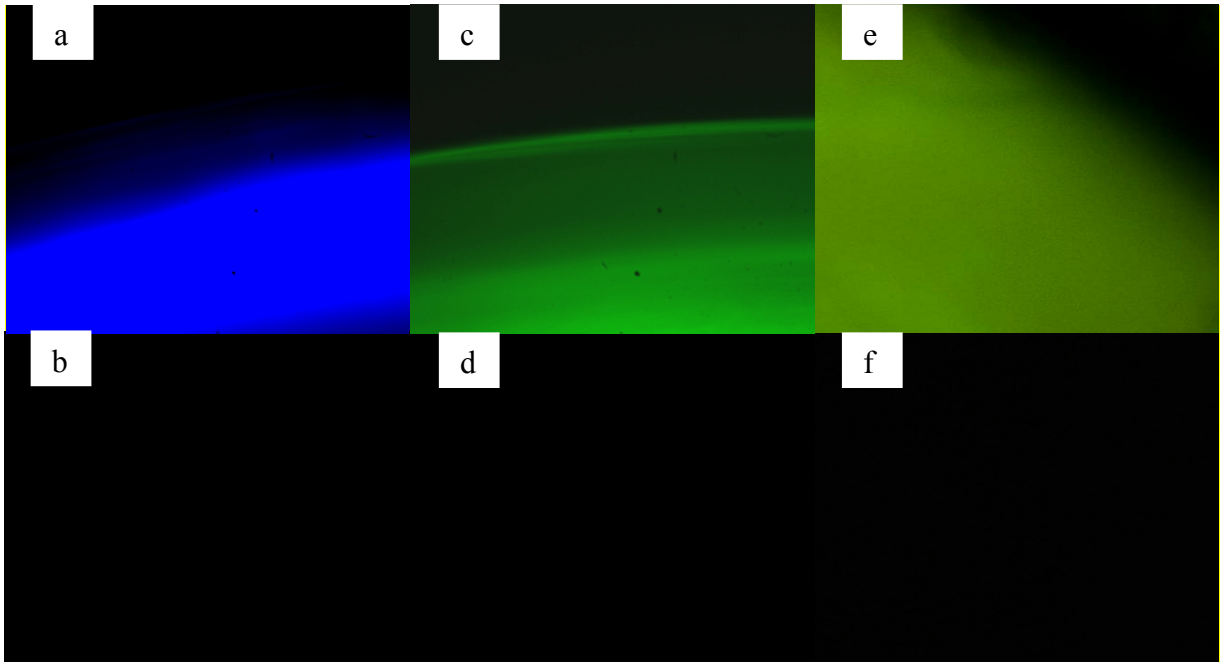


Figure 3.8. Fluorescence microscope images of functionalized hydrogels (a) with 4-Azido-N-ethyl-1,8-naphthalimide (**12**) (b) control of a without catalyst (c) with biotin azide (**14**)-streptavidin (d) control of c without catalyst (e) with bodipy azide (**16**) (f) control of e without catalyst

The images were taken at the edge of the hydrogel for contrast purposes. For the demonstration of an enzyme immobilization, the hydrogels that were treated with biotin azide (**14**) in the presence and absence of the catalyst system were incubated with FITC labeled streptavidin. The resulting images of streptavidin immobilization are shown in Figure 3.7 (c) and 3.7 (d).

To establish the relationship between number of remaining alkyne groups in the hydrogel and the ability to functionalize, hydrogels 6KG3 (1:4)-D were first reacted with same amounts of biotin azide (**14**) and then incubated with FITC labeled streptavidin. Each hydrogel was accompanied by the control gel, going through the same treatments without the catalyst system. Relative fluorescence values were calculated via J image 1.41 software

and plotted in Figure 3.9. As can be seen clearly from the graph, there was a direct correlation between the number of alkynes in the hydrogel and fluorescence after functionalization.

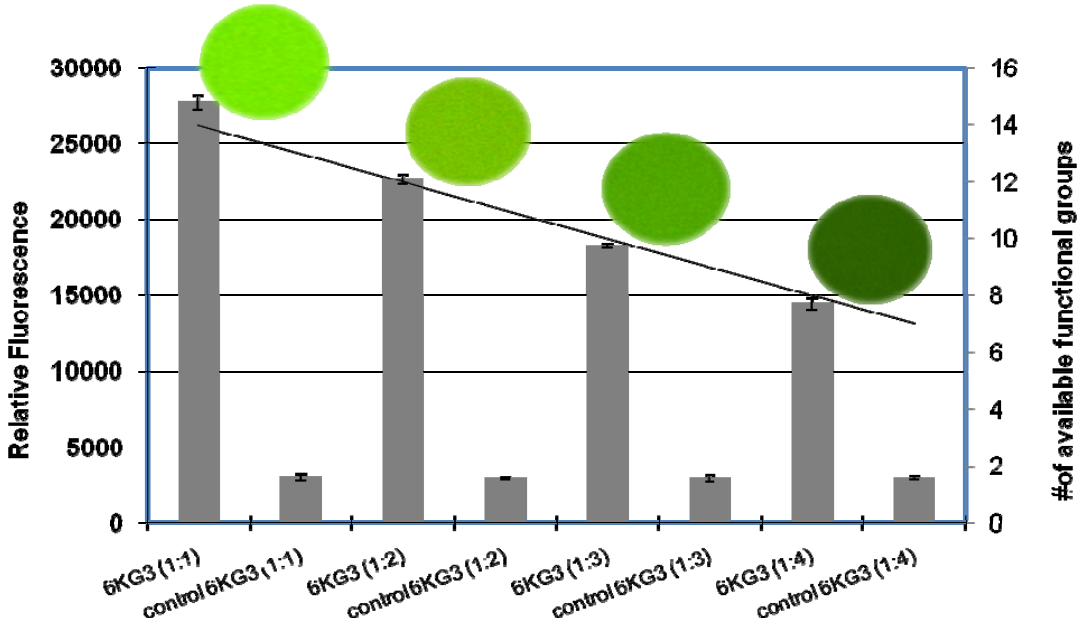


Figure 3.9. Relative fluorescence values of functionalized hydrogels with streptavidin and their controls that contain descending amount of functional groups.

3.4. Swelling Properties of Hydrogels

The swelling properties of the hydrogels were examined. Water uptake of two different constructs in which the length of the hydrophilic section was varied is shown in Figure 3.10.

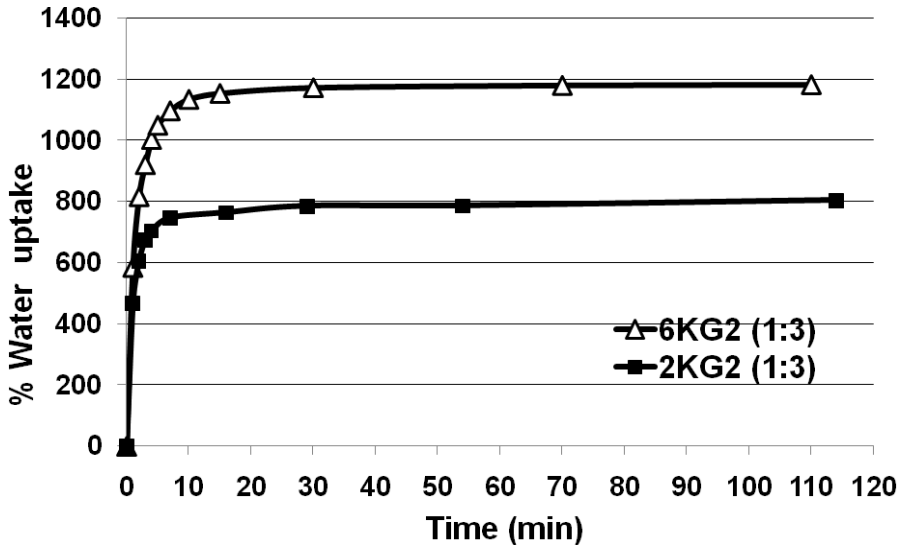


Figure 3.10. Water uptake comparison of 2KG2 (1:3) and 6KG2 (1:3) hydrogels

The hydrogel formation of the constructs 2KG2 (1:3) and 6KG2 (1:3) having PEG molecular weights of 2000 and 6000 respectively, was achieved with the same mol ratio of the diazide compound (1:3 alkyne:azide) and water uptake measurements were undertaken as explained. As can be seen in Figure 3.10, increase in PEG length has resulted in an increase in amount of water uptake as expected.

Comparative swelling behavior of 6KG2 and 6KG3 hydrogels were investigated as a function of time and the equilibrium hydration levels were reported in Table 1. The hydrogels prepared with more equivalents of the diazide **11**, namely 6KG2 (1:3) and 6KG3 (1:4), showed higher degree of swelling than the less crosslinked counterparts 6KG2 (1:1) and 6KG3 (1:1) (Figure 3.11 and 3.12). The increasing amount of the diazide, though increasing the crosslinking density, would also decrease the amount of hydrophobicity thus making the hydrogel more hydrophilic and ready to absorb water.

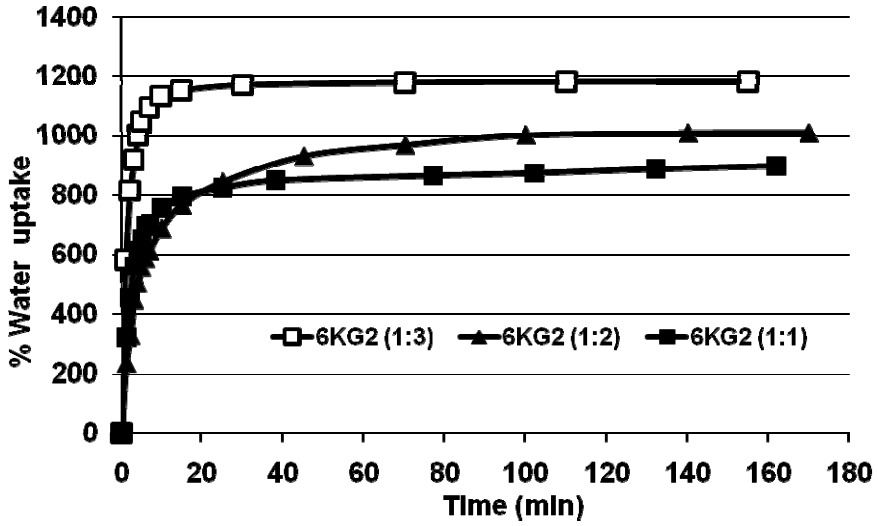


Figure 3.11. Water uptake comparison of 6KG2 (1:3) (1:2) and (1:1) hydrogels

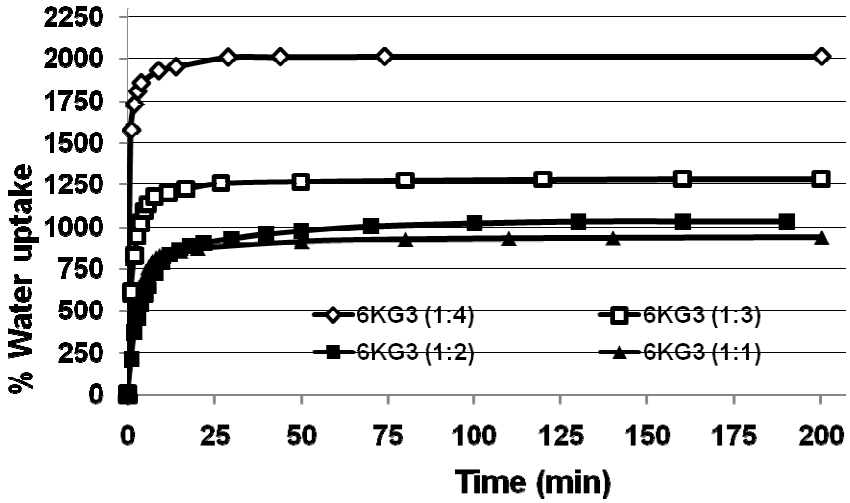


Figure 3.12. Water uptake comparison of 6KG3 (1:4), (1:3), (1:2), (1:1) hydrogels

3.5. Temperature Effect on Swelling Properties of Hydrogels

Percent change in water uptake of hydrogel 6KG2 (1:1) versus temperature is shown in Figure 3.13. Hydrogel 6KG2 (1:1) was placed in a water bath with increasing temperature after reaching hydration equilibrium at 0 ^{o}C. As expected, increasing the temperature of the water resulted in shrinking of the hydrogel.

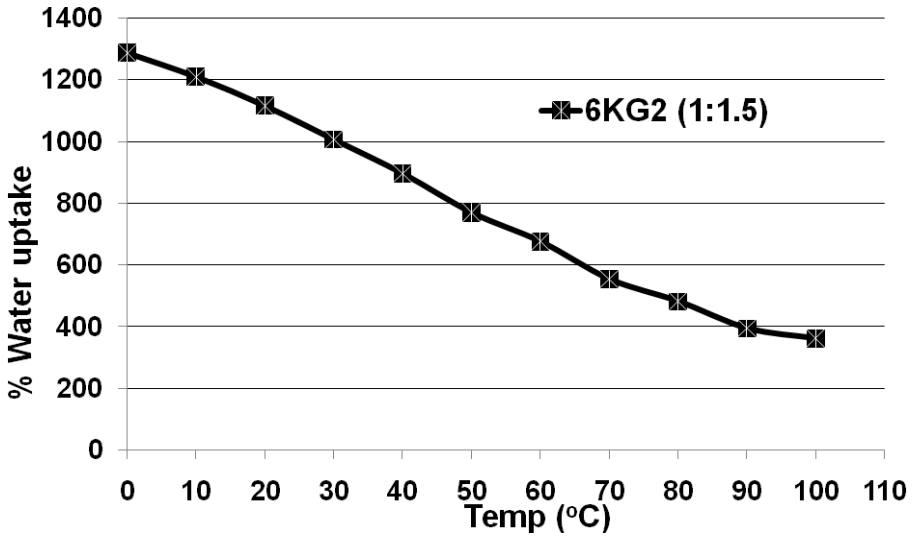


Figure 3.13. Temperature dependence of water uptake

3.6. Morphological Studies

SEM micrographs of dry and swollen hydrogels are presented in Figure 3.14 – 3.18. The morphological differences between dry and wet state of hydrogel can be clearly observed. No visible pores are seen in the "wet" pictures (Figure 3.14). Dry hydrogel presents a porous structure with 2.0 Micrometer-sized pores along with the smaller holes can be seen in the SEM images of 6KG3 (1:3) (Figure 3.18).

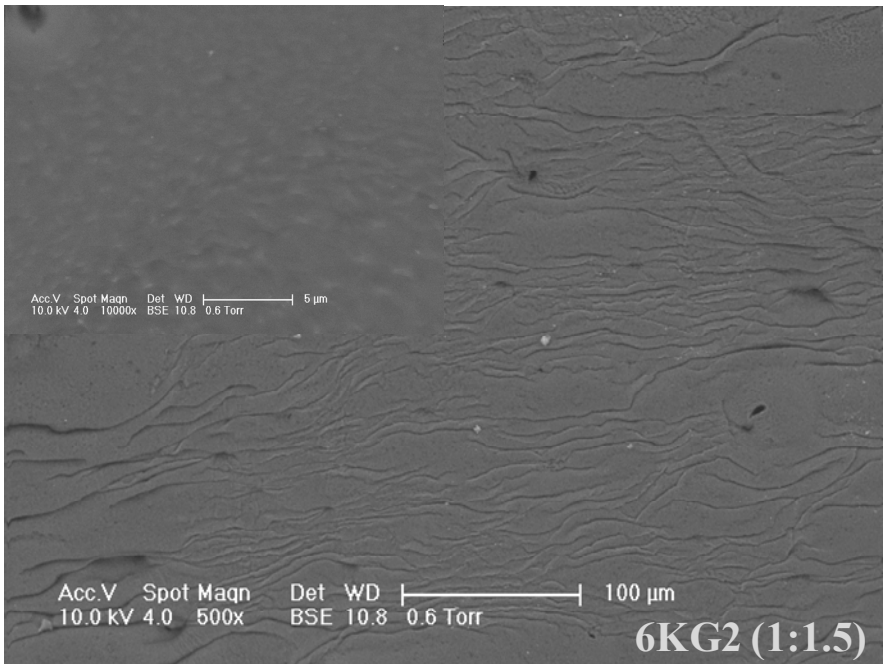


Figure 3.14. SEM images of wet hydrogel 6KG2 (1:1.5)

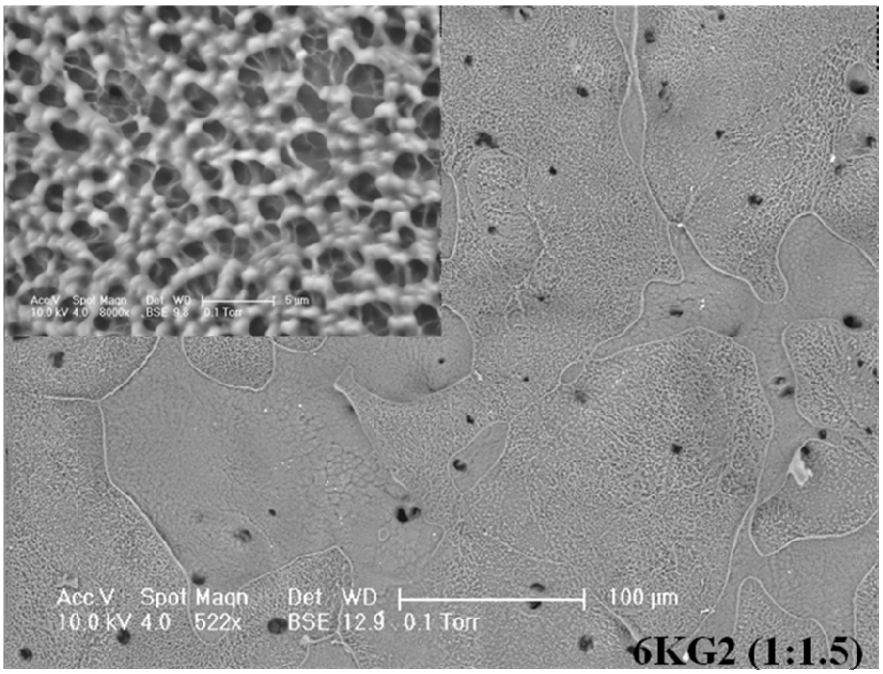


Figure 3.15. SEM images of dry hydrogel 6KG2 (1:1.5)

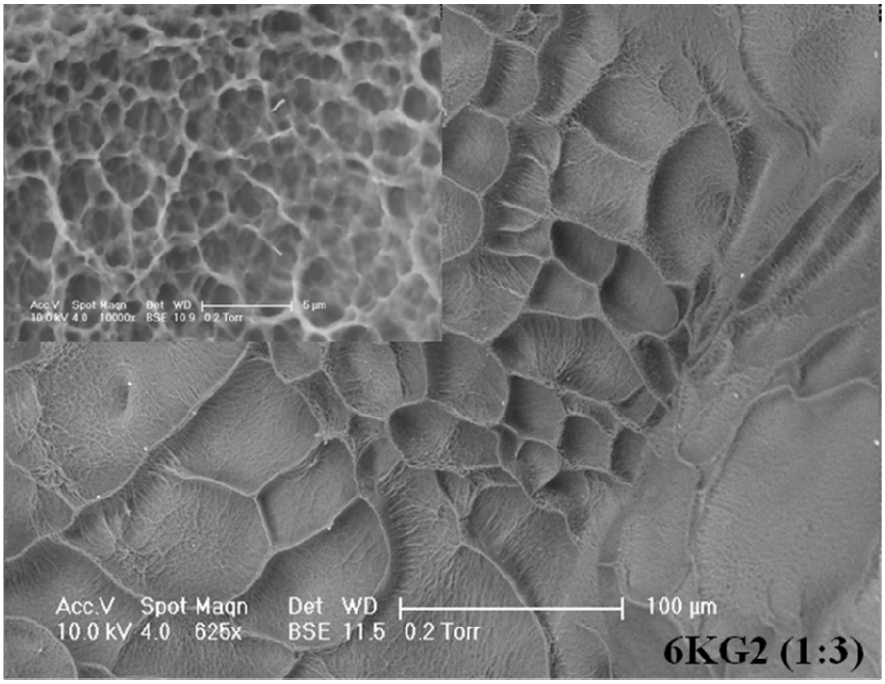


Figure 3.16. SEM images of dry hydrogel 6KG2 (1:3)

