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Tandem Mass Spectrometry (MS/MS) for Determination of Architecture of Synthetic Polymers

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**Tandem Mass Spectrometry (MS/MS) for Determination of
Architecture of Synthetic polymers**

Shoumik Saha

Department of **Chemical Engineering**

Honors Research Project

Submitted to

The Honors College

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Executive Summary

In this paper, the architecture of synthetic polymers was studied using Tandem Mass Spectrometry. The molecules being studied were difluorene-N₃, 3-difluorene-N₃ and VPOSS-3-difluorene, VPOSS stands for vinyl polyhedral oligomeric silsesquioxanes containing different numbers of dialkylfluorene groups. The compounds were synthesized at the University of Akron and they were examined in this paper to verify their structures. To do that, the compounds were first analyzed using single stage mass spectrometry to get information about their composition and then, each part of the structure was studied using fragment analysis by tandem mass spectrometry.

A m/z value of 869.6 Da ($z = 1$) corresponds to the difluorene-N₃, a m/z of 3116.8 Da corresponds to the silverated molecular ion of 3-difluorene-N₃ while the m/z of 3846.9 Da corresponds to silverated molecular ion of VPOSS-3-difluorene. Presence of dialkylfluorene groups were analyzed using tandem mass spectrometry discussed further in this paper. It can be concluded that the synthesis of VPOSS-3-difluorene was complete which was verified by the peak of 3846.9 Da in the mass spectrum. This molecule has enhanced features for electrochemical applications.

As I have worked under the guidance of the chemistry department, I can use my expertise of mass spectrometry along with my chemical engineering skills to further synthesize and analyze these compounds that I can use later in my career. With the compounds having electrochemical properties, they can be used in fuel cells which is a prominent topic of research. I can further use my skills when I try to achieve my doctorate degree in chemical engineering.

While this was done on a limited time, further research can be done to demonstrate the electrochemical properties of such molecules and try to enhance them with further addition of other side chains in the molecule.

Introduction and Purpose

The purpose of this research paper is to determine the architecture of branched polymers. Tandem Mass Spectrometry (MS/MS) was applied using laser-induced fragmentation (LIFT) to study the structure of the polymers. Matrix-Assisted Laser Desorption/Ionization (MALDI) was the soft ionization technique used with MS/MS. A branched polymer is one where a C-H group of the polymer is replaced with another polymer chain via covalent bonds to create a branched structure.¹ Branching provides significant improved properties to the polymer material in general.¹ The polymers being studied in this research contain different numbers of dialkylfluorene groups. The compounds are difluorene-N₃, 3-difluorene-N₃ and VPOSS-3-difluorene, (vinyl polyhedral oligomeric silsesquioxane (VPOSS)). These polymers are from a family of polymers called conjugated polymers. Such polymers are organic macromolecules that have a spatially extended π -bonding system² on the backbone to give them useful optical and electrical properties. Synthesis of the VPOSS-3-difluorene molecules, which is a sphere-rod conjugate, was the ultimate aim of the synthesis process. The self-assembly of such sphere-rod conjugates can potentially result in the formation of supramolecular lattices which show dissimilar charge transport properties, hence demonstrate electrochemical properties.³ As the polymers were synthesized here at the University of Akron and no prior analytical data were available on their branching, tandem mass spectrometry should provide a better way to characterize the side chains and elucidate the architecture of the molecules synthesized compared to a single stage mass spectrometry. Tandem mass spectrometry

makes use of two mass analyzers to efficiently determine the molecular connectivity and also the different components/branches present. It allows for an easy analysis of macromolecules such as the conjugated polymers mentioned above. The report will include more background information on the instrumentation, the experimental procedures and the discussion of the results to verify the structure of the polymers produced.

Background

Mass Spectrometry (MS) is an analytical technique that is used to analyze compounds and find out more information about the primary structure of the particular compound of interest. The basic principle is to generate gas-phase ions from the molecule, then separate the ions by their mass-to-charge ratio (m/z) and finally detect them qualitatively and quantitatively.⁴ A mass spectrometer is used to perform this analysis via a simple 3-step process which are ionization, mass analysis and detection. These tasks are done in a high vacuum atmosphere in the instrument itself. A simple mass spectrometer consists of three parts: ion source, analyzer and detector⁴ to perform the simple tasks as mentioned above.

The results from the data system in the mass spectrometer is in the form of a mass spectrum.⁴ It is a two-dimensional graph of the signal intensity or abundance of the ions versus the mass-to-charge ratio (m/z).⁴ From the mass spectrum, analysis can be done either by hand or with the aid of a computer to gather information about the entire molecule being studied. The peak with the highest m/z is referred to the molecular ion peak which corresponds to the intact ionized molecule. Several other peaks might show up due to fragmentation of the original molecule. All this information combined gives an overall idea of the molecule, which in this report, is used to

identify the entire molecule itself by studying the masses of the different fragments and the distance between the peaks in the tandem mass spectrum.

There have been numerous advancements in mass spectrometers for each stage described above. The form of mass spectrometry that was used in this study is called Tandem Mass Spectrometry. The simple type of mass spectrometry delineated above is referred to as single-stage mass spectrometry⁵ or one-dimensional MS of which the m/z value can be used to obtain molecular weight of a known polymer. However, in this paper, a new polymer was created for which data from mass spectrum alone cannot be used to determine the architecture of the compound. So, two-dimensional or Tandem Mass Spectrometry (MS/MS or MS²) was carried out coupled with MALDI as the ion source and Time-of-flight (ToF) as the mass analyzer.

In MALDI, the sample is first dissolved into a proper solvent and then mixed with a matrix which is a solution of small organic molecules. The matrix solution has a strong absorption at a laser wavelength. The matrix molecules absorb the laser power and evaporate and take the analyte molecules into a gas phase and protects the analyte from breaking apart by the laser. Usually the matrix is mixed with the analyte in a 1:100~10,000 ratio to disperse the analyte and not have them in a cluster. Since the matrix on the target plate is heated rapidly inside the instrument, consequent ablation and vaporization⁶ takes place which keeps the analyte molecule intact in the gas phase. The ionization process takes place when the analyte compound captures a proton from the ionized matrix. Occasionally charge exchange between matrix ions and analyte molecules takes place, forming analyte radical ions. For molecules that do not ionize easily this way, a salt solution is often added to the analyte-matrix mixture on the target plate, which in this experiment was a silver (Ag^+) salt.

A Time-of-Flight (ToF) mass analyzer is used in the instrument. This technique measures the flight time of the ions from the ion source to the ion detector. Ions from the ion source are accelerated by a potential (V_s) to gain kinetic energy and go through the field-free region of distance (L) to reach the detector. An electric potential energy (E_{el}) is applied to the ions which is equal to the charge of ions ($q = ze$) multiplied by V_s . This E_{el} is then converted to the kinetic energy (E_k) needed for the ions to exit the ion source, shown by equation 1. The ions travel across the field-free region at a constant velocity (v) to reach the detector. The corresponding flight time is calculated by equation 2. Combination of equation 1 and 2 gives equation 3 which describes the relationship between m/z and measured flight time (t).

$$E_{el} = qV_s = mv^2/2 = E_k \quad \text{equation 1}$$

$$L = vt \quad \text{equation 2}$$

$$m/z = 2t^2eV_s/L^2 \quad \text{equation 3}$$

The instrumentation used for tandem mass spectrometry was a Bruker UltraFlex III MALDI-ToF/ToF Mass Spectrometer, equipped with Nd: YAG laser (355nm). In MS/MS, ions of a specific m/z ratio from a specific precursor are isolated and energetically activated which then leads to diagnostic fragmentation.⁵ The instrument then detects these fragments and allows the user to reconstruct the entire compound. The process of isolation and fragmentation takes place physically in the instrument giving it a name of MS^2 in space. This is achieved via two mass analyzers. The first analyzer transmits only selected ions (precursor ions) of a specific m/z ratio. These precursor ions are formed at increased laser power, so that they can undergo unimolecular fragmentation. These fragments are post-accelerated upon entering the second analyzer where they are separated by their time of flight and are then detected to give a tandem mass spectrum.

Experimental Procedure

The polymer sample being studied was prepared first before the tandem mass spectrometry study was performed. A matrix solution of trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) was pre-made and used. A specific matrix should be chosen because of a certain number of characteristics it possesses. These include a high electronic absorption at the laser wavelength, high vacuum stability, low vapor pressure and a good solubility in the solvent that is used to dissolve the analyte as well.⁴ DCTB was chosen as a matrix in this experiment via multiple trial and error experiments to determine the proper matrix.

A salt solution is required because it helps with ionization of the analyte, in this instance a silver salt of silver trifluoroacetate (AgTFA) was used. Using an electronic balance, 0.8 mg of AgTFA was weighed and 80 μL of tetrahydrofuran (THF) was used to dissolve the AgTFA to create a 10 mg/mL salt solution. It was mixed well using a Vortex mixer.

Next, the sample was taken and diluted to a concentration of 10 mg/mL. 1mg of sample was weighed and 100 μL of chloroform (CHCl_3) was used to dilute the sample/analyte. The matrix and salt solution were mixed in a 10:1 ratio and mixed well using the vortex mixer.

