SYNTHESIS OF SEGMENT-BLOCK DENDRIMERS
VIA
CHEMICAL LIGATION

by
Muhammed Talha GÖKMEN
B.S. in Chemistry, Gazi University, 2003

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

Graduate Program in Chemistry
Boğaziçi University
2006

SYNTHESIS OF SEGMENT-BLOCK DENDRIMERS

VIA

CHEMICAL LIGATION

APPROVED BY:

Asst. Prof. Rana SANYAL ....

(Thesis Supervisor)

Prof. Dr. İlknur DOĞAN ....

Asst. Prof. Ebru TOKSOY ....

DATE OF APPROVAL: 27.01.2006

to my father

ACKNOWLEDGEMENTS

First of all I would like to express my sincere gratitude to my mentor Asst. Prof. Rana SANYAL for her support and for the most important thing for me; accepting me as her student.

I am also grateful to Dr. Amitav SANYAL for his invaluable advice, discussions on the mechanisms of reactions and help during the laboratory work.

I would like to express my thanks to Prof. İlknur DOĞAN for her constructive review of the final manuscript and for her care during my masters program.

I wish to extend my thanks to Dr. Ebru TOKSOY for her serving in my committee and for mass spectroscopy analysis.

I would like to thank Burcu SELEN ÇAĞLAYAN and Ayla TÜRKEKUL for their great patience and help running a large number of NMR experiments supporting my laboratory work.

I would like to extend my gratitude to all my instructors and all the faculty members for their understanding for borrowing glassware and chemicals. I would like to thank my labmates Volkan YEŞİLYURT, Ali Hikmet KARAYEL, Gonca SEBER and Tuğba DIŞPINAR for making the laboratory life entertaining. Finally I would like to thank Zahid MELEP and Hülya METİNER for their love and help throughout my study at Boğaziçi University.

This research has been supported by Boğaziçi University (BAP 04HC503) and TÜBİTAK Kariyer Grant (104 T185).

I dedicate this work to my parents, Satılmış - İkbal BAYKUŞ for everything especially for their love and encouragement for my masters program.

ABSTRACT

Dendrimers are unique, monodisperse macromolecules with well defined structure and molecular weight arising from their multi-step, iterative synthesis. Recently, they have been utilized for drug delivery applications. The aim of this study is to synthesize block dendrimers which consist of two different dendritic constituents (dendrons).Potentially, one constituent of a particular block dendrimer can be designed as the drug carrier and the other constituent can bear targeting functionality. 'Chemical ligation' (thiaproline ligation) is employed for the coupling of the dendrons. 'Thiaproline ligation' is an efficient coupling method designed for peptide synthesis. It does not require any reagents, works in aqueous media at room temperature with high yields. This method seems to be useful for coupling of two different dendrons to obtain a segment-block dendrimer for further applications.

Two different dendrons, poly(amido amine) and polyether dendrons, were synthesized via divergent method up to fourth generation and convergent method up to second generation respectively. These dendrons were equipped with the necessary functional groups and 'thiaproline ligation' was utilized for the coupling of dendrons. All compounds were identified by IR, ^{1}H NMR and ^{13}C NMR spectroscopy techniques.

ÖZET

Dendrimerler, çok basamaklı ve tekrar eden sentez stratejilerinden kaynaklanan, kesin olarak tanımlanmış yapıya ve molekül ağırlığına sahip makromoleküllerdir. Son yıllarda, dendrimerler ilaç taşıma sistemleri olarak kullanılmaktadırlar. Bu çalışmanın amacı, iki farklı dendritik bileşenlerden (dendron) oluşan blok dendrimerler sentezlemektir. Potansiyel olarak, dendronlardan birisi ilaç moleküllerini taşıyıcı, diğer dendron ise hedefleyici moleküller taşıyıcı özellikte hazırlanabilir. ‘Chemical ligation’ (thiaproline ligation) adlı metot, dendronları birleştirme işleminde kullanılmıştır. ‘Thiaproline ligation’ peptit sentezi için dizayn edilmiş yüksek verimli bir tepkimedir. Herhangi bir katalizöre ihtiyaç duymadan, su ortamında ve oda sıcalığında yüksek verimle çalışır. Bu yöntem, dendronların birleştirilip dilimli-blok dendrimerler elde edilmesinde kullanılmaya çok elverişli görünmektedir.

İki farklı dendron, poli(amido amin) ve polieter dendronlar, sırasıyla içten dışa doğru dördüncü jenerasyona kadar ve dıştan içe doğru ikinci jenerasyona kadar sentezlenmiştir. Bu dendronlar gerekli fonksiyonel gruplarla donatılmış ve birbirlerine bağlanmaları için ‘thiaproline ligation’ tepkimesi uygulanmıştır. Sentezlenen tüm bileşikler IR, ^{1}H NMR ve ^{13}C NMR spektroskopi yöntemleri ile karakterize edilmişlerdir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

J	Coupling constant
σ	Chemical shift
υ	Frequency
Cys	Cysteine
DCC	Dicyclohexylcarbodiamide
DMF	Dimethyl formamide
G	Generation
HOMO	Highest occupied molecular orbital
IR	Infrared
MHz	Mega hertz
NHS	N-Hydroxy succinimide
NMR	Nuclear magnetic resonance
OLED	Organic light emitting diode
PAMAM	Poly(amido amine)
tBoc	Tertiarybutyloxycarbonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Tetramethyl sylane
Trt	Triphenylmethyl
UV	Ultraviolet

1. INTRODUCTION

Macromolecules can be classified into four major groups, namely linear, cross-linked, branched and dendritic (Figure 1.1) [1]. The first three groups are traditional synthetic polymers which do not have exact structures and molecular weigths, and hence they are polydisperse. Dendrimers are the last class of macromolecules which have perfect monodispersity and regular highly-branched structure. These properties give large dendrimers their unique globular 3-D shape. Synthesis of this type of molecules has emerged in the literature about 20 years ago and reached about 10000 articles to date [2]. The unique molecular weigths and more precise structures of dendrimers arise due to the step-by-step, ordered, iterative reaction sequences employed during their synthesis.

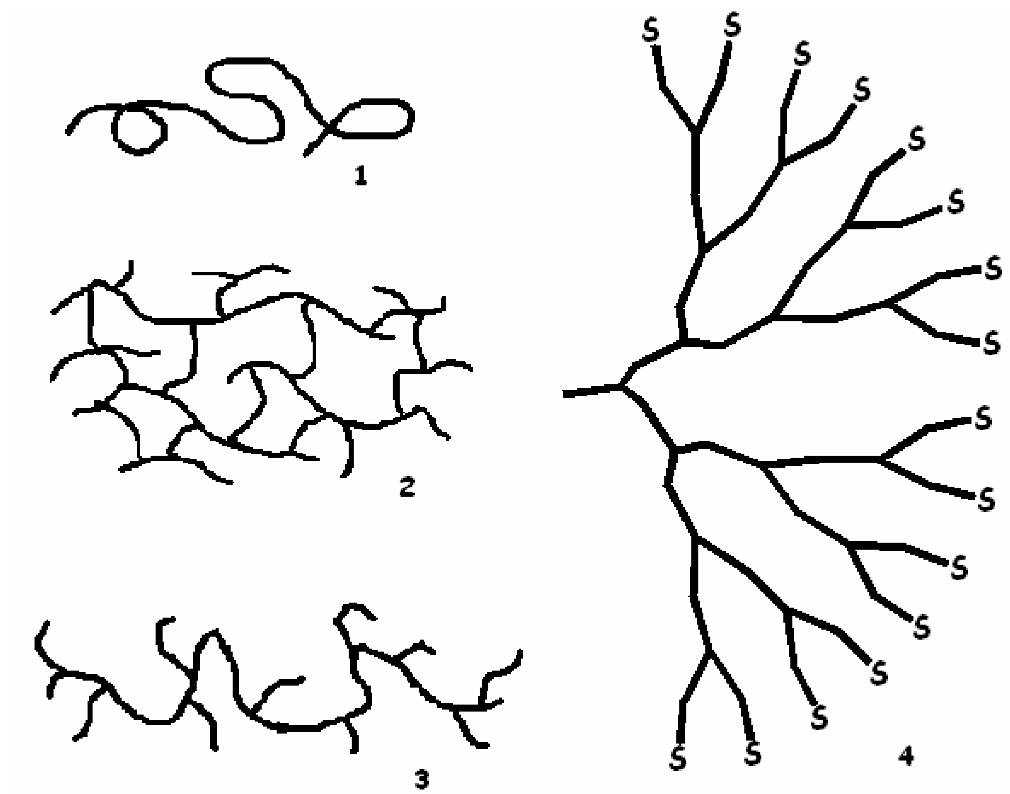
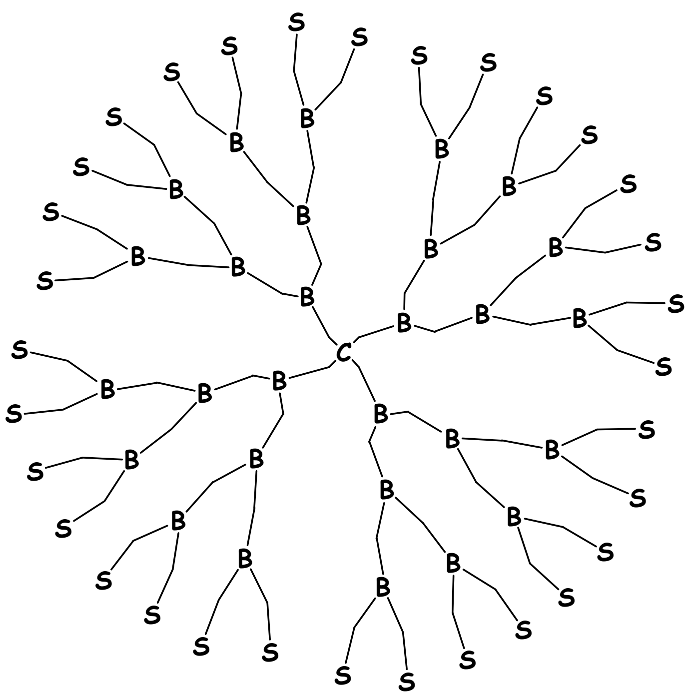


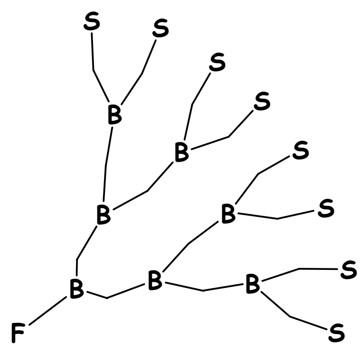
Figure1.1. Four major classes of macromolecules: **1** linear, **2** cross-linked, **3** branched, **4** dendritic

1.1. Structure of Dendrimers

Dendrimers consist of three main parts: a multifunctional core (**C**), branching units (**B**) and surface groups (**S**, also called peripheral groups) (Figure 1.2). Number of functional groups on the core determines the multiplicity of the dendrimer. Branching units are again multifunctional components which provide growth of the dendrimer. These units form the regular branched structure of the dendrimer, giving the dendritic shape. All dendritic architectures end up with a multiple number of surface groups. In the three dimensional structure, surface groups form the outer surface of the globular dendritic architecture. As inner layers and core are highly sterically hindered chemical activity of dendrimers mostly depend on peripheral groups. In the structure of a dendron, instead of the core there is a focal point. Usually the focal point of a dendron bears a reactive group which can eventually be utilized to couple the dendron onto a multifunctional core to form a dendrimer. Recent studies have focused on synthesis of different dendrons followed by their surface modification or combination to form dendrimers [3-4].



5 dendrimer



6 dendron

Figure 1.2. Schematic representation of dendrimers and dendrons. **C**: core, **B**: branching units, **S**: surface groups, **F**: focal point

Dendrimers have a three-dimensionally layered structure. Layers consist of repeating branching units called generations (Figure 1.3) [5]. First dashed circle represents the core2, outer circles represent the generations. For every increase in generation number, number of surface groups doubles for a bifunctional branching unit. For a tri-functional branching unit, the number of surface groups will be triple with every increase in generation.

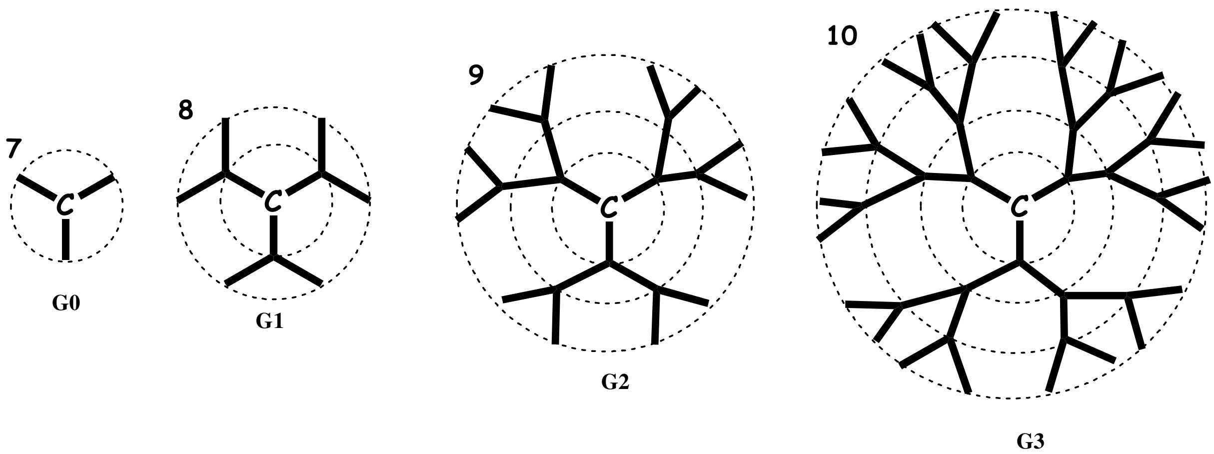
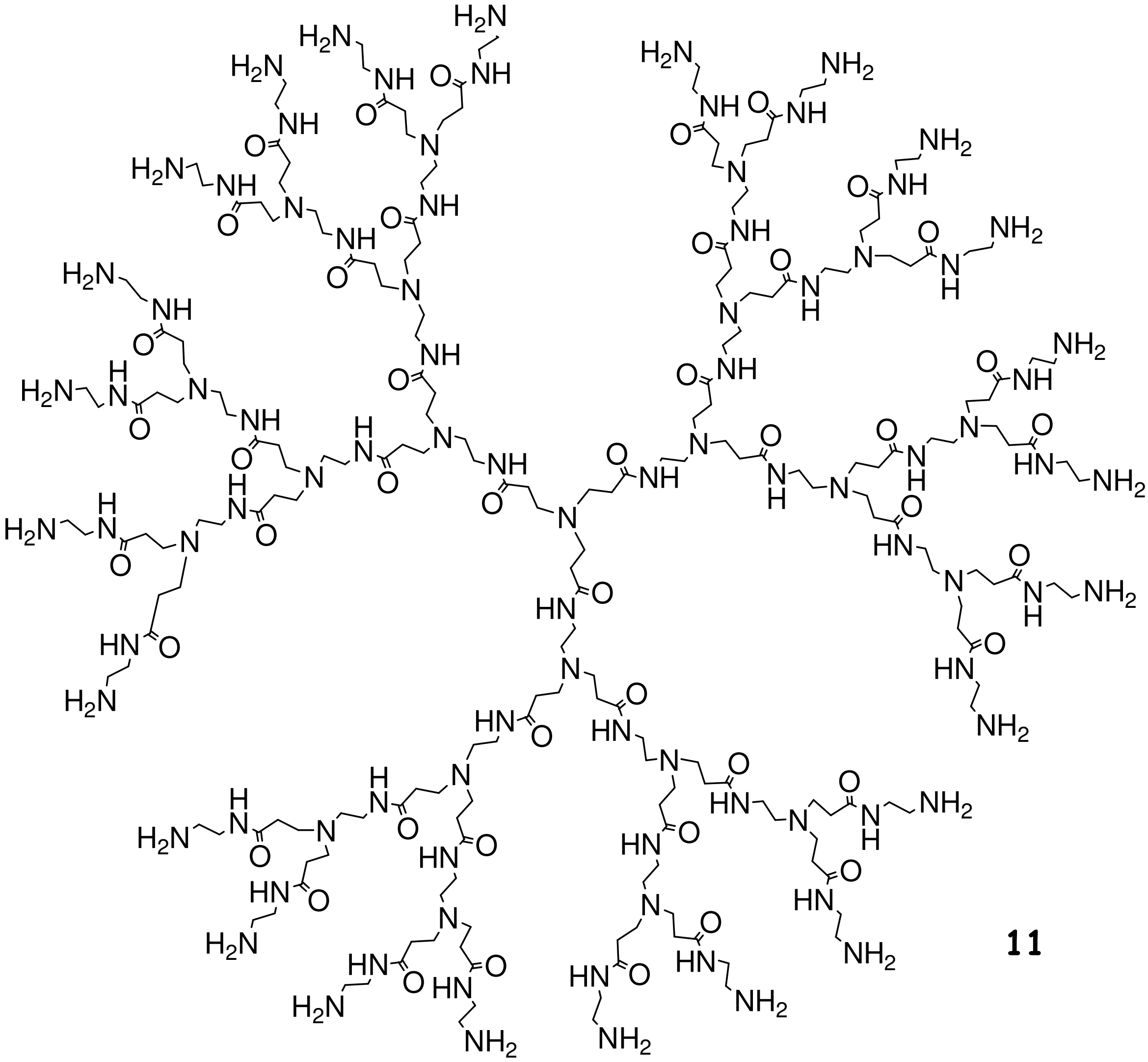


Figure 1.3. Generations of a dendrimer

1.2. Historical Development

Synthesis of dendritic molecules was pioneered by Vögtle [6] in the late 70's and he named his strategy as 'cascade synthesis'. A few years later, Denkewalter and coworkers [7-9] patented the synthesis of dendritic L-lysine based structures up to high generations but there was no detailed characterization of these macromolecules. In 1985, the word 'dendrimer' was introduced by Tomalia and coworkers [2] into the literature. The term was coined for the polyamidoamine dendrimer (Figure 1.4) which was abbreviated as PAMAM and has attracted the highest attention in dendrimer field thus far. In the same year, Newkome and coworkers' [10] 'arborols' appeared in the literature which were also dendritic molecules. These two studies attained great attention because they were including detailed characterization of these macromolecules. Consequently, the year 1985 can be accepted as the birth date of dendrimers.



11

Figure 1.4. Tomalia's ammonia core, third generation PAMAM dendrimer

One of the most important features of dendrimers is multi-functionality. For instance, Tomalia's third generation PAMAM dendrimer (Figure 1.4) has 24 exactly same primary amino groups on the surface. Because of this multi-functionality at the surface of the macromolecule, dendrimers have been under investigation in a variety of scientific areas such as modification of surfaces, transportation of different molecules into biological cells, model light harvesting systems etc.

1.3. Synthesis Strategies

Dendrimer synthesis is accomplished by following either a ‘convergent’ or ‘divergent’ construction approach. First synthetic strategies described for constructing a dendrimer utilized the divergent method. In divergent method the synthesis starts from the core, followed by the construction of branching units and finished by the addition of surface groups. The other major strategy which was developed later is called the convergent method. Convergent method starts with coupling of surface groups onto a branching unit. Coupling of these small dendrons onto a new branching unit is the following step. Repetition of these coupling of smaller dendrons onto branching units to obtain one higher generation dendron is the growth mechanism of convergent method. Finally these dendrons are stitched together, usually around a core molecule to obtain dendrimers. These two major strategies of dendrimer synthesis will be explained in detail below.

1.3.1. Divergent Method

Dendrimer synthesis using divergent method starts with what will become the core of dendrimer (Figure 1.5 structure **12**). Core must be multi-functional to provide the dendritic hyper-branched structure. This multi-functionality will also determine the number of dendrons on the final shape. Growth of dendrimer continues with addition of monomers **13** which will form inner layers of the final structure. Monomers are generally chosen as carrying one active and two or more inactive ends. The triangle on the structure of monomer **13** represents the active end and the ellipse represents the inactive functionality. This inactive end generally keeps the multi-functionality that will form the branching points of dendrimer. After coupling of these monomers onto core, protected dendrimer **14** is obtained and then activation step follows. First coupling and activation steps yield first generation dendrimer **15**. By repetition of coupling and activation steps, dendrimer grows up to very high molecular weights. After a number of coupling and activation steps synthesis stops and last added monomer molecules become surface groups of the dendrimer. For instance, PAMAM (Figure 1.4) and poly(propylene imine) (Figure 1.6) dendrimer families are being synthesized by divergent method.

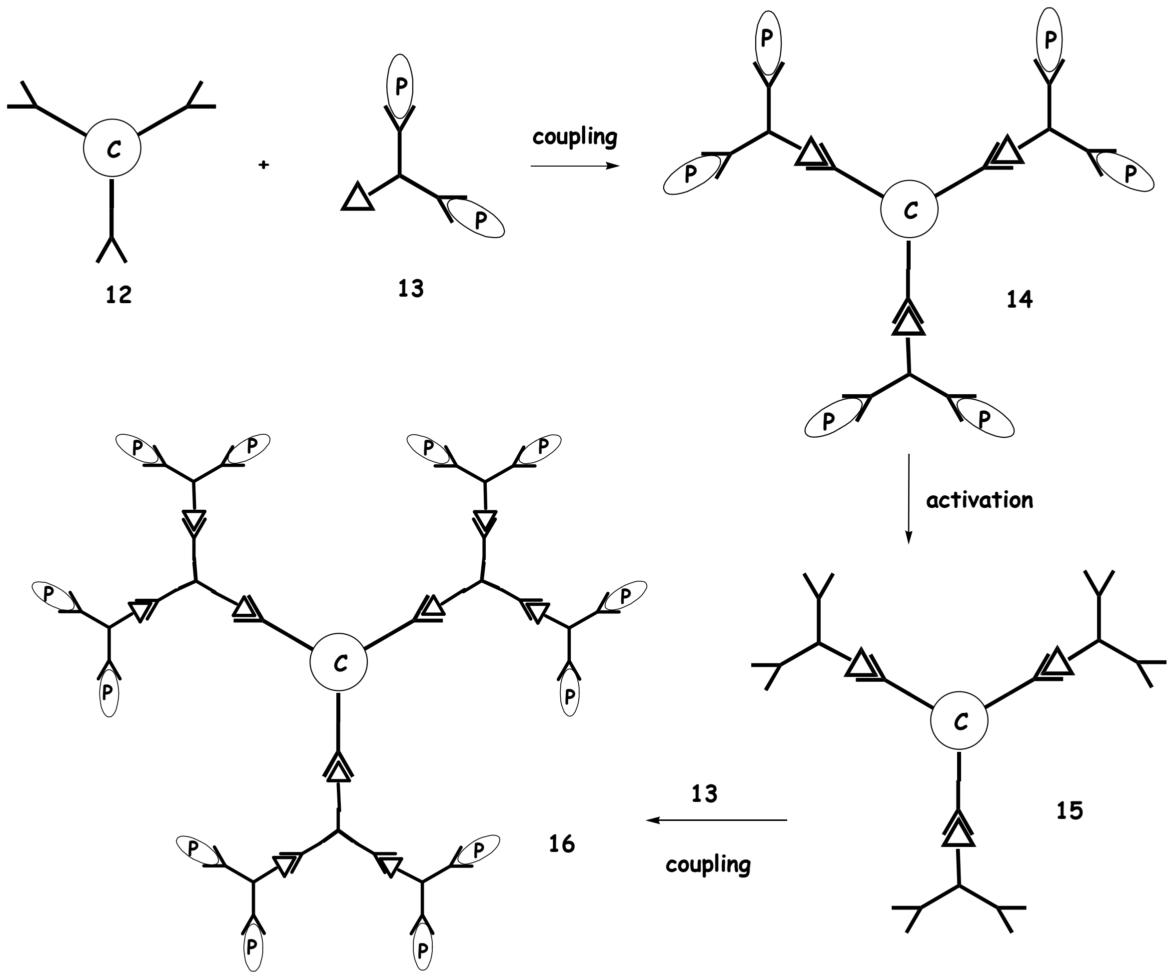
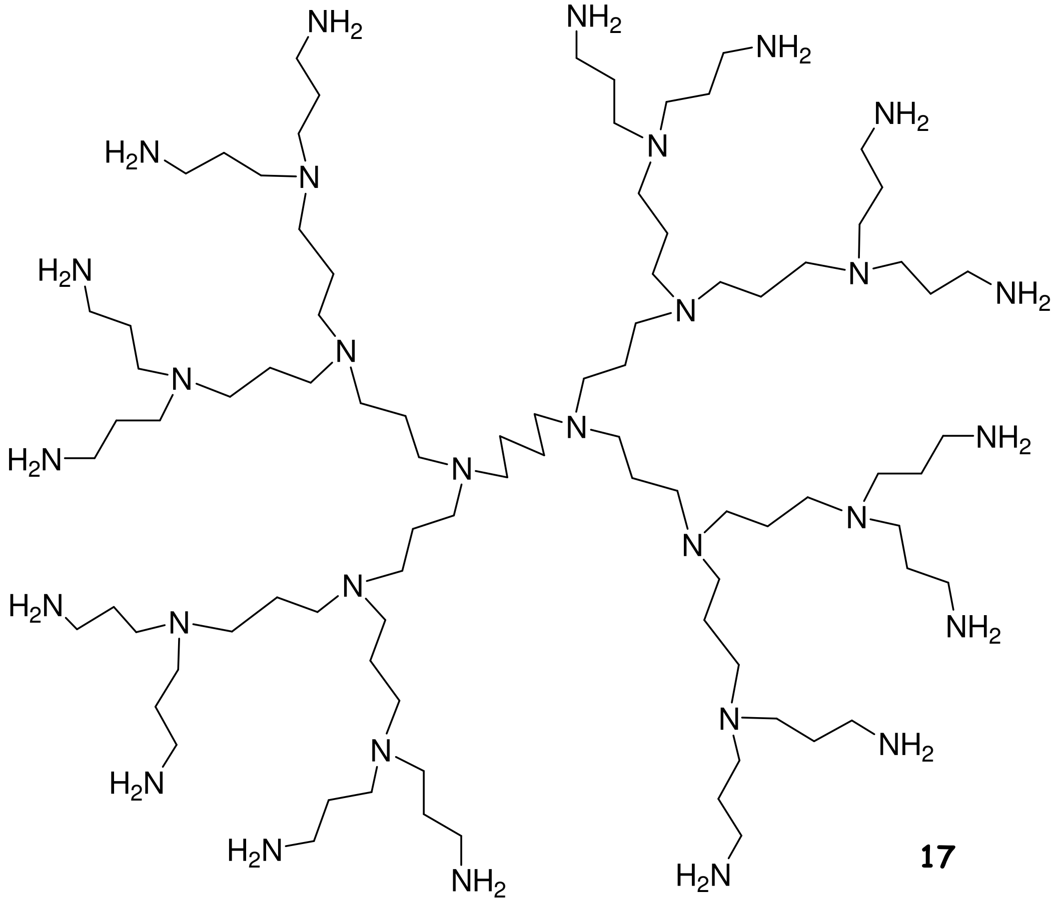


Figure 1.5. Divergent dendrimer synthesis where **C** is core and **P** is protecting group

Divergent method is suitable for large scale dendrimer synthesis. For coupling step (Figure 1.5) excess of monomer is being used for completion. After obtaining the desired product, small monomers can be removed by simple techniques like distillation, precipitation, or ultrafiltration. Another advantage of divergent method is doubling of molecular weight in every coupling step.

Loss of structural control especially when reached to high generations is the most important drawback of divergent method. As the generation increases, the number of coupling reactions needed to be accomplished per one step increases exponentially. For example, the number of coupling reactions between monomers and the third generation PAMAM dendrimer (Figure 1.4) necessary for the formation of fourth generation dendrimer is 48. Complete conversion for 48 reactions is very difficult and causes



17

Figure 1.6. Poly(propylene imine) dendrimer.

structural defects which cause polydispersity. In addition, detection of incomplete conversion is highly challenging.

1.3.2. Convergent Method

In 1990, Hawker and Fréchet [11] introduced the convergent method to the dendrimer synthesis field. In contrast to divergent method, convergent method starts with what will become the peripheral groups of dendrimer and proceeds toward core. Briefly, it is preparation of dendrons and coupling them onto a multi-functional core.

In convergent method, first step is the coupling of multiple surface groups **13** onto a multi-functional branching unit **17** to obtain **18** (Figure 1.7). Branching unit should have a protected focal point. As in the case of divergent method, activation step follows the coupling step. As a result of first coupling and activation steps, dendron **19** is obtained. Up to the generation which is desired, coupling and activation steps follow each other. Final step of convergent method is coupling of preformed dendrons **19** onto a multi-functional core **12** to obtain the desired dendrimer **20**.

In convergent method, purification is not as easy as in the case of divergent method. The reason will be obvious if the coupling reactions are compared. As explained above, in divergent method excess of monomers can easily be removed after coupling because dendrimer is at least 4-5 fold heavier then monomers in terms of molecular weight. As the generation increases this difference increases exponentially. However, in convergent method the molecular weight difference between starting dendron and obtained dendron after coupling is only 2 fold. Thus, column chromatography is utilized for purification.

The requirement of chromatographic techniques in convergent method makes this approach harder, but at the same time provides a better control on purity. Purification of dendrons in each step by column chromatography does not allow structural defects. As a reminder, separation of regular48 armed divergently grown fourth generation PAMAM dendron from irregular 47 armed one is almost impossible. Mass characterization results show that convergent dendrimers are exactly monodisperse, devoid of any structural defects. Consequently, the invention of convergent method can be accepted as a revolutionary step in the dendrimer science.

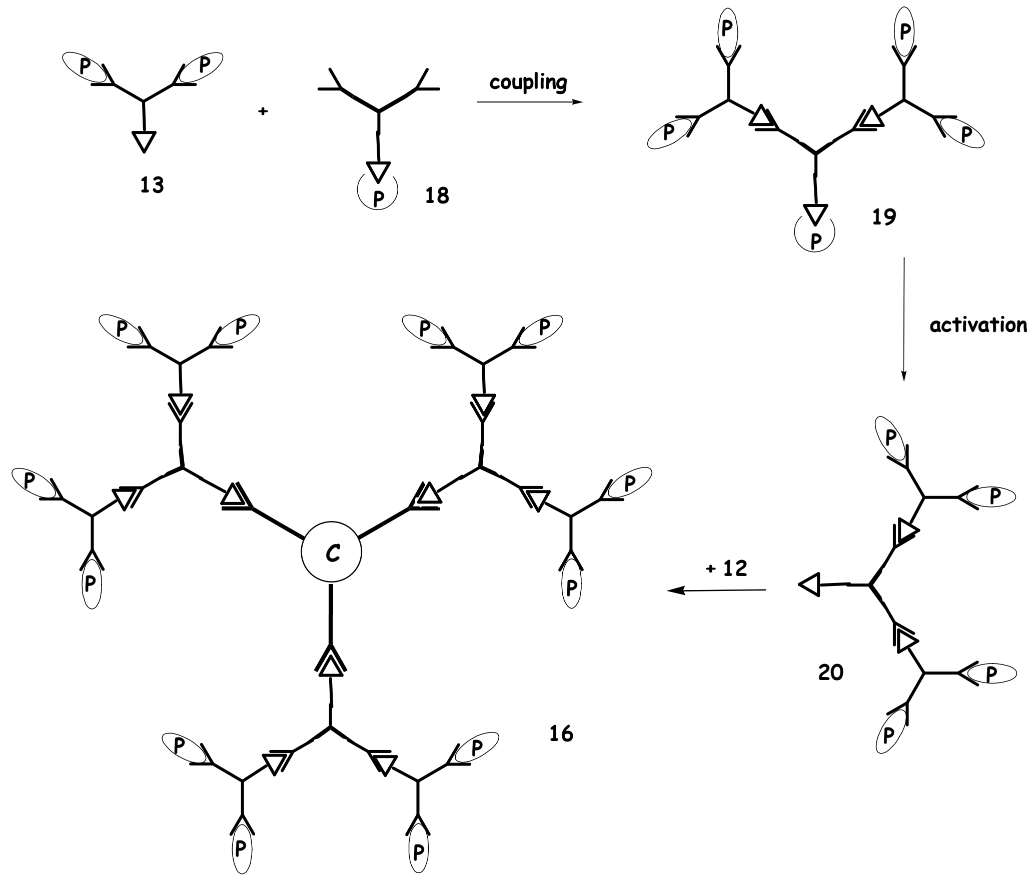
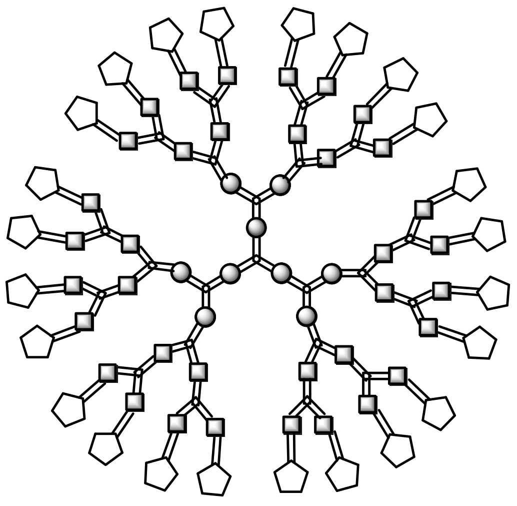


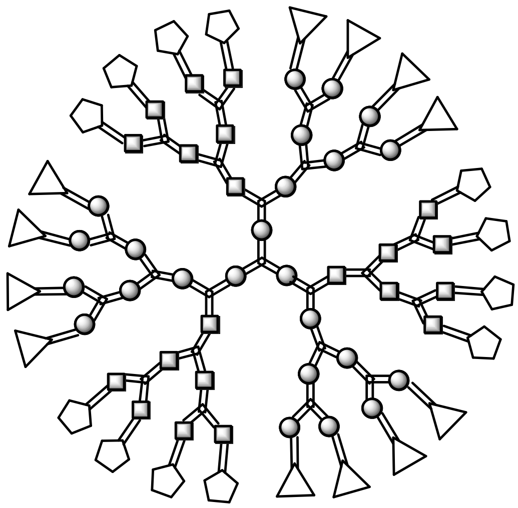
Figure 1.7. Convergent dendrimer synthesis where **C** is core and **P** is protecting group

2. BLOCK DENDRIMERS

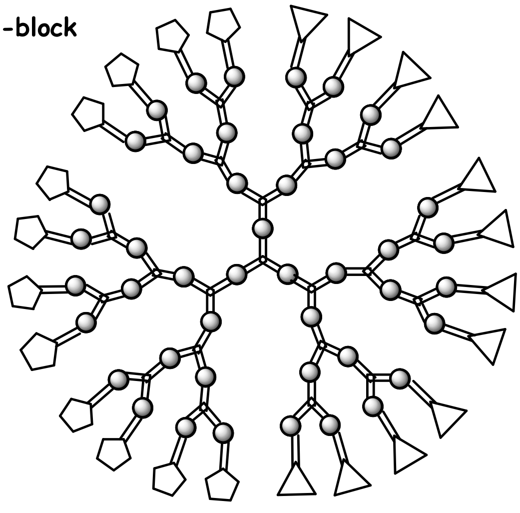
Dendrimers are useful carriers for a variety of molecules. To accommodate a number of different molecules on each one, dendrimers bearing different functional peripheral groups are constructed. A dendrimer composed of different building blocks is called a block dendrimer. These kinds of dendrimers can be classified in three major groups: layer-block **21**, segment-block **22** and surface-block **23** dendrimers [12] (Figure 2.1).



21 layer-block



22 segment-block



23 surface-block

Figure 2.1. Types of block dendrimers

2.1. Layer-Block Dendrimers

Layer-block dendrimers have different branching units from core to periphery which means that during the synthesis branching unit was changed at least once. It can be seen that layer-block dendrimers have same functional groups on the surface like normal symmetrical dendrimers. Layer-block dendrimers can be synthesized by both convergent and divergent methods. The first synthesis of a layer-block dendrimer (Figure 2.2) was reported by Hawker and Fréchet [13] via convergent attachment of poly(benzyl ether) dendron **24** to a poly(benzyl ester) monomer **25**. The obtained dendron **26** was then coupled to a triphenolic core **27** to obtain the layered dendrimer **28**.

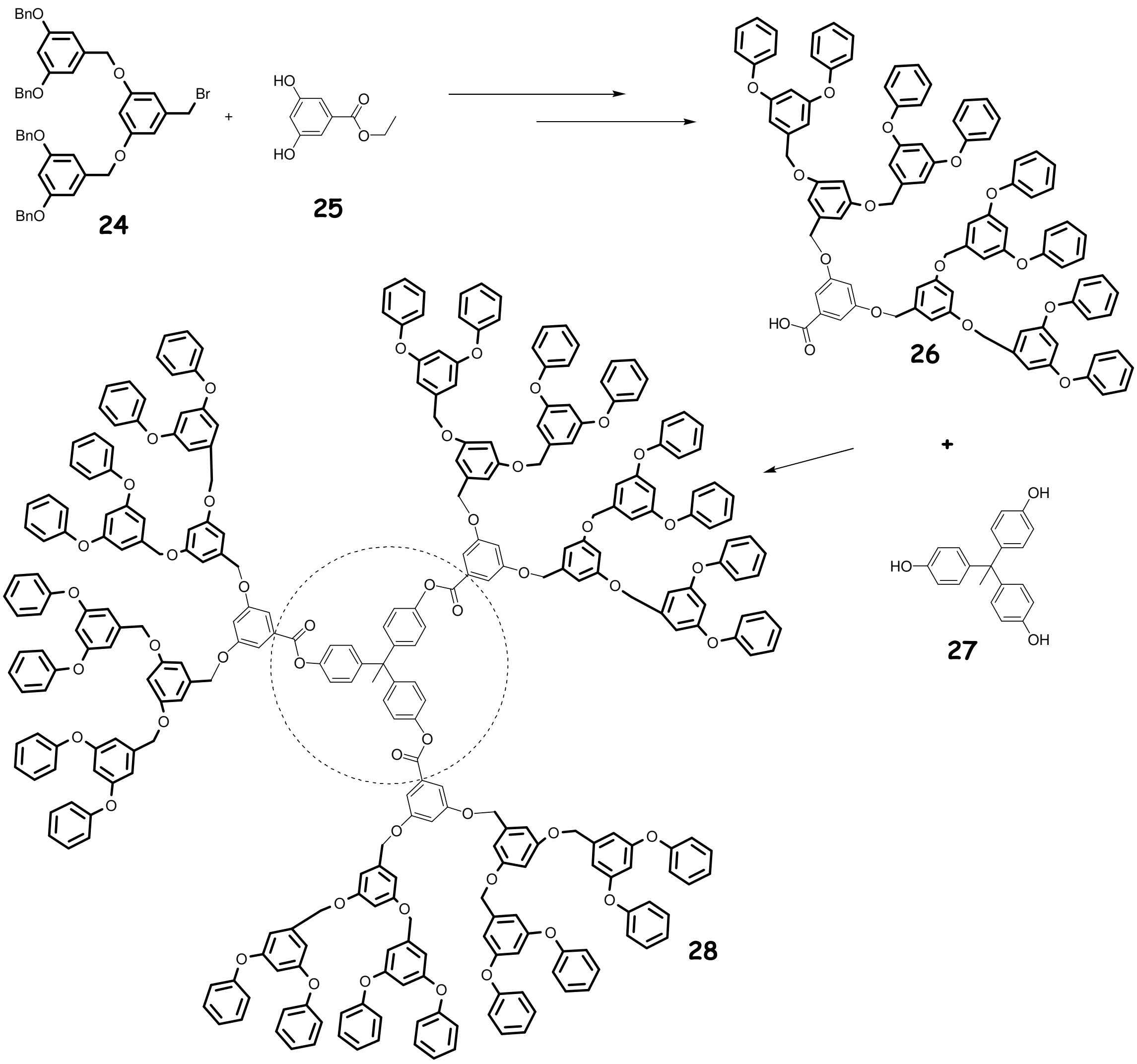
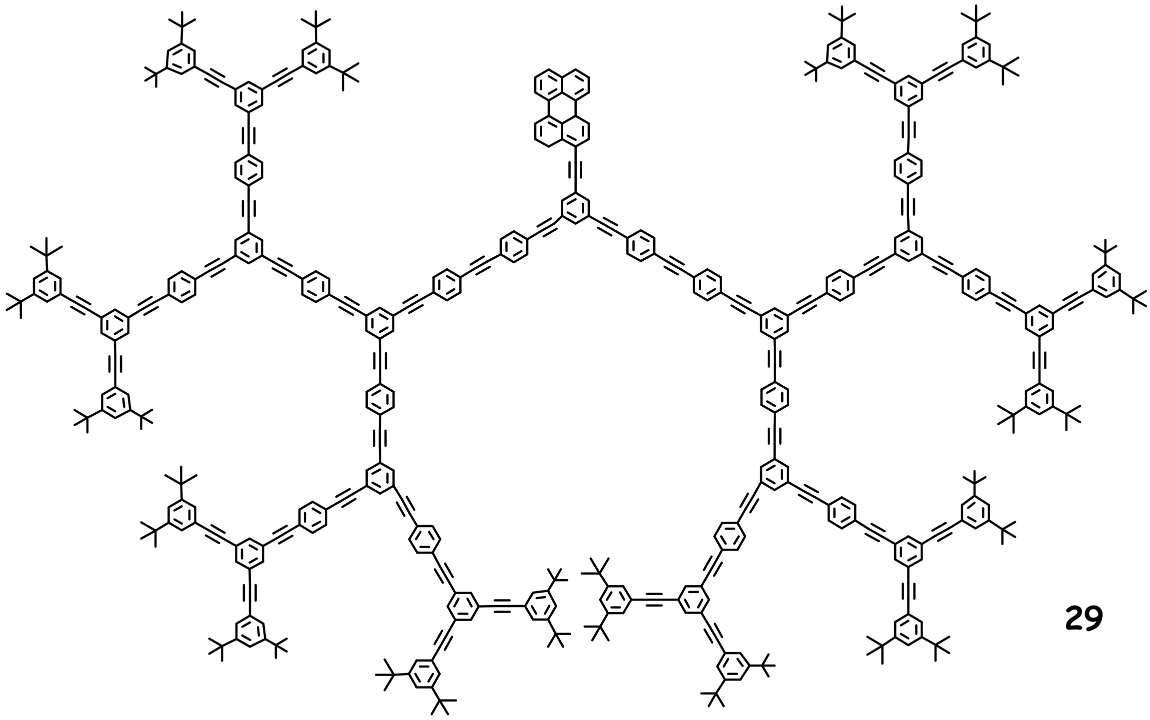


Figure 2.2. Synthesis of the first layer-block dendrimer

The constructed dendrimer has ether linkages at the outer layers and ester linkages at the inner layers which are seperated by the dotted circle.

