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Synthesis of XTH inhibitors

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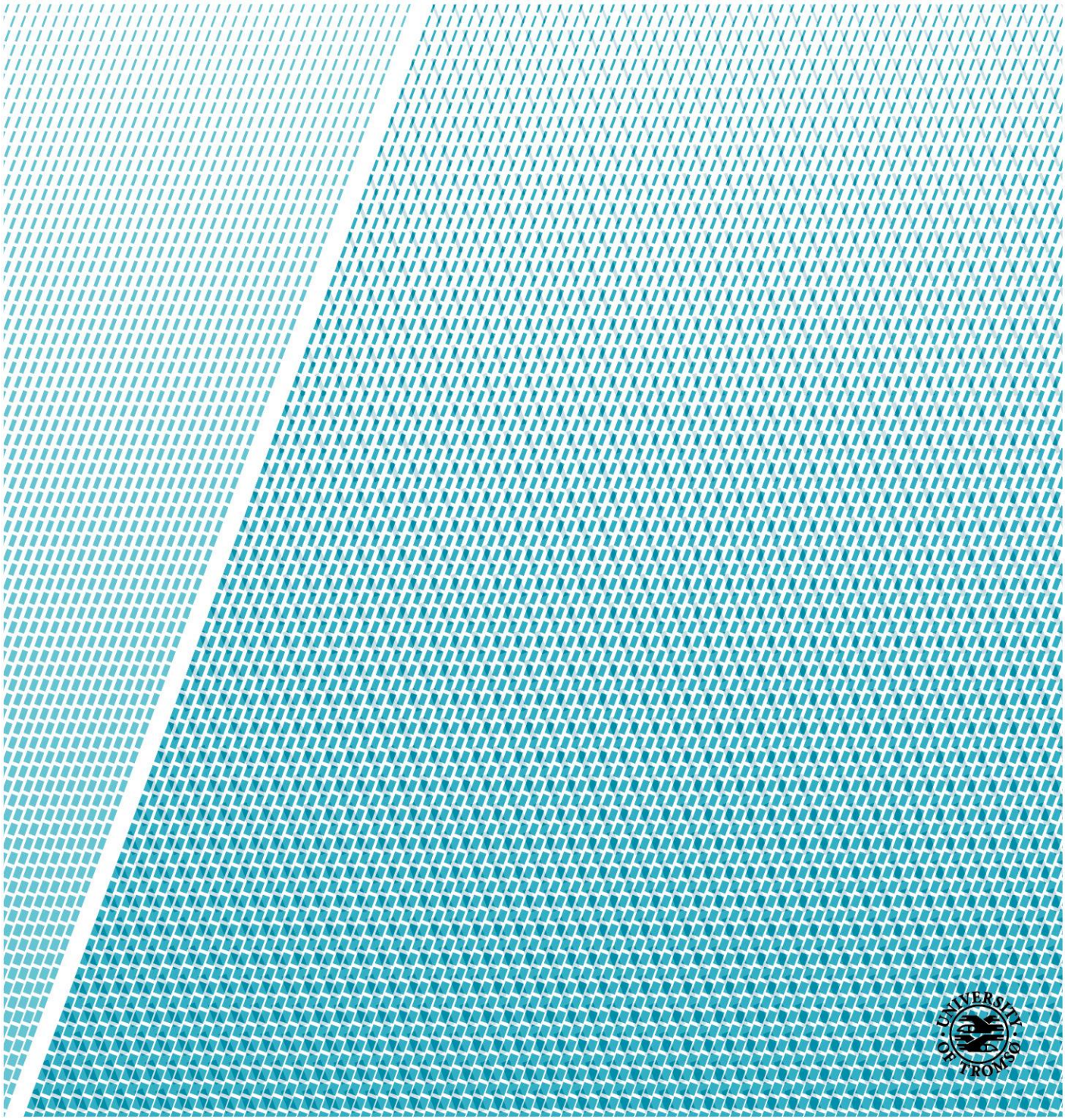

I Abstract

XTHs are a group of enzymes that were discovered in the early 1990s and are possibly involved in the infection of tomato plants by the *Cuscuta* parasite. In a recent study done by UIT arctic and marine biology shows that Brilliant Blue R250 works as a good inhibitor for these enzymes.

The focus of the project is to synthesize structurally similar molecules to potentially increase water solubility and inhibitor activity. Therefore, the current goal is to synthesize analogous molecules with other functional groups on the aromatic ring and molecules where the sulfonate groups have either been removed or been substituted by other functional groups such as nitro and phosphonate. The reason for this was to determine which parts of the molecule is needed to bind the enzyme and see what effect this will have on the XTH activity and water solubility.

Most of the planned work of synthesizing Brilliant Blue R250 derivatives were performed and showed traces of the desired molecules, exept the phosphonated derivatives. The compounds that showed traces except for some nitrated derivatives have been tested for effect on XTH activity.

II Acknowledgments

First of I would like to thank my Supervisor Professor Tore Lejon at University of Tromsø for helping and supervising me through this challenging project and for coming up with new ideas when stuck in the lab. I enjoyed the discussions about the project, how to solve the problems and the funny stories he had to tell. It's been a pleasure working under you on this project.

Next, I would like to thank my co-supervisor Truls Ingebrigtsen for helping me with the NMR, writing and providing me with some reading material for use in this thesis. Another thanks go to Frederick Alan Leeson for ordering and providing me with compounds that was used for synthesis in this thesis.

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I would also like to thank my family for helping, supporting and cheering on me when working on this thesis.

III Abbreviations

^{13}C NMR	Carbon 13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
BB-FCF	Brilliant Blue FCF
BB-G250	Brilliant Blue G250
BB-R250	Brilliant Blue R250
BSA	Bovine serum albumin
D	Doublet
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMSO	Dimethyl sulfoxide
ESI	Electronspray ionisation
G	Gram
GGB	Guinea Green B
HRMS	High resolution mass spectrometry
IR	Infrared spectroscopy
LC-MS	liquid chromatography mass spectrometry
M	Multiplet
M	Molar
m/z	Mass over charge
Mg	Milligram
mL	Milliliter
mM	Millimolar
mmol	Millimol
Ms	Millisecond
NMR	Nuclear magnetic resonance
pH	Potential hydrogen
Ppm	Parts per million
PVDF	Polyvinylidene difluoride
Q	Quartet
S	Singlet
S_N2	Nucleophilic bimolecular substitution
T	Triplett
THF	Tetrahydrofuran
TLC	Thin layer chromotography
UV	Ultraviolet
V-200	Coomassie Violet R200
XEH	Xyloglucan endohydrolase
XET	Xyloglucan endotransglucosylase
XGO	Xyloglucan oligosaccharide
XTH	Xyloglucan endotranshydrolase
Å	Angstrom

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1 Introduction

About 20 families in the plant kingdom are parasitic plants which consists of about 3000-5000 different species (Aly and Plant 2007). One of these families is called Cuscutaceae and consists of about 200 different species (Kaiser, Vogg et al. 2015). In this family a genus of parasitic plants called *Cuscuta* is a growing problem in agriculture resulting in huge financial losses and crop yields (Marambe, Wijesundara et al. 2002, Yoshida, Cui et al. 2016). However, a recent study performed at Artic and Marine Biology at UIT shows that by coating the species *Cuscuta Refleksa* whit Coomassie Brilliant Blue R250 (see figure 1), the amount of XET in haustoria penetrating the host plants was reduced and 1/3 of the haustoria produced by the parasite was not able penetrate the coated part of the host plant (Olson and Krause 2017).

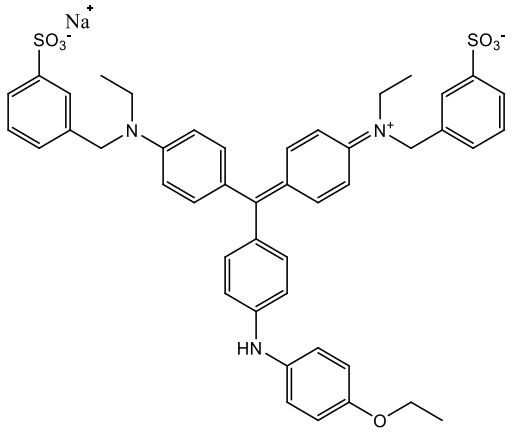


Figure 1: Overall chemical structure of Brilliant Blue R250.

2 Background

2.1 Cuscuta reflexa

Cuscuta reflexa is a parasitic plant that is common in Asia and most widespread in India (Aly and Plant 2007). The *Cuscuta* is known to be one of the fastest growing parasites and have evolved a lifestyle that requires it to feed water and nutrients from a host plant to survive (Heide-Jørgensen 2013). Due to their parasitic lifestyle they have no roots and consist mostly of the stem that grows in spirals around the host plant (Johnsen, Striberny et al. 2015). The seedlings of the parasite are able to sense and direct growth towards the host plant. When contact with the host is achieved an specialised connection organ called haustorium grows, penetrates the cell wall and then enters the vascular system allowing them to leech water, carbohydrates and nutrients from the host (Hegenauer, Fürst et al. 2016, Yoshida, Cui et al. 2016).

Figure 2: The parasitic plant Cuscuta reflexa (photo: Dinesh Valke, Wikimedia Commons)

2.2 XTH function

XTH also known as xyloglucan endotranshydrolase is an group of enzymes that found in most plants and is thought to have an important role in constructing, breaking up and redesigning of the cell wall(Schröder, Atkinson et al. 1997, Hara, Yokoyama et al. 2014). This group of enzymes has two main functions, one of them being XET (xyloglucan endotransglucosylase) and the other being XEH (xyloglucan endohydrolase) (Chormova, Franková et al. 2015). When

active, the XET function splits one xyloglucan chain and then use the energy from the splitting to form a new xyloglucan chain (Fry 1997). The XET does this by using nonhydrolytic cleavage, meaning water is not needed to break up the xyloglucan chains (Eklöf and Brumer 2010). The XEH function is when xyloglucan is incubated without available XGO (Xyloglucan oligosaccharide), which produces new reducing termini (K C Rose, Braam et al. 2003).

2.3 Biological testing

2.3.1 Protein Binding

The binding of the compounds where put to the test by staining them on polyvinylidene difluoride membranes that have been spotted with bovine serum albumin and protein that had been extracted from the *Cuscuta reflexa*. The membranes were prepared using 5 µL BSA standards or *Cuscuta reflexa* extract that were spotted on membranes that was dipped in methanol and then equilibrated in water and then were allowed to dry. During the staining process the membranes with bound proteins were wetted in methanol an equilibrated in water one more time before they were stained for 5 minutes in 40% methanol/7% acetic acid with 300 µM of the compound that is to be tested. Destaining was performed using 50% methanol/7% acetic acid for 7 minutes, then the membranes were washed with water for a couple of minutes, then dried. The compounds abilities to bind protein was determined by the visibility of the color of the spots where the protein was spotted on the membranes. In other words the stronger color the better binding to the protein. This method can only be used to test ability to bind protein (Aaron, Sandra et al. 2017).

2.3.2 XET inhibition

The labeling of xyloglucan oligosaccharides with sulforhodamine was performed according to the methods described by Kosik and Frakas (Kosik and Farkas 2008). XET test papers that were coated with xyloglucan and sulforhodamine-labelled xyloglucan olgisaccharides was performed using the methods described by Fry in 1997 (Fry 1997). In order to test the compounds effect on the XET activity of *Cuscuta reflexa*, extracts of 0.1x with 10mM or 50mM compound solutions were added, before spotting the XET test papers or control papers with 3 µL at 4°C. the papers were then incubated at 21°C for 1 hour and then the background

fluorescence was the carefully washed away using moderate agitation in ethanol: formic acid: water (1:1:1) for 2 hours. Destained papers were washed in water and then pictures were taken of the dry papers using a ChemiDoc Mp Imaging System (Bio-Rad) by using a Cy3 application with 400 ms exposure time. If the spot has a strong fluorescent signal the XET activity is high and lower if the opposite is true. Then the global background-adjusted volumes of the fluorecent spots were calculatet with the Image Lab software.

2.4 Triphenylmethane derivatives

Triphenylmethanes, also called triaryl derivatives, is a group of molecules commonly used in the industry as dyes (Ajaikumar and Pandurangan 2008) and as a way to treat infection in pisciculture (López-Gutiérrez, Romero-González et al. 2013). They have also shown positive biological results in antimicrobial and nervous system disorders (Mondal and Panda 2014). A common synthesis for these compounds involves a condensation reaction using aromatic aldehydes and aniline derivatives (Pasha and Nagashree April 2013). The overall structure of a triphenylmethane derivative is shown in figure 3.

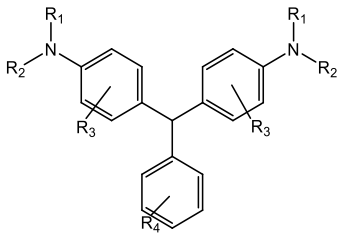


Figure 3: General structure of a triaryl system where R_{1-4} can be any alkyl, vinyl group or other functional groups.

3 Results and Discussion

This part of the thesis describes the work and the ideas towards the synthesis of Brilliant Blue R250 derivatives. The synthesized molecules were sent for screening of activity and the results are included in this part. The blue numbers in the headline for the reactions refferes to the obtained spectra in the appendix. Integration was done on the entire aromatic region and multiplet analysis was done peak by peak. All the synthesized compound turned out to be poorly soluble in most tested solvent. The poor solubility of the products resulted in column chromotography not being a very viable method for purification. This again resulted in difficulties purifying the obtained products and elucidating the obtained NMR spectra. As a result, excess protons were found in the aromatic region, from unreacted starting material and biproducts, when processing the spectra.

3.1 Functionalisation of Guinea Green B

In order to functionalise GGB a nitration reaction was chosen. The reason for this was to potentially increase the water solubility and activity of the compound and to se if it would be possible to controll regioselectivity of the the reaction. If the regioselectivity is controllable, it would give the possibility for subsequent reaction via NO_2 → NH_2 → N_2^+ → X where X can be Cl, Br or I. This would make it possible to substitute in any group of choice.

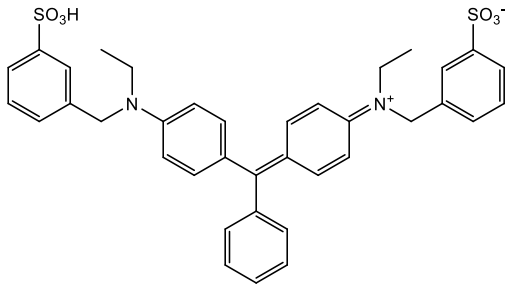
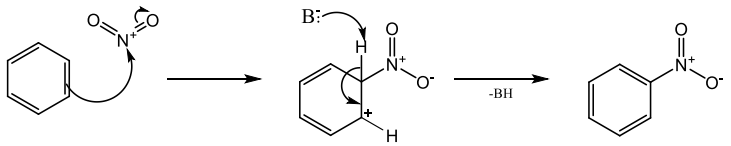


Figure 4: Chemical structure of GGB (Guinea Green B)

As a model compound GGB was chosen (see figure 4) due to it having the simplest structure of the comercially available molecules.

3.1.1 Nitration reactions

The nitration of an aromatic ring is the substitution of one hydrogen on the aromatic ring with a nitro group. These types of reactions are also called electrophilic aromatic substitution reactions. To perform the aromatic substitution a nitronium ion is needed. A traditional way is to use nitric acid in concentrated sulphuric acid to generate NO_2^+ (Ege 1999). The nitronium is also commercially available as salts (NO_2BF_4 and NO_2PF_6).



Scheme 1: Mechanism of electrophilic aromatic substitution where B = any base

As shown in scheme 1 the mechanism for the aromatic substitution starts off with the aromaticity of the ring breaking up to form a positively charged intermediate. Then the acidic proton gets deprotonated by any weak base to regenerate the aromaticity of the ring. The mechanism of the nitration reaction can be found in any organic chemistry book for example Ege (Ege 1999).

3.1.1.1 Sulphuric acid and nitric acid (7.1.1)

The chosen starting point for the nitration of GGB was to use a mixture of sulphuric and nitric acid. This regent was chosen while waiting for the commercially bought regents to arrive. The reaction was run under neat conditions to see what would happen.

According to the recorded spectra, the desired product was not obtained. In the ^{1}H NMR there are some peaks of intrest. When integrating the quartet at 3.80 ppm to give 4 protons the triplet at 1.33 ppm does not integrate to the expected 6 protons. In the 7.00-7.90 ppm area all the peaks add upp to more then the expected 20 protons. This means that some impurities in the sample are likely contrebuting with excess protons to the integrals. In the ^{13}C NMR spectra low consentration of the sample resulted in no visible peaks. In the HRMS spectra the mass of the compound was not found. This means that it is possible to say that something has been produced, but it is most likely not the target molecule.

Due to the results shown in the recorded spectra, it is not possible to say if the target product had been produced. Since the ^{13}C NMR spectra and the HRMS specter doesn't provide any information of the product. For the ^{13}C NMR this is most likely due to low solubility of the product resulting in low concentration of the NMR sample. For the HRMS, the product may not undergo ionization and therefore may not being detected, but based on the HRMS spectra of similar compounds in this thesis they do ionize so therefore the desired product does most likely not exist in the mixture. When adding the acid to the mixture a change of collor from very dark green to brown was observed. This could be due to very acidic conditions or fast addition of the regent resulting in increased temprature and the formation of the thermodynamic product. A sugestion to this problem would be to either find a new regent or lower the temprature.

3.1.1.2 Nitronium tetrafluoroborate in sulfolane (7.1.2)

Due to the bad results of the previous synthesis another approach would be needed. Therefore, nitronium tetrafluoroborate was chosen to avoid acidic conditions. Sulfolane was also chosen

solvent due to nitronium tetrafluoroborate not reacting with the solvent. The reaction temperature was kept the same as in **3.1.1.1** to compare under similar conditions.

The obtained spectra showed nothing to indicate that the wanted product had been produced. In the ^{1}H NMR there are some peaks that does not match with the target molecule. Due to poor solubility of the product only one peak at 48.8 ppm showed in the ^{13}C NMR spectra. This peak is most likely the result of some impurity in the sample due to the strong signal and no other peaks were visible. The mass of the target molecule was not detected in the HRMS. This means the target molecule most likely has not been produced.

When trying to nitrate using the nitronium tetrafluoroborate interesting results were obtained. In this reaction the obtained product was attempted purifying using column chromatography. First TLC was used to find an appropriate eluent, then it was used to monitor the progress of the column chromatography. Separation using column chromatography proved hard due to many different products had been produced (se figure 5).

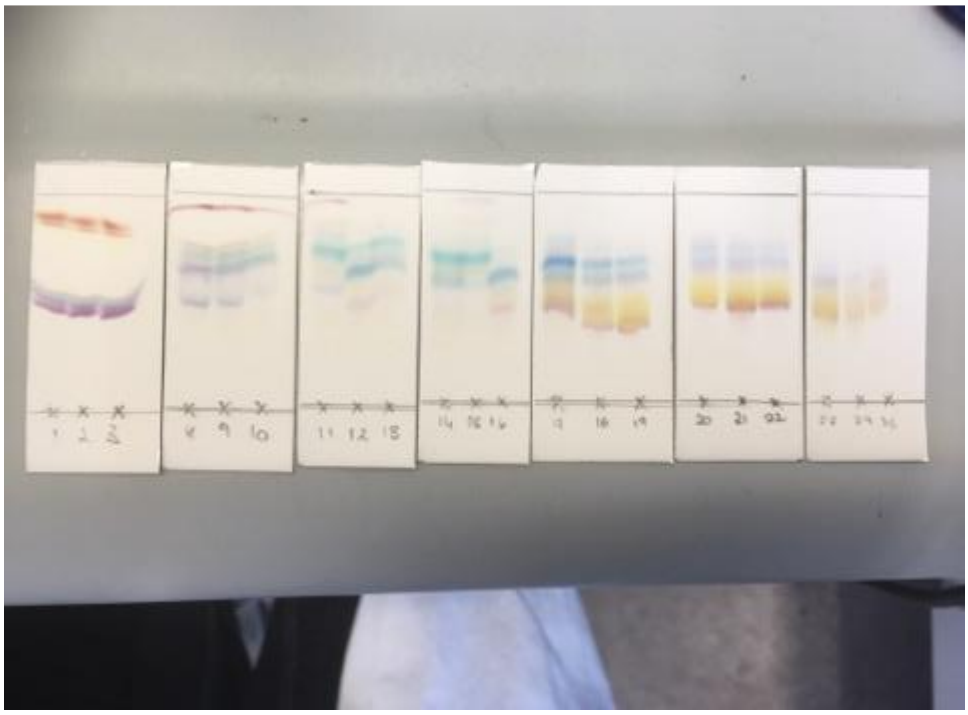


Figure 5: TLC monitoring of the column chromatography

The reason for the separation shown in figure 5 may be that the GGB (see figure 4) have many possible sites where a nitration can occur. This will result in multiple different regioisomeric products thus making it very hard to purify using traditional column chromatography. The reaction conditions in this synthesis were based on Zieger and Lees nitration with nitronium tetrafluoroborate in sulfolane (Zieger and Lee 1990). The high boiling point of sulfolane (285 °C) makes it hard to fully remove from the mixture (Yu, He et al. 2001). Therefore, lower boiling point solvents were tested (methanol, chloroform, DCM and diethyl ether), but all the tested solvents reacted vigorously with the nitronium tetrafluoroborate and was therefore not optimal. Based on the recorded spectra it's not possible to say if the desired product has been produced, but it is definitely possible to say that there have been produced several different products based on the TLC plates from figure 5.

3.1.1.3 Nitronium hexafluorophosphate (7.1.3)

After another failiure to nitrate GGB nitronium hexafluorophosphate was tested due to it not reacting with DCM (Olah, Ramaiah et al. 1997). This gives the opportunity to not use sulfolane as solvent effectively eliminating a problem from the last synthesis. The temperature of the reaction was changed to -78 °C to test if it would yield fewer regioisomers.

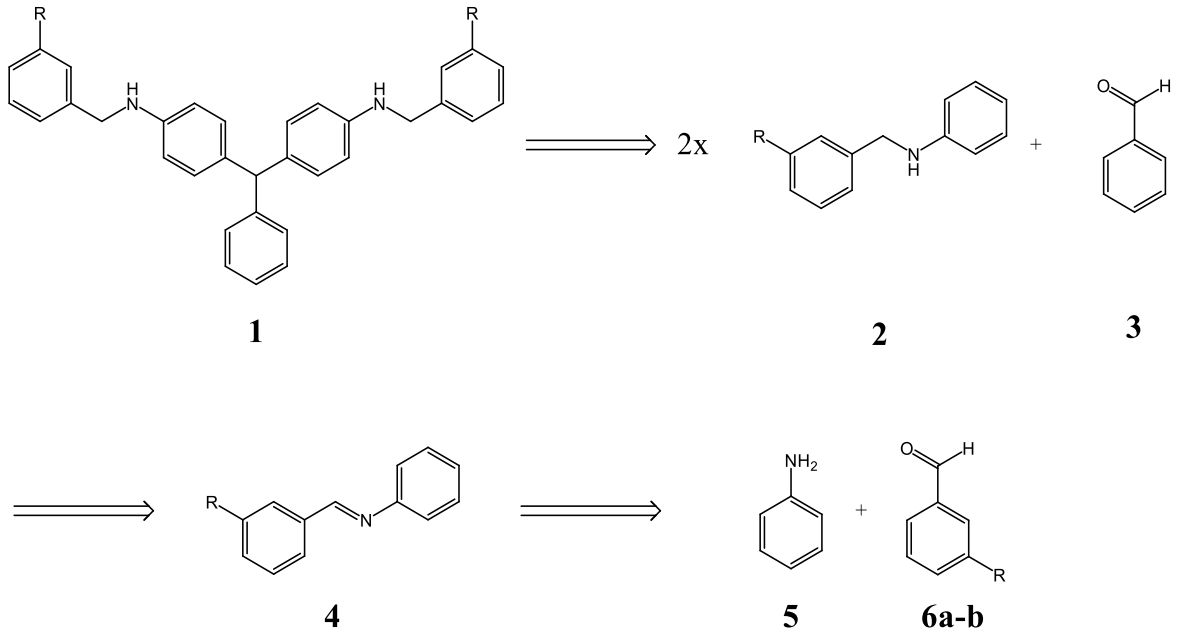
The obtained spectra didn't show any peaks that might belong to the expected product. The ^{1}H NMR shows nothing other than the solvent and one peak at 3.37 ppm. This peak does most likely not belong to the target molecule. For the ^{13}C NMR nothing other then the solvent peaks are visible. The mass of the wanted product was not detected in the HRMS spectra.

This nitration was done a little different than the others. Where in this the nitronium hexafluorophosphate was dissolved in DCM and then the GGB was disolved in DCM and slowly added at -78°C. This was due to the DCM barely dissolving the nitronium hexafluorophosphate. In theory this shuld result in fewer regioisomers being formed. This ended up not being the case, the obtained results were about the same as using nitronium tetrafluoroborate. A possible solution to this could be to lovering the temprature even more using liquid nitrogen. After another failed reaction it was decided that the nitration of GGB

would be abandoned and two new retrosynthesises were proposed. This was due to the product proving hard to purify and time would be better spent working on something else.

3.1.2 Synthesis of Brilliant Blue R250 derivatives

In order to synthesize multiple derivatives of Brilliant Blue a strategic retrosynthesis was developed as shown in scheme 2.

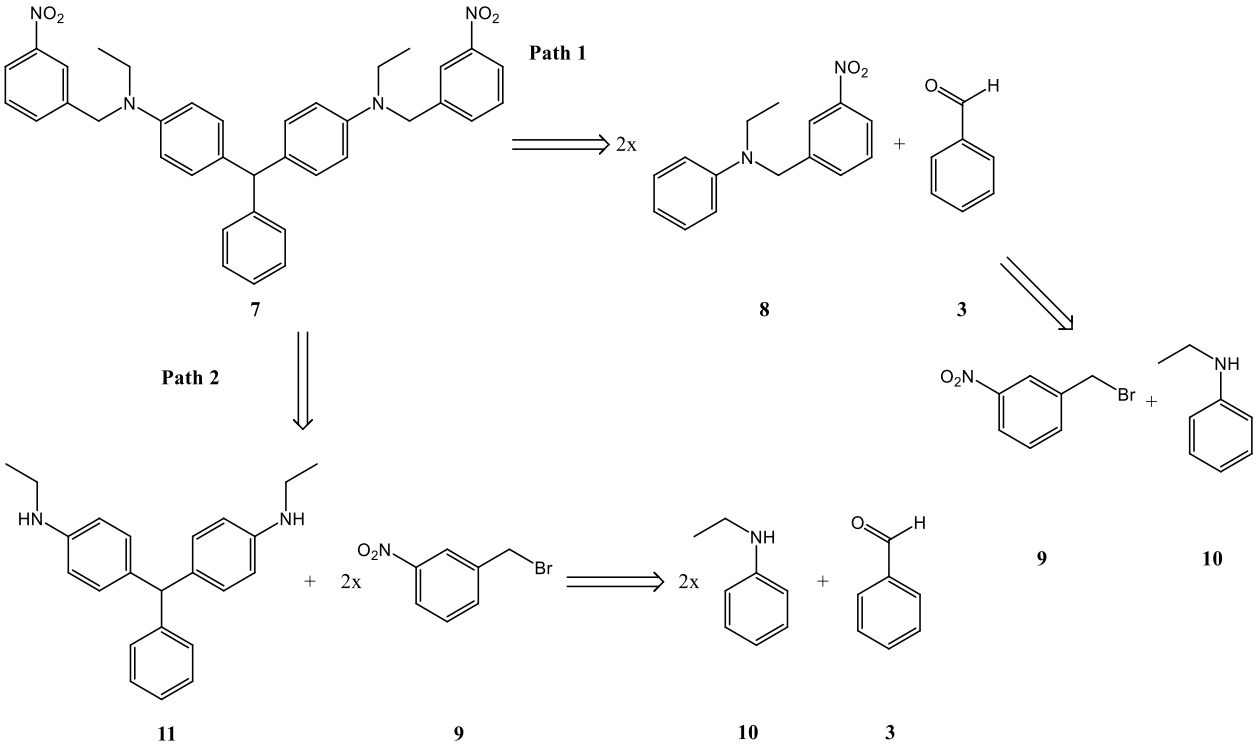


Scheme 2: Retrosynthesis of Brilliant Blue R250 derivatives R = NO_2 and SO_3H

In the first part of the retrosynthesis the compound is separated in to tre parts. These consist of one benzaldehyde (**3)** and two *N*-benzylaniline(**2)** derivatives where R = SO_3H, NO_2 or PO_3H_2. In order to achieve this in the forward direction, a condensation of the aldehyde to the benzylaniline was planned (Ajaikumar and Pandurangan 2008, Wang and Wang 2009). Moving on with disconnection of **2** gives **4**. The planned approach for this synthesis is using a traditional sodium borohydride reduction reaction (Lopez, de la Cruz et al. 2017). Disconnection of **4** gives aniline (**5)** and a *meta* benzaldehyde(**6a-b**). This step can be performed in two ways, either by using a Dean-Stark apparatus whit toluene as solvent to create an azeotrop that boils at 85°C (Dibble, Kurakake et al. 2018, May 2006). The other method that was the preferred method of choice was using molecular sieves to trap water and push the equilibrium towards the imine (Paniagua, Yadava et al. Feb-April 2018).

3.1.3 Synthesis of nitro substituted Brilliant Blue R250 derivatives using nucleophilic substitution

An attempt to make nitro substituted Brilliant Blue derivatives using nucleophilic substitution will also be sugested. There are two different retrosynthetic proposals, both shown in Scheme 3.



Scheme 3: The two possible retrosynthetic paths

The first disconnection in the **path 1** direction happens on the middle carbon and gives benzaldehyde (**3**) and two parts of *N*-ethyl-*N*-(3-nitrobenzyl)aniline (**8**). To form the nitro substituted Brilliant Blue derivative from **8** and **3** a triaryl condensation may be the most optimal strategy (Ajaikumar and Pandurangan 2008). Further disconnection of **8** reveals 1-(bromomethyl)-3-nitrobenzene (**9**) and *N*-ethylaniline (**10**). To perform the transformation from **9** and **10** to **8** a standard S_N2 substitution reaction was suggested (Ege 1999). In **path 2** the sugested disconnection of the final product reveals 4,4'-(phenylmethylene)bis(*N*-ethylaniline) (**11**) and 1-(bromomethyl)-3-nitrobenzene (**9**). In order to do this transformation a standard S_N2 substitution was sugested (Ege 1999). Further decomposition of 4,4'-(phenylmethylene)bis(*N*-ethylaniline) (**11**) gives the compounds *N*-ethylaniline (**10**) and benzaldehyde (**3**). The suggested approach to this could be to do a triaryl condensation reaction with **10** and **3** (Ajaikumar and Pandurangan 2008).

3.2 Synthesis of building blocks

In order to synthesise nitro Brilliant Blue derivatives, the basic building blocks as shown in figure 6 had to be made. The reason for synthesizing these molecules was to use the as precursors for further synthesis. This would potentially give an interesting molecule for biological testing. Due to the sulfonate groups not being present and what effect this may have on activity and solubility.

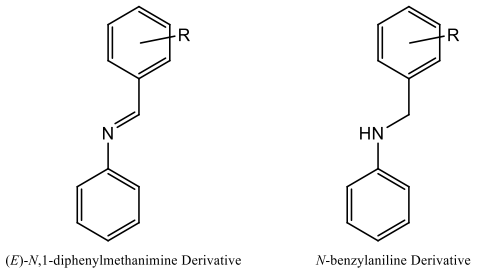
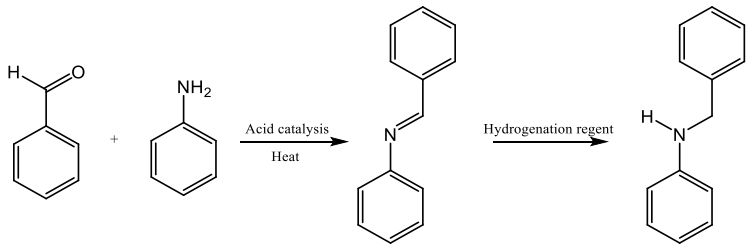


Figure 6: Shows the structure of the imines and benzylanilines R = Br and NO_2

In order to synthesise nitro and phosphonic Brilliant Blue derivatives some *N*-benzylaniline derivatives had to be made for further synthesis. These compounds were not commercially available and therefore had to be synthesized via an imine (see figure 6). The sulfonic *N*-benzylaniline was not synthesized due to the sulfonic benzaldehydes being very expensive compared to other starting materiales.

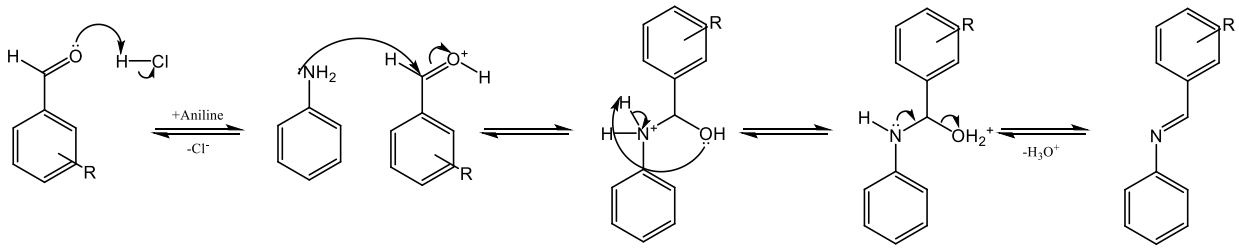
3.2.1 Reductive amination

Reductive amination is a simple method where an aldehyde and an amine react together in two steps to form a secondary or tertiary amine. In order to do a reductive amination a ketone and an amine will need to react and form an imine followed by hydrogenation. A standard reductive amination reaction is shown in Scheme 4 (Carey. and Giuliano. 2017).



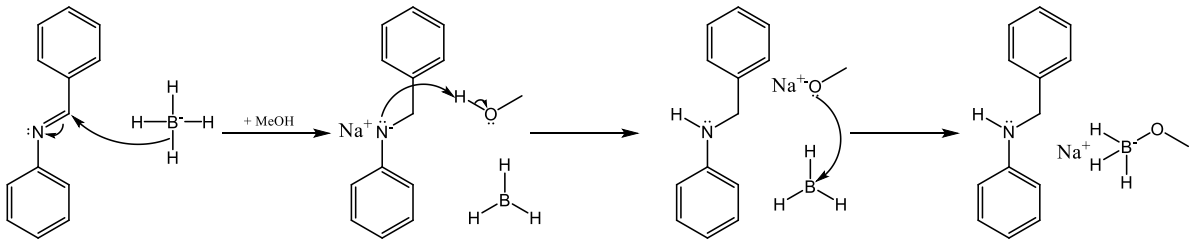
Scheme 4: Standard reductive amination reaction steps

Imines (also called Schiff bases) are relatively easily formed using an acid catalyzed condensation reaction (March. and Smith. 2007). As shown in Scheme 5 the acid starts off by protonating the oxygen activating the carbonyl for nucleophilic attack from aniline. The nucleophilic attack happens and the electrons on the carbonyl moves up to the oxygen to form a hydroxyl group. The deprotonation of the amine is likely to occur through the solvent, but is simplified in to a one step mechanism. Then a electronic push from the lonepair on the amine makes the water leave to form the imine (Lowry. and Richardson. 1976, Carey. and Giuliano. 2017).



Scheme 5: Imine formation mechanism (R = NO_2, SO_3H or Br)

The second step of the reductive amination is to reduce the imine to an amine. This can be done using metal catalysts for example iridium catalyst and hydrogen gas (Sui, Mao et al. 2017). Another option could be to slowly add sodium borohydryde while stirring. In this synthesis, sodium borohydride may be the perfect reducing agent due to it being a mild reducing agent (Carey. and Giuliano. 2017). This means that the risk of a reduction also happening at the R group is not that likely. For the reduction of the imine the proposed mechanism is shown in Scheme 6.



Scheme 6: Imine reduction mechanism

At the start of the reaction there is a hydride transfer from the borohydride to the electrophilic carbon of the imine. Then two of the electrones that help form the imine bonding moves on to the nitrogen giving it a negative charge that is stabilized by the positively charged sodium from the borohydride. Then the nucleophilic nitrogen attacks methanol to form the amine and the

methoxy salt. Then the methoxy attack the empty orbital on the borane preparing it for more reduction (Ege 1999).

3.2.1.1 Nitration of *N*-benzyl-*N*-ethylaniline using nitronium hexafluorophosphate (7.1.4)

This nitration reaction was preformed in an atempt to make a potential starting material for a triaryl condensation with benzaldehyde. Nitronium hexafluorophosphate was the regent of choice due to it being slightly soluble in DCM and to avoid the use of sulfolane. The reaction temprature was -78°C to test if it would be possible to get one regioisomer. C18 TLC plates were used to find a suitable eluent for the reverse phase column chromatography.

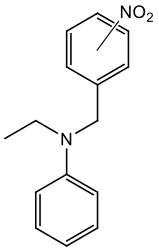


Figure 7: Structure of target molecule

The ^{1}H NMR spectra showed something that looked like two of everything. For example, the double triplet at 1.12-1.16 ppm and dubble quartet at 3.27-3.54 ppm. This is not a splitting or a result of two rotameres, but rather two different products. All these peaks gave a reason to belive that two different products may have been formed. The obtained ^{13}C NMR spectra of the nitration of *N*-benzyl-*N*-ethylaniline showed only the solvent peaks. In order to figure out if the peaks in the ^{1}H NMR was a result of splitting, rotameres or two product a LC-MS was used. The obtained LC-MS spectra showed the mass of the target compound and two peaks meaning there are two different regioisomers in the NMR sample. Another LC-MS scan also revealed a third product with two nitro groups substituted to *N*-benzyl-*N*-ethylaniline (see figure 8). The mass of the product was also detected using HRMS and the elemental composition of the target molecule was calculated.

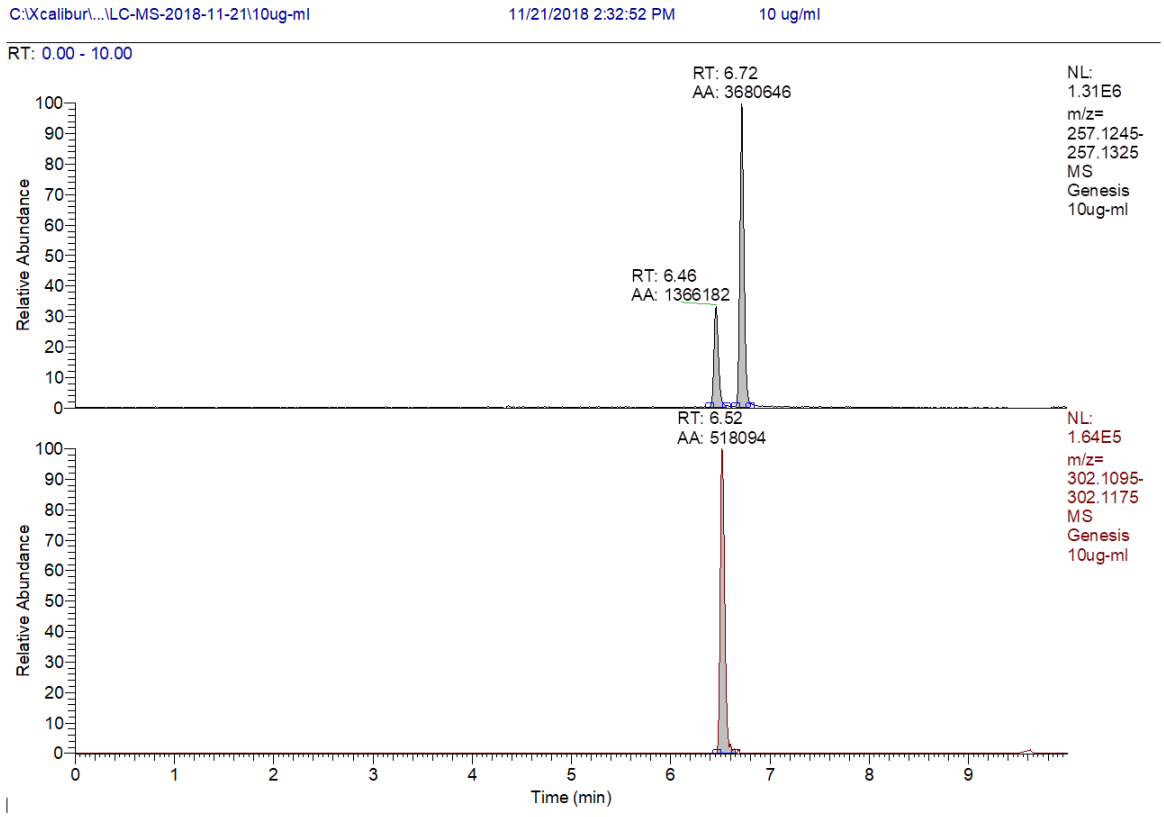


Figure 8: LC-MS spectra from the nitration of N-benzyl-N-ethylaniline using nitronium hexafluorophosphate

During the nitration of *N*-benzyl-*N*-ethylaniline the goal was to make *N*-ethyl-*N*-(3-nitrobenzyl)aniline that could be used in a triaryl condensation reaction with benzaldehyde to form a nitrated Brilliant Blue derivative. After reverse phase column chromatography, the obtained fractions only showed one spot on the C18 TLC plate. However, in the recorded spectra it is possible to see that two different regioisomers have been formed.

LC-MS also revealed a third product which is the double nitrated *N*-benzyl-*N*-ethylaniline. The nitration most likely happened on the most activated aromatic ring of the *N*-benzyl-*N*-ethylaniline. This is the aromatic ring that is connected to the nitrogen. If the nitration took place in the *para* position of this ring any further triaryl condensation reaction would not be possible due to nitro group blocking the position. Furthermore, the nitration of *N*-benzyl-*N*-ethylaniline was abandoned due to the nitro group most likely not substituting to the wanted phenyl ring and which makes any further reaction not possible if substituted to the *para* position. Another reason for abandoning is due to the low purity of the product, difficulties with purifying and the low yield.

3.2.1.2 Synthesis of (*E*)-1-(3-nitrophenyl)-*N*-phenylmethanimine (7.2.1.1)

Moleclar sieves and Dean-Stark apparatus was not needed for this synthesis due to the product formed easily without the removal of water. The reason for choosing hydrochloric acid as a catalyst was because it's a strong acid and it should easily activate the carbonyl of the benzaldehyde. Cold methanol was used to wash away unreacted starting material. This reaction was performed two times to get more product for further synthesis.

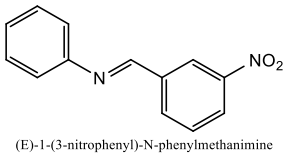


Figure 9: Structure of (E)-1-(3-nitrophenyl)-N-phenylmethanimine

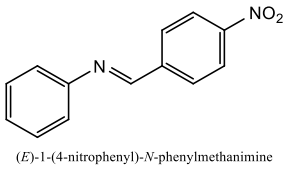
The ^{1}H NMR spectra of this reaction looks very clean except for some small peaks in the base line, that most likely is some traces of startingmatereal, as seen from the peak at 10 ppm being an aldehyde proton (Lampman, Pavia et al. 2010). The integrals in the proton spectra also look nice and the number of protons add upp to the target product. In the ^{13}C NMR spectra it is also possible to spot some starting material on the baseline. The peak at 190 ppm further support unreacted starting material due to it being a carbonyl shift (Lampman, Pavia et al. 2010). Other than that, 11 main peaks are to be expected due to symmetry in the product (Reich 2018). The mass of the target product was also found in the HRMS spectra and the elemental composition was calculated.

For the synthetic approach for the imine the recrystallization step was skipped due to the product being relatively pure. If the product would need further purification it will be enough to wash it with cold methanol. When the imine is forming there is also a possibility to form two different configurational isomers. This does not really matter due to later hydrogenation of both isomers gives will the same product.

3.2.1.3 Synthesis of (*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine (7.2.1.2)

Due to the product formed easily without the removal of water the previous molecular sieves and Dean-Stark aparatus was skipped. Heating the mixture was done to see if it could potenitaly

increase the reaction speed. The reason for doing a big batch in this synthesis was to make enough product to last for the entire lab work. The reaction was left over night to be efficient.



(*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine

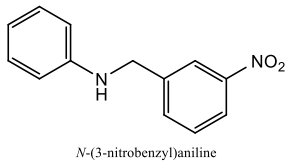
Figure 10: Structure of of (E)-1-(4-nitrophenyl)-N-phenylmethanimine

The obtained spectra for the synthesis of (*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine all looked good. In ^{1}H NMR spectra there are no noice or impurities on the base line, meaning the product have a high purity. It is possible to see the *para* splitting in the spectra that indicates an aromatic ring with two groups *para* to eachother (Lampman, Pavia et al. 2010). Integrating all the peaks gives 10 protons which corresponds to the number of protons in the target product. In the ^{13}C NMR spectra 10 different carbons were recorded. This is due to symmetry in the two aromatic rings making some carbons identical and therefore gives the same signal in the spectra (Reich 2018). Further to the HRMS spectra the mass of the wanted product is present and the elemental composition of the molecule was successfully calculated.

In this synthesis a different approach was used to make the imine. This approach involved changing the solvent to DCM and heating with refluxing. This was done to potentially increase the yield. As shown in the experimental part this did not happen. The reason for this is probably not due to the increase in temperature, but rather to much methanol being used effectively washing away the product. Removal of water was not needed due to the imine seemed to form easily.

3.2.1.4 Synthesis of *N*-(3-nitrobenzyl)aniline (7.2.3.1)

This reaction was done twice to get enough *N*-(3-nitrobenzyl)aniline for further synthesis. A two neck roud bottom flask was used to easily add sodium borohydride to the solution. Borohydride was slowly added until the reaction was complete. Other solvent was testet (THF and DCM), but was not as efficient as methanol. The reaction was monitored using TLC to determine when the reaction was completed. The reason for adding water was to quench leftover hydride and make use for extraction with ethyl acetate.



N-(3-nitrobenzyl)aniline

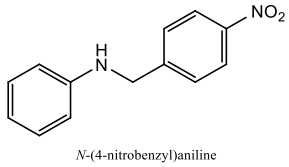
Figure 11: Structure of N-(3-nitrobenzyl)aniline

The obtained spectra for the synthesis of *N*-(3-nitrobenzyl)aniline everything looked good. In the ^{1}H NMR spectra all the expected peaks are present, and intergating them gives a total of 12 protons indicating the right product had been produced. The sample seems relatively pure except for a minor peak in the base line, most likely from either unreacted starting material or biproducts formed during the reaction. In the ^{13}C NMR spectra there are 11 signals due to symmetry in the final product (Reich 2018). There are 10 aromatic carbons in the 110-150 ppm region and 1 carbon found at 47.6 ppm suporting a carbon connected to a nitrogen (Lampman, Pavia et al. 2010). The HRMS managed to find the mass for wanted product and the expected elemental composition was calculated.

During the workup of the product only one extraction was performed due to further extraction didn't yield any more product. When looking at the peaks in the sample from 1-4 ppm it's possible to see a triplet, quartet and a singlet. These peaks are leftover ethyl acetate from the extraction of the product (SDBS 1999). During the reduction of the imine, borohydride was added until the reaction was completed and was monitored using TLC.

3.2.1.5 Synthesis of *N*-(4-nitrobenzyl)aniline (7.2.3.2)

Due to the high purity of the previous reaction, this reduction was carried out the same way as in chapter **3.2.1.4**.



N-(4-nitrobenzyl)aniline

Figure 12: Structure of N-(4-nitrobenzyl)aniline

In the obtained ^{1}H NMR spectra all the integrals look good and integration of the peaks gives a total of 11 protons. The proton that belongs to the nitrogen is not present in the spectra due to

the choice of solvent being methanol-*4d*. When looking at the IR spectra it show a N-H stretch at 3417 cm^{-1} supporting that the proton exists in molecule (Lampman, Pavia et al. 2010). The base line of the ^{1}H NMR spectra seems to have no noise and some impurities that is most likely unreacted starting material. However, there are some peaks in 1-4 ppm that are not supposed to be there. These peaks are as earlier mentioned leftover ethyl acetate from the extraction step (SDBS 1999). In the ^{13}C NMR spectra all the expected peaks are present. However, some peaks are leftover ethyl acetate. This leaves 9 peaks which fits well since there are some symmetry in the target compound where carbons with the same surroundings give the same signals (Reich 2018). The HRMS detected the mass of the wanted product and managed to calculate the expected elemental composition of the target molecule.

In this reaction there are some similarities from the previous reaction (**3.2.1.4**). There is some unreacted starting material in the sample which easily could have been removed with further purification. Further purifcations was not done due to the product already having a high purity. The leftover ethyl acetate could potentially be removed using a high vacuum pump overnight. Other than that, the synthesis seemed to have been a success and the obtained spectra look nice and the target compound have been obtained.

3.2.1.6 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline using iminium pathway (7.2.5.1)

This reaction was an attempt to get a version of the precursor as a tertiary amine. Molecular sieves were used for the removal of water and sodium borohydride was the reducing agent of choice due to it being a mild agent. Water was used to quench leftover hydride in the mixture. only one extraction was needed due to more not resulting in higher yield.

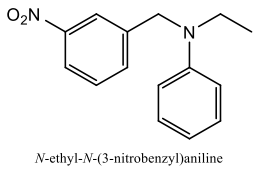


Figure 13: Structure of N-ethyl-N-(3-nitrobenzyl)aniline

In this synthesis at first glance the obtained spectra look promising. However, a closer look at the obtained spectra proved otherwise. The first suspicion started when looking at the recorded

HRMS spectra. The expected mass of the product was not found in the HRMS. There are severaø possible reasons why this can happen. The sample may not be concentrated enough for the HRMS, but this is less likely. When looking at the ^{1}H NMR spectra all the integrals seemed to be in order, which didn't make any sense considering the product not being found using the HRMS. In order to decide if the product had been produced a ^{1}H NMR spectrum of both the starting materials was recorded and stacked with the product (see figure 14).

A closer look at figure 14 reveals that the N-ethylaniline had not reacted with the nitrobenzaldehyde to form the iminium ion, but rather the 3-nitrobenzaldehyde have been reduced by the sodium borohydride to form (3-nitrophenyl)methanol. This also explains why the wanted product is nowhere to be seen in the mass spectra. This also means that the ^{13}C NMR does not show the shifts of the target molecule.

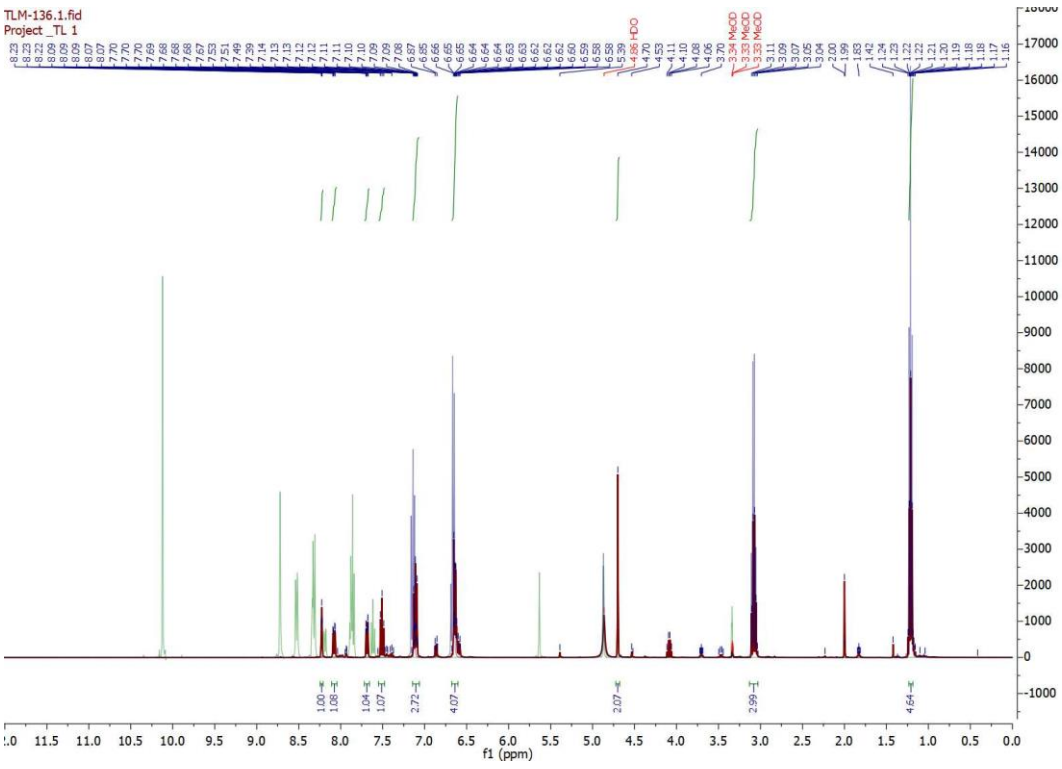


Figure 14: The stacked spectra of the recorded product (Red), 3-Nitrobenzaldehyde (Green) and N-ethylaniline (Blue).

Regarding the overall synthesis of the product, improvements could have been made. Longer reaction time to help the formation of the iminium before hydrogenation may have been an

improvement. It would also be possible to use drier solvent to push the equilibrium towards the iminium. The reaction could also have been run under inert gas to keep the water in the atmosphere from entering the system. Molecular sieves would still be in use to remove water produced in the reaction. Other counter ions for the iminium cation could also have been explored like Cl^- or SO_4^{2-}.

3.2.1.7 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline using S_N2 reaction (7.2.5.2)

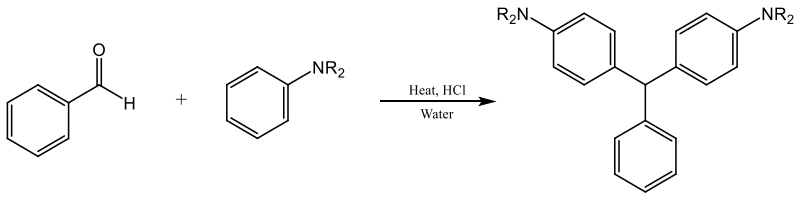
Due to not being able to obtain *N*-ethyl-*N*-(3-nitrobenzyl)aniline in the previous reaction a different approach was needed. The sodium carbonate solution was used to neutralise the acid forming during the reaction. The long reaction time was chosen to be sure everything had converted.

For this synthesis the obtained ^1H NMR spectra looks good all the peaks look nice and when integrated adds upp to 16 protons. The base line of the spectra looks good indicating a high purity of the product and the integrals all seem to be in order. The ^{13}C NMR spectra also looks relatively clean even though there seem to be a few impurities in the NMR sample. for the HRMS the mass of the target product was found, and the machine managed to calculate the elemental composition of the product.

Combined, the target molecule seems to have been obtained and the spectra indicate high purity. Therefore, no purification was needed for subsequent synthesis.

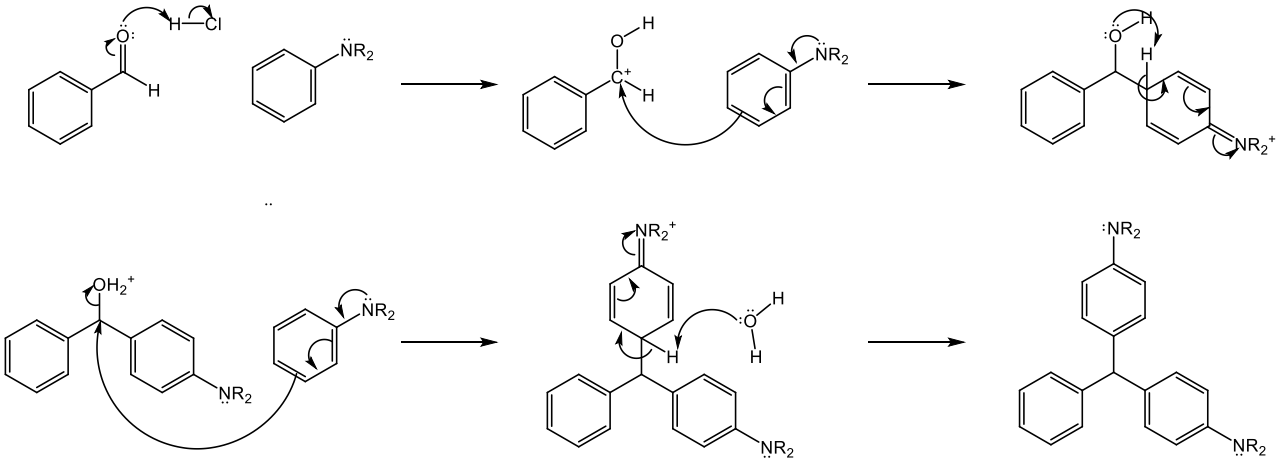
3.2.2 Triaryl condensation

The triaryl condensation is when a benzaldehyde and two primary or secondary aniline derivatives reacts under strongly acidic conditions to form a triaryl methane compound as shown in Scheme 7 (Weisz, Milstein et al. 2007, Nusser, Geiger et al. 2010).



Scheme 7: Standard triaryl condensation R = alkyl substituent

The tertiary amine can also undergo triaryl condensation where strongly acidic environment is not needed due to it being impossible to form an iminium ion.

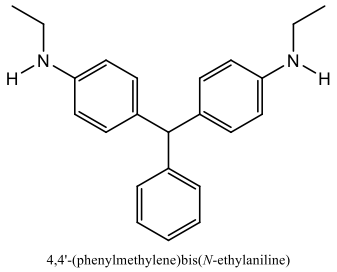


Scheme 8: Triaryl condensation mechanism R = alkyl substituent

As shown in Scheme 8 the triaryl condensation starts with the protonation of the aldehyde making the carbonyl more electrophilic. Then the free electron pair on the amine moves down and pushes the aromatic electrons to activate the *para* position and a nucleophilic attack happens on the carbonyl carbon. Then the alcohol electrons attack the acidic proton to regain the aromaticity of the ring. Another amine comes inn and the electrons on the nitrogen moves down, activates the *para* position in the amine and preformes a nucleophilic attack on the central carbon resulting in water leaving. The aromatic ring is then regained by water deprotonating the acidic proton, resulting in the triarylic system being formed (Ajaikumar and Pandurangan 2008, Pasha and Nagashree April 2013).

3.2.2.1 Synthesis of 4,4'-(phenylmethylene)bis(*N*-ethylaniline) (7.2.4)

This synthesis of 4,4'-(phenylmethylene)bis(*N*-ethylaniline) was preformed to test if it would be possible to use in a S_N2 reaction to form nitro Brilliant Blue derivatives (see scheme 3).



