

Spring 2018

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## Recommended Citation

Ganos, Maria, "Multi-dimensional Mass Spectrometry for the Characterization of Glycopolymers" (2018). *Honors Research Projects*. 691.

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**Multi-dimensional Mass Spectrometry for the Characterization of Glycopolymers**

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**3150 497- 001**

**April 27, 2018**

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**ABSTRACT:** Several glycopolymers synthesized by Activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP), were characterized by mass spectrometry to elucidate their microstructure and architecture. Analyses were performed using Matrix Assisted Laser Desorption Ionization- Time of Flight/ Time of Flight (MALDI-ToF/ToF) and Electrospray Ionization Mass Spectrometry (ESI-MS) instruments. The linear isopropylidened glycopolymer (P65B1) analyzed with ESI-MS also was examined with multi-stage mass spectrometry ( $MS^n$ ) using collision induced dissociation (CID) to examine the tetramer parent ion. This glycopolymer was found to be either cyclic or linear (sans end groups) due to the data being fit for both forms of the glycopolymer. The CL546A1 (isopropylidene protected), and CL552a1 (acetyl protected) both showed the glycopolymer to polymerize, yet without end groups on the polymer, meaning it had cyclized. The acetyl protected polymer also showed acetyl losses in the mass spectrum. The ideal MALDI-MS conditions for this glycopolymer were for the matrix DHB, the solvent of acetonitrile (ACN), and sodium trifluoroacetate (NaTFA), which was used as a cationizing agent. In ESI-MS, ACN and NaTFA were also used. Overall synthesis of glycopolymers is known to be difficult, making characterization difficult as well, as seen in the results shown.

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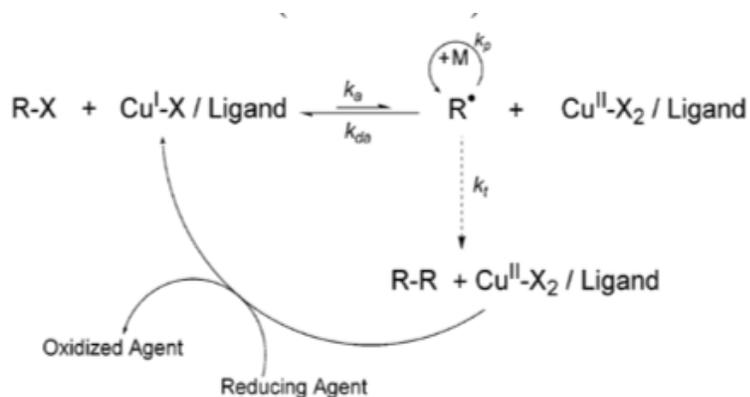
## i. Introduction

Glycopolymers have played an important role in biological studies in the fields of bioscience and polymer science.<sup>1</sup> Glycopolymers are polymers that contain carbohydrate moieties that can either be found naturally within the body or can be synthetically made.<sup>2</sup> Every cell is covered with a glycan coat, also called the glycocalyx or the pericellular matrix, which is

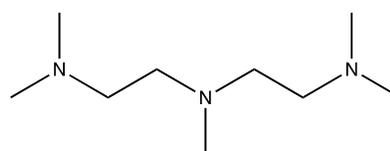
involved in cell communication between the exterior and interior of the cell.<sup>3</sup> While the glycocalyx is involved in cell adhesion, its most important role is in cell recognition events which is believed to be caused by carbohydrate-binding protein interactions.<sup>2</sup> Glycans, glycolipids, glycoproteins, and now, glycopolymers, all have sugars that are involved in these cell recognition events and adhesion.<sup>4</sup> Lectins, cell surface protein receptors, are where glycopolymers bind and allow for signals to go into the cell.<sup>2</sup> These lectins allow signal transduction to occur.<sup>3</sup> Multivalent sugar containing moieties attach to lectins strongly, while monovalent lectin-glycan interactions are weak resulting in weak biological signals.<sup>5</sup> When glycolipids interact with protein, they bind better in comparison to glycans due to the formation of compact rafts and caveolae.<sup>4</sup> Proteins bind specifically to these rafts, which are lateral assemblies that are composed of glycosphingolipids and cholesterol.<sup>6</sup> Caveolae create small cavity-like pockets on the cell surface and are considered to be rafts that have certain configurations.<sup>6</sup> Consequently, stronger receptor binding allows for stronger cell signals, and in this respect, current glycopolymers do not produce strong cell signals due to their weak binding in comparison to glycoproteins and glycolipids. Research has shown that when multiple saccharides are clumped together it allows for a higher affinity and results in better/stronger cell signaling called the glyco-cluster effect.<sup>4</sup> Synthetic glycopolymers should induce the cluster glycoside effect by behaving as multivalent sugars. Thus, synthetic glycopolymers can mimic natural glycoproteins and glycolipids within cells. They are normally used for biomedical and biological applications in regard to drug delivery.<sup>4</sup>

The two most common ways to synthesize glycopolymers are polymerization of glycomonomers or post functionalization of precursor polymers.<sup>7</sup> Atom-transfer radical polymerization, ATRP, is a controlled polymerization method which was used in this study to

form the glycopolymers examined.<sup>8</sup> This technique is typically used to synthesize polymers with specific molecular weights and narrow molecular distributions.<sup>8</sup> Copper is normally used as the transition metal to initiate the synthesis reaction.<sup>8</sup> An alkyl halide initiator is necessary since the halogen caps one end of a polymer chain allowing for further end group modification yielding the desired functional chain.<sup>8</sup> In this study ARGET ATRP, or Activators Regenerated by Electron Transfer ATRP, was used for the synthesis of the glycopolymers. The synthesis reaction begins when the metal ligand cleaves the halide from the initiator.<sup>8</sup> This results in a radical and an oxidized metal. The radical then permits for a monomer to be added on allowing for a propagation.<sup>8</sup> The newly synthesized chain is deactivated once the metal gets reduced.<sup>8</sup> Due to ATRP having excess metal appearing in the final product, ARGET ATRP can be used to reduce any excess Cu (II) that was synthesized in the end reaction.<sup>8</sup> The ATRP method was used to synthesize all the glycopolymers as seen below in *Scheme 1*.



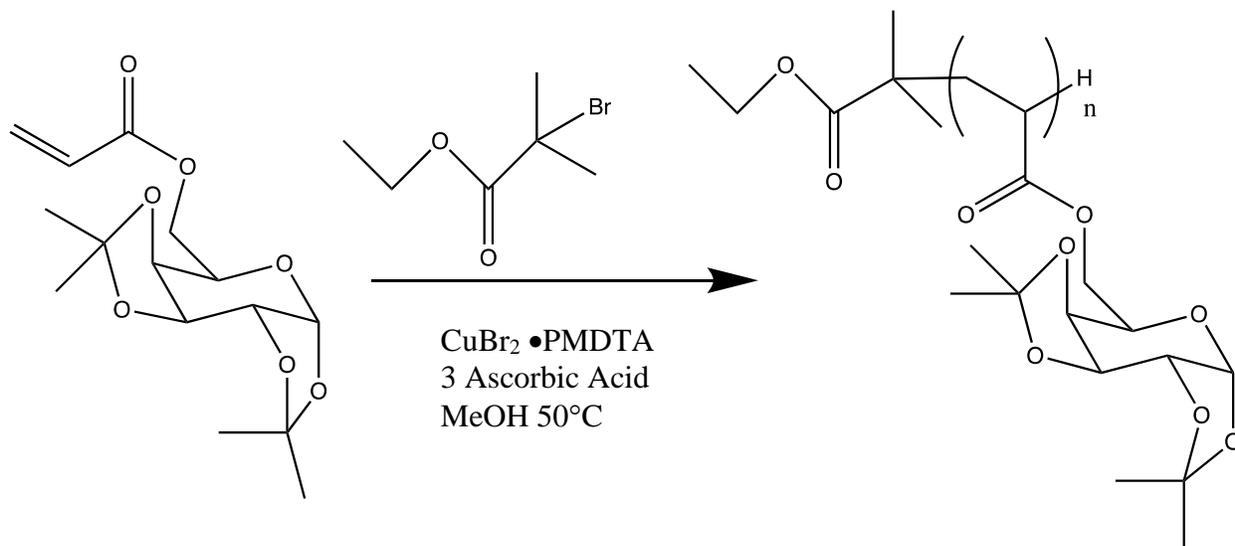
The halide was bromine and the reducing agent was Ascorbic acid. The ligand used was PMDTA as seen below.



