

Chapter 5

Towards the Synthesis of Glycodendrimers for Drug Delivery Applications

5.1 Introduction

Dendrimers are spherical macromolecules with multiple arms extending from a central core that are extremely branched.^{1,2} Dendrimer structure's atomistic aspect has lagged behind the rapid progress in design and synthesis.³ Dendrimers can be divided into more than fifty families, each with its own set of features, because the interior, core and surface can be modified to suit a variety of purposes.⁴ Low molecular weight and protein-based therapies have been found to have improved pharmacokinetic and pharmacodynamic properties when derivatized with polymers.^{5,6} The medicinal application is one of the most brilliant application of nanotechnology and the field of "nanomedicine" as a whole seems promising.⁷ Nanomedicine is critical for drug delivery and various other applications. Dendrimers having tree-like embranchment are nano-sized, highly functional three dimensional symmetric molecules with well-defined, monodisperse, and homogenous composition.^{8,9} These distinctiveness make them as ideal candidates for use in nanotechnology and a variety of biological applications.¹⁰⁻¹² Dendrimer structures are made up of a core, which is a fundamental atom or set of atoms and "dendrons" rise from this central core through a variety of chemical reactions.⁸ When compared to typical linear polymers, dendrimers have significantly enhanced chemical and physical properties. Important features of dendrimers are nano-size, monodispersity, biocompatibility, pharmacokinetics and periphery charge.¹³⁻¹⁷

Recent developments in characterization technologies and synthetic chemistry have made rapid growth of dendritic novel carriers possible. A range of dendritic scaffolds with finely tuned nano size and a large number of functional ending groups have also become presented.¹⁸

Dendrimer is derived from the Greek words “Dendron,” which means “tree or branch,” and “meros,” which means “part”.¹⁹ Dendrimer is made up of three parts: a central core, ending groups and repeating branching groups. The rise in the branching units number, which leads to the creation of globular structures, determines the “Generation number” of the dendrimers.²⁰⁻²³ The inclusion of functional groups in the dendrimer’s surface also allows the addition of additional moieties that can actively target diseases and enhance delivery, which is now commonly employed as targeting of tumor,²⁰ according to Palmerston Mendes L, Pan J, Torchilin VP 2017. They are ideal carriers for the targeted delivery of medicinal and diagnostic substances due to their specific characteristics.²⁴

5.1.1 Dendrimers have several advantages over traditional anti-cancer drugs

Dendrimers have a nanosize range of 1-100 nm for a pre-detectable release report, targeting potential and attractive pharmacokinetics.²⁵ On the exterior of dendrimers, there are numerous functional groups that can be used to connect vector devices to target to a specific spot in the body. Tumor cell-specific deliverance is facilitated by the presence of multiple functional groups on the surface of dendrimers.

5.1.2 Toxicity and its control

All kinds of dendrimers are known to have hemolytic and cytotoxic characteristics, raising concerns about their safety. The toxicity of dendrimers is determined by their properties, which can be linked primarily to the peripheral end groups and chemistry of the core also.^{20,26} Modification on the periphery of dendrimers can help to increase their safety, and the medication is simple to do by conjugation molecules with the reactive ending groups of dendrimers.

5.1.3 Carriers for drug delivery

- Dendrimers serve as a drug carrier for the anti-cancer drug delivery by encapsulating the drug within the dendrimer or covalently conjugating the drug to form a macromolecular prodrug.²⁷
- Dendrimers based on melamine were used to reduce the toxicity of the drug and solubilize the anticancer medicines methotrexate and 6-mercaptopurine.²⁸
- The deficit of controlled drug release kinetics in these delivery systems is a fundamental flaw. As a result, drug-encapsulated dendrimer systems may be best injected directly into the tumor.

5.1.4 Various forms of dendrimers are used to deliver drugs

Dendrimers' value is well known because to their ability to cross several delivery barriers employing two concepts, passive and active tumor targeting.

- Passive targeting relies on the EPR effect, in which dendrimers extravasate and aggregate in tumor tissue selectively.
- The conjugation of particular targeting ligands on dendrimer surfaces can promote their selective connection to over expressed receptors on particular tumor cells, which is known as active targeting.

Dendrimers are usually known for their guest-host properties and regular, well-defined, highly branched structures with a large number of reactive terminal groups.²⁹ The terminal groups are constricted in space and located at the molecule's periphery. As a result, dendrimers become exclusive starting materials for a variety of chemical alterations. As a result of synergistic effects, the alteration of various functional groups

on the dendrimer's surface can result to changes in, or improvement of, some of their features.³⁰⁻³² We can find various evidence of molecular recognition processes in biological systems that are aided by complex interactions within the molecule. This type of multivalent consequence is widely recognized in carbohydrate-protein interactions,³³ where the clustering of the saccharide molecule can considerably increase saccharide binding affinity to protein receptors.³⁴ Various sugar residues, connected to the branching point by sufficient lengths of linker arms via covalent bonds, may also be able to create significantly stronger structures with receptor proteins than individual saccharides, according to one theory. This theory has sparked interest in multivalent carbohydrate structure synthesis.³⁵ With these goals in mind, dendritic molecules, rather than proteins based carriers, could be considered excellent candidates for prospective oligosaccharide transporters in the creation of neoglyconjugates. Roy and colleagues recently released a series of publications on the chemical functionalization of dendrite-like macromolecules with the goal of producing pharmacologically important glycodendrimers.

5.1.5 Dendrimers: A very special role in drug delivery

Dendrimers are the dendritic polymers with well specified nanostructures and precise control over size, peripheral functionality and branching density. They are effective nanoscale transporters for the delivery of drugs and genes. Dendrimers can be used to create both hydrophobic and hydrophilic medicinal compounds. They can be used in oral, nasal, Intravenous, ocular, pulmonary and transdermal therapeutics techniques. Dendrimers offer immense potential as nanocarrier systems as they can penetrate cell barriers by both transcellular and paracellular pathways. The capacity

to statistically adjust and optimize the ratio of dendrimer peripheral groups that affect biodistribution, therapeutic dose, receptor-mediated targeting and controlled drug release as from dendrimer interior. Dendrimers have a 3-D structure that gives them a range of interesting properties, like well-defined exterior functional groups, nanoscaled globular shape, hydrophilic or hydrophobic cavities in the interior, and amazingly low polydispersity,³⁶ allowing them to be used in a variety of applications. Most of dendrimers, for example, have globular shapes with molecular diameters of less than 10 nm that can be modified by different dendrimer generations. As a result of this feature, dendrimers have similar sizes and forms to certain biomolecules, making them ideal biomimics.³⁷ Dendrimers' branching structure enriches the dendritic frameworks with well-defined numbers of peripheral functional groups, targeting groups, allowing medicinal molecules, solubilizing groups to be present on the periphery in a multivalent form.^{38,39} Furthermore, the hydrophilic and hydrophobic spaces in the inside of dendrimers/nanocarriers make them ideal candidates for encapsulating guest molecules, particularly medicines.^{40,41} Moreover, dendrimers' low polydispersity ensures that biodistribution of polymeric drug carriers employing them as frameworks is reproducible.^{42,43} Nanoparticles with a diameter of 1–10 nm have the ability to penetrate tumour cells. This helps to address constraints associated with free drug treatment, like poor in vivo/in vitro correlation, as well as other probable tumour resistances. Dendrimers are a non-viral gene transfer technology that is extremely useful. They have a significant advantage over the other gene transfer vectors in terms of being able to transfect cells without causing toxicity and being able to be tailored for stimuli-induced targeted delivery. Dendrimer's well-

defined functionalized architecture has prompted scientists to investigate the possibilities of using dendritic macromolecules like coenzyme B₁₂ and O₂-carrying haemoproteins to mimic protein functions. Dendrimers can thus be designed to be stimuli-responsive nanocarriers, having high payload efficacy, comprise molecular tags, reduce dose requirements, suppressing side effects, localized delivery and reduce drug migration during medication treatment. Dendrimers have a unique nanoscale geometry that provides structural advantages over linear polymers, liposomes and bigger nanoparticles. Rapid cellular entrance, targetability, lower macrophage absorption, and easier movement through biological membranes by transcytosis are only a few of the benefits.⁴⁴ Due to the presence of significant reactive functional groups at periphery, dendrimers are suitable drug transporters with increased drug loading capacities as compare to linear polymers.⁴⁵ Drugs encapsulated in PEGylated dendrimers have been shown to have improved penetration and retention (EPR). To summarise, low polydispersity, the ability to selective deliver the functional form of drug specifically at its targeted site of action and a high-density active drug loading are all significant aspects of dendritic polymeric materials for drug administration. As a result, extensive in vivo toxicity testing of dendrimers is critical and can aid in the development of customized dendrimer-mediated CNS drug carriers. They can be synthesized and tailored to individual needs. They are good drug delivery carriers due to their feasible structure, dimensions and functionality; also, they have very close size to several significant biological polymeric materials and complexes such as proteins and DNA, which are biologically optimal.^{46,47} The covalent binding of pharmaceuticals to the peripheral groups of dendrimers via hydrolytic or

biodegradable links improves the drug's pharmacological characteristics and allows for better drug release control.

5.1.6 The benefits of dendrimers

Dendrimers have the following qualities that make them an ideal transporter for administration of drugs, treating, and analysis.

1. **Low polydispersity index:** Due to strict synthesis control, their polydispersity index is quite lower. As branch density rises, the outermost limbs form spheres around a lower density interior, and the exterior density rises, yet most of the space stays hollow as it approaches the core. This area can be used to entrap a wide range of drugs.⁴⁶
2. **The effect of increased retention effect and permeability:** Dendrimer size (Generation 4-4.4 nm) is in the nanometer range. Cancer cells have porous membranes, making anticancer medicines more biopermeable. Dendrimers may have a higher permeability and retention impact (depending on their M.W) than tiny molecules, allowing them to target tumour cells more efficiently. The lymph system is a one-way system and drug-loaded dendrimers may be kept within it.⁴⁸
3. **Permeability is high:** This feature enhances drug intracellular transport. Dendrimers have the ability to overcome biobarriers such as the blood-brain barrier and cell membrane. The nanosize uniformity improve their ability to penetrate cell membranes and reduce the possibility of unwanted clearance from the body via the spleen or liver.^{49,50}

4. **Long-lasting/extensive influence:** Dendrimers release the drug over a long period of time. PAMAM dendrimers released more slowly, accumulated more in solid tumours, and had less toxicity. The surface of these dendrimers has been conjugated with Polyethylene glycol to prevent non-specific contact with plasma proteins. To obtain the desired therapeutic effect, an increase in circulation of blood time is required.⁵¹
5. **Higher solubilization:** Dendrimers' solubility-enhancing property is thought to be mediated by hydrogen bonding, ionic interactions, and hydrophobic interactions. Most anticancer medicines have poor solubility, which can be improved by loading them onto dendrimers.^{52,53} Dendrimers can improve the solubility, efficacy and biodistribution of a variety of treatments. It can also serve as diagnostic and imaging agents in brain tumor-bearing animal models.
6. **High purity and uniformity:** Dendrimers with a consistent size range, well-defined peripheral functionality, and minimal impurity are produced using the synthetic approach. Dendrimers that are monodispersed would help us achieve controlled drug delivery.^{54,55}
7. **Platform with several functions:** On the outer surface of dendrimers, there are multiple functional groups that can be used to link vector components for targeting to a specific spot in the body. To restructure individual receptors, ending groups can also be changed. Dendrimers that have their surfaces modified could be used to mimic biological substrates, exo-receptors, cofactors or inhibitors. By utilizing cross linking agents, free surface groups can create complexes or conjugates with drug-like compounds or ligands. Dendrimers can have solubility modifiers, stealth compounds and ligands attached to their surfaces.^{38,56}