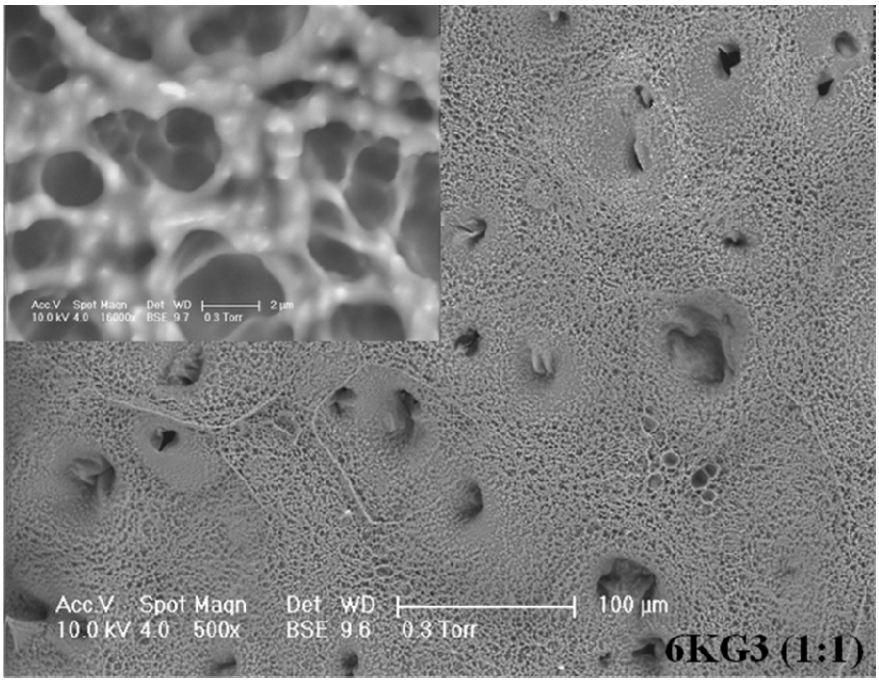


Figure 3.17. SEM images of dry hydrogel 6KG3 (1:1)

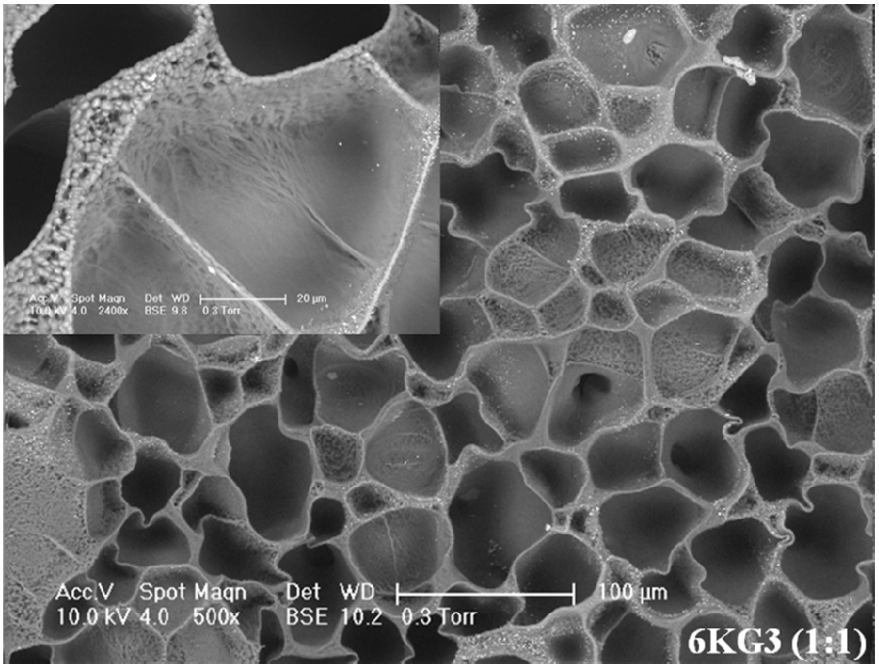


Figure 3.18. SEM images of dry hydrogel 6KG3 (1:1)

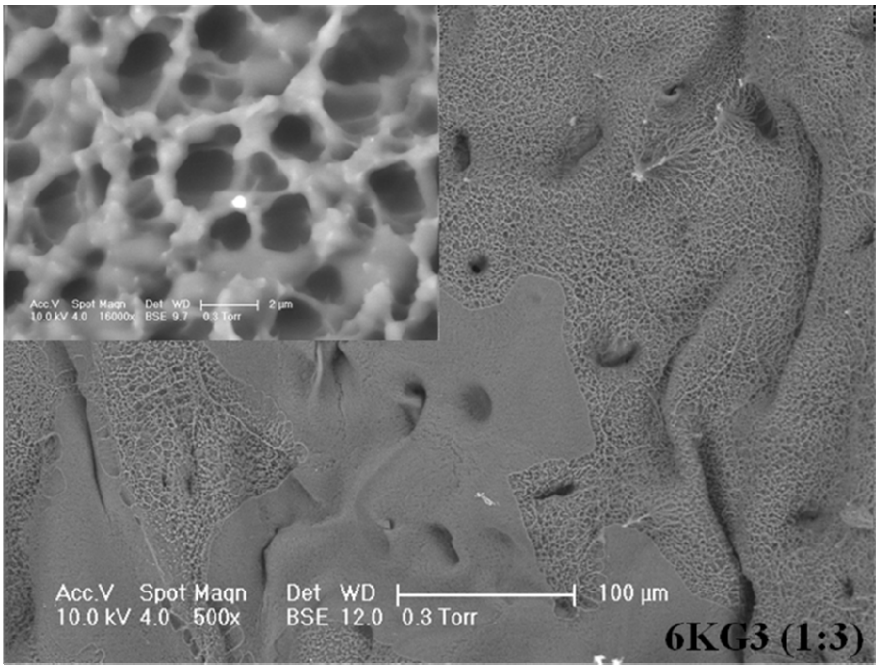


Figure 3.19. SEM images of dry hydrogel 6KG3 (1:3)

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 glycol 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. Synthesis of dendrons **1** and **2** are given in supporting information. The monomer and copolymer characterizations involved 1H 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 S.p.A. FlashEA® 1112 Elemental Analyzer (CHNS separation column, PTFE; 2 m; 6x5 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 10 kV.

4.2. Synthesis of Dendrons

4.2.1. Synthesis of 2nd Generation Alkyne Functionalized Dendron (1)

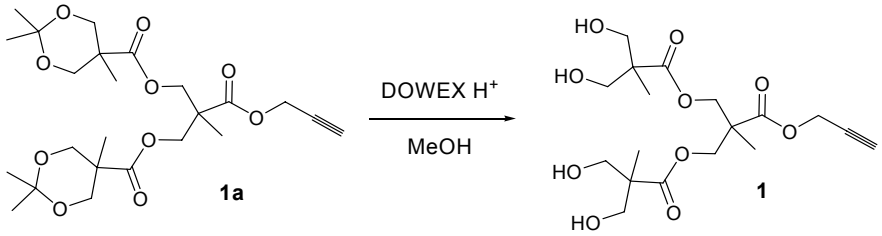


Figure 4.1. Synthesis of 2nd Generation of Dendron (**1**)

Alkyne functionalized dendrons **1a** and **2a** were synthesized according to previously reported literature procedures. [REF] Dowex X50WX2 (1.00g) was placed in a 100 mL Erlenmeyer flask and washed methanol (5 x 50 mL). Subsequently, Compound **1a**

(4.00 g, 0.019 mmol), Dowex X50WX2 (1.00 g) and methanol (50 mL) were added to a 100 mL rb flask and stirred for 24 h. After the reaction was completed Dowex was removed via filtration and the filtrate was dried under *vacuo* yielding compound **1** (3.17 g, 99%) as a white solid. ^{1}H NMR (CD_3OD, δ, ppm) 4.76 (d, 2H, *J* = 2.0 Hz), 4.30 (d, 2H, *J* = 12.2 Hz), 4.27 (d, 2H, *J* = 12.2 Hz), 3.68 (d, 4H, *J* = 11.0 Hz), 3.60 (d, 4H, *J* = 11.0 Hz), 2.96 (t, 1H, *J* = 2.0 Hz), 1.31 (s, 3H), 1.15 (s, 6H). FTIR (cm^{-1}): 3257, 1715. $C_{18}H_{28}O_{10}$ Calcd: C, 53.46; H, 6.98. Found: C, 53.80; H, 7.15.

4.2.2. Synthesis of 3rd Generation Alkyne Functionalized Dendron (2)

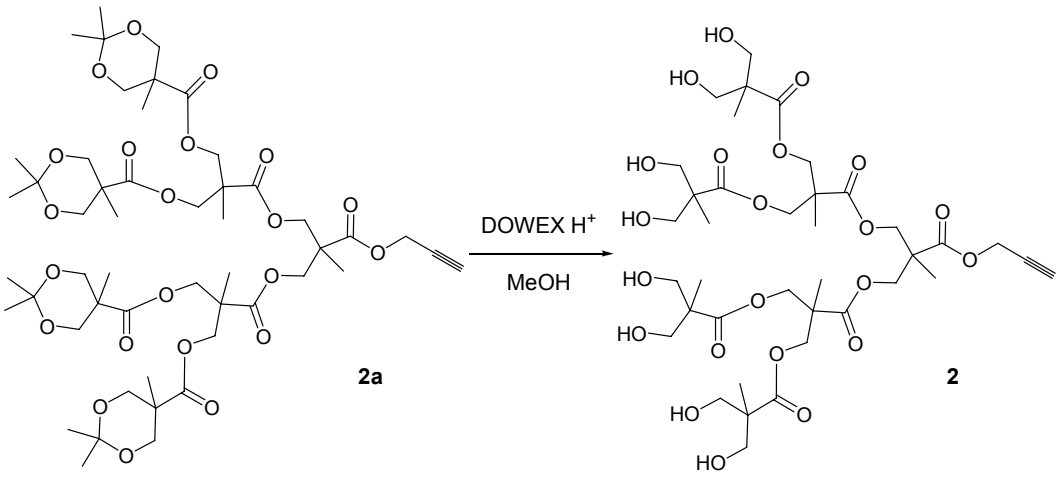


Figure 4.2. Synthesis of 2nd Generation of Dendron (2)

Dowex X50WX2 (1.00g) was placed in a 100 mL Erlenmeyer flask and washed methanol (5 x 50 mL). Subsequently, Compound **2a** (0.600 g, 1.24 mmol), Dowex X50WX2 (1.00 g) and methanol (50 mL) was added to a 100 mL rb flask and stirred for 24 h. After the reaction was completed Dowex was removed via filtration and the filtrate was dried under *vacuo* yielding compound **2** (0.495 g, 99%) as a white solid. ^{1}H NMR (CD_3OD, δ, ppm) 5.52 (d, 2H, *J* = 1.6 Hz), 4.39-4.28 (m, 12H), 3.68 (d, 8H, *J* = 10.8 Hz), 3.60 (d, 8H, *J* = 10.8 Hz), 3.01 (t, 1H, *J* = 1.6 Hz), 2.19 (s, 8H), 1.36 (s, 3H), 1.33 (s, 6H), 1.19 (s, 12H). FTIR (cm^{-1}): 3289, 1724. $C_{38}H_{60}O_{22}$ Calcd: C, 51.47; H, 7.19. Found: C, 51.48; H, 7.04.

4.3. Synthesis of PEG bis-azides (3 and 4)

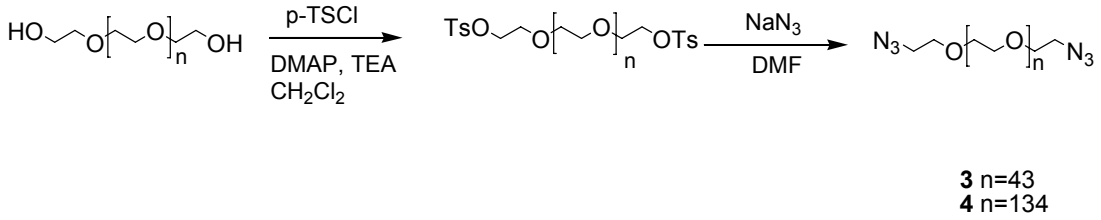


Figure 4.3. Synthesis of PEG bis-azides (**3** and **4**)

p-TsCl (3.82 g, 20 mmol) was added to a flask containing PEG 2K diol (4.0 g, 2 mmol), DMAP (2.44 g, 20 mmol) and triethylamine (5.5 g, 20 mmol) in CH_2Cl_2 (25 mL). The reaction was stirred at room temperature for 24 h. After the reaction was completed, the mixture was poured onto cold aq HCl (6M, 100 mL) and was extracted with CH_2Cl_2 (3 x 75 mL). Combined organic layers were dried over $NaSO_4$ and the solvent was removed under *vacuo.* Polymer was precipitated in cold diethyl ether and filtered. Dried polymer (3.9 g, 1.8 mmol) was dissolved in DMF (25 mL) and sodium azide (1.17 g, 18 mmol) was added to the solution. The reaction mixture was stirred for 24 h at 60 ^oC. The mixture was poured into cold aq HCl (6M, 100 mL) and was extracted with CH_2Cl_2 (3 x 75 mL). Solvent was evaporated under *vacuo.* Polymer was precipitated in cold diethyl ether. Product dried under high vacuum yielding 3.60 g (90%) of yellowish white solid. FTIR (cm^{-1}): 2884, 2100, 1102.

Synthesis of Compound **4.** Synthesized in a similar manner to Compound **3** starting from PEG 6K.

4.4. Synthesis of [G2]4OH[PEG2K] Copolymer (5)

PEG-2K-Diazide (**3**) (512 mg, 0.25 mmol) and propargyl [G2]4[OH] (**1**) (302 mg, 0.625 mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (3.6 mg, 0.025 mmol), PMDETA (0.5 μL, 0.025 mmol) in dry THF (2 mL) and purged with N_2.

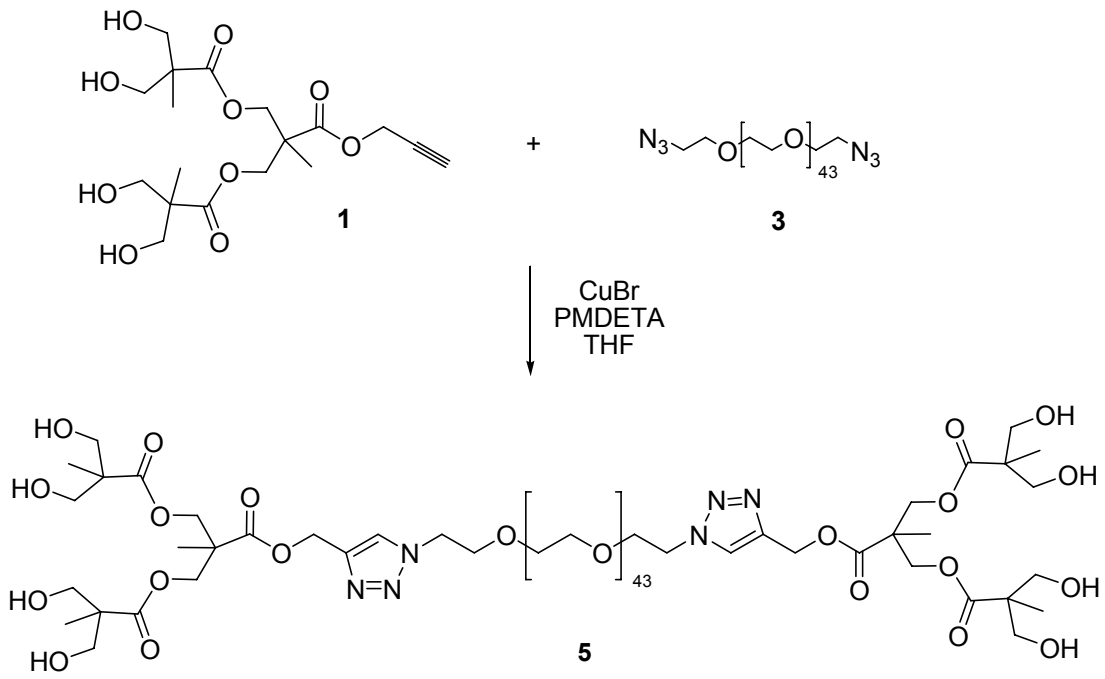


Figure 4.4. Synthesis of [G2]4OH[PEG2K] Copolymer (**5**)

The mixture was then transferred onto azide-propargyl alcohol solution and stirred at room temperature for 24 h. The solvent was then evaporated and the crude product was dissolved in CH_2Cl_2 (100 mL) and extracted with H_2O (25 mL) to remove copper salts. The solvent was concentrated under *vacuo* and the desired product was precipitated with Et_2O, filtered and dried under *vacuo* yielding compound **5** (680 mg, 95%) as a yellowish white solid. ^1H 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}): 3431, 2868, 1731. $C_{126}H_{236}N_6O_{64}$ Calcd: C, 52.97; H, 8.25 N, 2.94. Found: C, 53.01; H, 8.51 N, 3.00..

4.5. Synthesis of [G2]4OH[PEG6K] Copolymer (6)

PEG-6K-Diazide (**4**) (1000 mg, 0.167 mmol) and propargyl [G2]4[OH] (**1**) (73 mg, 0.183 mmol) were dissolved in dry THF (3 mL). In a separate flask were dissolved CuBr (2.4 mg, 0.017 mmol), PMDETA (3.5 μL, 0.017 mmol) in dry THF (2 mL) and purged with N_2. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at room temperature for 24 h.

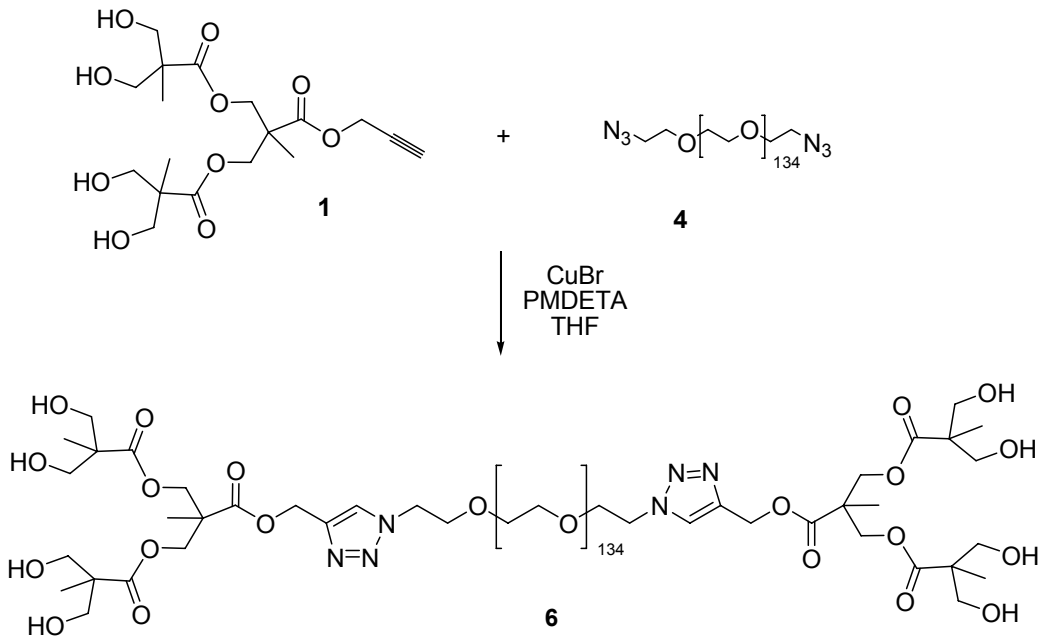


Figure 4.5. Synthesis of [G2]4OH[PEG6K] Copolymer (**6**)

The solvent was then evaporated and the crude product was dissolved in CH_2Cl_2 (100 mL) and extracted with H_2O (25 mL) to remove copper salts. The solvent was concentrated under *vacuo* and the desired product was precipitated with Et_2O, filtered and dried under *vacuo* yielding compound **6** (0.918 g, 80%) as a yellowish white solid ^{1}H NMR ($CDCl_3$, δ, ppm) 7.85 (s, 2H), 5.25 (s, 4H), 4.53 (t, 4H, J = 4.0 Hz), 4.34 (d, 4H, J = 10.8 Hz), 4.28 (d, 4H, J = 10.8 Hz), 3.86 (t, 4H, J = 4.0 Hz), 3.79-3.21 (m, 540H), 1.28 (s, 6H), 1.02 (s, 12H). FTIR (cm^{-1}): 3468, 2881, 1732. $C_{308}H_{6008}N_6O_{155}$ Calcd: C, 53.88; H, 8.78; N, 1.22. Found: C, 54.10; H, 9.01; N, 1.20.