*Scheme 1*. The ARGET ATRP synthesis scheme. The scheme is modified from that in reference 9.

ESI-MS was used to examine the linear isopropylindented glycopolymer that was synthesized using the ATRP reaction. The reaction carried out can be seen below in Scheme 2.

This ARGET ATRP reaction was performed using the ligand  $N,N,N',N'',N'''$ -pentamethyldiethylenetriamine, or PMDTA, a halo-initiator (bromo-isobutyrate), and a reducing agent, ascorbic acid. Characterization of the synthesized glycopolymer was achievable by MALDI-MS, ESI-MS, and multi-stage MS<sup>n</sup>.



*Scheme 2.* Reaction scheme of linear isopropylidened glycopolymer by ARGET ATRP.

Synthetic glycopolymers can be difficult to polymerize correctly into the desirable end product. Glycopolymers can become cyclic upon synthesis or other side reactions can take place leading to unwanted by-products. If glycopolymers are not synthesized properly, their use in drug delivery studies is not effective. Thus, glycopolymers must be characterized in order to determine which methods of synthesis are the best for further studies.

Characterization of samples can be accomplished by mass spectrometry. The general overview of mass spectrometry involves taking a sample and ionizing it into ions whose signal is then measured and recorded as intensity vs mass to charge ratio ( $m/z$ ). Based on the mass to

charge ratio, the composition can be determined. Mass spectrometry can be used to characterize large molecules with instruments such as matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF).<sup>10</sup> In this study both MALDI-ToF/ToF-MS and ESI- MS were used. This study focuses on the analysis of complex unknown glycopolymers by these methods.

MALDI-MS samples are prepared in accordance to the sample's properties in order to maximize its ionization efficiency. The samples are surrounded by a small, volatile organic compound called the matrix, which is spotted onto the MALDI plate either *with* the sample using the dry-droplet method, or *tiered with* the sample using the sandwich method. The matrix will absorb some of the energy from the laser pulses, which allows for intact molecular ions to be formed. It also disallows the sample to aggregate. When the matrix dries on the sample, or in the case of the sandwich method on top and below the sample, it causes for the sample to be trapped. The laser beam desorbs and ionizes the matrix which transfers charge to the desorbed sample. Once desorption and ionization are completed, the ions are accelerated at a fixed potential to enable separation by the mass to charge ratio ( $m/z$ ). In time of flight analyzers, the sample's  $m/z$  ratio is determined by quantifying the amount of time it takes to travel the length of the flight tube.<sup>10</sup>

ESI-MS is a solution state mass spectrometry technique, unlike MALDI, where one must allow the sample to dry on the MALDI plate before analysis. The sample in ESI is first sprayed into an aerosol off of a charged capillary, creating charged droplets.<sup>11</sup> The exact release mechanism to how this works is not exactly known, yet three models have been introduced as possible mechanisms: the ion evaporation model, the charged residue model, and the chain ejection model.<sup>12</sup> The ion evaporation model, IEM, states as the droplets of sample shrink due to evaporation, the field strength on the droplet becomes strong enough to expel sample ions from

each droplet.<sup>13</sup> The energy needed to increase the surface of the sample droplet as the ion is expelled is overcome by the energy provided by the electric field.<sup>13</sup> Geometric parameter, rate kinetics, and ion evaporation rate constants are all involved in this potential model.<sup>13</sup> The charge residue model, CRM, states that the electrospray produces droplets that continuously undergo fission until they only contain one ion. As the solvent evaporates from a droplet, it frees the ion.<sup>13</sup> The third model, the chain ejection model, or CEM, is based on the disorder of the chains, partial hydrophobicity, and the ability to bind to many charge carriers.<sup>12</sup> CEM states that one unfolded chain of the polymer will be ejected to the vapor phase and then the rest of the polymer will succeed this ejection.<sup>12</sup> Based on the glycopolymers studied, the CRM model is more probable for this study due to the size of the polymer, and makeup of the polymer itself. Once the sample leaves the electrospray tip, it enters the mass spectrometer where the sample is analyzed.<sup>11</sup> In tandem MS, a specific precursor ion is selected by its  $m/z$  and then undergoes collision-induced dissociation (CID) which fragments the chosen precursor ion and allows for daughter ions to be formed from parts of the precursor ion.<sup>13</sup> Multi-stage tandem mass spectrometry, or MS<sup>n</sup>, uses CID to fragment the “daughter” ions from the previous collision, as well as the fragments from *those* daughter ions, up to nine times (MS<sup>9</sup>).

The purpose of this study was to characterize various glycopolymers. The glycopolymers being used in these experiments were prepared by the research group of Professor Coleen Pugh at the University of Akron. Analysis was challenging due to difficulties in the synthesis of these glycopolymers and the extent of side reactions upon synthesis.

## ii. Methods and Materials

The samples analyzed by MALDI-MS were run at a concentration of 10 mg/mL in ACN. They were mixed with NaI (10 mg/mL in ACN) at a 1:10 v/v ratio. The MALDI-MS instrument used was a Bruker UltraFlex III ToF/ToF (Billerica, MA). Two different matrices were used: Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene], also known as DCTB (Sigma Aldrich, St. Louis, MO) and 2,5- dihydroxybenzoic acid, or DHB (Aldrich, Milwaukee, WI). Both were prepared at 20 mg/mL in ACN. The volume ratio of matrix: sample: salt was a 10:2:1  $\mu$ L. The samples were all applied to the MALDI plate using a sandwich method: the matrix was spotted on the MALDI plate, the sample was added on, and the final matrix covering coat was added.

For both MALDI- and ESI-MS, the solvents used were Fischer Chemical tetrahydrofuran (THF) and acetonitrile (ACN) (Fisher Scientific, St. Louis, MO).

To analyze the samples with MALDI-MS, the Bruker Daltonics MALDI plate was spotted with the samples using the sandwich method. For each sample tested, 3 spots were spotted and in between each sample a row of spots was left with no sample on the plate, to avoid sample contamination. Once the plate was dry, the plate was taken to the MALDI-MS and loaded into the instrument. The docking of the plate into the instrument was performed by pressing the green load button on the instrument. The camera was turned on and once the software read "Ready/In" the instrument was ready for analysis. RP 0-4 kDa reflectron positive mode method was used with a calibration tuned for lower molecular weight samples (under 10 kDa). The laser power was adjusted for the samples depending on the desired signal intensity. The calibration of the instrument was checked by using 2K and 4K Fluka Analytical poly(methyl methacrylate), or PMMA (St. Louis, MO), again following the same sample preparation above

for the samples being tested. A PMMA sample with DCTB and ACN was analyzed as a reference check. After calibration, the samples were examined and the laser power was adjusted. Spectra were summed until the intensity reached approximately  $1.0 \times 10^5$ , which allowed for intense peaks and high resolution. The spectrum was examined to characterize the sample. The samples that were characterized with MALDI were CL546a1, CL552a1, and P65B1. The CL546a1 sample was run with a matrix of DHB and the solvent of ACN. CL552a1 was run with a matrix of DHB and the solvent of THF. P65B1 was run under three different conditions. The first was with a matrix of DCTB and the solvent of THF. The second had a matrix of DHB and the solvent of ACN. The third had a matrix of DHB and the solvent of THF. Of these, it was found that the best parameters for this glycopolymer were a matrix of DHB, solvent of ACN, and the salt NaI. The data were analyzed with flexAnalysis software by Bruker.