Using a pipette, around 0.5 μL of the matrix-salt mixture was pipetted onto the MALDI target plate. The spot was allowed to evaporate. About 0.5 μL of the sample solution was pipetted on the same spot on the target plate and allowed to dry. Finally, another drop of matrix-salt mixture was pipetted on the same spot and dried again in what is called a sandwich method. The target plate is shown below. These steps were repeated on at least 4 spots of the target plate to achieve better results and allows us to repeat the experiments to get reproducible results.

Results and Discussion

Single-stage mass spectrometry (MS) was performed to determine the overall molecular weight of the molecules being studied. Each compound and its fragments were drawn and their respective masses were calculated using ChemDraw.

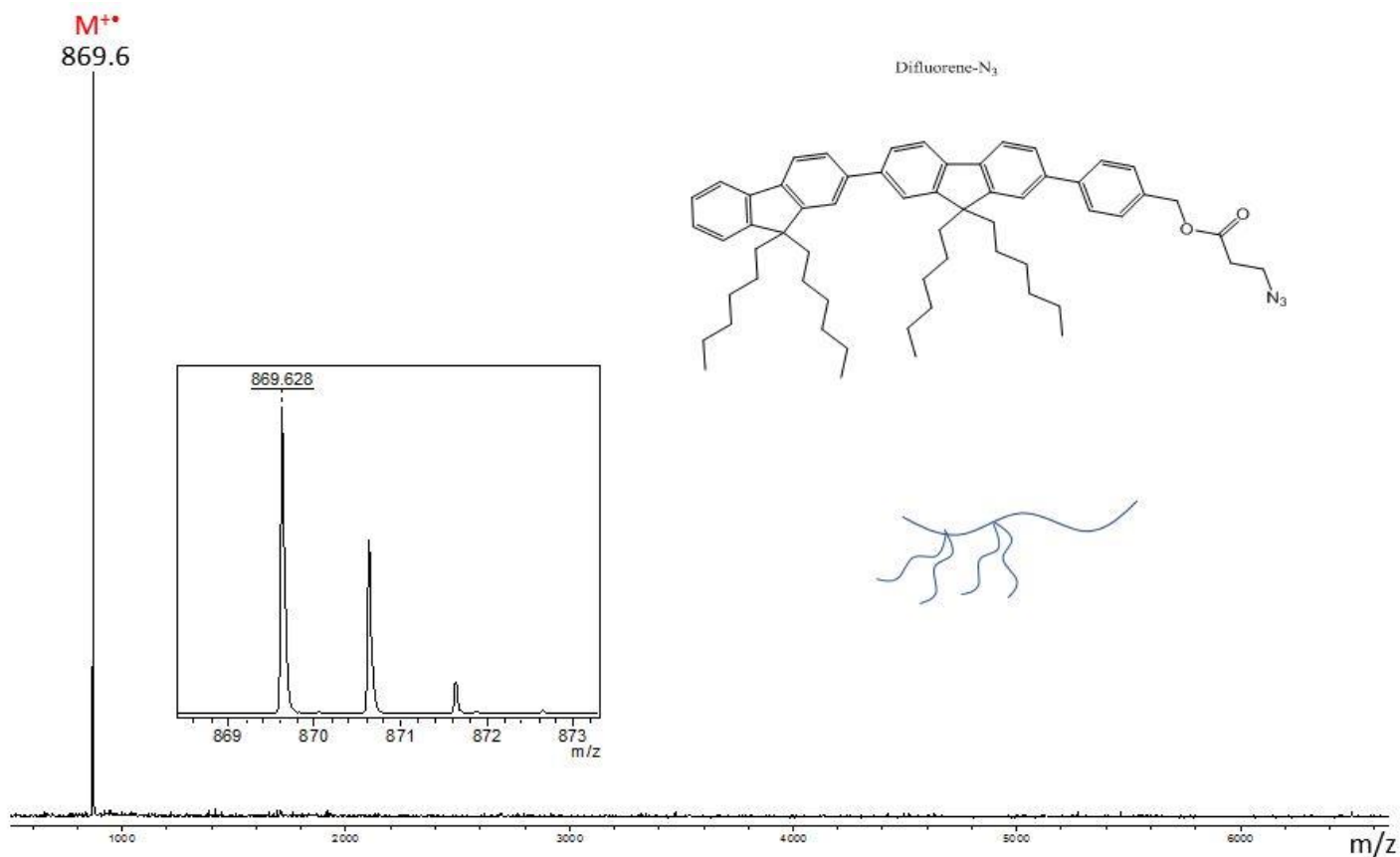


Figure 1: The MS of difluorene-N₃

The above mass spectrum shows the m/z value of 869.6 Da which corresponds to the radical ion of difluorene-N₃. This molecule was present in the form of a radical as shown by M^{•+} and no salt was required for the ionization process.

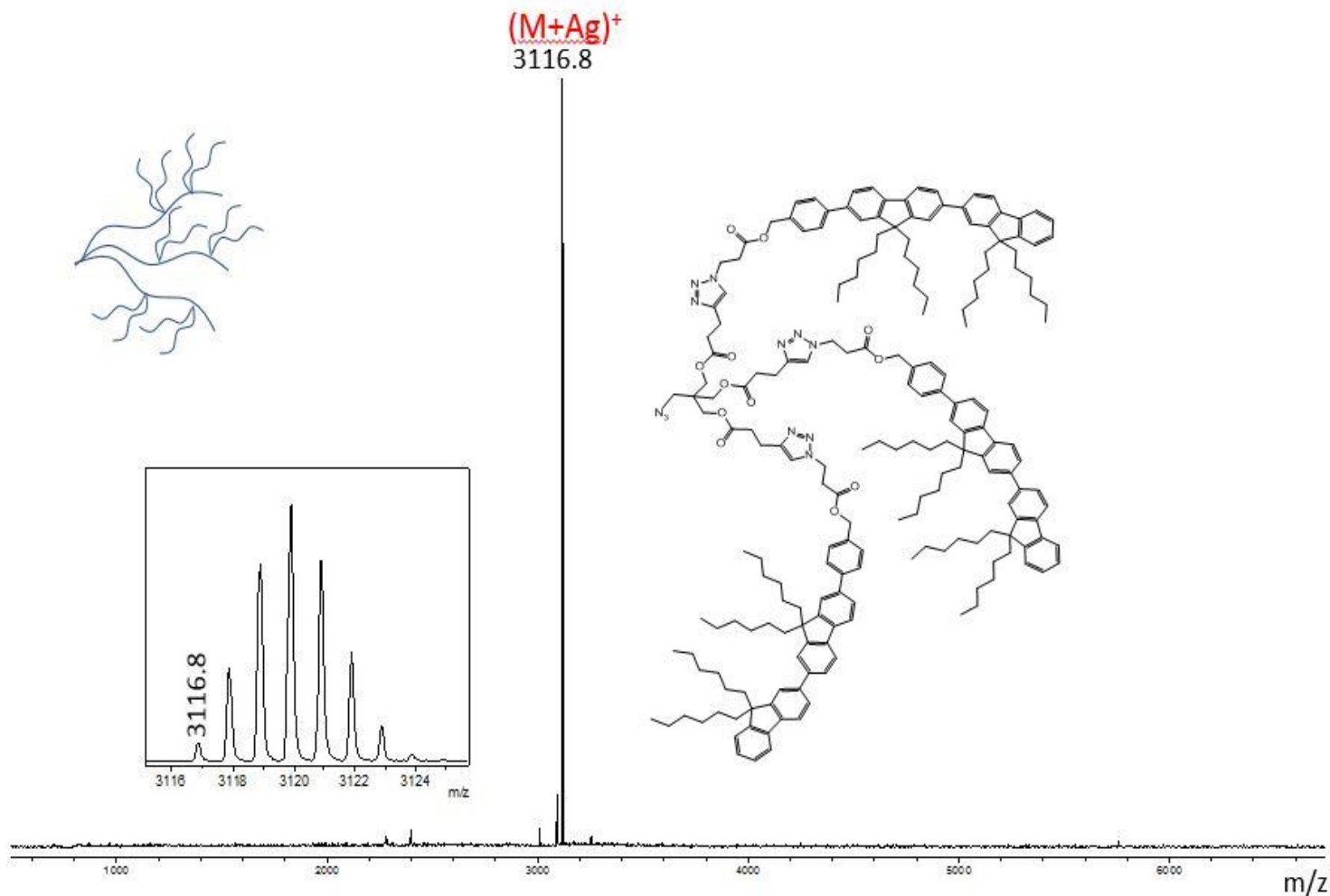


Figure 2: The MS of 3-difluorene-N₃

The above mass spectrum has the most intense peak at m/z value of 3116.8 Da which corresponds to the silverated molecular ion of 3-difluorene-N₃, shown as (M + Ag)⁺. The 3 in 3-difluorene-N₃ means the molecule contains three dialkylfluorene chains each containing 2 dialkylfluorene groups.