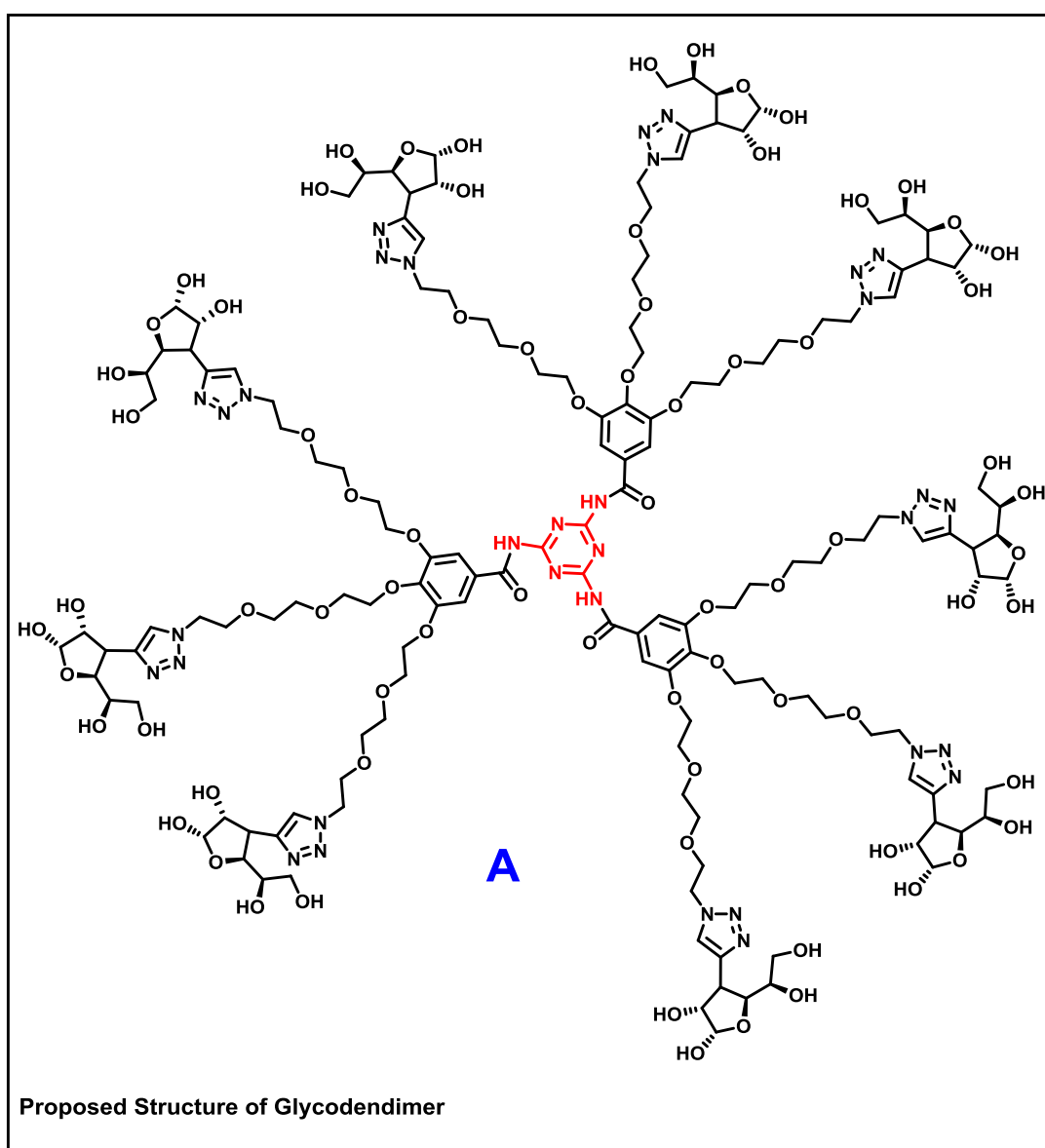
8. **Large capacity for loading:** By entrapment and absorption on the surface, dendrimers formations can also be used to store and carry a wide spectrum of inorganic and organic compounds. Drugs can become entrapped in interior spaces as well as electrostatically on dendrimer surfaces.⁵⁷
9. **Extremely stable:** The colloidal, physiological, and shelf-stability of dendrimers drug complexes or conjugates are all increased. Dendrimers have nanoscale particle sizes ranging from 1 to 100 nanometers, making them less sensitive to reticulum endothelial absorption.
10. **Low toxicity:** The majority of dendrimer compounds are low in cytotoxicity but high in biodegradability.⁴¹ If natural phospholipids are lacking in the dendritic system, PEGylation of the surface of the dendrimer can extend circulation time and minimize toxicity.

5.2 Literature survey

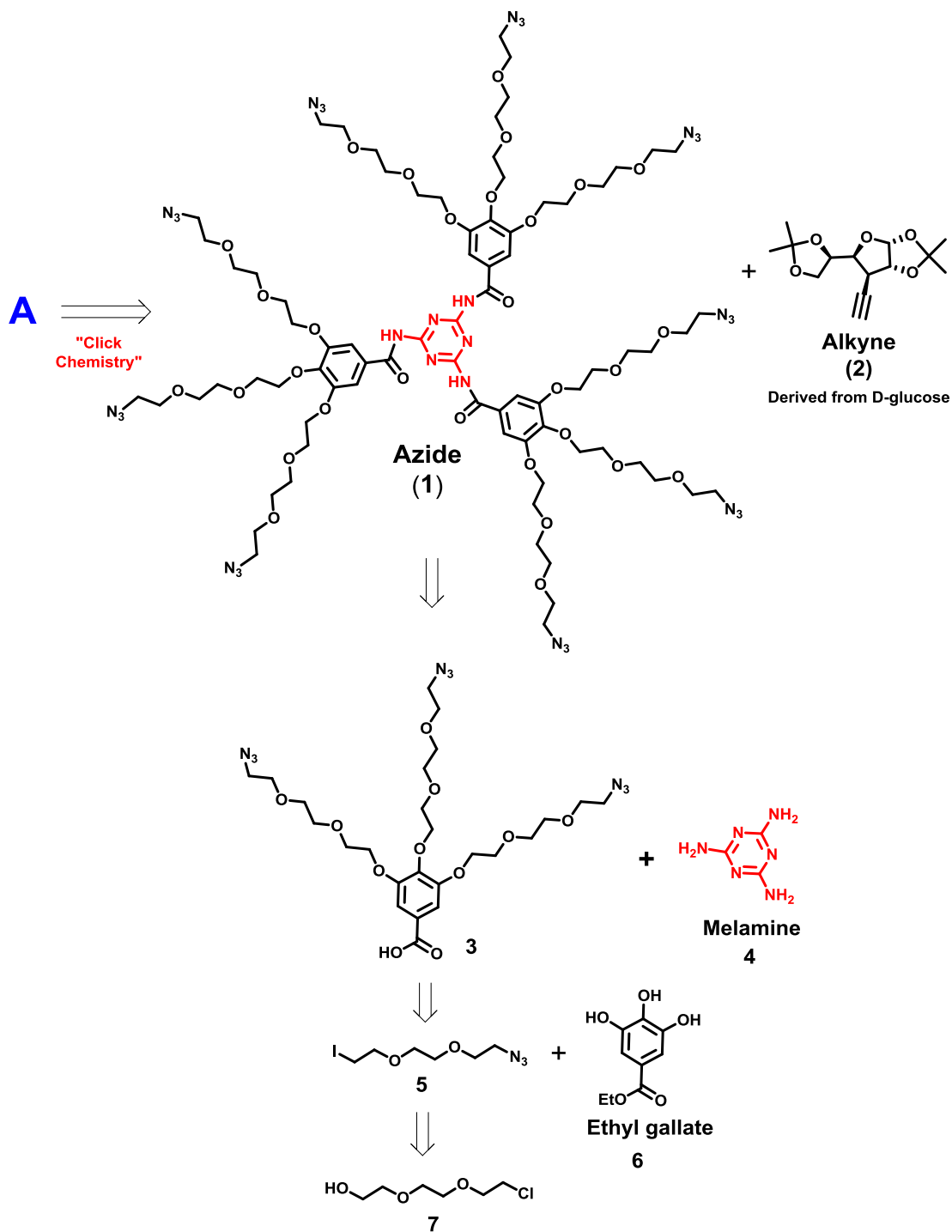
- Based on inspiration from biological systems, a broad variety of dendrimer topologies have been researched and generated for drug-delivery and biological applications over the last two decades.
- Monodispersity and multivalency have distinct strengths that are dependent on their surface functional groups, size and manufacturing.
- The first entire dendrimer family to be synthesized and commercialized is Poly(propylene imine) PPI.

5.3 Why we have chosen Melamine as a core for synthesis of glycodendrimers?

Commercially available, highly stable even at exposure of high temperature, flame retardant, moisture resistance and hence can enhance bioavailability of drugs.



5.4 Retrosynthetic analysis



Scheme 5.1

The proposed glycodendrimer (**A**) could be synthesized by doing 'click chemistry' reactions of azide (**1**) with alkyne (**2**). Azide **1** could be synthesized by the coupling of

acid (**3**) and melamine (**4**). Further compound (**3**) could be prepared by alkylation of 1-azido-2-(2-(2-iodoethoxy)ethoxy)-ethane (**5**) with ethyl gallate (**6**). Compound (**5**) could be synthesized from the starting material 2-(2-(2-chloroethoxy)ethoxy)ethanol (**7**) using sequential reactions.

5.4.1 Attempt towards the synthesis of azide (**1**)

We have started our synthetic journey for the preparation of azide (**1**). Accordingly 2-(2-(2-Chloroethoxy)ethoxy)ethanol (**7**) was converted into 2-(2-(2-Azidoethoxy)ethoxy)ethanol (**8**) by treating with sodium azide in the presence of DMF at 100°C (**Scheme 5.2**). The product formation was analyzed by TLC analysis. Further its formation was confirmed by ^1H NMR and ^{13}C NMR spectral analysis. The ^1H NMR spectrum of (**8**) showed multiplet at 3.03 ppm for $-\text{CH}_2\text{N}_3$ protons. The ^{13}C NMR spectrum of the compound showed a signal resonated at 50.7 ppm for azide attached carbon ($-\text{CH}_2\text{N}_3$). Compound (**8**) was also confirmed by matching its ^1H NMR spectrum with the reported data which was in consistent.