To analyze the sample of a linear isopropylidened glycopolymer, P65B1, ESI-MS and ESI-MS/MS<sup>n</sup> were performed. The samples analyzed by ESI-MS were run at a concentration of 1 ppm, or 0.001mg/mL in ACN. These samples were mixed with NaI (1 ppm solution in ACN) as a cationizing agent at a ratio of 1:10 (v/v). The ESI-MS instrument used was a Bruker HCT Ultra (Billerica, MA). Any glass vials used were first rinsed out with the solvent that was to be added to ensure no contamination. The solutions were vortexed to ensure the samples were dissolved within the solvent. To inject the samples, a 500  $\mu$ L Hamilton syringe was used. The NaI was mixed with the sample to help promote ionization. Tandem mass spectrometry experiments were performed on the selected parent ion and the daughter ions of the glycopolymers, up to MS<sup>4</sup>. Software used to analyze the ESI-MS data was the ESI Compass DataAnalysis software (Bruker Daltonics, Billerica, MA).

### iii. RESULTS/DISCUSSION

#### *MALDI-MS of glycopolymers CL546A1, and CL552A1*

The CL546A1 sample was an acetylated glycopolymer prepared using the ARGET ATRP synthesis. The matrix used for this sample was DHB, the solvent was ACN, and the sandwich method was used. This solvent and matrix showed the best intensities in the spectrum in comparison to the other matrices and solvents available and tested. The spectrum showed peaks at  $m/z$  values of 1230.078, 1632.580, 2035.009, 2437.395, 2839.769, 3242.142, 3644.545, and 4046.990 as seen in Appendix figure 1. From the spectrum, the difference between adjacent oligomers was found to be 402 Da as seen in (Fig. 1). Thus, the polymerization was successful and yielded a glycopolymer. The synthetic method used was expected to produce the glycopolymer seen in (Fig.2). There were no apparent end groups of 195 Da (initiator with bromine) or 128 Da (without bromine), corresponding to the end groups mass, present in the spectrum, conveying the formation of cyclics. Isometric linear oligomers with one saturated and one unsaturated acrylate chain end are also possible. The spectrum showed that acetyl losses were occurring.

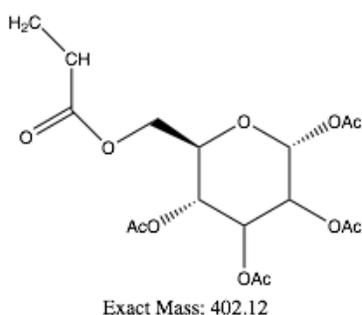


Figure 1. The structure of the repeating unit of the glycopolymer for samples CL546a1 and CL552a1.

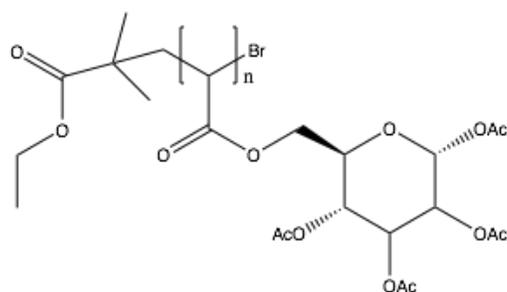


Figure 2. Structure of the CL546a1 and CL552a1 glycopolymers that were synthesized having end groups of 195 Da.

#### *MALDI-MS analysis of glycopolymer P65B1*

The P65B1 sample was an isopropylidened glycopolymer synthesized using ARGET ATRP. The first matrix used for this sample was DCTB, the solvent was THF, and the sandwich method was used. The spectrum showed peaks at  $m/z$  values of 1280.213, 1594.505, 1908.786, 2223.050, 2537.324, 2851.607, 3165.873, 3480.165, 379.459, 4108.765, 4423.086, 4737.423, 5051.778, 5366.153, 5680.550, 5994.975, 6309.456, 6623.911, 6938.394, 7252.931, 7567.509, 7882.093, 8511.302 as seen in Appendix figure 2. The difference between each oligomer peak was showing a repeating unit of  $\sim 314$  Da. The last peak showing an  $m/z$  of 8825.863 correspond to the 28-mer. The repeating unit of this glycopolymer can be seen in (Fig. 3).

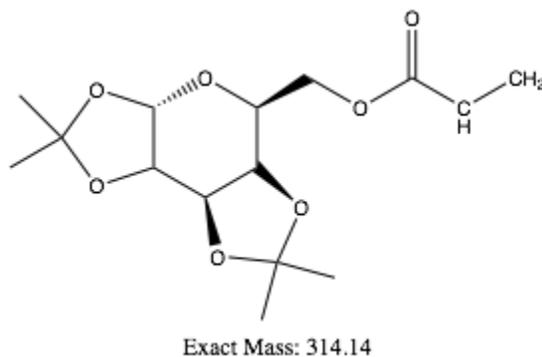


Figure 3. Structure of the carbohydrate moiety of the glycopolymer demonstrating the 314 Da repeating unit in the P65B1 polymer.

The second set of conditions set for the P65B1 glycopolymer had the following matrix DHB, the solvent ACN, salt NaI, and the sandwich method was used. This spectrum had peaks at  $m/z$  values of 617.192, 651.472, 965.591, 1279.769, 1593.969, 1908.173, 2222.359, 2536.553, 2850.746, 3164.940, 3479.136, 3793.337, 4107.542, 4421.762, 4736.000, 5050.254, 5364.518, 5678.740, 5993.046, 6307.448, 6936.212, 7565.115, 8193.955, and 8508.254, as seen in Appendix, figure 3. The largest oligomer detected was the 27-mer at 8508.254 Da with intensities up to  $1.0 \times 10^5$ . As seen in the previous P65B1 sample, the repeating unit was ~314 Da.

The last experimental study on P65B1 glycopolymer had the following conditions: matrix DHB, solvent THF, salt NaI, method sandwich. The spectrum contained peaks of  $m/z$  values: 965.796, 1093.909, 1594.284, 1908.547, 2537.076, 3479.828, 3794.081, 4108.332, 4422.610, 4736.899, 5051.205, 5679.899, 5994.240, 6308.649, 6623.185, and 7252.105 as shown in Appendix figure 4. From this spectrum, the difference between the peaks was shown to be again ~314 as seen for the other two conditions of the same polymer. The largest oligomer in this spectrum was at 8827.782 Da corresponding to the 28-mer of the polymer.

From these three sets of conditions, it was found that using the matrix of DHB with the solvent ACN afforded the best results. The DHB/ACN/NaI sample had the best resolution and the least amount of shots needed to obtain high intensity signals for the P65B1 sample. Overall, from the P65B1 sample, the minor distributions, but were 128 Da greater in mass than the major. This increment was arising from the mass of the initiating group without bromine.

### ESI-MS<sup>n</sup> of a Linear Isopropylidenedated glycopolymer

The linear isopropylidenedated glycopolymer (P65B1) examined was characterized using ESI-MS and tandem MS. The synthesis reaction can be seen in Scheme 2. Upon ESI-MS, the glycopolymer oligomers observed were a trimer followed by a tetramer, pentamer, hexamer, heptamer, and octamer. These resulted in the spectrum showing  $m/z$  values of 965.3, 1279.4, 1593.5, 1907.6, 2222.6, 2536.6 respectively as seen in Appendix figure 5. There were no acetyl losses, as seen in the acetylated glycopolymers, due to the acetyl group being replaced by a isopropylidene group in these samples. The tetramer was used as a parent ion for MS<sup>n</sup> fragmentation and further examination of the daughter ions. The MS<sup>2</sup> spectrum of the parent ion can be seen in Appendix figure 6 showing fragments of  $m/z$  values of 651.2, 805.2, 863.3, 921.3, 979.3, 1047.3, 1105.3, 1163.4, 1221.4, and the parent peak at 1279.4. From the spectrum, it appears that the parent ion of the tetramer was losing moieties of 58 Da. As seen in Fig. 4, it is proposed that the loss of 58 Da arose by the elimination of a C<sub>3</sub>H<sub>6</sub>O unit from the isopropylidene groups on the glycopolymer, presumably resulting in a lactone-like or ketone-like structure at the carbohydrate moiety. On the initial precursor ion, there were 8 isopropylidene groups present. In the initial fragmentation, 8 sequential mass losses of 58 Da were observed, corresponding to the isopropylidene groups present.