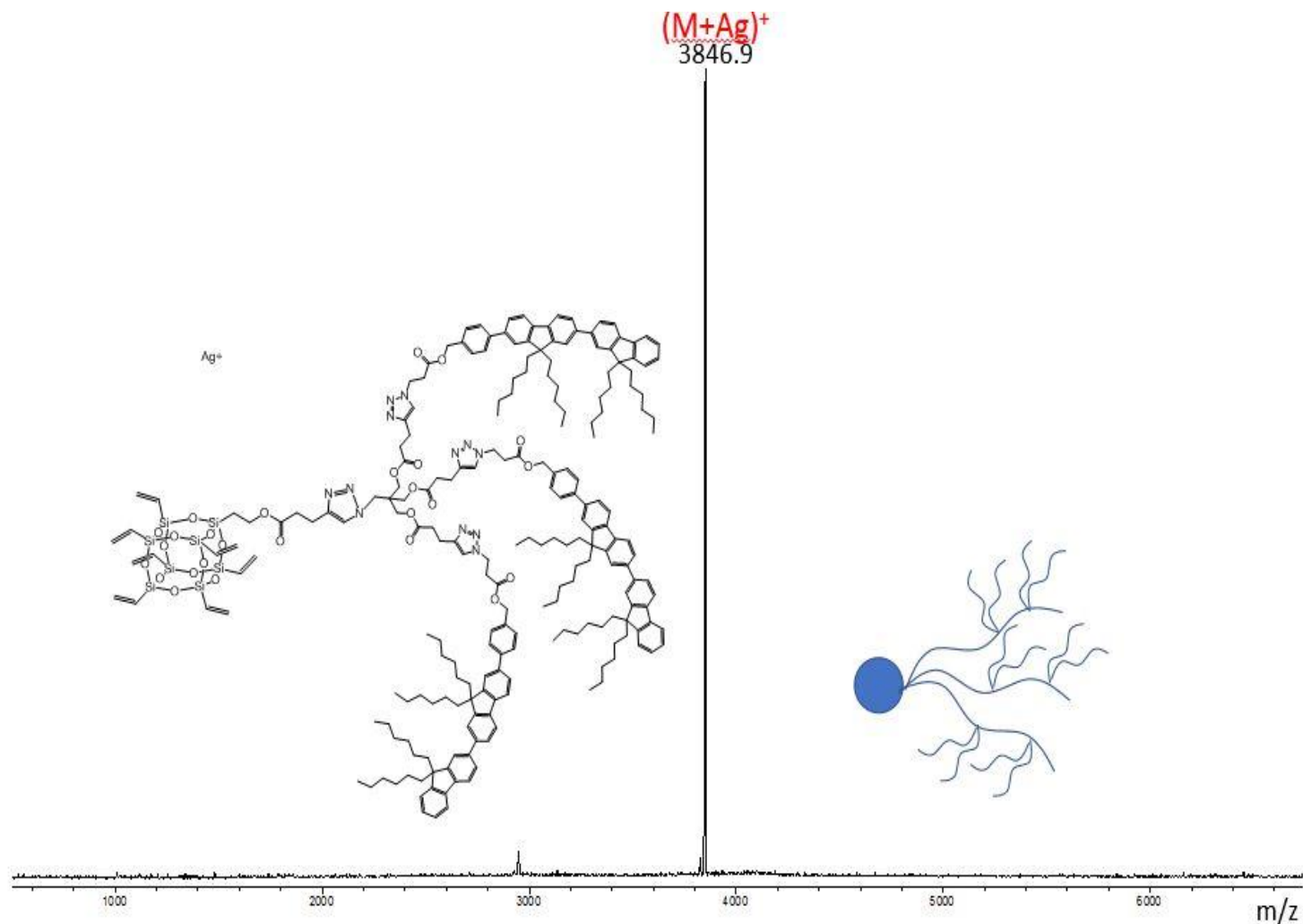


Figure 3: The MS of VPOSS-3-difluorene

The above mass spectrum has the most intense peak at the m/z value of 3846.9 Da which corresponds to the structure of VPOSS-3-difluorene. The silver salt was used to assist the ionization of the molecule. By subtracting the weight of the silver ion, the resulting mass of the molecule is 3739.1 Da. This molecule is the final product of the synthesis process and none of the chains contain a N_3 end group.

While single stage mass spectrometry (MS) gave the molecular weight of the molecules being studied, no structure information was obtained. Thus, tandem mass spectrometry (MS/MS) was performed to further study each of the chains present. This was done by studying the MS/MS

mass spectrum which shows the individual masses of each fragment that was produced in the fragmentation stage. The MS/MS mass spectra are shown below:

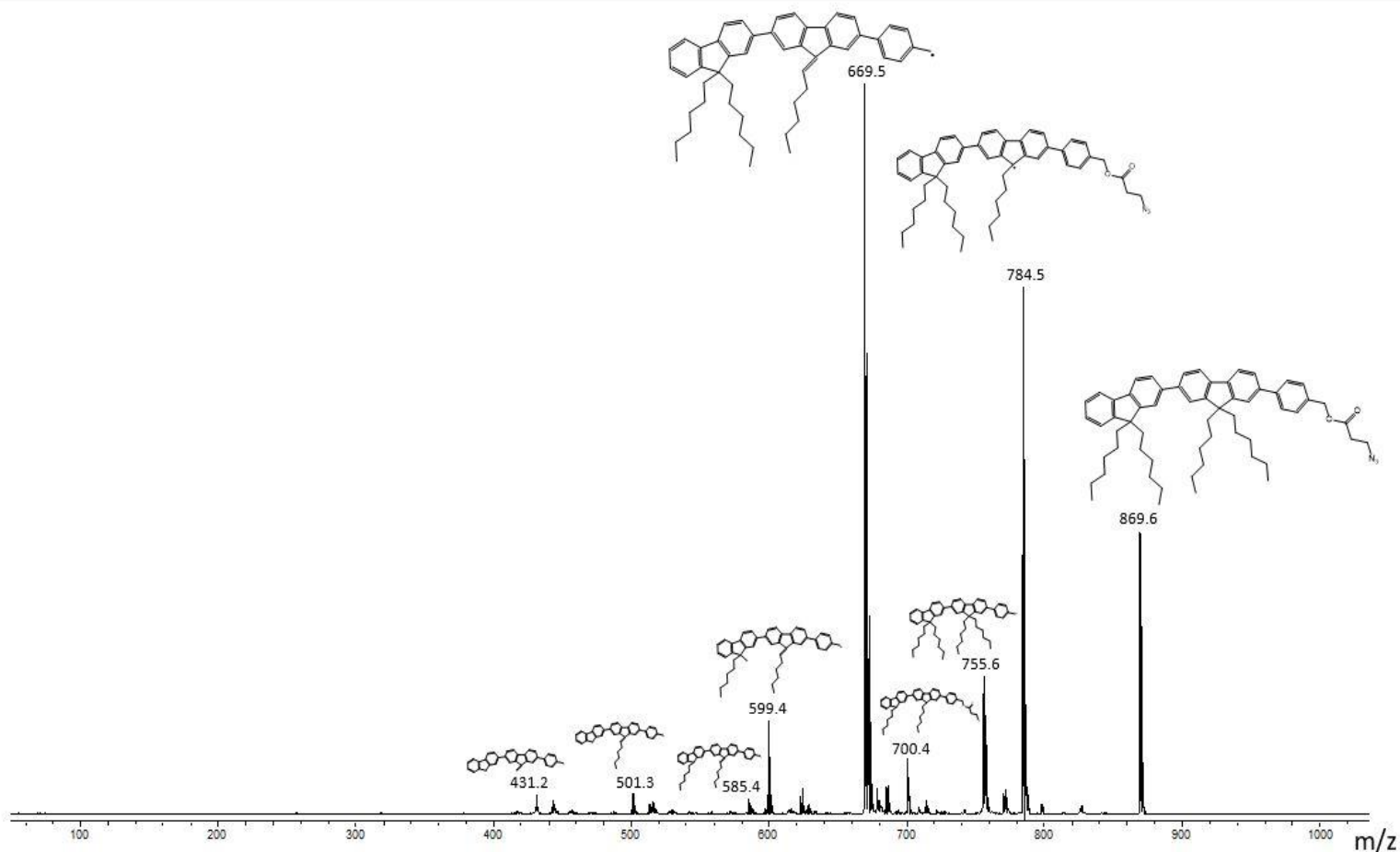


Figure 4: The MS/MS of difluorene-N₃

The above spectrum is MS/MS of difluorene-N₃. It shows the different m/z values detected and the corresponding fragment structures above each of the peaks. The peak at m/z of 869.6 Da is the parent molecule ion which was also detected by single stage mass spectroscopy. The peak at m/z of 784.5 Da is the fragment formed by loss of one alkyl chain from one of the fluorene as shown by the structure above that specific peak. Again, no salt was needed to produce this spectrum and the ions were in the form of radical ions.