4,4'-(phenylmethylene)bis(*N*-ethylaniline)

Figure 15: Structure of 4,4'-(phenylmethylene)bis(N-ethylaniline)

Highly acidic conditions with water as solvent was used due to no rection after 1 hour of stirring with toluene as the chosen solvent. The long reaction time was chosen to be certain the reactants would convert to the product. Sodium carbonate was used to neutralise the acidic mixture and crush the product out of solution as it proved easier to work with then sodium hydrogen carbonate. The reason for washing the product with plenty of water was to remove excess sodium carbonate.

When analysing the obtained ^{1}H NMR spectra an interesting pattern comes to light. There are two triplets and quartets in the 1.00-3.50 ppm region. This is most likely not due to the peak splitting, but rather due to there being two different stable rotameres of the product. this means if the energy needed to rotate the molecule is high, there will be blocked rotation and the molecule may occur as two different rotational isomers (Eliel, Wilen et al. 1994). In this case this is caused by the two phenyl rings blocking the free rotation close to the central carbon (see figure 16).

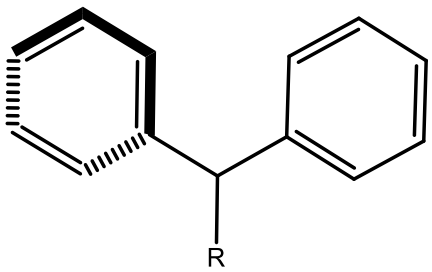


Figure 16: Chemical structure where the bold line goes out of the plane and the dashed line goes into the plane R = alkyle or vinyl group

Other than that, the integrals seem to be in order, but there are two protons missing from the spectra. These are most likely from the aromatic ring due to aromatic protons typically appear in the 6.00-8.00 region (Lampman, Pavia et al. 2010). As of the missing protons it is not possible to conclude that they don't exist due to the other spectra mainly HRMS. In the ^{13}C NMR spectra there are 4 peaks in the 10-40 ppm area, these 4 peaks are most likely the ethyl groups on the two nitrogens. Other than that, the spectra look to have a few impurities and a total of 13 main peaks which fits due to symetri in the product (Reich 2018). For the HRMS spectra the total mass for the product was found and the correct elemental composition was calculated for the expexted product.

The workup of the product proved hard due to the choice of solvent being water under very acidic conditions du to low acidic conditions yielded no product. This problem was solved using sodium hydrogen carbonate but was later swapped for sodium carbonate for easier workup. A high vacuum pump was also utilized for drying over night. Other than that, the recorded spectra seem to indicate that the wanted product had been produced and can be used for further synthesis.

3.3 Synthesis of Brilliant Blue derivatives

In order to synthesise Brilliant Blue derivatives a triaryl condensation reaction was chosen. this reaction was the prefered method due to it forming the base structure of Brilliant Blue (see figure 3). This reaction also gives the opportunity to add various different substituents to the aromatic ring originating from the benzaldehyde. Multiple different benzaldehydes were used to test what effect the final product may have on activity and water solubility.

3.3.1 Attempt to Synthesise 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline) (7.2.6.1)

This reaction was running under highly acidic conditions due to low acid consentration yielded no product after 1 hour of stirring. sodium carbonate was used for neutralization due to it proving easier to work with then sodium hydrogen carbonate. Water was used to wash away excess sodium carbonate.

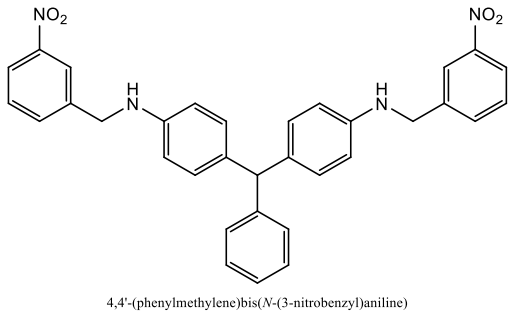


Figure 17: Structure of 4,4'-(phenylmethylene)bis(N-(3-nitrobenzyl)aniline)

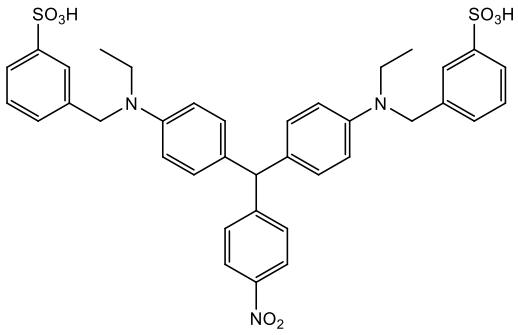
In the attempt to synthesise 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline) the spectra recorded gives reason to belive the target molecule exists in the sample. When looking at the ^{1}H NMR spectra the base line looks good with little to no noise. There are some peaks from 7.00-8.50 ppm, when integrating the peak at 5.66 ppm to 1 proton, these integrate to a total of 36 protons. This means there are some impurities contributing with excess protons. In the ^{13}C NMR spectra there are a few peaks in the 120-140ppm area which is most likely aromatic carbons other than that nothing of interest is visible (Lampman, Pavia et al. 2010). However, in the HRMS spectra the mass of the target product was detected and the HRMS managed to calculate the elemental composition of the wanted product. This means there are reason to belive there are traces of the target molecule in the sample.

When working with this synthesis acidic conditions were needed to get the reaction going. The conditions that proved most effective was water with added hydrochloric acid to get a pH roughly arround 1. This was done due to the HRMS not showing any product being formed after 1 hour of reaction time. The reason for the ^{13}C NMR spectra not showing anything of interest is most likely because of the sample not being concentrated enough due to poor solubility. Even if the product was not pure and only showed some traces of the target molecule

it was still sent for biological testing to see if there would be any effect. The nitro group was also chosen to see if other groups would bind to the enzyme.

3.3.2 Synthesis of 3,3'-(((((4-nitrophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.2)

4-nitrobenzaldehyde was chosen as startingmaterial for this reaction, to test the effect on water solubility and inhibitor activity. DMSO were the chosen solvent due to it could reach high temperatures without boiling. No acidic conditions were needed for this synthesis due to the product easily being produced without. Water was added to force the product out of solution. washing with water and ethyl acetate was done to remove leftover starting material and DMSO. Column chromatography was not used due to the product being poorly soluble.



3,3'-(((((4-nitrophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

Figure 18: Structure of 3,3'-(((((4-nitrophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

When looking at the obtained spectra there are some peaks of intrest. In the ^{1}H NMR spectra there seems to be a lot of leftover ethyl acetate from the workup even when left on a high vacuum pump over night to draw out any leftover solvent (SDBS 1999). Integrating the multiplet at 1.06 ppm to 6 protons gives 3 at 4.7 ppm and 1 5.73 ppm. The region at 6.50-8.50 ppm adds upp to 18 protons when integrated and 13 when doing multiplet analysis. This might be because of impurities in the sample meaning when integrating the peaks, they don't give the correct number of protons. In the ^{13}C NMR there is not much to see, mostly peaks corresponding to leftover ethyl acetate in the sample and a few peaks in the 120-150 ppm area that might be traces of the product (SDBS 1999). However, when looking at the HRMS spectra, the mass of the target product was detected. The HRMS also managed to calculate the elemental composition of the compound.

When working up the product there were two ways to force the product out of the DMSO solution. One of these was diluting it with water and the other was to dilute it with a mixture of 1:1 ethyl acetate and methanol. The mixture of ethyl acetate and methanol was chosen due to it being the easier solvent to dry after filtration of the product. When looking at the final spectra more vacuum pumping wound have been necessary to get rid of all the leftover ethyl acetate. Even if the product was not fully purified and the spectra didn't give much information, it was sent for biological testing.

3.3.3 Synthesis of 3,3'-(((((4-(dimethylamino)phenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.3)

4-(dimethylamino)benzaldehyde was chosen in an attempt to increase the inhibitor activity and water solubility of the product. The conditions were the same as the previous reaction. To force the product out of solution water was swapped for ethyl acetate and methanol solution because they are easier to dry. Washing with methanol and ethyl acetate was done to test if it could potentially increase the purity of the product. Column chromatography was not used due to the poor solubility of the product.

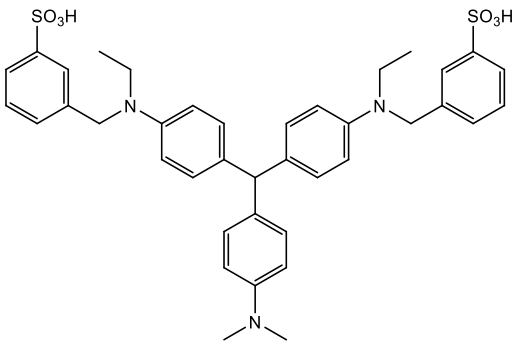


Figure 19: Structure of 3,3'-(((((4-(dimethylamino)phenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

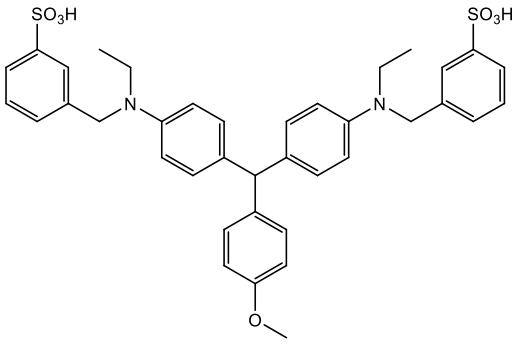
In this synthesis the spectra obtained show some peaks of interest. In ^{1}H NMR the base line looks clean and does not have much noise. Integrating the triplet at 1.06 ppm to 6 protons gives 4 protons for the quartet at 3.72 ppm. There are some aromatic signals in the 6.5-8.5 ppm area and these integrate to 18 protons when integrating and 15 when doing multiplet analysis peak by peak. It is also possible to see some leftover ethyl acetate from the workup of the product

(SDBS 1999). In the ^{13}C NMR spectra there are some peaks that might correspond to the target product, but it doesn't show all the peaks due to low consentration of the sample and poor solubility. When looking at the HRMS spectra the mass of the target product was found. The HRMS also managed to calculate the elemental composition of the target molecule. This means that the mass of the target product exists in the sample.

When working with the product the 4-(dimethylamino)benzaldehyde was chosen due to it having a polar nitrogen attached to the aromatic ring. This was done in hopes of making the product more water soluble. Ethyl acetate and methanol mixture was still the chosen mixture to force product out of the DMSO solution. Regardless of the spectra not giving much information to confirm the target product, some of it were sent for testing. The reasoning for this is that the spectra give some reason to think that the target product might have been obtained. This is based on some peaks in the ^{1}H NMR and the target mass from the HRMS. This means the molecule might exist in the sample and may give a positive biological effect.

3.3.4 Synthesis of 3,3'-(((((4-methoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.4)

The 4-methoxybenzaldehyde was chosen due to it having a polar group and was commercially available. The reaction conditions and the work up were the same as the previous reaction. Washing with cold methanol and ethyl acetate was done to potentially increase the purity.



3,3'-(((((4-methoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

Figure 20: Structure of 3,3'-(((((4-methoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

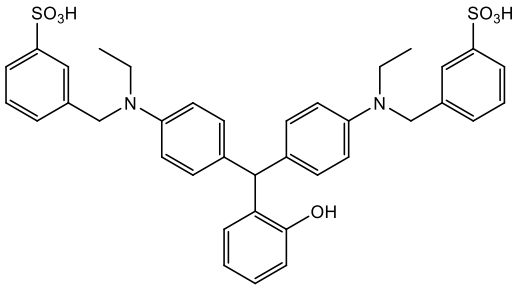
In the ^{1}H NMR spectra of this compound it is hard to tell if the wanted product had been produced. there are some peaks around 1.05 ppm that looks like it could be a triplet with some impurities, but are reported as a multiplet due to the possibility of the conformation having an impact on the measured signal (Eliel, Wilen et al. 1994). When integrating this peak to 6 protons, the other peaks they integrate to a total of 32 protons. Meaning a total of 6 protons is missing from the spectra. There should be a quartet in the spectra, but it is most likely hiding inside the broad water peak at 3.68-3.72 ppm. In the aromatic area from 6.50-8.00 ppm there are 2 protons in excess when integrating and 9 protons missing when doing multiplet analysis. This is most likely due to impurities in the sample making the integrals not add upp to the correct number of protons. For the ^{13}C NMR spectra there a few weak peaks that might be either the target molecule or impurities. In the HRMS spectra the target mass was found and the HRMS managed to calculate the elemental composition of the product. Meaning there are traces of something with the same mass and elemental composition as the target molecule in the obtained product.

When working up the product further purification could have been done to give a cleaner NMR spectrum. DMSO was the chosen solvent for the reaction due to it having a high boiling point making it possible heat the mixture to 120-130°C and easily dissolving the starting materials. When removing ethyl acetate from the product, vacuum was the preferred method of choice. Even if the spectra didn't give enough information to prove with certainty that the target molecule had been achived, biological testing was done. This was done due some peaks in the ^{1}H NMR fits well and the target mass and elemental composition was found in HRMS. This gave reason to belive the molecule had been produced and was therefore sent for testing.

3.3.5 Synthesis of 3,3'-(((((2-hydroxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.5)

The 2-hydroxybenzaldehyde was chosen to potentially increase the inhibitor activity and water solubility of the final product. The reaction conditions were not changed due to due to higher yield not being important for testing. Ethyl acetate and methanol was used to precipitate the

product out of solution. The product was washed with cold ethyl acetate and methanol to potentially increase the purity.



3,3'-(((((2-hydroxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

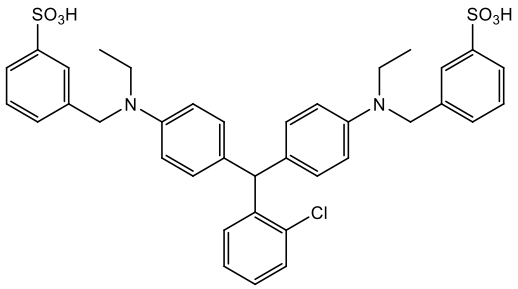
Figure 21: Structure of 3,3'-(((((2-hydroxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

In the ^{1}H NMR spectra there are existing peaks that looks like it can be the ethyl group of the target molecule. If this integrates to 6 protons the remaining peaks gives a total of 32 protons leaving only 3 protons for the two ethyl groups. This means there are impurities that gives excess protons when integrating the spectra. The leftover protons could be found in a quartet that is most likely hidden inside the broad water peak at 3.50-4.00 ppm. Doing a multiplet analysis the protons in the 6.50-8.00 ppm area gives 12 protons when the other peaks are integrated according to the target compound. This may be due to some impurities in the peaks that give incorrect amounts of protons when performing the analysis. In the ^{13}C NMR spectra there are some small peaks visible in the base line. This is most likely due to low concentration of the NMR sample and poor solubility. When looking at the HRMS spectra the target mass was found, and the elemental composition of the product was calculated. This combined with some peaks in the ^{1}H NMR spectra gives reason to believe the target product exist in the sample and was therefore sent for biological testing.

When working with this compound it seemed to rapidly oxidize or decompose when left in contact with air. To solve this problem the product was stored under argon as fast as possible after drying with high vacuum pump. Due to excess protons in the ^{1}H NMR spectra further purification of the product would have been needed to get a cleaner spectrum. For the ^{13}C NMR spectra a higher concentration of the product in the NMR sample would probably have given a better signal.

3.3.6 Synthesis of 3,3'-(((((2-chlorophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.6)

The reason for choosing the 2-chlorobenzaldehyde was for the potential for subsequent reactions and what effect the chlorine will have on inhibitor activity and water solubility. The reaction conditions remain unchanged due to the yield not being important for these reactions. The obtained powder was dried using a high vacuum pump to remove excess solvent.



3,3'-(((((2-chlorophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

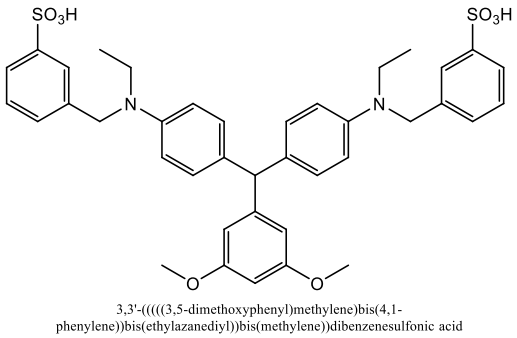
Figure 22: Structure of 3,3'-(((((2-chlorophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

Setting the multiplet at 1.06 ppm in ^{1}H NMR spectra to integrate to 6 protons gives a total of 32 protons. This leaves 3 protons for the quartet where it should be 4. Meaning there are some excess protons in the spectra, most likely from the 6.5-8 ppm area due to the other integrating close to the target number of protons and there only being 20 aromatic protons in the target compound. When doing multiplet analysis of this area it integrates to a total of 12 protons, further suporting som impureties in the sample. The quartet that is visible at arround 4 ppm is not the product, but rather leftover ethyl acetate from the workup (SDBS 1999). meaning the quartet for the wanted product is most likely hidden inside the broad water peak at 3.83 ppm. An easy way of fixing this problem would be to use a different solvent when doing NMR. The only problem with this is that all the obtained products with similar structure seems to be poorly soluble in anything that is not DMSO. When looking at the ^{13}C NMR spectra it is not possible to see much other than some very small peaks. This means the NMR sample was most likely not concentrated enough to give a reasonable ^{13}C NMR spectrum. However, the HRMS did find the mass of the expected product and managed to calculate the elemental composition of the target molecule. This combined with the peaks in the ^{1}H NMR spectra gives reason to believe the target molecule have been achieved and was therefore sent for biological testing.

If the target molecule has been achieved the chlorine would make it very interesting for further work. This is due to the possibility of further synthesis via palladium catalysis. For example doing a Hirao coupling to get a phosphonate group substituted to the compound(Belabassi, Alzghari et al. 2008). This reaction could also have been done with different halide (Br and I) benzaldehydes only changing the position (*ortho*, *meta* and *para*). This would give endless posibilities for designing this part of the molecule.

3.3.7 Synthesis of 3,3'-(((((3,5-dimethoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid (7.2.6.7)

The reason for choosing 3,5-dimethoxybenzaldehyde was to see what effect this will have on activity and water solubility of the final product. The reaction conditions were the same as the previos reactions due to the yield not being important for testing. Washing with water was done to remove leftover DMSO and ethyl acetate to try to remove leftover starting material.



3,3'-(((((3,5-dimethoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

Figure 23: Structure of 3,3'-(((((3,5-dimethoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

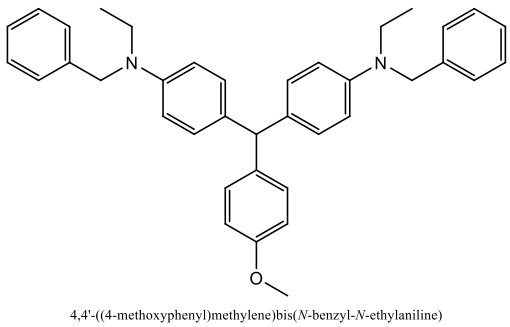
When integrating the multiplet at 1.04 ppm to 6 protons the other areas integrate close to the target molecule protons except the 6.00-8.00 ppm region where there is 1 proton in excess. When doing multiplet analysis this area integrates to a total of 10 protons. This is probably due to impurities in the sample adding. There is also a singlet at 3.66 ppm which may be the methoxy group protons, but it proved hard to integrate properly due to the other peaks. The quartet of the target molecule is once again most likely hidden in the broad peak in 3.60-3.90 ppm area. To get a much cleaner ^{1}H NMR sample further purification would have been needed. For the ^{13}C NMR spectra it's not posible to se much exept a few peaks. This is due to poor solubility of the product and the concentration of the sample being to low. For the HRMS the

mass of the target molecule was found, and the elemental composition was calculated. Meaning combined with the ^{1}H NMR spectra there are reason to believe the target molecule have been achieved and was therefore sent for biological testing.

When exposing the compound spotted on a TLC plate to UV light a color change was observed. The reason for this is most likely that UV light may catalyze the oxidation of the molecule. This gives the molecule a charge and in return makes it even more water soluble. When looking at the molecule that started this thesis (Brilliant Blue R250) it was also oxidized. However, the compounds were first sent for testing without oxidation to see if they would yield a positive result. If the results were good enough, oxidation would only be necessary to increase the water solubility of the compound.

3.3.8 4,4'-((4-methoxyphenyl)methylene)bis(*N*-benzyl-*N*-ethylaniline) (7.2.6.8)

The reason for choosing to synthesize a leuco base without the sulfonate groups was to see what effect this would have on the enzyme activity. This could also potentially reveal if the sulfonate groups would be needed to bind to the enzyme. Hydrochloric acid and water mixture were used as solvent due to no reaction after 1 hour of stirring in neutral conditions with DMSO. Washing with heptane was done to potentially remove leftover starting material. High vacuum pump was used to remove water from the sample. A glass rod was used to crush the solid into a green powder.



4,4'-((4-methoxyphenyl)methylene)bis(*N*-benzyl-*N*-ethylaniline)

Figure 24: Structure of 4,4'-((4-methoxyphenyl)methylene)bis(N-benzyl-N-ethylaniline)

When integrating the multiplet at 1.17 ppm in the ^{1}H NMR spectra to correspond to 6 protons. The other peaks integrate to correspond roughly to the target molecule. Except for the singlet

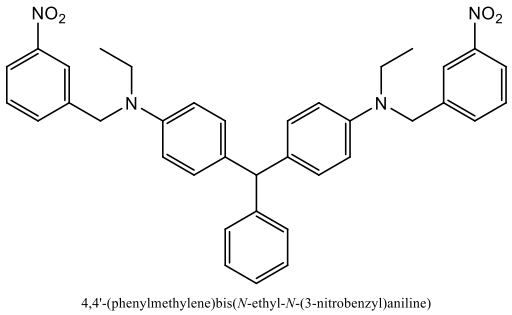
at 3.74 ppm and the area from 6.70-8.00 ppm. Then doing multiplet analysis the number of protons only change in the 6.70-8.00 ppm area to 19 protons. This is due to impurities in the sample and therefore giving the incorrect number of protons when integrating the peaks. These impurities would be possible to remove by further purifying the product. It is possible to see some leftover ethyl acetate from the workup of the reaction (SDBS 1999). When looking at the ^{13}C NMR spectra the peaks are relatively more visible then similar target molecules. There seem to be to manny peaks when considering there is some symmetry in the molecule, but a look at figure 16 tells that the symmetry may not be valid due to the orientation of the phenyl rings, meaning there could be some rotameres in the product (Eliel, Wilen et al. 1994). To get a better idea if there is some rotameric isomers in the sample further purification would be needed. When looking at the HRMS spectra, the mass of the target molecule was found and the HRMS managed to calculate the elemental composition of the target molecule. Combined with the ^{1}H NMR, ^{13}C NMR and the HRMS the compound was sent for testing because there is reason to believe that the target molecule has been achieved.

When choosing to make this molecule different approach had to be used. The same conditions as the sulfonic target molecules did not work this might suggest that the sulfonate groups might aid in catalysis of the reaction. Further testing revealed that very acidic conditions seemed to catalyse the reaction without the sulfonate groups (Ajaikumar and Pandurangan 2008). When choosing which molecule to make, the decision to make one without the sulfonate groups was made to se if this would have an effect on XTH activity.

3.3.9 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using the S_N2 reaction. (7.2.6.9)

An S_N2 approach to making a nitro brilliant blue derivative was chosen to see if it would be possible. The sulfonate groups have been substituted for a nitro group to see if it would have a positive effect on the inhibitor activity and water solubility. Toluene was used as solvent due to it easily dissolving the starting materiales. Triethylamine was used to neutralise the acidic conditions formed during the reaction. Washing with water was done to remove the triethylamine bromide salt formed during the reaction. The reason for not using as much starting

material as previous reactions is due to the 1-(bromomethyl)-3nitrobenzene being expensive compared to other starting materials.



4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline)

Figure 25: Structure of 4,4'-(phenylmethylene)bis(N-ethyl-N-(3-nitrobenzyl)aniline)

Looking at the obtained spectra there are some peaks that catch the eye. In the ^{1}H NMR there are most definitely a triplet (reported as multiplet due to impurities and could also be a result of a rotamer) and a quartet in the 1.16 and 3.04 ppm area. These are most likely the ethyl groups of the final product. Integrating the multiplet to 6 protons gives 4 to the quartet. Further integrating gives 28 protons for the 6.30-7.30 ppm area, this is not accurate considering it is supposed to have 21 protons. When doing multiplet analysis this area integrates to 12 protons. This means there are some impurities in the NMR sample that contributes to excess protons. In the ^{13}C NMR spectra there are not enough peaks for the target molecule. This can be due to symetri in the product, but could also be due to the sample not being consentrated enough due to poor solubility. In the HRMS the target molecule mass was found, and the elemental composition was calculated for the molecule. This meaning the molecule or something with the wanted elemental composition exists in the mixture.

In order to get a cleaner ^{1}H NMR spectra, further purifications would have been needed. For example, recrystallization with a solvent the product is poorly soluble in. For the ^{13}C NMR spectra a different solvent could have been used to get a stronger NMR sample. At the moment of writing the product have not been sent for testing due to not being obtained in a solid powder form.

3.3.10 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using *N*-ethyl-*N*-(3-nitrobenzyl)aniline (7.2.6.10)

Acidic conditions in water was used due to no reaction after 1 hour with heating in DMSO in a previous synthesis. The mixture was neutralised using sodium carbonate due to it proving easy to work with and to force the product out of solution. The powder was washed with water to remove excess sodium carbonate. The reason for using *N*-ethyl-*N*-(3-nitrobenzyl)aniline was to see if keeping the ethyl group in the final product would effect the activity and water solubility.

The ^{1}H NMR spectra gives some information of the obtained product. There are some singlets in the spectra, but they most likely don't belong to the target molecule. In the ^{13}C NMR spectra there are some peaks, but not likely the target molecule. Both these spectra might be because the obtained product was poorly soluble in all solvents tested exept chloroform which looked promising, but when looking at the HRMS spectra the mass of the target molecule was found, and its elemental composition was calculated. However, there are two peaks one at 599 and one at 601 and they are aprox 1:1 ratio. The 601 peak is the leuco product and 599 is the oxidized version of the molecule lacking two protons (see figure 26).

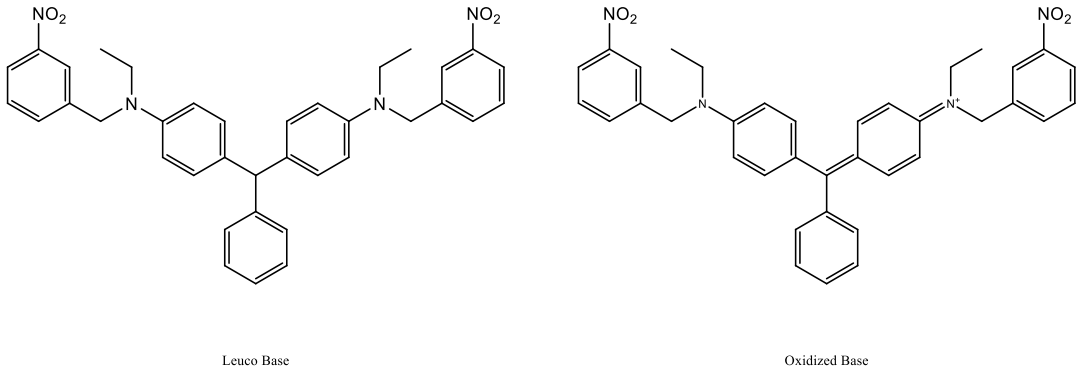
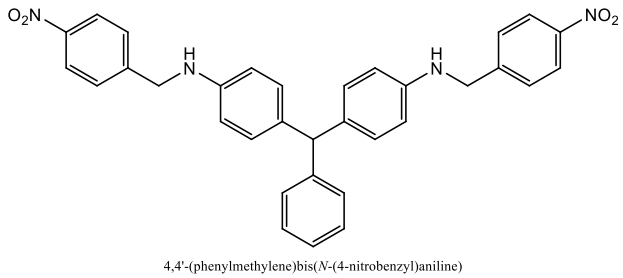


Figure 26: The leuco and the oxidized versions of the product.

This product has not been sent for testing as when writing the thesis due to more work would be needed. The reason for this is that the product most likely consists of the leuco and the oxidized versions of the product and it would be beneficial to only have one of them in the mixture when testing the molecule. For further work on the product an oxidation using DDQ might fully convert the leuco product (Wang, An et al. 2014). When doing NMR of the product further purification of the product and a stronger sample would most likely give a stronger spectrum.

3.3.11 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline) (7.2.6.11)

Moving the nitro group to the *para* position of the molecule was done to see what effect it would have on water solubility and activity. Hydrochloric acid and water mixture were used due to a previous synthesis proving no reaction after 1 hour in DMSO. Sodium carbonate was used to neutralise the solution due to it proving easy to work with. The final product was washed using plenty of water to remove remaining sodium carbonate.



4,4'-(phenylmethylene)bis(*N*-(4-nitrobenzyl)aniline)

Figure 27: Structure of 4,4'-(phenylmethylene)bis(N-(3-nitrobenzyl)aniline)

When looking at the obtained ^{1}H NMR spectra for the product there looks to be some impurities in the sample. These impurities are most likely starting matereal that have not been fully converted. The reason for this suspicion is due to the peak at 10.02 ppm that most likely is the aldehyde proton from unreacted benzaldehyde in the mixture (Lampman, Pavia et al. 2010). When looking at the ^{13}C NMR spectra it is possible to se some peaks, but not all the expected peaks. This is most likely due to poor solubility resulting in the NMR sample not being consentrated enough to give a good signal. When looking closer at the spectra it is possible to se a shift at 190 ppm that can confirm an aldehyde is present in the sample, most likely leftover benzaldehyde from the reaction (Lampman, Pavia et al. 2010). In the obtained HRMS spectra, the mass of the target molecule was found, and the sodium salt of the molecule was also found. Both of the elemental compositon of these molecules were also calculated. Due to the mass was found in the HRMS there is a reason to belive the wanted molecule had been produced.

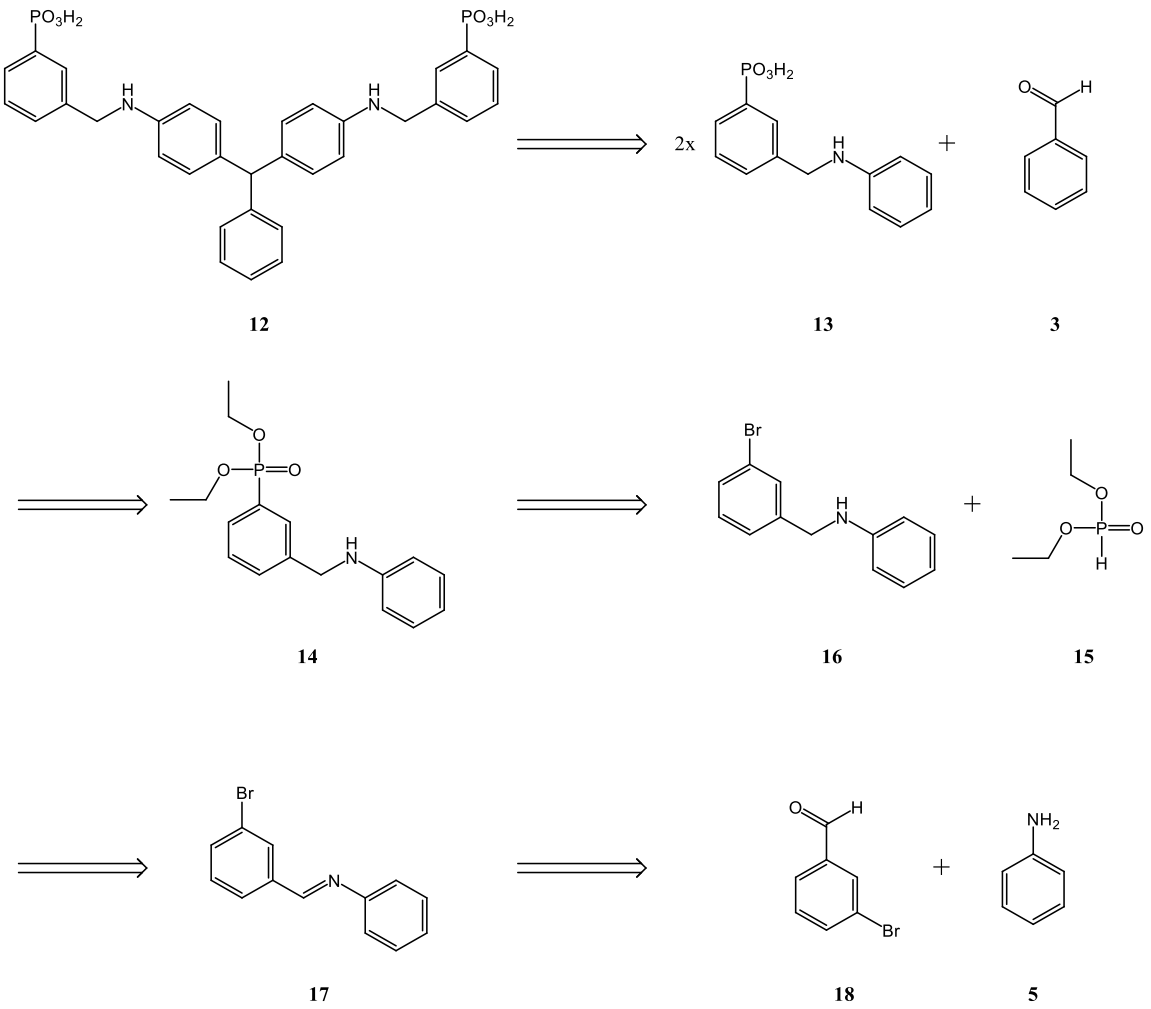
The reason the compound was mostly obtained as a sodium salt is most likely due to the neutralization process where sodium carbonate was used. This means the compound may have been deprotonated and a sodium has taken its place to form a salt. At the moment of writing the results from testing have not been recived and is therefore not included in this report.

3.4 Phosphorus containing Brilliant Blue derivatives

In order to test if other functional groups can bind to the XTH a phosphonic group was chosen. the reason for this was to see if substituting the sulfonate for a phosphonate would have an effect on enzyme binding, enzyme activity and water solubility of the final product. The reason for using bromobenzaldehyde was because phosphonic benzaldehyde was not commercially available and to attempt a Hirao coupling and then hydrolysis to get a phosphonic acid group (Hirao, Masunaga et al. 1980).

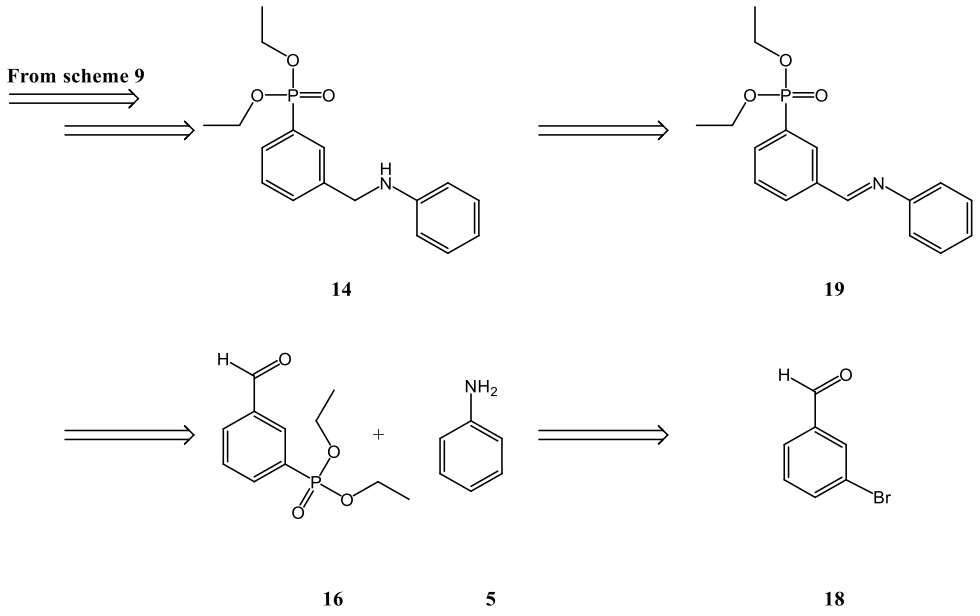
3.4.1 Retrosynthesis of Phosphonic Brilliant Blue derivatives

For phosphonic Brilliant Blue derivatives a new retrosynthesis had to be proposed due to the fact that (3-formylphenyl)phosphonic acid was not commercially available. The proposed retrosynthesis is shown in Scheme 9.



Scheme 9: Retrosynthesis of phosphonic acid Brilliant Blue R250 derivatives

In the proposed retrosynthesis the phosphonic Brilliant Blue derivative is disconnected to give benzaldehyde (**3**) and (3-((phenylamino)methyl)phenyl)phosphonic acid (**13**). This step could easily be completed by performing a triaryl condensation with **13** and **3** (Ajaikumar and Pandurangan 2008). Further down the retrosynthetic pathway diethyl (3-((phenylamino)methyl)phenyl)phosphonate (**14)** is suggested. In order to achieve this reaction a simple hydrolysis of the phosphite group was proposed (Keglevich, Grün et al. 2012). From compound **14** further disconnection were made to give diethyl phosphonate (**15**) and *N*-(3-bromobenzyl)aniline (**16**). In order to produce **14** from **15** and **16** a Hirao cross coupling using tetrakis(triphenylphosphine)palladium(0) was suggested (Hirao, Masunaga et al. 1980, Keglevich, Grün et al. 2012). Further disconnection gives (*E*)-1-(3-bromophenyl)-*N*-phenylmethanimine (**17**), to reduce the double bond a simple hydrogenation using sodium borohydride were proposed (Lopez, de la Cruz et al. 2017). More disconnection reveals aniline and 3-bromobenzaldehyde this was envisioned using toluene as solvent and Dean-Stark water trap to push the equilibrium towards the imine (Dibble, Kurakake et al. 2018).



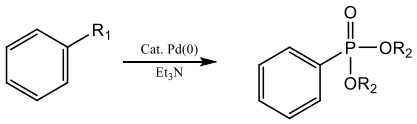
Scheme 10: Retrosynthesis of phosphonic acid Brilliant Blue R250 derivatives option 2

A diverging route is a retrosynthesis shown in Scheme 10 from **14** diethyl (*E*)-(3-((phenylimino)methyl)phenyl)phosphonate is proposed. To form **14** from **19** imine reduction using sodium borohydride was chosen. Further down the retrosynthesis Aniline (**5**) and diethyl (3-formylphenyl)phosphonate (**16**) appears. To perform this transformation an imine condensation using molecular sieves were suggested (Paniagua, Yadava et al. Feb-April 2018). Further disconnection of **16** reveals 3-bromobenzaldehyde (**18**). To transform **18** to **16** a

standard Hirao cross coupling using diethyl phosphonate and tetrakis(triphenylphosphine)palladium(0) was the suggested reaction (Hirao, Masunaga et al. 1980, Keglevich, Grün et al. 2012).

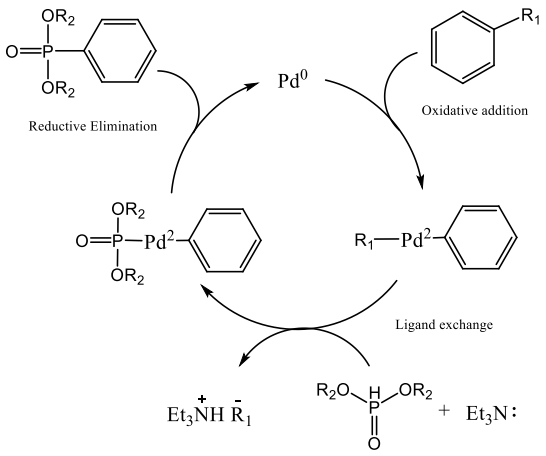
3.4.2 Hirao Cross coupling

The Hirao Cross coupling was discovered and developed in 1980 by Toshikazu Hirao (Hirao, Masunaga et al. 1980). Since the reaction was developed it has been extensively used to develop phosphites and phosphonic acids (Montchamp 2015). The basics of the Hirao coupling are shown below in Scheme 11 (Hirao, Masunaga et al. 1980, Belabassi, Alzghari et al. 2008). In the reaction any vinyl halide reacts with alkyle phosphites when catalyzed by Pd(0) (Montchamp 2015).



Scheme 11: Standard Hirao coupling reaction using any paladium 0 catalysts R_1 = Cl, Br or I, R_2 = any alkyl group.

The catalytic cycle of the Hirao coupling is described in Scheme 12. The reaction starts with an oxidative addition of the vinyl halide to the Pd(0) catalyst. Then a ligand exchange happens where the phosphite comes in and the triethylamine halide salt leaves the reaction. This leaves the vinyl phosphite complex to do reductive elimination and form the vinyl phosphite (Henyecz and Keglevich 2018).



Scheme 12: Catalytic cycle of the Hirao coupling R_1 = Cl, Br or I R_2 = alkyl group

3.4.3 Synthesis of (*E*)-1-(3-bromophenyl)-*N*-phenylmethanimine (7.2.1.3)

The reason for choosing this reaction was to use it for subsequent reactions to get a phosphonic brilliant blue derivative. This reaction was first tested not using molecular sieves or Dean-Stark aparatus, but it didn't seem to fully convert the starting materiales when monitored using TLC. Therfore, both molecular sieves and Dean-Stark have been tested. Dean-Stark had to be swapped for a distillation to try to distill the azeotrope out of the mixture. This was done due to toluene proving difficult to evaporate in the Dean-Stark apparatus.

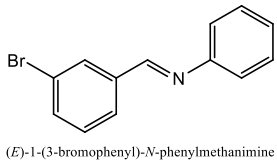


Figure 28: Structure of (E)-1-(3-bromophenyl)-N-phenylmethanimine

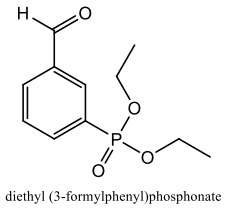
In the obtained spectra in the synthesis of (*E*)-1-(3-bromophenyl)-*N*-phenylmethanimine there seems to be a lot of small peaks in the base line. These peaks are most likely originated from unreacted starting material. In the ^{1}H NMR there are peaks that corresponds to the expected product. When integrating the peaks, they give a total of 10 protons which is to be expected. For the ^{13}C NMR spectra the same is also true, meaning some unreacted starting material is present in the sample. The peaks in the ^{13}C NMR spectra add upp to 13 carbons which fitts nicely with the target molecule. For the HRMS spectra the wanted mass was found and the machine calculated the expected elemental composition of the target product.

For this synthesis the change from nitro to bromine made the reaction slower. This is most likely due to the bromine working as a donating group making the protonated carbonyl less likely to undergo nucleophilic attack by aniline. In combination with the water forming during the reaction the equilibrium should be heavily favored towards the 3-bromobenzaldehyde and aniline. For the reaction to proceed removal of water would be necessary. This means adding either molecular sieves at room temperature or using dean stark apparatus with toluene as solvent (Dibble, Kurakake et al. 2018, Paniagua, Yadava et al. Feb-April 2018). The Dean Stark apparatus was eventually changed to a distillation due to toluene proved hard to evaporate in the apparatus. The idea was to distill the azetrope out of the reaction pushing the equilibrium towards the imine. Both methods were tried, but based on observations using TLC none of the methods seemed to fully convert the starting materials. The reason for this might be that the

reaction time was too short. If the reaction time was longer the nucleophilic attack would be more likely to happen and the water formed would have more time to be removed from the reaction mixture. This would make the method of using molecular sives the better one due to not having to regularly add toluene to the mixture. This imine was not reacted any further due to difficulties with purification and fully converting the starting materials.

3.4.4 Attempt to synthesis diethyl (3-formylphenyl)phosphonate (7.2.7)

This attempt was made to test if it would be possible to do a coupling directly on 3-bromobenzaldehyde and then use the product for subsequent reaction to produce a phosphoinc Brilliant Blue derivative. Tetrakis(triphenylphosphine)palladium(0) was used as a source for Pd(0) and a two neck round bottom flask was used to easily add diethyl phosphite to the mixture while stirring under argon. Triethyl amine was used as a base to form a halogen salt during the reaction. The mixture was washed with water to remove the salts that had been formed.



diethyl (3-formylphenyl)phosphonate

Figure 29: Structure of diethyl (3-formylphenyl)phosphonates

When first looking at the ^{1}H NMR spectra it is possible to see a benzylalcohol proton at 5.76 ppm (Lampman, Pavia et al. 2010). There are also a doublet at 4.88 ppm which is a result of a phosphorus coupling, which also is a benzylic proton (Claridge 2009). The quartet and the triplet from the two ethyl groups is visible at 3.89 ppm and 1.07 ppm. In the ^{13}C NMR spectra there is a lot of splitting that is caused by the phosphorus resulting in doublets with coupling constants varying from 3, 5 and 163 Hz. Stronger (broader peaks) splitting means the carbon is closer to the phosphorus (Pretsch, Buhlmann et al. 2000, Claridge 2009). In the HRMS spectra the wanted mass was not found and the mass spectra didn't manage to calculate the elemental composition of the product, but it is possible to see the bromine is still present in the product meaning the reaction has not happened in the target position and produced. most likely the oxidative addition has happened at the aldehyde which would explain the benzylic alcohol proton in the ^{1}H NMR spectra. This reaction has been done by Pandi referring to the spectra in their appendix (Pandi, Chanani et al. 2012).

To fix this problem a protection group for the aldehyde would be needed. For example, making an acetal either using methanol or ethylenglykol then hydrolyse back to the aldehyde after the phosphorus coupling reaction (Diness and Meldal 2015). This extra step was not explored due to time limitations.

3.5 Tested compounds

The top 5 of compounds in figure 30 where commercially obtained. As seen in figure 30 all these compounds (Brilliant Blue R250, Brilliant Blue G250, Guinea Green B, Brilliant Blue FCF and Coomassie Violet R200) seemed to bind proteins very well. The starting material CAS-101-11-1 for the sulfonic leuco bases (3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid) was also supposed to be tested, but was not tested due to the compound being colorless.

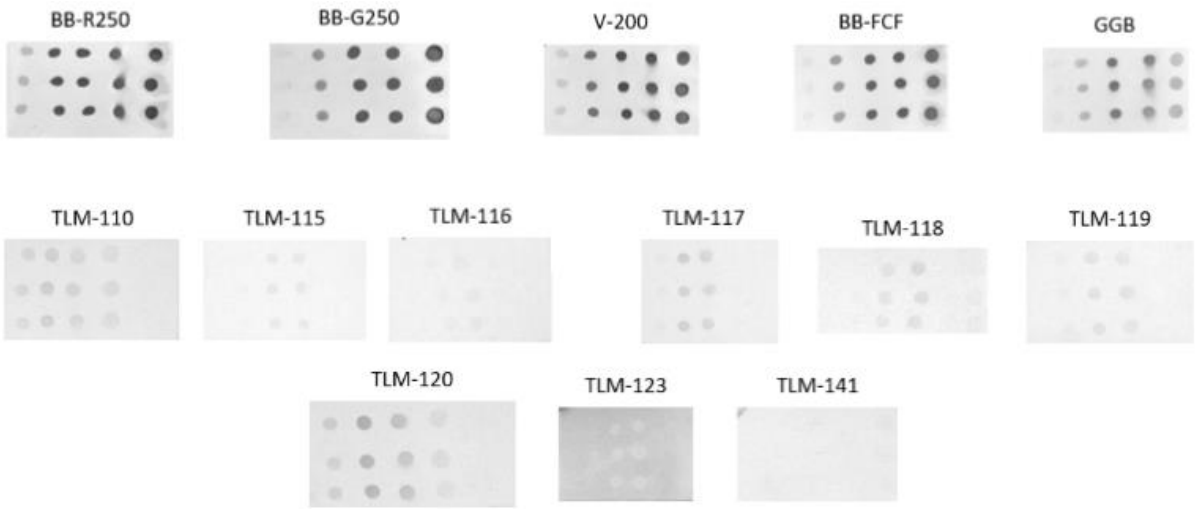


Figure 30: PVDF membranes after staining with the synthesized compounds used for determening protein binding.

When testing the commercially obtained molecules for XET activity (see figure 31) all exept 3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid seemed to inhibit activity when exposed at 1 mM and 5 mM consentration. However, Brilliant Blue FCF (see figure 32) was not as good of an inhibitor as the others. At the time of testing it was not clear why this might have been the case, but later testing of the synthesized compounds revealed that the sulfonate group might play a role in binding to the protein. This resulted in the creation of a hypothesis that the third sulfonate group on Brilliant Blue FCF is the reason to why the others are better inhibitors. The third sulfonate group may bind to the enzyme and inhibiting other due to geometry resulting in less/none inhibition. The inhibition that is seen for Brilliant Blue FCF may be for only the two other sulfonate groups.

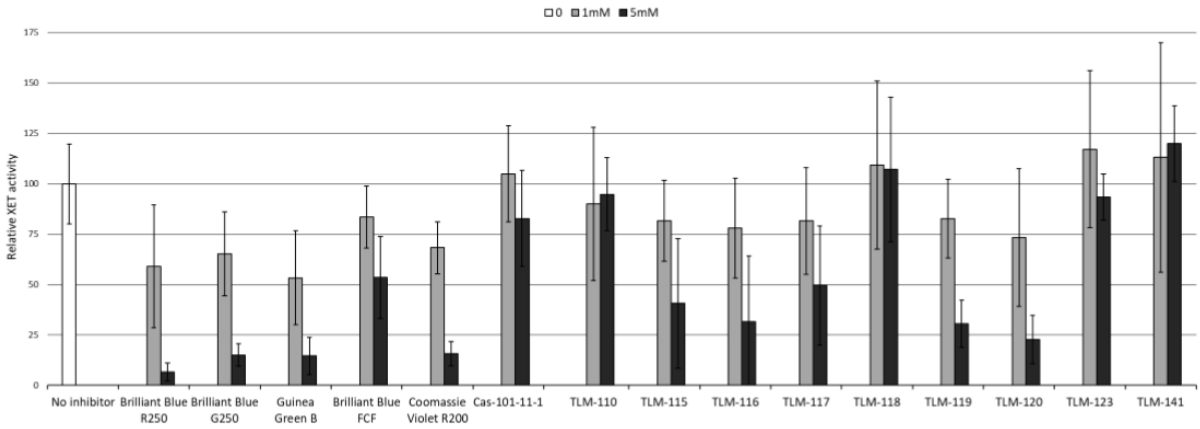


Figure 31: The X axis shows the fluorecent signals that represents the amount of XET activity in C. reflexa and the Y axis shows the represented compounds.

As for the synthesized compounds (see figure 33) all except TLM-141 and TLM-123 seemed to bind proteins. Which is interesting considering it's the only of the synthesized compounds which does not have a sulfonate groups in the molecule. This sparked the reason to believe that the sulfonate groups may play a bigger role in binding to the protein then previously though. Looking at the strength of the colors of the spots for the commercially obtained compounds, it seemed to bind stronger to the protein. Meaning an oxidation of the synthesized molecules may increase the binding strength even further. This may easily be done by a simple DDQ oxidation of the synthesized leuco bases (Wang, An et al. 2014). When looking at the measured effect on XET activity (see figure 31). It is possible to see that TLM-110, TLM-118, TLM-123 and TLM-141 have no inhibition when exposed for either 1 mM or 5 mM solution. Which is interesting due to TLM-110 and TLM-120 should be the same compound. The answer for this question is most likely that under the reaction conditions of both TLM-110 and TLM-120. The only difference between the two reaction conditions were the temperature used. TLM-110 was synthesized a temperature of 180°C and TLM-120 at a temperature of 120°C with stirring. Due to the high temperature in TLM-110 a desulfonation reaction might have occurred effectively removing the sulfonate groups by heating to very temperature and forming a desulfonated product (Huston and Ballard 1934).

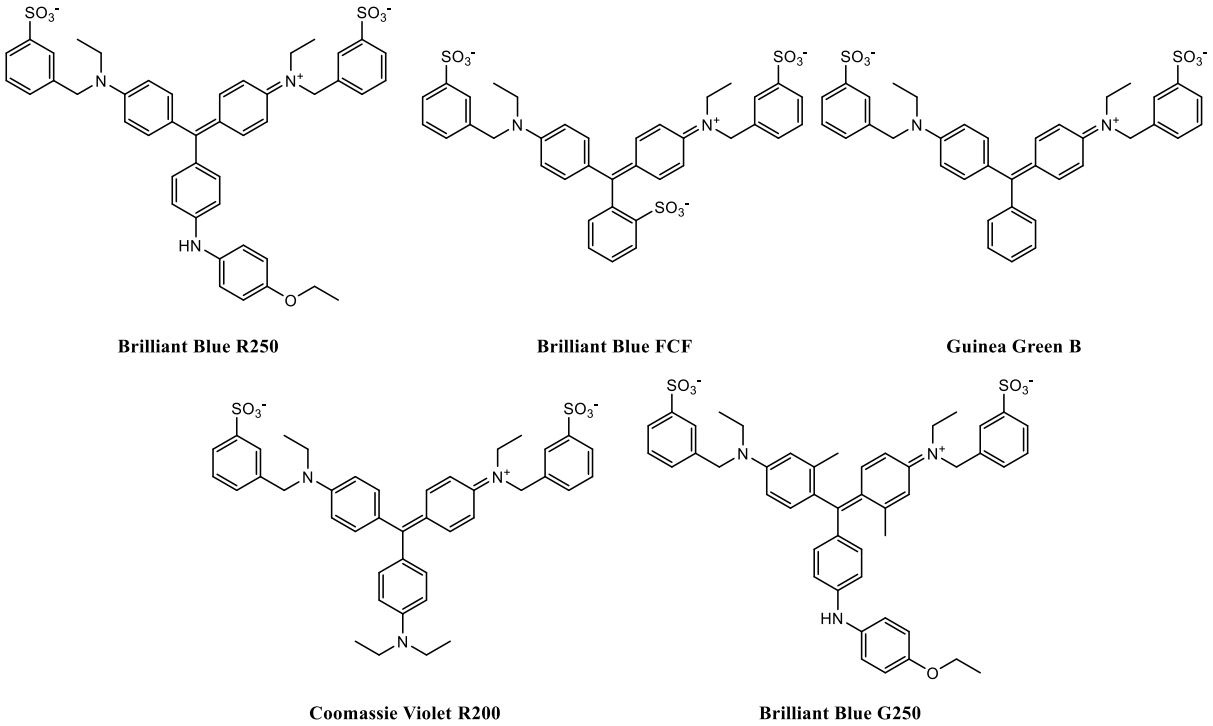


Figure 32: Commercially obtained molecules (Brilliant Blue R250, Brilliant Blue G250, Guinea Green B, Brilliant Blue FCF and Coomassie Violet R200).

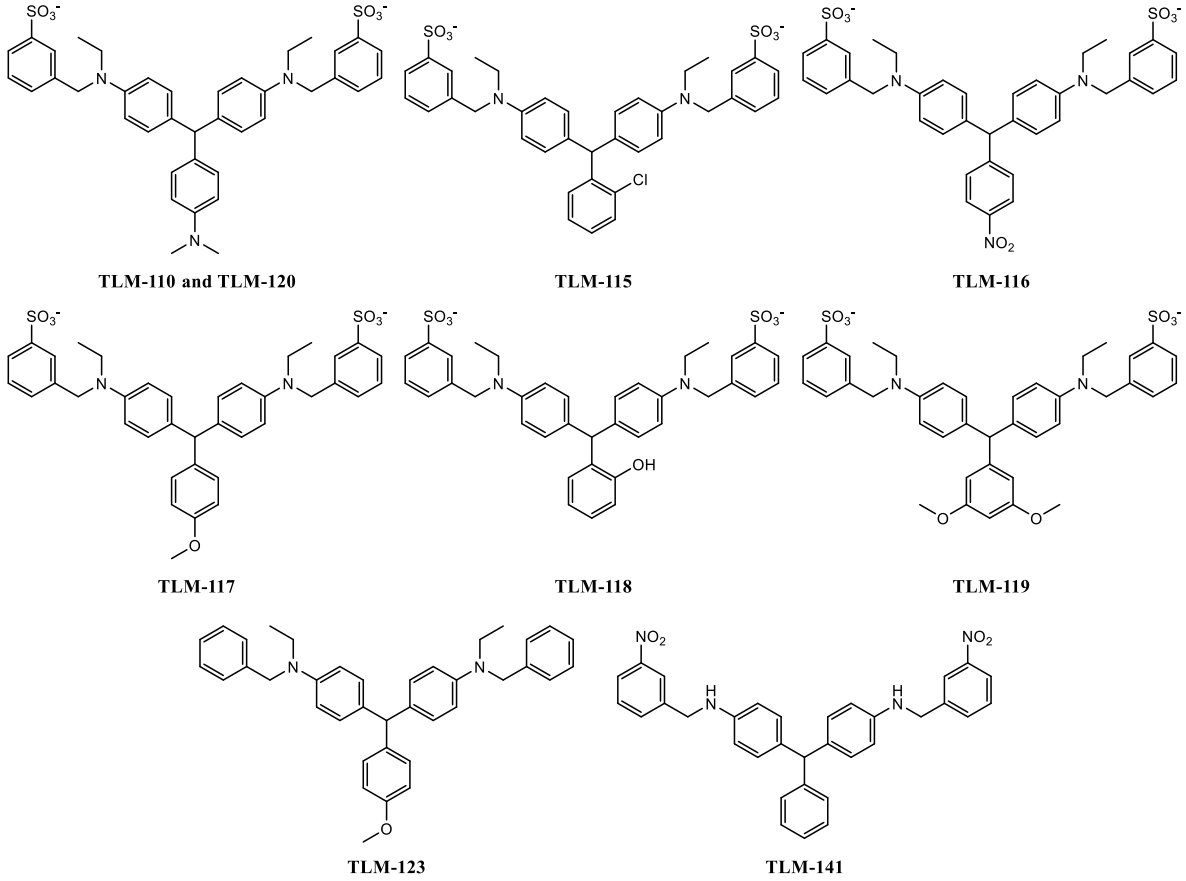


Figure 33: Structure and labeling of the different synthesized compounds

TLM-118 was also discovered to bind protein, but didn't seem to have any effect on XET activity. This gives opportunity to test if protein binding is enough to influence the level of Haustorium that penetrates the cell wall of the Tomato plants. The compound TLM-123 and TLM-141 does not have any effect on inhibition of the XET activity. Meaning by only removing the sulfonate group or swapping it for something else the activity increases to the levels of no inhibition. Which strengthens the hypothesis that the sulfonate group plays a bigger role when binding to the enzyme.

3.6 Further work

For further synthesis the 3-formylbenzenesulfonic acid would be an interesting benzaldehyde to work with. Due to the possibility of synthesizing sulfonic acid leuco bases without the ethyl group. It would also be interesting to oxidize the synthesized bases to potenitaly increase water solubility. Something also worth testing is if a protecting group for the bromobenzaldehyde would work to potentially make (3-formylphenyl)phosphonic acid to then do further synthesis with.