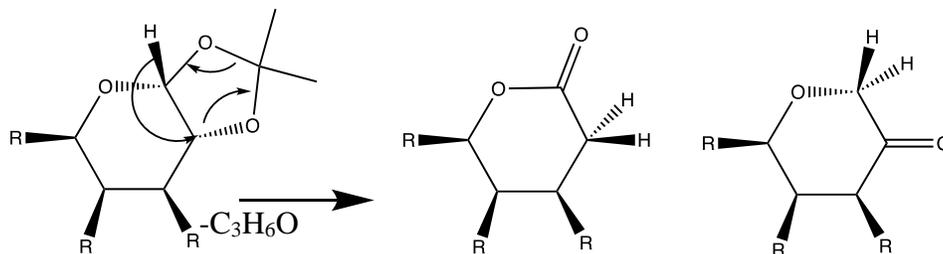


Figure 4. Proposed mechanism for the 58 Da loss from isopropylidenedated glycopolymers. This 58 Da loss most likely corresponds to acetone, which stemmed from the isopropylidene groups.

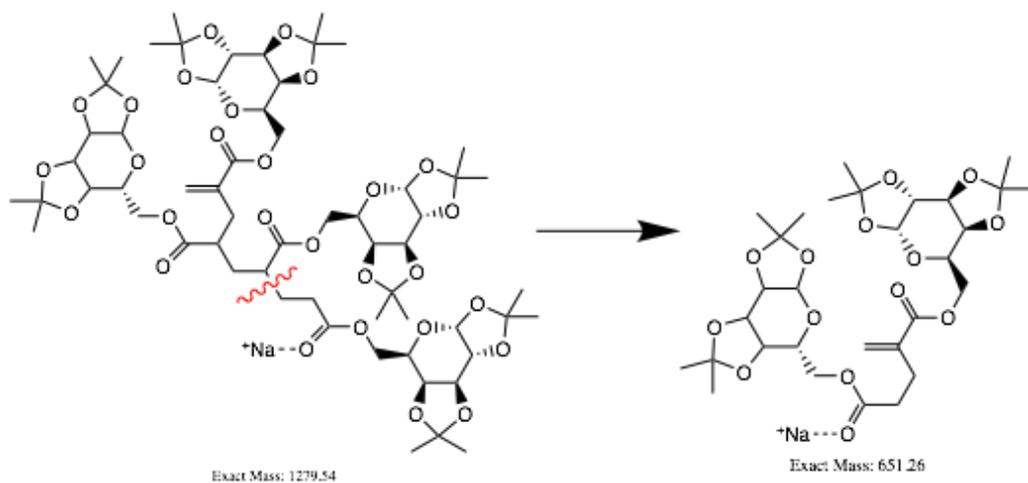


Figure 5. This shows the 651.2 Da dimer of the linear isopropylidinated glycopolymer that was sodiated after breakage from the tetramer.

The MS<sup>2</sup> spectrum also showed a peak at 651.2 *m/z* which was determined to be the dimer as seen in Fig. 5. Once the MS<sup>2</sup> spectrum was rationalized, the MS<sup>3</sup> spectrum of the fragment ion at *m/z* 1221.4 was examined. The spectrum can be seen in Appendix figure 7 and had *m/z* values of 661.2, 863.2, 921.3, 979.3, 1021.3, 1047.3, 1105.3, 1163.3, and 1221.4. This daughter peak had 6 losses of the 58 Da. The MS<sup>4</sup> of the MS<sup>3</sup> fragment at *m/z* 1163.3 showed 4 respective losses of 58 Da. The spectrum had peaks at *m/z* values of 661.2, 863.2, 921.2, 989.2, 1047.3, 1105.3, and 1163.3 as shown in Appendix figure 8. In this the spectrum, the 931.2 *m/z* could not be seen unless the spectrum was enlarged due to the intensity being low, yet it was present. The 931.2 *m/z* corresponds to a structure that contains two intact isopropylidened groups, with all other ones being lost in the form of acetone. A possible structure can be seen in Fig 6. These data confirm the occurrence of successive acetone losses from the glycopolymers.

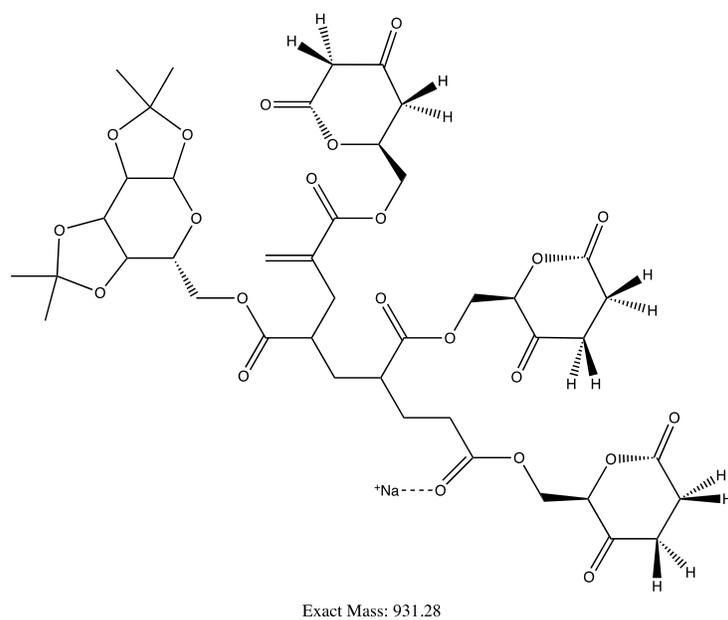


Figure 6. This shows the 931.2 Da fragment in the MS<sup>4</sup> spectrum was formed after 4 successive losses of 58 Da.

From the tandem MS results, it was determined that the glycopolymer was either cyclic or linear for the tetramer parent ion that was used to perform CID. The mass to charge ratios correlated for either structure. Fig. 7 and Fig. 8 show the cyclic and linear trimer forms of the glycopolymer. Either structure is consistent with the MS<sup>4</sup> results.

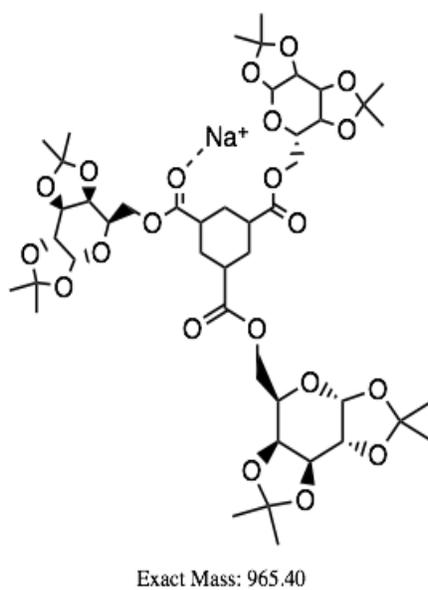


Figure 7. The cyclic form of the trimer.