One of the most noteworthy applications of layer-block dendrimers has been their use in light-harvesting devices. Moore and co-workers [14-15] have synthesized layer-block dendrons like dendron **29** based on phenylacetylene and investigated their intramolecular energy transfer abilities (Figure 2.3). They found that light-harvesting ability of these compounds increases with increasing generation which is related to the increase in molar extinction coefficient. Another result which they found was the decrease of energy transfer with increasing generation in the perylene series.



29

Figure 2.3. Dendritic light-harvesting device prepared by Moore et al.

Another approach which is similar to the previous one has been utilized by Morikawa et al. [16] in the synthesis of graded poly(ether ketone) dendrons by the convergent method using aromatic nucleophilic substitution reactions (Figure 2.4). They have used four compounds with different phenylene lengths as building blocks to obtain dendron **30**. As seen in Figure 2.4, from periphery to the dendron core, the number of phenylene units has increased by one for every generation. By this synthesis strategy they

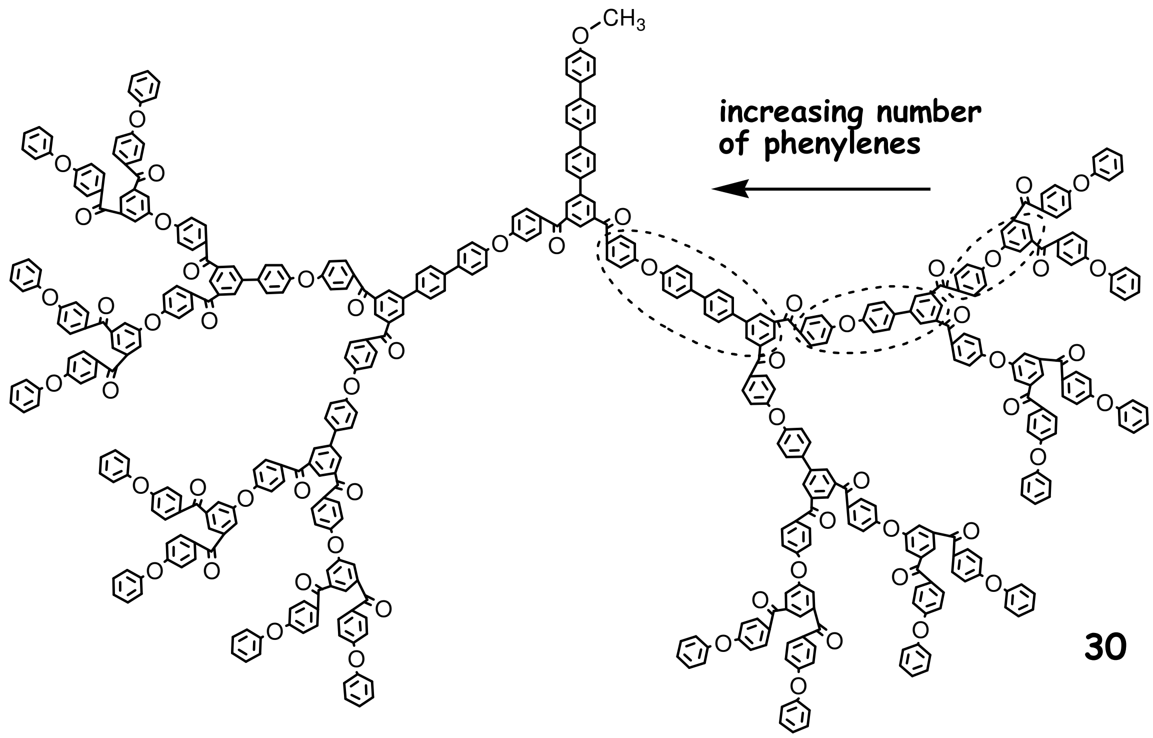


Figure 2.4. Morikawa and coworkers' graded dendron

obtained dendrons with increasing layer length from periphery to core and named these structures as graded poly(ether ketone) dendrons.

2.2. Segment-Block Dendrimers

Segment-block dendrimers are the hybrid molecules which different types of dendrons are present on the final unsymmetrical dendritic structure. Unlike the layer-block dendrimers, segment-block ones have different surface groups depending on the number of segments. Convergent approach is more suitable for the preparation of this kind of dendrimers because two or more different dendrons can be synthesized seperately and then coupled.

First example of this kind of architecture was accomplished by Hawker and Fréchet [17]. It was based on the poly(benzyl ether) and poly(benzyl ester) repeat units as contrasting dendritic components (Figure 2.5). The second generation Fréchet-type bromide dendron **24** was reacted with an excess of the poly(benzyl ester) monomer **31** to

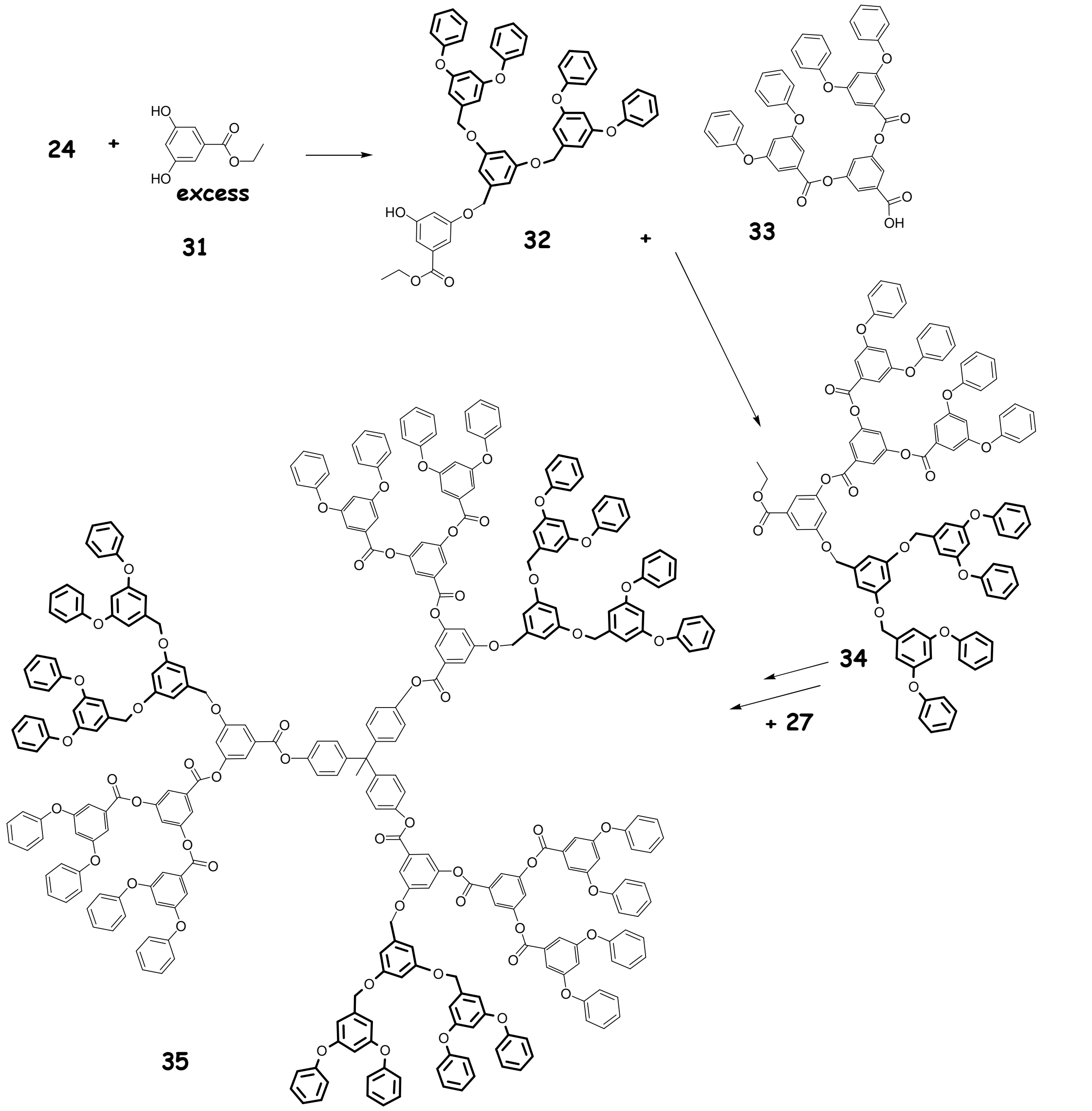


Figure 2.5. Synthesis of the first segment-block dendrimer

give the singly coupled product **32**. The remaining phenol was coupled to the second generation poly(benzyl ester) carboxylic acid **33** by a DCC-mediated esterification, yielding the unsymmetrical hybrid dendron **34**. After activation of the focal point, three of these dendrons coupled to a trisphenolic core **27** to yield dendrimer with three pairs of hybrid ester-ether dendritic wedges **35**.

Yamamato et al. assembled a segment-block dendrimer based on dehydration reaction between two different divergently synthesized aromatic dendrons (Figure 2.6) [18]. This segment-block dendrimer can be used for organic light-emitting diode (OLED)

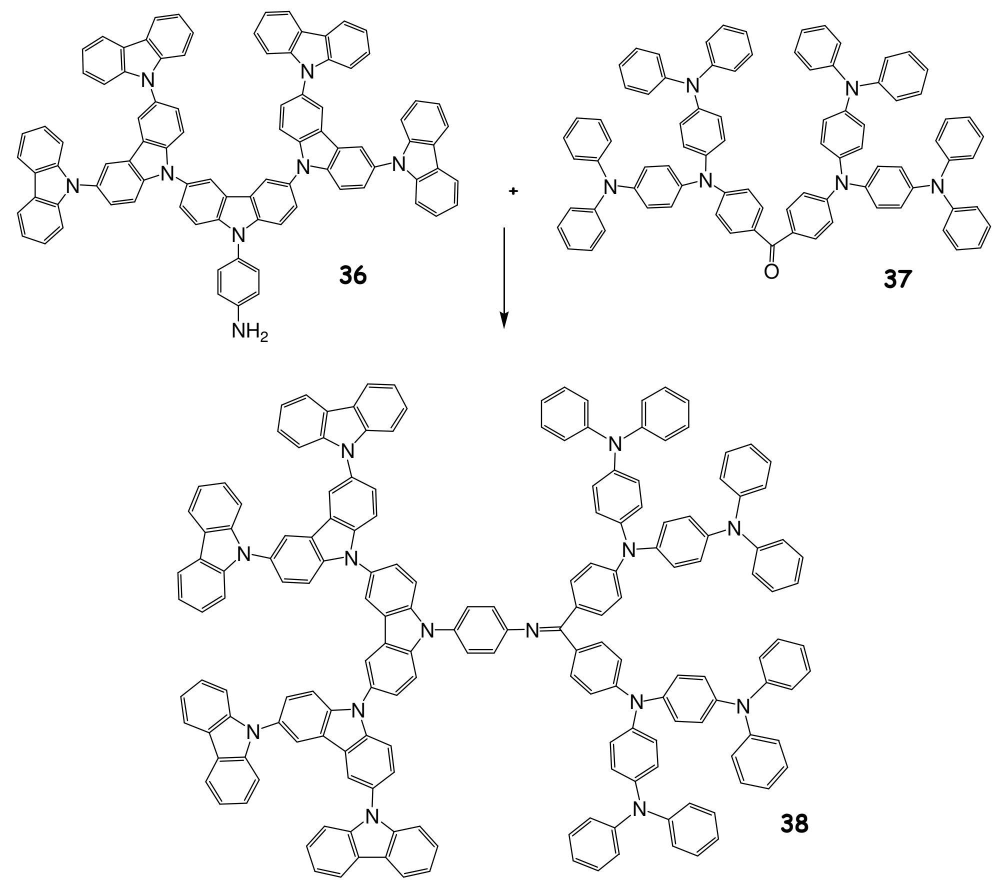


Figure 2.6. A segment-block dendrimer prepared for OLED applications.

applications. They found that the highest HOMO value was obtained when the highest generations of the dendrons were coupled.

A different approach to obtain layer-block and segment-block dendrimers was utilized by Chow et al. [19]. They introduced optical activity into block dendrimer field (Figure 2.7). The layer-block dendrimer **39** consists of the same structure of layers with different chirality and the surface-block dendrimer **40** consists of the same structure of segments with different chirality. They also investigated chiroptical properties of their block dendrimers.

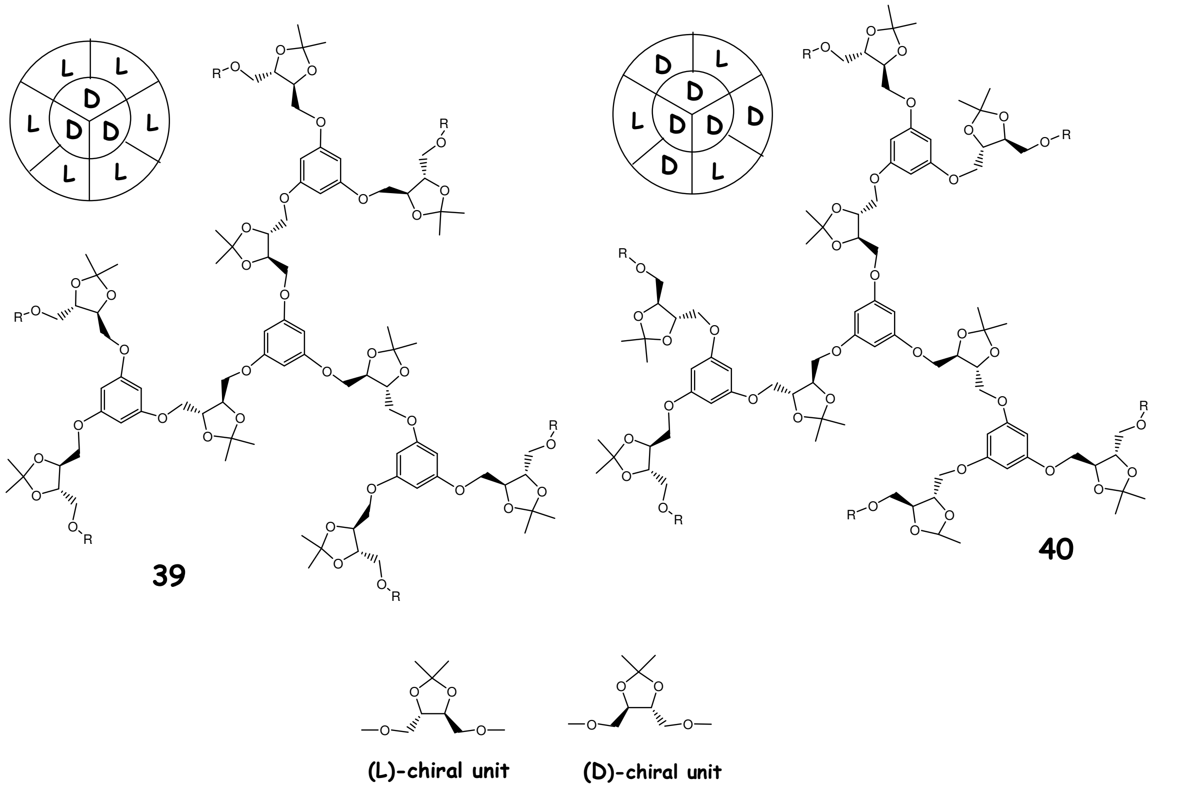


Figure 2.7. Chow's optically active block dendrimers

2.3. Surface-Block Dendrimers

Although very appealing in terms of physical and chemical properties, due to the involved synthesis there are not many examples of segment-block dendrimers in literature. Instead most research has focused on surface-block dendrimers which could be utilized for similar purposes as the segment-blocks due to the fact that physical properties of the dendrimers are governed by the surface groups. On the contrary to segment-block dendrimers, surface-block dendrimers have only one dendritic skeleton but similar to segment-blocks, at least two different groups of surface functionalities. So they are similar in having more than one surface functionality but different in inner shell homogeneity. In terms of synthesis surface-block dendrimers can be obtained more easily. Because, as in the Figure 2.8 one can synthesize only one dendron by one of the convergent or divergent methodologies then modify the surface to obtain two differentiated dendrons. To build up the final block dendrimer those two different dendrons must be coupled. On the other hand, for a segment-block dendrimer two different types of dendrimers should be synthesized separately and then coupled like in Figure 2.6. Unfortunately in the literature there is not an exact differentiation between these two types of dendrimers.

In the study by Okada et al. [20] hemisphere **41** was constructed by a divergent-growth procedure with a half-protected initiator core (Figure 2.8), followed by modification of terminal functional groups dually. Activation of the focal points yielded differentiated dendrons **42** and **43**. Finally the two hemispherical blocks were coupled at their reactive cores to obtain the surface-block dendrimer **44**. The sugar residues attached to the hydrophilic block possess cell recognition function and the other hemisphere which was protected by phtalic anhydride suggested for additional applications. Their strategy was named as divergent/convergent joint approach.

Wooley et al. [21] synthesized a surface-block dendrimer which was strongly dipolar (Figure 2.9). To achieve this property they prepared Fréchet type benzyl-ether dendrons which differ in surface groups. One of them was carrying electron-withdrawing cyano groups and the other electron-donating benzyloxy groups. Coupling of these two dendrons yielded the desired strongly dipolar dendrimer. They calculated dipole moment values from measurements of capacitance and refractive index and found that dipole moment increases with increasing molecular weight.

Surface-block concept was also utilized for building up ‘unimolecular micelles’. These kind of dendrimers contained benzyl ether end groups in one hemisphere and methyl esters in the other. Saponification of the esters produced the dendritic amphiphile which demonstrated micellar properties (Figure 2.10). Because of the well-segregated polar and nonpolar regions within the dendrimer, it is believed that these structures orient themselves at the interface between hydrophilic and hydrophobic solvents [22].

Twyman et al. synthesized of a surface-block PAMAM dendrimer naming ‘divergent-divergent joint approach’ (Figure 2.11) [23]. First a third generation PAMAM dendron **47** was synthesized and its surface was modified with unreactive isobutyl groups **48**. Next step was deprotection of the focal point at **48** affording the dendron **49** and addition of new amide and amine groups to build up second hemisphere **50**. Construction of the ester terminated hemisphere **51** was stopped at the third generation.

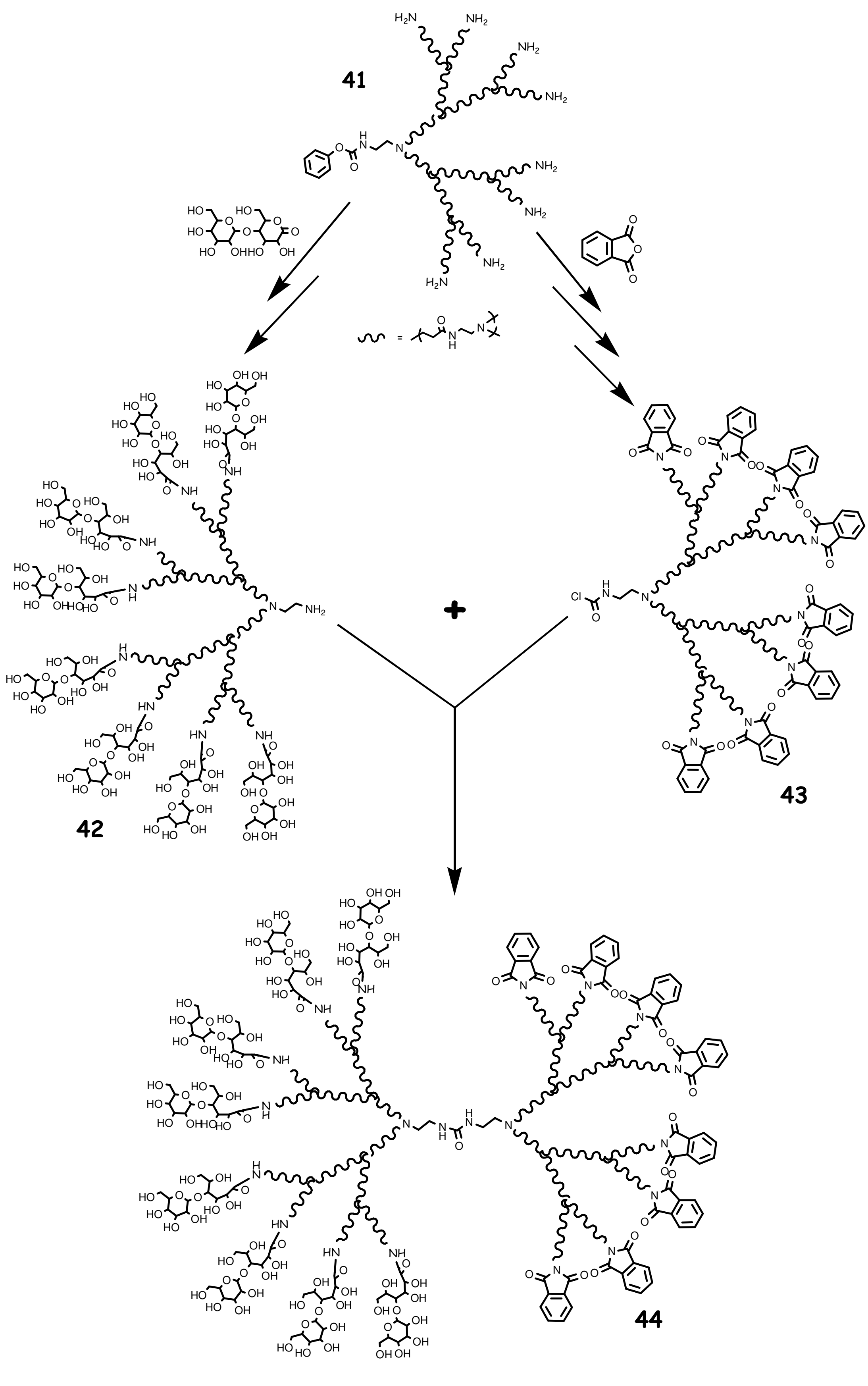
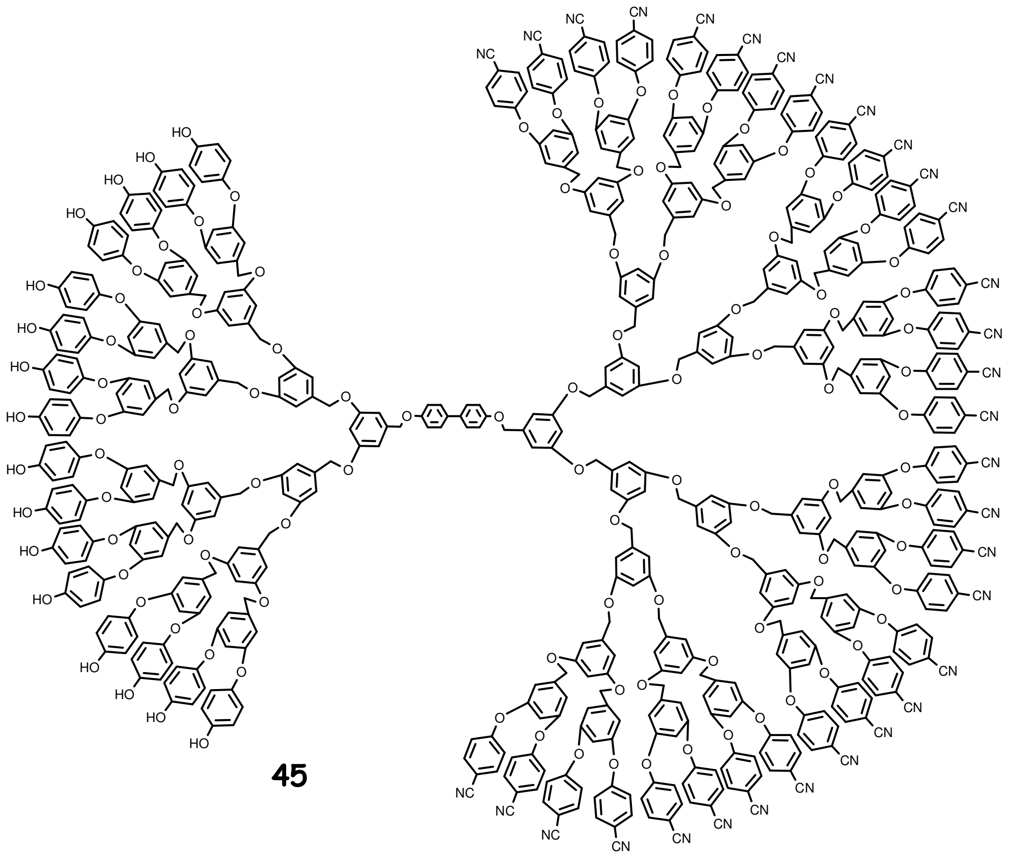
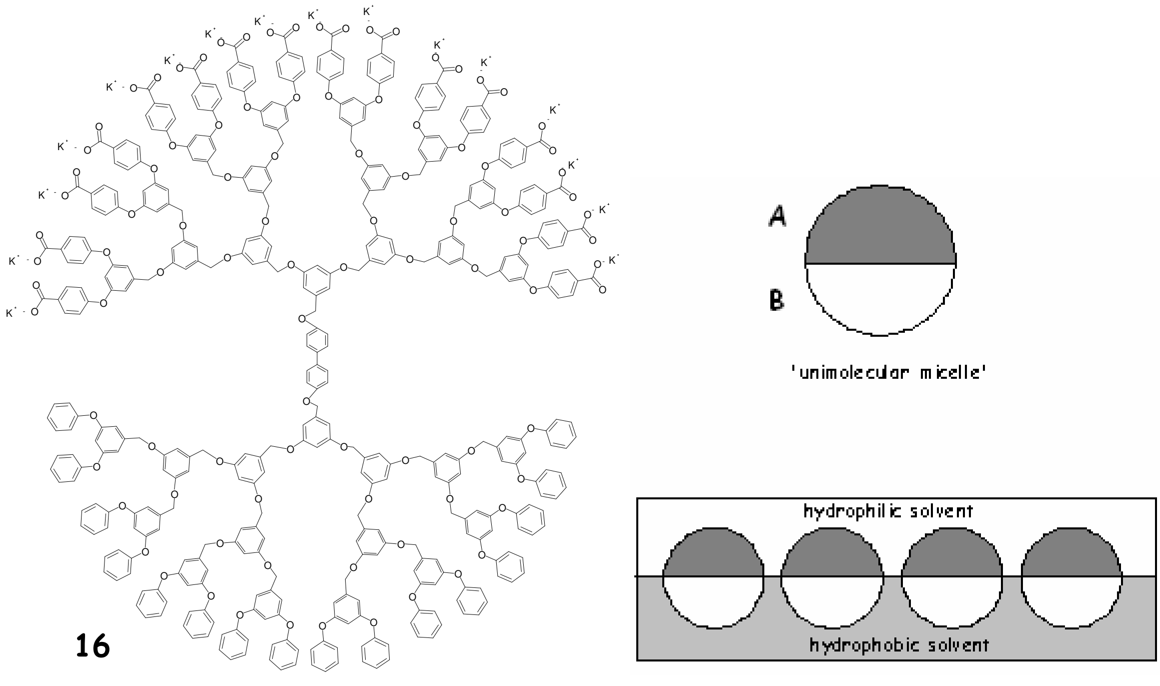


Figure 2.8. Synthesis strategy of Okada's surface-block PAMAM dendrimer



45

Figure 2.9. Wooley et al. synthesized a strongly dipolar surface-block dendrimer



16

Figure 2.10. Structure of dendritic unimolecular micelle

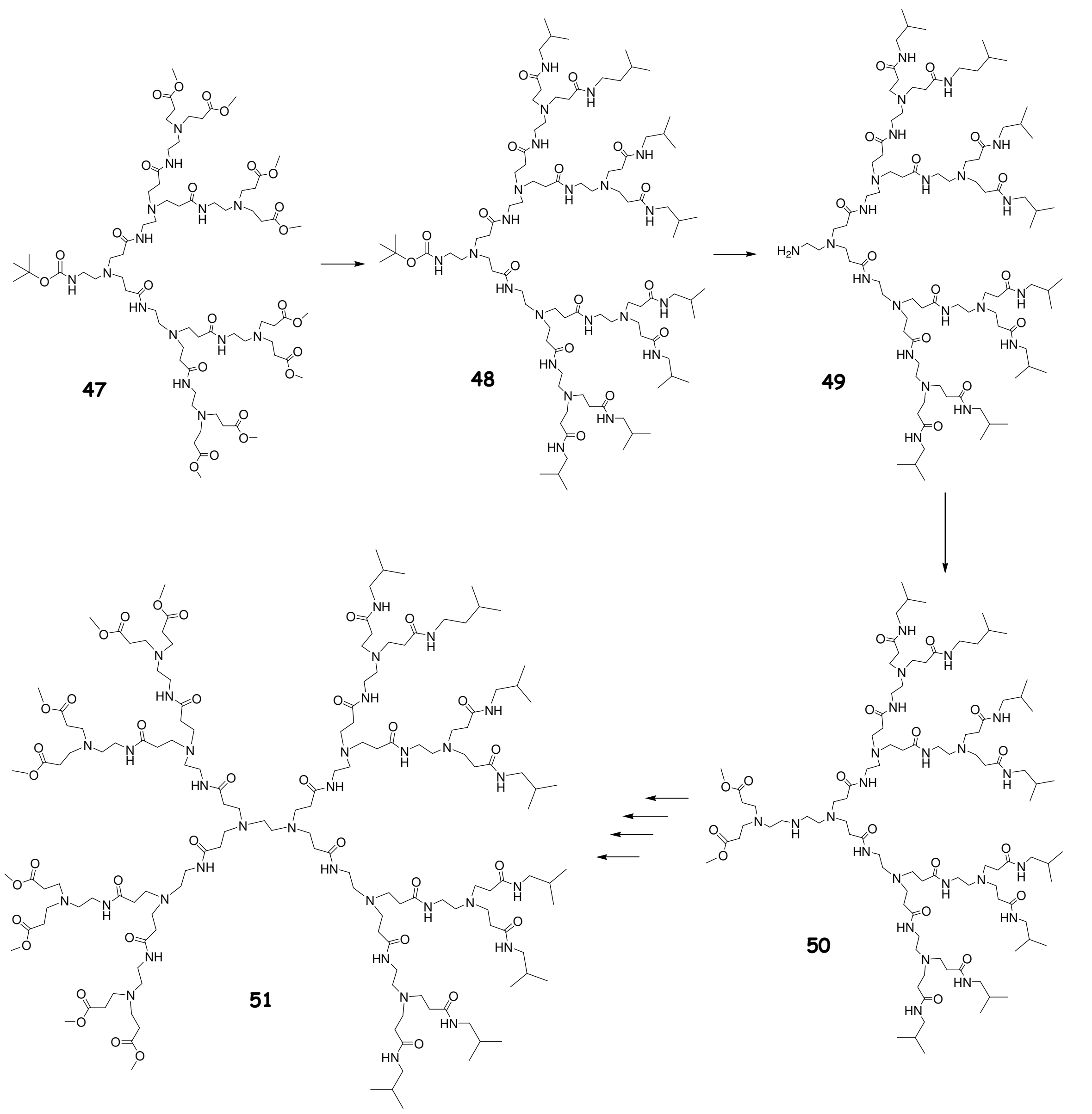
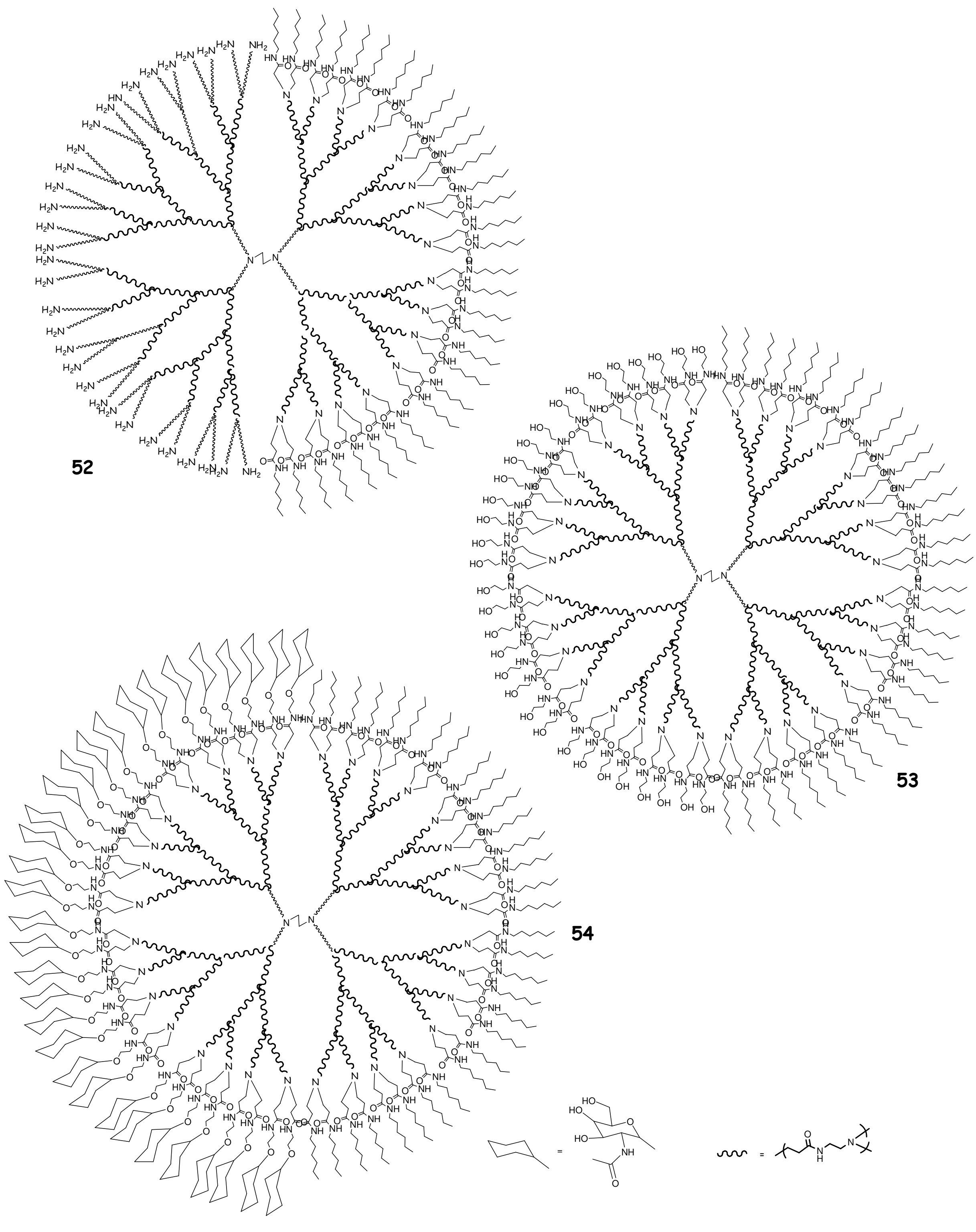


Figure 2.11. 'Divergent-divergent joint approach' developed by Twyman et al. for construction of surface-block PAMAM dendrimers

In a study yielding new 'unimolecular micelles', Okada et al. [24] employed surface-block PAMAM dendrimers. Three different block PAMAM dendrimers were synthesized via divergent-divergent method. These dendrimers were G4 amino/hexyl PAMAM dendrimer **52**, G4 hydroxyl/hexyl PAMAM dendrimer **53**, G4 glucosamine/hexyl PAMAM dendrimer **54** which are shown in Figure 2.12. Amphiphilic characteristics of these hybrids along with in situ adlayer formation and adsorption kinetics on solid substrates were investigated.



52

53

54

Figure 2.12. Three different surface-block PAMAM dendrimers prepared by Okada et al.

Fréchet et al. [25] synthesized ‘bow-tie hybrids’, a surface-block dendrimer, for drug delivery applications which had a polyester skeleton appended with polyethylene glycol tails (Figure 2.13). One hemisphere had protecting groups on the periphery and the

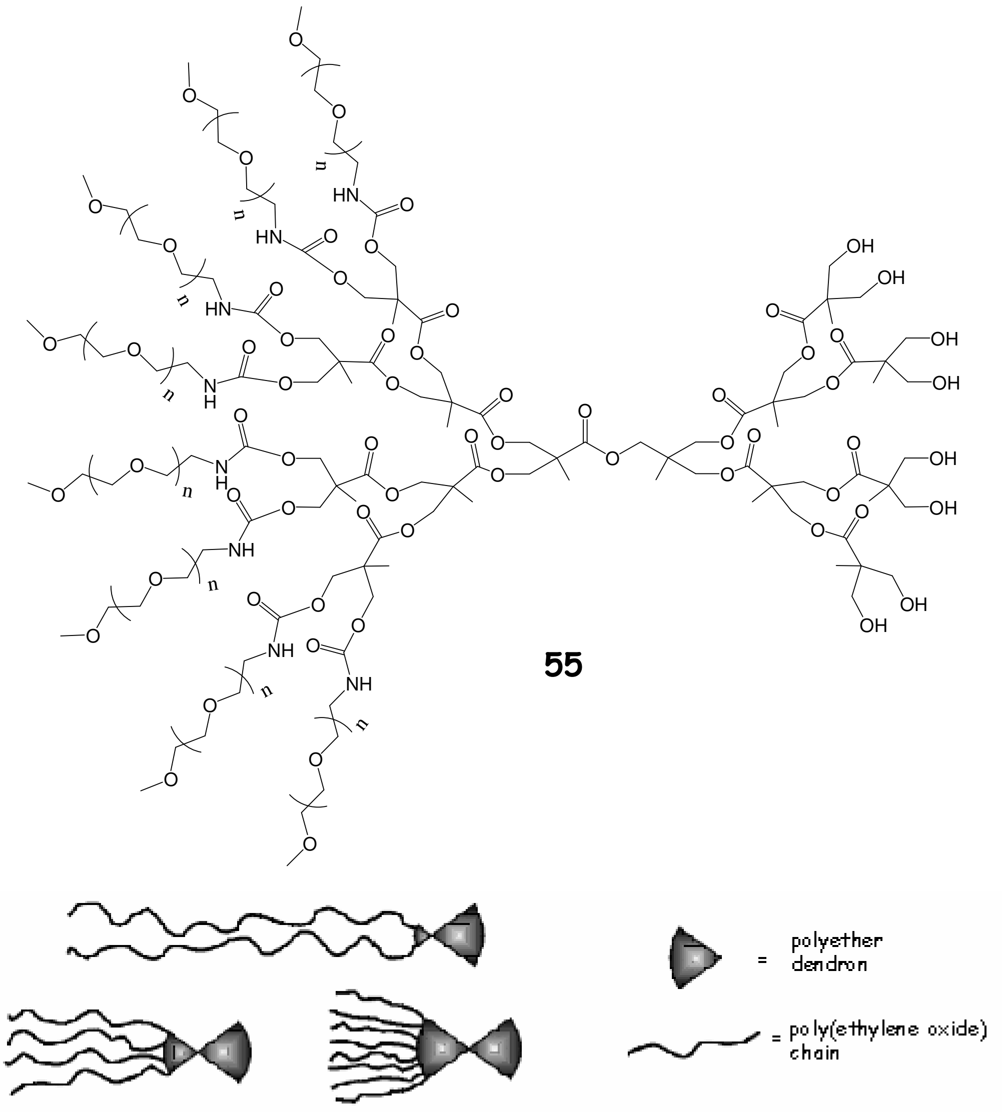


Figure 2.13. Fréchet's bow-tie hybrids

other did not. They attached different numbers of poly (ethylene glycol) chains in different lengths to the unprotected hemisphere. Then the protected hemisphere was deprotected and modified for different purposes.

An efficient synthesis of 'desymmetrized' polyphenylene dendrimer was reported by Müllen et al. [26]. They used Diels-Alder reaction to build up the block dendrimer. First tetraphenylcyclopentadienones **56** carrying various functional groups (A) were synthesized and were treated with tetrakis(4-ethynylphenyl)methane **57** to obtain mono-functionalized core **58**. Next step was coupling excess amount of tetraphenylcyclopentadienones **59** carrying new different functional groups (B) with preformed mono-functionalized cores **58** (Figure 2.14). This yielded the first generation surface-block dendrimer **60**. The dendrimers, in which (B) group is TMS protected triple bond, were deprotected and again treated with different tetraphenylcyclopentadienones to obtain second generation dendrimer. It is claimed in the study that this type of rigid surface-block dendrimers can be used in tailoring new nanoamphiphiles.