4.6. Synthesis of [G3]8OH[PEG6K] Copolymer (7)

PEG-6K-Diazide (**4**) (0.100 g, 0.167 mmol) and propargyl [G3]8[OH] (**2**) (0.332 g, 0.383 mmol) were dissolved in dry THF (4 mL). In a separate flask were dissolved CuBr (2.4 mg, 0.017 mmol), PMDETA (3.5 μL, 0.017 mmol) in dry THF (3 mL) and purged with N_2. The mixture was then transferred onto azide-propargyl alcohol solution and stirred at room temperature for 24 h.

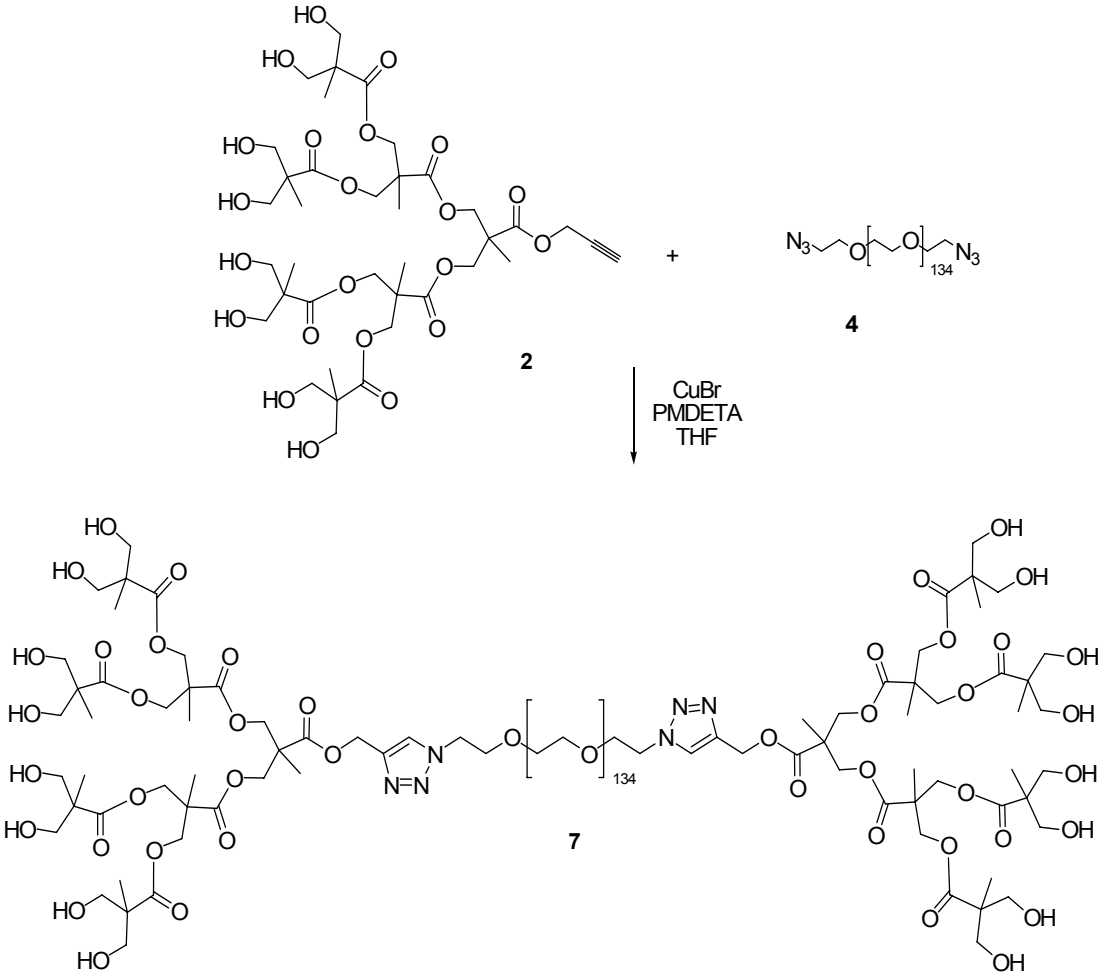


Figure 4.6. Synthesis of [G3]8OH[PEG6K] Copolymer (**7**)

The solvent was then evaporated and the crude product was dissolved in CH_2Cl_2 (100 mL) and extracted with H_2O (25 mL) to remove copper salts. The solvent was concentrated under *vacuo* and the desired product was precipitated with Et_2O, filtered and dried under *vacuo* yielding compound **7** (0.92 g, 71%) as a yellowish white solid. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.91 (s, 1H), 5.27 (s, 2H), 4.54 (t, 2H, *J* = 4 Hz), 4.28-4.19 (m, 12H), 3.85 (t, 2H, *J* = 4 Hz), 3.80-3.42 (m, 270H), 1.25-1.17 (m, 12H), 1.06 (s, 9H). FTIR (cm^{-1}): 3435, 2881, 1732. $C_{348}H_{664}N_6O_{179}$ Calcd: C, 53.61; H, 8.58 N, 1.08. Found: C, 53.22; H, 8.68; N, 1.45.

4.7. Functionalization of the [G2]4OH[PEG2K] Copolymer (8)

[G2]4OH[PEG2K] (**5**) (0.100 g, 0.035 mmol), pyridine (0.30 mL) and DMAP (0.003 g, 0.028 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL round bottom

flask. To the stirring reaction mixture was added 5-pentynoic acid anhydride (0.075 g, 0.42 mmol) and continued stirring for 24 h at room temperature under N_2.

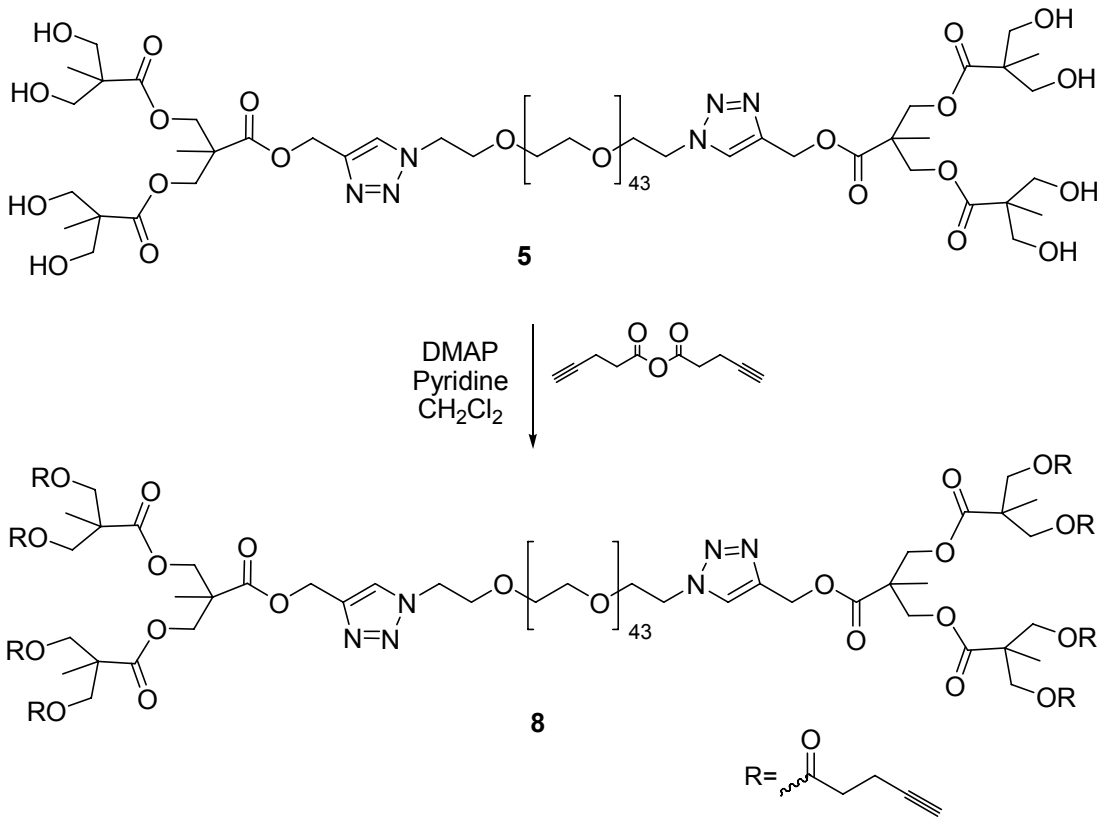


Figure 4.7. Synthesis of [G2]4OR[PEG2K] Copolymer (**8**)

Pyridine:water solution (1 mL, 1:1) was added to the reaction mixture and stirred at room temperature for 5 h. Reaction mixture was diluted with CH_2Cl_2 (30 mL) and then extracted with 1 M $NaHSO_4$ (3 x 30 mL), 10% Na_2CO_3 (3 x 30 mL) and then with brine (1 x 30 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and the residue was concentrated in *vacuo*. Crude product was purified by precipitation in diethyl ether to give 90 mg of **8** as a yellowish brown viscous liquid (74 % yield). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.79 (s, 2H), 5.21 (s, 4H), 4.52 (t, 4H, *J* = 5.0 Hz), 4.23-4.13 (m, 24H), 3.84 (t, 4H, *J* = 5.0 Hz), 3.78-3.40 (m, 180H), 2.51 (t, 16H, *J* = 6,6 Hz), 2.43 (t, 16H, *J* = 6,6 Hz), 1.95 (s, 8H), 1.20 (s, 6H), 1.17 (s, 12H). FTIR (cm^{-1}): 3262, 2868, 1736. $C_{166}H_{278}N_6O_{72}$ Calcd: C, 56.84; H, 7.93; N, 2.40. Found: C, 56.82; H, 7.60; N, 2.45.

4.8. Functionalization of the [G2]4OH[PEG6K] Copolymer (9)

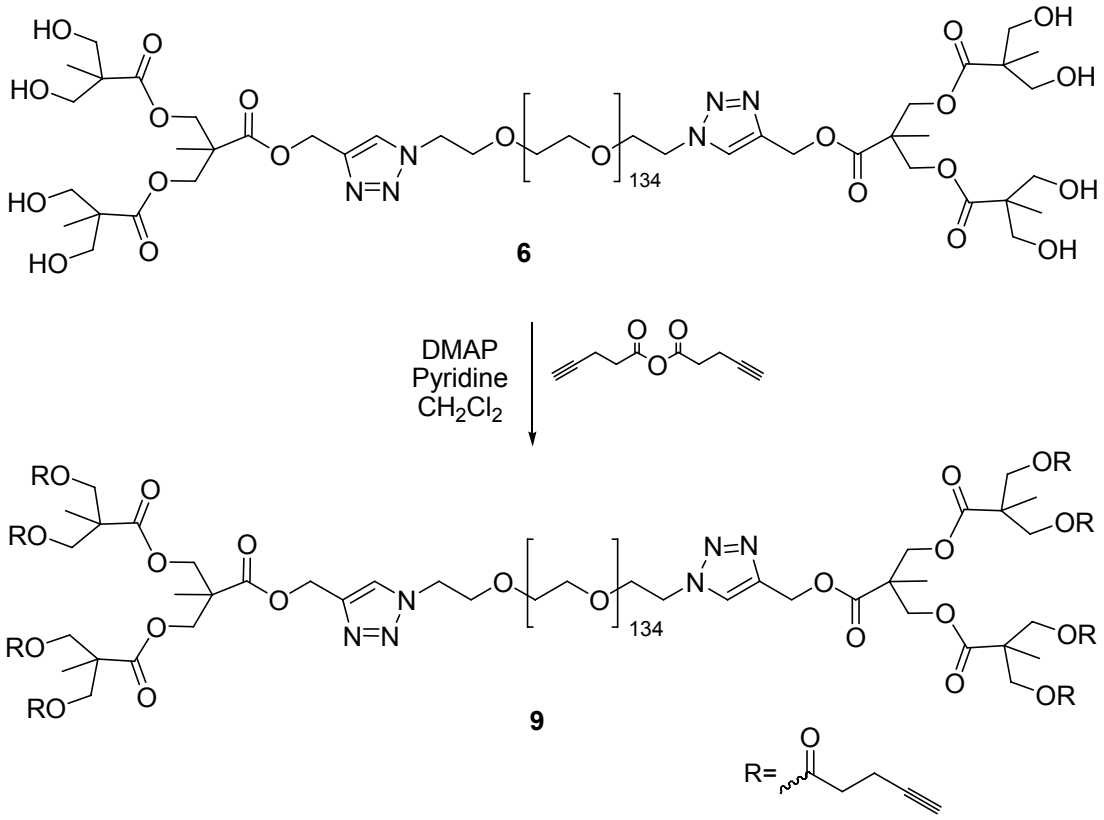


Figure 4.8. Synthesis of [G2]4OR[PEG6K] Copolymer (**9**)

[G2]4OH[PEG6K (**6**) (0.250 g, 0.036 mmol), pyridine (0.30 mL) and DMAP (0.004 g, 0.029 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL round bottom flask. To the stirring reaction mixture was added 5-pentynoic acid anhydride (0.078 g, 0.440 mmol) and continued stirring for 24 h at room temperature under N_2. Pyridine:water solution (1 mL, 1:1) was added to the reaction mixture and stirred at room temperature for 5 h. Reaction mixture was diluted with CH_2Cl_2 (50 mL) and then extracted with 1 M $NaHSO_4$ (3 x 50 mL), 10% Na_2CO_3 (3 x 50 mL) and then with brine (1 x 50 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and the residue was concentrated in *vacuo*. Crude product was purified by precipitation in diethyl ether to give 0.227 g of **9** as a yellowish white solid (83 % yield) ^{1}H NMR ($CDCl_3$, δ, ppm) 7.78 (s, 2H), 5.20 (s, 4H), 4.51 (t, 4H, *J* = 5.0 Hz), 4.22-4.12 (m, 24H), 3.84 (t, 4H, *J* = 5.0 Hz), 3.77-3.32 (m, 540H), 2.51 (t, 16H, *J* = 6,8 Hz), 2.43 (t, 16H, *J* = 6,8 Hz), 1.94 (s, 8H), 1.19 (s, 6H), 1.16 (s, 12H). FTIR (cm^{-1}): 3265, 2882, 1740. $C_{348}H_{632}N_6O_{163}$ Calcd: C, 55.68; H, 8.46; N, 1.12. Found: C, 55.05; H, 8.67; N, 0.84.

4.9. Functionalization of the [G3]8OH[PEG6K] Copolymer (10)

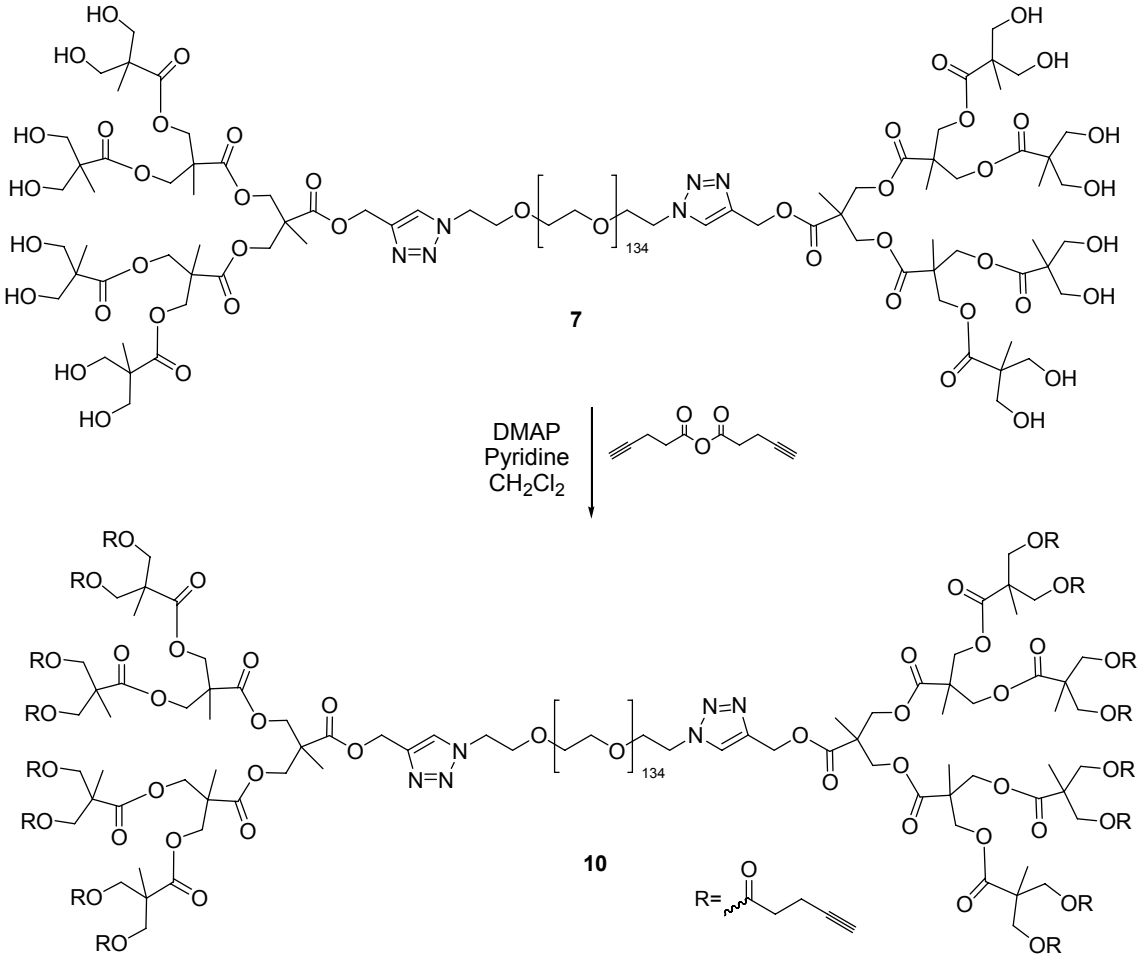


Figure 4.9. Synthesis of G3]8OR[PEG6K] Copolymer (**10**)

[G3]8OH[PEG6K] (**7**) (0.043 g, 0.0055 mmol), pyridine (0.30 mL) and DMAP (0.005 g, 0.0044 mmol) dissolved in dry CH_2Cl_2 (5 mL) in a 10 mL round bottom flask. To the stirring reaction mixture was added 5-pentynoic acid anhydride (0.024 g, 0.135 mmol) and continued stirring for 24 h at room temperature under N_2. Pyridine:water solution (1 mL, 1:1) was added to the reaction mixture and stirred at room temperature for 5 h. Reaction mixture was diluted with CH_2Cl_2 (30 mL) and then extracted with 1 M $NaHSO_4$ (3 x 30 mL), 10% Na_2CO_3 (3 x 30 mL) and then with brine (1 x 30 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and the residue was concentrated in *vacuo*. Crude product was purified by precipitation in diethyl ether to give 65 mg of **10** as a yellowish white solid (79 % yield) ^{1}H NMR ($CDCl_3$, δ, ppm) 7.80 (s, 2H), 5.23 (s, 4H), 4.53 (t, 4H, *J* = 5.0 Hz), 4.25-4.18 (m, 56H), 3.86 (t, 2H, *J* = 5.0 Hz), 3.80-3.42 (m, 540H), 2.54 (t, 32H, *J* = 6,8 Hz), 2.45 (t, 32H, *J* = 6,8 Hz), 1.97 (s, 16H), 1.24 (s, 6H),

1.22 (s, 24H), 1.19 (s, 6H). FTIR (cm^{-1}): 3279, 2882, 1739. $C_{428}H_{728}N_6O_{195}$ Calcd: C, 56.63; H, 8.08; N, 0.93. Found: C, 56.51; H, 8.27; N, 1.27.

4.10. General Synthesis of Hydrogels via [3+2] Huisgen "Click" Chemistry

Formation of hydrogel with click chemistry was achieved according to literature example with minor changes. To a small vial was added poly (ethylene glycol)-bis-tetraacetylene **9** (20 mg, 2.67 μmol), tetra (ethylene glycol) diazide **11** (0.652 mg, 2.67 μmol) in H_2O (18 μL) and ethanol (50 μL). To the vial was added deionized H_2O (110 μL) containing sodium ascorbate (1.0 mg, 5.04 μmol) and the mixture was mixed under ultrasound to give a clear solution. Copper sulfate (1.0 mg, 6.28 μmol) in water (25 μL) was added and after stirring for 10 seconds, the reaction mixture was poured into a teflon O-ring with 1.5 mm height and 1.0 cm diameter. The bottom of the ring was capped with a Teflon rod and upon addition of the reaction mixture; the ring was covered with a glass slide. The solution was allowed to react for 10 min at room temperature and then the glass slide was removed. The formed gel was taken out of the ring with the help of the Teflon rod and then was submerged into an aq EDTA solution (5%, pH ~7-8) to extract the trapped $CuSO_4$ and ethanol and finally was washed with deionized water.