For further biological testing TLM-118 is the most interesting molecule for infection trails on the coated host plants as described by Olson and Krause (Olson and Krause 2017). As it seems to bind the protein, but does not inhibit any XET activity. This could reveal if it is the protein-binding or the XET inhibition that prevents the haustoria from penetrating the host plant. Other suitable candidates would be TLM-123 and TLM-141, these would function as negative controls because they are not binding protein and they do not inhibit XET activity.

4 Conclusion

The nitration reactions proved to be hard and not an effective method to introduce a nitro group in one specific position of Guinea Green B. The nitration seemed to have yielded multiple different products that is difficult to purify using traditional column chromatography. Nitration of *N*-Benzyl-*N*-ethylaniline yield a mixture of tree different products two monosubstituted and one disubstituted as shown in figure 8. Furthermore, the nitration of Guinea Green B and *N*-Benzyl-*N*-ethylaniline was abandoned due to difficulties with substituting the nitro group in to the wanted position and difficulties with purification of the final product.

When working with making the *N*-Benzyl-*N*-ethylaniline derivatives with nitro and phosphonate in the *meta* position of the benzylic ring. The nitro substituted version was successfully made with and without the ethyl groups. It was then used for further synthesis in an attempt to make the leuco base with the nitro groups substituted for the sulfonate groups. The results obtained from the spectra was not that good, but the HRMS detected the mass of the target molecules and calculated the expected elemental compositions and was therfore tested. For the phosphonate derivative, direct coupling to the 3-bromobenzaldehyde resulted in a coupling between the carbonyl and the phosphite. Therefore, an attempt to make the imine to get a coupling at the bromine position was chosen. The imine also proved hard to form even with a water trap and molecular sieves. The final phosphonic *N*-Benzyl-*N*-ethylaniline and Brilliant Blue derivatives were therefore not produced.

When working with the sulfonic leuco bases all the produced compounds seemed to be found in traces in the HRMS and it was possible to se a few peaks corresponding to the overall structure of the molecule in the NMR spectras. Most of the leuco bases prooved hard to dissolve in various different solvents making purification and recording a ^{13}C NMR spectra difficult. According the recorded spectra there is reason to believe that all the target molecules have been achived and therefore they were sent for testing. However, it's not possible conclude with absolute certainty.

Regarding the biological testing of the molecules. All the commercially obtained molecules inhibited the XET function. All the molecules had about the same level of inhibition, except Brilliant Blue FCF which have an extra sulfonic group. This gave some reason to think the

sulfonate group might play a bigger role in binding to the enzyme. For the synthesized compounds (see figure 33) all except TLM-118, TLM-123 and TLM-141 influenced activity, but TLM-118 seemed to bind without any inhibition. in TLM-123 the sulfonate groups were removed and resulted in no inhibition. Which sparked the hypothesis of the sulfonate groups may play an important role in binding to the enzyme. This hypothesis may also be supported by TLM-141 not binding to the enzyme or changing the level of enzyme activity. This means its posible to conclude that there is good reason to belive that the sulfonate groups in the leuco bases play a bigger role in binding to the enzyme and inhibition.

5 Experimental Methods

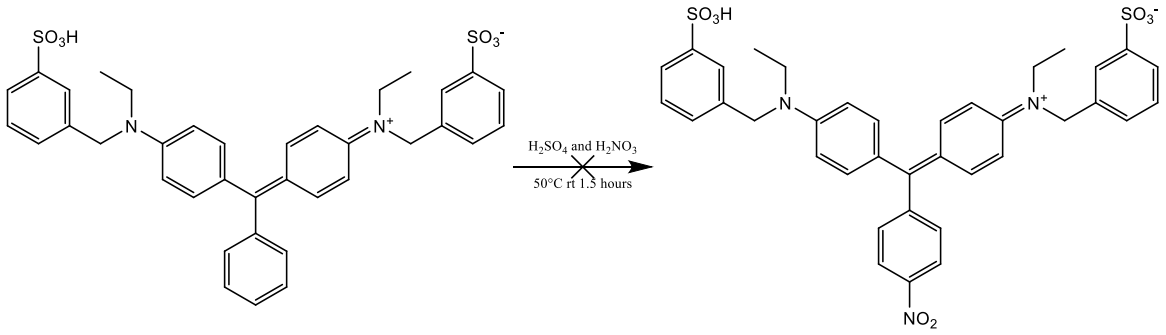
All the starting materials used in this project was purchased and obtained from Sigma Aldrich and VWR and used without purifications. The silica gel used when performing column chromatography was silica gel 60 (0.040 – 0.063 mm). the TLC plates used for monitoring reactions and the eluent during column chromatography was silica gel 60 F_{254} aluminium sheet and the stains on the plates were revealed using UV-light.

All the NMR spectra was recorded using an Ascent 400. the software used for processing the NMR spectra was MestreNova version 12.0.1 and the chemical shifts was reported in parts per million.

The HRMS was an LTQ orbitrap XL with ESI as an ionisation source and the mass was reported in mass over charge (m/z). The LC-MS spectra was obtained using a Thermo sientific Accella Autosampler. All the IR spectra was obtained using an agilent Technologies Cary 630 FTIR.

5.1 Nitration reactions

5.1.1 Nitration of GGB using Sulphuric and Nitric acid



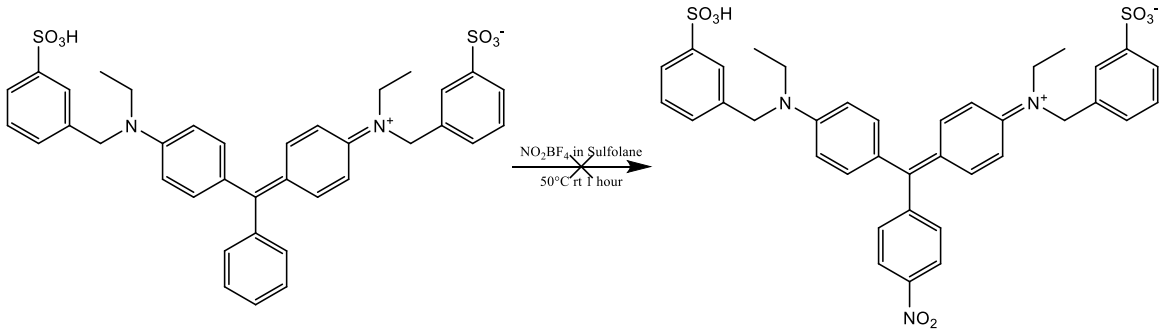
Guinea Green B (100mg, 0.145 mmol) was transferred over to a 25 mL round bottom flask, then heated in a 50°C water bath while stirring. when the reaction mixture was heating a mixture of 1:1 sulphuric acid and nitric acid was prepared using an ice bath by slowly adding sulphuric acid (5.00 mL, 93.8 mmol) to nitric acid (3.91 mL, 93.8 mmol). Of this mixture 13 µL (0.144 mmol) was carefully added and then the mixture was allowed to stir for 1 hour and 30 minutes. After stirring, a filtration of the solution was performed. Then the solution was neutralised using sodium hydrogen carbonate. The solution was then extracted using 3x50 mL of DCM. The DCM was then evaporated leaving a yellow powder (32 mg, 31.9%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.65 (m, 6H), 7.57 (t, J = 7.5 Hz, 2H), 7.44 (q, J = 8.2, 7.7 Hz, 2H), 7.41 – 7.36 (m, 4H), 7.36 – 7.25 (m, 6H), 7.05 (t, J = 9.5 Hz, 4H), 4.95 (s, 5H), 3.80 (q, J = 7.1 Hz, 4H), 1.33 (t, J = 7.0 Hz, 5H), 1.18 (q, J = 6.4 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) not concentrated enough to give signal

HRMS (ESI) m/z: [M^-] mass not found

5.1.2 Nitration of GGB using nitronium tetrafluoroborate



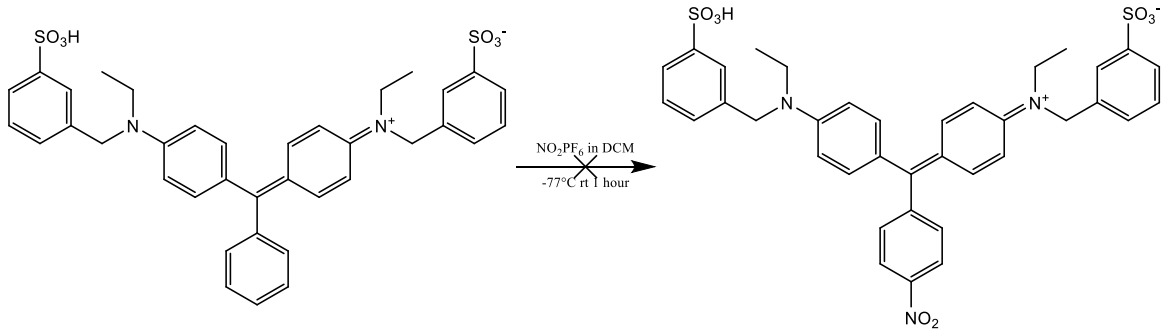
Guinea Green B (100mg, 0.145 mmol) was transferred over to a 25 mL round bottom flask and placed over a 50°C water bath while stirring. during a period of 30 minutes a 2.28 mL solution of nitronium tetrafluoroborate in sulfolane (0.5 M, 0.144 mmol) was slowly dripped to the reaction mixture. After adding the regent, the mixture was allowed to stir for 1 hour. After stirring purification using column chromatography was attempted. The column was monitored using TLC and gave a mixture of different compounds. The rest of the mixture was lost due to further purification (0 mg, 0%)

^{1}H NMR (400 MHz, Deuterium Oxide) δ 6.47 (t, *J* = 6.2 Hz, 2H), 6.08 – 5.96 (m, 1H), 5.42 (s, 1H), 5.17 (t, *J* = 6.1 Hz, 2H), 4.93 – 4.82 (m, 1H), 4.77 – 4.59 (m, 11H), 4.70 (s, 2H), 3.95 (d, *J* = 3.5 Hz, 1H).

^{13}C NMR (101 MHz, Deuterium Oxide) δ 48.8.

HRMS (ESI) m/z: [M^-] mass not found

5.1.3 Nitration of GGB using nitronium hexafluorophosphate



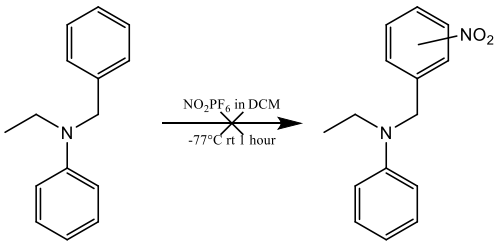
Nitronium hexafluorophosphate (100mg, 0.145 mmol) was placed in a 25 mL round bottom flask, added 10mL of DCM and then put on inert gas. The mixture was cooled to -78°C and slowly added 0.718g Guinea Green B dissolved in DCM during a 30-minute period while stirring. After the addition of the starting material the reaction mixture was set to stir in 1 hour at a constant -78°C. After 1 hour of contiguous stirring the mixture was slowly heated to room temperature. The mixture was then washed whit 20 mL of water to get rid of excess nitronium hexafluorophosphate. After washing the the solvent was evaporated leaving a blue oil. (40 mg, 39.9%)

^{1}H NMR (400 MHz, Methanol-d_4) only solvent peaks found and not concentrated enough to give a signal

^{13}C NMR (101 MHz, Methanol-d_4) not concentrated enough to give a signal

HRMS (ESI) m/z: [M^-] mass not found

5.2 Nitration of *N*-benzyl-*N*-ethylaniline using nitronium hexafluorophosphate



Nitronium hexafluorophosphate (450mg, 2.37 mmol) was added to a 25 mL round bottom flask whit 10 mL DCM, cooled to -78°C and added inert gas. After the reaction mixture was cooled to -78°C N-benzyl-N-ethylaniline (500 mg, 2.37 mmol) was added to the mixture in a period of 30 minutes. When the addition of *N*-benzyl-*N*-ethylaniline was completed the reaction was allowed to stir for 1 hour. the reaction mixture was then washed whit water and then the organic phase was prepared for column chromatography. After the column a C18 biotage column was used (8:2, water:etanol solution) for further purification. After completion of the C18 column all the fractions were monitored using TLC. The fractions containing the product were then combined, and the product was extracted with ethyl acetate using a contiguous extraction device. After extraction the ethyl acetate was evaporated and gave a yellow powder (35 mg, 5,8%)

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.47 (d, J = 2.8 Hz, 1H), 8.13 – 8.05 (m, 1H), 7.98 – 7.88 (m, 2H), 7.26 – 7.06 (m, 11H), 6.69 – 6.55 (m, 2H), 4.61 (s, 2H), 4.48 (s, 2H), 3.54 (q, J = 7.1 Hz, 2H), 3.27 (q, J = 7.1 Hz, 2H), 1.16 (t, J = 7.1 Hz, 4H), 1.12 (t, J = 7.1 Hz, 3H).

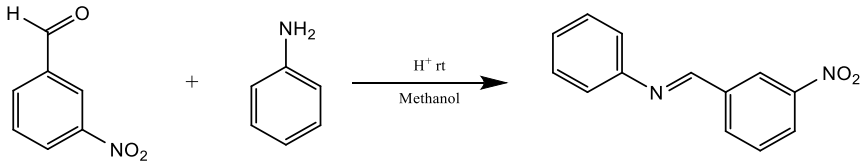
^{13}C NMR (101 MHz, Methanol-d_4) not concentrated enough to give signal

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{15}H_{17}O_2N_2$ 257.1285, mass found 257.1284

5.3 Synthesis of *N*-Benzylaniline derivatives

5.3.1 Synthesis of imines using Aniline and benzaldehydes

5.3.1.1 (*E*)-1-(3-nitrophenyl)-*N*-phenylmethanimine



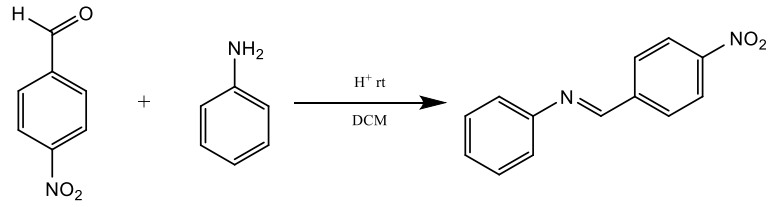
3-nitrobenzaldehyde (500 mg, 3.31 mmol) and Aniline (308 mg, 3.31 mmol) was mixed together and and added 10 mL of methanol. One drop of concentrated hydrochloric acid was added to the mixture and it was stirred for 10 minutes. After 10 minutes a yellow white powder had precipitated in the mixture. The powder was washed with cold methanol to give (*E*)-1-(3-nitrophenyl)-*N*-phenylmethanimine (611 mg, 81.6%)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.70 (t, *J* = 2.0 Hz, 1H), 8.51 (s, 1H), 8.28 (d, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.22 (d, *J* = 7.8, 1.3 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 2H), 7.28 – 7.20 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.5, 150.8, 148.7, 137.8, 134.2, 129.9, 129.4, 127.0, 125.6, 123.5, 121.0, 50.4.

HRMS (ESI) m/z: [M-H^{+}] calculated for $C_{13}H_{11}O_2N_2$ 227.0821, mass found 227.0802

5.3.1.2 (*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine



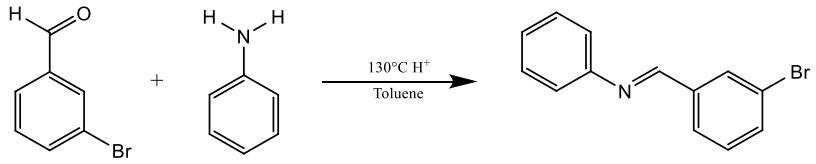
4-nitrobenzaldehyde (2.00g, 0.0132 mol) and aniline (1.23g, 0.0132 mol) was mixed together in a 50 mL round bottom flask and dissolved in 10 mL of DCM. Then one drop of concentrated hydrochloric acid was added, and the mixture was then put on reflux at 50 °C over night. After heating the mixture was cooled in an ice bath and the imine crystallized out of the mixture. After cooling, the imine was filtered and washed using cold methanol (953mg, 31.9%).

^{1}H NMR (400 MHz, Acetone-d_6) δ 8.77 (s, 1H), 8.36 (d, J = 8.4 Hz, 2H), 8.22 (d, J = 8.4 Hz, 2H), 7.52 – 7.24 (m, 5H).

^{13}C NMR (101 MHz, Acetone-d_6) δ 205.3, 158.1, 151.2, 149.4, 142.0, 129.5, 129.3, 126.9, 123.9, 121.2.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{13}H_{11}O_2N_2$ 227.0815, mass found 227.0807

5.3.1.3 (*E*)-1-(3-bromophenyl)-*N*-phenylmethanimine



3-bromobenzaldehyde (500 mg, 2.7 mmol) and aniline (252 mg, 2.7 mmol) was added to a round bottom flask and added 20 mL of toluene. then the round bottom flask was attached to a distillation apparatus and the mixture was heated to 130 °C. When the amount of toluene in decreased more was added. After distilling for a while, the reaction was stopped, and the remaining toluene was evaporated using the rotary evaporator leaving an orange oily residue (234 mg, 33.3%)

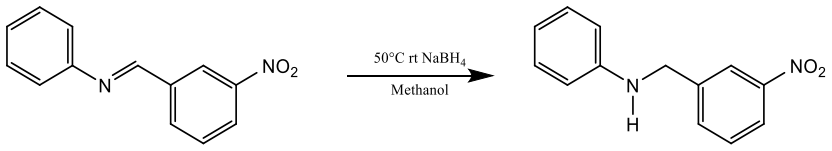
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.33 (s, 1H), 8.01 (t, *J* = 1.8 Hz, 1H), 7.74 (d, *J* = 7.7, 1.3 Hz, 1H), 7.54 (d, *J* = 8.0, 2.1, 1.1 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.24 – 7.15 (m, 3H).

^{13}C NMR (101 MHz, Methanol-d_4) δ 159.1, 151.0, 138.2, 133.9, 130.9, 130.3, 129.0, 127.5, 126.3, 122.6, 120.8.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{13}H_{11}N^{81}Br$ 262.0049, mass found 262.0050

5.3.2 Hydrogenation of imines using sodium borohydride

5.3.2.1 *N*-(3-nitrobenzyl)aniline



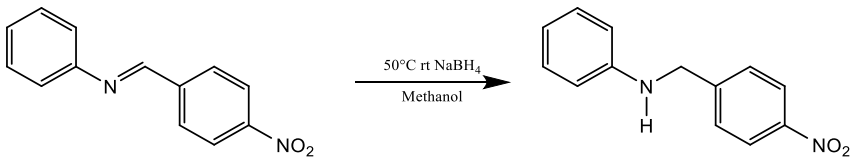
(*E*)-1-(3-nitrophenyl)-*N*-phenylmethanimine (500mg, 2.21 mmol) was added to a 100 mL two neck round bottom flask and dissolved in 20 mL of methanol. The reaction mixture was heated to 50°C then slowly added sodium borohydride. During the addition of sodium borohydride, the reaction was monitored using TLC until complete conversion were observed. The reaction mixture was then quenched with water and extracted with 20 mL of ethyl acetate. The solvent was then evaporated to give *N*-(3-nitrobenzyl)aniline (476 mg, 94.4%)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (t, *J* = 2.1 Hz, 1H), 8.03 (d, *J* = 8.2, 2.3 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.12 – 7.05 (m, 2H), 6.69 – 6.62 (m, 1H), 6.55 – 6.49 (m, 2H), 4.37 (s, 2H), 4.15 (s, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.6, 147.4, 142.1, 133.3, 129.6, 129.4, 122.3, 122.1, 118.2, 113.00, 47.6.

HRMS (ESI) m/z: [M-H^{+}] calculated for $C_{13}H_{13}O_2N_2$ 229.0977, mass found 229.0973

5.3.2.2 *N*-(4-nitrobenzyl)aniline



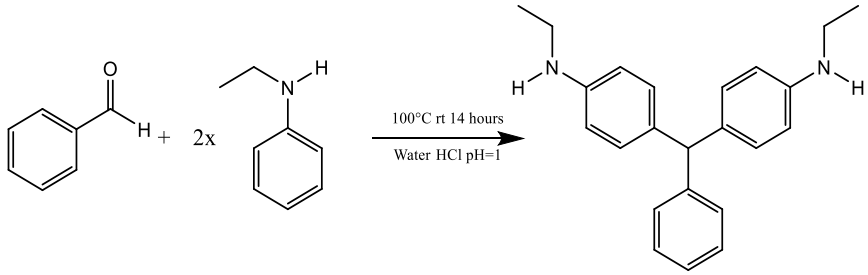
(*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine (953 mg, 4.21 mmol) was added to a 50 mL two neck round bottom flask and dissolved in 15 mL of methanol. The mixture was then put on reflux and heated to 50°C. then sodium borohydride was slowly added to the mixture while monitoring the reaction with TLC. When TLC showed that all the imine had been converted 20 mL of distilled water was added to the mixture. Then the product was extracted from the aqueous solution using 20 mL of ethyl acetate. Then the ethyl acetate was evaporated using a rotovap to get an orange oil (734 mg, 76.4%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.07 (d, 2H), 7.49 (d, 2H), 7.06 (t, 2H), 6.67 – 6.53 (m, 3H), 4.38 (s, 2H).

^{13}C NMR (101 MHz, Methanol-d_4) δ 148.7, 148.1, 146.8, 128.8, 127.6, 123.2, 116.9, 112.6, 60.3, 47.5, 47.3, 46.6, 13.1.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{13}H_{13}O_2N_2$ 229.0972, mass found 229.0962

5.4 Synthesis of 4,4'-(phenylmethylene)bis(*N*-ethylaniline)



N-ethyl aniline (500 mg, 4.13 mmol) and benzaldehyde (219 mg, 2.06 mmol) was added to a 25 mL round bottom flask and dissolved in a hydrochloric acid water solution (pH = 1). Then the mixture was put on reflux at a constant 100°C for 14 hours. After refluxing the acid mixture was neutralised using sodium carbonate and a green sluggish precipitate crushed out of the solution. the green precipitate was then filtered and washed with plenty of water to get rid of excess sodium carbonate. Then the precipitate was transferred to a 50 mL round bottom flask and dried using a high vacuum pump overnight (386 mg, 56.7 %).

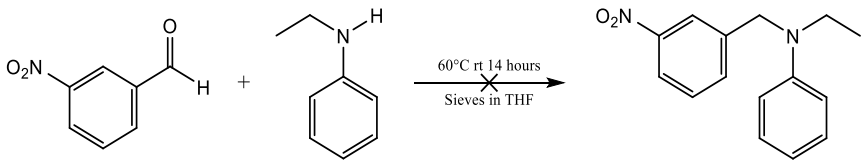
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.21 – 7.05 (m, 5H), 6.87 (d, 2H), 6.64 (d, 2H), 6.56 (d, 2H), 5.29 (s, 1H), 3.08 (q, *J* = 7.2 Hz, 2H), 3.03 (q, *J* = 7.1 Hz, 2H), 1.20 (t, *J* = 7.2 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, Methanol-d_4) δ 148.8, 146.8, 145.6, 133.8, 129.8, 129.1, 128.7, 127.7, 125.5, 117.0, 113.1, 113.1, 55.3, 38.6, 38.2, 13.6, 13.6.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{23}H_{27}N_2$ 331.2174, mass found 331.2171

5.5 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline

5.5.1 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline using iminium pathway



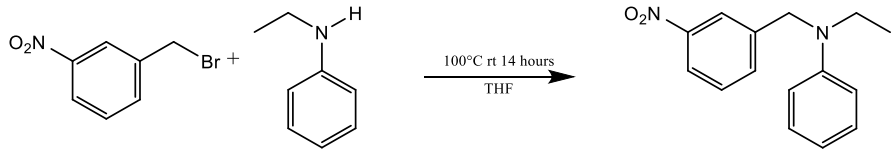
N-ethylaniline (400 mg, 3.30 mmol) and 3-nitrobenzaldehyde (500 mg, 3.30 mmol) was added to a 100 mL round bottom flask and dissolved in 20 mL of THF and applied 5g of molecular sieves (5 Å). Then 0.190 mL of acetic acid was added to the mixture and it was heated under reflux at a constant 60°C for 14 hours. After heating the sieves was filtered out of solution and the mixture was transferred to a 100 mL two neck round bottom flask and heated at a constant 40 °C while adding sodium borohydride to the solution. After hydrogenation, water was added to the solution and the product was extracted using ethyl acetate. The ethyl acetate was evaporated leaving an orange oil. (828 mg, 97.9%)

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.23 (s, 1H), 8.08 (d, 1H), 7.69 (d, 1H), 7.51 (t, J = 7.9 Hz, 1H), 7.11 (t, 3H), 6.68 – 6.60 (m, 4H), 4.70 (s, 2H), 3.08 (q, J = 7.1 Hz, 3H), 1.21 (t, J = 7.2 Hz, 5H).

^{13}C NMR (101 MHz, Methanol-d_4) δ 148.9, 148.3, 144.1, 132.4, 129.6, 129.2, 128.7, 121.6, 120.9, 116.9, 113.0, 62.6, 47.6, 47.3, 47.1, 38.3, 38.2, 13.6, 13.2.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{15}H_{17}O_2N_2$ 257.1290, mass not found

5.5.2 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline using a S_N2 reaction



1-(bromomethyl)-3-nitrobenzene (180 mg, 0.833 mmol) and *N*-ethylaniline (101 mg, 0.833 mmol) was added to a 100 mL round bottom flask and dissolved in 20 mL of THF. The mixture was heated at a constant 100°C under reflux for 14 hours. After the time had passed the mixture was slowly cooled down and washed with saturated sodium carbonate solution. The organic solvent was then evaporated leaving an orange oil (210 mg, 98.4%).

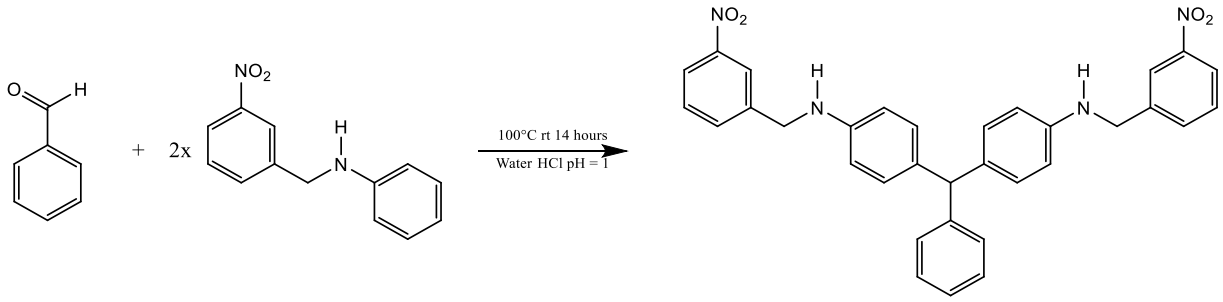
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 (s, 1H), 7.92 (d, 1H), 7.44 (d, *J* = 7.7, 1.4 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.10 – 7.02 (m, 2H), 6.60 – 6.51 (m, 3H), 4.44 (s, 2H), 3.37 (q, *J* = 7.1 Hz, 2H), 1.09 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.7, 148.0, 142.2, 132.9, 129.7, 129.5, 122.1, 121.6, 117.0, 112.6, 53.8, 45.7, 12.2.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{15}H_{17}O_2N_2$ 257.1290, mass found 257.1285

5.6 Synthesis of Brilliant Blue R250 derivatives

5.6.1 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline)



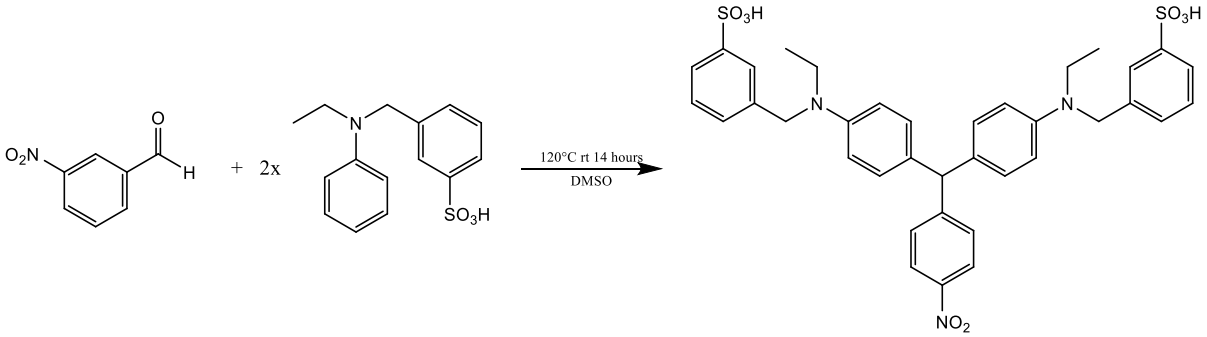
N-(3-nitrobenzyl)aniline (105 mg, 0.460 mmol) and benzaldehyde (24.0 mg, 0.230 mmol) was mixed together in a 25 mL round bottom flask and added 10 mL pH ~ 1 hydrochloric acid water solution. The mixture was then stirred under inert gas at 100°C for 14 hours. Then the solution was slowly cooled down to room temperature and neutralized using sodium carbonate to give a blue precipitate in the aqueous solution. The blue precipitate was then filtered out and washed with plenty of water. The powder and the filter paper were then putt in a 100 mL round bottom flask and dried using a high vacuum pump. (92 mg, 73.4%)

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.28 (s, 1H), 8.24 – 8.16 (m, 5H), 8.08 (d, J = 10.3 Hz, 3H), 7.84 – 7.77 (m, 4H), 7.75 (d, J = 7.6 Hz, 1H), 7.64 – 7.57 (m, 5H), 7.53 – 7.40 (m, 2H), 7.40 – 7.33 (m, 2H), 7.30 – 7.11 (m, 11H), 6.95 (d, J = 7.5 Hz, 2H), 5.66 (s, 1H), 4.68 (s, 5H).

^{13}C NMR (101 MHz, Methanol-d_4) δ 136.6, 136.4, 130.9, 130.2, 130.2, 129.8, 128.9, 128.5, 125.3, 124.2, 123.1, 122.8, 22.83.

HRMS (ESI) m/z: [M-H$^+$] calculated for $C_{33}H_{29}O_4N_4$ 545.2189, mass found 545.2177

5.6.2 3,3'-(((((4-nitrophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



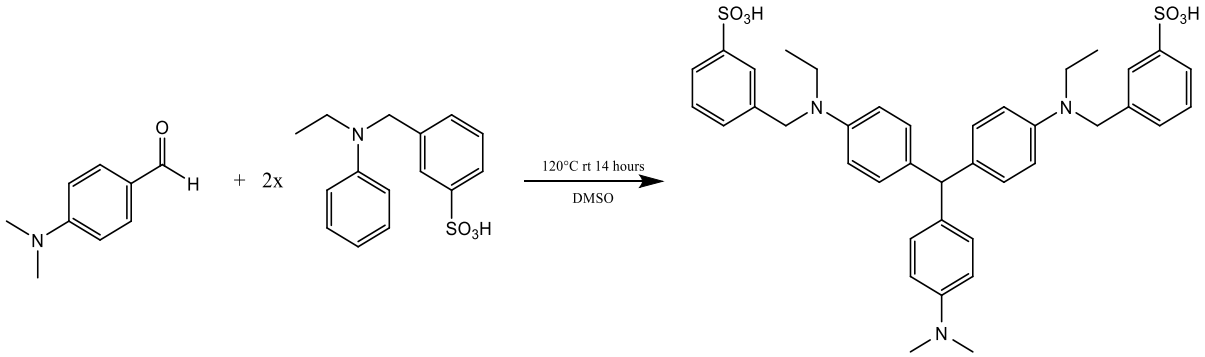
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 4-nitrobenzaldehyde (130 mg, 0.860 mmol) was measured in a 50 mL round bottom flask and dissolved in 3 mL of DMSO. The mixture was putt on stirring at a constant 120 °C for 14 hours. After the time passed the mixture was added 20 mL of water and a green powder precipitated out of solution. The precipitate was then filtered and washed with 3x20 mL of water. After washing with water, the precipitate was washed with 3x20 mL of ethyl acetate. After washing with ethyl acetate, the product was allowed to dry and gave a green powder (442 mg, 71.9%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 8.8, 1.7 Hz, 1H), 7.61 – 7.47 (m, 3H), 7.31 (d, J = 8.8, 1.9 Hz, 1H), 7.26 (t, J = 7.8, 1.8 Hz, 2H), 7.20 – 7.09 (m, 2H), 7.05 (s, 3H), 6.94 (s, 1H), 5.73 (s, 1H), 4.70 (s, 3H), 1.13 – 0.98 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 149.0, 130.8, 128.3, 124.2, 60.2, 54.2, 40.9, 21.5, 21.2, 14.5.

HRMS (ESI) m/z: [M^-] calculated for $C_{37}H_{35}O_8N_3S_2$ 356.5938, mass found 356.5935

5.6.3 3,3'-(((((4-(dimethylamino)phenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



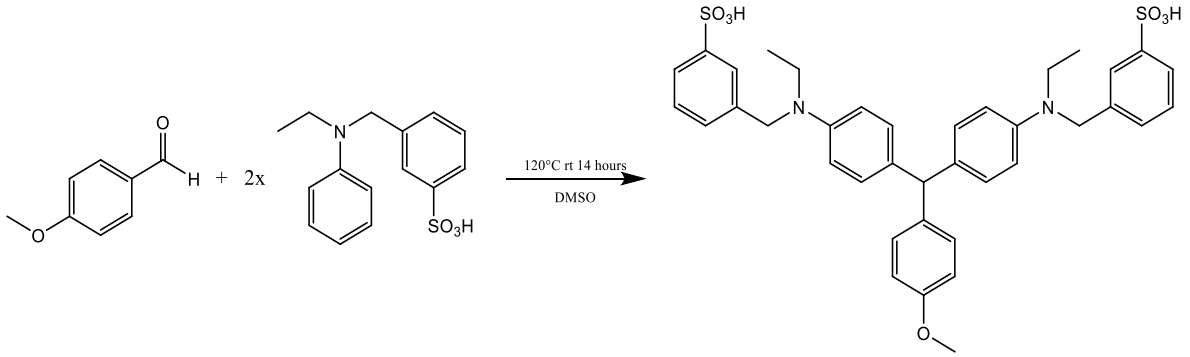
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 4-(dimethylamino)benzaldehyde (128 mg, 0.860 mmol) was mixed together in a 50 mL round bottom flask and dissolved in 3 mL of DMSO. the mixture was then stirred under a constant 120 °C for 14 hours. After the time had passed the mixture was slowly cooled to room temperature. After cooling 10 mL of methanol and 20 mL of ethyl acetate was added to the mixture and a blue precipitate crushed out. The precipitate was filtered out of the mixture and washed with cold methanol and ethyl acetate solution. The blue powder was then allowed to dry over night to give (266 mg, 43.4%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 2H), 7.58 (d, J = 7.8, 1.4 Hz, 2H), 7.48 – 7.38 (m, 7H), 7.24 (t, J = 7.6 Hz, 2H), 7.06 (d, J = 7.7, 1.5 Hz, 2H), 3.72 (q, J = 7.1 Hz, 4H), 2.57 (s, 1H), 1.06 (t, J = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 148.8, 130.4, 128.4, 49.1, 40.9, 10.9.

HRMS (ESI) m/z: [M^-] calculated for $C_{39}H_{41}O_6N_3S_2$ 355.6224, mass found 355.6221

5.6.4 3,3'-(((((4-methoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



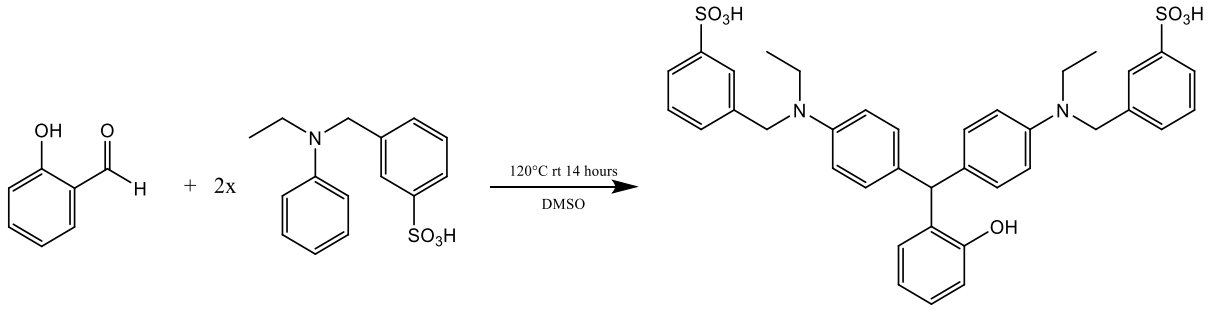
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 4-methoxybenzaldehyde (117 mg, 0.860 mmol) was mixed together in a round bottom flask and dissolved in 3 mL of DMSO and heated at a constant temprature of 120 °C while stirring for 14 hours. After heating the reaction mixture was slowly cooled down to room temperature. Then 10 mL of methanol and 20 mL of ethyl acetate was added to the mixture and a green powder precipitated from the mixture. The precipitate was then filtered from the solution and washed with cold methanol and ethyl acetate solution. The green powder pumped dry over night using a high vacuum pump (413 mg, 68.6%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (s, 1H), 7.54 (t, 2H), 7.43 – 7.28 (m, 0H), 7.27 (s, 0H), 7.23 (t, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.04 (s, 2H), 6.96 – 6.80 (m, 3H), 5.51 (s, 1H), 4.72 (s, 4H), 1.11 – 0.99 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 149.0, 130.6, 130.4, 128.2, 114.4, 55.5, 40.9.

HRMS (ESI) m/z: [M^-] calculated for $C_{38}H_{38}O_7N_2S_2$ 349.1066, mass found 349.1063.

5.6.5 3,3'-(((((2-hydroxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



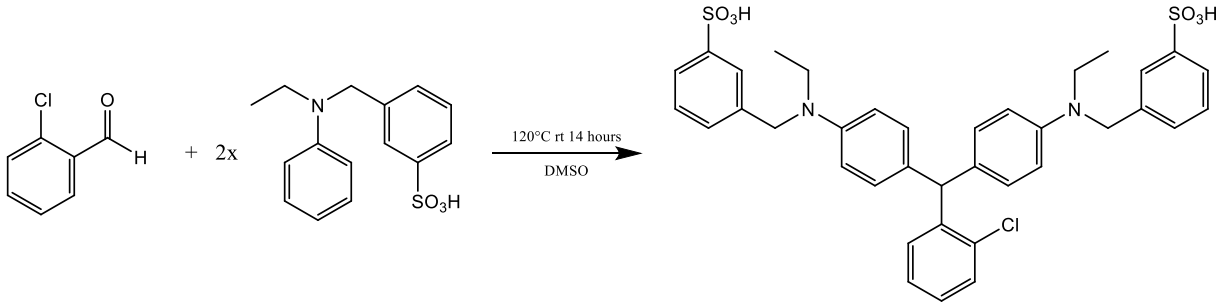
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 2-hydroxybenzaldehyde (105 mg, 0.86 mmol) was mixed together in a 50 mL round bottom flask and dissolved in 3 mL of DMSO. The mixture was then refluxed under a constant 120 °C for 14 hours. After heating the mixture was allowed to cool to room temperature and then 10 mL of methanol and 20 mL of ethyl acetate was added to the mixture and a green powder precipitated out of the mixture. Then the precipitate was filtered and washed with cold methanol and ethyl acetate solution. the powder was then pumped dry using a high vacuum pump, and then put on argon. (98 mg, 16.6%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (s, 1H), 7.55 (t, J = 7.9 Hz, 2H), 7.23 (t, J = 7.6 Hz, 2H), 7.17 (d, J = 8.0 Hz, 1H), 7.10 – 6.94 (m, 5H), 6.80 (d, 1H), 6.74 (t, J = 7.5 Hz, 1H), 6.55 (d, J = 7.5 Hz, 0H), 5.71 (s, 1H), 4.72 (s, 4H), 1.12 – 0.98 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 149.0, 130.7, 128.2, 49.1, 40.9.

HRMS (ESI) m/z: [M^-] calculated for $C_{37}H_{36}O_7N_2S_2$ 342.0987, mass found 342.0985.

5.6.6 3,3'-(((((2-chlorophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



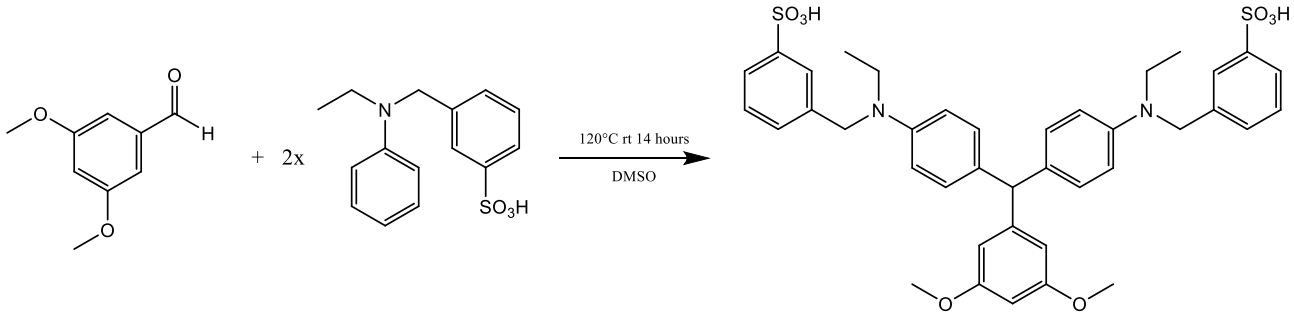
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 2-chlorobenzaldehyde (121 mg, 0.860 mmol) was added in a 50 mL round bottom flask and dissolved in 3 mL DMSO. The mixture was then put on reflux for 2 hours at a constant 120 °C. After heating the mixture was slowly cooled to room temperature, then added 10 mL of methanol and 20 mL of ethyl acetate and a cyan powder precipitated out of solution. the cyan precipitate was then filtered and washed with water and cold ethyl acetate. The precipitate was then dried using a high vacuum pump (187 mg, 30.9%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (s, 1H), 7.58 – 7.51 (m, 2H), 7.44 (d, J = 7.7, 1.4 Hz, 1H), 7.37 – 7.14 (m, 4H), 7.07 – 6.94 (m, 3H), 6.89 – 6.65 (m, 1H), 5.82 (s, 1H), 4.72 (s, 4H), 1.11 – 1.02 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 149.0, 133.8, 130.8, 128.2, 60.2, 40.9, 21.2, 14.6.

HRMS (ESI) m/z: [M^-] calculated for $C_{37}H_{35}O_6N_2ClS_2$ 351.0818, mass found 351.0816

5.6.7 3,3'-(((((3,5-dimethoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid



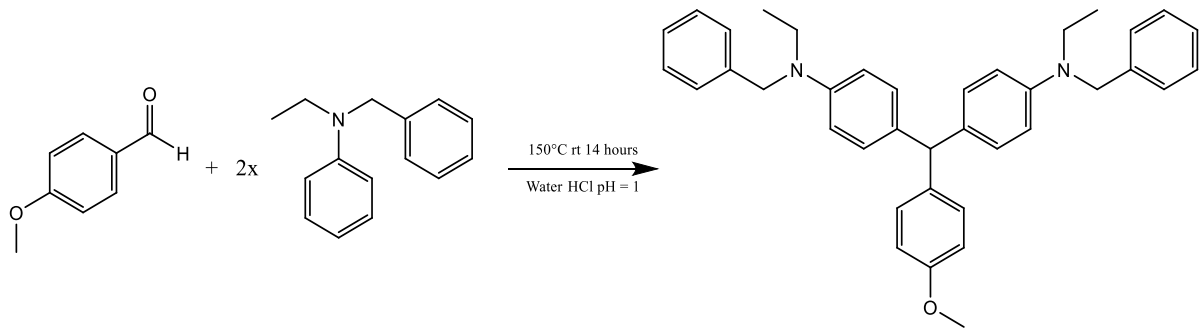
3-((ethyl(phenyl)amino)methyl)benzenesulfonic acid (500 mg, 1.72 mmol) and 3,5-dimethoxybenzaldehyde (143 mg, 0.86 mmol) was added to a 50 mL round bottom flask and disolved in 3 mL of DMSO. The mixture was then put on reflux and heated at a constant 120°C over night. After heating the reaction mixture was cooled down to room temperature, then 10 mL of methanol and 20 mL of ethyl acetate was added to the mixture and a green powder precipitated out of the solution. the precipitate was then filtered and washed with water and cold ethyl acetate. After washing the powder was dried using a high vacuum pump (343 mg, 54.6%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 2H), 7.60 – 7.49 (m, 2H), 7.44 – 7.34 (m, 2H), 7.21 (t, 2H), 7.04 (d, J = 7.6 Hz, 1H), 6.37 (t, J = 2.3 Hz, 1H), 6.17 – 6.10 (m, 1H), 5.50 (s, 0H), 4.74 (s, 4H), 3.66 (s, 4H), 1.10 – 1.01 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 160.9, 149.0, 130.7, 130.2, 128.3, 128.2, 107.9, 55.6, 40.9.

HRMS (ESI) m/z: [M^-] calculated for $C_{39}H_{40}O_8N_2S_2$ 364.1119, mass found 364.1115.

5.6.8 4,4'-((4-methoxyphenyl)methylene)bis(*N*-benzyl-*N*-ethylaniline)



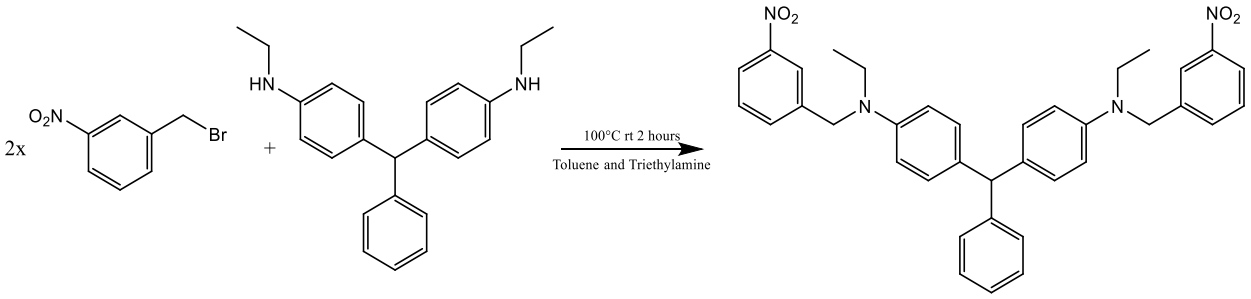
N-benzyl-*N*-ethylaniline (500 mg, 2.37 mmol) and 4-methoxybenzaldehyde (161 mg, 1.18 mmol) was was transferred to a 50 mL round bottom flask and disolved in 10 mL of pH = 1 hydrochloric acid and water mixture. After that the reaction mixture was put on reflux at a constant 150°C for 14 hours. When the reaction was complete a green goo had settled at the bottom of the flask. The acid water solution was poured off in to an Erlend Meyer flask and the green goo was washed with heptane. After washing the goo was put on a high vacuum pump overnight. The next day the solid were crushed using a glass rod to give a green powder (519 mg, 81.4%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, 1H), 7.67 – 7.57 (m, 3H), 7.46 (d, J = 7.0 Hz, 3H), 7.37 – 7.19 (m, 5H), 7.17 – 7.09 (m, 1H), 6.95 (d, J = 8.4 Hz, 3H), 6.92 – 6.74 (m, 3H), 5.56 (s, 1H), 4.83 – 4.75 (m, 5H), 3.74 (s, 4H), 1.21 – 1.09 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 191.8, 158.3, 135.2, 132.3, 130.6, 130.4, 130.4, 130.3, 130.1, 128.7, 128.6, 128.5, 127.1, 126.9, 123.8, 115.0, 114.4, 114.3, 60.3, 56.2, 55.6, 54.0, 53.7, 46.7, 21.6, 21.3, 14.6, 11.3.

HRMS (ESI) m/z: [M+H$^+$] calculated for $C_{38}H_{41}ON_2$ 541.3219, mass found 541.3218.

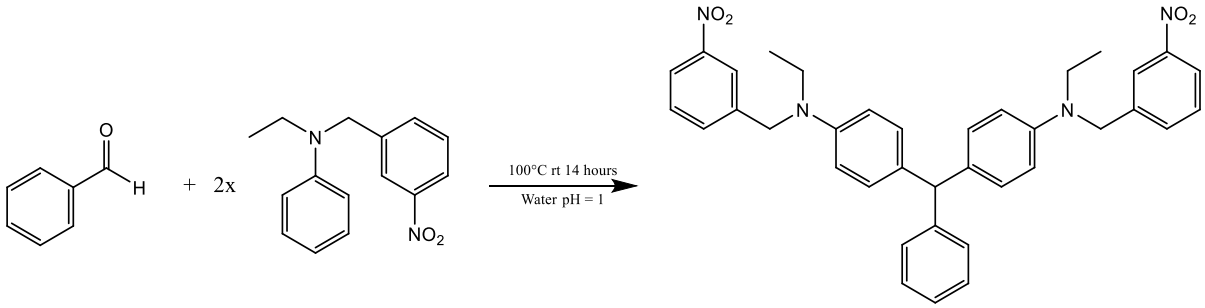
5.6.9 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using the S_N2 reaction.



4,4'-(phenylmethylene)bis(*N*-ethylaniline) (100 mg, 0.303 mmol) and 1-(bromomethyl)-3-nitrobenzene (131 mg, 0.606 mmol) where mixed together in a 50 mL round bottom flask, dissolved in 15 mL of toluene and added 2 mL of triethylamine. The mixture was then refluxed at a constant 100 ℃ for 2 hours. the solvent was then washed using water and then the toluene was evaporated to yield a brown tar (52 mg, 28,6%)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 – 7.13 (m, 2H), 7.12 – 7.07 (m, 2H), 7.07 – 7.02 (m, 2H), 6.99 – 6.89 (m, 1H), 6.86 – 6.80 (m, 2H), 6.54 – 6.48 (m, 0H), 6.45 (d, 2H), 6.42 – 6.35 (m, 1H), 5.25 (s, 1H), 3.04 (q, *J* = 7.2, 2.3 Hz, 4H), 2.09 (s, 4H), 1.19 – 1.11 (m, 6H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 146.6, 133.5, 130.2, 130.1, 129.5, 129.4, 129.4, 129.1, 128.3, 128.1, 128.1, 127.9, 125.8, 125.3, 122.1, 112.9, 112.6, 112.4, 55.2, 45.6, 38.7, 30.9, 14.0, 12.2.
HRMS (ESI) m/z: [M+H$^+$] calculated for $C_{37}H_{37}O_4N_4$ 601.2815, mass found 601.2807.

5.6.10 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using *N*-ethyl-*N*-(3-nitrobenzyl)aniline



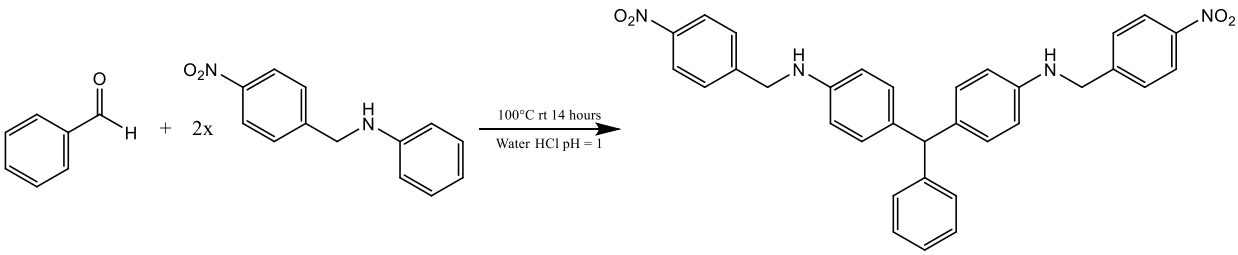
N-ethyl-*N*-(3-nitrobenzyl)aniline (207 mg, 0.807mmol) and Benzaldehyde (42.8mg, 0.403 mmol) where mixed together in a 50 mL round bottom flask. The mixture was then added 10 mL hydrochloric acid water mixture (pH = 1) and refluxed at 100 °C for 14 hours. After refluxing, the mixture was neutralised using sodium carbonate and a brown powder crushed out of solution. the powder was filtered and washed with plenty of water to yield a brown powder (102 mg, 42.2%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.42 (s, 3H), 2.10 (s, 8H), 1.27 – 1.15 (m, *J* = 5.7, 4.3 Hz, 4H), 0.81 (t, *J* = 6.7 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 207.0, 60.4, 50.9, 31.9, 30.9, 29.0, 22.7, 14.2, 14.1.

HRMS (ESI) m/z: [M+H^{+}] calculated for $C_{37}H_{37}O_4N_4$ 601.2815, mass found 601.2798. [M+H^{+}] calculated for $C_{37}H_{35}O_4N_4$ 599.2658, mass found 599.2652.

5.6.11 Attempt to synthesize 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline)



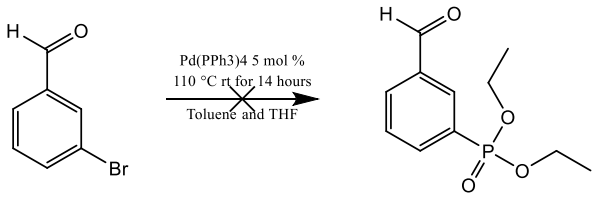
N-(4-nitrobenzyl)aniline (100 mg, 0.438 mmol) and benzaldehyde (0.023 mg , 0.219 mmol) was mixed together in a 50 mL round bottom flask and added 0.1M hydrochloric solution and was heated to 100 ℃ under reflux for 14 hours. after the 14 hours had passed the mixture was neutralised using sodium carbonate and a green powder crushed out of solution. the green powder was then filtered and washed with plenty of water and was then put on high vacuum pump over night to give a dark green powder (70 mg, 56.2%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.08 (m, 5H), 7.88 (d, 1H), 7.56 – 7.53 (m, 5H), 7.25 – 7.18 (m, 4H), 7.01 (d, *J* = 5.7, 2.3 Hz, 2H), 6.91 (d, *J* = 8.3 Hz, 2H), 6.88 – 6.81 (m, 2H), 6.78 (d, *J* = 8.2 Hz, 3H), 5.37 (s, 1H), 4.45 (s, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 192.5, 147.7, 143.7, 134.6, 130.5, 130.3, 130.2, 129.9, 129.7, 129.3, 129.1, 128.6, 128.5, 126.5, 124.1, 123.9, 123.9, 55.4.

HRMS (ESI) m/z: [M+H^{+}] calculated for $C_{33}H_{29}O_4N_4Na$ 567.2008, Mass found 567.2003

5.7 Attempt to synthesize diethyl (3-formylphenyl)phosphonate



3-Bromobenzaldehyde (100 mg, 0.540 mmol) and Tetrakis(triphenylphosphine)palladium(0) (31mg , 0.027 mmol) was mixed together in a 50 mL two neck round bottom flask. The mixture was then added 5 mL of triethyl amine and 20 mL of 1:1 Toluene THF solution. the mixture was then heated to 110 °C and then slowly added diethyl phosphite (75 mg, 0.540 mmol) disolved in 1:1 Toluene THF solution. the mixture was then refluxed for 14 hours. After heating the mixture was allowed to cool and the mixture was washed with water. Then the solvent was evaporated leaving a yellow oil (125 mg, 95.6 %)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (s, 1H), 7.29 – 7.22 (m, 2H), 7.03 (t, *J* = 7.8 Hz, 1H), 5.76 (s, 1H), 4.88 (d, *J* = 13.2 Hz, 1H), 4.00 – 3.78 (m, 4H), 1.07 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 140.1, 130.6 (d, *J* = 3.2 Hz), 130.0 (d, *J* = 5.5 Hz), 129.6 (d, *J* = 2.6 Hz), 125.8 (d, *J* = 5.5 Hz), 122.0 (d, *J* = 3.0 Hz), 69.5 (d, *J* = 163.5 Hz), 63.2 (d, *J* = 3.3 Hz), 63.2 (d, *J* = 3.6 Hz), 16.3 (d, *J* = 5.7 Hz).

