REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS OF DENDRONIZED POLYMERS BY RING OPENING METATHESIS POLYMERIZATION TECHNIQUES AND INVESTIGATION OF BIOLOGICAL ACTIVITIES

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DOCTOR OF PHILOSOPHY THESIS

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Program of Organic Chemistry

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A thesis submitted by Muhammad Nazrul ISLAM in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** is approved by the committee on 20.07.2020 in the Department of Chemistry, Program of Organic Chemistry.

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Muhammad Nazrul ISLAM

Signature

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Dedicated to Farhana

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Muhammad Nazrul ISLAM

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LIST OF SYMBOLS

d	Day
°C	Degree Centigrade
g	Gram
h	Hour
kV	Kilovolt
µg	Microgram
µL	Microliter
mV	Millivolt
M_n	Number Average Molecular Weight
Đ	Polydispersity Index
V	Volt
W	Watt
λ	Wavelength
ζ	Zeta Potential

LIST OF ABBREVIATIONS

BOC	Di-*tert*-dibutyldicarbonate
DCM	Dicholoromethane
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
DP	Degree of Polymerization
EDA	Ethylenediamine
EDA-BOC	*tert*-butyl N-(2-aminoethyl)carbamate
FT-IR	Fourier Transform Infrared Spectroscopy
G	Generation
GPC	Gel Permeation Chromatography
Grubbs 3rd	Dichloro-di(3-bromopyridino)N,NDimesitylenoimidazolino RuCHPh)
kDa	Kilo Dalton
LPR	Liquid Phase Polymer Based Retention
mL	Mililiter
MIC	Minimum Inhibitory Concentration
MWCO	Molecular Weight Cut-Off
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ppm	Parts Per Million
PAMAM	Poly(amidoamine) Dendrimer
PPI	Poly(propylene imine) Dendrimer
ROMP	Ring Opening Metathesis Polymerization
RPM	Rotation Per Minutes

TFA	Trifluroacetic Acid
TLC	Thin Layer Chromatography
THF	Tetrahydrofuran
UV-Vis	Ultraviolet Visible

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ABSTRACT

Synthesis of Dendronized Polymers by Ring Opening Metathesis Polymerization Techniques and Investigation of Biological Activities

Muhammad Nazrul ISLAM

Department of Chemistry

Doctor of Philosophy Thesis

Advisor: Prof. Dr. Metin TÜLÜ

In this thesis, *exo*-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride was successfully synthesized from furan and maleic anhydride by Diels-Alder reaction. Diels-Alder adduct was conjugated with hydrazine hydrate, ethylenediamine, BOC-protected ethylenediamine, 1,4-diaminobutane and 1,6-diaminohexane linker to get amine surfaced core. Percentage of yield of the product was found higher with the increase of chain length of linker. Percentage of yield for ethylenediamine, 1,4-diaminobutane and 1,6-diaminohexane linker core was 10%, 40%, and 52% respectively. When ethylenediamine was incorporated directly to oxanorbornene, a mixture of exo-endo products was observed which was not easily separable. Pure *exo*-isomer was required to polymerize the monomer later on. One NH_2 of ethylenediamine was protected by di-*tert*-butyl-dicarbonate (BOC) to get EDA-BOC.EDA-BOC was incorporated to *exo*-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride to get pure exo isomer. BOC was deprotected by

trifluoroacetic acid (TFA) to get NH_2 surface. Oxanorbornene cored amine surfaced compounds are extended as half generation dendritic compound by treating with methyl acrylate by Michael addition reaction. This process is laborious, multistep and percentage of product quite low. Same compound was synthesized by convergent strategy through 0.5 generation dendron from ethanolamine which was incorporated with furan-maleimide through Mitsunobu reaction. Homopolymer of two carbon liker 0.5 generation dendritic monomer has been synthesized by Grubbs 3rd and 2nd generation catalyst. Similarly, 0.5, 1.5 and 2.5 generation dendritic monomers were synthesized from six carbon linker core through divergent strategy by methyl acrylate and ethylenediamine. Homopolymers from all monomers were synthesized by Grubbs 3rd and 2nd generation catalyst. Targeted molecular weight of homopolymers was 20 kg/mol which was further investigated by GPC and ^{1}H NMR end group analysis. Cu-PAMAM-ROMP nanoparticles were prepared and catalytical activities of this nanoparticle for conversion 4-nitrophenol to 4-aminophenol were investigated by UV-vis absorption.

Again, *exo*-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride, reacts with pyridine-3-ylmethanamine to get pyridine containing monomer which was further converted to pyridinium salt by 1-bromohexane. Finally random and block copolymer of two and six carbon linker 0.5 generation dendritic monomer with pyridinium based quarternary ammonium monomer were synthesized through ROMP by Grubbs third generation catalyst. Targeted molecular weight of copolymers was 10 kg/mol, which were further investigated by GPC in DMSO solvent and ^{1}H NMR end group analysis. After then ester terminated polymers surface were treated by EDA-BOC to get BOC surfaced copolymers. These polymers were modified to be water soluble cationic polymer by TFA to get antibacterial polymers.

Antibacterial activities and hemolytic tests of synthesized copolymers have done by serial dilution method. Six carbon linker copolymers were observed high antibacterial activity against Gram-positive bacteria (*S. aureus*), whereas they were inactive against Gram-negative bacteria (*E. coli*). Moreover, though molecular weight and monomers feed ratio of monomers kept same, block copolymer shows

MIC 32μg/mL but random copolymer shows MIC 64 μg/mL. Hemolytic data shows all the copolymers, except two exemption, are non-toxic (>1000 μg/mL) against human blood cell. *S. aureus* incubated with active and inactive polymer and Zeta potential were measured to see relationship between the MIC and membrane surface charge density. Zeta potential of copolymers was found +5.7 mV to +16.3 mV but *S. aureus* incubated active polymer's zeta potential found -3.5 mV. Scanning Electron Microscope (SEM) image confirms damage of the bacterial cell wall after implementation of our antimicrobial polymer.

Keywords: ROMP, dendron, nanoparticle, catalytic, antibacterial.

ÖZET

Halka Açılım Metatez Polimerizasyon Tekniği ile Dendrize Polimerlerin Sentezi ve Biyolojik Aktivitelerin İncelenmesi

Muhammad Nazrul ISLAM

Kimya Bölümü

Doktora Tezi

Danışman: Prof. Dr. Metin TÜLÜ

Bu çalışma kapsamında furan ve maleik anhidritin Diels-Alder reaksiyonu ile başarılı bir şekilde ekzo-7-okzabisiklo[2.2.1]hept-5-ene-2,3-dikarboksilik anhidrit sentezlenmiştir. Amin yüzeyli bir çekirdek elde etmek amacıyla Diels-Alder katılma ürünü; hidrazin hidrür, etilendiamin, BOC ile korunmuş etilendiamin, 1,4-diaminobutan ve 1,6-diaminoheksan bağlayıcı ile konjüge edilmiştir. Elde edilen ürünün yüzde veriminin, bağlayıcının artan zincir uzunluğu ile doğru orantılı olarak artığı görülmüştür. Ürünün yüzde verimi kullanılan bağlayıcılara göre sırasıyla etilendiamin için %10, 1,4-diaminobutan için %40 ve 1,6-diaminoheksan için %52 olarak bulunmuştur. Etilendiamine doğrudan okzanorbornenin yapısına katıldığında, ortamda ayırması kolay olmayan ekzo ve endo karışımlarının oluştuğu gözlenmiştir. Sadece ekzo-izomer yapısının elde edilmesi için monomerin daha sonra polimerize edilmesi gerekmiştir. Etilendiaminin (EDA) bir $-NH_2$ grubu di-tert-butil-dikarbonat (BOC) ile korunup EDA-BOC kompleksi elde edilmiştir. EDA-BOC kompleksi ekzo-7-[2.2.1]hept-5-ene-2,3-dikarboksilik anhidrit ile bağlanarak saf ekzo-izomer elde edilmiştir. Bu kompleks trifloroasetik asit (TFA)

ile muamele edilerek koruyucu gruplar (BOC) uzaklaştırılmış ve yüzeye $-NH_2$ grupları bağlanmıştır. Yüzeyine amin grupları bağlanmış olan okzanorbornen çekirdeği ile metakrilat arasındaki Michael katılması reaksiyonu ile yarım jenerasyon dendritik yapı elde edilmiştir. Bu işlem zor, birçok aşamadan oluşan bir işlemdir ve ürün yüzde verimi oldukça düşük bulunmuştur. Aynı yapı, Mitsunobu yöntemiyle etilendiamine furan-maleimid bağlanarak elde edilen yarım jenerasyon dendrondan yakınsak yöntem kullanılarak sentezlenmiştir. İki karbon ara zincirli yarım jenerasyon dendritik monomerden ikinci ya da üçüncü jenerasyon Grubbs katalistleri kullanılarak homopolimerler sentezlenmiştir. Benzer şekilde, yarım, 1.5 ve 2.5 jenerasyon dendritik monomerler altı karbon ara zincirli çekirdeğe metakrilat ya da etilendiaminkatılarak ıraksak yöntemle sentezlenmiştir. Bütün homopolimerler ilgili monomerlerin ikinci ya da üçüncü jenerasyon Grubbs katalizörlüğünde reaksiyona girmesi ile sentezlenmiştir. Sentezlenen homopolimerlerin molekül ağırlıkları 20 kg/mol olarak hedeflenmiş ve elde edilen molekül ağırlıkları GPC ve ^1H NMR analizleri kullanılarak son grup analizi yapılarak hesaplanmıştır. Cu-PAMAM-ROMP nanopartikülleri hazırlanmış ve bu nanopartikülerin katalitik aktiviteleri 4-nitrofenol'ün 4-aminofenol'e dönüşümünde kullanılarak UV-Vis absorpsiyonu ile incelenmiştir.

Ekzo-7-okzabisiklo[2.2.1]hept-5-ene-2,3-dikarboksilik anhidrit 3-(aminometil)piridinile reaksiyona sokulmuş ve piridin içerikli monomer elde edilmiştir, piridin içerikli bu monomer daha sonra 1-bromohekzan ile reaksiyona sokularak piridinyum tuzuna dönüştürülmüştür. En sonunda fenil bazlı kuaterner amonyum içeren iki ya da altı karbon ara zincirli yarım jenerasyon dendritik monomerlerden halka açılma metatezi polimerizasyonu (ROMP) ile üçüncü jenerasyon Grubbs katalizörlüğünde blok ve rastgele kopolimerler sentezlenmiştir. Sentezlenen kopolimerlerin molekül ağırlığı 10 kg/mol olarak hedeflenmiştir ve DMSO solventi içerisinde GPC ve ^1H NMR analizleri yapılmış ve son grup analizi ile gerçek molekül ağırlıkları hesaplanmıştır. Daha sonra ester yüzeyli polimerler EDA-BOC ile muamele edilerek BOC yüzeyli kopolimerler elde edilmiştir. Bu polimerler TFA ile muamele edilmiş suda çözünür katyonik polimerlere modifiye edilerek antibakteriyel özellik kazandırılmıştır.

Sentezlenen kopolimerlerin antibakteriyel aktivite ve hemolitik aktivite testleri seri dilüsyon metodu kullanılarak yapılmıştır. Altı karbon ara zincirli kopolimerler gram-pozitif *S. aureus* bakterisine karşı yüksek antibakteriyel etkiyi göstermiş, ancak gram-negatif *E. coli* bakterisine karşı inaktif kalmışlardır. Ayrıca, aynı monomer oranı ve molekül ağırlığına sahip olan, blok ve rastgele kopolimerlerin MİK değerleri sırasıyla 32 μg/mL ve 64 μg/mL olarak bulunmuştur. Hemolitik aktivite sonuçları, iki kopolimer dışında diğer bütün kopolimerlerin kırmızı insan kan hücrelerine karşı toksik olmadığını (>1000 μg/mL) göstermiştir. Aktif ve inaktif polimerler ile inkübe edilen *S. aureus* bakterilerinin zeta potansiyel değerleri ölçülerek membran yüzey yük yoğunluğu ile MİK değerleri arasındaki ilişki incelenmiştir. Kopolimerlerin zeta potansiyelleri +5.7 mV ile +16.3 mV arasında bulunmuş; ancak *S. aureus*' a karşı aktif olan polimer ile *S. Aureus* inkübasyon sonrası ölçülen zeta potansiyel değeri -3.5 mV olarak bulunmuştur. Taramalı elektron mikroskop (SEM) görüntüleri sentezlediğimiz antimikrobiyal polimerler ile bakteri hücrelerinin etkileşimi sonucunda bakteri hücrelerinin hasar gördüğünü göstermektedir.

Anahtar kelimeler: ROMP, dendron, nanopartikül, katalitik, antibakteriyel.

1
INTRODUCTION

1.1 Literature Review

Dendrimers are three dimensional, globular shapes, extremely branched, multifunctioned surface groups on the periphery with relatively monodisperse macromolecules. They have drawn great concentration in recent times for widespread applications including sensors [1], [2], magnetic resonance imaging (MRI) agent [3]–[5], metal nanoparticles, drug delivery devices, solubility enhancement catalysis [6]–[8] antibacterial agents [9], [10] and many more. Poly(amidoamine) (PAMAM) dendrimers and poly(propylene imine) (PPI) dendrimer are most common types of dendrimers that are synthesized by conventional methods and microwave assisted synthesis. Dendrimers are generally synthesized by two different techniques:-divergent and convergent methods. Ethylenediamine and 1,4-diaminobutane cored PAMAM dendrimers are commercially available. 5-amino-8-hydroxyquinoline, p-xylylenediamine used as 2- directional core. Similarly, ammonia and of tri(ethanol)amine has used as 3-directional core to synthesize dendrimer. Dendronized polymers [11]–[13] can synthesize from further polymeric core of dendronized macromonomer that also has drawn researchers' attention in recent years [14]–[16].

On the other hand, in ring opening metathesis polymerization (ROMP) method, cycloalkenes are converted to linear polymers having double bond in the main chain for the synthesis of controlled, water soluble and biologically active polymers. Molecules having higher strain can easily polymerize with high yield as ring strain is the motive power behind ROMP of cyclic olefins that reliant on the release of ring strain energy. Norbornene and oxanorbornene derivatives are most common to synthesize ROMP. Oxanorbornene cored dendrimers are also reliable to synthesized dendrimer based ROMP and length of crosslinker in between dendritic branching and ROMP able oxanorbornene [17], [18] play significant rule

to synthesized such dendrimer based ROMP. Polymerization of the endo isomer of norbornene is effectively shielded the alkene from the Grubbs catalyst that the polymerization did not proceed even after extended reaction condition [19]. To get only exo isomer BOC-protected ethylenediamine is used and then deprotected by TFA to get free NH_2 terminated core as amines are not compatible with Grubbs catalyst [20].

First paper on dendritic macromolecules published by Tomalia *et al.* [21] where they describe the synthesis a new class of starburst macromolecules referred to as dendrimer. Synthesis of polyamidoamine dendrimer has done through Michael addition from a primary amine interior core followed by amidation with excess ethylenediamine. Simultaneous repeating the reactions done for growth of higher generation by controlled branching and terminal groups. This paper clearly showed intrinsic viscosities, hydrodynamic volumes, molecular weight, and number of terminal groups for each generation dendrimers.

Newkome and co-workers have [22] reported tree like three dimensional architectural model macromolecules having polar functional groups on outer surface. Reaction started from typical alkyl halide with tris-ester followed by reducing by $LiAlH_4$ to get triol. From one to thrre branching is producing in each repeated reaction through divergent techniques. These novel cascade molecules construction opens new fields of dendrimer like macromolecules.

Tomalia and Newkome types of dendrimer synthetic techniques were divergent process. The first Fréchet-type convergent synthesis of dendrimer was achieved by using 3,5-dihydroxybenzoyl alcohol (DHBA) monomer that formed benzyl ethers from phenols and benzylic halides in quantitative yields [23]. Aida and coworkers covalently attached Fréchet-type dendron to the zinc and iron containing 5,10,15,20-tetrakis(3',5'-dihydroxyphenyl)porphyrin core, by using the convergent methodology [24]. Helms and coworkers have been synthesized trivalent core containing three 4-(dimethylamino) pyridine (DMAP) analogs dendrimer [25]. Periphery of polysilane dendrimers was functionalized with diaminoarylnickel(II) complexes by Koten and coworkers that was the first dendrimer based catalytic system [26]. Fréchet and coworkers reported a rapid and scalable synthesis of

alkynes and protected aldehydes containing bifunctional dendrimers [27]. In 1989 Rebrov and coworkers firstly reported dendrimers containing heteroelements, in which they synthesized a silicon based fourth generation dendrimer that had siloxane linkages in the backbone and forty eight ethoxy groups at its periphery [28], [29]. Takahashi and coworkers were discovered the preparation of organo-platinum dendrimers of various sizes based on the Pt-acetylide linkages using convergent, divergent, or a combination of the two synthetic methodologies [30].

Kose *et al.* [31] has reported segment block dendrimers consisting of polyester and polyaryl ether dendrons via Diels-Alder cycloaddition. They synthesized oxanorbornene cored acetal-protected polyester dendrimer divergently up to third generation. Extention from first generation to second generation has done by deprotection of the acetal groups followed by treatment of thus-obtained diol with anhydride monomer and finally converted to maleimide functionalized dendron at high temperature with good yield via retro Diels-Alder reaction.

Murat Tonga *et al*. [32] has been synthesized furan-protected maleimide cored third-generation polyester dendron. After that anthracene-appended styrene polymer was incorporated with dendrons containing furan-protected maleimide copolymer via [4+2] cycloaddition reaction resulting in dendron-grafted polymer. Though the group made anthracene-dendron graft polymer, dendritic ROMP also could be well attempt form the double bond of norbornene group by using Grubbs catalyst.

H. Jung and coworker [17] have investigated the effect of dendric monomers generation on ROMP by using Grubbs third generation catalyst. They found first generation ring opening metathesis polymerizable poly(amide) dendronized monomer polymerization rate is higher than the second generation monomer. The rate of polymerization of 2^{nd} generation of the same dendritic monomer is considerably relies on initial concentration of the monomer. They also explore the effect of the cross-linker length in between polymerizable norbornene group and dendritic part on polymerization rate and found with the increase of the length of the crosslinker improve the polymerization rate.

Dendronized diblock copolymer of third generation aryl ether and second generation ester via ring opening metathesis polymerization has been studied by Sridhar Rajaram *et al.* [19]. Initially, they tried to polymerize endo isomer of norbornene of the poly aryl ether dendron's focal point but failed even in extended time. Finally, the group was able to polymerize exo isomer of norbornene containing poly aryl ether dendron by ROMP technique using Grubbs 3rd generation catalyst. They also polymerize polyester dendron and made diblock.

As both azides and alkynes are not well-suited with Grubbs catalyst, Jeremiah *et al.* [20] designed alkyl chloride terminated branching for simplistic modification to an azide after ROMP polymerization step. Later, they have done azide-alkyne click reaction by using copper catalyst to conjugate doxorubicin (DOX)-alkyne analogous to the central azide. In this article, they included six carbon linker in between the norbornene part and 3˚-amide side chain for decreasing steric obstacle to form polymer backbone and branches. This linker could assist consequent modification of side chain, such as conversion of azide from alkyl chloride and CuAAC.

Florent Allais *et al*. [33] has reported synthesis of polyphenolic dendronized polymer from diastereopure endo norbornene via ROMP using first and second generation Grubbs catalyst. Dendronized side chain were prepared in a convergent fashion using Steglich carbodiimide-mediated esterification as coupling reaction and benzyl ether/t-butylesters as a orthogonal protecting groups. Finally, ROMP has done from double bond of highly strain norbornene with highly stereospecific organization of the poly(norbornene) polymer backbone with coil-like spatial distribution of the dendrons. They found broad polydispersities of synthesized polymers by using Grubbs second generation catalyst that indicates a higher rate of propagation than initiation with Grubbs second generation catalyst. Finally, the research group generates stable helical and coil-like conformations of the backbone by molecular modeling using Cerius 2 and Material Studio from Accelrys with Universal Force Field methods.

Kyung Oh Kim *et al.* [18] reported dendronized rod-like homopolymers, block copolymers and gradient copolymers by living ROMP. They synthesized acetal-

protected polyester dendrimer divergently up to fourth generation along with ROMP polymerizable different endo-tricycle[4.2.2.0]deca-3,9-diene (TD) core by a macromonomer strategy. Among cyclobutene and bicyclo[2,2,2]oct-2-ene, only cyclobutene polymerized through ROMP possessing high ring strain. Nevertheless, bicyclo[2,2,2]oct-2-ene was not polymerized as it sterically hindered compound. Firstly, they tried to polymerize fourth generation dendron of TD macromonomers without using any spacer via ROMP. But the attempt was not successful because of the harsh steric bulkiness of fourth generation large dendron. After then they use ethyl and biphenyl linker to connect pendent dendron with ROMP able TD and became successful polymerization even rigid biphenyl spacer gives much better result with narrower PDIs. As poly(TD)s have much higher rigidity than polynorbornenes, the Lps of the dendronized polymers synthesized from TD macromonomers were larger in size (16.8–31.2 nm) than those of dendritic polymers synthesized from norbornene bearing fifth generation ester dendron (8 nm) determined by light scattering analysis. They also synthesized diblock copolymers and gradient copolymers by consecutive addition of the two monomers and simultaneous addition of monomers respectively. Finally, both copolymers were visualized by high-resolution atomic force microscopy (AFM).

Gina M. Stewart and Marye Anne Fox [34] have studied dendrimer- linear polymer hybrids through ROMP. In this study, initially they have been synthesized acyl–terminated first generation dendron by condensing 3,5-dihydroxyacetophenone with the naphthyl-capped. After that, the dendron was attached to exo-cis-norbornenediol and then polymerize by ring opening metathesis techniques. They also polymerize 2^{nd} generation dendron containing several naphthyl chromophores and found low polydespersity of 1.3. Finally, steady state fluorescence and time resolved fluorescence were investigated before and after polymerization.

Andrew J. Boydston *et al*. [35] polymerized dendronized macromonomer via ring expansion metathesis polymerization techniques by using two different Ruthenium based metathesis catalyst. As N-heterocyclic carbine backbone saturation influence catalyst activity they used different tether length of N-

heterocyclic carbine backbone and different monomer/catalyst concentration was applied to see the effect of degree of polymerization. Interestingly a reverse relationship was found between reaction concentration and degree of polymerization. Finally, atomic force microscopy (AFM) image is applied to confirm cyclic topology of uniform cyclic features with no detectable linear polymer contamination.

Meina Liu and coworker [36] have reported on polymerization of highly functional ROMP active *exo*-oxanorbornene based monomers via thiol based Michael addition and ROMP polymerization. Mono hydroxyl terminated core was synthesized from *exo*-3,6-Epoxy-1,2,3,6-tetrahydrophthalic and ethanolamine during 12 h refluxing. From that compound ester linked di-alkyl acetal groups terminated compound has synthesized that finally converted to di-hydroxy terminated monomer with Dowex H^+ resin (first generation). Similarly, second generation tetra hydroxyl terminated cored was synthesized by repeating the same procedure. Again surface was modified di and tetra vinyl ester from G1 and G2 monomer by using acryloyl chloride and acrylic acid. Finally, sugar, POSS and pyrrole functional, terminated dendron monomers have synthesized by R-SH as thiol-Michael adducts. All monomers have been polymerized by Grubbs catalyst generation one and Grubbs catalyst generation three. They found competitive conversion by using Grubbs catalyst third generation than Grubbs first generation catalyst. Finally, they prepared the fluorinated derivative and polymerize by Grubbs 3rd generation catalyst and found it more challenging to polymerize.

An article published by A. S. Erturk and coworkers [37] on copper nanoparticle synthesis through PAMAM dendrimer encapsulation. Third and fourth generation PAMAM dendrimers were prepared from the dendrimers were ethylenediamine, diethylenetriamine, and jeffamine core and tris and carboxyl surfaced. These nanoparticles were characterized by UV-Vis spectrometry and maximum metal loading capacity was investigated PAMAM-TRIS and PAMAM-COOH by spectrometric titration. Size of copper clusters was observed at 570 nm in UV-spectra.

Gürbüz *et al* [38] has synthesized dendrimer encapsulated silver nanoparticle by using Jeffamine cored fourth generation PAMAM and characterized them UV-Vis spectroscopy and high resolution transmission electron microscopy. They reported particle size of 4.71 nm with polydispersity 17.94 %. These dendrimers encapsulated silver nanoparticle was used for the interconversion from 4-nitrophenol towards 4-aminophenol with sodium borohydride ($NaBH_4$) and observed good catalytic activity with rate constant $k=0.12\times10^{-2}s^{-1}$.

Reported work by M. Nemanashi and R. Meijboom [39] showed synthesis of dendrimer encapsulated copper, silver and gold nanoparticle from 4–6 generations of PAMAM-OH and PAMAM-NH_2 dendrimer templating agents. They found the average particle size of metal nanoparticle decreased with the increasing generation of dendrimer. These dendrimer encapsulated metal nanoparticles were used for the reduction of nitrophenol to aminophenol along with sodium borohydride ($NaBH_4$) to correlate the rate constant and dendrimer generation. It was observed that with the increase of dendrimers generation, rate constant of reduction reaction decreased. Concentration of sodium borohydride also plays an important role on rate constant and increased with the increase of $NaBH_4$ concentration.

N. Charles and R. Meijboom [40] have reported PAMAM-OH dendrimer encapsulated ruthenium nanoparticles of size 1.1 to 2.2 nm. The catalytical activity of the nanoparticle was monitored by lowering in absorbance peak of 4-nitrophenol at λ 400 nm and growth of 4-aminophenol absorbance peak at λ 300 nm with $NaBH_4$. The kinetic data were modulated by to the Langmuir-Hinshewood equation and established relationship with particle size with catalytical activity. They demonstrated that smaller particles had higher catalytical activities.

J. Noh and R. Meijboom [41] have studied the effect of size and synthetic method on reducing ability of dendrimer templated and thiol capped Pd and Pt nanoparticles with sodium borohydride. Firstly they prepared hydroxyl terminated dendrimer templated Pd and Pt nanoparticles as well as thiol capped nanoparticle by reverse microemulsion and found dendrimer templated nanoparticle (1.9-2.5 nm) are comparatively lower in size than reverse

microemulsion method nanoparticles (3.5-5.1 nm). After then catalytical activity was investigated through aminophenol from nitrophenol by model reduction reaction. The effect of surface area was studied and found Kvalue off at the higher surface area reason. It was observed that dendrimer template nanoparticle has better catalytical activity.

Antibacterial activities of quaternary ammonium based poly(propyleneimine) (PPI) dendrimers were studied by Chris Zhisheng Chen *et al.* [10]. NH_2 terminated full generation dendrimers were quaternized by different chain length alkyl group to investigate their antibacterial efficiency. Antibacterial properties of surface modified PPI dendrimers depend on generations, chain length of hydrophobic group and charge densities. They have shown fourth generation dendrimers are more potent than lower generation dendrimers and ten carbon containing alkyl chain connected to quaternary ammonium group is highly active against studied bacteria.

Water soluble chitosan based poly(amidoamine) dendrimer nanoparticle and quaternized carboxymethyl chitosan were synthesized to investigate antibial activity and reported that chitosan based poly(amidoamine) dendrimer nanoparticles were strongly active against Gram-negative bacteria [42]. Yan *et al.* [43] has been reported carboxymethyl chitosan/poly(amidoamine) dendrimer cored nanoparticle for antibacterial activity against *E. coli* using cell membrane integrity, outer membrane permeability, inner membrane permeability along with electron microscopy. They also investigate the antibacterial mode of action by considering molecular chain conformation along with positively charge groups electrostatic interaction of carboxymethyl chiston/poly(amidoamine) dendrimer.

Tulu *et al.* [44] has synthesized 0-3 generation jeffamine cored water soluble PAMAM dendrimers with -NH_2 and –COOH surface functionality and disk diffusion method was used for antibacterial activity test. It was observed that NH_2 terminated first and fourth generation showed highest activity against some bacteria.

There are many reports that have been published on quaternary ammonium based antibacterial polymers [45]–[47]. For polymer design ROMP is an important method for the preparation of well-defined water-soluble polymer architectures [48]–[52] and biologically active polymers [53]–[55]. The Tew group studied ammonium-based polymers derived via ROMP and they found that antibacterial and hemolytic activity strongly dependent on alkyl chain length of repeating monomers [56]. The same group reported that the polymer bearing the guanidine functionality is more selective [57]. It was shown that with increasing number of amine groups as part of the hydrophobic polymeric group significantly decrease hemolytic activities [58], [59]. Moreover, Eren *et al*. demonstrated that ethyl or butyl substituent cationic pyridinium polymers synthesized via ROMP are weakly active and not hemolytic. Compared to these findings, when the alkyl group was longer, the polymers turned out to be effective and generally toxic [60]. It was also reported that aryl phosphonium bearing polymers had higher biocidal activity than the alkyl functional analogous [52]. Cationic charge density threshold was also crucial for the activity against *S. aureus* [51]. Surface and solution properties of the pyridinium-based homopolymers might also exhibit different biocidal activity [61].

Moreover, Eren *et al*. demonstrated that ethyl or butyl substituent cationic pyridinium polymers synthesized via ROMP are weakly active and not hemolytic. Compared to these findings, when the alkyl group was longer, that is the increasing hydrophobicity, the polymers turned out to be effective and generally toxic [60]. It was also observed that cationic charge density threshold also crucial for the activity against *S. aureus* [51]. Increasing the double charge density in each repeating unit resulted in high selectivity. Kaymaz *et al.* reported that 1,4-diazabicyclo[2.2.2]octane (DABCO) bearing polymer possessing double charge showed higher antimicrobial activity with *S. aureus* (minimum inhibitory concentration, MIC, 8 μg/mL) than pyridine containing mono charge polymer (MIC 128 μg/mL) in each repeating unit though both types of polymers were nonhemolytic (hemolytic concentration, HC_{50}, >2000 μg/mL). Previous report shows that hexyl pyridinium functionalized ROMP based polymer showed high

biocidal activity (MIC=4μg/mL) however it was toxic (HC_{50}=202μg/mL) [60]. Al-Badri *et al.* established that increasing the number of cationic groups per monomer with higher hydrophobic group have balance to increase the selectivity [58]. Furthermore, many articles reported that the presence of hydrogen bonding domain e.g., amide (–CONH–) seems to be precious in the structures of antimicrobials for the selective binding to bacteria through the positional alignment of the amide hydrogen bonds [62], [63]. It is previously reported that disubstituted amino amides for short cationic synthetic antimicrobial peptides should contain two cationic charged groups and two lipophilic bulky groups [64]–[66]. These findings directed to the design rule of antibacterial copolymers that the cationic charge density, molecular weight of polymers and cationic–hydrophobic balance should be considered to maximize their antimicrobial activities and reduce their hemolytic concentration [67], [68].

1.2 Objective of the Thesis

The findings reported in this dissertation are divided into synthesis and application parts. In the first part involves the synthesis of various generations oxanorbornene cored PAMAM dendrimer based monomers and their homopolymers prepared by ROMP techniques as well as their characterization by ^{1}H NMR and GPC. At the same time, some water soluble cationic random copolymers and block copolymers have synthesized to see biological activities. The second part represents the catalytical applications of the synthesized dendrimers encapsulated Cu nanoparticles. These applications are the investigation reduction capability of 4-nitrophenol to 4-aminophenol. In the biological activity test, MIC and HC_{50} has been reported of the synthesized cationic surfaced dendritic and hexyl-pyridinium based ROMP copolymers.

1.3 Hypothesis

Dendrimers are new class of polymers having monodispersity, wide variety of branching and high density on the surface functional groups. In the branch of

polyamidoamine (PAMAM) dendrimers have high density of tertiary amine that can bind easily with metal. Dendrimer encapsulate metal nanoparticle is well known and highly used for catalysis. ROMP is an astute strategy for biologically active controlled polymer synthesis. New types of ROMP based dendritic polymers could be chosen as a template for metal nanoparticle. These ROMP based dendritic polymer metal nanoparticle further be a potent candidate for catalytical activity.

Moreover, surface of dendrimers like polyamidoamine (PAMAM) dendrimers can be converted to quaternary ammonium or alkyl-ammonium groups by surface modification. Quaternary ammonium compounds have antimicrobial activities that widely used in antiseptics and disinfectants. Furthermore, presence of hydrogen bonding domain e.g., amide (–CONH–) seems in PAMAM branching to be precious in the structures of antimicrobials for the selective binding to bacteria through the positional alignment of the amide hydrogen bonds. Antimicrobial properties of these compounds have strong correlation between the lengths of the linker chain and antimicrobial activity that can be studied by different generation dendrimers. ROMP based dendrimers could open new era as an antibacterial agent. Dendrimer based norbornene backbone monomer could best choice for synthesizing dendritic ROMP polymer. ROMP based dendrimers synthesized in this dissertation to be used as an antibacterial agent too.

2
POLYMERS

2.1 History of Polymers

The word “polymer” was introduced by the Swedish chemist Jhon Jacob Berzelius [69] in 1833 in his book the "Jahres-Bericht" to express comparatively bigger molecules having identical empirical formula but very different chemical and physical properties [70]. For example, molecular formula of benzene and acetylene is C_6H_6 and C_2H_2 respectively. Empirical formula of both compounds is (CH) and he considered benzene is polymer of acetylene. Though present connotation of polymer is different than J. J. Berzelius’s definition, it gives a general concept of bigger molecules and isomerism. August Kekule provided a solid foundation for polymerism [71] and advanced hypothesis for organic substances originated from nature. Those are most closely related with life such as proteins, starch, and cellulose-may consist of very long chains which develop their special properties of the structure. According to Flory, proteins and carbohydrates were made up of a number of species differing from one another with respect to the degree of molecular condensation [72]. In 1986 A. V. Lourenco reported an adduct of ethylene glycol and ethylene dihalide of two to six repeating unit which was early example of polymer preparation [70]. Hermann Staudinger, on developing a new and simple preparation of the monomer, studied the polymerization of isoprene as early as 1910 [73] and fully concentrate on study of polymers and dedicate his life polymeric materials, preparation and their properties study. Hermann Staudinger who proposed the name "macromolecules” and contributes for establishment of macromolecular chemistry received the Nobel Prize in 1953 [74]. There are many chemists and scientists are prominent for their contribution in the field of polymer science such as Charles Goodyear for discovery of vulcanization process of rubber, Herman Francis Mark for X-ray diffraction work, Paul John Flory for understanding the polymer solution behavior (achieved the Nobel Prize in Chemistry in 1974),

Wallace Hume Carothers for invention of nylon and Karl Waldemar Ziegler, Ziegler-Natta catalyst for ring polymerization (won the Nobel Prize in Chemistry in 1963).

The term polymer consists of two Greek roots: poly means many and meros means part. Thus the word polymer means many parts and indicating a big molecule made up by combination of some smaller repeating unit called monomer. Polymer contains thousands to millions atoms in a molecule to make long chain or even complex structure. Simplest example polyethylene is a polymer which is formed by repeating unit of ethylene exposed in Figure 2.1.

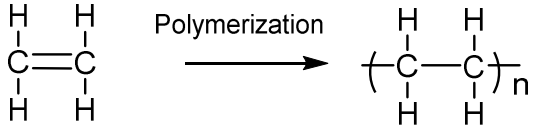


Figure 2.1 Reaction scheme of polyethylene

Herein, "n" is the number of monomer repeating unit. It might be few hundred to several thousand depending of molecular weight of polymer.

The word monomer refers to the repeating unit of a polymer that linking one after another to form polymeric chain. Monomers are simple organic molecules from which polymer molecules are made and usually named after poly of that monomer. In the previous example (scheme 2.1) it is clear that repeating of ethylene polymer chain is called polyethylene. Again number of repeating unit of the polymer is defined as degree of polymerization and represented by DP. Relationship between molecular weight (M) and degree of polymerization (DP) is as-

$$M = (DP)M_0 \tag{2.1}$$

Herein, M_0 represents the molar mass of each repeating monomer.

2.1.1 Polymerization Process

There are two basic types of polymerization mechanism- i) Addition polymerization and ii) Condensation polymerization.

2.1.1.1 Addition Polymerization

Addition polymer is the polymer that is formed by direct addition of two or more monomers by radical reaction and with no resulting water or small molecular by-product. In this polymerization, a small and low molecular weight simple molecule possessing a double bond is preferred as a monomer. So that double bond of the monomer opens and form free valences join with those of same molecules or other molecules to form polymer chain. For example, Teflon or polytetrafluoroethylene is formed by polymerization of tetrachloroethene given in Figure 2.2. No side products are formed in addition polymerization.

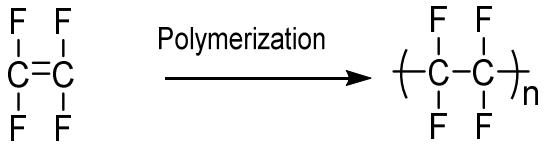


Figure 2.2 Synthesis scheme of Teflon

2.1.1.2 Condensation Polymerization

Condensation polymers are formed by condensation reaction where two complementary end groups of two monomers condense to form a linkage in the polymer chain by losing water or methanol like small molecules. The reaction leads successively from monomer to dimer, trimer, tetramer and so on stepwise. This polymer is also called step growth polymer as polymerization occurs by stepwise reaction until forming large polymer molecules. Formation of polyester is an example of addition polymerization exposed as follows-

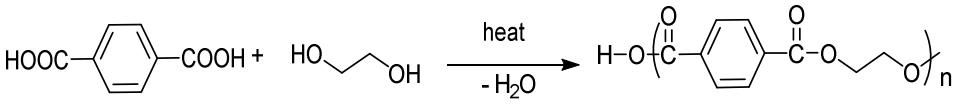


Figure 2.3 Synthesis scheme of polyester

2.2 Polymer Architecture

Polymers may be composed of either only one repeating unit or two different repeating units named as homopolymers and copolymers respectively.

2.2.1 Homopolymers

Polymers which have just a single repeating monomer on the compositions of the polymer structure are defined as homopolymers. An example of homopolymer is exposed in Figure 2.4.

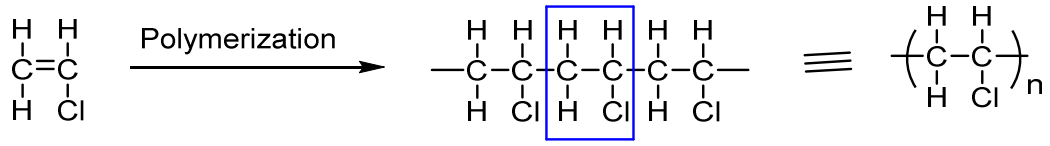


Figure 2.4 PVC- an example of homopolymer

2.2.2 Copolymers

Polymers bearing two or more different types of repeating monomer units in the composition of polymer structure are known as copolymers. Styrene-acrylonitrile copolymer is an example of simple copolymer which is formed by polymerization of styrene and acrylonitrile at a time in a reactor at certain reaction condition.

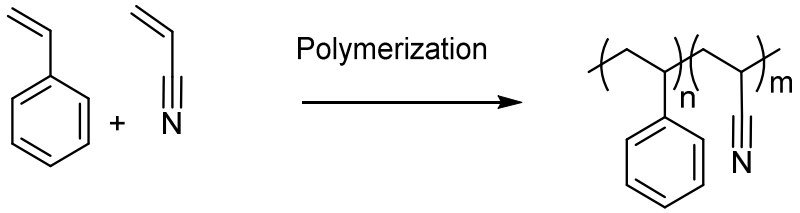


Figure 2.5 Synthesis scheme of copolymer

It is not always necessary to be same as polymer structure with monomer compounds that were made of repeating monomers. Some polymers such as nylon-6,6 have same repeating units that originated from more than one monomer structural unit -these polymer structures are still well known as homopolymers. Reaction of nylon-6,6 given as bellow-

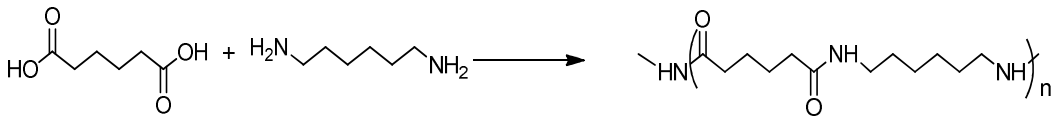


Figure 2.6 Synthesis scheme of nylon-6,6

The repeating units on the backbone in the copolymer structure might be organized in a variety of degrees of arrangements. It is also allowed that, only single nature of monomer repeating unit in the backbone and another type of monomer repeating unit might have in branches to get desired copolymer. There are various types of copolymers depending on its chain structure:-

2.2.2.1 Random Copolymer

The arrangement of repeating monomer units of these types of copolymer is random on the chain molecule without following any kind of order or regular agreement. If the repeating monomers unit represented by X and Y, then the random copolymer structure can be shown as Figure 2.7.

Y–X–X–Y–Y–Y–Y–X–X–X–Y–Y–X–X–X–Y–X–X

Figure 2.7 Arrangement of repeating units in random copolymer

2.2.2.2 Alternating Copolymer

In this type of copolymer, there is an alternating order of monomers unit in the arrangement of two monomers in the polymer chain–monomers are repeating one after another in the polymer backbone that is shown below.

X–Y–X–Y–X–Y–X–Y–X–Y–X–Y–X–Y–X–Y–X–Y

Figure 2.8 Arrangement of repeating units in alternating copolymer

2.2.2.3 Block Copolymer

In block copolymer, the structure is composed of comparatively longer arrangement of one monomer unit to get a block and then start repeating second monomer unit to prepare another block and each block chemically again bound together.

X–X–X–X–X–X–X–X–X–Y–Y–Y–Y–Y–Y–Y–Y

Figure 2.9 Arrangement of repeating units in block copolymer

2.2.2.4 Graft Copolymer

In graft copolymer, one monomer sequence is repeating to be made linear backbone and another monomer unit repeating as a branch on the backbone i.e, grafted onto a backbone. An ordinary structure of graft copolymer is shown below-

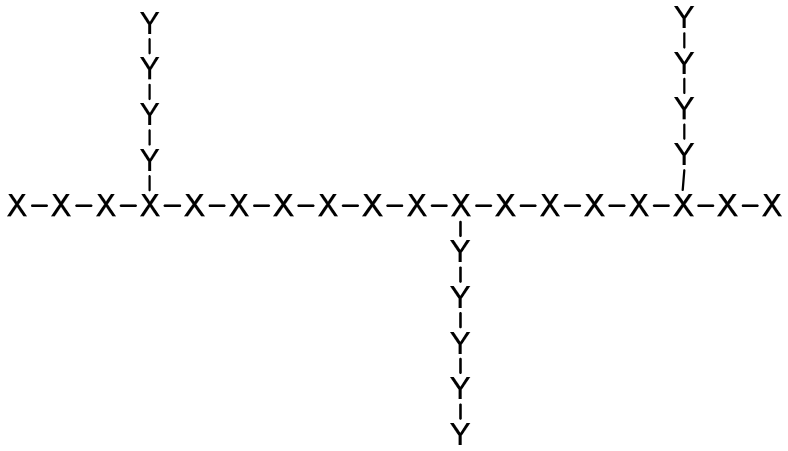
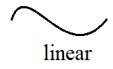
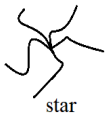
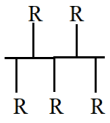
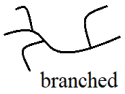
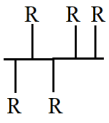
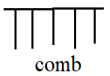
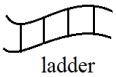
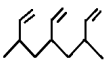
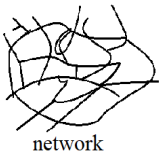


Figure 2.10 Arrangement of repeating units in graft copolymer

The structure of polymer can be formed by the reaction of monomers in certain principles to be connected with each other by any possible style. Monomers having two functional groups are able to link with two other monomers through chemical boding. That is why, the pattern of combination of bifunctional monomers is generally grown as linear chain and obtained polymer is known as linear polymer. Nevertheless, monomers having multiple functional groups can react in varieties of patterns that might form nonlinear polymer chain. Sometimes, polymerization reaction of a chain terminates before linked with another chain that might form branched polymer. Moreover, some polymer chain is grown up to linking with another polymer chain which is defined as cross-linked polymer. Schematic views of all types of molecular architecture of polymers are given in Table 2.1.

Table 2.1 Schematic view of all types of molecular architecture

Topology	Composition	Functionality	Micostructure
 linear	 homopolymer	 end-functional polymer	 isotactic
 star	block copolymer	 telechelic polymer	 syndiotactic
 branched	random copolymer	 site specific polymer	 heterotactic
 comb	alternating copolymer	 macromonomer	 cis-1,4-linkage
 ladder	 graft copolymer		 trans-1,4-linkage
 cyclic			 1,2-linkage
 network			

2.3 Dendronized Block Copolymers

Dendronized block co-polymers are block copolymer in which highly branched different generations dendrons are linked as pendant groups onto the backbone of a linear polymer [75]. Mainly three routes are used synthesize dendronized polymers- graft-to method, graft-from method and graft- through method.

2.3.1 Grafting From

Preparation of grafting from dendronized block co-polymers begins with the synthesis of main backbone of polymer containing multi-initiator (predetermined number) sites on surface which are afterward used to initiate further block of co-polymer. Based on polymerization methodology followed by both steps, initially

polymerization has done and after then initiating groups incorporate directly or protected form [76], [77]. In grafting from synthesis method, composition and length can be controlled as the backbone is synthesized first. Nevertheless, from the initiating surface active groups and backbone initiate significant steric effects which sometimes directed to introduce polymer initiation [78], [79].

2.3.2 Grafting Onto

During grafting onto synthesis technique, both main chain and dendronized branch are prepared individually. The procedure associates the reaction of branched functional polymers with main chain of polymer having many supportive functional groups. As main chain and branches are synthesized separately, the phenomenon and properties control is good if appropriate process of polymerization can be adopted to synthesize them from particular monomers. Nevertheless, in grafting onto techniques also might have some limitations. As two giant molecules are combined through a reaction of small active sites, probability of steric effect is more than grafting from synthesis. Again the technique is thermodynamically and kinetically unfavorable due to reaction of the chain ends of largely branched polymer and main chain functional groups and relatively low synthesis has done by grafting onto method. Though it is difficult to purify the final dendric graft polymers by repeated fractionation to get rid of unreacted linear chains, reactive side chains can be used in excess amount to increase the grafting density. Two parameters should keep in mind during the “grafting onto” synthesis for increasing the grafting density, there is the use a reactive polymeric side chain with a “thinner” structure, that can reduce the steric hindrance during grafting reactions [80].

2.3.3 Grafting Through

Grafting through approach consists of two synthetic steps: firstly, preparation of polymers having a further polymerizable group in the chain ends; secondly,

polymerization of the polymerizable chain end group to get the dendric grafts structures.

As the side chains are prepared through proper polymerization strategy first, polymer architecture can be tailored according to grafting onto approach and prepared controlled manner polymer. Grafting density tailored such a way that each monomer moiety (end functional group of polymers) attached to main chain of polymer that bears a certain number of branches. If end polymerizable group is polymerized by living polymerization process in 2nd step, then length of main chain remains under control. As a result, it provides molecular grafting with highly controlled architecture among the techniques. Specific early effort implements in grafting through techniques to prepare molecular grafting unable to show significant response due to difficulty of polymerization of end group of branches. Systematic synthesis of grafting from, grafting onto, grafting through polymerization techniques are shown in Figure 2.11.

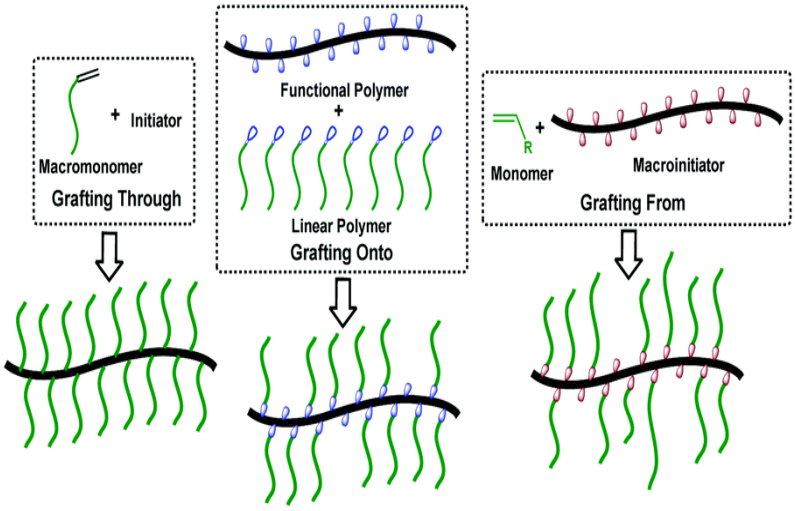


Figure 2.11 Grafting from, grafting onto, grafting through polymerization techniques [81]

2.4 Dendrimers

Dendrimers are highly branched polymeric materials with low polydispersity and three-dimensional globular architectural design. The term "dendrimer" is taken from the Greek words consisting dendron and meros; dendron means tree and

meros means part is meant to underscore the tree-like branched structure of this class of compounds which original designation of cascade molecules. The concept of repeating growth with branching was first reported in 1978 by Vogte (University of Bon, Germany) to produce low molecular amine [82]. The earliest paper was published by describing dendrimer by using ethylenediamine and ammonia as a core in 1985 by Donald A. Tomalia, Dow chemical, USA [21]. In the same year, Newkome *et al.* of Louisiana State University reported about three dimensional macromolecules synthesis with polar functional groups in the surface that they named Arborols which also means tree in a communication [22]. Though both of these initial routes were synthesized step-wise addition of monomeric units in a radially outward fashion (divergent approach) but Newkome's series were grown by the use of [1 → 3]-C-branched monomers; and Tomalia's dendrimers were built by the use of [1 → 2]-C-branched monomers [21], [22]. Up to now at least 10000 articles have been published in this field and dendrimer became a unique branch of chemistry.

Dendrimers are highly branched polymer structure by secondary architecture and network to form globular in shape from a central compound with multiple functional groups. Central part of the dendrimer from where side networking started is known as core. Regular arm of repeating units of polymer is interior (or branches) and functional groups at the end of globular structure are periphery or (end groups). An ordinary structure of a dendrimer is given in Figure 2.12

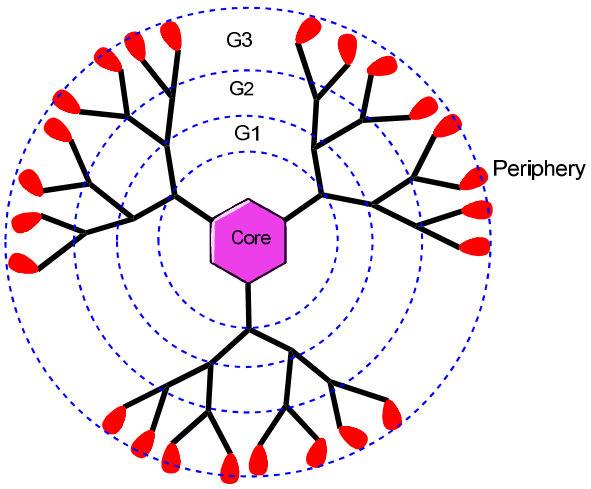


Figure 2.12 Structure of dendrimer

Molar mass of the dendrimer can be predicted by mathematical formula [83], [84]-

$$M = M_c + n_c.\left[M_m\left(\frac{n_m^G - 1}{n_m - 1}\right) + M^t.n_m^G\right] \quad (2.2)$$

where, Mc is the molecular weight of core, M_m is the molecular weight of side branches monomer, M_t is the molecular weight of the outer surface group, n_c is the multiplicity of central core, n_m is the multiplicity of side branching-juncture, G represents numbers of generation.

$$\text{Terminal groups } Z = n_c n_m^G \quad (2.3)$$

2.4.1 Dendrimer Synthetic Strategy

Dendrimers are generally synthesized by using either a divergent method or a convergent one. In divergent synthesis branches grown up from a multifunctional central core, one after another by a successive addition of layers of monomer in out word fashion. Vogtle's cascade molecules, Newkome's arbors, and Tomalias dendrimer was the divergent approach of dendrimer synthesis [21], [22], [82]. Divergent method is known as "outside in" method. In this method, initially core has been selected or synthesized and after then dendrimers are progressing like growing up a tree- from root to branch one after another sequentially outward way. This process is repeated in each generation to reached desired generation. In this synthesis method, with the increasing generation, functionality in the surface also increases. This leads to exponential reactions to be performed and this is difficult for even highly efficient reactions. Separation of the unreacted starting compounds, by-products and incomplete generation small dendrons are usually difficult for divergent method as their size, mass and other characteristics are very similar to perfect dendrimer especially in higher generation synthesis [85].

On the other hand, Hawker and Frechet in 1989-1990 introduced convergent method of dendrimer synthesis [86] which is further followed by Miller [87]. In convergent approach synthesis start from the periphery to central core by inwards growth. Initially, arms are synthesized by inwards process and then attached to multicultural core molecules [86]. Convergent synthesis techniques of dendrimers

provides greater control of the structure than the divergent techniques because of having relatively lower number of coupling reactions at each growth step, which permit finding pure dendritic products and functional versatility [88], [89]. The capability of specifically placed functional groups all over the structure, selectively modify the focal point or the chain ends, and prepare well-defined unsymmetrical dendrimers are among the most attractive features of the convergent techniques. Convergent approach is less readily scaled up than the divergent synthesis that is why; commercialization of convergent approach is presently limited, just only one family of polyether dendrons produce by Tokyo Kasei Co., Ltd. in Japan [86]. Because of having ability to modify dendrons at both the focal point and the chain ends by convergent approach is also used for synthetic versatility of dendrimers [90].

2.4.2 Types of Dendrimer

First dendrimer synthesized by Tomalia, was built through divergent approach by the use of [1→2]-C-branched monomers [21], [22]. On the other hand, Newkome's series were grown by the use of [1→3]-C-branched monomers through divergent approach. In 1990 Hawker and Frechet introduced convergent method of dendrimer synthesis [86]. So according to synthetic strategy mainly dendrimers are two types - i) divergent and ii) convergent that discussed in previous section. According to branching from monomer dendrimers are [1→2]-C-branched [21], [22] and [1→3]-C-branched dendrimers [91], [92]. Two most common types of commercially available dendrimers are i) PAMAM dendrimers and ii) PPI dendrimers. Divergent method is used for the synthesis of PAMAM dendrimers through a core ammonia or ethylenediamine. They are constructed using reactive sequences, consisting of double Michael addition reaction of methyl acrylate to amino group and amidation reaction by excess amount of ethylenediamine through a carbomethoxy intermediate. In polyamdoamine dendrimers containing tertiary amine as branching point and amide group (–NH-C=O) PPI dendrimer also synthesized from ethylene core through a divergent process. Initially,

ethylenediamine is reacts with acrylonitrile followed by heterogeneous hydrogenation through Raney nickel catalyst [93].

2.4.3 Dendrimers Encapsulated Metal Nanoparticles (DEMNs) and Catalysis

Among dendrimers, PAMAMs are the earliest dendrimer family which are successfully synthesized, characterized and commercialized [94]. High density of nitrogen ligands in PAMAM dendrimer allows them to behave like a container or template for the transition metals which are used for dendrimers-encapsulated metal nanoparticles (DEMNs) [95]. Dendrimer encapsulated nanoparticles (DENs) was firstly reported by Crooks *et al*. [96] and after then similar the procedure used parallelly by Tomalia [97]. Different types of Pt, Pd, Au, Ag, Cu, Ni, Ru, Mn, and Fe DEMNs were synthesized in the literature [98]–[104]. Dendrimers-encapsulated metal nanoparticles (DEMNs) were first studied as dendrimer intramolecular complexes between PAMAM dendrimer and copper (II) [105]. These metal PAMAM complexes can be simply determined by color changes, UV-VIS spectra and EPR spectra. In water solutions Cu^{2+} ions with hexahydrate show a weak absorption band at 810 nm stemming from d-d transition in the absence of PAMAMs [96], [106]. Reduction of Cu^{2+}dendrimer metal complex with a reducing agent i,e $NaBH_4$ results in the formation of intramolecular Cu clusters of dendrimer (Cu-DEN). After addition of excess $NaBH_4$ into the dendrimer, Cu^{2+} solution immediately changes the solution colour from blue to golden brown.

Dendrimer encapsulated metal nanoparticles (DEMNs) have gained great interest for use in catalysis [26], [107]–[109]. Rahim *et al.* [110] has been reported PAMAM-encapsulated Pd nanoparticles to use as catalysts in the Heck reaction. They demonstrated that the nanoparticle is a highly competent catalyst providing high percentage of yields at phosphine-free environment and with 200−400 times less Pd than usual. Yanhui Niu and Richard M. Crook [111] has been reported dendrimers templated transition metal nanoparticle and their uses to catalysis such as hydrogen addition, Heck reaction, Suzuki reactions in organic solvents and supercritical CO_2.

DENMS are used as good catalyst with $NaBH_4$ to reduce 4-nitrophenol to the 4-aminophenol [112], [113]. Nitrophenols which mostly used industrial and agricultural activities are common sources of organic pollutants. This compound also involves in manufacturing explosives, dyestuffs, insecticides, and other products [114], [115]. Interestingly, 4-aminophenol is key intermediate for the production of analgesic and antipyretic drugs in industry, one of which is paracetamol [116]. Thus, reduction of 4-nitrophenol to the 4-aminophenol by the direct catalyst is urgent and essential request by many industries as it could [38]. Only $NaBH_4$ can not reduce 4-nitrophenol to the 4-aminophenol even at long reaction time.

2.5 Ring Opening Metathesis Polymerization

The word "metathesis" generally means redistribution, but in organic chemistry, it is articulated as molecular regenerations by the redistribution of chemical bonds. So, olefin metathesis is carbon-carbon double bond (C=C) exchange reaction done by transition metal catalyst [117]. In olefin metathesis, alkenes fragment interchange in such a way that scissor double bond and reattach in reverse way. Eleuterio first reported MoO_3-Al_2O_3 catalyzed ring-opening polymerization of cycloolefins by alkene metathesis. Banks and Bailey reported a new type of catalytic reaction at high temperature called olefin disproportionation in 1964. Natta, *et al.* reported molybdenum and tungsten metal mediated catalyst for the ring-opening polymerization of cycloolefins. Chauvin, Grubbs and Schrock won the Nobel Prize in chemistry in 2005, for the discovery of metathesis method in organic chemistry.

ROMP is a kind of olefin metathesis polymerization technique where cyclic olefins are converted to olefins containing linear polymers where olefins are in the main chain [118]. The mechanism of the ROMP is generally based on typical olefin addition polymerizations. An ordinary mechanism of ROMP according to Chauvin's original proposal [119], is shown in Figure 2.13. Mechanism of ROMP follows initiation propagation and termination like an ordinary polymerization technique. Initiation of polymerization reaction starts by coordination of a transition metal

alkylidene complex to a cycloalkenes. As a result [2+2]-cycloaddition make a four membered metallacyclobutane intermediate that efficiently forms the starting of chain growth of polymer. This intermediate experience a cycloreversion reaction to form a new metal alkylidene. Though the size of newly formed complex has increased because of incorporated monomer, its reactivity regarding cyclic olefins is alike to the initiator. Therefore, these steps are progressing repeatedly throughout the propagation stage up to polymerization eases by consuming all monomers through reaching reaction equilibrium and finally terminated the reaction. Termination of ROMP polymerization is commonly done by adding ethyl vinyl ether to remove metal from growing polymer chain by reacting with metal-carbene sites of propagating polymer chain [120]. Except this step, leads to polymer degradation and metal contamination in desired product.

Like most other catalysts system, olefin metathesis catalysts are designed on stability, selectivity, and activity of the catalyst. Olefin metathesis reaction begins by transition metal chloride catalysis. But it is difficult to control molecular weight of the polymer by transition metal chloride. To control molecular weight transition metal complexes modified by organic ligands that work homogeneously. Titanacyclobutane complexes are the earliest example of single-component catalyst able of catalyzing living ROMP by control manner with small polydispersity indices (PDIs) [122]. Tantalum catalyst also reported for polymerization of norbornene by ROMP [123] but titanium metal based compounds are highly reactive with many other functional groups except olefins i,e do not react selective way [124]. Tungsten chloride-based catalysts polymerize strained cyclic olefins in a non-living style. Nevertheless, well-defined W–carbene catalyze living ROMP of norbornene [124], and tungsten based catalysts have ester groups tolerance. Molybdenum catalysts have high activity but wider range of functional groups stability such as ester, amide, imide, ketal, ether, cyano, trifluoromethyl and halogen groups [125].

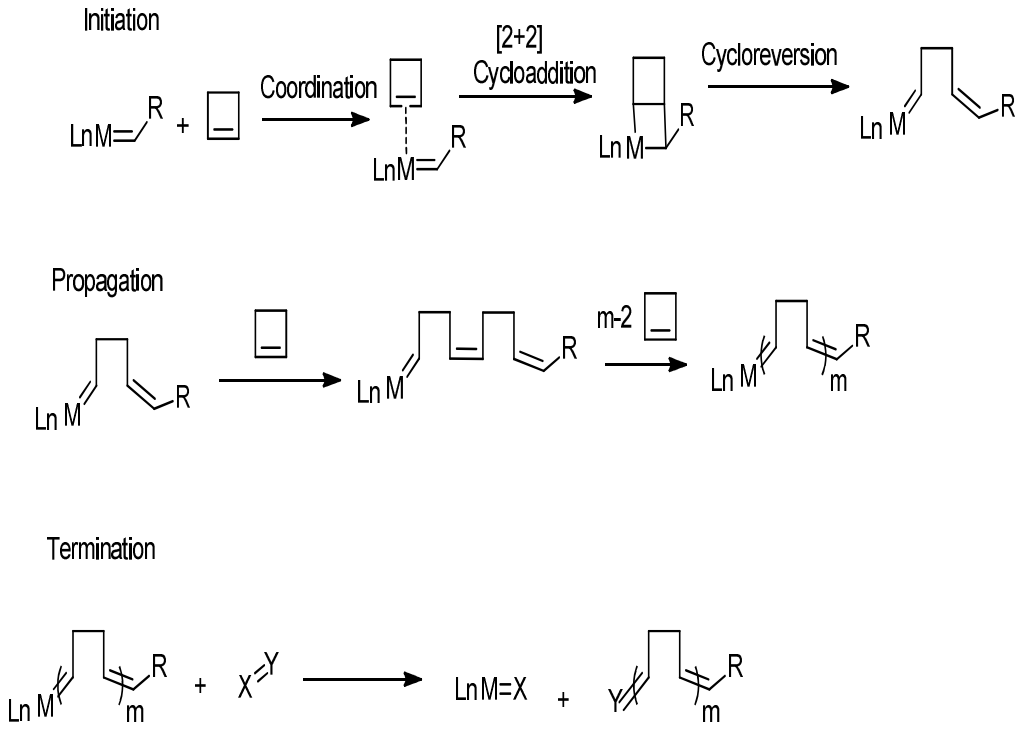


Figure 2.13 Reaction mechanism scheme of ROMP [121]

During mid 1990 decades, ruthenium mediated catalyst implemented newly by the synthesis of ruthenium benzylidene compounds [126]. First commercially available Ruthenium based catalyst is Grubbs first generation catalyst where two tricyclohexylphosphines and two chlorides coordinate to the ruthenium benzylidene moiety. To increase the activity and selectivity in metathesis reaction, one phosphine ligand in the first generation Grubbs catalysts are replaced by a bulky *N*-heterocyclic carbene (NHC) ligand [127]. Systems incorporating an imidazol(in)-2-ylidene [128] ligand are known as Grubbs second generation catalysts [129]. When remaining phosphine of Grubbs 2nd generation catalyst is replaced by pyridine, it increases initiation rates of third generation Grubbs catalyst [130]. Grubbs presented the history of Grubbs catalyst in his Noble lecture [131] and a complete review on Ruthenium-based olefin metathesis catalysts [132] are landmark for Ruthenium-based olefin metathesis catalysts. Structure of 1st, 2nd and 3rd generatıon Grubbs catalyst is as Figure 2.14.

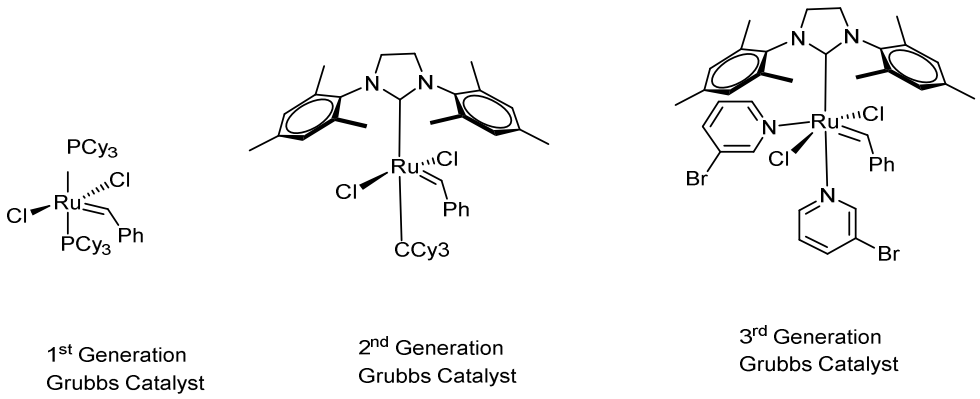


Figure 2.14 Structural formula of Grubbs catalysts [133]

Typically, ROMP is a thermodynamically favors process and driving force behind ROMP of cyclic olefins is reliant on the release of ring strain energy. Molecules having higher strain can easily polymerize with high yield and lower monomer concentration [134]. To polymerize lower strain olefins it is necessary decrease reaction temperature and increase monomer concentration. Ring strain olefins are as bellow-

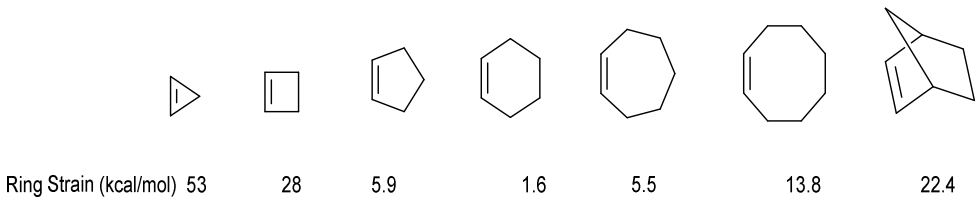


Figure 2.15 Ring strain of cyclic olefins

Cyclohexene has lowest strain energy and higher stability than any other cycloalkene. So, it is very unwilling to polymerize with considerable yield as the relief of ring strain is a major driving force for the reaction. Norbornenes that can easily synthesize through Diels-Alder reaction are favorite monomers for ROMP due to lower stability with high ring strain.

German chemistry professor Otto Diels and his student Kurt Alder at University of Kiel discovered a cycloaddition reaction in between electron rich dienes and electron deficient dienophile later came to known as Diels-Alder reaction. In 1950 Otto Paul Hermann Diels and Kurt Alder achieved noble prize in chemistry for this extraordinary and most useful synthetic reaction in organic chemistry [135]. In

cycloaddition addition reaction, two π bonds break down and form two new σ bonds to form a concentrated ring. An ordinary Diels-Alder reaction scheme has shown in Figure 2.16.

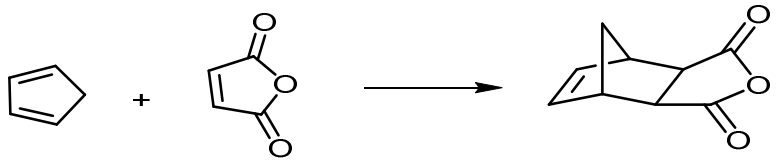


Figure 2.16 Diels-Alder reaction scheme

Furan is a 4-π electron component used as dienes that acts a nucleophile and maleic anhydride is a 2-π electron system behaves as dienophile. Compounds having at least one electron with drawing group and relatively weak double or triple bond acts as a good dienophile. Maleic anhydride has two electron withdrawing C=O groups attach to double bonds can be used as an excellent dienophile. Diels-Alder reaction of furan with maleic anhydride is very well known and form crystalline product [136]. But difficulty is to control the stereo-isomer of the products -among exo and endo which would be formed. Endo product is kinetically favored and formed initially as twice faster than endo product. Though endo compound produce in the beginning of the reaction quickly disappears from mixture of products at room temperature and final product is in exo-configuration [137].

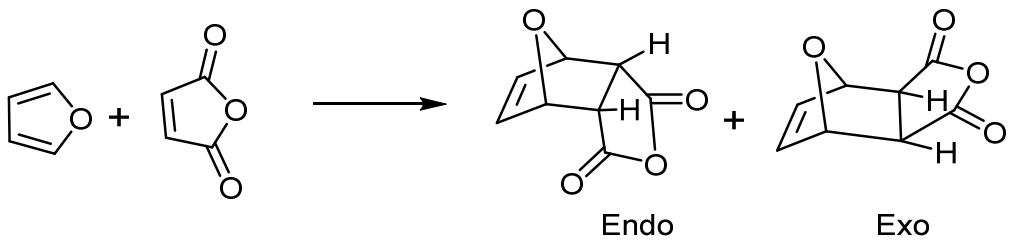


Figure 2.17 Reaction scheme of exo and endo products of a Diels-Alder reaction

Diels-Alder cycloaddition is a metal-free green approach in organic synthesis and an attractive strategy for cycloaddition with quantitative yield and no by-products. This cycloaddition in water solvent is used for the conjugation of biomolecules [138].

2.6 Liquid Phase Polymer Based Retention Technique

Water soluble polymers might contain unreacted monomers or incomplete low molecular weight polymers that can be separated by using membrane. Water soluble dendrimers are also containing incomplete branching or generation which molecular weight is smaller than the expected generation. Membrane filtration procedure is effectively used for isolation of small molecules and for their enrichment from dilute solutions by the help of a water soluble polymer. This process is called the liquid phase polymer based retention technique (LPR). So, LPR method is a hybrid membrane separation process by the combination of ultrafiltration membrane and retention properties of different water soluble polymers to isolate low molecular weight monomer or compound remained in water [139].

Ultrafiltration is rapid promising as a novel, flexible and adaptable procedure in separation technology. Through ultrafiltration, water-soluble, high molecular weight polymers can be purified from lower molecular weight electrolytes or molecule such as metal ions, incomplete generations or unreacted monomers. Before using in the LPR method, the polymers are fractionated by the same method using different membranes of known molecular weight exclusion limit. For LPR separation techniques, the biggest molecular weight fractions are normally used in combination with a low molecular weight exclusion limit membrane to make sure no polymers remain in the ultrafiltration cell. Metal ions that have high tendency to interact with polymer are remaining with polymer, these metal ions are unable to pass through the ultrafiltration system but other ions are able to pass through the membrane (Figure 2.18).

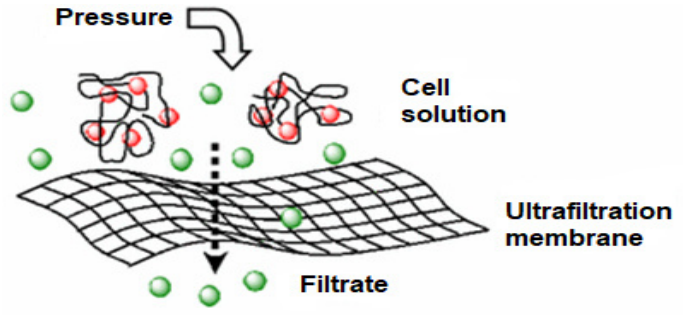


Figure 2.18 Ultrafiltration principle

The fundamental features of LPR technique are glass made filtration cell containing stirring system through magnet and a specific molecular weight cut-off (MWCO) membrane filter, a reserve tank and an external source of pressure such as a nitrogen cylinder[140], (Figure 2.19). In perfect cases, solution in ultrafiltration cell keep constant by supplying constant flow of solvent from reservoir tank by pressure of nitrogen. So that rate of exclusion of the eluent is equal to solvent flow of supply by pressure. Eluent is collected in a beaker which contains smaller molecules, metal ion, unreacted monomers or incomplete generation of dendrimer. High molecular weight polymers remain inside the cell that taken out by outlet. Essential factors are the molecular weight exclusion rate with broad pH range from 1 to 12, proper per minutes flow rate is 0.5–12 mL, retention volume is 2–50 mL and nitrogen pressure is 300 kPa. Usual MMCO range from 500 to 30,0000 Daltons (Da) based on molecular weight of the expected macromolecules [141]. A general exclusion rate of 10 kg/mol proved to be reliable for macromolecules having a molar mass from 30,000 to 50,000 Da.

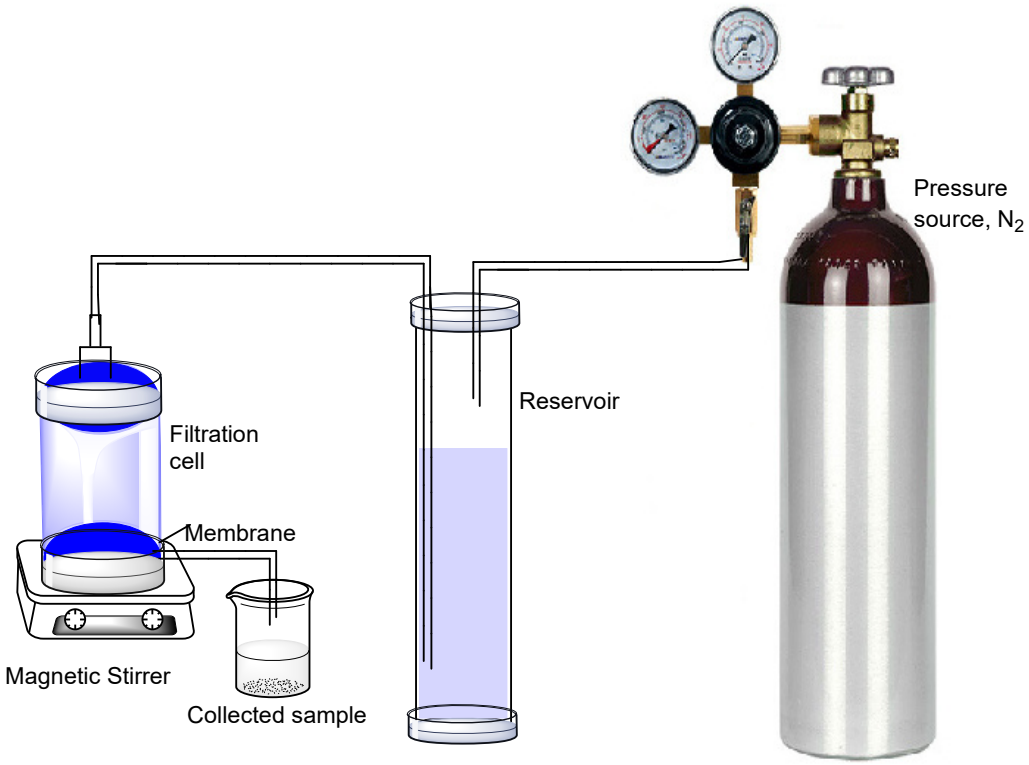


Figure 2.19 LPR methods set-up

LPR technique is usually have done by two different methods - i) washing method and ii) enrichment method. Efficiency of methods depends on the interaction between water soluble high molecular weight polymer and the separable impurities or small molecules in polymer solutions and the progressive retention by an ultrafiltration membrane [142]. The nature of interaction varies on the chemical structure of the water soluble polymer functional groups that occur fundamentally for electrostatic force and coordinate bond formation. The variables that affect the water soluble impurities or small molecules interaction can be divided into two groups: intrinsic to the polymer and extrinsic to the polymer. Intrinsic to the polymer types is the nature of the atoms present in the polymer main chain, types of the functional groups attached to the polymer backbone, the structure and composition of the polymer, molar mass and PDI index, length of linker in between the functional groups and the main chain, the degree of side chains, etc. Extrinsic to the polymer types are the charge and types of metal ion, the pH of the solution, the ionic strength, the temperature, and the dielectric constant of the medium.

The most common ultrafiltration membranes [141] are based on cellulose acetate, polysulfone, poly(ether sulfone), polyacrylonitrile and polyvinyldiene. Though cellulose acetate-based membranes are less prone to fouling and typically have a higher flux than polysulfone membranes at equivalent rejection, polysulfone membranes are used in various applications having higher stability. Furthermore, hydrophobic polymers and membranes can be modified to increase flux and reduce fouling.

2.7 Polymer Characterization

Characterization of polymers [143] is essential after the discovery of new polymers to know its properties, performance and prospective uses. It provides information about chain structure and molecular mass, polymer morphology, thermal & mechanical properties and additive contents. Polymer characterization is difficult and multi-dimensional procedure that should be done by experienced personnel with strong qualificational backgrounds and well equipped polymer laboratory.

There are various approaches for polymer characterization. The most common strategies for polymer characterization are as follows-

Molecular structure determination methods are-

i) Fourier Transform Infrared Spectroscopy (FTR)
ii) Nuclear Magnetic Resonance Spectroscopy (NMR)

Molecular weight of polymers are often analyzed by the following techniques-

i) Gel Permeation Chromatography (GPC)
ii) Dilute Solution Viscosity Testing (DSVT)
iii) Melt Flow Index Testing (MFI)
iv) End Group Analysis of Proton NMR

Determining the morphology ensures crystalline properties, phase separation and domain. Following analytical methods are commonly used for determination of polymer morphology-

i) Scanning Electron Microscopy (SEM)
ii) Transmission Electron Microscopy (TEM)
iii) Atomic Force Microscopy (AFM)

For determining thermal properties such as melting point, filler content, deformation with temperature and pressure following testing technique can be used.

i) Differential Scanning Calorimetry (DSC)
ii) Rheology Testing
iii) Thermogravimetric Analysis (TGA)
iv) Dynamic Mechanical Testing (DMA)

To determine the size and size distribution of the synthesized polymers zeta sizer is used by dynamic light scattering measurement and charge density of polymer is determined by zeta potential.

2.8 Molar Mass of Polymer

For a given known compound molecular weight (molar mass) is the sum of the atomic masses of the constituent atoms in the molecular formula. The empirical definition is the weight of a sample of the compound divided by the number of moles in that sample as determined in an experiment. The usual result of polymerization is a mixture of molecules identical in chemical structure of the chains but differing widely in the number of monomers incorporated into the chains. Determination molecular weight of the macromolecules is important for the implementation of large industrial purposes. Interesting and useful mechanical properties of the polymer material, such as materials is due to their large molecular weight. There are various kinds of molecular weight determination process such as number average molecular weight (Mn), viscosity average molecular weight (Mv), Mw: weight average molecular weight (Mw) and Z-average molecular weight (Mz).

2.8.1 Number Average Molecular Weight

The number average molecular weight is represented by M_n and defined as the sample molecular weight is divided by the total number of moles of all the various chain lengths.

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \tag{2.4}$$

The number-average molecular weight M_n, is obtained using SEC.

2.8.2 Weight Average Molecular Weight

The weight-average molecular weight is represented by Mw and defined as the sum of the molar masses of all fractions (i.e. macromolecules of a certain chain length) times the relative contribution of the individual fractions (weight) into the overall distribution of molar mass of the polymer.

$$M_w = \frac{\sum N_i (M_i)^2}{\sum N_i M_i} \tag{2.5}$$

The weight-average molecular weight, M_W, is obtained using static light scattering, SLS.

2.9 Polydispersity Index

Polydispersity index (P.D.I.) as a measure of the width of a mass distribution which is calculated by the weight-average molecular weight, *Mw*, divided by the number-average molecular weight, M_n

$$P.D.I. = Mw / M_N \tag{2.6}$$

Molar Mass of polymer is determined by gel permeation chromatography (GSC) or size exclusion chromatography (SEC) [144] and end group analysis of ^{1}HNMR spectroscopy.

2.10 Gel Permeation Chromatography

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) [145] is a method by which the separation of chemical components has done on the basis difference in molecular weight or size. It is one of the efficient techniques used to isolate and analyze the bio-macromolecular substances for the determination of molar mass of the polymer.

Size exclusion chromatography (SEC) separates dissolved molecules based on their size by pumping these molecules through specialized columns packed with a microporous material (gel). Porous polymer matrix is used as stationary phase whose pores are absolutely filled with a mobile phase such as a solvent or mixture of solvents. The pore size of polymer matrix is extremely important as the principle of separation mostly dependant on it. Polymer molecules higher than a certain size are totally excluded from the column pores, and smaller molecules stuck into the column partly or completely that make slower flow of these molecules. The flow of solvent as mobile phase will cause larger molecules to pass through the column without any hindrance and not penetrating the gel matrix,

whereas smaller molecules will be retarded due to their penetration of the porous matrix (Figure 2.20).

GPC components-

i) Stationary Phase
ii) The Mobile Phase
iii) The Columns
iv) The Pump
v) Detectors

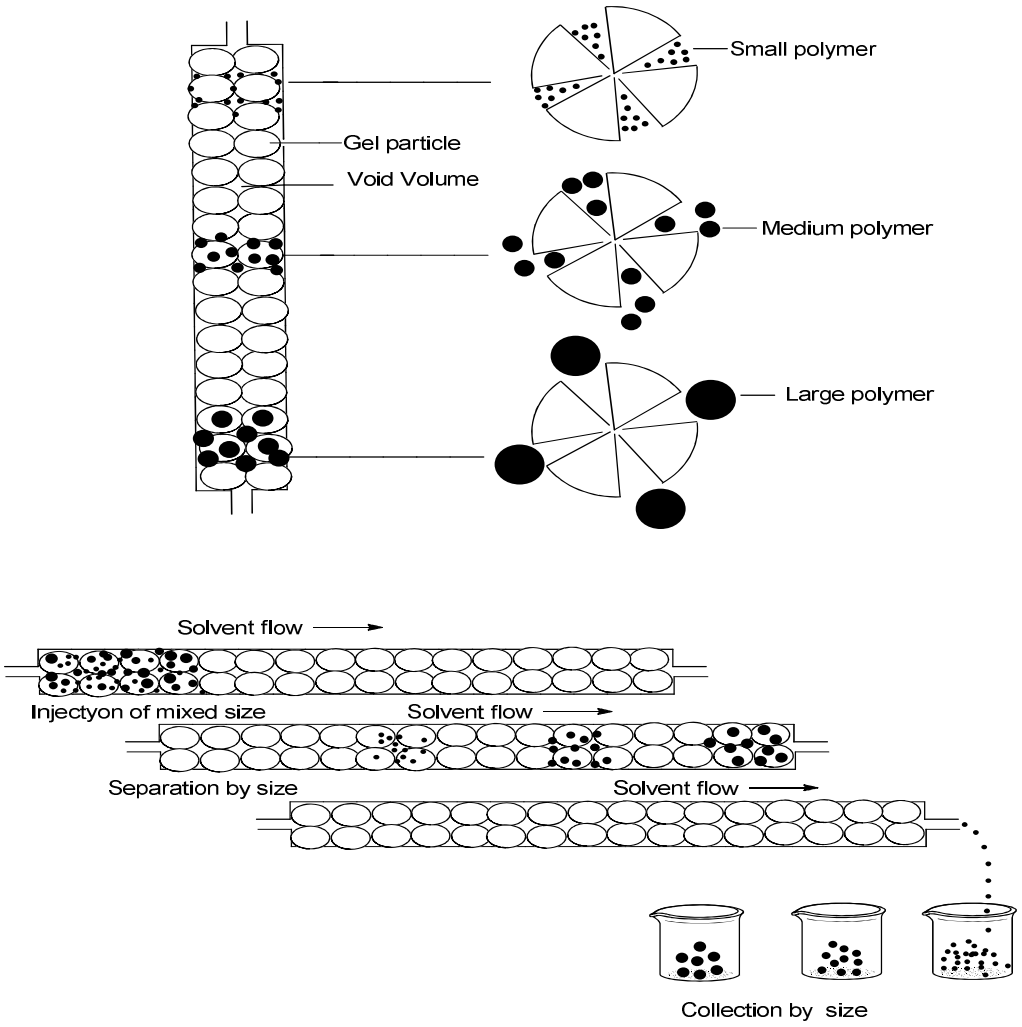


Figure 2.20 GPC: molecules separation technique based on their size

3
BACTERIA AND ANTIBACTERIAL POLYMERS

3.1 Bacteria: Cause of Infectious Diseases

Infectious diseases are caused by bacteria, viruses, parasites or fungi like pathogenic microbes which are transmitted directly or indirectly one person to another [146]. These kinds of diseases are the course of human civilization and have causes immense misery and death [147]. More than 25% of global annual deaths (about 15 million out of 57 million) are estimated to be related directly to infectious diseases and this figure will be higher after consideration of the mortality associated with as a consequence of past infection and morbidity [148]. Bacterial infections [146], [149], [150] are the most challenging global issue due to zoonotic transmittance [151], [152], hospitals secondary transmittance [153], [154] appearance of new pathogens [155], [156] and antibiotic drug resistance [157]–[159]. Global burden of infectious disease caused by bacterial infection is a global problem, especially resistance acquired by bacteria against antibiotics; there is an urgent need for novel antibiotics to combat against microbe. Moreover, to overcome bacterial infection occurred from medical devices, antibacterial polymers, biomaterials and bio-films have introduced or given directly on the outer surfaces of surgery equipment.

Bacteria are single-celled organisms with prokaryotic cell structure, which are invisible through naked eye and can be detected by microscopy. Bacteria have both beneficial and harmful functions. These creatures are capable of killing a person or an animal and also have a number of useful functions that are currently being considered.

3.1.1 History of Bacteria

Bacteria were first observed by Antony Van Leeuwenhoek in water droplet by a simple light microscope. The size of the bacteria observed by light microscopy and these bacteria was 1-2 μm wide and 2-10 μm length [160]. Antony Van Leeuwenhoek named them “animalcules” and published their observations in a series of letters to the Royal Society [161]. The name Bacterium was later introduced in 1838 by Christian Gottfried Ehrenberg. Bacterium is a Latin word which means singular. Although bacteria were known to be the cause of many diseases in the 19th century, there was no antibacterial treatment. In 1910, Paul Ehrlich discovered a compound that selectively kill this pathogen. So Paul Ehrlich developed antibiotic for the first time and won Nobel Prize in 1908 for his work on immunity [162].

Scientists who have contributed to the development of microbiology [163], [164] are as follows-

Hippocrates (Greece, BC 460 - 377 BC): The drug is considered one of the most important figures in history. Diseases are not the curse of the gods but causes from living, eating style and environmental conditions.

Marcus Terentius Varro (Rome, BC 116- BC 27): Some microorganisms are too small and not visible by the naked eyes and have suggested the idea of disease.

Jacobo Forli and Alexandro Benedetti (Italy, 14-15th century): They suggested that some particles exist in air can cause disease if they enter human body through inhaling.

Antonie van Leeuwenhoek (The Netherlands, 1632-1723): He is known as the father of microbiology. Bacteria were first observed by Antonie van Leeuwenhoek in 1676, using his own designed single-lens microscope. He called them "animalcules".

Christian Gottfried Ehrenberg (Germany, 1795-1876): He was one of the most important scientists who introduced us Bacterium Christian in 1838.

Louis Pasteur (France, 1822-1895): In 1885 he developed the first vaccine for rabies. He said that the fermentation event took place in 1859 by the growth of

microorganisms. He and Robert Koch proposed the idea that diseases caused germs.

Robert Koch (Germany, 1843-1910): He was awarded the Nobel Prize in 1905 after providing his theory of germs.

Carl Woese (USA, 1928-2012): He is known for his of discovery the group of single cell prokaryotic organism and structures different from normal bacteria.

Joseph Lister (United Kingdom, 1827-1912): The technique of antiseptic (protection from undesirable microorganisms) has led to surgery. This scientist has been able to alleviate the problems that doctors had suffered from postoperative inflammation by using dressings soaked in antiseptic matter.

Hans Christian Joachim Gram (Denmark, 1853-1938): He stained the bacteria with the dyeing method referred to by his name and divided them into two types as Gram positive and Gram negative. This method is still used to identify bacteria.

Paul Ehrlich (Germany, 1854-1915) He developed Salvarsan, the first antibiotic to treat the fuzzy and awarded the Nobel Prize for research in immunology in 1908.

Dmitri Iosifovich Ivanovsky (Russia, 1864-1920): He found small living things that passed through filters that bacteria could not pass and caused tobacco mosaic disease. Thus, the virus is separated from bacteria.

3.1.2 Structure of Bacteria

The outer layer or cell envelope of bacteria is composed of cell wall and cytoplasmic membrane. Cell wall is made of rigid components and cytoplasmic or plasma membrane exist under the cell wall. Cell envelope is composed of protoplasm, cytoplasm, cytoplasmic inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body [165].

Cell wall: Under the outer structures is the cell wall which is composed of a protein sugar molecule named peptidoglycan. This part is rigid which provides the shape of the cell by surrounding cytoplasmic membrane. Its main task of cell wall

is to protecting the cell from expanding and ultimate bursting when there are large differences in osmotic pressure due to water uptake in between cytoplasm and the environment. Cell wall composed a remarkable part of cells' dry weight which is necessary for growth and division of bacteria. Cell wall can not stain with simple stain and even not possible to monitor by light microscopy directly.

Outer membrane: Outer membrane is composed of single layer of peptidoglycan which is found only in Gram negative bacteria [166]. It acts as a primary barrier to the environment and consists of lipopolysaccharide (LPS) and phospholipids. The LPS present on the cell walls of Gram negative bacteria account for their endotoxic activity and antigen specificity. Outer membrane of Gram negative bacteria is tough and permeable that help bacteria adhere to animal cell and causes diseases.

Cytoplasmic membrane: Cytoplasmic membrane is present in both Gram positive and Gram negative bacteria immediately under the cell wall. It is highly organized and asymmetric two sides thin layer lining the internal part of cell wall which separates it from cytoplasm. It works like semipermeable membrane to control the metabolite flow and protoplasm formation.

Cytoplasm: The cytoplasm is a gel-like matrix possessing various solutes along with water and salts. Some other components are enzymes, nutrients and gases. The function of cell growth, metabolism and replication are carried out in cytoplasm. They are rich in ribosomes, DNA and fluid. DNA is coiled and polyamines haploid is located in nucleoid.

Ribosomes: They are the microscopic factories found in bacteria cell like all other cells which are centers of protein synthesis and translate the genetic code. They are a little smaller than the ribosomes of eukaryotic cells. There are significant differences between ribosomes of bacteria and eukaryotic ribosomes that some antibody will inhabit bacterial cells function but not eukaryotic ribosomes.

Mesosomes: In cytoplasm there are various tubules made by invagination of plasma membrane known as mesosomes. They are major respiratory enzyme that works for division of cells.

Cytoplasmic inclusions: The cytoplasmic inclusion storage granules phosphates, organic polymers and nutrients substances. Polymetaphosphates store energy and phosphate for cell metabolism and which are also known as metachromatic granules.

Nucleus: There is no nuclear membrane or nucleolus in the nucleus. It carries genetic material consist of DNA. The cytoplasmic carriers of genetic information are termed plasmids or episomes.

Capsule: Capsule is extracellular layer of bacteria that exists outermost part of bacteria. It is a condensed well defined layer made up of polysaccharides that closely surrounding the cell. They are most impermeable to save from external environment. The capsule protect against drying out to protect it from engulfing.

Flagella: Flagella are 3 to 12 μm long and 12 to 30 nm in diameter spiral filaments like as hair growing from cell cytoplasmic membrane. The location and number of flagella rely on specific bacteria and used to classify bacterial species. Some bacteria have only one flagella at single pole and some other bacteria have multiple flagella at both poles or all over of the cell surface. Flagella constructed by filament, hook and the basal body.

Pili/Fimbriae: Alike to hair and thin appendages are grown from bacteria cell membrane to outer environment are known as pili which are also called fimbriae. They are more rigid, thin, short and abundant than flagella and they do not have motility function. The fimbriae is consists of a subunit known as pilin. There are two types pili named as common pili which are also known as non-sex pili and the sex pili. Common pili are abundant in number and mediate their adhesion whereas the sex pili help to attach male to female bacteria at the time of conjugation.

Spore: Some bacteria have capacity to form resistance during resting known as spores that work for sustaining difficult environment and tough situations. Bacterial spore have no metabolic activity and do not play any role in reproduction. It is highly resistance to heat, radiation, disinfectant and dehydration. Spores have three different layers such as core, cortex and spore coat.

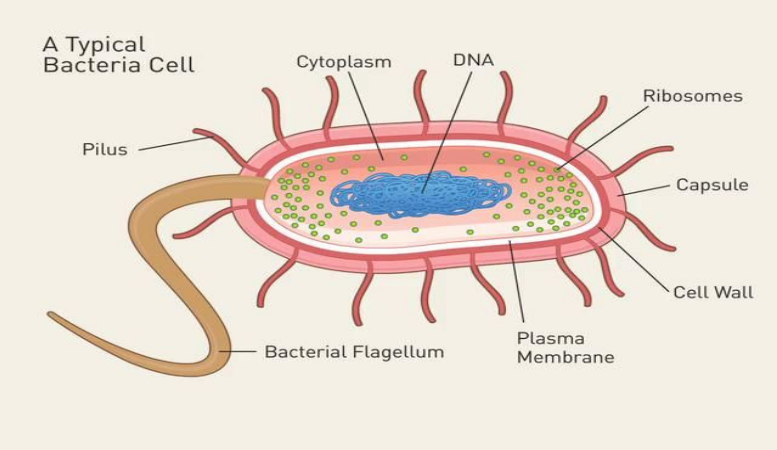


Figure 3.1 A typical bacterial structure [167]

3.1.3 Classification Systems of Bacteria

The classification of bacteria has done using varieties of different functions and many different typing schemes where an organism classified by a group but same microbe may define by another class depending on mode of classification. Presently the classification method used by clinical microbiologists based on morphology of bacteria, staining capacity of microbes, essentiality of O_2 for growth along with some other biochemical tests.

Gram stain and bacterial morphology: Among all other classification techniques, the Gram stain test is most useful method presented by H.C. Gram in 1884. Based on this classification system, bacteria can be classified as either Gram positive or Gram negative depending on their structural morphology and differential staining properties. When slides are successively stained by crystal violet iodine, then destained by alcohol and counter-stained with safranin, Gram positive bacteria stain blue-purple and Gram negative bacteria stain red. The difference between the two groups of bacteria is believed to be due structural characteristics of their cell walls of bacteria. Due to having much larger peptidoglycan (cell wall) in Gram positives bacteria, iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with the Gram negatives where the crystal violet is readily eluted from the bacteria. So, depending on bacterial morphology and staining properties, it can be differentiate either Gram positive or Gram

bacteria. For example, *Streptococcus pneumoniae* is a Gram positive bacterium, but *Escherichia coli* and *Vibrio cholerae*, are Gram negative bacteria.

Growth Requirements: Bacteria can be grouped as aerobic and anaerobic depending on their oxygen necessity to grow. An aerobic organism or aerobe is microorganism that can survive and grow in environment where oxygen is available whereas, an anaerobic microorganism (anaerobe) is any organism for which oxygen does not require for growth. In short, aerobic bacteria can grow in presence of oxygen but anaerobic bacteria grow only in an environment where there is no oxygen present. *Helicobacter pylori* and *E. coli* is the example of aerobic and anaerobic bacteria respectively.

Shape of Bacteria: On the basis of shape, bacteria can be classified into several varieties

i. Cocci (from kokkos meaning berry) are round or oval cells, sometimes a little bit flattened during adjacent to another.

ii. Bacilli (from baculus meaning rod) are rod like shape cells

iii. Vibrios possessing comma shaped curved rods and choose their name from their characteristics vibratory motility.

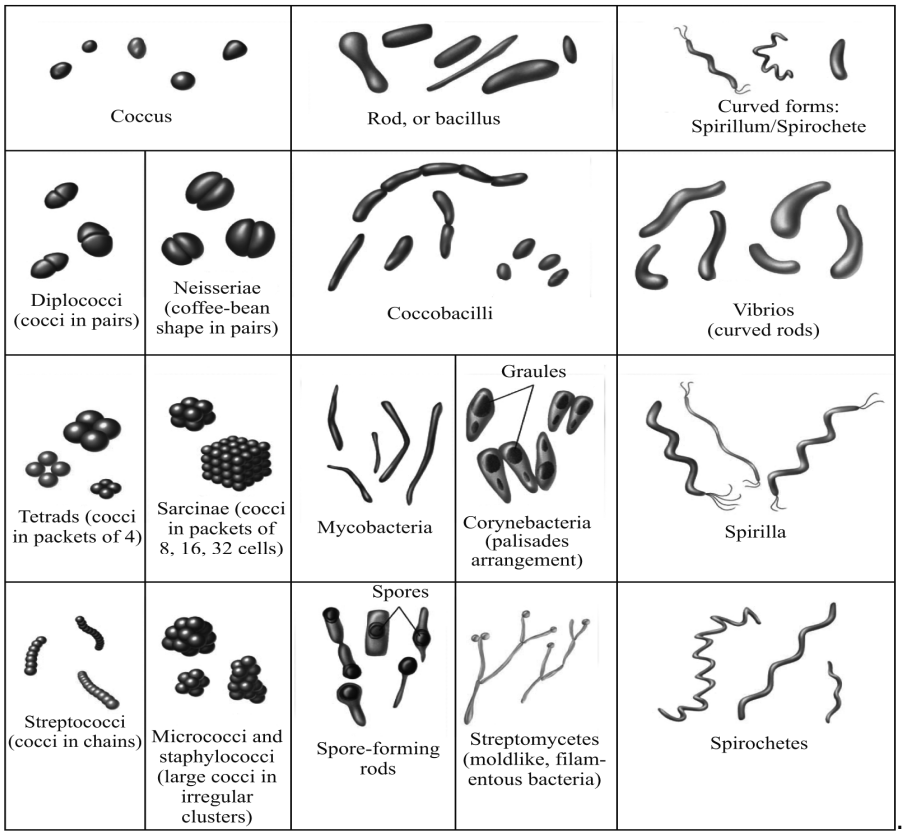
iv. Spirilla are rigid spiral shaped and able to move.

v. Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms

vi. Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from the act is meaning ray and makes meaning fungus)

vii. Mycoplasmas do not possess any stable morphology. These are cell wall deficient bacteria.

Table 3.1 Schematic view of different shapes of bacteria [168]



Nutritionally based classification: Nomenclature of bacteria can be done based on nutritionally, depending on essentiality of energy for growth and required metabolites synthesizing capability. Bacteria capable to gain energy from sunlight are named phototrophs, bacteria able to absorb energy through chemical reactions usually known chemotrophs. Bacteria capable to synthesise essential organic compounds named autotrophs and those are unable to synthesize their metabolites classified as heterotrophs.

3.2 Antibacterial Polymer

In recent years, the large numbers of polymers and copolymers have been synthesized and significantly active as antipathogenic agents through either quaternized on surface or functionalized with bioactive groups. Similarly,

quaternize primary, secondary or tertiary amine groups containing hydrophobic polymeric compound have been discovered which showed comparatively stronger activities against microbes.