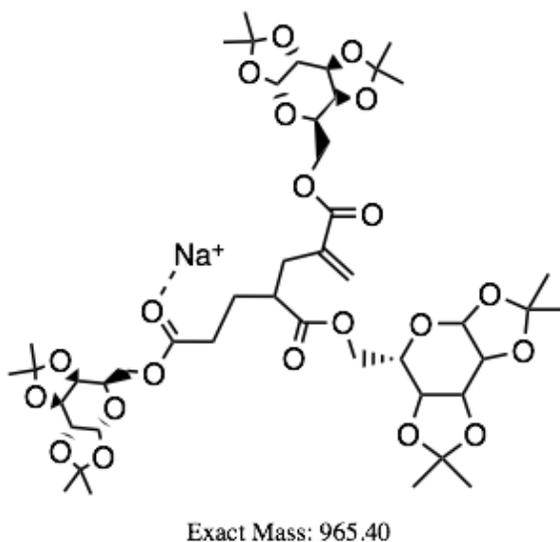


Figure 8. The linear form of the trimer.

Overall from the ESI-MS and multi-stage CID data, it was determined that the “linear” isopropylidened glycopolymer could have either a cyclic or linear architecture without end groups due to the spectral results appearing fit for either structure. The MS data showed oligomer species with the proposed repeat unit, meaning that polymerization was successful, but not always with the end groups proposed. The minor distribution of the ESI-MS also showed monomer units, but they were 128 Da greater than the major. This was due to the mass of the initiating group without bromine.

#### iv. Conclusion

The characterization of CL546a1, CL552a1, and P65B1 was carried out using MALDI- and ESI-MS. From analyzing the results, it was found that the synthesis reactions of ARGET-ATRP did form the correct glycopolymers, but some had other side reactions taking place. As described in other studies, synthesizing a pure polymer without any side reactions is difficult.

This was apparent, as end groups were detected only in small amounts in the mass spectra. The lack of enol groups in the major distribution observed suggested cyclization or chain transfer during polymerization. Enhancing preparation parameters throughout experimentation allowed for the analysis of the various glycopolymers. Glycopolymers CL546a1, CL552a1, and P65B1 were found to yield the best spectrums with DHB/ACN/NaI for MALDI-MS. P65B1 in ESI-MS was found to yield the best spectrum with ACN and NaI. Thus, from these glycopolymers it was determined isopropylidened and acetylated glycopolymers performed well with the solvent of ACN, the salt of NaI, and a matrix of DHB. It was difficult for these glycopolymers to ionize and produce high intensity, non-contaminated spectra, however, the structures were able to be analyzed by using multi-dimensional mass spectrometry techniques. The multi-stage tandem mass spectrometry data allowed for a fragmentation mechanism to be proposed as to how the linear isopropylidened glycopolymer underwent successive losses of C<sub>3</sub>H<sub>6</sub>O units. Overall mass spectrometry analysis showed that ARGET ATRP synthesis is promising for forming chains of acetylated and isopropylidened glycopolymers.

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## **Appendices**

- I. Safety Considerations
- II. Mass Spectrometry Spectrums Figs. 1-8

## Appendix 1: Safety Considerations

In order to ensure safety when performing the experiment, as in any laboratory environment, goggles and gloves were worn at all times upon handling the synthesized compounds, solvents, and matrices. It was important that the work environment was clean before and after performing the experiment. So, chemicals had to be disposed of properly at the end of performing each experiment. The chemicals were placed in the waste hood to evaporate, and any glass was placed in the glass waste containers. If any glass vials broke, they were disposed of cautiously using a broom. The mass spectrometry instruments all had to be cleaned properly after each use to ensure the instruments would be precise for the next experiment. Time was always left at the end of the experiment to thoroughly clean the instrument so no residues or samples were still present within the experiment.

Some of the chemicals used in this experiment had specific irritations that had to be made aware of before use. THF is known to be a flammable solvent that can cause skin and eye irritation . It can react with oxidants leading to hazard and fire . ACN, like THF, is considered a flammable liquid and causes skin and eye irritation. NaI is not flammable, yet it does irritate the skin and eyes. If it is inhaled it can cause irritation in the throat leading to coughing and a headache.<sup>14</sup> If ingested, vomiting and nausea will be induced. DHB is known to cause skin and eye irritation and can affect breathing. DCTB is another skin and eye irritant that can affect breathing as well. <sup>15</sup> When handling these chemical compounds and solvents, even when gloves and goggles were worn, they were handled in caution and were dealt under the hood to avoid inhalation.

## Appendix 2: Mass Spectrometry Spectrums Figs. 1-8

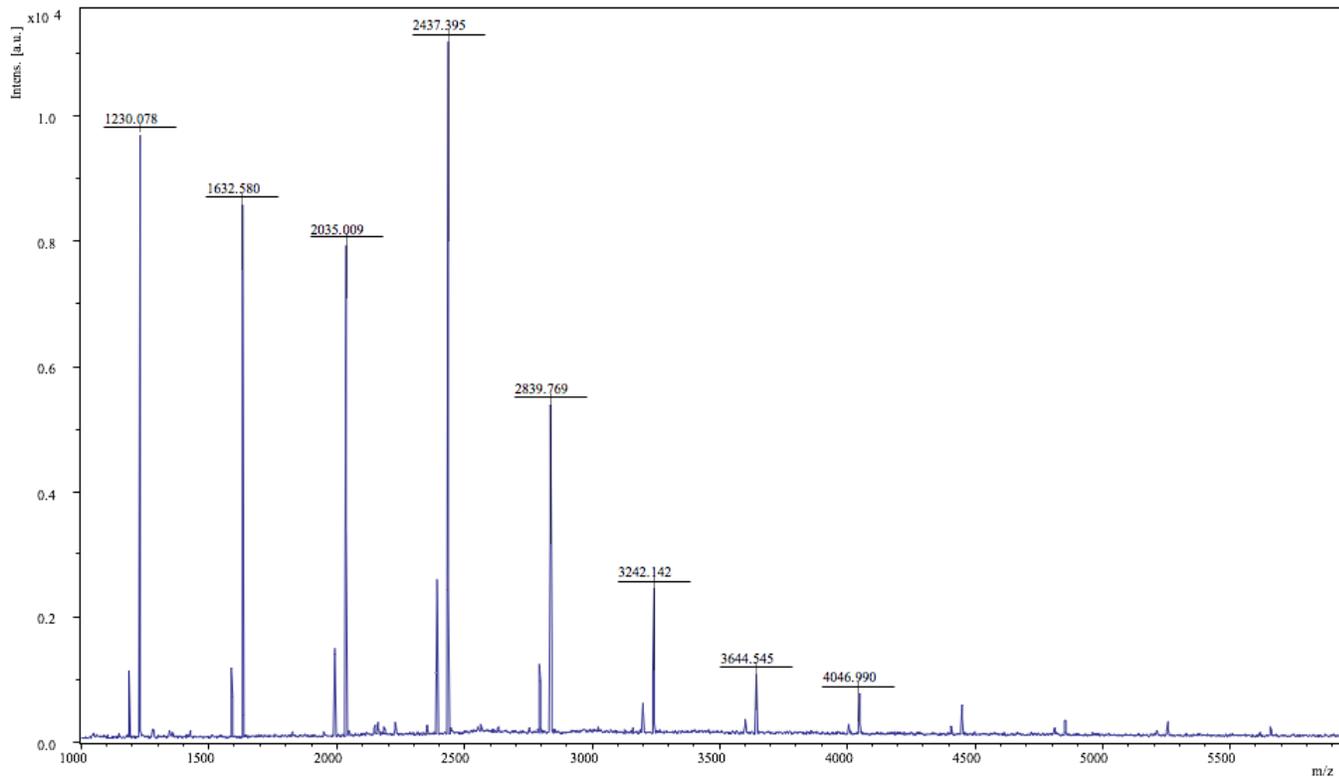


Figure 1. The MALDI-MS major distribution corresponds to a linear acetylated glycopolymer, CL546A1, without the proposed end group. The minor distribution is from acetyl losses.