HRMS (ESI) m/z: [M+H^{+}] calculated for $C_{11}H_{16}O_4BrNaP$ 344.9862, Mass found 344.9853

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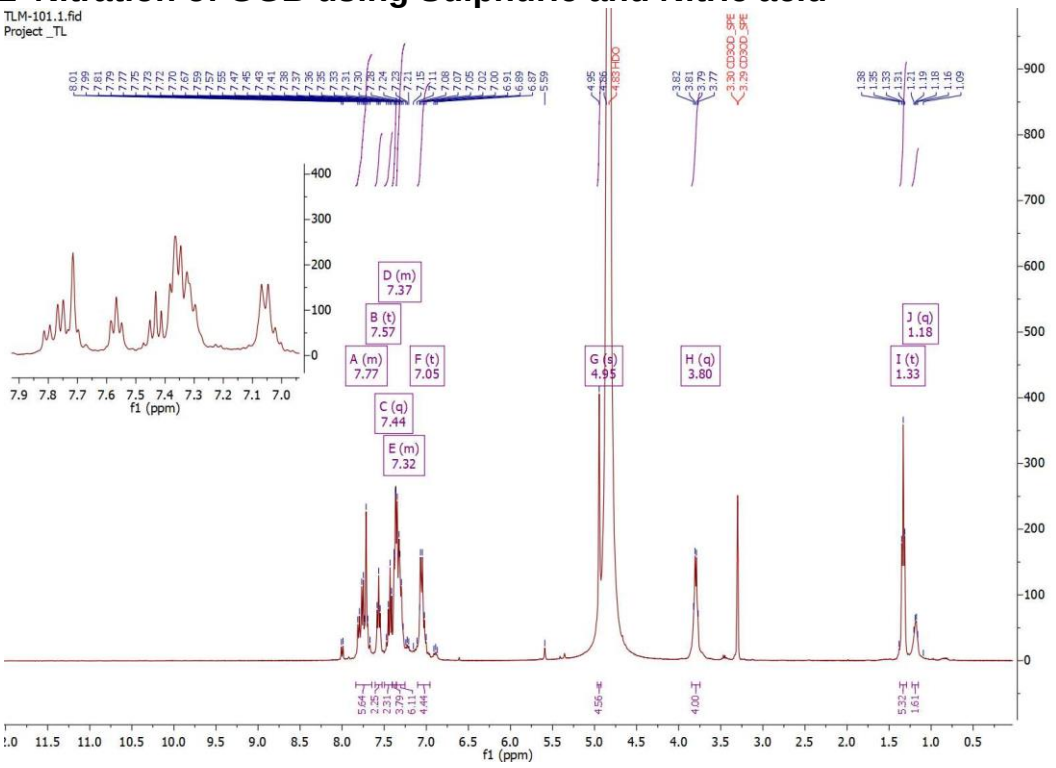
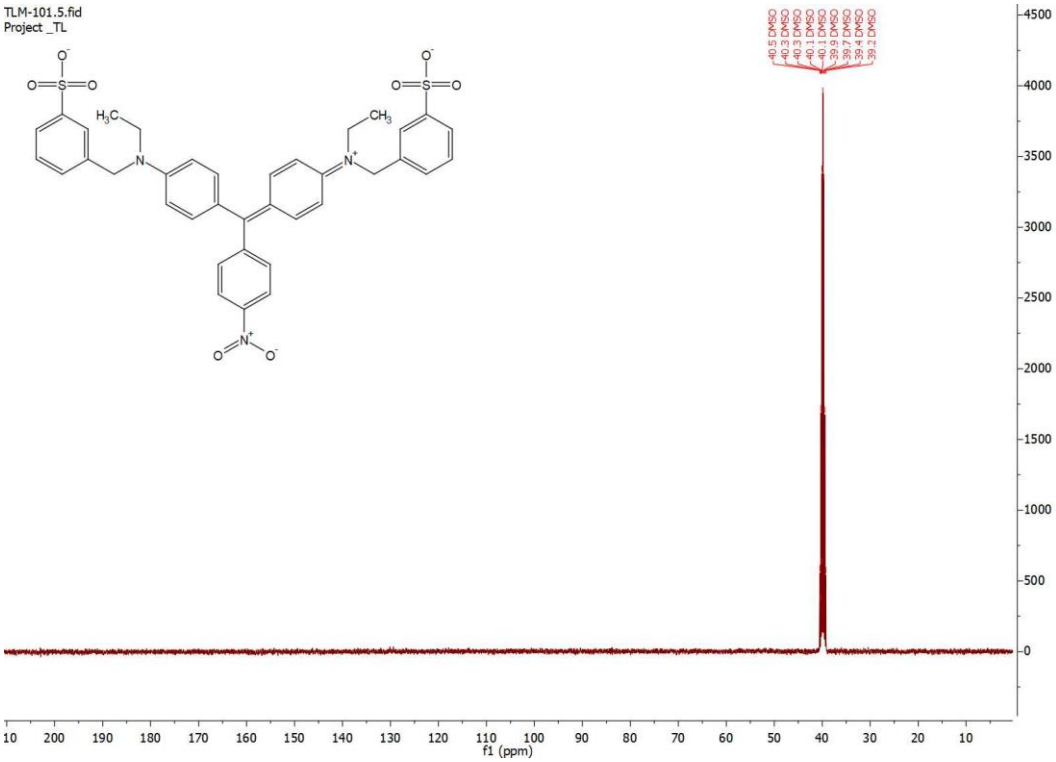
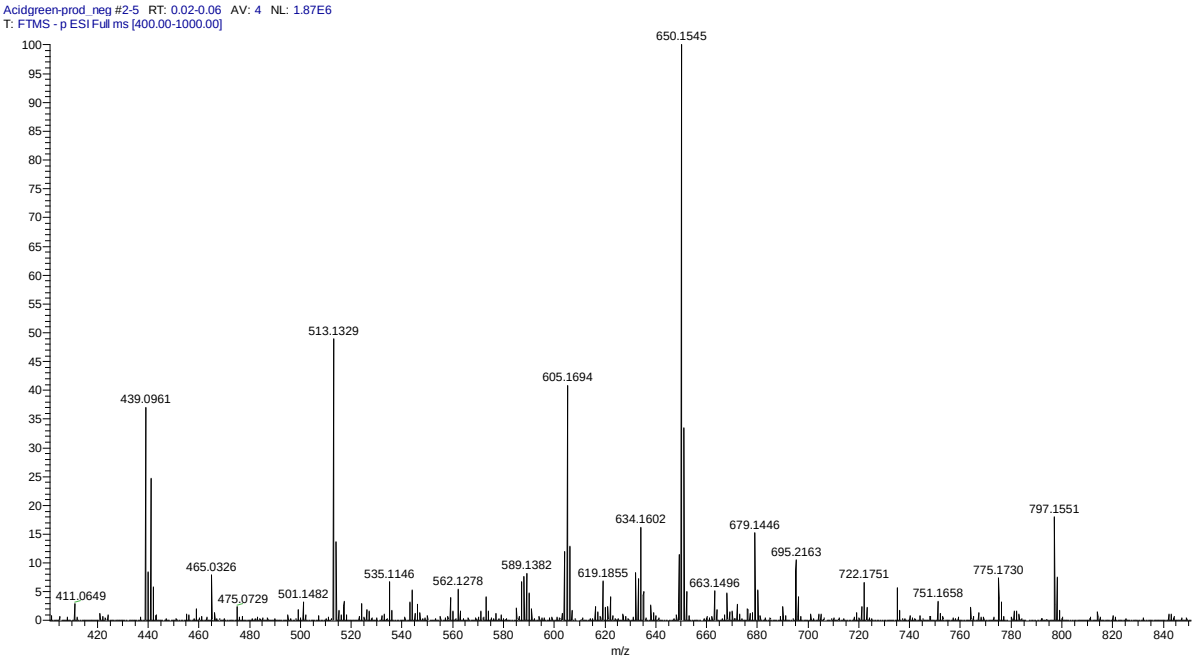
7 Appendix

7.1 Nitration Reactions

7.1.1 Nitration of GGB using Sulphuric and Nitric acid



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605.1694
439.0961
634.1602
679.1446
797.1551
695.2163
465.0326
589.1382
619.1855
722.1751
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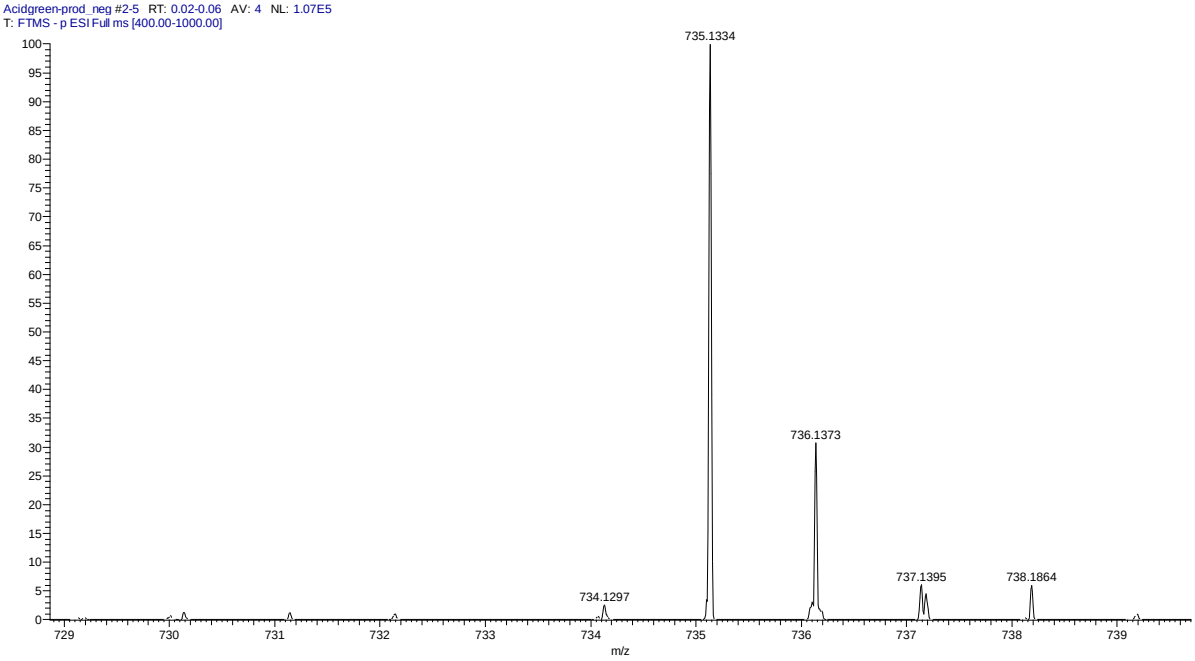
535.1146
562.1278
663.1496
751.1658
411.0649
475.0729
501.1482
m/z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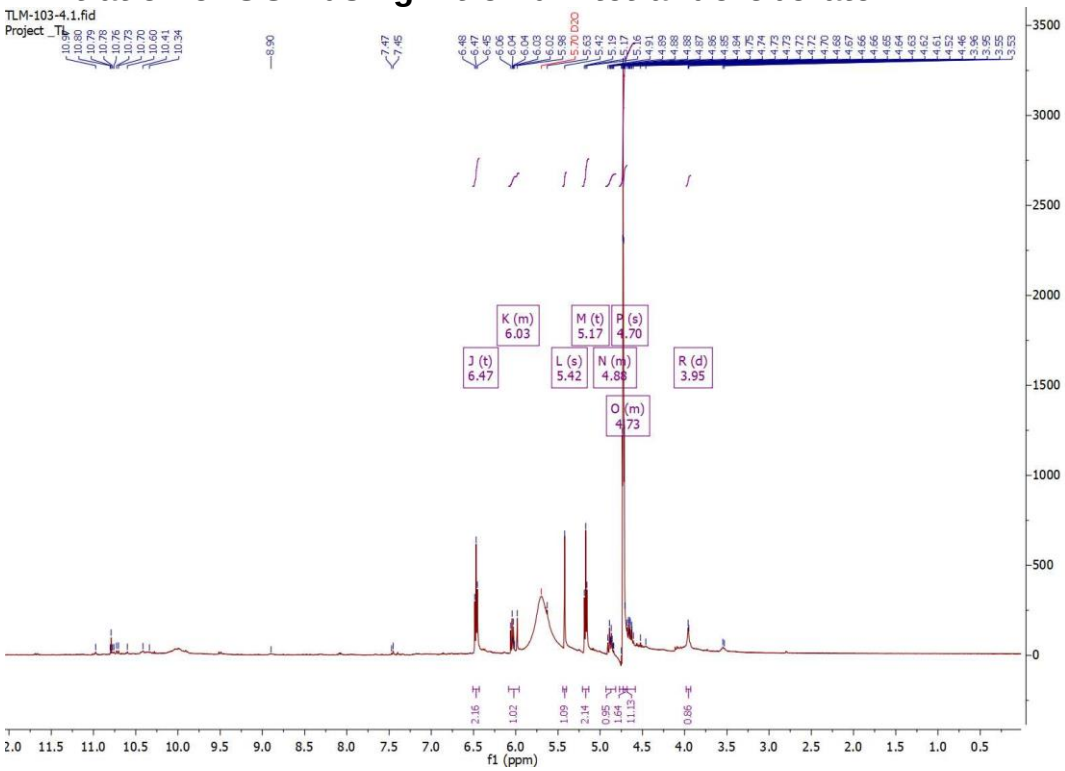
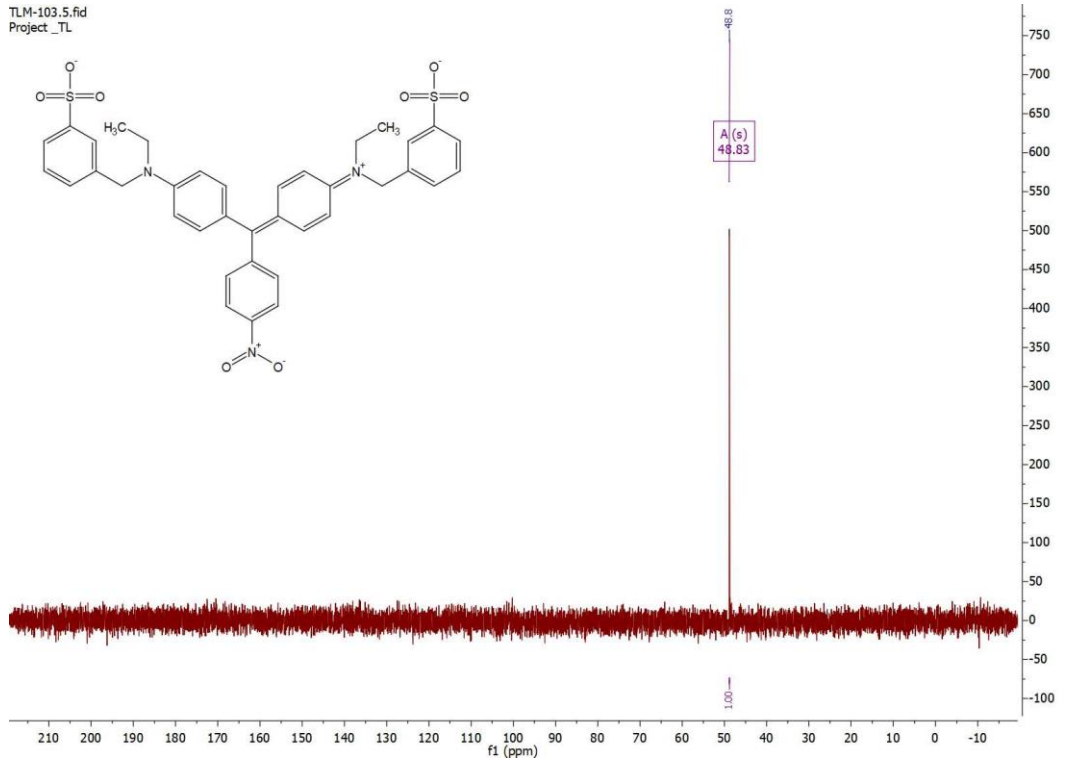
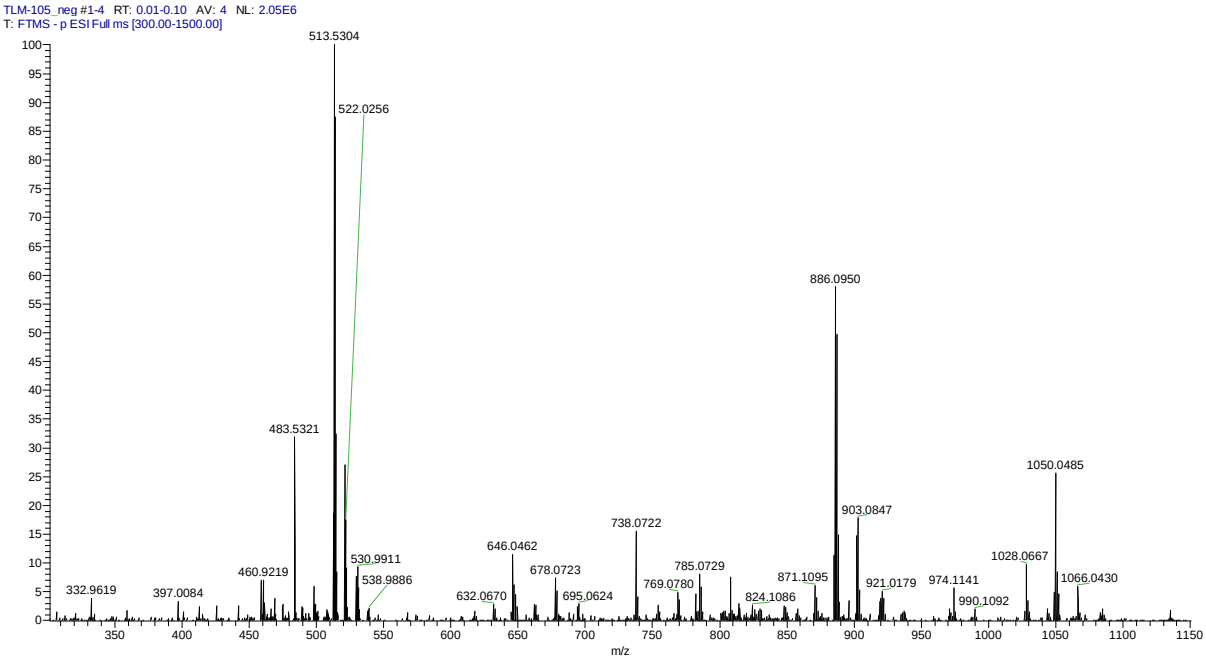
734.1297
m/z

7.1.2 Nitration of GGB using nitronium tetrafluoroborate



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886.0950
483.5321
1050.0485
903.0847
738.0722
646.0462
530.9911
538.9886
678.0723
785.0729
1028.0667
460.9219
769.0780
871.1095
921.0179
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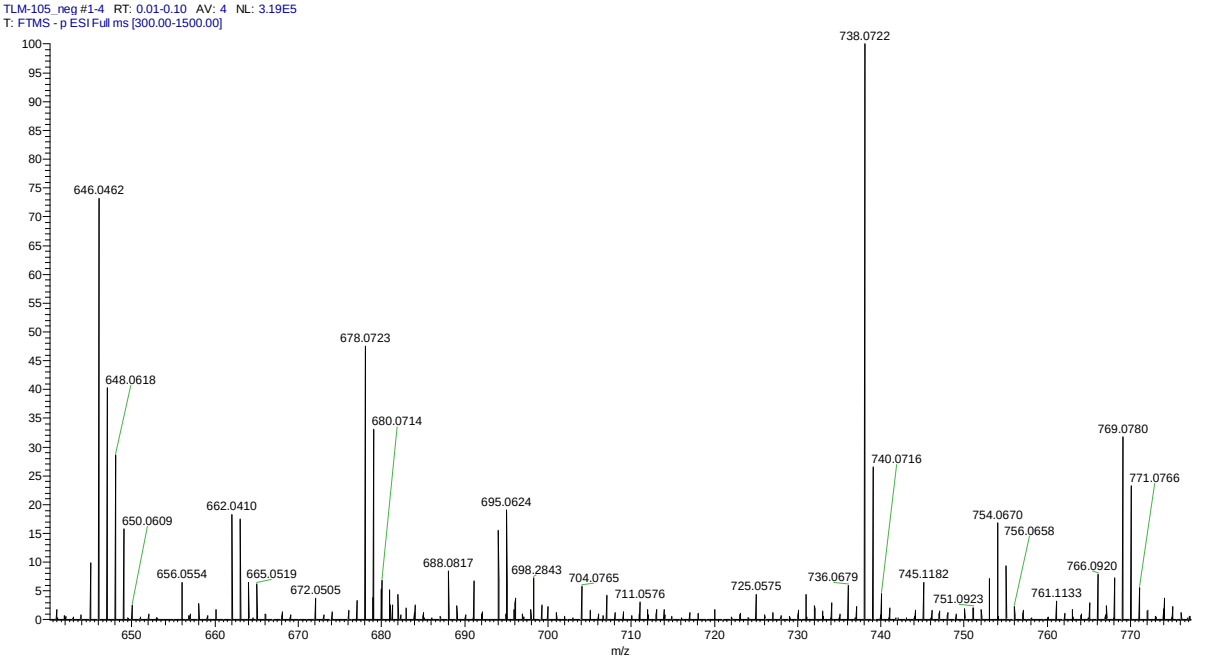
m/z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648.0618
680.0714
769.0780
740.0716
771.0766
695.0624
662.0410
650.0609
754.0670
756.0658
656.0554
665.0519
688.0817
698.2843
704.0765
672.0505
711.0576
725.0575
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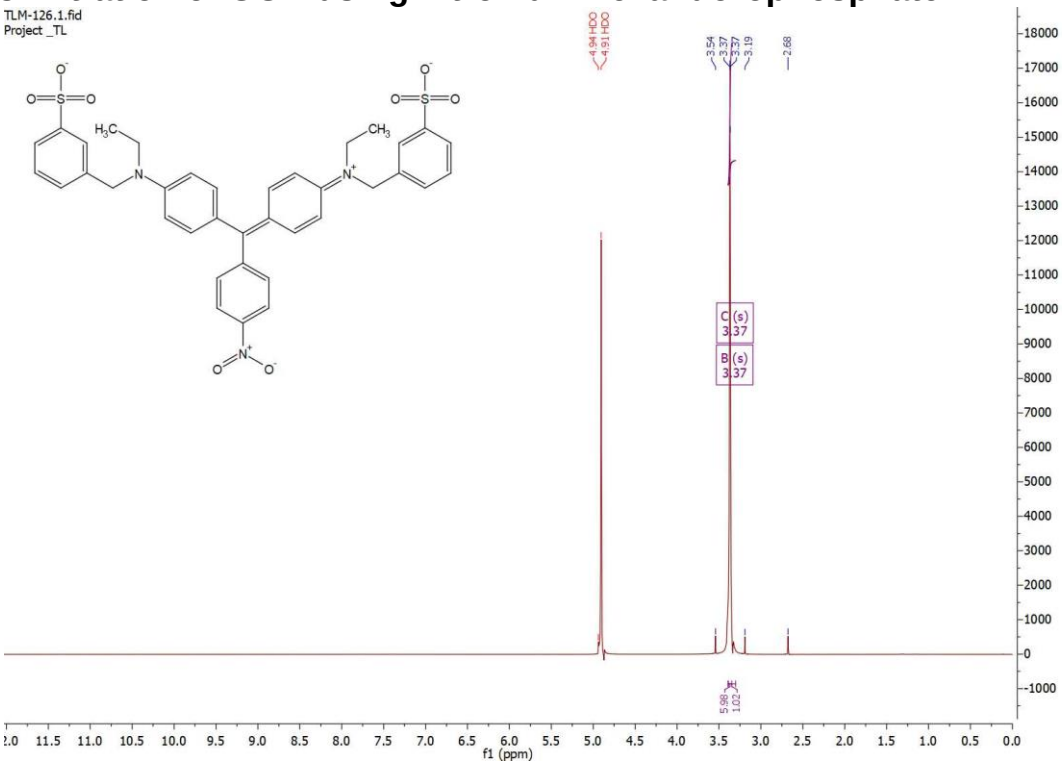
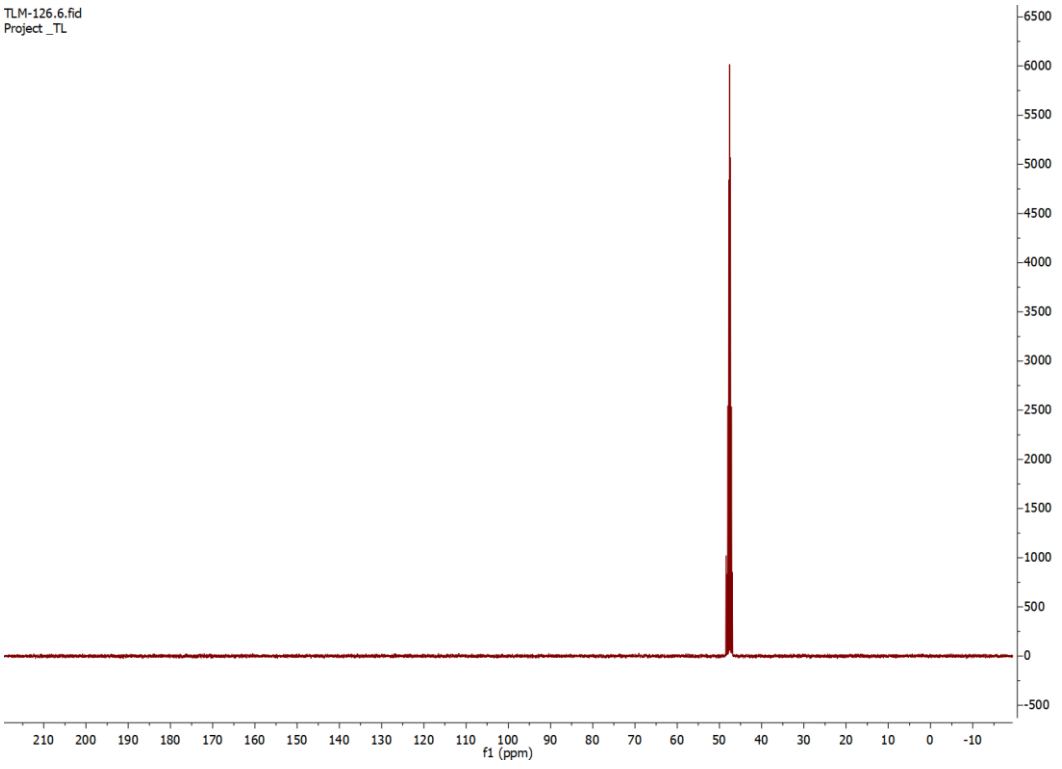
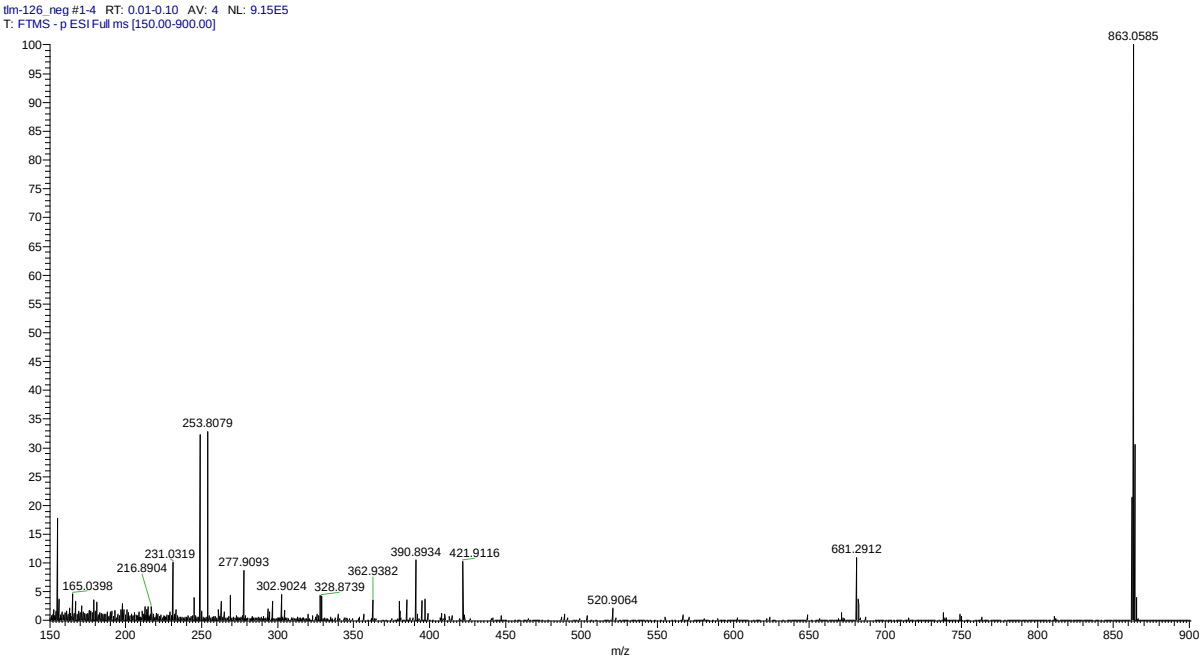
m/z

7.1.3 Nitration of GGB using nitronium hexafluorophosphate



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231.0319
216.8904
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302.9024
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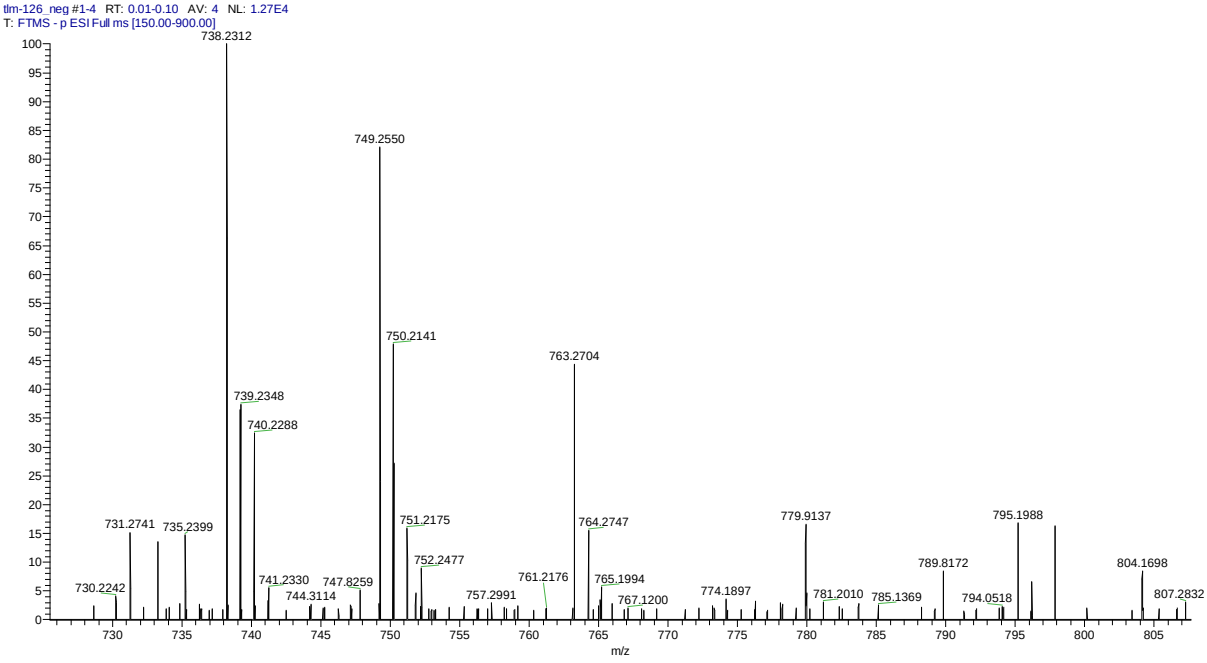
362.9382
390.8934
421.9116
520.9064
681.2912
m/z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763.2704
739.2348
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731.2741
735.2399
751.2175
764.2747
752.2477
779.9137
795.1988
789.8172
804.1698
730.2242
741.2330
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765.1994
757.2991
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774.1897
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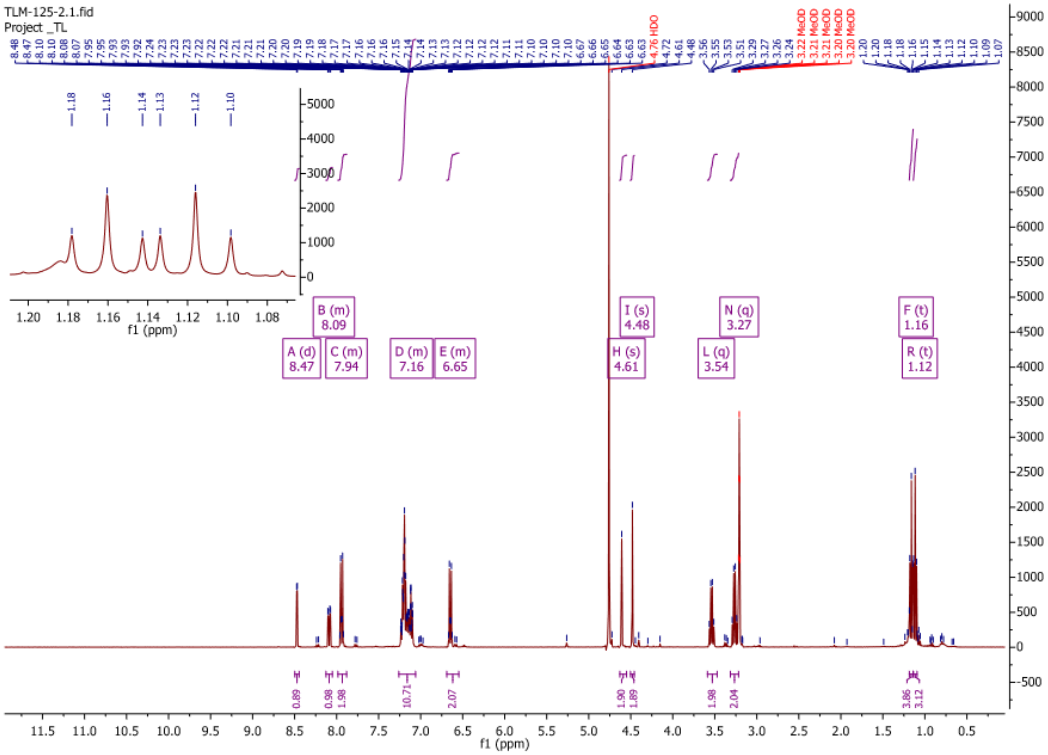
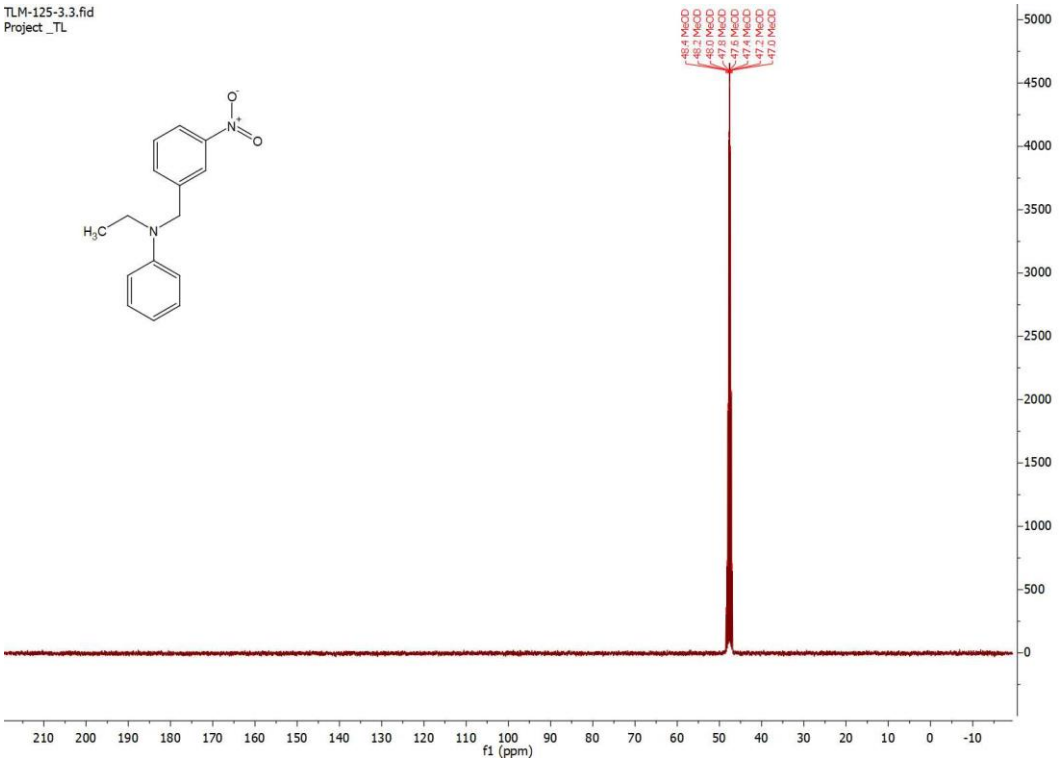
m/z

7.1.4 Nitration of *N*-Benzyl-*N*-ethylaniline using nitronium hexafluorophosphate





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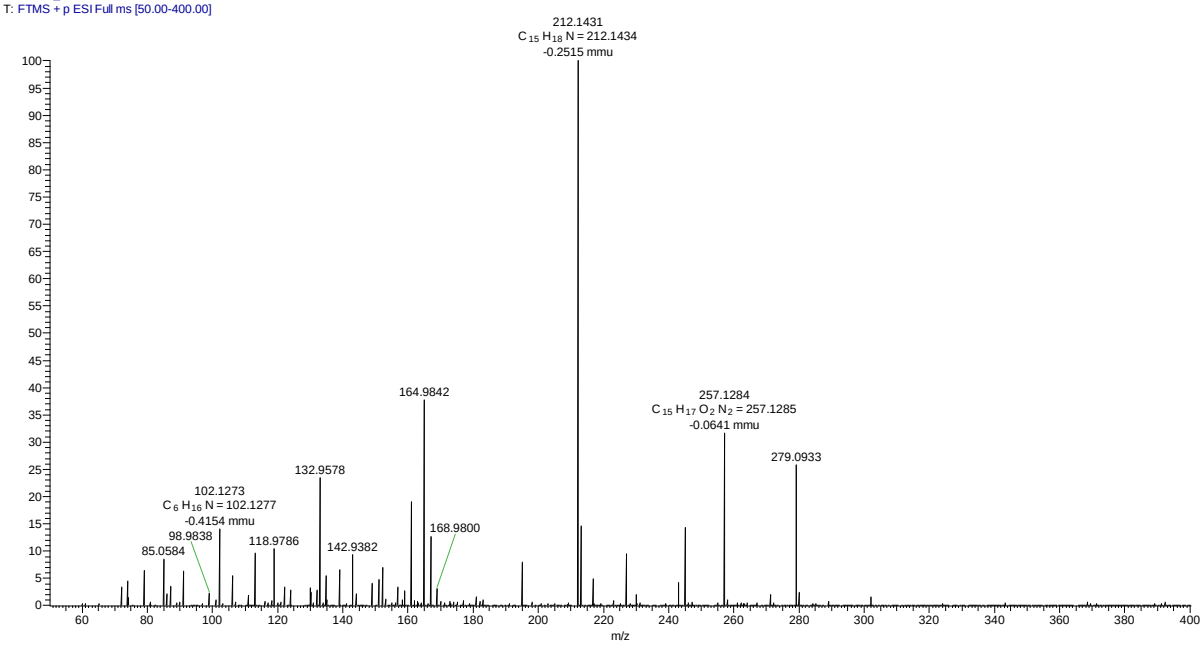

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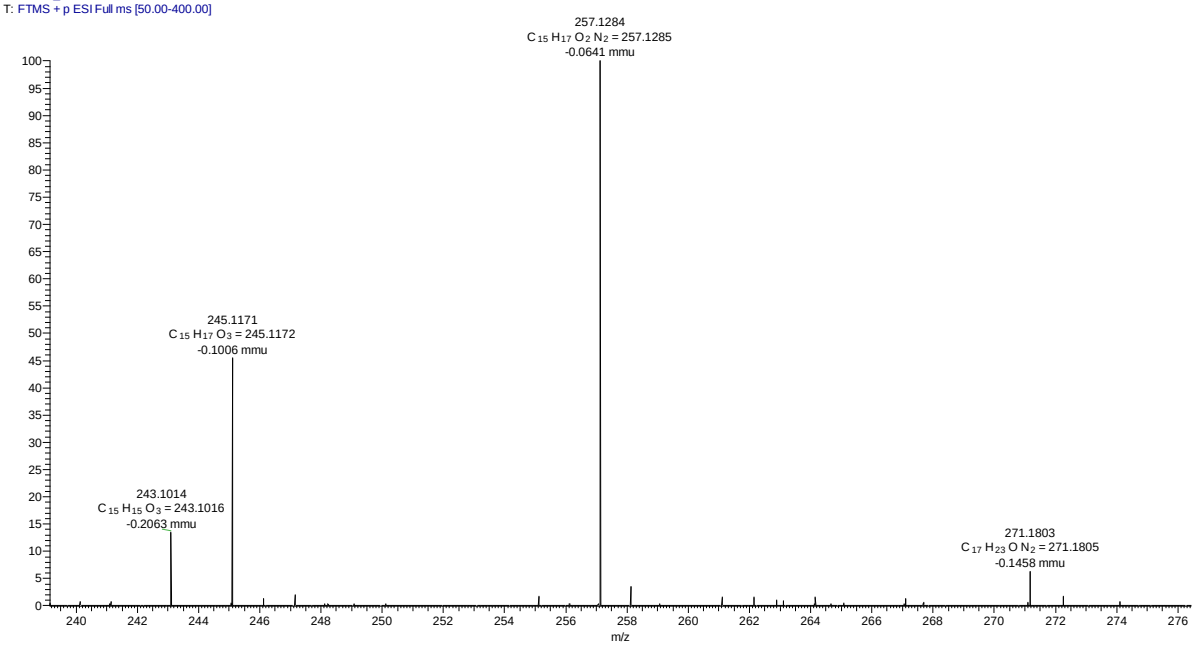
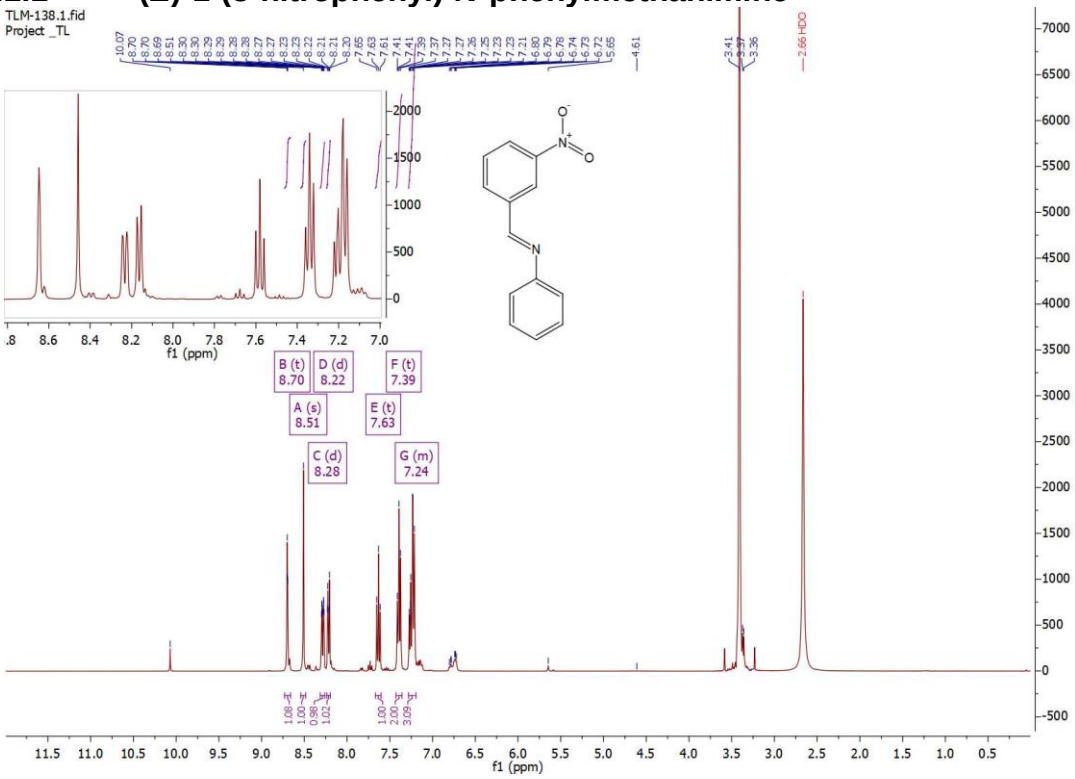
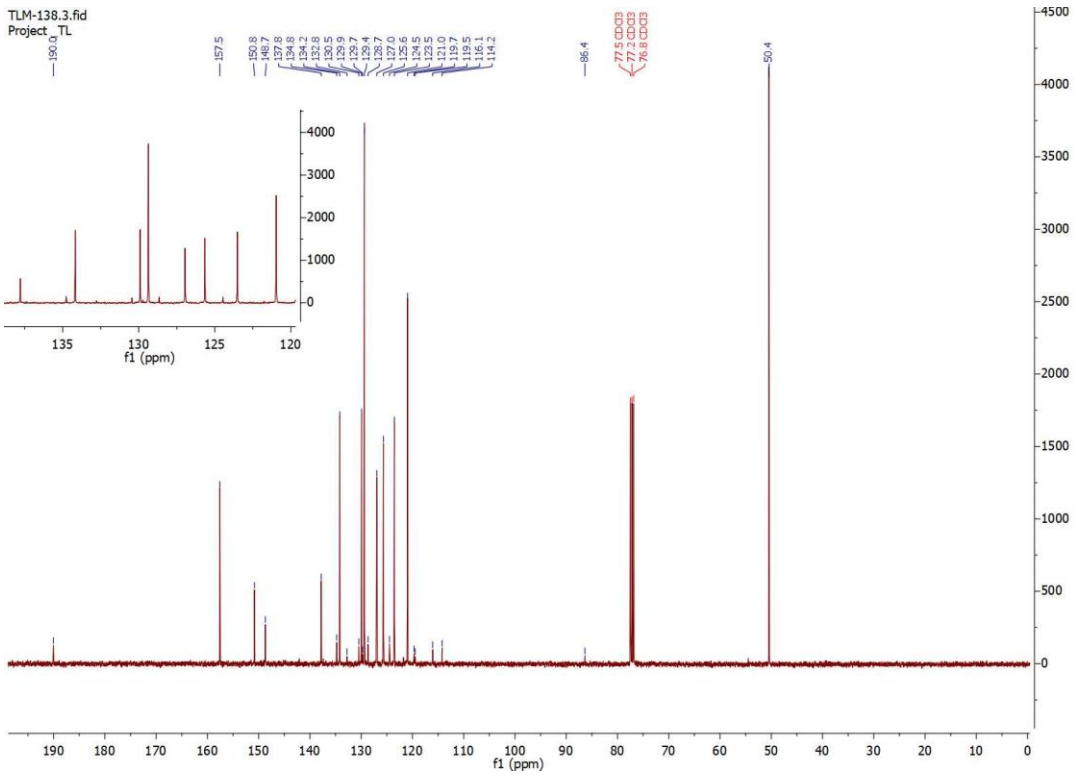


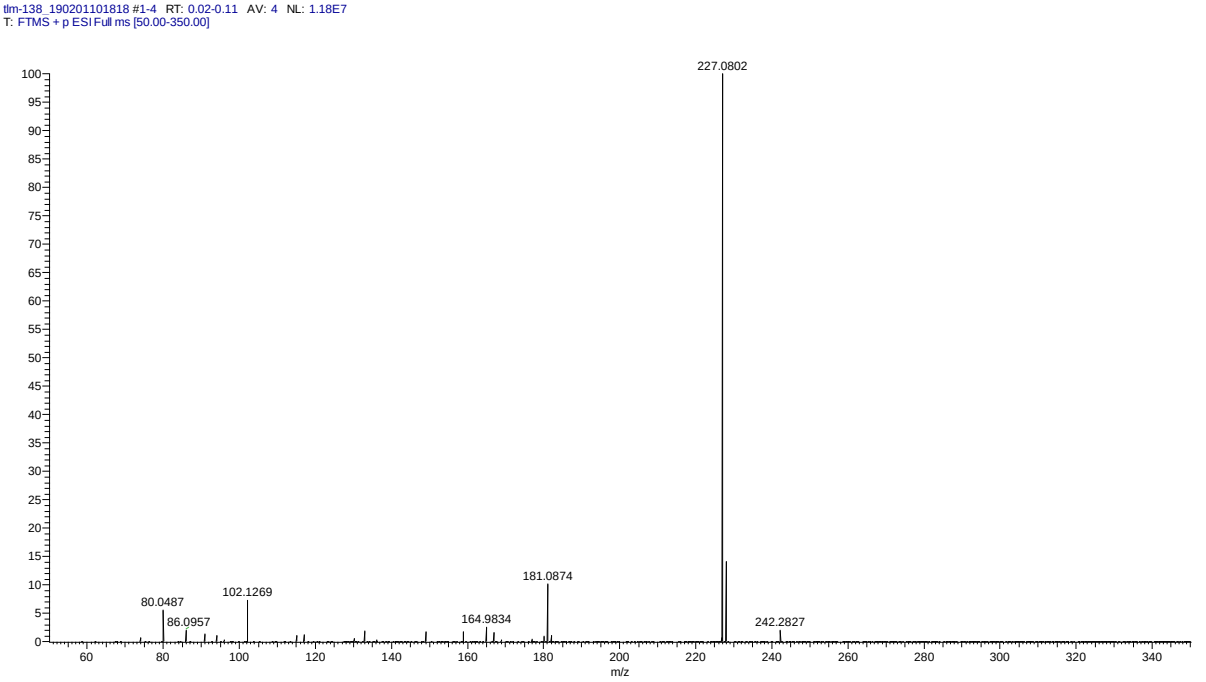
7.2 Synthesis of *N*-Benzylaniline derivatives

7.2.1 Synthesis of imines

7.2.1.1 (*E*)-1-(3-nitrophenyl)-*N*-phenylmethanimine



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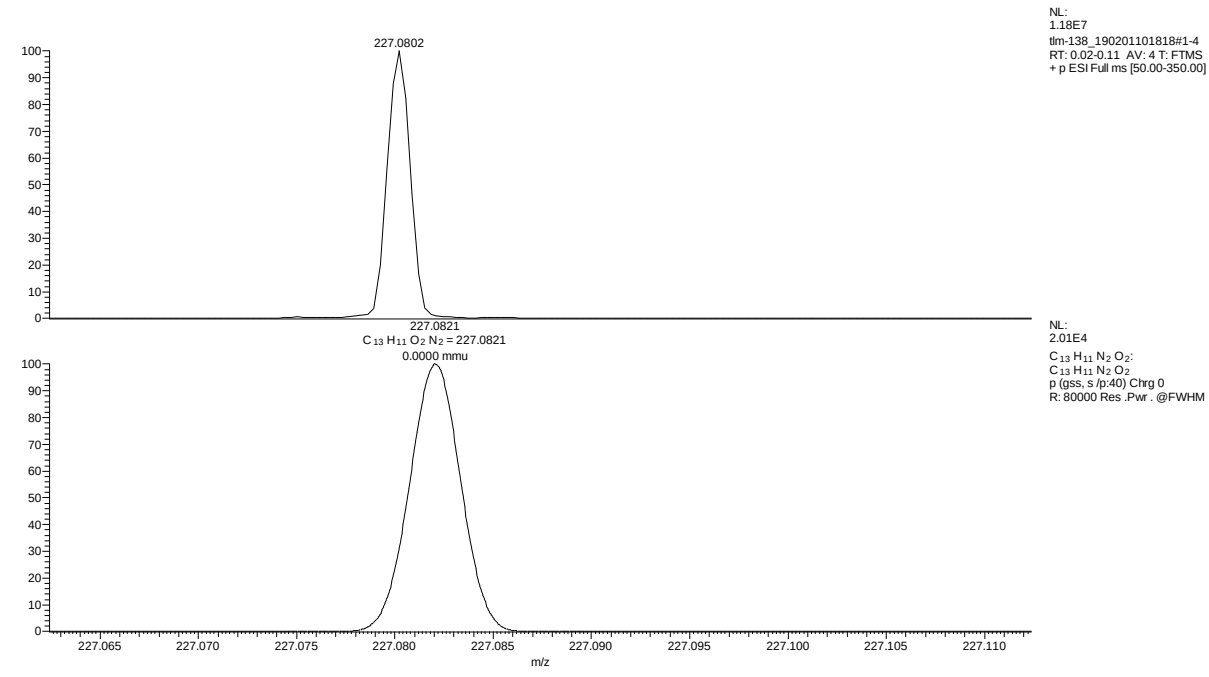
86.0957
164.9834
242.2827
m/z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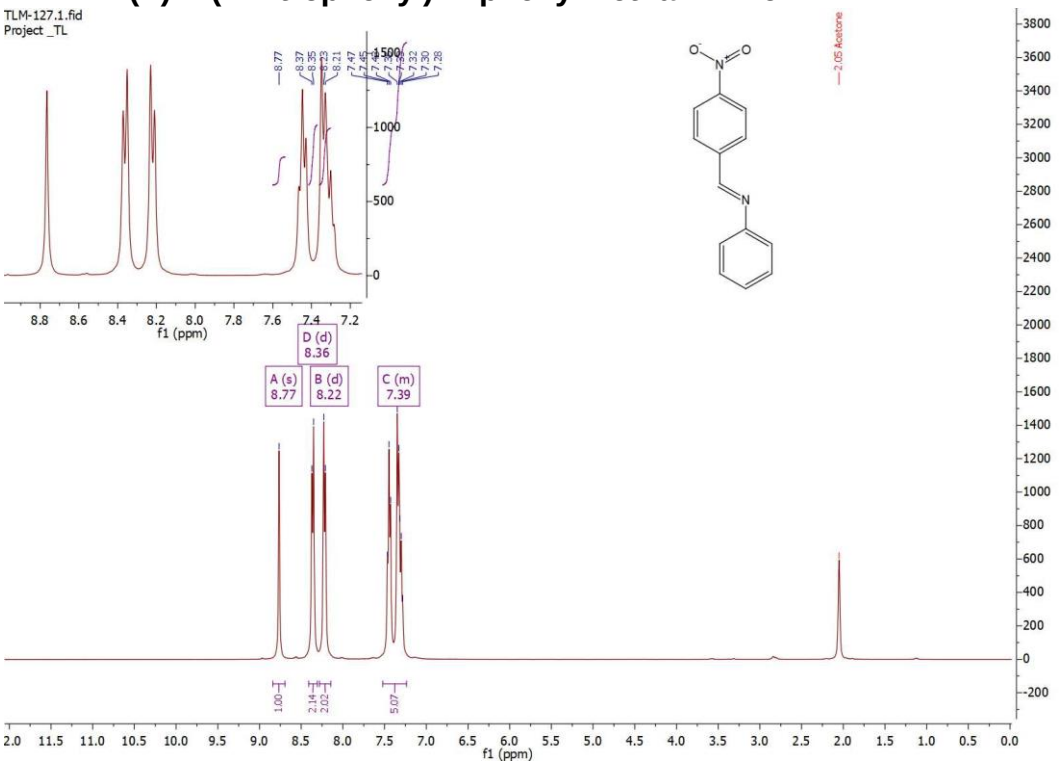
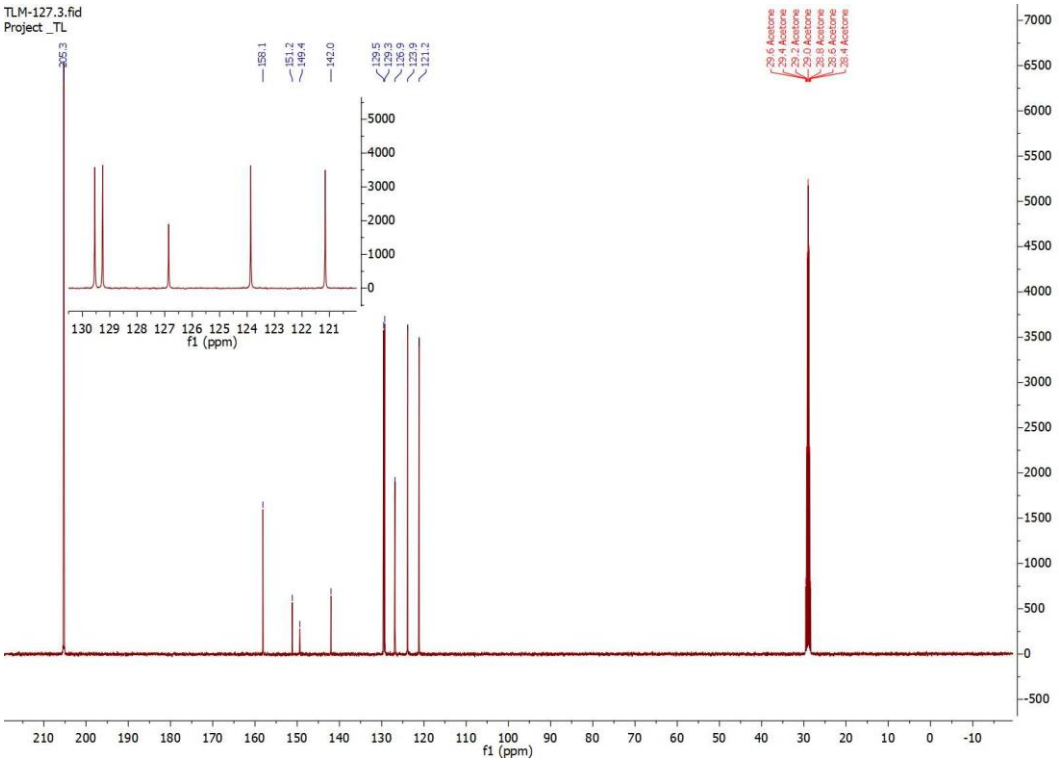
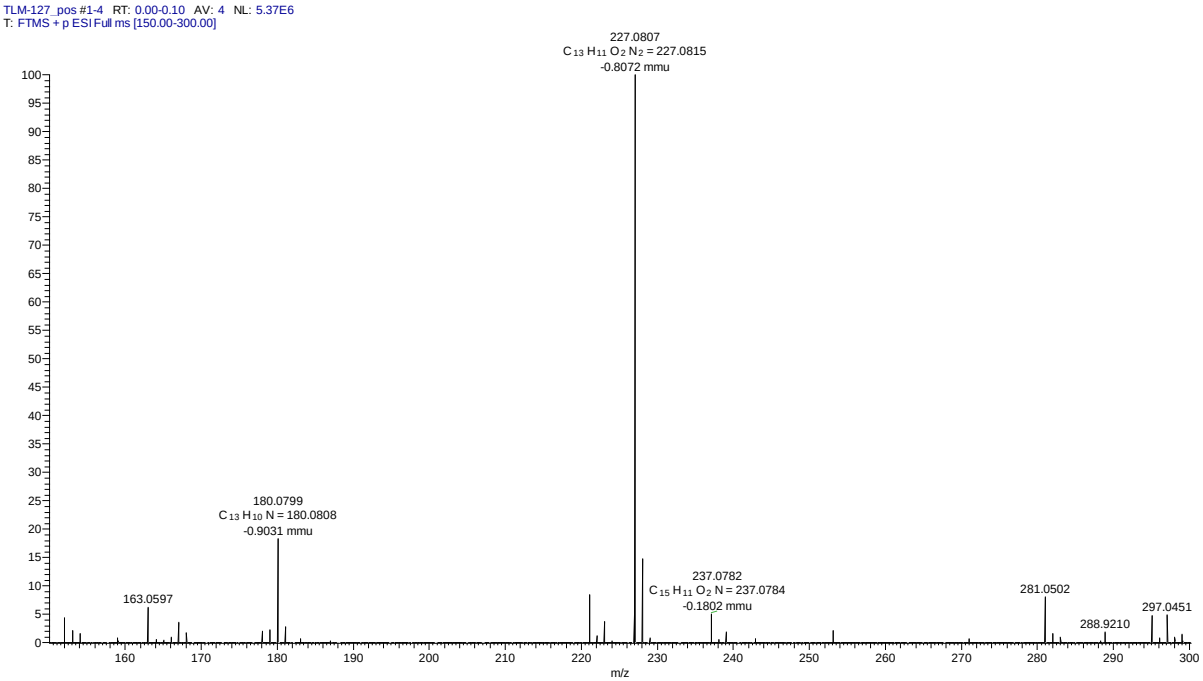
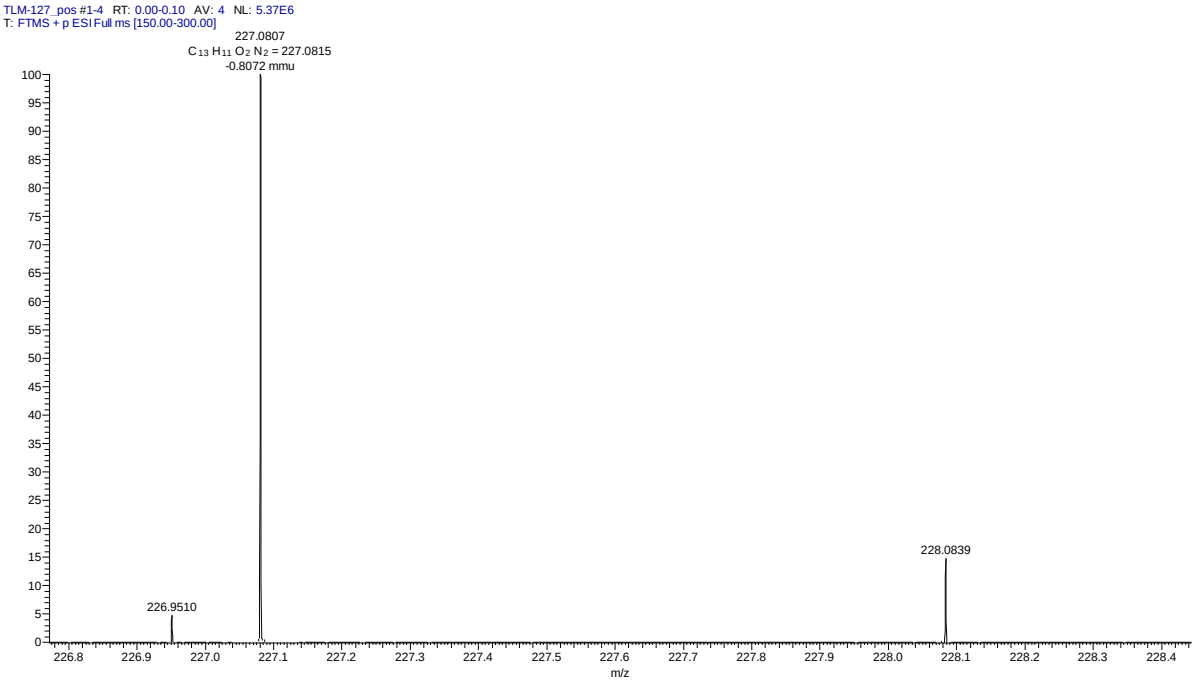
227.0821
C 13 H 11 O 2 N 2 = 227.0821
0.0000 mmu
NL:
2.01E4
C 13 H 11 N 2 O 2:
C 13 H 11 N 2 O 2
p (gss, s /p:40) Chrg 0
R: 80000 Res .Pwr . @FWHM
m/z