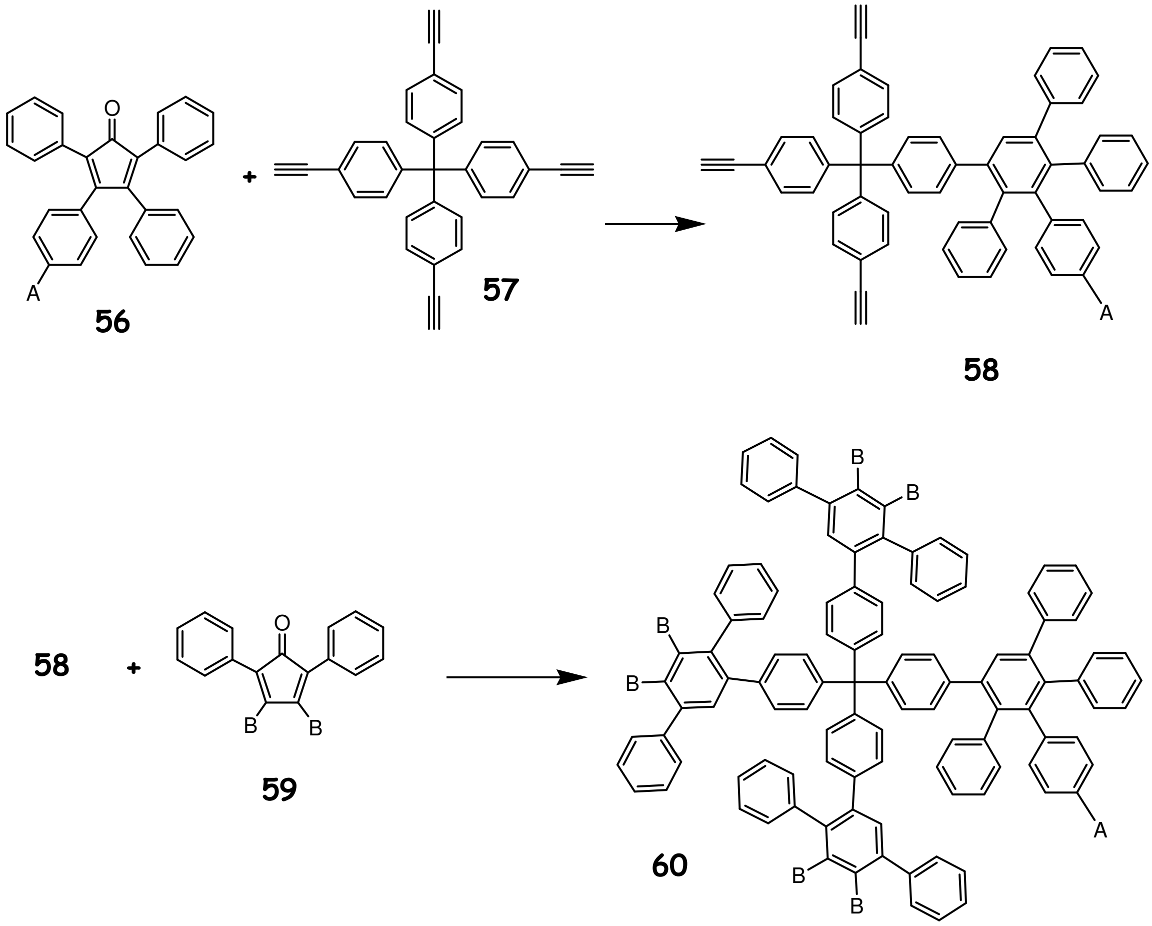


Figure 2.14. ‘Desymmetrized’ dendrimers

Phosphorus, nitrogen and sulfur containing block-dendrimers synthesized Majoral and coworkers are very heterogeneous in terms of elements [27]. A second generation dendron **61** carrying a double bond at its focal point and phenyl rings at its periphery was the first block of their surface-block dendrimer. Michael-type addition of methylhydrazine to the focal point of this dendron was the start of growth of second block. Continuation of dendron synthesis by again divergent method up to fourth generation afforded the desired surface-block dendrimer **62** bearing -CHO groups as surface groups on the newly formed hemisphere. Their hybrid molecules were named as di-block dendrimers. (Figure 2.15)

Even though there are a number of examples in literature about the synthesis of various types of block dendrimers, there is still room for improved approaches as most synthesis is not convergent. This results in lover overall yields and poor control over purity. The perfect construction of a block dendrimer will compose of individual synthesis of the respective blocks and then coupling of the two dendrons with a high yielding reaction where conditions will not affect any potentially viable surface functionality. Such a coupling strategy could utilize ‘chemical ligation’ reaction and will be discussed in the next chapter.

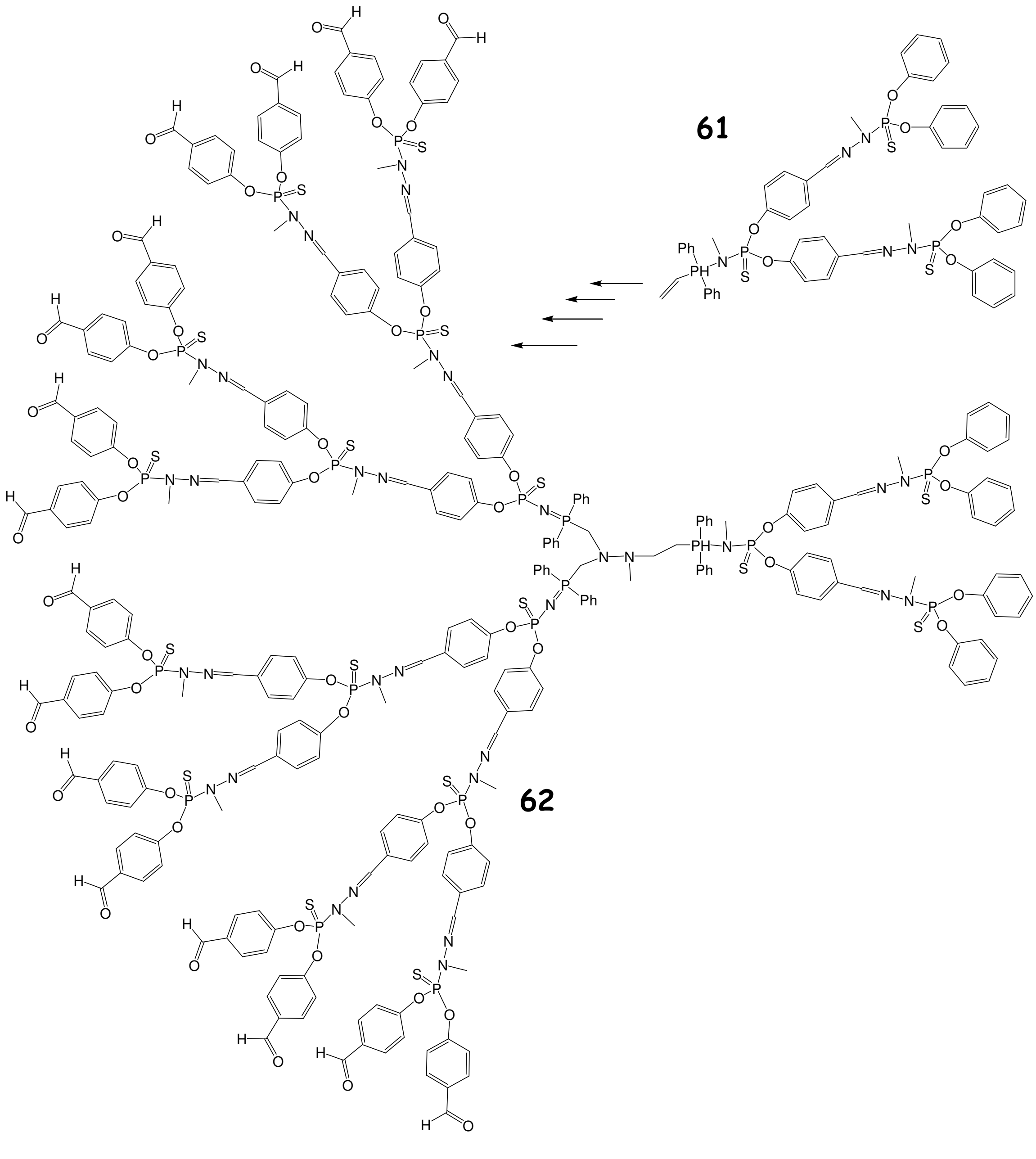
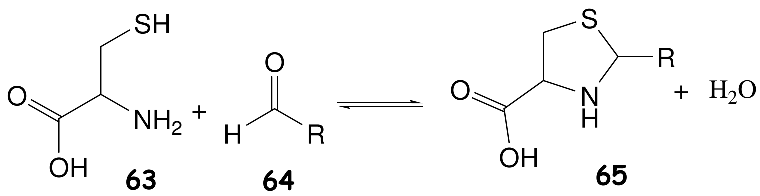


Figure 2.15. A phosphorus and sulfur containing surface-block dendrimer

3. THIAPROLINE LIGATION

The reaction between cysteine and formaldehyde is very well known for decades [28]. Cysteine **63** is one of the natural 20 essential amino acids and formaldehyde is the simplest acyl group carrying molecule. Other aldehydes **64** can also be coupled with cysteine (Figure 3.1). The product obtained **65** is a heterocylic ring called thiazolidine. Reaction works in aqueous media and does not need any other reagents.



63 **64** **65**

Figure 3.1. Reaction of cysteine with aldehydes

In 1994, Tam and coworkers utilized this reaction for peptide synthesis [29]. They first synthesized a peptide segment **66** and converted its C terminal to glycoaldehyde **67** (Figure 3.2). The next step was coupling of this first segment with an N-terminal cysteine peptide sequence **68**. The expected thiazolidine ring **69** formed. However, the reaction did not stop but continued to give a rearranged product because of the presence of electrophilic acyl group in the σ position of the nucleophilic nitrogen atom of the thiazolidine ring. The attack of this nucleophilic nitrogen to the acyl carbon formed an amide bond resulting in the new thiazolidine **70**. This strategy where the coupling step is followed by a rearrangement is termed as 'chemical ligation' by Tam. The revolutionary point of this study was the proposal of a new method for peptide coupling. Coupling of peptides to obtain longer peptide chains is a very important tool in organic chemistry as it allows convergent synthesis of long peptides. The selectivity of the coupling reaction is vital as formation of more than one product will yield inseparable mixtures. Therefore, the former reaction where any of the amine or thiol side chain bearing amino acid sequences can react with the aldehyde 64 is not a very efficient reaction. Requirement of a terminal cysteine and formation of an irreversible rearrangement product make this reaction unique.

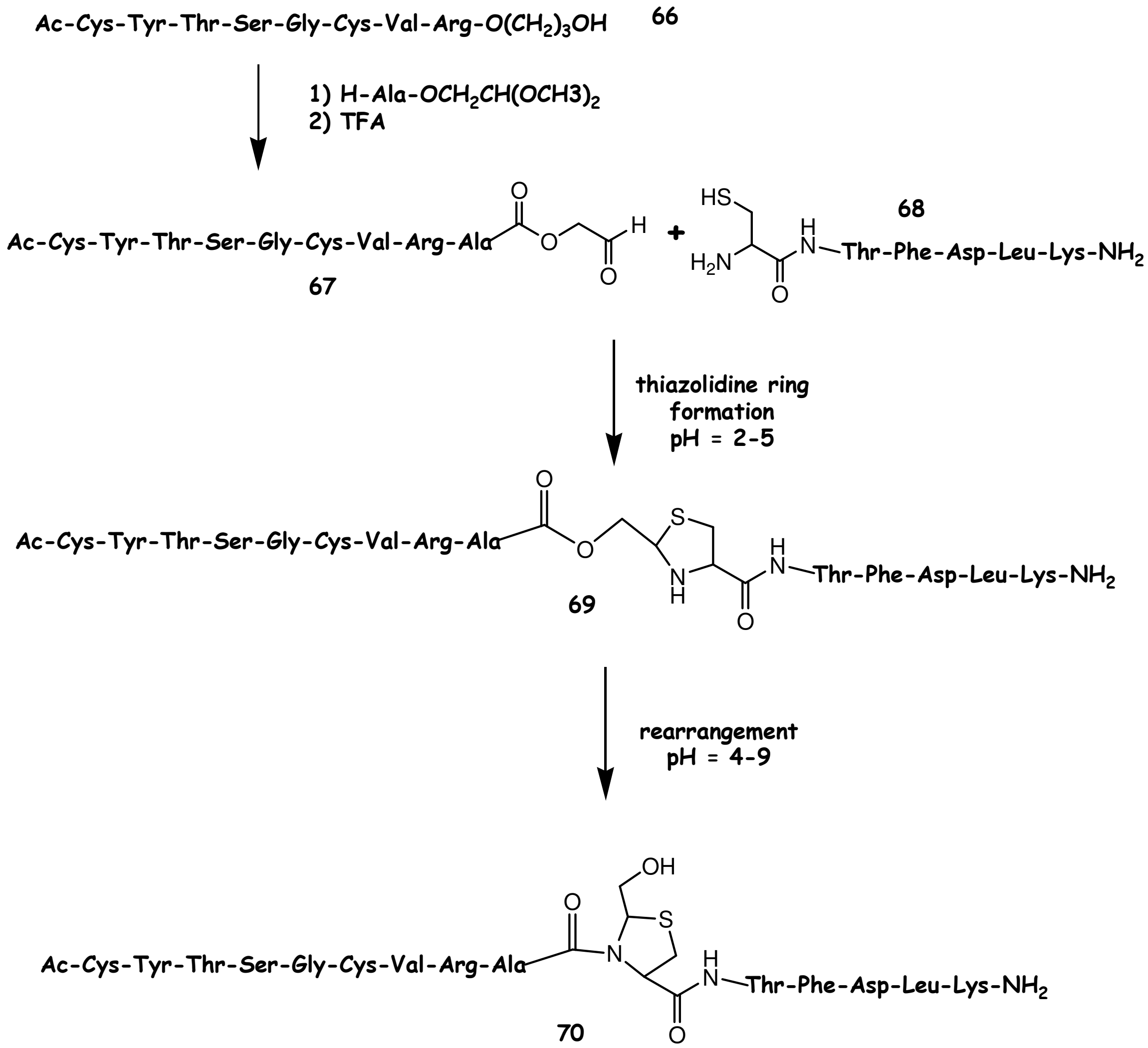


Figure 3.2. ‘Chemical ligation’ method

The mechanism of ‘chemical ligation’ (Figure 3.3) starts with formation of imine **74** between cysteine residue **72** and aldehyde **71** by dehydration reaction in two steps. Even though the sulfur atom is more nucleophilic than the nitrogen atom, the attack of sulfur to acyl carbon does not make any contribution to the reaction because of the irreversibility of the sulfur attack. After formation of the imine, thiazolidine ring **75** forms via attack of sulfur atom to the preformed double bond. As mentioned before, rearrangement follows the coupling because of the position of electrophilic acyl group to nucleophilic nitrogen atom. Attack of nitrogen atom to the acyl carbon yields the rearranged product **77** via a five membered ring transition state **76**.

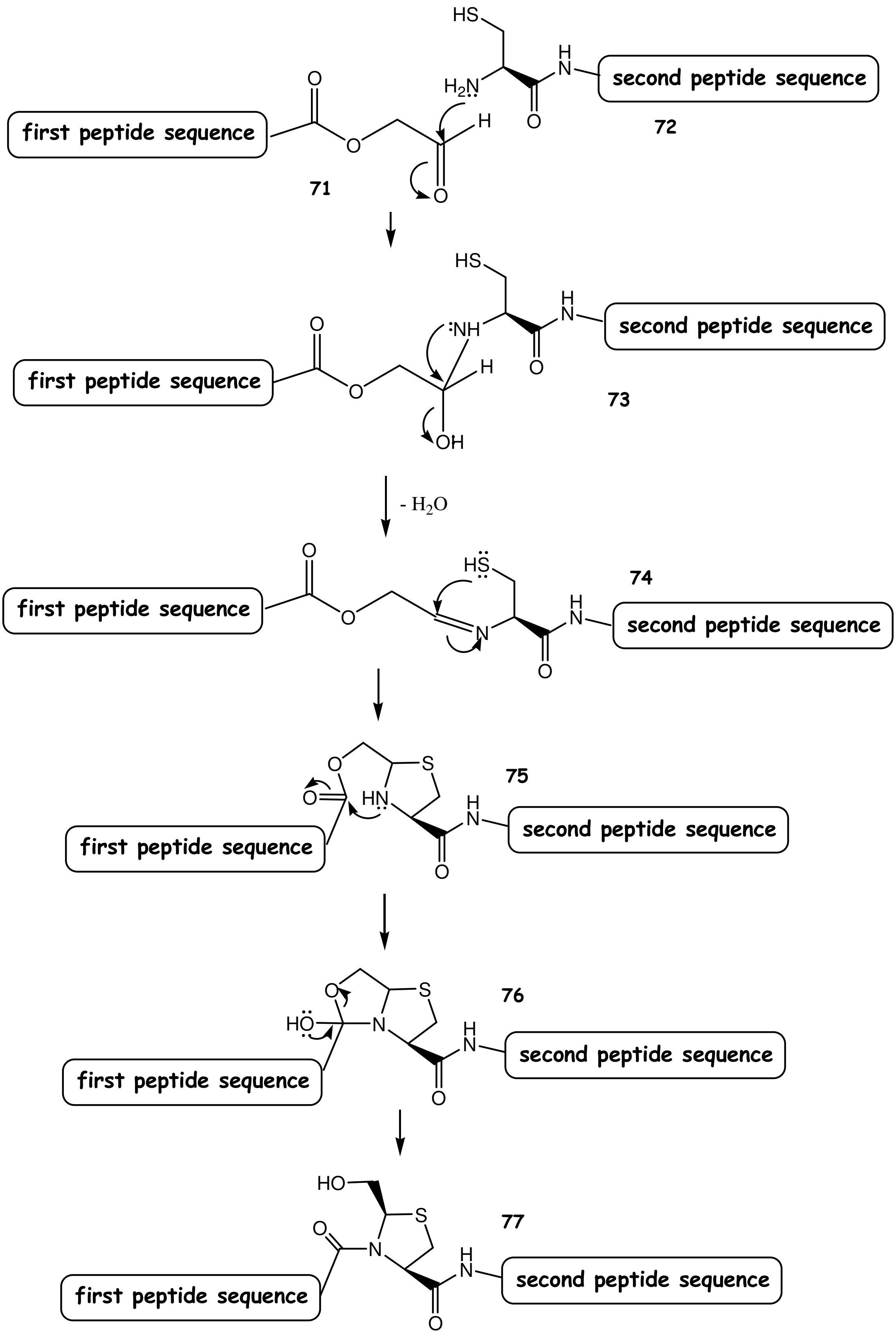


Figure 3.3. Mechanism of the ‘chemical ligation’ reaction

In this study, coupling is discovered to be the fast step. At pH = 5, coupling goes to completion in less than 15 minutes. Lower the pH, slower the rate of coupling. On the other hand, rearrangement is very slow compared to coupling and depends on steric hinderence of the segments and pH of the media. To prevent the slowing effect of steric hinderence of the C component on coupling, Glycin and Alanin were preferred. It has been found that, more hindered amino acids such as Valine decreases the rate of rearrangement by more than 10 fold. The effect of pH on rearrangement is similar to the coupling step. With an increase of pH from 6 to 9, the rate of rearrangement increased nearly 10 fold.

Two years later, Tam and coworkers [30] published another report about same ligation method for the synthesis of HIV-1 protease analogs (Figure 3.4). In this study, they ligated different peptide segments. The difference in this study was the usage of cosolvents. As can be seen from the mechanism, the by product of the ring formation step is water thus the decrease in water concentration should speed up this step. They found that, water miscible solvents (i.e. acetonitrile) accelerated the ring formation step by 6 fold. Yield of the ring formation step was varying from 60% to 80% and for the rearrangement step it was found to be > 90% after four days.

In 1999, Tam and coworkers [31] published a review article about peptide ligation strategies. In this review, the same ligation method mentioned above was named 'thiaproline ligation' method this time because of the resemblance of the ligation product of the cysteine and aldehyde to the proline. Proline is another member of the natural essential amino acid family. The prefix 'thia' represents the sulfur atom in the thiazolidine ring. The comparison of a proline **82** residue in a peptide backbone and thiazolidine ring **83** can be seen in Figure 3.5.

Later Tam and coworkers found that 'thiaproline ligation' also worked in nonaqueous media such as pyridine-acetic acid mixtures [32]. Optimal reaction conditions for this particular example was 10 hours at pH = 5,3 for the ring formation and 20 hours at pH = 6,6 for the rearrangement to afford the rearrenged product in 92,5 % yield (Figure 3.6). Rearrengement was confirmed by FT-IR spectroscopy by showing absence of ester peaks in 1710-1780 cm^{-1} since the ester group formed in the ring formation step disappears after rearrangement.

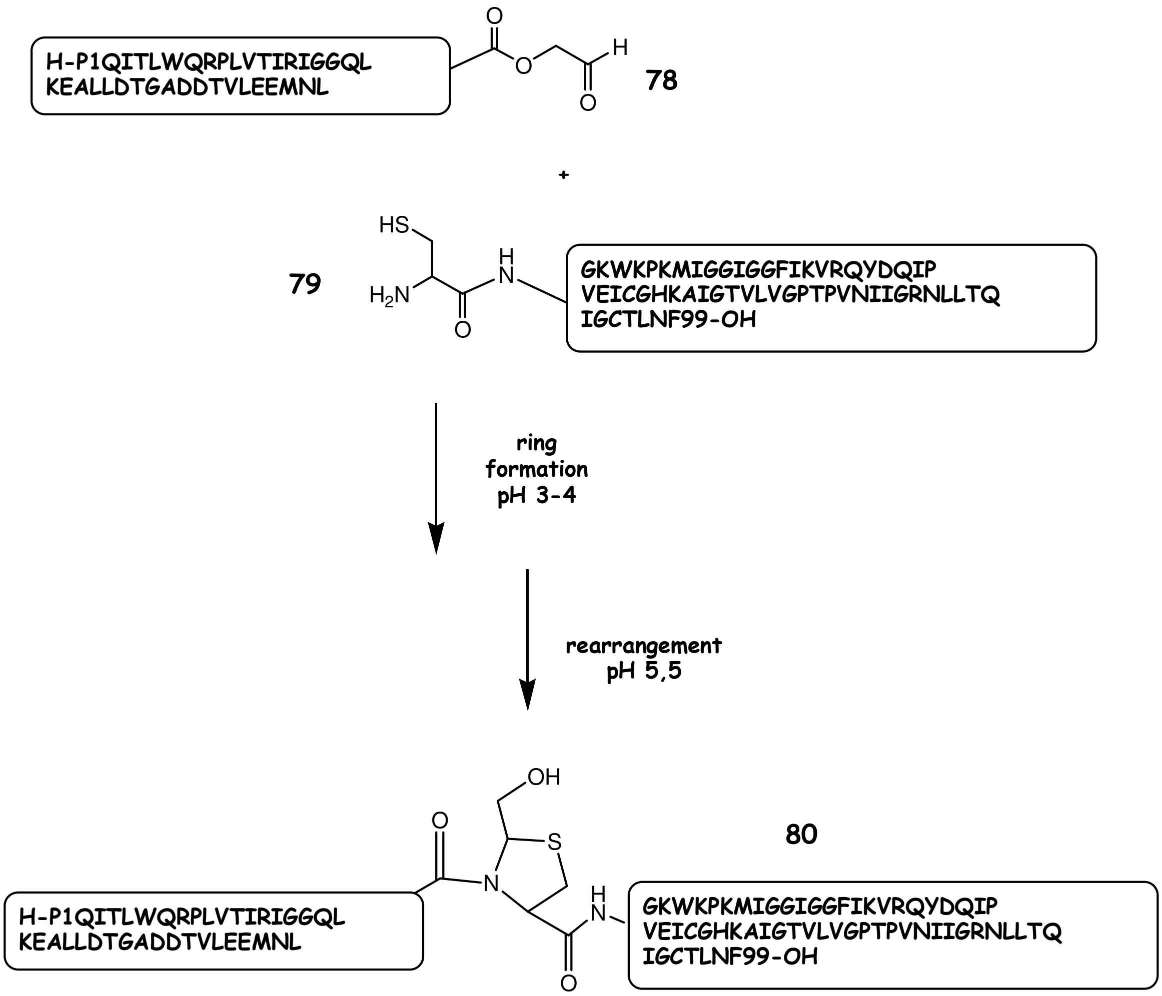
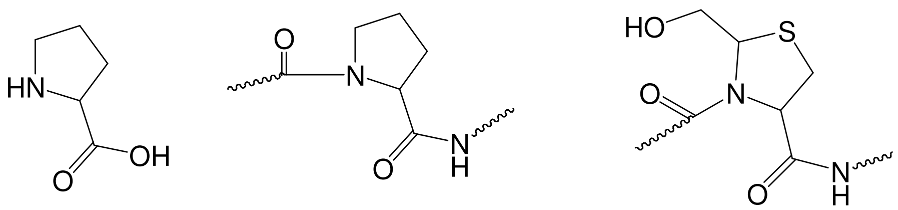


Figure 3.4. Recombinant HIV-1 protease prepared via ‘chemical ligation’ method



81 proline — **82 proline residue on a peptide backbone** — **83 thiazolidine structure (thiaproline)**

Figure 3.5. Comparison of the structures of proline residue and thiazolidine ring

‘Thiaproline ligation’ is a useful tool to couple large, multifunctional molecules. In our work, where we will be synthesizing dendrimers bearing various surface functionalities including biological molecules, this methodology seems to be promising to combine two dendrons. The requirement for this ligation method is the availability of cysteine

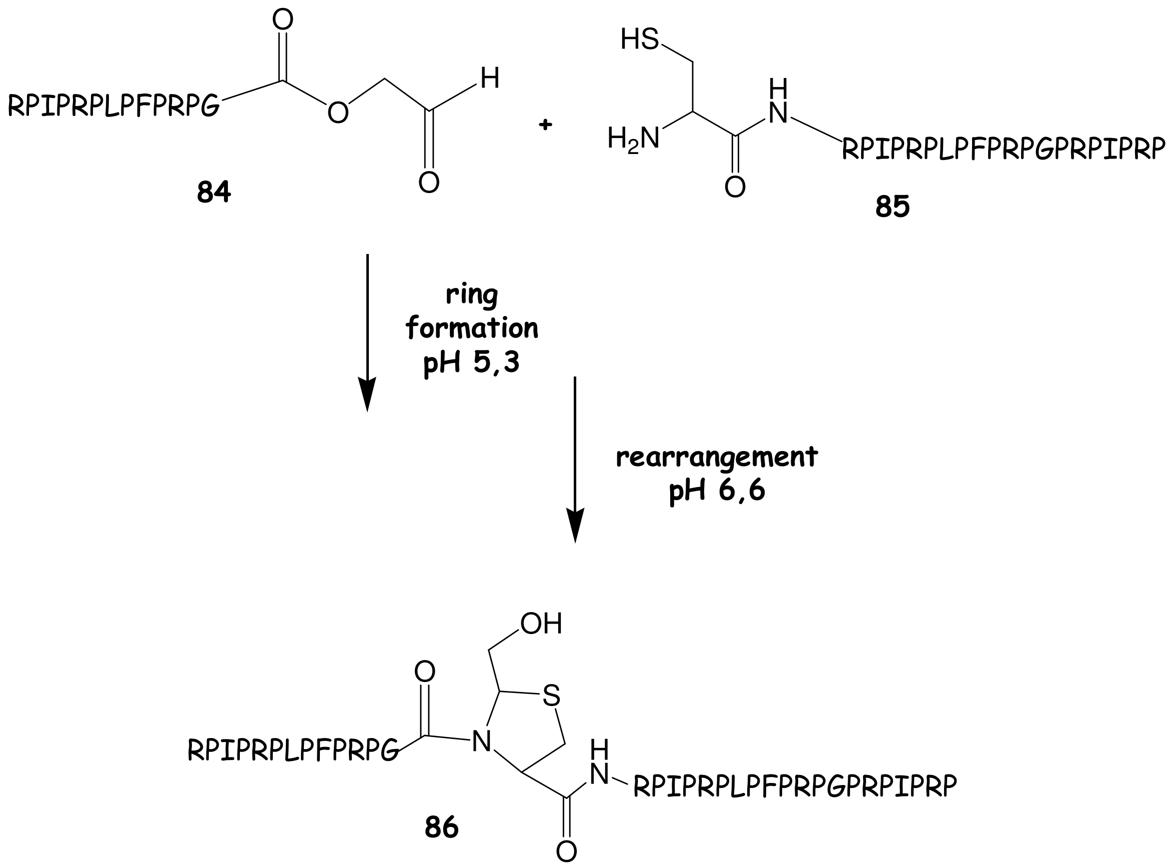


Figure 3.6. The peptide synthesized via 'thiaproline ligation' in nonaqueous media

functionality and the other one with an aldehyde. The utilization of this methodology for the synthesis of segment-block dendrimers will be discussed in the next chapter.

4. RESULTS AND DISCUSSION

4.1. General Methods and Materials

^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury-Mx operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, using deuterated chloroform as solvent in the Advanced Technologies Research and Development Center at Boğaziçi University. Infrared spectra were recorded on KBr plate on a Perkin-Elmer 1600 FT-IR Spectrophotometer. Flash chromatography was performed using silicagel-60 (43-60 nm). Thin layer chromatography was performed using silica gel plates (Kiesel gel 60 F_{254}, 0.2 mm thick, Merck). Plates were either viewed under an ultra violet lamp at 254 nm or immersed in a $KMnO_4$ solution and treated with heat for non-UV active compounds.

Chemicals were used as received (Merck, Aldrich, Lancaster, Alfa Aesar, Avocado). Solvents, CH_3OH, CH_2Cl_2, $CHCl_3$ and toluene (Merck, Riedel de Haen), were used as received. THF (Riedel de Haen) was distilled over sodium/benzophenone prior to use. CH_3CN and DMF (Riedel de Haen) were distilled over molecular sieves (4 A^0). Solvents used for purification, hexane, ethylacetate, CH_3OH and CH_2Cl_2 were distilled prior to use (Akkimya).

4.2. Synthesis of Dendrons

4.2.1. Poly(amido amine) Dendron (tBoc-PAMAM)

As explained in chapter 1, PAMAM is the first fully characterized and first commercialized dendrimer family. It is water soluble, biocompatible for low generations, easy to synthesize and cheap in terms of raw materials. Due to these properties, there are a large number of investigations regarding PAMAM in literature especially for drug delivery applications. It is synthesized by divergent method. Building blocks of PAMAM are methyl acrylate and ethylene diamine. The PAMAM dendrons reported here were synthesized by adaptation of procedures used for PAMAM dendrimer synthesis [1].

In our study, the ethylene diamine core is mono protected with tert-butyloxycarbonyl (tBoc) group. Construction of the dendron starts with the treatment of ethylene diamine **88** with ditertbutyldicarbonate **87** yielding the mono tBoc-protected ethylene diamine **89** which will become the focal point of the tBoc-PAMAM dendron (Figure 4.1).The next step is addition of two molecules of methylacrylate **90** to the mono tBoc-protected ethylene diamine **89** via Michael addition reaction to yield the tBoc-PAMAM G0.5 **91**. tBoc-PAMAM G0.5 **91** is then converted to tBoc-PAMAM G1 **92** via amidation with two molecules of ethylene diamine (**88**). Michael addition of four molecules of methylacrylate (**90**) to tBoc-PAMAM G1 **92** yields the tBoc-PAMAM G1.5 **93**. The next step is the amidation of the tBoc-PAMAM G1.5 **93** with four molecules of ethylene diamine (**88**) to yield the tBoc-PAMAM G2 **94** (Figure 4.2). The tBoc-PAMAM G2.5 **95** is obtained via Michael addition of eight molecules of methylacrylate (**90**) to the tBoc-PAMAM G2 **94**. The next step is conversion of the tBoc-PAMAM G2.5 **95** to the tBoc-PAMAM G3 **96** via amidation with eight molecules of ethylene diamine (**88**). Synthesis is terminated with production of the tBoc-PAMAM G3.5 **97** from the tBoc-PAMAM G3 **96** via Michael addition of sixteen molecules of methylacrylate (**90**).

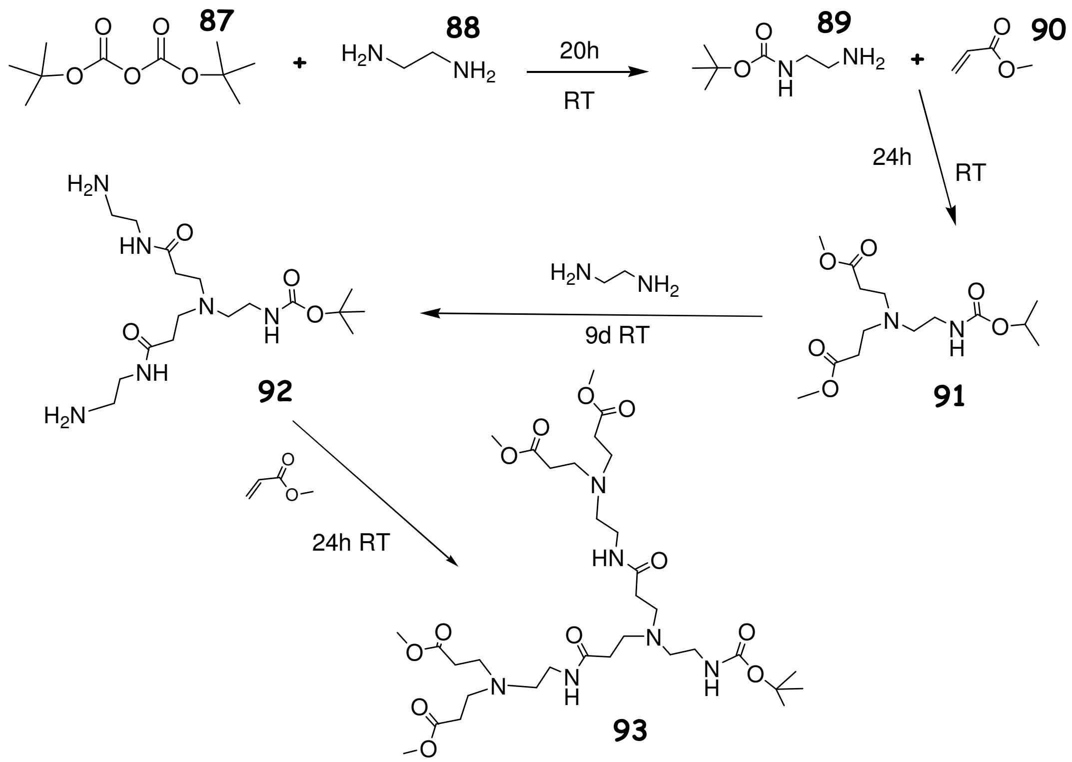


Figure 4.1. tBoc-PAMAM dendron synthesis: Low generations

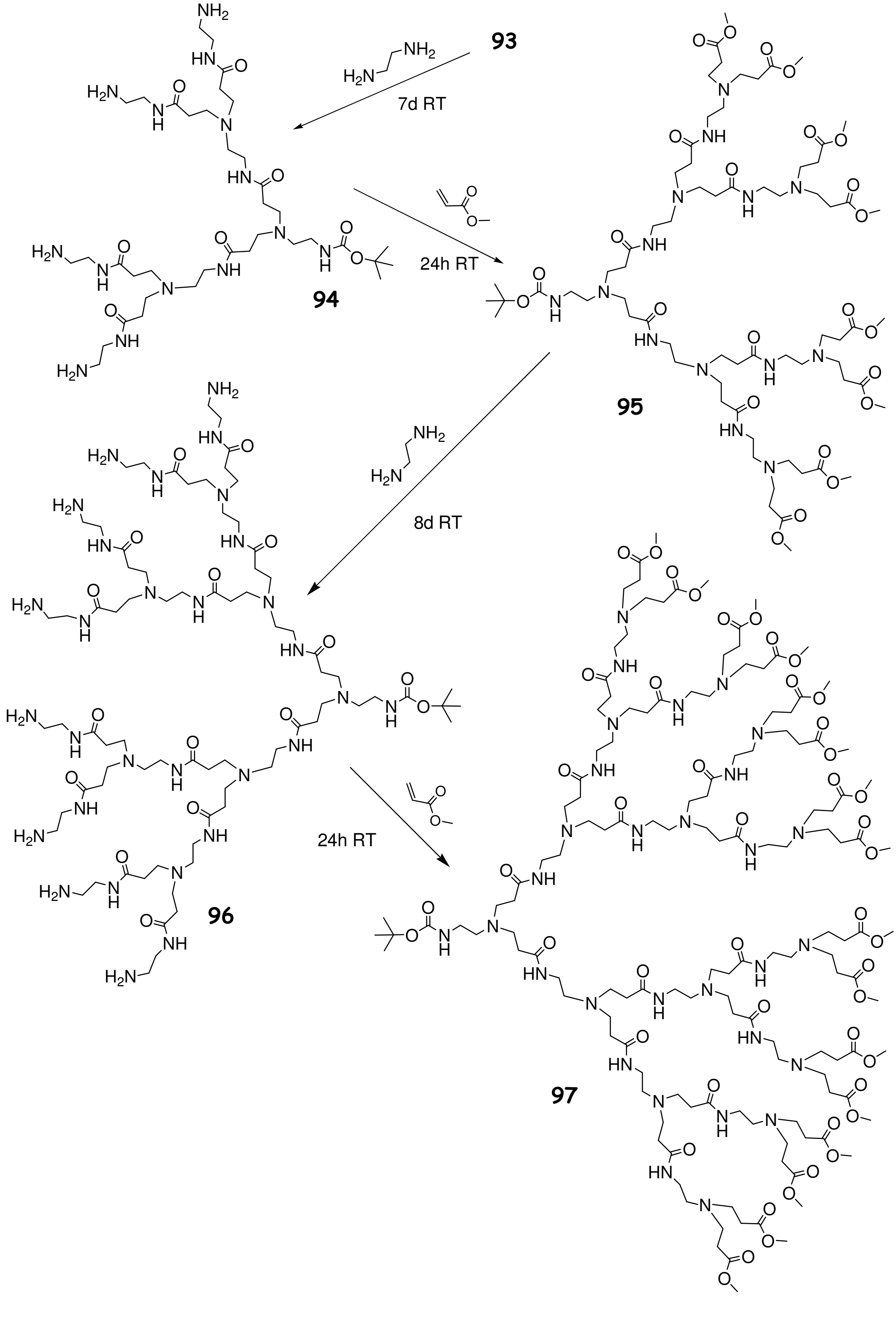
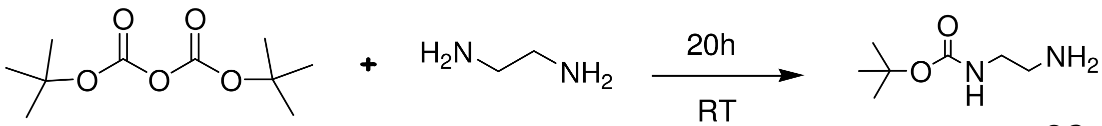


Figure 4.2. tBoc-PAMAM dendron synthesis: Higher generations

As can be seen from the procedure, Michael addition of methylacrylate and amidation with ethylene diamine are employed alternately for the construction of PAMAM dendron. Utilization of one of each reaction corresponds to an increase of one generation. Amine ended dendrons are integer generations and ester ended ones are half generations. Synthesis steps will be explained in detail below.

4.2.1.1. Synthesis of Mono tBoc-Protected Ethylene Diamine. To a stirred solution of ethylene diamine (**88**, 5.33 mL, 80.0 mmol) in $CHCl_3$ (75 mL) was added a solution of ditertbutyldicarbonate **87** (1.65 g, 7.6 mmol) in $CHCl_3$ (40 mL) over 4 h at 0 ^{0}C and the reaction mixture was stirred for 16h at room temperature under N_2. Organic layer was washed first with brine (6 x 25 mL) then with water (1 x 25 mL). Organic layer was dried over Na_2SO_4 and the solvent was removed under *vacuo* to obtain tBoc-protected ethylene diamine **89** as a pale yellow oil (0.7 g, 90 % yield). ^{1}HNMR (400 MHz, $CDCl_3$) δ 4.87 (bs, 1H), 3.18 (td, 2H, *J* = 6.0 Hz), 2.81 (t, 2H, *J* = 6.0 Hz), 1.44 (s, 9H) corresponding to the literature values [33] (Figure A.1).



87 **88** **89**

Figure 4.3. Synthesis of mono tBoc-protected ethylene diamine

4.2.1.2. Synthesis of tBoc-PAMAM G0.5. To a stirred solution of methyl acrylate (**90**, 5.5 mL, 61.1 mmol) in CH_3OH (5.5 mL) was added a solution of tBoc-protected ethylene diamine **89** (3.93 g, 24,5 mmol) in CH_3OH (75 mL) over 2 h at 0 ^{0}C, stirred for an additional 30min at 0 ^{0}C and then warmed up to room temperature for 24 h under N_2. All volatiles were removed under *vacuo* at 40 ^{0}C for 1 h and then the crude was left under high *vacuo* at room temperature for 12 h to obtain tBoc-PAMAM G0.5 **91** as a yellow-orange oil (7.0 g, 86 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (bs, 1H), 3.68 (s, 6H), 3.16 (bs, 2H), 2.74 (t, 4H, *J* = 6.4 Hz), 2.51 (bs, 2H), 2.42 (t, 4H, *J* = 6.4 Hz), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 155.7, 78.3, 52.7, 51.2, 48.8, 37.7, 32.3, 28.1. IR: υ = 3400, 1738, 1708 cm^{-1} (Figure A.2, A.3, A.4).

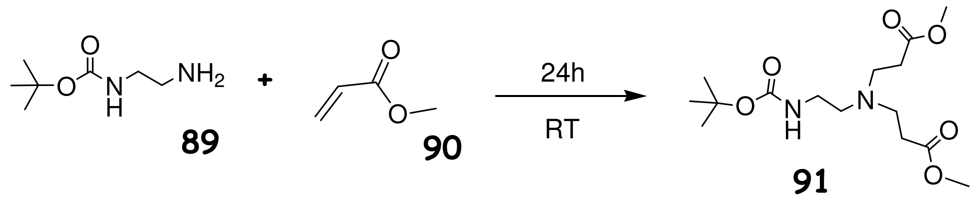


Figure 4.4. Synthesis of tBoc-PAMAM G0.5

<u>4.2.1.3. Synthesis of tBoc-PAMAM G1.</u> To a stirred solution of ethylene diamine (**88**, 12.3 mL, 183.6 mmol) in CH_3OH (15 mL) was added a solution of tBoc-PAMAM G0.5 **91** (2.3 g, 7.3 mmol) in CH_3OH (6 mL) over 1 h at 0 ^{0}C and then warmed up to room temperature for 9 d under N_2. Solvent was removed under *vacuo* at 38 ^{0}C. Toluene-methanol (9:1) mixture was added (5x30 mL) and volatiles were removed under *vacuo* at 38 ^{0}C and then methanol was added (5x20mL) and volatiles were removed under *vacuo* at 38 ^{0}C. Then crude was left under high *vacuo* at 42 ^{0}C for 40 h to obtain tBoc-PAMAM G1 **92** as a yellow oil (2.83 g, 99 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (bs, 2H), 6.41 (bs, 1H), 3.31-3.29 (m, 4H), 3.17-3.16 (m, 2H), 2.86 (t, 4H, *J* = 5.2 Hz), 2.72 (t, 4H, *J* = 5.6 Hz) 2.52 (bs, 2H), 2.34 (t, 4H, *J* = 6.4), 1.44 (s, 9H). IR: υ = 3285, 1700, 1646 cm^{-1} (Figure A.5, A.6).

