

APPLICATIONS OF TRIAZINE CHEMISTRY: EDUCATION,
REMEDICATION, AND DRUG DELIVERY

A Thesis

by

SUSAN E. HATFIELD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2007

Major Subject: Chemistry

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Approved by:

Chair of Committee,	Eric E. Simanek
Committee Members,	David E. Bergbreiter
	Stephen A. Miller
	Scott Senseman
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ABSTRACT

Applications of Triazine Chemistry: Education,
Remediation, and Drug Delivery. (May 2007)

Susan E. Hatfield, B.S., University of Arkansas at Monticello

Chair of Advisory Committee: Dr. Eric E. Simanek

Triazine chemistry has many applications from industrial usage, such as melamine resins, to academic interests in dendritic structures which may one day come to fruition as pharmaceutically applicable molecules. Organic synthesis, using the 1,3,5-triazine derivative 2,4,6-trichlorotriazine, cyanuric chloride, for practical applications was investigated. By utilizing the selective reactivity of cyanuric chloride, a plethora of targets from small molecules to large dendrimers may be synthesized.

Triazine chemistry was adapted to an educational application for the development of an undergraduate laboratory to synthesize simazine, a widely used herbicide. The laboratory was designed to foster a sense of the applications of chemistry in the world and its effect on the environment and society.

The modification of chitosan for herbicide remediation has been accomplished using triazine chemistry, as well. Treatment of chitosan iteratively with cyanuric chloride followed by piperazine produces dendritic grafts from these flakes. Dendrons of generation one through three were synthesized on chitosan backbones of low,

medium, and high molecular weights. The piperazine derivatives were shown to sequester more than 99% of atrazine from an aqueous 100 ppb solution in a 24 h period.

Drug delivery applications of triazine-based dendrimers were investigated. Pegylated G3 dendrimers with molecular weights of 18 and 34 kDa with 9% and 17% iodine content by weight, respectively, were synthesized as potential macromolecular contrast agents. The development of macromolecular contrast agents is of great interest to counteract the drawbacks associated with currently used, small molecule contrast media, including toxicity, extravasation into the extracellular space, and rapid clearance from the bloodstream. Dendrimers are well suited for use as macromolecular media due to the unique properties of these molecules, including monodispersity and multivalency.

DEDICATION

To my loving husband Freddie

ACKNOWLEDGMENTS

I would like to acknowledge my committee, Dr. David Bergbrieter, Dr. Stephen Miller, Dr. Scott Senseman, and Dr. Eric Simanek. Thank you for serving on my committee. I would also like to thank former group members of the Simanek group, in particular Dr. Alona Umali, Dr. Mackay Steffensen, Dr. Sergio Gonzalez, Dr. Megan Tichy, Dr. Eric Acosta, Dr. Hui-ting Chen, Dr. Izabela Owsik, Dr. Emily Hollink, and Dr. Michael Neerman. You were all great friends and wonderful mentors, and I'm glad we got to know each other and work together. To the current Simanek group members, Hannah Crampton, Karlos Moreno, Jong-doo Lim, Meredith Mintzer, Vince Venditto, and Dr. Abdellatif Chouai, I am grateful to have been your co-worker and your friend. Good luck!

Thank you to Dr. Shane Tichy and the Laboratory for Biological Mass Spectrometry at Texas A&M University. To the TAMU Chemistry Department GSO staff, Julie Wilson, Joy Monroe, and Sandy Manning, thanks for listening to me, supporting me, and giving me advice whenever it was needed. You helped to keep me sane whenever things were hectic. Most of all I would like to thank all of my family and friends who have continuously supported me throughout my graduate school experiences. My husband Freddie has been a great source of strength and love, and I don't think I would have made it without him. I love you, and thank you!

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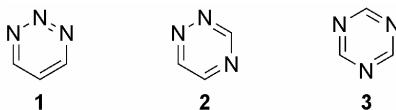
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CHAPTER I

INTRODUCTION: TRIAZINE APPLICATIONS

Triazines are six-membered aromatic heterocycles comprised of three carbon and three nitrogen atoms. The three isomers shown below in Chart 1 are 1,2,3-triazine (**1**), 1,2,4-triazine (**2**), and 1,3,5-triazine (**3**). The 1,3,5-triazines are the oldest and most extensively studied of the isomeric forms.^{1,2} Given that 1,3,5-triazine is a symmetrical molecule, compounds of this type are often referred to as *s*-triazines. The use of the term triazine in this work will solely refer to the 1,3,5-derivatives.

Chart 1. Triazine isomers

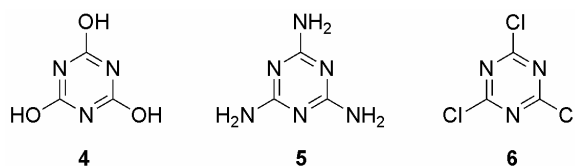


1,3,5-triazine was unknowingly first synthesized by Nef in 1895 by treating hydrogen cyanide with ethanol in an ether solution saturated with hydrogen chloride. The resulting salt was then treated with base and distilled to give 1,3,5-triazine in low yields, 10%. Nef incorrectly identified the product as a dimeric species. However, in 1954, Grundmann and Kreutzberger proved the compound to be a trimer of hydrogen cyanide, *s*-triazine.^{1,3}

This thesis follows the style of the *Journal of the American Chemical Society*.

Triazine is thermally stable unless heated to above 600 °C where it decomposes to form hydrogen cyanide. The triazine ring is fairly resistant to electrophilic substitution. However, it may readily undergo ring cleavage with nucleophiles and is very sensitive to hydrolysis by water and other hydroxyl-compounds to a lesser degree.^{1,3} A variety of heterocycles can be prepared from 1,3,5-triazine by treatment with bifunctional amines or related compounds, and it may be used as an alternative for HCN in reactions.³ The most commonly used triazine derivatives are shown below in Chart 2, cyanuric acid (**4**), melamine (**5**), and cyanuric chloride (**6**).

Chart 2. Common triazine derivatives



Triazines were first synthesized as the compound known as cyanuric acid (**4**). In 1776, Scheele produced cyanuric acid through the pyrolysis of uric acid. The common name of cyanuric acid was developed because, at that time, the compound was considered to be composed of cyanide groups, and it had been produced from uric acid. In 1820, Serullas repeated the work of Scheele to obtain cyanuric acid from cyanogens in water. It was discovered in 1830 that the two products were indeed the same, and the structure was elucidated by Liebig and Wohler.¹

Cyanuric acid is only slightly water soluble and is thermally unstable. At high temperatures, it decomposes to form toxic cyanic acid.¹ Cyanuric acid exists

preferentially as the oxo tautomer (**4b**) rather than the hydroxy form (**4a**).⁴ (Figure 1) Commercial applications of cyanuric acid include stabilizers of swimming pool disinfectants, household bleach, industrial cleaners, dishwasher detergents, and general sanitizers. Industrial preparation of cyanuric acid is accomplished through the pyrolysis of urea (**5**).⁴ (Scheme 1) Cyanuric acid has been found to naturally occur in nature in soil humus.¹

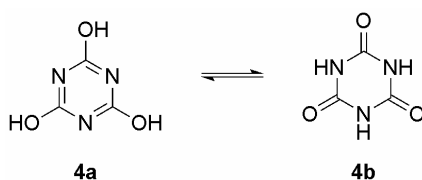
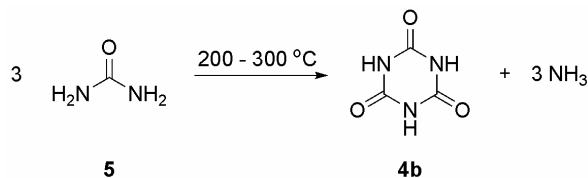


Figure 1. Tautomerism of cyanuric acid

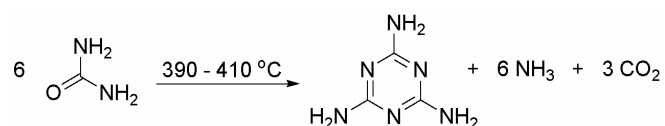
Scheme 1. Industrial synthesis of cyanuric acid



Melamine (**5**) was prepared in 1834 by Liebig by fusing potassium thiocyanate with ammonium chloride. The product was mostly melamine thiocyanate, but treating it with base provides the free melamine.^{1,3,5} It took over a century before commercial applications were fully realized. Melamine is now produced in large quantities, mostly for the formation of resins. In 1994, over 610,000 tons were produced worldwide. Currently industrial synthesis of melamine is from urea at 390–410 °C either non-

catalytically under high pressure processes or catalytically using low-pressure processes. The net reaction is the same using any of these processes as shown in Scheme 2.⁵ Naturally occurring melamine has been discovered in several meteorites which have reached earth.³

Scheme 2. Industrial synthesis of melamine

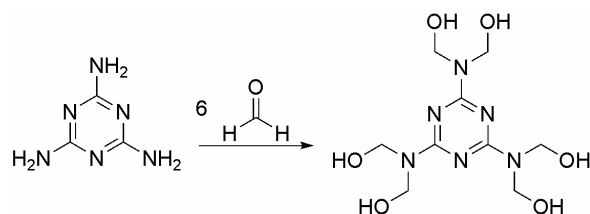


Melamine is most often reacted with formaldehyde for the production of resins. This practice began in 1935 in Germany.⁶ The polymers formed from melamine-formaldehyde resins have excellent chemical and physical properties. The resins have numerous applications including laminates, glues and adhesives, molding compounds, coatings, and paper and textiles.⁶ The reaction of melamine with formaldehyde is shown below in Scheme 3. Anywhere from one to six of the hydrogen atoms on the amine groups of melamine may be replaced by methylol groups. Methylolmelamines are unstable due to the possibility of further condensation or resinification, as shown in Scheme 4 with a monomethylolmelamine, but similar reactions occur with the other di- or tri-methylolmelamines.⁵

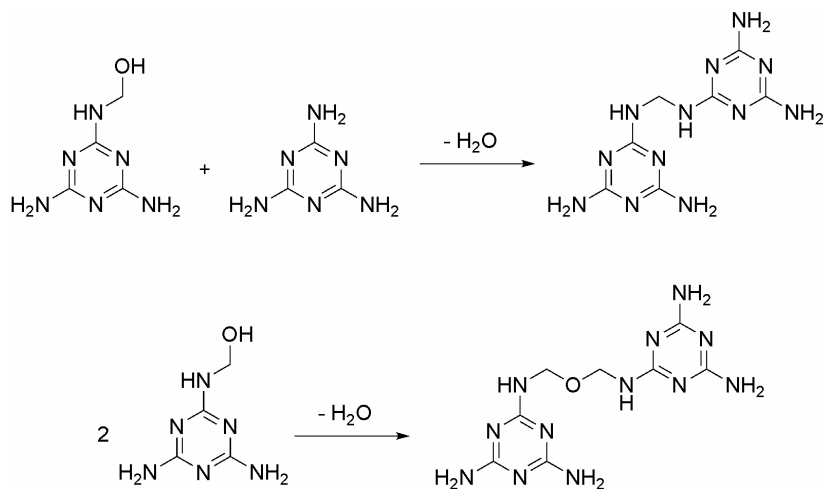
The oldest application of melamine resins is adhesives for wood such as plywood or particle board.⁶ Creating a foamed resin produces hard, yet flexible, lightweight materials which are used for sound insulation, fire protection, and cleaning products.^{7,8}

Other applications include impregnating resins to treat papers for decorative purposes, paper auxiliaries to enhance wet tensile strength, leather tanning agents, strengtheners for building materials, concrete additives, ion-exchange resins, and wood preservatives.⁶

Scheme 3. Initial reaction of formaldehyde and melamine



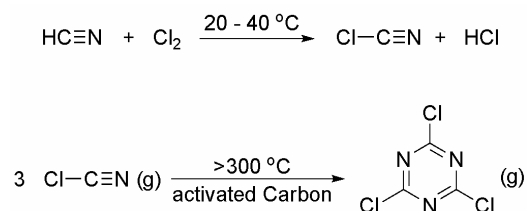
Scheme 4. Condensation and resinification of methylolmelamine



Cyanuric chloride, trichlorotriazine, (**6**) was first synthesized in 1827 by Serullas, who converted cyanogen chloride to cyanuric chloride using sunlight. However, the structure was not fully understood until 1867. Thought to be the trimer of cyanogen chloride for many years, Liebig determined the correct structure by passing chlorine over

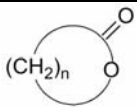
dry potassium thiocyanate.¹ Today, cyanuric chloride is produced by the trimerization of chlorinated hydrocyanic acid.⁹ (Scheme 5)

Scheme 5. Industrial synthesis of cyanuric chloride



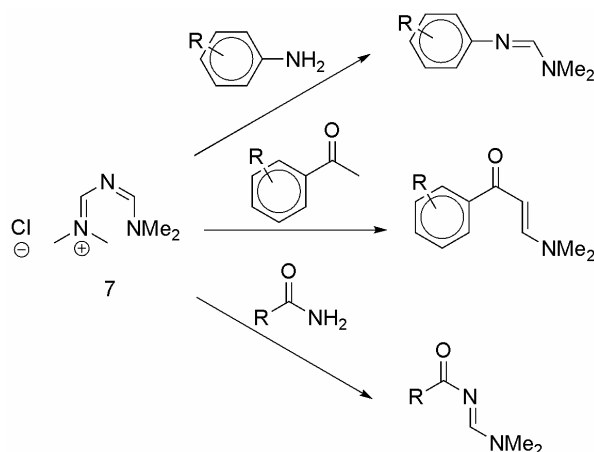
Cyanuric chloride has the most numerous applications of the triazine derivatives shown in Chart 2. Arguably the most important attribute of cyanuric chloride is its ability to undergo nucleophilic aromatic substitution ($\text{S}_{\text{N}}\text{Ar}$). It may be used as an analogous acid chloride equivalent in many organic transformations, including chlorination, dehydration, and coupling reactions.⁹⁻¹² Table 1 summarizes some of the reactions in which trichlorotriazine may be used.

Table 1. Reactions using cyanuric chloride as a reagent

Reactant	Product
$\text{R}-\text{CO}-\text{OH}$	$\text{R}-\text{CO}-\text{Cl}$
$\text{R}_1\text{R}_2-\text{CH}-\text{OH}$	$\text{R}_1\text{R}_2-\text{CH}-\text{Cl}$
$\text{R}-\text{CH}=\text{N}-\text{OH}$	$\text{R}-\text{C}\equiv\text{N}$
$\text{HO}-(\text{CH}_2)_n-\text{COOH}$	

Cyanuric chloride can also be used as an oxidizing agent using Swern oxidation conditions utilizing DMSO. The DMSO reacts with cyanuric chloride forming a complex which can be used to oxidize alcohols to give the carbonyl compound and dimethyl sulfide.¹⁰ The reaction of cyanuric chloride with DMF has been shown to produce a compound known as Gold's salt (7). The imminium salt prepared by Gold in 1960 is a Vilsmeier-Haak type reagent. The reagent transforms amines to *N,N*-dimethylamidines, ketones to *N,N*-dimethylenamino ketones, and amides to *N'*-acyl-*N,N*-dimethylformamidines as shown below in Scheme 6.^{9,10,13,14}

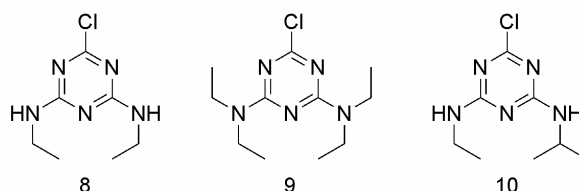
Scheme 6. Reactions of Gold's salt



Another important use of trichlorotriazine is for coupling reactions such as peptide coupling. The compound may be used as itself or as the 2-chloro-4,6-dimethoxy-1,3,5-triazine derivative (CDMT), with or without the addition of *N*-methylmorpholine. The reagents may be in solution or attached to a solid-support. The reactions proceed with high diastereoselectivity and enantioselectivity to obtain high

chlorazine (**9**).²⁰ Alkylaminotriazines are among the most widely used herbicides today. The most predominantly used derivative being atrazine (**10**).^{9,12}

Chart 3. Triazine herbicides

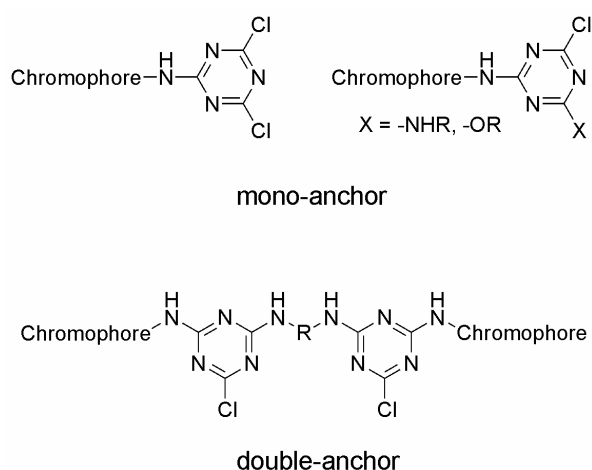


Reactive dyes are synthesized from cyanuric chloride through substitution with one or more alkoxy- or amino-containing chromophores. Reactive dyes, in general, contain functional groups that can undergo addition or substitution reactions with the amino or hydroxy groups present in the fiber. Rattee and Stephen were able to use basic aqueous conditions to fix triazine dyes onto cellulose. This was a breakthrough that led to commercial development. Procion-MX dyes were introduced in 1956 by ICI followed by Cibacron dyes from Ciba-Geigy in 1957.

There are two main categories of reactive triazine dyes, mono- or double-anchor dyes. Dyes such as Procion MX, Procion H, Procion P, or Cibacron are mono-anchor dyes, and Procion HE is an example of a double-anchor dye. (Chart 4) The dichlorotriazine dyes are more reactive and may be applied without any heat. However, these dyes are also more sensitive to hydrolysis, and an appropriate buffer is usually added to increase the stability. Due to reduced activity of the monochlorotriazine derivatives, these compounds require heat for fixation and are well suited for printing

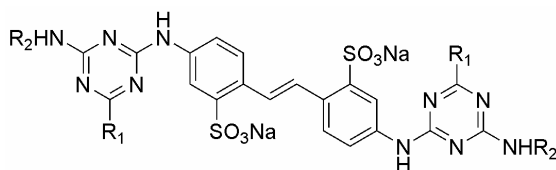
processes. The double-anchor compounds contain two monochlorotriazine units linked by a bridging group such as a diamine. The synthetic strategy utilized to produce these compounds allows the incorporation of two different chromophores into a single system. This greatly expands the range of attainable colors.^{12,21}

Chart 4. Mono-anchor and double-anchor triazine reactive dyes



Trichlorotriazine is also the basis for optical brighteners or fluorescent whitening agents. These compounds are employed to absorb ultraviolet light and emit blue fluorescent light. They are often used to mask the undesirable yellow tint in white substrates such as fabric or paper. These compounds are also used in detergent formulations to make laundered fabrics whiter or colors brighter. The most common derivatives contain 4,4'-diaminostilbene-2,2'-disulfonic acid (**11**) and have been used since 1941. A wide variety of amino, hydroxy, sulfo, or alkoxy groups may be

incorporated into the compound, and the triazine may have a chlorine retained in the structure or the chlorine may be substituted by amines.^{1,12}



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Due to the ability to substitute a wide variety of nucleophiles onto trichlorotriazine, as well as the capability to vary the groups attached during each substitution, interest in triazine dendrimers has grown.²² Dendritic structures have been investigated for years. The perfectly branching structures are reminiscent of a motif found in nature including, among others, snowflakes, trees, and neurons.²³ Dendrimers are highly branched, globular, multivalent, monodisperse molecules with synthetic versatility and many possible applications ranging from catalysis to electronics and drug delivery.^{22,24-26}

The concept of dendrimers was introduced in 1978 by Vögtle who synthesized branched amines through repetitive reactions.²⁷ In 1985, two groups, Tomalia and Newkome, independently reported the divergent synthesis of dendritic systems in which the synthesis begins at the core and reactions are carried out on the periphery until the desired dendrimer is reached.^{28,29} In 1990, the concept of convergent synthesis of dendrimers was introduced by Fréchet and co-workers. Using the convergent route, the

synthesis begins with the periphery groups, and a dendron of the desired size is coupled to a core in the final step.^{30,31} Both synthetic strategies are illustrated in Figure 2.

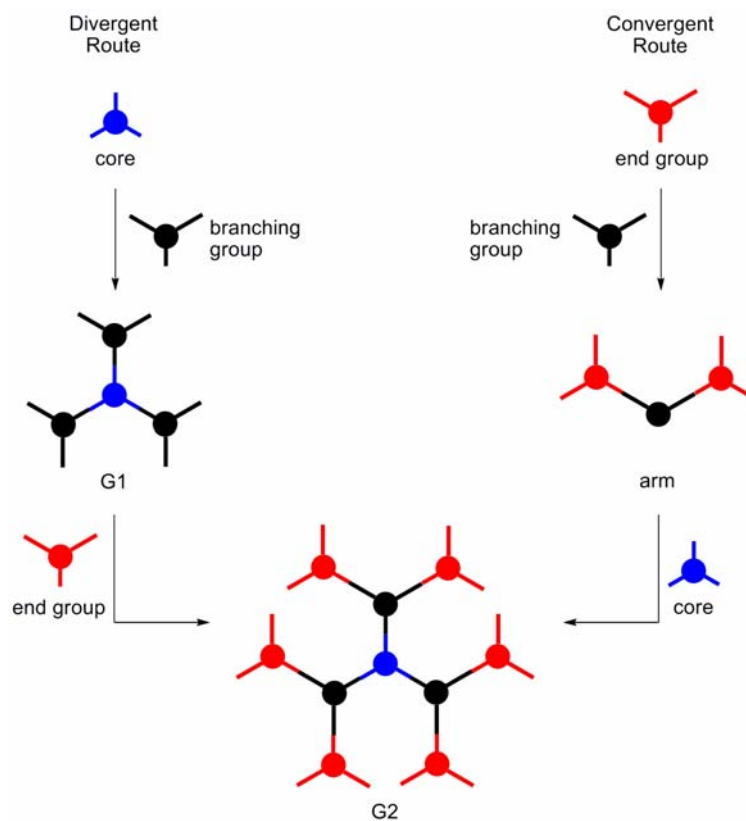
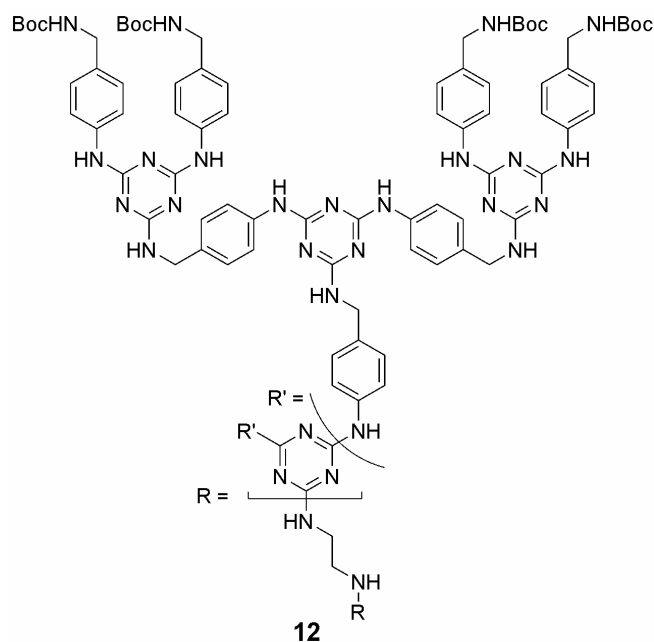


Figure 2. Divergent and convergent dendrimer synthetic strategies

The synthesis of triazine-based dendritic structures from cyanuric chloride for use as Gd chelating ligands in magnetic resonance imaging (MRI) applications were reported in 1996.³² Accounts of dendrimers synthesized from trichlorotriazine by nucleophilic aromatic substitution were first published in 2000 by the Simanek group at Texas A&M University. The melamine based dendrimer (**12**) was synthesized using

both the convergent and divergent route.^{33,34} Other groups have since reported triazine dendrimers and this subject has been reviewed by Steffensen and co-workers.^{22,35}

The work in the Simanek group has focused on improving melamine dendrimer synthetic strategies. This has been accomplished by exploiting the differential reactivity of cyanuric chloride to incorporate diverse reactive groups for further manipulations and by utilizing orthogonal protecting groups or diamines with chemoselective reactivity.³⁶⁻³⁸ Improvements in synthetic strategies are necessary for applications of these dendrimers to be widespread.



Applications pursued in the Simanek group have included endeavors to educate students on dendrimer chemistry itself, as well as triazine chemistry and its applications.^{39,40} Another field of interest has been environmental remediation by

attachment of triazine dendrons to solid supports.⁴¹⁻⁴⁴ Biologically relevant applications, such as drug delivery, have been a major focus with investigations of disulfide exchange for covalent conjugation of drugs,⁴⁵⁻⁴⁷ encapsulation of drugs,^{48,49} and the *in vitro* and *in vivo* studies of melamine dendrimer toxicity and biodistribution.⁵⁰⁻⁵² The limits of triazine dendrimer chemistry have not yet been reached as new synthetic strategies have been pursued⁵³ and the possibility of large scale syntheses is being explored for these multivalent molecules which may be readily tailored to the application of interest.

CHAPTER II

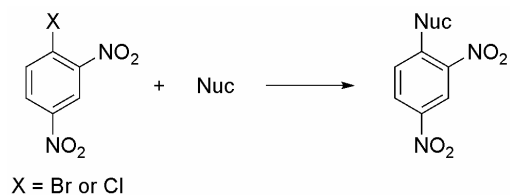
SYNTHESIS OF A TRIAZINE HERBICIDE AS AN UNDERGRADUATE LABORATORY EXERCISE

Introduction

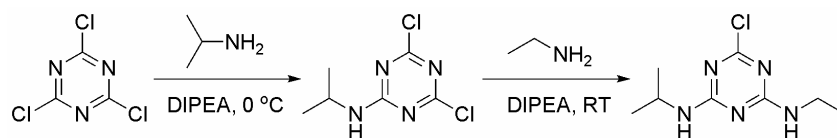
For most students who take undergraduate chemistry courses, it is often difficult to make a connection between concepts learned and real life. Many don't realize the numerous practical applications of chemistry that surround them. When a correlation is made between the world around us and the material learned, it can serve to bring about a whole new level of understanding.

Nucleophilic aromatic substitutions are less commonly performed in undergraduate laboratories than electrophilic aromatic substitution reactions. In addition, the products of these reactions often do not seem to have relevance to life outside of the laboratory for the students. Triazine herbicides are synthesized using nucleophilic aromatic substitution and have very important agricultural, economical, and environmental impacts in the world today.

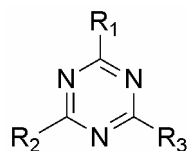
The most commonly studied nucleophilic aromatic substitution reactions involve 2,4-dinitrobromobenzene or 2,4-dinitrochlorobenzene as shown in Scheme 9. The electron withdrawing groups activate this reaction because the intermediate is stabilized by resonance, and the charge can be delocalized.

Scheme 9. Nucleophilic aromatic substitution

The synthesis of atrazine, as shown in Scheme 10, is very similar. It also involves nucleophilic aromatic substitution and resonance stabilization. Atrazine is synthesized from cyanuric chloride by the sequential reaction with isopropylamine and ethylamine. Other herbicides based on 1,3,5-triazine are summarized in Table 2.

Scheme 10. Synthesis of Atrazine

The triazine herbicide atrazine has been one of the most widely used herbicides in the production of corn, sorghum, and other crops for forty years in the U.S. It is a pre-emergent herbicide used to control broadleaf and grassy weeds. Its mode of action is through selective disruption of photosynthesis to kill weeds, resulting in increased crop production.⁵⁴

Table 2. Common 1,3,5-triazine herbicides

Herbicide	R ₁	R ₂	R ₃
Atrazine	Cl		
Cyanazine	Cl		
Simazine	Cl		
Terbutylazine	Cl		
Trietazine	Cl		
Prometon	O-CH ₃		
Terbumeton	O-CH ₃		
Ametryn	S-CH ₃		
Desmetryn	S-CH ₃		
Prometryn	S-CH ₃		
Simetryn	S-CH ₃		
Methoprotrotryne	S-CH ₃		

Due to the Safe Drinking Water Act of 1974, the EPA is required to determine the concentration of chemicals which may cause health problems. Atrazine is

moderately water-soluble and can be present in runoff from crop fields.⁵⁵ Atrazine is commonly detected in ground and drinking water and has been linked to cancer and endocrine gland disruption in people. For atrazine, the maximum contaminant level (MCL) is set at 3 parts per billion, ppb or $\mu\text{g/L}$. Short-term exposure at levels above the MCL may cause heart, lung, or kidney congestion along with low blood pressure, muscle spasms, adrenal gland damage, or weight loss. The long-term effects range from weight loss and cardiovascular damage to muscle degeneration or cancer.³⁵

The EPA has considered restricting or banning its use, but long-term costs would range from \$295-\$665 million a year.⁵⁴ Estimates have shown that nonuse of herbicides would increase production costs for the grower by \$21 billion annually and crop yields would be reduced by 5-67%.⁵⁶ The money lost from yield reductions or more expensive herbicide replacements would be passed down to livestock producers and eventually to consumers. Financial and agricultural disadvantages must be weighed against environmental concerns.

Although average concentrations in streams and wells rarely exceed the safe water standards, water monitoring has shown that atrazine is found in two-thirds of all samples from agricultural streams. In shallow ground water where runoff must travel further, providing more chances for absorption or degradation, the most common compounds found included atrazine and one of its degradation products deethylatrazine (DEA).⁵⁷ Atrazine and its metabolites are often found at levels exceeding the MCL in runoff in the days following application, up to 100 days for atrazine and up to 50 days for DEA and deisopropylatrazine (DIA).⁵⁸

Triazine herbicides are also used on residential lawns and golf courses. Although atrazine usage is regulated by the EPA for agricultural application, regulations can not be easily enforced for residential application. In this case recommendations are given for maximum application, especially regarding exposure of children to treated lawns.¹⁰ In urban streams and shallow ground water, atrazine, DEA, and simazine are among the most frequently detected herbicides.⁵⁷ There are no extensive studies on the effects on DEA and DIA, although these metabolites are commonly found in the same locations as atrazine. There are no set MCL values for DEA and DIA, and the lack of consideration to these metabolites' impact on the environment underestimates atrazine's total impact.⁵⁸ Despite all of this data, the EPA has recently issued a reregistration eligibility decision for atrazine. The 2006 statement concluded that although the herbicides have a neuroendocrine toxicity mechanism which could result in developmental and reproductive complications, the cumulative effects present no reasonable harm to any general population of the U.S.⁵⁹

Herbicides are classified according to selectivity, time of application, translocation in the plant, or mechanism of action. For mechanism of action, there are seven major classes of herbicides: growth regulators, seedling growth inhibitors, photosynthetic inhibitors, amino acid synthesis inhibitors, lipid synthesis inhibitors, cell membrane disruptors, and pigment inhibitors. Atrazine is a photosynthetic inhibiting herbicide.⁶⁰

Photosynthesis is the net reaction in plants which converts carbon dioxide and water to carbohydrates, oxygen, and water using energy from sunlight. Photosynthesis is

a two-part process: the light-dependent reactions in which chlorophyll and other pigments absorb light energy for the formation of ATP, NADPH, and O₂, and the carbon-assimilation or carbon-fixation reactions, sometimes referred to as the dark reactions, in which the ATP and NADPH are used to reduce CO₂ to form carbohydrates.⁶¹

Photosynthesis occurs in the chloroplasts of leaves. Inside this organelle is a fluid, stroma, and vesicles called thylakoids which are arranged into stacks known as grana. It is in the thylakoid membranes that the light reaction centers are located. The primary light absorbing molecules, chlorophyll *a* and *b*, are associated with specific binding proteins forming light-harvesting complexes (LHC's). In the LHC, chlorophyll molecules are fixed in relation to the thylakoid membrane, to other necessary protein complexes, and to each other. Other light absorbing pigments such as β -carotene and lutein are referred to as accessory pigments. These pigments absorb light at wavelengths not absorbed by the chlorophylls.⁶¹

Light-absorbing pigments are arranged into photosystems. Some of these pigments are considered light-harvesting or antenna molecules because they are not directly associated with the reaction center. Instead, these pigments absorb light energy and transfer it to the reaction center. When antenna chlorophyll, for example, absorb energy, the excited molecule then transfers the energy to a neighboring chlorophyll exciting this new molecule as the original one returns to its ground state. This continues until the photochemical reaction center is reached. When the excited chlorophyll molecule in the reaction center transfers an electron to a nearby electron acceptor, an

electron from a nearby electron donor is transferred to the reaction center chlorophyll. At this point, the electron acceptor in the chain is negatively charged and the electron donor in the chain is positively charged, and the charge separation initiates the oxidation-reduction reactions of photosynthesis.⁶¹ The thylakoid membranes contain four membrane proteins: photosystem II (PSII), cytochrome *b₆f*, photosystem I (PSI), and the ATP synthase complex.^{62,63} (Figure 3)

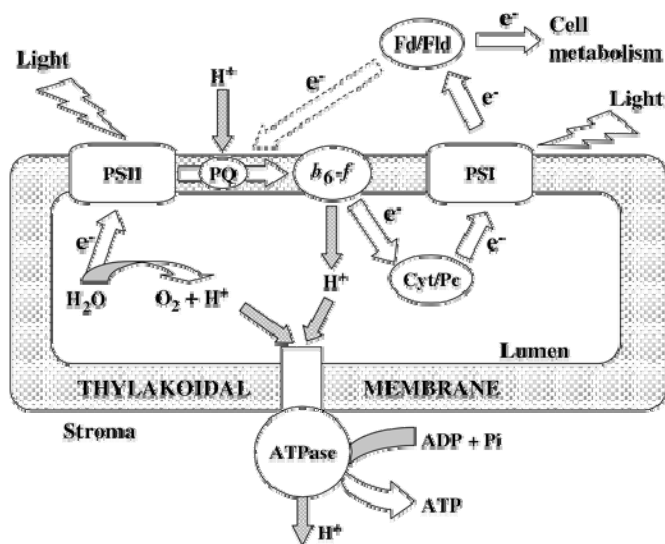


Figure 3. Thylakoid membrane-bound proteins for photosynthetic electron transport*

As shown in the Z-scheme in Figure 4, excitation of the PSII reaction center P680 transfers an electron to pheophytin which passes the electron to a protein-bound plastoquinone, Q_A , which passes it to another plastoquinone, Q_B , which is more loosely bound. Q_A and Q_B are bound into the D2 and D1 proteins, respectively. Q_B accepts two

* Reproduced with permission from "Electron Transfer between Membrane Complexes and Soluble Proteins in Photosynthesis" Hervás, M.; Navarro, J. A.; De La Rosa, M. A., *Acc. Chem. Res.* **2003**, *13*, 799. Copyright 2007 American Chemical Society.

electrons from Q_A and two protons from water to form fully reduced plastoquinone (PQH_2). The binding affinity of PQH_2 to this site is low. Another plastoquinone molecule may displace PQH_2 to bind to the D1 protein and is then referred to as Q_B . PQH_2 passes the electrons on to the cytochrome b_6f complex, from which they will continue on to PSI.^{62,64} Figure 5 shows PSII and the binding site for Q_B on the D1 protein that triazine herbicides may bind to block the electron flow, preventing ATP production. The triazine serves as a non-reducible analog to plastoquinone.^{61,65,66}

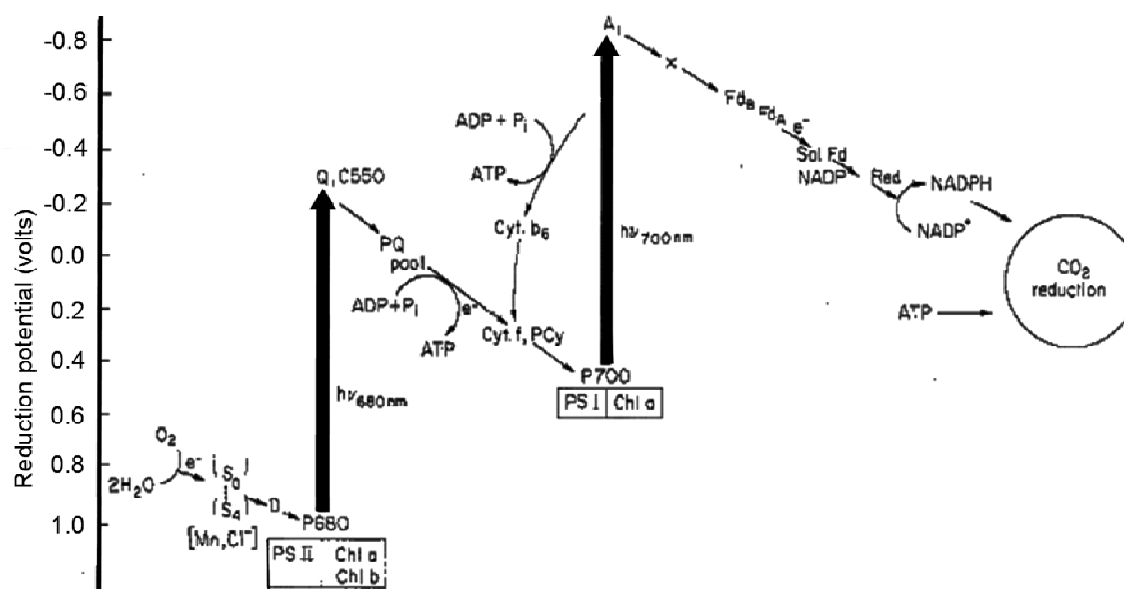


Figure 4. Z-scheme showing flow of electrons through photosystems[†]

[†] Reproduced with permission from "A Role for Manganese in Oxygen Evolution in Photosynthesis" Sauer, K., *Acc. Chem. Res.* **1980**, *13*, 250. Copyright 2007 American Chemical Society.

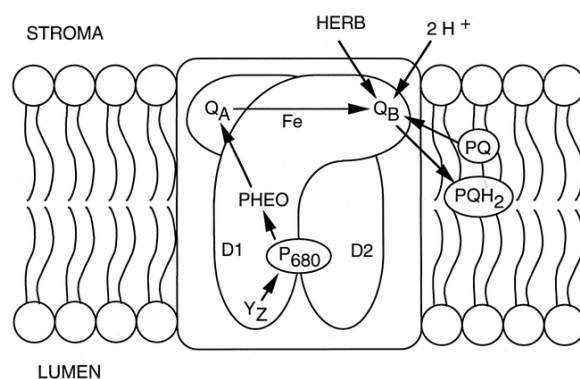


Figure 5. PSII complex showing electron transport and site of action of triazine herbicides[‡]

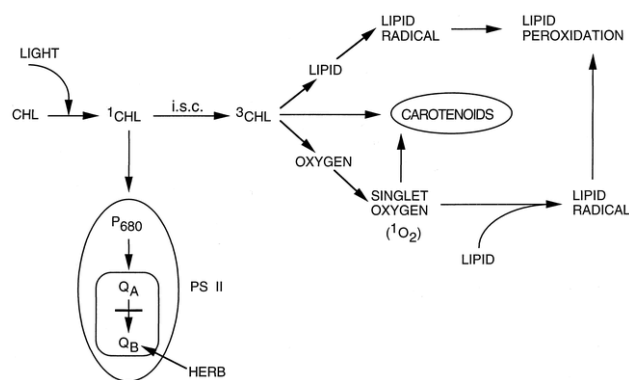
The PSII binding site was identified using photoaffinity labeling studies by Arntzen and co-workers in 1981. Azido-atrazine was used to covalently bind to the site in the receptor protein. UV irradiation of thylakoids in the presence of azido[¹⁴C]atrazine produces a reactive nitrene that covalently links the azido-atrazine to the chloroplast membranes. The membrane polypeptides may then analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, SDS-PAGE. Subsequent fluorography locates the ¹⁴C label and identifies the association of the azido-atrazine with the 34- to 32-kDa polypeptide size class. Binding did not occur in triazine-resistant chloroplasts.⁶⁷ This polypeptide is now known to be the D1 protein.⁶⁶ The crystal structure of the PSII system with atrazine bound has been resolved. The structure shows H-bonding and hydrophobic interactions to be the major contributors to the binding of atrazine in the Q_B site.⁶⁸

When plants are treated with atrazine, the Q_B binding site is blocked and the flow of electrons through PSII are therefore blocked. This causes an accumulation of singlet

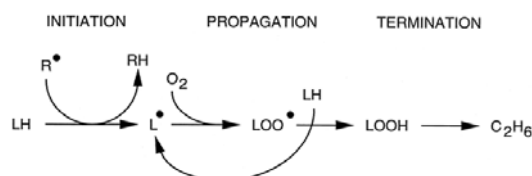
[‡] Reproduced with permission from “Light-dependent herbicides: an overview” Hess, F. D., *Weed Sci.* **2000**, *48*, 161. Copyright 2007 BioOne.

chlorophyll since the excited molecules cannot transfer an electron to PSII. Some singlet chlorophyll is converted to triplet chlorophyll through intersystem crossing (i.s.c.). Triplet chlorophyll may then directly initiate lipid peroxidation or may react with oxygen to form singlet oxygen which would initiate lipid radicals in the polyunsaturated fatty acids eventually causing membrane damage and leading to plant death. Schemes 11 and 12 below show the possible pathways of singlet chlorophyll upon activation and the lipid peroxidation process.⁶⁶ Triplet chlorophyll and singlet oxygen are normally produced during photosynthesis but are typically produced in small quantities and are adequately quenched by carotenoids in the membrane.^{65,66}

Scheme 11. Excitation of chlorophyll to ^1CHL and the possible pathways including electron transfer to PSII, quenching of ^3CHL or $^1\text{O}_2$ by carotenoids, and initiation of lipid radicals[§]



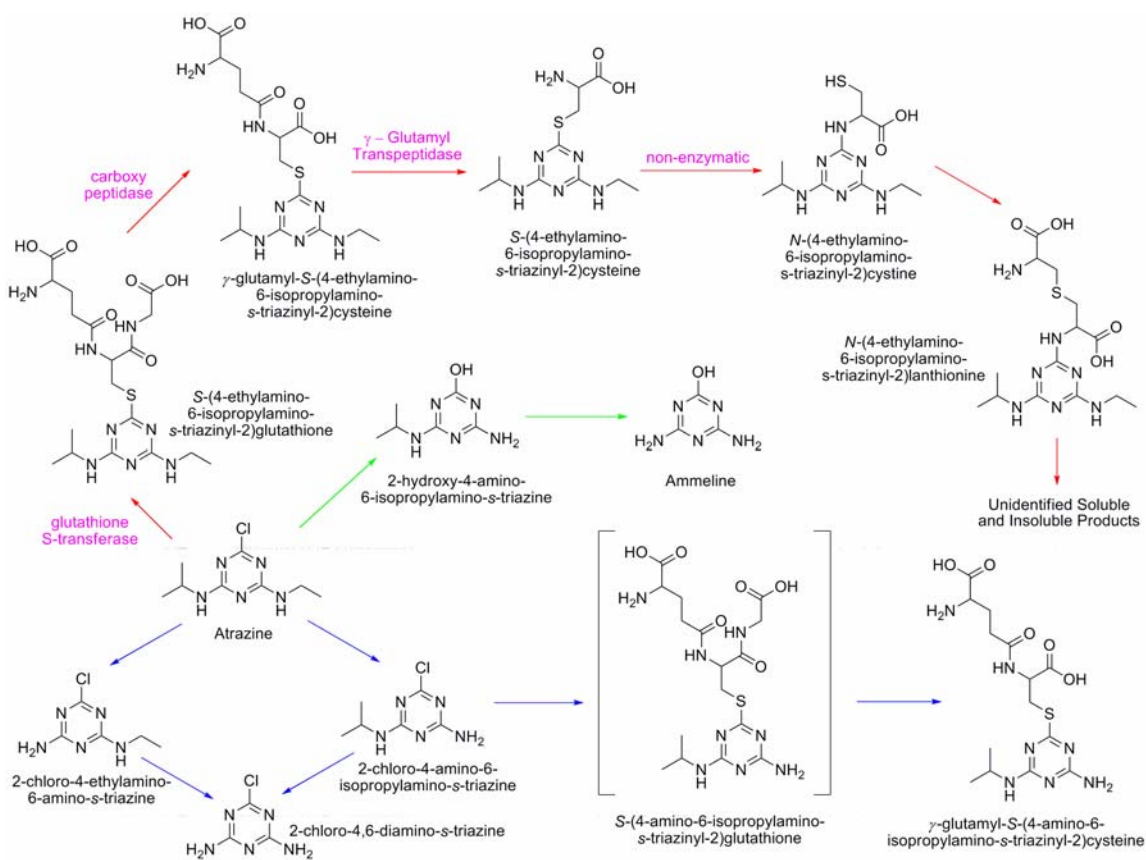
Scheme 12. Lipid Peroxidation of plant membrane polyunsaturated fatty acids[§]



[§] Reproduced with permission from “Light-dependent herbicides: an overview” Hess, F. D., *Weed Sci.* **2000**, *48*, 161, 168. Copyright 2007 BioOne.

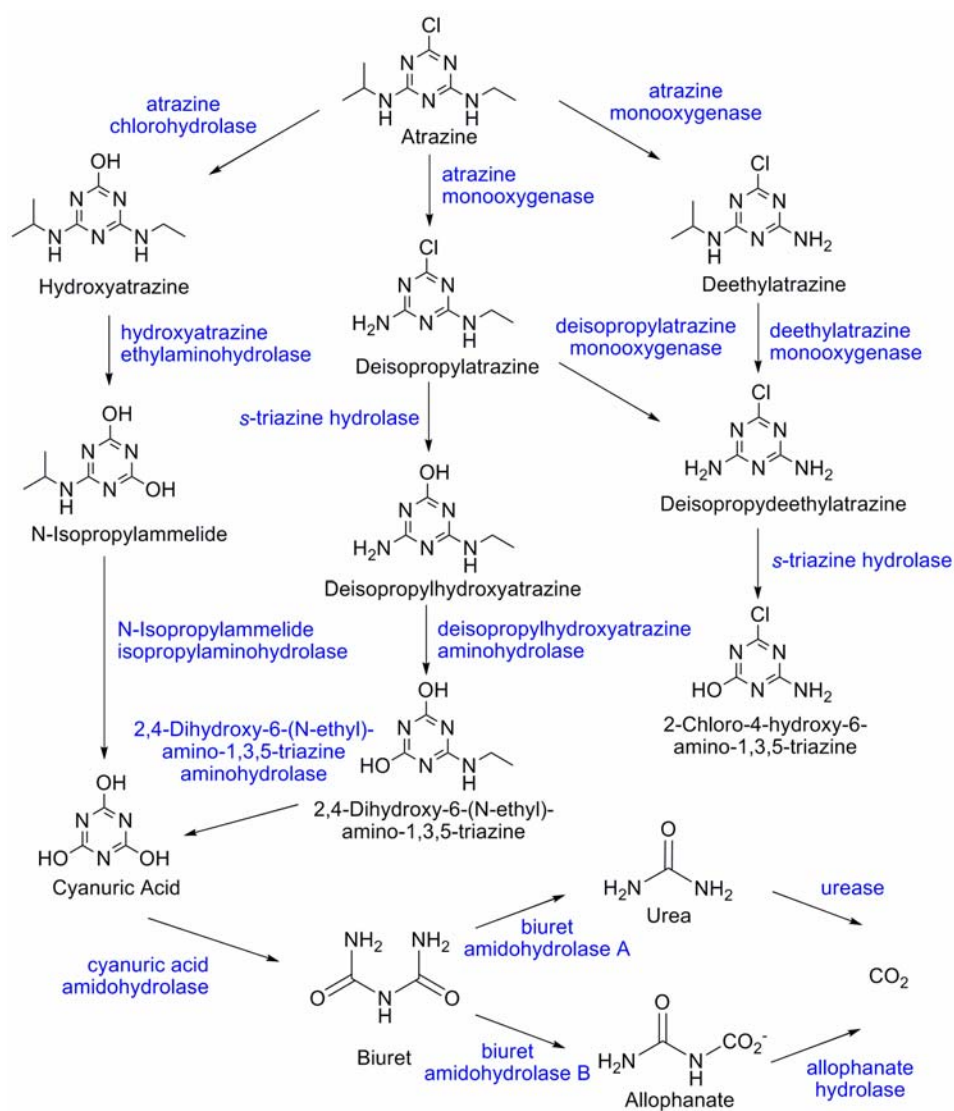
Resistance to triazine herbicides has been shown to be caused by a single point mutation which codes for substitution of a glycine residue in place of a serine residue in the binding pocket.⁶⁸⁻⁷⁰ However, atrazine is metabolized and detoxified in corn and other resistant species by three primary routes: 2-hydroxylation, *N*-dealkylation, and glutathione conjugation.⁷¹⁻⁷³ Scheme 13 provides the metabolic pathways of atrazine in sorghum.⁷¹

Scheme 13. Metabolic pathway of atrazine in sorghum. Major pathways are shown with red arrows, blue arrows indicate minor pathways, and the green arrows indicate a hypothesized reaction. The structure in brackets was not isolated.



The enzymatic breakdown of atrazine by microorganisms had been extensively studied. Scheme 14 was determined by Professor Larry Wackett at the University of Minnesota.⁷⁴ Atrazine can be degraded to DEA, DIA, dealkylatrazine (DAA), and hydroxyatrazine. Both bacteria and fungi found in soil have been shown to degrade atrazine to various metabolites.⁷⁵

Scheme 14. Atrazine degradation pathway map



Experimental

Materials and Methods. ACS-grade solvents were used for all the synthetic preparations. Distilled water was obtained in-house. All reagents and solvents were purchased from Acros Organics or Aldrich Chemical Co. and were used without further purification. ^1H NMR and proton decoupled ^{13}C NMR spectra were acquired on a Varian 300 MHz spectrometer using CDCl_3 or DMSO-d_6 . NMR chemical shifts are listed relative to tetramethylsilane in parts per million (ppm) and were referenced to the residual proton or carbon peak of the solvent.

Synthesis of Atrazine. Add cyanuric chloride (350 mg, 1.90 mmol) to 1 mL of acetone in a round bottom flask while stirring to obtain a slurry solution. Add isopropyl amine (0.17 mL, 1.86 mmol), place the flask in an ice bath, and allow it to cool while stirring for approximately five minutes. Then, a white precipitate is formed by adding 5.6 M NaOH (0.39 mL, 2.17 mmol). The reaction mixture is stirred while in the ice bath for 20-30 minutes, checking the progress of the reaction by TLC. When the substitution is complete as determined by TLC, the flask is placed in a water bath and heated at 35-40 °C. Upon heating, ethylamine, 70% in water, (0.16 mL, 1.96 mmol) is added. A very thick white precipitate is formed by adding 5.6 M NaOH (0.28 mL, 1.57 mmol), and the reaction mixture is allowed to stir for 20-30 minutes while checking the progress by TLC. When the reaction is complete, distilled water (2.50 mL) is added to precipitate the product, and the flask is allowed to cool to room temperature. The product is then filtered, rinsing well with distilled water, dried, and weighed. Product acquired is a white powder. Yield: 356-373 mg (87-91%). Melting point 165-168 °C. ^1H NMR

(300 MHz, CDCl₃) δ : 6.33 (s, NH), 6.02 (s, NH), 5.40 (d, $J=7.15$, NH), 5.25 (s, NH), 5.12 (d, $J=8.25$, NH), 4.15 (m, 1H), 3.40 (m, 2H), 1.20 (m, 9H) ¹³C NMR (75.5 MHz, CDCl₃) δ : 168.06, 165.57, 164.80, 42.86, 35.79, 22.88, 22.38, 14.64.

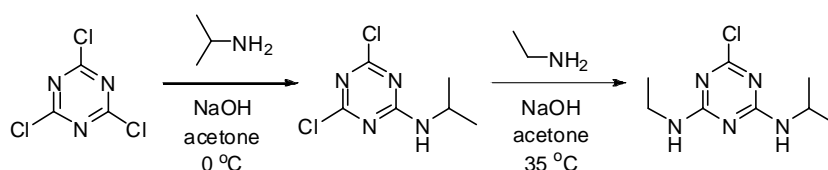
Synthesis of Simazine. A 0.5 M cyanuric chloride stock solution (5 mL, 2.5 mmol) is placed in a round bottom flask and into an ice bath to cool for about 10 min. while stirring. To this solution, a 1.0 M ethylamine solution in aqueous acetone (10 mL, 10.0 mmol) is added. After 5 min., the ice bath is removed and the reaction is allowed to stir for 15 min. as it comes to room temperature. The addition of distilled water (30 mL) precipitates the simazine which can be collected by filtration, rinsed well with distilled water (2 x 30 mL), dried, and weighed. Product obtained is a white solid. Yield: 449 mg (89%). Melting point: 225-227 °C. TLC: $R_f=0.25$ using 3:1 hexanes:ethyl acetate. Procedure modification: Allow reaction to stir for 30 min. at room temperature, and also, when filtering, final rinse should be with methanol (1 x 30 mL). ¹H NMR (300 MHz, DMSO-d₆) δ : 7.78 (t, $J= 4.52$ Hz, NH), 7.73 (t, $J=5.13$ Hz, NH), 7.67 (t, $J=5.01$ Hz, NH), 7.49 (m, NH), 3.24 (dd, $J=5.98$ Hz, 2H), 3.24 (dd, $J=6.96$ Hz, 2H), 1.05 (m, 6H) ¹³C NMR (75.5 MHz, DMSO-d₆) δ : 168.12, 167.48, 165.36, 165.13, 164.74, 35.00, 34.96, 34.86, 14.83, 14.65, 14.37, 14.28.

Results and Discussion

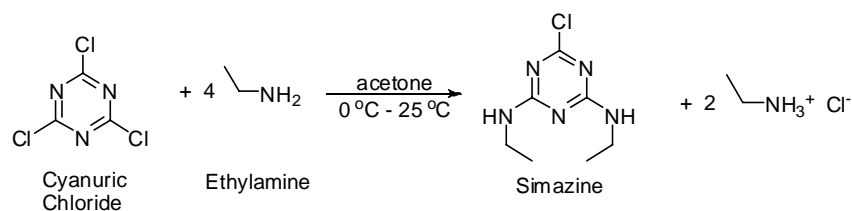
The first method investigated for developing the undergraduate laboratory was adopted from U.S. patent 4,166,909 using sodium hydroxide as the base.⁷⁶ (Scheme 15) Due to time constraints in the allotted laboratory time period, this method proved to be too lengthy to be completed in a normal 3 hour lab session. Modifications of the

procedure were made to simplify the synthesis. The synthetic target was altered to afford the triazine herbicide simazine rather than atrazine. Being symmetrical, simazine requires no monitoring of reaction progression in the middle of the procedure. Using this method, four equivalents of ethylamine may be added to serve as the reactant and to preclude the use of an exogenous base. (Scheme 16)

Scheme 15. Synthesis of atrazine using sodium hydroxide as an exogenous base.



Scheme 16. Synthesis of simazine by the reaction of cyanuric chloride with ethylamine.



During the first semester the laboratory was implemented, the preparation of simazine went fairly well when performed by students. Approximately 85% reported characterization data consistent with simazine as the major product (mp \sim 225-227 °C, R_f \sim 0.25). Approximately 54% of the students observed two compounds present by TLC, the second with R_f \sim 0.55. In most cases, the second spot was barely visible.

The second product is in most cases the dichlorotriazine that results from a single substitution on the triazine ring. The two possible causes for its appearance are

inadequate reaction times, or an error in measurements resulting in stoichiometric differences. Our solution to this problem, which has been adopted in subsequent semesters, is to run the reaction for 30 minutes at room temperature instead of 15 minutes. Students using a 30 minute reaction time achieved a higher success rate in that fewer reported two spots visible by TLC.

The product obtained is difficult to dry in the allotted time. When the melting points and yields must be calculated within the three hour period, a methanol rinse of the product removes residual water, as well as most of the unwanted dichlorotriazine. Recrystallization of the product from methanol is recommended for optimal purity but has not been implemented into the lab procedure at Texas A&M University. When the students are careful, utilize the full 30 min. reaction time at room temperature, and rinse well with methanol, little to no dichlorotriazine is detected in the final product.

Comments from students concerning the laboratory exercise were overwhelmingly positive when considering the ease of synthesis, clarity of procedural steps, interest level, and practicality. Negative comments from students were of the amount of time necessary to perform the experiment. However, the students were able to complete the lab in the allotted 3 hour time period with no difficulty when kept on task.

The background material available to the students on the mode of action and degradation of atrazine allows incorporation of biochemistry and simple biology to the curriculum. This works particularly well for the intended course of this exercise, an introductory biochemistry and organic chemistry course for non-science majors. Since

the development of this laboratory experiment, it has been adopted into the Texas A&M University chemistry curriculum. Also, the experiment sparked interest in the development of an entire course based on the chemistry and biology involved with Texas agriculture. The students synthesize simazine and propose studies to test the effectiveness of a simazine formulation. They then plant seeds according to their proposed methods and make observations on the effectiveness of their plan versus the effectiveness of the herbicide. Other studies in the course involve computer modeling to design novel triazine herbicides, synthesis of novel herbicides, and another round of testing on plants. The final study is ELISA assays of water samples from various sites in Texas, models of the ELISA strips to demonstrate their knowledge of the topic, and discussions about the results of the test as it applies to environmental impacts and regional importance of atrazine.

Conclusions

This experiment achieves several goals. It allows the students to perform a simple synthesis involving nucleophilic aromatic substitution and obtain a product in relatively high yield and purity. The product obtained is also very interesting and important to society today. This experiment may allow students to debate over whether economics and food production are more important than environmental concerns, or vice versa. A wide variety of discussions ranging from photosynthesis to farming techniques enhances a multi-disciplinary lesson which broadens the students' knowledge base. Most importantly, it allows students to see an example of how chemistry is relevant to and impacts the world.

CHAPTER III

MODIFICATION OF CHITOSAN FOR ATRAZINE SEQUESTRATION

Introduction

Chitosan is the deacetylated derivative of chitin, the second most abundant naturally occurring polysaccharide after cellulose. Chitosan is inexpensive, readily available, biodegradable, non-toxic, and may be easily functionalized at the amine group in the C-2 position. Chitosan and its derivatives have found applications in many different fields including antimicrobial agents, textile chemicals, biomedical applications, chromatographic separations, food and nutrition, cosmetics, and wastewater treatment.⁷⁷⁻⁸² Table 3 gives examples of the use of chitosan or its derivatives as adsorbents of undesirable pollutants.

The primary amines of chitosan offer a convenient handle for grafting functional polymers. To this end, several groups have investigated dendrimer-chitosan hybrid materials. Roy, Sashiwa, and Aiba *et al.* have reported the attachment of various PAMAM dendrimers and dendrons to chitosan. They also reported the attachment of polypropyleneimine dendrimers and dendrons of gallic acid and tri(ethylene glycol) to a chitosan backbone. These materials showed no activity per se, but were well characterized by NMR and GPC.⁸³⁻⁹⁰ Tsubokawa *et al.* report the divergent synthesis of PAMAM dendrons on chitosan powder. Again, while no activity data was provided, the materials were characterized by IR and SEM.⁹¹ The modification of chitosan as a solid support to perform atrazine sequestration from aqueous solutions is accomplished by the

incorporation of constrained secondary amines. Multiple amine groups may be easily incorporated by the divergent growth of highly branched dendritic molecules based on melamine.

Table 3. Chitosan adducts as adsorbents of pollutants.

Guest	Author	B/C*	Chitosan formulation	Material Removed
Dyes	Blackburn ⁹²	B	Flakes	Reactive Red, Direct Black, and Acid Blue
	Chiou ⁹³	B	Crosslinked beads	Reactive Red
	Chiou ⁹⁴	B	Crosslinked beads	Reactive, Direct, and Acid Dyes
	Juang ⁹⁵	B	Ground flakes	Reactive Red, Yellow, and Blue
Metals	Lasko ⁹⁶	B,C	Flakes	Ag ⁺ , Ag(NH ₃) ₂ ⁺ , Ag(SCN) ₃ ²⁻ , and Ag(S ₂ O ₃) ₂ ³⁻
	Boddu ⁹⁷	B,C	Coated onto ceramic alumina	Cr(VI)
	Peniche-Covas ⁹⁸	B,C	Mercaptan derivative	Hg ²⁺
	Prasher ⁹⁹	B	Flakes	Zn, Cu, Cd, and Pb
	Pedrosa ¹⁰⁰	B	Microspheres with PVA and sulphonated Cu(II)-phthalocyanine	Fe, Zn, Cu, and Mn
	Lasko ¹⁰¹	B	N- and S- derivatives	Cu ²⁺ , Cd ²⁺ , Pb ²⁺ , and Fe ²⁺
	Yen ¹⁰²	B	Coated onto sand	Cu ²⁺
	Wan Ngah ¹⁰³	B	Beads and crosslinked beads	Cu(II)
	Wang ¹⁰⁴	B	Crosslinked with crown ethers	Pb ²⁺ , Cu ²⁺ , Cr ³⁺ , and Ni ²⁺
	Kartal ¹⁰⁵	B	Flakes and powder	Cu, Cr, and As
	Prasher ¹⁰⁶	B	Flakes	Cu ²⁺ , Pb ²⁺ , Cd ²⁺ , and Zn ²⁺
	Cervera ¹⁰⁷	B	Ground flakes	Cd and Cr
	Modrzejewska ¹⁰⁸	C	Membranes (modified)	Cr(VI)
	Other	Fávere ¹⁰⁹	B,C	Microspheres
Yoshizuka ¹¹⁰		B	Crosslinked particles	Methyl parathion (pesticide) with/w/o Ag ⁺
Krysteva ¹¹¹		B,C	Flakes	Organic acids and polyphenols from wine production (vinasse)

*The assay of interest employs either a (B)atch or (C)olumn method

Atrazine is one of the most widely used herbicides for the production of corn, sorghum, and cotton in the U.S.¹¹² Due to its wide usage, atrazine is commonly detected in ground and drinking water, often at levels exceeding the EPA drinking water limit of 3 ppb.¹¹³ Atrazine has also been linked to health risks in humans and animals.

However, economic concerns and lack of a suitable alternative have allowed for its continued use.¹¹⁴ For this reason, methods to remove atrazine from drinking water or prevent atrazine from entering ground water are very important. The EPA approved method of removal for atrazine from drinking water is granular activated charcoal.⁴¹ However, this non-selective adsorbent is expensive, and its activity is dependent on the type of carbon used and the characteristics of the wastewater.⁹²

The Simanek group has demonstrated that atrazine readily undergoes nucleophilic aromatic substitution particularly well with constrained secondary amines. The amines, when placed on a solid support, provide a method for covalent atrazine sequestration (Equation 1).⁴¹ A wide range of solid supports have been investigated including commercially available polystyrene beads intended for peptide synthesis,⁴¹ polystyrene subsequently modified with isonipecotic acid,⁴⁴ silica gels,¹¹⁵ thermomorph polyacrylamides (a transiently solid support),¹¹⁶ and organoclays.¹¹⁷

Equation 1



Experimental

Materials and Methods. Cyanuric chloride 99% was purchased from Acros Organics. Piperazine, *N,N*-diisopropylethylamine (DIPEA), ninhydrin, and chitosan

with molecular weights of approximately $50,000 \text{ g mol}^{-1}$ (low), $200,000 \text{ g mol}^{-1}$ (medium), and $1,000,000 \text{ g mol}^{-1}$ (high) were purchased from Aldrich. The atrazine concentrations were measured by atmospheric pressure chemical ionization (APCI) using a Thermofinnigan LC Q Deca mass spectrometer. Quantitation was performed by comparing the relative area of the unknown peaks to a standard curve over a linear region of 5-125 ppb concentration of atrazine. Characterization of the chitosan materials was achieved using IR spectroscopy on a PerkinElmer Spectrum One FTIR spectrometer equipped with an Attenuated Total Reflectance sampling accessory. XPS measurements were taken with a Kratos Axis Ultra Imaging X-ray photoelectron spectrometer using a powder holder. TGA measurements were taken with a Netzsch TG 209 °C TGA. The materials were heated under nitrogen from 25 to 850 °C at a ramp of 10 °C per minute.

Synthesis and Characterization. Samples of chitosan flakes (2.0 g) of low, medium, and high molecular weights are washed with 1 M sodium hydroxide solution and rinsed with three 100-mL portions of water, or until the filtrate is no longer basic. A cold solution (100 mL) of five equivalents (0.5 M) of cyanuric chloride and five equivalents of Hünig's Base in tetrahydrofuran is added to the chitosan, and the reaction mixture is shaken at 0 °C overnight. The chitosan is then rinsed with three 100-mL portions of methanol and one 100-mL portion of tetrahydrofuran. The sample is treated with a solution of ten equivalents of piperazine (100 mL of 1.0 M) and ten equivalents of Hünig's Base in a 9:1 solution of tetrahydrofuran:methanol, and shaken overnight at room temperature. The samples are then washed with three 100-mL portions each of methanol and water followed by one 100-mL portion of ethyl acetate. Iterative reactions

of the material in this manner result in dendron growth on the chitosan surface. Upon completion of each synthetic transformation, 300-mg samples are taken. The samples are analyzed using ATR-FTIR. A ninhydrin test is performed by placing a few flakes of each sample into individual vials, adding ninhydrin solution, heating, and observing any color change.

Acid hydrolysis of the derivatized chitosan materials was accomplished by placing 20 mg of the material in 1 mL of 0.1 M HCl and heating at 75 °C for two weeks. A control sample containing a G1 dendron was also exposed to the same conditions. Upon cooling, the solution is added to approximately 1 mL of water, and 3M NaOH is added dropwise while swirling the solution until a pH of approximately 7 is reached. The water is removed by rotary evaporation, the solid is washed with methanol, and the salts are removed by filtration.

Powdered chitosan was synthesized using methods of Tsubokawa, *et al.*¹¹⁸ Samples of chitosan flakes (3.0 g) of low, medium, and high molecular weights are placed in 300 mL of water and 30 mL of 0.5 M HCl is added. The mixture is allowed to stir for about one hour at which time the solution is filtered to remove any undissolved material, and 10 M NaOH is added until a precipitate forms and the solution reaches pH 10. The precipitate is then filtered, washed with approximately 300 mL water, or until the filtrate is neutral, washed with approximately 100 mL of acetone, and dried under vacuum.

Atrazine Sequestration. Thirty milligrams of each sample is added to individual 5 mL fritted syringes containing 3 mL of 100 ppb aqueous atrazine solution.

This is done in triplicate. The syringes are capped and shaken for 24 h. using a wrist action shaker. The solutions are collected, filtered through 0.2 μm syringe filters, and analyzed for the amount of atrazine remaining in solution by liquid chromatography (LC)-MS at the Laboratory for Biological Mass Spectrometry at Texas A&M University.

Adsorption—Desorption Experiments. The chitosan samples from the atrazine study were placed in glass vials, 5 mL of acetone is added to each vial, and the vials were shaken for 24 h using a wrist action shaker. The solutions were collected and filtered through 0.22 μm syringe filters. The solvent was evaporated, and the samples are redissolved in distilled water. The samples were then analyzed for the amount of atrazine remaining by UV—Vis absorption at 221 nm, compared to a standard calibration curve. The results of these studies were inconclusive.

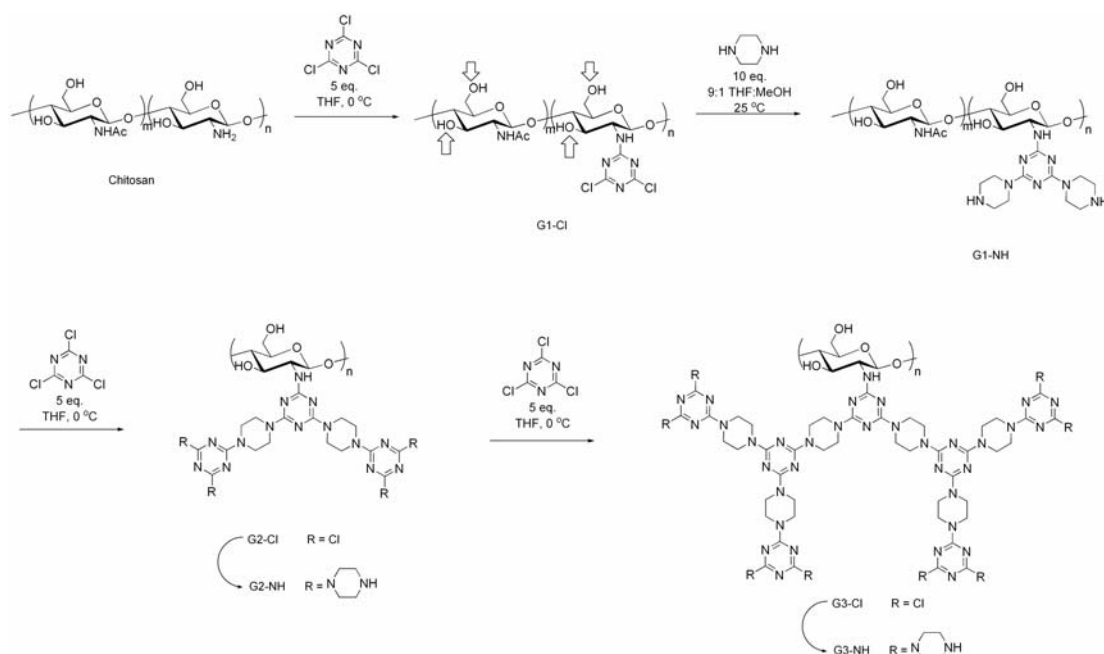
Results and Discussion

Nomenclature. For clarity, the grafted polymers on chitosan are referred to by their size using the common term "generation" abbreviated "G" with a number "1-3". To distinguish between the dichlorotriazine and piperazine adducts, we use the abbreviations "Cl" and "NH" respectively.

Synthesis and Characterization. The synthesis of the grafted polymers is shown in Scheme 17. For clarity, the idealized structure, a dendrimer graft, is shown. These grafts are grown stepwise in an iterative fashion such that reaction of chitosan with cyanuric chloride will produce some amount of dichlorotriazine, **G1-Cl**, which upon reaction with piperazine produces **G1-NH**. Chitosan flakes and powders were treated similarly. These materials were suspended in tetrahydrofuran and incubated with

an excess of reagent. The triazine chemistry was performed at 0 °C, a protocol adopted from earlier work on other supports, which we presume reduces the rate of hydrolysis or undesirable side reactions with the hydroxyl groups of the biopolymer that could lead to crosslinking (indicated with arrows). Reaction of the dichlorotriazine with an excess piperazine was performed at 25 °C, a temperature at which solution phase substitutions proceed readily.

Scheme 17. Synthesis of the desired grafts



Possible cross-linking of chitosan chains and structural defects from incomplete substitutions, especially as steric hindrance increased, were two difficulties encountered by using divergent dendron synthesis. The divergent strategy adopted here significantly reduces the burden of synthesis in comparison with the convergent strategy, which

requires that the dendrimer be first prepared in solution and then grafted to the support. When both strategies were compared for silica gel supported materials, it was found that materials afforded by the divergent approach were better sequesterants.¹¹⁵ Both issues suggest that these materials obtained by divergent pathway are of significantly greater interest.

While the chemistry is pedestrian, monitoring these reactions proved to be remarkably challenging. While chitosan shows some solubility in aqueous acetic acid, the modifications intended cannot be run under these conditions as hydrolysis actively competes with nucleophilic aromatic substitution. Unfortunately, upon modification, the solubility of the graft products is further diminished such that solution phase analysis including NMR spectroscopy was not possible. X-ray photoelectron spectroscopy proved to be highly dependent on sample preparation and surface non-uniformity. Thermogravimetric analysis which is often useful for organic-inorganic hybrid materials gave no quantitative information due to the presence of only organic material. Differences in the weight loss curves did indicate a change in the structure of the materials, however. Acid hydrolysis of the chitosan adducts could be effected, but no useful structural information was available presumably due to the low abundance of material due to our strategy of surface derivatization. When identical conditions were employed for a melamine dendron with a similar structure, no decomposition was observed.

Colorimetric analysis, however, proved remarkably useful. Figure 6 shows the result of treatment of these materials with a solution of ninhydrin affords a dark color

(purple or brown) in the presence of amines. The solution remains yellow in the absence of amines. The iterative nature of the synthesis, masking amines with dichlorotriazines and then providing secondary amines of piperazine, in principle should provide an oscillating positive and negative colorimetric test. Numerically quantifying the test is difficult because the dye absorbs to the chitosan flakes.

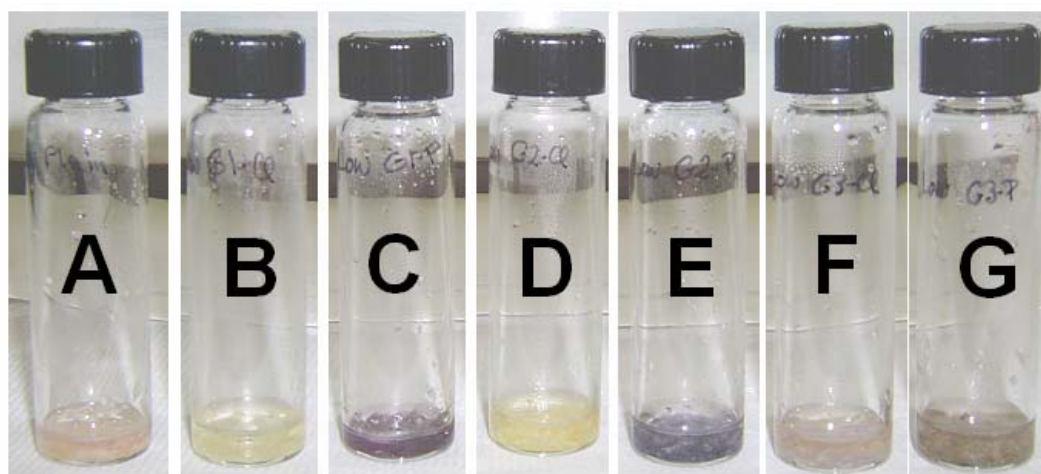


Figure 6. Colorimetric analysis of derivatized chitosan flakes.
A) Chitosan. B) G1-Cl. C) G1-NH. D) G2-Cl. E) G2-NH. F) G3-Cl. G) G3-NH.

Indirectly, the colorimetric tests reveal limitations in the synthesis. That is, the outline synthesis described in Scheme 17 where perfectly branched materials are prepared is not entirely accurate. Instead, incomplete reaction likely occurs, especially at generation 3 as evident from the pink-tinge that the solution of **G3-Cl** affords: free amines are present. As thorough washing is part of the protocol, we believe that the color is a result of the structural defects similar to those observed with the silica gel supported materials.¹¹⁵ For the powdered chitosan adducts, the ninhydrin results were

similar, but it appeared that the reaction may have proceeded more readily to completion as evidenced by an absence of a pink or purple hue for the **G3-Cl** powdered material as seen in Figure 7.



Figure 7. Colorimetric analysis of powdered materials.
A) Chitosan. B) G1-Cl. C) G1-NH. D) G2-Cl. E) G2-NH. F) G3-Cl. G) G3-NH.

The materials were also subjected to attenuated total internal reflection Fourier transform infrared spectroscopy (ATR-FTIR). Bands in the region of $1400\text{-}1600\text{ cm}^{-1}$ are introduced after the first reaction sequence. These bands are attributed to the triazine and melamine-type structures on the chitosan. Evidence of incomplete growth is given by the less pronounced changes in this region as the dendron growth progresses to higher generations. The iterative nature of the synthesis is revealed with the intensity of the broad absorption band centered at 3400 cm^{-1} attributed to N-H and O-H stretching. The

band is more pronounced in chitosan, **G1-NH**, **G2-NH** and **G3-NH** materials than in the corresponding dichlorotriazines, **G1-Cl**, **G2-Cl**, and **G3-Cl**. (Figure 8)

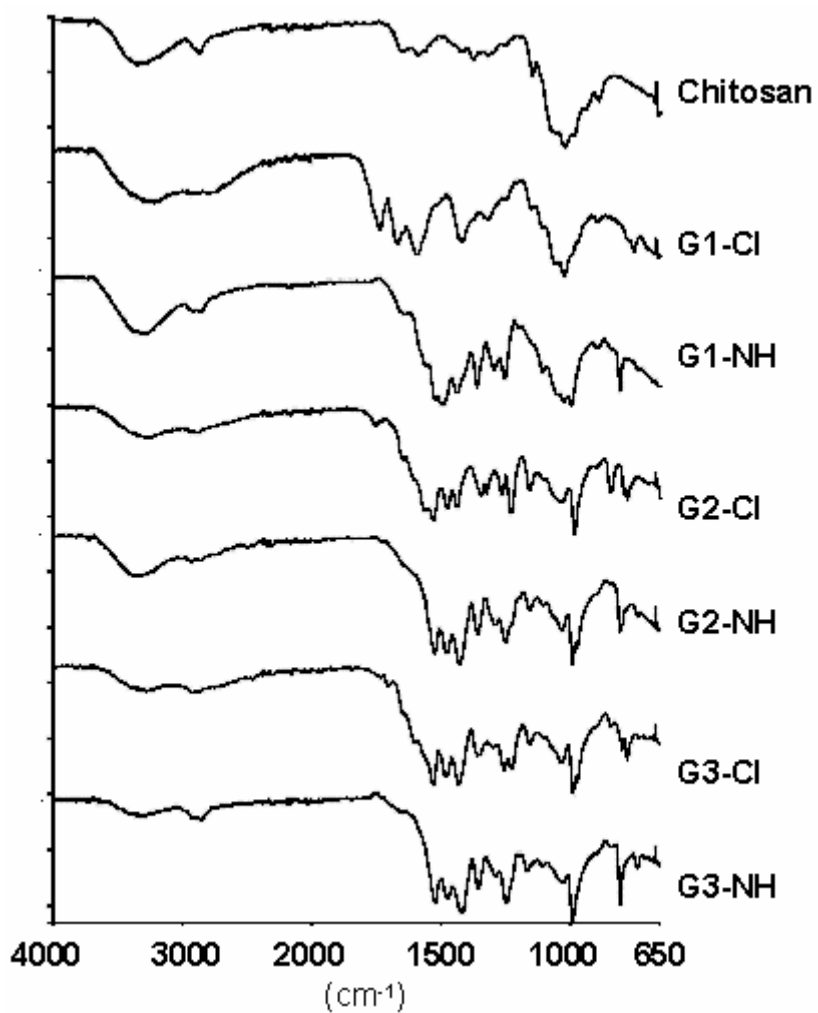


Figure 8. ATR-IR spectra of the materials

Atrazine Sequestration. The hypothesis for the presence of nucleophilic, constrained secondary amines displayed on chitosan was born out in sequestration studies. Figure 9 summarizes the results of batch equilibration studies performed using

chitosan flakes of low, medium and high molecular weights. Suspended chitosan, before or after washing with aqueous sodium hydroxide, shows only modest sequestration potential. Upon installation of the dichlorotriazine, **G1-Cl**, the sequestration potential drops off precipitously due presumably to the absence of nucleophilic amines. This result is consistent with other studies, but somewhat surprising given that we might expect that the hydrophobic character of atrazine, as evident from its low solubility in water, would promote adsorption onto the more hydrophobic support. Upon installation of the piperazine groups, all three supports sequester more than 99% of the atrazine from a 100 ppb solution as evaluated by LC-MS. This activity drops off as the amines are masked with triazines of **G2-Cl**, but remains greater than unmodified chitosan or **G1-Cl**. We attribute this to the onset of hydrophobic character, as colorimetric studies suggest a lack of nucleophilic amines. This trend continues through the subsequent materials. Quantifying results from samples derived from powdered chitosan was not possible using LC-MS due to the presence of materials sufficiently sized to foul the instrument, but small enough to elute through dialysis membranes. We believe this material is chitosan derived from the powdering process. Efforts to wash these samples continuously for one week did not provide remedy.

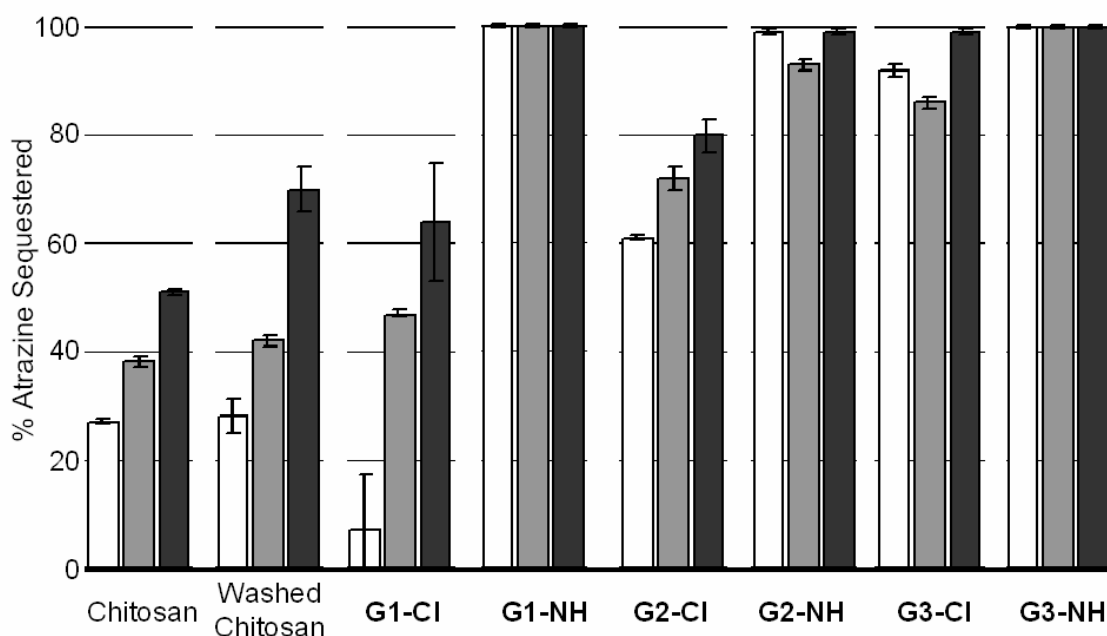


Figure 9. Atrazine sequestration by the materials using low (white), medium (gray) and high (black) molecular weight chitosan flakes

Conclusions

Chitosan, which has many potential uses for environmental remediation, can be modified to perform efficient sequestration of triazine herbicides such as atrazine. Dendritic molecules, such as dendrimers based on melamine, can be synthesized on solid chitosan flakes. The dendrimers increase the number of amine groups on the surface of the chitosan and increase the amount of atrazine sequestered. The use of chitosan as a solid support has an advantage of its ability to be woven into fabric and possibly coupled with erosion control for sequestration in the field. The combination of chitosan's ability to adsorb materials such as metals and dyes with pesticides leads to many future possible uses of chitosan for remediation and pollution control.

CHAPTER IV

SYNTHESIS OF MELAMINE DENDRIMERS FOR DRUG DELIVERY APPLICATIONS

Introduction

The term “magic bullet” was first introduced by Paul Ehrlich. Ehrlich’s work with immunology led to his receiving the Nobel Prize in Physiology or Medicine in 1908. Among his contributions was the belief that compounds with specific structures could be found which would recognize and bind a specific disease-causing target in an organism to provide therapeutic action to this target without causing harm to the organism itself – a magic bullet.¹¹⁹ Among many other accomplishments, Ehrlich’s work contributed to the establishment of chemotherapeutic techniques.¹²⁰

Pharmacologically active polymers have been recognized as important targets for decades, especially in the field of cancer therapeutics due to the non-specificity of many drugs. These “magic bullets” could enter tumor cells and deliver a payload of drug without harming the individual. In 1975, Ringsdorf defined the ideal structure and properties of polymeric drug agents.¹²¹ He described a carrier with a biostable or biodegradable backbone comprised of three parts. First, the polymer carrier should have a group that renders the macromolecule soluble and nontoxic. The second group is for the attachment of the drug, which would be performed under mild conditions and would incorporate a spacer to separate the drug from the polymer. These conditions would need to be met in order to ensure there would be no adverse effects on the drug’s biological

activity once attached to the polymeric carrier. The linkage between the polymer and the drug would need to be stable under normal body conditions but able to release the drug rapidly by hydrolysis or enzymatic processes once the site of action is reached. The third group would transport the entire carrier to the target cells by use of a homing device or through nonspecific enhancement of cellular-uptake.

Polymers, and therefore polymer drug conjugates, enter cell membranes by endocytosis rather than by diffusion. The macromolecules are engulfed by the plasma membrane and form endosomes which can fuse with enzyme-containing lysosomes. Provided the linker connecting the drug and carrier meets the aforementioned conditions, the linker may be cleaved to release the drug either enzymatically or by hydrolysis induced by the decrease in pH from 7.4 in the cytoplasm to pH 5 found in the lysosome.

Macromolecules show specificity to tumor cells due to a phenomenon known as the enhanced permeability and retention effect (EPR).¹²² Maeda and co-workers coined the term EPR effect in 1986.¹²³ They attributed the EPR effect to two main factors: leaky tumor vessels allowing entry of macromolecules, an activity which is not usually allowed in normal tissues, and an ineffective tumor lymphatic drainage system which prevents clearance of the macromolecules and promotes their accumulation. (Figure 10)

The typical value for the molecular weight of macromolecules which can exploit the EPR effect is greater than 40 kDa,¹²⁴ but studies have shown polymers with molecular weights between 20 and 800 kDa are able to access tumors. Studies of non-uniform accumulation of polymers in tumor tissue demonstrate that the threshold of

vascular permeability actually varies with polymer architecture, tumor size and type, and even vessel to vessel in the local microenvironment of the tumor.¹²⁵

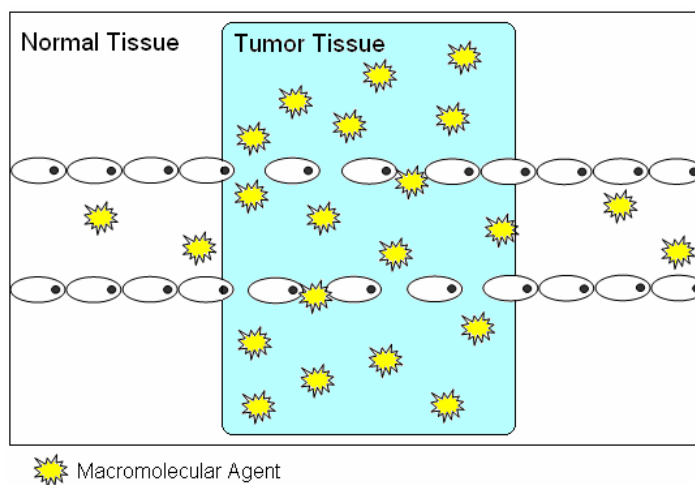


Figure 10. The EPR effect.

As mentioned in Chapter I, dendrimers are highly branched, symmetric, synthetic polymers emanating from a central core. These multivalent molecules are globular in shape, monodisperse, and allow control of molecular weight, surface groups, and interior groups.²⁸⁻³¹ Due to these properties, dendrimers are attractive targets for drug delivery agents.^{122,126-134} Figure 11 shows the possible uses of the multivalency of dendrimers. Synthetic manipulations to the functional groups in the core of the dendrimers would facilitate noncovalent encapsulation of drugs in the interior. Also, covalent attachment of solubilizing groups, targeting moieties, and drugs to the periphery of dendrimers is possible with modifications of the surface groups.

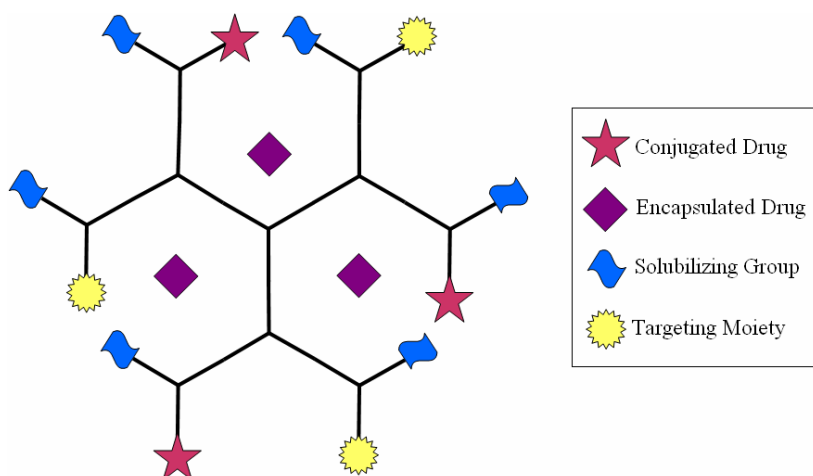


Figure 11. Possible functionalization of a dendritic drug delivery agent.

Noncovalent encapsulation of drugs with dendrimers can be accomplished using hydrogen bonding, hydrophobic, or electrostatic interactions between the guests and host.¹³⁵ One method of encapsulation is the construction of dendritic ‘unimolecular micelles’.¹³⁶⁻¹³⁹ Conventional polymeric micelles are amphiphilic block copolymers which form thermodynamic aggregates in proper solvents above the critical micelle concentration (c.m.c.). This property is also the limiting factor in micellar drug delivery applications. Under physiological conditions, the concentration may drop below the c.m.c. causing dissociation of the micelle into free polymer chains. With unimolecular micelles, the hydrophobic and hydrophilic segments are covalently bound together imparting stability to the micellar structure. Hydrophobic drugs can be solubilized in the hydrophobic core of the dendrimer while the hydrophilic portion, usually polyethylene glycol (PEG) chains, on the periphery solubilizes the entire carrier.¹³¹

Interactions between dendrimers and guests to afford encapsulation have been exploited by many groups.¹⁴⁰⁻¹⁵¹ Twyman, *et al.* synthesized water-soluble, hydroxyl terminated poly(amido amine) (PAMAM) dendrimers capable of solubilizing several small, acidic, hydrophobic model compounds including benzoic acid and salicylic acid. The complexes were stable at pH 7, but under acidic conditions, precipitation of the model hydrophobic compounds occurred. It was thought that protonation of the internal tertiary amines of the dendrimer interrupted the noncovalent interactions with the acidic guests causing this dissociation.¹⁵²

The effect of pH on dendrimer structure has also been investigated. Newkome and co-workers found that for a series of acid terminated cascade dendrimers of generation 1-5, the hydrodynamic radii decreased as pH decreased.¹⁵³ While for PAMAM dendrimers, a simulation study of generations 0-11 found the radius of gyration to increase with decreasing pH due to protonation of primary amines and interior tertiary amines. As the amines are protonated, electrostatic repulsions are created between the primary and tertiary amine sites pushing the arms apart and out. The calculated radii of gyration were in good agreement with the available SAXS and SANS data in this study. The structure was also shown to be more open to solvent as pH decreased allowing water molecules to more easily penetrate the interior of the dendrimer.¹⁵⁴ A study by Turro and co-workers showed that the binding specificity of PAMAM dendrimers is affected by pH. At neutral or slightly acidic pH, 2-naphthol has the ability to hydrogen bond to interior tertiary amines, but as the pH is lowered,

protonation of the amines coupled with an opening of the interior of the dendrimer causes a reduction in binding until eventually no binding occurs.¹⁵⁵

Simanek and co-workers have reported the synthesis of dendrimers based on melamine for noncovalent encapsulation of anti-cancer agents. Zhang, *et al.* reported the ability of a melamine dendrimer to efficiently encapsulate 10-hydroxycaptotecin and a novel bisindolemethane. However, encapsulation efforts between indomethacin, an anionic drug, and the cationic dendrimer provided a precipitated complex rather than facilitating solubilization of the molecule, and another anionic drug, methotrexate, experienced neither a solubilization increase nor a precipitation event. These studies suggest that the composition of the dendrimer plays an important role in the encapsulation ability.¹⁵⁶ Subsequent efforts in this laboratory reported by Neerman, *et al.* show that a variant of this dendrimer is able to effectively solubilize and reduce the hepatotoxicity of methotrexate and 6-mercaptopurine.¹⁵⁷

Covalent attachment of drugs to dendrimers is the second method for the preparation of drug-macromolecule complexes. Fréchet and co-workers synthesized several structures to this end. PEG-poly(aryl ether) dendrimers were synthesized for the attachment of model drug compounds. The PEG chains were selected as the solubilizing groups. The model compounds cholesterol, phenylalanine, and tryptophan were conjugated to the periphery of the dendrimer to illustrate the conjugation of drugs with various functionalities: hydroxyl, carboxylic acid, and amino groups, respectively.¹⁵⁸ A second polyether dendrimer was synthesized with terminal ester groups. The esters were converted to hydrazides to conjugate folate or

methotrexate residues to the periphery.¹⁵⁹ A three-arm poly(ethylene oxide) (PEO) star with terminal polyester dendrons was synthesized, also, and the anti-cancer drug doxorubicin was covalently bound to the periphery of the dendritic wedges by the formation of acid labile hydrazones. The compound was evaluated for *in vitro* and *in vivo* activity showing promising activity that should further advance the development of such macromolecular drug conjugates.^{134,160}

Another example of covalent conjugation is Baker's work in which antibodies are attached to dendrimers for use as targeting moieties.^{161,162} Monoclonal antibodies show promise for use as cancer-targeting agents because the surface antigens expressed by tumor cells are specific to the cancer or are overexpressed on cancerous cells. Efforts to form immunoconjugates in which a drug is directly conjugated to the antibody found limitations such as reduced drug activity or reduced antibody affinity. Conjugation of the drug and the antibody to a macromolecular carrier may be useful for overcoming these limitations.¹⁶²

Simanek and co-workers have reported the synthesis of dendrimers based on melamine with incorporation of multiple orthogonal functional groups. The differential reactivity of the primary building block of these dendrimers, cyanuric chloride, allows for incorporation of different groups with each substitution if desired, as discussed in Chapter I. The work of Steffensen and Simanek provided a dendrimer with five orthogonally reactive groups.³⁸ Modifications of this dendrimer allows for a multitude of possibilities. The incorporation of peptides to increase cellular uptake, small molecules such as biotin to increase binding, dyes to follow cellular uptake, or addition

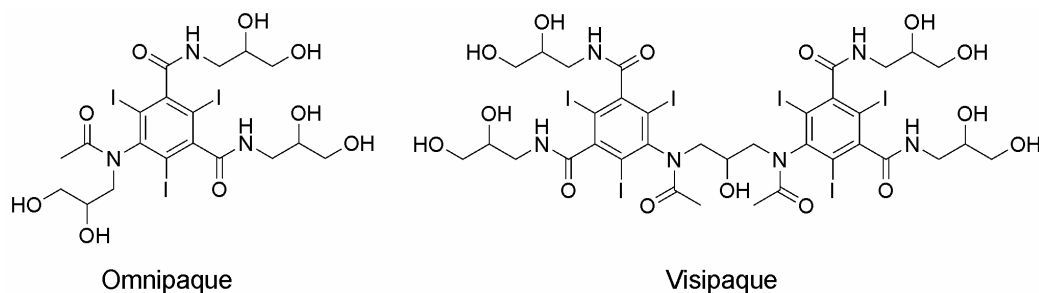
of an alkyne to partake in 'click chemistry' are but a few of the possibilities set out by this multifunctional dendrimer. Lim and Simanek followed suit with a similar dendrimer containing four functional groups which could be used for the conjugation of drugs, PEGylation to enhance solubility and biocompatibility, incorporation of "tags" to monitor biodistribution, and attachment of targeting moieties.¹⁶³

Another major aspect of dendrimers for drug delivery applications is for imaging. Dendrimers are of great interest as macromolecular carriers of contrast agents for MRI, scintigraphy, ultrasound, and X-ray, including computed tomography (CT).^{164,165} Contrast agents are used to modify the response of the signal produced to improve the sensitivity and specificity of the imaging method being employed. Ideal contrast agents have little interaction with the organism and are well tolerated.¹⁶⁴ However, this is not always the case. As said by Dr. Milos Sovak in *The Handbook of Experimental Pharmacology-Radiocontrast Agents*, "Contrast media are drugs by default...The position of CM in pharmacology is unique. First, there is the unusual requirement of biological inertness. An ideal CM should be completely inert, i.e., stable, not pharmacologically active, and efficiently and innocuously excretable. Because they fail to meet these requirements, CM must be considered drugs."¹⁶⁶

Contrast agents have been used since shortly after the discovery of X-rays by C. W. Roentgen in 1895. Iodine is often the element of choice for contrast agents due to the fact that iodinated contrast agents produce positive contrast during an X-ray. The presence of the higher atomic weight atoms compared to the atoms in the biological tissue causes the attenuation of the radiation to be higher for the contrast agent than the

surrounding tissue. The first contrast agent was NaI and was fairly toxic. Subsequent research efforts attempted to mask the iodine to reduce toxicity by binding the iodine to an organic moiety. The first commercially available contrast agent was introduced in 1929, uroselectan, which contained one iodine atom bound to a non-aromatic six-membered ring. Subsequent contrast agents utilized benzene rings as the iodine carriers to incorporate 2 or 3 iodine atoms per molecule. Contrast agents then progressed from ionic monomers to nonionic monomers and eventually to dimers of the ionic or nonionic compounds. Most of the present-day contrast agents are nonionic monomers containing polyol segments. The non-iodine portion of the contrast agent serves to increase solubility, form stable covalent bonds to iodine, and mask the iodine atoms making them “biologically invisible” to the body.^{164,167} (Chart 5)

Chart 5. Commonly used X-ray contrast agents.



