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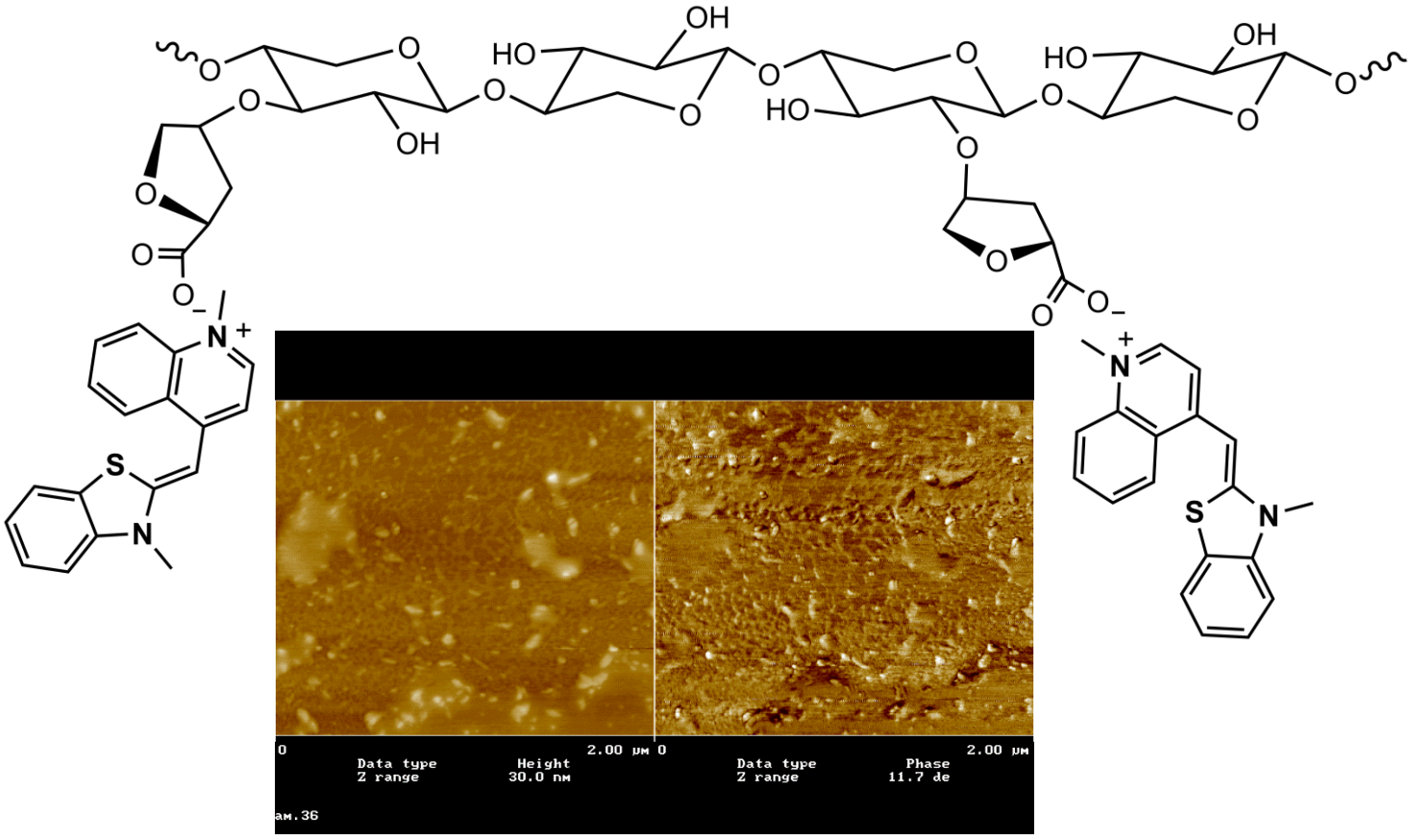

Oxidized Hemicellulose as a Carrier of Cyanine Dyes to DNA

Bachelor Science Thesis

Department of Chemistry and Chemical Engineering
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Cover: On the top is a picture of oxidized arabinoxylan bonding to thiazole orange. On the bottom is an AFM picture of oxidized arabinoxylan.

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ABSTRACT

The aim of this project was study the interactions between the cyanine dyes TO and BO together with the oxidized carbohydrates oxAX and MeGlcA. TO and BO was to be synthesized and AX and MeGlc was to be oxidized. It was desired to investigate and study the formation of H aggregates that can arise when the dyes bond to a carbohydrate matrix. The ability to form H aggregates was also to be tested with TO together with AX and xylan. The dyes ability to bond to DNA also needed to be proven. Furthermore the oxidized carbohydrates were to be investigated as carriers for the cyanine dyes to DNA.

The analysis of the bonding between the cyanine dyes, the carbohydrates and DNA was done using fluorescence spectroscopy and UV-vis spectroscopy. The qualitative analysis of oxAX, MeGlcA, TO and BO was done using FT-IR, ^{1}H NMR and ^{13}C NMR. The oxidation was performed using TEMPO with BAIB as co-oxidant.

The results from this project was that TO together with oxAX can form H aggregates, but not with BO. MeGlcA does not form H aggregates. AX and xylan together with TO gave H aggregates, which could imply that the carbohydrates might not have to be oxidized for this phenomena to occur. Also both TO and BO can bond to DNA which results in high intensities of fluorescence. The cyanine dyes showed a higher affinity for bonding to the DNA rather than the carbohydrate when both materials are present.

The conclusions of this project is that oxAX, AX and xylan together with TO can form H aggregates in solution. BO and TO bonds to DNA and that oxAX can be a potential carrier for DNA. But further research is needed to study this in depth and specify the exact models of the carriers.

Keywords: H aggregate, cyanine dye, hemicellulose, fluorescence, DNA, carrier

SAMMANFATTNING

Målet med denna rapport var att undersöka interaktionerna mellan cyaninfärgämnena BO och TO med oxiderad hemicellulosa. MeGlcA användes också som en jämförelse. Oxidation av dessa kolhydrater skulle utföras och cyaninfärgämnena skulle syntetiseras. Dessa ämnen i lösning ska kunna ge upphov till H aggregat som även ska studeras i denna rapport. Förmågan att bilda H aggregat testades även med xylan och AX. Cyaninfärgämnenas förmåga att binda till DNA skulle studeras. Denna studie utfördes för att utvidga kunskaperna om cyaninfärgämnenas egenskaper och dess beteende tillsammans med oxiderade kolhydraterna. Slutgiltigen så skulle de oxiderade kolhydraternas förmåga att fungera som bärare av cyaninfärgämnena till DNA undersökas.

Analyserna av de oxiderade kolhydraterna, cyaninfärgämnena och DNA:t utfördes med fluorescens- och absorptionsspektroskopi. Den kvalitativa analysen av TO, BO, MeGlcA och oAX utfördes med FT-IR, ^{1}H NMR och ^{13}C NMR. Oxidationen av kolhydraterna utfördes med TEMPO tillsammans med BAIB som sekundärt oxidantsmedel.

Resultatet från denna rapport gav att TO tillsammans med oxAX uppvisar H aggregat i lösning, men inte med MeGlcA. BO uppvisar inga H aggregat. AX och xylan tillsammans med TO ger H aggregat i lösning. Det har även bevisats att BO, TO, oxAX och MeGlcA har försumbar fluorescens själva i lösning. Men när DNA tillsätts så binder BO och TO till DNA:t istället vilket ger upphov till en hög intensitet av fluorescens.

Det har visat sig att oxAX kan vara en potentiell bärare av cyaninfärgämnen till DNA. Detta stödjs av att cyaninfärgämnena bildar en svag bindning till kolhydraten men har högre affinitet för att binda till DNA när denna tillsätts. Ytterligare studier av detta krävs för att studera mekanismerna.

ABBREVATIONS

AFM	Atomic Force Microscopy
AX	Arabinoxylan
BO	(Z)-1-methyl-4-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)pyridin-1-ium 4-methylbenzenesulfonate
FT-IR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
MeGlc	Methyl-α-D-Glucopyranoside
MeGlcA	Methyl-α-D-Glucuronic Acid
oxAX	Oxidized Arabinoxylan
TO	Thiazole Orange

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1 INTRODUCTION

1.1 Background

Carbohydrates are attractive materials due to their properties as biodegradable, renewable and non-toxic. These properties can further improve the practice of green chemistry. The chemical industries are always searching for new raw materials for different applications and carbohydrates extracted from plant material are generally seen as environmentally friendly alternative to petroleum based chemicals. There is ongoing work to transform them to fuels, alcohols such as ethanol but also higher alcohols. Other fields are as components in composites and biomaterials such as films for packaging materials. More advanced applications are found in medicinal applications. [1]

Using different polysaccharides as carrier for drugs is one way to increase the use of renewable chemicals and drugs while also enhancing the effect of the drug. One large challenge in pharmacological technology is the use of carriers for drugs. The effects of the drug is dependent on many factors, such as solubility, local toxicity, metabolism and more. If the drug can be attached to an inert carrier substance some of these factors can be easily changed and the overall effect of the drug can be changed. [2]

The fact that many of the known drugs contain amines which can be protonated to give them a positive charge may allow the use of negatively charged carriers to form an ion-aggregate. [3] Hemicellulose and other carbohydrates can be oxidized into carboxylic acid which are negatively charge when deprotonated. This could allow the molecules to bond together and when the aggregate reaches its target area the drug can be released. To study this behavior *in vitro* cyanine dyes can be used as a model compound instead of the drug. The cyanine dyes are positively charged and show little to no fluorescence on their own, but combined with DNA they may be able to form fluorescent aggregates which can be detected by fluorescence. [4]
Cyanine dyes were first synthesized more than a century ago and in the beginning they were applied in the photography field. Newer research has found more potential uses for them such as a photorefractive material, light absorbing compounds and more. [5] Cyanine dyes has during recent years also been given attention as a fluorescent probe for biological systems. The cyanine dyes have been shown to bind to DNA and create fluorescent aggregates. Fluorescent marker

technology has been applied in the detection of cancer and AIDS and shows great promise to be developed further. It could be a cheap and gentler alternative to other known methods such as radioactive probes and other detection methods. [4]

The use for polysaccharides together with cyanine dyes could also have other potential applications such as increases the stability of water-based solutions. Stock solutions of cyanine dyes are unstable and needs to be prepared immediately prior to use to prevent degradation. If it was possible to increase the stability of the molecules with polysaccharides this would lead to more practical and easier use of cyanine dyes, with less errors in the measurements.