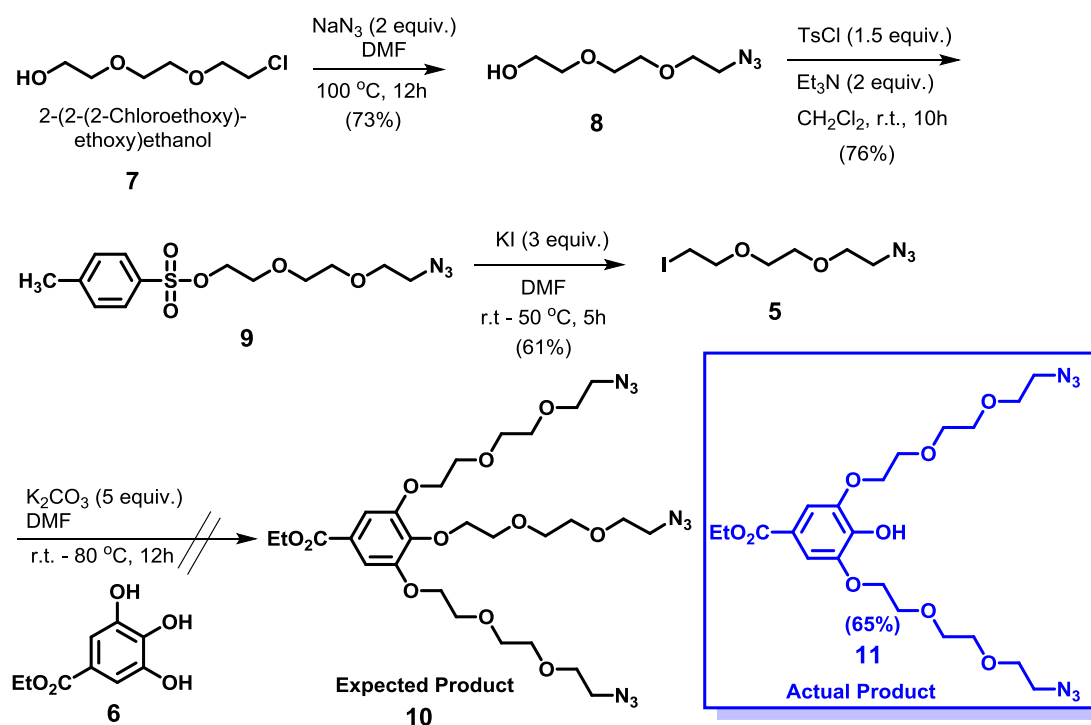
Now (**8**) was converted into (**9**) by reacting it with tosylchloride and triethylamine in the presence of DCM at room temperature (**Scheme 5.2**). The product formation was analysed by TLC analysis. Further it was confirmed by ^1H NMR and ^{13}C NMR spectral analysis. The ^1H NMR spectrum of compound **9**, hydroxyl peak ($-\text{OH}$) at 2.11 ppm got disappeared and new doublet peaks for aromatic protons at 7.80 and 7.34 ppm, respectively were appeared for 4 protons because of tosyl protons, also a new singlet for methyl protons ($-\text{CH}_3$) at 2.44 ppm was observed. The ^{13}C NMR spectrum of the compound showed new signals in the product resonated at 144.9 ppm for one aromatic carbon (1C, $\text{Ar}-\text{C}-\text{SO}_3^-$), 133.0 ppm for one aromatic carbon (1C, $\text{Ar}-\text{C}-\text{CH}_3$), 129.9

ppm for two aromatic carbons (2C, Ar-C), 128.0 ppm for two aromatic carbons (2C, Ar-C) and a signal at 21.7 ppm for one methyl carbon (1C, Ar-C-CH₃) confirmed the formation of the compound **(9)**. It was further confirmed by matching its ¹H NMR spectrum with the reported data which was in consistent.

We have next planned to substitute tosyl group with iodo. Accordingly compound **(9)** was treated with potassium iodide in the presence of DMF at room temperature to 50°C (**Scheme 5.2**). The product formation was analysed by TLC analysis. Further its confirmation was done by ¹H NMR and ¹³C NMR spectral analysis. The structures of the synthesized compound 1-azido-2-(2-(2-iodoethoxy)ethoxy)ethane (**5**) was confirmed by its ¹H NMR spectrum, where aromatic peaks for tosyl group get disappeared and new multiplet peak at 3.17 ppm for two protons (m, 2H, -CH₂I) was observed. The ¹³C NMR spectrum of the compound showed new signal in the product resonated at 3.5 ppm for one carbon (1C, ICH₂-) while the aromatic peaks for tosyl group at 144.9 ppm for one aromatic carbon (1C, Ar-C-SO₃-), 133.0 ppm for one aromatic carbon (1C, Ar-C-CH₃), 129.9 ppm for two aromatic carbons (2C, Ar-C), 128.0 ppm for two aromatic carbons (2C, Ar-C) and a signal at 21.7 ppm for one methyl carbon (1C, Ar-C-CH₃) were disappeared which confirmed the formation of the compound **(5)**. Further the spectral data of compound **(5)** in consistent with the reported one.

Now **(5)** was tried to alkylate with ethyl gallate. Accordingly **(5)** was treated with ethyl gallate in the presence of potassium carbonate and DMF and mixture was heated upto 80°C (**Scheme 5.2**). The product formation was analysed by TLC analysis which showed new spot has been developed. After careful analysis of ¹H NMR and ¹³C NMR

spectral data, it was concluded that expected product (**10**) was not formed, but the actual product was ethyl 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxy benzoate (**11**). The reason behind this is that the middle hydroxyl protons were not taken up by base due to steric hindrance. The structures of the compound (**11**) was confirmed by its ^1H NMR spectrum, where signal intensities were in consistent with the bi- alkylation and a new broad singlet at 8.00 ppm for aromatic hydroxyl proton (s, 1H, -OH) confirms the formation of compound (**11**) and not (**10**).

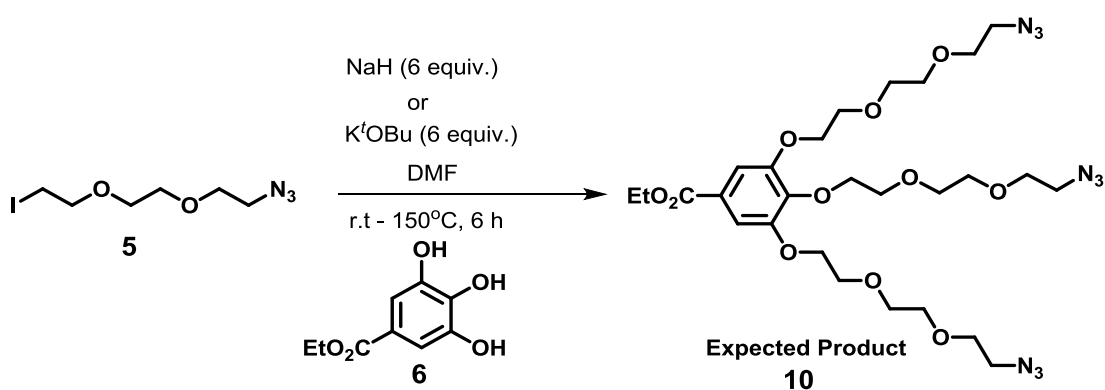


Scheme 5.2

The ^{13}C NMR spectrum of the compound showed the signal in the reactant resonated at 3.5 ppm for one carbon (1C, ICH_2 -) was disappeared while the new aromatic peaks at 171.1 ppm for one carbonyl carbon (1C, Ar-C-C=O), at 149.9 ppm for two aromatic carbons (2C, Ar-C-O), at 138.7 ppm for one aromatic carbon (1C, Ar-C-OH), 125.8 ppm for one aromatic carbon (1C, Ar-C-C=O), 110.2 ppm for two aromatic carbons

(2C, Ar-C), at 73.4 ppm for one carbon (1C, -CH₂CH₃) and a signal at 29.7 ppm for one methyl carbon (1C, -CH₂CH₃) were appeared which further supports the formation of the compound ethyl 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoate (**11**).

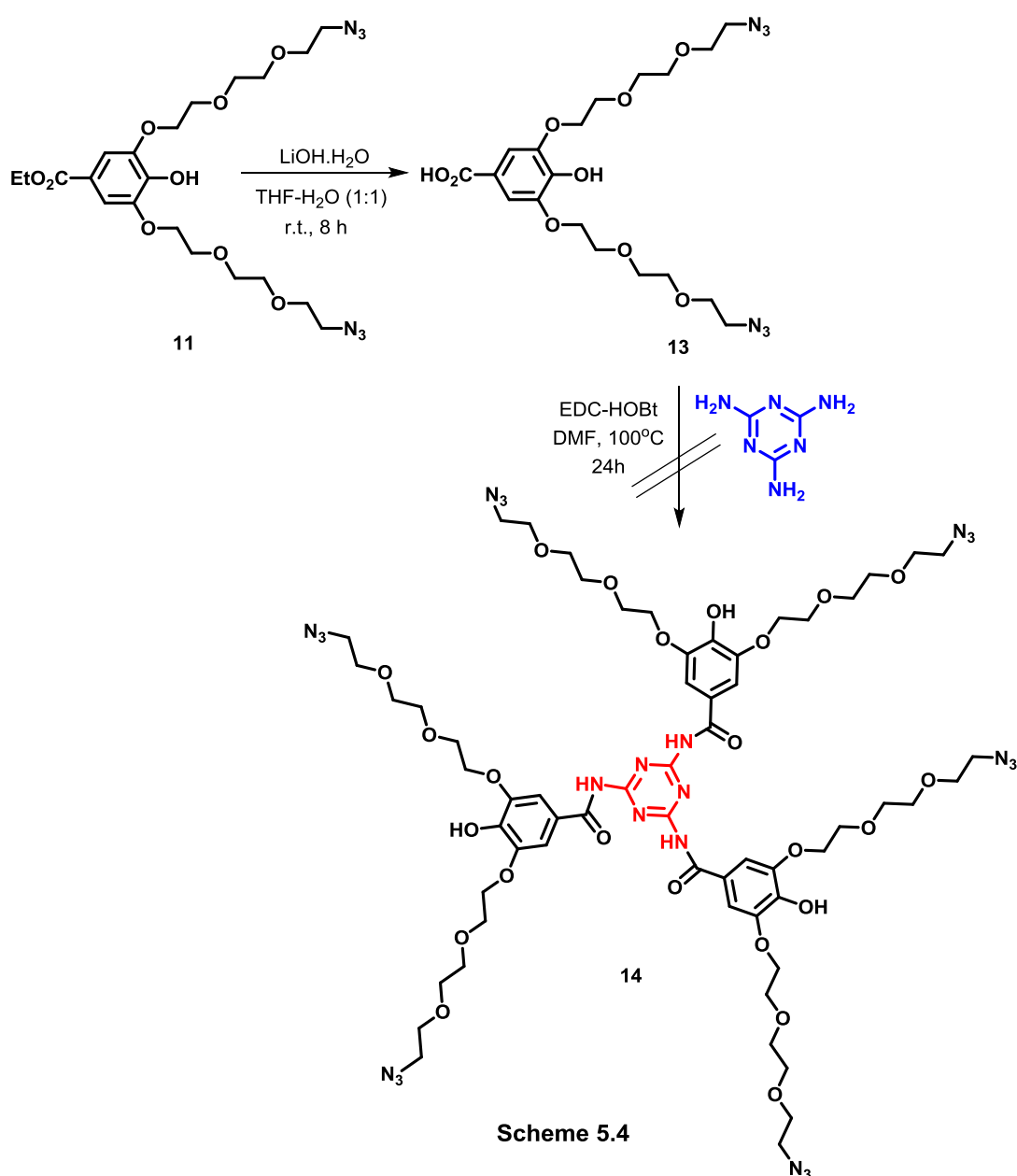
Further to afford the required product 3,4,5-tris(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoate (**10**), we have changed the reaction conditions and strong basic condition was used. Compound (**5**) was reacted in presence of ethyl gallate and strong base NaH or K^tOBu in the presence of DMF and temperature was increased up to 150°C (**Scheme 5.3**). The progress of reaction was monitored with the help of TLC analysis. The new isolated product was analyzed by ¹H NMR and ¹³C NMR spectroscopy, which showed that iodo compound (**5**) under gone elimination reaction and (2-(2-azidoethoxy)ethoxy)ethene (**12**) was obtained as only product (**Scheme 5.3**).