For CT imaging, large amounts of contrast agent are needed, approximately 30-50 g of iodine which is equivalent to about 70-120 g of drug. The solutions injected into the patients are very concentrated, usually about 200-400 mg/mL for a total volume of 100-150 mL. This creates high osmotic potentials causing more toxicity effects.^{164,168}

As shown in Figure 12 below, the contrast agent creates an increase in plasma osmolality in the blood stream and extravascular water is drawn into the vessels. At the same time, the hypertonicity of the plasma causes water within red blood cells to be drawn into the vascular space as well. This can cause toxic effects in the body, in addition to diluting the concentration of contrast agent.¹⁶⁶

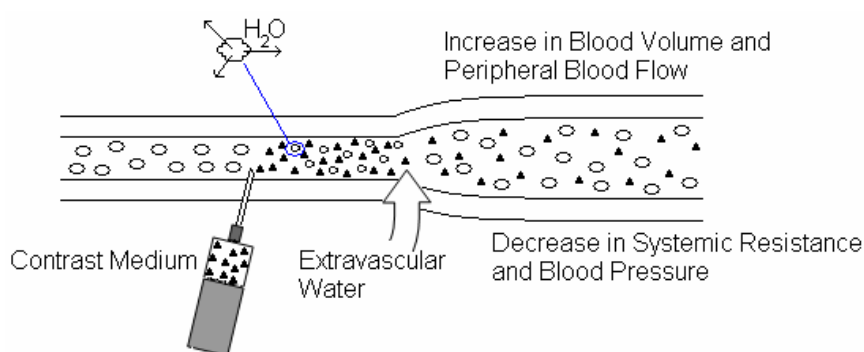


Figure 12. Effect of injection of contrast medium with high osmolality into bloodstream

More than 600 million X-ray examinations are conducted annually with approximately 75 million of these employing contrast agents. Approximately 1% of the patients receiving contrast agents experience adverse reactions which is equivalent to 750,000 patients per year. Adverse reactions can range from mild reactions including nausea and itching to severe reactions including anaphylactic shock or renal failure. Severe reactions occur in 0.01%-0.04% or 7,000-30,000 patients each year.¹⁶⁸ But adverse reactions have been shown to more likely develop in patients with a history of allergy to contrast media, asthma, or those who are medically unstable or debilitated.

Also, the introduction of low-osmolality agents has caused a significant decrease in non-fatal reactions and renal impairment.¹⁶⁹

Another factor to consider with currently used contrast agents is extravasation which causes the contrast agent to diffuse into the extracellular space. (Figure 13) This occurs because blood vessels contain pores approximately 12 nm in diameter through which small molecules can easily pass. Molecules larger than approximately 20 kDa cannot pass through these pores. Extravasation causes the contrast media to be rapidly distributed throughout the body after injection. This results in an extremely short imaging window and limits the possibility of accurate imaging to the first passage of the agent through the area of interest.¹⁶⁴

The employment of macromolecular contrast agents would reduce the dosage needed due to longer circulation times and lack of extravasation. In addition, macromolecules would reduce the osmotic potential, thereby reducing toxicity of the contrast agents, by increasing the number of iodine atoms on a molecule. Macromolecular agents could also aid in the detection of tumors due to the increased accumulation in tumor tissue caused by the EPR effect.¹⁶⁴

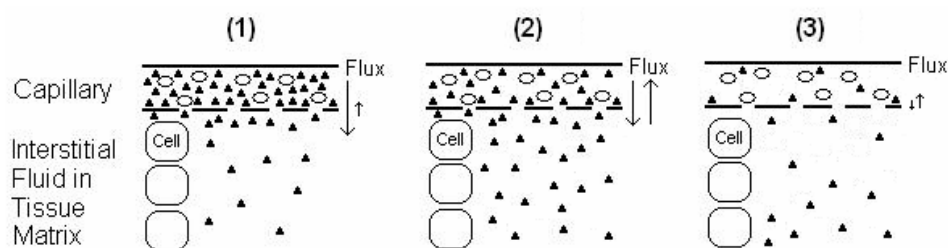


Figure 13. Extravasation of contrast agent out of capillary into extracellular space (1) during bolus, (2) shortly after bolus, and (3) a longer period after bolus.

Polymeric and dendritic contrast agent delivery systems have been reported but each has its drawbacks, whether it be synthetic problems such as drug uniformity, reproducibility, or purity of the compounds produced, or the economic feasibility of the synthesis on a large scale.^{164,165,167,170-172} A few other examples have come close to an ideal structure. Brasch and co-workers have synthesized a macromolecular iodinated contrast agent having a PEG core and lysine dendrons on both ends. This method allows for a monodisperse molecule with a predetermined weight.¹⁷³ The molecule with molecular weight of approximately 40 kDa and an iodine content of 27% by weight has been shown to have good activity as a contrast agent and exhibits the ability to characterize tumor vasculature when compared to a commonly used small molecule contrast agent, iohexol (Omnipaque).¹⁷⁴ Lysine dendrimers, however, are not readily amenable to large-scale, economically cost-efficient syntheses.

Another example which was synthesized by Le Lem, *et al.* is called P743.^{175,176} This monodisperse, dendritic, iodinated macromolecule has a molecular weight of approximately 13 kDa and an iodine content of 35% by weight and has been shown to have lower attenuation but longer circulation times than the standard contrast agent iobitridol.¹⁷⁷⁻¹⁷⁹ Data reported for tumor studies were a bit inconclusive stating that the use of iodinated contrast agents to image tumors is feasible.¹⁸⁰ Since this molecule is slightly below the value normally associated with the EPR effect, perhaps the activity was not marked enough to warrant a conclusive study.

Experimental

Materials and Methods. ACS-grade solvents were used for all the synthetic preparations. Distilled and deionized water was obtained in-house. 2,2'-Oxybis(ethylamine) was purchased from TCI Organic Chemicals, and 2,4-diiodoaniline was purchased from Spectra Group Limited, Inc. NHS-m-dPEG of molecular weight 1214 was purchased from Quanta BioDesign, Ltd., and m-PEG-SPA-2000 was purchased from Nektar Therapeutics. All other reagents and solvents were purchased from Acros Organics or Aldrich Chemical Co. and were used without further purification. ^1H NMR and proton decoupled ^{13}C NMR spectra were acquired on a Varian 300 MHz spectrometer using CDCl_3 , CD_3OD , Acetone- d_6 , or D_2O . NMR chemical shifts are listed relative to tetramethylsilane in parts per million (ppm) and were referenced to the residual proton or carbon peak of the solvent. MS analysis was performed by the Laboratory for Biological Mass Spectrometry at Texas A&M University. Elemental analysis was performed by Atlantic Microlab, Inc. in Norcross, GA. CHN analysis was performed by combustion using automatic analyzers. Chlorine and iodine analysis was performed by flask combustion followed by ion chromatography. Analysis gave percent by weight determination. Gel Permeation Chromatography (GPC) data was obtained using a multi-detector system with a Visco-GEL, mixed-bed, I-MBMMW-3078 column 7.8 mm x 30 cm with a flow rate of 1.000 mL/min, injection volume of 100 μL , detector temp. of 27.0 $^\circ\text{C}$, and column temp. of 30.0 $^\circ\text{C}$ using HPLC-grade THF as the mobile phase. The detectors employed were Viscotek: UV/Vis detector model VE3210 with readings taken at 265 nm, RI detector

model VE3580, and RALS/LALS/DP model 270 dual detector. Data was viewed and manipulated using OmniSEC software. Thin-layer chromatography was performed using EMD silica gel 60 F254 pre-coated glass plates (0.25 mm), and preparative chromatography was performed using EMD silica gel 60 (0.040 mm particle size).

Isosteric Dendrimer Nomenclature. For compounds having the structure $\text{RHN}(\text{CH}_2)_2\text{X}(\text{CH}_2)_2\text{NHR}'$ with R and R' being either a Hydrogen or other substituent and X being a Boc-protected amine, oxy, or methylene group, compounds will be denoted as N, O, or C, respectively.

Isosteric Dendrimer Synthesis and Characterization.

Compound 1. A solution of diethylene triamine (3.16 mL, 29 mmol) in 58 mL of DCM (0.5 M) was placed in an ice bath, and a solution of ethyl trifluoroacetate (7.28 mL, 61 mmol) in 30.5 mL DCM (2M) was added dropwise. The solution was stirred at 0 °C for 30 min. then further allowed to stir for an hour at room temperature. At this time, a solution of triethylamine (8.51 mL, 61 mmol) and di-*tert*-butyl dicarbonate (13.32 g, 61 mmol) in 30.5 mL DCM (2 M) was added dropwise. The reaction was allowed to stir at room temperature for 18 hours. Then, the reaction mixture was extracted with saturated sodium bicarbonate (2 x 150 mL), dried over magnesium sulfate, and rotovapped to one third of the volume. At this point, the same volume of hexanes was added and the solution was placed in the freezer for 18 hours. The white crystalline product was filtered, washed with hexanes, and dried under vacuum. Yield: 7.60 g (66%). ^1H NMR (300 MHz, CDCl_3) δ : 7.74 (s, NH), 7.22 (s, NH), 3.49 (s, 8H),

1.44 (s, 9H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 157.10, 117.63, 113.82, 99.84, 82.06, 47.16, 46.08, 40.20, 39.06, 28.01. ESI-MS (m/z): calcd 395.1280, found 396.1359 $[\text{M}+\text{H}]^+$.

Compound **2**. **1** (1.02 g, 2.6 mmol) was dissolved in 14 mL ethanol and 3M NaOH (10 mL, 30 mmol) was added. The solution was stirred for 18 hours, rotovapped to remove the ethanol, and the residue was extracted with DCM (3 x 100 mL). Then, the sample was dried over magnesium sulfate, rotovapped, and dried under vacuum. Yield: 429 mg (82%). ^1H NMR (300 MHz, CDCl_3) δ : 3.17 (s, br, 4H), 2.73 (t, $J=6.46$, 4H), 1.41 (s, NH) 1.35 (m, 9H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 155.88, 79.46, 50.49, 40.50, 28.22. ESI-MS (m/z): calcd 203.1634, found 204.1516 $[\text{M}+\text{H}]^+$, 226.1421 $[\text{M}+\text{Na}]^+$

Compound **3**. 1,1'-carbonyl diimidazole (3.21 g, 19.8 mmol) and *tert*-butanol (2.0 mL, 19.9 mmol) were dissolved in 70 mL toluene in a pressure flask. The solution was heated at 65 °C for three hours. Upon cooling, diethylene triamine (1.1 mL, 9.9 mmol) was added, and the solution was again heated to 65 °C for 3 hours. The mixture was then cooled, transferred to a round bottom flask, rotovapped, dissolved in DCM, and extracted with water (3 x 100 mL). The combined organic layers were dried over sodium sulfate, rotovapped, and dried under vacuum. Yield: 1.63 g (54%). ^1H NMR (300 MHz, CDCl_3) δ : 5.02 (s, NH), 3.17 (q, $J=5.78$, 2H), 2.68 (t, $J=5.78$, 2H), 1.87 (s, NH), 1.40 (s, 9H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 156.17, 79.17, 48.75, 40.23, 40.21, 28.35. ESI-MS (m/z): calcd 303.2158, found 304.2139 $[\text{M}+\text{H}]^+$.

Compound **4**. To a cold solution of cyanuric chloride (347 mg, 1.9 mmol) and Hünig's Base (0.75 mL, 4.3 mmol) in 10 mL THF, a solution of **3** (1.13 g, 3.7 mmol) in 10 mL THF was added. The reaction was stirred at 0 °C for about 3 hours and then allowed to warm to room temperature and stir for 18 hours. The solution was filtered, and the filtrate was rotovapped, dissolved in DCM, extracted with water (3x 100 mL), dried over magnesium sulfate, and rotovapped. The product was then purified by silica gel column chromatography using a gradient of DCM—4:1 (DCM:EtOAc). Yield: 1.05 g (79%). ¹H NMR (300 MHz, CDCl₃) δ: 5.62 (s, NH), 5.21 (s, NH), 3.58 (m, 8H), 3.25 (m, 8H), 1.36 (m, 36H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 168.82, 165.15, 156.17, 79.17, 79.10, 78.96, 47.86, 47.31, 47.12, 39.37, 38.57, 37.55, 28.37, 28.23. MALDI-MS (m/z): calcd 717.3940, found 718.3904 [M+H]⁺, 740.3672, [M+Na]⁺.

Compound **5-N**. **2** (137 mg, 0.7 mmol) and Hünig's Base (0.1 mL, 0.6 mmol) were added to a solution of **4** (219 mg, 0.3 mmol) in 3 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to MeOH gradient. Yield: 178 mg (66%). ¹H NMR (300 MHz, CDCl₃) δ: 5.59 (s, NH), 5.44 (s, NH), 3.57 (s, 8H), 3.42 (s, 4H), 3.27 (d, *J*=4.95, 8H), 3.11 (s, 4H), 2.93 (s, NH), 1.40 (m, 45H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 168.62, 165.67, 165.62, 156.22, 79.14, 79.10, 47.45, 47.37, 46.83, 40.05, 38.45, 28.43. MALDI-MS (m/z): caclcd 884.5807, found 885.6851 [M+H]⁺, 907.6590 [M+Na]⁺, 923.6322 [M + K]⁺.

Compound **5-O**. Oxy-ethylene diamine (0.16 mL, 1.4 mmol) and Hünig's Base (0.25 mL, 1.5 mmol) was added to a solution of **4** (524 mg, 0.7 mmol) in 7 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to MeOH gradient. Yield: 370 mg (65%). ¹H NMR (300 MHz, CDCl₃) δ: 5.95, (s, NH), 5.70 (s, NH), 5.59 (s, NH), 5.23 (s, NH), 3.57 (m, 10H), 3.48 (s, 4H), 3.27 (s, 8H), 2.86 (s, 2H), 2.19 (s, NH), 1.38 (s, 36H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.71, 165.48, 156.11, 79.07, 78.84, 72.85, 69.74, 47.32, 46.57, 41.58, 40.45, 38.27, 28.35. MALDI-MS (m/z): cacl'd 785.5123, found 786.4912 [M+H]⁺, 808.4731 [M+Na]⁺, 824.4530 [M+K]⁺.

Compound **5-C**. Pentane diamine (0.17 mL, 1.4 mmol) and Hünig's Base (0.25 mL, 1.4 mmol) was added to a solution of **4** (520 mg, 0.7 mmol) in 7 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to MeOH gradient. Yield: 370 mg (65% Yield). ¹H NMR (300 MHz, CDCl₃) δ: 6.05 (s, NH), 5.77 (s, NH), 5.62 (s, NH), 4.91 (s, NH), 3.53 (m, 8H), 3.30 (m, 10H), 2.66 (s, 2H), 2.01 (s, NH), 1.53 (m, 4H), 1.37 (s, br, 38H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.70, 165.55, 165.47, 156.121, 79.00, 78.74, 47.29, 46.50, 41.72, 40.47, 40.38, 38.26, 38.20,

32.92, 30.94, 29.43, 28.36, 24.11. MALDI-MS (m/z): caclcd 783.5331, found 784.6586 [M+H]⁺, 806.6439 [M+Na]⁺, 822.6312 [M+K]⁺.

Compound **6-N**. **5-N** (215 mg, 0.2 mmol) was added to a cold solution of cyanuric chloride (18 mg, 0.09 mmol) and Hünig's Base (0.04 mL, 0.2 mmol) in 3 mL THF. The reaction was stirred at 0 °C for about 3 hours and then allowed to warm to room temperature and stir for 18 hours. The solution was filtered, and the filtrate was rotovapped, dissolved in DCM, extracted with water (3 x 25 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a gradient of DCM—1:1:1% (DCM:EtOAc:MeOH). Yield: 153 mg (84%). ¹H NMR (300 MHz, CDCl₃) δ: 6.15 (s, NH), 5.79 (s, NH), 5.57 (s, NH), 5.32 (s, NH), 3.56-3.29 (br, 48H), 1.40 (s, 90H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.72, 165.40, 164.83, 156.25, 155.42, 78.13, 48.75, 47.40, 45.90, 39.92, 28.46. MALDI-MS (m/z): caclcd 1880.1239, found 1882.3485, [M+H]⁺, 1904.3252 [M+Na]⁺, 1919.2969 [M+K]⁺.

Compound **6-O**. **5-O** (207 mg, 0.3 mmol) was added to a cold solution of cyanuric chloride (25 mg, 0.1 mmol) and Hünig's Base (0.07 mL, 0.4 mmol) in 2 mL THF. The reaction was stirred at 0 °C for about 3 hours and then allowed to warm to room temperature and stir for 18 hours. The solution was filtered, and the filtrate was rotovapped, dissolved in DCM, extracted with water (3 x 25 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a gradient of DCM—1:1:1% (DCM:EtOAc:MeOH). Yield: 151 mg (34%). ¹H NMR (300 MHz, CDCl₃) δ: 5.83 (s, NH), 5.63 (s, NH), 5.26 (s, NH),

3.56 (s, 32H), 3.26 (s, 16H), 1.38 (s, 72H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.56, 165.61, 165.47, 156.16, 79.11, 79.05, 69.82, 69.73, 69.51, 69.09, 47.41, 47.36, 46.61, 40.57, 40.31, 40.26, 38.35, 38.22, 28.49, 28.32, 28.22. MALDI-MS (m/z): calcd 1681.9871, found 1683.3053 $[\text{M}+\text{H}]^+$, 1705.2670 $[\text{M}+\text{Na}]^+$, 1721.2533 $[\text{M}+\text{K}]^+$.

Compound **6-C**. **5-C** (187 mg, 0.2 mmol) was added to a cold solution of cyanuric chloride (24 mg, 0.1 mmol) and Hünig's Base (0.07 mL, 0.4 mmol) in 2 mL THF. The reaction was stirred at 0 °C for about 3 hours and then allowed to warm to room temperature and stir for 18 hours. The solution was filtered, and the filtrate was rotovapped, dissolved in DCM, extracted with water (3 x 25 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a gradient of DCM—1:1 (DCM:EtOAc). Yield: 132 mg (67%). ^1H NMR (300 MHz, CDCl_3) δ : 5.85 (s, NH), 5.76 (s, NH), 5.65 (s, NH), 5.04 (s, NH), 3.53 (s, 16H), 3.26 (s, 24H), 1.55 (s, 12H), 1.36 (s, 72H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.73, 165.51, 165.32, 156.12, 79.02, 78.78, 78.76, 47.35, 46.63, 46.57, 46.42, 40.57, 40.42, 40.35, 38.38, 38.21, 38.12, 28.30. MALDI-MS (m/z): calcd 1678.0285, found 1679.9914 $[\text{M}+\text{H}]^+$, 1700.9791 $[\text{M}+\text{Na}]^+$, 1716.9474 $[\text{M}+\text{K}]^+$.

Compound **7-N**. **2** (47 mg, 0.23 mmol) and Hünig's Base (0.05 mL, 0.29 mmol) was added to a solution of **6-N** (145 mg, 0.08 mmol) in 4 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to 9:1:1%

(DCM:MeOH:NH₄OH) gradient. Yield: 50 mg (32%). ¹H NMR (300 MHz, CDCl₃) δ: 6.26 (s, NH), 5.62 (s, NH), 3.54-3.26 (br, 56H), 2.91 (s, NH), 1.39 (m, 99H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.68, 165.62, 165.58, 165.52, 156.39, 156.25, 156.20, 156.17, 156.12, 156.09, 79.06, 79.03, 78.92, 78.91, 78.83, 47.64, 47.59, 47.44, 47.35, 47.32, 47.22, 47.03, 47.01, 39.87, 39.79, 39.74, 39.67, 39.65, 28.43, 28.32. MALDI-MS (m/z): caclcd 2047.3106, found 2049.4480 [M+H]⁺, 2061.4207 [M+Na]⁺, 2071.4383 [M + K]⁺.

Compound **7-O**. Oxy-ethylene diamine (0.04 mL, 0.39 mmol) and Hünig's Base (0.07 mL, 0.40 mmol) was added to a solution of **6-O** (221 mg, 1.3 mmol) in 1.3 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to 9:1:1% (DCM:MeOH:NH₄OH) gradient. Yield: 139 mg (61%). ¹H NMR (300 MHz, CDCl₃) δ: 5.71 (s, br, NH), 3.58 (s, 38H), 3.30 (s, 16H), 2.71 (s, NH), 2.08 (s, 2H), 1.41 (s, 72H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.46, 156.15, 78.86, 69.76, 47.33, 46.58, 40.88, 40.34, 40.21, 38.20, 28.35, 28.30. MALDI-MS (m/z): caclcd 1750.1054, found 1751.1823 [M+H]⁺, 1774.1702 [M+Na]⁺.

Compound **7-C**. Pentane diamine (0.03 mL, 0.27 mmol) and Hünig's Base (0.05 mL, 0.27 mmol) was added to a solution of **6-C** (153 mg, 0.09 mmol) in 1 mL THF in a pressure flask. The reaction was stirred at 70 °C for 48 hours, and upon cooling, the solution was filtered. The filtrate was then rotovapped, dissolved in DCM, extracted with water (3 x 50 mL), dried over magnesium sulfate, and rotovapped. The product

was then purified using silica gel column chromatography with a 19:1 (DCM: MeOH) to 9:1:1% (DCM:MeOH:NH₄OH) gradient. Yield: 97 mg (61%). ¹H NMR (300 MHz, CDCl₃) δ: 5.96 (s, NH), 5.83 (s, NH), 5.71 (s, NH), 5.26 (s, NH), 4.83 (s, NH), 3.52 (s, 16H), 3.26 (s, 24H), 2.95 (s, 2H), 1.71 (s, 2H), 1.51 (s, 12H), 1.36 (m, 72H), 1.18 (m, 6H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 165.32, 156.25, 79.07, 47.44, 46.63, 40.50, 38.23, 29.63, 29.19, 28.43, 28.37, 26.89, 24.23. MALDI-MS (m/z): cacl'd 1744.1676, found 1745.0729 [M+H]⁺, 1768.0572 [M+Na]⁺, 1784.0320 [M + K]⁺.

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Compound **8**. Diethanolamine (21.23 mL, 221.5 mmol) was dissolved in 40 mL of acetone and placed in an ice bath. A solution of cyanuric chloride (10.21 g, 55.4 mmol) in 25 mL of acetone was added dropwise. The reaction mixture was allowed to gradually warm to room temperature while stirring for 18 h. The solution was then rotovapped and distilled water (90 mL) was added forming a slurry which was stirred at room temperature for 3 h before placing in ice bath to precipitate product. The white powder was filtered and dried. Yield: 16.32 g (92%). ¹H NMR (300 MHz, DMSO-d₆) δ: 4.77 (m, 4H), 3.58 (s, 16H). ¹³C NMR (75.5 MHz, DMSO-d₆) δ: 168.16, 164.11, 58.56, 50.81, 50.36, 39.50. APCI-MS (m/z): cacl'd 321.1204, found 322.1 [M+H]⁺.

Compound **9**. Acetic anhydride (2.9 mL, 31.0 mmol) in 5 mL of DCM and acetyl chloride (2.2 mL, 31.0 mmol) in 5 mL of DCM is added dropwise to a slurry of **8** (985 mg, 3.1 mmol) in 15 mL of DCM. The reaction is then allowed to stir for 18 h obtaining a clear solution. Triethylamine (1.5 mL, 10.8 mmol) is added, and then the mixture is slowly added to a solution of saturated sodium bicarbonate and stirred until

the evolution of gas is no longer apparent. The organic layer is washed with saturated sodium bicarbonate (3 x 150 mL), dried with magnesium sulfate, filtered, and rotovapped. The product is further purified by silica gel column chromatography using 10:1 (CHCl₃:EtOAc) as the eluent. Yield: 1.39 g (92%) ¹H NMR (300 MHz, CDCl₃) δ: 4.26 (t, *J*=5.50, 8H), 3.81 (m, 8H), 2.04 (d, *J*=3.30, 12H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 170.084, 164.88, 99.85, 62.38, 61.64, 47.38, 47.24, 20.89, 20.84. ESI-MS (*m/z*): caclcd 489.1626, found 490.1674 [M+H]⁺, 512.1548 [M+Na]⁺.

Compound **10**. **9** (660 mg, 1.4 mmol) is dissolved in 15 mL of THF and placed in a dry-ice/isopropyl alcohol bath. *N*-aminoethylpiperazine (0.71 mL, 5.4 mmol) is added dropwise and the reaction is allowed to warm to room temperature while stirring for approximately 18 h. The reaction mixture is rotovapped and purified by silica gel column chromatography using a 19:1—9:1 (DCM:MeOH) gradient. Yield: 656 mg (84%). ¹H NMR (300 MHz, CDCl₃) δ: 4.24 (d, *J*=4.95, 8H), 3.76 (m, 12H), 2.84 (d, *J*=4.40, 2H), 2.43 (s, 8H), 2.03 (d, *J*=4.40, 12H). ¹³C NMR (75.5 MHz, CDCl₃) δ: 170.97, 165.11, 99.83, 62.63, 60.39, 53.12, 46.96, 42.98, 38.41, 20.95. ESI-MS (*m/z*): caclcd 582.3126, found 583.3261 [M+H]⁺.

Compound **11**. Cyanuric Chloride (87.0 mg, 0.47 mmol) is dissolved in 10 mL of THF and placed in an ice bath. DIPEA (0.24 mL, 1.4 mmol) and **10** (656 mg, 1.1 mmol) is added and the reactions is allowed to slowly come to room temperature while stirring for 18 h. The solution is then rotovapped and purified by silica gel column chromatography using 1:1:3% (CHCl₃:EtOAc:MeOH) as the eluent. Yield: 184 mg (31%). ¹H NMR (300 MHz, CDCl₃) δ: 6.05 (s, NH), 4.22 (t, *J*=5.64, 16H), 3.75-3.68

(m, 24H), 3.49 (d, br, 4H), 2.53 (d, br, 4H), 2.43 (s, 8H), 2.01 (s, 24H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 170.87, 165.40, 165.07, 164.63, 99.79, 62.57, 62.47, 56.27, 52.70, 46.91, 42.83, 37.25, 20.93. MALDI-MS (m/z): caclcd 1275.5875, found 1276.6317 $[\text{M}+\text{H}]^+$.

Compound **12**. A solution of *N*-aminoethylpiperazine (0.5 mL, 3.8 mmol) in 8 mL of DCM (0.5 M) was placed in an ice bath, and a solution of ethyl trifluoroacetate (0.5 mL, 4.2 mmol) in 2 mL DCM (2M) was added dropwise. The solution was stirred at 0 °C for 30 min. then further allowed to stir for an hour at room temperature. At this time, a solution of triethylamine (0.6 mL, 4.3 mmol) and di-*tert*-butyl dicarbonate (954 g, 4.2 mmol) in 2 mL DCM (2 M) was added dropwise. The reaction was allowed to stir at room temperature for 18 hours. Then, the reaction mixture was extracted with saturated sodium bicarbonate (2 x 100 mL), dried over magnesium sulfate, and rotovapped. Ethanol (19 mL) and 3M NaOH (15 mL) was added to the residue and the mixture was allowed to stir at room temperature for 6 h. At this time, the solution was rotovapped, dissolved in DCM, extracted with saturated NaCl (3 x 50 mL), dried over magnesium sulfate, filtered, and rotovapped. Yield: 692 mg (79%). ^1H NMR (300 MHz, CDCl_3) δ : 3.39 (m, 4H), 2.79 (t, $J=5.91$, 2H), 2.40 (m, 8H), 1.42 (s, 9H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 154.68, 79.57, 60.18, 52.93, 43.73, 43.24, 38.27, 28.35. ESI-MS (m/z): caclcd 229.1790, found 230.1813 $[\text{M}+\text{H}]^+$.

Compound **13**. Cyanuric chloride (226 mg, 1.2 mmol) was dissolved in 12 mL THF and **12** (1.94 g), 8.5 mmol) was added. The solution was heated to 70 °C and allowed to react for 1 week. The solution was then filtered, rotovapped, and purified

using silica gel column chromatography with a DCM—1:1:1% (DCM:EtOAc:MeOH) gradient. Yield: 126 mg (43%). ^1H NMR (300 MHz, CDCl_3) δ : 5.34 (s, NH), 3.41 (s, 18H), 2.51 (s, 6H), 2.39 (s, 12H), 1.44 (s, 27H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 168.08, 156.77, 154.66, 79.61, 69.24, 56.95, 52.93, 52.84, 52.66, 43.89, 43.60, 43.11, 37.05, 28.45, 28.37. ESI-MS (m/z): caclcd 762.5228, found 763.5485 $[\text{M}+\text{H}]^+$.

Compound **14**. **13** (107 mg, 0.14 mmol) is dissolved in DCM (2mL) and trifluoroacetic acid (2 mL) is added. The reaction is allowed to stir for 24 h at room temperature, rotovapped, and DIPEA is added (5 mL, 26 mmol).

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Compound **15**. Cyanuric chloride (1.04 g, 5.6 mmol), 1-Boc-piperazine (3.69 g, 19.8 mmol), and DIPEA (9.8 mL, 56.4 mmol) were dissolved in THF (56 mL) and placed in a pressure flask. The mixture was heated to 70° C and allowed to stir for 48 h. The solution was rotovapped, dissolved in DCM (50 mL), extracted with water (3 x 125 mL), dried over magnesium sulfater, filtered, and rotovapped. The residue was dissolved in a minimal amount of DCM, excess MeOH was added, and the solution rotovapped until it became slightly cloudy. More DCM was added and it was again rotovapped until the solution became slightly cloudy. At this time the flask was placed in the freezer to reprecipitate the product. The next day, the precipitate was filtered and dried. Yield: 2.98 g (83%). ^1H NMR (300 MHz, CDCl_3) δ : 3.72 (m, 12H), 3.42 (m, 12H), 1.46 (s, 27H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 164.68, 155.97, 79.30, 44.94, 37.32, 28.36, 27.69. ESI-MS (m/z): caclcd 633.3962, found 634.4021 $[\text{M}+\text{H}]^+$.

Compound **16**. **15** (1.55 g, 2.45 mmol) was dissolved in DCM (22 mL) and trifluoroacetic acid (22 mL) was added. The reaction was allowed to stir for 4 h at room temperature. The solution was then rotovapped repeatedly, rinsing well with DCM each time. 1M NaOH (35 mL) was added to the residue until a pH 14 was reached and then the solution was extracted with CHCl_3 (3 x 200 mL), or until UV activity was no longer detected in the aqueous layer. The combined portions were dried over magnesium sulfate, filtered, and rotovapped. Yield: 779 mg (95%). ^1H NMR (300 MHz, CDCl_3) δ : 3.69 (m, 12H), 2.82 (m, 12H), 1.85 (s, NH). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.24, 45.96, 44.19. ESI-MS (m/z): caclcd 333.2398, found 334.2334 $[\text{M}+\text{H}]^+$.

Compound **17**. Cyanuric chloride (2.0 g, 10.8 mmol) was dissolved in acetone (43 mL) to obtain a 0.25 M solution. 1-Boc-piperazine (1.8 g, 9.7 mmol) was dissolved in acetone (16 mL) and sodium bicarbonate (940 mg, 11.2 mmol) dissolved in water (18 mL) to obtain 0.6 M solutions of each. The three solutions were chilled, and water (65 mL) was placed in an ice bath to cool, also. A slurry of cyanuric chloride (0.1 M) was then made by adding the cyanuric chloride solution to the well stirred ice water. Then the cold solutions of Boc-piperazine and NaHCO_3 were added, and the reaction was allowed to stir at 0 °C for 2 h. Precipitation of the product occurs, and after the 2 h the reaction mixture is filtered. The precipitate is washed with cold water and dissolved in DCM (150 mL). The solution is extracted with water (150 mL), dried over magnesium sulfate, filtered, rotovapped, and dried. The product was then purified using silica gel column chromatography with 20:1 (CHCl_3 :EtOAc) as the eluent. Yield: 3.07 g (95%). ^1H NMR (300 MHz, CDCl_3) δ : 3.84 (m, 4H), 3.49 (m, 4H), 1.46 (s, 9H). ^{13}C NMR

(75.5 MHz, CDCl₃) δ : 170.39, 154.34, 80.63, 43.93, 28.31. ESI-MS (m/z): cacl'd 333.0759, found 334.0781 [M+H]⁺.

Compound **18**. Bis(3-aminopropyl)amine (1.62 mL, 11.3 mmol) and DIPEA (6.0 mL, 34.0 mmol) were dissolved in THF (25 mL), and BOC-ON (5.58 g, 22.7 mmol) was dissolved in THF (25 mL). Both solutions were cooled to 0 °C and then combined. The mixture was allowed to gradually warm to room temperature and stirred for 4 h. The solution was then rotovapped, dissolved in DCM (100 mL), extracted with 5% NaOH (3 x 100 mL), dried over magnesium sulfate, filtered, and rotovapped again. The residue was then reprecipitated from DCM with petroleum ether overnight in the freezer. The precipitate is then filtered and dried. Yield: 2.97 g (79%). ¹H NMR (300 MHz, CDCl₃) δ : 5.20 (s, NH), 3.18 (q, *J*=6.05, 4H), 2.62 (t, *J*=6.46, 4H), 1.62 (qd, *J*=6.33, 6.51, 4H), 1.41 (s, 18H). ¹³C NMR (75.5 MHz, CDCl₃) δ : 156.08, 78.95, 47.41, 38.92, 29.73, 28.40. ESI-MS (m/z): cacl'd 331.2471, found 332.2563 [M+H]⁺.

Compound **19**. Cyanuric chloride (2.04 g, 11.4 mmol) was dissolved in acetone (45 mL) to obtain a 0.25 M solution. **18** (3.4 g, 10.2 mmol) was dissolved in acetone (17 mL) and sodium bicarbonate (984 mg, 11.4 mmol) dissolved in water (19 mL) to obtain 0.6 M solutions of each. The three solutions were chilled, and water (69 mL) was placed in an ice bath to cool, also. A slurry of cyanuric chloride (0.1 M) was then made by adding the cyanuric chloride solution to the well stirred ice water. Then the cold solutions of **18** and NaHCO₃ were added, and the reaction was allowed to stir at 0 °C for 2 h. Precipitation of the product occurs, and after the 2 h the reaction mixture is filtered. The precipitate is washed with cold water and dissolved in DCM (150 mL). The

solution is extracted with water (150 mL), dried over magnesium sulfate, filtered, rotovapped, and dried. Yield: 4.35 g (89%). ^1H NMR (300 MHz, CDCl_3) δ : 5.04 (s, NH), 3.61 (t, $J=6.88$, 4H), 3.11 (q, $J=6.14$, 4H), 1.78 (m, $J=6.67$, 4H), 1.43 (s, 18H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 170.08, 164.66, 155.97, 79.29, 44.93, 37.31, 28.35, 27.67. ESI-MS (m/z): caclcd 478.1862, found 479.1988 $[\text{M}+\text{H}]^+$, 501.1783 $[\text{M}+\text{Na}]^+$.

Compound **20**. **19** (1.60 g, 3.3 mmol), DIPEA (1.74 mL, 10 mmol), and propargylamine (0.23 mL, 3.3 mmol) were dissolved in THF (40 mL) and allowed to stir at room temperature for 18 h. The solution was rotovapped, dissolved in DCM (100 mL), extracted with water (3 x 100 mL) and brine (1 x 100 mL), dried over magnesium sulfater, filtered, and rotovapped. The compound was then purified using silica gel column chromatography with 8:1 (CHCl_3 :EtOAc) as the eluent. Yield: 1.34g (81%). ^1H NMR (300 MHz, CDCl_3) δ : 6.29 (s, NH), 5.54 (s, NH), 4.93 (s, NH), 4.18 (m, 2H), 3.58 (m, 4H), 3.10 (m, 4H), 2.22 (s, 1H), 1.76 (m, 4H), 1.44 (m, 18H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 167.71, 165.02, 164.86, 156.11, 155.89, 79.66, 78.91, 71.39, 44.28, 43.70, 43.67, 37.63, 36.79, 30.77, 28.41, 27.89, 27.69. ESI-MS (m/z): caclcd 497.2517, found 498.2634 $[\text{M}+\text{H}]^+$.

Compound **21**. **16** (490 mg, 1.5 mmol) was dissolved in THF (50 mL), and **17** (1.53 g, 4.6 mmol) was separately dissolved in THF (50 mL) to give a slurry. The solutions were cooled to 0 °C and DIPEA (2.6 mL, 14.7 mmol) was added to the solution of **16**. The two solutions were then combined and stirred at 0 °C for 1 h then allowed to warm to room temperature and stir for 18 h. The reaction mixture was then rotovapped, dissolved in DCM (100 mL), extracted with water (3 x 100 mL), dried over

magnesium sulfater, filtered, and rotovapped. The compound was purified using silica gel chromatography with a 8:1—4:1 (DCM:EtOAc) gradient. Yield: 1.59 g (88%). ^1H NMR (300 MHz, CDCl_3) δ : 3.81 (s, br, 36H), 3.45 (s, br, 12H), 1.47 (s, 27H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 169.65, 167.31, 164.42, 154.59, 80.24, 43.29, 43.23, 43.01, 42.95, 42.88, 42.79, 42.74, 28.35. MALDI-MS (m/z): caclcd 1224.5367, found 1225.5638 $[\text{M}+\text{H}]^+$, 1248.5532 $[\text{M}+\text{Na}]^+$, 1263.5167 $[\text{M}+\text{K}]^+$.

Compound **22**. **21** (514 mg, 0.42 mmol), DIPEA (0.44 mL, 2.5 mmol), and 2-(2-aminoethoxy)ethanol (0.25 mL, 2.5 mmol) were dissolved in THF (4 mL) and placed in a pressure flask. The mixture was heated to 70 °C and allowed to stir for 24 h. The reaction mixture was then rotovapped, dissolved in DCM (50 mL), extracted with water (3 x 75 mL), dried over magnesium sulfater, filtered, and rotovapped. The product was then purified using silica gel column chromatography with 25:1 (DCM:MeOH) as the eluent. Yield: 385 mg (64%). ^1H NMR (300 MHz, CDCl_3) δ : 5.40 (s, NH), 3.73 (m, 36H), 3.59 (m, 18H), 3.41 (s, br, 12H), 1.46 (s, 27H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 166.18, 165.28, 165.04, 154.76, 79.89, 72.33, 70.11, 61.63, 42.97, 42.89, 40.46, 28.38. MALDI-MS (m/z): caclcd 1431.8436, found 1432.8631 $[\text{M}+\text{H}]^+$, 1454.8362 $[\text{M}+\text{Na}]^+$, 1470.8266 $[\text{M}+\text{K}]^+$.

Compound **23**. Concentrated HCl was added to a slurry of **22** in MeOH. The solution became clear and was allowed to stir at room temperature for 18 h. The solution was then rotovapped until only about 5 mL of water remained, and the residue was diluted with distilled water (20 mL). The solution was then extracted with DCM (3 x 25 mL). The aqueous layer was rotovapped and dried to form a white powder. Yield:

83.4 mg (99%). ^1H NMR (300 MHz, D_2O) δ : 4.14 (br, 12H), 3.95 (br, 24H), 3.69 (br, 18H), 3.64 (br, 6H), 3.34 (br, 12H). ^{13}C NMR (75.5 MHz, D_2O) δ : 161.9, 158.1, 156.0, 154.3, 71.7, 68.6, 60.5, 43.5, 43.1, 40.7, 40.3. MALDI-MS (m/z): caclcd 1131.6863, found 1132.8087 $[\text{M}+\text{H}]^+$, 1170.7723 $[\text{M}+\text{K}]^+$.

Compound **24**. **23** (83.4 mg, 0.06 mmol), DIPEA (0.2 mL, 1.1 mmol), and **20** (194 mg, 0.39 mmol) were dissolved in THF (4 mL) and placed in a pressure flask. The reaction is heated at 70 °C for 5 days at which time the mixture is rotovapped, dissolved in DCM, and extracted with water/saturated NaCl (3 x 75 mL). The organic phase is dried with magnesium sulfate, filtered, and rotovapped. Purification was attempted using silica gel column chromatography using a 19:1:10% (DCM:MeOH:EtOAc)—4:1 (DCM:MeOH) gradient. No appreciable amount of pure dendrimer was obtained. Presence of the dendrimer was confirmed by MALDI-MS; however, each fraction analyzed contained an impurity of unknown origin. MALDI-MS (m/z): caclcd 2515.5115, found 2517.4269 $[\text{M}+\text{H}]^+$, 2539.3878 $[\text{M}+\text{Na}]^+$.

Iodinated Dendrimer Synthesis and Characterization.

Compound **25**. **16** (268 mg, 0.81 mmol) was dissolved in THF (20 mL) and placed in an ice bath to cool. DIPEA (1.4 mL, 8.1 mmol) and then **19** (1.24 g, 2.58 mmol) was added, and the reaction was allowed to slowly warm to room temperature and stir for 18 h. The solution was rotovapped, dissolved in CHCl_3 (100 mL), extracted with water (3 x 100 mL), dried over magnesium sulfate, filtered, and rotovapped. The compound was then purified using silica gel column chromatography with 4:1 (DCM:EtOAc) as the eluent. Yield: 1.07 g (80%). ^1H NMR (300 MHz, CDCl_3) δ :

5.59 (s, NH), 4.84 (s, NH), 3.85 (s, 24H), 3.56 (m, 12H), 3.08 (m, 12H), 1.75 (m, 12H), 1.43 (s, 54H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 169.24, 165.15, 164.91, 164.25, 156.12, 155.81, 79.24, 78.80, 43.81, 43.36, 43.29, 42.83, 42.59, 37.70, 36.72, 28.38, 28.35, 27.82, 27.74. MALDI-MS (m/z): caclcd 1659.8675, found 1660.1431 $[\text{M}+\text{H}]^+$, 1682.1252 $[\text{M}+\text{Na}]^+$.

Compound **26**. **25** (685 mg, 0.41 mmol) was dissolved in THF (10 mL) and excess piperidine (0.4 mL, 4.1 mmol) was added. The mixture was allowed to stir at room temperature for 18 h. Then, the solution was filtered through a silica plug, rinsing well with THF (150 mL) to remove most of the piperidine. The solution was then rotovapped, dissolved in DCM (50 mL), extracted with water (3 x 75 mL), dried over sodium sulfate, and filtered through a second silica plug rinsing with 19:1 (DCM:MeOH) (400 mL). Yield: 737 mg (99%). ^1H NMR (300 MHz, CDCl_3) δ : 5.29 (s, NH), 3.80 (s, br, 24H), 3.72 (s, br, 12H), 3.59 (s, br, 12H), 3.06 (s, br, 12H), 1.70 (m, br, 12H), 1.59 (m, br, 18H), 1.41 (s, 54H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.87, 165.25, 164.80, 155.97, 78.93, 44.10, 43.15, 42.97, 41.52, 37.10, 36.24, 28.49, 27.59, 25.76, 24.88. MALDI-MS (m/z): caclcd 1807.2050, found 1808.6305 $[\text{M}+\text{H}]^+$, 1829.5946 $[\text{M}+\text{Na}]^+$.

Compound **27**. **26** (634 mg, 0.35 mmol) was placed in MeOH (8mL) to form a cloudy solution and concentrated HCl (4 mL) was added. The solution became clear and the mixture was allowed to stir at room temperature for 18 h. For isolation of the amine-HCl salt, the solution was then rotovapped, adding ethanol to help remove water. (Alternatively, the neutral compound may be isolated by adding 3M NaOH until pH 14

is reached and extracting with CHCl_3 until no UV activity is detected in the aqueous layer.) Yield: 536 mg (>99%) ^1H NMR (300 MHz, CDCl_3) δ : 3.78 (s, 24H), 3.70 (s, 12H), 3.62 (t, $J=6.60$, 12H), 2.67 (t, $J=5.91$, 12H), 1.71 (m, 12H), 1.61 (m, 6H), 1.53 (s, br, 12H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.67, 165.39, 164.93, 44.12, 43.11, 42.63, 39.21, 39.17, 31.46, 25.83, 25.02. MALDI-MS (m/z): caclcd 1206.8904, found 1207.7268 $[\text{M}+\text{H}]^+$.

Compound **28**. **27** (282 mg, 0.23 mmol) was dissolved in H_2O (2 mL). DIPEA (0.69 mL, 4.0 mmol) and EtOAc (3 mL) was added. **19** (960 mg, 2.0 mmol) was dissolved in DCM (9 mL) and added to the solution. The mixture was allowed to stir at room temperature for about 1 h. The solution was then rotovapped, and the residue was dissolved in DCM (50 mL). This solution was extracted with water (3 x 75 mL), dried over magnesium sulfate, filtered, and purified by silica gel column chromatography using 4:1:1% (DCM: EtOAc:MeOH). Yield: 541 mg (60%). ^1H NMR (300 MHz, CDCl_3) δ : 5.67-5.60 (br, NH), 4.99 (s, br, NH), 3.81 (s, br, 24H), 3.74 (s, br, 12H), 3.63-3.55 (br, 36H), 3.38 (s, br, 12H), 3.06 (s, 24H), 1.84-1.55 (br, 72H), 1.42-1.40 (d, br, 108H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.31, 165.29, 165.05, 164.81, 156.10, 155.83, 79.05, 78.73, 44.24, 44.04, 43.37. 43.25, 43.07, 37.79, 37.43, 36.73, 27.86, 25.77. 24.88. MALDI-MS (m/z): caclcd 3860.1476, found 3865.1311 $[\text{M}+\text{H}]^+$, 3887.0812 $[\text{M}+\text{Na}]^+$, 3903.0288 $[\text{M}+\text{K}]^+$.

Compound **29**. **28** (203 mg, 0.05 mmol) was dissolved in THF (10 mL) and excess piperidine (0.1 mL, 1.0 mmol) was added. The mixture was allowed to stir at room temperature for 18 h. The solution was filtered through a silica gel plug rinsing

with THF (125 mL), rotovapped, dissolved in DCM (25 mL), extracted with water (3 x 50 mL), dried over sodium sulfate, filtered, and rotovapped. Yield: 215 mg (98%). ^1H NMR (300 MHz, CDCl_3) δ : 5.25 (s, NH), 3.79 (s, br, 24H), 3.68 (s, br, 36H), 3.53 (s, br, 36H), 3.34 (s, 12H), 3.02 (s, 24H), 1.81 (s, 12H), 1.66-1.54 (m, br, 78H), 1.39 (s, 108H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 165.83, 165.34, 164.93, 164.76, 155.96, 78.79, 44.08, 43.07, 42.09, 41.81, 37.08, 28.55, 28.28, 27.66, 25.75, 24.99. MALDI-MS (m/z): caclcd 4154.8224, found 4157.1251 $[\text{M}+\text{H}]^+$, 4179.9701 $[\text{M}+\text{Na}]^+$.

Compound **30**. **29** (137 mg, 0.03 mmol) was placed in MeOH (2 mL) to form a cloudy solution and concentrated HCl (1 mL) was added. The solution became clear and the mixture was allowed to stir at room temperature for 18 h. For isolation of the amine-HCl salt, the solution was then rotovapped, adding ethanol to help remove water. Yield: 113 mg (>99%). ^1H NMR (300 MHz, CDCl_3) δ : 3.80 (s, 24H), 3.72-3.56 (br, 72H), 3.32 (s, 12H), 2.64 (s, 12H), 2.26 (s, 24H), 1.80-1.50 (br, 78H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 167.83, 165.95, 165.34, 165.15, 164.74, 43.94, 43.80, 43.79, 43.76, 42.94, 42.90, 42.87, 38.88, 31.19, 25.57, 24.76. MALDI-MS (m/z): caclcd 2954.1932, found 2955.1557 $[\text{M}+\text{H}]^+$.

Compound **31**. Cyanuric chloride (278 mg, 1.5 mmol) and 2,4-diiodoaniline (1.04 g, 3.0 mmol) were dissolved in THF (15 mL) and allowed to stir at room temperature for 1 h. The reaction mixture was then filtered to remove precipitated salts. The filtrate was rotovapped, dissolved in CHCl_3 (25 mL), extracted with 0.05 M HCl (2 x 50 mL) and brine (1 x 50 mL), dried with sodium sulfate, filtered, and rotovapped until a minimal amount of CHCl_3 remained. At this time, hexanes was added and the flask

was placed in the freezer. The next day the precipitate was isolated by filtration and dried. Yield: 633 mg (85%). ^1H NMR (300 MHz, Acetone- d_6) δ : 9.65 (s, NH), 8.32 (d, $J=1.93$, 1H), 7.86 (d, $J=1.93$) and 7.84 (d, $J=1.93$) 1H, 7.41 (s) and 7.38 (s) 1H. ^{13}C NMR (75.5 MHz, Acetone- d_6) δ : 166.44, 165.22, 147.73, 139.13, 130.44, 98.90, 93.29.

Compound **32**. Excess piperazine (240 mg, 2.8 mmol) was dissolved in DCM (20 mL) and placed in an ice bath under a nitrogen atmosphere. NHS-m-d-PEG with molecular weight of 1214 Da (518 mg, 0.43 mmol) was dissolved in DCM (15 mL) and quickly added to the piperazine solution. The reaction was allowed to stir under nitrogen, slowly warm to room temperature, and stir for 18 h at room temperature. The solution was then rotovapped and purified using silica gel column chromatography with 9:1:1% (DCM:MeOH: NH_4OH) as the eluent. Yield: 492 mg (98%). ^1H NMR (300 MHz, CDCl_3) δ : 3.61 (m, br, 97H), 3.35 (s, 2H), 2.60 (t, $J=6.88$, 4H), 2.46 (s, br, 4H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 71.84, 70.58, 70.47, 70.34, 58.96, 33.48. MALDI-MS (m/z): caclcd 1185.44, found 1185.2719 $[\text{M}+\text{H}]^+$, 1207.2245 $[\text{M}+\text{Na}]^+$, 1223.1993 $[\text{M}+\text{K}]^+$.

Compound **33**. Excess piperazine (212 mg, 2.4 mmol) was dissolved in DCM (20 mL) and placed in an ice bath under a nitrogen atmosphere. mPEG-SPA-2000 with molecular weight of approximately 2000 Da (700 mg, 0.35 mmol) was dissolved in DCM (15 mL) and quickly added to the piperazine solution. The reaction was allowed to stir under nitrogen, slowly warm to room temperature, and stir for 18 h at room temperature. The solution was then rotovapped and purified using silica gel column chromatography with 9:1:1% (DCM:MeOH: NH_4OH) as the eluent. Yield: 607 mg

(88%). ^1H NMR (300 MHz, CDCl_3) δ : 3.57 (m, br, 169H), 3.31 (s, 2H), 3.08 (br, 2H), 2.97 (br, 2H), 2.56 (t, $J=6.19$, 4H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : 71.72, 70.55, 70.39, 70.35, 70.31, 70.21, 70.07, 67.72, 58.86. MALDI-MS (m/z): calcd 1966.35, found 2286.7140 (median of mass range from 1670.6244-2852.7119).

Compound **34**. **30** (177 mg, 0.056 mmol) was placed in DCM (10 mL) and MeOH (1 mL) to obtain a slurry. DIPEA (15.3 mL, 3.4 mmol) and **31** (531 mg, 1.1 mmol) were added. The solution was allowed to stir at room temperature for 48 h. The solution was then rotovapped, dissolved in CHCl_3 (80 mL), extracted with water (3 x 150 mL), dried over sodium sulfate, filtered, and rotovapped. The compound was purified using silica gel column chromatography using 100:1 (CHCl_3 :MeOH) as the eluent. Yield: 396 mg (82%). ^1H NMR (300 MHz, CDCl_3) δ : 9.82 (s, NH), 8.03-7.19 (br, 36H), 6.93 (br, NH), 6.53 (br, NH) 5.46 (br, NH), 3.77-3.26 (br, 132H), 1.93-1.40 (br, 90H). ^{13}C NMR (75.5 MHz, CDCl_3) δ : . MALDI-MS (m/z): calcd 8423.9560, found a series of peaks with differences of 125 or 127 indicating loss of iodine with a median of 8036.32. Combustion elemental analysis (CHN/I): theoretical C 35.46, H 3.44, N 19.93, I 36.12; found C 35.26, H 3.42, N 18.94, I 35.61.

Compound **35**. **34** (48 mg, 0.006 mmol), **32** (119 mg, 0.1 mmol), and DIPEA (0.03 mL, 0.14 mmol) were dissolved in THF (2.9 mL) and placed in a pressure flask. The reaction mixture was heated to 70 °C for 3 days. The solution was then rotovapped, dissolved in a minimal amount of deionized water, and filtered through a 0.2 μm syringe filter to remove any undissolved particles. The solution was then placed into 10 kDa molecular weight cut-off dialysis membrane tubing, clamped on both ends, and placed

into a stirred 4 L container of deionized water for one week. The water was removed and replaced 3 times a day for the first day, 2 times a day for days 2-5, and 1 time on days 6 and 7. After ensuring removal of excess PEG was complete by HPLC-RI analysis, the sample was rotovapped and dried. Yield: 79 mg (62%). MALDI-MS (m/z): calcd 22220.6, found: series of peaks ranging from approximately 15-22 kDa with a median value of 18746.67.

Compound **36**. **34** (17.5 mg, 0.002 mmol), **33** (75 mg, 0.038 mmol), and DIPEA (0.01 mL, 0.057 mmol) were dissolved in THF (1 mL) and placed in a pressure flask. The reaction mixture was heated to 70 °C for 3 days. The solution was then rotovapped, dissolved in a minimal amount of deionized water, and filtered through a 0.2 µm syringe filter to remove any undissolved particles. The solution was then placed into 25 kDa molecular weight cut-off dialysis membrane tubing, clamped on both ends, and placed into a stirred 4 L container of deionized water for ten days. The water was removed and replaced 3 times a day for the first day, 2 times a day for days 2-6, and 1 time on days 7-10. After ensuring removal of excess PEG was complete by HPLC-RI analysis, the sample was rotovapped and dried. Yield: 57 mg (86%). MALDI-MS (m/z): calcd 31727.0, found: series of peaks ranging from approximately 30-36 kDa with a median value of 34639.44.

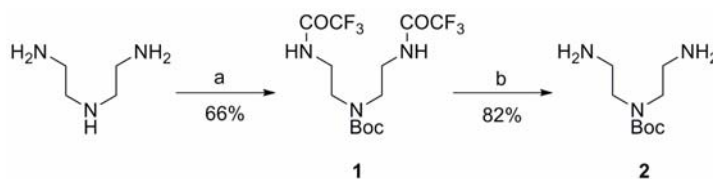
Results and Discussion

Isosteric Dendrimer Synthesis and Characterization. The synthesis of a dendrimer based on melamine containing isosteric diamine linkers was attempted to investigate the effect of differing atom electronegativities on the encapsulation of

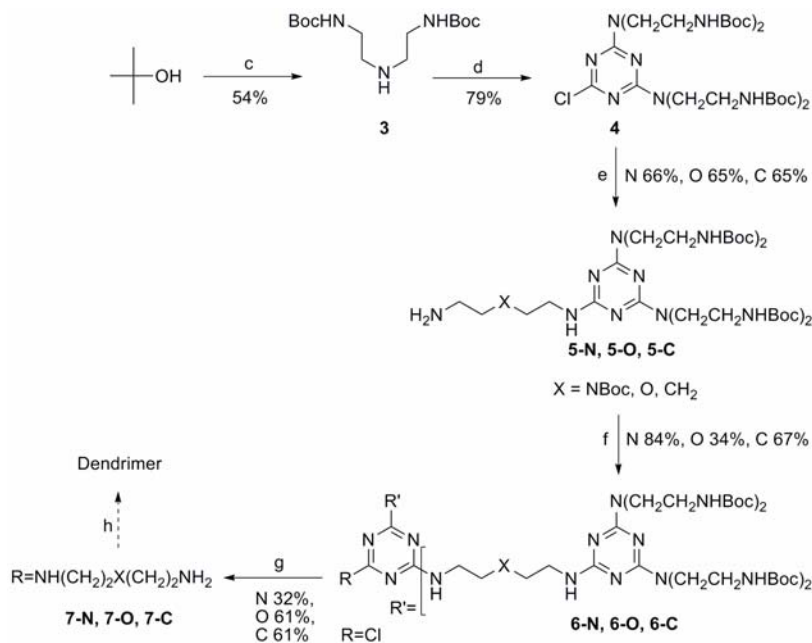
molecules such as drugs. The convergent synthesis would yield a G2 dendrimer with 24 primary amine groups which could then be further synthetically modified. The synthesis proceeded through iterative reactions of cyanuric chloride with the diamine linkers. For this study, the linkers chosen differed in the nature of the central atom. Linkers of the form $\text{NH}_2(\text{CH}_2)_2\text{X}(\text{CH}_2)_2\text{NH}_2$ were utilized where $\text{X} = \text{N-Boc}$, O , or CH_2 for diethylene triamine, 2,2'-oxybis(ethylamine), and 1,5-diaminopentane, respectively.

The synthesis of these dendrimers proved to be rather arduous with dimerizations and incomplete substitutions due to steric hinderence and reduced reaction rates causing low yields at each step. Through a series of protections and deprotections, Boc protection of the secondary amine of diethylene triamine can be achieved in order to assure substitution of the primary amine onto the triazine ring using a method reported by Koščová, *et al.*¹⁸¹ (Scheme 18) The amine derivative, the most interesting of the three, was carried through to near completion with only one step remaining. Attempts to synthesize dendrimers with oxy and methylene derivatives were unsuccessful. The final coupling step of the dendrimer synthesis proved to be quite difficult with many side products and incomplete substitutions. (Scheme 19)

Scheme 18. Synthesis of mono-Boc triamine linker. a) CF_3COOEt , DCM, $0\text{ }^\circ\text{C}$ —RT 1.5 h, then $(\text{Boc})_2\text{O}$, Et_3N , RT, 18 h. b) NaOH , EtOH , RT, 18 h.



Scheme 19. Synthesis of isosteric dendrimers. c) CDI, Toluene, 65 °C, 3 h, then diethylene triamine, 65 °C, 3 h. d) C₃N₃Cl₃, THF, DIPEA, 0 °C-RT, 18 h. e) H₂N(CH₂)₂X(CH₂)₂NH₂, THF, DIPEA, 70 °C, 48 h. f) C₃N₃Cl₃, THF, DIPEA, 0 °C-RT, 18 h. g) H₂N(CH₂)₂X(CH₂)₂NH₂, THF, DIPEA, 70 °C, 48 h. h) C₃N₃Cl₃, THF, DIPEA, 70 °C, 1 week.



Upon completion of the synthesis, further studies would investigate the relationship of these differing atoms upon the noncovalent encapsulation of drug molecules into the interior of the dendrimer. This would be a proof-of-concept experiment to demonstrate the best linker-type for encapsulation. Presumably the most electronegative group, the amine, would demonstrate the greatest proficiency for noncovalent interactions with guest molecules followed by the oxy, and then the methylene group.

A problem with the initial target dendrimer is the presence of amine groups on the periphery which are Boc-protected. This leaves no selectivity for deprotection of

only the interior amines and would hinder any studies of drug encapsulation for fear of the exterior amines interfering with the interior amine interactions. Changing the periphery groups to hydroxyls could remedy this, however the synthesis would still encounter similar problems of dimerization and incomplete substitutions, lowering yields and frustrating purification efforts.

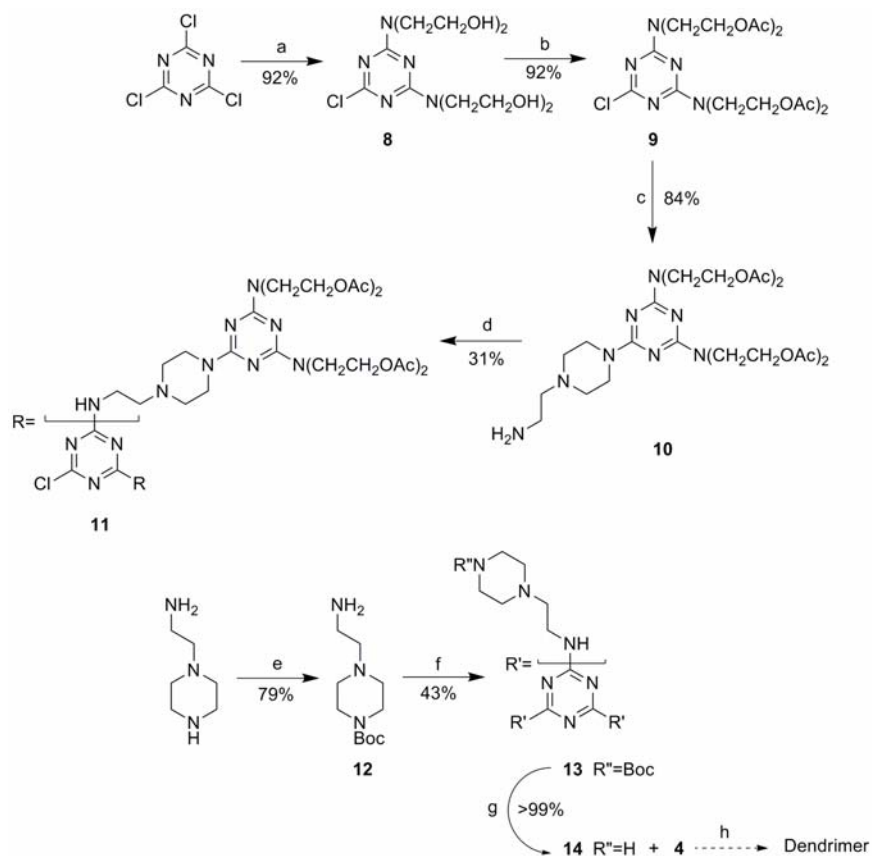
pH Responsive Dendrimer Synthesis and Characterization. The synthesis of a G2 dendrimer containing nine additional protonation sites in the interior due to the incorporation of the linker aminoethyl piperazine was attempted. The dendrimer would include 24 hydroxyl groups on the periphery which may be further synthetically modified to provide solubility, attach targeting moieties, etc. The dendrimer would allow a study of how the electrostatic interactions between guest molecules and a melamine dendrimer would vary with pH, specifically an anionic guest such as the anti-cancer drug methotrexate.

The synthesis of this dendrimer, too, proved to be quite difficult presumably due to the extra effect of the basic tertiary amine incorporated with the linker. The presence of the hydroxyl group along with the choice of linker made isolation of amine intermediates from impurities nearly impossible. Acetylation of the hydroxyls solved this issue, but the incorporation of acetyl groups caused additional side products which were attributed to acetyl transfer.

A core comprised of three mono-Boc protected amino ethylpiperazines coupled to cyanuric chloride was synthesized with some degree of difficulty given that unexpected and unwanted side products were produced during the Boc protection of the

linker. A G1 dendron was then to be coupled to the deprotected core in a hypercore type reaction as shown in Scheme 20. No product was obtained, either due to sterics or absence of a strong enough exogenous base. This method of dendrimer synthesis appeared to be of little utility.

Scheme 20. Incorporation of 3° amine linker to melamine dendrimer. a) diethanolamine, acetone, 0 °C—RT, 18 h. b) acetic anhydride, acetyl chloride, DCM, RT, 18 h, then triethylamine. c) *N*-aminoethylpiperazine, THF, -50 °C—RT, 18 h. d) C₃N₃Cl₃, DIPEA, THF, 0 °C—RT, 18 h. e) CF₃COOEt, DCM, 0 °C—RT 1.5 h, then (Boc)₂O, Et₃N, RT, 18 h. f) C₃N₃Cl₃, THF, 70 °C, 1 week. g) TFA/DCM 1:1, RT, 24 h. h) THF, DIPEA, 70 °C, 3 days

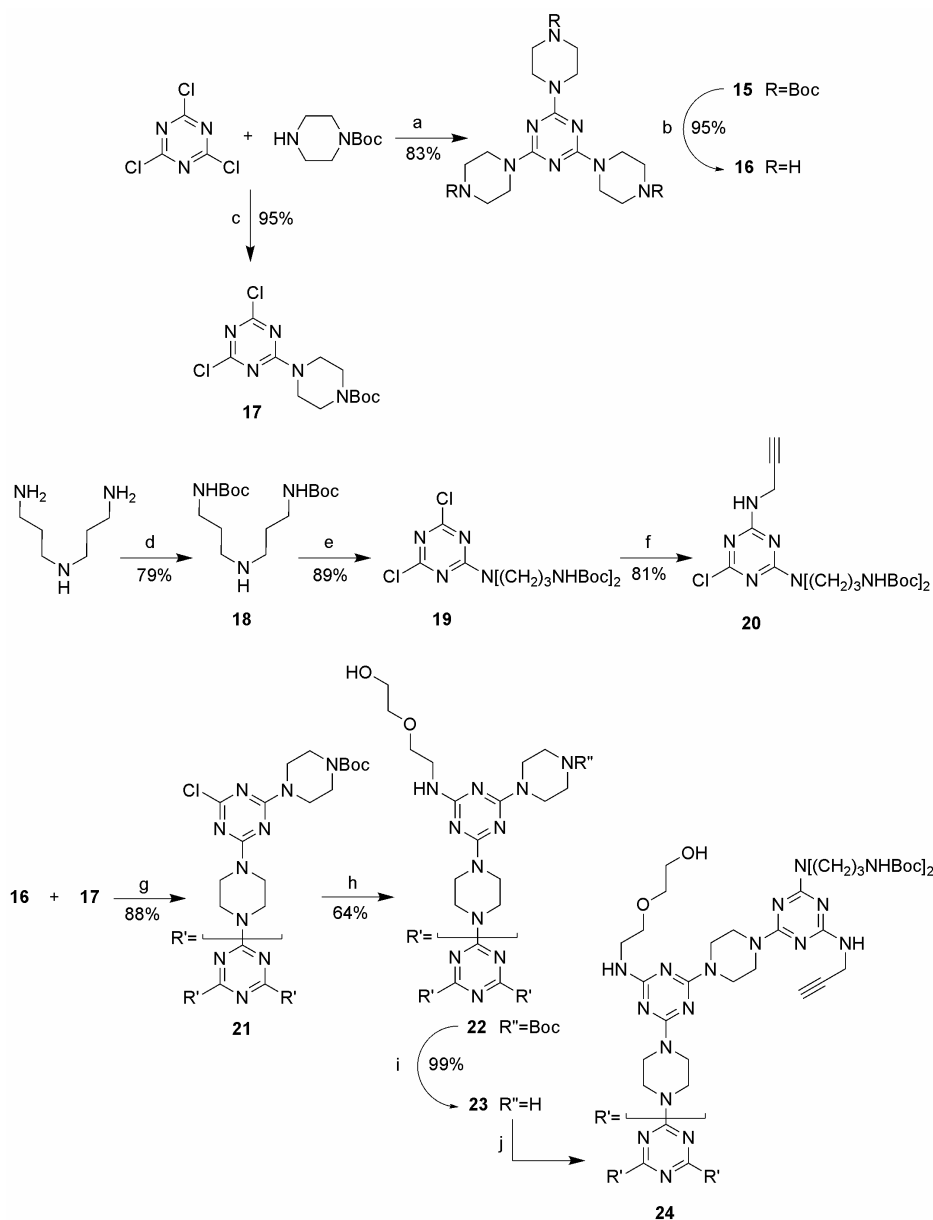


‘Divergent Diversity’ Dendrimer Synthesis and Characterization. Through the work of Dr. Emily Hollink, a ‘new’ route to synthesize melamine dendrimers was developed. The dichlorotriazine method proceeds rapidly and requires less harsh conditions than the traditional routes employed in the Simanek group. The method plays upon the differential reactivity of cyanuric chloride to afford monodisperse dendrimers divergently while still allowing for incorporation of various groups.⁵³ Synthesis of the dichlorotriazine building blocks may be accomplished using green methods adopted from Lowe and co-workers.¹⁸² The reactions are performed in an acetone/water mixed solvent-system employing sodium bicarbonate as an exogenous base, and generally, precipitation of the product occurs allowing simple filtration of the product.

Using this method a dendrimer was synthesized containing three reactive groups – hydroxyl, amine, and alkyne. The G2 dendrimer would allow the attachment of drug molecules, fluorescent markers, and PEG chains for solubility and biocompatibility with three hydroxyl groups, three propargyl groups, and six amines. (Scheme 21)

Problems with this synthesis occurred, however, when installing propargyl groups. When treating a monochlorotriazine with propargyl amine, unwanted side products formed. The propargyl amine must be appended as the second triazine substitution for the reaction to readily proceed. However, this produces a situation in which attachment of a monochlorotriazine to the dendrimer must occur necessitating heating, long reaction times, and the use of a strong base to produce dendrimer in good yield. The dendrimer was synthesized as shown by a crude mass spectrometry analysis, but purification afforded no appreciable amount of pure compound.

Scheme 21. Synthetic strategy incorporating three functional groups. a) DIPEA, THF, 70 °C, 48 h. b) TFA/DCM (1:1), RT, 4 h. c) NaHCO₃, Acetone/Water, 0 °C, 2 h. d) BOC-ON, DIPEA, THF, 0 °C—RT, 4 h. e) C₃N₃Cl₃, NaHCO₃, Acetone/Water, 0 °C, 2 h. f) propargylamine, DIPEA, RT, 18 h. g) DIPEA, THF, 0 °C—RT, 18 h. h) aminoethoxyethanol, DIPEA, THF, 70 °C, 24 h. i) HCl (conc.)/MeOH (2:1), RT, 18 h. j) **20**, DIPEA, THF, 70 °C, 5 days.



Iodinated Dendrimer Synthesis and Characterization. Melamine dendrimers have been synthesized as potential macromolecular CT imaging agents. The synthesis

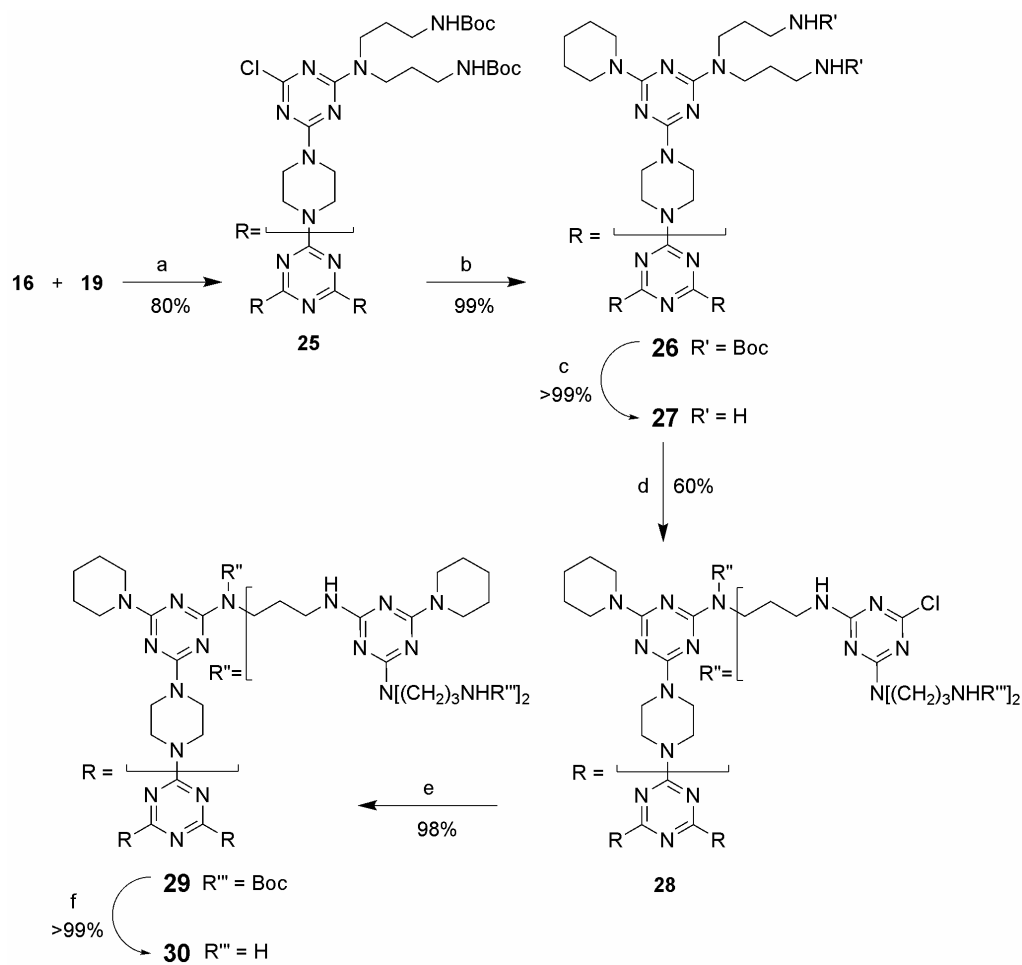
proceeded through a divergent pathway utilizing dichlorotriazine building blocks and commercially available 2,4-diiodoaniline. The synthetic strategy was similar to that of the ‘divergent diversity’ dendrimer mentioned above. Dendrimers are synthesized in good yield and with excellent purity using this method. Formation of the G1-Cl is accomplished at room temperature. The next step is what we refer to as “capping” in which the reactive chloride is barred from further reactive steps by treating with piperidine. This reaction, too, is carried out under ambient conditions in good yield within 18 h. The steps are then iterative to form the second generation. (Scheme 22) After deprotection, introduction of the iodine moiety occurs.

A dichlorotriazine was synthesized with diiodoaniline. To facilitate the reaction, two equivalents of diiodoaniline were necessary rather than using an exogenous base. Within one hour at room temperature, the dichlorotriazine was formed. Filtration, followed by reprecipitation from dichloromethane with hexanes provided the desired dichlorotriazine in good yield. Treating the G2-amine with the dichloro-diiodoaniline, again, at room temperature provided a dendrimer (**34**) with a molecular weight of 8423 Da. (Scheme 23)

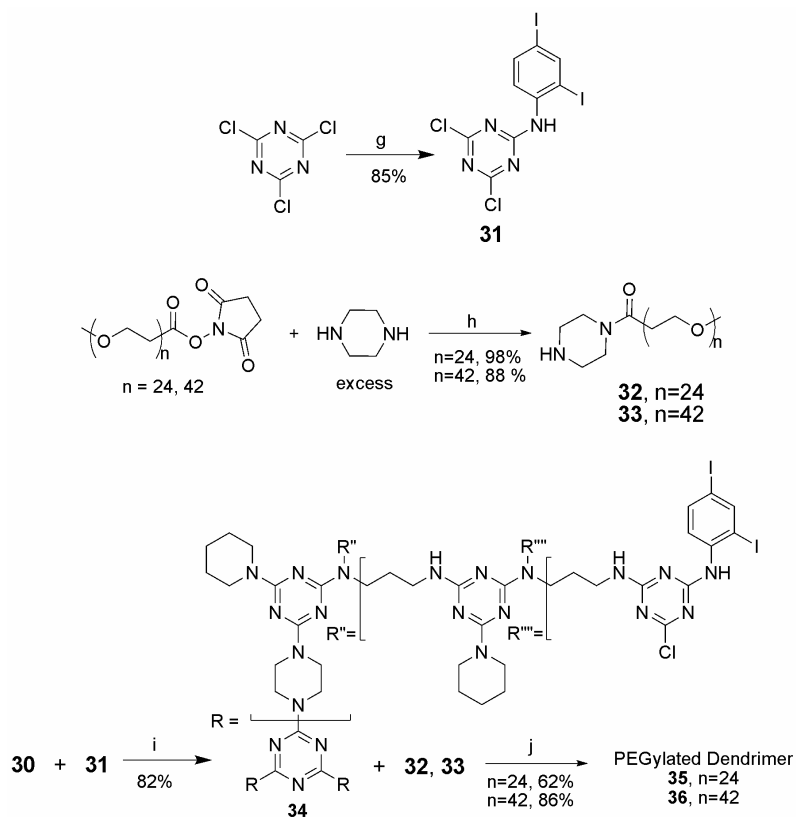
This dendrimer (**34**) was shown to be monodisperse by multi-detector GPC analysis. Taking into account that this technique will not give definitive data for dendrimers due to standardization with linear polystyrene, the evidence is fairly strong that dendrimers based on melamine synthesized by the dichlorotriazine route are monodisperse molecules. The compound had a \bar{M}_n of 8,272 Da and a \bar{M}_w of 8,300 Da, which were in close agreement with the calculated molecular weight, and a

hydrodynamic radius of 1.757 nm. The elemental analysis obtained for this compound was in fairly good agreement with expected calculated values. Discrepancies can most likely be attributed to the encapsulation of solvent molecules such as hexanes. MALDI mass spectrometry for this molecule shows peaks indicating ionized loss of iodine, making characterization by this method difficult.

Scheme 22. Divergent synthesis of precursor dendrimer. a) DIPEA, THF, 0 °C—RT, 18 h. b) piperidine, THF, RT, 18 h. c) MeOH:HCl (conc.) (2:1), RT, 18 h. d) **19**, DIPEA, DCM/EtOAc/H₂O, RT, 1 h. e) piperidine, THF, RT, 1 h.



Scheme 23. Synthesis of iodinated dendrimer. g) 2,4-diiodoaniline, THF, RT, 1 h. h) N₂, DCM, 0 °C—RT, 18 h. i) DIPEA, DCM:MeOH (10:1), RT, 48 h. j) DIPEA, THF, 70 °C, 3 days.



Subsequent pegylation of the dendrimer **34** with PEG₁₀₀₀ or PEG₂₀₀₀ piperazine derivatives at 70 °C for 3 days yields G3 dendrimers with 24 iodine molecules and molecular weights of approximately 18 and 34 kDa, respectively, as determined by MALDI mass spectrometry. (Scheme 23) Upon completion of dialysis using either 10 kDa or 25 kDa molecular weight cut-off dialysis membranes, respectively, size exclusion chromatography/refractive index HPLC data was recorded to determine if any free PEG remained. Each dendrimer showed a single peak for the desired product.

For the 34 kDa dendrimer, GPC data was also obtained. The PDI was found to be 1.051 which was undoubtedly higher than the parent dendrimer due to the introduction of 12 PEG chains, each of which are not precisely monodisperse. The molecular weight determination was in good agreement with mass spectral data. The dendrimer had a \bar{M}_n of 34,618 Da and a \bar{M}_w of 36,367 Da and a hydrodynamic radius of 4.281. This synthesis affords pure material in good yields with only the PEGylation step requiring harsh reaction conditions or long reaction times.

These dendrimers have an iodine content of approximately 17% and 9% by weight for **(35)** and **(36)**, respectively. The difference in iodine content paired with a difference in molecular weight would make for an interesting comparison during a CT scanning experiment. If the dendrimers were compared to a commonly used, small molecule contrast agent such as Omnipaque, one would find that the dendrimers would have a longer retention time in the bloodstream allowing for longer evaluation times, increasing accuracy of diagnoses. For the smaller of the two dendrimers with a higher iodine content compared to a larger molecular weight dendrimer with a lower iodine weight content, the results would be indicative of the importance of both of these factors combined.

Conclusions

Dendrimers based on melamine have many potential applications in the drug delivery realm. Whether it is noncovalent encapsulation or covalent attachment, these dendrimers are promising vehicles for anti-cancer treatment and macromolecular contrast agents, among other applications. With the proper choice of linker, and a

synthetic route that limits harsh reactions conditions, prolonged reaction times, or laborious purification, the possibilities are limitless for incorporation of various groups of choice onto monodisperse molecules to fulfill the application at hand.

CHAPTER V

CONCLUSION

This work focused on organic synthesis using the 1,3,5-triazine moiety, specifically cyanuric chloride for the development of chemistry with practical applications for education, remediation, and drug delivery. From the education perspective, triazine chemistry was adapted for the development of a new organic undergraduate laboratory experiment. The students synthesized a widely used and environmentally controversial herbicide, simazine. The experiment is currently being performed by over 1000 students each year at Texas A&M University. In addition to reinforcing nucleophilic aromatic substitution chemistry, this laboratory was intended to encourage discussion about the impact that simple chemistry has on society and the environment.

The potential for environmental remediation was realized by the development of materials to sequester pollutants such as atrazine. The modification of chitosan for herbicide sequestration was accomplished using triazine chemistry. Dendrons of generation one through three were synthesized on chitosan. Piperazine groups installed during the synthesis effectively sequester atrazine from an aqueous 100 ppb solution, presumably irreversibly through covalent bond formation mediated by nucleophilic aromatic substitution. Surprisingly, the first generation graft is as effective as subsequent generations at sequestering atrazine. More than 99% of the atrazine is removed from an aqueous solution during a 24 hour period. The coupling of efficient herbicide sequestration to chitosan's well documented ability for removal of metal

contaminants from wastewater and its ability to be woven into fibers, presents many possible formulation for this chemistry.

Dendrimers have found many potential applications in the biomedical field, particularly as drug delivery vehicles and imaging agents. The development of macromolecular contrast agents is of great interest to counteract the drawbacks associated with currently used, small molecule contrast media. The use of macromolecular contrast agents may reduce the osmotic potential, thereby reducing the toxicity of the medium, by increasing the number of iodine atoms on a single molecule. Macromolecules may also reduce the dosage necessary as the drug circulates longer, and could aid in the detection of cancerous tissue due to preferential accumulation in tumor cells. Triazine dendrimers were synthesized as potential imaging agents. The synthesis proceeded through a divergent pathway utilizing dichlorotriazine building blocks and commercially available 2,4-diiodoaniline. The dendrimers were synthesized in good yield with excellent purity using this method. For triazine-based dendrimers which utilize the differential reactivity of cyanuric chloride, any number of targets may be envisioned and some are synthetically tractable. The judicious choice of amine linkers and development of more facile synthetic routes ease the synthetic burden.

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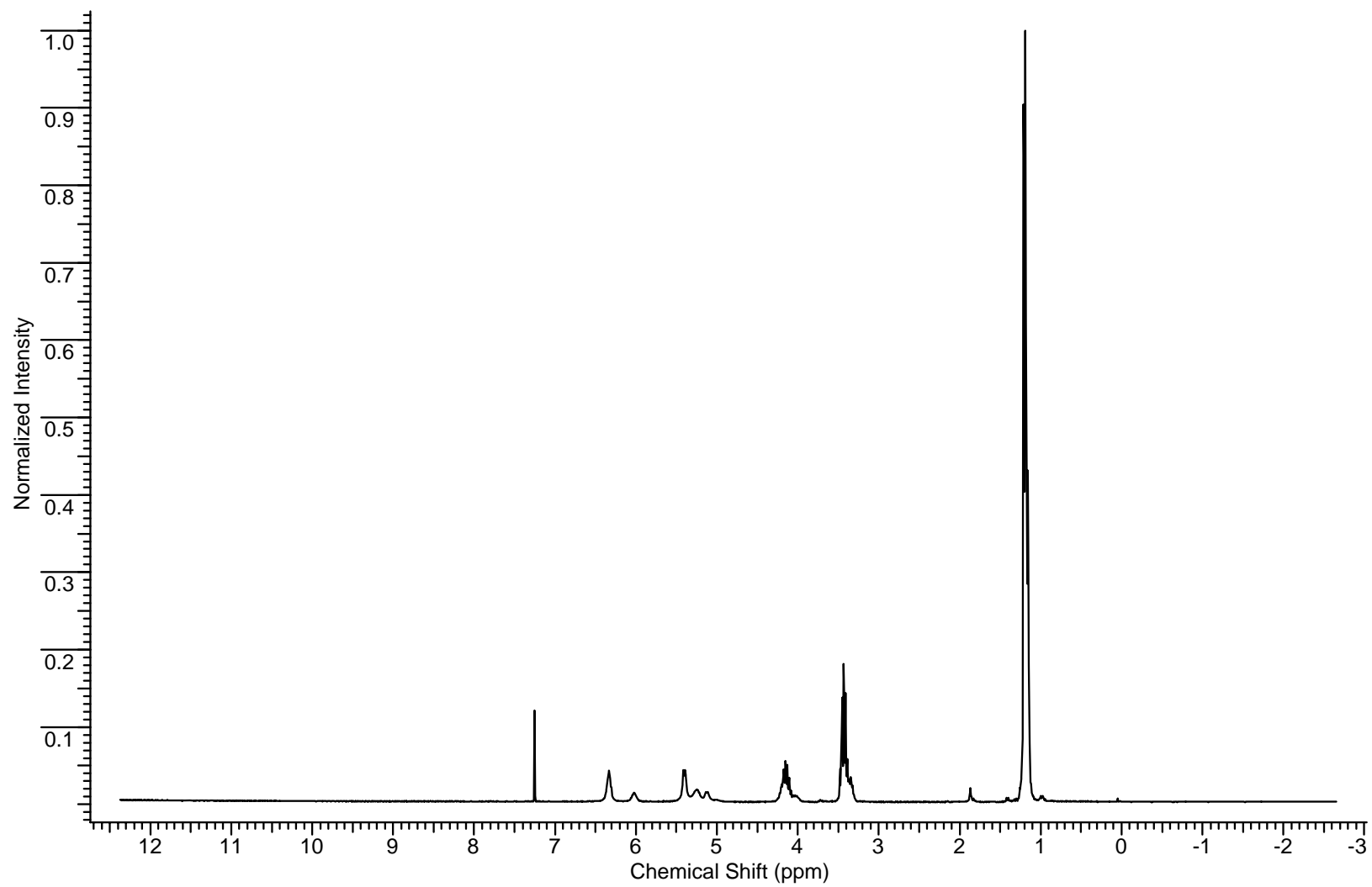
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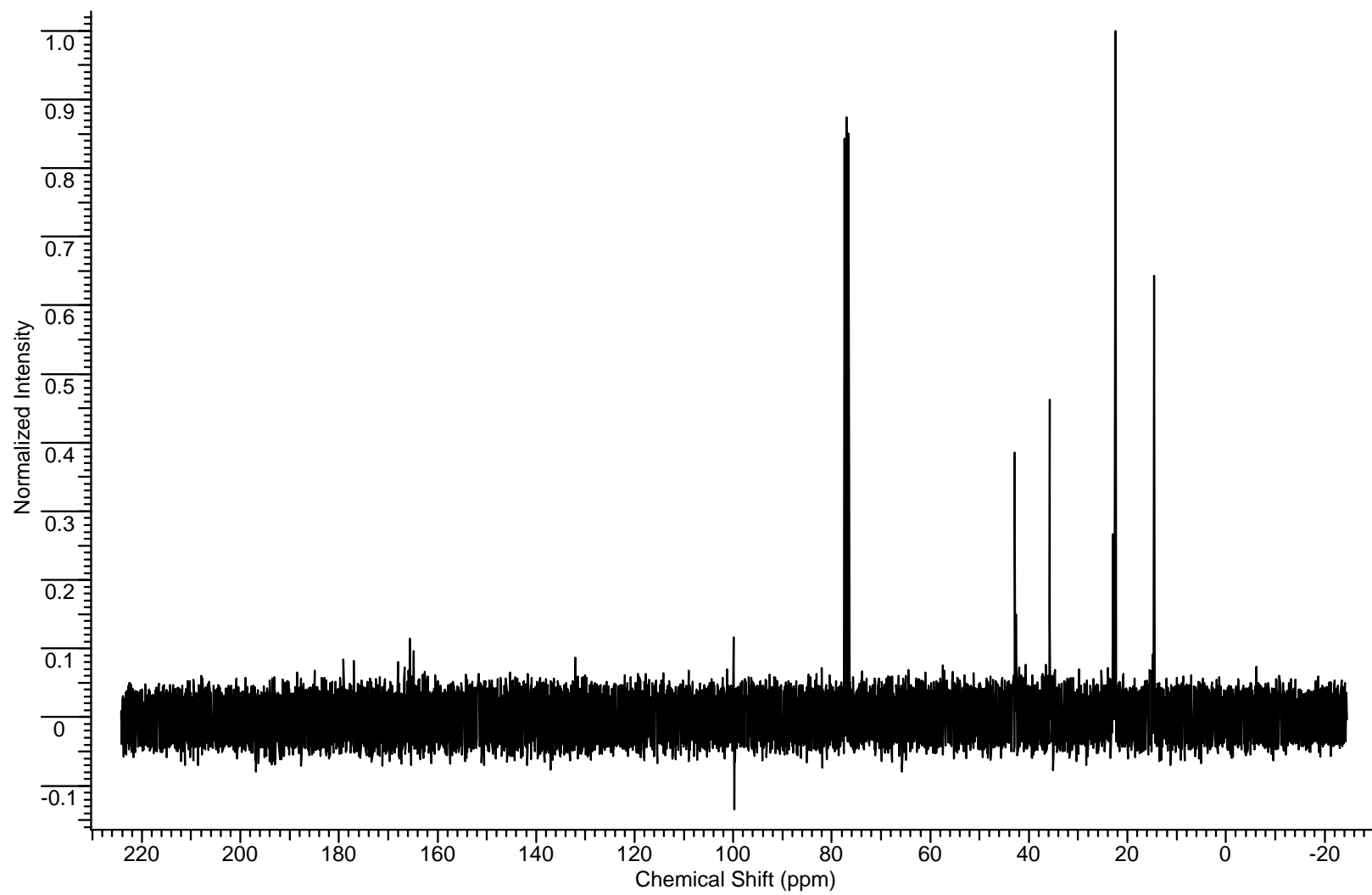
APPENDIX A

CHAPTER II: SPECTRA AND LABORATORY EXPERIMENT

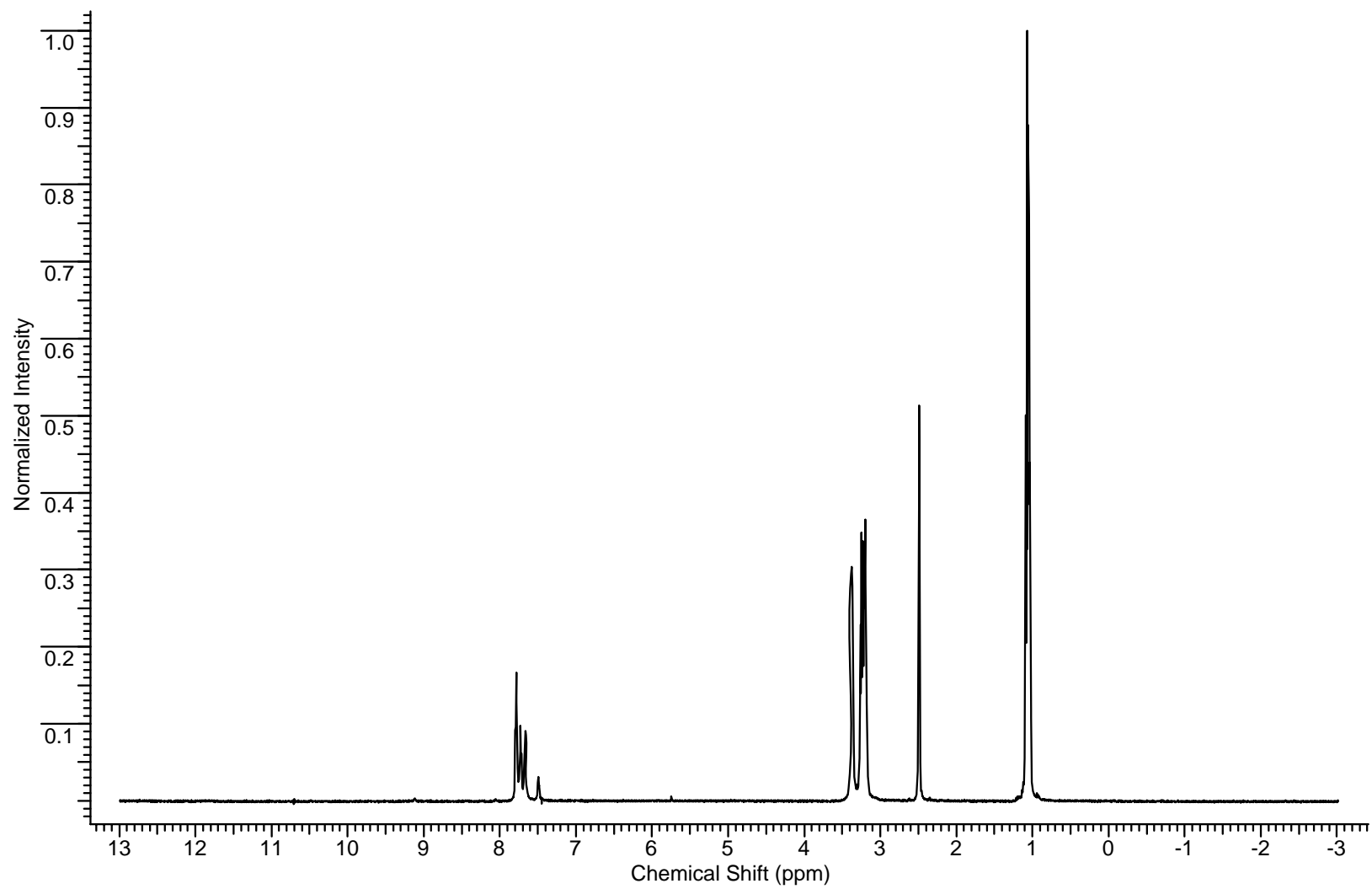
Atrazine, ^1H NMR



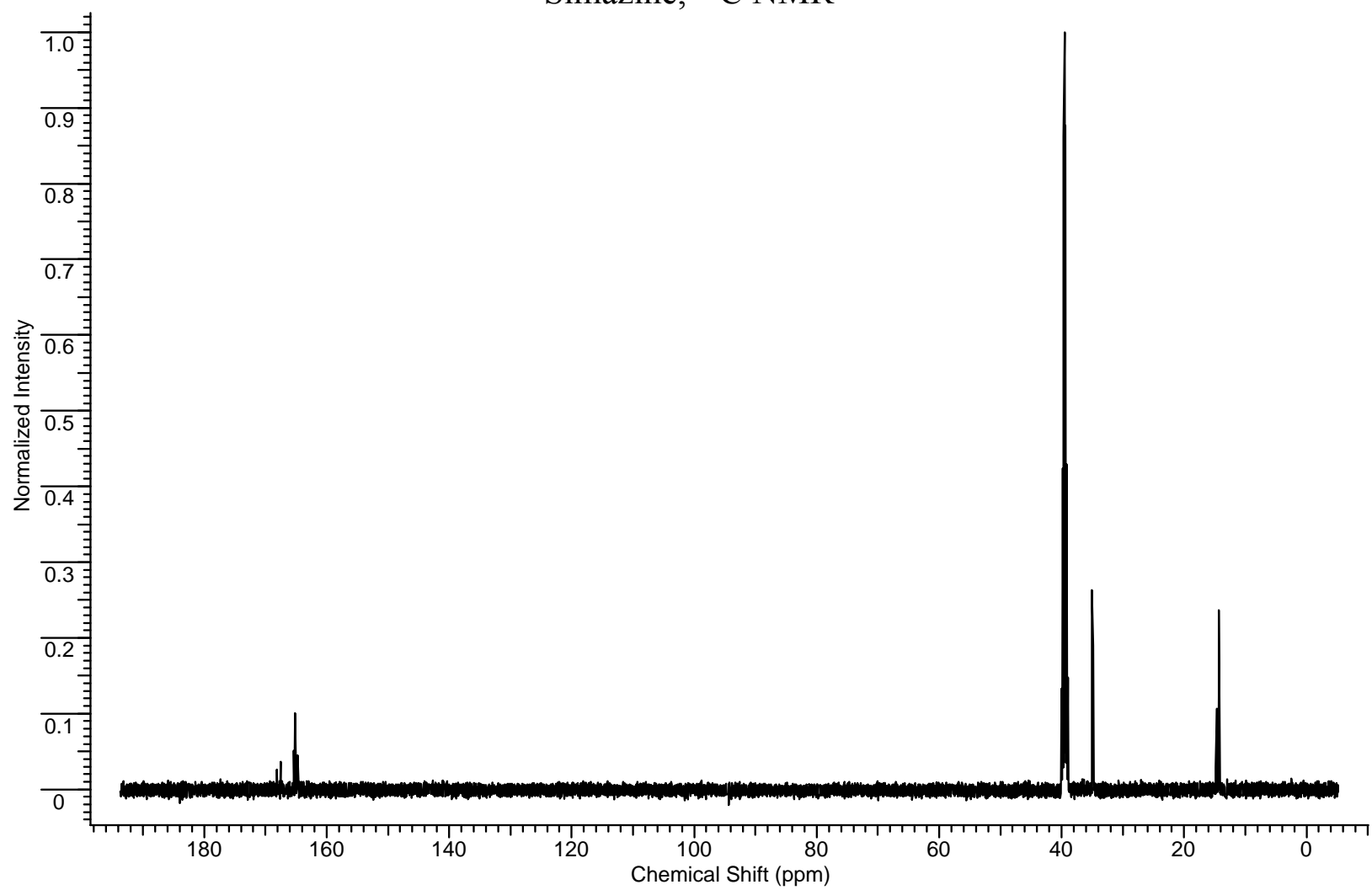
Atrazine, ^{13}C NMR



Simazine, ^1H NMR



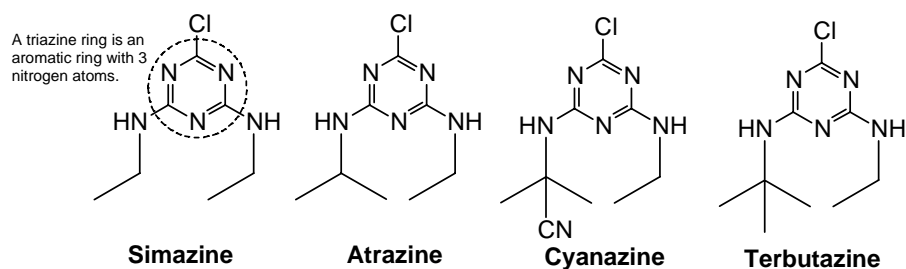
Simazine, ^{13}C NMR



Preparation of a Triazine Herbicide

Introduction

The triazine herbicides are the most commonly used herbicides to control weeds during the production of corn, sorghum, and sugarcane in the U.S. More than 100,000,000 lbs of these herbicides are used every year in the United States. These herbicides all have a similar structure. The most common triazine herbicides are shown below.