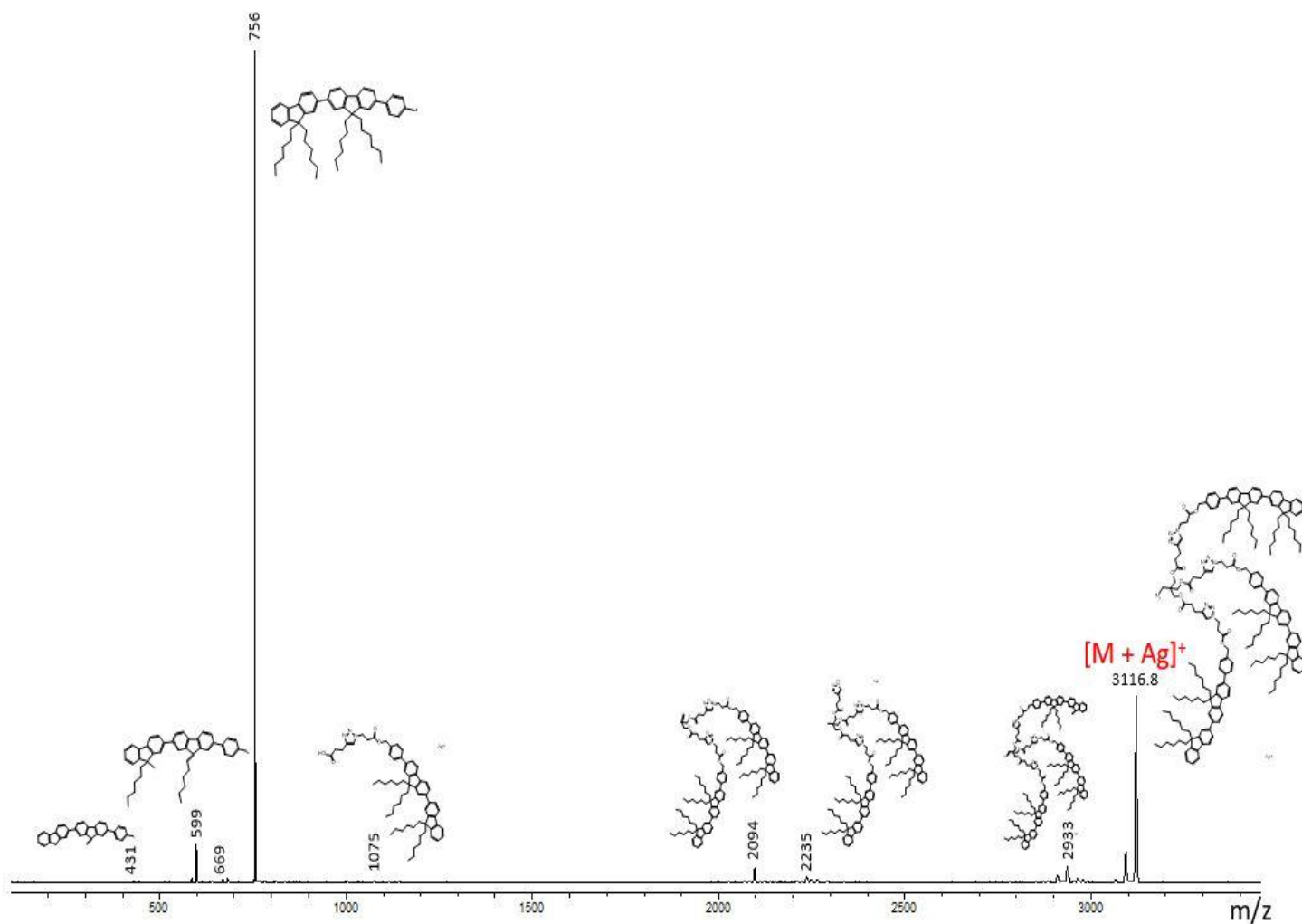


Figure 5: The MS/MS of 3-difluorene-N₃

The spectrum above is for 3-difluorene-N₃. Silver salt was used to assist the ionization of the molecule. The peak at m/z of 3116.8 Da shows the silverated molecule ion as also verified by MS. The m/z of 2094 Da shows the loss of one arm while the m/z of 1075 Da corresponds to one arm in the molecule. The other peaks in the spectrum are for different fragments that were produced, and each structure is shown in the appendix.

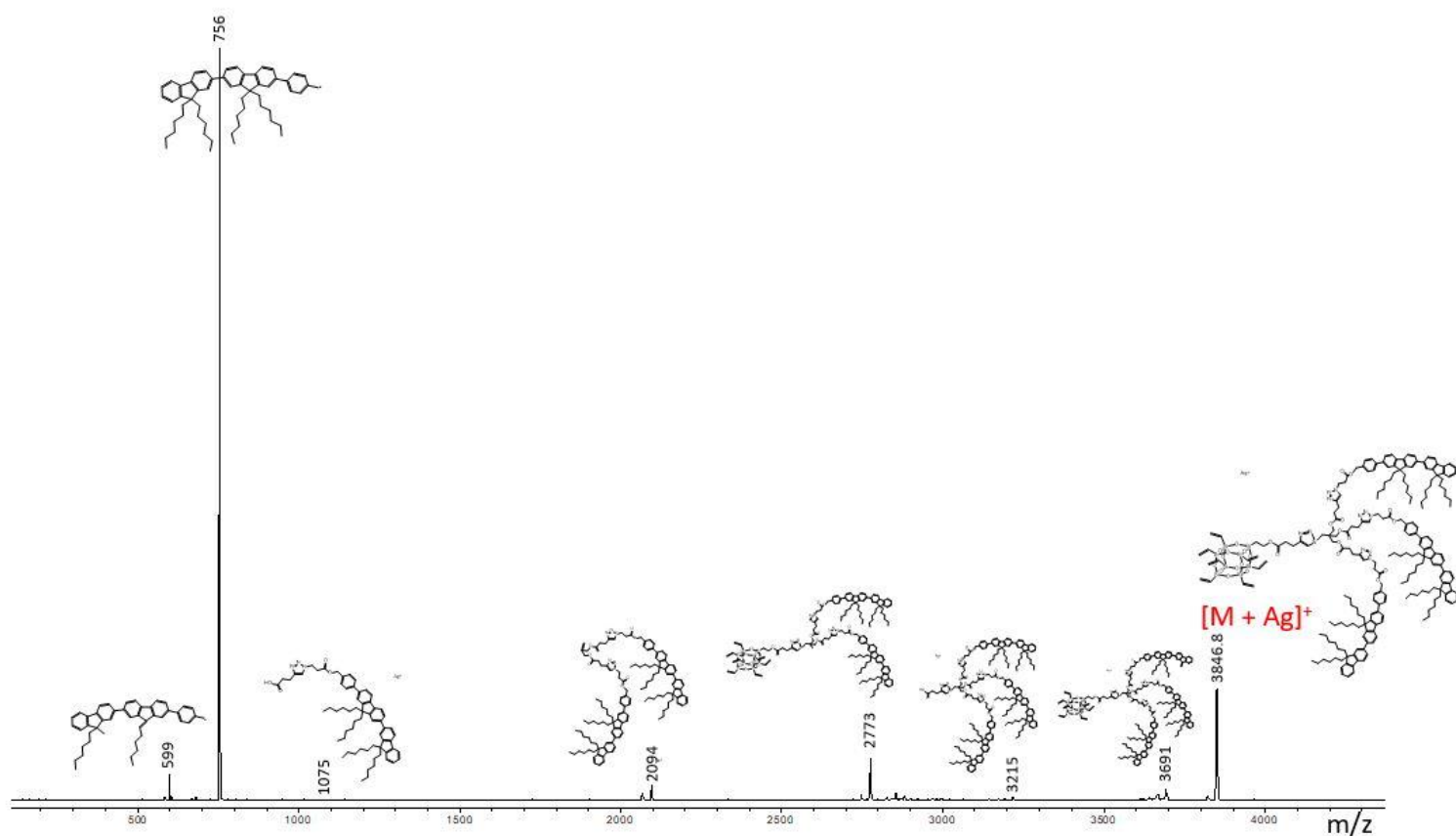


Figure 6: The MS-MS of VPOSS-3-difluorene

The spectrum above is for VPOSS-3-difluorene. The peak at m/z of 3846.8 Da is the silverated molecular ion and its structure is shown above the peak which is also verified by MS. The peak with a m/z of 3215 Da corresponds to the fragment with the loss of the VPOSS. The peak at m/z of 2773 Da corresponds to the fragment with the loss of one arm. Finally, the peak at m/z of 2094 Da shows the fragment with the loss of one arm and the VPOSS part of the molecule. A table is attached in the appendix which shows all the peaks with their m/z value and the respective fragment structures. These 6 mass spectra give information on the structure of each of the molecules that were synthesized and studied in this research paper.