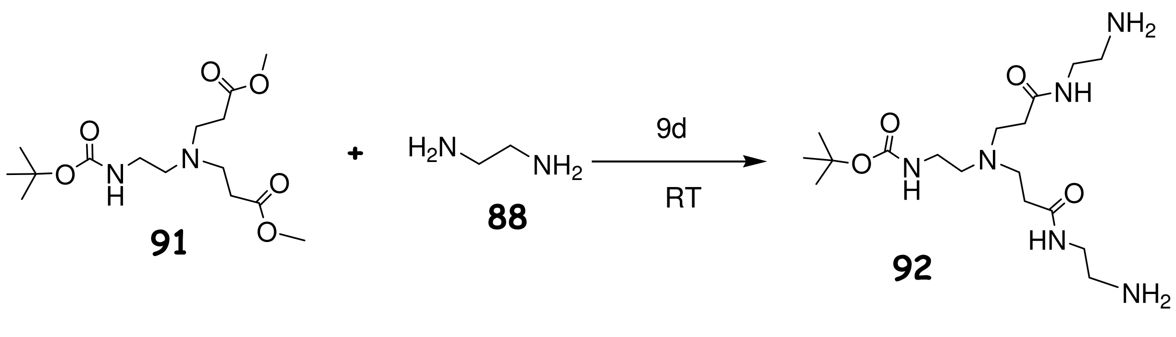


Figure 4.5. Synthesis of tBoc-PAMAM G1

<u>4.2.1.4. Synthesis of tBoc-PAMAM G1.5.</u> To a stirred solution of methyl acrylate (**90**, 3.1 mL, 34.2 mmol) in CH_3OH (5 mL) was added a solution of tBoc-PAMAM G1 **92** (2.66 g, 6.8 mmol) in CH_3OH (9 mL) over 2 h at 0 ^{0}C, kept at 0 ^{0}C for 1 h and then warmed up to room temperature for 24 h under N_2. All volatiles were removed under *vacuo* at 40 ^{0}C for 1 h. The crude was left under high *vacuo* at room temperature 12 h to obtain tBoc-PAMAM

G1.5 **93** as a yellow-orange oil (4.60 g, 92 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (bs, 2H), 5.47 (bs, 1H), 3.65 (s, 12H), 3.29-3.28 (m, 4H), 3.15 (bm, 2H), 2.73 (t, 12H, *J* = 6.8 Hz), 2.52 (t, 6H, *J* = 4.8 Hz), 2.41 (t, 8H, *J* = 6.4 Hz), 2.33 (t, 4H, *J* = 6.4 Hz), 1.40 (s, 9H). IR: υ = 3338, 1731, 1654 cm^{-1} corresponding to the literature values [34] (Figure A.7, A.8).

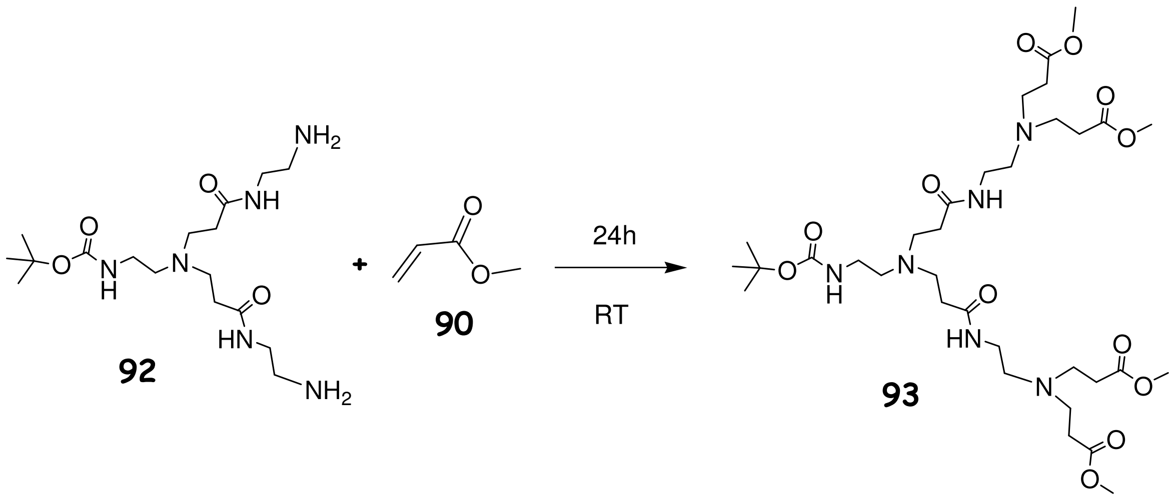


Figure 4.6. Synthesis of tBoc-PAMAM G1.5

<u>4.2.1.5. Synthesis of tBoc-PAMAM G2.</u> To a stirred solution of ethylene diamine (**88**, 67.6 mL, 1014 mmol) in CH_3OH (104 mL), was added a solution of tBoc-PAMAM G1.5

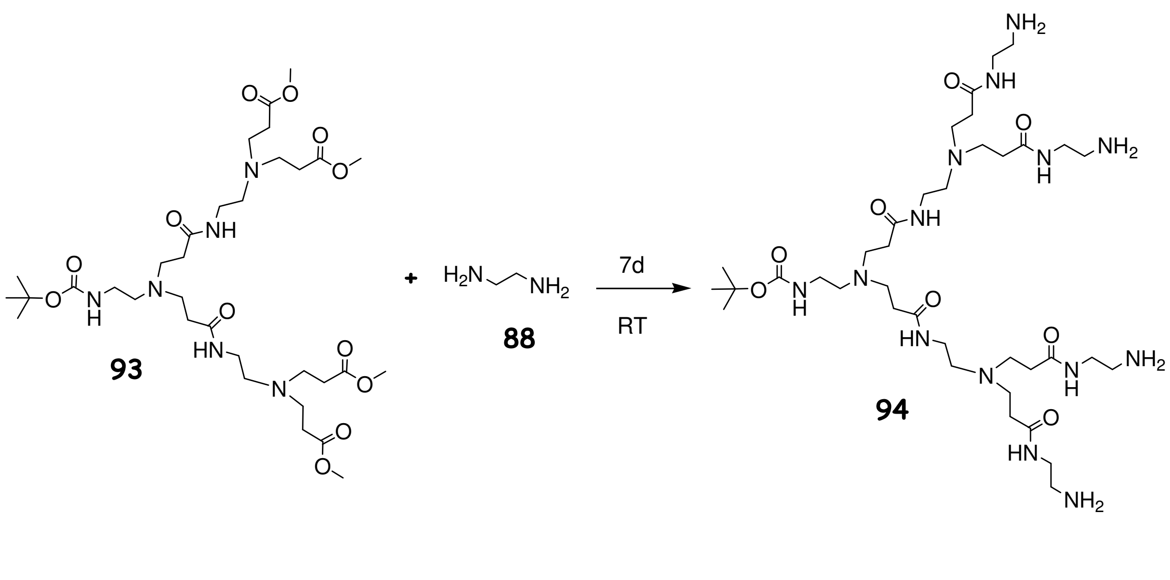


Figure 4.7. Synthesis of tBoc-PAMAM G2

93 (3.86 g, 5.3 mmol) in CH_3OH (39 mL) over 1 h at 0 ^{0}C and then warmed up to room temperature for 7 d under N_2. Solvent was removed under *vacuo* at 38 ^{0}C. Toluene-methanol (9:1) mixture was added (5x30 mL) and volatiles were removed under *vacuo* at 38 ^{0}C and then methanol was added (5x20mL) and volatiles were removed under *vacuo* at 38 ^{0}C. Then crude was left under high *vacuo* at 42 ^{0}C for 40 h to obtain tBoc-PAMAM G2 **94** as an orange viscous oil (4.66 g, 95 % yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (bs, 2H), 7.72 (bs, 1H), 7.60 (s, 2H), 3.28-3.12 (m, 14H), 2.80 (t, 8H, J = 5.2 Hz), 2.70 (t, 12H, J = 6.8 Hz), 2.50 (bt, 6H), 2.34-2.29 (m, 12H), 1.40 (s, 9H). IR: υ = 3277, 1638, 1546 cm^{-1} (Figure A.9, A.10).

<u>4.2.1.6. Synthesis of tBoc-PAMAM G2.5.</u> To a stirred solution of methyl acrylate (**90,** 4.35 mL, 48.3 mmol) in CH_3OH (10.5 mL), was added a solution of tBoc-PAMAM G2 **94** (4.08 g, 4.83 mmol) in CH_3OH (20 mL) over 2 h at 0 ^{0}C, kept at 0 ^{0}C for additional 30 min and then warmed up to room temperaturE for 24 h under N_2. All volatiles were removed under *vacuo* at 40 ^{0}C for 1h. The crude was left under high vacuo at room temperature for48 h to obtain tBoc-PAMAM G2.5 **95** as an orange-brown viscous oil (4.65 g, 96 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (bs, 4H), 3.65 (s, 24H), 3.27 (bm, 12H), 3.15

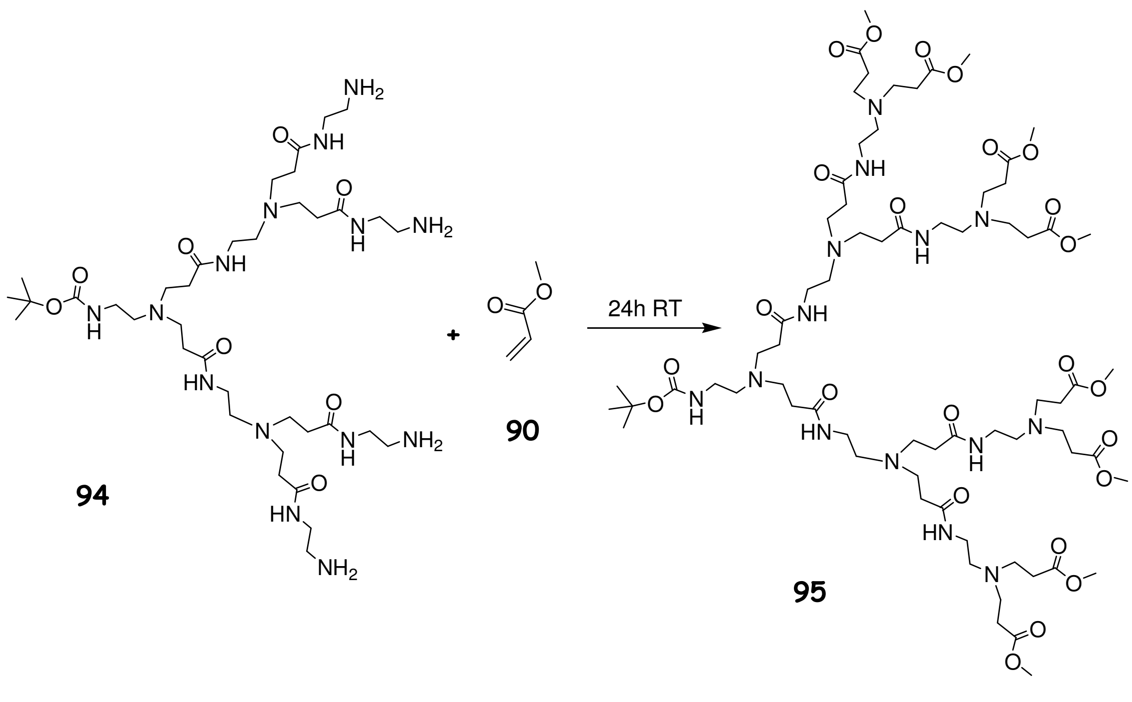


Figure 4.8. Synthesis of tBoc-PAMAM G2.5

(bm, 2H), 2.79-2.71 (m, 28H), 2.59-2.52 (m, 14H), 2.43-2.35 (m, 28H), 1.40 (s, 9H). IR: υ = 3285, 1731, 1646 cm^{-1} (Figure A.11, A.12).

<u>4.2.1.7. Synthesis of tBoc-PAMAM G3.</u> To a stirred solution of ethylene diamine (**88**, 27.3 mL, 409.5 mmol) in CH_3OH (35 mL) was added a solution of tBoc-PAMAM G2.5 **95** (3.2 g, 1.8 mmol) in CH_3OH (14 mL) over 1 h at 0 ^{0}C and then warmed up to room temperature for 8 d under N_2. Solvent was removed under *vacuo* at 38 ^{0}C. Toluene-methanol (9:1) mixture was added (4x50 mL + 3x35 mL) and volatiles were removed under *vacuo* at 38 ^{0}C and then methanol was added (1x60 mL + 5x30 mL) and volatiles were removed under *vacuo* at 38 ^{0}C. Then crude was left under high *vacuo* at 42 ^{0}C for 40h to obtain tBoc-PAMAM G3 **96** as an orange highly viscous oil (3.80 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (bs, 6H), 7.71 (bs, 6H), 7.50 (bs, 2H), 3.25-3.14 (m, 30H), 2.80-2.70 (m, 44H), 2.50 (m, 14H), 2.34 (m, 28H), 1.40 (s, 9H). IR: υ = 3277, 1638, 1546 cm^{-1} corresponding to the literature values [34] (Figure A.13, A.14).

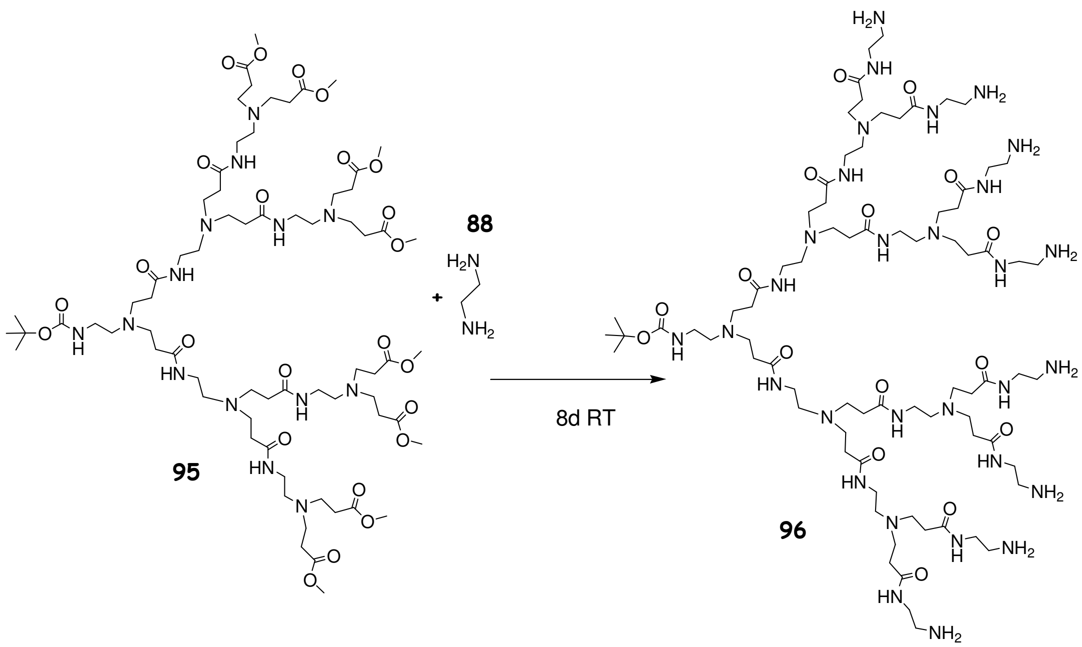


Figure 4.9. Synthesis of tBoc-PAMAM G3

<u>4.2.1.8. Synthesis of tBoc-PAMAM G3.5.</u> To a stirred solution of methyl acrylate (**90**, 3.3 mL, 36.4 mmol) in CH_3OH (6 mL) was added a solution of tBoc-PAMAM G3 **96** (3.20 g,

1.82 mmol) in CH_3OH (12 mL) over 2 h at 0 ^{0}C, kept at 0 ^{0}C for additional 1 h and then warmed up to room temperature for 24 h under N_2. All volatiles were removed under *vacuo* at 40 ^{0}C for 1h. The crude was left under high *vacuo* at room temperature for 48 h to obtain tBoc-PAMAM G3.5 **97** as a orange-brown viscous oil (5.94 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (bs, 3H), 7.68 (bs, 32H), 7.10 (bs, 6H), 3.65 (s, 48H), 3.26-3.13 (m, 30H), 2.76-2.71 (m, 60H), 2.53-2.52 (m, 30H), 2.43-2.34 (m, 60H), 1.39 (s, 9H). IR: υ = 3300, 1738, 1646 cm^{-1} (Figure A.15, A.16).

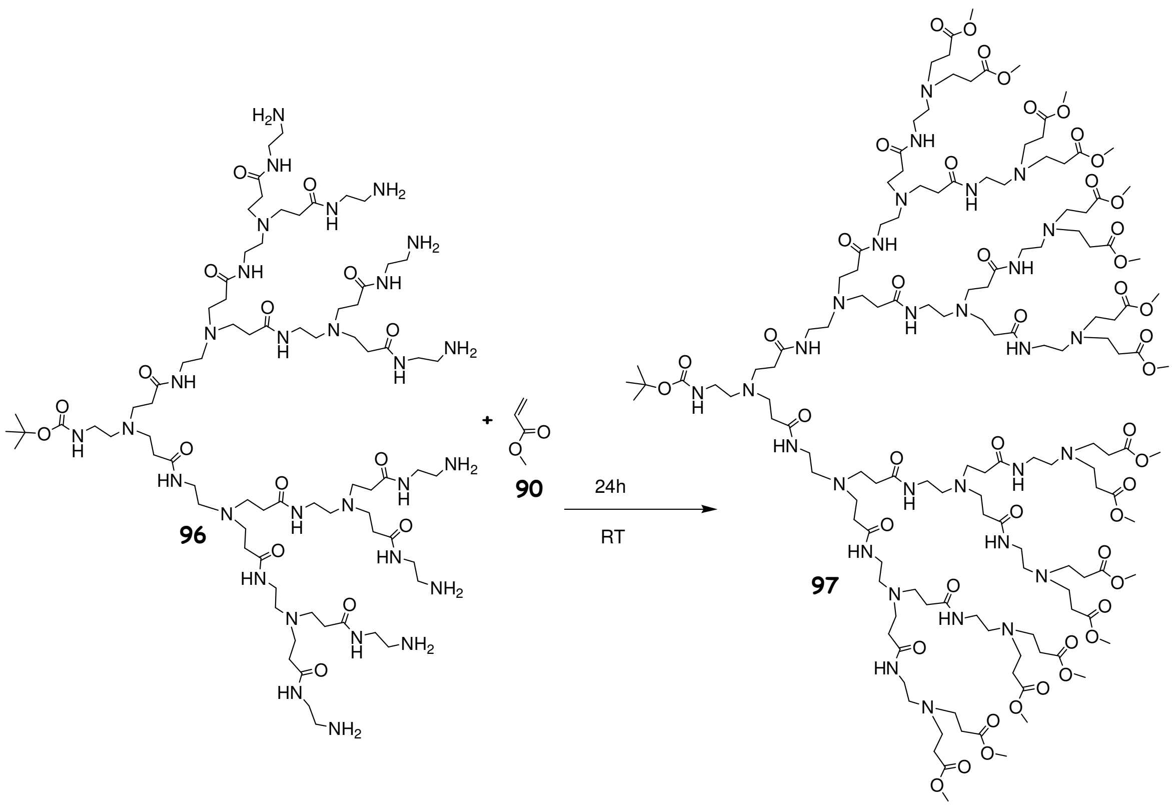


Figure 4.10. Synthesis of tBoc-PAMAM G3.5

4.2.2. Polyether Dendron

Polyether dendron was chosen as a model fragment since it is a water soluble, dendron synthesized using convergent method. The synthesis procedure was adopted from a report by Lee et al. who synthesized a similar dendron based upon diethyleneglycol monomethylether [35]. Synthesis starts with the S_N2 reaction between an alcohol which will become surface groups of the dendron and 3-chloro-2-chloromethyl propene. Mono substituted product is always presents as by-product which after isolated was resubjected to alkylation conditions to obtain higher yields. The second step is the hydroboration of the alkene dendron with BH_3 to obtain a hydroxyl group at the focal point.

Briefly, the synthetic steps involve substitution of chlorine atoms of 3-chloro-2-chloromethyl propene **99** with diethyleneglycol monoethylether (**98**) to yield the ether G0.5 **100** (Figure 4.11). The primary alcohol functionalized ether G1 **101**, is obtained via

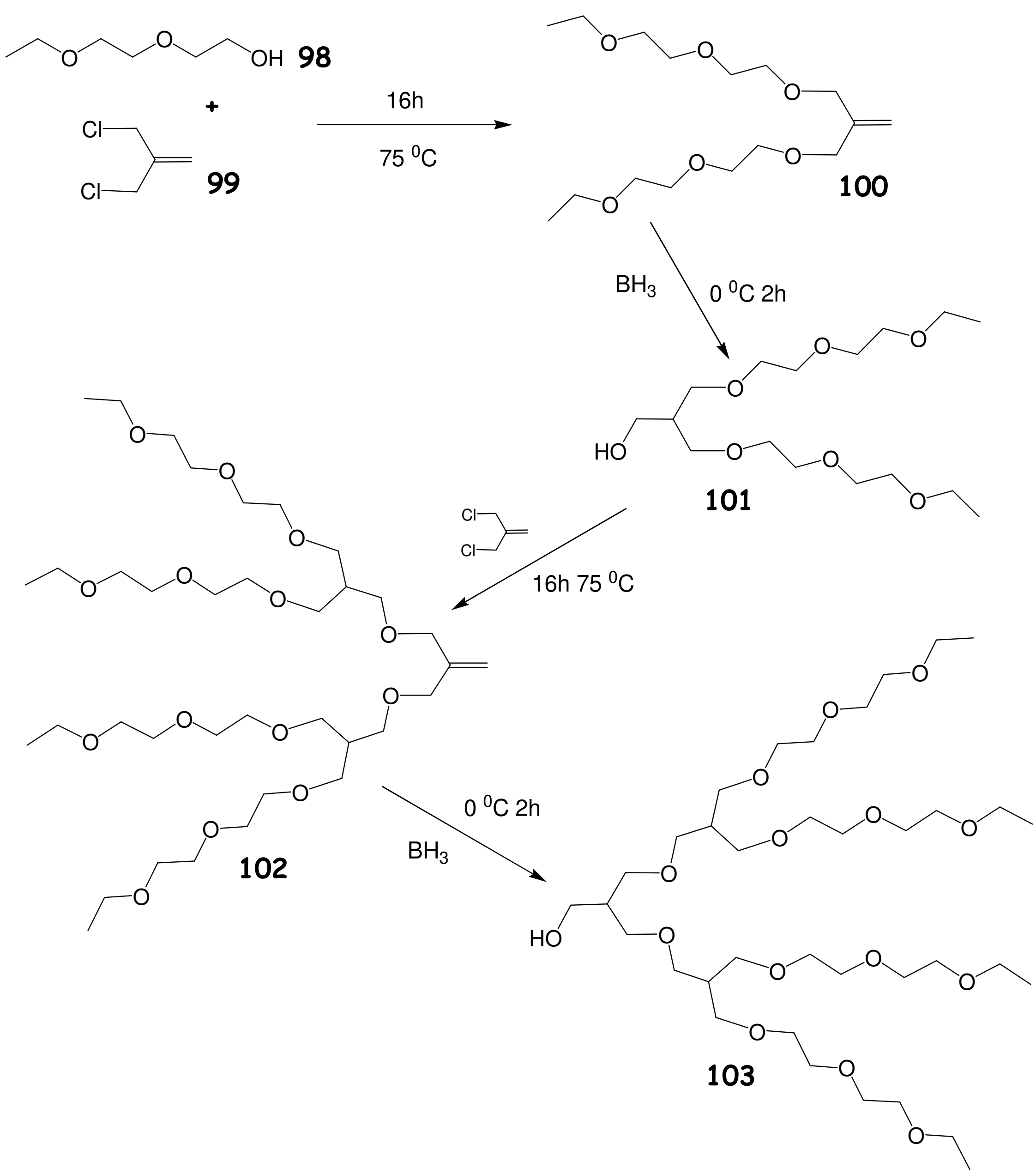


Figure 4.11. Ether dendron synthesis

hydroboration of the ether G0.5 **100** with BH_3. Thus obtained alcohol undergoes S_N2 reaction with 3-chloro-2-chloromethyl propene **99** to yield the ether G1.5 **102**. The next step is hydroboration of the ether G1.5 **102** to yield the ether G2 **103** as a primary alcohol.

As seen from the procedure, S_N2 reaction between an alcohol and 3-chloro-2-chloromethyl propene and hydroboration of obtained alkene with BH_3 to obtain a primary alcohol follow each other for the construction of ether dendron. Utilization of one of each corresponds to increase one generation. Alcohol functionalized dendrons are integer generations and double bond functionalized ones are half generations. Synthesis steps are explained in detail in subsequent sections.

4.2.2.1. Synthesis of Ether G0.5. Solid NaH (60 %, 3.0 g, 75 mmol) was washed with freshly distilled THF (10 mL) at room temperature under N_2 and dissolved in THF (25 mL). To this slurry was added 3-chloro-2-chloromethyl propene **99** (1.85 mL, 16.0 mmol) followed by slow addition of diethyleneglycol monoethylether **98** (8.5 g, 63.3 mmol) while stirring. The solution was stirred at 75 ^{0}C for 16 h. Distilled H_2O (2 mL) was added and all volatiles were removed under *vacuo*. H_2O (20 mL) was then added and the solution was extracted with CH_2Cl_2 (5x20mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (3:7) as eluent to obtain the ether G0.5 **100** as a colorless oil (10.0 g, 94 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (t, 2H, J = 0.8 Hz), 4.00 (dd, 4H), 3.65-3.56 (m, 16H), 3.51 (q, 4H, J = 6.8 Hz), 1.19 (t, 6H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 113.9, 71.7, 70.6, 70.4, 69.7, 69.4, 66.5, 15.0 (Figure A.17, A.18).

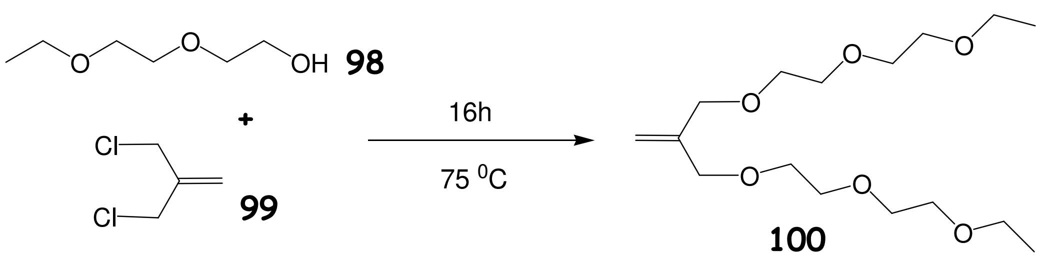


Figure 4.12. Synthesis of ether G0.5

4.2.2.2. Synthesis of Ether G1. To a stirred solution of ether G0.5 **100** (4.60 g, 14.4 mmol) in freshly distilled THF (7 mL), a solution of BH_3 in THF (1M, 23.0 mL) was added very slowly at 0 ^{0}C under N_2. The mixture was stirred for 150 min at 0 ^{0}C then was added 3 M aqueous solution of NaOH (8.5 mL) very slowly. After being stirred for 15 min, an aqueous solution of H_2O_2 (30%, 8.5 mL) was added and stirred for 30 min at room temperature. Organic layer was removed under *vacuo* and remaining aqueous mixture was saturated with Na_2CO_3 (5 g) and extracted with ethyl acetate (5x50 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (3:1) as eluent to obtain ether G1 **101** as a colorless oil (4.69 g, 97 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (bs, 2H), 3.61-3.48 (m, 24H), 2.97 (bs, 1H), 2.11 (m, 1H), 1.19 (t, 6H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.4, 70.3, 70.2, 70.1, 69.5, 66.3, 62.2, 41.0, 14.8 (Figure A.19, A.20).

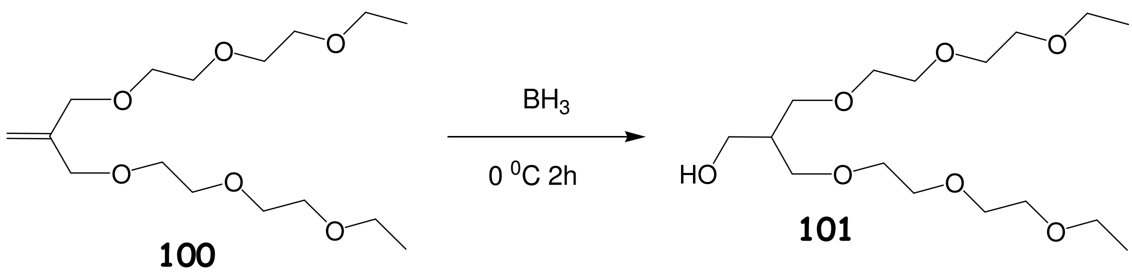


Figure 4.13. Synthesis of ether G1

4.2.2.3. Synthesis of Ether G1.5. Solid NaH (60 %, 0.44 g, 11.0 mmol) was washed with freshly distilled THF (5 mL) at room temperature under N_2 and dissolved in THF (15 mL). To this slurry was added 3-chloro-2-chloromethyl propene **99** (.26 mL, 2.2 mmol) followed by slow addition of G1 **101** (3.1 g, 9.2 mmol) while stirring. The solution was stirred at 75 ^{0}C for 16 h. Distilled H_2O (2 mL) was added and all volatiles were removed under *vacuo*. H_2O (20 mL) was then added and the solution was extracted with CH_2Cl_2 (5x20mL). The combined layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (7:3) as eluent to obtain the ether G1.5 **102** as a colorless oil (0.5 g, 29 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (s, 2H), 3.90 (s, 4H), 3.63-3.48 (m, 48H), 3.42 (d, 4H, J = 6 Hz), 2.20-2.14 (m, 2H), 1.20 (t, 12H, J = 7.2 Hz) (Figure A.21).

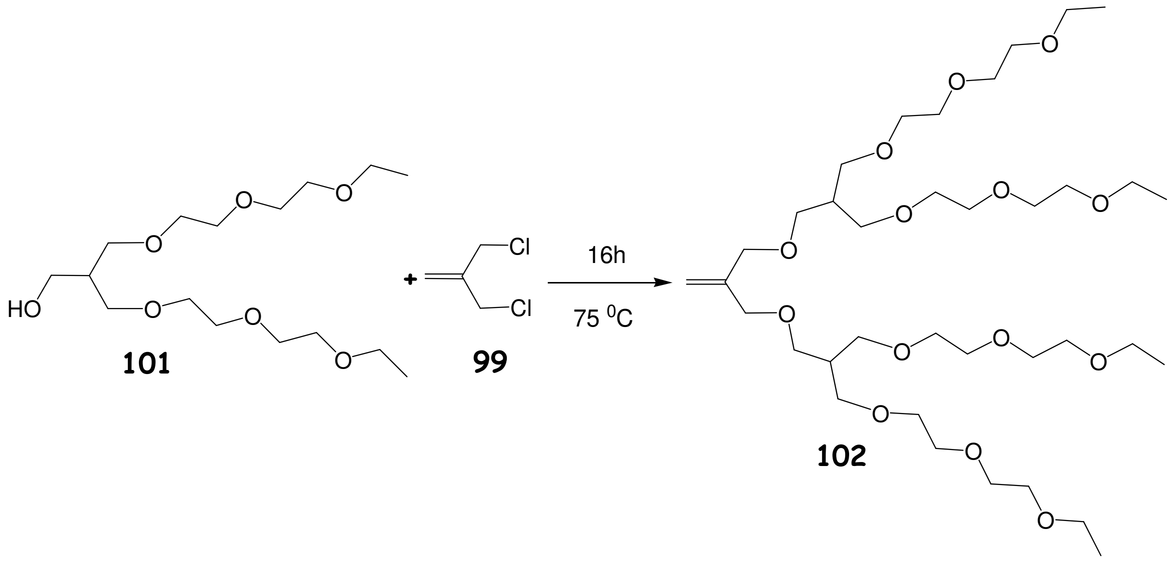


Figure 4.14. Synthesis of ether G1.5

4.2.2.4. Synthesis of Ether G2. To a stirred solution of ether G1.5 **102** (0.2 g, 14.4 mmol) in freshly distilled THF (0.16 mL), a solution of BH_3 in THF (1M, 0.16 mL) was added very slowly at 0 ^{0}C under N_2. The mixture was stirred for 2 h at 0 ^{0}C then was added 3 M aqueous solution of NaOH(0.15 mL) very slowly. After being stirred for 15 min, an aqueous solution of H_2O_2 (30 %, 0.15 mL) was added and stirred for 30 min at room temperature. Organic layer was removed under *vacuo* and remaining aqueous mixture was

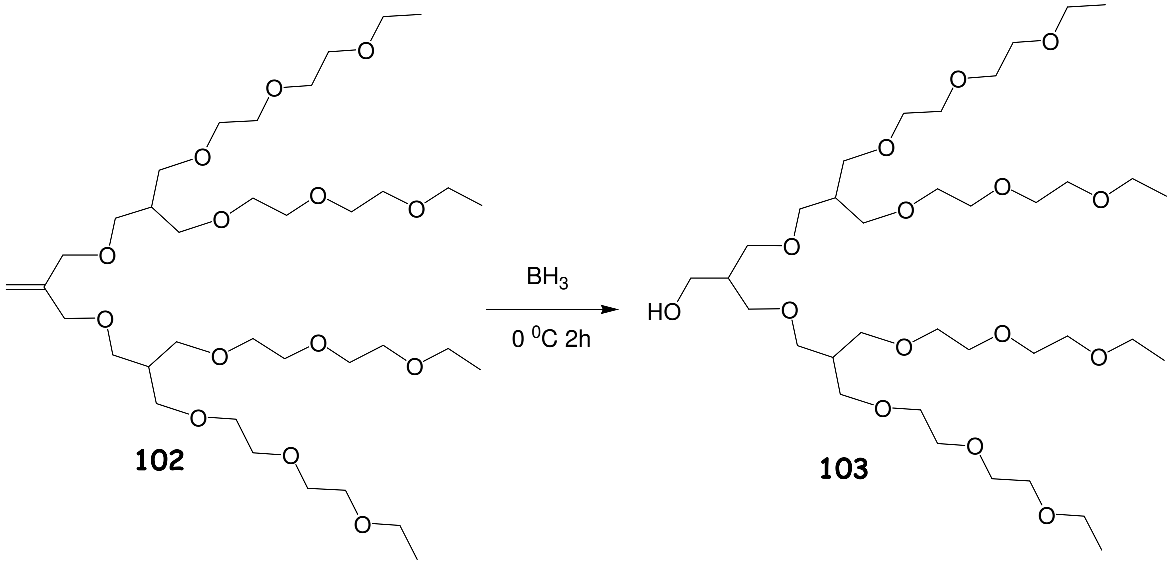


Figure 4.15. Synthesis of ether G2

saturated with Na_2CO_3 (2 g) and extracted with ethyl acetate (5x30 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (4:1) as eluent to obtain ether G2 **103** as a colorless oil (0.12 g, 56 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (d, 2H, J = 5.6 Hz), 3.62-3.39 (m, 56H), 2.28 (bs, 1H), 2.18-2.12 (m, 2H), 2.07-2.04 (m, 1H), 1.18 (t, 12H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.9, 70.6, 70.5, 70.4, 69.8, 69.6, 66.6, 63.6, 41.3, 40.0, 15.1 (Figure A.22, A. 23).

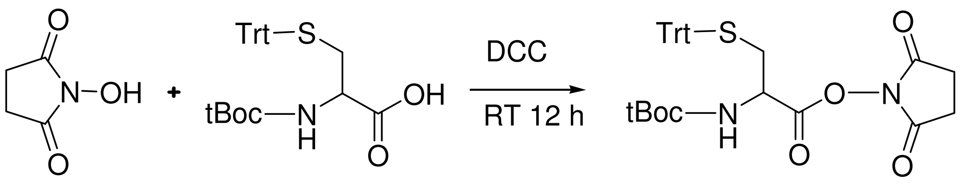
4.3. Functionalization of Dendrons

It is required to modify dendrons with aldehyde or cysteine functionality because the aim of this study is to couple these dendrons via 'thiapoline ligation'. For this purpose, PAMAM dendrons are functionalized with cysteine and ether dendrons are functionalized with an aldehyde group.

4.3.1. Functionalization of PAMAM Dendrons

To functionalize PAMAM dendrons with cysteine, first requirement is deprotection of focal point of tBoc protected dendrons. For this purpose trifluoroacetic acid (TFA) is used. After deprotection, free dendrons were coupled with N-hydroxy succinimide activated N-(Boc) S-(Trt) cysteine. Only ester ended dendrons were employed because amine ended generations cannot be used because of interference of surface amine groups along with focal amine. Before coupling with aldehyde tBoc and Trt groups were deprotected with TFA and triethylsilane and immediately used.

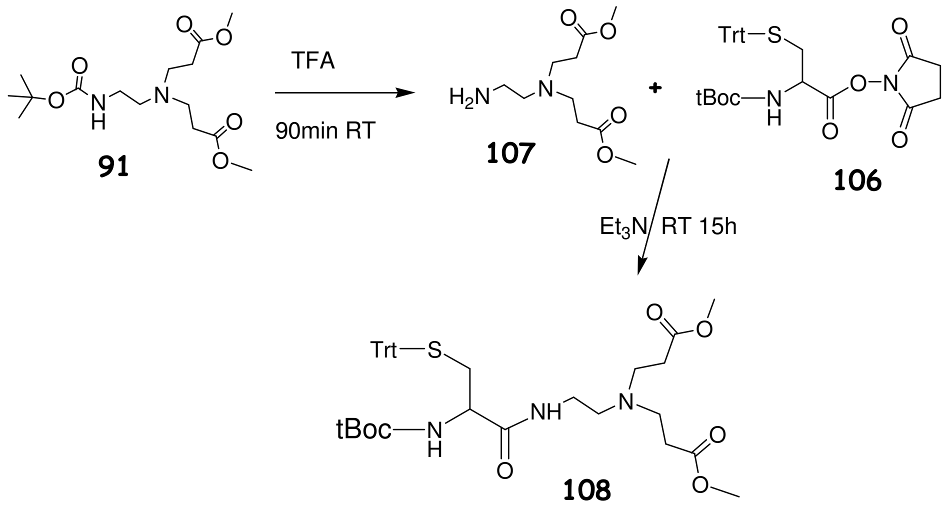
4.3.1.1. Synthesis of N-Hydroxy Succinimide Activated Cysteine. N-Hydroxy succinimide **104** (0.40 g,3.44 mmol), Boc-Cysteine(Trt)-OH **105** (1.60 g, 3.44 mmol) and DCC (0.71 g, 3.44 mmol) were added to ethyl acetate and stirred at room temperature for 12 h. Resulting solution was filtered and solvent was removed under *vacuo* to yield Boc-Cysteine(Trt)-NHS **106** (2.07 g). No additional attempts to further purify **106** were made due to its instability. The compound was used as such in the next reaction.



104 105 106

Figure 4.16. Synthesis of Boc-Cysteine(Trt)-NHS

<u>4.3.1.2. Functionalization of tBoc-PAMAM G0.5.</u> To a solution of tBoc-PAMAM G0.5 **91** (0.4 g, 1.2 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.5 mL) at 0 ^{0}C and stirred for 90 min at room temperature. All volatiles were removed under *vacuo* to yield TFA salt of PAMAM G0.5 **107**. To a solution of PAMAM G0.5 **107** (0.3 g) and N-hydroxy succinimide activated cysteine **106** (0.72 g, 1.3 mmol) in CH_2Cl_2 (10 mL)was added triethylamine (0.36 mL) and the reaction mixture was stirred for 15 h at room temperature under N_2. All volatiles were removed under *vacuo* and the crude was purified by column chromatography with ethyl acetate:hexane (3:2) as eluent to obtain the S(Trt)N(Boc)Cys-PAMAM G0.5 **108** as a colorless oil (0.15 g, 18 % yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, 6H), 7.24 (t, 6H, J = 6.8), 7.18 (t, 3H), 6.77 (bs, 1H), 5.20 (d, 1H), 3.64 (s, 6H), 3.33 (bt, 2H), 2.76-2.54 (m, 6H), 2.47 (q, 2H, J = 5.2 Hz), 2.36 (t, 4H, J = 7.2 Hz), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 170.2, 155.1, 144.5, 129.5, 127.9, 126.6, 79.7, 66.6, 53.3, 53.0, 51.7, 49.1, 37.2, 34.8, 32.6, 28.3 (Figure A.24, A. 25).



91 107 106 108

Figure 4.17. Functionalization of tBoc-PAMAM G0.5

4.3.2. Functionalization of Ether Dendrons

To furnish the ether dendrons with aldehyde functionality, ether G1 and ether G2 were first converted into acid via treatment with succinic anhydride. The second step was conversion of dendritic acids into acetals via treatment with bromomethyl dimethylacetal. These dendritic acetals were being stored in cold and deprotected by TFA to yield corresponding aldehyde and used immediately for the coupling reaction.

<u>4.3.2.1. Synthesis of Ether G1 Acid.</u> To a solution of ether G1 **101** (1.5 g, 4.4 mmol) in CH_3CN (30 mL), were added succinic anhydride **109** (0.62 g, 6.2 mmol) and dimethylaminopyridine (0.22 g, 1.8 mmol) were added and refluxed for 10 h under N2. All volatiles were removed under *vacuo and* the crude product was purified by column chromatography with methanol:methylene chloride (1:19) as eluent to obtain ether G1 acid **110** as a yellow oil (1.87 g, 96 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (d, 2H, *J* = 5.6 Hz), 3.62-3.44 (m, 24H), 2.62 (m, 4H), 2.25-2.19 (m, 1H), 1.19 (t, 6H, *J* = 3.6 Hz). IR: υ = 1731 cm^{-1} (Figure A.26, A.27).

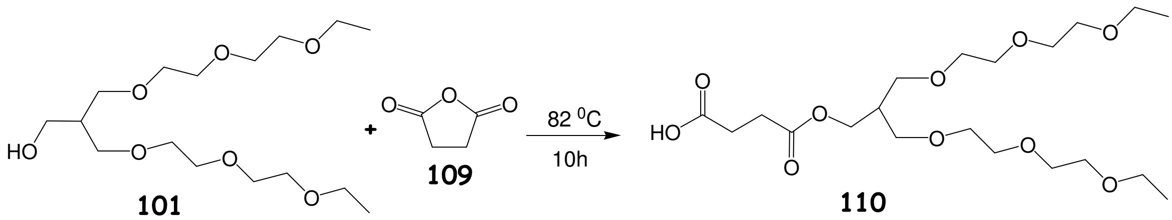


Figure 4.18. Synthesis of ether G1 acid

<u>4.3.2.2. Synthesis of Ether G1 Acetal.</u> To a solution of ether G1 acid **110** (0.75 g, 1.7 mmol) in DMF (4 mL), Cs_2CO_3 (0.28 g, 0.9 mmol) and bromomethyl dimethylacetal **111** (0.6 mL, 5.13 mmol) were added and stirred for 2 d at 70 ^{0}C under N_2. All volatiles were removed under *vacuo* then 20 mL brine was added and extracted with CH_2Cl_2 (6x20 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (7:3) as eluent to obtain ether G1 acetal **112** as a yellow oil (0.58 g, 64 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (t, 1H, *J* = 5.2 Hz), 4.15 (d, 2H, *J* = 5.2 Hz), 4.10 (d, 2H, *J* = 5.2 Hz), 3.61-3.43 (m, 24H), 3.37 (s, 6H), 2.65-2.61 (m, 4H), 3.38 (s, 6H),

2.25-2.23 (m, 1H), 1.19 (t, 6H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.8, 101.0, 70.6, 70.5, 70.3, 69.7, 69.0, 66.5, 63.7, 63.0, 53.8, 39.0, 28.84, 28.77, 15.0. IR: υ = 1738 cm^{-1} (Figure A.28, A.29, A.30).