There are two basic types of polymers depending on their mode of actions - i) active ii) passive. Passive polymer forms a surface film to restrict adhesion of microbes by decreasing adsorption of the protein on its surface, that's why repelling the bacteria without actively interacting with it. Due to having hydrophobic and negatively charged properties of bacteria, passive polymers should be either (1) hydrophilic; (2) positively-charged; or (3) have a low surface free energy. So, generally polymers that work passive way comprise (1) self-healing, slippery liquid-infused porous surface (SLIPS), such as poly(dimethyl siloxane); (2) uncharged polymers, such as poly(ethylene glycol) (PEG), poly(2-methyl-2- oxazoline), polypeptoid, polypoly(n-vinyl-pyrrolidone), and poly(dimethyl acrylamide); and (3) charged polyampholytes and zwitterionic polymers, such as phosphobetaine, sulfobetaine, and phospholipid polymers. Examples of passive antibacterial polymers are poly(ethylene glycol), poly(sulfobetaine methacrylate), polyphenols, poly(dimethyl acrylamide) etc.

Nevertheless, active polymers actively kill bacteria after functionalization of polymers surface with cationic biocides, antibacterial peptides or antibiotics like biocidal agents. Nisinimmobilized organosilicon, polyurethane accommodated quaternary ammonium, poly(N,N-diethylethylene diamine-coyrosol-based acrylic), organosilicon containing quaternary ammonium chloride and poly(2-(dimethylamino)ethyl methacrylate)tethering quaternary ammonium are some examples of active polymers [8–11].

The mode of action of bioactive polymers depends on function of surface functionalized groups incorporated to polymer backbone or branches. For example, positively charged quaternary ammonium is mostly used that binds with bacterial cell wall and destroys the cytoplasmic membrane ensuing leakage of intracellular components and subsequent cell death. Mode of actions of active cationic polymers are as follows- (i) adsorption onto the bacterial cell surface (ii) diffusion through the cell wall (iii) binding to the cytoplasmic membrane (iv) disruption of the

cytoplasmic membrane (v) release of K^+ ions and other cytoplasmic constituents and (vi) precipitation of cell contents and the death of the cell [169]. As bacterial cell membranes are highly negatively charged as compared to human cell membranes, the polymers are expected to selectively bind to bacteria over human cells.

3.2.1 Basic Requirement for Antimicrobial Polymer

To act as an antimicrobial agent, polymer should have some standard characteristics-

(1) Simple synthesized procedure and economically not expensive.

(2) Should be stable for long term use and easy to store.

(3) Should be insoluble in water for a water purification application,

(4) Should not break down and or release harmful by-products,

(5) Should be nontoxic or nonirritating during handling it,

(6) Should be reproducible for reuses and

(7) Biocidal to a wide range of microorganisms and works as biocides in a short time of contact.

3.2.2 Factors Affecting the Antimicrobial Activity

There are numerous factors and cofactors which influence antibacterial activities and mode of action for antimicrobial polymers. Molecular weight of polymer, length of spacer or linker in between biologically active functionality and polymer backbone, hydrophilicity-hydrophobicity balance, and types of counterions are most important for antibacterial polymer [170], [171].

Effect of molecular weight: The molecular weight of polymers acts a significant role in antimicrobial activities. Investigation of molecular weight dependence on antibacterial activities has drawn attention by many research groups. Tulu *et al*

synthesized Jeffamine cored PAMAM dendrimers where highest generation anionic dendrimers showed lowest cytotoxicity on L929 cells and first generation water soluble PAMAM showed higher antibacterial activity. So jeffamine cored PAMAM dendrimers antibacterial properties showed best at lower molecular weight that height molecular weight [44], [172], [173]. Y. Shin and coworkers in found chitosans with high molecular weights showed high antibacterial properties than chitosans with lower molecular weight [174]. Ikeda *et al* reported antibacterial activity of polyacrylates homopolymers containing biguanide units and poly(vinylbenzyl ammonium chloride) [169], [175]. They establish that antibacterial activity of the polymethyl acrylate possessing branched biguanide groups against *S. aureus* was significantly reliant on molar mass of polymers. Molar mass of polymer from 5×10^4 to 1.2×10^5 g/mol found standard for antibacterial activities. Antibacterial activity raised with higher molecular weight up to molecular weight lower than 5×10^4 g/mol, whereas the biocidal activity dropped stridently with the raise of the molar mass of the macromolecules over 1.2×10^5 g/mol.

Eren and coworkers investigated the molecular weight and charge density dependence on antibacterial activities of mono- and double-charge containing, pyridine, and DABCO based ROMP polymers and found charge density increased with the increase of molecular weight and hence antibacterial activities against *S. aureus* increased [52].

Karen Lienkamp and coworker showed that lower molecular weight ROMP based oligomers are efficient against *E. coli*, and activity starting oligomers molecular weight 6900 g/mol, a clear rise of bactericidal activities against *E. coli* 100 µg/mL was seen whereas maximized for propyl_10k of 3.75 µg/mL. For *S. aureus*, the reverse trend was reported where best activities were noted for the small oligomers of molecular weight 1080 g/mol and activity is progressively lost as the molar mass increases up to 10 kDa [55].

The antibacterial activities were observed to increase monotonically with molecular weight up to 7.7×10^4 g/mol, the maximum molecular weight experiment during the study. Nevertheless, antibacterial activities of the quaternary

ammonium salts possessing fractionated polymeric material against *S. aureus*, *B. subtilis*, *E. coli*, *A. aerogenes*, and *P. aeruginosa* were reported slight dependency on molecular weight. Kanazawa and co-workers [176] reported the molar mass dependence of poly(tributyl 4-vinylbenzyl phosphonium chloride) against *S. aureus* in saline solution and noted that bactericidal activity increased with the increase of molecular weight from 1.6×10^4 to 9.4×10^4 Da.

Kanazawa and co-workers [177] reported phosphonium salts containing polycationic biocides and make a comparison with their bactericidal activity with the corresponding monomers. They have established that the activity of polymers was increased with the increasing molecular weight. Chen and co-workers [10] prepared quaternary ammonium functionalized poly(propyleneimine) dendrimers and shown that biocidal activity of these dendrimers have parabolic relationship on molecular weight. Tokura and co-workers published an article representing similar observations.

Nevertheless, Panarin and co-workers [178] have shown that the bactericidal activities have no reliance on molecular weight for copolymers of vinylamine, methyl acrylate, and *N*-vinyl pyrrolidone with branched cationic ammonium functionality. To understand the molecular weight discrepancy appropriately, bacteria structure needs to take under consideration. Most of the investigators found that *S. aureus* disruption depends on molecular weight. Studies represented that macromolecules having molar mass from 5×10^4 to 9×10^4 g/mol do not show any difficulty diffusing across the cell wall of the *S. aureus* like Gram positive bacterium. However, for *E. coli like* Gram negative bacterium, cell membrane diffusion is highly complex because of having of an external membrane.

Effect of counter ion: Kanazawa and co-workers [177] reported poly[tributyl(4-vinylbenzyl)phosphonium] salts counteranion dependence against *S. aureus*. It was found that the nature of the counter anion affects antibacterial activity. The biocidal properties were little for a counteranion, which tends to form a tight ion-pair with phosphonium ion whereas it was high for those facilitating ionic dissociation to free ions. The antimicrobial properties were in the order of chloride

> tetrafluoride > perchlorate > hexafluorophosphate, which could be correlated with the solubility products of the polymers.

The biocidal efficiency of the quaternary ammonium dendrimers developed by Chen *et al.* showed reliance on the counterion. They observed that the bromide anions containing biocides are more potent than those possesses chloride anions. On the other hand, the research carried out by Panarin and co-workers on the preparation of vinylamine and methyl methacrylate homopolymers having quaternary ammonium pendant groups do not show any effect for chloride, bromide, and iodide counter anions on the antibacterial activities [178].

Shandil *et al*. studied the effect of counter anion of 4-vinyl pyridine based polymers on antimicrobial behavior and reported polymers containing butyl alkyl chain and OH^-counter ion exhibited strong activity over SO_4^{2-}, HSO^{3-}, NO_3^- or CH_3COO^- against *C. freundii*, and *M. circinelloides* [179].

Karen Lienkamp and coworker synthesized diamine containing homopolymer to investigate the effect of various organic counteranions on bactericidal activities. Hexanoate, dodecanoate, benzoate, and tosylate salt was introduced to change the hydrophobicity were introduced and reported existence of the organic counteranions does indeed influence the bactericidal properties of polymers [180].

Effect of spacer length and alkyl chain: Antimicrobial activity is dependent on alkyl chain of polymer that is present on side chain. Spacer length in the structures of quaternary ammonium and phosphonium salts affect bioactivity due to the change in both hydrophobicity and charge density of the polymer. Hydrophobicity and charge density, consequently affect antibacterial activity mechanism with the cytoplasmic membrane. For a polymeric quaternary ammonium chloride biocide, the hydrophilic lipophilic balance influences the antimicrobial properties. Though Panarin *et al.* [178] showed that the biocidal properties of synthesized polymers unable to show any diffidence on chain lengths, Ikeda *et al.*discovered bactericidal properties of poly(trialkylvinylbenzylammonium chloride) was maximum for the longest chain (C12) among their study. Sawada and co-workers were synthesized

perfluoro-propylated and perfluoro-oxaalkylated end-capped 2-(3-acrylamidopropyldimethylammonio) ethanoate (APDMAE) [181] and investigated their biocidal properies against *S. aureus* and *P. aeruginosa*. Because of possessing longer chain in prefluoro-oxaalkylated APDMAE polymer, it was found more active against both *S. aureus* and *P. aeruginosa*. Recently, Nonaka and co-workers prepared methacryloylethyl trialkyl phosphonium chlorides, *N*-isopropylacrylamide copolymers. They reported that the bactericidal properties of the copolymers against *E. coli* were higher for longer alkyl chain length in the phosphonium groups in the copolymer [182]. Relationship of alkyl chain length and biocidal activity could be parabolic but still not beyond doubt.

The polymer having maximum selectivity for bacterial over mammalian cells is 3kDa ethyl, with nice efficiency for both *E. coli* and *S. aureus*. Methyl containing polymers are inactive and nontoxic but butyl chain containing polymers are active but toxic. From butyl_3 kDa to hexyl_3kDa the biocidal efficiency reduces again up to the polymers become inactive [55].

In a study, amphiphilic polyoxanorbornene with various quaternary alkyl pyridinium side chains were synthesized. The biocidal activities of these polymers, with different alkyl derivatives, were examined by bacterial growth inhibition experiment and hemolytic activity (HC_{50}) against human red blood cells (RBCs) to provide selectivity of these polymers for bacterial over mammalian cells. A series of polymers containing various alkyl chain substituents (ethyl, butyl, hexyl, octyl, decyl and phenylethyl) and two different molecular weights (3 kDa and 10 kDa) were prepared. The influence of alkyl chain length are categorized into two to observe biological activity: those with an alkyl substituent containing four or fewer carbons had MIC of 200 mg/mL and a HC_{50} greater than 1650 mg/mL whereas those with six or more carbons had lower MICs 12.5 mg/mL and HC_{50} 250 mg/mL [60].

Eren *et al*. [60] investigated the effect of length of alkyl substituent on the surface activity of ROMP based biocidal macromolecules. Among pyridinium, ethyl pyridinium, hexyl pyridinium and octyl pyridinium based ROMP cationic polymer hexyl containing pyridinium unit exhibited the highest antibacterial efficiency

against *Escherichia coli* on solid surface killing 99% of the bacteria in 5 min. On the other hand, phenyl and octyl functionalized quaternary pyridinium groups showed lower antibacterial activities on the solid surfaces compared to their solution phase biocidal properties [61].

3.3 Methods for Evaluating Antimicrobial Activity Test

3.3.1 Disk-Diffusion Method

Disk-diffusion or Kirby-Bauer test is commonly used microbiology techniques developed in 1940, is used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Though all bacteria can not be tested accurately by this method, disk diffusion method is probably the most widely used method for determining antibacterial resistance around the world due to convenience efficiency and cost effectiveness. Approximately 1×10^8-2×10^8 suspension is isolated and prepared particular McFarland standard then spread evenly in a Petri dish through an appropriate agar such as Muller–Hinton agar. The agar typically contains 30% beef infusion, 1.75% casein hydrolysate, 0.15% starch and 1.70% agar and pH adjusted to neutral at 25 °C.

During the experiment, the dishes are impregnated through different known concentrations of various antibacterial compound are placed on to the surface of agar. A multichannel disc dispenser can speed up placement of the dishes. After 16-24 h incubation at 35 °C zones of growth inhibition around each of the antibacterial compound are measured to the nearest millimeter. A circular transparent area of no growth in the immediate vicinity of disc indicates susceptibility to antibiotics. The size of the zone can be related to the MIC by using reference table.

However, as this method determines bacterial growth inhibition that does not mean bacterial death. So, the disk diffusion method cannot distinguish bactericidal and bacteriostatic effects. Moreover, by using the agar disk-diffusion method minimum inhibitory concentration (MIC) can not determine appropriately, as it is impossible to quantify exact amount of the antibiotics diffused into the agar

medium. An approximate MIC can be estimated for some microorganisms and antibacterial agents by comparing the inhibition zones' diameter with stored algorithms. Though disk-diffusion assay does not offer exact MIC but the technique has many advantages over other methods such as simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents.

3.3.2 Broth Dilution Method

Dilution methods are most authentic experimental procedure for the MIC values determination. Among other dilution methods, Broth micro- or macro-dilution is fundamental antimicrobial susceptibility testing method. The procedure involves preparing two-fold dilutions of the antimicrobial agent (e.g. 1, 2, 4, 8, 16, 32 and 64 mg/mL) in a liquid growth medium dispensed in tubes containing a minimum volume of 2 mL (macro dilution) or with smaller volumes using 96-well microtitration plate (microdilution). Then, each tube or well is inoculated with amicrobial inoculum prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarlandscale. After well-mixing, the inoculated tubes or the 96-well microtitration plate are incubated (mostly without agitation) under suitable conditions depending upon the test microorganism.

The minimum inhibitory concentration (MIC) is the lowest concentration of biocidal agent that completely inhibits growth of the organism in tubes or micro-dilution wells. The main limitation of the macrodilution method are the laborious, manual handling, errors and contamination risk in the preparation of antimicrobial solutions for each test, and the comparatively large amount of reagents and space required.

4
EXPERIMENTALS

4.1 Experimental

4.1.1 Materials and Chemicals

Furan, maleic anhydride, maleimide, hydrazine hydrate, ethylenediamine (EDA), cobalt acetate, trifluoroacetic acid, triethylamine, 1,4-diaminobutane, 1,6 diamnohexane, di-*tert*-butyl dicarbonate (BOC), ethanolamine, methyl arylate, diisopropylazodicarboxylate (DIAD), triphenylphosphine (Ph_3P), Grubbs catalyst 3rd generation, inhibitor remover, magnesium sulfate (anhydrous), potassium permanganate, and common solvents such as methanol, tetrahydrofuran (THF), dichloromethane (DCM), n-hexane, DMF, DMSO, N,N-dimethylacetamide, ethyl acetate, N,N-dimethylformamide, n-butanol, t-butanol, toluene, benzine etc were used during experiments. Furan was purchased from Acros Organics and used as received. 1,6-diaminohexane, 1-bromohexane, maleic anhydride and methyl acrylate (MA) were taken from Aldrich and were used directly. Di-*tert*-butyl dicarbonate (BOC) and triethylamine were purchased from Sigma-Aldrich and ethylenediamine (EDA) was purchased from Carlo Erba Reagents. EDA-BOC was synthesized in the laboratory by a standard method as previously described [183]. Exo-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride was synthesized from maleic anhydride and furan in THF solvent by Diels-Alder reaction according to a published article [184]. 3-(aminomethyl)-pyridine was purchased from Merck and used as received. Second generation Grubbs catalyst & 3-bromopyridine were received from Sigma-Aldrich. Third generation Grubbs catalyst was prepared according to the literature [130]. Tetrahydrofuran (THF) and dichloromethane (DCM) is distilled via conventional techniques and stored over molecular sieves.

4.1.2 Instrumentation and Software

Oil bath: Oil bath is prepared by using silicon oil in a fan.

Magnetic stirrer: Magnetic stirrer supplied by Heidolph type MR 3001AC 230/240V, 625W, serial No. 100478499, made in Germany.

Rotary evaporator: Rotary evaporator produced by Heidolph, made in Germany. Heating control by water bath and cooling controller by a cooler produced by Thermoset and vacuum controlled by a motor (ILMVAC SL No 132805).

Centrifuge: Mistral 2000 centrifuge machine is used for centrifuge at 2000 rpm which is a fully microprocessor controlled multipurpose benchtop centrifuge

Dialysis Membrane: Dialysis membrane produced by Sterlitech Corporation. NADIR NP010 (item no 300789, MWCO 1000Da) and NADIR NP030 (item no 300786, MWCO 500Da) membrane have been used for ultrafiltration.

Vacuum Oven: Binder vacuum drying oven, VD series, temperature range from 5 °C to 200 °C.

FT-IR spectrometer: The IR spectra (4000–400 cm^{-1}, resolution were recorded with a Perkin Elmer Spectrum One (Serial No: C68739) in ATR.

NMR instrument: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz spectrometer.

GPC: Standard size-exclusion chromatography (SEC) was performed with a system composed of a Waters 515 pump, a RI Waters 2410 detector and PSS GRAM 1000 column. DMSO (with LiBr, 5 g/L) was used as the mobile phase (flow rate of 0.9 mL/min) at 60 °C. Pullulans from PSS (Polymer Standard Service (PSS), Mainz, Germany) were used as standards. For data acquisition and evaluation of the measurements, PSS WinGPC UniChrom 8.2 was used.

UV – Vis Absorption: UV-vis absorbance spectra were obtained using a PG T 70 Spectrometer (PG Instruments England) and a quartz cuvette having an optical path length of 1.00 cm.

Zeta Sizer: The zeta potential of copolymers was measured with a zeta sizer (Nano ZS (red badge), model number ZEN 3600, Malvern Instruments Limited, UK). The

samples were prepared and diluted with phosphate buffered solution, and folded capillary cell was used in the zeta potential measurement. The samples were placed to the analyzer chamber and readings were taken at 25 °C. Experiment for each sample was repeated thrice, and data are recorded as the mean ± standard deviation (SD).

Scanning Electron Microscope: ZEISS® brand EVO MA 10 scanning electron microscope (SEM) was used for cell membrane visualization. In SEM EVO MA 10, tungsten filament is used as electron source with the help of thermoluminescence radiation. SEM morphological analysis of samples was examined under vacuum below 5×10^{-5} mbar. The coated samples were attached to the sample holding containers with the help of carbon conductive tape locked to the axial steering table within the SEM device. Samples were imaged by using 15 kV acceleration voltage over a working distance of 4.5 mm to 6.0 mm. Scattered over the sample electrons collected by the help of the secondary electron detector surface images were obtained.

4.2 Monomer Synthesis

4.2.1 Core Synthesis

4.2.1.1 Synthesis of Oxanorbornene by Diels-Alder Reaction

33 g (0.33mol) of maleic anhydride was taken in a 1000 mL conical flask and dissolved in a 100 mL THF solvent. 24.50 mL (0.40 mol) of furan was added to maleic anhydride solution in nitrogen environment with continuous stirring for 10 min [184]. Mixture of the conical flask was sealed and covered by aluminum foil and kept in a cabinet in absence of light for four days. After four days big crystal of desired product was found. Product was isolated by suction filtration and washed with cold THF and dry crystal was collected and stored. Percentage of yield is found 92.81.

FT-IR (cm^{-1}): 3001 (=C-H), 1782 (O=C-O-C=O), 1084 (C-O-C). ^{1}H NMR: 6.59 (s, 2H), 5.36 (s, 2H), 3.32 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 171.8, 137.3, 82.1, 49.2.

4.2.1.2 Synthesis of *Exo*-3, 6- tetrahydrophthalide by Furan Maleimide Diels-Alder Reaction

The starting product was synthesized following standard method [185]. 5.36 g (0.0552 mol) of maleimide was dissolved in 15 mL ethyl acetate in a sealed tube under nitrogen condition. 3.80 mL (0.0558 mol) of furan was taken out under nitrogen and added to the sealed tube. Sealed tube was filled with nitrogen with pressure and transferred to oil bath. Let the reaction stirring for 24 h at 90 ℃. After 24 h, newly formed product precipitated on bottom of the sealed tube. Ethyl acetate was taken out by a Pasteur pipette and sedimented product was washed by 100-150 mL cold diethyl ether. Trace amount of diethyl ether is removed by vacuum oven during overnight.

FT-IR (cm^{-1}): 3066 (=C-H), 1700 (O=C-N-C=O (amide), 1090 (C-O-C, ether). ^{1}H NMR: 8.08 (s, 1H), 6.77(s, 2H), 5.31(s, 2H), 2.89 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm):175.85, 136.56, 80.96, 48.69.

4.2.2 Synthesis of Different Generation Dendritic Monomer without Linker

4.2.2.1 Synthesis of NH_2 Terminated Monomer Core by Oxanorbornene and Hydrazine Hydrate

Synthesis of hydrazine hydrate derivative of *exo*-3,6-epoxy-1,2,3,6-tetrahydropathalic-anhydride was synthesized according to a published article [186] by slightly modified way. Benzene solvent (15 mL) was added to 5.0 g (0.03 mol) of exo-3,6-epoxy-1,2,3,6 tetrahydropathalic-anhydride was mixed with to make suspension. 1.90 g (0.03 mol) of hydrazine hydrate was added to benzene suspension dropwise with continuous stirring. After half an hour white precipitate was found and rotation continued for two hours for completion of reaction. Solvent was filtrate with suction filtration and air dried overnight. Crystalline white product was washed by chloroform with centrifuge and filtrate by suction filtration. Percentage of yield of the product is 83.50.

FT-IR (cm^{-1}): 3338-3309 (-NH_2), 3030 (=C-H), 1682 (O=C-N-C=O (amide), 1054 (C-O-C, ether). ^1H NMR (DMSO-d_6, 500 MHz): 6.54 (s, 2H), 5.04 (s, 2H), δ 2.86 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.8, 136.6, 80.3, 45.4.

4.2.2.2 Synthesis 0.5 Generation Dendritic Monomer from NH_2 Terminated Oxanorbornene

Azo-Michael addition reaction tried to by following work of a former researcher of our laboratory [173]. Methyl acrylate was purified by inhibitor remover resin. Inhibitor remover resin was poured in a small column and methyl acrylate was passed through the resin of the column and collected as a pure form and purity was confirmed by proton ^1H NMR. 0.50 g monomer core was dissolved in DMF and added to 1 mL methyl acrylate in a flask which was kept in magnetic stirring for two days. TLC showed no new product.

This reaction tried to proceed in DMSO and acetonitrile solvent but no product was formed. As Michael addition is carried out in protic solvent and basic medium [187]. So, monomer core is dissolved in DMF, and pH of the solution was measured and pH found 5.3. So, to make the solution basic, trimethylamine was added to the reaction medium and reaction was carried out for three days. Remarkable product was not found. To carry out the reaction in protic solvent, monomer core was dissolved in DMF and 2 mL methanol was added to maintain protic solvent medium but no desired product was found.

4.2.3 Synthesis of Two Carbon Linker Different Generation dendritic Monomers

4.2.3.1 Synthesis of Two Carbon Linker Core by Oxanorbornene Ethylenediamine (EDA)

Ethylenediamine (14.9 g, 201.0 mmol) and 3a,4,7,7a-tetrahydro- 4,7-epoxyisobenzofuran-1,3-dione (oxanonorbornene) (4.80 g, 28.90 mmol) were taken in a 100 mL flux and refluxed at 80 °C for 2 h [188]. The product mixture was poured into distilled H_2O and organic layer was extracted with DCM (2 × 30 mL).

Remained water was dried from extracted organic part by Na_2SO_4 overnight. Soild Na_2SO_4 was removed by filter paper and organic solution was concentrated by rotary evaporator. A translucent solid was obtained by recrystallization from a mixture of DCM and ether (1:3 v/v) (yield 9.90%).

^{1}H NMR : 6.54 (s, 2H), 5.04 (s, 2H), 3.48 (t, 2H), 2.80 (t, 2H), 1.01 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.8, 136.6, 80.3, 45.4.

4.2.3.2 Di-*tert*-butyl Dicarbonate (BOC) Protected Ethylenediamine (EDA)

tert-Butyl *N*-(2-aminoethyl)carbamate or N-Boc-EDA was synthesized according to published literature [189]. Ethylenediamine (8.25 g, 137.50 mmol) was dissolved in DCM (20 mL) into a 100 mL two necked flask with continuous stirring at -5°C to 0 °C (lower temperature was maintained by using ice bath). Then, 5 mL (22.90 mmol) of BOC was dissolved in 40 mL DCM. BOC solution was added dropwise slowly in the cold reaction mixture at N_2 atmosphere. After the addition of all BOC solution, the reaction was continued for 22 h at air temperature, and a white suspension was obtained. The white suspension was separated by filtration and concentrated the filtrate by vacuum evaporator at lower pressure to get a yellowish oily liquid. Then the liquid was washed extensively with saturated brine solution. Filtrate was extracted with chloroform three times, and dried by Na_2SO_4 overnight and filtered. Excess solvents were dried by rotary evaporation to find N-Boc-EDA as yellowish oil of 3.12 g (Yield 85.3%).

FT-IR (cm^{-1}): 3351 (-NH_2), 2931-2975 (-C-H), 1687 (O=C-NH-, amide), 1165 (-O-C=O, ester).^{1}H NMR ($CDCl_3$, 500 MHz): δ4.85 (s, 1H), 3.15 (t, 2H), 2.71 (t, 2H), 1.45 (s, 12H), 1.20 (s, 2H).

4.2.3.3 Synthesis of Two Carbon Linker BOC Functioned Monomer Core by Oxanorbornene and BOC Protected EDA

A literature procedure [190] for the cobalt acetate catalyzed *exo*-3,6-Epoxy-1,2,3,6-tetrahydrophthalic anhydride transformation was tailored for the preparation of monomer. Monoprotected ethylenediamine (EDA-BOC) (1.80 g, 30 mmol) was

added to oxanorbornene (5 g, 30 mmol) in *N*,*N*-dimethylacetamide (6 mL) at 60 °C and stirred by a magnetic stirrer for 20 min. 0.1 mmole of cobalt acetate was dissolved by DMA and then added to this mixture, then acetic anhydride (5 mmol) also added immediately. After then mixture of the reaction was stirred for 4 h at 80 °C. The product mixture cooled at ordinary temperature. Solvent extraction process done by ethyl acetate and finally extracted organic portion was cleaned by dilute HCl, dried by $MgSO_4$. Solvent was removed by rotary evaporation. Product monitor by TLC with ethyl acetate: n-hexane = 4:1 purified by column chromatography. Percentage of yield was 30%.

FT-IR (cm^{-1}): 2990-2947 (-C-H), 1687 (O=C-NH-, amide), 1165 (-O-C=O, ester). ^1H NMR: 6.45 (s, 2H), 5.20 (s, 2H), 4.71 (s, 1H), 3.57 (t, 2H, *J* = 5 Hz), 3.24 (d, 2H, *J* = 5 Hz), 2.79 (s, 2H), 1.35 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 176.4, 155.9, 136.4, 80.9, 79.2, 47.3, 38.8, 38.4, 28.2.

4.2.3.4 Synthesis of Two Carbon Linker NH_2Terminated Monomer Core BOC Deprotected with TFA

To get free NH_2 group in the terminal chain, BOC has deprotected [191] from monomer core. The compound was dissolved in DCM and trifluoroacetic acid (TFA) was added to the reaction mixture and stirring at room temperature for 48 h. Solvent was removed by rotary evaporator to get free NH_2 terminated monomer core. After then the product was washed by diethyl ether and centrifuged several times. Trace amount of diethyl ether was removed by vacuum oven. Structure of the product is elucidated by ^1H NMR and ^{13}C NMR spectroscopy.

FT-IR (cm^{-1}): 3300 (-NH_2), 3034 (=C-H), 2990-2947 (-C-H), 1682 (O=C-NH-, amide), 1125 (C-O-C, ether). ^1H NMR: 6.59 (s, 2H), 5.21 (s, 2H), 3.80 (t, 2H, *J* = 5 Hz), 3.16 (t, 2H, *J* = 5 Hz), 3.01 (s, 2H). ^{13}C NMR (75 MHz, D_2O, δ, ppm): 179.4, 162.7, 136.4, 117.8, 81.4, 47.8, 37.9, 36.5.

4.2.3.5 Synthesis of Two Carbon Linker 0.5 Generation Dendritic Monomer

Azo-Michael addition reaction tried to proceed according to work of a former researcher of our laboratory with slight modified way [173]. 1.0 g (3.1 mmol) of BOC deprotected monomer NH_2 terminated core was dissolved in 9 mL methanol. Then 3.0 g (29.6 mmol) triethylamine was added to the core solution. After then 9.0 g (10.45 mmol) methyl acrylate was added to the reaction mixture dropwise and continued the reaction mixture stirring for five days. Solvent was removed by rotary evaporator at 50 °C bath temperature and reduced pressure. Concentrated product was monitored by FT-IR, ester peak was observed. Synthesis of 0.5 generation dendritic monomer by this procedure was successful. Structure of the product is elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy.

FT-IR (cm^{-1}): 2984-2829 (-C-H), 1725 (O-C=O, ester), 1687 (O=C-NH-, amide), 1142 (C-O-C, ether).^{1}H NMR: 6.51 (s, 2H), 5.25 (s, 2H), 3.65 (s, 6H), 3.53 (t, 2H, *J* = 5 Hz), 2.90 (s, 2H, *J* = 5 Hz), 2.75 (t, 4H, *J* = 5Hz), 2.60 (t, 2H, *J* = 5 Hz), 2.39 (t, 4H, *J* = 5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 176.2, 172.6, 136.6, 81.0, 51.3, 50.6, 49.5, 47.6, 36.9, 32.9.

4.2.3.6 Synthesis of 0.5 Generation Dendron from Ethanolamine

3.0 g ethanolamine was taken in 15 mL methanol solvent in a 250 mL round bottom flask and then 15 mL methyl acrylate was added to ethanolamine solution dropwise and continued stirring for three days at room temperature [192], [193]. After then methanol and excess methyl acrylate are removed by rotary evaporator at 60 °C with high vacuum. Product is yellowish oily dense liquid with nearly 95% yield. Product is monitored by FT-IR and proton NMR in $CDCl_3$.

FT-IR (cm^{-1}): 3459 (-OH), 2952-2834 (-C-H), 1720 (-O-C=O, ester), 1172 (C-O).^{1}H NMR ($CDCl_3$): δ 3.7 (s, 6H), 3.6 (t, 2H), 3.0 (s, 1H), 2.8 (t, 4H), 2.6 (2H, t), 2.4 (4H, t).

4.2.3.7 Synthesis of Two Carbon Linker 0.5 Generation Dendritic Monomer by Combination of Furan Maleimide and Dendron

The reaction was carried out through Mitsunobu reaction [194]. 3.0 g of furan maleimide was dissolved in 55 mL dry THF at 60 °C at nitrogen condition. After then solution was cooled at room temperature. 8.50 g of 0.5 generation dendron was dissolved in 10 mL dry THF and added to furan maleimide solution by a syringe at room temperature under nitrogen condition. 4.70 g triphenylphosphine (PPh_3) was dissolved in 10 mL dry THF and added to the reaction mixture through a syringe. Reaction flask was transferred into ice bath to make 0 °C. 3.90 mL diisopropylazodicarboxylate (DIAD) was measured by syringe and added to cool reaction mixture slow dropwise during half an hour. After then reaction flask is covered by aluminum foil and kept stirring for 48 h at ordinary temperature. After then THF was removed by evaporating and precipitate in cold petroleum ether through product dropping in cold petroleum ether of a 250 mL beaker. Pure product was isolated by a column against silica gel with ethylacetate and hexane mixture 1:6. Percentage of yield is 75%.

FT-IR (cm^{-1}): 2984-2829 (-C-H), 1725 (O-C=O, ester), 1687 (O=C-NH- (amide), 1142 (C-O-C, ether). ^1H NMR: 6.51 (s, 2H), 5.25 (s, 2H), 3.65 (s, 6H), 3.53 (t, 2H, *J* = 5 Hz), 2.90 (s, 2H, *J* = 5 Hz), 2.75 (t, 4H, *J* = 5Hz), 2.60 (t, 2H, *J* = 5 Hz), 2.39 (t, 4H, *J* = 5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 176.2, 172.6, 136.6, 81.0, 51.3, 50.6, 49.5, 47.6, 36.9, 32.9.

4.2.3.8 Synthesis of Two Carbon Linker 1.0 Generation Dendritic Monomer

To synthesize full generation dendritic monomer, 0.5 generation monomer (2.55 g, 6.71 mmol) was dissolved in 50 mL methanol in a 100 mL flask. Ethylenediamine (8.05 g, 134.16 mmol) was also dissolved in 30 mL methanol. Ethylenediamine solution was added to 0.5 generation dendric monomer solution with continuous stirring by magnetic rotator. The reaction was continued at normal temperature for 72 h and unreacted starting compound and solvent were removed under 200 mBar vacuums at 50 °C bath temperature. Though methanol was removed by

rotary evaporator, ethylenediamine still remains in the product. Excess ethylenediamine was removed by addition of 50 mL n-butanol by azeotropic solvent was removed by rotary evaporator at high vacuum. This process has done several times for removal of all ethylenediamine. Very few EDA might have in the reaction mixture; these have removed by liquid phase polymer retention (LPR) process. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy. FT-IR (cm^{-1}): 3339-3384 (NH_2), 1701 (O=C-NH-C=O, amide), 1603 and 1445 (NHC=O, amide), 1027 (C-O-C, ether). ^{1}H NMR: 8.32 (2H, s), 6.55 (2H, s), 5.11 (2H, s), 3.78 (2H, t), 3.16 (4H, t), 3.08 (2H, t), 2.84 (2H, s), 2.62 (4H, t), 2.56 (4H, t), 2.50 (4H, t).

4.2.3.9 Synthesis of Two Carbon Linker 1.5 Generation Dendritic Monomer

2.50 g (29.14 mmol) methyl acrylate was dissolved in 20 mL methanol which was further added to a stirred solution of previously synthesized amine terminated 1.0 dendric monomer (2.12 g, 4.85 mmol) in methanol (20 mL). The final mixture was stirred at room temperature for 48 h and excess reagents and solvent were dried by a vacuum evaporator under vacuum of 300 mBar at 45 °C bath temperature. Finally synthesized 1.5 generation dendrimer product found as yellowish gel. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 3318 (NH), 1733 (C=O), 1656 (HNC=O), 1537 (HNC=O); ^{1}H NMR : 8.71 (s, 2H), 6.56 (s, 2H), 5.11 (s, 2H), 3.67 (t, 8H), 3.62 (s,12H), 3.17 (t, 2H), 2.98 (t, 4H), 2.84 (s, 2H), 2.82(t, 4H), 2.69 (t, 2H), 2.46 (t, 8H).

4.2.4 Synthesis of Four Carbon Linker Different Generation Dendritic Monomer

4.2.4.1 Synthesis of Four Carbon Linker Monomer Core by Oxanorbornene and 1,4-Diaminobutane

Four carbon linker monomer core was synthesized by following standard procedure described in a published article [195]. The oxanorbornene (2.05 g, 12.33 mmol) and 1,4-diaminobutane (5.96 g, 74.03 mmol) were heated together at

80 °C for 2h in a flask. The reaction mixture was cooled down at open air temperature and 25 mL of DCM was added to dilute crude product. The solution was washed with distilled water for five times (25 mL × 5) and finally washed with brine (25 mL x 3). The organic layer was dried over $MgSO_4$ and concentrated by vacuum in a rotary evaporator to get pure product. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 3452-3375 (NH_2), 3012 (=C-H), 2939-2864 (-C-H), 1725, 1691 (O=C-NH- (amide), 1151 (C-O-C, ether). ^{1}H NMR: 6.54 (s, 2H),5.29 (s, 2H), 3.51 (t, 2H, *J*=5Hz), 2.79 (s, 2H), 2.77 (t, 2H, *J* =5 Hz), 1.67 (t, 2H, *J* = 5 Hz),1.52 (t, 2H, *J* = 5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 180.61, 136.56, 80.93, 53.44, 47.44, 46.23, 31.27, 26.07.

4.2.4.2 Synthesis of Four Carbon Linker 0.5 Generation Dendritic Monomer

Azo-Michael addition has done by following a published article of the former researcher of our laboratory [173]. A homogenous solution of methyl acrylate (2.21 mmol, 25.73 mmol) in methanol (20 mL) was mixed to a clear solution of amine terminated four carbon linker core (1.52 g, 6.43 mmol) in methanol (20 mL). The reaction mixture was continued magnetic stirring at environmental temperature for 48 h and excess methyl acrylate and solvent were dried under vacuum at 45 °C bath temperature by a rotary evaporator and finally synthesized 0.5 generation dendritic monomer products were found as turbid oily liquid. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 2951-2827 (-C-H), 1733 (O-C=O, ester), 1693 (O=C-NH-, amide), 1026 (C-O-C, ether). ^{1}H NMR: δ 6.51 (s, 2H), 5.26 (s, 2H), 3.67 (s, 6H), 3.48 (t, 2H, *J* = 5 Hz),2.85 (s, 2H),2.74 (t, 4H, *J* = 5 Hz), 2.44 (t, 2H, *J* = 5 Hz), 1.55 (t, 2H, *J* = 5 Hz), 1.40 (t, 2H, *J* = 5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ176.36, 172.98, 136.53, 80.9, 52.98, 51.56, 49.19, 47.41, 38.61, 32.44, 25.26, 25.25.

4.2.4.3 Synthesis of Four Carbon Linker 1.0 Generation Dendritic Monomer

To synthesize full generation, 0.5 generation dendritic monomer (1.69 g, 4.14 mmol) was dissolved in methanol (30 mL) in a 100 mL flask. Ethylenediamine (4.96 g, 82.75 mmol) was also dissolved in 20 mL methanol. Ethylenediamine methanol solution was added to 0.5 generation dendritic monomer solution with continuous stirring by magnetic rotator. The reaction was continued at air temperature for 72 h and unreacted reagents and solvent were removed under 200 mBar vacuums at 50 °C bath temperature. Though all methanol solvent was removed by rotary evaporator but ethylenediamine still remain in the product. Unreacted EDA was removed by addition of 50 mL n-butanol by azeotropic mixture was removed by rotary evaporator. Conversion of desired product was ensured by FT-IR and ^{1}H NMR.

FT-IR (cm^{-1}): 3352-3292 (NH_2), 2935-2918 (-C-H), 1701 (O=C-NH-, amide), 1603 (O=C-NH-, amide), 1027 (C-O-C, ether). ^{1}H NMR: 6.53 (s, 2H), 5.28 (s, 2H),3.49 (t, 2H, *J* = 5Hz),3.0 (s, 2H),2.85 (s, 2H), 2.75 (t, 2H, *J* = 5 Hz), 1.37 (m, 4H, *J* = 5 Hz).

4.2.4.4 Synthesis of Four Carbon Linker 1.5 Generation Dendritic Monomer

2.50 g (29.06 mmol) methyl acrylate was dissolved in 20 mL methanol that was added to amine terminated 1.0 dendritic monomer (2.25 g, 48.43 mmol) in methanol (20 mL) solution. The reaction mixture was continued to stirring at ambient temperature for 48 hours and unreacted reagents and solvent were removed by vacuum evaporator under vacuum at 45 °C bath temperature and finally synthesized 1.5 generation dendrimer product is yellowish gel. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 2984-2829 (-C-H), 1725 (O-C=O, ester), 1687 (O=C-NH- (amide), 1142 (C-O-C, ether). ^{1}H NMR: δ 1.18-1.40 (8H, m), 2.18-3.12 (32 H, m), 3.85 (12H, s),5.12 (2H, s), 6.65 (2H, s), 7.57 (2H, s).

4.2.4.5 Synthesis of Four Carbon Linker 2.0 Generation Dendritic Monomer

1.5 generation dendric monomer (3.19 g, 3.94 mmol) was dissolved in methanol (50 mL) in a 200 mL flask. 10 equivalent weight of ethylenediamine (9.46 g 157.72 mmol for each ester group was also dissolved in 50 mL methanol. Ethylenediamine methanol solution was added to 1.5 generation dendric monomer solution with continuous stirring by magnetic rotator to get 2.0 generation dendritic monomer. The reaction was continued at ambient temperature for 4 d and unreacted reactants and methanol solvent were removed by a rotary evaporator at 50 °C bath temperature. Though all methanol was removed by rotary evaporator but ethylenediamine still remain in the product. Excess ethylenediamine was removed by addition of 50 mL butanol that made azeotropic solvent of lower boiling point was removed by rotary evaporator at high vacuum. This process has done several times for removal of all ethylenediamine. Conversion of desired product was ensured by FT-IR. Very few EDA might have in the reaction mixture; these will remove by liquid phase polymer retention (LPR) process by using membrane MWCO 500 Da. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 3281 (NH_2), 3032 (=C-H), 2928-2864 (-C-H), 1699 (O=C-NH-, amide), 1643 (O=C-NH-, amide), 1568 (amide), 1032 (C-O-C, ether).^{1}H NMR: 7.93 (s, 6H), 6.55 (s, 2H), 5.12 (s, 2H), 3.16 (t, 2H), 3.05 (m, 16H), 2.55 (m, 12H), 2.43 (t, 2H), 2.20 (m, 12H),1.07-1.05 (m, 4H).

4.2.4.6 Synthesis of Four Carbon Linker 2.5 Generation Dendritic Monomer

Methyl acrylate (4.5 g, 52.37 mmol) was dissolved in methanol (50 mL) that was added to amine terminated generation 2.0 dendritic monomer (4.02 g, 4.36 mmol) in methanol (50 mL). The final mixture was continued to stirring at ambient temperature for 48 hours and unreacted reagents and solvent were evaporated under vacuum at 45 °C bath temperature. Finally synthesized 2.5 generation dendrimer monomer product was found as yellowish gel. Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy.

FT-IR (cm^{-1}): 2951-2825 (-C-H), 1731 (O-C=O, ester), 1644 (O=C-NH- (amide), 1534 (O=C-NH- (amide), 1174 (C-O-C, ether). ^1H NMR: 7.95 (s, 6H), 6.55 (s, 2H), 5.12 (s, 2H), 3.87 (s, 24H), 3.58 (t, 2H), 2.91 (s, 2H), 2.68 (t, 2H), 2.66 (m, 28H), 2.42 (m, 12H), 2.32 (m, 28H), 2.22 (m, 12H), 1.40 (m, 4H).

4.2.5 Synthesis of Six Carbon Linker Different Generation Dendritic Monomer

4.2.5.1 Synthesis of Six Carbon Linker Monomer Core

Six carbon linker monomer core was synthesized by following a published article [195]. The *exo*-oxanorbornene (2.73 g, 16.5 mmol) and 1,6-diaminohexane (11.50 g, 98.7 mmol) were heated together at 80 °C for 2h. The product mixture was cooled and dissolved in 25 mL of DCM. The product solution was purified with water (25 mL x 5) and saturated NaCl solution (25 mL × 3). The extracted organic portion was dried by $MgSO_4$ and solvent was removed by vacuum in rotary evaporator to get pure product. Amount of the product was found 2.23 g, yield 51.29%.

FT-IR (cm^{-1}): 3368-3283 (NH_2), 3067 (=C-H), 2930-2867 (-C-H), 1692 (O=C-NH- , amide), 1152 (C-O-C, ether). ^1H NMR: 6.53 (s, 2H), 5.28 (s, 2H), 3.49 (t, 2H, J = 5Hz), 3.0 (s, 2H), 2.85 (s, 2H), 2.75 (t, 2H, J = 5 Hz), 1.37 (m, 4H, J = 5 Hz).^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm): 176.3, 136.5, 80.9, 47.4, 41.4, 38.8, 31.8, 27.5, 26.3, 26.0.

4.2.5.2 Synthesis of Six Carbon Linker 0.5 Generation Dendritic Monomer

Azo-Michael addition was done according to a published article from our laboratory [173]. A clear solution of methyl acrylate (2.07 g, 24.09 mmol) in methanol (20 mL) was added to clear solution of amine terminated core (2.01 g, 8.03 mmol) in methanol (20 mL). The reaction mixture was continued to stirring air temperature for 72 h and unreacted starting compound and solvents were removed by rotary evaporator under vacuum at 45 °C bath temperature and at the end synthesized 0.5 generation dendrimer product was yellowish powder and product yield was obtained as 95%.

FT-IR (cm^{-1}): 2934-2857 (-C-H), 1733 (O-C=O, ester), 1693 (O=C-NH-, amide), 1165 (C-O-C, ether). ^{1}H NMR : 6.53 (s, 2H), 5.29 (s, 2H), 3.69 (s, 6H), 3.49 (t, 2H, *J* = 5 Hz), 2.85 (s, 2H), 2.77 (t, 4H, *J* = 5 Hz), 2.44 (t, 2H, *J* = 5 Hz), 1.46 (m, 4H, *J* = 5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 176.3, 172.9, 136.5, 80.9, 53.4, 51.6, 49.2, 47.2, 38.9, 32.2, 27.6, 27.3, 26.7, 26.5, 26.0.

4.2.5.3 Synthesis of Six Carbon Linker 1.0 Generation Dendritic Monomer

Full generation dendrimer was synthesized by amidation reaction according to a published article from our laboratory [173]. 0.5 generation dendric monomer (2.51 g, 5.75 mmol) was dissolved in methanol (50 mL) in a 200 mL flask. Ethylenediamine (6.90 g, 115.01 mmol) was also dissolved in 50 mL methanol. EDA methanol solution was added to 0.5 generation dendric monomer solution with continuous stirring by magnetic rotator to get 1.0 generation six carbon linker dendritic monomer. The reaction was continued at ambient temperature for 72 h and unreacted reagents and solvents were removed by a rotary evaporator under vacuum and 50 °C bath temperature. Though all methanol was removed by rotary evaporator but EDA still remains in the product. Excess EDA was removed by addition of 50 mL butanol that made azeotropic solvent of lower boiling point was can be removed by rotary evaporator at high vacuum. This process has done several times for removal of all ethylene diamine. But still trace amount of EDA might remain that removed by LPR method through a membrane of MWCO 200 Da. Finally yield of the product was obtained 85%.

FT-IR (cm^{-1}): 3339-3284 (NH_2), 2924-2853 (-C-H), 1698 (O=C-NH- (amide), 1657 (O=C-NH-), 1560 (O=C-NH-), 1040 (C-O-C, ether). ^{1}H NMR: 1.26-1.33 (8H, m), 2.17-3.18 (8H, m), 4.98 (2H, s), 6.44 (2H, s), 7.86 (2H, s).

4.2.5.4 Synthesis of Six Carbon Linker 1.5 Generation Dendritic Monomer

Magnetic stirred saturated solution of methyl acrylate (3.49 g, 40.55 mmol) in methanol (20 mL) was added to amine terminated 1.0 generation dendritic monomer (3.33 g, 6.75 mmol) in methanol (20 mL). Reaction mixture was

continued to stirring at ordinary temperature for 48 h and after then unreacted methyl acrylate and solvent were removed by rotary evaporator under vacuum and 50 °C bath temperature. Atthe end, synthesized 1.5 generation dendrimer product was yellowish gel. Percentage of the yield found 92%.

FT-IR (cm^{-1}): 2934-2860 (-C-H), 1733 (O-C=O, ester), 1700 (O=C-NH-, amide), 1666 (O=C-NH-, amide), 1436 (O=C-NH-, amide), 1172 (C-O-C, ether).^{1}H NMR (500 MHz, DMSO-d_6): δ7.67 (s, 2H), 6.55 (s, 2H), 5.12 (s, 2H), 3.58 (s, 12H), 3.31 (m, 12H), 2.65 (m, 4H), 2.39 (m, 8H), 1.40 (m, 4H), 1.18 (m, 4H).

4.2.5.5 Synthesis of Six Carbon Linker 2.0 Generation Dendritic Monomer

Full generation dendrimer was synthesized by amidation reaction according to a published article from our laboratory [173]. Six carbon linker 1.5 generation dendritic monomer (4.02 g, 4.80 mmol) was dissolved in 50 mL methanol in a round bottom flask. EDA (11.54 g, 192.35 mmol) was also dissolved in 50 mL methanol in a separate flask. Ethylenediamine methanol solution was added to 0.5 generation dendric monomer solution with continuous stirring by magnetic rotator to get full generation monomer. The reaction was continued at air temperature for 5 days and unreacted EDA and solvent were removed under 200 mBar vacuum at 50°C bath temperature. Though all methanol was removed by rotary evaporator but EDA still remains in the product. Excess EDA was removed by addition of 50 mL butanol, which made azeotropic solvent of low boiling point was removed by rotary evaporator at high vacuum. This process has done several times for removal of all ethylenediamine. But still trace amount of EDA might remain that removed by LPR method through a membrane of MWCO 1000 Da.

FT-IR (cm^{-1}): 3350-3281(NH_2), 2860-2937 (-C-H), 1692 (O=C-NH-, amide), 1632(O=C-NH-, amide), 1551 (O=C-NH-, amide), 1195 (C-O-C, ether). ^{1}H NMR: 7.90 (s, 6H), 6.55 (s, 2H), 5.12 (s, 2H), 3.03 (m, 14H), 2.62 (t, 2H), 2.56 (s, 2H), 2.21 (m,16H), 121-1.34 (m, 8H).

4.2.5.6 Synthesis of Six Carbon Linker 2.5 Generation Dendritic Monomer

A clear solution of methyl acrylate (3.29 g, 38.30 mmol) in methanol (20 mL) was mixed to amine terminated 2.0 generation dendritic monomer (4.54 g, 4.79 mmol) in methanol (20 mL). Reaction was continued to stirring at ambient temperature for 48 h and unreacted reagents and methanol solvent were removed by a rotary evaporator under vacuum at 45 °C bath temperature. At the end, synthesized 2.5 generation dendritic monomer product was found as yellowish gel.

FT-IR (cm^{-1}): 2951-2828 (-C-H), 1731 (O-C=O, ester), 1643 (O=C-NH- (amide), 1543 (O=C-NH-, amide), 1198 (C-O-C, ether). ^{1}H NMR : 7.93 (s, 6H), 6.55 (s, 2H), 5.12 (s, 2H), 3.61 (s, 24H), 3.58 (14H), 3.10 (t, 2H), 2.75 (t, 2H), 2.71(s, 2H), 2.42 (m, 4H), 2.22 (m, 12H), 1.38-1.23(m, 8H).

4.2.6 Synthesis of Pyridinium Based Monomer

4.2.6.1 Synthesis of Pyridine Based Oxanorobornene

A published method [60] for maleic anhydride-maleimide analogous compound [26, 27] by using sodium acetate catalyst was tailored for the preparation of the aminopyridine derivative (4-pyridine-3-ylmethyl- 10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione, 2). Furan-maleic anhydride adduct (1.92 g, 11 mmol) was dissolved in DMAc, (6 mL) and heated at 60 ˚C to make it hot. Pyridin-3-ylmethanamine (2.40 mL, 22 mmol) was mixed to a furan-maleic anhydride adduct solution in hot furan-maleic anhydride adduct solution and stirred for 20 minutes. Small amount sodium acetate (0.50 g, 5 mmol), was dissolved in 12 mL acetic anhydride. Sodium acetate suspension was added to reaction mixture was stirred for 2 h at 90 °C. After then stopped stirring and cooled down the crude productatair temperature. This crude product solution was diluted by ethyl acetate and purified by saturated NaCl solution. Aqueous layer was washed by ethyl acetate several times (5 times) to make sure all product goes to organic layer. Brine solution was used to wash combined organic layer and dried by $MgSO_4$. Organic layer was dicant and evaporated under reduced pressure by

rotary evaporation at room temperature. The pure product was separated by column chromatography THF: hexane (3:1, v/v) against basic aluminum oxide.

FT-IR (cm^{-1}): 3024 (=C-H) 2999-2860 (-C-H), 1697 (O=C-NH- (amide), 1596 (C=C, conjugate)1176 (C-O-C, ether). ^{1}H NMR: 8.52 (m, 2H), 7.65 (m, 1H, *J* = 5 Hz), 7.25 (m, 1H, *J* = 5 Hz), 6.52 (s, 2H), 5.30 (s, 2H), 4.65 (s, 2H), 2.88 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 175.7, 149.8, 149.1, 136.6, 135.9, 131.1, 123.4, 80.9, 47.6, 39.8.

4.2.6.2 Synthesis of Hexyl-Pyridinium Based Oxanorobornene

To synthesize the product, solution of pyridinium based oxanorbornene (0.13 g, 0.47 mmol) in 2 mL dried acetonitrile, 1-bromohexane (0.67 g, 4.7 mmol) was mixed together under nitrogen condition [60]. The mixture was heated to 60 °C for overnight where a reflux condenser on top of the flask to overcome solvent evaporation. Quaternized products were obtained by precipitation in diethyl ether through putting reaction mixture solution dropwise in a beaker of full of ether. Quaternary salt was purified by centrifuge against diethyl ether several times.

FT-IR (cm^{-1}): 3024 (=C-H) 2999-2860 (-C-H), 1697 (O=C-NH- (amide), 1639 (C=C, conjugate) 1195 (C-O-C, ether). ^{1}H NMR: 9.29 (m, 2H), 8.41 (m, 1H), 8.09 (m,1H, *J* = 5 Hz), 6.53 (s, 2H), 5.26 (s, 2H), 4.94 (s, 2H), 3.20 (s, 2H), 1.99 (t, 2H, *J* = 5Hz), 1.31 (m, 8H, *J* = 5 Hz), 0.86 (t, 3H, *J* = 0.75 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 170.7, 138.9, 138.3, 131.5, 131.1, 123.1, 75.8, 57.0, 43.0, 33.8, 26.5, 25.8, 20.4, 17.1, 8.7.

4.3 Homopolymer Synthesis

4.3.1 Synthesis of Grubbs 3rd Generation Catalyst

0.20 g of 2nd generation Grubbs catalyst was weighted in a septum vial and 0.5 mL of 3-bromopyridine was added to the 2nd generation Grubbs catalyst under nitrogen condition and stirred for 10 minutes by a magnetic stirrer (red colour of 2nd generation Grubbs catalyst converted to green) [130]. 10 mL pentane was poured into the vial and filtrate by a suction crucible at air pressure in a dark

room. Washed the product by pentane at least five times and dried by N_2 gas. Synthesized and dried 3rd generation Grubbs catalyst was taken in a small vial and covers with aluminum foil and stored in +4 ˚C in a fridge.

4.3.2 Amount of Catalyst Calculation for Desired Molecular Weight of Polymer

Expected molecular weight of polymer is 20,000 g/mol

Molecular weight of two carbon linker dendritic monomer 308.39 g/mol

Weight of two carbon linker dendritic monomer taken = 0.1 g,

$$\text{So, [Monomer]} = \frac{Amount\ of\ monomer}{Molecular\ weight\ of\ monomer} \quad (4.1)$$

$$= 0.1/308.39$$

$$= 2.628881\times10^{-4}$$

$$\text{Degree of polymer (DP)} = \frac{Molecular\ weight\ of\ \ polymer}{Molecular\ weight\ of\ monomer} \quad (4.2)$$

$$= 20{,}000/308.39$$

$$= 52.57761$$

$$\text{Again, } DP = \frac{[Monomer]}{[Catalyst]} \quad (4.3)$$

$$\Rightarrow 52.57761 = ([2.628881\times10^{-4}])/([\text{Catalyst}])$$

$$\Rightarrow [\text{Catalyst}] = ([2.628881\times10^{-4}])/52.57761$$

$$\Rightarrow [\text{Catalyst}] = 5 \times 10^{-6}$$

Molecular weight of Grubbs 3rd generation catalyst = 884.47 g/mol

$$Concentration\ of\ Catalyst = \frac{Amount\ of\ catalyst}{Molecular\ weigt\ of\ catalyst} \quad (4.4)$$

$$Amount\ \ of\ Catalyst = 884.47 \times 5 \times 10^{-6}$$

$$= 0.004422\ \text{g}$$

4.3.3 Polymerization Procedure of Monomer of Two Carbon Linker 0.5 Generation by Grubbs 3rd Generation Catalyst

0.10 g monomer was taken in a vial and dissolved it with 2 mL dry DCM. 0.0044 g Grubbs 3rd generation catalyst was weighted in a separate vial and dissolved by 0.5 mL dry DCM. Monomer solution was kept under nitrogen condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at room temperature. Reaction was terminated by adding ethyl vinyl ether (30% solution in DCM) and continued stirring more half an hour. Polymer product was precipitated by adding diethyl ether and precipitate polymer was washed several times by centrifuging over diethyl ether. Diethyl ether was decanted and precipitated solid polymer is dried by nitrogen flow. Polymer was confirmed by ^{1}H NMR spectroscopy.

^{1}H NMR: 7.65-7.35 (m, 5H), 6.05 (s, br, 1H), 5.80 (s, br, 1H), 4.86 (s, br, 1H), 4.34 (s, br, 2H), 3.59 (6H), 3.39 (2H), 2.68 (2H), 2.55 (6H), 2.38 (4H).

4.3.4 Polymerization Procedure of Monomer of Two Carbon Linker 1.5 Generation by Grubbs 3rd Generation Catalyst

0.10 g monomer was taken in a vial and dissolved it with 2 mL dry DCM. 0.0044 g Grubbs 3rd generation catalyst was weighted in a separate vial and dissolved by 0.5 mL dry DCM. Monomer solution was kept under nitrogen condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at ambient temperature. Polymerization reaction was terminated by adding ethyl vinyl ether (30% solution in DCM) and continued stirring more half an hour. Polymer product was precipitated by adding diethyl ether and precipitate polymer was washed several times by centrifuging over diethyl ether. Diethyl ether was decanted and precipitated solid polymer is dried by nitrogen flow. Polymer was confirmed by ^{1}H NMR spectroscopy.

^{1}H NMR : 7.65-7.35 (m, 5H), 6.05 (s, br, 1H), 5.80 (s, br, 1H), 4.86 (s, br, 1H), 4.34 (s, br, 2H), 3.59 (6H), 3.39 (2H), 2.68 (2H), 2.55 (6H), 2.38 (4H).

4.3.5 Polymerization of Six Carbon Linker 0.5 Generation Dendritic Monomer by Grubbs 2nd Generation Catalyst

0.10 g monomer was taken in a vial and dissolved it with 2 mL dry DCM. 0.0044 g Grubbs 2nd generation catalyst was weighted in a separate vial and dissolved by 0.5 mL dry DCM. Monomer solution was kept under nitrogen condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at ambient temperature. Polymerization was terminated by adding ethyl vinyl ether (30% solution in DCM) and continued stirring more half an hour. Precipitate the polymer by adding diethyl ether and washed several times by centrifuging over diethyl ether. Diethyl ether was decanted and precipitated polymer was dried by nitrogen flow. Polymer vial was rapped by aluminum foil and stored in dark place. Synthesized polymer was confirmed by ^{1}H NMR.

^{1}H NMR : (7.65-7.35 (m, 5H), 6.05 (s, br, 1H), 5.82 (s, br, 1H), 4.92 (s, br, 1H), 4.44 (s, br, 2H), 4.51 (br, 1H), 3.59 (6H), 3.39 (2H), 2.68 (2H), 2.55 (2H), 2.42 (4H), 2.30 (4H), 1.51 (2H),1.32 (6H), 1.2 (4H).

4.3.6 Polymerization of Six Carbon Linker 0.5 Generation Dendritic Monomer by Grubbs 3rd Generation Catalyst

0.20 g monomer was taken in a vial and dissolved it with 2 mL dry DCM. 0.0088 g Grubbs 3rd generation catalyst is weighted in a separate vial and dissolved by 0.50 mL dry DCM. Monomer solution is kept at N_2 condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at ambient temperature. Polymerization was terminated by adding ethyl vinyl ether (30% solution in DCM) and continued stirring more half an hour. Polymer was precipitated by adding diethyl ether and washed several times by centrifuging over diethyl ether. Diethyl ether was decanted and precipitated solid polymer was dried by nitrogen flow. Polymer vial is rapped by aluminum foil and stored in dark place.

^{1}H NMR : (7.65-7.35 (m, 5H), 6.05 (s, br, 1H), 5.82 (s, br, 1H), 4.92 (s, br, 1H), 4.44 (s, br, 2H), 4.51 (br, 1H), 3.59 (6H), 3.39 (2H), 2.68 (2H), 2.55 (2H), 2.42 (4H), 2.30 (4H), 1.51 (2H),1.32 (6H), 1.2 (4H).

4.3.7 Polymerization of Six Carbon Linker 1.5 Generation Dendritic Monomer by Grubbs 3rd Generation Catalyst

0.20 g monomer was taken in a vial and dissolved it with 2 mL dry trifluoroethanol. 0.0088 g Grubbs 2nd generation catalyst was weighted in a separate vial and dissolved by 0.5 mL dry DCM. Monomer solution was kept under nitrogen condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at room temperature. Reaction was quenched by adding ethyl vinyl ether (30% solution in DCM) and continued stirring more half an hour. Synthesized polymer was precipitated by adding diethyl ether and washed several times by centrifuging over diethyl ether. Diethyl ether is decanted and precipitated solid polymer was dried by nitrogen flow. Polymer vial is rapped by aluminum foil and stored in a dark place.

4.3.8 Polymerization of Six Carbon Linker 2.5 Generation Dendritic Monomer by Grubbs 2nd Generation Catalyst

0.20 g monomer was taken in a sealed tube and dissolved it with 2 mL dry trifluoroethanol. 0.0088 g Grubbs 3rd generation catalyst was weighted in a separate vial and dissolved by 0.5 mL dry dichloromethane. Monomer solution was kept under nitrogen condition and catalyst solution was added to monomer solution at a time and kept vigorous stirring over five days at 80 ˚C temperature. After 5 days heating system stopped and reaction is quenched by adding ethyl vinyl ether (30% solution in DCM) at room temperature and continued stirring more half an hour. Synthesized polymer was precipitated by adding diethyl ether and washed several times by centrifuging over diethyl ether. Diethyl ether is decanted and precipitated solid polymer was dried by nitrogen flow. Polymer vial is rapped by aluminum foil and stored in a dark place.

^{1}H NMR : 8.4-7.9 (6H), 7.65-7.35 (m, 5H), 6.21 (s, br, 1H), 5.55 (s, br, 1H), 4.68 (s, br, 1H), 4.31 (s, br, 2H), 4.00-3.51 (26H), 3.51-3.05 (40H), 3.00-2.50 (16H), 2.49-2.00 (26H), 1.5-1.00 (8H).

4.4 Dendrimer Based Metal Nanoparticle Synthesis and Their Catalytic Activity

4.4.1 Synthesis of Dendrimer Encapsulated Copper Nanoparticle

The synthesis of copper encapsulated dendrimer based ROMP nanoparticle was carried out by following a standard procedure from the published article [196]. According to the literature by Crooks et al, one Cu^{2+} ion will coordinate with the maximum four tertiary amine groups. Total molecular weight of six carbon linker 2.5 generation dendrimer based ROMP polymer is 20,021 and monomer molecular weight is 1638 so repeating is unit is 12 and each repeating unit contains 7 tertiary amine, total number of tertiary amine 96. So number of titration point is 12. Dendrimer based ROMP solution was prepared by the addition of distilled water (15 mL) which results in an aqueous dendrimer based ROMP solution with a concentration of 10 μM. The pH of the aqueous dendrimer solution was adjusted to 5.7 before the addition of the $CuSO_4$. The correct amount of aqueous $CuSO_4$ was calculated from the binding studies was added to each generation of prepared aqueous dendrimer solution [44]. Molar ratios of Cu^{2+} ions used in the case of 2.5 generation dendrimer based PAMAM, were 12. The solution was stirred for 20 min to allow the metal ions to complex to the tertiary amine groups of PAMAM-ROMP. 20 M excess of aqueous $NaBH_4$ was added dropwise to this solution. The Cu^{2+} ions were reduced to zero-valent Cu atoms, which resulted in the formation of the desired Cu encapsulated PAAM-ROMP. Immediate colour change from blue to golden brown in solution is straight forward evidence for the reduction of Cu^{2+}ions into PAMAM dendrimer branching cavity.

4.4.2 Reduction Ability Test of Synthesized Dendritic ROMP Encapsulated Copper Nanoparticle

An ideal reduction reaction of 4-nitro phenol to 4-aminophenol by using $NaBH_4$ as the hydrogen source was taken to estimate the catalytic activity of the synthesized dendrimer based ROMP Cu-nanoparticle [39]. Before investigating the catalytic activity of dendrimer based ROMP Cu nanoparticle, $NaBH_4$ was added to the 4-

nitrophenol solution without synthesized nanoparticle and UV-Vis spectrum was taken immediately and after 24 h to show that only $NaBH_4$ can not reduce 4-nitrophenol as UV-Vis measurement, insignificant decrease in the adsorption band of 4-nitrophenol was observed. After then, dendrimer based ROMP Cu nanoparticle catalyst was added to $NaBH_4$ and 4-nitrophenol solution and UV-Vis absorption was measured immediately in repeated measurement which shows gradual drop in the absorption band λ 400 nm while a new characteristic absorption band occurrence and increase at around 295 nm, which is the characteristic peak of 4-aminophenol. Successful reduction of the 4-nitrophenol to 4-aminophenol was observed from UV-Vis absorption peak [197], [198].

4.5 Synthesis of Copolymers

4.5.1 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8 kDa:2 kDa

4.5.1.1 Required Amount of Monomer and Catalyst Calculation

Expected total molecular weight of random copolymer is 10,000 g/mol

Molecular weight of two carbon linker dendritic monomer is 380.39 g/mol

Weight of two carbon linker dendritic monomer is taken = 0.2g

According to equation 4.1, [monomer] = $0.2/380.39 = 5.2577\times10^{-4}$

DP for two carbon linker dendritic monomer = 8,000/380.39=21.0310

By using equation 4.3 catalyst concentration $=5.26\times10^{-4}/21.0310 = 2.4999 \times 10^{-5}$

By using equation 4.4, amount of catalyst=$884.47 \times 2.4999 \times 10^{-5} = 0.02211$ g

Again, for pyridinium monomer, expected molecular weight is 2000 Da and molecular weight of pyridinium monomer is 421.33 g/mol.

So, using equation 4.2, DP of pyridinium monomer$= \frac{2000}{421.33} = 4.7468$

According to equation 4.3, [Monomer]$=4.7468\times[2.4999 \times 10^{-5}]=1.1866 \times 10^{-4}$

Hence, amount of pyridinium monomer = $1.1866 \times 10^{-4}\times421.33 = 0.0499\ g$

4.5.1.2 Copolymer Synthesis Procedure

0.20 g of two carbon linker dendric monomer was dissolved in 1.5 mL dry DCM. 0.0499 g of pyridinium monomer was dissolved in 1 mL dry methanol. Both the monomer solutions were mixed with each other in vial that was covered by aluminum foil and start stirring at N_2 condition and air temperature. 0.02211 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture was stirred and continued for 5 days at normal temperature. After 5 days, polymerization was quenched by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM) followed by stirring for half an hour more for complete termination process. Then the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven and elucidated by ^{1}H NMR spectroscopy.

P1_a: ^{1}H NMR: 9.07 (br, 2H), 8.53 (br, 1H), 8.15 (br, 1H), 7.47-7.30 (m, 5H), 5.97 (br, 1H), 5.75 (br, 1H), 4.81 (br, 1H), 4.64 (br, 2H), 4.51 (br, 1H), 3.57 (2H), 3.39 (s, 1H), 3.35 (s, 6H), 2.66 (4H), 2.50 (4H), 2.34 (2H) 1.89 (2H),1.26 (6H), 0.83 (4H).

4.5.1.3 Synthesis Of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8 kda:2 kDa

Synthesized 8 kDa:2 kDa random copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer (7 kDa:3 kDa) was dissolved in of 1 mL DCM and 1 mL methanol solvent. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then ice bath was removed and reaction mixture stirred properly under ambient temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_a: ^{1}H NMR: 9.23-8.06 (br, m, 4H), 7.47-7.30 (m, 5H), 5.76 (br, 1H), 5.74 (br, 1H), 4.94 (br, 1H), 4.78 (br, 1H), 4.47 (2H), 3.66 (2H), 3.29 (8H), 2.93 (1H), 2.74 (2H), 2.62 (4H), 2.38 (4H) 1.98 (2H), 1.44 (18H), 1.31 (16H), 0.83 (2H).

4.5.1.4 Synthesis of Quaternary Amine Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8 kDa:2 kDa

0.10 g of BOC surfaced random copolymer was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution has remained under magnetic stirring for 24 h for BOC deprotection. After 24 h BOC deprotected solution of polymer was separated from crude product by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}HNMR spectroscopy.

P3_a: ^{1}H NMR : 8.64-7.89 (br, m, 4H), 7.39-7.24 (m, 5H), 6.00 (br, 1H), 5.58 (br, 1H), 4.93 (br, 1H), 4.53 (br, 2H), 4.48 (br, 1H), 3.84 (2H), 3.62 (2H), 3.43 (8H), 3.00 (6H), 2.82 (1H), 1.96 (4H) 1.17 (6H), 0.71 (4H).

4.5.2 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 7 kDa:3 kDa

4.5.2.1 Required Amount of Monomer and Catalyst Calculation

Two carbon linker dendritic monomer concentration = 0.20/380.39 = 5.2577×10^{-4}

By using equation 4.2, DP for two carbon linker monomer= 7,000/380.39=18.4021

From equation 4.3 catalyst concentrations,=([5.26×10^{-4}])/18.40= 2.856×10^{-5}

By using equation 4.4 catalyst amount = $884.47 \times 2.8571 \times 10^{-5}$ = 0.02527 g

Again, from equation 4.2, for pyridinium based polymer, DP = $\frac{3000}{421.33}$ =7.1203

Monomer concentration =$7.1203\times[2.8571 \times 10^{-5}]$ =2.0343×10^{-4}

Using equation 4.4, amount of pyridinium monomer=$2.0343\times 10^{-4}\times421.33$

Hence, required amount of pyridinium based monomer = 0.08571 g

4.5.2.2 Random Copolymer Synthesis Procedure

0.20 g of two carbon linker dendritic monomer was dissolved in 1.5 mL dry DCM. 0.08571 g of pyridinium based monomer was dissolved in 1 mL dry methanol. Both the monomer solutions were mixed with each other in vial that was covered by aluminum foil and start stirring at N_2 condition and ambient temperature. 0.02527 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and let the reaction mixture stirring for 5 d at room temperature. After 5 days, polymerization reaction was quenched by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM) followed by stirring for half an hour more for complete termination process. Then polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate of polymer was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P1_b: ^{1}H NMR: 9.06 (br, 2H), 8.50 (br, 1H), 8.15 (br, 1H), 7.50-7.29 (m, 5H), 5.97 (br, 1H), 5.76 (br, 1H), 4.80 (br, 1H), 4.61 (br, 1H), 4.36 (br, 2H), 3.57 (2H), 3.39 (s, 1H), 3.35 (s, 6H), 2.66 (4H), 2.50 (4H), 2.34 (2H) 1.89 (2H),1.27 (6H), 0.85 (4H).

4.5.2.3 Synthesis of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 7 kDa:3 kDa

Synthesized 7 kDa:3 kDa random copolymers have free ester group on the surface that reacts with BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer (7 kDA:3 kDa) was dissolved in a solvent 1 mL DCM and 1 mL methanol solvents. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then reaction mixture continued stirring under laboratory temperature for 5 d. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_b: ^{1}H NMR: 9.21-8.04 (br, m, 4H), 7.43-7.06 (m, 5H), 5.99 (br, 1H), 5.89 (br, 1H), 4.93 (br, 1H), 4.75 (br, 2H), 4.46 (1H), 3.64 (2H), 3.29 (8H), 2.94 (1H), 2.74 (4H), 2.62 (4H), 2.36 (2H) 2.05 (2H), 1.44 (18H), 1.31 (16H), 0.87 (2H).

4.5.2.4 Synthesis of Quaternary Amine Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 7 kDa:3 kDa

0.10 g of BOC surfaced random copolymer was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}H NMR spectroscopy.

P3_b: ^{1}H NMR: 8.66-7.89 (br, m, 4H), 7.34-7.25 (m, 5H), 6.02 (br, 1H), 5.58 (br, 1H), 4.97 (br, 1H), 4.48 (1H), 3.87 (4H), 3.61 (8H), 3.42 (8H), 2.74 (4H), 3.00 (4H), 2.82 (2H) 1.88 (2H), 1.17 (6H), 0.81 (2H).

4.5.3 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7 kDa:3 kDa

4.5.3.1 Required Amount of Monomer and Catalyst Calculation

Two carbon linker dendritic monomer concentration = $0.2/380.39 = 5.26\times10^{-4}$

DP for two carbon linker dendritic monomer = $7,000/380.39=18.40$

From equation 4.3 concentration of catalyst $=([5.26\times10^{-4}]/18.40 = 2.8571 \times 10^{-5}$

From equation 4.4 amount of catalyst $= 884.47 \times 2.86 \times 10^{-5} = 0.02527$ g

According to equation 4.2, DP of pyridinium based polymer $= \frac{3000}{421.33} = 7.1203$

By using equation 4.3, monomer concentration $=7.1203\times[2.86 \times 10^{-5}] =2.03\times 10^{-4}$

From equation 4.1, amount of pyridinium monomer $=2.03\times 10^{-4}\times421.33= 0.0857g$

4.5.3.2 Block Copolymer Synthesis Procedure

Block copolymer was synthesized according to a published article[199].0.0857 g of pyridinium monomer was dissolved in 1 mL dry methanol in vial that was covered by aluminum foil and start stirring at N_2 condition and ambient temperature. 0.02527 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5mL DCM. All catalyst solution was added to monomer solutions by a Pasteur pipette and reaction mixture stirring continued for 4 h. 0.2 g of two carbon linker dendritic monomer was dissolved in 1.5 mL dry DCM in a different vial. After complete polymerization of pyridinium monomer and two carbon linker dendrite monomer solution was added to reaction mixture and let reaction stirring for 5 d. After 5 days polymerization was terminated by adding 0.5 mL ethyl vinyl ether solution (30% solution in dichloromethane). Let the reaction stirring for half an hour more for complete termination process. Then the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid ppt was dried by using vacuum oven and structure elucidated by ^{1}H NMR.

P1_c: ^{1}H NMR: 9.08 (br, 2H), 8.53 (br, 1H), 8.15 (br, 1H), 7.49-7.29 (m, 5H) 5.97 (br, 1H), 5.75 (br, 1H), 4.81 (br, 1H), 4.64 (br, 2H), 4.37 (br, 1H), 3.57 (2H), 3.39 (s, 1H), 3.35 (s, 6H) 2.66 (4H), 2.50 (4H), 2.34 (2H) 1.89 (2H), 1.26 (6H), 0.83 (4H).

4.5.3.3 Synthesis of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7 kDa:3 kDa

Synthesized Block copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.1 g of block copolymer (7 kDa:3 kDa) was dissolved in 1 mL DCM and 1 mL methanol solvent. Polymer solution was kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then ice bath was removed and mixture of the reaction stirring continued under air temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid

polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_c: ^{1}H NMR: 9.36-8.06 (br, m, 4H), 7.37-7.00 (m, 5H), 6.09 (br, 1H), 5.77 (br, 1H), 5.01 (br, 1H), 4.78 (br, 2H), 4.40 (1H), 3.66 (2H), 3.39 (4H), 3.29 (4H), 2.93 (1H), 2.74 (4H), 2.36 (4H), 2.35 (2H), 1.98 (2H), 1.43 (18H), 1.37 (16H), 0.86 (2H).

4.5.3.4 Synthesis of Quaternary Amine Functionalized Two Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7kDa:3kDa

0.1 g of BOC surfaced block co-polymer 7 kDa:3 kDa was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 hours at room temperature for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}H NMR.

P3_c: ^{1}H NMR: 8.65-7.90 (br, m, 4H), 7.30-7.23 (m, 5H), 6.01 (br, 1H), 5.81 (br, 1H), 4.93 (br, 1H), 4.56 (2H), 4.45 (1H), 3.87 (4H), 3.58 (2H), 3.39 (8H), 3.05 (4H), 3.00 (4H), 2.79 (8H) 1.86 (2H), 1.14 (6H), 0.69 (2H).

4.5.4 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 5 kDa:5 kDA

4.5.4.1 Required Amount of Monomer and Catalyst Calculation

Two carbon linker dendritic monomer concentration =0.2/380.39 = 5.26×10^{-4}

DP for two carbon linker dendritic polymer = 5,000/380.39=13.14

By using equation 4.3 catalyst concentration= 5.2577×10^{-4} /13.14= 4.00012×10^{-5}

By using equation 4.4, amount of catalyst = $884.47 \times 4.00012 \times 10^{-5}$ = 0.0353 g

DP of polymers of pyridinium monomer = $\frac{5000}{421.33}$ =11.8671

From equation 4.3, [pyridinium monomer]=$11.8671\times[4.0 \times 10^{-5}] = 4.7469 \times 10^{-4}$

Using equation 4.1, amount of pyridinium monomer= $4.75 \times 10^{-4} \times 421.33 = 0.2\ g$

4.5.4.2 Copolymer Synthesis Procedure

0.20 g of two carbon linker dendric monomer was dissolved in 1.5 mL dry DCM. 0.2 g of pyridinium monomer was dissolved in 1 mL dry methanol. Both the monomer solutions were mixed with each other in vial that is covered by aluminum foil and start stirring at inert condition at air temperature. 0.0353 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and the reaction mixture stirring continued for 5 days at air temperature. After 5 days, polymerization was quenched by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM). Let the reaction stirring for half an hour more for complete termination process. Then the polymer is precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR.

P1_d: ^{1}H NMR: 9.08 (br, 2H), 8.52 (br, 1H), 8.15 (br, 1H), 7.50-7.32 (m, 5H), 5.97 (br, 1H), 5.77 (br, 1H), 4.81 (br, 1H), 4.64 (br, 1H), 4.36 (br, 2H), 3.56 (2H), 3.41 (s, 1H), 3.33 (s, 6H), 2.66 (4H), 2.51 (4H), 2.35 (2H) 1.91 (2H), 1.27 (6H), 0.85 (4H).

4.5.4.3 Synthesis Of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 5 kDa:5 kDa

Synthesized 5 kDa:5 kD random copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer (5 kDa:5 kDa) was dissolved in 1 mL DCM and 1 mL methanol solvent. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then ice bath is removed and let the reaction mixture stirring under room temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid

polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_d: ^{1}H NMR: 9.03-8.07 (br, m, 4H), 7.48-7.06 (m, 5H), 5.77 (br, 1H), 5.58 (br, 1H), 4.81 (br, 2H), 4.66 (1H), 3.29 (4H), 3.15 (4H), 2.94 (1H), 2.74 (4H), 2.59 (4H), 2.37 (2H), 2.03 (2H), 1.44 (18H), 1.41 (16H), 0.87 (2H).

4.5.4.4 Synthesis of Quaternary Amine Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 5 kDa:5 kDa

0.10 g of BOC surfaced random copolymer was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer was confirmed by ^{1}H NMR spectroscopy.

P3_d: ^{1}H NMR: 8.68-7.92 (br, m, 4H), 7.40-7.26 (m, 5H), 5.87 (br, 1H), 5.56 (br, 1H), 4.96 (br, 1H), 4.70 (2H), 4.47 (1H), 3.89 (4H), 3.62 (2H), 3.49 (8H), 3.05 (4H), 2.76 (8H), 2.73 (1H), 1.86 (2H), 1.17 (6H), 0.71 (2H).

4.5.5 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 4 kDa:6 kDA

4.5.5.1 Required Amount of Monomer and Catalyst Calculation

Two carbon linker dendritic monomer concentration = $0.2/380.39 = 5.2577\times10^{-4}$

DP for two carbon linker dendritic monomer= $4{,}000/380.39=10.5155$

From equation 4.3 catalyst concentration $=[5.2577\times10^{-4}]/10.5155 = 5.00 \times 10^{-5}$

By using equation 4.4 amount of catalyst $= 884.47 \times 5.00 \times 10^{-5} = 0.04422$ g

Again, from equation 4.2, DP of pyridinium monomer $= \frac{6000}{421.33} = 14.2406$

By using equation 4.3, [pyridinium monomer]$=14.2406\times[5.0 \times 10^{-5}]=7.1204 \times 10^{-4}$

From equation 4.1, amount of pyridinium monomer= $7.1204 \times 10^{-4} \times 421.33 = 0.3\ g$

4.5.5.2 Copolymer Synthesis Procedure

0.20 g of two carbon linker dendric monomer was dissolved in 1.5 mL dry DCM. 0.3 g of pyridinium monomer was dissolved in 1 mL dry methanol. Both the monomer solutions were mixed with each other in vial that was covered by aluminum foil and start stirring inert condition at air temperature. 0.0422 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture stirring continued for 5 days at ambient temperature. After 5 d, polymerization was terminated by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM). Let the reaction stirring for half an hour more for complete termination process. Polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR.

P1_e: ^{1}H NMR : 9.09 (br, 2H), 8.52 (br, 1H), 8.16 (br, 1H), 7.52-7.30 (m, 5H), 5.97 (br, 1H), 5.75 (br, 1H), 4.81 (br, 1H), 4.65 (br, 1H), 4.37 (br, 2H), 3.55 (2H), 3.37 (1H), 3.36 (s, 6H), 2.64 (4H), 2.51 (4H), 2.33 (2H) 1.89 (2H), 1.26 (6H), 0.85 (4H).

4.5.5.3 Synthesis of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 4 kDa:6 kDa

Synthesized 4 kDa:6kDa random copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer (7 kDa:3 kDa) was dissolved in 1 mL DCM and 1 mL methanol solvent. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then reaction mixture continued stirring under air temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR.

P2_e: ^{1}H NMR: 9.00-8.03 (br, m, 4H), 7.46-7.22 (m, 5H), 5.77 (br, 1H), 5.50 (br, 1H), 5.22 (2H), 4.79 (br, 1H), 4.73 (1H), 3.65 (2H), 3.27 (4H), 2.92 (br,8H), 2.74 (1H), 2.58 (4H), 2.44 (2H), 2.02 (2H), 1.44 (18H), 1.31 (16H), 0.87 (2H).

4.5.5.4 Synthesis of Quaternary Amine Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 4 kDa:6 kDa

0.10 g of BOC surfaced random copolymer was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Synthesized polymer structure was elucidated by ^{1}H NMR.

P3_e: ^{1}H NMR: 8.67-7.92 (br, m, 4H), 7.40-7.25 (m, 5H), 5.73 (br, 1H), 5.58 (br, 1H), 4.91 (2H), 4.47 (br, 1H), 3.62 (1H), 3.43 (2H), 3.27 (4H), 3.10 (br,8H), 2.85 (1H), 1.86 (2H), 1.17 (6H), 0.71 (2H).

4.5.6 Synthesis of Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 3 kDa:7 kDA

4.5.6.1 Required Amount of Monomer and Catalyst Calculation

Two carbon linker dendritic monomer concentration = $0.2/380.39 = 5.2577\times10^{-4}$

DP for two carbon linker dendritic monomer= $3{,}000/380.39=7.89$

From equation 4.3, concentration of catalyst= $[5.2577\times10^{-4}]/7.89= 6.6667 \times 10^{-5}$

By using equation 4.4, amount of catalyst = $884.47 \times 6.6667 \times 10^{-5} = 0.0589$ g

From equation 4.2, DP of pyridinium monomer, $= \frac{7000}{421.33} = 16.6141.$

By using equation 4.3, [monomer] $=16.6141\times\times 6.6667 \times 10^{-5} = 1.1076 \times 10^{-3}$

From equation 4.1, amount of pyridinium monomer= $1.1 \times 10^{-3}\times421.33 = 0.4666\ g$

4.5.6.2 Copolymer Synthesis Procedure

0.20 g of two carbon linker dendric monomer was dissolved in 1.5 mL dry DCM. 0.4666 g of pyridinium monomer was dissolved in 1 mL dry methanol. Both the monomer solutions were mixed with each other in a vial that is covered by aluminum foil and starts stirring inert condition at air temperature. 0.0589 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and l reaction mixture stirring continued for 5 days at air temperature. After 5 d, polymerization was terminated by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM). Let the reaction stirring for half an hour more for complete termination process. Then the polymer is precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven and elucidated by ^{1}H NMR.

P1_f: ^{1}H NMR: 9.10 (br, 2H), 8.52 (br, 1H), 8.15 (br, 1H), 7.50-7.29 (m, 5H), 5.95 (br, 1H), 5.76 (br, 1H), 4.81 (br, 1H), 4.68 (br, 1H), 4.51 (br, 2H), 3.59 (2H), 3.41 (s, 1H), 3.31 (s, 6H), 2.67 (4H), 2.51 (4H), 2.34 (2H) 1.91 (2H), 1.28 (6H), 0.87 (4H).

4.5.6.3 Synthesis of BOC Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 3 kDa:7 kDa

Synthesized 3 kDa:7 kDa random copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer (3 kDa:7 kDa) was dissolved in 1 mL DCM and 1 mL methanol solvent. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then ice bath is removed and let the reaction mixture stirring under laboratory temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_f: ^{1}H NMR: 8.99-8.08 (br, m, 4H), 7.50-7.05 (m, 5H), 6.19 (br, 1H), 5.67 (br, 1H), 5.08 (2H), 4.75 (br, 2H), 3.65 (2H), 3.36 (4H), 2.99 (br,8H), 2.77 (1H), 2.43 (4H), 2.05 (2H), 1.44 (18H), 1.40 (16H), 0.87 (2H).

4.5.6.4 Synthesis of Quaternary Amine Surfaced Two Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 3 kDa:7 kDa

0.10 g of BOC surfaced random copolymer is taken in a small vial and 2 mL net trifluoroacetic acid is added to it. Polymer solution kept stirring for 24 h for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}H NMR spectroscopy.

P3_f: ^{1}H NMR: 8.66-7.91 (br, m, 4H), 7.37-7.26 (m, 5H), 5.73 (br, 1H), 5.56 (br, 1H), 4.93 (1H), 4.75 (br, 2H), 4.47 (1H), 3.62 (4H), 3.46 (br, 8H), 3.04 (8H), 2.86 (1H), 1.86 (2H), 1.16 (6H), 0.70 (4H).

4.5.7 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8 kDa:2kDa

4.5.7.1 Required Amount of Monomer and Catalyst Calculation

Expected total molecular weight of random copolymer is 10,000 g/mol

Molecular weight of six carbon linker dendritic monomer is 436.50 g/mol

Amount of six carbon linker dendritic monomer taken for polymerization = 0.20 g,

Six carbon linker dendritic monomer concentration= $0.20/436.50 = 4.5819\times10^{-4}$

DP of six carbon linker dendritic monomer = 8,000/836.50=18.3276

According to equation 4.3, [catalyst] = $[4.5819\times10^{-4}]/18.3276 = 2.5 \times 10^{-5}$

By using equation 4.4, amount of catalyst $= 884.47 \times 2.5 \times 10^{-5} = 0.02211$ g

From equation 4.1, DP of quaternary pyridinium based monomer $= \frac{2000}{421.33} = 4.75$

Quaternary pyridinium monomer concentration =4.75×[2.5 × 10^{-5}]=1.86 × 10^{-4}

So, amount of quaternary pyridinium monomer = 1.87 × 10^{-4}×421.33= 0.0499 g

4.5.7.2 Random Polymerization Procedure

Copolymer was synthesized according to a published article [200]. 0.20 g of six carbon linker dendritic monomer and 0.0499 g of quaternary pyridinium based monomer were weighted in separate vial; and dissolved in 1.5 mL dry DCM and 1 mL dry methanol respectively. Both the monomer solutions were mixed with each other in vial that was covered by aluminum foil to protect from light. Stirring started vigorously under N_2 condition at room temperature by using magnetic stirrer. 0.02211 g of Grubbs catalyst 3rd generation was weighted in a new vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and let the reaction mixture stirring for 5 days at ambient temperature. After 5 days, polymerization was terminated by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM). Let the reaction stirring for half an hour more to complete termination process. Then the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Precipitated solid polymer was dried by using vacuum oven and analyzed by proton ^{1}H NMR.

P1_g: ^{1}H NMR : 9.07 (br, 2H), 8.51 (br, 1H), 8.13 (br, 1H), 7.51-7.29 (m, 5H), 5.96 (br, 1H), 5.75 (br, 1H), 4.85 (br, 1H), 4.61 (br, 2H), 4.38 (br, 1H), 3.56 (2H), 3.40 (s, 1H), 3.39 (s, 6H) 2.63 (4H), 2.50 (4H), 2.37 (2H) 1.91 (2H), 1.46-1.21 (m, 16H), 0.85 (4H).

4.5.7.3 Molecular Weight Determination from ^{1}H NMR End Group Analysis

Integration for five protons of phenyl group (7.3-7.6 ppm) that from Grubbs catalyst is 1.0; So, integration for each proton of phenyl group that from Grubbs catalyst is 1.0/5= 0.2. Again, integration for four protons of pyridine (8.0-9.5 ppm) of pyridinium based monomer is 4.47; So, integration for each proton pyridine of the monomer=1.1175

Therefore, the number of repeating unit of pyridinium based monomer (DP=m) is 1.1175/0.2= 5.58 (pyridinium based monomer). Integration for two protons (5.6-6.2ppm) of polymer backbone (a/b) is 11.16,

Integration for one proton of polymer backbone (a/b) 5.58, the backbone formed from both monomers. So, DP (m+n)= 5.58/ 0.2 = 27.9 (six carbon linker dendritic monomer & pyridinium based monomer)

DP(n)= (27.9- 5.58) =22.32 (six carbon linker dendritic monomer)

MW of polymer = $MW\ of\ monomer\ 1 \times DP(n)$ + MW of monomer $3 \times DP\ (m)$

$=(436.50 \times 22.32) + (421.33 \times 5.5875)$ = 9742.52+2354.18 = 12096.70 g/mol

4.5.7.4 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8 kDa:2 kDa

Synthesized copolymers have free ester group on the surface that reacts with free NH_2 group of BOC protected EDA to form ester bond and BOC surface. 0.10 g of random copolymer was dissolved in 1 mL DCM and 1 mL methanol. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 °C and stirring half an hour more. After then ice bath was removed from reaction medium and the reaction was continued stirring under ambient temperature for 5 days. After 5 d, the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^1H NMR.

4.5.7.5 Synthesis of Quaternary Ammonium Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 8kDa:2kDa

0.10 g of BOC surfaced polymer was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h for BOC deprotection. After 24 h BOC deprotected quarternize cationic polymer was separated by adding diethyl ether and purified by centrifuge several times.

Remaining trace amount solvent was removed through evaporator. Desired product structure was elucidated by ^{1}H NMR.

4.5.8 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 7 kDa :3 kDa

4.5.8.1 Required Amount of Monomer and Catalyst Calculation

Six carbon linker dendritic monomer concentration = $0.2/436.50 = 4.5819\times10^{-4}$

DP of six carbon linker dendritic monomer = $7{,}000/436.50=16.04$

By using equation 4.3, catalyst concentrations=$[4.5819\times10^{-4}]/16.04=2.857\times10^{-5}$

From equation 4.4, amount of catalyst $= 884.47 \times 2.8571 \times 10^{-5} = 0.0252$ g

According to equation 4.2, DP for pyridinium monomer $= \frac{3000}{421.33} = 7.12$

By using equation 4.3, monomer concentrations=$7.12\times[2.86\times10^{-5}] = 2.03\times10^{-4}$

So, amount of pyridinium monomer= $2.03\times10^{-4}\times421.33 = 0.0857$ g

4.5.8.2 Random Copolymer Synthesis Procedure

0.20 g of six carbon linker monomer was dissolved in 1.5 mL dry DCM. 0.0857 g of monomer was dissolved in 1 mL dry methanol. Both the monomer solutions are mixed with each other in vial that was covered by aluminum foil and start stirring under absence of air at room temperature. 0.02521 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture stirring continued for 5 days at ambient temperature. After 5 days, polymerization was terminated by adding 0.5 mL ethyl vinyl ether (30% solution in DCM). Let the reaction stirring for half an hour more for complete termination process. Then the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven and structure of the product elucidated by ^{1}H NMR.

P1_h: ^{1}H NMR : 9.08 (br, 2H), 8.51 (br, 1H), 8.15 (br, 1H), 7.50-7.30 (m, 5H), 5.95 (br, 1H), 5.73 (br, 1H), 4.81 (br, 1H), 4.62 (br, 2H), 4.38 (br, 1H), 3.58 (2H), 3.37 (s, 6H), 2.62 (2H), 2.37 (4H), 2.31 (4H) 1.90 (2H), 1.46-1.21 (m, 16H), 0.85 (4H).

4.5.8.3 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 7 kDa:3 kDa

Synthesized copolymers have free ester group on the surface that reacts with free NH_2 of BOC protected EDA to form ester bond and BOC surface. 0.1 g of random copolymer (7 kDa:3 kDa) was dissolved in 1 mL DCM and 1 mL methanol. Polymer solution flask was kept on ice bath for half an hour and 3 mL EDA-BOC was added to polymer solution at 0 ˚C and stirring half an hour more. After then ice bath was removed and stirring of reaction mixture continued under air temperature for 5 days. After 5 days, the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by proton ^{1}HNMR spectroscopy.

P2_h:^{1}H NMR: 9.27-8.03 (br, m, 4H), 7.44-7.07 (m, 5H), 6.07 (br, 1H), 5.78 (br, 1H), 5.31 (2H), 4.76 (br, 1H), 4.63 (1H), 3.66 (2H), 3.26 (br,8H), 2.90 (1H), 2.76 (4H), 2.44 (8H), 2.04 (2H), 1.44 (18H), 1.31 (16H), 0.88 (2H).

4.5.8.4 Synthesis of Quaternary Amine Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Randon Copolymer 7kDa:3kDa

0.1 g of BOC surfaced random copolymer (7 kDa:3 kDa) was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h at air temperature for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethylether and purified by centrifuge several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}H NMR spectroscopy.

P3_h: ^{1}H NMR: 8.65-7.92 (br, m, 4H), 7.91-7.27 (m, 5H), 5.86 (br, 1H), 5.71 (br, 1H), 5.31 (2H), 4.78 (br, 1H), 4.47 (1H), 3.67 (br, 2H), 2.39 (8H), 3.09 (4H), 3.03 (8H), 2.83 (1H), 2.72 (4H), 1.86 (2H), 1.62-1.10 (14H), 0.71 (4H).

4.5.9 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7kDa:3 kDa

4.5.9.1 Required Amount of Monomer and Catalyst Calculation

Six carbon linker monomer monomer concentration = $0.2/436.50 = 4.5819\times10^{-4}$

Degree of polymer (DP) for six carbon linker monomer = 7,000/436.50=16.0366

According to equation 4.3, [catalyst] $=[4.5819\times10^{-4}]/16.0366 = 2.857 \times 10^{-5}$

By using equation 4.4, amount of catalyst = $884.47\times2.8571\times10^{-5} = 0.0252$ g

From equation 4.2, DP of pyridinium monomer $= \frac{3000}{421.33} = 7.1203$

From equation 4.3, [pyridinium monomer] $=7.1203\times[2.86 \times 10^{-5}] =2.0343 \times 10^{-4}$

So, amount of pyridinium monomer= $2.0343 \times 10^{-4}\times421.33 = 0.0857$ g

4.5.9.2 Block Copolymer Synthesis Procedure

Block copolymer was synthesized according to a published article [199]. 0.0857 g of pyridinium monomer was dissolved in 1 mL dry methanol in vial that was covered by aluminum foil and start stirring under nitrogen condition at normal temperature. 0.0252 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to pyridinium monomer solutions by a Pasteur pipette and reaction mixture stirring continued for 4 h. 0.2 g of Six carbon linker dendritic monomer was dissolved in 1.5 mL dry DCM in a different vial. After complete polymerization of pyridinium monomer and six carbon linker dendritic monomer solution was added to reaction mixture and let reaction stirring for 5 d. After 5 days polymerization was terminated by adding 0.5 mL ethyl vinyl ether solution (30% solution in DCM). Let the reaction stirring for half an hour more for complete termination process. Then

the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid ppt is dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P1_i: ^{1}H NMR : 9.04 (1H), 8.99 (1H), 8.37 (m, 1H), 8.14 (m, 1H,), 7.50-7.29 (m, 5H) 5.95 (br, 1H), 5.75 (br, 1H), 4.86 (br, 1H), 4.86 (br, 2H), 4.41 (br, 1H), 3.58 (2H), 3.35 (s, 6H), 2.64 (2H), 2.57 (1H), 2.37 (4H), 2.32 (4H), 1.90 (2H), 1.47-1.21 (m, 16H), 0.87 (4H).

4.5.9.3 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7 kDa:3 kDa

Synthesized block copolymers have free ester group on the surface that could react with BOC protected EDA to form ester bond and BOC surface. 0.1 g of block copolymer (7 kDa:3 kDa) was dissolved in of 1 mL DCM and 1 mL methanol. Polymer solution kept on ice bath for half an hour and 3 mL EDA-BOC was taken to polymer solution at ice temperature and stirring half an hour more. After then ice bath is removed and reaction mixture stirring continued under room temperature for 5 days. After 5 days the polymer was precipitated by adding diethyl ether and purified by centrifuge against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_i: ^{1}H NMR: 9.34-8.03 (br, m, 4H), 7.44-7.07 (m, 5H), 6.11 (br, 1H), 5.80 (br, 1H), 5.32 (2H), 4.80 (1H), 4.78 (br, 1H), 3.68 (1H), 3.28 (2H), 2.91 (1H), 2.43 (br, 8H), 2.43 (8H), 2.02 (2H), 1.42 (18H), 1.30 (16H), 0.86 (2H).

4.5.9.4 Synthesis of Quaternary Amine Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Block Copolymer 7kDa:3kDa

0.1 g of BOC surfaced block co-polymer 7 kDa:3 kDa is taken in a small vial and 2 mL net trifluoroacetic acid is added to it. Polymer solution kept stirring for 24 hours at room temperature for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge

several times. Trace amount of solvent is removed by vacuum evaporator. Structure of polymer is elucidated by ^{1}H NMR spectroscopy.

P3_i: ^{1}H NMR: 8.66-7.90 (br, m, 4H), 7.98-7.24 (m, 5H), 5.71 (br, 1H), 5.61 (br, 1H), 4.87 (br, 1H), 4.47 (br, 1H), 3.62 (2H), 2.39 (8H), 3.08 (4H), 3.02 (8H), 2.82 (1H), 2.72 (4H), 1.85 (2H), 1.63-1.15 (14H), 0.69 (4H).

4.5.10 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 5 kDa :5 kDa

4.5.10.1 Required Amount of Monomer and Catalyst Calculation

Six carbon linker dendritic monomer concentration = $0.2/436.50 = 4.5819\times10^{-4}$

DP of six carbon linker dendritic monomer monomer = $5{,}000/436.50=11.45$

From equation 4.3, catalyst concentration $=4.5819\times10^{-4}/11.45 = 4.00002 \times 10^{-5}$

By using equation 4.4, amount of catalyst $= 884.47 \times 4.00002 \times 10^{-5}= 0.03537$ g

DP of polymer pyridinium monomer $= \frac{5000}{421.33} =11.8671$

Pyridinium monomer concentration$=11.8671\times4.00002 \times 10^{-5} = 4.75 \times 10^{-4}$

By using equation 4.1, amount of pyridinium monomer$= 4.75 \times 10^{-4}\times421.33= 0.2$ g

4.5.10.2 Random Copolymer Synthesis Procedure

0.20 g of six carbon linker monomer taken in 1.5 mL dry DCM. 0.2 g of pyridinium based monomer was dissolved in 1 mL dry methanol. Both the monomer solutions are mixed with each other in vial that was covered by aluminum foil and start stirring at inert condition at air temperature. 0.03537 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture stirring continued for 5 days at ambient temperature. After 5 days, polymerization was terminated by adding 0.5 mL ethylvinylether (30% solution in DCM). Reaction mixture continued stirring for half an hour more for complete termination process. Then the polymer was precipitated by adding

diethylether and purified by centrifuzed against diethylether several times. Solid precipitate was dried by using vacuum oven. Structure of the product elucidated by ^{1}H NMR spectroscopy.