Scheme 5.3

The structure of (**12**) was confirmed by its ¹H NMR spectrum, where a broad singlet at 8.00 ppm for aromatic hydroxyl proton (s, 1H, -OH) and peaks for other aromatic protons were

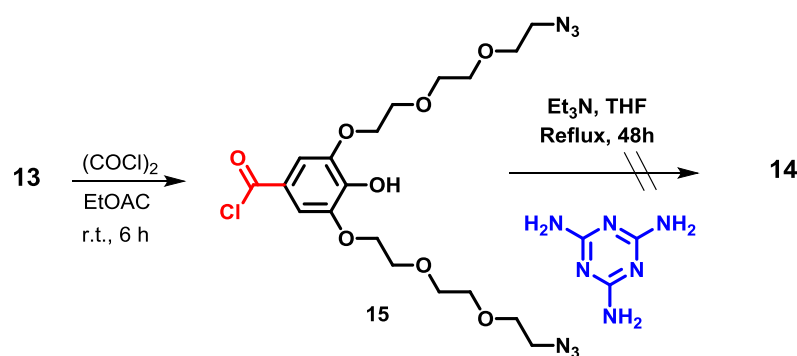
disappeared while new peaks of the product i.e. a doublet of doublet at 6.51 ppm for one olefinic proton (dd, 1H, $\text{CH}_2=\text{CHO}-$), a multiplet at 4.31-3.92 for two protons (m, 2H, $\text{CH}_2=\text{CHO}-$) were appeared. The ^{13}C NMR spectrum of the compound showed that the peak at 171.1 ppm for one carbonyl carbon (1C, $\text{Ar}-\text{C}-\text{C}=\text{O}$), for aromatic carbons were disappeared and new peaks at 151.6 ppm for one carbon (1C, $\text{CH}_2=\text{CHO}-$), at 86.7 ppm for one carbon (1C, $\text{CH}_2=\text{CHO}-$), which confirmed the formation of the compound (**12**).



After many efforts and failure towards the synthesis of expected product **(10)**, we have decided to move ahead with the substrate **(11)** for the synthesis towards glycodendrimers. Accordingly, ester **(11)** was hydrolyzed with LiOH.H₂O in THF-H₂O mixture (**Scheme 5.4**).

The formation of polar spot in the TLC, confirmed the hydrolysis reaction. After work-up, the crude acid product **(13)** was subjected for coupling reaction with melamine under EDC and HOBt reaction condition (**Scheme 5.4**). The TLC analysis was disappointing because of multiple spots were appeared.

Finally we have attempted to convert acid **(13)** to acyl chloride **(15)** by heating it with oxalyl chloride in ethyl acetate (**Scheme 5.5**). The crude acyl chloride was used as such for coupling reaction with melamine in presence of triethylamine (**Scheme 5.5**). The TLC analysis further showed the appearance of multiple spots, confirms the formation of complex mixture.



Scheme 5.5

5.5 Conclusion

Synthetic studies towards the synthesis of glycodendrimer has been carried out. The tri O-alkylation of ethyl gallate with iodo compound **(5)** could not take place under various

reaction conditions. Subsequently, the coupling reaction of melamine with bi-alkylated acid (**13**) / acyl chloride (**15**) was performed, but unfortunately did not yield satisfactory results. It therefore needs to be further optimized before proceeding to achieve the proposed glycodendrimer synthesis. The effort in this direction are presently being perused in our laboratory.

5.6 Experimental

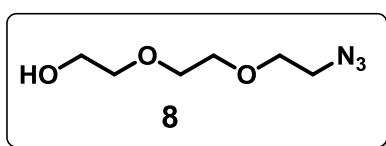
5.6.1 Materials

2-(2-(2-Chloroethoxy)ethoxy)ethanol, sodium azide and analytical grade solvents were purchased from Sigma Aldrich. Melamine, tosyl chloride and sodium sulphate were purchased from Central Drug House and used as such. Distillation of solvents was done before its use.

5.6.2 Methods

Nuclear Magnetic Resonance (NMR) spectra (^1H) and (^{13}C) were recorded on Bruker 400 MHz and 101 MHz spectrometer, respectively. The samples were made in chloroform-d using tetramethylsilane (TMS) as the internal standard and chemical shift values were measured in ppm on a scale downfield from TMS and the coupling constant J are in Hz.

Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethanol (**8**)

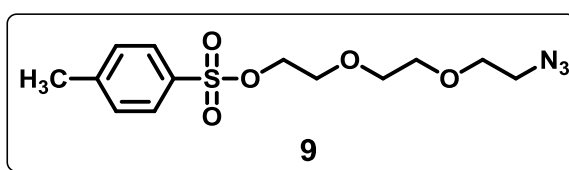


In an oven dried two necked round bottom flask 2-(2-(2-chloroethoxy)ethoxy)ethanol (5.0 g, 30 mmol) and sodium azide (3.9 g, 60 mmol) were added in dry DMF (40 mL)

and resulting mixture was stirred at 100°C for 12 h. The progress of the reaction was monitored with the help of TLC. After 12h, the TLC showed complete consumption of starting material 2-(2-(2-chloroethoxy)ethoxy)ethanol. Now the mixture was transferred to separating funnel and the organic layer (EtOAc) was collected and the aqueous layer was further extracted with ethyl acetate (EtOAc) for three times. The organic layer was combined, washed with brine, dried with Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was further purified by column chromatography to get the pure product 2-(2-(2-azidoethoxy)ethoxy)ethanol (3.1 g, 72%) as a colorless oil.

¹H NMR (80 MHz, Chloroform-*d*) δ 3.76-3.40 (m, 10H, -OCH₂CH₂O-), 3.03 (m, 2H, -OCH₂CH₂N₃), 2.11 (s, 1H, -OH); ¹³C NMR (20 MHz, Chloroform-*d*) δ 70.7 (1C, -OCH₂-), 70.6 (1C, -OCH₂-), 70.4 (1C, -OCH₂-), 70.0 (1C, -OCH₂-), 61.7 (1C, HOCH₂-), 50.7 (1C, -CH₂N₃).

Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (9):

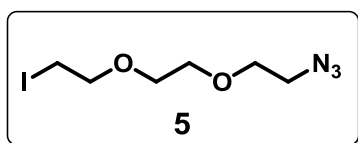


In an oven dried two necked round bottom flask compound **8** (2.0 g, 11.4 mmol) and TEA (2.31 g, 22.8 mmol) were added in DCM (20 mL) at 0 °C and further tosyl chloride (3.26 g, 17.1 mmol) was added by portions. Then the resulting mixture was stirred and allowed to heat up to room temperature and then further stirred for 10 h. The progress of the reaction was monitored with the help of TLC. After 10 h, the TLC showed complete consumption of starting material 2-(2-(2-Azidoethoxy)ethoxy)ethanol. Now the mixture was transferred to separating funnel and the organic layer

(DCM) was collected and the aqueous layer was further extracted with DCM for three times. The organic layer was combined, washed with 1 M HCl, brine, dried with Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was further purified by column chromatography with eluent (PE/EA = 4:1, v/v) to get the pure product 2-(2-(2-Azidoethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (2.6 g, 69%) as a colorless oil.

¹H NMR (80 MHz, Chloroform-*d*) δ 7.80 (d, 2H, *J* = 8.0 Hz, Ar-**H**), 7.34 (d, 2 H, *J* = 8.0 Hz, Ar-**H**), 4.17 (t, 2H, *J* = 4.0 Hz, -O₂SOCH₂CH₂O-), 3.75-3.35 (m, 10 H, -OCH₂CH₂-), 2.44 (s, 3 H, CH₃); ¹³C NMR (20 MHz, Chloroform-*d*) δ 144.9 (1C, Ar-**C**-SO₃-), 133.0 (1C, Ar-**C**-CH₃), 129.9 (2C, Ar-**C**), 128.0 (2C, Ar-**C**), 70.8 (1C, -OCH₂-), 70.6 (1C, -OCH₂-), 70.1 (1C, -OCH₂-), 69.8 (1C, -OCH₂-), 68.8 (1C, -OCH₂-), 50.7 (1C, -CH₂N₃), 21.7 (1C, Ar-**C**-CH₃).

Synthesis of 1-azido-2-(2-(2-iodoethoxy)ethoxy)ethane (5)

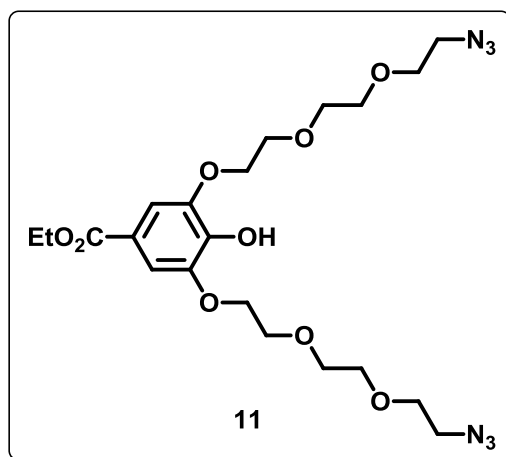


In an oven dried two necked round bottom flask compound **9** (2.0 g, 6.08 mmol) was added in dry DMF (10 mL) and then sodium iodide (2.73 g, 18.24 mmol) was added by portions, then the resulting mixture was stirred and heated at 50 °C for 5 h. The progress of the reaction was monitored with the help of TLC. After 5 h, the TLC showed complete consumption of starting material 2-(2-(2-azidoethoxy)ethoxy)ethyl-4-methylbenzenesulfonate. Now the mixture was transferred to separating funnel and the organic layer (EtOAc) was collected and the aqueous layer was further extracted with

ethyl acetate (EtOAc) for three times. The organic layer was combined, washed with brine, dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was further purified by column chromatography with eluent (PE/EA = 6:1, v/v) to get the pure product 1-Azido2-(2-(2-iodoethoxy) ethoxy) ethane (1.25 g, 72%) as a colorless oil.

^1H NMR (80 MHz, Chloroform-*d*) δ 3.87-3.17 (m, 12H, $-\text{CH}_2-$); ^{13}C NMR (20 MHz, Chloroform-*d*) δ 72.1 (1C, $-\text{OCH}_2\text{CH}_2\text{I}$), 70.5 (1C, $-\text{OCH}_2-$), 70.3 (1C, $-\text{OCH}_2-$), 70.0 (1C, $-\text{OCH}_2-$), 50.9 (1C, $-\text{CH}_2\text{N}_3$), 3.5 (1C, ICH_2-).