Conclusion

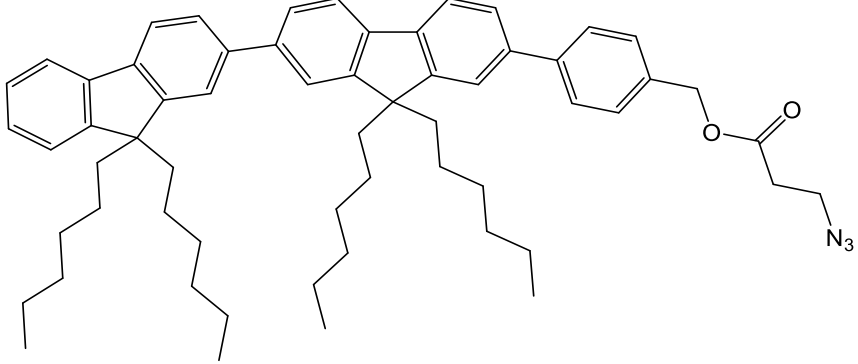
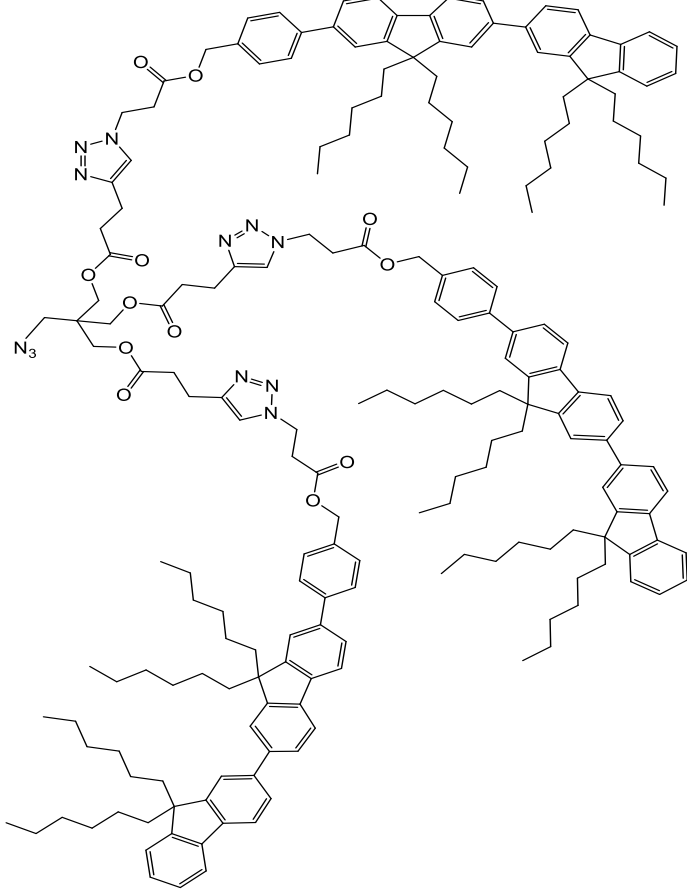
A study was done to determine the architecture of the synthetic monodisperse polymers difluorene-N₃, 3-difluorene-N₃ and VPOSS-difluorene. These polymers are from a family of polymers called conjugated polymers as they show useful electrical properties. At first, a single stage mass spectrometry (MS) experiments were performed to show the exact molecular weight of each of these molecules and confirm their monodispersity. After the molecular weight was verified, Tandem Mass Spectrometry (MS/MS) was performed with MALDI as a soft ionization technique and LIFT as the dissociation technique. Tandem Mass spectrometry makes use of two mass analyzers and helps to give a better picture of the constituents of the molecules at hand. The MS/ MS step shows the different components of the macromolecule by causing the molecule to dissociate into different fragments which are then detected in the MS/MS mass spectrum from the instrument. DCTB was used as the matrix to allow even ionization inside the instrument while a silver salt was used to help this ionization process for bigger molecules containing benzene rings. The analysis of each of the spectra verified the structure of the molecules synthesized. All safety precautions were followed such as wearing PPE to prevent any accidents that might affect the person conducting the experiments.

Appendix 1

Safety was of paramount importance in the lab while conducting the experiments. Personal Protective Equipment (PPE) was worn during the entire duration of the experiments. Gloves and safety goggles were worn to protect the hands and eyes from the reactive chemicals. Long pants and closed-toed shoes were worn to protect the legs and feet from spills. Finally, a laboratory apron was worn to protect oneself from any imminent spills. The desk station was always kept clean and a written procedure was followed, and experiments were performed under the supervision of an experienced lab assistant. There were proper waste containers for each specific chemical. Different pipette heads were used whenever different solutions needed to be transferred to prevent contamination of chemicals. Prior knowledge of other safety aspects like the eye-wash station, shower and fire extinguishers were acquired in the case of any fatal incidents that would require those devices.

All the chemicals used in the experiments performed are harmful if inhaled or came into contact with the skin. They might cause skin or eye irritation. If the chemical came into contact with the skin, the person must wash with plenty of soap and water. If on the skin, the person must rinse their eyes in the eye wash station. If inhaled, the person must be moved to a well-ventilated area with fresh air and must consult a doctor for a checkup for any after effects.

Appendix 2

m/z (Da)	Structure
869.6	 <p>The structure shows a dendritic molecule with a central core of three phenyl rings. Each phenyl ring is substituted with a propyl chain. The propyl chains are further substituted with phenyl rings, which are in turn substituted with more propyl chains. This branching continues, resulting in a complex, multi-ring structure. The terminal group is an azide group (-N₃) attached to a propyl chain.</p>
3009	 <p>The structure shows a highly branched dendritic molecule. It features a central core of three phenyl rings, which are substituted with propyl chains. These propyl chains are further substituted with phenyl rings, which are in turn substituted with more propyl chains. This branching continues, resulting in a complex, multi-ring structure. The terminal group is an azide group (-N₃) attached to a propyl chain.</p>

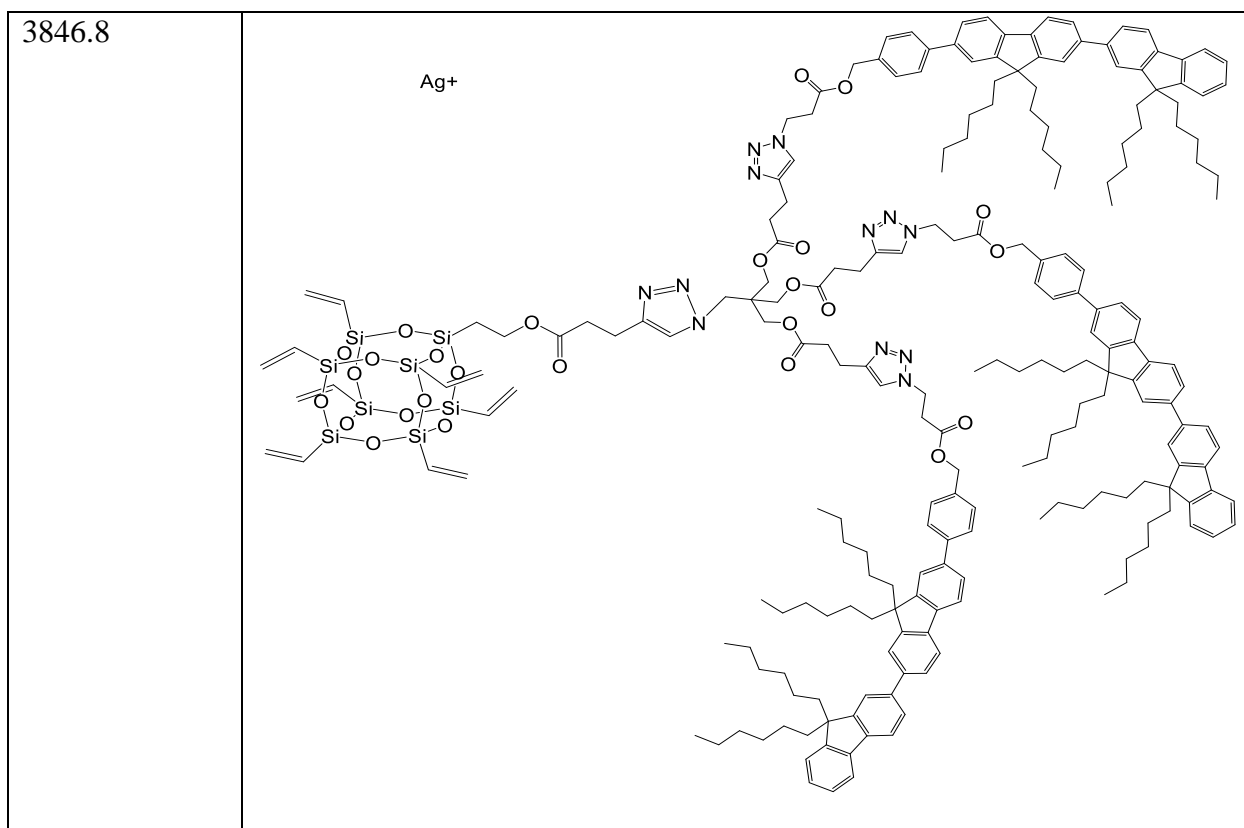


Table 1: The structure of the three molecules being studied and their m/z values from the MS.