1.2 Objectives

The aim of this project was to oxidize two types of carbohydrates, arabinoxylan (AX) and methyl-α-D-glucopyranoside (MeGlc), to their respective carboxylic acid by using TEMPO. MeGlc was to be used as a reference to the arabinoxylan to be able to compare the results.

In addition these carbohydrates were to be investigated as potential carriers of cyanine dyes, BO and TO, to DNA. These cyanine dyes were also to be synthesized. The properties of the cyanine dyes, DNA and carbohydrates were studied using absorption spectroscopy and fluorescence spectroscopy. The synthesized chemicals were analyzed with NMR and IR to verify the synthesis methods.

2 THEORY

2.1 Arabinoxylan (AX)

AX is a hemicellulose that consists of xylose linked together in a β(1-4) chain which are substituted with arabinose linked (1-2) or (1-3) to the xylose. [6] The side branches contains small amounts of other carbohydrates such as α-D-glucuronic acid, xylopyranose, 4-O-methyl-α-D-glucuronic acid and more. [7] The water solubility increases with a higher degree of substitution with arabinose. [6] AX is mainly found in cereal grains such as wheat, rye, barley, oat and more. AX have a vital part for the structure of the cell walls, but they are also important for humans as a form of dietary fiber. [7, 8]

2.2 TEMPO-Mediated Oxidation

TEMPO (2,2,6,6-Tetramethylpiperidin-1-oxyl) and its analogues are water soluble and stable nitroxyl radicals. TEMPO is often preferred as an oxidant for its ability to under mild conditions selectively oxidize C6 primary hydroxyl group to form aldehydes and carboxylic acids while being unreactive to secondary hydroxyl groups. [9, 10]

The ability to oxidize the substrate under mild conditions allows the polysaccharides to be oxidized without cleaving the polysaccharide chain and the arabinose units in AX. [11] TEMPO is normally used in catalytical amounts with the presence of a co-oxidant such as sodium hypochlorite or (diacetoxyiodo)benzene (BAIB), which converts the hydroxyl amine back into the reactive nitrosonium salt. [9,12]

2.3 Cyanine Dyes

The basic structure of a cyanine dye consists of two nitrogen bounded to two different aromatic groups where one of the nitrogen atoms is positively charged. These nitrogen groups are then linked together by a conjugated carbon chain. [13] Cyanine dyes are sensitive to light and degrades gradually when exposed. [13] Previous studies of cyanine dyes shows that cyanine have neglictable fluorescence on their own. [14] The cyanine dyes are aromatic cations with a planar structure which allows them to spontaneously bond non-covalently to DNA. When bonded to DNA they show a strong fluorescence, even at low concentrations of dye. [4]

2.4 H and J Aggregates

The intermolecular van der Waal forces between cyanine dyes make the dyes form different aggregates in solution where the molecules stack together to form dimer and bigger aggregates. What type of aggregates that is formed depends on the conditions. [15] In general the monomer and dimer have no fluorescent properties. [14] When comparing the absorption bands from these aggregates with the monomeric species, aggregates have different absorption bands than the monomers. There are two main types of aggregates H and J aggregates. J stands for Jelly, the man who discovered this phenomena and H stands for hypsochromic. It is commonly agreed that both of the aggregates consists of parallel dye molecules stacked together and form two-dimensional dye crystals. [13]

The molecules can either be stacked side-by-side, which are known as H-aggregates. The absorption bands of the H-aggregates give a hypsochromic shift relative to the monomer peak, sometimes known as blue shifts, which means that the absorption peak increases in energy respective to the monomer peak. The dye molecules can also be stacked head-to-tail as in J aggregates. This leads to a bathochromically-shifted absorption band relative to the monomer peak, sometimes known as red shifts, where the absorption peak decreases in energy. [13,15] Neither of the H and J-aggregates fluoresce on their own. [14] J aggregates can only appear with photographic cyanine dyes. [13]

The dyes can either self-associate to form these aggregates in solution or the dyes can attach themselves to a solid matrix in solution which leads to the formation of H and J aggregates. [13] Previous studies shows that cyanine dyes show this behavior when a polysaccharide, hyaluronic acid, is added to the dyes. [16]

3 EXPERIMENTAL

3.1 Materials

3.1.1 Chemicals

Methyl D-glucopyranoside from Acros Organics

BAIB previously synthesized

Arabinoxylan previously extracted from barley husk

TEMPO from Sigma Aldrich

DNA calf thymus from Sigma Aldrich

3.2 Methods

3.2.1 Methyl-α-D-Glucuronic Acid

Methyl- α-D-glucopyranoside (1.05 g, 5.18 mmol), which can be seen in figure 1 was dissolved in a 3:1 mixture of acetonitrile and water (6.5 ml acetonitrile 2.2 ml water) in an Erlenmeyer flask. The mixture were put in an ice-water bath and BAIB (3.46 g, 10.8 mmol) and TEMPO (0.133 g, 0.854 mmol) was added while stirring. The mixture were left in the ice-water bath for 2 hours while stirring. After this time the mixtures were put in room temperature for an additional 4 hours before evaporation on a rotary evaporator. The liquid product of methyl- α-D-glucose was washed in a separatory funnel with diethyl ether. The sample of methyl- α-D-glucose was freeze-dried before IR and NMR, even though no solids were obtained.

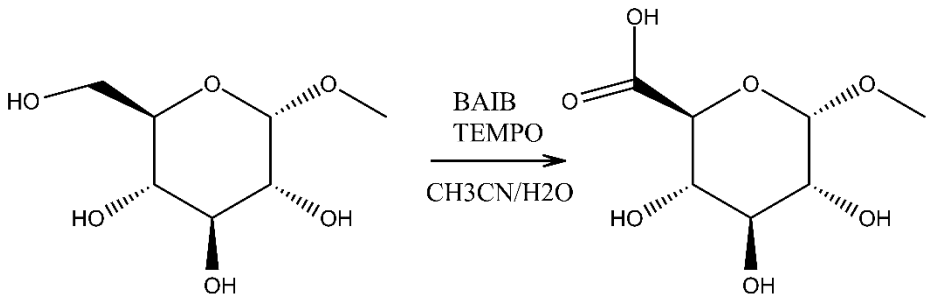

Figure 1. *TEMPO-mediated oxidation of MeGlc to its corresponding acid, MeGlcA, with BAIB as co-oxidant.*

^{1}H NMR (D2O): δ 3.31 (3H, s), 3.43 (1H, dd, J= 9.03, 10.04), 3.48 (1H, dd, J=6.4, 9.60), 3.57 (1H, dd, J=9.46, 10.78), 4.01 (1H, d, J=9.8), 4.73 (1H, d, J=3.7)
^{13}C NMR (D2O): δ 55.37 (OCH_3), 70.55, 70.72, 71.32, 72.55, 99.55 (CH), 176.70 (CO)

The sample contained impurities of acetonitrile which was used as a solvent.

3.2.2 Oxidized Arabinoxylan

Arabinoxylan (0.686 g, 5.19 mmol) was added to a 3:1 mixture of acetonitrile and water (6.5 ml and acetonitrile 2.2 ml water) in an Erlenmeyer flask. The arabinoxylan was only slightly soluble and formed a light brown suspension. The mixture were put in an ice-water bath and BAIB (3.46 g, 10.8 mmol) and TEMPO (0.133 g, 0.854 mmol) was added while stirring. The mixture was left in the ice-water bath for 2 hours while stirring. After this time the mixture were put in room temperature for an additional 4 hours before evaporation on a rotary evaporator. The solid product was gravity filtered and washed with cold ethanol. The product was freeze-dried before IR.

3.2.3 (Z)-1-methyl-4-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)pyridin-1-ium 4-methylbenzenesulfonate (BO)

3-methyl-2-(methylthio)benzo[d]thiazol-3-ium benzenesulfonate (342 mg, 1.2 mmol) and 1,4-dimethylpyridin-1-ium benzenesulfonate (747 mg, 2mmol), which can be seen in figure 2, was added to a round bottom flask and was suspended in 10 ml dichloromethane. Triethylamine (0.6 ml, 4.3 mmol) was added to form a yellow mixture which was stirred for 20 h at room temperature. Ethyl acetate was added to precipitate out the crude product and it was allowed to stir for 15 minutes. The mixture was filtered and washed with additional ethyl acetate. The mixture was heated in approximately 10 ml of ethyl acetate and was then allowed to cool down before filtering again. The reactants were supposed to be 2 mmol each, but error in the calculation gave 1.2 mmol instead, this gave the final yield of 71 % (373mg).

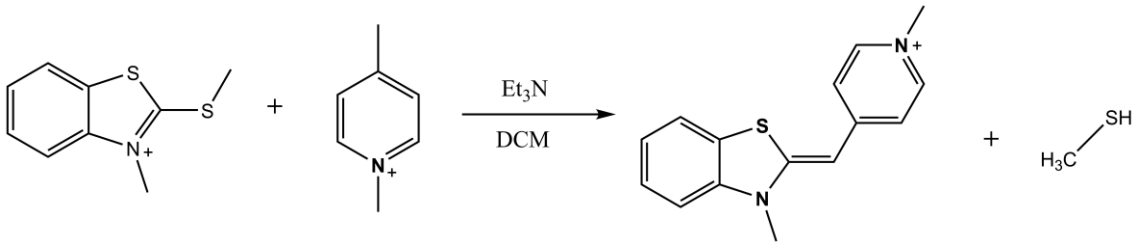

Figure 2. *Synthesis of BO.*

^{1}H NMR (CD_3OD): δ 2.35 (3H, s), 3.73 (3H), 3.99 (3H, s), 6.14 (1H, s), 7.21 (2H, d, J=8.0), 7.30 (1H, t, J=14.8), 7.40 (2H, d, J=7.2), 7.48 (1H, d, J=8.8), 7.52 (1H, t, J=15.6), 7.69 (2H, d, J=11.2), 7.76 (1H, d, J=8.0), 8.08 (2H, d, J=7.6)
^{13}C NMR (CD_3OD): δ 19.93, 31.8, 44.1, 89.13, 111.42, 118.67, 121.87, 123.57, 125.44, 127.75, 128.42, 141.38

A more detailed interpretation of the ^{1}H NMR for BO can be seen in figure B1 and table B1.