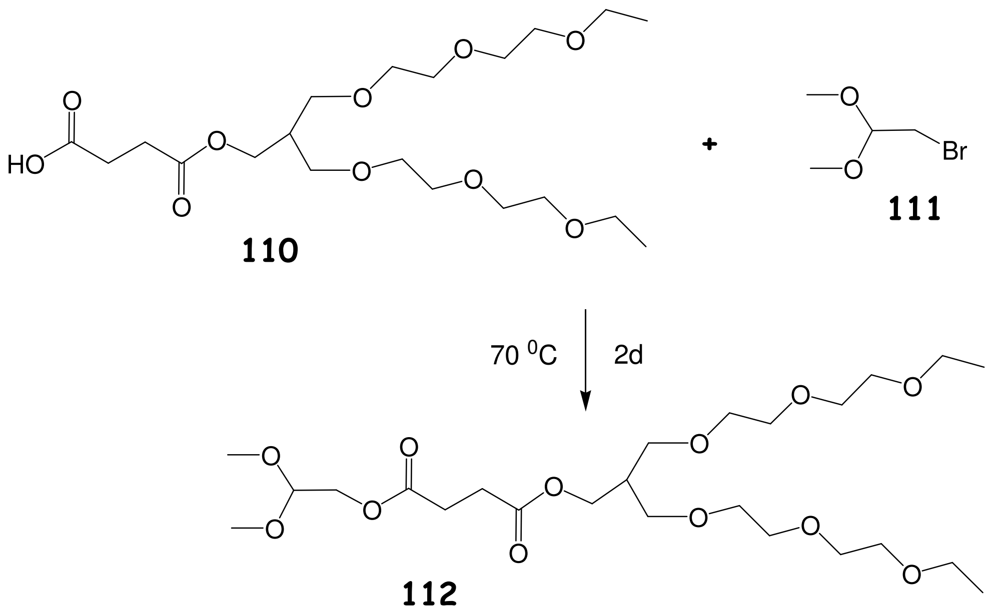


Figure 4.19. Synthesis of ether G1 acetal

<u>4.3.2.3. Synthesis of Ether G2 Acid.</u> To a solution of ether G2 **103** (1.35 g, 1.8 mmol) in CH_3CN (12 mL), succinic anhydride **109** (0.25 g, 2.5 mmol) and dimethylaminopyridine (0.09 g, 0.7 mmol) were added and refluxed for 10 h under N_2. All volatiles were removed under *vacuo* and the crude product was filtered by column chromatography with methanol:methylenechloride (1:19) as eluent. Solvent was removed under *vacuo* and then the crude was dissolved in ethyl acetate (30 mL) and extracted with $NaHCO_3$ solution (30 mL). The aqueous layer was neutralized with hydrochloric acid and extracted with CH_2Cl_2 (5x30 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo* to obtain ether G2 acid **113** (0.85 g, 56 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (d, 2H, J = 5.6 Hz), 3.63-3.55 (m, 30H), 3.51 (q, 8H, J = 7.2 Hz), 3.46 (d, 8H, J = 6.4 Hz), 3.43-3.32 (m, 8H), 2.59 (s, 4H), 2.19-2.12 (m, 3H), 1.19 (t, 12H, J = 7.2 Hz). IR: υ = 1731 cm^{-1} (Figure A.31, A.32).

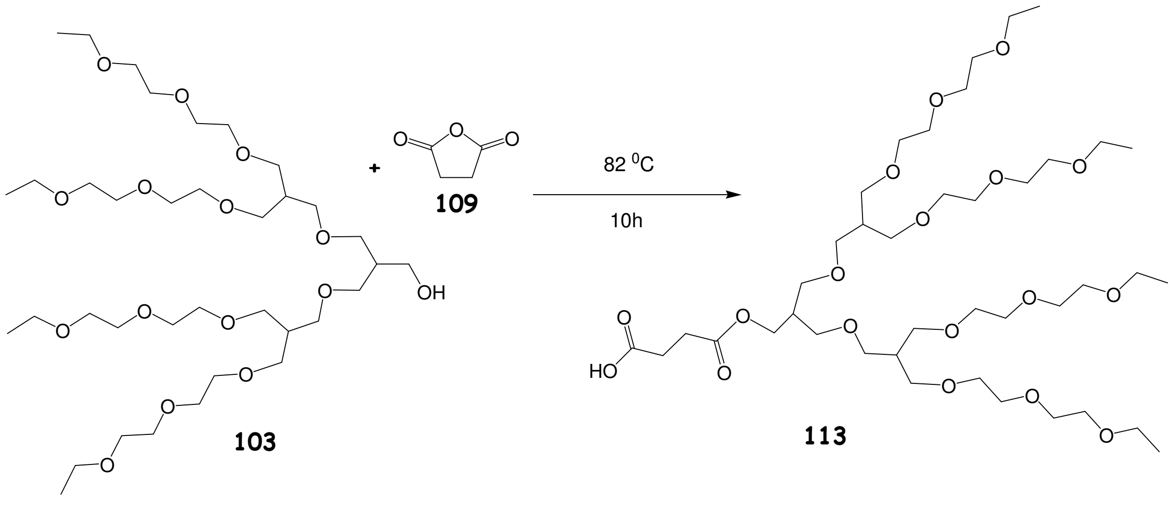


Figure 4.20. Synthesis of ether G2 acid

4.3.2.4. Synthesis of Ether G2 Acetal. To a solution of ether G2 acid **113** (0.71 g, 0.8 mmol) in DMF (4.5 mL), were added Cs_2CO_3 (0.2 g, 0.6 mmol) and bromomethyl dimethylacetal **111** (0.3 mL, 2.5 mmol) and stirred for 2 d at 70 ^{0}C under N_2. All volatiles were removed under *vacuo* then 20 mL brine was added and extracted with CH_2Cl_2 (6x20 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate as eluent to obtain ether G2 acetal **114** as a colorless oil (0.58 g, 64 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.48 (t, 1H, *J* = 5.2 Hz), 4.05 (d, 2H, *J* = 6 Hz), 4.04 (d, 2H, *J* = 5.2 Hz), 3.58-3.30 (m, 62H), 2.61-2.53 (m, 4H), 2.14-2.03 (m, 3H), 1.13 (t,

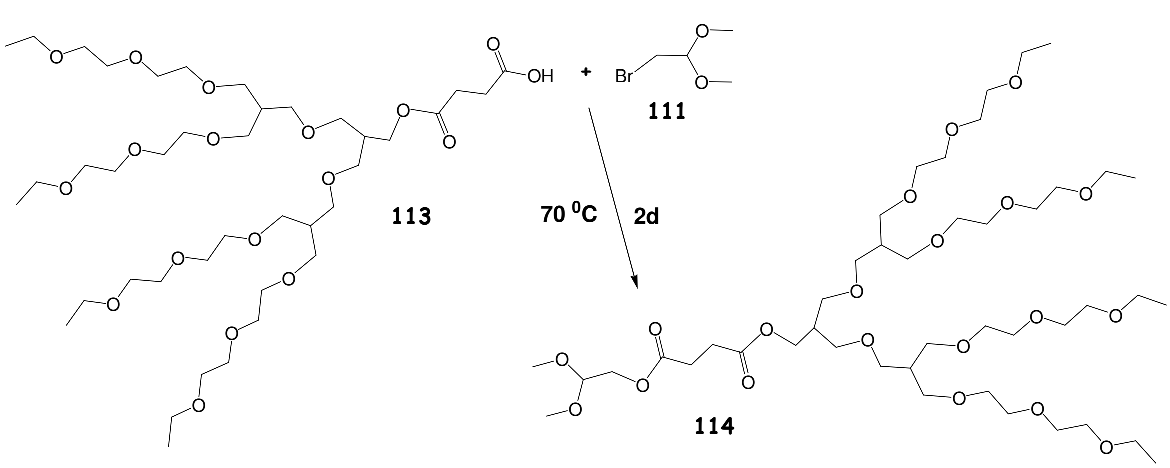


Figure 4.21. Synthesis of ether G2 acetal

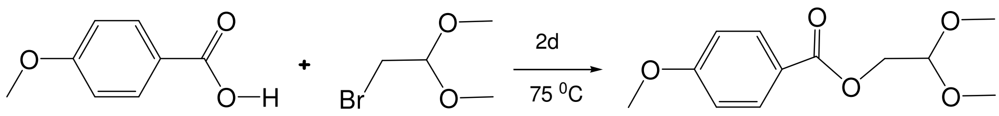
12H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.8, 101.0, 70.6, 70.4, 70.3, 69.7, 69.5, 69.4, 68.9, 66.5, 63.1, 63.0, 53.8, 40.0, 39.2, 28.85, 28.79, 15.0. IR: υ = 1738 cm^{-1} (Figure A.33, A.34, A.35).

4.4. Coupling Reactions

4.4.1. Synthesis of Model Compunds

As a model study 'thiaproline ligation' of aldehyde functionalized anisic acid and cysteine functionalized benzylamine was undertaken

4.4.1.1. Synthesis of Anisic Acetal. To a solution of anisic acid **115** (0.5 g, 3.3 mmol) in DMF (4 mL), Cs_2CO_3 (0.54 g, 1.64 mmol) and bromomethyl dimethylacetal **111** (1.2 mL, 9.84 mmol) were added and stirred for 2 d at 75 ^{0}C under N_2. All volatiles were removed under *vacuo* then 25 mL brine was added and extracted with CH_2Cl_2 (6x30 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with ethyl acetate:hexane (1:9) as eluent to obtain anisic acetal **116** as a colorless oil (0.3 g, 38 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 2H, J = 8.8 Hz), 6.90 (d, 2H, J = 8.8 Hz), 4.68 (t, 1H, J = 5.6 Hz), 4.30 (d, 2H, J = 5.6 Hz), 3.84 (s, 3H), 3.42 (s, 6H) (Figure A.36).



115 **111** **116**

Figure 4.22. Synthesis of anisic acetal

4.4.1.2. Synthesis of S(Trt)N(Boc)Cys Benzyl Amine. To a solution of benzyl amine **117** (0.2 g, 1.87 mmol) in CH_2Cl_2 (4 mL), N-hydroxy succinimide activated cysteine **106** (1.05 g, 1.87 mmol) and triethylamine (0.5 mL) were added in a flask and stirred for 15 h at room temperature under N_2. All volatiles were removed under *vacuo* and the crude was purified by column chromatography (silicagel) with ethyl acetate:hexane (17:3) as eluent to obtain the S(Trt)N(Boc)cys- Benzyl Amine **118** as a white solid (0.7 g, 66 % yield). ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.45 (d, 6H), 7.29-7.18 (m, 14H), 6.61 (bs, 1H), 5.12 (d, 1H), 4.35 (m, 2H), 3.98 (bs, 1H), 2.73 (q, 1H), 2.61 (q, 1H) (Figure A.37).

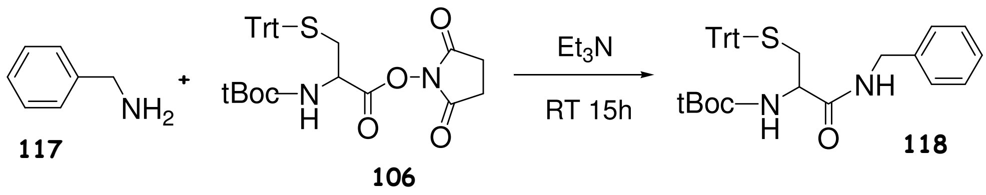


Figure 4.23. Synthesis of S(Trt)N(Boc)Cys benzyl amine

4.4.2. Model Coupling Reaction

To a stirred solution of S(Trt)N(Boc)cys-benzyl amine **118** in TFA (0.5 mL) were added H_2O (0.02 mL) and Et_3SiH (0.02mL) at 0 °C and allowed to warm up in 1 h. Then H_2O (2 mL) was added and the solution was extracted with diethyl ether (2x2 mL) to obtain cys-benzyl amine **120** in the water phase. To anisic acetal **116** was added TFA (0.5 mL) was added and stirred for 1 h at room temperature to obtain anisic aldehyde **119** which was used without purification. To the cys-benzyl amine **118** solution was added a solution of anisic aldehyde **119** in CH_3CN (2 mL) and pH was adjusted to 4 by adding sodium acetate trihydrate. The mixture was stirred for 90 min at room temperature to obtain the coupled product **121**. After coupling pH was adjusted to 8 by adding $NaHCO_3$ and the mixture was stirred for 24 h at room temperature. The resulting mixture was then extracted with ethyl acetate (3x5 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with CH_2Cl_2: CH_3OH (99:1).

4.4.3. Coupling of Dendrons

To a stirred solution of S(Trt)N(Boc)cys-PAMAM G0.5 **108** in TFA (0.5 mL) were added H_2O (0.02 mL) and Et_3SiH (0.02 mL) at 0 °C and allowed to warm up in 1 h then H_2O (1 mL) was added and the solution was extracted with diethyl ether (2x2 mL) to obtain PAMAM G0.5 cysteine **124** in the water phase. To ether G1 acetal **112** was added TFA (0.5 mL) and stirred for 1 h at room temperature to obtain ether G1 aldehyde **123** which

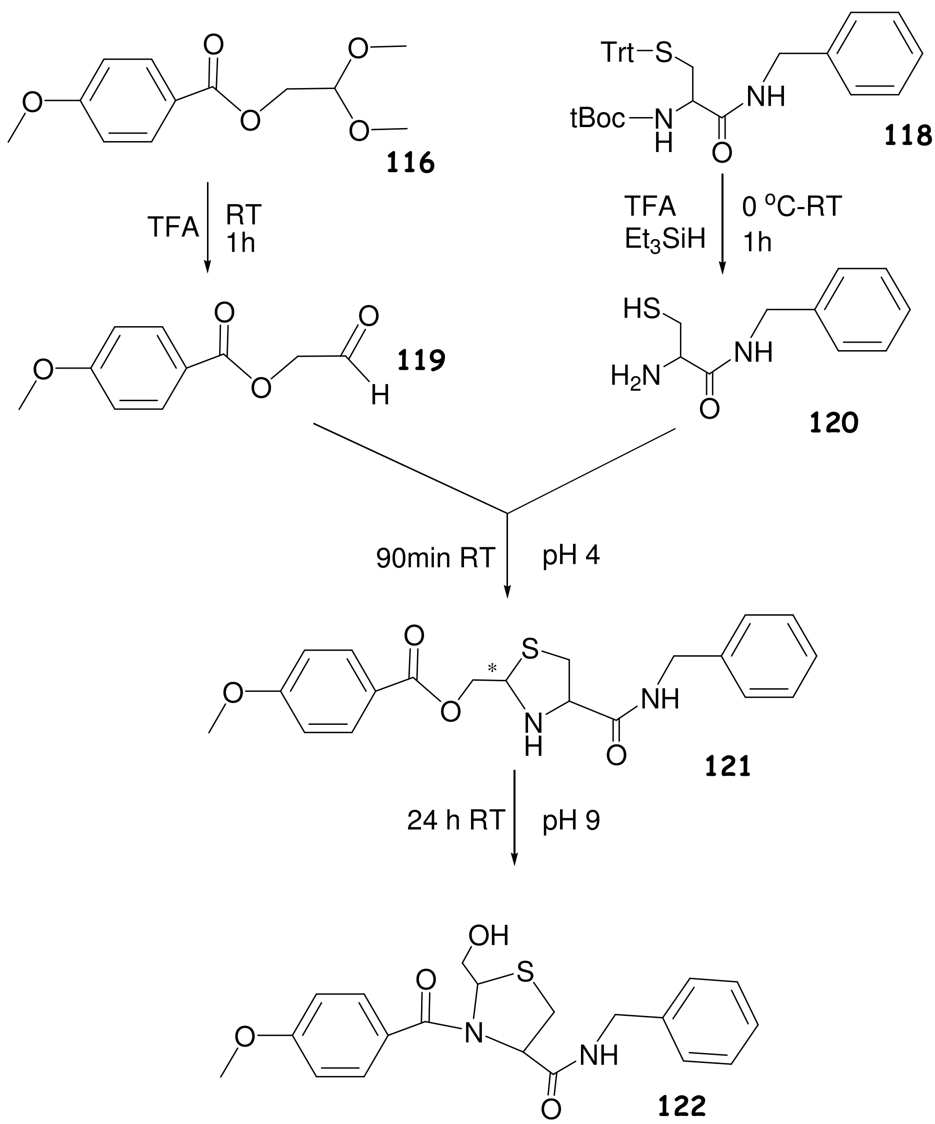


Figure 4.24. Ligation of anisic aldehyde with benzyl amine cysteine

was used without purification. To the PAMAM G0.5 cysteine **124** was added a solution of ether G1 aldehyde **123** in CH_3CN (2 mL) and pH was adjusted to 4 by adding sodium acetate trihydrate. The mixture was stirred for 90 min at room temperature to obtain the coupled product **125**. After coupling pH was adjusted to 8 by adding $NaHCO_3$ and the mixture was stirred for 24 h at room temperature. The resulting mixture was then extracted with ethyl acetate (3x5 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and solvent was removed under *vacuo*. The crude product was purified by column chromatography with CH_2Cl_2: CH_3OH (99:1). ^1H NMR studies showed that, dendrons were coupled but not rearranged. As it is seen from the Figure 4.25 coupled product has a new chiral center. Obtained product is a mixture of these diastereomers.

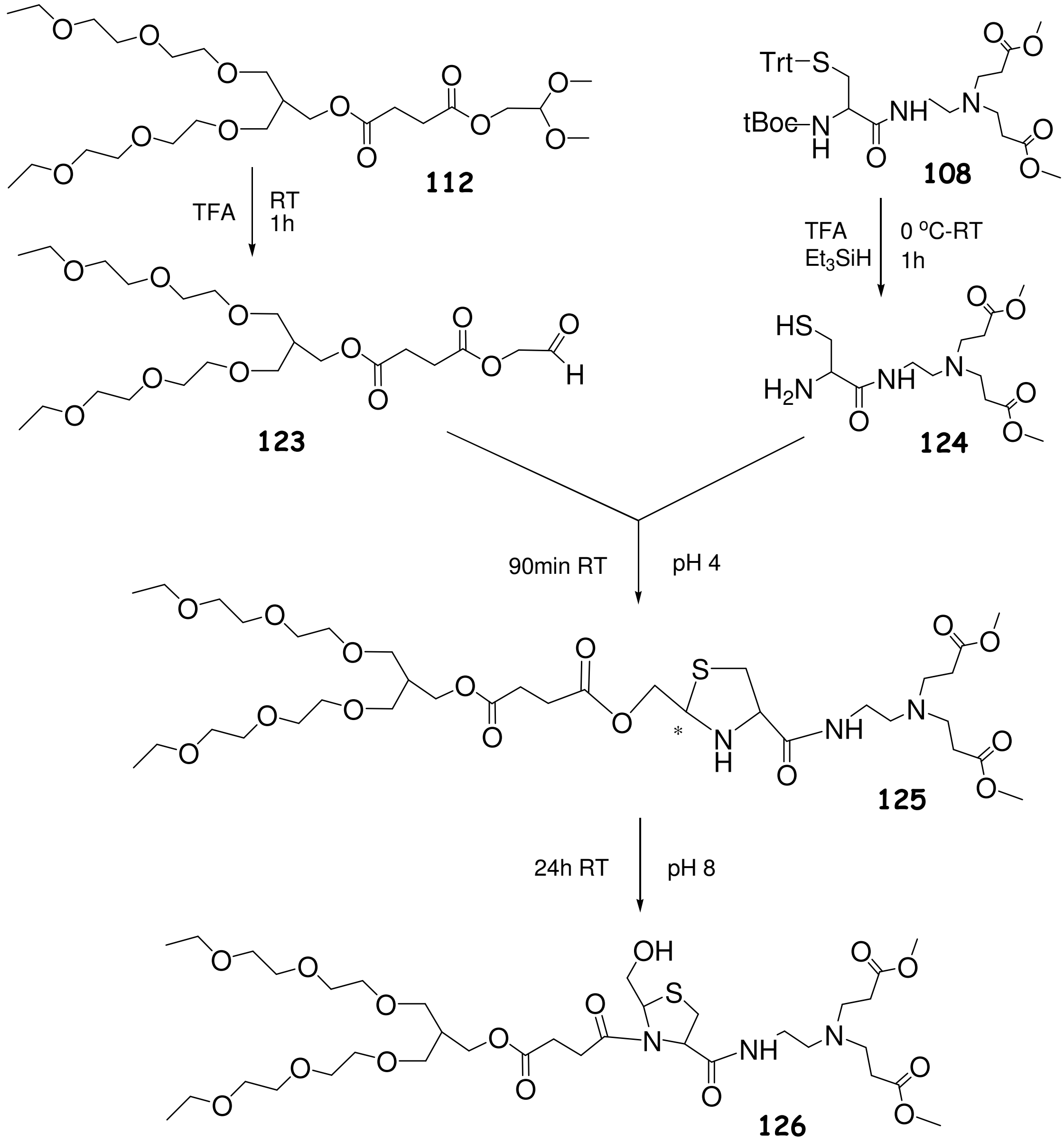


Figure 4.25. Ligation of ether G1 aldehyde with PAMAM G0.5 cysteine

5. CONCLUSIONS

Poly(amido amine) and polyether dendrons were synthesized to obtain a segment-block dendrimer via ‘thiaproline ligation’. For poly(amido amine) dendron, divergent method is used and up to Generation 3.5 dendron was synthesized. For polyether dendron, convergent method is used and reached up to Generation 2 dendron was synthesized. Model compounds, anisic acetal and benzyl amine cysteine, suitable for ‘thiaproline ligation’ were also synthesized. Ligation of these model compounds was performed and successful coupling was observed. Selected generations of dendrons were modified for ‘thiaproline ligation’. These modifications include coupling of poly(amido amine) dendron with cysteine and converting polyether dendrons to first acid and then acetal functionalities. Preliminary studies on coupling of these modified dendrons were also performed.

^{1}H NMR studies for the ligation of poly(amido amine) G0.5 cysteine with polyether G1 aldehyde showed that, of the two step procedure, the first step namely the coupling was successful. Whereas the second step, the rearrangement was not accomplished. Thus the obtained product is a mixture of diastereomers of unrearranged thiazolidine compounds and these diastereomers are inseparable via column chromatography. Because of the absence of rearrangement, coupling yields were also low due to the reversible character of the coupling reaction. The applied conditions must be optimized to facilitate the rearrangement and when the right conditions are obtained for small generation dendrons they can be applied for higher generation dendrons.

APPENDIX A: SPECTROSCOPY DATA

^{1}H NMR, ^{13}C NMR and IR spectroscopy data for the synthesized compounds are given below. Needed regions of NMR data were expanded.