m/z	Structure of fragments
869.6	
784.5	
755.6	

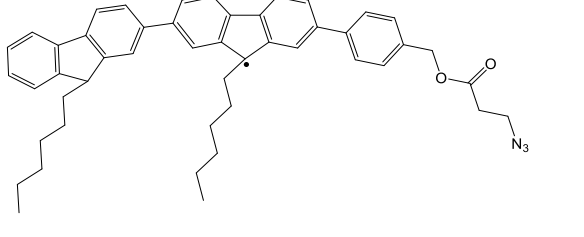
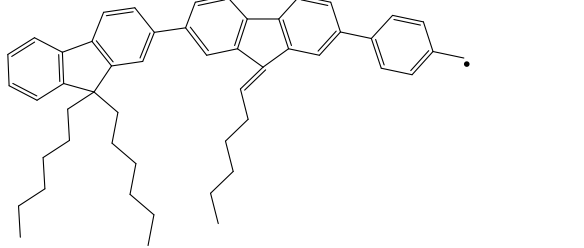
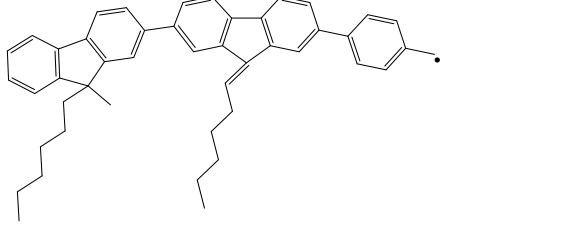
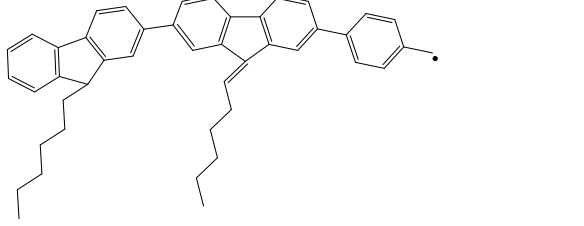
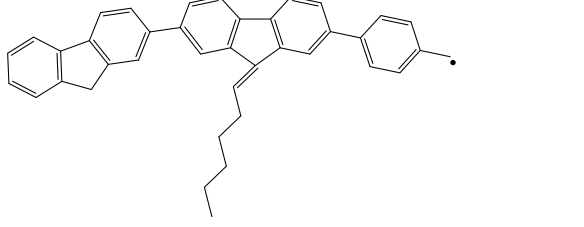
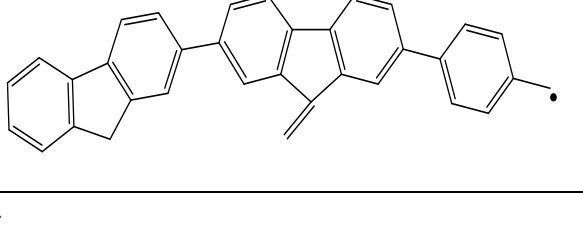
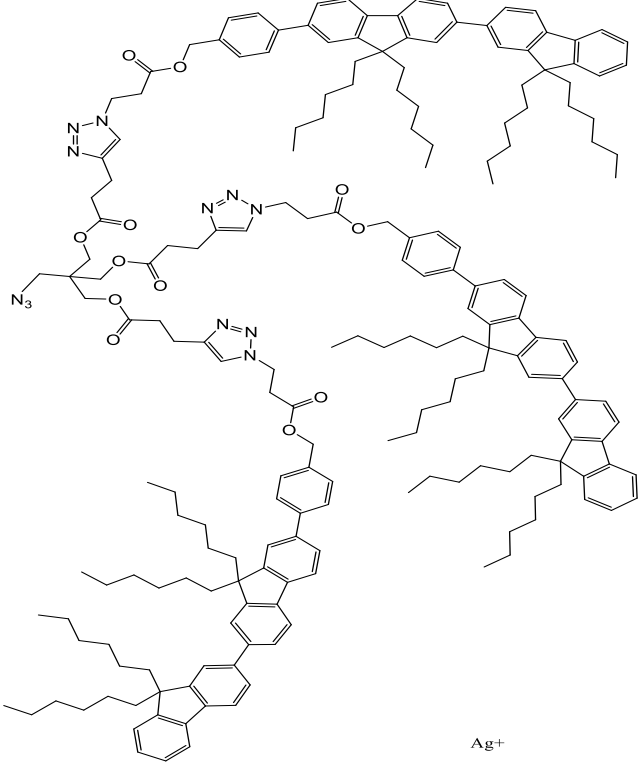
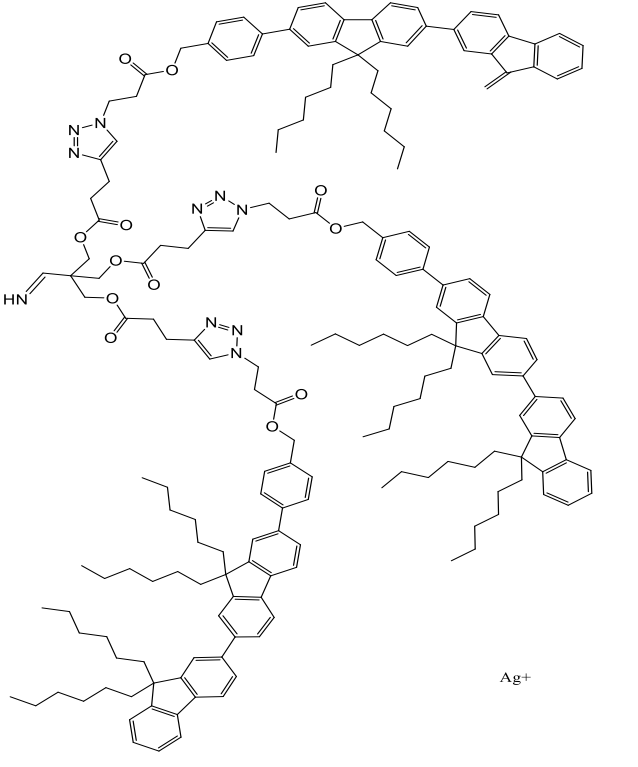
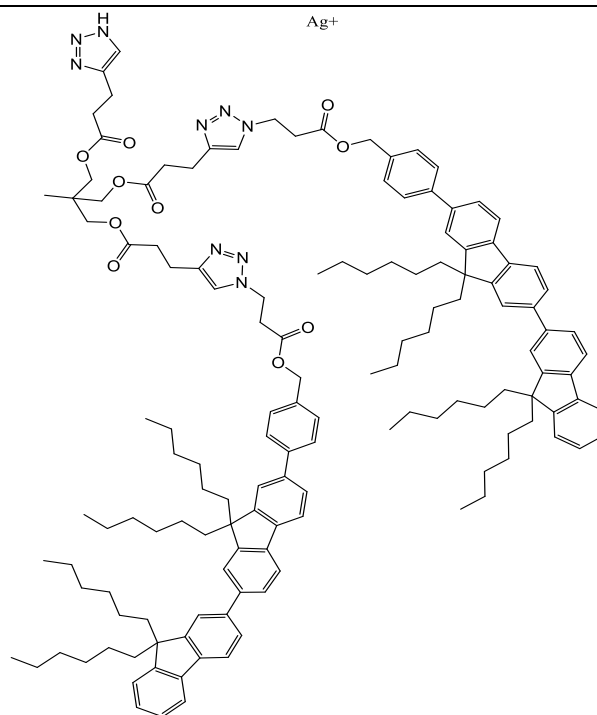
700.4	
669.5	
599.4	
585.4	
501.3	
431.2	

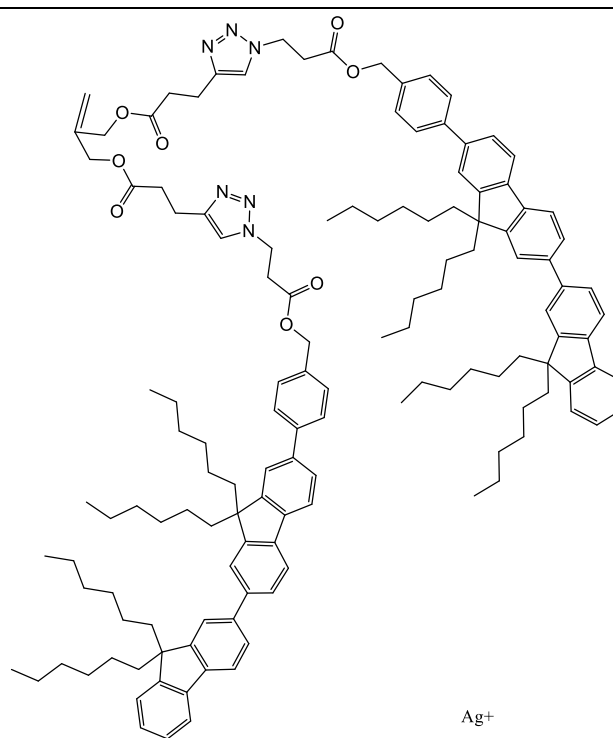
Table 2: The fragments from the MS/MS of Difluorene-N₃.

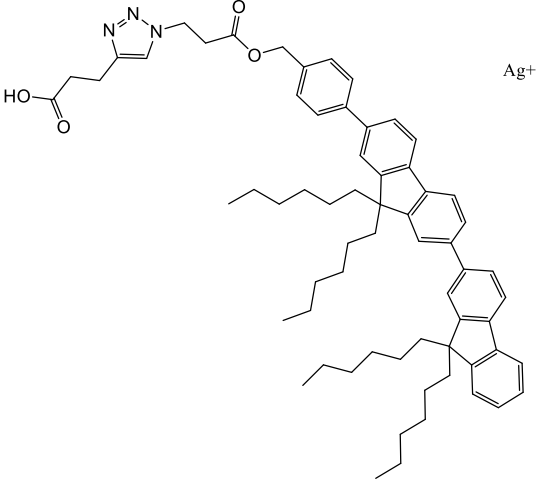
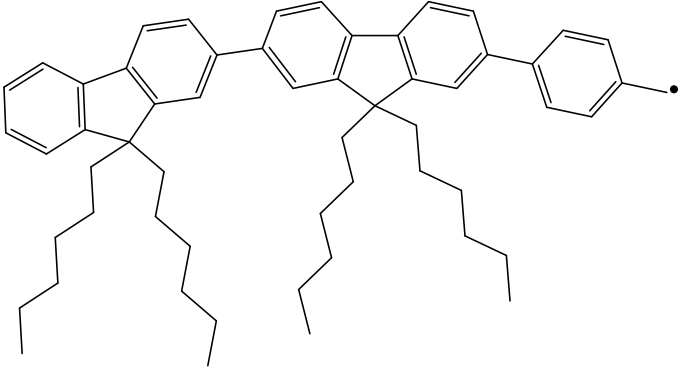
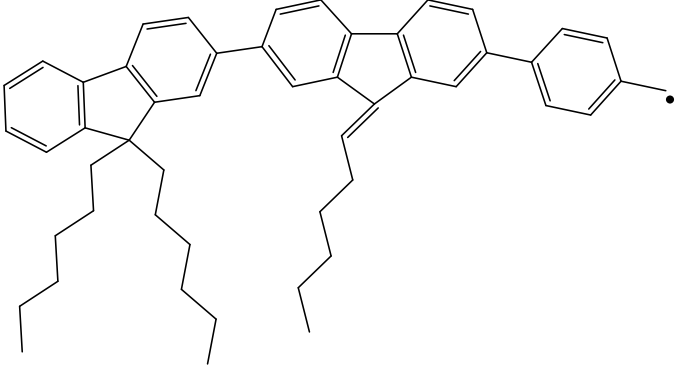
m/z	Structure of fragments
3116.8	 <p>Ag⁺</p>
2933	 <p>Ag⁺</p>