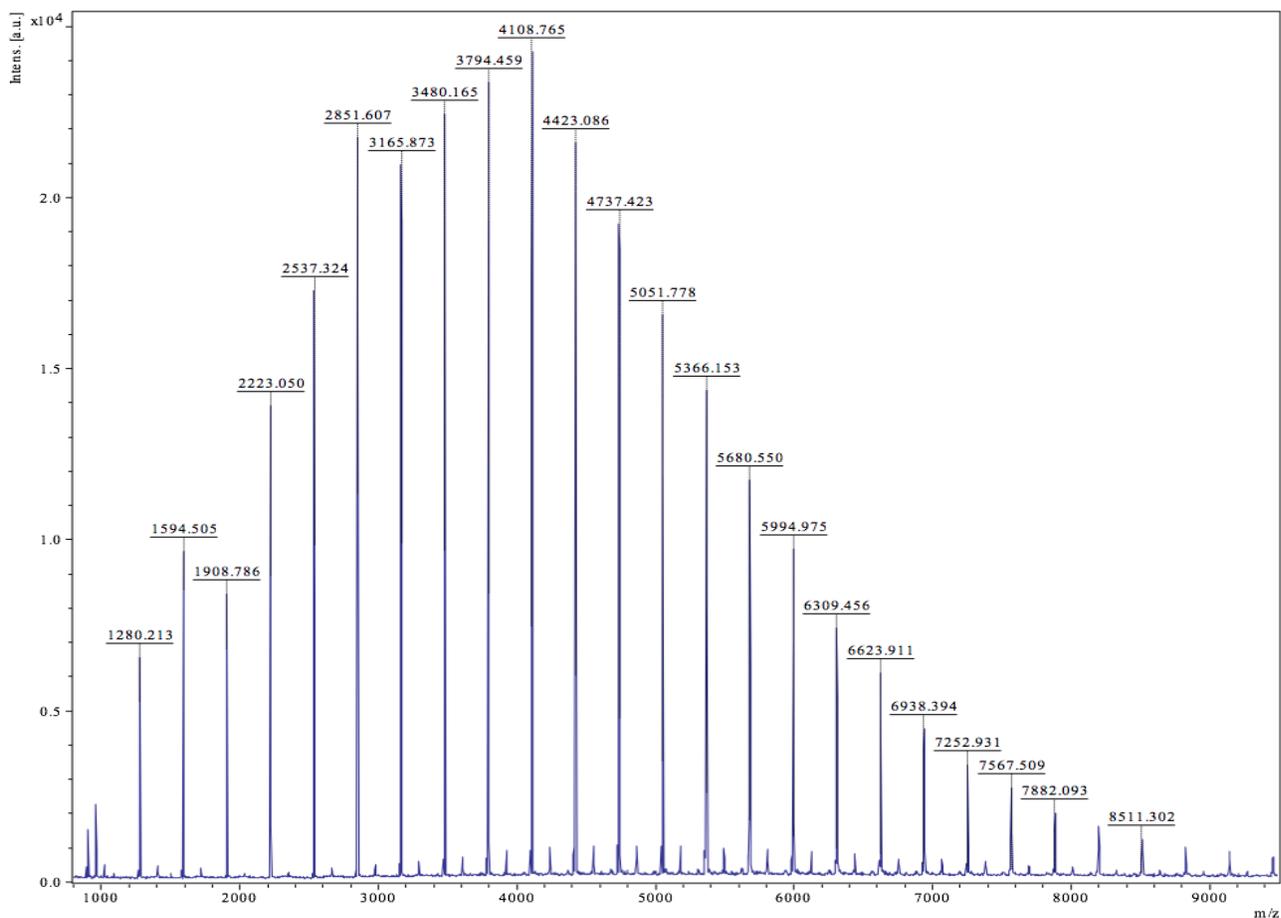


Figure 2. MALDI- MS spectrum for P65B1 with a matrix of DCTB and a solvent of THF. The major distribution corresponds to the isopropylidenated glycopolymer without end groups. The minor distribution also contains the monomer units, but is 128 Da (plus H) greater than the major. This corresponds to the mass of the initiating group, without bromine.

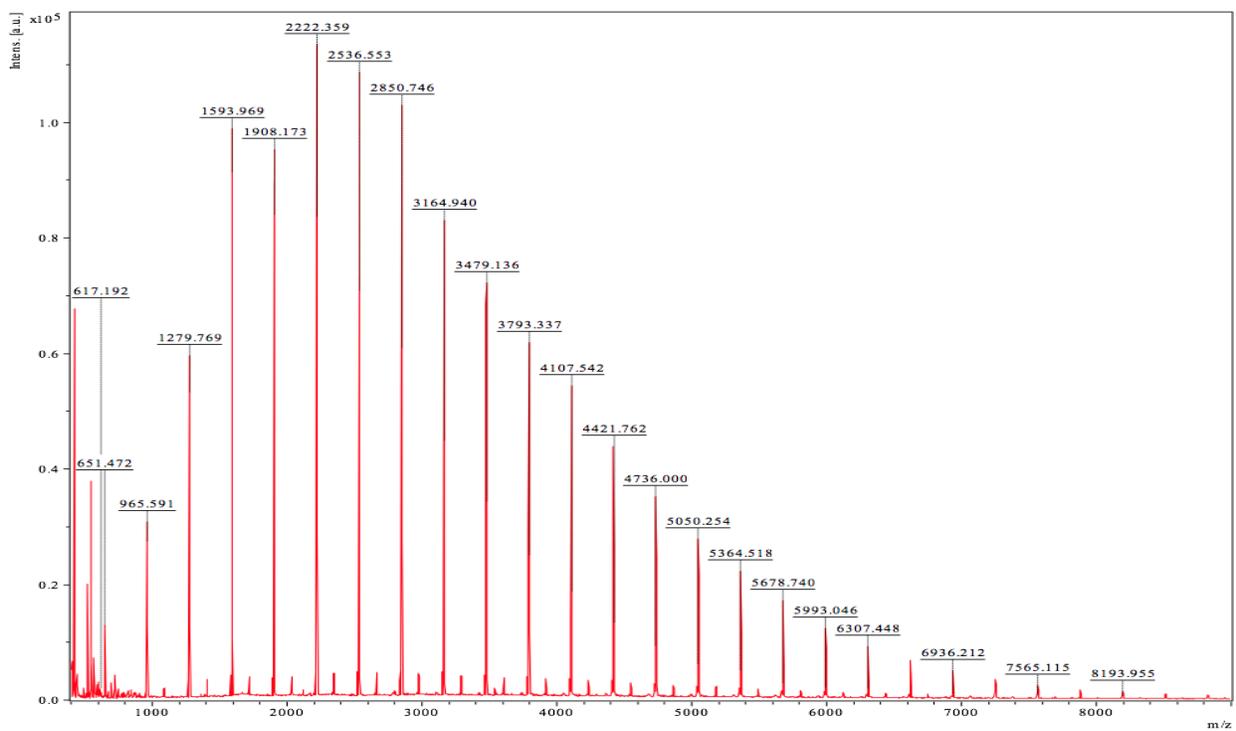


Figure 3. MALDI-MS spectrum for P65B1 with a matrix of DHB with a solvent of ACN. The MALDI-MS major distribution corresponds to a isopropylidenated glycopolymer without end groups. The minor distribution is the same as the major, with the projected end groups.