Synthesis of ethyl 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoate (11)

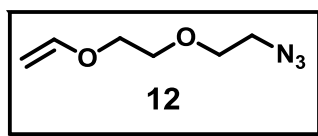


In a two necked round bottom flask compound 1-azido2-(2-(2-iodoethoxy)-ethoxy) ethane, **5** (1.0 g, 3.51 mmol) was added in dry DMF (10 mL) and then further ethyl gallate (231.8 mg, 1.17 mmol) and K_2CO_3 (2.42 g, 17.55 mmol), were added to the reaction mixture and then further the resulting solution was stirred at r.t. and heated upto 80°C for 12 h. The progress of the reaction was monitored with the help of TLC. After 12 h, the TLC showed complete consumption of starting material 1-Azido2-(2-(2-iodoethoxy) ethoxy) ethane. Now the mixture was transferred to separating funnel and

the organic layer (EtOAc) was collected and the aqueous layer was further extracted with ethyl acetate (EtOAc) for three times. The organic layer was combined, washed with brine, dried with Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was further purified by column chromatography with eluent (PE/EA = 6:1, v/v) to get the pure product ethyl 3,4,5-tris(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoate but actual product obtained was ethyl 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoate **11** (1.1 g, 61%) as a brown colored viscous liquid.

¹H NMR (80 MHz, Chloroform-*d*) δ 8.00 (s, 1H, -OH), 7.15 (s, 2H, Ar-H), 4.28 (m, 4H, -ArOCH₂CH₂O-), 3.76-3.31 (m, 16H, -OCH₂CH₂O-), 2.91 (m, 4H, -OCH₂CH₂N₃), 2.06 (m, 2H, -CH₂CH₃) 1.36 (t, 3H, -CH₃); ¹³C NMR (20 MHz, Chloroform-*d*) δ 171.1 (1C, Ar-C=O), 149.9 (2C, Ar-C-O), 138.7 (1C, Ar-C-OH), 125.8 (1C, Ar-C-C=O), 110.2 (2C, Ar-C), 73.4 (1C, -CH₂CH₃), 70.7 (2C, -OCH₂-), 70.6 (2C, -OCH₂-), 70.3 (2C, -OCH₂-), 70.1 (2C, -OCH₂-), 70.0 (2C, -OCH₂-), 50.7 (2C, -CH₂N₃), 29.7 (1C, -CH₂CH₃).

Formation of unexpected eliminated product (2-(2-azidoethoxy)ethoxy)ethene (**12**)

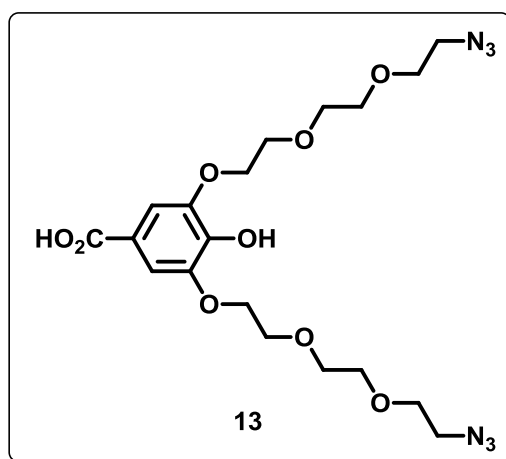


To obtain the required product **10**, we tried to change the reaction condition, to a stirred solution of compound **5** (1.0 g, 3.51 mmol) in dry DMF (10 mL) was added ethyl gallate (231.8 mg, 1.17 mmol) and NaH or K^tOBu (21.06 mmol), then the resulting solution was stirred at 150 °C for 6 h. The solvent was evaporated under reduced pressure. The residue was washed with water and extracted with EA for three times.

The organic layer was combined, washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with eluent (PE/EA = 6:1, v/v) to afford ethyl 3,4,5-tris(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoate but actual product obtained was the eliminated product (2-(2-(2-azidoethoxy)ethoxy)ethene **12** (0.4 g, 72%) as a brown colored viscous liquid.

^1H NMR (80 MHz, Chloroform-*d*) δ 6.51 (dd, 1H, $\text{CH}_2=\text{CHO}-$), 4.31-3.92 (m, 2H, $\text{CH}_2=\text{CHO}-$), 3.88-3.33 (m, 8H, $-\text{OCH}_2\text{CH}_2\text{N}_3$); ^{13}C NMR (20 MHz, Chloroform-*d*) δ 151.6 (1C, $\text{CH}_2=\text{CHO}-$), 86.7 (1C, $\text{CH}_2=\text{CHO}-$), 70.0 (1C, $-\text{OCH}_2-$), 69.5 (1C, $-\text{OCH}_2-$), 67.3 (1C, $-\text{OCH}_2-$), 50.6 (1C, $-\text{CH}_2\text{N}_3$).

Synthesis of 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoic acid (**13**)



3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoate, **11** (1.0 g, 1.95 mmol) was hydrolyzed by $\text{LiOH}\cdot\text{H}_2\text{O}$ in THF- H_2O (10 mL), then the resulting solution was stirred at r.t. for 8 h. The progress of the reaction was monitored with the help of TLC. After 8h, the TLC showed complete consumption of starting material 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoate. Now the mixture was transferred to separating funnel and the organic layer (EtOAc) was collected and the aqueous layer

was further extracted with ethyl acetate (EtOAc) for three times. The organic layer was combined, washed with brine, dried with Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was used as such for further reaction.

Attempted synthesis of *N,N',N''*-(1,3,5-Triazine-2,4,6-triyl)tris(3,5-bis(2-(2-(2-azidoethoxy)-ethoxy)ethoxy)-4-hydroxybenzamide) (14)

To a stirred solution of compound 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoic acid, **13** (100 mg, 0.206 mmol) in dry DMF (1 mL) was added melamine (8 mg, 0.068 mmol), EDC (43 mg, .023 mmol) and HOBt (31 mg, 0.23 mmol), then the resulting solution was stirred at 100°C for 24 h. The progress of reaction was monitored with the help of TLC, which showed multiple spots. Therefore this reaction was shut down.

Next we attempted to convert acid to acyl chloride by reacting 3,5-bis(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-4-hydroxybenzoic acid, **13** (0.05 g, 0.103 mmol) with oxalyl chloride (0.015 g, 0.113 mmol) in ethyl acetate (1 mL) at 0°C to room temperature, then the resulting solution was stirred at r.t. for 6h. The progress of reaction was monitored with the help of TLC, which showed multiple spots once again. Therefore we could not proceed further.

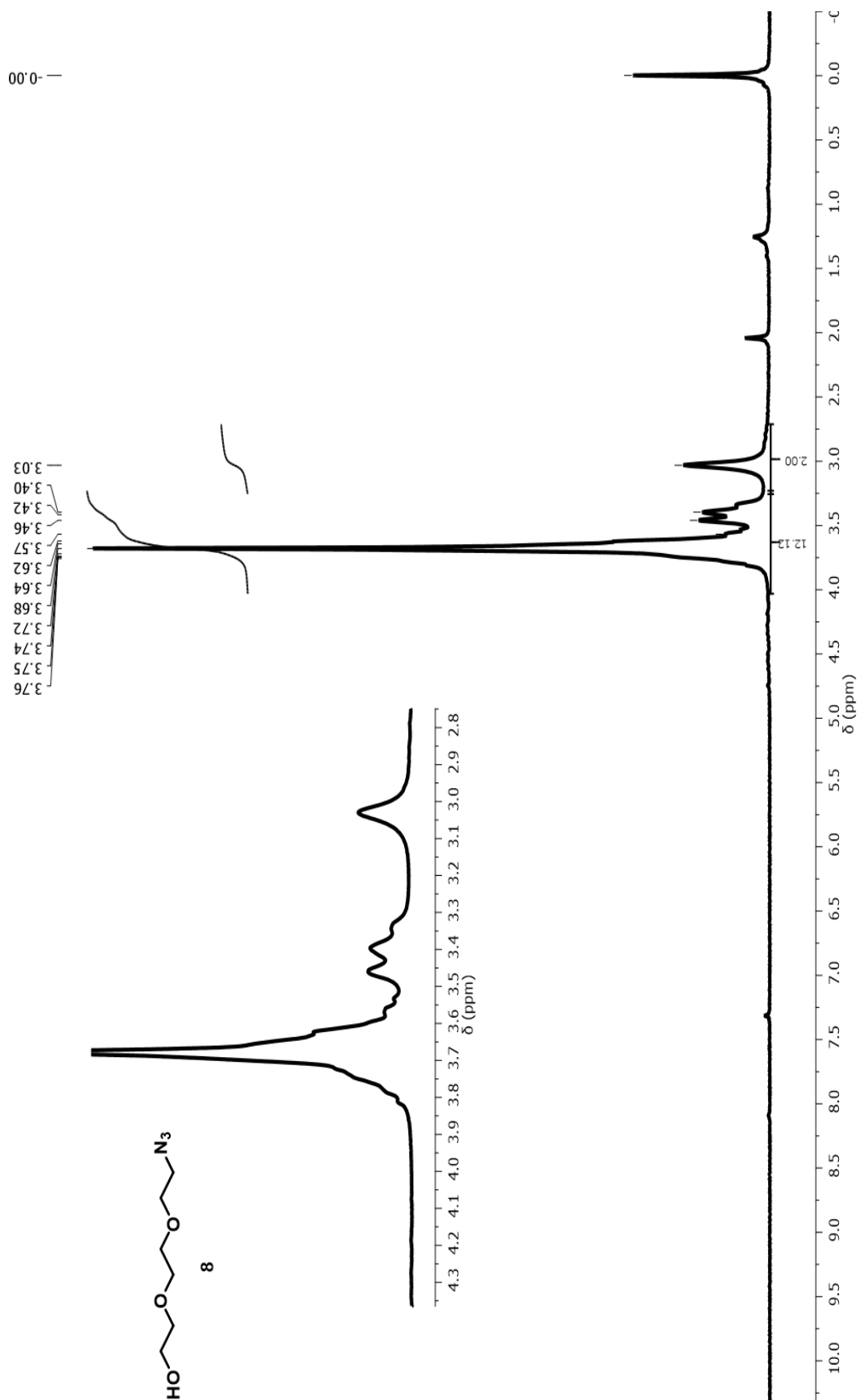


Figure 5.1. ^1H NMR spectrum of **8** in CDCl_3 (80 MHz)