P1_j: ^{1}H NMR: 9.09 (br, 2H), 8.52 (br, 1H), 8.15 (br, 1H), 7.48-7.29 (m, 5H), 5.95 (br, 1H), 5.75 (br, 1H), 4.82 (br, 1H), 4.63 (br, 2H), 4.39 (br, 1H), 3.55 (2H), 3.36 (s, 6H), 2.62 (2H), 2.37 (4H), 2.31 (4H) 1.89 (2H), 1.45-1.20 (m, 14H), 0.84 (4H).

4.5.10.3 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 5 kDa:5 kDa

Synthesized copolymers have free ester group on the surface that reacts with free NH_2 of BOC protected EDA to form ester bond and BOC surface. 0.1 g of random copolymer (5 kDa:5 kDa) was dissolved in mixture of 1 mL DCM and 1 mL methanol solvent. Polymer solution flask was kept on ice bath for half an hour and 3 mL EDA-BOC was taken to polymer at 0 ˚C and stirring half an hour more. After then reaction mixture stirring continued under ambient temperature for 5 days. After 5 days, the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by proton ^{1}HNMR spectroscopy.

P2_j: ^{1}H NMR: 9.33-8.07 (br, m, 4H), 7.48-7.06 (m, 5H), 5.56 (br, 1H), 5.32 (2H), 5.30 (br, 1H), 4.80 (1H), 4.78 (br, 1H), 3.66 (2H), 3.33 (8H), 3.28 (2H), 2.97 (1H), 2.75 (br, 4H), 2.44 (8H), 2.03 (2H),1.43 (18H), 1.30 (16H), 0.88 (2H).

4.5.10.4 Synthesis of Quaternary Amine Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Randon Copolymer 5kDa:5kDa

0.1 g of BOC surfaced random copolymer (5 kDa:5 kDa) was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h at laboratory temperature for BOC deprotection. After 24 h BOC deprotected polymer was precipitated by adding diethyl ether and purified by centrifuge

several times. To overcome solvent NMR peak, all solvent was dried by vacuum oven. Structure of polymer is confirmed by ^{1}H NMR spectroscopy.

P3_j: ^{1}H NMR (500 MHz, D_2O, δ, ppm): 8.66-7.90 (br, m, 4H), 7.38-7.24 (m, 5H), 5.72 (br, 1H), 5.61 (br, 1H), 4.96 (br, 1H), 4.48 (br, 1H), 3.62 (2H), 2.39 (8H), 3.08 (4H), 3.02 (8H), 2.82 (1H), 2.70 (4H), 1.85 (2H), 1.62-1.05 (14H), 0.70 (4H).

4.5.11 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 4 kDa : 6 kDa

4.5.11.1 Required Amount of Monomer and Catalyst Calculation

Six carbon linker monomer concentration = $0.2/436.50 = 4.5819\times10^{-4}$

DP of six carbon linker monomer = $4{,}000/436.50=9.1638$

By using equation 4.3, catalyst concentration $=[4.5819\times10^{-4}]/9.1638= 5.0 \times 10^{-5}$

By using equation 4.4 catalyst amount $= 884.47 \times 5.0 \times 10^{-5} = 0.044223$ g

According to equation 4.2, DP of pyridinium monomer $= \frac{6000}{421.33} = 14.2406$

From equation 4.3, [pyridinium monomer]$=14.2406\times5.0 \times 10^{-5} =7.1203 \times 10^{-4}$

Using equation 4.1, amount of pyridinium monomer$=7.1203\times 10^{-4}\times421.33 = 0.3\ g$

4.5.11.2 Random Copolymer Synthesis Procedure

0.20 g of six carbon linker monomer was dissolved in 1.5 mL dry DCM. 0.3 g of pyridinium based monomer was dissolved in 1 mL dry methanol. Both the monomer solutions are mixed with each other in vial that was covered by aluminum foil and start stirring under nitrogen condition at ambient temperature. 0.044223 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture stirring continued for 5 days at room temperature. After 5 days, polymerization was terminated by adding 0.5 mL ethylvinylether (30% solution in DCM). Reaction mixture continued stirring for half an hour more for complete termination process. Then the polymer was

precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven. Structure of desired product was elucidated by ^{1}H NMR spectroscopy.

P1_k: ^{1}H NMR: 9.08 (br, 2H), 8.52 (br, 1H), 8.15 (br, 1H), 7.48-7.28 (m, 5H) 5.96 (br, 1H), 5.75 (br, 1H), 4.82 (br, 1H), 4.63 (br, 2H), 4.38 (br, 2H), 3.56 (2H), 3.36 (s, 6H), 2.63 (2H), 2.37 (4H), 2.31 (4H) 1.89 (2H), 1.45-1.20 (m, 14H), 0.85 (4H).

4.5.11.3 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 4 kDa:6 kDa

Synthesized copolymers have free ester group on the surface that reacts with free NH_2 of BOC protected EDA to form ester bond and BOC surface. 0.1 g of random copolymer (4 kDa:6 kDa) was dissolved in mixture of 1 mL DCM and 1mL methanol solvent. Polymer solution flask was kept on ice bath for half an hour and 3 mL EDA-BOC was mixed to polymer at ice temperature and stirring half an hour more. After then ice bath was removed and reaction mixture stirring continued under room temperature for 5 days. After 5 days, the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by proton NMR spectroscopy.

P2_k: ^{1}H NMR: 9.21-8.06 (br, m, 4H), 7.48-7.06 (m, 5H), 5.76 (br, 2H), 5.31 (br, 2H), 4.74 (2H), 3.68 (2H), 3.27 (2H), 3.08 (4H), 2.91 (1H), 2.76 (4H), 2.43 (4H), 2.38 (4H), 2.03 (2H), 1.42 (18H), 1.31 (16H), 0.88 (2H).

4.5.11.4 Synthesis of Quaternary Amine Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Randon Copolymer 4 kDa:6kDa

0.1 g of BOC surfaced random copolymer (4 kDa:6 kDa) was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h at room temperature for BOC deprotection. Next day BOC deprotected cationic polymer was separated by adding diethyl ether and purified by centrifuge

several times. Trace amount of solvent was removed by vacuum evaporator. Structure of polymer is confirmed by ^{1}H NMR spectroscopy.

P3_k: ^{1}H NMR (500 MHz, D_2O, δ, ppm): 8.66-7.90 (br, m, 4H), 7.32-7.25 (m, 5H), 5.88 (br, 1H), 5.62 (br, 1H), 4.72 (br, 1H), 4.47 (br, 1H), 3.63 (2H), 2.39 (8H), 3.09 (4H), 3.04 (8H), 2.82 (1H), 2.72 (4H), 1.87 (2H), 1.62-1.17 (16H), 0.71 (4H).

4.5.12 Synthesis of Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 3 kDa :7 kDa

4.5.12.1 Required Amount of Monomer and Catalyst Calculation

Six carbon linker monomer concentration = $0.2/436.50 = 4.5819\times10^{-4}$

Degree of polymer (DP) of the dendritic monomer = $3{,}000/436.50=6.8728$

Using equation 4.3, catalyst concentration = $4.5819\times10^{-4}/6.8728 = 6.6667 \times 10^{-5}$

By using equation 4.4, amount of catalyst $= 884.47 \times 6.6667 \times 10^{-5} = 0.05895$ g

DP of pyridinium monomer $= \frac{7000}{421.33} = 16.6141$

By using equation 4.3, [pyridinium monomer]$=16.61\times6.67 \times 10^{-5} = 1.11 \times 10^{-3}$

Hence, amount of pyridinium monomer$= 1.11076 \times 10^{-3}\times421.33 = 0.4667\ g$

4.5.2.2 Random Copolymer Synthesis Procedure

0.20 g of six carbon linker monomer was dissolved in 1.5mL dry DCM. 0.4667 g of pyridinium based monomer was dissolved in 1 mL dry methanol. Both the monomer solutions are mixed with each other in vial that was covered by aluminum foil and start stirring at inert condition at ambient temperature. 0.05895 g of Grubbs catalyst 3rd generation was weighted in a separate vial and dissolved in 0.5 mL DCM. All catalyst solution was added to monomer mixture solution by a Pasteur pipette and reaction mixture stirring continued for 5 days at ambient temperature. After 5 days, polymerization was terminated by adding 0.5 mL ethyl vinyl ether (30% solution in DCM). Reaction mixture continued stirring for half an hour more for complete termination process. Then the polymer was

precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid precipitate was dried by using vacuum oven. Structure of the product was elucidated by ^{1}H NMR spectroscopy.

P1_l: ^{1}H NMR: 9.24 (br, 2H), 8.52 (br, 1H), 8.14 (br, 1H), 7.47-7.26 (m, 5H), 5.94 (br, 1H), 5.74 (br, 1H), 4.81 (br, 1H), 4.64 (br, 2H), 4.51 (br, 1H), 3.53 (2H), 3.35 (s, 6H), 2.61 (2H), 2.49 (4H), 2.33 (4H) 1.87 (2H), 1.24 (14H), 0.82 (4H).

4.5.12.3 Synthesis of BOC Surfaced Six Carbon Linker Dendritic and Quaternary Pyridinium Based Random Copolymer 3 kDa:7 kDa

Synthesized copolymers have free ester group on the surface that reacts with free NH_2 of BOC protected EDA to form ester bond and BOC surface. 0.1 g of random copolymer (3 kDa:7 kDa) was dissolved in 1 mL DCM and 1 mL methanol solvent. Polymer solution flask was kept on ice bath for half an hour and 3 mL EDA-BOC was taken to polymer solution at zero degree and stirring half an hour more. After then ice bath was removed and reaction mixture stirring continued under room temperature for 5 days. After 5 days, the polymer was precipitated by adding diethyl ether and purified by centrifuged against diethyl ether several times. Solid polymer precipitate was dried by using vacuum oven and analyzed by ^{1}H NMR spectroscopy.

P2_l: ^{1}H NMR: 9.26-8.10 (br, m, 4H), 7.47-7.05 (m, 5H), 5.63(br, 2H), 5.12 (2H), 4.75 (2H), 3.66 (2H), 3.35 (4H), 3.22 (4H), 2.99 (1H), 2.77 (4H), 2.44 (4H), 2.05 (2H),1.43 (18H), 1.20-0.90 (18H).

4.5.12.4 Synthesis of Quaternary Amine Functionalized Six Carbon Linker Dendritic and Quaternary Pyridinium Based Randon Copolymer 3kDa:7kDa

0.1 g of BOC surfaced random copolymer (3 kDa:7 kDa) was taken in a small vial and 2 mL net trifluoroacetic acid was added to it. Polymer solution kept stirring for 24 h at air temperature for BOC deprotection. Next day BOC deprotected cationic polymer was separated by adding diethyl ether and purified by centrifuge several

times. Trace amount of solvent was removed by vacuum oven. Structure of the polymer is confirmed by ^{1}H NMR spectroscopy.

P3_l: ^{1}H NMR: 8.66-7.91 (br, m, 4H), 7.41-7.25 (m, 5H), 5.69 (br, 1H), 5.58 (br, 1H), 4.95 (br, 1H), 4.47 (br, 1H), 3.62 (2H), 2.39 (8H), 3.09 (4H), 2.99 (8H), 2.82 (1H), 1.86 (2H), 1.61-1.16 (16H), 0.70 (4H).

4.6 Molecular Weight of Polymers Determined By GPC and ^{1}H NMR End Group Analysis

Molecular weight of homopolymers and copolymers were determined by GPC in different solvent depending on solubility of the polymer. 0.5 generation dendritic homopolymers molecular weight was determined in THF solvent. 1.5 and 2.5 generation dendritic homopolymers molecular weight were determined in H_2O solvent. Nevertheless, homopolymers molecular weight was determined in DMSO solvent. Molecular weight of all polymers was calculated from end group analysis of ^{1}H NMR**.** Molecular weights determined by GPC don't agree with the molecular weight determined by ^{1}HNMR end group analysis even with theoretical value. Molecular weight that found by GPC is very low than theoretical value while molecular weight determined by ^{1}HNMR end group analysis is much closer to theoretical value.

4.7 Determination of Minimum Inhibitory Concentration Values

The MIC values of synthesized water soluble cationic copolymers were investigated through microdilution technique which is standard for antibacterial susceptibility test. In this testing method, polymer solution was used to investigate the visible growth on microtiter plates on agar surfaces. MIC values of the antibacterial polymers were recognized as minimum concentration where bacteria cannot grow up at visible manner. 16 mg of polymer was dissolved in 1 mL distilled water after than 125 µL of polymer solution and 875 µL distilled water was taken to prepare stock solutions of 2 mg/mL concentration of each polymer.

Bacteria suspensions of *E. coli* (ATCC25922) and *S. aureus* (ATCC29213) were incubated for 24 hours at 37 °C. For bacteria suspension 0.5 McFarland (1 ×10 ^{8} CFU/mL) solutions were prepared and diluted in 1:200 (5 ×10^{5} CFU/ mL). 0.1 mL of LB broth, and 0.01 mL bacterial solution were placed into well-plates. Prepared polymer stock solution was diluted serially maintaining ratio 0.5 in ten rows of the wells. To see negative control, only bacterium has placed into eleventh row of wells and for positive control, a known antibiotic such as ciprofloxacin was added to 12th raw of the well. Then the well plate was vortexed and kept 24 hours at certain temperature for bacterial incubation. Next day, polymer containing wells were examined to see appearance of turbidity which indicates bacteria has grown whereas clear well without any turbidity indicates interruption of growth of bacteria. These wells were identified and concentration of transparent solution measured as the MIC value.

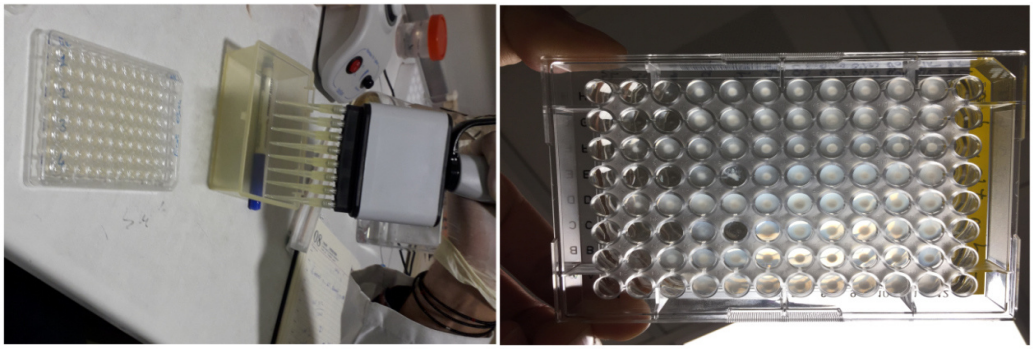


Figure 4.1 MIC values determination-a) microdilution process, b) monitoring bacterial growth and inhibition by turbidity and transparent wells respectively.

4.8 Investigation of Hemolytic Concentration (HC_{50})

Hemolytic concentration of polymers represents the toxicity arises on cells of the antimicrobial macromolecules [10]. Hemolytic activities of synthesized polymers were determined by using human fresh red blood cells of Rhesus factor negative, (RBCs, Rh-). 50 µL red blood cells were suspended in 10 mL 0.05 M, pH 7.4 phosphate buffered saline. RBC was clean several times (usually 3 times) by using 1500 rpm centrifugation for 4 minutes at 4 °C. After then RBC has suspended again in buffer solution to find yield 0.5% (v/v) solution. Water soluble cationic

polymeric solutions of concentration 16 mg/mL in distilled water were made and additional dilution has done using phosphonate buffer saline. 100 µL polymer solution was transferred in 96- well plate and made concentration difference by a way that final volume reached 200 µL in each well. For positive control, a known hemolytic compound TritonX-100 (0.2% in concentration) was added to RBCs whereas for negative control only RBC in PBS was taken in wells. Prepared 96-well plates were incubated for half an hour and further used centrifuge through 1500 rpm for 10 minutes at 4 °C. After then supernatant of every well plate was shifted to a new well. Synergy microplate reader was used to measure the absorbance released by treated RBC to calculate hemolysis. The HC_{50} value was identified through average concentration of cationic macromolecules that was able to do 50% hemolysis relative to the used positive control. All experiments were repeated three times.

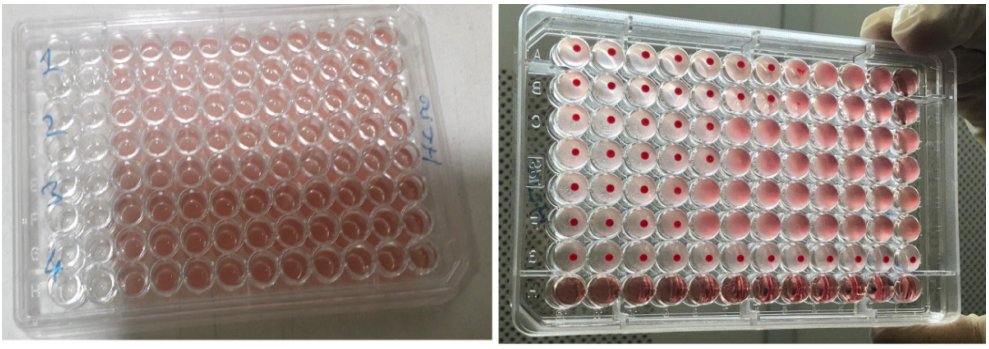


Figure 4.2 Hemolytic concentration determination- a) Human red blood cell (RBC) solution b) After putting polymer in red blood cell and centrifuge

4.9 Preparation of Cells for SEM

Surface morphology of *E.coli* and *S. aureus* were investigated by scanning electron microscopy (SEM) through treating with an active and an inactive polymer. SEM analysis has done by the microdilution method same as "Determination of MIC Value. For both polymers, MIC and supra MIC concentration solution has been prepared and 11.5 mL of each solution prepared and taken to Eppendorf tubes. Prepared polymer solution was centrifuged at 3000 rpm for 5 minutes and

removes supernatant. Remained pellets inside of Eppendorf tubes were cleaned twice by 2.0 mL PBS solution for every pellet. After then, the pellets for active and inactive polymer and control group were taken into consideration. Glass plates were used for SEM sample preparation. The collected pellets were smeared on clean glass plates as tips of pipette and covered with 2 mL of 2.5% glutaraldehyde. After then the prepared glass plates are incubated for 2 hours at open air temperature. After washing by PBS buffer during one minute dehydration treatment of glass plate has done through 50% ethanol, 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol, and 100% ethanol, respectively for ten minutes in each case. Dihydrated glass plates were incubated at ambient temperature for two hours. Finally, control cells and treated E. *Coli* and *S. aureus* cells were monitored and taken image with various magnifications by SEM.

4.10 Effective Diameter and Zeta Potential Measurement

Phosphate-buffered saline (PBS) (pH: 7.0) was freshly prepared and solutions were filtered through 22-µL filters to remove dust particles. Polymer's stock solution was prepared by using this PBS at a concentration of 2 mg/mL. Finally, 0.2 mg/mL polymer solution was prepared by taking 100 µL of these stock solutions and diluted by 900 µL of PBS. Zeta sizer and zeta potential of all polymer solutions were measured. One active and one inactive polymer were used for bacterial test. 100 µL PBS / 900 µL Bacteria solution (10^9 cell/mL Bacteria solution) mixture were used as two different blanks, 100 µL of stock solutions (2 mg/mL) were diluted with 100 µL PBS to give a concentration of 1 mg/mL. Finally, zeta potentials of solutions were measured where 100 µL of solution (1 mg/mL) was mixed to 900 µL of bacteria solution.

5
RESULTS AND DISCUSSION

5.1 Core Synthesis by Diels-Alder Reaction

Two different bicyclic compounds have been synthesized by Diels-Alder reaction to being used as starting core compound for monomer synthesis.

5.1.1 Oxanorbornene Synthesis

Oxanorbornene was synthesized from furan and maleic anhydride in THF solvent and kept in a dark place for 5 days. Crystalline white product found with very good yield. Purity depends on vigorous washing by cold THF. Extensive washing might lose some product but less washing will remain unreacted starting compounds. Reaction is given in Figure 5.1 and mechanism of the reaction is highly studied and well established.

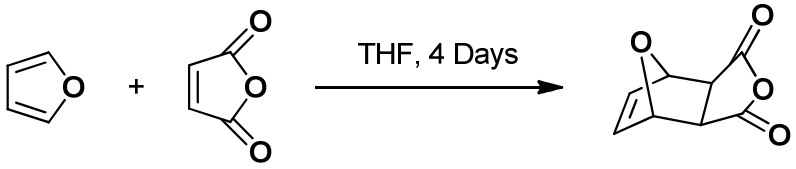


Figure 5.1 Core synthesis scheme by Diels-Alder reaction

FT-IR was taken to see the conversion of the product and compared with starting compounds shown in Figure 5.2. Anhydride of maleic anhydride shows two bands at 1800-1830 and 1740-1775 cm^{-1} for C=O and furan shows two double bonds at 3030 and ether C-O at 1070-1150 cm^{-1}. In FT-IR of Diels-Alder adduct shows both anhydride and ether peaks.

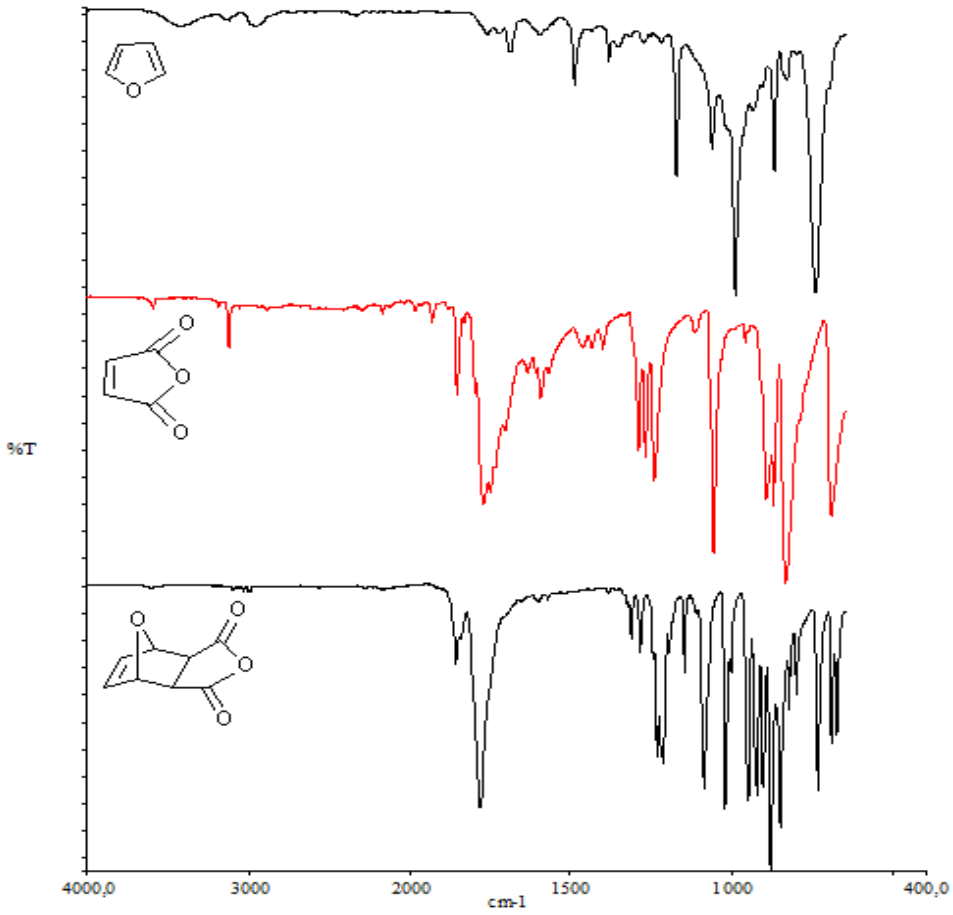


Figure 5.2 FT-IR of oxanorbornene, furan and maleic anhydride

To confirm synthesized product purity, both ^{1}H NMR and ^{13}C NMR spectrum were elucidated. In ^{1}H NMR protons of C=C shows singlet at 6.59 ppm, protons of HC-O-CH at 5.36 ppm and rest of the protons are at 3.32 ppm indicates the *exo*-product. As position of the singlet is not in 3.9 ppm, synthesized product must be exo-3,6-epoxy-1,2,3,6-tetrahydropathalic-anhydride. Usually, endo product, hydrogen of endo configuration bands is in 3.9 ppm [137]. Moreover, if endo-isomer remains with exo procut, it also gives small peak nearby or along with 6.5 and 5.33 ppm. ^{1}H NMR and ^{13}C NMR of exo-3,6-epoxy-1,2,3,6-tetrahydropathalic-anhydride is given in Figure B1 and B2 in appendix part. In ^{13}C NMR, spectra, 171.8 ppm for C of C=O, 137.3 ppm for c of C=C, 82.1 ppm for C adjacent to C-O-C and 49.2 ppm for rest C of

synthesized Diels-Alder adduct. To proceed on further reaction solubility test of the product has investigated and given in Table 5.1 that indicates synthesized oxanorbornene is soluble in acetone, DMSO and N, N-dimethylacetamide.

Table 5.1 Solubility test of synthesized oxanorbornene

Solvent	Acetone	H_2O	$CHCl_3$	CH_3OH	DMSO	N-N dimethyl acetamide
Solubility	+	-	-	-	+	+

5.1.2 *Exo*-3, 6-tetrahydrophthalide

Pure exo isomer of 3, 6-exo-tetrahydrophthalide was synthesized by the combination of furan and maleimide in ethylacetate through Dilels-Alder cycloaddition (reaction scheme is in Figure 5.3). Precipitated product was washed by cold diethyl ether through centrifuge. Chemical structure of synthesized product was elucidated using FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy. In FT-IR spectra 3066 cm^{-1} (=C-H), 1700 cm^{-1} for (O=C-N-C=O (amide) and 1090 cm^{-1} for bridge C-O-C (ether). Olefinic protons of oxanorbornene were observed at 6.52 ppm as singlet, NH proton of amide was observed at 8.08 ppm as broad in the corresponding ^{1}H NMR spectrum. ^{13}C NMR for the olefinic carbon and carbonyl carbon peaks were present at 136.56 and 175.85 ppm, respectively, confirming the formation of the exo stereoisomer structure of the Diels-Alder adduct.

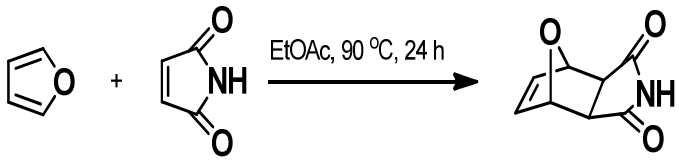


Figure 5.3 Furan-maleimide adduct scheme by Diels-Alder reaction

FT-IR, ^{1}H NMR and ^{13}C NMR spectra were given in Figures as appendix A2, B3 and B4 respectively. Powderly white product with good yield also exo isomer.

5.2 Synthesis of Different Generation Monomer without Carbon Linker in between Oxanorbornene and Dendritic Part

To get amine terminated oxanorbornene derivative hydrazine hydrate was used and after then methyl acrylate has been used for desired dendritic monomer.

5.2.1 Synthesis of Monomer Core by Oxanorbornene and Hydrazine Hydrate

NH_2 terminated oxanorbornene derivative was synthesized in benzene. Within 15 min the white product sediment was found in the bottom of the flask. To ensure the reaction completion, stirring continued up to half an hour. Crystalline white products' percentage of yield was found 83.5. Reaction scheme is shown in Figure 5.4 and FT-IR spectra show very clear terminal $-NH_2$ peaks at 3338 cm^{-1} and 3309 cm^{-1} (Figure A3 in appendix part). Structure of the product has explained by ^1H and ^{13}C NMR in DMSO-d_6 solvent. Olefinic protons of oxanorbornene part were observed at 6.56 ppm as singlet in the corresponding proton NMR spectrum. ^{13}C NMR for the olefinic carbon and carbonyl carbon peaks were present at 136.7 and 174.8 ppm, respectively, represents the formation of desired product. ^1H and ^{13}C NMR spectra of the product is shown in appendix B5 and B6 sequentially in appendix part.

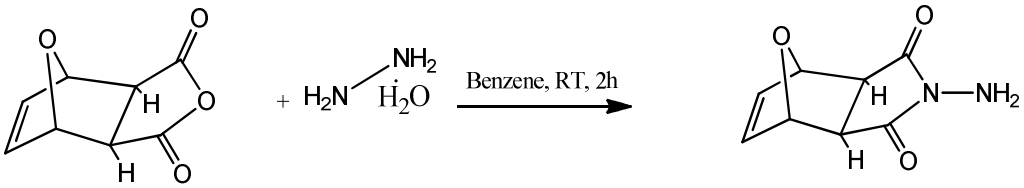


Figure 5.4 Core synthesis scheme by Diels-Alder adduct and hydrazine hydrate

The heteronuclear single quantum correlation experiment (HSQC) provides an indication of which proton is attached to a particular carbon center by data from ^1H signal detection and ^{13}C-NMR spectra. Most prominent signal in the HSQC spectrum (Figure 5.5) arises at the intercept of the signal at 45 ppm in the ^{13}C axis and 2.8 in the ^1H spectrum axis, confirming the proton and C and two protons adjacent to C=O. There are two peaks near 5 to 5.1 in proton axis but in ^{13}C axis only one peak near 80 ppm that means two protons attached to a carbon and rest

two protons peaks come from NH_2. Carbon-carbon double bond peaks is at 136 ppm of C axis and about 6.5 ppm for protons that attached to C=C. Form HSQC spectra it is clear that each proton is attached to a carbon except two protons at 5 ppm. So, it is clear that another proton is attached with N of NH_2.

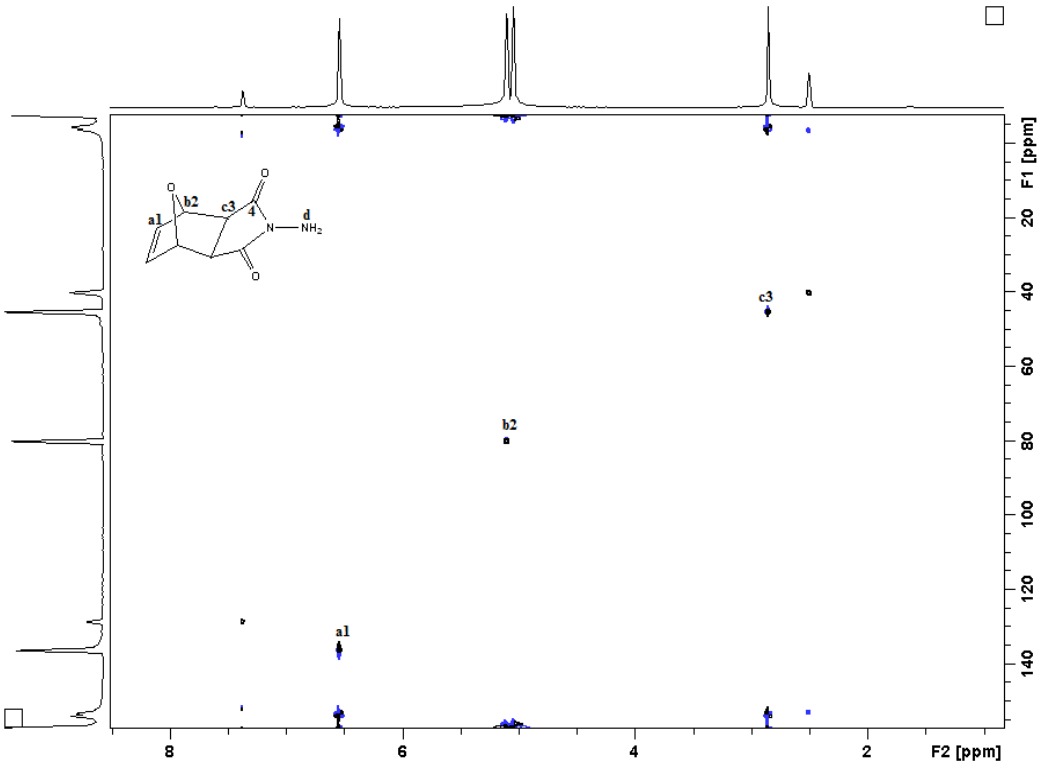


Figure 5.5 HSQC NMR of NH_2 terminated monomer core with no carbon linker

5.2.2 Attempt to Synthesize 0.5 Generation Dendritic Monomer without Linker

Michael addition is carried out in protic solvent and basic medium [187] and in most of the cases, methanol is used as solvent. But NH_2 terminated oxanorbornene was not soluble in methanol solvent. Reaction was carried out with newly synthesized NH_2 terminated oxanorbornene derivative and methyl acrylate in DMF solvent through magnetic stirring for two days at room temperature. TLC test indicates that there was no new product formed. This reaction was tried to proceed in DMSO and acetonitrile solvent but no product is formed. So, DMF solution of NH_2 terminated oxanorbornene monomer core was prepared to

measure pH and found 5.3. So, to make the solution basic, trimethylamine is taken to it. Reaction was proceeding on for three days and remarkable product was not found. To carry out the reaction in protic solvent, NH_2 terminated oxanorbornene monomer core was dissolved in DMF and 2 mL methanol was added to maintain protic solvent medium but no desired product was found.

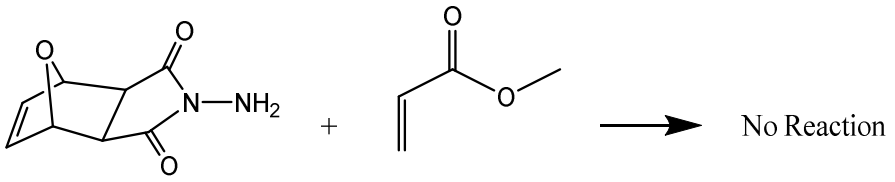


Figure 5.6 Attempts to synthesize of 0.5 generation monomer without carbon linker

5.3 Synthesis of Different Generation Monomers with Two Carbon Linker in Between Oxanorbornene and Dendron

To get amine terminated oxanorbornene derivative ethylenediamine and EDA-BOC were used and after then BOC was deprotected. Methyl acrylate and EDA have been used one after another repeatedly for the synthesis of different generation PAMAM dendritic monomers. This synthetic strategy is well known as divergent techniques. Convergent strategy has been taken to combine ethanolamine cored dendrimer with oxanorbornene through Mitsunobu reaction.

5.3.1 Synthesis of Two Carbon Linker Core by Oxanorbornene and Ethylenediamine (EDA)

To synthesized NH_2 terminated monomer core with two carbon linker, ethylenediamine was directly reacted with oxanorbornene [188]. Percentage of yield of the reaction found low and even both exo and endo isomer form together (Figure 5.7). Column chromatography was used to isolate one of the stereoisomer by using methanol, dichloromethane and ammonium-hydroxide (1:5:0.3,v/v) [201] but unable to get pure compound.

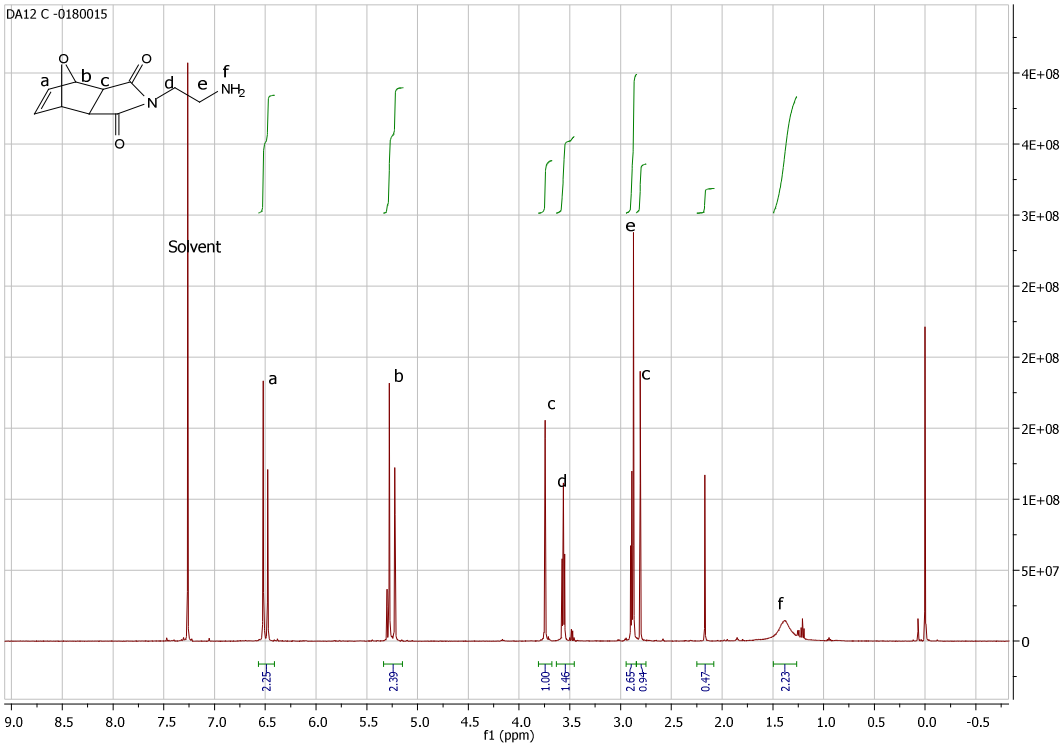


Figure 5.7 ^{1}H NMR of two carbon linker NH_2 terminated core

In ^{1}H NMR spectra products bands at 6.5 (2H, s), 5.5 (2H, s), 3.3 (2 H, s) indicates the exo-product. As position of a singlet is in 3.75 ppm and doublet in 6.5 and 5.5 ppm indicate the co-existence of endo product too. This assumption could be true due to having one extra peak at 6.5 and 5.5 respectively. Usually, endo product, hydrogen of endo configuration bands is in 3.9 ppm [137]. Probability of having two independent molecules (exo/endo) reported by L. A. Mitchell et al [188]. Triplet at 3.55 ppm and 2.8 ppm indicate two CH_2 group of ethylene side chain.

5.3.2 Synthesis of Di-*tert*-butyl Dicarbonate (BOC) Protected Ethylenediamine (EDA)

EDA-BOC was synthesized from ethylenediamine and di-*tert*-butyl dicarbonate (BOC) in DCM solvent. Though it is a well known reaction, but tricky. BOC solution should be added to excess amount than EDA and BOC should be added very slowly at dilute form under cold temperature. Otherwise, both the amine group of EDA

will be protected and white solid product will form. Moreover, EDA-BOC is highly air sensitive; synthesized product must be stored in frize under nitrogen at 4 °C. As excess amount of EDA is used, after vacuum evaporation EDA still remains. To remove excess EDA, washed with brine several times and extracted in ethyl acetate. EDA-BOC was found as yellowish oily liquid with yield of 85.3%. Reaction scheme is given in Figure 5.8.

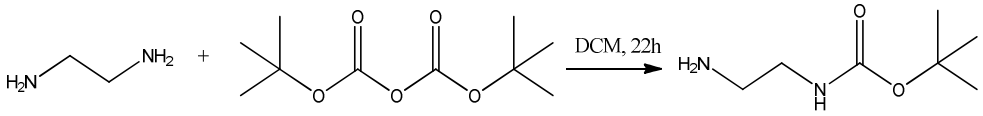


Figure 5.8 Di-*tert*-butyl dicarbonate (BOC) protection of ethylenediamine (EDA)

Structure was elucidated by FT-IR and ^{1}HNMR shown in appendix A4 and B7 respectively. In FT-IR, NH_2 peaks at 3351 cm^{-1} and NHC=O peak at 1687 cm^{-1} and 1453 cm^{-1} are clear indication of free amine and amide group. In ^{1}H NMR spectra, NH singlet broad peak at 4.86 ppm, two different triplet at 3.10 ppm and 2.73 ppm is for two different CH_2, and a big singlet peak at 1.38 ppm for twelve protons of BOC. Reaction mechanism scheme of EDA-BOC is given in Figure 5.9.

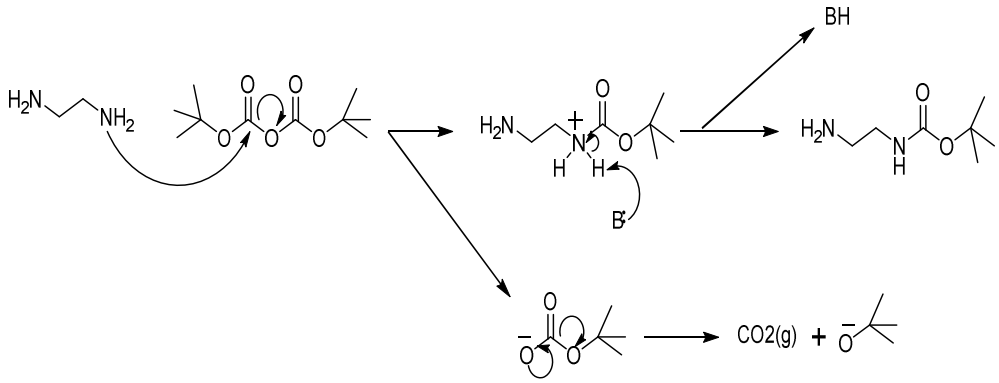


Figure 5.9 Reaction mechanism scheme of EDA-BOC

5.3.3 Synthesis of Two Carbon Linker BOC Functioned Monomer Core by Oxanorbornene and BOC-EDA

Monoprotected ethylenediamine (EDA-BOC) and oxanorbornene reacted at 80 °C in *N*,*N*-dimethylacetamide in presence of catalytical amount of cobalt acetate and

acetic anhydride. Crude product found with a mixture of compounds (reaction scheme is in Figure 5.10).

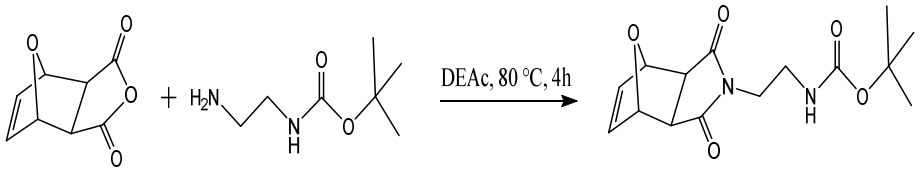


Figure 5.10 Synthesis of two carbon linker from oxanorbornene and BOC-EDA

Product monitor by TLC with ethyl acetate: n-hexane = 4:1 to get desired product. Pure product was obtained by column chromatography and percentage of yield was only 30%. Product chemical structure was explained by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy in $CHCl_3$ solvent (Appendix Figure A5, B8 and B9). Olefinic protons of oxanorbornene was observed at 6.52 ppm as a singlet, HC-O-CH proton was observed at 5.20 ppm as in the corresponding ^{1}H NMR spectrum. Twelve proton spectra of BOC at 1.35 ppm amide peak at 4.71 ppm represent that successful combination of EDA-BOC and oxanorbornene. ^{13}C NMR for the olefinic carbon, carbonyl and amide carbon peaks were present at 136.4, 176.4 and 1559 ppm, respectively.

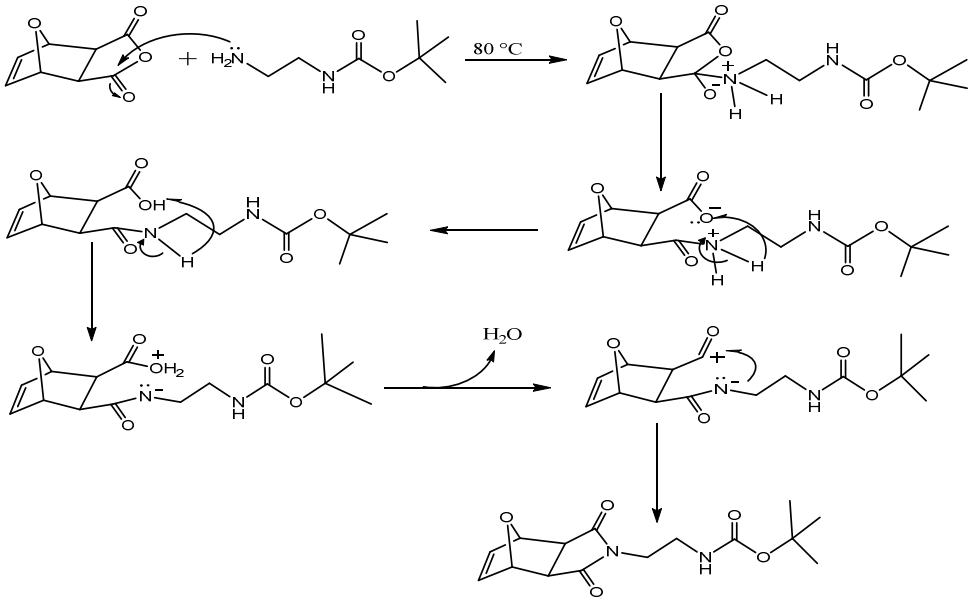


Figure 5.11 Reaction mechanism scheme for synthesis of two carbon linker from oxanorbornene and EDA-BOC

As the product was synthesized at higher temperature there was a probability to form exo endo mixture but product oxanorbornene parts proton ^{1}H NMR do not show doublet and very nice and clear singlet is agreement for pure exo product. Reaction mechanism for synthesis of two carbon linker from oxanorbornene and EDA-BOC is as follows

5.3.4 Synthesis of Monomer BOC Deprotected Salt

To get free NH_2 terminated core BOC was deprotected by TFA in DCM (1:1) during 24 h at room temperature. Percentage of yield of BOC salt was nearly 97%. Extensive washing in diethyl ether through centrifuge is required to get pure salt product. It is formed TFA salt of primary amine, reaction scheme is shown in Figure 5.12.

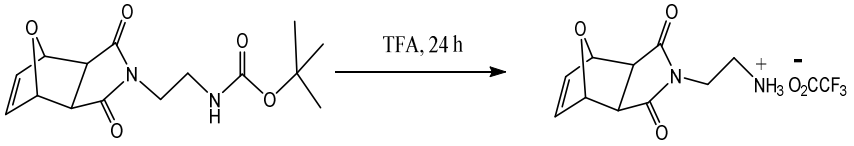


Figure 5.12 BOC deprotection scheme by TFA

Structure of the synthesized compound was elucidated by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy although NH_2 group is not well observed by FT-IR (Appendix Figure B10 and B11). Reaction mechanism scheme is in Figure 5.13.

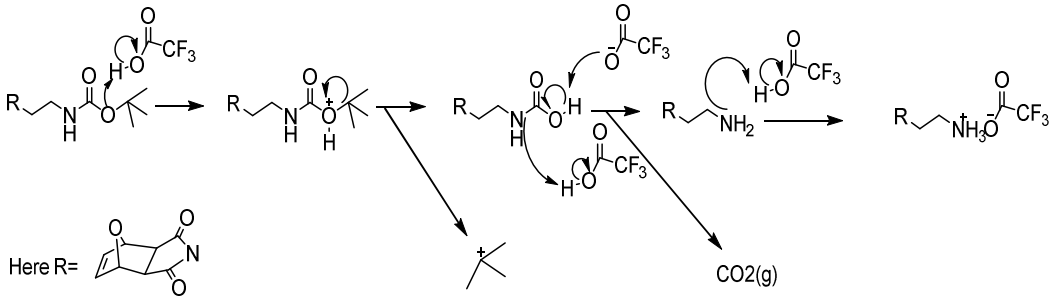


Figure 5.13 Reaction mechanism scheme of BOC deprotection

^{1}H NMR spectra of the BOC surfaced and BOC group removed monomer core is given in Figure 5.14. BOC group removal is confirmed by total disappearance of

trimethyl 12H peaks at 1.5 ppm and other peaks remain unchanged. In ^{13}C NMR for the olefinic carbon and carbonyl carbon peaks were present at 136.4 and 179.4 ppm, respectively. TFA salt carbonyl peak is at 162.7 ppm and 117.8 ppm is for C of CF_3.

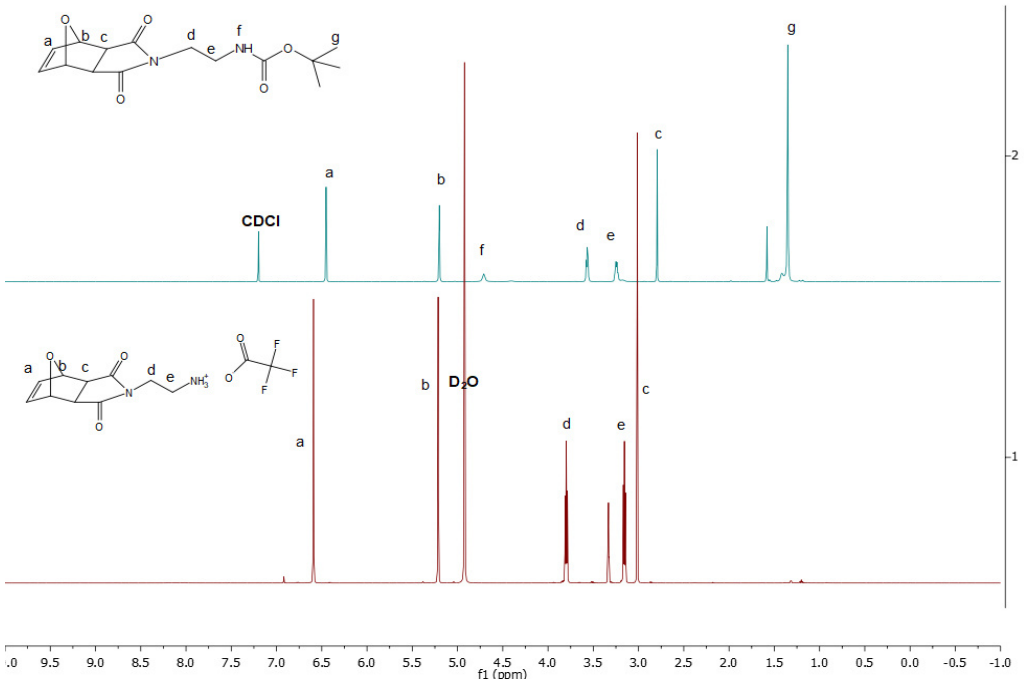


Figure 5.14 ^{1}H NMR comparison of BOC deprotection

5.3.5 Synthesis of Two Carbon Linker 0.5 Generation Dendritic Monomer

Azo-Michael addition reaction tried to proceed according to a published article of a former researcher of our laboratory [173]. After BOC deprotection, compound remains as a TFA salt. So, triethylamine has been used to remove this acidic salt. Excess amount of triethylamine and methyl acrylate were used for this reaction. To purify the product, crude product was dissolved in 4 mL THF and centrifuged three times. Clear THF solution taken out in a round bottom flask from centrifuge tube and evaporate to remove solvent. But still trace amount of triethylamine remained that removed by column chromatography by solvent mixture hexane and ethylacetate (1:4). White crystalline product was found and structure of the compound was elucidated by FT-IR and NMR spectroscopy

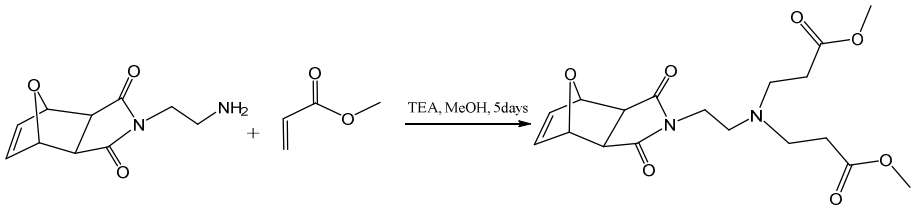


Figure 5.15 Reaction scheme of two carbon linker 0.5G dendritic monomer

FT-IR spectra of the compound seem successful progress of reaction (Figure A8 in appendix). In FT-IR spectra, alkyl C-H stretch spectra are in between 2850 to 2950 cm^{-1}. Spectra at 2985 cm^{-1} and 2829 cm^{-1} are the C-H bond of methane groups. FT-IR spectra of isolated double bond is usually nearly 1640 but no spectra is appeared in this region indicate the double bond of mehylacrylate disappeared by Michael addition reaction with primary amine. C=O stretch of ester and amides shows peak at 1750-1735 cm^{-1} and 1690-1630 cm^{-1}. Spectra at 1725 cm^{-1} come from the ester group of methyl acrylate and 1687 cm^{-1} is the indication of C=O stretch of amide group of the core. C-O-C stretch of ether is in the region 1000-1300 cm^{-1} and C-N bond stretch 1200-1025 cm^{-1}. Two broad spectra in this region indicate these bonds.

In ^{1}H NMR, protons of C=C at 6.51 ppm as singlet, protons adjacent to ether bridge at 5.75 ppm and protons of CHC=O o peaks at 2.9 ppm, surface methoxy protons are at 3.54 ppm, protons of CH_2N at 2.6 and 3.53 ppm and rest protons are at 2.4 and 2.8 ppm respectively (Figure B12). To ensure of the structure of the product ^{13}C NMR also recorded and given in Figure B13. ^{13}C NMR was recorded to see appropriate carbon structure in the backbone of the product. In ^{13}C NMR spectra, 176.2 ppm for C of C=O in oxanorbornene core, 172.6 ppm for C of C=O of ester, 136.6 ppm for C of C=C, 81 ppm for C adjacent to C-O-C, 50 ppm for rest C of oxanorbornene, 37 ppm and 32 ppm for C of two CH_2 groups of 0.5 dendric part (Appendix Figure B13). ^{1}H NMR of NH_2 terminated core and 0.5 generation dendrimer is shown in a single Figure and compared with each other in Figure 5.16.

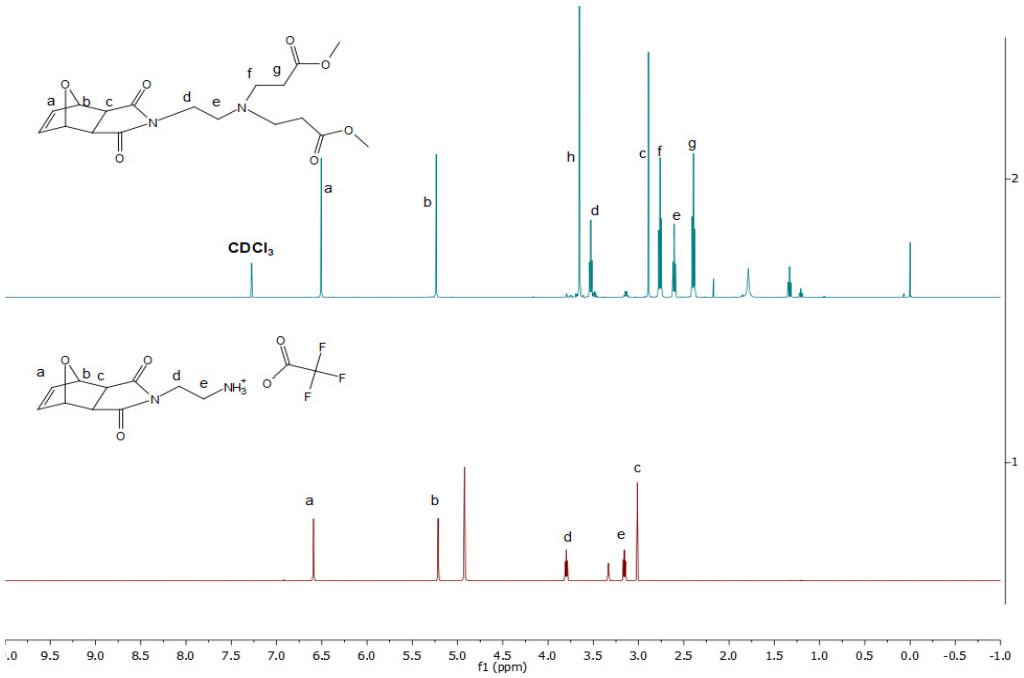


Figure 5.16 ^{1}H NMR comparison of core and 0.5 generation

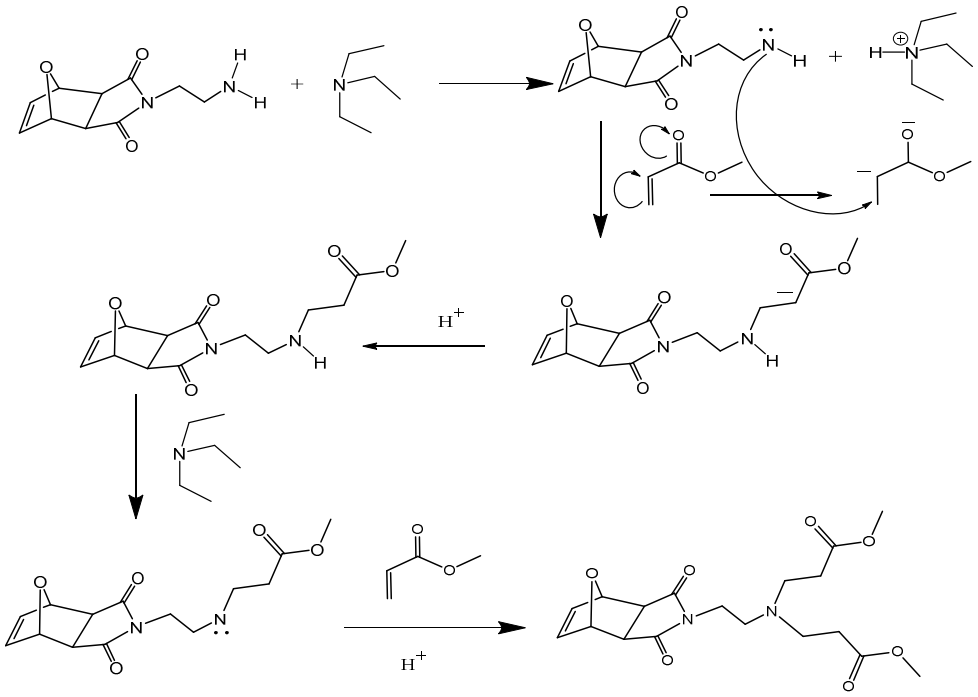


Figure 5.17 Reaction mechanism scheme for synthesis of two carbon linker from furan-meladimide and 0.5 generation dendron [187]

5.3.6 Synthesis of 0.5 Generation Monomer from Ethanolamine

Ethanolamine cored 0.5 generation dendron has been synthesized in methanol solvent at room temperature and found yellowish oily dense liquid product with nearly 95% yield (reaction scheme given in Figure 5.18). Product is monitored by FT-IR and proton NMR in $CDCl_3$. In Figure 5.19 disappearance of NH_2 IR peaks at 3200-3400 cm^{-1} from ethanolamine and formation of an ester peak at 1730 cm^{-1} ensures the successful conversion of 0.5 generation dendron. Proton NMR in $CDCl_3$ methoxy singlet peaks at 3.69 ppm for six hydrogen two ester branch, $-OCH_2$ peaks at 3.59 and NCH_2 peak at 2.59 ppm. Branching NCH_2 peak at 2.80 and protons at $CH_2C{=}O$ at 2.47 ppm and their integration confirm the successful conversion of the dendron.

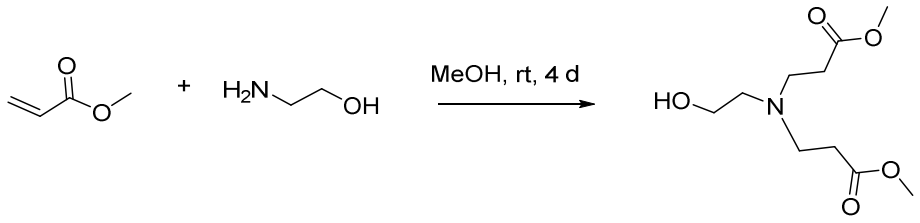


Figure 5.18 Half generation dendron synthesis scheme from ethanolamine

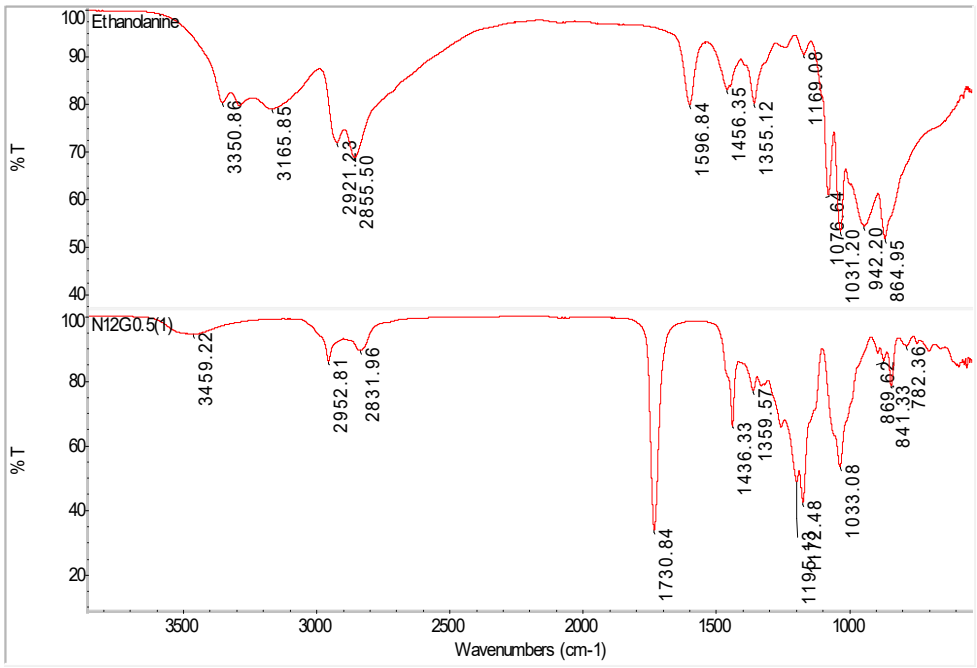


Figure 5.19 FT-IR spectra of 0.5 generation dendron and ethanolamine

5.3.7 Synthesis of Monomer by Combination of Furan Maleimide and Dendron

This combination in between furan-maleimide Diels-Alder adduct and ethanolamine cored 0.5 generation small dendron has done in presence of triphenylphosphine and diisopropylazodicarboxylate (DIAD) (reaction scheme given in Figure 5.20) by using Mitsunobi reaction [194]. Desired product has been separated by using column chromatography against basic alumina with ethyl acetate and hexane (1:6). Percentage of yield is 48%. Reaction mechanism is quite complex.

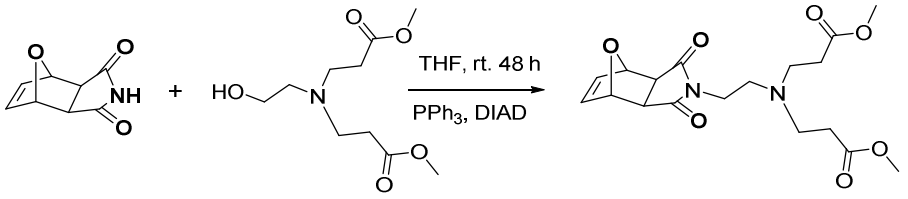


Figure 5.20 Reaction scheme of half generation dendron with furan-maleimide

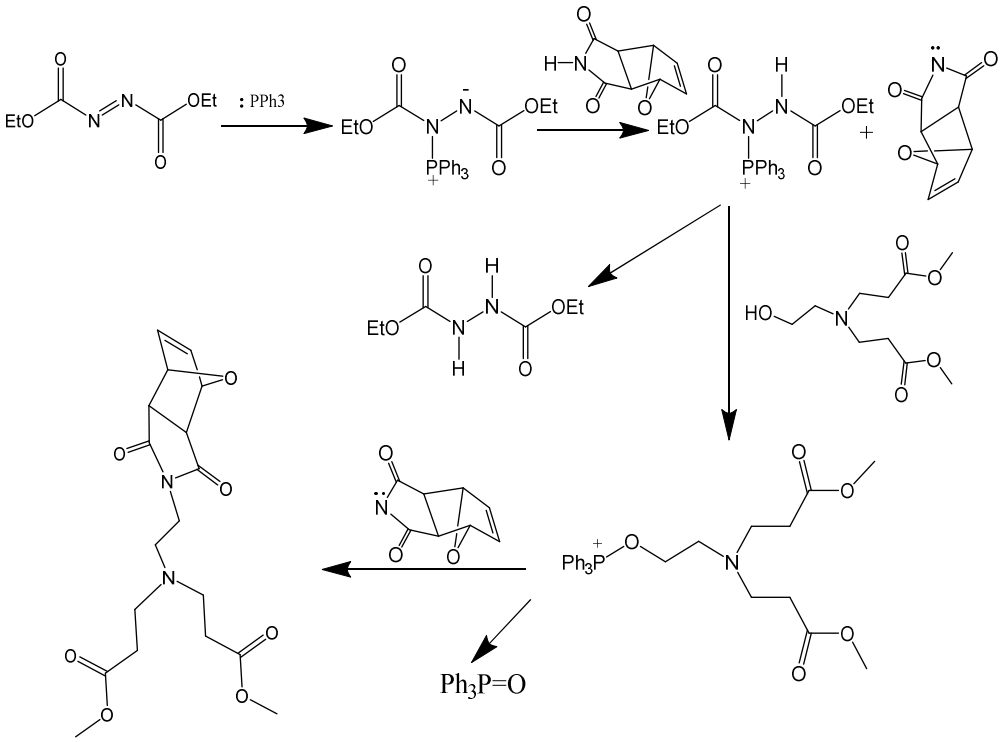


Figure 5.21 Reaction mechanism scheme of Mitsunobi reaction

This compound also synthesized through divergent process by using oxanorbornene and EDA-BOC followed to BOC deprotection and finally methyl acrylate Michael addition. The process is multisteps, low yield and time consuming. Synthesis of the compound by Mitsunobi reaction is comparatively easy with high yield.

The final structure was also explained by ^{1}H NMR, ^{13}CNMR and mass spectroscopy. Within the ^{1}H NMR spectrum of two carbon linker 0.5 generation monomer, the peak at 6.51 ppm represents the signal of the olefin protons, 2.60 ppm corresponds to N–CH_2 protons, 2.39 ppm represents to O=C-CH_2 protons and methoxy protons peaks were found at 3.64 ppm (Figure B12). In ^{13}C NMR spectrum, ^{13}C peaks at 136.6 ppm for C=C of oxanorbornene, 50.6 ppm for CH_3=O of ester, 32.9 ppm for $\underline{C}H_2C$=O and 49.5 ppm for N-CH_2 could be assigned (Appendix Figure B13). Finally, mass spectroscopy analysis of the monomer revealed a mass peak of 381.16 g/mol for the molecular formula ([$C_{18}H_{24}N_2O_7$]+H)+, while the theoretical mass of the monomer is 380.39 g/mol, which confirmed the successful synthesis (Figure B14).

5.3.8 Synthesis of Two Carbon Linker 1.0 Generation Dendritic Monomer

Full generation dendrimer has been synthesized by using EDA through amidation reaction. In this step, excess amount of EDA has been used and serried 4 days at room temperature to get 1.0 generation dendritic monomer (reaction scheme given in Figure 5.22). Unreacted EDA was removed through rotary evaporator at 60 °C along with high vacuum. Removal of all unreacted EDA is not possible by simply rotary evaporation even at high vacuum. Azeotropic mixture of toluene-methanol or n-butanol and EDA of lower boiling point has made to remove EDA but unable to remove completely. So, membrane filtration method is used for good purification for the small portion of incomplete dendrimers and excessof EDA. We used 100 Da MWCO membranes to remove excess EDA form the product.

IR spectra of the synthesized product have recorded and seen the ester group successfully converted to amide group, amide-I peak 1548 and amide-II is in 1643

cm^{-1} C=O at 1770, HNC=O at 1685 cm^{-1} one free amine spectra of NH_2 is 3200-3400 cm^{-1} and sp^3 hybridized C-H bond of CH_2 groups is in 2864 cm^{-1} and 2928 cm^{-1}.

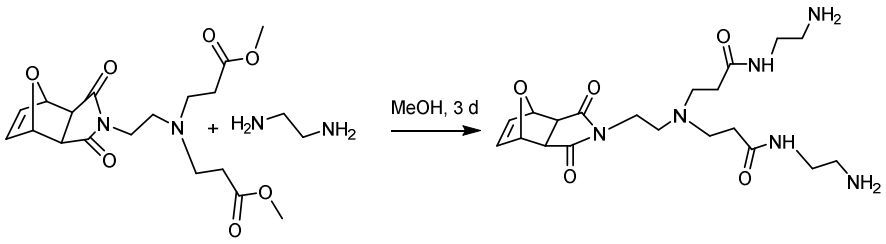


Figure 5.22 Synthesis scheme of two carbon linker 1.0G dendritic monomer

To explain the structure of the product ^1H NMR also recorded. Removal of methoxy peak at 3.60 ppm from 0.5 generation dendron and appearance of amide peak at 8.32 ppm confirmed the successful conversion of the product. Olefinic proton at 6.55 ppm and proton attached with bridge oxygen at 5.11 ppm support the presence of oxanorbornene attached with the dendritic group. An unexpected peak at broad peak at 4.5 ppm appeared for water in methanol that was used for ultrafiltration but unfortunately, some of them remain after evaporation (Appendix Figure B16)

5.3.9 Synthesis of Two Carbon Linker 1.5 Generation Dendritic Monomer

1.5 generation dendrimer has been synthesized by using methyl acrylate with NH_2 terminated 1.0 generation dendritic monomer at room temperature through Michael addition reaction**.** Excess amount of unreacted methyl acrylate was removed by rotary evaporator. Reaction is shown in scheme 5.6 and product is elucidated by using FT-IR, ^1H NMR spectroscopy.

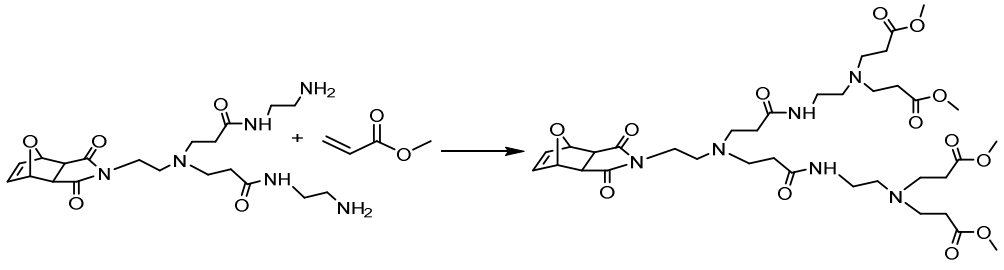


Figure 5.23 Synthesis scheme of two carbon linker 1.5G dendritic monomer

IR spectra of isolated double bond is usually nearly 1640 cm^{-1} but no spectra is appeared in this region indicate the double bond of methyl acrylate disappeared by Michael addition reaction with primary amine. C=O stretch of ester and amides shows peak at 1750-1735 cm^{-1} and 1690-1630 cm^{-1}. Spectra at 1725 cm^{-1} comes from the ester group of methyl acrylate and 1687 cm^{-1} is the indication of C=O stretch of amide group of the core. C-O-C stretch of ether is in the region 1000-1300 cm^{-1} and C-N bond stretch 1200-1025 cm^{-1}. Two broad spectra in this region indicate these bonds. In ^{1}H NMR spectra appearance of methoxy peak at 3.62 ppm and O=CNH peak at 8.71 ppm represent the 1.5 generation dendritic monomer. Oxanorbornene olefinic peak at 6.55 ppm and linker peaks similar as before and dendritic peaks indicate the successful conversion of the monomer (Appendix B17).

Table 5.2 Summary of two carbon linker monomers

Experiment No	Linker in between	Generation	Molecular weight	Number of terminated ester	Number of terminated amine	Number of tertiary amines
1	2	0	208.21	-	1	-
2	2	0.5	380.39	2	-	1
3	2	1.0	436.51	-	2	1
4	2	1.5	780.88	4	-	3

5.4 Synthesis of Different Generation Monomer with Four Carbon Linker Inbetween Oxanorbornene and Dendron

To get amine terminated oxanorbornene derivative 1,4-diamonobutane was used and after then methyl acrylate and EDA has been used one after another repeatedly for dendritic monomer synthesis

5.4.1 Synthesis of Four Carbon Linker Core by Diels-Alder Adduct and 1,4-Diaminobutane

Oxanorbornene was reacted to 1,4-diaminobutane to get four carbon linker NH_2 terminated core (synthesis scheme is in Figure 5.24). FT-IR spectra of the synthesized product have been recorded and seen the anhydride group successfully converted to amide group, C=O at 1691 cm^{-1}, HNC=O at 1401 cm^{-1}, terminal free amine spectra of 1,4-diaminobutane is at 3452 cm^{-1}, 3375 cm^{-1} and sp^3 hybridized C-H bond of CH_2 groups are in 2864 cm^{-1} and 2939 cm^{-1} (Appendix Figure A11).

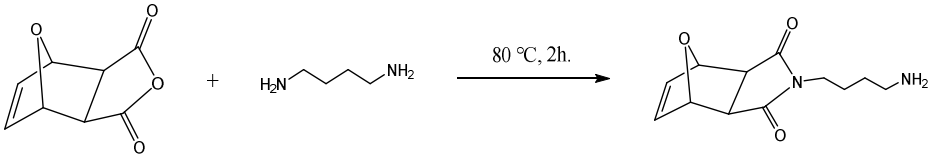


Figure 5.24 Synthesis scheme of four carbon liker monomer core

The characteristic ^1H NMR peak of the compound was observed at 6.54 ppm for C=C of oxanorbornene and 3.51-1.52 ppm for alkyl chain. In the ^{13}C NMR spectrum, representing peak at 136.56 ppm for the olefinic carbon, 180.61 ppm for carbonyl and 50-30 ppm alkyl carbon. Interpretations of all proton peaks of the product are shown in appendix part as Figure B18 and ^{13}C NMR peak as B19.

5.4.2 Synthesis of Four Carbon Linker 0.5 Generation Dendritic Monomer

Methyl acrylate was added to the amine-terminated four carbon linker core in order to get 0.5 generation dendritic monomer. The reaction proceed during 48 h in methanol at ambient temperature. Excess amount of unreacted methyl acrylate was removed by using rotary evaporator.

In FT-IR spectra, alkyl C-H stretch spectra are in between 2850 to 2950 cm^{-1}. Spectrum at 2951 cm^{-1} and 2827 cm^{-1} are the C-H bond of methane groups. IR spectra of isolated double bond is usually nearly 1640 cm^{-1} but no spectra is appeared in this region indicate the double bond of methyl acrylate disappeared by

Michael addition reaction with terminated NH_2 of the core. C=O stretch of ester and amides shows peak at 1750-1735 cm^{-1} and 1690-1630 cm^{-1}. Spectra at 1733 come from the ester group of methyl acrylate and 1693 cm^{-1} is the indication of C=O stretch of amide group of the core. C-O-C stretch of ether is in the region 1300-1100 cm^{-1} and C-N bond Stretch 1200-1025 cm^{-1}. Two broad spectra in this region indicate these bonds (Appendix Figure A12). The final structure was also elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy. Within the ^{1}H NMR spectrum of monomer, the peak at 6.51 ppm represents the signal of the olefin protons, 2.60 ppm corresponds to N–CH_2 protons, 2.39 ppm represents to O=C-CH_2 protons and methoxy protons peaks were observed at 3.67 ppm (Appendix Figure B20). In the ^{13}C NMR spectrum, ^{13}C peaks at 176.36 for C=O and 136.6 ppm for C=C of oxanorbornene. Spectra at 172.98 for C=O of ester terminal, spectra 5156.6 ppm for CH_3=O of ester, spectra at 32.44 ppm for $\underline{C}H_2C$=O and 49.19 ppm for N-CH_2could be assigned (Appendix Figure B21).

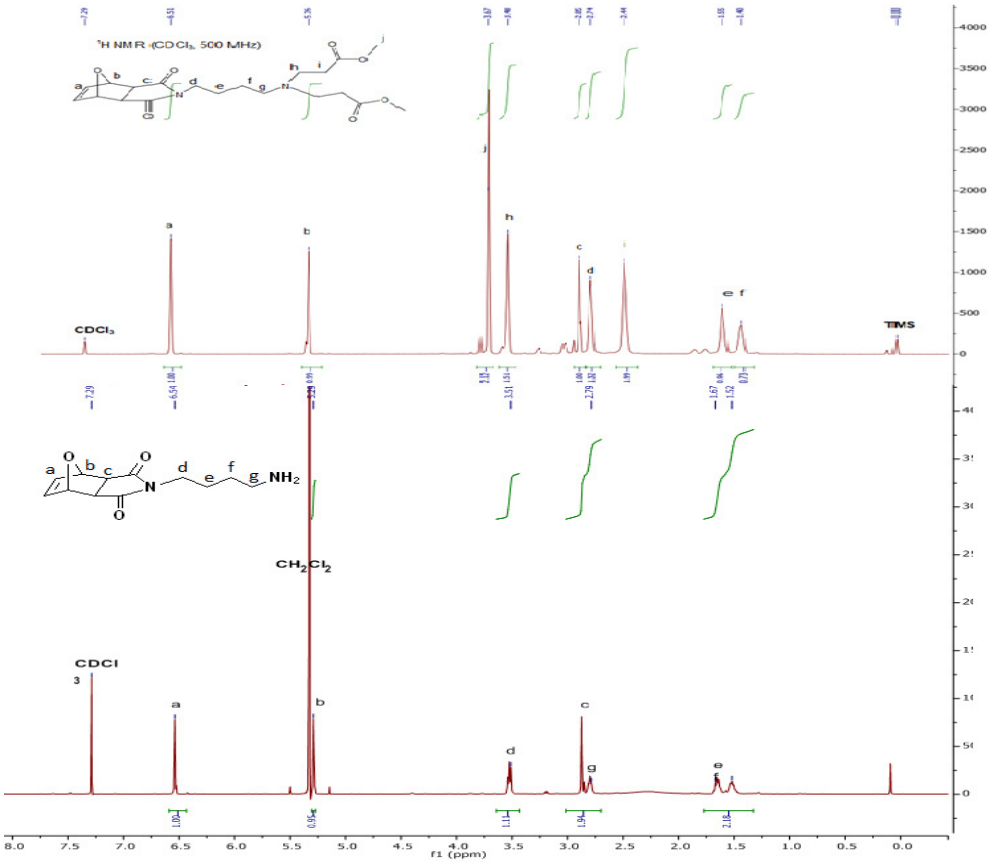


Figure 5.25 ^{1}H NMR comparison of four carbon linker core and 0.5 generation

5.4.3 Synthesis of Four Carbon Linker 1.0 Generation Dendritic Monomer

Full generation dendrimer has been synthesized by using EDA through amidation reaction. In this step excess amount of EDA has been used and serried 4 days at room temperature to get 1.0 generation dendritic monomer (reaction scheme is in Figure 5.26). Unreacted EDA was removed through rotary evaporator at 60 °C at high vacuum. Removal of all unreacted EDA was not possible by simply rotary evaporation even at high vacuum. Azeotropic mixture of toluene-methanol or n-butanol and EDA of lower boiling point has made to remove EDA but unable to remove completely. So, membrane filtration method is used for good purification for the small portion of incomplete dendrimers and excess of EDA. MWCO 100 Da membrane was used to remove excess EDA form the product.

Conversion of desired product was ensured by FT-IR spectra of the synthesized product has recorded (Appendix Figure A13) and seen the ester group successfully converted to amide group, amide-1 peak 1445 cm^{-1} and amide II is in 1603 cm^{-1}, C=O at 1701, one free amine spectra of NH_2 terminated full generation dendritic compound is at 3352 cm^{-1}, 3292 cm^{-1} and sp^3 hybridized C-H bond of CH_2 groups is in 2935 cm^{-1} and 2818 cm^{-1}. Products' chemical structure was elucidated by ^1H NMR spectroscopy. Within the ^1H NMR spectrum of monomer, the peak at 7.86 ppm represents the corresponding amide peak, spectra at 6.44 ppm represents the signal for protons attached to C=C, proton at 3.18 ppm represents corresponding proton adjacent to amide and tertiary nitrogen atoms, spectra at 1.33 and 1.26 ppm corresponds other alkyl protons of the linker (Appendix Figure B22).

5.4.4 Synthesis of Four Carbon Linker 1.5 Generation Dendritic Monomer

Four carbon linker 1.5 generation dendritic monomer has been synthesized by using methyl acrylate with 1.0 generation four carbon linker dendritic compound in methanol solvent at room temperature (reaction scheme is given Figure 5.26). Excess amount of methyl acrylate and methanol has been removed by using rotary evaporator at 60 °C under high vacuum. Structure of the product was confirmed by FT-IR and ^1H NMR spectroscopy.

In FT-IR spectra, spectrum at 2952 cm^{-1} and 2859 cm^{-1} represents the C-H bond alkyl chain. C=O stretch of ester and amides shows peak at 1733 cm^{-1} and 1692 cm^{-1} respectively. Spectra at 1172 cm^{-1} comes from the ester group of methyl acrylate and 1693 cm^{-1} is the indication of C=O stretch of amide group of the core and C-O-C stretch of ether shows the spectra at 1172 cm^{-1} (Appendix Figure A14). The final structure was also established by ^{1}H NMR spectroscopy. In proton NMR of four carbon linker 1.5 generation dendritic monomer, spectra at 7.67 ppm is representing proton of amide, singlet at 6.55 ppm is representing alkene proton, singlet at 5.12 ppm and 2.91 ppm is representing other protons of oxanorbornene ring. Terminal methoxy group protons show singlet at 3.58 ppm, protons of dendritic branch show spectra at 3.33-2.18 ppm, and four carbon linker protons spectra at 1.40-1.18 ppm. (Appendix Figure B23).

5.4.5 Synthesis of Four Carbon Linker 2.0 Generation Dendritic Monomer

Oxanorbornene cored four carbon linker 2.0 generation dendritic compound was synthesized by using EDA through amidation reaction with 1.5 generation dendritic monomer in methanol solvent at room temperature (reaction scheme is given in Figure 5.26). Unreacted EDA was removed through rotary evaporator at 60 °C under high vacuum. All unreacted EDA can not remove easily and finally used azeotropic mixture of toluene-methanol to remove trace amount of remaining EDA. To ensure all EDA removal membrane dialysis has done for 48 hours through liquid phase polymer retention (LPR) techniques.

Conversion of desired product was ensured by FT-IR and ^{1}H NMR spectroscopy. In IR spectra, 3320 cm^{-1} and 3288 cm^{-1} representing terminal NH_2 group, 2939 cm^{-1} representing –C-H of alkyl chain. For amide group, amide-1 peak 1632 and amide II is in 1556 cm^{-1} (Appendix Figure A15). Within the ^{1}H NMR spectrum of 1.0 dendritic compound, the peak at 7.92 ppm represents the signal of amide –NH proton, the peak at 6.55 ppm represents the signal of the olefin protons, 5.12 ppm represents the signal of CH-O of oxanorbornene, 3.09-2.43 ppm corresponds to dendritic branch protons and 1.34-1.21 ppm for the protons of the four carbon linker (Appendix Figure B24).

5.4.6 Synthesis of Four Carbon Linker 2.5 Generation Dendritic Monomer

To synthesize four carbon linker 2.5 generation dendritic monomer, methyl acrylate was added to 2.0 generation four carbon linker dendritic compound in methanol solvent and stirred 5 days at room temperature (reaction scheme is given in Figure 5.26). Unreacted methyl acrylate and methanol has been removed by using rotary evaporator at 60 °C under high vacuum. Structure of the product was confirmed by FT-IR and ^{1}H NMR spectroscopy.

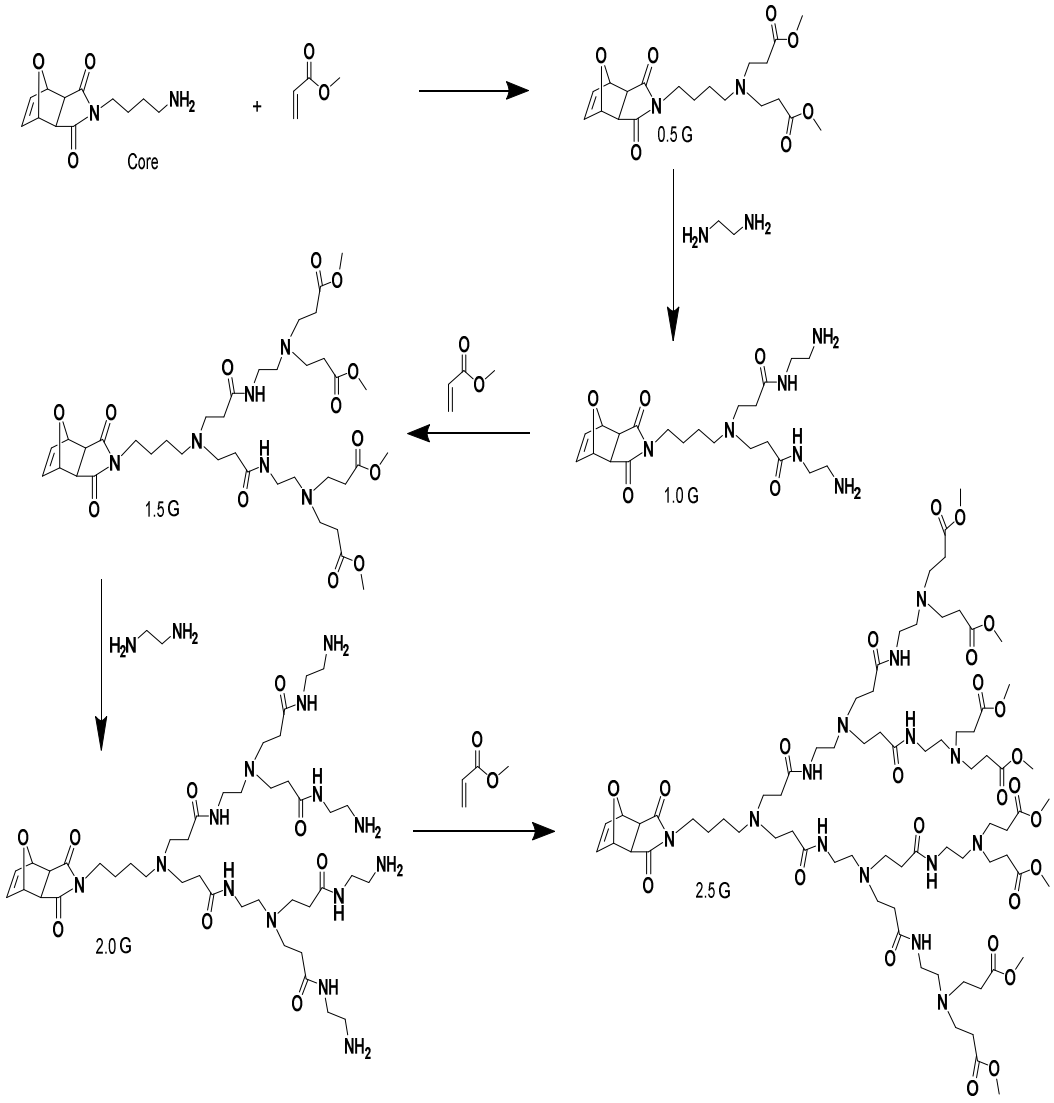


Figure 5.26 Synthesis scheme of four carbon linker 0.5 - 2.5G dendritic monomer

In FT-IR spectra, alkyl C-H stretch spectra is in between 2850 to 2950 cm^{-1}. Spectrum 2984 and 2829 are the C-H bond of methane groups. IR spectra of isolated double bond is usually nearly 1640 but no spectra appears in this region indicate the double bond of methyl acrylate disappeared by Michael addition reaction with primary amine. C=O stretch of ester and amides shows peak at 1750-1735 and 1690-1630. Spectra at 1725 come from the ester group of methyl acrylate and 1687 is the indication of C=O stretch of amide group of the core. C-O-C stretch of ether is in the region 1000-1300 and C-N bond stretch 1200-1025. Two broad spectra in this region indicate these bonds.

To confirm our product NMR spectra was recorded and given in appendix part. Proton NMR for product bands at 6.5 (2 H, multiplet), 5.3 (2 H, multiplet), 3.0 (2 H, singlet) indicates the oxanorbornene core and surface methoxy groups at 3.8. As 2.5 generation is highly branched it is difficult to elucidate peaks for each proton individually (Appendix Figure B.25).

Table 5.3 Summary of four carbon linker monomers

Experiment No	Linker in between	Generation	Molecular weight (g/mol)	Number of terminated ester	Number of terminated amine	Number of tertiary amines
1	4	0	236.26	-	1	-
2	4	0.5	408.44	2	-	1
3	4	1.0	464.56	-	2	1
4	4	1.5	808.92	4	-	3
5	4	2.0	921.14	-	4	3
5	4	2.5	1609.86	8	-	7

5.5 Synthesis of Different Generation Monomer with Six Carbon Linker Inbetween Oxanorbornene and Dendritic Part

To get amine terminated oxanorbornene derivative 1,6-diaminohexane was used and after then methyl acrylate and EDA has been used one after simultaneously to get different generation dendritic monomer.

5.5.1 Synthesis of Six Carbon Linker Monomer Core

To synthesize six carbon linker monomer core, 1,6-diaminohexane treated with oxanorbornene at 80 °C for 2 h. Percentage of yield of the product was found 51.29% as white powder. FT-IR spectra of the synthesized product have recorded and seen the anhydride group successfully converted to amide group one free amine spectra of hexamethylene diamine is in 3337 cm^{-1} and 3307 cm^{-1} and sp^3 hybridized C-H bond of CH_2 groups is in 2929 and 2857 cm^{-1} (Appendix Figure A17).

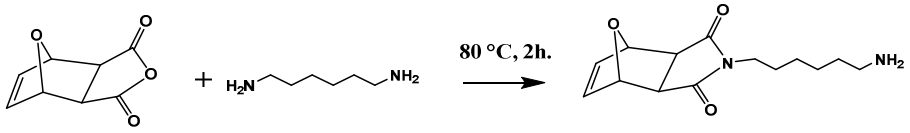


Figure 5.27 Synthesis scheme of six carbon linker monomer core

To confirm the structure of the product proton NMR has been recorded in $CDCl_3$ solvent that has shown in appendix as Figure B26 with accurate indication of each proton. The representative peaks were found at 6.53 ppm in the ^1H NMR spectrum and at 136.5 ppm within the ^{13}C NMR spectrum for the olefinic hydrogen of the product. The carbon signals of the alkyl linker were observed at 3.50-1.30 ppm and 47.4-26.0 ppm, respectively (Appendix Figure B26, B27)

5.5.2 Synthesis of Six Carbon Linker 0.5 Generation Dendritic Monomer

Six carbon linker 0.5 generation dendritic monomer was synthesized by the reaction of NH_2 terminated core compound and methyl acrylate through Michael

addition. Synthesis of this dendritic monomer procced in methanol at ambient temperature for 3 days. Reaction scheme is given in Figure 5.30.

To see the conversion FT-IR spectra have been recorded. Alkyl C-H stretch spectra are in between 2850 to 2950 cm^{-1}. Spectrum at 2934 cm^{-1} and 2857 cm^{-1} is the C-H bond of methane groups. IR spectra of isolated double bond is usually nearly 1640 cm^{-1} but no spectra have appeared in this region indicate the double bond of methyl acrylate disappeared by Michael addition reaction with primary amine. C=O stretch of ester and amides shows peak at 1750-1735 $cm^{-1}$and 1690-1630 cm^{-1} respectively. Spectra at 1733 cm^{-1} come from the ester group of methyl acrylate and 1693 cm^{-1} is the indication of C=O stretch of amide group of the core. C-O-C stretch of ether is in the region 1300-1000 cm^{-1} and C-N bond stretch 1200-1025 cm^{-1}. Two broad spectra in this region indicate these bonds (Appendix Figure A18).

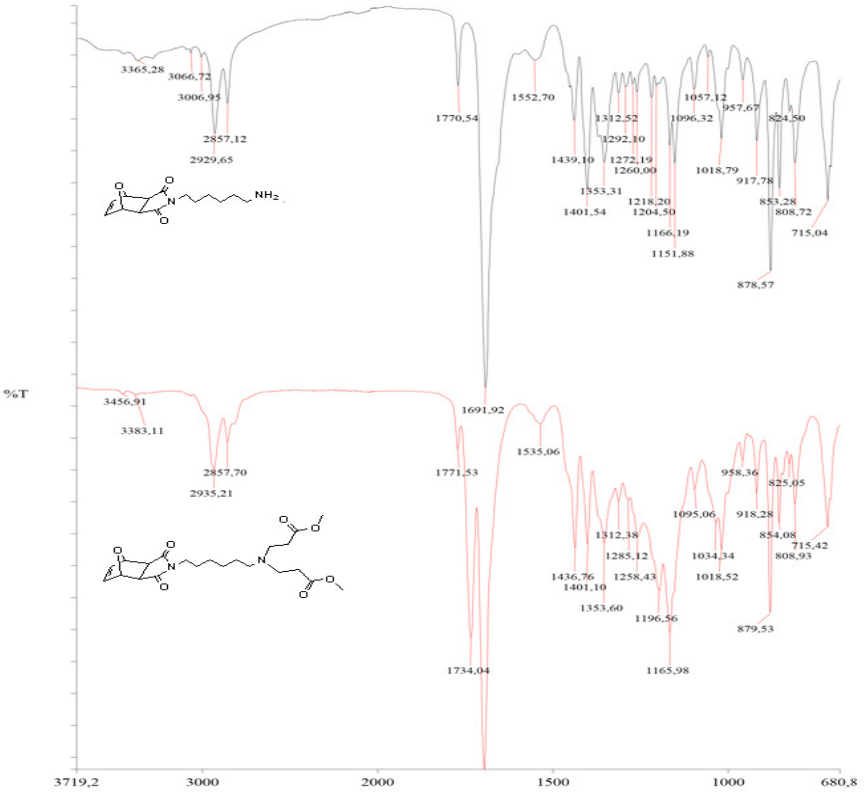


Figure 5.28 FT-IR of six carbon linker core and 0.5 generation dendritic monomer

Conversion of the product by comparing core FT-IR spectra is shown in Figure 5.28. Structure of the monomer was established by ^{1}H and ^{13}C NMR spectroscopy and mass spectrum analysis. In ^{1}H NMR spectrum of six carbon linker dendritic monomer, the peak at 6.53 represents the signal for the olefin protons, 2.77 ppm corresponds to the N–CH_2 protons, 2.45 ppm represents to O=C-CH_2 protons and methoxy protons peaks were observed at 3.69 ppm (Appendix Figure B28).

In the ^{13}C NMR spectrum, the ^{13}C signals at 136.5 ppm belonged to the C=C of oxanorbornene ring, 51.6 ppm to $\underline{C}H_3C=O$ of the ester moiety, 32.2 ppm to CH_2-N and 53.4 ppm to $\underline{C}H_2C=O$ (Appendix Figure B29). Mass analysis of the monomer revealed mass signal of observed as 437.22 g/mol for the molecular formula ($[C_{22}H_{32}N_2O_7]+H)+$, while theoretical molar mass of the monomer was determined to 436.50 g/mol (Appendix Figure B30).

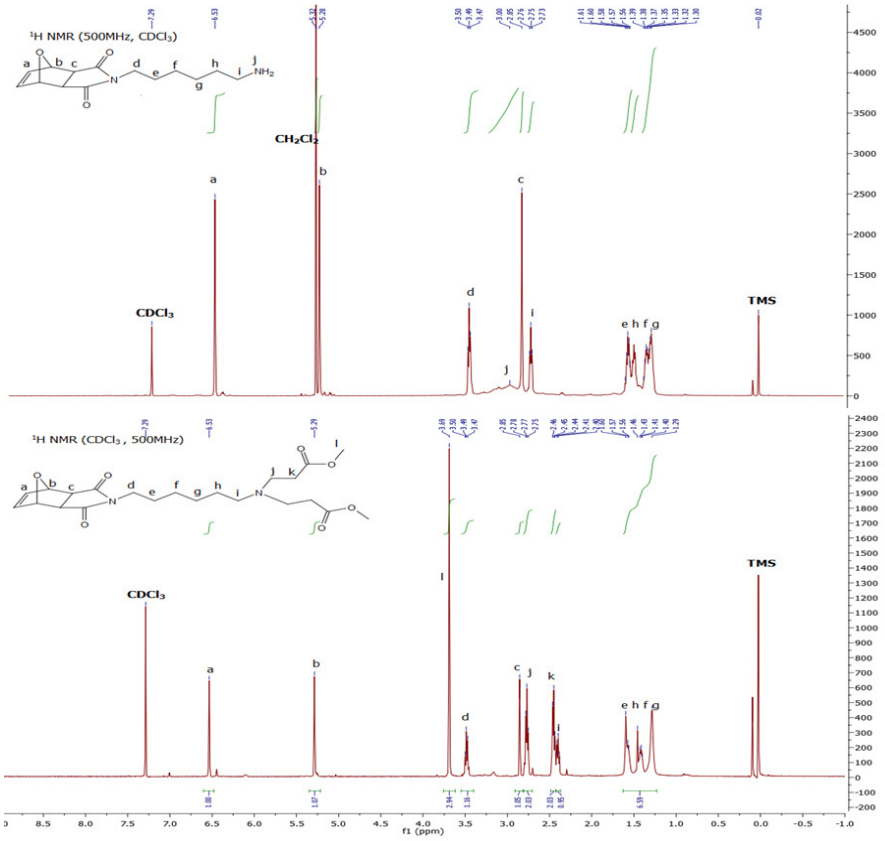


Figure 5.29 ^{1}H NMR comparison of core and 0.5 generation

5.5.3 Synthesis of Six Carbon Linker 1.0 Generation Dendritic Monomer

Six carbon linker 1.0 generation dendritic monomer has been synthesized from 0.5 generation monomer with EDA in methanol solvent (reaction scheme is given in Figure 5.30). Excess methanol and EDA were removed by rotary evaporator with high vacuum at 50 °C. Though methanol was removed by rotary evaporator but trace amount of EDA still remain in the product. Remaining EDA was removed by adding 50 mL n-butanol, azeotropic mixture was removed by rotary evaporator at elevated temperature and high vacuum. This process has done several times for removal of all ethylene diamine. Conversion of desired product was monitored by FT-IR. Very few ethylenediamine might have in the reaction mixture, these will remove by liquid phase polymer retention (LPR) process in final step.

Structure of the product was established by FT-IR and ^{1}H NMR spectroscopy. In FT-IR spectra, 3362 and 3292 cm^{-1} represent corresponding terminal primary amine, 2935 cm^{-1}, 2818 cm^{-1} represent corresponding C-H peak. Amide I and amide II shows spectra at 1603 and 1495 cm^{-1} (Appendix Figure A19)

5.5.4 Synthesis of Six Carbon Linker 1.5 Generation dendritic Monomer

Methyl acrylate was added to methanol to prepare clear solution which was added to a homogenous solution of amine terminated 1.0 dendric monomer in methanol (synthesis scheme is given in Figure 5.30). The final mixture was kept under magnetic stirring at ambient temperature for 48 hours and excess reagents and methanol were removed under vacuum of 300 mBar at 45 °C bath temperature and finally synthesized 1.5 generation dendrimer products was yellowish gel.