3.2.4 1-Methyl-4-[(3-methyl-2(3H)-benzothiazolylidene)methyl]quinolinium tosylate (TO)

2,3-Dimethyl-1,3-benzothiazol-3-ium tosylate 671 mg, 2 mmol) and 1-Methylquinolinium tosylate (631 mg, 2 mmol), which can be seen in figure 3, was added to a round bottom flask and suspended in 10 ml dichloromethane. Triethylamine (0.6 ml, 4.3 mmol) was added which caused the solids to dissolve and the mixture became deep red. After stirring 20 h at room temperature, 4 ml ethyl acetate was added to precipitate out the crude product and it was allowed to stir for 15 min. The mixture was then filtered and the red solids were washed with small portions of ethyl acetate. The solids were then suspended in 8 ml ethyl acetate and heated for a few minutes. After cooling to room temperature the suspension were filtered and the solids were washed with small portions of ethyl acetate to give thiazole orange (TO) (255 mg, 27%).

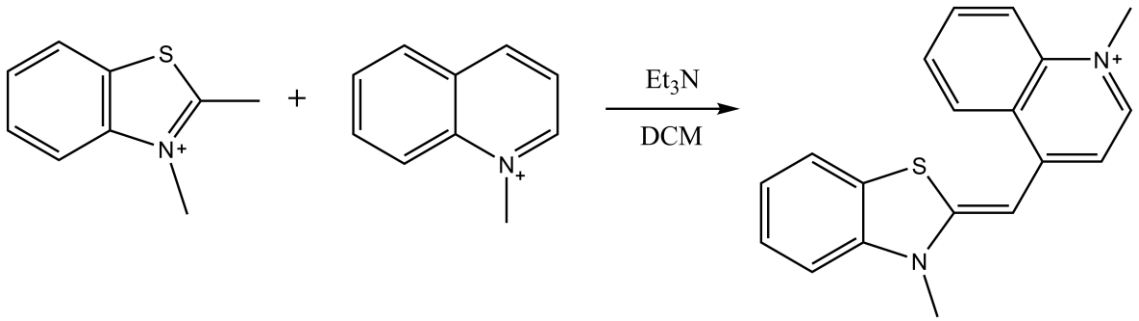

Figure 3. *Synthesis of thiazole orange (TO).*

^{1}H NMR (CD_3OD): δ 2.34 (3H, s), 3.98 (3H, s), 4.17 (3H, s), 6.91 (1H, s), 7.21 (2H, d, J=7.9), 7.40 (1H, t, J=15.2), 7.45 (1H, d, J=7.2), 7.59 (1H, t, J=15.2), 7.64 (1H, d, J=8.4), 7.69 (2H, d, J=8.2), 7.76 (1H, t, J=15.0), 7.87 (1H, d, J=8.0), 7.98 (1H, t, J=15.6), 8.01 (1H, d, J=6.8), 8.37 (1H, d, J=7.2), 8.65 (1H, d, J=8.4).
^{13}C NMR (CD_3OD): δ 19.85, 32.57, 41.73, 87.62, 108.29, 112.31, 117.52, 122.28, 124.41, 124.94, 125.64, 126.89, 128.06, 128.31, 133.08, 144.42.

A more detailed interpretation of the ^{1}H NMR for TO can be seen in figure B2 and table B2

4 ANALYSIS AND CHARACETERISATION

4.1 Fourier Transformation Infrared Spectroscopy (FT-IR)

FT-IR was used to determine the functional groups of the oxidized carbohydrates. 2mg of sample was mixed with 300mg and potassium bromide which then was ground to a fine powder. The powder was pressed under vacuum to form pellets. The FT-IR spectrum was then recorded using 32 scans per sample between the ranges of 400-4000 cm^{-1}. The peak of most interest in this case was the peak at around 1750 cm^{-1}. This peak indicates the presence of a carbonyl carbon. Spekwin32 was used to study the spectra and create the pictures.

4.2 Fluorescence and UV-vis Spectroscopy

For all the dilution and as a solvent a 25 µM disodium hydrogen phosphate (S in water was used as a buffer solution. Stock solutions of the carbohydrates and the cyanine dyes were prepared using the buffer solution. To obtain the desired concentration the stock solutions were mixed directly into the cuvette and in some cases addition buffer solution was added. The UV-vis absorption bands were recorded between 200 nm and 800 nm. Microsoft Excel was used for making the graphs.

4.3 Atomic Force Microscopy (AFM)

AFM was performed to determine the structure of the hemicellulose with hopes of being able to see the aggregates between the cyanine dye and the hemicellulose. The samples were diluted

to 0.001% with deionized water. Next the samples were put in ultrasonification for 15 minutes. One drop from the sample was put on a mica plate which then was allowed to dry for approximately 40 minutes. The AFM analysis gave no further results, there was no difference between pure oxAX and oxAX with cyanine dyes. The AFM analysis was performed by Anders Mårtensson, Polymer Technology, Chalmers University of Technology.

5 RESULTS AND DISCUSSION

5.1 FT-IR Analysis

FT-IR analysis of the oxidized carbohydrates to further support the chemical structure of the samples. The FT-IR spectra of arabinoxylan and oxidized arabinoxylan can be seen in figure 4 The wide OH-stretch peak around approximately 3400 cm^{-1} indicates the presence of an alcohol or carboxylic acid and the peak at around 2900 cm^{-1} indicates the presence of methyl groups. The peak at 1740 cm^{-1} indicates the presence of a carbonyl carbon and this peak is only present in the oxidized arabinoxylan, which supports the formation of a carboxylic acid in the oxidized arabinoxylan.

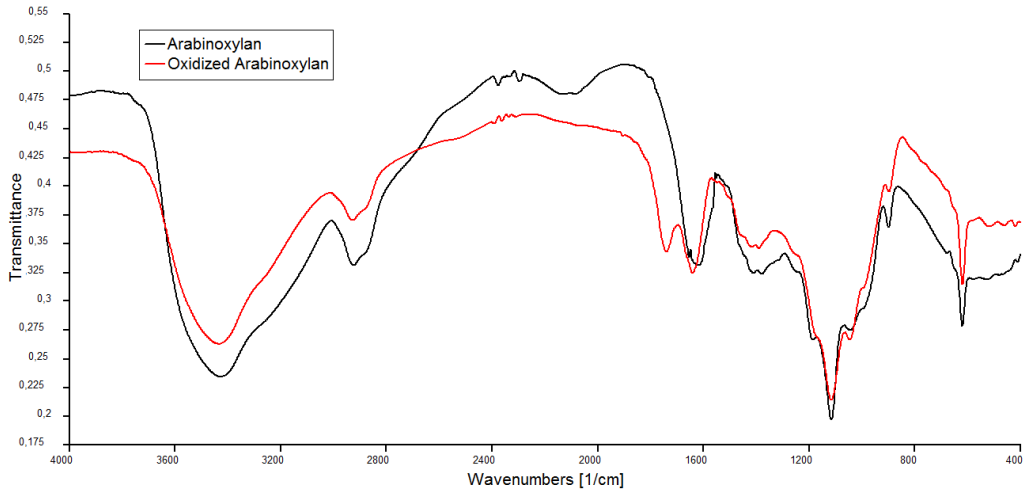

Figure 4. *FT-IR spectra of arabinoxylan and oxidized arabinoxylan*

5.2 UV-vis and Fluorescence Spectroscopy

To be able to study the formation of H aggregates absorption spectroscopy was performed. As can be seen in figure 5 the peak for TO alone in solution has its peak just below 500 nm. If studied closely the peak is split into two smaller peaks, which are the monomer to the right and the dimer to the right. When the concentration of TO increases the monomer can be seen to

decrease in size while the dimer peak increases in size. This behavior is intuitional since when the concentration increases there are more hydrophobic molecules of dye in the solution which want to clump together to avoid the water and thus forming dimers. When oxAX is added the peak is hypsochromically shifted in the diagram and the peak is now at around 440 nm. This supports the formation of H aggregates and implies that the TO in some way binds to the oxAX.

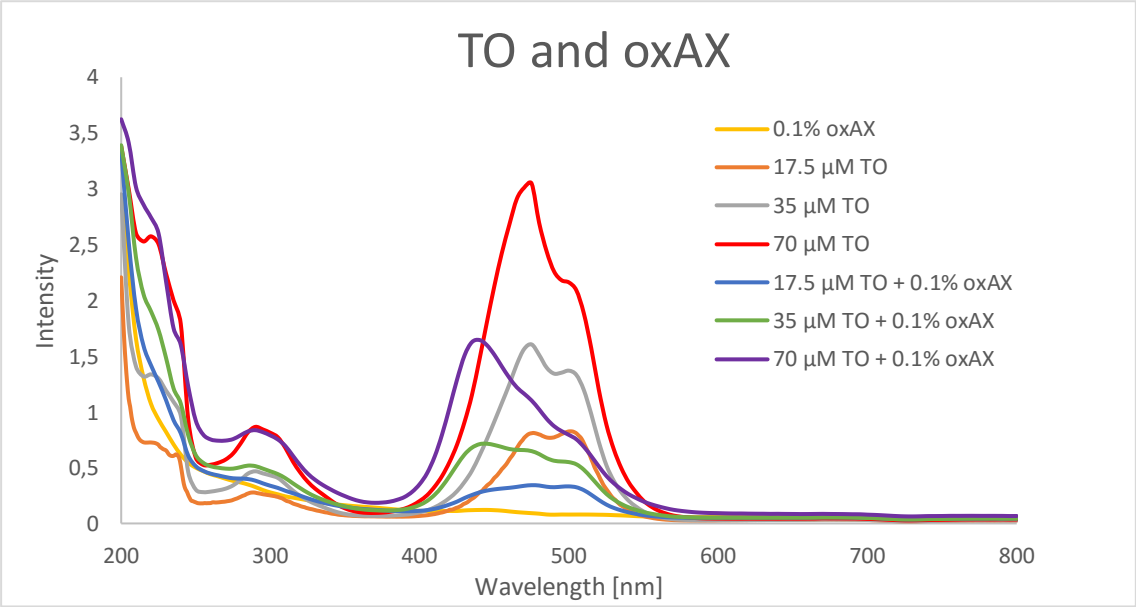

Figure 5. *Absorption spectra with varying concentrations of TO together with 0.1 wt% oxAX.*

The absorption bands oxAX with BO in figure A1, TO with MeGlcA in figure A2 and BO with MeGlcA in figure A3 does not show displacements of the peaks and no H aggregate peak can be seen. It is likely since MeGlcA is such a small molecule, that the cyanine dyes cannot bond to in the same way as with the large oxAX molecule. BO is also a smaller molecule with less hydrophobicity, which is something that could lead to the molecule not being able to form H aggregates. Further studies are needed for the correct model to be determined.