4.11. Functionalization of Hydrogels

The macro monomer units of synthesized hydrogel, dendron-polymer dendron-conjugate have alkyne groups at the periphery of the dendrons and were utilized for cross linking via Huisgen-type click reaction with a small bisazide containing molecule to afford the hydrogel. The residual alkyne groups remain available for efficient post functionalization of the hydrogel functionalization of hydrogel is experienced and proved by using three different Fluorogenic probes.

4.11.1. Functionalization with 4-Azido-N-ethyl-1,8-naphthalimide

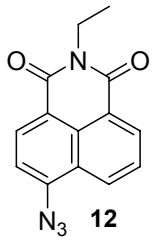


Figure 4.10. Structure of 4-Azido-N-ethyl-1,8-naphthalimide (**12**)

Functionalization with 4-Azido-N-ethyl-1,8-naphthalimide (**12**). Two identical cylindrically shaped hydrogels (6KG2 (1:1)) were synthesized (each about 20 mg) and placed into two different vials. 4-Azido-*N*-ethyl-1,8-naphthalimide (**12**, 0.00094 mmol, 0.25 mg), deionized water (2.5 mL) and ethanol (2.5 mL) were added to each of the vials. Sodium ascorbate (1.00 mg, 0.005 mmol) and $CuSO_4$ (1.00 mg, 0.004 mmol) was added to vial #1 for copper catalyzed cycloaddition. Vial#2 was left without catalyst as a control for nonspecific adsorptions. The reactions were stirred at room temperature for 12 h. After the reaction was completed, hydrogels were first transferred to an aq EDTA solution (5%, pH ~7-8) to extract the trapped $CuSO_4$ and ethanol and finally washed with deionized water.

4.11.2. Functionalization with Bodipy azide

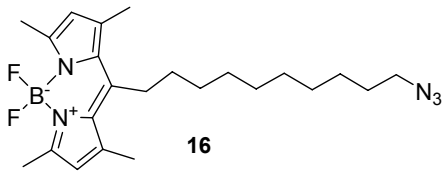
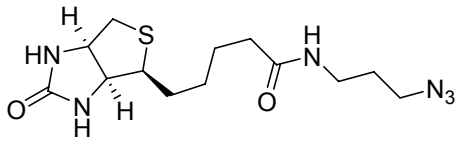


Figure 4.11. Structure of Bodipy azide (**16**)

Two identical cylindrically shaped hydrogels (6KG3 (1:3)-6KG3 (1:1)) were synthesized (each about 20 mg) and placed into two different vials. Prior to the reaction the hydrogels were washed with THF to remove water. Bodipy azide **16** (0.25 mg, 0.00058 mmol) in THF (2.5 mL) was added to each of the vials. PMDETA (1.20 □L, 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 as control for nonspecific adsorptions. The reactions were stirred at room temperature for 12 h. After the reaction

was completed hydrogels were washed with THF to remove any trapped dye molecules and then washed with an aq EDTA solution (5%, pH ~7-8) to extract the trapped Cu(I)Br and finally washed with deionized water.

4.11.3. Functionalization with Biotin Azide



14

Figure 4.12. Structure of Biotin Azide (**14**)

6KG2 (1:1) hydrogel (containing 0.016 mmole alkyne) is clicked overnight with biotin azide in the presence of $CuSO_4$ (1 mg, 0.0063 mmole) and sodium ascorbate (1 mg, 0,005 mmole). 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 rt 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. After the click reaction the hydrogels are incubated with FITC labeled streptavidin (0.1 mg/mL of PBS buffer, pH 7.4) for half an hour. After incubation hydrogels are rinsed with PBS and are kept in water for fifteen minutes, washed with water several times.

4.12. Measurements

4.12.1. Scanning Electron Microscopy (SEM) Analysis of the Hydrogel Samples

The hydrogel samples were first equilibrated in H_2O solution at room temperature to reach an equilibrium state. The equilibrated hydrogel samples were quickly frozen and further freeze-dried under vacuum until the solvent was sublimed. The freeze-dried samples were then studied by using a scanning electron microscope. Wet sample was studied without lyophilization step.

4.12.2. Physical Property Analysis (Water Uptake)

Physical property analysis (water uptake) was done according to the previously reported methods. The swelling behaviour of the hydrogels was characterized as a function of time. The experiments were carried out by measuring the weight gain as a function of immersion time in 10 mL of deionized water. At given times the disks were removed from the water, blotted with absorbent tissue paper to eliminate excess of water and weighed. Measurements were taken until equilibrium hydration degree was reached, considered when three consecutive determinations gave the same weight (±0.001 g). The ability for swelling was expressed as the swelling ratio percent, W, via Equation (1) in which M_w and M_d are the weights of wet and dry samples:

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

4.12.3. Temperature Dependence of Swelling Ratio

The temperature dependence of the swelling ratio was examined to evaluate the thermal properties of the PEG-dendron based hydrogels. Firstly hydrogel is completely saturated by swelling water at room temperature. Then the fully swollen hydrogel was placed in a vial at 0 ^{o}C. After 30 min, the hydrogel disk was removed from the water, blotted with absorbent tissue paper to eliminate excess of water and weighed. This process was repeated for every 10 ^{o}C increase in temperature till to reach 100 ^{o}C.

5. CONCLUSIONS

Synthesis of “click”able hydrogels has many novel properties and combines all of the advantages of used materials and reactions; Telechelic PEG polymer chain is reacted with G1 and G2 dendrons. The usage of “Click” reaction for dendron-polymer conjugation and synthesis of functional hydrogels by using copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction with dendron bearing telechelic PEGs are achieved and reported in this study.

Full characterization of molecules which are used in all steps are reported and functionality of synthesized hydrogels proved by using four different methods and procedures.

Synthesized hydrogels have a great potential for controlled drug delivery, cell growth, artificial tissue engineering and diagnostics, because functional groups of the hydrogels can be used to bind any azide bearing drugs, peptides and chemicals that can facilitate the cell attachment and growth.