2235



2094



1075	 <p>Chemical structure of a silver ion (Ag^+) complex. The complex consists of a central silver ion coordinated to a large, multi-ring ligand system. The ligand system includes a central benzene ring substituted with two long alkyl chains and two other rings. One of these rings is a benzimidazole derivative with a carboxylic acid group ($\text{HO}-\text{C}(=\text{O})-\text{CH}_2-\text{CH}_2-$) attached to its 2-position. The other ring is a benzene ring substituted with a long alkyl chain. The silver ion is coordinated to the nitrogen atoms of the benzimidazole ring and the oxygen atom of the carboxylate group.</p>
756	 <p>Chemical structure of a radical complex. The complex consists of a central benzene ring substituted with two long alkyl chains and two other rings. One of these rings is a benzimidazole derivative with a long alkyl chain attached to its 2-position. The other ring is a benzene ring substituted with a long alkyl chain. The silver ion is coordinated to the nitrogen atoms of the benzimidazole ring and the oxygen atom of the carboxylate group.</p>
669	 <p>Chemical structure of a radical complex. The complex consists of a central benzene ring substituted with two long alkyl chains and two other rings. One of these rings is a benzimidazole derivative with a long alkyl chain attached to its 2-position. The other ring is a benzene ring substituted with a long alkyl chain. The silver ion is coordinated to the nitrogen atoms of the benzimidazole ring and the oxygen atom of the carboxylate group.</p>

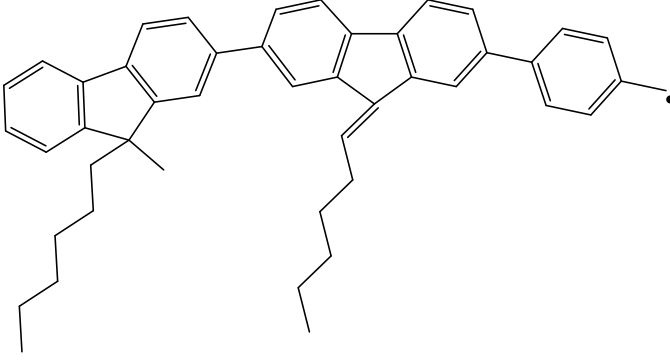
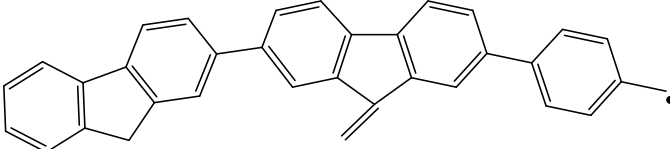
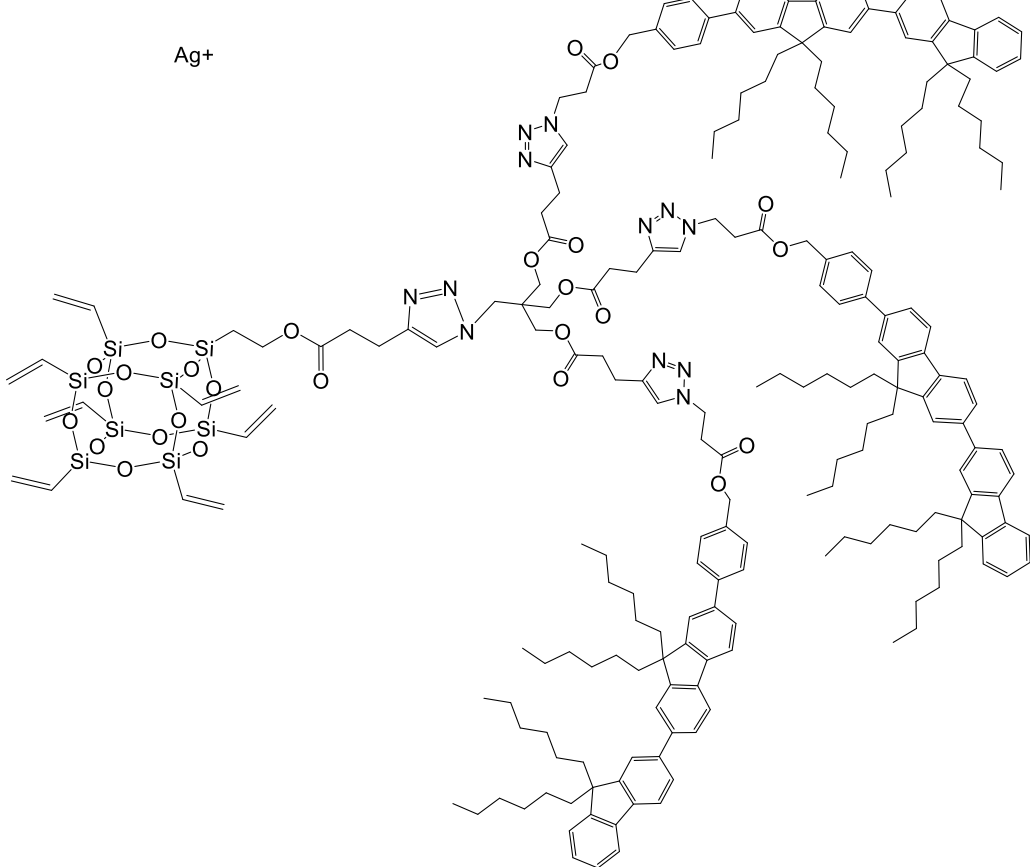
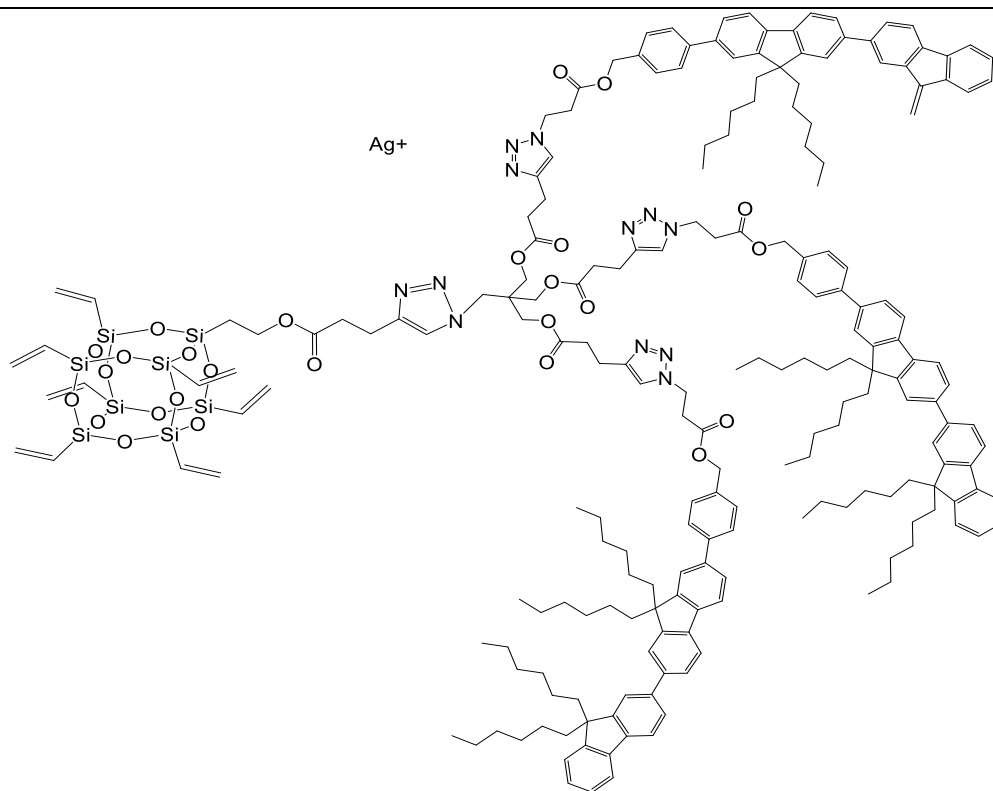
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431	