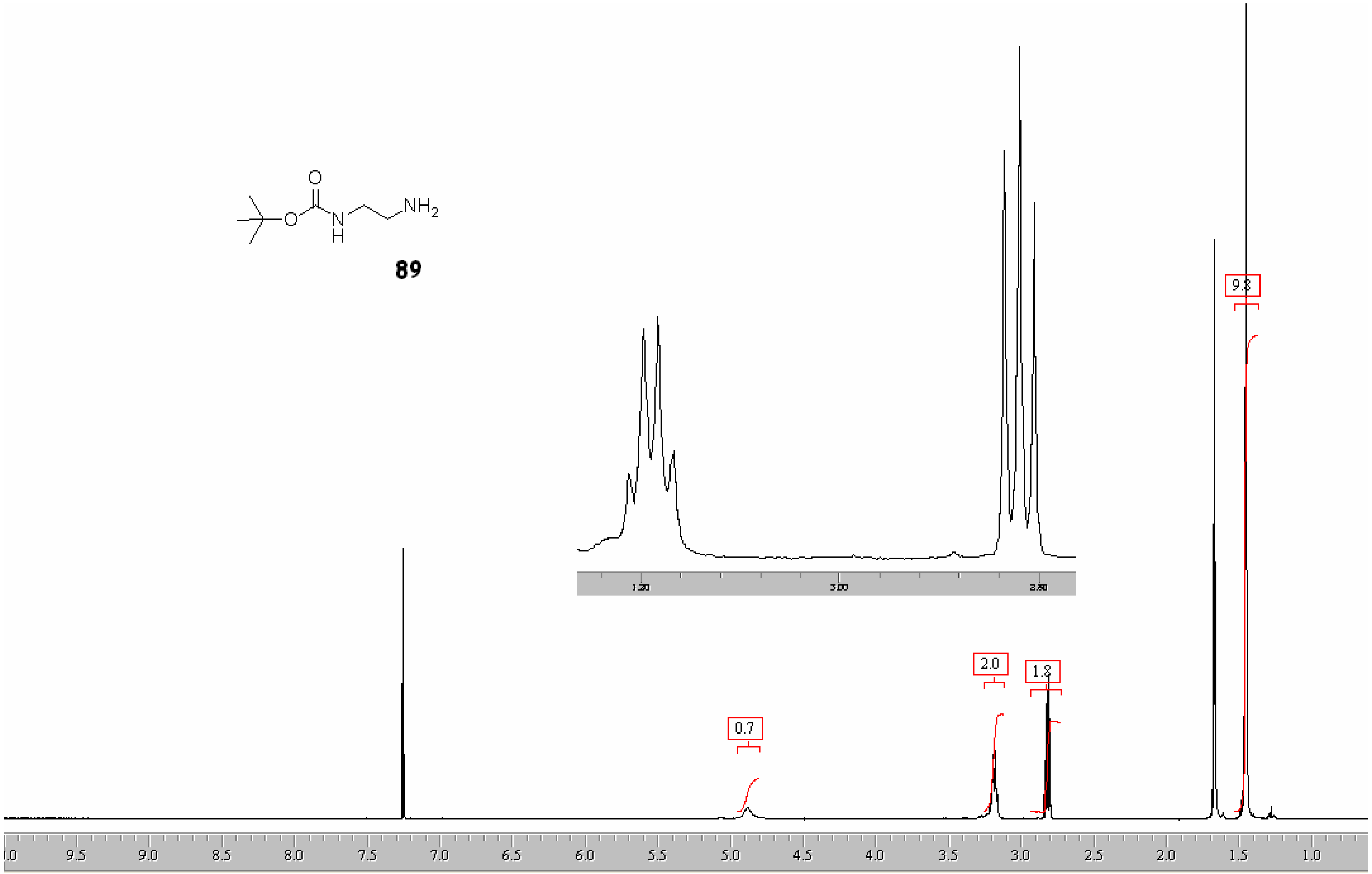


Figure A.1. ^{1}H NMR spectrum of mono tBoc protected ethylene diamine **89**

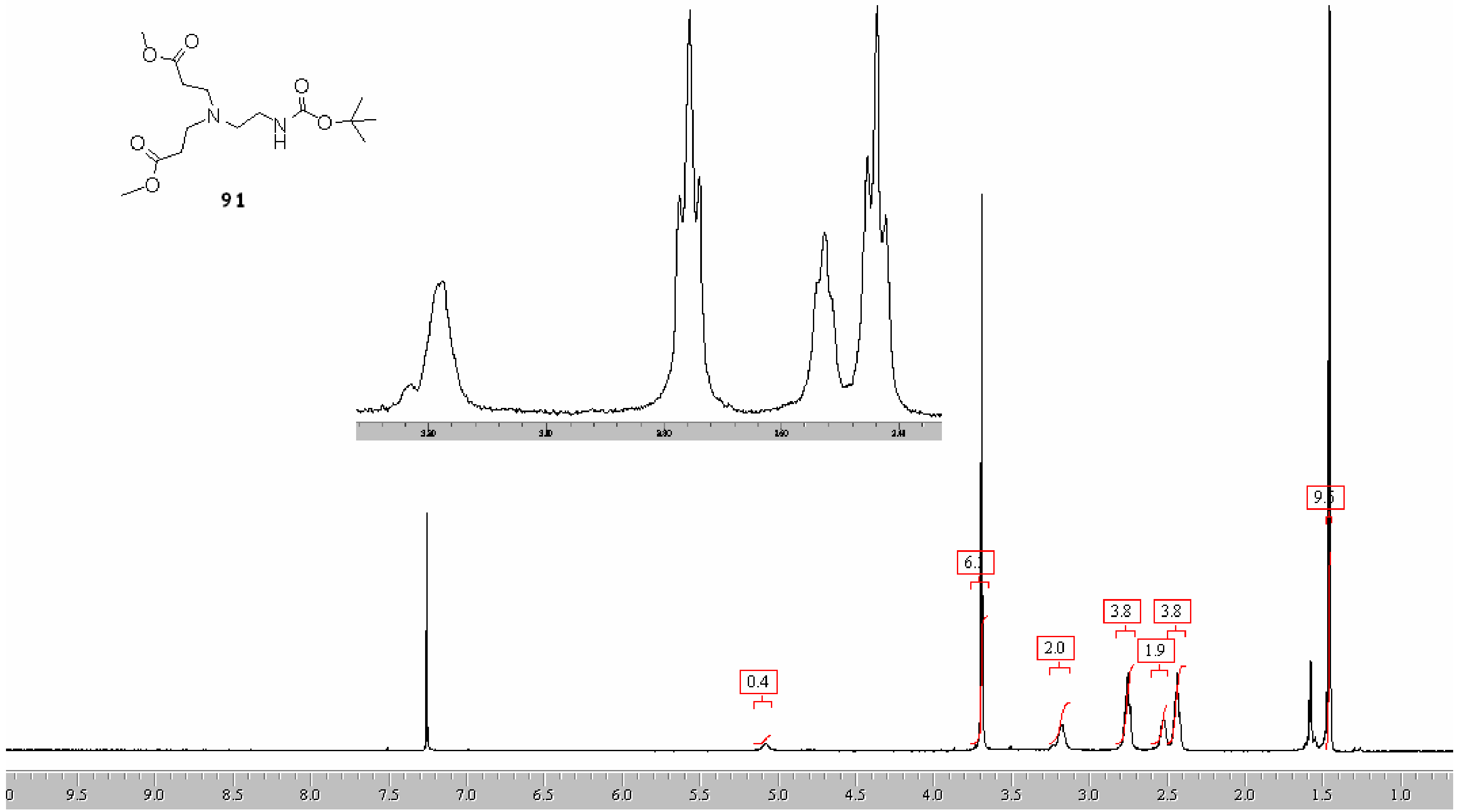


Figure A.2. ^{1}H NMR spectrum of PAMAM G0.5 **91**

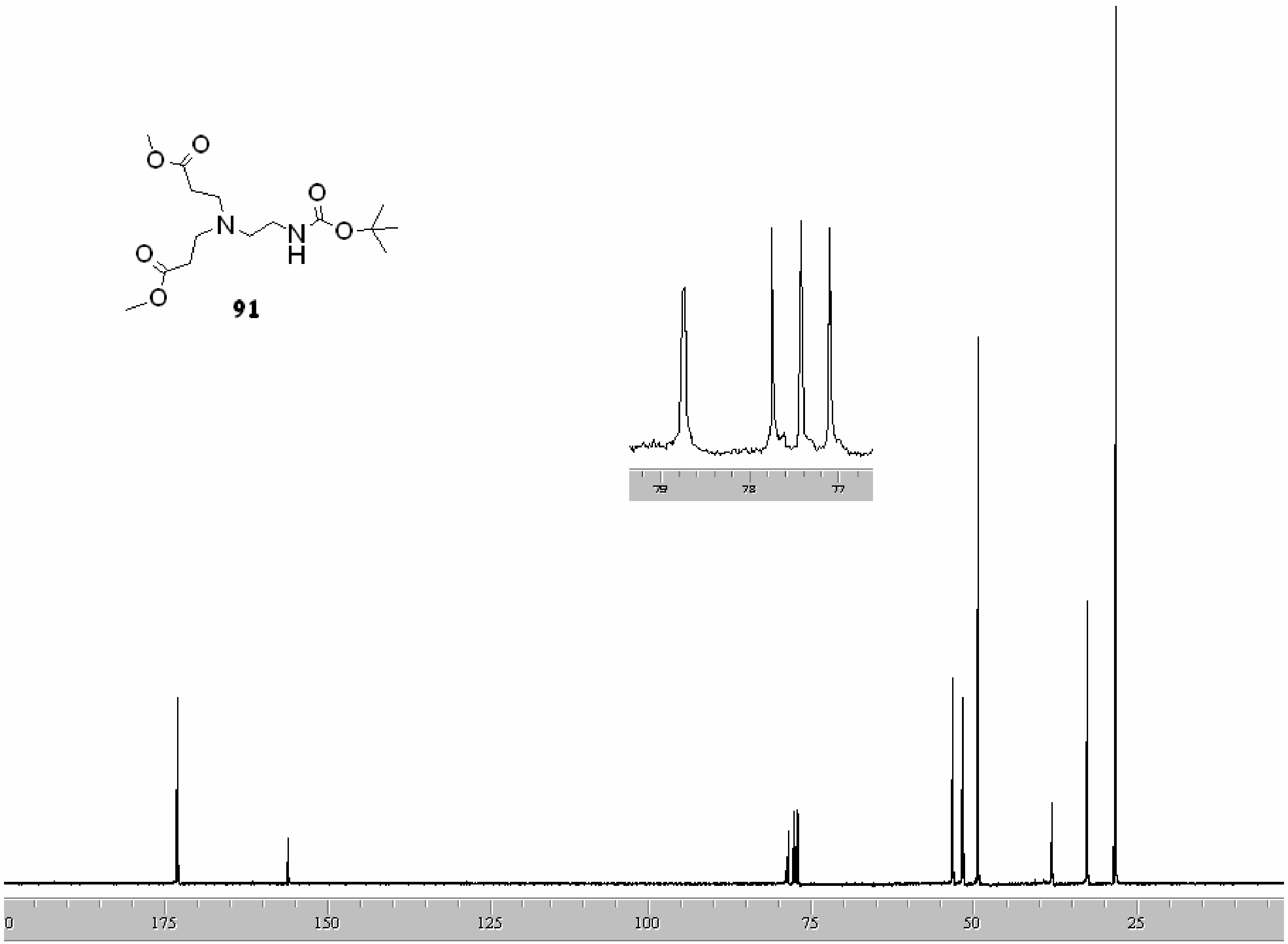


Figure A.3. ^{13}C NMR spectrum of PAMAM G0.5 **91**

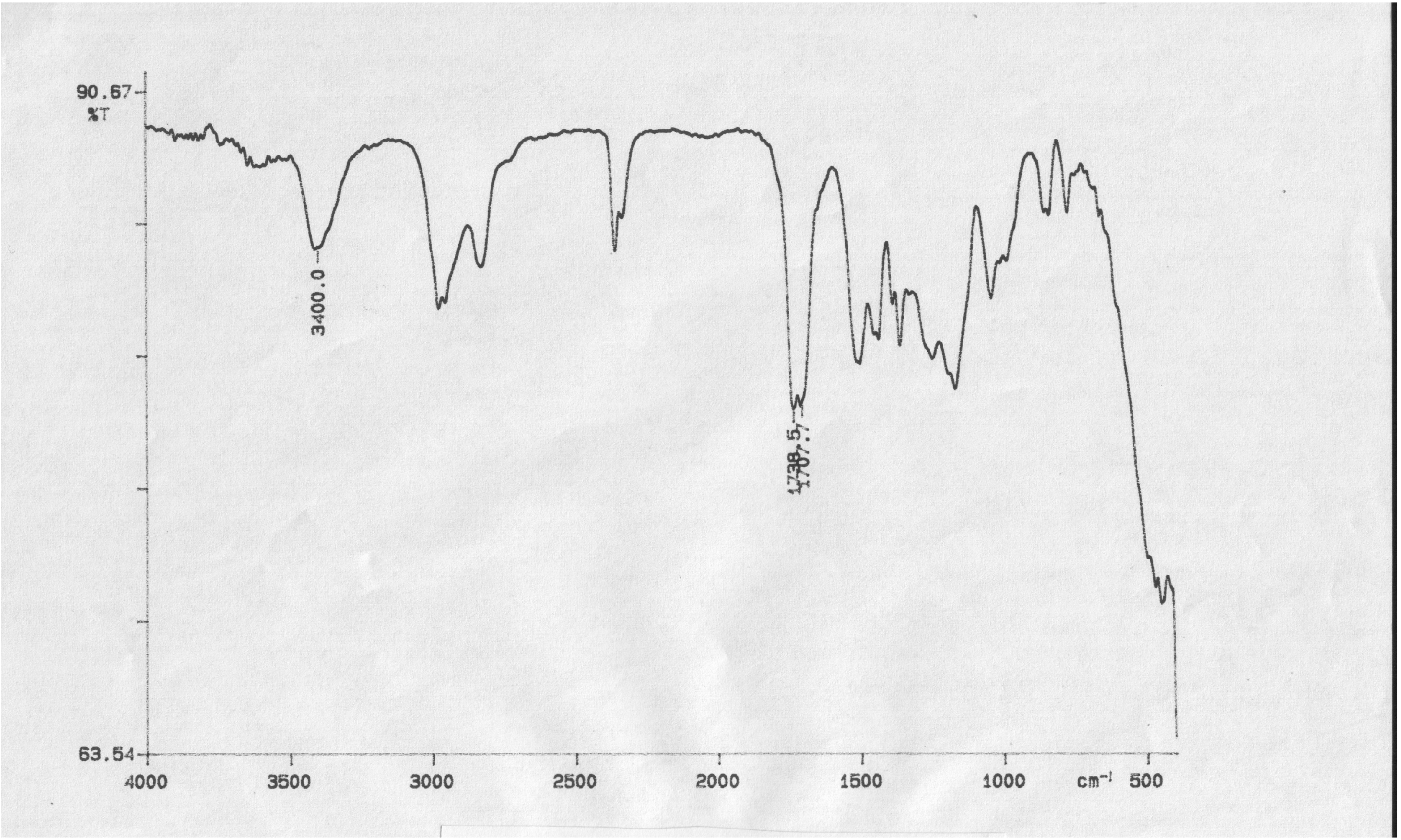


Figure A.4. IR spectrum of PAMAM G0.5 **91**

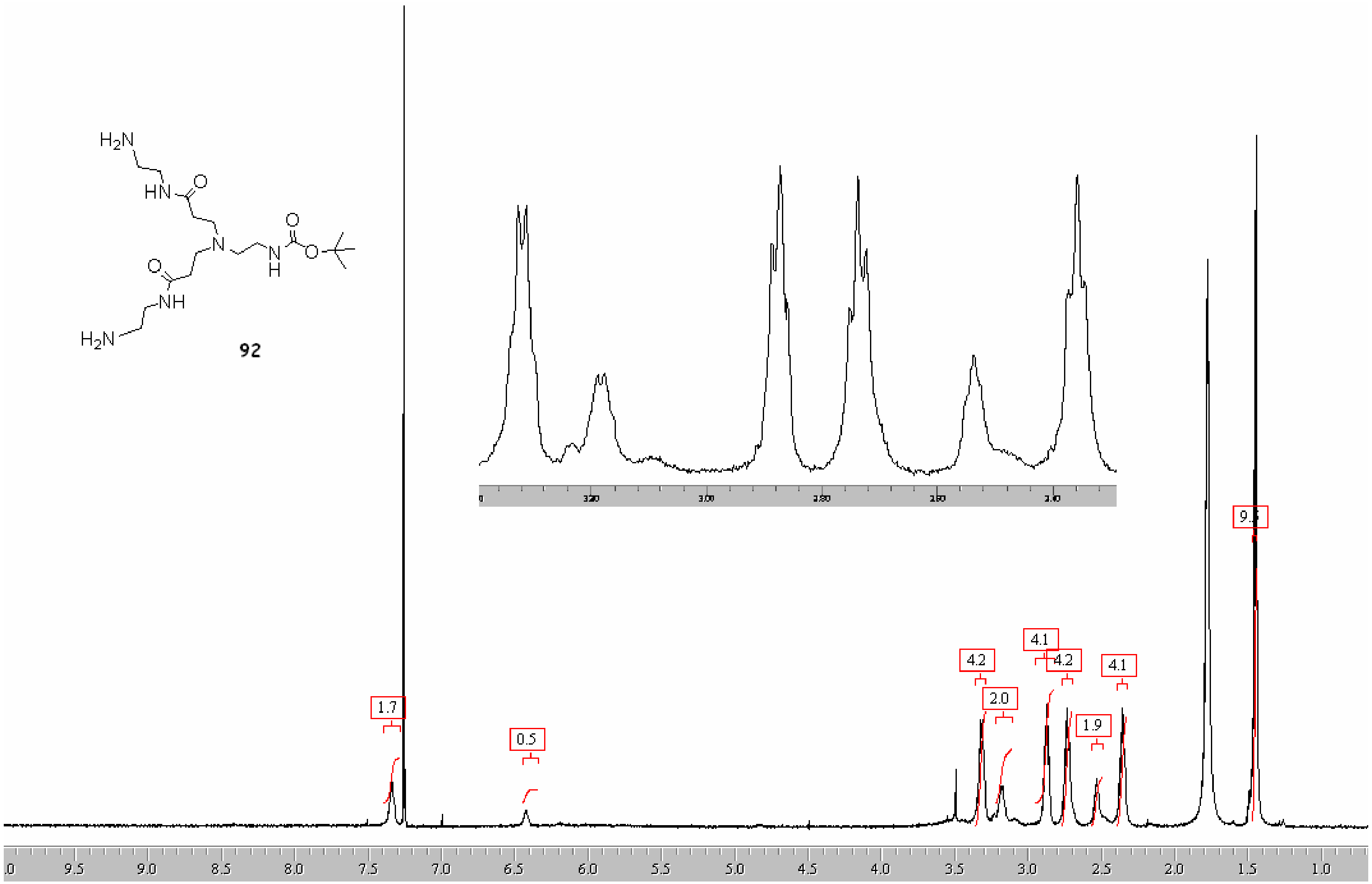


Figure A.5. [1]H NMR spectrum of PAMAM G1 **92**

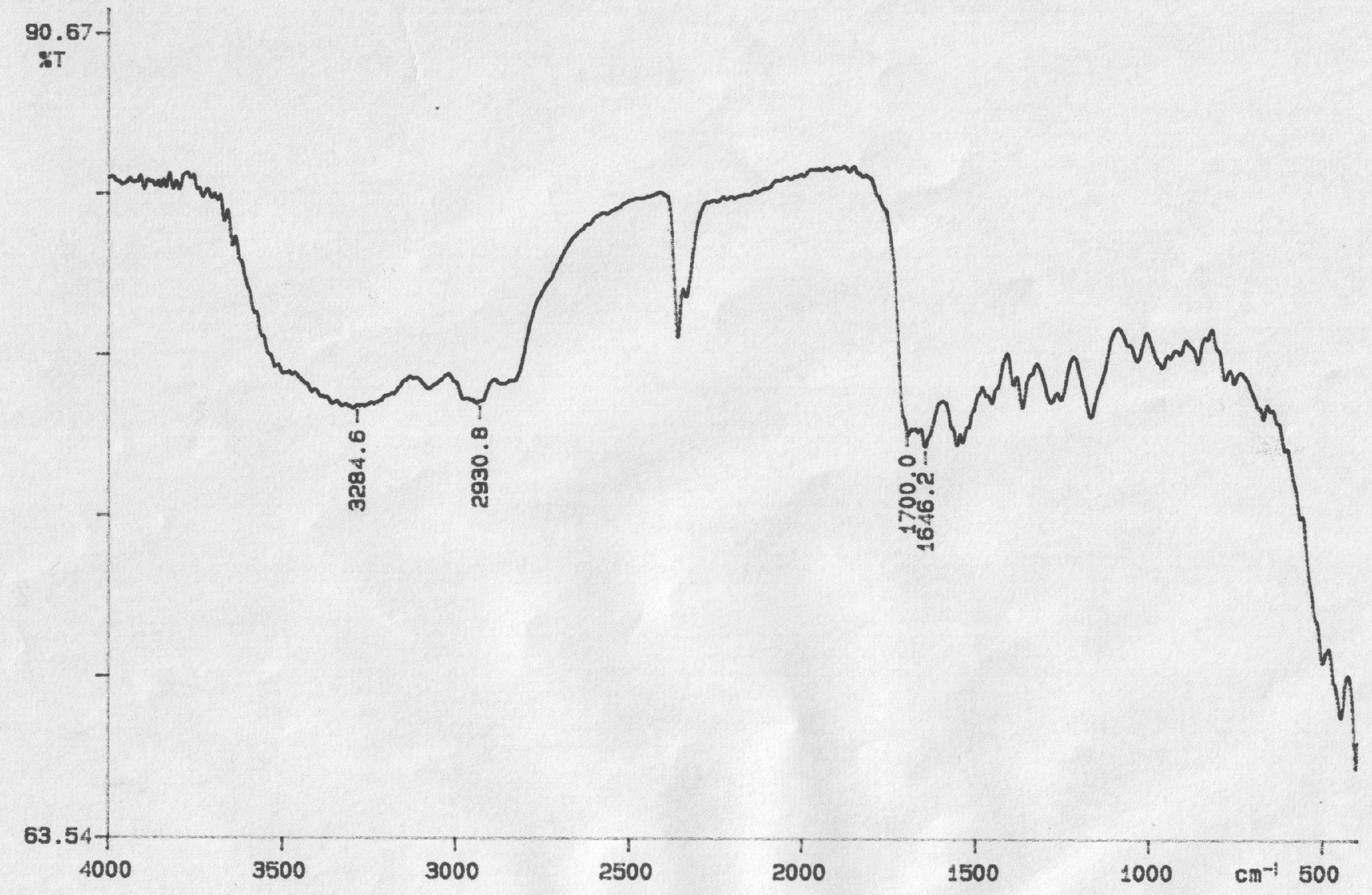


Figure A.6.IR spectrum of PAMAM G1 **92**

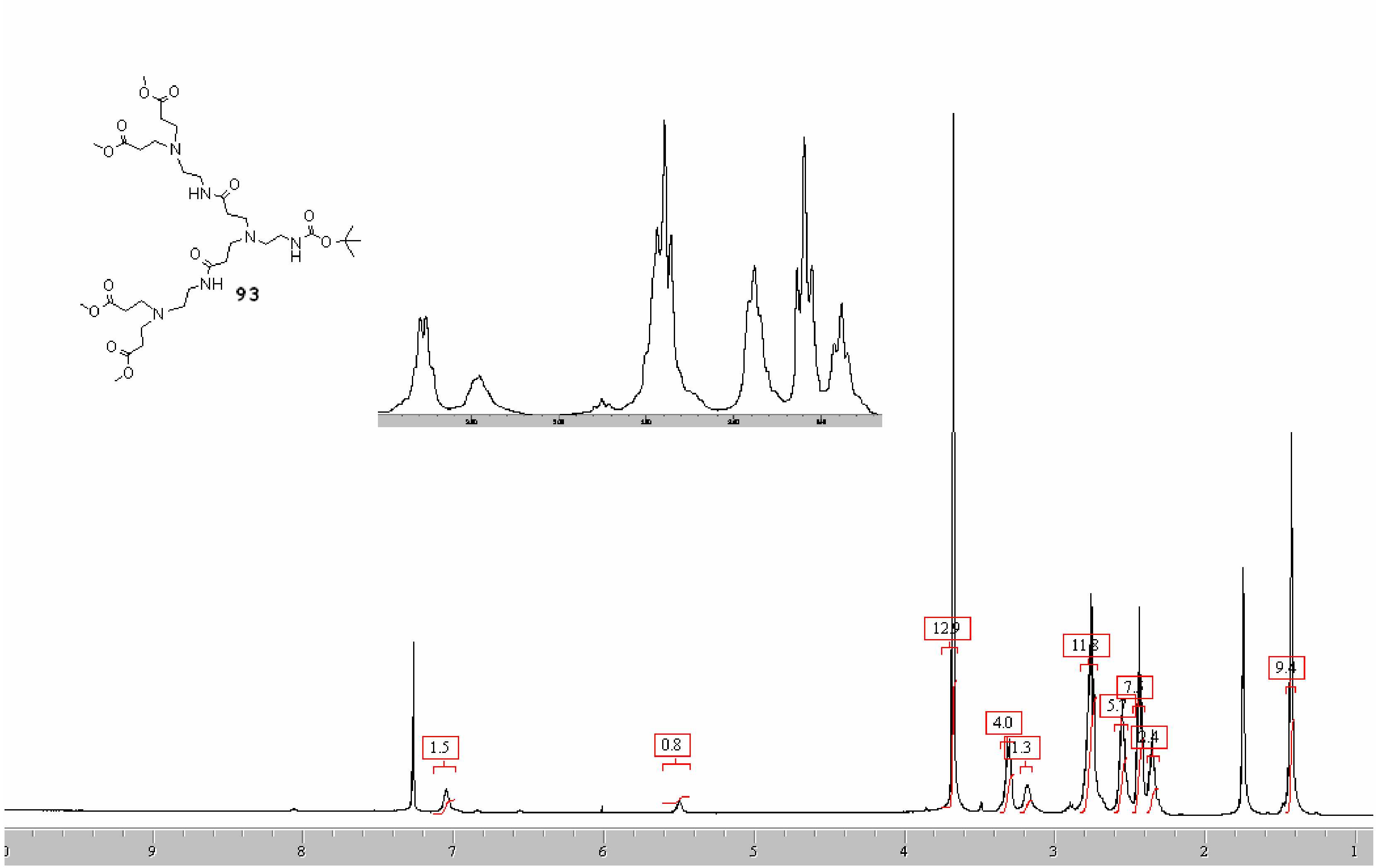


Figure A.7. ^{1}H NMR spectrum of PAMAM G1.5 **93**

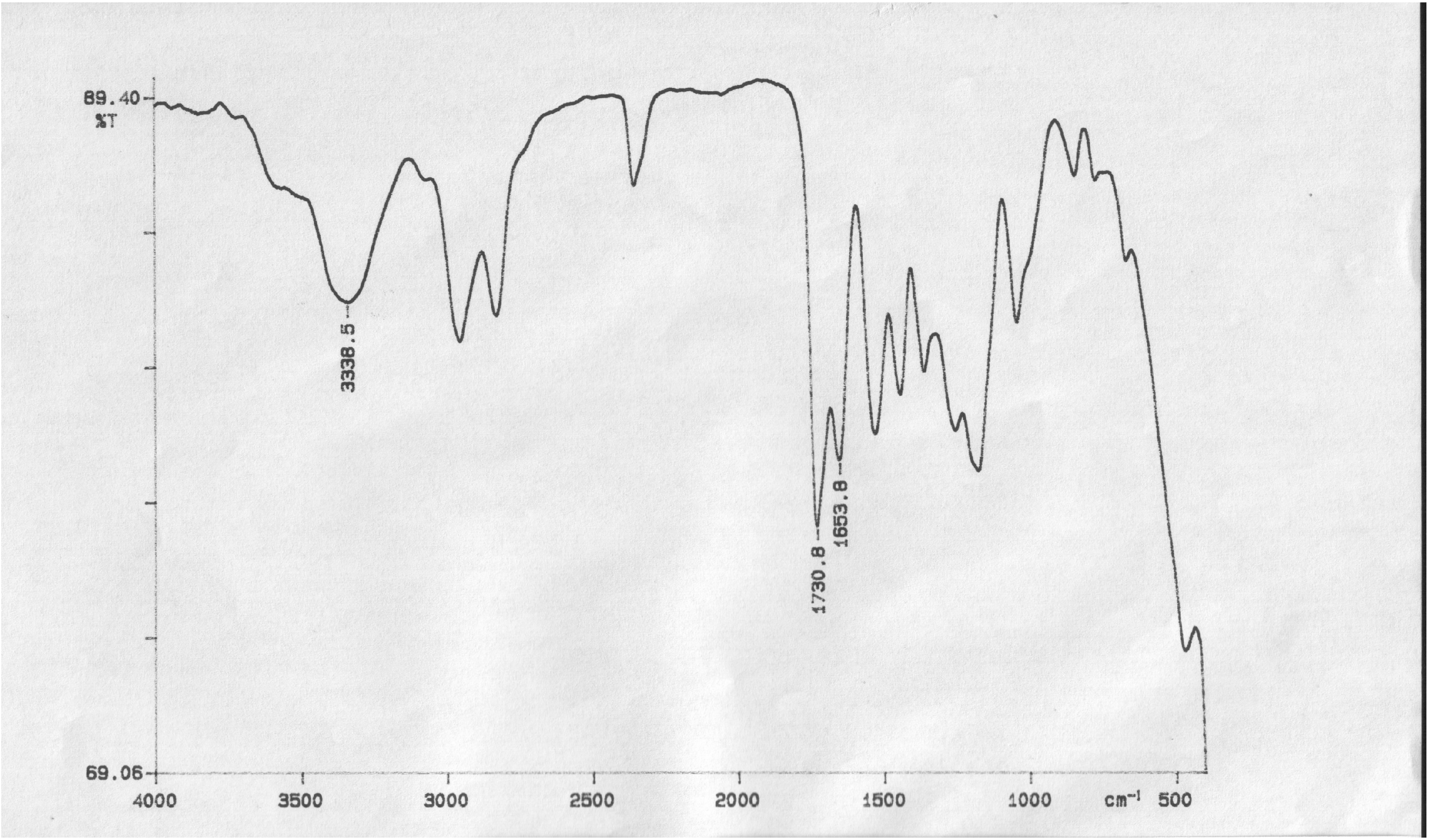


Figure A.8. IR spectrum of PAMAM G1.5 **93**

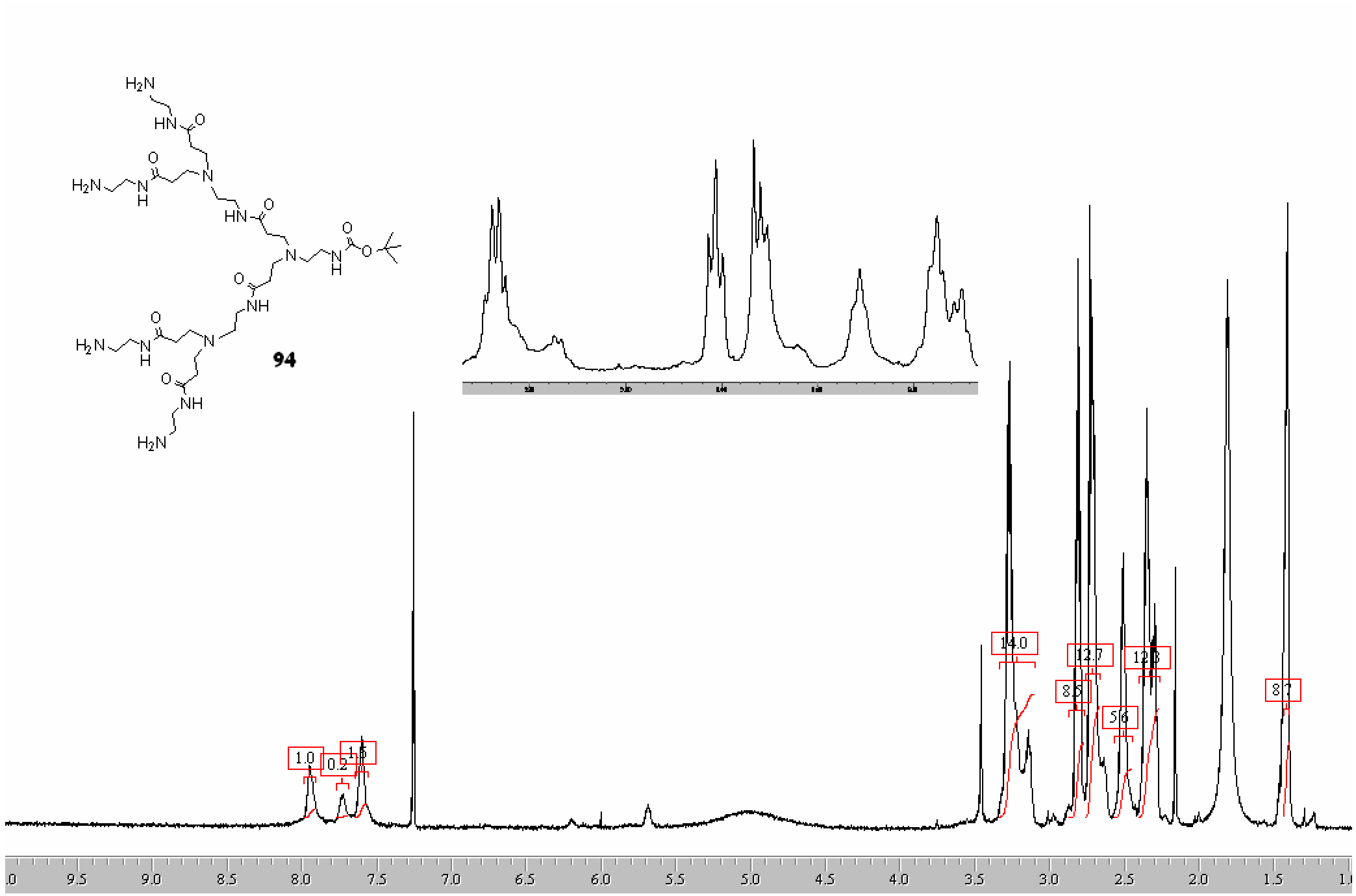


Figure A.9. ^{1}H NMR spectrum of PAMAM G2 **94**

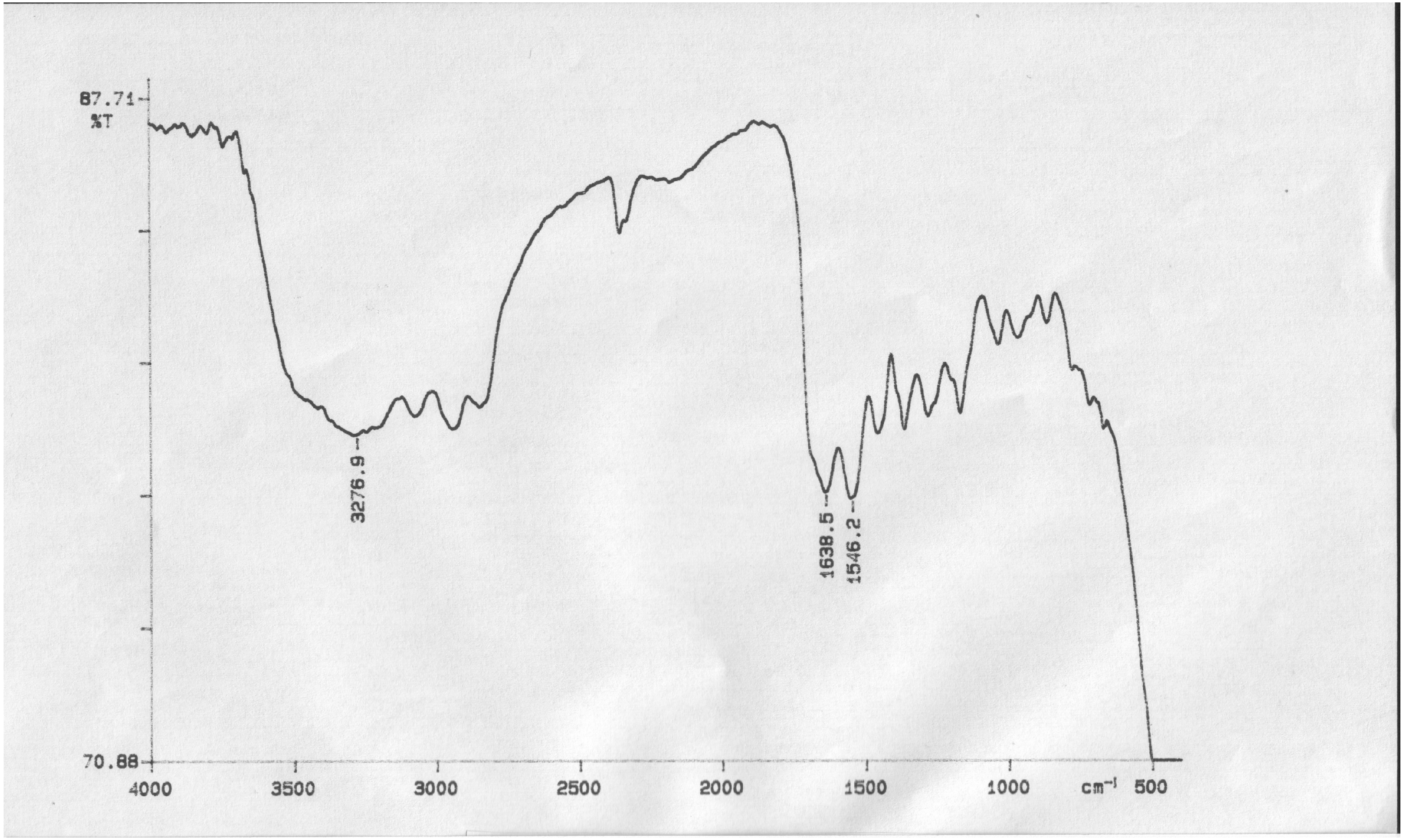


Figure A.10. IR spectrum of PAMAM G2 **94**

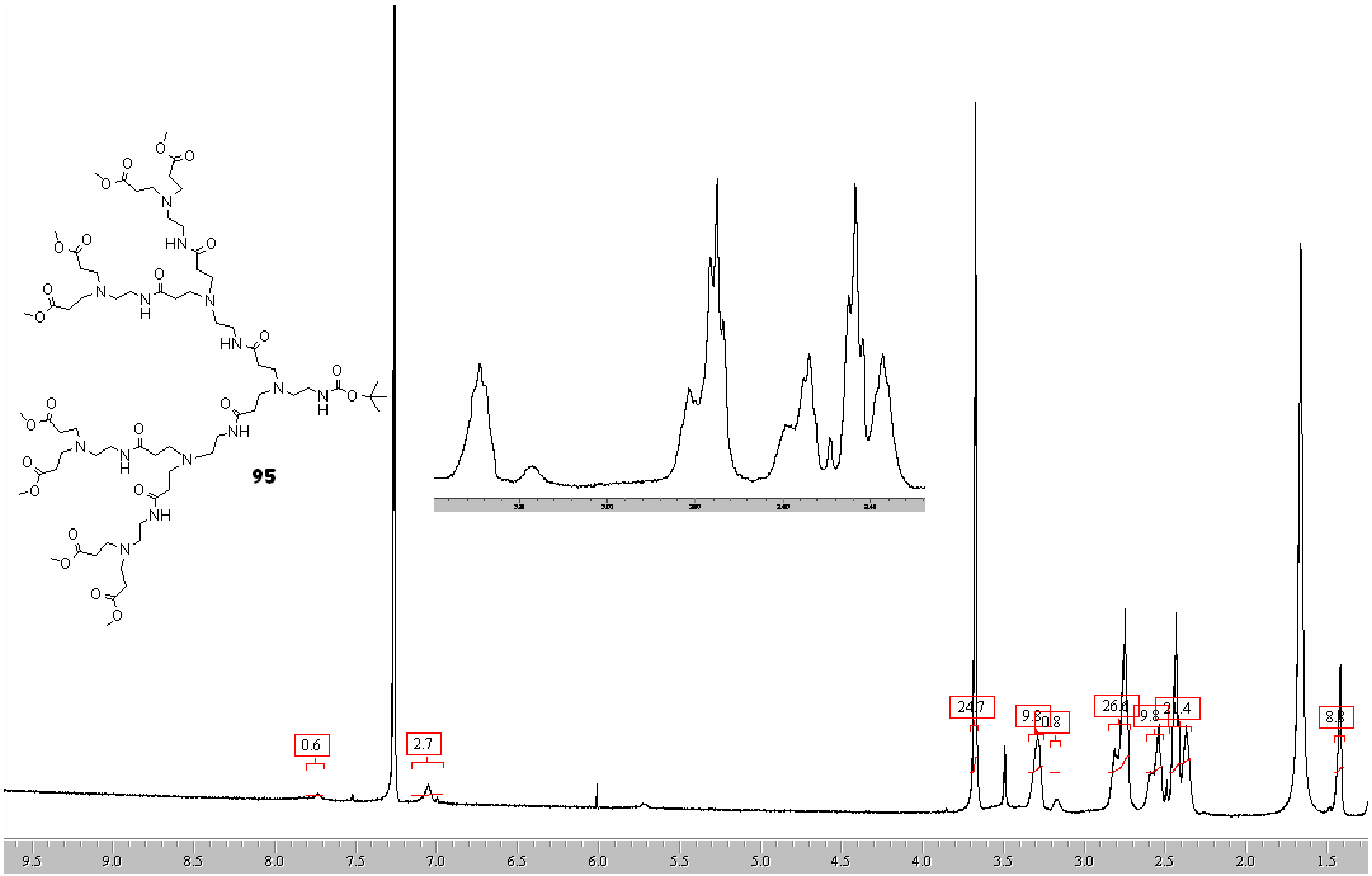


Figure A.11. ^{1}H NMR spectrum of PAMAM G2.5 **95**

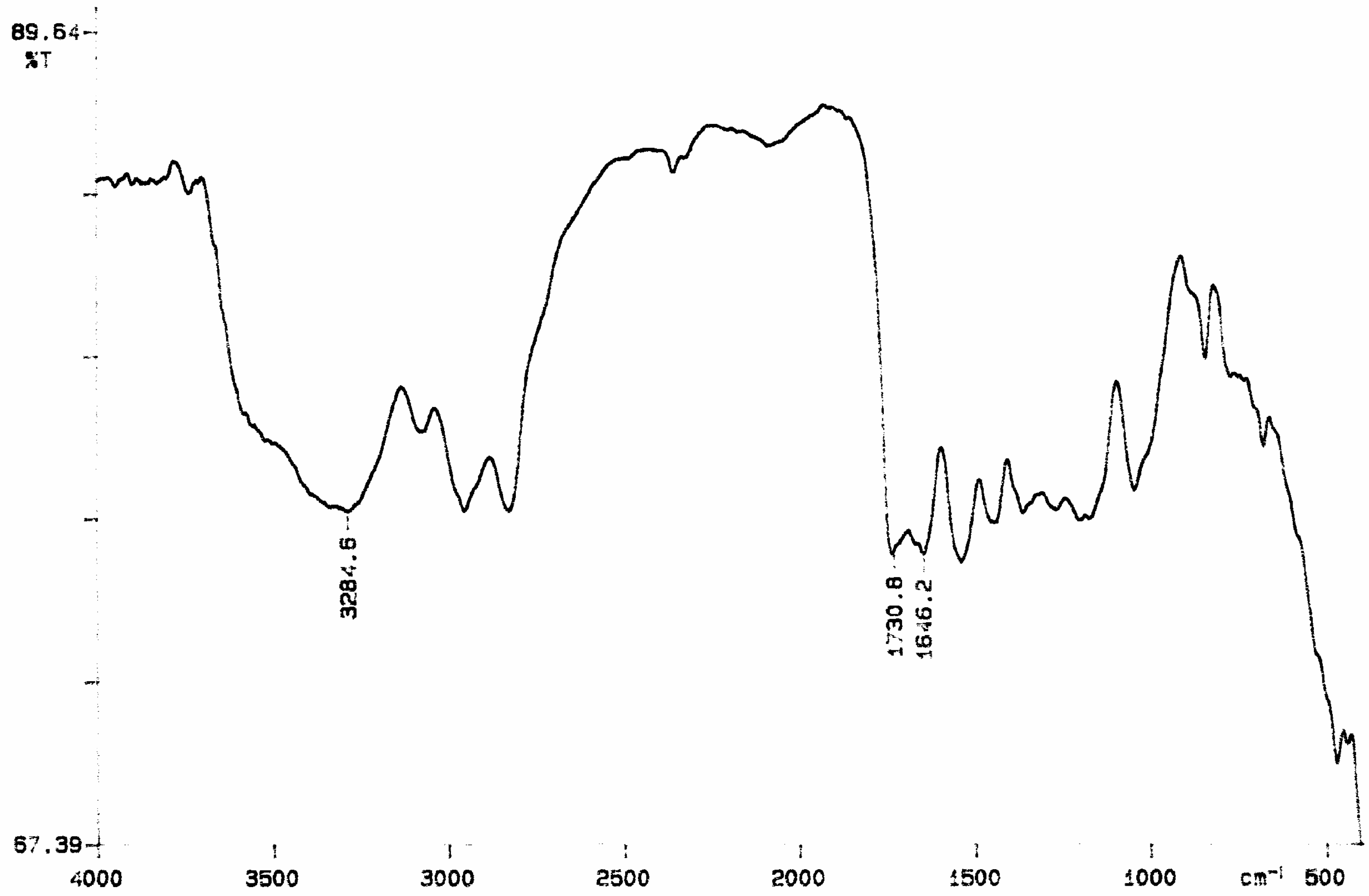


Figure A.12. IR spectrum of PAMAM G2.5 **95**

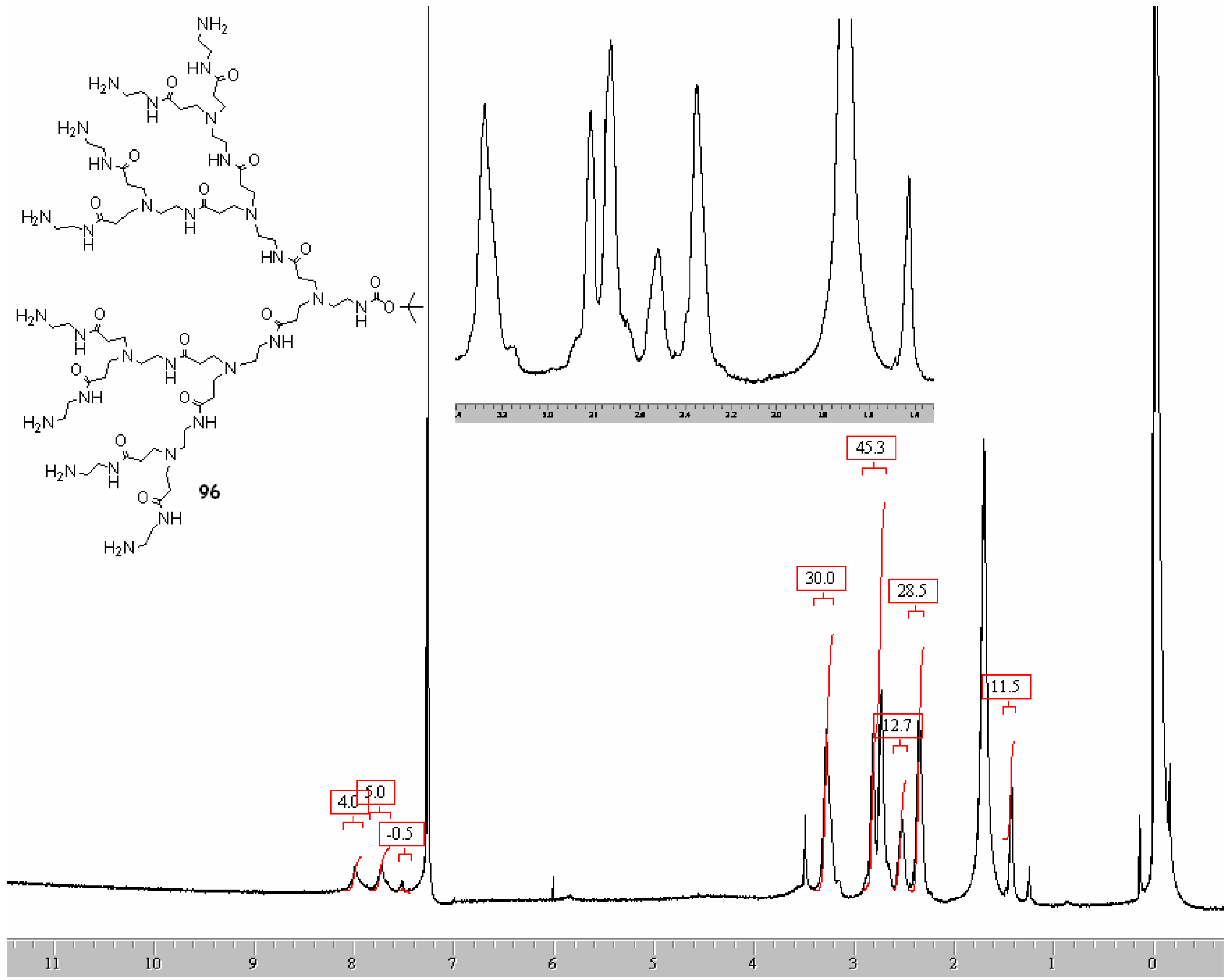


Figure A.13. ^{1}H NMR spectrum of PAMAM G3 **96**

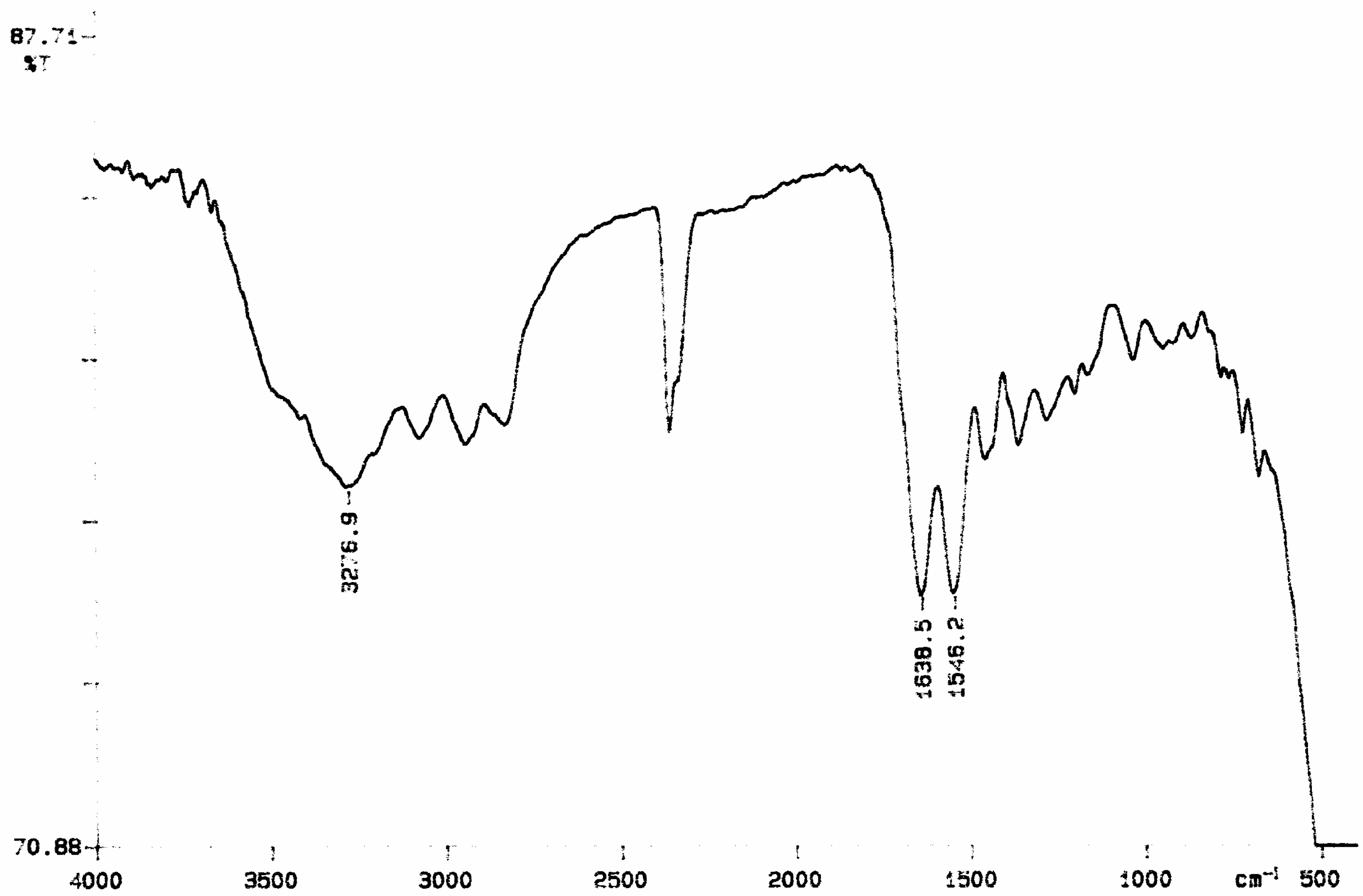


Figure A.14. IR spectrum of PAMAM G3 **96**

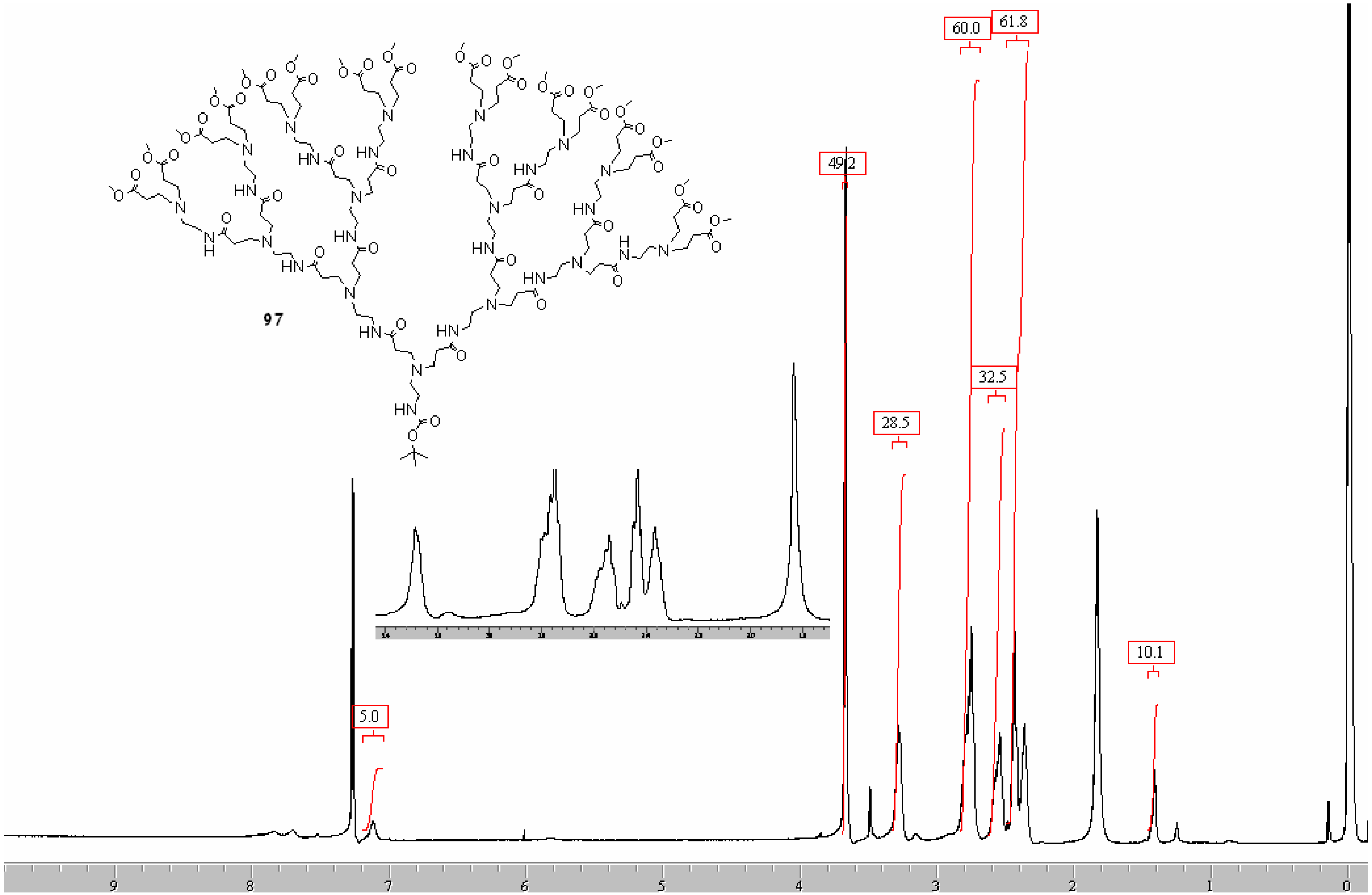


Figure A.15. ^{1}H NMR spectrum of PAMAM G3.5 **97**

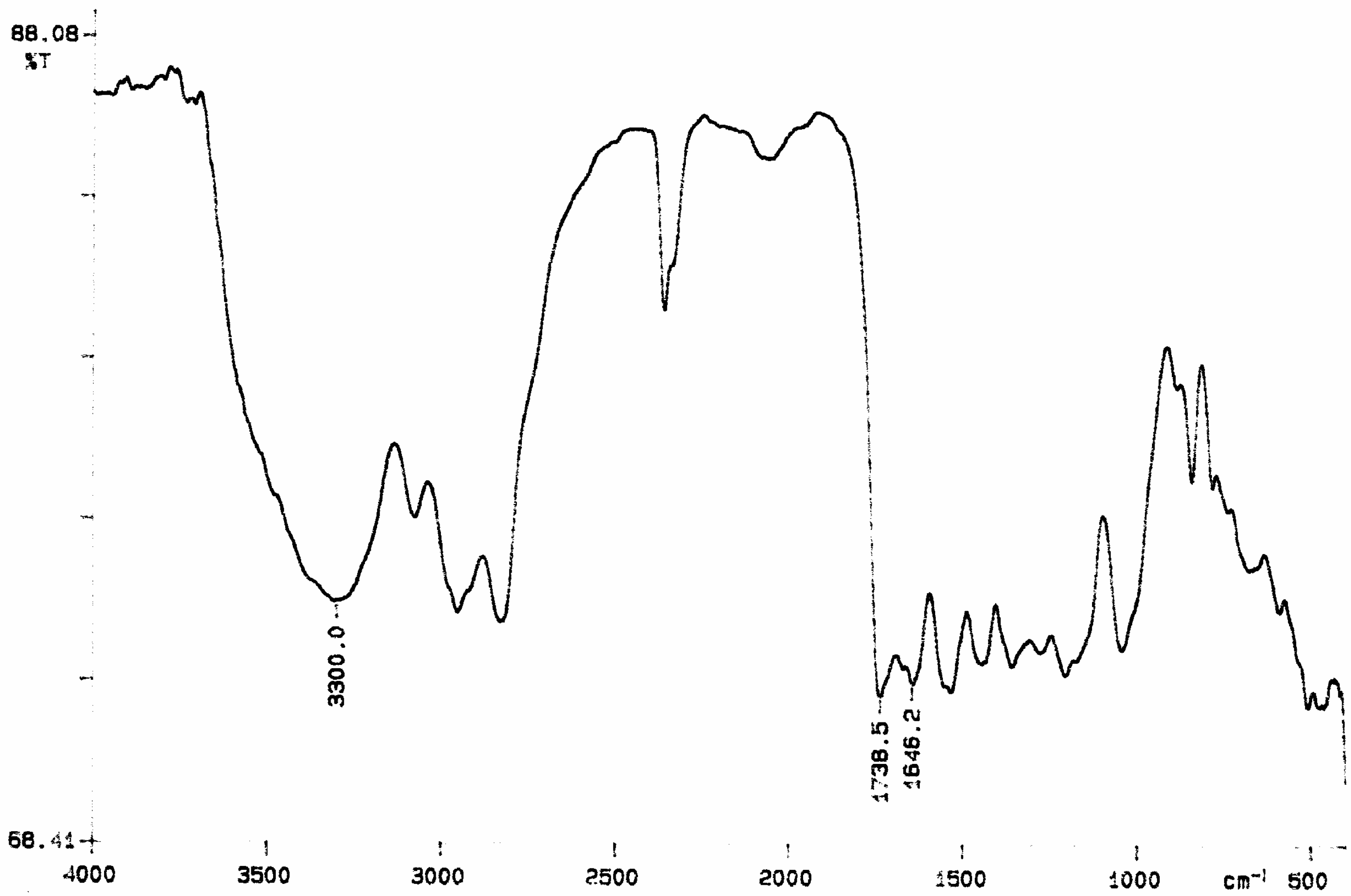


Figure A.16. IR spectrum of PAMAM G3.5 **97**

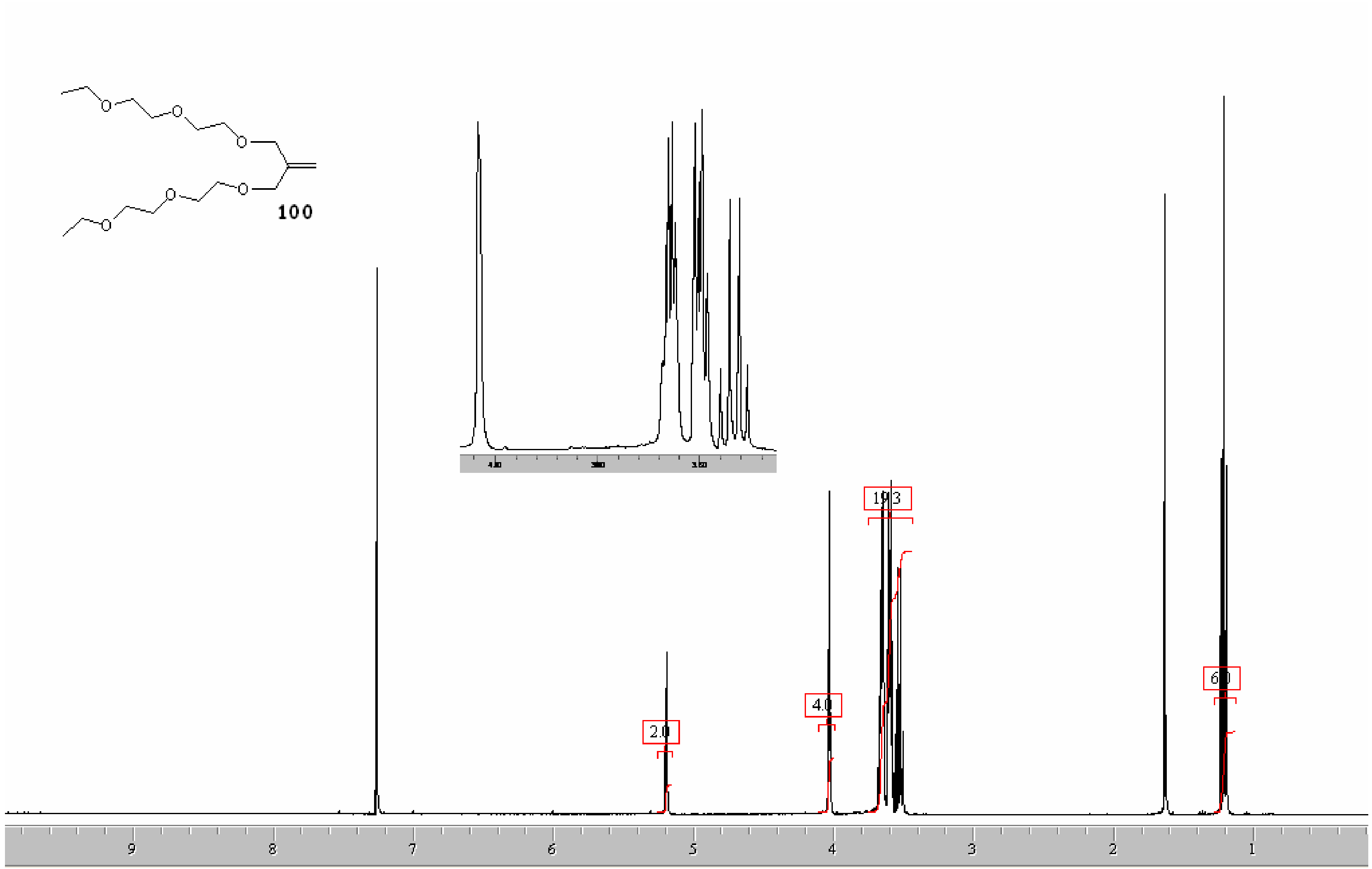


Figure A.17. ^{1}H NMR spectrum of ether G0.5 **100**

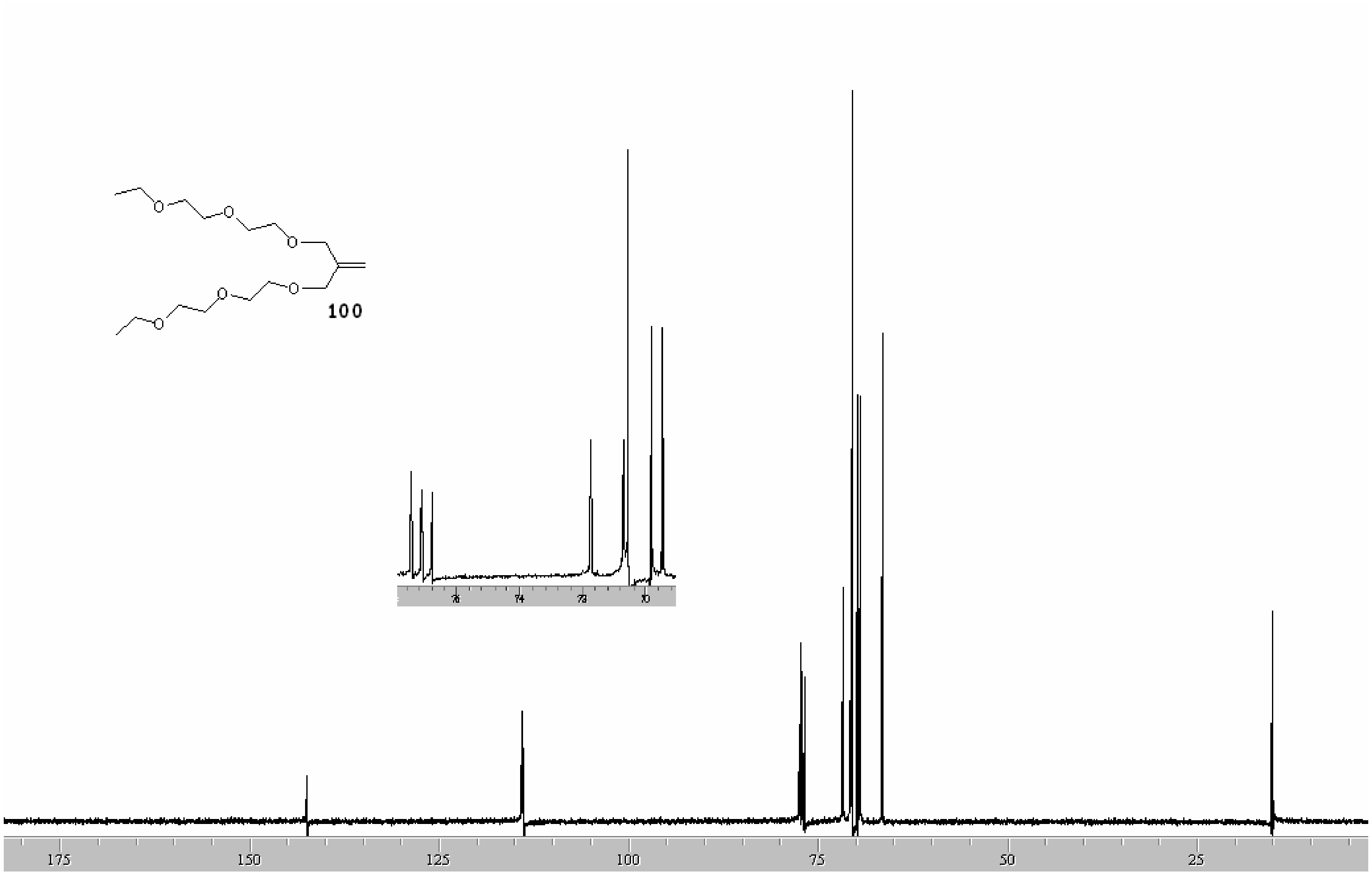


Figure A.18. ^{13}C NMR spectrum of ether G0.5 **100**

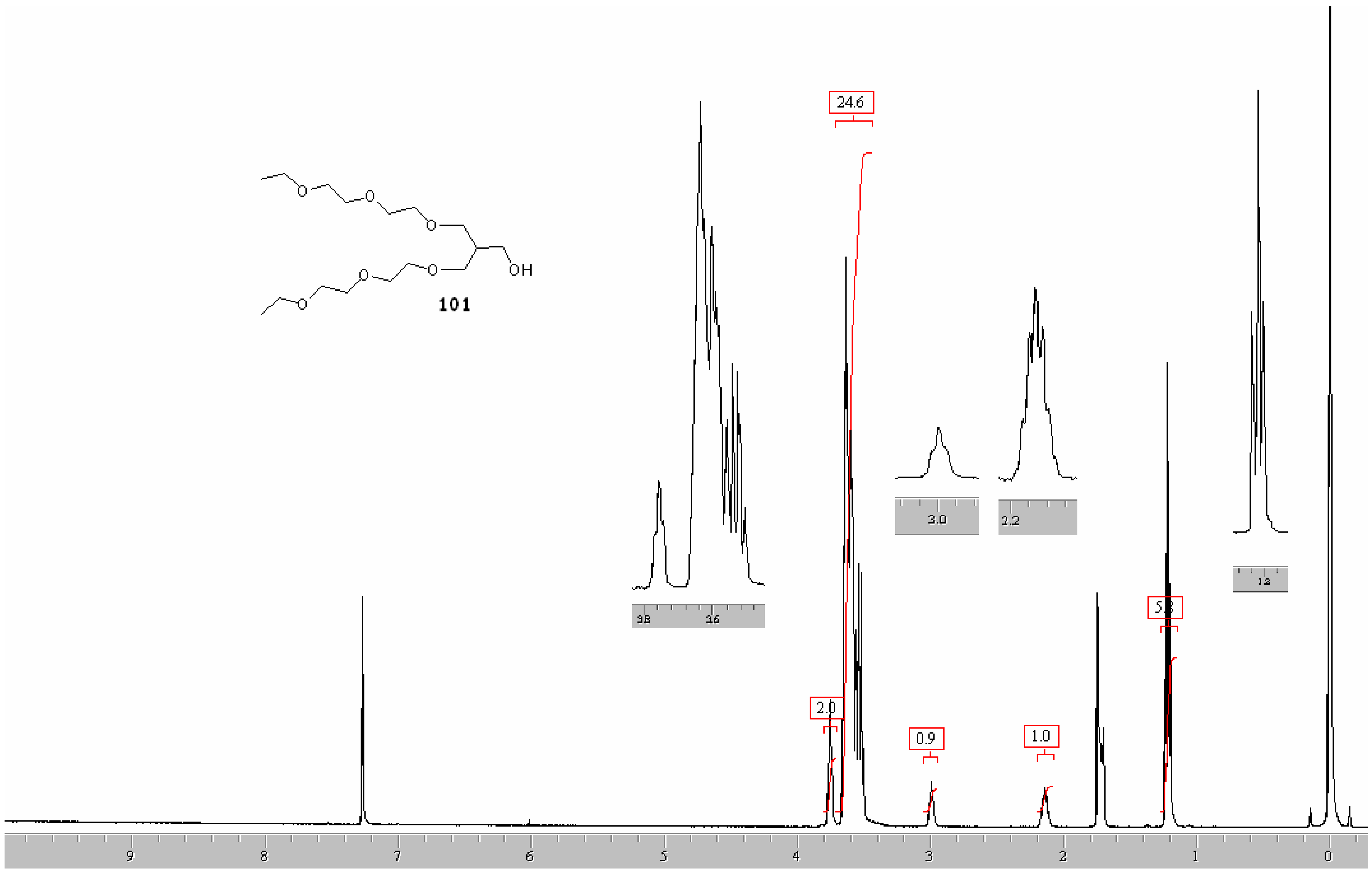


Figure A.19. ^{1}H NMR spectrum of ether G1 **101**

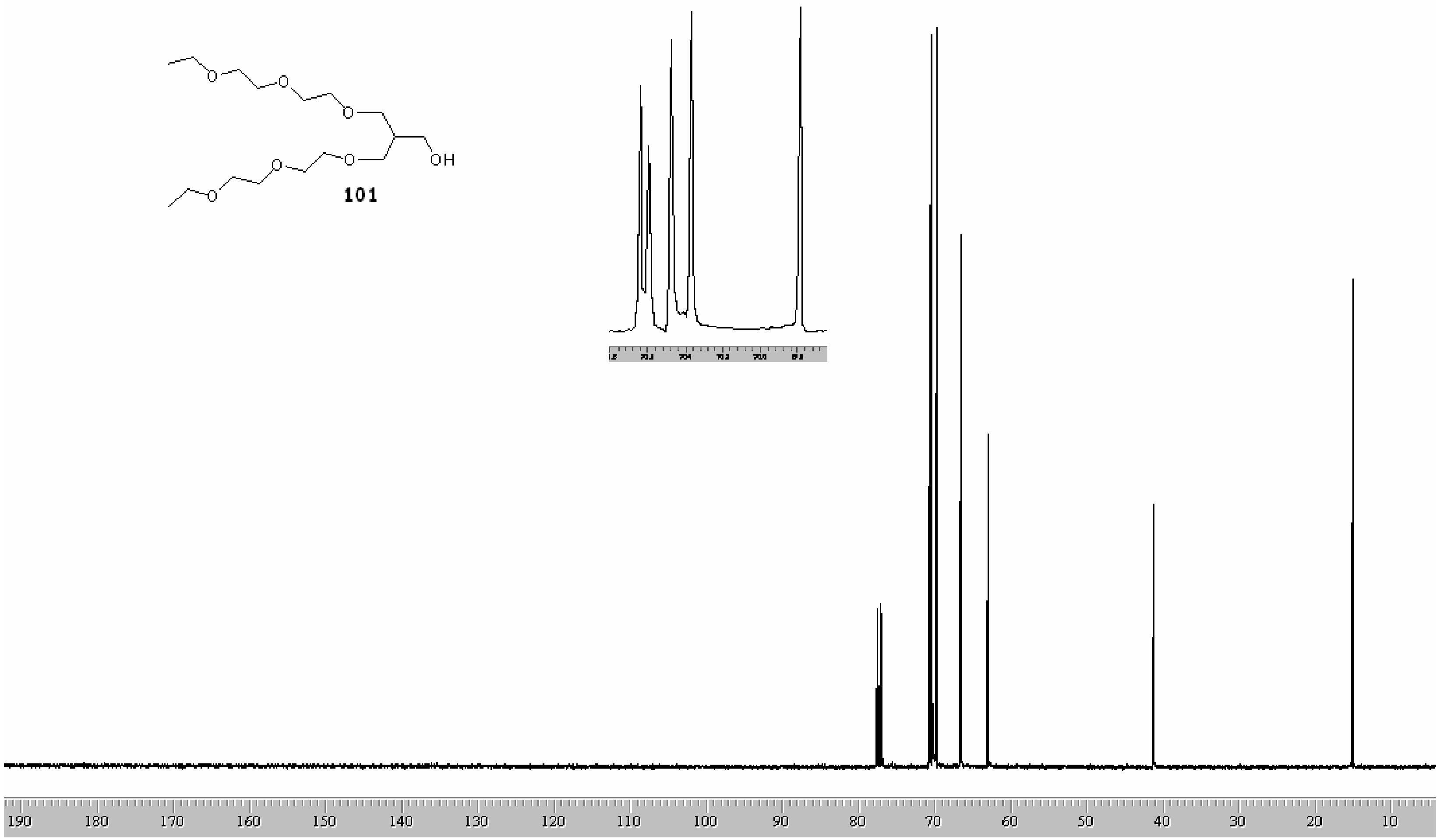


Figure A.20. ^{13}C NMR spectrum of ether G1 **101**

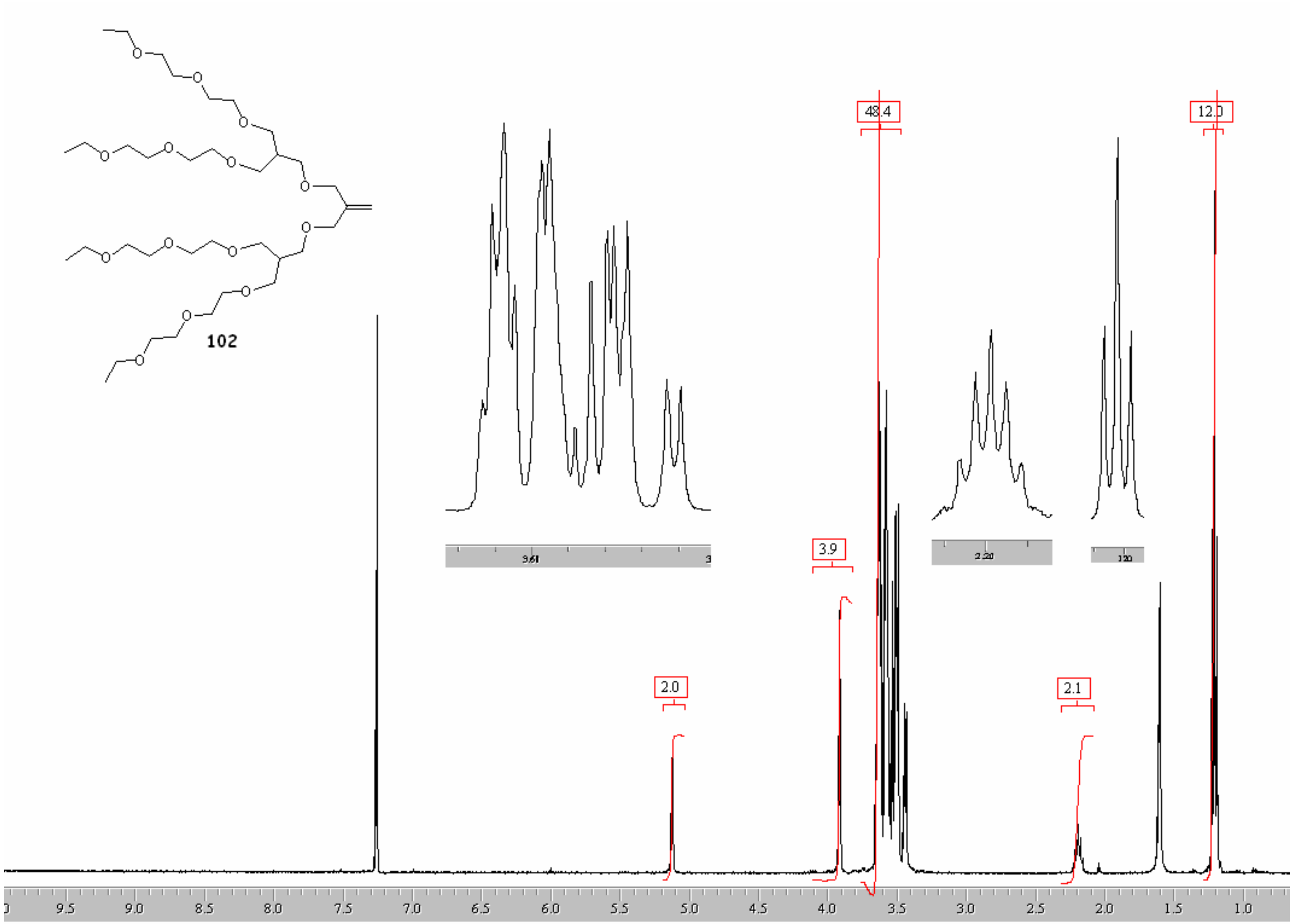


Figure A.21. ^{1}H NMR spectrum of ether G1.5 **102**

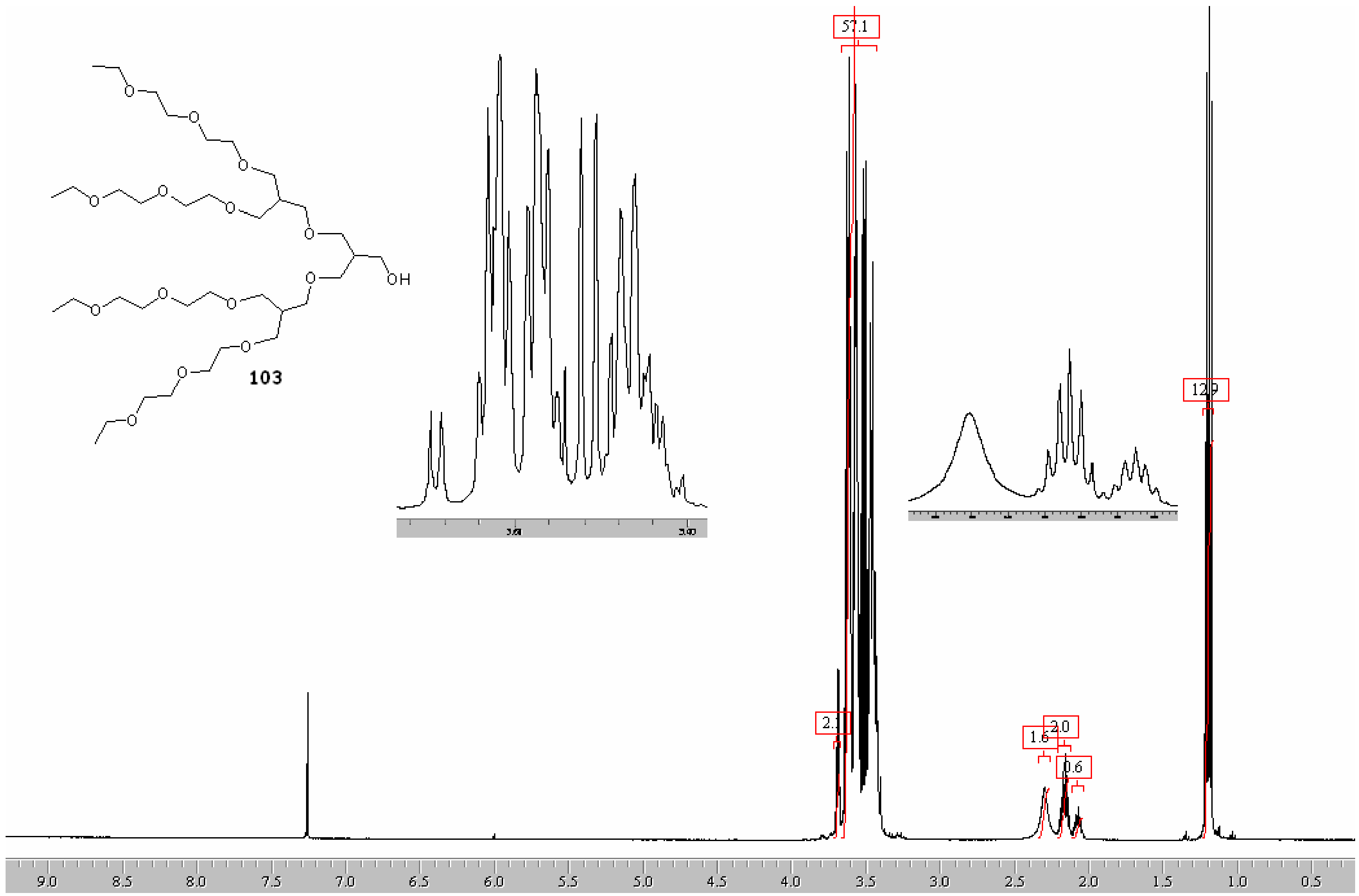


Figure A.22. ^{1}H NMR spectrum of ether G2 **103**

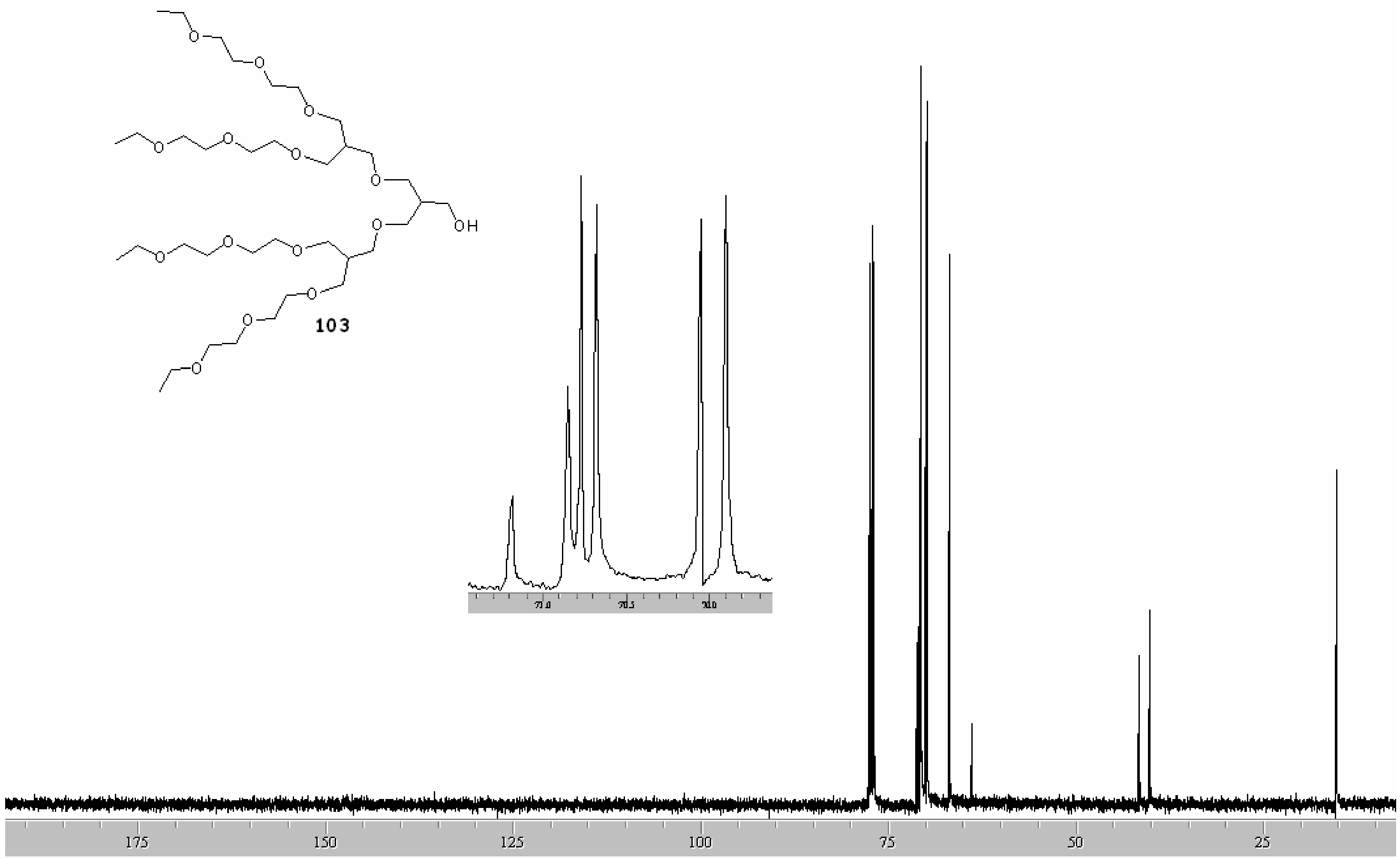


Figure A.23. ^{13}C NMR spectrum of ether G2 **103**

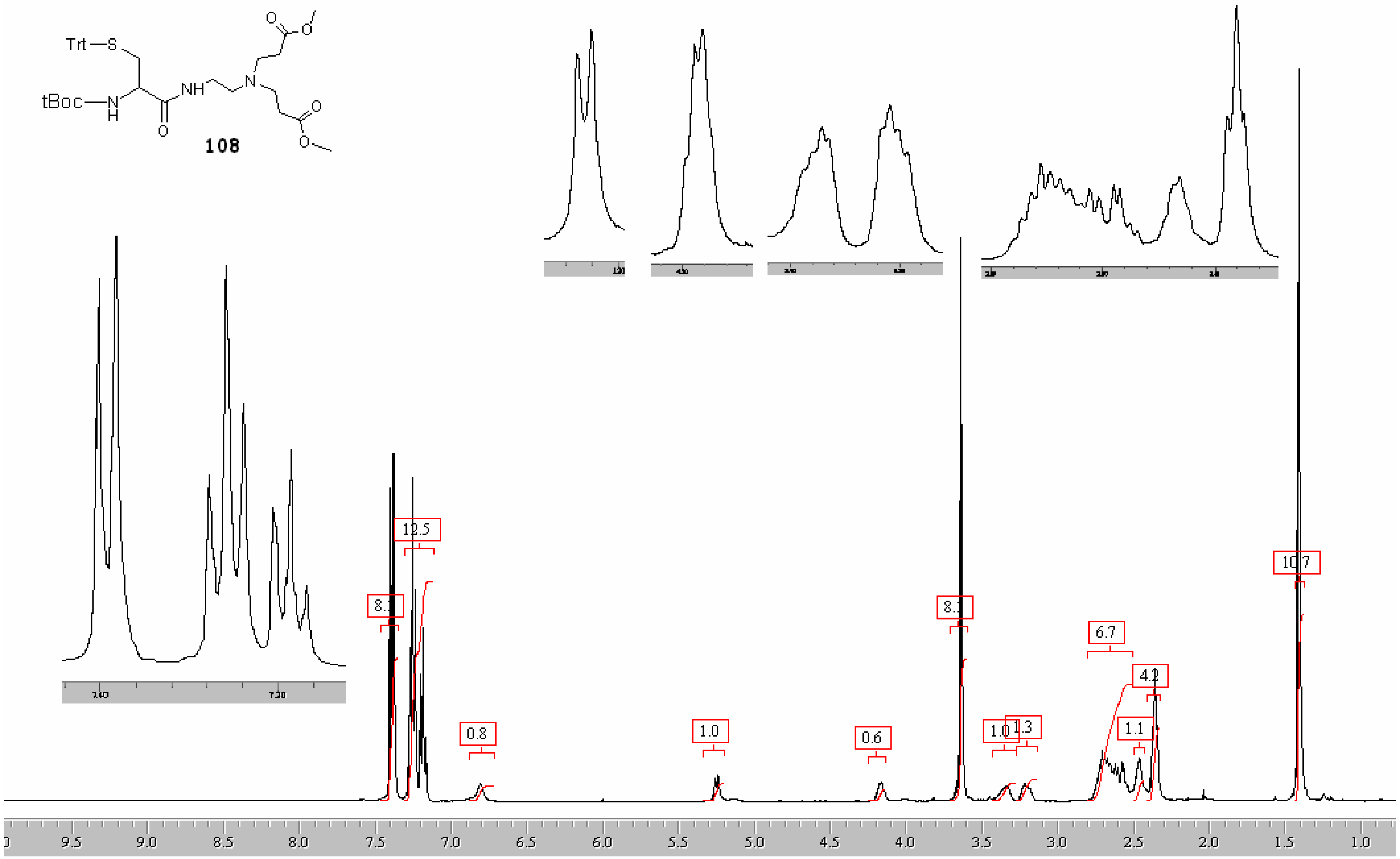