The original hypothesis was that the carboxylic acid groups on the carbohydrates can be deprotonated and attract the positively charged nitrogen on the cyanine dye. To support this and experiments with the same concentrations of TO but this time using non-oxidized carbohydrates was carried out. The carbohydrates used was AX and xylan which can be seen in figure A4 and figure A5 respectively. Xylan and AX both gave some shifts in the absorption bands when added. At 70 µM TO and 0.1% AX the peak is hypsochromically shifted which means that H aggregates were formed. It could be that the carbohydrates do not need to be oxidized for the cyanine dye to bond and they simple bond together in some other way. Further research is needed to explain this.

Since oxAX and TO gave H aggregates it was desired to further study this behavior. In figure 6 the concentration of TO was kept constant at 35 µM while the concentration of oxAX was varied between 0.1% and 0.001%. Observe that concentrations of 0.1 and 0.001 wt% oxAX gives H aggregates but with a concentration of 0.001 wt% oxAX no H aggregates can be seen. This means that if the ratio of oxAX to TO is to low the H aggregates cannot be observed. This was done to find a suitable concentration for the measurements with DNA. If the concentration is to low the H aggregates cannot be observed and if the concentration is too high the fluorescence spectrophotometer cannot measure intensities that high. The concentration which seemed to work good in both cases was 0.05% oxAX with 35 µM TO.

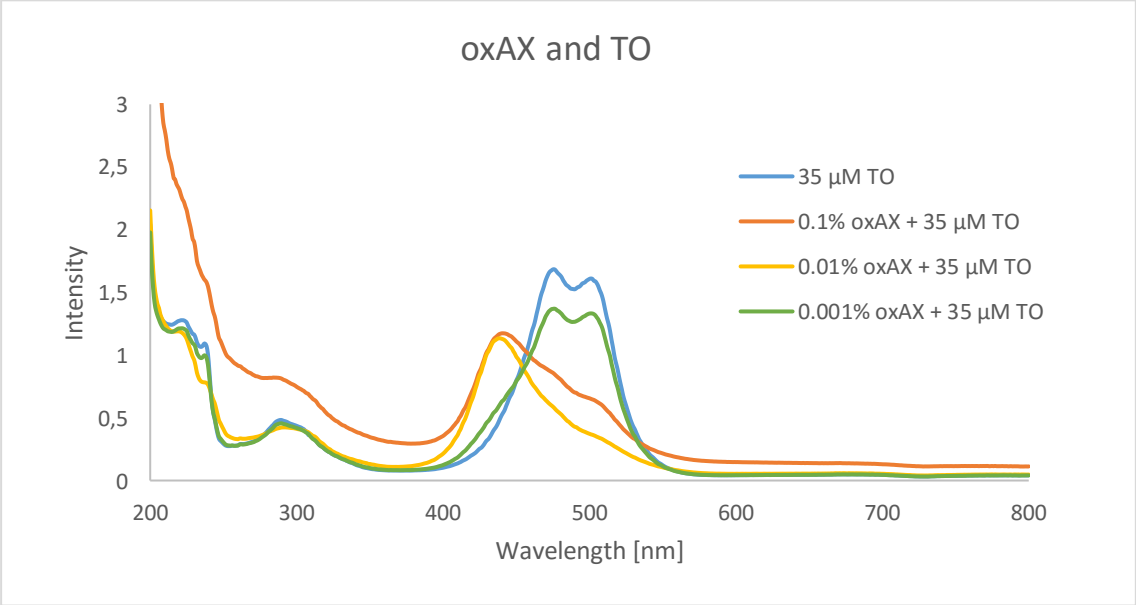

Figure 6. *Absorption spectra of 35 µM TO with varying concentration of oxAX*

Another objective of this report was to investigate if TO and BO could bond to the carbohydrate and then, when DNA was added the dyes should be released from carbohydrate and bind to DNA instead. To prove this samples containing TO or BO, DNA and oxAX or MeGlcA was prepared. To investigate the equilibriums the solutions was added in different orders. Absorption spectra's of this can be seen in figure 7, where DNA, TO and oxAX was used. The peak at around 440 nm represents only TO and oxAX, without the addition of DNA. The four peaks close together at around 500 nm are samples containing DNA, TO and oxAX, but with different methods of preparing the solutions.

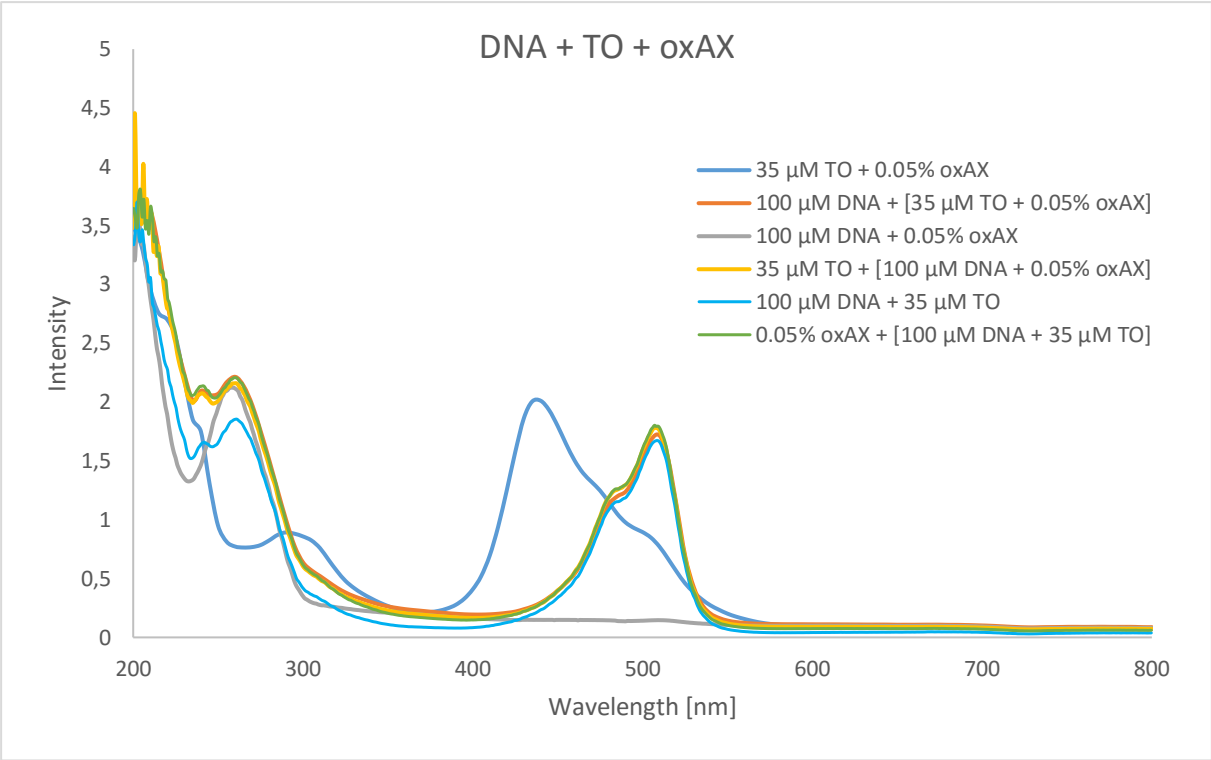

Figure 7. *Absorption spectra with different order of addition of DNA, TO and oxAX. The two solution specified inside the brackets was first prepared and the third solution was added after waiting for a couple of minutes.*

Fluorescence was also used to analyze the samples. The dyes themselves are shown to have no fluorescent properties alone in solution, as can be seen in figure A6 for BO and figure A7 for TO. When TO or BO are mixed with MeGlcA or oxAX, which can be seen in figure A8 and figure A9, figure A10 and figure A11, still gives insignificant fluorescence. The fluorescent properties arise when the dye are sterically hindered, such as when bonded to DNA. This behavior with DNA, BO or TO and oxAX or MeGlcA can be seen in figure A12, figure A13, figure A14 and figure A15. Observe that in figure A12 the concentration of TO is 35 µM and 0.05% oxAX while in the remaining the concentration of TO is 1 µM and 0.005% oAX. The emission bands containing DNA together with TO or BO give a high fluorescence while the other solutions gives little to no fluorescence. The lines which corresponds to DNA and TO or BO in solution are almost identical to each other and the order of which the substances are added does not seem to have any effect on the results. This supports that both TO and BO has a higher affinity for bonding to the DNA than to oxAX and MeGlcA.

It should be noted that the intensities of the lines in the different figures are not reliable to compare. The graphs was made on different days and the difference in intensity is due to the

face that cyanine dyes can degrade in solution over time. To avoid this the stock solutions of cyanine dyes would have to be prepared just before the measurements.

6 CONCLUSIONS

The results from this study shows that H aggregates of TO, but not BO, can be formed with oxidized arabinoxylan as a solid matrix. This result was also achieved with arabinoxylan and xylan which could mean that the arabinoxylan does not have to be oxidized for the cyanine dyes to be able to bond. Further studies are needed to determine the exact mechanisms and properties. In addition the oxidized arabinoxylan can work as a carrier of TO to DNA with a high rate of delivery.