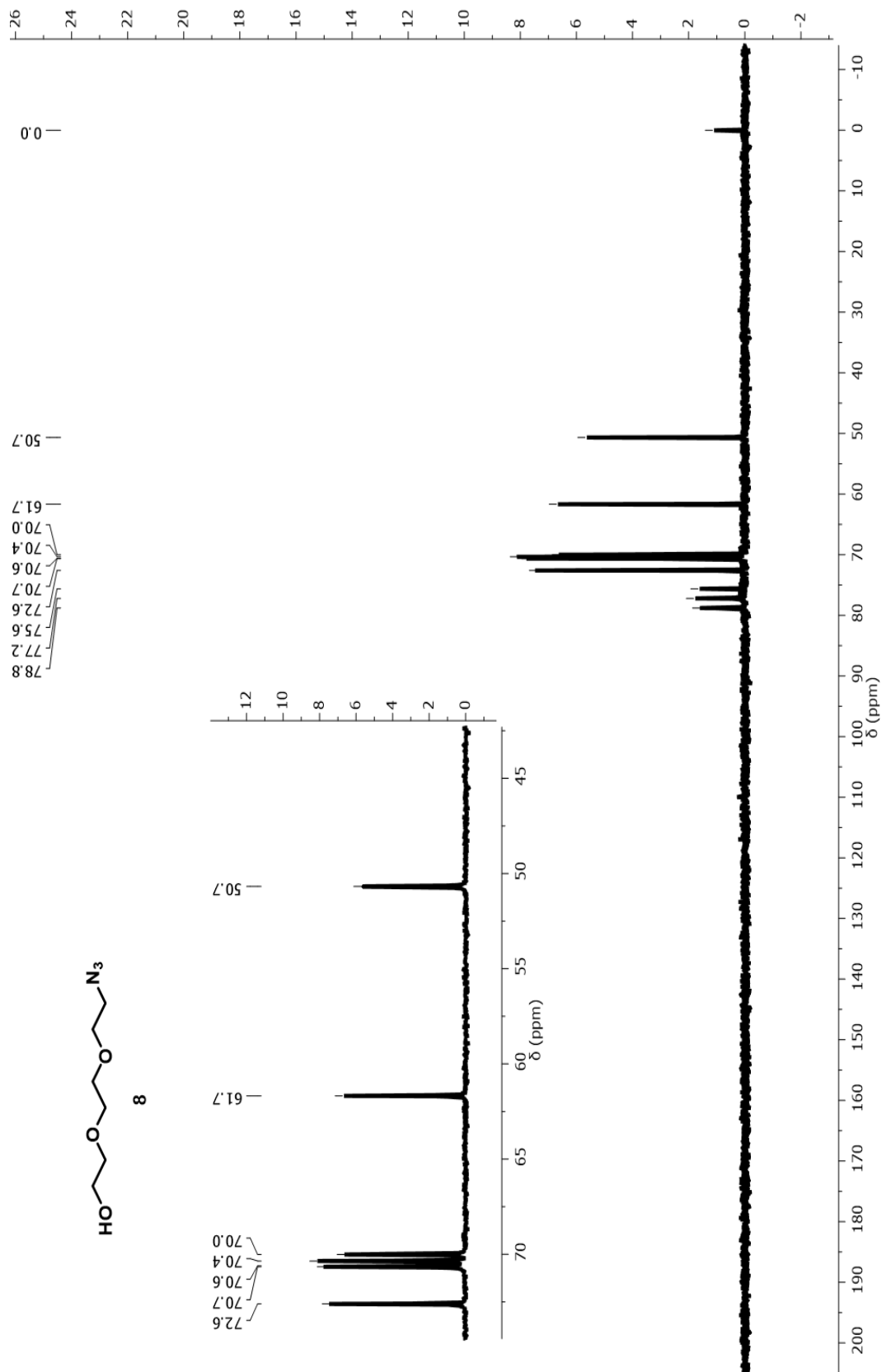


Figure 5.2. ^{13}C NMR spectrum of **8** in CDCl_3 (20 MHz)

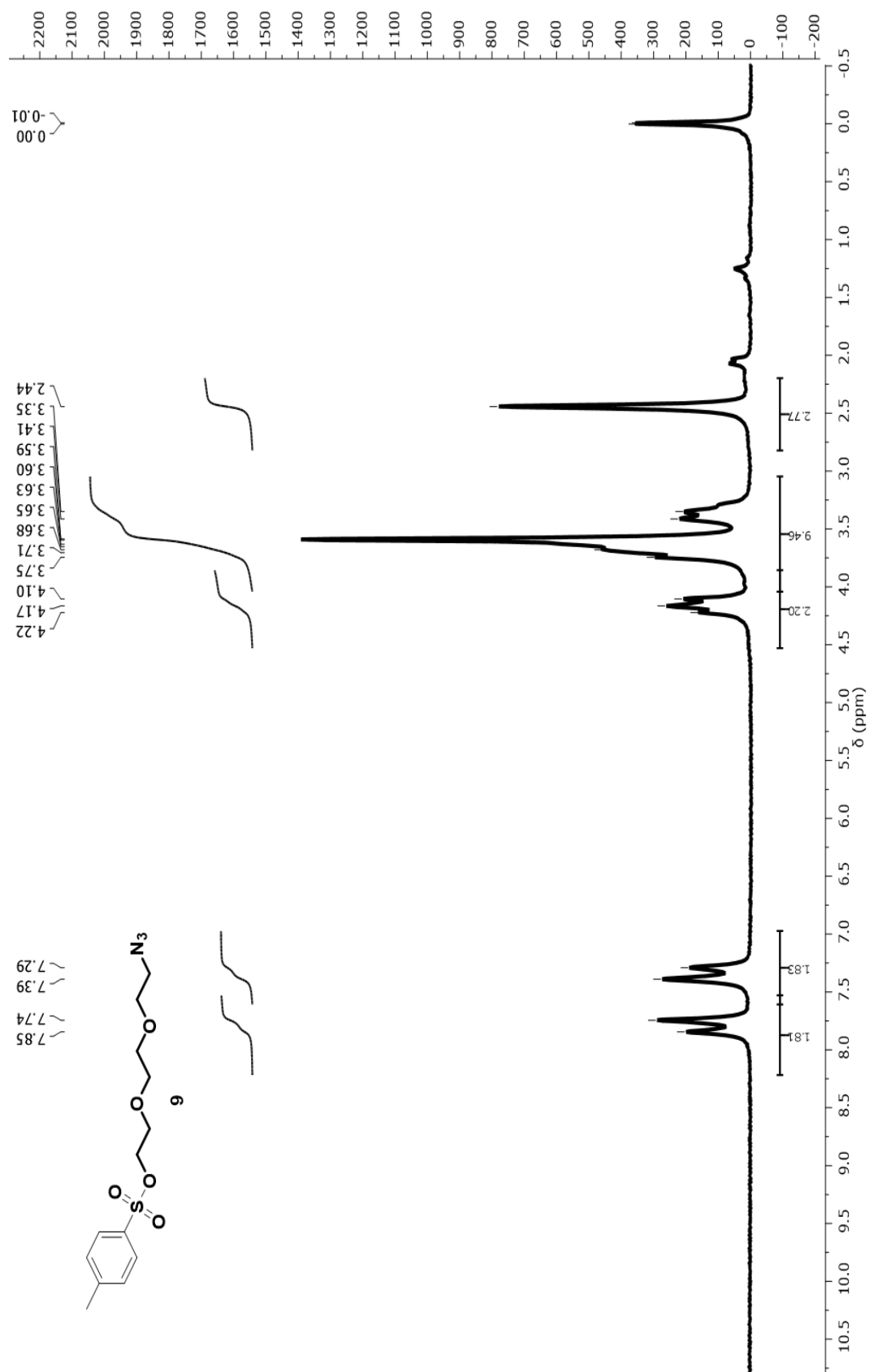


Figure 5.3. ^1H NMR spectrum of **9** in CDCl_3 (80 MHz)

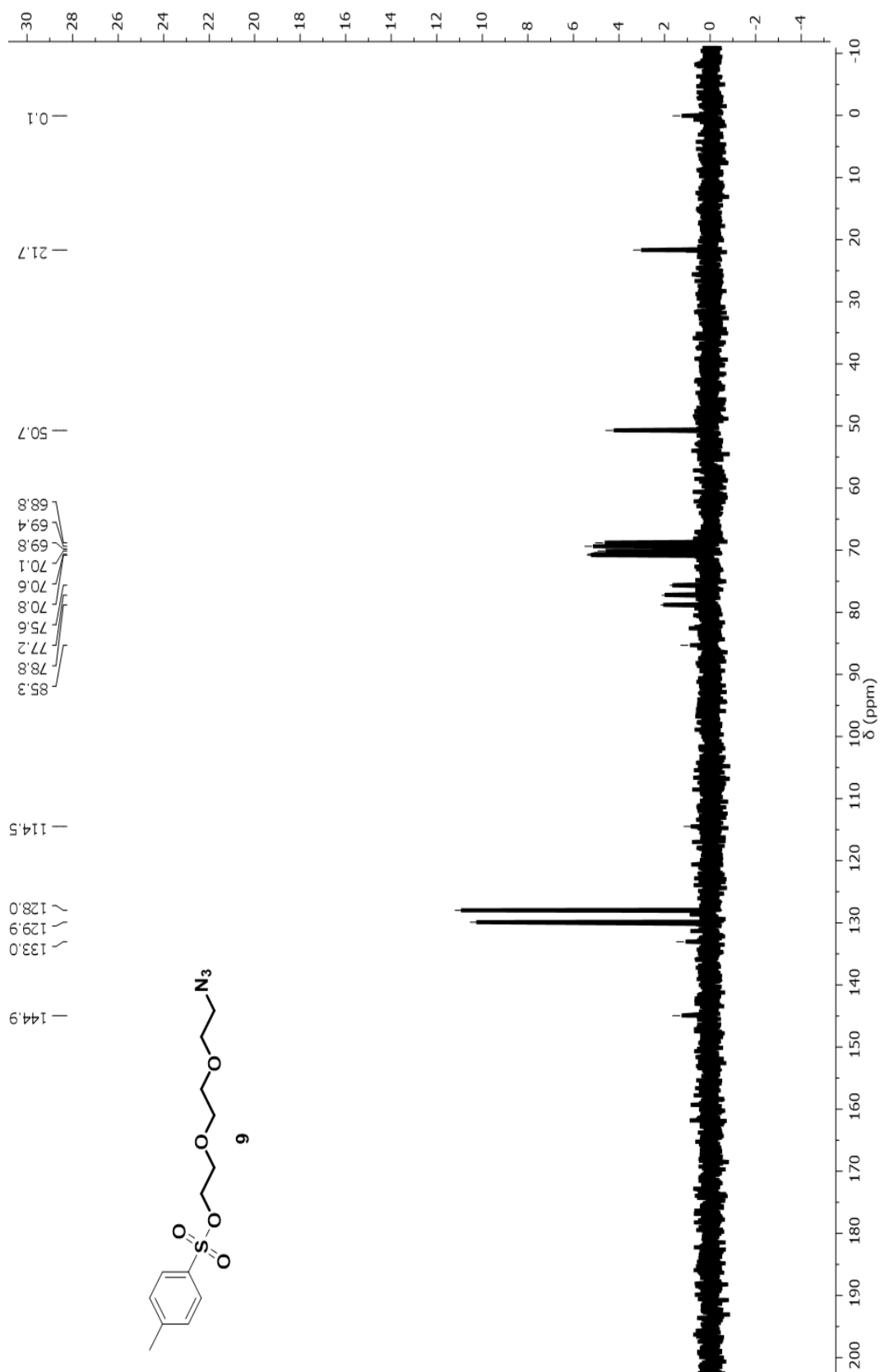


Figure 5.4. ^{13}C NMR spectrum of **9** in CDCl_3 (20 MHz)