7.2.1.2 (*E*)-1-(4-nitrophenyl)-*N*-phenylmethanimine

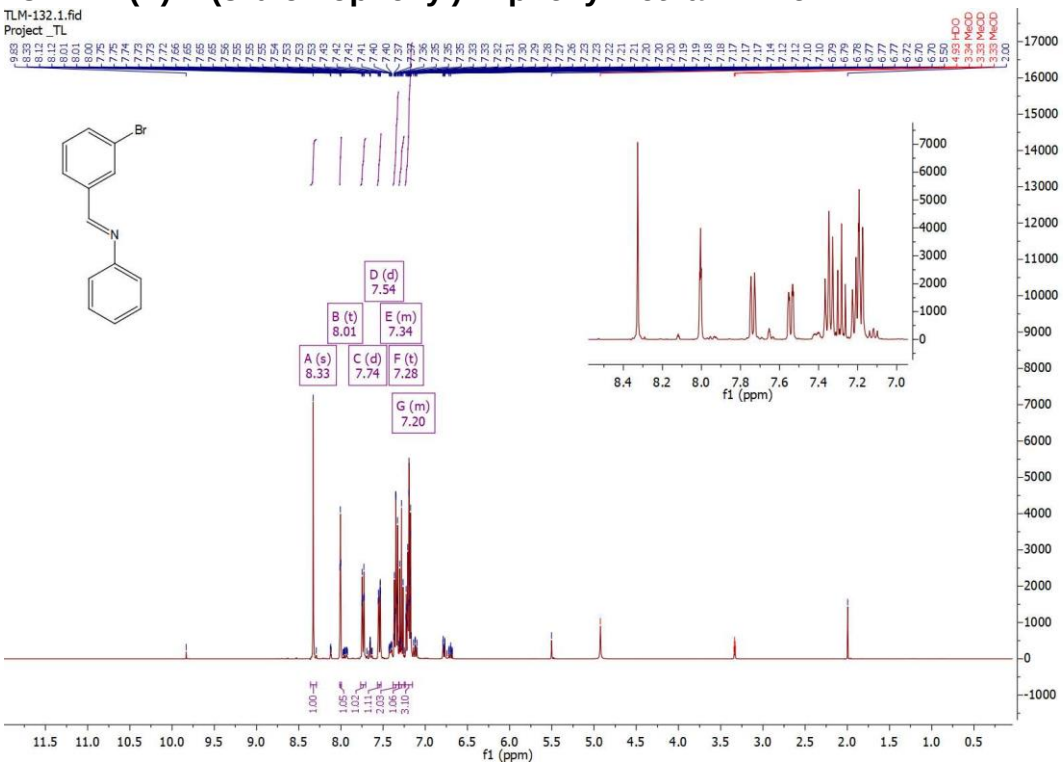


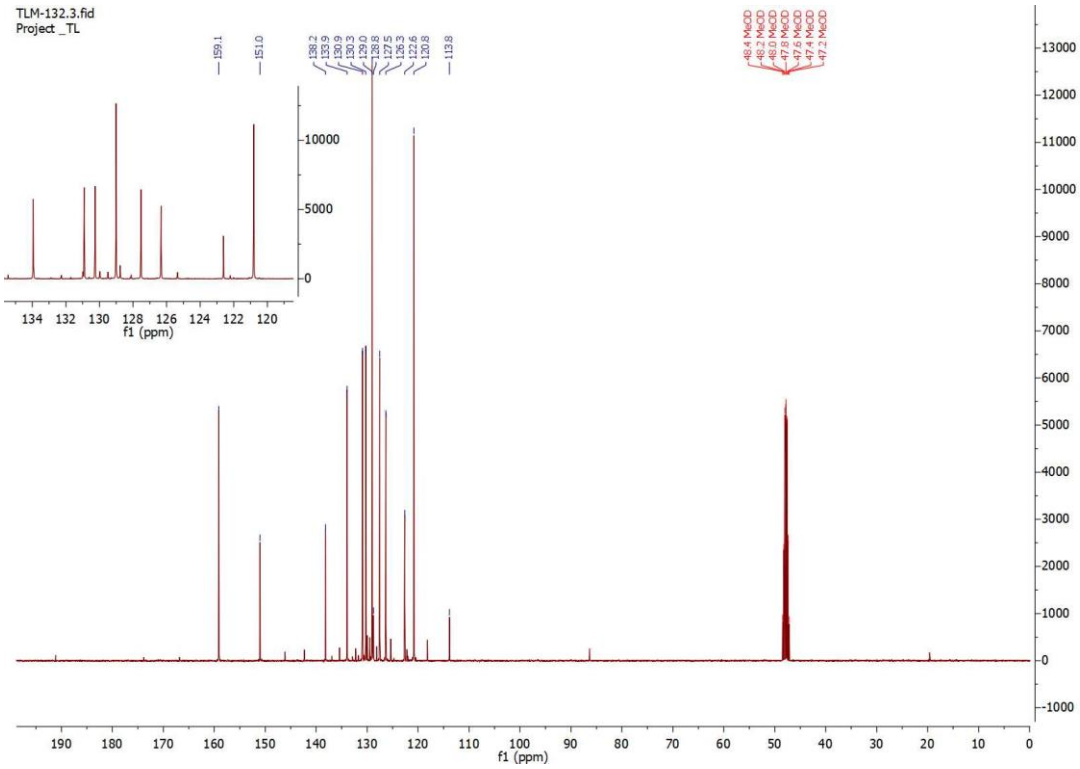


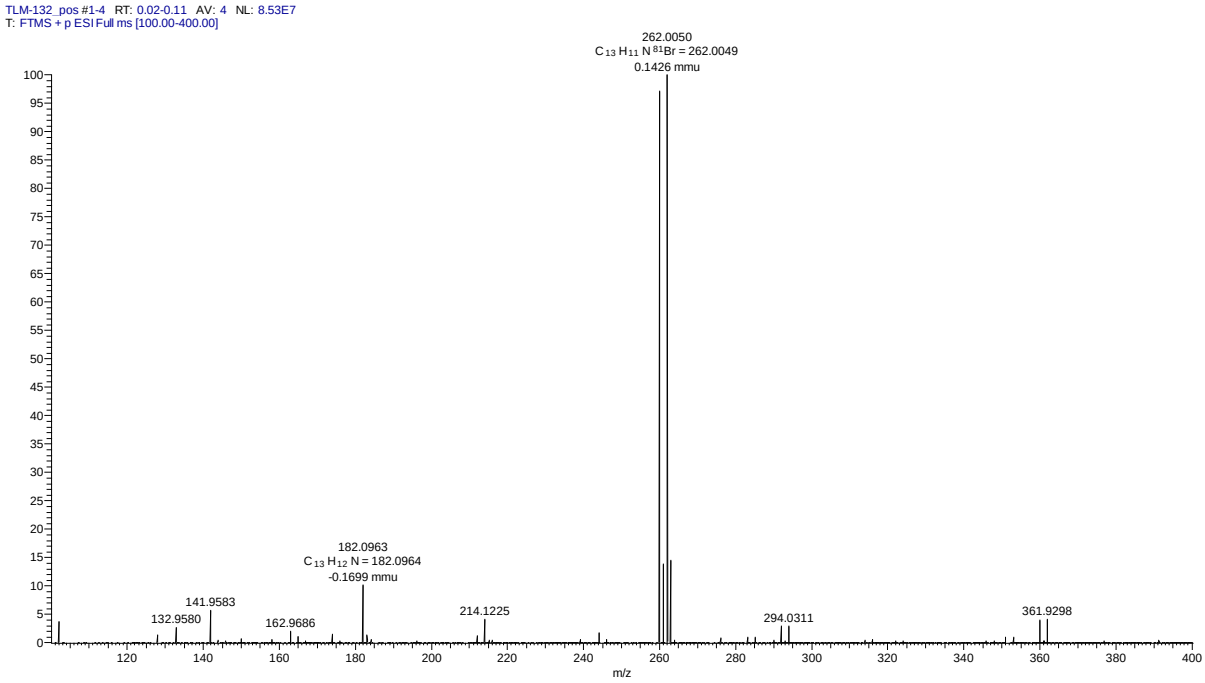

TLM-127_pos #1-4 RT: 0.00-0.10 AV: 4 NL: 5.37E6
T: FTMS + p ESI Full ms [150.00-300.00]
227.0807
C 13 H 11 O 2 N 2 = 227.0815
-0.8072 mmu
180.0799
C 13 H 10 N = 180.0808
-0.9031 mmu
163.0597
237.0782
C 15 H 11 O 2 N = 237.0784
-0.1802 mmu
281.0502
288.9210
297.0451
m/z

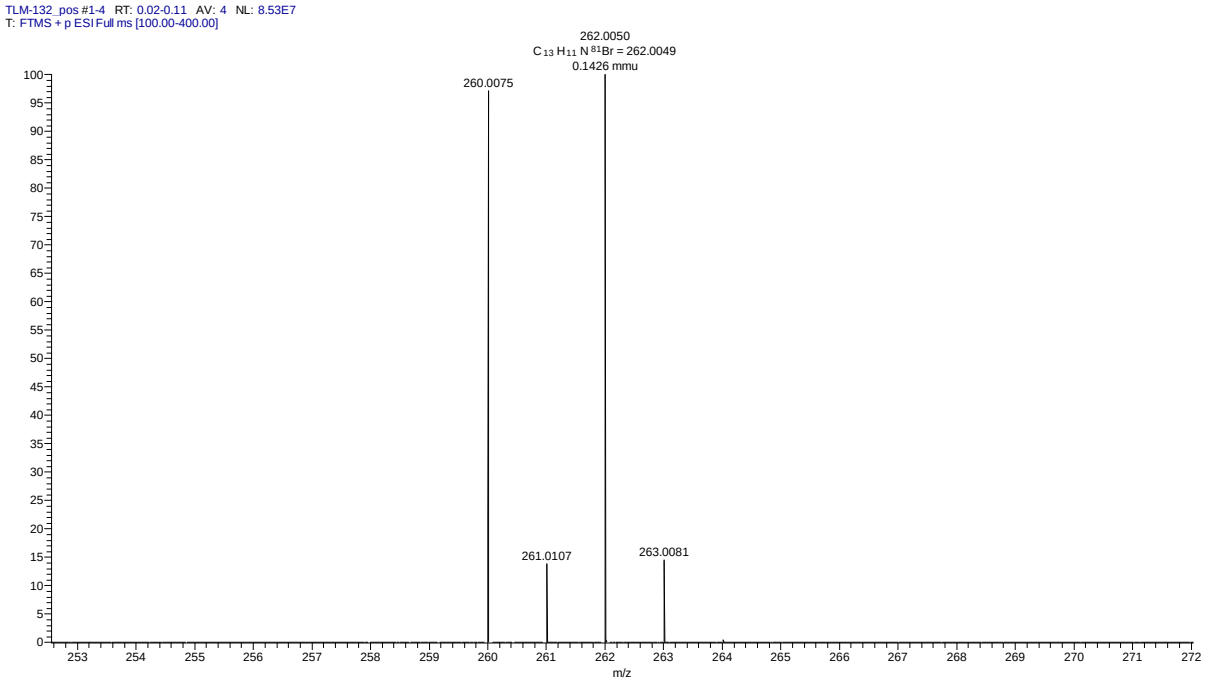

TLM-127_pos #1-4 RT: 0.00-0.10 AV: 4 NL: 5.37E6
T: FTMS + p ESI Full ms [150.00-300.00]
227.0807
C 13 H 11 O 2 N 2 = 227.0815
-0.8072 mmu
228.0839
226.9510
m/z

7.2.1.3 (*E*)-1-(3-bromophenyl)-*N*-phenylmethanimine



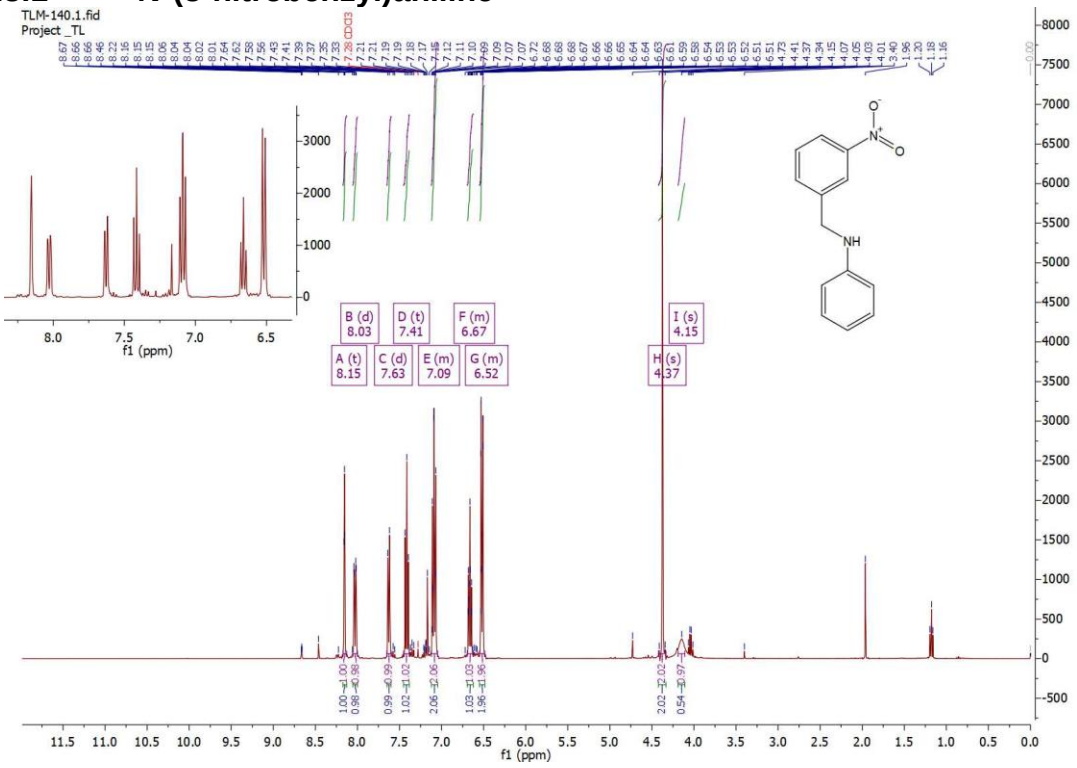


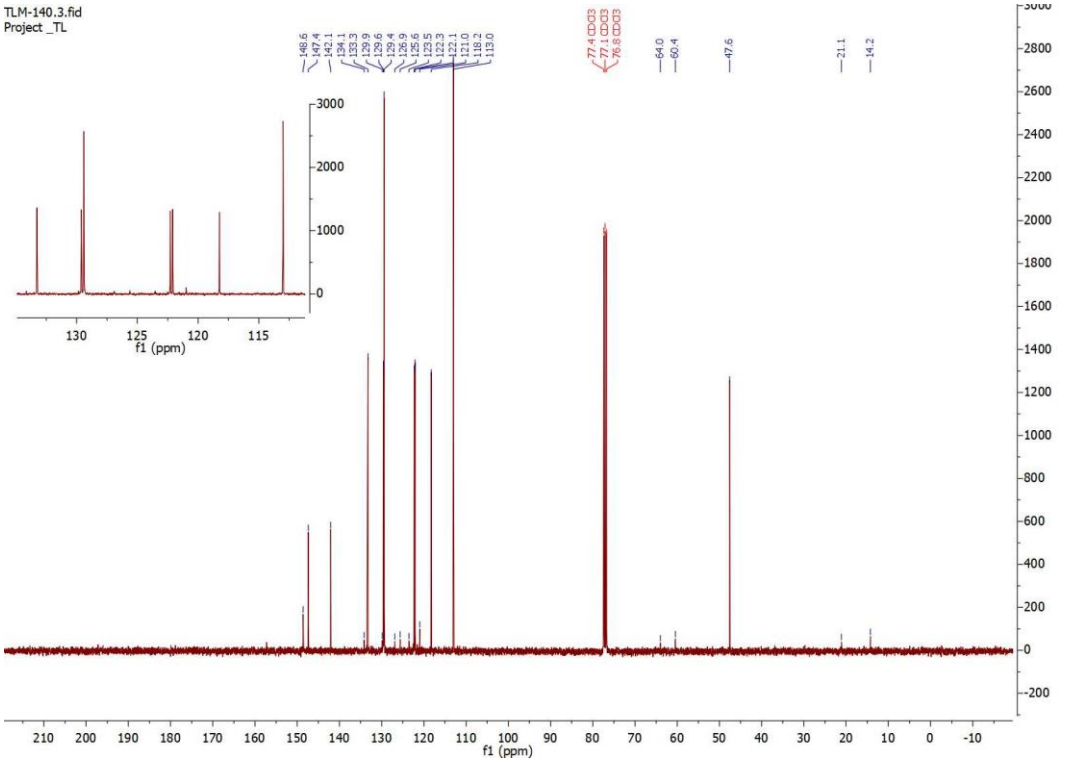

TLM-132_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 8.53E7
T: FTMS + p ESI Full ms [100.00-400.00]
262.0050
C 13 H 11 N 81Br = 262.0049
0.1426 mmu
182.0963
C 13 H 12 N = 182.0964
-0.1699 mmu
141.9583
132.9580
162.9686
214.1225
294.0311
361.9298
m/z

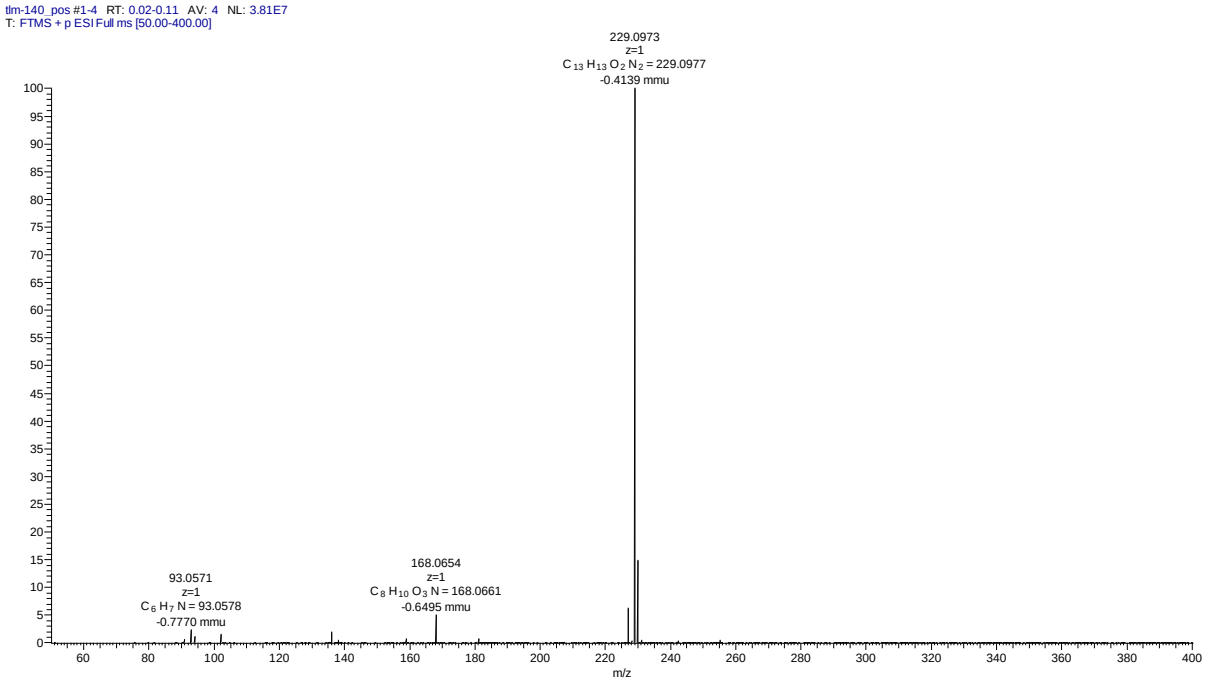

TLM-132_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 8.53E7
T: FTMS + p ESI Full ms [100.00-400.00]
262.0050
C 13 H 11 N 81Br = 262.0049
0.1426 mmu
260.0075
261.0107
263.0081
m/z

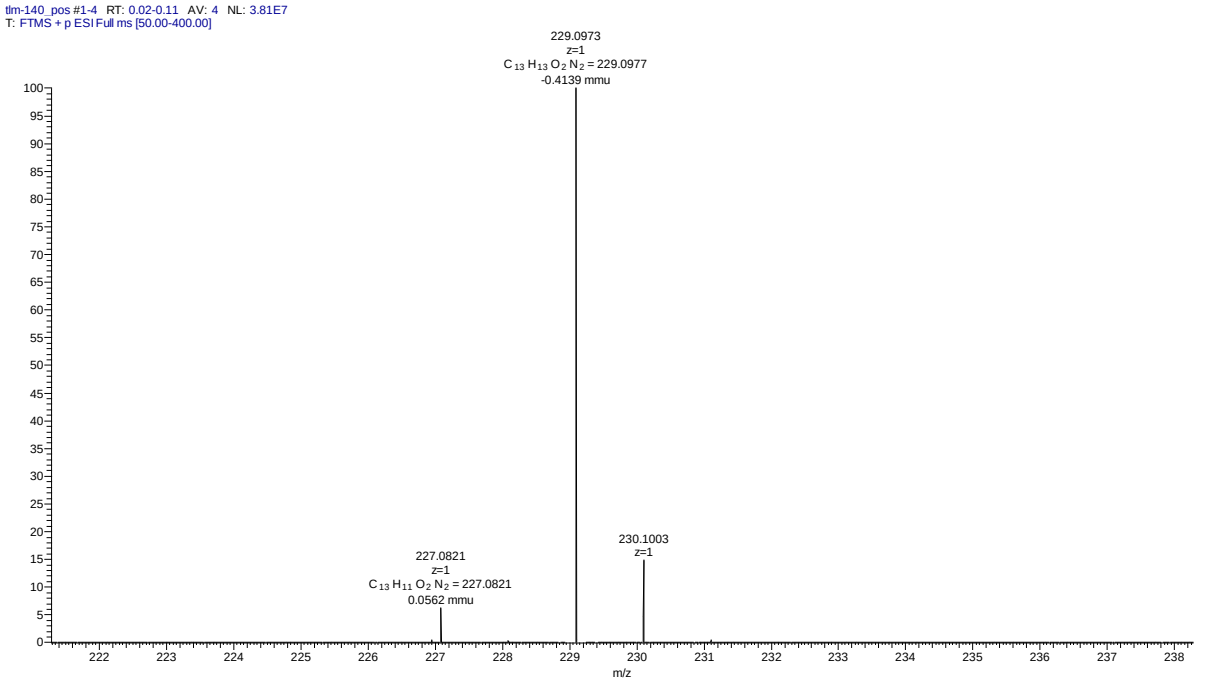
7.2.3 Hydrogenation of imines using sodium borohydride

7.2.3.1 *N*-(3-nitrobenzyl)aniline

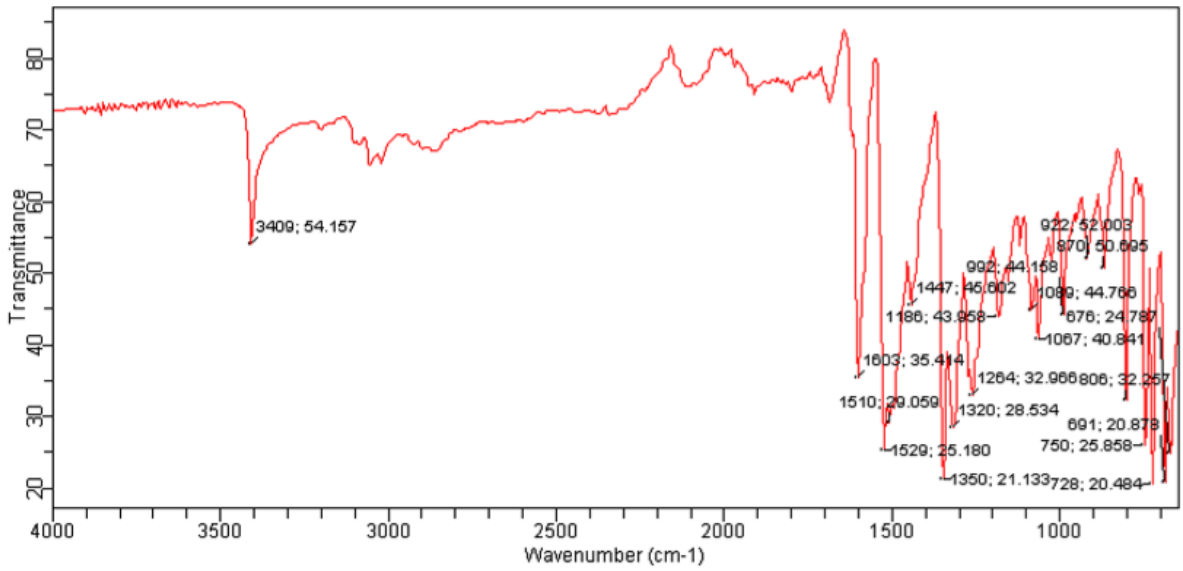




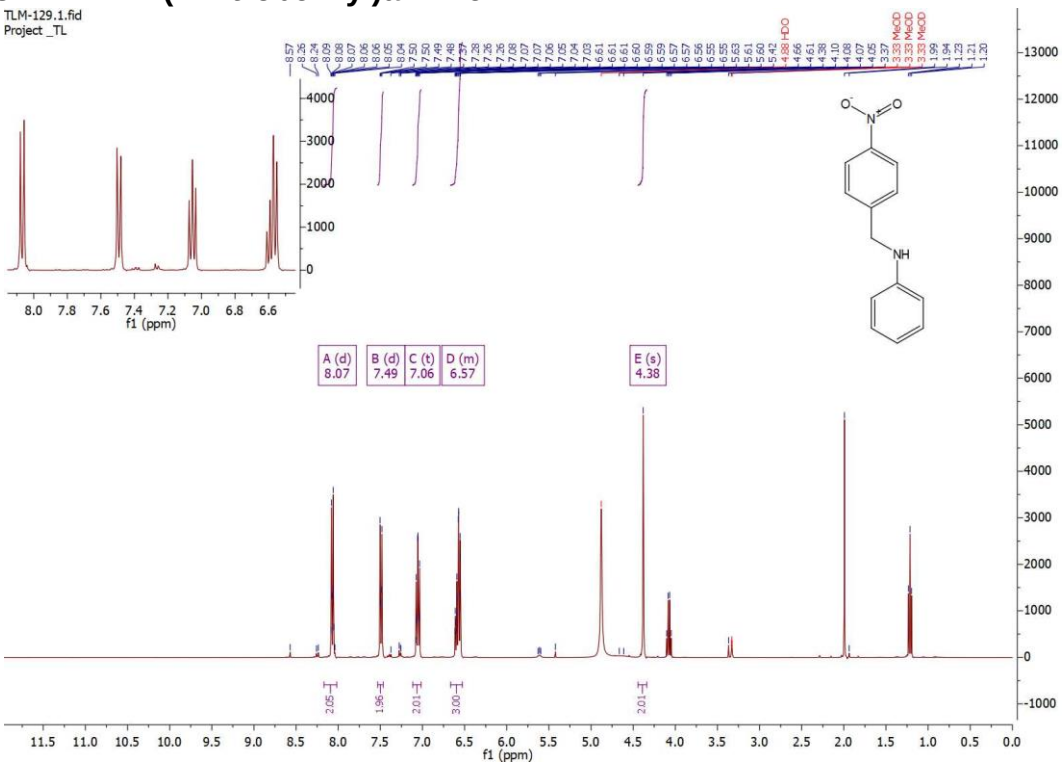

tlm-140_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 3.81E7
T: FTMS + p ESI Full ms [50.00-400.00]
229.0973
z=1
C 13 H 13 O 2 N 2 = 229.0977
-0.4139 mmu
93.0571
z=1
C 6 H 7 N = 93.0578
-0.7770 mmu
168.0654
z=1
C 8 H 10 O 3 N = 168.0661
-0.6495 mmu
m/z

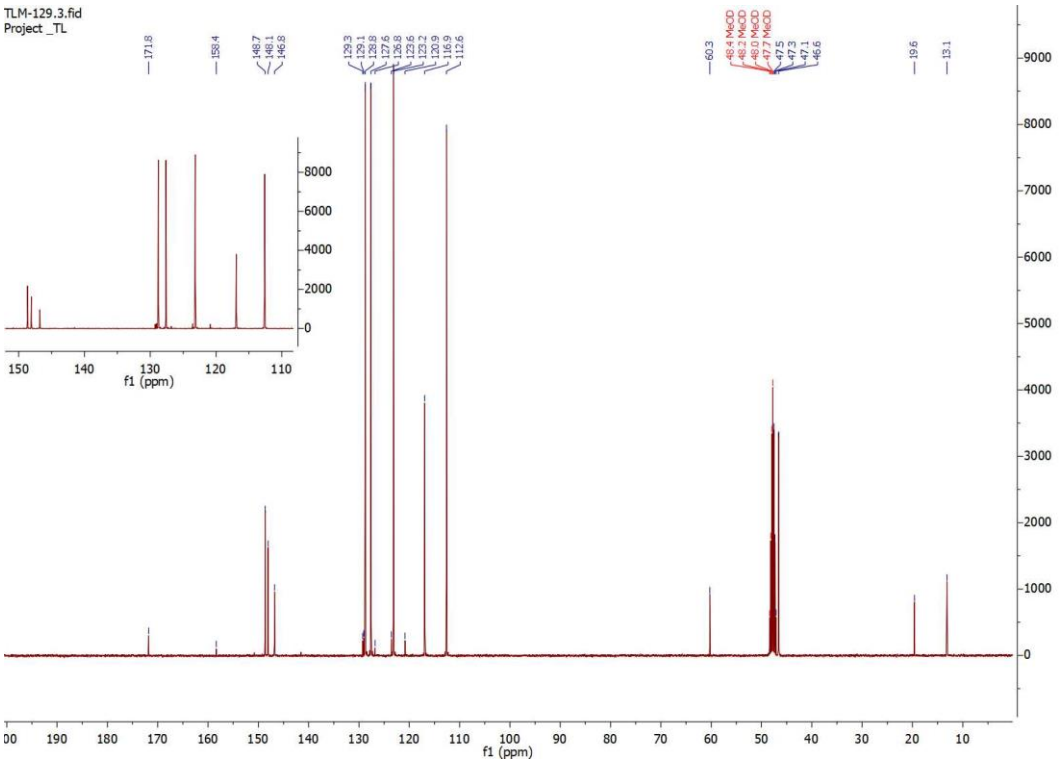

tlm-140_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 3.81E7
T: FTMS + p ESI Full ms [50.00-400.00]
229.0973
z=1
C 13 H 13 O 2 N 2 = 229.0977
-0.4139 mmu
230.1003
z=1
227.0821
z=1
C 13 H 11 O 2 N 2 = 227.0821
0.0562 mmu
m/z

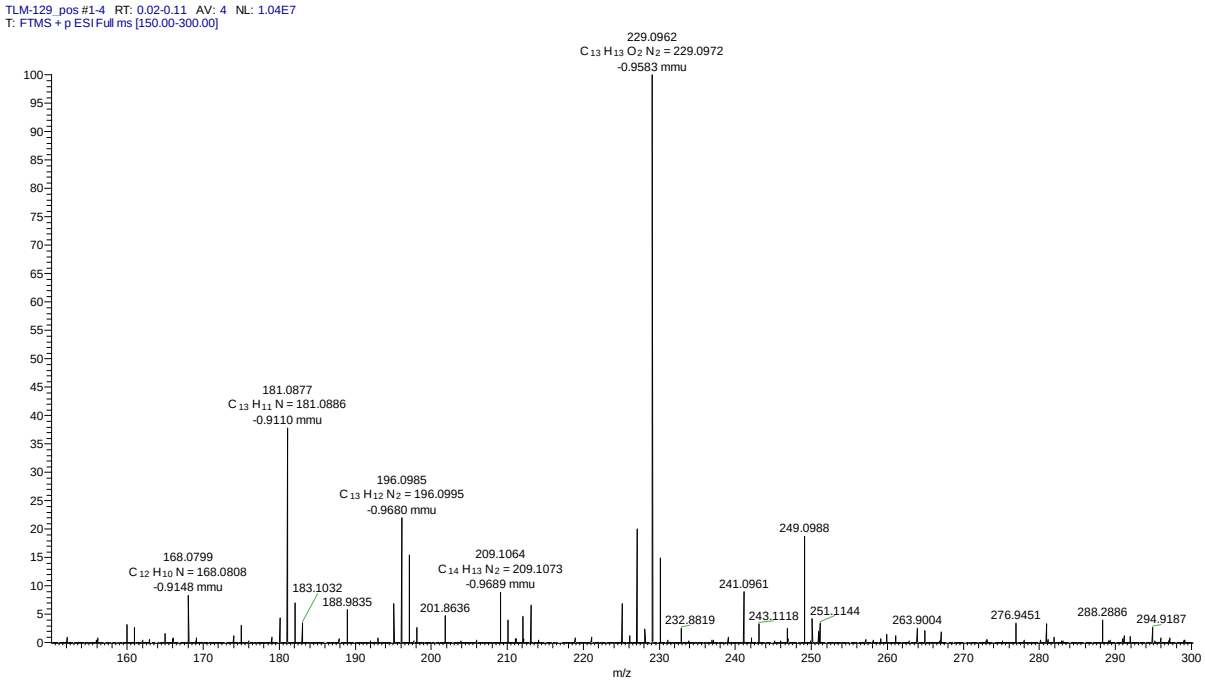
Sample ID:	tlm-140	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 4:48:43PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-140_2019-04-30T16-49-45.a2r		

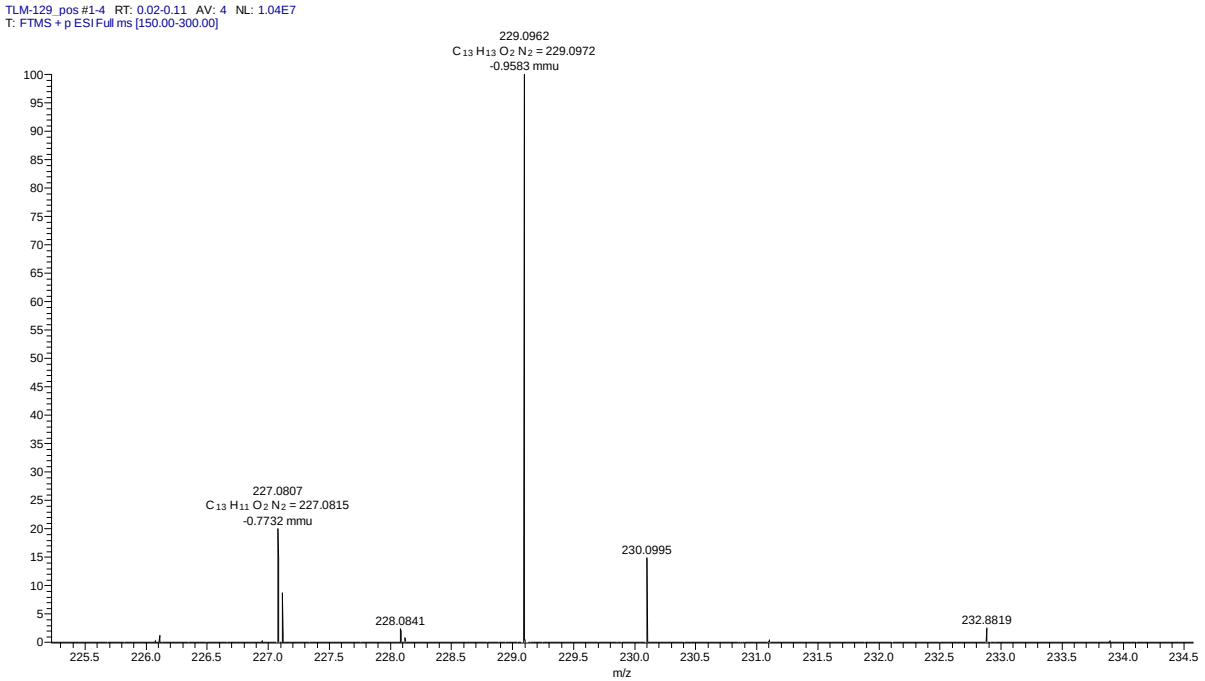


7.2.3.2 *N*-(4-nitrobenzyl)aniline

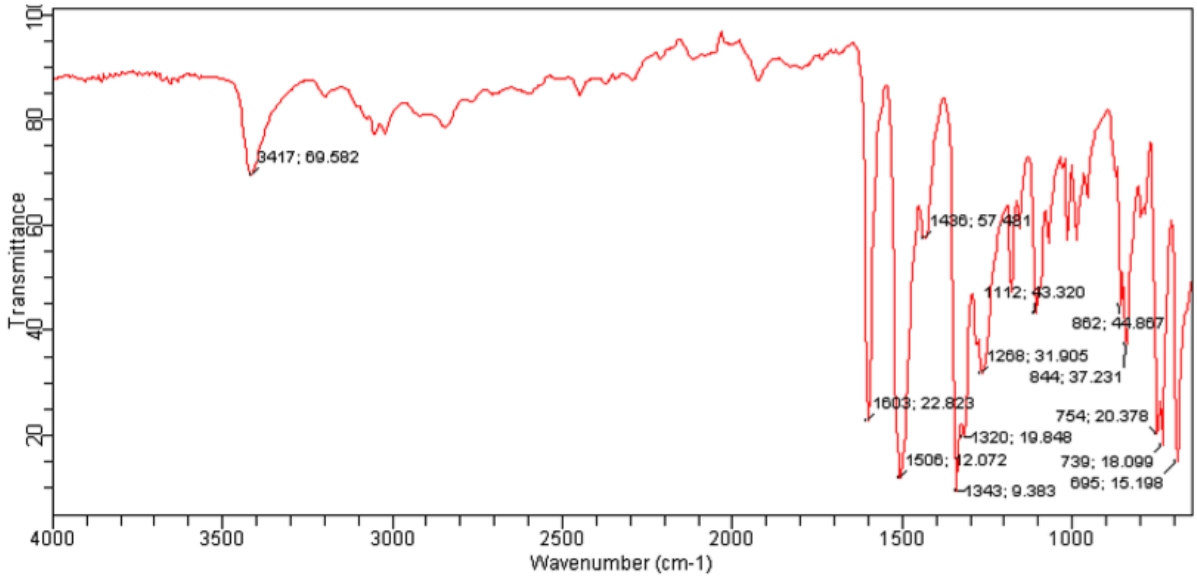




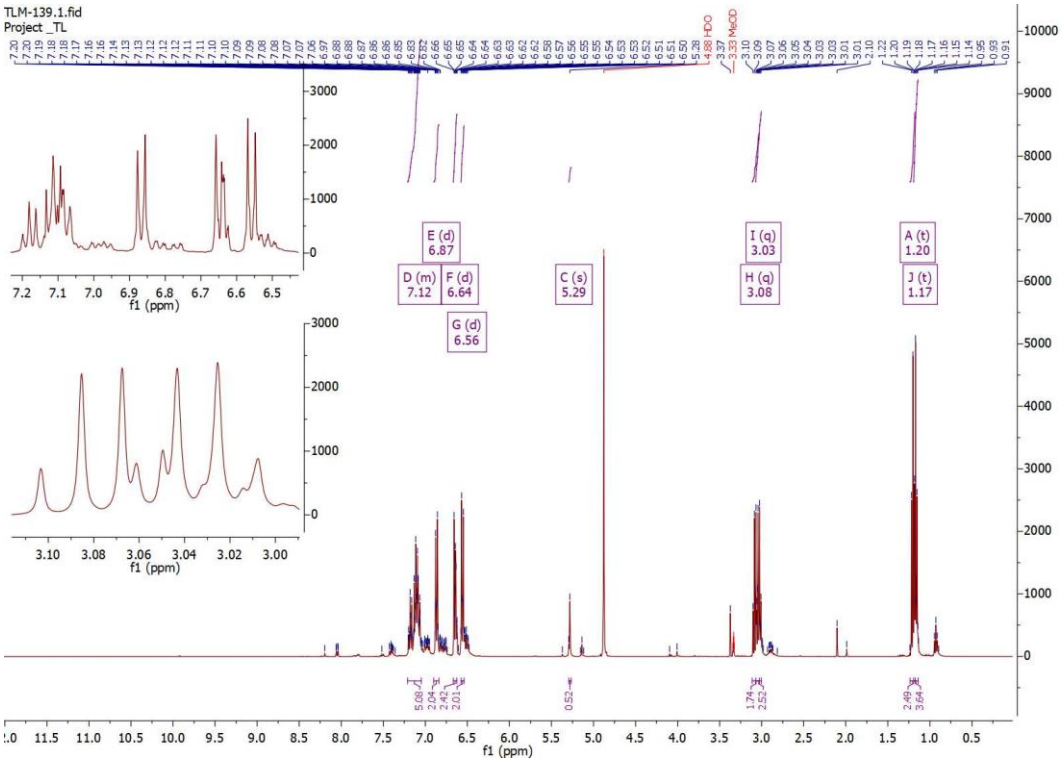

TLM-129_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 1.04E7
T: FTMS + p ESI Full ms [150.00-300.00]
229.0962
C13 H13 O2 N2 = 229.0972
-0.9583 mmu
181.0877
C13 H11 N = 181.0886
-0.9110 mmu
196.0985
C13 H12 N2 = 196.0995
-0.9680 mmu
249.0988
168.0799
C12 H10 N = 168.0808
-0.9148 mmu
209.1064
C14 H13 N2 = 209.1073
-0.9689 mmu
241.0961
183.1032
188.9835
201.8636
232.8819
243.1118
251.1144
263.9004
276.9451
288.2886
294.9187
m/z

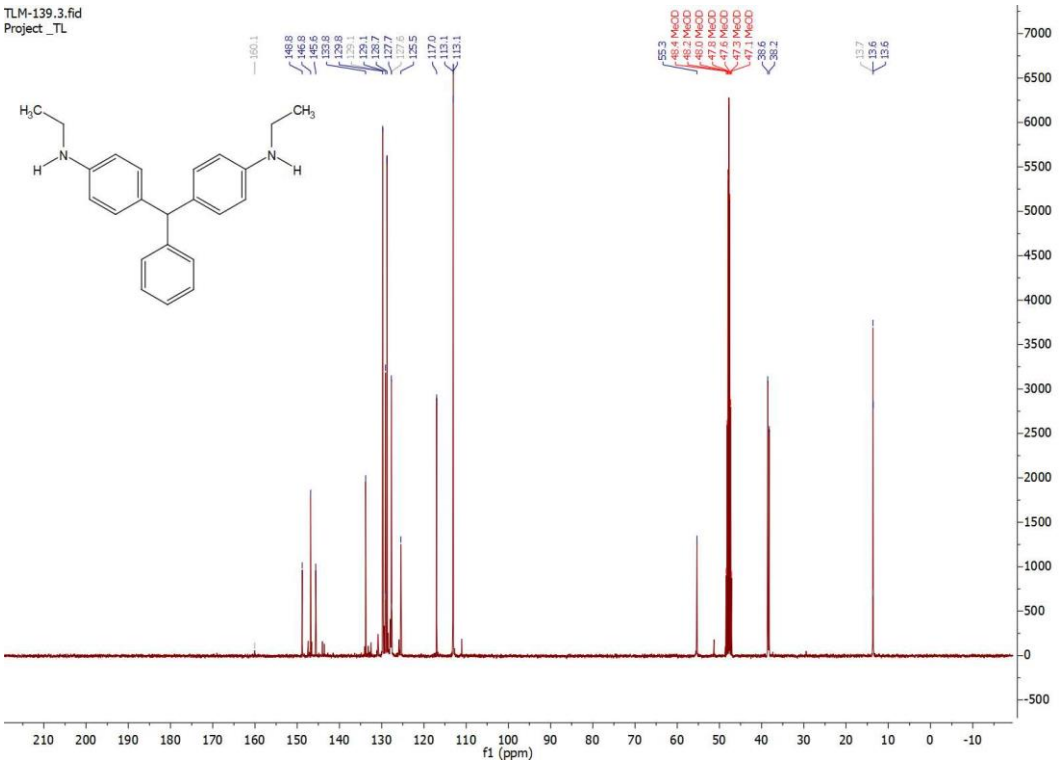

TLM-129_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 1.04E7
T: FTMS + p ESI Full ms [150.00-300.00]
229.0962
C13 H13 O2 N2 = 229.0972
-0.9583 mmu
227.0807
C13 H11 O2 N2 = 227.0815
-0.7732 mmu
230.0995
228.0841
232.8819
m/z

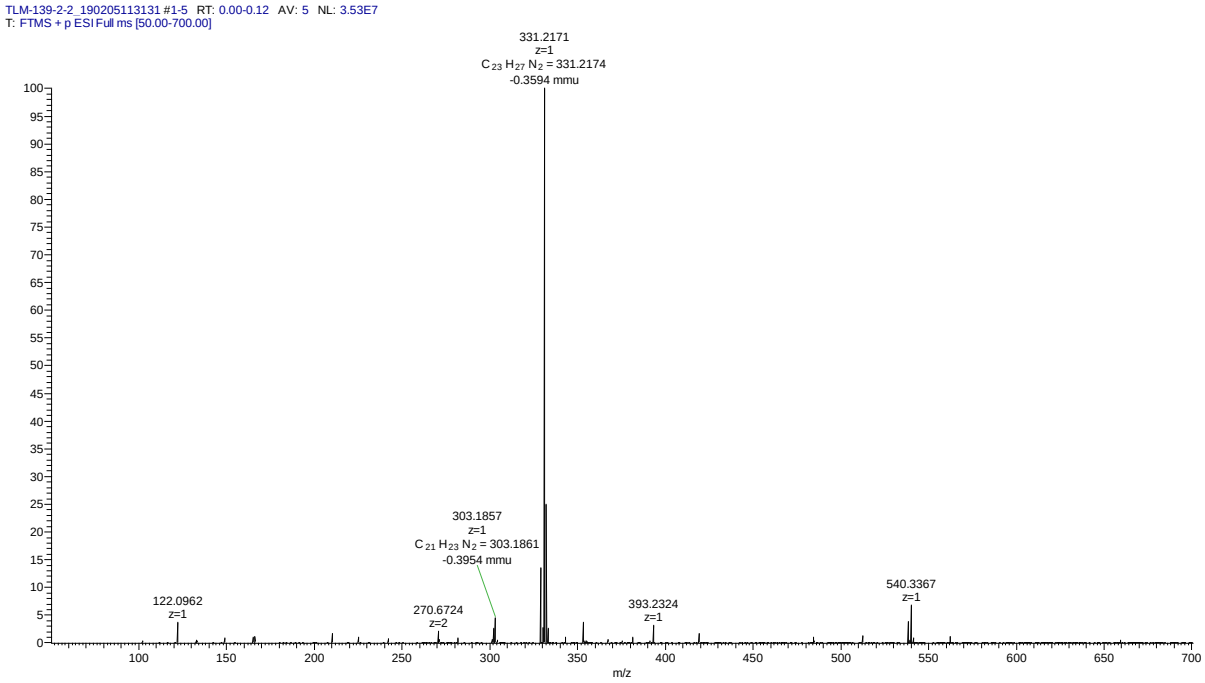
Sample ID:	tlm-129	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 4:44:19PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-129_2019-04-30T16-45-26.a2r		

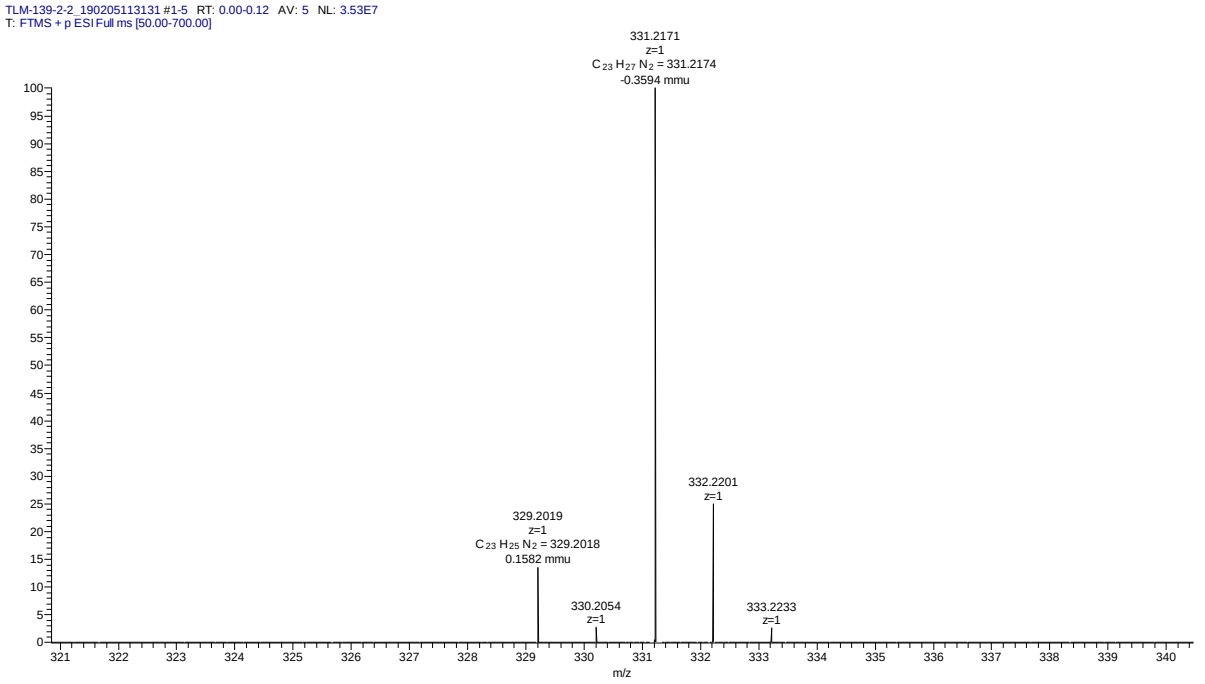


7.2.4 4,4'-(phenylmethylene)bis(*N*-ethylaniline)



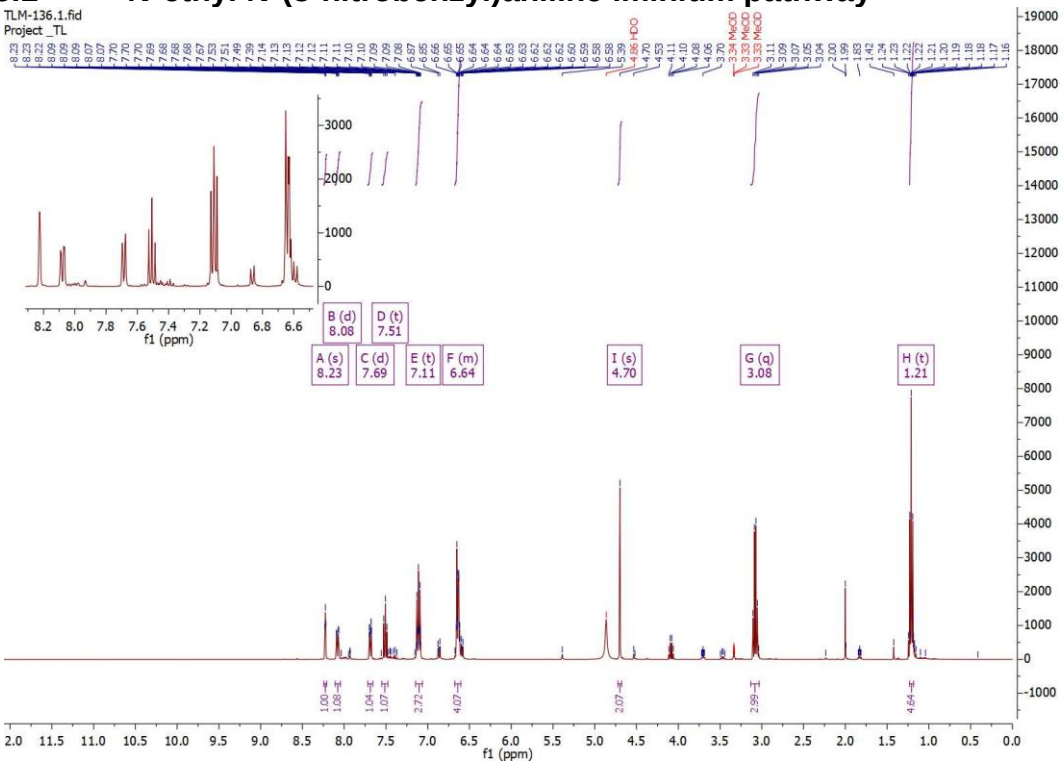


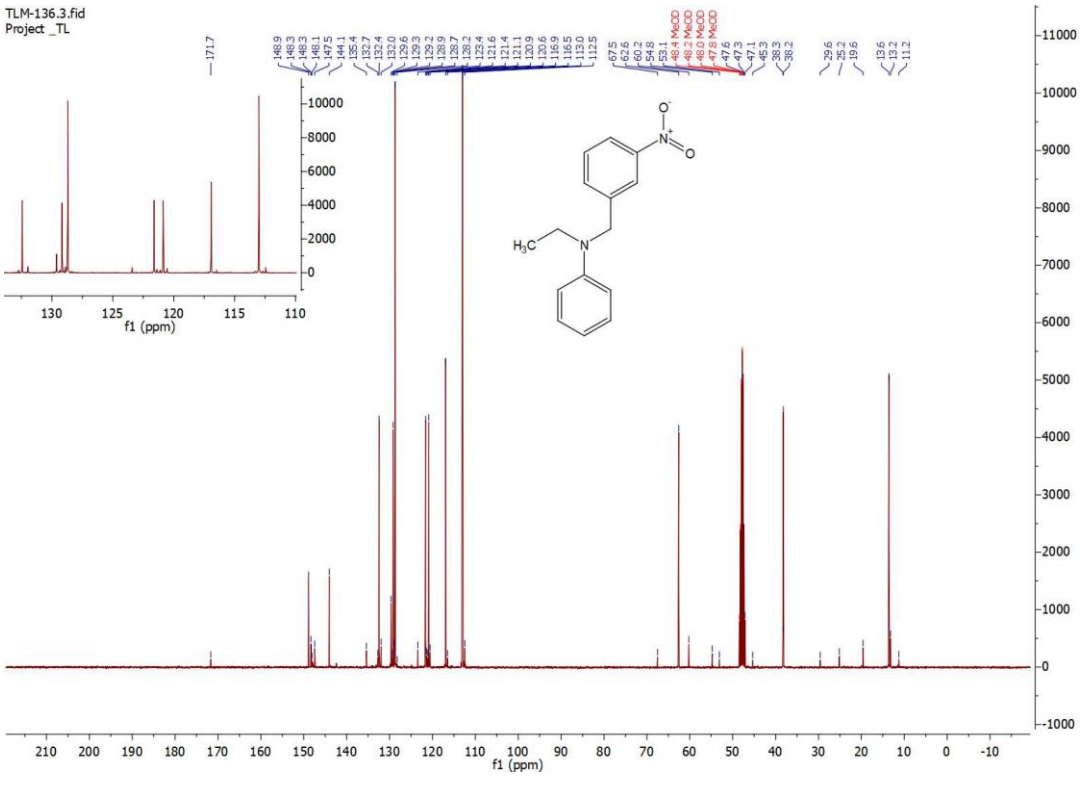

TLM-139-2-2_190205113131 #1-5 RT: 0.00-0.12 AV: 5 NL: 3.53E7
T: FTMS + p ESI Full ms [50.00-700.00]
331.2171
z=1
C 23 H 27 N 2 = 331.2174
-0.3594 mmu
303.1857
z=1
C 21 H 23 N 2 = 303.1861
-0.3954 mmu
122.0962
z=1
270.6724
z=2
393.2324
z=1
540.3367
z=1
m/z