These herbicides work by disrupting photosynthesis in broadleaf weeds. Without an ability to do photosynthesis, the weeds die, and crop production increases. Unfortunately, these herbicides are commonly detected in ground and drinking water. These molecules have been banned in Europe because their use has been linked to cancer and endocrine gland disruption in people.^{1, 2} The EPA has considered restricting or banning their use in this country, but longterm costs would range between \$295-\$665 million a year.²

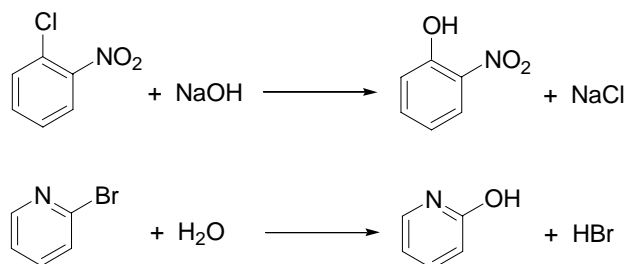
The triazine herbicides are prepared by nucleophilic aromatic substitution. Let's break this down:

nucleophile: a molecule that donates electrons to form a bond

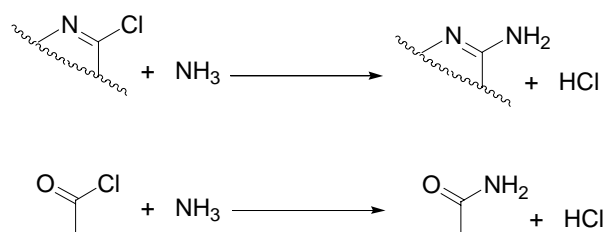
aromatic: a ring of 6 atoms with alternating double and single bonds (like benzene)

substitution: the replacement of one atom for another

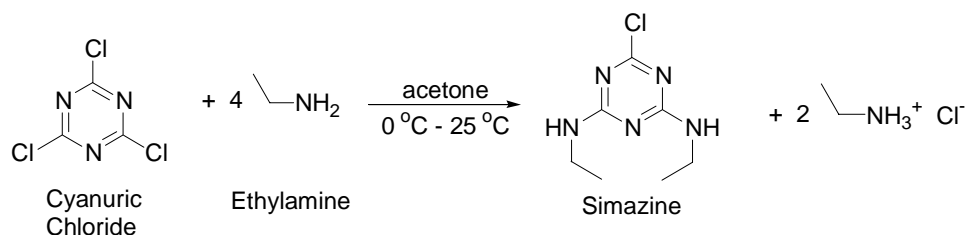
In most nucleophilic aromatic substitution reactions, atoms with lone pairs of electrons like nitrogen or oxygen replace halogens like Br, Cl, F, or I. Here are some examples of nucleophilic aromatic substitution reactions.



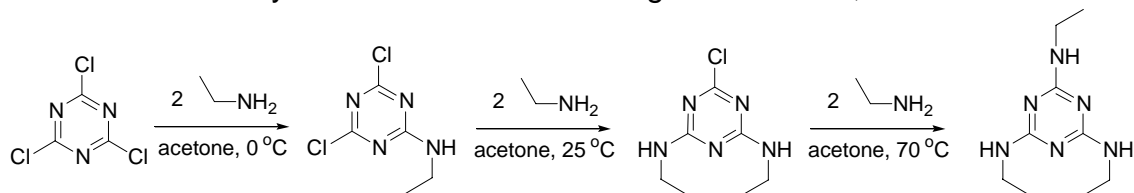
These reactions are very similar to the nucleophilic substitution of acid chlorides. When the nucleophile is added to the molecule, the chloride ion is released to form the substitution product.



In this laboratory experiment, we will synthesize simazine. This reaction sequence involves the substitution of two chlorine atoms of cyanuric chloride with two ethylamine groups as shown below.



Since HCl is produced as a by-product of this reaction, excess base (ethylamine) is necessary to neutralize the acid (HCl) produced. The substitution of cyanuric chloride is temperature dependent. By controlling the amount of nucleophile added and the reaction temperature, one, two, or even all three chlorides of cyanuric chloride can undergo substitution, as shown below.



Objectives

1. To complete a laboratory synthesis of the herbicide simazine using nucleophilic aromatic substitution.
2. To use previously acquired laboratory techniques to characterize the product obtained.

Procedure

Preparation of Simazine

1. Prepare an ice bath using a 400 mL plastic beaker and place the bath on magnetic stir plate.
2. In a 100-mL round-bottomed flask, place a stir bar and add 5 mL of the 0.5 M cyanuric chloride solution using the autodispenser in the hood. Cover the flask with foil to prevent evaporation of the solvent.
3. Clamp the flask in the ice bath, and allow the solution to sit and cool in the ice bath for about 10 min. while stirring with the magnetic stirrer.
4. From the volume of cyanuric chloride solution obtained and its concentration, calculate the theoretical yield of simazine. Record this value.
5. Obtain 10 mL of ethylamine solution (1.0 M in acetone/water) in a graduated cylinder from the autodispenser in the hood. Using a long stem funnel, carefully add the ethylamine while the solution is stirring. After 5 min., remove the ice bath and continue to stir the reaction for an additional 30 min. as it comes to room temperature.

CAUTION: Always wear appropriate eye protection. Cyanuric chloride will irritate your nose and eyes. Ethylamine will irritate your nose, eyes, and skin. Handle these chemicals with care and dispense in the hood.

6. Adding water will precipitate the simazine out of solution. Add about 30 mL of distilled water to the flask and stir for 5 min.
7. Collect your sample by filtering using a Büchner funnel and a 250-mL filter flask connected to a water aspirator for suction. Be sure to wet the filter paper with distilled water before adding your sample. Carefully pour the contents of your reaction flask into the funnel. Rinse the flask well with at least two 30 mL portions of distilled water and one 30 mL portion of methanol filtering each through the funnel to wash the product.
8. Continue suction through your sample for several minutes in order to dry it. Disconnect the rubber tubing from the filter before turning off the water.

9. Using a spatula, remove the sample from the filter paper and place it in a piece of folded filter paper and press until it is dry.
10. Weigh a watch glass. Add your dried sample and reweigh. Calculate the weight of simazine obtained. Determine the percent yield. Record these values.

Characterization of Simazine

1. Melting Point

- a) Obtain two melting point capillary tubes.
- b) Place a small amount of your compound onto a clean watch glass. Load your compound into the capillary tubes by pressing the open end of the tube into the solid, inverting the tube, and gently tapping the closed end on the bench top until the solid moves to the bottom.
- c) Once you have obtained about 2 to 3 mm of sample in the bottom of the tubes, place the melting point capillary with your sample into the Mel-Temp apparatus with the open end up. Begin heating at a rate of 10 °C/min. Carefully observe the solid within the melting point capillary and record the temperature at the first sign of melting (when the solid starts to turn to a liquid). Also record the temperature when the entire solid has melted (when it is completely liquid).
- d) Turn off the Mel-Temp and allow the temperature to cool to about 15 °C below the point at which you first observed melting. (This may be done quickly by passing compressed air over the apparatus.)
- e) Using the second melting point capillary tube containing your compound you prepared, determine the melting point by allowing the sample to heat at a rate of 1 to 2 °C/min. Record the melting point range obtained.

2. Thin Layer Chromatography

- a) Put approximately 6 mL of the TLC developing solvent (mixture of 75% hexanes and 25% ethyl acetate) into the TLC jar. Replace the lid and shake gently to saturate the air in the jar with the vapor of the solvent.
- b) Draw a light line with a pencil 1/2-inch from the bottom of the TLC plate. Be sure the line will be above the solvent level in the TLC jar.
- c) Dissolve a small quantity of your sample (about the size of a pea) in about 8 mL of acetone found in the fume hood. Use a TLC spotting capillary tube to apply a small dot of the solution on the line and allow the spot to dry.
- d) Put the TLC plate in the TLC jar, quickly replace the lid, and leave the jar undisturbed while the plate develops. Allow the solvent to ascend the plate until it is about 1/2-inch from the top of the plate. Remove the plate and quickly mark the top of the solvent line with a pencil.

- e) After the solvent has evaporated, observe the TLC plate under a UV lamp. Lightly draw a circle with a pencil around any spot(s) you can see under the UV lamp.
- f) Determine the R_f value of the spot(s) observed, and record the value(s).

Clean-up Procedures:

Dispose of solid crystals, filter paper, and TLC plates in solid waste container provided. Dispose of liquid waste in appropriately labeled liquid waste bottle.

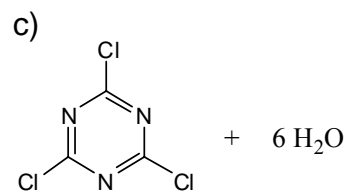
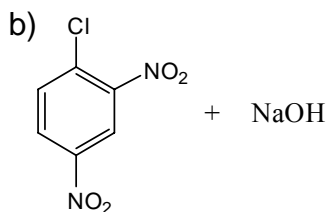
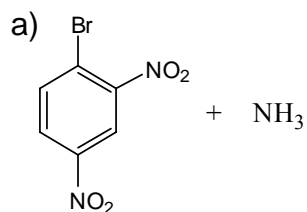
¹ Baumann, P.A.; Ketchersid, M. Texas Agricultural Extension Service Report L-5204; Texas A&M University: College Station, TX, Oct 1999.

² Ribaud, M.O.; Bouzahr, A. Agricultural Economic Report No. 699; Resources and Technology Division, Economic Research Service, U.S. Department of Agriculture: Washington, D.C., 1994.

This Lab Experiment was developed by Dr. Eric Simanek and Susan Hatfield, Texas A&M University

Pre-lab Questions

- Calculate the molecular weights of cyanuric chloride and simazine.
- Predict the products of the following nucleophilic aromatic substitution reactions.



In-lab Observations

- Volume of cyanuric chloride: _____ mL
- Concentration of cyanuric chloride: _____ M (mol/L)
- Moles of cyanuric chloride: _____ mols
- Theoretical Yield: _____ g Simazine

5. Volume of ethylamine: _____ mL
6. Weight of watch glass: _____ g
7. Weight of simazine sample and watch glass: _____ g
8. Weight of simazine sample: _____ g
9. Percent Yield: _____ %
10. Melting Point determination #1: _____ °C
- Melting Point determination #2: _____ °C
11. R_f value(s) _____

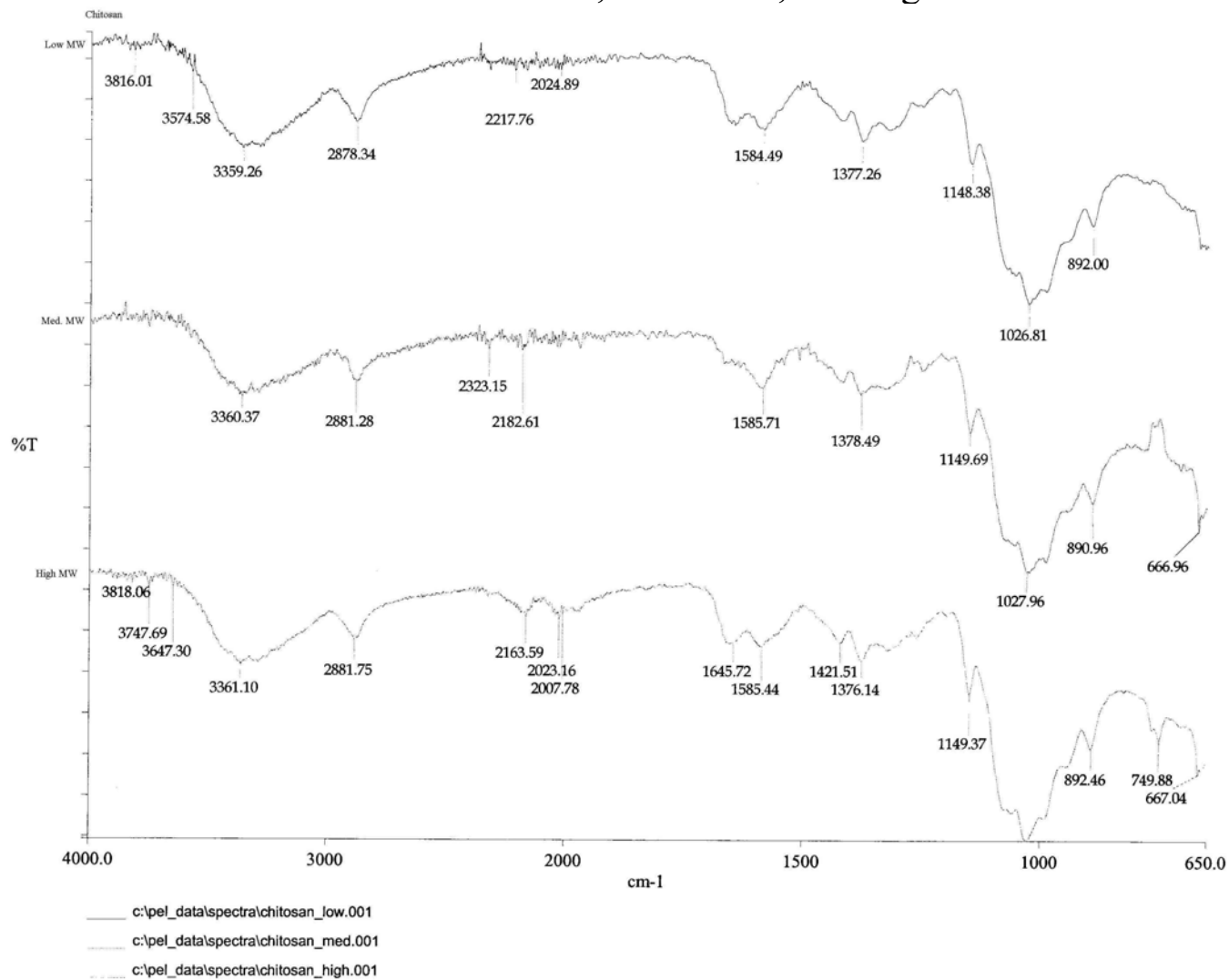
Post-lab Questions

1. Draw the side product that could be formed during the synthesis of simazine if:
 - a) Not enough amine was added
 - b) Too much amine was added and the solution was heated
 - c) Sodium Hydroxide was present (Several different products are possible here. Just draw one.)
2. The melting point of pure simazine is 225 - 227 °C. If the melting point of your product is different, why?
3. What are possible causes for obtaining a low percent yield in this reaction?
4. What are the possible causes of obtaining a percent yield greater than 100%?
5. What happens to the HCl produced during the synthesis of simazine?

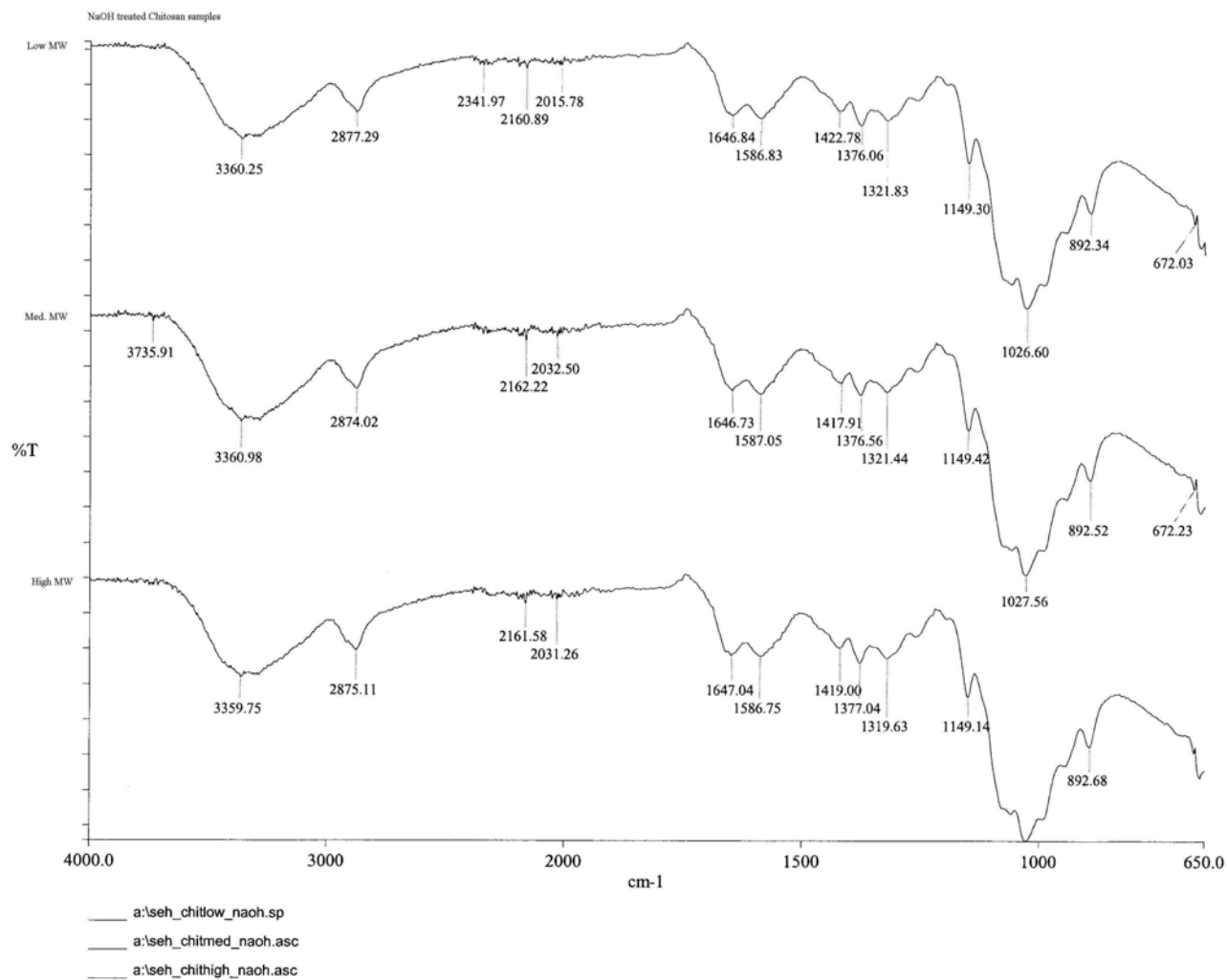
APPENDIX B

CHAPTER III: SPECTRA

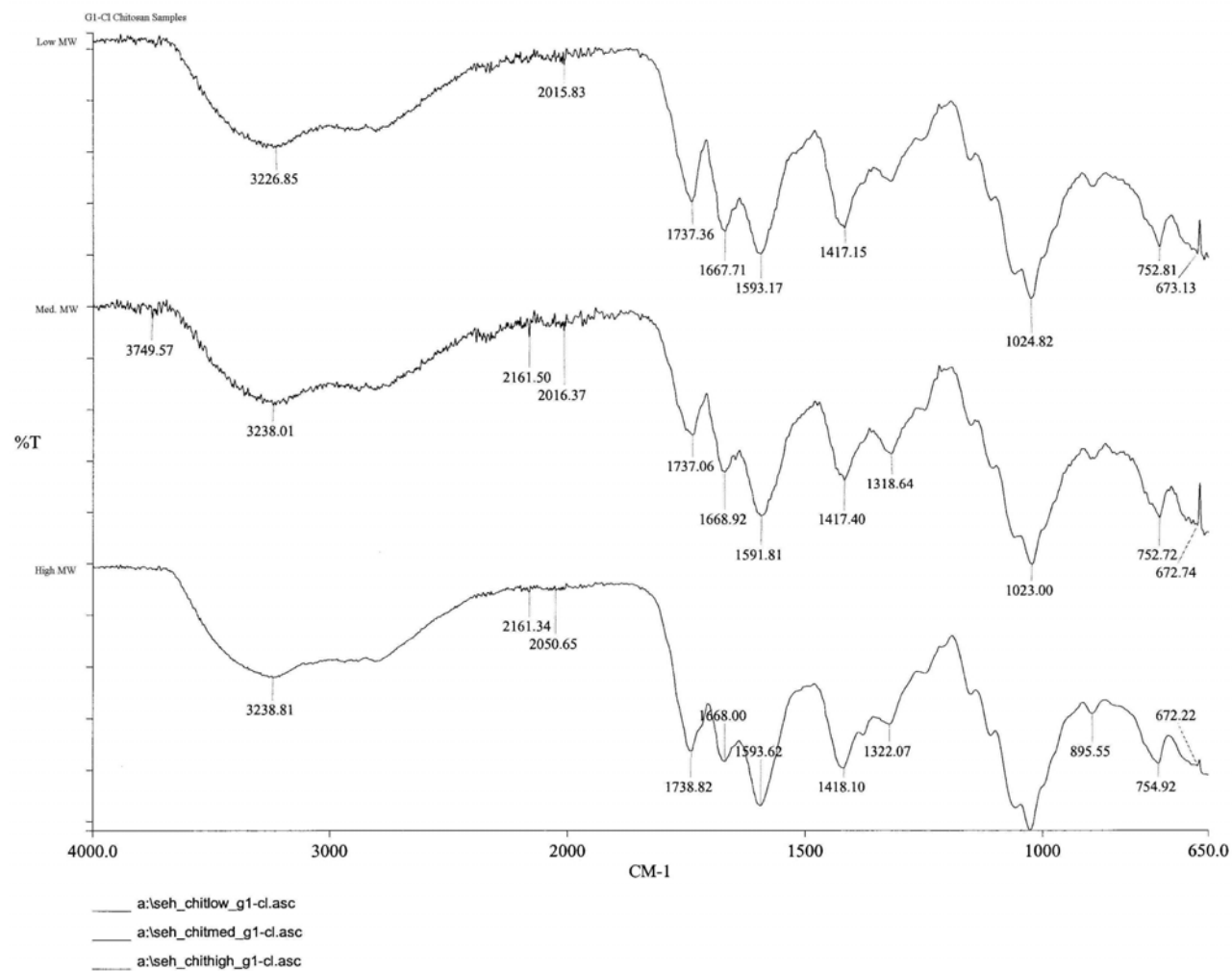
IR-Chitosan: Low MW, Med. MW, and High MW



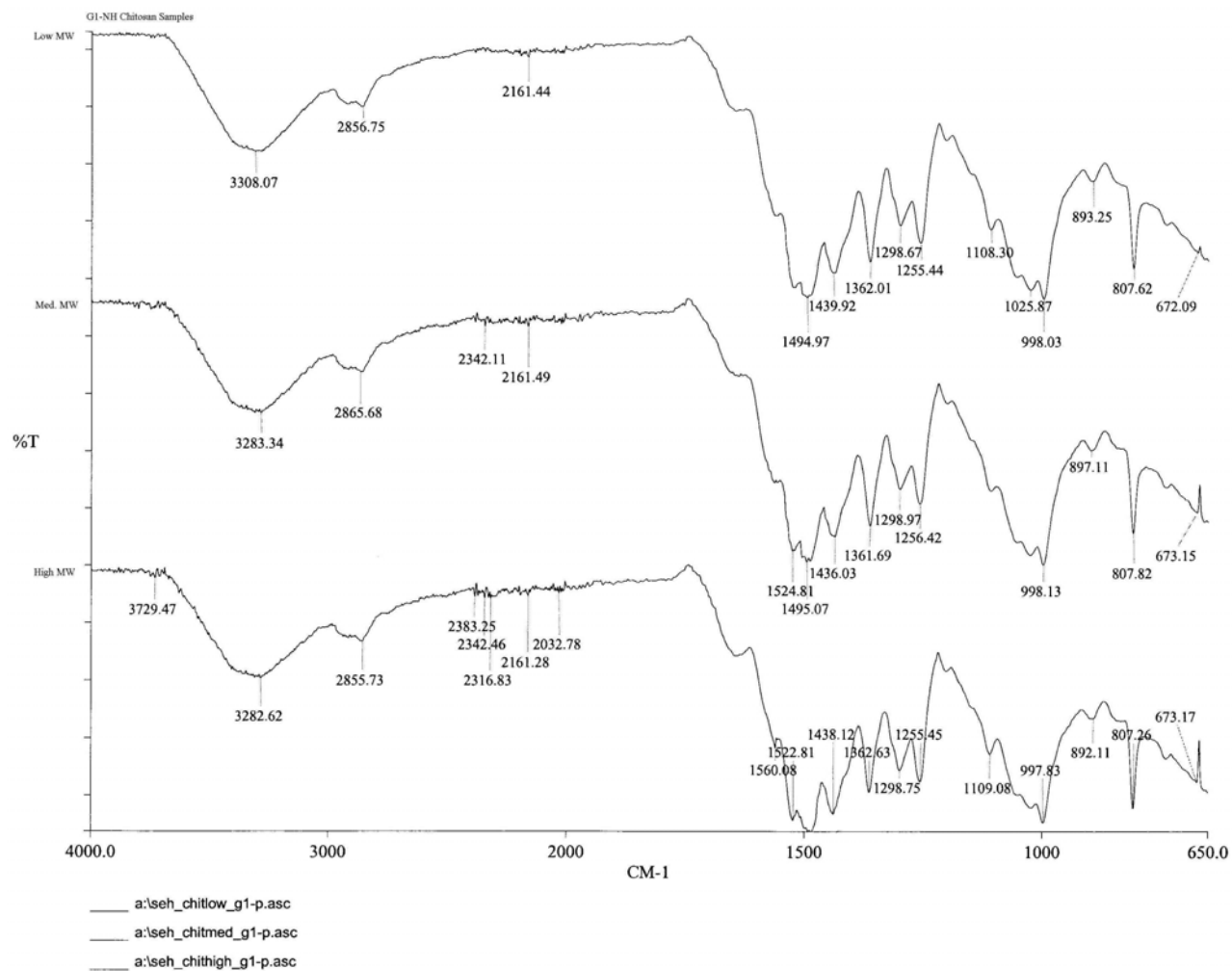
IR—NaOH-treated Chitosan: Low MW, Med. MW, and High MW



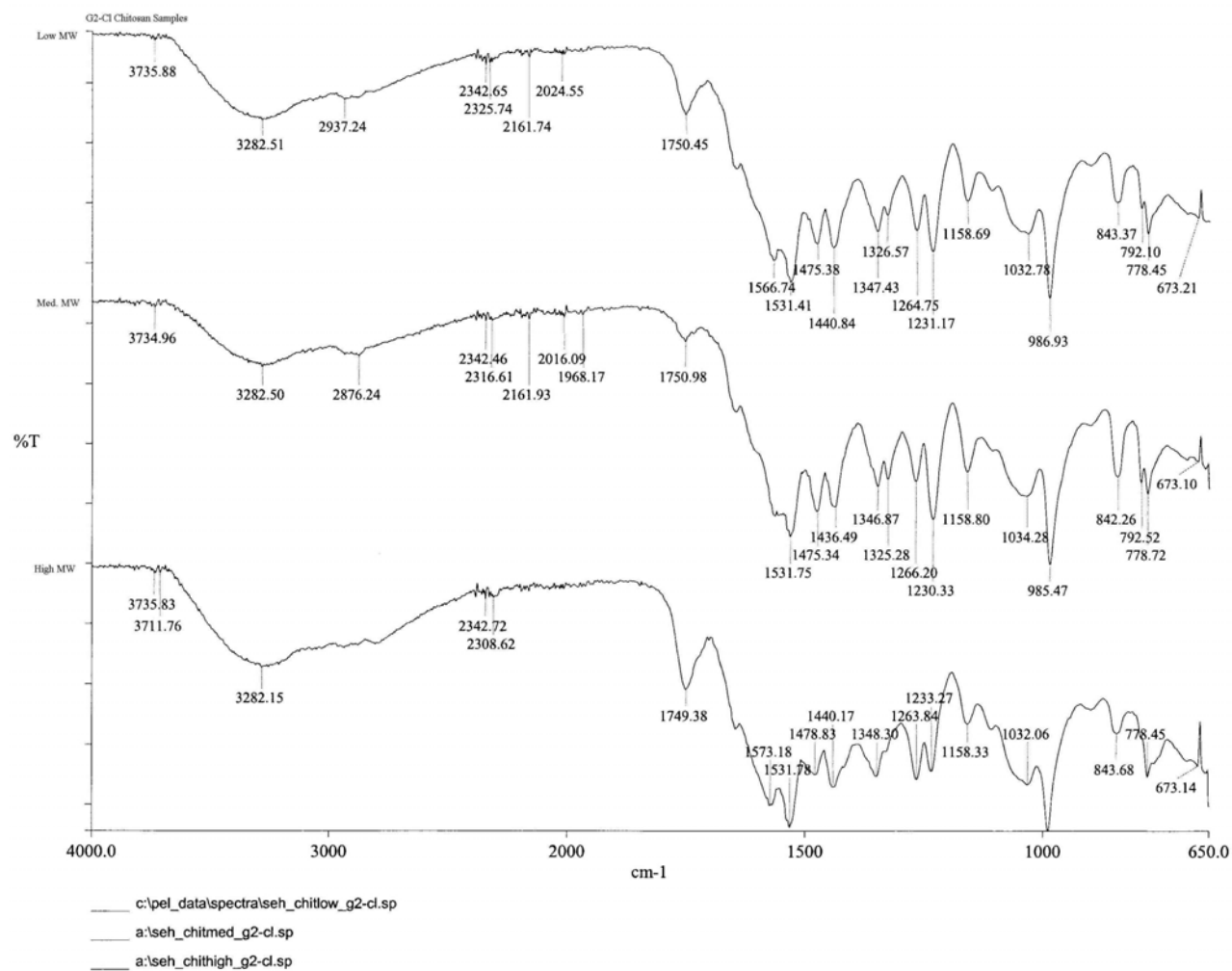
IR-Chitosan G1-Cl: Low MW, Med. MW, and High MW



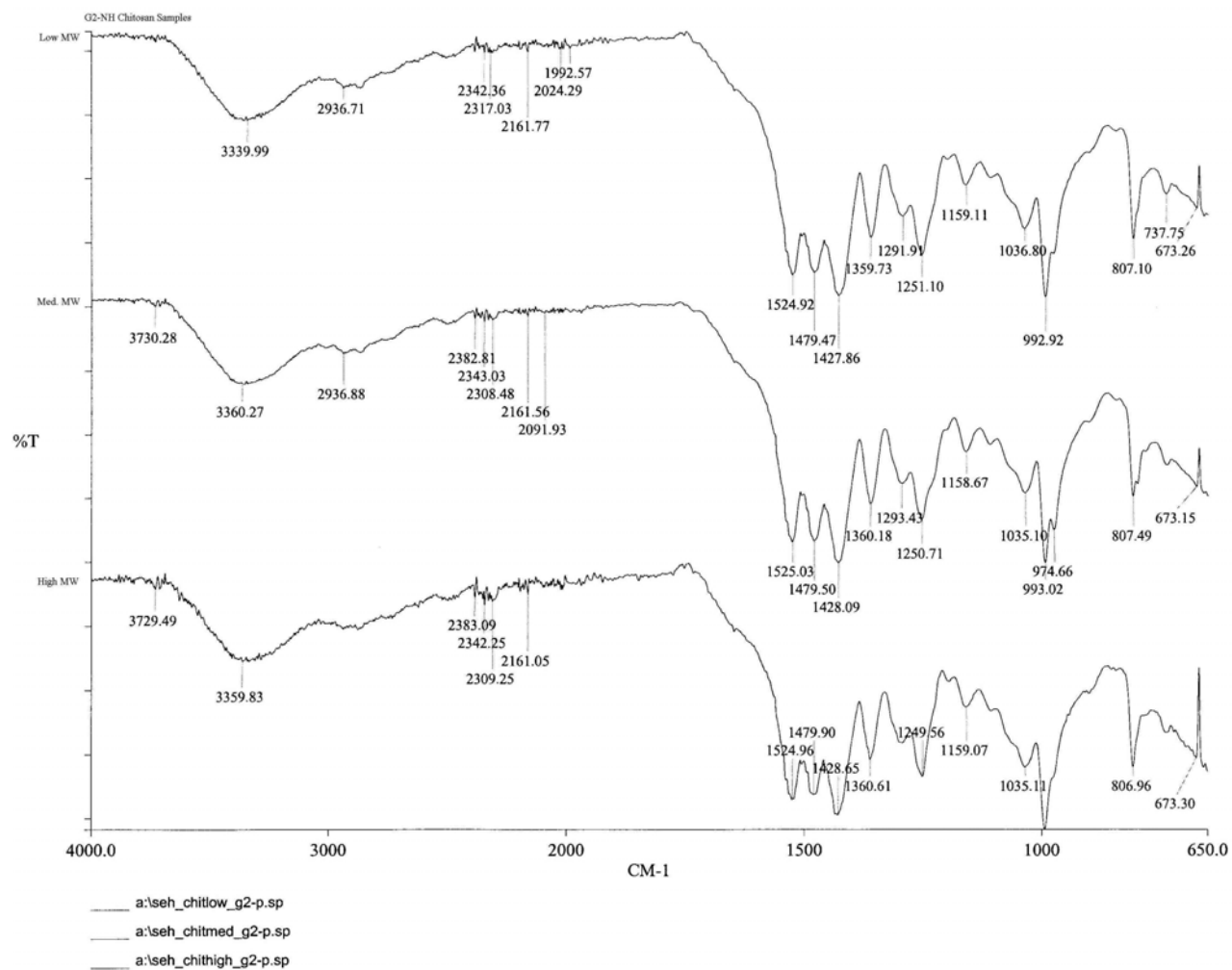
IR-Chitosan G1-NH: Low MW, Med. MW, and High MW



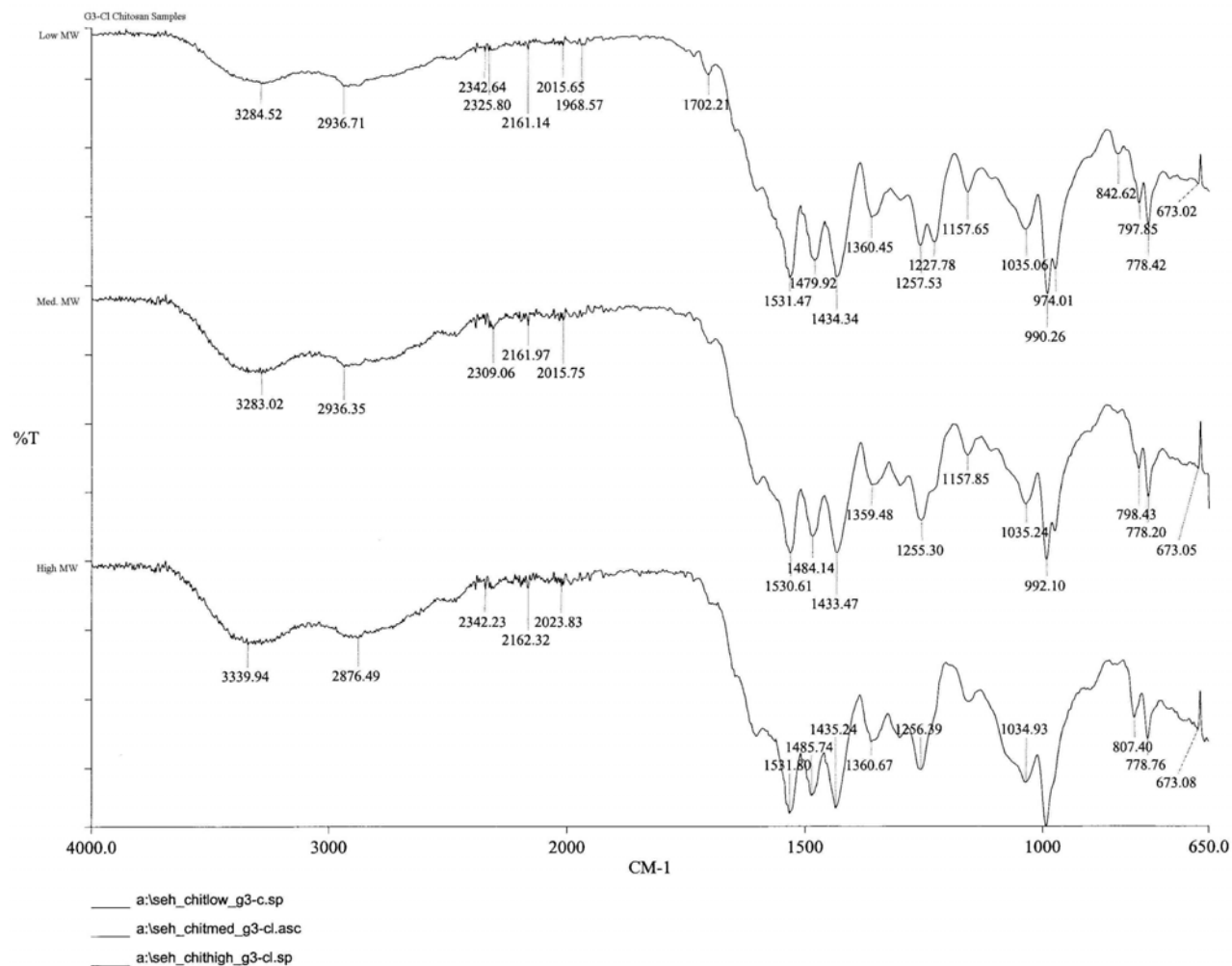
IR-Chitosan G2-Cl: Low MW, Med. MW, and High MW



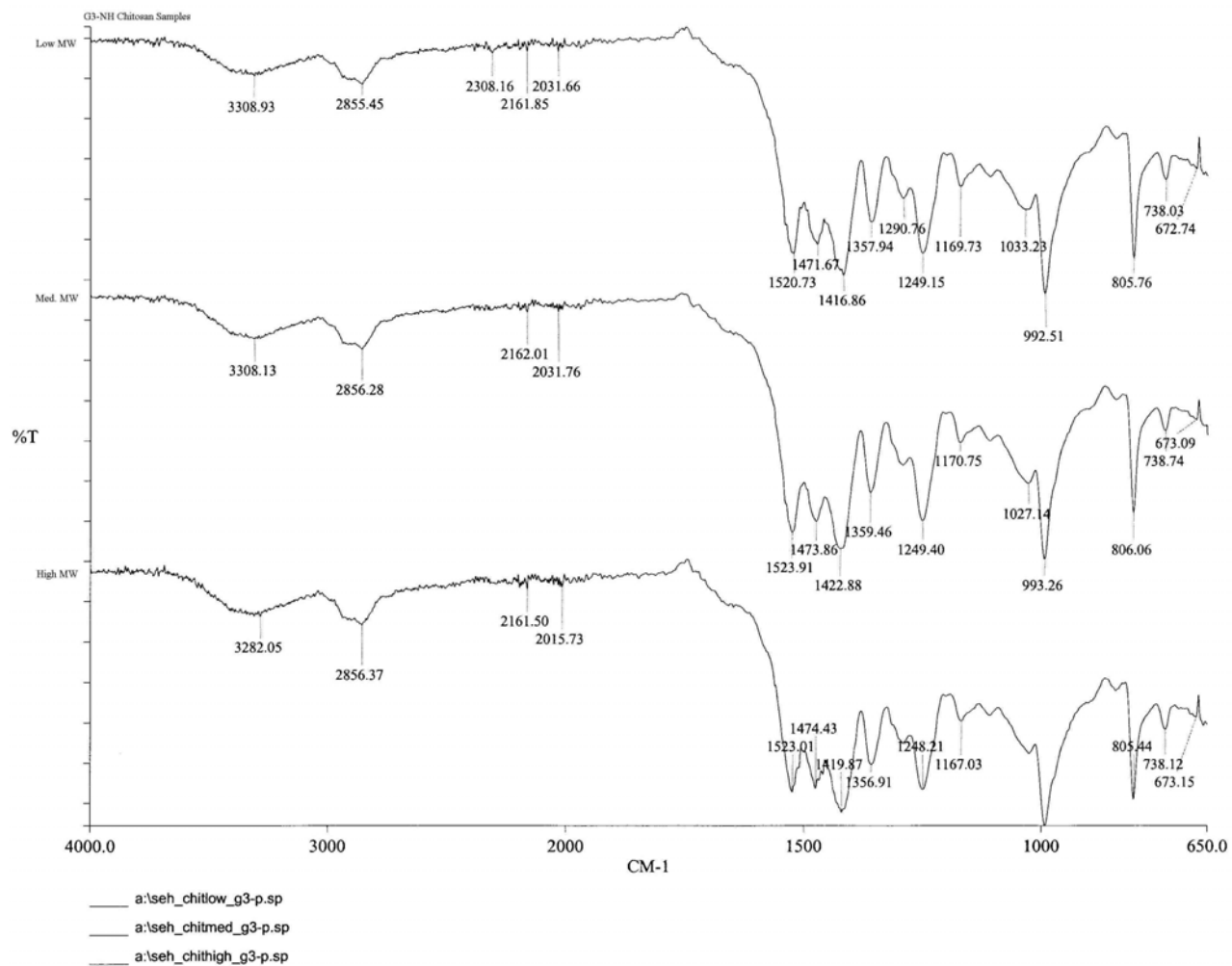
IR-Chitosan G2-NH: Low MW, Med. MW, and High MW



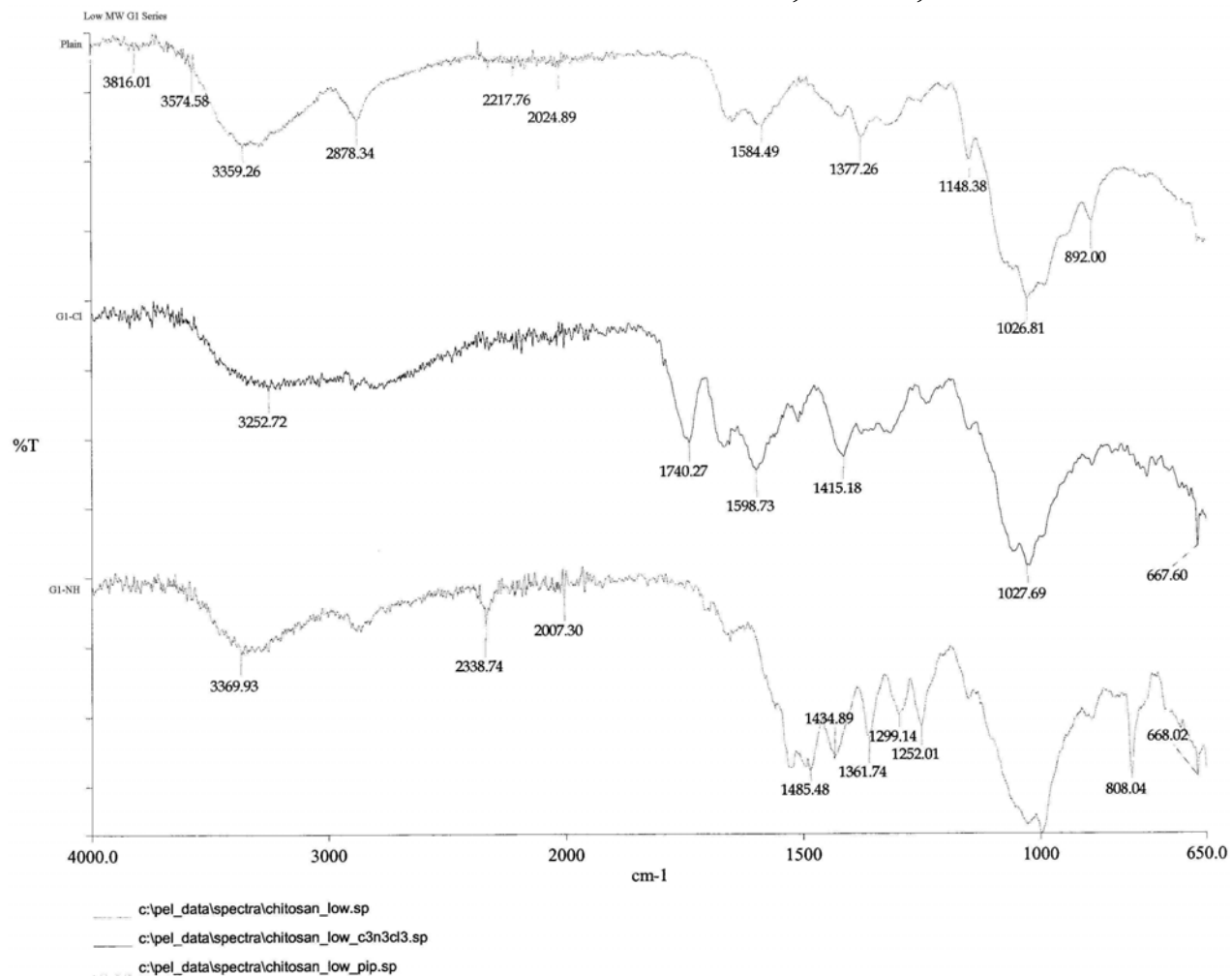
IR-Chitosan G3-Cl: Low MW, Med. MW, and High MW



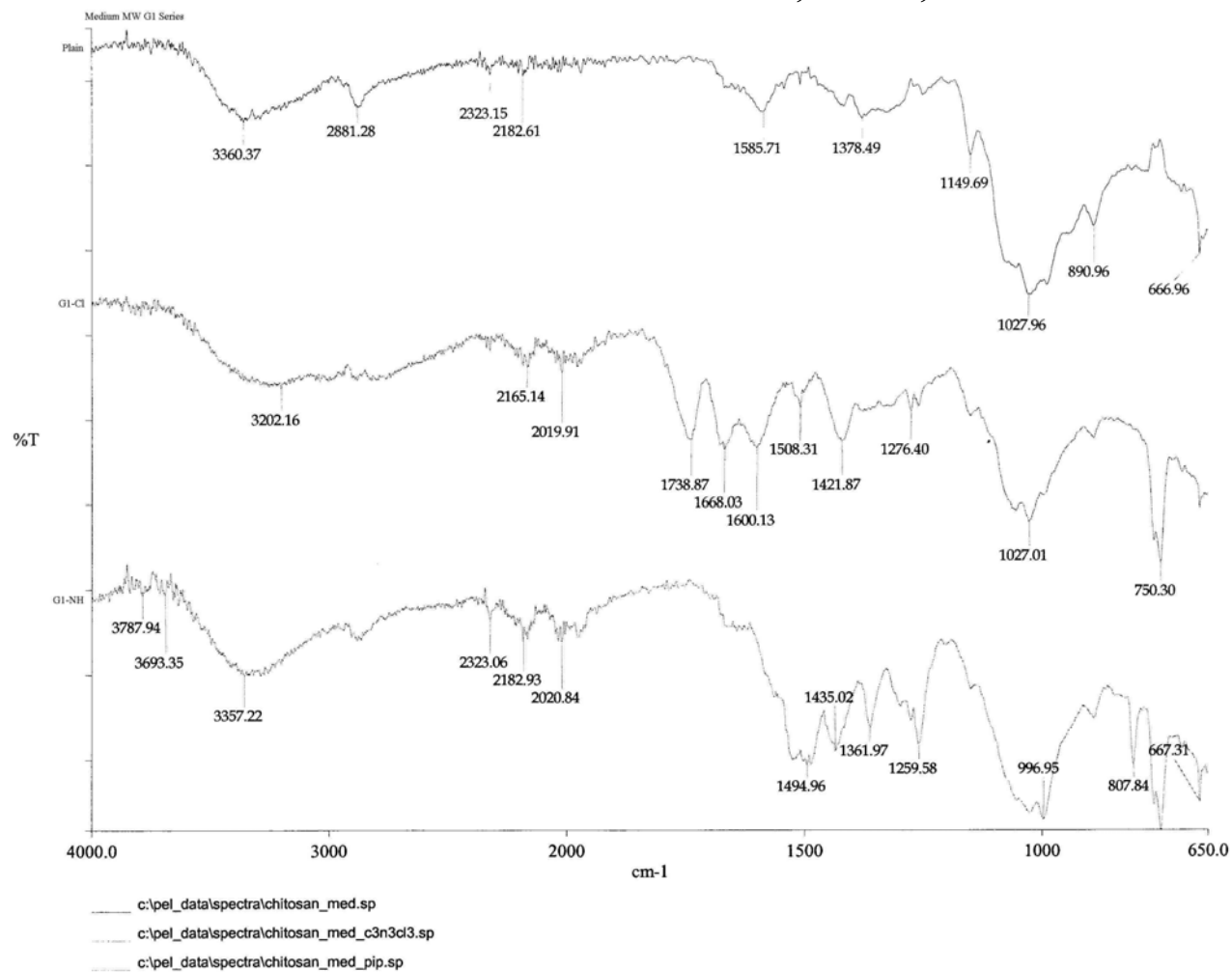
IR-Chitosan G3-NH: Low MW, Med. MW, and High MW



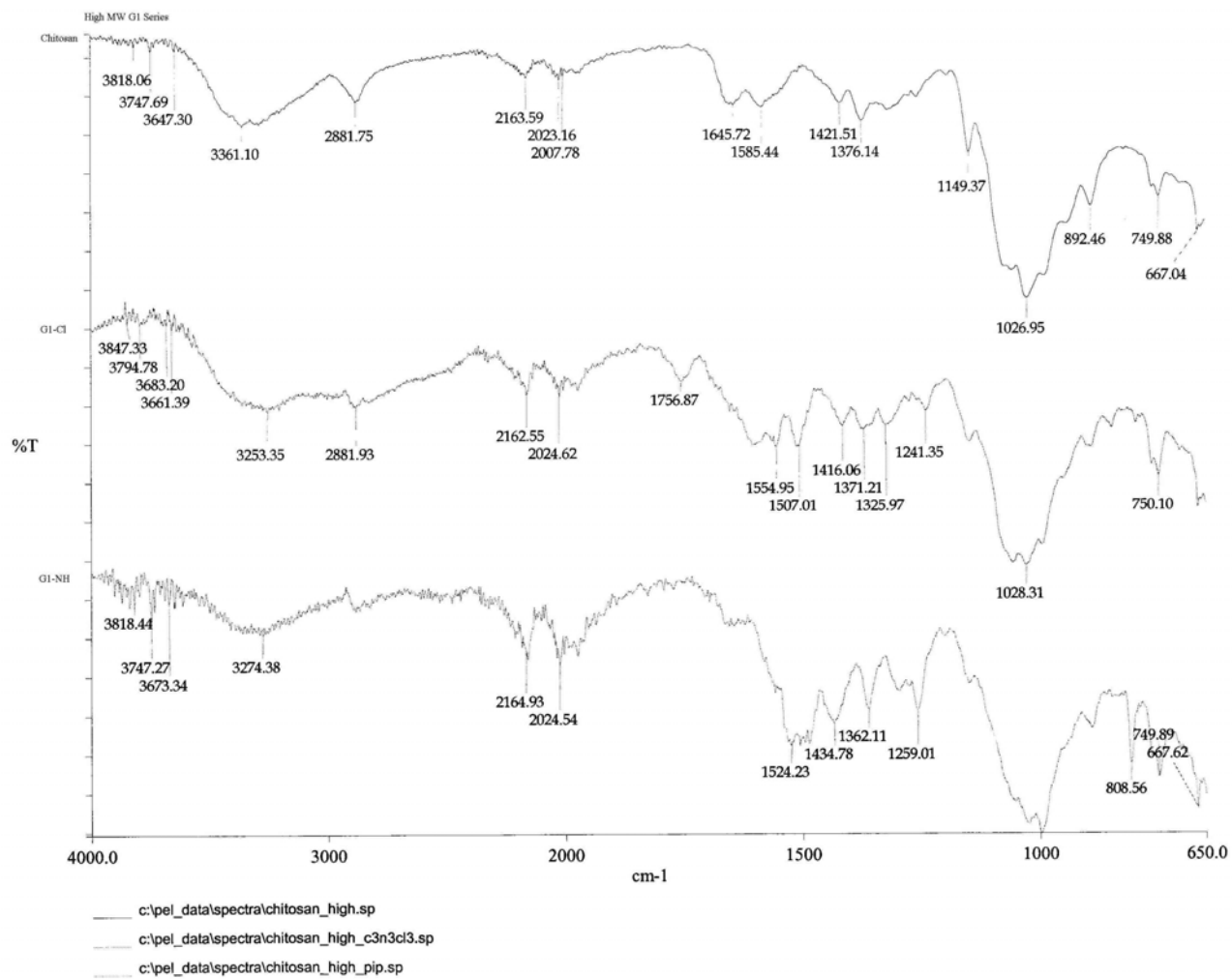
IR-Low MW Chitosan: Unmodified, G1-Cl, G1-NH



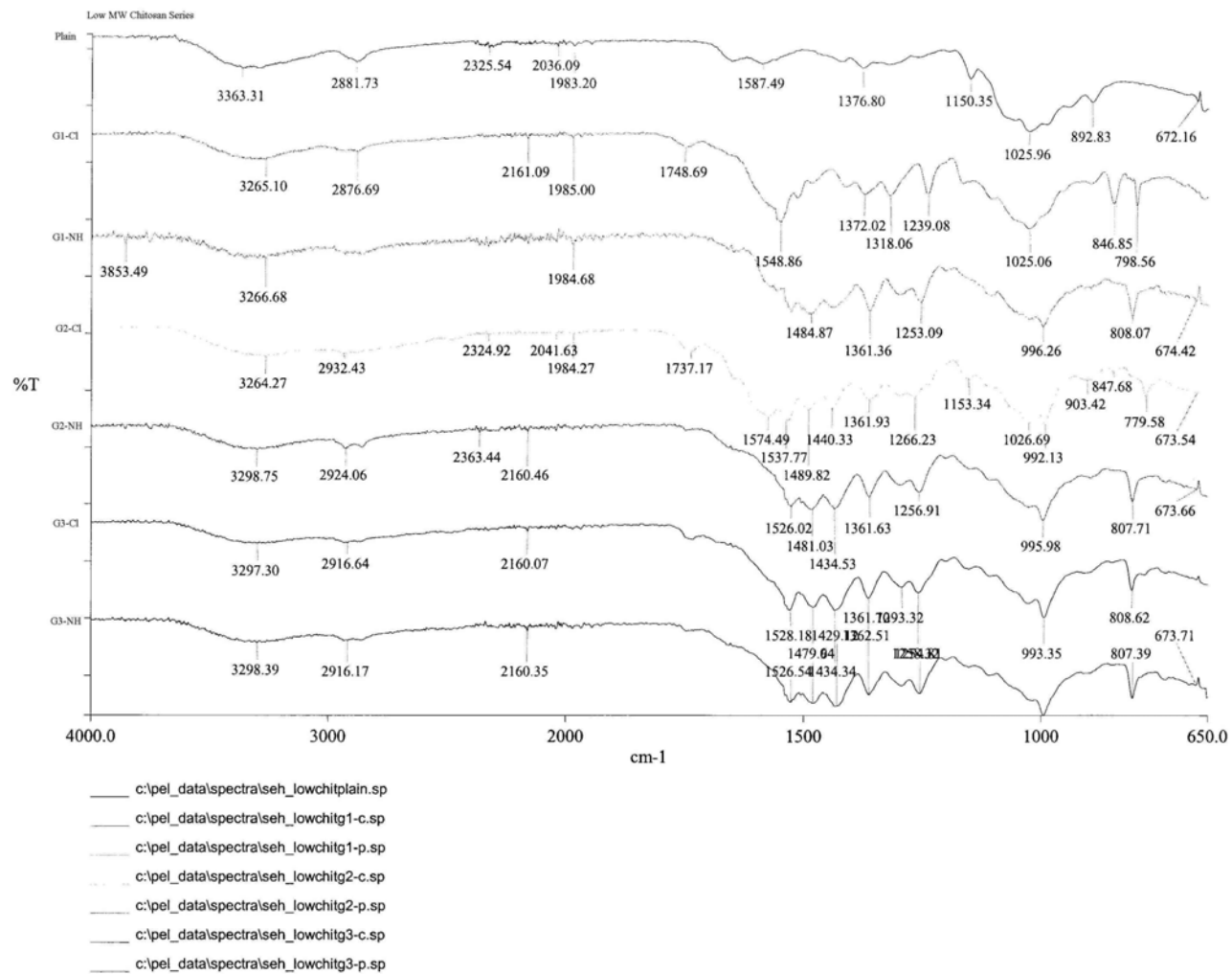
IR-Med MW Chitosan: Unmodified, G1-Cl, G1-NH



IR-High MW Chitosan: Unmodified, G1-Cl, G1-NH

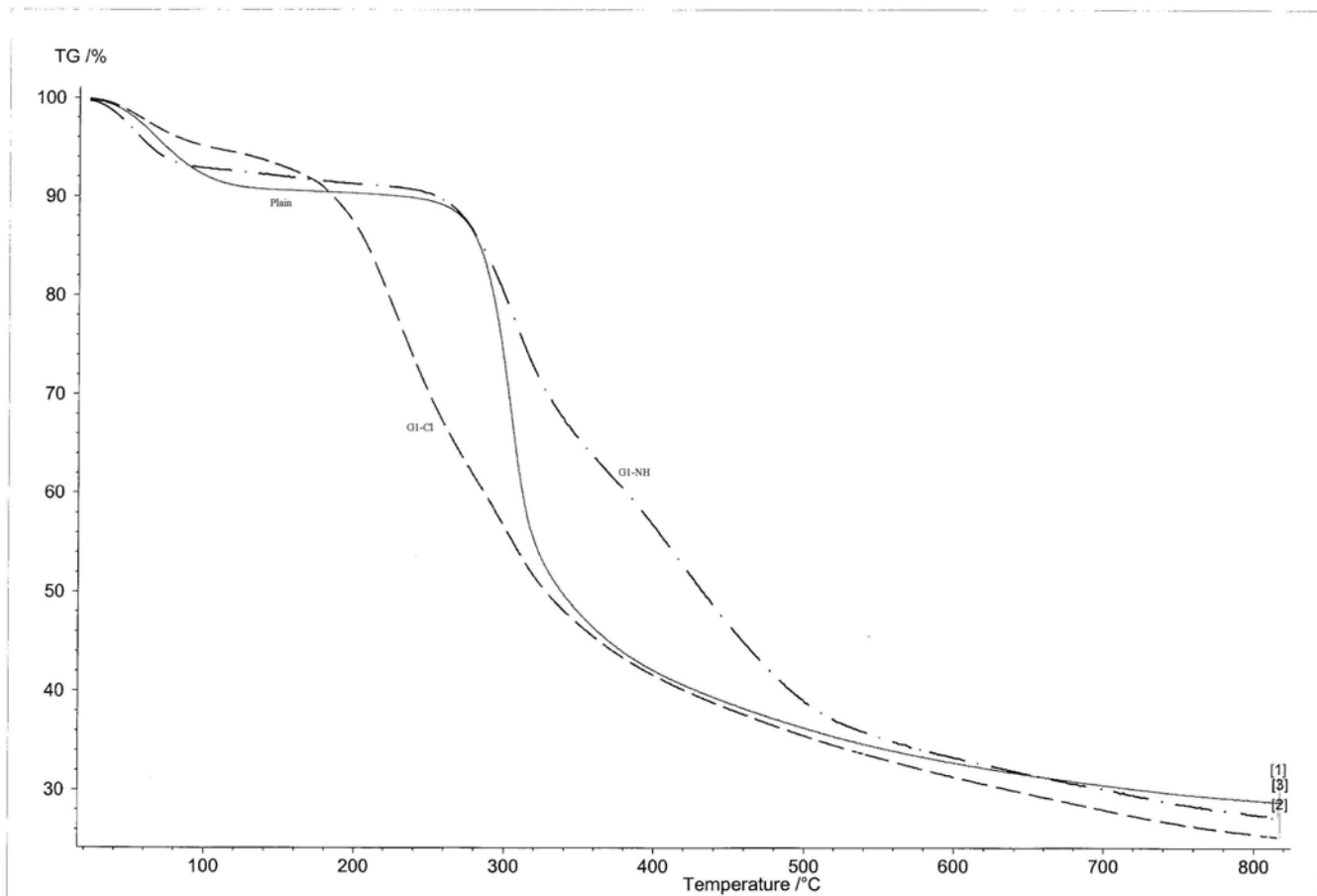


IR–Low MW Chitosan: Unmodified, G1-Cl, G1-NH, G2-Cl, G2-NH, G3-Cl, G3-NH



TGA Low MW Chitosan: Unmodified, G1-Cl, G1-NH

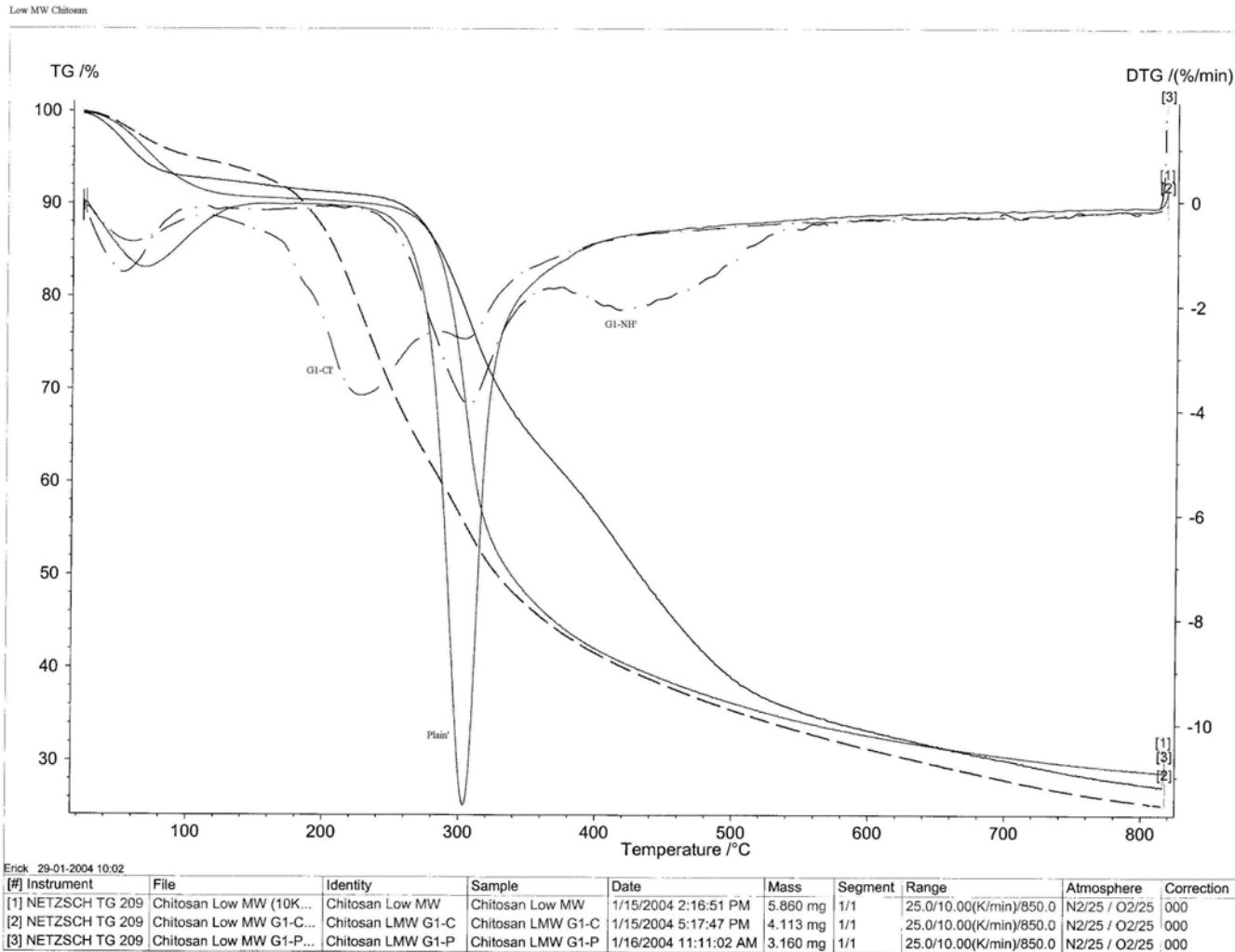
Low MW Chitosan



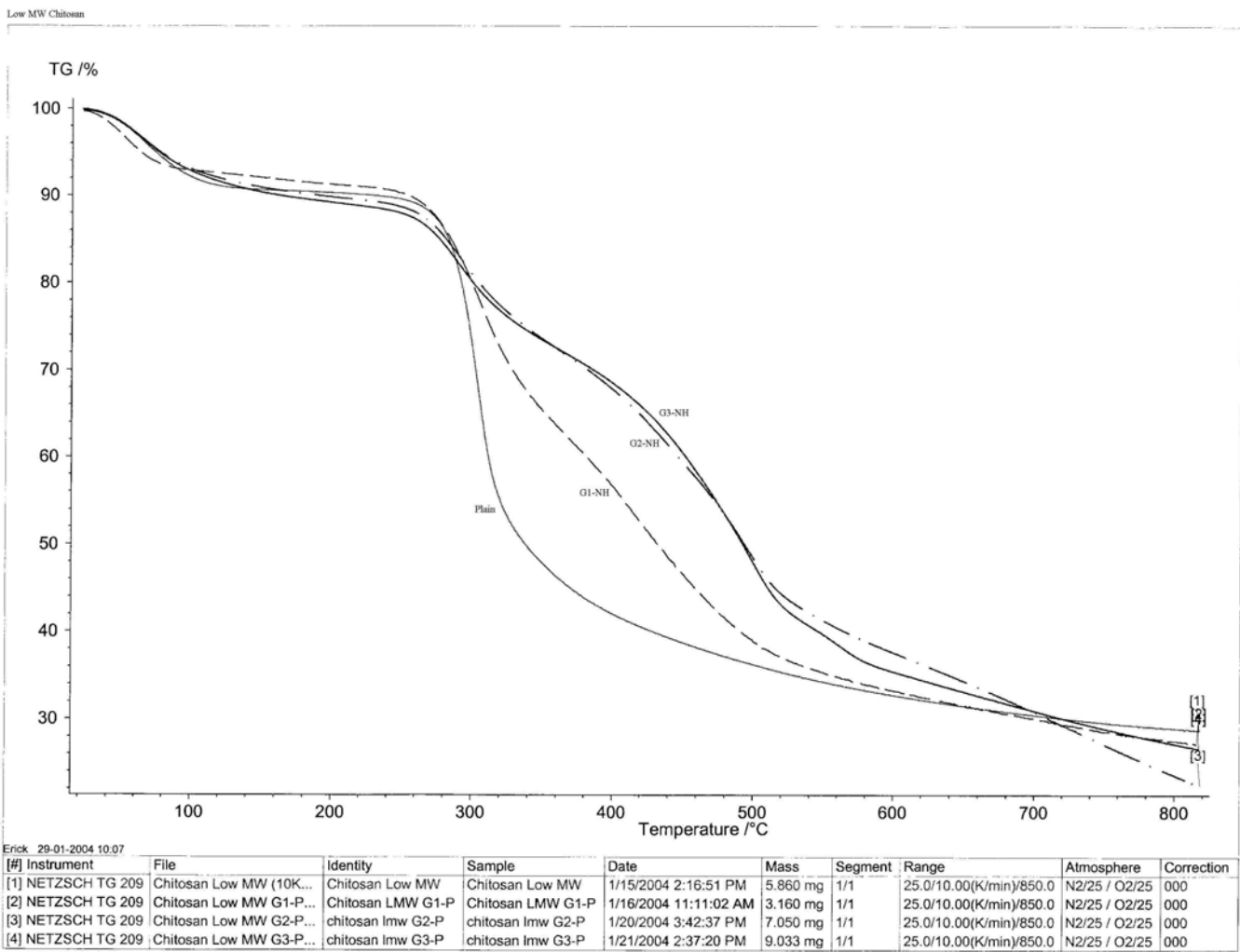
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[#] Instrument	File	Identity	Sample	Date	Mass	Segment	Range	Atmosphere	Correction
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[2] NETZSCH TG 209	Chitosan Low MW G1-C...	Chitosan LMW G1-C	Chitosan LMW G1-C	1/15/2004 5:17:47 PM	4.113 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000
[3] NETZSCH TG 209	Chitosan Low MW G1-P...	Chitosan LMW G1-P	Chitosan LMW G1-P	1/16/2004 11:11:02 AM	3.160 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000

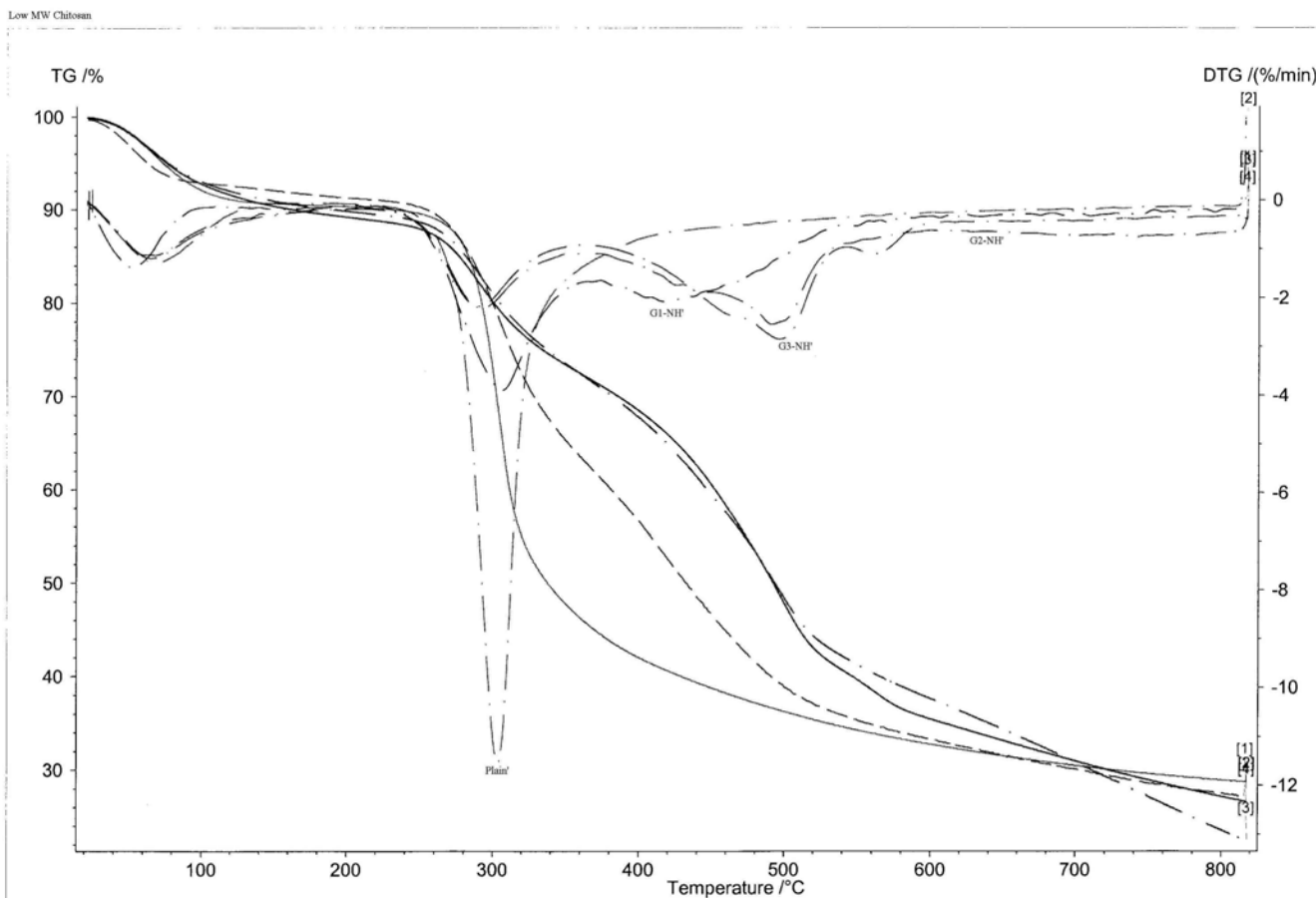
TGA Low MW Chitosan Derivative: Unmodified, G1-Cl, G1-NH



TGA Low MW Chitosan: Unmodified, G1-NH, G2-NH, G3-NH



TGA Low MW Chitosan Derivative: Unmodified, G1-NH, G2-NH, G3-NH



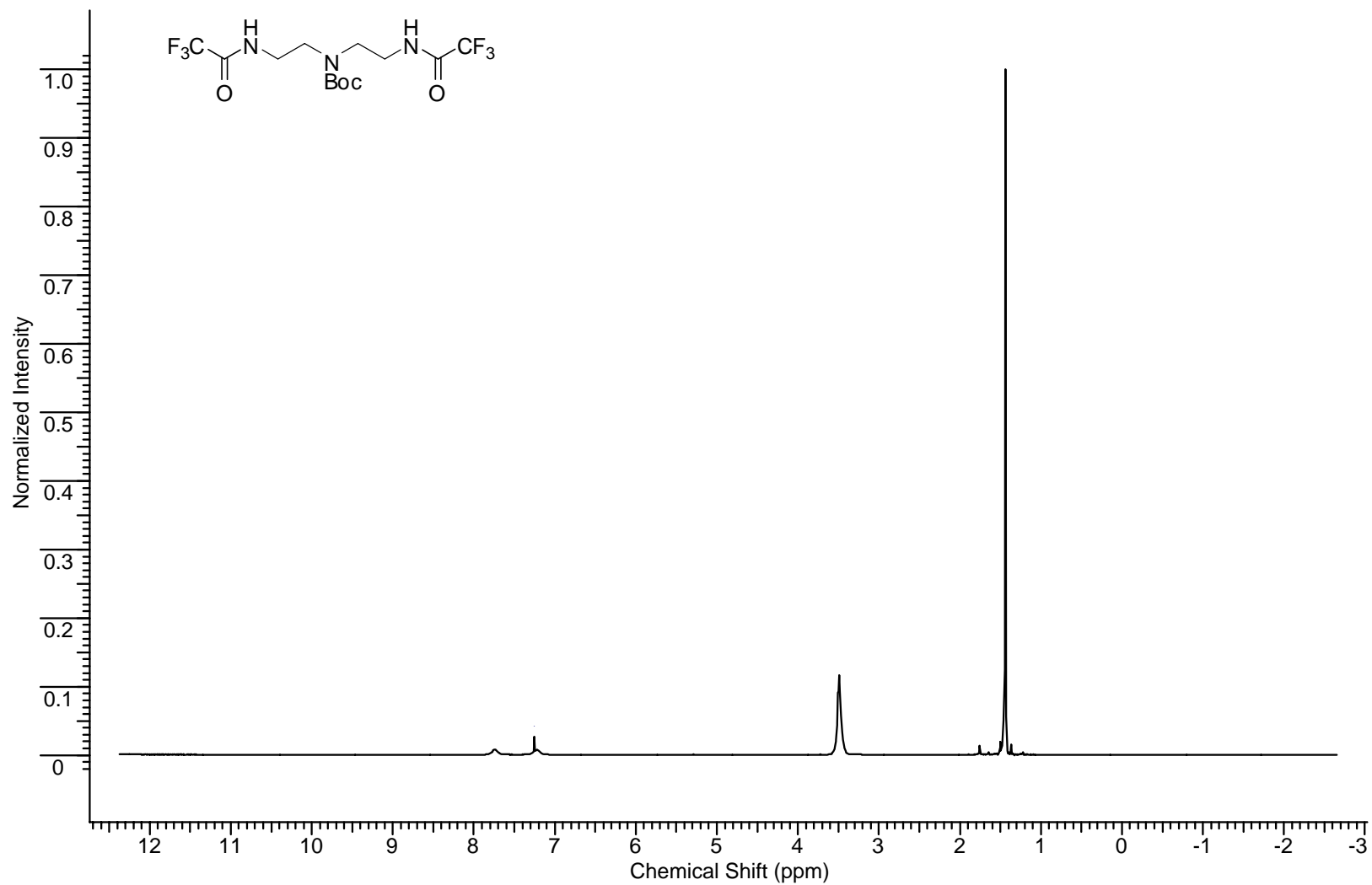
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[#] Instrument	File	Identity	Sample	Date	Mass	Segment	Range	Atmosphere	Correction
[1]	NETZSCH TG 209	Chitosan Low MW (10K...	Chitosan Low MW	1/15/2004 2:16:51 PM	5.860 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000
[2]	NETZSCH TG 209	Chitosan Low MW G1-P...	Chitosan LMW G1-P	1/16/2004 11:11:02 AM	3.160 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000
[3]	NETZSCH TG 209	Chitosan Low MW G2-P...	chitosan lmw G2-P	1/20/2004 3:42:37 PM	7.050 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000
[4]	NETZSCH TG 209	Chitosan Low MW G3-P...	chitosan lmw G3-P	1/21/2004 2:37:20 PM	9.033 mg	1/1	25.0/10.00(K/min)/850.0	N2/25 / O2/25	000

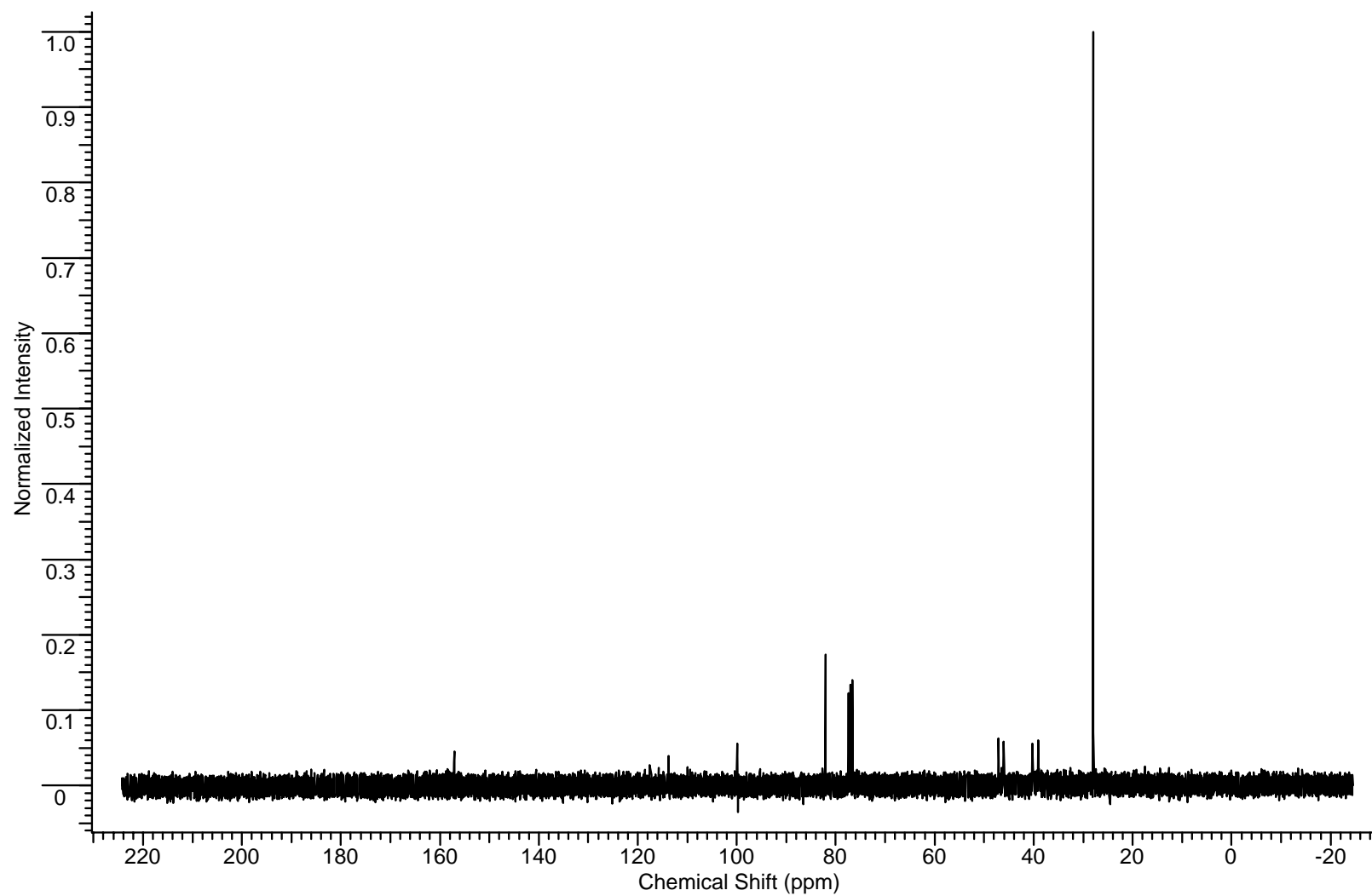
APPENDIX C

CHAPTER IV: SPECTRA

Isosteric Dendrimer Synthesis: Compound 1, ^1H NMR

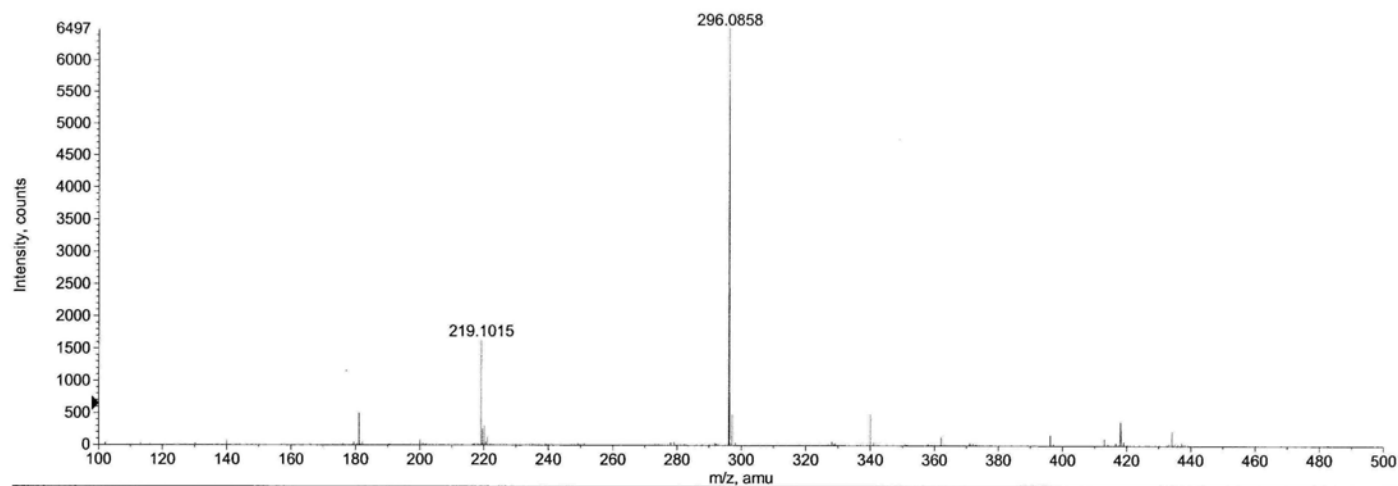


Isosteric Dendrimer Synthesis: Compound 1, ^{13}C NMR



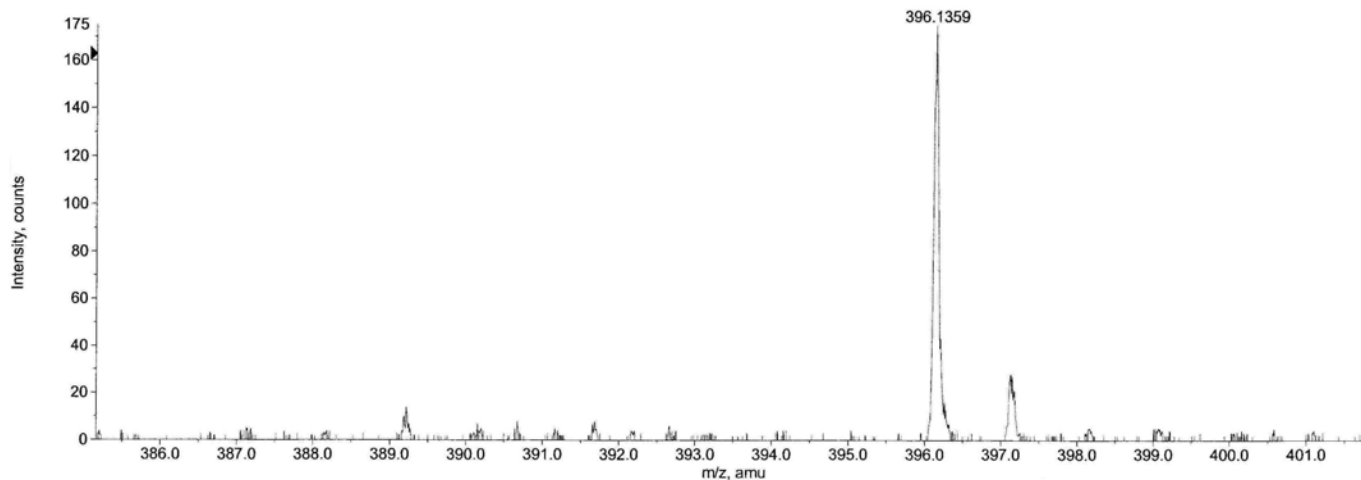
Isosteric Dendrimer Synthesis: Intermediate 1, ESI-MS

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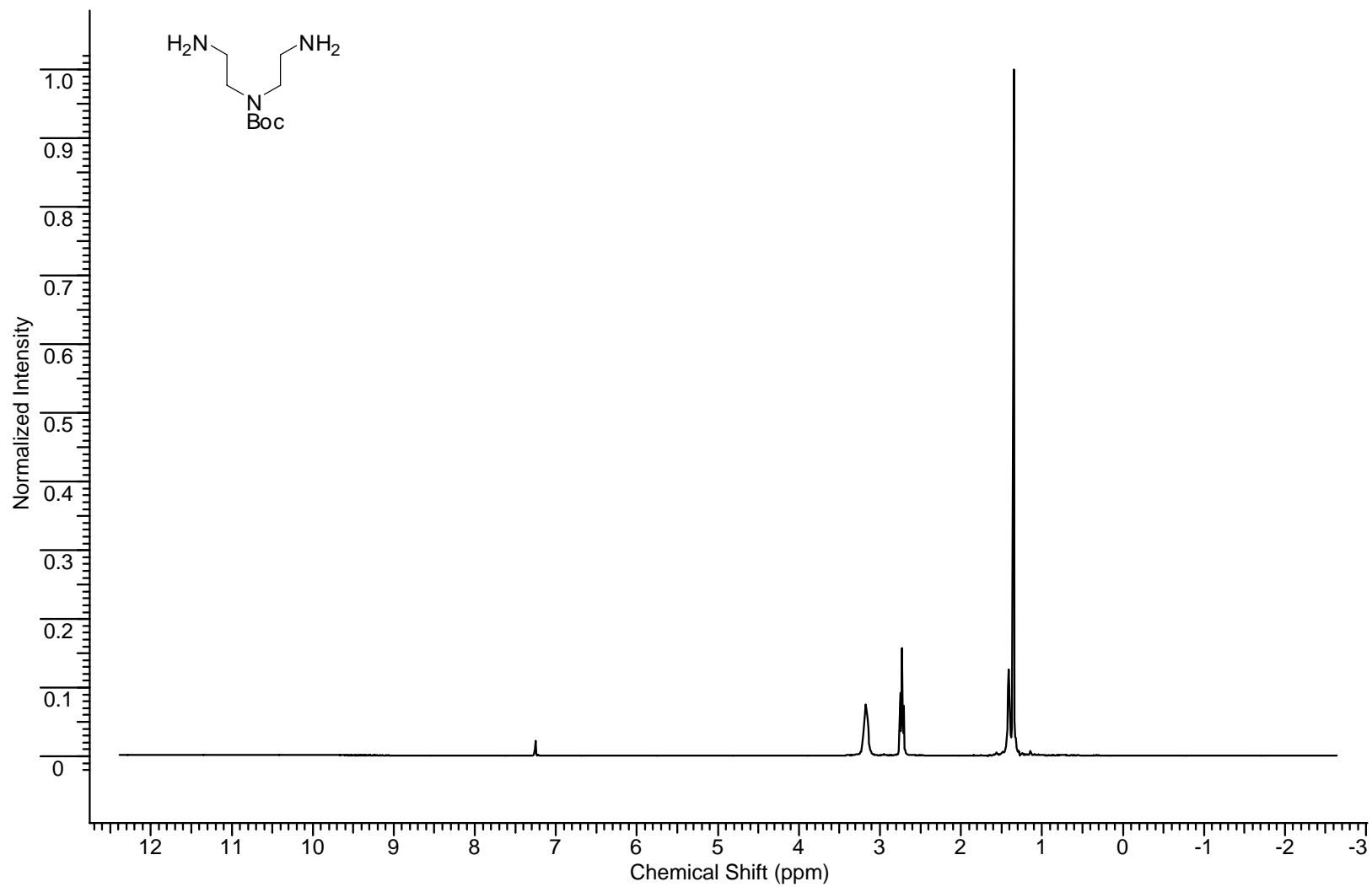