APPENDIX: SPECTROSCOPY DATA

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

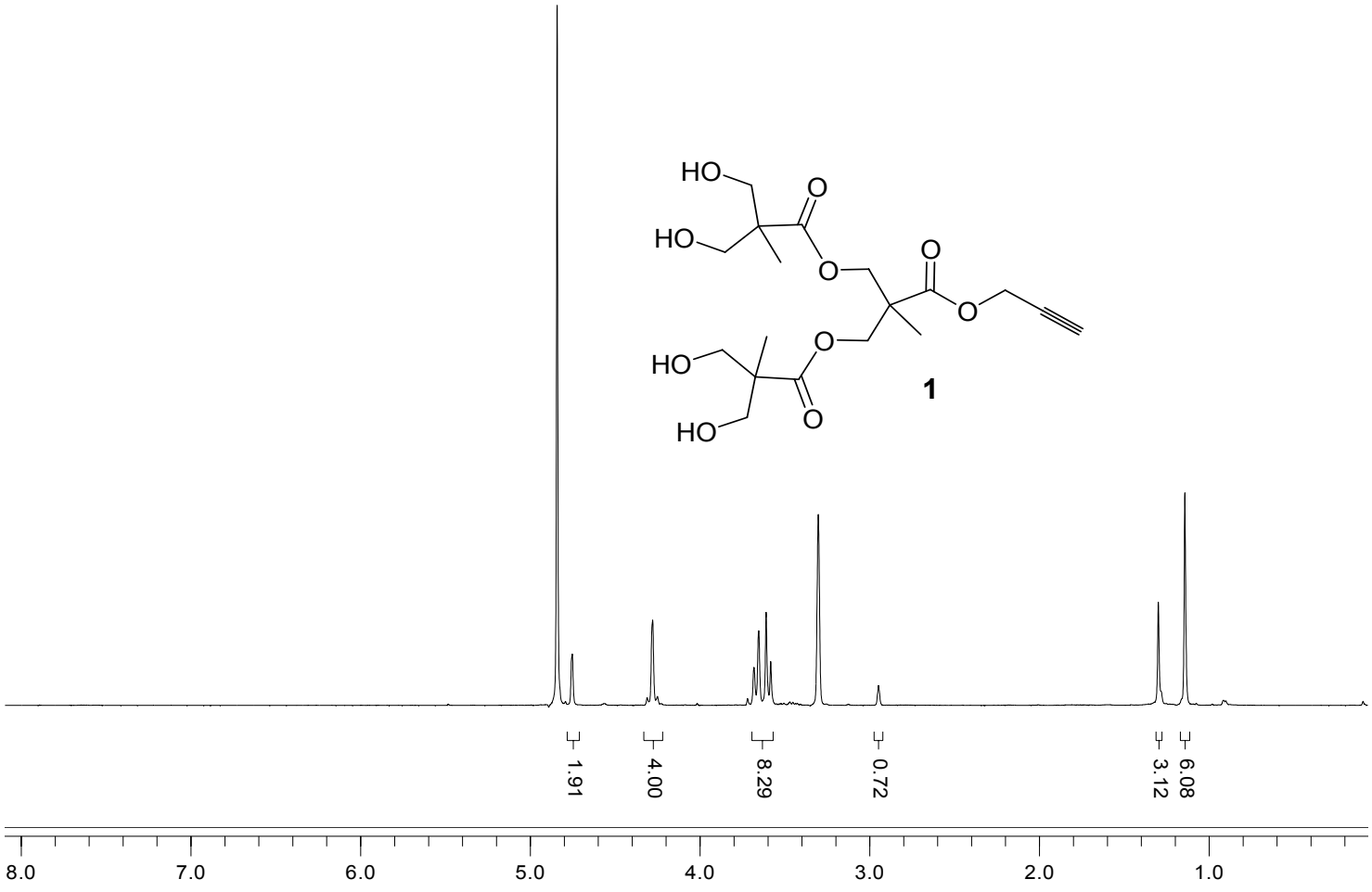


Figure A.1. ^{1}H NMR spectrum of 2nd generation polyester dendron **1**

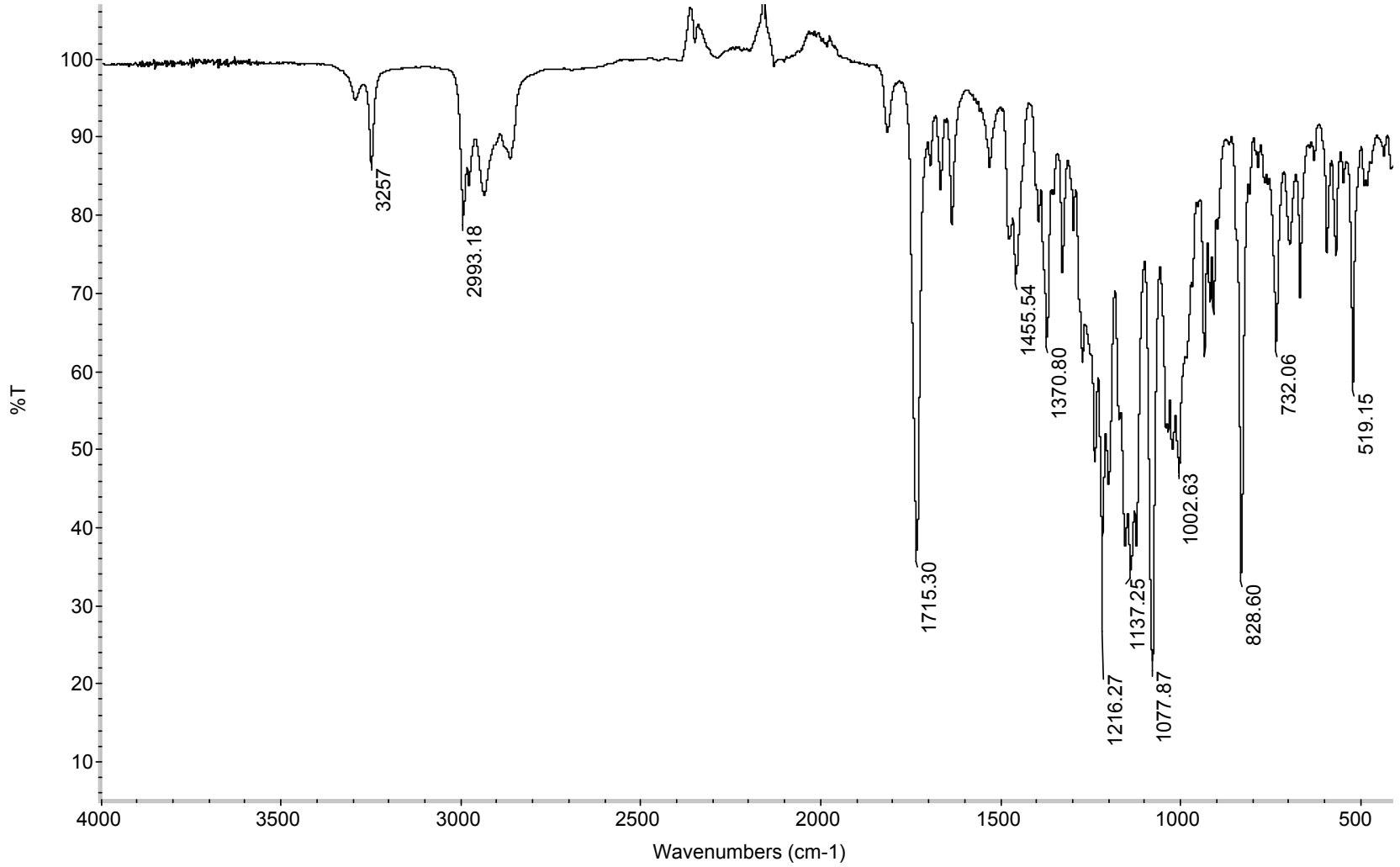


Figure A.2. ATR FTIR spectrum of 2nd generation polyester dendron **1**

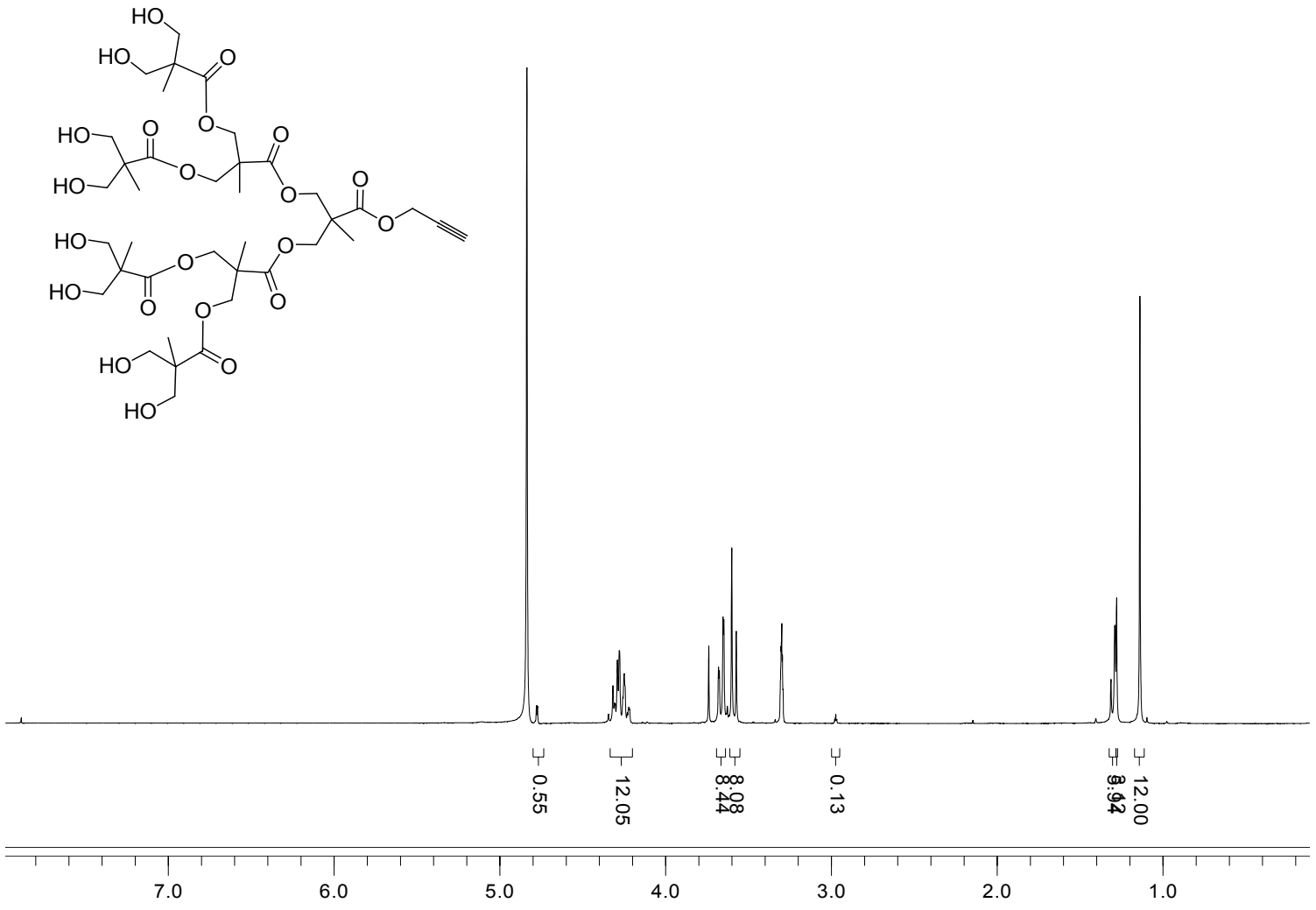


Figure A.3. ^{1}H NMR spectrum of 3rd generation polyester dendron **2**

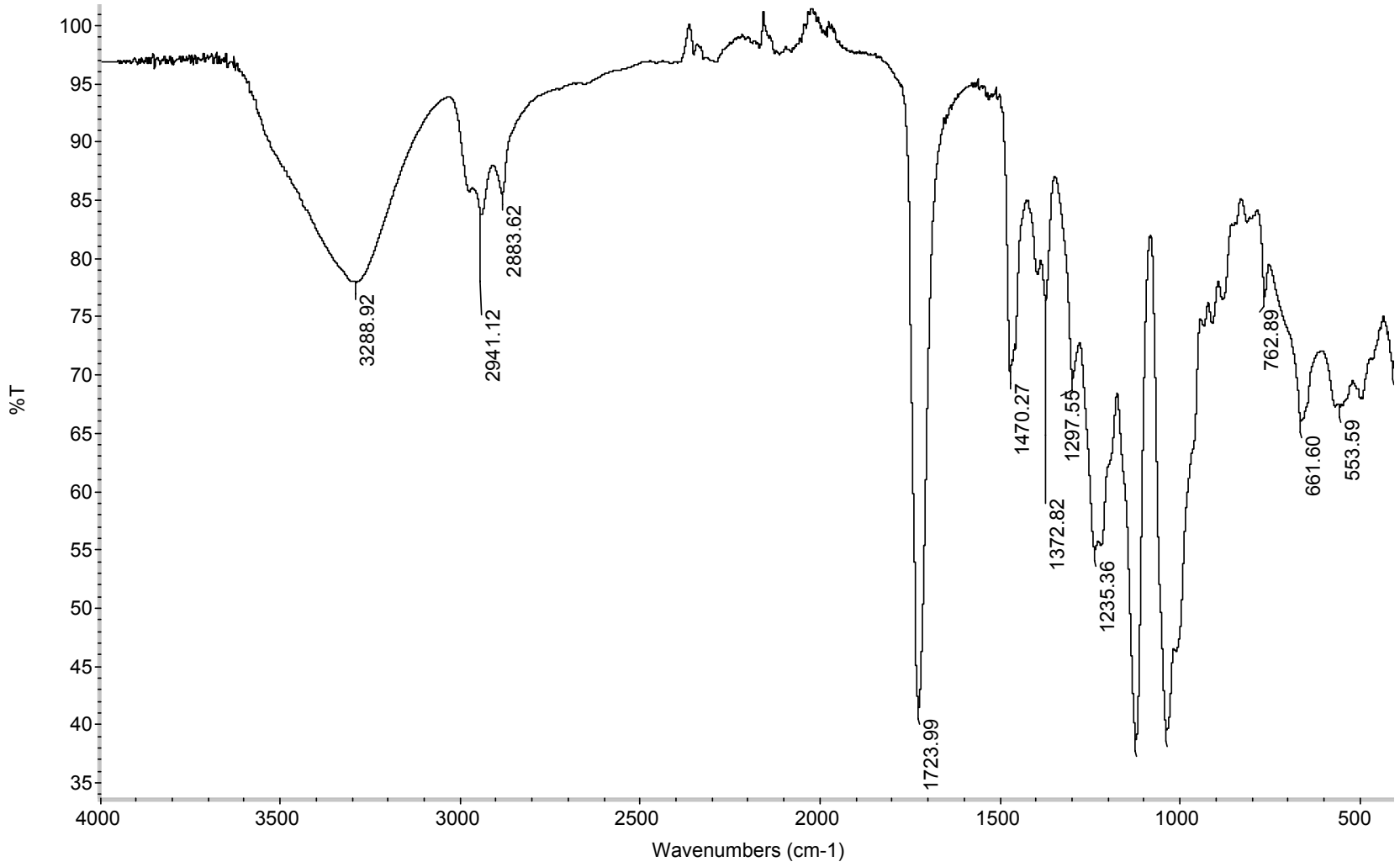


Figure A.4. ATR FTIR spectrum of 3rd generation polyester dendron **2**

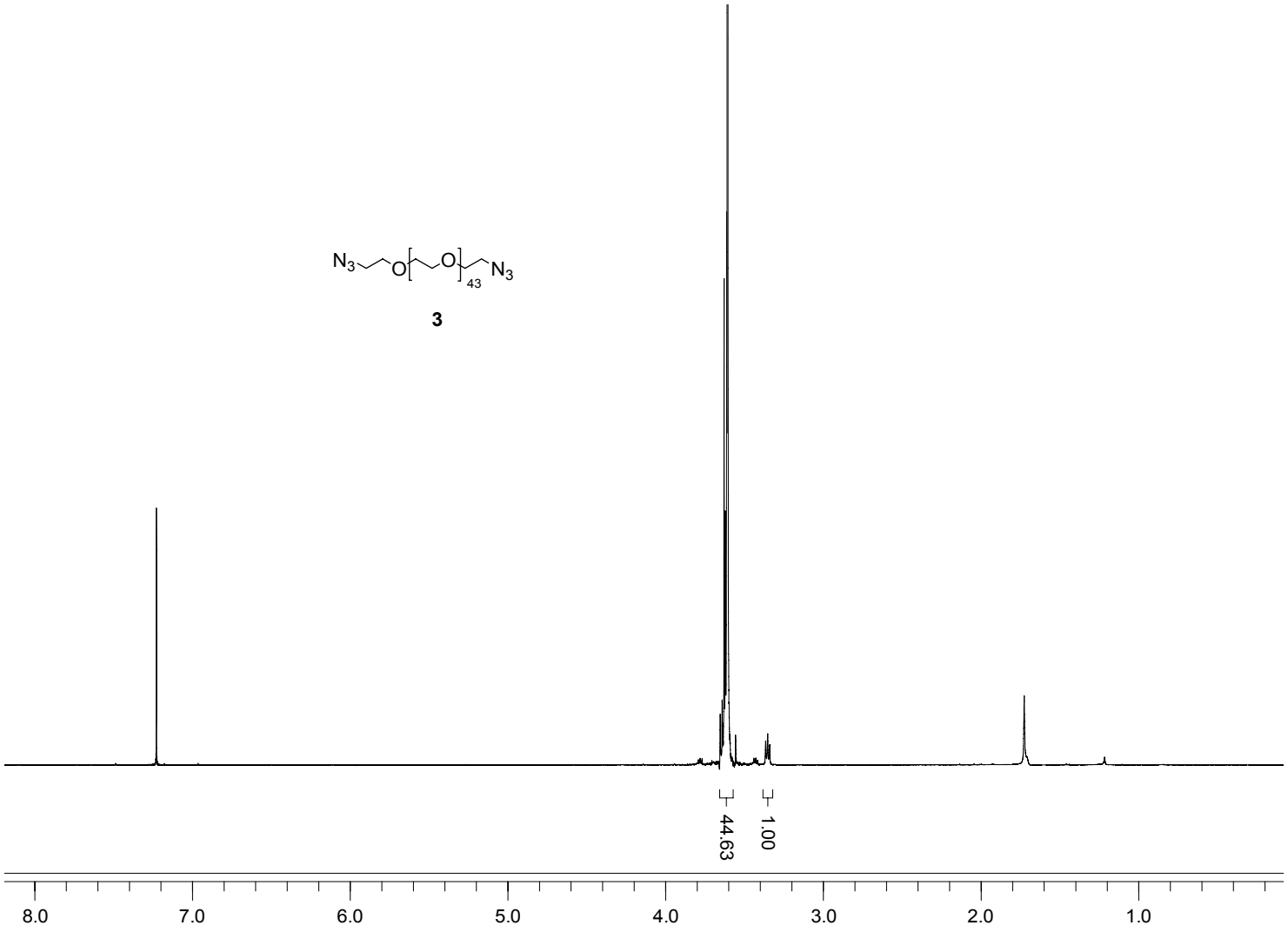


Figure A.5. ^{1}H NMR spectrum of PEG 2K bis azide **3**

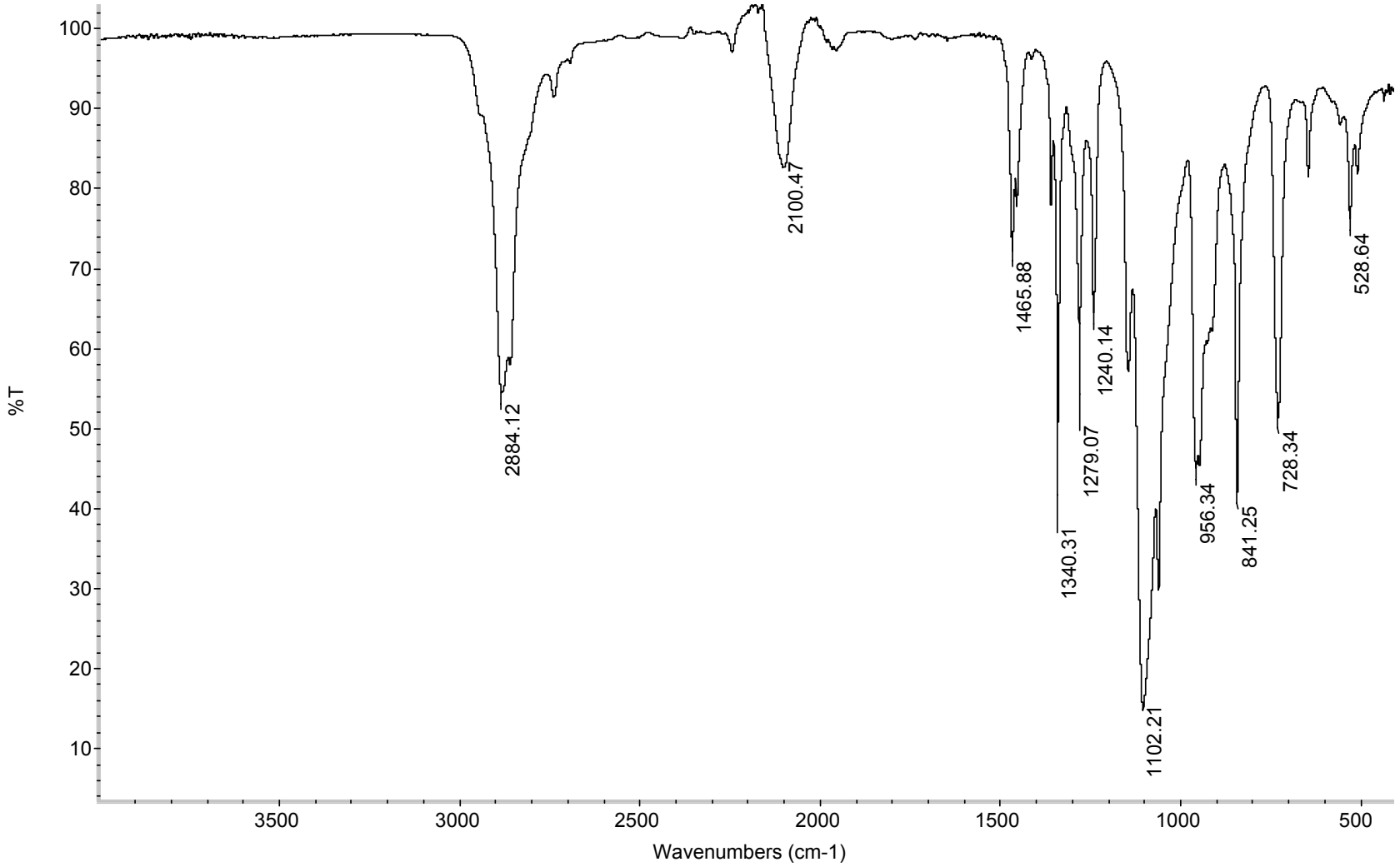


Figure A.6. ATR FTIR spectrum of PEG 2K bis azide **3**

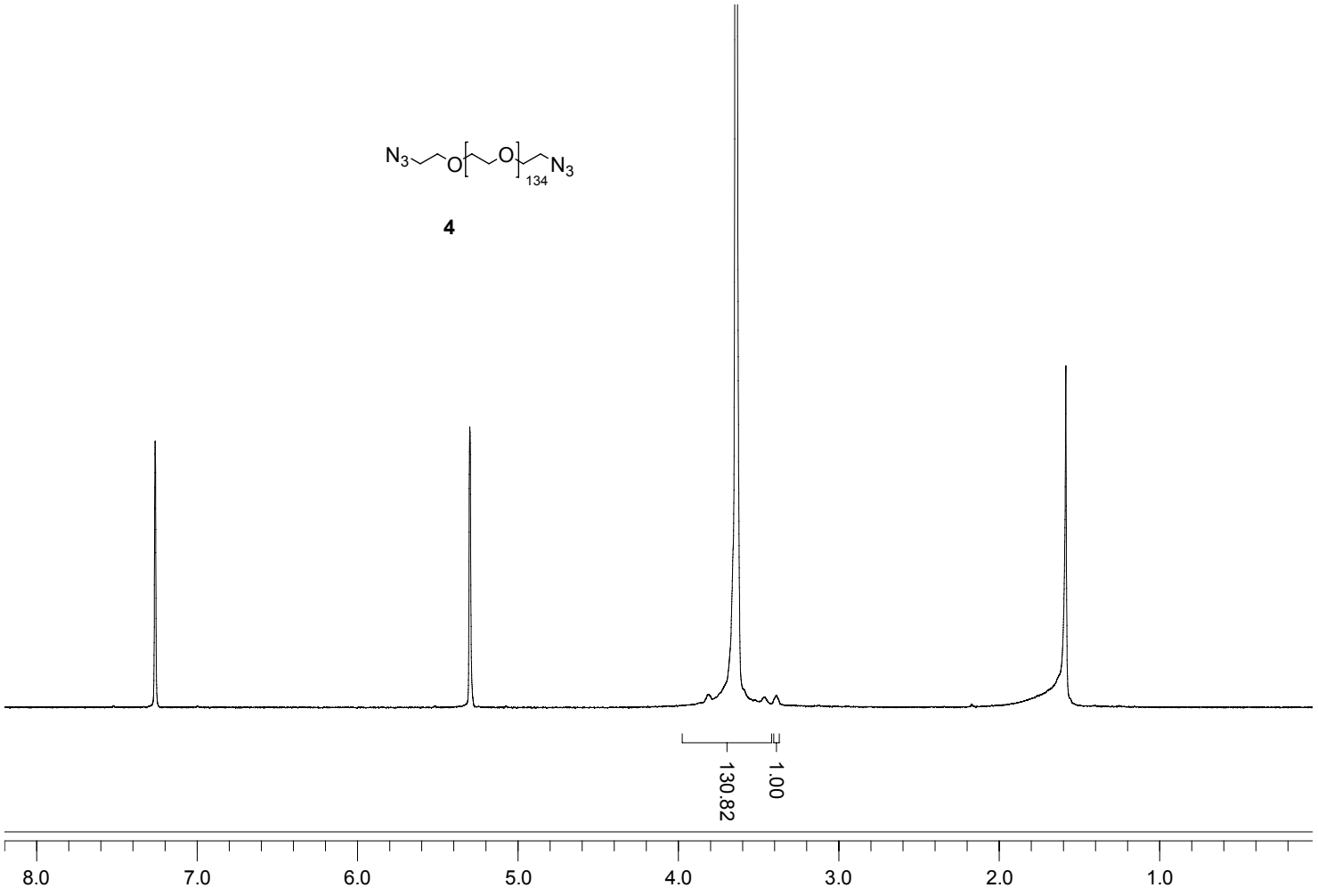


Figure A.7. ^{1}H NMR spectrum of PEG 6K bis azide 4

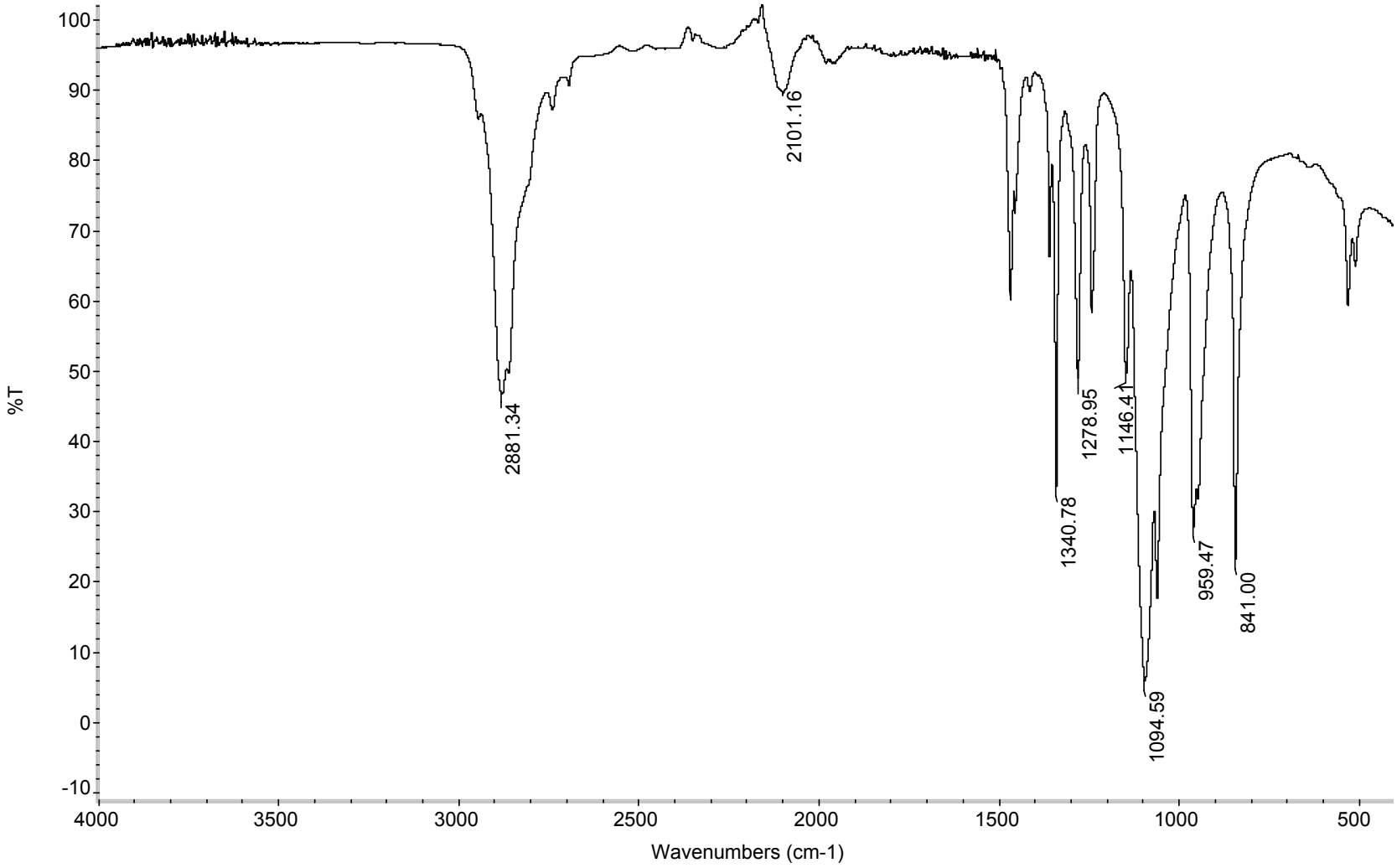
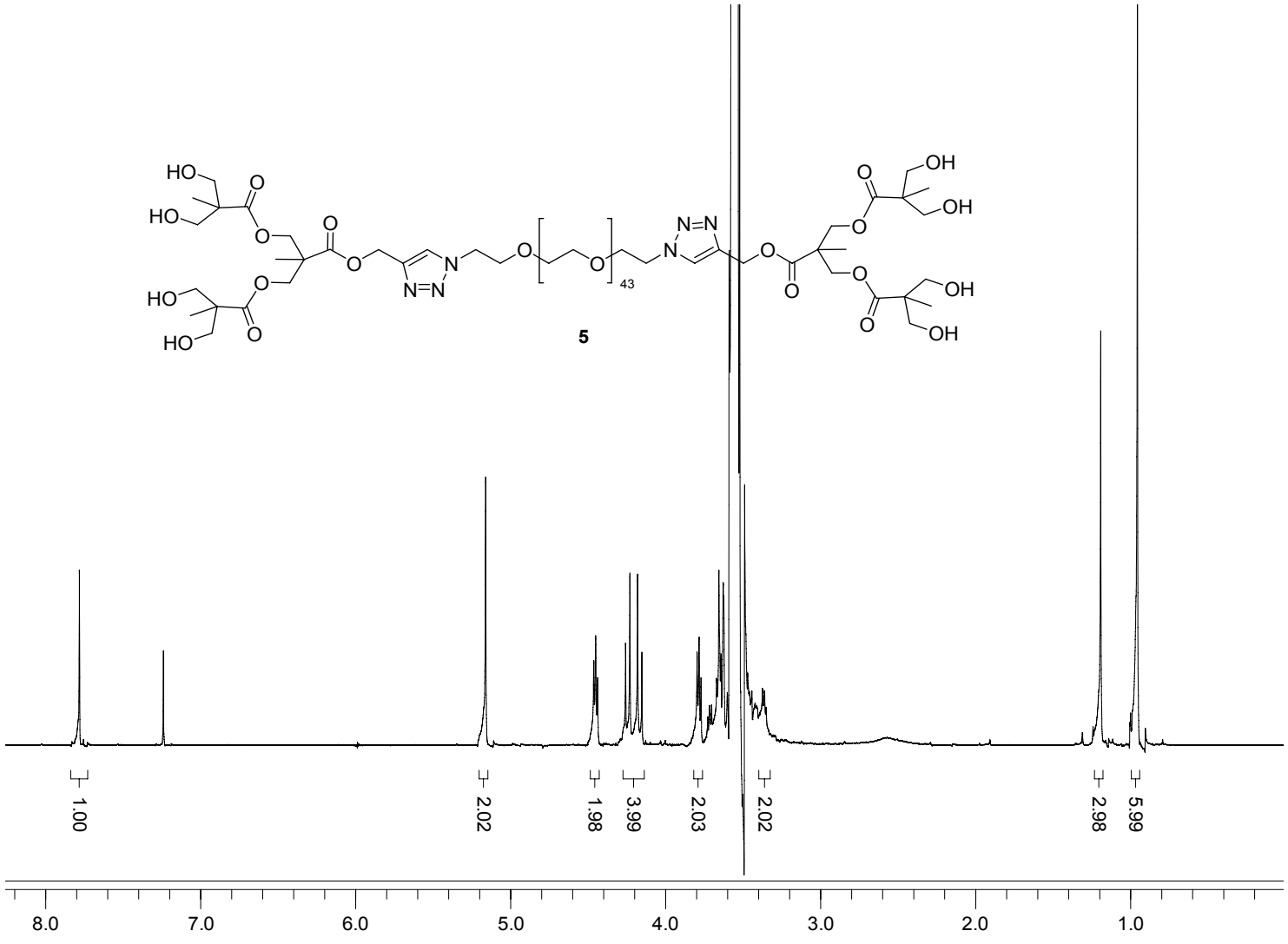