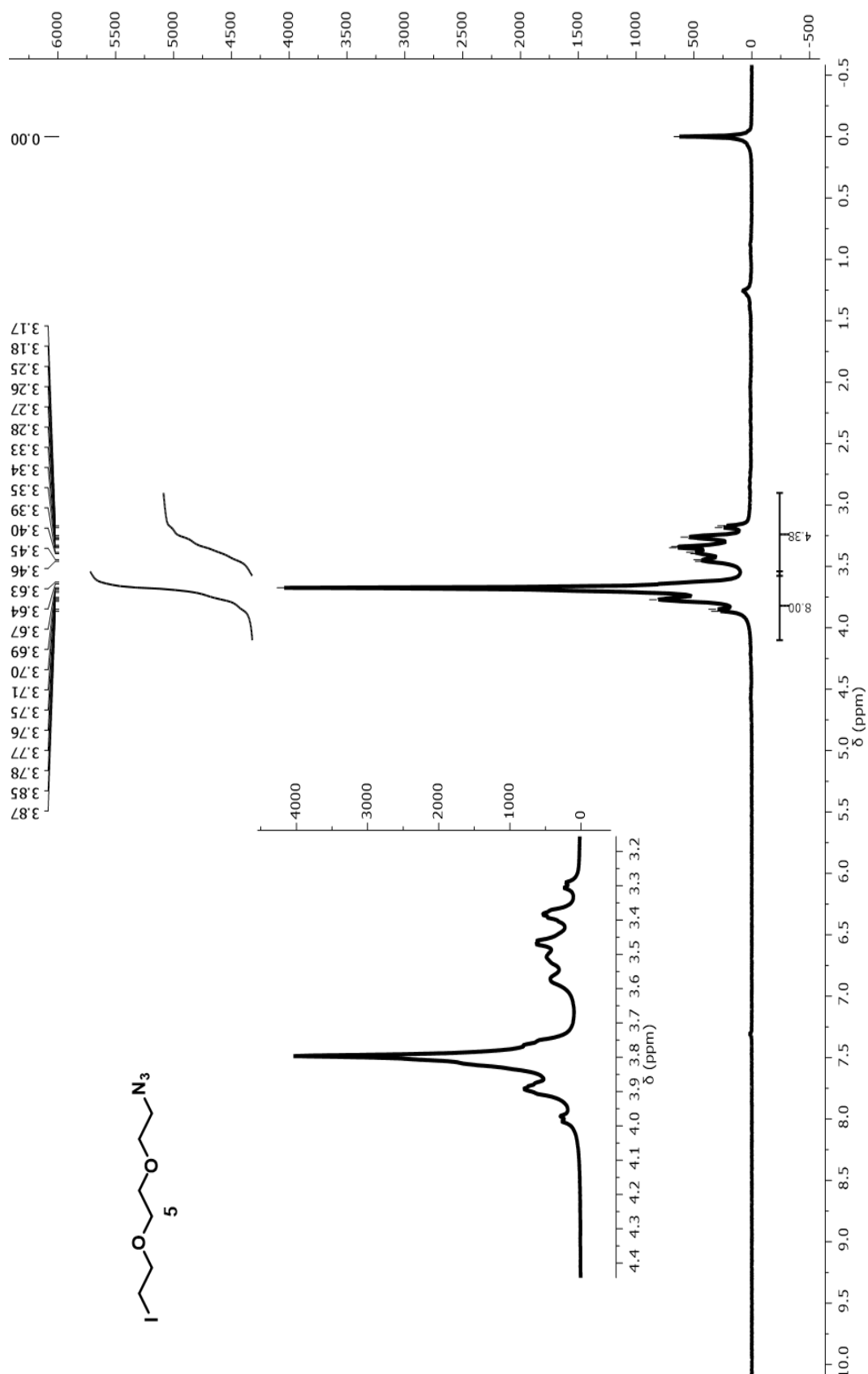


Figure 5.5. ^1H NMR spectrum of **5** in CDCl_3 (80 MHz)

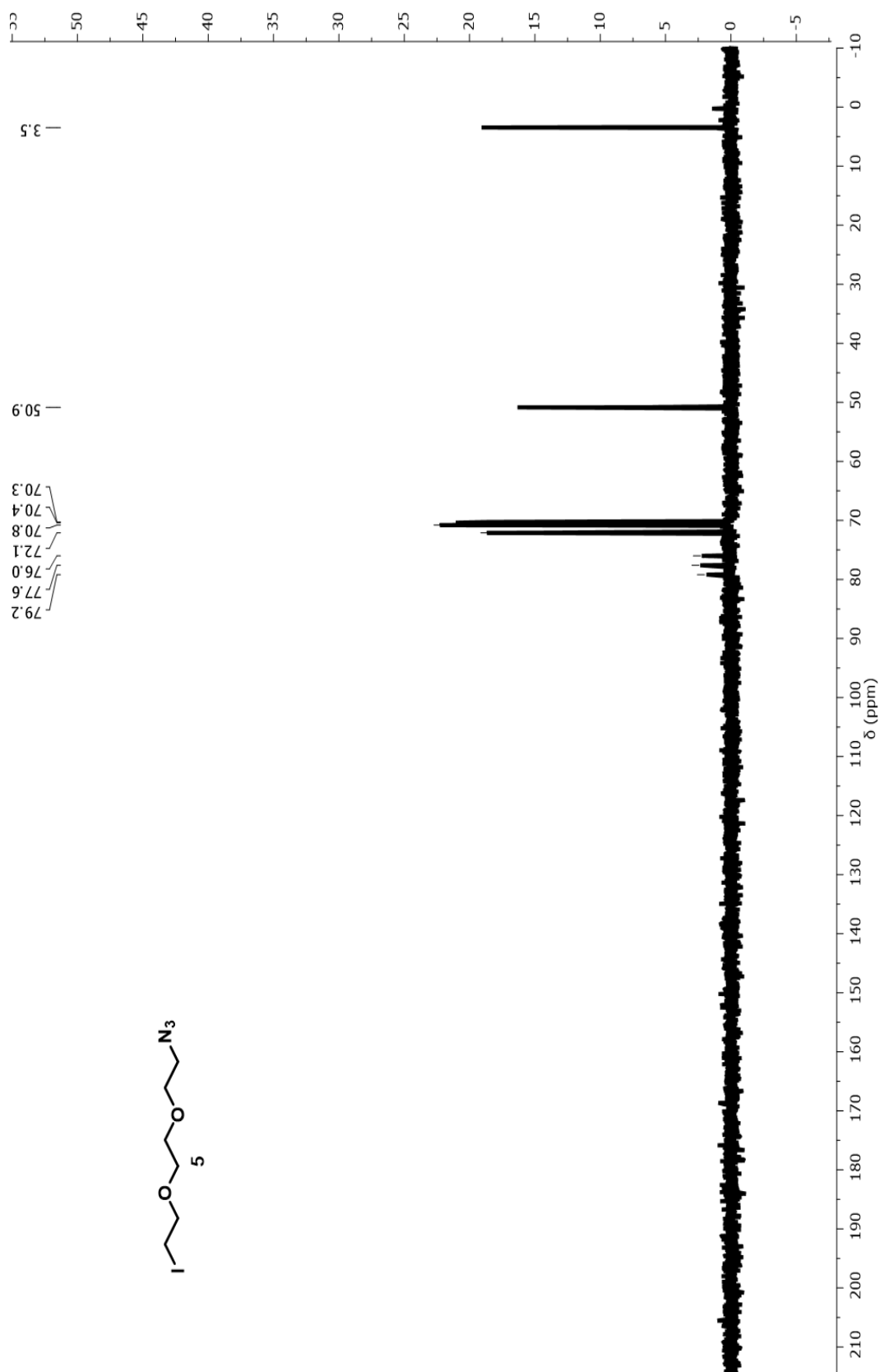
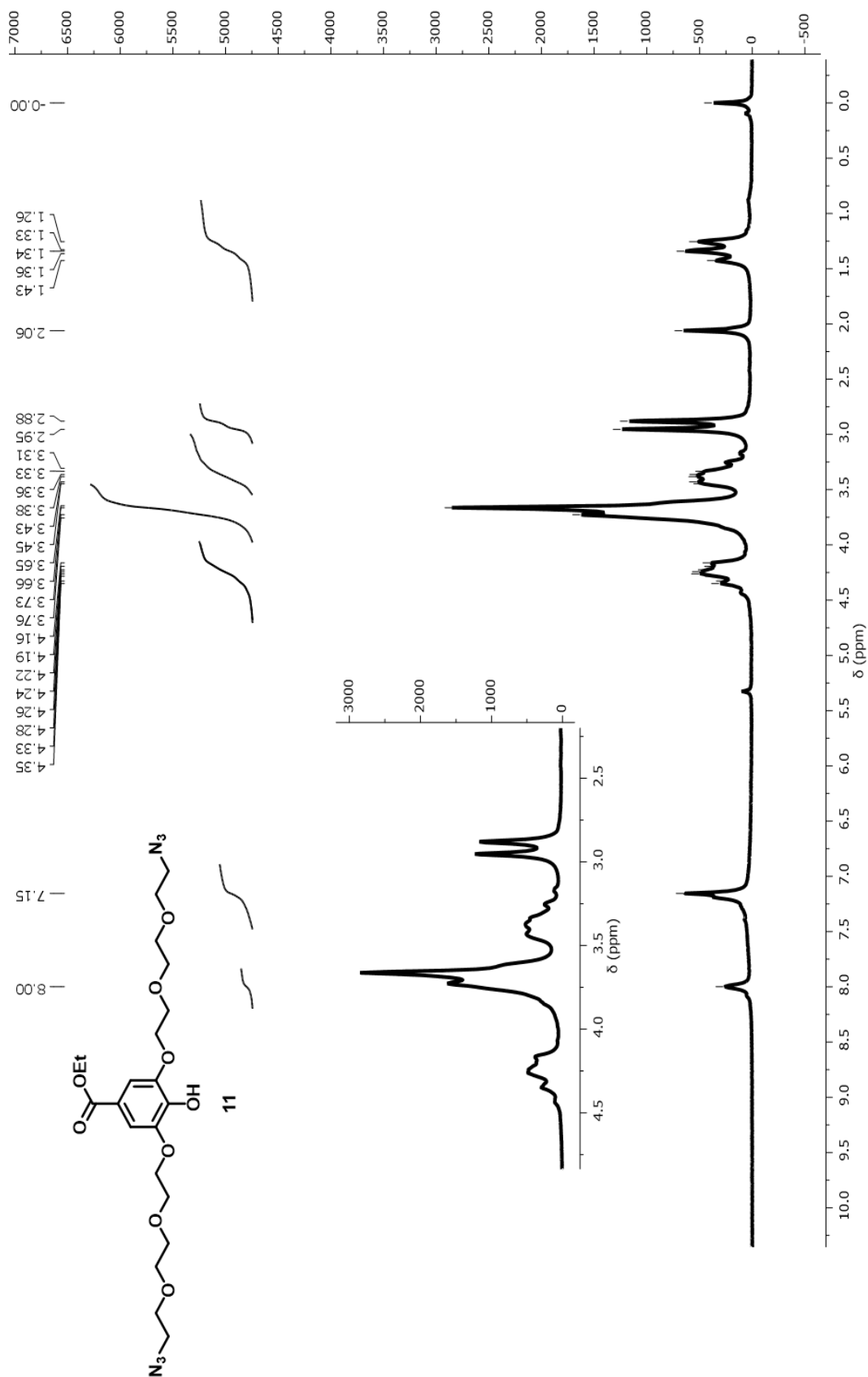


Figure 5.6. ¹³C NMR spectrum of 5 in CDCl₃ (20 MHz)