Synthesized product was monitored by FT-IR and ^{1}H NMR spectroscopy. Spectra at 2952 $cm^{-1}$and 2859 $cm^{-1}$is for –C-H bond, 1733 $cm^{-1}$is for terminates C=O of ester group, 1700 cm^{-1} is for N-C=O $cm^{-1}$of amide group and 1172 cm^{-1} for ether of the compound (Appendix Figure A20). In ^{1}H NMR spectrum of six carbon linker 1.5 generation dendritic monomer, the peak at 6.55 ppm represents the signal for the olefin protons, and 5.12 ppm and 2.91 ppm is another protons of oxanorbornene ring. 3.58 ppm corresponds to protons of methoxy group, 3.33-2.18 ppm

corresponds to dendritic branch protons, and 1.40-1.81 ppm represents the alkyl chain linker (Appendix Figure B32).

5.5.5 Synthesis of Six Carbon Linker 2.0 Generation Dendritic Monomer

2.0 generation dendrimer has been synthesized from 1.5 generation dendritic monomer by reacting with EDA through amidation reaction in methanol solvent at room temperature (reaction scheme is specified in Figure 5.30). Excess amount of unreacted EDA was removed through rotary evaporator at 60 °C under high vacuum. Here, liquid phase polymer retention (LPR) technique was used as significant purification for the small portion of incomplete dendrimers and excess of EDA. MWCO 200-500 Da membrane was used to remove excess EDA form the product.

Product structure was confirmed by FT-IR and ^{1}H NMR spectroscopy. In FT-IR spectra, terminal NH_2 show spectra at 3326 cm^{-1} and 3284 cm^{-1}, -C-H spectra at 2937 $cm^{-1}$and 2818 cm^{-1}, amide bonds show spectra at 1632 cm^{-1} and 1552 cm^{-1}; ether shows spectra at 1172 cm^{-1} (Appendix Figure A21). In ^{1}H NMR spectrum of six carbon linker 2.0 generation dendritic monomer, the peak at 7.90 ppm represents the signal for amide proton, 6.55 ppm represents the signal for the olefin protons, and 5.12 ppm and 2.91 ppm is other protons of oxanorbornene ring. 3.58 ppm corresponds to protons of methoxy group, 3.65-2.21 ppm corresponds to dendritic branch protons, and 1.34-1.21 ppm represents the alkyl chain linker (Appendix Figure B33).

5.5.6 Synthesis of Six Carbon Linker 2.5 Generation Dendritic Monomer

Methyl acrylate was mixed in methanol and transferred to clear solution of amine terminated six carbon linker 2.0 generation dendritic monomer in methanol. Reaction scheme is shown in Figure 5.30. Unreacted methyl acrylate and methanol were dried through rotary evaporator by of 300 mBar at 45 °C bath temperature and finally synthesized 2.5 generation dendrimer products were yellowish gel.

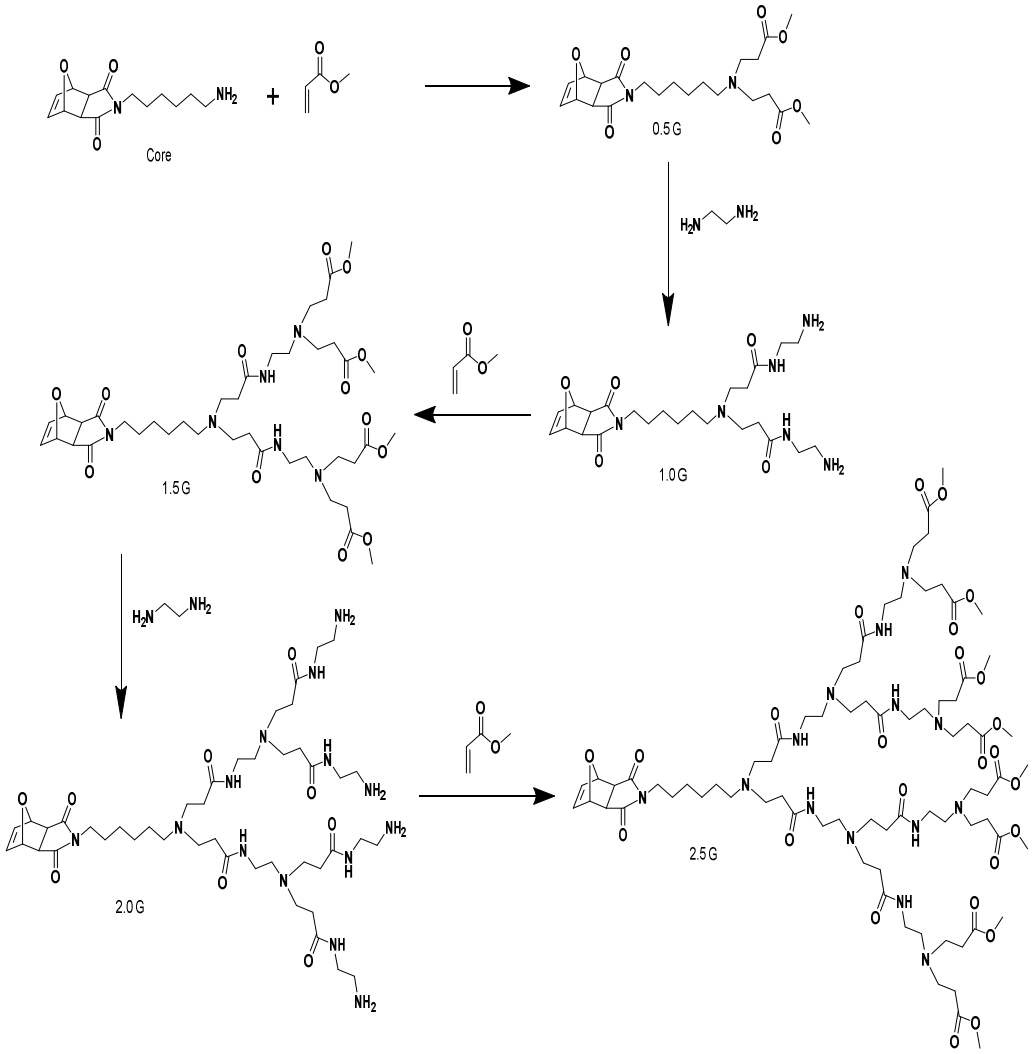


Figure 5.30 Synthesis scheme of six carbon linker 0.5- 2.5G dendritic monomer

Conversion of the product is monitored by FT-IR and ^{1}H NMR. After conversion 2.5 generation, ester peak appears at 1731 cm^{-1} and amide peaks are at 1643 cm^{-1} and 1543 cm^{-1}. Spectrum 2951 and 2828 cm^{-1} are the C-H bond of methane groups (Appendix Figure A22). Comparison of FT-IR of 2.0 generation and 2.5 generation dendritic monomer is shown in Figure 5.31.

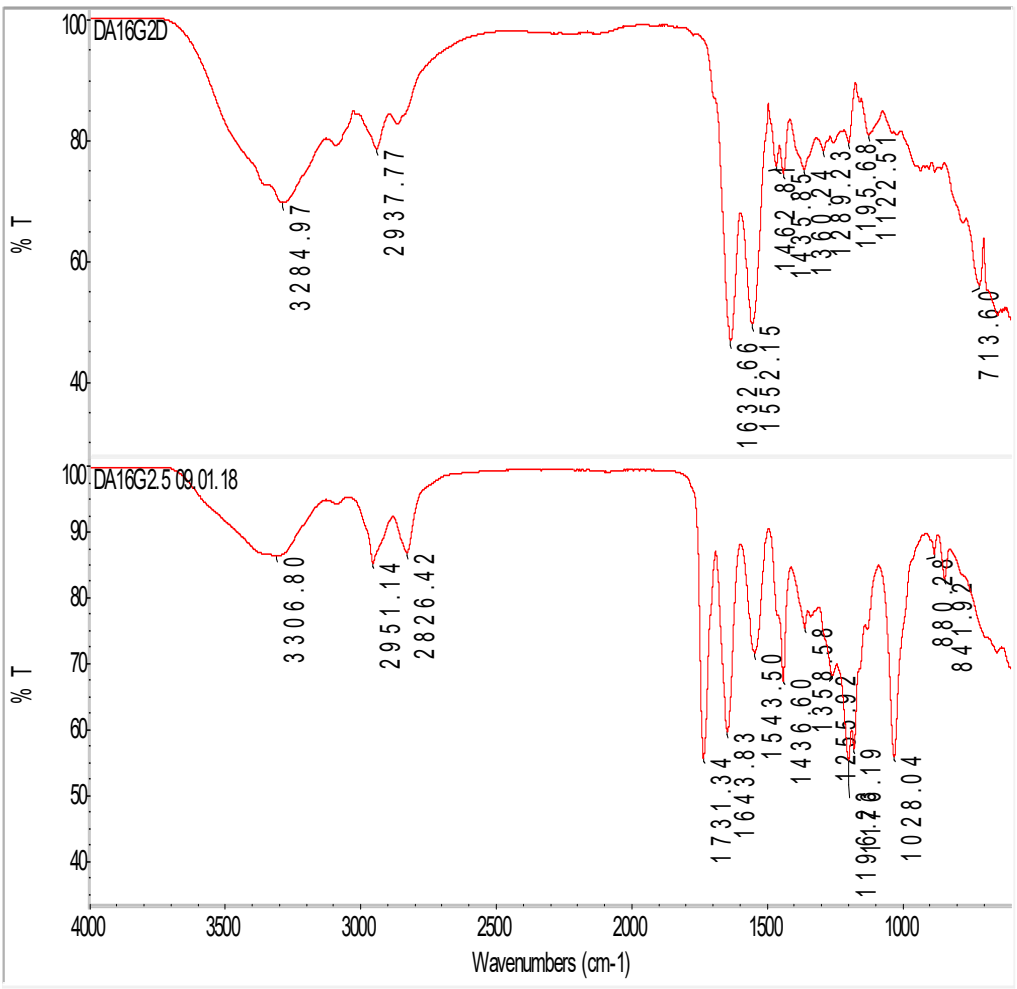


Figure 5.31 FT-IR of six carbon linker 2.5 generation dendritic monomer

To confirm our product ^{1}H NMR spectra were recorded and elucidated corresponding peaks for each proton. In ^{1}H NMR spectrum of 2.5 generation dendritic monomer, the peak at 7.93 ppm represents the signal for amide proton, 6.55 ppm represents the signal for the olefin protons, 3.61 ppm corresponds to protons of methoxy group, 2.75-2.22 ppm corresponds to dendritic branch protons, and 1.38-1.23 ppm represent corresponding the six carbon linker alkyl chain linker protons (Appendix Figure B34).

To summarize, comparison of FT-IR of 0.5, 1.0, 1.5, 2.0, 2.5 generation dendritic monomer is shown in Figure 5.32.

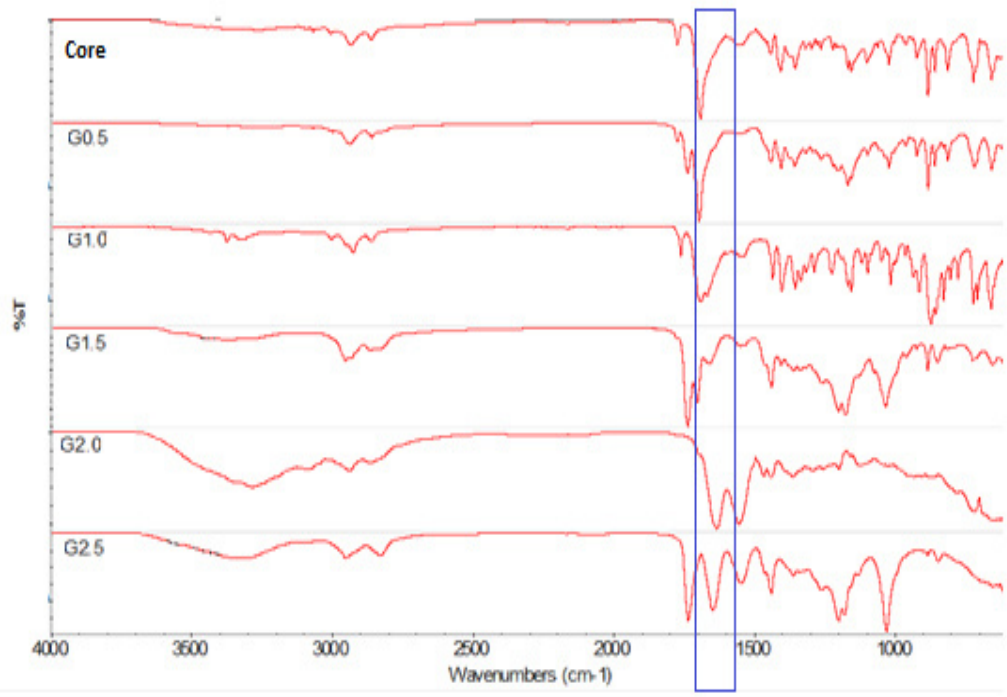


Figure 5.32 FT-IR of six carbon linker dendritic 0.5-2.5G dendritic monomers

Table 5.4 Summary of six carbon linker monomers

Experiment No	Linker in between	Generation	Molecular weight (g/mol)	Number of terminated ester	Number of terminated amine	Number of tertiary amines
1	6	0	264.32	-	1	-
2	6	0.5	436.50	2	-	1
3	6	1.0	492.61	-	2	1
4	6	1.5	836.97	4	-	3
5	6	2.0	949.19	-	4	3
6	6	2.5	1637.91	8	-	7

5.6 Synthesis of Pyridinium Based Monomer

Pyridiniumn based oxanorbornene monomer has been synthesized by two steps. Initially oxanorbornene was combined with aminomethylpyridine to get pyridine based oxanorbornene. After then the product reacted with 1-bromohexane by S_N2 reaction for quaternization to get hexyl-pyridinium based monomer [60].

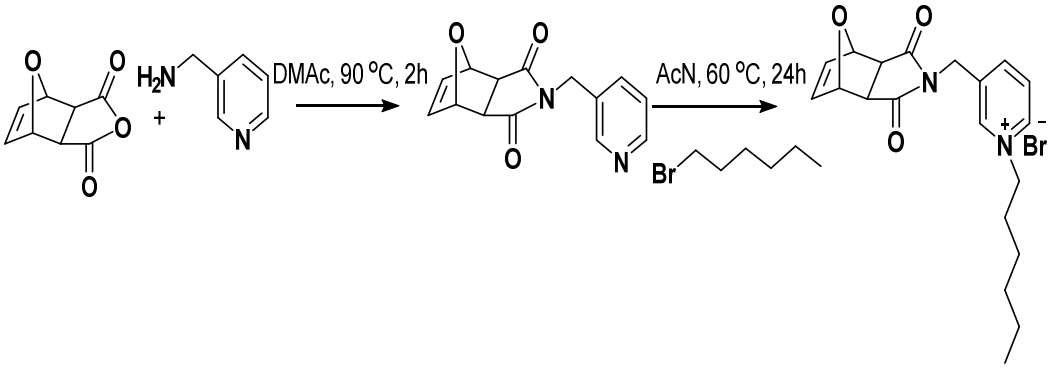


Figure 5.33 Pyridinium based oxanorbornene

In the ^1H NMR spectrum of the pyridine based product given as Appendix Figure B35, the signals correspond to the olefin hydrogen at 6.53 ppm, the peak of CH_2-N was observed at 4.65 ppm, and peaks belonging to the pyridine ring were observed in the range of 7.45-8.66 ppm. ^{13}C NMR peaks were observed in the range of 123–150 ppm corresponding to the pyridine ring of pyridine based oxanorbornene compound given in the corresponding spectrum in appendix Figure B36.

Pyridine based oxanorbornene compound was treated with bromohexane for the preparation of corresponding hexyl-pyridinium salt-based monomer (reaction scheme is given in Figure 5.33) [60]. The structure of **M3** was established via ^1H NMR where pyridine moieties could be assigned to the signals at 8.00-9.50 ppm, the olefin hydrogen signals at 6.53 ppm, and the hexyl peaks at 0.50-2.00 ppm (appendix Figure B37). The carbon signals for **M3** determined via ^{13}C NMR spectroscopy could be observed in the range of between 123–150 ppm corresponding to the pyridine ring, while the CH_3 group of the hexyl unit was observed at 8.7 ppm (Appendix Figure B38).

5.7 Homopolymer Synthesis

5.7.1 Synthesis of 3rd Generation Grubbs Catalyst

Grubbs third generation catalyst was synthesized by adding 3-bromopridine with the Grubbs second generation catalyst under nitrogen condition through magnetic stirring. Synthesized catalyst was washed by pentane in a dark room and dried under nitrogen gas. Third generation catalyst was green colour where was Grubbs second generation was red, so conversion just monitored by colour change. Synthesized product is well established and need to further experimental test. Third generation Grubbs catalyst was stored in a vial by covering with aluminum foil at +4 °C in a fridge. Reaction scheme is shown in Figure 5.34.

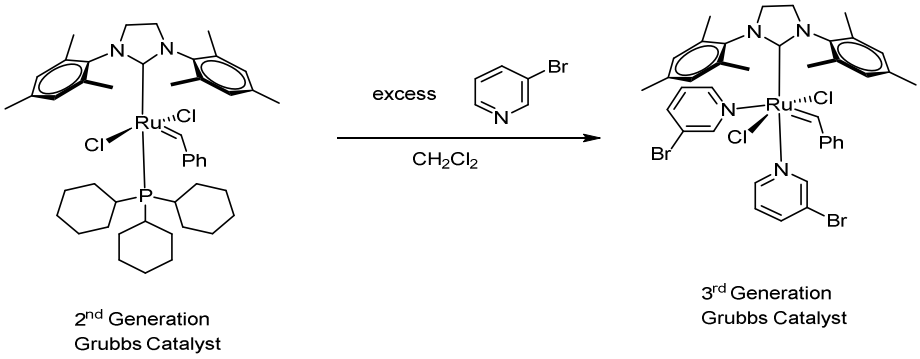


Figure 5.34 Synthesis scheme of 3rd generation Grubbs catalyst

5.7.2 Polymerization of Two Carbon Linker 0.5 Generation Dendritic Monomer by Grubbs 3rd Generation Catalyst

Two carbon linker dendritic monomer was polymerized in DCM by Grubbs 3rd generation catalyst at room temperature. Monomer solution was kept under nitrogen condition with vigorous stirring over five days. Polymerization reaction was terminated by adding ethylvinylether (30% solution in DCM) and polymer precipitate by using diethyl ether and ppt is washed several times by centrifuging over diethyl ether. Polymerization reaction scheme is shown in Figure 5.35.

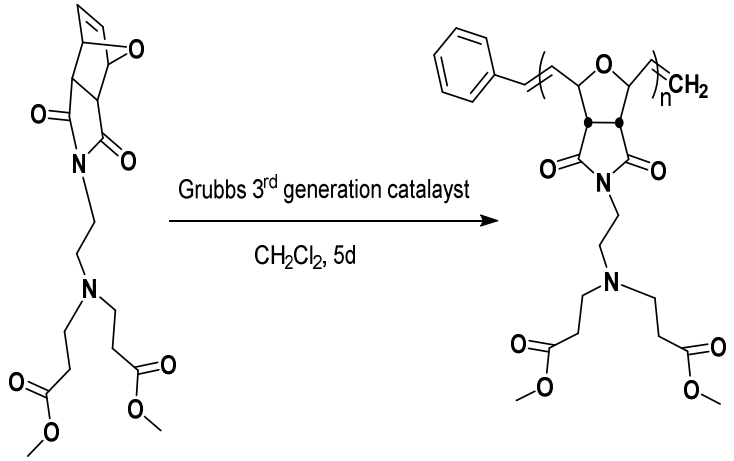


Figure 5.35 Polymerization of two carbon linker 0.5G dendritic monomer

Polymer was confirmed by proton NMR analysis in DMSO-d_6 solvent. Proton NMR of the polymer and monomer is given in Figure 5.36. Polymerization was confirmed by total disappearance of olefine peaks at 6.3-6.6 ppm and new peak appears at 4.0-5.0 ppm form double bond of polymer backbone.

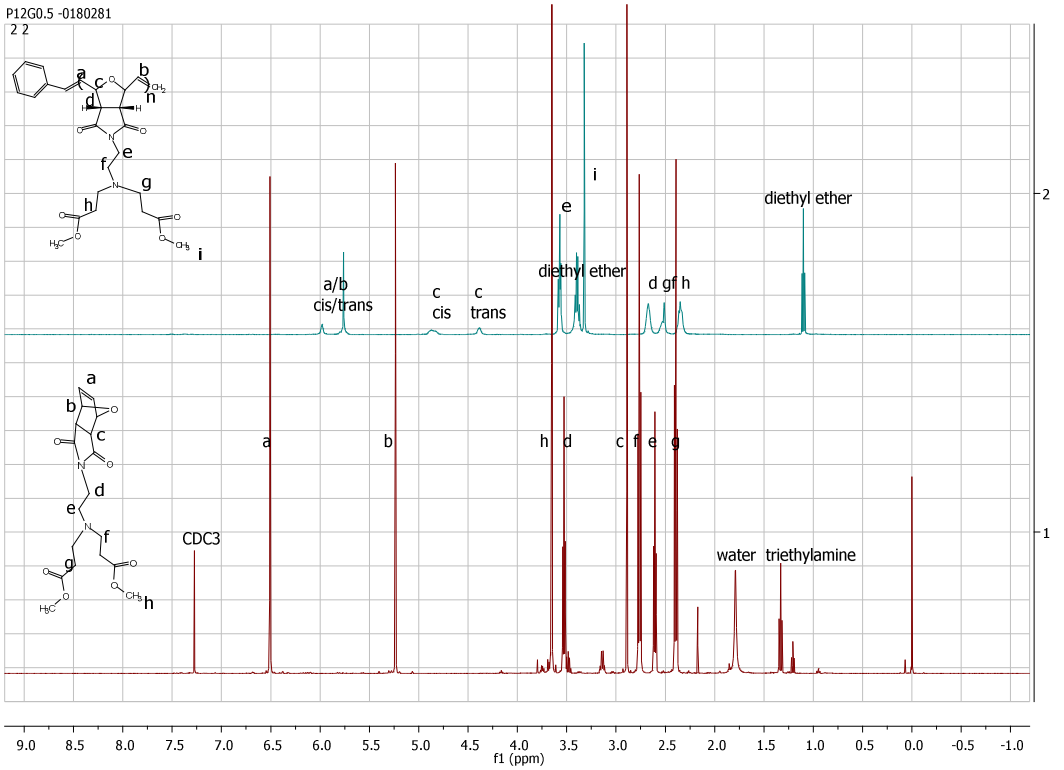


Figure 5.36 ^{1}H NMR of two carbon linker 0.5 generation monomer and its polymer

The manifestation of vinyl group attached hydrogens newly appeared by the ROMP techniques formed both cis- and trans-double bond. Relative integration of these two broad ^{1}H NMR signals represents cis/trans ratio occurred in the polymer compound (Appendix Figure B39). Obtained homopolymer possess 57% trans residue. Mn of this homopolymer was reported by applying end group analysis of the purified polymer by comparing to the assumed molar mass using ^{1}H NMR [202]. Degree of polymerization is calculated from (-CH=CH-$_{cis,trans-}$) peaks integration relative to the peaks of styrene end group comes from the Grubbs catalyst in the ^{1}H NMR spectra of the polymer. End phenyl group connected to the catalyst carbene moiety is visible at catalyst approximately 7.2 to 7.5 6 ppm. Other branching peaks remain same. Integration for phenyl group is 1.0 and therefore for each proton integration is 0.2. Integration of the (-CH=CH-$_{cis,trans}$) peaks relative to the styrenic end group from the Grubbs catalys is 20.07 for two proton and 10.03 is for each proton. Hence number of repeating unit is 50.175. Molecular weight of monomer is 380.39 and degree of polymer is 50.175. Molecular weight is calculated by multiplying degree of polymerization and molecular weight of monomer that is found 19086 g/mol. Our targeted molecular weight was 20,000 g/mol which is very close to our calculated molecular weight.

5.7.3 Polymerization of Six Carbon Linker 0.5 Generation Dendritic Monomer by Grubbs 2nd Generation Catalyst

Six carbon linker 0.5 generation dendritic monomer was polymerized in DCM by Grubbs second generation catalyst at room temperature under nitrogen condition with vigorous stirring over five days. Polymerization was terminated by adding ethylvinylether (30% solution in DCM) followed by half an hour more stirring. Synthesized polymer was precipitated by using diethyl ether and precipitate is washed several times by centrifuging over diethyl ether. Polymerization reaction scheme is shown in Figure 5.37.

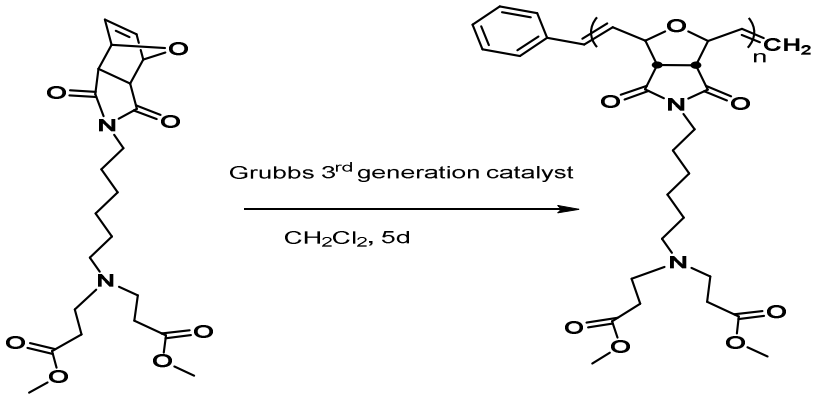


Figure 5.37 Polymerization reaction scheme of six carbon linker 0.5 generation dendritic monomer

Proton NMR the polymer was analyzed in DMSO-d_6 solvent. Polymerization was confirmed by total disappearance of olefine peaks at 6.3-6.6 ppm and new peak appears at 4.0-5.0 ppm form double bond of polymer backbone (Figure 5.38) [60].

The manifestation of vinylic hydrogens newly appeared by the ROMP reaction formed both cis- and trans-double bond. Relative integration of these two broad ^{1}H NMR signals represent cis/trans ratio in the polymer main chain (Figure 5.40). The synthesized homopolymers contained 70% trans residue. Mn of the polymer was calculated by applying end group analysis of the purified polymer in comparison to the assumed molar mass using ^{1}H NMR. Degree of polymerization is calculated from (-CH=$CH_{cis,trans}$–) peaks integration relative to the peaks of styrenic end group comes from the Grubbs catalyst in the polymer. The phenyl end group connected to the original carbene moiety from the catalyst is visible at catalyst approximately 7.2 to 7.5 6 ppm. Other branching peaks remain same. Integration for phenyl group is 1 and therefore for each proton integration is 0.2. Integration of the (-CH=CHcis,trans–) peaks relative to the styrenic end group from the Grubbs catalyst is 19.26 for two proton and 9.63 is for each proton. Hence number of repeating unit is 48.15. Molecular weight of monomer is 436.22 g/mole and degree of polymer is 48.15. Therefore calculated molecular weight is 21003 g/mole and our targeted molecular weight was 20000 g/mol which is much closer to our calculated molecular weight.

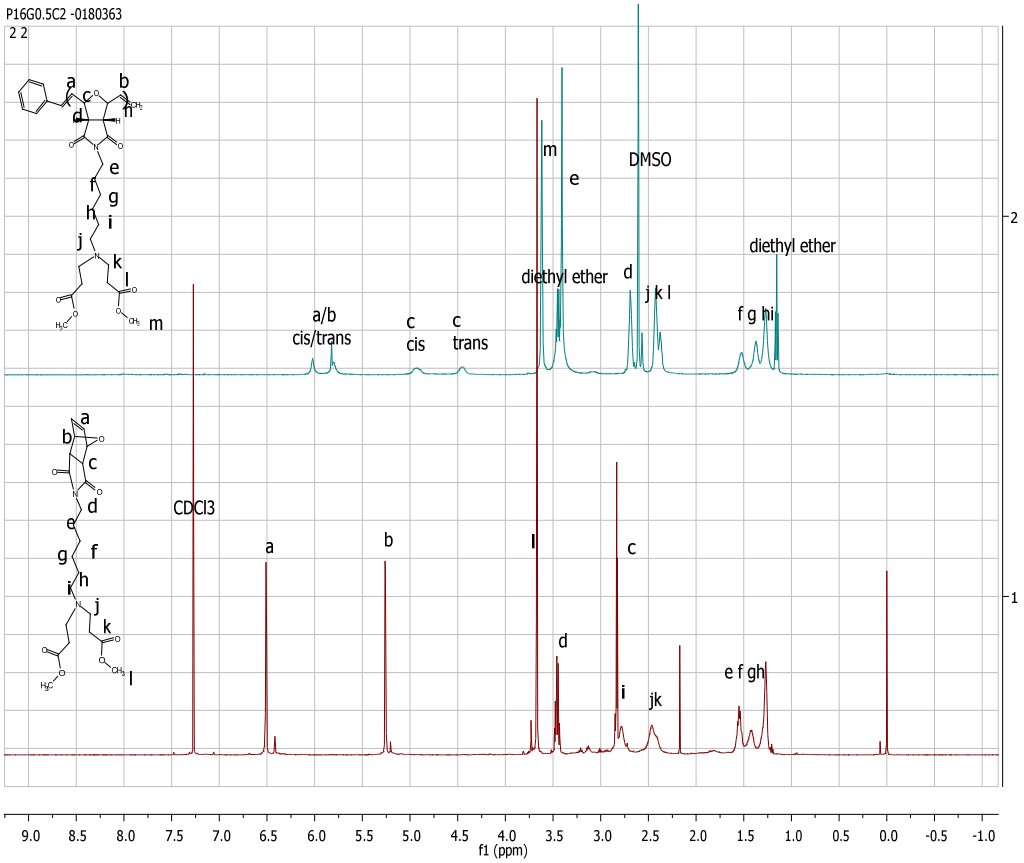


Figure 5.38 ^{1}H NMR of six carbon linker 0.5 generation and its polymer by Grubbs 2nd Generation catalyst

5.7.4 Polymerization of Six Carbon Linker 0.5 Generation Dendritic Monomer by Grubbs 3rdGeneration Catalyst

Six carbon linker 0.5 generation dendritic monomer was also was polymerized by Grubbs second generation catalyst. Reaction condition and procedure was as same as before just using 3rd generation Grubbs catalyst was used instead of 2nd generation Grubbs catalyst. Successful conversion of polymer was confirmed by proton NMR analysis in DMSO-d_6 solvent. Total disappearance of olefin peaks at 6.3-6.6 ppm and new peak appears at 4.0-5.0 ppm form double bond of polymer backbone indicate confirm polymerization (Figure 5.39) [60].

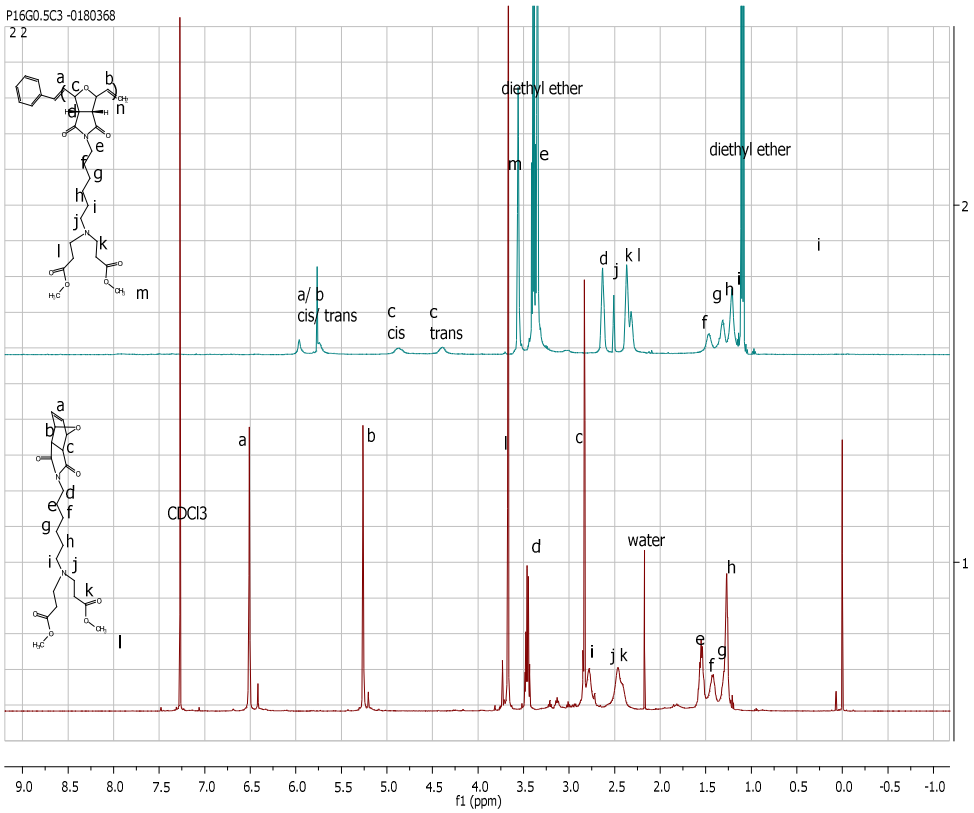


Figure 5.39 ^{1}H NMR of six carbon linker 0.5 generation monomer and its polymer by Grubbs 3rd generation catalyst

The manifestation of vinylic hydrogens newly appeared by the ROMP polymerization formed both cis- and trans-double bond. Broad peaks integration in ^{1}H NMR represent the relative cis/trans ratio in the polymer structure. Newly synthesized this homopolymer possessing 50% trans residue. Mn of the synthesized polymer was calculated by applying end group analysis of the purified polymer by comparing to the assumed molar mass using ^{1}H NMR (Figure 5.40). Degree of polymerization is calculated from (-CH=CHcis,trans–) peaks integration relative to end styrenic group comes from the Grubbs' catalyst in the polymer. The phenyl end group connected to the original carbene moiety from the catalyst is visible at catalyst approximately 7.2 to 7.5 6 ppm. Other branching peaks remain the same.

Integration for phenyl group is 1 and therefore for each proton integration is 0.2. Integration of the (-CH=CH$_{cis,trans}$–) peaks relative to the styrenic end group from

the Grubbs' catalyst is 20.66 for two proton and 10.33 is for each proton. Hence number of repeating unit is 49.65. Molecular weight of monomer is 436.22 g/mol and degree of polymer is 49.65. Therefore, calculated molecular weight is 21658 Da and targeted molecular weight (20000 Da) is close to each other.

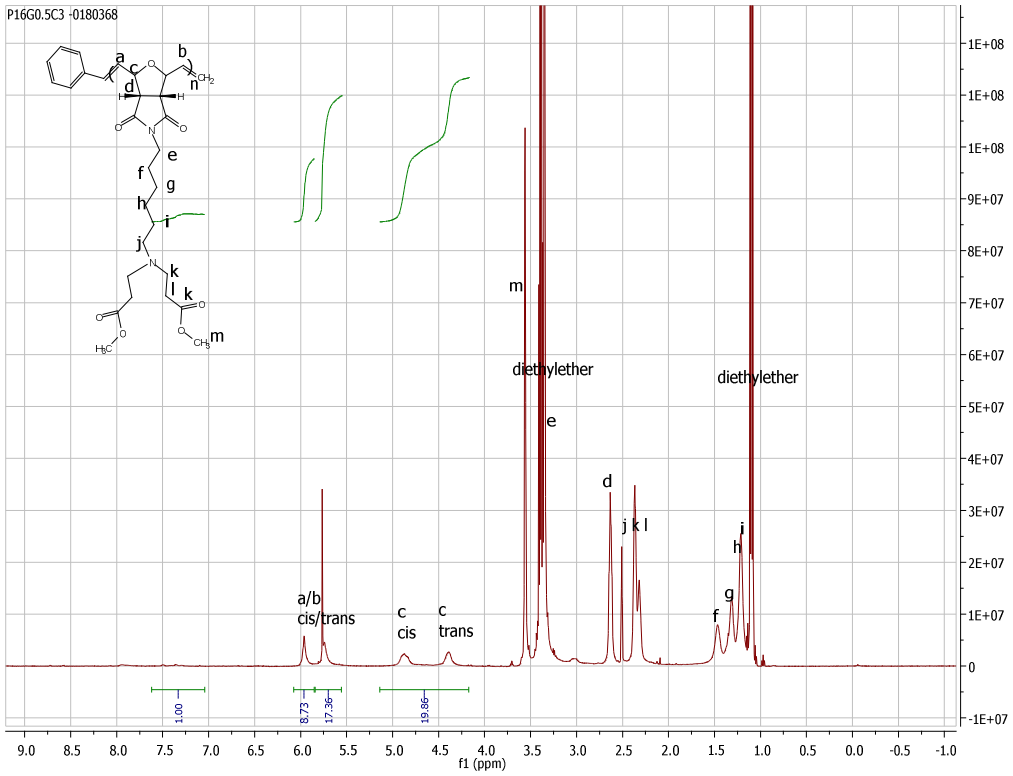


Figure 5.40 Calculation of M_n from ^1H NMR of polymer

5.7.5 Synthesis of Polymer of Two Carbon Linker 1.5 Generation Dendritic Monomer byGrubbs 2nd Generation Catalyst

1.5 generation of two carbon linker dendritic monomer was polymerized inside a sealed tube in trifluoroethanol solvent at 80 ˚C by Grubbs 2nd generation catalyst for 48 h reaction. Polymerization was successful observed by ^1H NMR spectroscopy. But molecular weight determined by GPC was very low. So, probably its formed only small oligomer. This polymer was not soluble in THF but soluble in water.

5.7.6 Polymerization of Six Carbon Linker 1.5 Generation Dendritic Monomer by Grubbs 2nd Generation Catalyst

1.5 generation of two carbon linker dendritic monomer was polymerized inside a sealed tube in trifluoroethanol solvent at 80 ˚C by Grubbs 2nd generation catalyst for 48 h reaction. Polymerization was successful observed by ^{1}H NMR spectroscopy. But molecular weight determined by GPC was very low. So, probably it's formed only small oligomer because of higher generation dendron containing dendronized monomer with usually form low degrees of polymerization [13], [203]. This polymer was not soluble in THF but soluble in water.

5.7.7 Polymerization of Six Carbon Linker 2.5 Generation Dendritic Monomer by Grubbs 2nd Generation Catalyst

2.5 generation of two carbon linker dendritic monomer was polymerized in a sealed tube at 80 ˚C by Grubbs 2nd generation catalyst for 48 h reaction in trifluoroethanol solvent. Reaction scheme is shown in Figure 5.41. Polymerization was successful, observed by ^{1}H NMR spectroscopy.

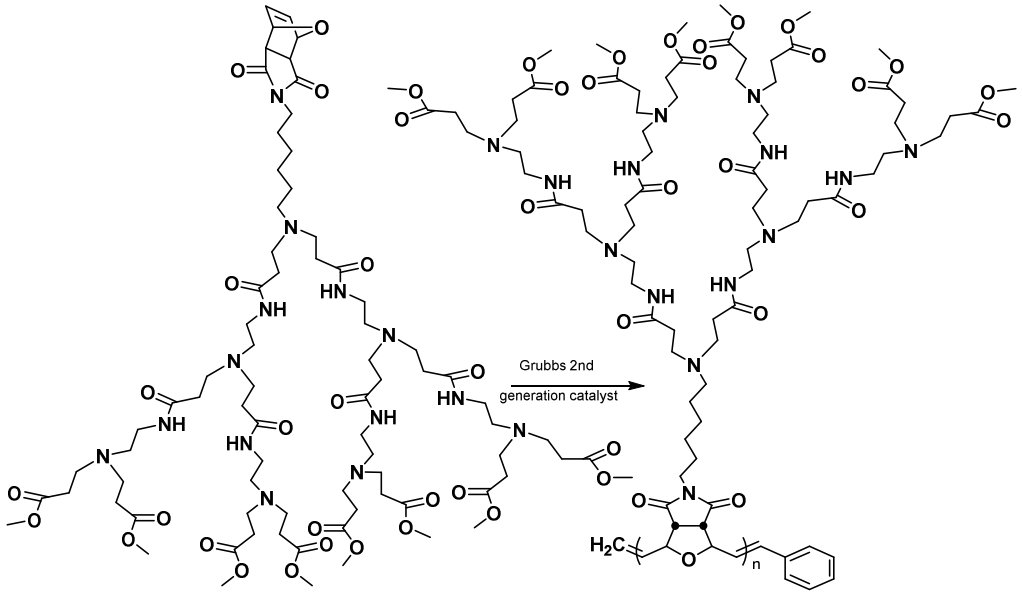


Figure 5.41 Polymerization rection scheme of six carbon linker 2.5 generation dendritic monomer

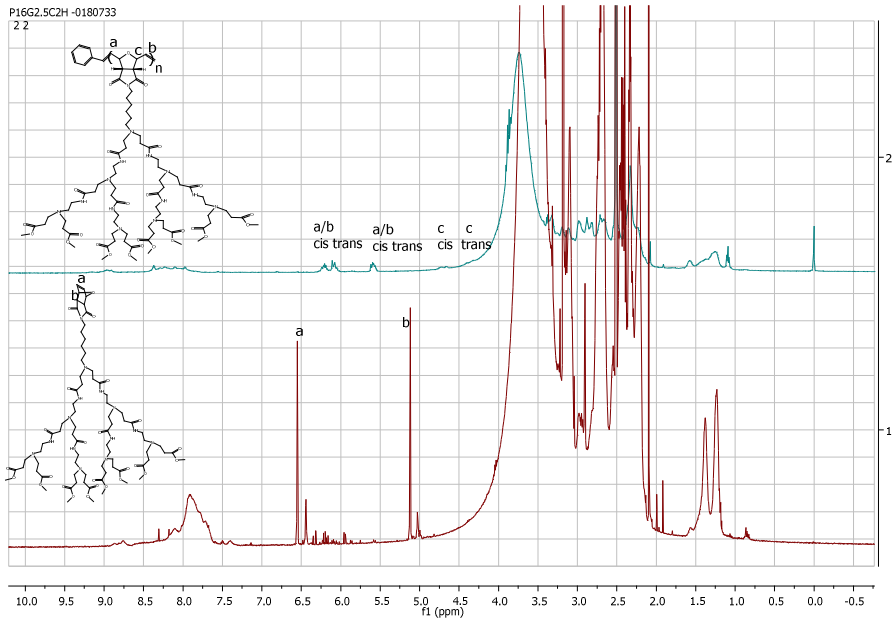


Figure 5.42 ^{1}H NMR of 2.5 generation dendritic monomer and its polymer

Polymerization was confirmed by total disappearance of olefine peaks of monomer at 6.3-6.6 ppm and new peak appears at 5.5-6.3 ppm form newly formed double bond of polymer backbone (Figure 5.42). ^{1}H NMR spectra of monomer and polymer is given in the same figure to show the conversion of polymer. Polymerization reaction mechanism scheme of ROMP is given in Figure 5.44.

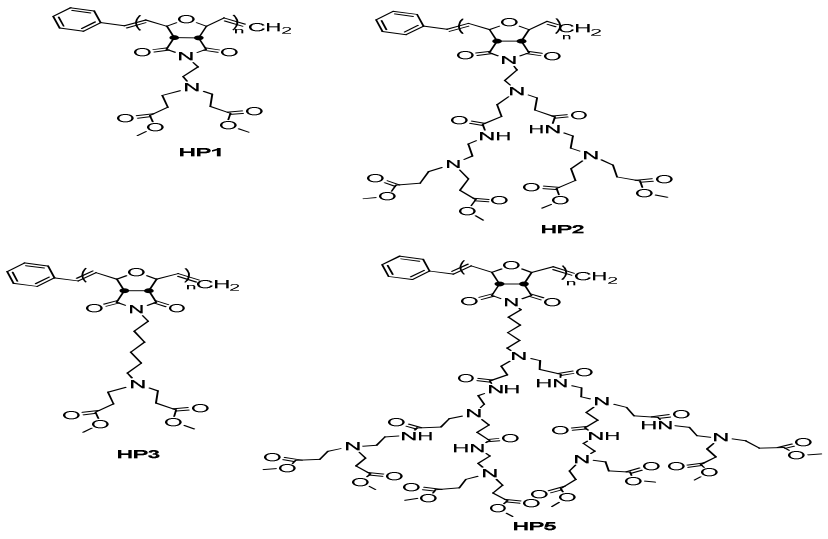


Figure 5.43 Dendritic ROMP homopolymers

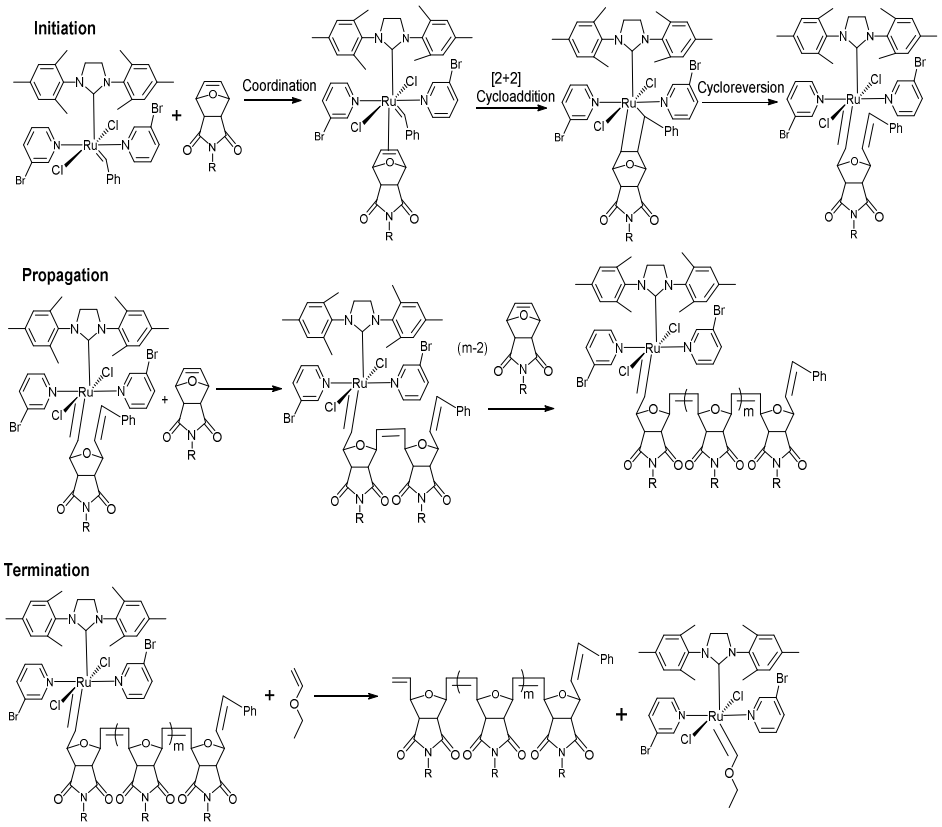


Figure 5.44 Polymerization reaction mechanism scheme of ROMP

Table 5.5 Molecular weight of polymers determined by GPC and NMR

Polymers	Targated Mn (g/mol)[a]	Solubility	Mn NMR (g/mol)[b]	Mn GPC (g/mol)[c]	PDI
P12G0	20000	THF	19086	14573	1.28
P16G0.5C2	20000	THF	21003	12389	2.04
P16G0.5C3	20000	THF	21658	12315	1.57
P16G2.5		H_2O	20908	10857	1.43

[a]theoretical number average molecular weight, [b]number average molecular weight calculated by NMR end group analysis, [c]number average molecular weight determined by GPC

5.7.8 Summary of Polymerization Attempts in Different Solvent and Conditions

During dendronized polymer synthesis from macromonomer, various factors such as nature of solvents, concentration of monomer, generation of dendronized monomer and nature of polymerizable group need to take under consideration. [75], [204]. For polymer synthesis from other monomer, we tried various solvents and reaction conditions but not able to polymerize all of them. All attempts are summarized in Table 5.6

Table 5.6 Summary of polymerization attempts in different solvent and conditions

Monomer	Solvent	Catalyst	Temperature	Reaction time	Conversion
DA12G0.5	Trifluroethanol	Grubbs 3rd	RT	2h	No
DA12G0.5	Dichloromethane	Grubbs 3rd	RT	5 d	Yes
DA16G0.5	Trifluroehanol	Grubbs3rd	RT	2 h	No
DA16G0.5	Dichloromethane	Grubbs 3rd	RT	5 d	Yes
DA16G0.5	Dichloromethane	Grubbs 2nd	RT	5 d	YES
DA16G1.5	Trifluroethanol	Grubbs 3rd	60 ˚C	2h	No
DA16G1.5	Trifluroethanol	Grubbs 2rd	60 ˚C	2h	No
DA16G2.5	Trifluroethanol	Grubbs 3rd	RT	2h	No
DA16G2.5	Dry THF	Grubbs 3rd	RT	24h	No
DA16G2.5	Dry THF	Grubbs 3rd	60 ˚C	24h	No
DA16G2.5	Trifluroethanol	Grubbs 3rd	RT	5 d	No
DA16G2.5	Trifluroethanol	Grubbs 3rd	60 ˚C	24h	No
DA16G2.5	Trifluroethanol	Grubbs 2nd	60 ˚C	24h	Partial

Note: DA represents core synthesized by Diels Alder reaction, 12 is for ethylene, 16 is for diaminohexane,G for generation as well as number after G represents specific generation and P represents polymer in the table.

5.8 ROMP-PAMAM Encapsulated Copper Nanoparticle Synthesis

PAMAM encapsulated copper nanoparticle studies were focused on intra dendrimer complexes between PAMAM dendrimer and Cu^{2+}. Copper nanoparticle can easily characterize by color changes and interpretable from UV-VIS spectra. It is well known that maximum four tertiary amine form complex with a Cu ion. Appropriate number of metal ion ration must be calculated to be synthesized ROPM-PAMAM dendrimer encapsulated Cu nanoparticle. Initially, ROMP-PAMAM solution was stirring for 20 minutes with $CuSO_4$ solution to form PAMAM Cu^{2+} complex which is turned to ROMP based PAMAM encapsulated Cu nanoparticle after reduction by the addition excess amount of $NaBH_4$. This phenomenon monitored by UV-Vis spectra and changes of colours of solution as well. Synthesized six carbon linker 2.5 generation polymer solution is colourless but after formation of Cu^{2+} complex it formed yellowish colour. After adding excess $NaBH_4$ Cu^{2+} reduced and formed Cu encapsulated PAMAM nanoparticle. Clolour of the ROMP-PAMAM encapsulated copper nanoparticle was green. By observing these colour changes, formation of nanoparticle easily observed. In the absence of PAMAMs, Cu^{2+} ions in aqueous solutions with hexahydrate show a weak absorption band centered at 810 nm stemming from d-d transition

5.9 Catalytic Activities ROMP-PAMAM Encapsulated Cu Nanoparticle

Sodium borohydride ($NaBH_4$) is a well know reducing agent but it cannot reduce 4-nitrophenol to 4-aminophenol by itself. To verify this statement initially UV-Vis absorption spectra of 4-nitrophenol was taken and immediately after adding $NaBH_4$ and after 24 hours long waiting. Results showed there was no significant decrease of nitro group and did not form UV-Vis absorption peaks amino group (Figure 5.45). So synthesized PAMAM dendrimer based ROMP polymer template Cu nanoparticle is used as a catalyst along with $NaBH_4$ to reduce 4-nitrophenol to 4-aminophenol.

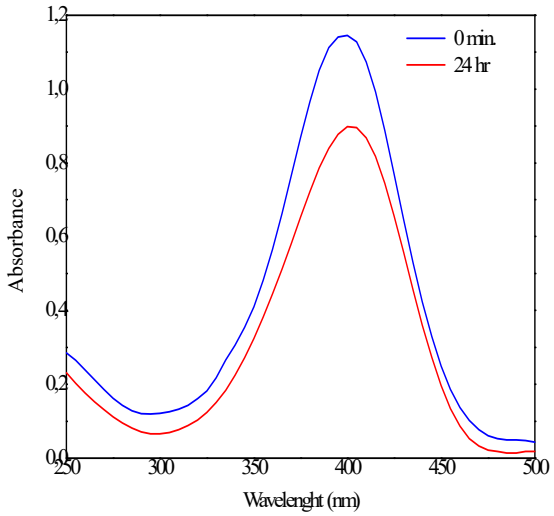
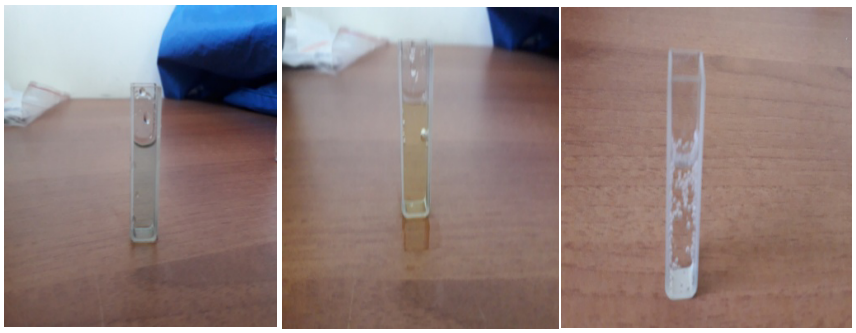


Figure 5.45 UV-Vis absorption spectra of 4-nitrophenol after addition of $NaBH_4$

Cu-PAMAM complex was blue with absorption 300 nm but after addition of $NaBH_4$ the colour of the solution turns to immediately to yellow and absorption lowered [38], [96] the catalytic activity of ROMP-PAMAM encapsulated Cu was investigated with a general reduction of 4-nitrophenol to 4-aminophenol with sodium borohydride ($NaBH_4$) as source of hydrogen catalyzed by our nanoparticle [39].



A **B** **C**

Figure 5.46 Colour changes during PAMAM encapsulated Cu nanoparticle and catalytic activities A) Cu^{2+} ROMP-PAMAM complex B) Cu ROMP-PAMAM nanoparticle C) After 4-nitrophenol to 4-aminophenol [37]

Actual absorption of 4-nitrophenol and 4-aminophenol is 400 nm and 310 nm respectively. In presence of ROMP-PAMAM encapsulated Cu nanoparticle catalyst, addition of excess $NaBH_4^-$ resulted gradual decrease of absorption λ400 nm and increase the absorption band 310 nm. Although, it is provided in most of literature simply, but the reaction mechanism is quite complex. Theoretically, it was expected that the absorption band at λ 310 nm will increase gradually but in our case it was not observed sharp increase due to some side reaction or probable reduction of double bond present in ROMP backbone. From UV-Vis spectra it is clear that NO_2 reduced as absorption peak follow gradual decrease (Figure 5.47).

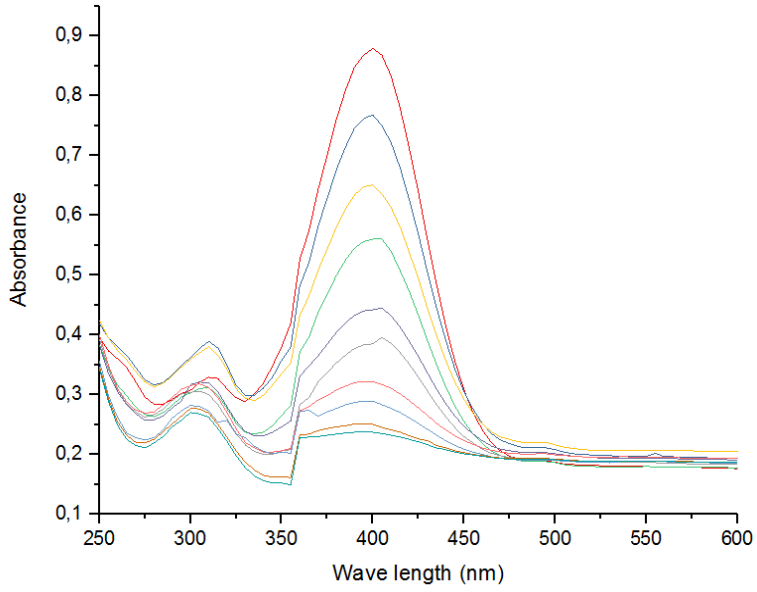


Figure 5.47 Absorption based on time in the UV-Vis spectra for the reduction of 4-nitrophenol to 4-aminophenol by ROMP-PAMAM encapsulates Cu nanoparticle in presence of $NaBH_4$

5.10 Synthesis of Copolymers

Two different types of copolymers (**P3_a-l)** were synthesized using the branched monomers, two carbon linker 0.5 generation dendron (**M1)** or six carbon linker 0.5 generation dendron **(M2)** and the pyridinium-containing monomer **M3**. Third generation Grubbs catalyst [130] was used to polymerize the corresponding monomers in dry DCM and methanol solvent mixture (1:1, v/v) at air temperature. Total molecular weight of the copolymers was targeted to be 10 kg/mol with

different ratio i.e. 8 kDa: 2 kDa, 7 kDa: 3 kDa, 5 kDa: 5 kDa, 4 kDa: 6 kDa, 3 kDa: 7 kDa kg/mol molecular weight of both monomers by using the proper monomer to catalyst concentration (Reaction scheme is given in Figure 5.48).

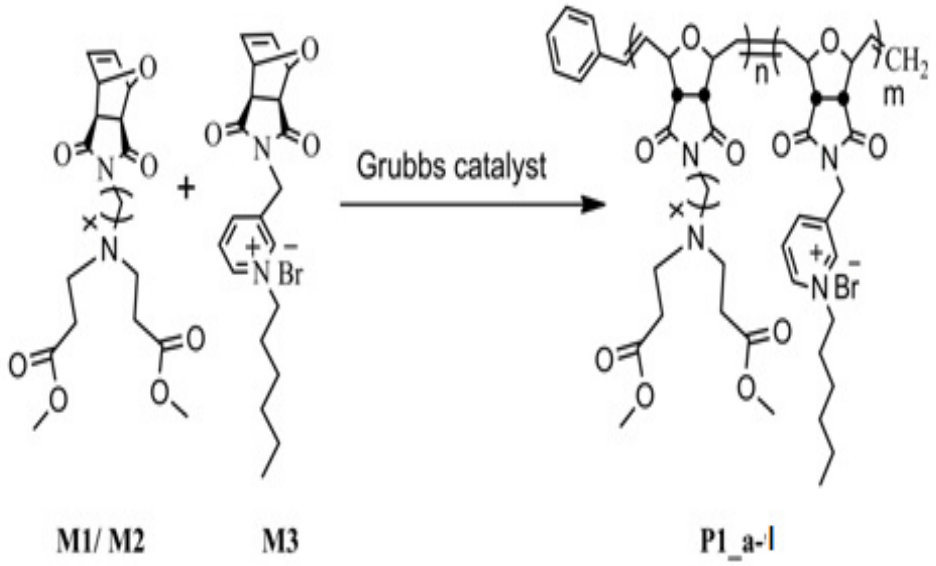


Figure 5.48 Synthesis scheme of copolymer

For random copolymers, both monomers were mixed together prior to the addition of the catalyst whereas for the preparation of the block copolymer pyridinium-containing monomer was first polymerized followed by sequential addition of the branched monomer two carbon linker 0.5 generation (**M1)**or six carbon linker 0.5 generation (**M2)**. The polymerization was terminated by adding ethylvinylether. So concluded from corresponding ^{1}H NMR, that olefin signals of both monomers originally at 6.5 ppm are vanished, while two new broad peaks appeared at 5.6-6.2 ppm, which proved the successful polymerization of both monomers. The corresponding monomer conversion of two carbon linker 0.5 generation, **M1** and pyridinium hexyl monomer (**M3)** to copolymer was also compared in ^{1}H NMR spectroscopy (Figure 5.49).

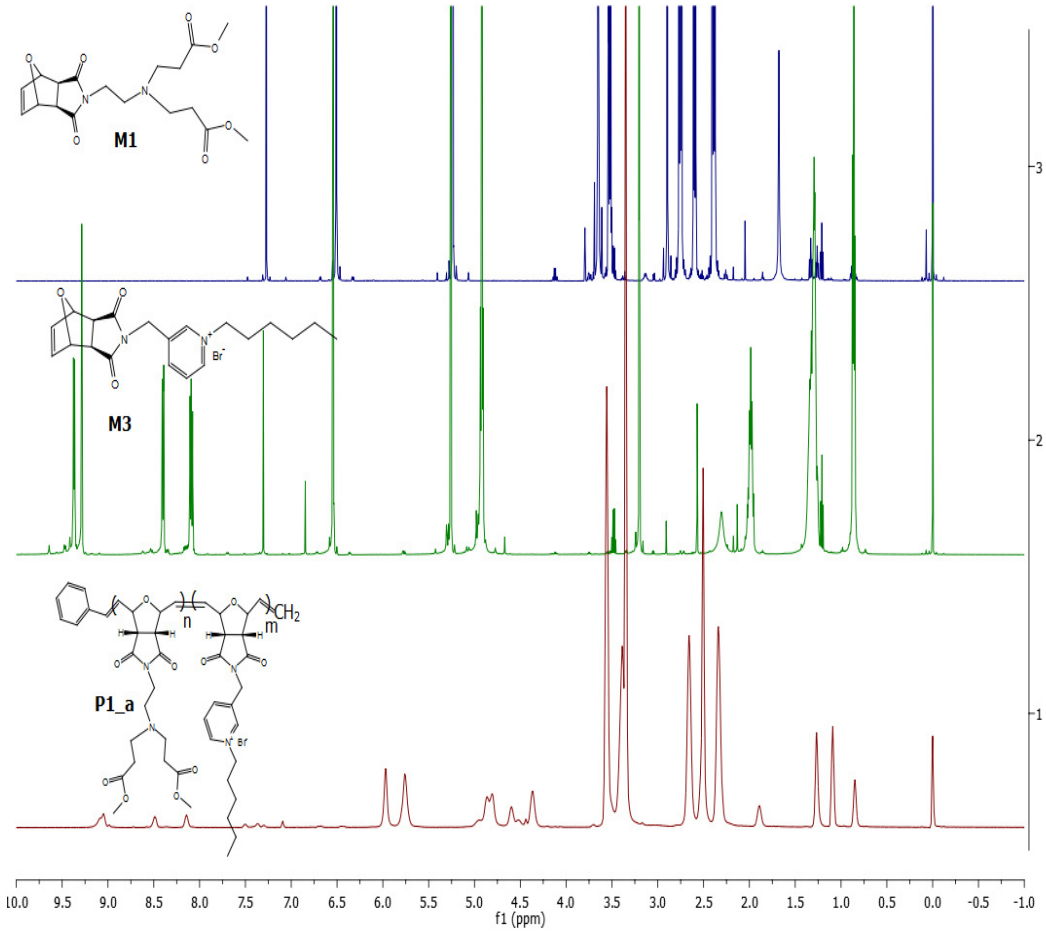


Figure 5.49 ^{1}H NMR of two carbon linker 0.5 generation monomer (M1), pyridinium-hexyl monomer (M3) and copolymer of both monomers

Table 5.7 indicates the targeted and observed molecular weights of the obtained copolymers for the series of **P1_a_l**. M_n of the polymers was calculated by end group analysis of the purified polymer by comparing to the assumed molar mass using ^{1}H NMR and GPC. The degree of polymerization was calculated via identification of the (-CH=CH-cis, trans-) peaks relative to the styrenic end group stemming from the Grubbs catalyst and the pyridinium peaks in the ^{1}H NMR spectra of the copolymer. The phenyl end group attached to main carbene moiety from the catalyst became visible at 7.2 to 7.5 ppm while pyridine signals were at 8.0-9.3 ppm. For example, M_n was calculated by determining the degree of polymerization for sample **P1_a** from ^{1}H NMR peak integration (Appendix Figure B42). The number of repeating unit of pyridinium based monomer was obtained

by comparison of phenyl group's five protons (at 7.3-7.6 ppm) with four protons of the pyridine unit as part of the polymer chain (at 8.0-9.5 ppm). Molecular weight of pyridinium based polymer moieties was calculated to be 2196 Da after multiplying average number of repeating unit (observed as 5.21) with the molecular weight of the monomer (421.33 g/mol). The total degree of polymerization for copolymer was found to be 29 by comparison of cis-trans protons of increasing C=C signal from both monomers in the polymer backbone with integration of five protons of phenyl group that from Grubbs catalyst (at 7.3-7.6 ppm). Average number of repeating unit (observed as 24) of dendritic based monomer was calculated by the number of pyridinium based monomer of repeating unit from total degree of polymerization. Molecular weight of dendritic polymer moieties was 9169 Da after multiplying the number of repeating unit and molecular weight of dendritic base monomer (380.15 g/mol). However, the average molecular weight (M_n) was not in agreement with M_n values determined by GPC. Most of the cases M_n determined by GPC was underestimated compared to values derived by NMR end group analysis [205]. Suer *et al.* also observed same phenomenon for polar phosphonium based ROMP polymer [51]. GPC calibration with linear, non-ionic pullulan standards resulted in different hydrodynamic volumes with respect to the cationic based copolymers, which can lead to a strong deviation [206]. However, GPC determines weight average molecular weight and number average molecular weight ratio and it indicated the broad distribution for the obtained copolymers [207] (Table 5.5) and featuring Đ of polymers were 1.34 to 4.78 (see the GPC spectra at Appendix Figure B81). In particular, dendron-based copolymers having 6 carbon linkers appeared to have a broad distribution. We used the methanol: dichloromethane mixture for the polymerization route and solubility of the growing chain as well as monomers in this solvent could affect the metathesis polymerization efficiency and cause broad distribution.

Further, the ester-terminated moieties of copolymers (**P1_a-l**) were treated with mono BOC-protected ethylenediamine at room temperature for 5 days (**P2_a-l**). Deprotection step was performed by the addition of TFA and lead to cationic polymers (**P3_a-l**) (reaction scheme is given in Figure 5.50) [208].

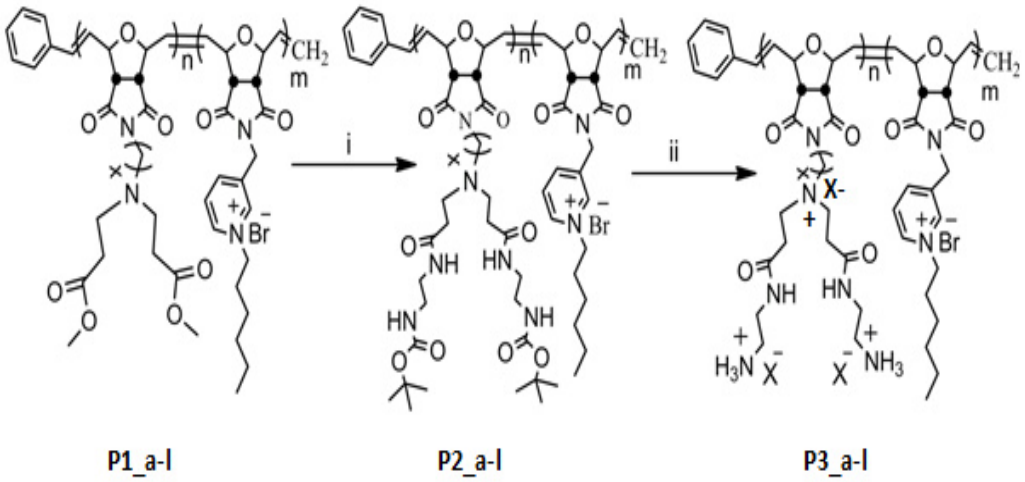


Figure 5.50 Surface modifications of copolymers i) EDA-BOC, DCM, methanol, 5d, rt, ii) TFA, 24h, rt. Here, P3_a (x=2, theoretical molecular weight of n and m =8 kDa and 2 kDa, random), P3_b (x=2, theoretical molecular weight of n and m =7 kDa and 3 kDa, random), P3_c (x=2, theoretical molecular weight of n and m =7 kDa and 3 kDa, block), P3_d (x=2, theoretical molecular weight of n and m =5 kDa and 5 kDa, random), P3_e (x=2, theoretical molecular weight of n and m =4 kDa and 6 kDa, random), P3_f (x=2, theoretical molecular weight of n and m =3 kDa and 7 kDa, random), P3_g (x=6, theoretical molecular weight of n and m =8 kDa and 2 kDa, random), P3_h (x=6, theoretical molecular weight of n and m =7 kDa and 3 kDa, random) and P3_i (x=6, theoretical molecular weight of n and m =7 kDa and 3 kDa, block), P3_j (x=6, theoretical molecular weight of n and m =5 kDa and 5 kDa, random), P3_k (x=6, theoretical molecular weight of n and m =4 kDa and 6 kDa, random), P3_l (x=6, theoretical molecular weight of n and m =3 kDa and 7 kDa, random) X^{-}=CF_3COO^{-}

Conversions were monitored by ^{1}H NMR spectroscopy as the BOC peak appeared at 1.33 ppm (Appendix Figure B54-B65) and complete disappearance of this peak was observed after treatment with TFA (Appendix Figure B66-B77). Tertiary amine at the dendritic structure was also protonated under the condition. Model compound **M1** was chosen to see the protonation by TFA under the same condition as polymer deprotection. It was observed that after deprotection methylene substituted tertiary cation (-CH_2-N+) switch to 0.41-0.64 ppm higher field (Appendix Figure B80).

Table 5.7 Molecular weight determined by GPC and end group analysis using ^{1}H NMR spectroscopy

Polymers	M_n[a] (g/mol)	Dendritic:pyridinium targeted feed (g/mol)[b]	Dendritic:pyridinium observed feed (g/mol)	M_n[c] (g/mol)	M_n[d] (g/mol)	**Đ**[e]
P1_a	10000	8k:2k	9169 : 2196	6561	11365	1.57
P1_b	10000	7k:3k	8854 : 3697	6523	12550	1.61
P1_c*	10000	7k:3k	8667: 3696	12610	12364	1.34
P1_d	10000	5k:5k	4626 : 5014	5334	9640	1.61
P1_e	10000	4k:6k	3466 : 5661	4150	9127	1.74
P1_f	10000	3k:7k	2930 : 7935	2808	10865	1.98
P1_g	10000	8k:2k	9734 : 2354	5983	12088	4.78
P1_h	10000	7k:3k	7851 : 3607	5630	11459	3.24
P1_i*	10000	7k:3k	6984 : 4950	10840	11934	1.86
P1_j	10000	5k:5k	5376 : 6646	3828	12022	3.18
P1_k	10000	4k:6k	5856 : 6012	4365	11868	3.46
P1_l	10000	3k:7k	3372 : 8791	2151	12163	2.41

[a]Targeted molecular weight, [b] Polymer series of P1_a-P1_f and P1_g-P1_l represent copolymer possessing linker x=2 carbon and x=6 carbon, respectively. [c]Observed number average molecular weight by GPC, [d]Observed molecular weight via end group analysis by ^{1}H NMR spectroscopy, [e]Polydispersity index (**Đ**) determined by GPC , *block copolymer.

Moreover, the solubility of obtained polymers before and after deprotection significantly differed. In more detail, the ester functionalized random and block polymers (**P1_a-l** series) were soluble in DMSO but only partially soluble in THF after sonication. Moreover, random polymers were found to be soluble in THF, while block polymers were not soluble in THF, but they were soluble in DMF. After treatment with EDA-BOC, polymers with BOC functionalized moieties (**P2_a-l** series) were soluble in chloroform. When the polymers were deprotected by addition of TFA, cationic polymers (**P3_a-l**) were no longer soluble in chloroform and began to dissolve in water.

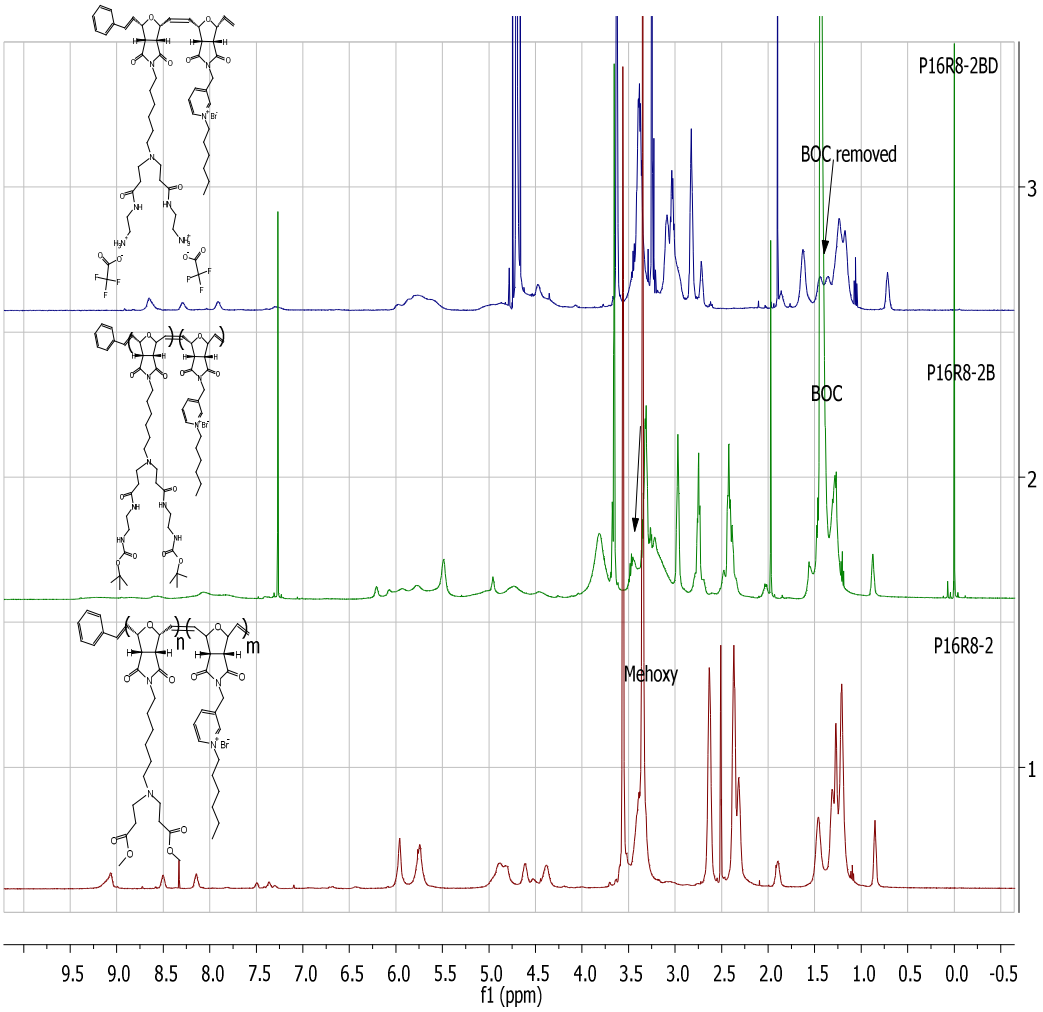


Figure 5.51 ^{1}H NMR of polymer, BOC surfaced polymer and BOC deprotected polymer

5.11 Antimicrobial and Hemolytic Activities of Copolymers

Antibacterial activities of synthesized water soluble cationic polymers were explored against a Gram-negative and a Gram-positive bacteria. *Escherichia Coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are taken as representative of Gram-negative and Gram-positive bacteria respectively. The minimum inhibitory concentration (MIC) of the polymers was determined by optical density (OD) measurements as the concentration where growth of bacteria was not observed [209] and result is tabulated in Table 5.8.

Table 5.8 Antimicrobial and hemolytic activities of copolymers

Polymers	*E. coli* (MIC μg/mL)	*S. aureus* (MIC μg/mL)	HC_{50} (μg/mL)	Selectivity (HC_{50}/MIC) against *S. aureus*
P3_a	>256	>256	>1000	>3.9
P3_b	128	>256	>1000	>3.9
P3_c	>256	>256	>1000	>3.9
P3_d	>256	256	>1000	>3.9
P3_e	>256	128	512	4.0
P3_f	>256	128	>1000	>7.8
P3_g	>256	256	>1000	>3.9
P3_h	256	64	>1000	>15.6
P3_i	>256	32	>1000	>31.2
P3_j	>256	128	4	0.03
P3_k	>256	>256	8	>0.03
P3_l	>256	128	>1000	7.8

All the copolymers were inactive against *E. coli.* Copolymers with two carbon linker dendritic group and hexyl pyridinium salts, **P3_a-P3_f series**, are inactive both against *E. coli* and *S. aureus.* The effect of chain length carbon linker was examined with six carbon linkers dendritic groups in copolymer series **P3_g-P3_l**. The dendritic and hexyl pyridinium groups in the copolymer were found to be 7 kDa: 3 kDa by weight, increasing the effectiveness against *S.aureus* with a MIC of 64 μg/mL and 32 μg/mL for **P3_h** and **P3_i** respectively. These polymers possessing random and block architecture did not exhibit a significant difference in biocidal activity. Although we wanted to examine block (**P3_i**) and random copolymer (**P3_h)**, architecture effect on biocidal activity, characterization of the polymer structure needs attention. GPC spectrum of block copolymer (see Appendix Figure 81) indicated broad distribution. These might be due to the

inefficient polymerization for these monomers and solvent mixture to get well defined architecture. Although we targeted block copolymer architecture, side reaction via cross metathesis and backbiting could also cause the imperfect block architecture. Inefficient catalyst initiation and slow propagation rate can also cause the size distribution. There are some report on the block copolymer efficiency over random copolymers using same cationic/hydrophobic monomer compositions[210] in spite of this we take care of about the assumption of block strategy at here. Increasing the dendritic units in the copolymer series (**P3_g**) drops in activity against to *S. aureus* (256 μg/mL). Increasing the hexyl pyridinium units in copolymer series, **P3_j**, **P3_k** and **P3_l** also showed a drop in activity. These could be because of poor solubility of these polymers in buffer that may cause a phase separation or agglomeration in the test environment. Consequently, inefficient of interaction between bacteria membrane and polymers might cause loss of biocidal activity.

When the whole structure is examined, it is seen that the balance between hydrophobic units and cationic charge density are important for biocidal activity. The antimicrobial activities of two and six carbon linkers possessing dendron based copolymer series were inactive against *E. coli*. The reason for this is explained below. Hexyl pyridinium and six carbon linkers feed in copolymer sets are crucial in activity against *S. aureus.* Copolymer series **P3_h** and **P3_i** showed selectivity between Gram-positive bacteria and Gram-negative bacteria. Similar selectivity was also reported in previous studies [58]. Whittaker group also demonstrated that twelve carbon tail containing low molecular weight polymer shown high antimicrobial activity against *S. aureus*, but decreased the activity against the Gram-negative bacteria [211]. The difference in activity against Gram-positive and Gram-negative bacteria explained from structure of cell wall [212]. Presence of high micelle layer with a single with phospholipid bilayer exist in Gram-positive bacteria. However, Gram-negative bacteria possess double phospholipid bilayer and their large interaction. Meanwhile, active polymer should also pass the lipopolysaccharide to interact with phospholipid bilayer. Biocidal activity of cationic polymers generally based on electrostatic interaction with negatively charged bacteria membrane and hydrophilic compounds of

phospholipid tail groups. These interaction causes destability the membrane and result in death of bacteria. We thought that dendron based copolymer with possessing two positive charge in each dendritic unit and hexyl pyridinium units stuck in the lipopolysaccharide and outer membrane layer. This causes loosing activity against *E. coli*. In spite of increasing the overall hydrophobicity with six carbon dendritic unit and hexyl pyridinium salt in the feed copolymer caused the penetration of polymer rising molecular layer and destroying the *S. aureus* membrane. Raising the hydrophobicity enhances the capacity of the polymers to bind onto the lipid membrane, increasing the concentration of bound polymer which leads to enhanced membrane perturbation and eventually cell death [213].

The presence of the CF_3COO^- counteranion form weak ion pairs and would disperse far from the polymer main chain. This will enhance the increase the overall positive charge and increase the polymer solubility and electrostatic interaction with the negatively charged bacteria membrane [180]. However, it should be taken into consideration that high hydrophobic ratio may affect the solubility in the test environment and could drop in the activity.

The hemolytic concentration (HC_{50}) of the polymers toward freshly collected human red blood cells (RBCs) was tested according to a published article [56]. Hemolysis was observed by identifying the absorbance of the released hemoglobin at 405 nm using a plate reader. Antibacterial selectivity defined as HC_{50}/MIC ratio. In Table 5.9, it seems none of the copolymers are hemolytic and hemolytic activity values found over 1000 μg/mL for each copolymer except the copolymer series **P3_j** (HC_{50} = 4 μg/mL) and **P3_k** (HC_{50} = 4 μg/mL). For these copolymer series, increasing the hydrophobicity resulted in increase in toxicity. Findings could correlate with Al-Badri *et al.* as increase number of cationic groups significantly reduced its hemolytic activity in their study as well [58]. However, most hydrophobic polymer possessing highest hexyl pyridinium moiety and six carbon linkers (P3_l) resulted in increase the HC_{50} values (HC_{50} = 128 μg/mL). This contradiction may be explained as the polymer may have affected the toxicity test result due to poor solubility in the test medium.

5.12 Biophysical Characterization

To recognize the interaction of bacterial plasma membrane with antimicrobial substances**,** atomic force microscopy (AFM), confocal microscopy, fluorescence spectroscopy, and solid state NMR like biophysical techniques are most common and effective. Bacterial membranes possess a large percentage of anionic lipids such as cardiolipin and phospholipids containing phosphatidylglycerol. Bacterial membranes superficial negative charge can be neutralized upon titration with cationic antibacterial polymers and this phenomenon can be followed by Zeta potential (ζ-potential) [214]. Therefore analysis of zeta potential of polymers and polymer interactions at the membrane level is significant parameter to know mode of action of antimicrobial polymers.

We investigated zeta potential of all synthesized polymer (**P3_a-l**) that is shown in Table 5.9. At the same time one inactive (**P3_a**) and one active (**P3_i**) polymer incubated with *S. aureus* to investigate bacterial cell disruption through zeta potential measurement. First, all the polymers zeta potential and size values of all the polymers were evaluated and it was observed that they are higher than +11 mV and 874 nm. All the polymers sizes are nearly μm due to positively charged polymers aggregation as shown in Figure 5.52. The Z-average diameters are almost micron in solution that indicates polymer aggregate in solution by liquid-liquid separation due to the weak association of polymer chains, rather than the conventional core-shell type micelles or vesicles [215].

However, it should keep in mind that block copolymer architecture might not be well established as discussed above. Increasing the dendron units also resulted in higher hydrodynamic radius of the polymer coil. Similar conclusion can be drawn from six carbon linker polymer **P3_g-l** as well.

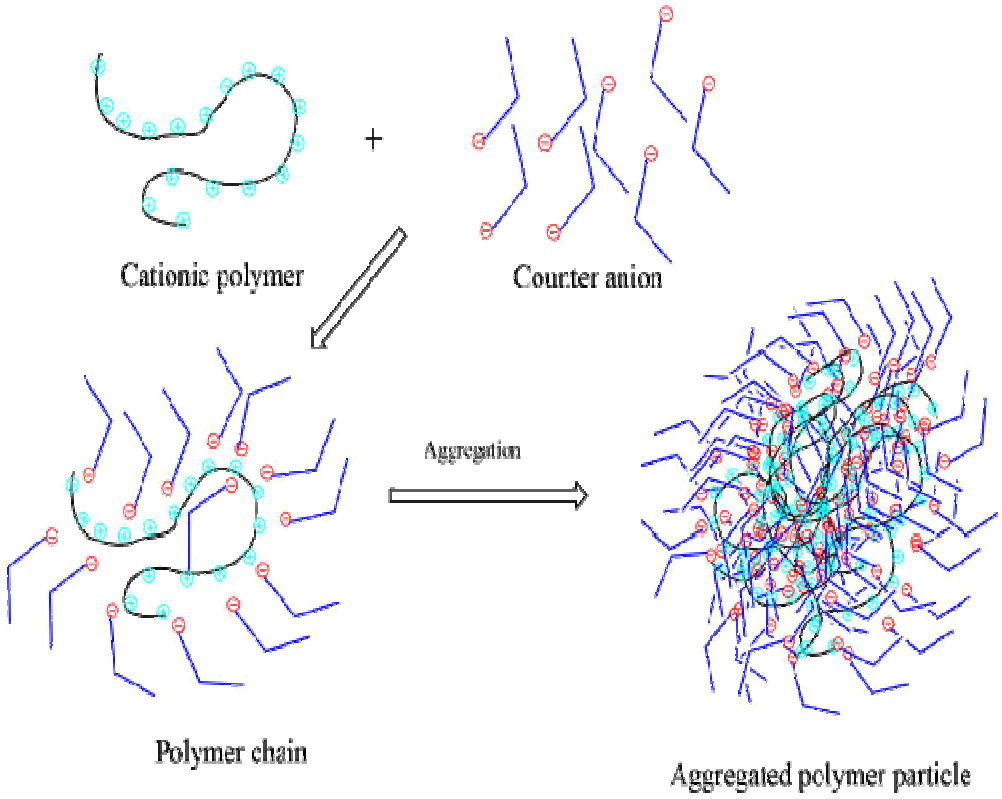


Figure 5.52 Schematic illustration of polymer aggregation

Zeta potential with high mV is considered strongly cationic in increase their stability in aqueous environment [216]. After the evaluation of polymers, the interaction of polymers against *S. aureus* were then investigated. It was observed that bare *S. aureus* in buffer gave a -15.68 mV due to the presence of negative charge bilayer. Values of zeta potential significantly decreased after the interaction of **P3_a** and **P3_i** against to *S. aureus*. The decrease in zeta potential may be explained by considering the release of negatively charged phospholipids, such as cardiolipin and posphatidylethanolamine from the *S. aureus* membrane disruption and complexation with cationic charge units of polymers. Interaction of *S. aureus* against to active (**P3_i**) and inactive polymer (**P3_a**) were used to investigate the membrane interaction. However, there is not big difference observed in between zeta potential of active and inactive polymer interaction with *S. aureus*.

Table 5.9 Zeta sizer and zeta potential of polymers

Polymers	Z-average (nm)	Zeta potential (mV) ± STD
P3_a	944	11.9±1.47
P3_b	1374	13.1±0.30
P3_c	1154	16.3±0.67
P3_d	350	5.7±0.65
P3_e	1171	8.24±0.71
P3_f	913	8.9±0.68
P3_g	858	11±0.40
P3_h	986	13.1±1.57
P3_i	1054	14.2±1.14
P3_j	1066	11.2±0.17
P3_k	1151	10.5±0.96
P3_l	1455	14.5±1.12
S. aureus		-15.68±0.49
P3_i + S. aureus		-3.51±0.37
P3_a+ S. aureus		-3.30±0.43

This phenomenon could explain from MIC values of inactive and active polymers. MIC values of inactive (**P3_a**) and active (**P3_i)** were 256 μL/mL and 32 μL/mL. As we used 200 μL/mL concentration polymer solution for zeta measurement so most of *S. aureus* also die with inactive polymer due to very high concentration of polymer. In fact zeta potential varies some other factors such as concentration, salt counter ion, hydrodynamic radious etc [217]. The effect of phosphate-buffer solution was also tested and a minor change in the zeta potential value of *S. aureus* was observed.

Scanning electron microscopy (SEM) was also used to examine the ultrastructural changes and detailed mechanisms of cell death of bacteria by antibacterial agent concentrations [52], [218]. In negative control both *E. coli* and *S. aureus* cells seems

very clear and good in condition where displayed a smooth and intact surface in Figure 5.53. The surface of the cells under SEM looked destroy after incubation with polymer at MIC value (Figure 5.53B and 5.53E). After incubation of polymer supra-MICs, the bacteria drastically damage all cell membrane (Figure 5.53C and 5.53F). Polymer is more active against *S. aureus* rather than *E. coli* that also observed from SEM image

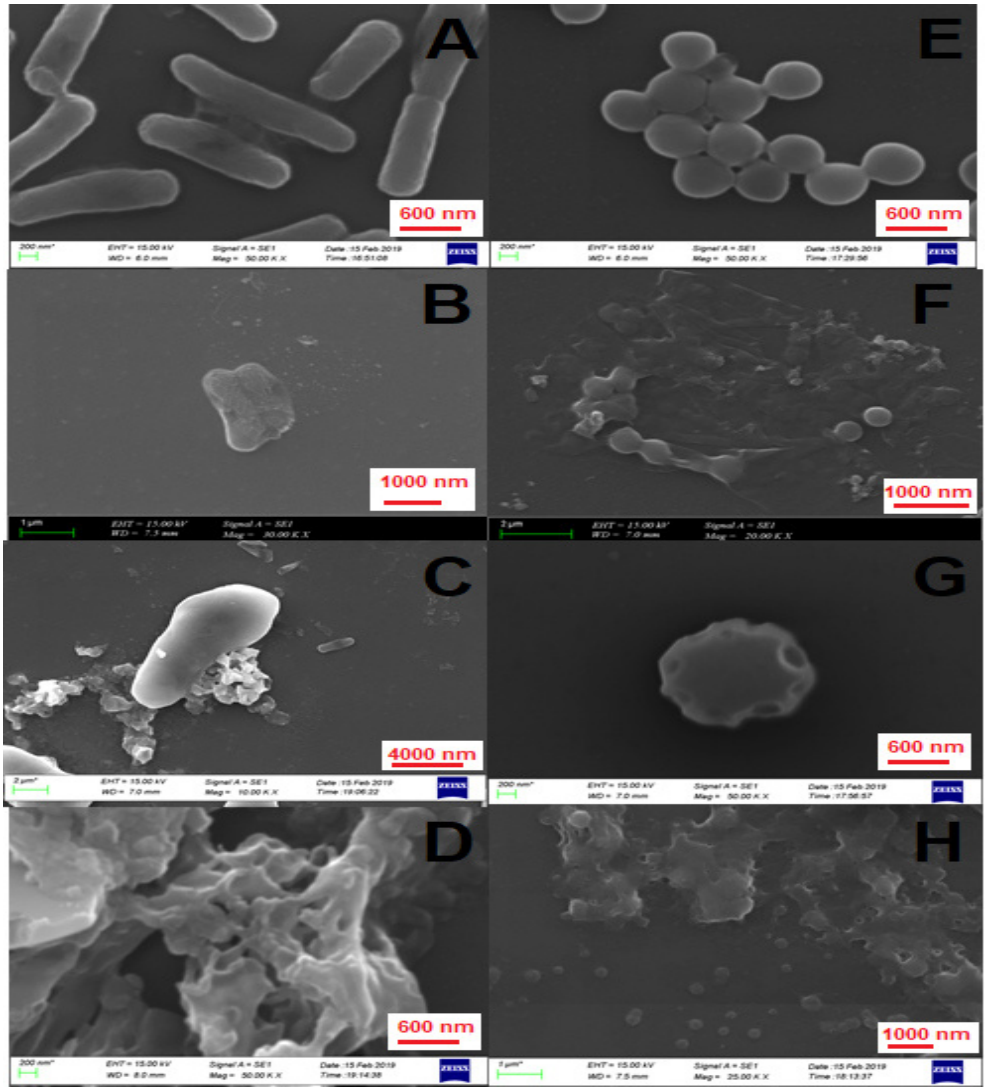


Figure 5.53 SEM micrographs of untreated *E. coli* and *S. aureus* on glass plate. (A) Negative control – only *E. coli* cell, (B). Positive control - *E. coli* treated by hexyl-phenyl based homopolymer (C) *E. coli* treated by copolymer at MIC value (256 µg/mL) (D) *E. coli* treated by copolymer at supra-MIC, (512 µg/mL), (E) Negative control – only *S. aureus* cell, (F). Positive control - *S. aureus* cell treated by hexyl-phenyl based homopolymer (G) *S. aureus* treated by copolymer at MIC value, (32 µg/mL), (H) *S. aureus* treated by copolymer at supra-MIC, (64 µg/mL).

5.13 Conclusion

In total four different ROMP homopolymers were synthesized and characterized. It was observed that monomer with higher generation containing six carbon chain linker can polymerize easy that the monomer containing two carbon linker. Moreover, exo isomer of oxanorbornene polymerize rather than endo isomer of oxanorbornene containing dendrimer. Molecular weight of polymers was determined by GPC and NMR end group analysis. Polydispersity of the polymer found 1.2 to 2.04. ROMP PAMAM encapsulated Cu nanoparticle was synthesized for 2.5 generation dendritic polymer and monitored by UV-Vis spectroscopy. Catalytic activity of the particle with $NaBH_4$ for reducing 4-nitrophenol to 4-aminophenol was observed significantly.

Moreover, twelve different water soluble cationic copolymers were successfully synthesized via ROMP with controlled molecular weight, hydrophobicity and charge densities. Antibacterial activities were conducted in phosphonate buffer through serial dilution method and it was found that six carbon linker containing 10 kg/mol block co-polymer had high activity against *S. aureus* (MIC = 32 μg/mL), and but inactive against *E. coli*. Newly synthesized two and six carbon linker dendron grafted and pyridinium based copolymers do not show toxicity against human blood cell as seen from HC_{50}. Biophysical techniques such as scanning electron microscopy and zeta potential have done to understand bacterial membrane surface mode of action and correlate with MIC value. These observed that polymer substance active to destroy the bacteria cell membrane. These new macromolecules with potent antibacterial activity combined with relatively low toxicity to human cells, which is desirable and possibly impactful in the development of new materials that can limit the spread of antibiotic-resistant microbes.