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APPENDIX A

A. Fluorescence and UV-vis spectras

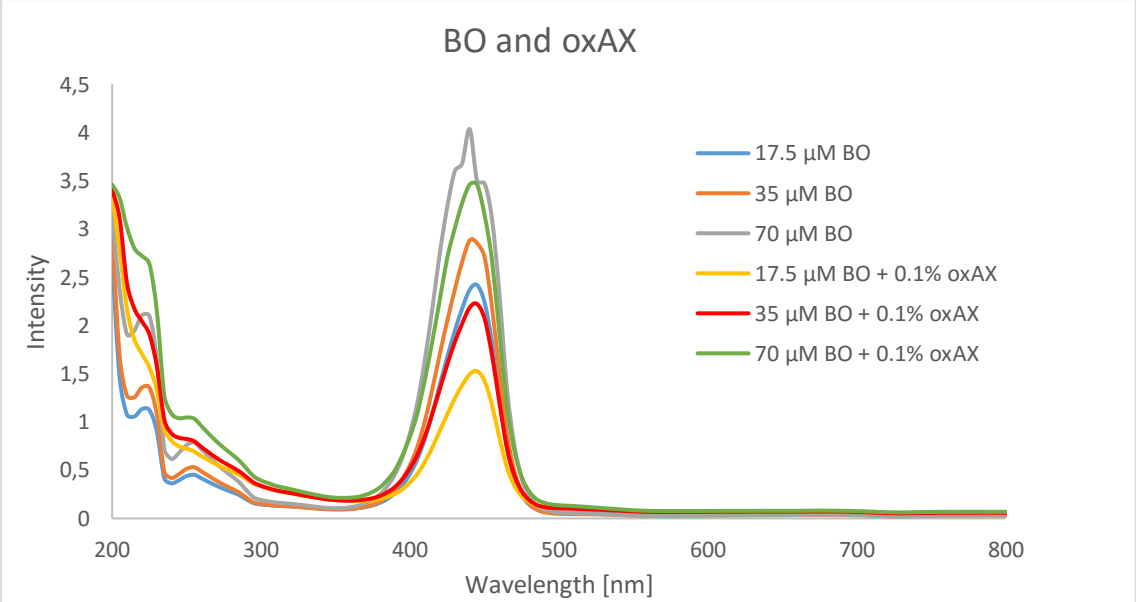

Figure A1. *Absorption spectra containing different concentrations of BO together with 0.1 wt% oxAX.*

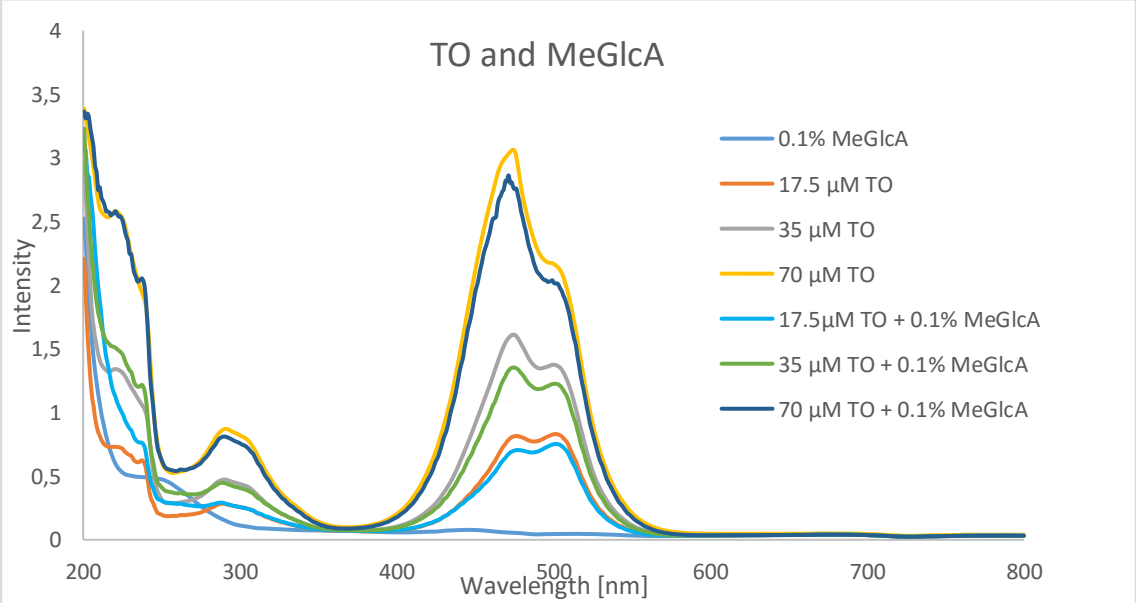

Figure A2. *Absorption spectra containing different concentrations of TO together 0.1 wt% MeGlcA.*

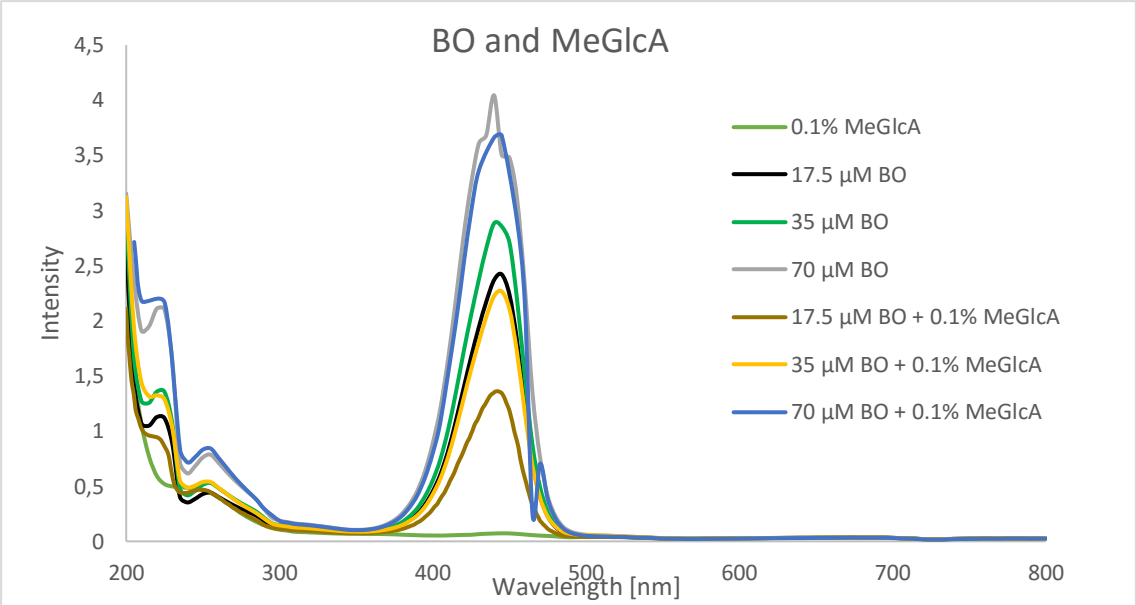

Figure A3. *Absorption spectra containing different concentrations of BO together with 0.1 wt% MeGlcA.*

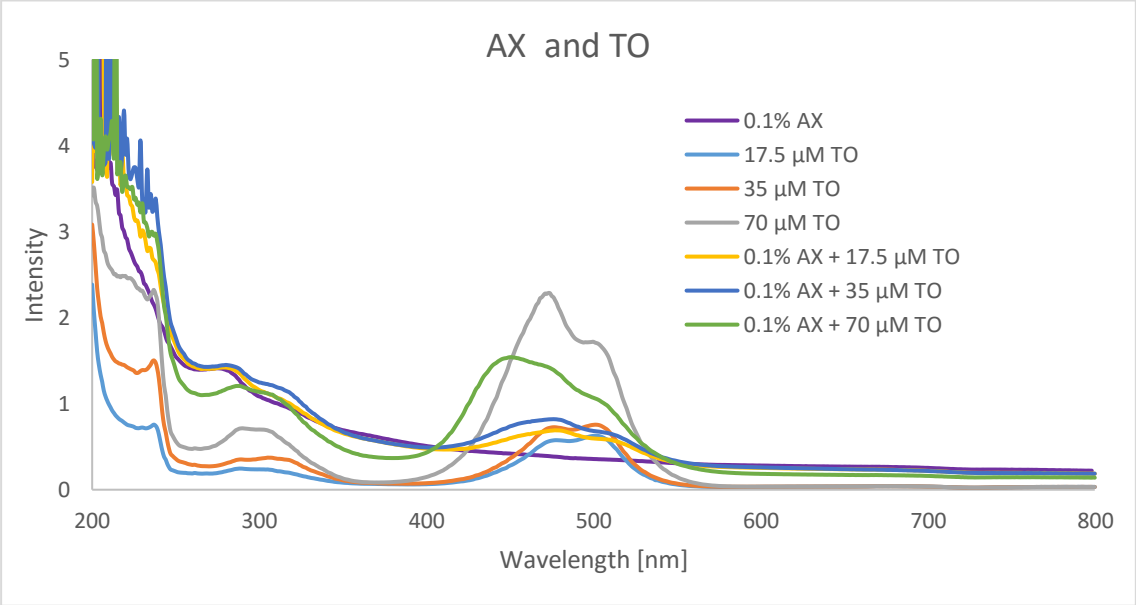

Figure A4. *Absorption spectra containing different concentrations of TO together with 0.1 wt% AX.*