+TOF MS: 29 MCA scans from Sample 2 (sh2-64) of 06100514.wiff
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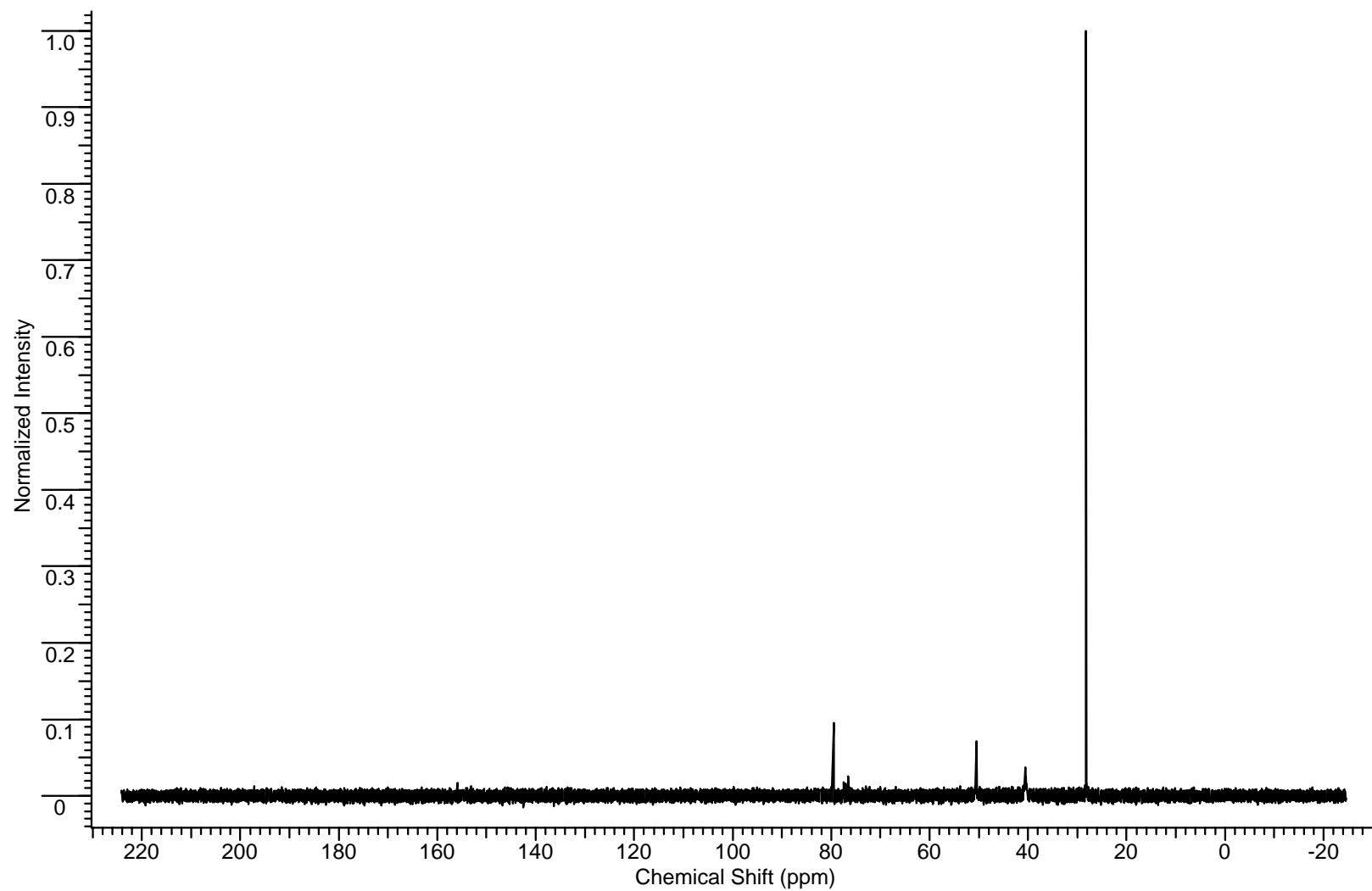
Max. 6497.0 counts



Isosteric Dendrimer Synthesis: Compound 2, ^1H NMR

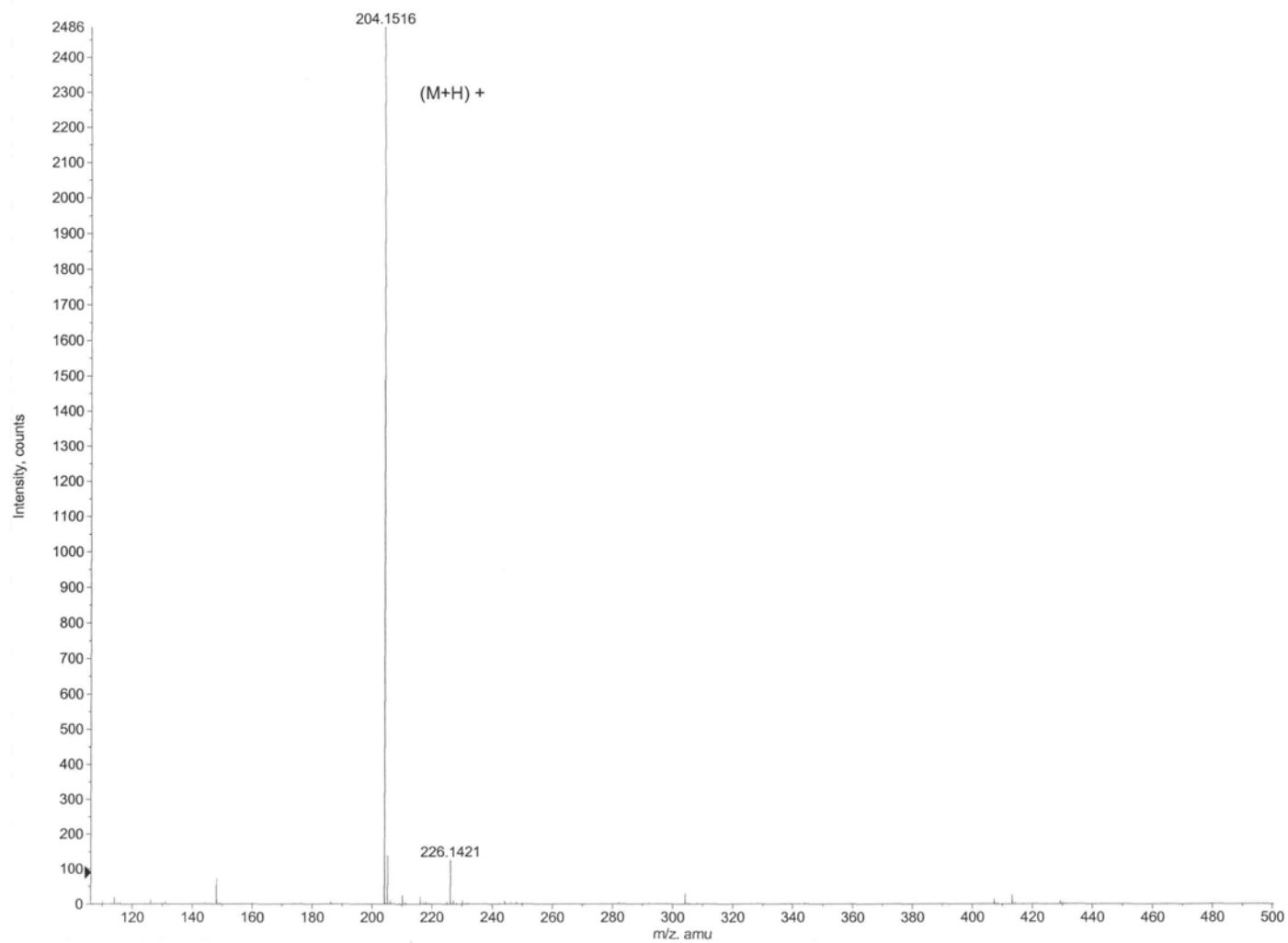


Isosteric Dendrimer Synthesis: Compound **2**, ^{13}C NMR

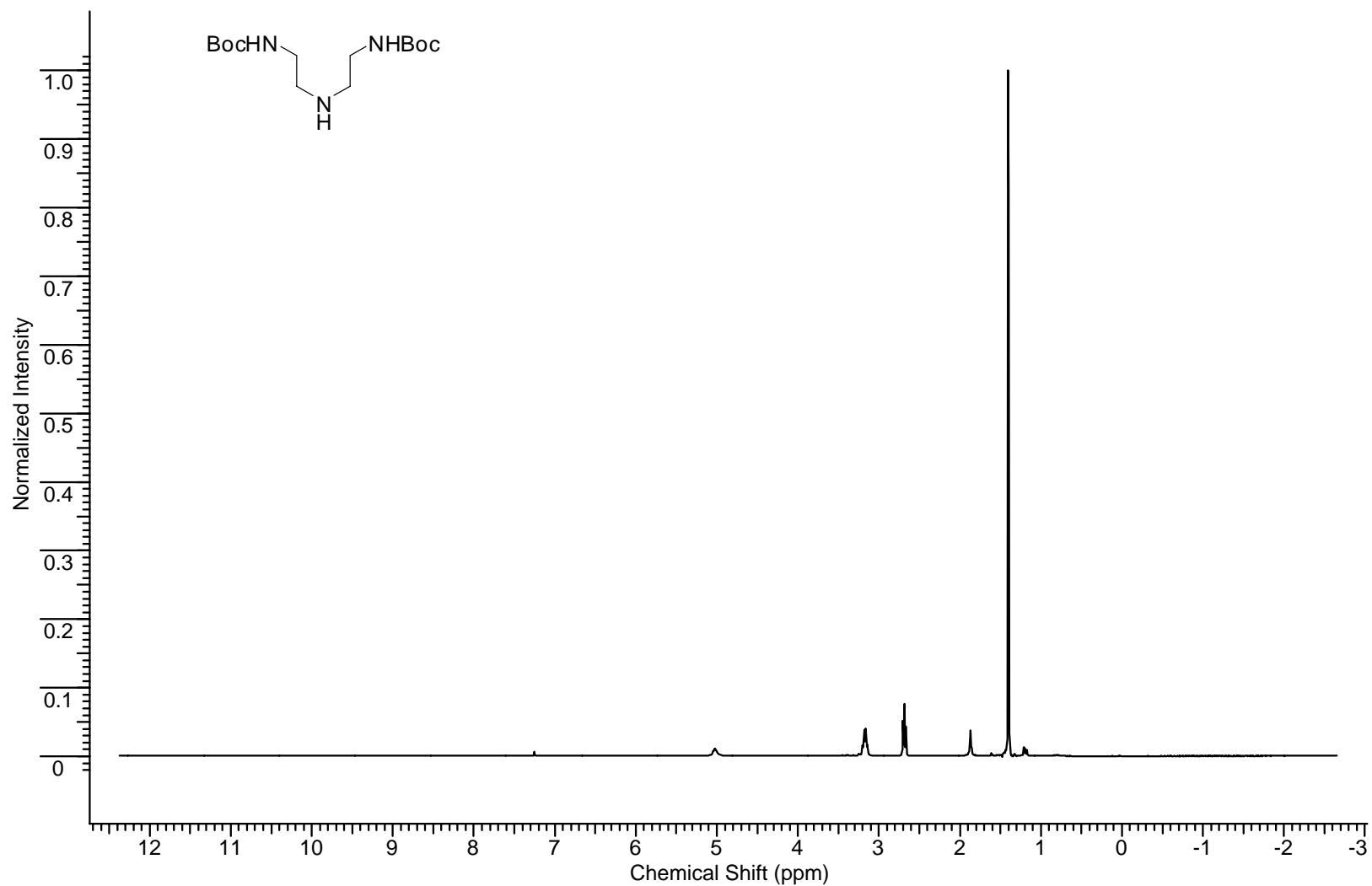


Isosteric Dendrimer Synthesis: Compound 2, ESI-MS

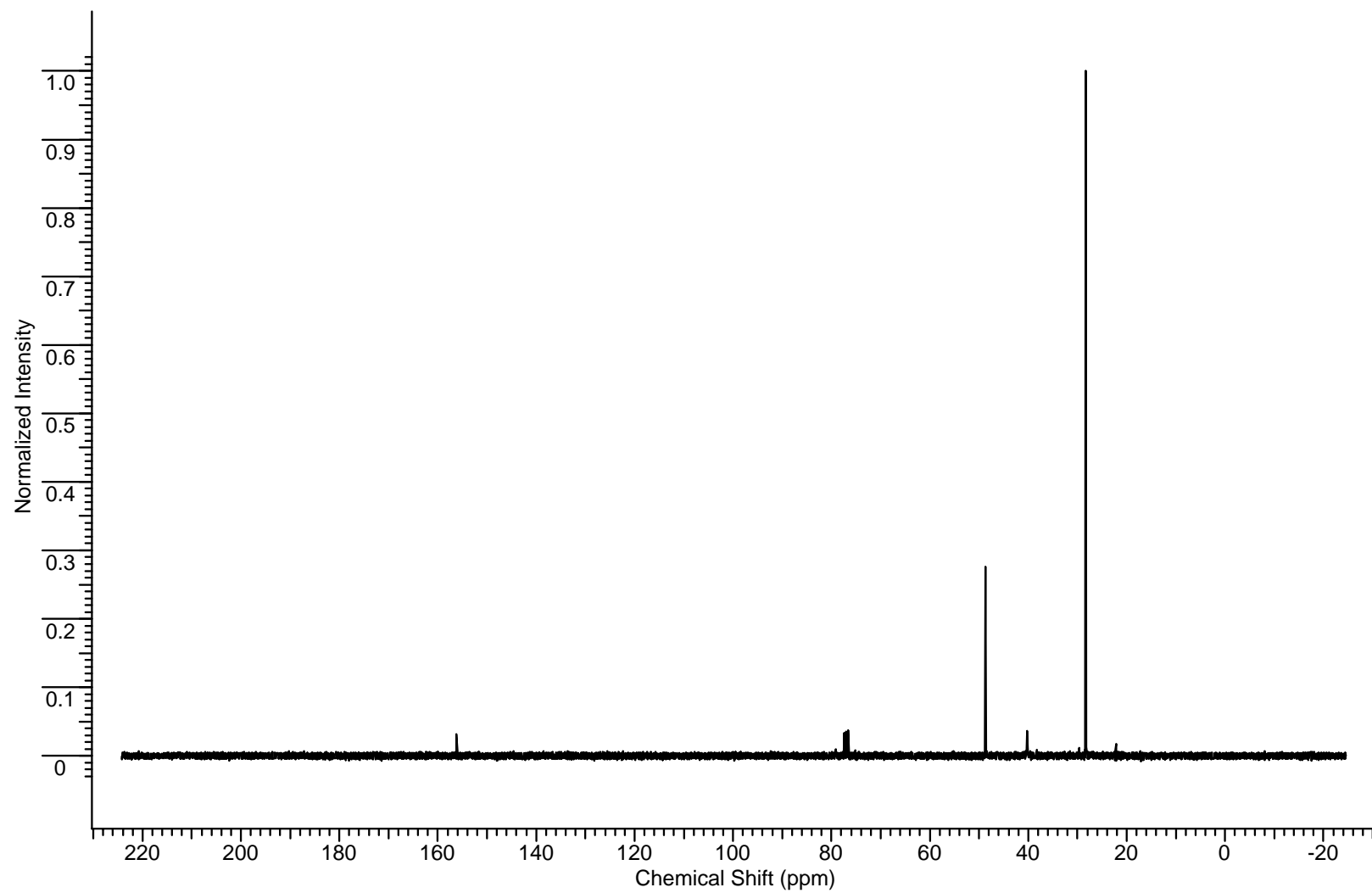
+TOF MS: 4 MCA scans from 03080505.wiff
a=3.55285049760, b=50e-004, t0=4.67077648975791820e+001



Isosteric Dendrimer Synthesis: Compound **3**, ^1H NMR

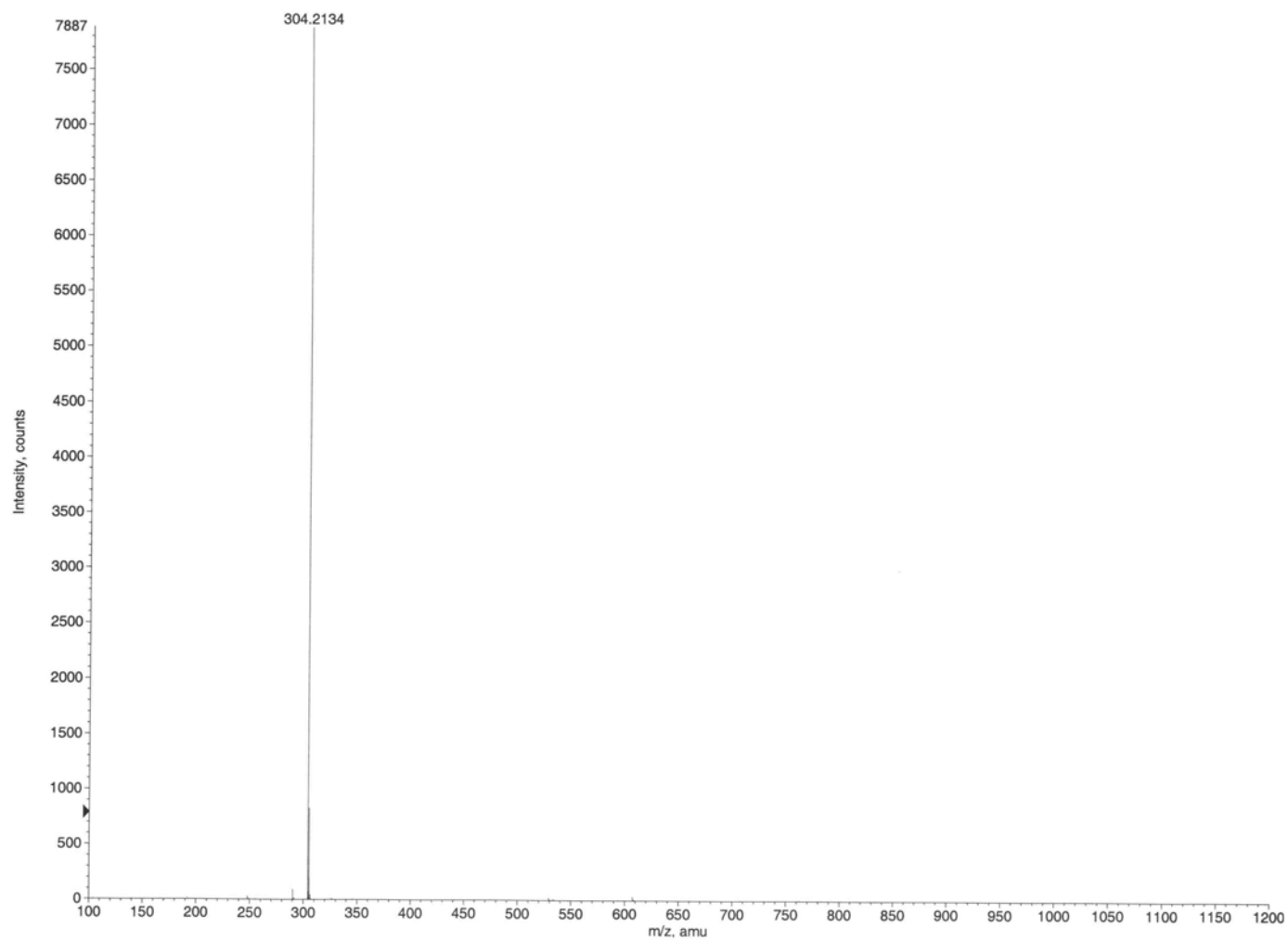


Isosteric Dendrimer Synthesis: Compound **3**, ^{13}C NMR

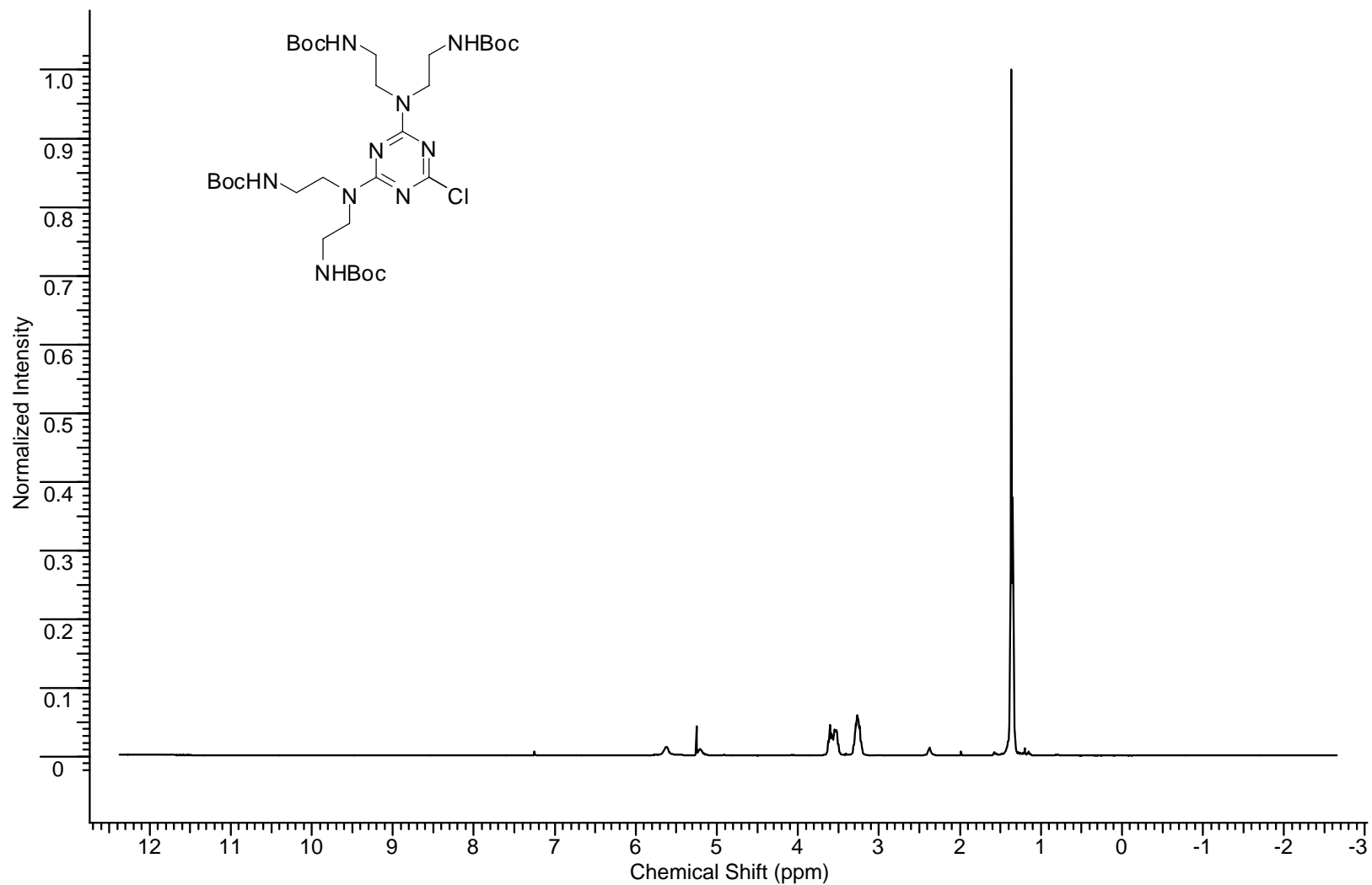


Isosteric Dendrimer Synthesis: Compound **3**, ESI-MS

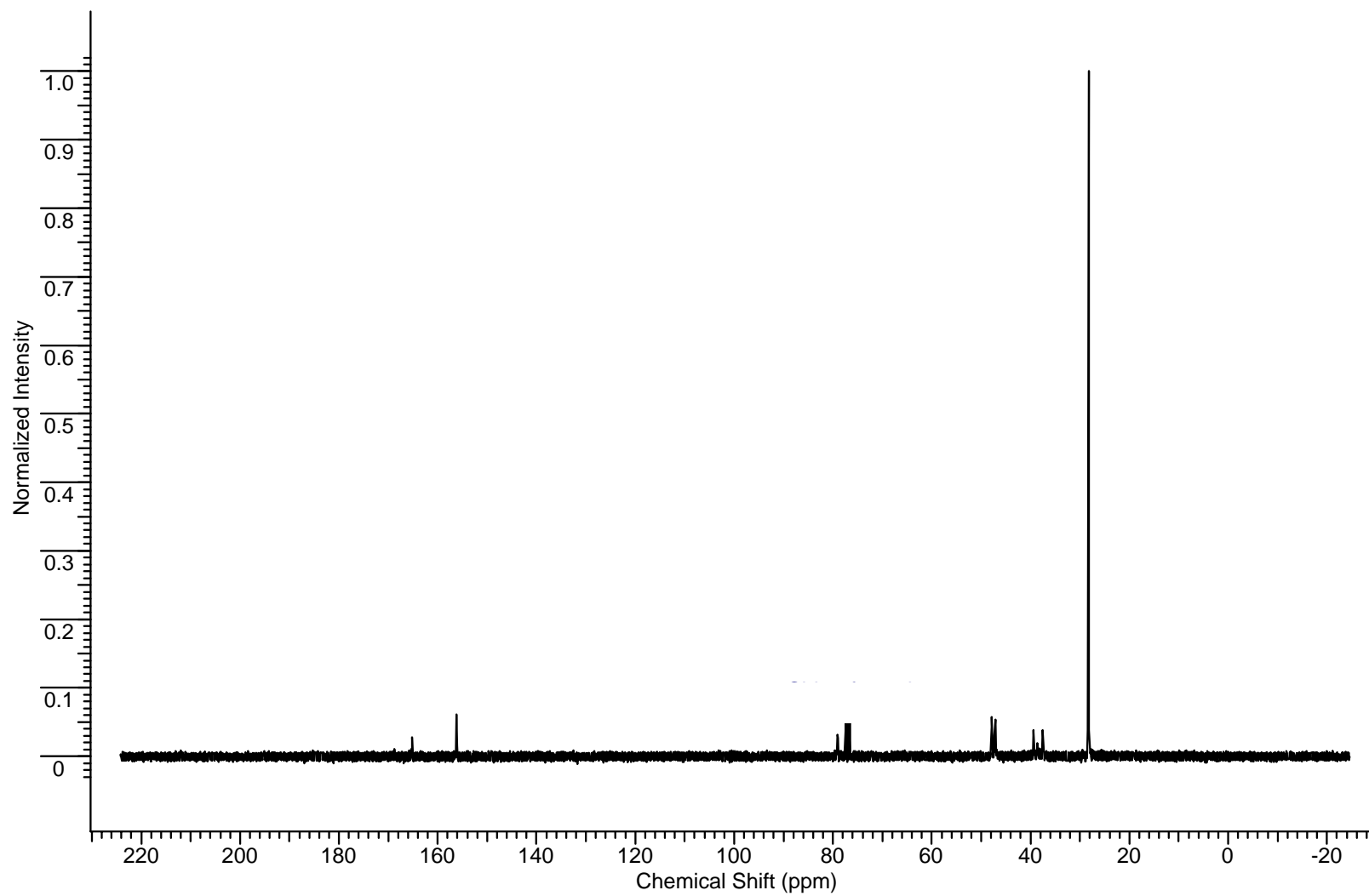
+TOF MS: 3 MCA ns from 01100512.wiff
a=3.55269219334. 20e-004, t0=4.53057312806668050e+001



Isosteric Dendrimer Synthesis: Compound 4, ^1H NMR

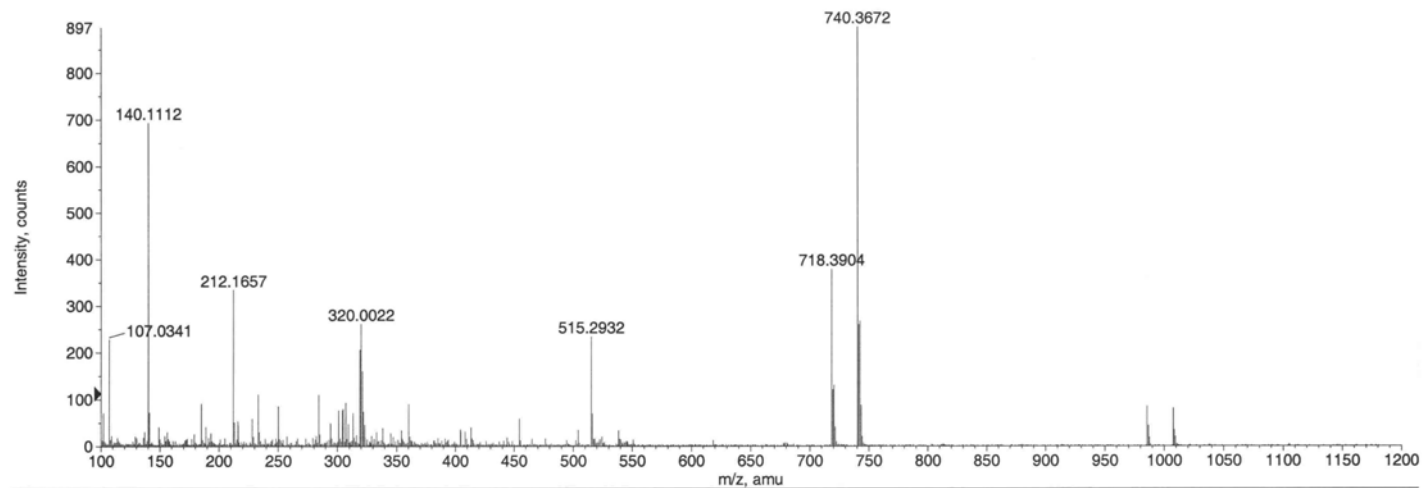


Isosteric Dendrimer Synthesis: Compound 4, ^{13}C NMR

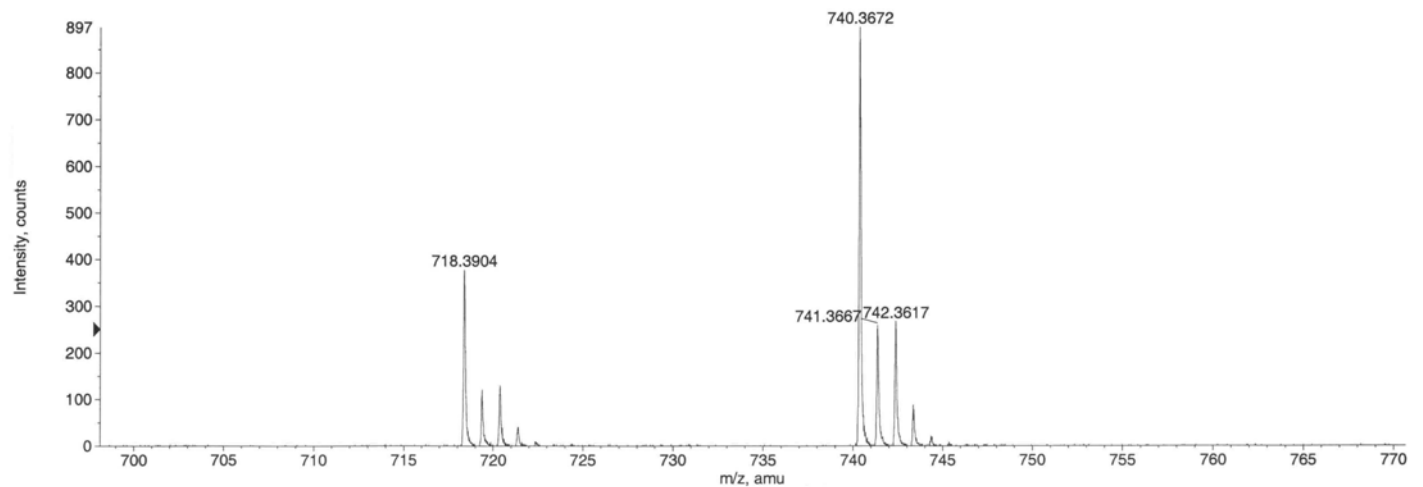


Isosteric Dendrimer Synthesis: Compound 4, ESI-MS

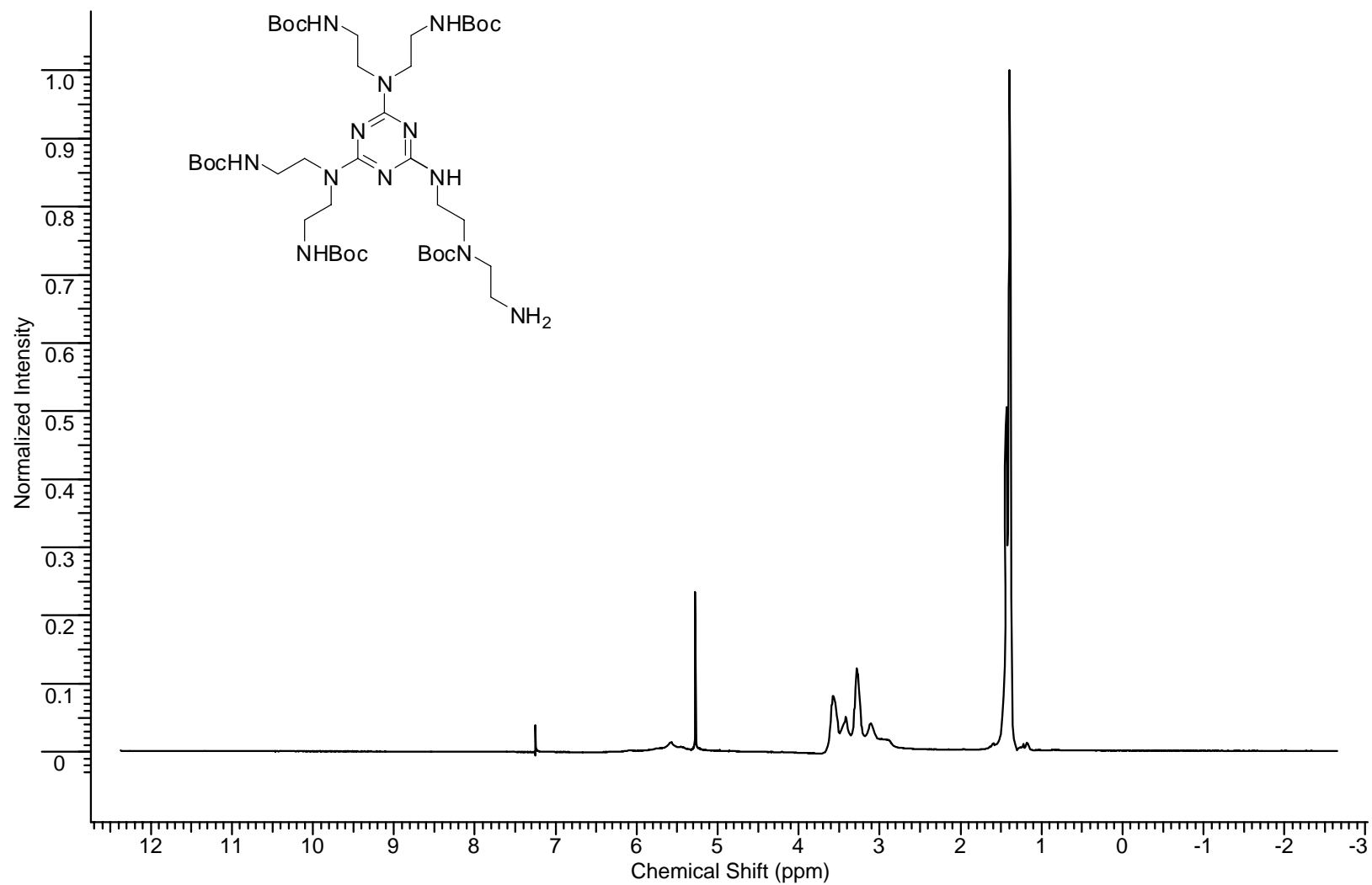
+TOF MS: 136 MCA scans from 01100511.wiff
a=3.55269219334 t0=4.53057312806668050e+001



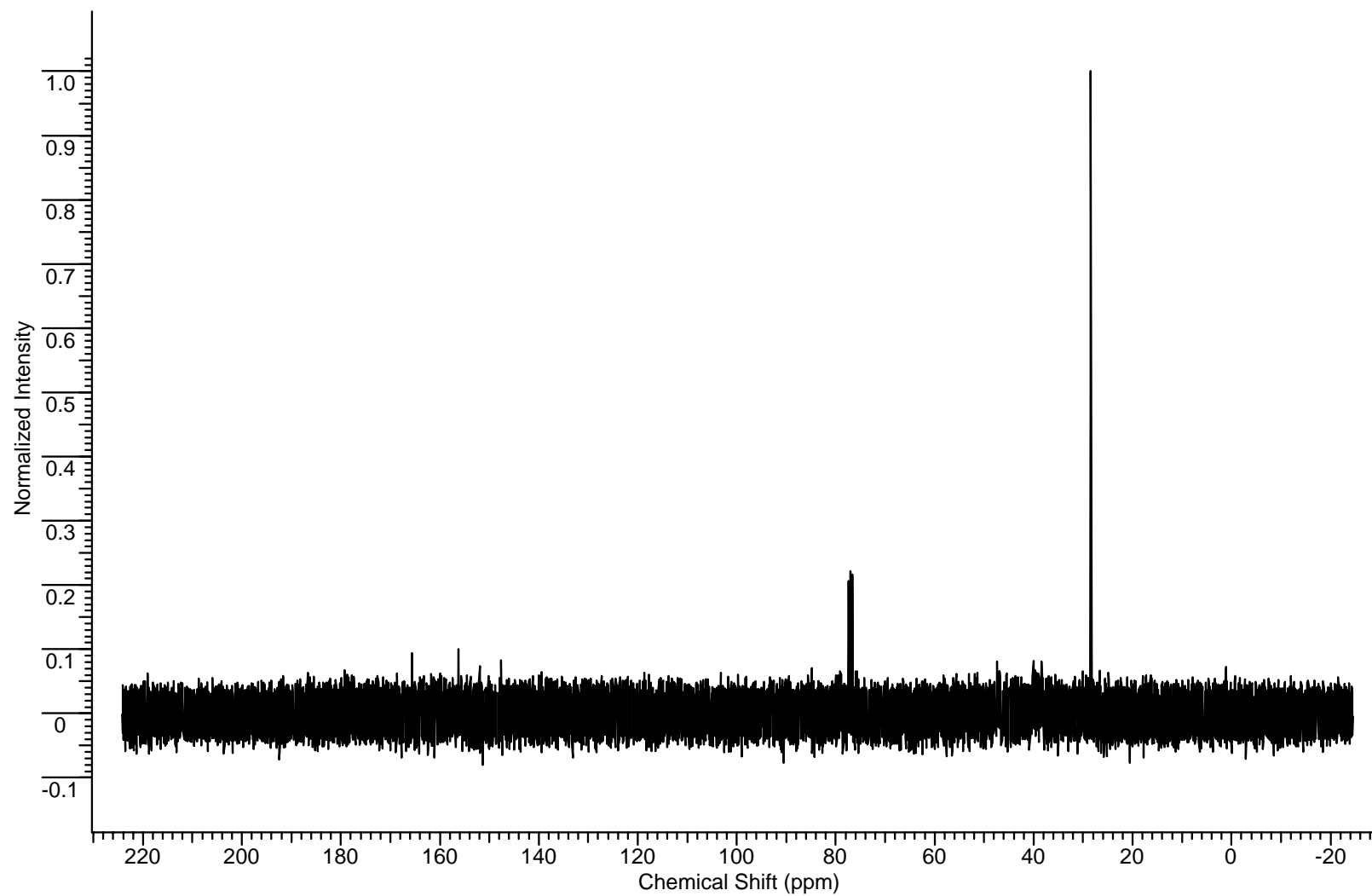
+TOF MS: 136 MCA scans from 01100511.wiff
a=3.55269219334770520e-004, t0=4.53057312806668050e+001



Isosteric Dendrimer Synthesis: Compound 5-N, ^1H NMR

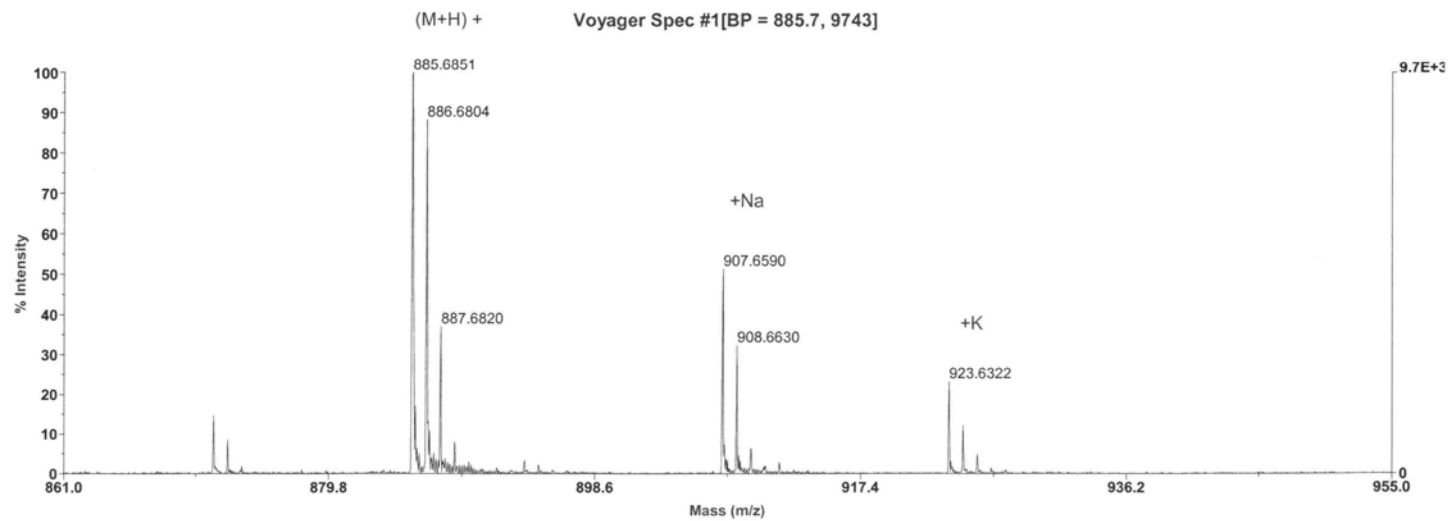
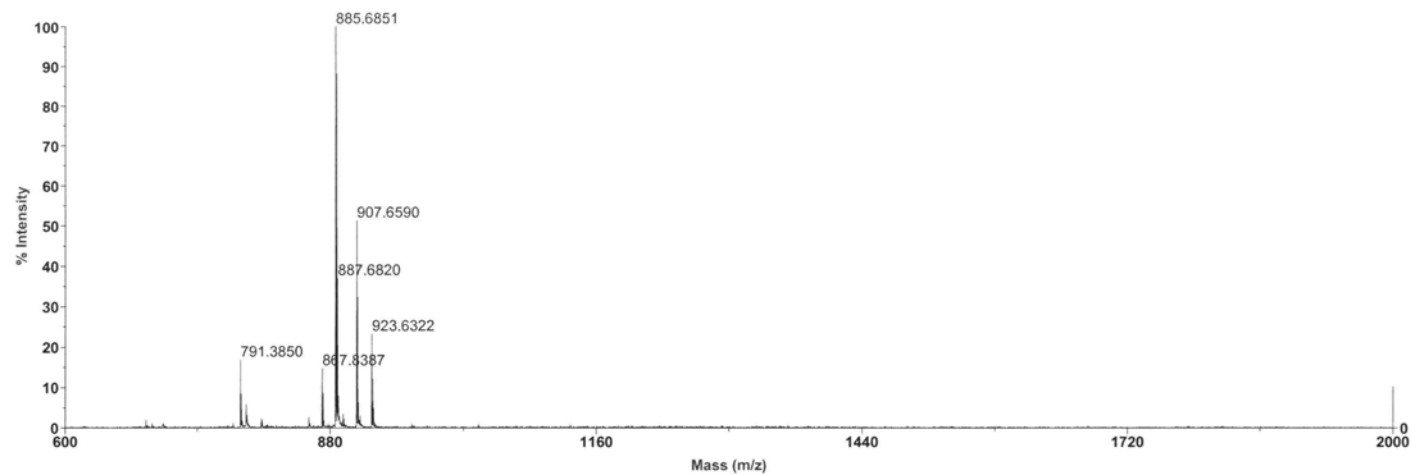


Isosteric Dendrimer Synthesis: Compound **5-N**, ^{13}C NMR

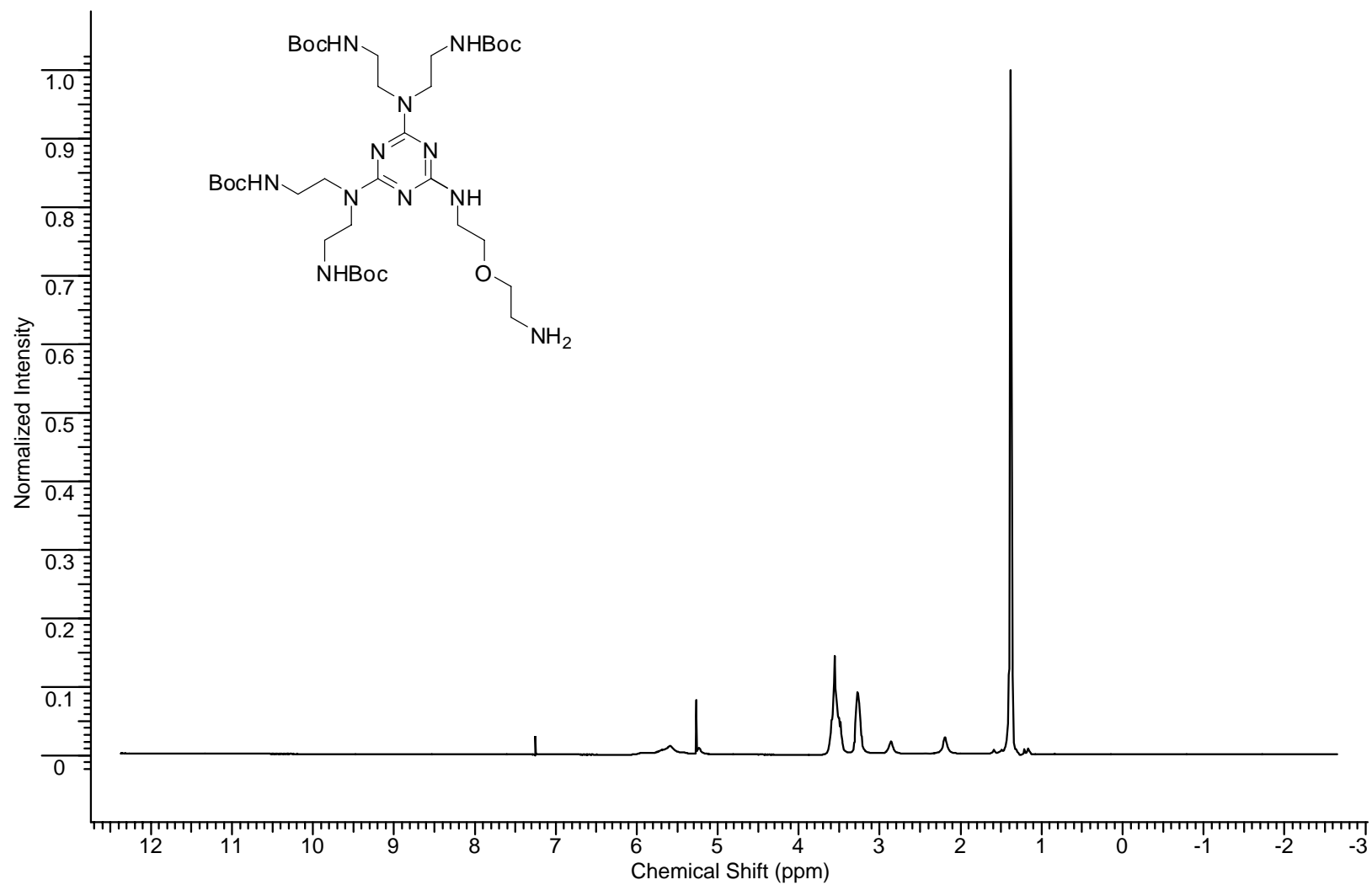


Isosteric Dendrimer Synthesis: Compound 5-N, MALDI-MS

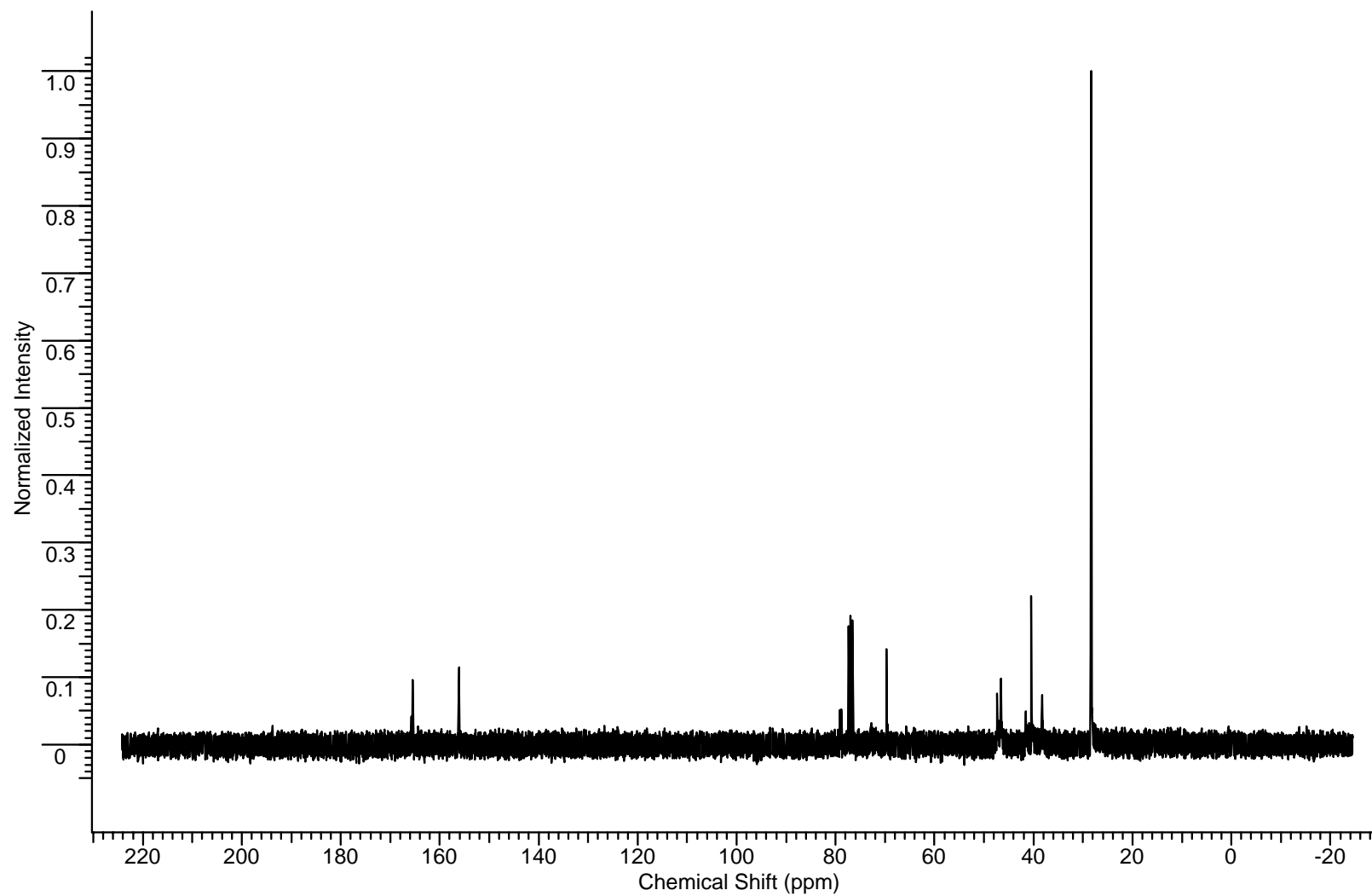
Voyager Spec #1 [BP = 885.7, 9743]



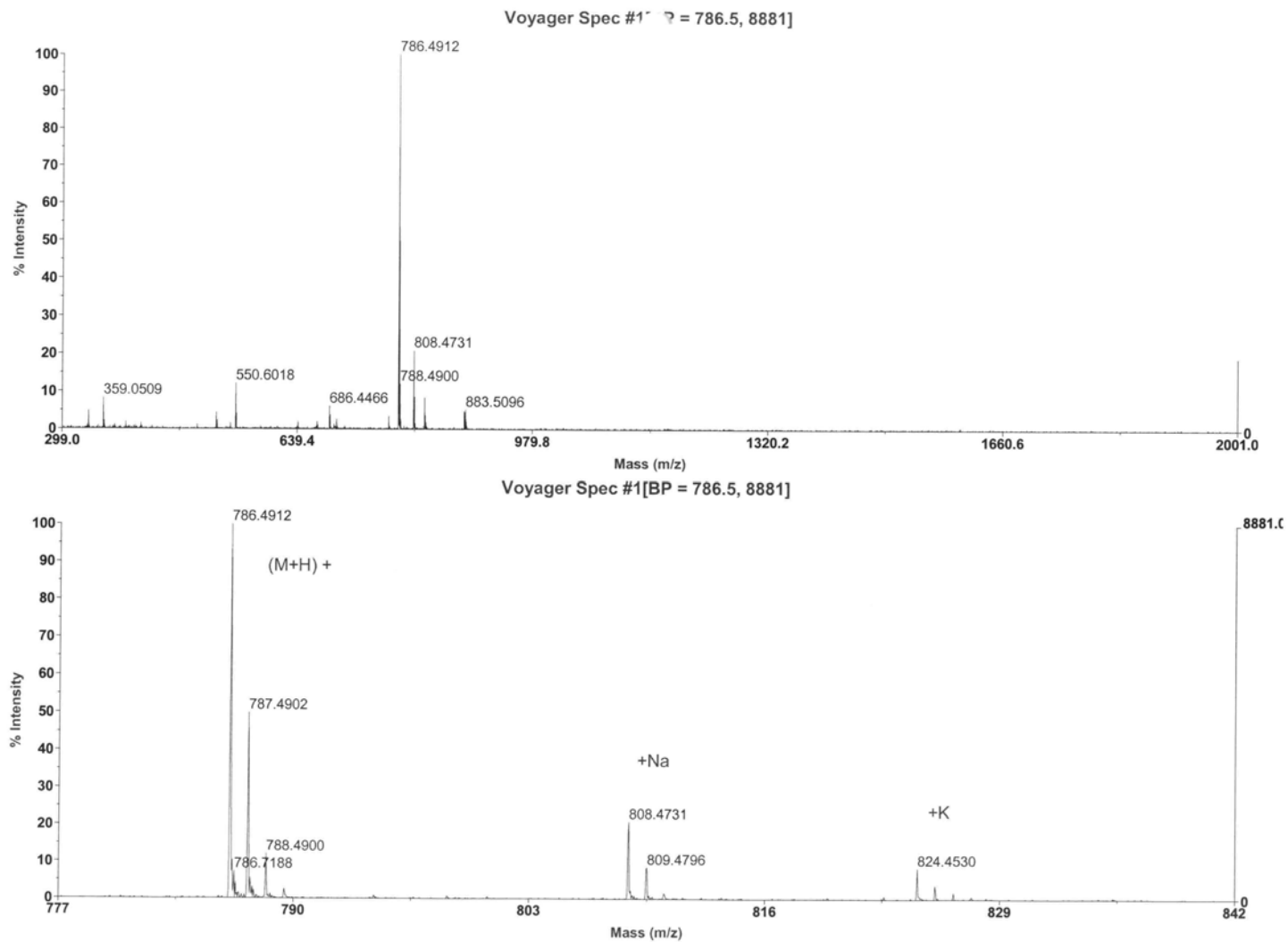
Isosteric Dendrimer Synthesis: Compound **5-O**, ^1H NMR



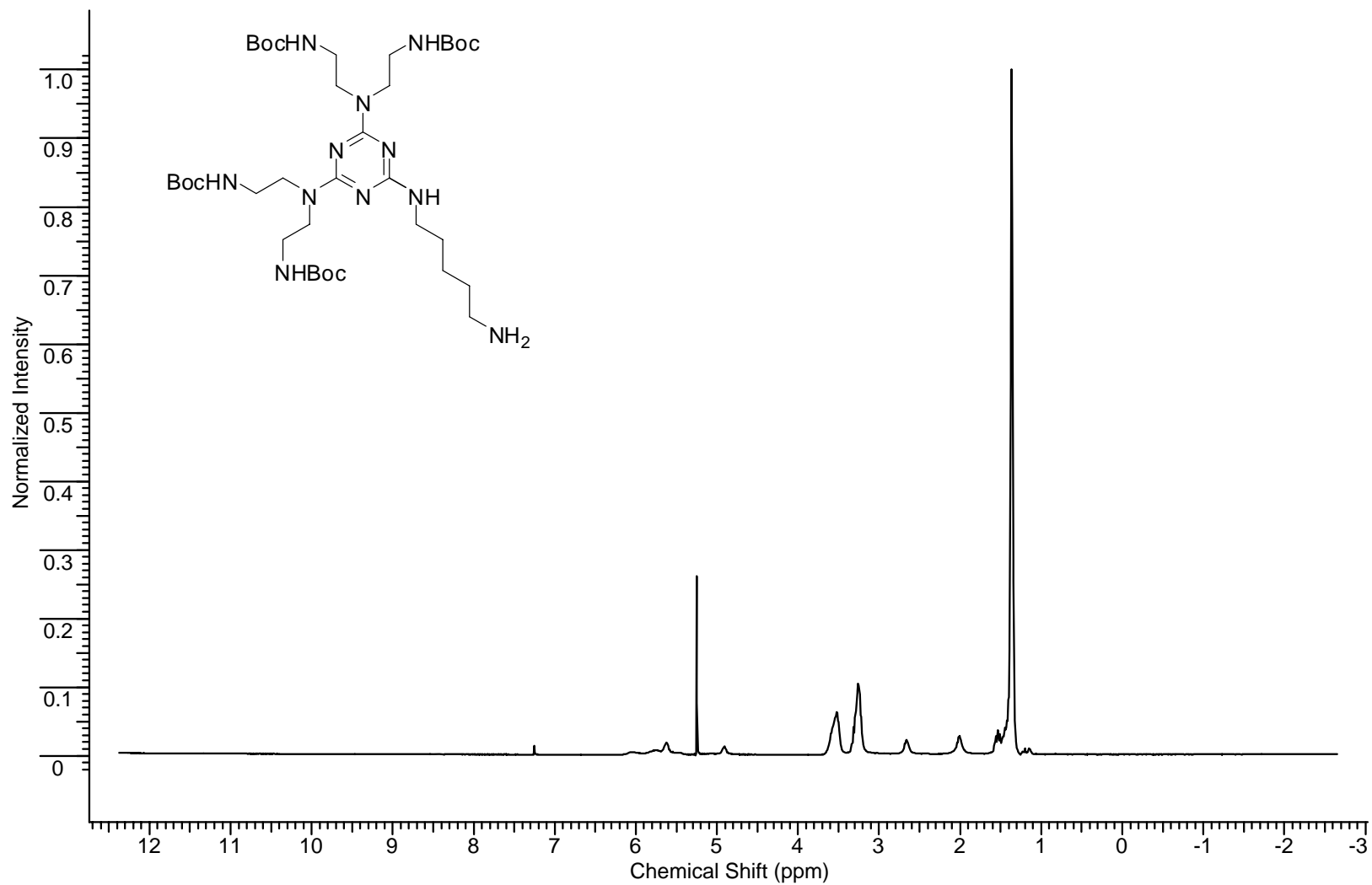
Isosteric Dendrimer Synthesis: Compound **5-O**, ^{13}C NMR



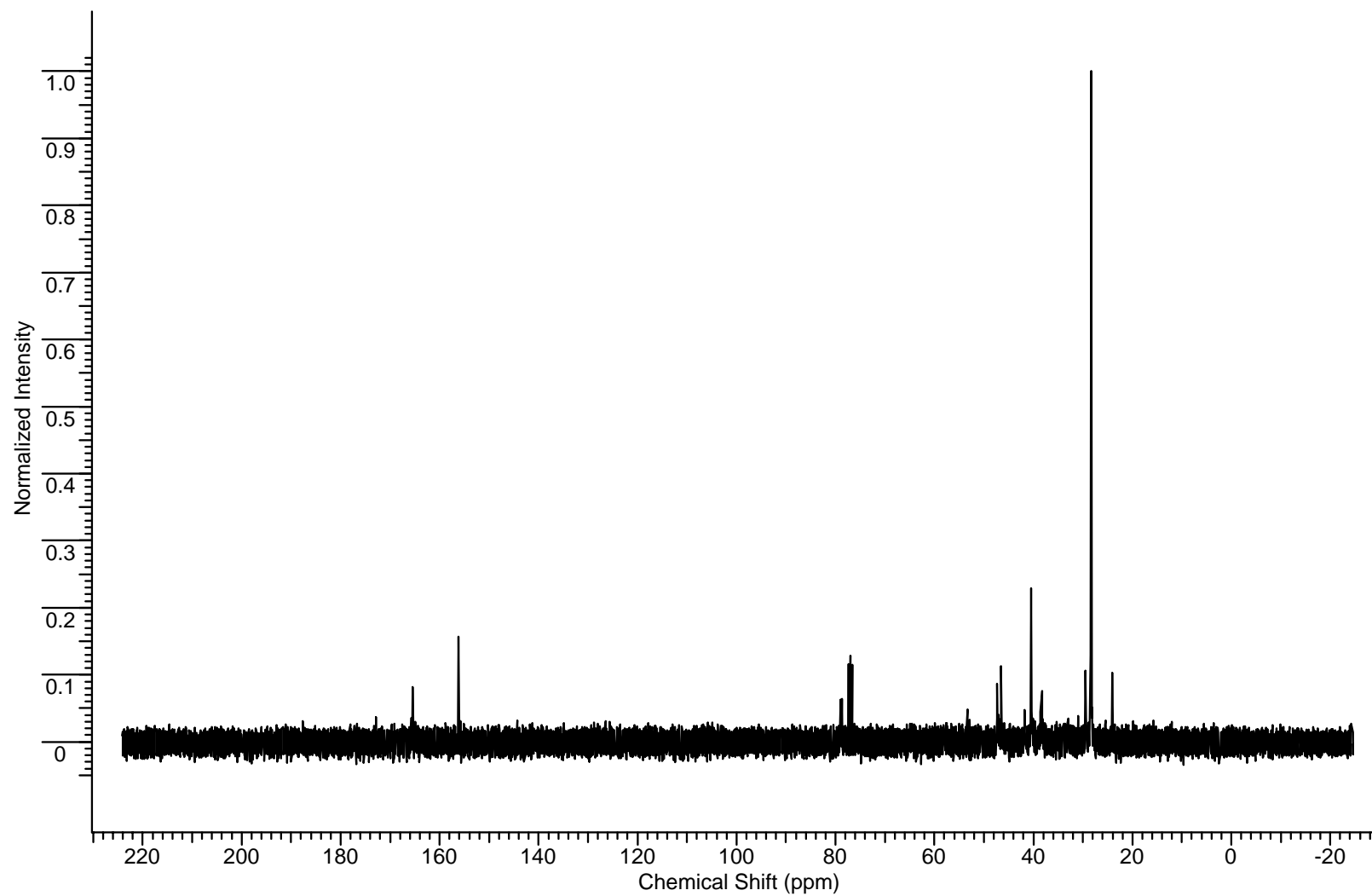
Isosteric Dendrimer Synthesis: Compound 5-O, MALDI-MS



Isosteric Dendrimer Synthesis: Compound 5-C, ^1H NMR

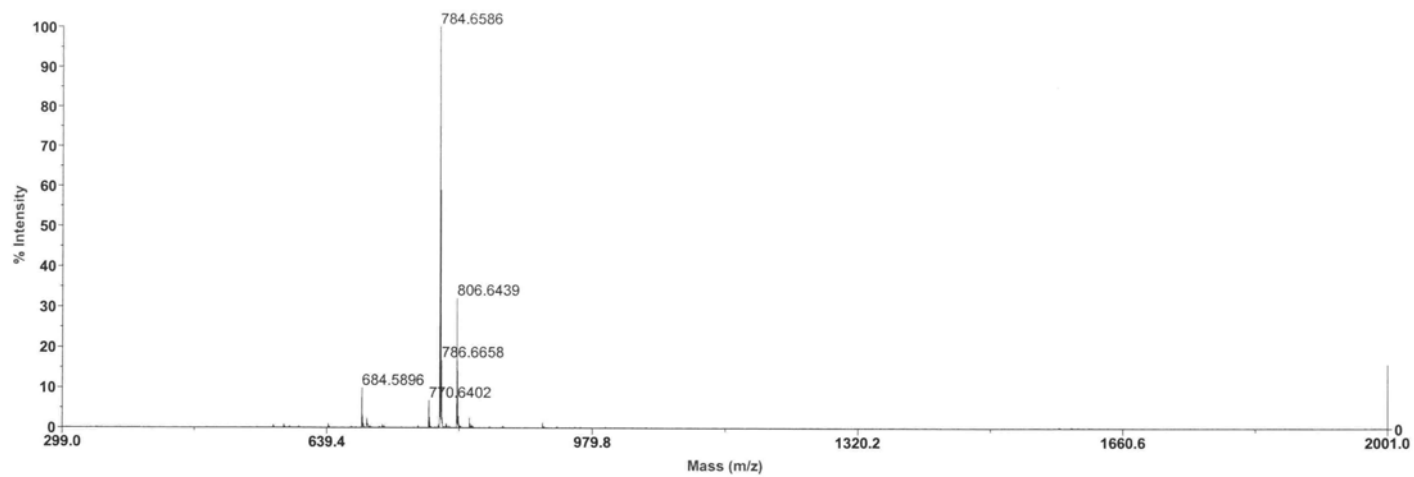


Isosteric Dendrimer Synthesis: Compound **5-C**, ^{13}C NMR

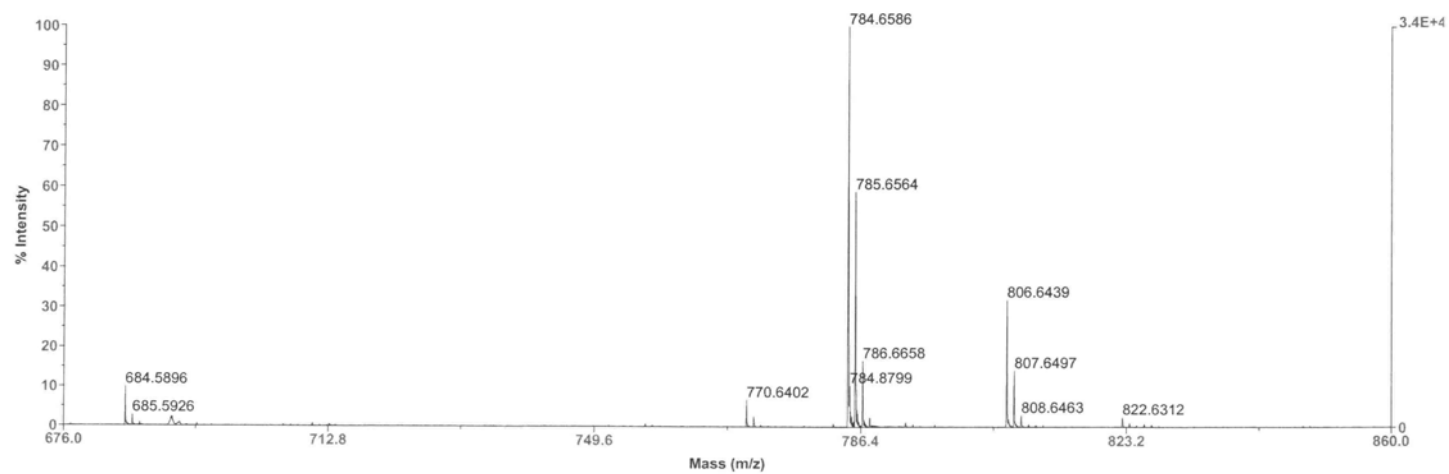


Isosteric Dendrimer Synthesis: Compound 5-C, MALDI-MS

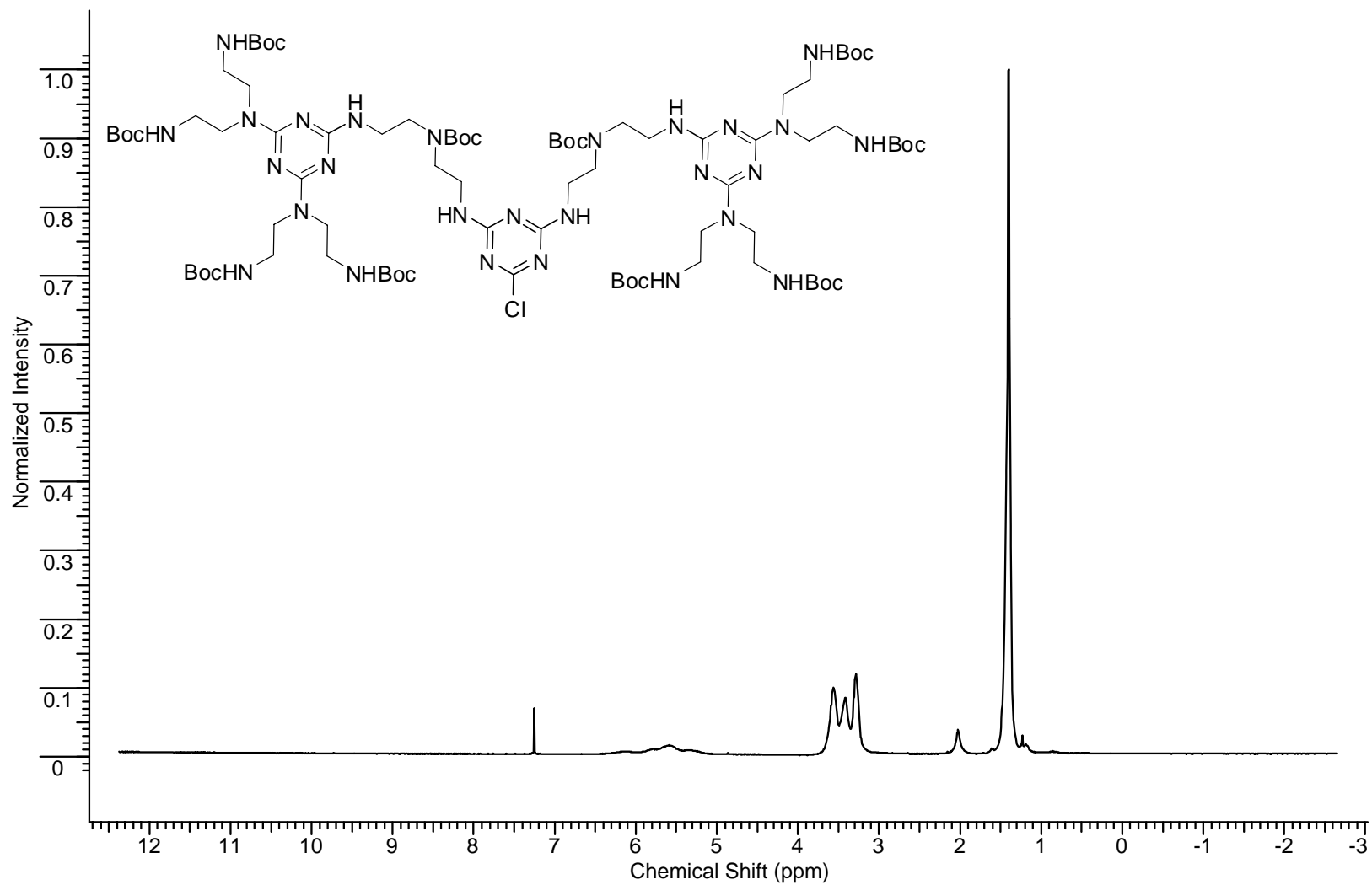
Voyager Spec #1[BP = 784.7, 33971]



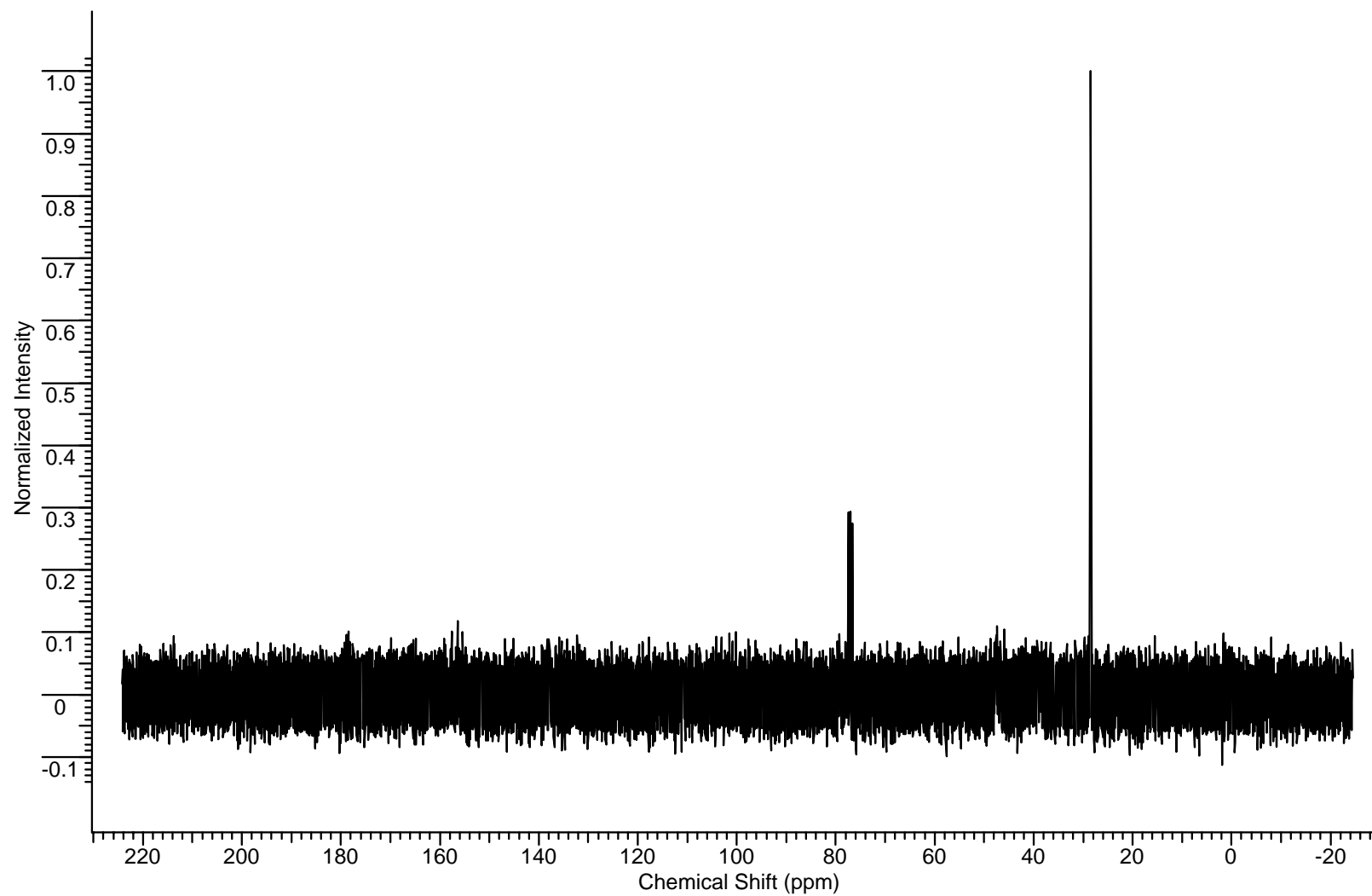
Voyager Spec #1[BP = 784.7, 33971]



Isosteric Dendrimer Synthesis: Compound 6-N, ^1H NMR

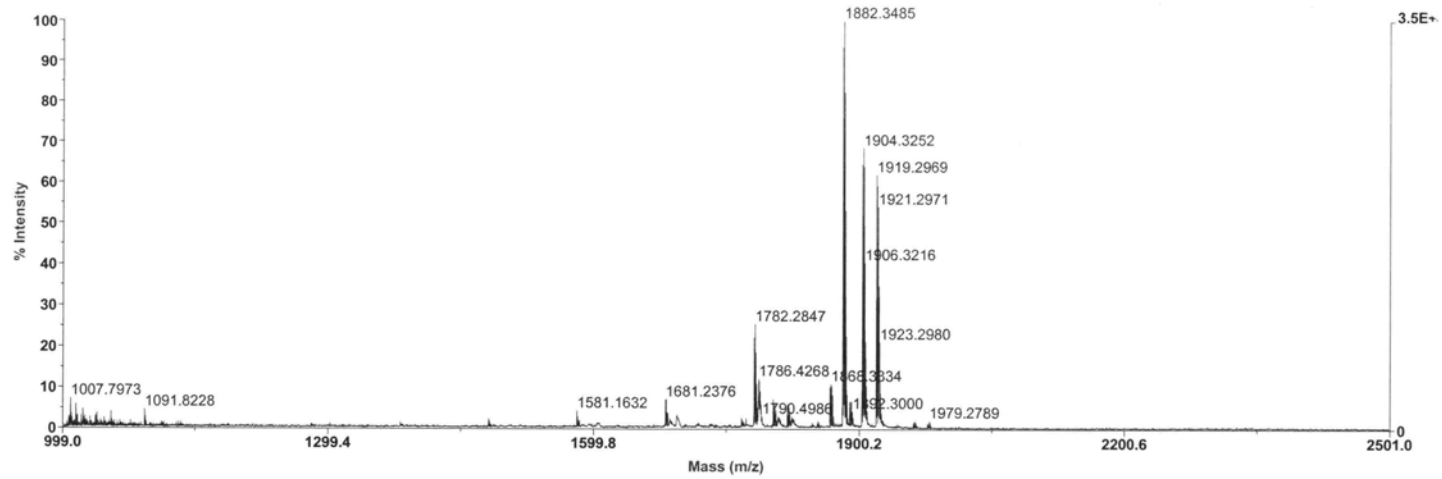


Isosteric Dendrimer Synthesis: Compound **6-N**, ^{13}C NMR

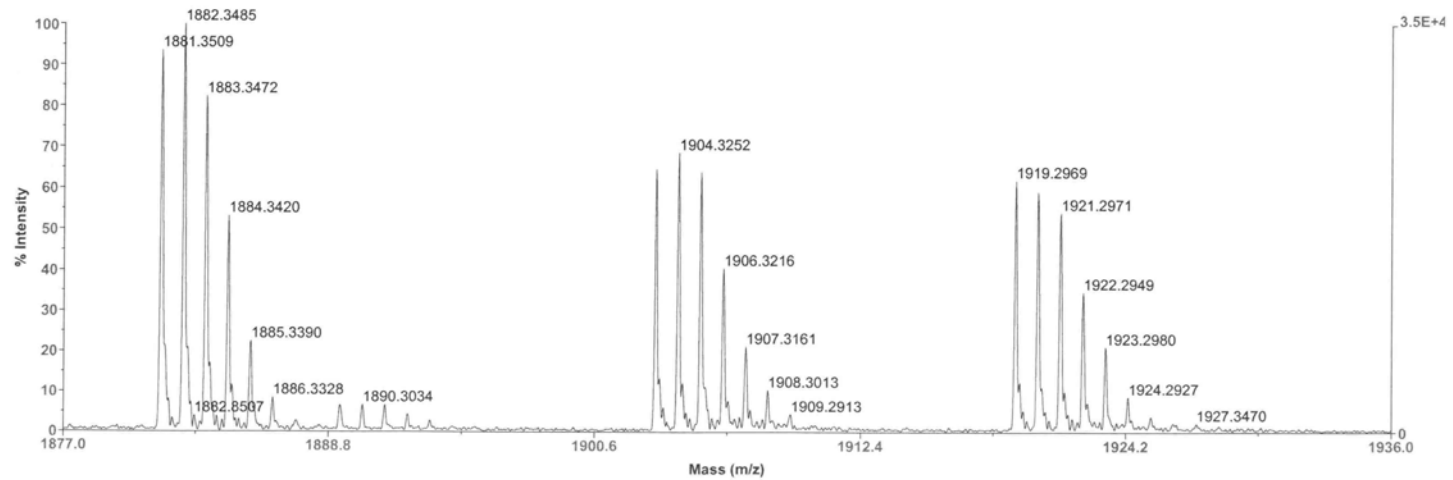


Isosteric Dendrimer Synthesis: Compound 6-N, MALDI-MS

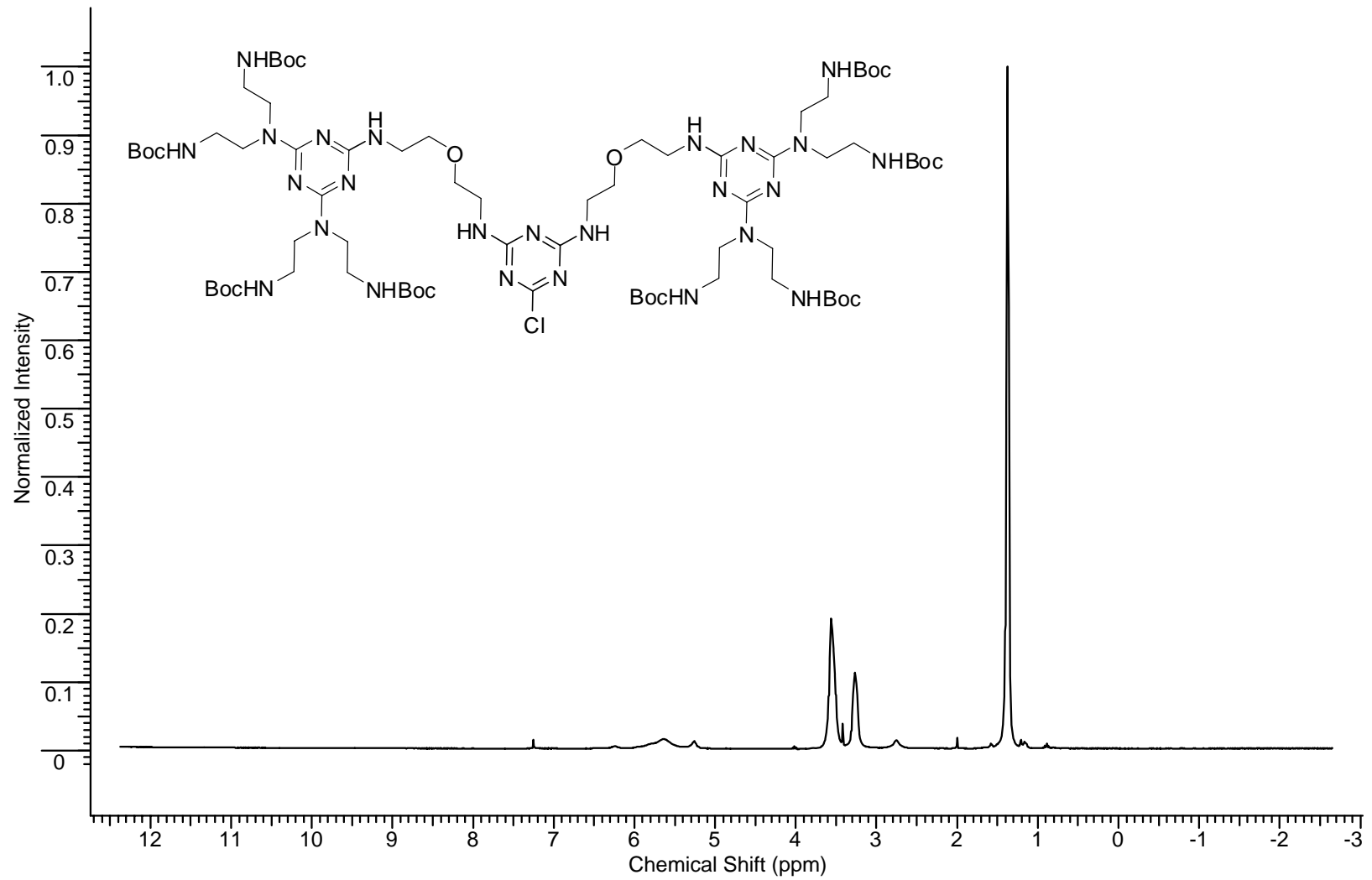
Voyager Spec #1 [BP = 1882.4, 35077]



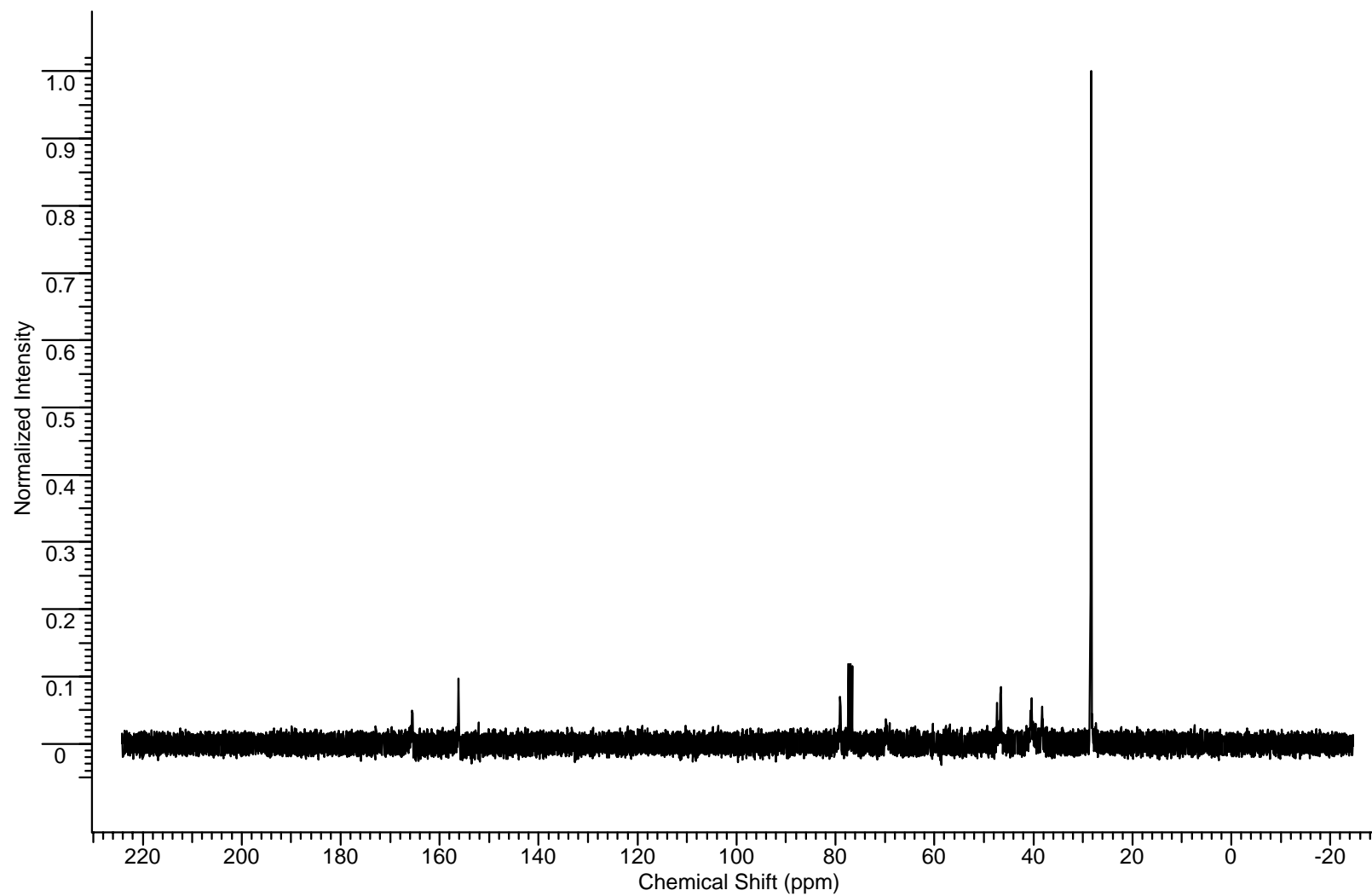
Voyager Spec #1 [BP = 1882.4, 35077]



Isosteric Dendrimer Synthesis: Compound **6-O**, ¹H NMR

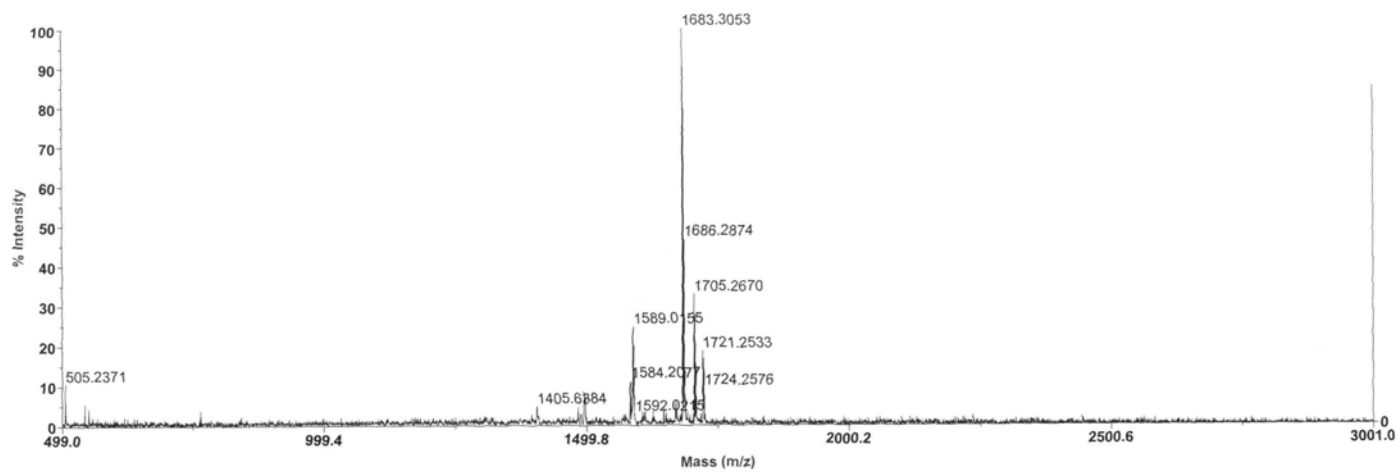


Isosteric Dendrimer Synthesis: Compound **6-O**, ^{13}C NMR

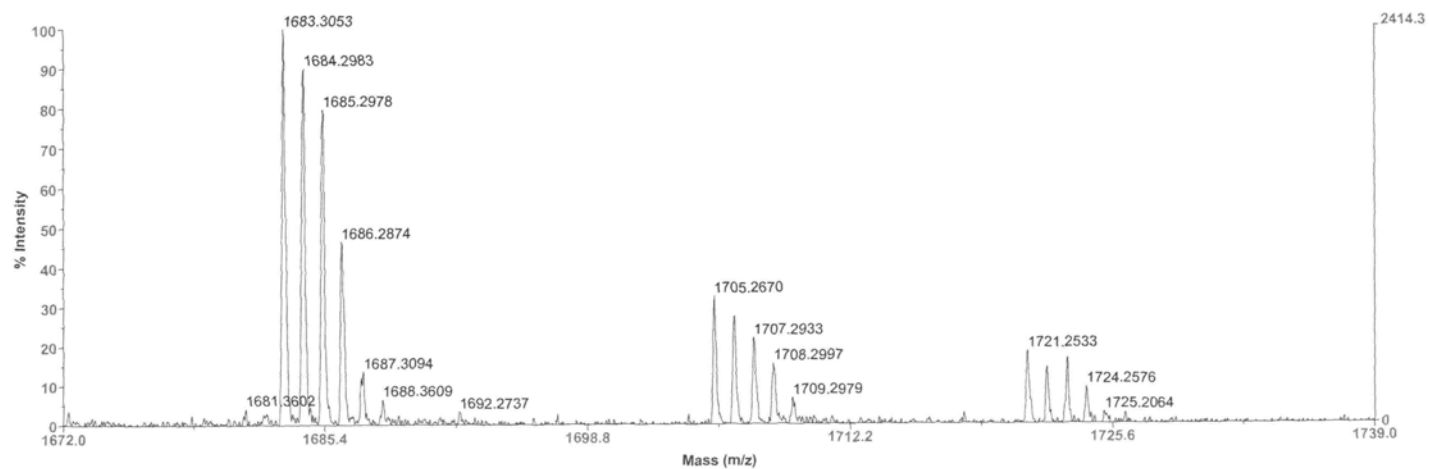


Isosteric Dendrimer Synthesis: Compound 6-O, MALDI-MS

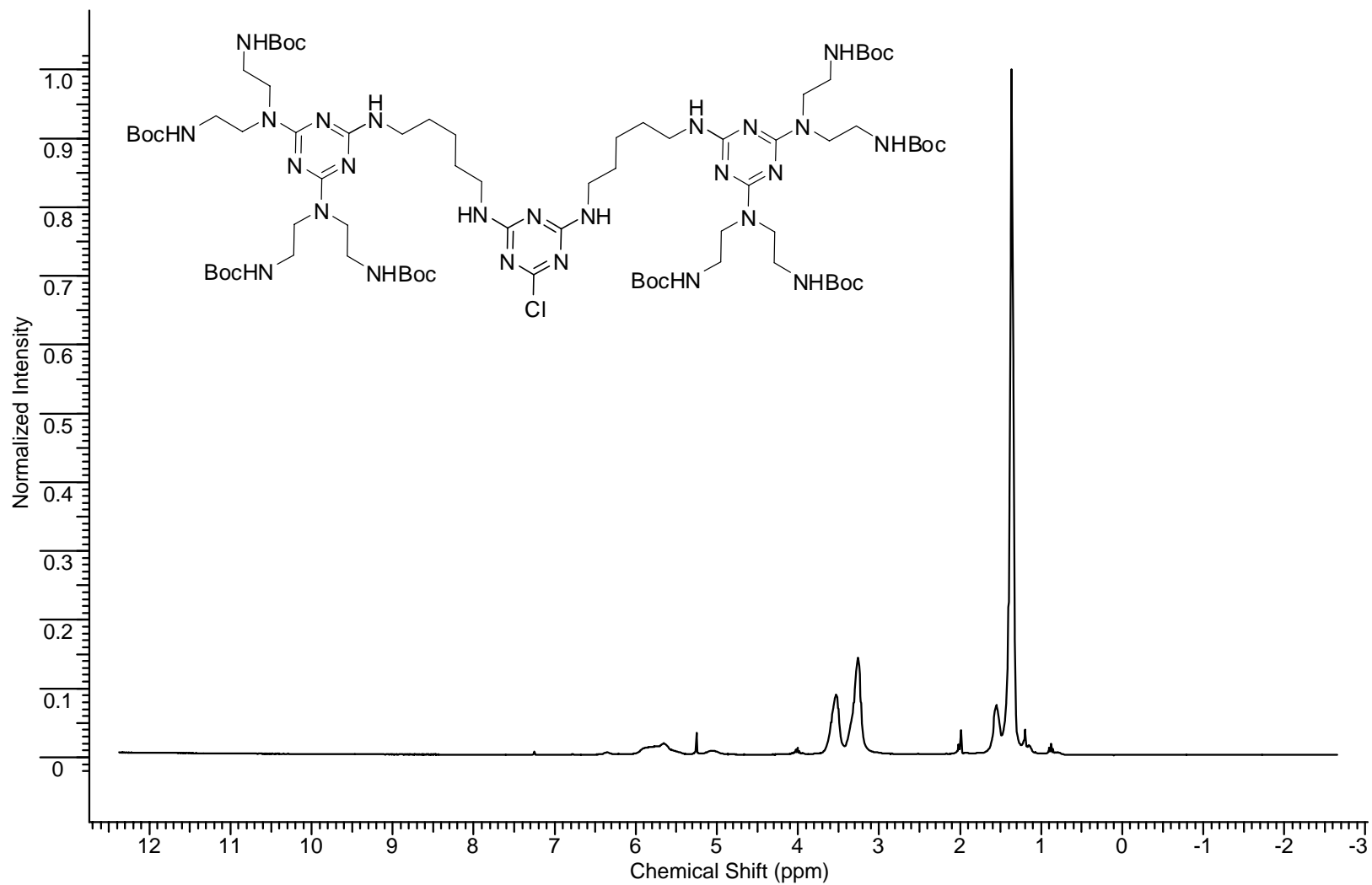
Voyager Spec #1=>AdvBC(2², 5, 0, 1)[BP = 1683.3, 2414]