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A
FT-IR SPECTRA

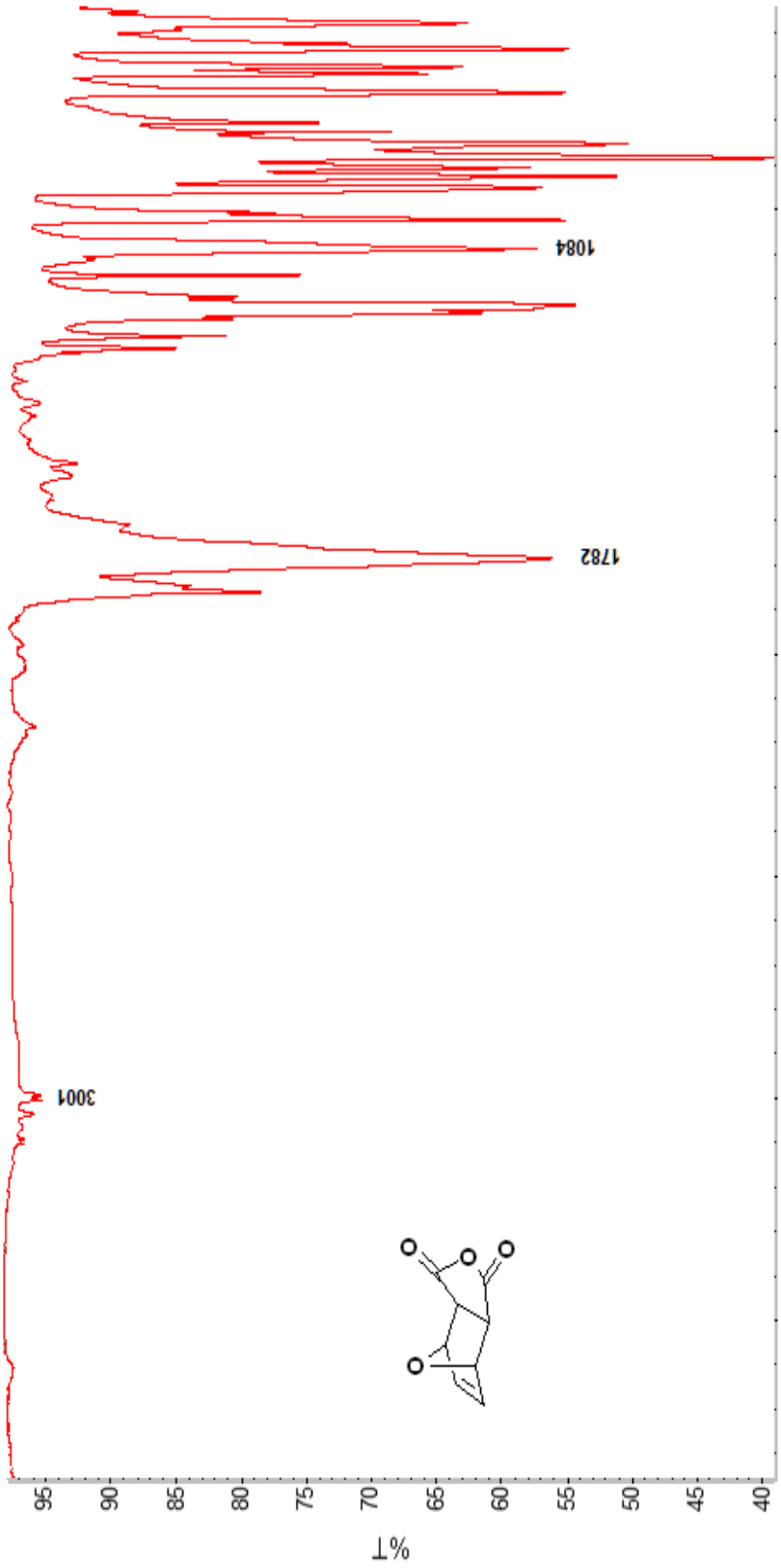

Figure A1 FT-IR of oxanorbornene

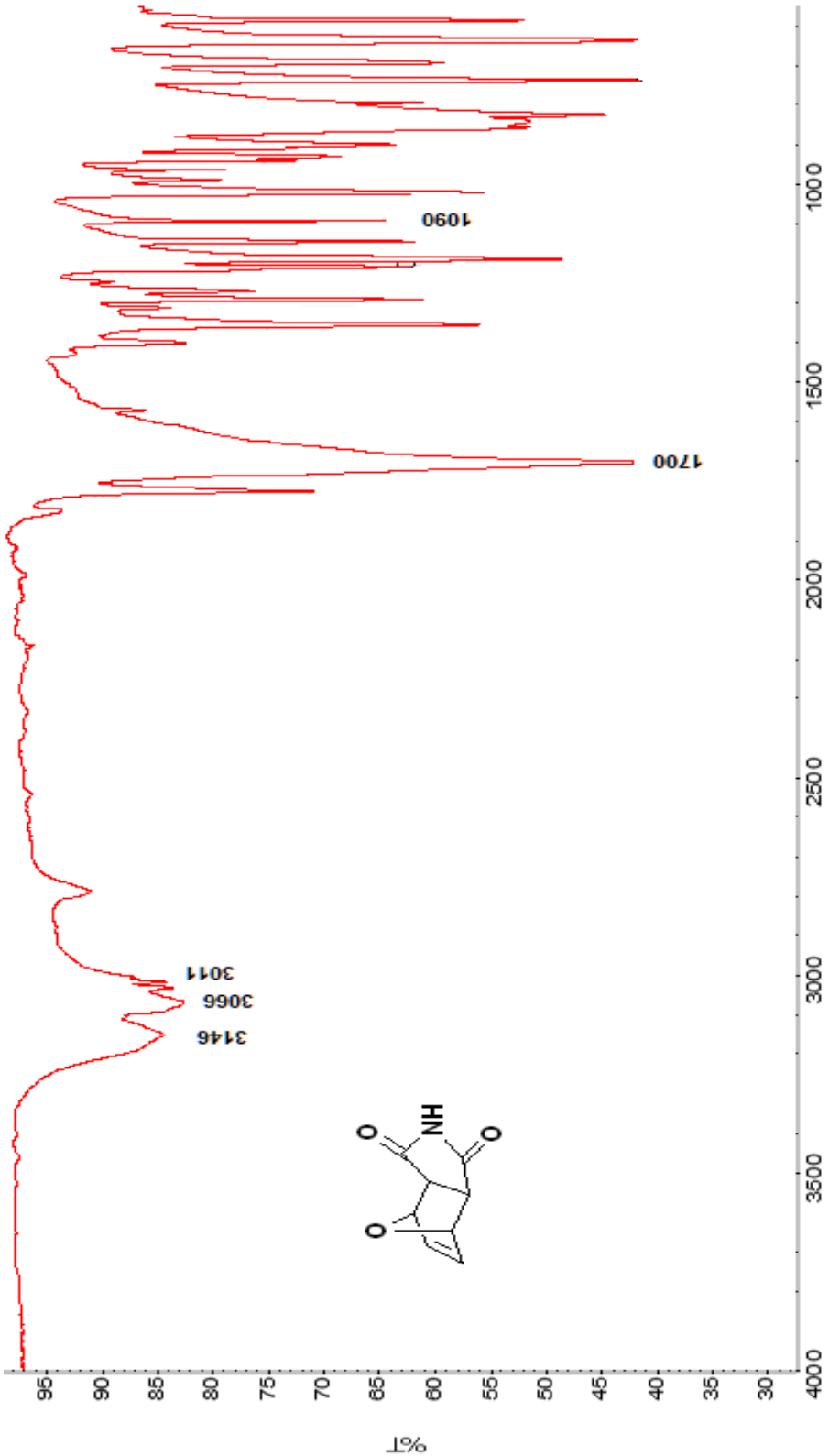

Figure A2 FT-IR of exo-3, 6-tetrahydrophthalide

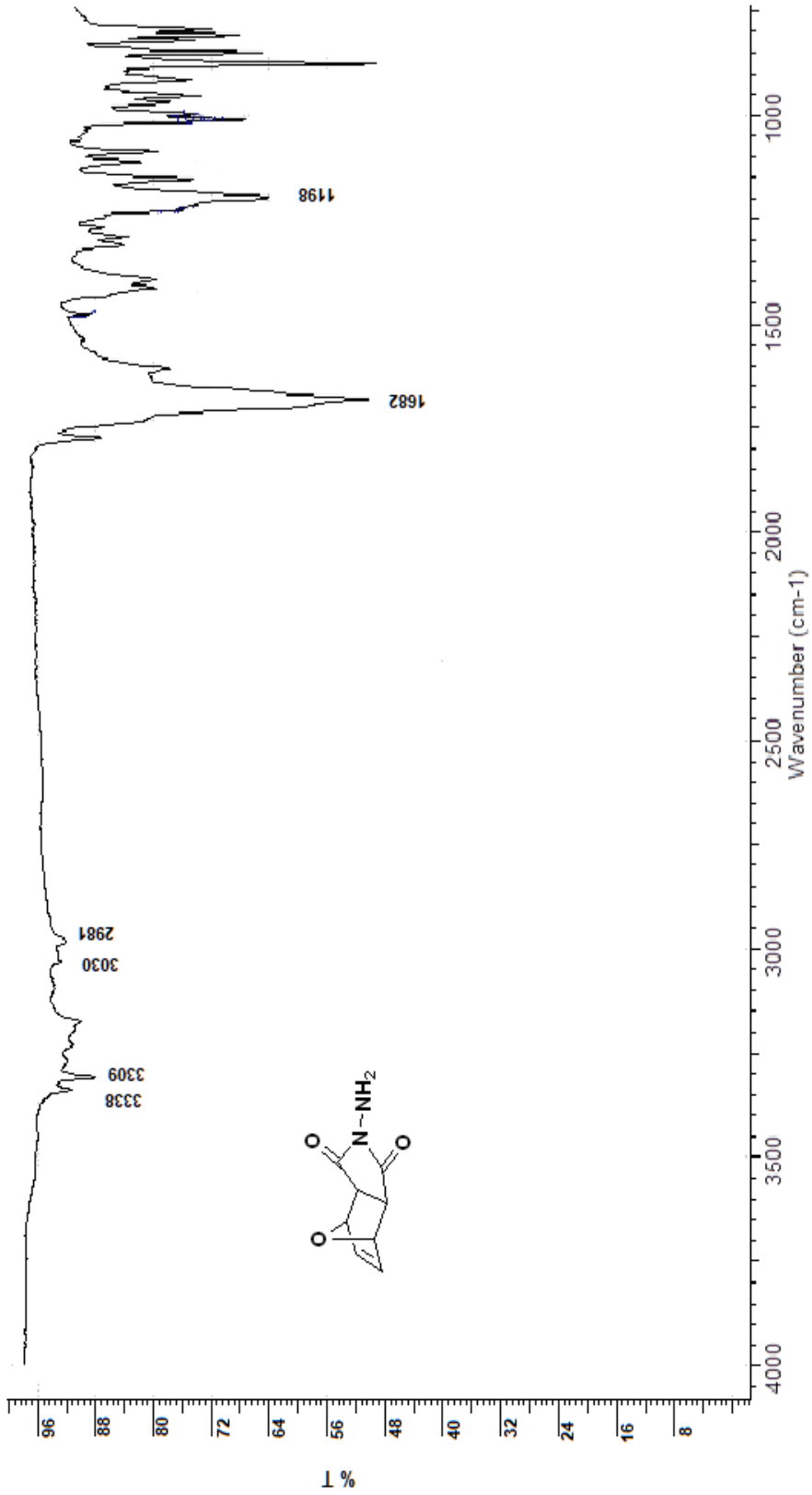

Figure A3 FT-IR monomer core without carbon linker

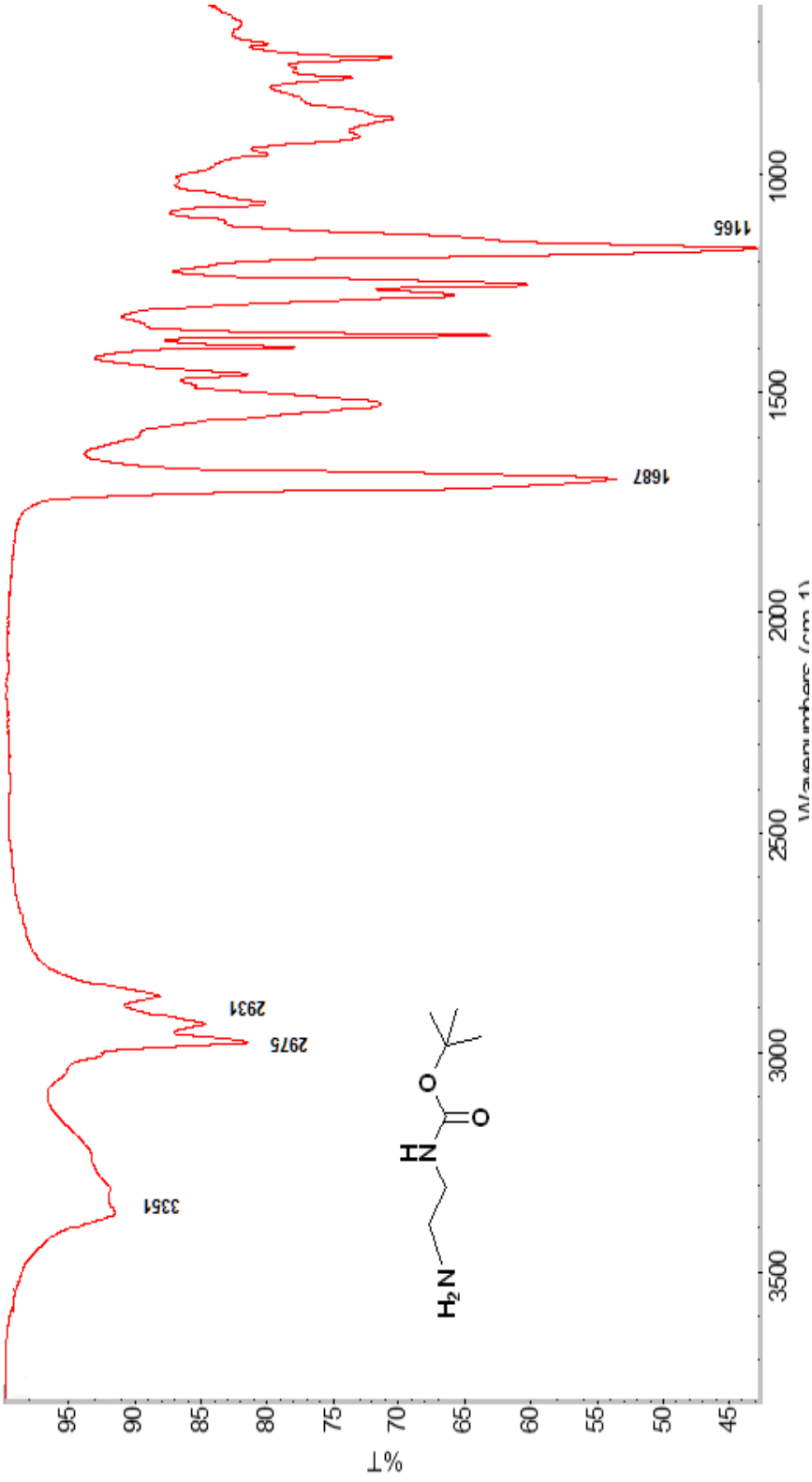

Figure A4 FT-IR of EDA-BOC

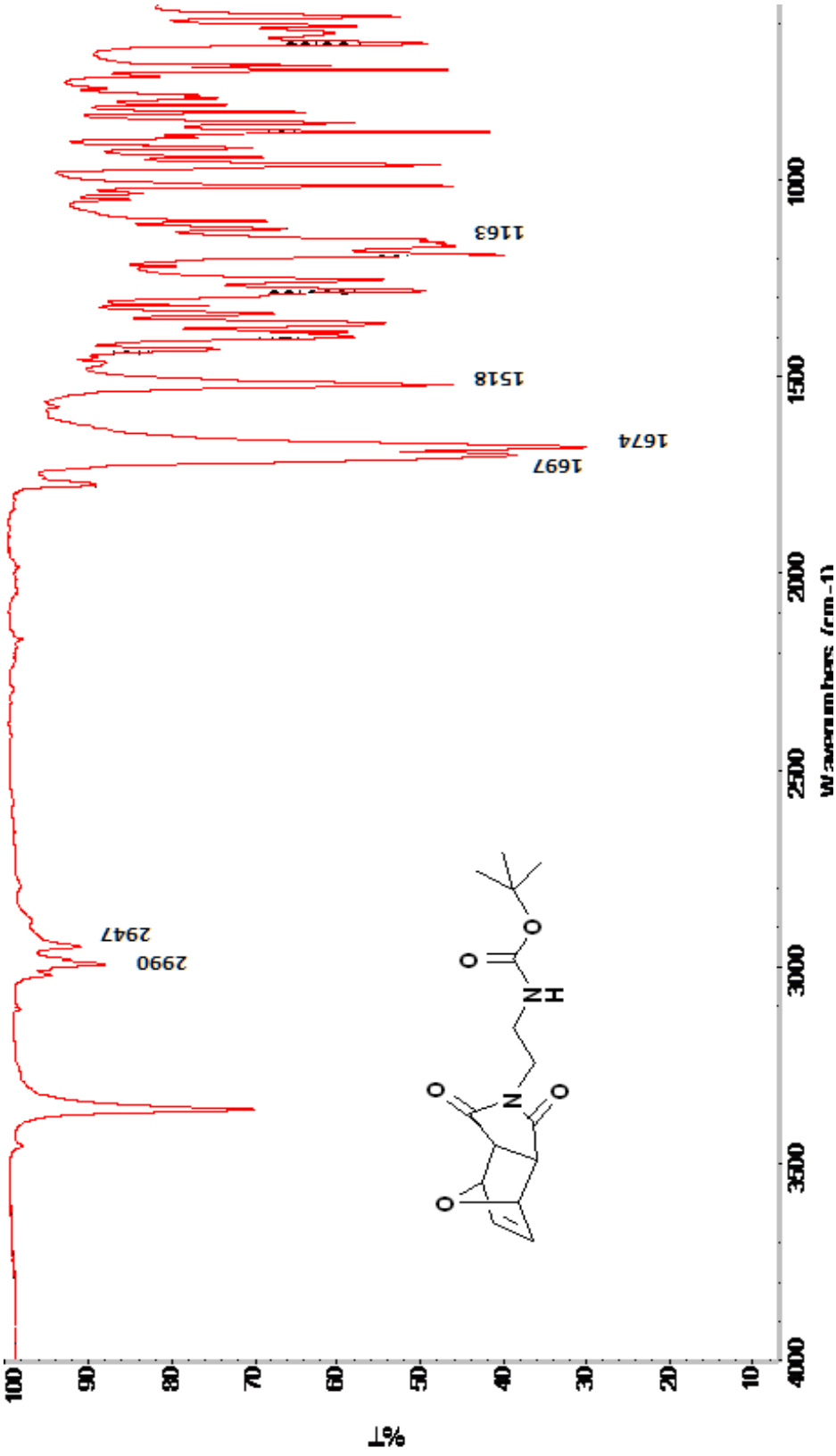

Figure A5 FT-IR of BOC functionalized two carbon linker monomer

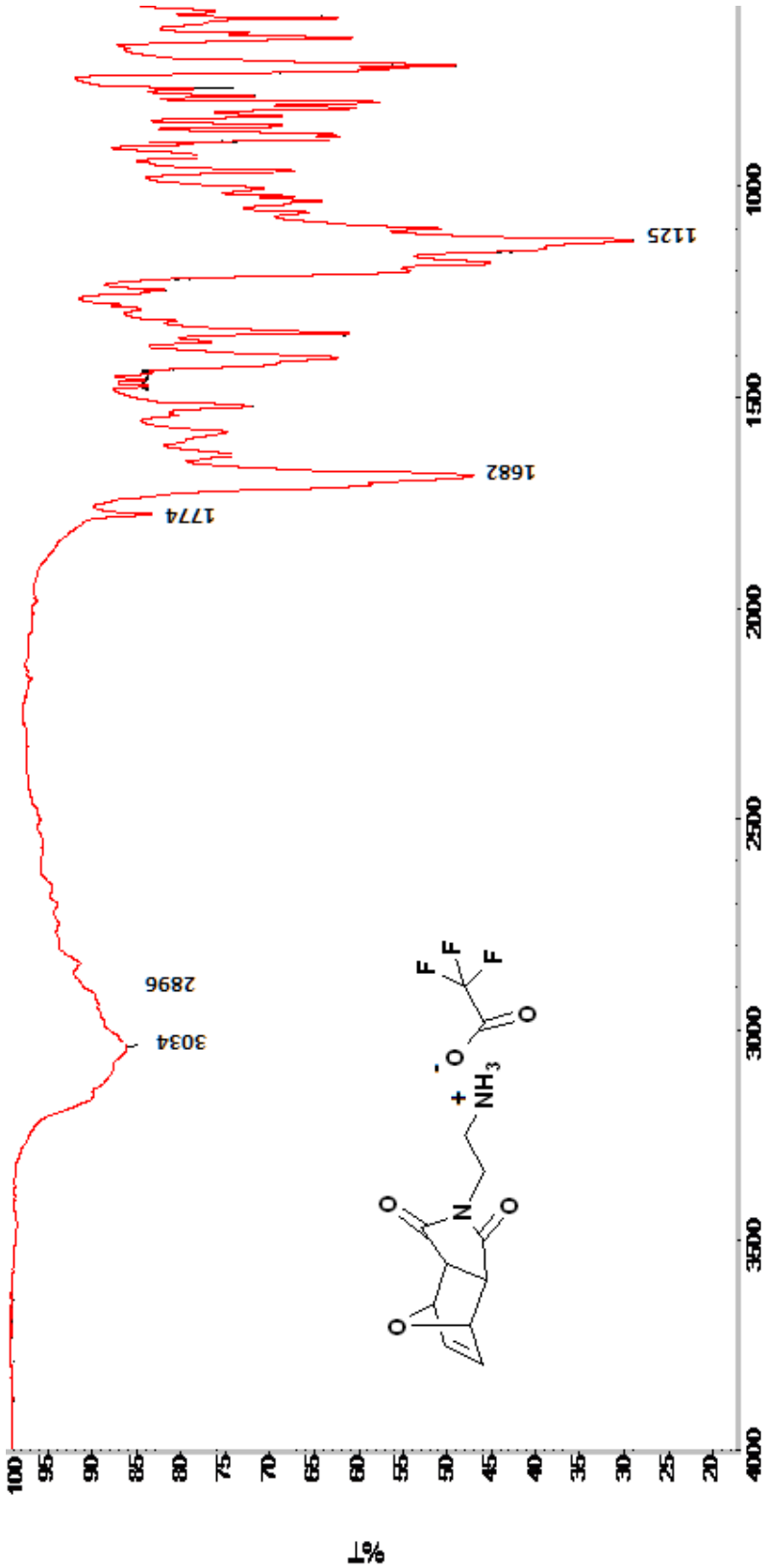

Figure A6 FT-IR of TFA salt of two carbon linker monomer

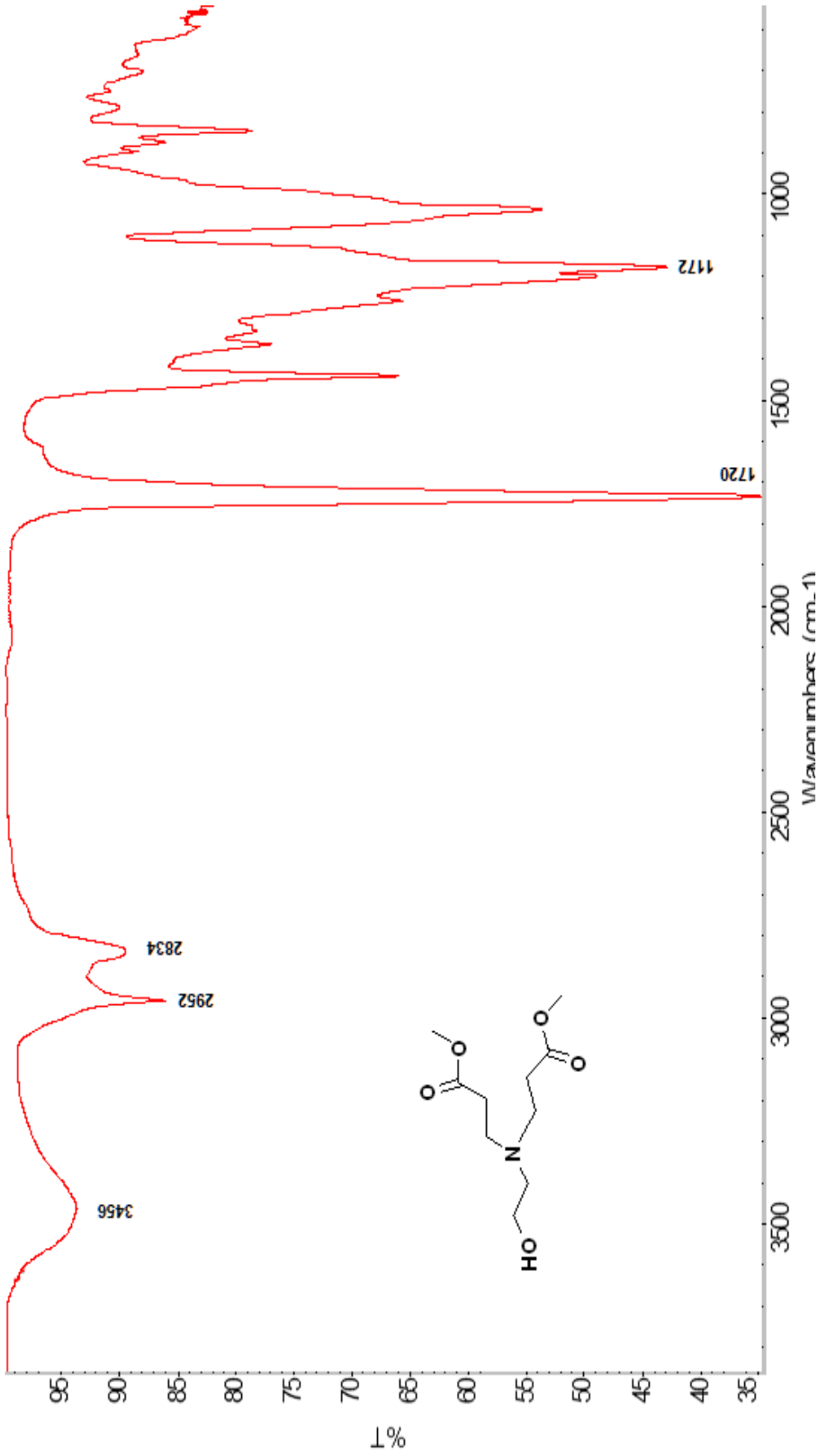

Figure A7 FT-IR of ethanolamine cored 0.5 generation dendritic monomer

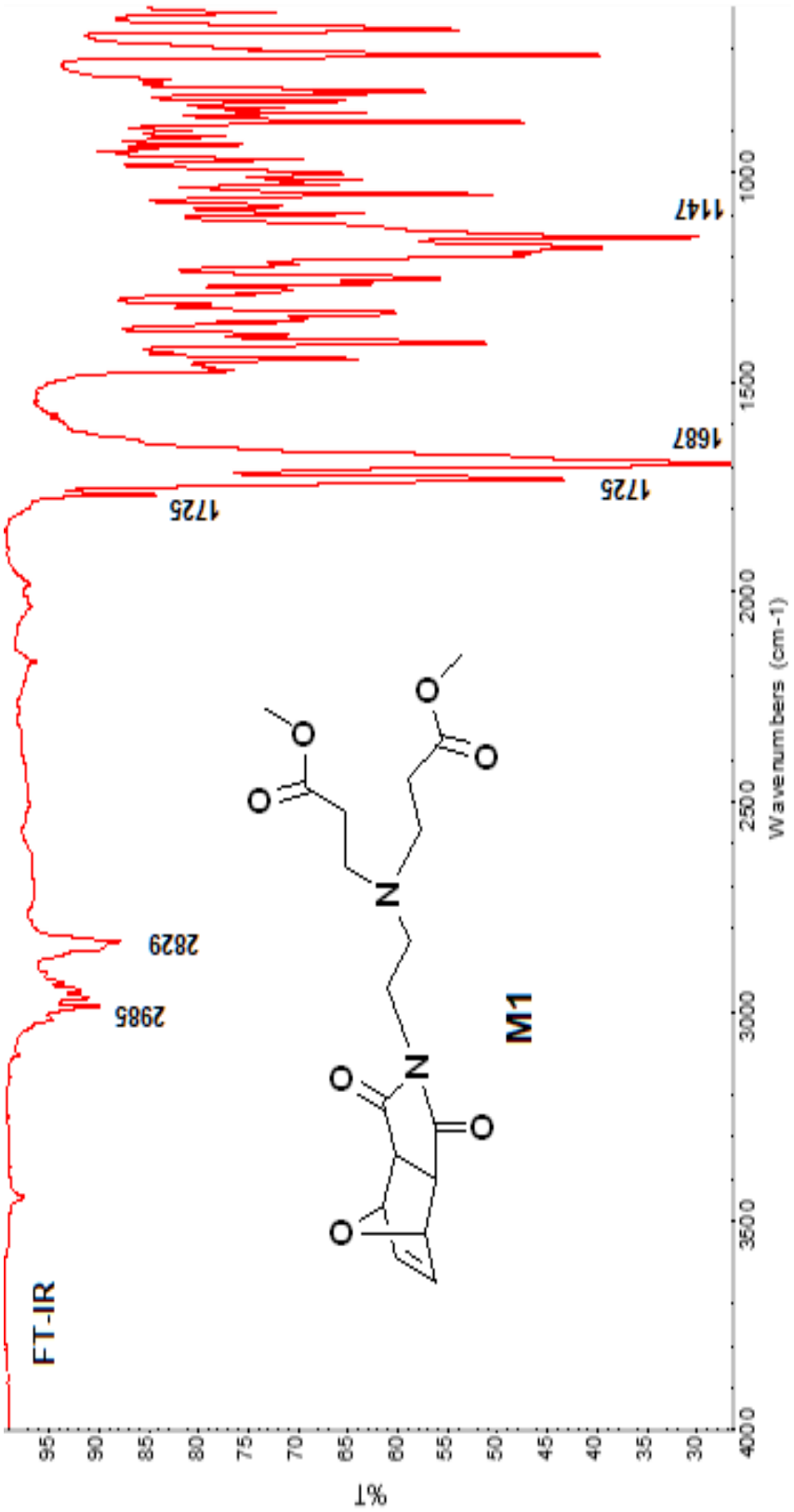

Figure A8 FT-IR of two carbon linker 0.5 generation dendritic monomer

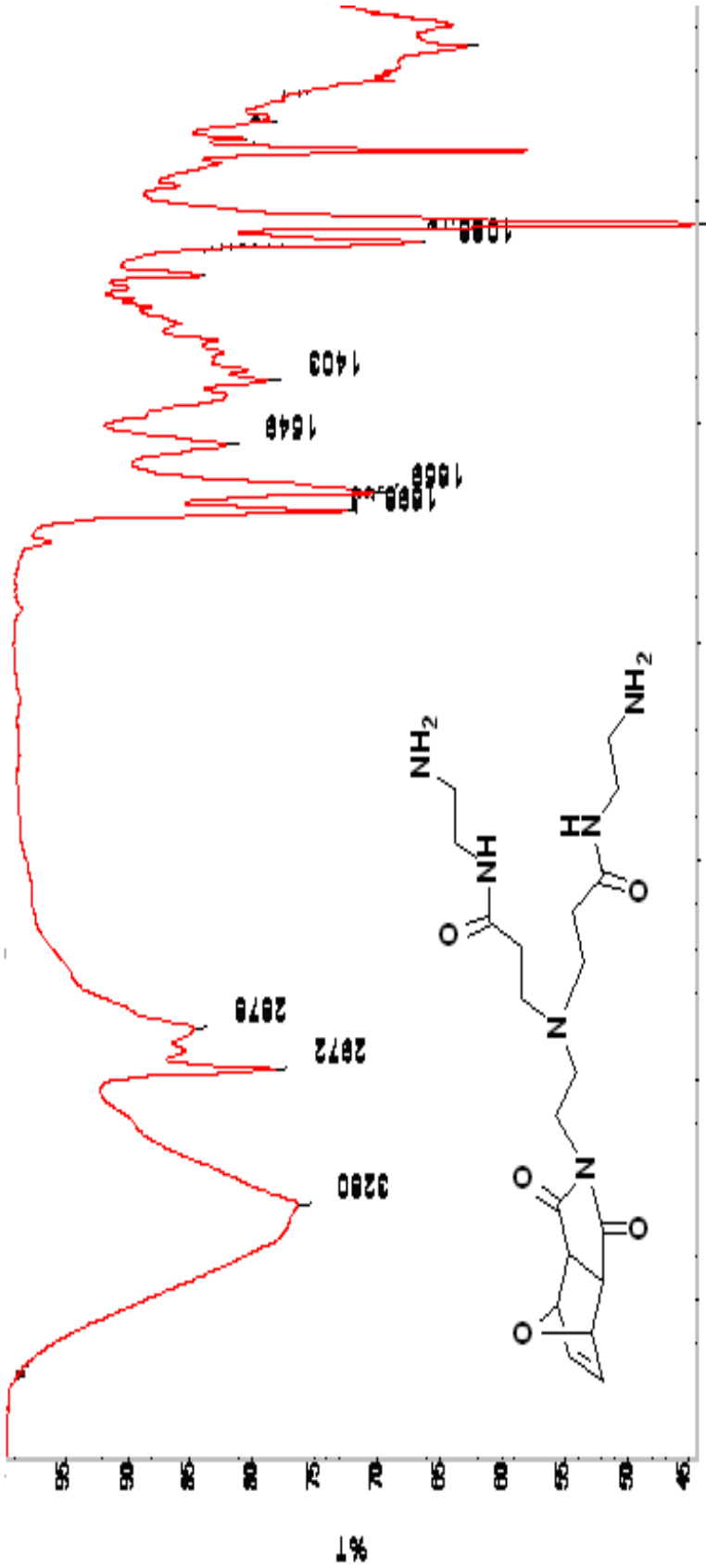

Figure A9 FT-IR of two carbon linker cored 1.0 generation dendritic monomer

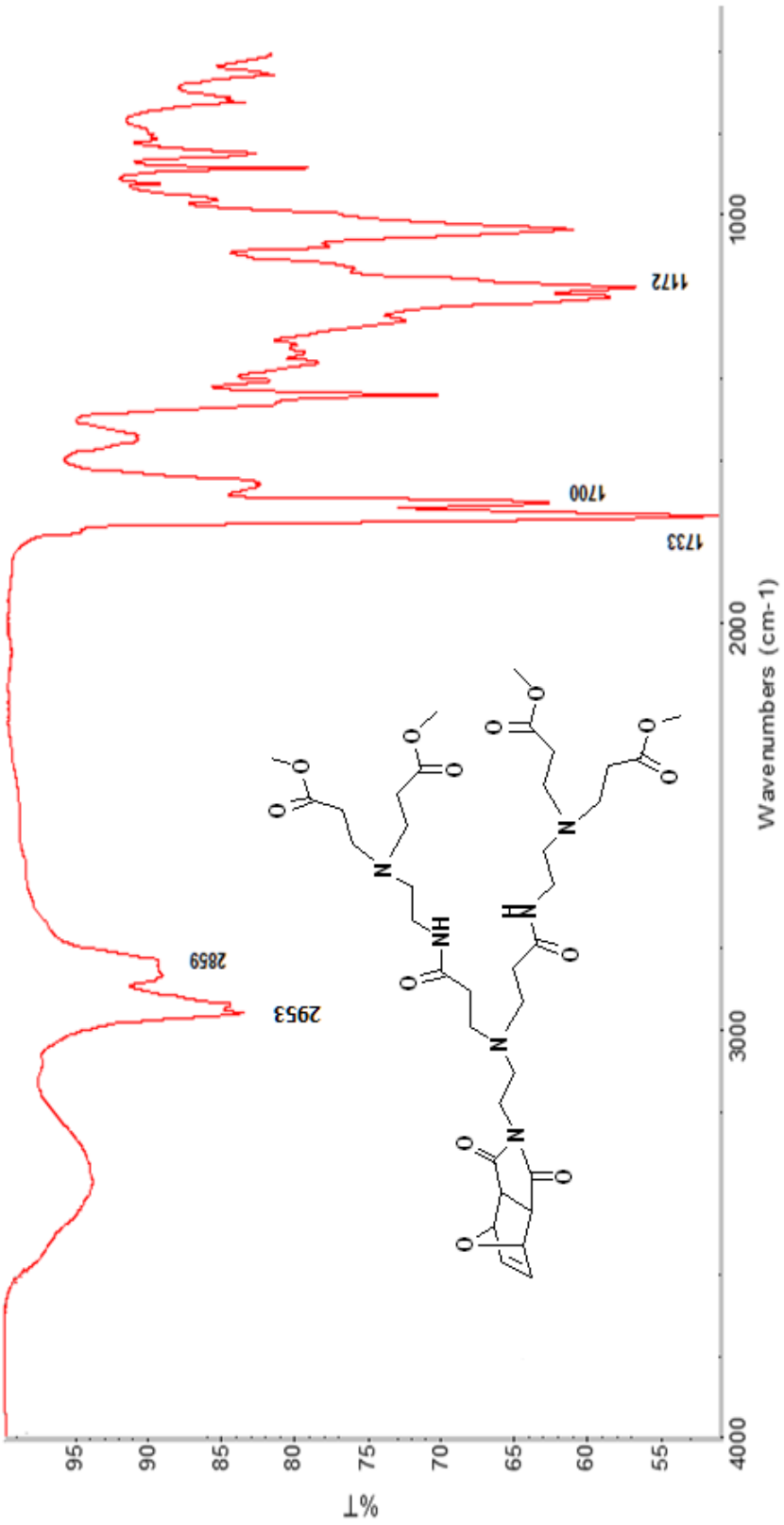

Figure A10 FT-IR of two carbon linker cored 1.5 generation dendritic monomer

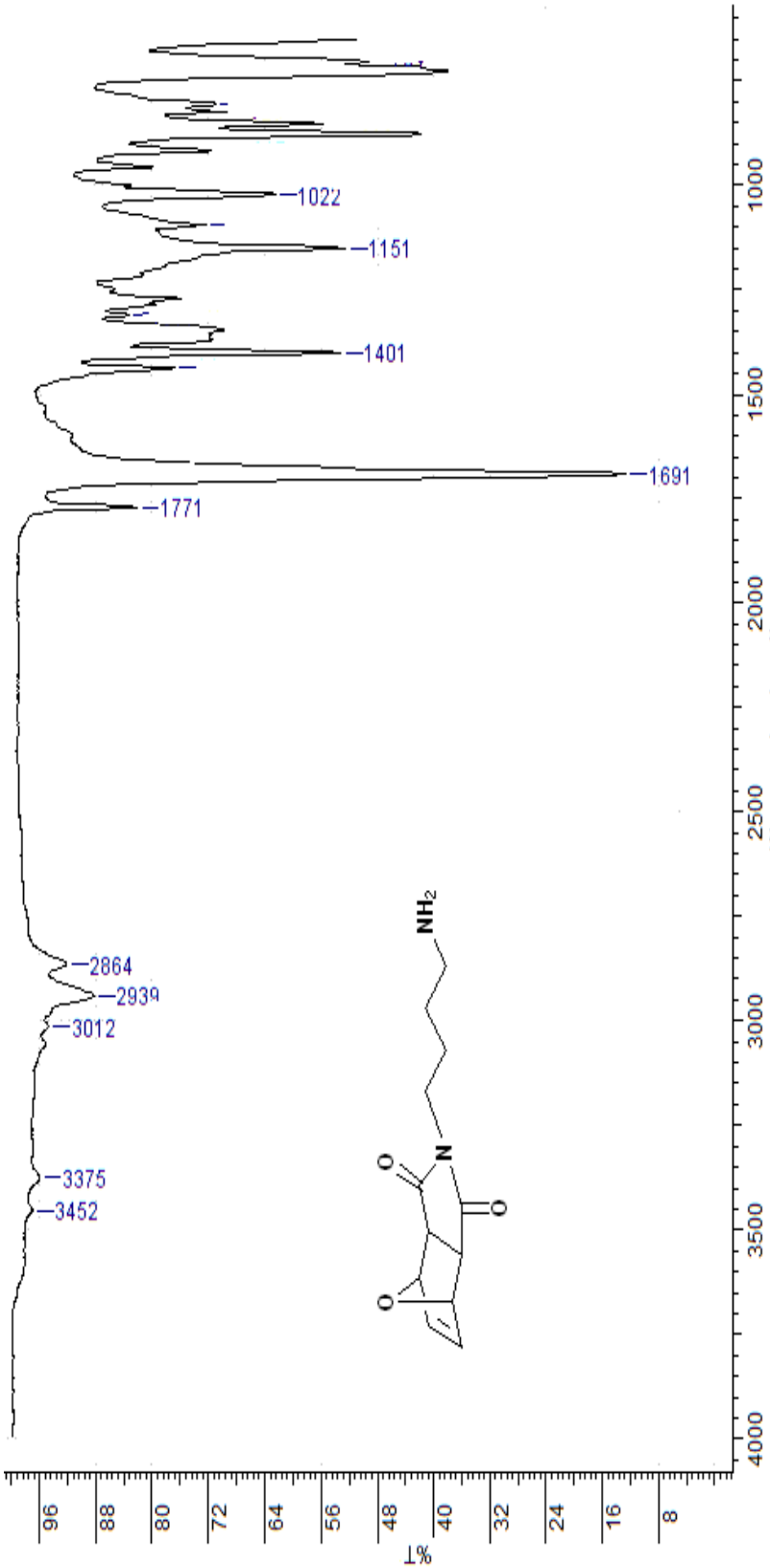

Figure A11 FT-IR of four carbon linker core

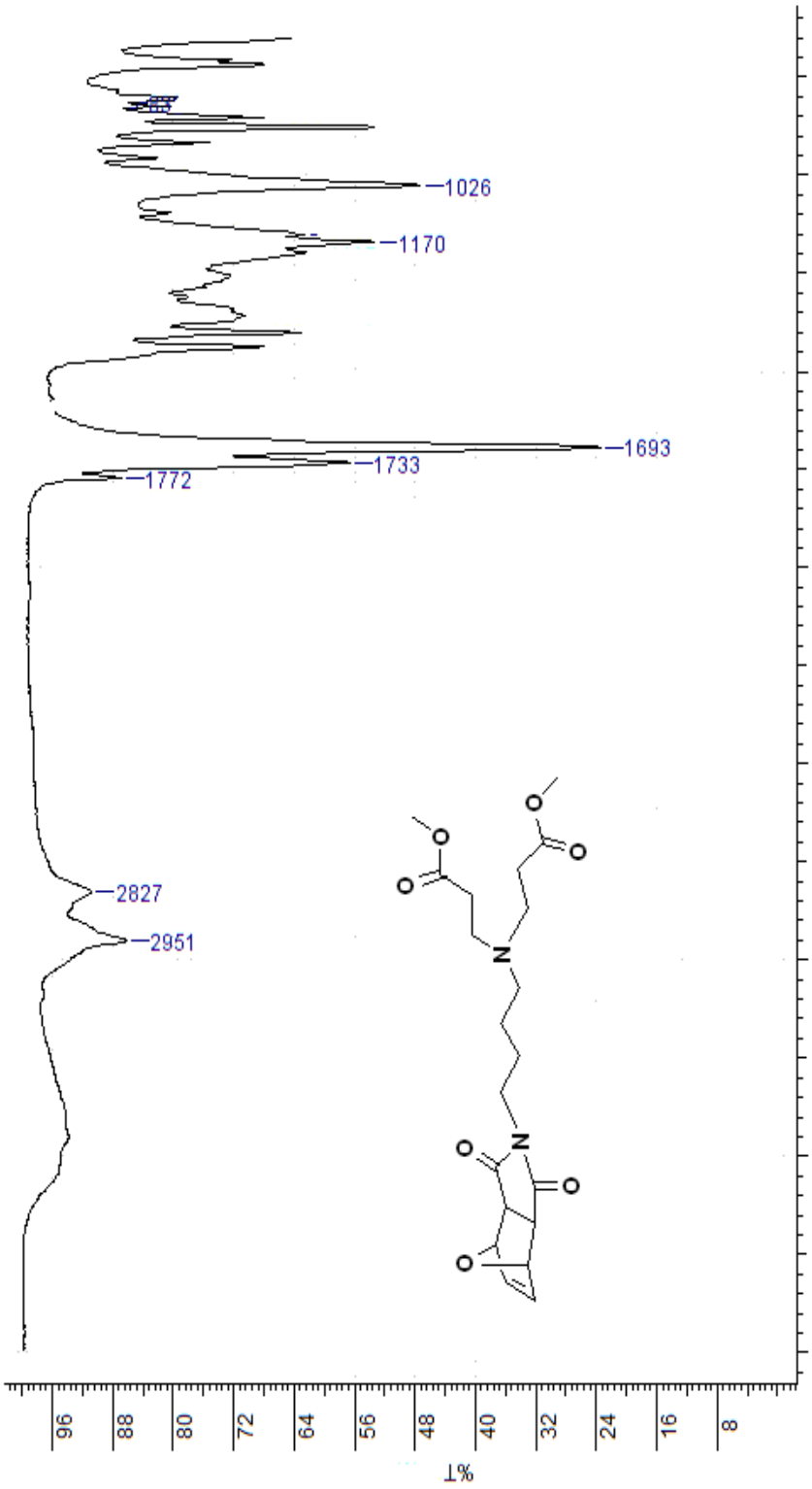

Figure A12 FT-IR o of four carbon linker 0.5 generation dendritic monomer

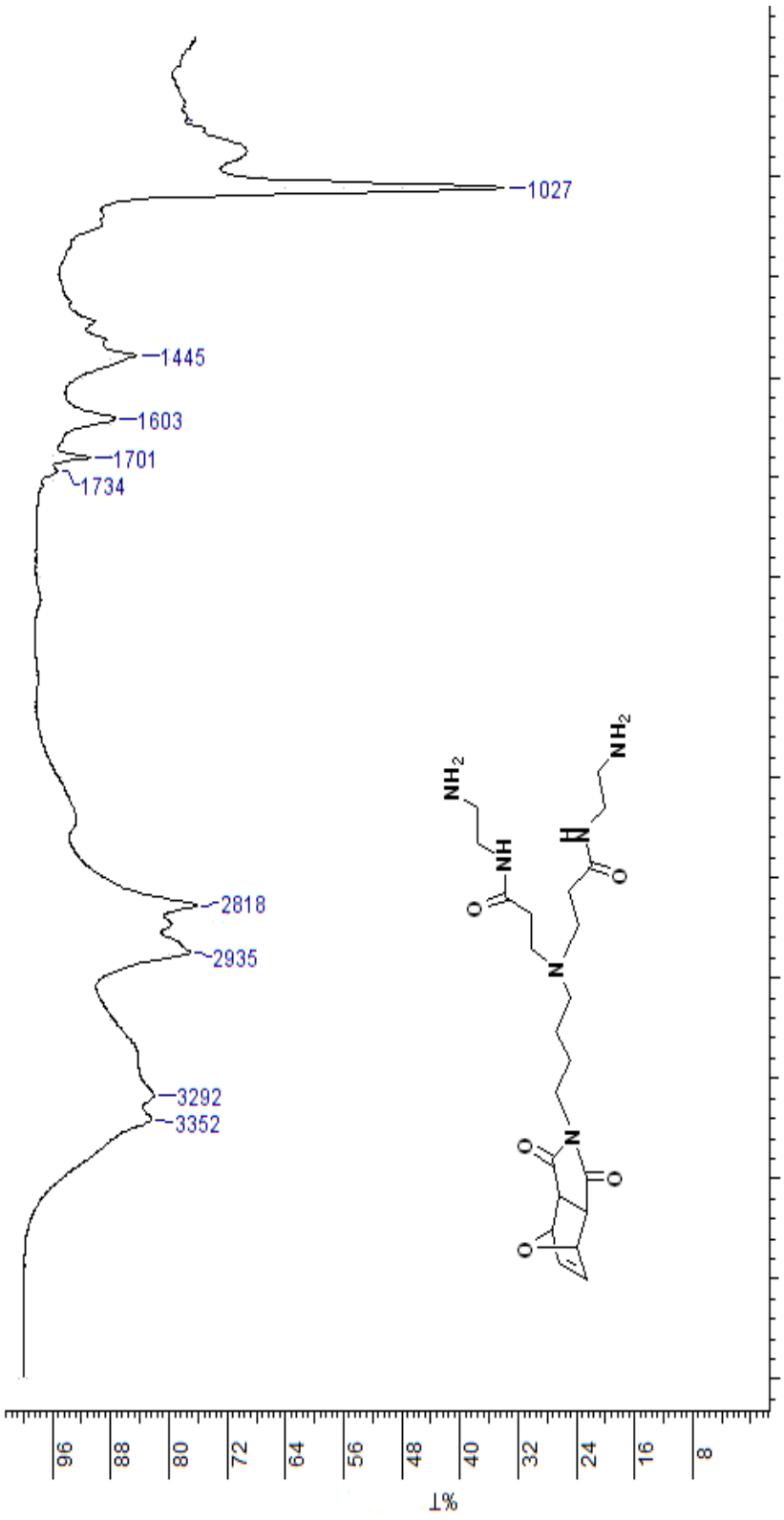

Figure A13 FT-IR of four carbon linker 1.0 generation dendritic monomer

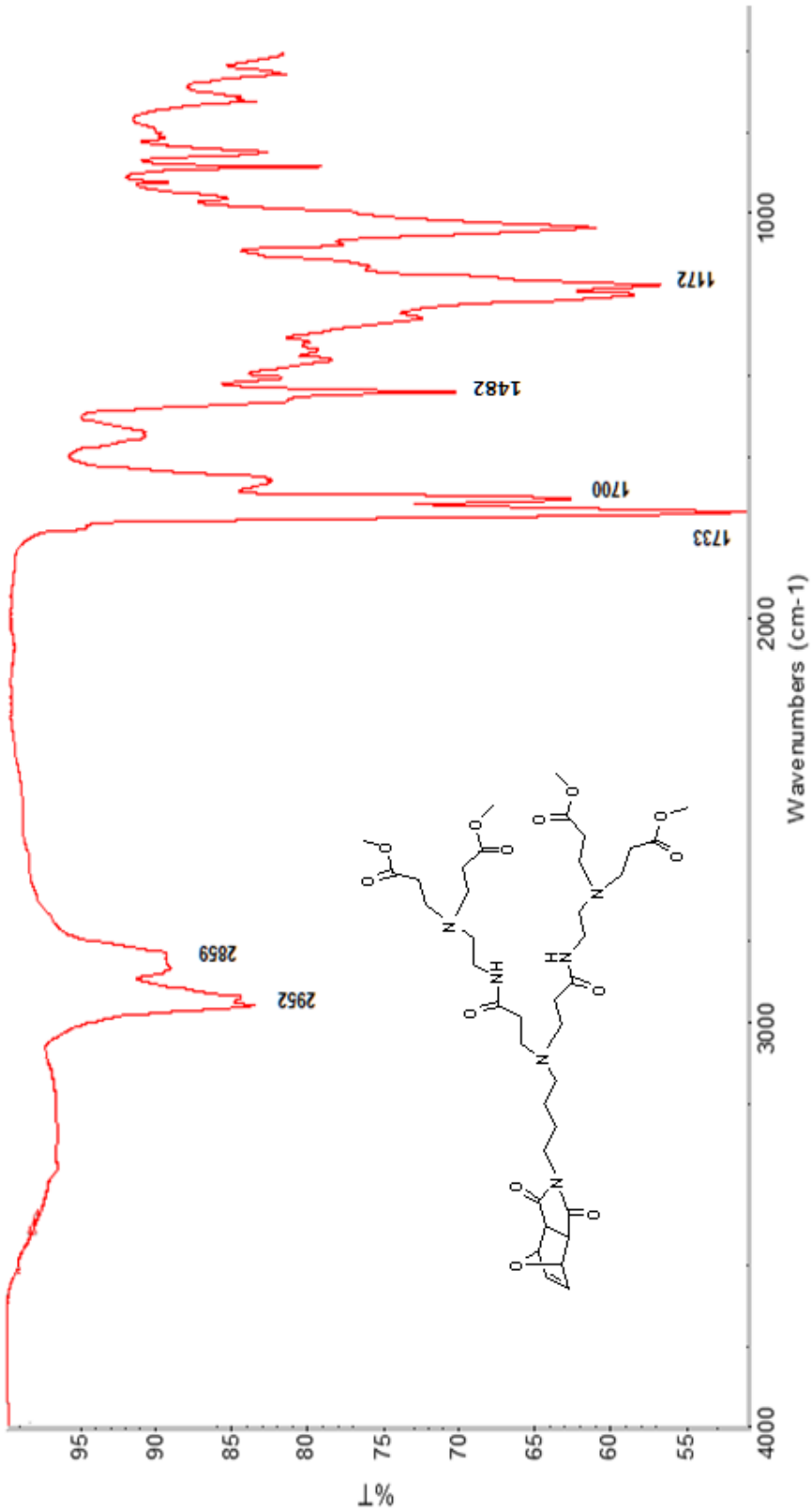

Figure A14 FT-IR of four carbon linker 1.5 generation dendritic monomer

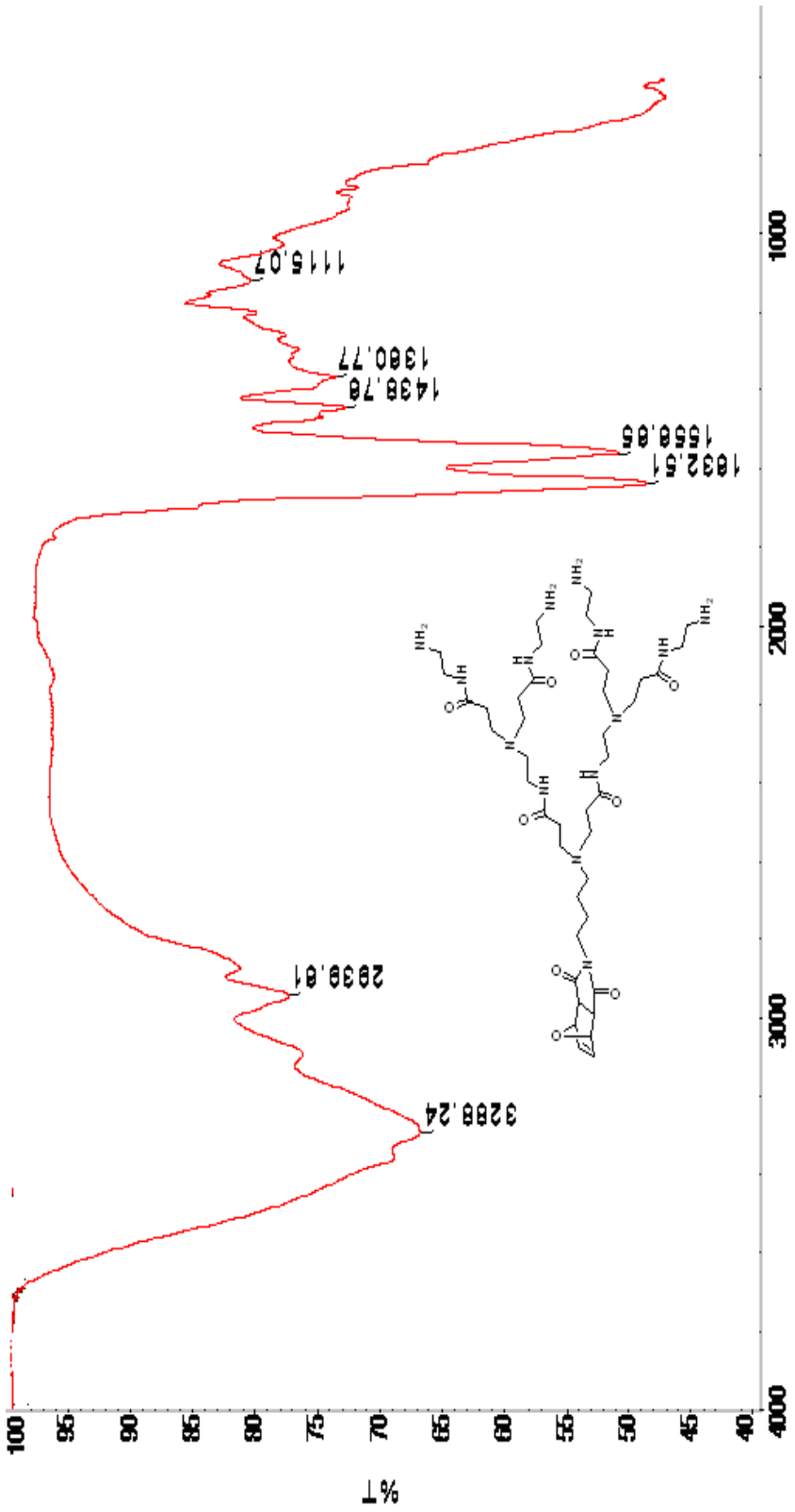

Figure A15 FT-IR of four carbon linker 2.0 generation dendritic monomer

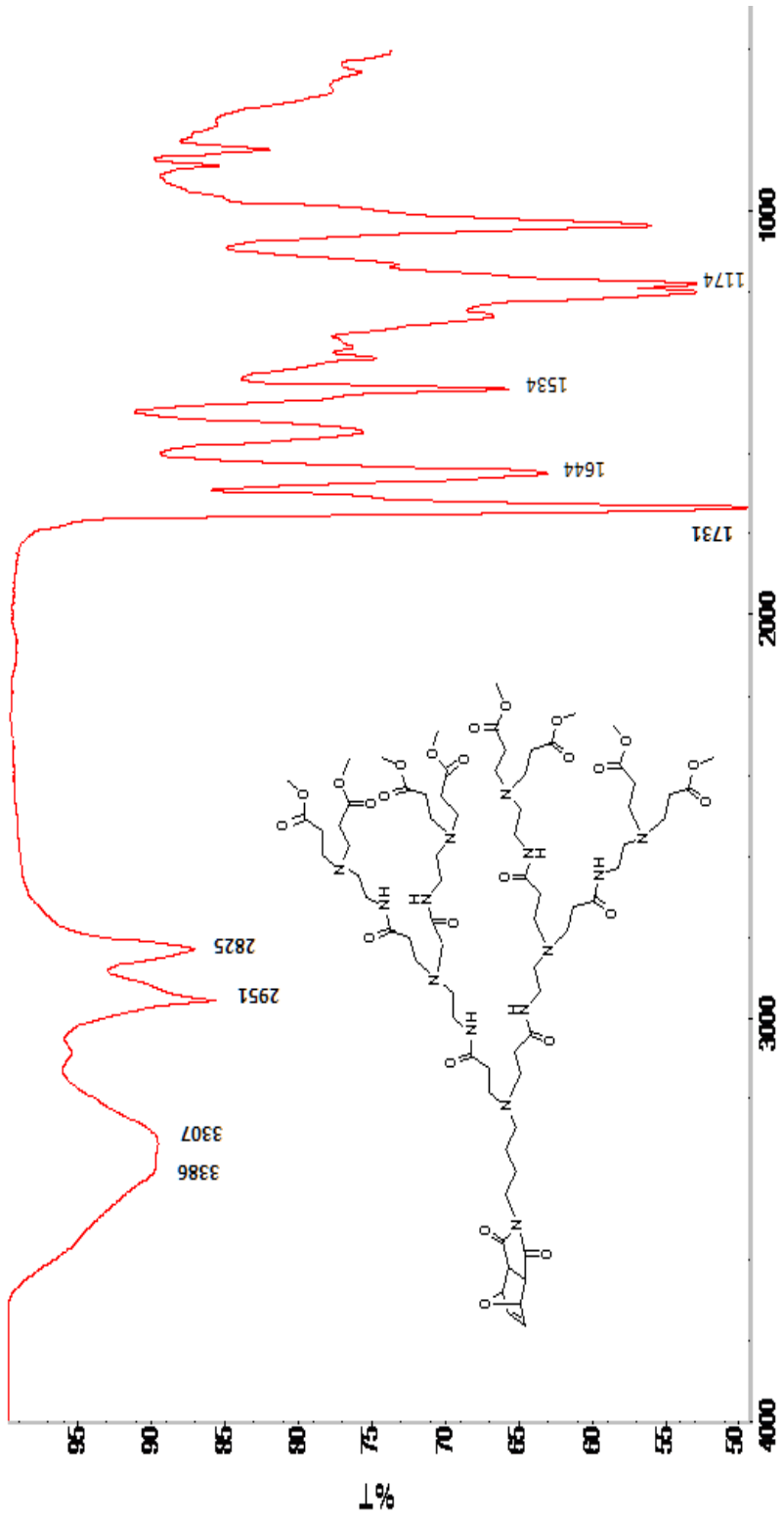

Figure A16 FT-IR of four carbon linker 2.5 generation dendritic monomer

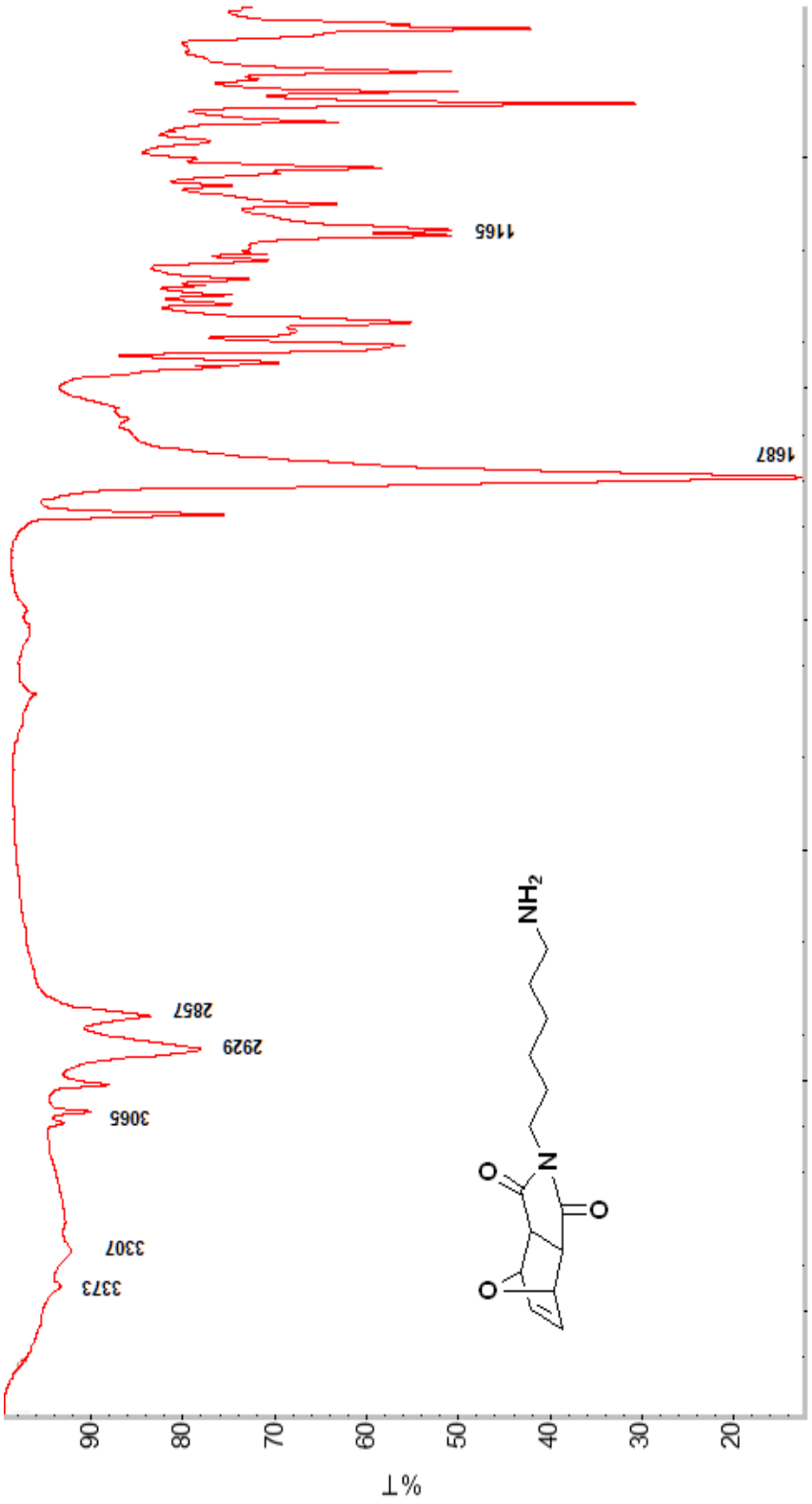

Figure A17 Six carbon linker monomer core

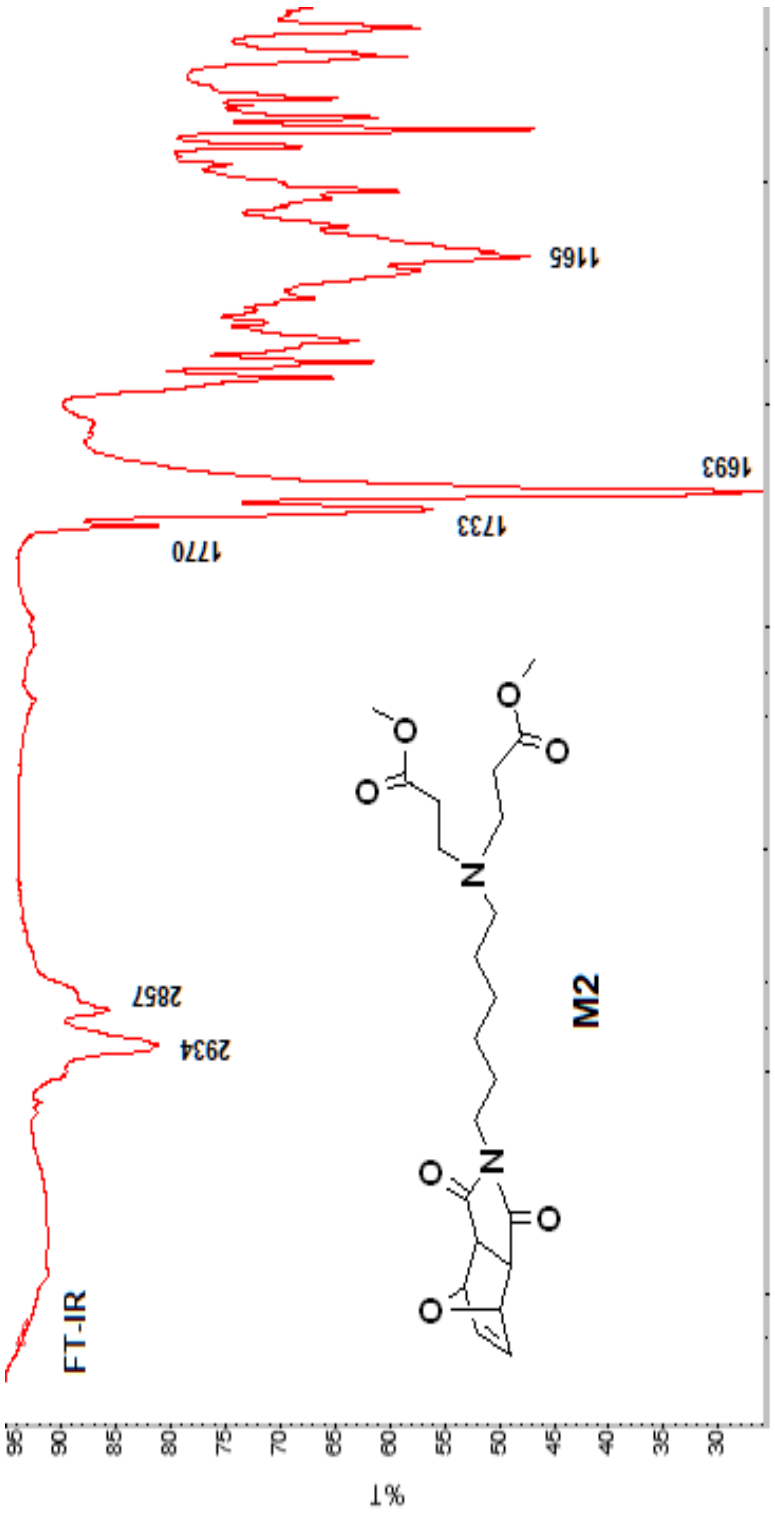

Figure A18 Six carbon linker 0.5 generation dendritic monomer

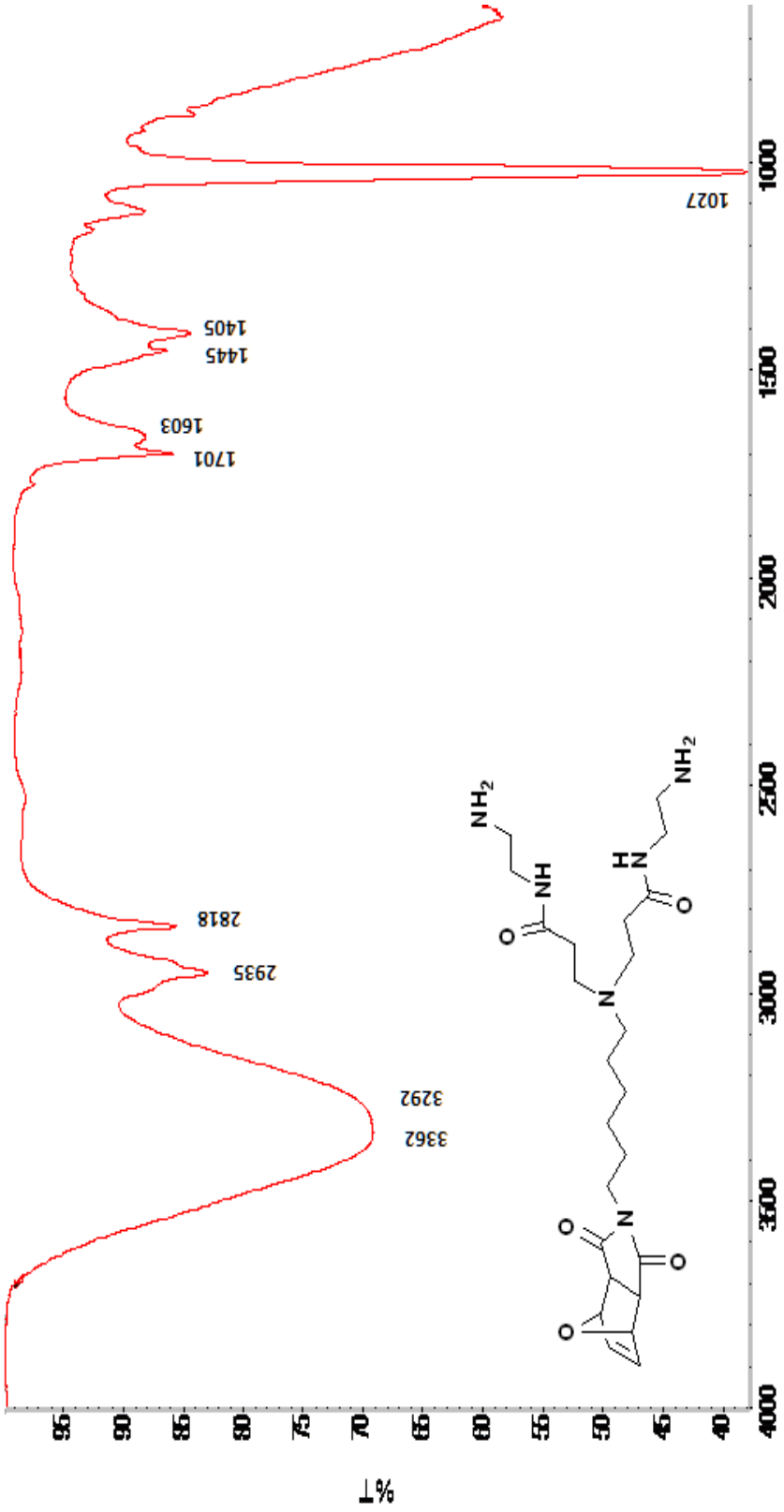

Figure A19 Six carbon linker 1.0 generation dendritic monomer

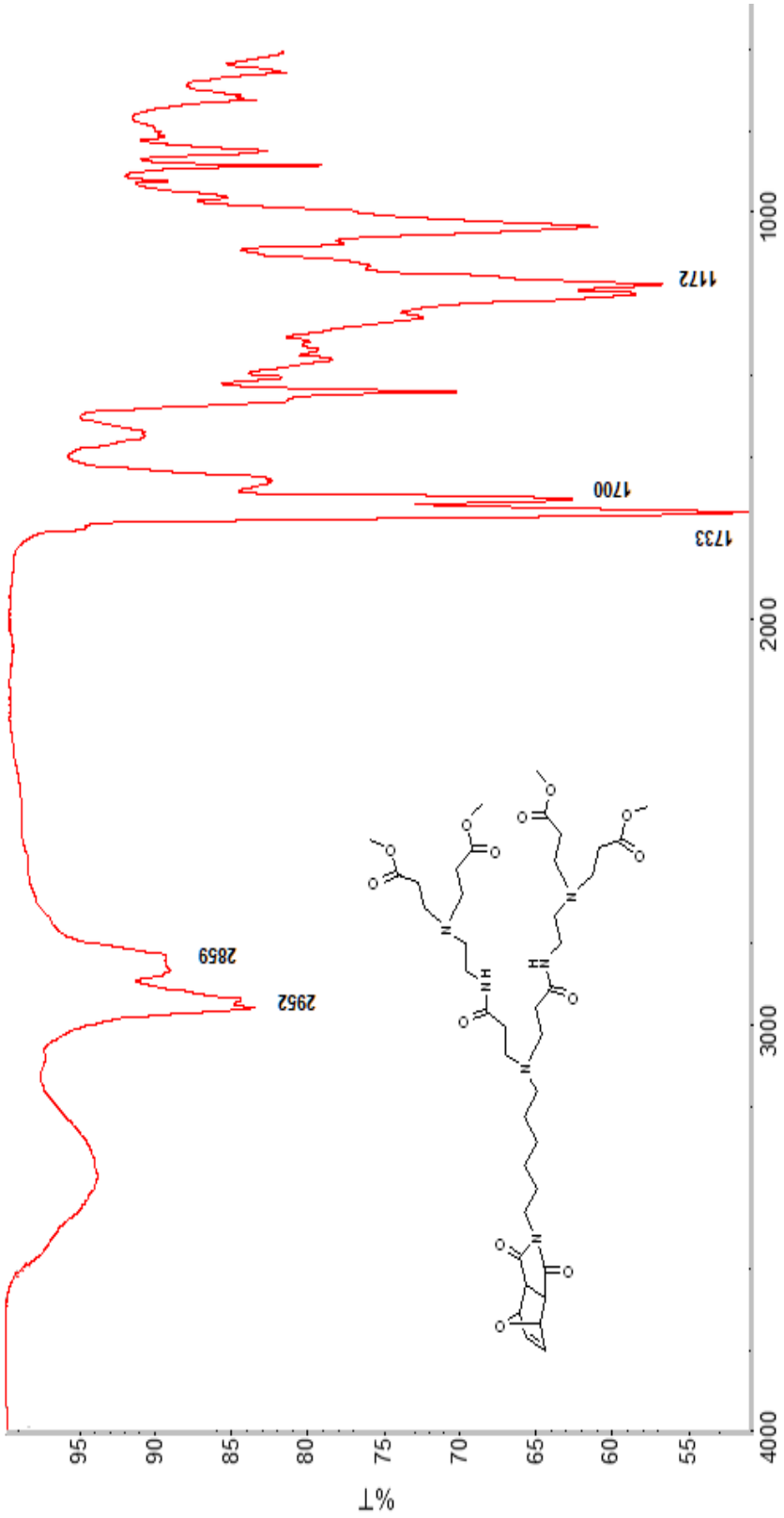

Figure A20 FR-IR of six carbon linker 1.5 generation dendritic monomer

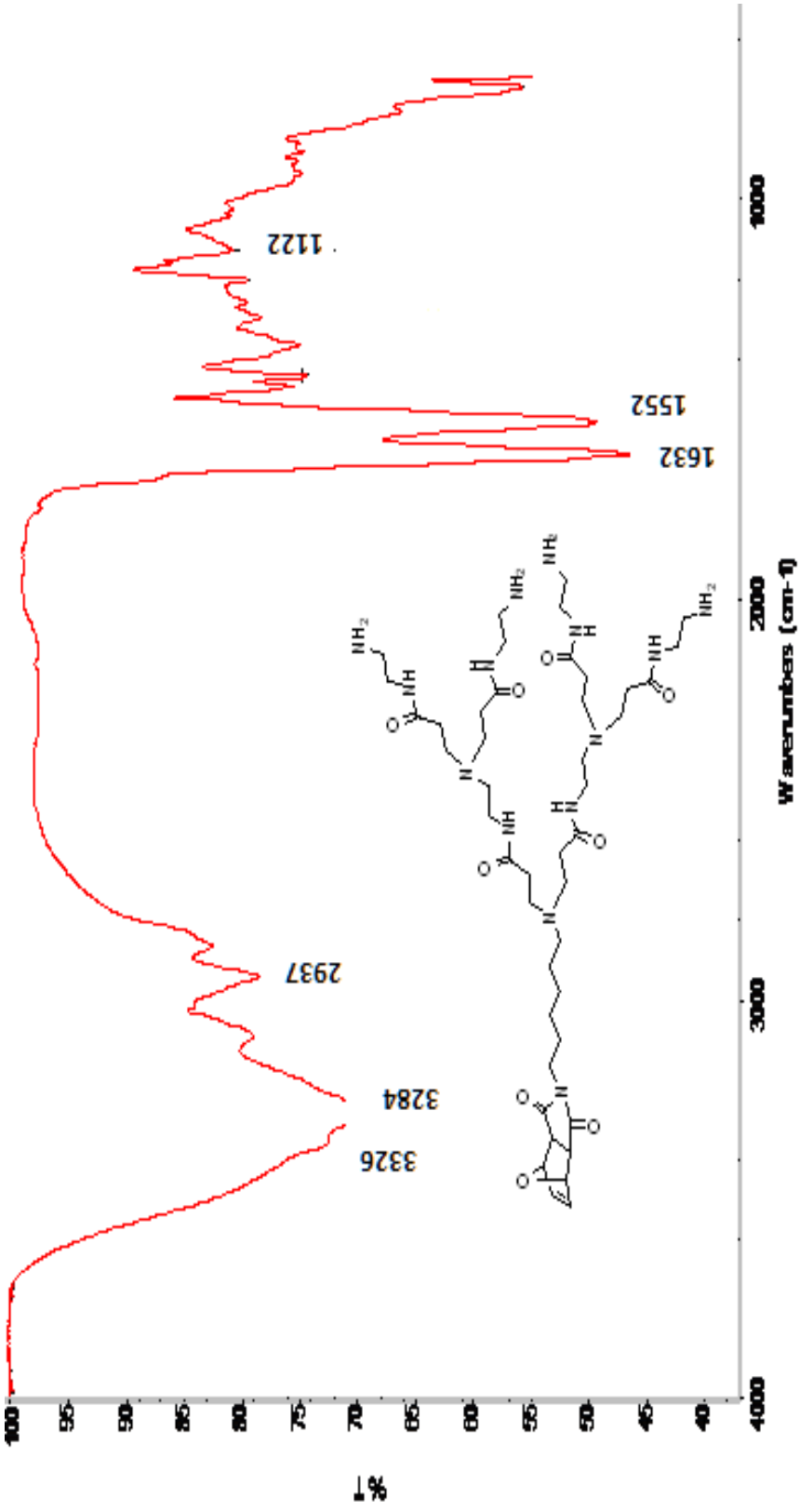

Figure A21 FR-IR of six carbon linker 2.0 generation dendritic monomer

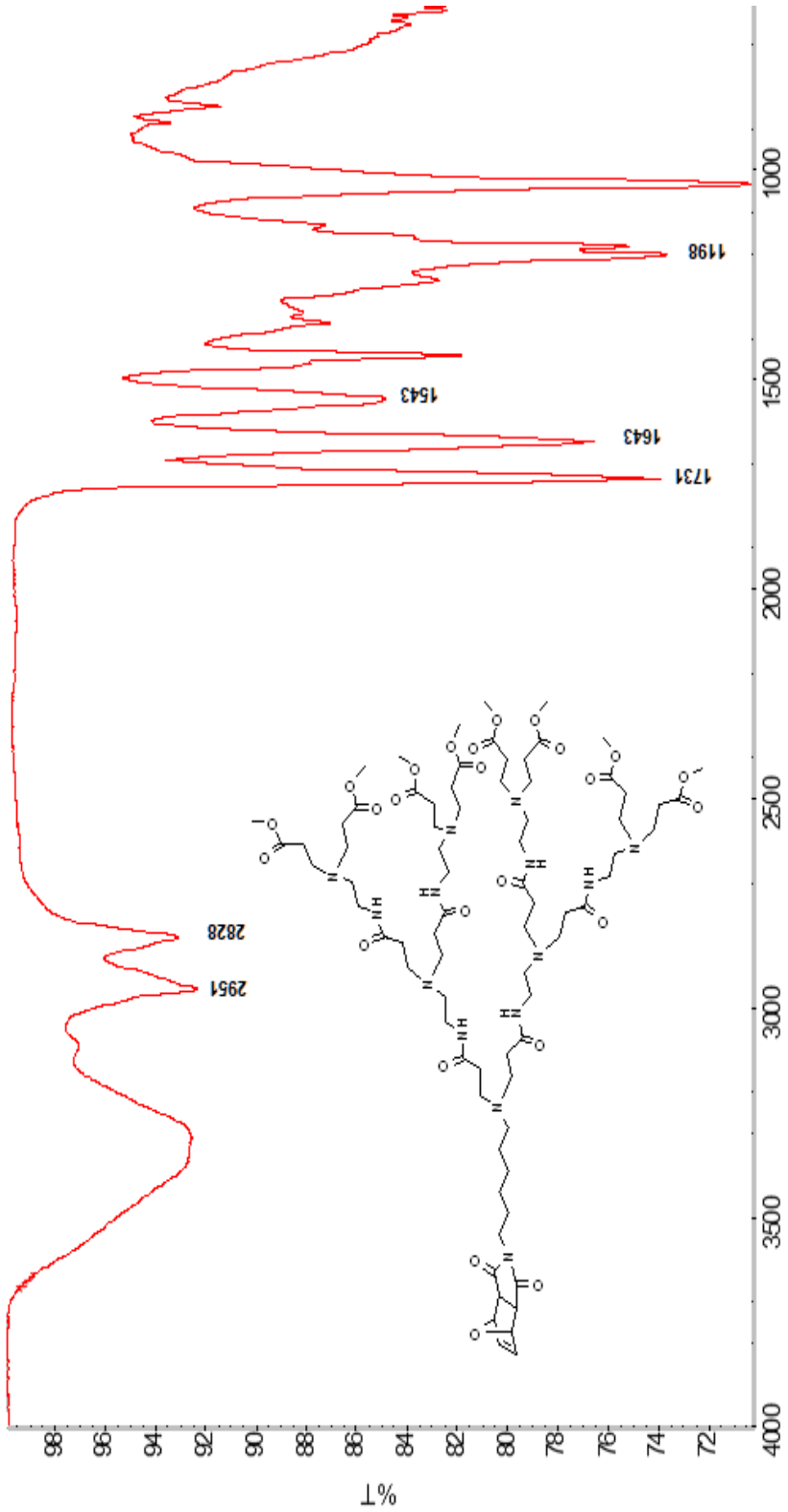

Figure A22 FR-IR of six carbon linker 2.5 generation dendritic monomer

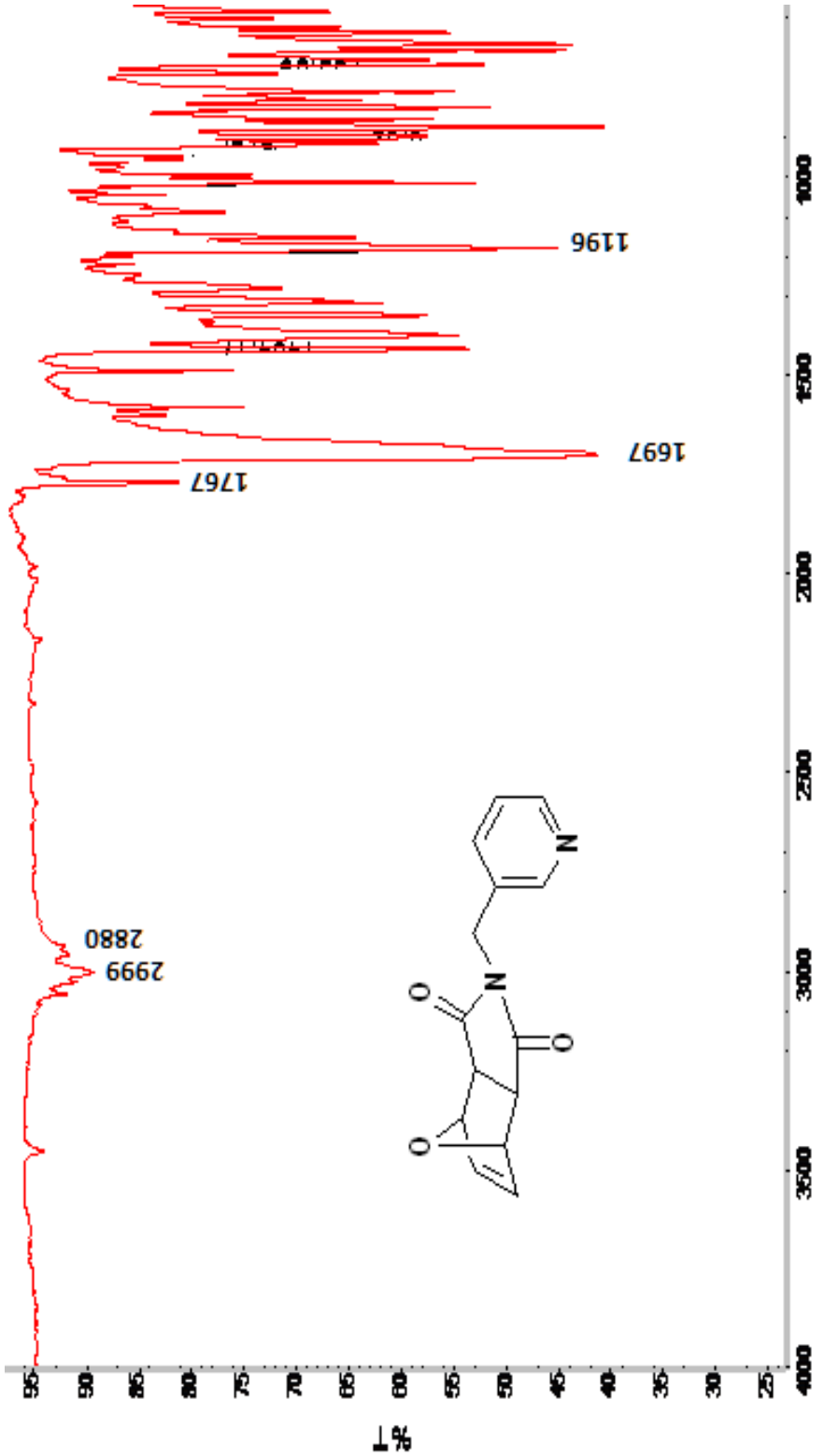

Figure A23 FR-IR of pyridine based oxanorbornene

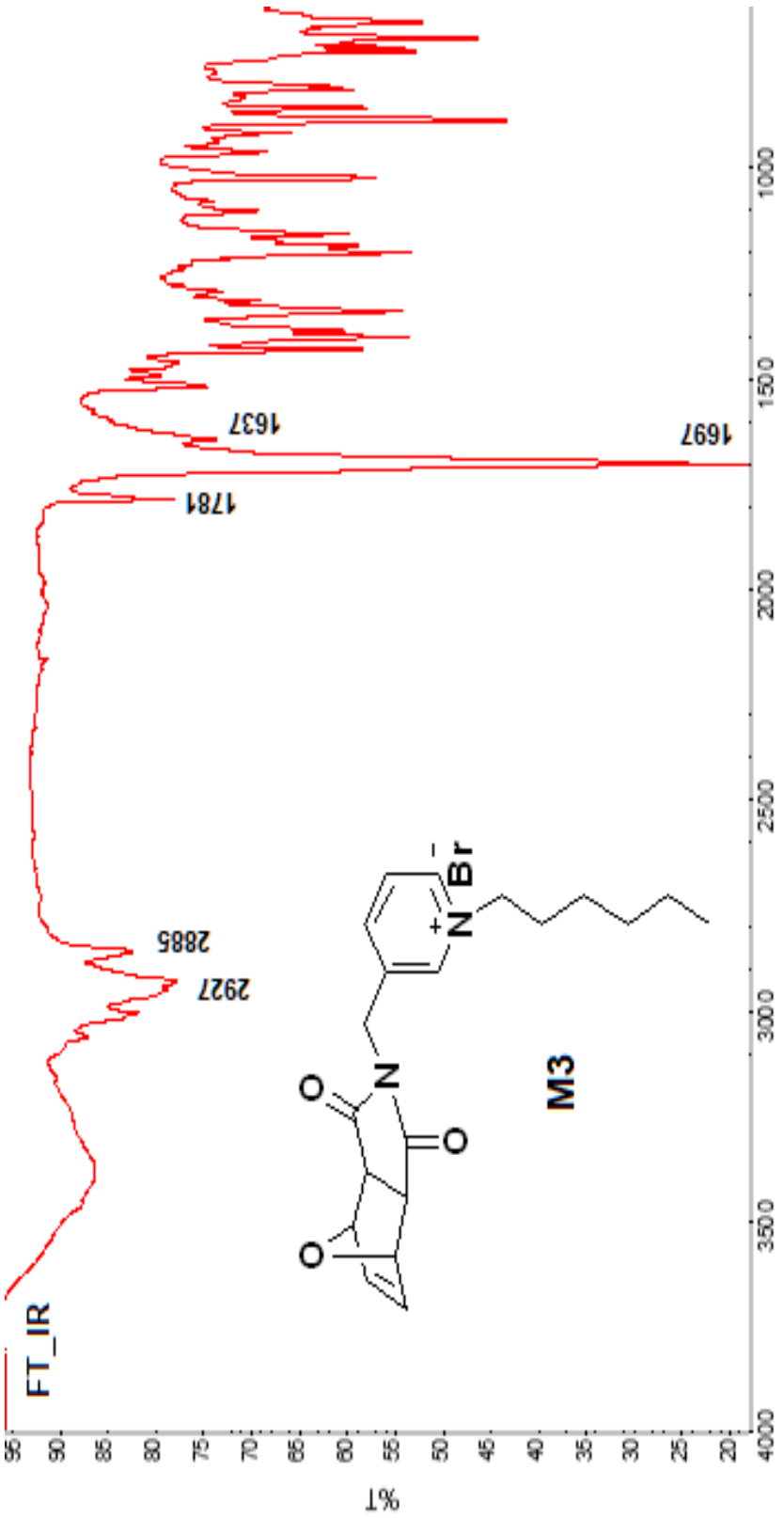