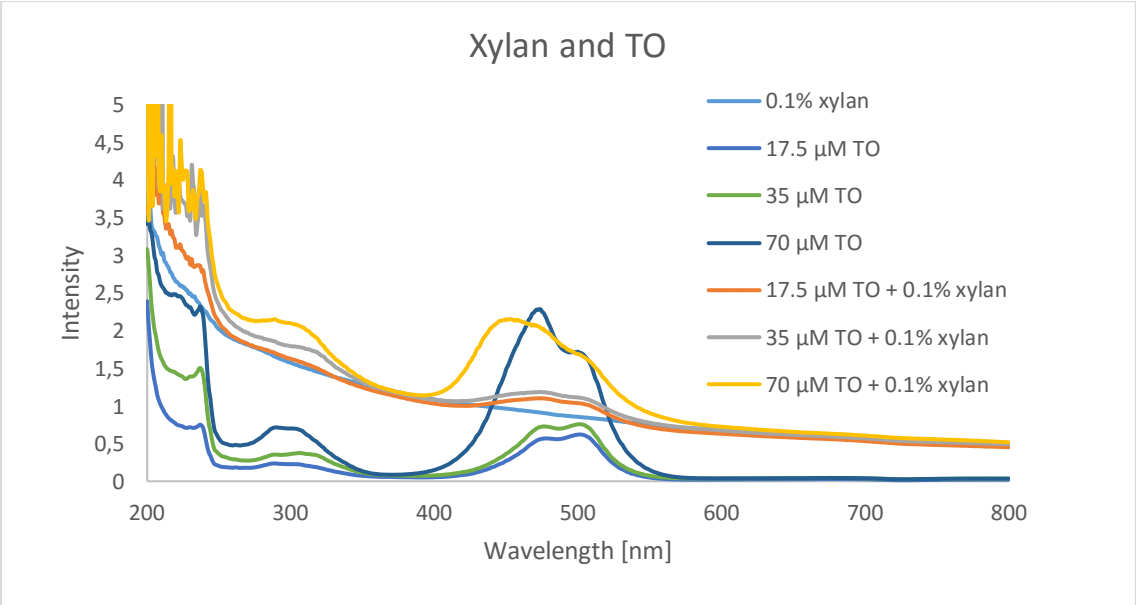

Figure A5. *Absorption spectra containing different concentrations of TO together with 0.1 wt% Xylan.*

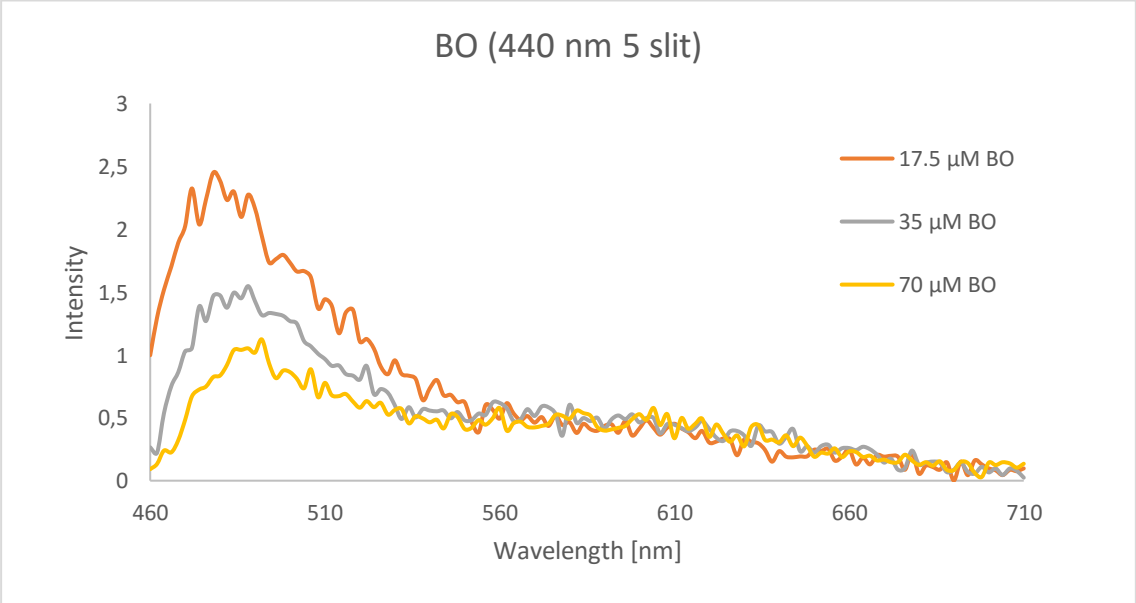

Figure A6. *Emission spectra of different concentrations of BO excited at 440 nm using a 5 slit emission and excitation slits.*

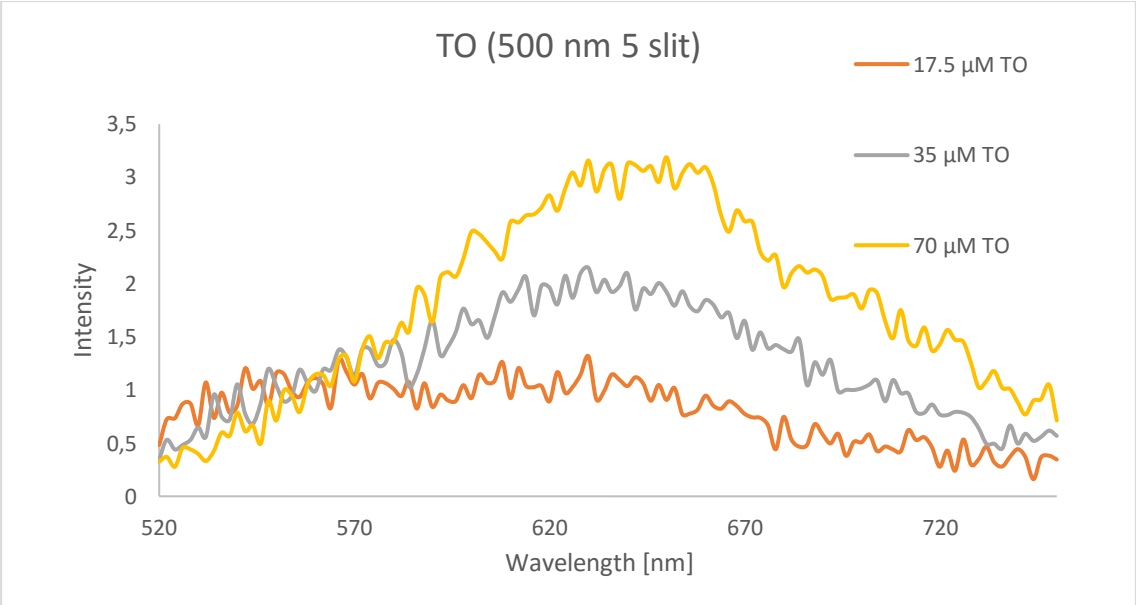

Figure A7. *Emission spectra of different concentrations of TO excited at 500 nm using a 5 slit emission and excitation slits.*

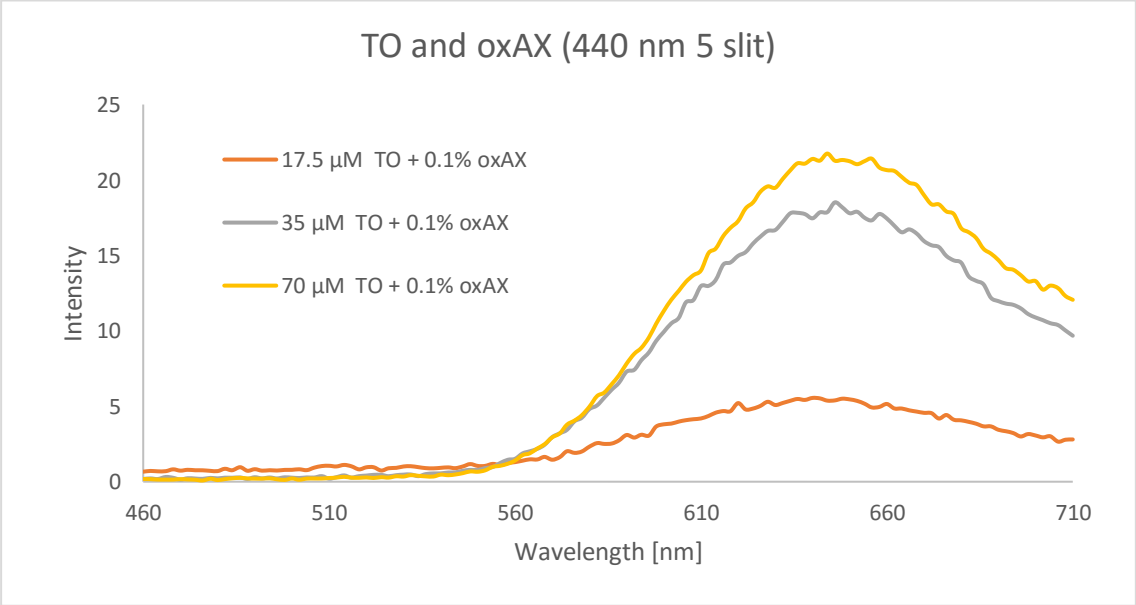

Figure A8. *Emission spectra of different concentrations of TO with 0.1 wt% MeGlcA excited at 500 nm using a 5 slit emission and excitation slits.*

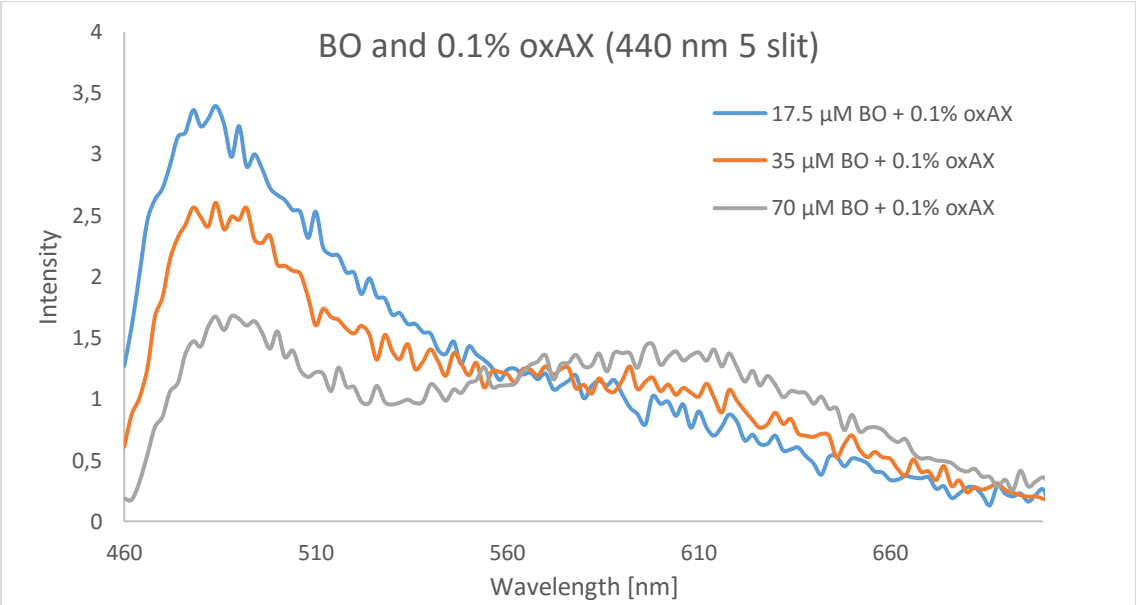

Figure A9. *Emission spectra of different concentrations of BO with 0.1 wt% oxAX excited at 500 nm using a 5 slit emission and excitation slits.*