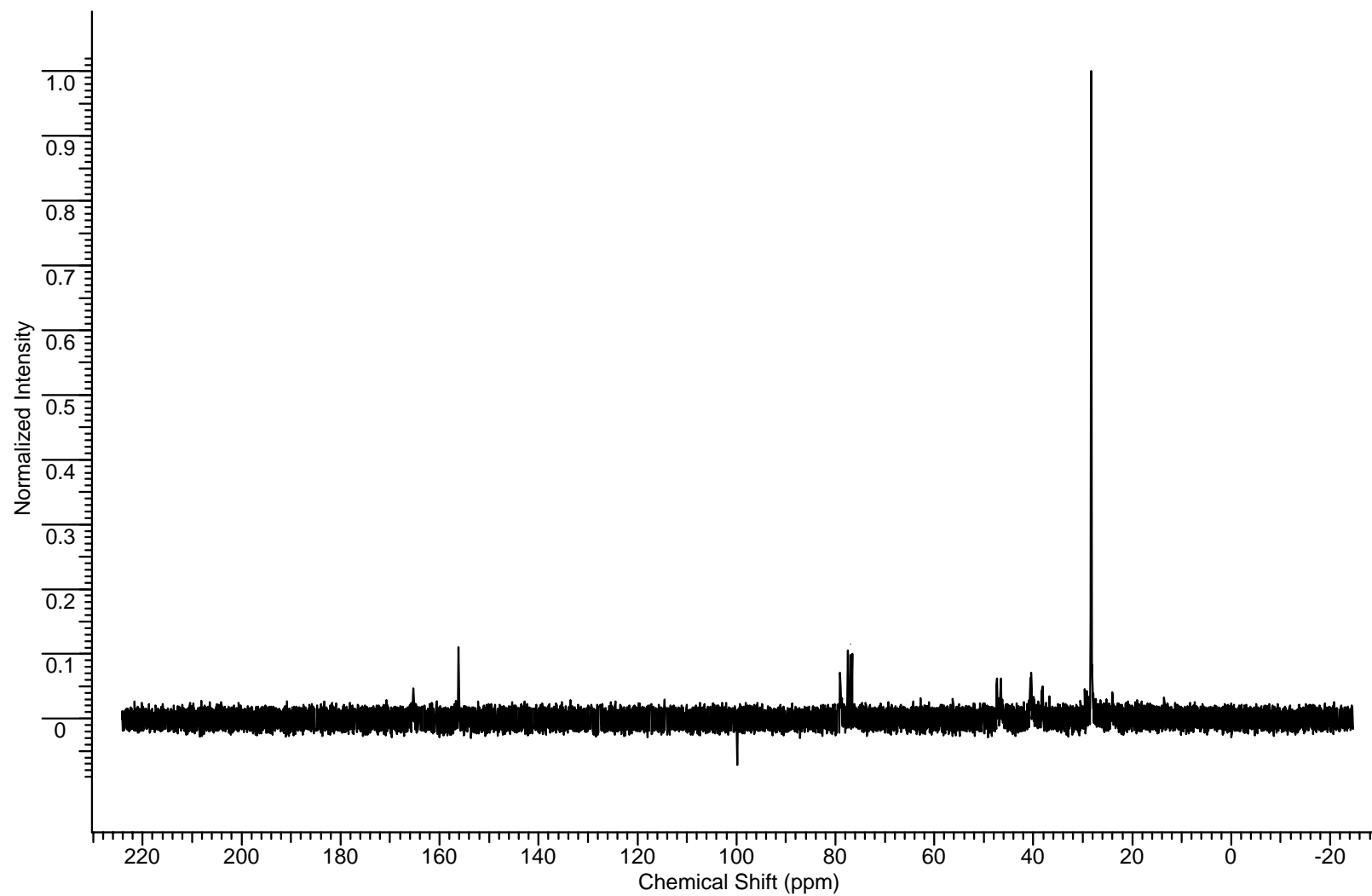
Voyager Spec #1=>AdvBC(32, 0, 5, 0, 1)[BP = 1683.3, 2414]



Isosteric Dendrimer Synthesis: Compound 6-C, ^1H NMR

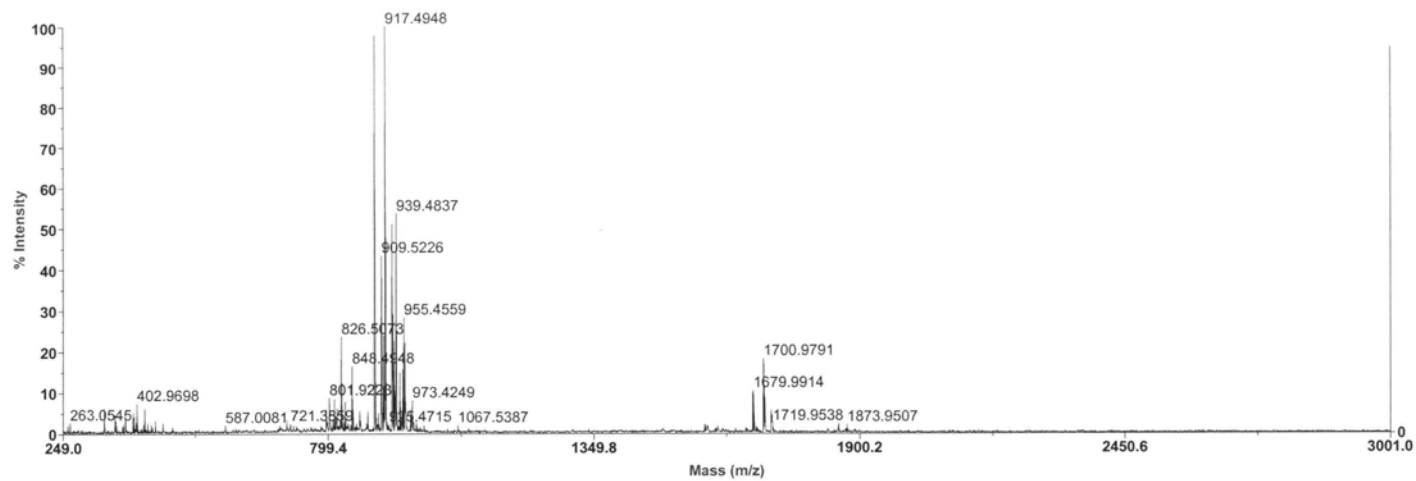


Isosteric Dendrimer Synthesis: Compound **6-C**, ^{13}C NMR

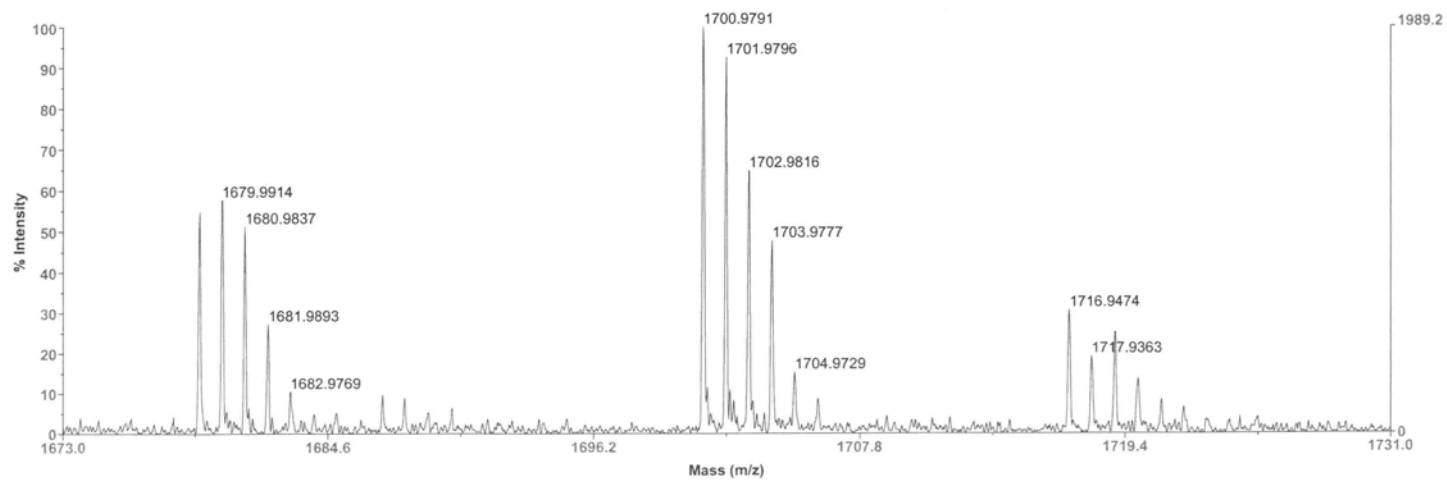


Isosteric Dendrimer Synthesis: Compound 6-C, MALDI-MS

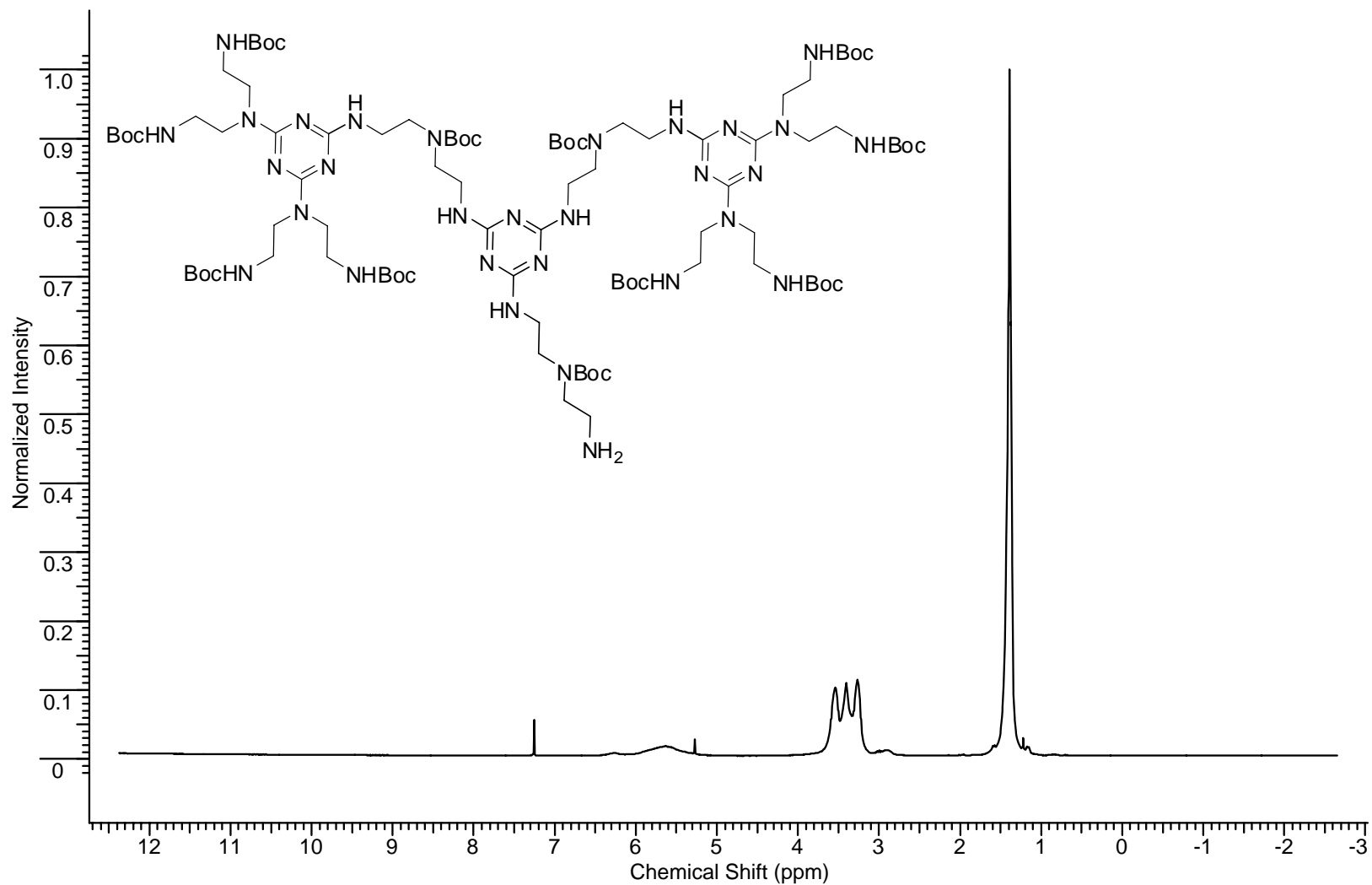
Voyager Spec #1 [BP = 917.5, 10867]



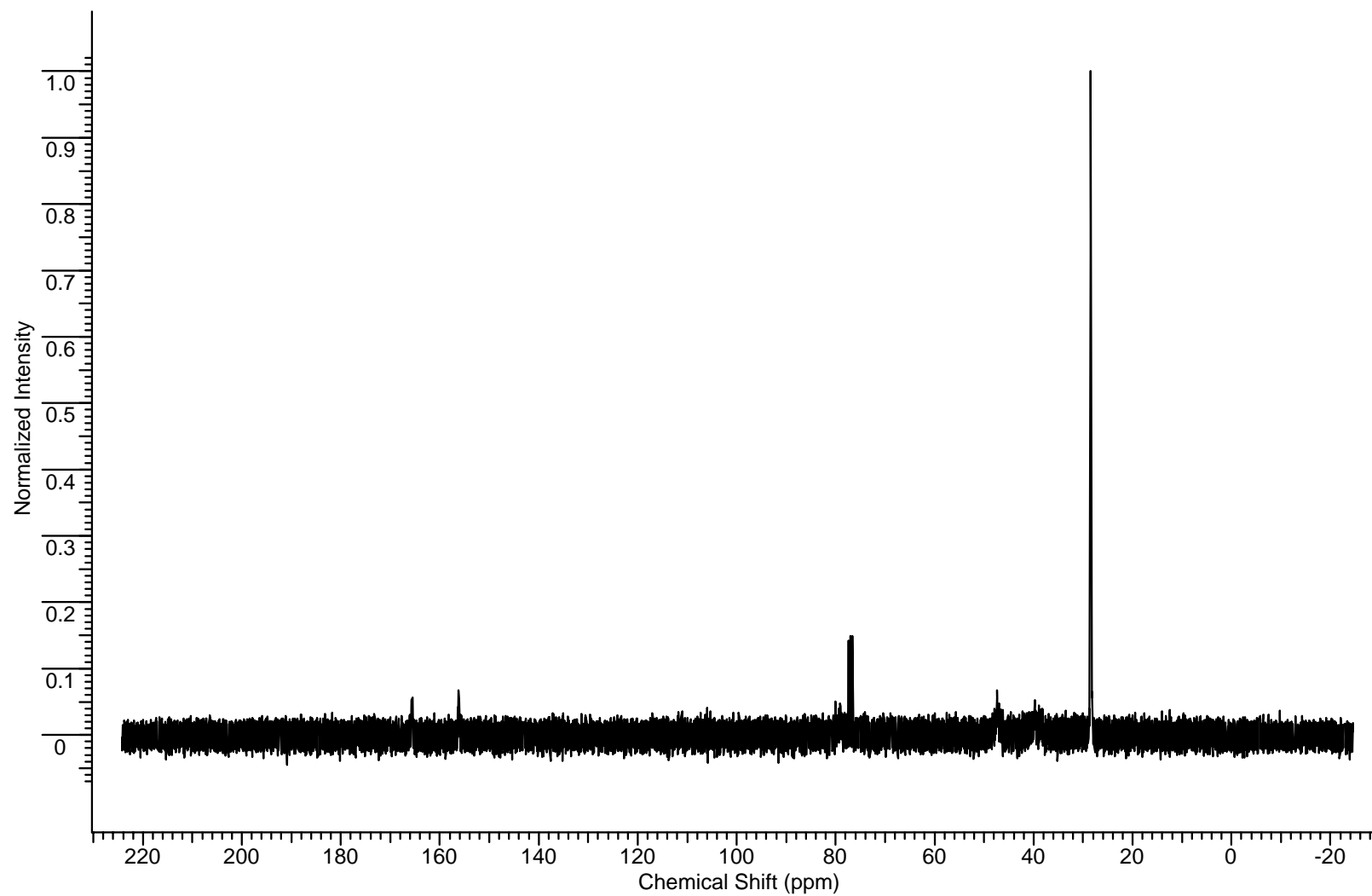
Voyager Spec #1 [BP = 917.5, 10867]



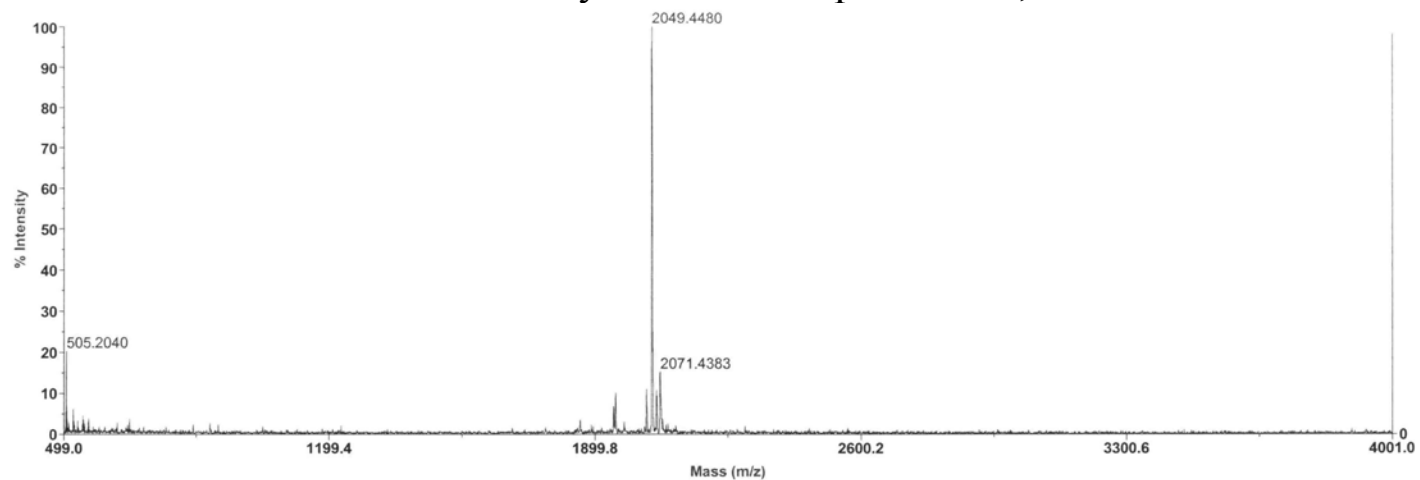
Isosteric Dendrimer Synthesis: Compound 7-N, ^1H NMR



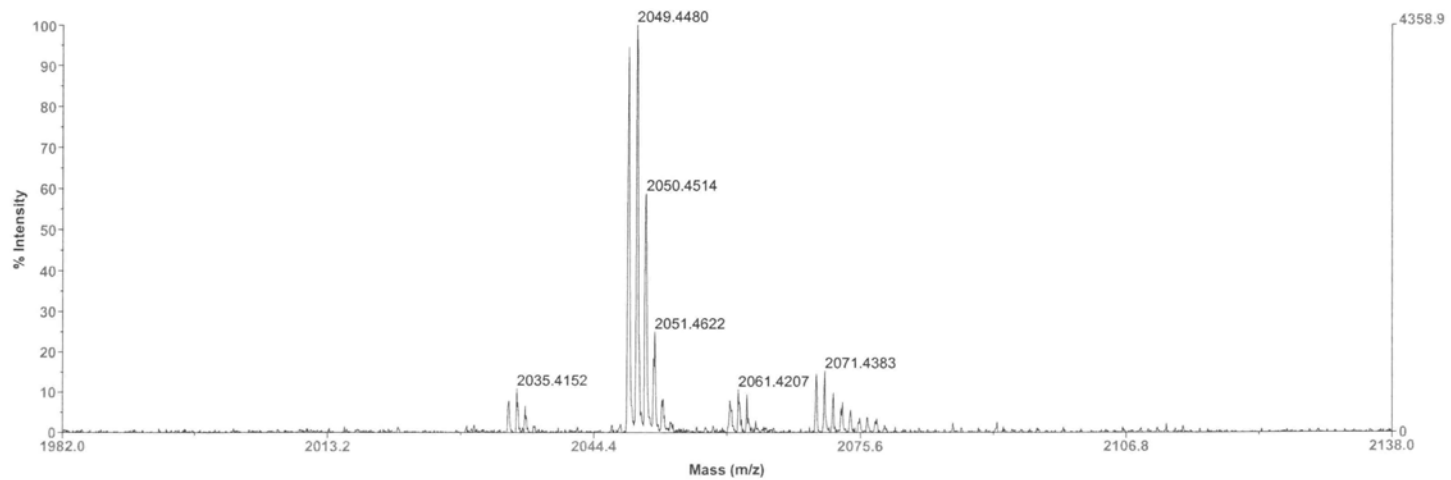
Isosteric Dendrimer Synthesis: Compound 7-N, ^{13}C NMR



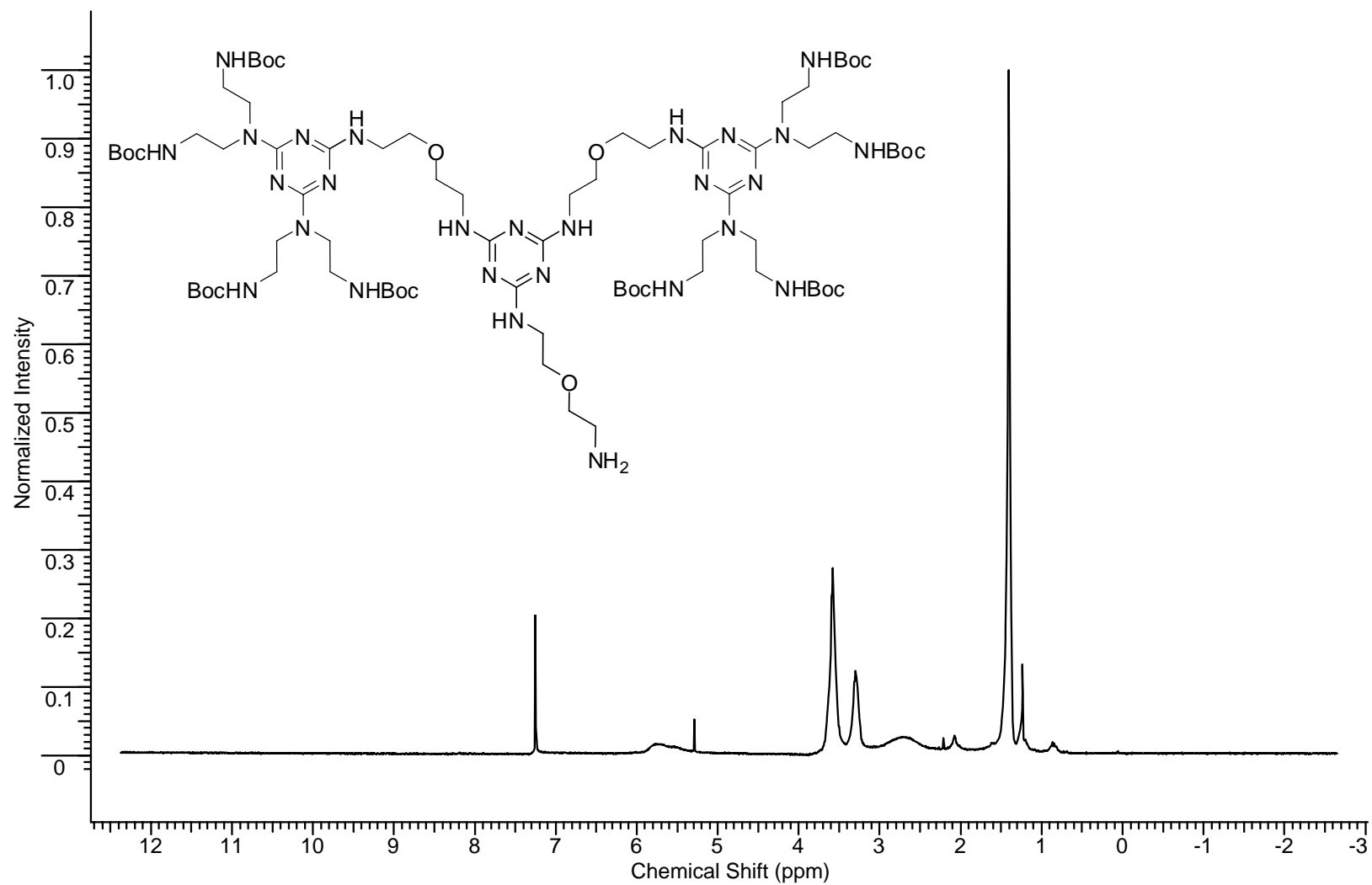
Isosteric Dendrimer Synthesis: Compound 7-N, MALDI-MS



Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 2049.4, 4359]



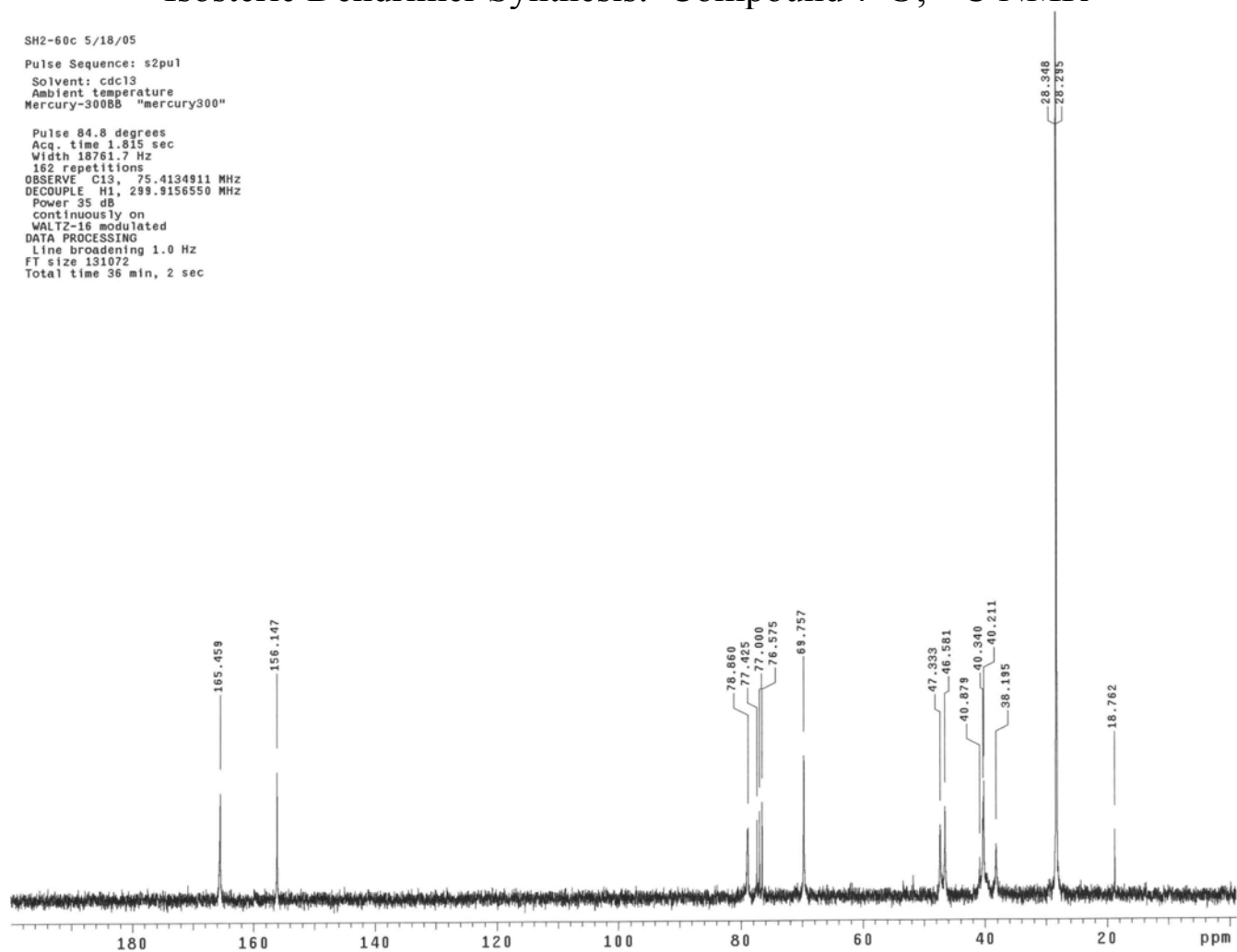
Isosteric Dendrimer Synthesis: Compound 7-O, ^1H NMR



Isosteric Dendrimer Synthesis: Compound 7-O, ¹³C NMR

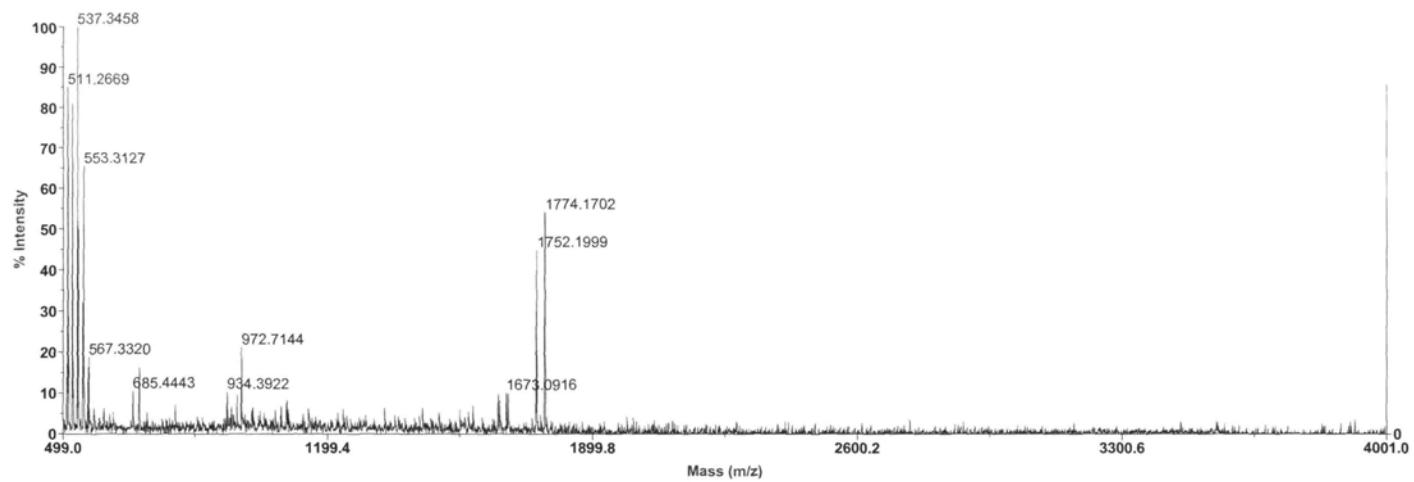
SH2-60c 5/18/05
Pulse Sequence: s2pu1
Solvent: cdc13
Ambient temperature
Mercury-300BB "mercury300"

Pulse 84.8 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
162 repetitions
OBSERVE C13, 75.4134911 MHz
DECOUPLE H1, 299.9156550 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 36 min, 2 sec

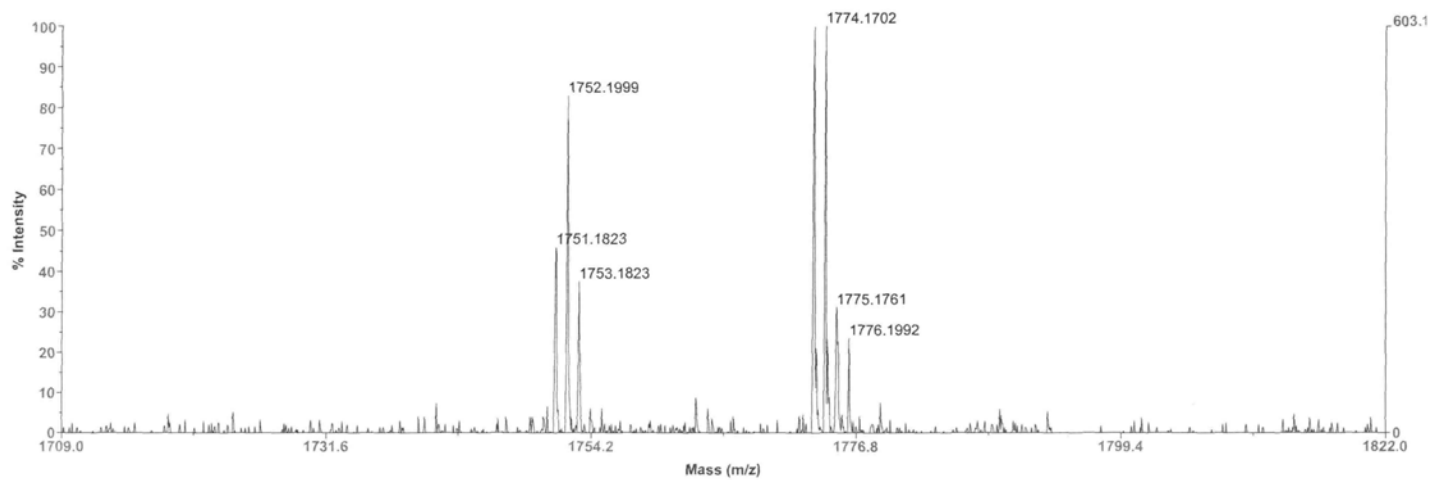


Isosteric Dendrimer Synthesis: Compound 7-O, MALDI-MS

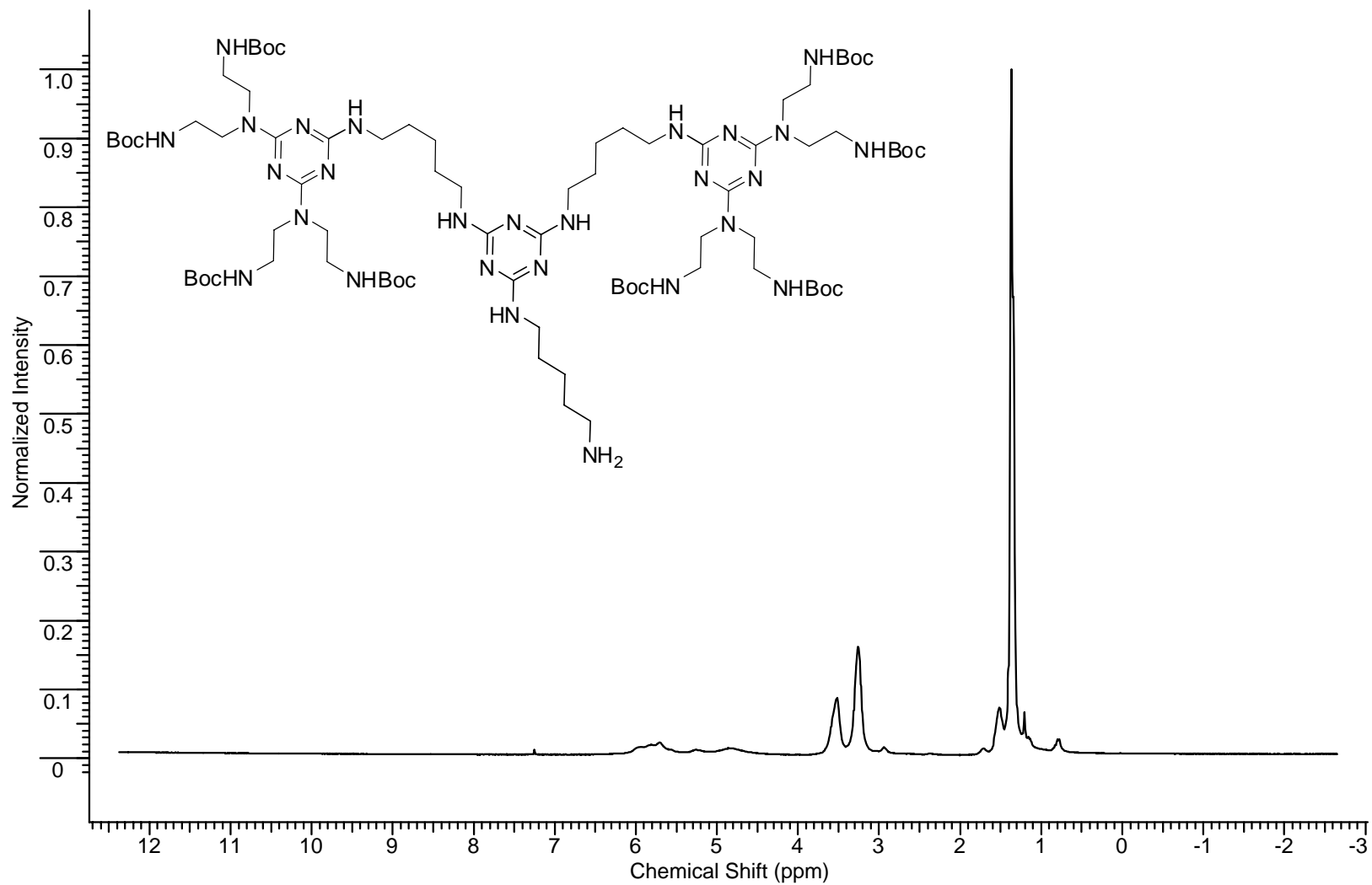
Voyager Spec #1=>AdvBC(22,0.5,0.1)[BP = 537.3, 1116]



Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 537.3, 1116]



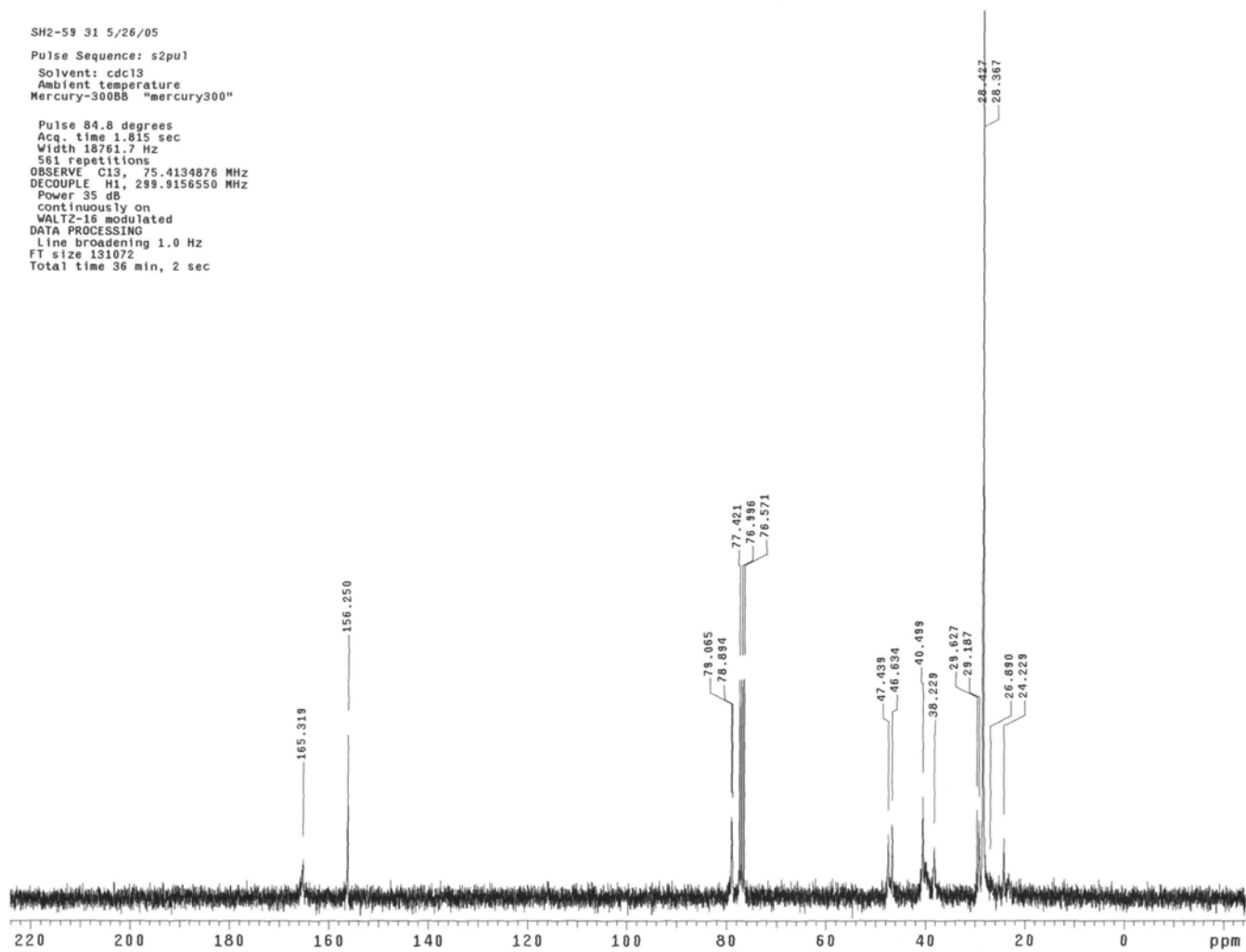
Isosteric Dendrimer Synthesis: Compound 7-C, ^1H NMR



Isosteric Dendrimer Synthesis: Compound 7-C, ^{13}C NMR

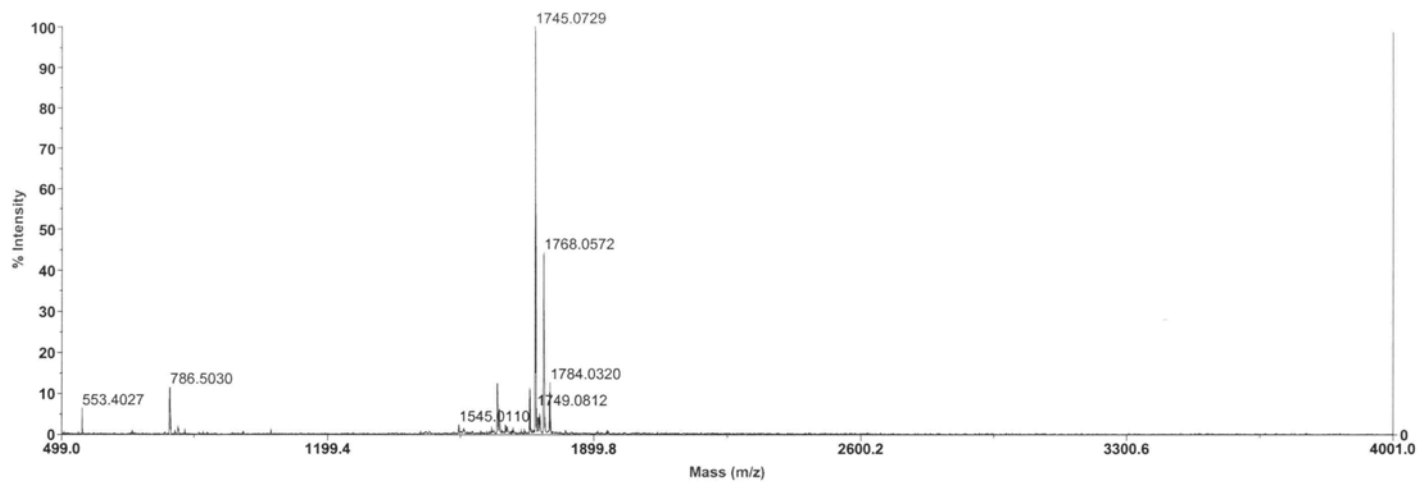
SH2-59 31 5/26/05
Pulse Sequence: s2pu1
Solvent: cdcl3
Ambient temperature
Mercury-300BB "mercury300"

Pulse 84.8 degrees
Acq. time 1.615 sec
Width 18761.7 Hz
561 repetitions
OBSERVE C13, 75.4134876 MHz
DECOUPLE H1, 299.9156550 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 36 min, 2 sec

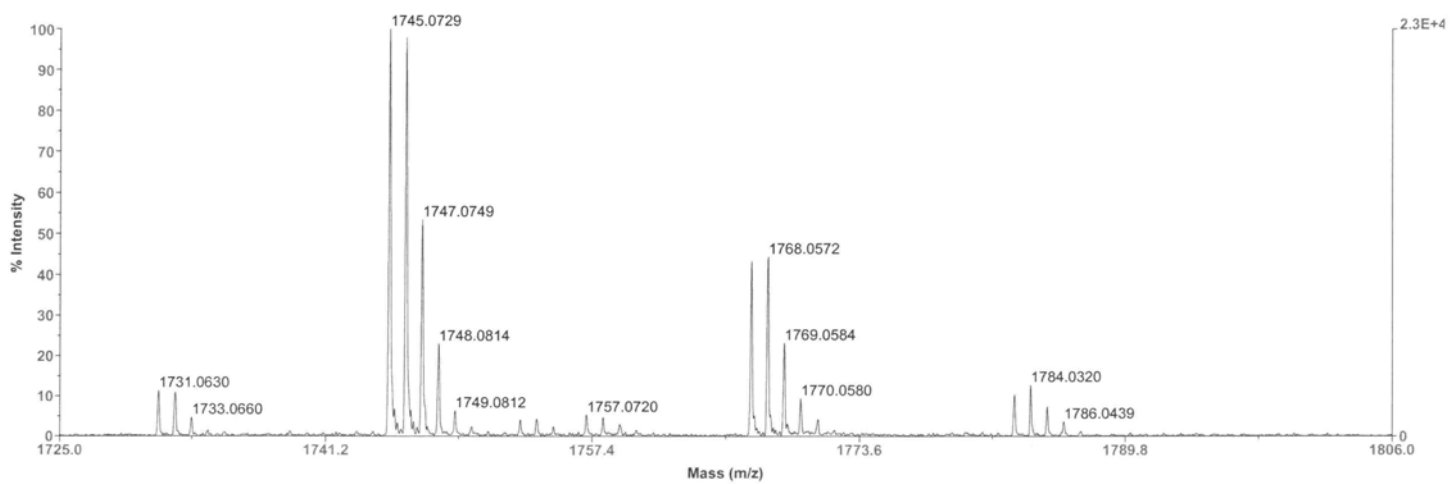


Isosteric Dendrimer Synthesis: Compound 7-C, MALDI-MS

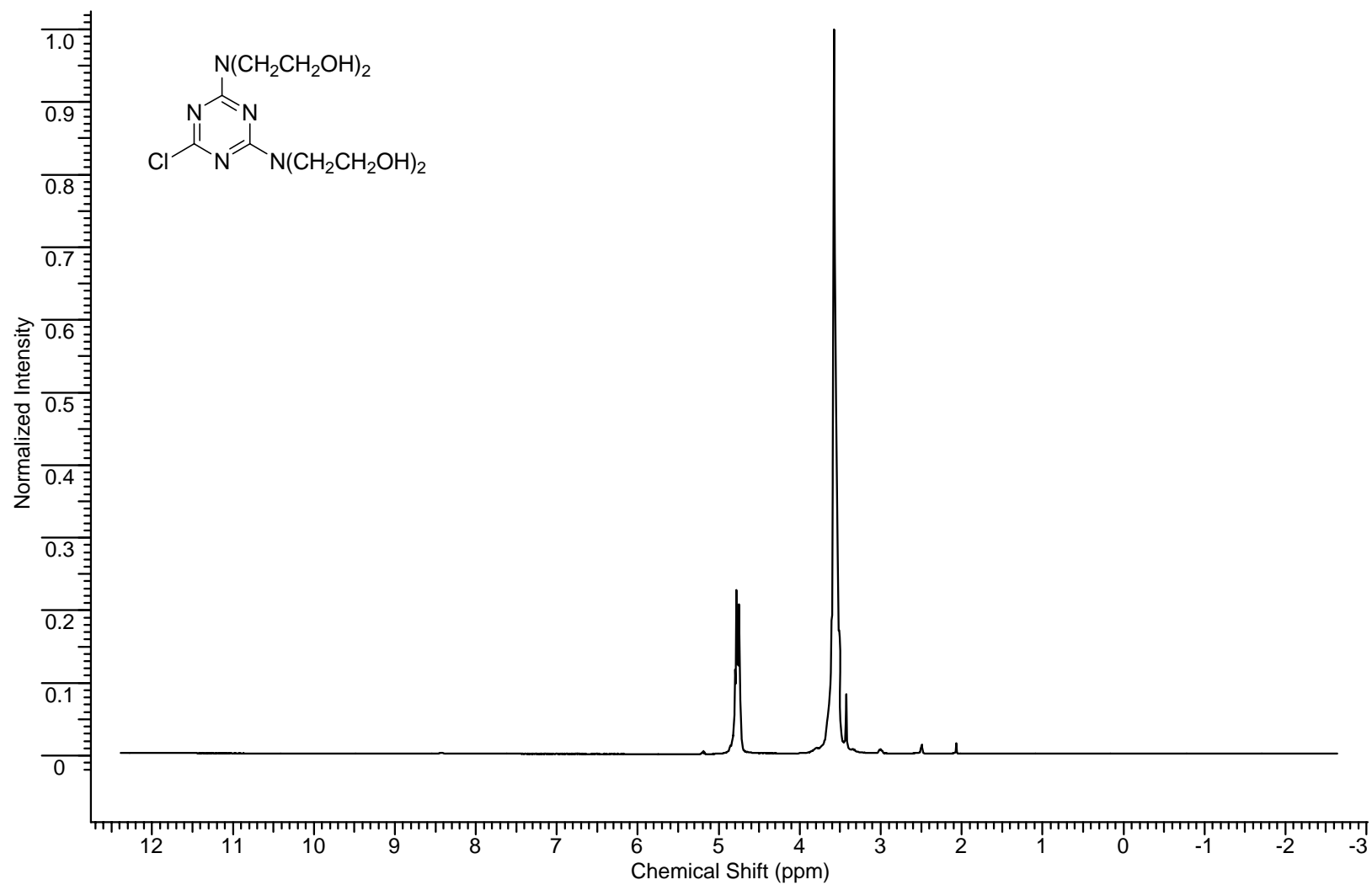
Voyager Spec #1[F⁻ = 1745.1, 22633]



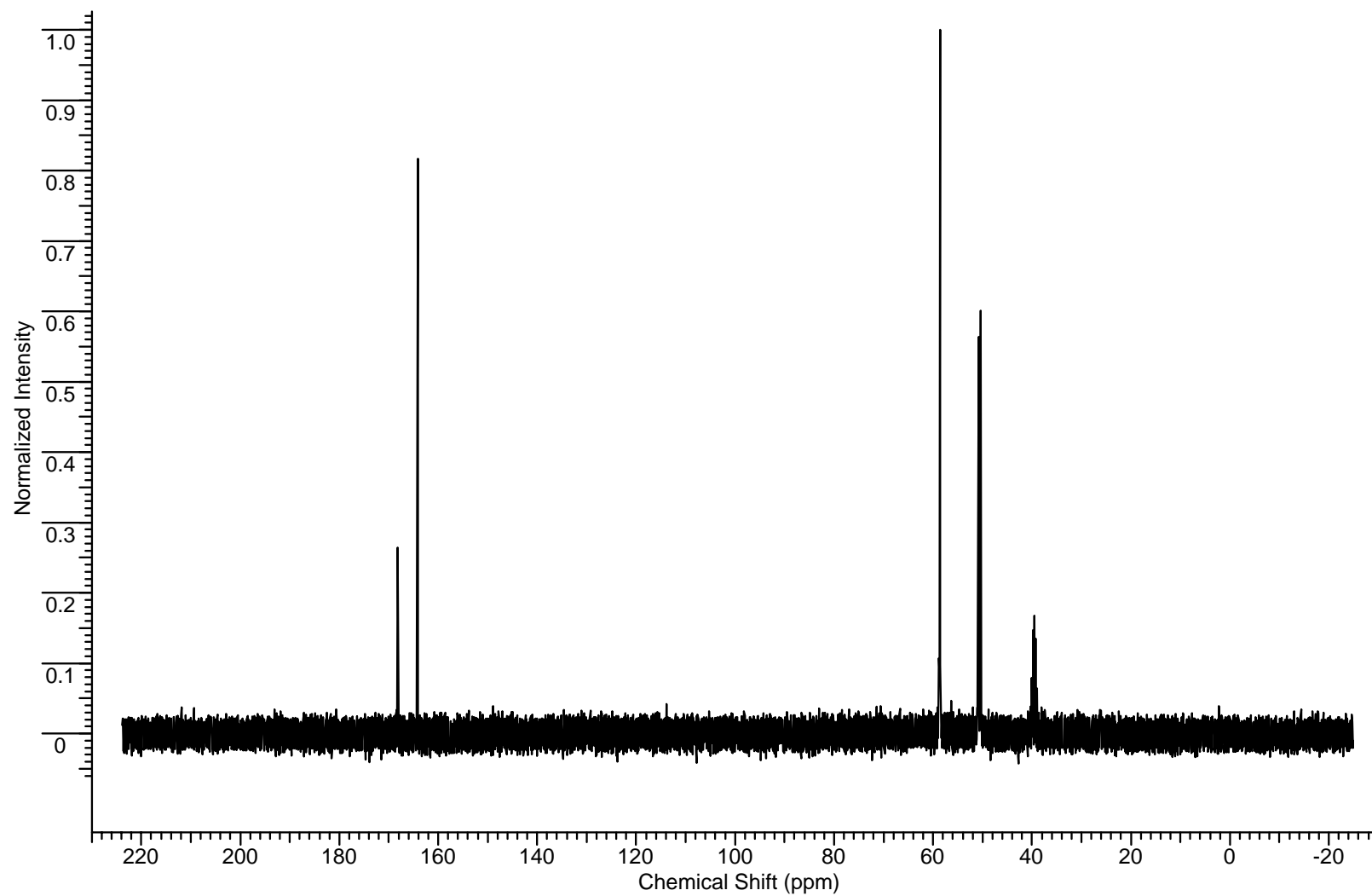
Voyager Spec #1[BP = 1745.1, 22633]



pH Responsive Dendrimer: Compound **8**, ^1H NMR



pH Responsive Dendrimer: Compound **8**, ^{13}C NMR



pH Responsive Dendrimer: Compound **8**, APCI-MS

C:\SERVICE L...2005\07-05\07150503c

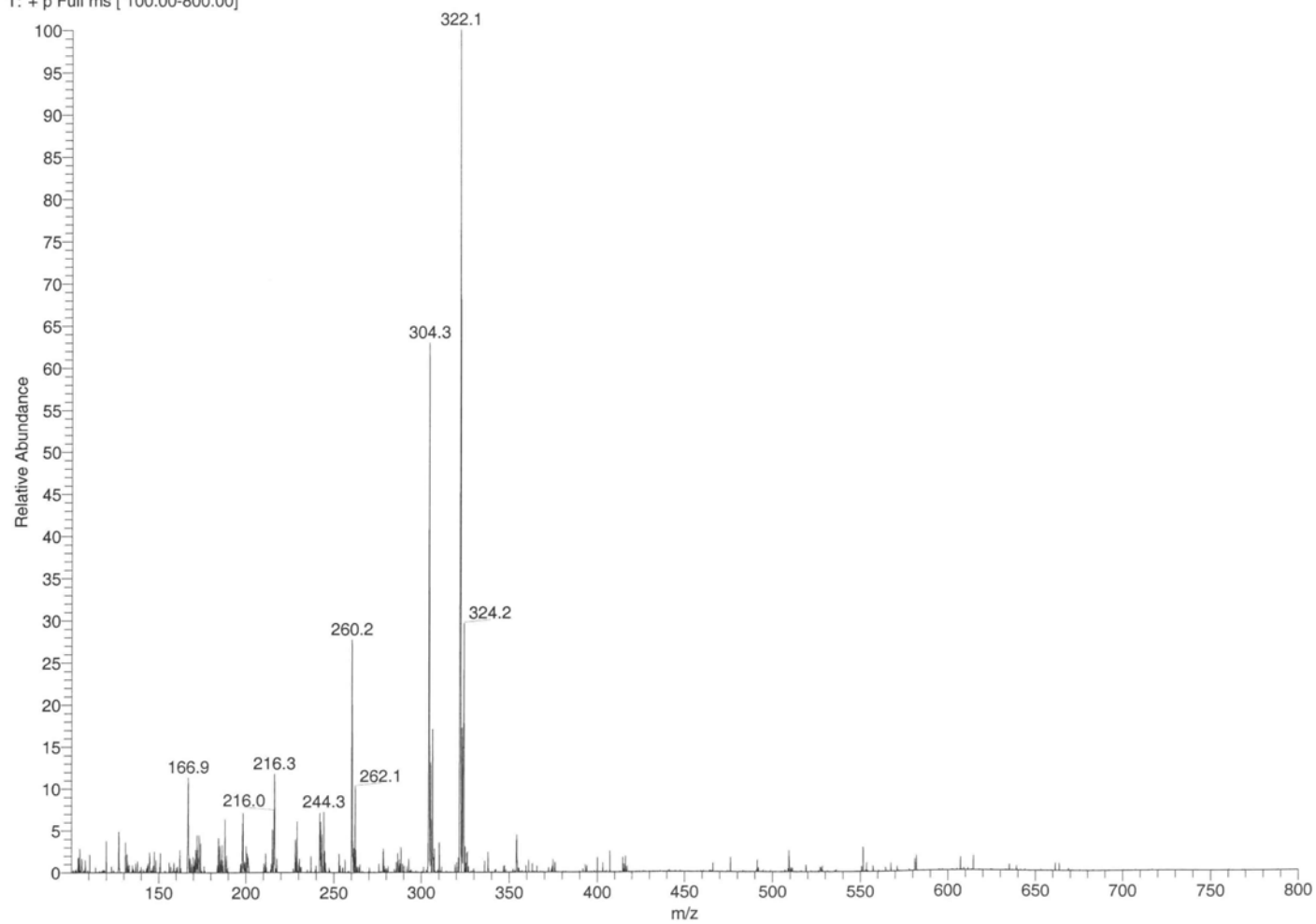
07/15/2005 04:46:11 PM

SH2 - 76

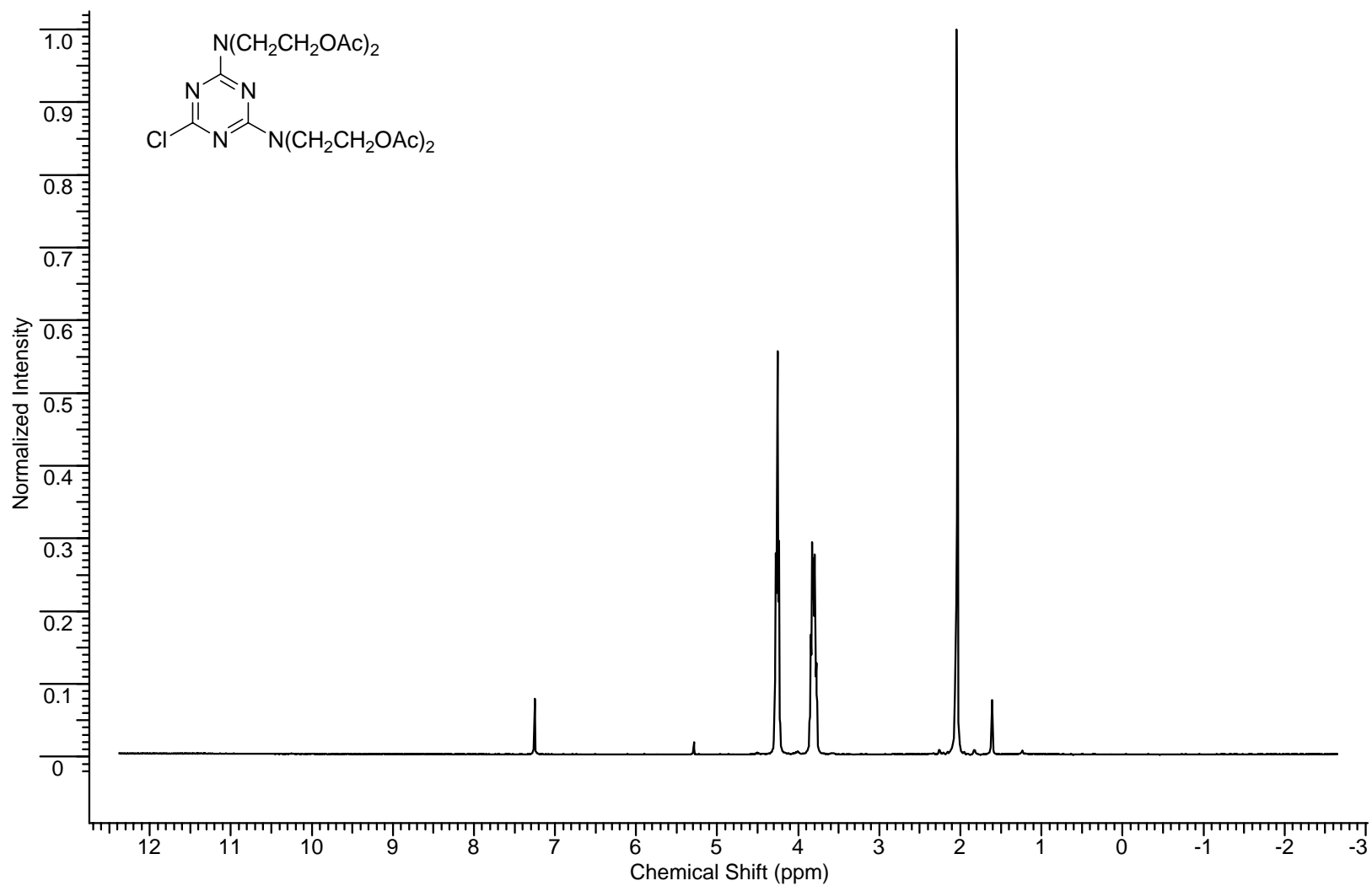
APCI at 450C

07150503c #31 RT: 0.43 AV: 1 SB: 46 0.00-0.39, 0.52-0.75 NL: 9.93E5

T: + p Full ms [100.00-800.00]



pH Responsive Dendrimer: Compound **9**, ^1H NMR



pH Responsive Dendrimer: Compound 9, ^{13}C NMR

SH2-98

Pulse Sequence: s2pu1

Solvent: cdc13

Ambient temperature

Mercury-300BB "mercury300"

Pulse 84.8 degrees

Acq. time 1.815 sec

Width 18761.7 Hz

158 repetitions

OBSERVE C13, 75.4134859 MHz

DECOUPLE H1, 299.9156550 MHz

Power 35 dB

continuously on

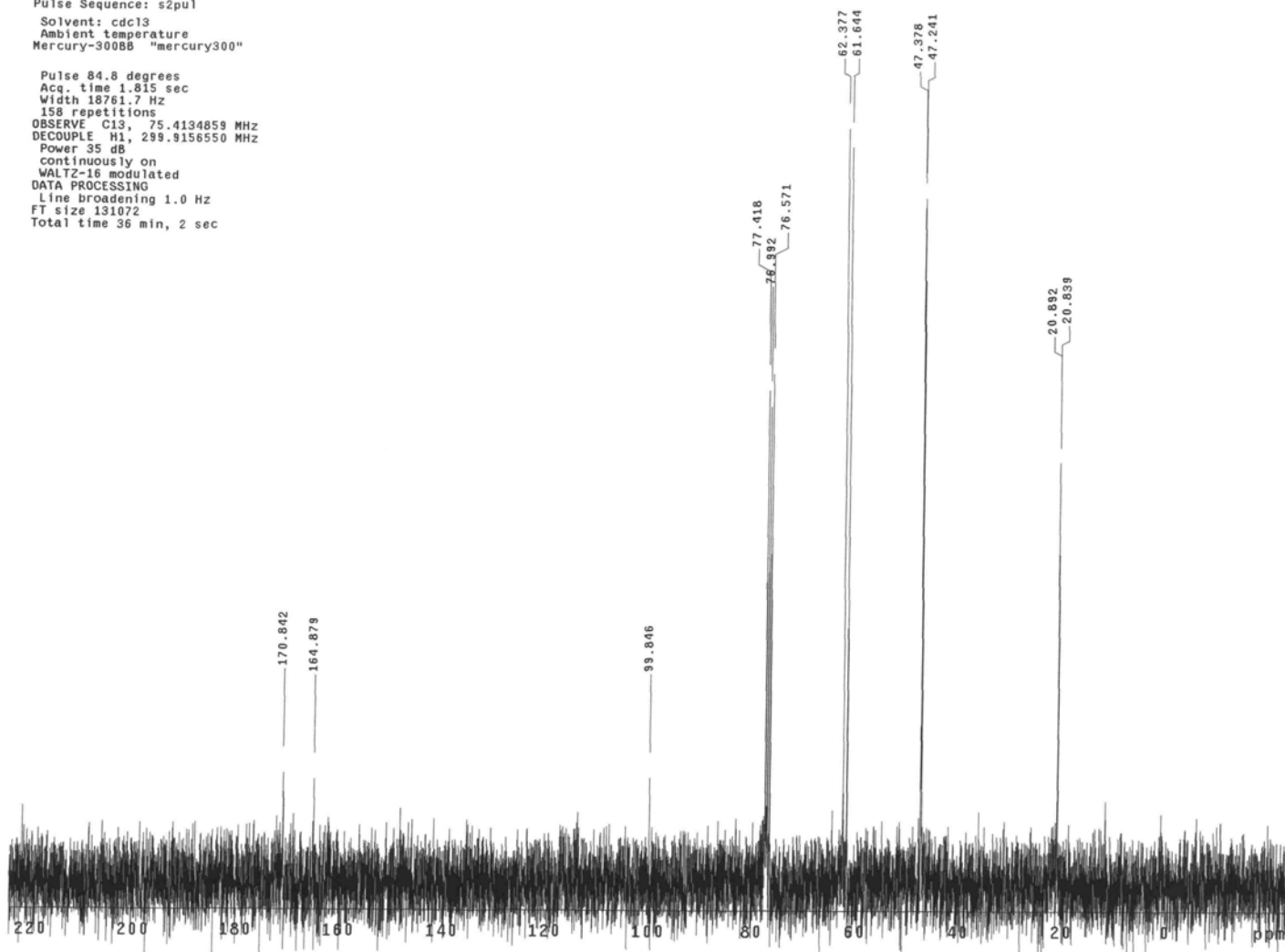
WALTZ-16 modulated

DATA PROCESSING

Line broadening 1.0 Hz

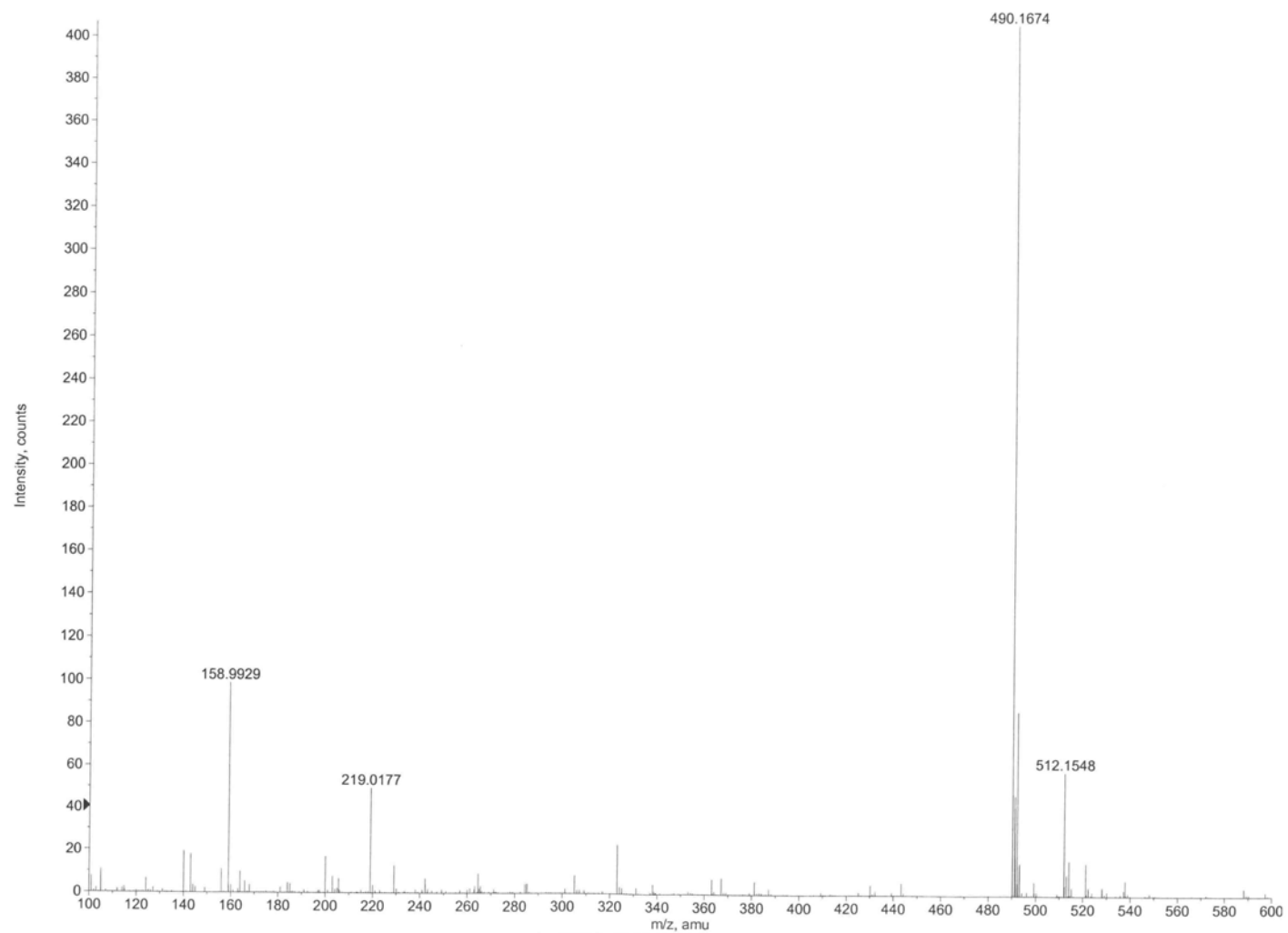
FT size 131072

Total time 36 min, 2 sec

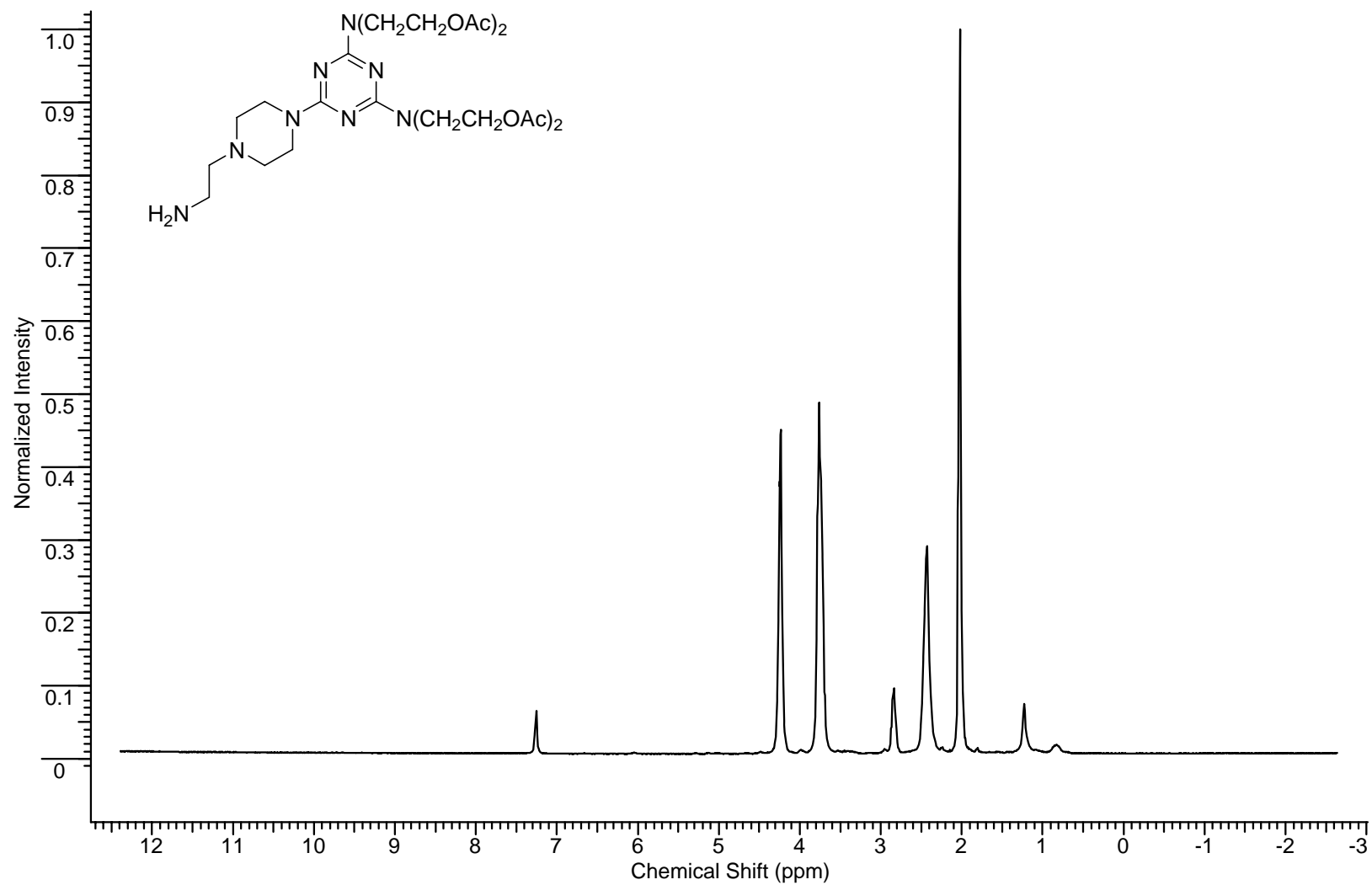


pH Responsive Dendrimer: Compound 9, ESI-MS

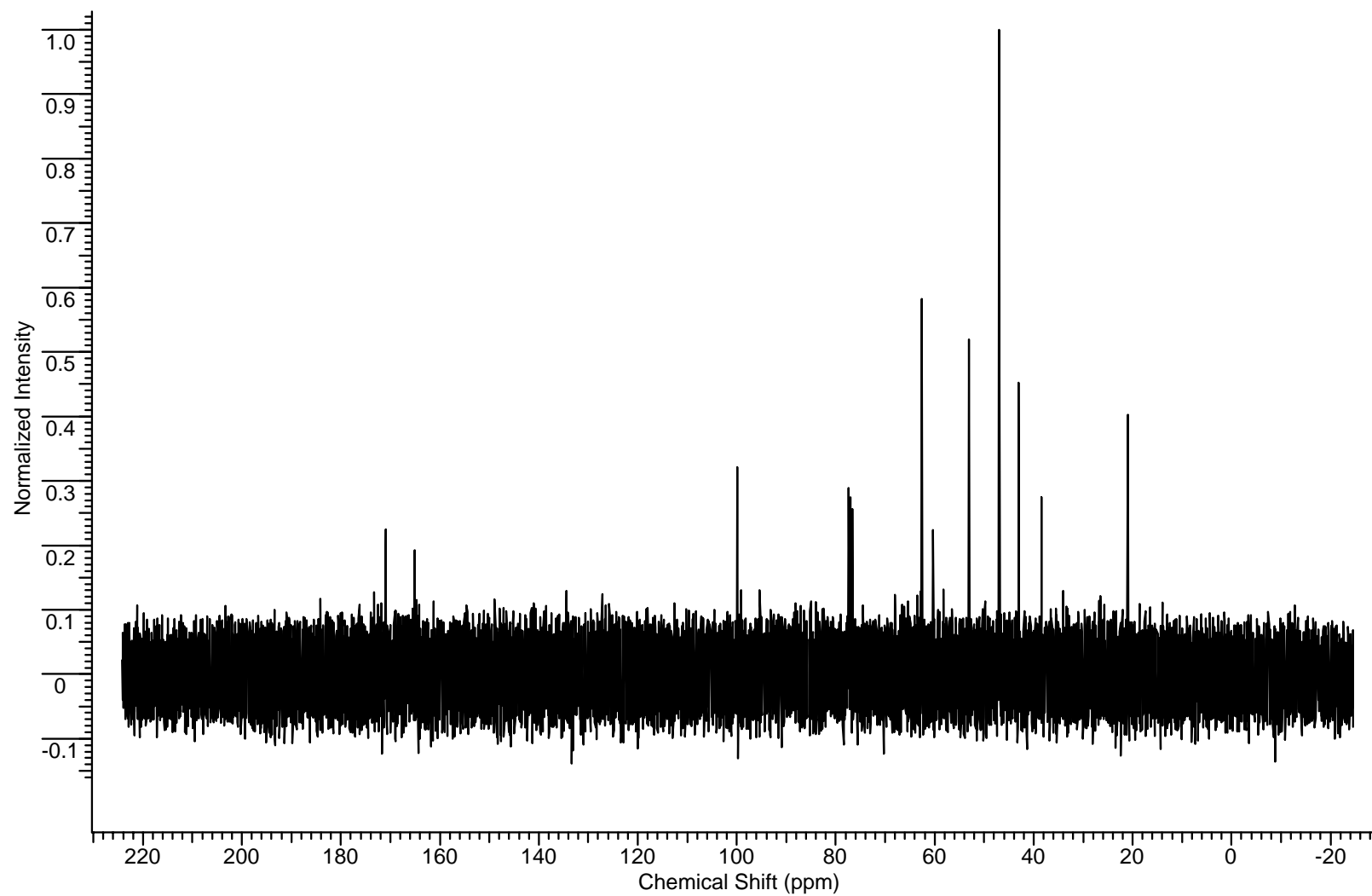
+TOF MS: 0.017 tr ^ 333 min from Sample 1 (SH2-98) of 09160510.wiff
a=3.55273976359 10e-004, t0=4.50160129713622150e+001



pH Responsive Dendrimer: Compound **10**, ^1H NMR

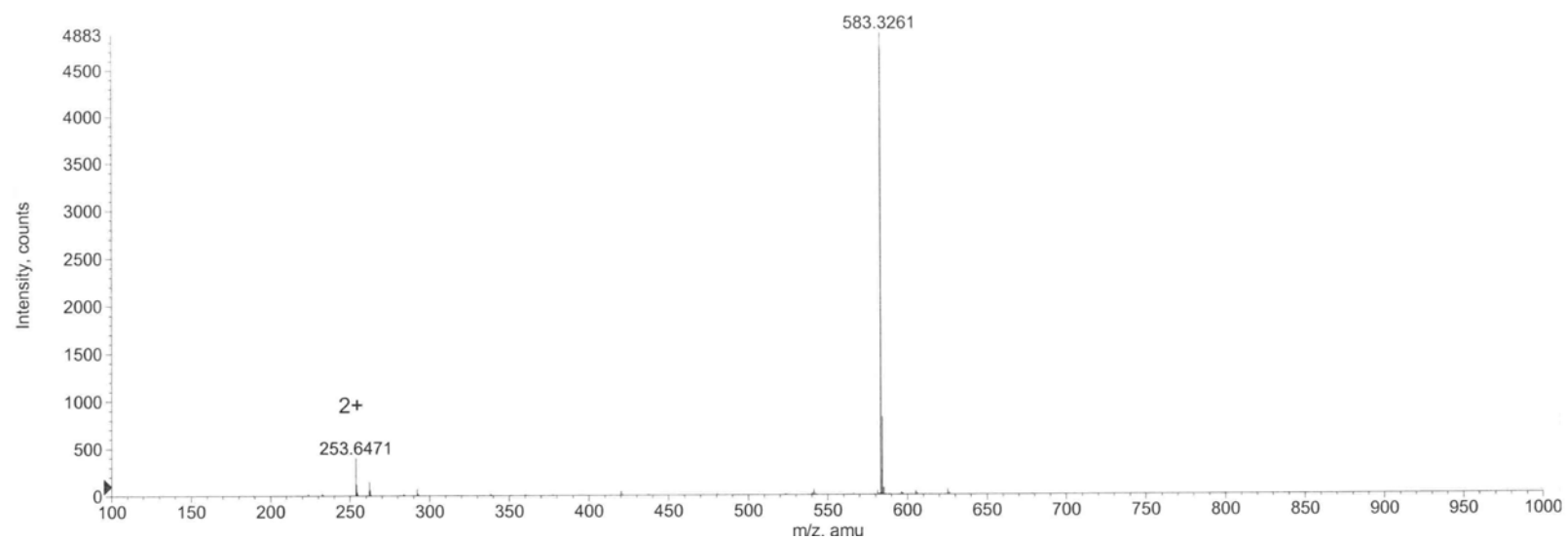


pH Responsive Dendrimer: Compound **10**, ^{13}C NMR

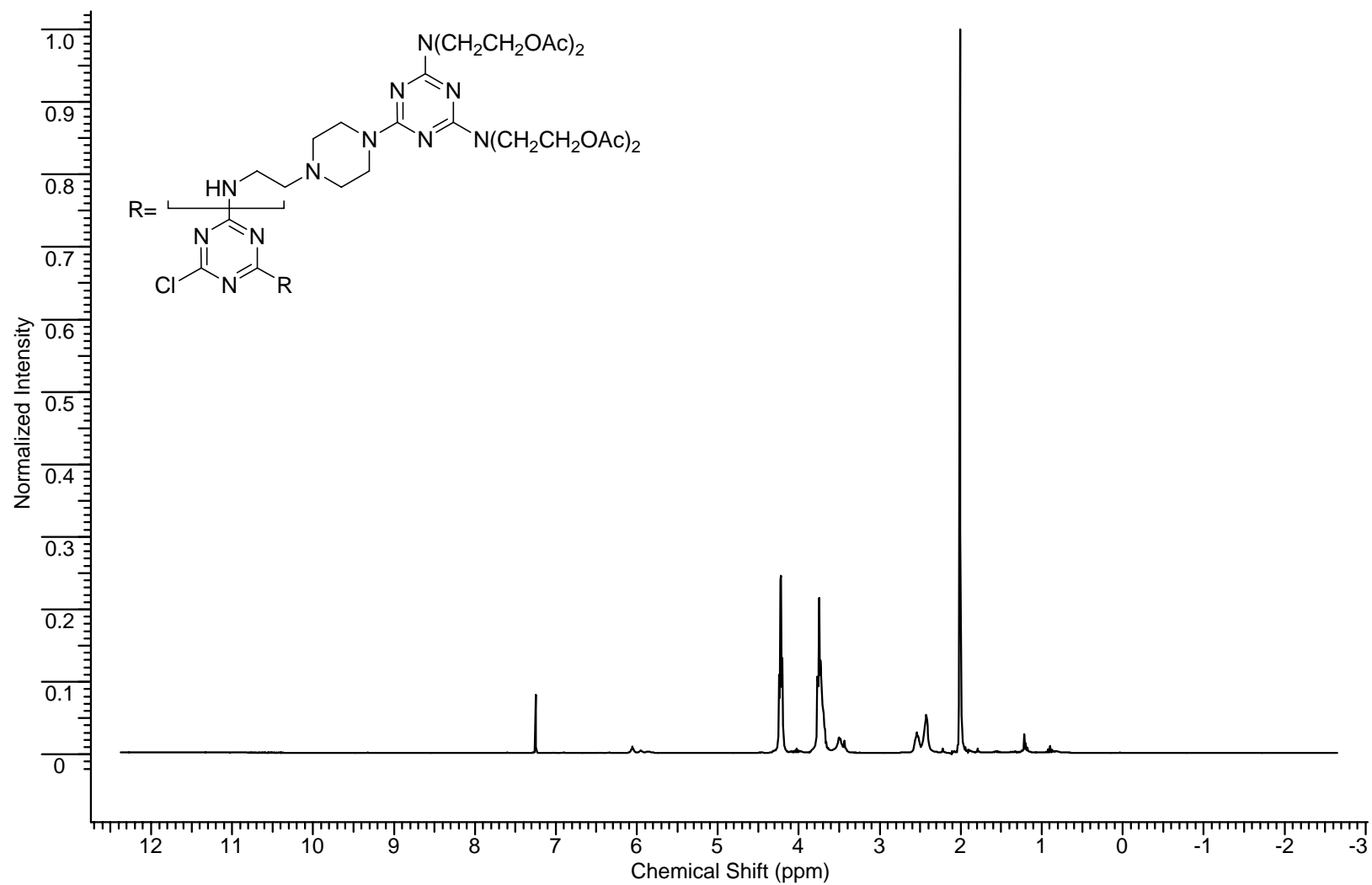


pH Responsive Dendrimer: Compound **10** ESI-MS

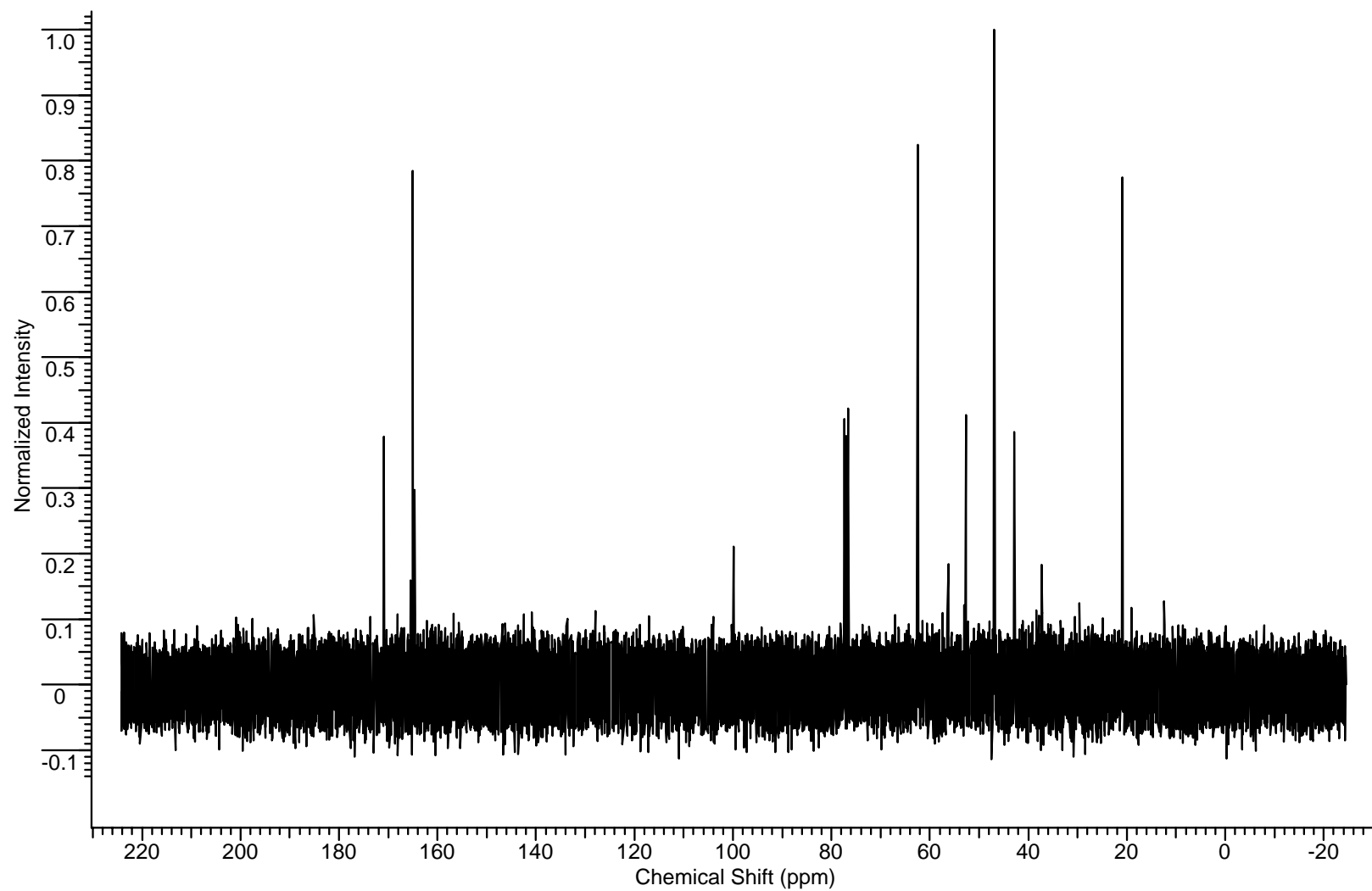
+TOF MS: 7 MCA scans from 09300502.wiff
a=3.55267488851, t0=4.50380410135476270e+001



pH Responsive Dendrimer: Compound **11**, ^1H NMR

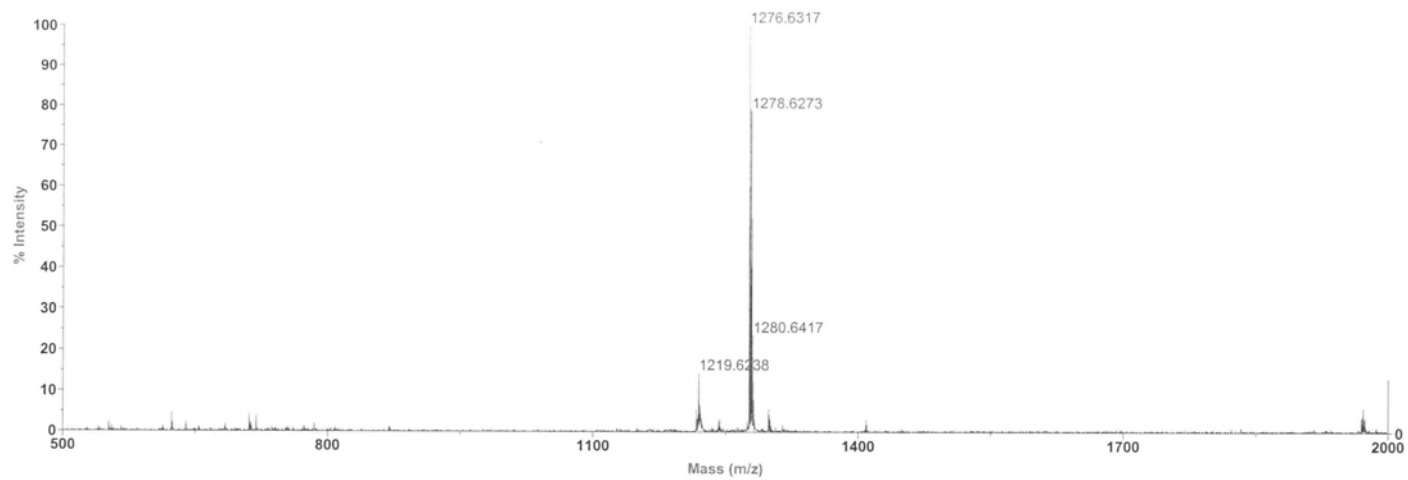


pH Responsive Dendrimer: Compound **11**, ^{13}C NMR

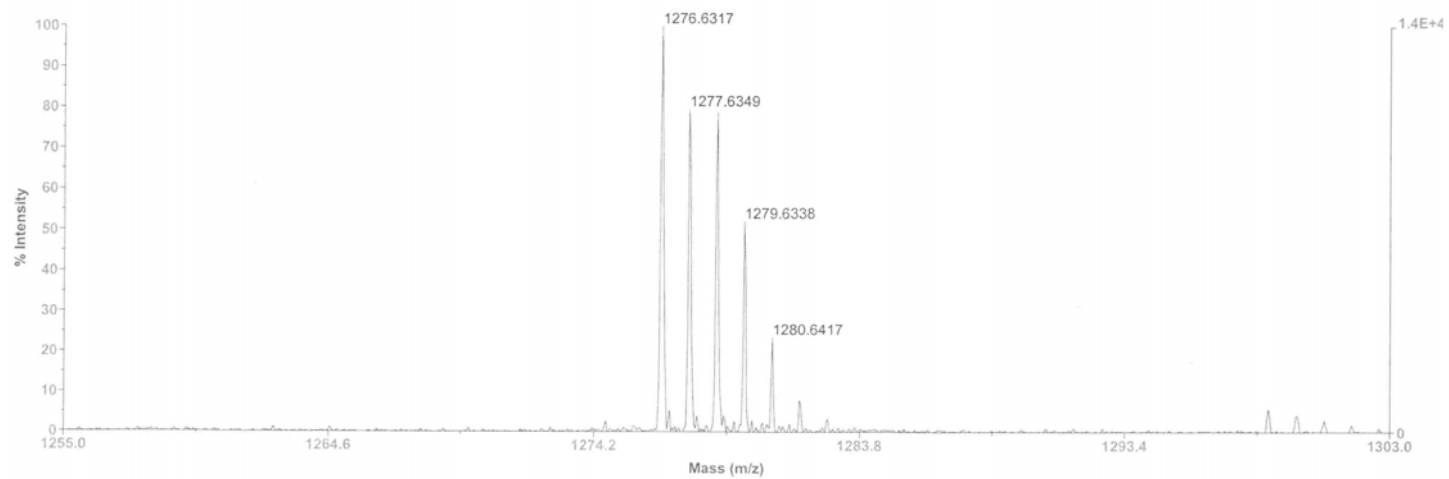


pH Responsive Dendrimer: Compound 11, MALDI-MS

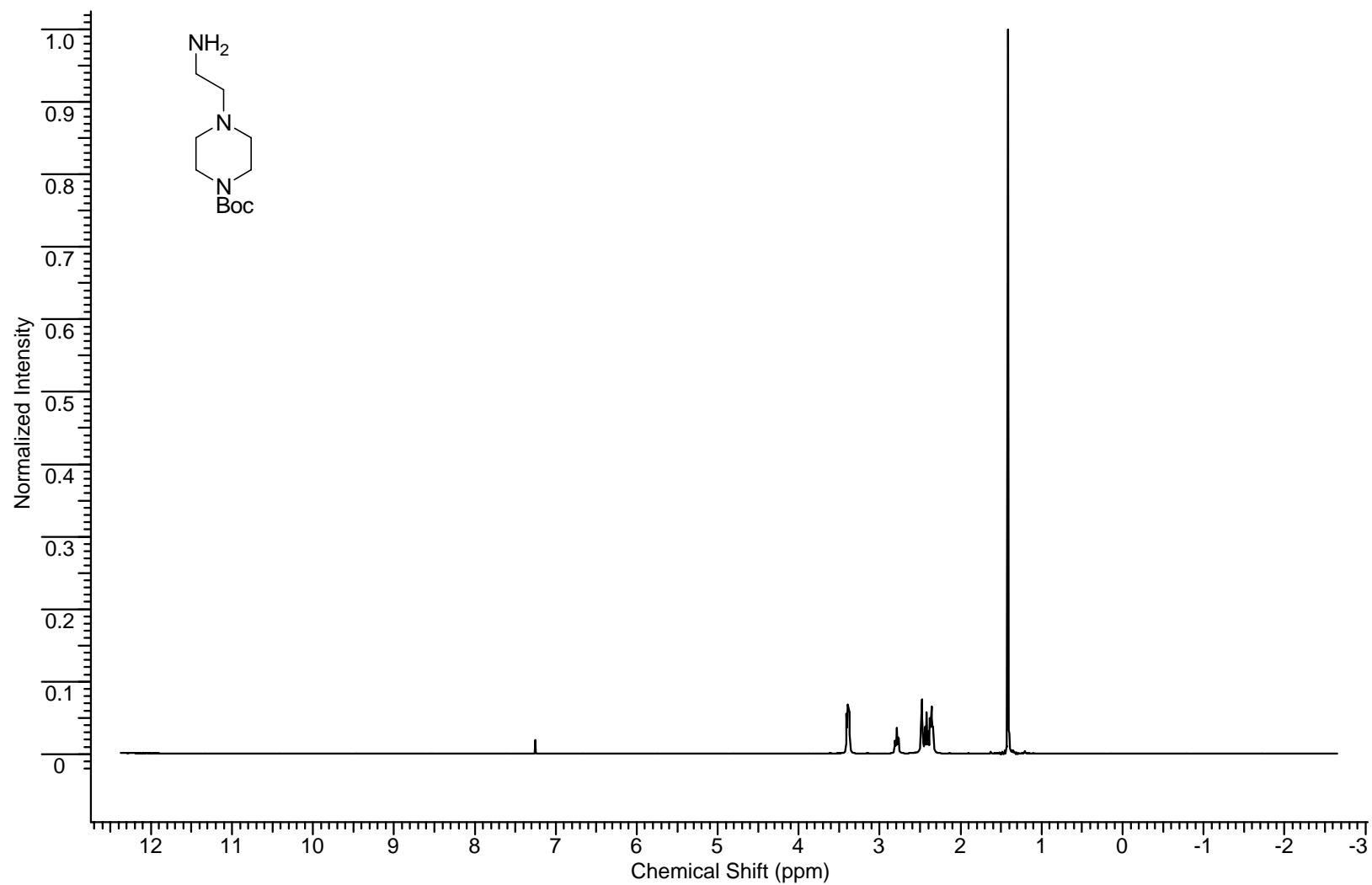
Voyager Spec #1=>AdvBC(2⁺, 5, 0.1)[BP = 1276.6, 13977]