Figure A24 FR-IR of hexyl pyridinium based oxanorbornene

B

^{1}H NMR, ^{13}C NMR, AND MASS SPECTRA

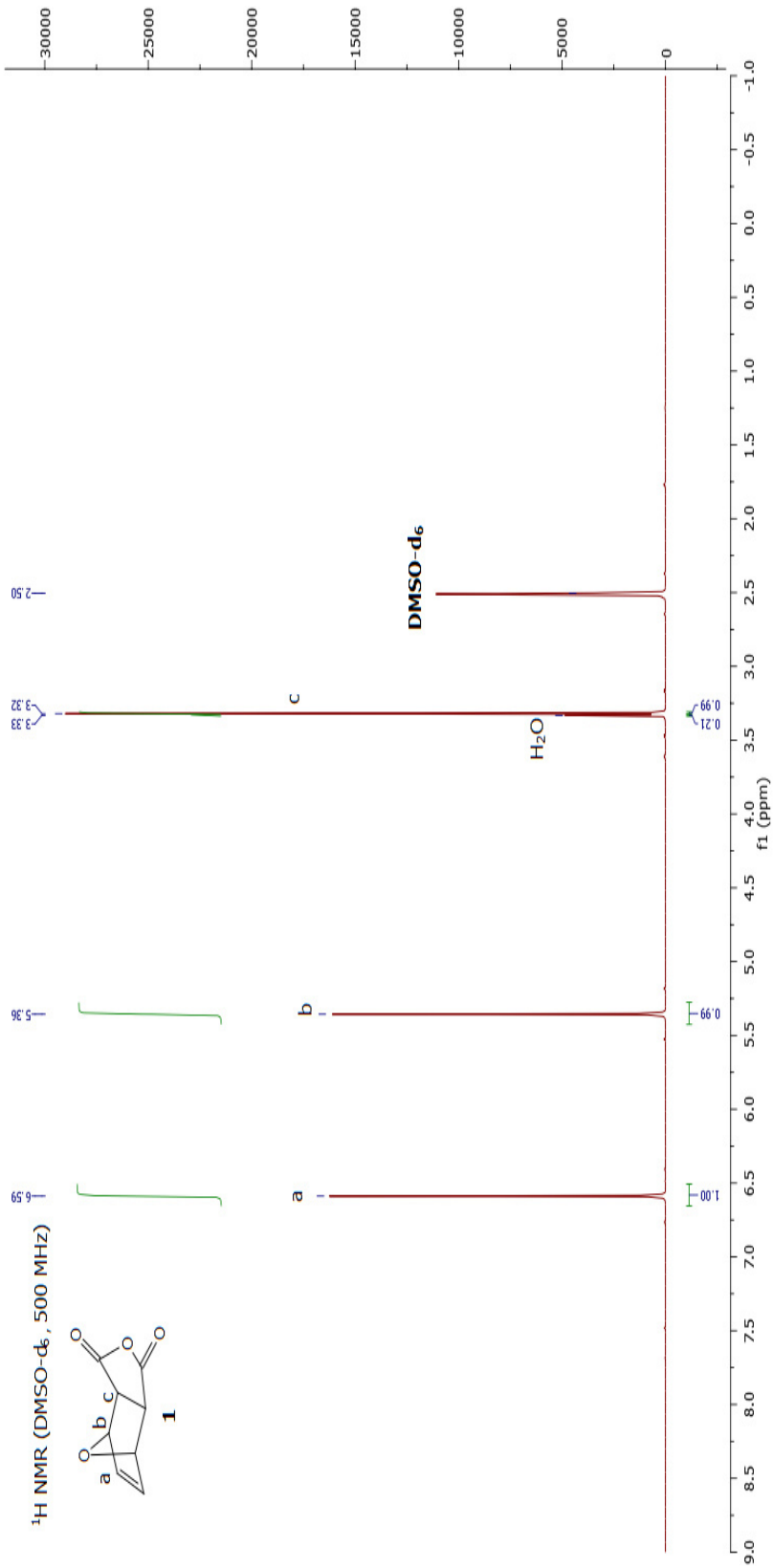

Figure B1 ^{1}H NMR of oxanorbornene

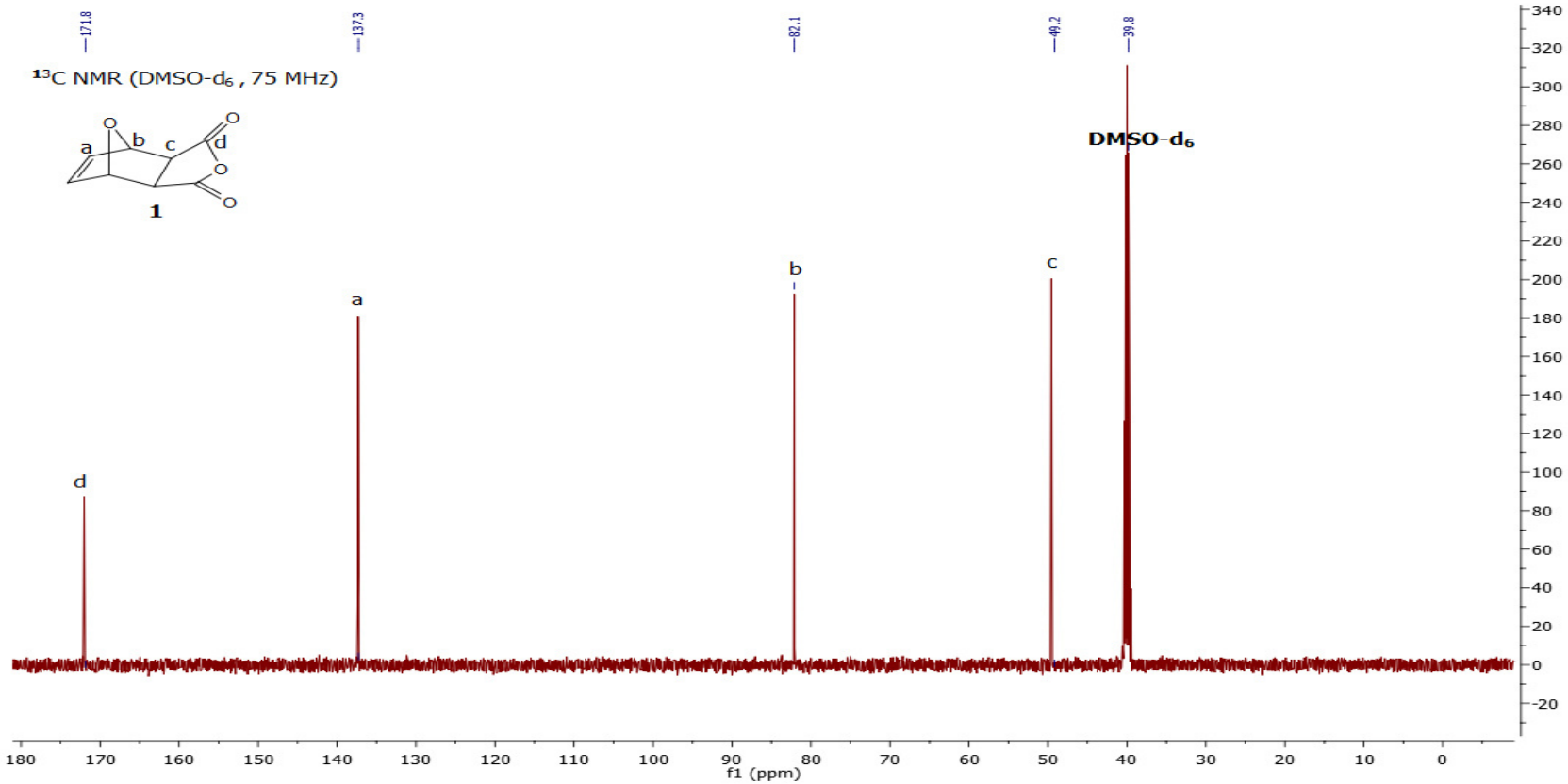

Figure B2 ^{13}C NMR of oxanorbornene

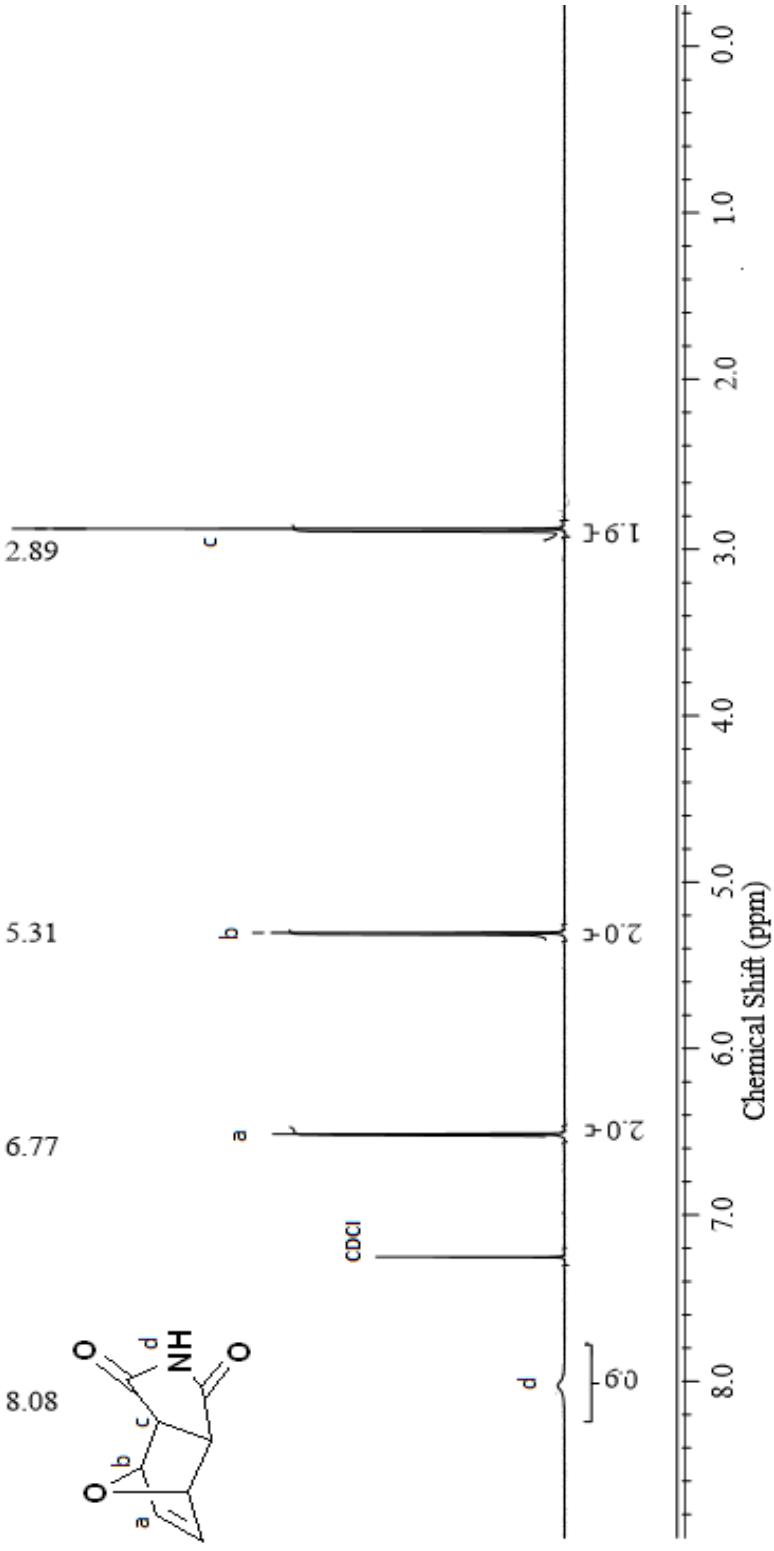

Figure B3 ^{1}H NMR of 3,6- exo-tetrahydrophthalide

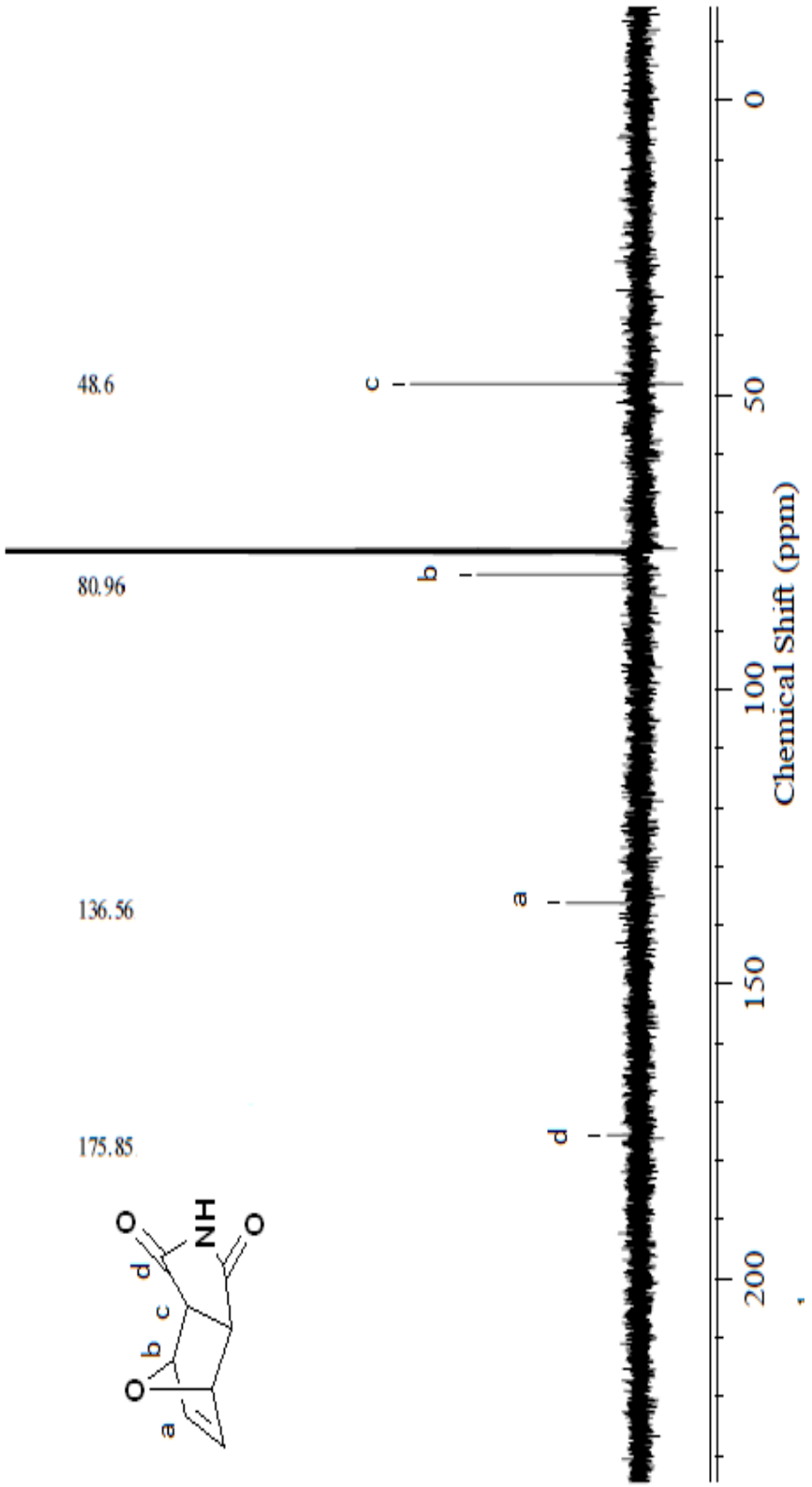

Figure B4 ^{13}C NMR of 3, 6- exo-tetrahydrophthalide

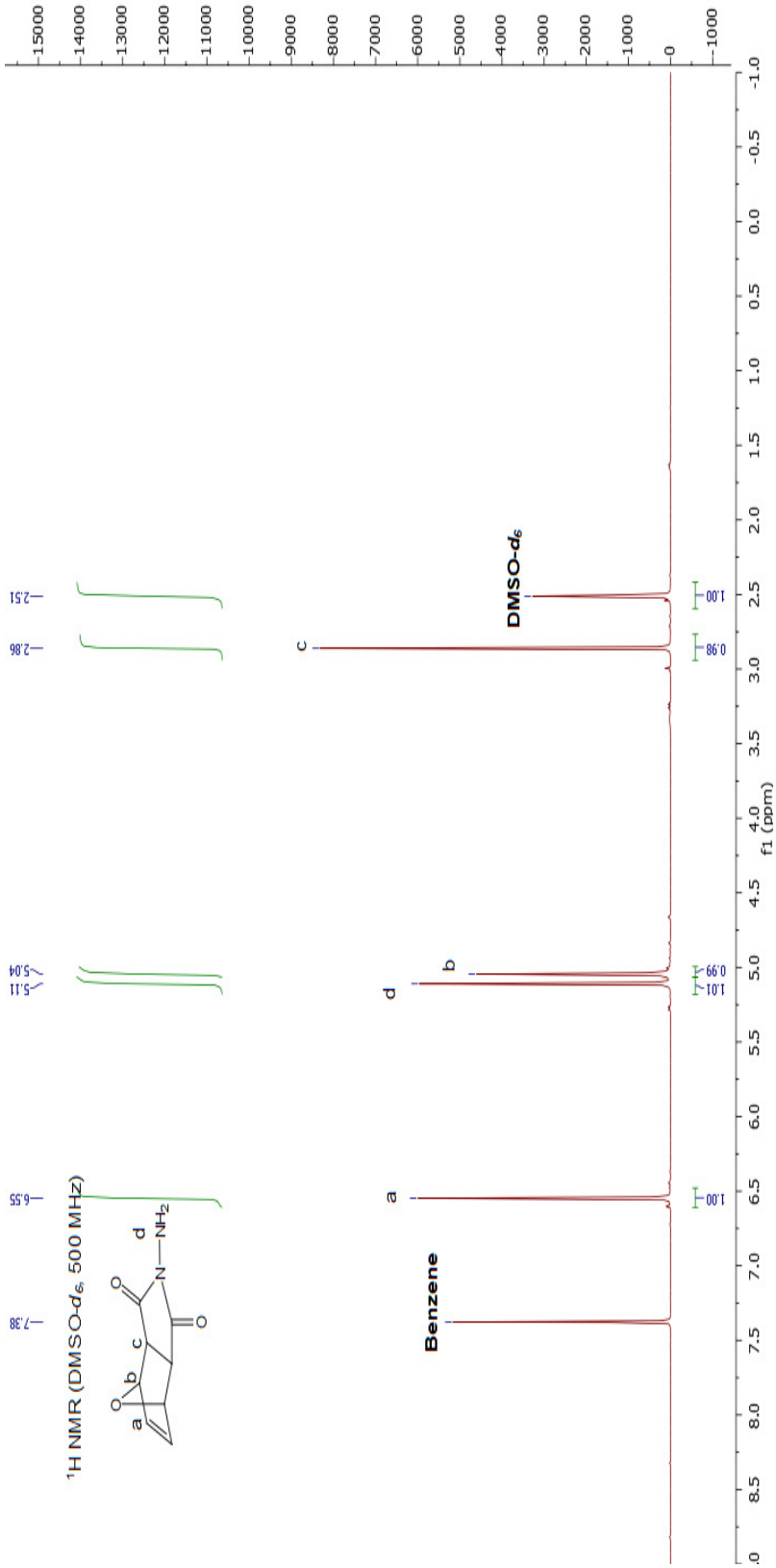

Figure B5 ^{1}H NMR of monomer core without carbon linker

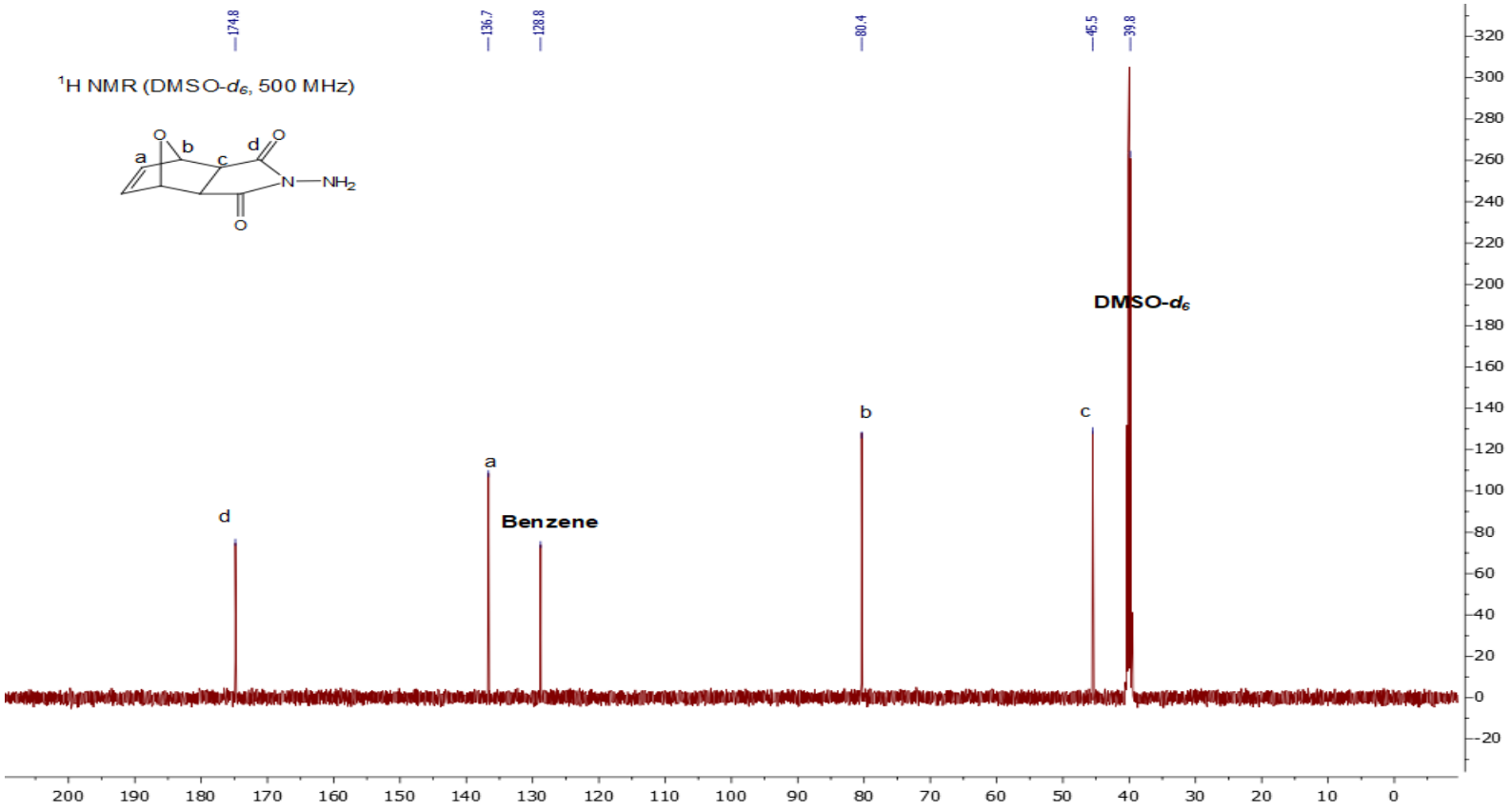

Figure B6 ^{13}C NMR of monomer core without carbon linker

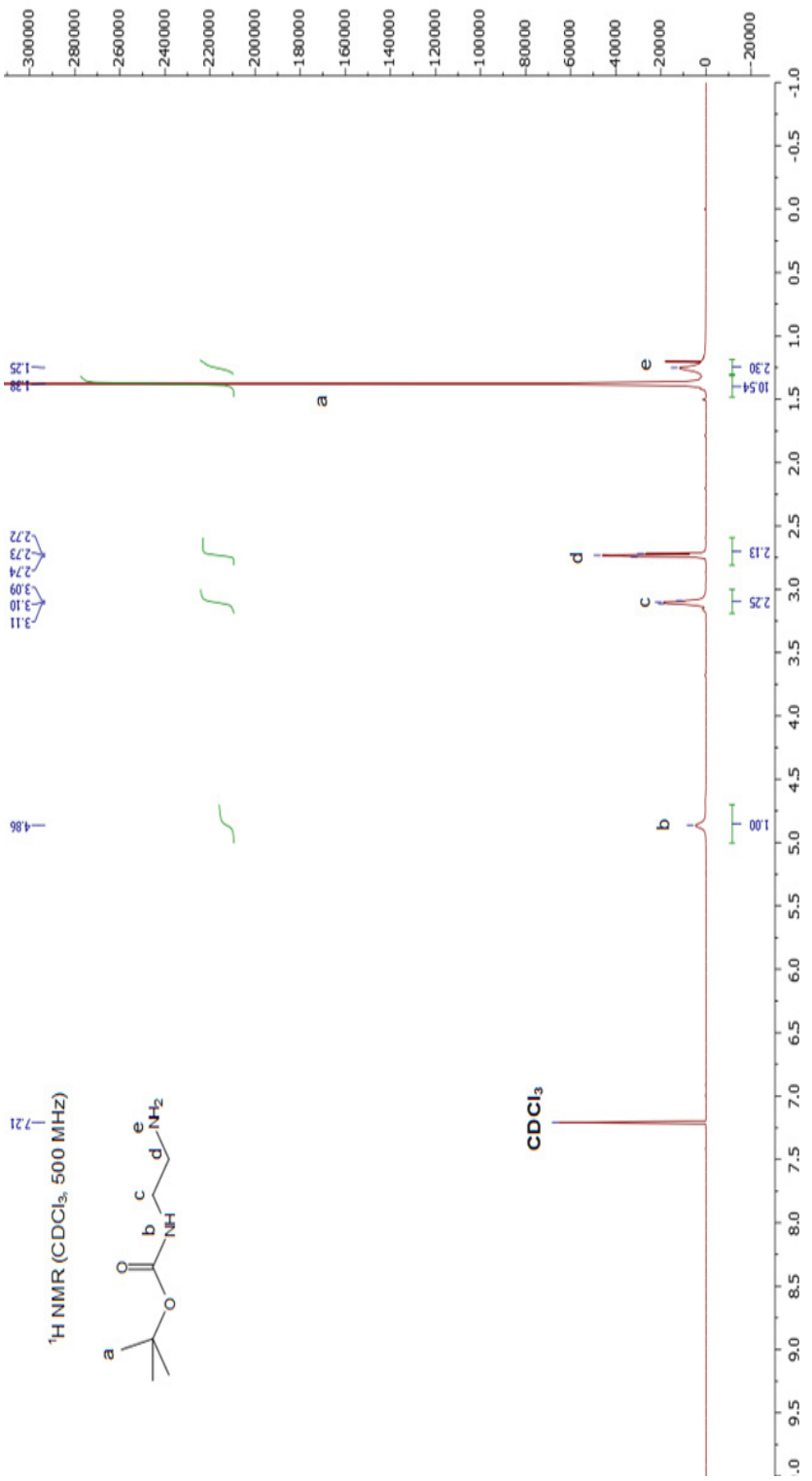

Figure B7 ^{1}H NMR of compound EDA-BOC

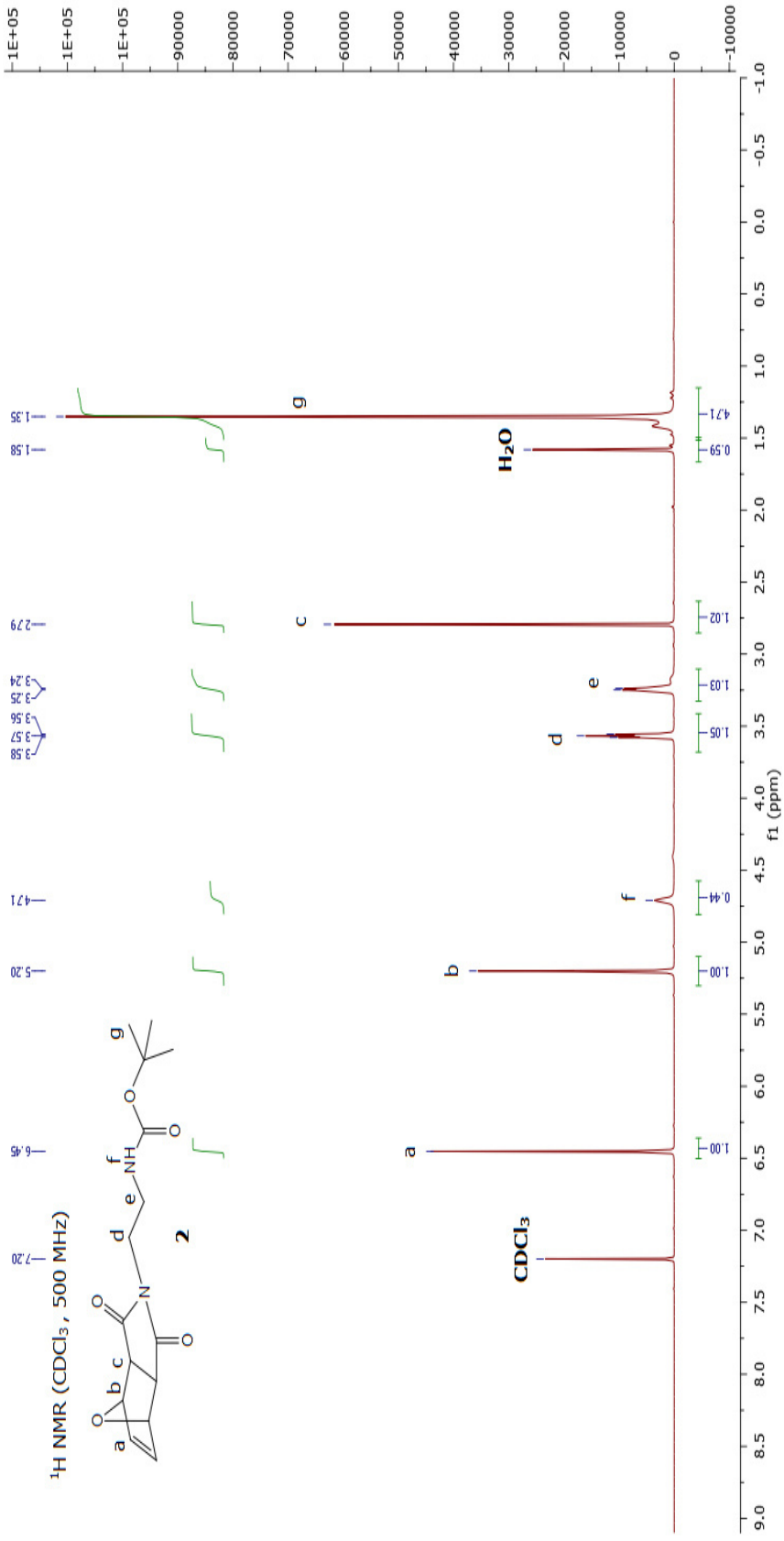

Figure B8 ^{1}H NMR of EDA-BOC incorporated oxanorbornene

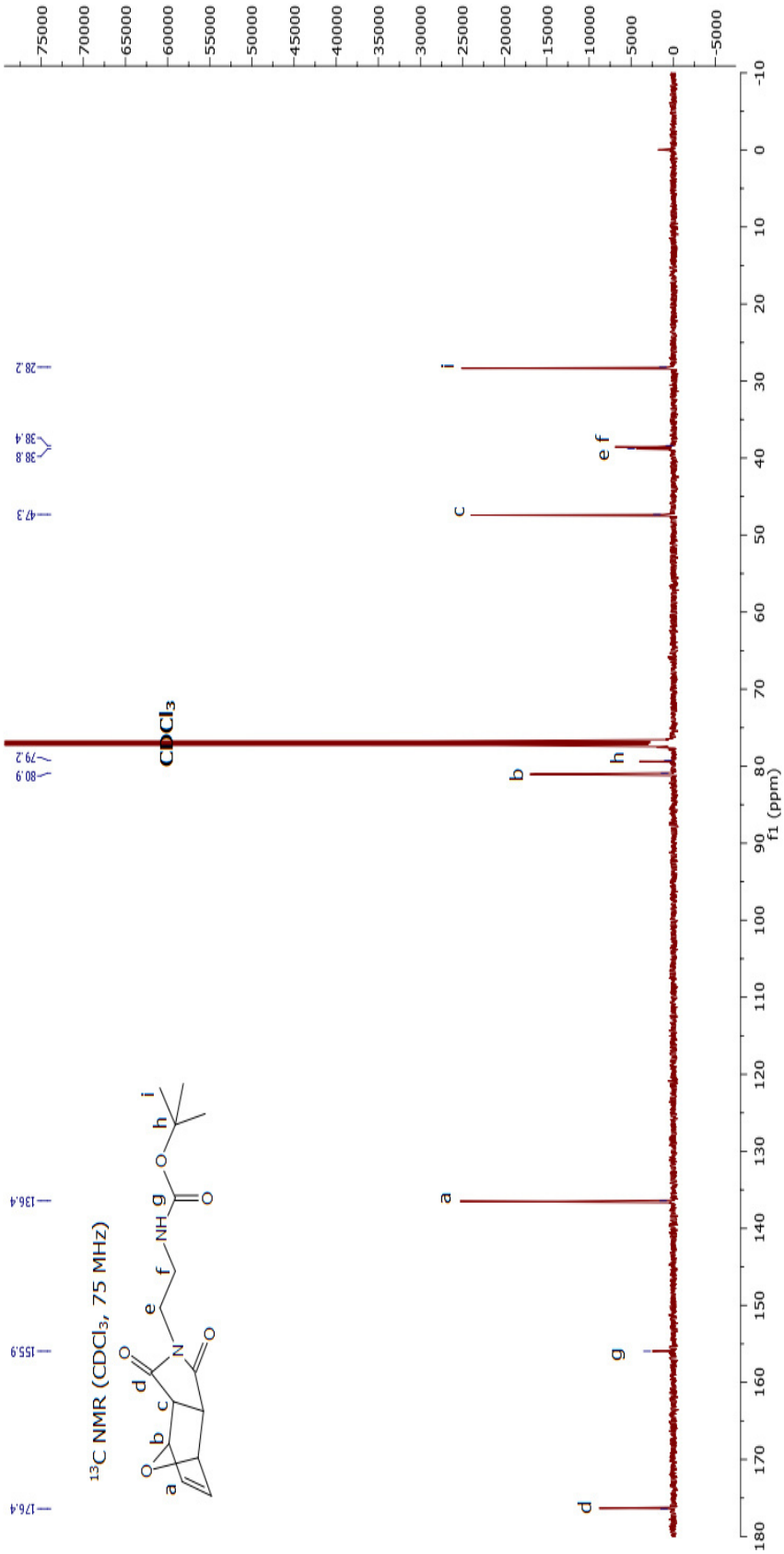

Figure B9 ^{13}C NMR of EDA-BOC incorporated oxanorbornene

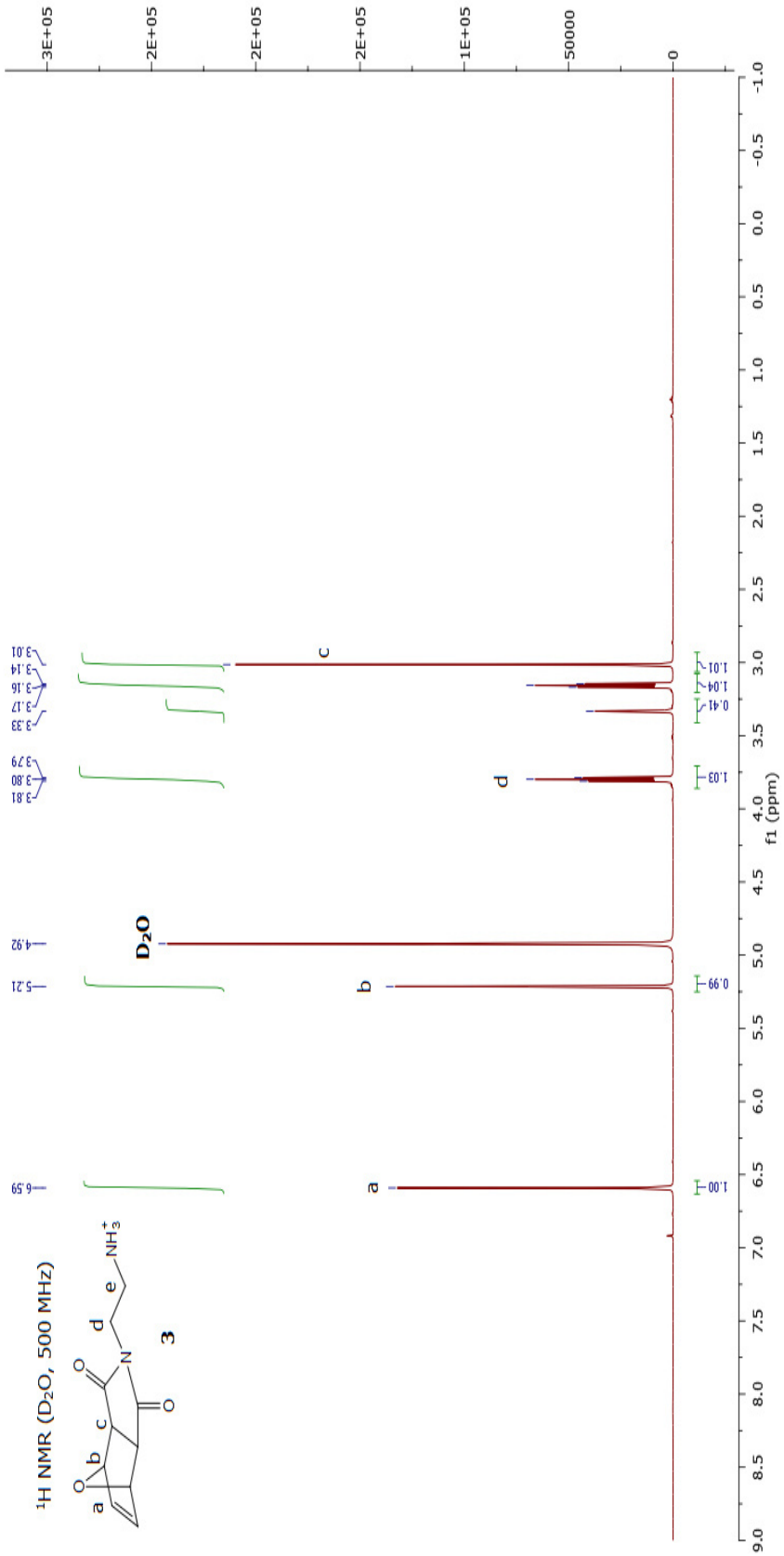

Figure B10 ^{1}H NMR of BOC deprotected two carbon spacer NH_2 terminated oxanorbornene

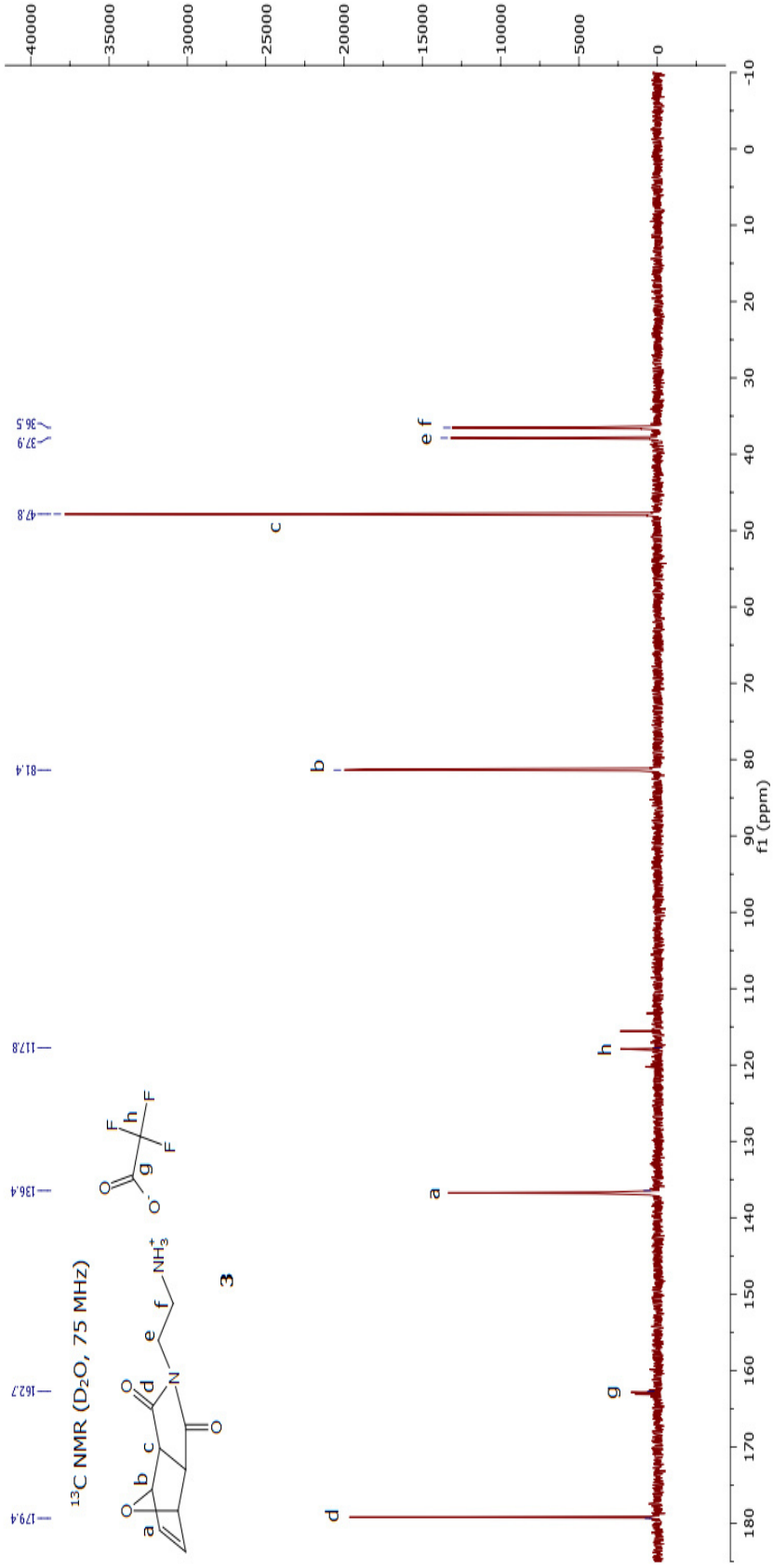

Figure B11 ^{13}C NMR of BOC deprotected two carbon spacer NH_2 terminated oxanorbornene

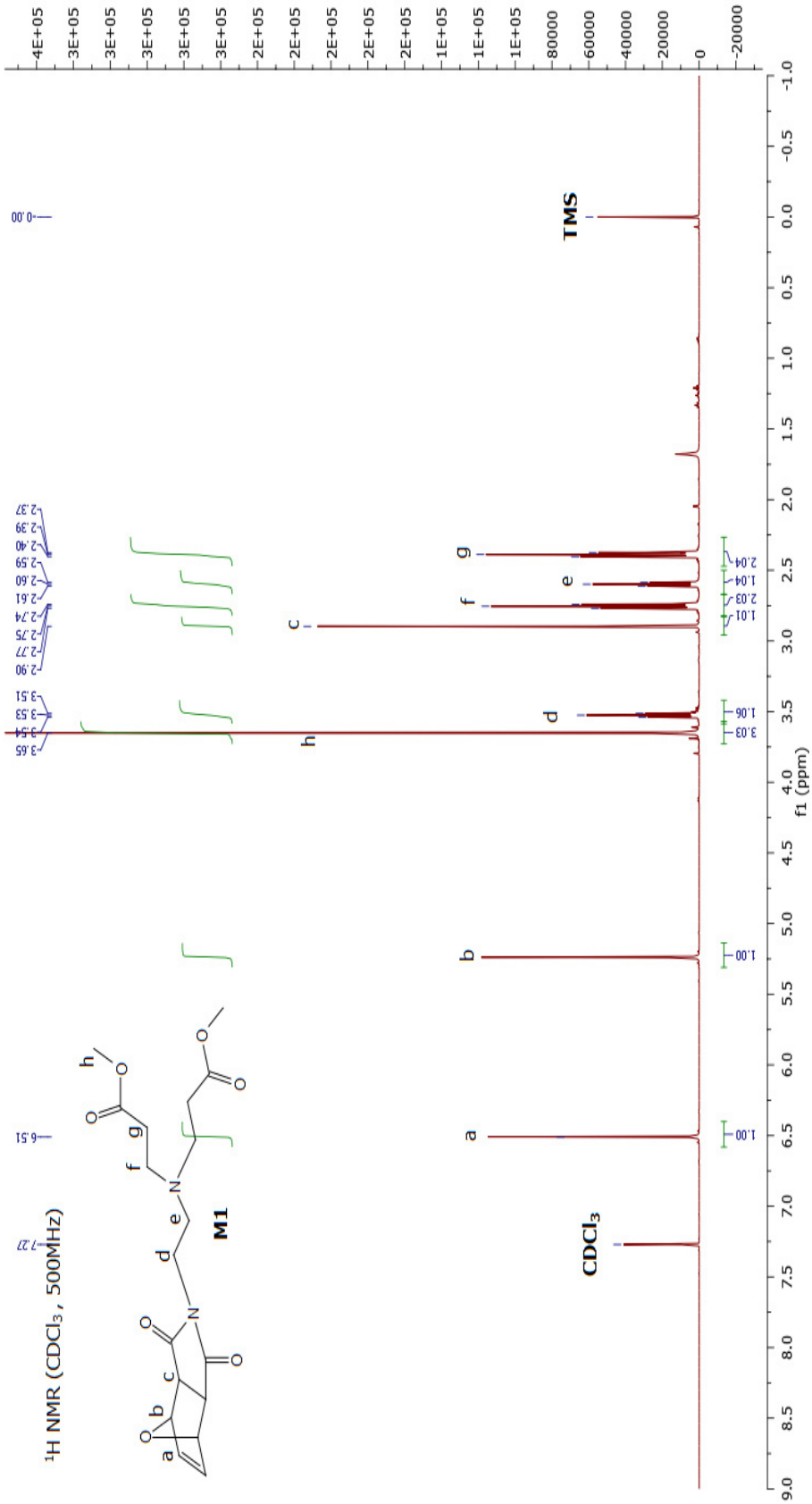

Figure B12 ^{1}H NMR of two carbon linker 0.5 generation dendritic monomer

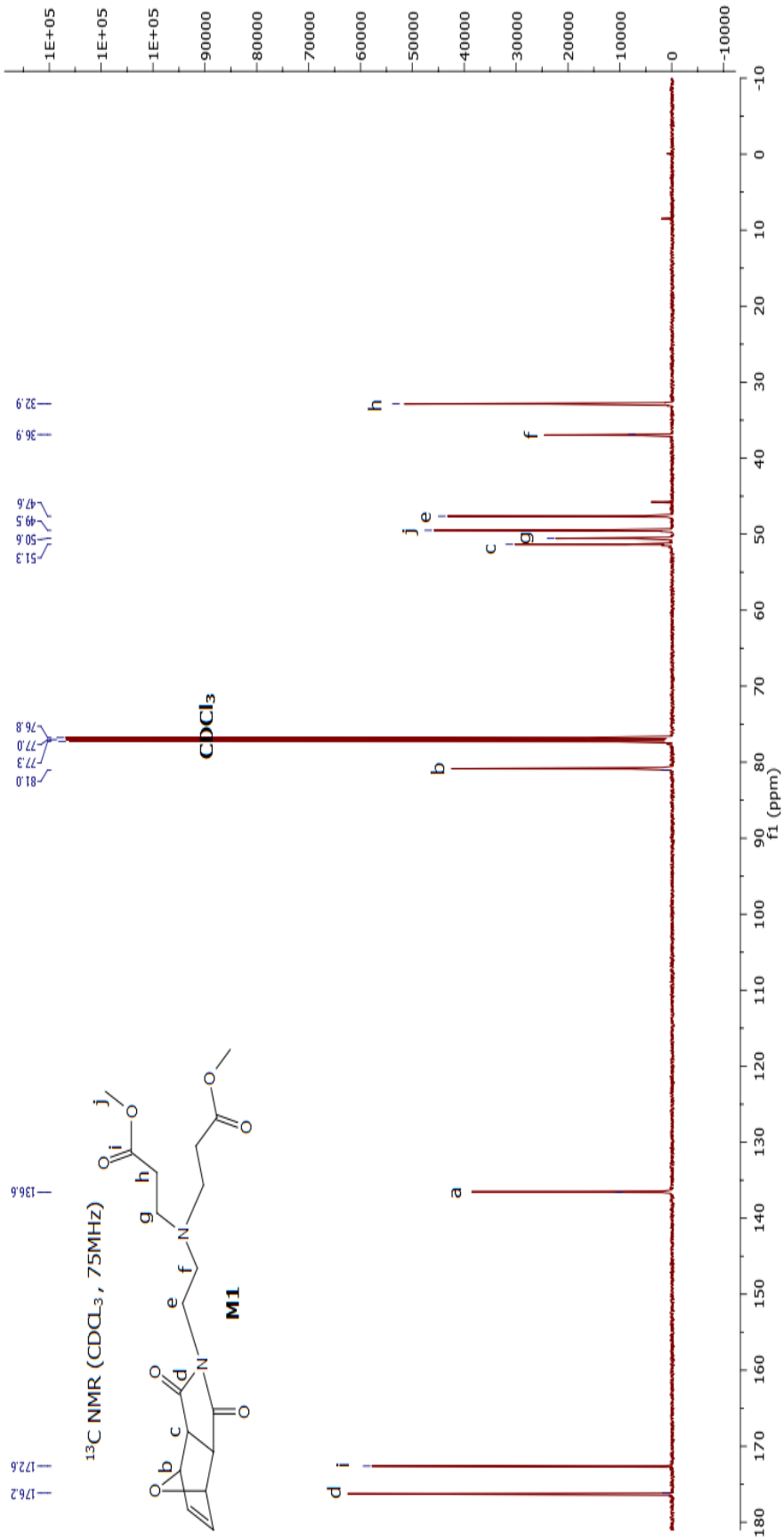

Figure B13 ^{13}C NMR of two carbon linker 0.5 generation dendritic monomer

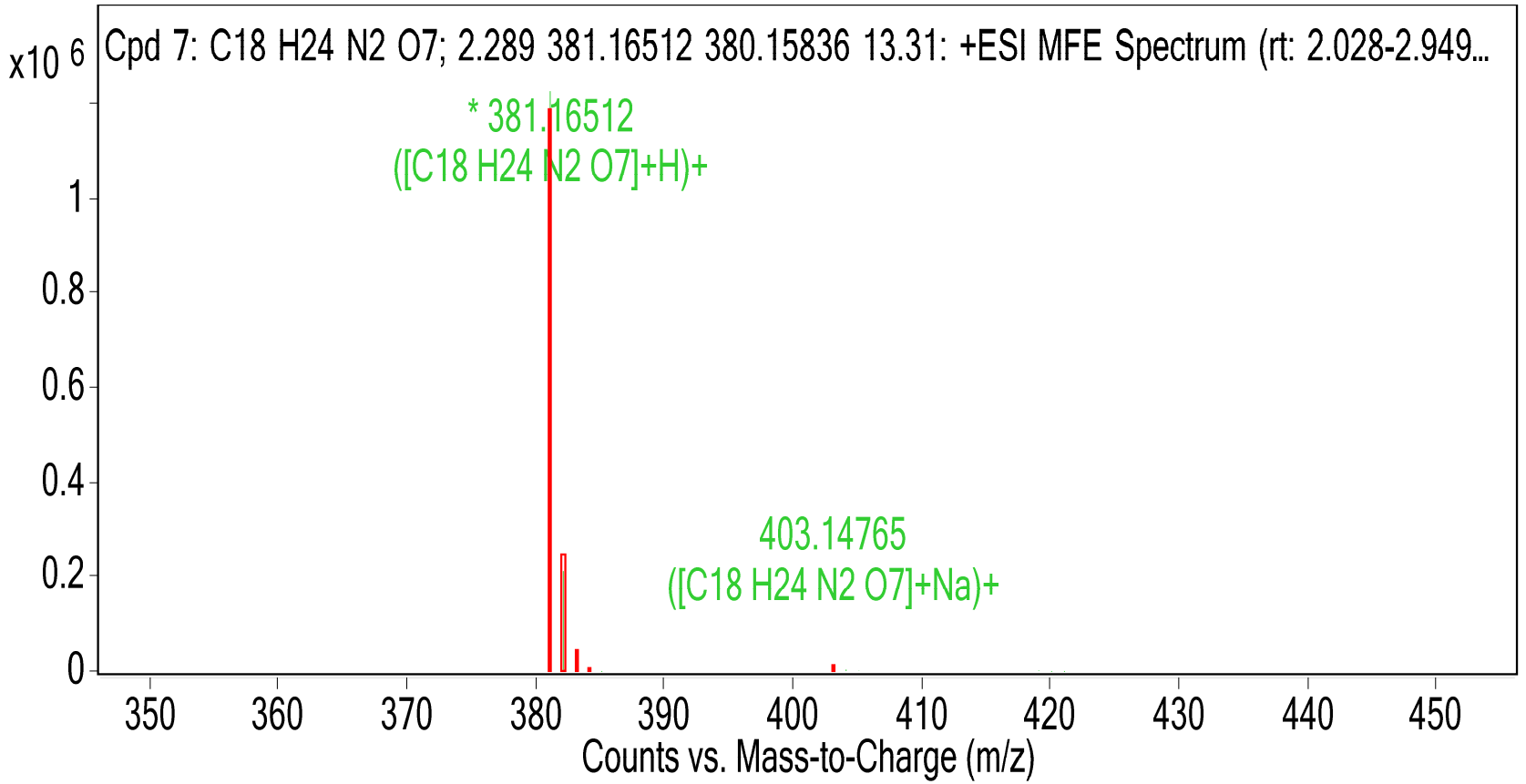

Figure B14 Mass spectra of two carbon linker 0.5 generation dendritic monomer

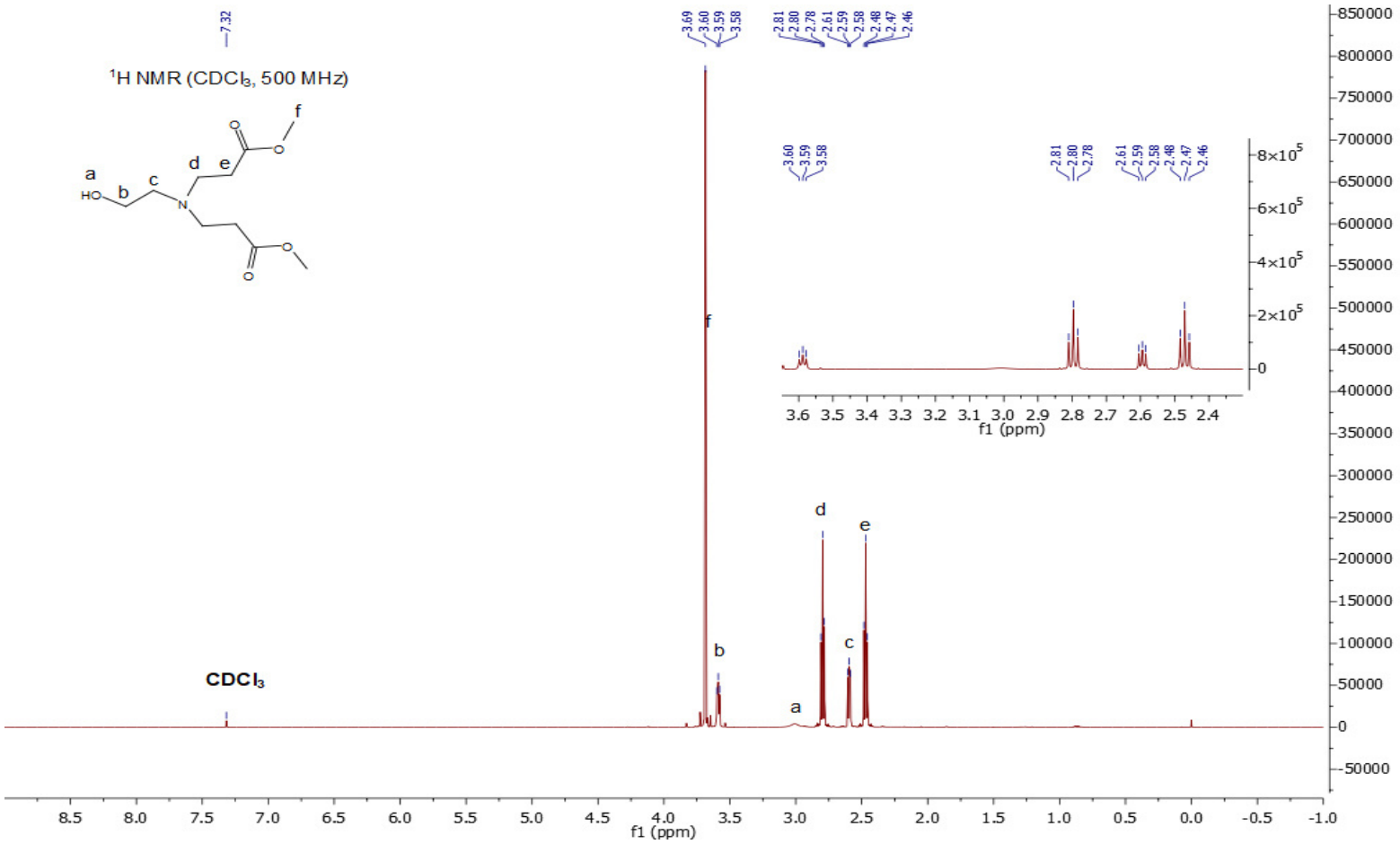

Figure B15 ^{1}H NMR of ethanolamine cored 0.5 generation dendron

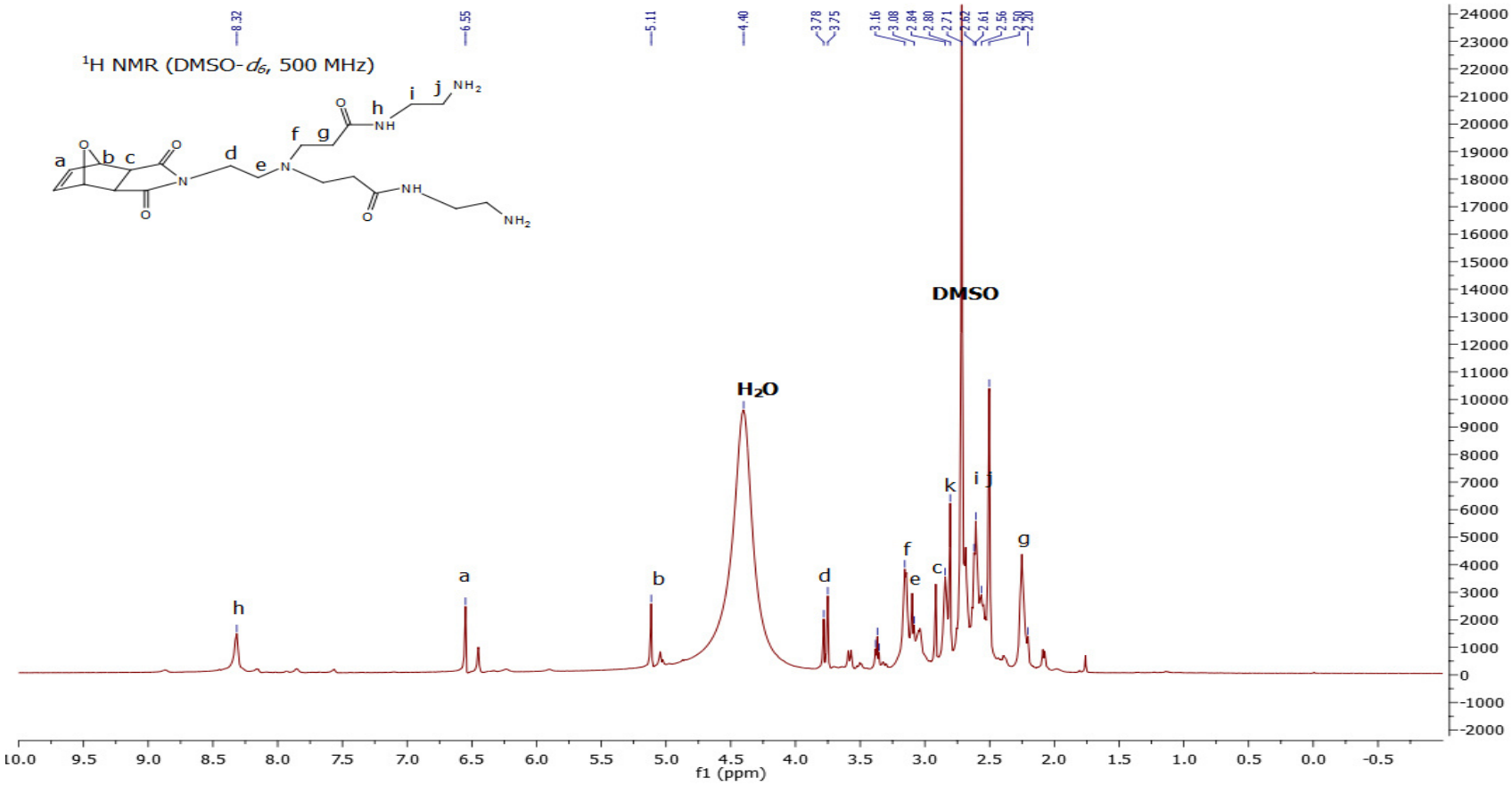

Figure B16 ^{1}H NMR of two carbon linker 1.0 generation dendritic monomer

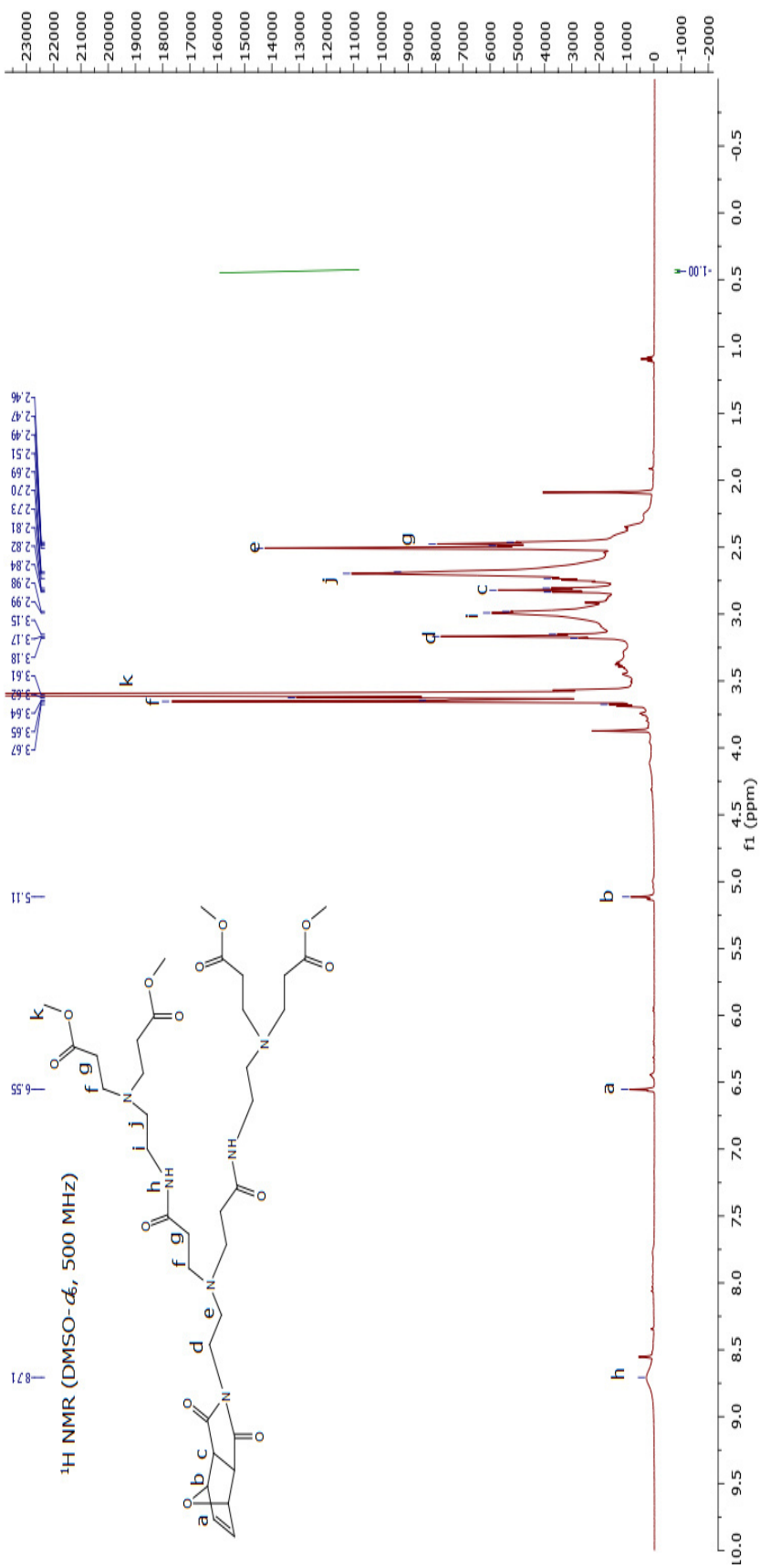

Figure B17 ^{1}H NMR of two carbon linker 1.5 generation dendritic monomer

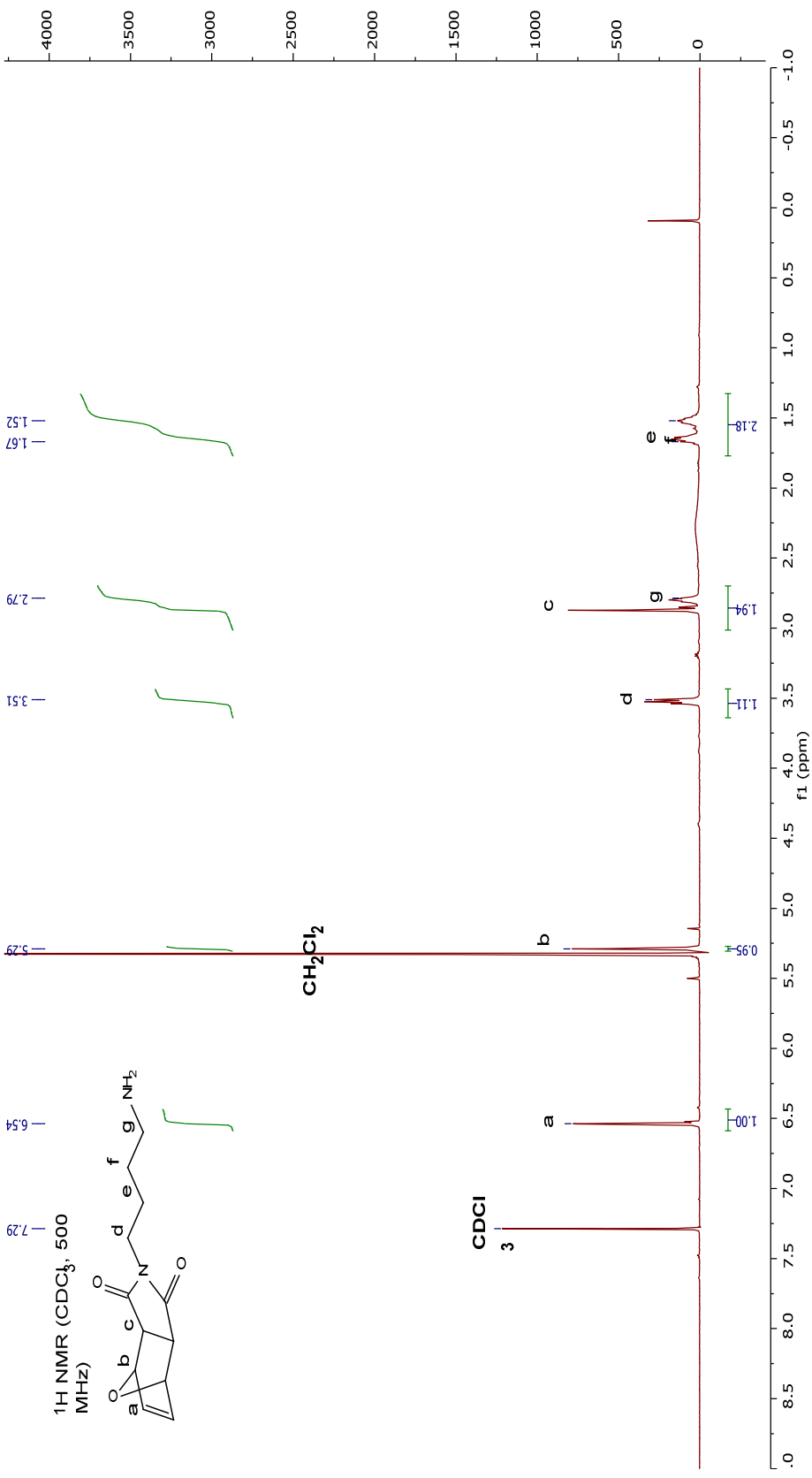

Figure B18 ^{1}H NMR of four carbon linker monomer core

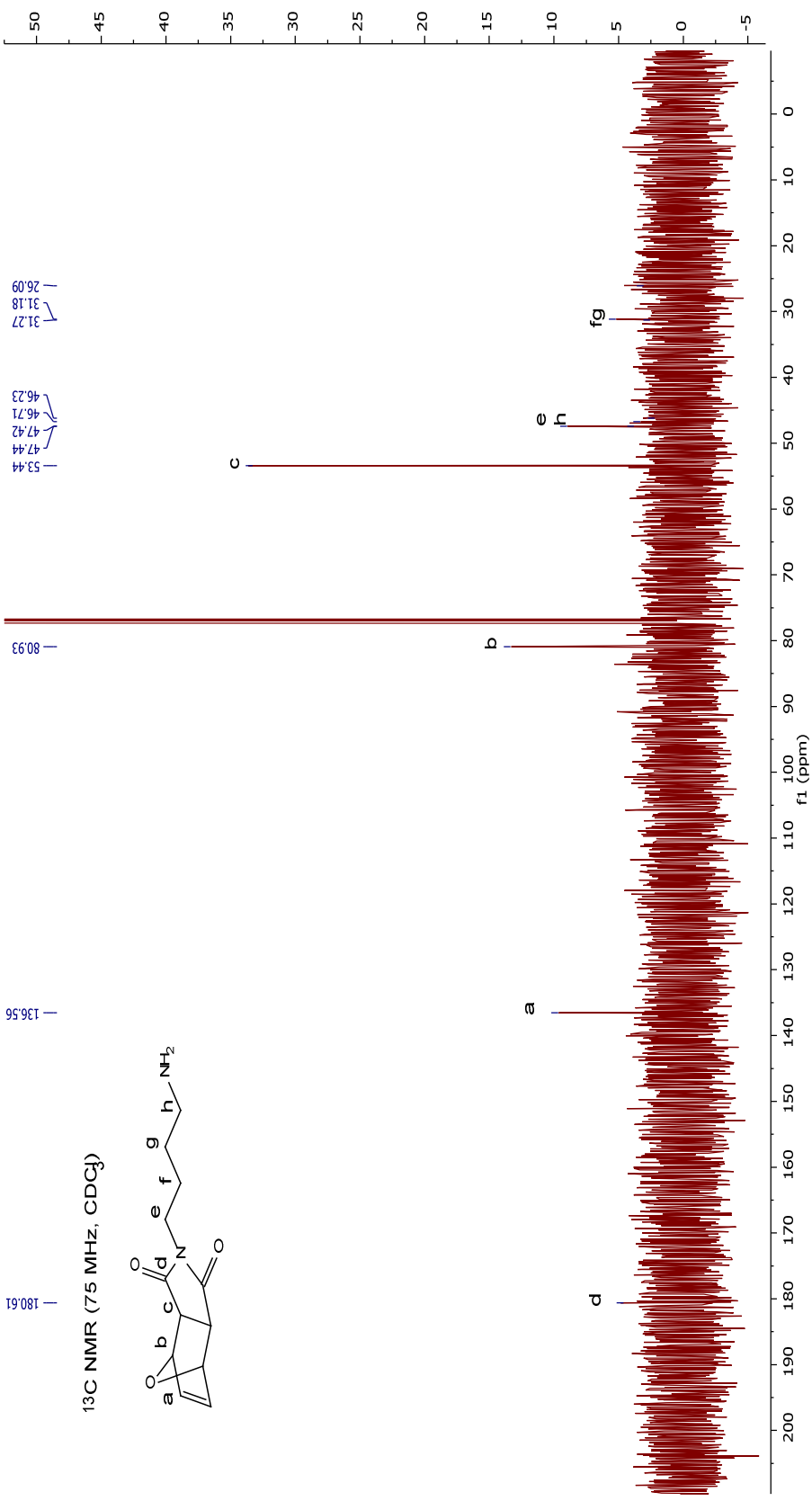

Figure B19 ^{13}C NMR of four carbon linker monomer core

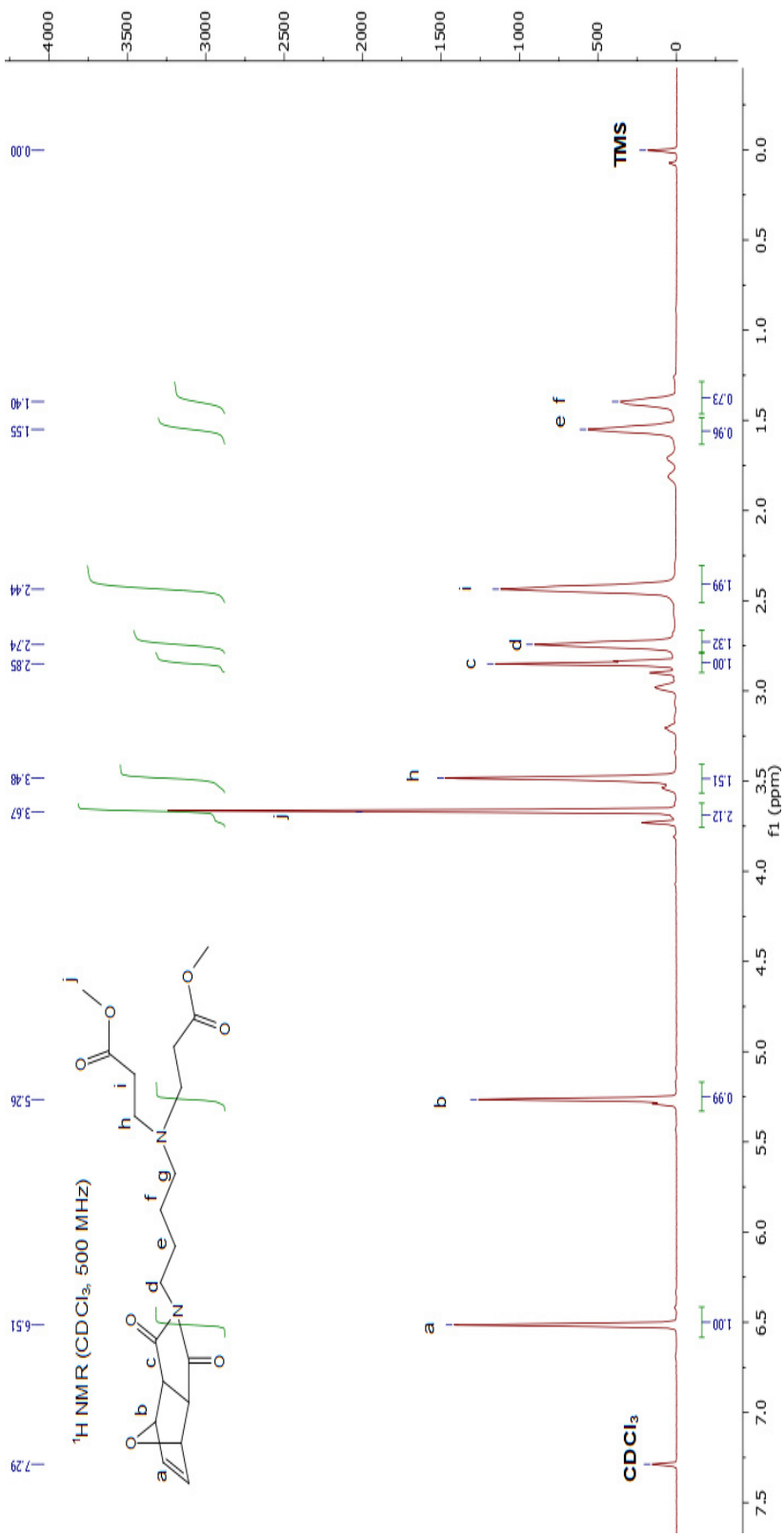

Figure B20 ^{1}H NMR spectra of four carbon linker 0.5 generation dendritic monomer

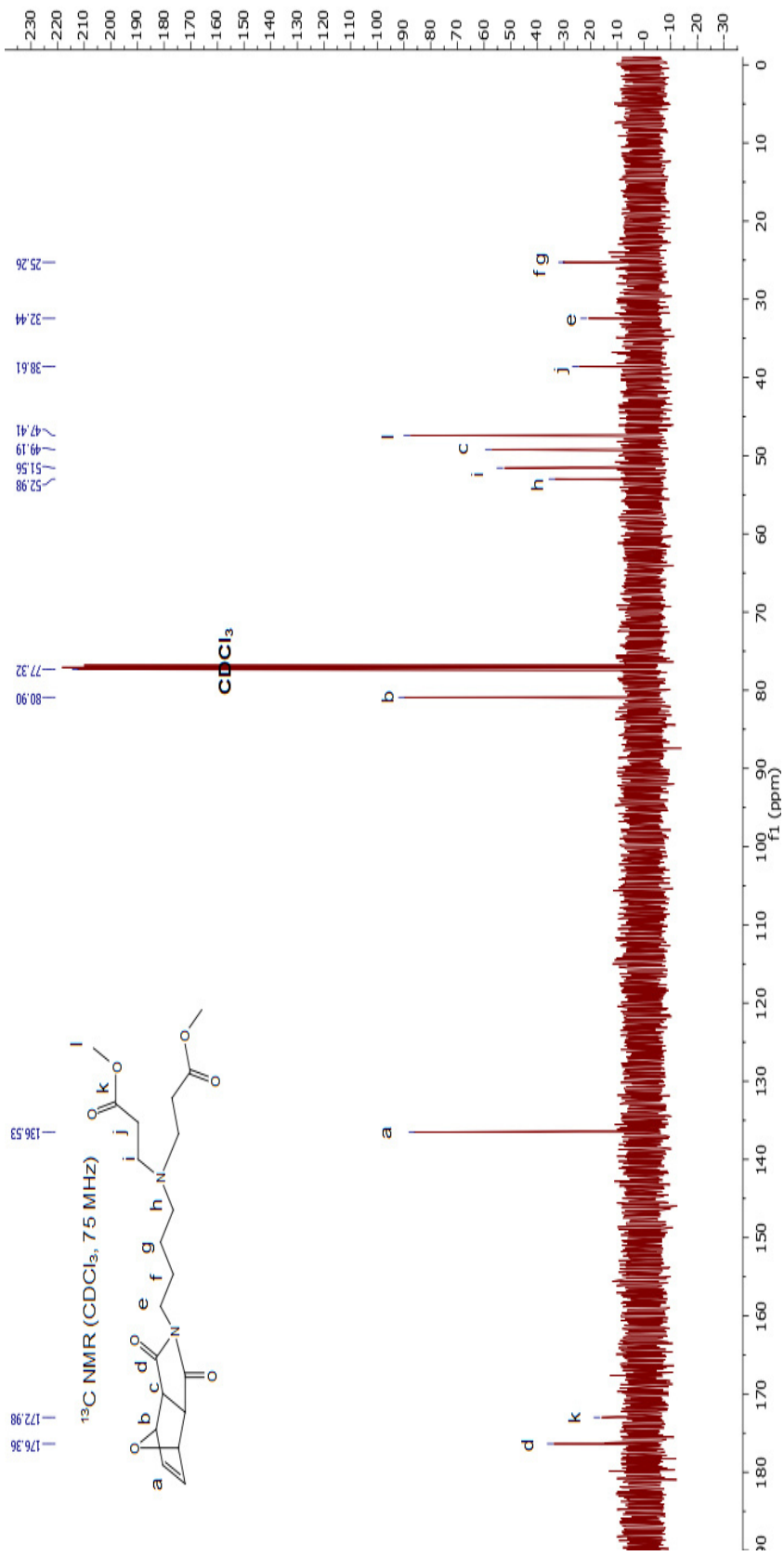

Figure B21 ^{13}C NMR spectra of four carbon linker 0.5 generation dendritic monomer

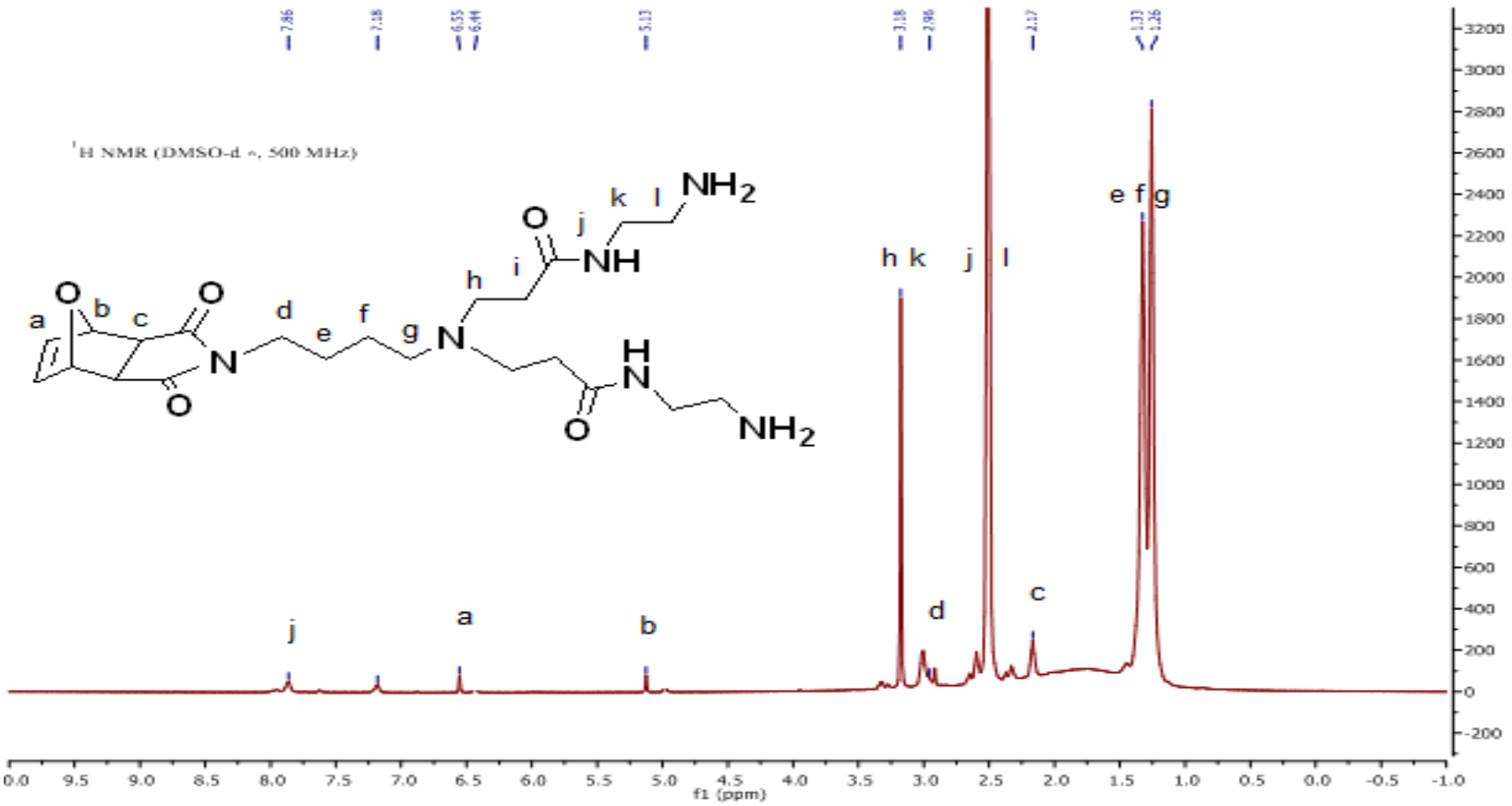

Figure B22 ^{1}H NMR spectra of four carbon linker 1.0 generation dendritic monomer