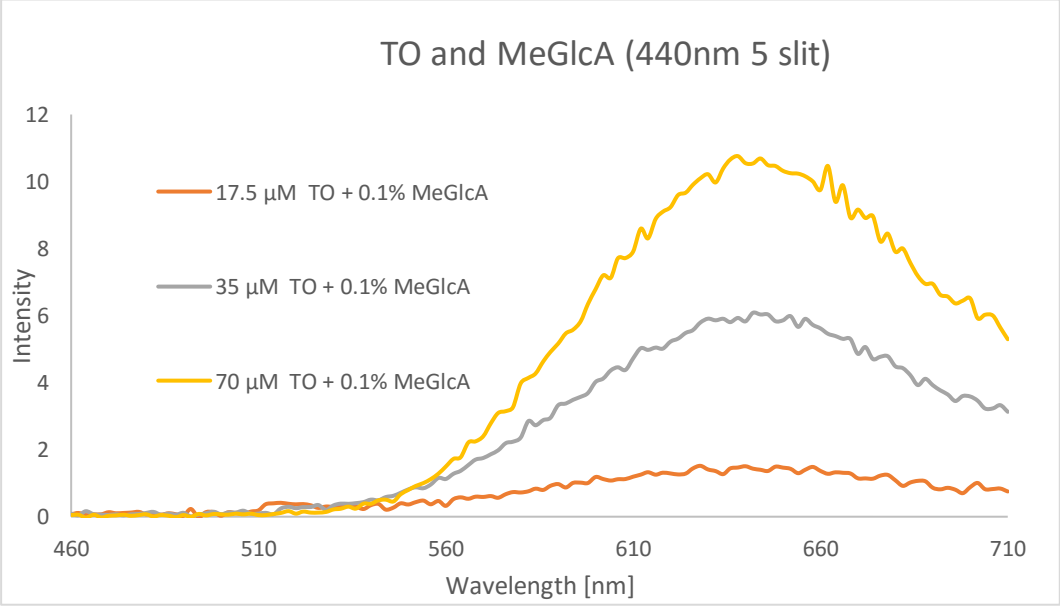

Figure A10. *Emission spectra of different concentrations of BO with 0.1 wt% MeGlcA excited at 440 nm using a 5 slit emission and excitation slits.*

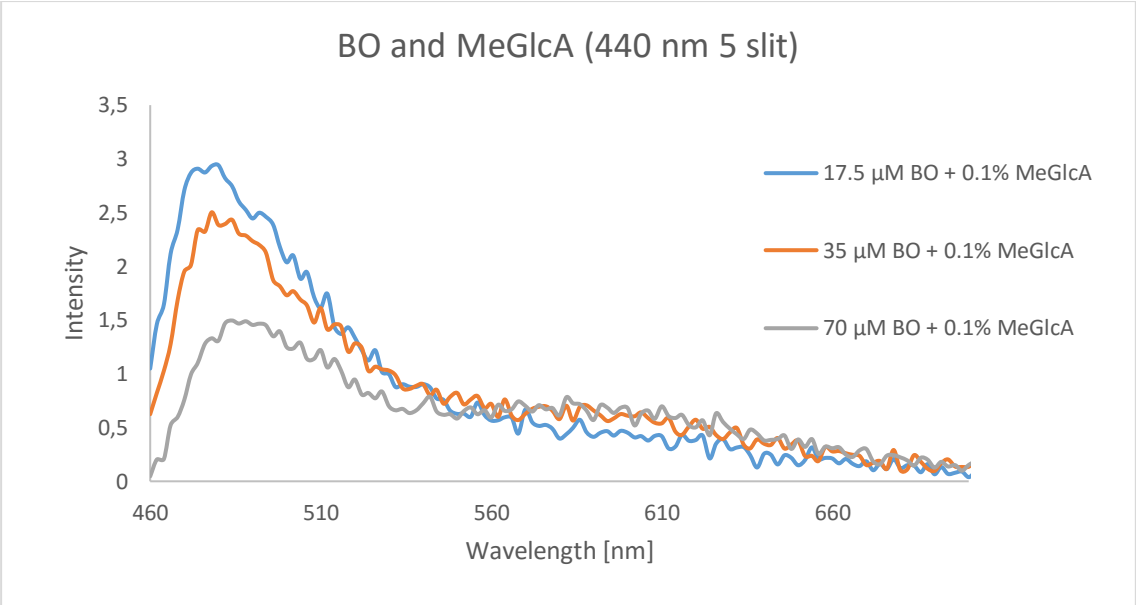

Figure A11. *Emission spectra of different concentrations of BO with 0.1 wt% MeGlcA excited at 440 nm using a 5 slit emission and excitation slits.*

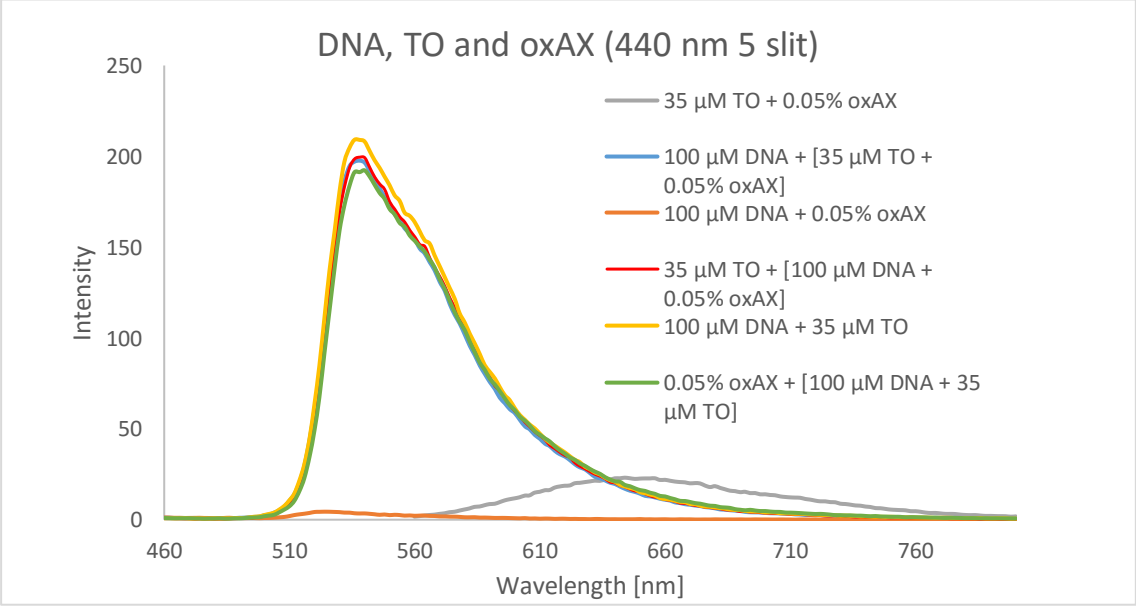

Figure A12. *Emission spectra of 100 µM DNA, 35 µM TO and 0.05 wt% oxAX added in different orders at 440 nm and 5 slit size of excitation and emisson. The two solutions inside the brackets was prepared first and the third one was added later.*

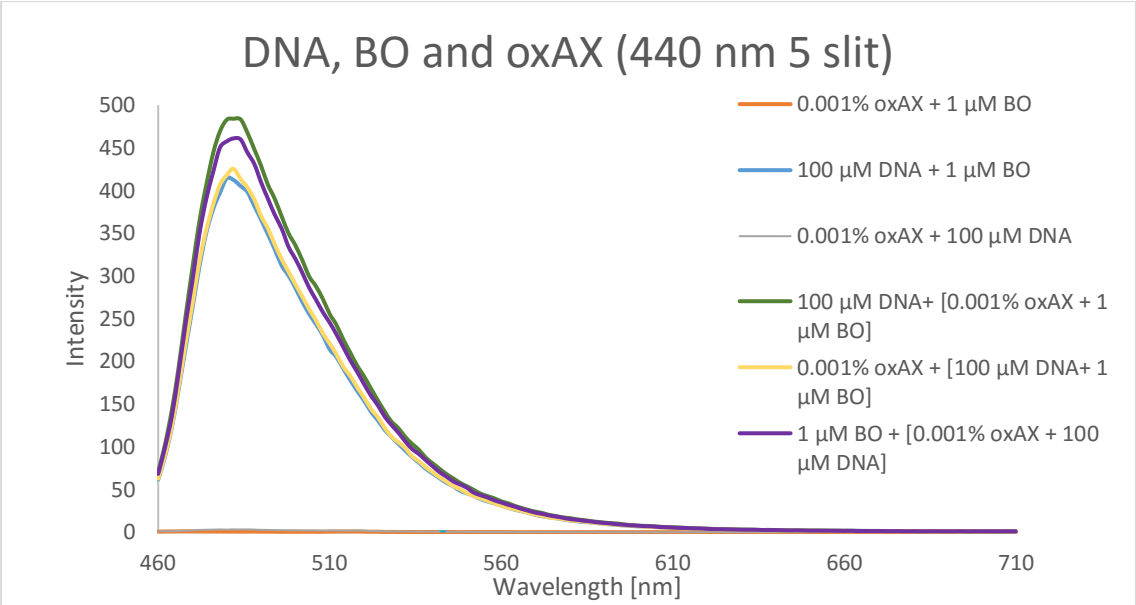

Figure A13. *Emission spectra of 100 µM DNA, 1 µM BO and 0.001 wt% oxAX added in different orders at 440 nm and 5 slit size of excitation and emisson. The two solutions inside the brackets was prepared first and the third one was added later.*