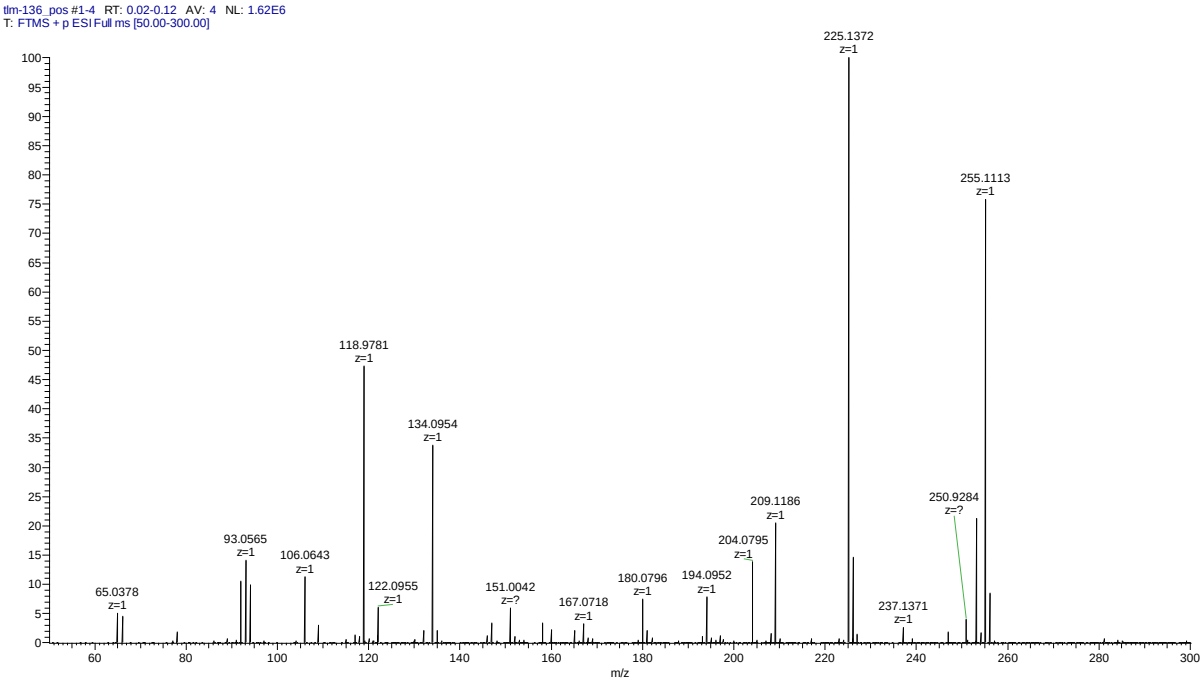

TLM-139-2-2_190205113131 #1-5 RT: 0.00-0.12 AV: 5 NL: 3.53E7
T: FTMS + p ESI Full ms [50.00-700.00]
331.2171
z=1
C 23 H 27 N 2 = 331.2174
-0.3594 mmu
332.2201
z=1
329.2019
z=1
C 23 H 25 N 2 = 329.2018
0.1582 mmu
330.2054
z=1
333.2233
z=1
m/z

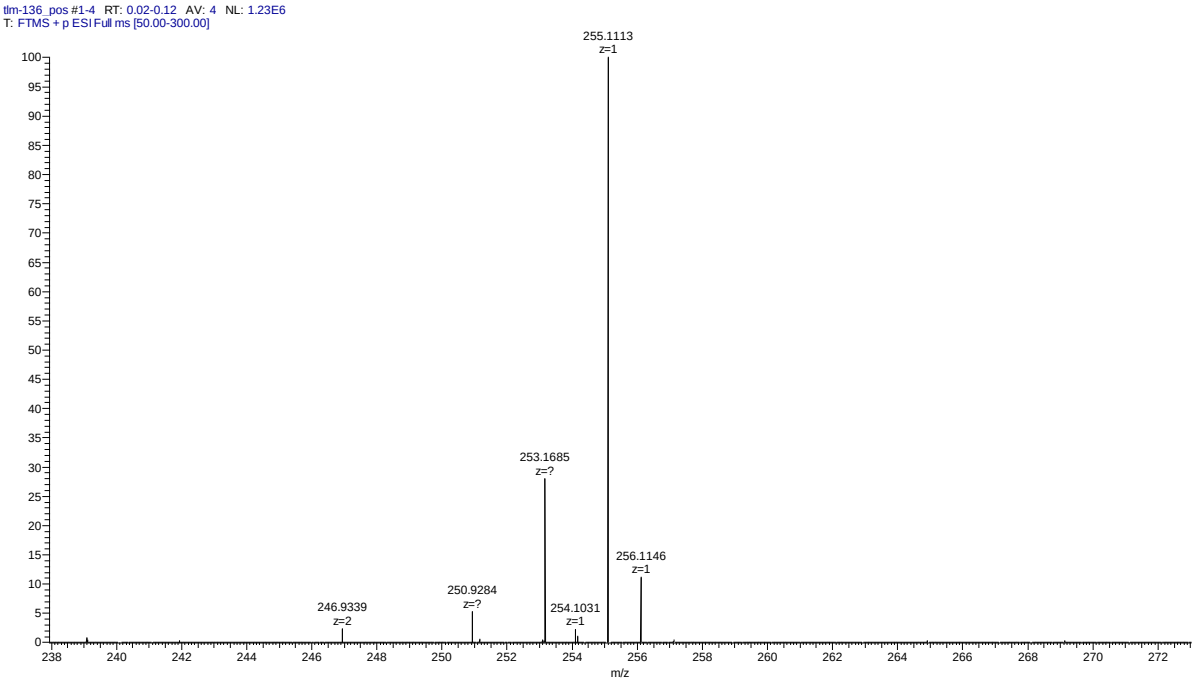
7.2.5 Attempt to synthesize *N*-ethyl-*N*-(3-nitrobenzyl)aniline

7.2.5.1 *N*-ethyl-*N*-(3-nitrobenzyl)aniline iminium pathway

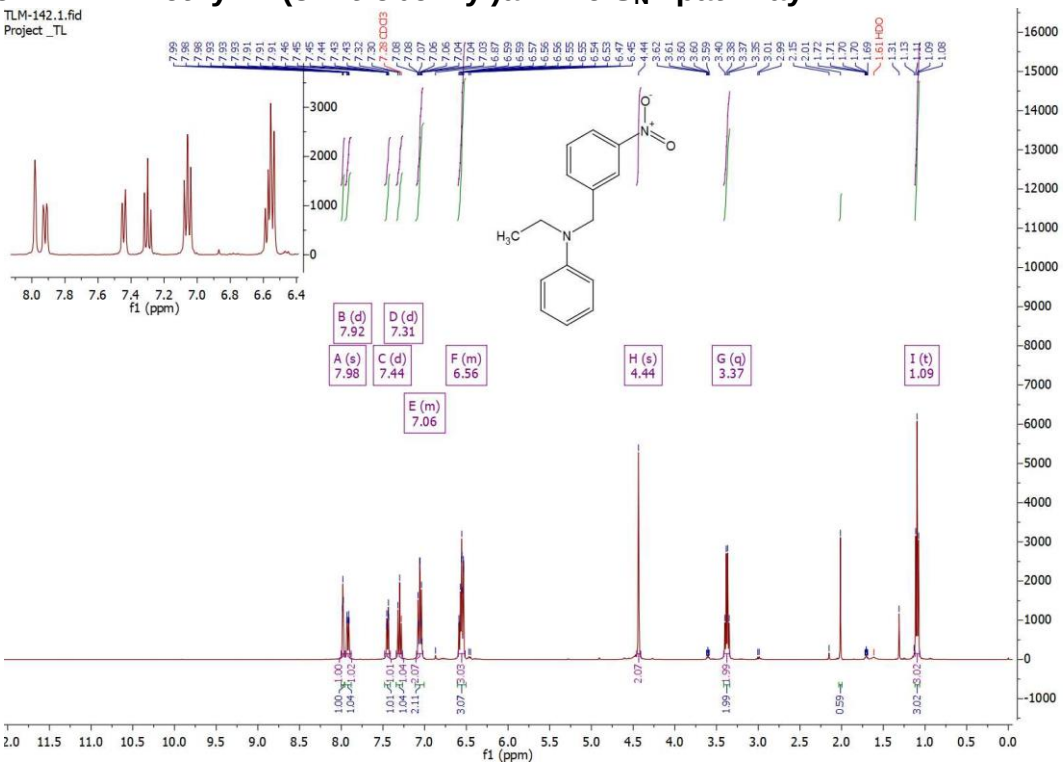


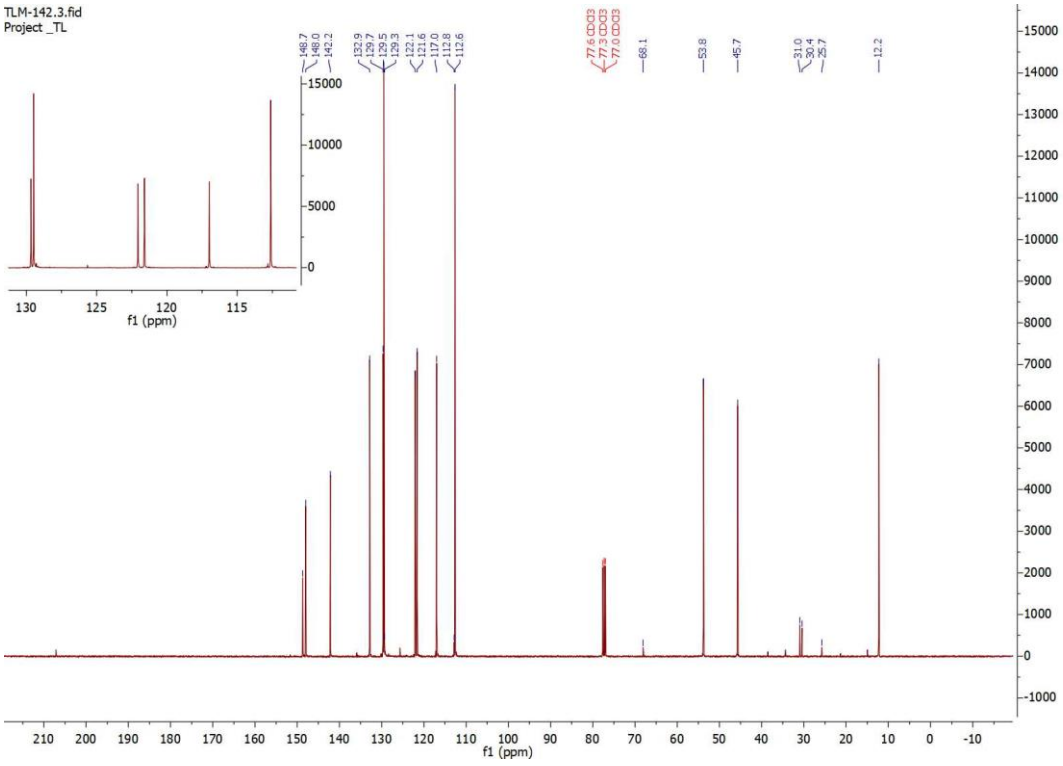


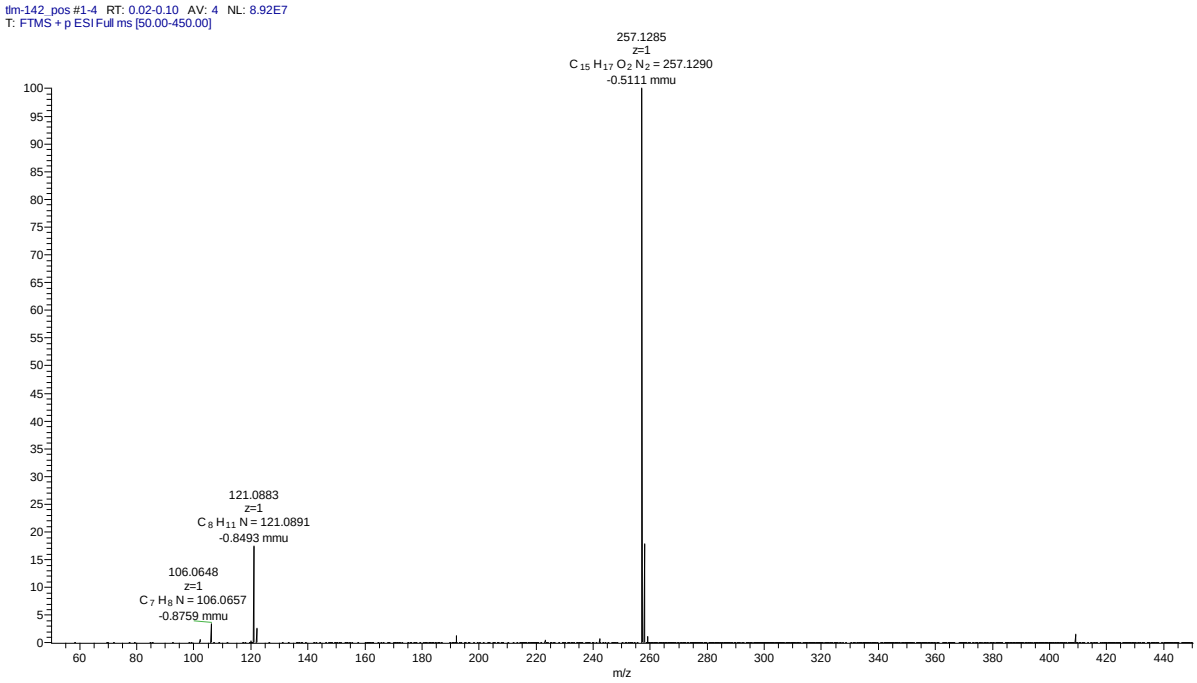

tlm-136_pos #1-4 RT: 0.02-0.12 AV: 4 NL: 1.62E6
T: FTMS + p ESI Full ms [50.00-300.00]
225.1372
z=1
255.1113
z=1
118.9781
z=1
134.0954
z=1
209.1186
z=1
250.9284
z=?
93.0565
z=1
106.0643
z=1
204.0795
z=1
65.0378
z=1
122.0955
z=1
151.0042
z=?
180.0796
z=1
194.0952
z=1
167.0718
z=1
237.1371
z=1
m/z

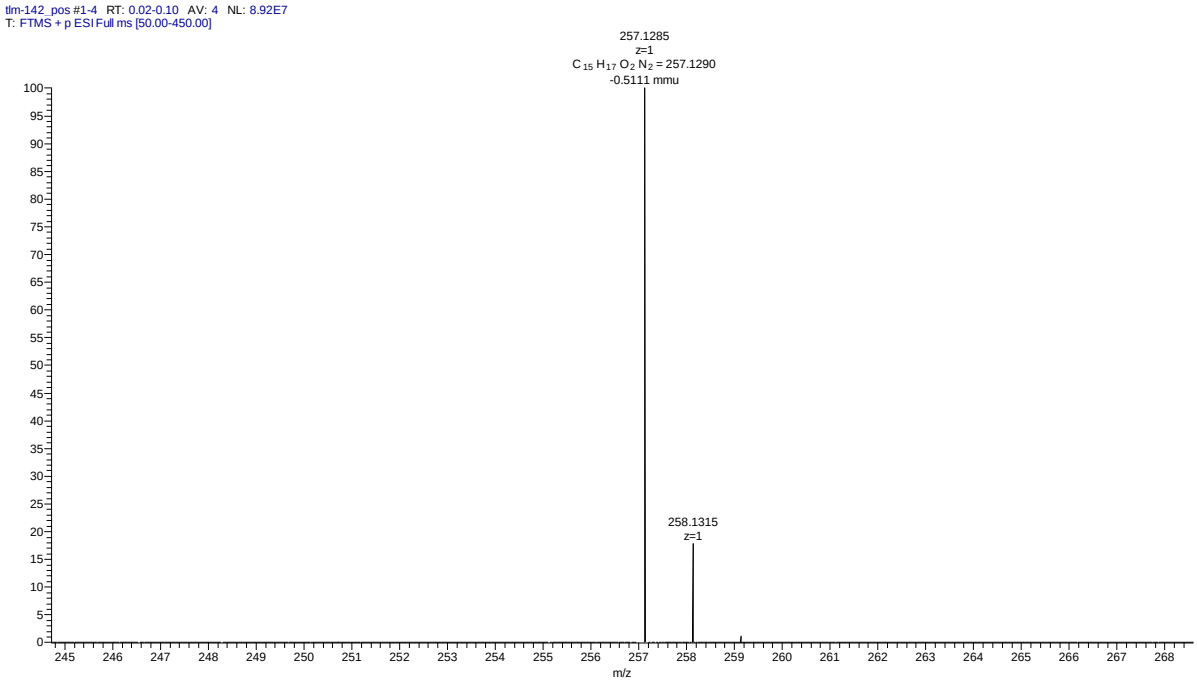

tlm-136_pos #1-4 RT: 0.02-0.12 AV: 4 NL: 1.23E6
T: FTMS + p ESI Full ms [50.00-300.00]
255.1113
z=1
253.1685
z=?
256.1146
z=1
250.9284
z=?
246.9339
z=2
254.1031
z=1
m/z

7.2.5.2 *N*-ethyl-*N*-(3-nitrobenzyl)aniline S_N2 pathway



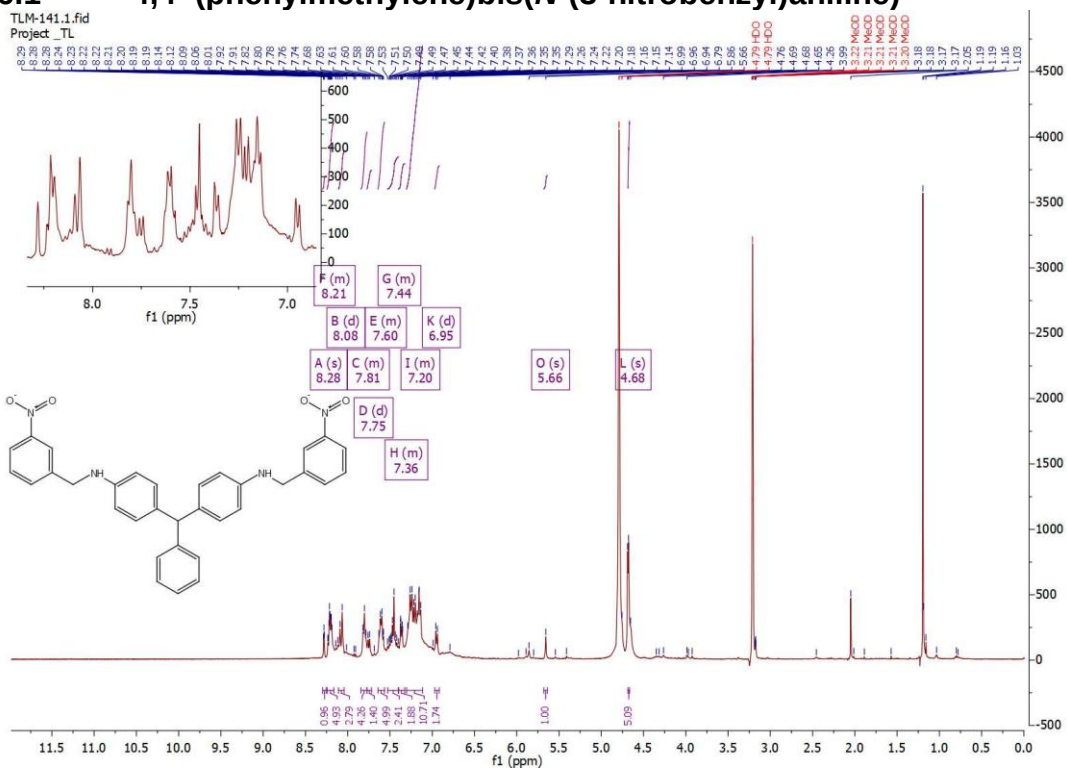


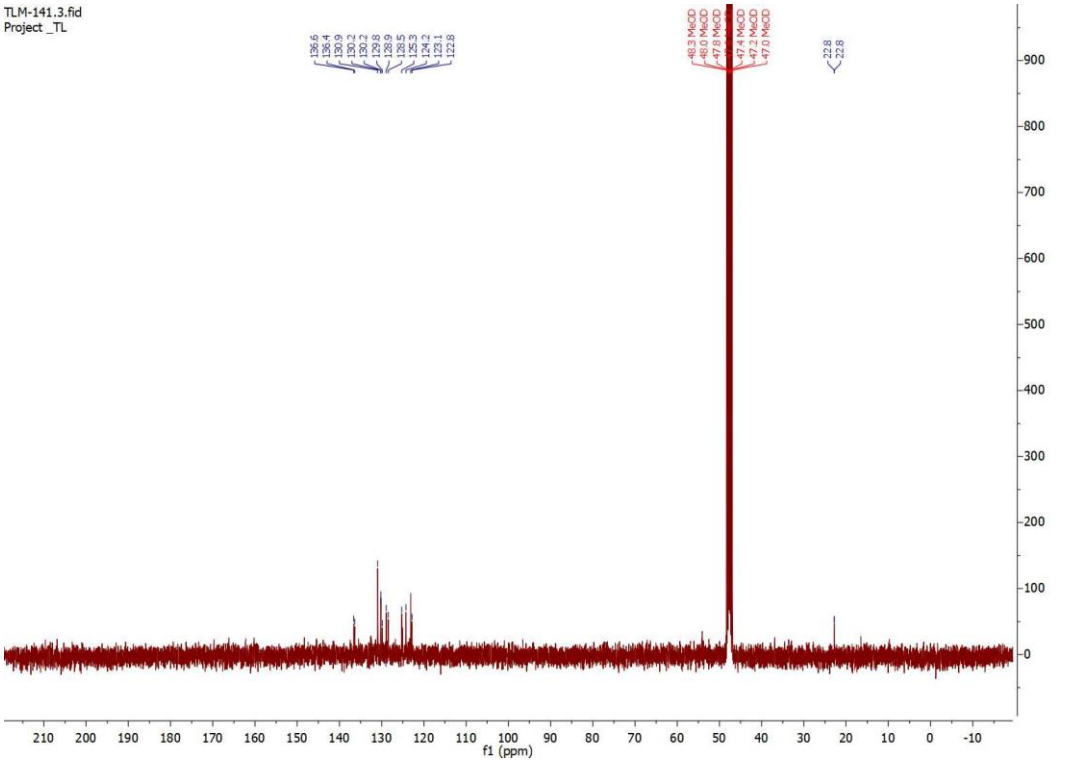

tlm-142_pos #1-4 RT: 0.02-0.10 AV: 4 NL: 8.92E7
T: FTMS + p ESI Full ms [50.00-450.00]
257.1285
z=1
C 15 H 17 O 2 N 2 = 257.1290
-0.5111 mmu
121.0883
z=1
C 8 H 11 N = 121.0891
-0.8493 mmu
106.0648
z=1
C 7 H 8 N = 106.0657
-0.8759 mmu
m/z

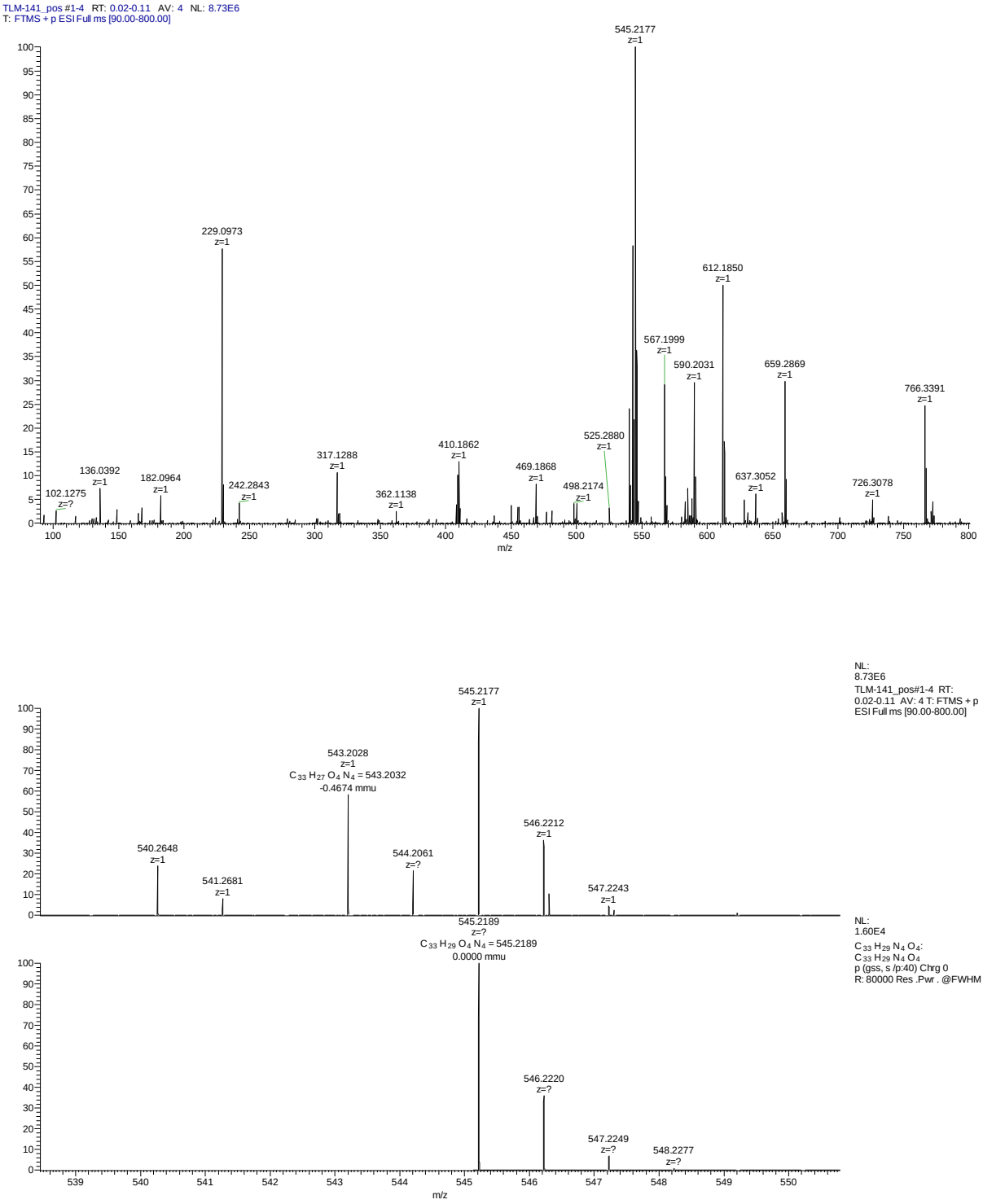

tlm-142_pos #1-4 RT: 0.02-0.10 AV: 4 NL: 8.92E7
T: FTMS + p ESI Full ms [50.00-450.00]
257.1285
z=1
C 15 H 17 O 2 N 2 = 257.1290
-0.5111 mmu
258.1315
z=1
m/z

7.2.6 Synthesis of Brilliant Blue R250 derivatives

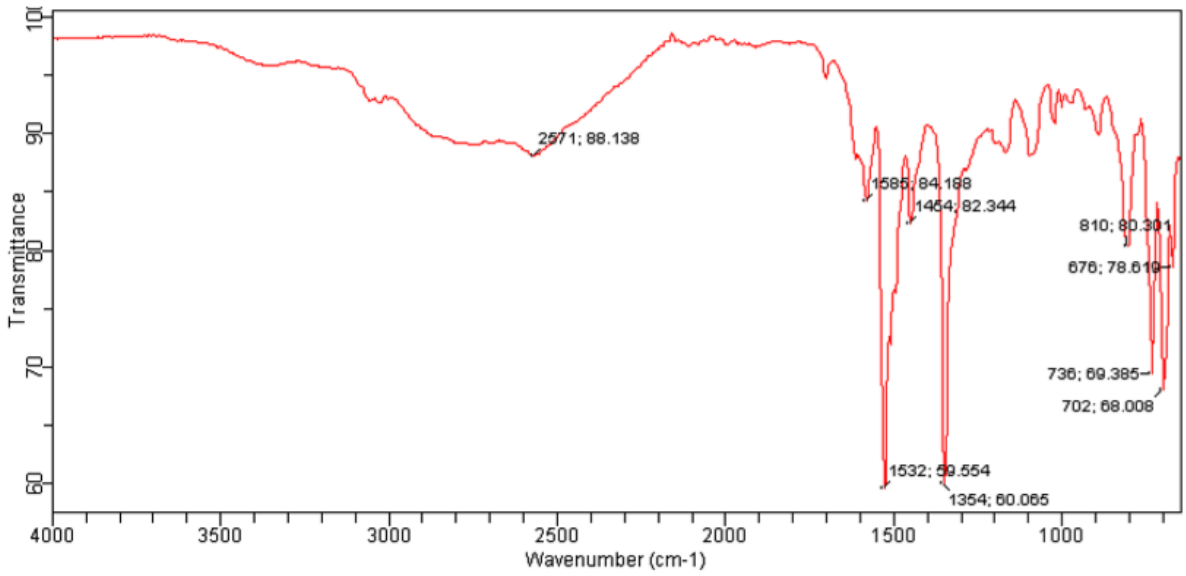
7.2.6.1 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline)



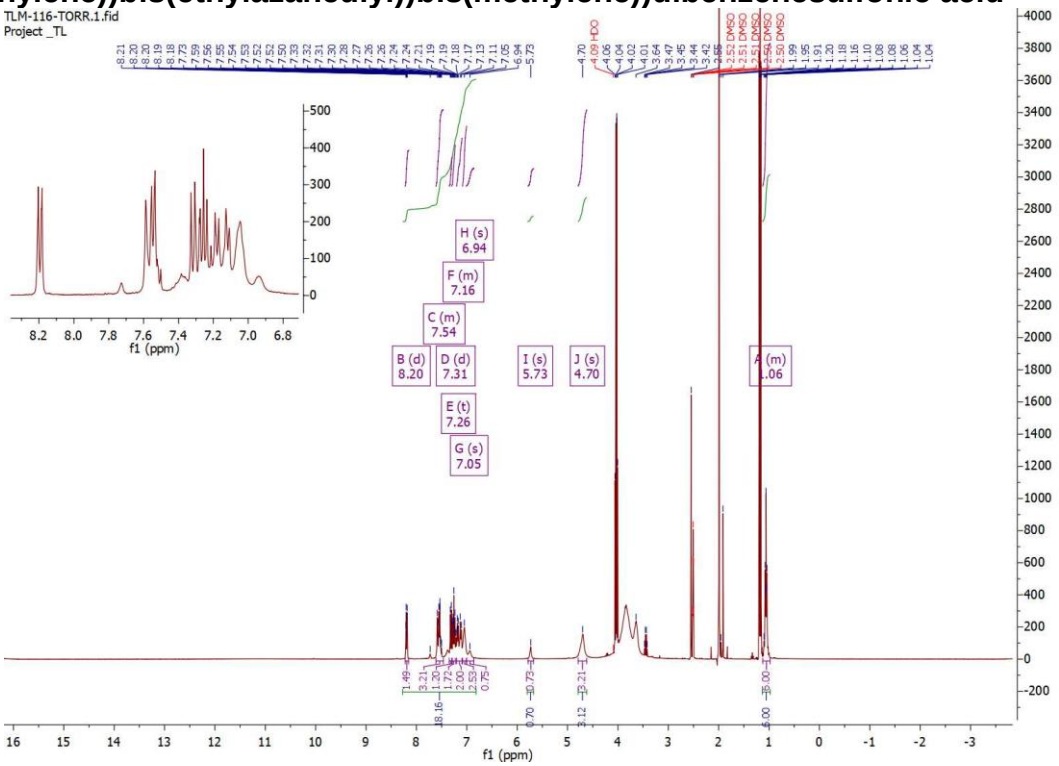


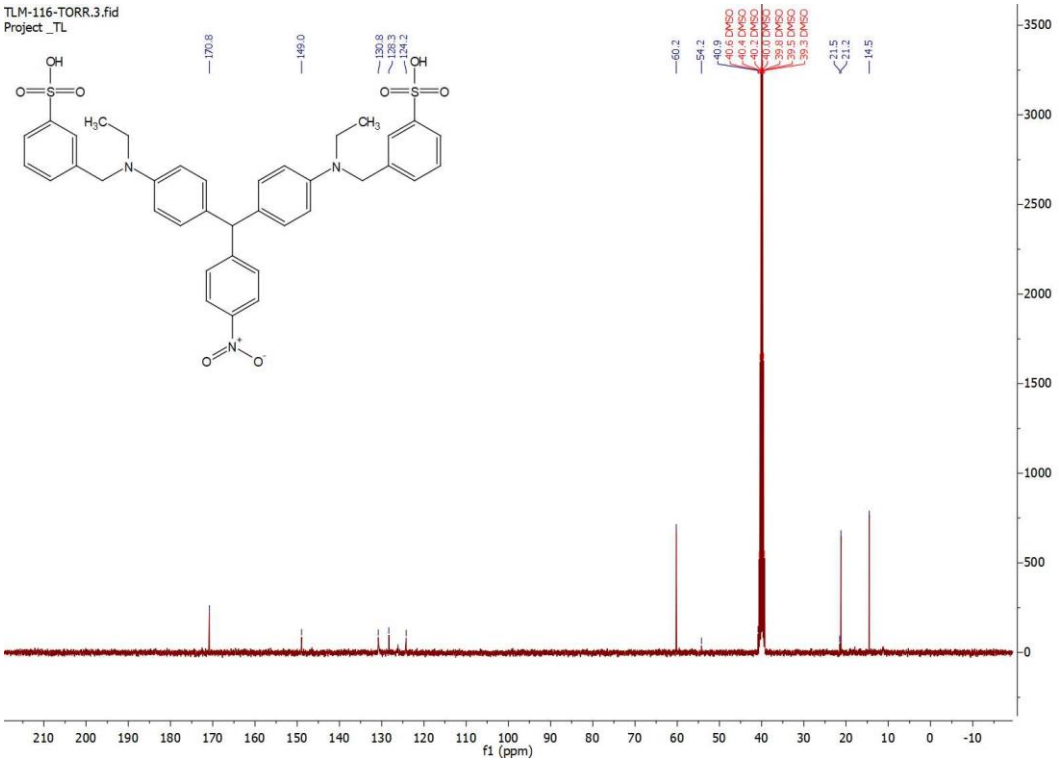

TLM-141_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 8.73E6
T: FTMS + p ESI Full ms [90.00-800.00]
545.2177 z=1
229.0973 z=1
612.1850 z=1
567.1999 z=1
590.2031 z=1
659.2869 z=1
766.3391 z=1
525.2880 z=1
410.1862 z=1
317.1288 z=1
469.1868 z=1
136.0392 z=1
182.0964 z=1
637.3052 z=1
498.2174 z=1
726.3078 z=1
242.2843 z=1
362.1138 z=1
102.1275 z=?
m/z
NL: 8.73E6
TLM-141_pos#1-4 RT: 0.02-0.11 AV: 4 T: FTMS + p ESI Full ms [90.00-800.00]
545.2177 z=1
543.2028 z=1
C33 H27 O4 N4 = 543.2032
-0.4674 mmu
546.2212 z=1
540.2648 z=1
544.2061 z=?
541.2681 z=1
547.2243 z=1
545.2189 z=?
C33 H29 O4 N4 = 545.2189
0.0000 mmu
NL: 1.60E4
C33 H29 N4 O4: C33 H29 N4 O4
p (gss, s /p:40) Chrg 0
R: 80000 Res .Pwr . @FWHM
546.2220 z=?
547.2249 z=?
548.2277 z=?
m/z

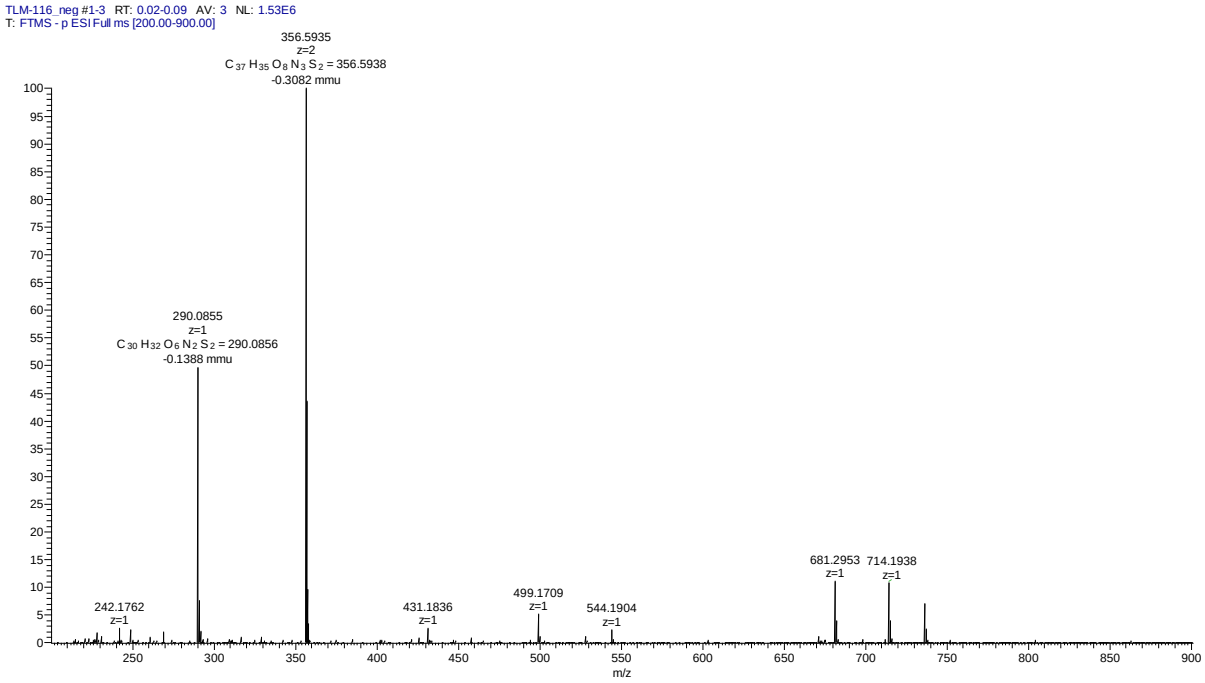
Sample ID:	tlm-141	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 4:52:12PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-141_2019-04-30T16-53-12.a2r		

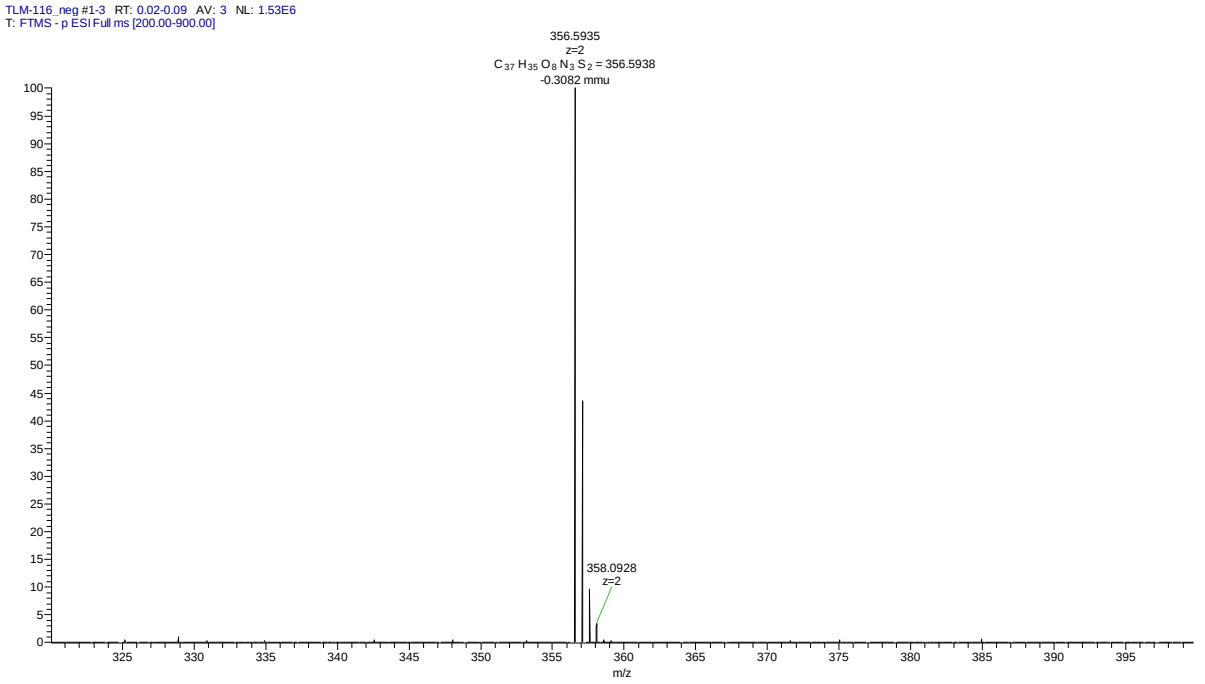


7.2.6.2 3,3'-(((((4-nitrophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

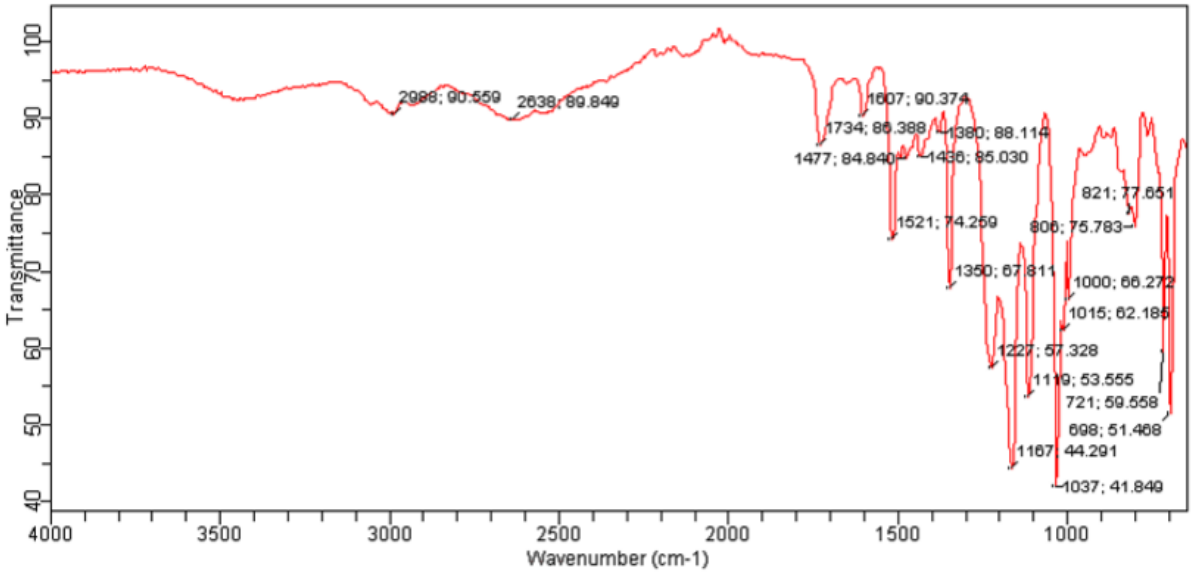




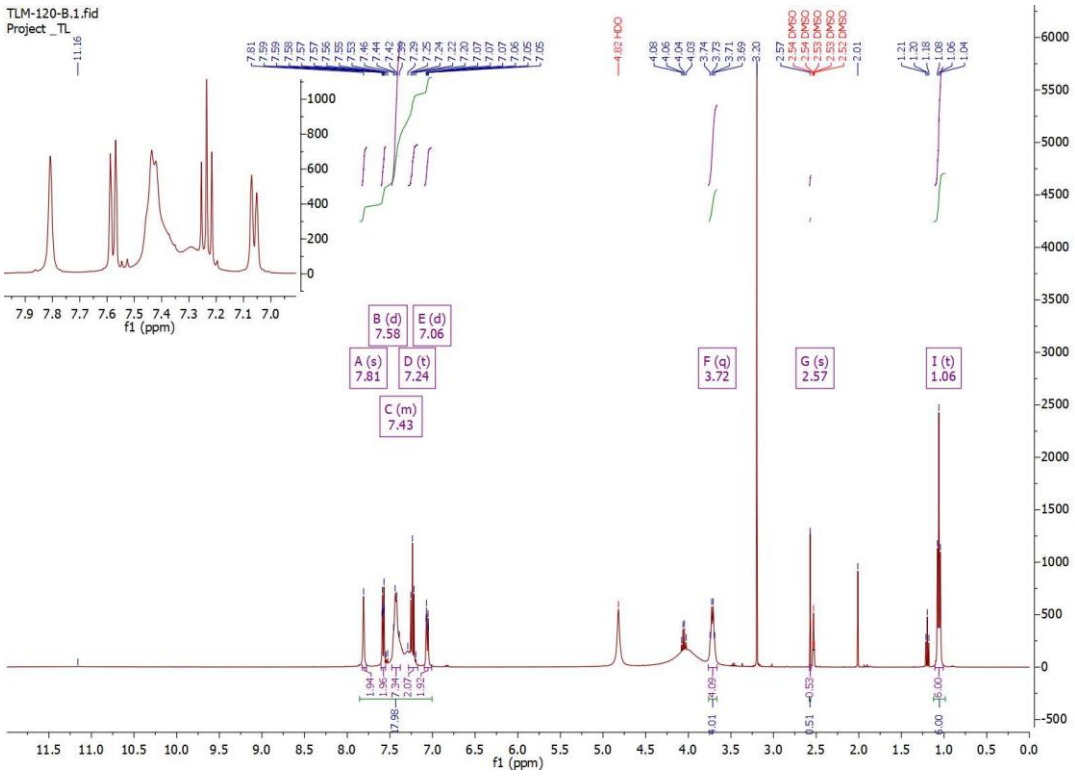

TLM-116_neg #1-3 RT: 0.02-0.09 AV: 3 NL: 1.53E6
T: FTMS - p ESI Full ms [200.00-900.00]
356.5935
z=2
C 37 H 35 O 8 N 3 S 2 = 356.5938
-0.3082 mmu
290.0855
z=1
C 30 H 32 O 6 N 2 S 2 = 290.0856
-0.1388 mmu
242.1762
z=1
431.1836
z=1
499.1709
z=1
544.1904
z=1
681.2953
z=1
714.1938
z=1
m/z

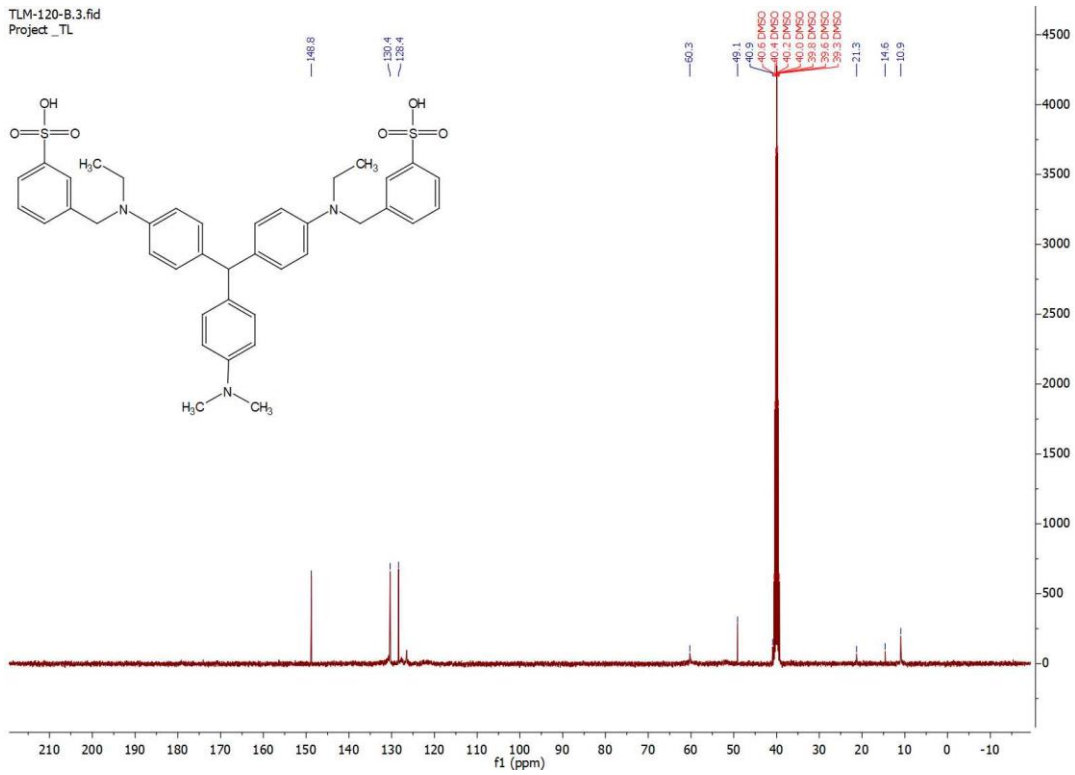

TLM-116_neg #1-3 RT: 0.02-0.09 AV: 3 NL: 1.53E6
T: FTMS - p ESI Full ms [200.00-900.00]
356.5935
z=2
C 37 H 35 O 8 N 3 S 2 = 356.5938
-0.3082 mmu
358.0928
z=2
m/z

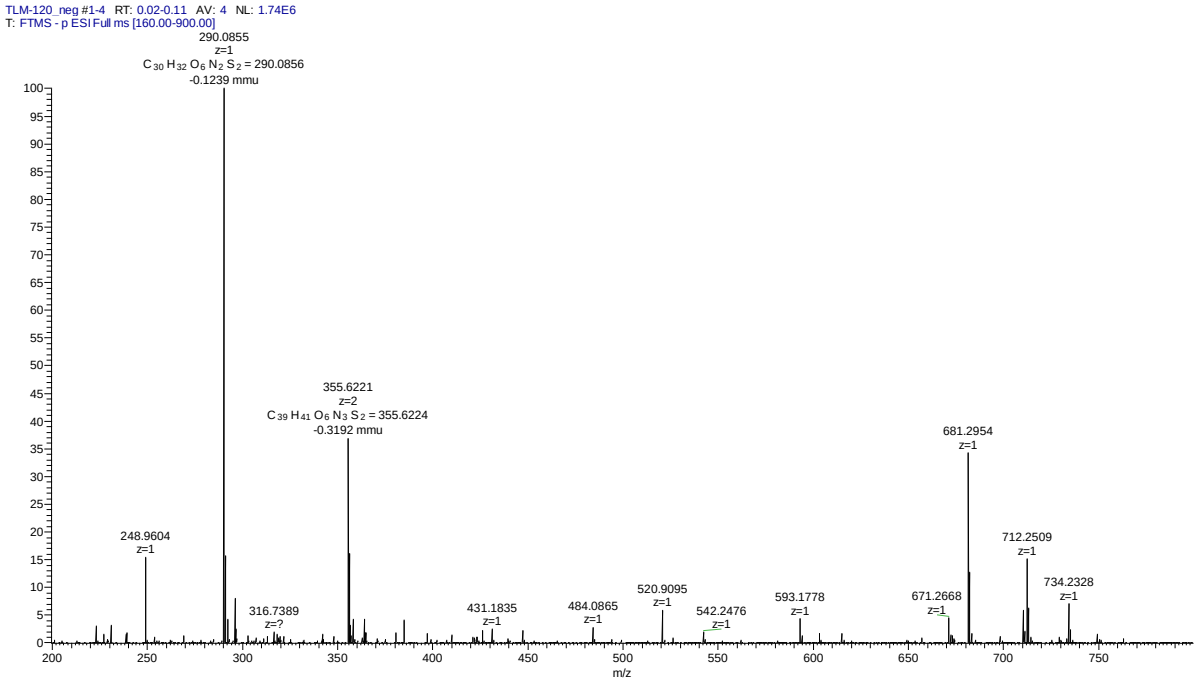
Sample ID:	tlm-116	Method Name:	ATR-default
Sample Scans:	16	User:	IK
Background Scans:	16	Date/Time:	10/23/2018 10:29:20AM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-116_2018-10-23T10-31-08.a2r		

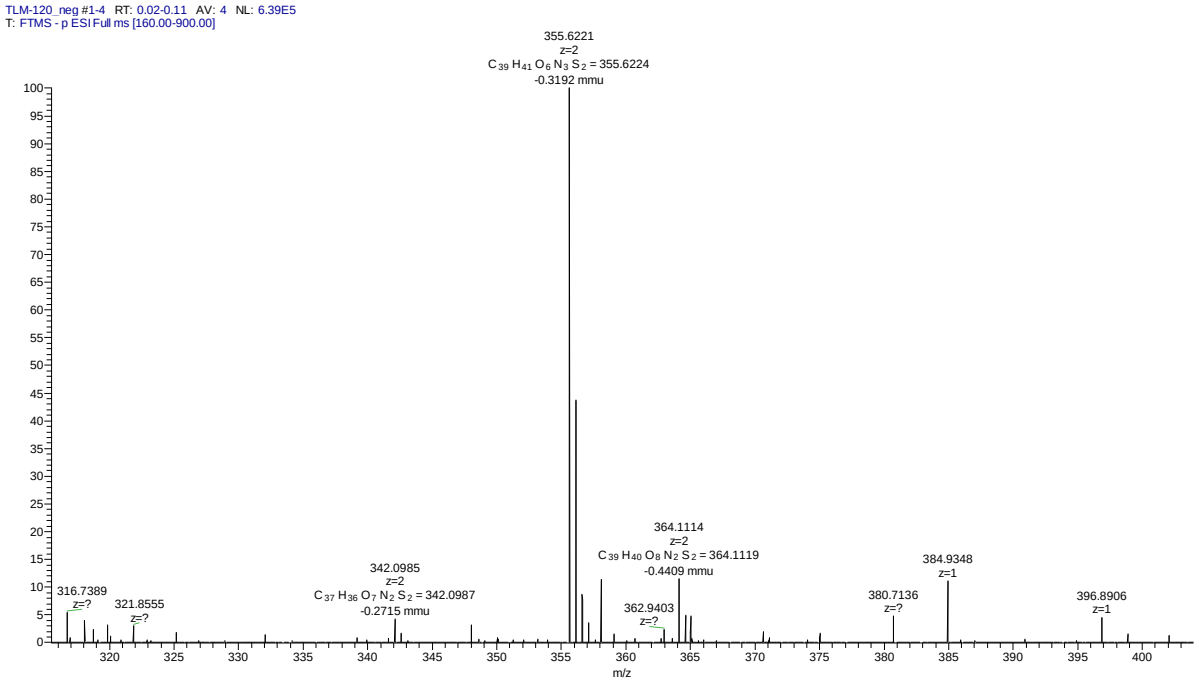


7.2.6.3 3,3'-(((((4-(dimethylamino)phenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

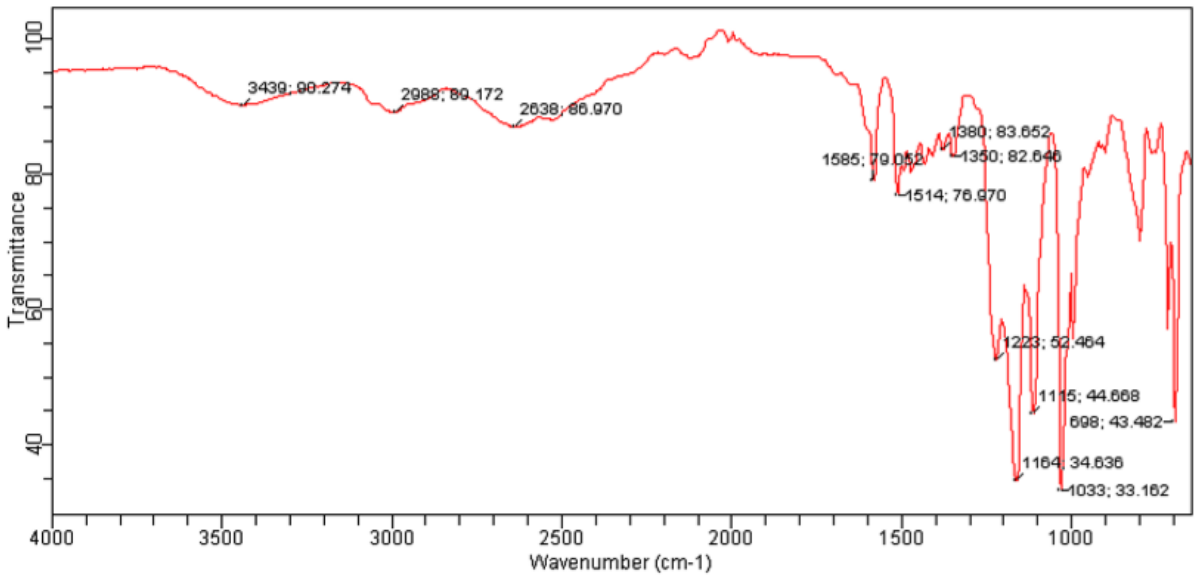




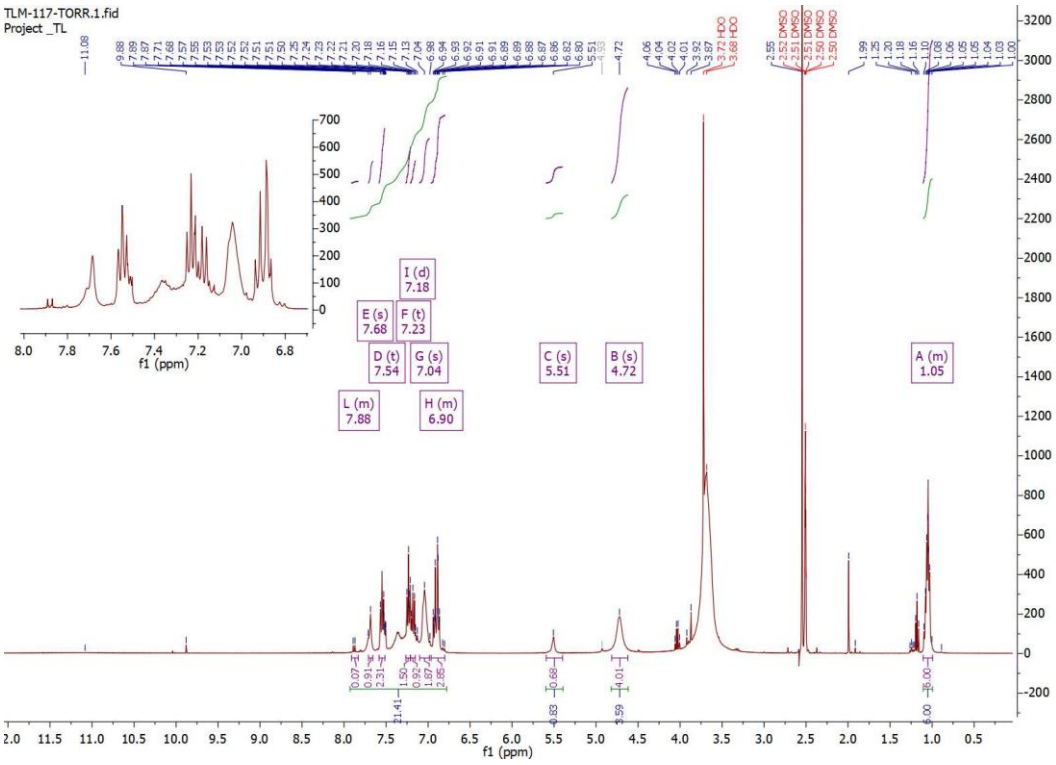

TLM-120_neg #1-4 RT: 0.02-0.11 AV: 4 NL: 1.74E6
T: FTMS - p ESI Full ms [160.00-900.00]
290.0855
z=1
C 30 H 32 O 6 N 2 S 2 = 290.0856
-0.1239 mmu
355.6221
z=2
C 39 H 41 O 6 N 3 S 2 = 355.6224
-0.3192 mmu
681.2954
z=1
248.9604
z=1
712.2509
z=1
734.2328
z=1
316.7389
z=?
431.1835
z=1
484.0865
z=1
520.9095
z=1
542.2476
z=1
593.1778
z=1
671.2668
z=1
m/z