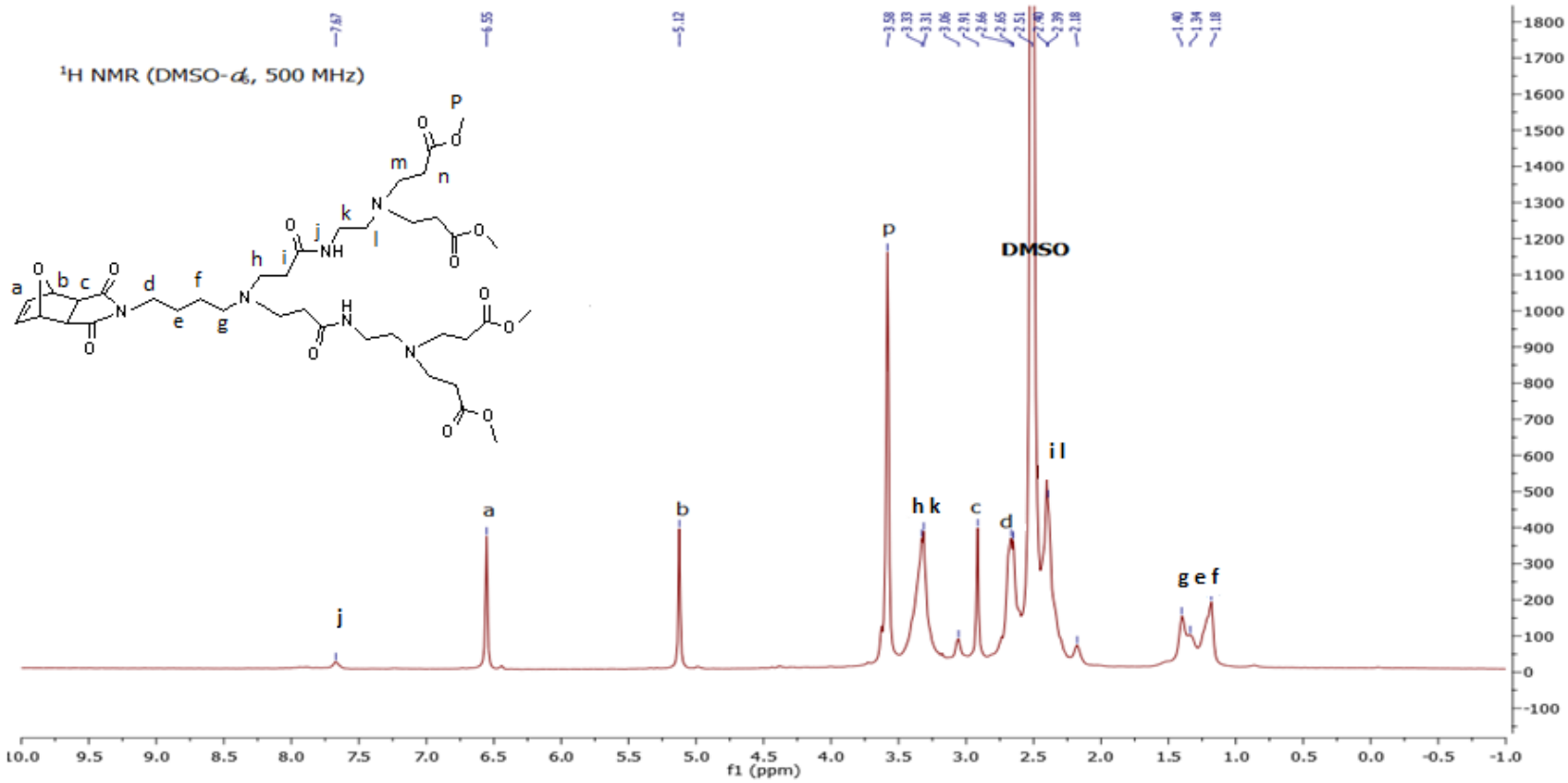

Figure B23 ¹H NMR spectra of four carbon linker 1.5 generation dendritic monomer

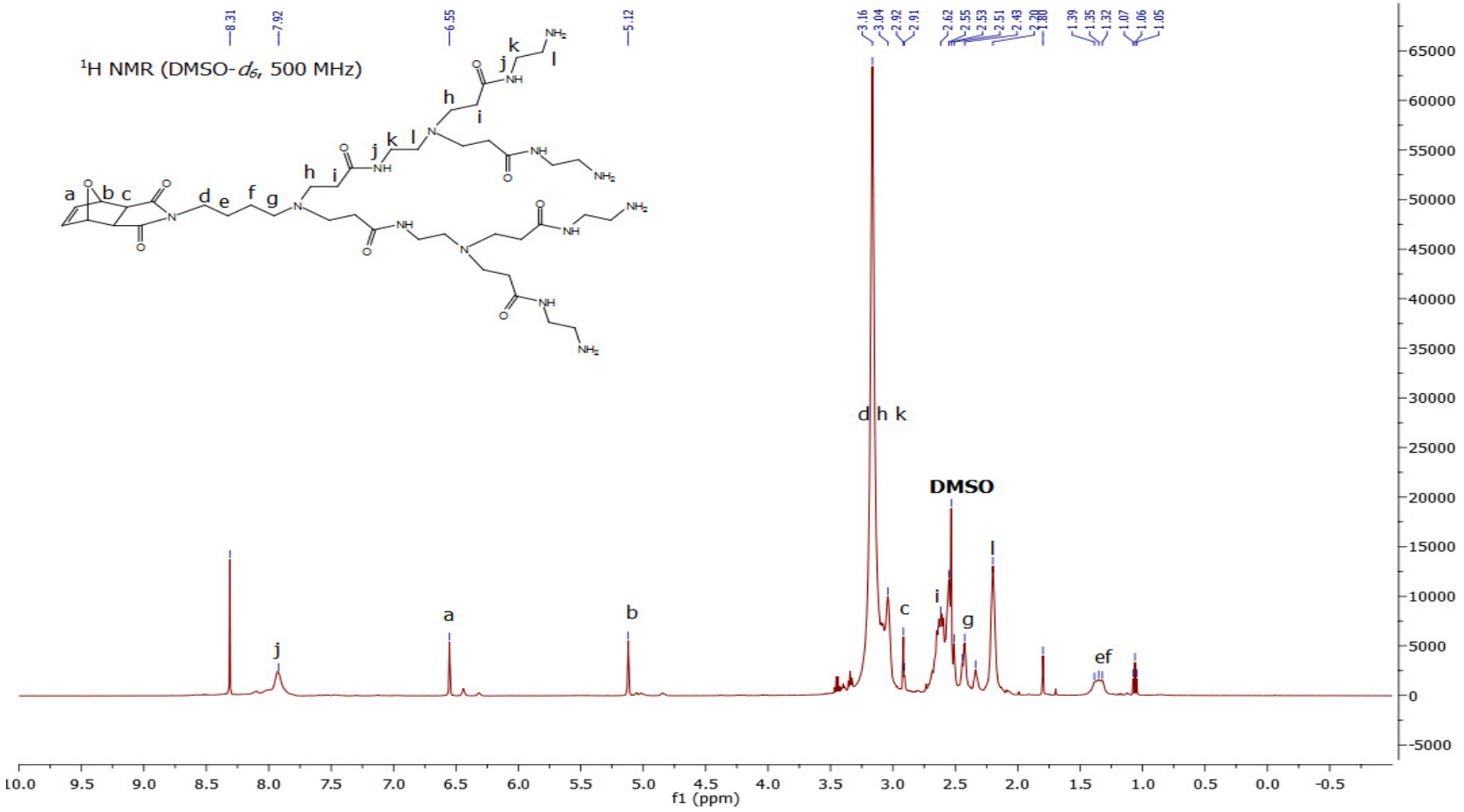

Figure B24 ¹H NMR of four carbon linker 2.0 generation dendritic monomer

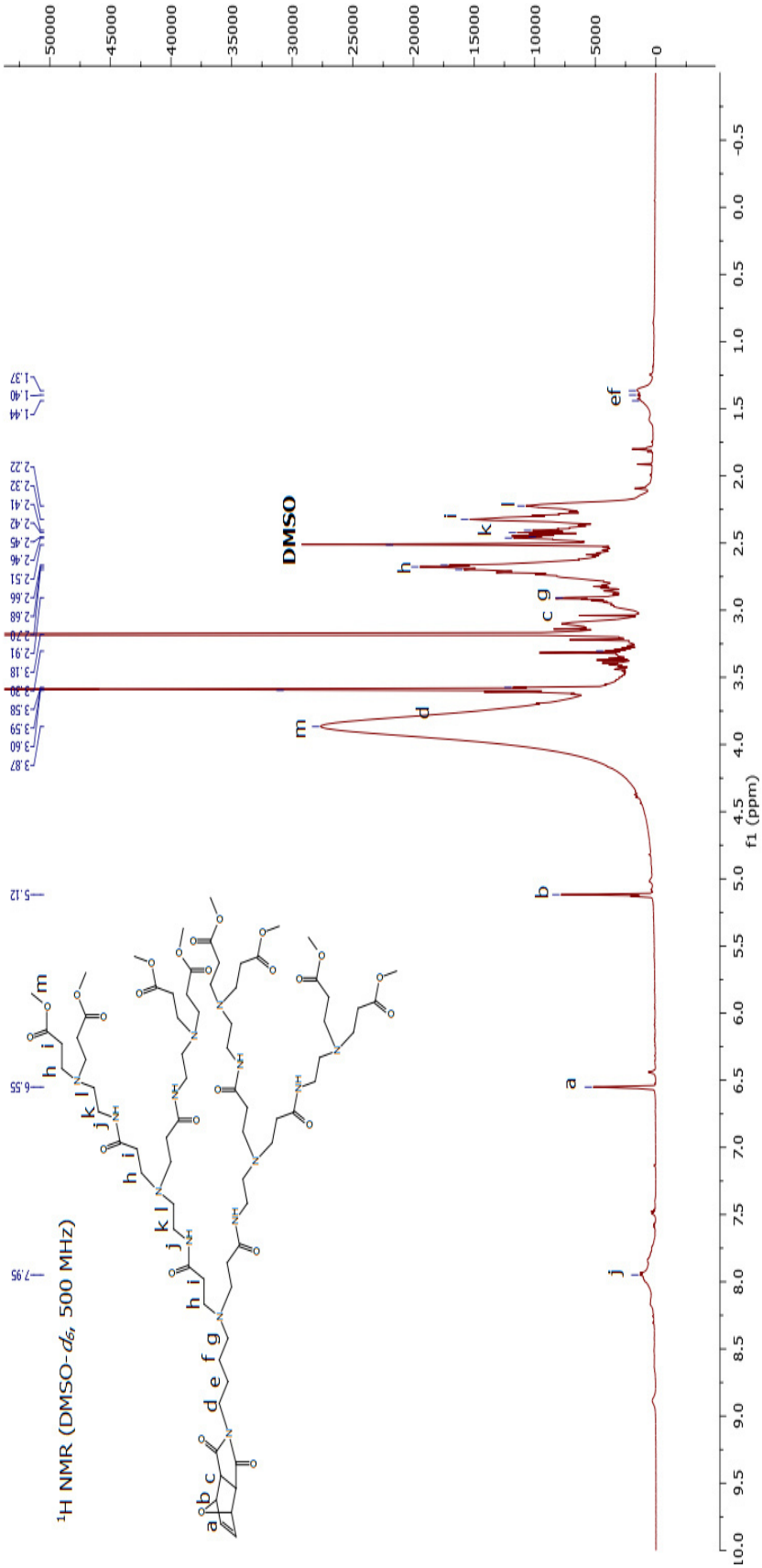

Figure B25 ^{1}H NMR of four carbon linker 2.5 generation dendritic monomer

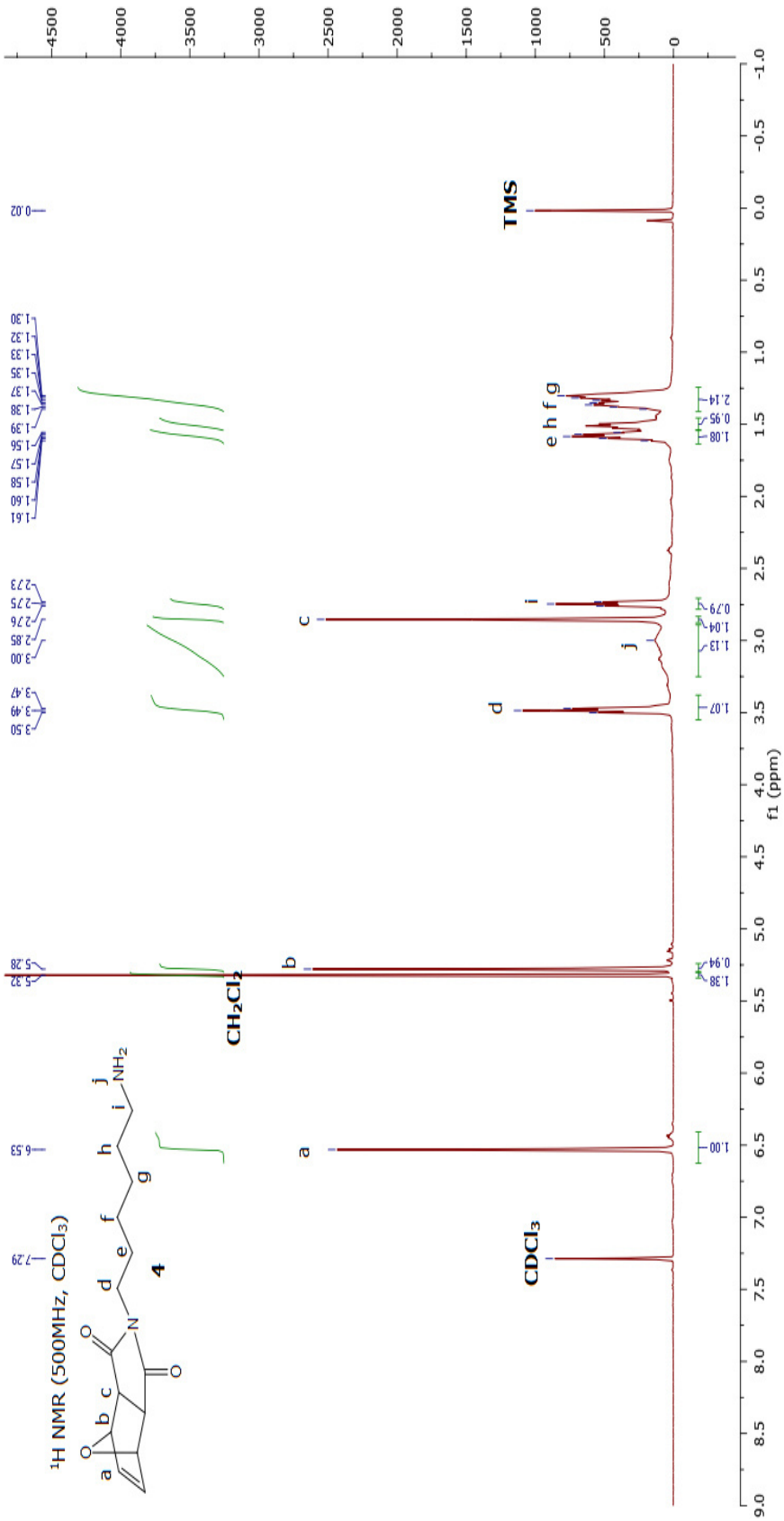

Figure B26 ^{1}H NMR of six carbon linker core

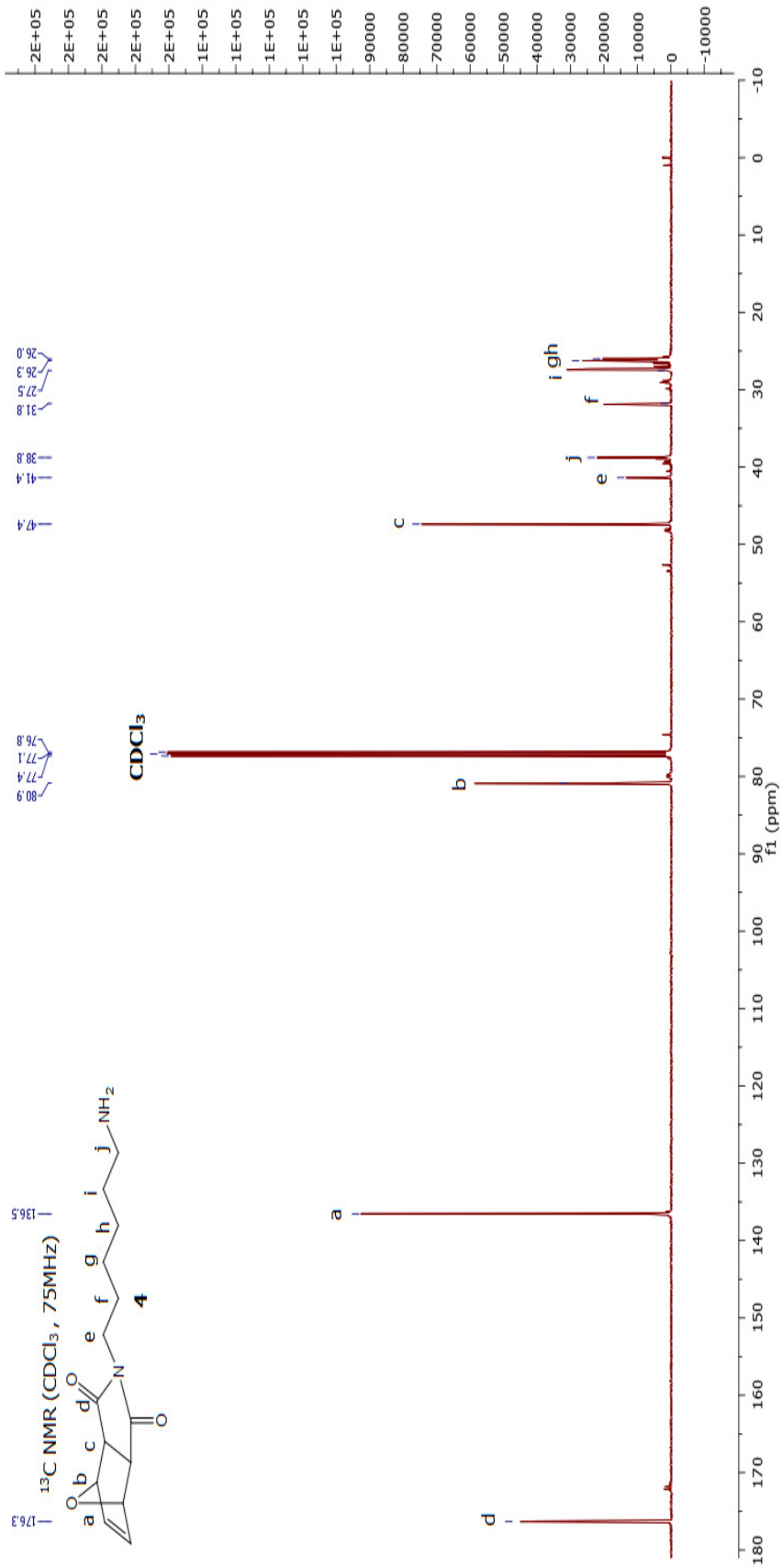

Figure B27 ^{13}C NMR of six carbon linker core

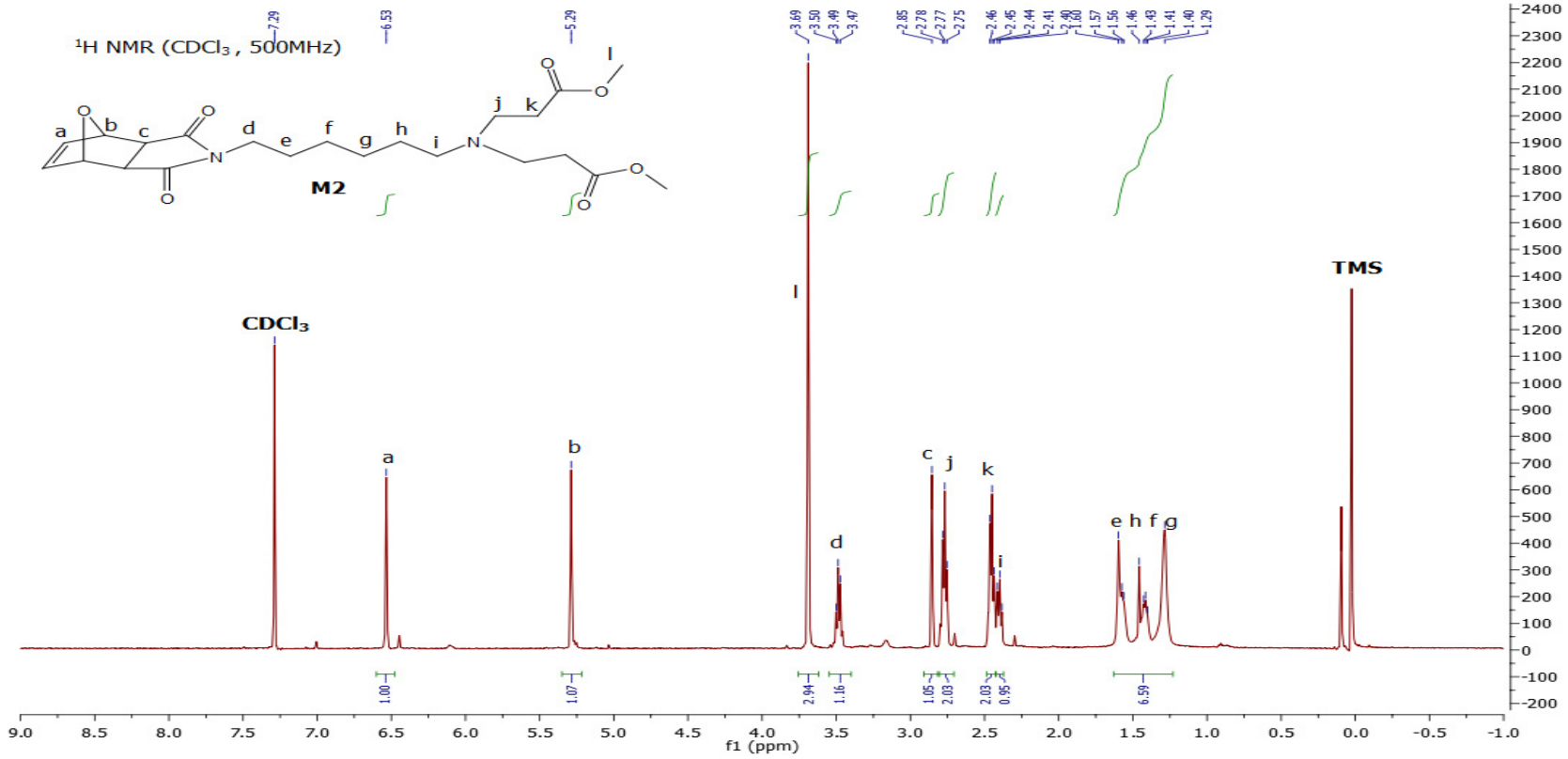

Figure B28 ¹H NMR of six carbon linker 0.5 generation dendritic monomer

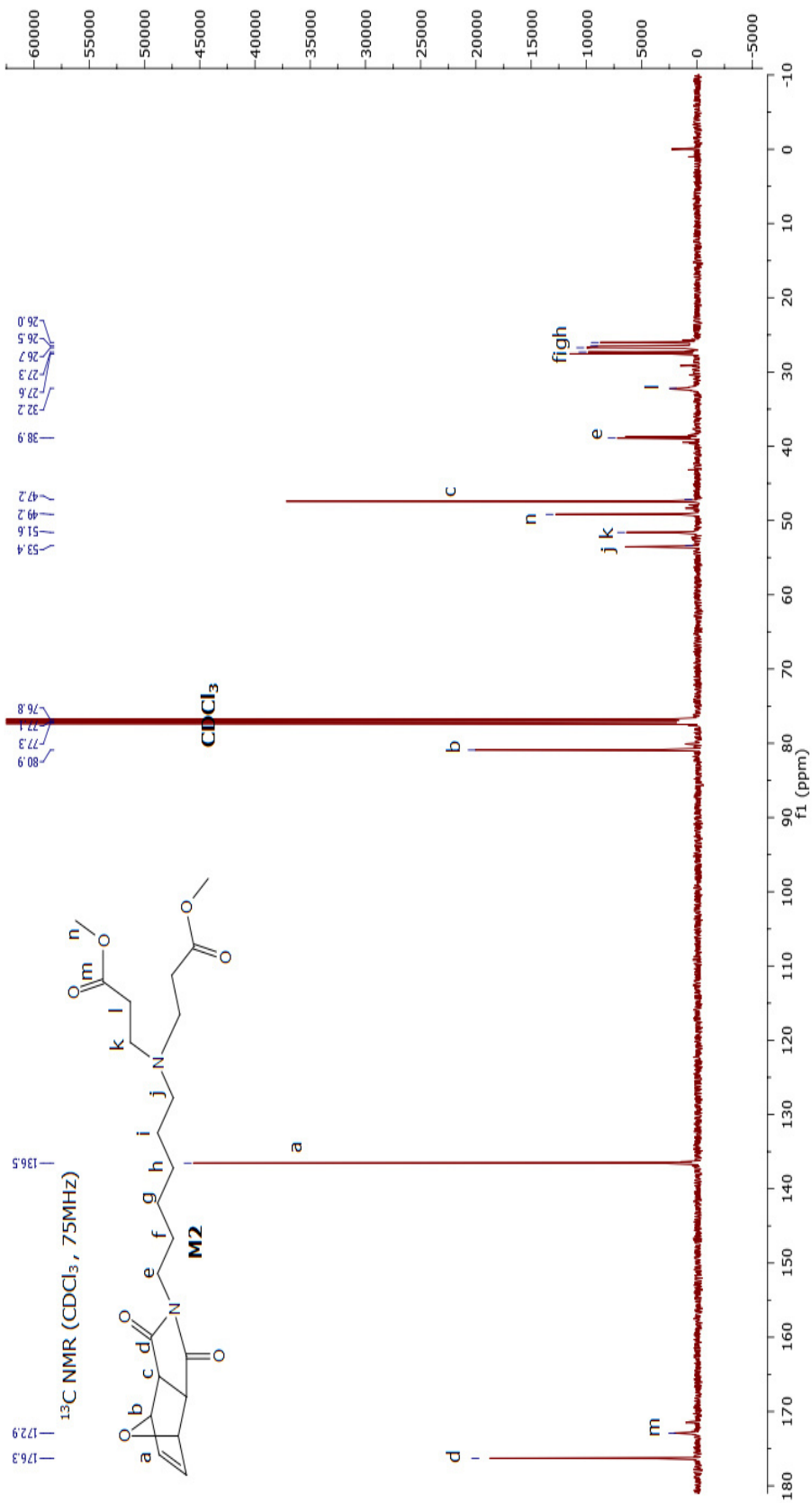

Figure B29 ^{13}C NMR of six carbon linker 0.5 generation dendritic monomer

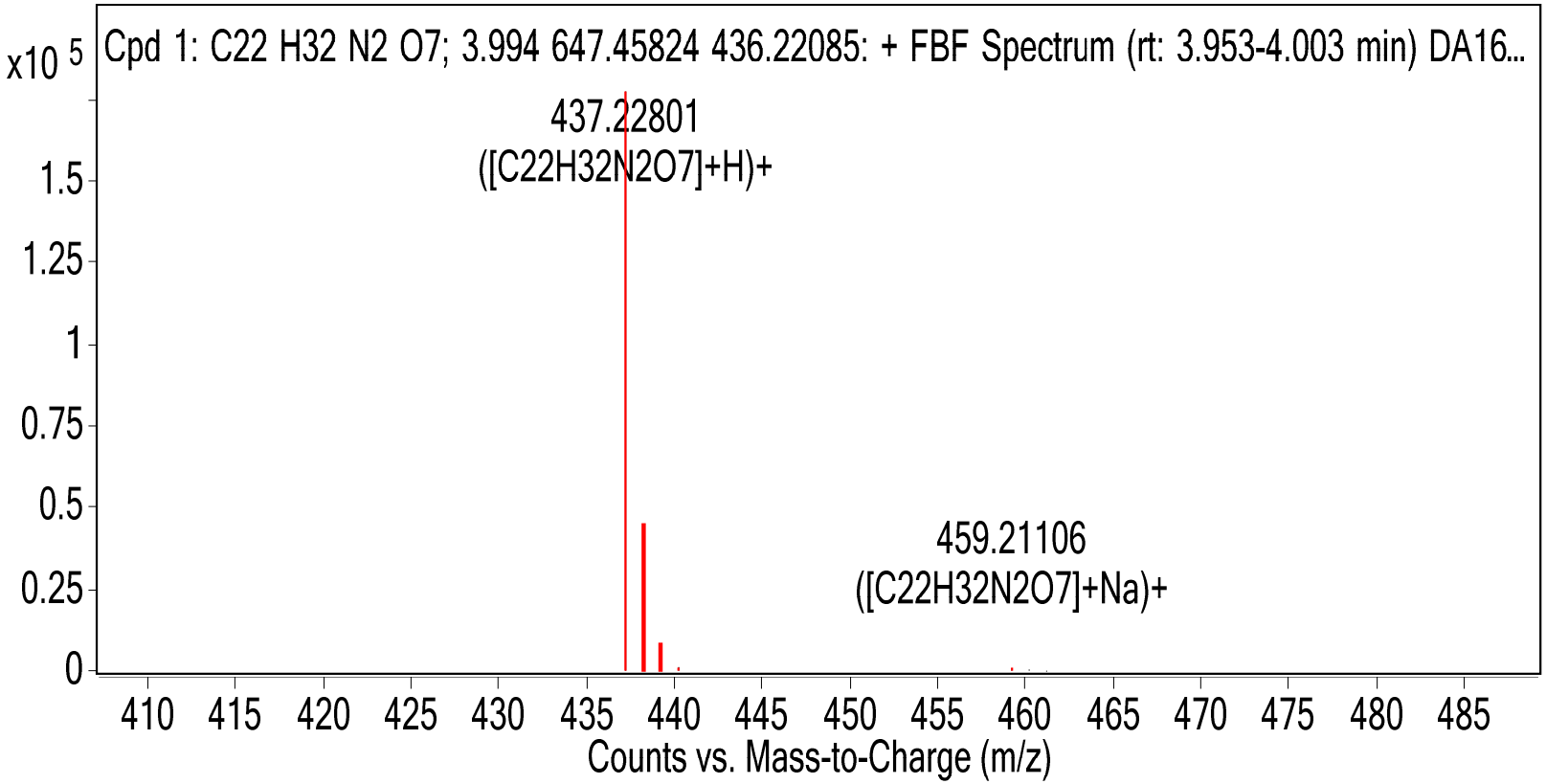

Figure B30 Mass spectra of six carbon linker 0.5 generation dendritic monomer

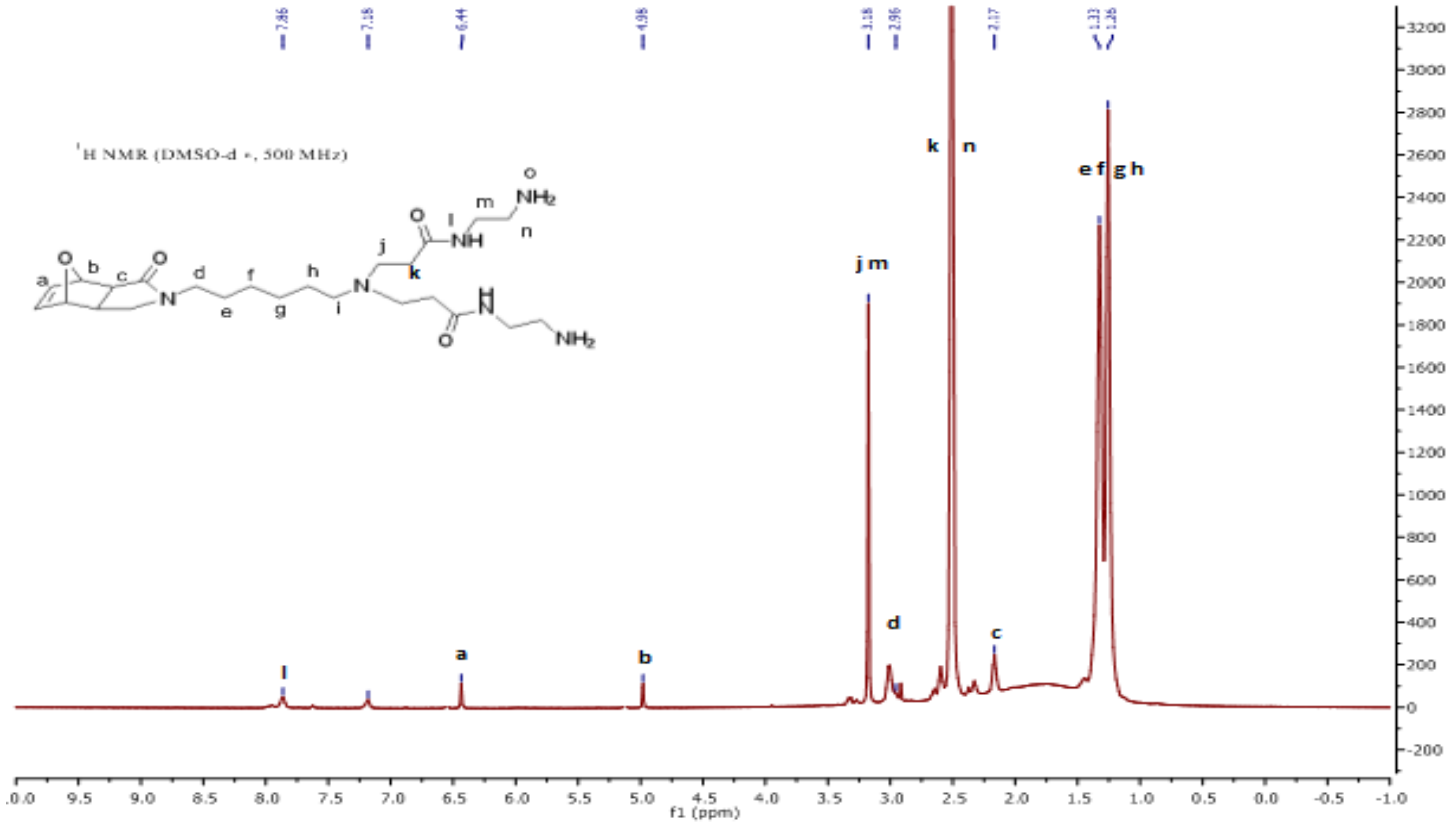

Figure B31 ^{1}H NMR of six carbon linker 1.0 generation dendritic monomer

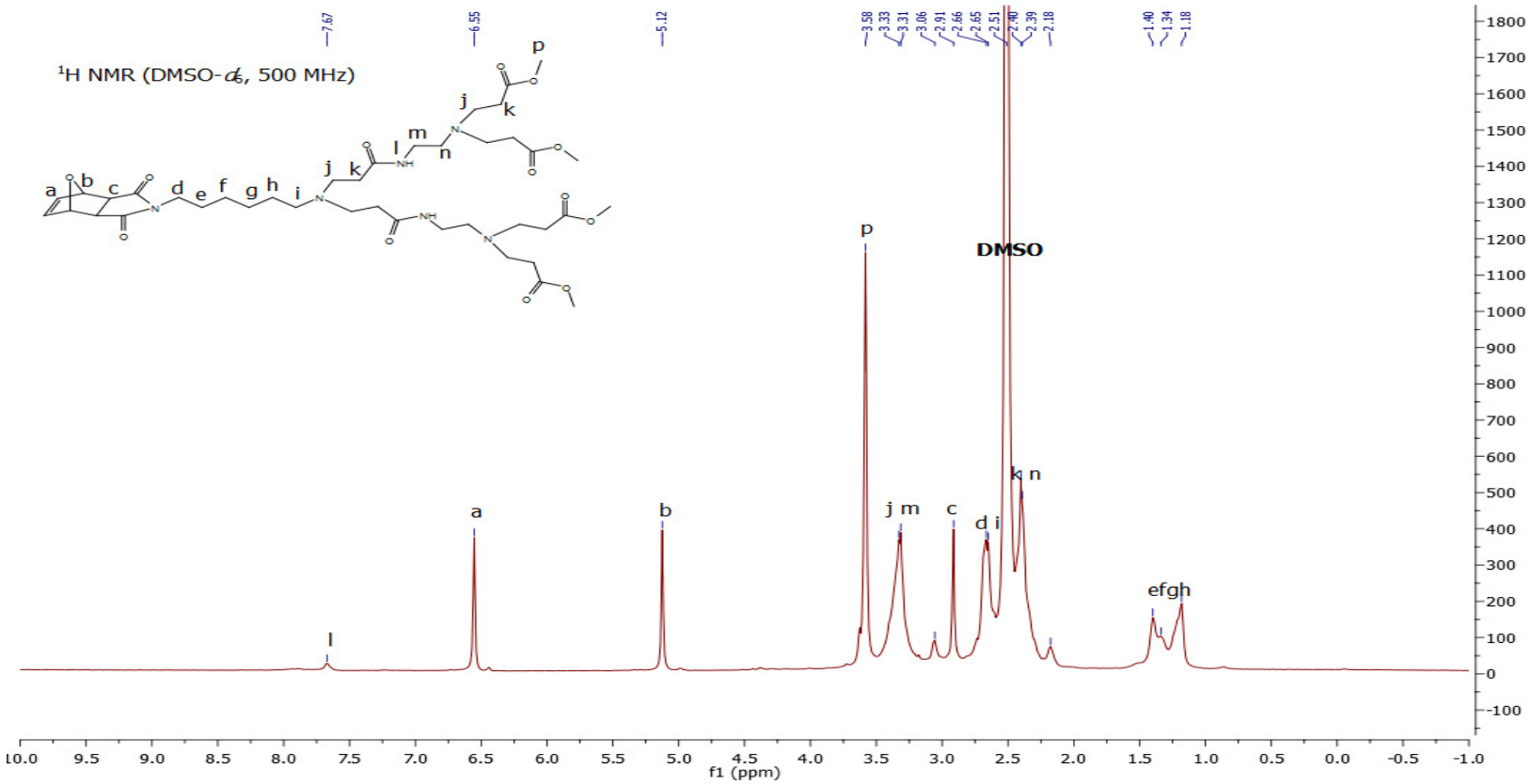

Figure B32 ¹H NMR of six carbon linker 1.5 generation dendritic monomer

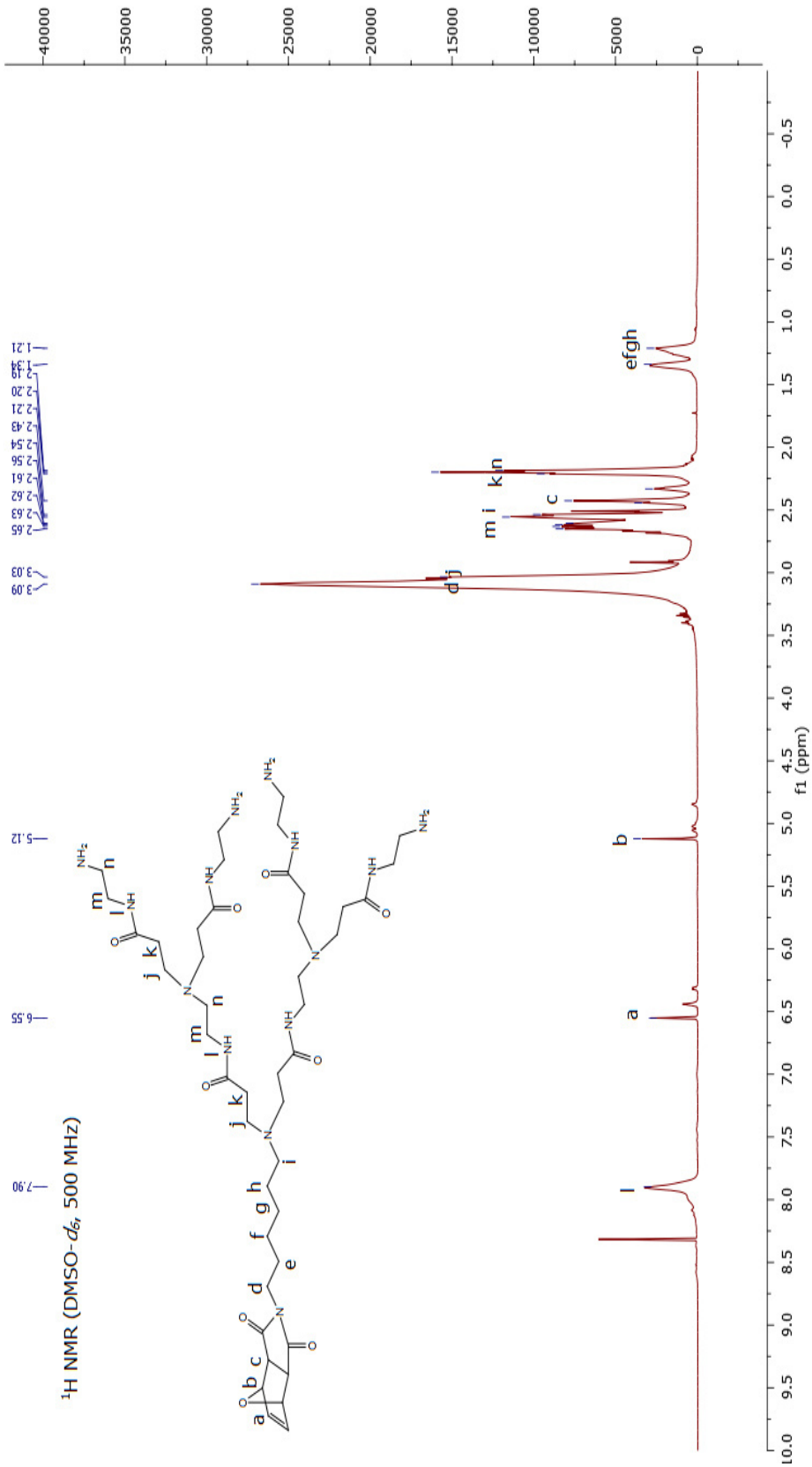

Figure B33 ^{1}H NMR of six carbon linker 2.0 generation dendritic monomer

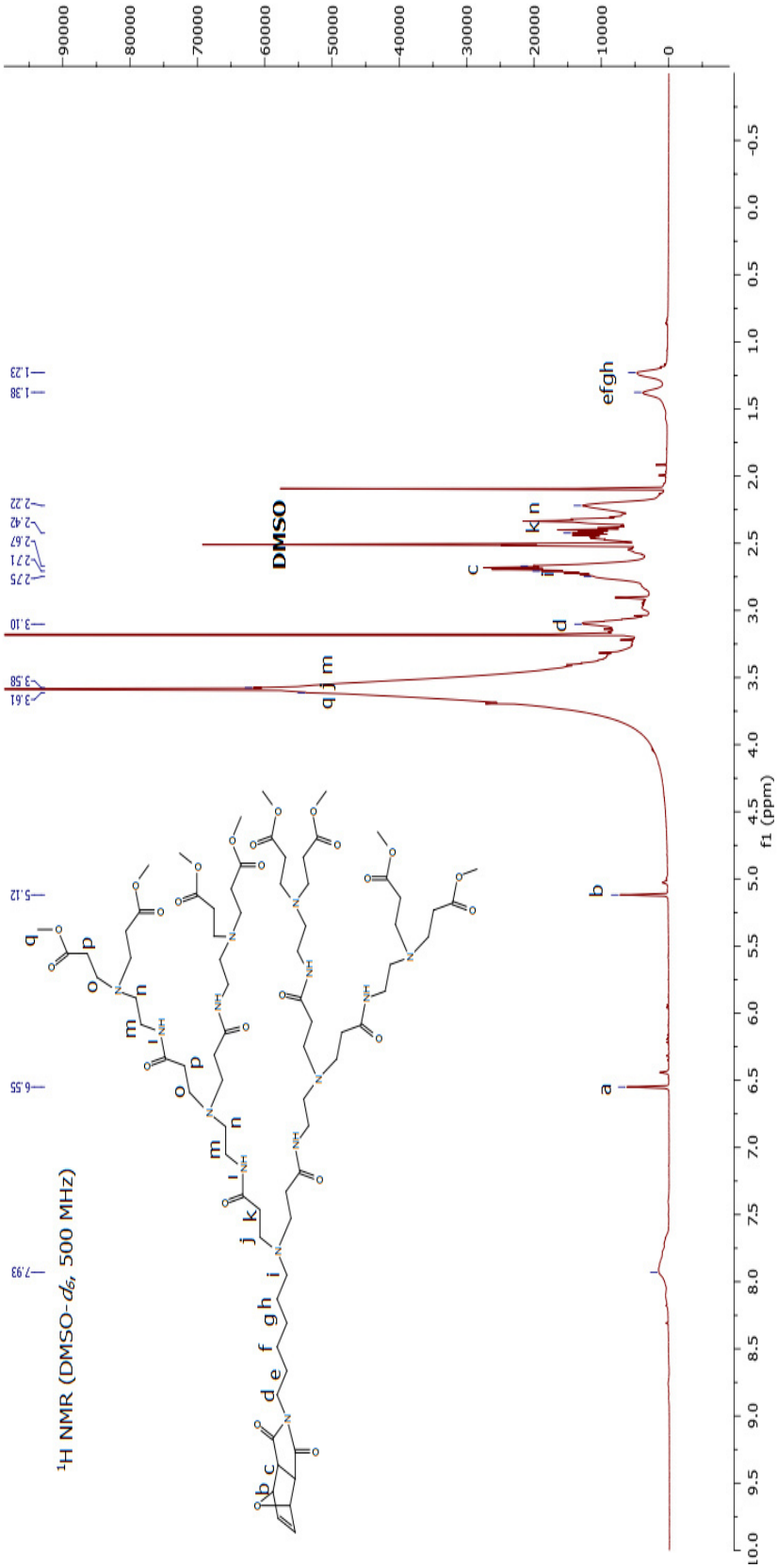

Figure B34 ^{1}H NMR of six carbon linker 2.5 generation dendritic monomer

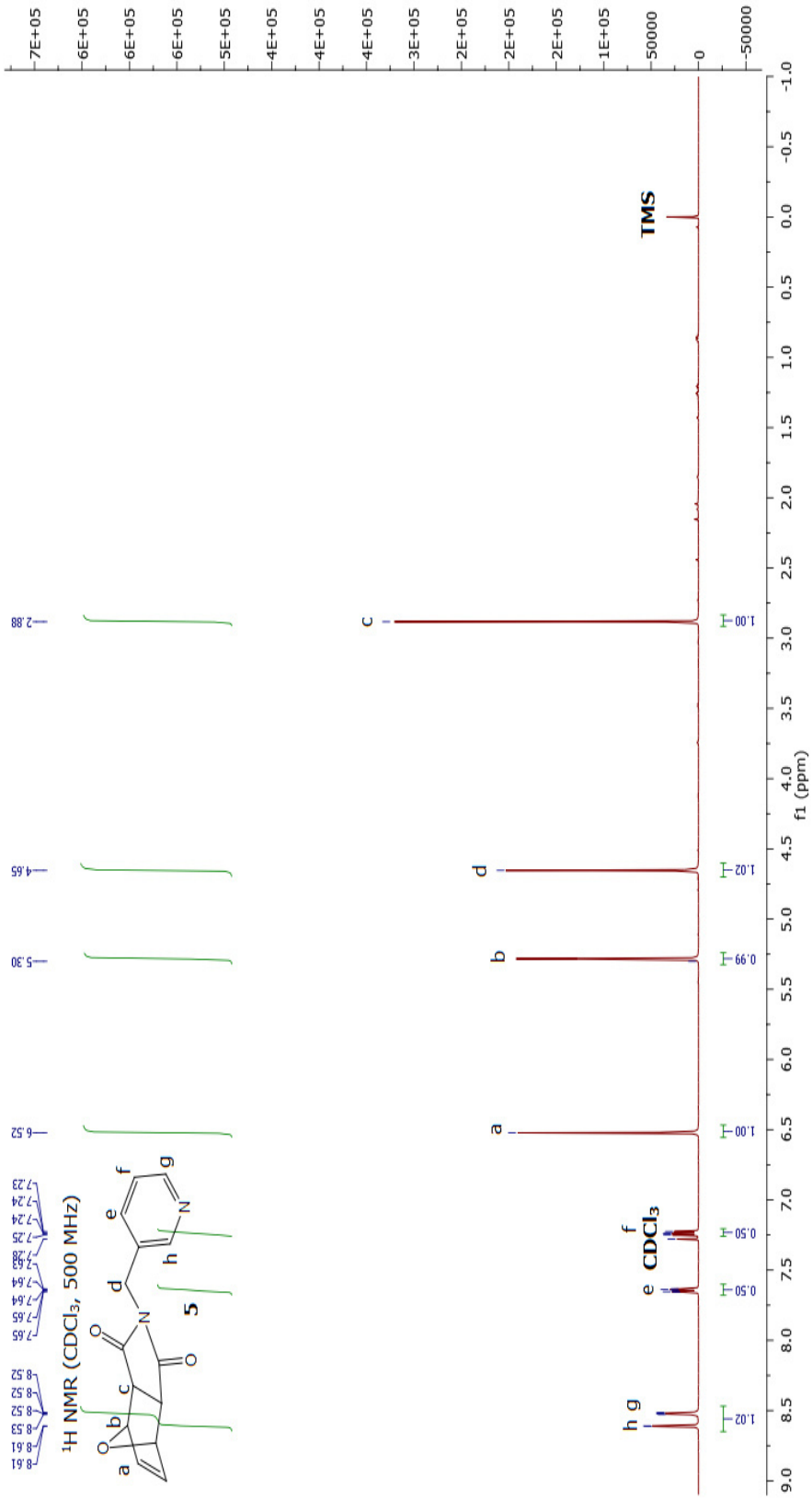

Figure B35 ^{1}H NMR of pyridine based monomer

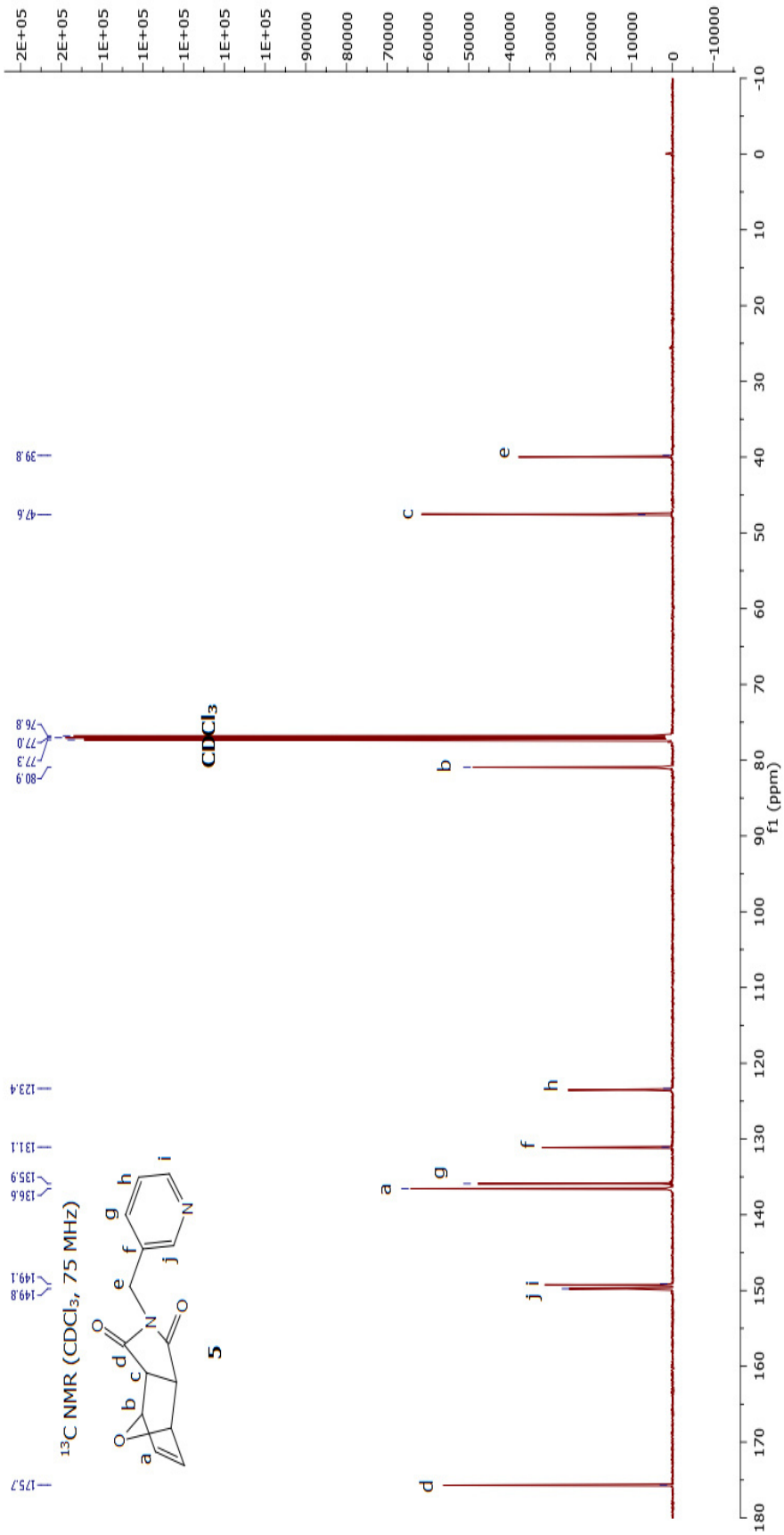

Figure B36 ^{13}C NMR of pyridine based monomer

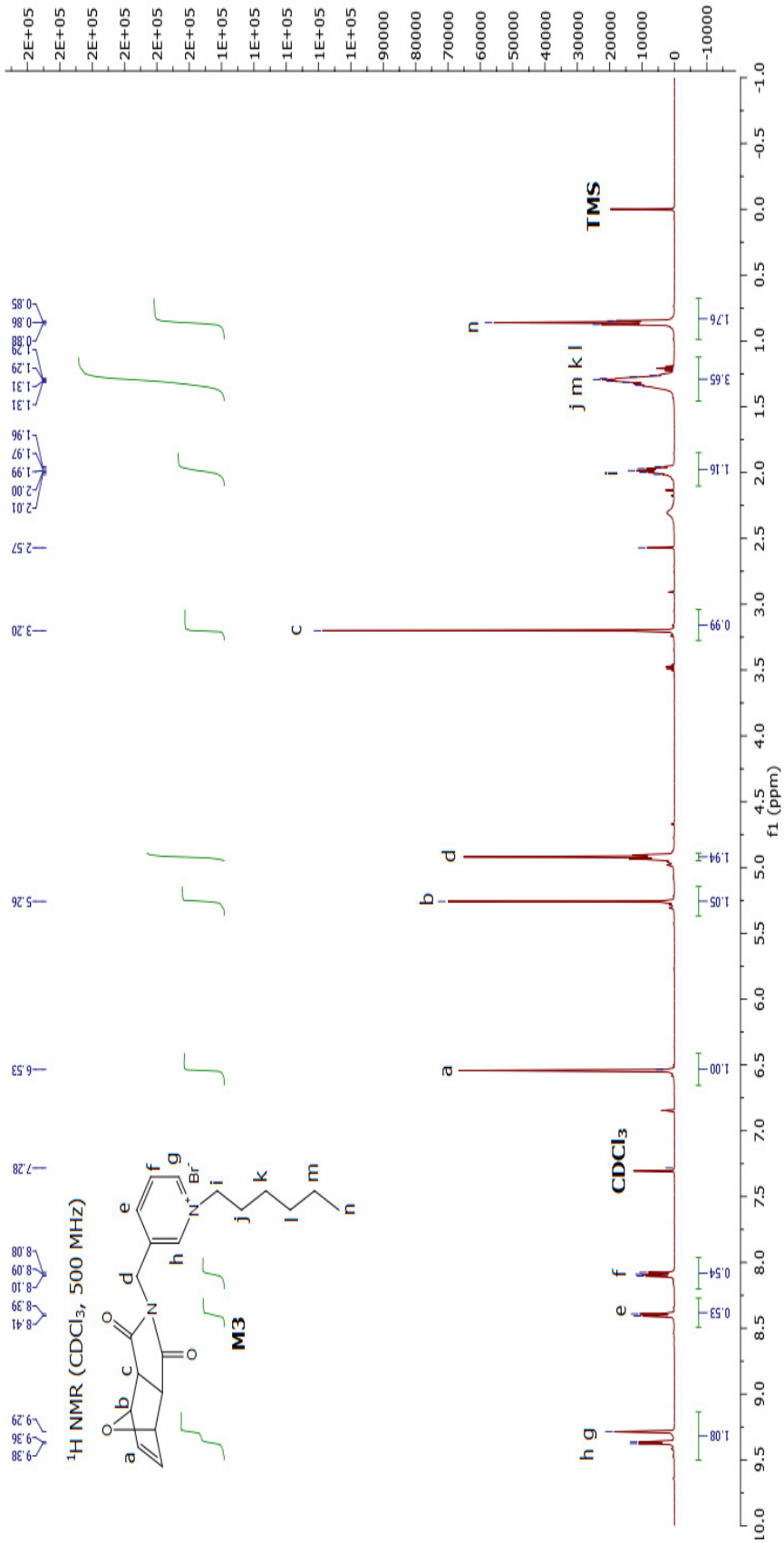

Figure B37 ^{1}H NMR of pyridinium based monomer

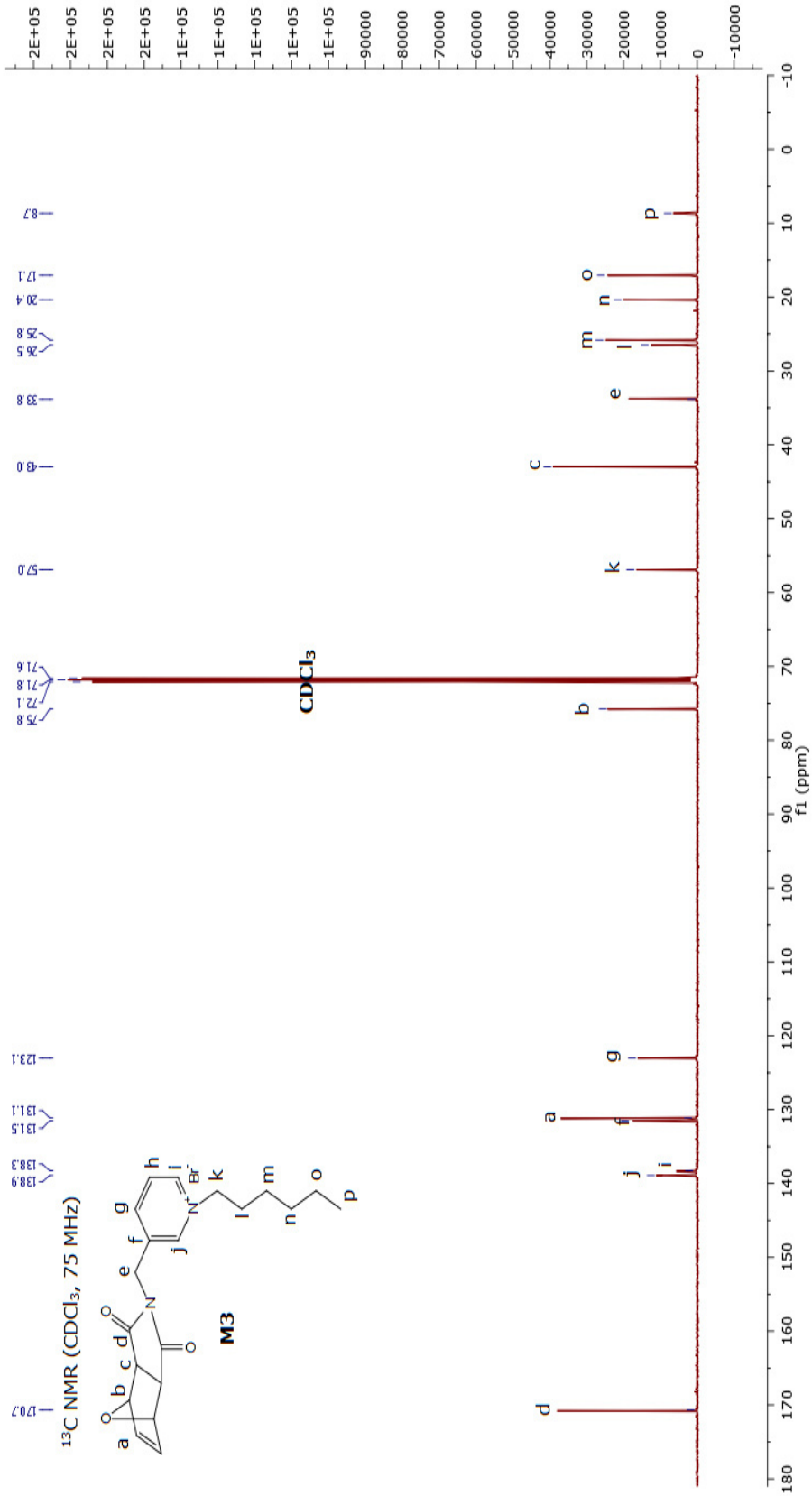

Figure B38 ^{13}C NMR of pyridinium based monomer

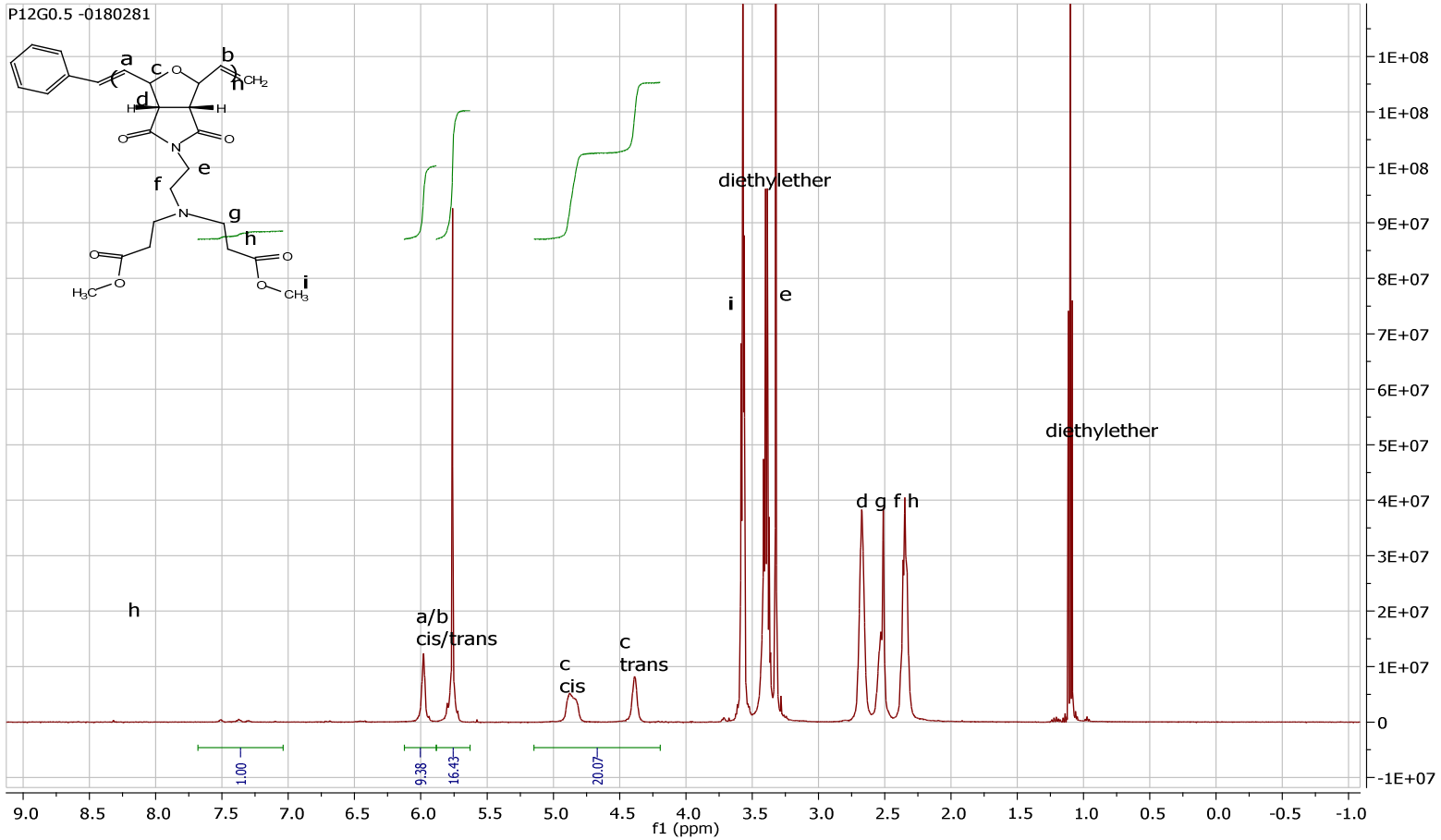

Figure B39 ^{1}H NMR of polymer synthesized from two carbon linker 0.5 generation dendritic monomer

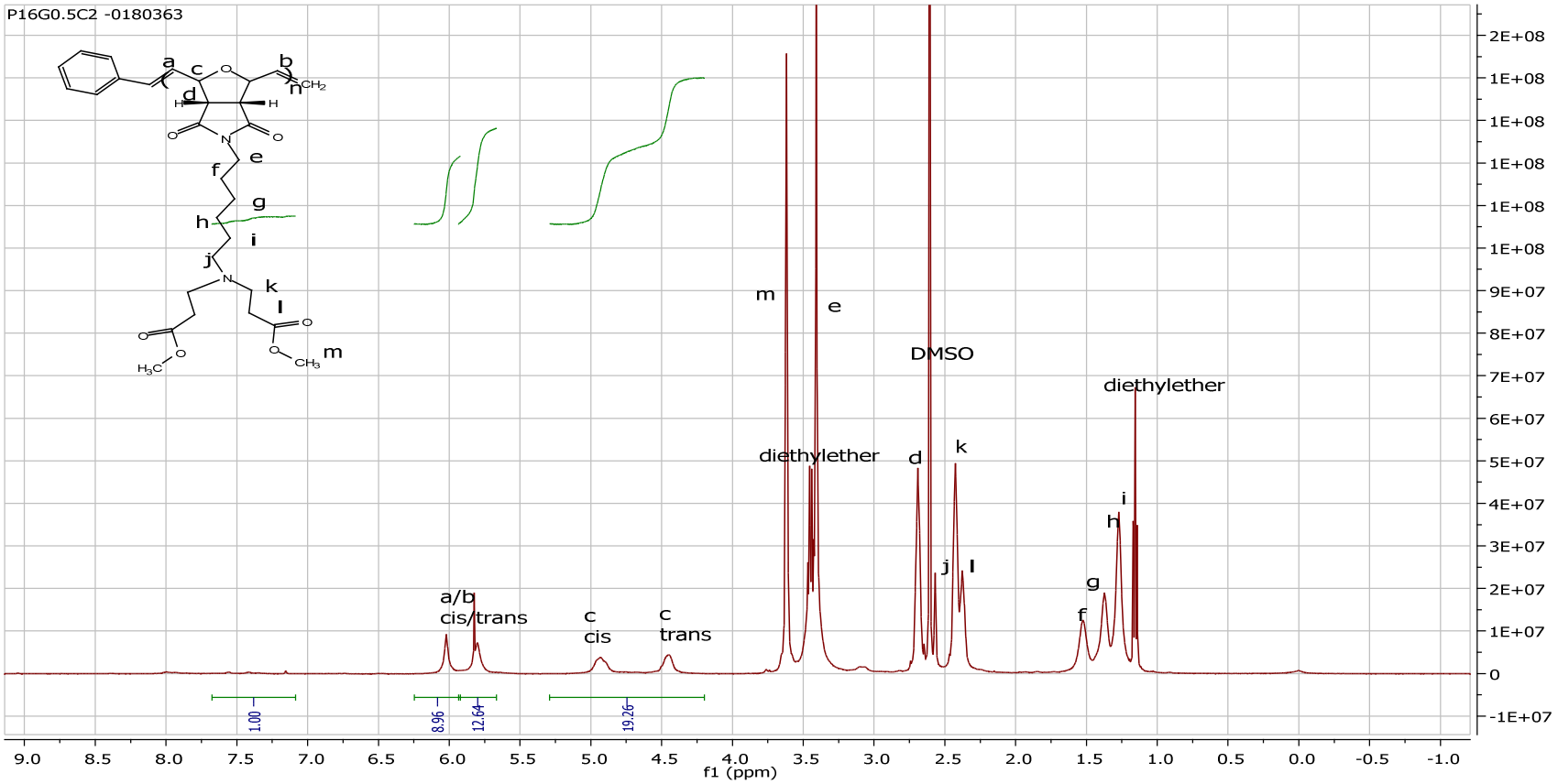

Figure B40 ^{1}H NMR of polymer synthesized from six carbon linker 0.5 generation dendritic monomer and M_n calculation

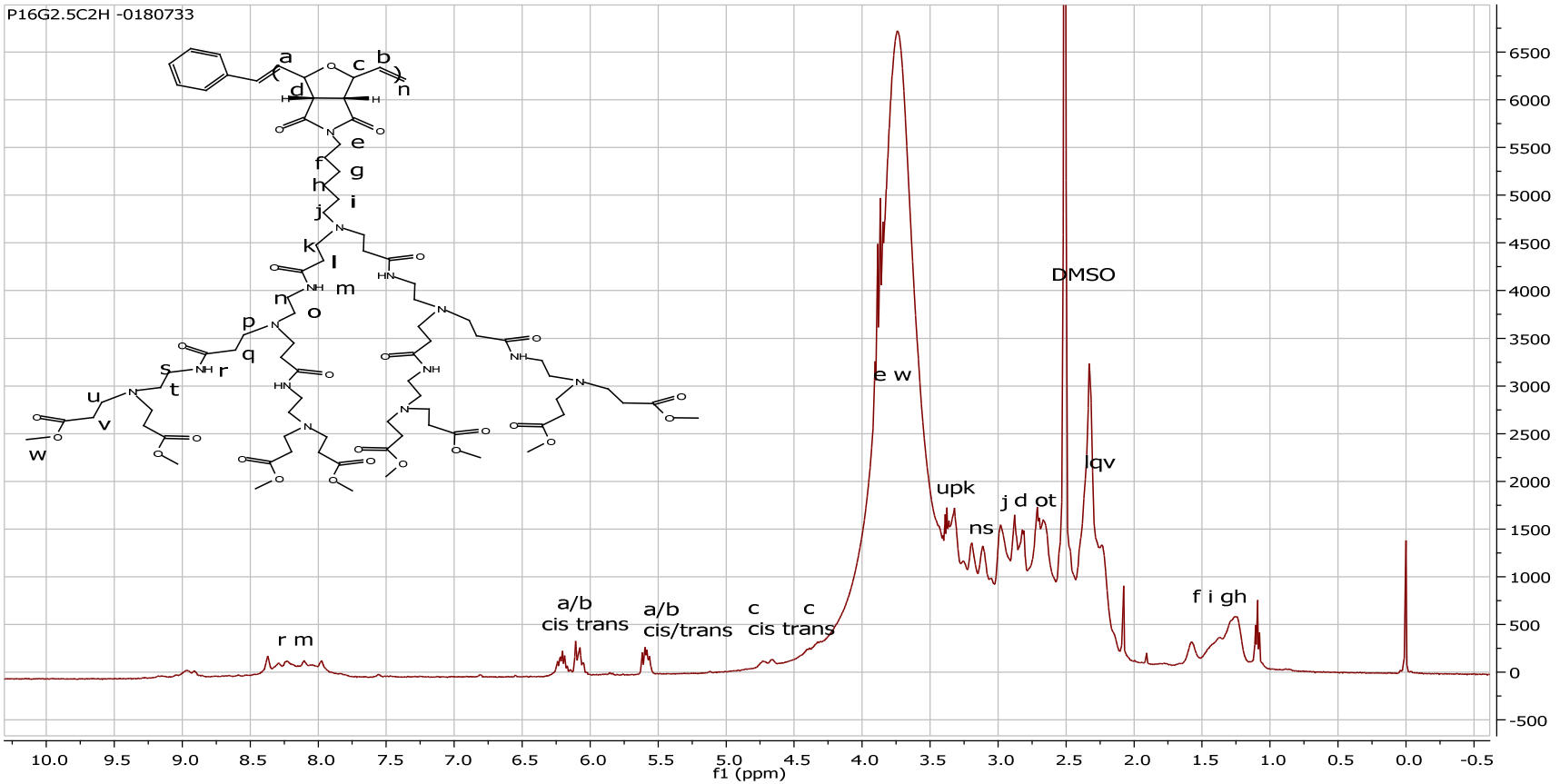

Figure B41 ^{1}H NMR of polymer synthesized from six carbon linker 2.5 generation dendritic monomer

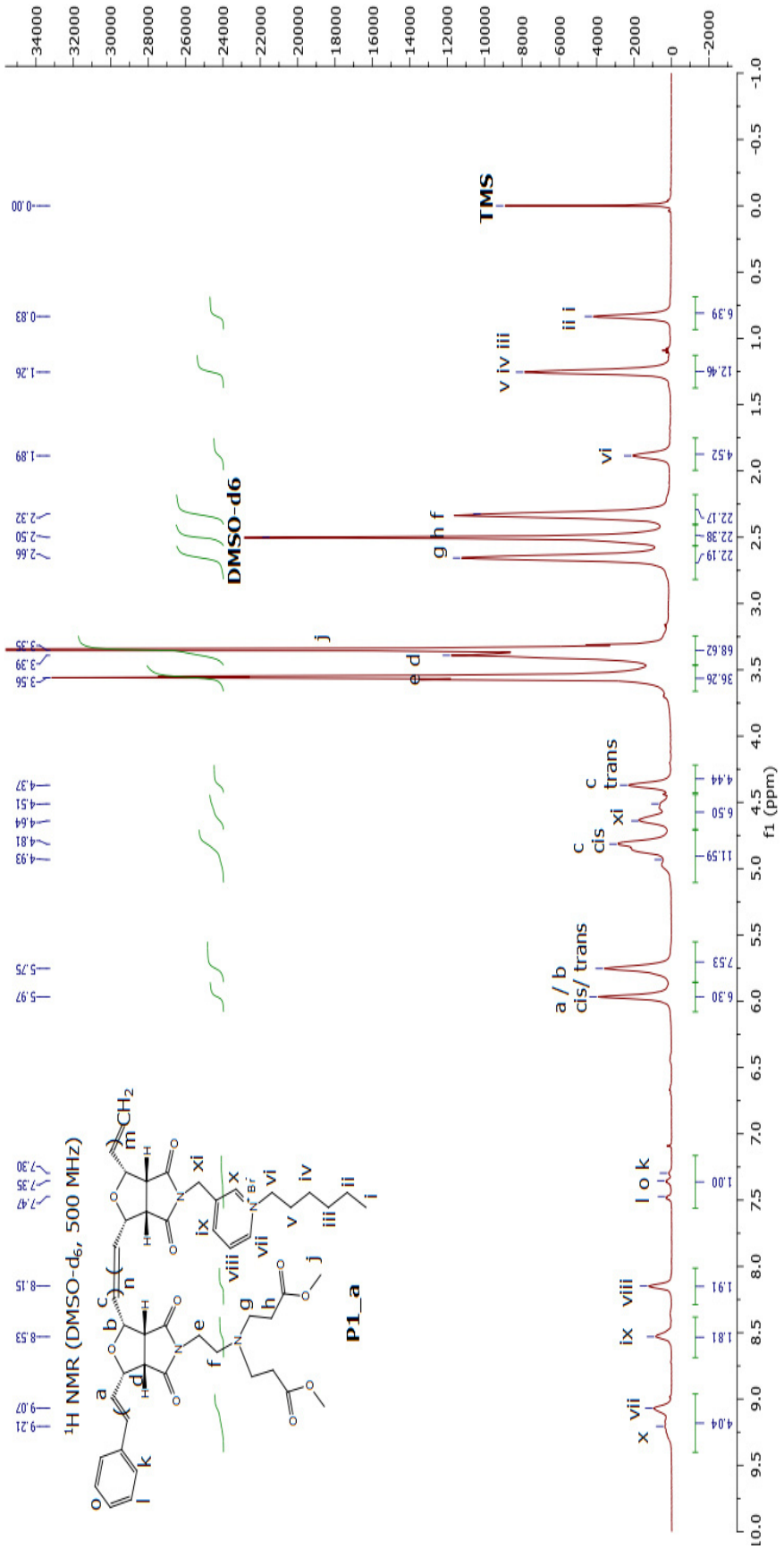

Figure B42 ^{1}H NMR of random copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (8k:2k)

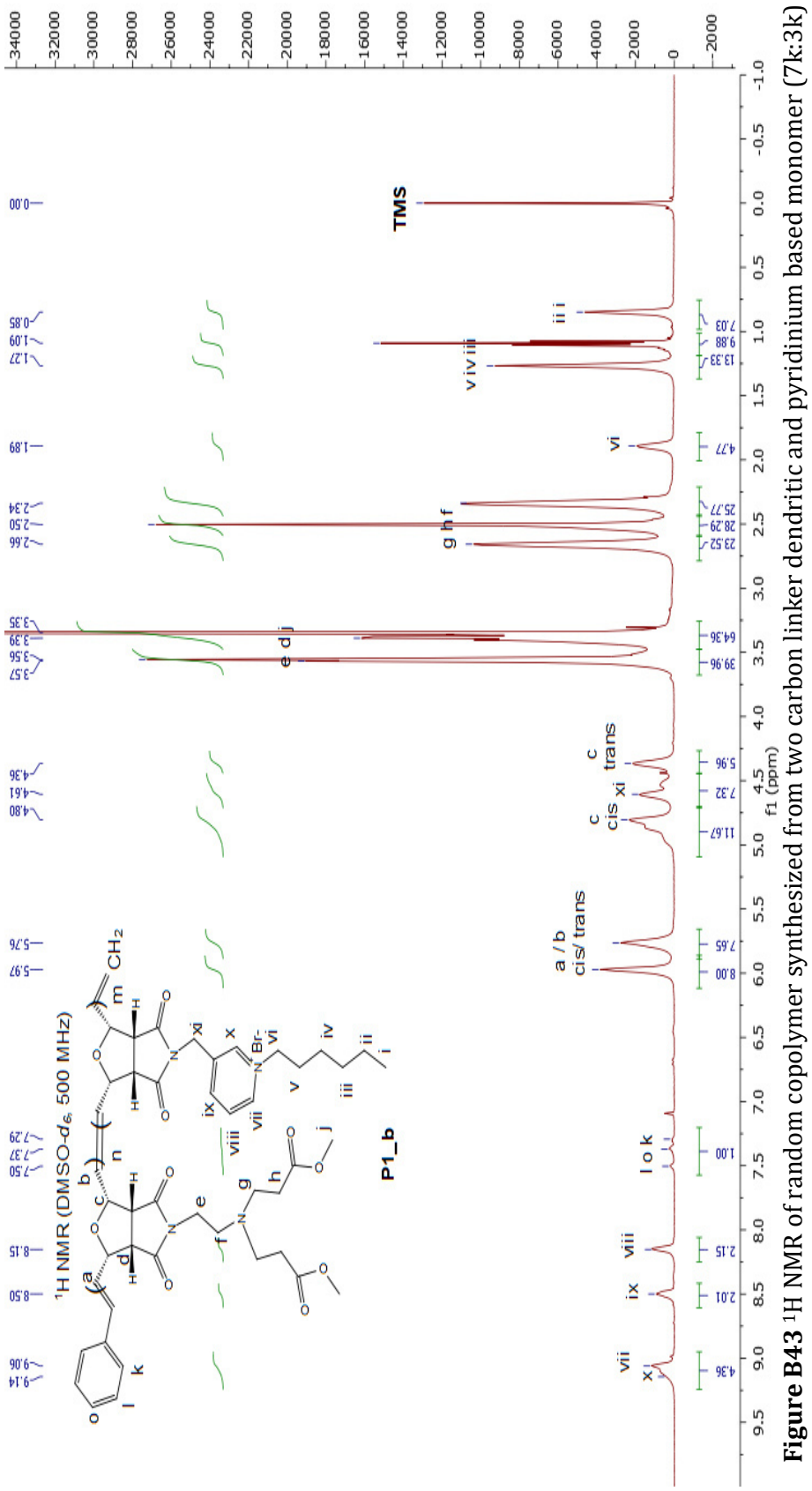

Figure B43 ^{1}H NMR of random copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (7k:3k)

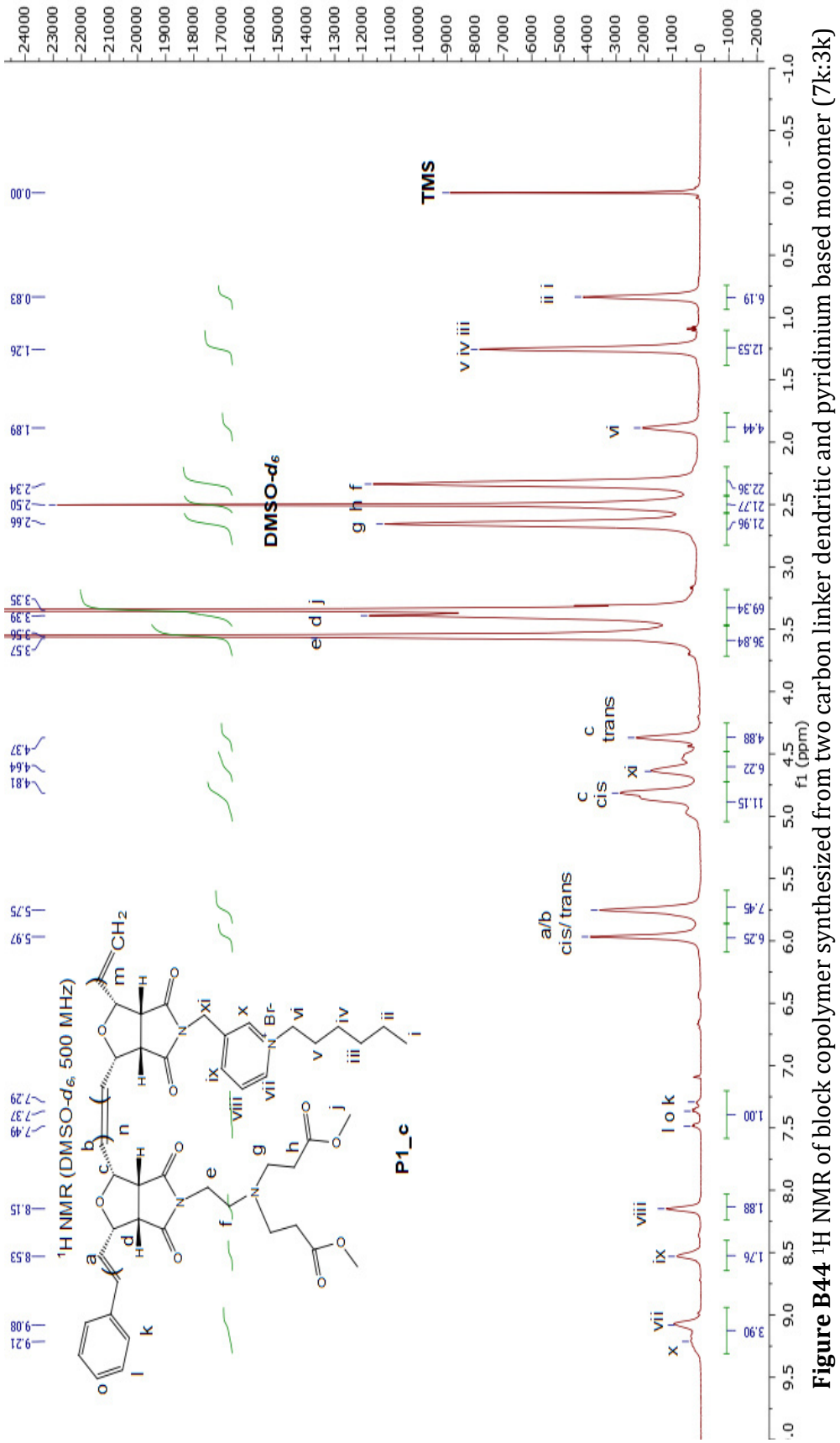

Figure B44 ^{1}H NMR of block copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (7k:3k)

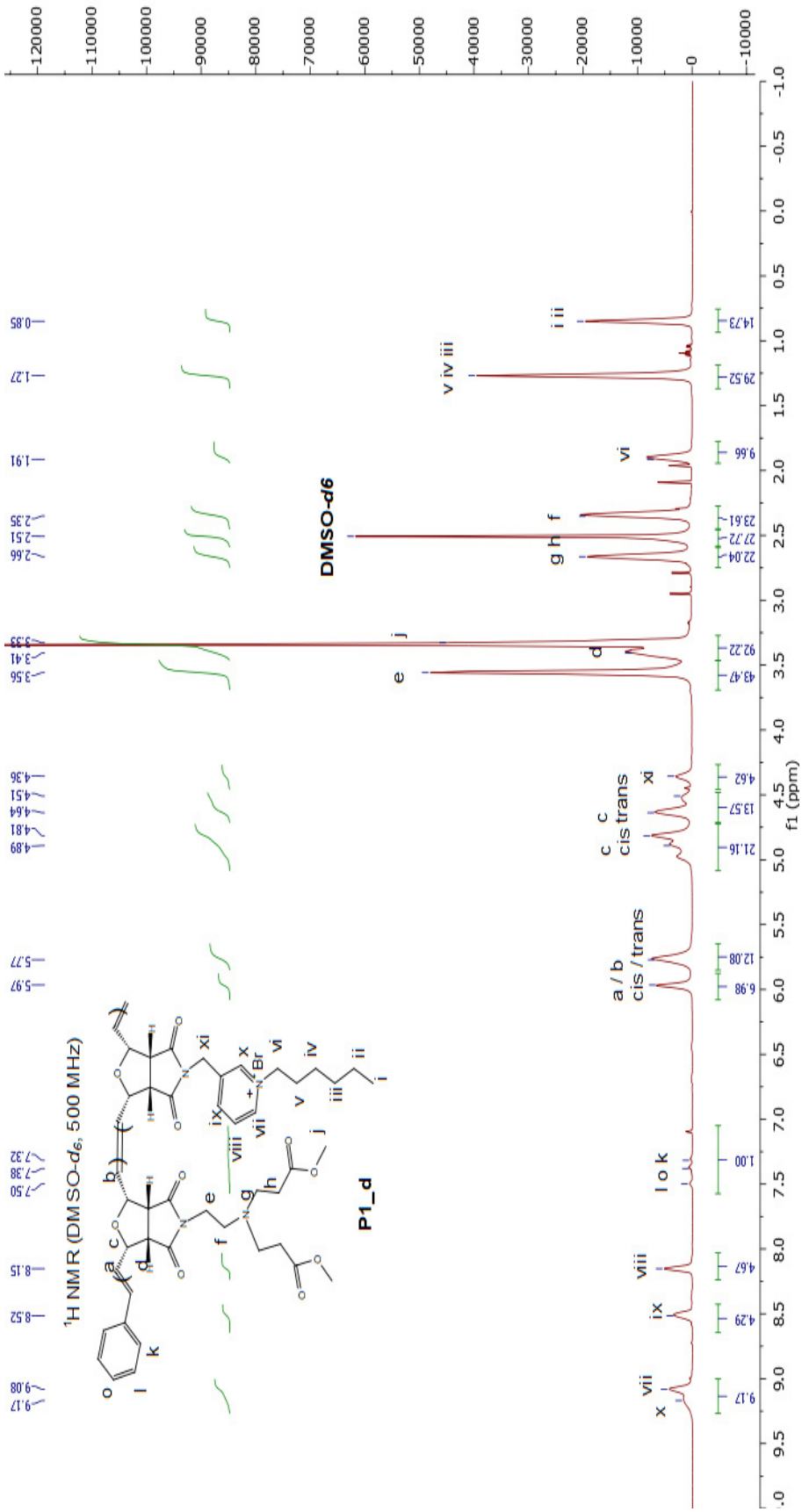

Figure B45 ^{1}H NMR of random copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (5k:5k)

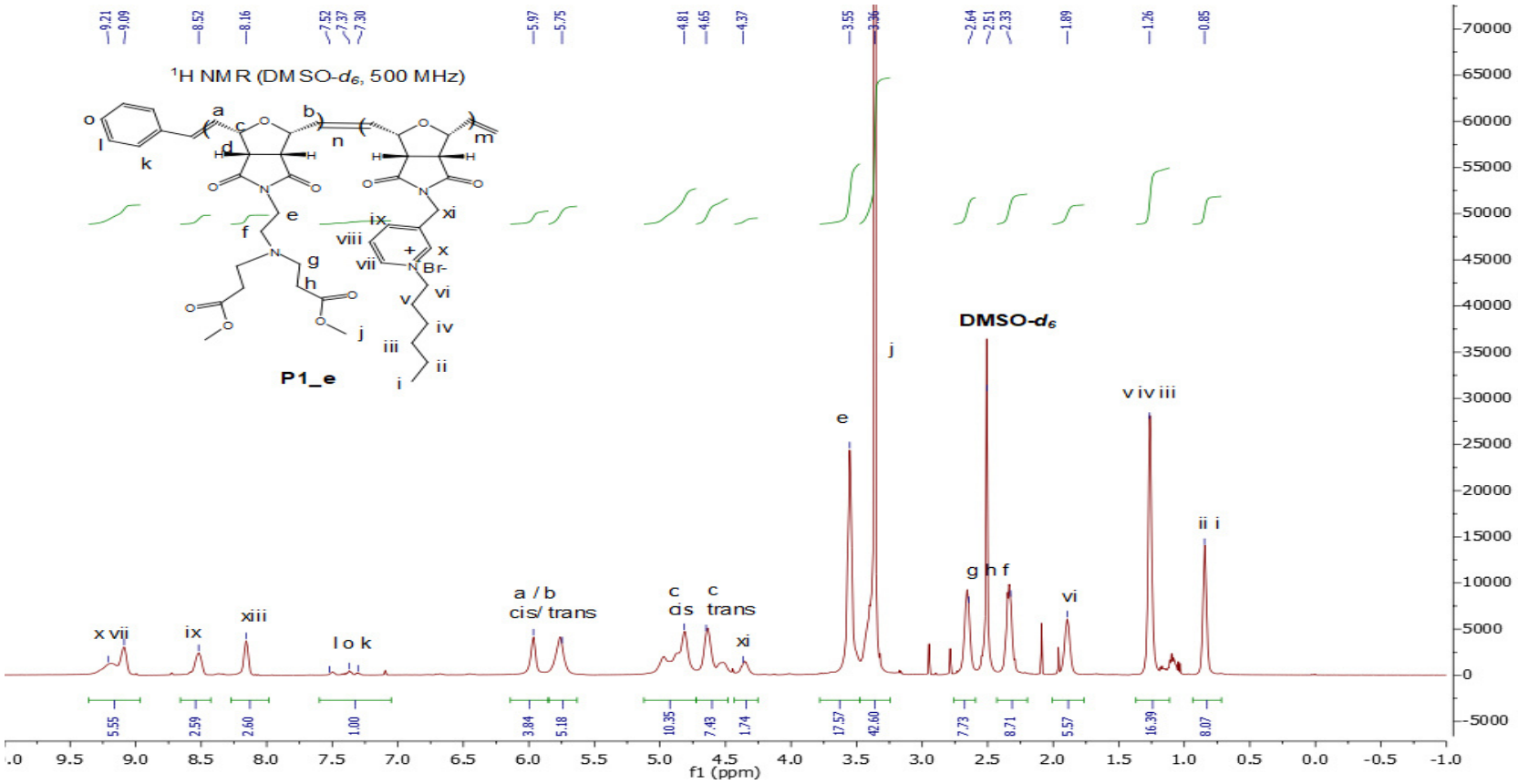

Figure B46 ^{1}H NMR of random copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (4k:6k)

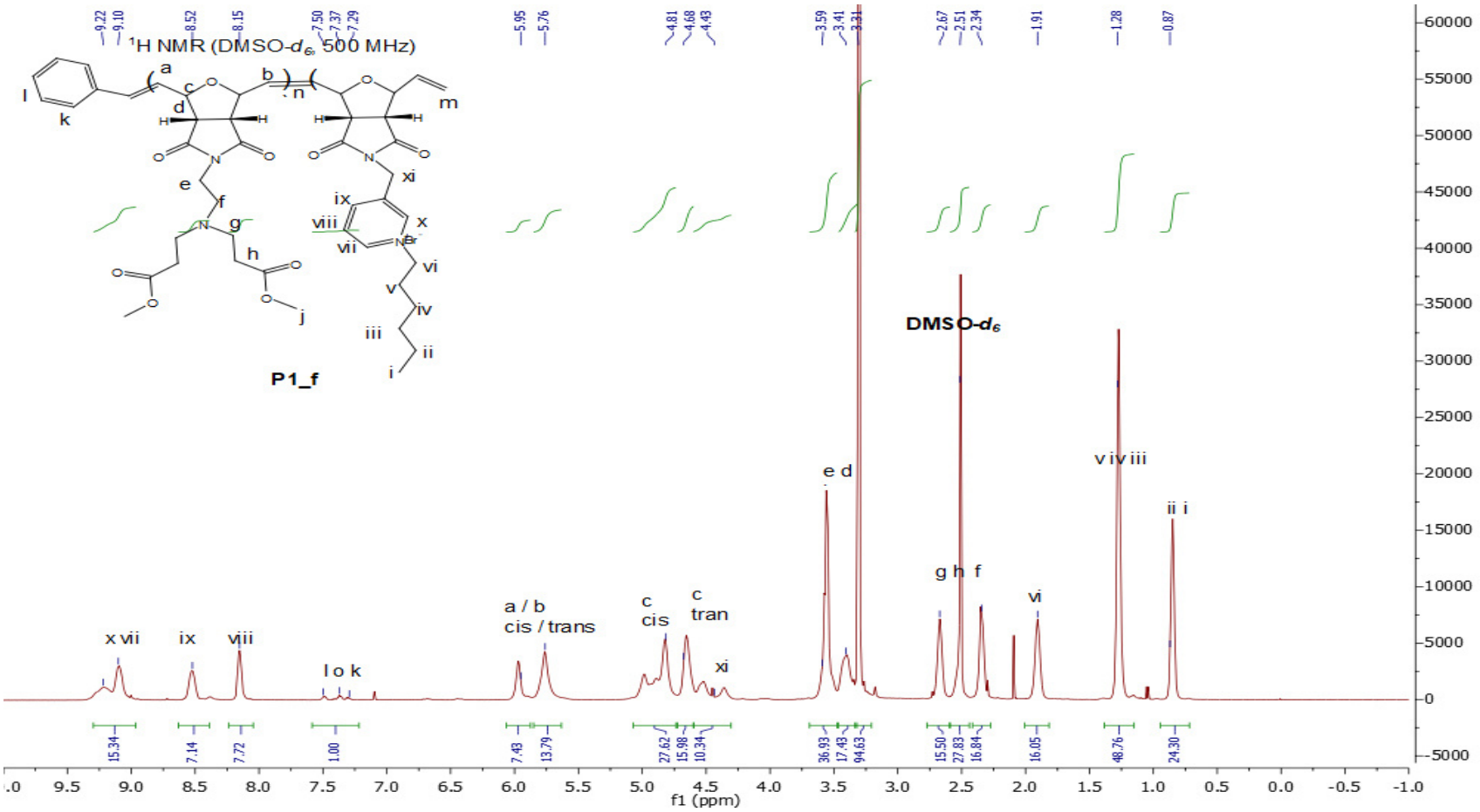

Figure B47 ^{1}H NMR of random copolymer synthesized from two carbon linker dendritic and pyridinium based monomer (3k:7k)

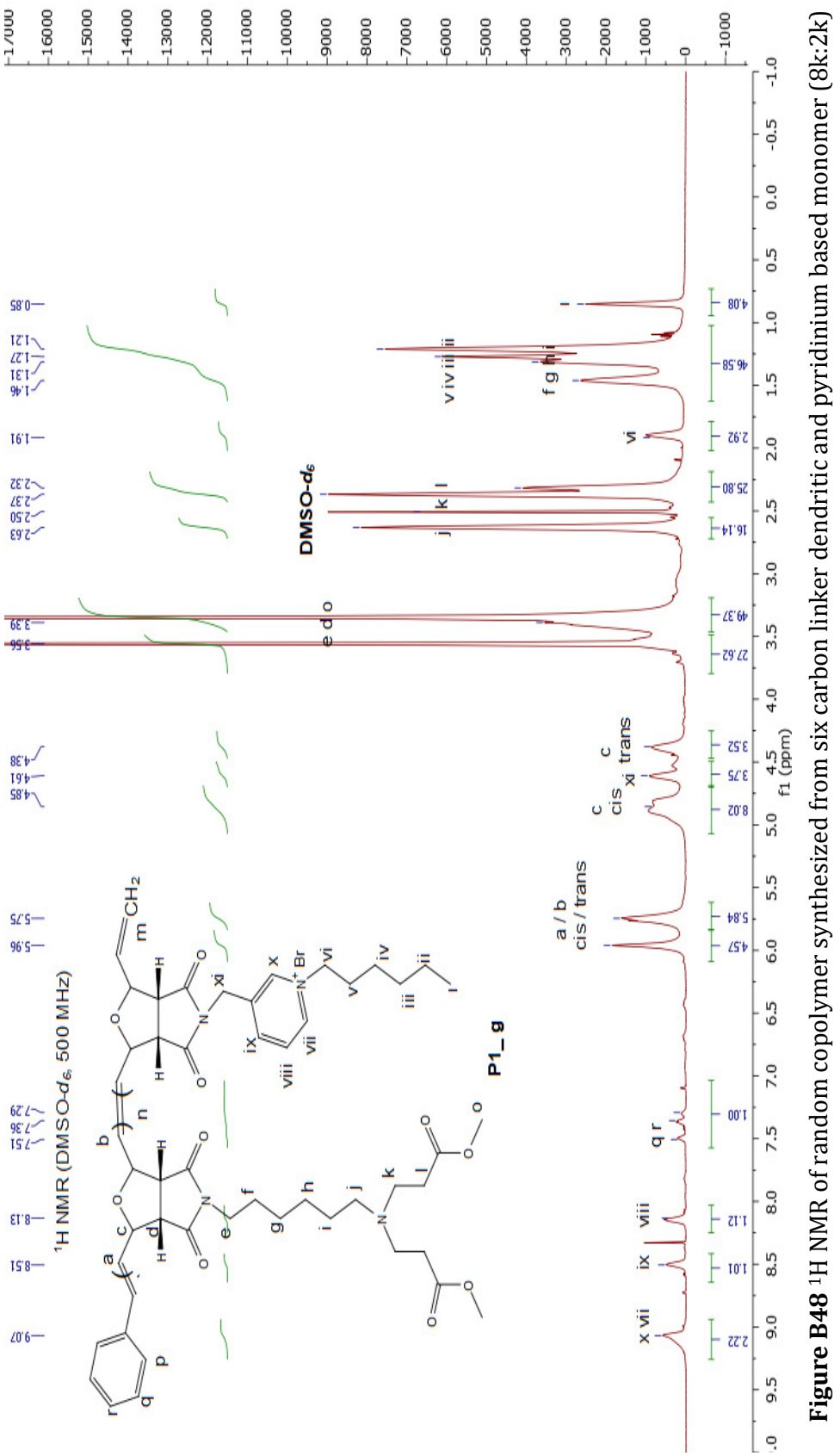

Figure B48 ^{1}H NMR of random copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (8k:2k)

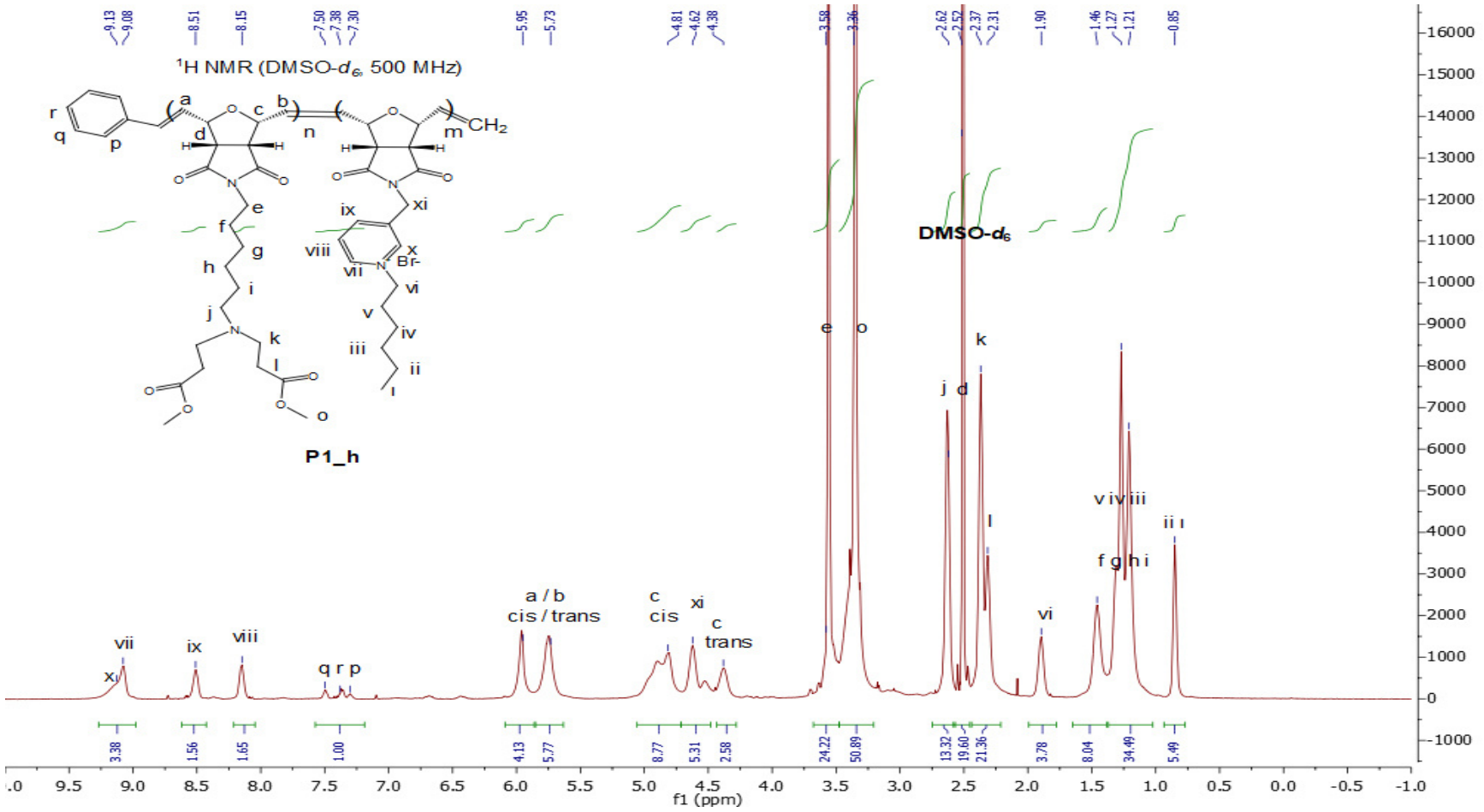

Figure B49 ^{1}H NMR of random copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (7k:3k)

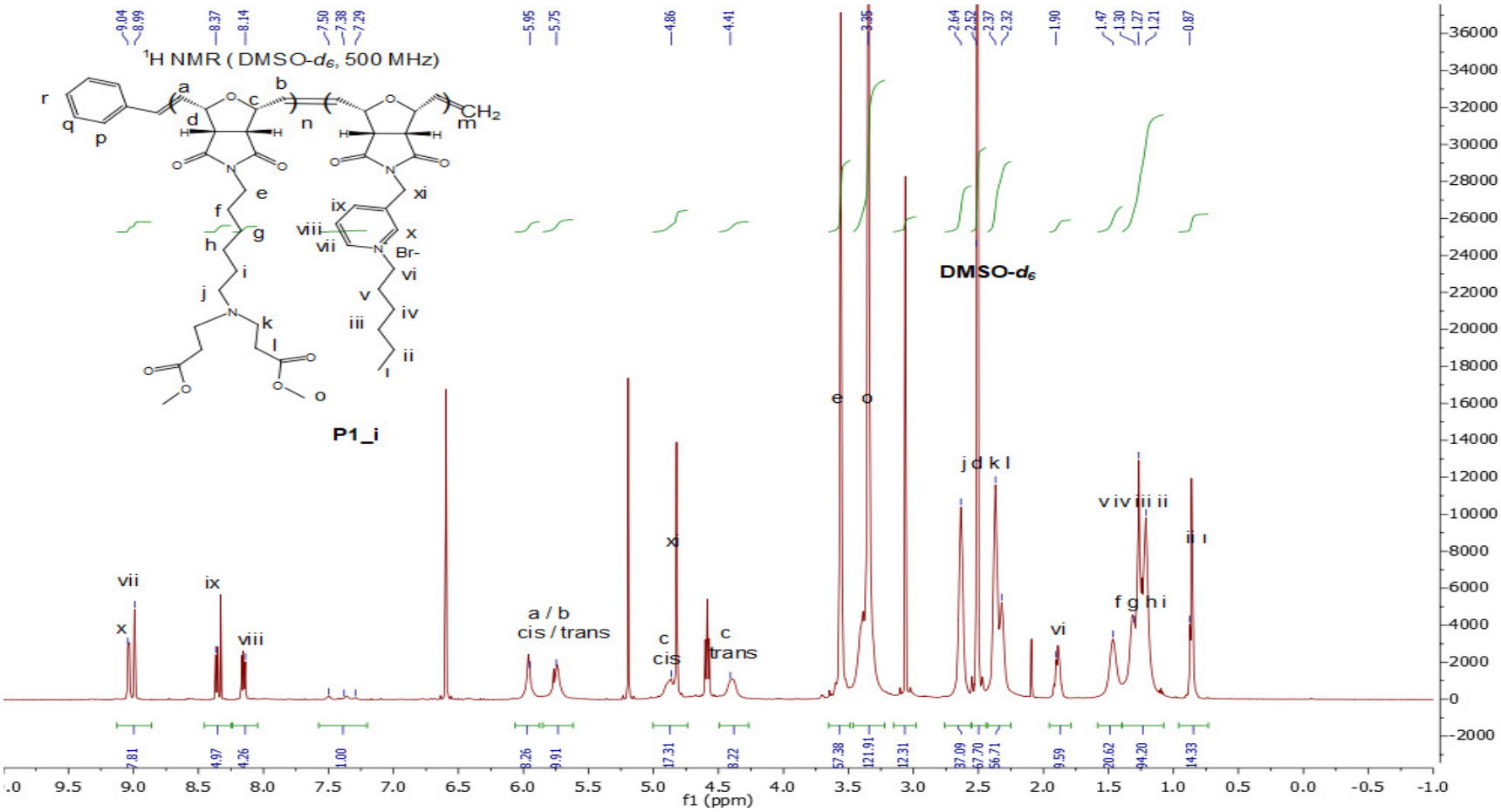

Figure B50 ^{1}H NMR of block copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (7k:3k)

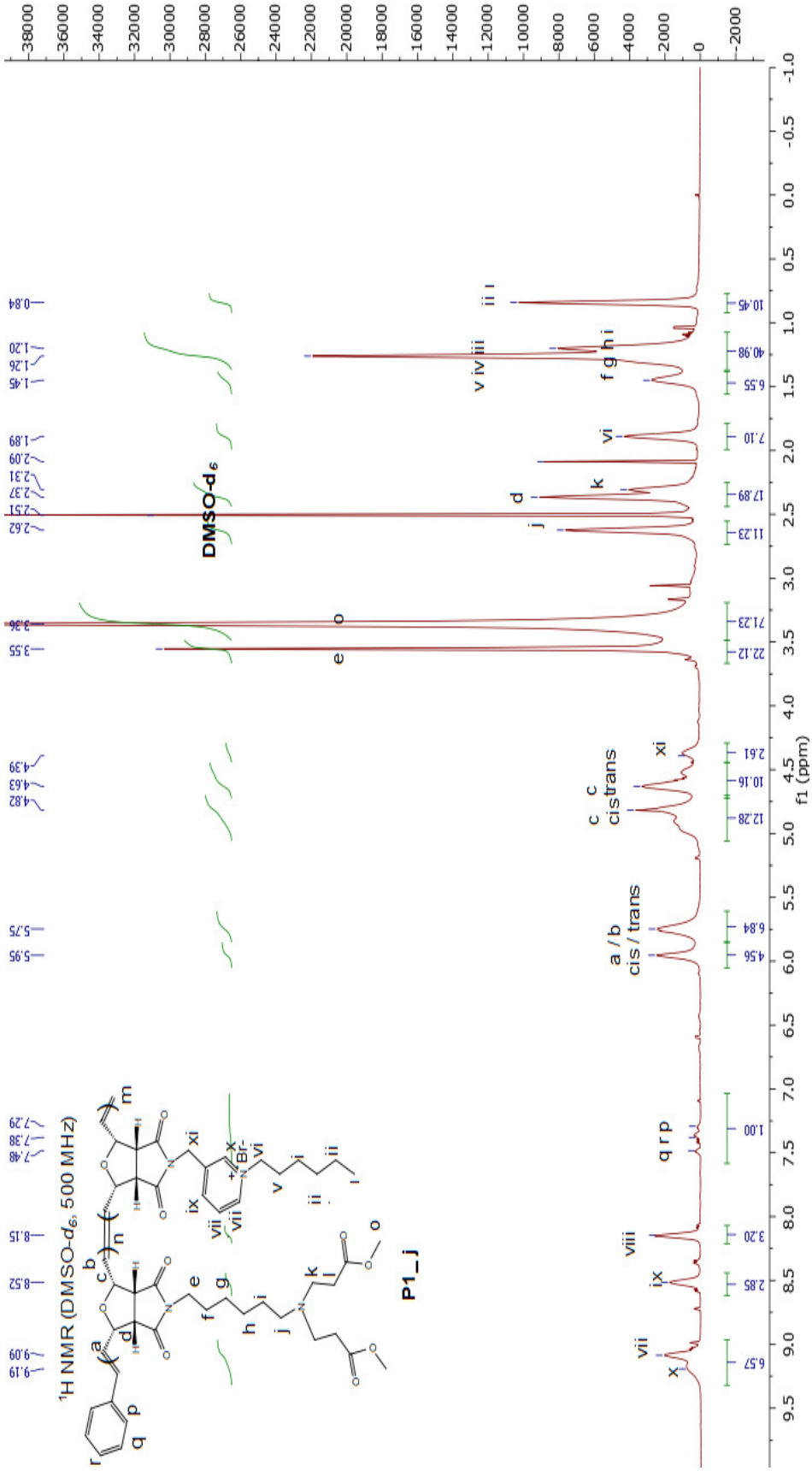

Figure B51 ^{1}H NMR of random copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (5k:5k)

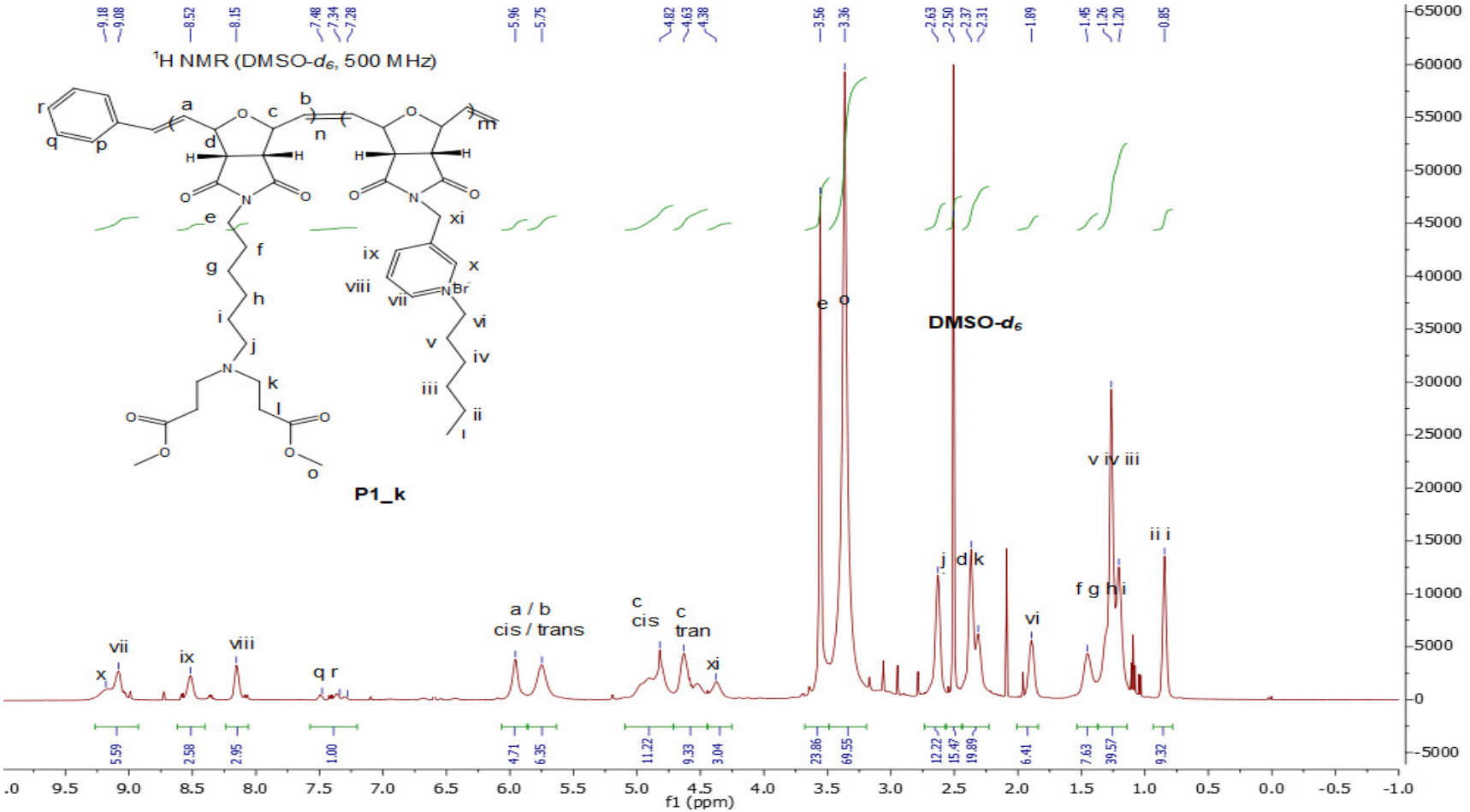

Figure B52 ^{1}H NMR of random copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (4k:6k)

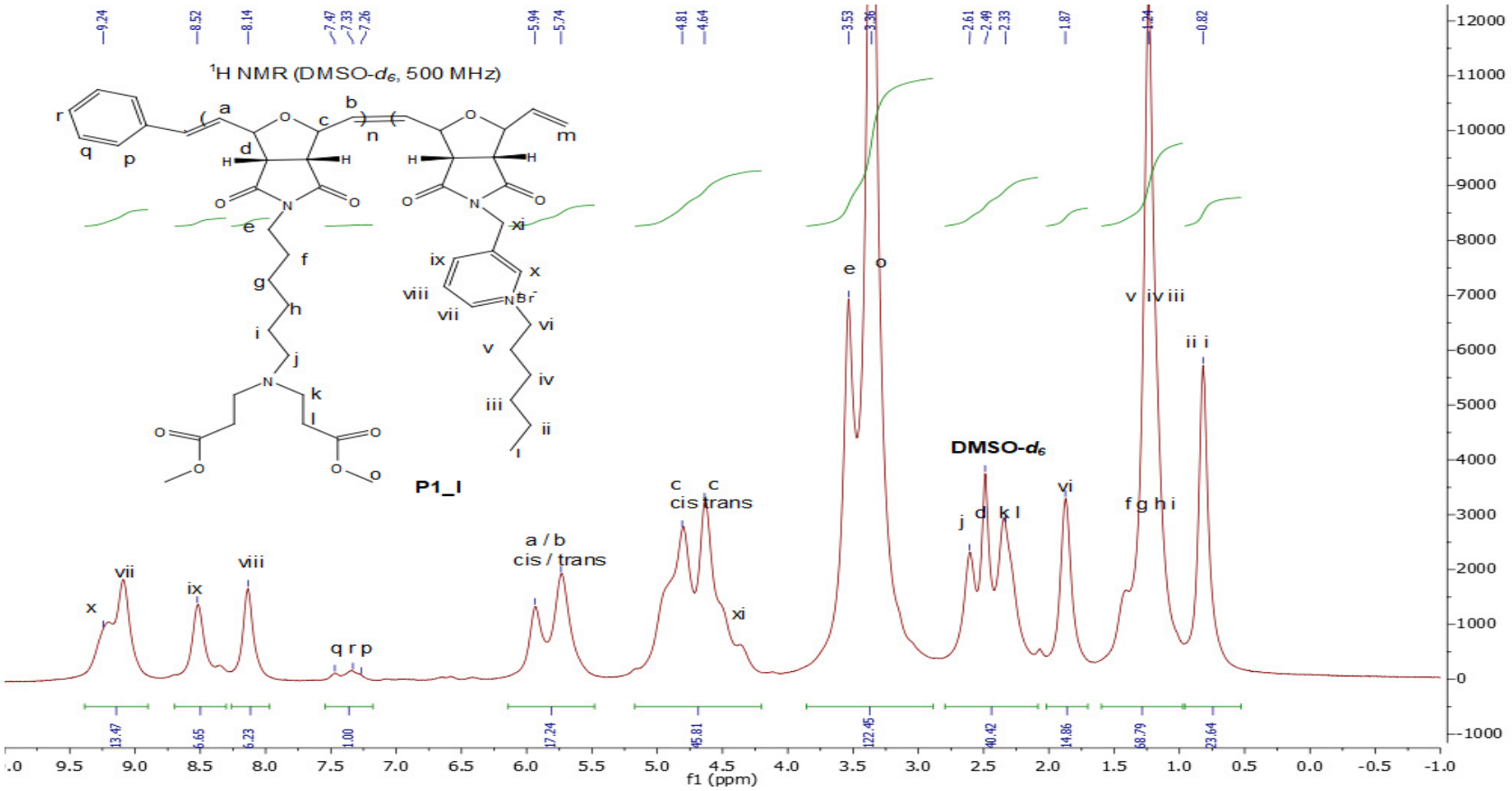

Figure B53 ^{1}H NMR of random copolymer synthesized from six carbon linker dendritic and pyridinium based monomer (3k:7k)

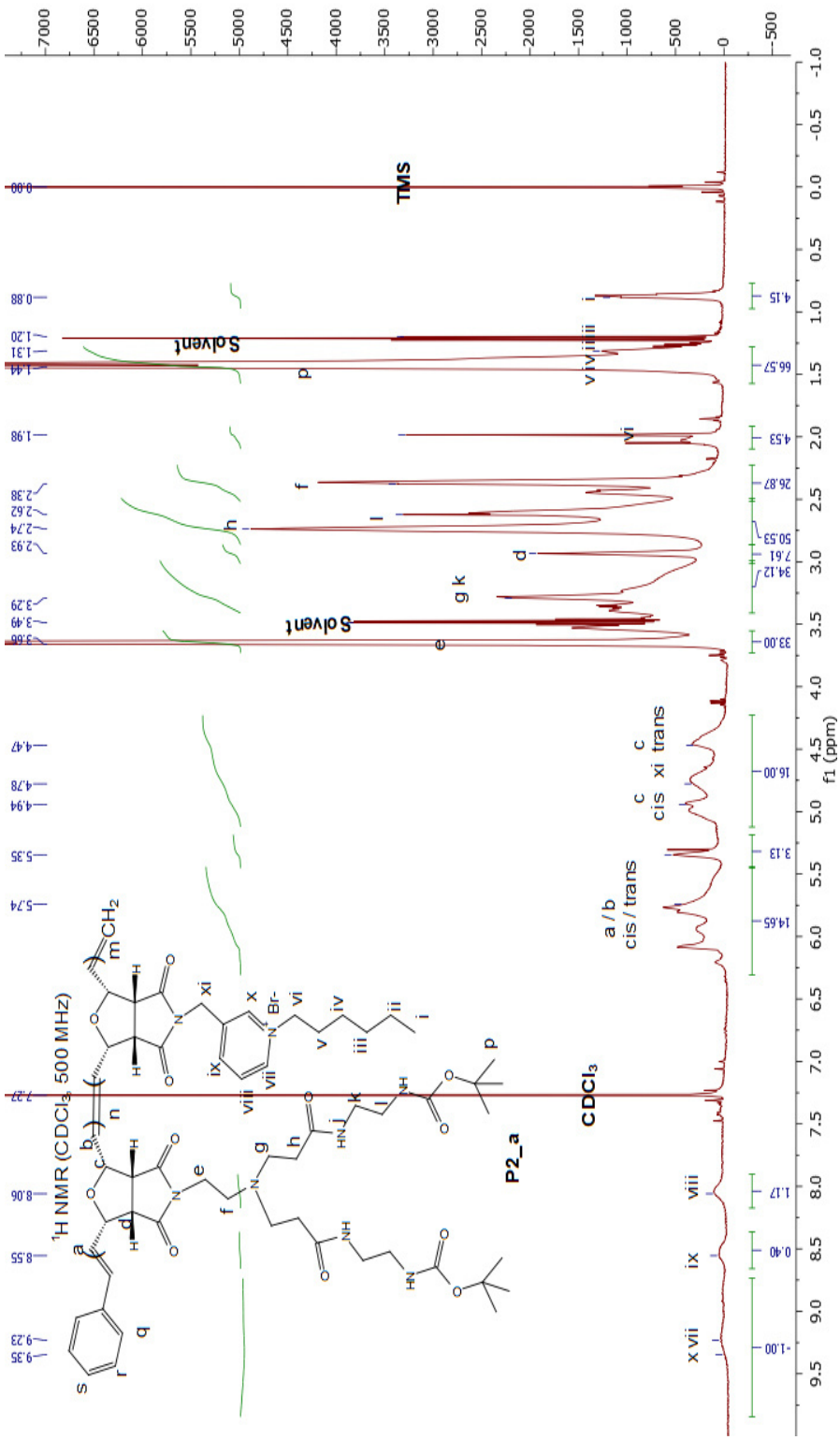

Figure B54 ^{1}H NMR of BOC surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (8k:2k)

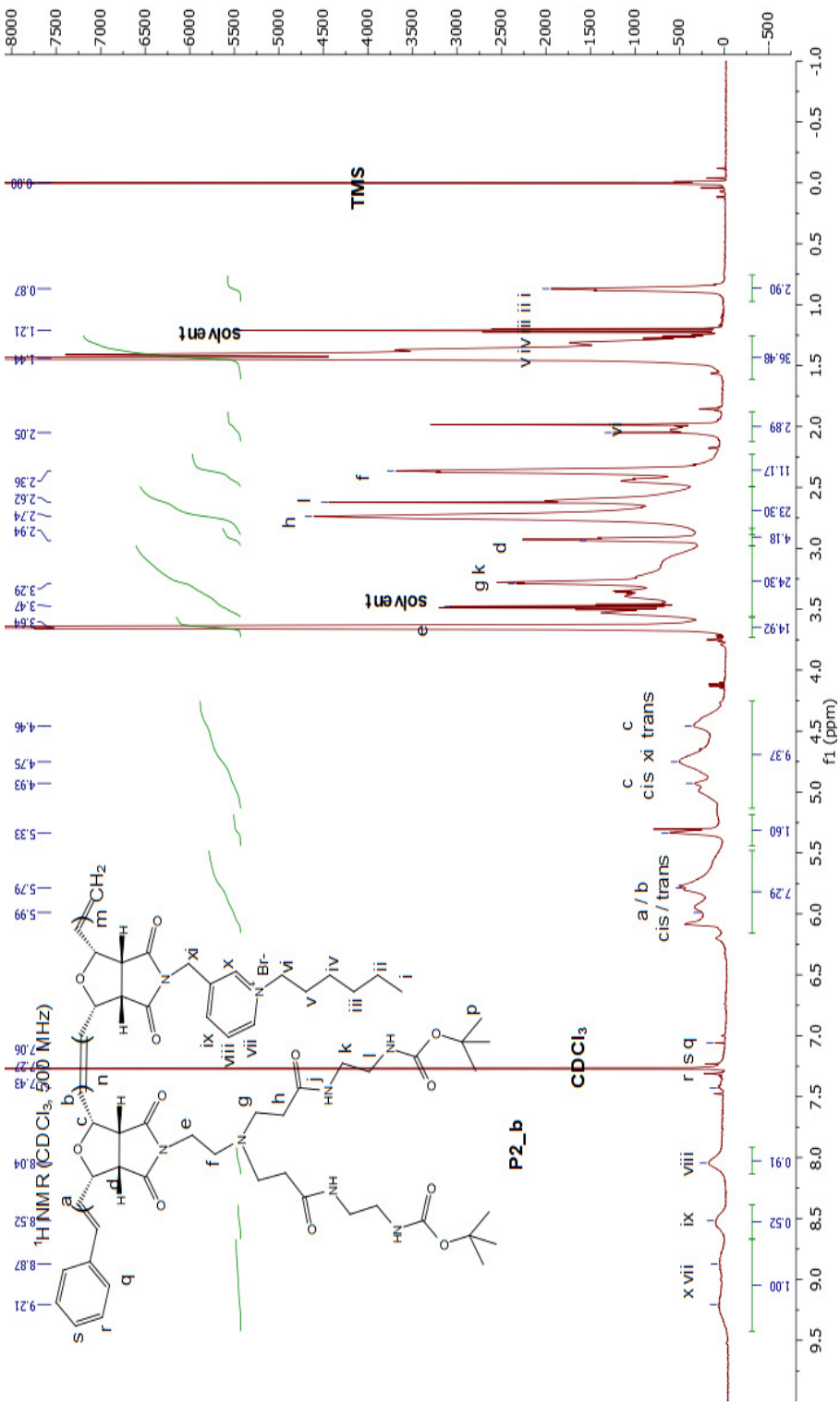

Figure B55 ^{1}H NMR of BOC surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