Figure A.24. ^{1}H NMR spectrum of S(Trt)N(Boc)Cys-PAMAM G0.5 **108**

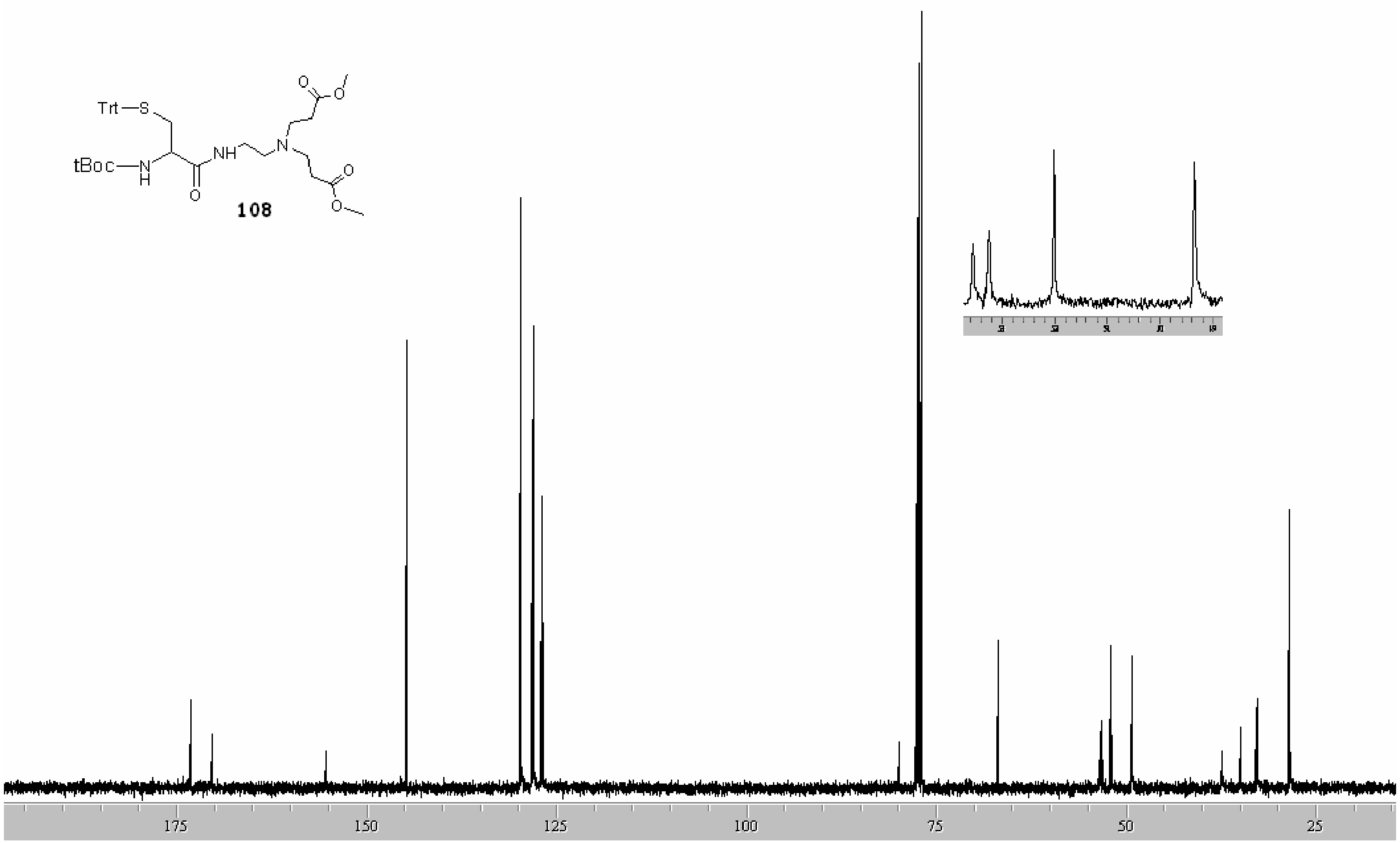


Figure A.25. ^{13}C NMR spectrum of S(Trt)N(Boc)Cys-PAMAM G0.5 **108**

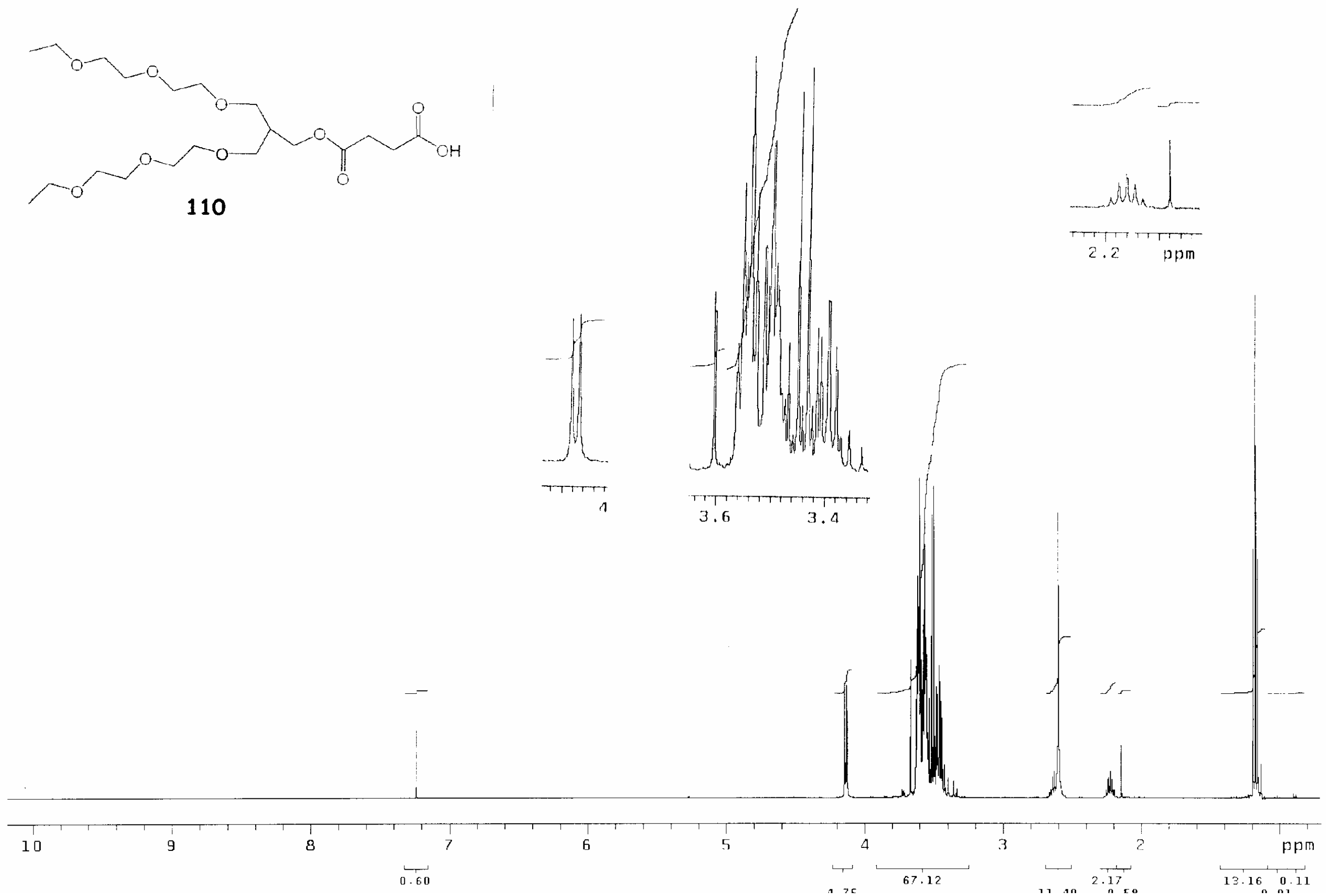


Figure A.26. ^{1}H NMR spectrum of ether G1 acid **110**

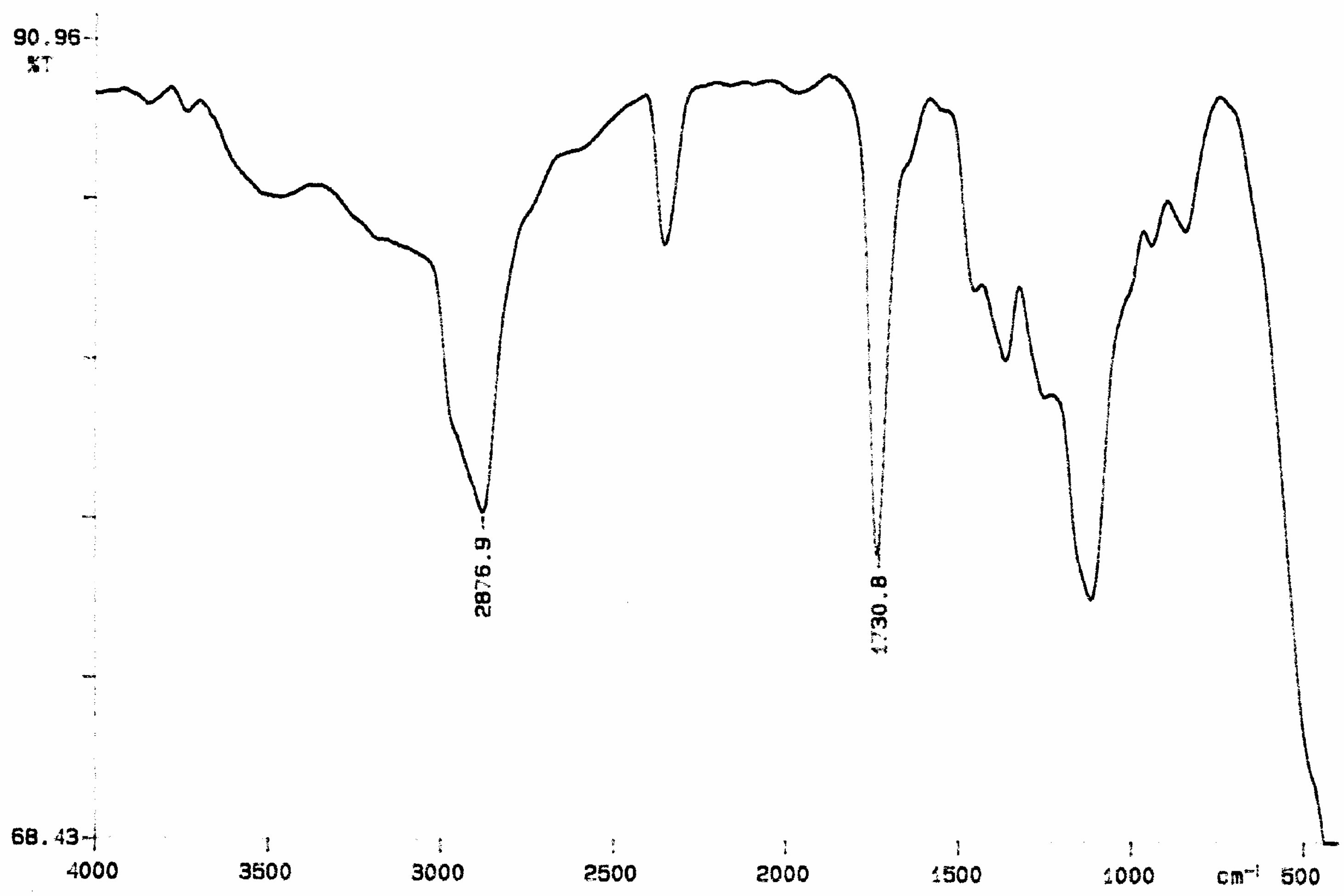


Figure A.27. IR spectrum of ether G1 acid **110**

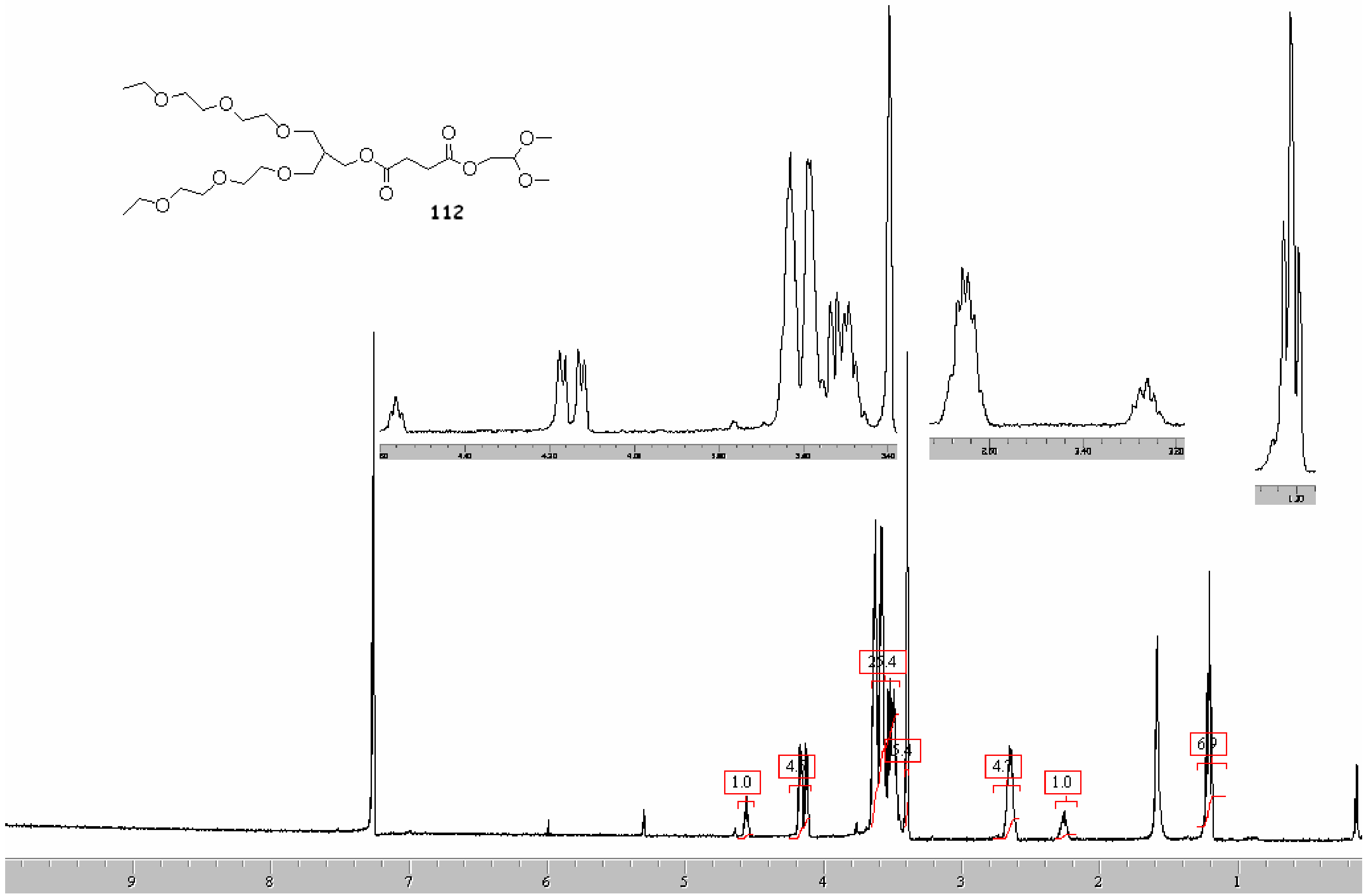


Figure A.28. ^{1}H NMR spectrum of ether G1 acetal **112**

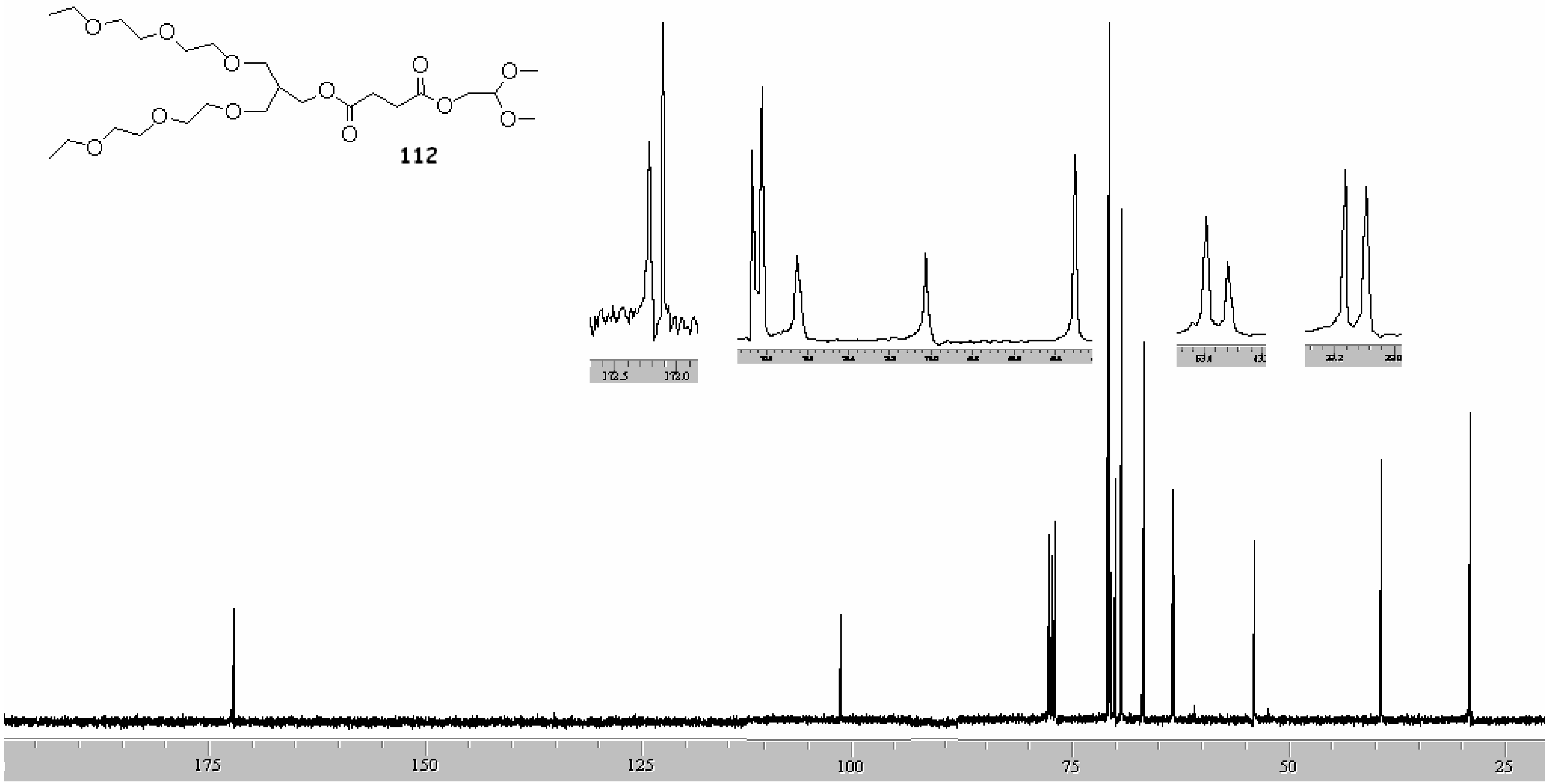


Figure A.29. ^{13}C NMR spectrum of ether G1 acetal **112**

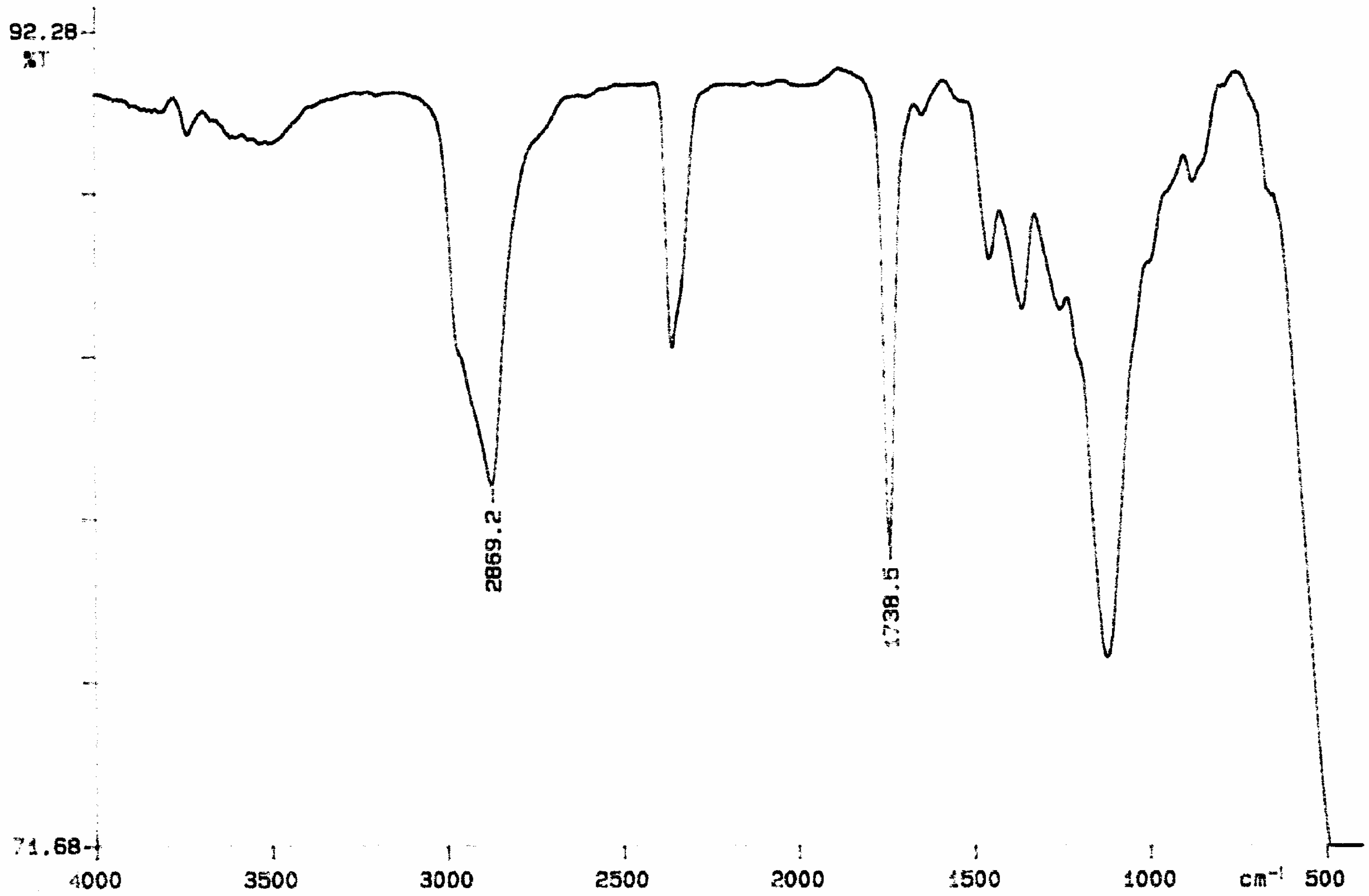


Figure A.30. IR spectrum of ether G1 acetal **112**

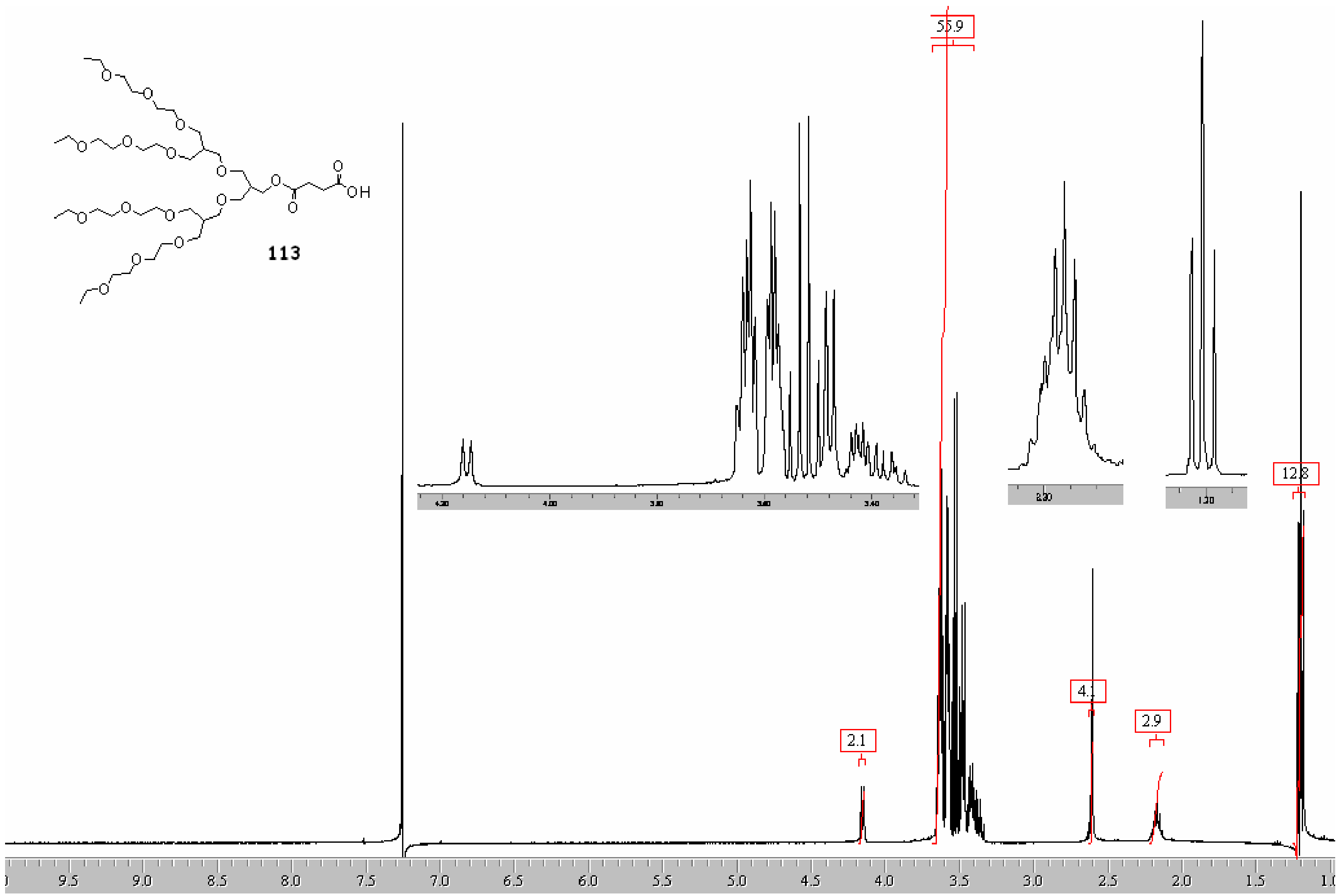


Figure A.31. ^{1}H NMR spectrum of ether G2 acid **113**

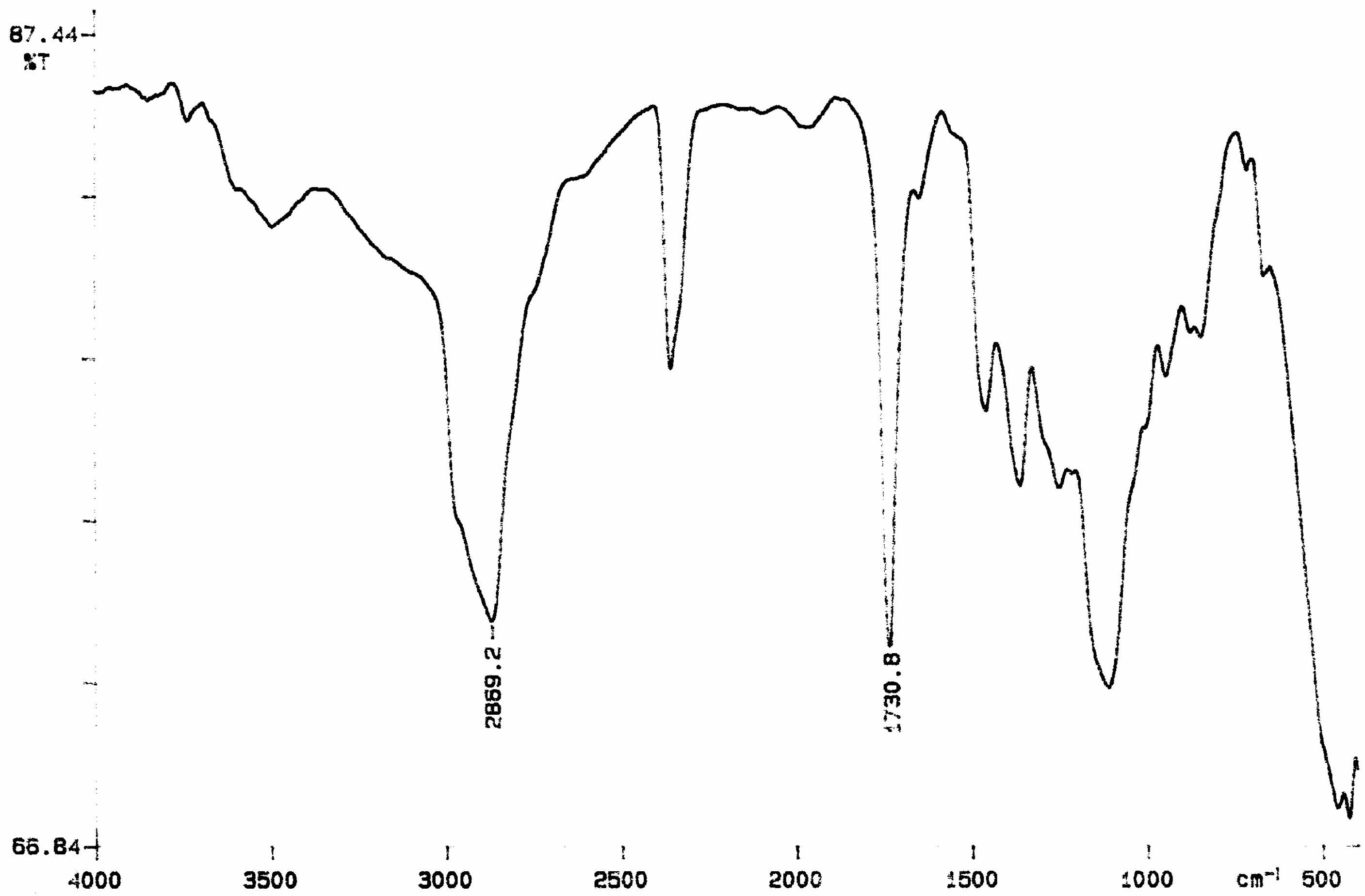


Figure A.32. IR spectrum of ether G2 acid **113**

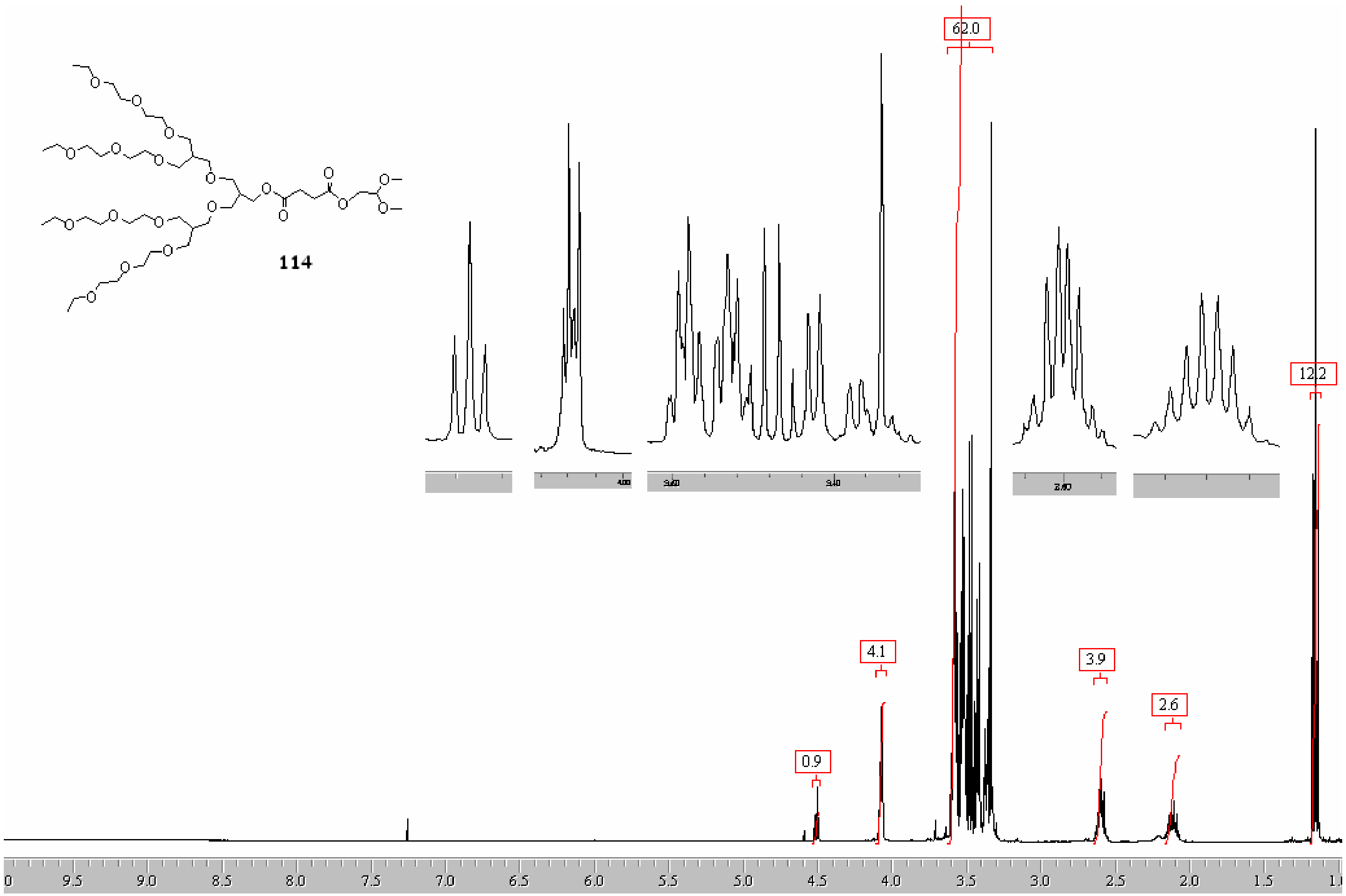


Figure A.33 ^{1}H NMR spectrum of ether G2 acetal **114**

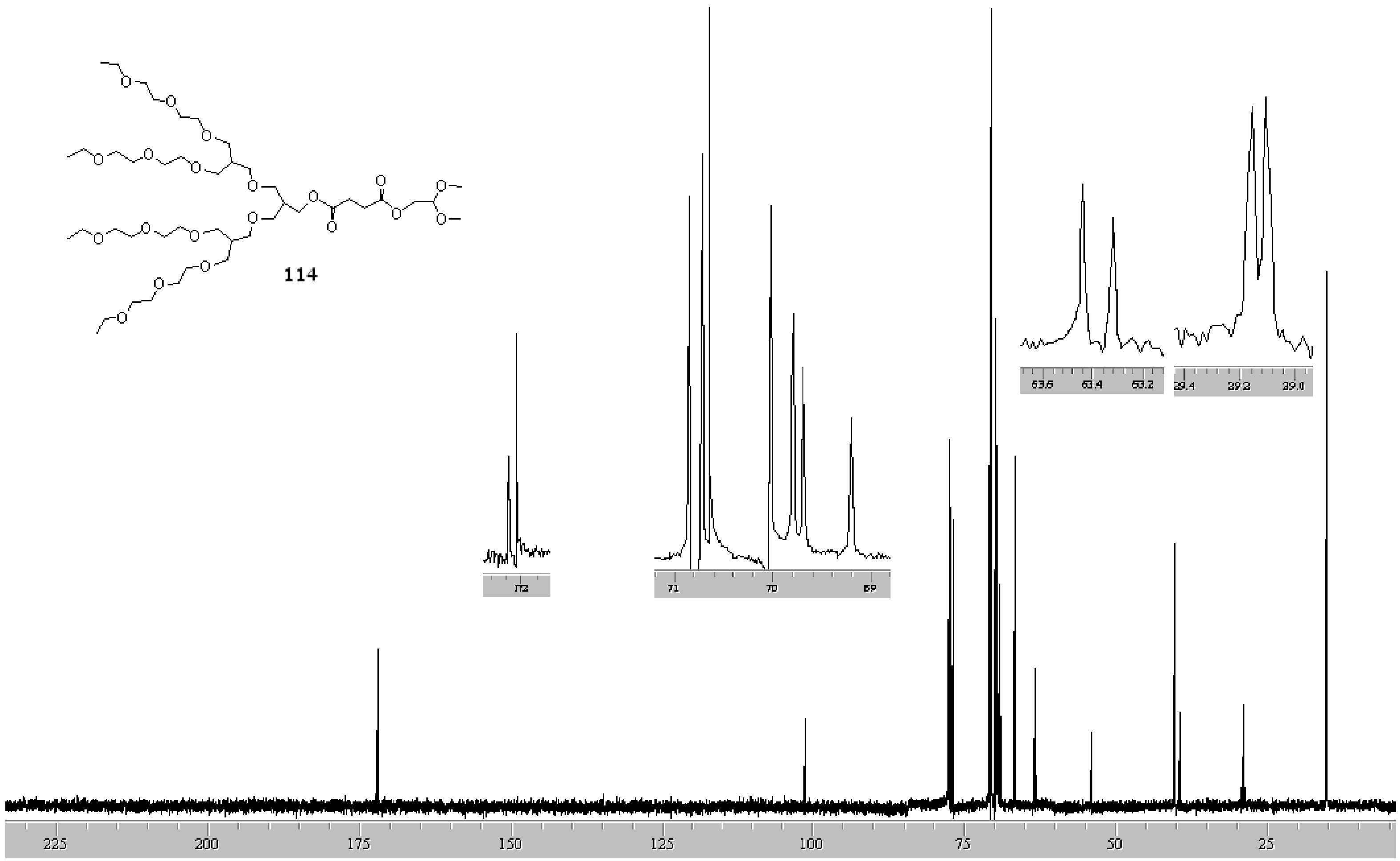


Figure A.34 ^{13}C NMR spectrum of ether G2 acetal **114**

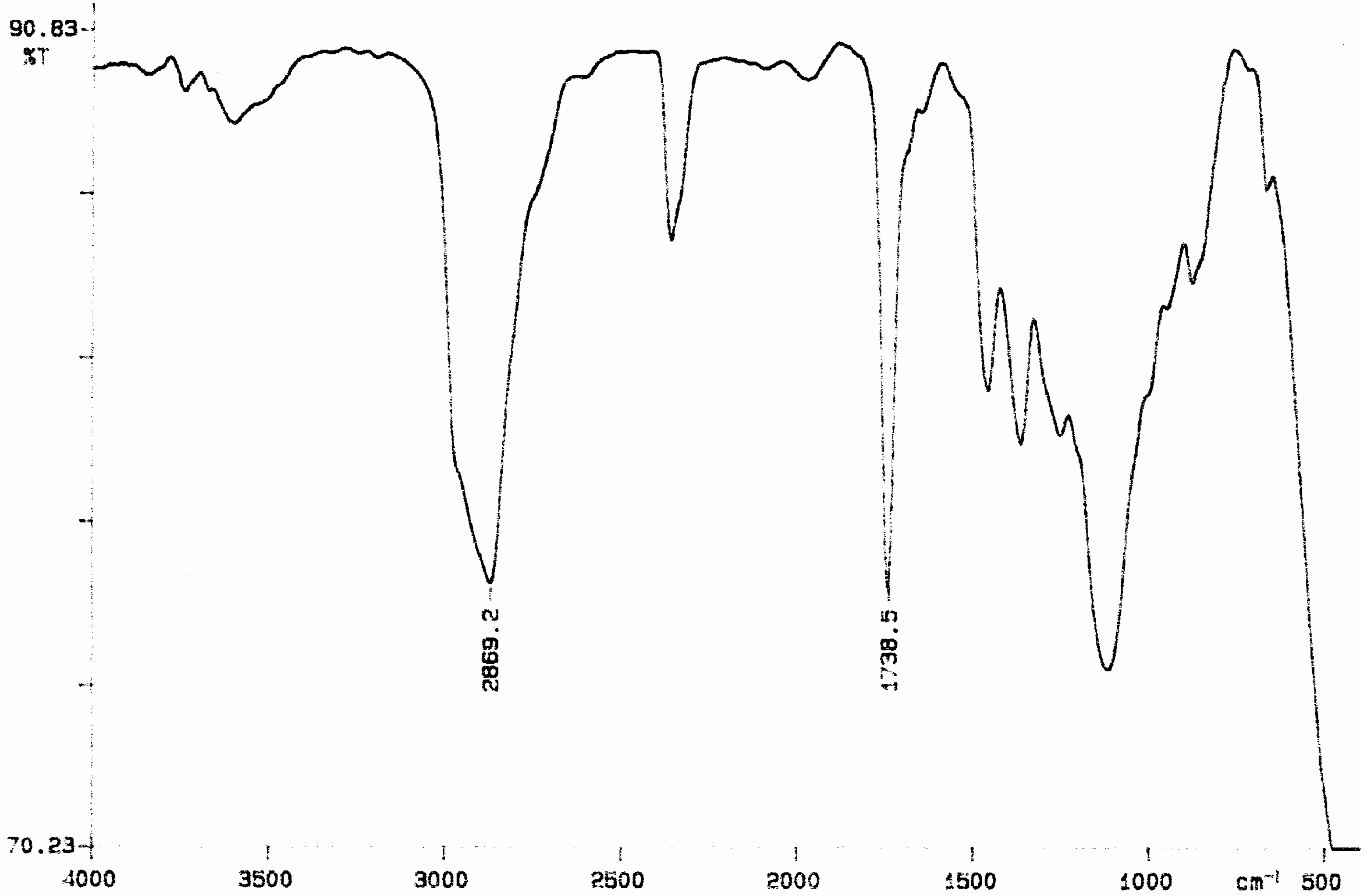


Figure A.35 IR spectrum of ether G2 acetal **114**

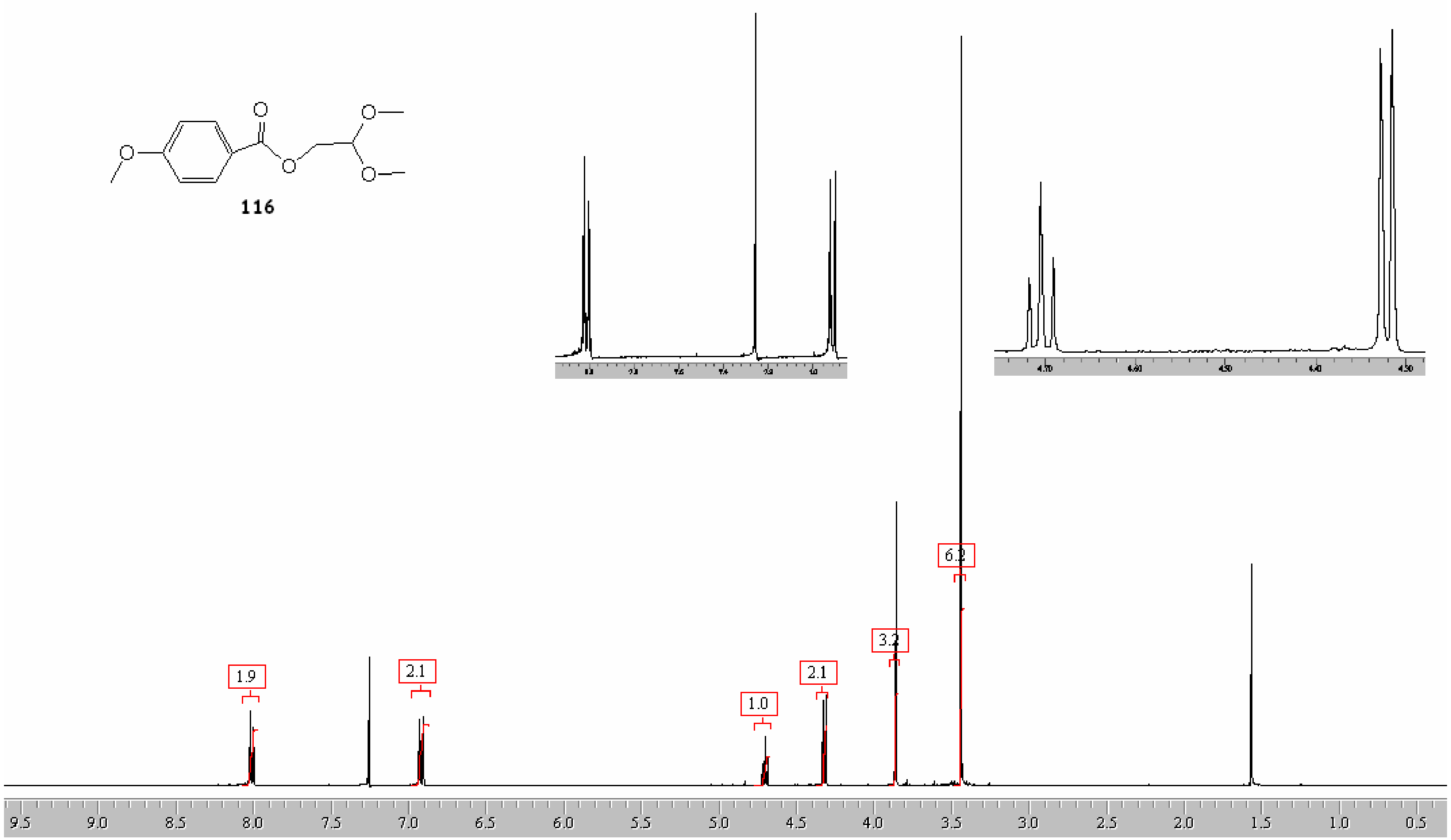


Figure A.36 ^{1}H NMR spectrum of anisic acetal **116**

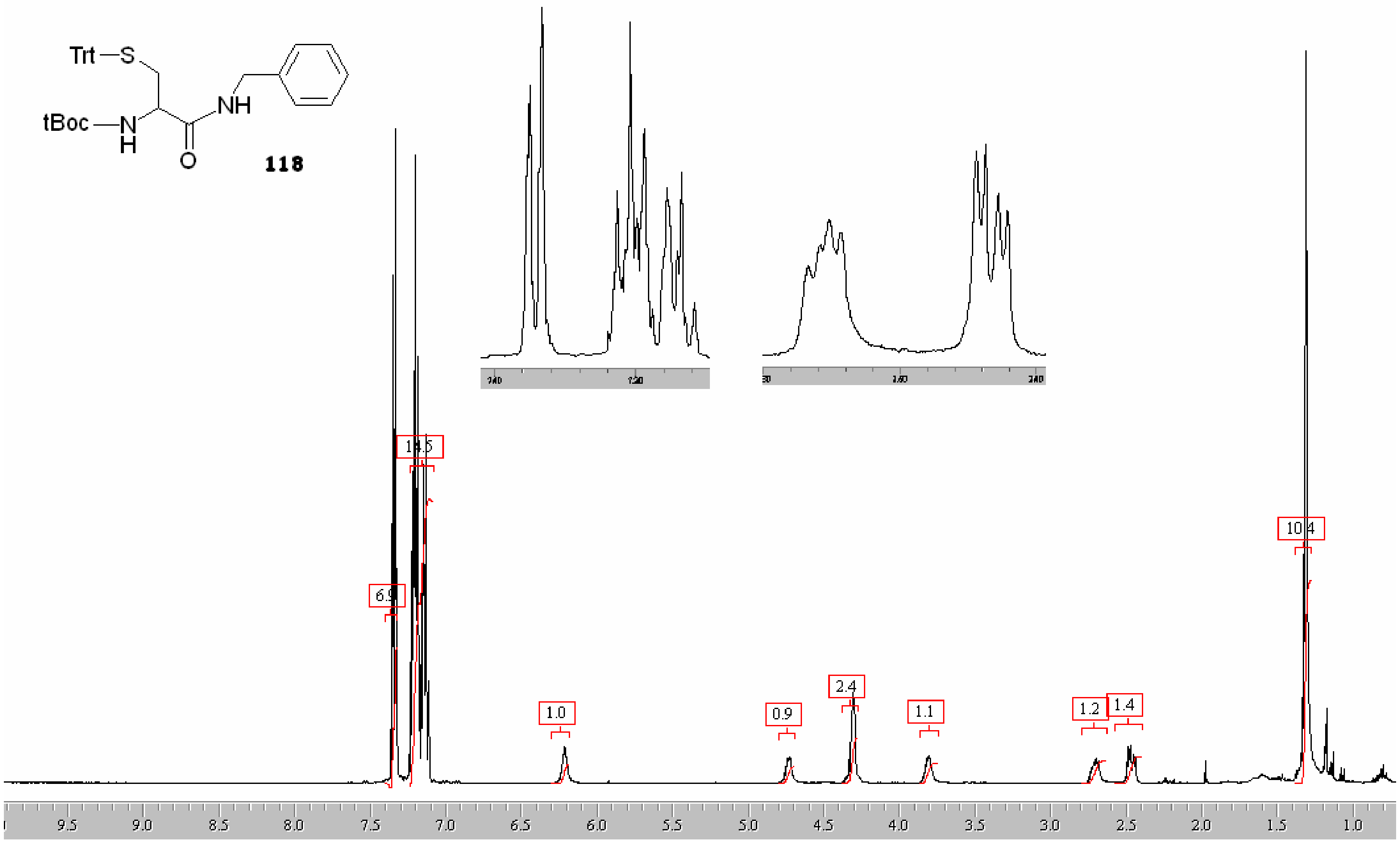


Figure A.37 ^{1}H NMR spectrum of S(Trt)N(Boc) benzylamine **118**

REFERENCES

1. Tomalia, D. A. and J. M. J. Fréchet, Dendrimers and Other Dendritic Molecules, John Wiley and Sons Ltd., West Sussex, 2001.

2. Tomalia, D. A., H. Baker, J.R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Polym. J. (Tokyo)* 1985, *17*, 117-132.

3. Aulenta, F., W. Hayes and S. Rannard, *Eur. Poly. J.* 2003, 39, 1741–1771.

4. Fréchet, J. M. J. and S. M. Grayson, *Chem. Rev.* 2001, *101,* 3819-3867.

5. Tomalia, D. A., A. M. Naylor and W. A. Goddard III, *Angew. Chem. Int. Ed.* 1990, *29*, 138-175.

6. Buhleier, E. W., W. Wehner, and F. Vögtle, *Synthesis* 1978, 155-158.

7. Denkewalter, R. G., J. Kolc and W. J. Lukasavage, U.S. Pat. 4,-289,872, 1981.

8. Denkewalter, R. G., J. Kolc and W. J. Lukasavage, U.S. Pat. 4,-360,646, 1982.

9. Denkewalter, R. G., J. Kolc and W. J. Lukasavage, U.S. Pat. 4,-410,688, 1983.