Figure A.8. ATR FTIR spectrum of PEG 6K bis azide **4**


HO
HO
HO
HO
HO
HO
O
O
O

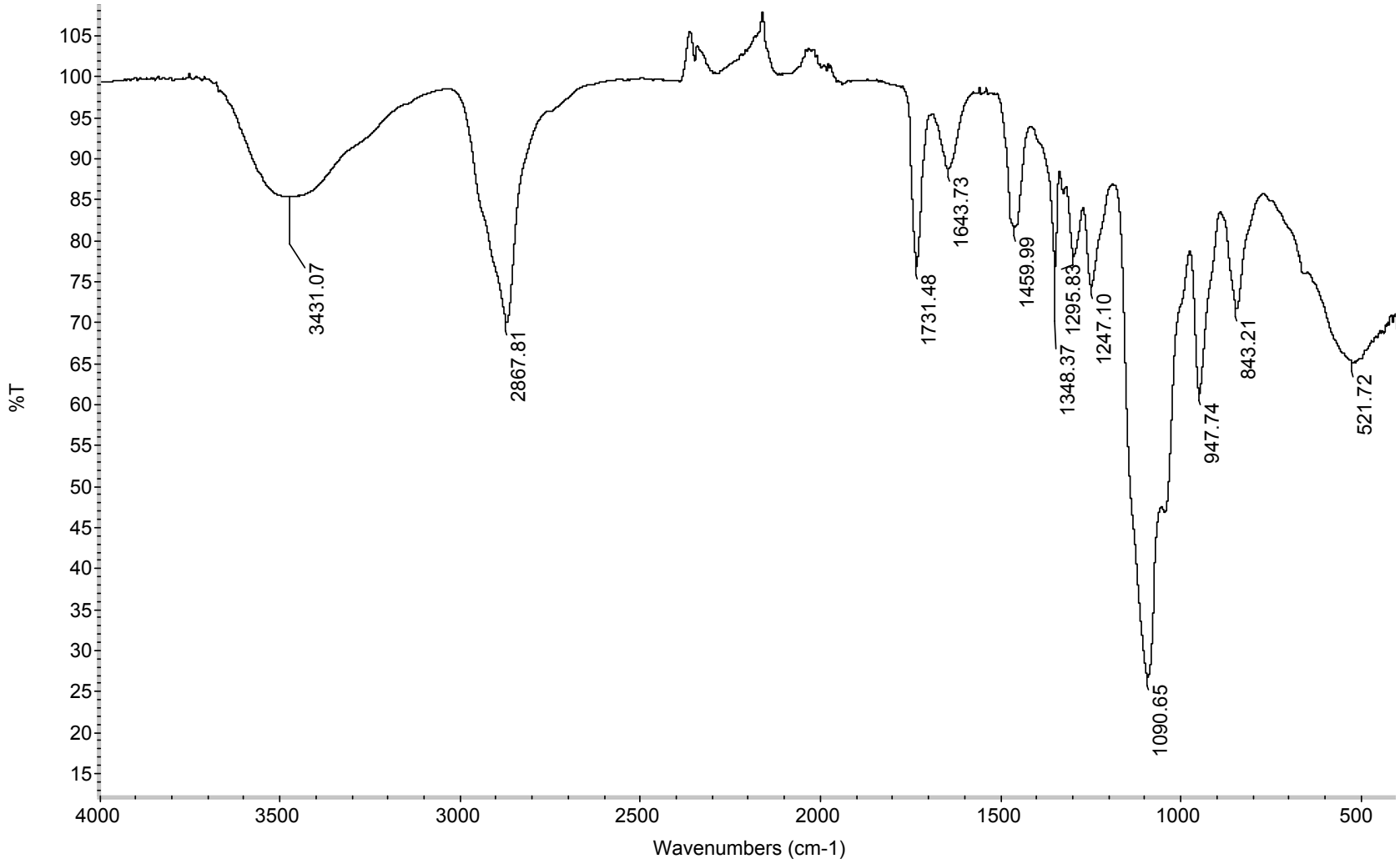


Figure A.10. ATR FTIR spectrum of [*G2]4OH[PEG2K]* **5**

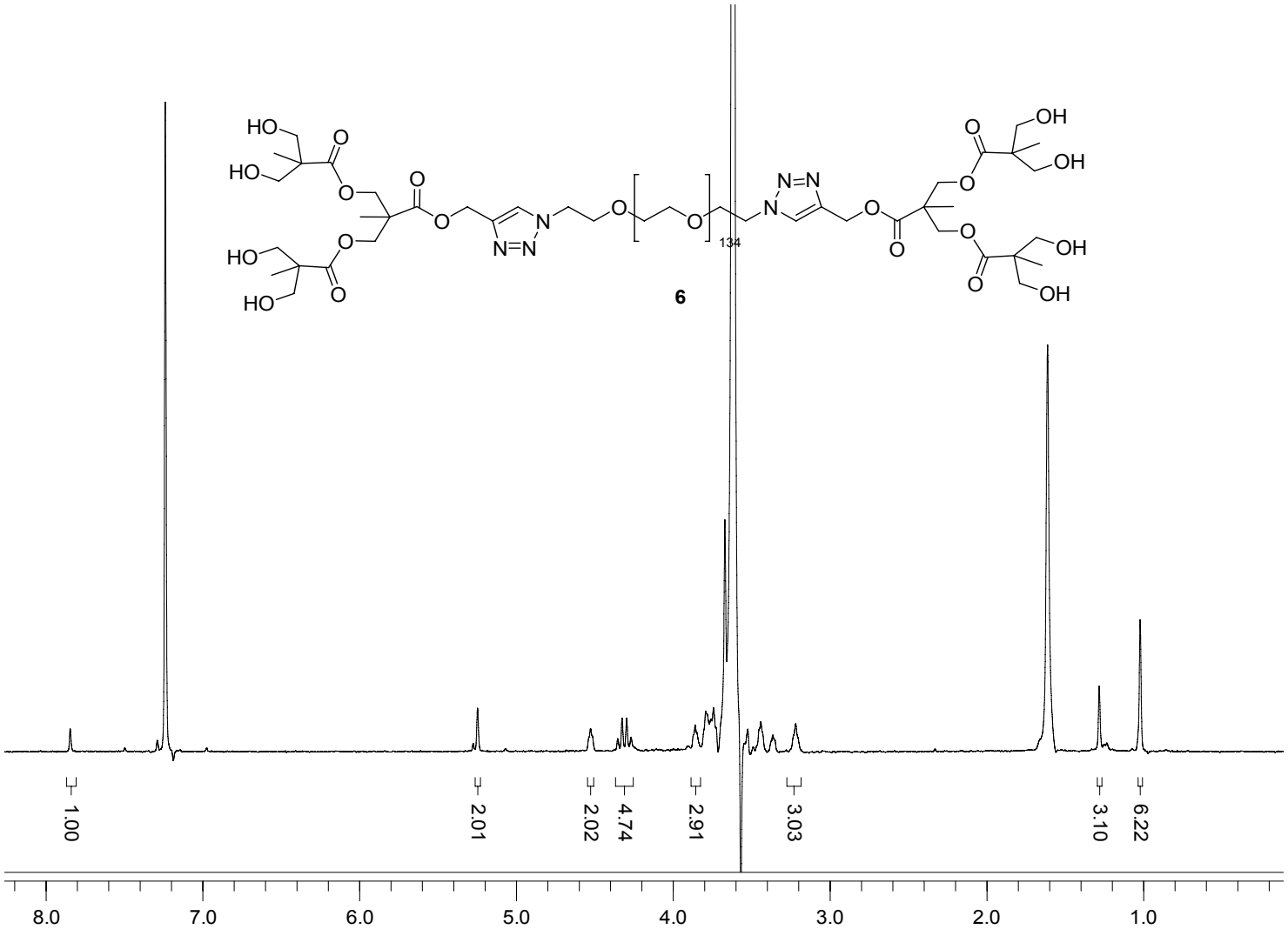


Figure A.11. ^{1}H NMR spectrum of *[G2]4OH[PEG6K]* **6**

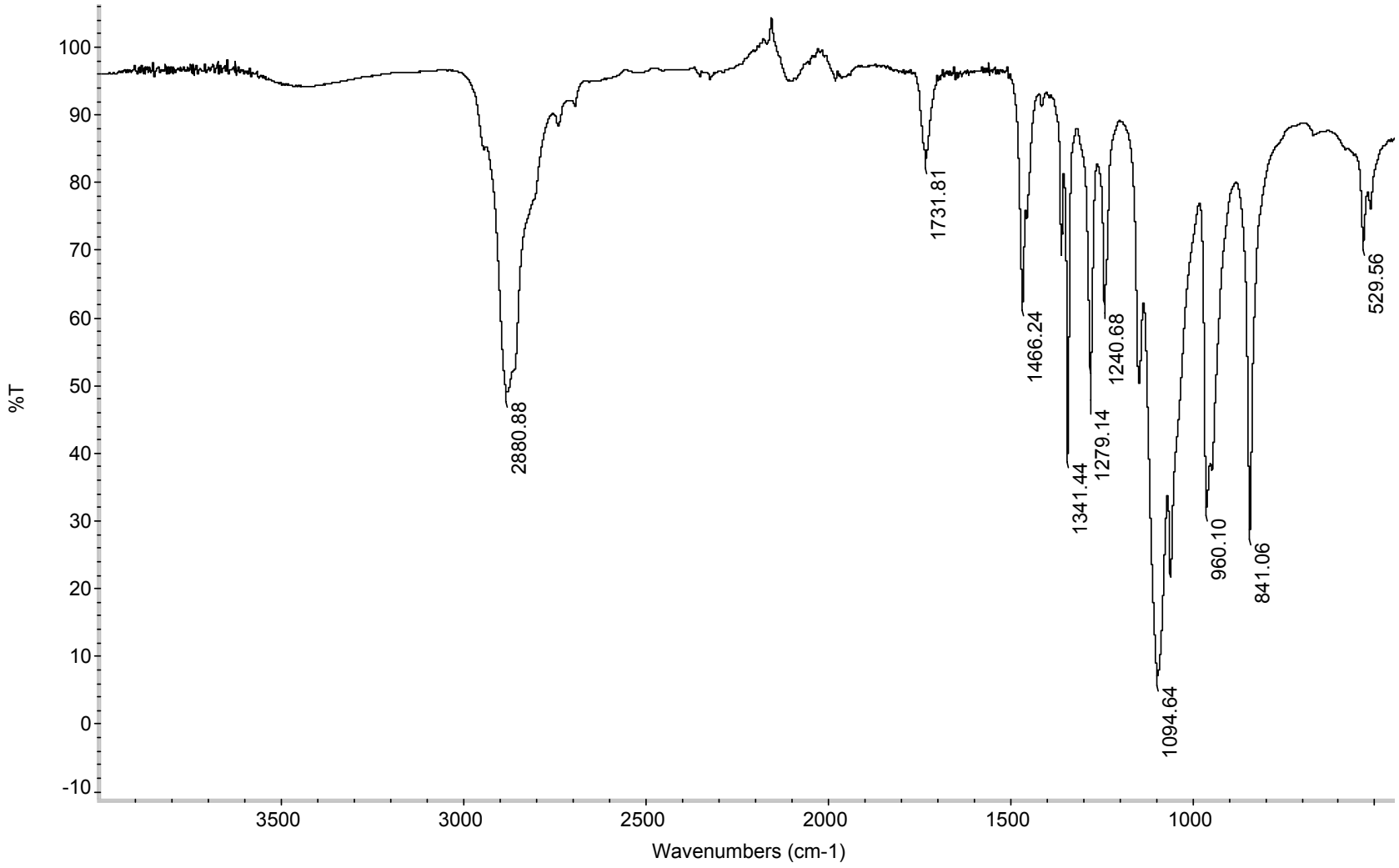


Figure A.12. ATR FTIR spectrum of *[G2]4OH[PEG6K]* **6**

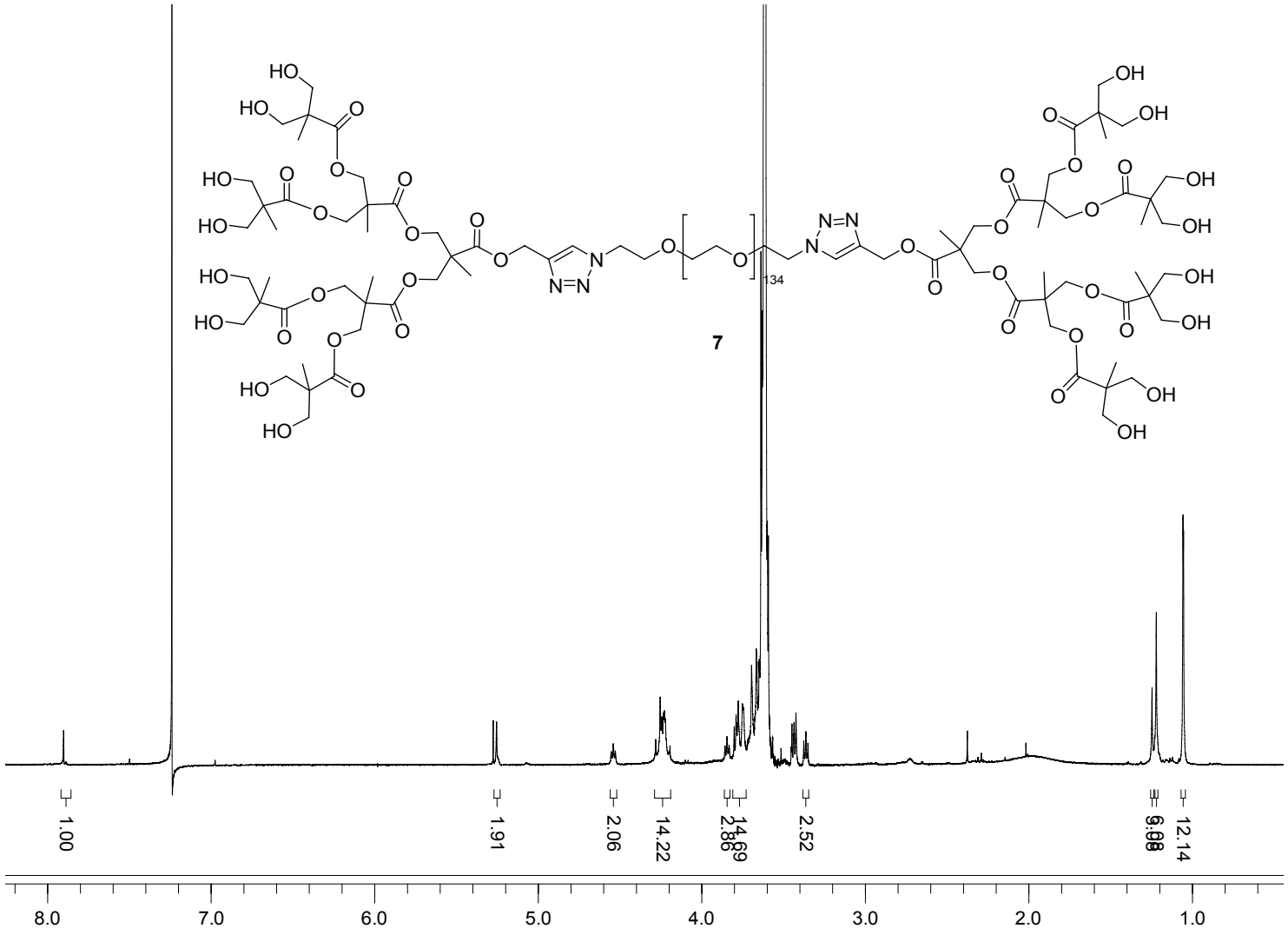


Figure A.13. ^{1}H NMR spectrum of *[G3]8OH[PEG6K]* **7**

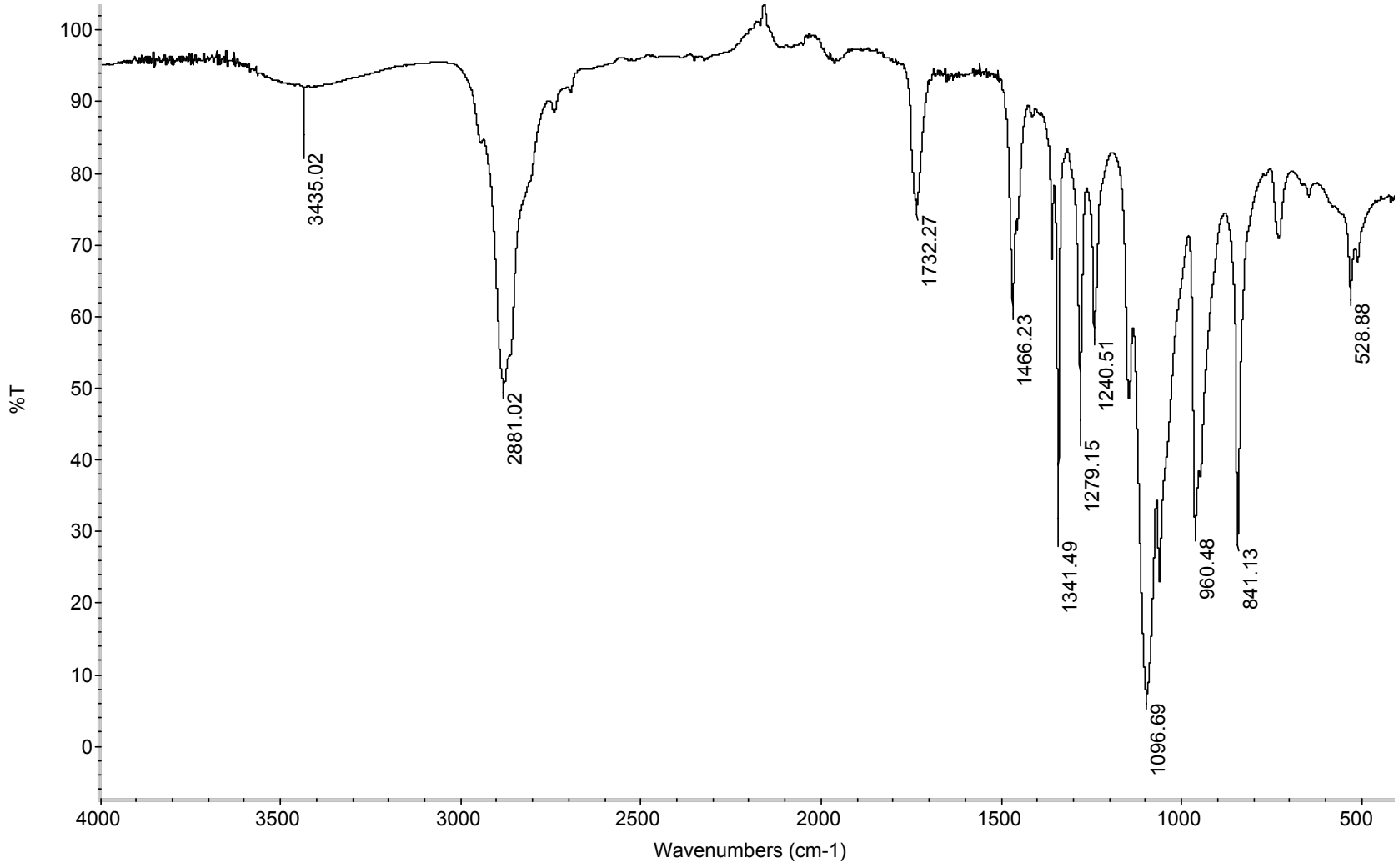


Figure A.14. ATR FTIR spectrum of *[G3]8OH[PEG6K]* **7**

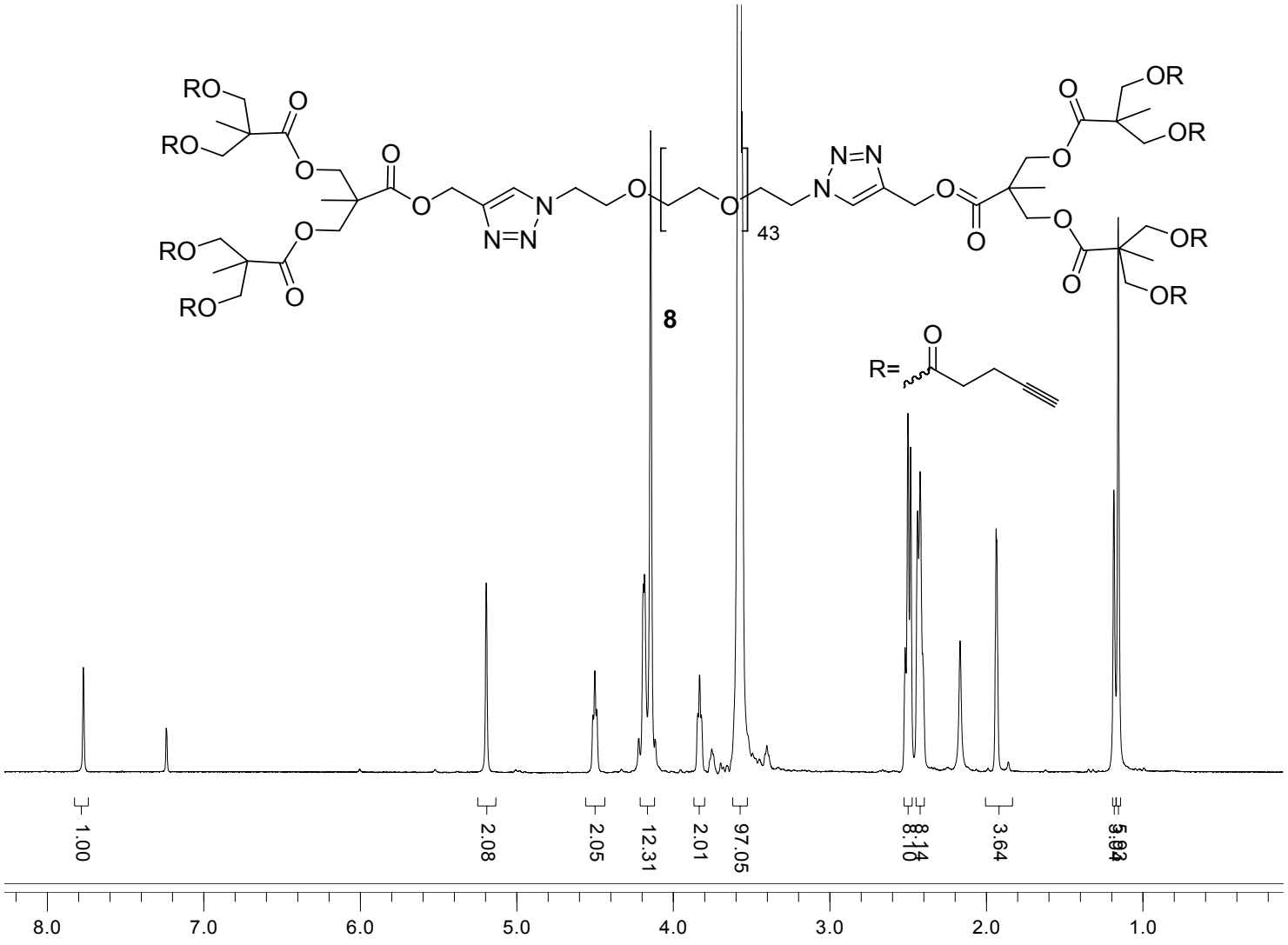


Figure A.15. ^{1}H NMR spectrum of *[G2]4OR[PEG2K]* **8**

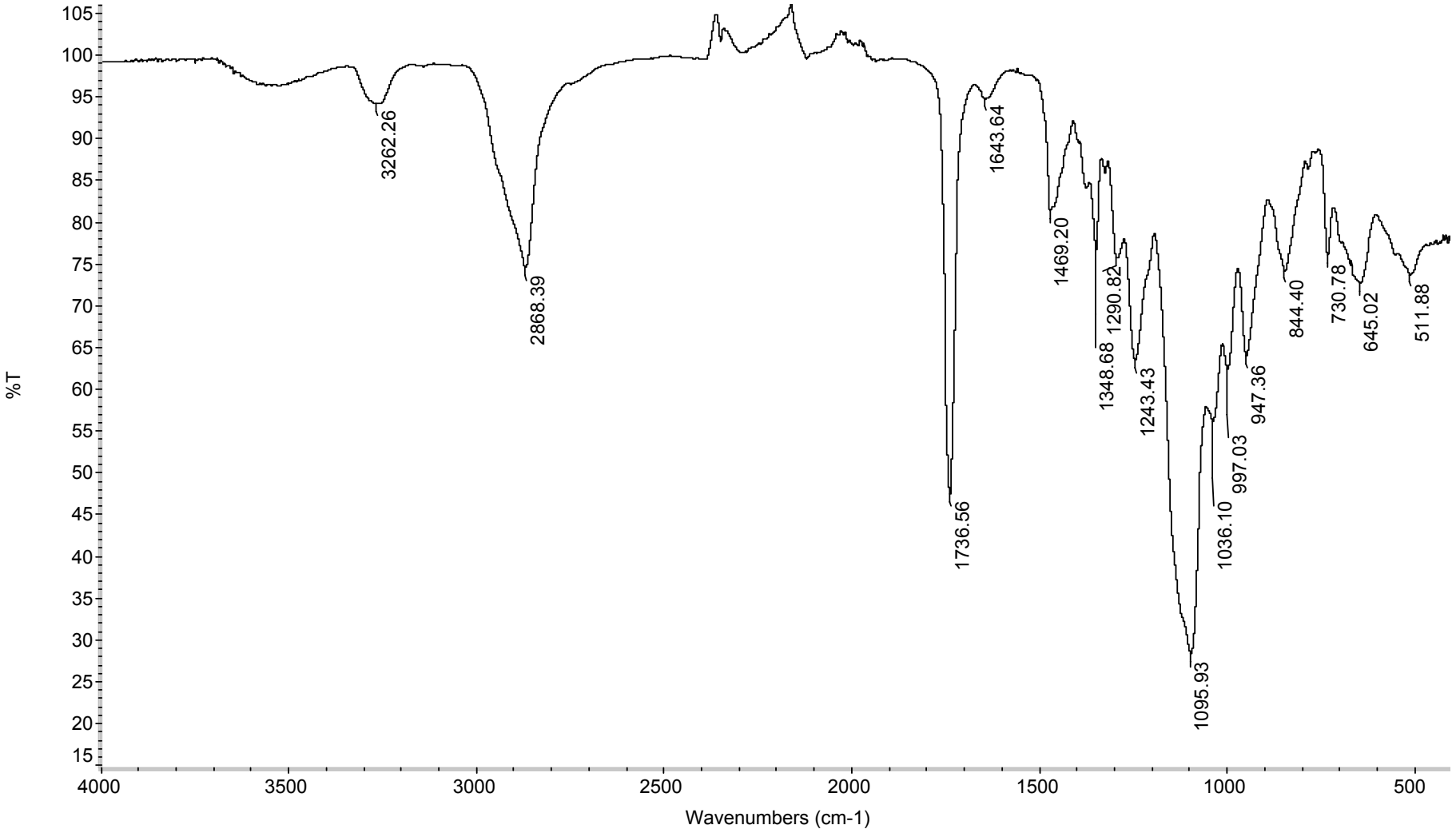


Figure A.16. ^{1}H NMR spectrum of *[G2]4OR[PEG2K]* **8**

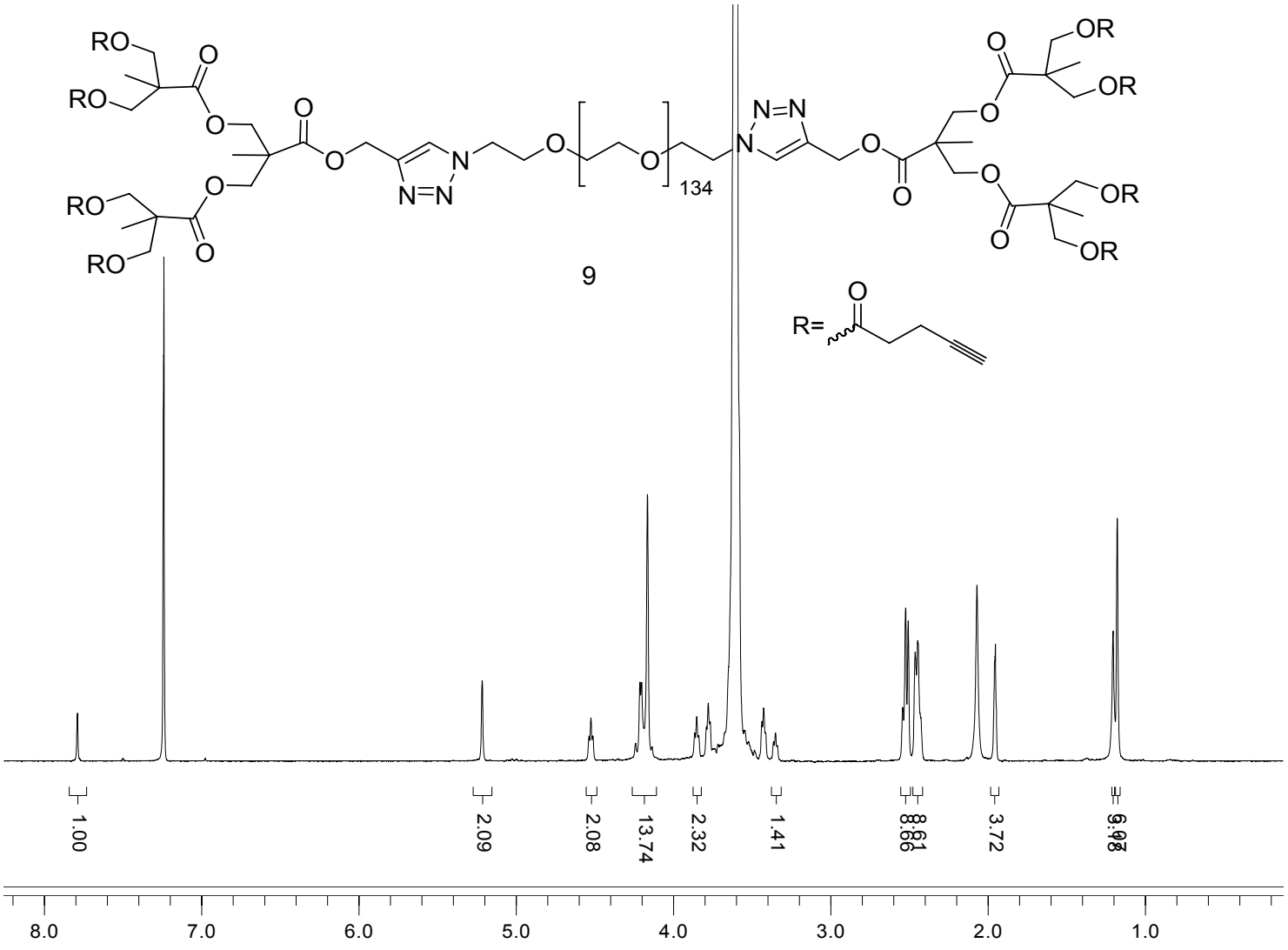


Figure A.17. ^{1}H NMR spectrum of *[G2]4OR[PEG6K]* **9**

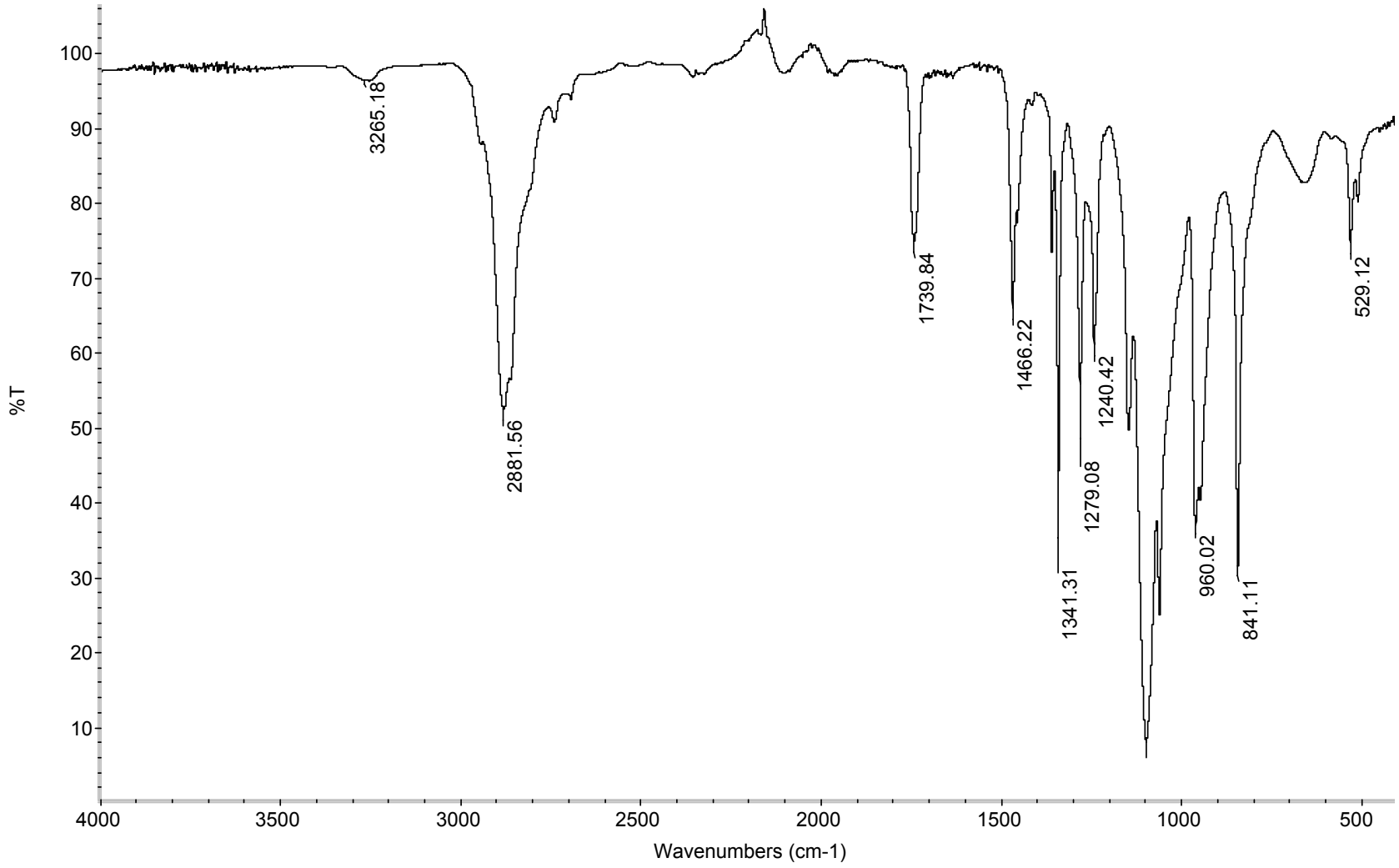


Figure A.18. ATR FTIR spectrum of *[G2]4OR[PEG6K]* **9**

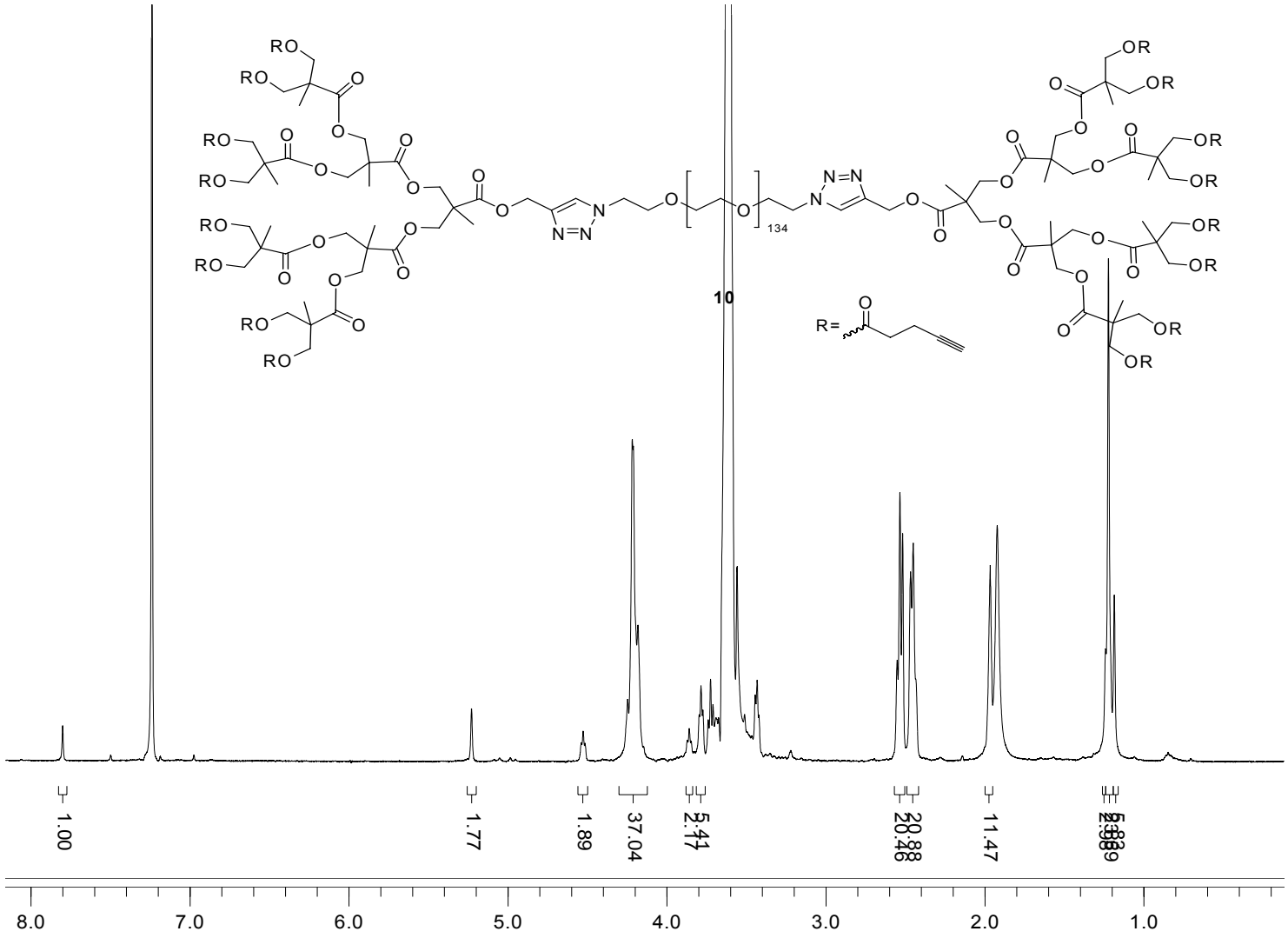


Figure A.19. ^{1}H NMR spectrum of *[G3]8OR[PEG6K]* **10**

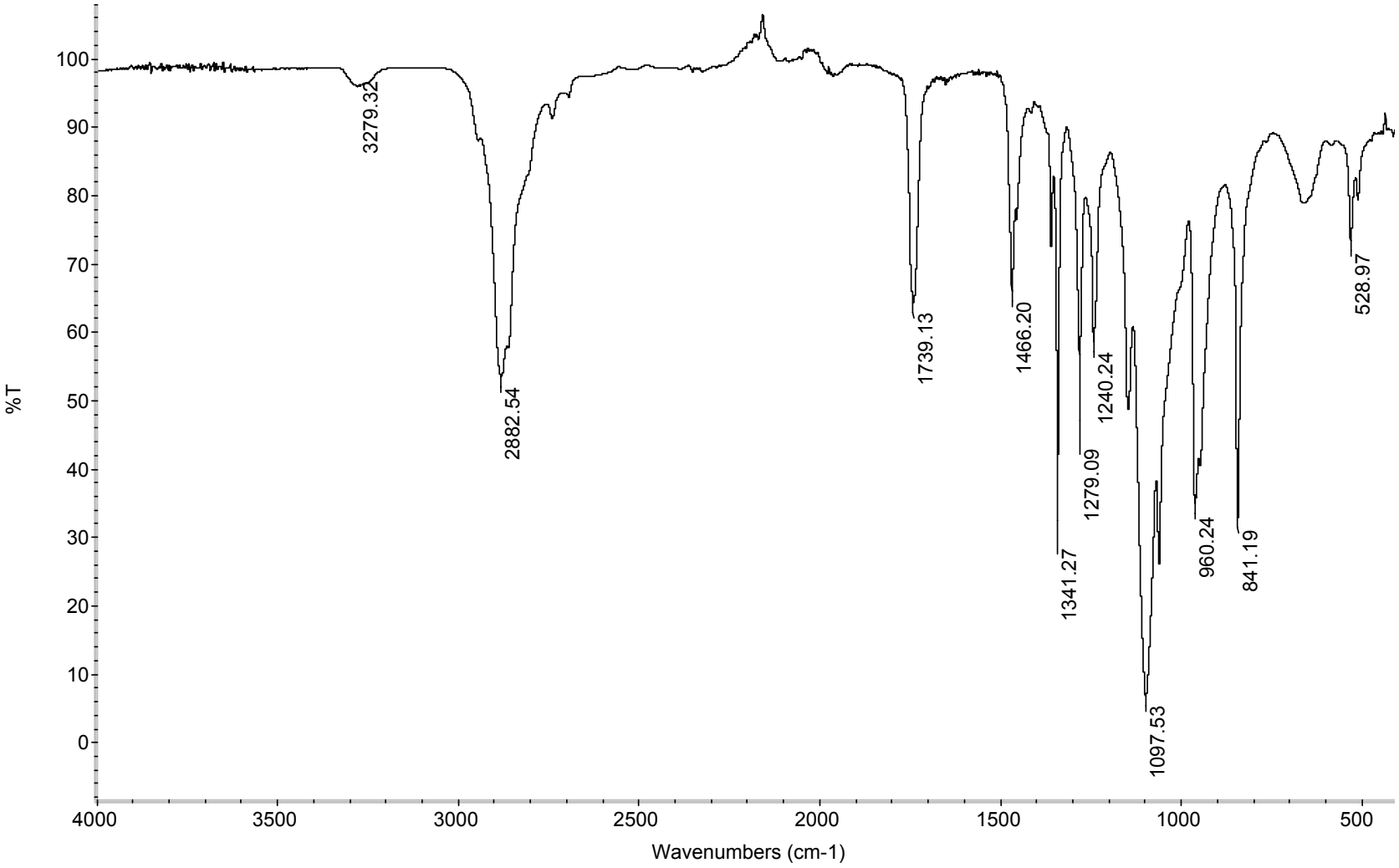


Figure A.20. ATR FTIR spectrum of *[G3]8OR[PEG6K]* **10**

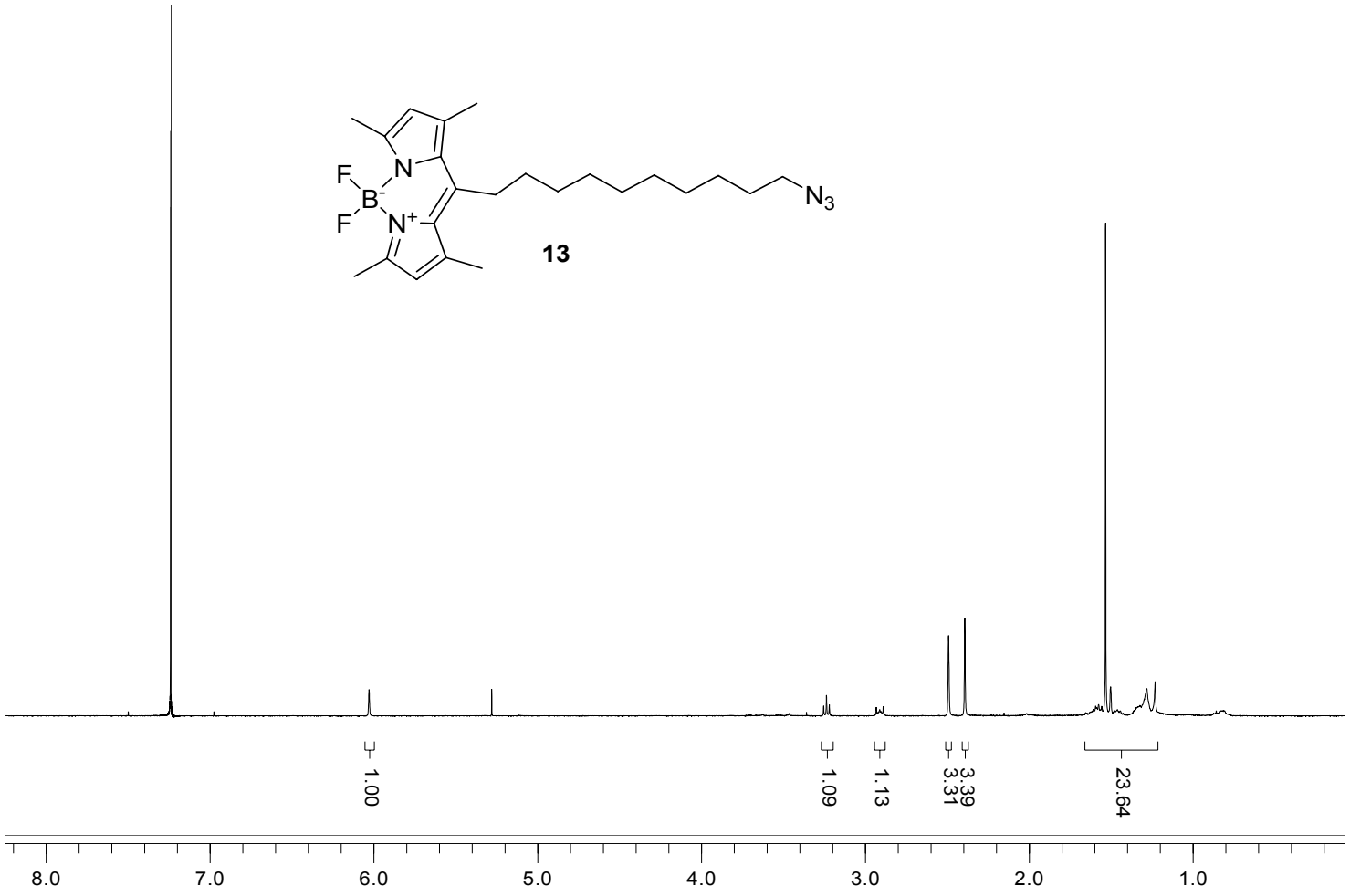


Figure A.21. ^{1}H NMR spectrum of Bodipy azide **16**

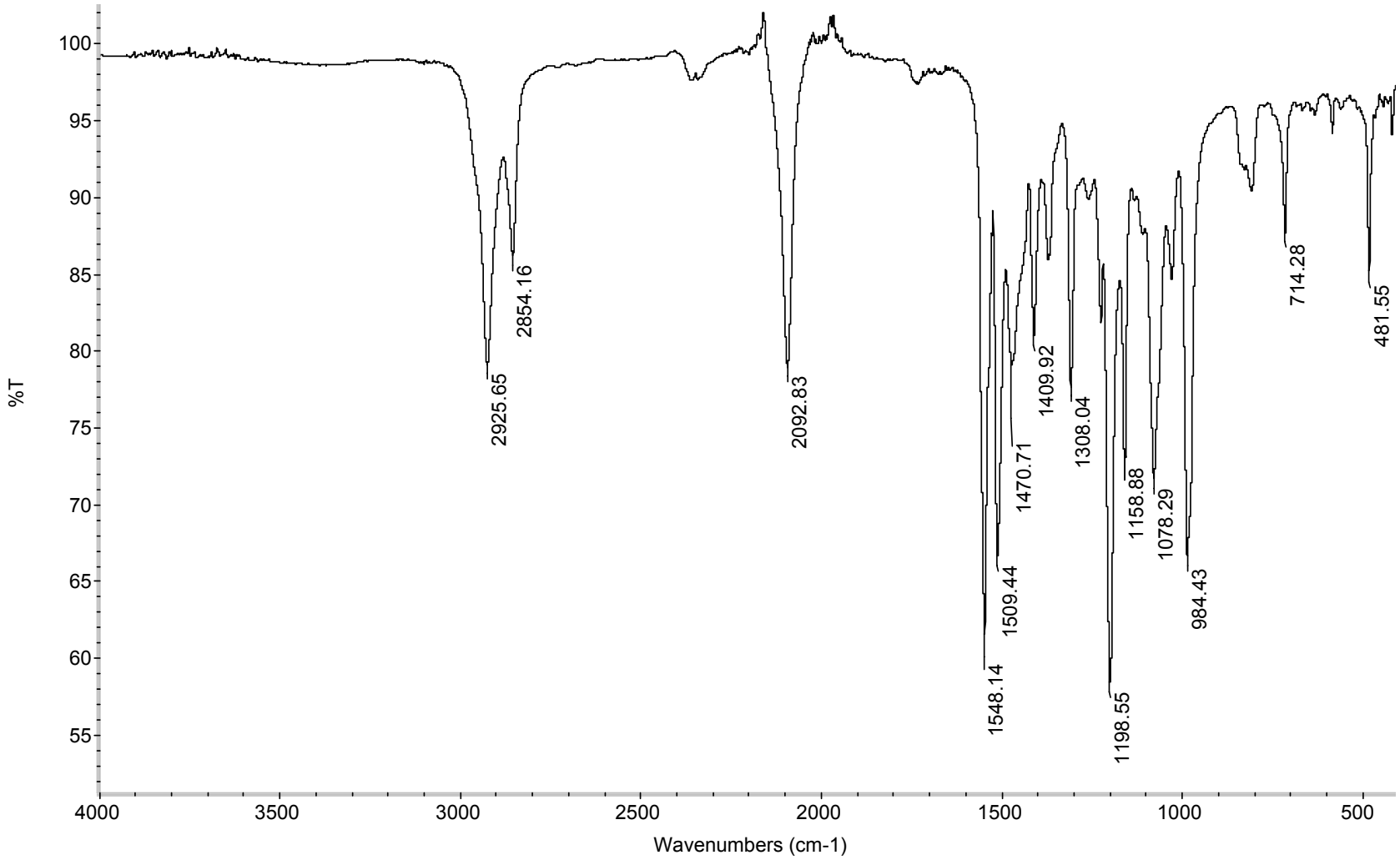


Figure A.22. ATR FTIR spectrum of Bodipy azide **16**

6. REFERENCES

1. Tabata, Y. "The importance of drug delivery" *Pharm. Sci. Technol. Today*, Vol. 3, pp. 80-89, 2000.

2. Park, K. "Biodegradable Hydrogels for Drug Delivery", Lancaster: Technomic Publishing Co., 1993.

3. Taipale, J., Keski-Oja, J. "Growth factors in the extracellular matrix", *FASEB J.* Vol. 11, pp. 51-59, 1997.

4. Tabata,Y., Ikada,Y. "Protein release from gelatin matrices", *Adv.Drug Deliv. Rev.,* Vol. 31, pp. 287-301, 1998.

5. Tabata,Y. "Surfactant-free preparation of biodegradable hydrogel microspheres for protein release", *J. Bioact. Compat. Polym.* Vol. 14, pp. 371–384, 1999.

6. Amanda, K., Silva, A., Richard, C., Bessodes, M., Scherman, D., Merten O.-W. "Growth Factor Delivery Approaches in Hydrogels" *Biomacromolecules*, Vol. *10,* pp. 9–18, 2009.

7. Galaev, I.Y., Mattiasson, B. "Smart polymers and what they could do in biotechnology and medicine". *Trends Biotechnol*., Vol. 17, pp. 335-340, 1999.

8. Baldwin, S.P., Saltzman, W.M. "Materials for protein delivery in tissue engineering**"**, *Adv. Drug Deliv. Rev.*, Vol. 33, pp. 71-86, 1998.

9. Gombotz, W.R., Pettit, D.K. "Biodegradable polymers for protein and peptide drug delivery**",** *Bioconjug. Chem.,* Vol. 6, pp. 332-351, 1995.

10. Peppas, N.A., Bures, P., Leobandung, W., Ichikawa H. “Hydrogels in pharmaceutical formulations”, *Eur. J. Pharm. Biopharm.*, Vol. 50, pp. 27-46, 2000.

11. Peppas, N.A., Huang, Y., Torres-Lugo, M., Ward, J.H., Zhang, J. “Physicochemical foundations and structural design of hydrogels in medicine and biology”, *Annu. Rev. Biomed. Eng.,* Vol. 2, pp. 9-29, 2000.

12. Gehrke, S.H., “Synthesis and properties of hydrogels used for drug delivery**”,** *Drugs Pharm. Sci.,* Vol. 102, pp. 473-546, 2000.