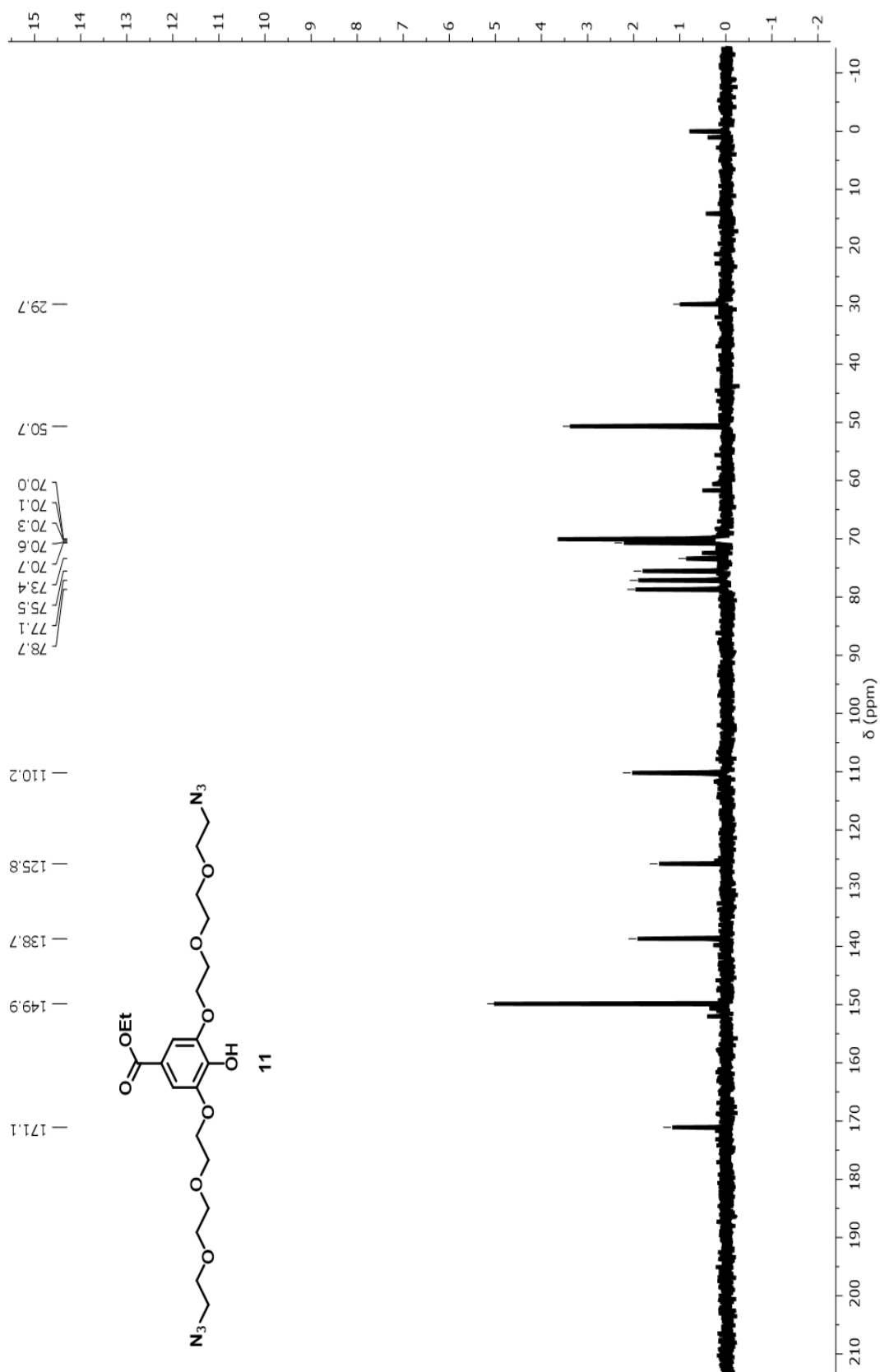


Figure 5.8. ^{13}C NMR spectrum of **11** in CDCl_3 (20 MHz)

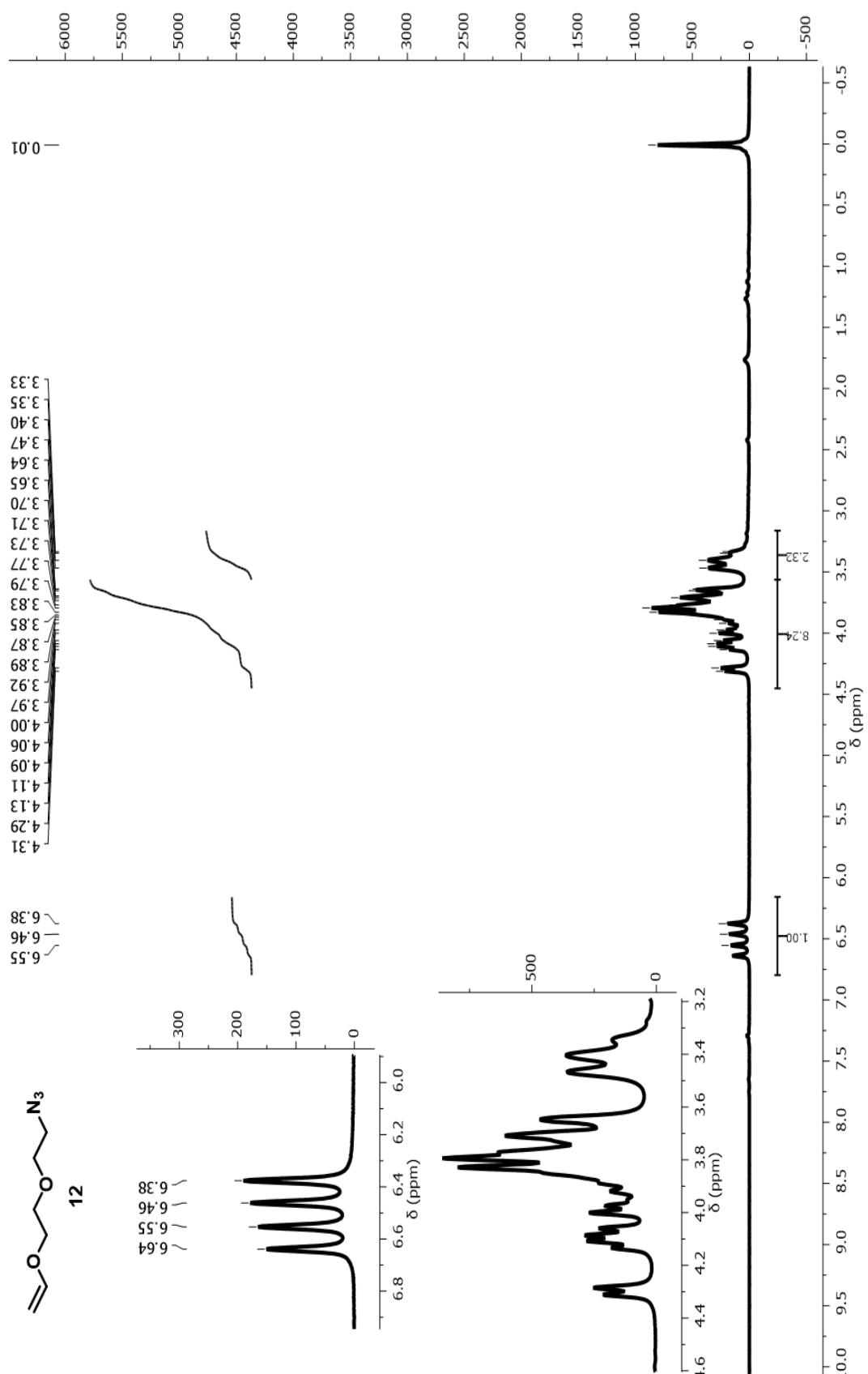


Figure 5.9. ¹H NMR spectrum of **12** in CDCl₃ (80 MHz)

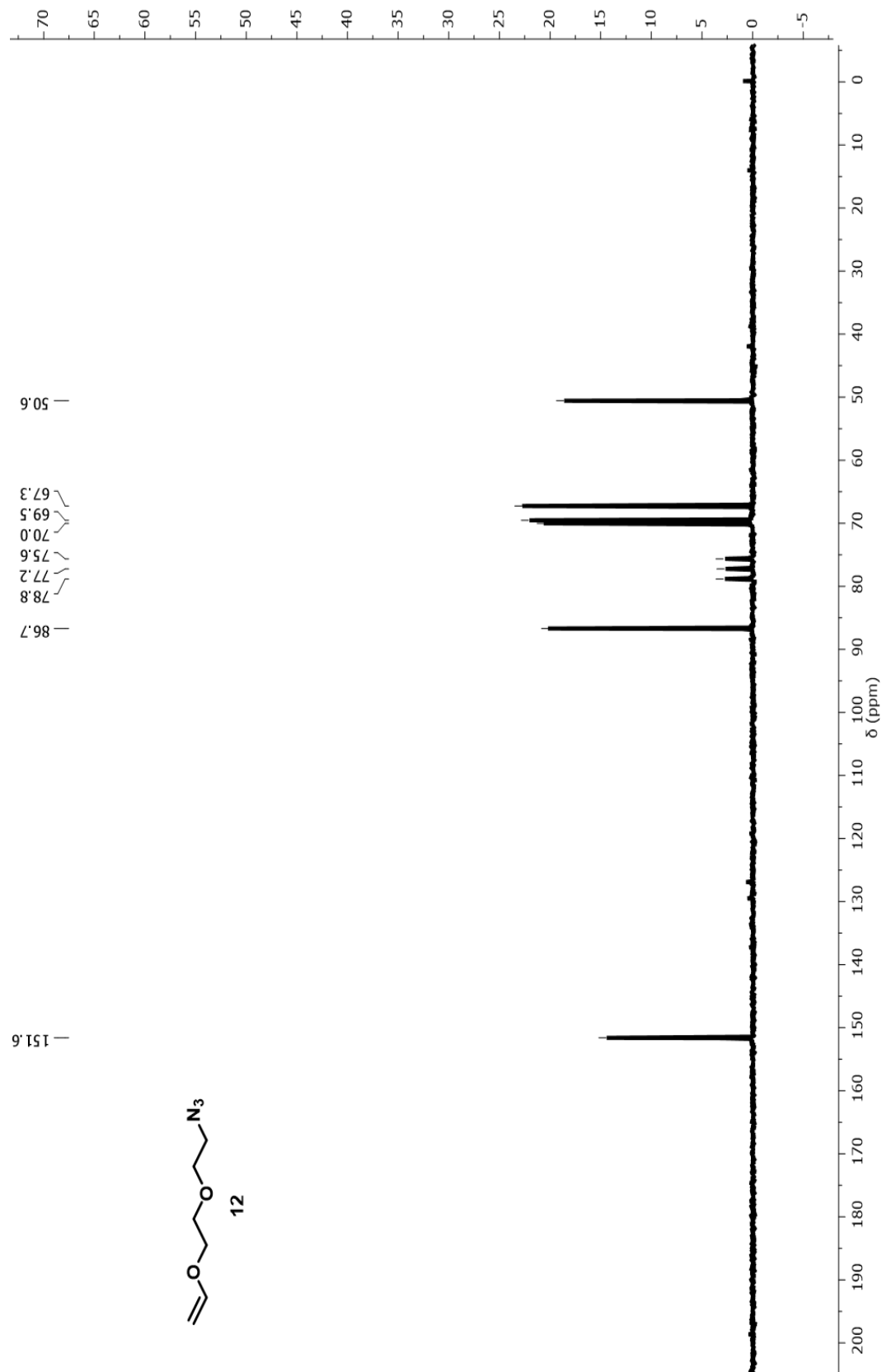


Figure 5.10. ^{13}C NMR spectrum of **12** in CDCl_3 (20 MHz)

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