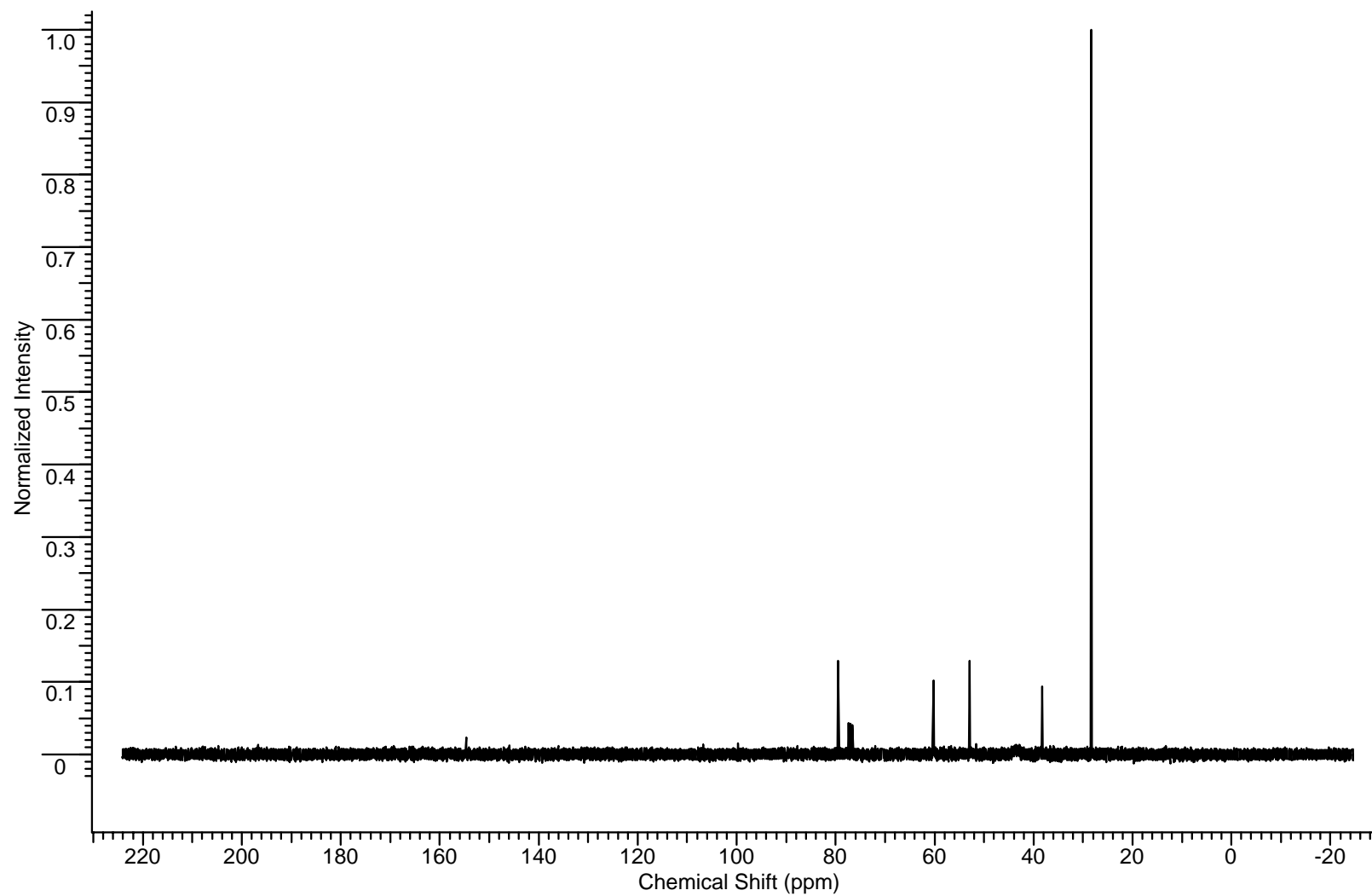
Voyager Spec #1=>AdvBC(32, 0.5, 0.1)[BP = 1276.6, 13977]



pH Responsive Dendrimer: Compound **12**, ^1H NMR

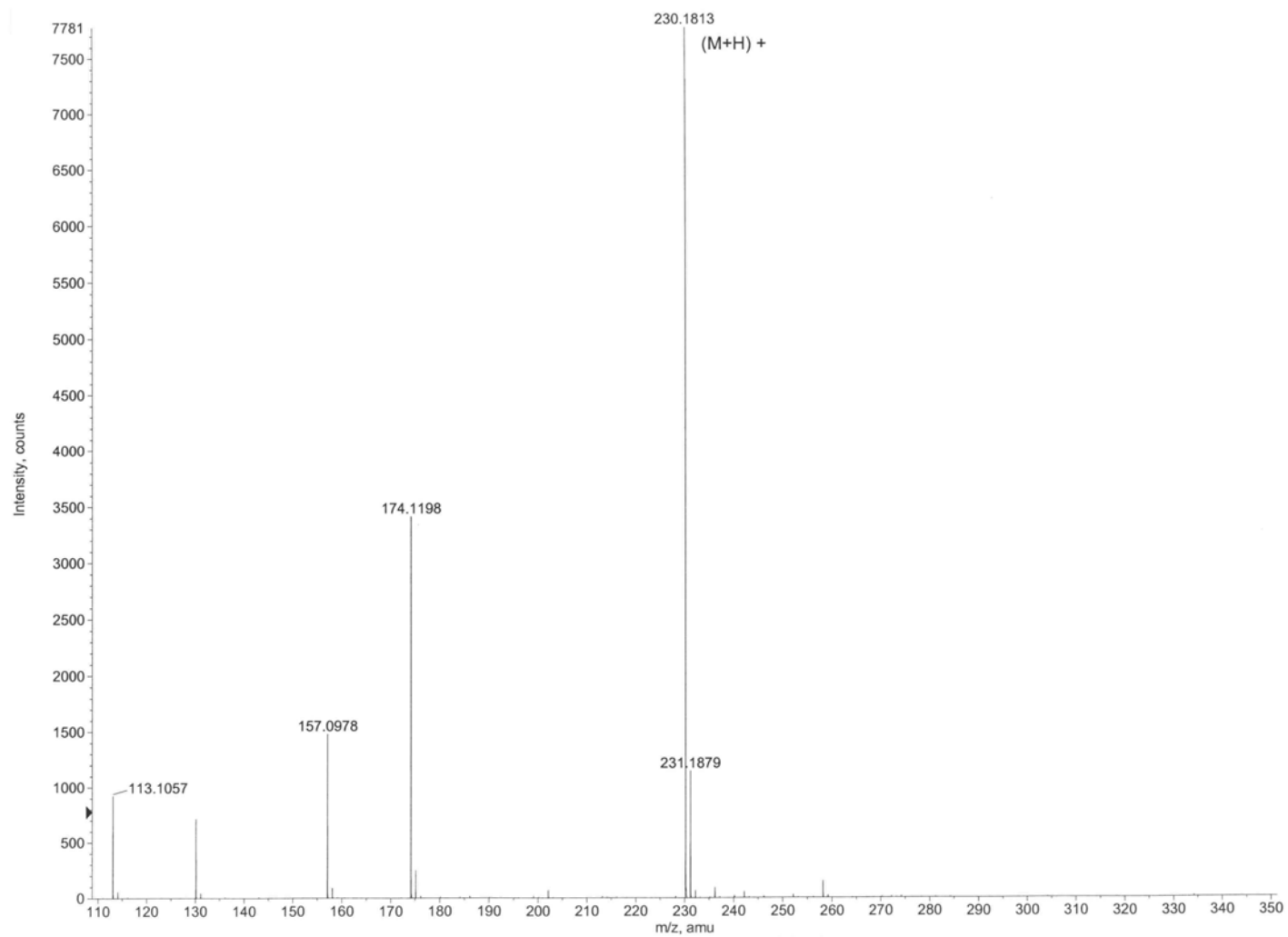


pH Responsive Dendrimer: Compound **12**, ^{13}C NMR

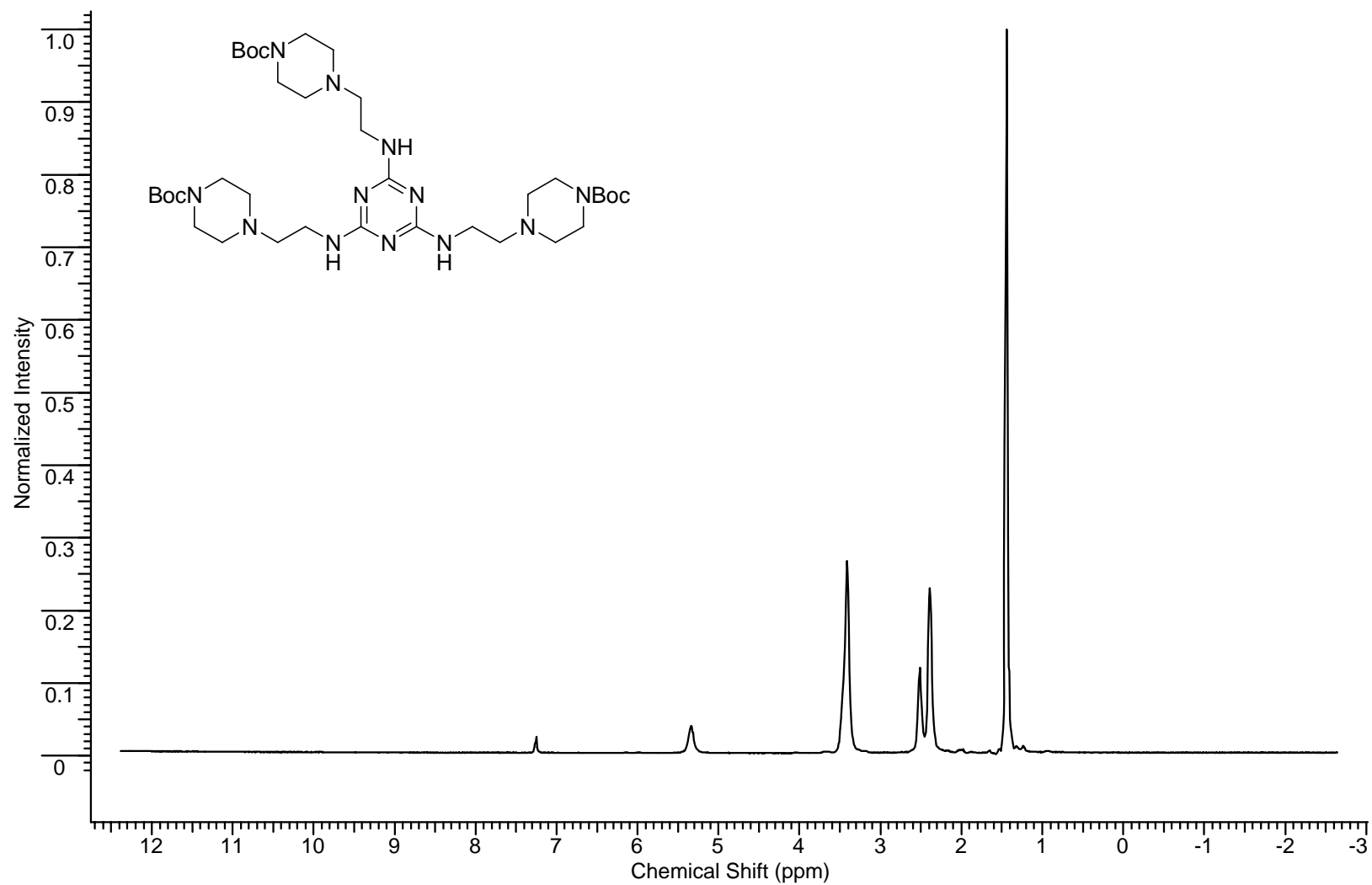


pH Responsive Dendrimer: Compound 12, ESI-MS

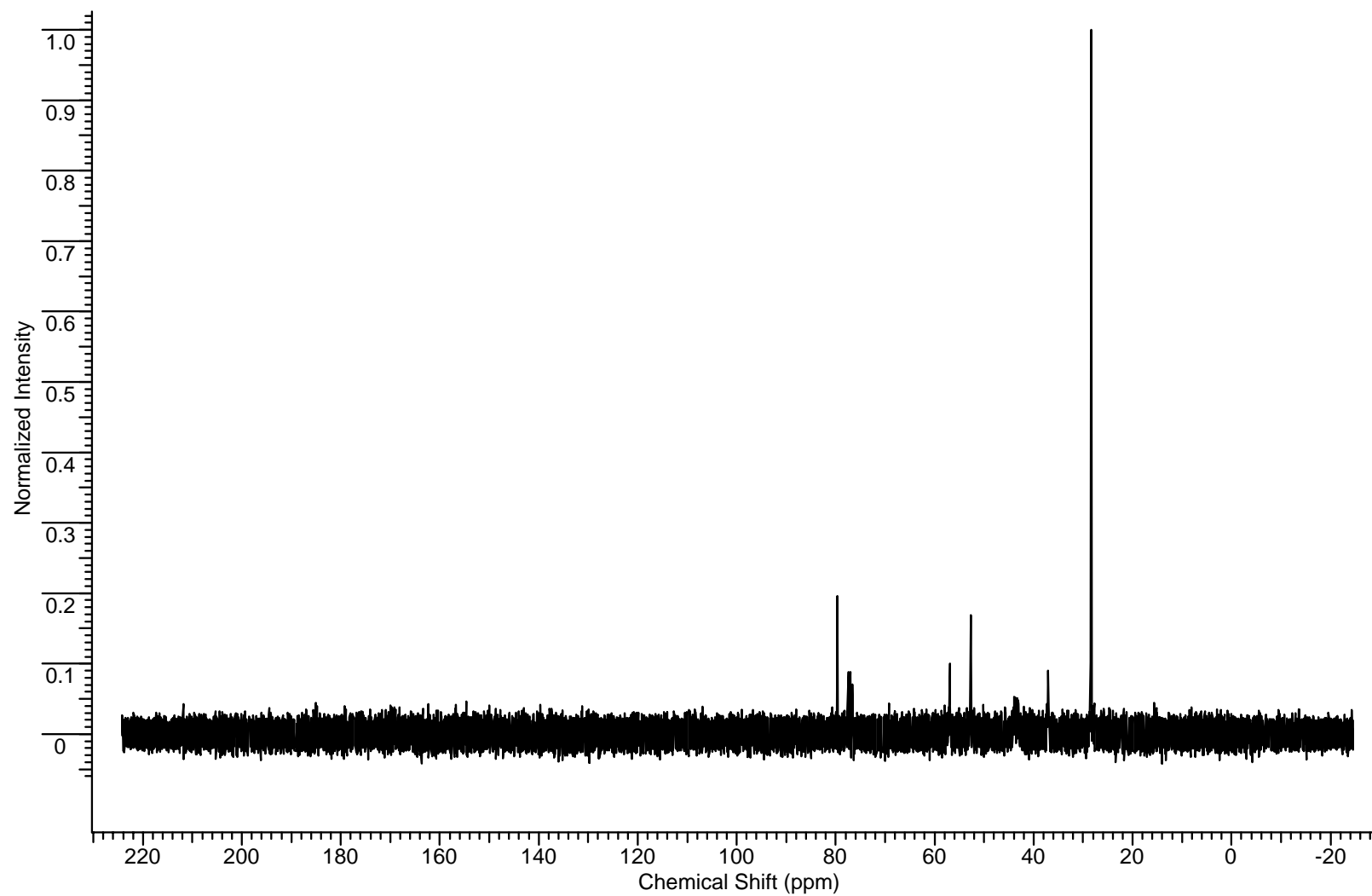
+TOF MS: 0.050 to 117 min from 12090514.wiff
a=3.55297799935 30e-004, t0=4.52305266737530470e+001



pH Responsive Dendrimer: Compound **13**, ^1H NMR

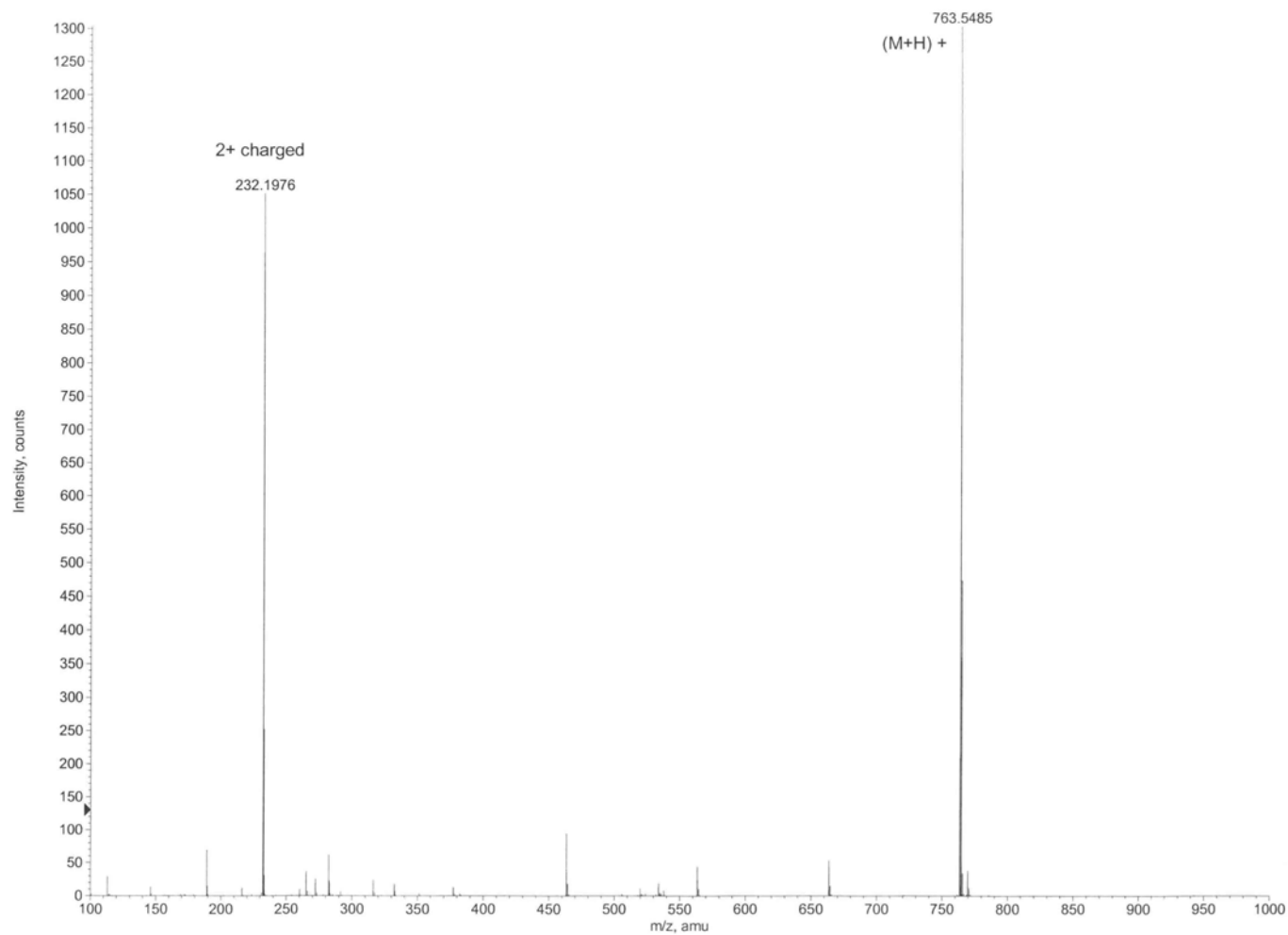


pH Responsive Dendrimer: Compound **13**, ^{13}C NMR

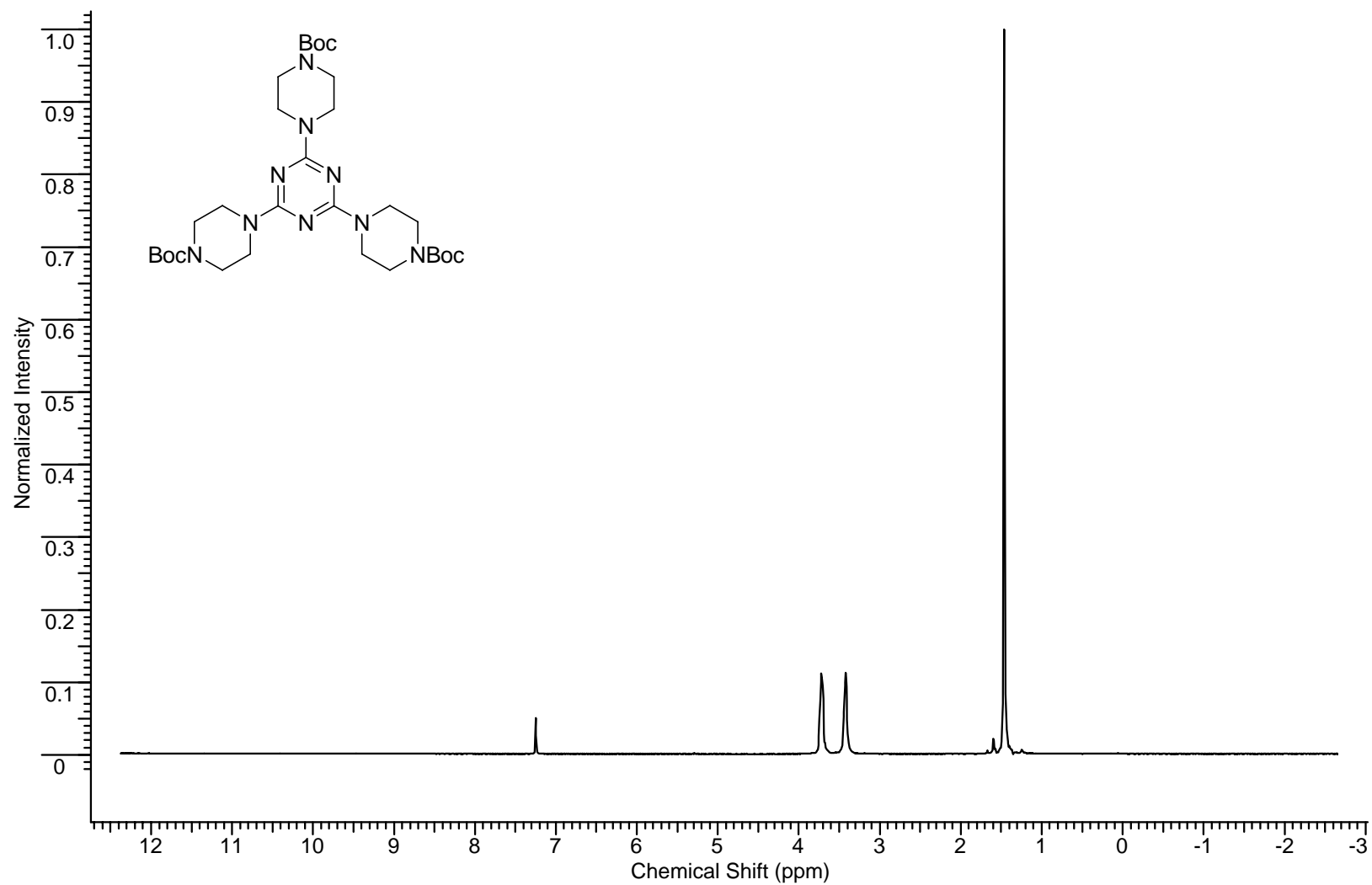


pH Responsive Dendrimer: Compound 13, ESI-MS

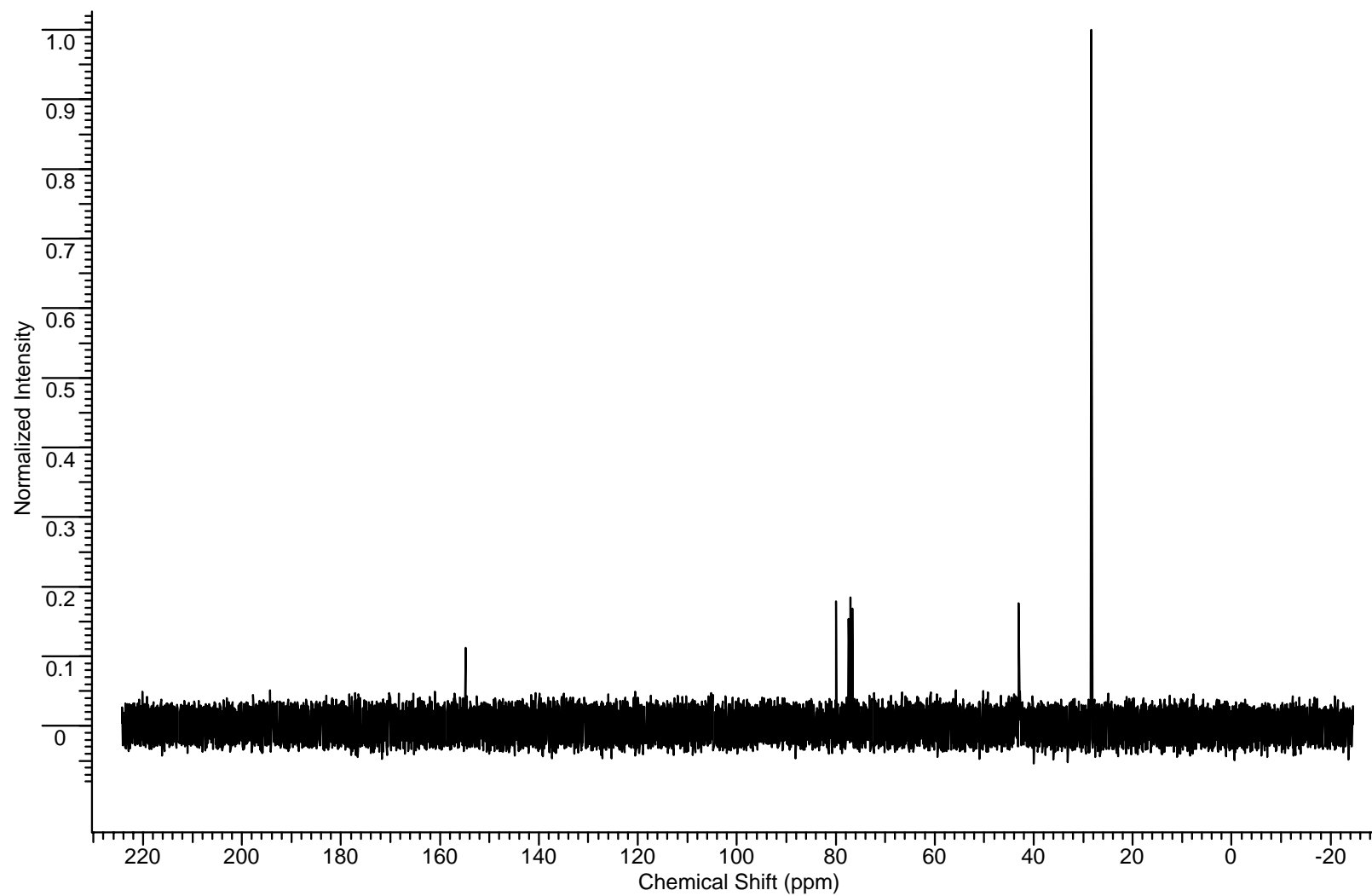
+TOF MS: 0.300 tr ^ 467 min from 09190507.wiff
a=3.55259403108 10e-004, t0=4.49300537365306810e+001



Divergent Diversity Dendrimer: Compound **15**, ^1H NMR

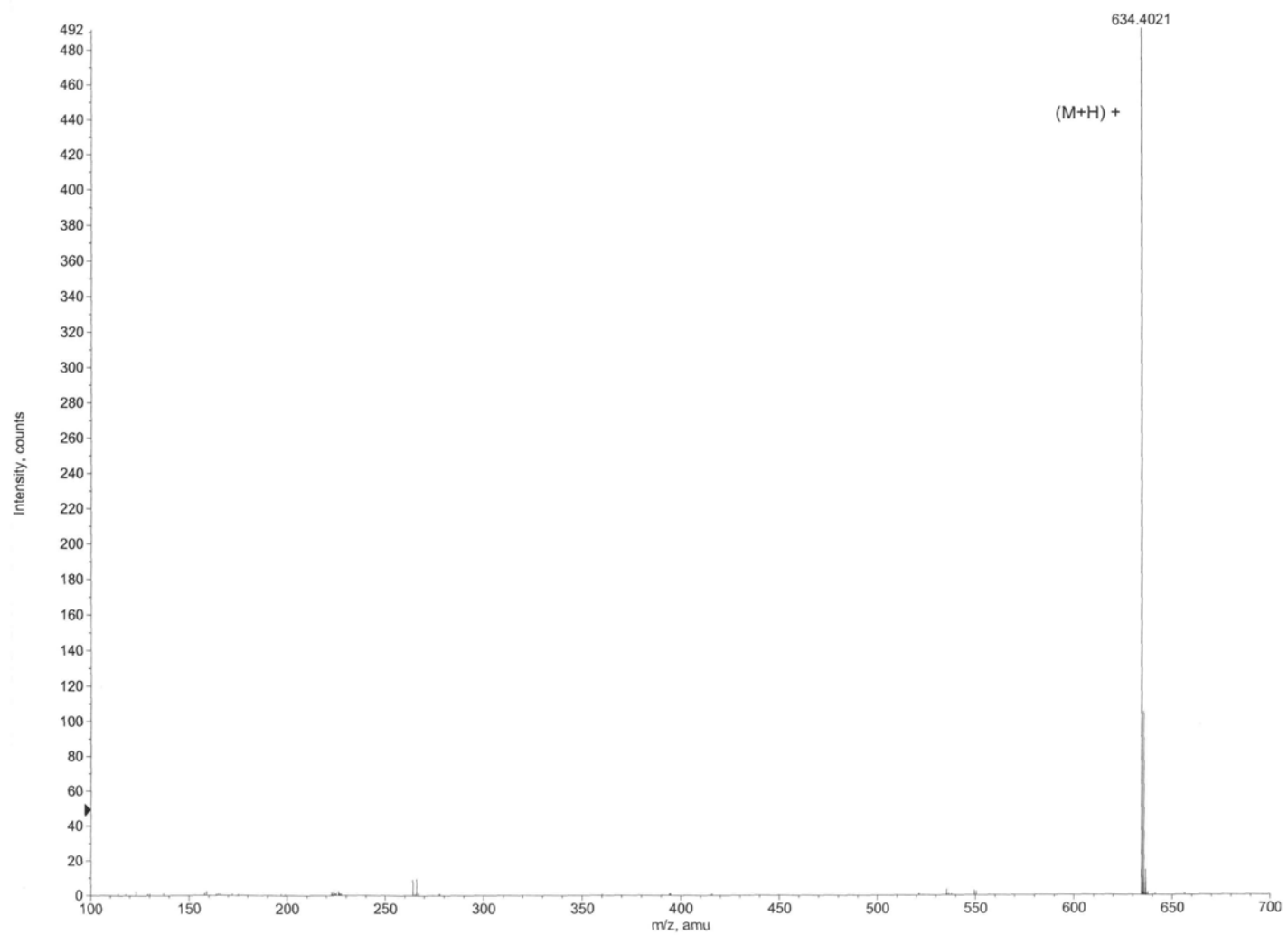


Divergent Diversity Dendrimer: Compound **15**, ^{13}C NMR

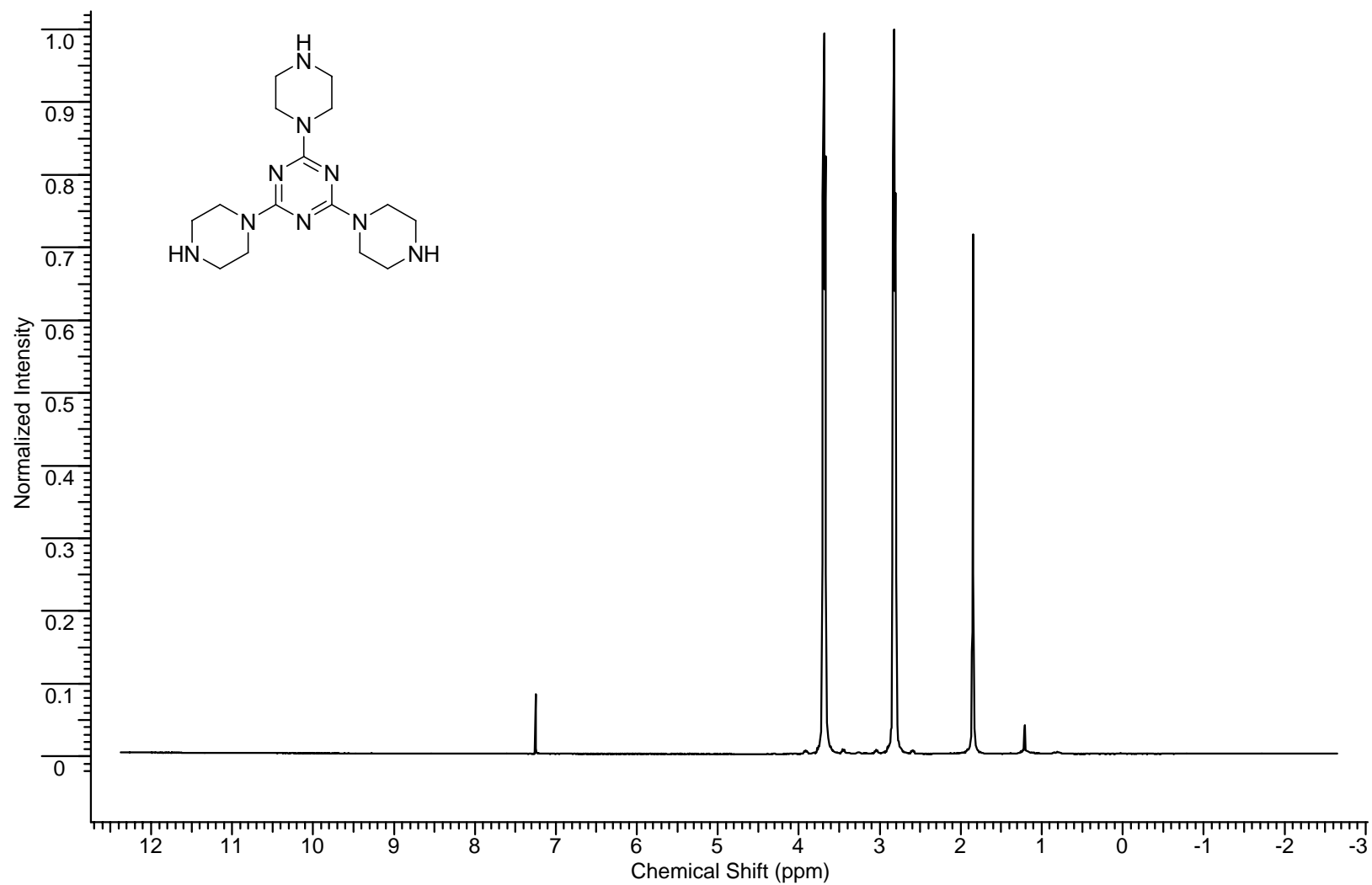


Divergent Diversity Dendrimer: Compound **15**, ESI-MS

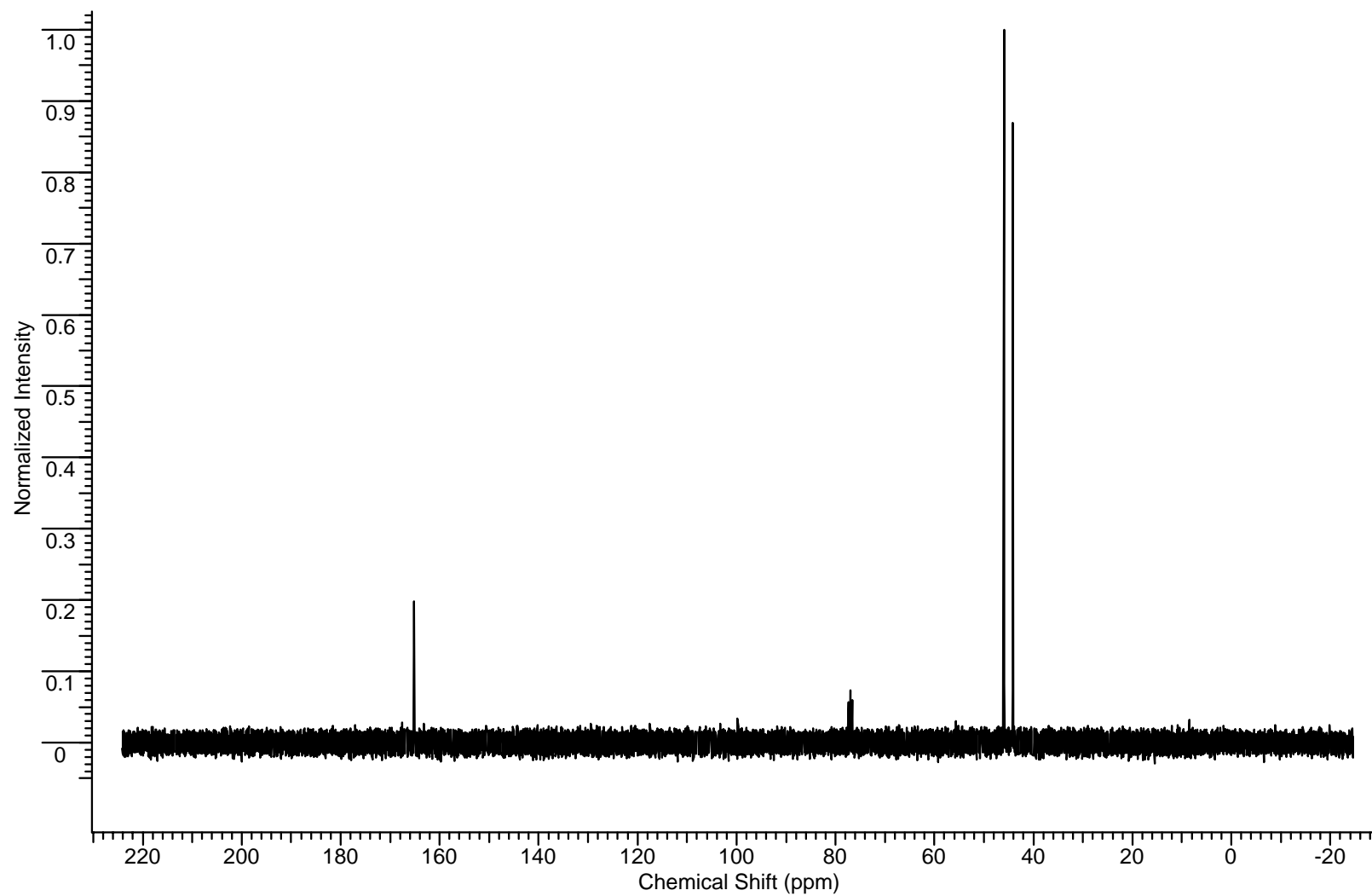
+TOF MS: 0.033 tr 0.050 min from 11210505.wiff
a=3.5527756154! 080e-004, t0=4.53476318629982420e+001



Divergent Diversity Dendrimer: Compound **16**, ^1H NMR

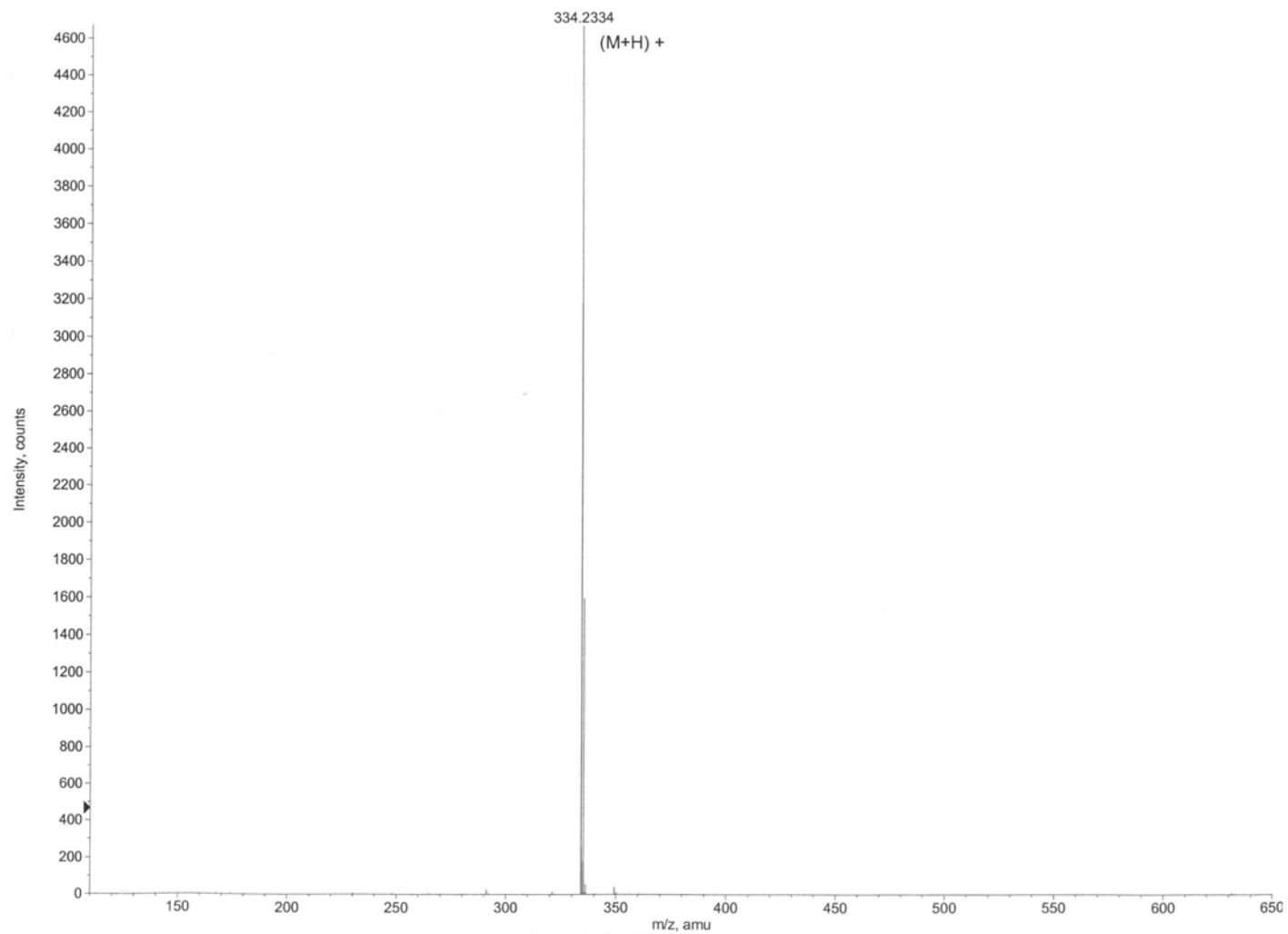


Divergent Diversity Dendrimer: Compound **16**, ^{13}C NMR

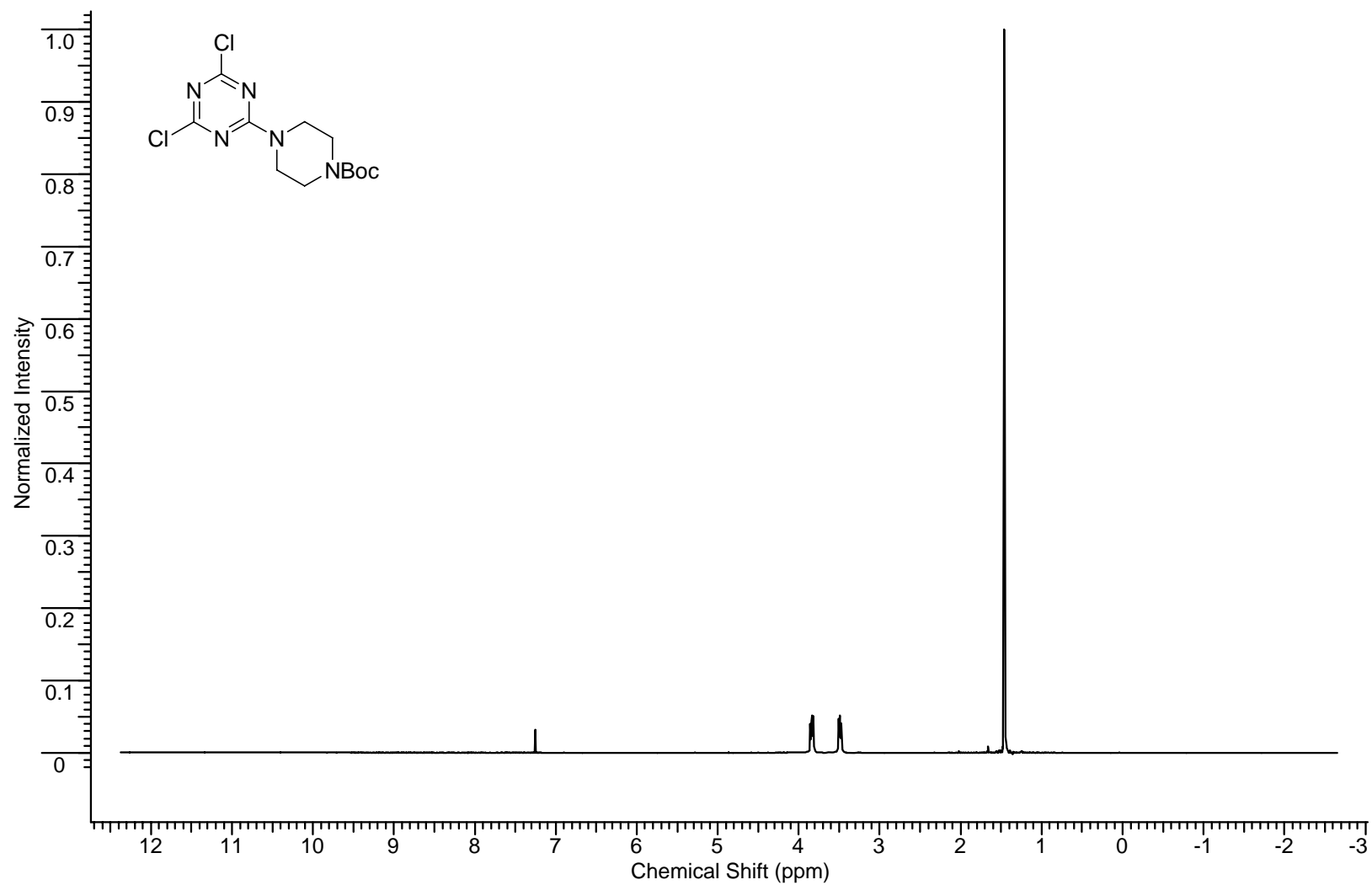


Divergent Diversity Dendrimer: Compound 16, ESI-MS

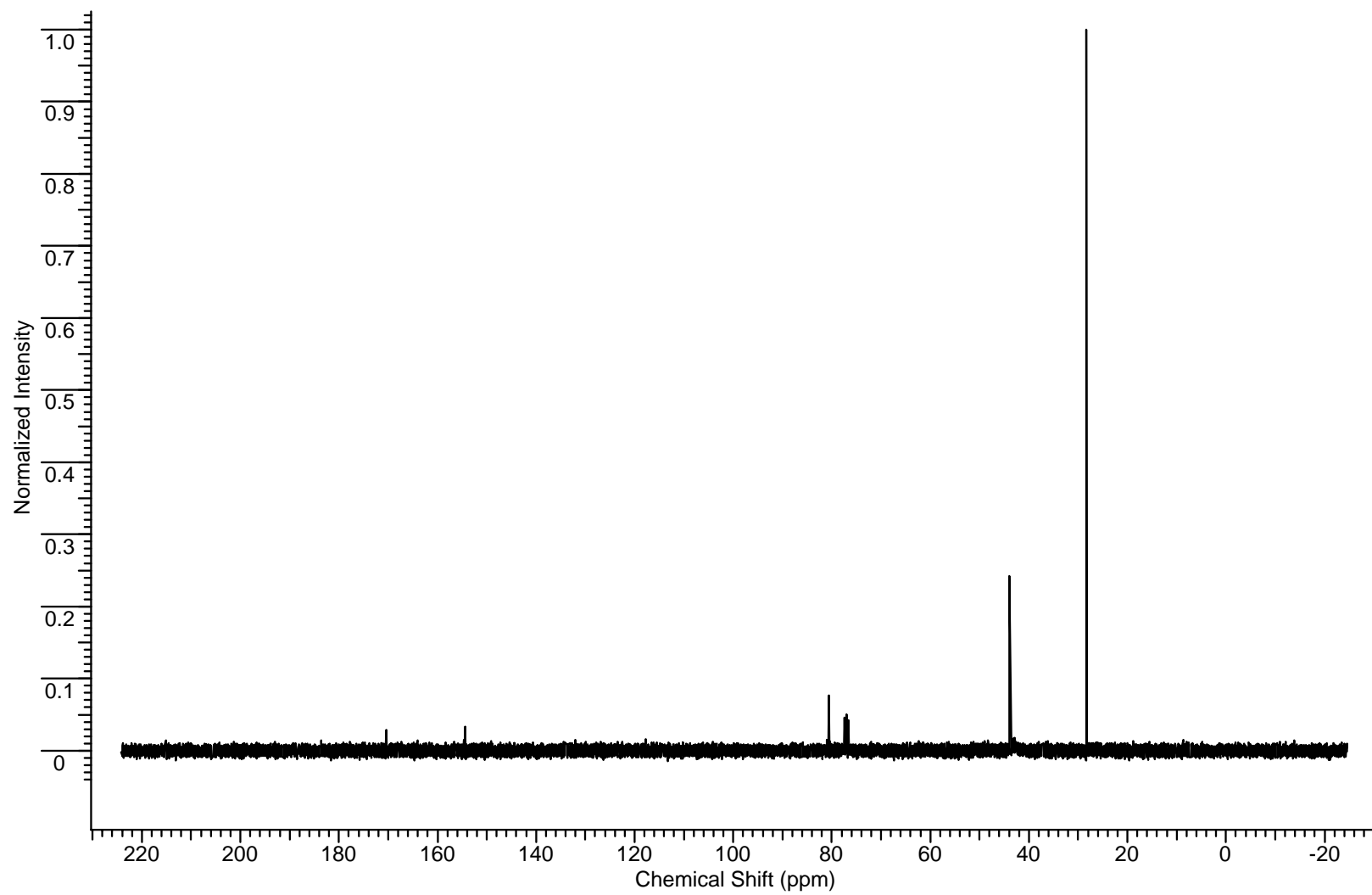
+TOF MS: 0.033 s⁻¹ 067 min from 12090516.wiff
a=3.55297799935 80e-004, t0=4.52305266737530470e+001



Divergent Diversity Dendrimer: Compound **17**, ^1H NMR

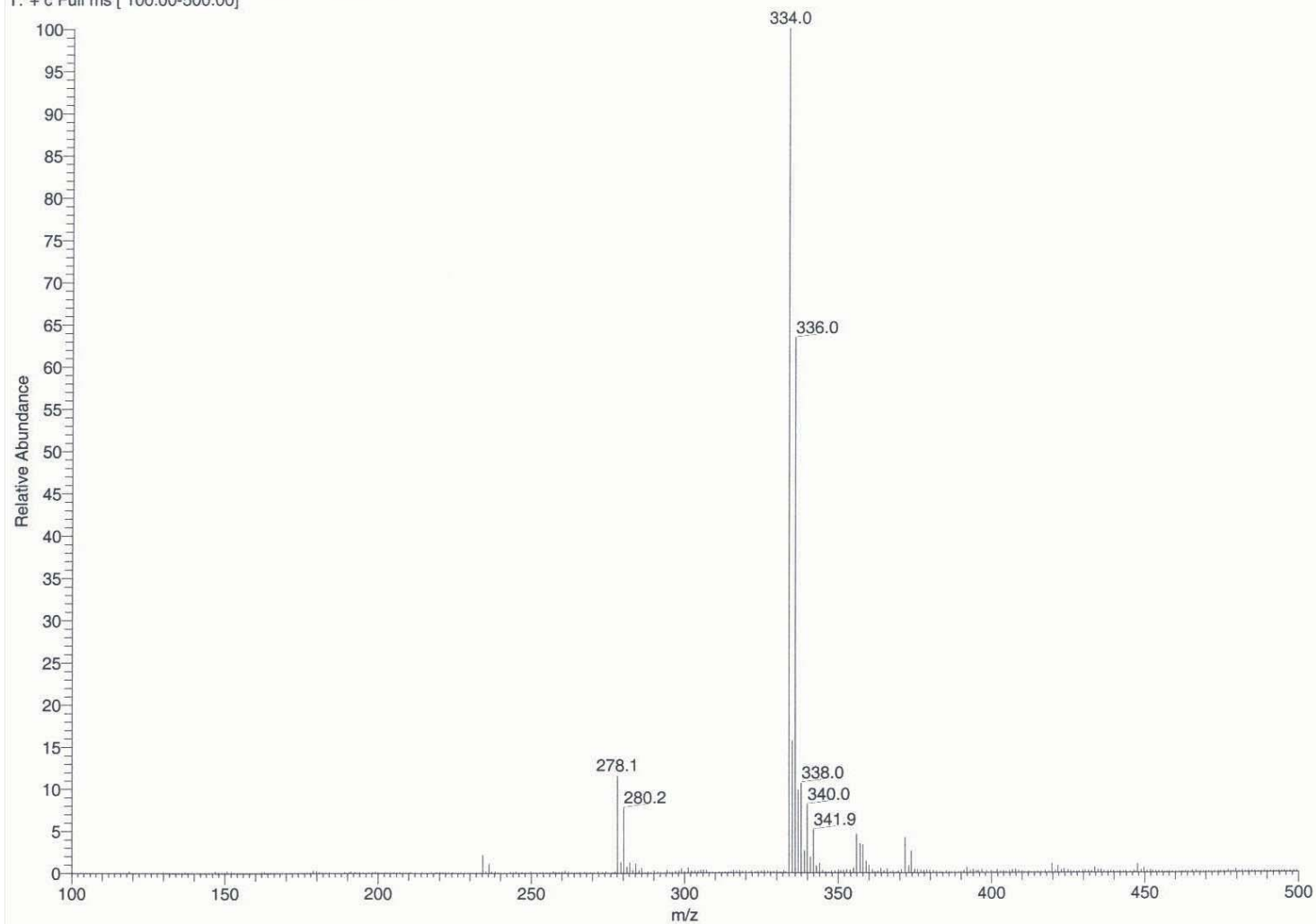


Divergent Diversity Dendrimer: Compound **17**, ^{13}C NMR

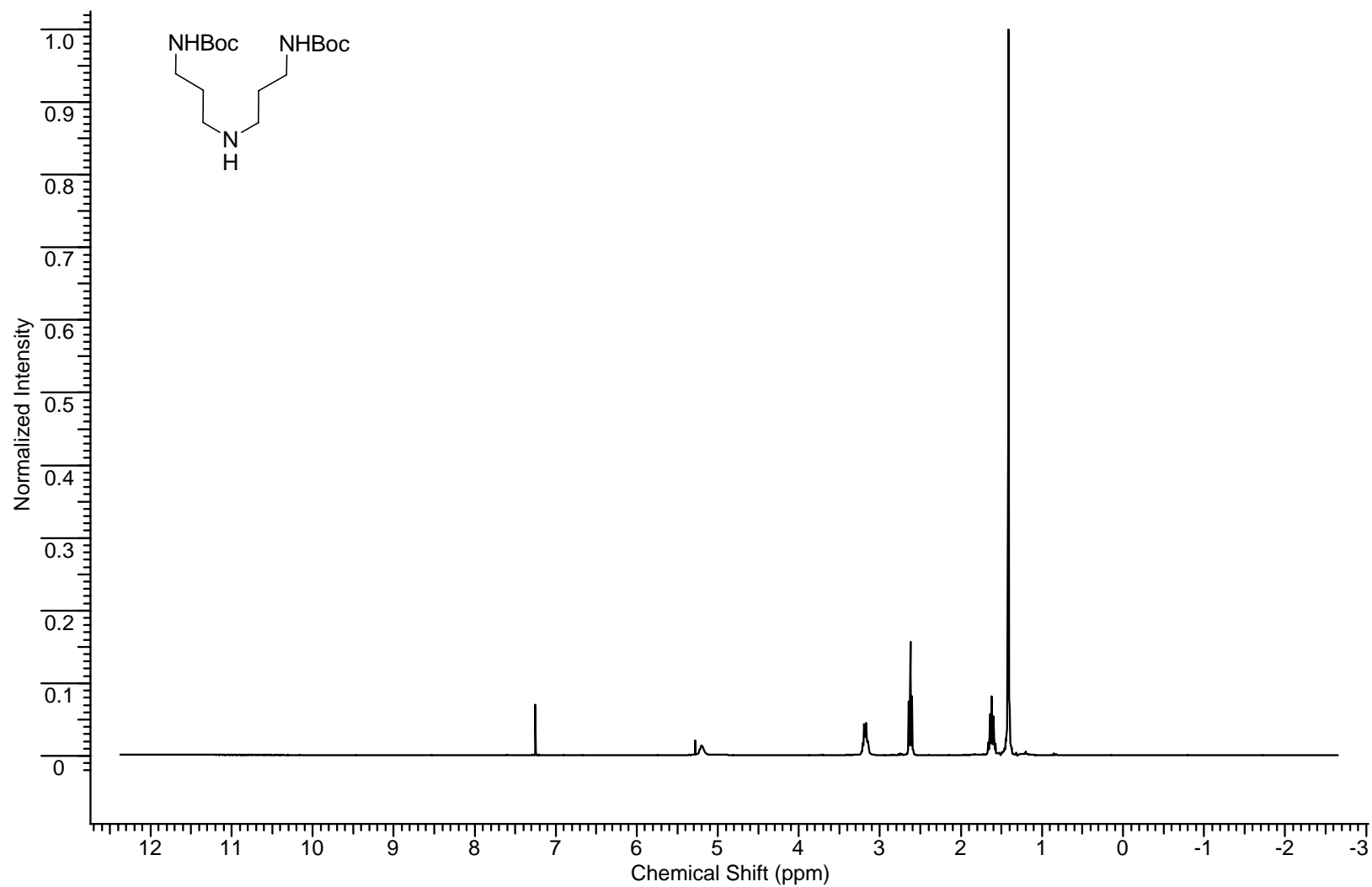


Divergent Diversity Dendrimer: Compound 17, ESI-MS

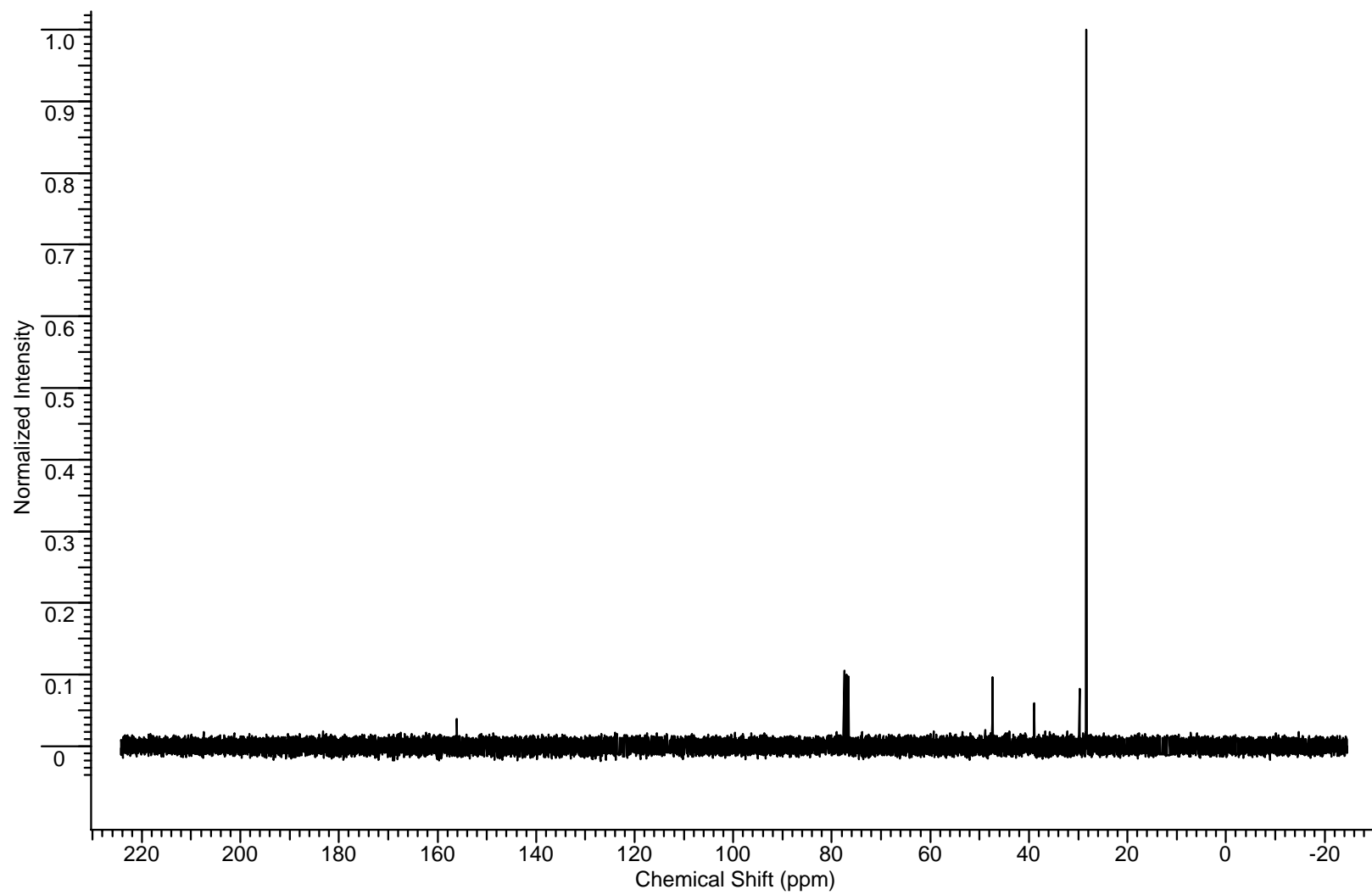
12070504 #2-20 RT: 0.01-0.19 AV: 19 NL: 7.81E7
T: + c Full ms [100.00-500.00]



Divergent Diversity Dendrimer: Compound **18**, ^1H NMR

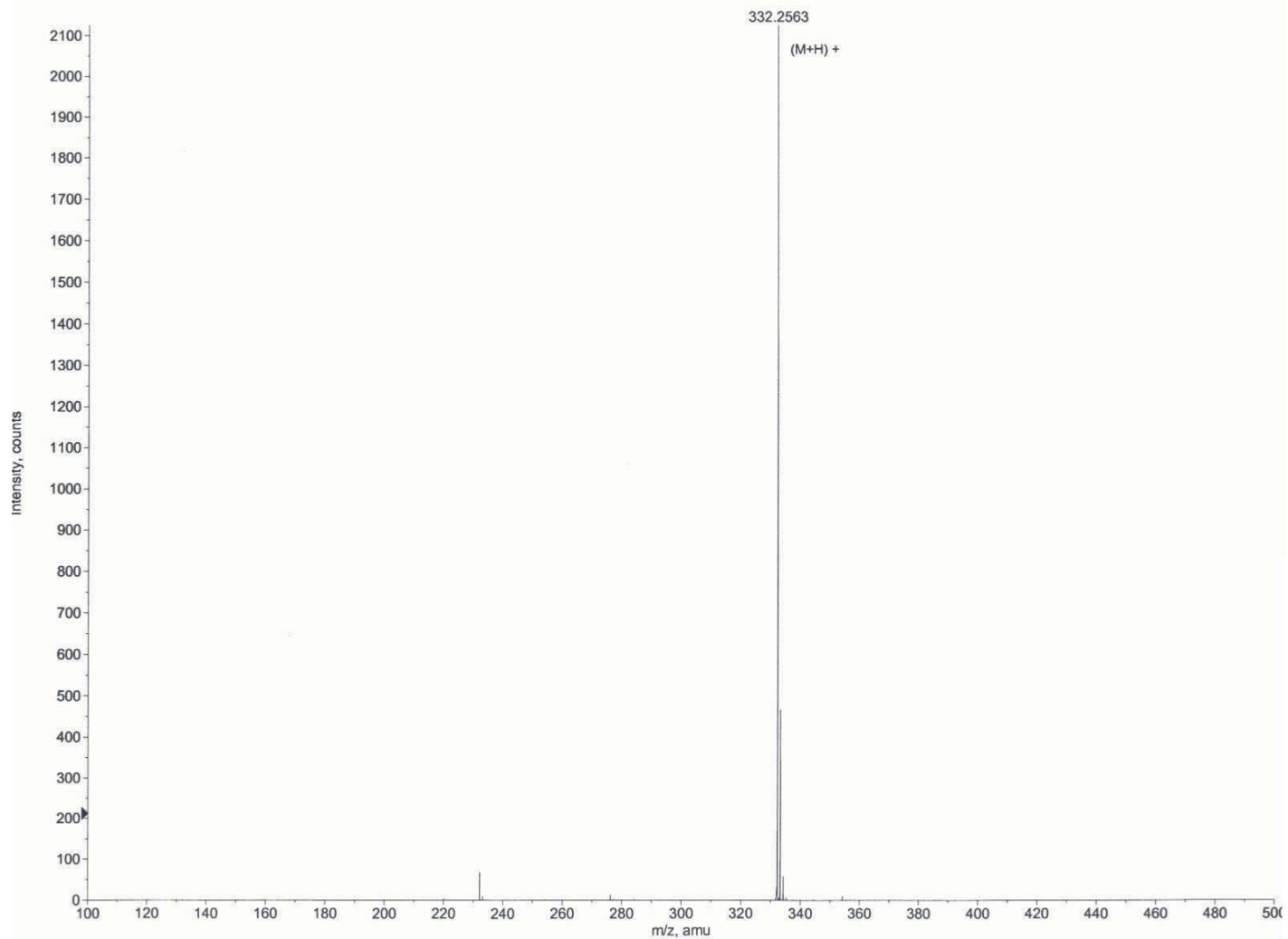


Divergent Diversity Dendrimer: Compound **18**, ^{13}C NMR

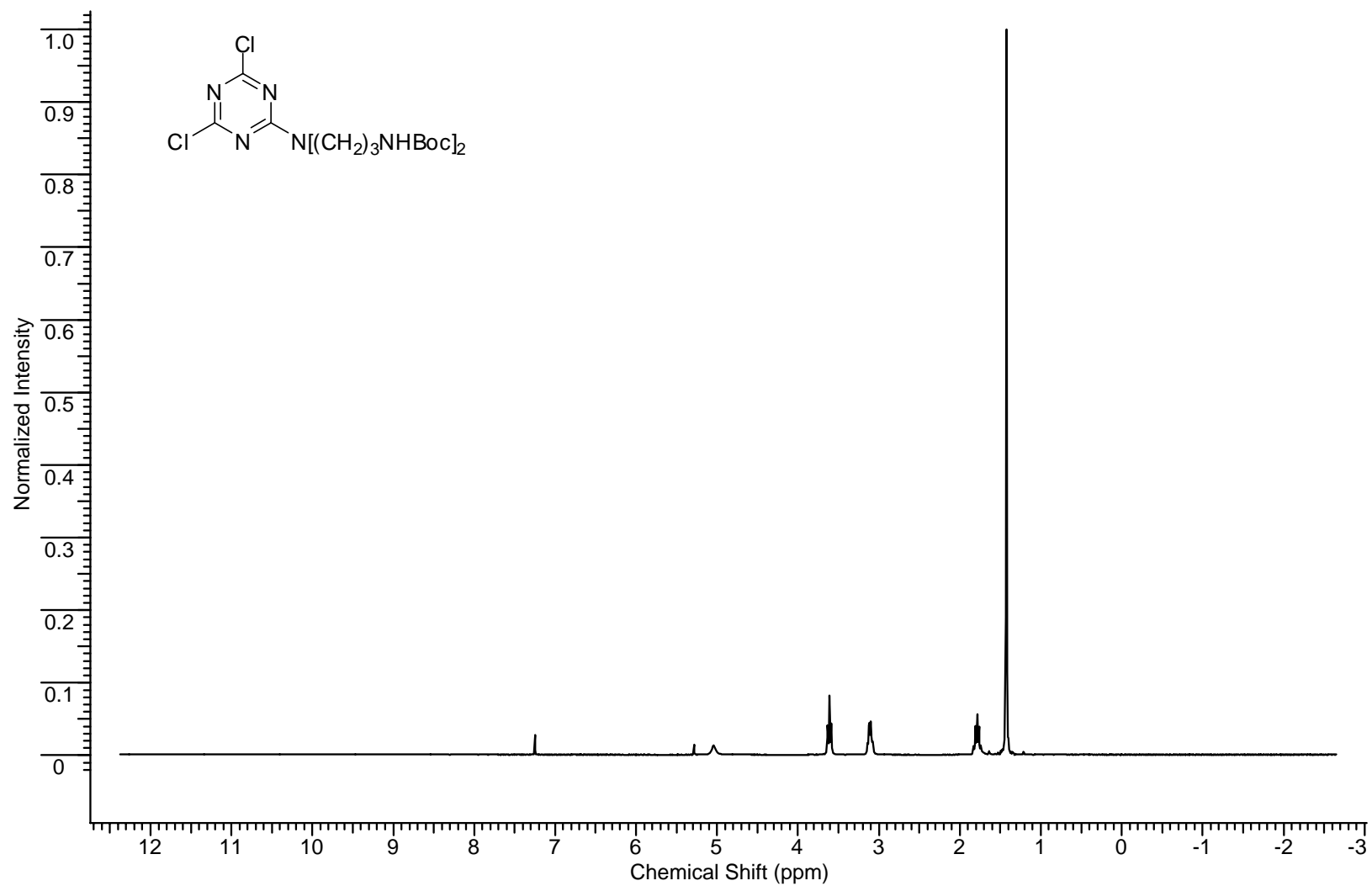


Divergent Diversity Dendrimer: Compound 18, ESI-MS

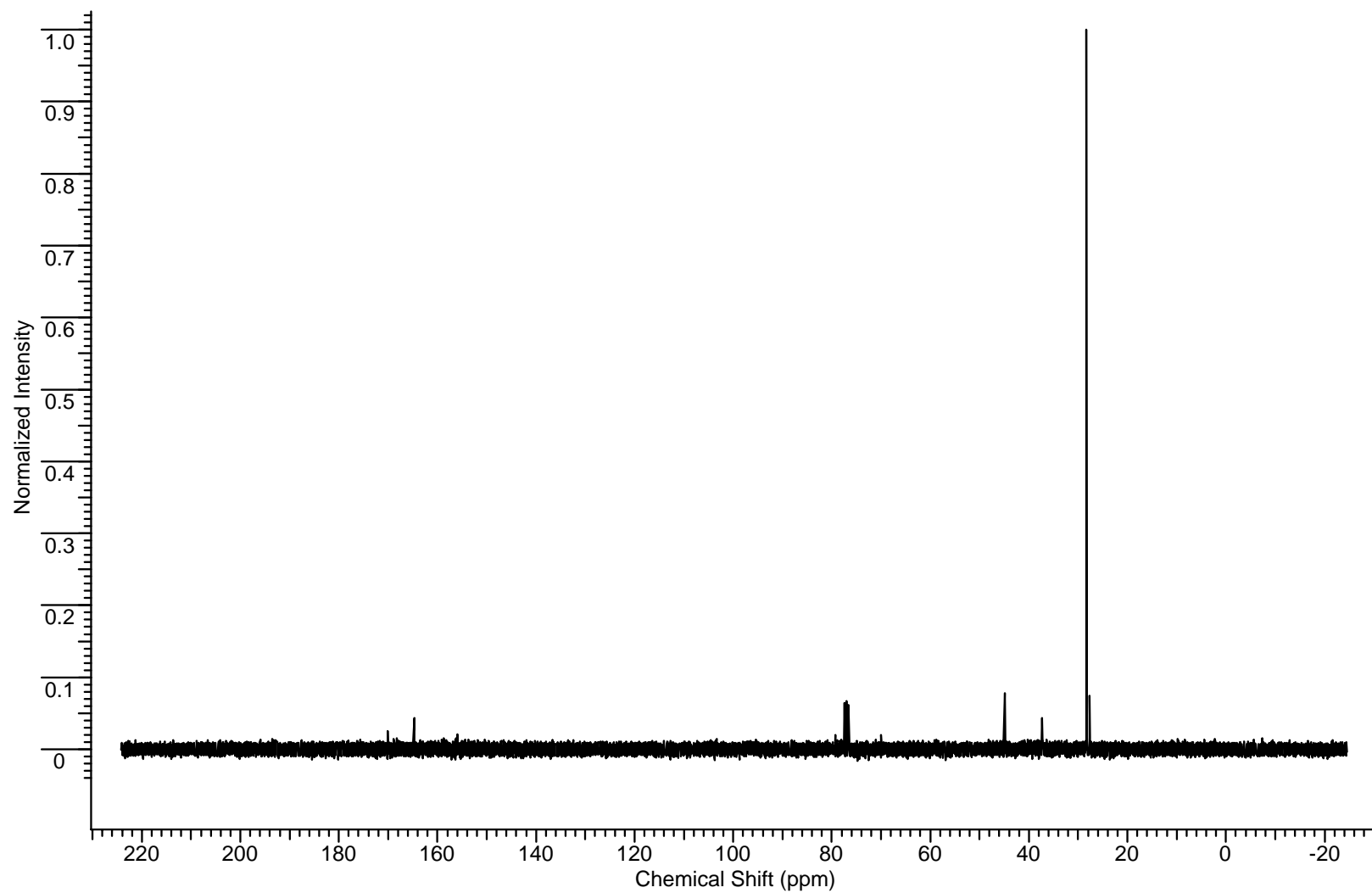
+TOF MS: 1.833 to 4.933 min from Sample 2 (sh3-54) of 02160604.wiff
t=3.55259115544 s, s0e-004, t0=4.70672986167664930e+001



Divergent Diversity Dendrimer: Compound **19**, ^1H NMR

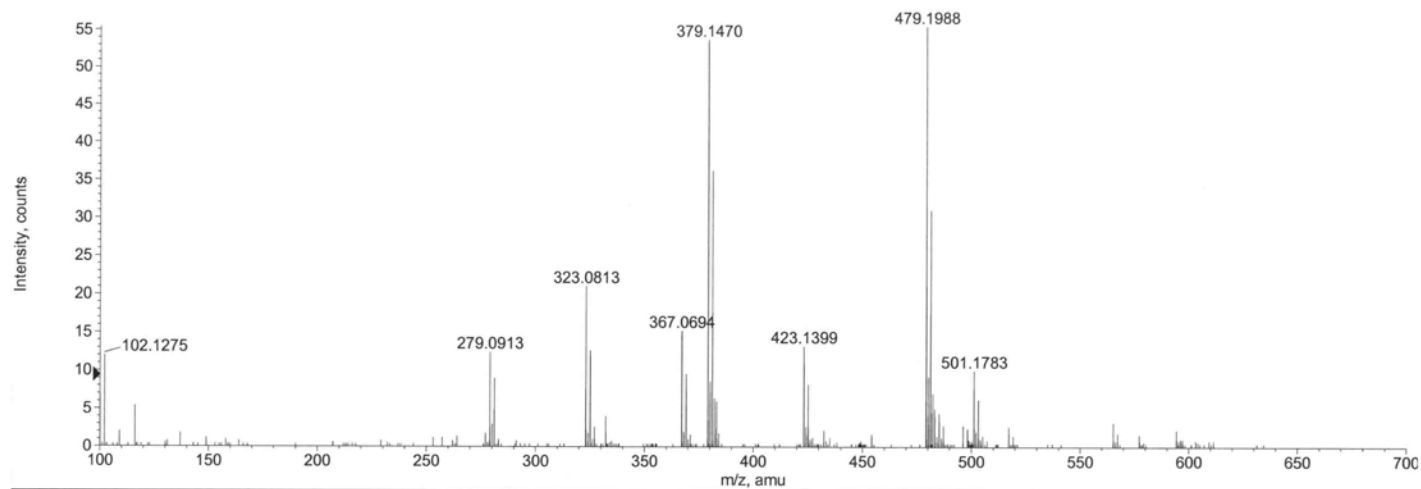


Divergent Diversity Dendrimer: Compound **19**, ^{13}C NMR



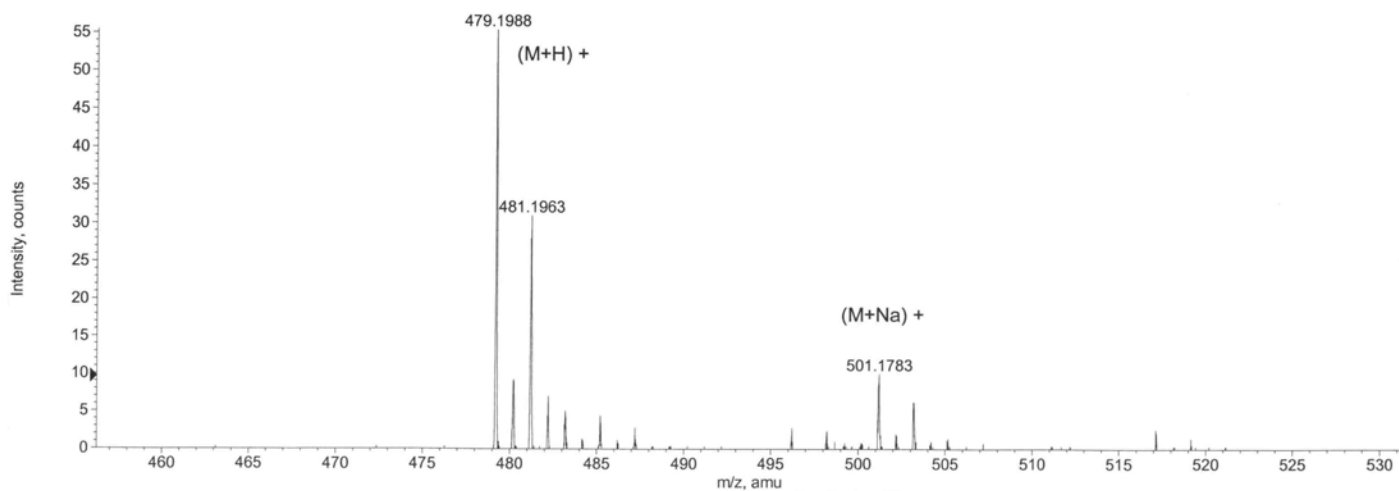
Divergent Diversity Dendrimer: Compound 19, ESI-MS

+TOF MS: 1.500 to 1.567 min from 11040511.wiff
a=3.55274758355 20e-004, t0=4.51038252762409680e+001

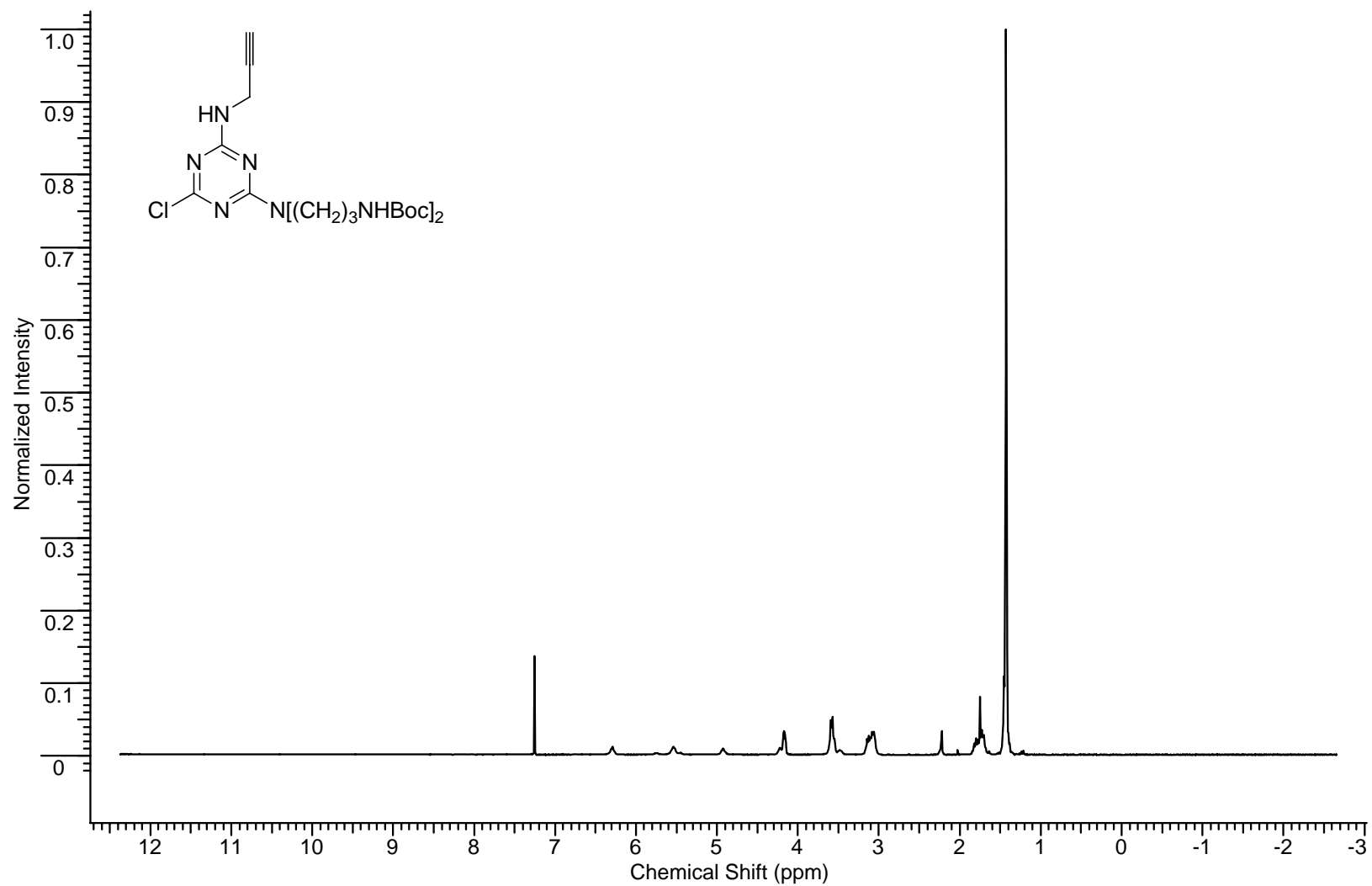


+TOF MS: 1.500 to 1.567 min from 11040511.wiff
a=3.55274758355959020e-004, t0=4.51038252762409680e+001

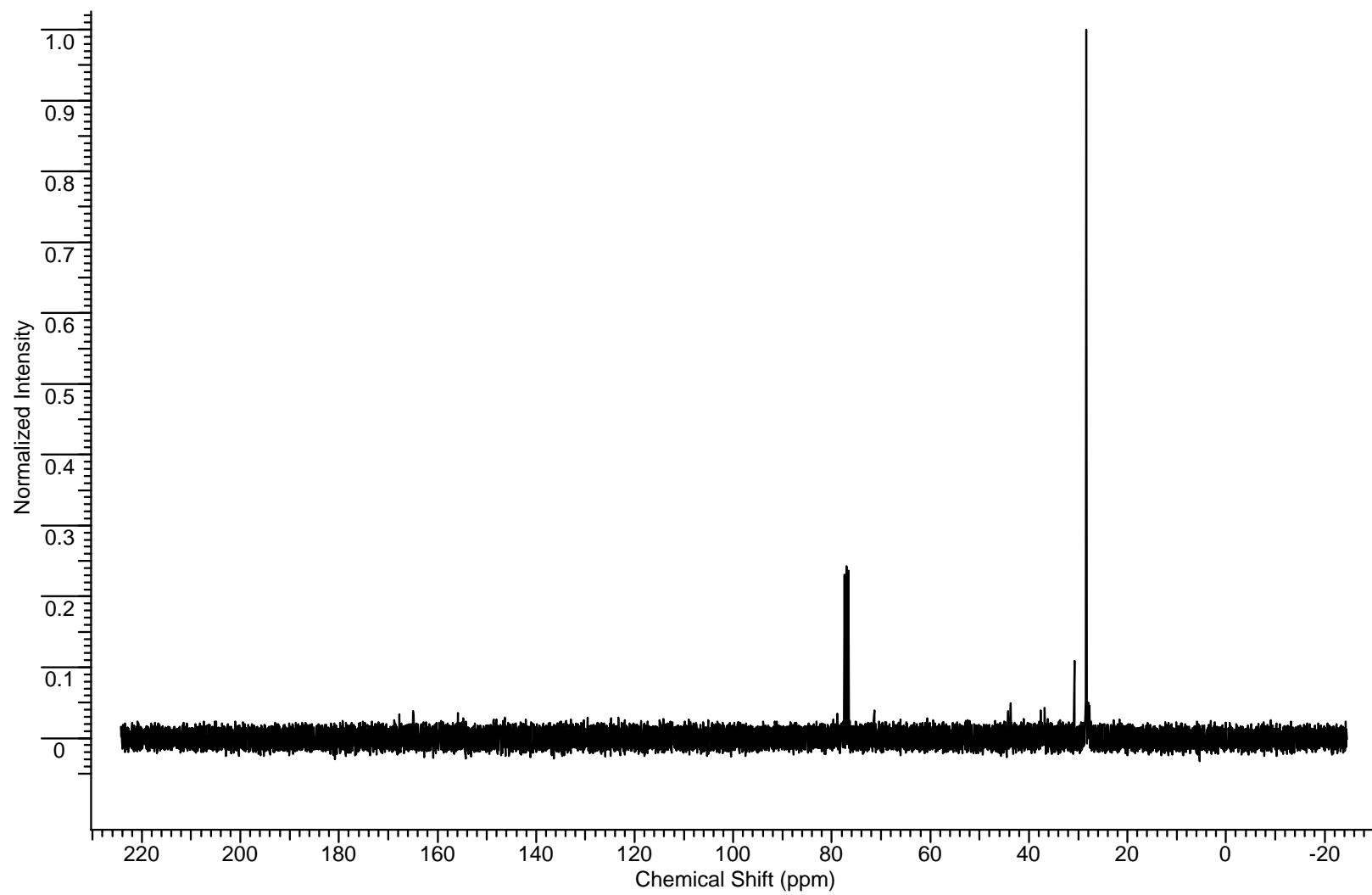
Max. 55.4 counts



Divergent Diversity Dendrimer: Compound **20**, ^1H NMR

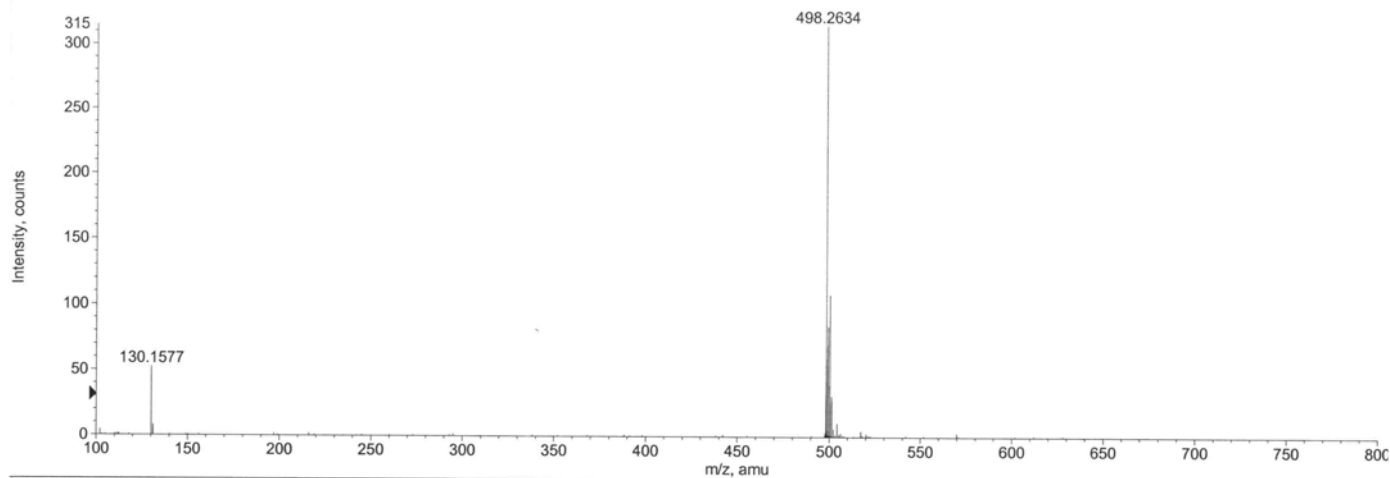


Divergent Diversity Dendrimer: Compound **20**, ^{13}C NMR

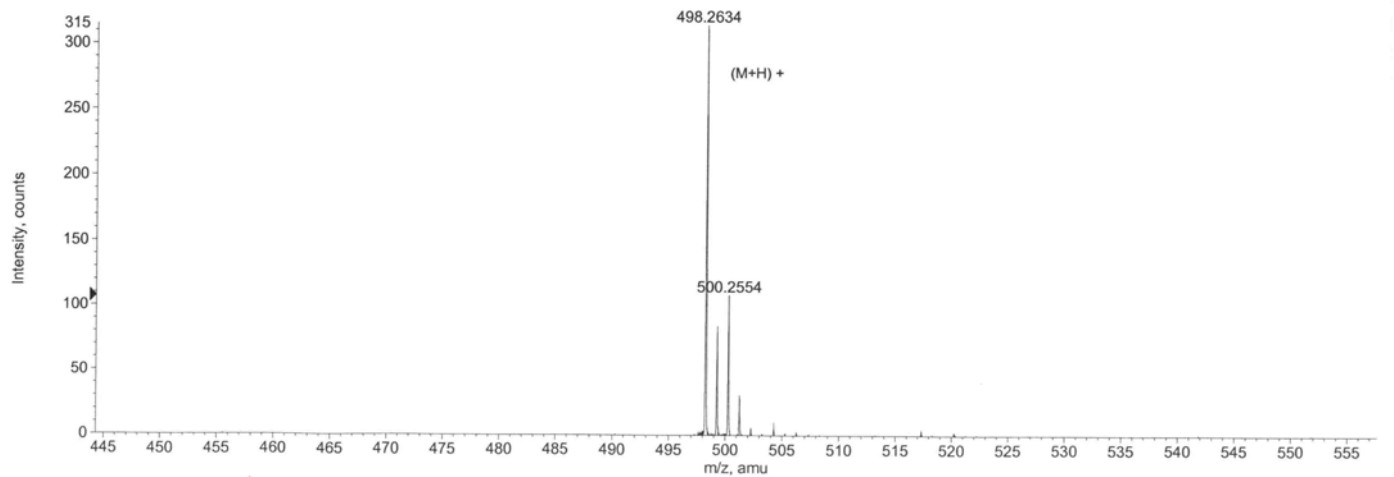


Divergent Diversity Dendrimer: Compound **20**, ESI-MS

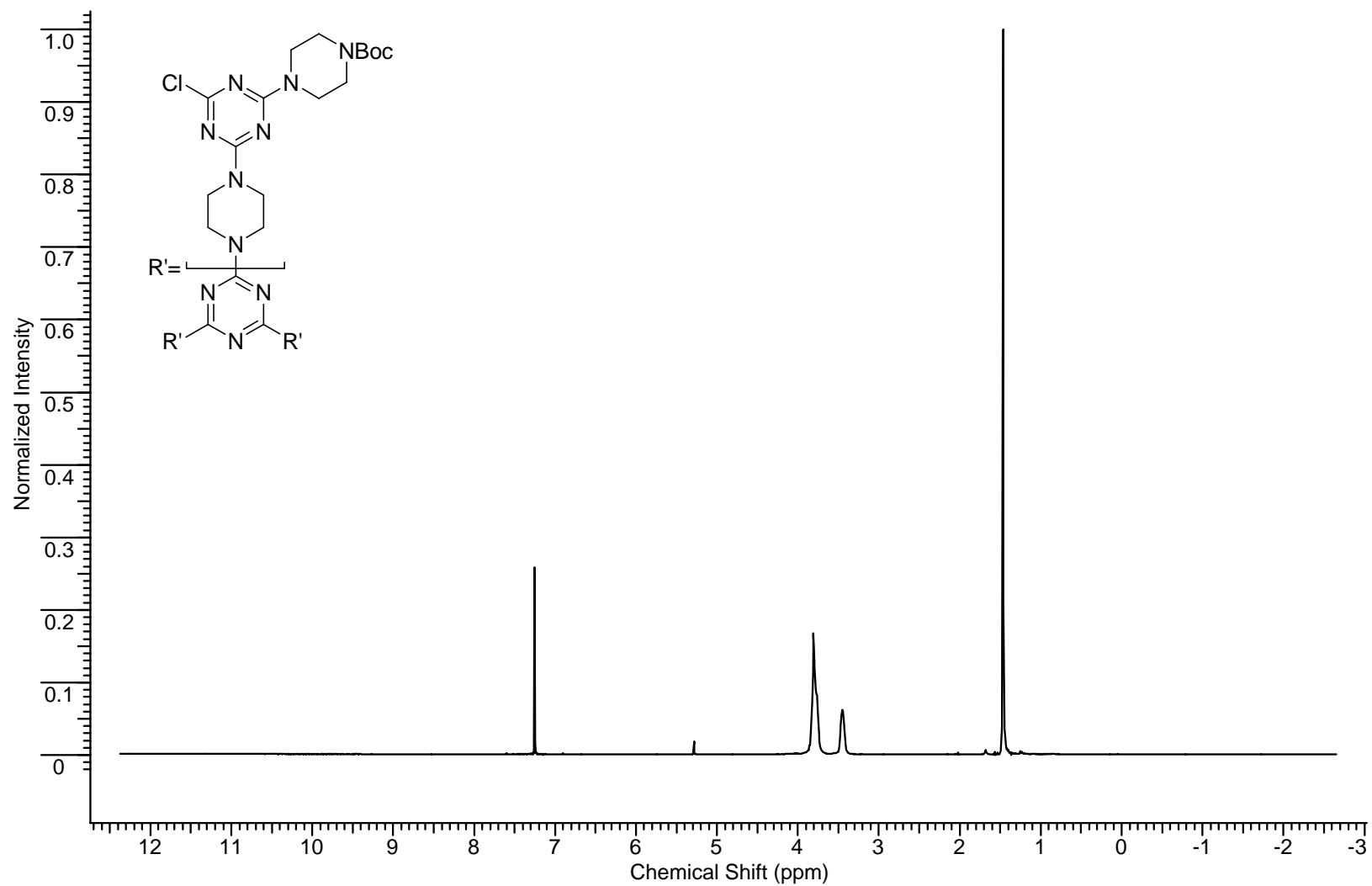
+TOF MS: 0.050 to 0.067 min from 02080604.wiff
a=3.55261755241, t0=4.76746673941051990e+001