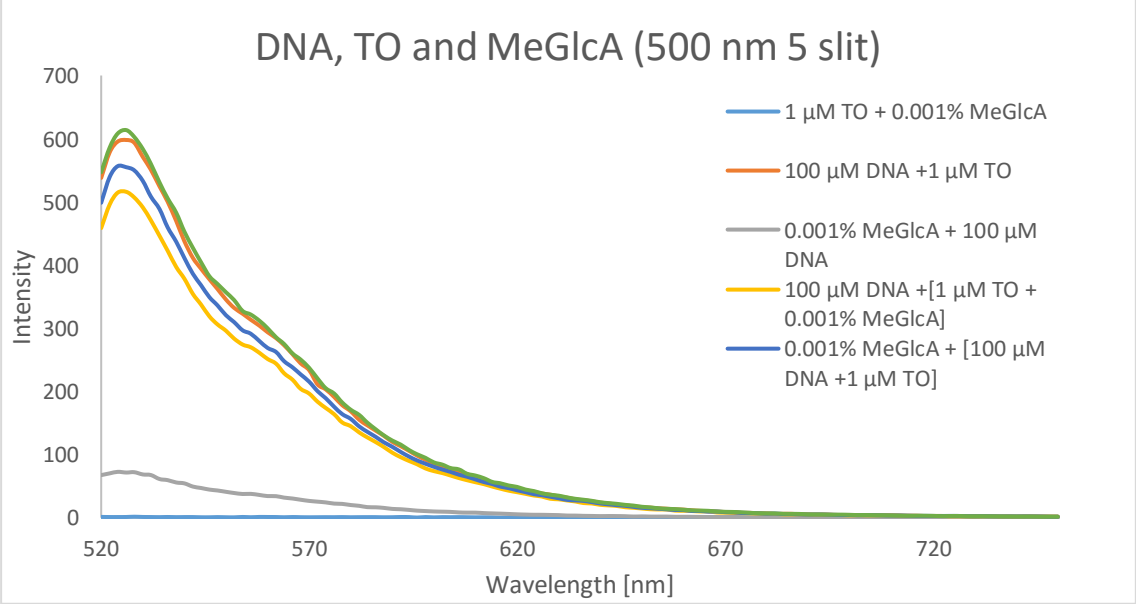

Figure A14. *Emission spectra of 100 µM DNA, 1 µM TO and 0.001 wt% MeGlcA added in different orders at 500 nm and 5 slit size of excitation and emisson. The two solutions inside the brackets was prepared first and the third one was added later.*

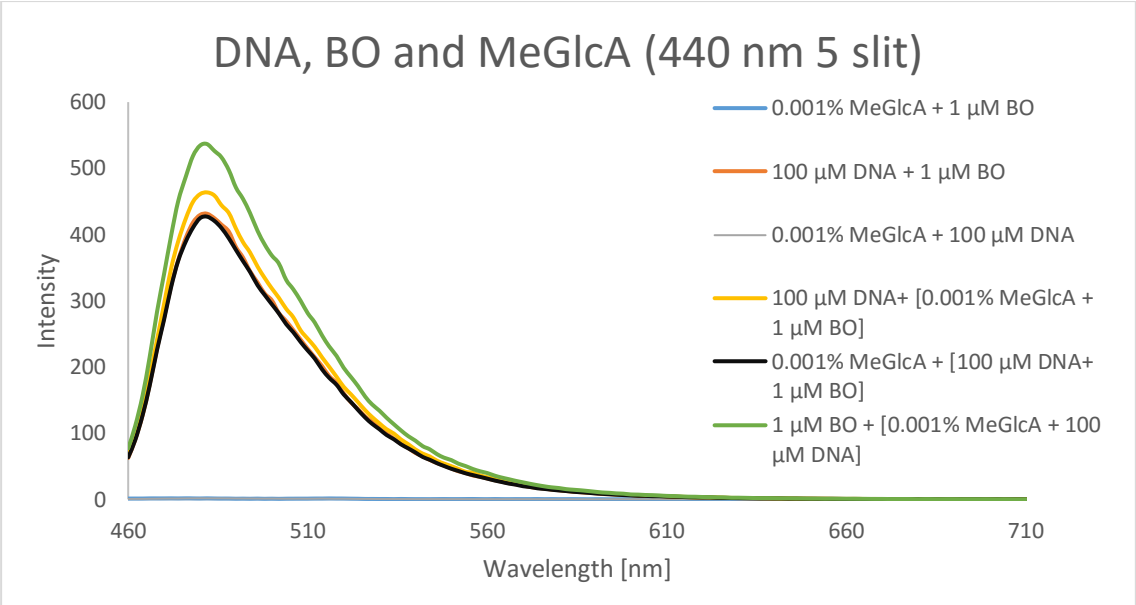

Figure A15. *Emission spectra of 100 µM DNA, 1 µM BO and 0.001 wt% MeGlcA added in different orders at 440 nm and 5 slit size of excitation and emisson. The two solutions inside the brackets was prepared first and the third one was added later.*

APPENDIX B

B. ^{1}H NMR figures and tables

Table B1. *^{1}H NMR chemical shifts in ppm for BO tosylate.*

	^{1}H NMR chemical shifts in ppm
H_A	8.08
H_B	7.40
H_C	6.14
H_D	7.52
H_E	7.48
H_F	7.30
H_G	7.76
H_H	7.69
H_I	7.21

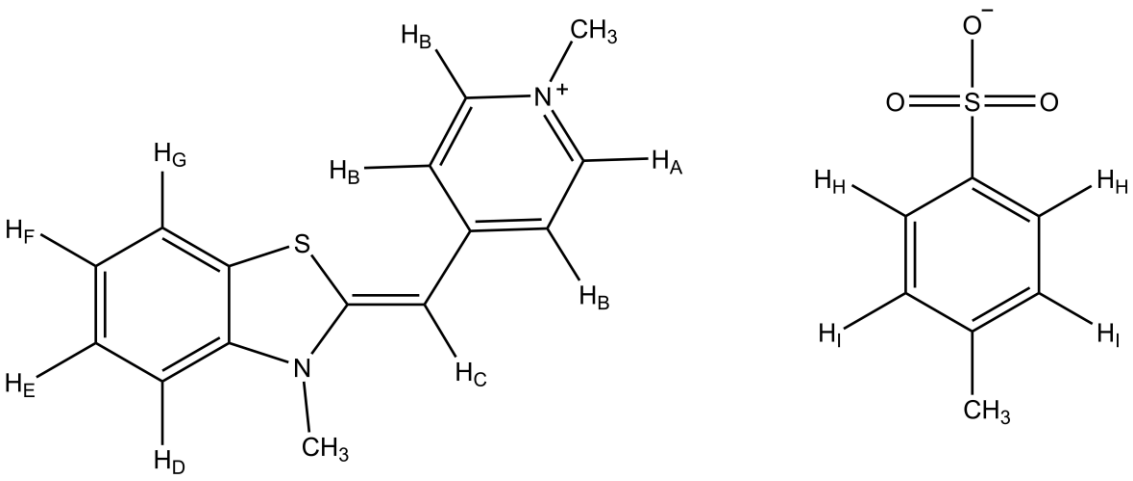

Figure B1. Chemical structure of BO tosylate with hydrogens drawn and named. Hydrogen with the same chemical shifts have the same name. The methyl groups have not been named.

Table B2. *^{1}H NMR chemical shifts in ppm for BO tosylate.*

	^{1}H NMR chemical shift in ppm
H_A	8.37
H_B	7.45
H_C	6.91
H_D	8.65
H_E	7.76
H_F	7.98
H_G	8.01
H_H	7.69
H_I	7.21
H_a	7.87
H_b	7.40
H_c	7.59
H_d	7.65

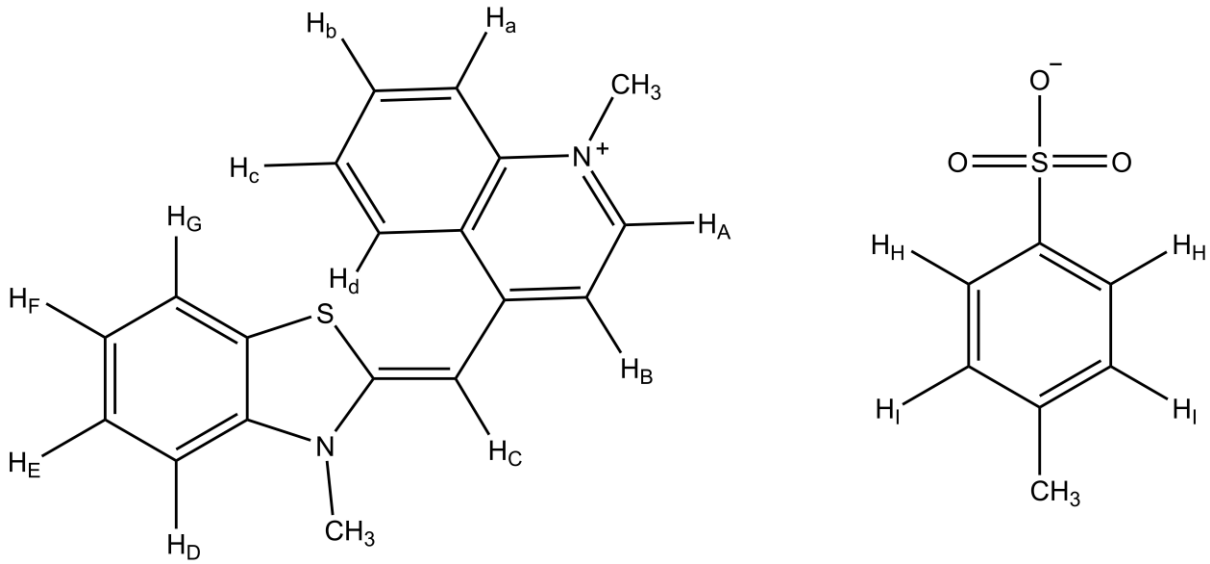

Figure B1. Chemical structure of TO tosylate with hydrogens drawn and named. Hydrogen with the same chemical shifts have the same name. The methyl groups have not been named.