10. Newkome, G. R., Z. Q. Yao, G. R. Baker and K. Gupta, *J. Org. Chem.* 1985, *50*, 2003-2004.

11. Hawker, C. J. and J. M. J. Fréchet, *J. Am. Chem. Soc.* 1990, *112*, 7638-7647.

12. Hawker, C. J., K. L. Wooley, and J. M. J. Fréchet, *Macromol. Symp.* 1994, *77*, 11-20.

13. Hawker, C. J. and J. M. J. Fréchet, *J. Am. Chem. Soc.* 1992, *114*, 8405-8413.

14. Xu, Z. and J. S. Moore, *Acta Polym.* 1994, *45*, 83-87.

15. Devadoss, C., P. Bharathi and J. S. Moore, *J. Am. Chem. Soc.* 1996, *118*, 9635-9644.

16. Morikawa, A. and K. Ono, *Macromolecules* 1999, *32*, 1062-1068.

17. Hawker, C. J. and J. M. J. Fréchet, *J. Am. Chem. Soc.* 1992, *114*, 8405-8413.

18. Kimoto, A., J. Cho, M. Higuchi and K. Yamamoto, *Macromolecules* 2004, *37,* 5531-5537.

19. Chow, H. F. and C. C. Mak, *Pure Appl. Chem.* 1997, *69*, 483-488.

20. Aoi, K., K. Itoh and M. Okada, *Macromolecules* 1997, *30*, 8072- 8074.

21. Wooley, K. L., C. J. Hawker and J. M. J. Fréchet, *J. Am. Chem. Soc.* 1993, *115*, 11496-11505.

22. Hawker, C. J., K. L. Wooley and J. M. J. Fréchet, *J. Chem. Soc., Perkin Trans. 1* 1993, 1287-1297.

23. Martin, I. K. and L. J. Twyman, *Tetrahedron Lett.* 2001, 1119-1121.

24. Ito, M., T. Imae, K. Aoi, K. Tsutsumiuchi, H. Noda and M. Okada, *Langmuir*, 2002, *18,* 9757-9764.

.

25. Fréchet, J. M. J. *J. Am. Chem. Soc.* 2002,14137.

26. Mihov, G., I. Scheppelmann and K. Müllen, *J. Org. Chem.* 2004, *69*, 8029-8037.

27. Maraval, V., R. M. Sebastian, F. Ben, R. Laurent, A. M. Caminade and J. P. Majoral, *Eur. J. Inorg. Chem.* 2001, 1681-1691.

28. Ratner, S. and H. T. Clarke, *J. Am. Chem. Soc.* 1937, *59*, 200-206.

29. Tam, J. and C. F. Liu, *J. Am. Chem. Soc.* 1994, *116*, 4149-4153.

30. Liu, C., C. Rao and J. Tam, *J. Am. Chem. Soc.* 1996, *118*, 307-312.

31. Tam, J., Q. Yu and Z. Miao, *Biopolymers* 1999, *51*, 311-332.

32. Miao, Z. and J. Tam, *J. Am. Chem. Soc.* 2000, *122*, 4253-4260.

33. Eisenfu, A., P. S. Arora, G. Sengle, L. R. Takaoka, J. S. Nowick and M. A. Famuloka, *Bioorg. Med. Chem.*, 2003, *11*, 235–249.

34. Wang, B., Y. Luo, X. Jia, Y. Gong, Y. Ji and L. Yang, *Acta Polym. Sinica*, 2004, 304-308.

35. Lee, M., Y. S. Jeong, B. K. Cho, N. K. Oh and W. C. Zin, *Chem. Eur. J.* 2002, *8*, 876-883.