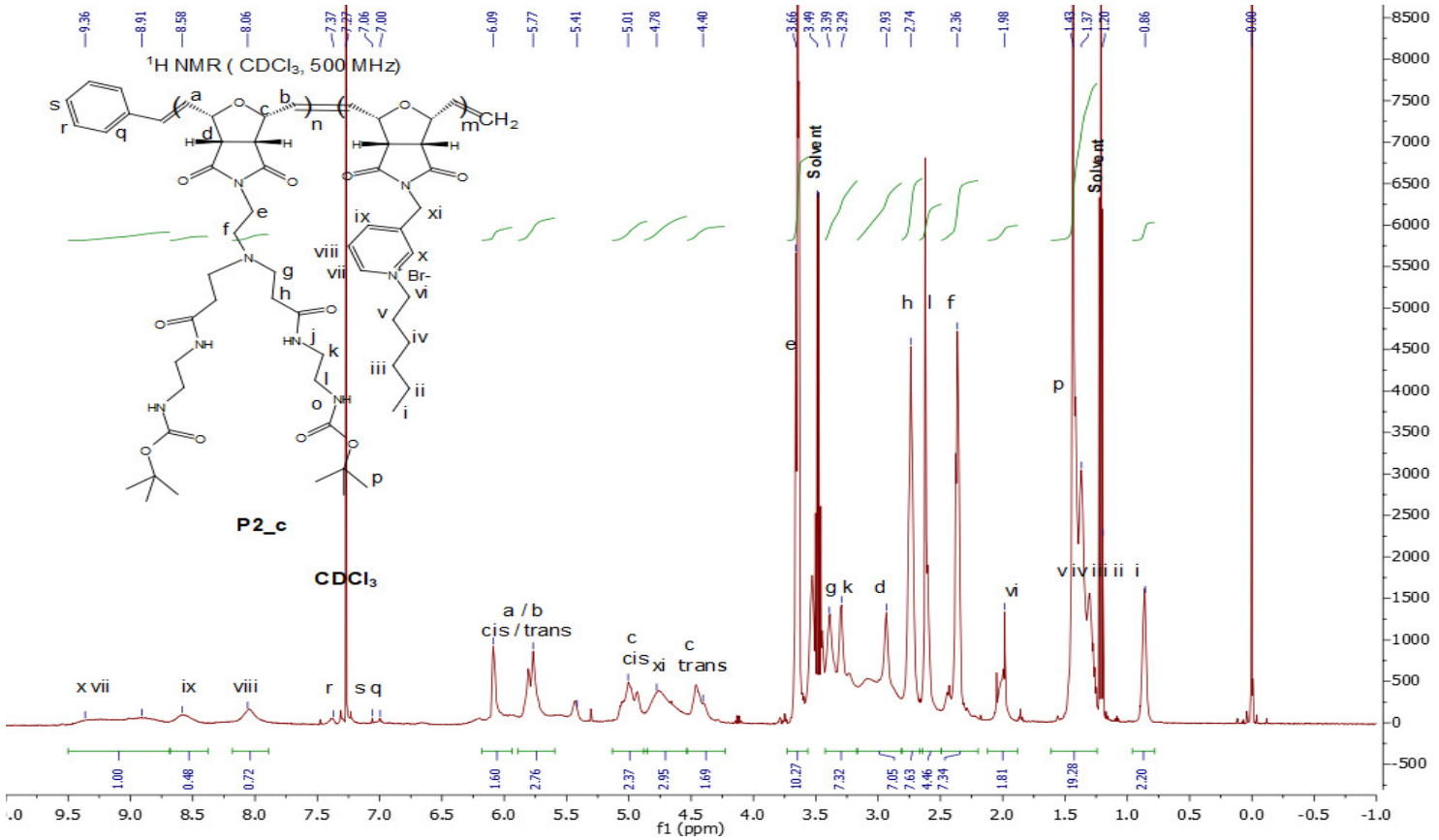

Figure B56 ^{1}H NMR of BOC surfaced block copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

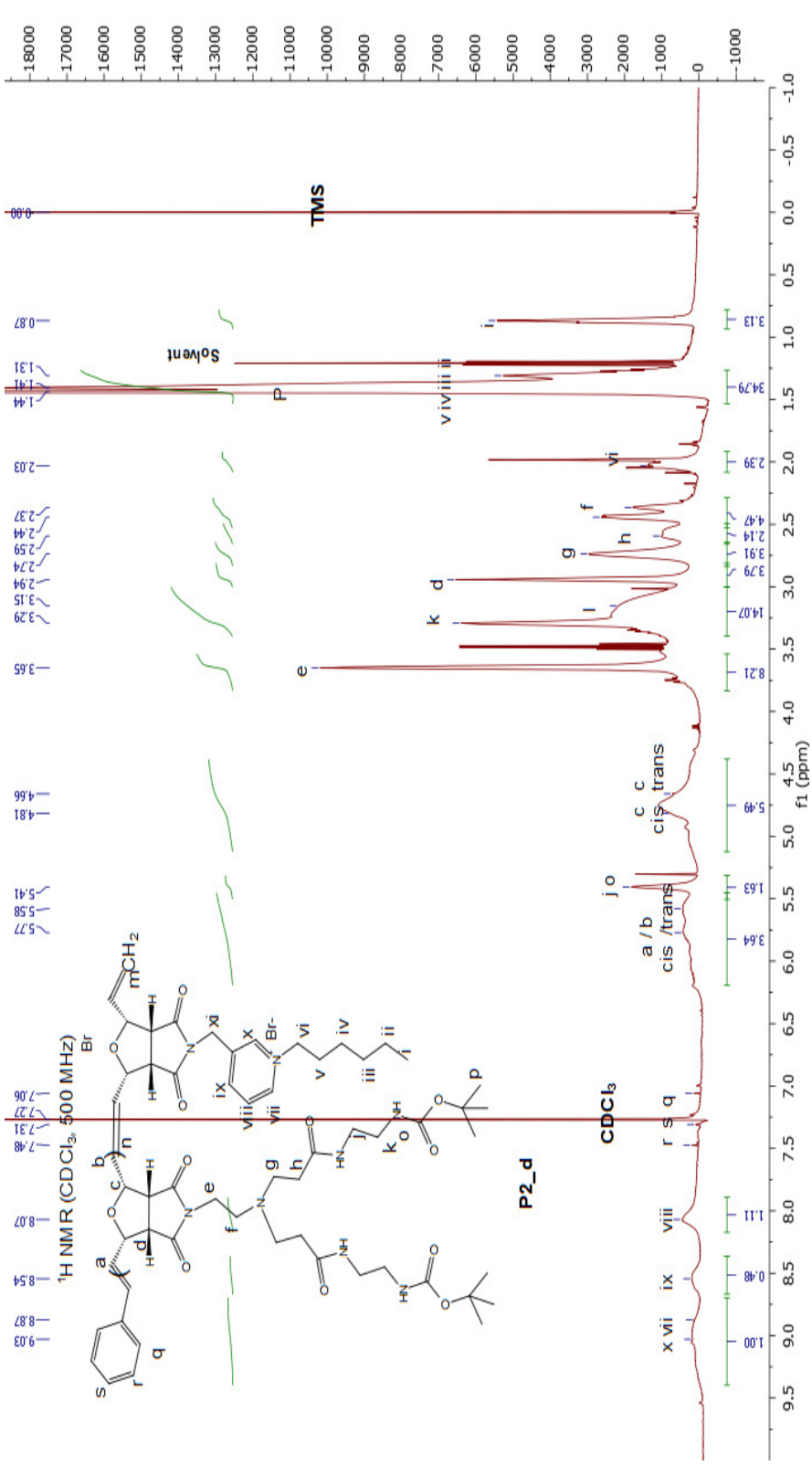

Figure B57 ^{1}H NMR of BOC surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (5k:5k)

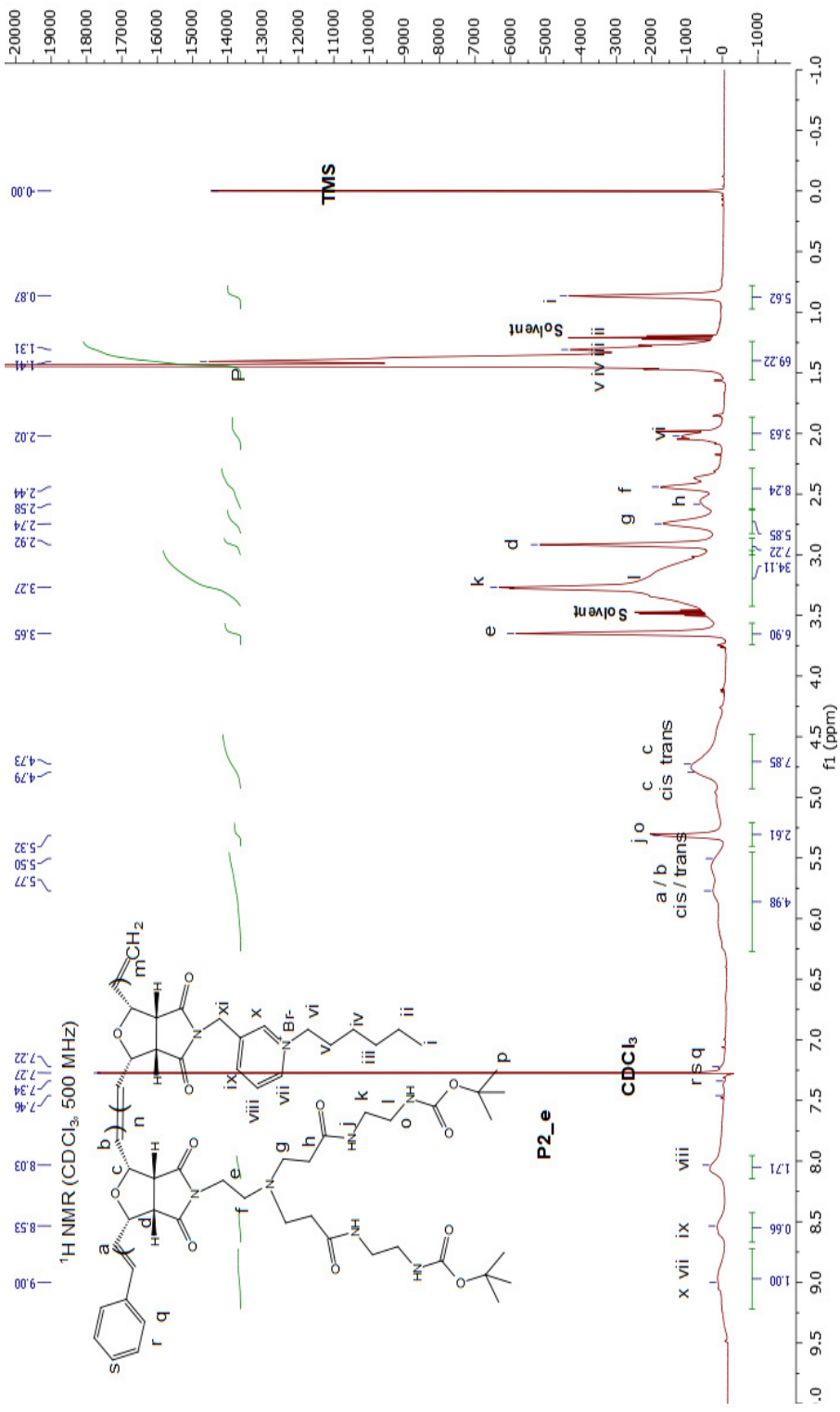

Figure B58 ^{1}H NMR of BOC surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (4k:6k)

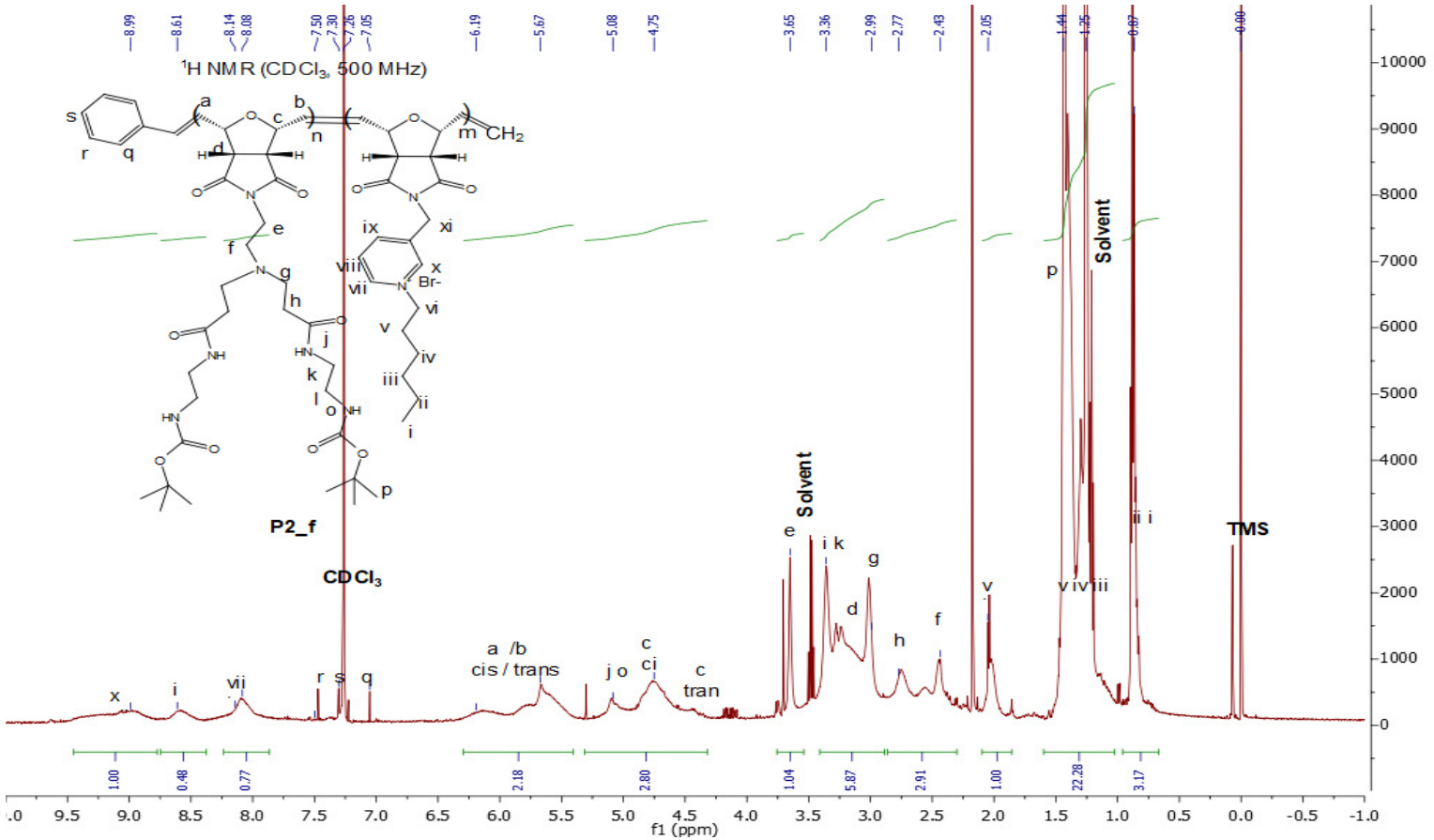

Figure B59 ¹H NMR of BOC surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (3k:7k)

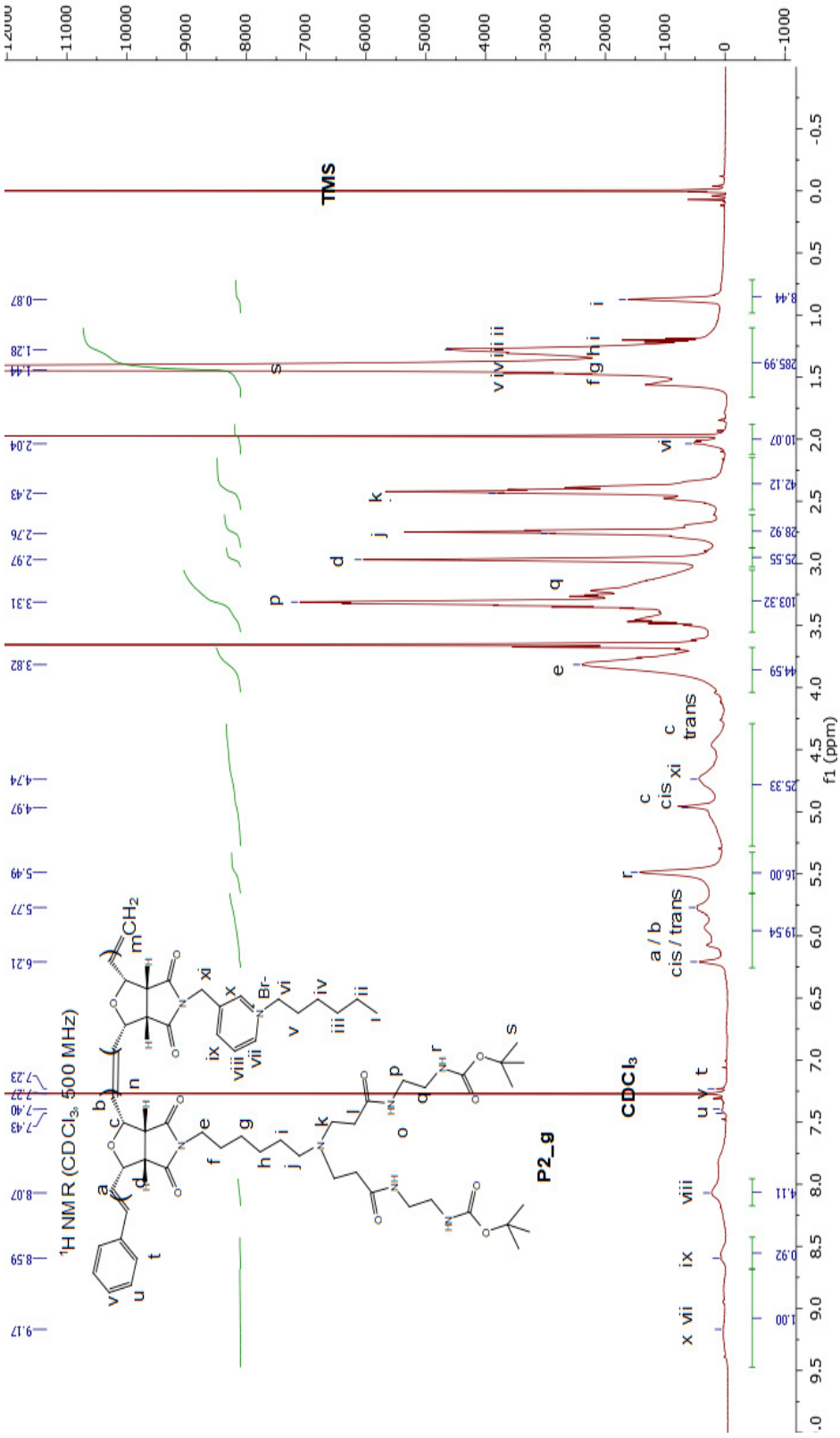

Figure B60 ^{1}H NMR of BOC surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (8k:2k)

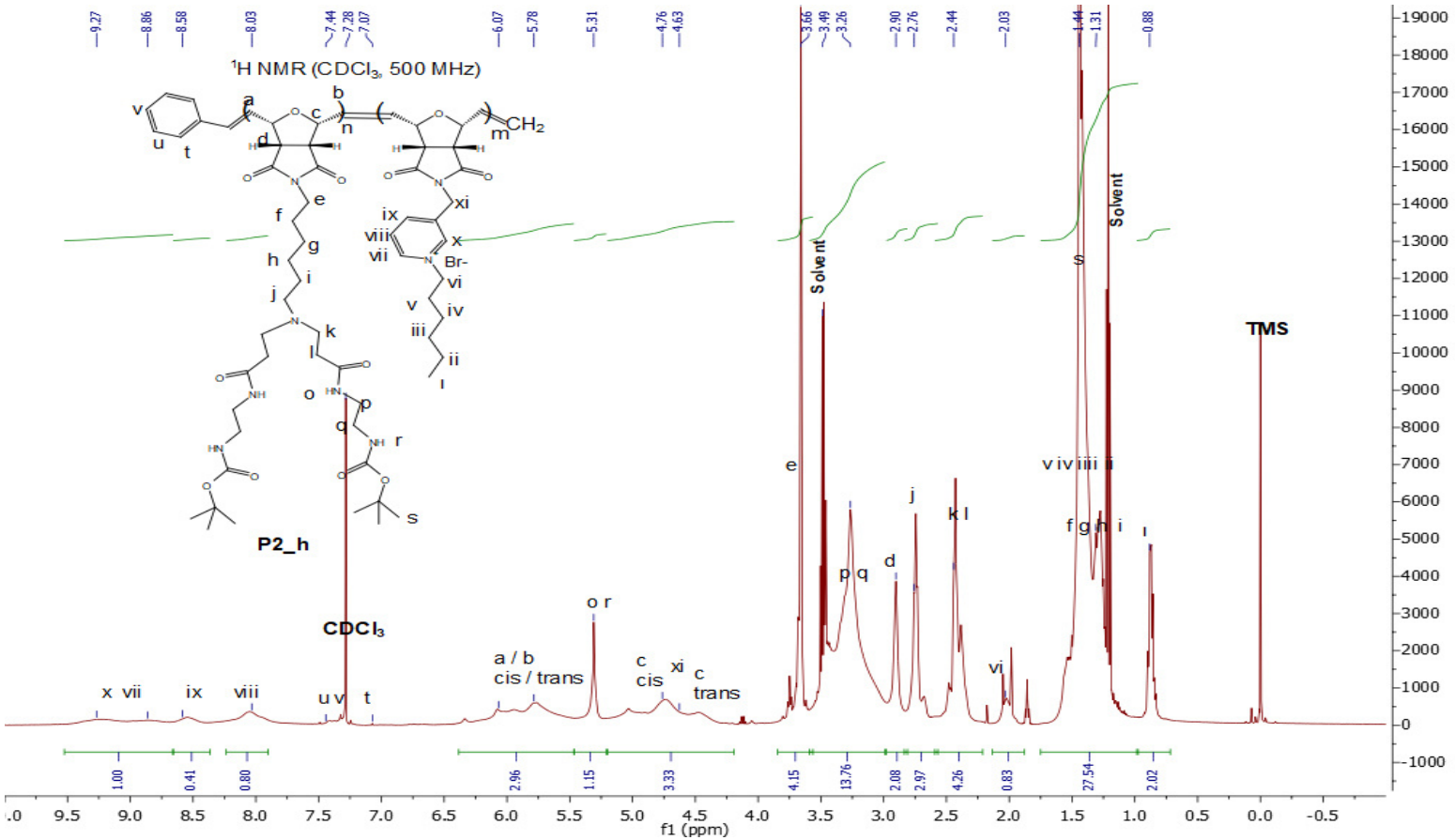

Figure B61 ¹H NMR of BOC surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (7k:3k)

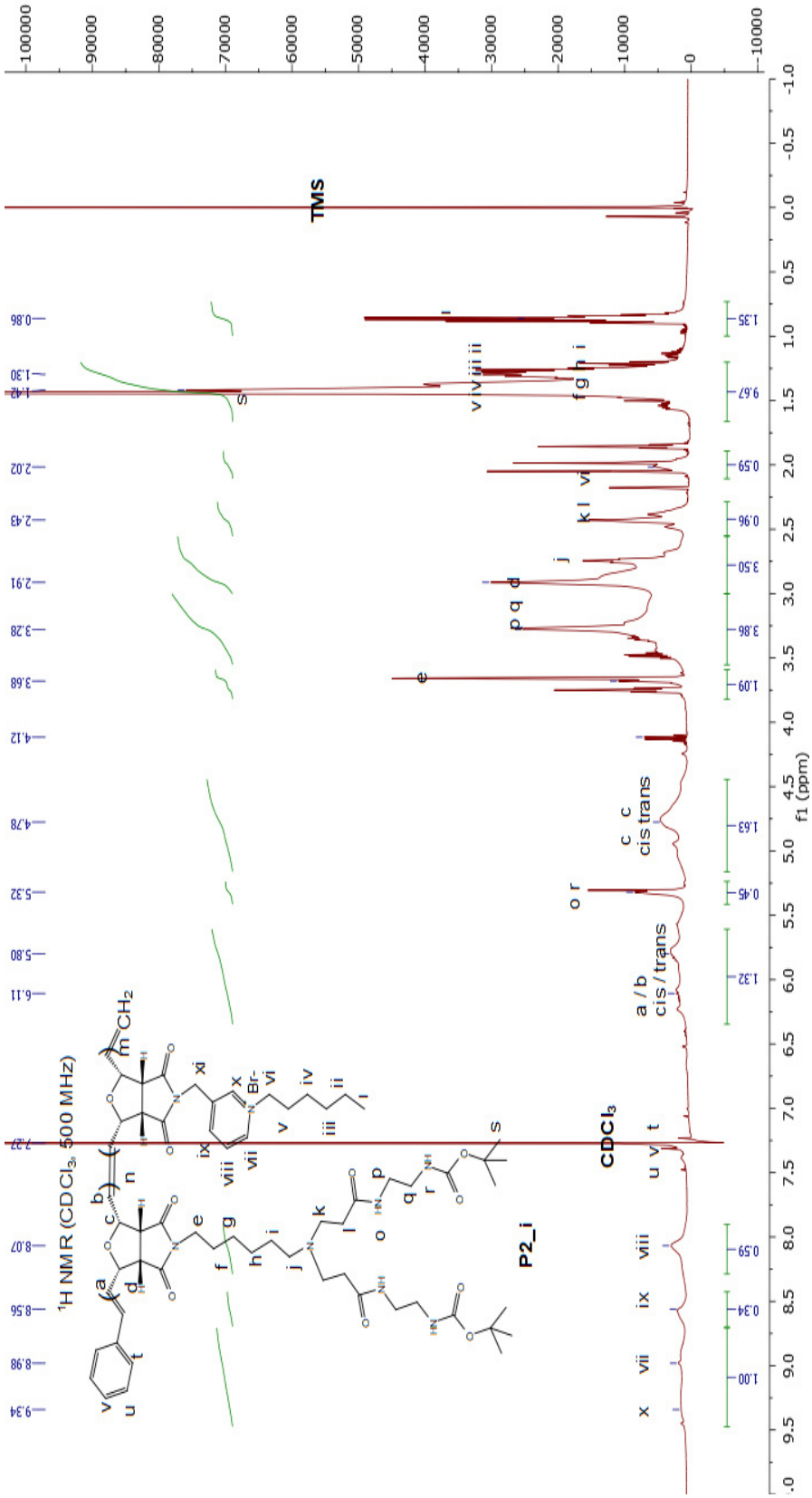

Figure B62 ^{1}H NMR of BOC surfaced block copolymer synthesized from six carbon linker dendritic and pyridinium monomer (7k:3k)

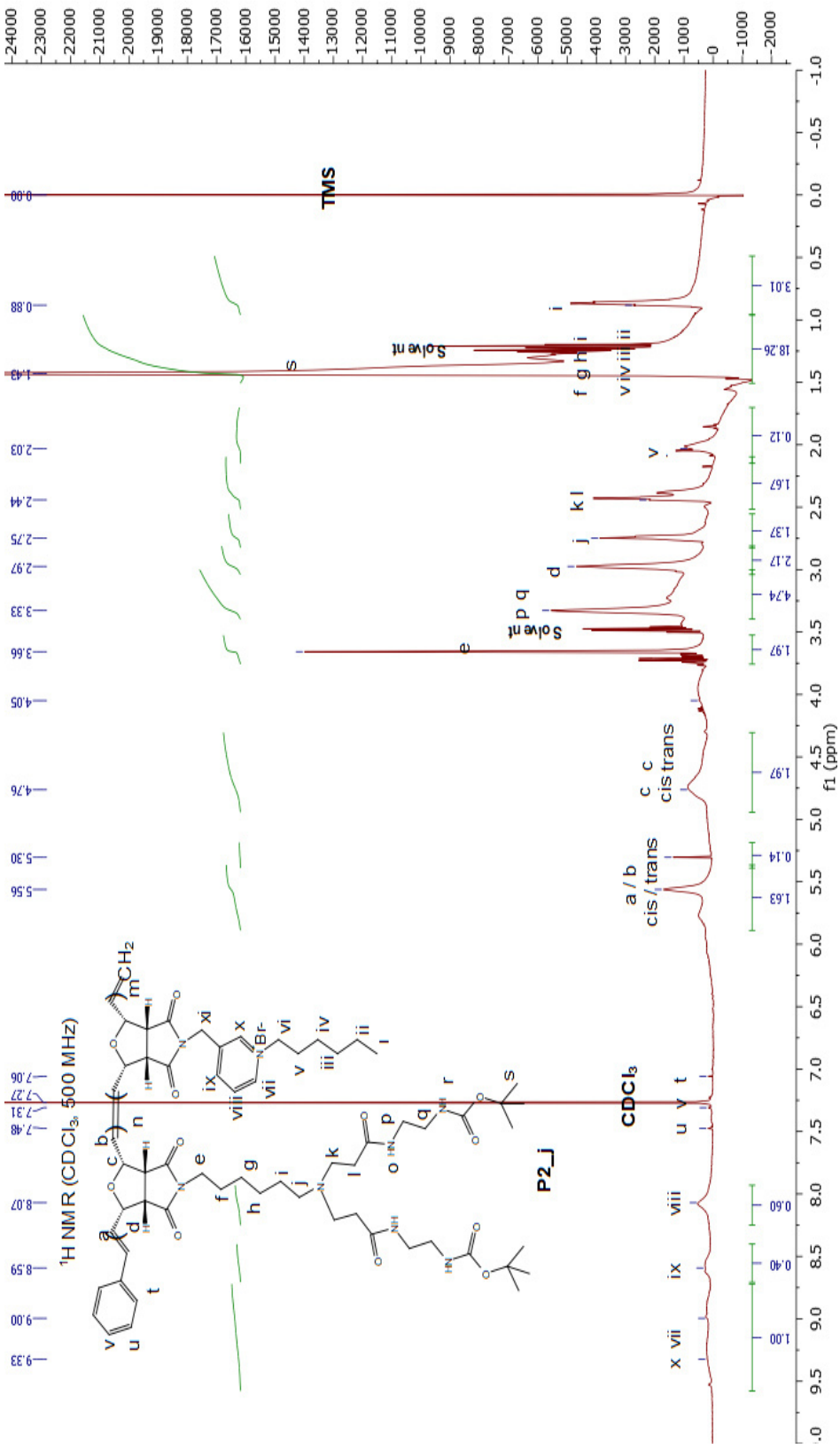

Figure B63 ^{1}H NMR of BOC surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (5k:5k)

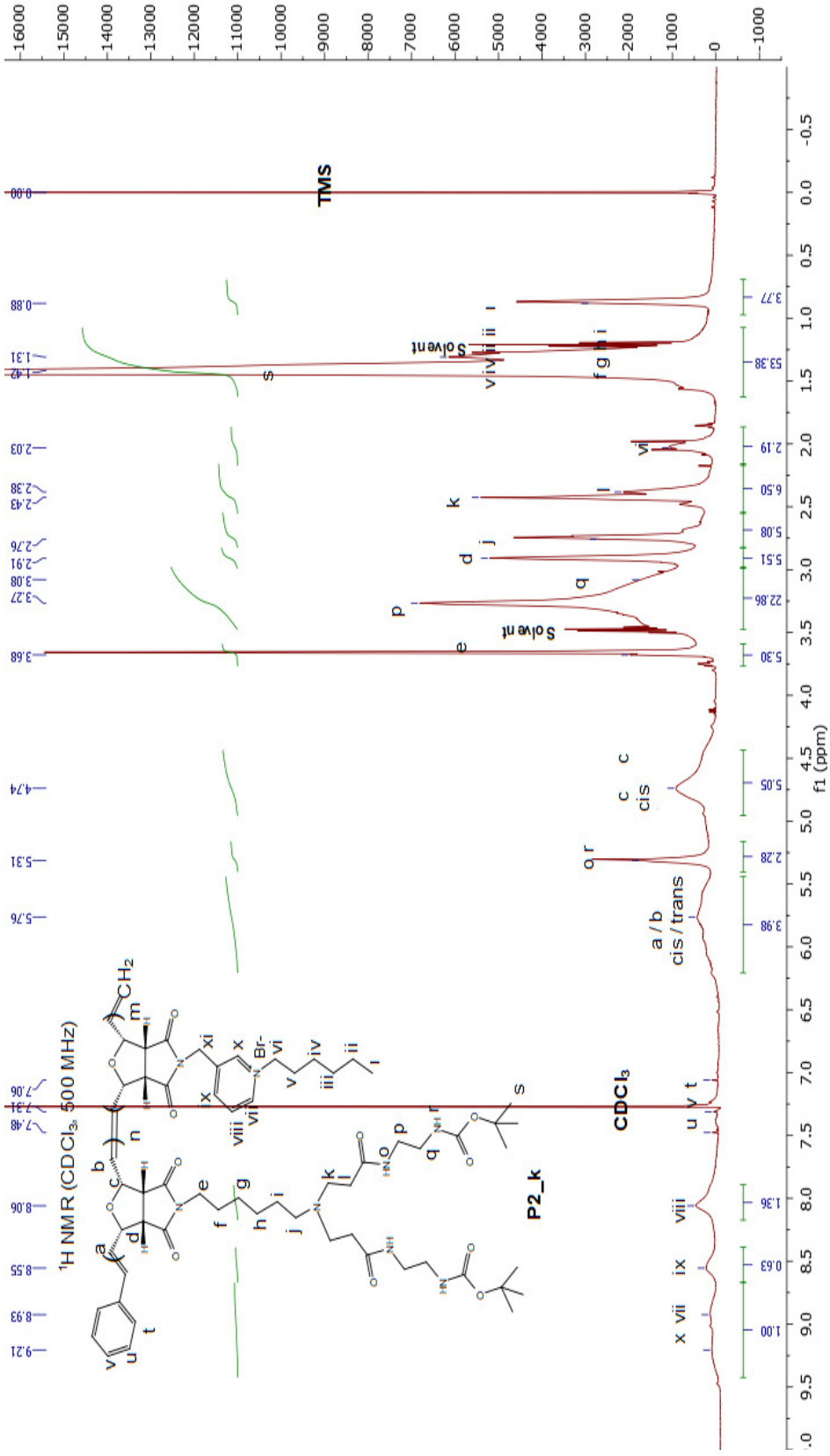

Figure B64 ^{1}H NMR of BOC surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (4k:6k)

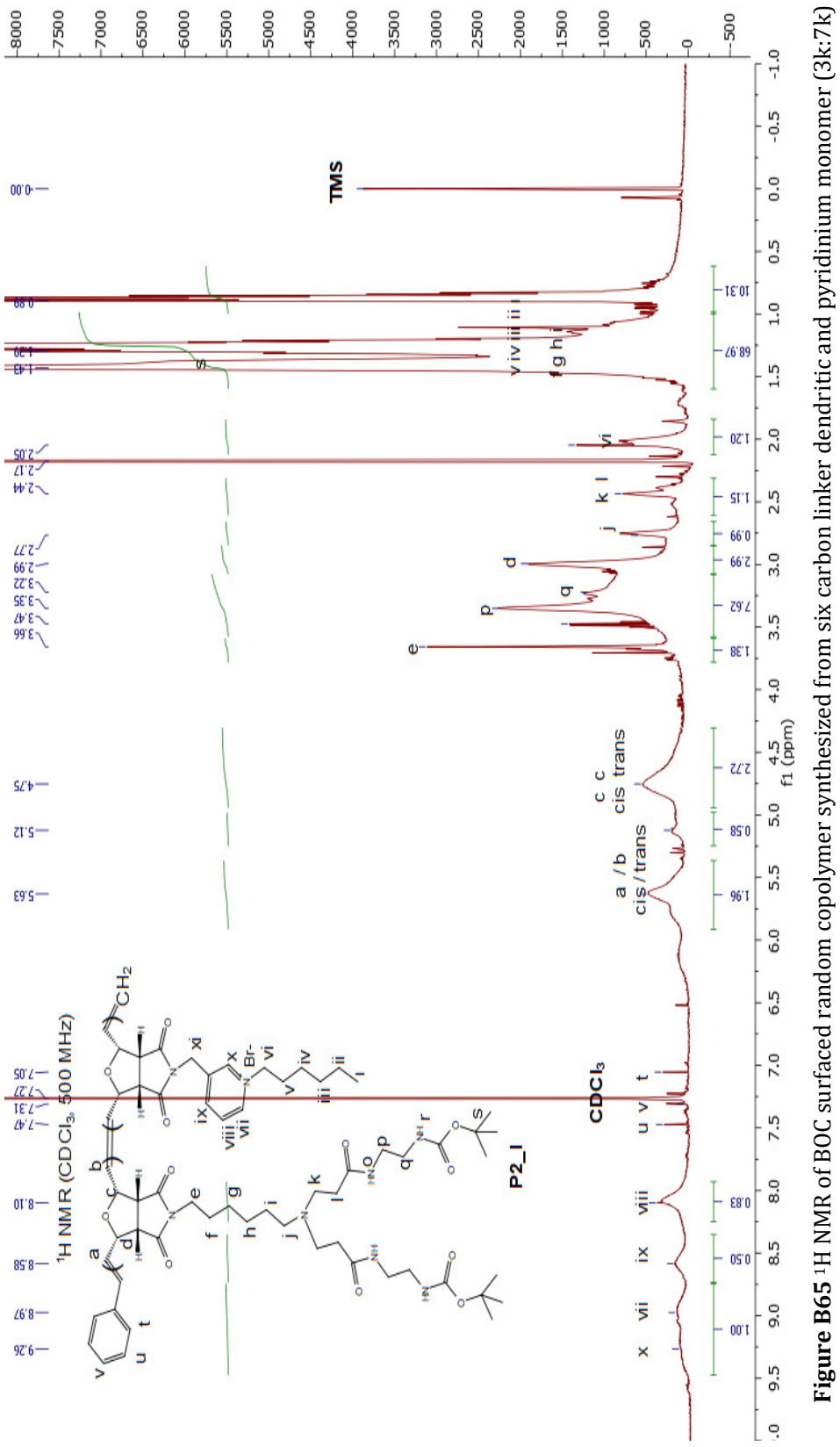

Figure B65 ^{1}H NMR of BOC surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (3k:7k)

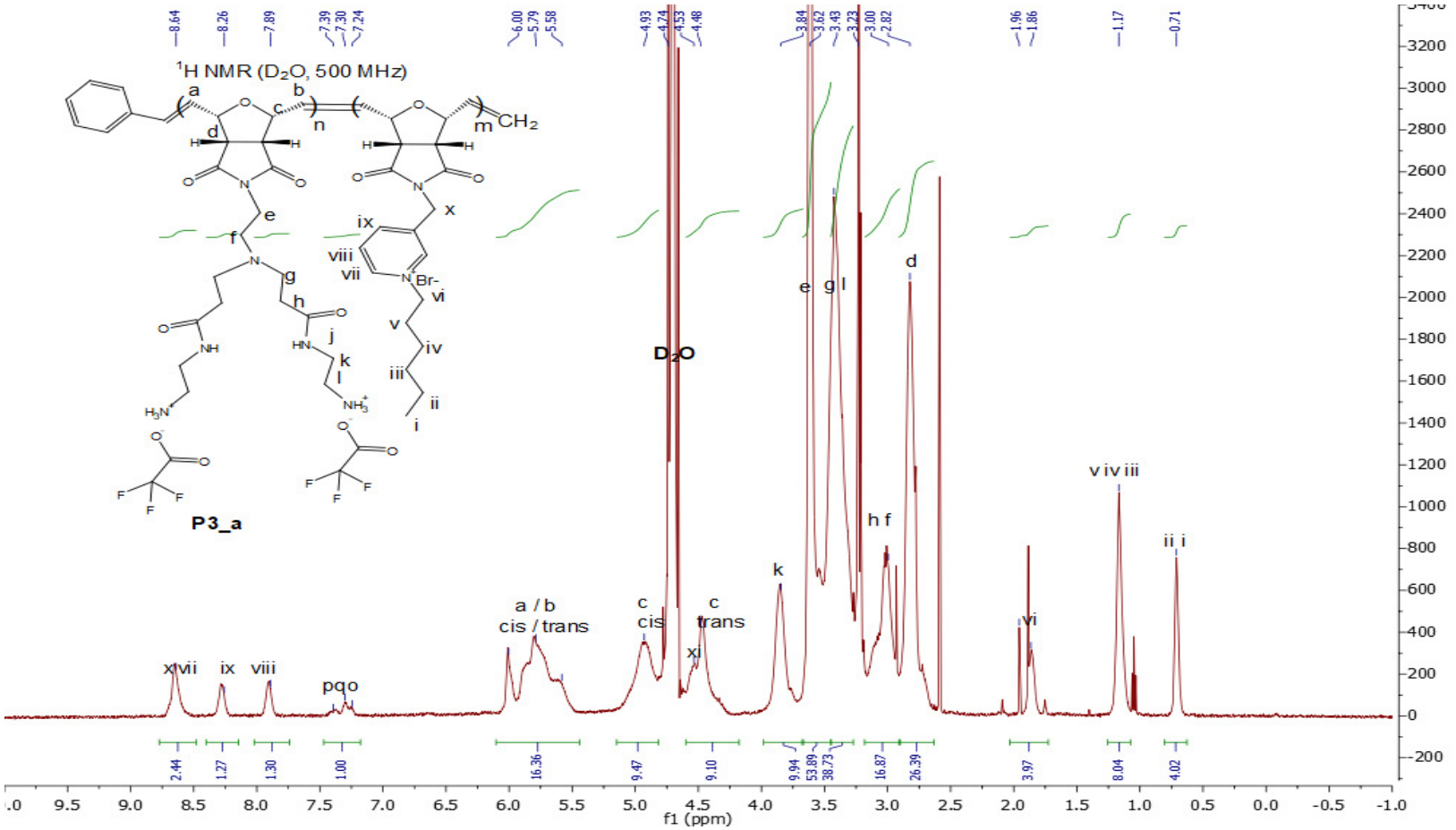

Figure B66 ¹H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (8k:2k)

a

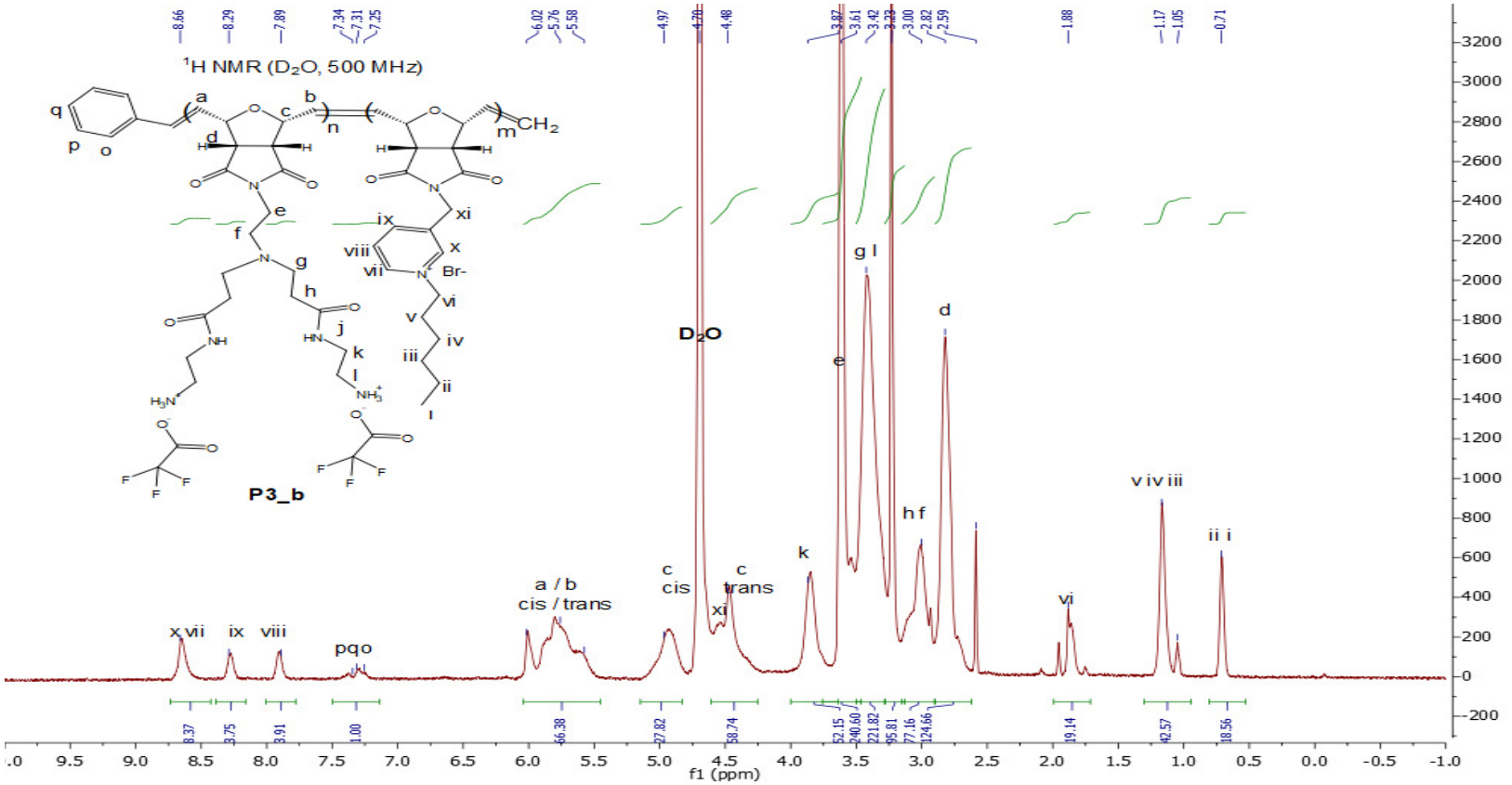

Figure B67 ¹H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

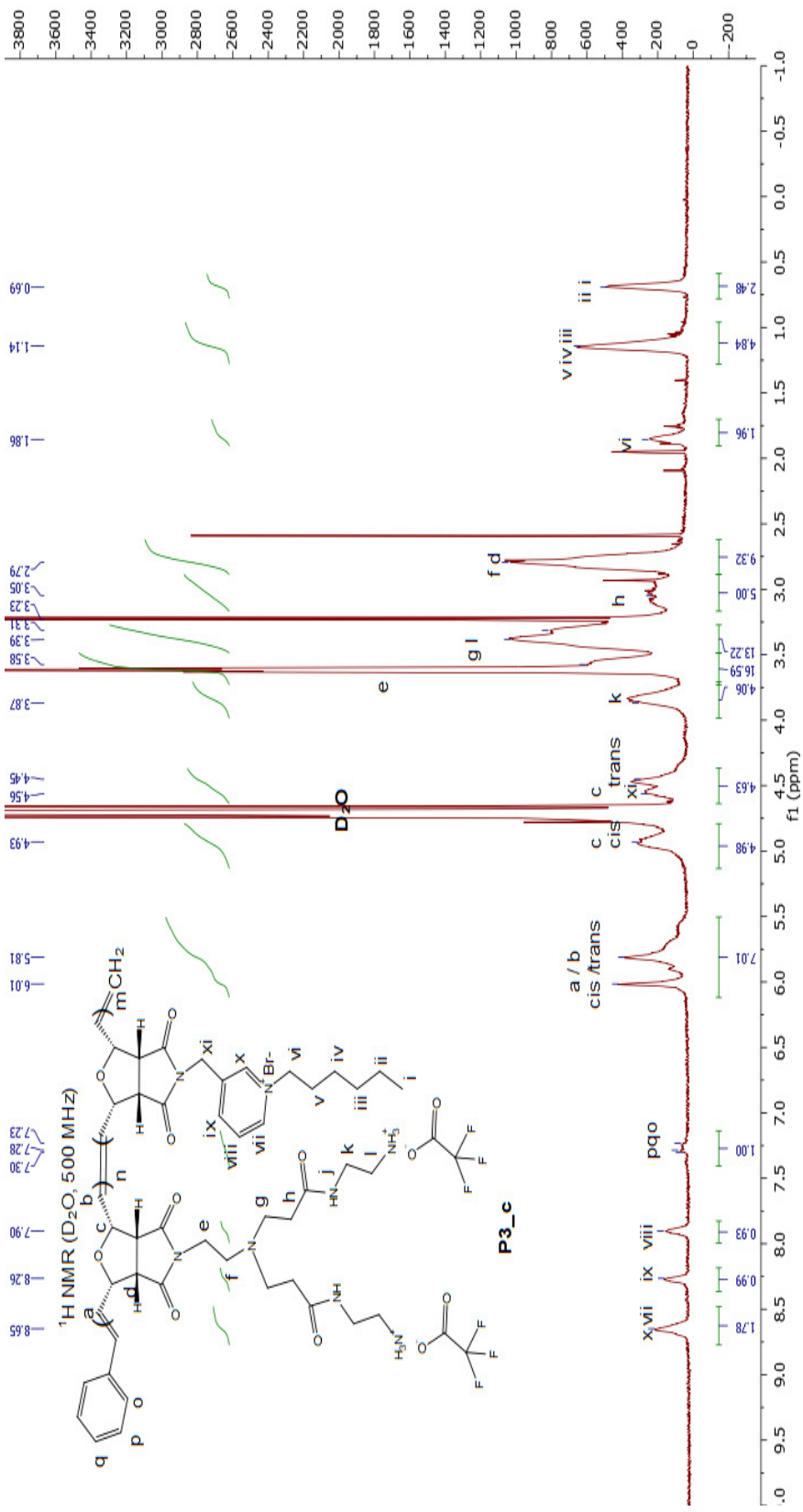

Figure B68 ^{1}H NMR of TFA salt surfaced bock copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

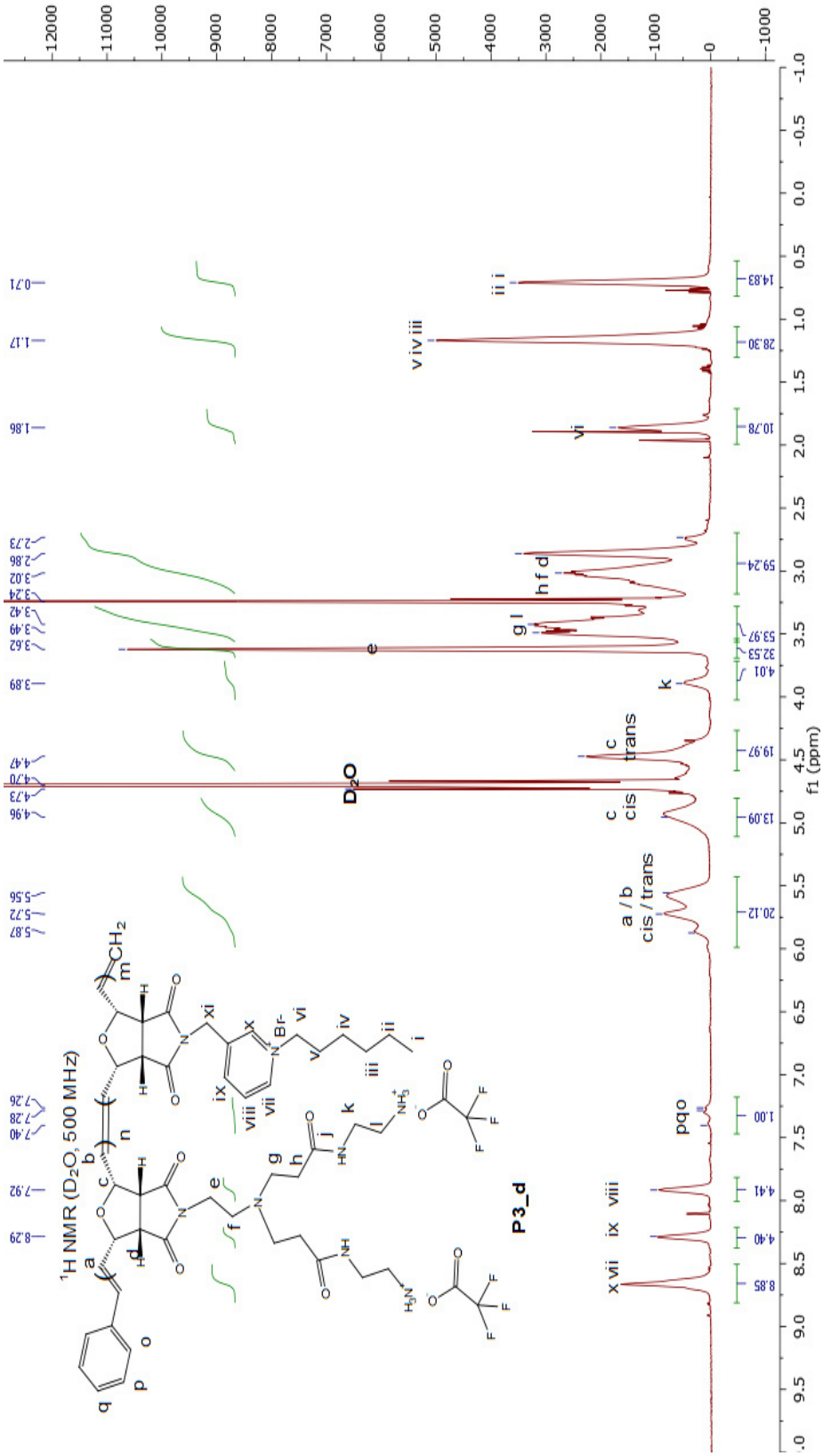

Figure B69 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (5k:5k)

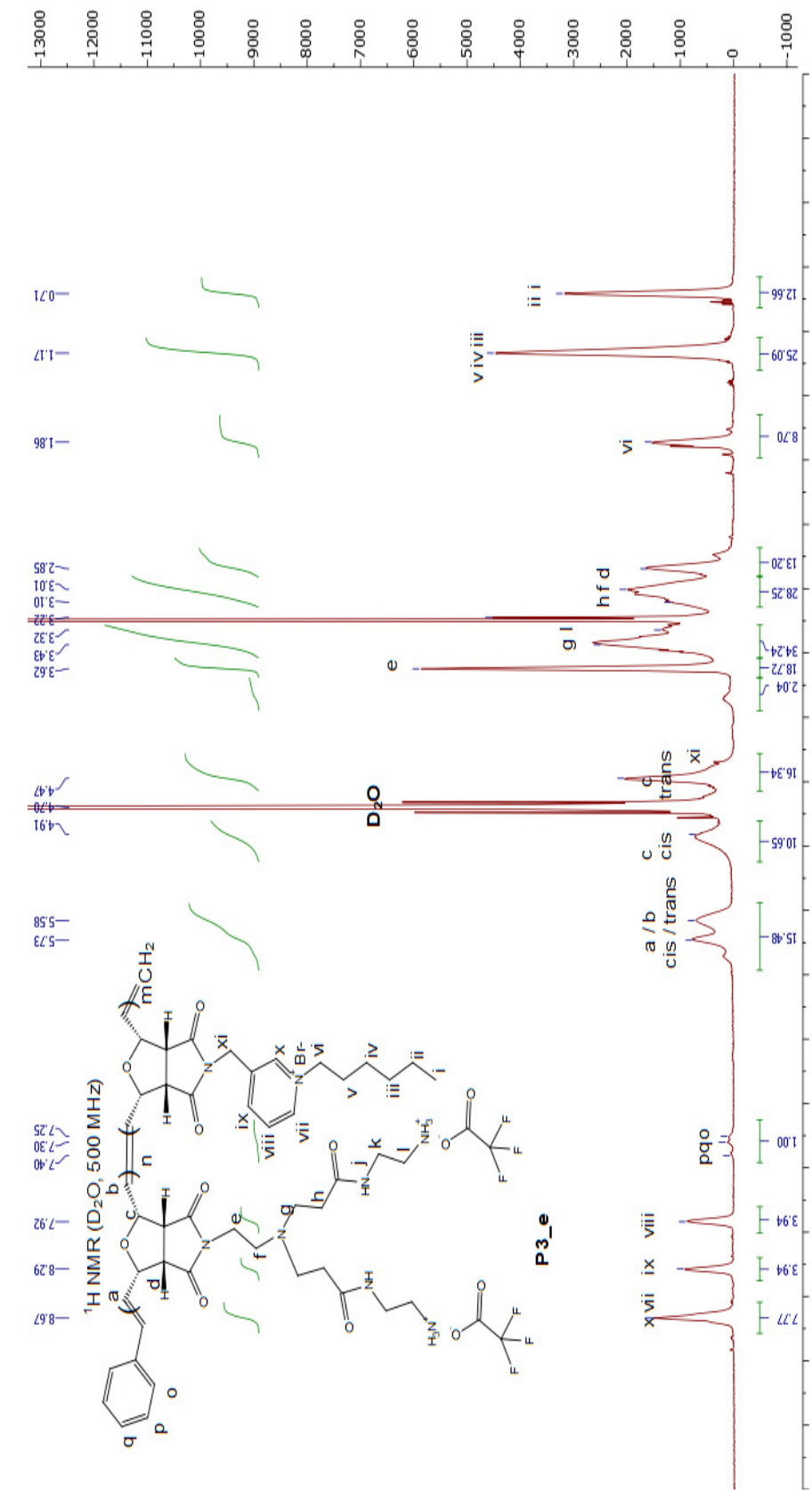

Figure B70 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (4k:6k)

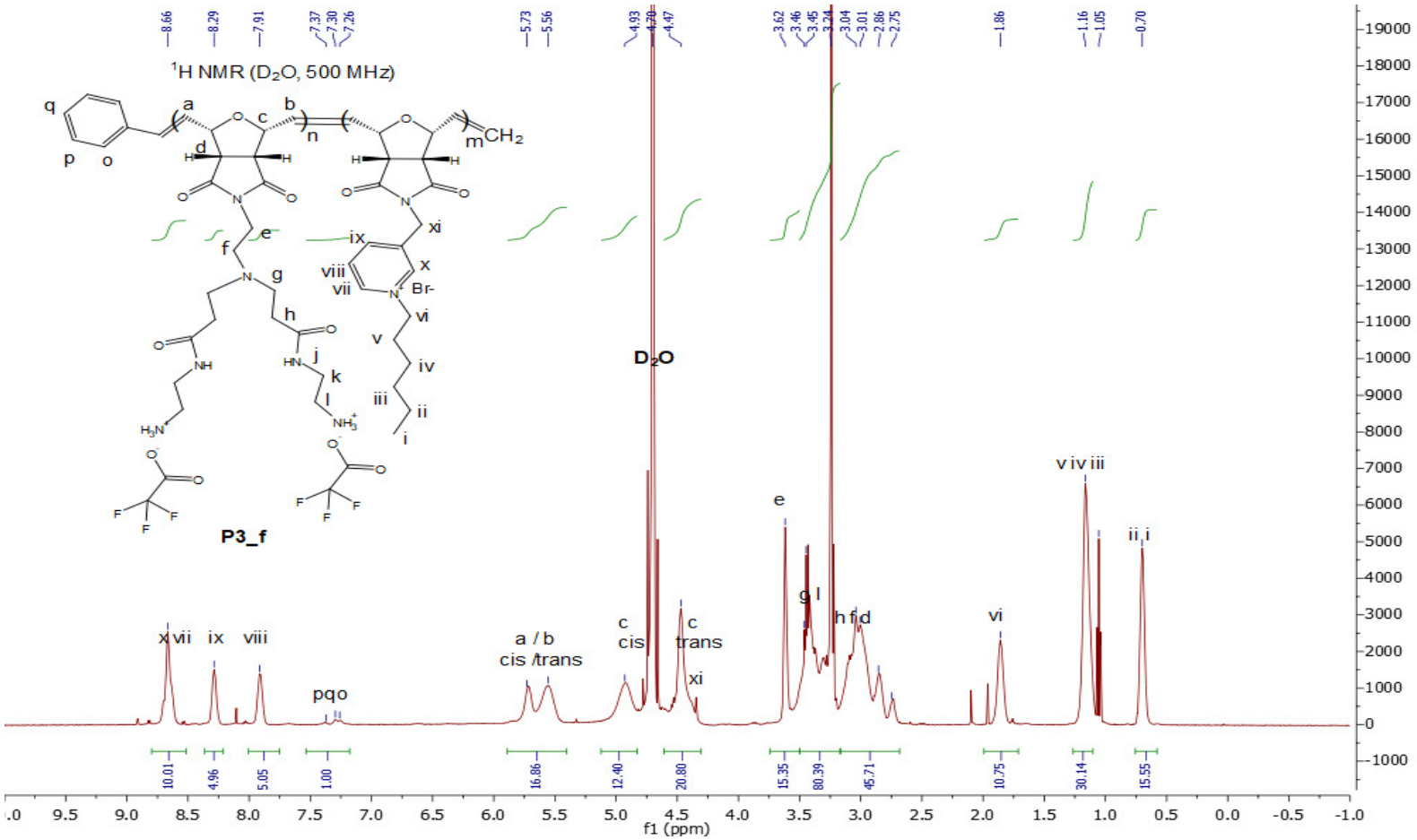

Figure B71 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (3k:7k)

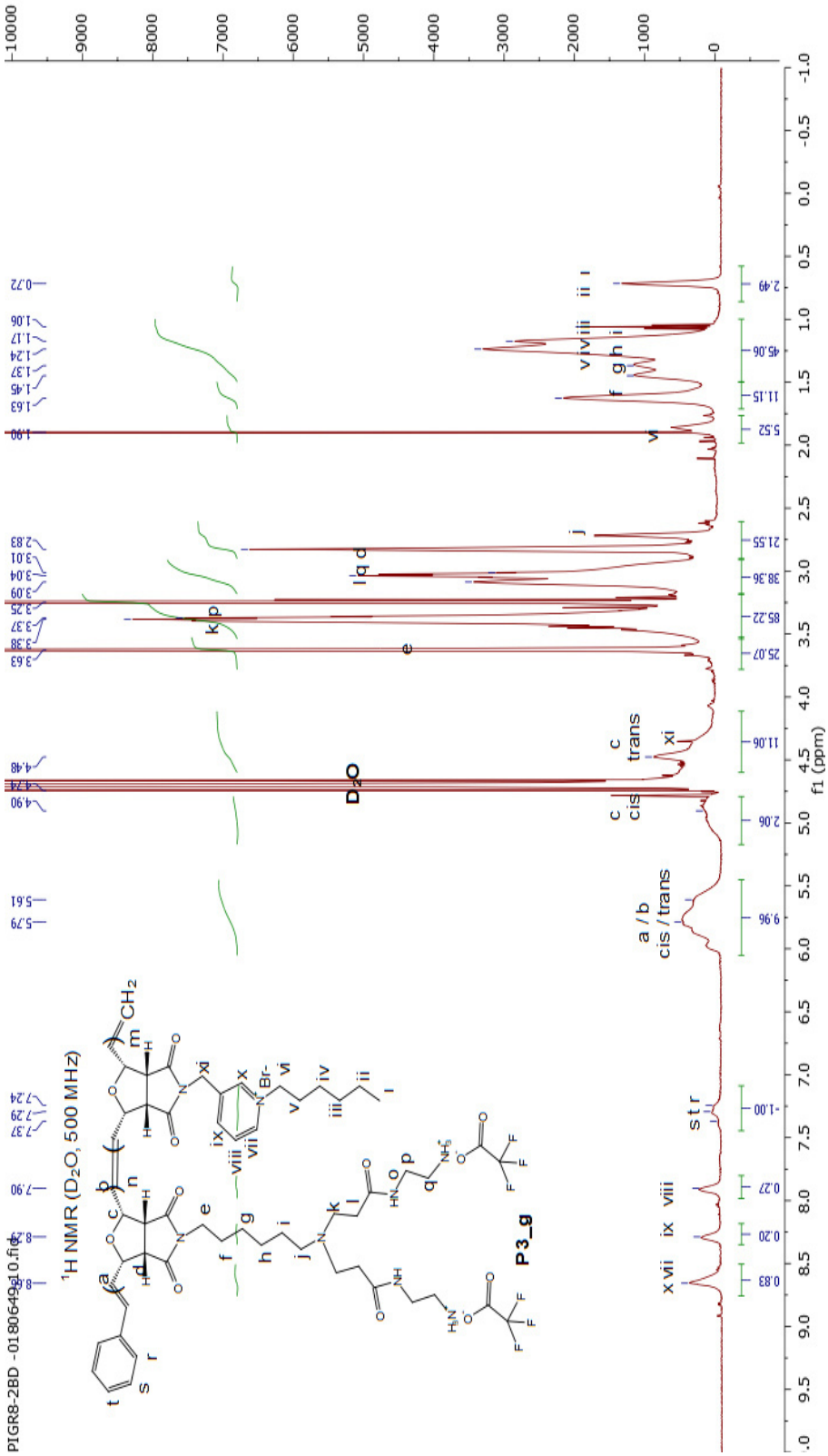

Figure B72 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from six carbon linker dendritic and pyridinium monomer (8k:2k)

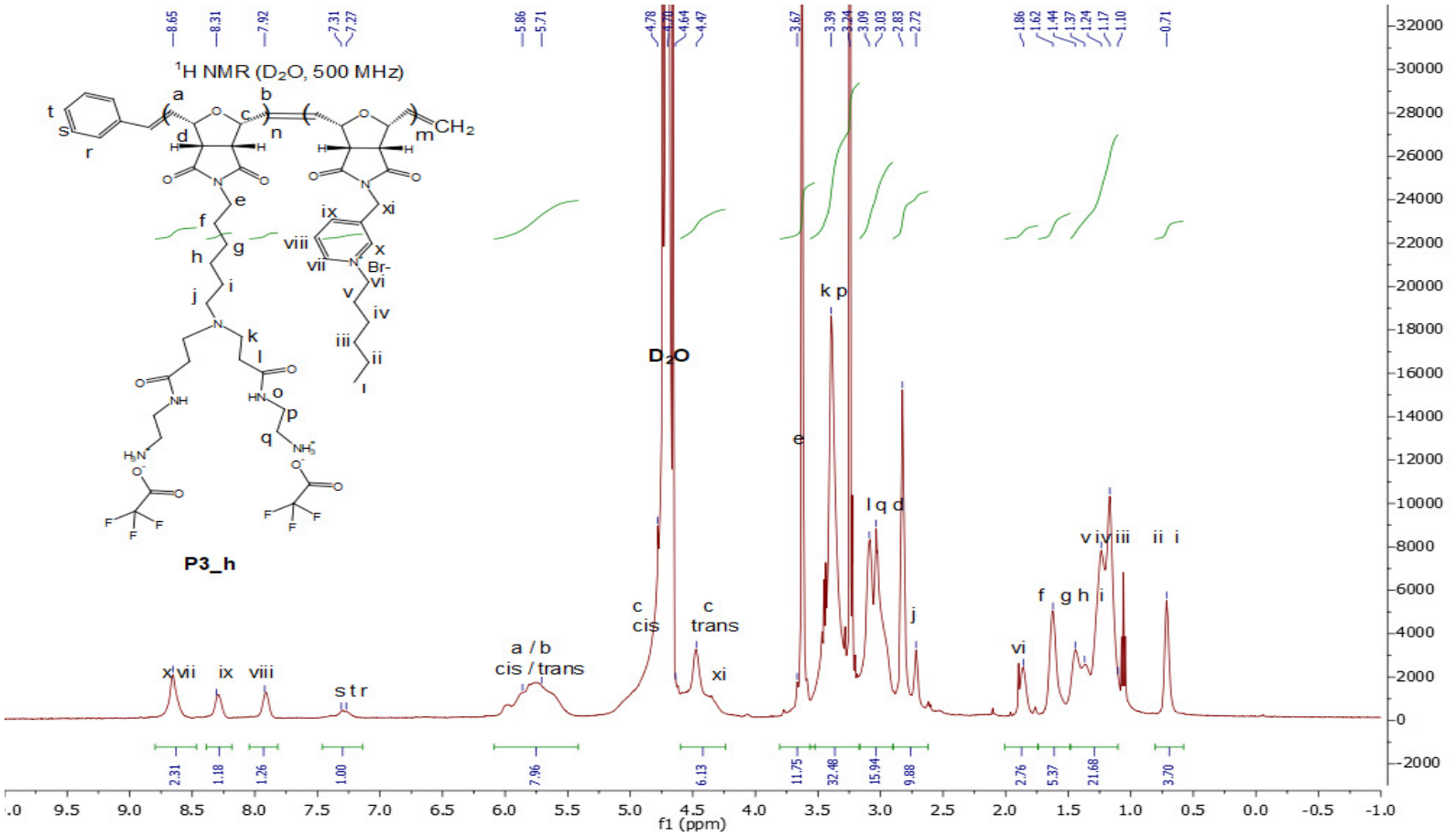

Figure B73 ¹H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

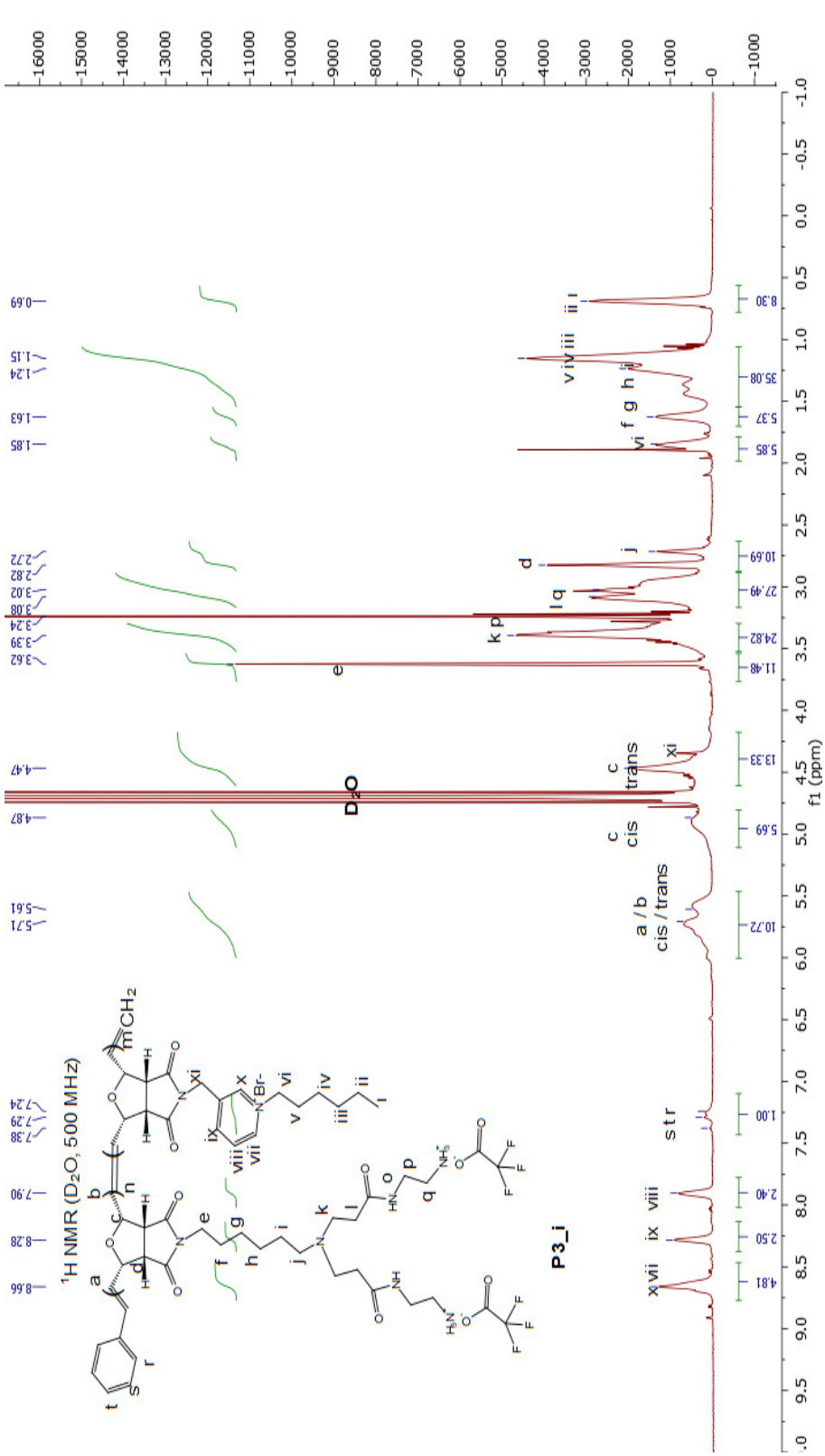

Figure B74 ^{1}H NMR of TFA salt surfaced block copolymer synthesized from two carbon linker dendritic and pyridinium monomer (7k:3k)

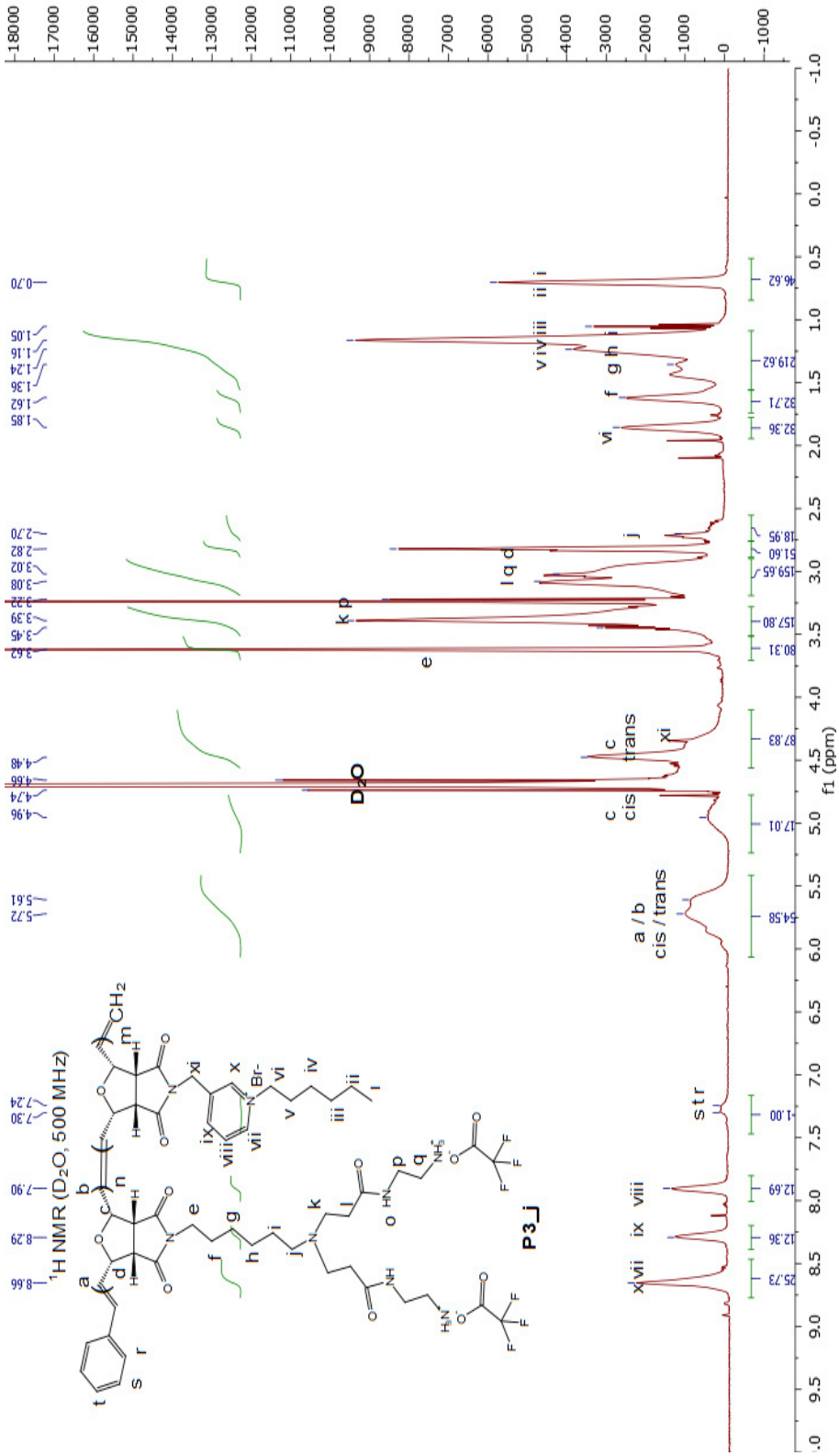

Figure B75 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (5k:5k)

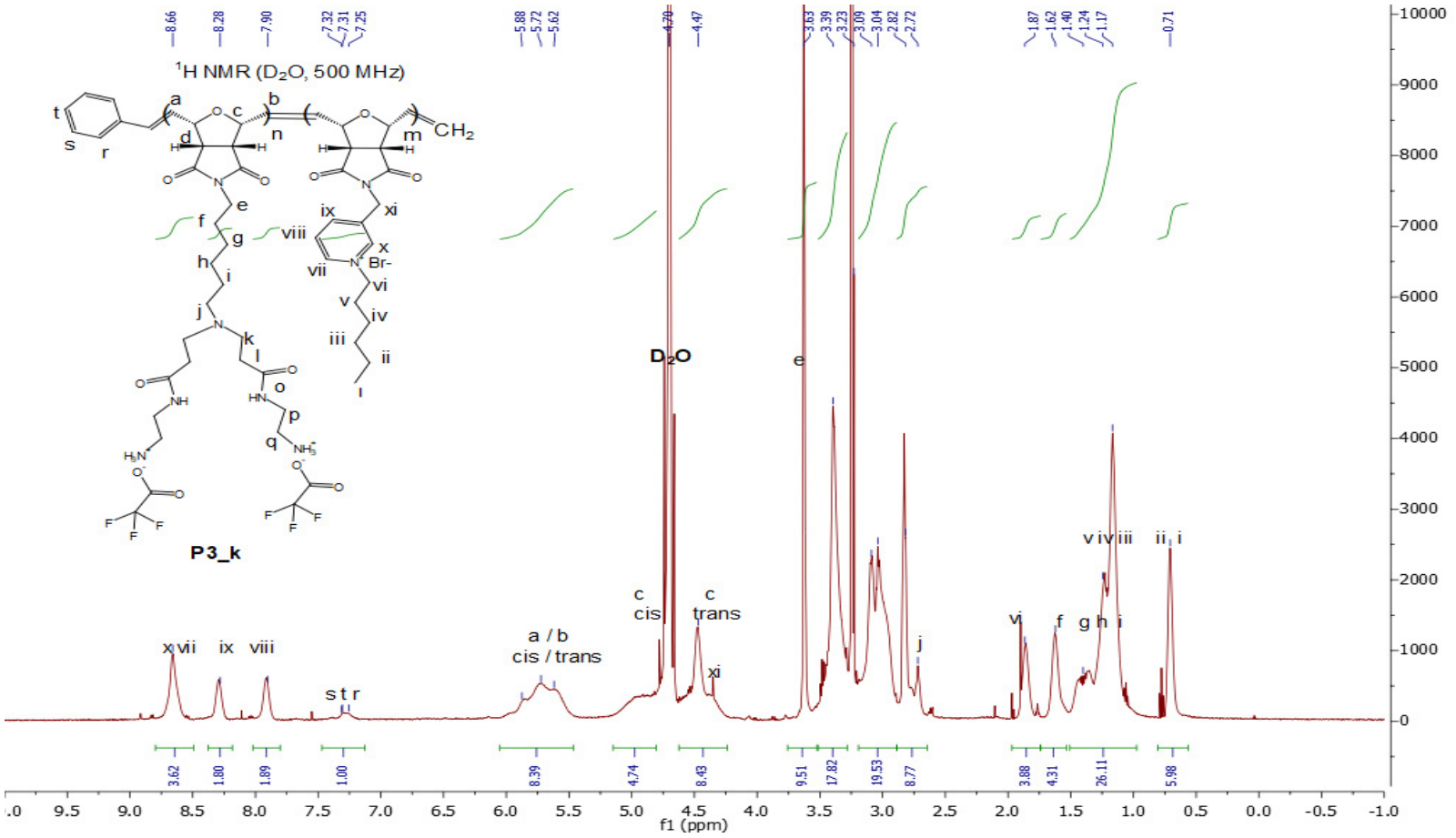

Figure B76 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (4k:6k)

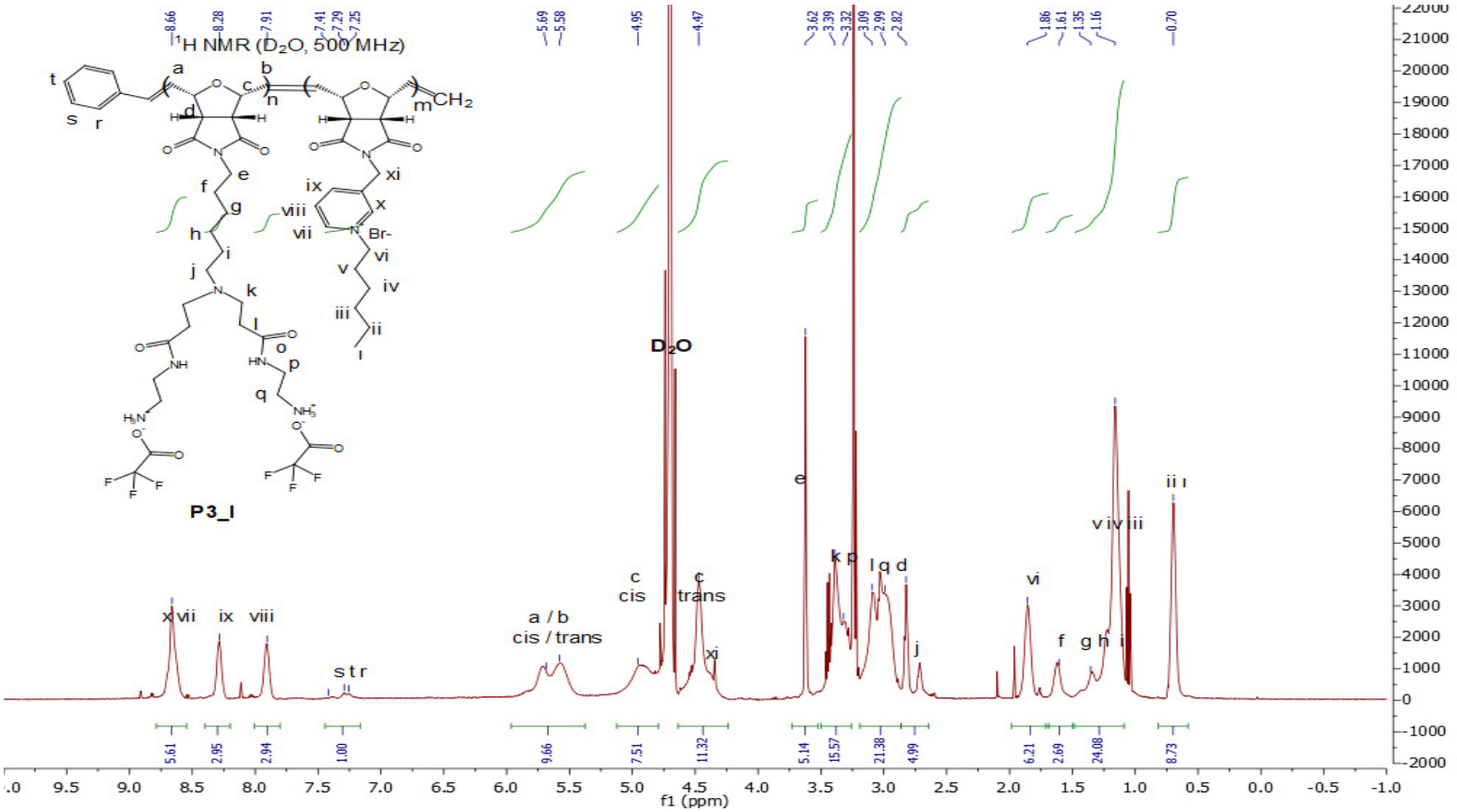

Figure B77 ^{1}H NMR of TFA salt surfaced random copolymer synthesized from two carbon linker dendritic and pyridinium monomer (3k:7k)

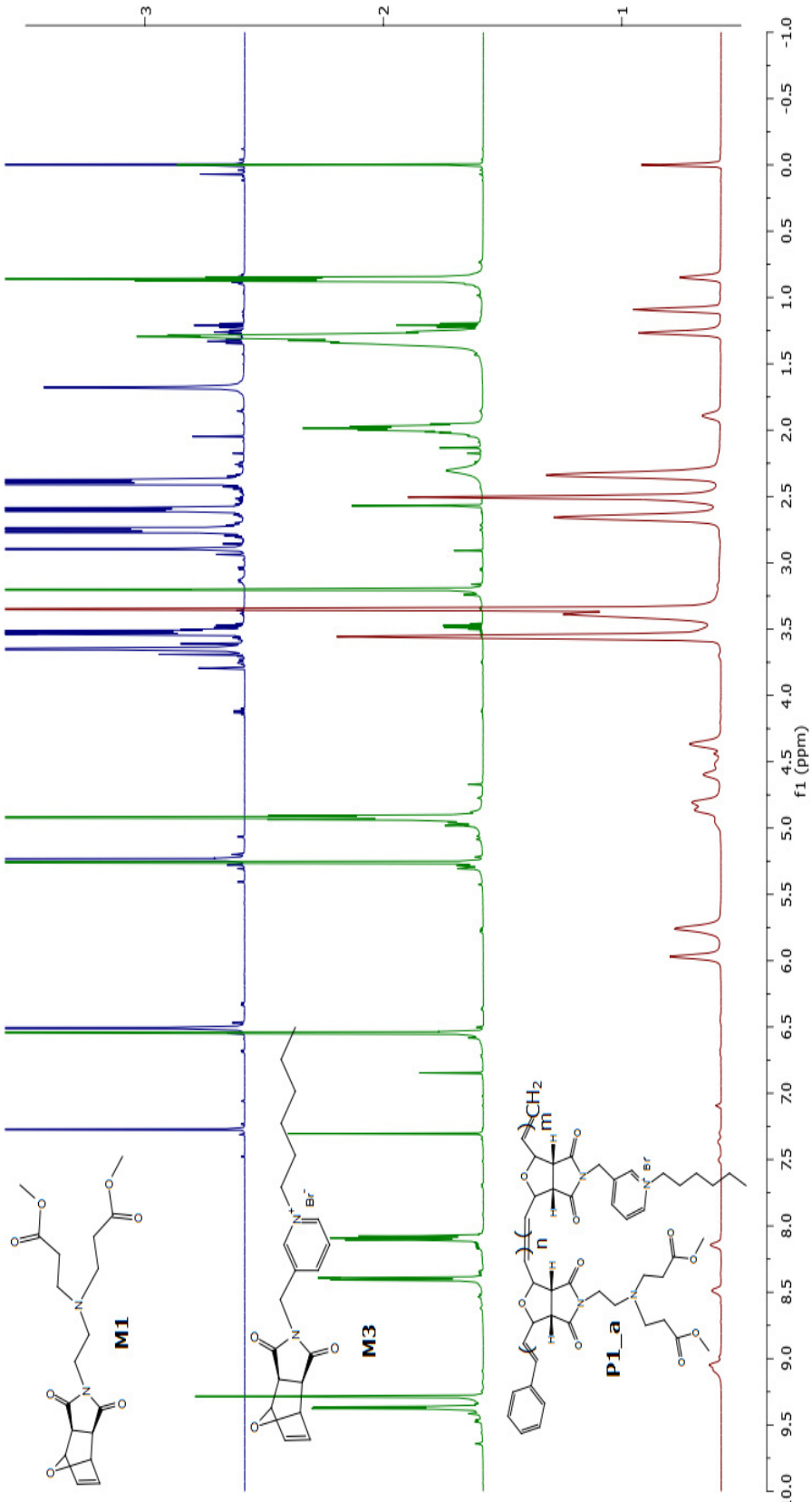

Figure B78 ^{1}H NMR of monomer M1, monomer M3 and copolymer of both monomers

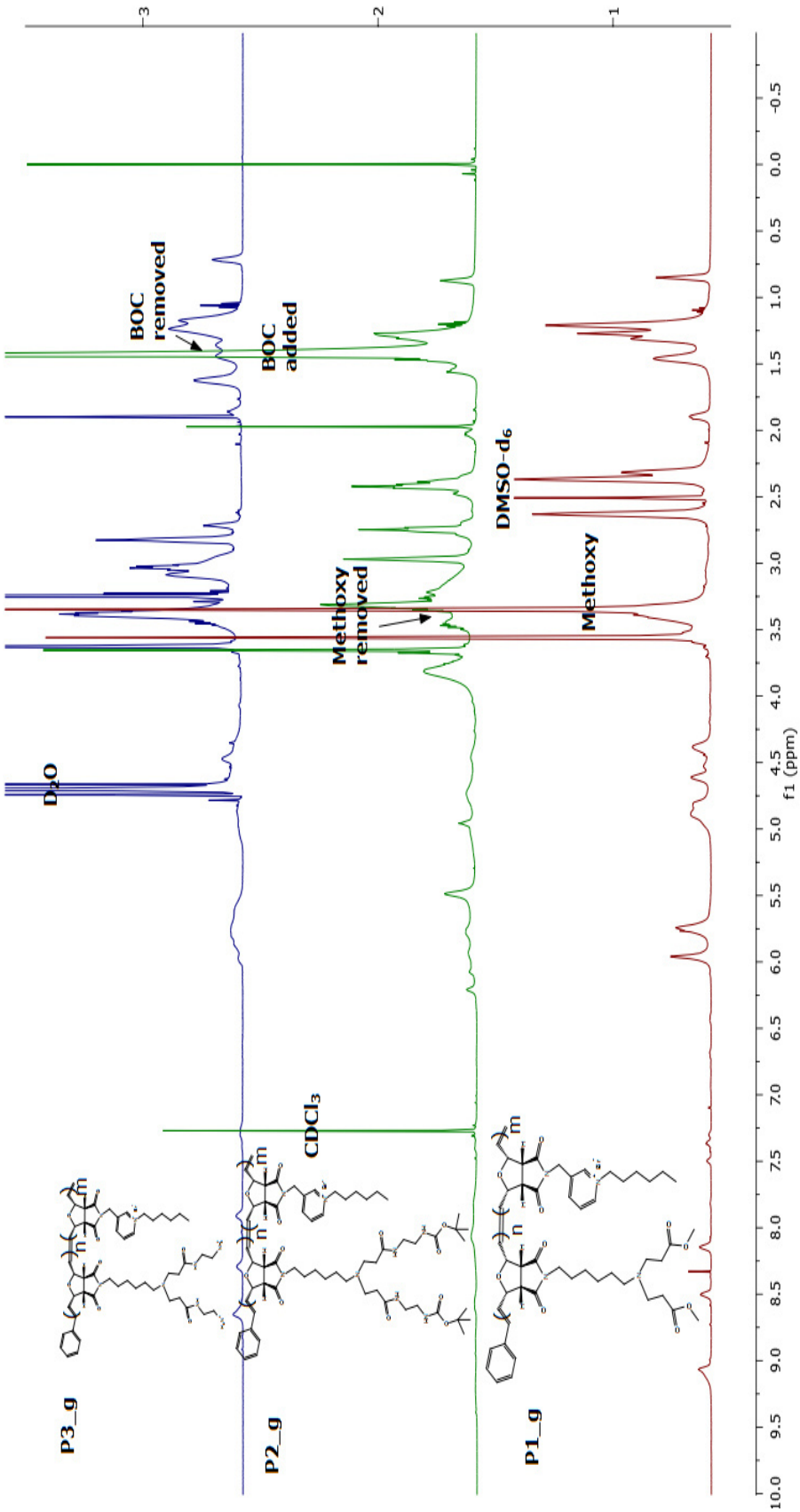

Figure B79 ^{1}H NMR of polymer, BOC surfaced polymer and BOC deprotected polymer

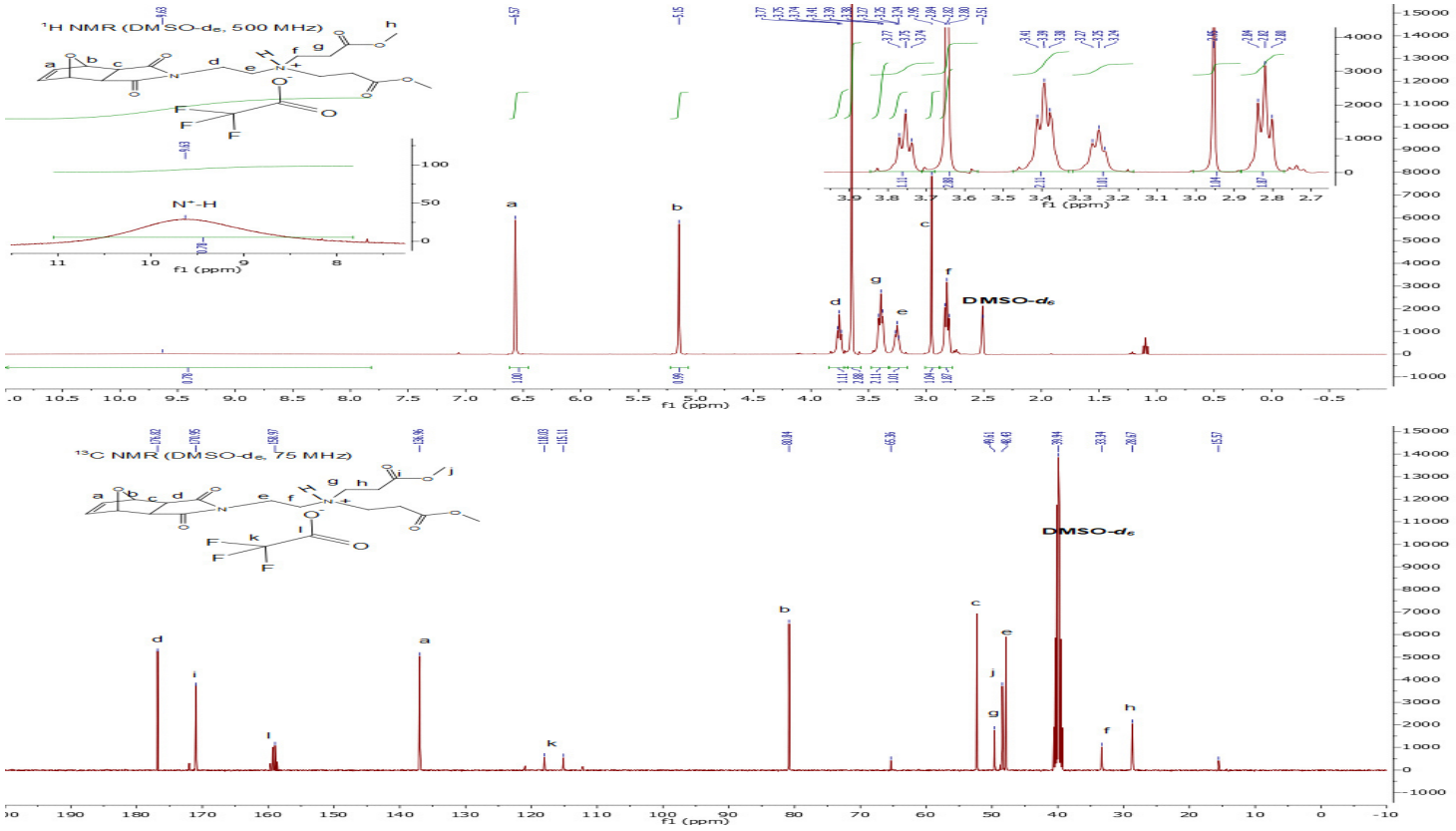

Figure B80 ^{1}H and ^{13}C NMR of a model compound M1 after protonation by TFA

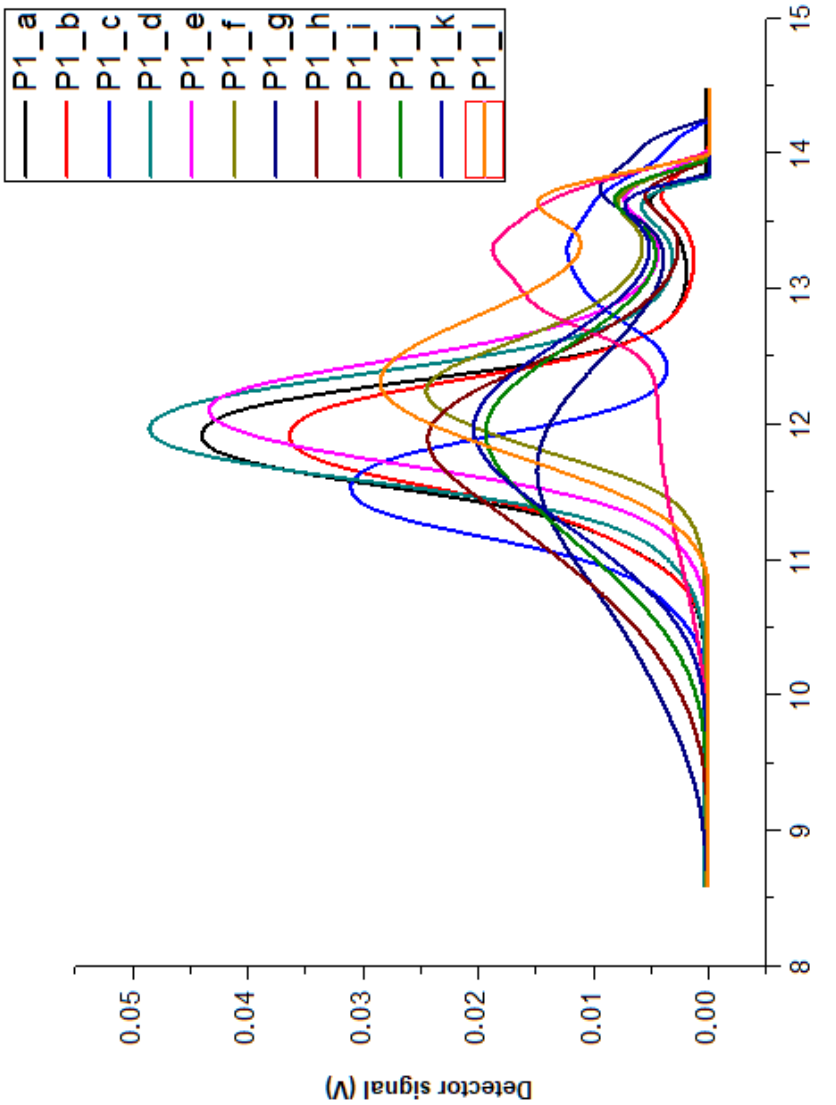

Figure 81 GPC of polymers (P1_a-l)

PUBLICATIONS FROM THE THESIS

Contact Information: nazrul_bd@yahoo.com

Papers

1. M. N. Islam, B. Aksu, M. Güncü, M. Gallei, M. Tulu and T. Eren, "Amphiphilic Water Soluble Cationic Ring Opening Metathesis Copolymer as an Antibacterial Agent", *Journal of Polymer Science*, vol. 58, no. 6, pp. 872-884, 2020.

2. M. N. Islam,T. Eren and M. Tulu, "Synthesis of Poly(amidoamine) (PAMAM) Dendronized Graft Polymers via ROMP and Their Catalytic Activities" *[Under Review]*

Conference Papers

1. M. N. Islam, T. Eren and M. Tulu, "Poly(amidoamine) (PAMAM) Dendrimer based Ring Opening Metathesis Polymer (ROMP) Synthesis for Catalytic Application", *31st National Chemistry Congress***,** 10-13 September 2019, Yildiz Technical University, Istanbul, Turkey.

2. M. N. Islam, B. Aksu, T. Eren and M. Tulu, "Dendron Grafted Ring Opening Metathesis Polymer (ROMP) as an Antibacterial Agent", *14th International Conference on Materials Chemistry (MC14),* 8-11 July 2019, Birmingham, United Kingdom.

3. M. N. Islam, B. Keskin and M. Tulu, "Preparation Graphene oxide-poly(amidoamine) dendrimer nanohybrid material modified electrode for enhancing capacitance, *13th International Conference on Electrochemistry*, 27- 28 May 2019 Barcelona, Spain.

Awards

1. Turkish Government Scholarship

2. Royal Society of Chemistry Conference Grants

3. Erasmus Mobility Grants