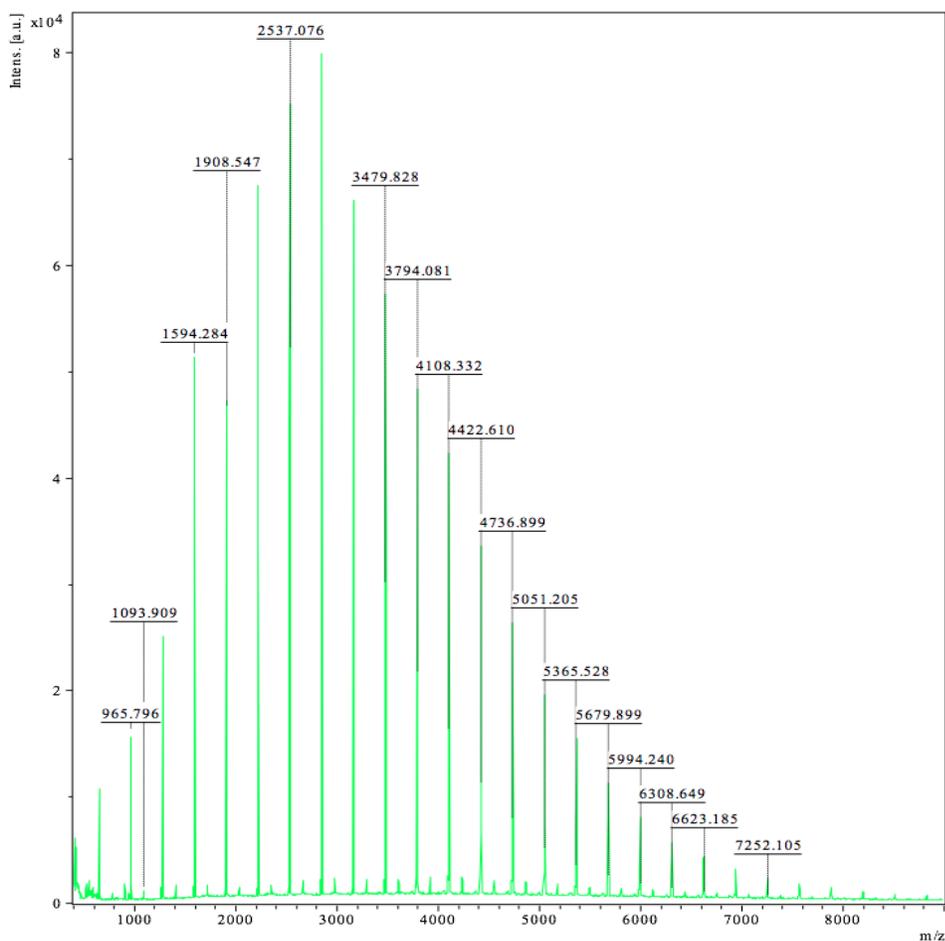


Figure 4. MALDI-MS spectrum for P65B1 in the following conditions: matrix (DHB), solvent (THF), salt (NaI), method (sandwich). The major distribution corresponds to the isopropylidened glycopolymer without end groups. The minor distribution is the same as the major, with the projected end groups.

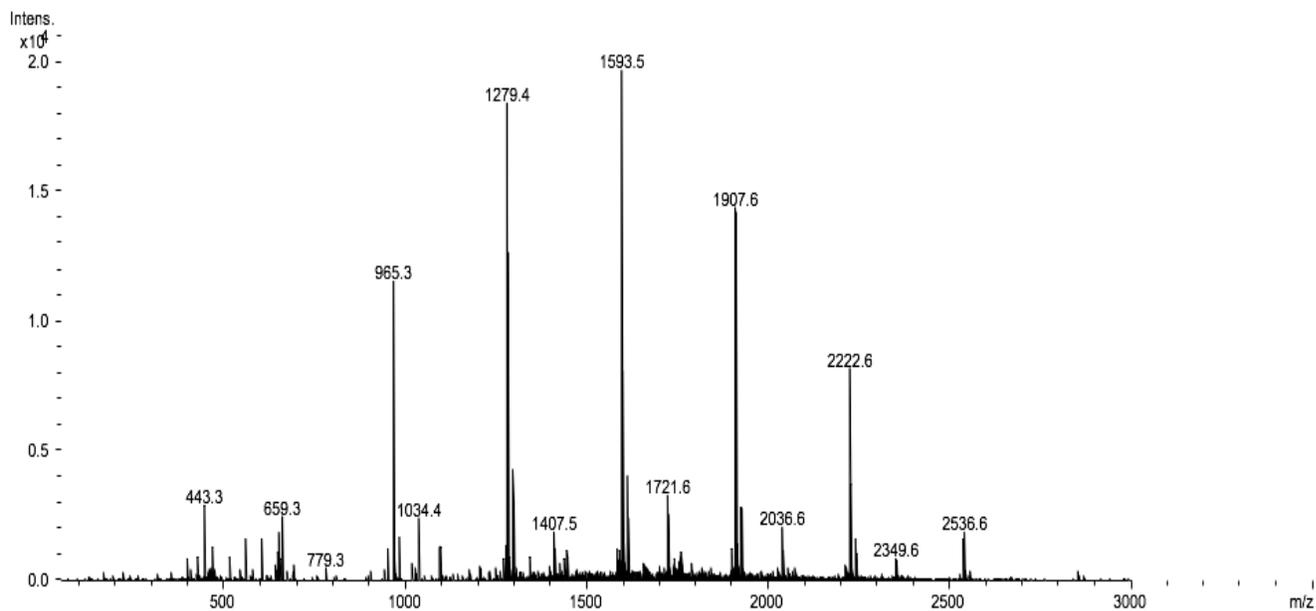


Figure 5. ESI-MS major distribution for the linear isopropylidened glycopolymer showed trimer, tetramer, pentamer, hexamer, heptamer, and octamer  $m/z$  values of 965.3, 1279.4, 1593.5, 1907.6, 2222.6, and 2536.6 respectively. The minor distribution also contains the monomer units, but is 128 Da (plus H) greater than the major. This corresponds to the mass of the initiating group, without bromine.