13. Park, K., Shalaby, W.S.W., Park H. “Biodegradable Hydrogels For Drug Delivery”, Basle: Technomic, 1993.

14. Smetana K. “Cell biology of hydrogels”, *Biomaterials,* Vol. 14, pp. 1046-1050, 1993.

15. Anderson, J.M., Langone, J.J. “Issues and perspectives on the biocompatibility and immunotoxicity evaluation of implanted controlled release systems”, *J. Controlled Release,* Vol. 57, pp. 107-113, 1999.

16. Anderson J.M., “In vivo biocompatibility of implantable delivery systems and biomaterials”, *Eur. J. Pharm. Biopharm.,* Vol. 40, pp. 1-8, 1994.

17. Wichterle, O., Lim, D. “Hydrophilic gels for biological use”, *Nature,* Vol. 185, pp. 117-118, 1960.

18. Langer, R.S., Peppas, N.A. “Present and future applications of biomaterials in controlled drug delivery systems”, *Biomaterials,* Vol. 2, pp. 201-214, 1981.

19. Bettini, R., Colombo, P., Peppas, N.A. “Solubility effects on drug transport through pH-sensitive, swelling-controlled release systems: Transport of theophylline and metoclopramide monohydrochloride”, *J. Controlled Release,* Vol. 37, pp. 105-111, 1995.

20. Cicek, H., Tuncel, A. “Immobilization of α-chymotrypsin in thermally reversible isopropylacrylamide-hydroxyethylmethacrylate copolymer gel”, *J. Polym. Sci. A Polym. Chem.,* Vol. 36, pp. 543-552, 1998.

21. Gombotz, W.R., Wee, S.F. “Protein release from alginate matrices”, *Adv. Drug Deliv. Rev.,* Vol. 31, pp. 267-285, 1998.

22. Polk, A., Amsden, B., Yao, K., Peng, T., Goosen, M.F.A. “Controlled release of albumin from chitosan-alginate microcapsules”, *J. Pharm. Sci.,* Vol. 83, pp. 178-185, 1994.

23. Liu, L.S., Liu, S.Q., Ng, S.Y., Froix, M., Ohno, T., Heller, J. “Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres”, *J. Controlled Release,* Vol. 43, pp. 65-74, 1997.

24. Murakami, Y., Yokoyama, M., Okano, T., Nishida, H., Tomizawa, Y., Endo, M., Kurosawa, H. “A novel synthetic tissue-adhesive hydrogel using a crosslinkable polymeric micelle”, *J. Biomed. Mater. Res.,* Vol. 80, pp. 421-427, 2007.

25. Quaglia, F. “Bioinspired tissue engineering: The great promise of protein delivery technologies”, *International Journal of Pharmaceutics,* Vol. 364, pp. 281-297, 2008.

26. Hennink, W.E., van Nostrum, C.F. “Novel crosslinking methods to design hydrogels”, *Adv. Drug Deliv. Rev.,*Vol.54, pp. 13-36, 2002.

27. Huisgen, R. "Cenetary Lecture - 1,3-Dipolar Cycloadditions". *Proceedings of the Chemical Society of London,* pp. 357, 1961.

28. Kolb, H.C., Finn, M.G., Sharpless, K.B. “Click Chemistry: Diverse Chemical Function from a Few Good Reactions” *Angew. Chem. Int. Ed*., Vol. 40, pp. 2004 – 2021, 2001.

29. Malkoch, M., Vestberg, R., Gupta, N., Mespouille, L., Dubois, P., Mason, A. F., Hedrick, J. L., Liao Q., Frank, C. W., Kingsbury, K., Hawker, C. J. "Synthesis of well-defined hydrogel networks using Click chemistry", *Chem. Commun*., pp. 2774-2776, 2006.

30. Ossipov, D.A., Hilborn, J. "Poly(vinyl alcohol)-Based Hydrogels Formed by Click Chemistry", *Macromolecules*, Vol. 39, pp. 1709-1718, 2006.

31. Xu, X.D., Chen, C.S., Wang, Z.C., Wang, G.R., Cheng, S.X., Zhang, X.Z., Zhuo, R.X. "Click chemistry for in situ formation of thermoresponsive P(NIPAAm-co-HEMA)-based hydrogels**"** *J. Polym. Sci. A: Polym. Chem.,* Vol. 46, pp. 5263-5277. 2008.