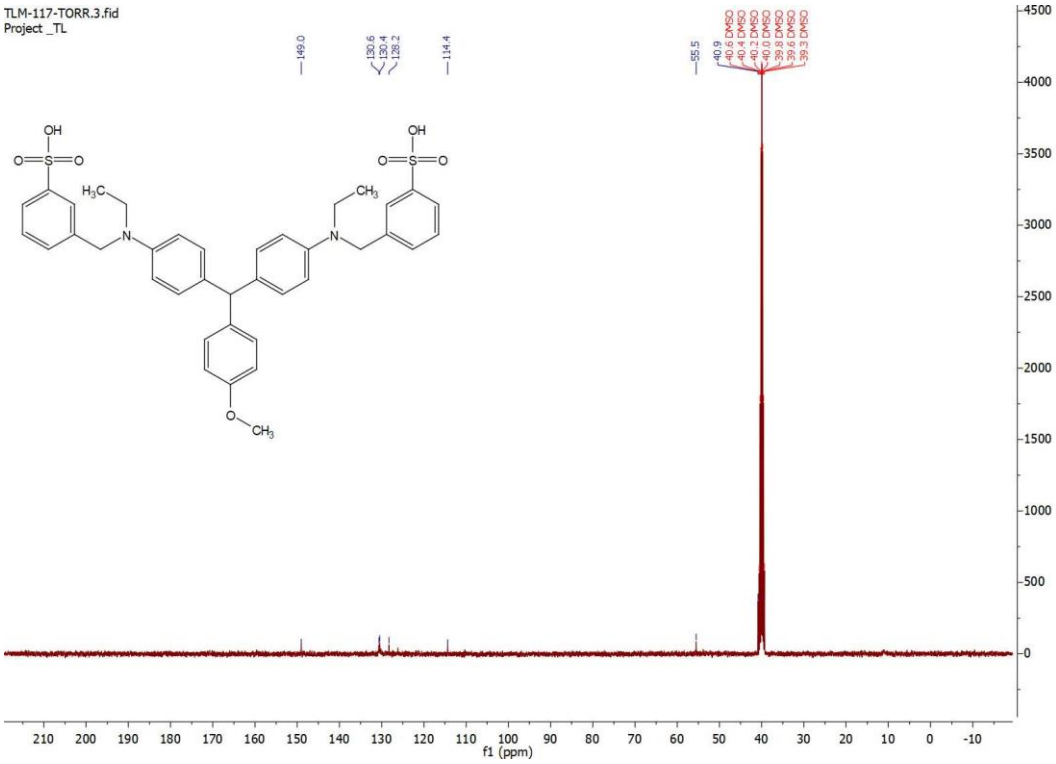

TLM-120_neg #1-4 RT: 0.02-0.11 AV: 4 NL: 6.39E5
T: FTMS - p ESI Full ms [160.00-900.00]
355.6221
z=2
C 39 H 41 O 6 N 3 S 2 = 355.6224
-0.3192 mmu
364.1114
z=2
C 39 H 40 O 8 N 2 S 2 = 364.1119
-0.4409 mmu
342.0985
z=2
C 37 H 36 O 7 N 2 S 2 = 342.0987
-0.2715 mmu
384.9348
z=1
316.7389
z=?
321.8555
z=?
362.9403
z=?
380.7136
z=?
396.8906
z=1
m/z

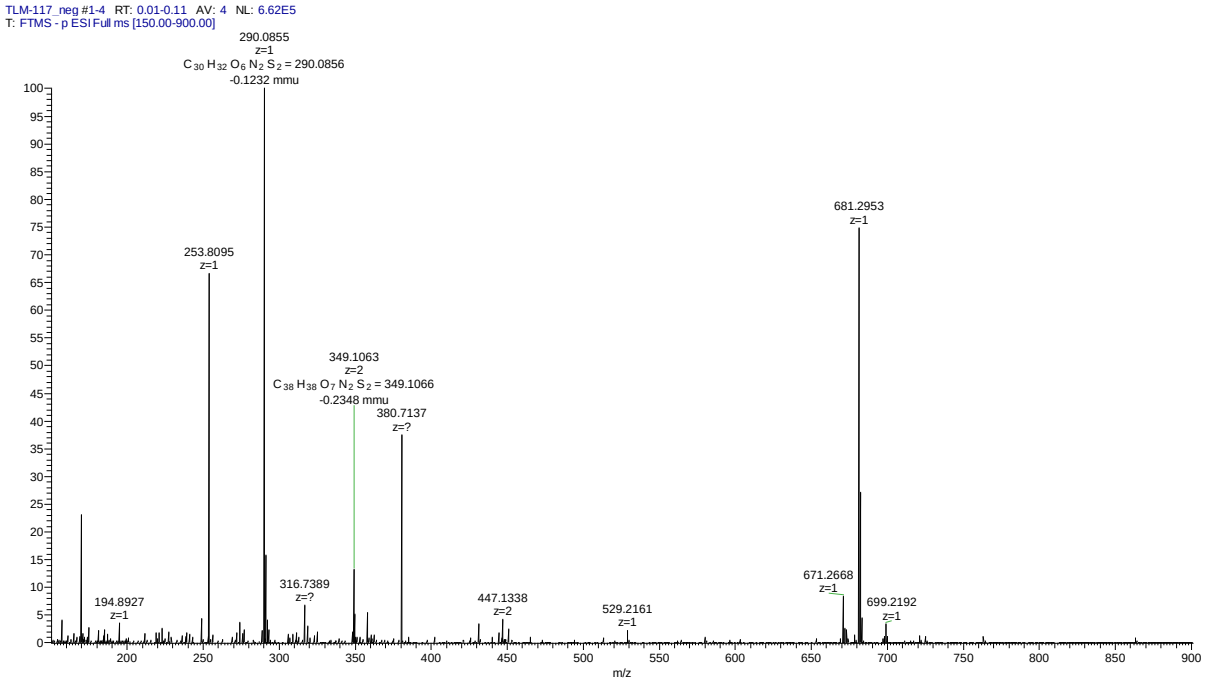
Sample ID:	tlm-120	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 4:59:28PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-120_2019-04-30T17-02-21.a2r		

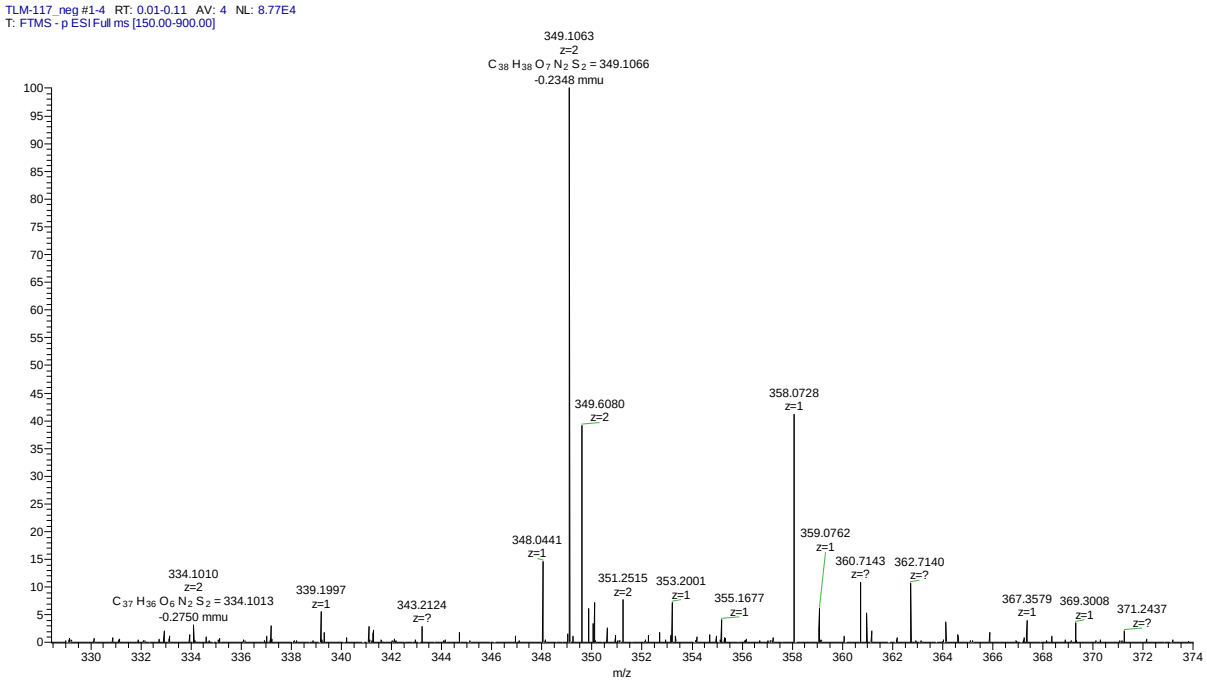


7.2.6.4 3,3'-(((((4-methoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

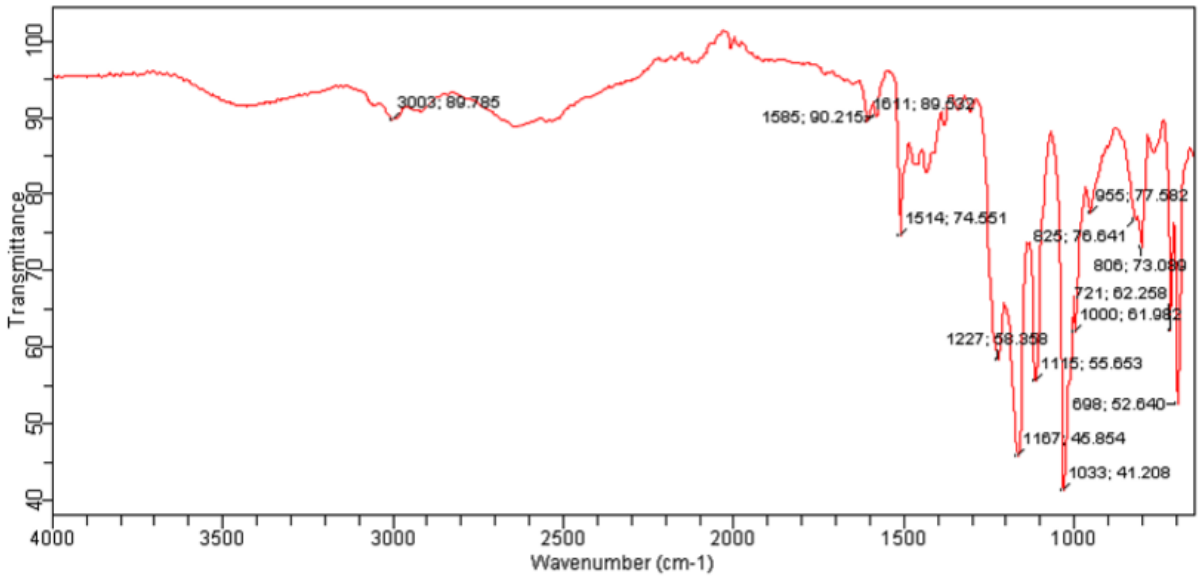




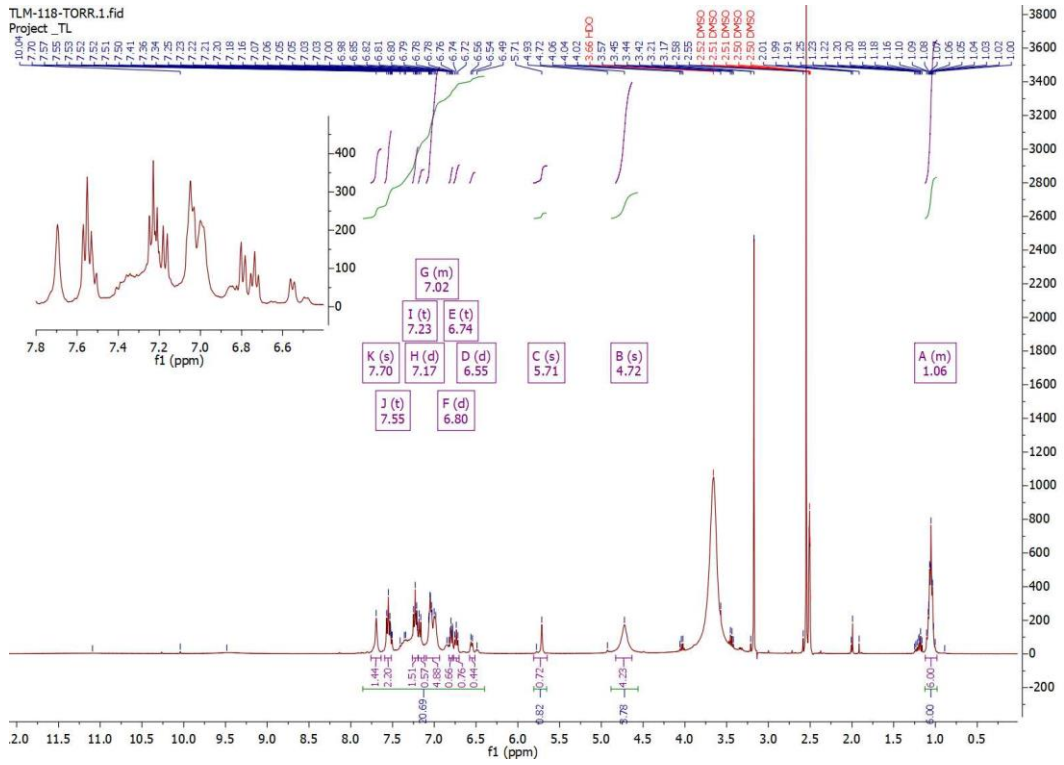

TLM-117_neg #1-4 RT: 0.01-0.11 AV: 4 NL: 6.62E5
T: FTMS - p ESI Full ms [150.00-900.00]
290.0855
z=1
C30 H32 O6 N2 S2 = 290.0856
-0.1232 mmu
253.8095
z=1
349.1063
z=2
C38 H38 O7 N2 S2 = 349.1066
-0.2348 mmu
380.7137
z=?
681.2953
z=1
194.8927
z=1
316.7389
z=?
447.1338
z=2
529.2161
z=1
671.2668
z=1
699.2192
z=1
m/z

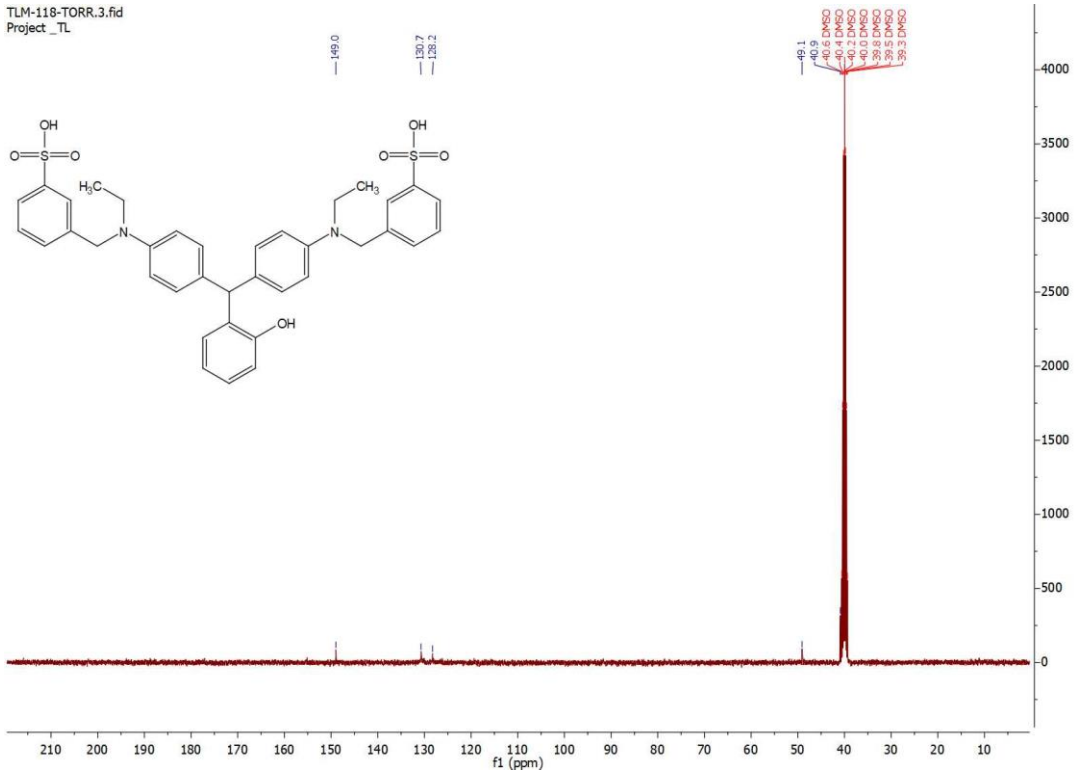

TLM-117_neg #1-4 RT: 0.01-0.11 AV: 4 NL: 8.77E4
T: FTMS - p ESI Full ms [150.00-900.00]
349.1063
z=2
C38 H38 O7 N2 S2 = 349.1066
-0.2348 mmu
349.6080
z=2
358.0728
z=1
348.0441
z=1
359.0762
z=1
334.1010
z=2
C37 H36 O6 N2 S2 = 334.1013
-0.2750 mmu
339.1997
z=1
343.2124
z=?
351.2515
z=2
353.2001
z=1
355.1677
z=1
360.7143
z=?
362.7140
z=?
367.3579
z=1
369.3008
z=1
371.2437
z=?
m/z

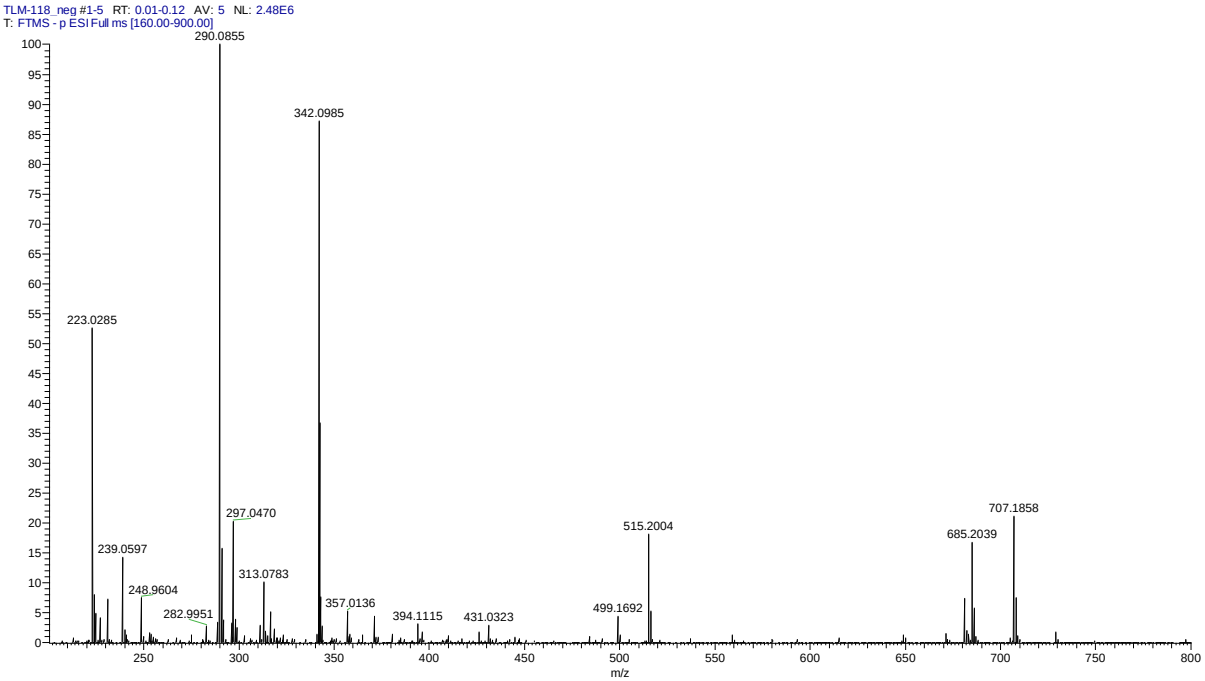
Sample ID:	tlm-117	Method Name:	ATR-default
Sample Scans:	16	User:	IK
Background Scans:	16	Date/Time:	10/23/2018 10:33:55AM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-117_2018-10-23T10-36-29.a2r		

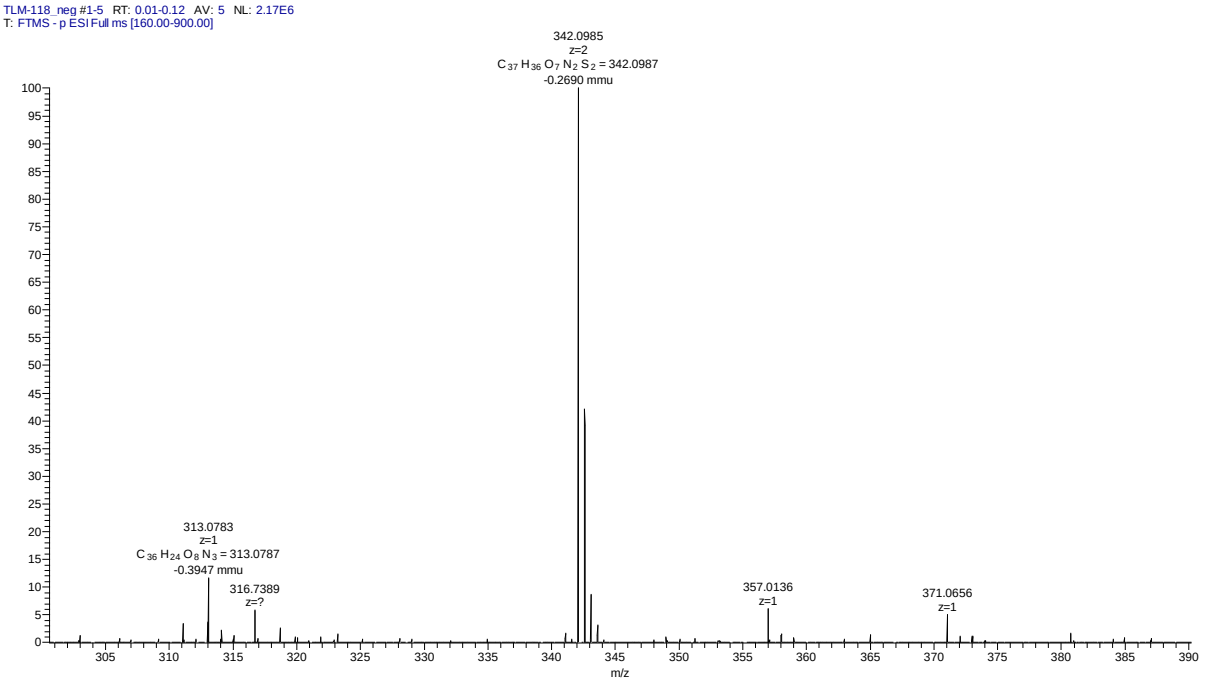


7.2.6.5 3,3'-(((((2-hydroxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

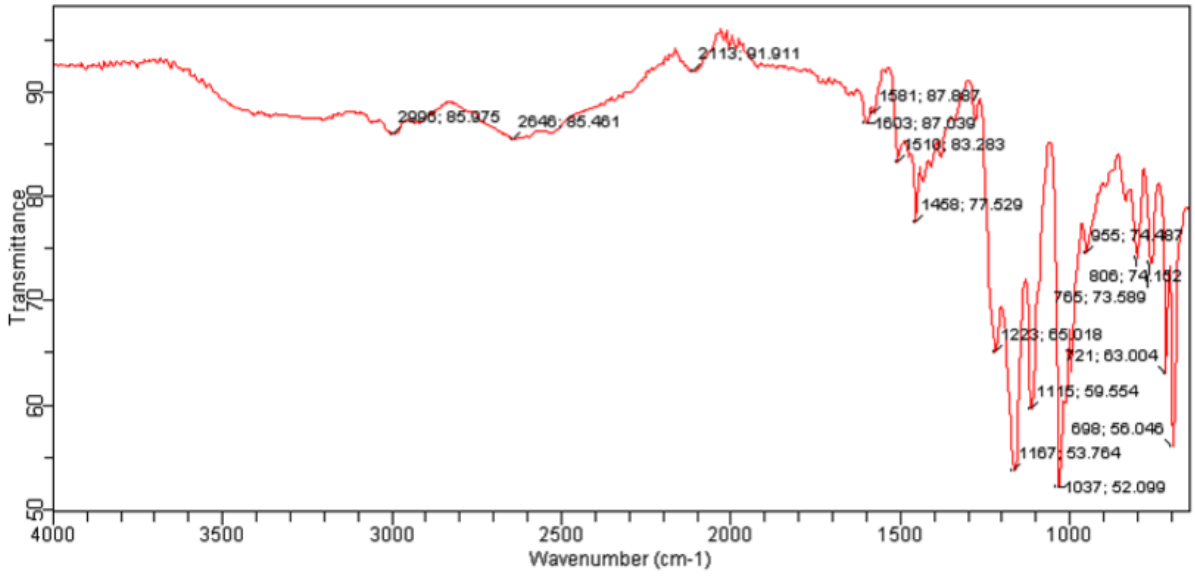




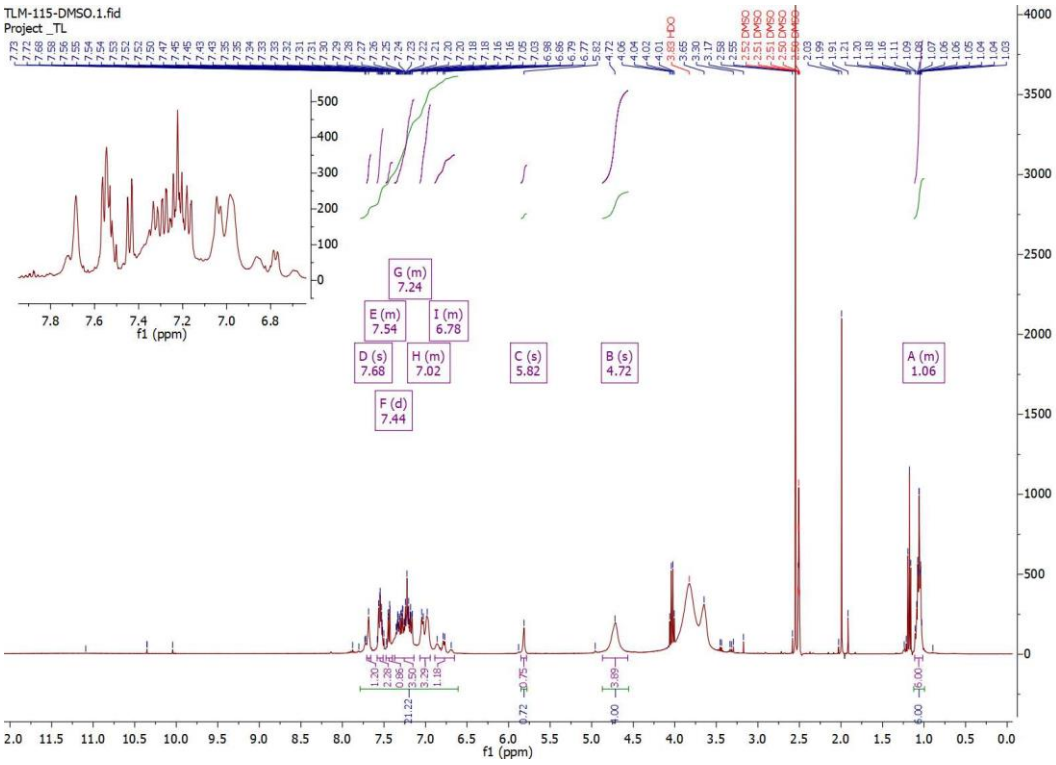

TLM-118_neg #1-5 RT: 0.01-0.12 AV: 5 NL: 2.48E6
T: FTMS - p ESI Full ms [160.00-900.00]
290.0855
342.0985
223.0285
297.0470
239.0597
313.0783
248.9604
357.0136
282.9951
394.1115
431.0323
499.1692
515.2004
685.2039
707.1858
m/z

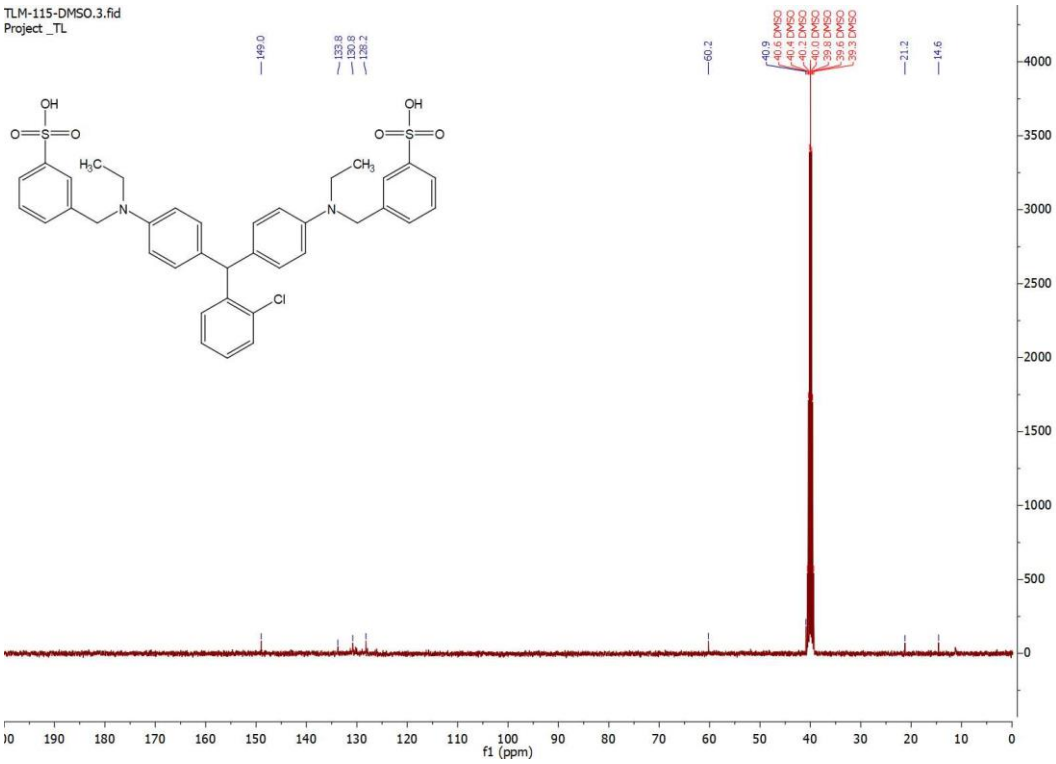

TLM-118_neg #1-5 RT: 0.01-0.12 AV: 5 NL: 2.17E6
T: FTMS - p ESI Full ms [160.00-900.00]
342.0985
z=2
C37 H36 O7 N2 S2 = 342.0987
-0.2690 mmu
313.0783
z=1
C36 H24 O8 N3 = 313.0787
-0.3947 mmu
316.7389
z=?
357.0136
z=1
371.0656
z=1
m/z

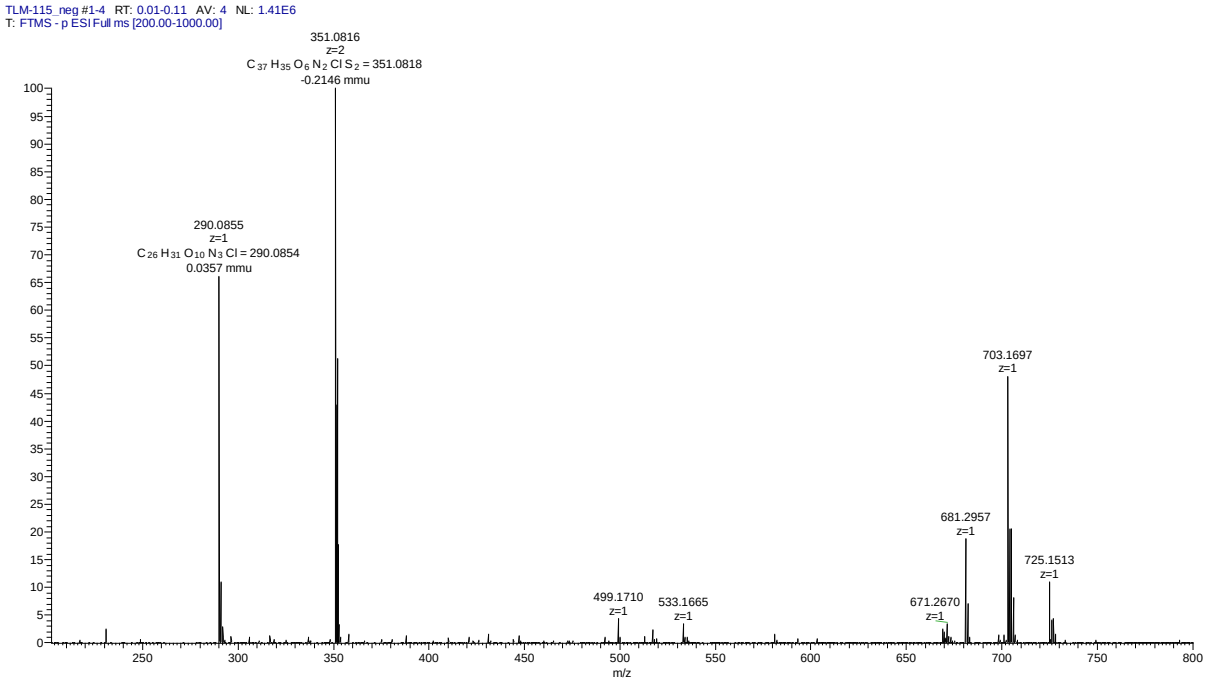
Sample ID:	tlm-118	Method Name:	ATR-default
Sample Scans:	16	User:	IK
Background Scans:	16	Date/Time:	10/23/2018 10:40:56AM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-118_2018-10-23T10-44-43.a2r		

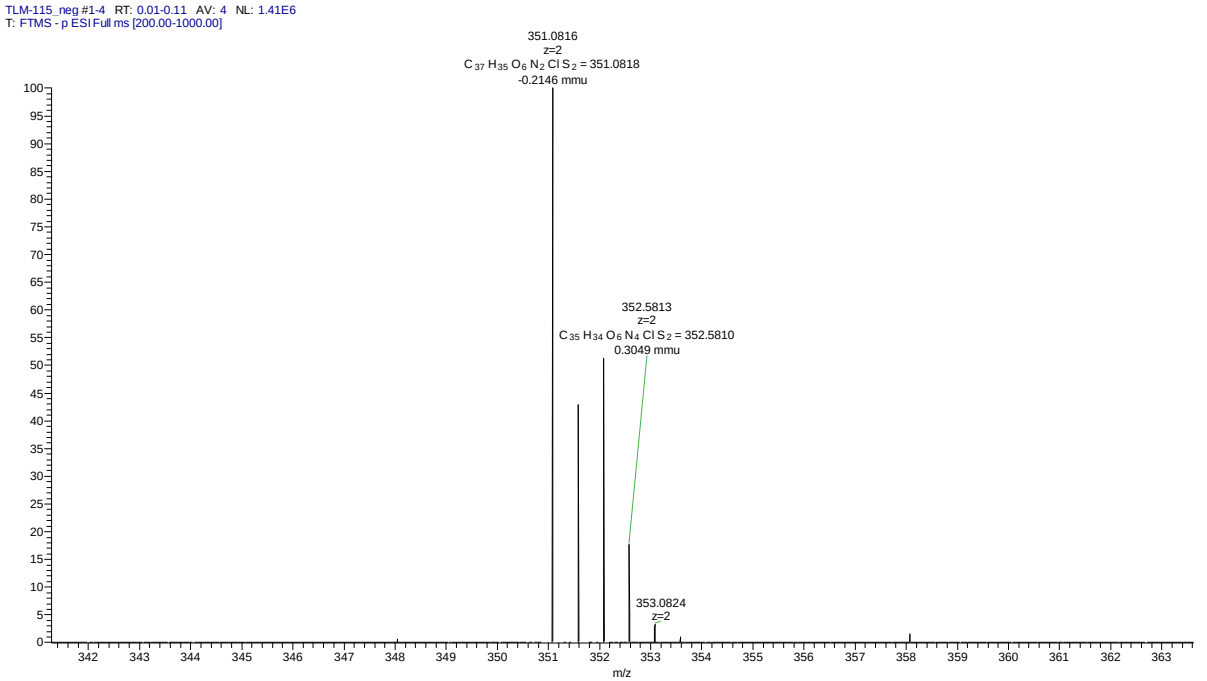


7.2.6.6 3,3'-(((((2-chlorophenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

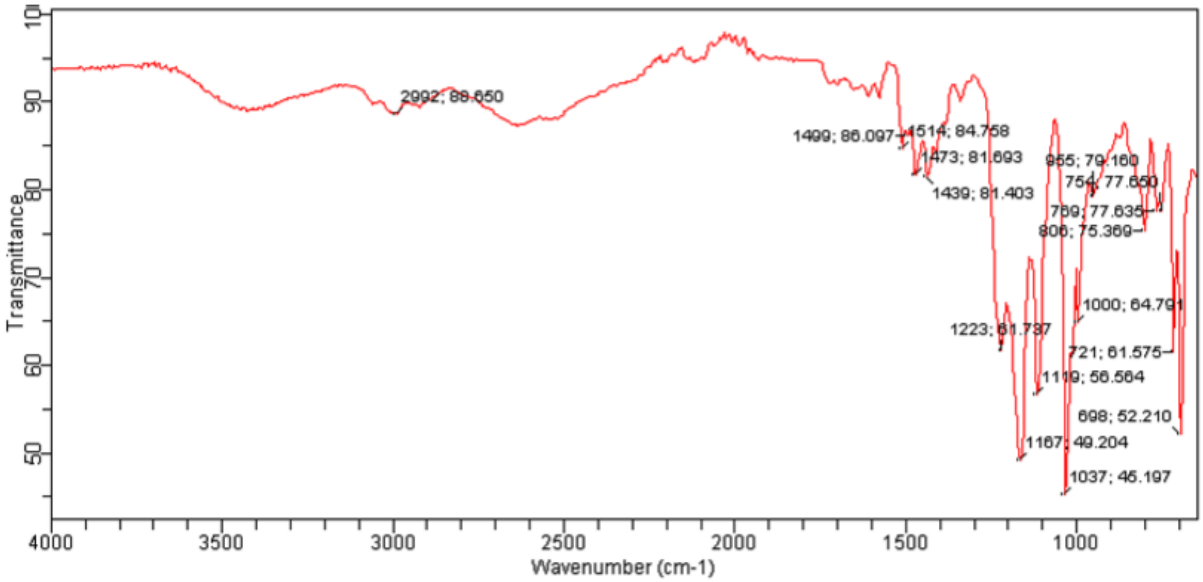




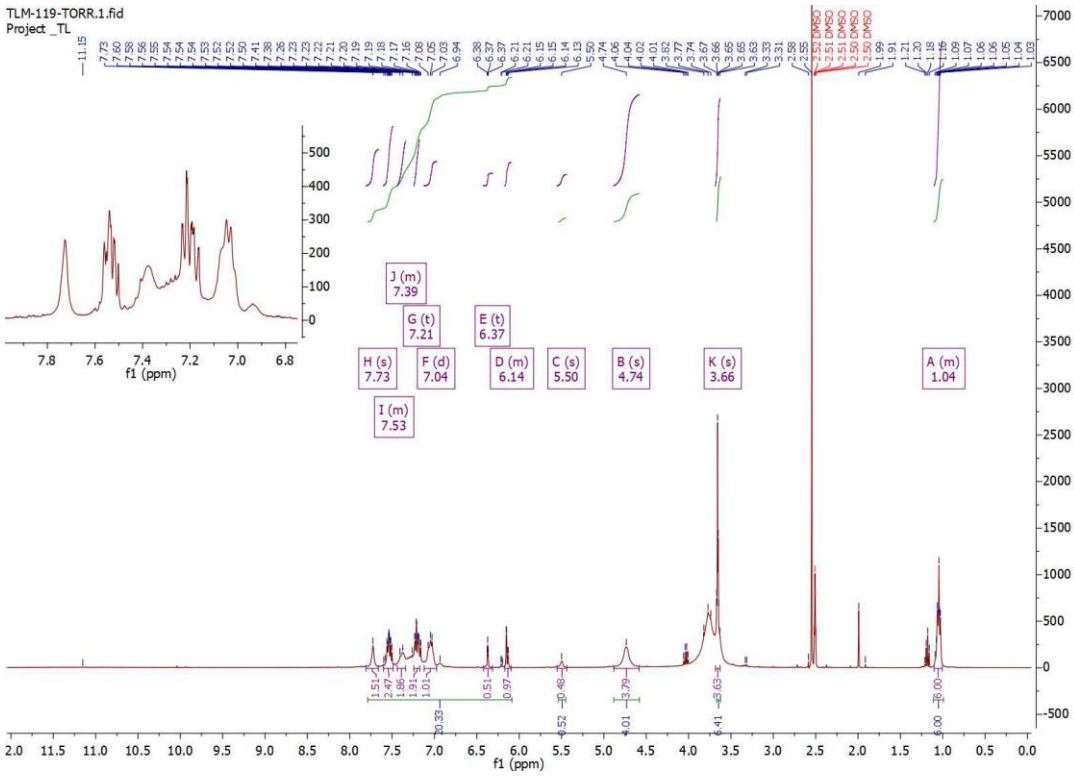

TLM-115_neg #1-4 RT: 0.01-0.11 AV: 4 NL: 1.41E6
T: FTMS - p ESI Full ms [200.00-1000.00]
351.0816
z=2
C 37 H 35 O 6 N 2 Cl S 2 = 351.0818
-0.2146 mmu
290.0855
z=1
C 26 H 31 O 10 N 3 Cl = 290.0854
0.0357 mmu
703.1697
z=1
681.2957
z=1
725.1513
z=1
499.1710
z=1
533.1665
z=1
671.2670
z=1
m/z

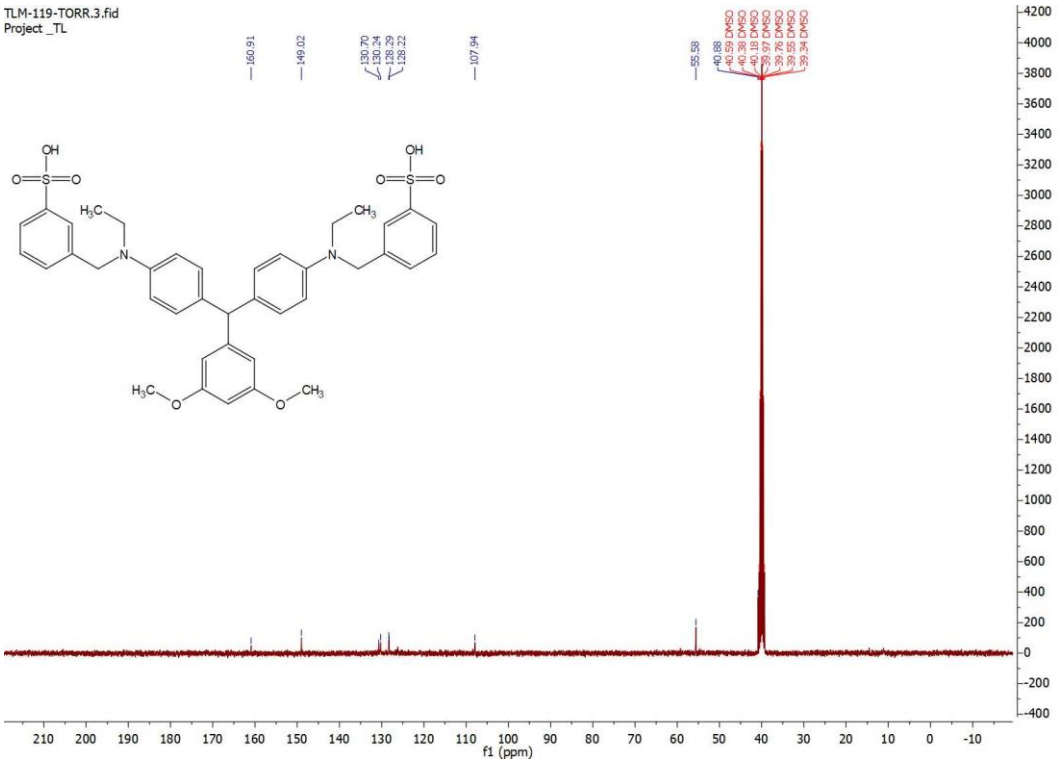

TLM-115_neg #1-4 RT: 0.01-0.11 AV: 4 NL: 1.41E6
T: FTMS - p ESI Full ms [200.00-1000.00]
351.0816
z=2
C 37 H 35 O 6 N 2 Cl S 2 = 351.0818
-0.2146 mmu
352.5813
z=2
C 35 H 34 O 6 N 4 Cl S 2 = 352.5810
0.3049 mmu
353.0824
z=2
m/z

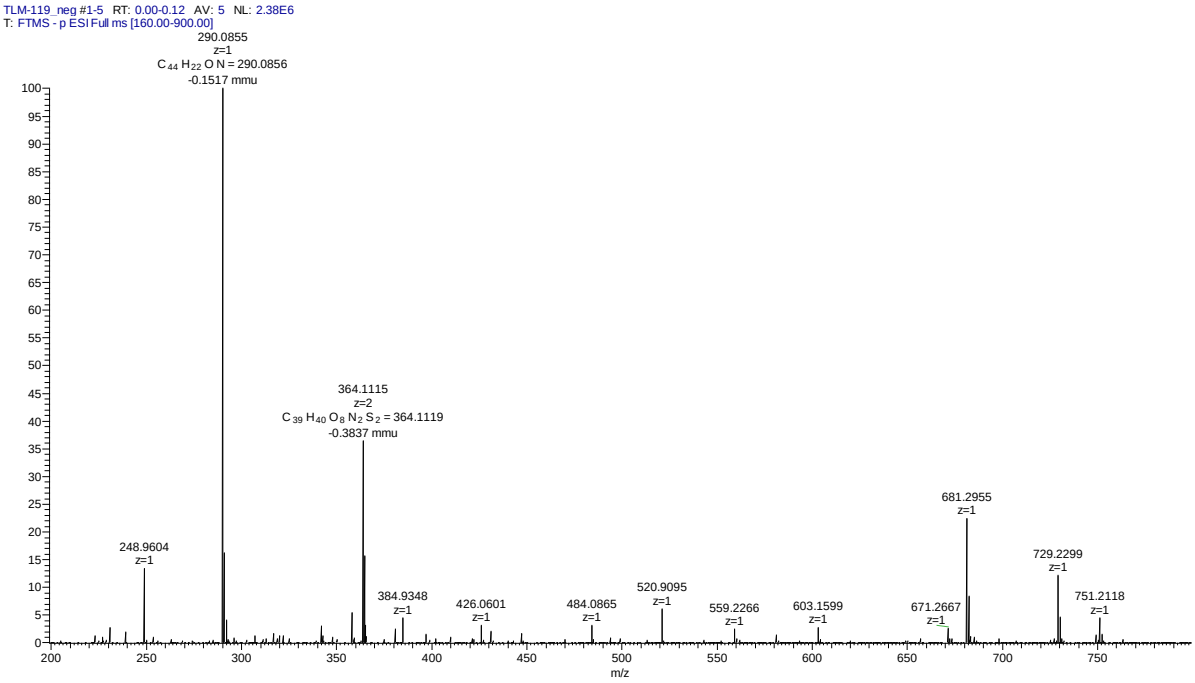
Sample ID:	tlm-115	Method Name:	ATR-default
Sample Scans:	16	User:	IK
Background Scans:	16	Date/Time:	10/23/2018 10:23:29AM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-115_2018-10-23T10-25-52.a2r		

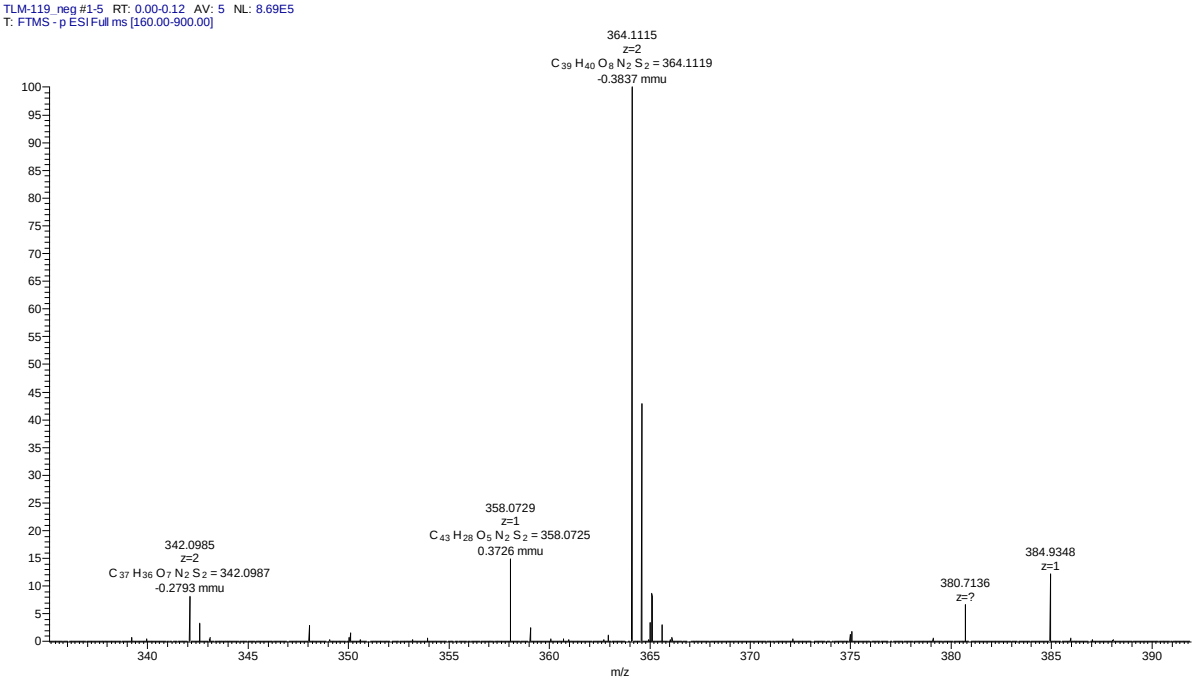


7.2.6.7 3,3'-(((((3,5-dimethoxyphenyl)methylene)bis(4,1-phenylene))bis(ethylazanediyl))bis(methylene))dibenzenesulfonic acid

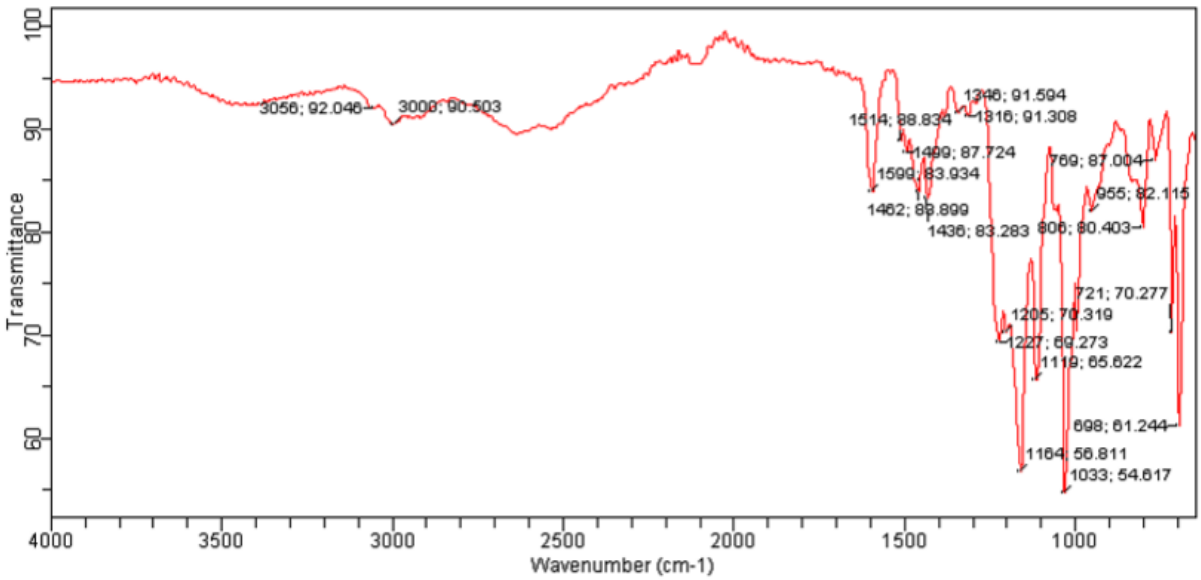




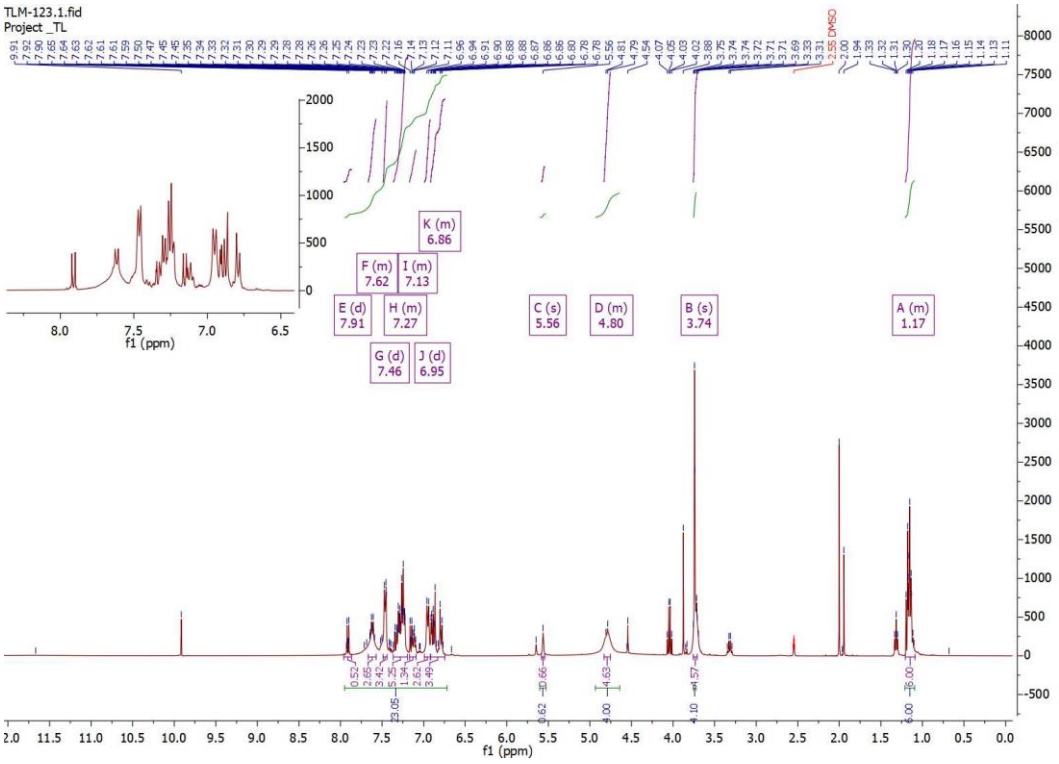

TLM-119_neg #1-5 RT: 0.00-0.12 AV: 5 NL: 2.38E6
T: FTMS - p ESI Full ms [160.00-900.00]
290.0855
z=1
C 44 H 22 O N = 290.0856
-0.1517 mmu
364.1115
z=2
C 39 H 40 O 8 N 2 S 2 = 364.1119
-0.3837 mmu
248.9604
z=1
384.9348
z=1
426.0601
z=1
484.0865
z=1
520.9095
z=1
559.2266
z=1
603.1599
z=1
671.2667
z=1
681.2955
z=1
729.2299
z=1
751.2118
z=1
m/z

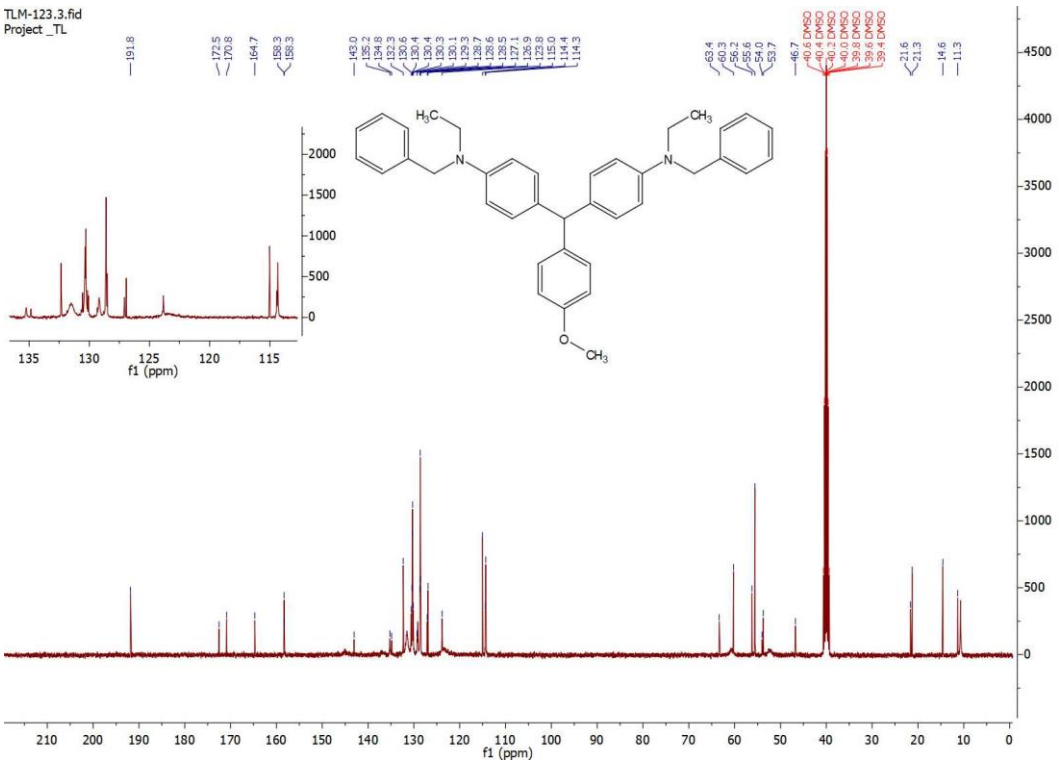

TLM-119_neg #1-5 RT: 0.00-0.12 AV: 5 NL: 8.69E5
T: FTMS - p ESI Full ms [160.00-900.00]
364.1115
z=2
C 39 H 40 O 8 N 2 S 2 = 364.1119
-0.3837 mmu
358.0729
z=1
C 43 H 28 O 5 N 2 S 2 = 358.0725
0.3726 mmu
342.0985
z=2
C 37 H 36 O 7 N 2 S 2 = 342.0987
-0.2793 mmu
380.7136
z=?
384.9348
z=1
m/z