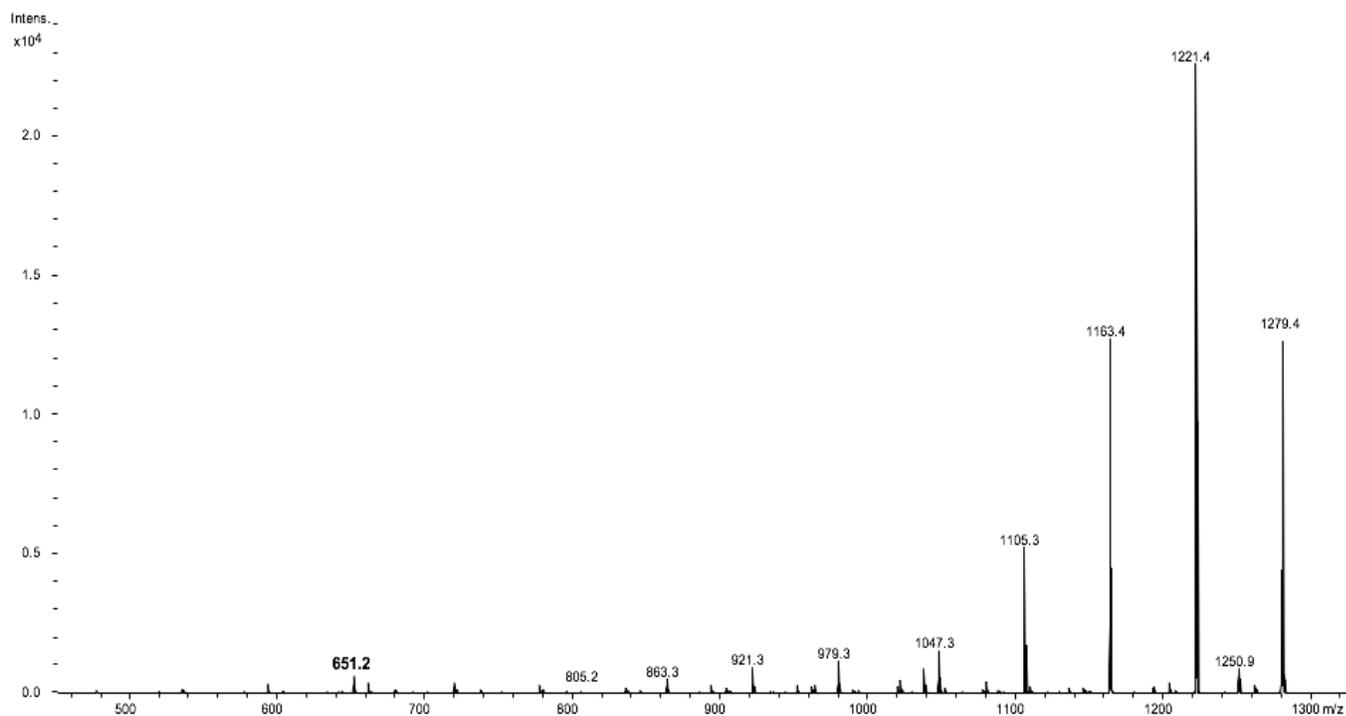


Figure 6. ESI-MS/MS spectrum of the linear isopropylidenated glycopolymer (P65B1). The parent ion was the tetramer at 1279.4  $m/z$ . This data shows successive losses of C<sub>3</sub>H<sub>6</sub>O eight times starting with  $m/z$  values of 1221.4, 1163.4, 1105.3, 1047.3, 979.3, 921.3, 863.3, and 805.2.

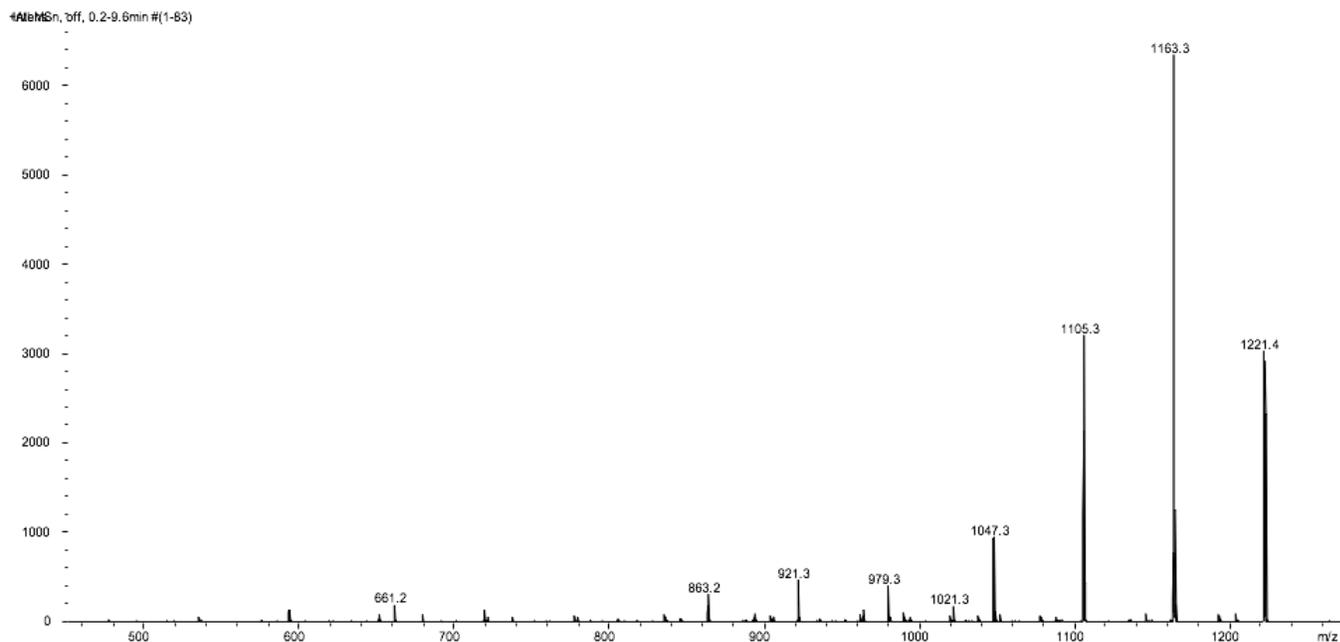


Figure 7. ESI-MS<sup>3</sup> spectrum for the linear isopropylidened glycopolymer (P65B1). The 1221.4 *m/z* ion from the initial tetramer MS/MS spectrum was used for further analysis. This data showed 6 successive losses of C<sub>3</sub>H<sub>6</sub>O starting with *m/z* values of 1163.3, 1105.3, 1047.3, 979.3, 921.3, and 863.2.

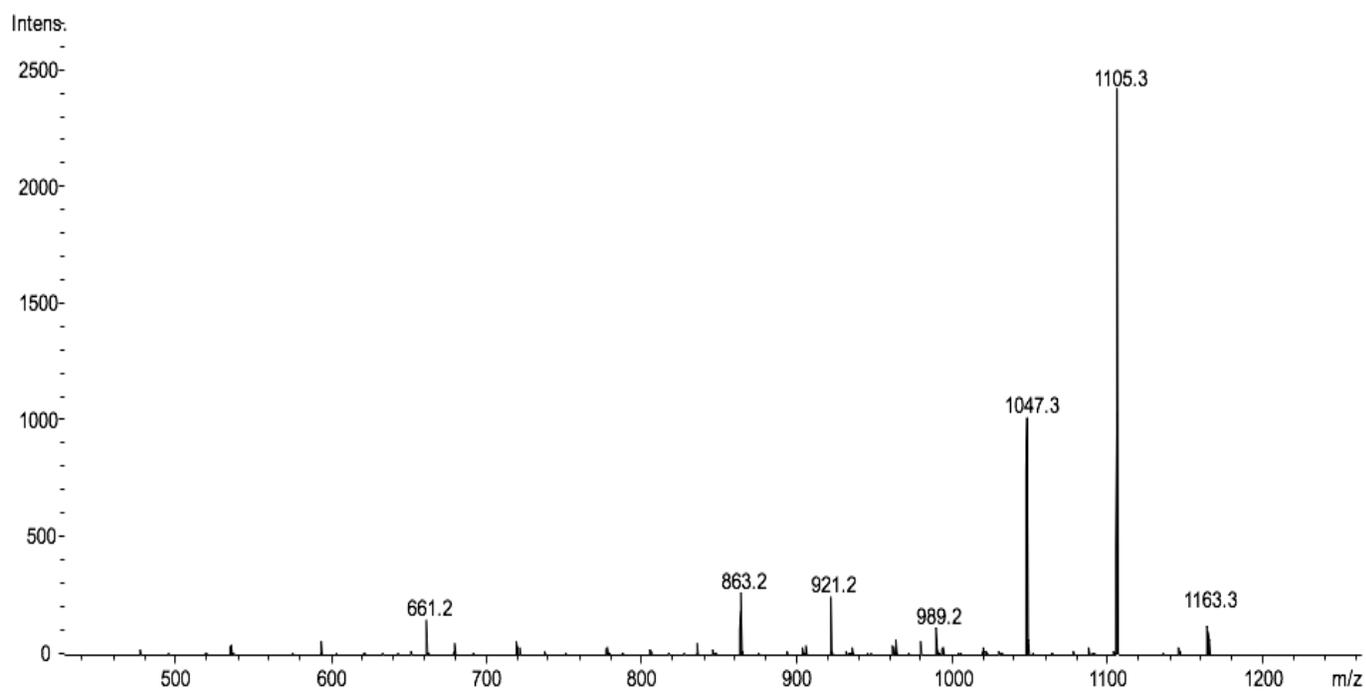


Figure 8. ESI-MS<sup>4</sup> spectrum for the linear isopropylidened glycopolymer (P65B1). The 1163.3 *m/z* ion was from the MS<sup>3</sup> spectrum was used for further analysis. From this precursor ion, there were 4 successive losses of C<sub>3</sub>H<sub>6</sub>O groups with *m/z* values at 1105.3, 1047.3, 989.2, and 931.2. The 931.2 *m/z* value was present but had a faint peak with a low intensity.