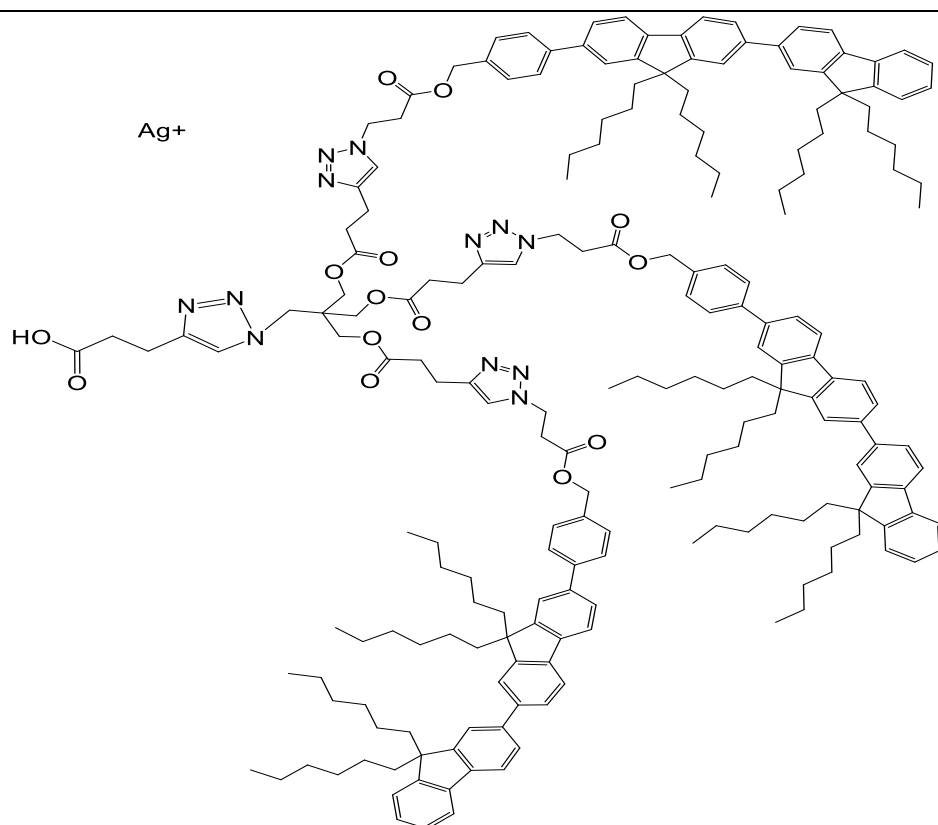
Table 3: The fragments from the MS/MS of 3-Difluorene-N₃.

m/z	Structure of Fragments
3846.8	<p data-bbox="516 1018 560 1050">Ag⁺</p> 

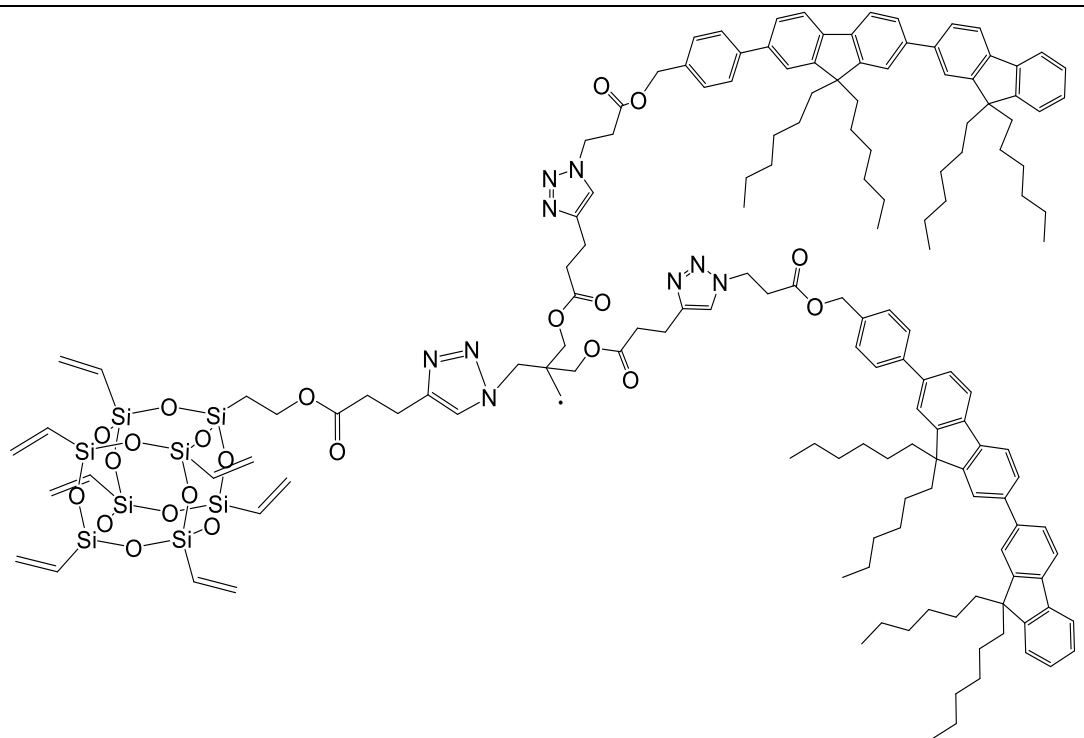
3691



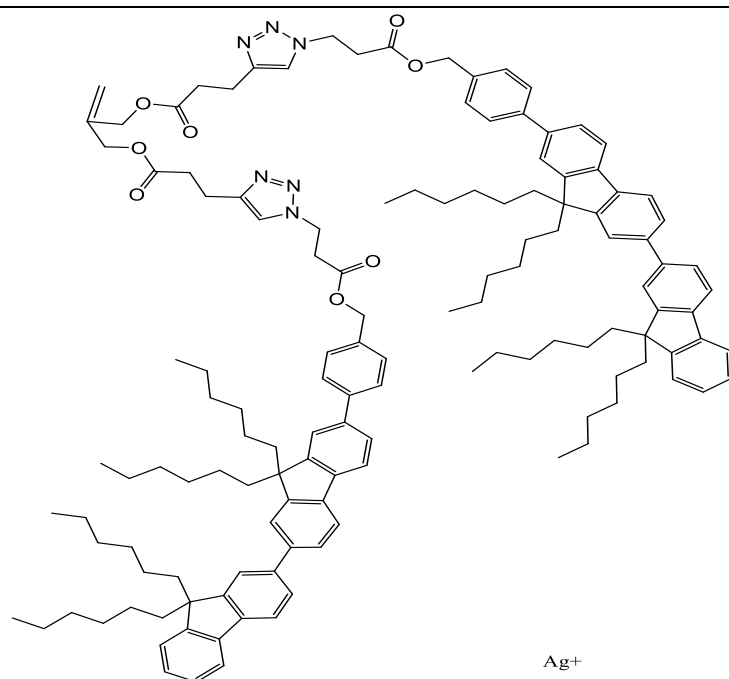
3215



2773



2094

Ag⁺

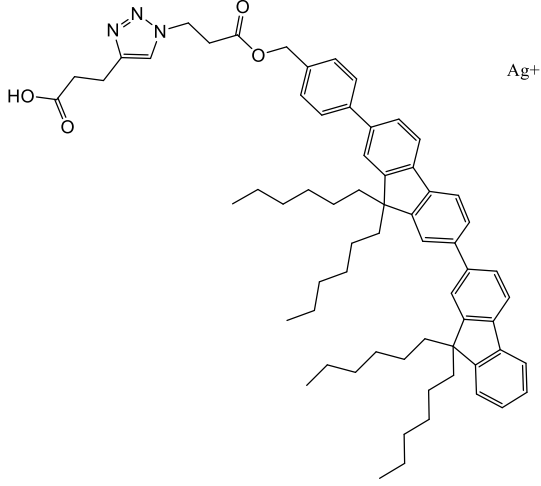
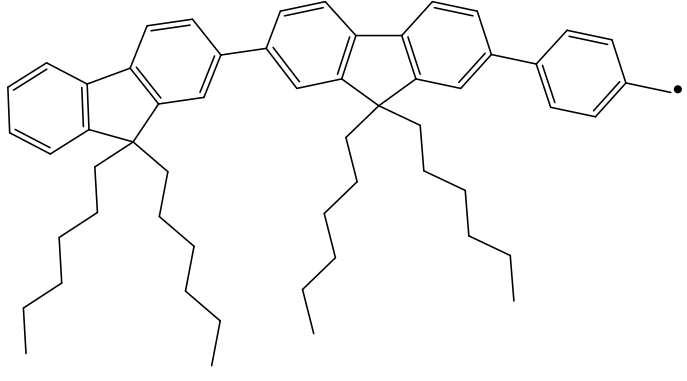
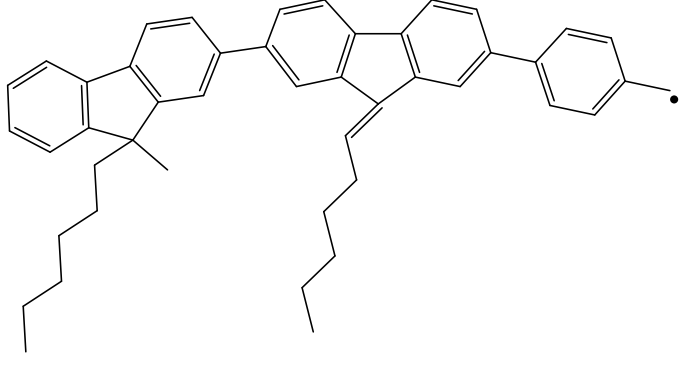
1075	 <p>Ag⁺</p>
756	
599	

Table 4: The fragments from the MS/MS of VPOSS-3-Difluorene.

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