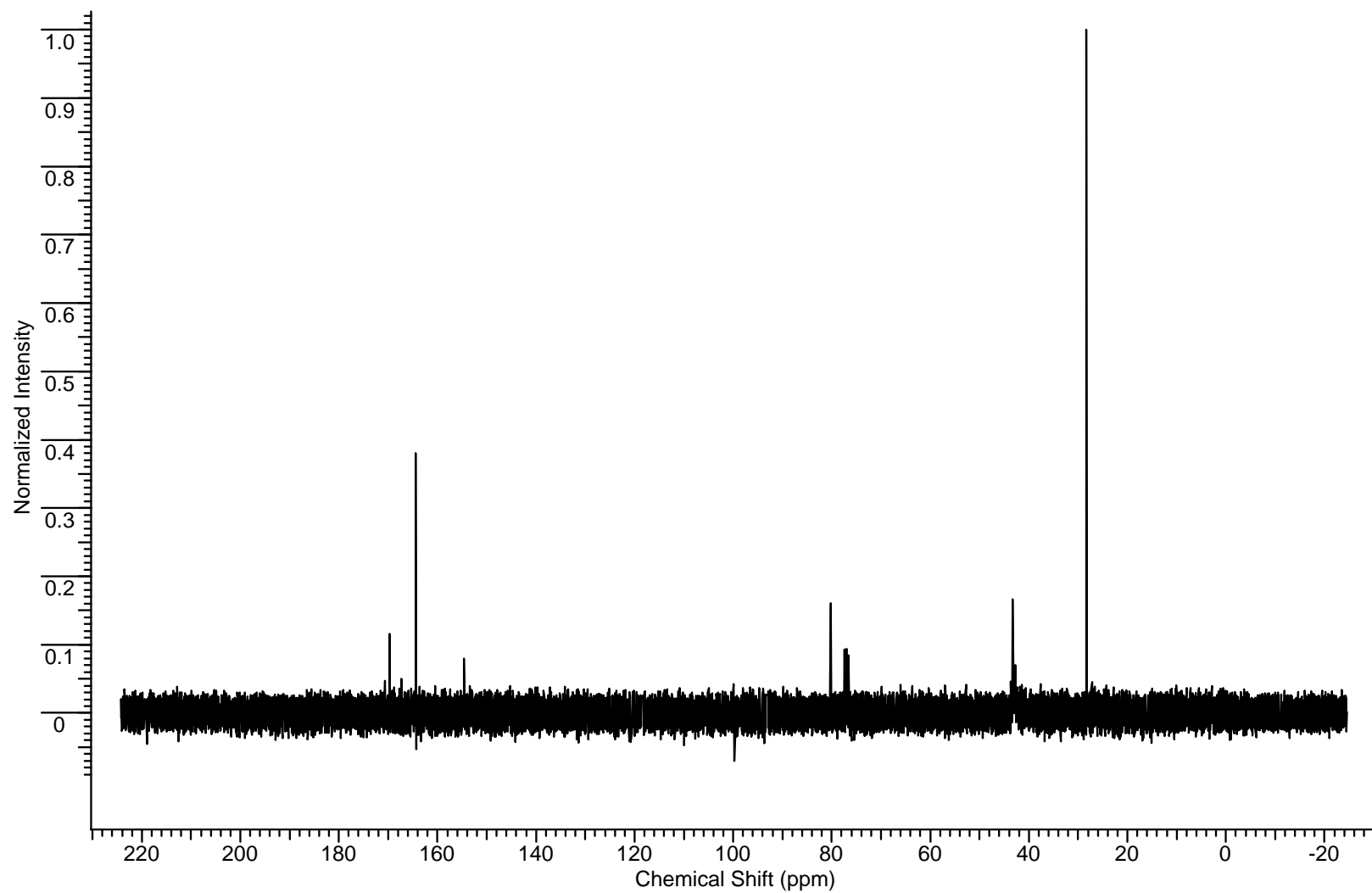
+TOF MS: 0.050 to 0.067 min from 02080604.wiff
a=3.55261755241757040e-004, t0=4.76746673941051990e+001



Divergent Diversity Dendrimer: Compound **21**, ^1H NMR

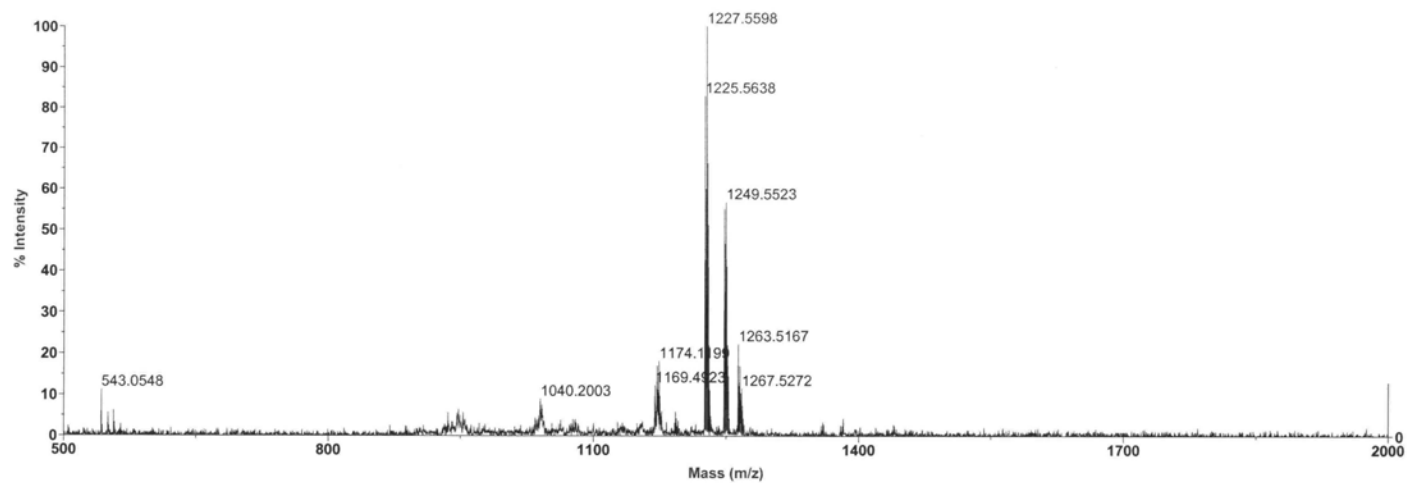


Divergent Diversity Dendrimer: Compound **21**, ^{13}C NMR

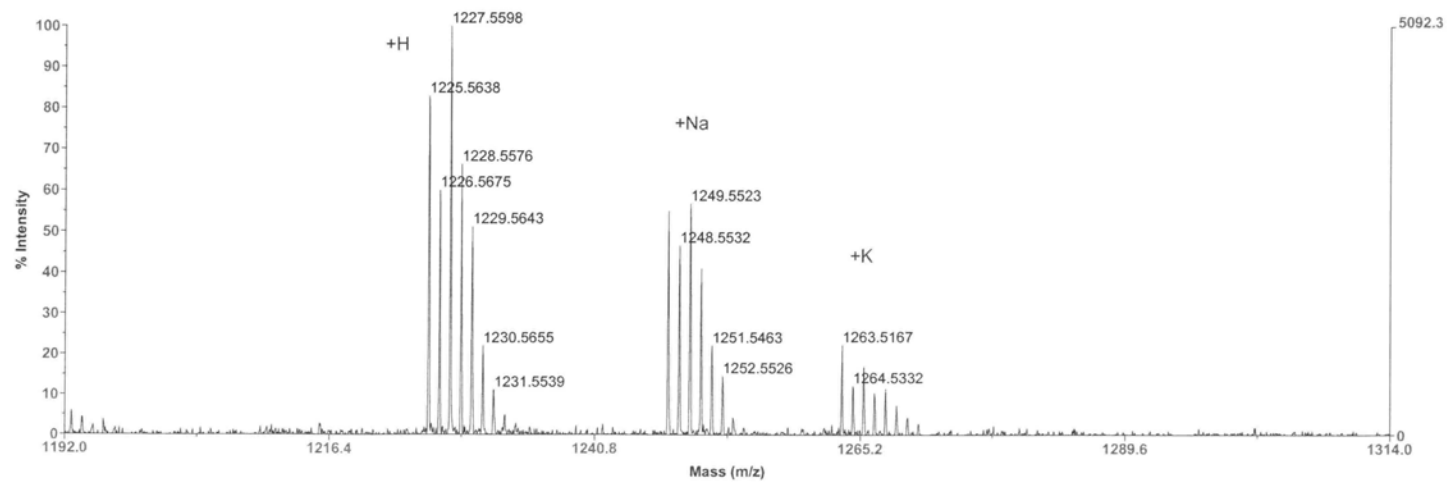


Divergent Diversity Dendrimer: Compound **21**, MALDI-MS

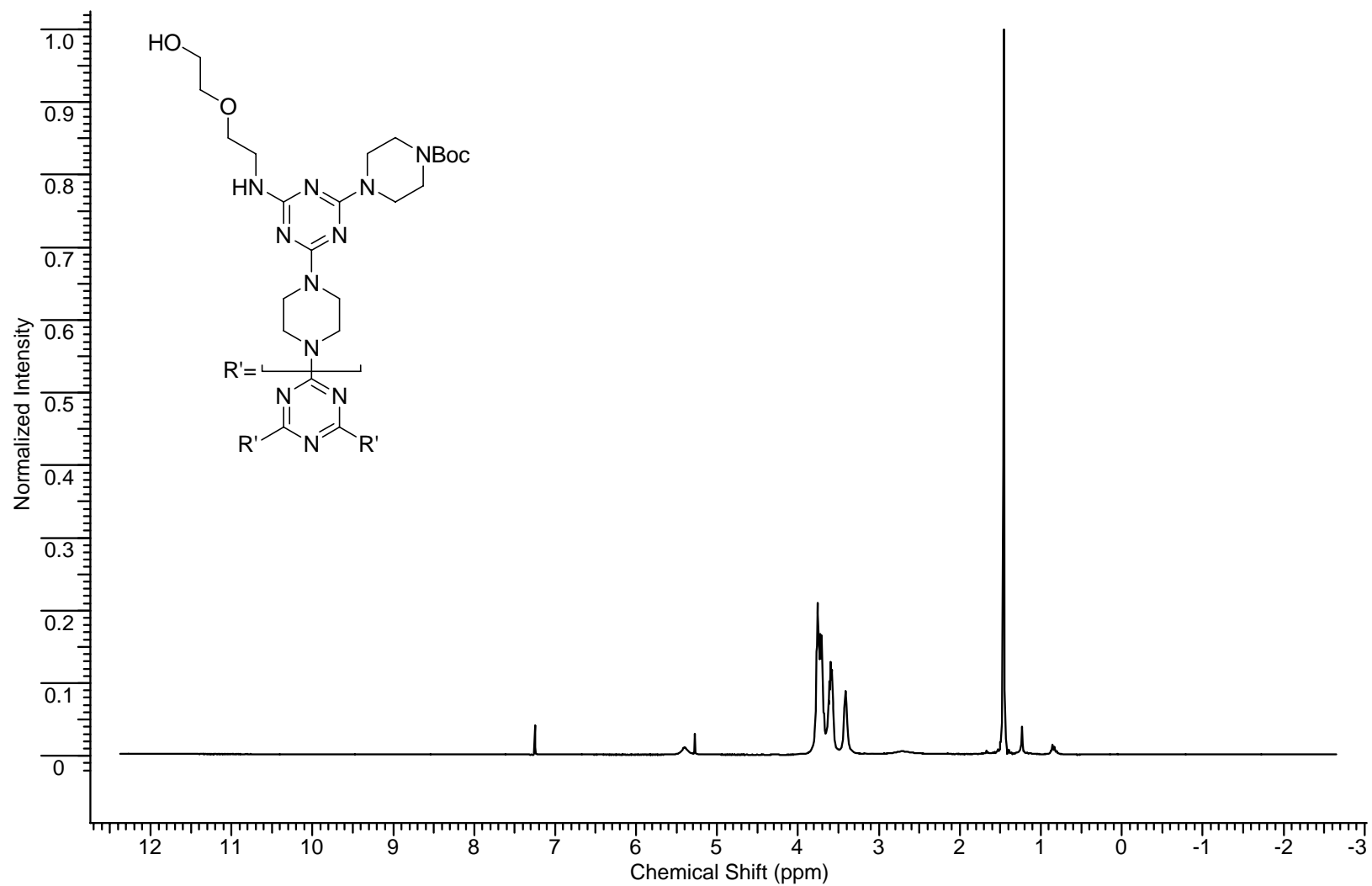
Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 1227.6, 5092]



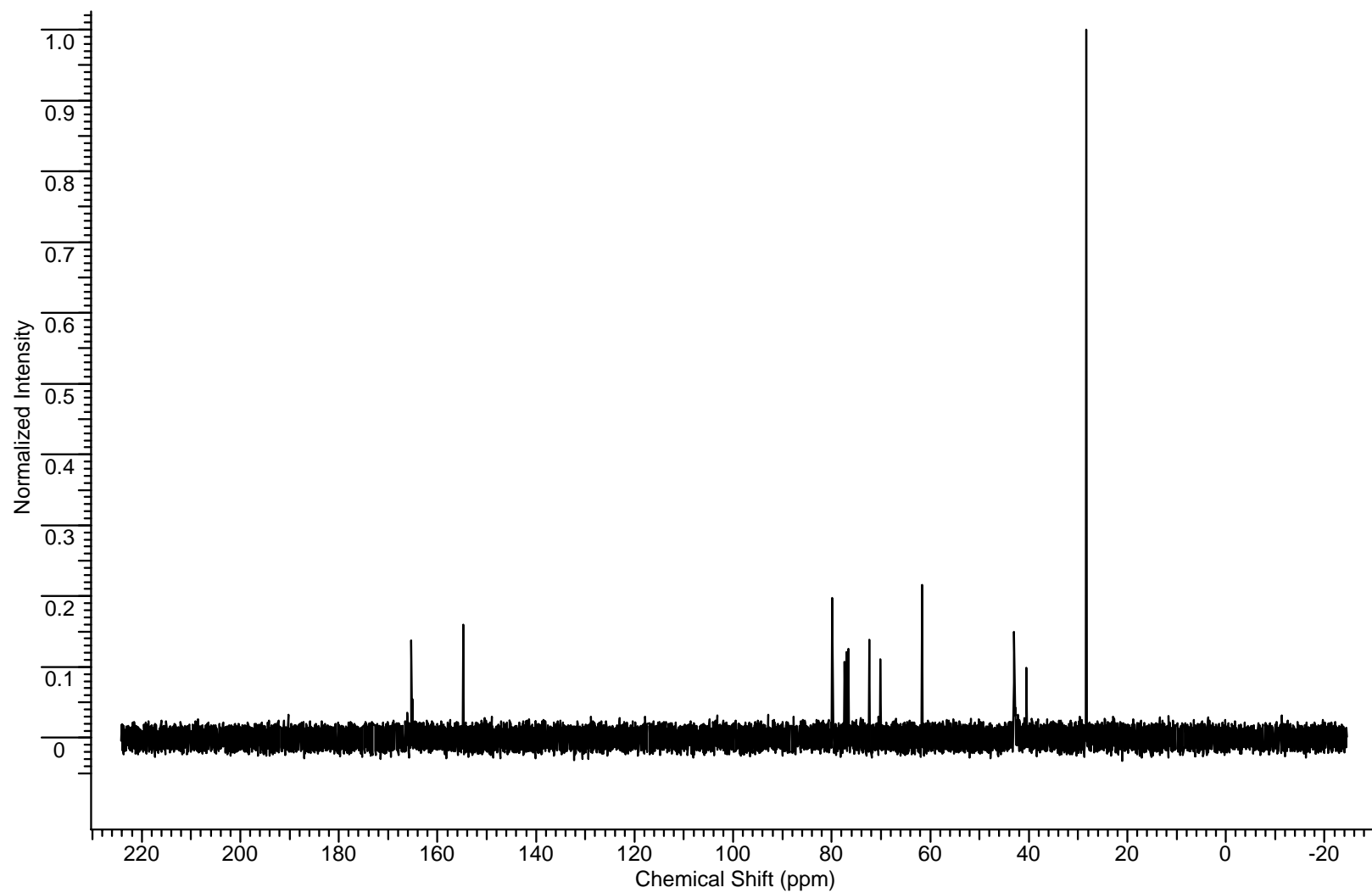
Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 1227.6, 5092]



Divergent Diversity Dendrimer: Compound **22**, ^1H NMR

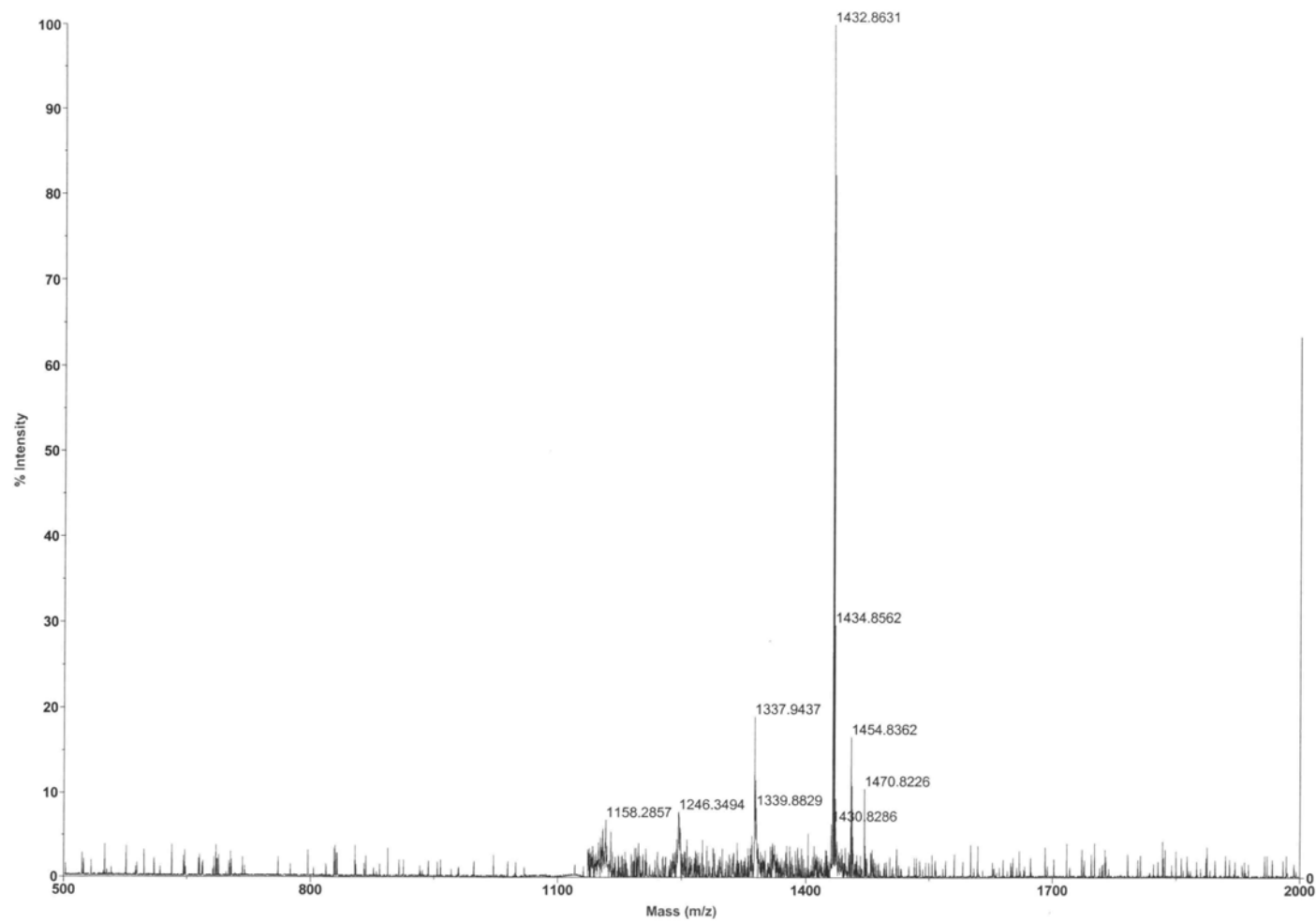


Divergent Diversity Dendrimer: Compound **22**, ^{13}C NMR



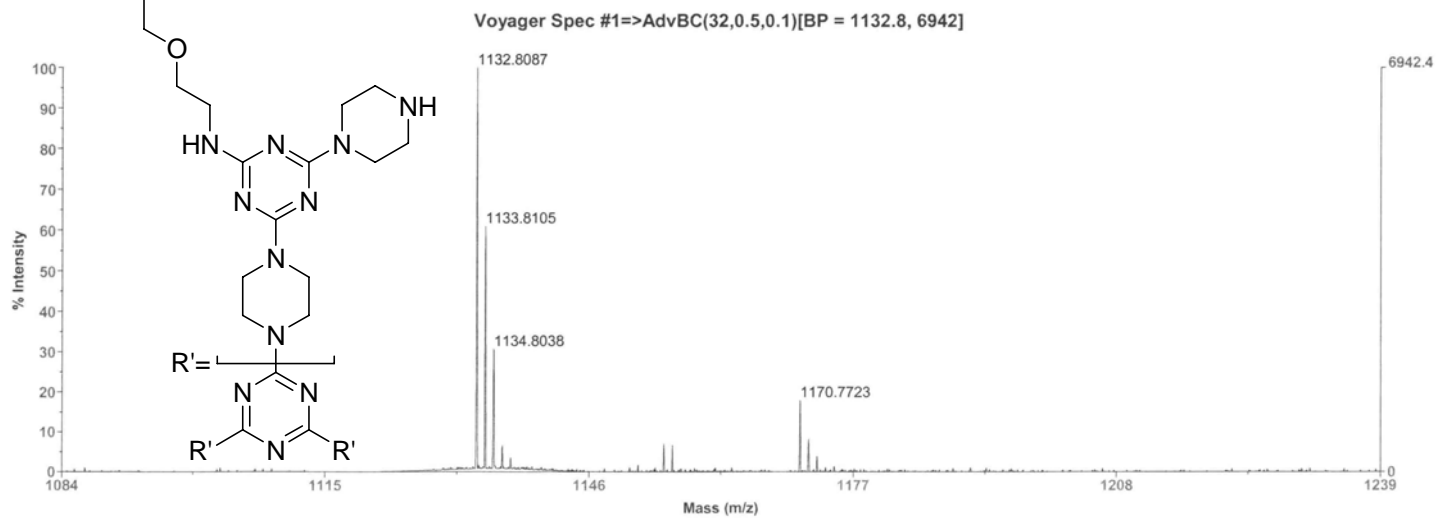
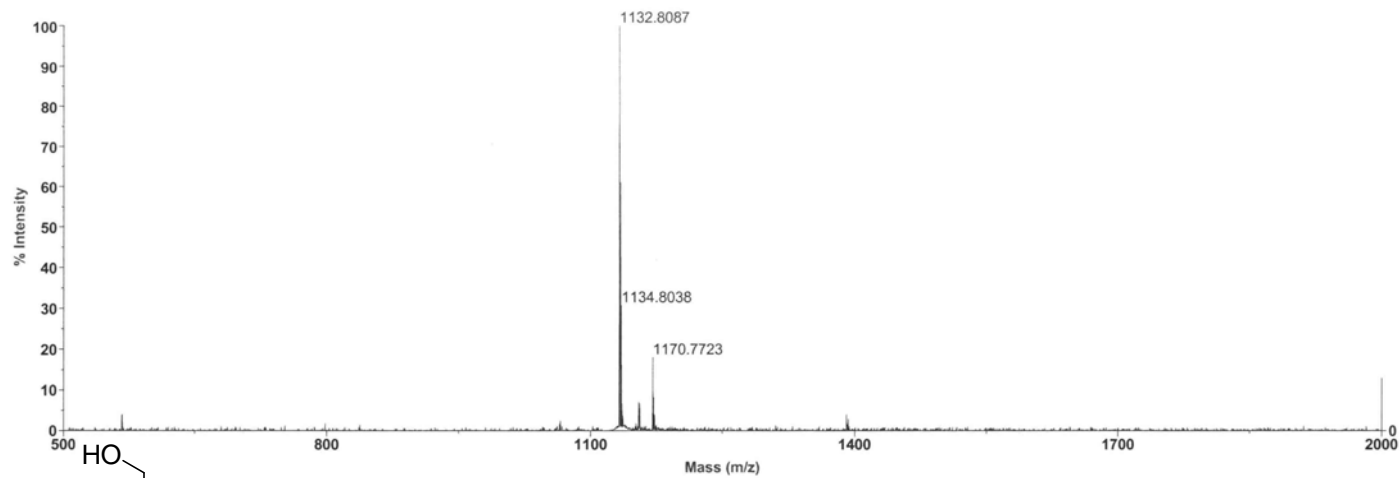
Divergent Diversity Dendrimer: Compound **22**, MALDI-MS

Voyager Spec #1=>AdvBC(32.1, 0.1)=>BC[BP = 1432.9, 1821]



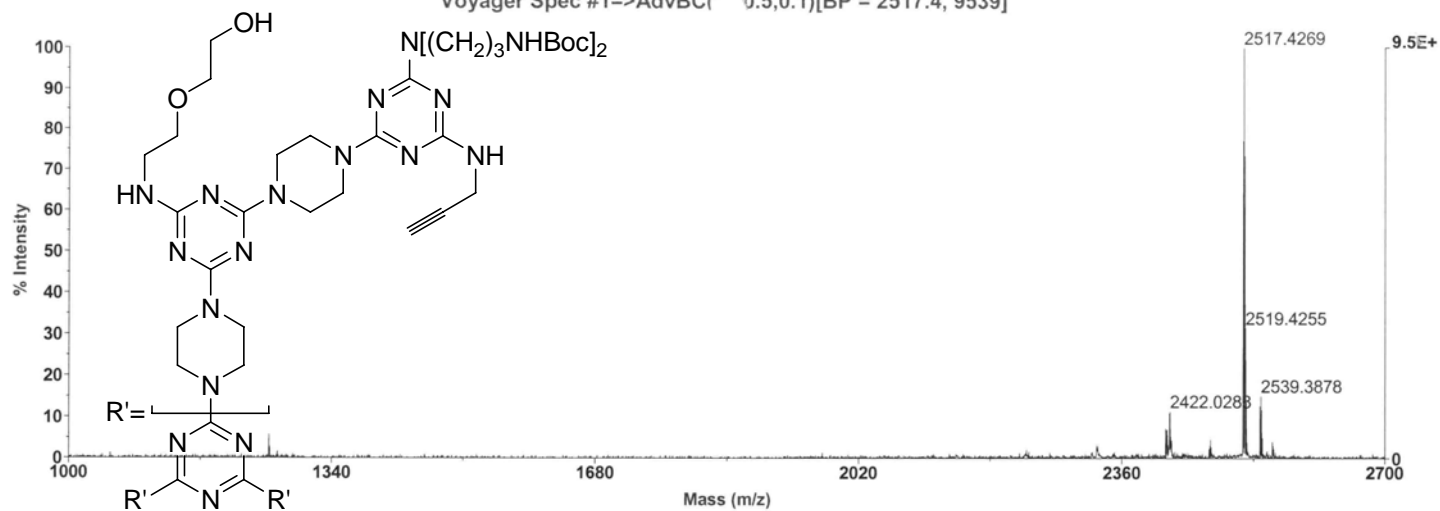
Divergent Diversity Dendrimer: Compound **23**, MALDI-MS

Voyager Spec #1=>AdvBC(5,0,1)[BP = 1132.8, 6942]

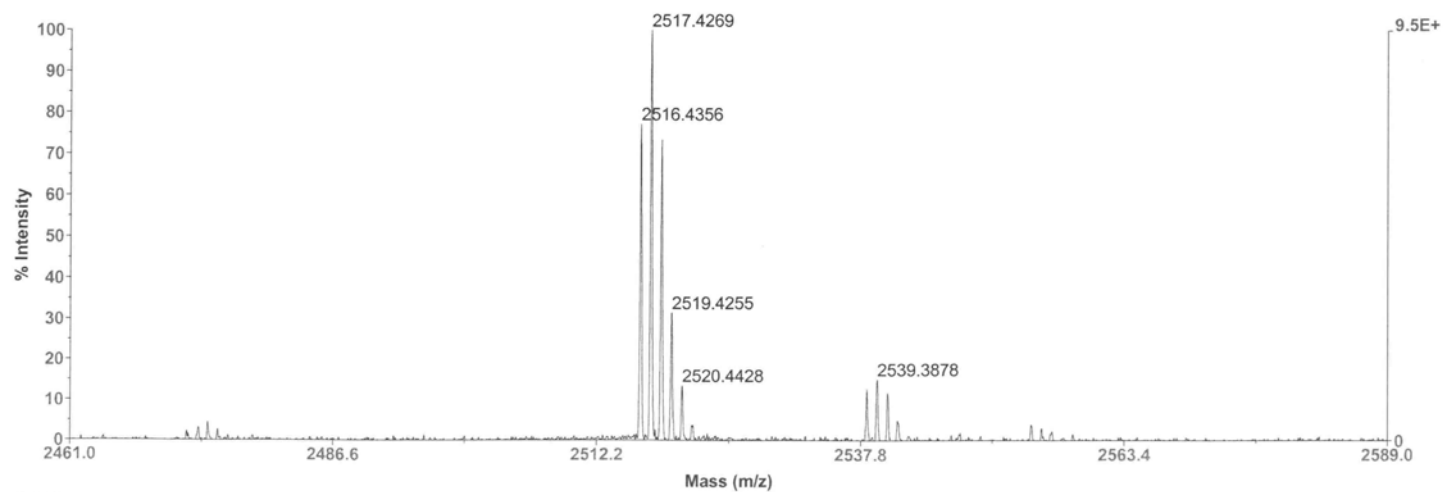


Divergent Diversity Dendrimer: Compound **24**, MALDI-MS

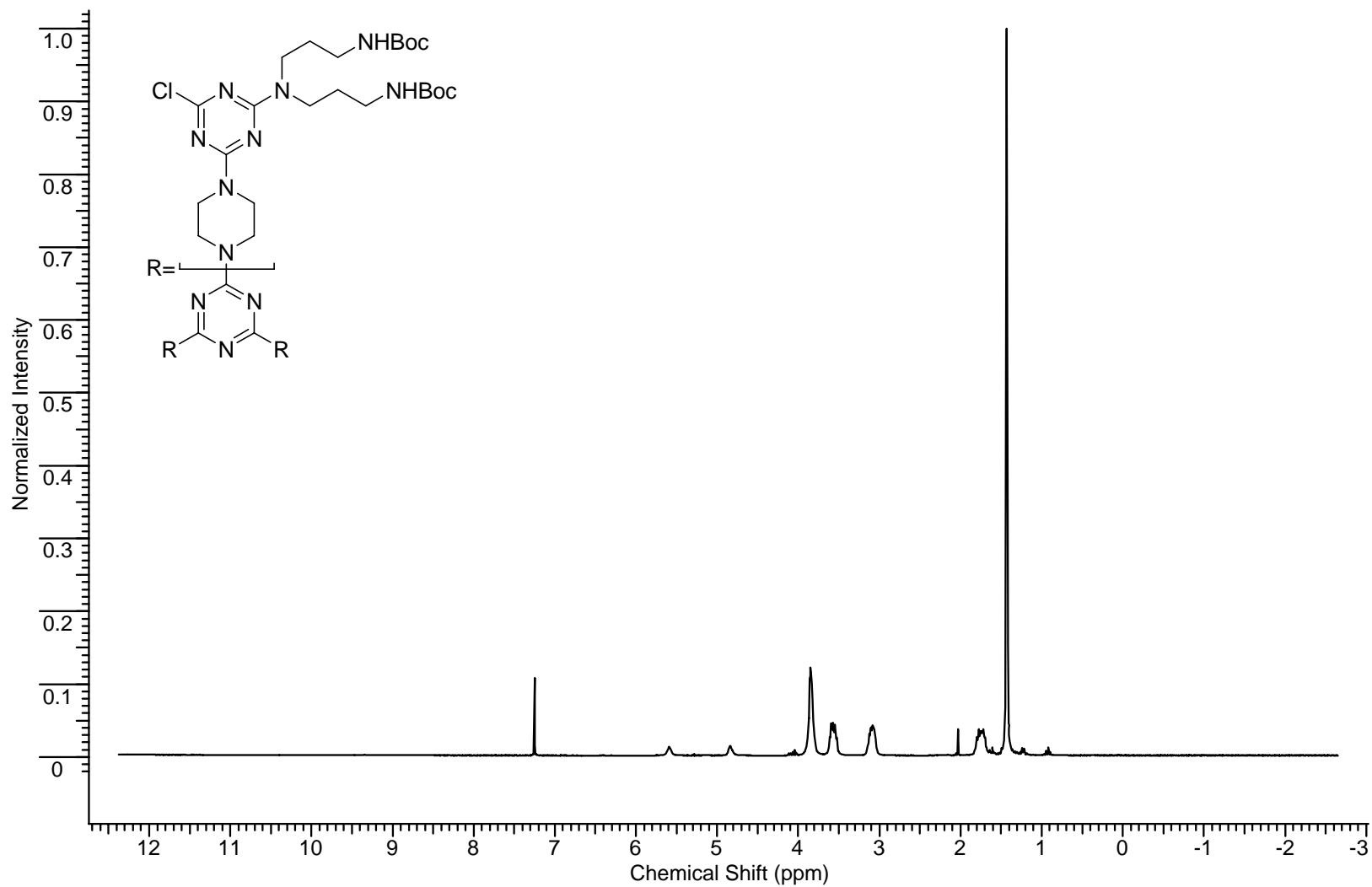
Voyager Spec #1=>AdvBC(0.5,0.1)[BP = 2517.4, 9539]



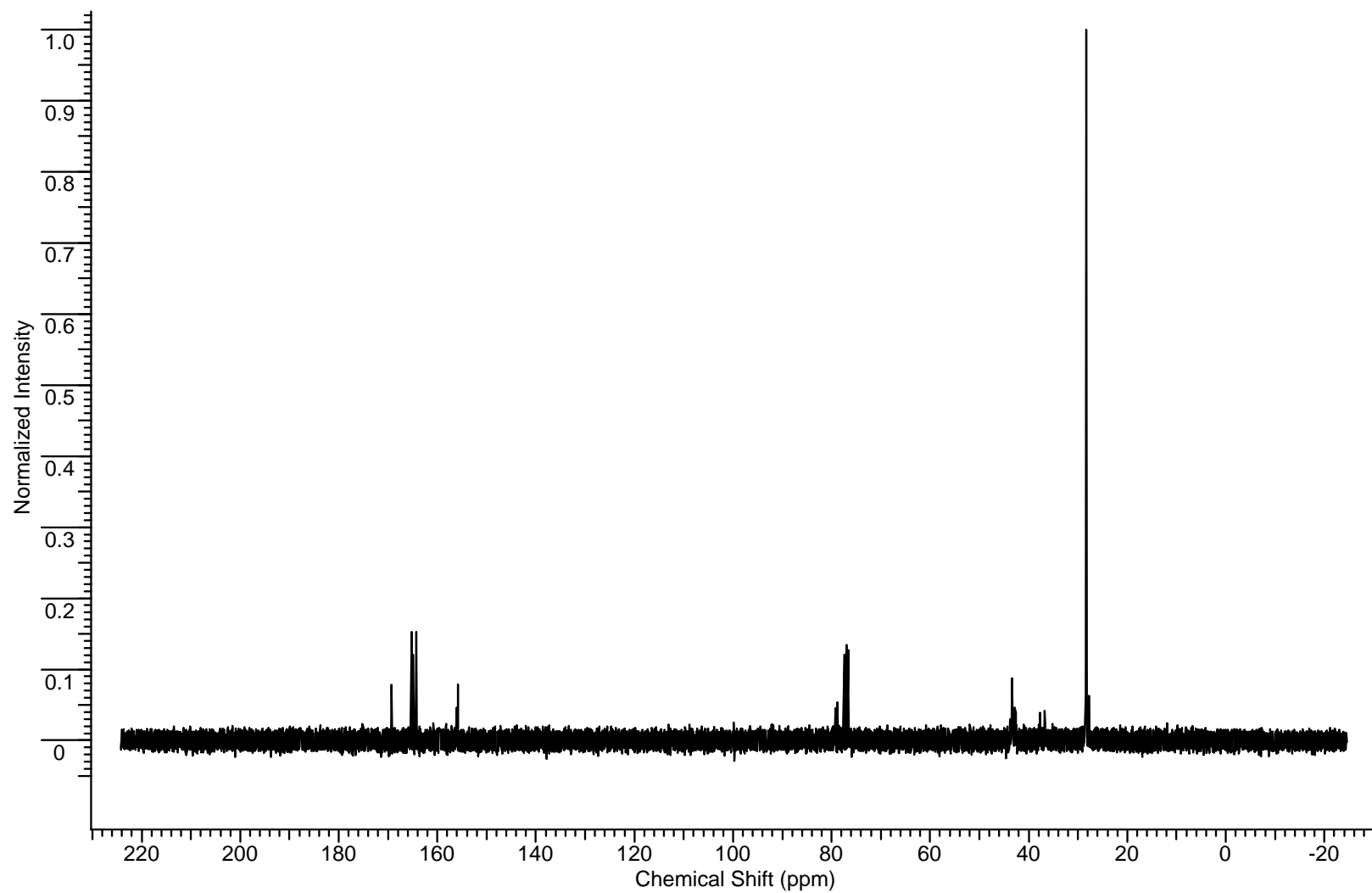
Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 2517.4, 9539]



Iodinated Dendrimer: Compound **25**, ^1H NMR

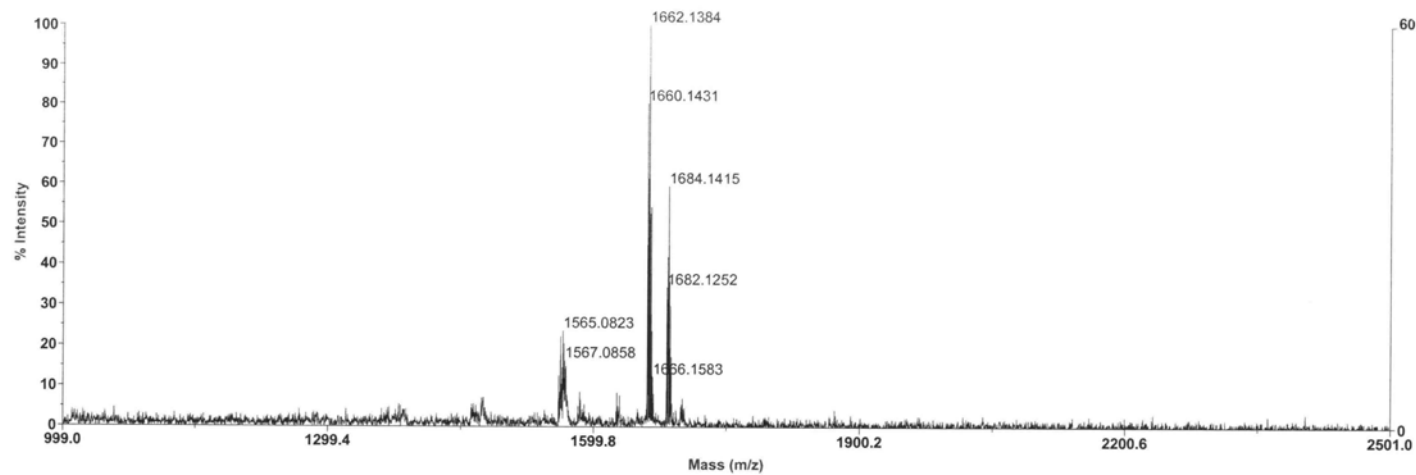


Iodinated Dendrimer: Compound **25**, ^{13}C NMR

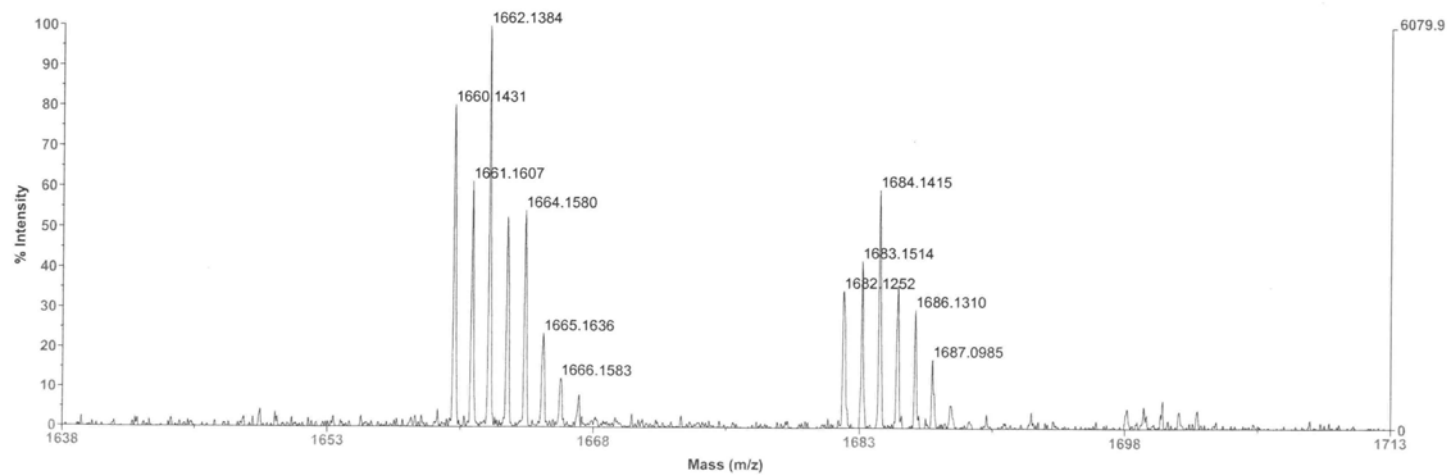


Iodinated Dendrimer: Compound **25**, MALDI-MS

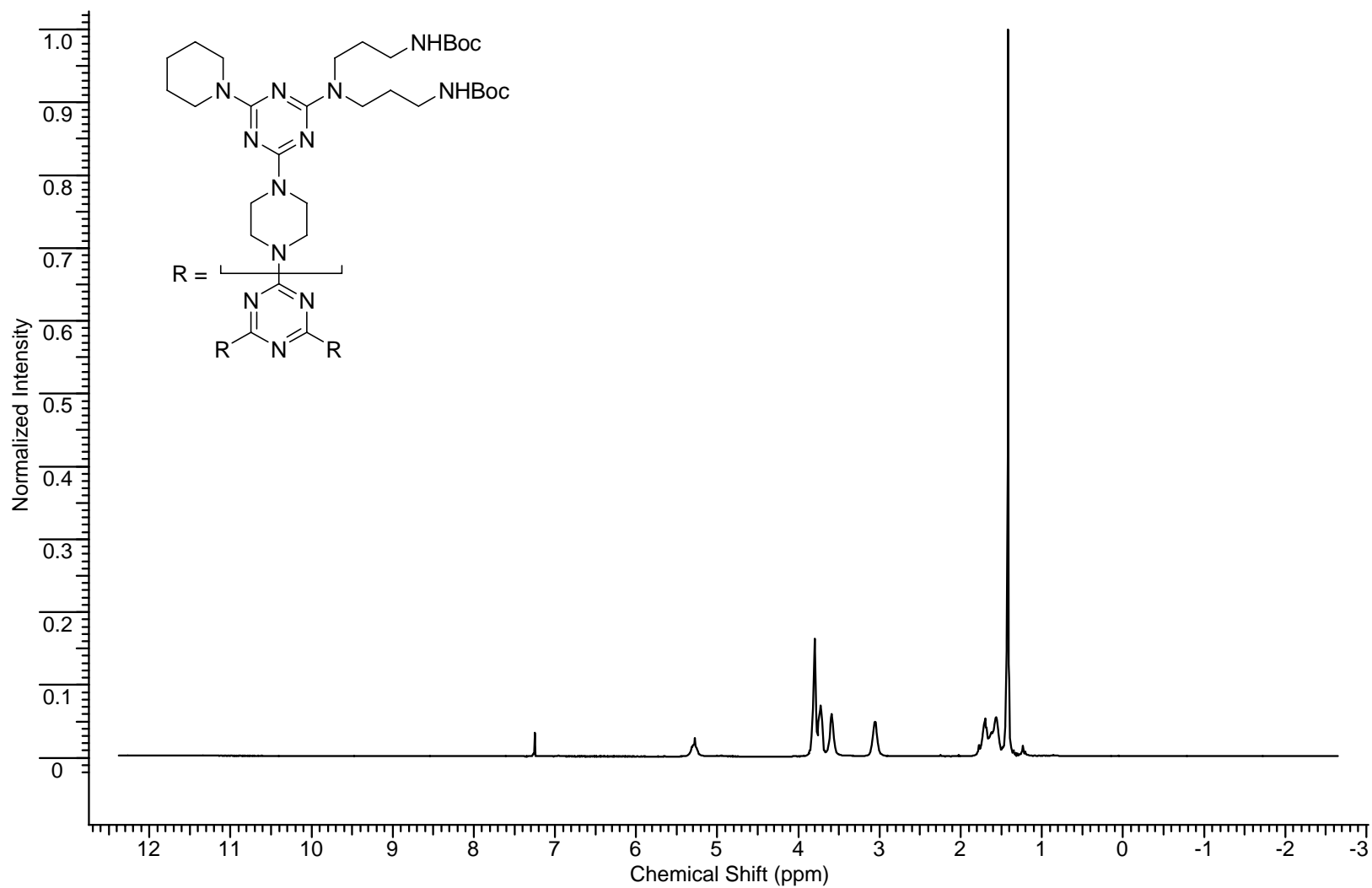
Voyager Spec #1=>AdvB[7,0.5,0.1][BP = 1662.1, 6080]



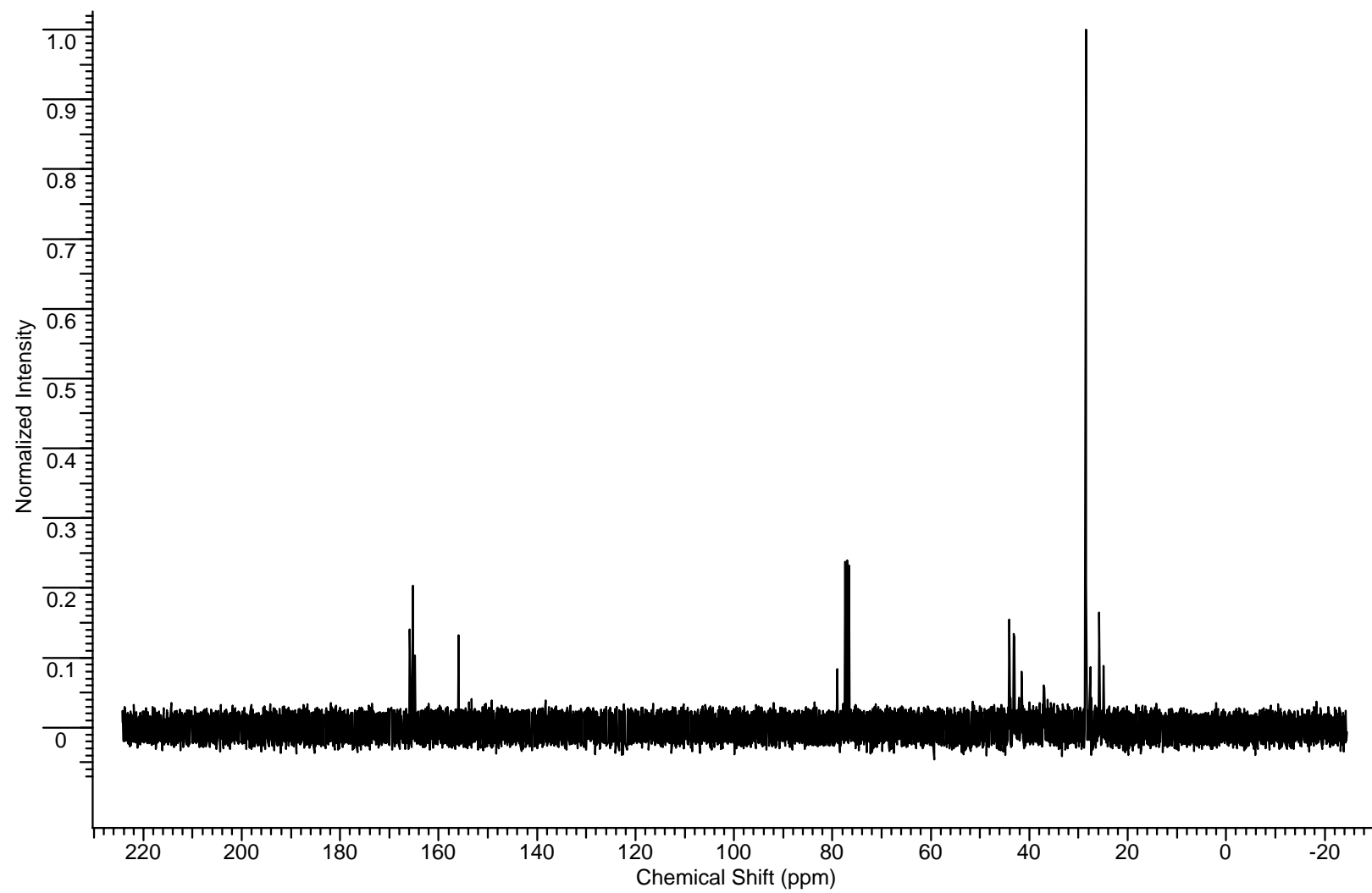
Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 1662.1, 6080]



Iodinated Dendrimer: Compound **26**, ^1H NMR

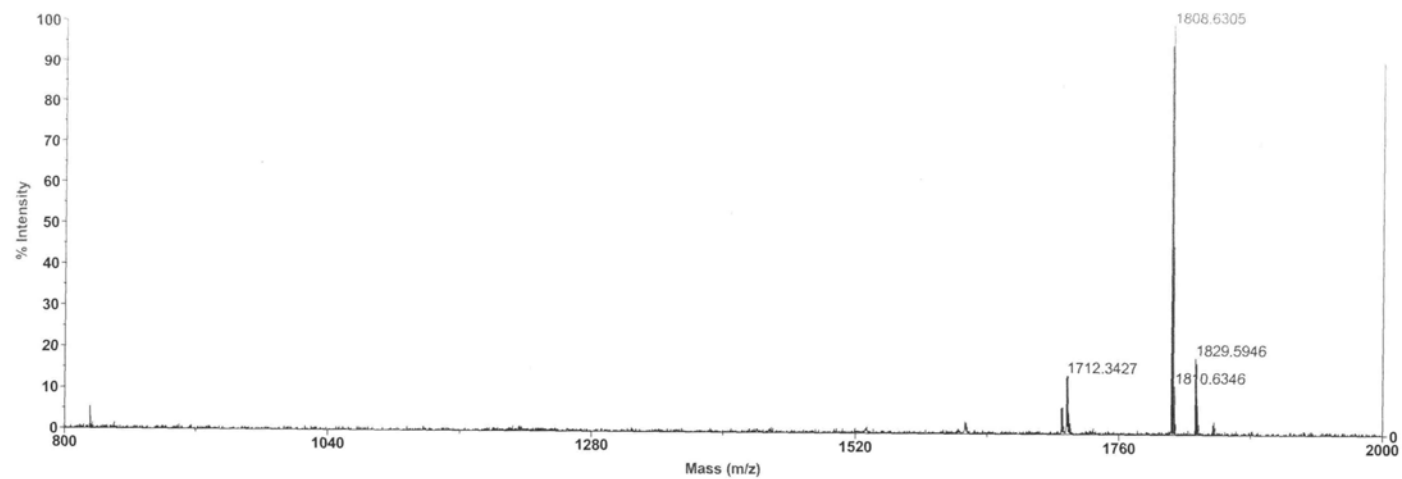


Iodinated Dendrimer: Compound **26**, ^{13}C NMR

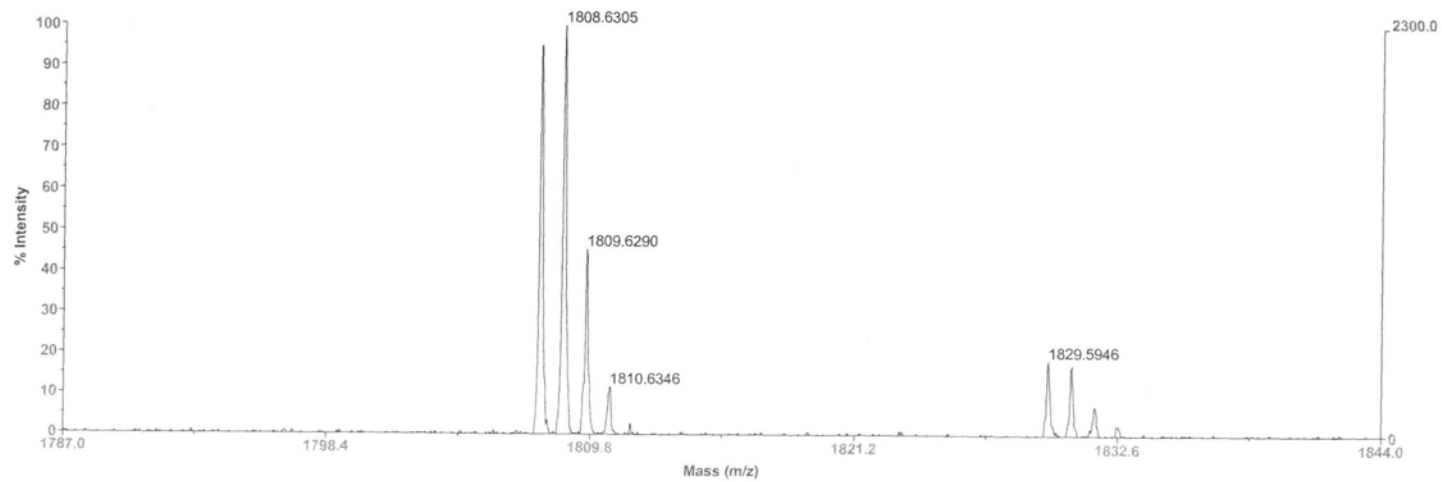


Iodinated Dendrimer: Compound **26**, MALDI-MS

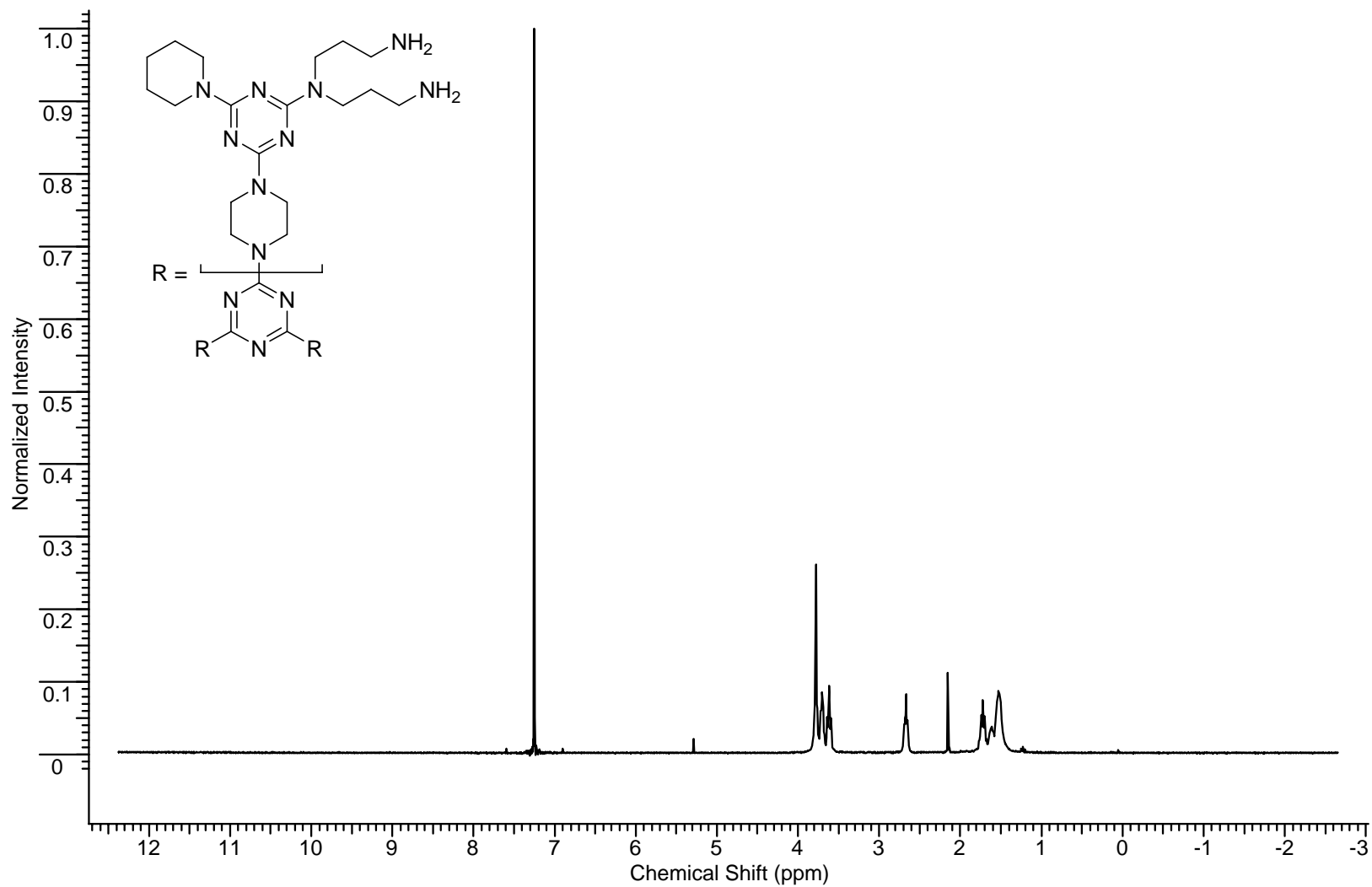
Voyager Spec #1[BP = 1808.6, 2300]



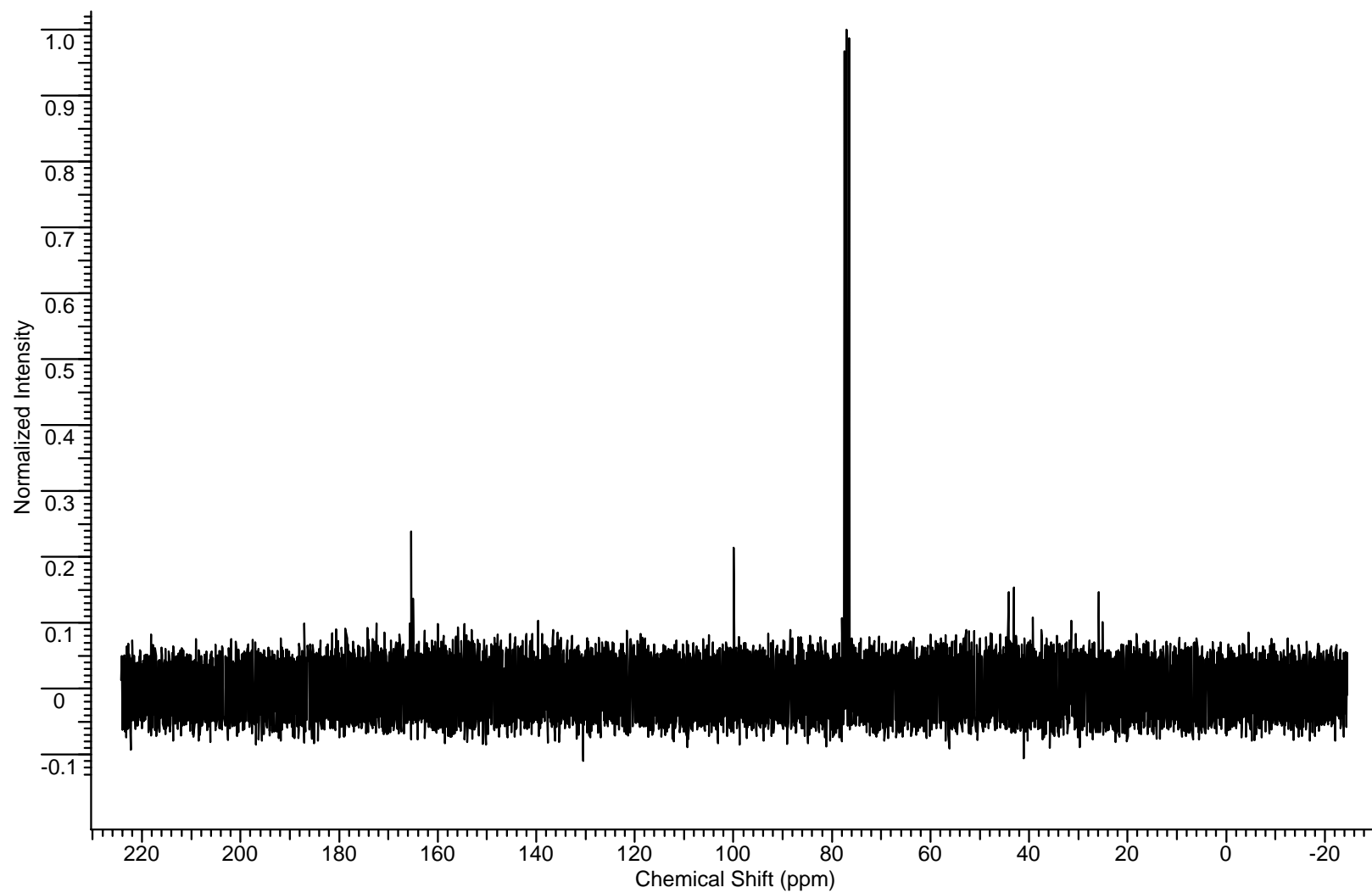
Voyager Spec #1[BP = 1808.6, 2300]



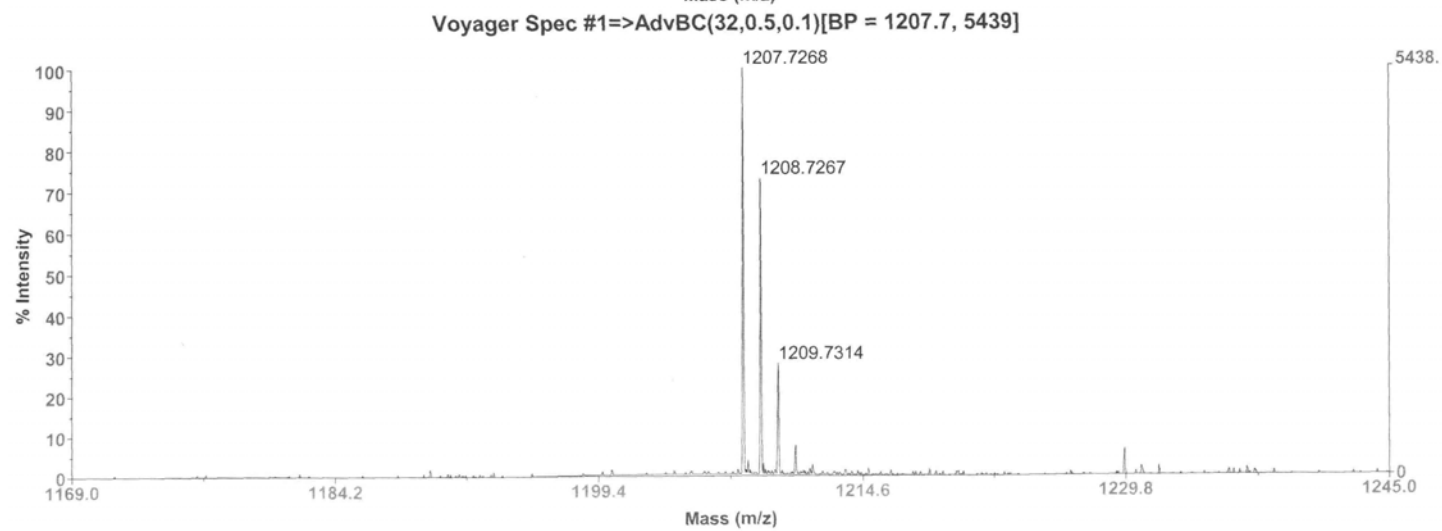
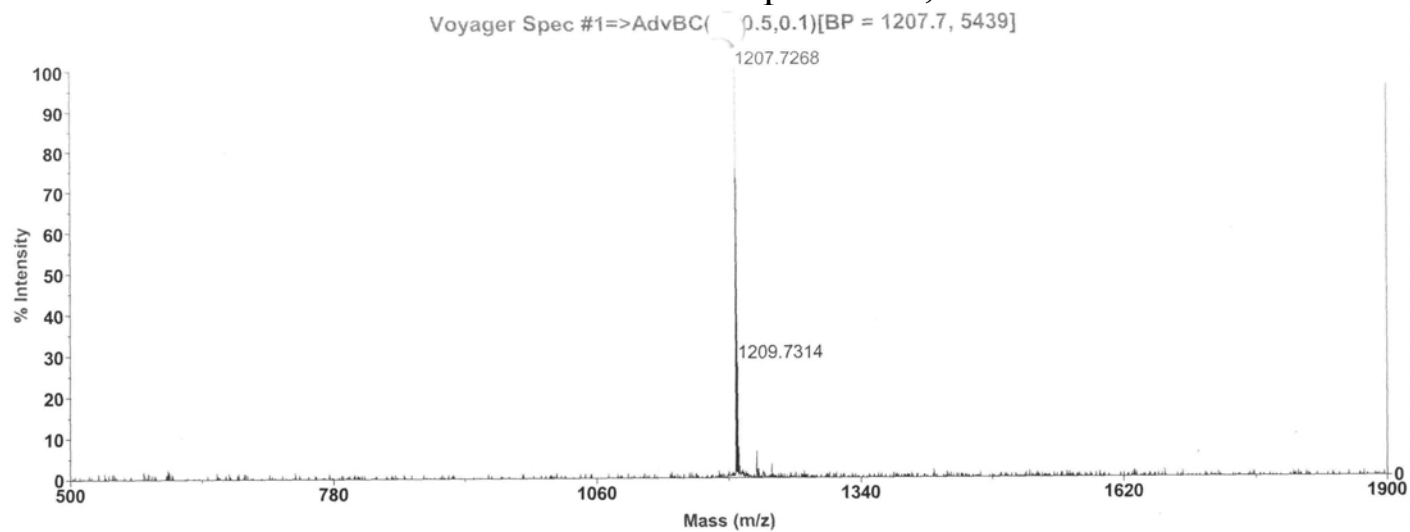
Iodinated Dendrimer: Compound 27, ^1H NMR



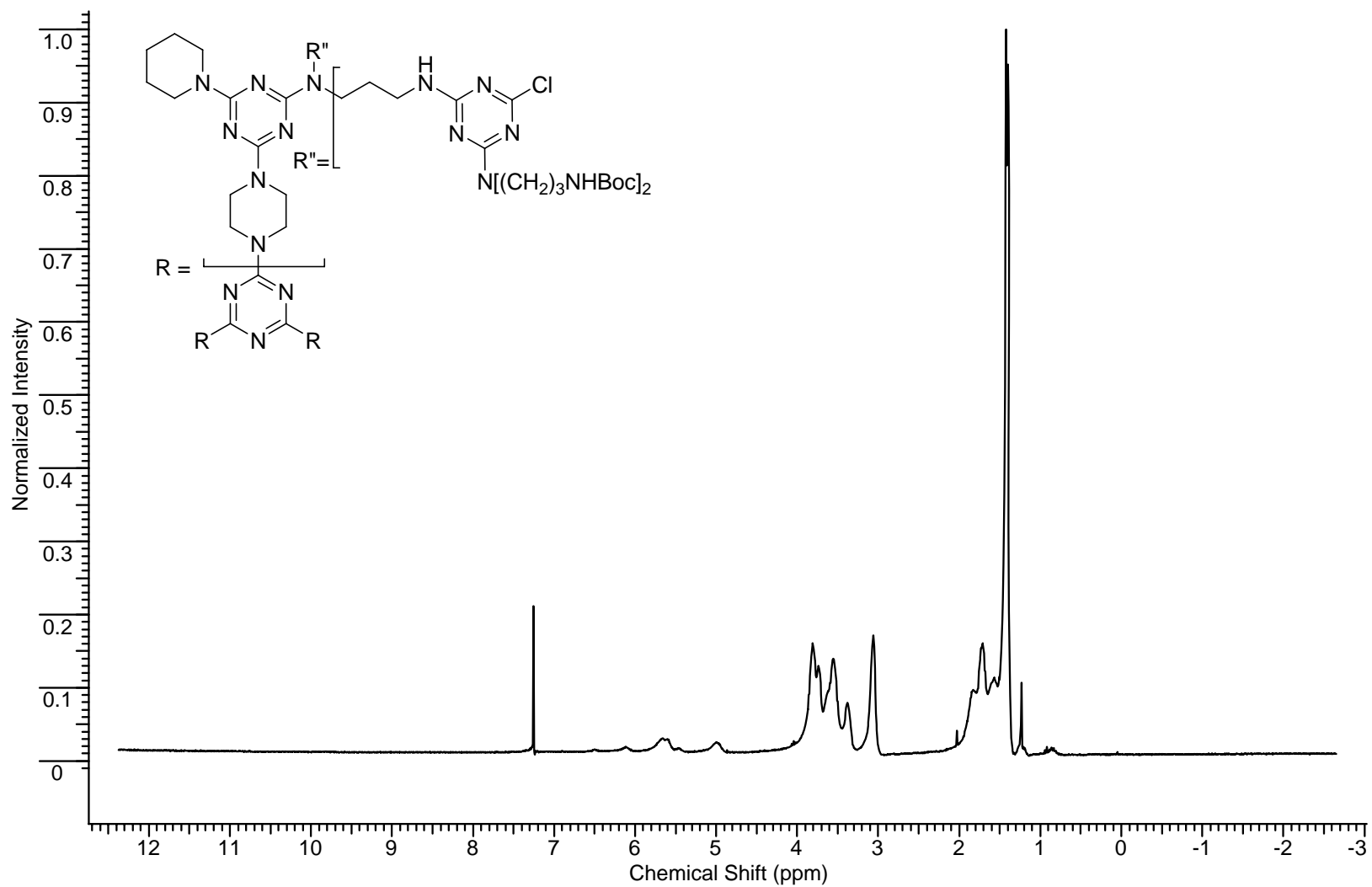
Iodinated Dendrimer: Compound **27**, ^{13}C NMR



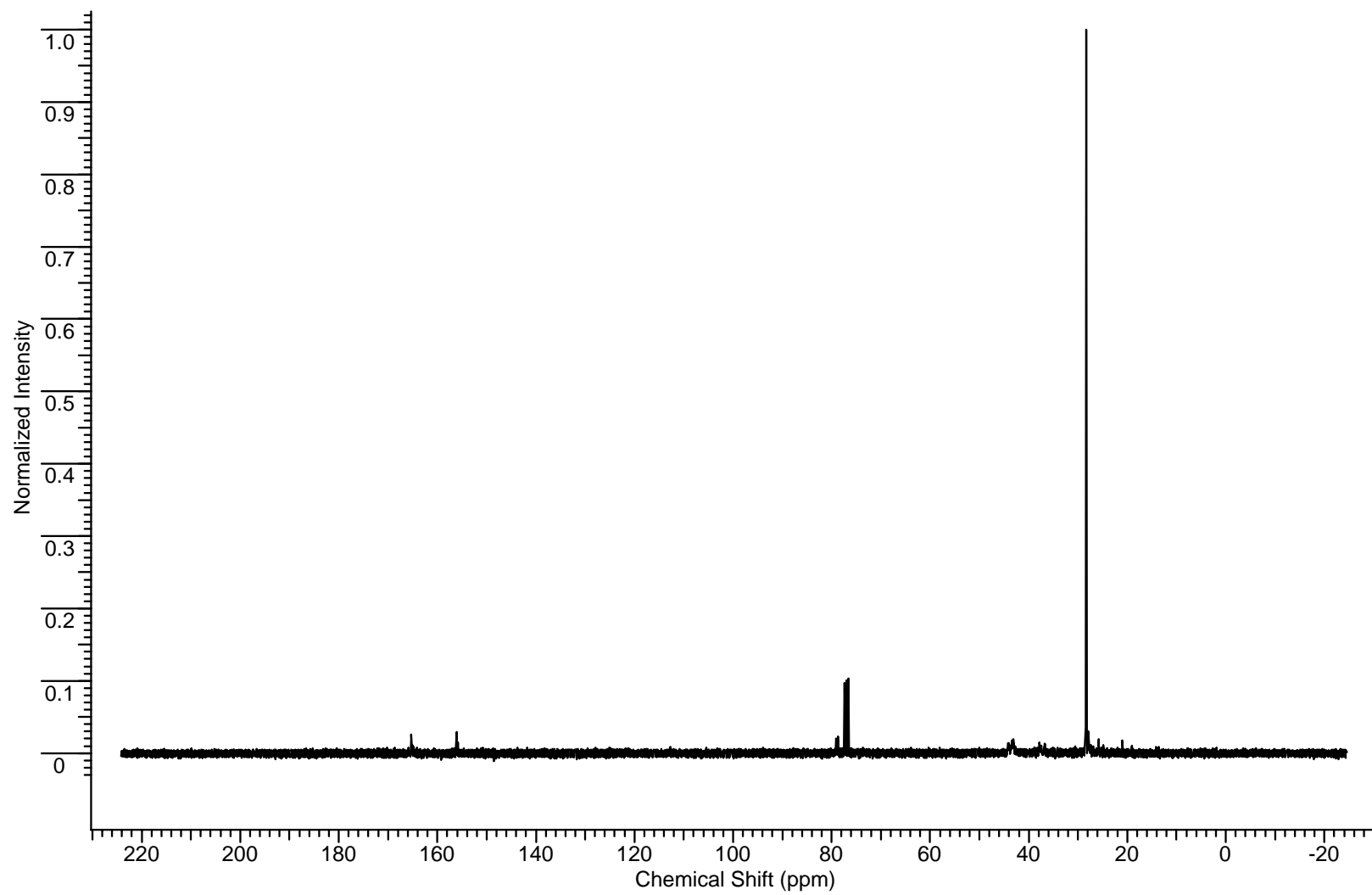
Iodinated Dendrimer: Compound 27, MALDI-MS



Iodinated Dendrimer: Compound **28**, ^1H NMR

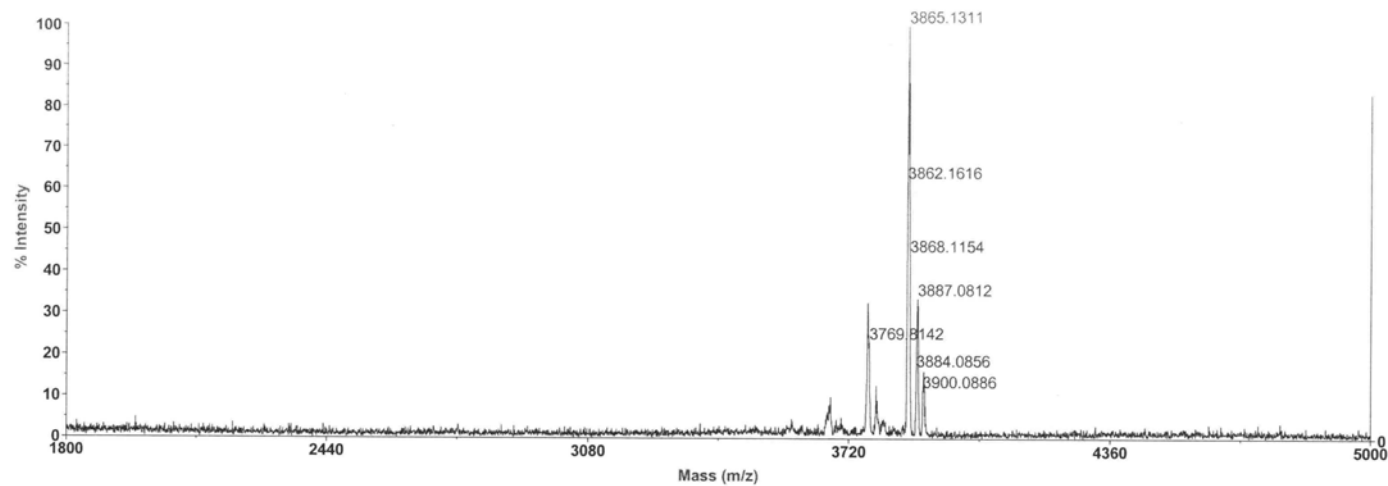


Iodinated Dendrimer: Compound **28**, ^{13}C NMR

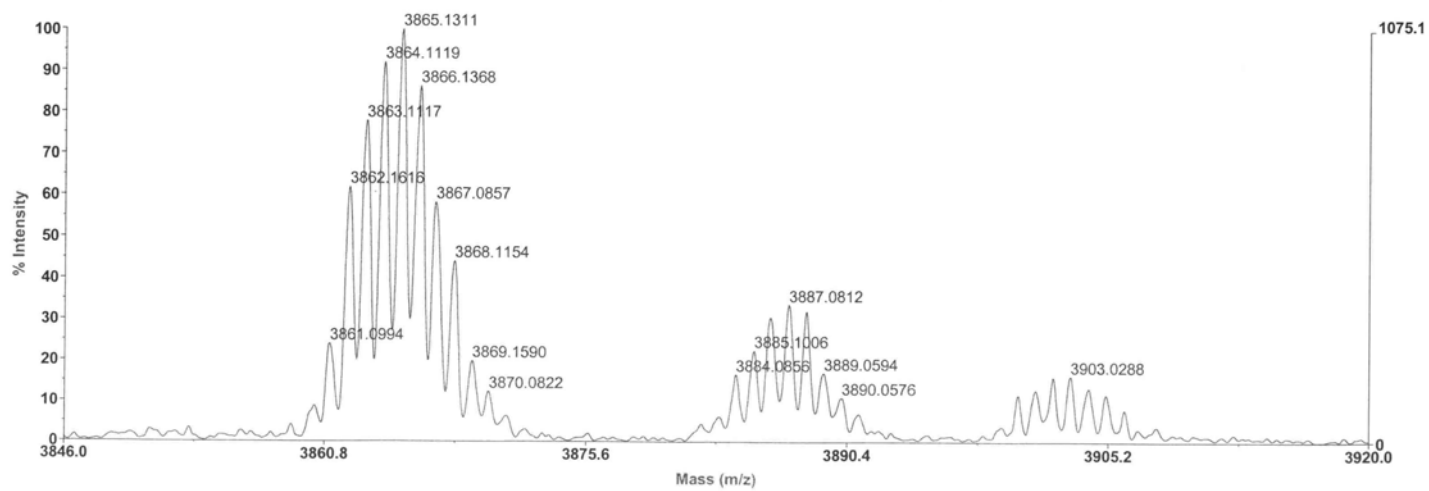


Iodinated Dendrimer: Compound **28**, MALDI-MS

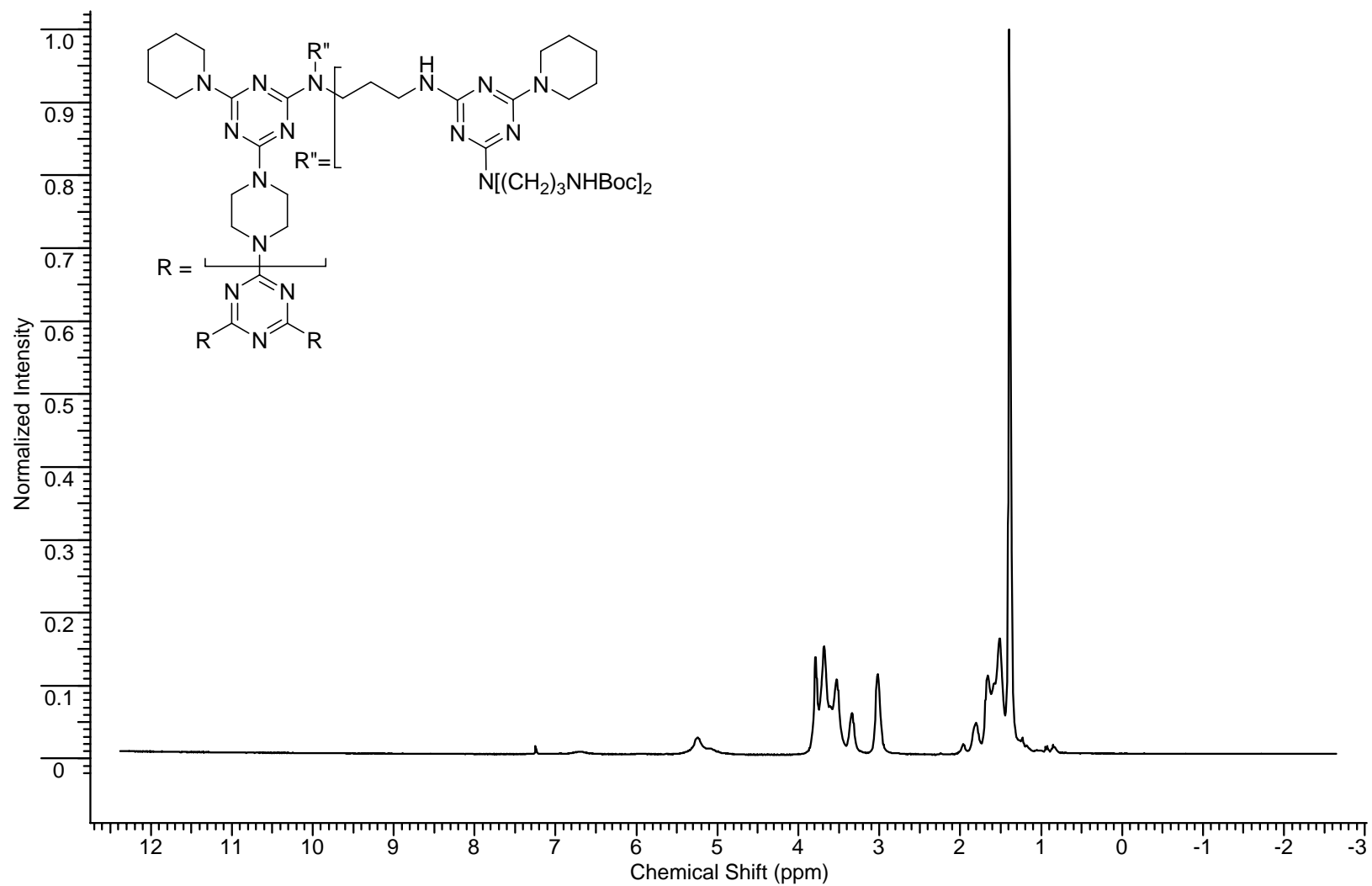
Voyager Spec #1=>RS')000[BP = 3865.2, 1075]



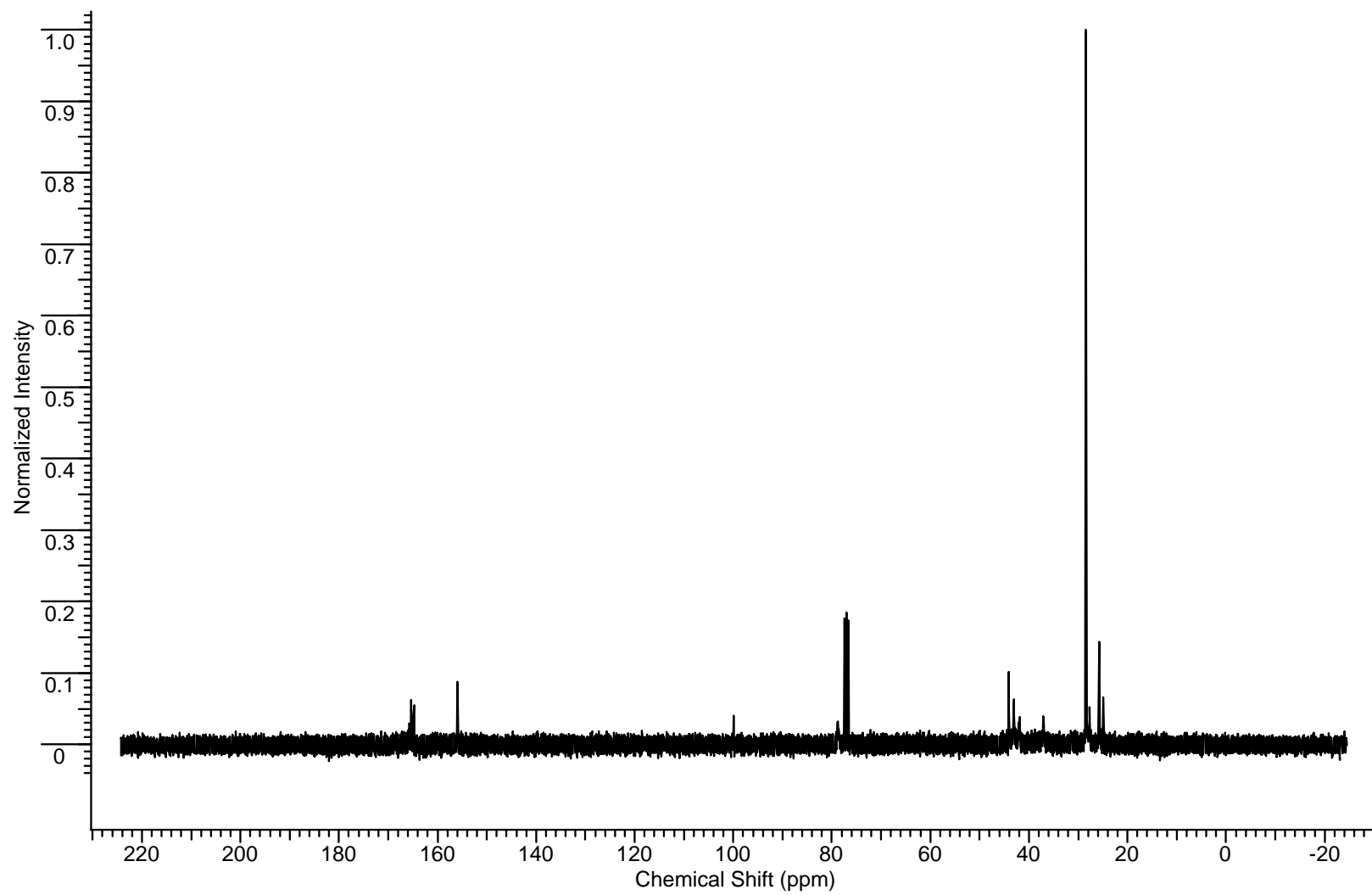
Voyager Spec #1=>RSM10000[BP = 3865.2, 1075]