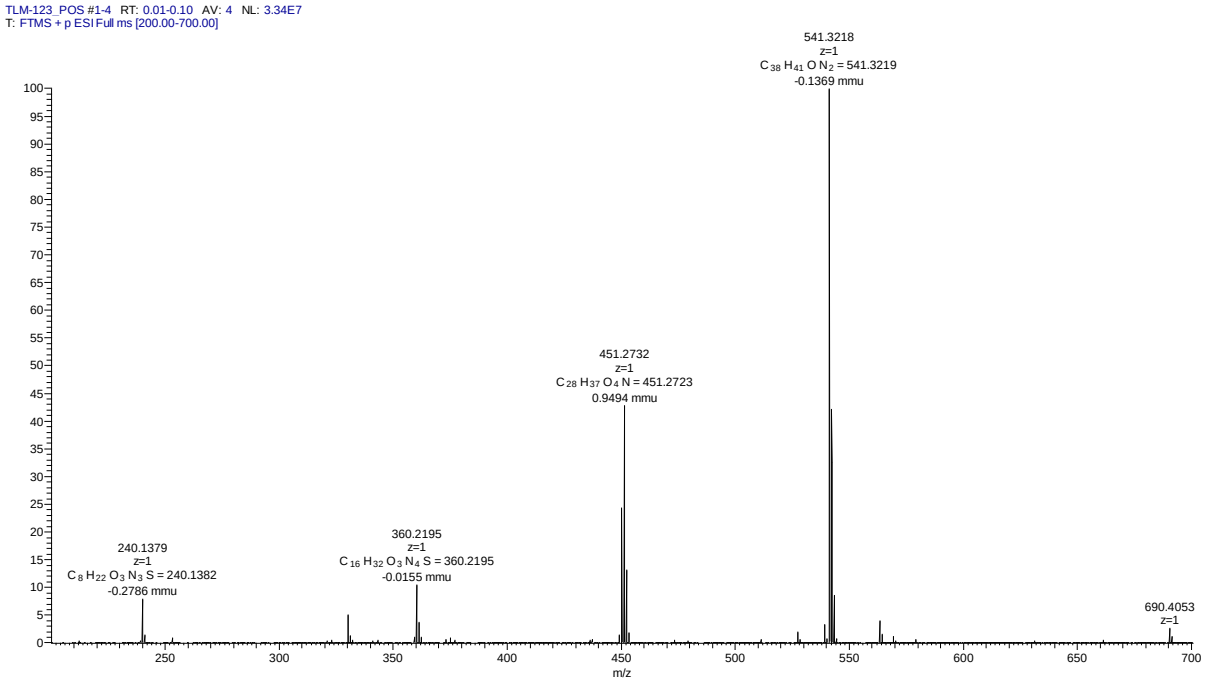
Sample ID:	tlm-119	Method Name:	ATR-default
Sample Scans:	16	User:	IK
Background Scans:	16	Date/Time:	10/23/2018 10:49:00AM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-119_2018-10-23T10-53-22.a2r		

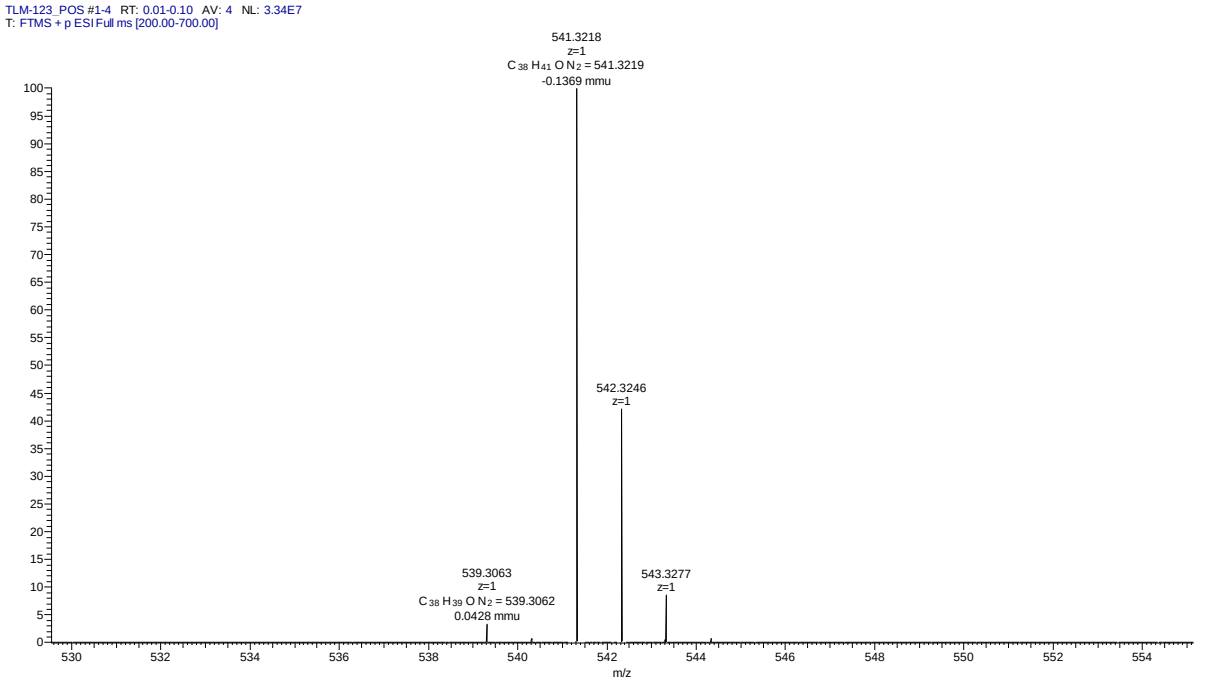


7.2.6.8 4,4'-((4-methoxyphenyl)methylene)bis(*N*-benzyl-*N*-ethylaniline)

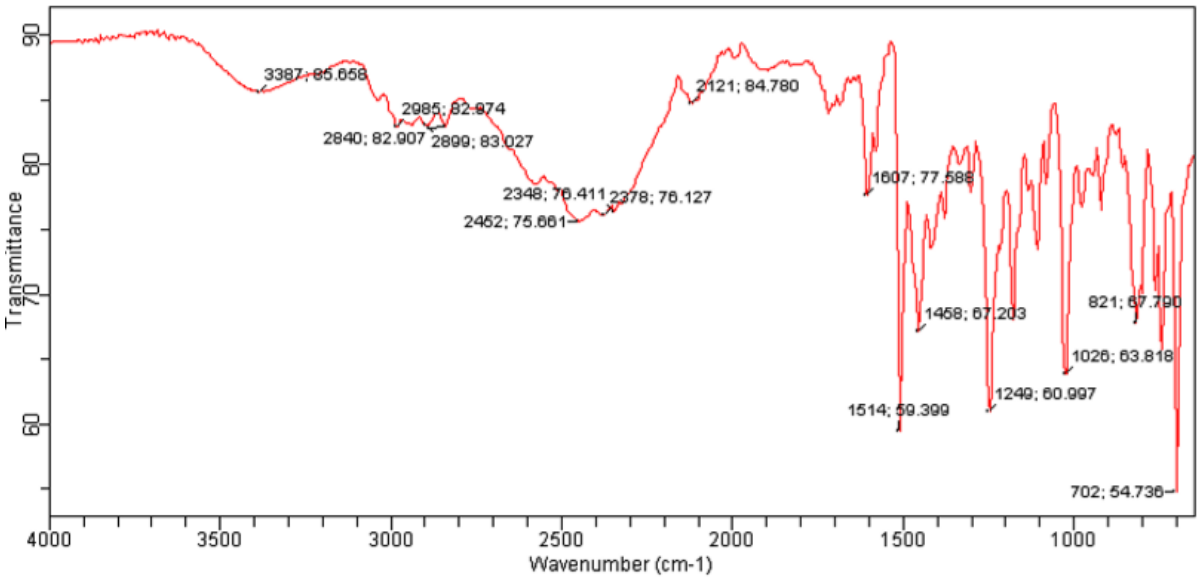




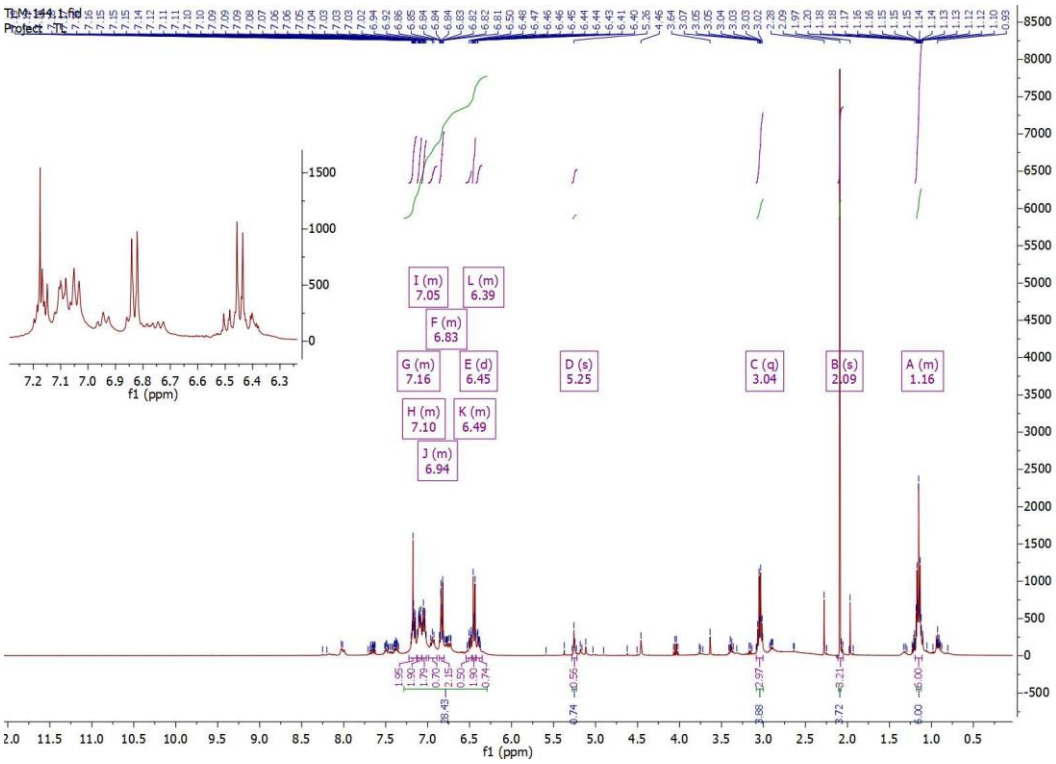

TLM-123_POS #1-4 RT: 0.01-0.10 AV: 4 NL: 3.34E7
T: FTMS + p ESI Full ms [200.00-700.00]
541.3218
z=1
C 38 H 41 O N 2 = 541.3219
-0.1369 mmu
451.2732
z=1
C 28 H 37 O 4 N = 451.2723
0.9494 mmu
360.2195
z=1
C 16 H 32 O 3 N 4 S = 360.2195
-0.0155 mmu
240.1379
z=1
C 8 H 22 O 3 N 3 S = 240.1382
-0.2786 mmu
690.4053
z=1
m/z

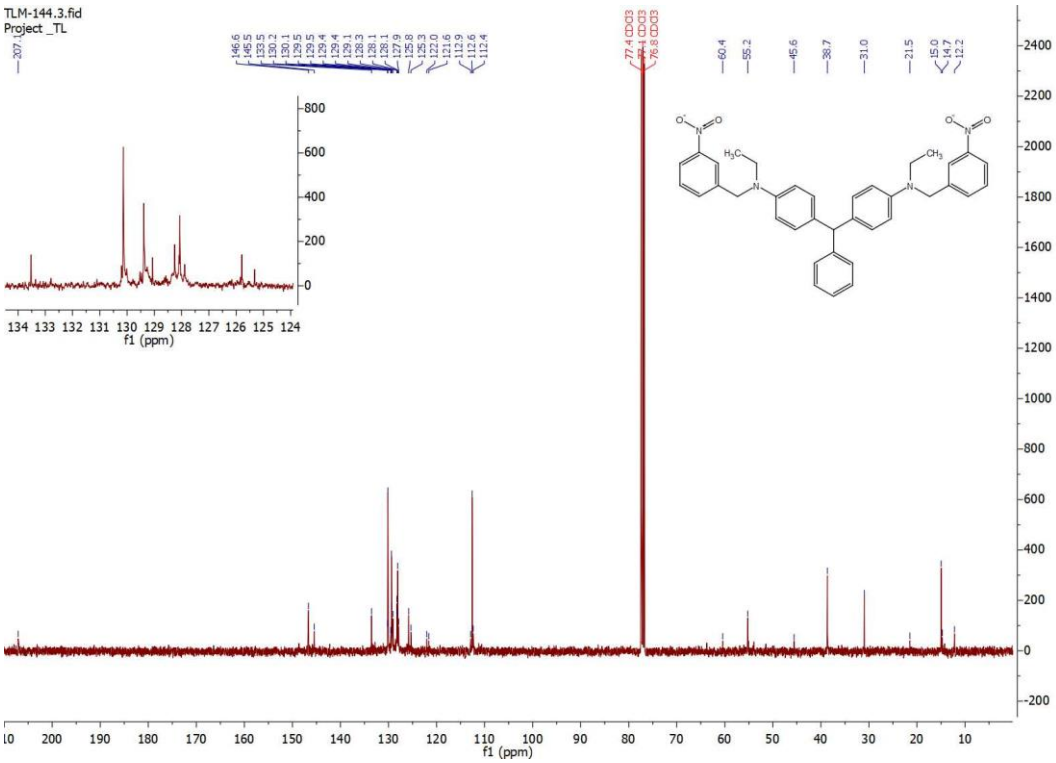

TLM-123_POS #1-4 RT: 0.01-0.10 AV: 4 NL: 3.34E7
T: FTMS + p ESI Full ms [200.00-700.00]
541.3218
z=1
C 38 H 41 O N 2 = 541.3219
-0.1369 mmu
542.3246
z=1
539.3063
z=1
C 38 H 39 O N 2 = 539.3062
0.0428 mmu
543.3277
z=1
m/z

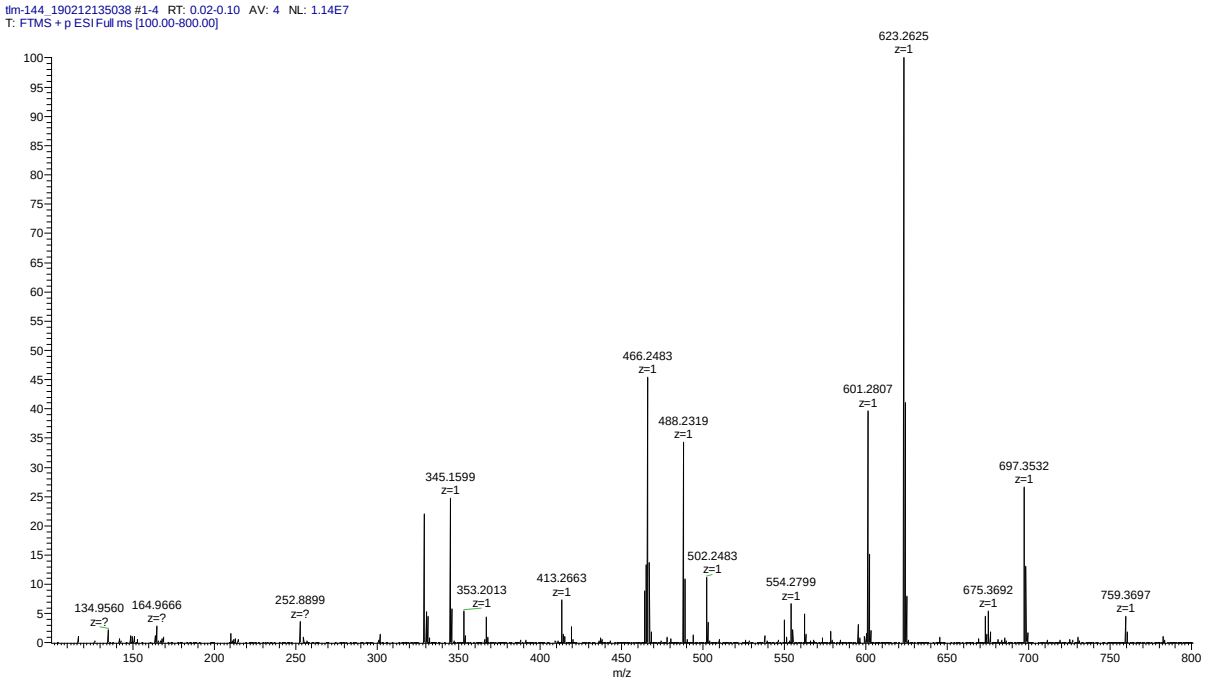
Sample ID:	tlm-123	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 5:06:58PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-123_2019-04-30T17-08-12.a2r		

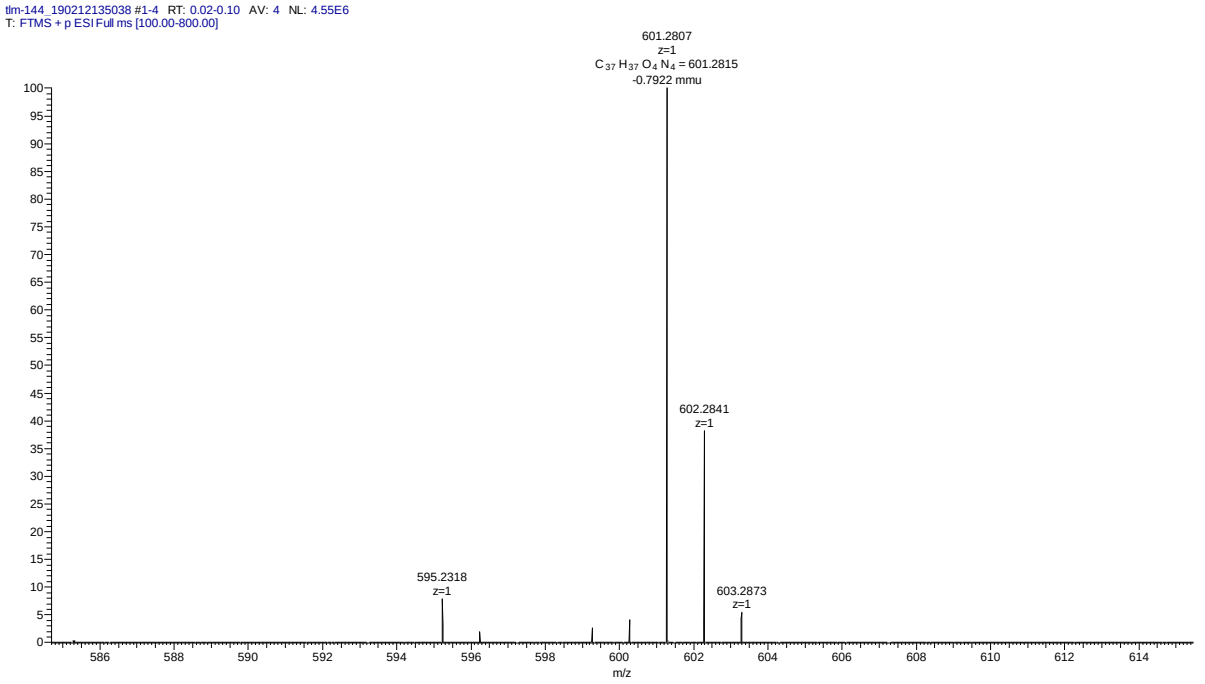


7.2.6.9 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using the S_N2 reaction

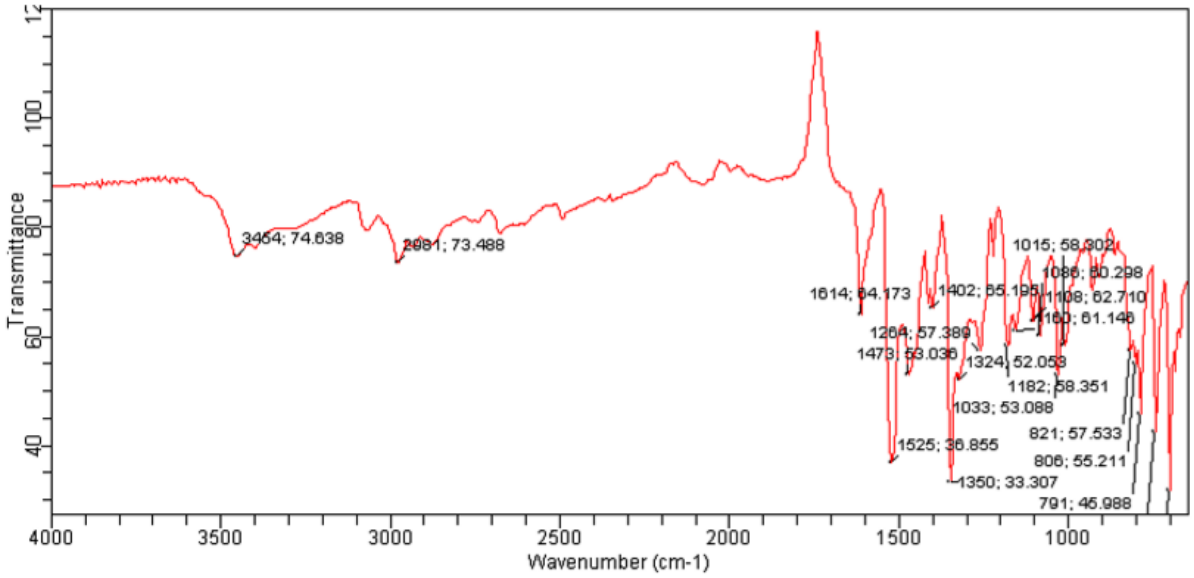




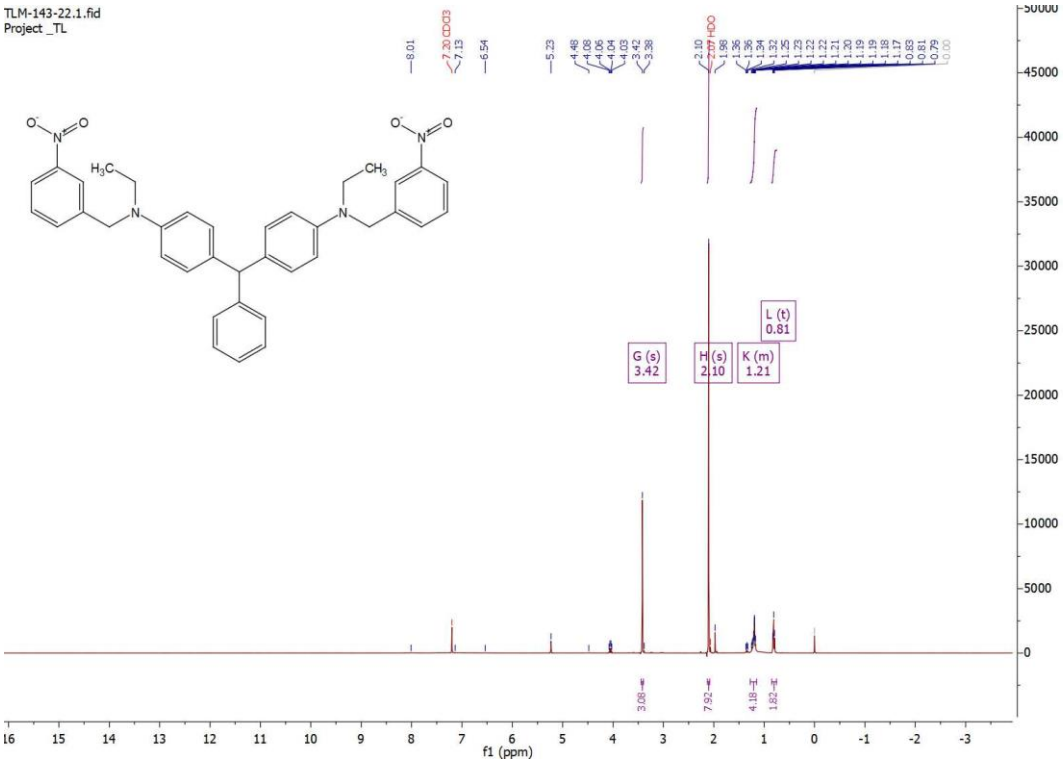

tlm-144_190212135038 #1-4 RT: 0.02-0.10 AV: 4 NL: 1.14E7
T: FTMS + p ESI Full ms [100.00-800.00]
623.2625 z=1
466.2483 z=1
601.2807 z=1
488.2319 z=1
345.1599 z=1
697.3532 z=1
502.2483 z=1
413.2663 z=1
353.2013 z=1
554.2799 z=1
675.3692 z=1
759.3697 z=1
134.9560 z=?
164.9666 z=?
252.8899 z=?
m/z

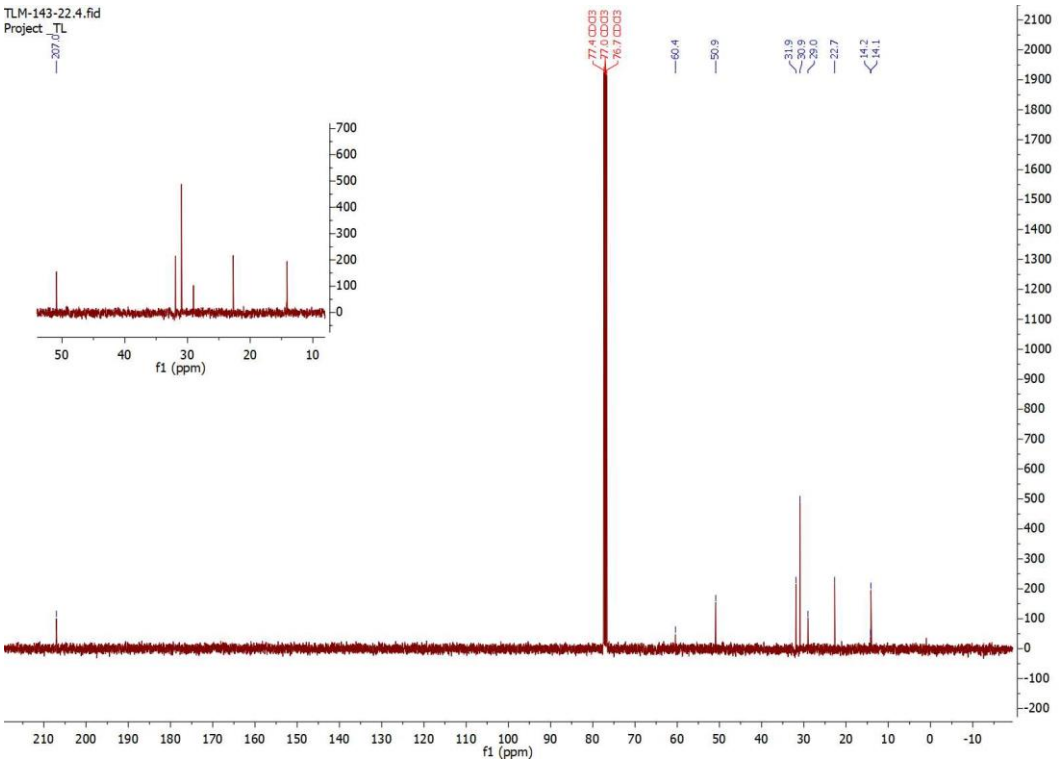

tlm-144_190212135038 #1-4 RT: 0.02-0.10 AV: 4 NL: 4.55E6
T: FTMS + p ESI Full ms [100.00-800.00]
601.2807
z=1
C37 H37 O4 N4 = 601.2815
-0.7922 mmu
602.2841 z=1
595.2318 z=1
603.2873 z=1
m/z

Sample ID:	tlm-144	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 5:18:30PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-144_2019-04-30T17-19-49.a2r		

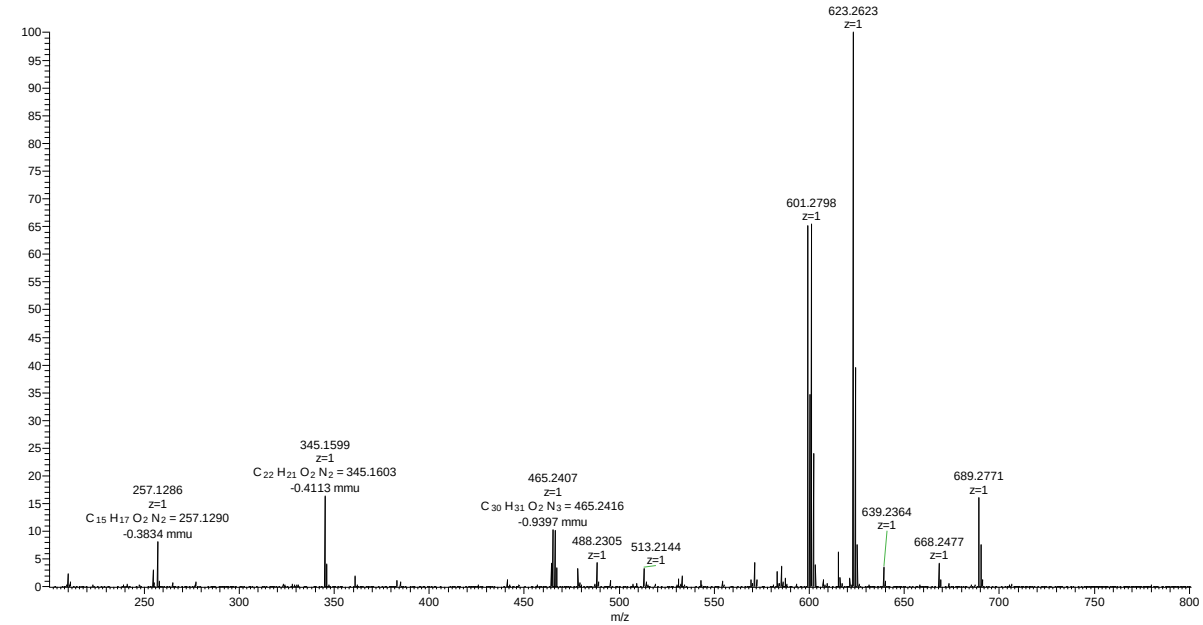


7.2.6.10 4,4'-(phenylmethylene)bis(*N*-ethyl-*N*-(3-nitrobenzyl)aniline) using *N*-ethyl-*N*-(3-nitrobenzyl)aniline

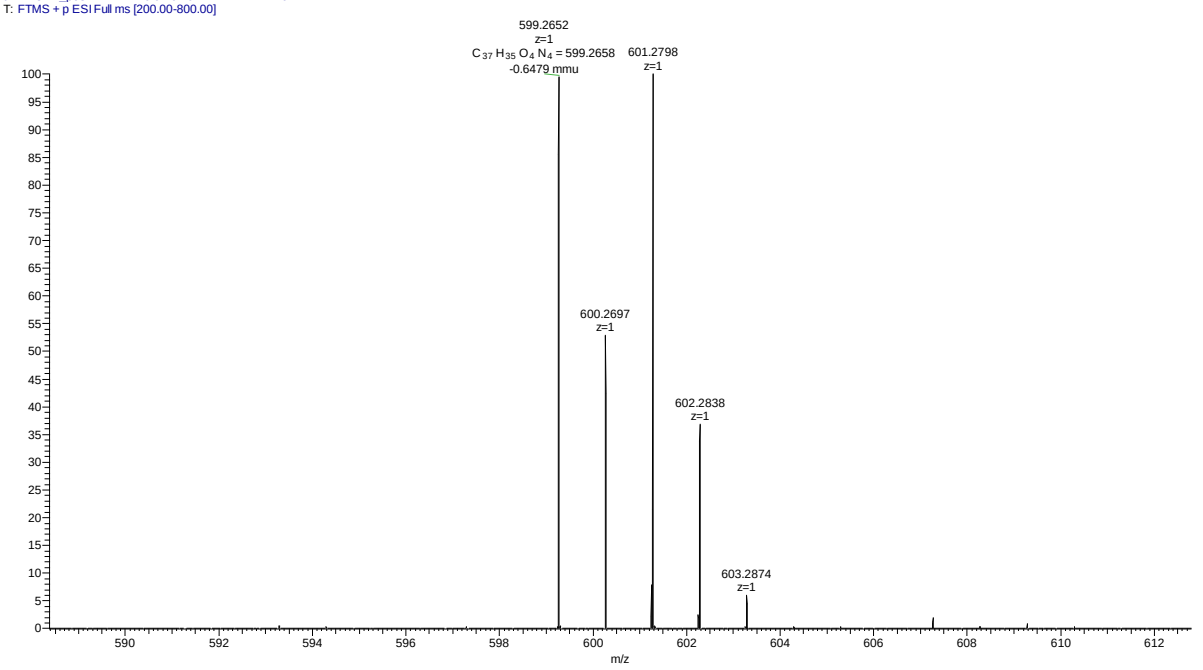




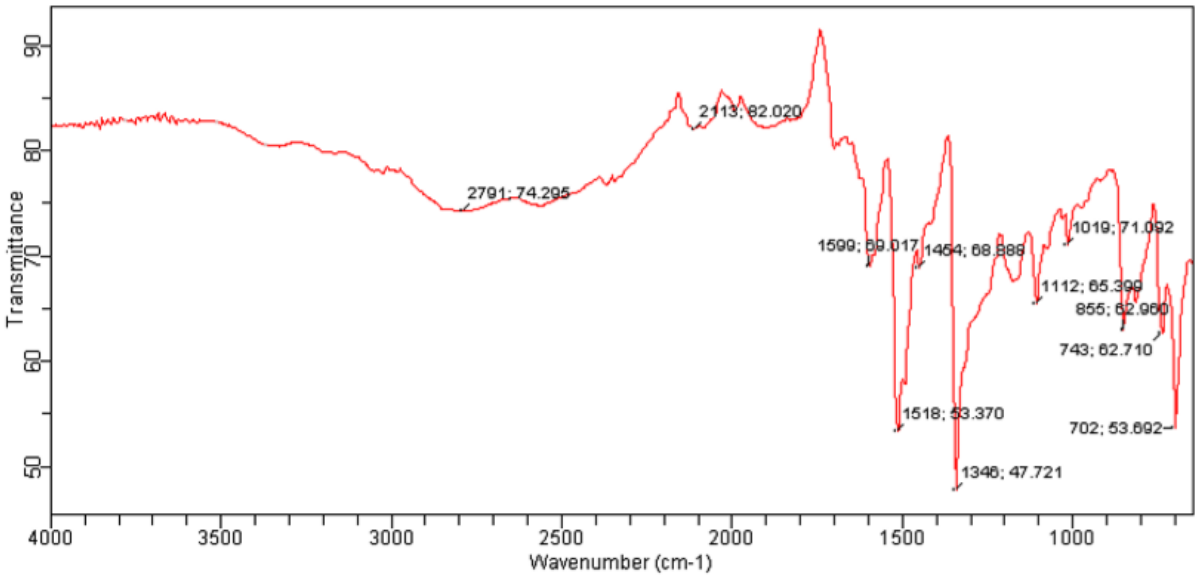
tlm-143-22_pos #1-4 RT: 0.02-0.10 AV: 4 NL: 3.35E7
T: FTMS + p ESI Full ms [200.00-800.00]



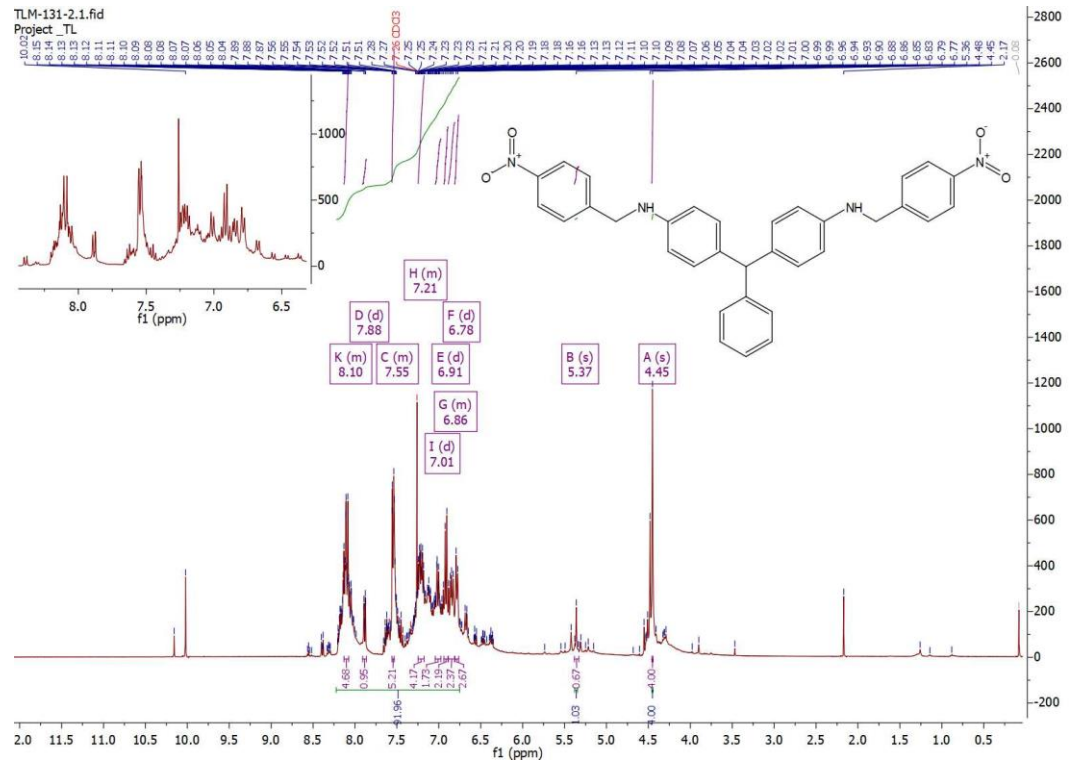
tlm-143-22_pos #1-4 RT: 0.02-0.10 AV: 4 NL: 2.20E7
T: FTMS + p ESI Full ms [200.00-800.00]

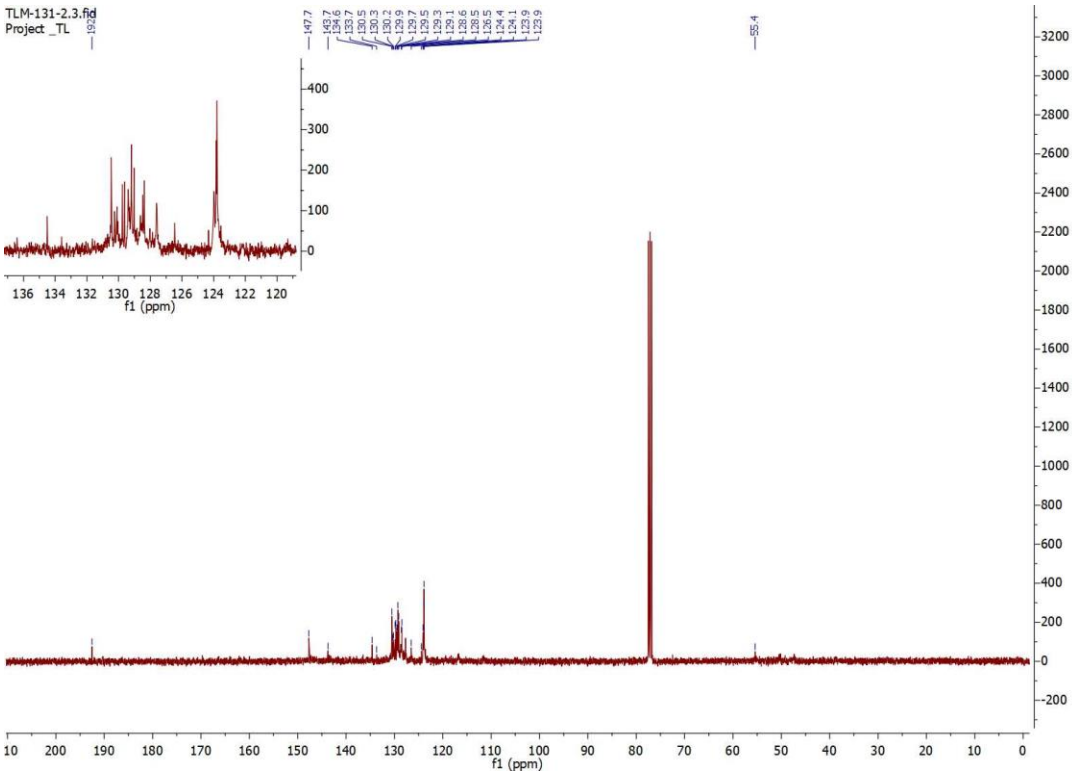


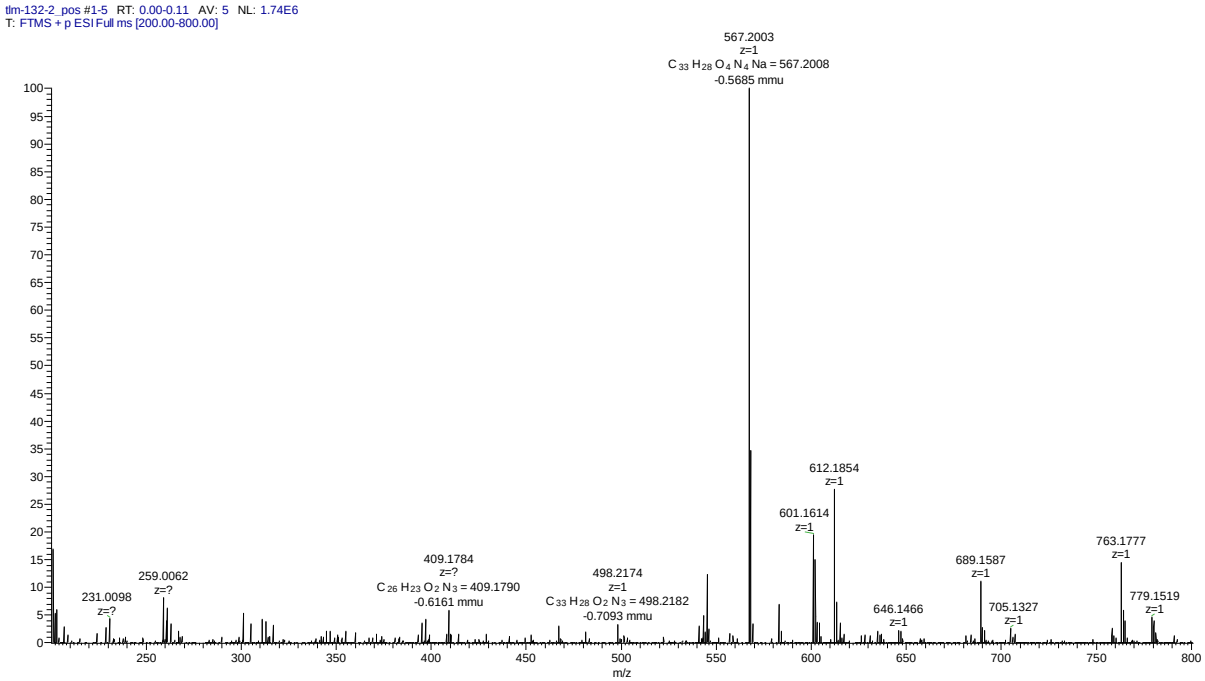
Sample ID:	tlm-143-2	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	4/30/2019 5:24:18PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\tlm-143-2_2019-04-30T17-25-38.a2r		

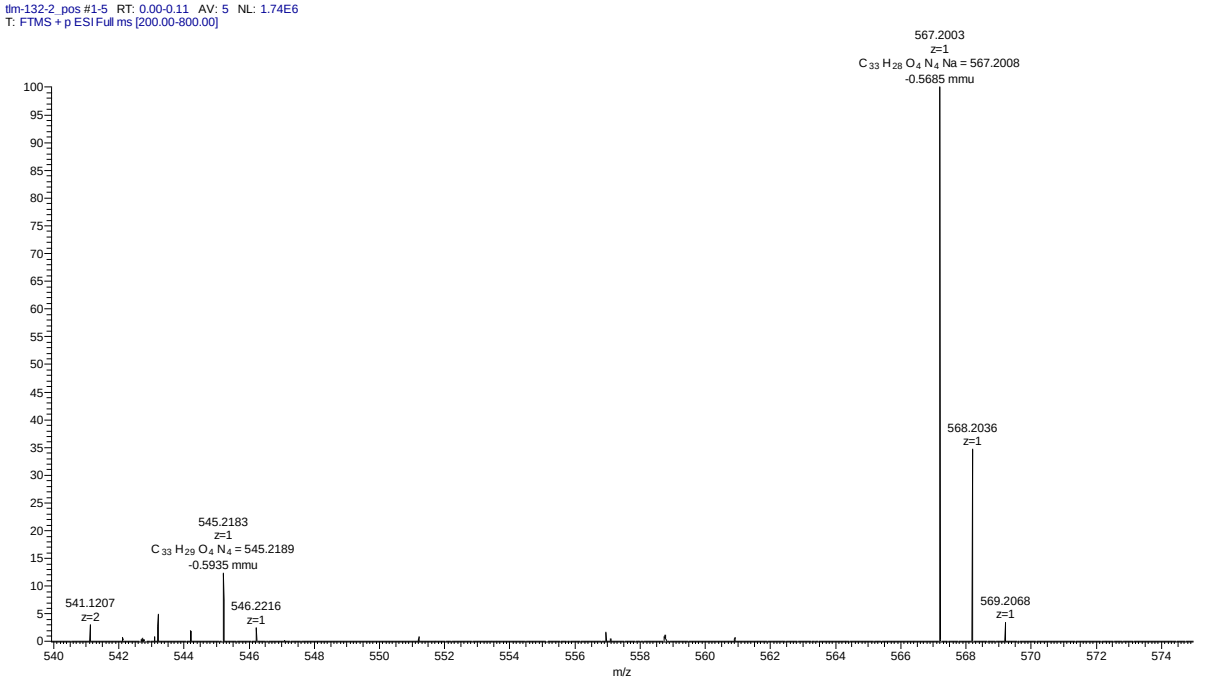


7.2.6.11 4,4'-(phenylmethylene)bis(*N*-(3-nitrobenzyl)aniline)

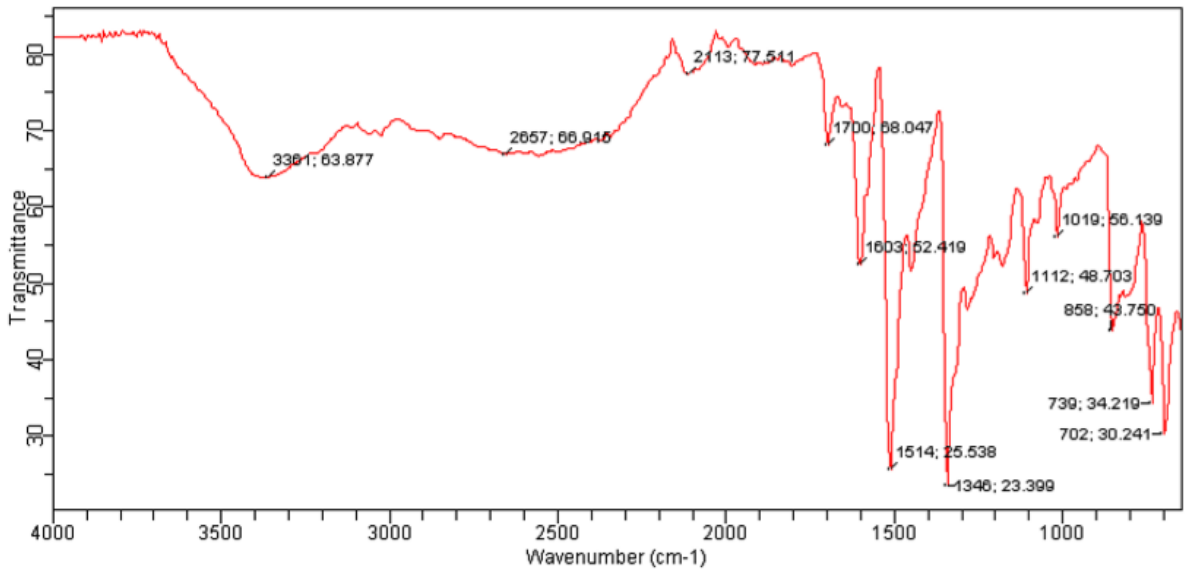




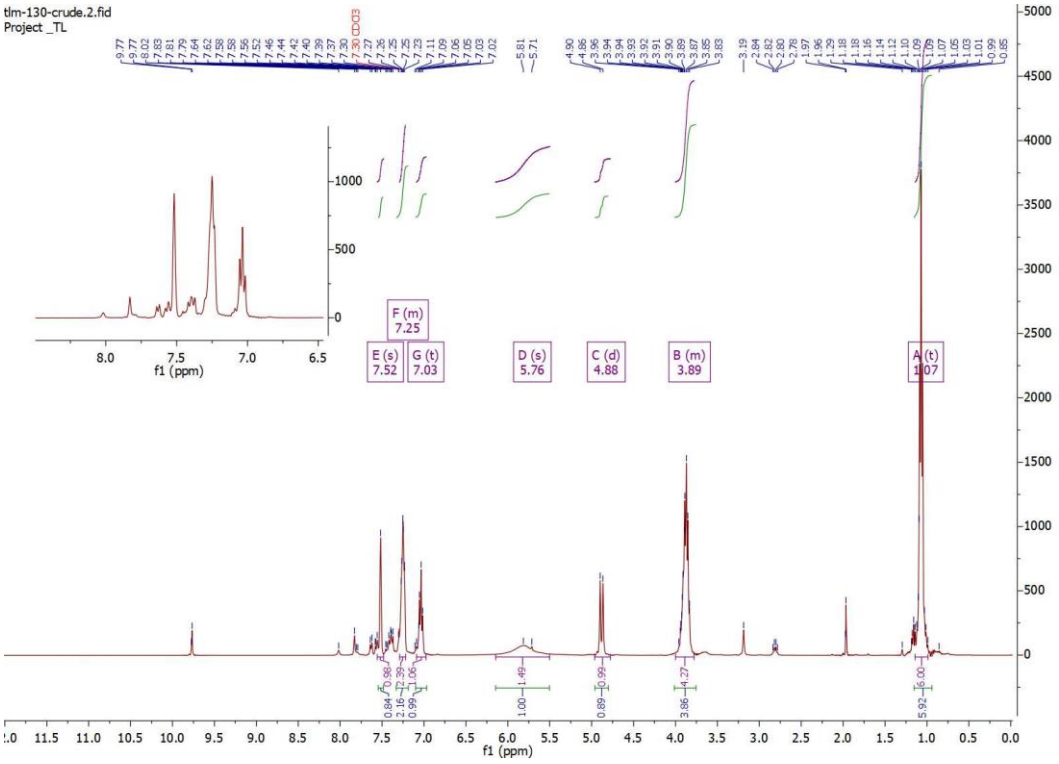

tlm-132-2_pos #1-5 RT: 0.00-0.11 AV: 5 NL: 1.74E6
T: FTMS + p ESI Full ms [200.00-800.00]
567.2003
z=1
C 33 H 28 O 4 N 4 Na = 567.2008
-0.5685 mmu
612.1854
z=1
601.1614
z=1
763.1777
z=1
689.1587
z=1
409.1784
z=?
C 26 H 23 O 2 N 3 = 409.1790
-0.6161 mmu
498.2174
z=1
C 33 H 28 O 2 N 3 = 498.2182
-0.7093 mmu
259.0062
z=?
231.0098
z=?
646.1466
z=1
705.1327
z=1
779.1519
z=1
m/z

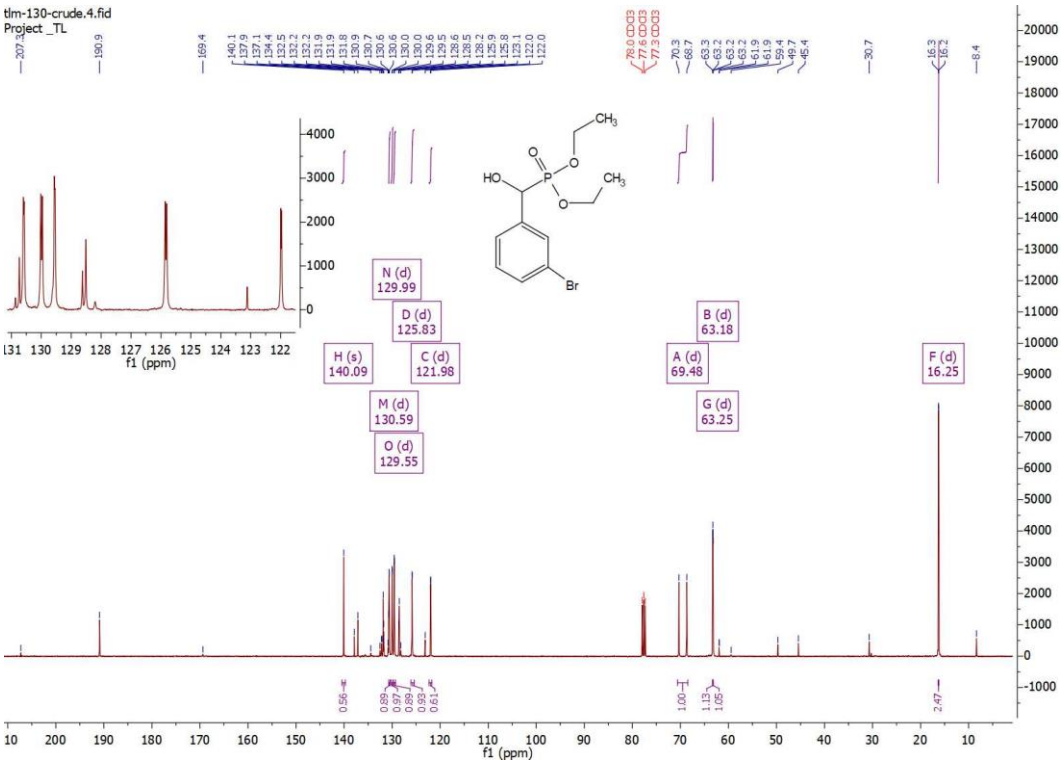

tlm-132-2_pos #1-5 RT: 0.00-0.11 AV: 5 NL: 1.74E6
T: FTMS + p ESI Full ms [200.00-800.00]
567.2003
z=1
C 33 H 28 O 4 N 4 Na = 567.2008
-0.5685 mmu
568.2036
z=1
545.2183
z=1
C 33 H 29 O 4 N 4 = 545.2189
-0.5935 mmu
541.1207
z=2
546.2216
z=1
569.2068
z=1
m/z

Sample ID:	TLM-131	Method Name:	Default
Sample Scans:	128	User:	IK
Background Scans:	128	Date/Time:	5/2/2019 3:50:38PM
Resolution:	8 cm-1	Range:	4,000.00 - 650.00
System Status:	Good	Apodization:	Happ-Genzel
File Location:	C:\Program Files\Agilent\MicroLab PC\Results\TLM-131_2019-05-02T15-51-55.a2r		

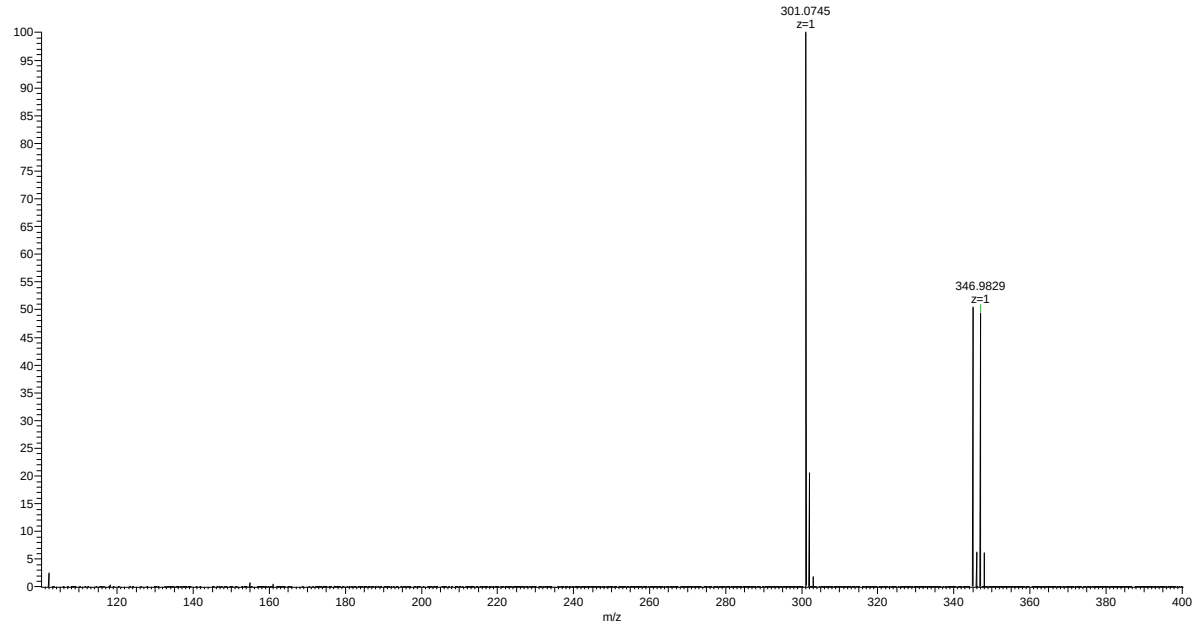


7.2.7 Attempt to synthesise (3-formylphenyl)phosphonate





TLM-130_POS #1-5 RT: 0.01-0.12 AV: 5 NL: 2.18E8
T: FTMS + p ESI Full ms [100.00-400.00]



TLM-130_POS #1-5 RT: 0.01-0.12 AV: 5 NL: 1.10E8
T: FTMS + p ESI Full ms [100.00-400.00]