32. Crescenzi, V., Cornelio, L., Di Meo, C., Nardecchia, S., Lamanna, R. "Novel hydrogels via click chemistry : Synthesis and potential biomedical applications" *Biomacromolecules*, Vol. 8, pp. 1844-1850, 2007.

33. Liu, S.Q., Lai, R.P., Ke, C.Y., Hedrick, J.L., Yang Y.Y. "Biodegradable poly(ethylene glycol)–peptide hydrogels with well-defined structure and properties for cell delivery", *Biomaterials*, Vol. 30, pp. 1453-1461, 2009.

34. Silva et al. "Growth Factor Delivery Approaches in Hydrogels", *Biomacromolecules*, Vol. 10, pp. 9-18. 2009.

35. D. Chacon et al. "Swelling and protein absorption/desorption of thermo-sensitive lactitol-based polyether polyol hydrogels", *Polymer*, Vol. 41, pp. 8257–8262, 2000.

36. T. Yu et al. "Functional Hydrogel Surfaces: Binding Kinesin-Based Molecular Motor Proteins to Selected Patterned Sites", *Adv. Funct. Mater*., Vol. 15, pp. 1303-309, 2005.

37. Hawker, C. J., Fréchet, J. M. J. "Preparation of polymers with controlled molecular architecture. A new convergent approach to dendritic macromolecules", *J. Am. Chem. Soc.,* Vol. 112, pp. 7638-7647, 1990.

38. Wu, P., Malkoch, M., Hunt, J., Vestberg, R., Kaltgrad, E., Finn, M.G., Fokin, V.V., Sharpless, K.B., Hawker, C.J. "Multivalent, bifunctional dendrimers prepared by click chemistry", *Chem. Commun*., pp. 5775–5777, 2005.

39. Veronese, F. M., Pasut, G. "PEGylation, successful approach to drug delivery", *Drug Discovery Today*, Vol. 10, pp. 1451-1458, 2005.

40. Ihre H., De Jesus O.L.P., Fréchet, J. M. J. "Fast and Convenient Divergent Synthesis of Aliphatic Ester Dendrimers by Anhydride Coupling", *J. Am. Chem. Soc.,* Vol. 123, pp. 5908-5917, 2001.

41. Fréchet, J. M. J., Gitsov, I., Monteil, T., Rochat, S., Sassi, J. F., Vergelati, C., Yu, D. "Modification of Surfaces and Interfaces by Non-covalent Assembly of Hybrid Linear−Dendritic Block Copolymers: Poly(benzyl ether) Dendrons as Anchors for Poly(ethylene glycol) Chains on Cellulose or Polyester", *Chem*. *Mater*., Vol. 11, pp. 1267-1274, 1999.

42. Gitsov, I.; Fréchet, J. M. J. "Stimuli-Responsive Hybrid Macromolecules: Novel Amphiphilic Star Copolymers With Dendritic Groups at the Periphery", *J. Am*. *Chem*. *Soc*., Vol. 118, pp. 3785-3786, 1996.

43. Liu, M., Kono, K., Fréchet, J. M. J. "Water-soluble dendrimer-poly(ethylene glycol) starlike conjugates as potential drug carriers" *J*. *Polym Sci. A*: *Polym Chem.*, Vol. *37*, pp. 3492-3503, 1999.

44. Namazi, H., Adeli, M. "Dendrimers of citric acid and poly (ethylene glycol) as the new drug-delivery agents" *Biomaterials*, Vol. 26*,* pp. 1175-1183, 2005.

45. Namazi, H., Adeli, M., Zarnegar, Z., Jafari, S., Dadkhah, A., Shukla, A. "Encapsulation of nanoparticles using linear–dendritic macromolecules", *Colloid. Polym. Sci.*, Vol. 285, pp. 1527-1533, 2007.

46. Megia, E.F., Correa, J., Riguera, R. "Clickable PEG-Dendritic Block Copolymers", *Biomacromolecules*, Vol. 7, pp. 3104-3111, 2006.

47. Grinstaf, M. W. "Dendritic Macromers for Hydrogel Formation: Tailored Materials for Ophthalmic, Orthopedic, and Biotech Applications", *J. Polym. Sci. A: Polym. Chem.,* Vol. 46, pp. 383-400, 2008.