Iodinated Dendrimer: Compound **29**, ^1H NMR

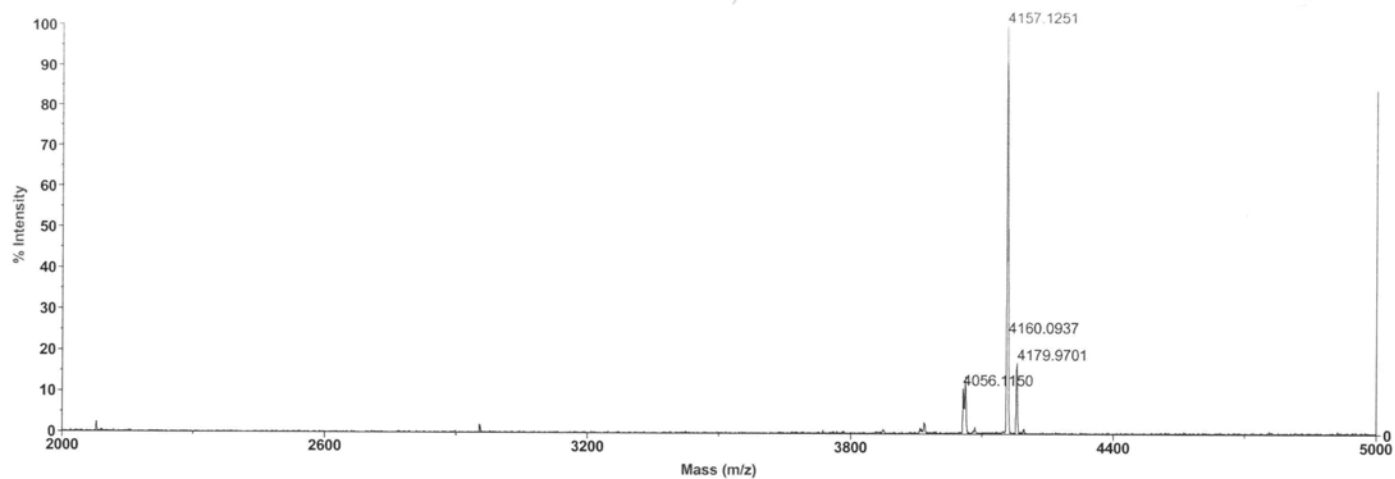


Iodinated Dendrimer: Compound **29**, ^{13}C NMR

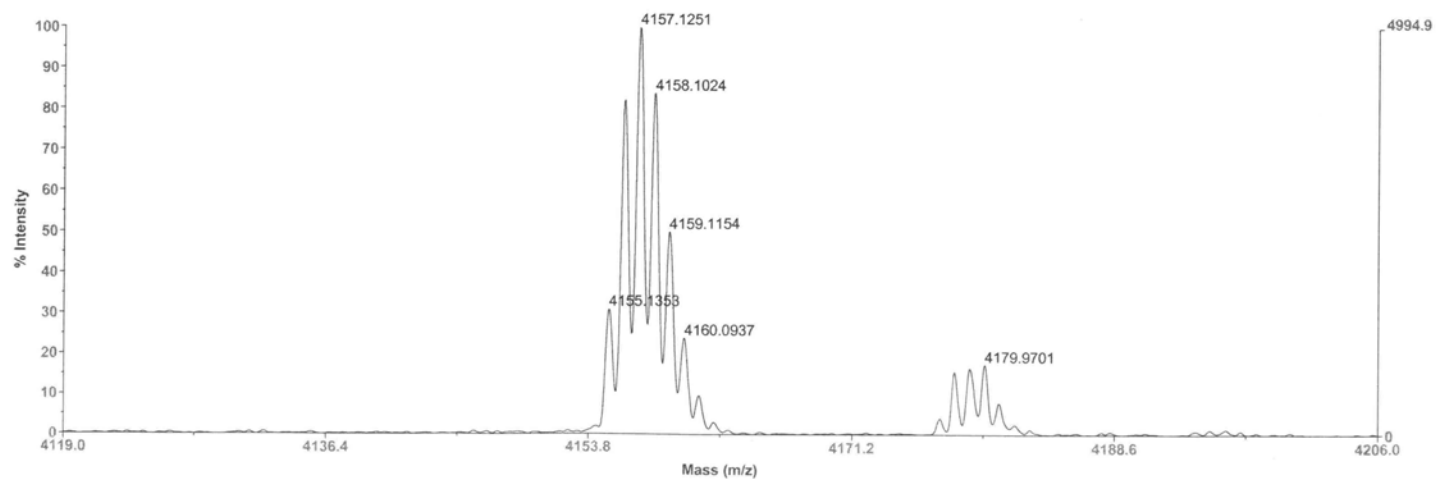


Iodinated Dendrimer: Compound 29, MALDI-MS

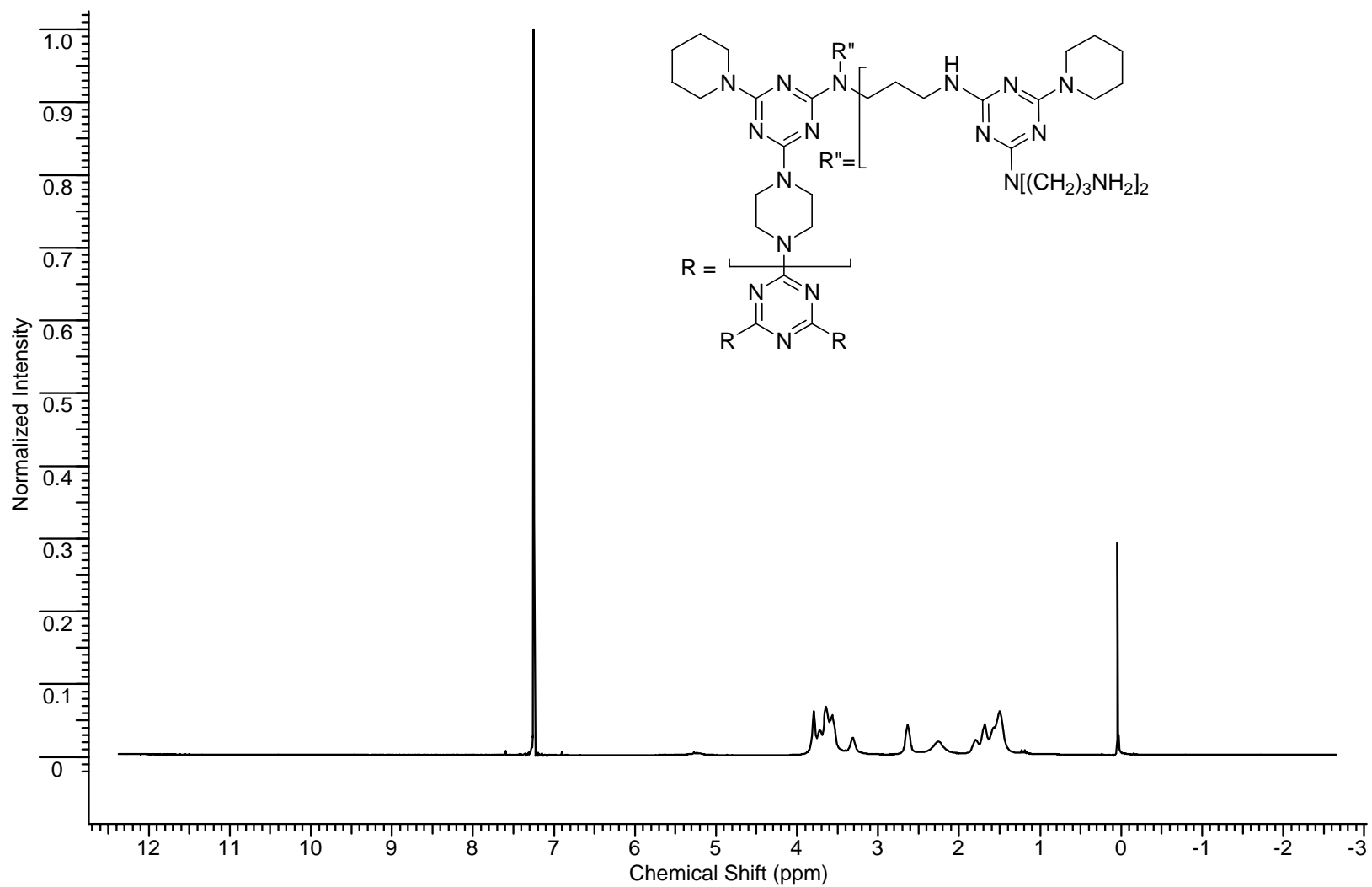
Voyager Spec #1=>RSM1000[BP = 4157.1, 4995]



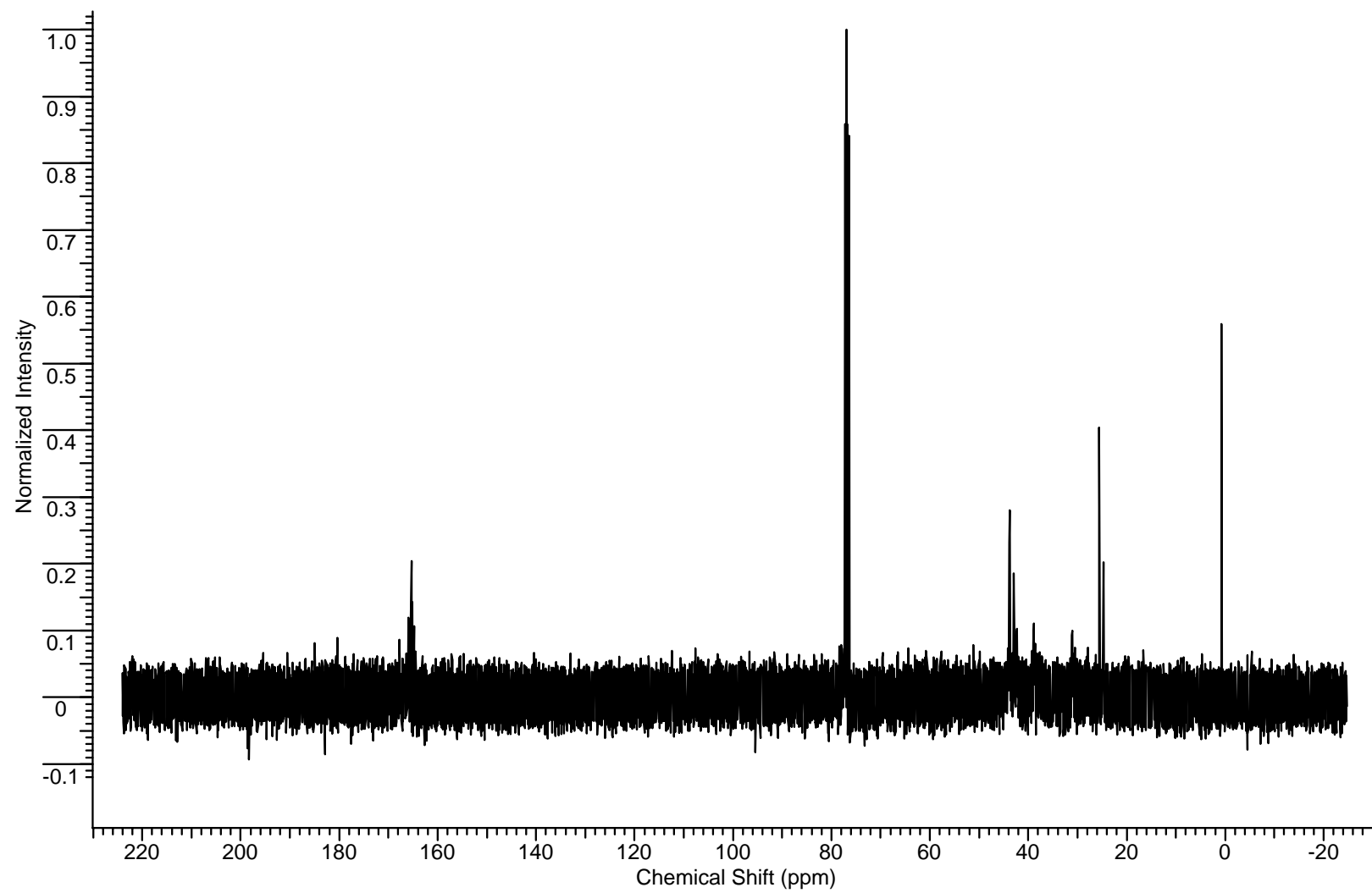
Voyager Spec #1=>RSM10000[BP = 4157.1, 4995]



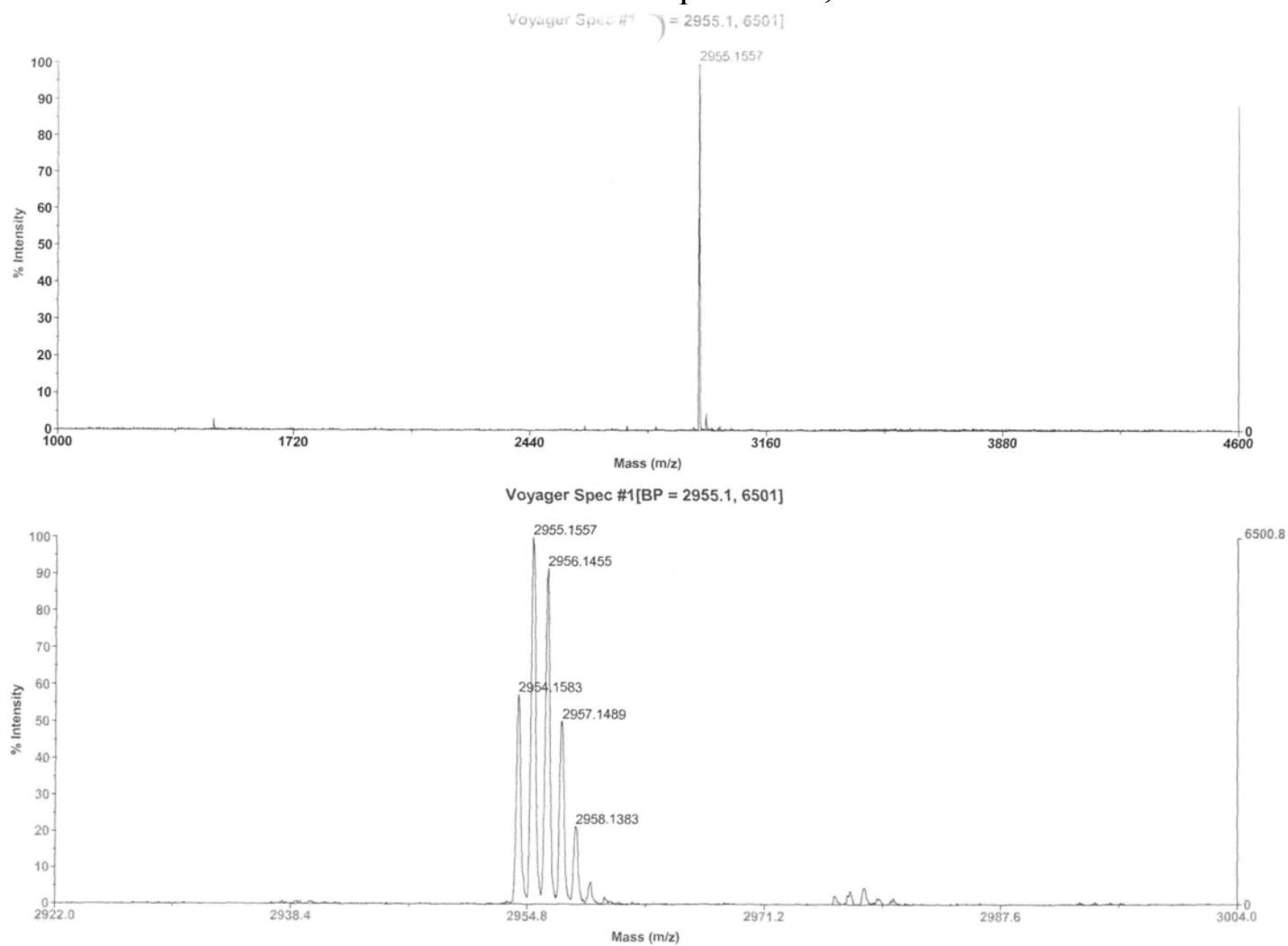
Iodinated Dendrimer: Compound **30**, ^1H NMR



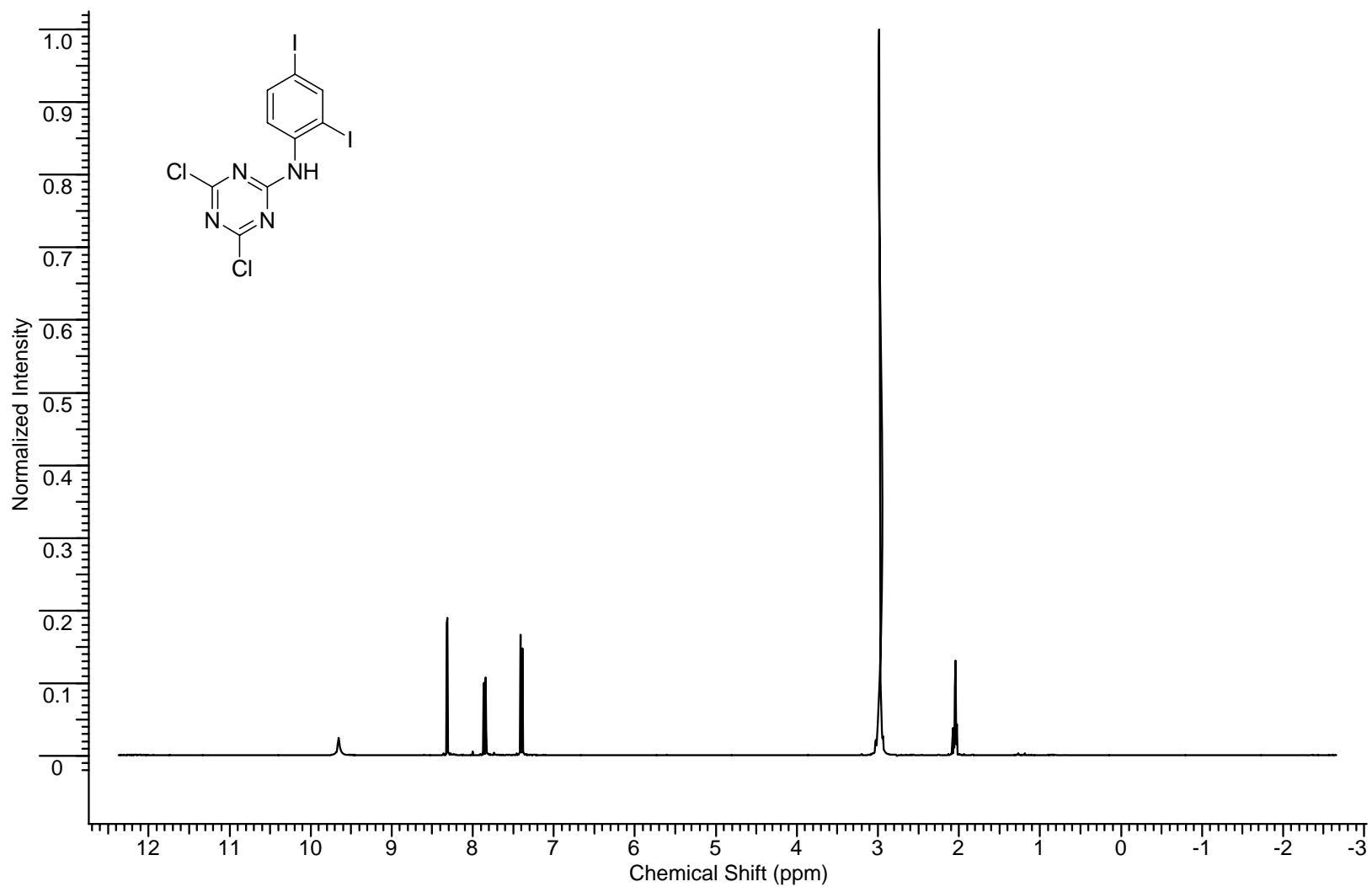
Iodinated Dendrimer: Compound **30**, ^{13}C NMR



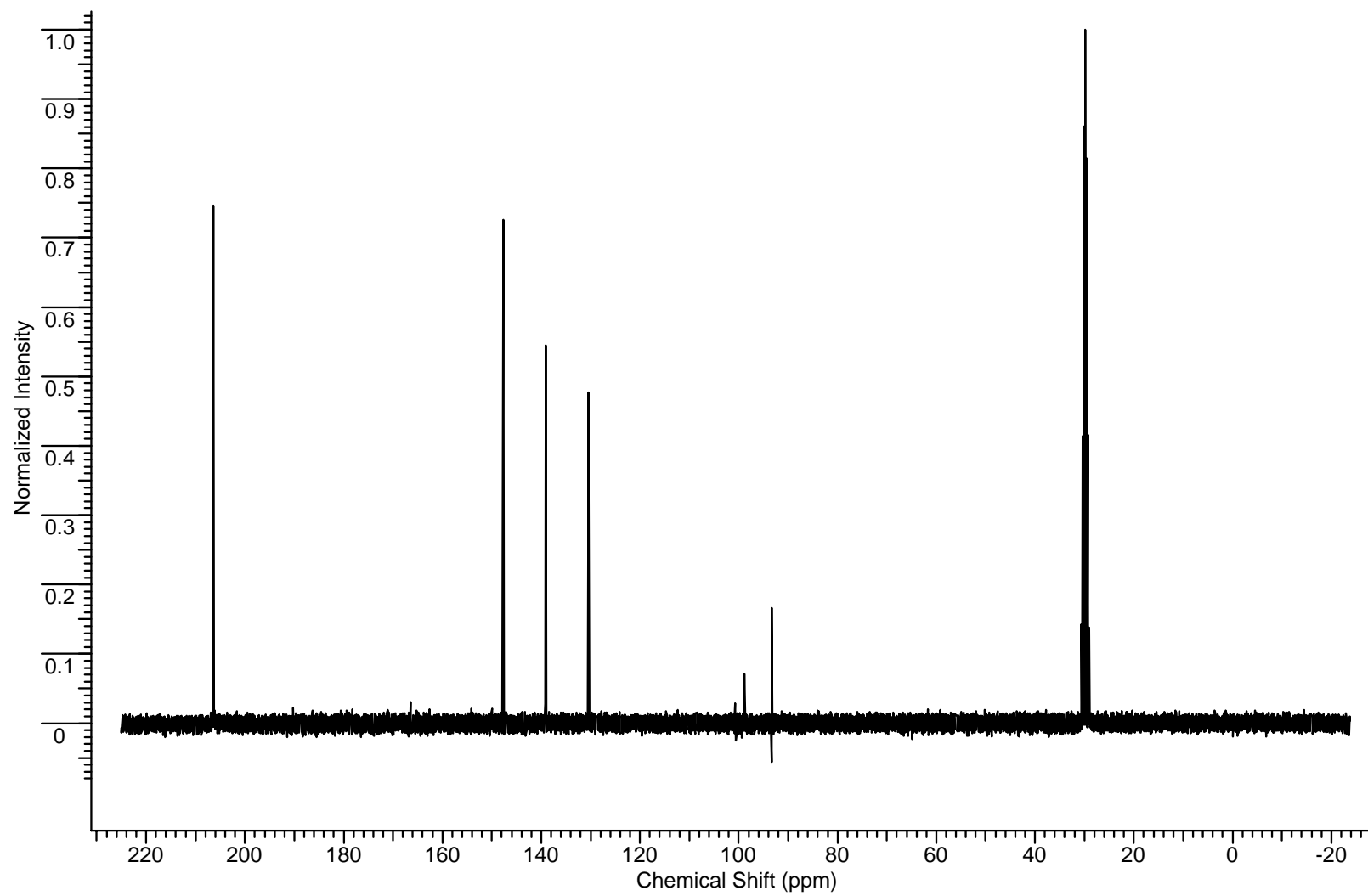
Iodinated Dendrimer: Compound **30**, MALDI-MS



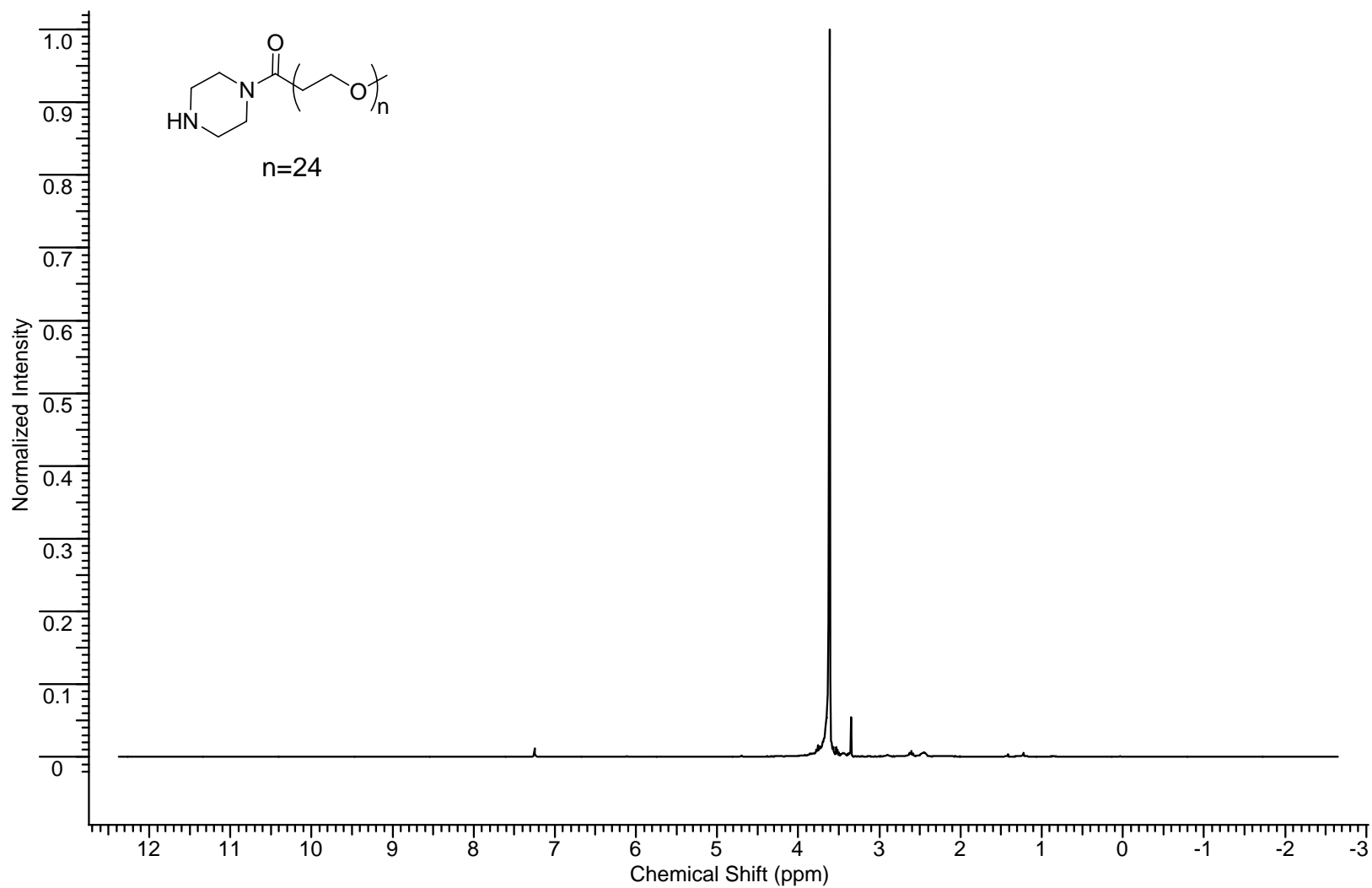
Iodinated Dendrimer: Compound **31**, ^1H NMR



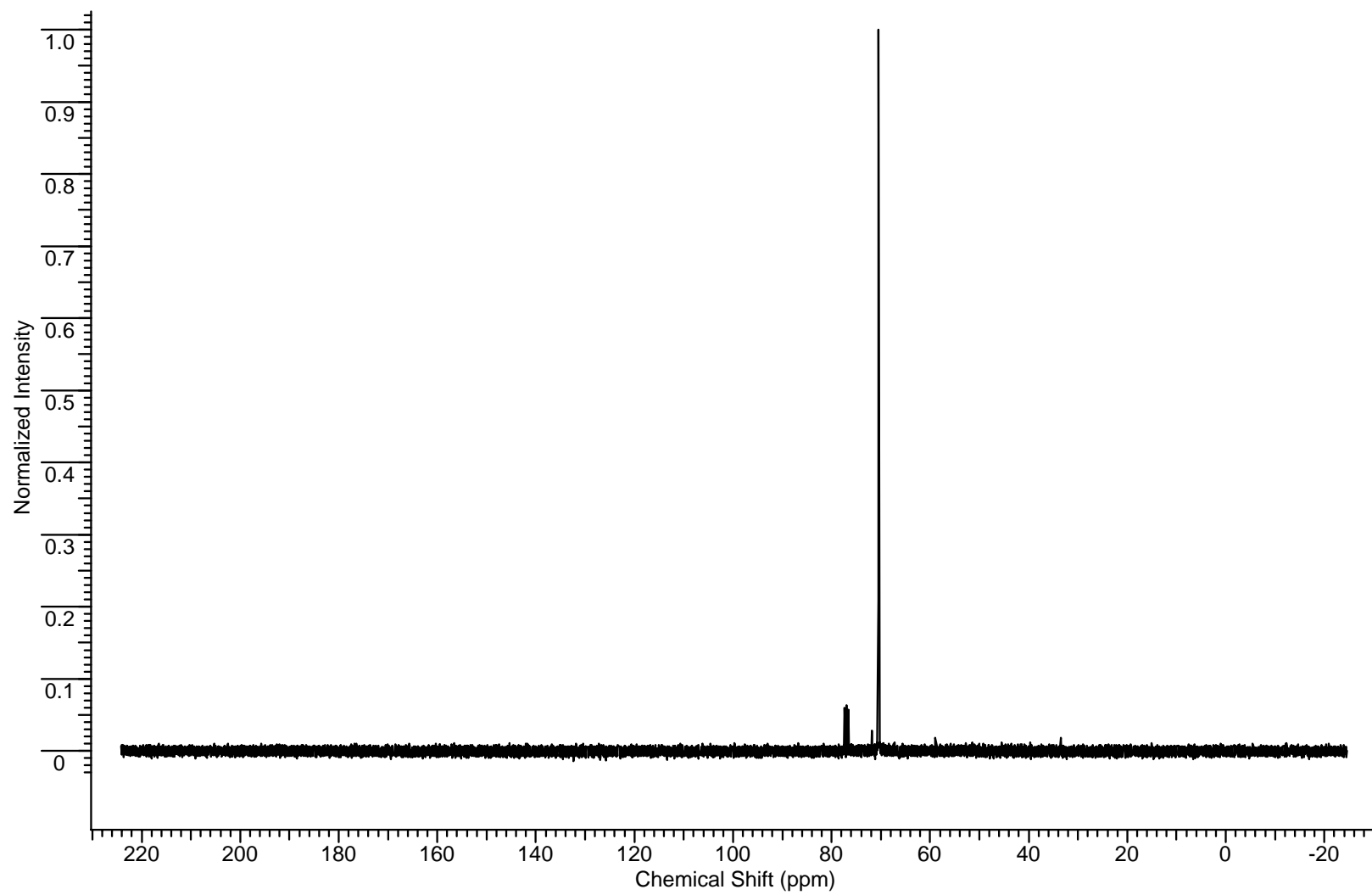
Iodinated Dendrimer: Compound **31**, ^{13}C NMR



Iodinated Dendrimer: Compound **32**, ^1H NMR

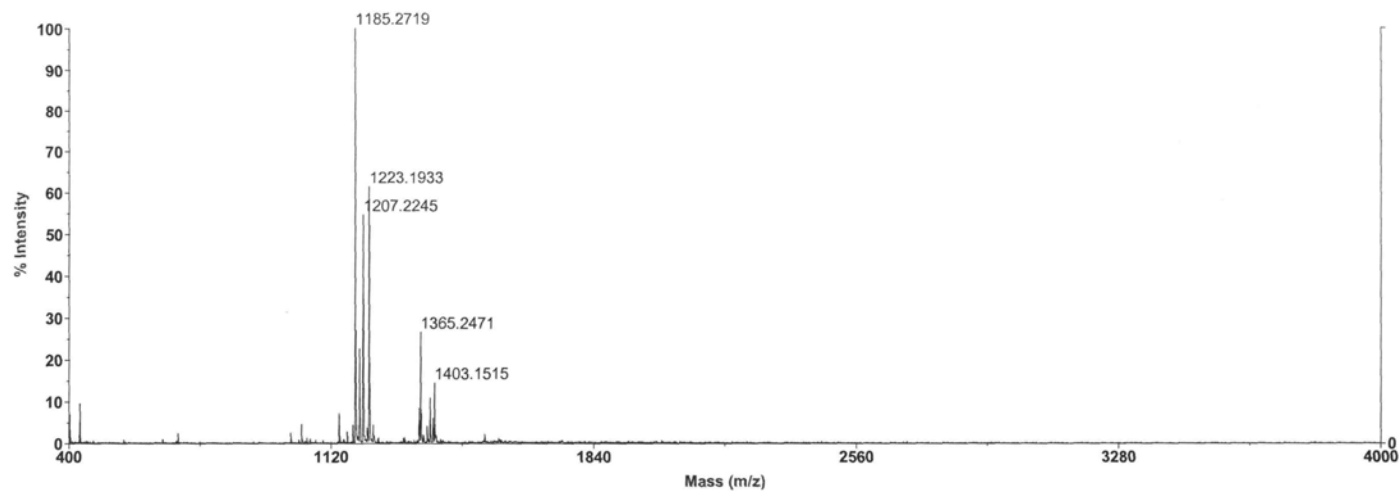


Iodinated Dendrimer: Compound **32**, ^{13}C NMR

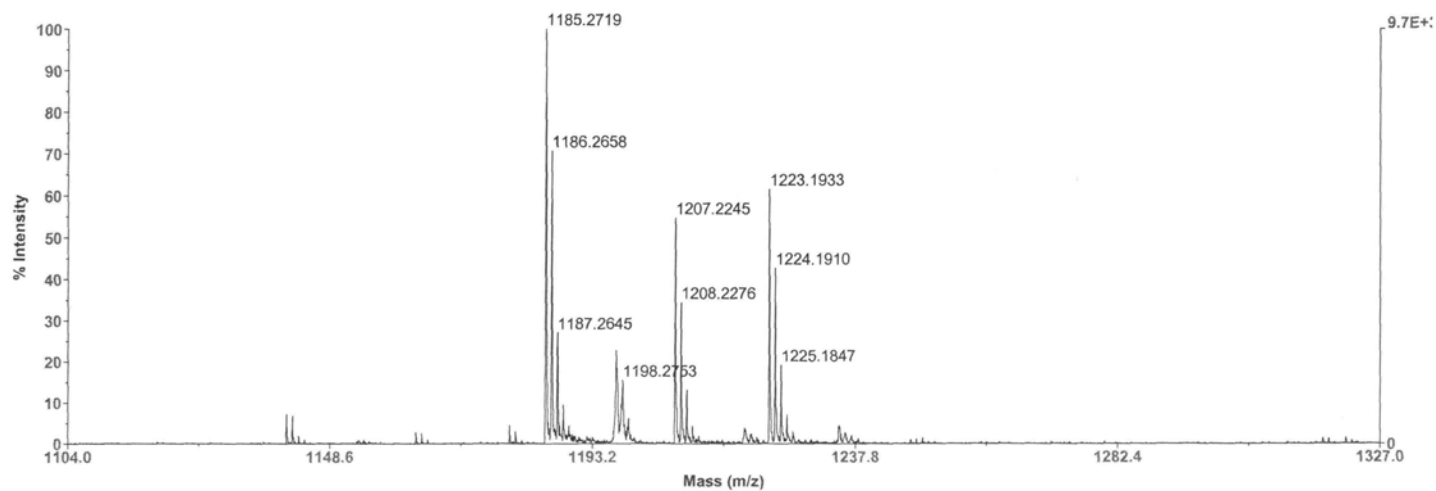


Iodinated Dendrimer: Compound **32**, MALDI-MS

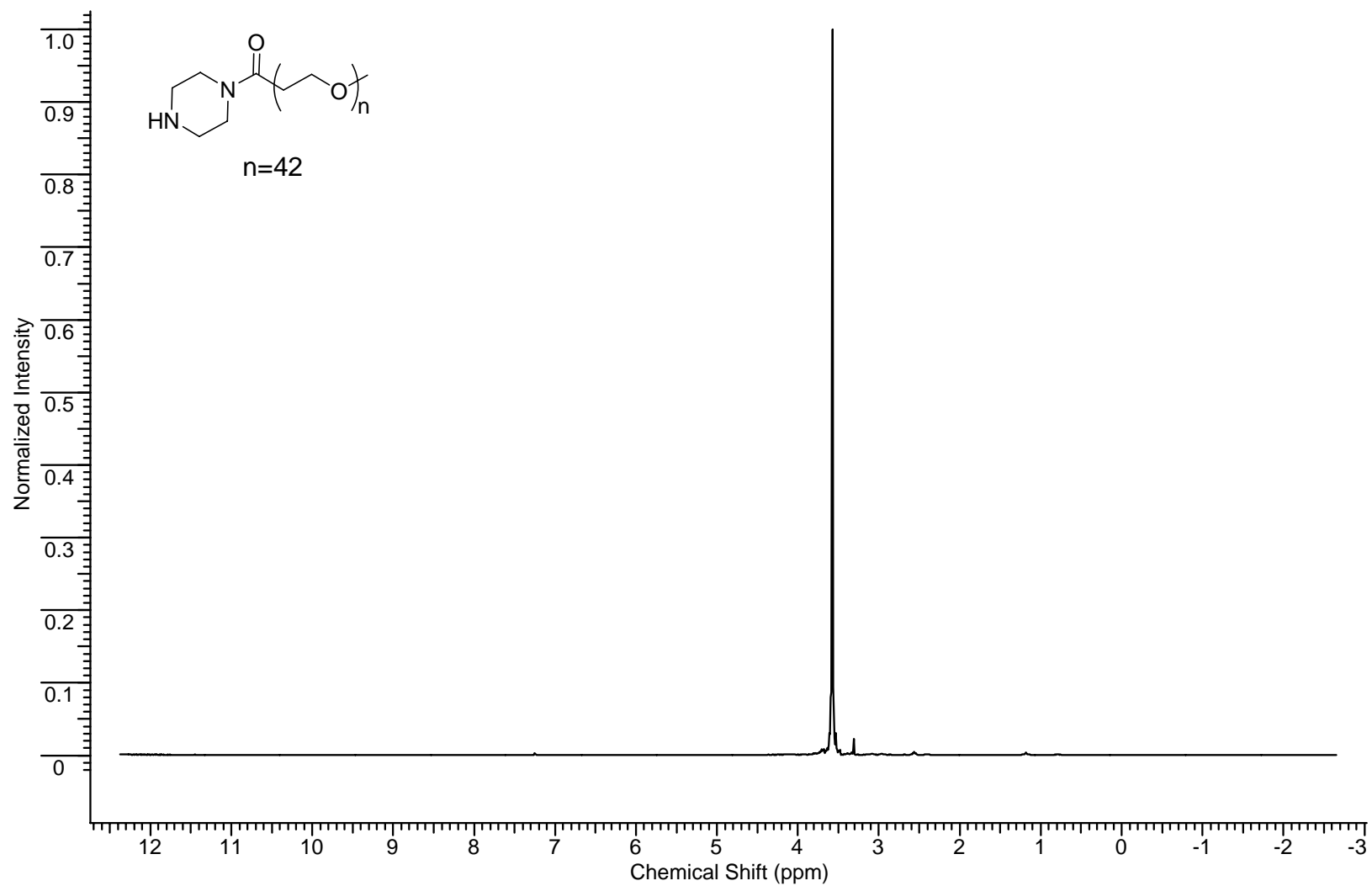
Voyager Spec #1[BP = 1185.3, 9718]



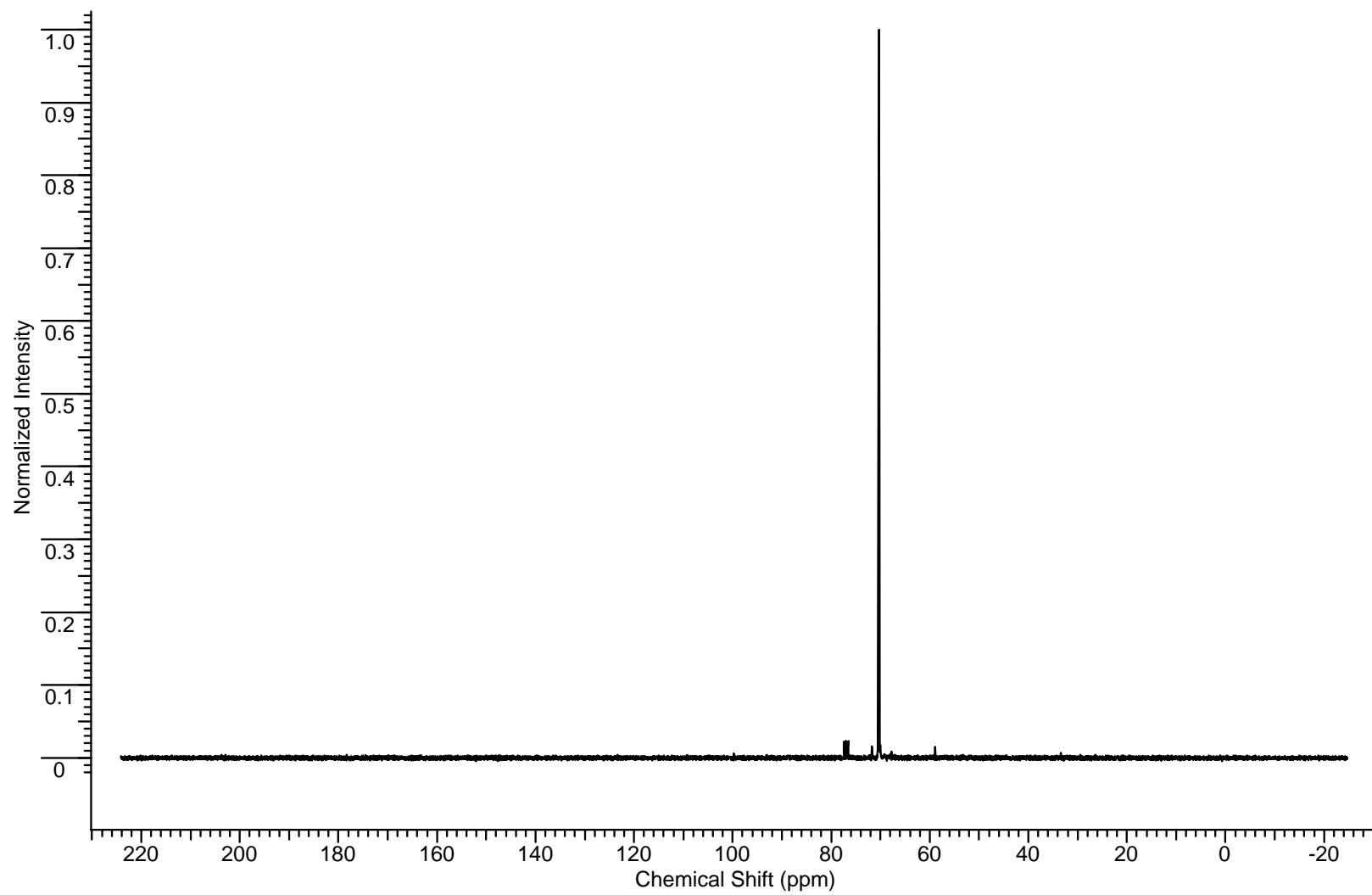
Voyager Spec #1[BP = 1185.3, 9718]



Iodinated Dendrimer: Compound **33**, ^1H NMR

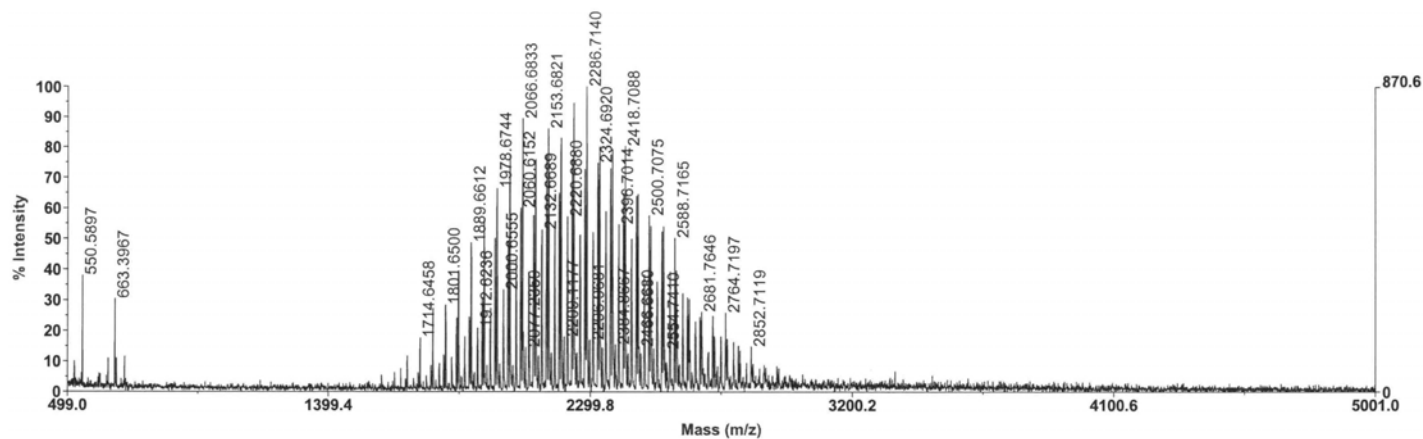


Iodinated Dendrimer: Compound **33**, ^{13}C NMR

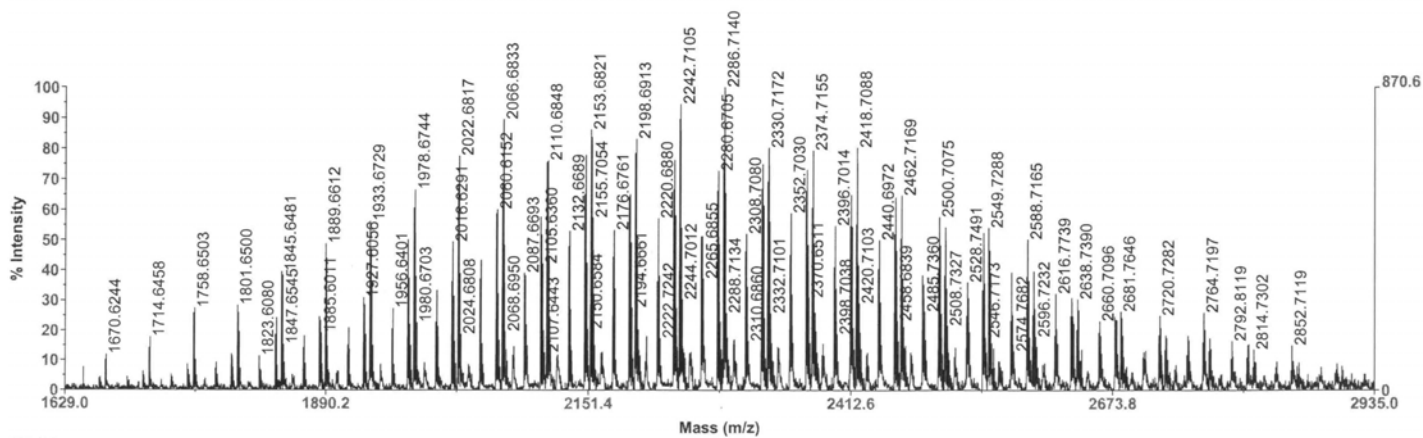


Iodinated Dendrimer: Compound **33**, MALDI-MS

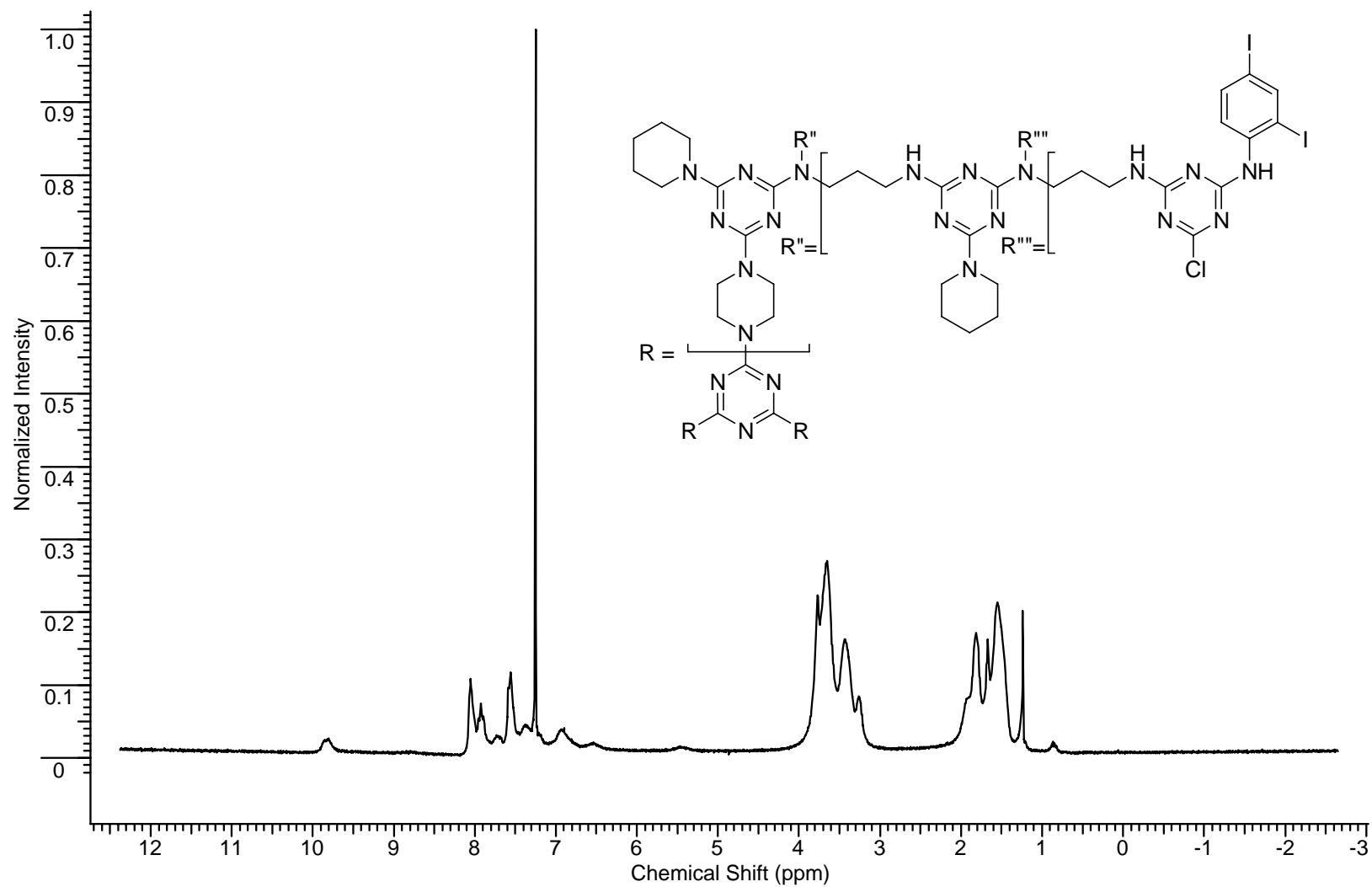
Voyager Spec #1=>AdvBC') 0,5,0,1)[BP = 2286.7, 871]



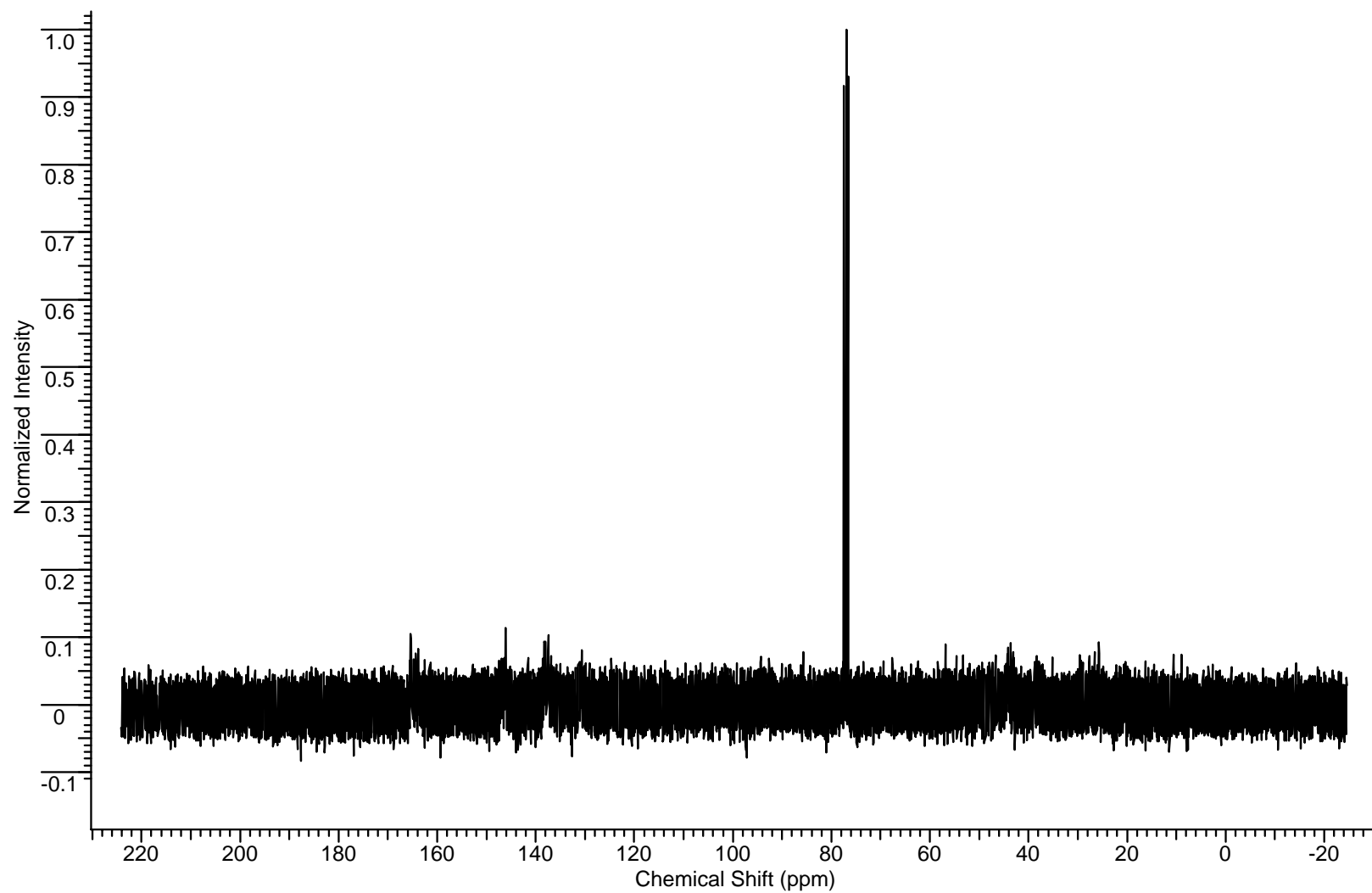
Voyager Spec #1=>AdvBC(32,0,5,0,1)[BP = 2286.7, 871]



Iodinated Dendrimer: Compound **34**, ^1H NMR

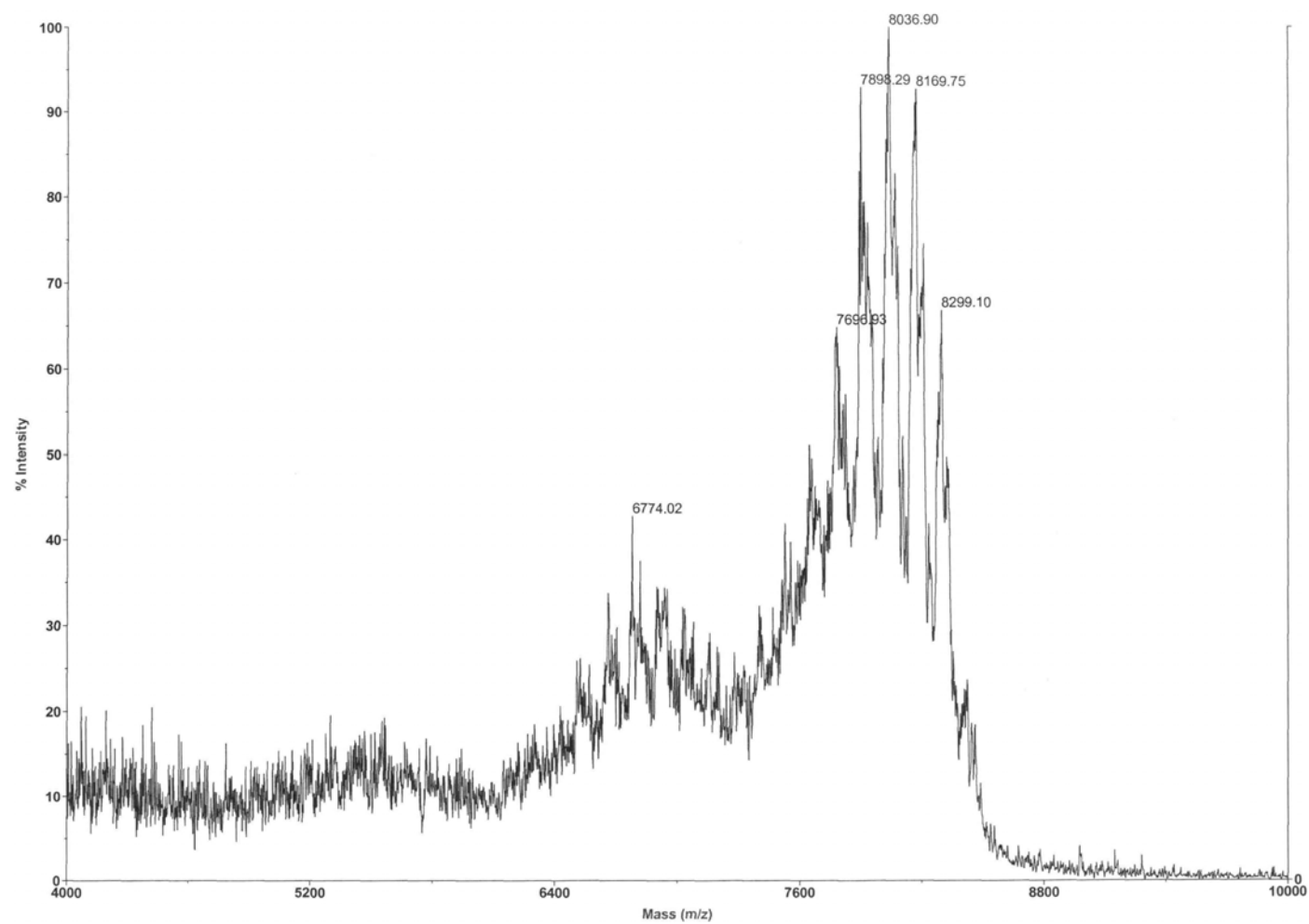


Iodinated Dendrimer: Compound **34**, ^{13}C NMR



Iodinated Dendrimer: Compound **34**, MALDI-MS

Voyager Spec #1=>RSM2000[BP = 8037.2, 446]



Iodinated Dendrimer: Compound 34, Elemental Analysis

ATLANTIC MICROLAB, INC.

Sample No. SH4-49

PO Box 2288
Norcross, Georgia 30091
(770) 242-0082

www.atlanticmicrolab.com

PROFESSOR/SUPERVISOR:
PO #: 456601

SUBMITTER

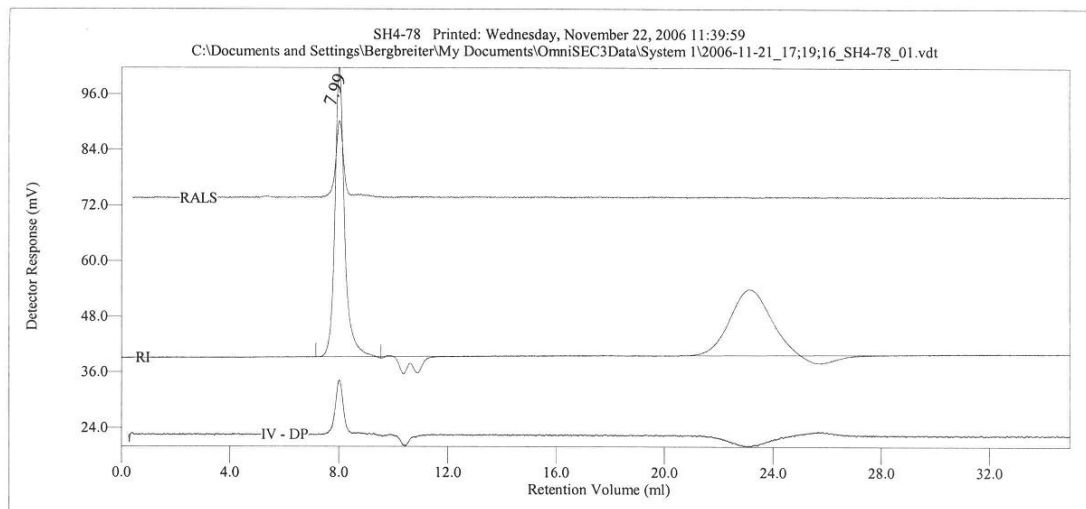
Company / School: Texas A&M Univ.
Address: Dept. of CHEMISTRY
P. O. Box 30012
College Station, TX 77842
NAME: Susan Hathfield DATE: 10-10-06
Simanek Res. group

Element	Theory	Found		Single <input checked="" type="checkbox"/> Duplicate <input type="checkbox"/>	
C	35.46	35.26	35.33	Elements Present: <u>C, H, N, Cl, I</u>	
H	3.44	3.42	3.44	Analyze for: <u>CHNI</u>	
N	19.93	18.94	18.90	Hygroscopic <input type="checkbox"/> Explosive <input type="checkbox"/> M.P. _____ B.P. _____	
I	36.12	35.61		To be dried: Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Temp. _____ Vac. _____ Time _____	
		NO CHARGE FOR DUPLICATES		FAX Service <input type="checkbox"/> FAX Phone # <u>Email</u>	
				Rush Service <input type="checkbox"/> (SEE CURRENT PRICE LIST) Phone Service <input type="checkbox"/> Phone No. _____	

Date Received OCT 12 2006 Date Completed OCT 13 2006

Remarks: achowai@mail.chem.tamu.edu

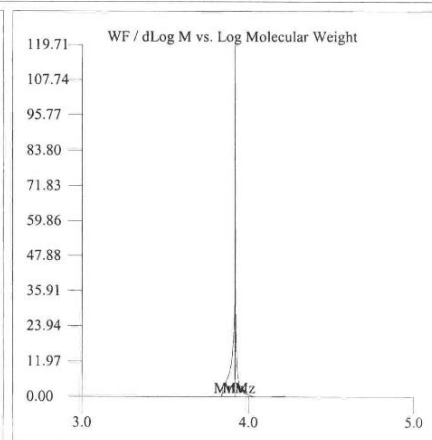
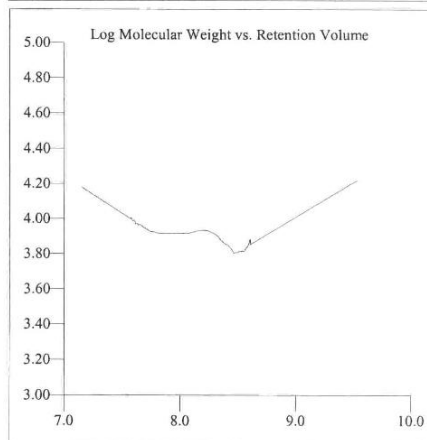
Iodinated Dendrimer: Compound 34, GPC Data



Multi-Detectors - Homopolymers : Results

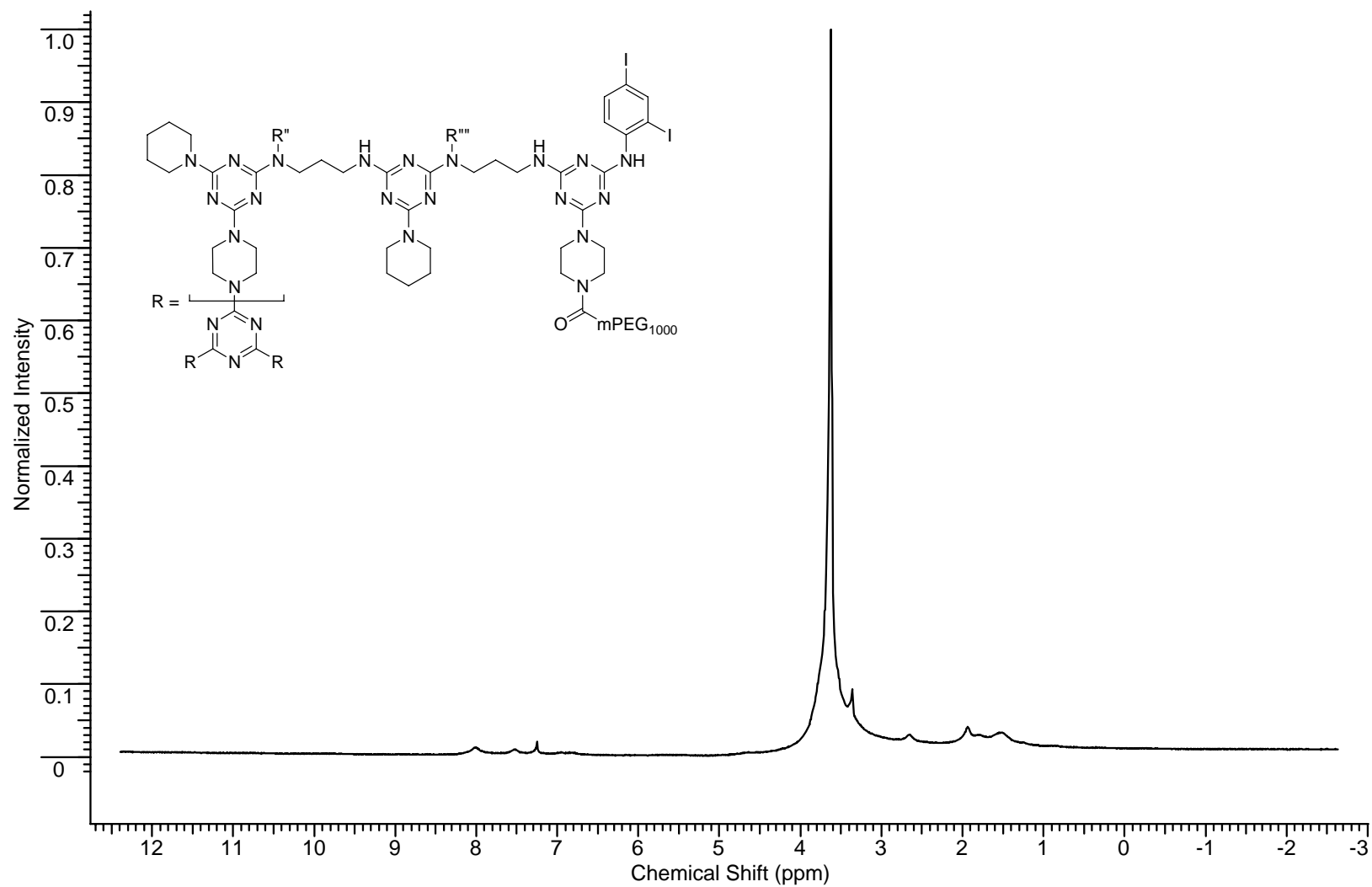
Peak RV - (ml)	7.990
Mn - (Daltons)	8,272
Mw - (Daltons)	8,300
Mz - (Daltons)	8,324
Mp - (Daltons)	8,277
Mw / Mn	1.003
Percent Above Mw:	0 0.000
Percent Below Mw:	0 0.596
IV - (dl/g)	0.0415
Rh - (nm)	1.757
Wt Fr (Peak)	1.000
Mark-Houwink a	0.000
Mark-Houwink logK	0.000
Branches	0.000
Branch Freq.	0.000
RI Area - (mvm)	25.60
UV Area - (mvm)	0.00
RALS Area - (mvm)	5.68
LALS Area - (mvm)	0.00
DP Area - (mvm)	4.06

Sample Parameters	Input	Calculated
Sample Conc - (mg/ml)	1.200	0.000
Sample Recovery (%)	0.000	0.000
dn/dc - (ml/g)	0.0000	0.1987
dA/dc - (ml/g)	0.0000	0.0000

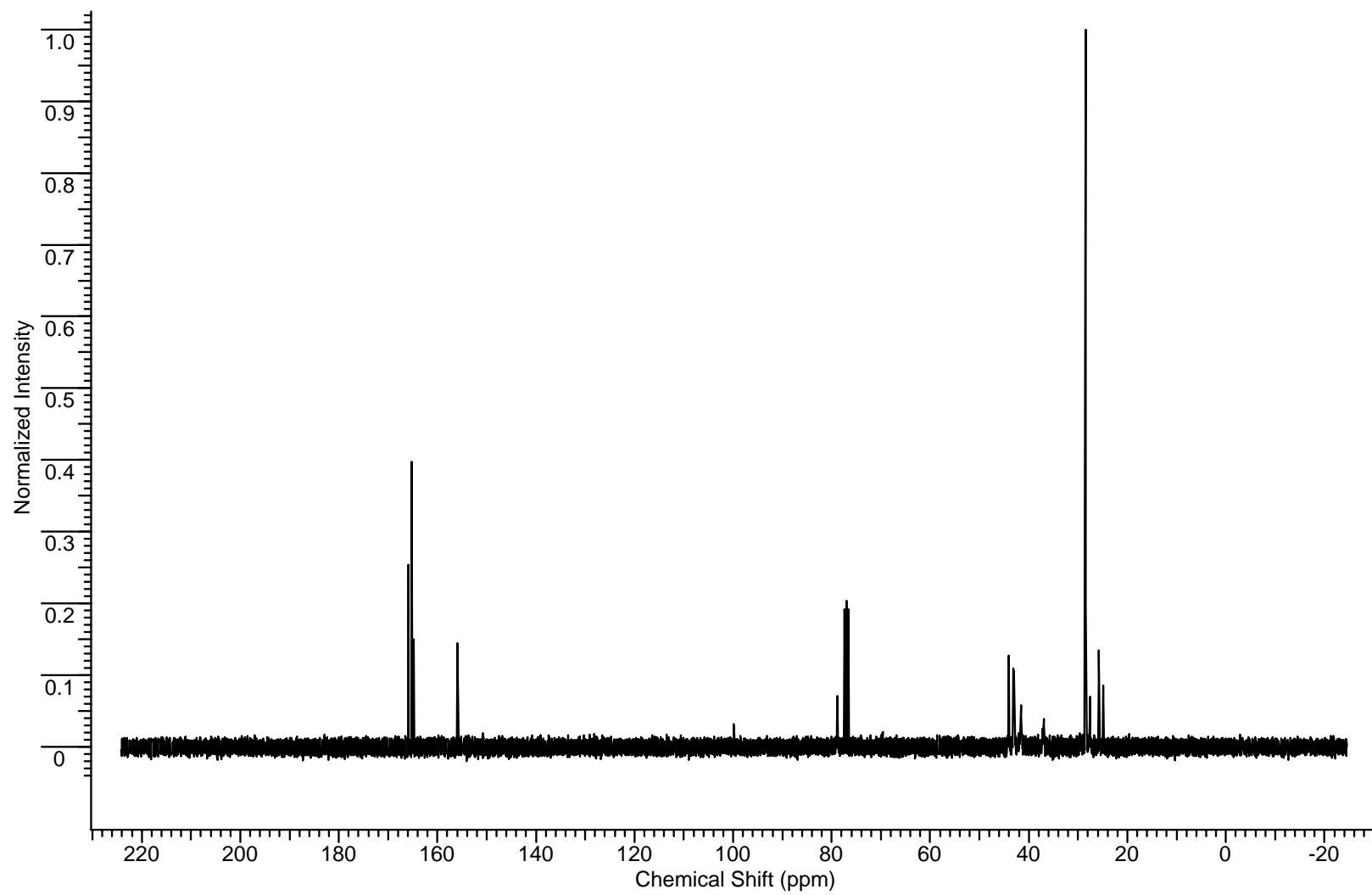


Annotation	
Method File	PS-THF-Multidetector-2-0001.vcm
Limits File	16_SH4-78_01-PS-THF-Multidetector-2-0001-0000.lim
Date Acquired	Nov 21, 2006 - 17:19:16
Solvent	THF
Acquisition Operator	admin : Administrator
Calculation Operator	admin : Administrator
Column Set	GMHxl
System	System 1
Flow Rate - (ml/min)	1.000
Inj Volume - (ul)	100.0
Volume Increment - (ml)	0.00333
Detector Temp. - (deg C)	27.0
Column Temp. - (deg C)	30.0
OmniSEC Build Number	210

Iodinated Dendrimer: Compound **35**, ^1H NMR

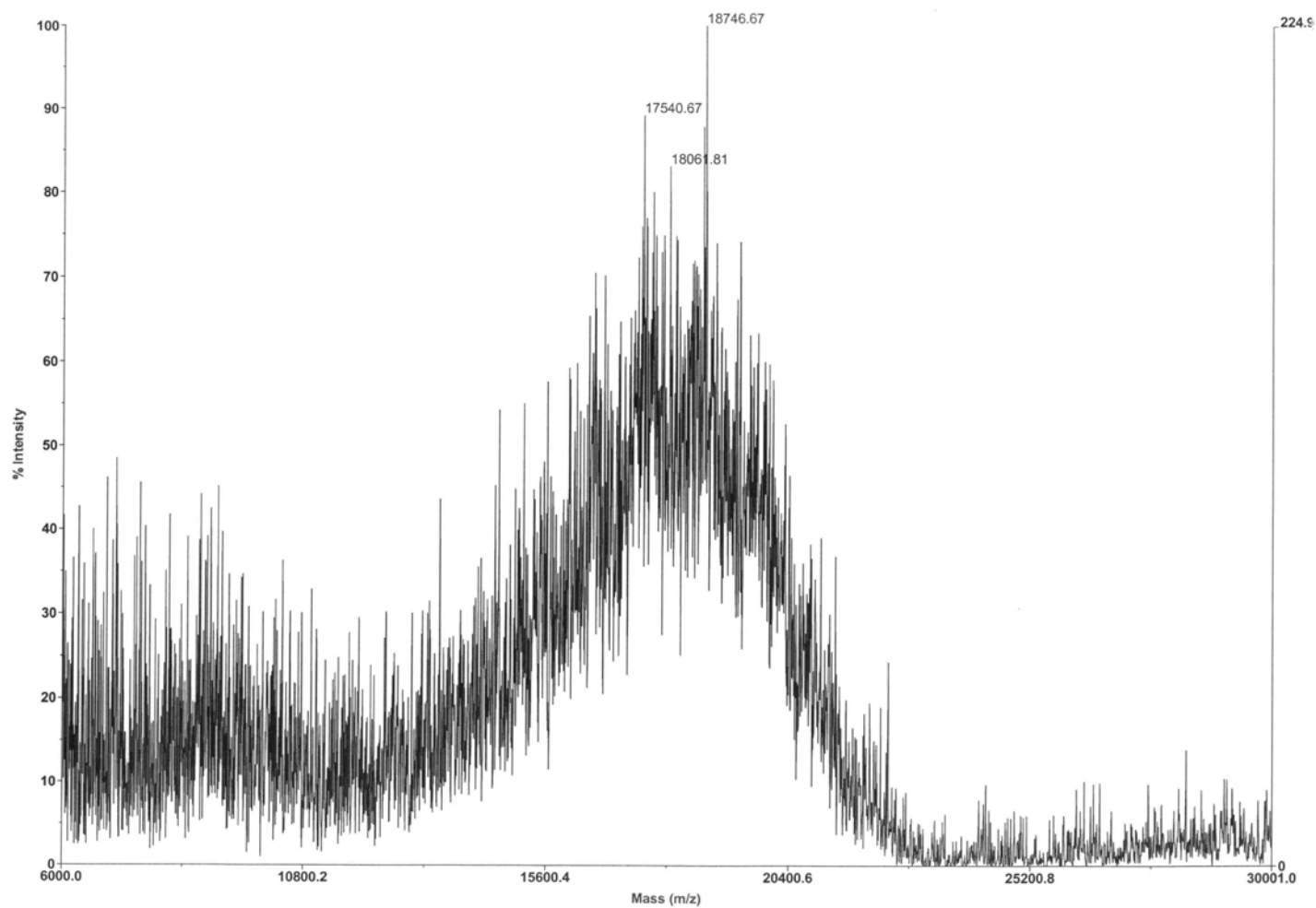


Iodinated Dendrimer: Compound **35**, ^{13}C NMR

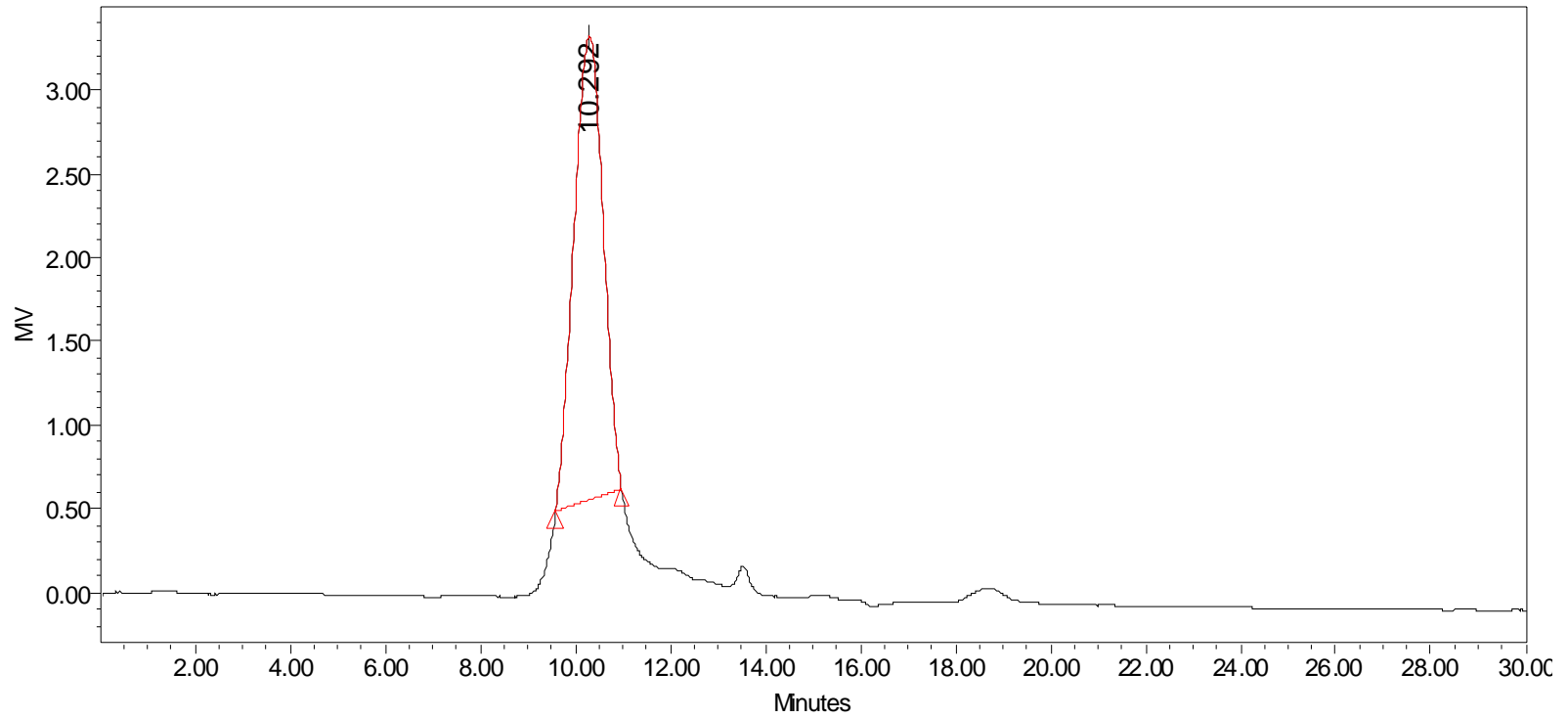


Iodinated Dendrimer: Compound **35**, MALDI-MS

Voyager Spec #1=>RSM2000[BP = 18747.8, 225]

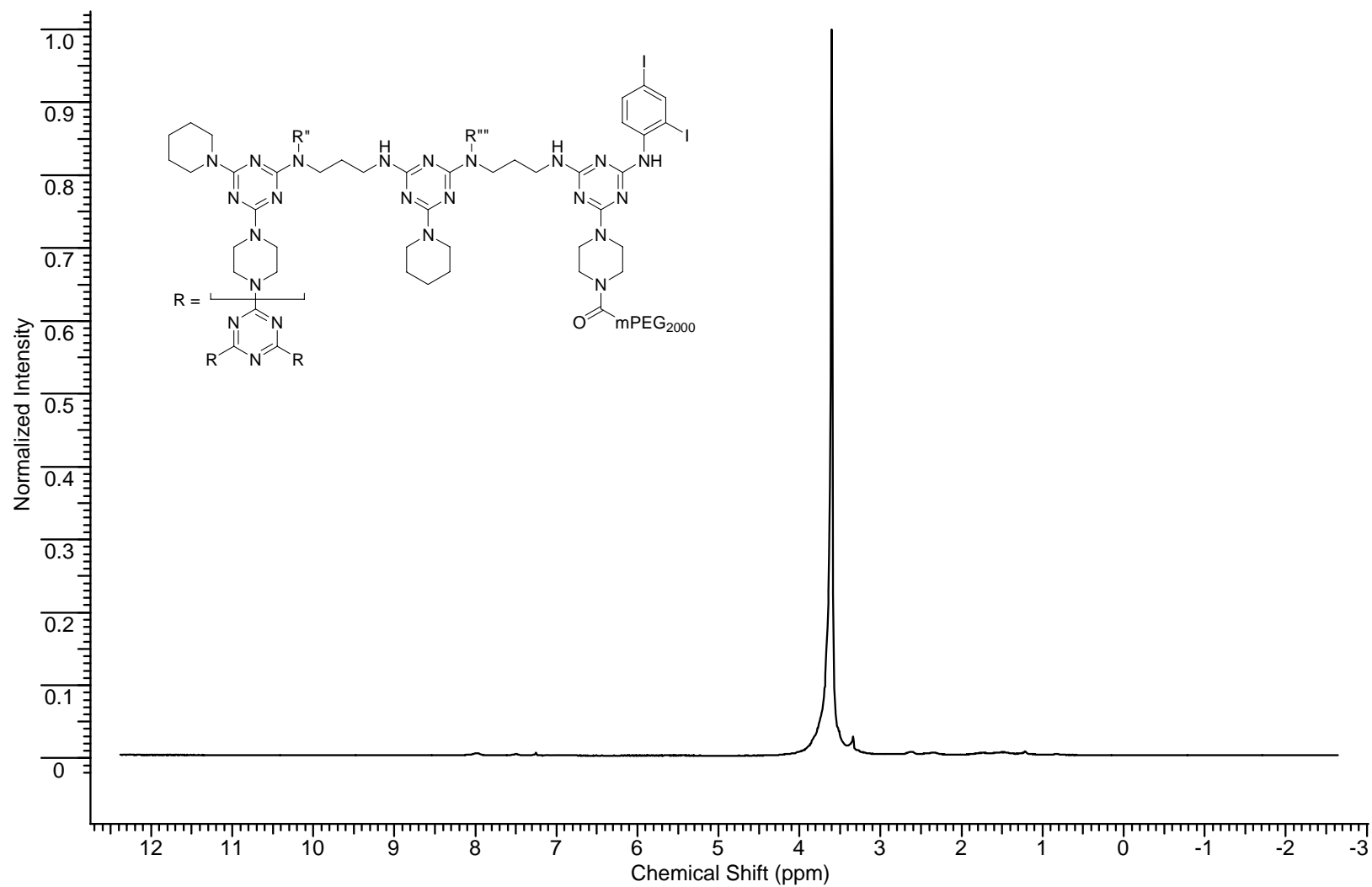


Iodinated Dendrimer: Compound 35, HPLC



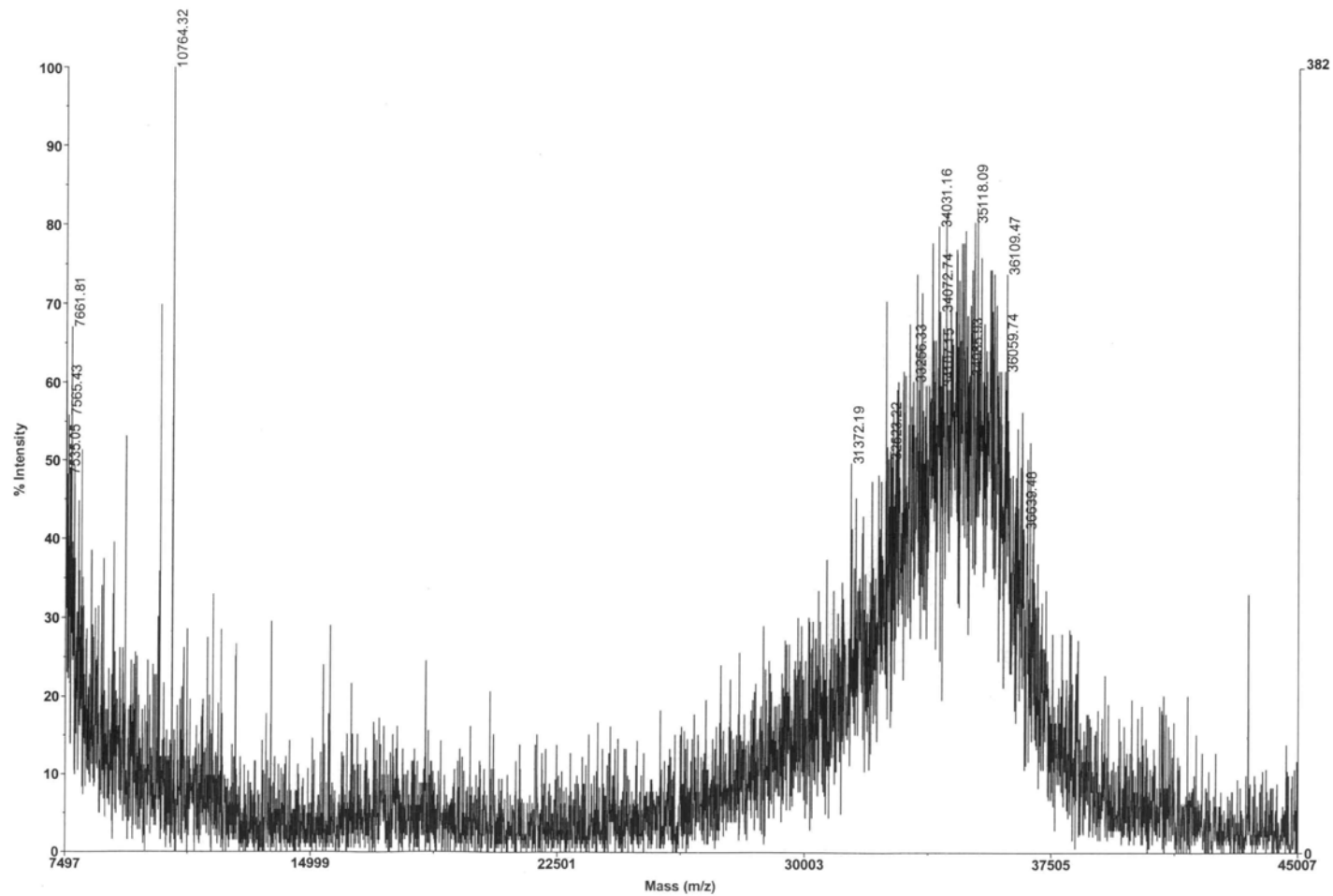
	Name	Retention Time	Area	% Area	Height	Int Type
1	SH4-83	10.292	118449	100.00	2771	BB
2	NaNO ₃	13.447				

Iodinated Dendrimer: Compound **36**, ^1H NMR

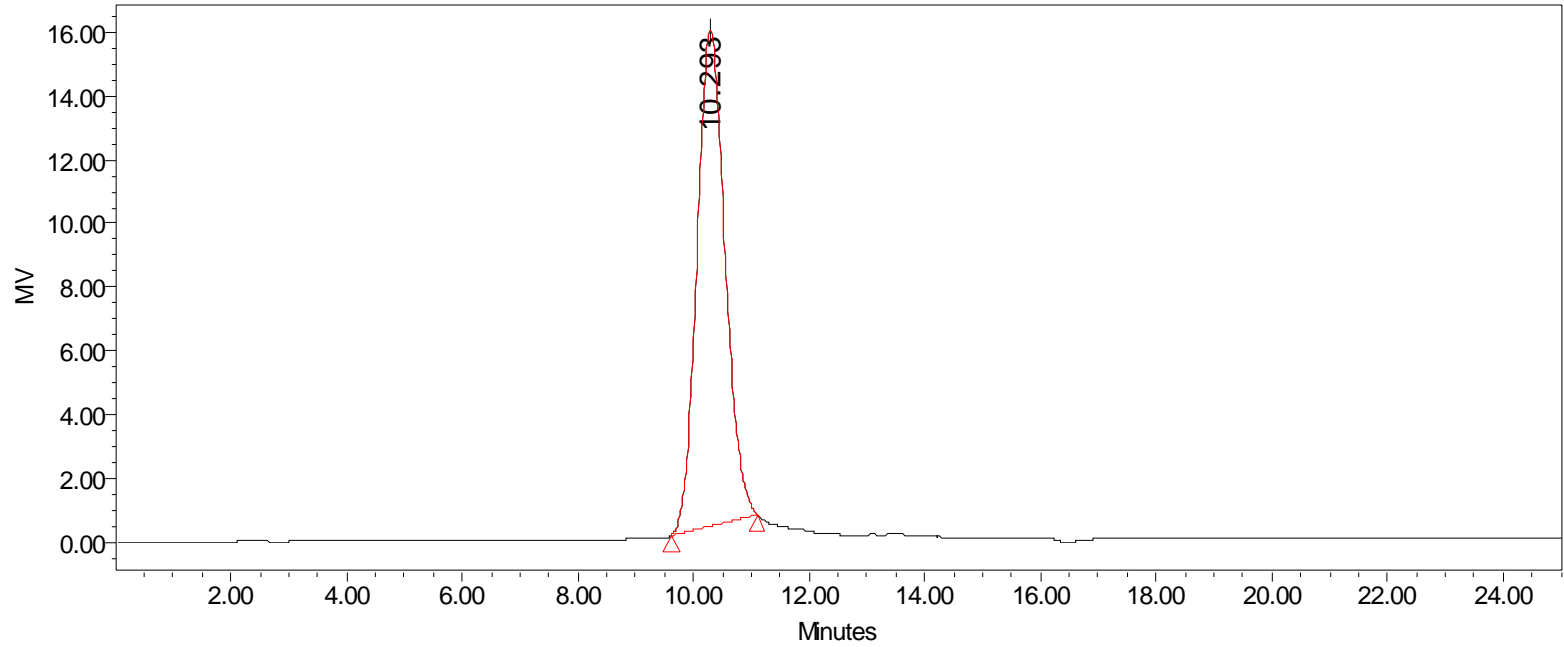


Iodinated Dendrimer: Compound **36**, MALDI-MS

Voyager Spec # [= 10764.3, 382]

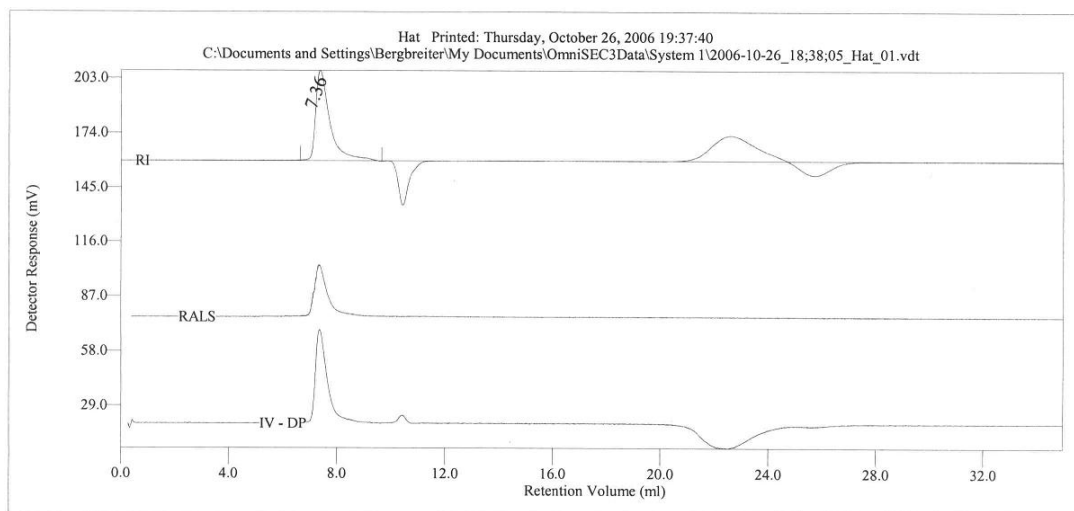


Iodinated Dendrimer: Compound 36, HPLC



	Name	Retention Time	Area	% Area	Height	Int Type
1	SH4-67	10.293	525739	100.00	15544	BB

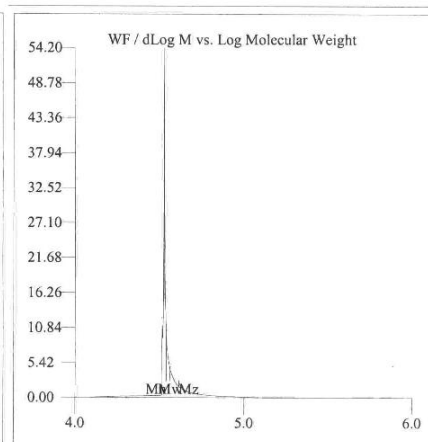
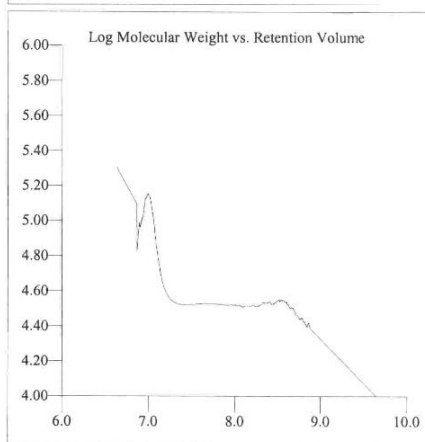
Iodinated Dendrimer: Compound 36, GPC data



Multi-Detectors - Homopolymers : Results

Peak RV - (ml)	7.357
Mn - (Daltons)	34,618
Mw - (Daltons)	36,367
Mz - (Daltons)	41,067
Mp - (Daltons)	33,586
Mw / Mn	1.051
Percent Above Mw:	0 0.000
Percent Below Mw:	0 0.000
IV - (dl/g)	0.1394
Rh - (nm)	4.281
WI Fr (Peak)	1.000
Mark-Houwink a	0.141
Mark-Houwink logK	-1.491
Branches	0.000
Branch Freq.	0.000
RI Area - (mvml)	29.01
UV Area - (mvml)	0.00
RALS Area - (mvml)	15.78
LALS Area - (mvml)	0.00
DP Area - (mvml)	26.61

Sample Parameters	Input	Calculated
Sample Conc - (mg/ml)	2.360	0.000
Sample Recovery (%)	0.000	0.000
dn/dc - (ml/g)	0.0000	0.1145
dA/dc - (ml/g)	0.0000	0.0000



Annotation	
Method File	PS-THF-Multidetector-2-0001.ver
Limits File	:38:05_Hat_01-PS-THF-Multidetector-2-0001-0001.lim
Date Acquired	Oct 26, 2006 - 18:38:05
Solvent	THF
Acquisition Operator	admin : Administrator
Calculation Operator	admin : Administrator
Column Set	GMHxl
System	System 1
Flow Rate - (ml/min)	1.000
lnj Volume - (ul)	100.0
Volume Increment - (ml)	0.00333
Detector Temp. - (deg C)	27.0
Column Temp. - (deg C)	30.0
OmniSEC Build Number	210

VITA

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